

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

WUHAN HEALTHGEN BIOTECHNOLOGY CORP.,
Petitioners

v.

VENTRIA BIOSCIENCE INC.,
Patent Owner

Case No. IPR2022-00712
Patent No. 10,618,951

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. 10,618,951 (the '951 patent) in view of the prior art. I understand that this Declaration accompanies a petition for *inter partes* review (IPR) of the '951 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 1-9 and 11-13 and would have had a reasonable expectation of success in doing so, based on the prior art publications identified below.

Combinations	Claims
Deeter in view of Tanaka	1-8, 11-13
Deeter in view of Tanaka and Meng	9
Deeter in view of Van Urk	1-8, 11-13
Deeter in view of Van Urk and Meng	9

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 involves investigating stem cell differentiation and modeling in cell culture, studying structure-function relationships of proteins, and biocatalyst engineering of proteins, including albumin.

8. My research experience also includes work with chaperone molecules and enzymes, including 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 relationship 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 275 papers, many dealing with enzyme applications and enzyme engineering, including optimization of enzyme stability, production, and properties. I have additional experience investigating enzyme structure and function using extensive chromatography, spectroscopic, and cell culture techniques.

10. Throughout my 36-year 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.

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**. 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 2009.

IV. BACKGROUND AND STATE OF THE ART

A. Recombinant Proteins in Cell Culture Media

1. Recombinant Human Serum Albumin (rHSA)

15. Human serum albumin (“HSA”) is the most abundant protein in human plasma. EX1048, 1. By 2009, HSA and recombinant human serum albumin (“rHSA”) had widely been used as a supplement in cell culture media. *See* EX1010, 5:36–6:28 (“It is known that albumin has a positive effect on the culture of cells”); EX1011, 1 (“Serum albumin stimulates growth of mammalian cells in synthetic and semisynthetic media.”).

16. Albumin serves several functions, and notably serves to deliver other molecules such as fatty acids to cells and to control osmotic pressure, which is important for regulating cells maintained in culture. EX1012, 1924, 1929.

17. As shown in the Figure below, albumin contains three domains (shown in blue, green, and red/tan), each of which has binding sites for various ligands. EX1048, 1.

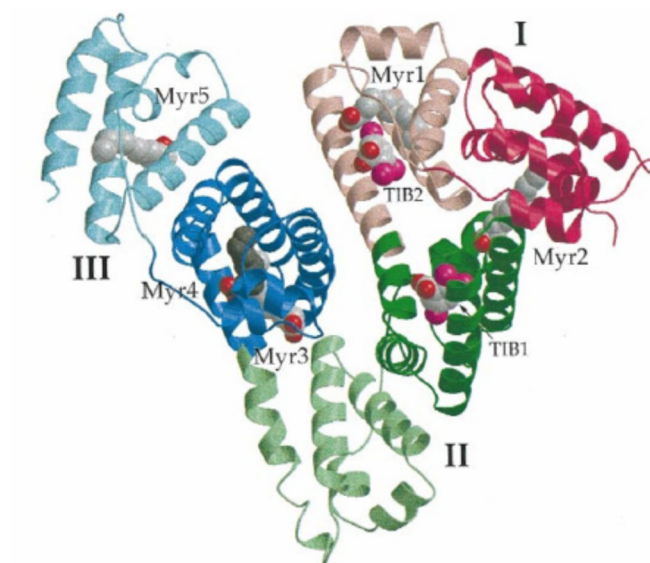


Figure 1. Domain Structure of HSA. *See EX1048, 1 (Fig. 1).*

18. HSA and rHSA have the same amino acid sequence and serve the same functions, including as a cell culture media supplement. The only difference between HSA and rHSA is their method of production—HSA is produced naturally and rHSA is expressed in a recombinant host that was engineered to express rHSA. As such, rHSA-supplemented serum-free medium was particularly well-known to be “an excellent alternative to standard serum-containing media for the cultivation of cells” because it posed no risk for contamination with pathogens originating from animals. EX1010, 2:22–25; EX1013, 29; EX1014, 13; EX1003 (Deeter), [0006]; EX1015, 1.

19. By at least 2009, several rHSA products had been commercialized, including rHSA produced in transgenic yeast and transgenic plants (e.g., rice). EX1016, 2; EX1014, 8, 13; EX1017, 8; EX1018; EX1045, 6. For example, rHSA

had been used as a cell culture media component in the manufacture of a new measles-mumps-rubella vaccine. EX1014, 8. In addition, Ventria's rHSA-based product, Cellastim™ (produced in transgenic rice), had been shown to promote growth in cell culture media. EX1006; EX1007; *see also* EX1003 (Deeter), [0118]–[0120], Figs. 23, 24.

2. Recombinant Human Lactoferrin

20. Like rHSA, recombinant human lactoferrin was also a known cell culture media supplement. EX1058 (Huang 2008), 1. Lactoferrin and its recombinant counterpart deliver iron, a key nutrient, to cells.

21. Huang discloses producing recombinant human lactoferrin (rhLF) in transgenic rice. EX1058, 1. Huang further discloses that all three forms of recombinant human lactoferrin (pis, apo, holo, which denote different levels of iron) promoted growth of intestinal cells. *Id.* Huang also reports that holo-rhLF promoted cell growth, reduced cell doubling time, and promoted greater production of antibodies compared to cells grown without rhLF. *Id.* Huang reports that rhLF was “as effective as human transferrin in promoting cell growth and antibody production.” *Id.*

22. Huang further discusses the benefits of using recombinant proteins instead of animal-sourced proteins, noting that by 2008 there was “a concern about the use of animal source[d] proteins and peptides in cell culture applications due to

potential contamination by adventitious infectious pathogens.” *Id.* As such “[r]ecombinant production of these proteins using a plant host provides a safe and cost effective alternative.” *Id.* Huang further notes that InVitria (a division of Patent Owner Ventria) was marketing recombinant human lactoferrin as Lacromin™. *Id.*, 2.

