

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

WUHAN HEALTHGEN BIOTECHNOLOGY CORP.,
Petitioner

v.

VENTRIA BIOSCIENCE INC.,
Patent Owner

Case No. IPR2023-00684

Patent No. 11,492,389

DECLARATION OF DOUGLAS S. CLARK, Ph.D.

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I. Introduction

1. I, Douglas S. Clark, Ph.D., have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, on behalf of Wuhan Healthgen Biotechnology Corp. as an independent expert to analyze certain claims of U.S. Patent No. 11,492,389 (the '389 patent) in view of the prior art. I understand that this Declaration accompanies a petition for *inter partes* review (IPR) of the '389 patent.

2. I am being compensated for the time I spend on this matter at my standard consulting rate, but my compensation is not contingent upon my opinions or the outcome of this or any other proceeding.

II. Summary of Opinions

3. I conclude that a person of ordinary skill in the art (POSA) would have been motivated to prepare the compositions of claims 1-29 of the '389 patent, and would have had a reasonable expectation of success in doing so, based on U.S. Patent Publication No. 2007/0166825 ("Hatsuyama," EX1004) in combination with WO 2007/002762 ("Deeter," EX1005) and the prior general knowledge of a POSA, including the knowledge that purified proteins such as albumin should preferably contain no more than 10 ppm detergent (as exemplified by U.S. Patent Publication No. 2006/0110399 ("Van Holten," EX1007), U.S. Patent Publication No. 2006/0194300 ("Schulz," EX1008), U.S. Patent Publication No. 2006/0008792 ("Viswanathan," EX1009), and U.S. Patent No. 5,094,960 ("Bonomo," EX1059).

III. Qualifications

4. I am currently Dean of the College of Chemistry and Professor in the Department of Chemical and Biomolecular Engineering at the University of California, Berkeley. I am also a faculty scientist at Lawrence Berkeley Laboratory, and I hold the endowed G.N. Lewis Chair.

5. Prior to my appointment as Dean, I served as Department Chair of Chemical and Biomolecular Engineering and Executive Associate Dean in the College of Chemistry at Berkeley. I have been a faculty member at Berkeley since 1986.

6. I received a Ph.D. in Chemical Engineering from the California Institute of Technology in 1983 and a B.S. in Chemistry, summa cum laude, from the University of Vermont in 1979.

7. My research is in the field of biochemical engineering, with particular emphasis on enzyme technology, biomaterials, and bioenergy. My laboratory research has involved investigating stem cell differentiation, culturing mammalian cells, studying structure-function relationships of enzymes, and molecular engineering of proteins, including chaperones.

8. My research experience also includes work with albumin. In a 2008 publication, my research group characterized the catalytic activities of bovine serum albumin (BSA) over a range of temperature profiles. We demonstrated that BSA has

robust biocatalytic activities at high temperatures, making it a useful model for thermophilic enzymes and use in a broad range of applications. I have also published a series of papers describing the structure-function relationships of chaperone proteins. Much of my work has utilized standard cell culture (including culture of stem cells and hybridomas), chromatography, and mass spectrometry techniques that were well known to practitioners in the field.

9. Since I was a graduate student in 1980, I have published more than 290 papers, many dealing with enzyme applications and enzyme engineering, including optimization of enzyme activity, stability, and production. I have additional experience investigating enzyme structure and function using extensive chromatography, spectroscopic, and analytical techniques.

10. Throughout my career, I have taught undergraduate and graduate courses in biochemical engineering and laboratory techniques, including enzyme assays, cell culture techniques, and protein purification.

11. I have served as the Editor-in-Chief of the journal *Biotechnology & Bioengineering* since 1996, and I have served on the editorial board of the journal *Extremophiles* since 1996 and on the journal *Enzyme and Microbial Technology* from 1994 to 2019. All these journals publish extensively on industrial biocatalysis, enzyme technologies, bioprocessing of starch and other biomass, and fermentation of the resulting sugars to produce alcohols and other products.

12. My textbook, *Biochemical Engineering*, contains material on enzyme kinetics, proteases, enzyme denaturation, fermentation, and bioproduct recovery, among other topics, including a discussion of the purification of recombinant proteins.

13. I have received a number of honors and awards for my work. I am a member of the National Academy of Engineering. I am a Fellow of the American Association for the Advancement of Science and the American Institute of Medical and Biomedical Engineers. I received both the Daniel I.C. Wang Award and the James E. Bailey Award from the Society of Biological Engineering; the Marvin J. Johnson Award in Microbial and Biochemical Technology from the American Chemical Society; the Food, Pharmaceutical, and Bioengineering Award of the American Institute of Chemical Engineers; the Amgen Award in Biochemical Engineering; the International Enzyme Engineering Award; and the NorCal Chemical Engineering Award—Industrial Research. I also received the Departmental Chemical Engineering Teaching Award and the Presidential Young Investigator Award (National Science Foundation).

14. My professional qualifications are described in further detail in my curriculum vitae, which is attached as **Appendix A**.

15. A list of the materials I considered in connection with my analysis is provided in **Appendix B**. I have also relied on my expertise and general knowledge of the art as of 2010.

IV. The '389 Patent

A. Overview

16. The '389 patent, titled “Cell Culture Media Containing Combinations of Proteins,” generally relates to cell culture media supplement compositions containing proteins, including preferably plant-produced heterologous proteins such as rHSA and recombinant human lactoferrin. *E.g.*, EX1001 (the '389 patent), 3:8-62.

17. The examples in the '389 patent concern the production and purification of recombinant human proteins rHSA expressed in transgenic rice (Examples 1-6), the use of such proteins in cell culture media (Examples 7 and 11-19), and the presence of rice heat-shock proteins in rHSA and their impact on cell viability (Examples 8-10). *See generally* EX1001, 62:59-96:44.

18. The '389 patent states that, in certain embodiments, the disclosed albumin contains less than about 1 EU endotoxin per mg albumin, less than about 10 ppm detergent, and less than about 2% aggregated albumin. EX1001, 45:43-60. The '389 patent acknowledges that “the other commercially available albumin

products may have similar overall purity, endotoxin and detergent levels to rice derived recombinant albumin.” EX1001, 75:5-8.

19. The '389 patent acknowledges that known methods of preparing and purifying albumin “provide those skilled in the art reading this disclosure with significant possible methods for preparing and purifying albumin,” citing several patents concerning the purification of albumin from various expression systems as examples, and states that “[i]n one aspect, the albumin for use in the present invention is purified using any of these art recognized processes listed above, and then mixed in aqueous solution with a heat shock protein.” EX1001, 50:1-12.

20. The '389 patent states that proteins produced in a plant will typically comprise “a different set of bound co-factors, lipids and contaminating proteins compared to the same protein produced in a different expression system.” EX1001, 50:38-41. A POSA would have appreciated that these co-factors, lipids, and contaminating proteins bound to albumin are from the plant host (i.e., they are plant lipids and proteins).

21. The '389 patent states that, in certain embodiments, the albumin has a “purity of greater than about 95%.” EX1001, 45:47-49. The '389 patent does not appear to define “purity.” However, in one instance, it equates purity with the “concentration of albumin per dry powder weight,” which was determined by HPLC. EX1001, 70:4-6. In another instance, the '389 patent indicates that purity was

determined by SDS-PAGE by first treating the sample with a reducing agent (Invitrogen NP004, dithiothreitol (DTT)), running a gel, and subtracting the bands attributed to impurities. In the context of SDS-PAGE, purity generally refers to the amount of the protein of interest versus other species (i.e., albumin versus host cell proteins).

B. Claims

22. The '389 patent has three independent claims that generally concern a cell culture media supplement comprising recombinant albumin (e.g., rHSA) produced in a transgenic plant having less than 1 EU endotoxin per mg albumin, where claim 1 further requires less than 10 ppm detergent and claim 24 further requires a plant carbohydrate or plant lipid. The independent claims of the '389 patent are provided below for reference.

The Independent Claims of the '389 Patent		
1. A cell culture media supplement or complete media composition for improving growth, viability, and/or productivity of cultured cells as compared to cells grown without said supplement or complete media composition, said cell culture media supplement or complete media composition comprising a plant-	24. A cell culture media supplement or complete media composition for improving growth, viability, and/or productivity of cultured cells as compared to cells grown without said supplement or complete media composition, said supplement or complete media composition comprising a plant-produced heterologous protein, wherein said plant-	26. A cell culture media supplement or complete media composition for improving the growth of a cell in cell culture comprising: a recombinant mammalian albumin wherein said albumin is: i) produced in a transgenic plant; and ii) has less than 1 EU of endotoxin/mg of albumin.

The Independent Claims of the '389 Patent		
produced heterologous protein, wherein said plant-produced heterologous protein is recombinant human albumin, said recombinant human albumin comprising: i) less than 1 EU of endotoxin/mg of albumin; and ii) less than 10 ppm detergent.	produced heterologous protein is recombinant human albumin, said recombinant human albumin comprising: i) less than 1 EU of endotoxin/mg of albumin; and ii) a monocot seed component, wherein said monocot seed component is a plant carbohydrate or plant lipid.	

23. The claims depending from claim 1 generally require that the rHA “is a sterile liquid or powder” (claim 2); that the rHA “has a molecular weight of 65-75 kDa” (claim 3); that the rHSA “has an isoelectric focusing point [pI] of 5.3” (claim 4); that the rHA “comprises albumin and fragments thereof provided that the fragments retain the biological or therapeutically beneficial activity of said albumin” (claim 5); that the rHA “comprises albumin dimers having a molecular weight of 130 kDa as detected by SDS-PAGE” (claim 6); that the rHSA “comprises less than 2% aggregates at around 250 kDa as detected by SDS-PAGE” (claim 7); that the rHSA “has an amino acid sequence homologous to native human albumin” (claim 8); that the rHA “is recombinant human serum albumin” (claim 9), “has an amino acid sequence that is at least 80% identical to secreted human serum albumin [HSA] comprising the sequence of SEQ ID NO: 1” (claim 10), or “comprises the amino

acid sequence of SEQ ID NO: 12” (claim 11); that the rHSA “comprises one or more bound lipids from a plant” (claim 12); that the rHSA “is a protein extract of a transgenic monocot plant” (claim 13), where the rHSA “comprises a monocot seed component” (claim 14) that “is a plant carbohydrate or lipid” (claim 15); that the rHSA “is a protein extract of transgenic seed” (claim 16); that the rHSA is “a protein extract of a transgenic rice seed” (claim 17), specifically, *Oryza sativa* (claim 18); that the composition “is free of serum derived albumin or yeast derived albumin” (claim 19); that the composition comprises “at least one additional protein” (claim 20), wherein “said an additional protein is selected from the group consisting of “growth factors, lactoferrin, transferrin, insulin, [and other proteins]” (claim 21), wherein “said additional protein is a heterologous plant-produced protein” (claim 22); and that the composition comprises “selenium and ethanolamine” (claim 23).

24. Claim 25 depends from claim 24 and requires that the rHA “has a purity of greater than 95%.” Claims 27-29 depend from claim 26 and further require that the albumin “is recombinant human serum albumin [rHSA]” (claim 27), that the transgenic plant “is a transgenic grain” (claim 28), specifically, “transgenic rice” (claim 29).

C. Priority Date

25. I understand that the ’389 patent is part of a series of patents and applications that claim priority to U.S. Application No. 12/708,462 (filed February

18, 2010) and Application No. 61/154,204 (filed February 20, 2009, and identified as EX1060).¹

26. I was informed that, to be entitled to rely on the date of the application filed in 2009 (“the 2009 application”), it must be shown that the 2009 application discloses the subject matter of the claims.

27. Here, the 2009 application does not disclose any rHSA containing “less than 1 EU endotoxin/mg albumin,” as required by all claims of the ’389 patent. EX1060 (the 2009 application). In fact, the word “endotoxin” does not appear in the 2009 application. *Id.* Relatedly, the 2009 application does not disclose the process for removing endotoxin set forth in Example 5 of the ’389 patent. In fact, the 2009 application’s examples regarding the production and purification of rHSA were

¹ As listed on the face of the ’389 patent, the ’389 patent issued from U.S. Application No. 17/834,026, which is a continuation of U.S. Application No. 17/212,403, filed on Mar. 25, 2021, which is a continuation of U.S. Application No. 16/847,337, filed on Apr. 13, 2020 (now Pat. No. 10,981,974), which is a continuation of U.S. Application No. 15/188,478, filed on Jun. 21, 2016 (now Pat. No. 10,618,951), which is a continuation of U.S. Application No. 12/708,462, filed on Feb. 18, 2010, now abandoned. The ’389 patent also identifies U.S. Provisional Application No. 61/145,204, filed February 20, 2009.

previously disclosed in Deeter (nearly verbatim). *Compare* EX1060, [0113]-[0115] (expression of rHSA in Example 3 and Table 1) *with* EX1005, [0103]-[0105] (same expression of rHSA in Example 3 and Table 1); *compare* EX1060, [0121]-[0122] (purification of rHSA in Example 5 and properties in Table 2) *with* EX1005, [0116]-[0117] (same purification of rHSA in Example 7 and properties in Table 3). In addition, the 2009 application does not contain the word “detergent” or disclose recombinant albumin containing less than 10 ppm detergent, as required by claims 1-24 of the ’389 patent.

28. As all claims of the ’389 patent require recombinant albumin comprising less than 1 EU endotoxin/mg albumin, I conclude that the 2009 application does not disclose the claimed compositions.

29. I have therefore analyzed the ’389 patent from the perspective of a POSA as of the filing date of Application No. 12/708,462, February 18, 2010.

30. However, in my view, the perspective of a POSA would not have changed from February 20, 2009, to February 18, 2010, as the subject matter claimed in the ’389 patent was known well before 2009.

D. Prosecution History

31. The ’389 patent was issued on November 8, 2022, which is more than twelve years after the patent’s disclosure was initially provided to the U.S. Patent and Trademark Office on February 18, 2010.

32. During prosecution, the Examiner stated that the closest prior art of record was Deeter (WO2007/002762 A2) and found that the applicant's declaration in a related application² showed that Deeter's compositions were "incapable of achieving the claimed less than 1 EU of endotoxin/mg of albumin and that the *levels of endotoxin in the cell culture medium were not appreciated at the time of Deeter's invention.*" EX1002, 281 (emphasis added).

33. The applicant's declaration contradicts the Examiner's assertion that "the levels of endotoxin in the cell culture medium were not appreciated at the time of Deeter's invention." *Compare* EX1002, 281 *with* EX1006, 698-703. In fact, the applicant's declaration states that "in the process carried out by Deeter et al. *endotoxin levels were observed in the final product.* However, *these values were not reported in the patent application at that time.*" EX1006, 698. Thus, it appears that the levels of endotoxin were "appreciated" by Deeter et al.—they were simply not reported. Moreover, any assertion that those in the art would not have recognized that Deeter's cell culture media would have been contaminated with endotoxin is incorrect, as exemplified by the numerous publications that I discuss in my

² Ventria submitted a declaration by Dr. Lorne Jordan during prosecution of U.S. Application No. 15/188,478 ("the '478 application") on November 17, 2019. EX1006, 702.

declaration. In short, while the precise levels of endotoxin were not reported, endotoxin contamination in Deeter's cell culture media was appreciated by Deeter et al. and by skilled artisans reading Deeter's disclosures.

34. Next, the Examiner erred in stating that Deeter's rHSA is "incapable" of achieving less than 1 EU of endotoxin/mg albumin. *See* EX1002, 281. Although the applicant's declaration asserted that "[t]echniques had to be researched and developed to reduce[]/remove endotoxin," in fact, such techniques were already known and published in the prior art. EX1006, 693. Some research—specifically, a review of the published literature—may have been advisable, but there is nothing new about the method that Ventria uses to purify rHSA in the '389 patent (i.e., a detergent wash followed by detergent removal). There is simply no evidence that Deeter's rHSA was "incapable" of being purified using routine techniques to achieve "less than 1 EU endotoxin/mg albumin." *Infra*, ¶¶ 140-154.

35. Indeed, in 1990, Aida³ discloses using detergent to purify albumin to achieve no detectable endotoxin (less than 0.001 EU of endotoxin per mg albumin),

³ Appendix B identifies the publications associated with the short names that I use in my declaration. For example, Appendix B, item no. 44, defines "Aida" as Y. Aida and M. Pabst, Removal of Endotoxin from Protein Solutions by Phase Separation using Triton X-114," *J. Immunological Methods*, 132 (1990) (191-195).

and later research established that technique as “a valuable tool” for decontaminating proteins in the laboratory, including recombinant proteins expressed in *E-coli* (i.e., proteins heavily contaminated with endotoxins). EX1044 (Aida), abstract, 4; EX1042 (Magalhaes), 10-11; EX1043 (Petsch), 10-11, 19. When the applicant’s declaration was submitted in 2019, the applicant had not submitted Aida, Magalhaes, or Petsch for the Examiner’s consideration. *See* EX1006, 775-794 (identifying publications submitted by the applicant).

36. It appears the applicant first submitted Aida on June 10, 2022, among numerous other documents. EX1002, 206 (listing Aida as a publication submitted); EX1002, 208 (showing date of submission as June 10, 2022); EX1002, 179-219 (listing documents submitted by the applicant). While the Examiner stated that all publications were considered (except where lined through), the teachings of Aida suggest the Examiner did not appreciate Aida’s teachings, which disclose the ease of purifying albumin to remove endotoxin and contradict the assertion that “the compositions of Deeter et al. are *incapable* of achieving less than 1 EU of endotoxin/mg albumin.” *See* EX1002, 281.

37. Moreover, the applicant does not appear to have submitted any publications describing the desirability of removing detergents used to purify proteins to less than 10 ppm or the routine removal of detergents from protein solutions using hydrophobic sorbents (such as sorbents designed to remove solvents

and detergent, e.g., “SDR” sorbents). For example, publications including Van Holten (EX1007), Schulz (EX1008), Viswanathan (EX1009), Burnouf (EX1049), Piquet (EX1050), Guerrier (EX1051), and Bonomo (EX1059) do not appear to have been disclosed. As such, the Examiner may not have appreciated that there was nothing novel or unexpected about achieving the levels of detergent contamination claimed in the ’389 patent.

38. Further, contrary to the applicant’s declaration, a careful inspection of the ’389 patent specification (which includes the same text as that in the priority ’478 application where the applicant’s declaration was filed during prosecution) shows it does *not* disclose the levels of endotoxin in Deeter’s rHSA. For context, the cited applicant’s declaration states as follows:

[T]he current application itself actually contains a comparison of the Deeter [WO 2007/002762]-produced albumin and the albumin in the current application. In fact, Example 5 of the current application is identical to the protocol described in Example 7 of Deeter[.] The current application designates this protocol as process ‘B000.’ Further, *Table E3 in the current application shows the endotoxin levels detected in albumin extracted and purified using the old ‘B000’ Deeter et al. process.*

EX1006, 693-694 (emphasis added).

39. As noted in the applicant's declaration quoted above, the specification of the '389 patent discloses Deeter's purification process in the first section of Example 5, where it identifies Deeter's process as "B000." EX1001, 65:57-66:3. However—somewhat confusingly—the specification of the '389 patent also identifies an alternative previously used process as "B000"—there are two B000 processes in the patent (i.e., Deeter's process and an "alternative" process). EX1001, Example 6, 69:31-61.

40. The specification of the '389 patent states that the comparison of endotoxin levels was based on rHSA purified by the "alternative process (B000)," which is detailed in Example 6. *Id.*, 69:26-30. This alternative B000 process includes mixing rHSA with 10g/L of CHAPS (a zwitterionic detergent) for one hour, then passing the solution through a Biorad SM-2 column. *Id.*, 69:31-62. This explains why the "old" Cellastim identified in Table E3 was contaminated with detergent—a reagent that Deeter does not disclose using at all. *See* EX1001, Table E3.

41. Thus, contrary to the applicant's declaration, Table E3 of the '389 patent compares rHSA purified by an alternative B000 process—not the process in Deeter—to the rHSA purified by the purportedly new process. EX1001, 69:20-62, Table E3. Thus, Table E3 of the '389 patent does **not** disclose the levels of endotoxin in Deeter's rHSA. Nevertheless, I note that Deeter's rHSA would have been expected to be contaminated with endotoxins, as Deeter does not disclose taking any

precautions to avoid endotoxin contamination (e.g., by using endotoxin-free water and cleaning equipment to remove endotoxins).

V. Legal Principles

42. The opinions I express in this declaration involve the application of my technical knowledge and experience to evaluating certain prior art with respect to the '389 patent. In preparing this declaration, certain patent law concepts have been explained to me by legal counsel, including the legal standard for interpreting claims, as well as those for assessing written description, enablement, and obviousness.

43. I understand that patentability must be analyzed from the perspective of a POSA in the same field as the challenged patent as of the “effective filing date” of the claims. I understand that a POSA is a hypothetical individual presumed to know the relevant art as of the effective filing date of the claims and has the same level of skill as the ordinary practitioner of the art at issue.

44. I understand that patent claims should be interpreted in accordance with their ordinary meaning as understood by a POSA at the time of the effective filing date in light of the claim language, patent specification, and prosecution history, unless the patentee unambiguously defines or otherwise modifies the meaning of a claim term.

45. I understand that a patent claim is unpatentable if the subject matter of the claim would have been obvious to a POSA as of the effective priority date.

46. I understand that when evaluating obviousness, I should consider (a) the scope and content of the prior art, (b) the differences between the prior art and the claimed invention, (c) the level of ordinary skill in the art, and (d) any “secondary considerations” of non-obviousness. I am informed that if present, these secondary considerations concern real-world evidence of nonobviousness of the claimed invention, and that the evidence must be related to the novel features of the claimed composition.

47. I further understand that a claim may be obvious based on multiple references combined with one another, or pursuant to the knowledge and skill of a POSA. I also understand there must have been a reason that would have prompted a POSA to combine the features of the prior art to arrive at the claimed subject matter, and that this is sometimes referred to as a “motivation to combine.”

48. I also understand that a POSA must have reasonably expected that the combination will work, which is sometimes referred to as a “reasonable expectation of success.”

VI. Level of Skill in the Art

49. I am informed that factors that may be considered when determining the level of skill in the art include (1) type of problems encountered in the art; (2) prior art solutions to those problems; (3) the rapidity with which innovations are

made; (4) sophistication of the technology; and (5) educational level of active workers in the field.

50. I am also informed that a POSA is a person of ordinary creativity and not an automaton, and that in many cases a POSA may be able to fit the teachings of the prior art together like pieces of a puzzle.

51. Here, the '389 patent generally concerns the production, purification, and subsequent use of recombinant proteins expressed in rice plants in cell culture media. I note that the methods of production in the '389 patent were already known, as exemplified by Deeter, which discloses the recombinant production of human growth factors, human lactoferrin, and human albumin in transgenic rice. EX1005, Examples 1-3, [0094]-[0104]. Indeed, in 2004 (even before Deeter), it was noted that “genetic engineering of plants is a mature technology.” EX1031 (Molecular Farming), 234. As the production of recombinant proteins in transgenic rice was already known, the '389 patent is primarily directed to removing endotoxin from rHSA and using plant-produced recombinant proteins in cell culture.

52. In my opinion, a person of ordinary skill in the art as of February 18, 2010, would have had a Ph.D. in chemistry, biochemistry, chemical engineering, or a related discipline and at least two years of experience involving the production and/or purification of proteins such as rHSA.

53. A POSA would have been familiar with common laboratory techniques and strategies for characterizing and purifying proteins. A POSA would have been familiar with the use of proteins, including albumin and rHSA, in cell culture media. A POSA would also have been knowledgeable of common uses of cell culture media, including the use of cell culture in biological research and for the production of biological products.

54. I have considered the prior art from the perspective of a POSA as of the 2010 effective filing date of the '389 patent. However, I note that the level of skill and knowledge of a POSA would have been essentially the same in 2009, and my opinions would be the same if the '389 patent were entitled to its 2009 priority date.

VII. Claim Construction

55. The '389 patent provides definitions for certain claim terms, which are summarized below.

Table 1: Claim Term Definitions in the '389 Patent	
Claim Term (claims)	'389 Patent
"cell culture media" (all claims)	"The terms 'cell culture medium,' 'cell culture media,' and 'culture medium' refer to the solutions used for growing, storing, handling and maintaining cells and cell lines." '389 patent, 16:46-48.
"cell culture media supplement" (all claims)	"The term 'cell culture media supplement' refers to a combination of one or multiple cell culture media components, with or without other ingredients, for addition to cell culture media." <i>Id.</i> , 17:16-19.

Table 1: Claim Term Definitions in the '389 Patent	
Claim Term (claims)	'389 Patent
“heterologous protein” (claims 1, 24) “recombinant” protein (all claims)	“The term ‘heterologous protein’ or ‘recombinant protein’ refers to a protein encoded in all or part by heterologous DNA, or a protein that is expressed from expression control sequences (such as a promoter, or enhancer) created in whole or part by the heterologous DNA which activates the expression of an endogenous gene.” <i>Id.</i> , 19:50-56.
“homologous” (claim 8)	“The term ‘homologous’ refers to the relationship between two proteins that possess a “common evolutionary origin”, including proteins from superfamilies (e.g., the immunoglobulin superfamily) in the same species of animal, as well as homologous proteins from different species of animal (for example, myosin light chain polypeptide, etc.; see Reeck et al., <i>Cell</i> , 50:667, 1987). Such proteins (and their encoding nucleic acids) have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions.” <i>Id.</i> , 20:13-23.
“identical” (claim 10)	Not explicitly defined; however: “As used herein, ‘identity’ means the percentage of identical nucleotide or amino acid residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions.” <i>Id.</i> , 21:1-5.
“monocot seed component” (claims 14, 15, 24)	“‘Monocot seed components’ refers to carbohydrate, protein, and lipid components extractable from monocot seeds, typically mature monocot seeds.” <i>Id.</i> , 23:18-20.
“produced in a transgenic plant” (claim 26)	Not explicitly defined, however: “With respect to recombinant proteins, the term ‘produced in a plant’ means that the recombinant protein has been expressed within a plant cell, and then purified from host cells proteins and lipids.” <i>Id.</i> , 26:28-31.

56. The '389 patent also provides a lengthy description of “albumin” in columns 37 to 39, which includes the following passages:

Fragments of native or synthetic albumin sequences may also have the desirable functional properties of the peptide from which they derived and may be used in any of the methods of the invention. The term “fragment” as used herein thus includes fragments of albumin provided that the fragment *retains the biological or therapeutically beneficial activity of the whole molecule* ['389 patent, 37:23-29 (emphasis added).]

The term “*derivative*” as used herein thus refers to albumin sequences or fragments thereof, which have modifications as compared to the native sequence ['389 patent, 37:55-57 (emphasis added).]

Thus, the albumin which may be used in any of the methods of the invention may have amino acid sequences which are substantially homologous, or substantially similar to the native albumin amino acid sequences, for example, to any of the native albumin gene sequences listed in Table DI. Alternatively, the *albumin may have an amino acid sequence having* at least 30% *preferably at least 40, 50, 60, 70, 75, 80, 85, 90, 95, 98, or 99% identity with albumin listed in Table DI.* In a preferred embodiment, the albumin for use in any of the methods of the present invention is at least 80% identical to the mature secreted human serum albumin (SEQ. ID. No. 1) as shown below (Swiss-Prot P02768) ['389 patent, 38:14-24 (emphasis added).]

Fusion proteins of albumin to other proteins are also included, and these fusion proteins may enhance[], activity, targeting, stability or potency. ['389 patent, 38:54-56 (emphasis added).]

Chemical modifications of the native albumin structure which retain or stabilize albumin activity or biological half-life may also be used with any of the methods described herein. Such chemical modification strategies include without limitation pegylation, glycosylation, and acylation ['389 patent, 38:57-61.]

. . . . All such variants, derivatives, fusion proteins, or fragments of albumin are included, may be used in any of the methods disclosed herein, and are subsumed under the term 'albumin'.
['389 patent, 39:3-7 (emphasis added).].

57. In view of the passages above, I believe that a POSA would understand that the term “albumin,” as used in the '389 patent, includes “variants, derivatives, fusion proteins, or fragments,” provided that they retain “the biological or therapeutically beneficial activity of the whole molecule.” *Id.*, 37:23-39:7. I note additionally that claim 10 requires that the claimed rHSA “has an amino acid sequence that is at least 80% identical to secreted human serum albumin comprising the amino acid sequence of SEQ ID NO: 1.” *Id.*, claim 10. Thus, the claimed recombinant albumin necessarily includes a fragment that may be missing up to 20% of the amino acid sequence of HSA (a fragment that would be expected to retain

some, but perhaps not all, of the beneficial activity of HSA). I thus interpret the “biological or therapeutically beneficial activity of the whole molecule” to require that the recombinant albumin retain beneficial activity associated with HSA. Combining these disclosures with the ’389 patent’s description of a “recombinant protein” (in the table above), a POSA would have understood that the claimed “recombinant human albumin” and “recombinant mammalian albumin” encompass:

- “recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it), [1] which has the amino acid sequence of a native [human or mammalian] albumin or [2] which is a variant, derivative, fusion protein, or fragment of native [human or mammalian] albumin (provided the fragment retains biological or therapeutically beneficial activity associated with the native [human or mammalian] albumin.”

58. Claim 5 of the ’389 patent recites cell culture media comprising “albumin and fragments thereof provided that the fragments retain the biological or therapeutically beneficial activity of said albumin.” EX1001, claim 5. The ’389 patent does not explicitly define “fragment.” However, the meaning of this term is informed, in part, by the meaning of recombinant albumin discussed above, which includes fragments that retain the beneficial biological activity associated with a native albumin such as HSA. *Supra*, ¶ 57. As a result, a POSA would have interpreted the added limitation of claim 5 as follows:

- “albumin and fragments thereof provided that the fragments retain the biological or therapeutically beneficial activity associated with native albumin.”

