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(54) **PHOTO-CROSSLINKED NUCLEIC ACID
HYDROGELS**

Publication Classification

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C07K 2/00 (2006.01)

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C07H 21/02 (2006.01)

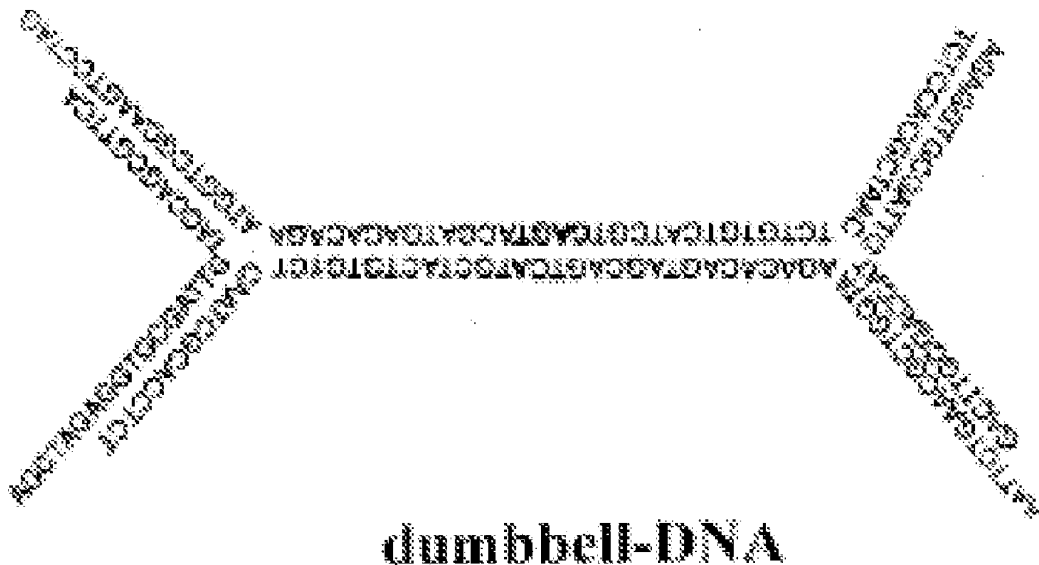
C07H 21/04 (2006.01)

(52) **U.S. Cl. 435/68.1; 536/23.1; 530/322; 530/358;
536/25.3**

(57)

ABSTRACT

Methods and compositions are provided for producing hydro-
gel nucleic acid structures using photo-crosslinking. Methods
of using the photo-crosslinked hydrogels for cell-free protein
production, and for encapsulating and delivering compounds,
are also provided.



dumbbell-DNA

Schematics of the synthesis of photocrosslinked DNA hydrogels

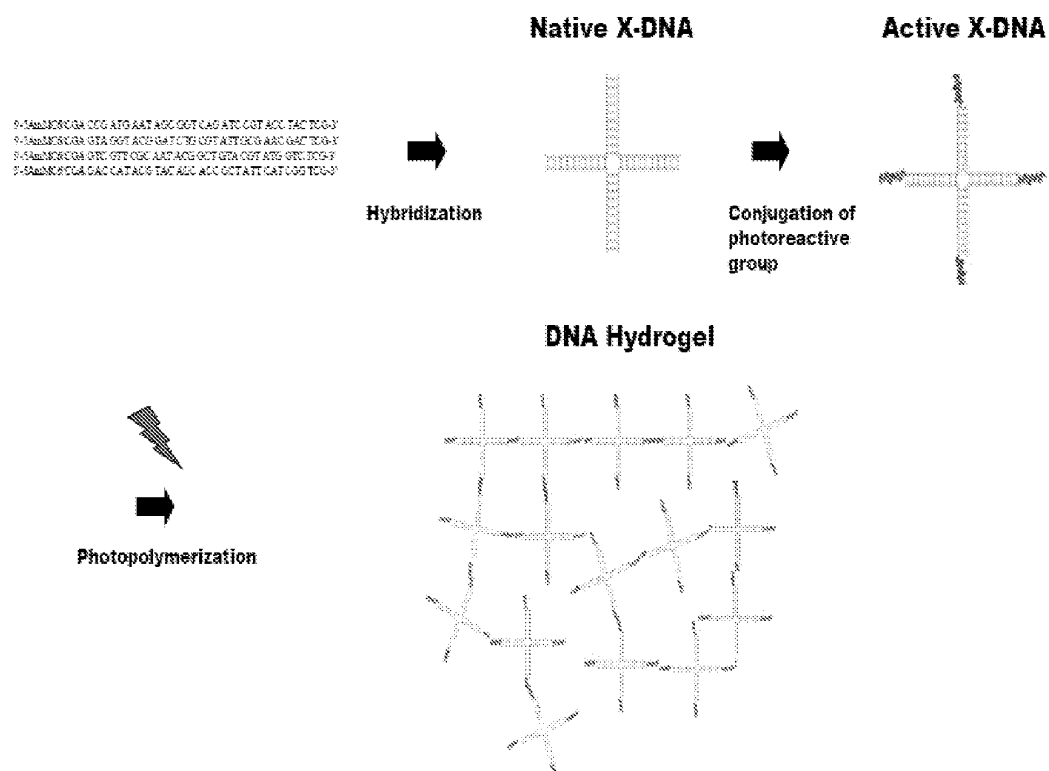


FIGURE 1

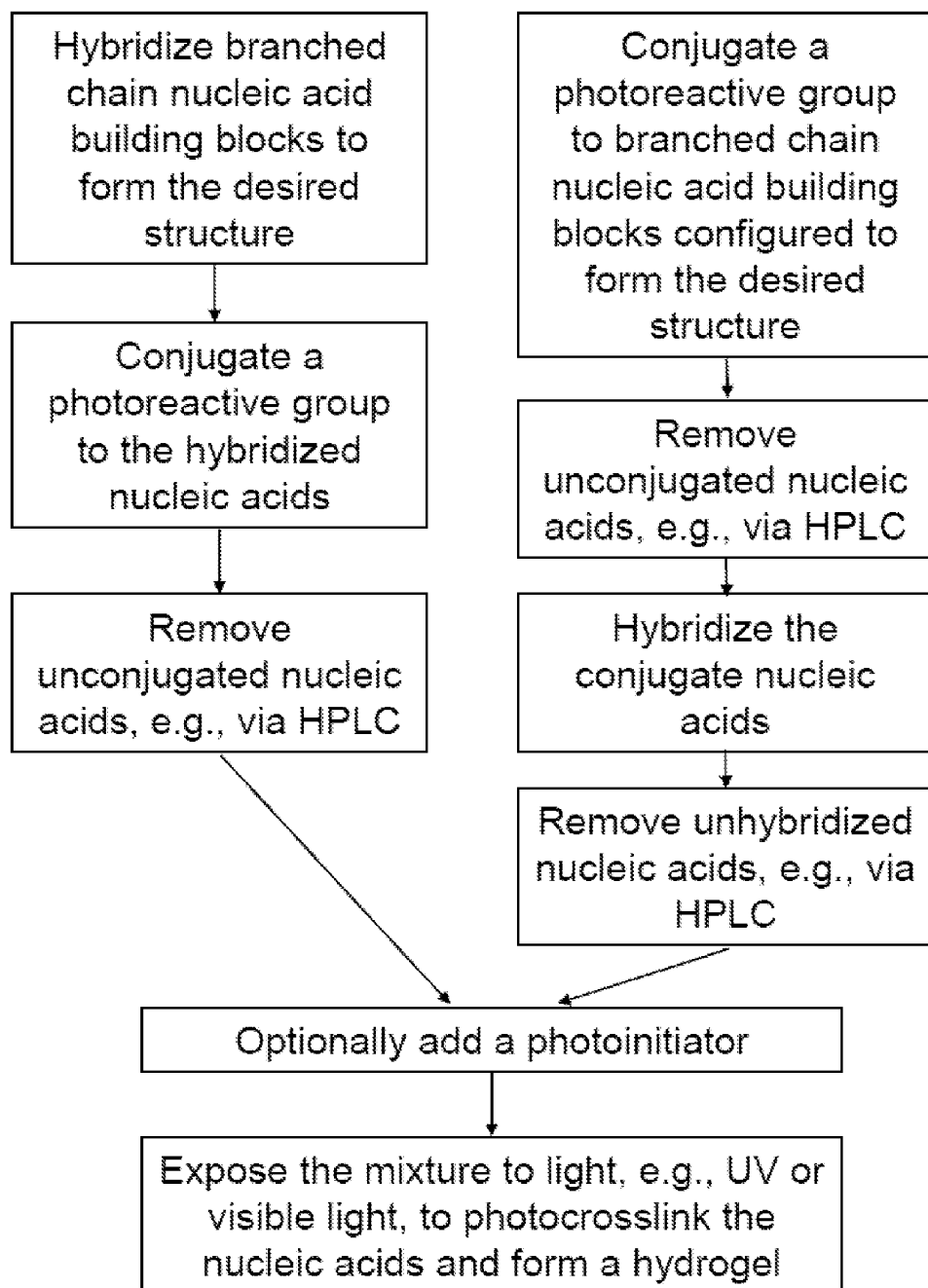


FIGURE 2

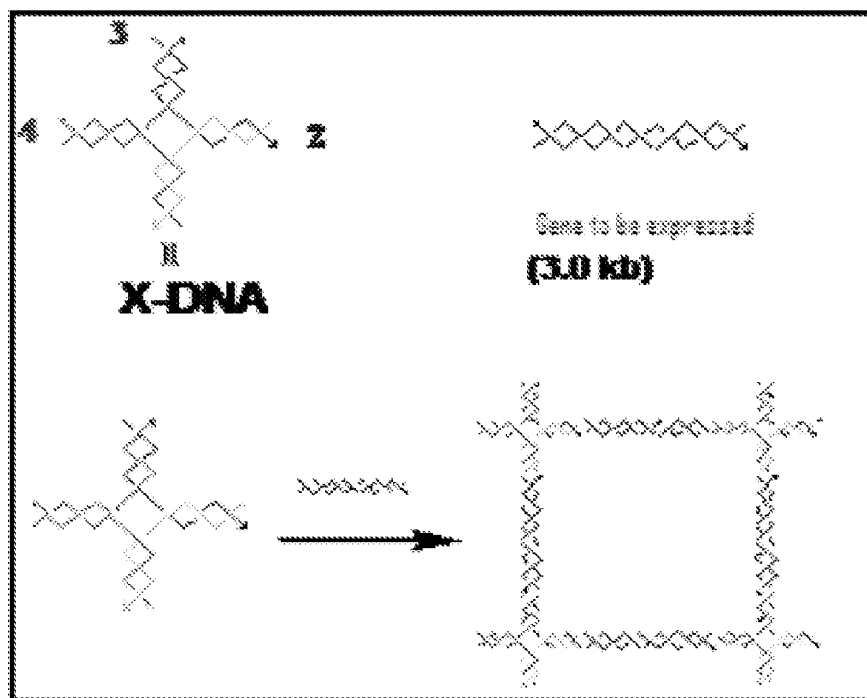


FIGURE 3A

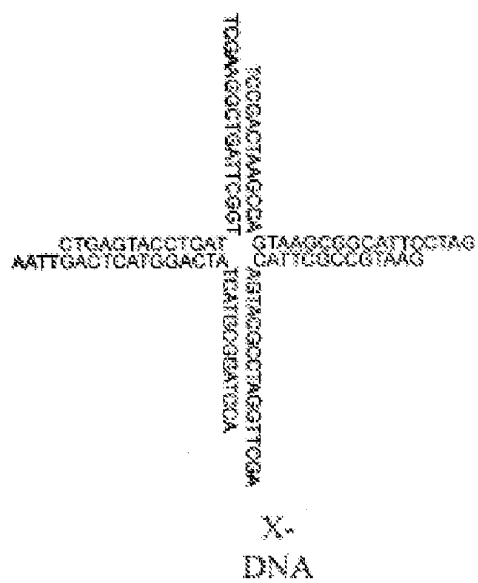


FIGURE 3B

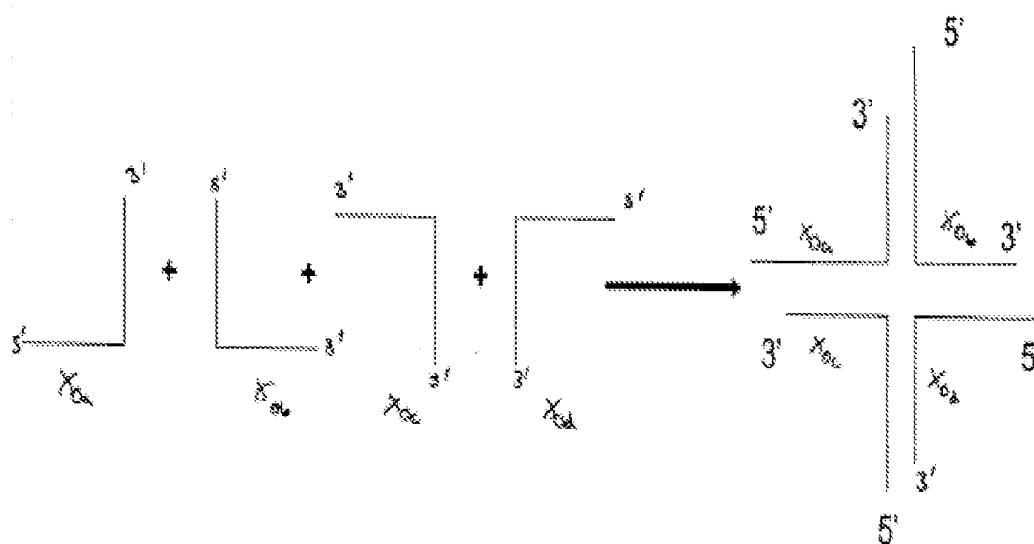


FIGURE 4

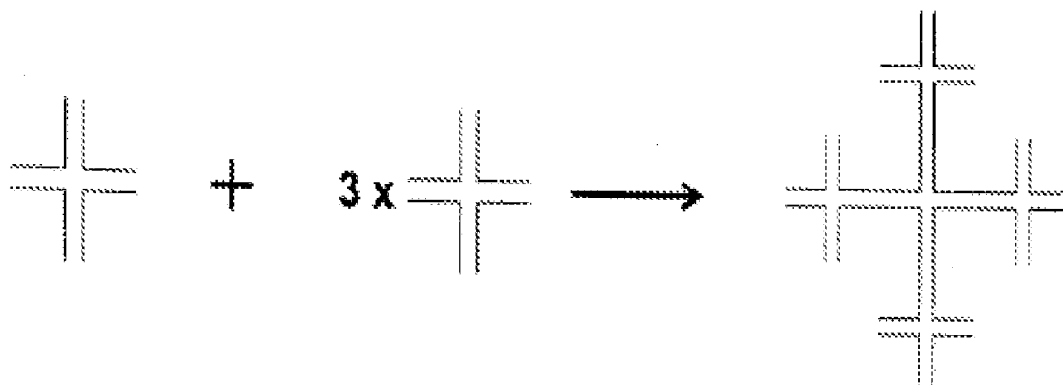


FIGURE 5

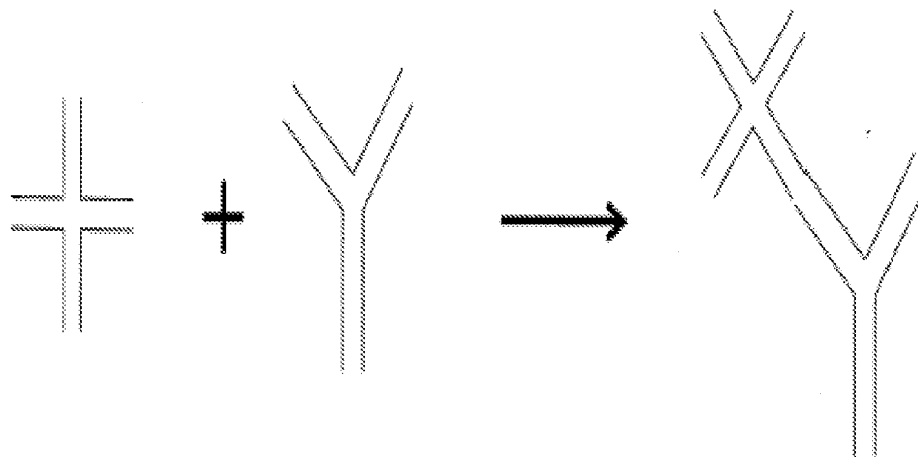


FIGURE 6A

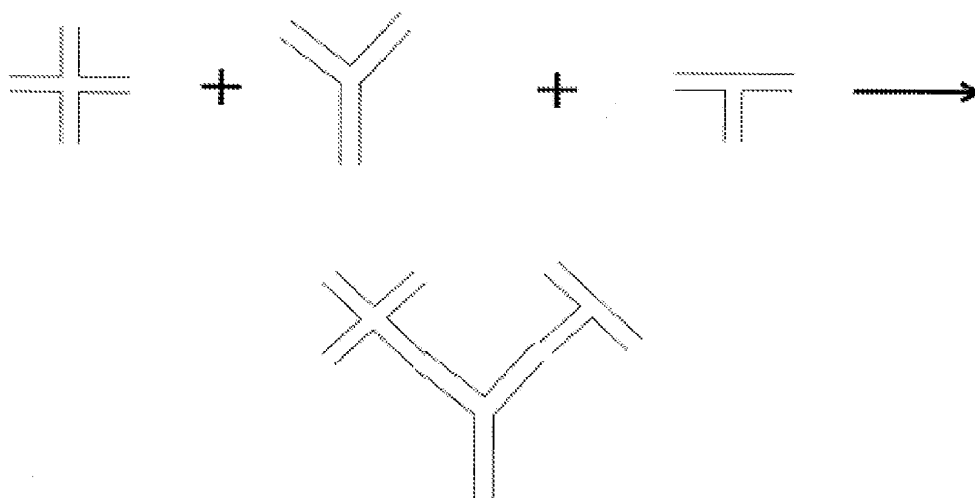


FIGURE 6B

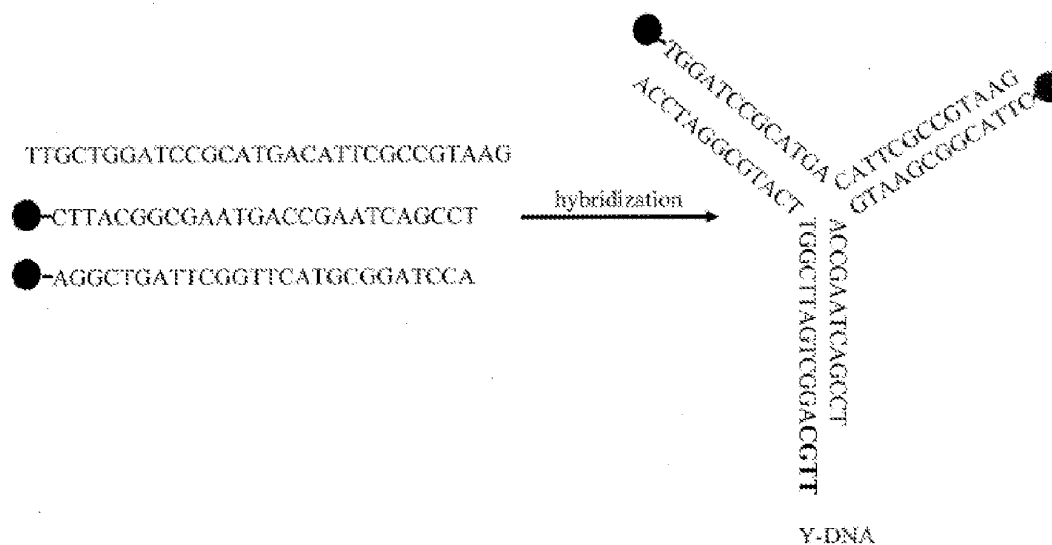


FIGURE 7A

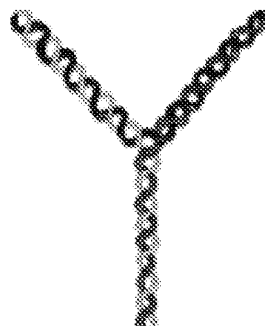
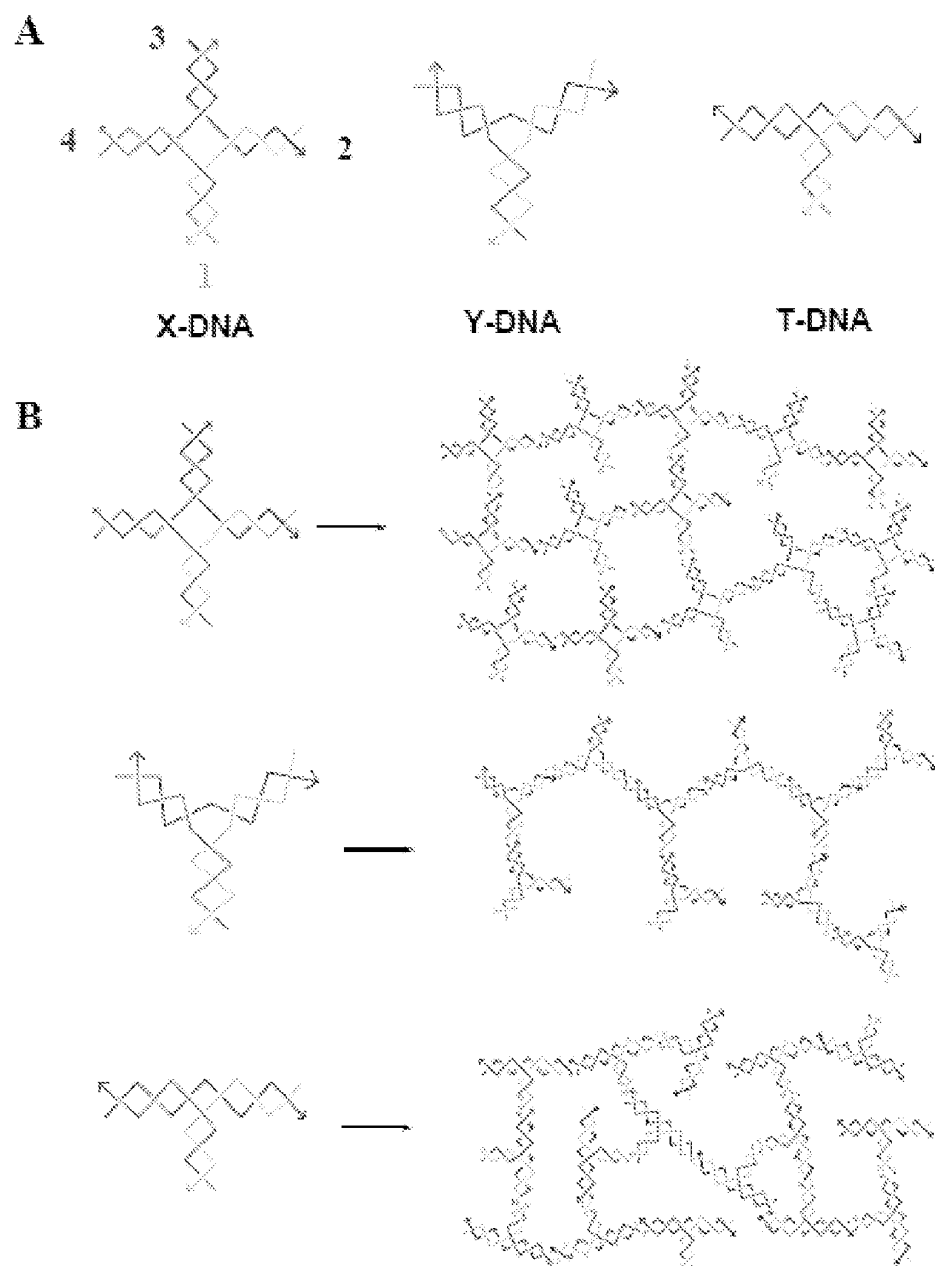


FIGURE 7B



FIGURES 8A and 8B

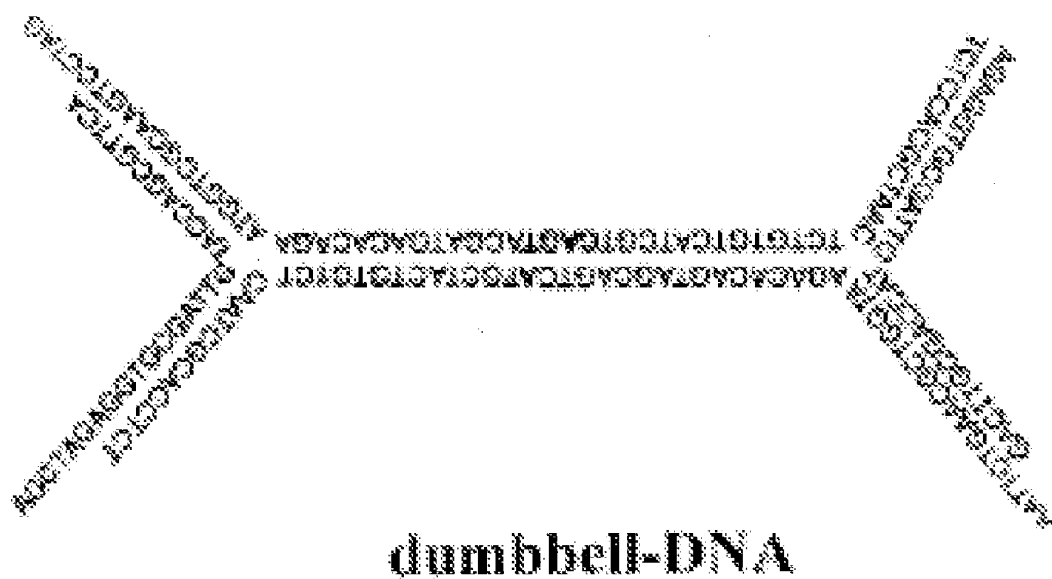


FIGURE 9

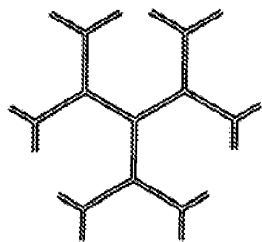


FIGURE 10A

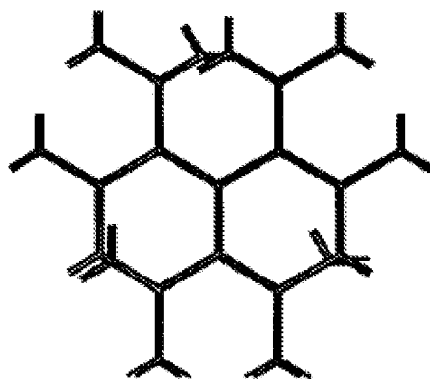


FIGURE 10B

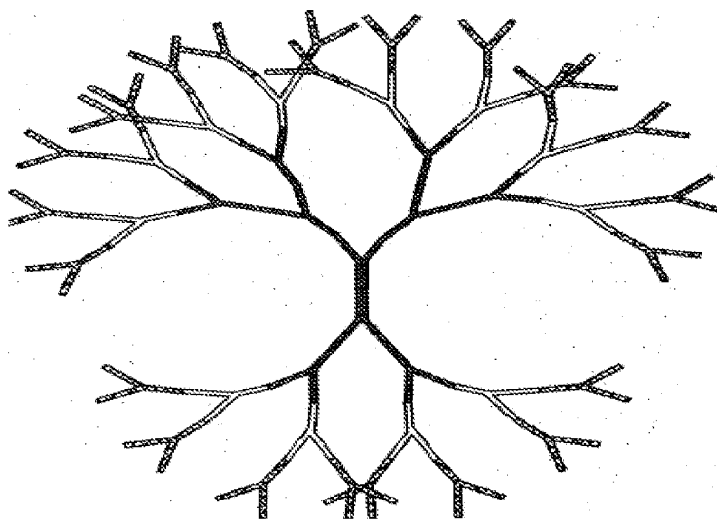


FIGURE 10C

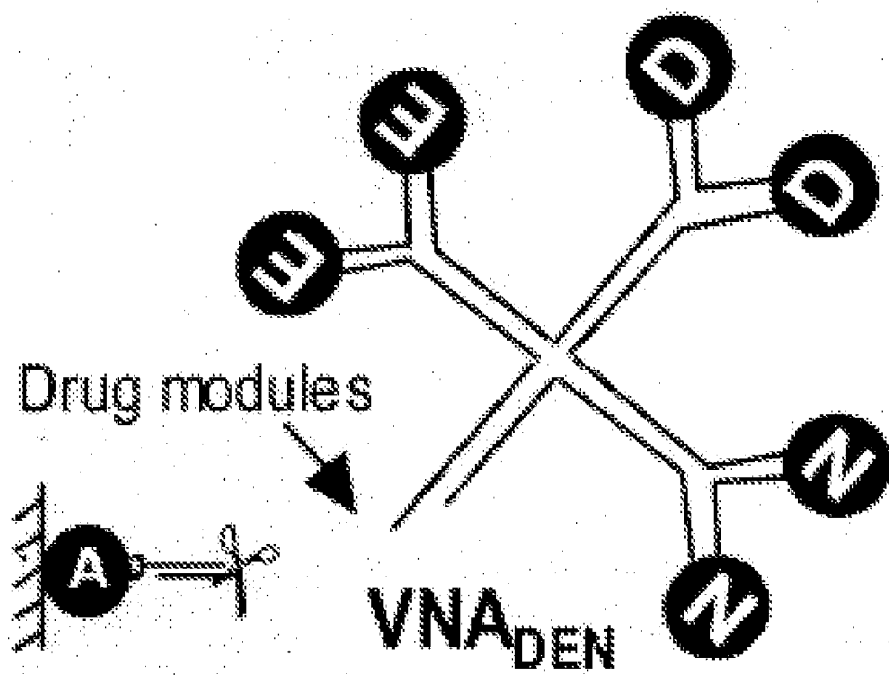


FIGURE 11

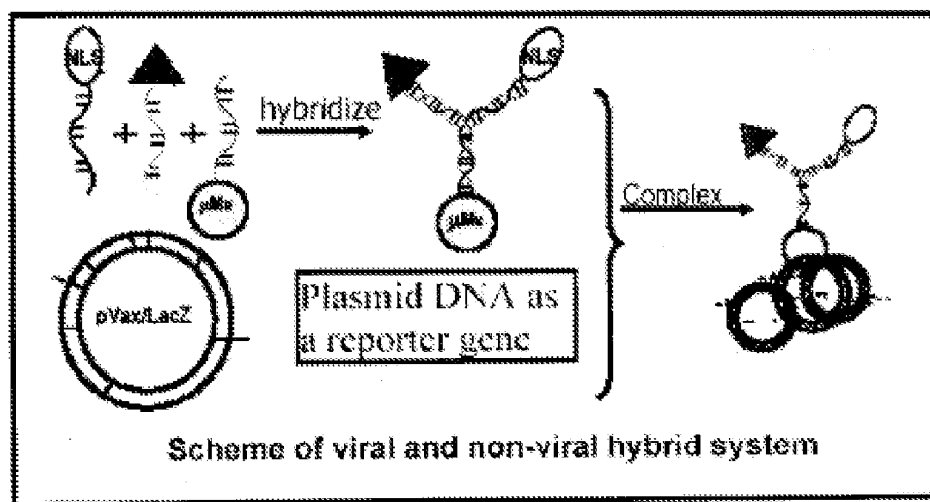


FIGURE 12

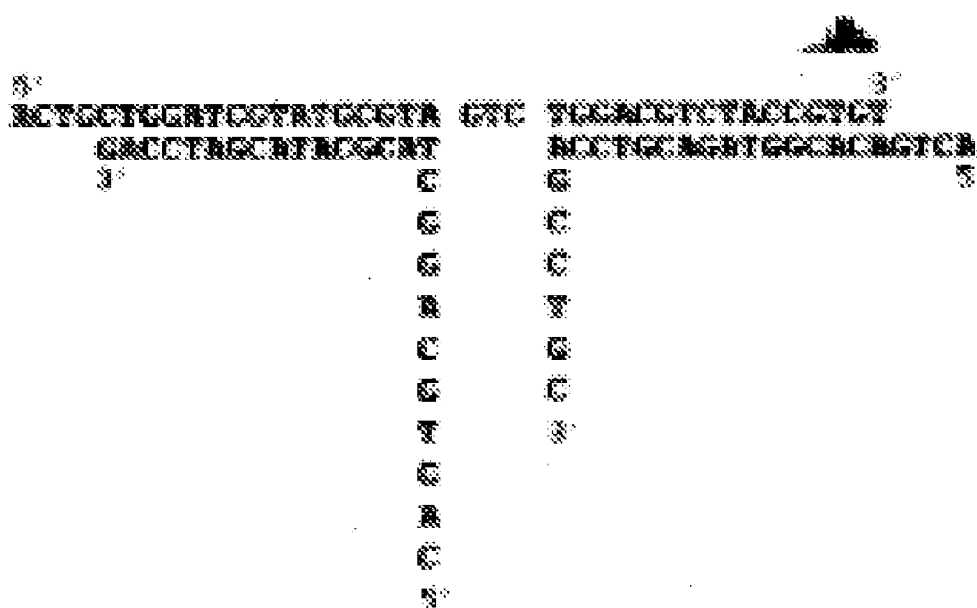


FIGURE 13

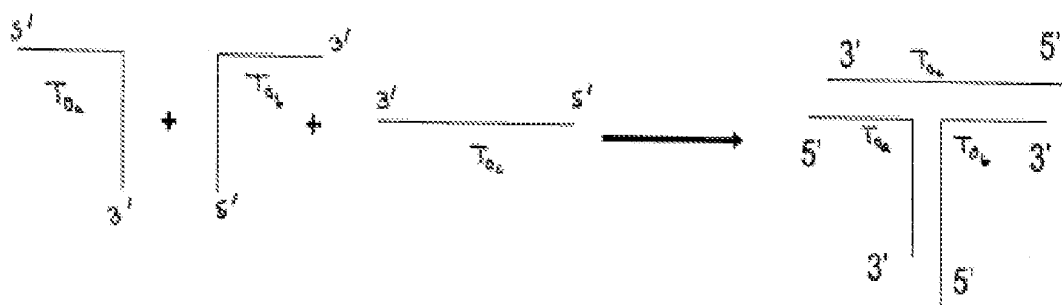


FIGURE 14

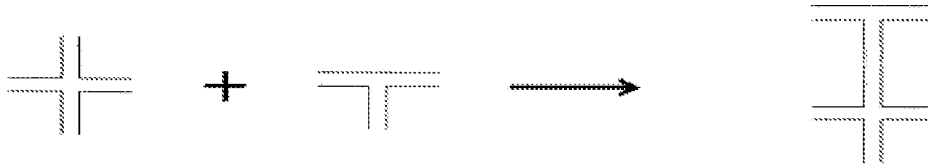


FIGURE 15A

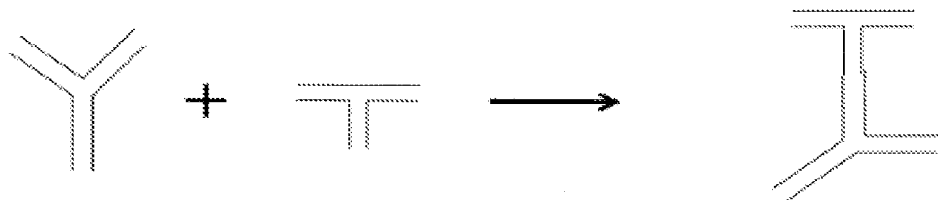


FIGURE 15B

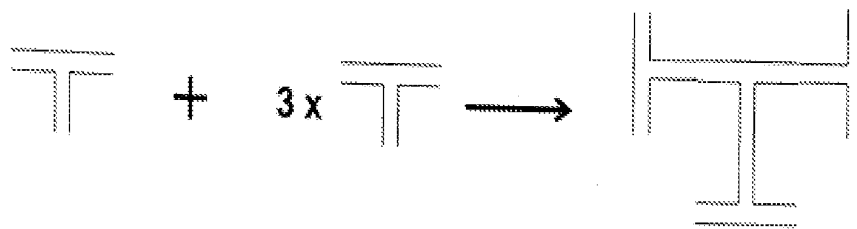


FIGURE 15C

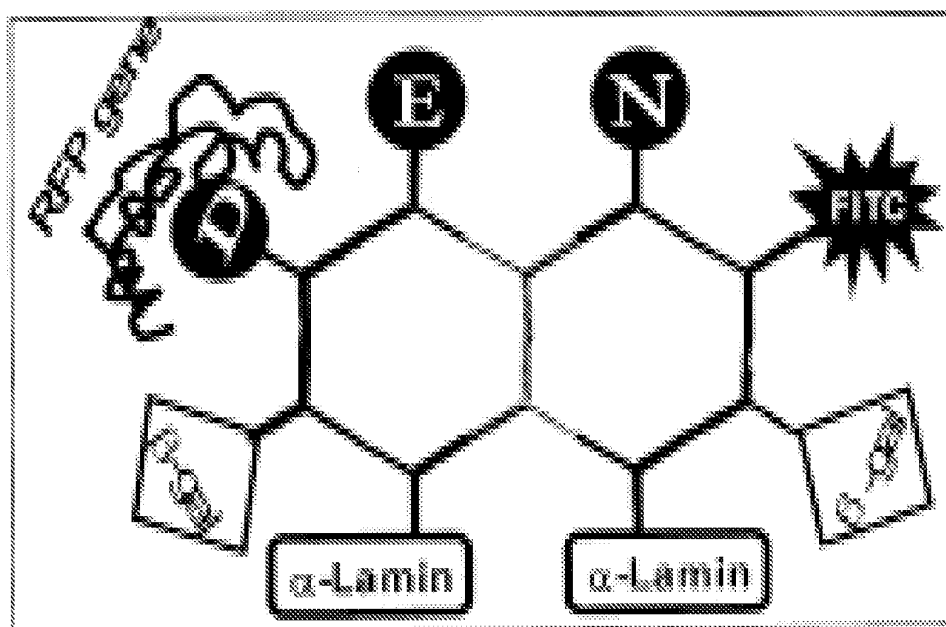


FIGURE 16

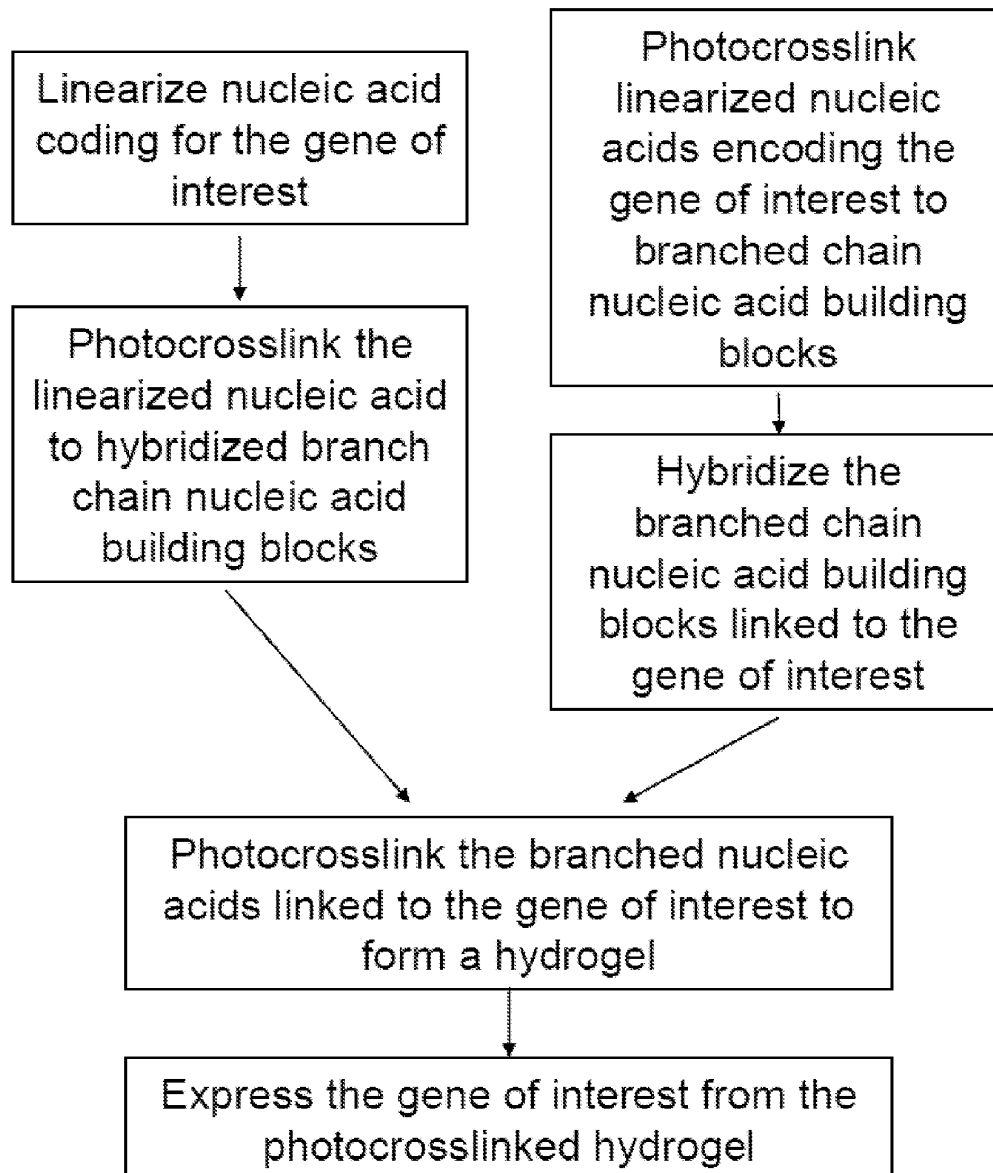
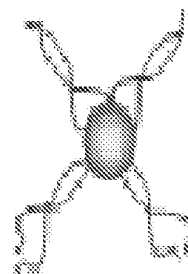
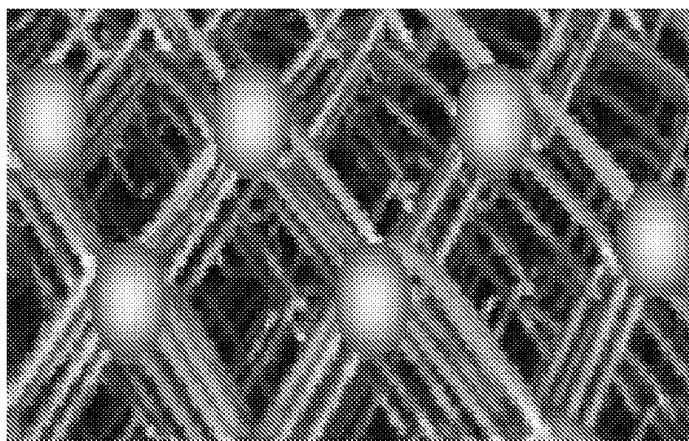