3. Recombinant Human Transferrin

23. Transferrin, including recombinant human transferrin, was also an established cell culture media component. EX1059 (Grosvenor). As Grosvenor explains, transferrin transports iron into cells, “which is essential for cell growth and the regulation of key metabolic processes, such as DNA synthesis and oxygen transport.” *Id.* Moreover, “[t]ransferrin has also been shown to play an important role in binding heavy metals in culture.” *Id.* Indeed, commonly used “cell lines such as CHO and NS0 require transferrin to attain optimal cell growth and productivity.” *Id.*

24. By 2008, recombinant human transferrin was commercially available and established as an effective alternative to animal-sourced transferrin in cell culture media. *Id.* As Grosvenor explains, “[a] recombinant analogue of human transferrin, CellPrime rTransferrin AF (rTransferrin), (Novozymes) has shown equivalence to hTf and superiority to bTf in stimulating cell growth and productivity across a number of cell lines.” *Id.*

4. Advantages of Plant-Derived Recombinant Proteins

25. Well before 2009, researchers recognized the advantages of using non-animal derived recombinant albumin in biological products. *See* EX1015, Summary; EX1020, 1; EX1010, 2; EX1012, 2; EX1021, Abstract; EX1022, 4:7–21; EX1023, Abstract; EX1003 (Deeter), [0006]-[0009].

26. Transgenic rice, a “well characterized” food crop, was deemed superior to other plant hosts as an attractive expression system for recombinant human proteins, including rHSA. EX1023, 10. For example, transgenic rice was reported to be superior to other transgenic plants because it does not produce any toxic secondary metabolites and it provides rHSA in high yield. EX1023, 10. Moreover, by 2009, general and specific techniques for producing proteins from transgenic rice cells had been well known, and recombinant proteins had been successfully produced in transgenic rice. *See* EX1003 (Deeter), [0094]-[0105]; EX1023, 3-5.

27. In addition, rice-derived rHSA (Cellastim) and recombinant human lactoferrin (Lacromin) had been sold and used as cell culture media supplements at least by 2008. EX1006; EX1007; EX1018; EX1058 (Huang 2008).

B. Endotoxin Was a Known Contaminant that Had Been Removed from albumin and rHSA

28. By 2009, researchers recognized that biological products and cell culture components should contain at most low levels of endotoxin (*e.g.*, less than

1 EU endotoxin per mg product or protein). *See, e.g.*, EX1025, 16–17; EX1026, 292; *see also* EX1027, 1:49–52 (“The presence of endotoxins in biologically derived products (biologicals) prepared for therapeutic use is of major concern due to the diverse and potentially harmful biological activities of these molecules.”); EX1028, 261 (“Endotoxin contamination of cell culture, especially via [fetal bovine serum], should be carefully avoided.”); EX1029, 3 (“There has been at least one call for ‘absolute absence’ of endotoxin in tissue culture media”); EX1047, 384 (only recombinant protein preparations that “contained <1 EU/endotoxin/g [recombinant protein] as determined by limulus amoebocyte lysate assay were used”); EX1050, 626 (“Endotoxin was removed to obtain low endotoxin CRCL preparations (<1 EU/mg protein).”); EX1052, 6811 (“The presence of endotoxin contamination represents a serious threat” to biological products.).

29. As a result, the presence of endotoxins in cell culture media supplements, such as rHSA, was a concern, as cell culture media was commonly used in the production of biological drug products. *See* EX1052, 6814 (“It is not uncommon to find high levels of endotoxin in biological products such as albumin.”). If present, endotoxins were known to complicate purification of downstream products produced in cell culture media.

30. Commercially available chromatography techniques had been widely used to purify albumin, including rHSA, to reduce the level of endotoxins, which,

if not strictly controlled could be harmful and/or complicate downstream products produced in cell culture media containing endotoxin-contaminated rHSA. *See, e.g.*, EX1004 (Van Urk), 43-55, 61-63, 72-73; EX1027, 49-52; EX1028, 255; EX1005, 6 and Figs. 1 and 2; EX1026, 10 (“ion exchange resins are the most common depyrogenation method for proteins.”); EX1030. Indeed, researchers acknowledged that “ideal” rHSA products should be free from contaminants such as endotoxins. EX1005, 1.

31. I note that endotoxins were well-known and common pyrogens. EX1064, 1. Relatedly, product that is free of pyrogens is necessarily free of endotoxins.

32. Techniques for removing endotoxin from proteins such as albumin were known. For example, in addition to the techniques of Tanaka and Van Urk (described below), Matejtschuk discloses that DEAE-Sepharose chromatography “consistently lowered” endotoxin levels in serum-derived albumin. EX1005, 6. Matejtschuk published in 2000, years before the ’951 patent application was filed.

33. As another example, Aida, titled “Removal of endotoxin from protein solutions by phase separation using Triton X-114,” discloses a simple process for removing endotoxin from bovine serum albumin (BSA). Aida explains that endotoxins may be removed by a simple two-phase extraction using the detergent Triton X-114, and further teaches that residual detergent may be removed using gel

filtration on Bio-Gel P-300 or absorption on SM-4 beads. EX1060 (Aida), 3. Aida reported that the purified BSA had no detectable endotoxin using the stringent *Limulus* assay. *Id.*, 3 (Table 1), 4. Aida explains that a “major advantage of the procedure was that it appeared not to harm the proteins.” *Id.*, 5. Aida suggests this process for removal of endotoxin from recombinant products and albumin. *Id.* Aida is Aida published in 1990, and I understand it is prior art to the ’951 patent.