59. A skilled artisan would have understood that such fragments need not retain the *full* or *complete* biological or therapeutically beneficial activity of full-length albumin (e.g., the claimed fragments retain at least some, but need not retain all, the beneficial biological activity of HSA). Indeed, claim 5 does not recite “full” or “complete” beneficial activity.

60. Finally, the independent claims (claims 1, 24, and 26) include introductory phrases that recite “improving growth, viability, and/or productivity of cultured cells as compared to cells grown without said supplement or complete media composition” (claims 1, 24) or “improving the growth of a cell in cell culture” (claim 26). I am informed that intended uses stated in the introduction of a claim (called the preamble) are generally not treated as claim requirements. However, even if the claims require “improving” growth in cell culture, the prior art teaches cell culture media comprising rHSA that provides such improvements in cell culture media.

61. I have applied these definitions and understandings in my analysis and have given all other claim terms their plain and ordinary meaning in the context of the '389 patent.

VIII. Background and State of the Art

62. By 2010 (or even 2009), there was a wealth of established knowledge regarding cell culture media, HSA, the benefits of recombinant proteins in cell culture media, transgenic hosts expressing rHSA, endotoxins—including the problems with endotoxin contamination in cell culture media, expected endotoxin contamination in recombinant proteins expressed in transgenic plants, and endotoxin removal—detergents used to purify proteins and the removal of such detergents from protein solutions, scientific principles relating to protein purification, and the similarities between rHSA, pHSA, and BSA. I have endeavored to summarize this extensive prior knowledge in the following sections of my declaration drawing on exemplary publications that may be generally grouped as follows:

- General knowledge of cell culture media, including serum-free media, e.g., a review of media for serum free culture by Jayme et al. (EX1010), a reference book titled *Animal Cell Technology* edited by Castilho et al. (EX1011);
- General knowledge of human serum albumin, its binding to other molecules, and the use and role of albumin in cell culture media, e.g., Exhibits EX1011 through EX1023, including a reference book titled *All About Albumin* by Peters et al (EX1013);
- General knowledge of the benefits of replacing animal-sourced proteins with recombinant proteins in cell culture media, e.g., Exhibits EX1004, EX1005, EX1024, EX1032;

- General knowledge regarding transgenic plants for the production of rHSA and other recombinant proteins, e.g., a prior patent assigned to Ventria (EX1024), a reference book titled *Molecular Farming* (EX1031), a review of recombinant proteins in transgenic plants (Kusnadi, EX1033), publications discussing the production of rHSA from transgenic plants and/or plant cells (Exhibits EX1034 through EX1036);
- General knowledge regarding the problems associated with endotoxins in cell culture and related protein preparations, e.g., Exhibits EX1025 through EX1030 and EX1037 and EX1045;
- General knowledge of endotoxin contamination in plant-produced recombinant proteins, particularly those grown in soil (dirt), e.g., Exhibits EX1038 through EX1041;
- General knowledge regarding the removal of endotoxin from protein solutions, including albumin, e.g., review articles published by Magalhaes et al. (EX1042) and Petsch (EX1043) and publications exemplifying such purification processes by Aida et al. (EX1044) and Reichelt et al. (EX1046);

- General knowledge regarding the detergents used in common protein purification steps, that such detergents should be removed from protein solutions to concentrations preferably below 10 ppm, and processes for their removal to less than 10 ppm, less than 5 ppm, and even less than 1 ppm, e.g., Exhibits EX1007 through EX1009, EX1047 through EX1051, EX1042 and EX1044, and EX1059;
- General knowledge regarding the principles of purifying native protein and recombinant proteins, e.g., the reference book, *Molecular Farming* (Exhibit EX1031);
- General knowledge regarding the strikingly similar properties of rHSA, pHSA, and BSA as they pertain to protein purification, e.g., Exhibits EX1005, EX1017, and EX1053 through EX1056.

63. I elaborate on the wealth of information known to a POSA in the following sections of my declaration.

A. Cell Culture Media

64. Cells grown in culture media require several nutrients for growth, proliferation, and productivity (e.g., producing biological products of interest). *See, e.g.,* EX1010 (Jayme), 2-4. As early as 1955, Eagle developed a “minimal essential medium” (MEM) formulation comprising salts, amino acids, sugars, vitamins, and other nutrients. *Id.*, 2. Eagle’s media required supplementation with plasma, serum,

or tissue extracts to support cell growth. *Id.* The amounts of nutrients in MEM were later increased to prepare Dubelco's modified MEM, called DMEM. *Id.*

65. Other nutrient mixtures were also common, including "Ham's Nutrient Mixtures (F-12 and F-10)." *Id.* F-12 was combined with DMEM to prepare a fortified basal medium. *Id.* This combination of DMEM/F-12 "emerged as the most widely utilized basal synthetic medium"; however, it still required supplementation with serum. *Id.*

66. A combination of insulin, transferrin, selenium, and ethanolamine (often abbreviated "ITSE" or "SITE") was later developed to support cell growth in media. *Id.*, 3. This "cocktail" of four additives had been commercialized by several suppliers by 1997 (when Jayme published its review). *Id.* ITSE was combined with DMEM/F-12 to "permit serum free cultivation of many cell types." *Id.*

67. Many modifications of these basal media were developed. *Id.*, 5. For example, for some applications, it was reported that lactoferrin was a superior substitute for transferrin (a component of ITSE). *Id.*

68. When serum is used in cell culture media, it provides numerous nutrients to cells, including albumin, transferrin, growth factors, insulin, growth hormones, and minerals such as iron, copper, selenium, and zinc, as summarized below.

Table 5.3 Serum components and their functions in the animal cells cultured <i>in vitro</i> (adapted from Freshney, 2005)	
Component	Probable function
Proteins	
Albumin	Osmotic control and buffering agent, transport of lipids, hormones and minerals, mechanical protection
Fetuin	Cell attachment
Fibronectin	Cell attachment
α_2 -Macroglobulin	Trypsin inhibitor
α_1 -Antitrypsin	Trypsin inhibitor
Transferrin	Binds iron
Growth factors	
Endothelial growth factor (ECGF)	Mitogen
Epidermal growth factor (EGF)	Mitogen
Fibroblast growth factor (FGF)	Mitogen
Insulin-like growth factors (IGF-1 and IGF-2)	Mitogen
Platelet-derived growth factor (PDGF)	Mitogen and major growth factor
Interleukin-1 (IL-1)	Induces interleukin-2 release
Interleukin-6 (IL-6)	Promotes differentiation
Hormones	
Hydrocortisone	Promotes attachment and proliferation
Insulin	Promotes glucose and amino acids uptake
Growth hormones	Mitogen (present in fetal serum)
Metabolites and nutrients	
Amino acids	Cellular proliferation
Glucose	Cellular proliferation
Keto-acids (e.g. pyruvate)	Cellular proliferation
Lipids (e.g. cholesterol)	Membrane synthesis
Minerals	
Iron, copper, zinc, selenium	Enzyme cofactors and constituents
Inhibitors	
γ -Globulin	
Bacterial toxins from prior contaminants	
Chalones (tissue-specific inhibitors)	

Figure 1. Serum components and their functions in animal cell culture media. EX1011 (Castilho), 118.

69. As Castilho explains, the metals supplied by serum were “usually added” to cell culture, “mainly if the medium is not supplemented with animal serum.” EX1011, 117.

70. Serum-free media comprise a more well-defined and less variable mixture of nutrients than serum. *See, e.g.*, EX1028, 1-2. By 1991, it was reported that serum-free media comprising a basal medium supplemented with animal-sourced proteins such as insulin, transferrin, and albumin combined with lipids and selenium “can promote growth of a number of different cell types ... from a number of different sources and species.” EX1028, 4. As Glassy explains, the major protein components in serum-free media were albumin, insulin, and transferrin, where albumin “functions mainly as a carrier for membrane precursors, fatty acids and lipids.” EX1012 (Glassy), 2. Glassy further notes that serum-free media were classified as “high or low in lipid or fatty acid content” and as “low or high protein” content. *Id.* Glassy acknowledges that because “albumin is normally present in concentrations up to three orders of magnitude higher than other protein supplements, the distinction between low and high protein is really a reflection of the albumin content.” *Id.* In serum-free media, albumin concentrations typically range from 0.5 to 5 mg/mL. *Id.*, 4.

71. Throughout the years, researchers developed numerous media optimized for various cell lines, many of which comprised albumin. For example, Glassy summarizes a variety of serum free media that were used to grow lymphocyte-derived cells and hybridoma cells, many of which comprised albumin, ethanolamine, insulin, β -mercaptoethanol, selenium, and/or transferrin in addition to

a basal medium. *Id.*, 5 (Table III, lymphocyte-derived cells). *Id.*, 9 (Table IV, hybridoma cells). Garcia-Gonzalo refers to a number of commercial serum-free media that were available, including “Knockout Serum Replacement,” which was a mixture of amino acids, vitamins and antioxidants, trace elements (including selenium), and proteins—specifically, transferrin, insulin, and lipid-rich albumin. EX1021 (Garcia-Gonzalo), 3-4.

72. Thus, while researchers had not developed a universal culture media that would work for all cell types, a vast amount of information was available to those seeking to develop and/or optimize cell culture media for the growth and/or productivity of various cell lines. *See, e.g.*, EX1011 (Castilho), Chapter 5 (pp. 111-125); EX1021 (Garcia-Gonzalo); EX1010 (Jayme); EX1013 (Peters), 329-331.

B. Human Serum Albumin

1. Structure

73. Human serum albumin (HSA or pHSA) is a globular protein having a molecular weight of about 66,500 Da (66.5 kDa), which accounts for about 60% of the total protein in blood serum. EX1034 (Sijmons), 1; EX1014 (Dockal), 1. As with all proteins, HSA is a polypeptide consisting of amino acids. EX1034, 1. HSA has 585 amino acids connected in a specific order that defines its primary structure (the sequence of atoms in its chemical structure). EX1014, 1.

74. This chain of 585 amino acids twists and folds to form albumin's secondary and tertiary structures (in a manner dictated by associations between the 585 amino acids in the chain), schematically depicted in Figure 2. HSA evolved from an “ancestral protein of about 190 amino acids by triplication of this primordial domain.” EX1014 (Dockal), 1. As a result, HSA is composed of “three structurally similar globular domains, each of which contains two subdomains, denominated IA, IB, IIA, IIB, IIIA, and IIIB.” EX1014 (Dockal), 1. The similarity in HSA's three domains (I, II, III) provides it with “striking internal homology,” where the domains share many common structural features. *Id.*, 2, 3, Fig. 2A-C (showing the similar structure of HSA's three domains).

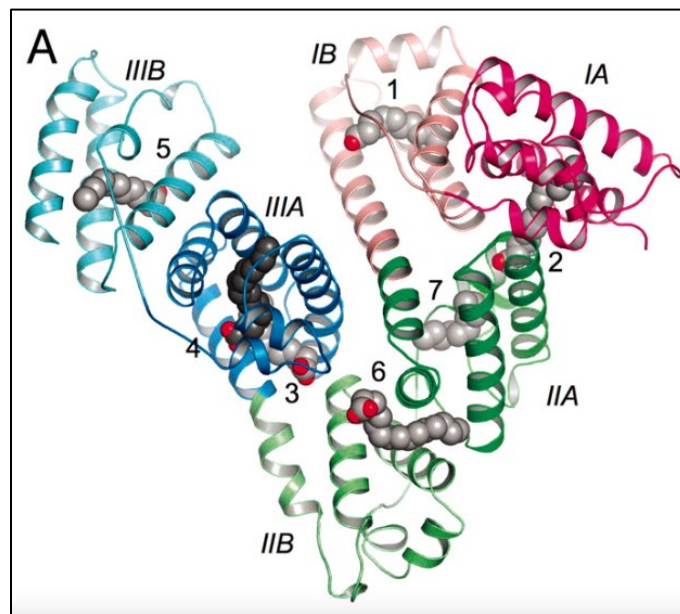


Figure 2. Structural depiction of HSA. EX1015, 2 (Fig. 1A).

75. The secondary and tertiary structure of albumin creates sites or pockets into which ligands such as fatty acids are bound. In the schematic above, the ribbons

represent the amino acid chain of albumin, and the gray spheres correspond to bound fatty acids. As Simard explains, “HSA is the primary transporter for delivering FA [fatty acids] to the tissues and possesses at least seven binding sites for this ligand.” EX1015, 1. The structural figure above also labels the domains (I, II, and III) and subdomains (A and B) of albumin (which often contain duplicative binding sites, e.g., each domain has a binding site for fatty acids).

76. In *All about Albumin*, Peters explains that HSA folds into its three domains by 17 intrachain disulfide bonds. EX1013 (Peters), 13. HSA also contains a free thiol on one cysteine (Cys-34). *Id.*, 72. The Cys-34 residue may also bond to a Cys-34 residue on another HSA molecule via a disulfide bridge, forming an albumin dimer (two albumins linked together), discussed further below. *Id.*, 74-75.

2. Albumin Binding to Ligands

77. HSA is capable of binding a variety of biomolecules, including fatty acids and other lipids, steroid hormones, a variety of pharmaceuticals, metals such as zinc, and enzymes, to name a few, which allows albumin to function as a carrier of essential nutrients to cells. EX1013 (Peters), 97-147; EX1019, 1 (Matejtschuk). In cell culture, “serum albumin has been recognized as a carrier of supplemented fatty acids and lipids.” EX1011 (Castilho), 92.

78. Peters explains that long-chain fatty acids are among the ligands that are “[m]ost strongly bound” to albumin. EX1013 (Peters), 97. Albumin in human plasma “typically carries 1 to 2 LCFA [long-chain fatty acid] molecules.” *Id.*, 101.

79. Researchers seeking to study albumin with low fatty acid content developed specialized techniques for their removal, including reducing the pH to 3 with formic acid to release bound fatty acids, which can be removed by a hydrophobic resin once they are detached from albumin (in this process, the pH is lowered to ensure that the fatty acids are protonated, as they would not otherwise bind effectively to the HIC sorbent). EX1013 (Peters), 337. As another example, it was reported that treating albumin (BSA) with charcoal reduced the fatty acid content to less than 0.1 mole of fatty acid per mole of albumin. EX1016 (Noel and Hunter), 6.

80. As noted above, HSA has been widely used as a supplement to promote the growth, viability, and/or productivity of cells in culture media. At times it is the nutrients by albumin, as opposed to the albumin itself, that promote the growth in cell culture. For example, the lipids naturally bound to albumin have been reported to be important for promoting the growth embryonic stem cells, such that albumin depleted of lipids did not promote growth, while albumin loaded with lipids did. EX1021 (Garcia-Gonzalo), Abstract.

81. As another example, Glassy reports that lipids or lipid precursors are important for hybridoma growth in serum-free media, noting that albumin is a source of such lipids. EX1012, 12. Castilho similarly notes that unsaturated fatty acids “have been shown to be essential for hybridomas grown in serum-free cultures, as they significantly enhance the cell yield and monoclonal antibody (mAb) productivity.” EX1011, 132.

3. Albumin Dimers and Larger Aggregates

82. Albumin may exist as a monomer (one albumin unit), a dimer (two albumin units), or oligomers (comprising three or more albumin units). *See* EX1017 (Atmeh), Abstract (“Albumin in solution exists as a monomer and higher aggregates.”).

83. Albumin aggregates tend to dissociate into monomers and/or dimers in solution. EX1017, Abstract, 7. As Atmeh explains, in a solution of albumin monomers, dimers, and larger aggregates at 8 °C, the larger aggregates dissociate into smaller forms “within a few hours,” leaving only albumin monomers and dimers. EX1017, Abstract; *id.*, 7 (“When the solutions of both BSA and HSA were stored for 4 months at 8 °C, oligomers dissociated into dimer[s] (17-23%) and monomer[s] (77 – 83%) with no higher aggregates.”); *see also* EX1054 (Jachimska), 3, 6, 7 (reporting that solutions of HSA and BSA contained “a significant fraction of protein dimers,” while no larger linear aggregates were observed).

84. The presence of aggregates in powdered samples of albumin was associated with the processes used to prepare the powders, e.g., lyophilization or freeze-drying, e.g.:

- “The presence of oligomers in the freshly prepared solutions of different commercial BSA and HSA albumin preparations can be attributed to the preparation procedures including lyophilization.” EX1017 (Atmeh), 11;
- “S-S linked dimers constitute the majority of the 5-10% polymeric forms found in most albumin preparations that have been lyophilized during production.” EX1013 (Peters), 75;
- “[A]ll the freeze-dried albumin fractions exhibited lower sulfhydryl content and higher dimer contents than the corresponding freshly eluted fractions” EX1016 (Noel and Hunter), 6;
- “[P]roteins that have been lyophilized (which is how research and pharmaceutical preparations are usually stored) undergo moisture-triggered aggregation,” which was an “undesirable phenomenon.” EX1018 (Griebenow & Klibanov), 1.

85. I note that the terms lyophilization and freeze-drying are often used synonymously. *See, e.g.*, EX1018 (Griebenow & Klibanov), 1 (referring to the “common laboratory and bioindustrial process of lyophilization or freeze-drying of

aqueous solutions of proteins.”); *id.* (describing lyophilization, which involves freezing protein solutions and drying with a freeze-drier at reduced pressure).

86. Griebenow and Klibanov report that the structural changes in lyophilized samples of rHSA (and other proteins) were reversible upon reconstitution in water. EX1018, 7.

87. Heat may also induce albumin aggregation, unless sufficient fatty acids are present. EX1020 (Brandt and Anderson), 1-2; EX1013 (Peters), 88 (noting that octanoate and long-chain fatty acids protect HSA from denaturation at 60 °C).

88. As Peters explains, when a solution of albumin is heated, “the fatty acids dissociate and move among albumin molecules,” and the albumins “that are temporarily fat free become denatured.” EX1013, 88-89. These fat-free albumins are “unprotected,” “readily susceptible to heat,” and “will aggregate and precipitate at 63 °C.” *Id.*, 88-89. By contrast, the remaining albumin molecules “accumulate fatty acids at higher molar ratios and become highly resistant to heat.” *Id.*

C. Non-Animal Cell Culture Media Supplements Were Recognized as Desirable

89. Historically, components of cell culture media were obtained from animal sources. EX1005 (Deeter), [0006]. However, cell culture components extracted from animals were known to have “several undesirable features,” including the risk of contamination with pathogens of animal and human origin such as mad cow disease (bovine spongiform encephalopathy, BSE), HIV, hepatitis virus,

and prions. EX1005 (Deeter), [0006]-[0007]; EX1024, 1:32-35 (“Production of blood proteins from plants mitigates contamination of the blood protein fraction with human viruses and other disease causative agents found in human or animal blood product fractions.”); *see also* EX1011 (Castilho), 121 (“Another factor to be taken into account when using animal serum is the potential risk for human health, due to the possible presence of adventitious agents, such as viruses and proteins as prions. In addition, serum can contain contaminants such as bacteria, fungi, and mycoplasmas (small bacteria without cellular walls), which can negatively affect cell culture.”). As Hatsuyama explains, the use of serum-free media comprising rHSA makes it “possible to reduce contamination with viruses and prion[s].” EX1004, [0016].

90. Such safety concerns prompted regulators to recommend replacing animal components with recombinant proteins in vaccine production and other applications of cell culture techniques. EX1005 (Deeter), [0007].

91. It was thus recognized that while animal-derived components could be used in cell culture media, it was “desirable to replace those components with equivalent materials that are not derived from animal sources.” EX1025 (Schenerman), 1. By 2003, “[o]ften it is possible to find a microbial or plant-derived component that meets or exceeds the desired functionality of the original animal component.” *Id.*

92. With regard to albumin specifically, Van der Valk explains, “Recombinant human albumin (r-HSA) can be used instead of purified human albumin. r-HSA is structurally identical to HSA and provides the following advantages: it is safe – free from viral and prior contamination; and it is consistent – greater homogeneity and high batch-to-batch consistency.” EX1032, 9.

D. Transgenic Hosts Expressing rHSA

93. By 2007, transgenic rice seeds had been used to express rHSA that was equivalent to pHSA at exceptionally high yields (up to 8.5 g/kg rice flour). EX1005, [0105], [0117]. *Molecular Farming* notes that transgenic rice “emerged as the model cereal species,” and many tools for using rice to express recombinant proteins had been developed. EX1031, 75.

94. In general, producing rHSA in transgenic plants such as rice seeds was expected to provide cost benefits. EX1039, 4 (“Cost benefits of plant production are enormous.”); EX1031 (*Molecular Farming*), 5 (“Over the last decade, plants have emerged as a convenient, safe and economical alternative to mainstream expression systems which are based on the large-scale culture of microbes or animal cells, or transgenic animals.”). Transgenic plants such as rice were touted as “potentially one of the most economical systems for large-scale production of recombinant proteins for industrial and pharmaceutical uses.” EX1033 (Kusnadi), 1. This was, in part, due to the high grain yield achievable with cereal crops such as rice. EX1031 (*Molecular*

Farming), 56. Plants in general were understood to “have numerous advantages over traditional expression technologies,” e.g., fermentation-based systems. *Id.*, 206. Indeed, it was estimated that proteins could be produced in plants at 2-10% of the cost of microbial fermentation systems (such as those used for yeast), as long as adequate yields were achieved (as they were in Deeter). *Id.* Moreover, the “start-up and running costs for molecular farming in plants are significantly lower than those of cell-based production systems because there is no need for fermenters or the skilled operators to run them.” *Id.* For example, “the costs involved in establishing small, non-sterile greenhouse facilities are dwarfed by those required for a sealed pilot fermentation plant.” *Id.*, 286. By contrast, for rHSA available as of 2004 (which would not have included rHSA expressed using Deeter’s technology), the “major disadvantage” was “the price.” EX1032 (Van der Valk), 9; EX1058, 150:8-11 (testimony of Ventria’s CEO Mr. Deeter that yeast-derived rHSA was “very, very expensive,” which “limited the market opportunity for the product, because it was just too expensive.”).

95. While tobacco- and potato-derived rHSA had also been prepared, the rHSA was expressed in very low yields of, e.g., 0.01 g/kg plant material. EX1035, 5; EX1034, 2; *see also* EX1031, 239 (reporting that plants had “relatively low expression yields” of “much less than 100 mg of active recombinant protein per kg of plant material”). These low yields were not considered cost effective, as explained

by Farran, which notes that the industry estimated that a cost-effective yield “appears to be ca. 0.1 g of HSA per kg of fresh weight.” EX1035, 9. As noted above, transgenic rice yielded rHSA at levels up to 8.5 g/kg rice flour. EX1005, [0105].

96. Transgenic tobacco had the additional problem of producing toxic secondary metabolites. EX1036 (Huang 2005), 10. Transgenic rice, by contrast, was “a well-characterized food crop that does not produce any toxic secondary metabolites.” *Id.* And transgenic seeds (e.g., rice seeds) provide a “better alternative” to plant leaves (e.g., tobacco leaves) as (1) seeds “contain a less complex mixture of proteins and lipids,” which is “an advantage for purification,” and (2) the stability of recombinant proteins was expected to be higher in seeds. EX1031 (Molecular Farming), 297.

E. Endotoxins

97. Endotoxins, or lipopolysaccharides (LPS), comprise part of the outer membrane of gram-negative bacteria such as *E-coli*. EX1023 (David), 1. A single *E-coli* cell contains about 2 million endotoxin molecules per cell. EX1027 (Ryan), 3. Bacteria constantly shed endotoxins, in small amounts when they are alive, and in large amounts when they die. *Id.*

98. Endotoxins have high heat stability and a tendency to aggregate in solution to form very large aggregates of, e.g., 1 kDa in aqueous solution. *Id.*

99. Endotoxins are everywhere. *E.g.*, EX1030 (Majde), 1. They are present in tap water, sera, and can bind to glassware and plasticware. EX1027 (Ryan), 4-5; EX1011 (Castilho), 114-15 (explaining that water is a source of endotoxin contamination and recommending the use of water meeting “strict purity standards” for use in cell culture, *e.g.*, water for injection).

100. As Majde explains, endotoxins ubiquitously contaminate all materials that are not processed using aseptic techniques, unless they are removed by certain procedures. EX1030 (Majde), 1; *see also* EX1025 (Schenerman), 1-4 (noting bacterial contamination in plant-produced proteins and recommending testing for, *e.g.*, endotoxins).

101. Notably, HSA binds endotoxin. *E.g.*, EX1022 (Jurgens), 1; EX1023 (David), 5 (noting that “serum albumin is a major LPS-binding factor,” where LPS means endotoxins); EX1011 (Castilho), 120 (noting that albumin “can act as a buffering or detoxifying agent (due to its binding to endotoxins), as well as serving as a carrier for vitamins, hormones, and other micronutrients.”).

102. As rHSA has the same structure as HSA (*e.g.*, the same amino acid sequence and thus the same folding and ligand binding site), it also binds endotoxins. EX1022 (Jurgens). For example, Jurgens reports that when endotoxin (LPS) is added to a solution of rHSA, “a high number of LPS molecules can be bound by each

albumin molecule,” with binding saturating at about 10 endotoxins per molecule of rHSA. *Id.*, 10.

F. The Art Recognized Problems Associated with Cell Culture Media Supplements Contaminated with Endotoxin

103. The problems with endotoxins in cell culture are two-fold.

104. First, endotoxins may contaminate a biological product of interest, posing a “serious threat to these products.” EX1026 (Gorbet), 1; EX1029 (Dawson),

1. For example, “[e]ndotoxin contamination of cell-based products is a major concern to the pharmaceutical industry, since endotoxins in vaccines and other cell-based parenteral drug products render them unfit for use.” EX1027 (Ryan), 8.

105. Second, endotoxins may cause significant variability in biological experiments, complicating efforts to draw reliable conclusions. EX1026 (Gorbet), 1; EX1027 (Ryan), 5-8. As Dawson explains, “contamination with endotoxin of some preparations commonly used in biology may give false results,” and by 1998, there had been “at least one call for the ‘absolute absence’ of endotoxin in tissue culture media.” EX1029 (Dawson), 3. As Bito explains, the use of proteins contaminated with endotoxins “in biological research can lead to erroneous conclusions and should, therefore, be avoided.” EX1037 (Bito), 1. Ryan echoes this concern, noting that while certain cell lines may have developed resistance to endotoxin over time (making contamination with endotoxins less important), in many systems, endotoxins affect cell growth and function and are “a source of significant

variability.” EX1027, 7, 8. As Magalhaes explains, endotoxins “are major contaminants found in commercially available proteins or biologically active substances, which often complicate study of the biological effects of the main ingredient.” EX1042, 1.

106. The problems associated with cell culture media contaminated with endotoxins have long been recognized in the art. For example, in 1977, Hewlett explained that when seeking to prepare serum-free media, the first “basic rule[]” is to use components “that have the lowest possible levels of bacterial endotoxin.” EX1028 (Hewlett), 2.

General principles

There are some **basic rules** that apply to the development of serum-free cell culture media whether or not the final medium is destined for a 5000 L fermenter tank or for a 5 mL culture flask:

1. **Use media and supplements that have the lowest possible levels of bacterial endotoxin.**
2. Prepare only minimal amounts of basal medium at any one time – a two week supply kept at 4°C is recommended. This avoids decomposition of the various components. Cells

Figure 3. Excerpt of Hewlett (EX1028), 2.

107. Peters similarly explains that albumin for use in cell culture should optimally be endotoxin free. EX1013 (Peters), 329 (“The albumin products should

optimally be those certified as nondenatured, endotoxin and Mycoplasma free, generally fat free, and optimally pretested for support of the tissue under study.”).

108. While it was well-known that cell culture media supplements should be essentially free of endotoxins, it was also known that cell culture media supplements at times failed to satisfy that requirement. *See* EX1026 (Gorbet), 1 (“Commercially available laboratory reagents used in cell culture . . . have also been found to contain endotoxin and induce a significant biological response.”); EX1030, 2 (noting that significant concentrations of microbial cell-wall products (MCWP), e.g., endotoxins, were found in common reagents such as non-USP serum albumin and recombinant hormones).

109. Majde explains that unless endotoxins are removed by certain procedures, they are “essentially ubiquitous contaminants of materials that are not processed using the aseptic good manufacturing practice (GMP) conditions employed by the pharmaceutical industry.” EX1030 (Majde), 1. While GMP-grade or USP-grade products certified for human use are generally free of detectable endotoxins, GMP and USP-grade manufacturing techniques are not commonly used for the production of research reagents. *Id.*

110. The art thus taught that where the level of endotoxin in a reagent for use in cell culture was not known, the product should be tested by the investigator prior to use.

- “Whenever possible, ask manufacturers to certify the endotoxin levels of their products; when in doubt test the media endotoxin levels both before and after adding any new components.” EX1027 (Ryan), 4;
- “We advocate routine testing of endotoxin on biomaterials and reagents used in evaluation of biomaterials and this should be the responsibility of every scientist to ensure the validity of any biomaterial study.” EX1026 (Gorbet), abstract. “If the certificate of analysis of a reagent or buffer does not mention endotoxin level, one should assume that it is contaminated.” EX1026, 4;
- “The endotoxin content of processed biological materials should be stated by the supplier, or should be tested by the investigator before they are used in biological research.” EX1037 (Bito), 3;
- “If you purchase peptide X, query the manufacturer as to whether X has been manufactured under GMP conditions or at least tested for endotoxin by the Limulus test. If the manufacturer is not familiar with these terms, then you must assume that X has been produced under conditions that could lead to MCWP [microbial cell-wall product, e.g., endotoxin] contamination. Batch inconsistencies are a strong indication that MCWP may be involved in the peptide response (23), regardless of source.” EX1030 (Majde), 3.