FIGURE 17



AuNP

FIGURE 18

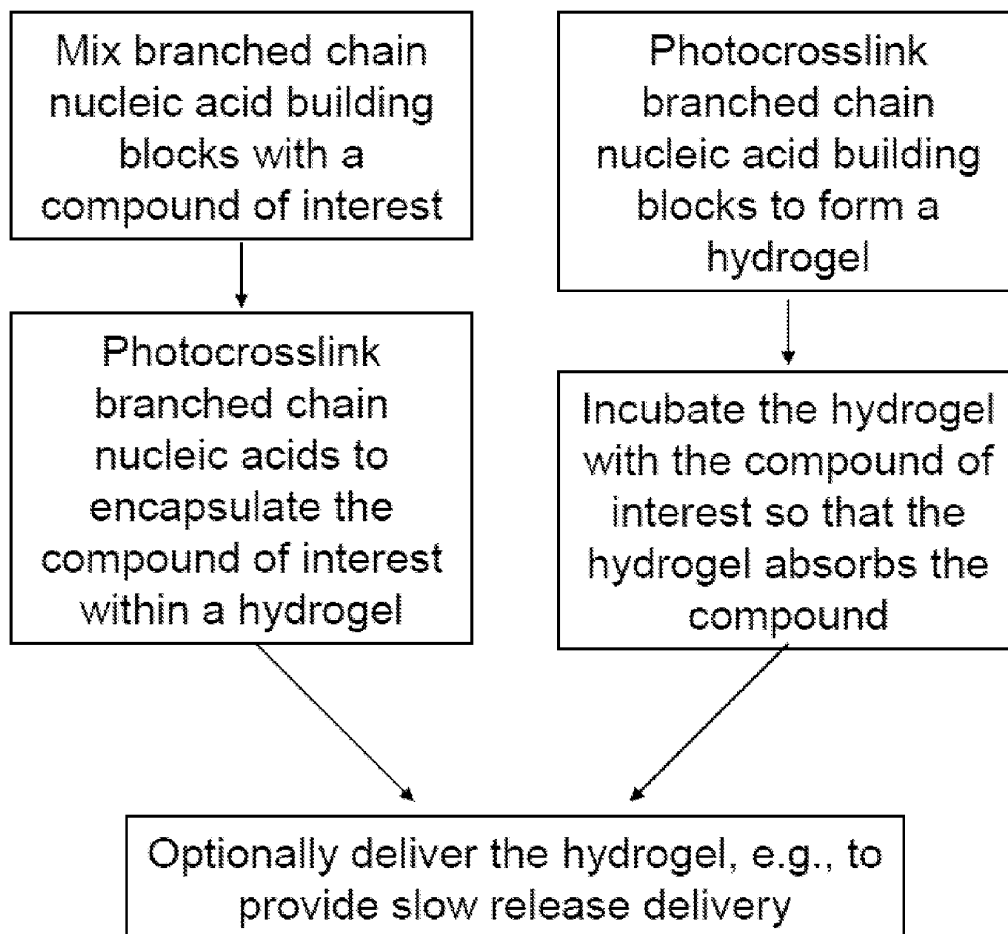


FIGURE 19

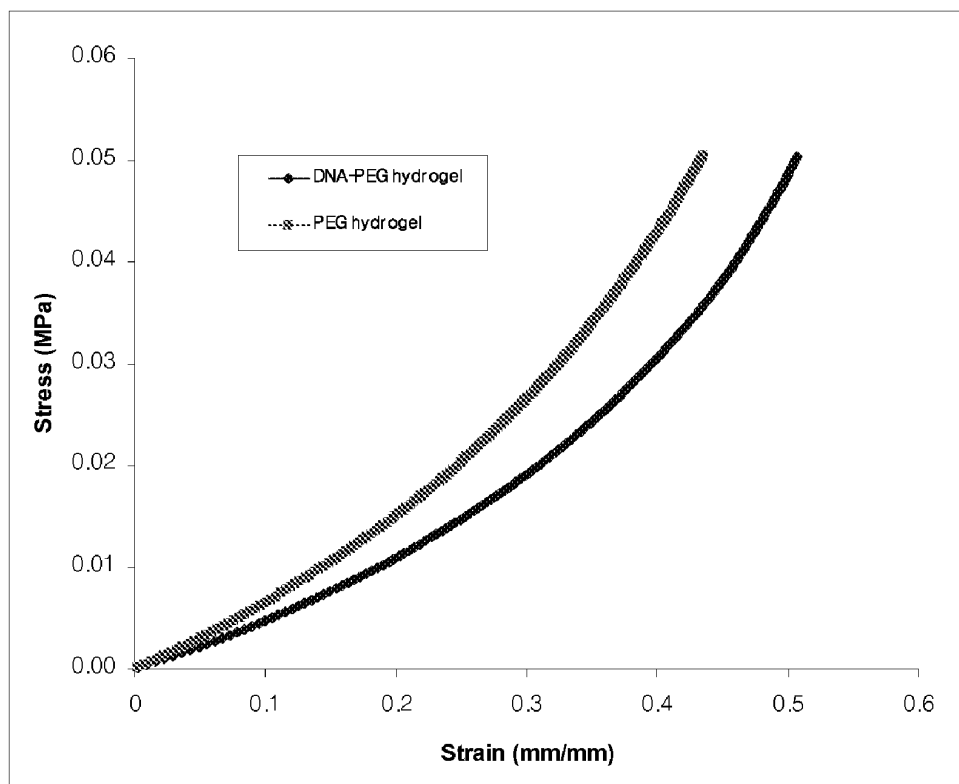
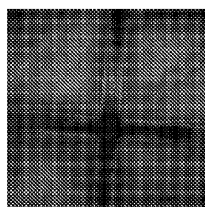
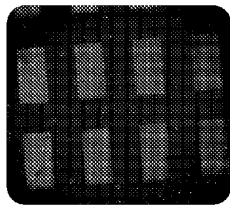


FIGURE 20

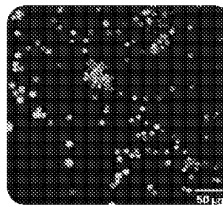
A)



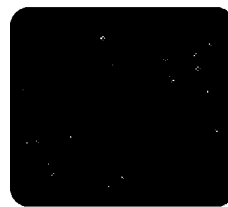
Micro thin films



Micropads



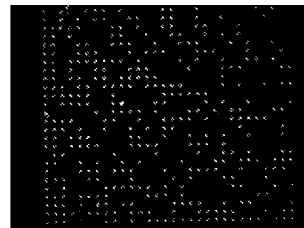
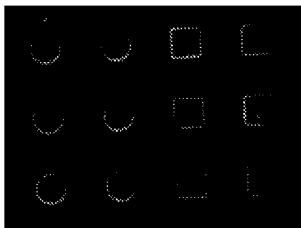
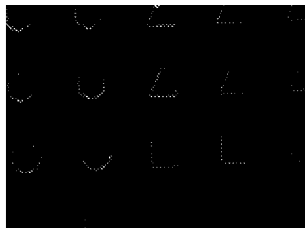
Microdroplet



Nanosphere

(1mm*1mm*20um)

B)



(All at 40X magnification)

C)

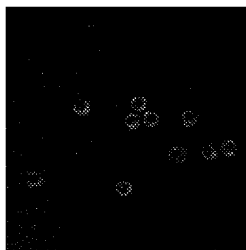
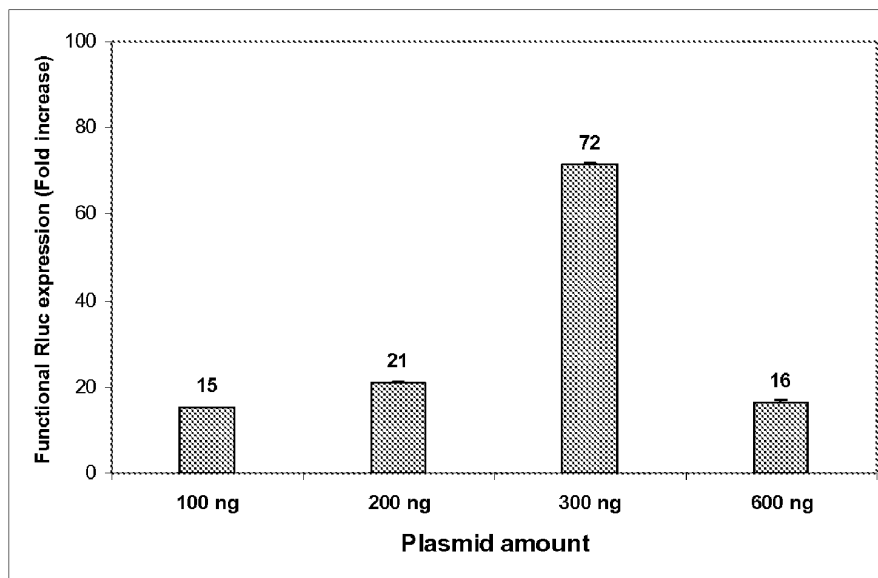


FIGURE 21

A)



B)

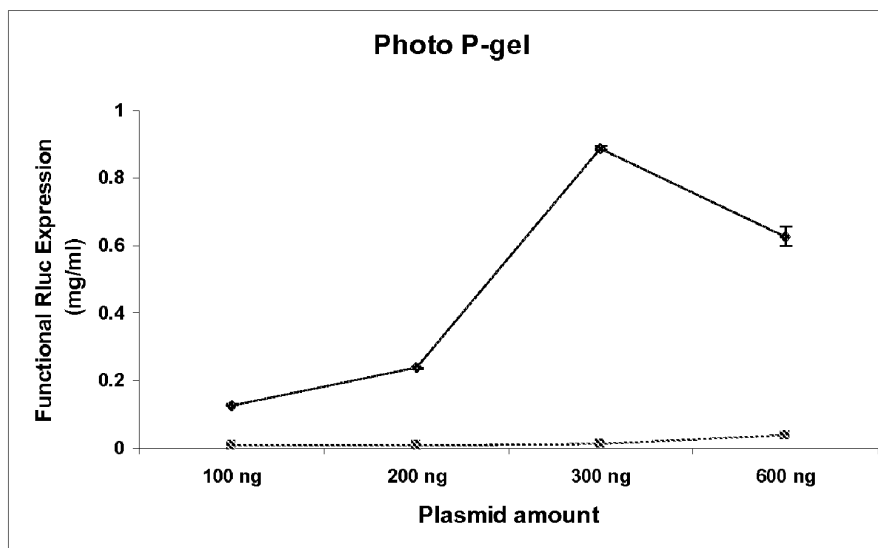


FIGURE 22

PHOTO-CROSSLINKED NUCLEIC ACID HYDROGELS

CROSS-REFERENCE

[0001] This application claims priority under 35 USC §119 to U.S. Provisional Application No. 61/086,423, filed Aug. 5, 2008 and entitled “PHOTO-CROSSLINKING-BASED METHOD FOR CREATING DNA HYDROGELS,” which application is incorporated herein by reference in its entirety. This application is related to U.S. patent application Ser. No. 11/464,184, filed Aug. 11, 2006 and entitled “NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION;” U.S. patent application Ser. No. 11/464,181, filed Aug. 11, 2006 and entitled “NUCLEIC ACID-BASED MATRIXES;” and U.S. patent application Ser. No. 11/423,633, filed Jun. 12, 2006 and entitled “DETECTION OF TARGET MOLECULES WITH LABELED NUCLEIC ACID DETECTION MOLECULES,” all of which applications are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] DNA molecules possess a distinct set of mechanical, physical, and chemical properties. From a mechanical point of view, DNA molecules can be rigid (e.g., when the molecules are less than 50 nm, the persistent length of double stranded DNA (Bouchiat, C. et al., *Biophys J* 76:409-13 (1999); Tinland et al., *Macromolecules* 30:5763-5765 (1997); Toth et al., *Biochemistry* 37:8173-9 (1998)), or flexible. Physically, DNA is small, with a width of about 2 nanometers and a length of about 0.34 nanometers per base pair (for B-DNA). In nature, DNA can be found in either linear or circular shapes. Chemically, DNA is generally stable, non-toxic, water soluble, and is commercially available in large quantities and in high purity. Moreover, DNA molecules are easily and highly manipulable by various well-known enzymes such as restriction enzymes and ligases. Under proper conditions, DNA molecules self-assemble with complementary strands of nucleic acid (e.g., DNA, RNA, or Peptide Nucleic Acid, (PNA)). Furthermore, DNA molecules can be amplified exponentially and joined, e.g., ligated, specifically. Thus, DNA is an excellent candidate for constructing nano-material.

[0003] There remains a need to develop efficient and fast methods to product compositions comprising nucleic acid molecules, which provide building blocks for forming new structures with a variety of applications.

SUMMARY OF THE INVENTION

[0004] The present invention provides compositions and methods directed to nucleic acid based structures, including hydrogels, which are produced through cross-linking, such as through photo-crosslinking.

[0005] In one aspect, the present invention provides a composition comprising a plurality of branched nucleic acid molecules, wherein at least a portion of the branched nucleic acid molecules are conjugated to a photoreactive group. In some embodiments, the at least a portion of the branched nucleic acid molecules are conjugated to the photoreactive group on their 5'-end. In some embodiments, the at least a portion of the branched nucleic acid molecules are conjugated to the photoreactive group on their 3'-end. In some embodiments, the at least a portion of the branched nucleic acid molecules are conjugated to the photoreactive group internally. In some

embodiments, at least a portion of the branched nucleic acid molecules are crosslinked through the photoreactive group.

[0006] In some embodiments, the plurality of branched nucleic acid molecules comprises deoxyribonucleic acid (DNA), ribonucleic acid (RNA), peptide nucleic acid (PNA), or a combination thereof. The branched nucleic acid molecules can comprise oligonucleotides. The nucleic acid molecules can comprise coding and non-coding nucleic acid molecules.

[0007] In some embodiments, the plurality of branched nucleic acid molecules has a branched chain structure comprising one or more of an X-shape, a Y-shape, a T-shape, a dumbbell shape or a dendrimer-like shape. The branched chain structure can be substantially X-shaped, substantially Y-shaped, substantially T-shaped, substantially dumbbell shaped, or substantially dendrimer-like.

[0008] In some embodiments, at least a portion of the nucleic acid molecules are linked to one or more additional compounds. Non-limiting examples of the one or more additional compounds include a peptide, a polypeptide, a protein, a lipid, a carbohydrate, an aptamer, an antibody, an antigen, a cell growth factor, a DNA binding agent, a detectable label, a selectable marker, biotin, a pharmaceutical agent, a drug, a small molecule, a therapeutic agent, a receptor molecule, a ligand, a nucleic acid molecule or a substrate.

[0009] Additional nucleic acid molecule include but are not limited to siRNA, miRNA, snRNA, an oligodeoxynucleotide (ODN), a gene sequence, an intron sequence, an exon sequence, a non-coding sequence, a peptide nucleic acid (PNA), or an mRNA sequence. The additional nucleic acid molecules can further comprise a coding region.

[0010] Additional peptides for use with the invention comprise an adenovirus core peptide, a synthetic peptide, an influenza virus HA2 peptide, a simian immunodeficiency virus gp32 peptide, an SV40 T-Ag peptide, a VP22 peptide, a Tat peptide, or a Rev peptide. In some embodiments, the additional peptide comprises a DNA condensing peptide, DNA protection peptide, endosomal targeting peptide, membrane fusion peptide, nuclear localization signaling peptide, or a protein transduction domain peptide.

[0011] Detectable labels for use with the invention include a radiolabeled probe, a fluorophore-labeled probe, a quantum dot-labeled probe, a chromophore-labeled probe, an enzyme-labeled probe, an affinity ligand-labeled probe, an electromagnetic spin labeled probe, a heavy atom labeled probe, or a nanoparticle light scattering labeled probe. In some embodiments, the detectable label comprises a chromophore, a fluorescent moiety, an enzyme, an antigen, a heavy metal, a magnetic probe, a dye, a nanocrystal, a phosphorescent group, a radioactive material, a chemiluminescent moiety, a scattering nanoparticle, a fluorescent nanoparticle, a Raman signal generating moiety, or an electrochemical detection moiety. In some embodiments, the detectable label comprises horseradish peroxidase, alkaline phosphatase, β -galactosidase, acetylcholinesterase, streptavidin, avidin, biotin, an aptamer, an antigen, an antibody, an immunoglobulin, an anti-immunoglobulin, umbelliferone, fluorescein, fluorescein isothiocyanate (FITC), rhodamine, tetramethyl rhodamine, TRITC, eosin, green fluorescent protein, erythrosin, coumarin, methyl coumarin, pyrene, malachite green, stilbene, lucifer yellow, Cascade Blue™, Texas Red, Phar-Red, allophycocyanin (APC), dichlorotriazinylamine fluorescein, dansyl chloride, R-phycoerythrin, phycoerythrin, a fluorescent lanthanide complex, Europium, Terbium, Cy3,

Cy5, Cy7, digoxigenin, dinitrophenyl, a molecular beacon, a fluorescent molecular beacon derivative, luminol, a light scattering material, a plasmon resonant material, gold, silver, a quantum dot, ^{14}C , ^{123}I , ^{124}I , ^{125}I , ^{131}I , Technetium-99m ($^{99\text{m}}\text{Tc}$), ^{35}S , ^{32}P or ^3H .

[0012] In some embodiments, the one or more additional compounds comprises a polymer. Non-limiting examples of applicable polymers include poly(ethylene glycol) (PEG), poly(N-isopropylacrylamide), poly(N-alkylacrylamide), poly(N-n-propylacrylamide), poly(N-isopropylmethacrylamide), a peptide, a polypeptide, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), poly(DTEC), dextran-poly lactide, elastin-like polypeptides, a polyester, polylactide, poly(L-lactic acid), poly(D,L-lactic acid), poly(lactide-co-glycolides), biotinylated poly(ethylene glycol-block-lactic acid), poly(alkylcyanoacrylate), poly(epsilon-caprolactone), polyanhydride, poly(bis(p-carboxyphenoxy)propane-sebacic acid), polyorthoester, polyphosphoester, polyphosphazene, polystyrene, polyurethane, poly(amino acid), poly(ethylene oxide), poly(ethylene oxide)-polypropylene-poly(ethylene oxide), poly(lactic acid)-g-poly(vinyl alcohol), poly(ethylene oxide)-poly(L-lactic acid), poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol), poly(L-lactide-ethylene glycol), poly(ethylene glycol)-co-poly(hydroxyl Acid), poly(vinyl alcohol), poly(lactic acid-co-lysine)-poly(aspartic acid), poly(-caprolactone-co-trimethylene carbonate), poly(L-lactic acid-co-glycolic acid-co-L-serine), poly(propylene fumarate), oligo(poly(ethylene glycol) fumarate), poly(propylene fumarate-co-ethylene glycol), poly(ethylene glycol)di[ethylphosphatidyl(ethylene glycol)methacrylate], poly(N-isopropylacrylamide)-poly(ethylene glycol), poly(N-isopropylacrylamide)-gelatin, poly(N-isopropylacrylamide-acrylic acid) or a derivative of any thereof.

[0013] In some embodiments, the one or more additional compounds comprises a natural or synthetic biocompatible material. Non-limiting examples of biocompatible materials include a poly(ethylene glycol) (PEG) hydrogel matrix, a N-isopropylacrylamide (NiPAAm) hydrogel matrix, a chitosan hydrogel matrix or a derivative of any thereof. Natural biocompatible materials include chitosan, methylcellulose, alginate, hyaluronic acid, agarose, fibrin, gelatin, collagen, dextran, or a derivative of any thereof. Synthetic biocompatible material include hydroxyethyl methacrylate, N-(2-hydroxypropyl)methacrylate, N-vinyl-2-pyrrolidone, N-isopropyl acrylamide, vinyl acetate, acrylic acid, methacrylic acid, polyethylene glycol acrylate/methacrylate, polyethylene glycol diacrylate/dimethacrylate, polyvinyl alcohol, propylene fumarate, or a derivative of any thereof.

[0014] In some embodiments, at least a portion of the nucleic acid molecules are linked to a substrate, e.g., a nanoparticle or a microparticle. In some embodiments, the substrate comprises one or more of a noble metal, a transition metal, a semiconductor material or a magnetic material. In some embodiments, the substrate comprises one or more of gold, silver, copper, palladium, platinum, cadmium sulfide (CdS), cadmium selenide (CdSe), titanium dioxide (TiO_2), zinc oxide (ZnO), carbon black, 4-phosphonooxy-2,2,6,6-tetramethylpiperidyl nitr-oxide, titanium dioxide, cobalt, nickel, iron, iron-cobalt, and magnetite (Fe_3O_4). In some embodiments, the substrate comprises glass or polydimethylsiloxane (PDMS).

[0015] The crosslinked nucleic acid molecules can form a nucleic acid hydrogel. The hydrogel can have a predeter-

mined geometric pattern. In some embodiments, the geometric pattern provides a plurality of pores. In some embodiments, the pores have a size that is less than about 15 nanometers. In some embodiments, the pores have a size selected from a group consisting of about 5 nanometers, about 10 nanometers, about 15 nanometers, about 20 nanometers, about 30 nanometers, about 40 nanometers, about 50 nanometers, and about 100 nanometers. In some embodiments, the pores have a size selected from a group of ranges consisting of about 0.1 micron to about 5 microns, about 5 microns to about 10 microns, about 10 microns to about 20 microns, about 20 microns to about 30 microns, about 30 microns to about 40 microns, about 40 microns to about 50 microns, about 50 microns to about 100 microns, and about 100 microns to about 200 microns.

[0016] The nucleic acid molecules can also form a three-dimensional structure. The three-dimensional structure can function as a macroscopic scaffold.

[0017] In another aspect, the present invention provides a method of crosslinking nucleic acid molecules, comprising: providing a plurality of nucleic acid molecules configured to form one or more branched chain structures; and photocrosslinking the plurality of nucleic acid molecules. In some embodiments, the method further comprises amplifying the nucleic acid molecules. In some embodiments, the method further comprises hybridizing the nucleic acid molecules before the photocrosslinking step.

[0018] In some embodiments, the method further comprises purifying the hybridized nucleic acid molecules. Such purification can comprise chromatography, e.g., high performance liquid chromatography (HPLC).

[0019] The methods of the invention can be used to synthesize or form, in whole or in part, any of the compositions described herein, e.g., using the nucleic acid building blocks, structures, and additional compounds described above. In some embodiments, the nucleic acid molecules are provided in an equimolar ratio.

[0020] In some embodiments, the method further comprises conjugating a photoreactive group to at least a portion of the nucleic acid molecules before the photocrosslinking step. The photoreactive group can be conjugated to one or more of the 5'-end of at least a portion of the nucleic acid molecules, the 3'-end of at least a portion of the nucleic acid molecules, and internally to at least a portion of the nucleic acid molecules. In some embodiments, the photoreactive group comprises a vinyl, acrylate, N-hydroxysuccinimide, amine, carboxylate or thiol moiety. In some embodiments, the photoreactive group is a primary amine modified group, a secondary amine modified group, or a tertiary amine modified group.

[0021] The photocrosslinking step can be performed under electromagnetic radiation, e.g., in the visible, ultraviolet (UV), near infrared, infrared, and/or microwave regions. The photocrosslinking can also be performed using gamma rays, X-rays, or radio waves as appropriate.

[0022] In some embodiments, the photocrosslinking is performed in the presence of a photoinitiator, including but not limited to Irgacure. The photocrosslinking can also be performed using a crosslinker, e.g., a UV crosslinker.

[0023] In some embodiments, a portion of the nucleic acid molecules are linked to one or more additional compounds, by photocrosslinking or other means. The one or more additional compounds include but are not limited to those described above.

[0024] In some embodiments, the methods of the invention are used to crosslink nucleic acid molecules, thereby forming a nucleic acid hydrogel. The hydrogel can have a structure, e.g., including one or more of a micro thin film, a micro pad, a micro thin fiber, a nanosphere or a microsphere. In some embodiments, the structures are fabricated by emulsification, photolithography, microfluidic synthesis, micro-molding, or micro-electrospinning, or a combination thereof. The methods can also be used to coat the nucleic acid hydrogel on the surface of a substrate.

[0025] In another aspect, the methods of the invention are used to rapidly crosslink nucleic acids, e.g., to create hydrogels. In some embodiments, the crosslinking is performed in 10 minutes or less. In some embodiments, the crosslinking is performed in 5 minutes or less. In still other embodiments, the crosslinking is performed in 1 minutes or less.

[0026] In another aspect, the present invention provides a method of encapsulating one or more compounds in a nucleic acid hydrogel, comprising: providing a plurality of nucleic acid molecules configured to create one or more branched chain structures; mixing the one or more compounds with the plurality of nucleic acid molecules; and photocrosslinking the plurality of nucleic acid molecules mixed with the one or more compounds to form a nucleic acid hydrogel, thereby encapsulating the one or more compounds in the nucleic acid hydrogel.

[0027] In a related aspect, the present invention provides a method of encapsulating one or more compounds in a nucleic acid hydrogel, comprising: providing a plurality of nucleic acid molecules configured to create one or more branched chain structures; photocrosslinking the plurality of nucleic acid molecules to form a nucleic acid hydrogel; and mixing the one or more compounds with the nucleic acid hydrogel, thereby encapsulating the one or more compounds in the nucleic acid hydrogel.

[0028] Non-limiting examples of the one or more encapsulated compounds include a protein, a peptide, a lipid, a nucleic acid, or a carbohydrate. In some embodiments, the one or more compounds comprise a therapeutic agent. The encapsulation of the therapeutic agent can be efficient, e.g., encapsulating the agent to at least about 90% efficiency. In some embodiments, the therapeutic agent is a small molecule, e.g., doxorubicin. In other embodiments, the one or more compounds comprises a cell, e.g., a mammalian cell. The methods can also be used to encapsulate a virus.

[0029] In another aspect, the present invention provides a method of delivering a compound, comprising: providing a plurality of nucleic acid molecules configured to create one or more branched chain structures; mixing the compound with the plurality of nucleic acid molecules; photocrosslinking the plurality of nucleic acid molecules mixed with the compound to form a composition comprising a nucleic acid hydrogel encapsulating the compound; and administering the composition to a subject, whereby the composition releases the compound in a time released manner, thereby delivering the compound.

[0030] In a related aspect, the present invention provides a method of delivering a compound, comprising: providing a plurality of nucleic acid molecules configured to create one or more branched chain structures; photocrosslinking the plurality of nucleic acid molecules to form a nucleic acid hydrogel; mixing the compound with the nucleic acid hydrogel to form composition wherein the compound is encapsulated within the nucleic acid hydrogel; and administering the com-

position to a subject, whereby the composition releases the compound in a time released manner, thereby delivering the compound.

[0031] In some embodiments, the delivered compound comprises a therapeutic agent. In some embodiments, the compound comprises a cell. The hydrogel can provide a three dimensional matrix on which the cell grows. The delivery can be to any appropriate matter, e.g., a cell, bodily fluid, tissue, organ or skin.

[0032] In some embodiments, the hydrogel used to deliver the agent comprises pores. Depending on the application, the pores can have a size greater than about 15 nanometers. Similarly, the pores can have a size of 15 nanometers or less.

[0033] In one aspect, the present invention provides a method of cell-free synthesis of one or more proteins, comprising: providing a plurality of nucleic acid molecules configured to create one or more branched chain structures; photocrosslinking the plurality of nucleic acid molecules to form a nucleic acid hydrogel; and expressing the one or more proteins from the nucleic acid hydrogel.

[0034] In some embodiments, the hydrogel comprises pores. In some embodiments, the size of the pores ranges from about 5 nanometers to about 500 nanometers, e.g., about 50 nanometers to about 500 nanometers. In some embodiments, the pores have a size of about 5 nanometers, about 10 nanometers, about 15 nanometers, about 20 nanometers, about 30 nanometers, about 40 nanometers, about 50 nanometers, and/or about 100 nanometers.

[0035] The hydrogel used to express the one or more proteins can comprise coding and non-coding nucleic acid molecules. The hydrogel can also comprise nucleic acid molecules and one or more macromolecules necessary for protein modification, thus producing modified proteins. Such modifications include but are not limited to phosphorylation, glycosylation, methylation, ubiquitination, biotinylation, alkylation, acetylation, glutamylation, glycylation, isoprenylation, lipoylation, phosphoantetheinylation, sulfation, citrullination, deamidation, isomerization, or a combination of any thereof.

INCORPORATION BY REFERENCE

[0036] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are used, and the accompanying drawings of which:

[0038] FIG. 1 provides a schematic outlining photocrosslinking of nucleic acid hydrogels.

[0039] FIG. 2 outlines two related processes for producing nucleic acid structures according to FIG. 1.

[0040] FIG. 3A illustrates a scheme for generating a network matrix comprising X-nucleic acids and linear nucleic acids encoding a protein of interest. FIG. 3B illustrates an exemplary X-DNA (SEQ ID NOs: 56 (i.e., 5' starting with

CTGA . . .), 57 (5' starting with ACCT . . .), 58 (5' starting with GAAT . . .) and 59 (5' starting with TCCG . . .).

[0041] FIG. 4 illustrates formation of an X-shape molecule.

[0042] FIG. 5 illustrates joining of several X-shape molecules.

[0043] FIG. 6A illustrates formation of matrixes comprised of X-shaped and Y-shaped molecules.

[0044] FIG. 6B illustrates formation of matrixes comprised of X-shaped, Y-shaped and T-shaped molecules.

[0045] FIGS. 7A and 7B illustrate Y-shaped DNA (SEQ ID NOs: 102, 108 and 112).

[0046] FIG. 8A illustrates X-, Y-, T-DNA building blocks. FIG. 8B shows the building blocks of FIG. 8A forming matrixes.

[0047] FIG. 9 illustrates a dumbbell-shape DNA (SEQ ID NOs: 102, 108 and 112 form Y-shapes that are joined end-to-end).

[0048] FIGS. 10A-C illustrate dendrimer like molecules and structures.

[0049] FIG. 11 illustrates a dendrimer like structure comprised of nucleic acids with terminal Y-shape arms with various compounds linked to the plurality of arms.

[0050] FIG. 12 illustrates a Y-shape DNA linked to various compounds, including a circular vector DNA linked to the Y-DNA via a μ Mu component.

[0051] FIG. 13 illustrates a T-shape DNA (SEQ ID NOs: 44-46).

[0052] FIG. 14 illustrates formation of a T-shape molecule.

[0053] FIGS. 15A-C illustrate matrixes comprised of T-shaped molecules linked to X-shaped (A), Y-shaped (B) and T-shaped (C) molecules.

[0054] FIG. 16 illustrates a multivalent nucleic acid dendrimer for delivery into a cell, and linked to various components.

[0055] FIG. 17 outlines a process for cell-free production of proteins using nucleic acid hydrogel structures.

[0056] FIG. 18 illustrates a networked matrix of X-shape nucleic acids, with AuNP integrated into the matrix.

[0057] FIG. 19 outlines a process for encapsulation and delivery of compounds using nucleic acid hydrogel structures.

[0058] FIG. 20 illustrates a comparison of the stress and strain of a photopolymerized DNA-PEG compared to a PEG hydrogel.

[0059] FIG. 21A illustrates fabricated shapes of DNA gels. FIG. 21B illustrates micropatterning of DNA gels. FIG. 21C illustrates a confocal image of DNA-PEG hydrogel coated to beads.

[0060] FIG. 22 illustrates the renilla luciferase protein expression using photo-polymerized DNA hydrogels. FIG. 22A shows the fold-change in luciferase activity with different concentrations of plasmid polymerized to the hydrogels compared to solution phase systems (SPS) control reactions. FIG. 22B also shows the effect of the total gene amounts on expression, determined by varying the number of photocrosslinked P-gel micropads in the reaction (Blue lines). The same amounts of the plasmid were used in solution phase systems (SPS) control reactions (Red lines). In terms of volumetric yield, the photocrosslinked P-gel produced up to about 1 mg/ml of functional protein.

DETAILED DESCRIPTION OF THE INVENTION

[0061] 1. Photo-Crosslinked Nucleic Acid Hydrogels

[0062] Photopolymerization, which exploits light-induced polymerization, is widely used to make relatively simple hydrogels. See, e.g., Peppas et al., *Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology*. Adv. Mater. 18:1345-60 (2006). DNA is a highly efficient material that can be controlled by various molecular tools, such as enzymes. See Luo, D. *The Road from Biology to Materials*. Mater. Today 6:38-43 (2003). Previously, we developed DNA hydrogels, DNA nanobarcodes, and dendrimer-like DNA nanostructures entirely from programmable self-assembled, branched DNA through enzyme ligation. See U.S. patent application Ser. Nos. 11/464,184, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION;" 11/464,181, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES." Our DNA hydrogels have been used as fundamental components in a variety of therapeutic applications. Recently, an enzyme-catalyzed DNA hydrogel has been used for cell-free protein synthesis, controlled drug delivery, and cell and tissue culture applications. See U.S. patent application Ser. Nos. 11/464,184, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION;" 11/464,181, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES." Although an enzyme-ligated approach to synthesizing DNA gels can yield a biocompatible, biodegradable, and inexpensive means to manipulating a product, this approach may use several hours of reaction time to produce a gel with soft gel properties. The present invention provides a method for rapidly producing DNA hydrogels using photo-crosslinking. The photo-crosslinked hydrogels can also have improved mechanical properties, e.g., increased hydrogel strength.

[0063] These photo-crosslinkable DNA hybrid hydrogels can be used to produce protein in specific micro-patterned regions. In some embodiments, DNA building blocks having photoreactive portions are conjugated onto gold nanoparticles (AuNP) to obtain modified AuNP-DNA conjugates after photoreaction. A variety of other nanoparticles can be used as well. These products have surface chemistry and gene delivery applications. In addition, cells within photo-crosslinked hydrogels are uniformly distributed and cultured. Various hydrogel matrices can be controlled by UV exposure time, photoinitiator concentration, and the properties of the DNA building blocks to modify cell behavior. These aspects make photo-cross-linked DNA hydrogels and particles valuable in making DNA gels more industrially feasible. These hydrogels can be used in novel bio-related applications, especially cell-free protein synthesis and controlled drug delivery.

[0064] In one aspect, the present invention provides for photo-crosslinking of self-assembled DNA molecules, a simple and fast method for creating DNA hydrogels and particles. Photo-crosslinking allows the DNA materials to be gelled in situ, enabling rapid gelation within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 minutes, and is able to utilize other materials including DNA nanoparticles ranging from the nanoscale (50-500 nm) to the microscale (20-30 μ m).

[0065] In one aspect, the present invention provides compositions of photo-crosslinked nucleic acid comprising a plurality of branched nucleic acid molecules, as described herein. At least a portion of the branched nucleic acid molecules comprise a photoreactive group, e.g., conjugated to their 5'-end, 3'-end, or internally. A group conjugated internally refers to a group that is not bound to either the 5' or 3' end of the nucleic acid molecule. In some embodiments, the

nucleic acids are conjugated to a photoreactive group at more than one location. In these embodiments, the photoreactive groups can all be the same group or can differ, even within one nucleic acid molecule. In a hydrogel composition, at least a portion of the branched nucleic acid molecules are crosslinked through the photoreactive group. The branched chain nucleic acid molecules can comprise any relevant form of nucleic acids, e.g., deoxyribonucleic acid (DNA), ribonucleic acid (RNA), peptide nucleic acid (PNA), or a combination thereof. In some embodiments, the plurality of branched nucleic acid molecules comprises oligonucleotides.

[0066] The nucleic acid molecules can also be crosslinked with other hydrogel molecules, including but not limited to a polyethylene glycol (PEG) hydrogel matrix, a N-isopropylacrylamide (NiPAAm) hydrogel matrix, a chitosan hydrogel matrix, or a combination thereof, thereby providing a versatile means to modify gel properties. In some embodiments, the other molecules are also photo-crosslinked to the nucleic acids. The pore size and mechanical strength of these modified hybrid nucleic acids hydrogels can be tailored by the molecular weight and polymer fraction of nucleic acid monomers by using other polymers. In some embodiments, the photopolymerization is performed using a batch process. In some embodiments, photopolymerization is performed using flow-through microfluidic schemes. Other approaches can be performed. The size and shape of molded nucleic acid-PEG hybrid hydrogels is controlled by combining photo-crosslinking with approaches such as micro-patterning in polydimethylsiloxane (PDMS) to produce micro thin films and micro pads, micro-electrospinning to produce micro thin fibers and microspheres and micro-emulsion in microfluidic channels to produce microspheres. Similar nanoscale structures are also provided. Precise tuning of the crosslinking degree and size of the nucleic acid particles is achieved by manipulating the nucleic acid building block size, the type of branched nucleic acid monomers and their initial concentration. Methods of tuning are provided herein.

[0067] In some embodiments, the nucleic acids are linked to one or more other molecules, e.g., a peptide, a polypeptide, an aptamer, an antibody, an antigen, a cell growth factor, a DNA binding agent, a detectable label, a selectable marker, a pharmaceutical compound, a therapeutic compound, a receptor molecule, a ligand or a nucleic acid molecule. In some embodiments, the other biological molecules are also photo-crosslinked to the nucleic acids. The nucleic acids can also be linked, e.g., via photo-crosslinks, with one or more polymers. Non-limiting examples of useful polymers poly(ethylene glycol) (PEG), poly(N-isopropylacrylamide), poly(N-alkylacrylamide), poly(N-n-propylacrylamide), poly(N-isopropylmethacrylamide), a peptide, a polypeptide, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), poly(DTEC), dextran-poly lactide, elastin-like polypeptides, a polyester, polylactide, poly(L-lactic acid), poly(D,L-lactic acid), poly(lactide-co-glycolides), biotinylated poly(ethylene glycol-block-lactic acid), poly(alkylcyanoacrylate), poly(epsilon-caprolactone), polyanhydride, poly(bis(p-carboxyphenoxy)propane-sebacic acid), polyorthoester, polyphosphoester, polyphosphazene, polystyrene, polyurethane, poly(amino acid), poly(ethylene oxide), poly(ethylene oxide)-polypropylene-poly(ethylene oxide), poly(lactic acid)-g-poly(vinyl alcohol), poly(ethylene oxide)-poly(L-lactic acid), poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol), poly(L-lactide-ethylene glycol), poly(ethylene glycol)-co-poly(hydroxy acid), poly(vinyl alcohol), poly(lactic

acid-co-lysine)-poly(aspartic acid), poly(-caprolactone-co-trimethylene carbonate), poly(L-lactic acid-co-glycolic acid-co-L-serine), poly(propylene fumarate), oligo(poly(ethylene glycol) fumarate), poly(propylene fumarate-co-ethylene glycol), poly(ethylene glycol)di[ethylphosphatidyl(ethylene glycol)methacrylate], poly(N-isopropylacrylamide)-poly(ethylene glycol), poly(N-isopropylacrylamide)-gelatin, poly(N-isopropylacrylamide-acrylic acid) or a derivative of any thereof.

[0068] The practice of various embodiments of the invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See Sambrook, Fritsch and Maniatis, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd edition (1989); *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY* (F. M. Ausubel, et al. eds., (1987)); the series *METHODS IN ENZYMOLOGY* (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *ANTIBODIES, A LABORATORY MANUAL*, and *ANIMAL CELL CULTURE* (R. I. Freshney, ed. (1987)).

[0069] As used in the specification and claims, the singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0070] As used herein, the terms “biologically active agent” or “bioactive agent” are used interchangeably and include but are not limited to a biological or chemical compound such as a simple or complex organic or inorganic molecule, peptide, peptide mimetic, protein (e.g. antibody, angiogenic, anti-angiogenic and cellular growth factors), an antigen or immunogen, liposome, small interfering RNA (siRNA), or a polynucleotide (e.g. vector, virus, viral vector, or anti-sense), therapeutic agents, organic or inorganic molecules can include a homogenous or heterogeneous mixture of compounds, including pharmaceuticals, radioisotopes, crude or purified plant extracts, and/or a cell, entities that alter, inhibit, activate, or otherwise affect biological or biochemical events, including classes of molecules (e.g., proteins, amino acids, peptides, polynucleotides, nucleotides, carbohydrates, sugars, lipids, nucleoproteins, glycoproteins, lipoproteins, steroids, growth factors, chemoattractants, aptamers, etc.) that are commonly found in cells and tissues, whether the molecules themselves are naturally-occurring or artificially created (e.g., by synthetic or recombinant methods). Such agents may be naturally derived or synthetic. “Therapeutic agents” include molecules or atoms which are useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, antibodies, antibody-drug conjugates, photoactive agents or dyes, and radioisotopes.

[0071] Examples of such agents include but are not limited to drugs, for example, small molecules, anti-cancer substances, analgesics, opioids, anti-AIDS substances, anti-cancer substances, immunosuppressants (e.g., cyclosporine), anti-viral agents, enzyme inhibitors, neurotoxins, hypnotics, anti-histamines, lubricants, tranquilizers, anti-convulsants, muscle relaxants and anti-Parkinson agents, anti-spasmodics and muscle contractants including channel blockers, miotics and anti-cholinergics, anti-glaucoma compounds, anti-parasite, anti-protozoal, and/or anti-fungal compounds, modulators of cell-extracellular matrix interactions including cell

growth inhibitors and anti-adhesion molecules, vasodilating agents, inhibitors of DNA, RNA or protein synthesis, anti-hypertensives, anti-pyretics, steroidal and non-steroidal anti-inflammatory agents, anti-angiogenic factors, anti-secretory factors, anticoagulants and/or antithrombotic agents, local anesthetics, ophthalmics, prostaglandins, targeting agents, neurotransmitters, proteins, cell response modifiers, and vaccines.

[0072] Preferably, though not necessarily, the drug is one that has already been deemed safe and effective for use by the appropriate governmental agency or body. For example, drugs for human use listed by the United States Food and Drug Administration (FDA) under 21 C.F.R. §§330.5, 331 through 361, and 440 through 460; drugs for veterinary use listed by the FDA under 21 C.F.R. §§500 through 589, incorporated herein by reference are all considered acceptable for use in accordance with compositions and methods disclosed herein.

[0073] The term “scaffold” can mean a three-dimensional structure capable of supporting cells. Cells may be encapsulated by the scaffold or may be disposed in a layer on a surface of the scaffold. The scaffold is formed by but not limited to the self-assembly of nucleic acid molecules described herein, that may include X-, Y-, T-, dumbbell-, or dendrimer-shape, as well as linear or circular shapes, or a combination thereof. It is also contemplated that the nucleic acid may be linked to a compound, such as a chemoattractant or a therapeutically active compound. The scaffold may be formed from one or more distinct molecular species of nucleic acids which are complementary and structurally compatible with each other. Nucleic acids containing mismatched pairs may also form scaffolds if the disruptive force is dominated by stabilizing interactions between the nucleic acids. Scaffolds are also referred to herein as matrixes, matrices, gels, nucleic acid hydrogel structures, nucleic acid gel structures, or hydrogel structures.

[0074] The terms “polynucleotide”, “nucleotide”, “nucleotide sequence”, “nucleic acid”, “nucleic acid molecule”, “nucleic acid sequence” and “oligonucleotide” are used interchangeably, and can also include plurals of each respectively depending on the context in which the terms are utilized. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides (DNA) or ribonucleotides (RNA), or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA, ribozymes, small interfering RNA, (siRNA), microRNA (miRNA), small nuclear RNA (snRNA), cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA (A, B and Z structures) of any sequence, PNA, locked nucleic acid (LNA), TNA (treose nucleic acid), isolated RNA of any sequence, nucleic acid probes, and primers. Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, are typically double-stranded RNA molecules of 20-25 nucleotides in length. siRNA can interfere with the expression of certain genes. LNA, often referred to as inaccessible RNA, is a modified RNA nucleotide. The ribose moiety of an LNA nucleotide is modified with an extra bridge connecting the 2' and 4' carbons. The bridge “locks” the ribose in the 3'-endo structural conformation, which is often

found in the A-form of DNA or RNA, which can significantly improve thermal stability. miRNAs are single-stranded RNA molecules of 21-23 nucleotides in length. miRNAs are typically partially complementary to one or more messenger RNA (mRNA) molecules, and hybridize thereto to down-regulate gene expression. Small nuclear RNA (snRNA) is a class of small RNA molecules that are found within the nucleus of eukaryotic cells. snRNA are involved in a variety of biological processes such as RNA splicing, regulation of transcription factors (7SK RNA) or RNA polymerase II (B2 RNA), and maintaining telomeres. They associate with specific proteins, and the complexes are referred to as small nuclear ribonucleoproteins (snRNP) or “snurps.”

[0075] A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components.

[0076] A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The nucleic acids, used in the various embodiments disclosed herein, may be modified in a variety of ways, including by crosslinking, intra-chain modifications such as methylation and capping, and by copolymerization. Additionally, other beneficial molecules may be attached to the nucleic acid chains. For example, photo-crosslinkable moieties can be attached to the nucleic acid chains. The nucleic acids may have naturally occurring sequences or artificial sequences. The sequence of the nucleic acid may be irrelevant for many aspects disclosed herein. However, special sequences may be used to prevent any significant effects due to the information coding properties of nucleic acids, to elicit particular cellular responses or to govern the physical structure of the molecule.

[0077] A “nucleotide probe” or “probe” refers to a polynucleotide used for detecting or identifying its corresponding target polynucleotide in a hybridization reaction. The nucleic acids may comprise intron and exon sequences, modified sequences, RNA, DNA, or analogs thereof.