34. By at least 2005, rHSA products containing low levels of endotoxin were commercially available. For example, a New Century Pharmaceuticals product catalogue from 2005 advertises the availability of rHSA with > 99% monomer content and low endotoxin. EX1017, 9; *see also* EX1016, 2 (using “Recombumin™, an ultrapure recombinant human albumin derived from *Saccharomyces cerevisiae*,” in cell culture). By 2006, an “endotoxin-free” rHSA product (Recombumin) was used in the manufacture of a new MMR vaccine. EX1014, 1, 8.

C. Chaperone and Heat Shock Proteins

35. By 2009, certain heat shock proteins were known to co-purify with recombinant proteins and be difficult to separate. *See* EX1031, 4; EX1049, 1. For example, it was known that heat shock proteins exist in all host cells and serve as “chaperone” proteins that bind to newly translated proteins and help such proteins adopt the same three-dimensional structure as their native counterparts. EX1031, 4;

EX1032, 1, 2; EX1033, 1. These host cell chaperones, including heat shock proteins, were reported to bind recombinant proteins due to their chaperone functionality. EX1031, 4.

36. It was also known by 2009 that rHSA and certain heat shock proteins have similar isoelectric points (pI) and molecular weights. For instance, the literature reported a pI for rHSA as about 5.3 and a pI for rice heat shock proteins generally ranging from about 4 to 7, with Hsp70 having a pI of about 5. EX1034, 4 (Fig. 1); EX1003 (Deeter), [0117] (Table 3).

37. In addition, the molecular weights of rHSA and Hsp70 were known to be ~67 kDa and 70 kDa, respectively. EX1003 (Deeter), 30, Table 3; EX1035, 1; EX1005, 1.

38. Accordingly, the “sticky” nature of heat shock proteins made them highly prevalent in rHSA preparations and difficult to separate from any bound rHSA using standard purification techniques. EX1041, 8 (noting that albumin itself is a “sticky” molecule).

D. The Prior Art References

1. Deeter

39. Deeter is a PCT publication assigned to Ventria that published on January 4, 2007. EX1003 (Deeter). Thus, I understand that it is prior art to the '951 patent.

40. Deeter describes the production and use of recombinant proteins produced by transgenic rice for improving growth and productivity of cells in cell culture media. EX1003 (Deeter), [0009].

41. Deeter states that cell culture media components derived from animals “have several undesirable features” and that “there is a need to produce components for incorporation into cell culture media that is not derived from, or produced by, animal sources, yet still retain their functional value for cell cultures.” EX1003 (Deeter), [0006], [0008]. To avoid the disadvantages of animal-derived cell culture media components, Deeter focuses on the “production and use of recombinant proteins produced by plant cells as components of cell culture media.” EX1003 (Deeter), [0002].

42. Deeter describes that “the invention comprises a method of transforming a rice cell by incorporating a DNA segment that encodes for components of cell culture media such as amino acids, growth factors, and other cell culture proteins including lactoferrin and human serum albumin.” EX1003 (Deeter), [0016]. Deeter further describes that the use of media supplemented with plant-derived components “improve[s] cell growth and productivity in cell cultures.” EX1003 (Deeter), [0019]. Deeter’s production system provides a very high yield of rHSA at up to 0.85% of brown flour weight (up to 8.5 grams rHSA/kg flour). EX1003 (Deeter), [0105]. Deeter further acknowledges that

“specific techniques for producing proteins from plant cells” were known and disclosed in patents and applications that Deeter incorporated into its own disclosure. EX1003 (Deeter), [0048].

43. Deeter recognizes that “specific techniques for producing proteins from plant cells” had been readily available and incorporated by reference to numerous earlier publications teaching producing recombinant proteins from transgenic plant cells. EX1003 (Deeter), [0048]. In Example 3, Deeter reports the successful production of rHSA from rice grain at high expression levels. EX1003 (Deeter), [0103]–[0105], Table 1. In Example 7, Deeter describes a process for purifying and characterizing rice-derived rHSA, which achieved at least 90% purity. EX1003 (Deeter), [0116]. Deeter shows that the biochemical and biophysical properties of the rice-derived rHSA were “equivalent” to plasma-derived HSA. EX1003 (Deeter), [0117].

44. Deeter also tests whether “[t]he effectiveness of recombinant human serum albumin from rice (rHSA) to stimulate cell growth [is] similar to plasma derived human serum albumin (pHSA).” EX1003 (Deeter), [0118] (Example 8). Deeter added 100 mg/L rHSA to cell culture media containing 5.5 µg/ml (5.5 mg/L) transferrin (among other components). *Id.* As depicted in Figure 23, Deeter reports that “cells grown in media with rHSA supplementation grew equivalently or better than cells grown in media with plasma derived HSA. EX1003 (Deeter),

[0118]. According to Deeter, these results indicate that rHSA from rice was “functionally equivalent” or “better than” pHSA in enhancing hybridoma cell growth in reduced-serum media.” EX1003 (Deeter), [0118].

45. In Example 9, Deeter tests the growth of AE1 hybridoma cells in supplemented serum-free media (SFM) in the presence of the rice-derived rHSA or plasma-derived HSA and concluded that “this example shows that plant derived recombinant human serum albumin can promote cell growth in serum-free media and replace animal derived albumin for use in tissue culture.” EX1003 (Deeter), [0120], Fig. 24.

46. Deeter also discloses that its invention comprises “at least one plant-produced heterologous protein as a cell culture component.” EX1003 (Deeter), [0081]. Deeter discloses that the heterologous proteins are “preferably human,” including “lactoferrin, transferrin, and serum albumin,” among others. EX1003 (Deeter), [0081].

47. Deeter details the production of human lactoferrin in transgenic rice ([0100]-[0102]), purifying said lactoferrin ([0106]-[0110]), and promoting cell growth with cell culture media supplemented with recombinant human lactoferrin ([0111]-[0115], Table 2, Figs. 20-22).