111. Thus, it was well-known that cell culture media should be essentially free of endotoxins, and where the level of endotoxins in a cell culture media

supplement was not known, it should be tested before using the supplement in biological research.

G. The Art Recognized that Unless Removed, Endotoxins Would Be Present in Proteins Produced in Plants

112. It was understood that alternatives to animal-derived raw materials, e.g., plant-derived substitutes, may be contaminated with endotoxins. EX1025 (Schenerman), 1, 2.⁴ For example, Schenerman cautioned that animal-free may not mean problem free. EX1025, 1.

113. Thus, while Schenerman advocates for the use of animal-free products, including plant-derived components that may meet or exceed the functionality of the original animal component, it acknowledges that such products may be a source of impurities such as endotoxin, and it specifically identifies contamination with bacteria—the source of endotoxin—as a risk factor for grains and seeds (e.g., rice). EX1025 (Schenerman), 4 (Table 2).

⁴ *Molecular Farming* similarly notes that “plant-derived products” will require purification, and GMP practices would require “quantification of endotoxins.” EX1031 (Molecular Farming), 300. Schenerman also recognizes that proteins expressed in grains or seeds may be contaminated with bacteria, e.g., *E. coli*, which are a source of endotoxins. EX1025, 4.

114. I note that while aseptic suspension cultures of plant cells in a bioreactor had a relatively low risk of endotoxin contamination, it was understood that the same was not true for plants grown in dirt. *See, e.g.*, EX1038 (McDonald), 2. For example, McDonald explains that the use of bioreactors (as opposed to plants grown in dirt) “reduced potential for endotoxin and mycotoxin contamination derived from the plant and soil source.” *Id.* Larrick also notes that where “the processed plant or seed” was “grown in dirt,” one would expect a contamination with endotoxins.⁵ EX1039 (Larrick), 4. *Molecular Farming* similarly reflects the understanding that transgenic plants grown in soil may be contaminated with, e.g., soil bacteria (a source of endotoxins). EX1031, 237.

115. Given the known risks of endotoxin contamination, techniques were employed to avoid introducing endotoxins during the purification of recombinant proteins expressed in transgenic plants, including transgenic wheat and transgenic rice. EX1040 (Brereton); EX1041 (Fujiwara).

⁵ While Larrick notes that “rapid processing and early filtration” may minimize endotoxin contamination (EX1039, 4), filtration would not have been expected to meaningfully separate endotoxins from albumin due to its size and ability to bind endotoxins. EX1043 (Petsch), 8.

116. For example, Brereton reports that extracts from transgenic wheat seeds “were prepared to avoid the introduction of exogenous endotoxin,” including by cleaning equipment with Etoxaclean and using sterile, endotoxin-free (pyrogen-free) water to prepare solutions. *Id.*, 3. Using these measures, Brereton reports that crude extracts from the mature seeds from a transgenic wheat line were found to contain about 11.4 mg/ml soluble protein (*id.*, 4) and about 150 endotoxin units (EU)/ml (*id.*, 6). Brereton explains that since steps were taken to avoid endotoxin contamination during extraction, the residual endotoxins were likely “derived from gram-negative bacteria resident on the wheat seeds during growth and storage,” and the residual endotoxin was expected to be removed “easily.” *Id.*, 7.

117. Similarly, Fujiwara reports taking steps to avoid introducing endotoxin contaminants into samples of recombinant proteins expressed in transgenic rice. EX1041, 5. For example, Fujiwara dry-heated all glassware at 250 °C for 90 minutes

to decompose any endotoxins and used Milli-Q water (endotoxin-free water)⁶ during purification. *Id.*, 2. Fujiwara notes that buffers “were prepared just before use with dry-heated glassware and fresh Milli-Q water.” *Id.* The authors reported that after purification by anion exchange chromatography, cation exchange chromatography,

⁶ As reported elsewhere, water purified using the Milli-Q system is endotoxin-free. EX1045 (Elsheikh), 3 (Table 4); *see also id.*, 4 (noting that cell culture media prepared using tap water containing more than 2.4 EU/ml had “a significantly lower proportion of hatched blastocysts compared with M-Q [Milli-Q] water.”). By contrast, purifying water by “distillation and deionizing columns does not remove endotoxin,” making laboratory water “the most conspicuous source of endotoxin during material synthesis in the laboratory.” EX1026 (Gorbet), 4.

and gel permeation, the proteins contained about 0.000024 EU of endotoxins/ μ g protein (i.e., 0.024 EU/mg).⁷ *Id.*, 5.

H. Methods of Removing Endotoxins Were Well Known and Readily Utilized

118. Techniques for removing endotoxins from numerous proteins were well-known, including techniques based on ultrafiltration, chromatography, and two-phase separation using detergents. EX1043 (Petsch), 7-19.

119. The selection of the proper purification technique was understood to depend in part on the physicochemical properties of the protein of interest (e.g., size, hydrophobic/hydrophilic character, isoelectric point). EX1043, 7, 8, 12. As such, I focus my discussion below on techniques that were successful for albumin.

⁷ I note that Fujiwara indicates that it was available on-line as of February 14, 2010, which is four days before the '389 patent application was filed on February 18, 2010. If there is a dispute over whether Fujiwara was available to a skilled artisan prior to the priority date of the '389 patent, Fujiwara still reflects the knowledge of those in the field regarding techniques for excluding endotoxins from recombinant proteins expressed in transgenic plants (i.e., it is evidence that those in the field were aware of and employed such techniques).

120. Chromatography columns having an affinity for endotoxins had also been developed and successfully used to remove endotoxins from BSA. EX1043, 12-14. For example, an endotoxin-selective sorbent (PLL-Sepharose 4B) was used to decontaminate a solution of 1 mg/ml BSA containing 2,500 EU endotoxin/ml of solution, where the endotoxin level was depleted to about 1-2 EU endotoxin/ml of solution, as shown in Fig. 5B of Petsch. EX1043 (Petsch), 12, Fig. 5B.

121. While affinity chromatography worked to some degree, the technique that proved most effective for albumin was phase separation using Triton X-114. EX1044 (Aida), Abstract, 4; EX1042 (Magalhaes), 10. As Magalhaes explains, this technique provides a convenient method for removing endotoxin from solutions of albumin, where endotoxin was reduced by 1,000-fold in the first cycle, and further cycles resulted in “***complete removal of endotoxin***” from albumin and other proteins (cytochrome *c* and catalase). EX1042, 10 (citing Aida, EX1044). In this technique, Triton X-114 “aids in the dissociation of endotoxin from the protein, while also providing a convenient phase separation capability for removing the dissociated endotoxin.” EX1042, 10. Moreover, “[p]roteins treated by this procedure retained normal functions.” EX1044, Abstract.

122. As Aida explains, the “[c]omplete removal of endotoxin” achieved means that endotoxins were “undetectable by the *Limulus* assay.” EX1044, 4. Given the sensitivity of the *Limulus* gel assay used by Aida, which can detect endotoxins

down to levels of 0.03 EU/ml (EX1027 (Ryan), 4), Aida's purified solution of 30 mg/ml albumin would have contained at most 0.001 EU endotoxin/mg albumin (calculated as the sensitivity of the assay, 0.03 EU/ml, divided by 30 mg/ml albumin). *See also* EX1026 (Gorbet), 4 (noting that the sensitivity of the *Limulus* gel clot assay is 0.03 EU/ml).

123. As an added benefit, each cycle of phase separation could be completed within 15 minutes. EX1044 (Aida), Abstract. Thus, phase separation "provides a rapid and gentle method for removing endotoxin from protein solutions." EX1044, Abstract. As Magalhaes notes, "[i]n addition to its simplicity," endotoxin removal using Triton X-114 "is cost effective, especially at a large scale." EX1042, 11. Indeed, the 15-minute cycles "can be repeated multiple times until endotoxin in the aqueous phase reaches a satisfactory level." *Id.*

124. Phase separation using Triton X-114 had been used to purify recombinant proteins expressed in *E-coli*, e.g., proteins expectedly contaminated with very high loads of endotoxin (as each *E-coli* contains about 2 million endotoxin molecules). EX1042, 10-11. Triton X-114 had also been used in combination with affinity chromatography to remove endotoxins from tagged recombinant proteins expressed in *E-coli* from levels of 2500 EU endotoxin/mg protein to as low as 0.4 to 0.2 EU endotoxin/mg protein. EX1046, 4.

125. Two-phase separation using detergents such as Triton X-114 was a useful technique for proteins like albumin, because it partitions the endotoxins into a detergent-rich micellar phase, while proteins like albumin preferentially remain in the micelle-poor aqueous phase, as depicted schematically below:

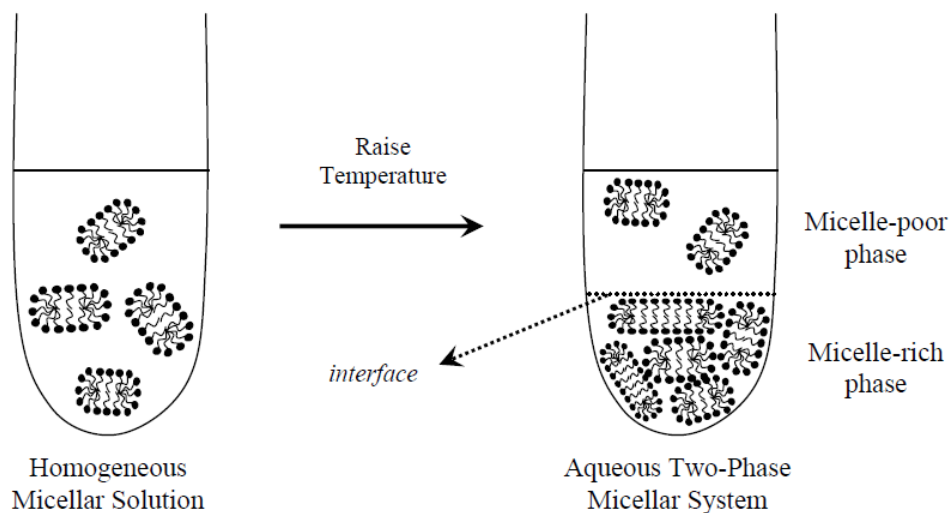


Figure 4. EX1042, 10 (Fig. 3).

126. Above left, the figure depicts the initial mixing of the protein solution contaminated with endotoxin to form micelles, where the endotoxins are inside the micellar structures and the water-soluble protein (albumin) is outside. *See* EX1043 (Petsch), 10. This mixing step is typically performed below the “cloud point” of Triton X-114 of 22 °C, e.g., at 4 °C. *See id.* As the temperature is increased to above the cloud point, “micelles aggregate to droplets with very low water content, by that forming a new phase.” *Id.* Endotoxins remain in the detergent-rich phase—the micelle-rich phase depicted above right (the lower portion of the diagram). *Id.*

I. It Was Well Known that Endotoxin-Removing Detergents Should Be Removed from Protein Preparations

127. It was established long before 2010 that detergents used to purify proteins must be removed. For example, Aida noted that residual Triton X-114 present at 0.018% was harmful to cells. EX1044, 4. Aida and Magalhaes thus teach that the detergents used for protein purification must be removed. *Id.*, 4-5; EX1042 (Magalhaes), 10. Indeed, it was reported that the effective concentration of Triton X-114 to kill half of the cells in a sample was 6.88 mg/L (6.88 ppm). EX1047, 4-5; *see also* EX1048, 4 (selecting Triton X-100 as a known, widely used detergent to compare different cytotoxicity assays, where Triton X-100 was already known to cause “rapid cell death by necrosis” caused by permeabilization of the cell membranes, and calculating EC₅₀ concentrations for Triton X-100 of 31-80 μ m, depending on the assay).

J. Techniques for Removing Detergents Were Well Known

128. Prior to the '389 patent, it was well-known in the art that hydrophobic interaction chromatography (“HIC”) could be used to remove detergents from protein solutions. EX1049, 1. As Bonomo explains, “[h]ydrophobic interaction chromatography (HIC) is a commonly used tool in the biochemists’ arsenal of molecular separation techniques.” EX1059, 3:31-33. The advantage of this technique was that it was “fast and it induces minimal impact on the protein composition.” EX1049, 1.

129. In a patent filed in 1988, Bonomo teaches that a column containing Bulk C-18 media (an HIC sorbent) reduced levels of Triton X-100 in plasma preparations to an average of 4.69 ppm (ranging from 1.5 ppm to 8.0 ppm). EX1059, Example 1 and Table 1 (column 9).

130. This technique had been widely employed prior to 2010. For example, in 1992, Piquet reports that castor oil and a hydrophobic “C18” resin successfully reduce the amount of a detergent (octoxynol 9) used to treat blood plasma by 2000-fold. EX1050, 2, 4. Piquet’s plasma samples comprised about 60% albumin and, after purification, comprised 55 g/l proteins (55 mg/ml), 0.88 EU endotoxins/ml plasma (about 0.02 EU endotoxin/mg protein), and less than 5 µg/ml detergent (less than 5 ppm detergent). EX1050, 4.

131. In 1995, Guerrier reports that another HIC sorbent, SDR⁸ HyperD, allows for the removal of detergents at “a high capacity in order to treat large amounts of [virally] inactivated biological fluids in small-sized columns.” EX1051, 1. Guerrier reports that SDS HyperD efficiently removes Triton X-100 from antithrombin III (ATIII), immunoglobulins G (IgG), and bovine serum. *Id.*, 5-6. Guerrier reports achieving 100% removal efficiency for ATIII and IgG, where the levels of Triton X-100 were less than 10 ppm. *Id.*, 6 (Table 1). The efficiency of

⁸ SDR stands for solvent-detergent removal.

removal for bovine serum was less (95.2%), which Guerrier attributes to the presence of hydrophobic or amphiphilic components in serum, although the efficiency was increased to 99.5% by loading a smaller amount of serum onto the column. *Id.*, 5-6. I note that albumin would not have been expected to contain the “hydrophobic or amphiphilic components” that reduced the efficiency of detergent removal in Guerrier’s experiment with bovine serum (which is a complex “soup” that comprises more than just BSA).

132. Guerrier further suggests the use of SDR HyperD to purify solutions of human albumin. EX1051, 5. For example, Guerrier discloses that when a 10 mg/ml human albumin was injected in large amounts onto the SDR HyperD column, about 0.2-0.8 mg/ml of albumin per ml packing material was non-specifically bound to the sorbent. EX1051, 5. Guerrier explains that this protein loss is minimal, “as 200-500 mg of proteins can be treated per ml of SDR HyperD sorbent.” *Id.* This translates to a protein loss of no more than 0.4%,⁹ indicating that nearly all HSA (and equivalent rHSA) purified in a SDR HyperD column should be recovered.

⁹ For example, dividing the highest level of HSA potentially adsorbed (0.8 mg/ml) by the lowest amount of protein treated (200 mg/ml) equals 0.4% protein loss.

133. By 2009, C18 and SDR HyperD were the “HIC sorbents most often used for removal of S/D [solvent/detergent] agents from therapeutic protein products.” EX1049 (Burnouf), 2.

134. Van Holten characterizes SDR HyperD as the “first and most preferable adsorbent” for removing Triton X-100 from blood products. EX1007, 17:10-11. Van Holten teaches that SDR HyperD and another resin (Amberchrom CG161C) both removed Triton X-100 to below 1 ppm from RhoGAM® (an immunoglobulin formulation). EX1007, 17:10-22; *id.*, 9:34-36 (noting that RhoGAM® is an immunoglobulin formulation). Van Holten further teaches that “protein-containing solutions comprising mammalian milk, ascites fluid, saliva, placental extracts, tissue culture extracts, products of fermentation, transgenic derived products and recombinant proteins can all be purified by these methods.” EX1007, 2:11-17.

135. Burnouf compares the efficacy of SDR HyperD (used by Guerrier and Van Holten) and C18 (used by Piquet and Bonomo) in the removal of reagents used in viral inactivation, e.g., Triton X-45 and Triton X-100. *Id.*, 2-4. Burnouf finds that both sorbents performed comparably in the removal of Triton X-45 (to below 2 ppm), while SDR HyperD was superior to removing Triton X-100 (achieving levels as low as 7 ppm). *Id.*, 4 (Table 2). Burnouf further explains that these HIC sorbents provided nearly complete recovery of the target protein. EX1049, 4-5.

136. A POSA would have reasonably expected the efficiency of HIC in the removal of Triton X-114 would fall between Triton X-45 and Triton X-100, as the properties of Triton X-114 fall between those of Triton X-45 and Triton X-100. EX1052 (Wang), 2 (reporting known properties of Triton detergents). For example, the Triton detergents have the same basic structure (see below), where the average number of ethylene units, cloud point, critical micelle concentration (CMC), and hydrophobic lipophilic balance (HLB) of Triton X-114 each fall between the respective values for Triton X-45 and Triton X-100. EX1052, 2.

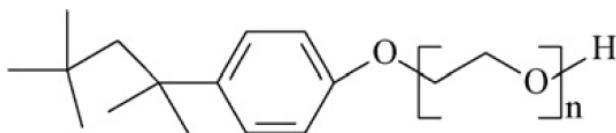


Fig. 1. Basic structure of Triton X series.

Table 1 Basic parameters of Triton X series				
Trade name	Average ethylene unit	Cloud point ^a (°C)	CMC (ppm)	HLB
Triton X-45	4.5	Dispersible	57	9.8
Triton X-114	7.5	25	100	12.3
Triton X-100	9.5	66	130	13.4
^a Aqueous solution containing 1% (v/v) nonionic surfactant.				

Figure 5. EX1052 (Wang), 2.

137. Thus, SDR HyperD would expectedly be effective for the removal of Triton X-114, just as it was effective for removing a slightly smaller Triton detergent (Triton X-45) and a slightly larger one (Triton X-100).

138. Of course, other HIC sorbents would have also been expected to be effective at removing detergents, including the Triton detergents. For example, Viswanathan teaches that certain grades of SEPABEADS® removed Triton X-100 to 5 ppm and 6.16 ppm. EX1009, [0070], [0073]-[0074], Tables 1-2. Viswanathan discloses optimal conditions for using SP825 SEPABEADS® to reduce the concentration of Triton X-100 to less than 1 ppm. EX1009, [0075]-[0076], Table 3 (explaining that a “proportion of 1:4 resin to plasma at 30 °C for 30-40 minutes was optimal for removal of solvent detergent” from plasma).

139. Thus, the prior art discloses suitable techniques for removing detergents to levels below 10 ppm, below 5 ppm, and even below 1 ppm from plasma (Piquet, Bonomo, Viswanathan) and blood proteins (Van Holten), including immunoglobulins (Guerrier, Burnouf), and further explains that HIC sorbents are suitable for purifying HSA (Guerrier) and recombinant proteins (Van Holten). *Supra*, ¶¶ 127-138.

K. Purifying Proteins in General

140. In general, the same techniques that successfully purify native proteins from non-host-specific contaminants can be used to purify recombinant proteins with similar or identical physicochemical properties, as such properties provide the basis for many purification techniques. EX1031 (Molecular Farming), 233 (noting that in downstream processing of recombinant proteins from different sources,

“there are more common steps than operations addressing expression system-specific problems or requirements” and that “the intrinsic features used for purification (affinity, hydrophobicity, etc.) are the same.”); *see also* EX1008 (Schulz), [0032] (noting that the disclosed purification technique is generally suitable for a protein (alpha-1-antitrypsin, A1AT) “obtained from plasma and its fractions or recombinant or transgenically expressed A1AT”); EX1007 (Van Holten), [0036] (explaining that the disclosed purification technique can be used to purify, e.g., blood proteins and “transgenic derived products and recombinant proteins.”).

141. As a result, “[w]ell-established procedures and protocols should therefore be utilized, and should be adapted to the special requirements of the source material *only when absolutely necessary*.” EX1031 (Molecular Farming), 233. (emphasis added). As noted in *Molecular Farming*, “*the majority of product-specific requirements for downstream processing are not associated with the particular expression system*,” leading to the conclusion that the potential economic advantages of plant production systems lie in the upstream rather than the downstream part of the process.” EX1031 (Molecular Farming), 235 (emphasis added).

142. For example, where a recombinant protein and its native counterpart have approximately the same molecular weight, isoelectric point (the pH at which the protein has a net neutral charge), and ligand binding capacity, the techniques for

purifying the native protein would have been expected to be generally applicable to purifying the recombinant protein to remove non-host specific impurities such as endotoxins and detergents.

143. This is particularly true for the techniques commonly used to purify proteins from the contaminants at issue here (endotoxins and detergents), as discussed further below. *Infra*, ¶¶ 149-153.

144. To be clear, purifying recombinant proteins expressed in transgenic plants may include many of the same techniques employed for their native counterparts. EX1031 (Molecular Farming), 243. As explained in *Molecular Farming*, protein purification involves three phases: (1) capture, (2) intermediate purification, and (3) polishing. *Id.*, 234-243. The capture phase “generally aims to concentrate the highly dilute starting material and remove bulk impurities, rather than achieving a high degree of purity by high resolution and selectivity,” and as such, “robust techniques such as ion exchange chromatography are often used to “capture” the target protein. *Id.*, 240. While the “capture” step may be optimized for a specific host (e.g., to remove the bulk plant impurities), the intermediate and polishing steps “will not significantly differ from established biotechnological processes.” EX1031 (Molecular Farming), 243.

L. rHSA, pHSA, and BSA Were Known to Share Similar and/or Equivalent Physicochemical Properties

145. rHSA expressed in transgenic rice was known to have physicochemical properties equivalent to pHSA (EX1005, Table 3), which in turn was well-known to have properties very similar to that of bovine serum albumin (BSA), as tabulated below. *See* EX1053, 1 (noting that BSA is “very similar to human albumin in terms of structure and physicochemical properties”).

Albumin	MW (kDa)	Amino Acids	pI
Rice-rHSA	66.8-67.9 ¹	585	5.3 ¹
pHSA	66.9-68.1 ¹	585	5.3, ¹ 5.1, ³ 4.7-4.9 ³
BSA	66.3 – 69 ² 67 ³	582 ²	5.1, ³ 4.7-5.1, ³ 4.7 ⁴
¹ EX1005, Table 3. ² EX1053, 1. ³ EX1054 (Jachimska). ¹⁰ ⁴ EX1055 (Brahma)			

146. Additionally, rHSA, pHSA, and BSA were all known to bind a large number of ligands, including fatty acids. EX1005, Table 3; EX1055, 2. This would have been expected based on the structural similarities in BSA and pHSA, which have domains that are either identical or differ only in one amino acid. EX1056, 4,

¹⁰ As Jachimska explains, small deviations in isoelectric points (pI) can be explained by the use of various buffers used to prepare protein solutions. EX1054, 6.

Fig. 4 (showing the similarities in amino acid sequences and domains in pHSA, BSA, α -fetoprotein (AFP)). As Gorin explains, BSA and pHSA have 27 disulfide bridges that create loops in the amino acid chains, where the length of each loop in BSA and pHSA is identical in 25 out of 27 instances, and in “no case was the difference in the loop length greater than 1 residue.” *Id.* (the loops for AFP were also similar, but it was missing some of the disulfide bridges and its loop lengths differed in 5 instances).

147. Brahma models the structure of BSA and compares it to pHSA, noting that a “remarkable feature of these structures is most of the surface residues were polar supporting their high solubility in aqueous solvents,” as shown in Brahma’s Figure 6 (below). EX1055, 8-9. Indeed, the locations of negatively charged amino acid residues (shown in red) and positively charged residues (shown in blue) appear to be remarkably similar:

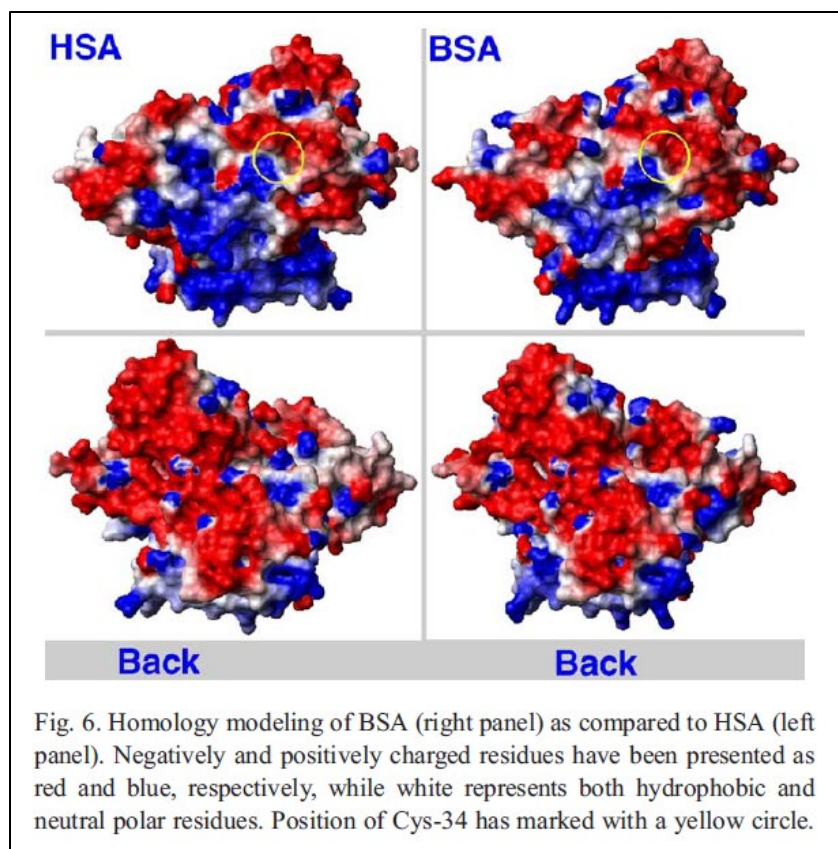


Figure 6. EX1055 (Brahma), Fig. 6.

148. As succinctly stated by Brahma, BSA is “a structural analog of human serum albumin.” EX1055, abstract.

149. In the context of protein purification, the similar physicochemical proteins of BSA and HSA (and relatedly, the equivalent rHSA) provide a strong expectation that a technique that effectively separates one of these albumins from a non-host specific impurity (e.g., endotoxin or detergent) would also work for another of these albumins.

150. In the context of endotoxin removal using a detergent, BSA, pHSA, and rHSA would all have been expected to behave in the same way, as the outer surfaces

of these albumins are predominantly hydrophilic (as shown above by the prominence of the red and blue charged residues in Figure 6, *supra*, (Brahma’s Fig. 6), meaning they would all be partitioned into the water-rich, micelle-poor phase/endotoxin-poor phase. *Supra*, ¶¶ 125-126. In other words, a skilled artisan would have appreciated that two-phase separation using a detergent such as Triton X-114 that successfully removed endotoxins from BSA (as shown in Aida) would also work for pHSA and Deeter’s equivalent rHSA.

151. The same holds true in the context of common hydrophobic sorbents used for detergent removal. Hydrophobic sorbents such as SDR HyperD have an affinity for hydrophobic molecules, and the largely hydrophilic outer surfaces of BSA and pHSA (and equivalent rHSA) would expectedly discourage binding of these proteins to a hydrophobic sorbent, meaning a skilled artisan would have recognized that detergents would be successfully removed from BSA, pHSA, and Deeter’s equivalent rHSA using hydrophobic sorbents.

152. I note that Ventria previously submitted comments regarding whether processes that worked for native proteins would work for recombinant proteins expressed in transgenic plants. *Wuhan Healthgen Biotechnology Corp. v. Ventria Bioscience Inc.*, IPR2022-00712, Paper No. 6, 29-34 (“IPR2022-00712, Paper No. 6”). For example, Ventria argued that there are “‘vast differences’ between the two albumin sources with different concerns that militate against the combination and

would not lead to an expectation of success.” *Id.*, 30. I disagree. While plasma is different from transgenic rice, the albumins produced in plasma and Deeter’s transgenic rice are “equivalent.” EX1005 (Deeter), Table 3. Techniques that successfully purify pHSA would thus also expectedly work for Deeter’s rHSA, as I explain above. Moreover, techniques that successfully purified more complex mixtures such as plasma or serum would also expectedly work for rHSA, as purifying a complex mixture was expectedly more difficult than purifying a target protein.

153. Ventria also argued that “‘recombinant mammalian albumin’ ‘produced in a transgenic plant’ confers the claimed albumin compositions with markedly different structural properties when compared with recombinant mammalian albumin produced in non-plant host cells, *including animals* and yeast.” IPR2022-00712, Paper No. 6, 31 (emphasis in original). Deeter contradicts this statement, as it reports that rHSA from transgenic rice and pHSA are “equivalent.” EX1005 (Deeter), Table 3; *see also* EX1032 (Van Der Valk), 9 (noting that in cell culture media, “[r]ecombinant human albumin (r-HSA) can be used instead of purified human albumin. ***r-HSA is structurally identical to HSA*** and provides the following advantages: it is safe – free from viral and prion contamination; and it is consistent – greater homogeneity and high batch-to-batch consistency.”) (emphasis added).