[0078] An “aptamer” refers in general to either an oligonucleotide of a single defined sequence or a mixture of said oligonucleotides, wherein the mixture retains the properties of binding specifically to the target molecule. Thus, as used herein “aptamer” denotes both singular and plural sequences of oligonucleotides. Structurally, the aptamers of the invention are specifically binding oligonucleotides. Oligonucleotides include not only those with conventional bases, sugar residues and internucleotide linkages, but also those which contain modifications of any or all of these three moieties. U.S. Pat. No. 5,756,291, incorporated herein by reference, provides a description of aptamers, methods of preparing and testing aptamers, and uses thereof. Oligonucleotide aptamers comprise both DNA and RNA. In addition, peptide aptamers are polypeptides that are designed to interfere with other protein interactions inside cells. They can consist of a variable peptide loop attached at both ends to a protein scaffold and display binding affinities similar to that of an antibody.

[0079] A “target molecule” includes a molecule specifically bound by a binding domain of a bispecific binding agent of the invention.

[0080] As used herein, the term “nanofiber” refers to a fiber having a diameter of nanoscale dimensions. Typically a nanoscale fiber has a diameter of 500 nm or less. According to

certain embodiments of the invention a nanofiber has a diameter of less than 100 nm. According to certain other embodiments of the invention a nanofiber has a diameter of less than 50 nm. According to certain other embodiments of the invention a nanofiber has a diameter of less than 20 nm. According to certain other embodiments of the invention a nanofiber has a diameter of between 10 and 20 nm. According to certain other embodiments of the invention a nanofiber has a diameter of between 5 and 10 nm. According to certain other embodiments of the invention a nanofiber has a diameter of less than 5 nm.

[0081] As used herein, the terms “isolated and/or purified” refer to in vitro preparation, isolation and/or purification of a nucleic acid molecule of the invention, so that it is not associated with in vivo substances, or is substantially purified from in vitro substances.

[0082] The following terms are used to describe the sequence relationships between two or more polynucleotides: (a) “reference sequence,” (b) “comparison window,” (c) “sequence identity,” (d) “percentage of sequence identity,” and (e) “substantial identity.”

[0083] As used herein, “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a segment of or the entirety of a specified sequence.

[0084] As used herein, “comparison window” makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may include additions or deletions (i.e., gaps) compared to the reference sequence (which does not include additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 5, 10, or 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence, a gap penalty can be introduced and is subtracted from the number of matches.

[0085] Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Preferred, non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller, *CABIOS*, 4:11 (1988), which is hereby incorporated by reference in its entirety; the local homology algorithm of Smith et al., *Adv. Appl. Math.*, 2:482 (1981), which is hereby incorporated by reference in its entirety; the homology alignment algorithm of Needleman and Wunsch, *JMB*, 48:443 (1970), which is hereby incorporated by reference in its entirety; the search-for-similarity-method of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 85:2444 (1988), which is hereby incorporated by reference in its entirety; the algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA*, 87:2264 (1990), which is hereby incorporated by reference in its entirety; modified as in Karh and Altschul, *Proc. Natl. Acad. Sci. USA*, 90:5873 (1993), which is hereby incorporated by reference in its entirety.

[0086] Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, Calif.); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software

Package, Version 8 (available from Genetics Computer Group (GCG), 575 Science Drive, Madison, Wis., USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins et al., *Gene*, 73:237 (1988), Higgins et al., *CABIOS*, 5:151 (1989); Corpet et al., *Nucl. Acids Res.*, 16:10881 (1988); Huang et al., *CABIOS*, 8:155 (1992); and Pearson et al., *Meth. Mol. Biol.*, 24:307 (1994), which are hereby incorporated by reference in their entirety. The ALIGN program is based on the algorithm of Myers and Miller, supra. The BLAST programs of Altschul et al., *JMB*, 215:403 (1990); *Nucl. Acids Res.*, 25:3389 (1990), which are hereby incorporated by reference in their entirety, are based on the algorithm of Karlin and Altschul supra.

[0087] Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (worldwideweb.ncbi.nlm.nih.gov). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues, always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached.

[0088] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0089] To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul et al., *Nucleic Acids Res.* 25:3389 (1997), which is hereby incorporated by reference in its entirety. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul et al., supra. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g. BLASTN for nucleotide sequences, BLASTX for proteins) can be used. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and

the BLOSUM62 scoring matrix. See worldwideweb.ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

[0090] Comparison of nucleotide sequences for determination of percent sequence identity to the sequences disclosed herein can be made using the BlastN program (version 1.4.7 or later) with its default parameters or any equivalent program. By “equivalent program” is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by the preferred program.

[0091] As used herein, “sequence identity” or “identity” in the context of two nucleic acid sequences makes reference to a specified percentage of residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window, as measured by sequence comparison algorithms or by visual inspection. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and, therefore, do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have “sequence similarity” or “similarity.” Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif.).

[0092] As used herein, “percentage of sequence identity” means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may include additions or deletions (i.e., gaps) as compared to the reference sequence (which does not include additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

[0093] The term “substantial identity” of polynucleotide sequences means that a polynucleotide includes a sequence that has at least 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, or 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, or 79%, preferably at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more preferably at least 90%, 91%, 92%, 93%, or 94%, and most preferably at least 95%, 96%,

97%, 98%, or 99% sequence identity, compared to a reference sequence using one of the alignment programs described using standard parameters.

[0094] Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions (see below). Generally, stringent conditions are selected to be about 5° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of about 1° C. to about 20° C., depending upon the desired degree of stringency as otherwise qualified herein.

[0095] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, sub-sequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0096] As noted above, another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. The phrase “hybridizing specifically to” refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular DNA or RNA. “Bind(s) substantially” refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

[0097] “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0098] The term “hybridized” as applied to a polynucleotide refers to the ability of the polynucleotide to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. The hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0099] As is known to one skilled in the art, hybridization can be performed under conditions of various stringency. Suitable hybridization conditions are such that the recognition interaction between the probe and target ER-stress related gene is both sufficiently specific and sufficiently

stable. Conditions that increase the stringency of a hybridization reaction are widely known and published in the art. See, for example, (Sambrook, et al., (1989), *supra*; Nonradioactive In Situ Hybridization Application Manual, Boehringer Mannheim, second edition). The hybridization assay can be formed using probes immobilized on any solid support, including but are not limited to nitrocellulose, glass, silicon, and a variety of gene arrays. A preferred hybridization assay is conducted on high-density gene chips as described in U.S. Pat. No. 5,445,934.

[0100] “Stringent hybridization conditions” and “stringent hybridization wash conditions” in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. Longer sequences hybridize specifically at higher temperatures. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution.

[0101] For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, *Anal. Biochem.*, 138:267 (1984), which is hereby incorporated by reference in its entirety; $T_m = 81.5^\circ \text{C.} + 16.6 (\log M) + 0.41 (\% \text{ GC}) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. T_m is reduced by about 1°C. for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C. lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH.

[0102] However, severely stringent conditions can use a hybridization and/or wash at 1, 2, 3, or 4°C. lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C. lower than the T_m ; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C. lower than the T_m . Using the equation, hybridization and wash compositions, and desired T, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T of less than 45°C. (aqueous solution) or 32°C. (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology Hybridization with Nucleic Acid Probes*, Part I Chapter 2 “Overview of Principles of Hybridization and the Strategy of Nucleic Acid Probe Assays,” Elsevier, New York (1993), which is hereby incorporated by reference in its entirety. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C. lower than the T_m for the specific sequence at a defined ionic strength and pH.

[0103] An example of highly stringent wash conditions is 0.15 M NaCl at 72°C. for about 15 minutes. An example of stringent wash conditions is a $0.2\times \text{SSC}$ wash at 65°C. for 15 minutes (see, Sambrook, *infra*, for a description of SSC

buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of a medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is $1\times \text{SSC}$ at 45°C. for 15 minutes. An example of a low stringency wash for a duplex of, e.g., more than 100 nucleotides, is $4-6\times \text{SSC}$ at 40°C. for 15 minutes. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.5 M, more preferably about 0.01 to 1.0 M, Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. and at least about 60°C. for long probes (e.g., >50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of $2\times$ (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This occurs, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

[0104] Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or Northern blot is 50% formamide, e.g., hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C. , and a wash in $0.1\times \text{SSC}$ at 60 to 65°C. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C. , and a wash in $1\times$ to $2\times \text{SSC}$ ($20\times \text{SSC}=3.0 \text{ M NaCl}/0.3 \text{ M trisodium citrate}$) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C. , and a wash in $0.5\times$ to $1\times \text{SSC}$ at 55 to 60°C.

[0105] The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. As used herein the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

[0106] As used herein, “expression” refers to the process by which a polynucleotide is transcribed into mRNA and/or the process by which the transcribed mRNA (also referred to as “transcript”) is subsequently being translated into peptides, polypeptides, or proteins. The transcripts and the encoded polypeptides are collectively referred to as “gene product.”

[0107] As used herein the term “ligation” refers to the process of joining DNA molecules together with covalent bonds. For example, DNA ligation involves creating a phosphodiester bond between the 3' hydroxyl of one nucleotide and the 5' phosphate of another. Ligation is preferably carried out at $4-37^\circ \text{C.}$ in presence of a ligase enzyme. Suitable ligases include *Thermus thermophilus* ligase, *Thermus aquaticus* ligase, *E. coli* ligase, T4 ligase, and *Pyrococcus* ligase.

[0108] The term “photochemical reaction” can refer to any chemical reaction initiated by the absorption of a quantum of electromagnetic radiation to form an excited state. “Photoreactive” refers to an entity that participates in a photochemical reaction. For example, a conjugate photoreactive crosslinker can be formed wherein photoreactive agents are coupled through a linking group. Photo-reactive amino acid analogs include photoreactive diazirine analogs to leucine and methionine. L-Photo-Leucine and L-Photo-Methionine are analogs of the naturally occurring L-Leucine and L-Methionine amino acids and can form crosslinks when exposed to UV light. As used herein, “photo-responsive” is used interchangeably with photoreactive.

[0109] “Photo-crosslinking” refers to bond formation that links one polymer chain to another upon exposure to light of appropriate wavelengths. For example, two nucleic acid polymers conjugated to a photoreactive group can be covalently photo-crosslinked by covalent bond formation between the photoreactive groups.

[0110] A “photoinitiator” typically includes an agent that forms free radicals when illuminated by light of appropriate wavelengths. For example, Igracure from CIBA is a photoinitiator for radical polymerization upon light exposure. Non-limiting example classes of compounds useful as photoinitiators include aromatic carbonyl compounds (e.g., benzoin derivatives, benziketals, acetophenone derivatives, hydroxyalkylphenones) and aromatic ketones (e.g., benzophenone and thioxanthone). Non-limiting examples of photoinitiators include Esacure from Lamberti spa, benzophenone, dimethoxyphenyl acetophenone, 2,2-dimethoxy, 2-phenylacetophenone and 2,2-diethoxyacetophenone, 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, ethyl eosin, eosin Y, fluorescein, 2,2-dimethoxy, 2-phenylacetophenone, 2-methyl, 2-phenylacetophenone, 12959, camphorquinone, rose bengal, methylene blue, erythrosin, phloxime, thionine, riboflavin, and methyl green. Other photoinitiators are listed and described in U.S. Pat. Nos. 3,715,293 and 3,801,329. Still other photoinitiators comprise 1-(4-Fluorophenyl)-2-methyl-2-morpholino-1-propanone, 1,7-bis(9-acridinyl)heptane, 1-Chloro-4-propoxythioxanthone, 1-Hydroxy cyclohexyl phenyl ketone, 2,2-Di ethoxy acetophenone, 2,3,4,4'-Tetrahydroxy Benzophenone, 2,3,4-Trihydroxybenzophenone, 2,4,6-Trimethyl benzoyl diphenyl phosphine oxide, 2,4,6-Trimethylbenzophenone, 2/4-Diethylthioxanthone, 2/4-Isopropylthioxanthone, 2-Benzyl-2-(dimethylamino)-1-[4-(4-morpholinyl)phenyl]-1-butanone, 2-Chlorothioxanthone, 2-Dimethyl-aminoethylbenzoate, 2-Ethylhexyl-4-dimethylaminobenzoate, 2-Hydroxy-2-methyl-phenyl-propan-1-one, 2-Hydroxy-4'-hydroxyethoxy-2-methylpropionophenone, 2-Isopropylthioxanthone, 2-Methyl Benzophenone, 2-Methyl-1-[4-(methylthio)phenyl]-2-morpholinopropanone-1,4-(4-Methylphenylthiophenyl)-phenylmethanone, 4,4'-Difluoro benzophenone, 4,4'-Dimethoxy benzophenone, 4-Chloro benzophenone, 4-Methyl acetophenone, 4-Methyl benzophenone, 4-Phenylbenzophenone, Benzil dimethyl ketal, Benzophenone, Benzophenone hydrazone, Bis(p-tolyl) iodonium hexafluorophosphate, Dimethyl Sebacate, Diphenyl Iodonium Hexafluorophosphate, Ethyl (2,4,6-trimethylbenzoyl)phenylphosphinate, Ethyl-4-(dimethylamino)benzoate, Methyl o-benzoyl benzoate, Methyl phenyl glyoxylate, N,N,N',N'-Tetraethyl-4,4-diaminobenzophenone, Phenyltribromomethylsulphone, acylphosphine oxide (APO) and bisacylphosphine oxide (BAPO), 1-[4-(2-Hydroxyethoxy)-

phenyl]-2-hydroxy-2-methyl-1-propane-1-one, 2,2-Dimethoxy-1,2-diphenylethan-1-one, hydroxy-cyclohexyl-phenyl-ketone, methylbenzoylformate, oxy-phenyl-acetic acid 2-[2 oxo-2 phenyl-acetoxy-ethoxy]ethyl ester, oxy-phenyl-acetic-2-[2-hydroxy-ethoxy]-ethyl ester, alpha-dimethoxy-alpha-phenylacetophenone, 2-Benzyl-2-(dimethylamino)-1-[4-(4-morpholinyl)phenyl]-1-butanone, diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide, phosphine oxide, bis(eta 5-2,4-cyclopentadien-1-yl), bis[2,6-difluoro-3-(1H-pyrrol-1-yl)phenyl]titanium, Iodonium, (4-methylphenyl)[4-(2-methylpropyl)phenyl]-hexafluorophosphate(1-), bis(2,6-dimethoxybenzoyl)-2,4,4-trimethyl pentylphosphineoxide. Photoinitiators also comprise related compounds and derivatives of these compounds.

[0111] A number of techniques for protein analysis are available in the art. They include but are not limited to radioimmunoassays, ELISA (enzyme linked immunoradiometric assays), “sandwich” immunoassays, immunoradiometric assays, in situ immunoassays (using e.g., colloidal gold, enzyme or radioisotope labels), western blot analysis, immunoprecipitation assays, immunofluorescent assays, and SDS-PAGE.

[0112] A “subject,” “individual” or “patient” is used interchangeably herein, which refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed.

[0113] In various embodiments of the invention, the nucleic acid-based matrixes can have nanoparticle, nanosphere, nanoshell, micelle, core-shell, multi-core shell, multi-layered, nanogel, microparticle, microsphere, microgel, macrogel, nanoscale, macroscale, macroscopic, block, branched, hyperbranched, hybrid, tree-like, comb-like, brush, grafting, vesicle, coil, global, coil-coil, coil-global, rod, membrane, film, coating, self-assembly, cyclic, microconduit, microchannel, nanochannel, porous, nonporous, tube, microtube, nanotube, semi-interpenetrating network, cross-linked, or a highly networked structure.

[0114] In certain aspects, the nucleic acid molecules provide monomer building blocks and/or cross-linkers that form a three-dimensional matrix or scaffold structure. A matrix of the invention can be comprised of nucleic acids that are X-shaped, Y-shaped, T-shaped, dumbbell-shaped nucleic acids, or a combination thereof. Collectively or singularly, one or more matrices of the present invention may be referred to herein as “biomaterial”, “matrix”, “dendrimer”, “dendrimer like”, hydrogel, gel or “scaffold”, and including plural forms thereof. Examples of various shape nucleic acids (e.g., DNA) are disclosed in U.S. patent application Ser. Nos. 10/877,697 and 60/756,453, which are incorporated by reference in their entirety.

[0115] Furthermore, in some embodiments the matrixes form gels that are molded into any desired shape and/or size. In one embodiment, such gels are formed entirely from branched DNA. In other embodiments, the gels are formed of linear and branched nucleic acids. In yet further embodiments, the linear or branched nucleic acids can be DNA, RNA, PNA, TNA, LNA or any combination thereof. For example, a gel can comprise branched DNA that form building blocks supporting the matrix and also linking linear DNA that encodes a protein(s) of interest. In another embodiment,

the hydrogel can be comprised entirely of RNA building blocks and protein-encoding sequences. In yet another embodiment, the gels are hydrogels. Such hydrogels are in large part comprised of H₂O molecules when hydrated, and thus are inexpensive, e.g., an average hydrogel costs less than \$5. In addition, fine tuning of the chemical and physical properties of these hydrogels can be easily accomplished by adjusting the concentrations and types of branched nucleic acid building blocks, thus allowing the hydrogels to comprise particular physical/chemical properties tailored for specific applications.

[0116] Nucleic acids have different rates of degradation, which may be modified and exploited. Additionally, particular degradation products may be desired (for instance, nucleic acids with a given sequence). The sites or timing of degradation may be modified so as to obtain these products. The type of nucleic acid selected may affect degradation as well as the different combination of types of nucleic acid. For example, RNA will likely degrade much more rapidly than DNA. Different DNA structures may have different degradation rates. This may also vary by the tissue in which the nucleic acid is used. Various disease states or injuries may also affect degradation. In some embodiments, the degradation rate can be controlled by selecting nucleic acids of a single shape, or a combination of different shapes (i.e., X, Y, T, dumbbell shapes).

[0117] In other embodiments, purified nucleic acids may be linked to other nucleic acids or other compounds to reduce degradation. Linking may be accomplished in a variety of ways, including hydrogen bonds, ionic and covalent bonds, π - π bonds, polarization bonding, van der Waals forces. As used herein, "link" and "cross-link" are used interchangeably. More than one type of crosslinking may be used within a given biomaterial. For example, use of a type of crosslinking easily degraded in a cell coupled with a more degradation resistant type of crosslinking may result in a biomaterial that is opened in two phases, one when the easily degraded crosslinks are broken and second when the more resistant crosslinks or the nucleic acid itself are degraded. In some embodiments, crosslinking is accomplished by UV radiation, esterification, hydrolysis, intercalating agents, neoplastic agents, formaldehyde, formalin, or silica compounds. Such methods are taught by U.S. patent application Ser. No. 11/464,181, filed Aug. 11, 2006 and entitled "Nucleic Acid-Based Matrixes." Examples of linking include but are not limited to the use of siloxane bridges as described in U.S. Pat. No. 5,214,134. The present invention further provides photo-crosslinking of the nucleic acids. In some embodiments, photoreactive groups are conjugated to the nucleic acids and linking occurs on exposure to light sufficient to link the photoreactive groups.

[0118] Crosslinking may occur between two strands of a double stranded nucleic acid or between the strands of two separate double strands. It may also occur between two separate single strands. Double strand to single strand crosslinking is also possible, as is crosslinking between different regions of one strand. Increased levels of crosslinking will generally slow degradation of nucleic acids. Linkers such as small organic molecules (esters, amines) or inorganic molecules (silicas, siloxanes), including microparticles or nanoparticles thereof, may be used to attach copolymers to nucleic acids. Any of the different shaped nucleic acids of the invention can be linked or cross-linked by one or methods described herein. Therefore, X-shaped, Y-shaped, T-shaped,

dumbbell shaped or any combination thereof can be linked to each other, as well as to other chemical moieties or polymeric compounds.

[0119] In addition, in certain aspects, the nucleic acids can be linked to biologically active agents, including drugs, selection markers, detectable signals, other therapeutic agents, peptides, such as signal or cell targeting peptides, nucleic acid sequences, proteins (including antibodies), plasmids, viruses, viral vectors, small molecules, inorganic compounds, metals or derivatives thereof. Additionally, any inorganic or organic molecules, including amino acids, silicas, cytokines, such as interleukins, biologics and drugs may be added to the nucleic acid polymers to produce certain biological effects. Nucleic acids provide a variety of molecular attachment sites and therefore facilitate covalent, ionic and hydrogen bonding, as well as Van der Waals attachments, or other forms of attachment. In some embodiments, these molecules are also linked to nucleic acids by photo-crosslinking.

[0120] In one embodiment, a nucleic acid-based matrix is strengthened by cross-linking nanoparticles or microparticles onto the nucleic acids of the matrix. In one embodiment, the nucleic acids are branched DNA molecules. In some embodiments, the nanoparticles or microparticles are gold, silver, copper, iron, carbon black, 4-phosphonooxy-2,2,6,6-tetramethylpiperidyl oxy nitr-oxide, titanium dioxide, and a magnetic material.

[0121] In addition, the nucleic acids may be methylated, ethylated, alkylated, or otherwise modified along the backbone to influence degradation rates. Generally, methylated, hemi-methylated, ethylated, or alkylated nucleic acids will degrade more slowly. Other backbone modifications affecting degradation rates include the use of heteroatomic oligonucleoside linkages as described in U.S. Pat. No. 5,677,437. Additionally, modifications may be used to prevent the nucleic acid from being transcribed or translated in a given tissue or organism. In addition, the nucleic acids may be capped to prevent degradation. Such caps are generally located at or near the termini of the nucleic acid chains. Examples of capping procedures are included in U.S. Pat. Nos. 5,245,022 and 5,567,810.

[0122] In certain embodiments, where the nucleic acids are DNA molecules, the quantity of DNA in a matrix can be measured by DNA specific labels. DNA-binding dye suitable for this application include SYBR green, SYBR blue, DAPI, propidium iodine, Hoechst, SYBR gold, ethidium bromide, acridines, proflavine, acridine orange, acriflavine, fluorocoumanin, ellipticine, daunomycin, chloroquine, distamycin D, chromomycin, homidium, mithramycin, ruthenium polypyridyls, anthramycin, and the like.

[0123] In another aspect, other fluorescent labels such as sequence specific probes can be employed in the amplification reaction to facilitate the detection and quantification of the amplified products. Probe-based quantitative amplification relies on the sequence-specific detection of a desired amplified product. It utilizes fluorescent, target-specific probes (e.g., TaqMan® probes) resulting in increased specificity and sensitivity. Methods for performing probe-based quantitative amplification are well established in the art and are taught in U.S. Pat. No. 5,210,015.

[0124] In further aspects of the invention, matrixes also include copolymers that maybe biodegradable or nonbiodegradable. Copolymers that are also biodegradable and non-toxic to mammals may be preferred. However, polymers in which only one polymer (e.g. the nucleic acid portion)

degrades, leaving a non-biodegradable framework may also be desirable in certain situations. Examples of materials that may be used as copolymers include but are not limited to, poly(amino acids), including PGA, PLA, PLGA and poly (proline), polysaccharides, such as cellulose, chitin and dextran, proteins, such as fibrin and casein, VICRYL®, MAXON®, PDS, poly(e-caprolactone), polyanhydrides, trimethylene carbonate, poly(beta.-hydroxybutyrate), poly (DTH imino carbonate), poly(bisphenol A iminocarbonate), poly(ortho ester), polycyanoacrylate, polyphosphazene, poly(N-isopropylacrylamide), poly(N-alkylacrylamide), poly(N-n-propylacrylamide), poly(N-isopropylmethacrylamide), poly(ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide), poly(DTEC), dextran-poly lactide, elastin-like polypeptides, a polyester, polylactide, poly(L-lactic acid), poly(D,L-lactic acid), poly(lactide-co-glycolides), biotinylated poly(ethylene glycol-block-lactic acid), poly (alkylcyanoacrylate), poly(epsilon-caprolactone), polyanhydride, poly(bis(p-carboxyphenoxy)propane-sebacic acid), polyorthoester, polyphosphoester, polyphosphazene, polystyrene, polyurethane, poly(amino acid), and hyaluronic acid, derivatives thereof, aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylene oxalates, polyamides, poly(iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, poly(anhydrides), polyphosphazenes, polyoxaamides and polyoxaesters containing amines and/or amido groups, polyanhydrides from diacids of the form HOOC—C₆H₄—O—(CH₂)_m—O—C₆H₄—COOH where m is an integer in the range of 2 to 8 and copolymers thereof with aliphatic alpha-omega diacids of up to 12 carbons are also suitable, and blends of any thereof.

[0125] Aliphatic polyesters include but are not limited to homopolymers and copolymers of lactide (which includes lactic acid, d-, l- and meso lactide), glycolide (including glycolic acid), ε-caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), alkyl derivatives of trimethylene carbonate, δ-valerolactone, β-butyrolactone, γ-butyrolactone, delta-decalactone, gamma-decalactone, hydroxybutyrate (repeating units), hydroxyvalerate (repeating units), 1,4-dioxepan-2-one (including its dimer 1,5,8,12-tetraoxacyclotetradecan 7,14-dione), 1,5-dioxepan-2-one, 6,6-dimethyl-1,4-dioxan-2-one 2,5-diketomorpholine, pivalolactone, alpha, alpha diethylpropiolactone, ethylene carbonate, ethylene oxalate, 3-methyl-1,4-dioxane-2,5-dione, 3,3-diethyl-1,4-dioxan-2,5-dione, 6,8-dioxabicyclooctane-7-one or any polymer material capable of linking to nucleic acids of the present invention.

[0126] Additional examples of copolymers that can be linked to the nucleic acid matrixes of the present invention include peptide sequences. Therefore, in some embodiments the nucleic acid and a copolymer such as peptide sequences are utilized to form a nucleic acid-peptide matrix. Examples of such peptides include but are not limited to sequences such as those reported in U.S. Pat. Nos. 5,670,483 and 5,955,343, and U.S. patent application Ser. No. 09/778,200, the contents of all of which are incorporated herein by reference. These peptide chains consist of alternating hydrophilic and hydrophobic amino acids that are capable of self-assembling to form an exceedingly stable beta-sheet macroscopic structure in the presence of electrolytes, such as monovalent cations.

[0127] The peptide chains are complementary and structurally compatible. The side-chains of the peptide chains in the structure partition into two faces, a polar face with charged

ionic side chains and a nonpolar face with alanines or other hydrophobic groups. These ionic side chains are self-complementary to one another in that the positively charged and negatively charged amino acid residues can form complementary ionic pairs. These peptide chains are therefore called ionic, self-complementary peptides, or Type I self-assembling peptides. If the ionic residues alternate with one positively and one negatively charged residue (−+−+−+), the peptide chains are described as “modulus I;” if the ionic residues alternate with two positively and two negatively charged residues (−++−++), the peptide chains are described as “modulus II.” In some embodiments, peptide sequences for use with the invention have at least 12 or 16 amino acid residues. Both D- and L-amino acids may be used to produce peptide chains. They may be mixed in the same chain, or peptide compositions may be prepared having mixtures of individual chains that themselves only include D- and L-amino acids. Exemplary peptide sequences for use with the invention include those listed in Table 1. Therefore, in various embodiments a nucleic acid matrix or gel can further comprise one or more peptides disclosed herein to form, for example, a DNA-peptide matrix. Such peptides can be associated with X-, Y-, T-, dumbbell- or dendrimer-shape nucleic acids to provide an additional level of control for providing matrixes of morphological and internal structures as desired.

TABLE 1

Representative Self-Assembling Peptides			
Name	Sequence (n-->c)	Modulus	SEQ. ID NO.
DAR16-IV*	n-DADADADARARARA-c	IV	1
DAR32-IV	n-(ADADADADARARARAR) ² -c	IV	2
EHK16	n-HEHEHKHKHEHEHKHK-c	N/A	3
EHK8-I	n-HEHEHKHK-c	N/A	4
VE20*	n-VEVEVEVEVEVEVEVEVEVE-c	N/A	5
RF20*	n-RFRFRFRFRFRFRFRFRFRF-c	N/A	6

N/A denotes not applicable

*These peptides form a β-sheet when incubated in a solution containing NaCl, however they have not been observed to self-assemble to form a macroscopic scaffolds.

[0128] Other self-assembling peptide chains may be generated by changing the amino acid sequence of any self-assembling peptide chains by a single amino acid residue or by multiple amino acid residues. Additionally, the incorporation of specific cell recognition ligands, such as RGD or RAD, into the peptide scaffold may promote the proliferation of the encapsulated cells. In vivo, these ligands may also attract cells from outside a scaffold to the scaffold, where they may invade the scaffold or otherwise interact with the encapsulated cells. To increase the mechanical strength of the resulting scaffolds, cysteines may be incorporated into the peptide chains to allow the formation of disulfide bonds, or residues with aromatic rings may be incorporated and cross-linked by exposure to UV light. The in vivo half-life of the scaffolds may also be modulated by the incorporation of protease cleavage sites into the scaffold, allowing the scaffold to be enzymatically degraded. Combinations of any of the above alterations may also be made to the same peptide scaffold.

[0129] Self-assembled nanoscale structures can be formed with varying degrees of stiffness or elasticity. While not wishing to be bound by any theory, low elasticity may be an important factor in allowing cells to migrate into the scaffold and to communicate with one another once resident in the scaffold. The peptide scaffolds described herein typically have a low elastic modulus, in the range of 1-10 kPa as measured in a standard cone-plate rheometer. Such low values permit scaffold deformation as a result of cell contraction, and this deformation may provide the means for cell-cell communication. In addition, such moduli allow the scaffold to transmit physiological stresses to cells migrating therein, stimulating the cells to produce tissue that is closer in microstructure to native tissue than scar. Scaffold stiffness can be controlled by a variety of means including changes in peptide sequence, changes in peptide concentration, and changes in peptide length. Other methods for increasing stiffness can also be used, such as by attaching a biotin molecule to the amino- or carboxy-terminus of the peptide chains or between the amino- and carboxy-termini, which may then be cross-linked.

[0130] Peptide chains capable of being cross-linked may be synthesized using standard f-moc chemistry and purified using high pressure liquid chromatography (Table 2). The formation of a peptide scaffold may be initiated by the addition of electrolytes as described herein. The hydrophobic residues with aromatic side chains may be cross-linked by exposure to UV irradiation. The extent of the cross-linking may be precisely controlled by the predetermined length of exposure to UV light and the predetermined peptide chain concentration. The extent of cross-linking may be determined by light scattering, gel filtration, or scanning electron microscopy using standard methods. Furthermore, the extent of cross-linking may also be examined by HPLC or mass spectrometry analysis of the scaffold after digestion with a protease, such as matrix metalloproteases. The material strength of the scaffold may be determined before and after cross-linking.

TABLE 2

Representative Self-Assembling Peptides			
Name	Sequence (n-->c)	Modulus	SEQ. ID. NO.
RAD16-I	n-RADARADARADARADA-c	I	7
RGDA16-I	n-RADARGDARADARGDA-c	I	8
RADA8-I	n-RADARADA-c	I	9
RAD16-II	n-RARADADARADADADA-c	II	10
RAD8-II	n-RARADADA-c	II	11
EAKA16-I	n-AEAKAEAKAEAKAEAK-c	I	12
EAKA8-I	n-AEAKAEAK-c	I	13
RAEA16-I	n-RAEARAEARAEARAEA-c	I	14
RAEA8-I	n-RAEARAEA-c	I	15
KADA16-I	n-KADAKADAKADAKADA-c	I	16
KADA8-I	n-KADAKADA-c	I	17

TABLE 2-continued

Representative Self-Assembling Peptides			
Name	Sequence (n-->c)	Modulus	SEQ. ID. NO.
KLD12	n-KLDLKLKDLKLDL-c		18
EAH16-II	n-AEAEAHAAEAEAHAAH-c	II	19
EAH8-II	n-AEAEAHAAH-c	II	20
EFK16-II	n-FEFEFKFKFEFEFKFK-c	II	21
EFK8-11	n-FEFKFEFK-c	I	22
KFE12	n-FKFEFKFEFKFE-c		23
KFE8	n-FKFEFKFE-c		24
KFE16	n-FKFEFKFEFKFEFKFE-c		25
KFQ12	n-FKFQFKFQFKFQ-c		26
KIE12	n-IKIEIKIEIKIE-c		27
KVE12	n-VKVEVKVEVKVE		28
ELK16-II	n-LELELKLKLELELKLK-c	II	29
ELK8-II	n-LELELKLK-c	II	30
EAK16-II	n-AEAEAKAKAEAEAKAK-c	II	31
EAK12	n-AEAEAEAEAKAK-c	IV/II	32
EAK8-II	n-AEAEAKAK-c	II	33
KAE16-IV	n-KAKAKAKAEAEAEAEA-c	IV	34
EAK16-IV	n-AEAEAEAEAKAKAKAK-c	IV	35
RAD16-IV	n-RARARARADADADADA-c	IV	36
DAR16-IV	n-ADADADADARARARAR-c	IV	37

[0131] Aggrecan processing sites, such as those underlined in Table 3, may optionally be added to the amino- or carboxy-terminus of the peptides or between the amino- and carboxy-termini. Likewise, other matrix metalloprotease (MMP) cleavage sites, such as those for collagenases, may be introduced in the same manner. Peptide scaffolds formed from these peptide chains, alone or in combination with peptides capable of being cross-linked, may be exposed to various proteases for various lengths of time and at various protease and peptide concentrations. The rate of degradation of the scaffolds may be determined by HPLC, mass spectrometry, or NMR analysis of the digested peptide chains released into the supernatant at various time points. Alternatively, if radiolabeled peptide chains are used for scaffold formation, the amount of radiolabeled material released into the supernatant may be measured by scintillation counting. For some embodiments, the beta-sheet structure of the assembled peptide chains is degraded sufficiently rapidly that it is not necessary to incorporate cleavage sites in the peptide chains.

TABLE 3

Representative Peptide Sequences Having AggreCan Processing Sites		
Name	Sequence (N-->C)	SEQ. ID. NO.
REE	RGDYRYDYTFREEE-GLGSRDYRGDY	38
KEE	RGDYRYDYTFKEEE-GLGSRDYRGDY	39
SELE	RGDYRYDYTASELE-GRGTRYDYRGDY	40
TAQE	RGDYRYDYAPTAEQ-AGEGPRDY-RGDY	41
ISQE	RGDYRYDYPTISQE-LGQRPRDYRGDY	42
VSQE	RGDYRYDYPTVSQE-LGQRPRDYRGDY	43

[0132] If desired, peptide scaffolds may also be formed with a predetermined shape or volume. To form a scaffold with a desired geometry or dimension, an aqueous peptide solution is added to a pre-shaped casting mold, and the peptide chains are induced to self-assemble into a scaffold by the addition of an electrolyte, as described herein. The resulting geometry and dimensions of the macroscopic peptide scaffold are governed by the concentration and amount of peptide solution that is applied, the concentration of electrolyte used to induce assembly of the scaffold, and the dimensions of the casting apparatus.

[0133] If desired, peptide scaffolds may be characterized using various biophysical and optical instrumentation, such as circular dichroism (CD), dynamic light scattering, Fourier transform infrared (FTIR), atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). For example, biophysical methods may be used to determine the degree of beta-sheet secondary structure in the peptide scaffold. Additionally, filament and pore size, fiber diameter, length, elasticity, and volume fraction may be determined using quantitative image analysis of scanning and transmission electron microscopy. The scaffolds may also be examined using several standard mechanical testing techniques to measure the extent of swelling, the effect of pH and electrolyte concentration on scaffold formation, the level of hydration under various conditions, and the tensile strength.

[0134] The type of nucleic acid polymer or copolymer used will affect the resulting chemical and physical structure of the polymeric biomaterial. The matrixes formed by nucleic acid polymers alone, or nucleic acid polymers and copolymers may be used for a variety of purposes, including controlled release of biologically active agents described herein (e.g., drug delivery), encapsulation and/or culturing cells, tissue engineering applications including, inter alia, to increase tissue tensile strength, as templates for tissue formation, to guide tissue formation, to stimulate nerve growth, to improve vascularization in tissues, as a biodegradable adhesive, as device or implant coating, or to improve the function of a tissue or body part. In addition, the matrixes formed from nucleic acid polymers alone, or nucleic acid polymers and copolymers can be used in a cell-free protein producing system.

[0135] Therefore, in one aspect, a matrix is comprised of branched building block nucleic acid molecules linked to at least one copolymer known in the art or disclosed herein

above, and said matrix comprises linear nucleic acid molecules that encode one or more proteins of interest to be expressed therein or therefrom.

[0136] Matrixes comprising nucleic acids and copolymers may be formed in a variety of ways, depending upon the copolymer used and the desired properties of the finished matrix(es). The copolymer may be attached to the nucleic acid by covalent, ionic or hydrogen bonds or by Van der Waals forces. Linkers such as small organic molecules (esters, amines) or inorganic molecules (silicas, siloxanes), including microparticles or nanoparticles thereof, may be used to attach copolymers to nucleic acids. The finished biomaterial may contain the nucleic acids and copolymers arranged in a variety of fashions including, substantially end-to-end, end-to-side, side-to-side, or any mixture thereof with one or more linkages securing such attachments. Copolymers may also fall into the general forms or block copolymers and graft copolymers. Furthermore, chemical and biological properties of nucleic acid polymers may be influenced by modifications of the copolymers, such as modification of the hydrophobicity or hydrophilicity of the polymers or copolymers.

[0137] Nucleic acid-based matrixes provide many important advantages over protein hydrogels and polymeric hydrogels. First, many different nucleic acid hydrogels with unique properties can be precisely designed and easily fabricated because of the availability of a great variety of branched nucleic acid building blocks of different shapes and different lengths. The different shapes and/or different lengths of nucleic acid monomers can result in different pore sizes of the matrix formed therewith, which in turn can control drug release rates, or provide different three-dimensional scaffolds for cell culturing or tissue engineering. Furthermore, matrixes comprised of nucleic acids of different shapes/lengths can also provide scaffolds for improved protein production, by providing anchoring points for macromolecules necessary for protein expression (e.g., polymerases), as well as a plurality of coding sequences for one or more proteins.

[0138] Therefore, in various embodiments, different release rates are readily obtained by selection Y-, T-, X-DNA or a combination of one or more Y-, T-, or X-DNA to compose a hydrogel.

[0139] Second, since the conditions for fabricating nucleic acid matrixes are very mild (e.g. at room temperature and with a neutral pH), in various embodiments the matrixes provide a unique tool that may be applied in many biotechnological or biomedical applications. For example, since many bioactive agents, including drugs and/or proteins, or cells (e.g., mammalian cells) can be dissolved or dispersed in an aqueous nucleic acid solution, encapsulation of such bioactive agents, e.g., drugs and live cells, is effected in situ, eliminating the need to load drugs into gels and also avoiding denaturing conditions that preclude, for example, encapsulation of live cells.

[0140] Consequently, bioactive agents and cells are contained in an aqueous, physiologically compatible environment, during the pre-gelling, thus allowing the efficiency of encapsulation of such agents to reach close to 100%. In addition, nucleic acid building blocks can also post-react with other chemicals such as nucleic acid-specific reagents, e.g., fluorescent dyes, enabling the tracing of gel matrixes, of biodegradation and distribution processes.

[0141] Furthermore, nucleic acid matrixes are biodegradable and non-immunogenic (e.g., DNA strands are synthesized de novo and lack the immuno-stimulative, bacterial

CpG motifs (Krieg et al., *Nature* 374, 546 (1995); D. Schwartz et al, *J Clin Invest* 100, 68 (1997))), making nucleic acid hydrogels ideal as controlled drug delivery carriers. Therefore, providing yet additional improvement over many of the existing platforms for biomaterials. Moreover, as alluded to previously, due to the mild gelling conditions, in situ encapsulation, and their intrinsic biocompatibilities and biodegradabilities, nucleic acid hydrogels can also encapsulate live cells, thus serve as matrices for 3D cell culture or tissue engineering. In addition, such matrixes can also serve as carriers in cell/tissue transplantation, whereby a bioactive agent and/or cell is delivered in effective concentrations to a desired target site in vivo.

[0142] In one aspect, the invention provides methods of making nucleic acid hydrogels using photocrosslinking. The method can be applied in the context of any of the hydrogels described here, e.g., using X-, Y-, T-, dumbbell building blocks, dendrimer-like shapes and the like. The method includes providing an appropriate mixture of nucleic acid molecules configured to form one or more branched chain structures as desired and photocrosslinking the nucleic acid molecules. The methods allow for rapid formation of hydrogels, e.g., the photocrosslinking can be carried out in about 10 minutes or less. In some embodiments, the photocrosslinking is performed in under 1 min, under 2 min, under 3 min, under 4 min, under 5 min, under 6 min, under 7 min, under 8 min, under 9 min, under 10 min, under 11 min, under 12 min, under 15 min, under 20 min, under 25 min, under 30 min, under 35 min, under 40 min, under 45 min, under 50 min, under 55 min, or under 1 h. If desired, the photocrosslinking reaction can be performed more slowly, e.g., in over 1 min, over 2 min, over 3 min, over 4 min, over 5 min, over 6 min, over 7 min, over 8 min, over 9 min, over 10 min, over 11 min, over 12 min, over 15 min, over 20 min, over 25 min, over 30 min, over 35 min, over 40 min, over 45 min, over 50 min, over 55 min, or over 1 h. If desired, the reaction can be performed in over 1 h, e.g., 90 minutes, 2 h, overnight, or more.

[0143] In some embodiments, the nucleic acid building blocks are first amplified, e.g., using PCR. The nucleic acids can also be hybridized prior before the photocrosslinking step. In some embodiments, the hybridized molecules are at least partially purified before crosslinking. Non-limiting examples of purification techniques include those that separate the nucleic acids by size, e.g., chromatography, electrophoresis or gel filtration. Chromatography techniques include high performance liquid chromatography (HPLC) or flow pressure chromatography (FPLC).

[0144] To facilitate the photo-crosslinking, the nucleic acids can be conjugated to a photoreactive group. In some embodiments, a photoreactive group is conjugated to at least a portion of the nucleic acid molecules before photocrosslinking. Commonly used classes of photoinitiated polymerization include radical photopolymerization through photocleavage (e.g., C—C, C—Cl, C—O, or C—S bonds cleavage), radical photopolymerization by hydrogen abstraction, cationic photopolymerization, condensation polymerization (amine+diacrylate), acrylate systems (acrylate+acrylate), charge transfer, and thiol-ene based photopolymerization system (—SH+acrylate). Non-limiting examples of photoreactive groups include those containing vinyl (e.g., acrylate), N-hydroxysuccinimide, amine, carboxylate and thiol terminated groups. The groups can comprise a primary amine modified

group, secondary amine modified group, tertiary amine modified group, etc. Other such groups are known to those of skill in the art.

[0145] It is further possible to link the photoreactive groups (e.g., amine, thiol, carboxylate, acrylate, etc) to modified nucleic acids. The nucleic acid molecules to be crosslinked can have an attachment point for the photoreactive group on their 5'-end, 3'-end, internally, or a combination thereof. Different photoreactive groups can be attached at different sites as desired to facilitate different hydrogel properties, e.g., strength, configuration, or attachment of other molecules. For example, the nucleic acid building blocks may be photocrosslinked at one end or both ends, and another molecule can be photocrosslinked at another site, e.g., an internal site of the nucleic acid building blocks. Non-limiting examples of other molecules comprise a polymer, a biocompatible agent, a peptide, a polypeptide, a protein, a lipid, a carbohydrate, an aptamer, an antibody, an antigen, a cell growth factor, a DNA binding agent, a detectable label, a selectable marker, biotin, a pharmaceutical agent, a drug, a small molecule, a therapeutic agent, a receptor molecule, a ligand, a nucleic acid molecule or a substrate, or a combination thereof can be. The molecules can be photocrosslinked through combinations of linkages, such as acrylate-polymer+amine-DNA, N-hydroxysuccinimide-polymer+amine-DNA, acrylate-polymer+thiol-DNA, thiol-polymer+acrylate-DNA, amine-polymer+thiol-DNA, thiol-polymer+amine-DNA, etc.

[0146] The photocrosslinking is carried out by exposing the hybridized nucleic acids to an appropriate source of electromagnetic radiation e.g., a source of ultraviolet (UV) or visible light, near infrared, infrared wavelengths and microwaves. In some embodiments, gamma rays, X-rays, radio waves are used. A variety of bulbs, lasers or fibers can be used to provide illumination. In some embodiments, light emitting diodes (LEDs) are used. Different wavelengths are possible. In some embodiments, different illumination sources are used to form one hydrogel matrix. In a non-limiting example, one source may be used to photocrosslink the nucleic acid scaffold, whereas another source is used to photocrosslink one or more compounds to the nucleic acids. Any such combination of photoreactive groups and light sources useful for creating the hydrogels of the present invention are within the scope of the invention.