48. In Table 2, Deeter compares hybridoma growth and monoclonal antibody (IgG) production in un-supplemented cell culture media (control), media

supplemented with transferrin (TF), media supplemented with recombinant human lactoferrin (rhLF), and media supplemented with 5% fetal bovine serum (FBS). EX1003, 30. As shown in Table 2 of Deeter (below), transferrin and recombinant human lactoferrin substantially increased the number of cells and the amount of monoclonal antibody produced relative to control media. *Id.*

Table 2. Effect of human transferrin (TF) and recombinant human lactoferrin (rhLF) on hybridoma growth and monoclonal antibody production.

	TF/rhLF	Cell count (X10 ⁶)		IgG level (ug/ml)	
Treatment	(ug/ml)	Mean	SD	Mean	SD
Control	0	0.39	0.18	15.78	5.63
TF	5	0.70	0.25	34.10	8.44
rhLF	5	0.63	0.10	43.58	8.24
TF	25	0.78	0.24	31.93	7.10
rhLF	25	0.81	0.20	36.10	7.54
TF	100	0.86	0.33	28.58	5.82
rhLF	100	0.78	0.26	29.73	4.28
5% FBS	0	0.98	0.12	23.88	5.50

Figure 2. Table 2 of Deeter. EX1003, 30.

2. Tanaka

49. Tanaka is titled “Purification of human albumin by the combination of the method of Crohn with liquid chromatography,” provides a simplified method for purifying albumin to remove pyrogens (e.g., endotoxin) and aggregated albumin. EX1053 (Tanaka). I understand Tanaka is prior art to the '951 patent, having published over 10 years earlier in 1998.

50. Tanaka discloses isolating a fraction of impure albumin from serum, then purifying the albumin using (1) DEAE-Sepharose chromatography, (2) fatty acid removal, (3) gel filtration on Sephacryl S-200 HR, and (4) viral inactivation. EX1053 (Tanaka), 2-4. Tanaka explains that the disclosed method is more time- and cost-efficient than prior methods for purifying albumin from serum. EX1053, 5. Tanaka's process is depicted below.

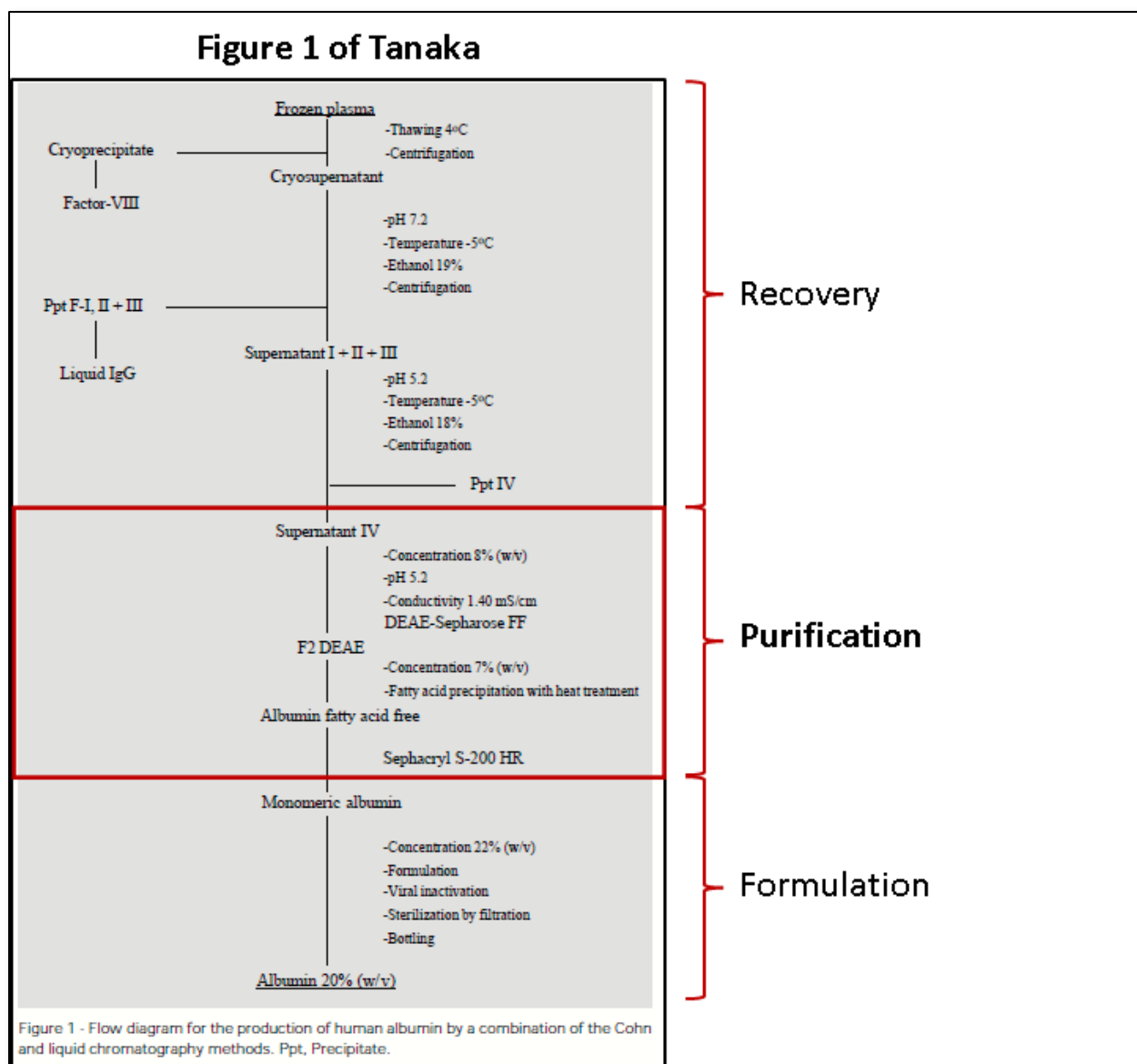


Figure 3. Tanaka’s process for recovery, purification, and formulation of albumin. EX1053, 2 (Fig. 1) (annotated).