154. I am not aware of any purported “structural” differences between Deeter’s rHSA and pHSA that would impact purification or the function of albumin. While different hosts may contain different impurities, non-host specific impurities (including endotoxins and detergents) would expectedly be removed from rHSA, pHSA, and more complicated mixtures comprising pHSA (e.g., blood plasma) using the same techniques. *Supra*, ¶¶ 118-151. Moreover, Deeter already teaches how to achieve 98% purity, indicating that the vast majority of contaminants (including host cell contaminants) can be successfully removed using routine techniques such as extraction, filtration, and chromatography. EX1005 (Deeter), [0116], [0117].

IX. Prior Art

A. Hatsuyama (EX1004): Cell Culture Media Comprising rHSA

155. “Hatsuyama” is a U.S. Patent Publication No. 2007/0166825, which was published on July 19, 2007. I understand that Hatsuyama is prior art to the ’389 patent.

156. Hatsuyama discloses serum-free cell culture media for hematopoietic stem cells and hematopoietic progenitor cells containing rHSA and a basal medium. EX1004 (Hatsuyama), Abstract. Hatsuyama explains that by using the media, hematopoietic cells “can be expanded in a more undifferentiated state,” with a “much higher” efficiency than when using serum-free media or media containing pHSA. *Id.*, [0016]. Hatsuyama further explains that the cell culture media reduces the

potential for “contamination with viruses and prion, which can be problematic in cell transplantation, because it enables the expansion of a hematopoietic cell using a serum-free medium.” *Id.*

157. Hatsuyama exemplified its cell culture media composition with rHSA having less than 0.5 EU per 250 mg rHSA (less than 0.002 endotoxin units (EU) per mg albumin). *Id.*, [0076], Table 1. Hatsuyama’s rHSA was expressed in yeast and comprised low levels of yeast-derived components (yeast proteins and polysaccharides). *Id.*, [0069], [0076]. I note that such low levels of contamination are expected, even for purified rHSA, as it is a “sticky” molecule known to bind other molecules. *Supra*, ¶¶ 77-78.

158. Hatsuyama teaches that the rHSA used in its cell culture compositions may be obtained from any recombinant host, including plants. EX1004, [0025]. Hatsuyama states that “in addition to hosts already described in the literature, those that will be developed in the future can also be utilized as appropriate.” *Id.*, [0026].

159. Hatsuyama further states that the rHSA is preferably “purified to a sufficient extent to permit its use as a pharmaceutical (injection)” and may be collected and purified using known techniques for separation and purification. EX1004, [0024], [0225]. For example, Hatsuyama notes that rHSA expressed in yeast may be purified by “cation exchanger treatment, hydrophobicity

chromatography, and anion exchanger treatment,” and the rHSA may be further purified by a “chelate resin treatment or boric acid/salt treatment.” EX1004, [0030].

160. A skilled artisan would have appreciated that suitability for use as an injectable would necessarily require far less than 1 EU/mg rHSA given the FDA’s stringent requirements of no more than 5 EU/kg body weight per hour (EX1057, 22¹¹) and the amount of albumin required for therapeutic use, e.g., “several hundred or thousand milligrams per kg body weight” (EX1043, 5-6). Even for administration of just 100 mg albumin/kg body weight (less than a therapeutic dose), the injectable would need to contain less than 0.05 EU/mg rHSA to meet FDA’s limit of 5 EU/kg body weight (5 EU/kg body weight divided by 100 mg rHSA/kg body weight).

161. Hatsuyama explains that the composition may further contain a lipid, vitamins, growth factors such as insulin, iron sources such as transferrin, or other components as necessary. *Id.*, [0044].

162. Hatsuyama explains that its cell culture media containing rHSA was superior to comparable media containing pHSA in terms of expansion of certain cell

¹¹ FDA’s guidance recommends that for any parenteral drug (which includes injectables), “the upper limit of acceptance criterion for endotoxin be 5 EU/kg body weight/hour,” and for intrathecally administered drugs (injected into the spinal column), the upper limit is 0.2 EU/kg body weight/hour. EX1057, 22.

lines. *Id.*, [0115]. The rHSA-containing medium also expanded certain cell lines “in a more undifferentiated state” compared to serum-containing media and media containing pHSA. *Id.*, [0034].

B. Deeter (EX1005): rHSA Expressed in Transgenic Rice

163. “Deeter” is PCT Publication by Deeter et al., WO 2007/002762, assigned to Ventria, which was published on January 4, 2007. EX1005 (Deeter). Thus, I understand that Deeter is prior art to the ’389 patent.

164. Deeter describes the production and use of recombinant proteins produced by transgenic rice for improving growth and productivity of cells in cell culture media. EX1005 (Deeter), [0009]. Deeter discloses that its invention comprises cell culture media with “at least one plant-produced heterologous protein as a cell culture media component.” *Id.*, [0010]. Deeter explains that such plant-produced cell culture media components avoid certain “undesirable features” of animal-derived components, including the risk of contamination with pathogens of animal and human origin. *Id.*, [0006]-[0008].

165. Deeter describes expressing rHSA in transgenic rice, yielding up to 8.5 grams rHSA/kg rice flour. *Id.*, [103]-[105]. Deeter isolated its rHSA by (1) dehusking the rice, grinding to flour, and mixing for 30 minutes with an extraction buffer at pH 4.9, (2) clarifying the solution by filtration (e.g., removing insoluble matter), and (3) flowing the filtrate through an anion exchange column (Q-

Sepharose) to yield rHSA that was approximately 90% pure by SDS-PAGE. *Id.*, [0116]. A skilled artisan would have recognized that this initial purification sequence was the “capture” step discussed in *Molecular Farming*. EX1031, 237-243. Deeter further states that an additional chromatography step (using Blue-Sepharose) “can then be used to increase the purity from 90% to approximately 98% on scanning analysis of SDS-PAGE gels.” *Id.* This second chromatography step is essentially the “intermediate purification” phase discussed in *Molecular Farming*. EX1031, 239-240. Deeter does not disclose taking any measures to exclude endotoxin contamination during these processing steps. *Id.*, [0103]-[0105]. For example, Deeter does not disclose using endotoxin-free water or cleaning equipment in contact with its rHSA to remove endotoxins. *Id.* Deeter also does not disclose any “polishing” steps to further purify its rHSA, e.g., the final steps to achieve purity for a pharmaceutical-grade protein. *See* EX1031 (*Molecular Farming*), 236, 237, 243.

166. Deeter states that the “purified” rHSA was “equivalent” to pHSA. *Id.*, [0117]. For example, Deeter’s rHSA and pHSA had approximately the same molecular weight, the same isoelectric focusing point (5.3), and the same thermal stability (midpoint: 65 °C). *Id.*, Table 3 (above [0118]).

167. Deeter defines the term “purifying” to include any step that enriches the component of interest and acknowledges that other components may remain after purification:

The term “purifying” is used interchangeably with the term “isolating” and generally refers to any separation of a particular component from other components of the environment in which it is found or produced. For example, purifying a recombinant protein from plant cells in which it was produced typically means subjecting transgenic protein-containing plant material to separation techniques such as sedimentation, centrifugation, filtration, and chromatography. The results of any such purifying or isolating step(s) may still contain other components as long as the results enrich for the component of interest.

Id., [0063].

168. Thus, as defined by Deeter, a “purified” rHSA may still contain substantial levels of impurities, provided at least some of the impurities have been removed. *Id.*, [0063].

169. Deeter further explains that the use of media supplemented with plant-derived components “improve[s] cell growth and productivity in cell cultures.” EX1005 (Deeter), [0019]. In Examples 8 and 9, Deeter evaluates the effectiveness of rHSA (referred to as CELLASTIM™) in stimulating the growth of hybridoma AE1 cells and concludes that rHSA from rice was “functionally equivalent” or “better than” pHSA in enhancing hybridoma cell growth in reduced-serum media (Example 8) and serum-free media (Example 9).” EX1005 (Deeter), [0118]-[0120], Figs. 23, 24. These results strongly suggest that Deeter’s rHSA was bound to rice-

derived lipids, e.g., rice-derived fatty acids, as fatty acids were known to be important for hybridoma growth in serum-free media. EX1011 (Glassy), 2-4, 12.

C. General Knowledge that Purified Proteins Should Comprise Less than 10 ppm of Detergents Employed During Purification

170. The general principle that detergents used to purify proteins must be removed to concentrations of less than 10 ppm was well-known to a POSA. This principle had been noted repeatedly in the prior art, as discussed below. Indeed, detergents (including Triton detergents) were commonly used to inactivate viruses in protein preparations, and it was established that such detergents must be removed after viral inactivation (as well as after endotoxin removal, as noted above). *Supra*,

¶ 127. For example:

- Van Holten explains that it was “important to be able to determine that the residual amount of the S/D [solvent/detergent] post removal is *less than about 10 ppm*” in blood products. EX1007, [0112] (emphasis added). Van Holten acknowledges that techniques to remove such detergents using HIC chromatography are applicable to blood proteins (e.g., albumin) and recombinant proteins. *Id.*, [0005].

- Schulz similarly explains that “[e]specially when producing medical preparations, *Triton X-100 concentrations* above 50 ppm, preferably *above 10 ppm should be avoided*, and it is generally *desirable to reduce the detergent contents as much as possible*.” EX1008, [0012] (emphasis added). Schulz thus describes a method to decrease the concentration of SD (solvent/detergent) reagents to *below 10 ppm*. *Id.*, [0044]. After purification, Schulz’s proteins comprised *less than 5 ppm* Triton X-100. *Id.*, [0047].
- Viswanathan explains that “detergents may readily destroy or damage living cells,” and further notes that “*to ensure living cells and proteins are not damaged . . . removal of detergents is imperative*.” EX1009, [0010] (emphasis added).
- Viswanathan notes that the British pharmacopeia limits the level of Triton X-100 to *less than 5 ppm* in virus-inactivated plasma products. *Id.*, [0069], [0101], Table 7. Viswanathan characterizes plasma preparations comprising 44 ppm, 25 ppm, 15 ppm, and 13.7 ppm Triton as unacceptable, and preparations comprising 5 ppm and 6.16 ppm as acceptable. *Id.*, [0071]-[0077], Tables 1-3.

- Bonomo states that removal of “organic solvents, detergents and other virus-inactivating agents from biological products is necessary if a particular substance is not well tolerated by humans or other biological systems in which it is to be used, e.g., *in tissue cultures*.” EX1059, 2:9-14. Bonomo thus reports reducing Triton detergent concentrations to 1.5 to 8.0 ppm. *Id.*, 9:50-67 (Table 1).

171. These publications reflect the general knowledge of a skilled artisan that detergents used in protein purification must be removed to less than 10 ppm, particularly when one intends to purify a protein to a sufficient degree for use as a pharmaceutical and/or when the protein is intended for use in tissue or cell culture media. Indeed, common detergents used in protein purification (e.g., Triton detergents) were known to be cytotoxic (harmful to cells). *Supra*, ¶ 127. A skilled artisan would thus have been well-aware that such detergents must be removed to the greatest extent possible—and certainly to below 10 ppm—before using a purified protein in cell culture media. Fortunately, techniques such as the use of HIC sorbents were well-known, efficient, and routinely used to purify protein preparations such that they contained less than 10 ppm, less than 5 ppm, and even less than 1 ppm of any detergent used during purification. *Supra*, ¶¶ 128-139.

X. Claims 1-29 Would Have Been Obvious over Hatsuyama and Deeter and the General Knowledge that Purified Proteins Should Comprise Less than 10 ppm of Detergents Employed During Purification

A. Overview

172. Hatsuyama discloses serum-free media for expanding hematopoietic cells (such as stem cells and progenitor cells) comprising rHSA and other nutrients, including lipids, insulin, and transferrin, where the media provided superior cell growth compared to media containing serum or pHSA. EX1004, [0016], [0034]-[0037], [0044]. Hatsuyama exemplifies its cell culture media using rHSA expressed in transgenic yeast (*id.*, [0069]) and teaches that any transgenic host may be used to prepare rHSA, including “transgenic plants” (*id.*, [0025]) and hosts developed in the future (*id.*, [0026]), where the rHSA has preferably been purified to a sufficient extent to permit its use as a pharmaceutical (*id.*, [0024]). As noted above, a skilled artisan would have understood that to be suitable as an injectable pharmaceutical, the rHSA would have needed to contain well below 1 EU/mg albumin, e.g., well below 0.05 EU/mg rHSA. *Supra*, ¶ 159. This is consistent with the level of endotoxin in Hatsuyama’s rHSA, which contained only 0.5 EU/250 mg albumin, i.e., 0.002 EU/mg rHSA. EX1004, Table 1 (below [0076]).

173. Like Hatsuyama, Deeter concerns the use of recombinant proteins, including rHSA, in cell culture media. EX1005, [0118]-[0120]. Deeter reports that rHSA was expressed at up to 8.5 g/kg rice flour, which appears to have been among

the highest (if not the highest) expression yield of a recombinant protein in a plant. *Id.*, [0105]; *supra*, ¶ 95. Deeter’s reduced-serum and serum-free media comprising rHSA (along with insulin, transferrin, selenite, and ethanolamine, among other components) promoted hybridoma cell growth equivalently or better than cells grown in media with pHSA. *Id.*, [0118], [0120]. Deeter concludes that plant-derived rHSA can “replace animal derived albumin for use in cell culture.” *Id.*, [0120].

174. As discussed further below, it is my opinion that a POSA would have been motivated to modify Hatsuyama to incorporate rHSA produced in transgenic rice (as taught by Deeter) and, when doing so, would have been motivated to take precautions to exclude and remove endotoxins (and any endotoxin-removing agents such as Triton X-114) to prepare serum-free cell culture media. A POSA would have had a reasonable expectation of success in making this simple substitution, as Hatsuyama specifically teaches that rHSA produced in a transgenic plant may be used in its cell culture media, and the art provides ample guidance on purifying proteins to remove endotoxins and detergents. *Supra*, ¶¶ 118-144.

B. Motivation to Combine

175. Hatsuyama and Deeter concern the same field of endeavor, specifically, the use of rHSA in cell culture media as a substitute for pHSA. A POSA would have appreciated the importance of replacing historically used animal-derived components in cell culture media, as taught by both Hatsuyama and Deeter. A POSA

would have also recognized the advantageous results reported in Hatsuyama and Deeter, as each independently disclosed that cell culture media comprising rHSA performed equivalently or better than pHSA in promoting the growth of cells in culture, specifically, stem cells in Hatsuyama and hybridoma cells in Deeter.

176. Given the known advantages of transgenic rice over yeast, a POSA would have been motivated to use plant-derived rHSA in Hatsuyama's cell culture media—as specifically suggested by Hatsuyama in paragraph 25. EX1004 (Hatsuyama), [0025]. For example:

- The expression of rHSA in transgenic rice had been shown to provide very high yields of rHSA, up to 8.5 g rHSA/kg rice flour. EX1005, [0105].
- The rHSA expressed in transgenic rice was “equivalent” to pHSA, e.g., both Deeter's rHSA and pHSA were reported to be non-glycosylated and exhibited comparable ligand binding. EX1005, Table 3.
- Producing rHSA in transgenic rice would have expectedly provided a more cost-effective system for expressing rHSA than fermentation using transgenic yeast. *Supra*, ¶ 94.

177. Based on the successful results of Hatsuyama and Deeter in stem cells and hybridoma cells, respectively, a POSA would have further recognized that cell

culture media comprising rHSA could be developed to promote the growth of a wide range of cell types.

178. A POSA would have been motivated to ensure that the plant-derived rHSA in Hatsuyama's cell culture media comprised well below 1 EU endotoxin/mg albumin, as taught by Hatsuyama, and less than 10 ppm of any detergent used for endotoxin removal. EX1004, [0025]; *supra*, ¶¶ 170-171. A POSA would have appreciated the importance of using rHSA with such high purity, as those in the art understood that even low levels of contaminating endotoxins and detergents could be problematic in cell culture media. *Supra*, ¶¶ 103-107, 127, 170-171.

179. A POSA would not have assumed (without evidence) that Deeter's rHSA had already achieved the level of purity taught by Hatsuyama (e.g., 0.002 EU endotoxin/mg albumin), as Deeter neither reports the level of endotoxins in its rHSA nor discloses taking any steps to exclude endotoxin contamination during processing. *Supra*, ¶¶ 108-117, 164. Thus, while Deeter's rHSA was "purified" (e.g., by two initial chromatography steps, akin to the "capture" and "intermediate" phases of protein purification), a POSA would have appreciated that endotoxin contaminants very likely remained (and indeed, were very likely introduced during Deeter's purification process given Deeter's lack of measures to exclude them). *Supra*, ¶¶ 108-117, 164. A POSA would thus have been motivated to improve upon Deeter's rHSA by taking measures to exclude and/or remove endotoxins, as the

exceptionally high expression yields and successful results in cell culture media in Deeter were quite appealing.

180. A POSA would have recognized many benefits from combining the teachings of Hatsuyama and Deeter to provide cell culture media comprising rHSA that was purified to the levels taught in Hatsuyama and expressed in transgenic rice as taught in Deeter, including:

- Cell culture media comprising rHSA that was superior to Deeter's media due to the reduction/elimination of endotoxin contaminants that may harm cells, complicate biological research, and contaminate biological products expressed in cell culture media;
- Cell culture media comprising rHSA that was more cost-effective than Hatsuyama's media (exemplified using rHSA expressed in yeast) due to Deeter's high expression yield of rHSA in transgenic rice, the potential for plants to provide the most efficient and economical source of recombinant proteins, and the known high cost of Hatsuyama's exemplary rHSA expressed in yeast;

- Cell culture media that may be used and/or optimized to promote the expansion or growth of a variety of cells, including stem cells and progenitor cells (as taught in Hatsuyama), hybridoma cells (as taught in Deeter), and a wide variety of other cells that benefit from the presence of albumin and/or lipids (e.g., fatty acids) bound to albumin, as Deeter reported that its rHSA can “replace animal derived albumin for use in tissue culture” (EX1005, [0128]);
- Cell culture media that may comprise other recombinant proteins expressed in transgenic rice, e.g., recombinant human lactoferrin, which provided superior results when compared to animal-derived transferrin, as taught in Deeter; and
- Serum-free cell culture media that reduced the risk of contamination with animal-associated viruses and prions, as taught in both Hatsuyama and Deeter.

181. Thus, for at least the reasons above, a POSA would have been motivated to modify Hatsuyama in view of Deeter.

C. Reasonable Expectation of Success

182. A POSA would have had a reasonable expectation of success in substituting Hatsuyama’s rHSA (exemplified by yeast-derived rHSA) with rice-derived rHSA, as Hatsuyama taught that its serum-free media could comprise rHSA from any host, including transgenic plants. EX1004, [0025]. This would have been a simple substitution, as Deeter enabled the production of rHSA in transgenic rice

(using the same method copied into the '389 patent). *Compare* EX1005, [0103]-[0104] *with* EX1001, 64:5-60. Indeed, long before 2010, the production of recombinant proteins in plants was a well-established and “mature field.” *Supra*, ¶¶ 51, 93-94.

183. A POSA would have also had a reasonable expectation of successfully achieving rHSA produced in transgenic rice with far less than 1 EU endotoxin per mg rHSA.

184. For example, a POSA would have been knowledgeable about the use of endotoxin-free (e.g., pyrogen-free) water and proper cleaning of glassware and other equipment to avoid introducing high levels of endotoxin contamination during rHSA purification. *Supra*, ¶¶ 115-117.

185. To the extent that the rHSA comprised residual endotoxin above acceptable levels (e.g., substantially above Hatsuyama’s 0.002 EU endotoxin/mg albumin), a POSA would have reasonably expected to remove such endotoxins using routine purification processes. For example, a POSA would have reasonably expected that a simple two-phase separation using a detergent such as Triton X-114 would effectively remove endotoxin to well below 1 EU endotoxin/mg albumin, with a 1000-fold decrease in endotoxin contamination reasonably expected during the first 15-minute cycle, and a reasonable expectation that further cycles would

provide endotoxin levels below the limit of detection (e.g., around 0.001 EU endotoxin/mg protein). *Supra*, ¶¶ 121-123.

186. A POSA would have expected that this two-phase separation would work for Deeter's rHSA, as Deeter's rHSA was equivalent to pHSA, and pHSA was well-known to be strikingly similar to BSA. *Supra*, ¶¶ 145-153. Particularly for the ability to partition into the aqueous phase (the property pertinent here), rHSA, pHSA, and BSA would have been reasonably expected to behave in the same manner to achieve the same successful depletion of endotoxins to far below 1 EU endotoxin/mg albumin.

187. Regarding detergent removal, a POSA would have been motivated to ensure that Deeter's rHSA comprised less than 10 ppm of any detergent used for endotoxin removal to avoid harming cells or tissues in cell culture, as reflected by the general knowledge of a POSA. *Supra*, ¶¶ 170-171. For example:

- Bonomo teaches that removal of organic solvents and detergents must be removed from biological products if the products are to be used in systems such as tissue cultures. EX1059, 2:9-14; *see also id.*, 9:50-67 (Table 1) (reporting that purified plasma preparations comprised 1.0 ppm to 8.0 ppm Triton), 10:1-29 (Table 2) (reporting the composition of plasma, including albumin);

- Viswanathan teaches that “removal of detergents is imperative” to “ensure that living cells and proteins are not damaged” (EX1009, [0010]), that the British Pharmacopeia sets the maximum amount of Triton X-100 at 5 ppm (*id.*, [0069]), and that plasma preparations comprising more than 5 ppm residual detergent were not acceptable (*id.*, [0070]-[0076], Tables 1-3);
- Schulz teaches that “[e]specially when producing medical preparations,” Triton X-100 concentrations should preferably be below 10 ppm, and that “it is generally desirable to reduce the detergent contents as much as possible.” EX1008, [0012] (emphasis added);
- Van Holten explains that it was “important to be able to determine that the residual amount of the S/D [solvent/detergent] post removal is *less than about 10 ppm*” in blood protein preparations. EX1007, [0112] (emphasis added).

188. These publications reflect the general understanding that detergents used in protein purification should be removed to the greatest extent possible, including to concentrations of less than 10 ppm or less than 5 ppm, particularly where the protein would be in contact with living cells and/or tissue cultures.

189. A POSA would have applied this general understanding to the purification of rHSA for use in Hatsuyama’s cell culture media, as a POSA would have sought to avoid introducing agents (e.g., Triton detergents) that could harm the living cells that the cell culture media are intended to support.

190. A POSA would have had a reasonable expectation of success using an HIC sorbent to reduce the levels of any residual detergent to below 10 ppm. For example, HIC sorbents successfully removed detergents to levels below 10 ppm—at times below 5 ppm and even below 1 ppm—from plasma (Piquet, Bonomo, Viswanathan) and blood proteins (Van Holten), including immunoglobulins (Guerrier, Burnouf), and it had been established that HIC sorbents were suitable for purifying HSA (Guerrier) and recombinant proteins (Van Holten). *See* EX1007 (Van Holten); EX1009 (Viswanathan); EX1049 (Burnouf); EX10050 (Piquet); EX1051 (Guerrier); EX1059 (Bonomo); *supra*, ¶¶ 128-139. To be clear, albumin is a blood protein and is present in human blood plasma at levels of about 60%. *See* EX1050 (Piquet), 4 (Table 4). Thus, techniques that worked for blood proteins, recombinant proteins, and plasma would have expectedly also worked for pHSA and Deeter’s equivalent rHSA.

191. HIC sorbents effectively removed a range of detergents, including octoxynol 9 (Piquet), Triton X-45 (Burnouf), and Triton X-100 (Burnouf, Van Holten, Viswanathan, Bonomo, Guerrier). *See supra*, ¶¶ 128-139; EX1007 (Van Holten); EX1009 (Viswanathan); EX1049 (Burnouf); EX10050 (Piquet); EX1051 (Guerrier); EX1059 (Bonomo). HIC sorbents thus would have been reasonably expected to work for Triton X-114 (Aida’s detergent), as Triton X-114 has properties between Triton X-45 and Triton X-100. *Supra*, ¶¶ 137-137, Fig. 5.

192. Moreover, it was well-known that any loss of pHSA on a hydrophobic sorbent such as SDR HyperD would be minimal, and the same result would have been expected for Deeter's rHSA (which was equivalent to pHSA). *Supra*, ¶¶ 132, 140-154.

193. I address additional considerations related to a POSA's motivation to combine Hatsuyama and Deeter and reasonable expectation of success in doing so below, in the context of specific claim limitations.

D. Claim by Claim Analysis

1. Claim 1

194. Claim 1 recites a “cell culture media supplement or complete media composition for improving the growth, viability, and/or productivity of cultured cells as compared to cells grown without said supplement or media composition” (the claim preamble) comprising (1) plant-produced recombinant human albumin, (2) less than 1 EU of endotoxin/mg albumin, and (3) less than 10 ppm detergent. EX1001, claim 1; *supra*, ¶ 22 (providing the full text of claim 1).

a. Claim Preamble: “cell culture media supplement or complete media composition *for improving the growth, viability, and/or productivity of cultured cells as compared to cells grown without said supplement or media composition*”

195. Both Hatsuyama and Deeter disclose cell culture media compositions, i.e., “solutions for growing, storing, handling and maintaining cell lines.” *Supra*, ¶¶ 155, 163; EX1001, 16:46-48 (defining cell culture media). Additionally, while the

italicized language in the preamble (“for improving . . .”) may not be limiting, both Hatsuyama and Deeter disclose cell culture media compositions comprising rHSA that improve the growth, viability, and/or productivity of cultured cells compared to cells grown without said rHSA, e.g., media comprising serum or pHSA (as in Hatsuyama) or media without rHSA (as in Deeter). EX1004, [0016]; EX1005, [0118]-[0120], Figs. 23, 24.

b. “plant-produced heterologous protein [that] is recombinant human albumin”

196. Hatsuyama’s cell culture media composition, as modified in view of Deeter, contains rHSA produced in a transgenic plant, where the rHSA satisfies the definitions of a “recombinant” protein and a “heterologous protein” provided in the ’389 patent, and where the rHSA has the amino acid sequence of native HSA (satisfying the requirement for “recombinant albumin”). *See* EX1005, [0103]-[0105]; EX1001, 19:50-56.

c. “less than 1 EU endotoxin/mg albumin”

197. Hatsuyama’s cell culture media composition, as modified in view of Deeter, comprises “less than 1 EU endotoxin/mg albumin.”

198. For example, Hatsuyama teaches that the rHSA is preferably “purified to a sufficient extent to permit its use a pharmaceutical (injection).” EX1004, [0024]. A POSA would have understood pharmaceutical grade rHSA for injection to comprise far less than 1 EU endotoxin/mg albumin. *Supra*, ¶ 159.

199. Moreover, Hatsuyama exemplified its cell culture media using yeast-derived rHSA containing 0.002 EU endotoxin/mg albumin, and a POSA would have been motivated to achieve a comparable level of purity in Deeter's rice-derived rHSA to avoid introducing unwanted contaminants into their cell culture media. EX1004, Table 1 (below [0076]).

200. A POSA would have thus been motivated to take precautions to avoid introducing endotoxins into Deeter's rice-derived rHSA, including by using endotoxin-free water and by cleaning equipment (e.g., glassware) to remove endotoxins.

201. In addition, a POSA would have been motivated to test rice-derived rHSA for endotoxin to confirm that the level of endotoxins present was sufficiently low, e.g., not substantially higher than the 0.002 EU endotoxin/mg albumin taught by Hatsuyama.

202. To the extent that an undesirable level of endotoxins remained, a POSA would have had a reasonable expectation of success in removing them to well below the claimed threshold of 1 EU endotoxin per mg albumin, as discussed above. *Supra*, ¶¶ 121-126, 140-154.

d. "less than 10 ppm detergent"

203. A POSA would have been motivated to remove any detergent (e.g., Triton X-114) used to remove endotoxins from Deeter's rHSA (to the extent that any

such purification was required after taking precautions to exclude endotoxins during extraction and purification) to concentrations of less than 10 ppm prior to using it in Hatsuyama's cell culture media.

204. It was common knowledge that detergents used in the purification of proteins should be subsequently removed to less than 10 ppm or even less than 5 ppm. *Supra*, ¶¶ 170-171. Indeed, detergents commonly used in protein purification were known to cause cell death, even at very low concentrations. *Supra*, ¶ 127. A POSA would thus have been motivated to remove such detergents to the greatest extent possible during rHSA purification.

205. A POSA would have reasonably expected to remove any detergent used in purification to below 10 ppm using standard hydrophobic sorbents, which had

been used to reduce the levels of detergents to less than 10 ppm, less than 5 ppm, and even less than 1 ppm.¹² *Supra*, ¶¶ 128-139.

206. A POSA would have been able to make routine modifications to the techniques known in the art for the removal of detergents, including common detergents such as Triton X-114, if appropriate, to achieve the desired results by routine optimization of chromatography conditions such as the pH and the ionic strength/conductivity of the buffer solution.

¹² While the reported EC50 of Triton X-114 was 6.88 ppm (reported in EX1047, 5), a skilled artisan would have appreciated that rHSA would comprise preferably about 0.5 to 10 w/v %, and more preferably about 1 to 5 w/v %, of Hatsuyama's cell culture media. EX1004 (Hatsuyama), [0047]; see also *id.*, [0078], [0079] (preparing media containing 2% (w/v) rHSA). As such, a skilled artisan would have reasonably expected that even if residual detergent (e.g., Triton X-114) were present at a concentration of 10 ppm in rHSA, the concentration of detergent in the cell culture media would have been much lower, e.g., at 1 ppm for media comprising 10 w/v % rHSA and at 0.1 ppm for media comprising 1% w/v rHSA. A POSA would have reasonably expected these extremely low residual detergent levels would not adversely impact cell culture media, including Hatsuyama's cell culture media.