[0147] In some embodiments, the reaction is carried out in the presence of an initiator, e.g., a photoinitiator that forms free radicals when illuminated by light of appropriate wavelengths. Irgacure from CIBA is a preferred photoinitiator in some embodiments because it is approved for use by the FDA. Other photoinitiators are described herein. Photoinitiators are desirable but optional depending on the design of the building blocks and reaction conditions.

[0148] FIG. 1 illustrates a general schematic for synthesizing hydrogels using photo-crosslinkable DNA building blocks. FIG. 2 outlines two related embodiments of the synthesis method. In one embodiment (the rightmost path shown in FIG. 2), single stranded DNA (ssDNA) is conjugated to a photoreactive group and then hybridized to form DNA building blocks. In another embodiment (the leftmost path in FIG. 2), hybridized DNA building blocks are first prepared without prior conjugation to photo-responsive groups, and then the photoreactive groups are conjugated to the building blocks. In either embodiment, unconjugated DNA building blocks are removed by an appropriate method, e.g., using a size separation technique such as HPLC, before crosslinking.

[0149] Photo-crosslinkable building blocks can be modified, e.g., by biocompatible poly(ethylene glycol) (PEG) chains. Purified DNA-PEG conjugates can be collected from the synthesized sample of DNA and PEG by an HPLC fraction collector or the like.

[0150] In some embodiments, the methods of the invention are used to coat and/or pattern the surface of a substrate. Such substrates include bulk glass, PDMS, and other materials. Similarly, the methods can be used to coat the surface of various types of nanoparticles and microparticles comprising gold, silver, copper, iron, carbon black, 4-phosphonooxy-2, 2,6,6-tetramethylpiperidyl-oxo-nitric-oxide, titanium dioxide. In some embodiments, the particles are magnetic particles. The hydrogel coatings can be configured to adjust various properties, e.g., size, thickness, and shape, depending on the desired design and properties.

[0151] The methods of the invention can incorporate means of linking molecules other than by photocrosslinking. For example, we have previously described methods of making hydrogels wherein the nucleic acid building blocks are joined by enzymatic techniques, e.g., via ligation. Such methods are taught by U.S. patent application Ser. Nos. 11/464,184, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION;" 11/464,181, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES." In some embodiments, the methods of the invention comprise hydrogel formation using a combination of enzymatic or chemical linkage along with photocrosslinking. In one non-limiting example, the nucleic acid hydrogel building blocks are photocrosslinked and the one or more additional compounds are linked to the building blocks using enzymatic or chemical techniques. Many useful additional compounds are disclosed herein, including non-building block nucleic acids, e.g., those having coding regions. In another non-limiting example, the nucleic acid hydrogel building blocks are ligated together and one or more additional compounds are photocrosslinked. In still another example, some building block nucleic acids are enzymatically linked whereas others are photocrosslinked. In any of these embodiments, the photocrosslinking and enzymatic/chemical linking can be performed concurrently or sequentially, or any combination thereof.

[0152] In some embodiments, the nucleic acid hydrogels are linked to another hydrogel material that comprises a natural or synthetic biocompatible material. Non-limiting examples include a poly(ethylene glycol) (PEG) hydrogel matrix, a N-isopropylacrylamide (NiPAAm) hydrogel matrix, a chitosan hydrogel matrix or a derivative of any thereof. Such linkage can serve to alter hydrogel properties. The alternate hydrogel material can be derived from a natural material, e.g., chitosan, methylcellulose, alginate, hyaluronic acid, agarose, fibrin, gelatin, collagen, dextran, or a derivative of any thereof. The alternate hydrogel material can also comprise a synthetic material, including but not limited to hydroxyethyl methacrylate, N-(2-hydroxypropyl)methacrylate, N-vinyl-2-pyrrolidone, N-isopropyl acrylamide, vinyl acetate, acrylic acid, methacrylic acid, polyethylene glycol acrylate/methacrylate, polyethylene glycol diacrylate/dimethacrylate, polyvinyl alcohol, propylene fumarate, or a derivative of any thereof. Combinations of hydrogel materials can also be used together. For example, a natural polymer and a synthetic monomer, a natural polymer and a synthetic monomer, two or more natural polymers, two or more synthetic polymers, and the like.

[0153] Depending on the desired application, the hydrogels of the invention can be fabricated into a variety of shapes, e.g., micro thin films, micro pads, micro thin fibers, nanospheres or microspheres. Microscale or nanoscale patterns can be fabricated by emulsification, photolithography, microfluidic synthesis, micromolding, or micro-electrospinning, or a combination thereof.

[0154] Building Blocks of Different Shapes

[0155] One aspect of the invention is directed to a matrix comprising nucleic acids that include X-shape, T-shape, Y-shape, dumbbell-shape or dendrimer shape, which nucleic acids can be used as building blocks for new, designer biomaterials. Thus the nucleic acid(s) have different shapes and one or more shapes can be used as a monomer or a crosslinker (e.g., building block) for constructing a matrix. In one embodiment, branched nucleic acids are all of one shape (X-, Y-, dumbbell- or T-shape), which nucleic acids are used as monomers or crosslinkers to form nucleic acid hydrogels. In some embodiments, branched nucleic acids are prepared through the hybridization of the complementary sequences of the pre-designed oligonucleotides. Appropriate branched chain nucleic acid sequences are disclosed herein, although any building blocks having appropriate sequences can be used. In some embodiments, the nucleic acids are DNA, RNA, PNA, LNA or TNA. In some embodiments, one or more combinations of such nucleic acids can be used as building blocks. In some embodiments, the monomers are linked to other monomers by photo-crosslinking according to the present invention. In further embodiments, the monomers are linked to other monomers by ligation. For example, the monomers can undergo a ligation reaction facilitated by a nucleic acid ligase. In some embodiments, more than one method of cross linking is used, e.g., both photocrosslinking and ligation.

[0156] Nucleic acids are capable of undergoing enzymatic reactions. In some embodiments, the reactions include reactions by enzymes, wherein said one or more enzyme is a DNA polymerase, RNA reverse transcriptase, terminal transferase, DNA ligase, RNA ligase, exonuclease, ribonuclease, endonuclease, polynucleotide kinase, DNA methylase, or DNA ubiquitinase. Furthermore, reactions include any reaction wherein one or more enzyme is an enzyme that shortens nucleic acids, lengthens nucleic acids, amplifies nucleic acids, labels nucleic acids, or a combination of reactions/enzymes thereof.

[0157] In one aspect of the invention, a matrix comprises different shape nucleic acid molecules, ratios for which can be selected to predetermine the geometrical pattern, chemical and physical properties for the resulting matrix. For example, a matrix can be comprised of a ratio of monomers of X- and Y-shaped, X- and T-shaped, X- and dumbbell-shaped, Y- and T-shaped, Y- and dumbbell-shaped, or T- and dumbbell shaped nucleic acids. In such embodiments, each monomer can be DNA, RNA, PNA, LNA, TNA or analogs thereof. In one embodiment, the matrix is comprised of DNA. In another embodiment, the matrix is comprised of RNA and DNA. In yet another embodiment, the matrix is comprised of RNA. Therefore, one or more matrixes can be entirely comprised of nucleic acids molecules of one type or a combination of types (e.g., DNA, RNA types, etc.).

[0158] In one embodiment, the resulting matrix is three-dimensional. In another embodiment, the resulting matrix is capable of gelling. In yet another embodiment, the resulting matrix is a hydrogel. In yet further aspects of the invention, as

disclosed herein, any one or more matrixes of the present invention can be linked to a copolymer or additional chemical moieties. Furthermore, the nucleic acid molecules of the one or more matrices of the present invention can be linear, X-shape, Y-shape, T-shape, dumbbell-shape, dendrimer shape or any combination thereof. The following non-limiting examples provide some of the characteristics for nucleic acid molecules (e.g., building blocks) that may be utilized in one or more compositions or methods disclosed herein.

[0159] X-Shape

[0160] In one aspect of the present invention, a matrix of the present invention is comprised entirely or at least in part of branched nucleic acids that are X-shape nucleic acids. In one embodiment, the X-shape nucleic acid is DNA. In yet another embodiment, the matrix is comprised of X-shape DNA and/or RNA, or analogs/derivative thereof. In another embodiment, the matrix is comprised of X-shape DNA, and linear DNA, RNA or PNA. In one preferred embodiment, the matrix is nearly entirely comprised of nucleic acids. In yet another embodiment, the X-shape nucleic acids are RNA.

[0161] In another aspect, the matrix is comprised of X-shape and linear nucleic acids and at least one copolymer. In one embodiment, the X-shape and linear nucleic acid is DNA. In another embodiment, the X-shape nucleic acid is DNA, while the linear nucleic acid is DNA, RNA or PNA. The at least one copolymer is selected from those known in the art or as disclosed herein above. In one embodiment, the copolymer is a peptide monomer.

[0162] In addition, certain aspects of the invention are directed to the X-shape nucleic acid being “reinforced” to produce a more stable and resilient scaffold or matrix, by linking a nanoparticle to the nucleic acid. In one embodiment, the X-shape nucleic acid is DNA. In another embodiment, the X-shape nucleic acid is RNA. In some embodiments, the X-shape DNA is linked to a nanoparticle or microparticle. The substrate can be composed of any appropriate material. For example, the substrate can be a metal substrate, e.g., a noble metal or a transition metal. Such metals include without limitation gold, silver, palladium, or platinum. The substrate can also be a semiconductor material, e.g., cadmium sulfide (CdS), cadmium selenide (CdSe), titanium dioxide (TiO₂), zinc oxide (ZnO). In some embodiments, magnetic materials are used as the substrate. Magnetic materials appropriate for use with the invention comprise cobalt, nickel, iron, iron-cobalt, and magnetite (Fe₃O₄). Other substrate materials are envisioned, e.g., carbon black or 4-phosphonooxy-2,2,6,6-tetramethylpiperidyl nitroxide.

[0163] In one embodiment, four different oligonucleotides with complimentary sequences, termed as X0a, X0b, X0c, and X0d (Table 5A), are hybridized with each other through an annealing process to achieve the final X-DNA. Furthermore, a plurality of said X-DNA can be linked via same or different linear DNA, which can be varied by sequence and/or size, to construct a unique matrix or networked matrix. See FIGS. 3A-B.

[0164] In certain aspects of the invention, the X-DNA terminal ends are designed with sticky ends that are capable of undergoing an enzymatic reaction. In one embodiment, the enzymatic reaction is a ligation reaction with a DNA ligase, which results in covalent linkage of two or more monomers. In yet a further embodiment, the DNA ligase is a T4 DNA ligase.

[0165] In one aspect, X-shaped nucleic acids used in building a matrix result in the matrix comprising a tensile modulus

of about 0.4 and tensile strength of about 42%. In some embodiments, the X-linked DNA comprises a tensile modulus of about 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, or about 0.7. In some embodiments, X-DNA used to build a matrix comprise tensile strength (ultimate elongation) of about 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50%, whereby the percentage refers to its own length (stretch).

[0166] In one embodiment, X-DNA molecules can be designed and synthesized in such a way that each arm of the X-DNA possesses a complimentary sticky end whose sequences are palindromic.

[0167] X-shaped nucleic acid molecules can be synthesized by mixing equal amounts of four oligonucleotide strands. The nomenclature is as follows: X0a, X0b, X0c, and X0d are the four corresponding single oligonucleotide chains that form a X0-nucleic acid molecule (X0). Similarly, X1a, X1b, X1c, and X1d are the four corresponding single oligonucleotide chains that form an X1-nucleic acid molecule (X1); and Xna, Xnb, Xnc, and Xnd are the four corresponding single oligonucleotide chains that form a Xn-shaped nucleic acid molecule (Xn). The reactions can be the following: X0a+X0b+X0c+X0d→X0, X1a+X1b+X1c+X1d→X1, and Xna+Xnb+Xnc+Xnd→Xn, etc. See FIGS. 4 and 5.

[0168] For the X-shaped nucleic acid molecule, the region 2 of each polynucleotide is complementary to region 3 of one of the other three polynucleotides. For example, with reference to the sequences in Tables 5A and 5B: region 2 of SEQ ID NO: 56 is complementary to region 3 of SEQ ID NO: 59, region 2 of SEQ ID NO: 57 is complementary to region 3 of SEQ ID NO: 56, region 2 of SEQ ID NO: 58 is complementary to region 3 of SEQ ID NO: 57; and region 2 of SEQ ID NO: 59 is complementary to region 3 of SEQ ID NO: 58.

[0169] In one embodiment, the length of each of the regions can vary. For example, in some embodiments, the second and/or third regions for the X-shaped nucleic acid molecule and the second and/or fourth regions of the T-shaped nucleic acid molecules are about 13 nucleotides each in length. In some embodiments, the lengths of these regions may be 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, these regions may be larger than 20 nucleotides in length, for example they may be about 25, 30, 35, 40, 45, or 50 nucleotides in length.

TABLE 4A

Sequences of Oligonucleotides					
Strand	SEQ ID NO:	Region 1	Region 2	Region 3	Region 4
T _{0a}	44	5'-ACTG	CTGGATCG TATGCGTA	GTC	TGGACGTCTAC CGTGT-3'
T _{0b}	45	5'-CAGT	GCAGGCT		ACGCATACCAT CCAG-3'
T _{0c}	46	5'-ACTG	ACACGGTA GACGTCCA		GCCTGC-3'

TABLE 4B

Sequence Table	
SEQ ID NO	Sequence
44	5'-ACTGCTGGATCGTATGCGTAGTCTGGACGTCTACCGTGT-3'
45	5'-CAGTGCAGGCTACGCATACCATCCAG-3'
46	5'-ACTGACACGGTAGACGTCCAGCCTGC-3'
47	5'-ACTG-3'
48	5'-CAGT-3'
49	5'-CTGGATCGTATGCGTA-3'
50	5'-GCAGGCT-3'
51	5'-ACACGGTAGACGTCCA-3'
52	5'-GTC-3'
53	5'-TGGACGTCTACCGTGT-3'
54	5'-ACGCATACCATCCAG-3'
55	5'-GCCTGC-3'

TABLE 5A

Sequences of Oligonucleotides				
Strand	SEQ ID NO:	Region 1	Region 2	Region 3
X _{0a}	56	3'-TCGA	AGGCTGATTCGGT	TAGTCCATGAGTC-5'
X _{0b}	57	3'-AATT	GACTCATGGACTA	TCATGCGGATCCA-5'
X _{0c}	58	3'-AGCT	TGGATCCGCATGA	CATTGCGCGTAAG-5'
X _{0d}	59	3'-GATC	CTTACGGCGAATG	ACCGAATCAGCCT-5'

TABLE 5B

Sequence Table	
SEQ ID NO	Sequence
56	3'-TCGAAGGCTGATTCGGTTAGTCCATGAGTC-5'
57	3'-AATTGACTCATGGACTATCATGCGGATCCA-5'
58	3'-AGCTTGGATCCGCATGACATTGCGCGTAAG-5'
59	3'-GATCCTTACGGCGAATGACCGAATCAGCCT-5'
60	3'-TCGA-5'
61	3'-AATT-5'
62	3'-AGCT-5'

TABLE 5B-continued

Sequence Table	
SEQ ID NO	Sequence
63	3'-GATC-5'
64	3'-AGGCTGATTCGGT-5'
65	3'-GACTCATGGACTA-5'
66	3'-TGGATCCGCATGA-5'
67	3'-CTTACGGCGAATG-5'
68	3'-TAGTCCATGAGTC-5'
69	3'-TCATGCGGATCCA-5'
70	3'-CATTGCGCGTAAG-5'
71	3'-ACCGAATCAGCCT-5'

[0170] Thus, X-DNA can be photocrosslinked with each other, resulting in a DNA hydrogel. The gels, irrespective of the kind of DNA building blocks used (e.g., X-, Y-, dumbbell- or T-shape), can be formed rapidly through this process, e.g., in minutes, e.g., 10 min or less. In some embodiments, the gels are formed in less than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 min. In some embodiments, linear nucleic acids, Y-shape, T-shape, dumbbell-shape or dendrimer shape nucleic acids can also be incorporated into a matrix or gel structure formed of X-shape nucleic acids. Therefore, in some embodiments, the matrix is comprised of X-shape and one or more other shapes in a ratio of each monomer that is preselected as desired. See FIG. 6A-B.

[0171] Y-Shape

[0172] In another aspect, the nucleic acids comprising a matrix are Y-shape nucleic acids, as illustrated by FIGS. 7A and 7B. In one embodiment, the Y-shape nucleic acid is DNA. In yet another embodiment, the matrix comprises Y-shape DNA and/or RNA, or analogs/derivatives thereof. In another embodiment, the matrix is comprised of Y-shape DNA, and linear DNA or RNA. In one embodiment, the matrix is comprised entirely of nucleic acids that are Y-shape. In a further embodiment, the matrix can comprise Y-shape and X-shape nucleic acids, in a ratio that is preselected as desired.

[0173] In one embodiment, dendrimer-line DNA (DL-DNA) is assembled by photocrosslinking of Y-DNA molecules, whose sequences are specifically designed so that crosslinking between Y_i and Y-DNA could only occur when i≠j, where i and j refer to the generation number n, for example, G1, G2, etc. In addition, the linking can only occur in one direction, that is, Y1->Y2->Y3->Y4, and so on. When Y0 is ligated to Y1 with a 1:3 molar stoichiometry, one Y0 is linked with three Y1, forming the first-generation DL-DNA. G1 is then ligated to six Y2 (one Y2 for each of the six free branches of G1), resulting in a second-generation DL-DNA (G2). The third (G3), fourth (G4), and higher generation DL-DNA are assembled in a similar way. Note that the assembled DL-DNA (G_n) has only one possible conforma

tion due to the unidirectional linking strategy. The general format of the nth-generation DL-DNA is $G_n = (Y_0)(3Y_1)(6Y_2) \dots (3 \cdot 2^{n-1}Y_n)$, where n is the generation number and Y_n is the nth Y-DNA. The total number of Y-DNA in an nth-generation DL-DNA is $3 \cdot 2^{n-2}$. The growth of DL-DNA from nth generation to (n+1)th generation requires a total of $3 \cdot 2^n$ new Y_{n+1} -DNA.

[0174] Three specific polynucleotides are combined to form each Y-DNA. Each polynucleotide may include three regions. A first region (region 1) of each polynucleotide may include nucleotides that will form a 5' sticky end when a Y-DNA is formed. A "sticky end" is a single-stranded overhang portion of one of the polynucleotides. In various embodiments, the sticky ends for any X-, Y-, T- or dumbbell-shape nucleic acid can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides. In some embodiments, a polynucleotide will not have this sticky end. In general, a shorter sticky end will allow for less selectivity in binding. For example, a polynucleotide lacking a sticky end would have little to no selectivity. The sticky end in some embodiments is a four nucleotide sticky end.

[0175] The sticky end in some embodiments is a four nucleotide sticky end. In some embodiments, the sticky end includes, or is, TGAC, GTCA, CGAT, ATCG, GCAT, ATGC, TTGC, GCAA, or GGAT (e.g., Tables 4-6).

[0176] The second region (region 2) of each polynucleotide is complementary to the third region (region 3) of one of the other two polynucleotides that form the Y-DNA. The third region of each polynucleotide is complementary to the second region of the other of the other two polynucleotides of Y-DNA. For example, with reference to the sequences in Tables 6A and 6B: region 2 of SEQ ID NOs 72-76, represented by SEQ ID NO:96, is complementary to region 3 of SEQ ID NOs 82-86, represented by SEQ ID NO:101, region 3 of SEQ ID NOs 72-76, represented by SEQ ID NO:97, is complementary to region 2 of SEQ ID NOs 77-81, represented by SEQ ID NO:98; and region 2 of SEQ ID NOs 82-86, represented by SEQ ID NO:100, is complementary to region 3 of SEQ ID NOs 77-81, represented by SEQ ID NO:99.

[0177] In some embodiments of the invention, the length of each of the regions can vary. For example, in some embodiments, the second and/or third regions are about 13 nucleotides each in length. In some embodiments, the lengths of the second and/or third regions may be 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments of the invention, the second and/or third regions may be larger than 20 nucleotides in length, for example, they may be about 25, 30, 35, 40, 45, or 50 nucleotides in length.

[0178] In one embodiment of the invention, each polynucleotide is 30 nucleotides in length, with the first region having 4 nucleotides, the second region having 13 nucleotides, and the third region also having 13 nucleotides. In some embodiments of the invention, the Y-shape polynucleotides include, essentially include, or are comprised of SEQ ID NOs:72-86, or SEQ ID NOs: 102-112. With respect to any of the nucleic acid building blocks described herein (e.g., X-, Y-, T-, dumbbell-, dendrimer-shape), in various embodiments, the 5' end can comprise a phosphorylation modification so as to include various labels disclosed here, including Alexa Fluor 488, B0630 (See, probes/labels, supra).

TABLE 6A

Sequences of Oligonucleotides				
Strand	SEQ ID NO:	Region 1	Region 2	Region 3
Y_{0a}	72	5'-TGAC	TGGATCCGCATGA	CATTCCCGTAAG-3'
Y_{1a}	73	5'-GTCA	TGGATCCGCATGA	CATTCCCGTAAG-3'
Y_{2a}	74	5'-ATCG	TGGATCCGCATGA	CATTCCCGTAAG-3'
Y_{3a}	75	5'-ATGC	TGGATCCGCATGA	CATTCCCGTAAG-3'
Y_{4a}	76	5'-GCAA	TGGATCCGCATGA	CATTCCCGTAAG-3'
Y_{0b}	77	5'-TGAC	CTTACGGCGAATG	ACCGAATCAGCCT-3'
Y_{1b}	78	5'-CGAT	CTTACGGCGAATG	ACCGAATCAGCCT-3'
Y_{2b}	79	5'-GCAT	CTTACGGCGAATG	ACCGAATCAGCCT-3'
Y_{3b}	80	5'-TTGC	CTTACGGCGAATG	ACCGAATCAGCCT-3'
Y_{4b}	81	5'-GGAT	CTTACGGCGAATG	ACCGAATCAGCCT-3'
Y_{0c}	82	5'-TGAC	AGGCTGATTCGGT	TCATGCGGATCCA-3'
Y_{1c}	83	5'-CGAT	AGGCTGATTCGGT	TCATGCGGATCCA-3'
Y_{2c}	84	5'-GCAT	AGGCTGATTCGGT	TCATGCGGATCCA-3'
Y_{3c}	85	5'-TTGC	AGGCTGATTCGGT	TCATGCGGATCCA-3'
Y_{4c}	86	5'-GGAT	AGGCTGATTCGGT	TCATGCGGATCCA-3'

TABLE 6B

Sequence Table	
SEQ ID NO	Sequence
72	5'-TGACTGGATCCGCATGACATTGCGCGTAAG-3'
73	5'-GTCATGGATCCGCATGACATTGCGCGTAAG-3'
74	5'-ATCGTGGATCCGCATGACATTGCGCGTAAG-3'
75	5'-ATGCTGGATCCGCATGACATTGCGCGTAAG-3'
76	5'-GCAATGGATCCGCATGACATTGCGCGTAAG-3'
77	5'-TGACCTTACGGCGAATGACCGAATCAGCCT-3'
78	5'-CGATCTTACGGCGAATGACCGAATCAGCCT-3'
79	5'-GCATCTTACGGCGAATGACCGAATCAGCCT-3'
80	5'-TTGCCTTACGGCGAATGACCGAATCAGCCT-3'
81	5'-GGATCTTACGGCGAATGACCGAATCAGCCT-3'
82	5'-TGACAGGCTGATTCGGTTTCATGCGGATCCA-3'
83	5'-CGATAGGCTGATTCGGTTTCATGCGGATCCA-3'
84	5'-GCATAGGCTGATTCGGTTTCATGCGGATCCA-3'
85	5'-TTGACAGGCTGATTCGGTTTCATGCGGATCCA-3'
86	5'-GGATAGGCTGATTCGGTTTCATGCGGATCCA-3'

TABLE 6B-continued

Sequence Table	
SEQ ID NO	Sequence
87	5'-TGAC-3'
88	5'-GTCA-3'
89	5'-CGAT-3'
90	5'-ATCG-3'
91	5'-GCAT-3'
92	5'-ATGC-3'
93	5'-TTGC-3'
94	5'-GCAA-3'
95	5'-GGAT-3'
96	5'-TGGATCCGCATGA-3'
97	5'-CATTCGCCGTAAG-3'
98	5'-CTTACGGCGAATG-3'
99	5'-ACCGAATCAGCCT-3'
100	5'-AGGCTGATTCGGT-3'
101	5'-TCATGCGGATCCA-3'
102	5'-TTGCTGGATCCGCATGACATTGCGCGTAAG-3'
103	5'-CGTTTGGATCCGCATGACATTGCGCGTAAG-3'
104	5'-ATGCTGGATCCGCATGACATTGCGCGTAAG-3'
105	5'-TGGATCCGCATGACATTGCGCGTAAG-3'
106	5'-GCATCTTACGGCGAATGACCGAATCAGCCT-3'
107	5'-GCAACTTACGGCGAATGACCGAATCAGCCT-3'
108	5'-CTTACGGCGAATGACCGAATCAGCCT-3'
109	5'-GCATAGGCTGATTCGGTTCATGCGGATCCA-3'
110	5'-TTGCAGGCTGATTCGGTTCATGCGGATCCA-3'
111	5'-AACGAGGCTGATTCGGTTCATGCGGATCCA-3'
112	5'-AGGCTGATTCGGTTCATGCGGATCCA-3'

[0179] In another aspect, the matrix is comprised of Y-shape and linear nucleic acids and at least one copolymer. In one embodiment, the Y-shape and linear nucleic acid is DNA. In another embodiment, the Y-shape nucleic acid is DNA, while the linear nucleic acid is DNA, RNA, TNA or PNA. Furthermore, the at least one copolymer is selected from those known in the art or as disclosed herein. In one embodiment, the copolymer is a peptide monomer, as known in the art or disclosed herein.

[0180] In certain aspects of the invention, the Y-DNA terminal ends are designed with sticky ends as described above that are capable of undergoing a reaction. In some embodiments, the reaction is attachment of a photoreactive moiety to allow photocrosslinking. In some embodiments, the reaction

is an enzymatic reaction. In some embodiments, wherein enzymatic reactions are used to link nucleic acids, the reaction is a ligation reaction with a DNA ligase. In yet a further embodiment, the DNA ligase is a T4 DNA ligase.

[0181] In one embodiment, Y-shape nucleic acid building blocks are joined end-to-end to produce a dumbbell shaped building block or dendrimer like nucleic acid. See, e.g., FIGS. 7 and 8B.

[0182] In one aspect, Y-shaped nucleic acid building blocks form a matrix having a tensile modulus of about 0.4 and tensile strength of about 42%. In some embodiments, the X-linked DNA comprises a tensile modulus of about 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, or about 0.7. In one embodiment, Y-nucleic acids utilized to build a matrix comprise tensile strength of about 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60%. In one embodiment, the Y-shaped nucleic acids are DNA. In another, embodiment, the Y-shaped nucleic acids are RNA.

[0183] In another aspect, the Y-shape nucleic acids of the invention are used to form a dendrimer structure (exemplary dendrimer structures are shown in FIGS. 10A-C). Branched nucleic acids described herein are dendrimer like, thus by combining such nucleic acids in a step-wise or all in one fashion, dendrimer structures are formed. Furthermore, in some embodiments, the various arms of said Y-shape nucleic acids, as comprised in a dendrimer structure, are linked to one or more biologically active agents, which agents are described herein. Thus, in one embodiment, the arms are linked to a targeting peptide or signal peptide, a selection marker, a detectable label, a small compound, a drug, a pharmaceutical or to a plasmid or viral vector, or virus. It should be apparent to one of skill in the art that the Y-shape nucleic acids forming said dendrimer afford attachment of multiple same or different compounds, as illustrated in FIGS. 11-12. In other words, the dendrimer structures are anisotropic and/or multivalent. In other embodiments, X-shaped, T-shaped or dumbbell-shaped nucleic acids are used to form dendrimer structures.

[0184] In one embodiment the Y-shape, X-shape, T-shape or dumbbell-shape arms are attached to a peptide moiety comprising an adenovirus core peptide, a synthetic peptide, an influenza virus HA2 peptide, a simian immunodeficiency virus gp32 peptide, an SV40 T-Ag peptide, a VP22 peptide, a Tat peptide, a Rev peptide, DNA condensing peptide, DNA protection peptide, endosomal targeting peptide, membrane fusion peptide, nuclear localization signaling peptide, a protein transduction domain peptide or any combination thereof.

[0185] In another embodiment, the Y-shape, T-shape, X-shape or dumbbell shape nucleic acids are linked to one or more biologically active agents, including the preceding peptides, one or more selection markers, one or more detectable labels, one or more drugs, small compounds, or nucleic acid sequences or one or more copolymer compounds.

[0186] T-Shape

[0187] In yet another aspect, the nucleic acids forming a matrix are T-shape nucleic acids (FIG. 13). In one embodiment, the T-shape nucleic acids are DNA. In yet another embodiment, the matrix comprises T-shape DNA and/or RNA, or analogs/derivatives thereof. In addition, a matrix can be comprised of T-shape and one or more different shapes of nucleic acids, including X-, Y-, dumbbell- or dendrimer-shape nucleic acids, as well as a combination thereof.

[0188] In one embodiment, the T-shape nucleic acids have a tensile strength selected from 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65%. In addition, the T-shape nucleic acids can have a degree of swelling selected from 100, 105, 110, 115, 120, 125, 130, or 135%. For the T-shaped nucleic acid molecule, the second region (region 2) of each polynucleotide is complementary to the fourth region (region 4) of one of the other two polynucleotides. The fourth region of each polynucleotide is complementary to the second region of the other of the other two polynucleotides of T-shaped nucleic acid molecule. The third region is either absent or is a linker to permit formation of the T-shaped configuration. For example, with reference to the sequences in Tables 4A and 4B: region 2 of SEQ ID NO: 46 is complementary to region 4 of SEQ ID NO: 44, region 4 of SEQ ID NO: 46 is complementary to region 2 of SEQ ID NO: 45, and region 2 of SEQ ID NO: 44 is complementary to region 4 of SEQ ID NO: 45.

[0189] T-shaped nucleic acid molecules can be synthesized by mixing equal amounts of three oligonucleotide strands. The nomenclature is as follows: T0a, T0b, and T0c are the three corresponding single oligonucleotide chains that form a T0-nucleic acid molecule (T0). Similarly, T1a, T1b, and T1c are the three corresponding single oligonucleotide chains that form a T1-nucleic acid molecule (T1); and Tna, Tnb, and Tnc are the three corresponding single oligonucleotide chains that form a Tn-shaped nucleic acid molecule (Tn). The reactions can be the following: T0a+T0b+T0c→T0, T1a+T1b+T1c→T1, and Tna+Tnb+Tnc→Tn, etc. See FIGS. 13 and 14.

[0190] In various embodiments, selection of X-, Y- or T-DNA can be used to design hydrogels of differing external morphologies and internal structure. Such morphologies are described in U.S. patent application Ser. Nos. 11/464,184, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION;" 11/464,181, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES," which applications are incorporated herein by reference in its entirety. As show therein, for example, in a dry state surface morphology revealed a tangled pattern for X-DNA gel, a fibrous form for Y-DNA gel, and a scale shape for T-DNA gel. Furthermore, X-DNA gels can exhibit two flat DNA gel strips tangled into a knot to form a large sheet with many wrinkles on the surface. Y-DNA gel exhibits fibrous a fibrous form spreading out from many branches. T-DNA gel exhibits puckers on a sheet. In a swollen state, the surface morphology of the gels exhibited a large number of various sized pores and channels, with obvious fibers of fractal-shapes on the periphery and perpendicularly erected, scale like structures for X-, Y- and T-DNAs.

[0191] In yet other embodiments, gels can be comprised of one or more differently shaped nucleic acids, including X-, Y-, T-, dumbbell- or dendrimer-shaped DNA (e.g., Y- and X-DNA, or Y- and T-DNA or X- and T-DNA). See FIGS. 15A-C. In yet further embodiments, applicable to any matrix disclosed herein, gels can be comprised of nucleic acids that include DNA, RNA, PNA, TNA, or a combination thereof.

[0192] Matrix Pore Size

[0193] In another aspect of the invention, by selecting a particular nucleic acid or combination of nucleic acids to construct a matrix, the resulting matrix comprises pores. In one embodiment, the pores are of a size selected from 5 nm, about 10 nm, about 15 nm, about 20 nm, about 30 nm, about 40 nm, about 50 nm, and about 100 nm. In yet another embodiment, said pores have a size selected from a group

consisting of about 0.1 micron to about 5 microns, about 10 microns, about 20 microns, about 30 microns, about 40 microns, about 50 microns, about 100 microns, about 200 microns, about 300 microns, about 400 microns, about 500 microns, 600 microns and about 1000 microns. Therefore, by selecting different length monomers and/or different shapes, a matrix can be constructed having substantially predetermined pore sizes.

[0194] In another aspect of the invention, methods are directed to producing three-dimensional matrices by utilizing branched nucleic acids, in particular X-, Y-, T-, dumbbell-, or dendrimer-nucleic acids as the building blocks that form said three-dimensional matrices. In one embodiment, the nucleic acids are DNA molecules. In another embodiment, the nucleic acids are RNA and/or PNA. Furthermore, the nucleic acids can be a combination of DNA, RNA or PNA, or any other nucleic acids disclosed herein. In another embodiment, said matrices comprise linear nucleic acids that encode one or more proteins.

[0195] In one aspect, the three-dimensional matrix structure is designed to comprise certain pore sizes based on selection of the particular nucleic-acid based building blocks, wherein a matrix (hydrogel) is comprised of a single shape monomer building block, which monomer can be X-, Y-, T-, dumbbell- or dendrimer-shape, or a combination thereof. In one embodiment, the nucleic acids are X and Y shapes and provided in predetermined ratios so as to provide a network matrix structure resulting in a predictable pore size or range of pore sizes. In another embodiment, the nucleic acids further include linear nucleic acids utilized concomitantly with the various shapes selected from X-, Y-, Y-, dumbbell-, dendrimer-shapes, or a combination thereof. In another embodiment, the network matrix structure is hydradable, whereby a dry compound comprising the nucleic acid-based matrix swells in volume (with water or similar liquid) from about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500%.

[0196] Hydrogel Swelling

[0197] DNA incorporation and the degree of swelling of the DNA hydrogels.

[0198] The swelling degree Q of the DNA hydrogel was calculated from:

$$Q = 1 + \rho_2 \cdot \left(\frac{m_{sw}}{m_d \cdot \rho_1} - \frac{1}{\rho_1} \right)$$

[0199] where m_{sw} is the weight of the sample in the swollen state, m_d is the weight of the dry extracted sample, and ρ_1 and ρ_2 are the specific densities of the swelling medium and the polymer, respectively. ρ_2 was determined by weighing a sample piece of precisely known length, width, and thickness (A. Lendin, A. M. Schmidt, R. Langer, *Proc Natl Acad Sci USA* 98, 842 (2001)).

[0200] The DNA gels comprising sequences shown in Table 7 were fabricated in a cylindrical mold with a known size and volume. The gels were thoroughly freeze-dried overnight and then weighed. For the swollen gels, 300 μ l of fresh water was added into the dried-gel tube and incubated for over a day in order for the DNA gels to swell. All values shown in Table 8 are an average of at least three replicates. N/D means "not determined" Mechanical properties of the X-, Y-, and T-gels are compared in Tables 8 and 9.

TABLE 7

Examples of Oligonucleotides used to construct X-, Y- and T-nucleic acid building blocks.									
Strand		Segment 1		Segment 2		SEQ. ID. NO.			
X01	5'-p-ACGT	CGA	CCG	ATG	AAT	AGC	GGT	CAG	113
		ATC	CGT	ACC	TAC	TCG	-3'		
X02	5'-p-ACGT	CGA	GTA	GGT	ACG	GAT	CTG	CGT	114
		ATT	GCG	AAC	GAC	TCG	-3'		
X03	5'-p-ACGT	CGA	GTC	GTT	CGC	AAT	ACG	GCT	115
		GTA	CGT	ATG	GTC	TCG	-3'		
X04	5'-p-ACGT	CGA	GAC	CAT	ACG	TAC	AGC	ACC	116
		GCT	ATT	CAT	CGG	TCG	-3'		
Ya	5'-p-ACGT	CGA	CCG	ATG	AAT	AGC	GGT	CAG	117
		ATC	CGT	ACC	TAC	TCG	-3'		
Yb	5'-p-ACGT	CGA	GTC	GTT	CGC	AAT	ACG	ACC	118
		GCT	ATT	CAT	CGG	TCG	-3'		
Yc	5'-p-ACGT	CGA	GTA	GGT	ACG	GAT	CTG	CGT	119
		ATT	GCG	AAC	GAC	TCG	-3'		
Ta	5'-p-ACGT	CGA	CAG	CTG	ACT	AGA	GTC	ACG	120
		ACC	TGT	ACC	TAC	TCG	-3'		
Tb	5'-p-ACGT	CGA	GTC	GTT	CTC	AAG	ACG	TAG	121
		CTA	GGA	CTC	TAG	TCA	GCT	GTC	
		G-3'							
Tc	5'-p-ACGT	CGA	GTA	GGT	ACA	GGT	CGT	CGT	122
		CTT	GAG	AAC	GAC	TCG	-3'		

[0201] Note that p represents the phosphorylation on the 5' end of the oligonucleotide.

TABLE 8

Hydrogel Swelling		
DNA building blocks (initial concentration)	DNA building blocks incorporated (%)	Q (%)
X-DNA gel (0.2 mM)	83.05 ± 2.10	693.79 ± 8.33
Y-DNA gel (0.2 mM)	81.07 ± 0.63	439.65 ± 7.30
T-DNA gel (0.2 mM)	80.12 ± 5.15	428.19 ± 10.14
X-DNA gel (0.1 mM)	79.66 ± 5.91	531.07 ± 24.17
Y-DNA gel (0.1 mM)	80.45 ± 3.28	281.04 ± 7.53
T-DNA gel (0.1 mM)	79.67 ± 1.56	278.13 ± 9.51
X-DNA gel (0.03 mM)	79.67 ± 2.78	N/D
Y-DNA gel (0.03 mM)	79.08 ± 0.60	137.05 ± 13.90
T-DNA gel (0.03 mM)	72.41 ± 4.76	132.64 ± 3.73

TABLE 9A

Tensile Strength			
DNA Building Blocks (initial concentration)	Specific Gravity	Tensile Modulus (mPa)	Tensile Strength (%) (Ultimate Elongation)
X-DNA gel (0.2 mM)	1.060	0.0415 = 0.0003	42.50 = 1.13
Y-DNA gel (0.2 mM)	1.033	0.0015-0.0001	48.89 = 0.59
T-DNA gel (0.2 mM)	1.012	0.0104 = 0.0007	57.93 = 0.00

[0202] The specific gravity of a substance is a comparison of its density to that of water. The percentage (ultimate elongation) indicates how close the measurements are made to the point at which the gel breaks.

TABLE 9B

Mechanical properties of dried and swollen hydrogels			
	Specific Gravity	Tensile Modulus (mPa)	Tensile Strength
X-DNA			
0.01 dried	N/A	N/A	N/A
0.01 swollen	N/A	N/A	N/A
0.03 dried	1.699	0.07472	35.795
0.03 swollen	6.830	0.008766	35.070
0.05 dried	2.070	0.01767	47.764
0.05 swollen	13.023	0.04174	41.909
Y-DNA			
0.05 dried	1.752	0.04660	34.404
0.05 swollen	17.706	0.001434	41.909
T-DNA			
0.05	2.07	0.03854	38.286
0.05 swollen	9.394	0.01010	57.917

[0203] To confirm the formation of these branched DNA building blocks, a gel electrophoretic migration-shift assay (GEMSA) coupled with a DNA-specific fluorescent dye (SYBR I) is employed. Such confirmation is described in U.S. patent application Ser. Nos. 11/464,184, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION;" 11/464,181, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES," which applications are incorporated herein by reference in its entirety. The size (molecular weight) and the purity (polydispersity) are assessed. Generally, due to their larger size, the mobility of complete X-DNA is slower. Incomplete hybridization of X01, X02, X03, and X04, on the other hand, resulted in a small amount of incomplete X-DNA. In general, lower salt concentrations can be used for more specific base-pairing, while higher salt concentrations favor strong electrostatic interactions. Ionic influences on DNA are familiar to one of ordinary skill in the art. (See, e.g., Macromolecules, 1997, 30: 5763; J. Phys. Chem. 2006; 110: 2918-2926; Biophys. J. 1996; 70: 2838-46.)

[0204] Similar experiments as above were also performed with Y- and T-DNA, which led to controlled-assembled hydrogels with different properties. In embodiments where ligation is used, the manufacturer's protocols were followed. Mg++ was added for ATP. Hydrogel gelation correlated with ligase activity. For example, by using twice the amount of ligase (e.g., 60 Units), the DNA hydrogel was completely formed within 30 minutes. Irrespective of the kind of DNA monomer (e.g., X, Y, or T), the gelation of all hydrogels was

completed at room temperature and neutral pH within 2 hours. A typical example of ligase reaction utilized Ligase 10x buffer which has a composition of 300 mM Tris-HCl (pH 7.8), 100 mM Mg Cl₂, 100 mM DTT and 10 mM ATP. T4 DNA ligase is supplied with 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol. In general, hydrogels formed by photocrosslinking are formed more rapidly and can display greater strength.

[0205] The amount of DNA building blocks incorporated can be calculated by subtracting the DNA concentration in the supernatant after gelation from the initial DNA concentration after gelation.

[0206] Dendrimer Structures

[0207] As almost all nucleic acid molecules are either linear or circular, to rationally construct nucleic acid biomaterials, additional shapes of nucleic acids as basic building blocks must be first constructed. In addition, these nucleic acid building blocks must be readily incorporated into larger structures in a controlled manner. Thus, in one aspect of the invention, dendrimer like nucleic acid structures are assembled to provide a biomaterial compound. See FIGS. 10A-C.

[0208] In other aspects of the invention branched or DL-DNAs are used to form dendrimer structures. Synthesizing monodisperse polymers demands a high level of synthetic control which is achieved through stepwise reactions, building the dendrimer up one monomer layer, or a "generation," at a time. Each dendrimer consists of a multifunctional core molecule with a dendritic wedge attached to each functional site. The core molecule is referred to as "generation 0." Each successive repeat unit along all branches forms the next generation, "generation 1," "generation 2," and so on until the terminating generation, as illustrated in FIG. 10C.

[0209] There are two defined methods of dendrimer synthesis, divergent and convergent. In the divergent method the molecule is assembled from the core to the periphery; while in the convergent method, the dendrimer is synthesized beginning from the outside and terminating at the core. In either method the synthesis requires a stepwise process, attaching one generation to the last, purifying, and then changing functional groups for the next stage of reaction. For example, in FIG. 10C, the shaded inner core represents one step, followed by the unshaded "Y" molecules as an additional and subsequent step, and finally the stippled "Y" molecules as a further additional and subsequent step. This functional group transformation is necessary to prevent unbridled polymerization. Such polymerization can lead to a highly branched molecule which is not monodisperse—otherwise known as a hyper-branched polymer.

[0210] In the divergent method, the surface groups initially are unreactive or protected species which are converted to reactive species for the next stage of the reaction. In the convergent approach the opposite holds, as the reactive species must be on the focal point of the dendritic wedge.

[0211] Due to steric effects, continuing to react dendrimer repeat units leads to a sphere shaped or globular molecule until steric overcrowding prevents complete reaction at a specific generation and destroys the molecule's monodispersity. The number of possible generations can be increased by using longer spacing units in the branches of the core molecule. The monodispersity and spherical steric expansion of dendrimers leads to a variety of interesting properties. The steric limitation of dendritic wedge length leads to small molecular sizes, but the density of the globular shape leads to

fairly high molecular weights. The spherical shape also provides an interesting study in molecular topology. Dendrimers have two major chemical environments, the surface chemistry due to the functional groups on the termination generation, which is the surface of the dendritic sphere, and the sphere's interior which is largely shielded from exterior environments due to the spherical shape of the dendrimer structure. The existence of two distinct chemical environments in such a molecule implies many possibilities for dendrimer applications.