51. Tanaka’s purification steps are shown in the center of the above schematic.

52. In the first purification step, DEAE-Sepharose FF chromatography, Tanaka discloses the conditions for eluting albumin, e.g., 25 mM acetate buffer,

pH 4.5, loading concentration (8% w/v), and flow rate (2 l/min). EX1053, 3 (Fig. 2 caption). I note that the selected pH of 4.5 is between the isoelectric points of endotoxin (~2) and albumin (5.3), facilitating removal of endotoxin from albumin on a DEAE-Sepharose column. *See* EX1027, 2:13-15 (noting that at pH levels above 2, endotoxin aggregates are negatively charged and will bind to positively charged surfaces). Tanaka shows the elution profile on the DEAE-Sepharose FF gel and provides the elution conditions in Figure 2, below.

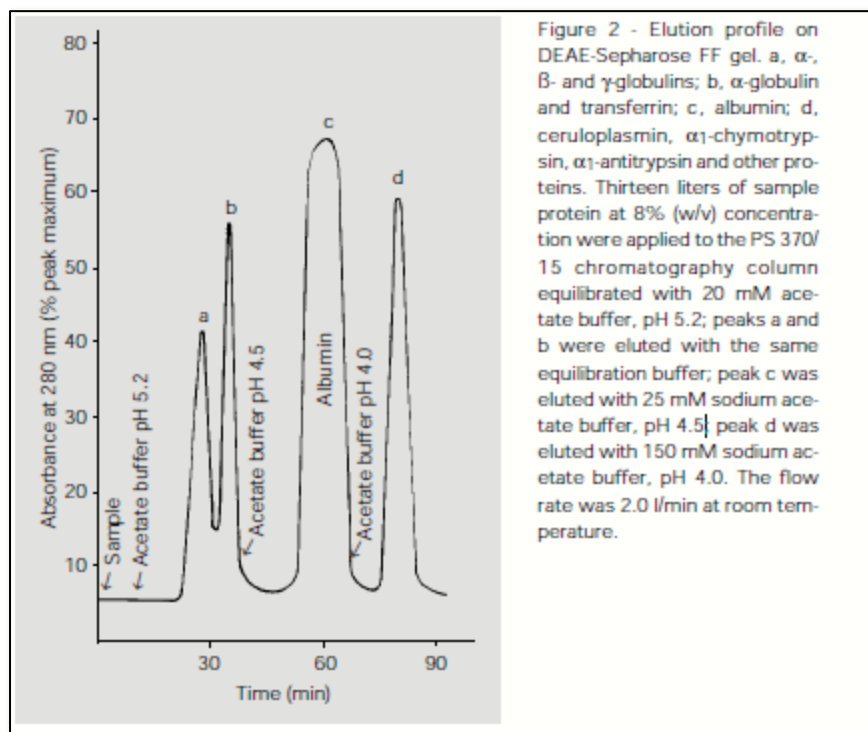


Figure 4. Figure 2 of Tanaka. EX1053, 3 (Fig. 2).

53. Tanaka discloses that the albumin eluted from the DEAE-Sepharose column was concentrated and the pH and conductivity of the solution was adjusted,

after which the solution was heated to 55 °C for three hours to precipitate fatty acids. EX1053, 3.

54. Next, Tanaka discloses further purifying the albumin by Sephacryl S-200 HR gel, again detailing the conditions for purification, e.g., albumin concentration (7% w/v), pH (6.0), flow rate (600 ml/min), and elution solution (50 mM sodium chloride). EX1053, 3, Fig. 3.

55. Tanaka then discloses that viral inactivation was performed by heating at 60 °C for 10 hours. *Id.*, 4.

56. Tanaka evaluated the level of aggregated albumin in the purified sample using gel filtration using two Superdex 200 HR 10/30 columns (Pharmacia Biotech) in series. *Id.*, 4. Tanka reported detecting 99.0% to 99.3% monomeric albumin, 0.7% to 1.0% dimers, and the absence of larger aggregates. *Id.*