207. Thus, claim 1 of the '389 patent would have been obvious to one of ordinary skill in the art.

2. Claim 2

208. Claim 2 depends from claim 1 and further requires that the “recombinant human albumin is a sterile liquid or powder.” EX1001, claim 2.

209. Hatsuyama discloses that its rHSA composition may be prepared as a sterile liquid or powder composition. EX1004, [0031]. For example, Hatsuyama teaches that rHSA may be subjected to “sterilization heat treatment.” *Id.*

210. Hatsuyama further teaches that the rHSA may be formulated with a pharmaceutically acceptable carrier to prepare a pharmaceutical composition in the form of an injection or liquid. *Id.*, [0041]. Hatsuyama explains that sterilization may be achieved “by filtration sterilization through a bacterial retention filter, combining with a bactericidal agent, or irradiation.” *Id.*, [0043].

211. Hatsuyama additionally teaches that the sterile injection containing rHSA “can be prepared as a sterile solid composition by lyophilization and the like,” where the powder form “can be used after being freshly dissolved in sterile distilled water for injection or another solvent.” *Id.*

212. I conclude that claim 2 would have been obvious in view of these disclosures in Hatsuyama and my analysis above for claim 1.

3. Claim 3

213. Claim 3 depends from claim 1 and further requires that the recombinant human albumin has a molecular weight of 65-75 kDa.

214. Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA having a molecular weight of 66.9 to 68.1 kDa. EX1005, Table 3 (page 31). Thus, claim 3 would have been obvious for all the reasons discussed above for claim 1.

4. Claim 4

215. Claim 4 depends from claim 1 and further requires that the recombinant human albumin has an isoelectric focusing point of 5.3.

216. Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA having an isoelectric focusing point of 5.3. EX1005, Table 3. Thus, claim 4 would have been obvious for all the reasons discussed above for claim 1.

5. Claim 5

217. Claim 5 depends from claim 1 and further requires that the recombinant human albumin comprises "albumin and fragments thereof provided that the fragments retain the biological or therapeutically beneficial activity of said albumin." EX1001, claim 5.

218. A POSA would have understood that this term means: "albumin and fragments thereof provided that the fragments retain the biological or therapeutically beneficial activity associated with native albumin." *Supra*, ¶¶ 58-59.

219. Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA and "fractions thereof." EX1005, [0061]. Specifically, Deeter defines the term "heterologous protein" to include "albumin . . . and fractions thereof." *Id.*

220. Deeter further explains that its plant produced heterologous proteins are "preferably human" and include "growth factors, lactoferrin, transferrin, *serum albumin*, insulin, growth hormone, *and fractions thereof . . .*" *Id.*, [0081].

221. A POSA would have expected a fragment (or fraction) of rHSA to retain the beneficial activity associated with native albumin, which itself includes fragments of native albumin. Albumin by nature has three homologous domains, each of which has beneficial biological activity (e.g., each domain may serve as a carrier of fatty acids and other nutrients). A POSA would have expected that even if albumin lost an entire domain or subdomain, it would have retained the beneficial activity associated with albumin.

222. As such, claim 5 would have been obvious over Hatsuyama and Deeter.

6. Claim 6

223. Claim 6 depends from claim 1 and further requires that the recombinant human albumin comprises "albumin dimers having a molecular weight of about 130 kDa as detected by SDS-PAGE." EX1001, claim 6.

224. A POSA would have reasonably expected that Hatsuyama's cell culture media modified in view of Deeter would comprise albumin dimers, particularly when lyophilized as suggested by Hatsuyama. Indeed, a POSA would have been motivated to subject Deeter's rHSA to lyophilization (a common and convenient technique), as specifically suggested by Hatsuyama, and would have reasonably expected that lyophilization would induce the formation of albumin aggregates, primarily albumin dimers (to the extent such dimers were not already present in Deeter's rHSA). EX1004, [0031]; *supra*, ¶ 84.

225. A POSA would have also understood that the dimers present and/or formed during processing of Deeter's rHSA would have had "a molecular weight of about 130 kDa as detected by SDS-PAGE," as recited in claim 6, because Deeter's rHSA had a molecular weight of 66 kDa and thus a dimer comprised of two full-length albumins would have been expected to be observed by SDS-PAGE around 130 kDa (or, more specifically, around 132 kDa).

226. Thus, claim 6 would have been obvious to one of ordinary skill in the art.

7. Claim 7

227. Claim 7 depends from claim 1 and further requires that the recombinant human albumin comprises "less than 2% aggregates at around 250 kDa as detected by SDS-PAGE." EX1001, claim 7.

228. A POSA would have been motivated to eliminate albumin oligomers (if present) when purifying rHSA for use in Hatsuyama's cell culture media, as it was understood that the monomeric form of albumin (the non-aggregated form) serves as the best source of beneficial cell nutrients in cell culture (due to its correctly folded structure, which permits binding and delivery of cell nutrients). By contrast, albumin aggregates (particularly those larger than a dimer) would have expectedly had fewer accessible binding sites for cell nutrients compared to monomeric albumin due to their aggregated state.

229. A POSA would have had a reasonable expectation of success in removing albumin oligomers from the rHSA preparation, as the use of techniques such as gel filtration (which separates proteins species based on size) were routinely used for this purpose. A POSA would have also understood that Hatsuyama's optional step of lyophilizing rHSA could be omitted to maximize the amount of monomeric rHSA. Alternatively, a POSA would have reasonably expected that any oligomers formed during lyophilization would be converted to monomeric and dimeric albumin when the lyophilized protein was reconstituted in water. *Supra*, ¶¶ 82-86.

230. Thus, claim 7 would have been obvious to one of ordinary skill in the art.

**a. Comments on the '389 Patent's Discussion of
Aggregates at 250 kDa**

231. For completeness, I note that the '389 patent asserts that the rHSA purified in the purportedly “new” process contained “significantly less aggregates at around 250 kDa than the old process samples have. (Average greater than 2% for the old process, average less than 1% for the new process).” EX1001, 72:42-44. This passage does *not* reflect the level of aggregates at 250 kDa in *Deeter's* rHSA, as I explain below.

232. As I explained above, the specification includes two “old” B000 processes (*supra*, ¶¶ 38-40), and it does not explicitly state which B000 process was used to purify the “old” Cellastim analyzed by SEC-HPLC or SDS-PAGE. EX1001, 71:43-72:47. However, Example 6 indicates that the rHSA prepared using the *alternative* process (not the process in Deeter) was used:

Methods: Albumin prepared using process B000C described in Example 5 [this is the new process], was compared to albumin prepared using *an alternative process (B000)* which was previously used to prepare recombinant albumin (Cellastim (Batches B202 to B217)).

EX1001, 69:26-29.

233. As this passage is the introduction to the comparisons in Example 6, which includes the SEC-HPLC and SDS-PAGE comparisons, I expect that the Cellastim purified by the *alternative* process—not by the process in Deeter—was

used in the comparisons contained in Example 6. As such, I do not expect that Deeter's rHSA necessarily contained more than 2% aggregates at 250 kDa as observed by SDS-PAGE. And even if Deeter's rHSA contained more than 2% aggregates at 250 kDa, a POSA would have been motivated to remove them, and would have reasonably expected to do so using routine purification techniques, as I explain above.

8. Claim 8

234. Claim 8 depends from claim 1 and further requires that the recombinant human albumin has “an amino acid sequence that is homologous to native human albumin,” i.e., recombinant human albumin and native human albumin that “have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions.” EX1001, claim 8; EX1001, 20: 20-23.

235. Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA having an amino acid sequence that is homologous to native human albumin. EX1005, [0103]-[0104], Fig. 17. For example, Deeter discloses codon-optimizing the “consensus sequence” of P02768 (Swiss-Prot) for “suitable expression of human serum albumin in rice grain.” *Id.*, [0103]. Deeter further discloses confirming the “correct DNA and protein sequences,” and using the

optimized gene to express rHSA. *Id.*, [0103]-[0105], Table 2, Fig. 17.¹³ For completeness, I note that codon optimization involves optimizing the DNA sequence to express the desired amino acid sequence; i.e., it is performed to optimize expression of the protein and does not alter the amino acid sequence of the protein. As Deeter explains, when expressing recombinant human lactoferrin, the “numbers of codons changed accounted for 22.46% of the entire sequence,” while the “amino acid composition remains identical to native human lactoferrin.” *Id.*, [0100].

236. Thus, claim 8 would have been obvious to one of ordinary skill in the art.

9. Claim 9

237. Claim 9 depends from claim 1 and further requires that the recombinant human albumin is “recombinant human serum albumin.” EX1001, claim 9. Hatsuyama’s cell culture media modified in view of Deeter would comprise recombinant human serum albumin (rHSA). EX1005, [0103]. Thus, claim 9 would have been obvious to a POSA.

¹³ I note that Figure 17 of Deeter is Figure 5B of the ’389 patent. EX1007, Fig. 17; EX1001, Fig. 5B.

10. Claim 10

238. Claim 10 depends from claim 9 and further requires that the rHSA “has an amino acid sequence that is at least 80% identical to secreted human serum albumin comprising the amino acid sequence of SEQ ID NO: 1.” EX1001, claim 10. SEQ ID No. 1 is the sequence of amino acids naturally present in mature secreted HSA identified as “Swiss-Prot P02768.” *Id.*, 38:24-53.

239. Hatsuyama’s cell culture media modified in view of Deeter would comprise rHSA that “has an amino acid sequence that is at least 80% identical to secreted human serum albumin comprising the amino acid sequence of SEQ ID NO: 1,” i.e., the sequence of “Swiss-Prot P02768.” Deeter discloses that its rHSA was expressed using a Gt1 signal peptide and a codon optimized HSA sequence “based on the native protein sequence derived from P02768 (Swiss-Prot).” EX1005, [0039]; *see also id.*, [0103], Fig. 17. Thus, Deeter’s rHSA would have been expected to include 100% of the amino acid sequence of HSA in Swiss-Prot P02768 (which is identified in the ’389 patent as SEQ ID NO: 1).

240. Thus, claim 10 would have been obvious to a POSA.

11. Claim 11

241. Claim 11 depends from claim 1 and further requires that the rHSA has “the amino acid sequence of SEQ ID NO: 12.” EX1001, 11. The ’389 patent provides “SEQ ID NO 12” in columns 121-124, where it identifies 585 amino acids using

standard three-letter codes. The '389 patent also refers to SEQ ID NO. 12 below Table E2, where it indicates that the sequence DAHKSE corresponds to residues 1-6 of SEQ ID No. 12. EX1001, 66:23. I note that each letter in DAHKSE would have been understood to correspond to an amino acid (e.g., "D" is aspartic acid, "A" is alanine). In other words, DAHKSE identifies each amino acid using a one-letter code.

242. SEQ ID NO. 12 also appears in Figure 5A of the '389 patent, where it is identified using one-letter codes. EX1001, Fig. 5A. For comparison, when SEQ ID NO. 12 (identified using 3-letter codes in columns 121-123) is converted to one-letter codes, the result is as follows:

Three to One results

```
>results for sequence "sequence 12" starting "AspAlaHisLys"
DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAE
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV
DVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP
KLDEL RDEGKASSAKQRLK CASLQKFGERA FKAWAVARLSQRFPKAEFAEVSKLVTDLT K
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA
DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVL LRLAKTYETTLEKC
CAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQLG EYKFQNAL LVRYTKKVPQVST
PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES
LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT
KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLV AASQAALGL
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Figure 7. SEQ ID NO. 12 after converting the three-letter codes to one-letter codes using an on-line tool available at

[https://www.bioinformatics.org/sms2/three to one.html](https://www.bioinformatics.org/sms2/three%20to%20one.html).

243. This one-letter code sequence appears both in Figure 5A of the '389 patent and Figure 17 of Deeter. EX1005, Fig. 17.

244. Thus, Hatsuyama's cell culture media modified in view of Deeter satisfies the limitation of claim 11, as the same sequence is disclosed in Deeter.

12. Claim 12

245. Claim 12 depends from claim 1 and further requires that the recombinant human albumin comprises "one or more bound lipids from a plant." EX1001, claim 12.

246. A POSA would have reasonably believed that Hatsuyama's cell culture media modified in view of Deeter comprised rHSA bound to one or more lipids from a plant, including fatty acids.

247. First, Deeter's rHSA and pHSA had equivalent thermal stability with a midpoint of 65 °C, which is consistent with the heat stability of albumin bound to fatty acids (which are lipids). EX1005, Table 3; *supra*, ¶¶ 87-88. A POSA would have appreciated that lipids such as fatty acids that were expectedly bound to Deeter's rHSA were "from a plant," as required by claim 12, as there is no other apparent source of fatty acids.

248. Second, Deeter's rHSA was as good as, or better than, HSA in promoting the growth of hybridoma cells in culture. EX1005, [0118]-[0200], Figs.

23-24. This result would have been attributed, at least in part, to the presence of fatty acids bound to albumin, as fatty acids were known to be important for hybridoma growth in culture, and Deeter's media did not contain any other source of fatty acids. EX1005, [0118]-[0200]; *supra*, ¶ 81.

249. Moreover, a POSA would have reasonably expected Deeter's rHSA would retain bound lipids/fatty acids, even if such rHSA were treated with a detergent to remove endotoxins and subjected to detergent removal using an HIC sorbent. The prior art taught that bound fatty acids should be released from albumin by treatment with acid (e.g., at pH 3.0) to allow for their removal by a hydrophobic resin, and the known, common techniques for removing endotoxins and detergents discussed above do not involve treatment with acid or otherwise lowering the pH to 3.0. *Supra*, ¶ 79; EX1013 (Peters), 337. Moreover, the prior art teaches that after phase separation with detergents and/or detergent removal using an HIC sorbent, proteins retain their normal functions (*supra*, ¶ 121), and a POSA would not have expected albumin to retain its normal functions if *all* bound fatty acids had been removed. *Supra*, ¶¶ 77-81, 88.

250. Thus, claim 12 would have been obvious over Hatsuyama and Deeter.

a. Additional Comments on “one or more bound lipids from a plant” Recited in Claim 12

251. To the extent that the Patent Owner argues that Deeter's rHSA would not have been expected to comprise “one or more lipids from a plant,” I disagree (as

explained above), and I note that such arguments would undermine the disclosures of the '389 patent.

252. For example, outside the claims, the '389 patent does not mention that the recombinant albumin comprises “one or more bound lipids from a plant,” as recited in claim 12 of the '389 patent. It thus appears that the inventors expected that skilled artisans would appreciate that rHSA produced in transgenic rice comprised bound lipids from a plant, even after purification, and even if the presence of such bound lipids was not explicitly stated. I agree, as skilled artisans were well-aware of the nature of albumin (and equivalent rHSA) and its strong affinity to bind lipids such as fatty acids. Indeed, binding fatty acids was known to be a basic characteristic of albumin, including HSA and equivalent rHSA.

253. I note that the specification acknowledges that, in general, proteins produced in plants “typically comprise” bound lipids. EX1001, 26:37-42 (“Proteins produced in a plant will typically comprise a different set of bound co-factors, lipids and contaminating proteins compared to the same protein produced in a different expression system, or purified from a different source.”). Yet this basic principle does not distinguish the claimed rHSA from Deeter’s rHSA, as both were prepared using the same transgenic plant.

254. The '389 patent also defines “monocot seed components” to include “carbohydrate, protein, and lipid components extractable from monocot seeds, typically mature monocot seeds.” EX1001, 23:18-20.

255. The same definition also appears in Deeter. EX1005, [0056].

256. It appears that the inventors appreciated that the disclosed rHSA would have been understood to comprise bound lipids from a plant, even if that were not explicitly stated. The same understanding would have applied to Deeter and been reinforced by the thermal stability of Deeter’s rHSA and the ability of Deeter’s rHSA to promote the growth of hybridoma cells in culture. *Supra*, ¶¶ 81, 87.

13. Claim 13

257. Claim 13 depends from claim 1 and further requires that the recombinant human albumin “is a protein extract of a transgenic monocot plant.” EX1001, claim 13. Hatsuyama’s cell culture media modified in view of Deeter would comprise a protein extract of a transgenic monocot plant, as Deeter discloses that its rHSA is an extract from transgenic rice grains, and rice is a monocot. EX1005, [0103], claim 3 (page 34).

258. Thus, claim 13 merely recites a feature of Rice-rHSA that was already known and disclosed in Deeter.

14. Claim 14

259. Claim 14 depends from claim 13 and further requires that the recombinant human albumin comprises “a monocot seed component.” EX1001, claim 14. As noted above, monocot seed components are defined as “carbohydrate, protein, and lipid components extractable from monocot seeds, typically mature monocot seeds.” EX1001, 23:18-20.

260. Hatsuyama’s cell culture media modified in view of Deeter would contain rHSA comprising “a monocot seed component,” i.e., a “carbohydrate, protein, and lipid components extractable from monocot seeds, typically mature monocot seeds.” EX1005, [0056]. This definition encompasses fatty acids (which are lipids). Thus, claim 14 would have been obvious for all the reasons discussed above for claim 12.

15. Claim 15

261. Claim 15 depends from claim 14 and further requires that the monocot seed component “is a plant carbohydrate or plant lipid.” EX1001, claim 15. This claim would have been obvious for all the reasons discussed above, as Hatsuyama’s cell culture media modified in view of Deeter would comprise rHSA expectedly bound to rice lipids, e.g., rice fatty acids, as explained above for claim 12.

16. Claim 16

262. Claim 16 depends from claim 13 and further requires that the recombinant human albumin “is a protein extract of a transgenic seed.” EX1001,

claim 16. Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA from transgenic rice seeds and thus claim 16 would have been obvious for all the reasons discussed above, e.g., for claims 1 and 13.

17. Claim 17

263. Claim 17 depends from claim 13 and further requires that the recombinant human albumin is a protein extract of a transgenic rice seed." EX1001, claim 17. Claim 17 would have been obvious for all the reasons discussed above (e.g., see discussion of claims 1 and 13), because Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA that is a protein extract from transgenic rice seeds. EX1005, [0105].

18. Claim 18

264. Claim 18 depends from claim 17 and further requires that the "rice is *Oryza sativa*." EX1001, claim 18.

265. A POSA modifying Hatsuyama's cell culture media in view of Deeter would have been motivated to extract rHSA from transgenic *Oryza sativa* rice seeds, as Deeter discloses using Taipei 309, a strain of *Oryza sativa*, *Japonica*, to produce recombinant proteins, specifically recombinant human epidermal growth factor (rhEGF), recombinant human insulin-like growth factor I (rhIGF), recombinant human intestinal trefoil factor (rhITF), and recombinant human lactoferrin (rhLF) in

transgenic *Oryza sativa* (Japonica) rice. EX1005, [0026], [0030], [0034], [0037], [0099]-[0100] (rhLF).

266. A POSA would have reasonably expected that Deeter used *Oryza sativa* to express rHSA given Deeter's use of this rice strain for other recombinant proteins and would have been motivated to use the same expression system given Deeter's high expression yields. Alternatively, Deeter would have motivated a POSA to select *Oryza sativa* as an appropriate transgenic host for the expression of rHSA, as *Oryza sativa* is the only type of rice disclosed in Deeter. Claim 18 would thus have been obvious to a skilled artisan prior to the filing of the '389 patent.

19. Claim 19

267. Claim 19 depends from claim 1 and further requires that the cell culture media supplement or complete media composition "is free of serum derived albumin and yeast derived albumin." EX1001, claim 19.

268. The cell culture media of Hatsuyama in combination with Deeter would have contained rHSA as the only albumin; e.g., it would not have contained serum derived albumin or yeast derived albumin. Indeed, Hatsuyama discloses that its rHSA may be expressed in any suitable host, including transgenic plants, and provides no reason to combine more than one type of albumin. EX1004, [0024]-[0026].

269. Thus, claim 19 would have been obvious for all the reasons discussed above for claim 1.

20. Claim 20

270. Claim 20 depends from claim 1 and further requires that the composition comprise “at least one additional protein.” EX1001, claim 20.

271. Both Hatsuyama and Deeter teach including at least one additional protein to the cell culture media composition. For example, Hatsuyama’s exemplary cell culture media contain rHSA combined with insulin and holotransferrin (which are both proteins). EX1004, [0078], [0079]. Similarly, Deeter’s exemplary cell culture media contain rHSA combined with insulin and transferrin. EX1005, [0118], [0119].

272. Thus, Hatsuyama in combination with Deeter teach the limitation of claim 20.

21. Claim 21

273. Claim 21 depends from claim 20 and further requires that the additional protein “is selected from the group consisting of growth factors, lactoferrin, *transferrin*, *insulin*, growth hormone,” EX1001, claim 21 (emphasis added).

274. As discussed for claim 20, both Hatsuyama and Deeter teach the limitation of claim 21.

22. Claim 22

275. Claim 22 depends from claim 20 and further requires that the additional protein “is a heterologous plant-produced protein.” EX1001, claim 22.

276. A POSA modifying Hatsuyama’s cell culture media in view of Deeter would have been motivated to replace Hatsuyama’s transferrin with Deeter’s recombinant lactoferrin, as specifically suggested by Deeter. EX1005, [0081]. For example, Deeter suggests using “*at least* one plant-produced heterologous protein as a cell culture component.” *Id.* Deeter thus specifically suggests combining plant-produced recombinant proteins in cell culture media.

277. Deeter further teaches that plant-produced recombinant human lactoferrin (rhLF), identified as LACROMIN™, “can replace animal-derived transferrin in cell culture media for hybridoma growth.” *Id.*, [0113]; *see also id.*, [0114]-[0115], Table 2.

278. A POSA would have been motivated to replace Hatsuyama’s transferrin with Deeter’s plant-produced recombinant human lactoferrin to avoid the known disadvantages of using proteins sourced from animals. *See* EX1005, [0006] (noting that cell culture media comprising lactoferrin, transferrin, and serum albumin derived from animal sources “have several undesirable features.”).

279. While Deeter demonstrates that rhLF “can replace transferrin derived from [an] animal source” using hybridoma cells, Deeter indicates that its method of

combining “at least one” recombinant protein produced in a plant is not limited to hybridoma cells but rather extends generally to cell culture media, including reduced serum and serum free media. *See* EX1005, claim 12. Deeter specifically notes that cell culture media include media for stem cells. *Id.*, [0002]. A POSA would thus have reasonably expected that plant produced recombinant lactoferrin would be an appealing addition to other cell culture media, including Hatsuyama’s.

280. A POSA would have thus been motivated to replace Hatsuyama’s transferrin with rhLF, as taught by Deeter. A POSA would have had a reasonable expectation of success in making this simple substitution based on Deeter’s teachings.

23. Claim 23

281. Claim 23 depends from claim 20 and further requires that the cell culture media comprise “selenium and ethanolamine.” EX1001, claim 23.

282. A POSA would have been motivated to try well-known cell culture media supplements such as selenium and ethanolamine when modifying Hatsuyama’s cell culture media in view of Deeter. Deeter discloses combining rHSA with insulin, transferrin, sodium selenite, and ethanolamine (e.g., ITSE, *supra*, ¶ 66) to promote the growth of hybridoma cells in cell culture media. EX1005, [0118], [0119]. A POSA would have understood that sodium selenite was a source of selenium, a common nutrient in cell culture media. *Supra*, ¶¶ 66, 68-69, 71.

283. A POSA would have been motivated to modify Hatsuyama's cell culture media comprising Deeter's Rice-rHSA to include selenium and ethanolamine, as taught by Deeter. EX1005, [0118], [0119]; *supra*, ¶ 173. Indeed, the addition of selenium and ethanolamine to cell culture media was quite common in the art. *Supra*, ¶¶ 66, 68-69, 71.

284. A POSA would have been motivated to make this minor modification, and would have had a reasonable expectation of success in doing so, based on Deeter's successful results and the general knowledge that selenium and ethanolamine promoted the serum-free cultivation of many cell types. EX1005, [0118], [0119]; *supra*, ¶¶ 66, 68-69, 71.

24. Claim 24

285. Claim 24 is an independent claim that recites a cell culture media supplement or complete media composition comprising plant-produced recombinant human albumin, where the recombinant human albumin comprises "i) less than 1 EU endotoxin/mg of albumin; and ii) a monocot seed component, wherein said monocot seed component is a plant carbohydrate or plant lipid." EX1001, claim 24.

286. Claim 24 requires features that are also required by claim 1, (e.g., recombinant human albumin comprising less than 1 EU endotoxin/mg albumin) and claims 14 and 15 (a monocot seed component that is a plant carbohydrate or plant

lipid). Thus, I conclude that claim 24 would have been obvious for all the reasons discussed above for claims 1, 14, and 15.

25. Claim 25

287. Claim 25 depends from claim 24 and further requires that said recombinant human albumin has a purity of greater than 95%. EX1001, claim 25. The '389 patent explains that protein purity may be evaluated by SDS-PAGE. *Id.*, 22:31-34.

288. Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA having a purity of greater than 95%, as Deeter discloses that its rHSA may be purified "to approximately 98% based on scanning analysis by SDS-PAGE gels." EX1005, [0116]. Thus, I conclude that claim 25 would have been obvious for all the reasons discussed above.

26. Claim 26

289. Claim 26 is an independent claim that recites a cell culture media supplement or complete media composition containing recombinant mammalian albumin (e.g., recombinant human albumin) that is "i) produced in a transgenic plant; and ii) has less than 1 EU endotoxin/mg of albumin. EX1001, claim 26. Claim 26 thus recites features of cell culture media that I have already addressed at length above. I conclude that claim 26 would have been obvious for all the reasons discussed for at least claims 1 of the '389 patent.

27. Claims 27-29

290. Claims 27-29 depend from claim 26 and further require that the albumin is rHSA (claim 27), that the transgenic plant is a transgenic grain (claim 28), specifically, transgenic rice (claim 29, which further depends from claim 28). These claims would have been obvious for all the reasons discussed above, as Hatsuyama's cell culture media modified in view of Deeter would comprise rHSA expressed in transgenic rice (a grain). EX1005, [00103]-[0105].

XI. Secondary Considerations

291. I understand that secondary considerations refer to real-world evidence concerning obviousness or non-obviousness. I am also informed that if the patentee provides evidence of secondary considerations, the evidence must relate to the novel features of the claims as opposed to claimed features already known in the prior art.

292. I am informed that secondary considerations may include:

- unexpected results, e.g., results obtained that would not have been expected;
- long felt, unmet need, e.g., a need in the art for the claimed composition that was not already met by the prior art;
- commercial success, e.g., evidence that a product practicing the claims was commercially successful;
- industry praise, e.g., praise for a product practicing the invention;

- copying, e.g., the existence of others copying a product practicing the invention.

293. I reserve the right to respond to any evidence of secondary considerations offered by the patentee in this proceeding.

294. Based on the '389 patent and the state of the prior art, I provide a few comments on secondary considerations below.

E. The Prior Art Enables the Production, Extraction, and Purification of Deeter's rHSA

295. Deeter enables the production of rHSA. Indeed, Deeter produced its rHSA using the same method that was later copied into the '389 patent application (nearly verbatim). *Compare* EX1005, [0103]-[0105], Table 1 *with* EX1001, 64:5-60 (Example 3 and Table E1).

296. A POSA would further have been readily able to extract Deeter's rHSA from transgenic rice using routine extraction techniques, as exemplified by Deeter (EX1005, [0116]) and U.S. Patent No. 7,304,208, which details a general method for extracting "blood proteins" such as rHSA from transgenic rice and obtaining a clarified filtrate using common, routine processing techniques. EX1024, 16:14-33.

297. The prior art further would have enabled a POSA to purify Deeter's rHSA to contain less than 1 EU endotoxin/mg albumin and less than 10 ppm detergent for all the reasons I discuss above. *Supra*, ¶¶ 140-154, 182-192.

F. No Unexpected Results

298. In my opinion, the '389 patent does not describe any results that would have been surprising or unexpected to a person of ordinary skill in the art familiar with the closest prior art that existed in February 2010 (or even 2009), which I believe would have been Hatsuyama and/or Deeter.

1. rHSA with “less than 1 EU endotoxin per mg of albumin” Was an Expected and Routine Result using Known Methods

299. The applicant's declaration submitted during prosecution of the '478 application asserts that the “ability to achieve recombinant albumin with such low endotoxin levels was unexpected.” EX1006, 700. I disagree. A POSA would have considered the removal of endotoxin from a solution of rHSA routine based on the teachings of at least Aida (EX1044) and Magalhaes (EX1042). *Supra*, ¶¶ 118-126, 140-154.

300. Indeed, the technique for removing endotoxin in the '389 patent appears to be a simplified (and less efficient) version of the technique used to purify albumin (BSA) in Aida. Aida discloses adding a detergent (Triton X-114) to a solution of albumin on ice (below the cloud point of Triton X-114), then warming to 37 °C (above the cloud point), “whereupon two phases formed.” EX1044, Abstract; EX1043 (Petsch), 10; *supra*, ¶ 126. The phase separation induced by Aida's temperature adjustment promotes endotoxin separation from the aqueous albumin-

containing phase. EX1043 (Petsch), 10. By comparison, the '389 patent states that in “a preferred method,” Triton X-114 (Aida’s detergent) or Triton X-100 is added to an albumin solution, after which the solution is mixed at room temperature for at least an hour—with no temperature adjustment. EX1001, 68:21-24. This process is exemplified in Example 5 of the '389 patent, which describes adding Triton X-100 to an albumin solution then mixing for one hour at 20 ± 2 °C, *without* subsequently heating to above the cloud point of Triton X-100 (66 °C). EX1001, 69:14-16; EX1052 (Wang), 2 (identifying the cloud point of Triton X-100). Although the inventors do not expressly disclose using a temperature adjustment to further promote the removal of endotoxins from the albumin solution (as disclosed in Aida, and summarized in a review published by Magalhaes), achieving rHSA comprising less than 1 EU endotoxin/mg of albumin would have been reasonably expected to be achieved using the methods of the prior art.

301. I note additionally that Aida’s albumin (BSA) and the rHSA of Deeter share strikingly similar physicochemical properties, such that a POSA would have reasonably expected that Aida’s technique would work well for Deeter’s rHSA. *Supra*, ¶¶ 140-154. Indeed, the inventors of the '389 patent recognized that techniques for purifying one albumin would work for another. EX1001, 50:1-12 (explaining that known methods of purifying albumin may be used to purify the “albumin of the present invention.”). Indeed, claim 26 broadly recites “recombinant

mammalian albumin” comprising “less than 1 EU endotoxin/mg of albumin,” indicating that the inventors appreciated that a technique that worked for rHSA would also work for other mammalian albumins, e.g., recombinant BSA.

302. The applicant’s declaration asserts that an initial attempt at purifying albumin using a detergent and hydrophobic resin did not achieve the claimed levels of endotoxin or detergent. EX1006, 700. This initial attempt at removing endotoxin from albumin used zwitterionic detergent (“CHAPS”) and a Biorad SM-2 column. EX1001, 70:56-59. Although the inventors do not disclose using Aida’s established method of endotoxin removal from albumin first, using Triton detergents for endotoxin removal remained well-established.

2. Endotoxin Contamination of rHSA Expressed in Transgenic Rice (as in Deeter) Was Not Unexpected

303. To the extent that Ventria may argue that endotoxin contamination in rHSA expressed in transgenic rice (as taught by Deeter) was unexpected, I disagree. Skilled artisans appreciated the risk of endotoxin contamination, particularly for recombinant proteins expressed in plants grown in dirt, as explained above. *Supra*, ¶¶ 114-117.

304. Indeed, by 2007, Ventria’s customers appear to have appreciated that rHSA expressed in rice (Cellastim) may be contaminated with endotoxin. EX1058, 151:20-152:12 (testimony of Ventria’s CEO Scott Deeter that in 2007, Ventria had problems satisfying customer requirements with respect to “the performance and the

endotoxin levels of the product and the quality of the product.”). As reflected in Mr. Deeter’s testimony, the “media industry” and “injectable drug industry” required low levels of endotoxin, and Ventria’s early version of Cellastim, while commercialized, contained too much endotoxin to satisfy the needs of at least some customers.
EX1058, 153:25-154:3

3. rHSA Comprising “less than 10 ppm detergent” Was an Expected and Routine Result Using Known Methods

305. The applicant’s declaration states that “we developed a protocol to absorb the detergent-endotoxin complex from the solution onto a hydrophobic detergent-binding resin.” EX1006, 700. I note that the declaration does not purport to say that protocols for removing detergents using a hydrophobic detergent-binding resin (an HIC sorbent) were surprising. Nor could it, as such protocols were already well-established in the prior art. *Supra*, ¶¶ 128-139.

306. The applicant’s declaration notes that an initial attempt at detergent removal was unsuccessful. EX1006, 700. However, that failed initial attempt (which would not have been known to a POSA) does not render the removal of detergents (especially Triton detergents) unexpected. *Supra*, ¶ 128-139.

4. The '389 Patent Does Not Describe Any Unexpected Results in Cell Culture Media

307. I disagree with the statements in the '389 patent indicating that “surprising” results were achieved with plant-produced recombinant proteins in cell culture media. For example, the '389 patent states:

- “plant derived recombinant cell culture component proteins surprisingly enhanced the cell growth and viability when added in specific mixtures to mammalian cells grown in culture.” EX1001, 31:50-53.
- “Surprisingly some combinations of proteins provide for a synergistic effect on cell growth and productivity which provides unexpected benefits compared to the use of the individual proteins.” *Id.*, 31:53-56.
- “Surprisingly the combination of Insulin, transferrin and plant derived recombinant human serum albumin produced superior cell growth compared to 10% serum, demonstrating the superior properties of these combinations of proteins.” *Id.*, 92:57-60.
- “Specifically the present experiments establish that the synergistic properties of human serum albumin with transferrin related proteins such as transferrin and lactoferrin is an unexpected and surprising property of rice derived recombinant human serum albumin.” *Id.*, 94:33-37.

308. Combinations of proteins, including recombinant proteins, were routinely used in cell culture, along with other components such as insulin, transferrin, and lactoferrin. *Supra*, ¶¶ 66, 68, 70, 71. Each supplement is expected to serve a different function in promoting cell growth and/or productivity. As a result, supplementing base media with combinations of supplements often produces greater benefits than adding the supplements individually.

309. Notably, the '389 patent provides data showing the effect of combining rHSA and recombinant lactoferrin; however, it does not appear to provide data showing the effect of combining HSA and lactoferrin for comparison. EX1001, 85:1-87:38. Thus, it is unclear whether any purported synergy is attributed to the use of plant-derived rHSA and rLF or simply the natural result of combining the native forms of such proteins. At most, the '389 patent includes comparative data for rHSA versus pHSA in combination with recombinant transferrin (rTF), where the combination of rHSA and rTF had a greater than additive effect, while pHSA and rTF did not. EX1001, 92:62-95:13. However, the claims of the '389 patent are not limited to combinations of rHSA and rTF. For example, claim 21 requires recombinant albumin combined with an additional protein selected from a list that includes transferrin but does not require that the additional protein be produced in a plant, and claim 22 requires recombinant albumin in combination with *any* plant-produced heterologous protein (not limited to transferrin). EX1001, claims 20-22.

310. Next, the '389 patent states that its rHSA had an overall purity of greater than 95%, while “the old method routinely produced albumin with a maximum purity of about 90%,” and asserts that “[s]urprisingly, despite the increased product purity [of new versus old batches of rHSA], these changes in processing also resulted in *enhanced* co-purification of heat shock proteins, (see below) with the recombinant albumin.” EX1001, 73:45-53. The '389 patent does not provide any data to support this assertion, e.g., it does not establish that Deeter’s rHSA contained fewer heat shock proteins than the rHSA purified in the '389 patent. Indeed, while the level of heat shock proteins was known to promote cell survival in culture media “under a variety of conditions” (as acknowledged in the '389 patent at col. 30, lines 41-47), the less purified version of rHSA (Cellastim) performed just as well as the more highly purified rHSA (also called Cellastim) at low concentrations (e.g., at 0.5 to 2 mg/ml) where endotoxin and detergent contamination would have been less detrimental. EX1001, col. 73 (Tables E3 and E4). In other words, the superior performance of the purportedly new rHSA at high concentrations is very likely due to the lower levels toxic contaminants, not any purported elevated level of heat shock proteins.

XII. Conclusion

311. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true. I further

declare that all statements made herein were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code.

DATE: March 31, 2023

By: 

Douglas S. Clark, Ph.D.

Appendix A

CURRICULUM VITAE OF DOUGLAS S. CLARK

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Education

Ph.D.	California Institute of Technology Advisor: James E. Bailey	1983	Chemical Engineering
B.S.	University of Vermont, <i>Summa Cum Laude</i>	1979	Chemistry

Professional Experience

G. N. Lewis Professor and Dean, College of Chemistry, University of California, Berkeley, California – July 2013 to present

Warren and Katharine Schlinger Distinguished Professor and Chair, Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California – July 2011 to June 2013

Executive Associate Dean, College of Chemistry, University of California, Berkeley, California – January 2008 to July 2011

Co-director, Berkeley Synthetic Biology Institute, University of California, Berkeley, California – April 2011 to December 2018

Vice-Chairman, Department of Chemical Engineering, University of California, Berkeley, California - July 1995 to July, 1998; July 2007 to December 2007

Professor of Chemical Engineering, University of California, Berkeley, California - July 1994 to present

Associate Professor of Chemical Engineering, University of California, Berkeley, California - July 1989 to 1994

Assistant Professor of Chemical Engineering, University of California, Berkeley, California - July 1986 to July 1989

Faculty Scientist, Lawrence Berkeley Laboratory - December 1986 to present

Assistant Professor of Chemical Engineering, Cornell University, Ithaca, New York - January 1984 to July 1986

Chemist, R&D, Gulf Science and Technology Company, Pittsburgh, Pennsylvania - June 1979 to September 1979

Honors and Professional Activities

California Academy of Bioengineering, inaugural class, 2021

D.I.C. Wang Award for Excellence in Biochemical Engineering, 2019

Member, National Academy of Engineering, Elected, 2019

Hohenshuh Lecturer in Chemical Engineering and Bioengineering, Washington State University, 2016

James E. Bailey Award of the Society for Biological Engineering, 2014

Division Lecturer, ASM General Meeting, 2012

Bioengineering Centennial Lecture, AIChE Annual Meeting, 2008

Department of Chemical Engineering Teaching Award, 2006

The Marvin J. Johnson Award in Microbial and Biochemical Technology (of the American Chemical Society), 2006

NorCal Chemical Engineering Excellence Award - Industrial Research, 2004
Fellow, American Association for the Advancement of Science, Elected 2003
Food, Pharmaceutical, and Bioengineering Award of the AIChE, 2003
International Enzyme Engineering Award, 2003
Amgen Biochemical Engineering Award, 2003
Lindsay Distinguished Lecturer, Chemical Engineering, Texas A&M University, 2002
Gwathmey Lecturer in Surface Science, Engineering, and Medicine, University of Virginia, 2001
Alan Berman Research Publications Award, 2000
Editor-in-Chief: *Biotechnology and Bioengineering*, 1996 to present
Editorial Board: *Extremophiles*, 1996 to present
Who's Who in Science and Engineering, Millennium Edition
International Advisory Board, *Encyclopedia of Catalysis*, 1998
Invited Speaker: 1998 Annual Meeting of the National Academy of Engineering
Fellow of the American Institute of Medical and Biomedical Engineers, Elected 1995
Associate Editor: *Biotechnology and Bioengineering*, 1990 to 1996
Editorial Board: *Enzyme and Microbial Technology*, 1994 to 2019
Named "Most Appreciated Faculty Member," AIChE Student Chapter, U.C. Berkeley, 1995.
Merck Centennial Lecturer in Biochemical Engineering, California Institute of Technology, 1992.
Who's Who Among Rising Young Americans, 1990, 1992
Editor: *Enzyme Engineering XI*, New York Academy of Sciences, 1991
 Applied Biocatalysis, Marcel Dekker, 1988
Director: Food, Pharmaceutical, and Bioengineering Division of the AIChE, 1991 -1994
Merck Faculty Development Award, 1988
Presidential Young Investigator Award, National Science Foundation, 1986
Hannah V. Howard Award: Outstanding Student of College of Arts and Sciences, University of Vermont, 1975-1979
Phi Beta Kappa, 1978

Professional Societies

Member: NAE, AIChE, ACS, AIMBE, AAAS

Consulting and Related Activities

Co-founder, Board of Directors, Solidus Biosciences, Inc. (2001 to 2016).
Co-founder, Board of Directors, and Scientific Advisory Board, EnzyMed, Inc. (1994 - 1999);
EnzyMed, Inc. was acquired by Albany Molecular Research, Inc. (Nasdaq: AMRI), 1999.

Other consulting activities have ranged from one to several days of contact per year and have included a variety of pharmaceutical and chemical companies (e.g., Millipore, Syntex, Engelhard, Mobil, ExxonMobil, Johnson Matthey, and Kosan Biosciences), along with expert witnessing.

Conference Organization

Biochemical Engineering Steering Committee, Biochemical and Molecular Engineering XXI Conference, Mont Tremblant, Quebec, Canada, July 2019.
Scientific Advisory Board, 5th International Conference on High Pressure Bioscience and Technology, 2008.

Program Co-Chairman: Engineering Foundation Conference, "Biochemical Engineering XII," Sonoma, CA 2001
Program Committee: 1st North American Symposium on Chemical Reaction Engineering, Houston, TX 2001
Executive Committee: Engineering Foundation Conference, "Enzyme Engineering XIII," San Diego, CA 1995