[0212] As such, hydrophobic/hydrophilic and polar/nonpolar interactions can be varied in the two environments. The existence of voids in the dendrimer interior furthers the possibilities of these two heterogeneous environments playing an important role in dendrimer chemistry. Therefore, in a further embodiment dendrimer structures can accept molecules in the void spaces in addition to or alternative to the linkage to one or more arm portions of one or more terminal monomer (e.g., Y-shape) nucleic acid molecules. Dendrimers have a number of uses, e.g., as molecular weight and size standards, gene transfection agents, as hosts for the transport of biologically important guests, and as anti-cancer agents, to name but a few. Much of the interest in dendrimers involves their use as catalytic agents, utilizing their high surface functionality and ease of recovery. Dendrimers' globular shape and molecular topology, however, make them highly useful to biological systems. Utilizing nucleic acid molecules as building blocks for dendrimer construction and further linked to biologically active agents provides wholly new opportunities in biotechnology and medicine.

[0213] In some aspects of the invention the dendrimer structures can be formed at any stage during a step-wise process of formulation to provide a multivalent structure. Such a dendrimer structure can be composed of multimers that are Y-, X-, T-, or dumbbell shape. In one embodiment, the multimers forming said dendrimer structure are DNA multimers. In another embodiment, the dendrimer structure is comprised of DNA and/or RNA. As indicated above, dendrimers are formed through step-wise addition of different nucleic acid monomers (i.e., building blocks), where for example, nucleic acid monomer ends provide overhangs for subsequent ligation reactions thus expanding the three-dimensional structure of the expanding dendrimer structure.

[0214] Therefore, in selection various nucleic acids, monomers of different length can be used to form dendrimers having a different internal and surface area network. Furthermore, the various sticky ends and/or monomer units can provide a substrate for linking to one or a plurality of biologically active agents. Such biologically active agents are known in the art or described herein.

[0215] In one aspect of the invention, a method is directed to controlled assembly of dendrimer-like DNA (DL-DNA) from Y-shaped DNA (Y-DNA). See FIGS. 10-12 and 15. In one embodiment, the resulting DL-DNA is stable and monodisperse. In a further embodiment, the multivalent DNA dendrimers are isotropic or anisotropic, thus capable of linkage to other compounds. See FIG. 11-12.

[0216] In certain embodiments, the dendrimer structures are linked or cross-linked to additional compounds selected from a group consisting of an adenovirus core peptide, a synthetic peptide, an influenza virus HA2 peptide, a simian immunodeficiency virus gp32 peptide, an SV40 T-Ag peptide, a VP22 peptide, a Tat peptide, and a Rev peptide. Such additional compounds are selected from a group consisting of

DNA condensing peptide, DNA protection peptide, endosomal targeting peptide, membrane fusion peptide, nuclear localization signaling peptide, a protein transduction domain peptide or a combination thereof. See, e.g., FIG. 12.

[0217] In one embodiment, the dendrimer structures are utilized in a method of delivering a biologically active agent to a cell, or to a subject. In another embodiment, the dendrimer structure comprises a linkage to a signal or targeting peptide as described herein above, and comprises a biologically active agent.

[0218] In yet another embodiment, the dendrimer comprises a targeting peptide, a biologically active agent, a selection marker and a detectable label. Selection markers include antibiotics which are known in the art for both eukaryotic and prokaryotic cells, or disclosed herein. Therefore, as noted, a dendrimer can provide a multivalent structure comprised of several distinct molecules that are bound to one or more arms of a one or more multimer nucleic acid molecules of which a dendrimer is composed. See FIGS. 11, 12 and 16.

[0219] Specific examples of detectable molecules include radioactive isotopes such as p32 or H3, fluorophores such as fluorescein isothiocyanate (FITC), TRITC, rhodamine, tetramethylrhodamine, R-phycoerythrin, Cy-3, Cy-5, Cy-7, Texas Red, Phar-Red, allophycocyanin (APC), epitope tags such as the FLAG or HA epitope, and enzyme tags such as alkaline phosphatase, horseradish peroxidase, β -galactosidase, and hapten conjugates such as digoxigenin or dinitrophenyl, etc (FIG. 16). Other detectable markers include chemiluminescent and chromogenic molecules, optical or electron density markers, etc. The probes can also be labeled with semiconductor nanocrystals such as quantum dots, described in U.S. Pat. No. 6,207,392. Qdots are commercially available from Quantum Dot Corporation.

[0220] Additional examples of reagents which are useful for detection include, but are not limited to, radiolabeled probes, fluorophore-labeled probes, quantum dot-labeled probes, chromophore-labeled probes, enzyme-labeled probes, affinity ligand-labeled probes, electromagnetic spin labeled probes, heavy atom labeled probes, probes labeled with nanoparticle light scattering labels or other nanoparticles or spherical shells, and probes labeled with any other signal generating label known to those of skill in the art. Non-limiting examples of label moieties useful for detection in the invention include, without limitation, suitable enzymes such as horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; members of a binding pair that are capable of forming complexes such as streptavidin/biotin, avidin/biotin, aptamer/target or an antigen/antibody complex such as, for exemplary purposes only, rabbit IgG and anti-rabbit IgG (other species and immunoglobulin classes or fragments thereof can be used); fluorophores such as umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, tetramethyl rhodamine, eosin, green fluorescent protein, erythrosin, coumarin, methyl coumarin, pyrene, malachite green, stilbene, lucifer yellow, Cascade BlueTM, Texas Red, dichlorotriazinylamine fluorescein, dansyl chloride, phycoerythrin, fluorescent lanthanide complexes such as those including Europium and Terbium, Cy3, Cy5, molecular beacons and fluorescent derivatives thereof, as well as others known in the art as described, for example, in Principles of Fluorescence Spectroscopy, Joseph R. Lakowicz (Editor), Plenum Pub Corp, 2nd edition (July 1999) and the 6th Edition of the Molecular Probes Handbook by Richard P. Hoagland; a luminescent material such as luminol; light

scattering or plasmon resonant materials such as gold or silver particles or quantum dots; or radioactive material include ^{14}C , ^{123}I , ^{124}I , ^{125}I , ^{131}I , Technetium-99m ($^{99\text{m}}\text{Tc}$), ^{35}S or ^3H . For antibody/antigen complexes and detection systems, one of skill in the art will recognize many detection systems are available. For example, antibodies from different species, non-limiting examples comprising mouse, rabbit and chicken. Different classes of antibodies can be used, e.g., IgA, IgD, IgG, IgM and IgE, and variants, fragments, and chimeras thereof. Monoclonal and/or polyclonal antibody preparations are also available. The term "monoclonal antibody" (mAb) refers to an antibody obtained from a population of substantially homogeneous antibodies; that is, the individual antibodies comprising the population are identical except for naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic determinant, also referred to as an epitope. In contrast, the antibodies in a preparation of polyclonal antibodies are typically a heterogeneous population of immunoglobulin isotypes and/or classes and also exhibit a variety of epitope specificity.

[0221] Examples of labels include, but are not limited to, chromophores, fluorescent moieties, enzymes, antigens, heavy metal, magnetic probes, dyes, phosphorescent groups, radioactive materials, chemiluminescent moieties, scattering or fluorescent nanoparticles, Raman signal generating moieties, and electrochemical detection moieties. Genotyping using a microarray can be performed using any of a variety of methods, means and variations thereof for carrying out array-genotyping analysis.

[0222] Furthermore, backbone labels are nucleic acid stains that bind nucleic acid molecules in a sequence independent manner. Examples include intercalating dyes such as phenanthridines and acridines (e.g., ethidium bromide, propidium iodide, hexidium iodide, dihydroethidium, ethidium homodimer-1 and -2, ethidium monoazide, and ACMA); some minor groove binders such as indoles and imidazoles (e.g., Hoechst 33258, Hoechst 33342, Hoechst 34580 and DAPI); and miscellaneous nucleic acid stains such as acridine orange (also capable of intercalating), 7-AAD, actinomycin D, LDS751, and hydroxystilbamidine. All of the aforementioned nucleic acid stains are commercially available from suppliers such as Molecular Probes, Inc. Still other examples of nucleic acid stains include the following dyes from Molecular Probes: cyanine dyes such as SYTOX Blue, SYTOX Green, SYTOX Orange, POPO-1, POPO-3, YOYO-1, YOYO-3, TOTO-1, TOTO-3, JOJO-1, LOLO-1, BOBO-1, BOBO-3, PO-PRO-1, PO-PRO-3, BO-PRO-1, BO-PRO-3, TO-PRO-1, TO-PRO-3, TO-PRO-5, JO-PRO-1, LO-PRO-1, YO-PRO-1, YO-PRO-3, PicoGreen, OliGreen, RiboGreen, SYBR Gold, SYBR Green I, SYBR Green II, SYBR DX, SYTO-40, -41, -42, -43, -44, -45 (blue), SYTO-13, -16, -24, -21, -23, -12, -11, -20, -22, -15, -14, -25 (green), SYTO-81, -80, -82, -83, -84, -85 (orange), SYTO-64, -17, -59, -61, -62, -60, -63 (red).

[0223] In another embodiment, the dendrimer structure is linked to a plurality of biologically active agents as described herein above, and said plurality of biologically active agents include targeting peptides. Thus the dendrimer of the invention can be linked to one or more peptides selected from an adenovirus core peptide, a synthetic peptide, an influenza virus HA2 peptide, a simian immunodeficiency virus gp32 peptide, an SV40 T-Ag peptide, a VP22 peptide, a Tat peptide, a Rev peptide, a DNA condensing peptide, DNA protection

peptide, endosomal targeting peptide, membrane fusion peptide, nuclear localization signaling peptide, a protein transduction domain peptide or a combination thereof.

[0224] In yet another aspect of the invention, the dendrimer is linked to nucleic acid vectors (e.g., plasmid or viral vectors or linear nucleic acid sequences), which are delivered into a cell or subject. Such vectors are known in the art or disclosed herein. Thus in such an embodiment, the dendrimer structures are used in method of effecting transfection or genetic modification of a cell. One central aspect of the dendrimer structures is anisotropic and multivalent.

[0225] 2. Protein Synthesis

[0226] In some aspects, the nucleic acid matrixes are directed to producing proteins in a cell-free system. Such matrixes simplify protein expression, because virtually all proteins, including toxic proteins or even multiple proteins, can now be expressed easily and efficiently from a protein-producing matrix ("P-gel") without any living organisms/cells. In addition, mutations of any gene can be studied directly at the protein level without transformation and selection. Further, a cell-free system provides an easier route to purifying final protein products. Protein expression efficiency is expected to be inordinately high and the cost to be extremely low due to the reusability of both enzymes and P-gels. The cost of protein production is further reduced by eliminating the need to feed live cells, maintain reactors, and perform post-expression purification.

[0227] In one aspect of the invention, a nucleic acid matrix is a P-gel matrix, which is constructed of two categories of nucleic acid molecules. First, nucleic acids are selected for providing structural support or for forming a networked matrix three-dimensional structure ("building blocks" or "monomers" or "cross-linkers"). In addition, the matrix comprises linear nucleic acids that encode a protein of interest. The matrix can be designed to provide protein-encoding nucleic acids that encode one or more proteins. Therefore, a particular matrix can encode a single or a plurality of proteins.

[0228] In one aspect, a method of cell-free synthesis of one or more proteins uses a plurality of nucleic acid molecules configured to create one or more branched chain structures, as described herein. A general scheme is outlined in FIG. 17. The plurality of nucleic acid molecules is photo-crosslinked according to the methods of the invention to form a nucleic acid hydrogel. The one or more proteins can be expressed from the hydrogel. A variety of configurations are possible. For example, the hydrogel can comprise both coding and non-coding nucleic acid molecules, e.g., the scaffolding may comprise non-coding regions. The hydrogel can also comprise one or more nucleic acids or other macromolecules necessary for protein modification, e.g., phosphorylation, glycosylation, methylation, ubiquitination, biotinylation, alkylation, acetylation, glutamylation, glycylation, isoprenylation, lipoylation, phosphoantetheinylation, sulfation, citrullination, deamidation, or isomerization. The proteins produced in these embodiments comprise modified proteins, having one of more of the listed modifications or the like.

[0229] In some embodiments, the P-gel matrixes can be comprised entirely of DNA or RNA or a combination of RNA and DNA, which combinations can comprise each type of nucleic acid as a building block or protein-encoding nucleic acid. Various macromolecules necessary for protein expression/translation are known in the art, such as rabbit reticulocyte, wheat germ and bacterial extracts. As such the matrix can alternatively provide DNA, RNA or a combination of

both, whereby the appropriate macromolecules are selected to provide either "coupled" transcription (of DNA) followed by translation into protein, or translation (of RNA) into protein.

[0230] Furthermore, various ranges for protein production are obtained by designing matrixes or P-gels with varying concentrations of building block nucleic acids, protein expressing nucleic acids, as well as different ratios of RNA to DNA encoding a protein.

[0231] In one embodiment, the building block monomer nucleic acids comprise X-shaped nucleic acid that provide a networked matrix, which network also comprises linear nucleic acids that encode at least one or more desired protein. In addition, the building blocks can comprise X-, Y-, dumb-bell-, T-, dendrimer-shapes or a combination thereof. Furthermore, the P-gel can be constructed of X- and Y-shape nucleic acids in a predetermined ratio to provide a particular P-gel geometry, whereby linear nucleic acids are also integrated into the resulting network matrix. In yet another embodiment, said building block monomers are DNA, or PNA. In addition, the linear nucleic acid is DNA, RNA, TNA, PNA or a combination thereof (including any two thereof).

[0232] In yet another embodiment, the P-gel is comprised entirely of DNA. In a further embodiment, a P-gel monomer has a molecular weight selected from about 50 kDa to about 500 MDa. In various embodiments, the molecular weight is 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 800, 900, 1000 kDa. In other embodiments, the molecular weight is 1, 5, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 or 550 MDa. In one preferred embodiment, a P-gel is a hydrogel.

[0233] In addition, the P-gel is hydradable whereby the dry form swells in volume, e.g., by addition of water by about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1500%.

[0234] Furthermore, the P-gel is comprised by nucleic acids having tensile strength selected that is about 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65%.

[0235] One aspect of the invention is directed to a nucleic acid matrix is comprises of nucleic acid molecules, branched and linear, and produces proteins in a cell-free environment. In one embodiment, the matrix forms a gel and is comprised entirely from DNA (linear genes as monomers and branched, X-shape DNA as crosslinkers). Proteins are produced directly from the gel via in vitro transcription coupled with translation (TNT). Post-expression purification is no longer a challenge since the system is cell-free and since the major components are expressed proteins. Also, both the gels and the TNT enzymes can be recycled and reused many times, further reducing costs. Maintenance of cells is no longer needed either. In one embodiment, the gel is hydrogel thus hydradable with water.

[0236] In another embodiment, the protein yield for the matrix is 7.9 mg from 1 cm³. In other embodiments, the yield is about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 mg from 1 cm³ of the gel.

[0237] Furthermore, in one embodiment, the cross-linker nucleic acid is DNA and further is X-DNA. In some embodiments, an X-DNA is about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nm in length. In yet a further embodiment an X-DNA is about 10 to 20 nm in length. A further embodiment is directed to an X-DNA that is 14 nm in length.

[0238] In one embodiment, the hydrogel comprises pores. The DNA building blocks can be configured to adjust the pore size. In one embodiment, the pores are from about 50 nanometers to 500 nanometers in size, e.g., the pores can have a size of about 5 nanometers, about 10 nanometers, about 15 nanometers, about 20 nanometers, about 30 nanometers, about 40 nanometers, about 50 nanometers, and about 100 nanometers. In some embodiments, the hydrogel comprises pores of multiple different sizes.

[0239] In yet another aspect, the gel is molded into a matrix forming a hollow structure with one closed end and one open end, or two closed ends, wherein the structure provides surface area internally and externally from which proteins can be transcribed. The concentration of genes a network format such as in a nucleic acid hydrogel provide higher concentrations of genes that kinetically increase the rate of transcription. In addition, the networked scaffolds of nucleic acids provide anchoring sites for more enzymatic activities and turnovers. Moreover, the hollow tube structure provides a concentrated solution of the necessary macromolecules necessary for translation or transcription-coupled translation thus enhancing expression yields for a particular gel or gels substantially.

[0240] In one embodiment, the hollow "close ended" networked matrix enhances the protein yield 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold as compared to an open matrix.

[0241] In various embodiments of the protein yielding matrixes or P-gels comprise nucleic acid building blocks that are DNA, RNA, PNA, TNA or a combination thereof. In one embodiment, the nucleic acid is entirely DNA, entirely RNA or a combination of RNA and DNA. In a further embodiment, the cross-linker DNA is selected from branched nucleic acids that are X-, Y-, T-, dumbbell- or dendrimer-shape and the protein-encoding nucleic acid is linear or circular.

[0242] In another aspect of the invention, the matrix constructed of nucleic acids of the present invention is further linked to at least one (or more) copolymer or additional compound, which are known in the art or described herein above. The nucleic acid molecules are capable of undergoing various enzymatic reactions, including DNA polymerase, RNA reverse transcriptase, terminal transferase, DNA ligase, RNA ligase, exonuclease, ribonuclease, endonuclease, polynucleotide kinase, DNA methylase, and DNA ubiquitinase. Therefore, the nucleic acid molecules can be readily modified or linked to said copolymer(s) or additional compound(s).

[0243] In some embodiments, the protein yielding matrixes yield protein at a rate of 10, 15, 20, 25, 30, 35, 40, 45 ng protein per 1 ng DNA or 1 ng RNA.

[0244] Post-Translational Modifications

[0245] A yet another aspect of the invention is directed to nucleic-acid based protein-yielding matrixes where the resulting protein is post-translationally modified. Glycosylation of proteins in most eukaryotes occurs commonly in the ER, i.e., yeast, insect, plant and mammalian cells share the features of N-linked oligosaccharide processing in the ER. Though the resultant glycoproteins in the ER have a near identical carbohydrate structure, with only the initial glycosylation in the ER, glycoproteins with a therapeutic efficacy cannot be fully produced. Therefore, in various embodiments a hydrogel can comprise the macromolecules necessary for post-translational modification of proteins produced in the cell-free protein synthesis system of the invention.

[0246] The production of premature glycoprotein, which does not undergo the complete post-translational modifica-

tion, may be caused by the deficiency of the terminal glycosylation machinery such as the Golgi apparatus. In other words, oligosaccharide processing by different cell types may diverge in the Golgi apparatus. The initial step in O-glycosylation by mammalian cells is the covalent attachment of N-acetylgalactosamine to serine or threonine. No O-glycosylation sequence has been identified analogous to the Asn-X-Ser/Thr template required for N-glycosylation. In further contrast to N-glycosylation, no preformed, lipid-coupled oligosaccharide precursor is involved in the initiation of mammalian O-glycosylation. Sugar nucleotides serve as the substrates for the first and all subsequent steps in O-linked processing. Following the covalent attachment of N-acetylgalactosamine to serine or threonine, several different processing pathways are possible for mammalian O-linked oligosaccharides in the Golgi. The oligosaccharide structures of glycoproteins can have a profound effect on properties critical to the human therapeutic use, including plasma clearance rate, antigenicity, immunogenicity, specific activity, solubility, resistance to thermal inactivation, and resistance to protease attack. Therefore, for a cell-free protein synthesis to be applied to the large-scale production of glycoprotein and for a rapid insight into the role of protein glycosylation to understand the relationship among stability, conformation, function of protein and glycosylation, an efficient cell-free completely post-translationally modified protein synthesis system in which protein is completely post-translationally modified can be implemented using the protein-yielding matrixes described herein.

[0247] For the production of proteins having the complete and correct structure, the present invention includes the combination of a cell-free protein synthesis system and co- and post-translational modification machinery containing organelles, separated from cells, relevant to co- and post-translational modification. Methods of producing proteins from hydrogels are disclosed by U.S. patent application Ser. No. 11/464,184, filed Aug. 11, 2006 and entitled "NUCLEIC ACID-BASED MATRIXES FOR PROTEIN PRODUCTION." The present invention provides photo-crosslinked P-gels. This method is suitable especially to large-scale production of efficacious and useful proteins. Additionally, this method can be applied directly to post-translational modification processes, required to produce a biologically active protein besides glycosylation.

[0248] As mentioned above, since the addition of only the ER cannot produce the completely post-translationally modified proteins, the addition of co- and post-translational modification machinery involved in terminal glycosylation is necessary. The addition of co- and post-translational modification machinery containing signal recognition particle, ER, Golgi apparatus, plasma membrane, and the like to the cell-free protein synthesis reaction mixture stimulates the production of completely post-translationally modified protein. A complete incubation mixture (containing the components of cell-free protein synthesis and co- and post-translational modification machinery) gives the completely post-translationally modified proteins. The events of the co- and post-translational modification process can be faithfully reproduced in vitro.

[0249] Cell sources for the preparation of the extract or lysate for the cell-free protein synthesis system and those for the co- and post-translational modification machinery may be the same or different. In the case of using the same cell, the extract or lysate for the cell-free protein synthesis system and

the co- and post-translational modification machinery may be prepared separately or together. Examples for methods of preparing such extracts are known in the art, as described in U.S. Pat. No. 6,780,607, which is incorporated by reference herein in its entirety.

[0250] The co- and post-translational modification machinery may be prepared from tissues and cultured cell lines. In glycosylation it is favorable to genetically engineer a cell source for the enhancement of the expression level of glycosylation related enzymes and/or for the enrichment of the pool of sugar nucleotides which serve as sugar donors in glycosylation. This type of genetic manipulation can be carried out by those skilled in the art; therefore, the detailed explanation is omitted in this specification.

[0251] As an example for obtaining the cell extract in the cell-free protein synthesis method, the preparation of nuclease-treated RRL and a crude homogenate from Chinese hamster ovary (CHO) cells, as well as the preparations of ER containing signal recognition particle, Golgi apparatus, and plasma membrane from a crude homogenate are described in detail in U.S. Pat. No. 6,780,607. Such extracts can be obtained from any relevant mammalian cell(s).

[0252] A glycoprotein produced by the cell-free protein synthesis using one or more matrixes of the invention, can be further modified through carbohydrate-adding reaction and/or carbohydrate-deleting reaction and/or carbohydrate-substituting reaction with enzymes relevant to the modification of side chains, e.g., glycosyltransferase, glycosidase, transglycosidase and so on. As such the addition, deletion, or substitution of carbohydrate side chains is affected. Furthermore, in another embodiment, one or more protein-yielding matrixes, in conjunction with the necessary macromolecules, can produce proteins with carbohydrate side chains not known in the general glycoprotein structures or produce novel glycoprotein structures synthesized artificially, and thus resulting in development of new glycoproteins. For example, in the carbohydrate-adding reaction resultant itself or the erythropoietin (EPO) separated from it, sialic acid is further attached to the terminal chain thereof by transglycosidase which is one of carbohydrate chain addition enzymes, and the efficacy of glycoprotein increases with the addition of sialic acid to the terminal chain thereof.

[0253] Therefore, in some embodiments, the protein-yielding matrixes can be applied to the production of proteins of therapeutic, commercial or research value. This includes proteins such as growth hormones, granulocyte colony stimulating factor, interleukin, interferon, thrombopoietin, tissue plasminogen activator and humanized monoclonal antibody. Additionally, in certain embodiments, kits are provided comprising nucleic acid matrixes for protein production of completely post-translationally modified protein as well as the necessary extracts discussed above that are necessary for post-translational modification thus enabling a research tool in the form of a co- and post-translational modification analyze protein functionality.

[0254] Recyclability

[0255] In other aspects of the invention, the protein yielding matrixes can be re-used at least 3 times and can last 7 days before the gel micropads are degraded by nucleases (from lysates). However, by linking the nucleic acid based matrixes of the invention with at least one copolymer or at least one additional compound, matrixes are constructed that are mechanically stronger gels. In one embodiment, doping with gold nanoparticles (AuNP) is utilized to make stronger gels,

where gold is attached either onto the DNA strands by direct crosslinking AuNP with DNA or between DNA strands by suspending AuNP in the gel. FIG. 18 shows a schematic drawing of crosslinking AuNP onto DNA, representing a gel that was constructed. In addition, nuclease activity can be significantly reduced by adding compounds known in the art (such as DNase, Exo Nuclease III, etc.), or achieved by either conventional protein fractions or by passing through Affinity columns to further purify extracts utilized for in vitro protein expression.

[0256] In another aspect of the invention, the nucleic acid matrixes can be further stabilized against degradation by modifications of the nucleic acid backbone. Such modifications are described herein or known in the art, such as those disclosed in U.S. Patent Publication Nos. 2005/32068, 2004/161844, 2001/49436, and U.S. Pat. Nos. 5,610,289; 5,965,721; 6,201,103 (teaching Peptide Nucleic Acid comprising modified backbone), or 6,025,482, the disclosure of each of which is incorporated herein by reference.

[0257] In yet another aspect, the matrix is further stabilized by linking nucleic acids of the matrix to a copolymer, which are known in the art or described herein. In one embodiment, a branched DNA-polystyrene hybrid molecule is constructed. Therefore, in some embodiments, a particular gel DNA P-gel is constructed either entirely from a DNA-copolymer hybrid molecule or from a mixture of X-DNA and DNA-polystyrene. Thereby, providing a hybrid DNA P-gel whose backbone consists of a nuclease-resistant polystyrene group. Additional copolymers that can be linked to nucleic acids are disclosed supra.

[0258] As such, the matrixes are significantly strengthened and become amenable to recycling. Thus, in one embodiment the protein yielding matrixes can be re-used 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 times.

[0259] 3. Encapsulation and Delivery

[0260] Another central aspect is directed to a matrix composed of nucleic acids described herein, so as to provide a structure for delivery of one or more biologically active agents. In one embodiment, the matrix can deliver cells, along with one or more biologically active agents. In another embodiment, the matrix can provide a scaffold for three-dimensional cell growth or tissue regeneration, either in vitro or in vivo. In yet another embodiment, the matrix providing a platform for cell growth or tissue generation concomitantly delivers one or more biologically active agents contained therein and released therefrom.

[0261] In one aspect, the invention provides a method of encapsulating one or more compounds of interest in a nucleic acid hydrogel. A general scheme is outlined in FIG. 19. The method uses a plurality of nucleic acid molecules configured to create one or more branched chain structures, as disclosed here. In some embodiments, the nucleic acid molecules are mixed with the one or more compounds of interest, and the plurality of nucleic acid molecules are photo-crosslinked to form a composition comprising a nucleic acid hydrogel encapsulating the one or more compounds within. In some embodiments, the plurality of nucleic acid molecules are photo-crosslinked to form a nucleic acid hydrogel, then the hydrogel is mixed with the compound and allowed to absorb the compound, thereby forming a composition comprising a nucleic acid hydrogel encapsulating the one or more compounds within. Multiple methods of encapsulating compounds can be used with one hydrogel preparation. In a related aspect, the invention provides for administering these

compositions to a subject. The compositions can release the compound in a time released manner to deliver the compound to the subject. The compound can be delivered to a variety of applicable, e.g., a cell, tissue, organ, skin or bodily fluid, e.g., blood or lymph. The compositions may be administered in a convenient manner. Non-limiting manners include by the oral, intravenous (where water soluble), intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes, or by implanting.

[0262] The DNA hydrogels of the invention can be formulated into diverse patterns at both millimeter and micrometer scale. By selecting different types of DNA monomers, e.g., selecting different building blocks described herein, or by varying the length, sequence or shape of said building blocks, one can tailor the surface morphology and the internal structures of the hydrogel, including the pore size of the gel and release kinetics. In some embodiments, the pore size is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nanometers. For example, the pore size can up to about 15 nanometers. For example, controlled insulin-release has been accomplished for more than one month with no burst effect, and a zero-order release has been achieved for the release of CPT from the gels. These biodegradable, biocompatible, nucleic acid hydrogels can be exploited in a variety of biomedical applications including sustained drug delivery, tissue engineering, 3D cell culture, cell transplant therapy, and other biomedical applications.

[0263] Due to the very mild, aqueous conditions and rapid and efficient gelation provided by the encapsulation methods of the invention, a variety of agents (from small molecules to proteins to even live cells) can be encapsulated within the gel in situ. This feature not only minimizes the post-gelation drug loading step, but also realizes a close to 100% encapsulation efficiency, e.g., at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or about 100% encapsulation efficiency. The drugs and live cells can be well preserved and protected because no organic solvents or harsh manipulations are used. In some embodiments, the compounds are biomolecules, e.g., protein, peptide, lipid, nucleic acid, carbohydrates or any combination thereof. In some embodiments, the one or more compounds comprise a therapeutic agent, e.g., small molecules or other drugs, toxins, immunomodulators, chelators, antibodies, antibody-drug conjugates, photoactive agents or dyes, and/or radioisotopes. In some embodiments, the small molecule drug of interest is doxorubicin. The methods can also be used to encapsulate living cells, e.g., mammalian cells. In some embodiments, the hydrogel provides a three dimensional matrix on which the cell grows. In some embodiments, the compounds of interest comprise a vector or virus, including a viral vector.

[0264] In one embodiment, the matrix is comprised of branched nucleic acids that are DNA. In another embodiment, the DNA is X-shape, Y-shape, T-shape, dumbbell-shape or dendrimer shape, or a combination thereof. In yet another embodiment, matrix comprises branched DNA and linear DNA or RNA. In yet another embodiment, the matrix comprises branched nucleic acids that include DNA and RNA. In yet a further embodiment, the matrix can comprise at least one copolymer known in the art or disclosed herein above. As such, the copolymer is linked to one or more building block nucleic acids of the matrix. Additional embodiments are directed to linking components or chemical moieties to the matrix, by cross-linking such additional components to the nucleic acids or copolymers of the matrix. Additional com-

ponents in this context have been disclosed herein, and include small molecules, nanoparticles, microparticles, nanofilaments, metals, or peptides. In one embodiment, the additional component is a nanoparticle that is a metal, e.g., gold, silver, iron or copper. In one embodiment, the additional component is a microparticle that is a metal, more preferably, gold, silver, iron, or copper. In some embodiments, the metals are magnetic. The additional component can also comprise carbon black, 4-phosphonooxy-2,2,6,6-tetramethylpiperidyl-oxy nitroxide, titanium dioxide.

[0265] Examples of biologically active agents that can be incorporated into a matrix or matrices include but are not limited to bioactive agents delivered alone or in combination with another compound and/or cell. Nonlimiting examples of bioactive agents include interferon, interleukin, erythropoietin, granulocyte-colony stimulating factor (G-CSF), stem cell factor (SCF), leptin (OB protein), interferon (alpha, beta, gamma), ciprofloxacin, amoxycillin, *lactobacillus*, cefotaxime, levofloxacin, cefipime, mebendazole, ampicillin, *lactobacillus*, cloxacillin, norfloxacin, tinidazole, cefpodoxime, proctil, azithromycin, gatifloxacin, roxithromycin, cephalosporin, anti-thrombogenics, aspirin, ticlopidine, sulfapyrazone, heparin, warfarin, growth factors, differentiation factors, hepatocyte stimulating factor, plasmacytoma growth factor, glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factor (FGF), transforming growth factor (TGF), platelet transforming growth factor, milk growth factor, endothelial growth factors, endothelial cell-derived growth factors (ECDGF), alpha-endothelial growth factors, beta-endothelial growth factor, neurotrophic growth factor, nerve growth factor (NGF), vascular endothelial growth factor (VEGF), 4-1 BB receptor (4-1BBR), TRAIL (TNF-related apoptosis inducing ligand), artemin (GFRalpha3-RET ligand), BCA-1 (B cell-attracting chemokine), B lymphocyte chemoattractant (BLC), B cell maturation protein (BCMA), brain-derived neurotrophic factor (BDNF), bone growth factor such as osteoprotegerin (OPG), bone-derived growth factor, thrombopoietin, megakaryocyte derived growth factor (MDGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), ciliary neurotrophic factor (CNTF), neurotrophin 4 (NT4), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (mCSF), bone morphogenetic protein 2 (BMP2), BRAK, C-10, Cardiotrophin 1 (CT1), CCR8, anti-inflammatory: paracetamol, salsalate, diflunisal, mefenamic acid, diclofenac, piroxicam, ketoprofen, dipyrone, acetylsalicylic acid, antimicrobials amoxicillin, ampicillin, cephalosporins, erythromycin, tetracyclines, penicillins, trimethoprim-sulfamethoxazole, quinolones, amoxicillin, clavulanate, azithromycin, clarithromycin, anti-cancer drugs aliteretinoin, altertamine, anastrozole, azathioprine, bicalutamide, busulfan, capecitabine, carboplatin, cisplatin, cyclophosphamide, cytarabine, doxorubicin, epirubicin, etoposide, exemestane, vincristine, vinorelbine, hormones, thyroid stimulating hormone (TSH), sex hormone binding globulin (SHBG), prolactin, luteotropic hormone (LTH), lactogenic hormone, parathyroid hormone (PTH), melanin concentrating hormone (MCH), luteinizing hormone (LHb), growth hormone (HGH), follicle stimulating hormone (FSHb), haloperidol, indomethacin, doxorubicin, epirubicin, amphotericin B, Taxol, cyclophosphamide, cisplatin, methotrexate, pyrene, amphotericin B, anti-dyskinesia agents, Alzheimer vaccine, antiparkinson agents, ions, edetic acid, nutrients, glucocorticoids, heparin, anticoagulation agents, anti-virus

agents, anti-HIV agents, polyamine, histamine and derivatives thereof, cystineamine and derivatives thereof, diphenhydramine and derivatives, orphenadrine and derivatives, muscarinic antagonist, phenoxybenzamine and derivatives thereof, protein A, streptavidin, amino acid, beta-galactosidase, methylene blue, protein kinases, beta-amyloid, lipopolysaccharides, eukaryotic initiation factor-4G, tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 (to 18) receptor antagonist (IL-1ra), granulocyte macrophage colony stimulating factor (GM-CSF), novel erythropoiesis stimulating protein (NESP), thrombopoietin, tissue plasminogen activator (TPA), urokinase, streptokinase, kallikrein, insulin, steroid, acetylsalicylic acid, acetaminophen, analgesic, anti-tumor preparation, anti-cancer preparation, anti-proliferative preparation or pro-apoptotic preparation.

[0266] In some aspects the matrixes of the present invention encapsulate a vector exclusively, or along with cells and/or other biologically active agents disclosed herein. Examples of vectors include adenoviral vectors, adenoviral associated vectors, retroviral vectors, and/or plasmid vectors.

[0267] In other aspects of the invention the nucleic acid vectors are deposited in the matrix of the invention and are delivered to a target cell or tissue. In other aspects, such vectors can encode a therapeutic protein or antisense mRNA. In yet other aspects of the invention, one or more vectors each encoding a different therapeutic capable agent delivered to cells or tissue via the device of the invention. Therefore, the device of the invention will controllably release vectors to effectuate gene delivery, such as in gene therapy. Gene delivery may be either endogenously or exogenously controlled. Examples of endogenous control include promoters which are sensitive to a physiological signal such as hypoxia or glucose elevation. Exogenous control systems involve gene expression controlled by administering a small molecule drug. Examples include tetracycline, doxycycline, ecdysone and its analogs, RU486, chemical dimerizers such as rapamycin and its analogs, etc.

[0268] In an alternative aspect of the invention, the device can deliver the small molecule drug, such as those in the preceding paragraph, where the device is utilized to deliver the vector and the inducible agent (e.g., small molecule drug), the vector alone or some combination thereof.

[0269] Vectors include derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combinations of functional mammalian vectors and functional plasmids and phage DNA. Eukaryotic expression vectors are well known, e.g. such as those described by P J Southern and P Berg, *J Mol Appl Genet.* 1:327-341 (1982); Subramini et al., *Mol. Cell. Biol.* 1:854-864 (1981), Kaufmann and Sharp, *J. Mol. Biol.* 159:601-621 (1982); Scahill et al., *PNAS USA* 80:4654-4659 (1983) and Urlaub and Chasin *PNAS USA* 77:4216-4220 (1980), which are hereby incorporated by reference. The vector used in one or methods disclosed herein may be a viral vector, preferably a retroviral vector. Replication deficient adenoviruses are preferred. For example, a "single gene vector" in which the structural genes of a retrovirus are replaced by a single gene of interest, under the control of the viral regulatory sequences contained in the long terminal repeat, may be used, e.g., Moloney murine leukemia virus (MoMuLV), the Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and the murine myeloproliferative sarcoma virus (MuMPSV), and avian retroviruses such as reticuloendotheliosis virus

(Rev) and Rous Sarcoma Virus (RSV), as described by Eglitis and Andersen, *BioTechniques* 6(7):608-614 (1988), which is hereby incorporated by reference.

[0270] Recombinant retroviral vectors into which multiple genes may be introduced may also be used with the matrixes or methods of the invention. As described by Eglitis and Andersen, above, vectors with internal promoters containing a cDNA under the regulation of an independent promoter, e.g. SAX vector derived from N2 vector with a selectable marker (noe.sup.R) into which the cDNA for human adenosine deaminase (hADA) has been inserted with its own regulatory sequences, the early promoter from SV40 virus (SV40) may be designed and used in accordance with methods disclosed herein or as known in the art.

[0271] In some aspects of the invention, the vectors comprising recombinant nucleic acid molecules are first introduced (e.g., transfected) into cells, which cells are deposited in the matrixes of the invention. For example, the vectors comprising the recombinant nucleic acid molecule are incorporated, i.e. infected, into the BM-MNCs by plating ~5e5 BM-MNCs over vector-producing cells for 18-24 hours, as described by Eglitis and Andersen *BioTechniques* 6(7):608-614 (1988), which is hereby incorporated by reference, and subsequently said cells are deposited into the reservoir portion of the device.

[0272] In some aspects of the invention the nucleic acid molecule encodes proteins such as growth factors, including but not limited to, VEGF-A, VEGF-C, PIGF, KDR, EGF, HGF, FGF, angiopoietin-1, and cytokines. In additional preferred embodiments, the nucleic acid molecule encodes endothelial nitric oxide synthases eNOS and iNOS, G-CSF, GM-CSF, VEGF, aFGF, SCF (c-kit ligand), bFGF, TNF, heme oxygenase, AKT (serine-threonine kinase), HIF.alpha. (hypoxia inducible factor), Del-1 (developmental embryonic locus-1), NOS (nitric oxide synthase), BMP's (bone morphogenic proteins), SERCA2a (sarcoplasmic reticulum calcium ATPase), beta 2-adrenergic receptor, SDF-1, MCP-1, other chemokines, interleukins and combinations thereof.

[0273] In additional aspects of the invention, the matrixes of the invention comprise genes which may be delivered in the autologous BM-MNCs using one or more methods disclosed herein include but are not limited to nucleic acid molecules encoding factor VIII/von Willebrand, factor IX and insulin, NO creating genes such as eNOS and iNOS, plaque fighting genes, thrombus deterrent genes, for example. Therefore, in such an example, the matrix of the invention contains cells that secrete the therapeutic agent from the pores of the matrix, wherefrom the therapeutic agent exits from the matrix into the surrounding cells (e.g., in vitro or in vivo). It will be appreciated that the preceding growth factors can also be delivered in the form of synthesized or recombinant proteins.

[0274] In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the nucleotide sequence of interest (e.g., encoding a therapeutic capable agent) can be ligated to an adenovirus transcription or translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the AQP1 gene product in infected hosts. (See e.g., Logan & Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659 (1984)).

[0275] Specific initiation signals can also be required for efficient translation of inserted therapeutic nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire therapeutic gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals can be needed. However, in cases where only a portion of the therapeutic coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See e.g., Bittner et al., *Methods in Enzymol.*, 153:516-544 (1987)).

[0276] Cell and Tissue Culture

[0277] Although the preceding description relates to therapeutic applications, the invention is also applicable to non-therapeutic applications such as cell culturing and tissue engineering, by providing a three-dimensional scaffold and/or delivery of biologically active agents (e.g., cell growth factors, angiogenic factors). Thus agents that can be controllably released by embodiments of the invention include therapeutic agents, cell culture agents and tissue engineering agents.

[0278] As such one aspect of the invention is directed to matrixes or methods, where the matrix is utilized to encapsulate a cell. In one embodiment, the matrix can be utilized to propagate and culture cells in vitro. Further, in vitro applications include tissue generation or regeneration, by utilizing the matrix either as a structural scaffold or as both a scaffold and source of growth promoting factors. In another embodiment, the matrix is implanted into a target site in a subject. The term "implanted" is used to mean any means of delivery known in the art and is not necessarily limited to invasive procedures (e.g., topical, or skin-based applications).

[0279] In one embodiment, the matrix is utilized in a cell culture to release a particular agent in a controlled manner to monitor the effects of such an agent on cells or tissue cultures. For example, the device of the invention can be utilized in a method of screening different agents to determine the mechanisms, by which such compounds induce cell differentiation, e.g., such as in studying effects on stem cells. Methods of utilizing cell and tissue culture are known in the art, such as disclosed in U.S. Pat. Nos. 7,008,634 (using cell growth substrates with tethered cell growth effector molecules); 6,972,195 (culturing potentially regenerative cells and functional tissue organs in vitro); 6,982,168 or 6,962,980 (using cell culture to assay compounds for treating cancer); 6,902,881 (culturing techniques to identify substances that mediate cell differentiation); 6,855,504 (culturing techniques for toxicology screening); or 6,846,625 (identifying validated target drug development using cell culture techniques), the disclosure of each of which is herein incorporated by reference. The matrixes of the invention are readily adaptable to such cell culturing techniques as would be evident to one of ordinary skill in the art.

[0280] In some aspects of the invention, the matrix encapsulates cells and a biologically active agent, whereby the matrix provides a three-dimensional scaffold on which cells grow/differentiate, either in vitro or in vivo. Furthermore, the

matrix nucleic acids can be linked to additional copolymers to provide a substrate surface defining a tissue contacting surface, whereby the surface is disposed with polypeptides or peptides which are cell/tissue growth potentiating. The matrix can release biologically active agents that are also cell/tissue growth potentiating, where such polypeptides/peptides include PDGF, EGF, FGF, TGF, NGF, CNTF, GDNF, VEGF and type I collagen peptides, or functionally active fragments and/or combinations thereof.

[0281] The nucleic acid matrixes or matrixes either without or further linked with additional polymers may be used for a variety of tissue engineering applications including, inter alia, to increase tissue tensile strength, improve wound healing, speed up wound healing, as templates for tissue formation, to guide tissue formation, to stimulate nerve growth, to improve vascularization in tissues, as a biodegradable adhesive, as device or implant coating, or to improve the function of a tissue or body part.

[0282] In some embodiments, the matrixes may also more specifically be used as sutures, scaffolds and wound dressings. The type of nucleic acid polymer or copolymer used may affect the resulting chemical and physical structure of the polymeric biomaterial.

[0283] In another embodiment, a matrix is placed in the or on a wound area, whereby the matrix controllably releases a desired therapeutic agent that promotes wound healing, exclusive of or in addition to providing a scaffold for cell regrowth/regeneration necessary for improved or faster healing. For example, the therapeutic agent can comprise cell growth or angiogenic factors, described herein, as one of several potential agents.

[0284] Target Sites for Delivery or Implantation

[0285] It will be appreciated that the matrixes of the invention can be implanted using methods known in the art, including invasive, surgical, minimally invasive and non-surgical procedures. Depending on the subject, target sites, and agent(s) to be delivered the microfabrication techniques disclosed herein, can be adapted to make the delivery scaffold of the invention of appropriate size and shape. The matrix described herein is suitable for use in various locations in the body. For example, they can be implanted on the surface of the skin, under the skin, or in or near internal tissues or organs. The scaffolds in some embodiments are located in or near a gastro-intestinal tract, airway tissue or organ, cardiovascular tissue or organ, or neuronal tissue or organ. Other examples of target sites for implantation include but are not limited to the eye, pancreas, kidney, liver, stomach, muscle, heart, lungs, lymphatic system, thyroid gland, pituitary gland, ovaries, prostate, skin, endocrine glands, ear, breast, urinary tract, brain or any other site in an animal.