57. Tanaka provides the results from capillary acetate electrophoresis in Figure 4, below.

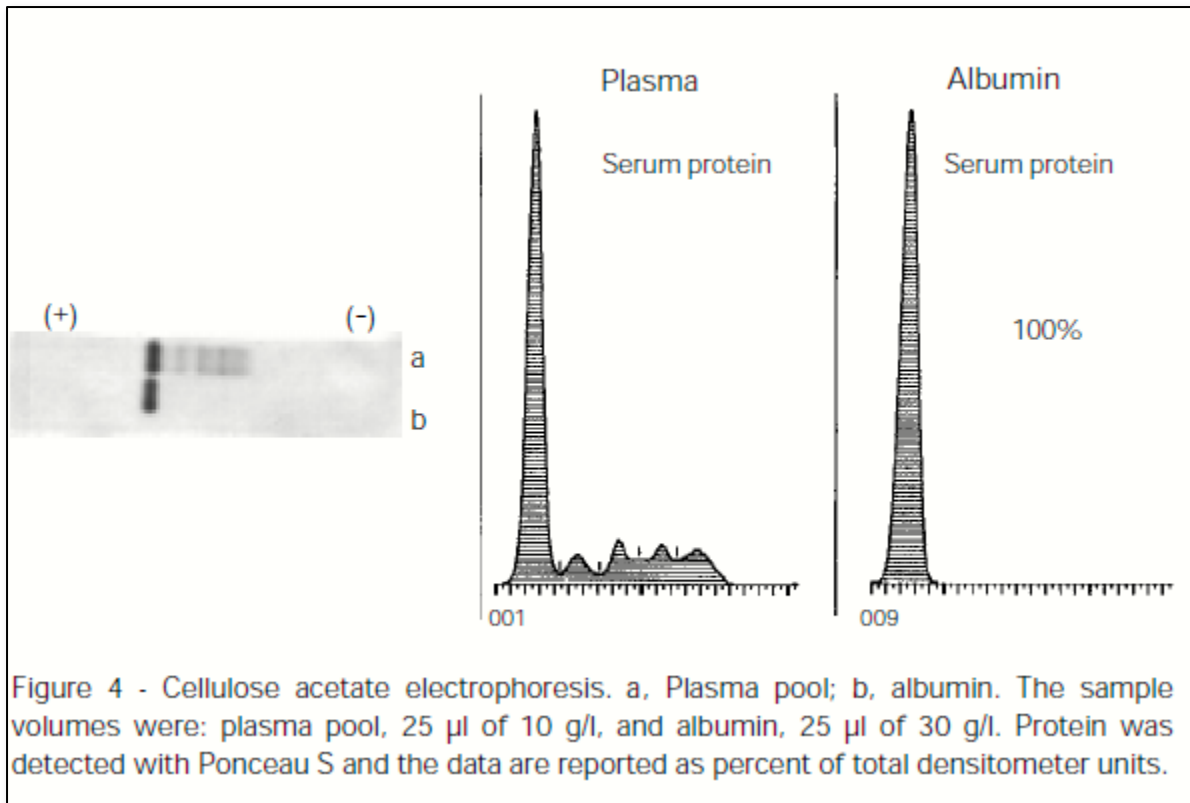


Figure 4 - Cellulose acetate electrophoresis. a, Plasma pool; b, albumin. The sample volumes were: plasma pool, 25 μ l of 10 g/l, and albumin, 25 μ l of 30 g/l. Protein was detected with Ponceau S and the data are reported as percent of total densitometer units.

Figure 5. Figure 4 of Tanaka.

58. Tanaka provides a summary of the characteristics of the purified albumin in Table 1, below.

Table 1 - Characteristics of 20% albumin obtained by the method described.		
Fifteen lots were analyzed. *Pharmacopée Européenne (13).		
	Results	Specifications*
Yield (g/l plasma)	25.0 ± 0.5	–
Protein concentration (w/v)	20.0 ± 1.0%	>95 to <105%
Purity (cellulose acetate electrophoresis)	>99.0%	>95%
Size distribution (Superdex 200)		
Monomers	99.0 to 99.3%	mono + dimers = 95%
Dimers	0.7 to 1.0%	
Polymers	not detected	polymers = 5%
Pyrogens	satisfactory	satisfactory
Abnormal toxicity	satisfactory	satisfactory
Sodium caprylate	0.16 mmol/g protein	–
Prekallikrein activator activity	<5 IU/ml	<35 IU/ml
Aluminum	<50 µg/l	<200 µg/l
pH	6.8 to 7.0	6.7 to 7.3
Sodium	140 ± 5 mmol/l	<160 mmol/l
Potassium	<0.05 mmol/g protein	<0.05 mmol/g protein
Heme (absorbance at 403 nm)	≤0.04	<0.15
Anti-HIV	negative	negative
Anti-HCV	negative	negative
HBS-Ag	negative	negative

Figure 6. Tanaka, Table 1, annotated.

59. As shown in Table 1 of Tanaka, the purified albumin was 99.0-99.3% monomeric, 0.7-1.0% dimeric, and no larger aggregates were detected. EX1053, 4.

60. Tanaka explains that the disclosed process provides “a high quality, *pyrogen-free* product with a reduction of viruses, as previously reported and validated by Pharmacia Biotech, associated with the process of viral inactivation

by pasteurization at 60°C for 10 h. This guaranteed the biological safety of the final product.” EX1053, 5 (emphasis added).

61. A POSA would have understood that Tanaka’s “pyrogen-free” albumin would be free of pyrogens, including endotoxin.

3. Van Urk

62. Van Urk is a PCT publication that published on August 3, 2000 reporting on the preparation of highly pure albumin with no detectable endotoxin. EX1004 (Van Urk). I understand that it is prior art to the ’951 patent.

63. Van Urk describes “a process for purifying ... recombinant human albumin (rHA) produced by transforming or transfecting an organism with a nucleotide coding sequence encoding the amino acid sequence of human serum albumin, including *rHSA produced using transgenic* animals or *plants*.” EX1004 (Van Urk), 1:1–8. Van Urk teaches that “the albumin may be obtained from a transgenic plant, such as tobacco, potato or corn (maize).” EX1004 (Van Urk), 22:27–28. Van Urk also states that “[t]he albumin of the present invention” “may be used ... in cell culture.” EX1004 (Van Urk), 23:26–24:2.

64. In Example 1, Van Urk describes processes for production of rHSA in yeast and rHSA purification. EX1004 (Van Urk), 32–55 (Example 1). Van Urk discloses a purification process for obtaining “highly pure albumin . . . from an impure albumin solution,” where the albumin may be obtained from yeast, plasma,

a transgenic animal, or a transgenic plant. EX1004 (Van Urk), 22. Van Urk defines the term “albumin” to mean “HSA and/or rHA [rHSA].” EX1004 (Van Urk), 1.

65. According to Van Urk, the rHSA of the invention is “characterised by extremely low levels of, or by being essentially free of ... endotoxin ... By essentially free is meant below detectable levels.” EX1004 (Van Urk), 21:5–10. Van Urk discloses that the endotoxin content of the purified rHSA was “assayed using Limulus amoebocyte lysate by kinetic turbidimetric measurement at 340 nm, at a temperature of 36.5-37.5° C using an automatic endotoxin detection system (e.g., LAL 5000E).” EX1004 (Van Urk), 72. Van Urk discloses that “[n]o endotoxin was detected” in the produced rHSA by the kinetic turbidimetric measurement. EX1004 (Van Urk), 73. Van Urk further discloses that its rHSA was non-pyrogenic, meaning free of pyrogenic agents including endotoxins. EX1004 (Van Urk), 63.