Executive Committee: Engineering Foundation Conference, "Enzyme Engineering XII," Dauville, France, 1993
Program Chairman: Engineering Foundation Conference, "Enzyme Engineering XI," Keauhou-Kona, Hawaii, 1991
Program Committee: Ninth International Biotechnology Congress, 1992
Biocatalysis and Biomimetics, ACS Symposium Series No. 392, 1989

Sessions Chaired at Meetings

Chairman, Session 1, Biocatalysis 2010, Puerto Morelos, Mexico, December 2010.
Co-chairman, "Proteins and Enzymes," 8th International Conference on Extremophiles, Ponta Delgada, Azores, Portugal, September 2010.
Moderator, Panel Discussion on Embryonic Stem Cells, ACS National Meeting, San Francisco, CA, March 2010.
Co-chairman, "Biotechnology & Bioengineering 50th Anniversary – Biochemical Engineering Past to Present," Biochemical Engineering XVI, Burlington, VT, July 2009
Co-chairman, "Biological Reaction Engineering and Fuel Cells," NASCRE-2, Houston, TX February 2007
Co-chairman, "Advances in Biocatalysis," Enzyme Engineering XVIII, Gyeong-ju, Korea, October 2005.
Chairman, "Enzymes in Unnatural Environments," Workshop on Biocatalysis in Non-conventional Media, Manchester, UK, April 2005.
Co-chairman, "Applied Biocatalysis and Biotransformations," ACS National Meeting, San Diego, CA, March 2005.
Chairman, "Extremophile Metabolic Enzymology I," Extremophiles 2004: 5th International Conference on Extremophiles, Cambridge, MD, September 2004.
Co-chairman, "Biocatalysis," Metabolic Engineering VI: Applied Systems Biology, Castelveccchio Pascoli, Italy, October, 2002.
Co-chairman, "Biocatalysis," Recent Advance in Fermentation Technology, Long Beach, CA, November, 2001.
Co-chairman, "Biochemical Reaction Engineering," 1st North American Symposium on Chemical Reaction Engineering, Houston, TX, January, 2001.
Co-chairman, "Biodiversity and Physiology," Third International Congress on Extremophiles, Hamburg, Germany, September, 2000.
Co-chairman, "Forty Years of *Biotechnology and Bioengineering*: Highlights and Reflections," ACS National Meeting, San Francisco, CA, March, 2000.
Co-chairman, "Biodiversity/New Enzyme Discovery," Enzyme Engineering XV, Kailua-Kona, Hawaii, October, 1999.
Co-chairman, "Discovery and Production of Bioactive Small Molecules," Biochemical Engineering XI, Salt Lake City, Utah, July, 1999.

Co-chairman, "Origins, Environments, and Organisms," Cold Spring Harbor Laboratory Program on Biochemistry at 100°C, Cold Spring Harbor, NY, December, 1998.

Co-chairman, "Advances in Biocatalysis and Protein Engineering," AIChE Annual Meeting, Miami, FL, November, 1998.

Co-chairman, "Enzymes," International Conference on High Pressure Bioscience and Biotechnology, Heidelberg, Germany, September, 1998.

Co-chairman, "Combinatorial Chemistry," ACS Annual Meeting, Boston, MA, August, 1998.

Co-chairman, "Artificial Enzymes," Enzyme Engineering XIV, Beijing, China, October, 1997.

Meeting co-chairman, "Extremophilic Organisms: Harvesting Biodiversity for Industrial Applications," San Diego, CA, August, 1997.

Co-chairman, "Frontiers in Biochemical Engineering," Biochemical Engineering X, Kananaskis, Canada, May, 1997.

Chairman, "Advances in Combinatorial Chemistry and High Throughput Screening," ACS Spring National Meeting, San Francisco, CA, April, 1997.

Co-chairman, "Advances in Combinatorial Chemistry and High Throughput Screening," ACS Spring National Meeting, New Orleans, LA, March, 1996.

Co-chairman, "Advances in Bioprocessing: Novel Bioreactor Systems," 1995 International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December, 1995.

Co-chairman, "Preparation and Properties of Designed Catalysts," Enzyme Engineering 13, San Diego, CA, October, 1995.

Co-chairman, "Biocatalysis and Protein Engineering," AIChE Annual Meeting, San Francisco, CA, November, 1994.

Co-chairman, "Advances in Biocatalysis and Protein Engineering," ACS National Meeting, San Diego, CA, March, 1994.

Chairman, "Reaction Mechanisms of New Biocatalysts," Enzyme Engineering 12, Deauville, France, September, 1993.

Co-chairman, "Biodiversity," Eighth International Conference on Biochemical Engineering, Princeton, NJ, July 1993.

Co-chairman, "Biocatalysis Under Extreme Conditions," ACS National Meeting, Denver, CO, March, 1993.

Co-chairman, "Biocatalysis in Synthesis and Biotransformation," 9th International Biotechnology Symposium, Washington, D.C., August, 1992.

Co-chairman, "Enzymes from Extremophiles," ACS National Meeting, San Francisco, CA, April, 1992.

Co-chairman, "Monitoring Cellular Physiology and Metabolism for Process Control and Optimization," Cell Culture Engineering 3, Palm Coast, FL, February, 1992.

Co-chairman, "Novel Directions in Biocatalysis," ACS National Meeting, Washington, D.C., August, 1990.

Chairman, "High Resolution Purification," Smith-Kline & French-Triton-UCLA Symposium on Protein Purification and Biochemical Engineering, Lake Tahoe, CA, March, 1990.

Co-chairman, "Biocatalysis and Biomimetics: Novel Applications in Synthesis," AIChE Annual Meeting, San Francisco, CA, November, 1989.

Co-chairman, "Regulation of Growth and Product Formation of Recombinant Cells," AIChE Annual Meeting, Washington, D.C., November, 1988.

Co-chairman, "Biosensor and Analytical Methods in Bioprocessing," Sixth International Conference on Biochemical Engineering, Santa Barbara, CA, October 1988.

Co-chairman, "Symposium on Biomimetics and Biocatalysis: Impact of Surface and Interfacial Structure on Enzyme Activity," ACS National Meeting, Toronto, June, 1988.

Co-chairman, "Separations," 27th Annual NorCal AIChE Symposium, Berkeley, March, 1988.

Co-chairman, "Bioprocessing Engineering," ACS National Meeting, New Orleans, September, 1987.

Co-chairman, "Fermentation Processes under Extreme Conditions," AIChE Summer National Meeting, Minneapolis, August, 1987.

Co-chairman, "Basic Advances in Physical Sciences," Fifth International Conference on Biochemical Engineering, Henniker, NH, July 1986.

Chairman, "Recent Advances in Biochemical Engineering," AIChE Annual Meeting, Chicago, November 1985.

Short Courses and Special Presentations

Short Course, Protein Engineering & Industrial Biocatalysis, Exxon Mobil, Annandale, NJ, June 2018.

"Biotechnology Update: From Miracles in the Lab to Money in the Bank," a presentation to the students of Banneker High School, Washington, D.C., to commemorate National Science and Technology Week, April, 1990. Sponsored by NSF.

"Separation and Purification of Proteins from Recombinant Organisms," Millipore Corporation, Bedford, MA, May, 1989.

"Mechanism and Kinetics of Enzyme Catalysis," an introductory course offered as part of the Conference on Biotechnology sponsored by the AIChE, AIChE National Meeting, New York City, New York, November, 1987.

Instructor, Biochemical Engineering Program, ASEE Summer School for Chemical Engineering Faculty, North Dartmouth, Massachusetts, August, 1987

"Biocatalytic Engineering", one of three lectures comprising a short course entitled "Modern Biotechnology: Scope and Recent Developments", Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, April, 1985. The short course was sponsored by the Union Carbide Agricultural Products Company and the University of North Carolina at Chapel Hill Department of Chemistry.

Textbooks

Biochemical Engineering, H.W. Blanch and D.S. Clark, Marcel Dekker, Inc., 1995, 704 pages.

Publications

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2. D.D. Do, D.S. Clark, and J.E. Bailey, "Modeling Enzyme Immobilization in Porous Solid Supports", *Biotechnol. Bioeng.*, **24**, 1527 (1982).
3. D.S. Clark and J.E. Bailey, "Structure-Function Relationships in Immobilized Chymotrypsin Catalysis", *Biotechnol. Bioeng.*, **25**, 1027 (1983).
4. K.E. Dennis, D.S. Clark, J.E. Bailey, Y.K. Cho, and Y.H. Park, "Immobilization of Enzymes in Porous Supports: Effects of Support-Enzyme Solution Contacting", *Biotechnol. Bioeng.*, **26**, 892 (1984).
5. D.S. Clark and J.E. Bailey, "Characterization of Heterogeneous Immobilized Enzyme Subpopulations Using EPR Spectroscopy", *Biotechnol. Bioeng.*, **26**, 231 (1984).
6. D.S. Clark, J.E. Bailey, R. Yen, and A. Rembaum, "Enzyme Immobilization on Grafted Polymeric Microspheres", *Enzyme and Microbial Technology*, **6**, 317 (1984).
7. D.S. Clark and J.E. Bailey, "Deactivation Kinetics of Immobilized α -Chymotrypsin Subpopulations", *Biotechnol. Bioeng.*, **26**, 1090 (1984).
8. D.S. Clark and J.E. Bailey, "Kinetic and EPR Spectroscopy Studies of Immobilized Chymotrypsin Deactivation", *Ann. N.Y. Acad. Sci.*, **434**, 31 (1984).
9. D.S. Clark and J.E. Bailey, "Deactivation of α -Chymotrypsin and α -Chymotrypsin-CNBr Sepharose 4B Conjugates in Aliphatic Alcohols", *Biochim. Biophys. Acta*, **788**, 181 (1984).
10. D.S. Clark, J.E. Bailey, and D.D. Do, "A Mathematical Model for Restricted Diffusion Effects on Macromolecule Impregnation in Porous Supports", *Biotechnol. Bioeng.*, **27**, 208 (1985).
11. J.E. Bailey and D.S. Clark, "EPR Studies of Immobilized Chymotrypsin", in *Methods in Enzymology*, **135**, K. Mosbach, Ed. (Academic, New York, 1987), 502- 512.
12. G.A. Marg, G.L. Millhauser, P.S. Skerker, and D.S. Clark, "Application of EPR Methods in Studies of Immobilized Enzyme Systems", *Ann. N.Y. Acad. Sci.*, **469**, 253 (1986).
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29. J.F. Miller, C.M. Nelson, J.M. Ludlow, N.N. Shah, and D.S. Clark, "High Pressure-Temperature Bioreactor: Assays of Thermostable Hydrogenase with Fiber Optics," Biotechnol. Bioeng., **34**, 1015 (1989).
30. G.A. Marg and D.S. Clark, "Activation of Glucose Isomerase by Divalent Cations: Evidence for Two Distinct Metal Binding Sites," Enzyme Microb. Technol., **12**, 367 (1990).
31. M.K. Murphy, E.J. Fernandez, and D.S. Clark, "A Comparison of Three Radio Frequency Coils for NMR Studies of Conductive Samples," Magn. Reson. Med., **12**, 382 (1989).
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33. C. Pina, D.S. Clark, and H.W. Blanch, "The Activity of PEG-Modified Chymotrypsin in Aqueous and Organic Media," Biotechnol. Techniques, **3**, 333 (1989).
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35. D. S. Clark and R. M. Kelly, "Hot Bacteria," Chemtech (invited paper), **20**, 655 (1990).
36. T.W. Randolph, H.W. Blanch, and D.S. Clark, "Biocatalysis in Supercritical Fluids," in *Biocatalysts for Industry*, J. Dordick, Ed. (Plenum Press, N.Y., 1991), p. 219.
37. E.J. Fernandez and D.S. Clark, "Noninvasive Studies of Bioreactors," Chemtech (invited paper), **21**, 99 (1991).
38. A.G. Michiels, A. Wang, D.S. Clark, and H.W. Blanch, "Inhibition of Dextranucrase by -D-Glucose Derivatives," Appl. Biochem. Biotechnol., **31**, 237 (1991).
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41. A. Mancuso, E.J. Fernandez, H.W. Blanch, and D.S. Clark, "A Nuclear Magnetic Resonance Technique for Determining Hybridoma Cell Concentration in Hollow Fiber Bioreactors," *Biotechnology*, **8**, 1282 (1990).
42. R. M. Guinn, P.S. Skerker, P. Kavanagh, and D.S. Clark, "Activity and Flexibility of Alcohol Dehydrogenase in Organic Solvents," *Biotechnol. Bioeng.*, **37**, 303 (1991).
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44. M.M. Fernandez, D.S. Clark, and H.W. Blanch, "Papain Kinetics in the Presence of a Water-Miscible Organic Solvent," *Biotechnol. Bioeng.*, **37**, 967 (1991).
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- S-2. G. Makrygiorgos, A.J. Berliner, F. Shi, D.S. Clark, A.P. Arkin, A. Mesbah, “Data-Driven Flow-Map Models for Data-Efficient Discovery of Dynamics and Fast Uncertainty Quantification of Biological and Biochemical Systems,” (submitted). <https://doi.org/10.1101/2022.02.19.481146>
- S-3. J.D. Adams, K.B. Sander, C.S. Criddle, A.P. Arkin, D.S. Clark, “Engineering osmolysis susceptibility in *Cupriavidus necator* and *Escherichia coli* for recovery of intracellular products,” (submitted). <https://doi.org/10.21203/rs.3.rs-2398573/v1>

Patents/Patent Applications

1. “Biocatalytic Methods for Synthesizing and Identifying Biologically Active Compounds,” J.Wesley Fox, Jonathan Dordick, and Douglas S. Clark (WO/1995/005475).
2. “Biocatalytic Methods for Synthesizing and Identifying Biologically Active Compounds,” J.Wesley Fox, Jonathan Dordick, and Douglas S. Clark (WO/1996/005511).
3. "Reacting an Enzyme in a Non-aqueous Solvent by Adding a Lyophilizate of Enzyme and Salt to the Solvent," Jonathan Dordick, Yuri Khmelnitsky, and Douglas S. Clark (Patent No. US 5,449,613; 1995).
4. “Biocatalytic Methods for Synthesizing and Identifying Biologically Active Compounds,” Jonathan S. Dordick, Douglas S. Clark, Peter C. Michels, and Yuri L. Khmelnitsky (WO/1997/010233; 1997).

5. "Biocatalytic Methods for Synthesizing and Identifying Biologically Active Compounds," Jonathan S. Dordick, Douglas S. Clark, Peter C. Michels, and Yuri L. Khmelnitsky (U.S. Patent 6,136,961; 2000).
6. "Two-Step Enzymatic Acylation," Yuri Khmelnitsky et al. (Patent No. US 6,261,813; 2001).
7. "Molecularly-Imprinted Material Made by Template-Directed Synthesis," Markowitz et al. (Patent No. US 6,310,110; 2001).
8. "Molecularly-Imprinted Material Made by Template-Directed Synthesis," Markowitz et al. (Patent No. US 6,713,416; 2002).
9. "Taxol Derivatives," Jonathan S. Dordick, Douglas S. Clark, Peter C. Michels, and Yuri L. Khmelnitsky (Patent No. US 6,465,625; 2002).
10. "Molecularly-Imprinted Material Made by Template-Directed Synthesis," Markowitz et al. (Patent No. US 6,583,191; 2003).
11. "Molecularly-Imprinted Material Made by Template-Directed Synthesis," Markowitz et al. (Patent No. US 6,660,780; 2003).
12. "Methods for Preparing Doxorubicin Derivatives," D.H. Altreuter, D.S. Clark, P. C. Michels, J.O. Rich, H. Meckler, J. S. Dordick (WO 2003/057687; 2003).
13. "Biocatalytic Solgel Microarrays," D.S. Clark and J.S. Dordick (US 7,267,958 (2007); WO 2003/038131; 2003).
14. "In Vitro Metabolic Engineering on Microscale Devices," J.S. Dordick, A. Srinivasan, J. Kim, D.H. Sherman, and D.S. Clark (WO 2003/038404; 2003).
15. "Three-Dimensional Cellular Array Chip and Platform for Toxicology Assays," J.S. Dordick, D.S. Clark, M.Y. Lee, and R. A. Kumar (WO 2007/053561; 2007).
16. "In Vitro Metabolic Engineering on Microscale Devices," J.S. Dordick, A. Srinivasan, J. Kim, D.H. Sherman, and D.S. Clark (US 7,427,497; 2008).
17. "Heterologous Expression of Extremophilic Heat Shock Proteins and Chaperones in Microorganisms to Increase Tolerance to Toxic Compounds," D. S. Clark et al. (WO/2008/106430).
18. "Biocatalytic Solgel Microarrays," D.S. Clark and J.S. Dordick (Japan 4365216; 11/2009).
19. "Biocatalytic Solgel Microarrays," D.S. Clark and J.S. Dordick (CA 2,466,172; 7/2010).
20. "Biocatalytic Solgel Microarrays," D.S. Clark and J.S. Dordick (US 7,846,747; 12/2010).

21. "Three-Dimensional Cellular Array Chip and Platform for Toxicology Assays," J.S. Dordick, D.S. Clark, M.Y. Lee, and R. A. Kumar (CA 2,628,219; 11/2012).
22. "Three-Dimensional Cellular Array Chip and Platform for Toxicology Assays," J.S. Dordick, D.S. Clark, M.Y. Lee, and R. A. Kumar (Japan 4959710; 3/2012).
23. "Heterologous Expression of Extremophilic Heat Shock Proteins and Chaperones in Microorganisms to Increase Tolerance to Toxic Compounds," D. S. Clark et al. (U.S. 8,685,729; 4/2014).
24. "Polypeptides for use in the Deconstruction of Cellulose," J. E. Graham, M. E. Clark, F. T. Robb, D. S. Clark, H. W. Blanch (US 9,334,515; 5/2016).
25. "Thermophilic Mutants of Trichoderma Reesei Endoglucanase I," H.A. Chokhawala, T.W. Kim, H.W. Blanch and D.S. Clark (U.S. 9,410,135; 8/2016).
26. "Methods to Produce Fuels," Z.C. Baer, H.W. Blanch, D.S. Clark, S. Sreekumar, F.D. Toste, G. Gunbas, K.A. Goulas, A.A. Gokhale, and J.B. Binder (US 9,790,444; 10/2017).
27. "Method to Convert Fermentation Mixture into Fuels," D.F. Toste et al. (U.S. 9,856,427; 1/2018).
28. "Artificial Metalloenzymes Containing Noble Metal-Porphyrins," J.F. Hartwig, H.M. Key, P. Dydio, and D.S. Clark (WO/2017/066562; 4/2017, US Patent Application 15953331; 10/2018). Also **U.S. Patent 11,518,768; 12/2022**.
29. "Thermophilic Mutants of Trichoderma Reesei Endoglucanase I," H.A. Chokhawala, T.W. Kim H.W. Blanch and D.S. Clark (Brazil, BR112013006315; 4/2017). Also U.S. Patent 9,410,135.
30. "Devices and Methods for Generating Oligodendrocyte Progenitor Cells," D.V. Schaffer, R. Muckom, and D.S. Clark (U.S. Patent Application No. 62/839,399; 4/2020, WO 2020/219696; 10/2020).
31. "Host Cells and Methods Useful for Producing Unnatural Terpenoids Using a Novel Artificial Metalloenzyme," J. Huang, Z. Liu, D.S. Clark, J.D. Keasling, A. Mukhopadhyay, J.F. Hartwig (U.S. Patent Appl. No. 17/200,715; 3/2021).
32. "Direct Air Capture and Bioelectrochemical Conversion of CO₂," D.S. Clark, O.M. Yaghi, P. Yang, A. Abel, J. Adams, S. Cestellos-Blanco, H.Lyu, A. Zhumekenov. (Provisional patent application, 2021).

Invited Presentations

D. S. Clark, D.I.C. Wang Award Lecture, From Artificial Cofactors to Synthetic Arrays: Teaching Old Enzymes New Tricks, 2019 AIChE Annual Meeting, Orlando, Florida, November 2019.

D. S. Clark, Plenary Lecture, 14th Asian Congress on Biotechnology (ACB 2019), From Artificial Cofactors to Protein Scaffolds: Teaching Old Enzymes New Tricks, Taipei, Taiwan, July 2019.

D. S. Clark, Distinguished Lecture, Global Frontiers in Science and Technology, Berkeley, CA, June 2018.

D. S. Clark, "Protein Engineering for New Biocatalysts and Advanced Biomaterials," *Plenary Lecture*, 2017 Korean Society for Microbiology and Biotechnology (KMB) International Symposium & Annual Meeting, Busan, South Korea, June 2017.

D. S. Clark, "Improving cellulase function at the extremes: Overcoming barriers of temperature and inhibition," Symposium on Biotechnology for Fuels and Chemicals, San Francisco, CA, May 2017.

D. S. Clark, "Thermostable Protein Templates for Biocatalysis and Functional Materials," BASF and CARA Enzyme Technology Workshop, Berkeley, CA, April 2016.

D. S. Clark, "Protein Engineering for New Biocatalysts and Advanced Biomaterials," Inaugural Paul Hohenshuh Lecture, Washington State University, Pullman, WA, February 2016.

D. S. Clark, "Metabolic and Protein Engineering for the Production of Fuels and Chemicals," BASF Corporation, Ludwigshafen, Germany, November 2015.

D. S. Clark, "A User Guide to the γ -Prefoldin--a Self-Assembling Protein Template--and an Update on the Limits of Cellulases," Genencor/Dupont Industrial Biosciences, Palo Alto, CA, October 2015.

D. S. Clark, "The Hyperthermophilic γ -Prefoldin: a Unique Stabilizing Influence with Material Aspirations," Army Biostability Symposium, Natick, MA, September 2015.

D. S. Clark, "Kinetic and Modeling Studies of Cellulase-Substrate Interactions: From Single Molecules to Cocktail Mixtures," Gordon Research Conference- Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes, Andover, NH, August 2015.

D. S. Clark, "The hyperthermophilic γ -prefoldin: a unique stabilizing influence with material aspirations," 2015 Colorado Protein Stability Conference, Breckenridge, Colorado, July 2015.

D. S. Clark, "The College of Chemistry at UC Berkeley: A Global Catalyst for Industrial Innovation," Japan Techno-Economics Society (JATES) Committee Meeting, July 2015.

D. S. Clark, "Controlled Assembly of Ultrastable Protein Parts for Nanomaterials and Biomechanical Devices," ShanghaiTech Advances in Research (STAR) Symposium, Shanghai, China, June 2015.

D. S. Clark, “Recent Progress in the Evolution of Biofuels (with Some Added Twists),” Chemical Engineering and Materials Science Research Forum 2015, Michigan State University, East Lansing, MI, May 2015

D. S. Clark, “A Personal Perspective on Biotechnology’s Progress in a Golden Era (and how Jay Bailey saw it all coming),” Bailey Award Lecture, AIChE National Meeting, San Francisco, November 2014.

D. S. Clark, “Tapping New Sources of Cellulases: Extremophiles Add to the Fray,” ACS National Meeting, San Francisco, August 2014.

D.S. Clark, “Conversion of Lignocellulose to Biofuels. Bioprocess Optimization: from Cellulose Hydrolysis to Product Fermentation,” BP Biofuels, San Diego, CA, July 2014.

D. S. Clark, “Sweetening the Deal for Biofuels: Engineering the Efficient Deconstruction of Biomass,” Invited webinar as part of the “Best in BIOT Award,” September 2013.

D. S. Clark, “Sweetening the Deal for Biofuels: Engineering the Efficient Deconstruction of Biomass,” *Plenary Lecture*, ACS National Meeting, New Orleans, April 2013 (awarded the “Best in BIOT Award” for best presentation).

D. S. Clark, “Cellulosic Biofuels: Sustainable Energy for the Future,” Cal Homecoming Lecture, Richard C. Blum Hall, UC Berkeley, October 2012.

D. S. Clark, “Breaking a Biofuels Bottleneck: Deconstructing Biomass from the Ground Up,” Keynote Lecture, 15th International Biotechnology Symposium (IBS 2012), Daegu, Republic of Korea, September 2012.

D.S. Clark, “Exploring the Limits of Biomass Degradation: From Sampling Pools to Pooling Genes,” *Division Lecture*, ASM General Meeting, San Francisco, CA, June 2012.

D.S. Clark, “From Biomass to Biofuels: Breaking the Cellulose Barrier,” Society of Biological Engineering (SBE) Webinar, October 2011.

D.S. Clark, “From Biomass to Biofuels: Breaking the Cellulose Barrier,” 8th European Congress of Chemical Engineering, Berlin, Germany, September 2011.

D.S. Clark, “Protein Structure and Function in Nearly Anhydrous Solvents: Can Biocatalysis Run Dry?” Gordon Research Conference on “Biological Molecules in the Gas Phase and in Solution,” August 2011.

D. S. Clark, “Discovery, Design, and Development of Thermophilic Cellulases,” Biocatalysis 2010, Puerto Morelos, Mexico, December 2010.

D. S. Clark, “Discovery, Design, and Development of Thermophilic Cellulases,” 8th International Conference on Extremophiles, Ponta Delgada, Azores, Portugal, September 2010.

D.S. Clark, “From Biomass to Biofuels: Breaking the Cellulose Barrier,” UCSF/UCB Bioengineering Conference, Lake Tahoe, CA, September 2010.

D.S. Clark and J. S. Dordick, “Catalytic Stimulus: Putting Enzymes to Work in Organic Solvents,” Symposium in Honor of Fred Heineken on the Occasion of His Retirement from NSF, ACS National Meeting, San Francisco, CA, March 2010.

D. S. Clark, “Self-Reporting and Self-Assembling Material Components Based on Extremophilic Proteins,” AFOSR Program Reviews, National Harbor, MD, January 2010.

D. S. Clark, “Engineering Proteins into the Fold: New Biocomponents for Nanoscale Engineering (Keynote Lecture),” Biochemical Engineering XVI, Burlington, VT, July 2009

D. S. Clark, “The Life and Times of B&B: Editorial Reflections on the First Half Century,” Biochemical Engineering XVI, Burlington, VT, July 2009

D. S. Clark, “Developing Improved Thermostable Cellulases: High-Throughput Cellulolytic Assays and Protein Engineering Strategies,” 31st Symposium on Biotechnology for Fuels and Chemicals, San Francisco, CA, May 2009.

D.S. Clark, “Past, Present, and Future of Biocatalysis,” AIChE Centennial Session: ChE Research and Technology – Past and Future, AIChE National Meeting, Philadelphia, PA, November 2008.

D.S. Clark, “Unraveling the Mechanisms of Piezophily in a Deep-Sea Thermophile,” 5th International Conference on High Pressure Bioscience and Technology, San Diego, CA, September, 2008.

D.S. Clark, “Enhancing Bacterial Solvent Tolerance by Expression of an Extremophilic Chaperone,” SIM Annual Meeting, San Diego, CA, August, 2008.

D.S. Clark, “Adapting to a Life of Stress: Why Do Some Enzymes Work Best Under Pressure?” Gordon Research Conference on Biopolymers, Newport, RI, June 2008.

D.S. Clark, “Improving the Safety of Drugs and Chemicals (without Harming a Hare),” College of Chemistry Dean’s Dinner, Berkeley, CA, April 2008

D.S. Clark, “Engineering Extremophilic Chaperones for Biocatalysis and Nanobiotechnology,” Biochemical Engineering XV, Quebec City, Canada, July 2007.

D.S. Clark, “High-Throughput Biocatalysis: Creating New Opportunities for Synthesis and Discovery, NASCRE-2, Houston, TX February 2007.

D.S. Clark, "From EPR in Micelles to NMR Inside Cells: Reflections on a Resonant Collaboration," AIChE National Meeting, San Francisco, CA, November 2006.

D.S. Clark, "Biochemical Technology Goes to Extremes: Exploring New Dimensions of Biological Function," Marvin J. Johnson Award Lecture, ACS National Meeting, San Francisco, CA, September 2006.

D. S. Clark, "Biocatalytic Microarrays for High-Throughput Synthesis and Toxicology Assays," Pacific Rim Summit on Industrial Biotechnology and Bioenergy," Honolulu, HI, January, 2006.

D.S. Clark, "New Discovery Routes to the Origins and Limits of Biocatalysis," Enzyme Engineering XVIII, Gyeong-ju, Korea, October, 2005.

D.S. Clark, "From the Earliest Organisms to the Latest Arrays: Old and New Prospects for Biocatalysis," Faculty Seminar for Homecoming Weekend, U.C. Berkeley College of Chemistry, September, 2005.

D.S. Clark, "Microfluidic Multiplex Systems for Multi-step Biotransformations and In-Situ Product Detection," Biochemical Engineering XIV, Harrison Hot Springs, BC, Canada, July 2005.

D.S. Clark, "Enzymes and Their Material Needs: Overcoming the Natural Limitations of Biocatalysis," Workshop on Biocatalysis in Non-conventional Media, Manchester, UK, April 2005.

D.S. Clark, "Life in Organic Solvents, Ammonia, and Supercritical Fluids," Workshop on the Limits of Organic Life in Planetary Systems, The National Academies, Washington, DC, May 2004.

D.S. Clark, "From the Earliest Organisms to the Latest Arrays: Old and New Prospects for Biocatalysis," NorCal ACS Meeting, Walnut Creek, CA, April 2004.

D. S. Clark, "Engineering Biocatalytic Systems for Multi-scale, Multi-phase Reactions," Biotech 2003, Lisbon, Portugal, December 2003.

D. S. Clark, "Characteristics of Dry Enzymes in Organic Solvents," Meeting on The Molecular Basis of Life: Is Live Possible Without Water? (organized by the Royal Society), London, UK, December 2003.

D. S. Clark, "Adventures in Biocatalysis: Engineering Enzymes to New Extremes" (Food, Pharmaceutical, and Bioengineering Award Lecture), Annual AIChE Meeting, San Francisco, CA, November 2003.

D. S. Clark, Enzyme Engineering Award Lecture (with J.S. Dordick), Enzyme Engineering XVII, Santa Fe, NM, November 2003.

D. S. Clark, "From the Earliest Archaea to the Latest Arrays: Old and New Prospects for Biochemical Engineering" (Amgen Award Lecture), Biochemical Engineering XIII, Boulder, CO, July 2003.

D. S. Clark, "Biocatalytic Synthesis of Naturally Inspired Libraries," Metabolic Engineering IV: Applied Systems Biology," Castelvechio Pascoli, Italy, October 2002.

D.S. Clark, "Enzyme Engineering: An Evolution in the Making," 224th National ACS Meeting, Boston, MA, August 2002.

D.S. Clark," "Biocatalyst Engineering for Drug Discovery," 7th Annual World Congress on Enzyme Technologies, San Francisco, CA, March, 2002.

D. S. Clark, "Engineering Bioactive Surfaces for Biotechnology," Gwathmey Lecture in Surface Science, Engineering, and Medicine; University of Virginia, 2001.

P.C. Michels and D.S. Clark, "Combinatorial Biocatalysis as a Practical, Integrated Solution for Pharmaceutical Chemistry," Drug Discovery by Design, Boston, MA, November, 2001

D. H. Altreuter, J. Ju, C.B. Park, and D.S. Clark, "Biocatalyst Engineering for Drug Discovery and Screening," Recent Advances in Fermentation Technology, Long Beach, CA, November, 2001.

D.S. Clark, "Relating Form to Function in Complex Systems: Molecular-Based Studies of Immobilized Enzymes and Cells, Annual AIChE Meeting, Reno, NV, November, 2001.

D.S. Clark, "Biotechnology of Extremophiles: Challenges and Opportunities," Center for Environmental Biotechnology, LBNL, Berkeley, CA, June, 2001.

D.S. Clark, "Reaction Engineering Strategies for the Rapid Discovery of New Bioproducts," 1st North American Symposium on Chemical Reaction Engineering, Houston, TX, January, 2001

D.S. Clark, M.M.C. Sun, R. Frankenberg, J.S. Beadell, F.T. Robb, and M. Lee, "Structure and Function of Multimeric Enzymes Near 100°C: Piecing Things Together Under Pressure," Pacifichem 2000, Honolulu, HI, December, 2000.

D.S. Clark, "Combinatorial Biocatalysis for the Generation of New Chemical Products: From Molecular Design to Reaction Engineering," AIChE Annual Meeting, Los Angeles, CA, November, 2000.

M.M.C. Sun, C.B. Park, M. Lee, R. Frankenberg, F.T. Robb, and D.S. Clark, "Stress, Pressure, and Performance: Cellular and Molecular Mechanisms of Barophily at High Temperatures," Third International Congress on Extremophiles, Hamburg, Germany, September, 2000.

D.S. Clark, "Pressure Stabilization of Proteins Near 100°C: Implications for Structure-Function and Structure-Stability Relationships," 2nd International Conference on Protein Stabilization, Lisbon, April, 2000.

D.S. Clark, J.D. Keasling, C. Wang, S.W. Bang, and N. Renninger, "Metabolic Engineering of Microorganisms for Heavy Metal Bioremediation," DOE-NABIR Workshop, Reston, VA, February, 2000.

D.S. Clark, "Integrating Biocatalysis into the Drug Discovery Pipeline," Biochemical Engineering XI, Salt Lake City, Utah, July, 1999.

D.S. Clark, "Pressure Stabilization of Proteins Near 100°C: Implications for Structure-Function Relationships," Cold Spring Harbor Laboratory Program on Biochemistry at 100°C, Cold Spring Harbor, NY, December, 1998.

D. S. Clark, "Challenges and Opportunities with Extremophiles and Their Enzymes" (Keynote Lecture), 48th Annual Meeting of the Canadian Chemical Engineering Conference, London, Ontario, October, 1998.

D.S. Clark, "Engineering Enzymes to New Extremes," IBC Conference on Directed Evolution of Industrial Enzymes, San Diego, CA, September, 1998.

D.S. Clark, "Biocatalysis in the P-T Plane," International Conference on High Pressure Bioscience and Biotechnology, Heidelberg, Germany, September, 1998.

D.S. Clark, "Tailoring Enzymes for Greater Efficiency in Combinatorial Synthesis," 1998 Gordon Conference on Green Chemistry, Meriden, NH, August, 1998.

D.S. Clark, "Combinatorial Biology," 20th Annual Engineering Industrial Liaison Program Conference," U.C. Berkeley, March 1998.

D. S. Clark, "Integration of Biocatalytic and Chemical Methods for Combinatorial Synthesis," CHI Conference on Exploiting Molecular Diversity: Small Molecule Libraries for Drug Discovery, Coronado, CA, March, 1998.

D. S. Clark, "Combinatorial Biocatalysis: A Natural Approach to Drug Discovery," IBC International Conference on Combinatorial Synthesis of Natural Products, San Francisco, CA, December, 1997.

M.M. Sun, F.T. Robb, and D. S. Clark, "Biocatalysis in the P-T Plane," Enzyme Engineering XIV, Beijing, China, October, 1997.

Y.L. Khmel'nitsky, P.C. Michels, J.S. Dordick, and D.S. Clark, "Combinatorial Biocatalysis," Enzyme Engineering XIV, Beijing, China, October, 1997.

D.S. Clark, "Barophilic Enzymes and How They Cope with Pressure," IBC International Conference on Extremophilic Organisms: Harvesting Biodiversity for Industrial Applications, San Diego, CA, August, 1997.

D.S. Clark, "Biocatalyst Engineering for Extreme Environments," Chemistry and Biochemistry Under Extreme Conditions, Santorini Island, Greece, June, 1997.

D.S. Clark, "Setting the Research Agenda in Biotechnology: The Evolving Synergy Between Chemistry and Engineering," Chancellor's Forum Program, University of California, Berkeley, November, 1996.

D.S. Clark, "Biocatalyst Engineering for Extreme Environments," Sixth Biochemical Engineering Conference," Yusong, Korea, September, 1996.

D.S. Clark, M. Sun, J. DiRuggiero, C. Vetriani, and F.T. Robb, "Temperature-Pressure Effects on Recombinant Production of Thermophilic Enzymes," 1996 SIM Annual Meeting, Research Triangle Park, NC, August, 1996.

D.S. Clark, "The Role of Pressure in Life at High Temperatures," 2nd Annual Symposium on German-American Frontiers of Science, Sponsored by Alexander von Humboldt-Stiftung and the U.S. National Academy of Sciences, Woods Hole, MA, June, 1996.

D.S. Clark, "Combinatorial Biocatalysis for Generating Small Molecule Libraries," Exploiting Molecular Diversity: Small Molecule Libraries for Drug Discovery," Coronado, CA, January, 1996.

M. Sun, L. Giarto, P.C. Michels, and D.S. Clark, "Pressuring Enzymes to Improve Their Behavior," 1995 International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December, 1995.

J.S. Dordick, J. Kim, Y. Khmelnitsky, V. Paradkar, and D.S. Clark, "Enzymes in High Salt and Solvent Environments," 1995 International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December, 1995.

D.S. Clark, M. Sun, L. Giarto, P.C. Michels, A. Matschiner, and F.T. Robb, "Activation and Stabilization of Thermophilic Enzymes by Pressure," International Conference on High Pressure Bioscience and Biotechnology, Kyoto, Japan, November, 1995.

D.S. Clark, "Adapting to a Life of Stress: Why do Some Enzymes Work Best Under Pressure," Thirteenth International Conference on Enzyme Engineering, San Diego, CA, October, 1995.

D.S. Clark, "Enzyme Activation in Nonaqueous Media," ARO Workshop on Biotechnological Aspects of Synthesis and Degradation, Cashiers, NC, May, 1995.

D.S. Clark, "Biocatalysis for Generating Libraries of Organic Compounds," Exploiting Molecular Diversity: Small Molecule Libraries for Drug Discovery," La Jolla, CA, January, 1995.

D. S. Clark, "Biotechnology and Chemical Engineering Education: Perspective from Academic Bioengineering," 1994 AIChE Annual Meeting, San Francisco, CA, November, 1994.

D.S. Clark, "Physiology of Thermophilic Marine Archaea at Extreme Temperatures and Pressures," 1994 SIM Annual Meeting, Boston, MA, August, 1994.

D.S. Clark, "Barophilic Enzymes from Extreme Thermophiles," at Extremozymes: Biocatalysis under Extraordinary Conditions," A Workshop Sponsored by NSF, Washington, D.C., May, 1994.

P.C. Michels and D.S. Clark, "Preliminary Characterization of an Extremely Thermophilic and Barophilic Protease from the Deep Sea Thermophile *M. jannaschii*," Leo J. Friend Award Symposium, ACS National Meeting, San Diego, CA, March, 1994.

D.S. Clark, "Physiology of Extreme Thermophiles at High Temperatures and Pressures," International Thermophiles '93 Conference, Hamilton, New Zealand, December, 1993.

D. S. Clark, "Probing Enzyme Stability with Pressure: Structure-Function Relationships Consistent with Pressure-Induced Stabilization," US/NZ Workshop on Extreme Thermophiles, Hamilton, New Zealand, December, 1993.