[0286] In certain embodiments, the gels, or scaffolds of the invention can be encased in a nonbiodegradable material, which materials are known in the art. For example, if a matrix structure of the invention is attached to a temporary implant, the matrix can be encased in a nonbiodegradable casing. Suitable materials for casings include but are not limited to poly(dimethylsiloxane), silicone elastomers, polyurethane, poly(tetrafluoroethylene), polyethylene, polysulfone, poly(methyl methacrylate), poly(2-hydroxyethyl methacrylate), polyacrylonitrile, polyamides, polypropylene, poly(vinyl chloride), poly(ethylene-co-(vinyl acetate)), polystyrene, poly(vinyl pyrrolidone), yellow wax, petrolatum cholesterol, stearyl alcohol, white wax, white petrolatum, methylparaben, propylparaben, sodium lauryl sulfate, propylene glycol, glycerol, and the like.

erogelatin, gelling agents such as carbomer 934, cellulose derivatives, natural gums, penetration enhancers such as dimethyl sulfoxide, ethanol propylene glycol, glycerin, urea, glycerol, coloring agents, lactose, stearic acid, starch glycolate, sugar, gelatin, fixed vegetable oils and fats, glycerin, propylene glycol, alcohol, ethyl oleate, isopropyl myristate, dimethyl acetamide, and mixtures or aqueous or oil based dispersions of these.

[0287] Selection of implantation sites for the matrixes (gels or scaffolds) are within the skill of one of skill in the art. For example, suitable sites for implantation in the eye include the anterior chamber, posterior chamber, vitreous cavity, suprachoroidal space, subconjunctiva, episcleral, intracorneal, epicorneal and sclera. Suitable sites extrinsic to the vitreous comprise the suprachoroidal space, the pars plana and the like. The suprachoroid is a potential space lying between the inner scleral wall and the apposing choroid. Matrixes implanted in a suprachoroid may deliver drugs to the choroid and to the anatomically apposed retina, depending upon the diffusion of the drug from the implant, the concentration of drug comprised in the implant and the like. Additional methods and procedures for implanting a device of the invention in various tissue/organ sites are known in the art, such as disclosed in U.S. Pat. Nos. 7,013,177; 7,008,667; 7,006,870; 6,965,798; 6,963,771; 6,585,763; 6,572,605; or 6,419,709, the disclosure of each of which is herein incorporated by reference.

[0288] In another embodiment the matrix provides a means for topical delivery, such as to skin. For example, the matrix or gel can be encased in a nondegradable casing (e.g., plastics or bandage or patch) providing an aperture or surface for contacting the target site (i.e., skin). Subsequently, the gel can release in a time controlled manner the desired drug to the target site.

[0289] One aspect of the invention is directed to utilization of the matrixes or scaffolds of the invention in wound healing. In general, the body is able to regenerate injured tissue to produce new tissue having properties similar to the original tissue. For example, small cuts heal without forming permanent scars, and clean fractures in bone are healed by the formation of new bone that binds the two fragments of bone together. However, connective tissue cells and other organ cells are anchorage dependent—they require a scaffold to exhibit normal physiological behavior. Where tissue damage is extensive or large gaps are present, cells migrating into the wound may not find proper anchorage and may produce scar tissue to bridge the gap between healthy tissue at the edges of the wound. Scar tissue does not have the same mechanical and biological properties as the original tissue. For example, scar tissue in skin is not as pliable as the original tissue. Scar tissue in bone is not as strong as uninjured bone and often provides a weak point where it is easier to break the bone again. Some tissues, such as articular cartilage, do not naturally regenerate, and healing only proceeds by the formation of scar tissue. In another embodiment, the matrix provides a scaffold for wound healing (e.g., burns, cuts, deep tissue trauma), which scaffold can be encased in a nondegradable or degradable casing, or applied without any such casing, to a selected target site. The scaffold can concomitantly release a desired drug compound while also providing a scaffold/support for cell growth and tissue (e.g., skin) regeneration.

[0290] Drugs of Use in the Invention

[0291] The methods and compositions of the invention include the study and use of drugs, e.g., insulin sensitizers,

and include performing association studies for determining genotypic and/or phenotypic traits associated with responsiveness to drugs, e.g., insulin sensitizers, screening individuals for predisposition to response to drugs, e.g., insulin sensitizers, e.g., adverse response, and/or administering or not administering drugs, e.g., insulin sensitizers to the individual based on such screening. This section describes certain drugs of use in embodiments of the invention. Further useful drugs for the invention are described in section IVC, Association studies and methods for classes of drugs.

[0292] Insulin Sensitizers

[0293] One class of drugs included in certain embodiments of the invention is an insulin sensitizer. The term “insulin sensitizer,” or “insulin sensitizing agent,” as used herein, refers to any agent capable of enhancing either secretion of or, more typically, tissue sensitivity to, insulin. Non-exclusive examples of insulin sensitizers include metformin, sulfonylureas, alpha glucosidase inhibitors and PPAR modulators, including thiazolidinediones. Further examples of insulin sensitizers are described below.

[0294] The thiazolidinediones are examples of PPAR modulators, which are one class of insulin sensitizers. The term “PPAR modulator,” as used herein, refers to peroxisome proliferator-activated receptor agonists, partial agonists, and antagonists. The modulator may, selectively or preferentially, affect PPAR alpha, PPAR gamma, or both receptors. Typically, the modulator increases insulin sensitivity. According to one aspect, the modulator is a PPAR gamma agonist. One PPAR gamma agonist used in embodiments of the invention is 5-[6-(2-fluorobenzyl)oxy-2-naphthyl]methyl]-2,4-thiazolidinedione; (MCC-555 or “netoglitazone”).

[0295] Insulin Sensitizers—PPAR Modulators

[0296] One class of insulin sensitizers of the invention is PPAR modulators, and in particular PPAR-gamma modulators, e.g., PPAR-gamma agonists. PPAR modulators include the PPAR-alpha, PPAR-delta (also called PPAR-beta), and PPAR-gamma agonists. Especially useful are the thiazolidinediones (TZDs), which were developed in the 70's and 80s by screening newly synthesized compounds for their ability to lower blood glucose in diabetic rodents. Three molecules from this class, troglitazone, rosiglitazone, and pioglitazone, were ultimately approved for the treatment of patients with Type II diabetes. Although these compounds were developed without an understanding of their molecular mechanism of action, by the early 90s evidence began to accumulate linking the thiazolidinediones to the nuclear receptor PPAR-gamma. It was ultimately demonstrated that these molecules were high affinity ligands of PPAR-gamma and that they increased transcriptional activity of the receptor. Without wishing to be bound by theory, multiple lines of evidence now indicate that the antidiabetic activities of the thiazolidinediones are mediated by their direct interaction with the receptor and the subsequent modulation of PPAR-gamma target gene expression.

[0297] Thiazolidinediones of use in the methods of the invention include: (1) rosiglitazone; (2) pioglitazone; (3) troglitazone; (4) netoglitazone (also known as MCC-555 or isaglitazone or neoglitazone); and (5) 5-BTZD.

[0298] Other PPAR modulators of use in the invention include modulators that have recently been the subject of clinical trials: (1) Muraglitazar (PPAR gamma and alpha agonist, Bristol-Myers/Merck); (2) Galida tesaglitazar (PPAR gamma and alpha agonist, AstraZeneca); (3) 677954 (PPAR gamma, alpha, and delta agonist, GlaxoSmithKline); (4)

MBX-102 (PPAR gamma partial agonist/antagonist, Metabolex); (5) T131 (PPAR gamma selective modulator, Tularik/Amgen); (6) LY818 (PPAR gamma and alpha partial agonist, Eli Lilly/Ligand); (7) LY929 (PPAR gamma and alpha agonist, Eli Lilly/Ligand); and (8) PLX204 (PPAR gamma, alpha, and delta agonist, Plexxikon). See, e.g., BioCentury, Jun. 14, 2004. Further PPAR modulators include LY 519818, L-783483, L-165461, and L-165041.

[0299] Additionally, the non-thiazolidinediones that act as insulin-sensitizing agents include, but are not limited to: (1) JT-501 (JTT 501, PNU-1827, PNU-7,6-MET-0096, or PNU 182716: 4-(4-(2-(5-methyl-2-phenyl-oxazol-4-yl)ethoxy)benzyl)isoxazolidine-3,5-dione; (2) KRP-297 (5-(2,4-dioxothiazolidin-5-ylmethyl)-2-methoxy-N-(4-(tri-fluoromethyl)benzyl)benzamide or 5-((2,4-dioxo-5-thiazolidinyl)methyl)-2-methoxy-N-((4-(trifluoromethyl)phenyl)methyl)benzamide); and (3) Farglitazar (L-tyrosine, N-(2-benzoylphenyl)-o-(2-(5-methyl-2-phenyl-4-oxazolyl)ethyl) or N-(2-benzoylphenyl)-O-(2-(5-methyl-2-phenyl-4-oxazolyl)ethyl)-L-tyrosine, or (S)-2-(2-benzoylphenylamino)-3-(4-(12-(5-methyl-2-phenyl-2-oxazo-4-yl)ethoxyphenyl)propionic acid, or GW2570 or G1-262570).

[0300] Other agents have also been shown to have PPAR modulator activity such as PPAR-gamma, SPPAR-gamma, and/or PPAR-alpha/delta agonist activity. Examples are: (1) AD 5075 (5-(4-(2-hydroxy-2-(5-methyl-2-phenyloxazol-4-yl)ethoxy)benzyl)-thiazolidine-2,4-dione); (2) R 119702 (or CI-1037 or CS 011); (3) CLX-0940 (peroxisome proliferator-activated receptor alpha agonist/peroxisome proliferator-activated receptor gamma agonist); (4) LR-90 (2,5,5-tris(4-chlorophenyl)-1,3-dioxane-2-carboxylic acid, PPAR alpha/gamma agonist); (5) CLX-0921 (PPAR gamma agonist); (6) CGP-52608 (PPAR agonist); (7) GW-409890 (PPAR agonist); (8) GW-7845 (2((S)-1-carboxy-2-(4-(2-(5-methyl-2-phenyl-oxazol-4-yl)-ethoxy)-phenyl)-ethylamino)-benzoic acid methyl ester, PPAR agonist); (9) L-764406 (2-benzene-sulphonylmethyl-3-chloroquinoline, PPAR agonist); (10) LG-101280 (PPAR agonist); (11) LM-4156 (PPAR agonist); (12) Risarestat (CT-112, (+)-5-(3-ethoxy-4-(pentyloxy) phenyl)-2,4-thiazolidinedione aldose reductase inhibitor); (13) YM 440 (PPAR agonist); (14) AR-H049020 (PPAR agonist); (15) GW 0072 ((+)-(2S,5S)-4-(4-(5-((dibenzyl carbomoyl)methyl)-2-heptyl-4-oxothiazolidin-3-yl)butyl)benzoic acid); (16) GW 409544 (GW-544 or GW-409544); (17) NN 2344 (DRF 2593); (18) NN 622 (DRF 2725); (19) AR-H039242 (AZ-242); (20) GW 9820 (fibrate); (21) GW 1929 (N-(2-benzoylphenyl)-O-(2-(methyl-2-pyridinylamino) ethyl)-L-tyrosine, known as GW 2331, PPAR agonist); (22) SB 219994 ((S)-4-(2-(2-benzoxazolylmethylamino) ethoxy)-alpha-(2,2,2-trifluoroethoxy)benzenepropanoic acid or 3-(4-(2-(N-(2-benzoxazolyl)-N-methylamino) ethoxy)phenyl)-2(S)-(2,2,2-trifluoroethoxy)propionic acid or benzenepropanoic acid, 4-(2-(2-benzoxazolylmethylamino) ethoxy)-alpha-(2,2,2-trifluoroethoxy)-, (alpha S)-, PPAR alpha/gamma agonist); (23) L-796449 (PPAR alpha/gamma agonist); (24) Fenofibrate (propanoic acid, 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-, 1-methylethyl ester, known as TRICOR, LIPCOR, LIPANTIL, LIPIDIL MICRO PPAR alpha agonist); (25) GW-9578 (PPAR alpha agonist); (26) GW-2433 (PPAR alpha/gamma agonist); (27) GW-0207 (PPAR gamma agonist); (28) LG-100641 (PPAR gamma agonist); (29) LY-300512 (PPAR gamma agonist); (30) NID525-209 (NID-525); (31) VDO-52 (VDO-52); (32) LG 100754 (peroxisome proliferator-activated receptor agonist);

(33) LY-510929 (peroxisome proliferator-activated receptor agonist); (34) bexarotene (4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)ethenyl)benzoic acid, known as TARGRETIN, TARGRETIN, TARGRETIN; also known as LGD 1069, LG 100069, LG 1069, LDG 1069, LG 69, RO 264455); and (35) GW-1536 (PPAR alpha/gamma agonist).

[0301] In some aspects of the invention, radioisotopes can be delivered via the implantable device of the invention. For example, it is well known in the art that various methods of radionuclide therapy can be used for the treatment of cancer and other pathological conditions, as described, e.g., in Harbert, "Nuclear Medicine Therapy", New York, Thieme Medical Publishers, 1987, pp. 1-340. A clinician experienced in these procedures will readily be able to adapt the implantable device described herein to such procedures to mitigate or treat disease amenable to radioisotope therapy thereof.

[0302] In some aspects the radio isotopes include but are not limited to isotopes and salts of isotopes with short half life: such as Y-90, P-32, I-131, Au 198. Therefore in one aspect of the invention, the implantable device can be utilized to deliver radioisotopes.

[0303] It is also well known that radioisotopes, drugs, and toxins can be conjugated to antibodies or antibody fragments which specifically bind to markers which are produced by or associated with cancer cells, and that such antibody conjugates can be used to target the radioisotopes, drugs or toxins to tumor sites to enhance their therapeutic efficacy and minimize side effects. Examples of these agents and methods are reviewed in Wawrzynczak and Thorpe (in Introduction to the Cellular and Molecular Biology of Cancer, L. M. Franks and N. M. Teich, eds, Chapter 18, pp. 378-410, Oxford University Press, Oxford, 1986), in Immunoconjugates. Antibody Conjugates in Radioimaging and Therapy of Cancer (C.-W. Vogel, ed., 3-300, Oxford University Press, New York, 1987), in Dillman, R. O. (CRC Critical Reviews in Oncology/Hematology 1:357, CRC Press, Inc., 1984), in Pastan et al. (Cell 47:641, 1986), in Vitetta et al. (Science 238:1098-1104, 1987) and in Brady et al. (Int. J. Rad. Oncol. Biol. Phys. 13:1535-1544, 1987). Other examples of the use of immunoconjugates for cancer and other forms of therapy have been disclosed, inter alia, in Goldenberg, U.S. Pat. Nos. 4,331,647, 4,348,376, 4,361,544, 4,468,457, 4,444,744, 4,460,459, 4,460,561 and 4,624,846, and in Rowland, U.S. Pat. No. 4,046,722, Rodwell et al., U.S. Pat. No. 4,671,958, and Shih et al., U.S. Pat. No. 4,699,784, the disclosures of all of which are incorporated herein in their entirety by reference.

[0304] Other thiazolidinedione and non-thiazolidinedione insulin sensitizers of use in the invention are described in, e.g., Leff and Reed (2002) Curr. Med. Chem.—Immun., Endoc., & Metab. Agents 2:33-47; Reginato et al. (1998) J. Biol. Chem., 278 32679-32654; Way et al. (2001) J. Biol. Chem. 276 25651-25653; Shiraki et al. (2005) JBC Papers in Press, published on Feb. 4, 2005, as Manuscript M500901200, and U.S. Pat. Nos. 4,703,052; 6,008,237; 5,594,016; 6,838,442; 6,329,423; 5,965,589; 6,677,363; 4,572,912; 4,287,200; 4,340,605; 4,438,141; 4,444,779; 4,572,912; 4,687,777; 4,725,610; 5,232,925; 5,002,953; 5,194,443; 5,260,445; 6,300,363; 6,034,110; and 6,541,493; U.S. Patent Application Publications 2002/0042441; 2004/0198774 and 2003/0045553; EP Patent Nos. 0139421 and 0332332; and PCT Publication Nos. WO 95/35314; WO 00/31055; WO 01/3640, all of which are incorporated by reference herein in their entirety.

[0305] Netoglitazone

[0306] One thiazolidinedione PPAR modulator for use in the methods of the invention is netoglitazone (5-[(6-(2-fluorobenzyl)oxy-2-naphthyl)methyl]-2,4-thiazolidinedione; MCC-555). Structures and methods of preparation of netoglitazone and various forms of netoglitazone of use in the invention are described in, e.g., U.S. Pat. Nos. 5,594,016; 6,541,493; 6,541,493; 6,838,442; U.S. Patent Application No. 2004/0198774 and 2003/045553; PCT Publication Nos. WO 00/31055; WO 01/36401; WO 03/018010, and WO 00/73252; Japanese Patent Unexamined Publication (KO-KAI) Nos. (Hei) 6-247945/1994 and (Hei) 10-139768/1998; Japanese Patents 2001172179 and 2003040877; and Reginato et al. (1998) J. Biol. Chem. 273: 32679-32684; all of which are incorporated by reference herein in their entirety.

[0307] It has been reported that netoglitazone is more efficacious than pioglitazone and troglitazone in lowering plasma glucose, insulin, and triglyceride levels and that it is about three-fold more potent than rosiglitazone. The activity of netoglitazone appears to be context-specific, as in some cell types it behaves as a full agonist of PPAR-gamma and as a partial agonist or antagonist in others. In addition, it appears to modulate PPAR-alpha and delta as well. See, e.g., U.S. Patent Application Publication No. 2004/0198774.

[0308] Forms of Drugs

[0309] Some compounds useful in the invention, including the TZD PPAR modulators such as netoglitazone, may have one or more asymmetric carbon atoms in their structure. In addition, stereochemically pure isomeric forms of the compounds as well as their racemates can also be delivered using one or more matrix disclosed herein. Stereochemically pure isomeric forms may be obtained by the application of art known principles. Diastereoisomers may be separated by physical separation methods such as fractional crystallization and chromatographic techniques, and enantiomers may be separated from each other by the selective crystallization of the diastereomeric salts with optically active acids or bases or by chiral chromatography. Pure stereoisomers may also be prepared synthetically from appropriate stereochemically pure starting materials, or by using stereospecific reactions.

[0310] Some compounds useful in the invention may have various individual isomers, such as trans and cis, and various alpha and beta attachments (below and above the plane of the drawing). In addition, where the processes for the preparation of the compounds according to the invention give rise to mixture of stereoisomers, these isomers may be separated by conventional techniques such as preparative chromatography. The compounds may be prepared as a single stereoisomer or in racemic form as a mixture of some possible stereoisomers. The non-racemic forms may be obtained by either synthesis or resolution. The compounds may, for example, be resolved into their components enantiomers by standard techniques, such as the formation of diastereomeric pairs by salt formation. The compounds may also be resolved by covalent linkage to a chiral auxiliary, followed by chromatographic separation and/or crystallographic separation, and removal of the chiral auxiliary. Alternatively, the compounds may be resolved using chiral chromatography. Unless otherwise noted the scope of the bioactive agents, that can be included in the matrix(es) disclosed herein, is intended to cover all such isomers or stereoisomers per se, as well as mixtures of cis and trans isomers, mixtures of diastereomers and racemic mixtures of enantiomers (optical isomers) as well.

[0311] In addition, compounds to be delivered by or included in the matrixes of the invention may be prepared in various polymorphic forms. For example, insulin sensitizers of use in the invention can occur in polymorphic forms, and any or all of the polymorphic forms of these insulin sensitizers are contemplated for use in the invention. Polymorphism in drugs may alter the stability, solubility and dissolution rate of the drug and result in different therapeutic efficacy of the different polymorphic forms of a given drug. The term polymorphism is intended to include different physical forms, crystal forms, and crystalline/liquid crystalline/non-crystalline (amorphous) forms. Polymorphism of compounds of therapeutic use has is significant, as evidenced by the observations that many antibiotics, antibacterials, tranquilizers etc., exhibit polymorphism and some/one of the polymorphic forms of a given drug may exhibit superior bioavailability and consequently show much higher activity compared to other polymorphs. For example, Sertraline, Frentizole, Ranitidine, Sulfathiazole, and Indomethacine are some of the pharmaceuticals that exhibit polymorphism.

[0312] Some embodiments of the invention include the use of netoglitazone in one of its polymorphic forms. Netoglitazone can be prepared in various polymorphic forms. Any polymorphic forms of netoglitazone known in the art may be used in the methods of the invention, either separately or in combination. Thus, the methods of the invention include association studies using any or all of the polymorphic forms of netoglitazone, as well as screening and treatment using any or all of the polymorphic forms of netoglitazone, compositions and kits based on these forms, and the like.

[0313] Polymorphic forms of netoglitazone include the A, B, C, D, E and amorphous crystal forms described in PCT Published Application No. WO 01/36401 and in U.S. Pat. No. 6,541,493; for example, the E form is described in PCT Published Application No. WO 01/36401.

[0314] Some of the compounds described herein may exist with different points of attachment of hydrogen coupled with double bond shifts, referred to as tautomers. An example is a carbonyl (e.g. a ketone) and its enol form, often known as keto-enol tautomers. The individual tautomers as well as mixtures thereof are encompassed within the invention.

[0315] Prodrugs are compounds that are converted to the claimed compounds as they are being administered to a patient or after they have been administered to a patient. The prodrugs are compounds of this invention, and the active metabolites of the prodrugs are also compounds of the invention.

[0316] Other agents useful in the methods of the invention include, but are not limited to:

[0317] 1. Biguanides, which decrease liver glucose production and increases the uptake of glucose. Examples include metformin such as: (1) 1,1-dimethylbiguanide (e.g., Metformin-DepoMed, Metformin-Biovail Corporation, or METFORMIN GR (metformin gastric retention polymer)); and (2) metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide monohydrochloride, also known as LA 6023, BMS 207 150, GLUCOPHAGE, or GLUCOPHAGE XR.

[0318] 2. Alpha-glucosidase inhibitors, which inhibit alpha-glucosidase, and thereby delay the digestion of carbohydrates. The undigested carbohydrates are subsequently broken down in the gut, reducing the post-prandial glucose peak. Examples include, but are not limited to: (1) acarbose (D-glucose, O-4,6-dideoxy-4-(((1S-(1alpha,4alpha,5beta,6alpha))-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclo-

hexen-1-yl)amino)-alpha-D-glucopyranosyl-(1-4)-O-alpha-D-glucopyranosyl-(1-4)-, also known as AG-5421, Bay-g-542, BAY-g-542, GLUCOBAY, PRECOSE, GLUCOR, PRANDASE, GLUMIDA, or ASCAROSE); (2) Miglitol (3,4,5-piperidinetriol, 1-(2-hydroxyethyl)-2-(hydroxymethyl)-, (2R (2alpha, 3beta, 4alpha, 5beta))- or (2R,3R,4R, 5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)-3,4,5-piperidinetriol, also known as BAY 1099, BAY M 1099, BAY-m-1099, BAYGLITOL, DIASTABOL, GLYSET, MIGLIBAY, MITOLBAY, PLUMAROL); (3) CKD-711 (0-4-deoxy-4-((2,3-epoxy-3-hydroxymethyl-4,5,6-trihydro-xycyclohexane-1-yl)amino)-alpha-b-glucopyranosyl-(1-4)-alpha-D-glucopyranosyl-(1-4)-D-glucopyranose); (4) emiglitate (4-(2-((2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)-1-piperidinyl)ethoxy) benzoic acid ethyl ester, also known as BAY o 1248 or MKC 542); (5) MOR14 (3,4,5-piperidinetriol, 2-(hydroxymethyl)-1-methyl-, (2R-(2alpha, 3beta, 4alpha, 5beta))-, also known as N-methyldeoxynojirimycin or N-methylmoranoline); and (6) Voglibose (3,4-dideoxy-4-((2-hydroxy-1-(hydroxymethyl)ethyl)amino)-2-C-(hydroxymethyl)-D-epi-inositol or D-epi-inosito-1,3,4-dideoxy-4-((2-hydroxy-1-(hydroxymethyl)ethyl)amino)-2-C-(hydroxymethyl)-, also known as A 71100, AO 128, BASEN, GLUSTAT, VOGLISTAT.

[0319] 3. Insulins include regular or short-acting, intermediate-acting, and long-acting insulins, injectable, non-injectable or inhaled insulin, transdermal insulin, tissue selective insulin, glucophosphokinase (D-chiroinositol), insulin analogues such as insulin molecules with minor differences in the natural amino acid sequence and small molecule mimics of insulin (insulin mimetics), and endosome modulators. Examples include, but are not limited to: (1) Biota; (2) LP 100; (3) (SP-5-21)-oxobis(1-pyrrolidinecarbodithioato-S,S') vanadium, (4) insulin aspart (human insulin (28B-L-aspartic acid) or B28-Asp-insulin, also known as insulin X14, INA-X14, NOVORAPID, NOVOMIX, or NOVOLOG); (5) insulin detemir (Human 29B-(N-6-(1-oxotetradecyl)-L-lysine)-(1A-21A), (1B-29B)-Insulin or NN 304); (6) insulin lispro ("28B-L-lysine-29B-L-proline human insulin, or Lys (B28), Pro (B29) human insulin analog, also known as lys-pro insulin, LY 275585, HUMALOG, HUMALOG MIX 75/25, or HUMALOG MIX 50/50); (7) insulin glargine (human (A21-glycine, B31-arginine, B32-arginine) insulin HOE 901, also known as LANTUS, OPTISULIN); (8) Insulin Zinc Suspension, extended (Ultralente), also known as HUMULIN U or ULTRALENTE; (9) Insulin Zinc suspension (Lente), a 70% crystalline and 30% amorphous insulin suspension, also known as LENTE ILETIN II, HUMULIN L, or NOVOLIN L; (10) HUMULIN 50/50 (50% isophane insulin and 50% insulin injection); (11) HUMULIN 70/30 (70% isophane insulin NPH and 30% insulin injection), also known as NOVOLIN 70/30, NOVOLIN 70/30 PenFill, NOVOLIN 70/30 Prefilled; (12) insulin isophane suspension such as NPH ILETIN II, NOVOLIN N, NOVOLIN N PenFill, NOVOLIN N Prefilled, HUMULIN N; (13) regular insulin injection such as ILETIN II Regular, NOVOLIN R, VELOSULIN BR, NOVOLIN R PenFill, NOVOLIN R Prefilled, HUMULIN R, or Regular U-500 (Concentrated); (14) ARIAD; (15) LY 197535; (16) L-783281; and (17) TE-17411.

[0320] 4. Insulin secretion modulators such as (1) glucagon-like peptide-1 (GLP-1) and its mimetics; (2) glucose-insulinotropic peptide (GIP) and its mimetics; (3) exendin and its mimetics; (4) dipeptyl protease (DPP or DPPIV) inhibitors such as (4a) DPP-728 or LAF 237 (2-pyrrolidin-

ecarbonitrile, 1-(((2-((5-cyano-2-pyridinyl)amino)ethyl)amino)acetyl), known as NVP-DPP-728, DPP-728A, LAF-237); (4b) P 3298 or P32/98 (di-(3N-((2S,3S)-2-amino-3-methyl-pentanoyl)-1,3-thiazolidine) fumarate); (4c) TSL 225 (tryptophyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid); (4d) Valine pyrrolidide (valpyr); (4e) 1-aminoalkylisoquinolinone-4-carboxylates and analogues thereof; (4f) SDZ 272-070 (1-(L-Valyl)pyrrolidine); (4g) TMC-2A, TMC-2B, or TMC-2C; (4h) Dipeptide nitriles (2-cyanopyrrolodides); (4i) CD26 inhibitors; and (4j) SDZ 274-444; (5) glucagon antagonists such as AY-279955; and (6) amylin agonists which include, but are not limited to, pramlintide (AC-137, Symlin, tripro-amylin or pramlintide acetate).

[0321] 5. Insulin secretagogues, which increase insulin production by stimulating pancreatic beta cells, such as: (1) asmitiglinide ((2S)-cis-octahydro-gamma-oxo-alpha-(phenylmethyl)-2H-isoindole-2-butanone acid, calcium salt, also known as mituglimide calcium hydrate, KAD 1229, or S 21403); (2) Ro 34563; (3) nateglinide (trans-N-((4-(1-methylethyl)cyclohexyl) carbonyl)-D-phenylalanine, also known as A 4166, AY 4166, YM 026, FOX 988, DJN 608, SDZ DJN 608, STARLIX, STARSIS, FASTIC, TRAZEC); (4) JTT 608 (trans-4-methyl-gamma-oxocyclohexanecarboxylic acid); (5) sulfonylureas such as: (5a) chlorpropamide (1-[(p-chlorophenyl) sulfonyl]-3-propylurea, also known as DIABINESE); (5b) tolazamide (TOLANASE or TOLANASE); (5c) tolbutamide (ORINASE or RASTINON); (5d) glyburide (1-[[p-[2-(5-chloro-o-anisamido) ethyl]phenyl]sulfon-yl]-3-cyclohexylurea, also known as Glibenclamide, DIABETA, MICRONASE, GLYNASE PresTab, or DAONIL); (5e) glipizide (1-cyclohexyl-3-[[p-[2-(5-ethylpyrazinecarboxamido) ethyl]phenyl]sulfonyl]urea, also known as GLUCOTROL, GLUCOTROL XL, MINODIAB, or GLIBENESE); (5f) glimepiride (1H-pyrrole-1-carboxamide, 3-ethyl-2,5-dihydro-4-m-ethyl-N-[2-[4-[[[4-methylcyclohexyl]amino]carbonyl]amino]sulfonyl]phenyl]-ethyl]-2-oxo-, trans-, also known as Hoe-490 or AMARYL); (5g) acetohexamide (DYMELOR); (5h) gliclazide (DIAMICRON); (5i) glipentide (STATICUM); (5j) gliquidone (GLURENORM); and (5k) glisulamide (DIABENOR); (6) K+ channel blockers including, but not limited to, meglitinides such as (6a) Repaglinide ((S)-2-ethoxy-4-(2-((3-methyl-1-(2-(1-piperidinyl)phenyl)butyl)amino)-2-oxoethyl)benzoic acid, also known as AGEE 623, AGEE 623 ZW, NN 623, PRANDIN, or NovoNorm); (6b) imidazolines; and (6c) α -2 adrenoceptor antagonists; (7) pituitary adenylate cyclase activating polypeptide (PACAP); (8) vasoactive intestinal peptide (VIP); (9) amino acid analogs; and (10) glucokinase activators.

[0322] Growth Factors such as: (1) insulin-like growth factors (IGF-1, IGF-2); (2) small molecule neurotrophins; (3) somatostatin; (4) growth hormone-releasing peptide (GHRP); (5) growth hormone-releasing factor (GHRF); and (6) human growth hormone fragments. Immunomodulators such as: (1) vaccines; (2) T-cell inhibitors; (3) monoclonal antibodies; (4) interleukin-1 (IL-1) antagonists; and (5) BDNF. Glucose resorption inhibitors such as those described in U.S. Patent Application No. 2003/0045553. Other antidiabetic agents: (1) Hu-Glucagon; (2) DHEA analogs; (3) carnitine palmitoyl transferase (CPT) inhibitors; (4) islet neurogenesis; (5) pancreatic β amyloid inhibitors; and (6) UCP (uncoupling protein)-2 and UCP-3 modulators.

[0323] In one aspect the matrix structures of the invention can be utilized to elicit an immune response in a subject.

Therefore, in one embodiment, the matrix releases an antigen or immunogen in a time controlled manner so as to elicit an immune response in a subject. Such an immune response can impart protective immunity or “vaccinate” an animal against the desired antigen or immunogen. In an alternative, embodiment, the antigen or immunogen can be linked to a portion of the nucleic acid matrix (or gel or scaffold), or to the nucleic acid matrix-copolymer structure.

[0324] Additional agents of use in the invention include any agents known in the art for treatment of disorder of blood glucose regulations and/or their complications. Such agents include, but are not limited to, cholesterol lowering agents such as (i) HMG-CoA reductase inhibitors (lovastatin, simvastatin and pravastatin, fluvastatin, atorvastatin, rivastatin and other statins), (ii) sequestrants (cholestyramine, colestipol and a dialkylaminoalkyl derivatives of a cross-linked dextran), (iii) nicotinic alcohol, nicotinic acid or a salt thereof, (iv) PPAR.alpha. agonists such as fenofibric acid derivatives (gemfibrozil, clofibrate, fenofibrate and benzafibrate), (v) inhibitors of cholesterol absorption for example beta-sitosterol and (acyl CoA:cholesterol acyltransferase) inhibitors for example melinamide and (vi) probucol; PPARdelta agonists such as those disclosed in WO97/97/28149; antiobesity compounds such as fenfluramine, dexfenfluramine, phentermine, sulbitramine, orlistat, neuropeptide Y5 inhibitors, and, $\beta 3$ adrenergic receptor agonist; and ileal bile acid transporter inhibitors.

[0325] Classes of Drugs

[0326] Drugs may be classed into mechanistic classes, structural classes, classes based on pharmacological effect, and other classes of drugs that are based on the chemical or biological nature of the drugs, or that are empirically based.

[0327] Mechanistic classifications are based on the mechanism of action of drugs, e.g., receptor targets or other targets of the drugs. For example, drugs that primarily act on the autonomic nervous system may be classed as cholinoreceptor-activating drugs, or cholinesterase-inhibiting drugs, or cholinoreceptor-blocking drugs, or adrenoceptor-activating drugs, or adrenoceptor-blocking drugs.

[0328] However, as is known in the art, often drugs do not have a known target or a precisely defined mechanism, and may be classed according to similarities in other aspects the drugs, such as similarities of the chemical structure that are thought to be important to the action of the drugs. Such similarities include structural components, optical isomerism, crystal structure, and the like.

[0329] Drugs may also be classed based on their major pharmacological action, e.g., lipid-lowering drugs, antidepressants, anxiolytics, and the like. The second drug may be placed in the same class as the first drug by in vitro and/or in vivo studies; in some embodiments, action through the same or similar mechanism may be predicted from structural analysis.

[0330] In some embodiments, drugs are classified based on their effects in one or more in vitro, cellular, tissue, organ, or animal models. Such effects may be molecular, supramolecular, cellular, tissue, organ, or whole-organism effects, or combinations thereof. In some embodiments, drugs are classified based on their effects in one or more animal models together with associations between genotypes and response in the animal models. For example, drug A may cause response M in a mammal, e.g., a rat, mouse, or primate, of genotype X (e.g., genotype at one or more SNPs), and may cause response N in a primate of genotype Y. If drug B is found to cause response

M in a mammal of genotype X and response N in a mammal of genotype Y, then drug B is considered to be in the same class as drug A. It will be appreciated that such classification may be greatly refined based on the number of genetic variations included in the genotype, the number of responses measured, and the like. The animal model allows a much wider range of drugs to be tested, as well as more invasive parameters to be measured as indications of response, and can allow a much more extensive database to be established in a relatively short time, compared to human testing.

[0331] In other embodiments, expression profiles for a drug in a model system may be used to classify the drug. For example, all, most, or some of the known drugs of a class of drugs that has an effect in humans (e.g., statins that lower the risk of heart disease) may be tested in an animal model. Animals administered the drug may show consistent profiles of gene expression in response to the drug (e.g., increases in expression of a gene or set of genes related to antiinflammatory activity). Other drugs of other classes may be tested in animal models. The expression profiles associated with the drugs in a particular class may be correlated. A new drug may be assigned to a drug class based on its expression profile in one or more animal models. The associations of one or more drugs in that class between one or more genetic variations and a response to the drug(s) may be used to modulate the use of the new drug, for example, in research (e.g., clinical trials) and/or in the clinical setting.

[0332] In some embodiments, a new drug in a class of drugs is first tested in a model, e.g. an animal model, in which other drugs in the class of drugs have been tested, and in which a genotype for the animal is used to predict responses to the new drug. The results of the animal studies can be used to refine predictions for the association between genetic variations and response to a new drug in humans. Animal models may be developed or existing animal models may be used. The animal model can be for a particular physiological, biochemical, or metabolic state, e.g., a disease or pathological state. Healthy or superhealthy states may also be modeled (e.g., decelerated aging).

[0333] Drugs may be further put into classes, or into subclasses of the same class, by classifications based on their mode administration (e.g., intravascular, intramuscular, subcutaneous, ocular, inhalation, oral, sublingual, suppository, skin, via pump, and the like), formulation type (e.g., rapid acting, sustained release, enterically coated, etc.), mode of uptake and delivery to site of action, metabolism (e.g., drugs metabolized through Phase I reactions such as oxidation via hepatic microsomal P450 system and subclasses thereof, through oxidation via nonmicrosomal mechanisms and subclasses thereof, through reduction, through hydrolysis and subclasses thereof; drugs metabolized through Phase II reactions such as glucuronidation, acetylation, mercapturic acid formation, sulfate conjugation, N—, O—, and S-methylation, trans-sulfuration; and combinations thereof), metabolic products and/or byproducts and their structure and/or function, pharmacokinetics, pharmacodynamics, elimination, and the like

[0334] It will be appreciated that these classifications are exemplary only, and that any means of classifying drugs that allows a non-random predictability of the effects of drugs in the class may be used. Further systems of drug classification and specific drugs within each class may be found in the art. See, e.g., Anderson, Philip O.; Knoben, James E.; Troutman, William G, eds., *Handbook of Clinical Drug Data*, Tenth

Edition, McGraw-Hill, 2002; Pratt and Taylor, eds., Principles of Drug Action, Third Edition, Churchill Livingstone, New York, 1990; Katzung, ed., Basic and Clinical Pharmacology, Ninth Edition, McGraw Hill, 20037ybg; Goodman and Gilman, eds., The Pharmacological Basis of Therapeutics, Tenth Edition, McGraw Hill, 2001; Remington's Pharmaceutical Sciences, 20th Ed., Lippincott Williams & Wilkins., 2000; Martindale, The Extra Pharmacopoeia, Thirty-Second Edition (The Pharmaceutical Press, London, 1999); all of which are incorporated by reference herein in their entirety.

[0335] Any suitable class of drugs for which genotyping and association studies are possible for at least one member of the class may be the subject of the described methods and compositions. Classes include the insulin sensitizers as described herein, e.g., PPAR modulators. Thus, in some embodiments, the invention provides a method for predicting an individual's responsiveness to an insulin sensitizer, e.g., a PPAR modulator based on the individual's genotype and the results of association studies between genotype and responsiveness to another insulin sensitizer, e.g., PPAR modulator. In some embodiments, the prediction of an individual's responsiveness to an insulin sensitizer, e.g., PPAR modulator is used to include or exclude the individual in a clinical trial.

In some embodiments, the prediction of an individual's responsiveness to an insulin sensitizer, e.g., PPAR modulator is used to modulate the individual's administration of another insulin sensitizer, e.g., PPAR modulator. In some embodiments such modulation occurs in a clinical trial. In some embodiments, the prediction of an individual's responsiveness to an insulin sensitizer, e.g., PPAR modulator is used to determine that the individual should be treated with a drug other than an insulin sensitizer, or in some embodiments a PPAR modulator.

[0336] Mechanistic Classes of Drugs

[0337] One non-exclusive exemplary classes of drugs for which genotyping and association studies with one member may be used to predict effects of another member include, mechanistic classes of drugs used in the treatment of diabetes (including PPAR modulators). This class of drugs also illustrates how drugs can also be subclassed by, e.g., mode of administration. For example, insulin and insulin analogs may be formulated for administration by injection, nasal spray, transdermal, oral or inhalation routes. Each type of formulation can have unique profiles of responses and associated genetic variations. An example of classifications of such drugs by mechanism, together with representative members of the mechanistic classes, is given in Table 10.

TABLE 10

Classes of Drugs for Treatment of Diabetes		
Class	Mechanism of Action	Examples
Peroxisome Proliferator-Activated Receptor (PPAR) Agonists	Target PPAR-gamma or PPAR-gamma and -alpha (see below). PPAR are nuclear receptors that help regulate glucose and lipid metabolism. Activation of PPAR-gamma improves insulin sensitivity and thus improves glycemic control.	Rosiglitazone, Pioglitazone, Balaglitazone, see also others described herein
Dual-Action Peroxisome Proliferator-Activated Receptor Agonists	Act on both PPAR-gamma and PPAR-alpha. PPAR-alpha activation has effects on cellular uptake of fatty acids and their oxidation, and on lipoprotein metabolism. May also act to reduce inflammatory response in vascular endothelial cells.	TAK-559, Muraglitazar, Tesaglitazar, Netoglitazone, see also others described herein
Biguanidines	Complete mechanism is not known. Reduces gluconeogenesis in the liver by inhibiting glucose-6-phosphatase.	Metformin, Metformin GR
Sulfonylureas	Induce insulin secretion by binding to cellular receptors that cause membrane depolarization and insulin exocytosis.	Glimepiride, Glyburide/glibenclamide, Glipizide, Gliclazide, Tobutamide
Insulin and Insulin Analogs (Injectable, Inhaled, Oral, Transdermal, Intranasal)	Supplements endogenous insulin. Insulin analogs have a variety of amino acid changes and have altered onset of action and duration of action, as well as other properties, compared to native insulin. Inhaled insulin is absorbed through the alveoli. Spray oral insulin is absorbed by the buccal mucosa and intranasal through the nasal mucosa. Transdermal insulin is absorbed through the skin.	Insulin lispro, Insulin aspart, Insulin glargine, Exubera, AERx Insulin Diabetes Management System, HIM-2, Oaralin, Insulin detemir, Insulin glulisine
Meglitinides	Are thought to bind to a nonsulfonylurea beta cell receptor and act to cause insulin secretion by mechanism similar to sulfonylureas	Repaglinide, Nateglinide, Mitiglinide
Alpha-Glucosidase Inhibitors	Inhibit carbohydrate digestion. Act at brush border of intestinal epithelium.	Acarbose, Miglitol, Voglibose
Glucagon-Like Peptide(GLP)-1 Analogs	Diabetic patients may lack native GLP-1, and analogs act as substitutes. GLP-1 is an intestinal peptide hormone that induces glucose-dependent insulin secretion, controls gastric emptying, inhibits appetite, and modulates secretion of glucagon and somatostatin.	Exenatide, Exenatide LAR, Liraglutide, ZP 10, BN51077,

TABLE 10-continued

Classes of Drugs for Treatment of Diabetes		
Class	Mechanism of Action	Examples
Dipeptidyl Peptidase (DPP)-IV Inhibitors	Inhibit DPP-IV, a ubiquitous enzyme that cleaves and inactivates GLP-1, thus inhibition of DPP-IV increases GLP-1 activity	LAF-237, p-32/98, MK-431, P3298, NVP LAF 237,
Pancreatic Lipase Inhibitors	Inhibits lipases, thus inhibiting uptake of dietary fat. This causes weight loss, improves insulin sensitivity and lowers hyperglycemia.	Orlistat
Amylin Analogs	Act to augment amylin, which acts with insulin by slowing glucose absorption from the gut and slows after-meal glucose release from liver.	Pramlintide
Dopamine D2 receptor agonists	Thought to act to alleviate abnormal daily variations in central neuroendocrine activity that can contribute to metabolic and immune system disorders.	Bromocriptine
Immunosuppressants	Suppress autoimmune response thought to be implicated in Type I and possibly Type II diabetes. Example: Humanized monoclonal antibody that recognizes and inhibits the alpha subunit of IL-2 receptors; humanized Mab that binds to T cell CD3 receptor to block function of T-effector cells that attack the body and cause autoimmune disease	Daclizumab, NBI 6024, TRX-TolerRx, OKT3-gamma-1-ala-ala
Insulin-like growth factor-1 agonists	Recombinant protein complex of insulin-like growth factor-1 and binding protein-3; regulates the delivery of somatomedin to target tissues. Reduces insulinitis severity and beta cell destruction	Somatomedin-1 binding protein 3
Insulin sensitizers	Insulin sensitizers, generally orally active	S15261, Dexlipotam, CLX 0901, R 483, TAK 654
Growth hormone releasing factor agonists	Mimic the action of native GHRF	TH9507, SOM 230
Glucagon antagonists	Inhibit glucagon action, stimulating insulin production and secretion, resulting in lower postprandial glucose levels	Liraglutide, NN 2501
Diabetes type 1 vaccine	Prevents destruction of pancreatic beta cells that occurs in type 1 diabetes	Q-Vax, Damyd vaccine
Sodium-glucose co-transporter inhibitor	Selectively inhibits the sodium glucose co-transporter, which mediates renal reabsorption and intestinal absorption of glucose to maintain appropriate blood glucose levels.	T 1095
Glycogen phosphorylase inhibitors	Inhibit glycogen phosphorylase, thus slowing release of glucose	Ingliforib
Undefined mechanisms	Drugs that act in ways beneficial to those with Type I or Type II Diabetes Mellitus, e.g., by reducing blood glucose and triglyceride levels, whose mechanisms have not been elucidated.	FK 614, INGAP Peptide, R 1439
Antisense oligonucleotides	Bind to RNA and cause its destruction, thereby decreasing protein production from corresponding gene.	ISIS 113715
Insulinotropin agonists	Stimulate insulin release	CJC 1131
Gluconeogenesis inhibitors	Inhibit gluconeogenesis, thus modulating blood glucose levels	CS 917
Hydroxysteroid dehydrogenase inhibitors	Inhibit hydroxysteroid dehydrogenase, which are responsible for excess glucocorticoid production and hence, visceral obesity	BVT 3498
Beta 3 adrenoceptor agonist	Agonist for beta 3 adrenoceptor, decreases blood glucose and suppresses weight gain	YM 178, Solabegron, N5984
Nitric oxide antagonist	Decreases effects of NO	NOX 700
Carnitine palmitoyltransferase inhibitor	Inhibits carnitine palmitoyltransferase	ST 1326

[0338] In other embodiments, mechanistic classes of drugs used in the treatment of abnormal cholesterol and/or triglyceride levels in the blood are used in conjunction with a method or composition of the invention. Broad mechanistic classes include the statins, fibrates, cholesterol absorption inhibitors, nicotinic acid derivatives, bile acid sequestrants, cholesteryl ester transfer protein inhibitors, reverse lipid transport path-

way activators, antioxidants/vascular protectants, acyl-CoA cholesterol acyltransferase inhibitors, peroxisome proliferator activated receptor agonists, microsomal triglyceride protein inhibitors, squalene synthase inhibitors, lipoprotein lipase activators, lipoprotein (a) antagonists, and bile acid reabsorption inhibitors. An example of classification of such drugs by mechanism, together with representative members of the mechanistic classes, is given in Table 11.