66. Van Urk discloses that its rHSA contains less than 1% aggregated albumin. For example, Van Urk discloses that in its rHSA, “[t]here may be up to 1% dimeric albumin but no larger polymers or aggregates are detectable.” EX1004 (Van Urk), 63. Van Urk discloses formulating its rHSA as a 25% w/v solution and then evaluating the level of aggregation using gel permeation high pressure liquid chromatography (gel permeation HPLC), which is another term for size-exclusion HPLC. EX1004 (Van Urk), 71. Van Urk’s formulated rHSA was “found to contain

less than 0.1% polymeric albumin,” such that “the formulation as described herein has no detrimental effect on the polymer/aggregate content of the purified albumin.” EX1004 (Van Urk), 71. Van Urk also discloses that when evaluated by two-dimensional gel electrophoresis, “only one spot was visible, indicating the presence of only one protein species.” EX1004 (Van Urk), 71-72.

67. For completeness, I note that Van Urk also evaluated its rHSA using SDS PAGE with a reducing agent (β -mercaptoethanol, BME) and observed that “the proportion of albumin present as a monomer is at least 99.9%.” EX1004 (Van Urk), 69. However, SDS-PAGE under reducing conditions does not reflect the total level of aggregated albumin, because any albumin aggregates bound by disulfide bonds are converted to individual albumin units.

4. Meng

68. Meng is European Patent No. EP 2,177,602, which was filed on November 1, 2008, and claims priority to an application filed on July 13, 2007. I understand that Meng is prior art to the '951 patent.

69. Meng discloses serum-free cell culture media for stem cell culture comprising transferrin and serum albumin (among other components). EX1054 (Meng), 5:17-21. Meng discloses that the serum albumin may be human serum albumin. *E.g.*, EX1054 (Meng), 4:28-30, 5:22-27.

70. Meng discloses that the rHSA preferably “be sufficiently purified to the extent to be used as a drug (injection solution).” EX1054 (Meng), [0024]. As explained by the Japan Patent Office, it would have been understood that “albumin used in the injectable drug does not contain endotoxin or aggregated albumin.” EX1055 (JPO), 2.

71. Meng further discloses that in its cell culture media, the concentration of transferrin is 0.1 mg/L to 10 mg/L, while the concentration of albumin is “not less than 0.1 g/L,” which is 100 mg/L. EX1054 (Meng), [0022]-[0023]. Thus, Meng teaches that the ratio of transferrin to rHSA should be at least 1:10 to 1:1000.

72. Meng also discloses stem cell culture media in which the ratio of transferrin to rHSA is 1:455. EX1054, 13:11-24 (disclosing media comprising 5.5 mg/L transferrin and 2.5 g/L rHSA (2,500 mg/L rHSA), where 2,500 divided by 5.5 is 455).

V. THE '951 PATENT

A. Overview

73. The '951 patent is titled “Cell Culture Media Containing Combinations of Proteins.”

74. The '951 patent generally relates to cell culture media supplement compositions containing proteins, including recombinant proteins and combinations of recombinant human lactoferrin and rHSA. *E.g.*, Patent 3:43-53.

75. The '951 patent describes the production of recombinant human lactoferrin in transgenic rice and processes for purifying the protein. Patent, 62:59-63:41, 64:63-65:52 (Examples 1 and 4).

76. The '951 patent further describes the production rHSA in transgenic rice and processes for purifying the protein. Patent, 64:6-60, 65:54-73:67 (Examples 3, 5 and 6).

77. The '951 patent acknowledges that “the other commercially available albumin products may have similar overall purity, endotoxin and detergent levels to rice derived recombinant albumin.” Patent, 75:10-13.

78. The '951 patent acknowledges that heat shock protein 70 (Hsp70) co-purifies with the rHSA due to their similar isoelectric points, abilities to bind hydrophobic molecules, and molecular weights. Patent, 50:39-51:6. As a result, techniques such as anion exchange chromatography (e.g., GE DEAE Sepharose), cation exchange chromatography, hydrophobic chromatography, gel filtration or size exclusion chromatography, and precipitation result in co-purification of rHSA and Hsp70. Patent, 50:39-51:6.

B. The Challenged Claims of the '951 Patent

79. I understand that Healthgen challenges the patentability of claims 1-9, and 11–13. These claims are presented below.

Challenged Claims of the '951 Patent
1. 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; ii) has less than 1 EU endotoxin/mg albumin; and iii) less than 2% aggregated albumin.
2. The composition of claim 1, wherein said recombinant albumin comprises at least 0.01 % wt/wt of a heat shock protein.
3. The composition of claim 2, wherein said heat shock protein is a rice heat shock protein.
4. The composition of claim 2, wherein said heat shock protein is selected from said group consisting of Rice HSP70 genes, and rice endosperm lumenal binding protein.
5. The composition of claim 2, wherein said heat shock protein is selected from the group consisting of Rice (gb1ACJ54890.11), EEC69073/OsI_37938, and AAB63469.
6. The composition of claim 2, wherein said supplement comprises at least 0.04% wt/wt HSP70.
7. The composition of claim 2, further comprising a recombinant transferrin related protein, wherein said transferrin related protein is lactoferrin.
8. The composition of claim 2, further comprising a recombinant transferrin related protein, wherein said transferrin related protein is transferrin.
9. The composition of claim 8, wherein said ratio of said transferrin to said recombinant albumin is 1:50 to 1:5000.
11. The composition of claim 1, wherein said albumin is recombinant human serum albumin.
12. The composition of claim 1, wherein said transgenic plant is a transgenic grain.
13. The composition of claim 12, wherein the transgenic grain is transgenic rice.

C. Priority Date

80. The '951 patent claims priority to application no. 12/708/462 (filed February 18, 2010) and application no. 61/154,204 (filed February 20, 2009).