D.S. Clark, "Probing the Limits of Life: Engineering Considerations for the Study and Application of Extreme Thermophiles," Eighth International Conference on Biochemical Engineering, Princeton, NJ, July, 1993.

D.S. Clark, "Thermophilic Enzymes as Industrial Catalysts," at Enzymes, A Billion Dollar Business by 2000: Fact or Fiction, Chicago, IL, June, 1993.

D.S. Clark, "Probing the Structural Basis for Modified Enzyme Activity in Nonaqueous Media," ARO Workshop on Biotechnology, Cashiers, NC, May, 1993.

D.S. Clark, "Hot Bacteria and Their Role in Biotechnology," Cal Open House, U.C. Berkeley College of Chemistry, April, 1992.

D.S. Clark, "Structure-Function Relationships of Enzymes in Novel Process Environments," Meeting on Research Opportunities in Biomolecular Engineering: The Interface Between Chemical Engineering and Biology (sponsored by the NIH), Washington, D.C., December, 1992.

D.S. Clark, "High-Pressure Biocatalytic Reactions," Industrial Research Technology Institute, Taipei, Taiwan, R.O.C., September, 1992.

D.S. Clark, P. Sears, and T.M. Spitznagel, "Progress Toward the Rational Design of Immobilized Protein Systems," 9th International Biotechnology Symposium, Washington, D.C., August, 1992.

D.S. Clark, "High-Pressure Biocatalysis: Experience with Enzymes from Deep-Sea Thermophiles," 1992 Gordon Conference on Biocatalysis, Meriden, NH, July, 1992.

D.S. Clark, "Natural Enzymes Under Unnatural Conditions: Grace Under Pressure," BRI Days 1992, sponsored by National Research Council Canada, Montréal, Canada, May, 1992.

D.S. Clark, "Unique Properties and Potential Applications of Organisms from Extreme Environments," Chemical Engineering and Chemistry Alumni Association Kickoff, Berkeley, CA, April, 1992.

A. Mancuso, S.T. Sharfstein, H.W. Blanch, and D.S. Clark, "Carbon-13 NMR Studies of Murine Hybridoma Metabolism," Cell Culture Engineering 3, Palm Coast, FL, February, 1992.

D. Hei, C. Nelson, and D.S. Clark, "Pressure Effects on the Activity and Stability of Enzymes from Marine Thermophiles," International Marine Biotechnology Conference, Baltimore, MD, October, 1991.

D.S. Clark, "Enzymes from Extremophiles: Properties and Potential Applications," Opportunities with Industrial Enzymes, Chicago, IL, October, 1991.

D.S. Clark, "Noninvasive Monitoring of Mammalian Cell Metabolism," Seventh International Conference on Biochemical Engineering, Santa Barbara, CA, March 1991.

C.M. Nelson, D. Hei, and D.S. Clark, "The Effects of Pressure on Bio-Activity and Stability at Elevated Temperatures," Thermophily Today: International Meeting on Cell Constituents, Genetics, and Biotechnological Applications of Thermophilic Micro-Organisms, Viterbo, Italy, May, 1990.

D.S. Clark, "Structural and Functional Heterogeneity of Immobilized Enzymes," 1990 Gordon Conference on Biomolecular Recognition and Immobilization, Plymouth, NH, August, 1990.

D.S. Clark, "The Use of NMR to Determine Metabolic Activity in Tissue and Cell Cultures," FASEB Annual Meeting, Washington, D.C., April, 1990.

D.S. Clark, "Noninvasive Techniques in Studies of Immobilized Cells," International Symposium on the Physiology of Immobilized Cells, Wageningen, The Netherlands, December, 1989.

D.S. Clark, "Publishing, Presenting, and the Pursuit of Tenure: Experiences of a Young Professor," Young Faculty Forum, AIChE Annual Meeting, San Francisco, CA, November, 1989.

D.S. Clark, P.S. Skerker, R.M. Guinn, and C.A. Haynes, "Structure and Dynamics of Enzymes in Nonaqueous Media," SIM Annual Meeting, Seattle, WA, August, 1989.

D.S. Clark, "ESR Studies of Protein Function in Extreme Environments," 1989 Gordon Conference on Reactive Polymers, Ion Exchangers, and Adsorbents, New Port, RI, August, 1989.

D.S. Clark, "NMR Spectroscopy as a Noninvasive Technique for Monitoring Metabolism," Bio-Instrumentation Workshop, Sponsored by the Biotechnology Process Engineering Center of M.I.T., Irvine, CA, June, 1989.

D.S. Clark, "Noninvasive Methods for Monitoring Cellular Function," Sixth International Conference on Biochemical Engineering, Santa Barbara, CA, October 1988.

P.S. Skerker, L. Creagh, J.M. Prausnitz, H.W. Blanch, and D.S. Clark, "Matrix Effects in Heterogeneous Enzyme Catalysis," ACS National Meeting, Toronto, June, 1988.

D.S. Clark, "Kinetic and Structural Investigations of Ligand Binding to Surface-Bound Proteins," AIChE Annual Meeting, New York City, NY, November, 1987.

D.S. Clark, "Conformational Analyses of Enzymes and Substrates in Processing Environments", Ninth International Enzyme Engineering Conference, Santa Barbara, CA, October 1987.

D.S. Clark, "Biocatalysis - Recent Advances and Future Directions", I&EC - ACS 22nd State-of-the-Art Symposium, Berkeley, CA, July 1987.

D.S. Clark, "Modern Biotechnology: Scope and Recent Developments", Lawrence Berkeley Laboratory High School Honors Program Seminar Series, Berkeley, CA, June 1987.

D.S. Clark, "Modern Methods for Molecular Studies of Proteins: Unique Tools for Some of Biochemical Engineering's Unique Problems", AIChE Annual Meeting, Miami Beach, FL, November 1986.

P.S. Skerker, E.J. Fernandez, R.B. Jagoda, and D.S. Clark, "Spectroscopic Studies of Structure-Function Relationships in Free and Immobilized Proteins", Fifth International Conference on Biochemical Engineering, Henniker, NH, July 1986.

S. Branner, G. Marg, and D.S. Clark, "Fundamental Studies of Glucose Isomerase Activation by Divalent Metals", New England Biotechnology Association Third Worcester Colloquium, Worcester, MA, May 1986.

D.S. Clark, "Structure-Function Relationships of Immobilized Enzymes and Immobilized Antibodies: New Insights Provided by EPR Spectroscopy", Cornell University Biotechnology Symposium, Ithaca, NY, October 1985.

Papers Presented at Conferences

D.S. Clark, "Enzymes and Their Material Needs: Overcoming the Natural Limitations of Biocatalysis," ACS National Meeting, San Diego, CA, March 2005.

M.Y. Lee, J.S. Dordick, and D.S. Clark, "Metabolizing Enzyme Toxicology Assay Chip (MetaChip) for in Vitro Drug Metabolism and Toxicity," ACS National Meeting, Anaheim, CA, March 2004.

T.C. Logan, M. Hajra, and D.S. Clark, "Toward the development of a microscale system for combinatorial biocatalysis," ACS National Meeting, Anaheim, CA, March 2004.

A.L. Meadows, H.W. Blanch, and D.S. Clark, "Quantifying the Metabolism of Human Breast Cancer: Applications to Drug Target Discovery," AIChE Annual, San Francisco, CA, November 2003.

M.Y. Lee, C.B. Park, A. L. Meadows, P. Reeder, D.S. Clark, and J.S. Dordick, "Development of a Metabolizing Enzyme Toxicology Assay Chip (MetaChip) for in Vitro Drug Metabolism and Toxicity," AIChE Annual, San Francisco, CA, November 2003.

U. Akbar, J.S. Dordick, and D.S. Clark, "Solid-Phase Biocatalytic Amplification of the Natural Product Bergenin," AIChE Annual Meeting, San Francisco, CA, November 2003.

B. B. Boonyaratanakornkit, A. J. Simpson, C. B. Park, C. Fraser, N. M. A. El-Sayed, and D. S. Clark, "Expression Profiling and High-Pressure Adaptation of the Deep-Sea Thermophile *Methanococcus jannaschii*," ACS National Meeting, New Orleans, LA, March, 2003.

U. Akbar, J.S. Dordick, and D.S. Clark, "Solid-Phase Biocatalytic Amplification of the Natural Product Bergenin," ACS National Meeting, New Orleans, LA, March, 2003.

B. B. Boonyaratanakornkit, A. J. Simpson, C. B. Park, C. Fraser, N. M. A. El-Sayed, and D. S. Clark, "Adaptation of the Deep-Sea Thermophile *Methanococcus jannaschii* to Extreme Temperatures and Pressures," AIChE Annual Meeting, Indianapolis, IN, November, 2002.

D.S. Clark and C.B. Park, "Biocatalyst Engineering for Synthesis and Screening of Chemical Libraries," 224th National ACS Meeting, Boston, MA, August, 2002.

J.P. Lindsay, D.S. Clark, and J.P. Lindsay, "Combinatorial Biocatalyst Preparation and High-Throughput Enzyme Screening for Improved Functional Properties," 224th National ACS Meeting, Boston, MA, August, 2002.

C. B. Park and D. S. Clark, "High Temperature-Pressure Chemostat for Cultivation of Deep-Sea Extremophiles," poster presented at 101st American Society for Microbiology Annual Meeting, Orlando, FL, May, 2001.

Y.-D. Kim, J. S. Dordick, and D. S. Clark, "Biocatalytic Polydimethylsiloxane Coatings," poster presented at Gordon Conference on Polymers (East), New London, CT, June, 2000.

M. T. Ru, S. Y. Hirokane, A. S. Lo, J. S. Dordick, J.A. Reimer, and D. S. Clark, "Optimization of Salt-Induced Activation of Enzymes in Organic Solvents," poster presented at ACS National Meeting, San Francisco, CA, March, 2000.

Y. Guo, Y. Yang, Y. D. Kim, and D. S. Clark, "High Throughput Screening of Hydrolytic Enzymes for Graywater Treatment and Prevention of Fouling," poster presented at ACS National Meeting, San Francisco, CA, March, 2000.

M.T. Ru, Wei-Ting Hsu, J.S. Dordick, J.A. Reimer, and D.S. Clark, "Role of Ion Hydration in the Salt-Induced Activation of Enzymes in Organic Media," AIChE Annual Meeting, Dallas, TX, November, 1999.

D.S. Clark, J.D. Keasling, S. C. Ozuna, and C.L. Wang, "Metabolic Engineering of Microorganisms to Precipitate Cadmium Wastes," AIChE Annual Meeting, Dallas, TX, November, 1999.

H. W. Blanch, D.S. Clark, and N.S. Forbes, "The Metabolic Response of Cancer Cells to Estradiol," AIChE Annual Meeting, Dallas, TX, November, 1999.

D. S. Clark, J.A. Morgan, I.A. Stergiades, and M. Tius, "Combinatorial Chemoenzymatic Synthesis of Novel Nucleoside Analogs for Pharmaceutical Screening," AIChE Annual Meeting, Dallas, TX, November, 1999.

H. W. Blanch, D.S. Clark, and K.W. Wicklund, "Characterization of MCF7 Human Breast Cancer Cells in Microcarrier Culture: Effects of Glucose and Hypoxia," AIChE Annual Meeting, Dallas, TX, November, 1999.

D.S. Clark, J.A. Reimer, M.T. Ru, and J.S. Dordick, "Optimizing the Salt-Induced Activation of Enzymes in Organic Solvents: Effects of Lyophilization Time and Water Content," AIChE Annual Meeting, Miami, FL, November, 1998.

M. M. Sun, C. Vetriani, N. Tolliday, D. Maeder, F. T. Robb, and D.S. Clark, "Improving the Stability of Hyperthermophilic Enzymes by Protein Engineering and Application of High Pressure," ACS Annual Meeting, Boston, MA, August, 1998.

D.H. Altreuter, J.S. Dordick, and D.S. Clark, "Biocatalysis for the Generation of Novel Doxorubicin Derivatives," ACS Annual Meeting, Boston, MA, August, 1998.

M. M. Sun, D.S. Clark, J. DiRuggiero, C. Vetriani, and F.T. Robb, "Pressure-Regulated Activity and Stability of Hyperthermophilic Enzymes," AIChE Annual Meeting, Los Angeles, CA, November, 1997.

D.S. Clark, J. S. Dordick, D. H. Altreuter, P. C. Michels, and Y. L. Khmelnitsky, "Combinatorial Biocatalysis for Drug Discovery and Optimization," Biochemical Engineering X, Kananaskis, Canada, May, 1997.

K. A. Yarema and D. S. Clark, "Electron Spin Resonance Studies of Epidermal Growth Factor/Epidermal Growth Factor Receptor Interactions," poster presented at Biochemical Engineering X, Kananaskis, Canada, May, 1997.

J.S. Dordick, Y.L. Khmelnitsky, P.C. Michels, and D.S. Clark, "Molecular Diversity Through High Speed Biocatalysis," ACS National Meeting, New Orleans, LA, March, 1996.

A. Matschiner, P.C. Michels, J. DiRuggiero, F. Robb, and D.S. Clark, "Recombinant Production of Hyperthermophilic Enzymes: Effects of High Temperature and Pressure," poster presented at Biochemical Engineering IX, Davos, Switzerland, May, 1995.

P.C. Michels, J.A. Baross, J.D. Keasling, and D. S. Clark, "Bioremediation Potential of Newly Isolated, Metal-Tolerant Archaea," poster presented at Biochemical Engineering IX, Davos, Switzerland, May, 1995.

P.C. Michels, J.S. Dordick, and D.S. Clark, "High Pressure-Temperature Biocatalysis in Aqueous and Organic Solvents," AIChE Annual Meeting, San Francisco, CA, November, 1994.

P.P. Wangikar, D.S. Clark, and J.S. Dordick, "Protein and Solvent Engineering of Enzyme-Substrate Hydrophobic Interactions," AIChE Annual Meeting, San Francisco, CA, November, 1994.

Y. Khmelnsky, P.P. Wangikar, V. Suzawa, P. Michels, J.S. Dordick, and D.S. Clark, "Approaching Aqueous-Like Activity in Nonaqueous Solvents: New Strategies for Activating Enzymes in Organic Media," poster presented at Enzyme Engineering 12, Deauville, France, September, 1993.

P. Michels, D.J. Hei, J.P. Amend, M. Domonoske, H.H. Helgeson, and D.S. Clark, "Bioreactors for Extremophiles and Their Enzymes," SIM-CSM Joint Annual Meeting, Toronto, August, 1993.

D. Hei, P. Michels, and D.S. Clark, "Structure-Stability Relationships in Thermophilic and Barophilic Enzymes," ACS National Meeting, Denver, CO, March, 1993.

D. Hei, P. Michels, and D.S. Clark, "Pressure Effects on the Activity and Stability of Enzymes from Extreme Thermophiles," ACS National Meeting, San Francisco, CA, April, 1992.

G.W. Hiller, D.S. Clark, and H.W. Blanch, "Cell Retention-Chemostat Studies of Hybridoma Cells: Antibody Productivity at Low Growth Rates," ACS National Meeting, San Francisco, CA, April, 1992.

S.T. Sharfstein, A. Mancuso, H.W. Blanch, and D.S. Clark, "*In vivo* Nuclear Magnetic Resonance Studies of Mammalian Cells Grown in Hollow Fiber Bioreactors," ACS National Meeting, San Francisco, CA, April, 1992.

P.S. Sears and D.S. Clark, "Dynamics of Proteins at Solid-Liquid Interfaces," AIChE Annual Meeting, Los Angeles, CA, November, 1991.

L.C.M. Auberson, D.S. Clark, and H.W. Blanch, "Primary Metabolism and Antibody Production in Hybridomas," AIChE Annual Meeting, Los Angeles, CA, November, 1991.

T.M. Spitznagel and D.S. Clark, "Surface-Density and Orientation Effects on Immobilized Antibodies and Antibody Fragments," AIChE Annual Meeting, Los Angeles, CA, November, 1991.

R. Affleck and D.S. Clark, "Solvent and Hydration Effects on Enzyme Activity and Dynamics," AIChE Annual Meeting, Los Angeles, CA, November, 1991.

D. Hei, C. Nelson, and D.S. Clark, "Pressure Effects on the Activity and Stability of Enzymes from Extreme Thermophiles," poster presented at Enzyme Engineering XI, Kailua-Kona, Hawaii, September, 1991.

R. Affleck, C.A. Haynes, and D.S. Clark, "Influence of Solvent Dielectric Constant on Protein Dynamics, poster presented at Enzyme Engineering XI, Kailua-Kona, Hawaii, September, 1991.

D. Hei, C. Nelson, and D.S. Clark, "Pressure-Temperature Effects on the Structure and Function of Enzymes from Deep-Sea Thermophiles," ACS National Meeting, Atlanta, GA, April, 1991.

A. Mancuso, L.C.N. Auberson, S.T. Sharfstein, H.W. Blanch, and D.S. Clark, "Non-invasive Examination of Hybridoma Metabolism with Nuclear Magnetic Resonance Spectroscopy," ACS National Meeting, Atlanta, GA, April, 1991.

C. Nelson, N. Shah, J. Ludlow, D. Hei, and D.S. Clark, "Catalytic and Structural Properties of Extremely Thermophilic Hydrogenases," AIChE Annual Meeting, San Francisco, CA, November, 1989.

A. Mancuso, E.J. Fernandez, H.W. Blanch, and D.S. Clark, "Noninvasive Measurements of Hybridoma Metabolism in a Hollow Fiber Reactor," AIChE Annual Meeting, San Francisco, CA, November, 1989.

R.M. Guinn, M. Fernandez, D.S. Clark, and H.W. Blanch, "Structure-Function Relationships of Proteases in Aqueous-Cosolvent Mixtures," AIChE Annual Meeting, San Francisco, CA, November, 1989.

D. Hei, C. Nelson, N. Shah, and D.S. Clark, "Catalytic and Structural Properties of Extremely Thermophilic Hydrogenases," poster presented at Enzyme Engineering X, Kashikojima, Japan, October 1989.

P. Skerker, R.M. Guinn, and D.S. Clark, "Structure-Function Relationships of Alcohol Dehydrogenase in Organic Solvents," AIChE Annual Meeting, Washington, D.C., November, 1988.

T.W. Randolph, D.S. Clark, H.W. Blanch, and J.M. Prausnitz, "Surfactant Assemblies in a Supercritical Fluid: Manganese and Nitroxide EPR Studies," AIChE Annual Meeting, Washington, D.C., November, 1988.

E.J. Fernandez, A. Mancuso, H.W. Blanch, and D.S. Clark, "NMR Studies of Hybridoma Metabolism in a Hollow-Fiber Reactor," AIChE Annual Meeting, Washington, D.C., November, 1988.

E.J. Fernandez, A. Mancuso, M.K. Murphy, L. Custer, H.W. Blanch, D.S. Clark, and D. Foxall, "Use of NMR Spectroscopy and Imaging to Study Hybridomas in a Hollow Fiber Reactor," ACS National Meeting, Los Angeles, CA, September, 1988.

A.L. Creagh, H.W. Blanch, D.S. Clark, and J.M. Prausnitz, "Enzymes in Reverse Micelles," poster presented at the 7th International Symposium on Surfactants in Solution, Ottawa, Canada, October, 1988.

P. Skerker, R.M. Guinn, and D.S. Clark, "Structure-Function Relationships of Alcohol Dehydrogenase in Organic Solvents," poster presented at the Symposium on Biocatalytic Synthesis of Organic Compounds, Saratoga Springs, NY, August, 1988.

E.J. Fernandez, A.M. Mancuso, and D.S. Clark, "Hollow Fiber Bioreactors for Long-Term NMR Studies of Mammalian Cell Metabolism," poster presented at the 13th International Conference on Magnetic Resonance in Biological Systems, Madison, WI, August 14-19, 1988.

E.J. Fernandez and D.S. Clark, "NMR Spectroscopy Studies of Hybridoma Metabolism," AIChE Annual Meeting, New York City, New York, November, 1987.

J.F. Miller, N.N. Shah, J.M. Ludlow, and D.S. Clark, "Pressure Effects on the Function of Thermostable Hydrogenase and the Extreme Thermophile Methanococcus jannaschii," AIChE Annual Meeting, New York City, New York, November, 1987.

J.F. Miller, J.M. Ludlow, and D.S. Clark, "A Novel Bioreactor for Studying the Structure and Function of Enzymes at Extreme Temperatures and Pressures", poster presented at Enzyme Engineering IX, Santa Barbara, California, October 1987.

J.F. Miller, N.N. Shah, and D.S. Clark, "Pressure-Temperature Relationships in the Growth of Methanococcus jannaschii, An Extremely Thermophilic Methanogen," AIChE Summer Meeting, Minneapolis, Minnesota, August 1987.

D.S. Clark, P.S. Skerker, and E.J. Fernandez, "Immobilization-Induced Conformational Changes in Proteins and Their Functional Consequences", AIChE Annual Meeting, Miami Beach, Florida, November 1986.

J.F. Miller, E.L. Almond, N.N. Shah, W.B. Streett, and D.S. Clark, "High Pressure-Temperature Bioreactor for Studying Pressure-Temperature Relationships in Bacterial Growth and Productivity", poster presented at Biochemical Engineering V, Henniker, New Hampshire, and at the Symposium on High Pressure in Biology, Toronto, Canada, July 1986.

S. Branner, G. Marg, P. Skerker, and D.S. Clark, "Molecular-Level Investigations of Immobilized Enzyme Catalysis by Electron Paramagnetic Resonance Spectroscopy", AIChE Annual Meeting, Chicago, Illinois, November 1985.

E.J. Fernandez, F.B. Fernandez, R.B. Jagoda, and D.S. Clark, "EPR Spectroscopy Studies of Immobilized Monoclonal Antibody Structure and Function", ACS Annual Meeting, Chicago, Illinois, September 1985.

P.S. Skerker, R.R. Miller, G.L. Millhauser, and D.S. Clark, "Structural and Functional Responses of Enzymes to Immobilization: New Insights from EPR Spectroscopy", poster presented at Enzyme Engineering VIII, Helsingor, Denmark, September 1985.

G.A. Marg, G.L. Millhauser, P.S. Skerker, and D.S. Clark, "Application of EPR Methods in Studies of Immobilized Enzyme Systems", poster presented at Fourth International Conference on Biochemical Engineering, Galway, Ireland, October 1984.

D.S. Clark and J.E. Bailey, "Detailed Characterization of Immobilized Chymotrypsin Deactivation in Aliphatic Alcohols", poster presented at Enzyme Engineering VII, White Haven, Pennsylvania, September 1983.

D.D. Do, M.M. Hossain, D.S. Clark, and J.E. Bailey, "Restricted Diffusion Effects on Enzyme Immobilization in Porous Solids", AIChE Annual Meeting, Los Angeles, California, November 1982.

Seminars

“Lessons from Life at the Extremes (and Teaching Old Enzymes New Tricks),”
Department JD Lindsay Seminar, Texas A&M University, virtual, February 2022.
Department of Bioengineering, University of California, Merced, April 2022.

“From Artificial Cofactors to Synthetic Arrays: Teaching Old Enzymes New Tricks,”
Department of Chemical and Biological Engineering, Princeton University, February 2020

“Continuing Stories Told & Untold Behind the Clark Deanasty,”
Staff Seminar, College of Chemistry, University of California, Berkeley, October 2019

“Research Stories Told & Untold Behind the Clark Deanasty,”
Staff Seminar, College of Chemistry, University of California, Berkeley, March 2018

“Protein Engineering for New Biocatalysts and Advanced Biomaterials,”
Department of Chemical Engineering and Materials Science Seminar, University of California- Irvine, March, 2017
Department of Chemical Engineering, Chemical Engineering 290 Seminar Series, University of California- Santa Barbara, November, 2016
Department of Chemical and Biomolecular Engineering, University of Delaware, April, 2016

“Breaking a Biofuels Bottleneck: Deconstructing Biomass from the Ground Up,”

Department of Chemical and Environmental Engineering, U.C. Riverside, September, 2012

Department of Food Science and Biotechnology, Seoul National University, Korea

Department of Chemical Engineering, MIT, April, 2012

“From Biomass to Biofuels: Breaking the Cellulose Barrier,”

Codexis, Redwood City, CA, November, 2011

Department of Chemical Engineering, Stanford University, April, 2011

Biomolecular Science and Engineering Program, UCSB, February, 2011

“Engineering Proteins for New Functions in Biomaterials: Some Assembly Required,”

Department of Biomedical Engineering, Tufts University, April, 2010

Department of Chemical Engineering, Columbia University, April, 2010

“Engineering Proteins into the Fold: New Biocomponents for Nanoscale Engineering,”

School of Chemical Engineering, Purdue University, March, 2009

“The Scale-Down of Cell Culture for High-Throughput Screening and Drug Discovery,”

Nanyang Technological University, Singapore, March, 2009

“Metabolism on a Chip for High-Throughput Toxicity Screening,”

Department of Nutritional Science and Toxicology, University of California, Berkeley, February, 2009

Department of Chemical and Biological Engineering, University of British Columbia, May, 2007

Department of Chemical and Biomolecular Engineering, UCLA, April, 2007

Department of Chemical and Biomolecular Engineering, Rice University, February, 2007

Department of Chemical Engineering, U.C. Santa Barbara, October, 2006

Department of Chemical Engineering, Texas Tech University, September, 2006

“Engineering in the (Bio)Material World: Overcoming the Natural Limitations of Biocatalysis,”

Department of Chemical Engineering, Princeton University, April, 2006

Institute for Biotechnology, Juelich, Germany, March, 2006

“Organic-Soluble Proteins (and Other Extreme Adaptations): Biocatalyst Engineering Enters a New Phase,”

Department of Chemical and Biological Engineering, Northwestern University, May, 2005

“Organic Soluble (and Sol-able) Proteins: Biocatalyst Engineering Enters a New Phase,”

Department of Chemical & Biological Engineering, University of Wisconsin, April, 2005

“Enzymes and Their Material Needs: Overcoming the Natural Limitations of Biocatalysis,”

Department of Chemical & Biomolecular Engineering, University of Illinois at Urbana-Champaign, October, 2004

“High-Throughput Biocatalysis: Creating New Opportunities for Synthesis and Discovery,”

- KAIST, Taejon, Korea; Seoul National University, Seoul, Korea, August, 2004
- “From the Earliest Organisms to the Latest Arrays: Old and New Prospects for Biocatalysis,”
Department of Chemical and Biomolecular Engineering, University of Pennsylvania,
April, 2004
- “Tapping New Sources of Biodiversity: Extremophiles Enter the Array,”
McGowan Institute for Regenerative Medicine, University of Pittsburgh, May, 2003
- “Engineering the Next Generation of Biocatalysts: Crossing Old Barriers with High Turnover,”
Department of Chemical Engineering, Georgia Institute of Technology, September, 2002
- “Built for Speed: Engineering the Next Generation of Biocatalysts,”
Department of Chemical Engineering, Texas A&M University, April, 2002
- “Biocatalyst Engineering: Crossing Old Barriers with High Turnover,”
Department of Chemical Engineering, Stanford University, April, 2002
Department of Chemical Engineering and Applied Chemistry, University of Toronto,
December, 2001
Department of Chemical Engineering, North Carolina State University, December, 2001
- “Multiscale Design and Optimization of Biocatalytic Materials,”
Department of Chemical Engineering, University of Southern California, October, 2001
Department of Chemical Engineering, University of Virginia, April, 2001
Department of Chemical Engineering, Carnegie Mellon University, March 2001
Department of Chemical Engineering, University of Pittsburgh, March 2001
Department of Chemical Engineering, University of Texas, Austin, March 2001
- “Biocatalyst Engineering: Crossing Old Barriers with High Turnover,”
Department of Chemical and Environmental Engineering, University of California,
Irvine, March, 2000
- “Engineering Biocatalysts to New Extremes,”
Department of Chemical Engineering, University of Houston, March, 1999.
- “Taking Biotechnology to Extremes: New Opportunities for Extremophiles,”
Center for Environmental Biotechnology, Lawrence Berkeley Laboratory, Berkeley, CA,
July, 1998.
- “Challenges and Opportunities with Extremophiles and Their Enzymes,”
USDA, Wyndmoor, PA, May, 1998
Mobil Research Center, Paulsboro, NJ, May, 1998
- “Engineering Biocatalysts to New Extremes,”
Department of Chemical Engineering, Washington State University, March, 1998.
Department of Chemical Engineering, Johns Hopkins University, September, 1997.

- Department of Chemical Engineering, University of Virginia, September, 1997.
- “Biocatalyst Engineering for Extreme Environments,”
Department of Chemical Engineering, MIT, April, 1997.
- “The Role of Pressure in Life at High Temperatures,”
Center of Marine Biotechnology, University of Maryland, February, 1997.
- “Biocatalyst Engineering for Extreme Environments,”
Department of Chemical and Biochemical Engineering, University of Maryland,
Baltimore County, February, 1997.
Department of Chemical Engineering, Notre Dame, October, 1996.
- “Combinatorial Biocatalysis for the Synthesis of Chemical Libraries,”
KAIST, Yusong, Korea, September, 1996.
- “Engineering the Discovery and Analysis of New Drugs,”
Cornell Bioengineering Program, Cornell University, May, 1996.
- “Combinatorial Biocatalysis,”
Western Regional Research Center, USDA, April, 1996.
- “Taking Biotechnology to Extremes: New Opportunities for Extremophiles,”
Department of Chemical Engineering, University of Pittsburgh, February, 1995.
Department of Chemical Engineering, Purdue University, December, 1994
Department of Microbiology, University of Illinois, November, 1994
- “Biocatalysis in the P-T Plane,”
Department of Chemical Engineering, University of Colorado, March, 1994.
Department of Chemical Engineering, University of Washington, February, 1994
Department of Chemical Engineering, Tulane University, January, 1994.
Department of Chemical Engineering, Stanford University, January, 1994.
- "Probing the Limits of Life: Engineering Considerations for the Study and Application of Extreme Thermophiles,"
Department of Chemical Engineering, University of California, Davis, November, 1993.
- "Natural Enzymes Under Unnatural Conditions: Grace Under Pressure,"
Department of Chemical Engineering, University of Delaware, May, 1993.
- "NMR Studies of Hybridoma Metabolism and Antibody Production in a Hollow Fiber Bioreactor,"
Biophysics Seminar Series, Cornell University, April, 1993.
Department of Chemical Engineering, Northwestern University, October, 1992.
- "NMR Spectroscopy Studies of Metabolic Regulation in Hybridomas,"

Biochemical Process R&D, Merck Sharp & Dohme Research Laboratories, West Point, Pa, May, 1992.

"Natural Enzymes Under Unnatural Conditions: Grace Under Pressure,"

Department of Chemical Engineering, California Institute of Technology (Merck Centennial Lecture Series), January, 1992.

Department of Chemical Engineering, University of Michigan, December, 1991.

"Biological Activity at Extreme Temperatures and Pressures,"

Department of Geology and Geophysics, University of California, Berkeley, May, 1991.

"Structure and Function of Proteins and Receptor Sites at Interfaces,"

Department of Chemical Engineering, Rensselaer Polytechnic Institute, March, 1991;

Department of Chemical Engineering, Yale University, March, 1991;

Department of Chemistry and Biochemistry, University of California, Santa Cruz, January, 1991;

Department of Chemical Engineering, University of Illinois, September, 1990.

"Improving Proteins for Process Applications,"

Department of Chemical and Materials Engineering, University of Iowa, April, 1990;

Department of Chemical and Petroleum Engineering, University of Pittsburgh, March, 1990;

Department of Chemical Engineering, University of Utah, February, 1990;

Department of Chemical and Biochemical Engineering, University of Iowa, April, 1990.

"Natural Enzymes Under Unnatural Conditions: Grace Under Pressure,"

Meeting of the Northern California Section of the AIChE, Berkeley, CA, February, 1990.

"Growth and Metabolism of Hybridomas in a Hollow Fiber Reactor," Dow Chemical Company, Midland, MI, January 1990.

"Structural and Catalytic Properties of Enzymes in Nonaqueous Media,"

Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN, November 1989.

"Temperature-Pressure Effects on the Metabolic and Enzymatic Activity of Extremely Thermophilic Bacteria,"

Center for Oceanography and Marine Biology, College Park, MD, November, 1989;

Applied Mechanics and Engineering Science Dept., U.C. San Diego, La Jolla, CA, May 1989;

Scripps Institution of Oceanography, La Jolla, CA, May 1989.

"Expanding the Dimensions of Biocatalysis: An Engineering Approach," School of Chemical Engineering, Cornell University, Ithaca, NY, May 1989.

- "Temperature-Pressure Relationships in the Metabolic and Enzymic Activity of a Deep-Sea Vent Archaeobacterium," Department of Chemical Engineering:
Virginia Polytechnic Institute, Blacksburg, VA, May 1988;
UCLA, Los Angeles, CA, May 1988.
- "Structural and Catalytic Properties of Enzymes in Novel Processing Environments," Department of Chemical Engineering:
University of Pennsylvania, Philadelphia, Pennsylvania, November 1987;
Rice University, Houston, Texas, January 1988;
University of Houston, Houston, Texas, January 1988;
University of California, Santa Barbara, February 1988;
Duke University Center for Biochemical Engineering, Durham, North Carolina, April 1988.
- "Non-Invasive Studies of Animal Cell Metabolism Using NMR Spectroscopy,"
Chiron Corporation, Emeryville, California, October 1987.
- "Spectroscopic Investigations of Soluble and Immobilized Glucose Isomerase,"
Allied/Signal Corporation, Des Plaines, Illinois, July 1987.
- "On Enzyme Immobilization and Bacterial Thermophily: What's Going On Here?,"
Stauffer Chemical Company, Richmond, California, January 1987.
- "EPR and EXAFS Studies of Immobilized Glucose Isomerase Catalysis," Department of Chemical Engineering:
University of California, Berkeley, California, February 1986;
Stanford University, Palo Alto, California, February 1986.
- "Biotechnological Applications of Immobilized Enzymes and Immobilized Cells: An Overview,"
Meeting of the Local Chapter of the AIChE, Rochester, New York, January 1986.
- "Use of Bound Enzymes in Food Processing: Experience with Glucose Isomerase,"
Department of Food Science, Cornell University, Ithaca, New York, October 1985.
- "Molecular-Level Investigations of Immobilized Enzyme Catalysis by Electron Paramagnetic Resonance Spectroscopy," Department of Chemical Engineering, University of California, Los Angeles, California, November 1984.
- "Macroscopic and Molecular Properties of Immobilized Enzymes,"
Washington University Biotechnology Seminar Series, St. Louis, Missouri.

Appendix B

Appendix B – Materials Considered

1. U.S. Patent No. 11,492,389
2. Prosecution History of U.S. Patent No. 11,492,389
3. The Board’s decision denying institution in *Wuhan Healthgen Biotechnology Corp. v. Ventria Bioscience Inc.*, IPR2022-00712, Paper No. 8 (Aug. 19, 2022)
4. U.S. Patent Publication No. 2007/0166825 (“Hatsuyama”)
5. WO 2007/002762 (“Deeter”)
6. Prosecution History of U.S. Patent No. 10,618,951
7. U.S. Patent Publication No. 2006/0110399 (“Van Holten”)
8. U.S. Patent Publication No. 2006/0194300 (“Schulz”)
9. U.S. Patent Publication No. 2006/0008792 (“Viswanathan”)
10. Jayme et al., “Basal Medium Development for Serum-Free Culture: a Historical Perspective,” *Cytotechnology*, 23:95-101 (1997) (“Jaymne”)
11. Castilho et al., “Animal Cell Technology: From Biopharmaceuticals to Gene Therapy,” (Castilho, Moraes, Augusto, and Butler, Eds.) (New York, Taylor & Francis Group, 2008) (“Castilho”)

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14. Dockal et al., “The Three Recombinant Domains of Human Serum Albumin,” *The Journal of Biological Chemistry*, vol. 274, no. 41, 29303-29310 (1999) (“Dockal”)
15. Simard et al., “Locating High-Affinity Fatty Acid-Binding Sites on Albumin by X-Ray Crystallography and NMR Spectroscopy,” *Proceedings of the Nat’l Acad. of Sci.*, vol. 102, no. 50, 17958=17963 (2005) (“Simard”)
16. J.K. Fuller Noel and M. J. Hunter, “Bovine Mercaptalbumin and Non-mercaptalbumin Monomers,” *J. Biological Chemistry*, vol. 247, no. 22, 7391-7406 (1972) (“Noel and Hunter”)
17. Atmeh et al., “Albumin Aggregates: Hydrodynamic Shape and Physico-Chemical Properties,” *Jordan J. of Chemistry*, vol. 2, no. 2, 169-182 (2007) (“Atmeh”)
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21. F. Garcia-Gonzalo et al., “Albumin-Associated Lipids Regulate Human Embryonic Stem Cell Self-Renewal,” *PLoS One*, (2008), Issue 1, e1384 (“Garcia-Gonzalo”)
22. Jurgens et al., “Investigation into the Interaction of Recombinant Human Serum Albumin with Re-lipopolysaccharide and Lipid A,” *J. of Endotoxin Research*, vol. 8, no. 2, 115-126 (2002) (“Jurgens”)
23. David et al., “Characterization of the Interaction of Lipid A and Lipopolysaccharide with Human Serum Albumin: Implications for an Endotoxin Carrier Function for Albumin,” *J. of Endotoxin Research*, vol. 2, 99-106 (1995) (“David”)
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60. U.S. Provisional Application No. 61/154,204
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63. A. Tresec et al., “Removal of Detergent and Solvent from Solvent-Detergent-Treated Immunoglobulins,” *J. of Chromatography A*, 852 (1999), 87-91
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