TABLE 11

Classes of Drugs for Treatment of Abnormal Cholesterol and/or Triglyceride Levels in the Blood		
Class	Mechanism of Action	Examples
Statins	Competitive inhibitors of HMG-CoA reductase	Atorvastatin, Simvastatin, Pravastatin, Fluvastatin, Rosuvastatin, Lovastatin, Pitavastatin, Cerivastatin (withdrawn), Fenofibrate, Bezafibrate, Gemfibrozil, clofibrate, ciprofibrate, Ezetimibe
Fibrates	PPAR α activators	
Cholesterol Absorption Inhibitors	May inhibit NCP1L1 in gut	
Nicotinic Acid Derivatives	Inhibits cholesterol and triglyceride synthesis, exact mechanism unknown	Niacin
Bile Acid Sequestrants	Interrupt the enterohepatic circulation of bile acids	Colesevelam, Cholestyramine, Colestimide, Colestipol
Cholesteryl Ester Transfer Protein Inhibitors	Inhibit cholesteryl ester transfer protein, a plasma protein that mediates the exchange of cholesteryl esters from antiatherogenic HDL to proatherogenic apolipoprotein B-containing lipoproteins	JTT-705, CETi-1, Torcetrapib
Reverse Lipid Transport Pathway Activators	Stimulate reverse lipid transport, a four-step process form removing excess cholesterol and other lipids from the walls of arteries and other tissues	ETC-216, ETC-588, ETC-642, ETC-1001, ESP-1552, ESP-24232
Antioxidants/Vascular Protectants	Inhibit vascular inflammation and reduce cholesterol levels; block oxidant signals that switch on vascular cellular adhesion molecule (VCAM)-1	AGI-1067, Probucol (withdrawn)
Acyl-CoA Cholesterol Acyltransferase (ACAT) Inhibitors	Inhibit ACAT, which catalyzes cholesterol esterification, regulates intracellular free cholesterol, and promotes cholesterol absorption and assembly of VLDL	Eflucimibe, Pactimibe, Avasimibe (withdrawn), SMP-797
Peroxisome Proliferator Activated Receptor Agonists	Activate PPARs, e.g., PPAR α , γ , and possibly δ , which have a variety of gene regulatory functions	Tesaglitazar, GW-50516, GW-590735, LY-929, LY-518674, LY-465608, LY-818
Microsomal Triglyceride Transfer Protein (MTTP) Inhibitors	Inhibit MTTP, which catalyze the transport of triglycerides, cholesteryl ester, and phosphatidylcholine between membranes; required for the synthesis of ApoB.	Implitapide, CP-346086
Squalene Synthase Inhibitors	Interfere with cholesterol synthesis by halting the action of liver enzymes; may also slow or stop the proliferation of several cell types that contribute to atherosclerotic plaque formation	TAK-475, ER-119884
Lipoprotein Lipase Activators	Directly activate lipoprotein lipase, which promotes the breakdown of the fat portion of lipoproteins	Ibrolipim (NO-1886)
Lipoprotein(a) Antagonists	Not yet established	Gembacene
Bile Acid Reabsorption Inhibitors	Inhibit intestinal epithelial uptake of bile acids.	AZD-7806, BARI-1453, S-8921

[0339] In other embodiments, mechanistic classes of drugs used in the treatment of depression are used in conjunction with a method or composition of the invention. Current or emerging antidepressant drugs act by a variety of mechanisms, e.g., selective serotonin reuptake inhibitors (SSRIs), serotonergic/noradrenergic agents, serotonin/noradrenergic/dopaminergic agents, tricyclic antidepressants, monoamine

oxidase inhibitors (MAOIs), noradrenergic/dopaminergic agents, serotonin antagonists, serotonin agonists, substance P antagonists, and beta3 adrenoreceptor agonists. An example of classification of such drugs by mechanism, together with representative members of the mechanistic classes, is given in Table 12.

TABLE 12

Classes of Drugs for Treatment of Depression		
Class	Mechanism of Action	Examples
Selective Serotonin Reuptake Inhibitor (SSRI)	Block presynaptic reuptake of serotonin. Exert little effect on norepinephrine or dopamine reuptake. Level of serotonin in the synaptic cleft is increased.	Escitalopram, Sertraline, Citalopram, Paroxetine, Paroxetine, controlled

TABLE 12-continued

Classes of Drugs for Treatment of Depression		
Class	Mechanism of Action	Examples
Serotonergic/noradrenergic agents	Inhibit both serotonin reuptake and norepinephrine reuptake. Different drugs in this class can inhibit each receptor to different degrees. Do not affect histamine, acetylcholine, and adrenergic receptors.	release, Fluoxetine, Fluoxetine weekly, Fluvoxamine, olanzapine/fluoxetine combination, Venlafaxine; Reboxetine, Milnacipran, Mirtazapine, Nefazodone, Duloxetine
Serotonergic/noradrenergic/dopaminergic agents	Several different mechanisms. Block norepinephrine, serotonin, and/or dopamine reuptake. Some have addictive potential due to dopamine reuptake inhibition.	Bupropion, Maprotiline, Mianserin, Trazodone, Dexmethylphenidate, Methyphenidate, Amineptine
Tricyclic Antidepressants	Block synaptic reuptake of serotonin and norepinephrine. Have little effect on dopamine. Strong blockers of muscarinic, histaminergic H ₁ , and alpha-1-adrenergic receptors.	Amitriptyline, Amoxapine, Clomipramine, Desipramine, Doxepin, Imipramine, Nortriptyline, Protriptyline, Trimipramine
Irreversible Monoamine Oxidase Inhibitors	Monoamine oxidase (MAO) metabolizes monoamines such as serotonin and norepinephrine. MAO inhibitors inhibit MAO, thus increasing levels of serotonin and norepinephrine.	Isocarboxazid, Phenelzine, Tranylcypromine, Transdermal Selegiline
Reversible Monoamine Oxidase Inhibitors	See above. Short acting, reversible inhibitor, inhibits deamination of serotonin, norepinephrine, and dopamine.	Moclobemide
Serotonergic/noradrenergic/dopaminergic reuptake inhibitors	Act to block all of serotonin, norepinephrine, and dopamine reuptake. May have addictive potential due to dopamine reuptake inhibition.	DOV-216303, DOV-21947
Noradrenergic/dopaminergic agents	Block reuptake of norepinephrine and dopamine	GW-353162
Serotonin Antagonists	Selective antagonist of one serotonin receptor (the 5-HT ₁ receptor)	Agomelatine
Serotonin Agonists	Partial agonist of the 5-HT _{1A} receptor.	Eptapirone, Vilazodone, OPC-14523, MKC-242, Gepirone ER
Substance P Antagonists	Modify levels of substance P, which is released during acute stress.	Aprepitant, TAK-637, CP-122721, E6006, R-763OPC-GW-597599
Beta ₃ Adrenoreceptor Agonists	Indirectly inhibit norepinephrine reuptake. Also being investigated for treatment of obesity and diabetes because they stimulate lipolysis and thermogenesis.	SR-58611

[0340] In other embodiments, mechanistic classes of drugs used in the treatment of multiple sclerosis are used in conjunction with a method or composition of the invention. These drugs can be classed as, e.g., recombinant interferons, altered peptide ligands, chemotherapeutic agents, immunosuppressants, corticosteroids, monoclonal antibodies,

chemokine receptor antagonists, AMPA receptor antagonists, recombinant human glial growth factors, T-cell receptor vaccines, and oral immunomodulators. An example of classification of such drugs by mechanism, together with representative members of the mechanistic classes, is given in Table 13.

TABLE 13

Classes of Drugs for Treatment of Multiple Sclerosis		
Class	Mechanism of Action	Examples
Recombinant interferons	IFN-beta has numerous effects on the immune system. Exact mechanism of action in MS not known	Interferon-beta-1b, Interferon-beta-1a
Altered peptide ligands	Ligands either templated on sequence of myelin basic protein, or containing randomly arranged amino acids (e.g., ala, lys, glu, tyr) whose structure resembles myelin basic protein, which is thought to be an antigen that plays a role in MS. Bind to the T-cell receptor but do not activate the T-cell because are not presented by an antigen-presenting cell.	Glatiramer acetate, MBP-8298, Tiplimotide, AG-284

TABLE 13-continued

Classes of Drugs for Treatment of Multiple Sclerosis		
Class	Mechanism of Action	Examples
Chemotherapeutic agents	Immunosuppressive. MS is thought to be an autoimmune disease, so chemotherapeutics that suppress immunity improve MS	Mitoxantrone, Methotrexate, Cyclophosphamide
Immunosuppressants	Act via a variety of mechanisms to dampen immune response.	Azathioprine, Teriflunomide, Oral Cladribine
Corticosteroids	Induce T-cell death and may up-regulate expression of adhesion molecules in endothelial cells lining the walls of cerebral vessels, as well as decreasing CNS inflammation.	Methylprednisolone
Monoclonal Antibodies	Bind to specific targets in the autoimmune cascade that produces MS, e.g., bind to activated T-cells	Natalizumab, Daclizumab, Alemtuzumab, BMS-188667, E-6040, Rituximab, M1 Mabs, ABT 874, T-0047
Chemokine Receptor Antagonists	Prevent chemokines from binding to specific chemokine receptors involved in the attraction of immune cells into the CNS of multiple sclerosis patients, and inhibiting immune cell migration into the CNS	BX-471, MLN-3897, MLN-1202
AMPA Receptor Antagonists	AMPA receptors bind glutamate, an excitatory neurotransmitter, which is released in excessive quantities in MS. AMPA antagonists suppresses the damage caused by the glutamate	E-2007
Recombinant Human Glial Growth Factor (GGF)	GGF is associated with the promotion and survival of oligodendrocytes, which myelinate neurons of the CNS. rhGGF may help myelinate oligodendrocytes and protect the myelin sheath.	Recombinant Human GGF2
T-cell Receptor Vaccine	Mimic the part of the receptor in T cells that attack myelin sheath, which activates regulatory T cells to decrease pathogenic T-cells.	NeuroVax
Oral Immunomodulators	Various effects on the immune response that can modulate the process of MS	Simvastatin, FTY-720, Oral Glatiramer Acetate, FTY-720, Pirfenidone, Laquinimod

[0341] In other embodiments, mechanistic classes of drugs used in the treatment of Parkinson's disease are used in conjunction with a method or composition of the invention. These classes include dopamine precursors, dopamine agonists, COMT inhibitors, MAO-B inhibitors, anticholinergic agents, mixed dopaminergic

agents, adenosine A2a antagonists, alpha-2 adrenergic antagonists, antiapoptotic agents, growth factor stimulators, and cell replacements. An example of classification of such drugs by mechanism, together with representative members of the mechanistic classes, is given in Table 14.

TABLE 14

Classes of Drugs for Treatment of Parkinson's Disease		
Class	Mechanism of Action	Examples
Dopamine Precursors	Act as precursors in the synthesis of dopamine, the neurotransmitter that is depleted in Parkinson's Disease. Usually administered in combination with an inhibitor of the carboxylase enzyme that metabolizes levodopa. Some (e.g., Duodopa) are given by infusion, e.g., intraduodenal infusion	Levodopa, Levodopa-carbidopa, Levodopa-benserazide, Etilevodopa, Duodopa
Dopamine Agonists	Mimic natural dopamine by directly stimulating striatal dopamine receptors. May be subclassed by which of the five known dopamine receptor subtypes the drug activates; generally most effective are those that activate receptors the in the D2 receptor family (specifically D2 and D3 receptors). Some are formulated for more controlled release or transdermal delivery.	Bromocriptine, Cabergoline, Lisuride, Pergolide, Pramipexole, Ropinirole, Talipexole, Apomorphine, Dihydroergocryptine, Lisuride, Piribedil, Talipexole, Rotigotin CDS, Sumanitrol, SLV-308

TABLE 14-continued

Classes of Drugs for Treatment of Parkinson's Disease		
Class	Mechanism of Action	Examples
COMT Inhibitors	Inhibits COMT, the second major enzyme that metabolized levodopa.	Entacapone, Tolcapone, Entacapone-Levodopa-Carbidopa fixed combination,
MAO-B Inhibitors	MAO-B metabolizes dopamine, and inhibitors of MAO-B thus prolong dopamine's half-life	Selegiline, Rasagiline, Safinamide
Antiglutamatergic Agents	Block glutamate release. Reduce levodopa-induced dyskinesia	Amantadine, Budipine, Talampandol, Zonisamide
Anticholinergic Agents	Thought to inhibit excessive cholinergic activity that accompanies dopamine deficiency	Trihexyphenidyl, Benztropine, Biperiden
Mixed Dopaminergic Agents	Act on several neurotransmitter systems, both dopaminergic and nondopaminergic.	NS-2330, Sarizotan
Adenosine A2a antagonists	Adenosine A2 antagonize dopamine receptors and are found in conjunction with dopamine receptors. Antagonists of these receptors may enhance the activity of dopamine receptors.	Istradefylline
Alpha-2 Adrenergic Antagonists	Not known.	Yohimbine, Idazoxan, Fipamezole
Antiapoptotic Agents	Can slow the death of cells associated with the neurodegenerative process of Parkinson's disease.	CEP-1347, TCH-346
Growth Factor Stimulators	Promote the survival and growth of dopaminergic cells.	GPI-1485, Glial-cell-line-derived Neurotrophic Factor, SR-57667, PYM-50028
Cell Replacement Therapy	Replace damaged neurons with health neurons.	Spheramine

[0342] The above classifications are exemplary only. It will be appreciated that a drug class need not be restricted to drugs used in the treatment of a single disease, but that a given mechanistic class may have members useful in the treatment of a number of diseases. For example, MAO-B inhibitors are useful in the treatment of both Parkinson's disease and depression; as another example, statins are useful in the treatment of dyslipidemias but are also being found to have more general use in diseases where inflammation plays a major role, e.g., multiple sclerosis and other diseases.

[0343] Further classifications of drugs by mechanism are known in the art; often these classifications may be further classified by structure. Non-exclusive examples of drug classes useful in the methods and compositions of the invention, and representative members of these classes, include:

[0344] Sedative-Hypnotic Drugs, which include drugs that bind to the GABAA receptor such as the benzodiazepines (including alprazolam, chlordiazepoxide, clorazepate, clonazepam, diazepam, estazolam, flurazepam, halazepam, lorazepam, midazolam, oxazepam, quazepam, temazepam, triazolam), the barbiturates (such as amobarbital, pentobarbital, phenobarbital, secobarbital), and non-benzodiazepines (such as zolpidem and zaleplon), as well as the benzodiazepine antagonists (such as flumazenil). Other sedative-hypnotic drugs appear to work through non-GABA-ergic mechanisms such as through interaction with serotonin and dopaminergic receptors, and include buspirone, isapirone, gepirone, and tandospirone. Older drugs work through mechanisms that are not clearly elucidated, and include chloral hydrate, ethchlorvynol, meprobamate, and paraldehyde.

[0345] In some embodiments, sedative-hypnotic drugs that interact with the GABA receptor, such as benzodiazepines and non-benzodiazepines, are further classified as to which

subunit or subunits of the GABAA receptor that they interact with, e.g., the α (which is further classified into six subtypes, including α -1,2,3, and 5), β (further classified as four different types), γ (three different types), δ , ϵ , π , ρ , etc. Such a classification can allow further refinement of associations between genetic variation and responsiveness to a given sedative-hypnotic that interacts with a particular subclass, and predictions for a new sedative-hypnotic that interacts with the same subclass of receptors.

[0346] Opioid analgesics and antagonists act on the opioid receptor. The majority of currently available opioid analgesics act primarily at the μ opioid receptor. However, interactions also occur with the δ and κ receptors. Similar to the sedative-hypnotics, in some embodiments opioid analgesics are further classed as to subtypes of receptors at which they primarily interact, thus allowing further refinement of the association between drug response and genetic variation, and higher predictability for a new drug, based on which receptor (s) it interacts with. Opioid analgesics include alfentanil, buprenorphine, butorphanol, codeine, dezocine, fentanyl, hydromorphone, levomethadyl acetate, levorphanol, meperidine, methadone, morphine sulfate, nalbuphine, oxycodone, oxymorphone, pentazocine, propoxyphene, remifentanyl, sufentanil, tramadol; analgesic combinations such as codeine/acetaminophen, codeine/aspirin, hydrocodone/acetaminophen, hydrocodone/ibuprofen, oxycodone/acetaminophen, oxycodone/aspirin, propoxyphene/aspirin or acetaminophen. Opioid antagonists include nalmefene, naloxone, naltrexone. Antitussives include codeine, dextromethorphan.

[0347] Nonsteroidal anti-inflammatory drugs act primarily through inhibition of the synthesis of prostaglandins, e.g., through inhibition of COX-1, COX-2, or both. Older

NSAIDs (e.g., salicylates) tend to be non-selective as to the type of COX inhibited, whereas newer drugs are quite selective (e.g., the COX-2 inhibitors). Non-selective COX inhibitors include aspirin, acetylsalicylic acid, choline salicylate, diclofenac, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, magnesium salicylate, meclofenamate, mefenamic acid, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, salsalate, salicylsalicylic acid, sodium salicylate, sodium thiosalicylate, sulindac, tenoxicam, tiaprofen, azapropazone, carprofen, and tolmetin. Selective COX-2 inhibitors include celecoxib, etoricoxib, meloxicam, rofecoxib, and valdecoxib.

[0348] Histamine agonists and antagonists are classified according to receptor subtype. H1 agonists or partial agonists include 2-(m-fluorophenyl)-histamine and antagonists include chlorpheniramine, scopolamine, mepyramine, terfenadine, astemizole, and triprolidine; further antagonists (which may be further classified by their chemical structures) include the ethanolamines carbinoxamine, dimenhydrinate, diphenhydramine, and doxylamine; the ethylaminediamines pyrilamine and tripelemnamine; the piperazine derivatives hydroxyzine, cyclizine, fexofenadine and meclizine; the alkylamines brompheniramine and chlorpheniramine; and miscellaneous antagonists cyproheptadine, loratadine, cetirizine. H2 agonists include dimaprit, impromidine, and amthamine; and antagonists (useful in the treatment of gastric acid secretion) include cimetidine, ranitidine, nizatidine, and famotidine; H3 agonists include R- α -methylhistamine, imetit, and immetip and antagonists include thioperamide, iodophenpropit, and clobenpropit; and H4 agonists include clobenpropit, imetit, and clozapine and antagonists include thioperamide. Available preparations include the H1 blockers azelastine, brompheniramine, buclizine, carbinoxamine, cetirizine, chlorpheniramine, clemastine, cyclizine, cyproheptadine, desloratadine, dimenhydrinate, diphenhydramine, emedastine, fexofenadine, hydroxyzine, ketotifen, levocabastine, loratadine, meclizine, olopatadine, phenindamine, and promethazine.

[0349] Drugs used in asthma include sympathicomimetics (used as "relievers," or bronchodilators) such as albuterol, albuterol/ipratropium, bitolterol, ephedrine, epinephrine, formoterol, isoetharine, isoproterenol, levalbuterol, metaproterenol, pirbuterol, salmeterol, salmeterol/fluticasone, terbutaline; aerosol corticosteroids (used as "controllers," or antiinflammatory agents) such as beclomethasone, budesonide, flunisolide, fluticasone, fluticasone/salmeterol, triamcinolone; leukotriene inhibitors such as montelukast, zafirlukast, zileuton; cormolyn sodium and nedocromil sodium; methylxanthines such as aminophylline, theophylline, dyphylline, oxtriphylline, pentoxifylline; antimuscarinic drugs such as ipratropium; and antibodies such as omalizumab.

[0350] Erectile dysfunction drugs include cGMP enhancers such as sildenafil (Viagra), tadalafil, vardenafil, and alprostadil, and dopamine releasers such as apomorphine.

[0351] Drugs used in the treatment of gastrointestinal disease act by a number of mechanisms. Drugs that counteract acidity (antacids) include aluminum hydroxide gel, calcium carbonate, combination aluminum hydroxide and magnesium hydroxide preparation. Drugs that act as proton pump inhibitors include esomeprazole, lansoprazole, pantoprazole, and rabeprazole. H2 histamine blockers include cimetidine, famotidine, nizatidine, ranitidine. Anticholinergic drugs include atropine, belladonna alkaloids tincture, dicyclomine,

glycopyrrolate, I hyoscyamine, methscopolamine, propantheline, scopolamine, tridihexethyl. Mucosal protective agents include misoprostol, sucralfate. Digestive enzymes include pancrelipase. Drugs for motility disorders and antiemetics include alosetron, cisapride, dolasetron, dronabinol, granisetron, metoclopramide, ondansetron, prochlorperazine, tegaserod. Antiinflammatory drugs used in gastrointestinal disease include balsalazide, budesonide, hydrocortisone, mesalamine, methylprednisone, olsalazine, sulfasalazine, infliximab. Antidiarrheal drugs include bismuth subsalicylate, difenoxin, diphenoxylate, kaolin/pectin, loperamide. Laxative drugs include bisacodyl, cascara sagrada, castor oil, docusate, glycerin liquid, lactulose, magnesium hydroxide [milk of magnesia, Epsom Salt], methylcellulose, mineral oil, polycarbophil, polyethylene glycol electrolyte solution, psyllium, sienna. Drugs that dissolve gallstones include monooctanoin, ursodiol.

[0352] Cholinergic-activating drugs, which act by activating muscarinic and/or nicotinic receptors include esters of choline (e.g., acetylcholine, metacholine, carbamic acid, carbachol, and bethanechol) and alkaloids (e.g., muscarine, pilocarpine, lobeline, and nicotine); cholinesterase-inhibiting drugs which typically act on the active site of cholinesterase include alcohols bearing a quaternary ammonium group (e.g., edrophonium), carbamates and related agents (e.g., neostigmine, physostigmine, pyridostigmine, ambenonium, and demecarium), and organic derivatives of phosphoric acid (e.g., echothiophate, soman, parathion, malathion); cholinergic-blocking drugs typically act as antagonists to nicotinic receptors (further classified as ganglion-blockers, such as hexamethonium, mecamylamine, tetraethylammonium, and trimethaphan; and neuromuscular junction blockers, see skeletal muscle relaxants) or antagonists to muscarinic receptors (e.g. atropine, propantheline, glycopyrrolate, pirenzepine, dicyclomine, tropicamide, ipratropium, bantropine, gallamine, methoctramine, AF-DX 116, telenzepine, trihexyphenidyl, darifenacin, scopolamine, homatropine, cyclopentolate, anisotropine, clidinium, isopropamide, mepenzolate, methscopolamine, oxyphenonium, propantheline, oxybutynin, oxyphenacylimine, propiverine, tolterodine, tridihexethyl), which can be further subclassed as to which muscarinic receptor is the primary site of the effect, e.g., M1, M2, M3, M4, or M5, allowing greater predictability for an association between a genetic variation and a response for a new drug based on its primary site of effect. Available preparations of antimuscarinic drugs include but are not limited to atropine; belladonna alkaloids, extract, or tincture; clidinium; cyclopentolate; dicyclomine; flavoxate; glycopyrrolate; homatropine; 1-hyoscyamine; ipratropium; mepenzolate; methantheline; methscopolamine; oxybutynin; pranteline; scopolamine; tolterodine; tridihexethyl; tropicamide. Available preparations of ganglion blockers include mecamylamine and trimethaphan. Available cholinesterase regenerators include pralidoxime.

[0353] Adrenoceptor-activating drugs and other sympathomimetic drugs may be classified according to the receptor or receptors that they activate, e.g., α -one type (including subtypes A, B, D), α -two type (including subtypes A, B, and C), β type (including subtypes 1, 2, and 3), and dopamine type (including subtypes 1, 2, 3, 4, and 5). Exemplary drugs include epinephrine, norepinephrine, phenylephrine, methoxamine, milodrine, ephedrine, xylometazoline, amphetamine, methamphetamine, phenmetrazine, methylphenidate, phenylpropanolamine, methylnorepinephrine, dobutamine,

clonidine, BHT920, oxymetazoline, isoproterenol, procaterol, terbutaline, metaproterenol, albuterol, ritodrine, dopamine, fenoldopam, bromocriptine, quinpirol, dexmedetomidine, tyramine, cocaine (dopamine reuptake inhibitor), apraclonidine, brimonidine, ritodrine, terbutaline, and modafinil. Available preparations include amphetamine, apraclonidine, brimonidine, dexmedetomidine, dexmethylphenidate, dextroamphetamine, dipivefrin, dobutamine, dopamine, ephedrine, epinephrine, fenoldopam, hydroxyamphetamine, isoproterenol, mephentermine, metaraminol, methamphetamine, methoxamine, methylphenidate, midodrine, modafinil, naphazoline, norepinephrine, oxymetazoline, pemoine, phendimetrazine, phenylephrine, pseudoephedrine, tetrahydrozoline, and xylometazoline.

[0354] Adrenoceptor antagonist drugs may be classified by receptor Type I in the same manner as adrenoceptor agonists, and include tolazoline, dibenamine, prazosin, terazosin, doxazosin, phenoxylbenzamine, phentolamine, rauwoscine, yohimbine, labetalol, carvedilol, metoprolol, acebutolol, alprenolol, atenolol, betaxolol, celiprolol, esmolol, propranolol, carteolol, penbutolol, pindolol, timolol, butoxamine, ergotamine, dihydroergotamine, tamulosin, alfuzosin, indoramin, urapidil, bisoprolol, nadolol, sotalol, oxprenolol, bopindolol, medroxalol, and bucindolol. Available preparations include: alpha blockers doxazosin, phenoxylbenzamine, phentolamine, prazosin, tamsulosin, terazosin, and tolazoline; and beta blockers acebutolol, atenolol, betaxolol, bisoprolol, carteolol, carvedilol, esmolol, labetalol, levobunolol, metipranolol, nadolol, penbutolol, pinolol, propranolol, sotalol, timolol; and synthesis inhibitor metyrosine.

[0355] Antihypertensive agents include drugs that work by a variety of mechanisms and thus overlap with other classifications. Agents can include diuretics such as thiazide diuretics, and potassium sparing diuretics; drugs that act on the central nervous system such as methyl dopa and clonidine; ganglion-blocking drugs, suprenal; adrenergic neuron-blocking agents such as guanethidine, guanadrel, bethanidine, debrisoquin, and reserpine; adrenoceptor antagonists such as propranolol, metoprolol, nadolol, carteolol, atenolol, betaxolol, bisoprolol, pindolol, acebutolol, and penbutolol, labetalol, carvedilol, esmolol, pazosin, phentolamine and phenoxylbenzamine; vasodilators such as hydralazine, minoxidil, sodium nitroprusside, diazoxide, fenoldopam, and calcium channel blockers (e.g., verapamil, diltiazem, amlodipine, felodipine, isradipine, nicardipine, nifedipine, and nisoldipine); ACE-inhibitors such as captopril, enalapril, lisinopril, benazepril, fosinopril, moexipril, perindopril, quinapril, ramipril, andtrandolapril; angiotensin receptor blocking agents such as losartan, valsartan, candesartan, eprosartan, irbesartan, and telmisartan. Preparations available include: beta adrenoceptor blockers acebutolol, atenolol, betaxolol, bisoprolol, carteolol, carvedilol, esmolol, labetalol, metoprolol, nadolol, penbutolol, pindolol, propranolol, timolol; centrally acting sympathoplegic drugs clonidine, guanabenz, guanfacine, methyl dopa; postganglionic sympathetic nerve terminal blockers guanadrel, guanethidine, and reserpine; alpha one selective adrenoceptor blockers doxazosin, prazosin, terazosin; ganglion-blocking agent mecamylamine; vasodilators diazoxide, fenoldopam, hydralazine, minoxidil, nitroprusside; calcium channel blockers amlodipine, diltiazem, felodipine, isradipine, nicardipine, nisoldipine, nifedipine, verapamil; ACE inhibitors benazepril, captopril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, andtrandolapril; and angiotensin receptor

blockers candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan.

[0356] Vasodilators used in angina pectoris include nitric oxide releasing drugs such as nitric and nitrous acid esters of polyalcohols such as nitroglycerin, isorbide dinitrate, amyl nitrite, and isorbide mononitrate; calcium channel blockers such as amlodipine, felodipine, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, bepridil, diltiazem, and verapamil; and beta-adrenoceptor-blocking drugs (see above). Available preparations include: nitrates and nitrites amyl nitrite, isorbide dinitrate, isorbide mononitrate, nitroglycerin; calcium channel blockers amlodipine, bepridil, diltiazem, felodipine, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, and verapamil; and beta blockers acebutolol, atenolol, betaxolol, bisoprolol, carteolol, carvedilol, esmolol, labetalol, levobunolol, metipranolol, nadolol, penbutolol, pinolol, propranolol, sotalol, timolol.

[0357] Drugs used in heart failure include cardiac glycosides such as digoxin; phosphodiesterase inhibitors such as inamrinone and milrinone; beta adrenoceptor stimulant such as those described; diuretics as discussed below; ACE inhibitors such as those discussed above; drugs that inhibit both ACE and neutral endopeptidase such as omaprilat; vasodilators such as synthetic brain natriuretic peptide (nesiritide) and bosentan; beta adrenoceptor blockers such as those described above. Available preparations include: digitalis digoxin; digitalis antibody digoxin immune Fab; sympathomimetics dobutamine and dopamine; ACE inhibitors captopril, enalapril, fosinopril, lisinopril, quinapril, ramipril, andtrandolapril; angiotensin receptor blockers candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan; beta blockers bisoprolol, carvedilol, and metoprolol.

[0358] Cardiac arrhythmia drugs include drugs that act by blocking sodium channels such as quinidine, amiodaron, disopyramide, flecainide, lidocaine, mexiletine, moricizine, procainamide, propafenone, and tocainide; beta-adrenoceptor-blocking drugs such as propranolol, esmolol, and sotalol; drugs that prolong the effective refractory period by prolonging the action potential such as amiodaron, bretylium, sotalol, dofetilide, and ibutilide; calcium channel blockers such as verapamil, diltiazem, and bepridil; and miscellaneous agents such as adenosine, digitalis, magnesium, and potassium. Available preparations include: the sodium channel blockers disopyramide, flecainide, lidocaine, mexiletine, moricizine, procainamide, propafenone, quinidine sulfate, quinidine gluconate, and quinidine polygalacturonate; the beta blockers acebutolol, esmolol, and propranolol; the action potential-prolonging agents amiodaron, bretylium, dofetilide, and sotalol; the calcium channel blockers bepridil, diltiazem, and verapamil; and adenosine and magnesium sulfate.

[0359] Diuretic agents include drugs that act as carbonic anhydrase inhibitors such as acetazolamide, dichlorophenamide, methazolamide; loop diuretics such as furosemide, bumetanide, torsemide, ethacrynic acid, and mercurial diuretics; drugs that inhibit NaCl transport in the distal convoluted tubule and, in some cases, also act as carbonic anhydrase inhibitors, such as bendroflumethiazide, benzthiazide, chlorothiazide, chlorthalidone, hydrochlorothiazide, hydroflumethiazide, indapamide, methyclothiazide, metolazone, polythiazide, quinethazone, and trichlormethiazide; potassium-sparing diuretics such as spironolactone, triamterene,

eplerenone, and amiloride; osmotic diuretics such as mannitol; antidiuretic hormone agonists such as vasopressin and desmopressin; antidiuretic hormone antagonists such as lithium and demeclocycline. Available preparations include acetazolamide, amiloride, bendroflumethiazide, benzthiazide, brinzolamide, bumetanide, chlorothiazide, chlorthalidone, demeclocycline, dichlorphenamide, dorzolamide, eplerenone, ethacrynic acid, furosemide, hydrochlorothiazide, hydroflumethiazide, indapamide, mannitol, methazolamide, methyclothiazide, metolazone, polythiazide, quinethazone, apironolactone, torsemide, triamterene, and trichlormethiazide.

[0360] Serotonin and drugs that affect serotonin include serotonin agonists such as fenfluramine and dexfenfluramine, buspirone, sumatriptan, cisapride, tegaserod; serotonin antagonists p-chlorophenylalanine and p-chloroamphetamine, and reserpine; and the serotonin receptor antagonists phenoxybenzamine, cyproheptadine, ketanserin, ritanserin, and ondansetron; serotonin reuptake inhibitors are described elsewhere herein. Serotonin receptor agonists include almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan, sumatriptan, and zolmitriptan.

[0361] Ergot alkaloids are useful in the treatment of, e.g., migraine headache, and act on a variety of targets, including alpha adrenoceptors, serotonin receptors, and dopamine receptors. They include bromocriptine, cabergoline, pergolide, ergonovine, ergotamine, lysergic acid diethylamide, and methysergide. Available preparations include dihydroergotamine, ergonovine, ergotamine, ergotamine tartrate, and methylergonovine.

[0362] Vasoactive Peptides include aprepitant, bosentan.

[0363] Eicosanoids include prostaglandins, thromboxanes, and leukotrienes. Eicosanoid modulator drugs include alprostadil, bimatoprost, carboprost tromethamine, dinoprostone, epoprostenol, latanoprost, misoprostol, montelukast, travaprost, treprostinil, unoprostone, zafirlucast, zileuton. Further eicosanoid modulators are discussed elsewhere herein as nonsteroidal antiinflammatory drugs (NSAIDs)

[0364] Drugs for the treatment of acute alcohol withdrawal include diazepam, lorazepam, oxazepam, thiamine; drugs for prevention of alcohol abuse include disulfiram, naltrexone; and drugs for the treatment of acute methanol or ethylene glycol poisoning include ethanol, fomepizole.

[0365] Antiseizure drugs include carbamazepine, clonazepam, clorazepate dipotassium, diazepam, ethosuximide, ethotoin, felbamate, fosphenytoin, gabapentin, lamotrigine, levetiracetam, lorazepam, mephényloin, mephobarbital, oxycarbazepine, pentobarbital sodium, phenobarbital, phenytoin, primidone, tiagabine, topiramate, trimethadione, valproic acid.

[0366] General anesthetics include desflurane, dexmedetomidine, diazepam, droperidol, enflurane, etomidate, halothane, isoflurane, ketamine, lorazepam, methohexital, methoxyflurane, midazolam, nitrous oxide, propofol, sevoflurane, thiopental.

[0367] Local anesthetics include articaine, benzocaine, bupivacaine, butamben picrate, chloroprocaine, cocaine, dibucaine, dyclonine, levobupivacaine, lidocaine, lidocaine and etidocaine eutectic mixture, mepivacaine, pramoxine, prilocalne, procaine, proparacaine, ropivacaine, tetracaine.

[0368] Skeletal muscle relaxants include neuromuscular blocking drugs such as atracurium, cisatracurium, doxacurium, metocurine, mivacurium, pancuronium, pipecuronium, rocuronium, succinylcholine, tubocurarine, vecuronium;

muscle relaxants (spasmolytics) such as baclofen, botulinum toxin type A, botulinum toxin type B, carisoprodol, chlorphenesin, chlorzoxazone, cyclobenzaprine, dantrolene, diazepam, gabapentin, metaxalone, methocarbamol, orphenadrine, riluzole, and tizanidine.

[0369] Antipsychotic agents include aripiprazole, chlorpromazine, clozapine, fluphenazine, fluphenazine esters, haloperidol, haloperidol ester, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, prochlorperazine, promazine, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, triflupromazine, ziprasidone; mood stabilizers include carbamazepine, divalproex, lithium carbonate, and valproic acid.

[0370] Agents used in anemias include hematopoietic growth factors such as darbopoetin alfa, deferoxamine, epoetin alfa (erythropoietin, epo), filgrastim (G-CSF), folic acid, iron, oprelvekin (interleukin 11), pegfilgrastim, sargramostim (GM-CSF), and vitamin B12.

[0371] Disease-modifying antirheumatic drugs include anakinra, adalimumab, auranofin, aurothioglucose, etanercept, gold sodium thiomalate, hydroxychloroquine, infliximab, leflunomide, methotrexate, penicillamine, sulfasalazine. Drugs used in gout include allopurinol, colchicine, probenecid, sulfinpyrazone.

[0372] Drugs used in disorders of coagulation include abciximab, alteplase recombinant, aminocaproic acid, anisindione, antihemophilic factor [factor VIII, AHF], anti-inhibitor coagulant complex, antithrombin III, aprotinin, argatroban, bivalirudin, cilostazol, clopidogrel, coagulation factor VIIa recombinant, dalteparin, danaparoid, dipyridamole, enoxaparin, eptifibatide, Factor VIIa, Factor VIII, Factor IX, fondaparinux, heparin sodium, lepirudin, phytonadione [K1], protamine, reteplase, streptokinase, tenecteplase, ticlopidine, tinzaparin, tirofiban, tranexamic acid, urokinase, warfarin.

[0373] Hypothalamic and pituitary hormones include bromocriptine, cabergoline, cetrorelix, chorionic gonadotropin [hCG], corticorelin ovine, corticotropin, cosyntropin, desmopressin, follitropin alfa, follitropin beta [FSH], ganirelix, gonadorelin acetate [GnRH], gonadorelin hydrochloride [GnRH], goserelin acetate, histrelin, leuprolide, menotropins [hMG], nafarelin, octreotide, oxytocin, pergolide, protirelin, sermorelin, somatrem, somatotropin, thyrotropin alpha, triptorelin, urofollitropin, vasopressin.

[0374] Thyroid and antithyroid drugs include the thyroid agents: levothyroxine [T4], liothyronine [T3], liotrix [a 4:1 ratio of T4:T3], thyroid desiccated [USP]; and the antithyroid agents: diatrizoate sodium, iodide, iopanoic acid, ipodate sodium, methimazole, potassium iodide, propylthiouracil [PTU], thyrotropin; recombinant human TSH.

[0375] Adrenocorticosteroids and adrenocortical antagonists include the glucocorticoids for oral and parenteral use: betamethasone, betamethasone sodium phosphate, cortisone, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, hydrocortisone [cortisol], hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide. Another class of adrenocorticoids are the mineralocorticoids, e.g., fludroc-

tisone acetate. The adrenal steroid antagonists include aminoglutethimide, ketoconazole, mitotane.

[0376] Gonadal hormones and inhibitors include the estrogens: conjugated estrogens, dienestrol, diethylstilbestrol diphosphate, esterified estrogens, estradiol cypionate in oil, estradiol, estradiol transdermal, estradiol valerate in oil, estrone aqueous suspension, estropipate, ethinyl estradiol; the progestins: hydroxyprogesterone caproate, levonorgestrel, medroxyprogesterone acetate, megestrol acetate, norethindrone acetate, norgestrel, progesterone; the androgens and the anabolic steroids: methyltestosterone, nandrolone decanoate, oxandrolone, oxymetholone, stanozolol, testosterone, testosterone aqueous, testosterone cypionate in oil, testosterone enanthate in oil, testosterone propionate in oil, testosterone transdermal system, testosterone pellets. Drugs may further be classed as antagonists and inhibitors of gonadal hormones: anastrozole, bicalutamide, clomiphene, danazol, dutasteride, exemestane, finasteride, flutamide, fulvestrant, letrozole, mifepristone, nilutamide, raloxifene, tamoxifen, and toremifene.

[0377] Agents that affect bone mineral homeostasis include Vitamin E, its metabolites and analogs: calcifediol, calcitriol, cholecalciferol [D3], dihydrotachysterol [DHT], doxercalciferol, ergocalciferol [D2], and paricalcitol; calcium: calcium acetate [25% calcium], calcium carbonate [40% calcium], calcium chloride [27% calcium], calcium citrate [21% calcium], calcium gluconate [6.5% calcium]; calcium gluceptate [8% calcium], calcium gluconate [9% calcium], calcium lactate [13% calcium], and tricalcium phosphate [39% calcium]; phosphate and phosphate binders such as phosphate and sevelamer; and other drugs such as alendronate, calcitonin-salmon, etidronate, gallium nitrate, pamidronate, plicamycin, risedronate, sodium fluoride, teriparatide, tiludronate, zoledronic acid.

[0378] Beta-lactam antibiotics and other inhibitors of cell wall synthesis include the penicillins, such as amoxicillin, amoxicillin/potassium clavulanate, ampicillin, ampicillin/sulbactam sodium, carbenicillin, dicloxacillin, mezlocillin, nafcillin, oxacillin, penicillin G benzathine, penicillin G procaine, penicillin V, piperacillin, piperacillin and tazobactam sodium, ticarcillin, and ticarcillin/clavulanate potassium; the cephalosporins and other beta-lactam drugs, such as the narrow spectrum (first generation) cephalosporins, e.g., cefadroxil, cefazolin, cephalixin, cephalothin, cephradine, and cephradine; the second generation (intermediate spectrum) cephalosporins, e.g., cefaclor, cefamandole, cefmetazole, cefonicid, cefotetan, ceftiofur, cefprozil, cefuroxime, and loracarbef; the broad spectrum (third- and fourth-generation) cephalosporins, e.g., cefdinir, cefditoren, cefepime, cefixime, cefoperazone, cefotaxime, cefpodoxime proxetil, ceftazidime, ceftibuten, ceftizoxime, and ceftriaxone. Further classes include the carbapenem and monobactam, e.g., aztreonam, ertapenem, imipenem/cilastatin, and meropenem; and other drugs such as cycloserine (seromycin pulvules), fosfomycin, vancomycin.

[0379] Other antibiotics include chloramphenicol, the tetracyclines, e.g., demeclocycline, doxycycline, methacycline, minocycline, oxtetracycline, and tetracycline; the macrolides, e.g., azithromycin, clarithromycin, erythromycin; the ketolides, e.g., telithromycin; the lincosamides, e.g., clindamycin; the streptogramins, e.g., quinupristin and dalbapristin; and the oxazolidinones, e.g., linezolid.

[0380] Aminoglycosides and spectinomycin antibiotics include amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, spectinomycin, streptomycin, and tobramycin.

[0381] Sulfonamides, trimethoprim, and quinolone antibiotics include the general-purpose sulfonamides, e.g., sulfadiazine, sulfamethizole, sulfamethoxazole, sulfanilamide, and sulfisoxazole; the sulfonamides for special applications, e.g., mafenide, silver sulfadiazine, sulfacetamide sodium. Trimethoprim include trimethoprim, trimethoprim-sulfamethoxazole [co-trimoxazole, TMP-SMZ]; the quinolones and fluoroquinolones include cinoxacin, ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, nalidixic acid, norfloxacin, ofloxacin, sparfloxacin, and trovafloxacin.

[0382] Antimycobacterial drugs include drugs used in tuberculosis, e.g., aminosalicylate sodium, capreomycin, cycloserine, ethambutol, ethionamide, isoniazid, pyrazinamide, rifabutin, rifampin, rifapentine, and streptomycin; and drugs used in leprosy, e.g., clofazimine, dapsone.

[0383] Antifungal agents include amphotericin B, butaconazole, butenafine, caspofungin, clotrimazole, econazole, fluconazole, flucytosine, griseofulvin, itraconazole, ketoconazole, miconazole, naftifine, natamycin, nystatin, oxiconazole, sulconazole, terbinafine, terconazole, tioconazole, tolnaftate, and voriconazole.

[0384] Antiviral agents include abacavir, acyclovir, adfovir, amantadine, amprenavir, cidofovir, delavirdine, didanosine, efavirenz, enfuvirtide, famciclovir, fomivirsen, foscarnet, ganciclovir, idoxuridine, imiquimod, indinavir, interferon alpha-2a, interferon alpha-2b, interferon-2b, interferon alpha-n3, interferon alfacon-1, lamivudine, lopinavir, ritonavir, nelfinavir, nevirapine, oseltamivir, palivizumab, peginterferon alpha-2a, peginterferon alpha-2b, penciclovir, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, tenofovir, trifluridine, valacyclovir, valgancyclovir, zalcitabine, zanamivir, and zidovudine.

[0385] Further antimicrobial agents, disinfectants, antiseptics, and sterilants include the miscellaneous antimicrobial agents, e.g., methenamine hippurate, methenamine mandelate, metronidazole, mupirocin, nitrofurantoin, polymyxin B; and the disinfectants, antiseptics, and sterilants, e.g., benzalkonium, benzoyl peroxide, chlorhexidine gluconate, glutaraldehyde, hexachlorophene, iodine aqueous, iodine tincture, nitrofurazone, oxychlorosene sodium, providone-iodine, silver nitrate, and thimerosal.

[0386] Antiprotozoal drugs include albendazole, atovaquone, atovaquone-proguanil, chloroquine, clindamycin, doxycycline, dehydroemetine, eflornithine, halofantrine, iodoquinol, mefloquine, melarsoprol, metronidazole, nifurtimox, nitazoxanide, paromomycin, pentamidine, primaquine, pyrimethamine, quinidine gluconate, quinine, sodium stibogluconate, sulfadoxine and pyrimethamine, and suramin.

[0387] Anthelmintic drugs include albendazole, bithionol, diethylcarbamazine, ivermectin, levamisole, mebendazole, metrifonate, niclosamide, oxamniquine, oxtel pamoate, piperazine, praziquantel, pyrantel pamoate, suramin, thiabendazole.

[0388] Immunopharmacological agents include abciximab, adalimumab, alefacept, alemtuzumab, anti-thymocyte globulin, azathioprine, basiliximab, BCG, cyclophosphamide, cyclosporine, daclizumab, etanercept, gemtuzumab, glatiramer, ibritumomab tiuxetan, immune globulin intrave-

nous, infliximab, interferon alfa-2a, interferon alfa 2b, interferon beta-1a, interferon beta-1b, interferon gamma-1b, interleukin-2, IL-2, aldesleukin, leflunomide, levamisole, lymphocyte immune globulin, methylprednisolone sodium succinate, muromonab-CD3 [OKT3], mycophenolate mofetil, pegademase bovine, peginterferon alfa-2a, peginterferon alfa-2b, prednisone, RHo(D) immune globulin microdose, rituximab, sirolimus, tacrolimus [FK506], thalidomide, and trastuzumab.

[0389] Heavy metal chelators include deferoxamine, dimercaprol, edetate calcium [calcium EDTA], penicillamine, succimer, and unithiol.

[0390] Structural Classes of Drugs

[0391] In another example of drug classification embodiments, a drug may be classified according to its structural class or family; certain drugs may fall into more than one structural class or family. Thus, in some embodiments, drugs are classified according to structure. Drugs that have a common action may have different structures, and often one of the best predictors of a drug's likely action is its structure. By way of example only, certain classes of drugs may be further organized by chemical structure classes presented herein. One non-limiting example is antibiotics. Table 15, below, presents non-limiting examples of antibiotics further classified by illustrative chemical structure classes.

TABLE 15

Structural Classes of Antibiotic Drugs	
Structure Class	Examples of Antibiotics within Structure Class
Amino Acid Derivatives	Azaserine, Bestatin, Cycloserine, 6-diazo-5-oxo-L-norleucine
Aminoglycosides	Armastatin, Amikacin, Gentamicin, Hygromycin, Kanamycin, Streptomycin
Benzochinonoids	Herbimycin
Carbapenems	Imipenem, Meropenem
Coumarin-glycosides	Novobiocin
Fatty Acid Derivatives	Cenulenin
Glucosamines	1-deoxynojirimycin
Glycopeptides	Bleomycin, Vancomycin
Imidazoles	Metroidazole
Penicillins	Benzylpenicillin, Benzathine penicillin, Amoxycillin, Piperacillin
Macrolides	Amphotericin B, Azithromycin, Erythromycin
Nucleosides	Cordycepin, Formycin A, Tubercidin
Peptides	Cyclosporin A, Echinomycin, Gramicidin
Peptidyl Nucleosides	Blasticidine, Nikkomycin
Phenicoles	Chloramphenicol, Thiamphenicol
Polyethers	Lasalocid A, Salinomycin
Quinolones	8-quinolinol, Cinoxacin, Ofloxacin
Steroids	Fusidic Acid
Sulphonamides	Sulfamethazine, Sulfadiazine, Trimethoprim
Tetracyclins	Oxytetracyclin, Minocycline, Doxycycline

[0392] In some embodiments, drugs are classed as optical isomers, where a class is two or more optical isomers, or racemate, of a compound of the same chemical formula. Thus, the invention includes methods and compositions for screening individuals for a genetic variation and/or phenotypic variation that predicts responsiveness to a first drug, and using this association to determine whether or not to modulate the treatment of an individual with a second drug, where the first and second drugs are optical isomers. In some embodiments, the first drug is a racemate and the second drug is a stereoisomer that is a component of the racemate. In some embodiments the first drug is a stereoisomer and the second drug is a racemate that includes the stereoisomer. In some

embodiments the first drug is a first stereoisomer and the second drug is a second stereoisomer of a compound.

[0393] In some embodiments, drugs are classed as different crystal structures of the same formula. Thus, the invention includes methods and compositions for screening individuals for a genetic variation and/or phenotypic variation that predicts responsiveness to a first drug, and using this association to determine whether or not to modulate the treatment of an individual with a second drug, where the first and second drugs are members of a class of drugs of the same chemical formula but different crystal structures.

[0394] In some embodiments, drugs are classed by structural components common to the members of the class. Thus, the invention includes methods and compositions for screening individuals for a genetic variation and/or phenotypic variation that predicts responsiveness to a first drug, and using this association to determine whether or not to modulate the treatment of an individual with a second drug, where the first and second drugs are members of a class of drugs that contain the same structural component. By way of example only, a drug may be structurally classified as an acyclic ureide; acylureide; aldehyde; amino acid analog; aminoalkyl ether (clemastine, doxylamine); aminoglycoside; anthracycline; azalide; azole; barbituate; benzodiazepine; carbamate (e.g., felbamate, meprobamate, emylcamate, phenprobamate); carbapenam; carbohydrate; carboxamide (e.g., carbamazepine, oxcarbazepine); carotenoid (e.g., lutein, zeaxanthin); cephalosporin; cryptophycin; cyclodextrin; diphenylpropylamine; expanded porphyrin (e.g., rubyrins, sapphyrins); fatty acid; glycopeptide; higher alcohol; hydantoins (e.g., phenylol); hydroxylated anthroquinone; lincosamide; lipid; lipid related compound; macrolide; mustard; nitrofurans; nitroimidazole; non-natural nucleotide; non-natural nucleoside; oligonucleotide; organometallic compound; oxazolidinone; penicillin; phenothiazine derivative (alimemazine, promethazine); phenylpiperidine; phthalocyanine; piperazine derivative (e.g., cetrizine, meclozine); platinum complex (e.g., cis-platin); polyene; polyketide; polypeptide; porphyrin; prostaglandin (e.g., misoprostol, enprostil); purine; pyrazolone; pyrimidine; pyrrolidine (levetiracetam); quinolone; quinone; retinoid (e.g., isotretinoin, tretinoin); salicylate; sphingolipid; steroid (e.g., prednisone, triamcinolone, hydrocortisone); substituted alkylamine (e.g., talastine, chlorphenamine); substituted ethylene diamine (mepyramine, thonzylamine); succinimide (ethosuximide, phenisuximide, mesuximide); sulfa; sulfonamide (sulfathiazole, mafenide); sulfone; taxane; tetracycline (e.g., chlortetracycline, oxytetracycline); texaphyrin (e.g., Xcytrin, Antrin); thiazide; thiazolidinedione; tocopherol, tocotrienol, triazine (e.g., lamotrigine); urea; xanthine (theobromine, aminophylline); and zwitterion.

EXAMPLES

Example 1

Preparation of DNA Building Block (E.G. X-DNA, Y-DNA, T-DNA)

[0395] A DNA building block (X-DNA, Y-DNA, or T-DNA) is chosen depending on the purpose of the experiment as described herein. Sequences for appropriate DNA building blocks are shown in Table 16.

TABLE 16

Oligonucleotide sequences of the DNA building blocks for photocrosslinked DNA hydrogels and DNA nanospheres				
Strand	SEQ ID	Segment 1	Segment 2	
X01	123	5'-5AmMC6/	CGA CCG ATG AAT AGC GGT	CAG ATC CGT ACC TAC TCG-3'
X02	124	5'-5AmMC6/	CGA GTA GGT ACG GAT CTG	CGT ATT GCG AAC GAC TCG-3'
X03	125	5'- /5AmMC6/	CGA GTC GTT CGC AAT ACG	GCT GTA CGT ATG GTC TCG-3'
X04	126	5'- /5AmMC6/	CGA GAC CAT ACG TAC AGC	ACC GCT ATT CAT CGG TCG-3'
Y0a	127	5'- /5AmMC6/	CGA CCG ATG AAT AGC GGT	CAG ATC CGT ACC TAC TCG-3'
Y0b	128	5'- /5AmMC6/	CGA GTC GTT CGC AAT ACG	ACC GCT ATT CAT CGG TCG-3'
Y0c	129	5'- /5AmMC6/	CGA GTA GGT ACG GAT CTG	CGT ATT GCG AAC GAC TCG-3'
T0a	130	5'- /5AmMC6/	CGA CAG CTG ACT AGA GTC	ACG ACC TGT ACC TAC TCG-3'
T0b	131	5'- /5AmMC6/	CGA GTC GTT CTC AAG ACG	TAG CTA GGA CTC TAG TCA
			GCT GTC G-3'	
T0c	132	5'- /5AmMC6/	CGA GTA GGT ACA GGT CGT	CGT CTT GAG AAC GAC TCG-3'

[0396] Three oligonucleotides are designed such that two have partial complementary sequences, thus forming one arm of a Y-DNA, and the remaining third oligonucleotide, have complementary sequences to the other. These three oligonucleotides should eventually hybridize, forming a Y-DNA (SEQ ID NOs 127-129). Other building blocks such as X-DNA (SEQ ID NOs 123-126) and T-DNA (SEQ ID NOs 130-132) have the same procedure and principle. These DNA building blocks should contain a primary amine modified group on its 5'-end group which can be used to attach a variety of photoreactive modifiers to an oligonucleotide.

[0397] The polyacrylamide gel electrophoresis-purified 5'-end phosphorylated and amine modified oligonucleotides are synthesized or purchased according to the designed sequences, then dissolved in an annealing buffer at a final concentration of 0.2 mM for DNA building block preparation.

[0398] X-DNA Gel

[0399] X-DNA branched nucleic acids are designed comprising sequences depicted in Table 16. The X01 to X04 were selected as four corresponding single oligonucleotides that formed an X-DNA. Without further purification, oligonucleotides (Integrated DNA technologies) were dissolved in an annealing buffer (10 mM Tris, pH=8.0, 1 mM ethylenediaminetetraacetic acid (EDTA), and 50 mM NaCl) with a final concentration of 50 mM. X-DNA was constructed by mixing four oligonucleotide components (with the same molar ratio) in sterile Milli-Q water with a final concentration of 40 mM for each oligonucleotide. Hybridizations were performed according to the following procedures: (i) Denaturation at 95°

C. for 2 min. (ii) Cooling at 65° C. and incubation for 2 min (iii) Annealing at 60° C. for 5 min. and (iv) Further annealing at 60° C. for 0.5 min with a continuous temperature decrease at a rate of 1° C. per min. The annealing steps were repeated a total of 40 times. The final annealed products were stored at 4° C.

[0400] Functionalization of Photoactive Parts onto the DNA Building Block

[0401] To synthesize photoreactive groups to each of the four arms of the X-DNA building block, a photoreactive group was conjugated to the DNA building block in a 10:1 molar ratio by mixing in a sterile 0.5 mL microcentrifuge tube. The reaction mixture was incubated overnight at room temperature. The synthesized DNA conjugates were obtained by removing the non-reacted DNA building blocks and functional groups using HPLC. The purified photocrosslinkable DNA conjugates were examined by gel electrophoretic migration shift assay (GEMSA). The DNAs are larger if crosslinked so move slower through the gel.

[0402] Conjugation of Polyethylene Glycol Monoacrylate (PEGA) onto Y-DNA

[0403] PEGA (0.5 mM, 3,400 Da) was added to water containing 5' amine-modified ss-DNA, Y-DNA, or X-DNA (0.2 mM). The reaction was carried out overnight at room temperature. Non-reacted amine-modified Y-DNA and PEGA were removed by HPLC equipped with a photodiode array detector (Waters).

[0404] Formation of DNA Nanospheres by Photo-Polymerization

[0405] The purified photocrosslinkable DNA conjugates were photo-polymerized with 265 nm UV light (8 mW cm⁻²) in an aqueous solution of 5 wt % photoinitiator Irgacure (Ciba Geigy) using a UV crosslinker (Spectronics Corporation, XL-1000) for 10 min.

[0406] Characterization of swollen DNA hydrogels. Atomic Force Scanning Microscopy (AFM) (Nanoscope III, Digital Instruments) was carried out in air using the tapping mode with rectangular cantilevers with tetrahedral tips (Olympus). Mica was chosen as a solid substrate and used immediately after cleavage in a clean atmosphere. For the sample preparation, surface modification was accomplished by a deposit of silane in distilled and deionized water (DDI water). Briefly, the fresh mica was placed in a container filled with 10 mL 3-aminopropyltriethoxysilane (APTES) solution (2% w/w) for 15 min, and then the APTES-derivatized mica was thoroughly washed with DDI water several times and dried with a gentle stream of nitrogen gas. A piece of DNA gel was loaded on the mica for 7 min, and then washed with DDI water several times and dried. For SEM imaging, strips cut from the dried DNA gel were placed into the top of the SEM holder with carbon tape and metal-coated with Au/Pd to obtain high resolution images.

[0407] Mechanical properties. The mechanical property measurements were performed on a Dynamic Mechanical Analyzer (DMA 2980, TA Instruments, Inc). The hydrogel was clamped between a parallel-plate compression clamp with a diameter of 1.0 cm. This test was conducted on a cylindrical shaped DNA gel with 7.0 mm in diameter and 3.0 mm in height. For tensile testing, DNA hydrogels were approximately 3.0 mm thick cast in a cylindrical mold (approximately 5 mm×5 mm)

[0408] FIG. 20 illustrates a comparison of the stress versus strain properties for a photo-crosslinked DNA-PEG hydrogel versus a PEG hydrogel alone. As seen in the Figure, the

DNA-PEG hydrogel was stronger, experiencing less strain at a given level of stress than the PEG hydrogel.

[0409] In vitro degradation assays. A dry gel was weighed first and then transferred to a microcentrifuge tube filled with PBS, pH 7.4. The tube was slowly shaken at 37° C. Supernatants of a gel sample were removed, immediately followed by a measuring of the weight of the sample. Fresh PBS buffer solution was added to the sample and then returned to the 37° C. shaker.

[0410] Biocompatibility

[0411] A 96-well microtiter plate was seeded with Chinese Hamster Ovarian (CHO) cells and 200 μ L, of growth medium in each well. The cells were incubated at 37° C. with 5% CO₂. The DNA gel was placed into the 96-well plate containing CHO cells one day after the cells were plated at 0.01, 0.05, 0.25, 1 and 5 nM. Cell viability was evaluated 36 hours later by using CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay kits (Promega). No significant differences were observed at the various concentrations.

[0412] Fabrication of Micropatterned DNA Gel

[0413] DNA gels in a micro-meter scale fixed shape are made by casting the shape into a PDMS mold fabricated by photolithography. The dimensions of the shape are on the order of μ m, e.g., the size of each shape can be about 500×400 μ m² with a height 20 μ m. DNA pre-gel solution (2 μ L) is dropped on an APTES modified glass slide, and the PDMS mold is put on the solution. After curing for 2 hours, the PDMS mold is peeled off. To visualize the DNA gel micropatterns, the molded gel is stained with the DNA specific dye SYBR I, and the fluorescence image is captured by a fluorescence microscope. FIG. 21A illustrates fabricated shapes of DNA gels.

[0414] To show that the swollen, millimeter-scale DNA hydrogel indeed contains DNA molecules, a DNA specific dye (SYBR I, Green: Ex/Em, 494 nm/520 nm) is used to stain the gel. After staining, intense green fluorescence indicates that the hydrogel is composed of DNA molecules. Similar results are obtained with a different fluorescent, DNA-specific dye (EtBr, Red: Ex/Em, 518 nm/605 nm).

[0415] These DNA gels can be formed into pre-selected and different shapes at a macroscopic scale. For example, DNA hydrogels with rectangular, round, triangular, cross, and star shapes can be made in the millimeter size (i.e., macroscopic). FIG. 21B illustrates micropatterning of DNA gels. In addition, DNA hydrogels can also be molded into complicated shapes at microscopic scale. A micrometer-sized DNA hydrogel in a given shape is fabricated using traditional photolithography. These DNA hydrogels repeatedly return to their original shapes even after successive drying and hydrating without collapsing to films or powders.

[0416] Different swelling profiles of DNA hydrogels can be achieved by adjusting the initial concentration and the types of DNA monomers (e.g., as shown in Table 16): The higher the initial concentration of the DNA monomers, the higher the degree of swelling of each hydrogel. For example, a Y-DNA based hydrogel (Y-DNA gel) might swell more than 400% at the highest initial concentration of DNA monomers (0.2 mM); while at the lowest initial concentration (0.03 mM), it might swell only about 100%. Besides the initial concentrations, the different types of DNA monomers also influence the degree of swelling. Generally, X-DNA based hydrogel (X-DNA gel) shows a higher swelling degree than both the Y-DNA gel and T-DNA gel.

[0417] The morphology and structure of the DNA hydrogel is further studied using a variety of visualization methods including AFM, FE-SEM, and confocal microscopy. FIG. 21C illustrates a confocal image of DNA-PEG hydrogel coated to beads. The surface morphology and the inner structure of each DNA hydrogel in dried and swollen states differ depending on the types of DNA monomer used. These techniques are used to reveal surface morphology (e.g., a woven pattern for X-DNA gel, a fibrous form for Y-DNA gel, and a scale shape for T-DNA gel). In the swollen state, inner structures of DNA hydrogels are optically sectioned and exposed using confocal microscopy and SYBR. Again, the inner structure of a DNA-hydrogel differs drastically with different types of DNA monomers. The more detailed inner structures of the DNA hydrogels are further evaluated using AFM at molecular resolution.

[0418] The imaging results demonstrate that the original shapes of DNA monomers have significant effect on both surface morphologies and inner structures of the final DNA hydrogels. More importantly, by selecting different shapes of DNA monomers and by adjusting the lengths of branched arms, one can design different DNA hydrogels with desired structures and properties.

[0419] DNA hydrogels fabricated from different DNA monomers have specific chemical and physical properties. The mechanical properties of DNA hydrogels are tested using a Dynamic Mechanical Analyzer (DMA 2980, TA Instruments), e.g., to measure tensile strength, as described above.

[0420] In addition, gel degradability can also be adjusted by selection of different types and/or different concentrations of DNA monomers. Degradation processes of empty DNA hydrogels are evaluated by measuring their daily DNA mass loss in the presence of various media. For example, gels are measured in phosphate buffered saline (PBS, pH 7.4) at room temperature for 10 days. The medium can also comprise 10% serum-supplemented media. The degradation processes are determined by the internal structures of DNA gels as well as the environment (e.g., in the presence of serum which is abundant in nucleases). For example, X-DNA hydrogel can be more stable than Y- and T-gels. Without being bound by theory, the X-DNA gel may have prevented DNA molecules from being easily accessed by nucleases. To validate this notion, a degradation test is performed at a low temperature (4° C.) when most enzymes were inactive. As expected, all DNA hydrogels showed little degradation even after a month. This result also points to another advantage of DNA hydrogels: they are stable and can be stored for a long period of time under a refrigerated condition (4° C.).

[0421] In addition, for in vivo animal tests (B57L mice), 250 μ g of blank DNA gels are injected into mice and compared with controls injected with PBS.

Example 2

Encapsulation and Delivery

[0422] Encapsulated DNA Nanospheres

[0423] Compounds to be delivered can be loaded after preparing DNA nanospheres for drug delivery applications. A positively charged agent, a DNA binding agent, a detectable label were encapsulated with this technique. In one set of experiments, preparations of doxorubicin loaded DNA hydrogels and DNA nanospheres were prepared using pho-

topolymerization. Photocrosslinked DNA hydrogels and DNA nanospheres were prepared according to the standards outlined in Example 1.

[0424] To perform doxorubicin loading, a macromer solution with photocrosslinked DNA hydrogels and DNA nanospheres was placed into a doxorubicin dissolved solution in a 1:5 molar ratio by mixing in water in a sterile 0.5 mL microcentrifuge tube.

[0425] The reaction mixture is incubated overnight at room temperature. The doxorubicin loaded DNA hydrogels and DNA nanospheres are obtained by removing the non-reacted doxorubicin after centrifuge.

[0426] Encapsulation During Photo-Polymerization

[0427] A variety of compounds, e.g., a pharmaceutical compound, a therapeutic compound, a nucleic acid molecule (a gene sequence, oligodeoxynucleotide, siRNA, miRNA, snRNA, mRNA, etc.), a peptide, a protein, a lipid, an antigen, an antibody, a cell growth factor, a selectable marker, a receptor molecule, a ligand can also be encapsulated during photo-reaction. In one set of experiments, a photo-crosslinkable gene was encapsulated.

[0428] Functionalization of Photoresponsive Parts onto the Gene

[0429] To conjugate photoreactive moieties to each of one or more parts of a gene, a photoreactive group is conjugated to the designed primer DNA sequence in a 5:1 molar ratio in water in a sterile 0.5 mL microcentrifuge tube. The reaction was carried out overnight at room temperature. Non-reacted DNA building blocks and photo-reactive groups are then removed by HPLC. The purified photo-crosslinkable genes are confirmed by gel electrophoretic migration shift assay (GEMSA).

[0430] Conjugation of Polyethylene Glycol Monoacrylate (PEGA) onto Gene

[0431] PEGA (1.0 mM, 3,400 Da) was added to water containing 5' and 3' amine-modified gene (0.2 mM). The reaction was carried out overnight at room temperature. Non-reacted amine-modified gene and PEGA were removed by HPLC equipped with a photodiode array detector (Waters).

[0432] Preparation of Gene Encapsulated DNA Nanospheres by Photo-Polymerization

[0433] Photo-crosslinkable DNA building blocks and genes are prepared according to the standards outlined in Example 1. The purified photocrosslinkable DNA building blocks and genes were photo-polymerized with UV light (8 mW cm⁻²) in an aqueous solution of 5 wt % photoinitiator Irgacure (Ciba Geigy) using a UV crosslinker (Spectronics Corporation, XL-1000) for 10 min.

[0434] The release of the above compounds is dependant on the types of DNA monomers. It will be apparent to one of skill in the art that a particular matrix of the invention can be designed to deliver a particular drug target to determine release rates, notwithstanding nucleic acid affinity characteristics for a candidate drug. For example, larger pore sizes may obviate or diminish any drug-nucleic acid interference characteristics that are present. Therefore, by designing a matrix comprising different nucleic acid molecules, as well as nucleic acid molecules of different shape, sequence or length, a designer gel can be produced for any target drug, including any bioactive agent, cells, viruses, small molecules, peptides, polypeptides and antibodies, for example. Indeed, DNA hydrogels are soft materials whose mechanical properties can be precisely controlled.

[0435] Since the gelation conditions were very mild and since no organic solvent or high temperature is used, the DNA gels can be used for encapsulating live mammalian cells. Chinese Hamster Ovarian (CHO) cells were encapsulated that were pre-stained with CellTracker™ Red CMTPX Probes (Molecular Probes, Carlsbad, Calif.) into 0.2 mM X-DNA gels. X-DNA building blocks were stained with a DNA specific dye (SYBR I, green) before gelation. The CHO cells are observed to divide, producing daughter cells even after being entrapped in the DNA hydrogel. The DNA hydrogel thereby provides a scaffold for cell transplant, 3D cell culture, and tissue engineering.

[0436] To assess the utility of the DNA hydrogel system for cell transplantation and also for in vivo administration of drugs, the biocompatibility of DNA hydrogels is evaluated. Cytotoxicity assays are performed with Chinese Hamster Ovarian (CHO) cells using the conventional CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, Wis.). Results revealed that all DNA hydrogels are non-toxic to cultured cells.

Example 3

Cell-Free Protein Production

[0437] A new DNA hydrogel was constructed utilizing nucleic acids, where linearized plasmid vector containing a gene for renilla luciferase was used to photocrosslink X-shaped DNA (X-DNA), incorporating the gene into the DNA hydrogel. *Renilla* luciferase is a 36 kDa monomeric protein and does not require a post-translational modification for activity. The linearized plasmid vectors were prepared by digesting the vector at a single site using Mlu I restriction enzyme. The X-DNA building blocks were prepared through complimentary hybridization of four different oligonucleotides. The gel electrophoresis result showed a complete linearization of the circular DNA after Mlu I digestion.

[0438] The photo-crosslinking of X-DNA to linearized plasmids was performed using one of two methods to link the renilla luciferase genes to the DNA hydrogels. In one method, the genes were crosslinked onto DNA building blocks wherein the genes were modified with one or more photo-responsive moieties. After which, the DNA-building blocks were hybridized. In a second approach, the DNA building blocks were first hybridized before the modified gene sequences were photo-crosslinked. Using either approach, the methods were carried out essentially as described in Example 1.

[0439] In vitro expression of *Renilla* luciferase (Rluc) protein from DNA gel pads was conducted using a coupled gene transcription and translation (TNT) kit (Promega). Conventional cell-free systems are solution phase systems (SPS), in which the gene templates are dispersed in solution. Here, SPS was used as a benchmark to evaluate the productivity (efficiency and yield) of protein produced using photocrosslinked P-gels. In preliminary experiments, we produced Rluc protein with the photocrosslinked P-gel using the same conditions as those for the SPS.

[0440] A comparison of a P-gel to a solution based system is shown in FIG. 22. FIG. 22A shows photocrosslinked protein-producing gel consists of genes as part of the gel scaffolding. The numbers above the bars indicate the fold increase in functional protein expression efficiency of Photocrosslinked P-gels over SPS controls. The results showed that a gene expression in DNA gel form had an efficiency about

72× greater than solution based systems. FIG. 22B shows the effect of the total gene amounts on expression, determined by varying the number of photocrosslinked P-gel micropads in the reaction (Blue lines). The same amounts of the plasmid were used in SPS control reactions (Red lines). In terms of volumetric yield, the photocrosslinked P-gel produced up to about 1 mg/ml of functional protein. Because the DNA gel pad is composed only of DNA, there were no non-specific bindings that may have caused lower protein expression in other solid phase systems. In other solid phase systems, biotin labeled linearized plasmids were immobilized onto avidin-covered solid beads where T7 RNA polymerase bound non-specifically to the biotin labeled linearized plasmids, disrupting gene expression.

[0441] Preparation of the linear renilla luciferase reporter gene. A plasmid DNA, pRL-null, containing *Renilla* luciferase driven by a T7 RNA polymerase promoter was purchased from Promega (Madison, Wis.). The plasmid DNA (pDNA) was amplified in competent *Escherichia coli* and purified by an Eppendorf Perfectprep® Plasmid Mini Kit (Westbury, N.Y.). The amplified and purified pDNA was digested with the restriction enzyme Mlu I (Promega), which cuts the plasmid at a single site before the T7 promoter.

[0442] Construction of the DNA building block and protein producing DNA hydrogel. The branched DNA sequences (Table 16) were designed and synthesized using commercially available oligonucleotide synthesis. The X₀₁ to X₀₄ single oligonucleotides that formed an X-DNA were amplified and functionalized as described in Example 1. To construct the protein producing X-DNA gel, the X-DNA (X-DNA concentration, 13 ug/ul) were similarly functionalized and mixed with the X-DNA. The photocrosslinking was carried out as in Example 1.

[0443] Fabrication of DNA gel micropads. A micro-meter scale DNA gel pad was prepared by molding the DNA pre-gel solution in a PDMS replica that was fabricated by photolithography. The dimensions of the gel micropads were 20 μm×200 μm×400 μm. A DNA pre-gel solution (1 μl) was dropped onto an APTES modified glass slide, and a PDMS replica was placed on the solution. After curing for 8 hours at

room temperature, the PDMS replica was peeled off. The DNA gel micro-patterns were visualized using a fluorescence microscope after staining with the DNA specific dye, SYBR I.

[0444] Characterization of swollen DNA hydrogels. AFM was carried out under water in a fluid cell on PicoPlus AFM (Molecular Imaging, Tempe, Ariz.) in MAC mode using type II MAClevers tips (Molecular Imaging, Tempe, Ariz.). Mica was chosen as a solid substrate and used immediately after cleavage in a clean atmosphere. For the sample preparation, surface modification was accomplished by a deposit of silane in MilliQ water. Briefly, the fresh mica was placed in a container filled with 10 ml 3-aminopropyltriethoxysilane (APTES) solution (2% w/w) for 15 min, and then the APTES-derivatized mica was thoroughly washed with MilliQ water several times and dried with a gentle stream of nitrogen gas. A piece of DNA gel was loaded onto the mica for imaging.

[0445] In vitro transcription and translation. A coupled transcription and translation was carried out using the TNT Coupled Transcription/Translation System (Promega, Madison, Wis.) in a volume of 50 μl. For protein producing DNA hydrogel, 15 gel pads produced from the micro-fabricated device were added to the expression solution. Incubation was carried out in a water bath at 30° C. for 75 min. All samples were stored at -80° C. before assaying for luciferase activity. The luciferase activity was evaluated by the *Renilla* Luciferase Assay System (Promega, Madison, Wis.) and measured with a luminometer.

[0446] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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Lys Ala Asp Ala Lys Ala Asp Ala
1 5

<210> SEQ ID NO 18
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 18

Lys Leu Asp Leu Lys Leu Asp Leu Lys Leu Asp Leu
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 19

Ala Glu Ala Glu Ala His Ala His Ala Glu Ala Glu Ala His Ala His
1 5 10 15

<210> SEQ ID NO 20
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 20

Ala Glu Ala Glu Ala His Ala His
1 5

<210> SEQ ID NO 21
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 21

Phe Glu Phe Glu Phe Lys Phe Lys Phe Glu Phe Glu Phe Lys Phe Lys
1 5 10 15

<210> SEQ ID NO 22
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 22

Phe Glu Phe Lys Phe Glu Phe Lys
1 5

<210> SEQ ID NO 23
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 23

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Phe Lys Phe Glu Phe Lys Phe Glu Phe Lys Phe Glu
1 5 10

<210> SEQ ID NO 24
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 24

Phe Lys Phe Glu Phe Lys Phe Glu
1 5

<210> SEQ ID NO 25
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 25

Phe Lys Phe Glu Phe Lys Phe Glu Phe Lys Phe Glu Phe Lys Phe Glu
1 5 10 15

<210> SEQ ID NO 26
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 26

Phe Lys Phe Gln Phe Lys Phe Gln Phe Lys Phe Gln
1 5 10

<210> SEQ ID NO 27
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 27

Ile Lys Ile Glu Ile Lys Ile Glu Ile Lys Ile Glu
1 5 10

<210> SEQ ID NO 28
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 28

Val Lys Val Glu Val Lys Val Glu Val Lys Val Glu
1 5 10

<210> SEQ ID NO 29
<211> LENGTH: 16

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 29

Leu Glu Leu Glu Leu Lys Leu Lys Leu Glu Leu Glu Leu Lys Leu Lys
1 5 10 15

<210> SEQ ID NO 30
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 30

Leu Glu Leu Glu Leu Lys Leu Lys
1 5

<210> SEQ ID NO 31
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 31

Ala Glu Ala Glu Ala Lys Ala Lys Ala Glu Ala Glu Ala Lys Ala Lys
1 5 10 15

<210> SEQ ID NO 32
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 32

Ala Glu Ala Glu Ala Glu Ala Glu Ala Lys Ala Lys
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 33

Ala Glu Ala Glu Ala Lys Ala Lys
1 5

<210> SEQ ID NO 34
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

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<400> SEQUENCE: 34

Lys Ala Lys Ala Lys Ala Lys Ala Glu Ala Glu Ala Glu Ala Glu Ala
1 5 10 15

<210> SEQ ID NO 35

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 35

Ala Glu Ala Glu Ala Glu Ala Glu Ala Lys Ala Lys Ala Lys Lys
1 5 10 15

<210> SEQ ID NO 36

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 36

Arg Ala Arg Ala Arg Ala Arg Ala Asp Ala Asp Ala Asp Ala Asp Ala
1 5 10 15

<210> SEQ ID NO 37

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 37

Ala Asp Ala Asp Ala Asp Ala Asp Ala Arg Ala Arg Ala Arg Ala Arg
1 5 10 15

<210> SEQ ID NO 38

<211> LENGTH: 26

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 38

Arg Gly Asp Tyr Arg Tyr Asp Tyr Thr Phe Arg Glu Glu Glu Gly Leu
1 5 10 15

Gly Ser Arg Tyr Asp Tyr Arg Gly Asp Tyr
20 25

<210> SEQ ID NO 39

<211> LENGTH: 26

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 39

Arg Gly Asp Tyr Arg Tyr Asp Tyr Thr Phe Lys Glu Glu Glu Gly Leu

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1	5	10	15
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Gly Ser Arg Tyr Asp Tyr Asp Gly Asp Tyr
20 25

<210> SEQ ID NO 40
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 40

Arg Gly Asp Tyr Arg Tyr Asp Tyr Thr Ala Ser Glu Leu Glu Gly Arg	
1 5 10 15	

Gly Thr Arg Tyr Asp Tyr Arg Gly Asp Tyr
20 25

<210> SEQ ID NO 41
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 41

Arg Gly Asp Tyr Arg Tyr Asp Tyr Ala Pro Thr Ala Gln Glu Ala Gly	
1 5 10 15	

Glu Gly Pro Arg Tyr Asp Tyr Arg Gly Asp Tyr
20 25

<210> SEQ ID NO 42
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 42

Arg Gly Asp Tyr Arg Tyr Asp Tyr Pro Thr Ile Ser Gln Glu Leu Gly	
1 5 10 15	

Gln Arg Pro Arg Tyr Asp Tyr Arg Gly Asp Tyr
20 25

<210> SEQ ID NO 43
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 43

Arg Gly Asp Tyr Arg Tyr Asp Tyr Pro Thr Val Ser Gln Glu Leu Gly	
1 5 10 15	

Gln Arg Pro Arg Tyr Asp Tyr Arg Gly Asp Tyr
20 25

<210> SEQ ID NO 44
<211> LENGTH: 39

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 44
actgctggat cgtatgcgta gtctggacgt ctaccgtgt 39

<210> SEQ ID NO 45
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 45
cagtcaggc tacgcatacc atccag 26

<210> SEQ ID NO 46
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 46
actgacacgg tagacgtcca gcctgc 26

<210> SEQ ID NO 47
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 47
actg 4

<210> SEQ ID NO 48
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 48
cagt 4

<210> SEQ ID NO 49
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 49
ctggatcgta tgcgta 16

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<210> SEQ ID NO 50
<211> LENGTH: 7
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 50

gcaggct 7

<210> SEQ ID NO 51
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 51

acacggtaga cgtcca 16

<210> SEQ ID NO 52
<211> LENGTH: 3
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 52

gtc 3

<210> SEQ ID NO 53
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 53

tggacgtcta cgtgt 16

<210> SEQ ID NO 54
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 54

acgcatacca tccag 15

<210> SEQ ID NO 55
<211> LENGTH: 6
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 55

gcctgc 6

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<210> SEQ ID NO 56
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 56

ctgagtacct gattggctta gtcggaagct 30

<210> SEQ ID NO 57
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 57

acctaggcgt actatcaggt actcagttaa 30

<210> SEQ ID NO 58
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 58

gaatgccgct tacagtacgc ctaggttcga 30

<210> SEQ ID NO 59
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 59

tccgactaag ccagtaagcg gcattcctag 30

<210> SEQ ID NO 60
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 60

agct 4

<210> SEQ ID NO 61
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 61

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ttaa 4

<210> SEQ ID NO 62
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 62

tcga 4

<210> SEQ ID NO 63
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 63

ctag 4

<210> SEQ ID NO 64
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 64

tggcttagtc gga 13

<210> SEQ ID NO 65
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 65

atcaggtact cag 13

<210> SEQ ID NO 66
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 66

agtacgccta ggt 13

<210> SEQ ID NO 67
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 67

gtaagcggca ttc

13

<210> SEQ ID NO 68

<211> LENGTH: 13

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 68

ctgagtacct gat

13

<210> SEQ ID NO 69

<211> LENGTH: 13

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 69

acctaggcgt act

13

<210> SEQ ID NO 70

<211> LENGTH: 13

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 70

gaatgccgct tac

13

<210> SEQ ID NO 71

<211> LENGTH: 13

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 71

tccgactaag cca

13

<210> SEQ ID NO 72

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 72

tgactggatc cgcattgacat tcgccgtaag

30

<210> SEQ ID NO 73

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 73

gtcatggatc cgcattgacat tcgccgtaag 30

<210> SEQ ID NO 74

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 74

atcgtggatc cgcattgacat tcgccgtaag 30

<210> SEQ ID NO 75

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 75

atgctggatc cgcattgacat tcgccgtaag 30

<210> SEQ ID NO 76

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 76

gcaatggatc cgcattgacat tcgccgtaag 30

<210> SEQ ID NO 77

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 77

tgaccttacg gcgaatgacc gaatcagcct 30

<210> SEQ ID NO 78

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 78

cgatcttacg gcgaatgacc gaatcagcct 30

<210> SEQ ID NO 79

<211> LENGTH: 30

<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 79
gcacctttacg gcgaatgacc gaatcagcct 30

<210> SEQ ID NO 80
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 80
ttgcctttacg gcgaatgacc gaatcagcct 30

<210> SEQ ID NO 81
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 81
ggacctttacg gcgaatgacc gaatcagcct 30

<210> SEQ ID NO 82
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 82
tgacaggctg attcgggttca tgcggatcca 30

<210> SEQ ID NO 83
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 83
cgataggctg attcgggttca tgcggatcca 30

<210> SEQ ID NO 84
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 84
gcacaggctg attcgggttca tgcggatcca 30

<210> SEQ ID NO 85

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<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 85

ttgcaggctg attcgggtca tgcggatcca 30

<210> SEQ ID NO 86
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 86

ggataggctg attcgggtca tgcggatcca 30

<210> SEQ ID NO 87
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 87

tgac 4

<210> SEQ ID NO 88
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 88

gtca 4

<210> SEQ ID NO 89
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 89

cgat 4

<210> SEQ ID NO 90
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 90

atcg 4

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<210> SEQ ID NO 91
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 91

gcac 4

<210> SEQ ID NO 92
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 92

atgc 4

<210> SEQ ID NO 93
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 93

ttgc 4

<210> SEQ ID NO 94
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 94

gcaa 4

<210> SEQ ID NO 95
<211> LENGTH: 4
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 95

ggat 4

<210> SEQ ID NO 96
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 96

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tg gatccgca tga 13

<210> SEQ ID NO 97
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 97

cattcgccgt aag 13

<210> SEQ ID NO 98
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 98

cttacggcga atg 13

<210> SEQ ID NO 99
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 99

accgaatcag cct 13

<210> SEQ ID NO 100
<211> LENGTH: 13
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 100

aggctgattc ggt 13

<210> SEQ ID NO 101
<211> LENGTH: 13
<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 105

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

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<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

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<210> SEQ ID NO 108

<211> LENGTH: 26

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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oligonucleotide

<400> SEQUENCE: 108

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<210> SEQ ID NO 126
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<212> TYPE: DNA
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<210> SEQ ID NO 135
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<400> SEQUENCE: 135

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<210> SEQ ID NO 136
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<400> SEQUENCE: 136

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1-116. (canceled)

117. A composition comprising a plurality of photo-crosslinked branched nucleic acid molecules, wherein at least a portion of the photo-crosslinked nucleic acid molecules provides a macroscopic three dimensional structure and wherein at least a portion of the photo-crosslinked nucleic acid molecules is double stranded.

118. The composition of claim **117**, wherein the nucleic acid molecules comprise DNA, RNA, PNA or a combination thereof.

119. The composition of claim **117**, wherein the branched nucleic acid molecules form a shape comprising X-shape, Y-shape, T-shape, dumbbell shape, a dendrimer shape or combinations thereof.

120. The composition of claim **117**, wherein the macroscopic three dimensional structure provide a macroscopic scaffold.

121. The composition of claim **120**, wherein the macroscopic scaffold has a predetermined geometric shape.

122. The composition of claim **117**, wherein the photocrosslinked branched nucleic acids form a macroscopic three dimensional structure having a tensile modulus of 0.45, 0.5, 0.55, 0.6, 0.65 or 0.7.

123. The composition of claim **117**, wherein at least a portion of the nucleic acid molecules are linked to one or more additional compounds selected from the group consisting of a peptide, a polypeptide, a protein, a lipid, a carbohydrate, an oligonucleotide, a polynucleotide, an aptamer, an antibody, an antigen, a cell growth factor, a DNA binding agent, a detectable label, a selectable marker, biotin, a pharmaceutical agent, a drug, a small molecule, a therapeutic agent, a receptor molecule, a ligand and a substrate.

124. The composition of claim **117**, wherein the nucleic acid molecule comprises a coding region and a non-coding region.

125. A method of forming a macroscopic three dimensional structure comprising photo-crosslinked branched nucleic acids comprising the steps of:

- a) providing a plurality of nucleic acid molecules, wherein a portion of the nucleic acid molecules are hybridized to provide branched chain nucleic acid building blocks and at least a portion of the branched nucleic acid molecules are conjugated to a photoreactive group; and
- b) photocrosslinking the branched nucleic acid molecules to provide a macroscopic three dimensional structure.

126. The method of claim **125**, wherein the branched chain nucleic acid building blocks are formed by photocrosslinking linear nucleic acids coding for a protein to hybridized branched chain nucleic acids.

127. The method of claim **125**, wherein the branched chain nucleic acid building blocks are formed by photocrosslinking linear nucleic acids coding for a protein to nucleic acids capable of forming branched chain hybridized building blocks and hybridizing said nucleic acids to form branched chain hybridized building blocks.

128. The method of claim **125**, wherein the at least a portion of the branched nucleic acid molecules are conjugated to a photoreactive group on their 5' end, 3' end, internally, or a combination thereof.

129. The method of claim **125**, wherein the branched chain nucleic acid building blocks comprise one or more of a shape selected from the group consisting of X-shape, Y-shape, T-shape, dumbbell shape and dendrimer-like shape.

130. The method of claim **125**, wherein the photoreactive group comprises a vinyl, acrylate, N-hydroxysuccinimide, amine, carboxylate or thiol moiety.

131. The method of claim **125**, wherein the photoreactive group is a primary amine modified group, a secondary amine modified group or a tertiary amine modified group.

132. The method of claim **125**, wherein the photocrosslinking is performed in the presence of a photoinitiator.

133. The method of claim **125**, wherein the macroscopic three dimensional structure is formed in 10 minutes or less.

134. A method of cell-free synthesis of one or more proteins comprising:

- a) providing the composition of claim **1** comprising a nucleic acid molecule encoding for a protein; and
- b) contacting the composition from a) with cell-free transcription and translation components such that cell-free synthesis of the protein occurs.

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