81. I am informed that to be entitled to rely on the date of the 2009 application, that application must disclose the subject matter of the claims.

82. Here, the 2009 application does disclose any rHSA containing “less than 1 EU endotoxin/mg albumin,” as required by all claims of the '951 patent. EX1061 (2009 application). In fact, the word “endotoxin” does not appear in the 2009 application. *Id.* Relatedly, the 2009 application does not disclose the “new” process for removing endotoxin set forth in example 5 of the '951 patent.

83. Additionally, the 2009 application does not disclose a composition comprising “less than 2% aggregated albumin,” as required by all claims of the '951 patent. EX1061 (2009 application). In fact, the 2009 application does not mention albumin aggregates or aggregation at all. *Id.*

84. As all claims of the '951 patent require less than 1 EU endotoxin/mg albumin and less than 2% aggregated albumin, I conclude that the 2009 application does not disclose the claimed compositions.

85. I am informed that the Application No. 12/708,462 filed on February 18, 2010, contains the same disclosures as the '951 patent. I have therefore

analyzed the '951 patent from the perspective of a POSA as of the filing date of Application No. 12/708,462, February 18, 2010.

86. 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 '951 patent was known well before 2009.

VI. Level of Ordinary Skill in the Art

87. I believe that a person of ordinary skill in the art as of the earliest February 20, 2009, filing date would have had a Ph.D. in chemistry, biochemistry, or chemical engineering, with at least two years of experience involving production and/or purification of proteins such as a rHSA and/or experience with compositions suitable for use in cell culture media.

VII. Claim Construction

88. I have been informed that Ventria and Healthgen agreed to certain interpretations of the '951 patent claims in a related litigation. *See* EX1036. I have reviewed and agree with the parties' agreed upon claim constructions in that proceeding, which are included below for reference. I have applied these constructions in my analysis.

Parties' Agreed Upon Claim Construction Proposals in Litigation		
Term	Claim(s)	Parties' Proposed Construction
"A cell culture media supplement or complete media composition [[<i>for improving the growth of a cell in cell culture</i>]]"	1	the preamble term "[a] cell culture media supplement or complete media composition" is limiting [in other words, it is a requirement of the claim]; preamble is otherwise not limiting [meaning the phrase "for improving the growth of a cell in cell culture" is <i>not</i> a claim requirement] "cell culture media supplement" is "a combination of one or multiple cell culture media components, with or without other ingredients, for addition to cell culture media"
"aggregated albumin"	1	Non-monomeric albumin (e.g., albumin dimers)
"less than 2% aggregated albumin"	1	less than 2% non-monomeric albumin by weight
"recombinant albumin comprises at least 0.01% wt/wt of a heat shock protein"	2	Plain and ordinary meaning
"heat shock protein"	2, 3	a naturally occurring or synthetic form of a member of the heat shock protein super family that retains anti-apoptotic activity

89. I am also informed that the parties disputed the meaning of two claim terms, which were subsequently construed by the presiding Administrative Law Judge in the litigation in Exhibit 1065. The parties' proposals and the adopted constructions are below. I agree with the adopted constructions and have applied them in my analysis.

Parties' Disputed Claim Construction Proposals and Adopted Constructions			
Term (claim)	Ventria's Proposed Construction	Healthgen's Proposed Construction	Construction adopted in litigation
“recombinant mammalian albumin” (claim 1)	Mammalian albumin having a molecular weight between 66-69 kDa that is exogenous (i.e., heterologous or foreign) to the transgenic plant and retains the biological or therapeutically beneficial activity of its corresponding native mammalian albumin	recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it) which has the amino acid sequence of a native mammalian albumin or which is a variant, derivative, fusion protein, or fragment of native mammalian albumin (provided the fragment retains the biological or therapeutically beneficial activity of the native mammalian albumin)	recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it) which has the amino acid sequence of a native mammalian albumin or which is a variant, derivative, fusion protein, or fragment of native mammalian albumin (provided the fragment retains the biological or therapeutically beneficial activity of the native mammalian albumin)
“produced in a transgenic plant” (claim 1)	The recombinant albumin has been expressed within a plant cell that has been engineered to stably express recombinant albumin	expressed within a host plant cell	expressed within a host plant cell into which a recombinant or heterologous nucleic acid molecule has been introduced

EX1065, 16-17, 24.

90. I note that the prior art rHSA of Deeter is identical to the “recombinant mammalian albumin” of the '951 patent, and it was also produced by

the same “transgenic plant.” Therefore, any proposed constructions capturing the purported invention were also disclosed by Deeter.

91. Claims 7 and 8 of the '951 patent recite “transferrin related protein.” The specification defines this term as “transferrin family proteins including transferrin, lactoferrin, melanotransferrin, and ovotransfer[r]in.” Patent, 39:54-56. I have applied this definition in my analysis.

92. Claim 9 of the '951 patent recites a range of ratios of transferrin to recombinant albumin, specifically, 1:50 to 1:5000. The specification describes a cell culture media supplement comprising “a ratio (wt/wt) of transferrin related protein to albumin of about 1 to 5000 to about 1 to 0.5.” *E.g.*, Patent, 5:1-2.

93. A POSA would have understood that in the context of the '951 patent, the claimed ratios reflect the weight ratio of transferrin to recombinant albumin, which would be the same as the ratio of the concentration of transferrin to the concentration of recombinant albumin in the composition. For example, a composition containing 1 mg/L transferrin and 500 mg/L recombinant albumin would have a ratio of 1:500.

94. The other terms in the challenged claims all have established meanings in the art. I have thus applied the plain and ordinary meaning of these terms as they would have been understood in the context of the '951 patent.

VIII. Legal Principles Applied

95. 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 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.

96. I understand that patentability must be analyzed from the perspective of “a person of ordinary skill in the art” (“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.

97. I understand that that the Patent Trial and Appeal Board (“PTAB”) applies the same claim construction standard used in district courts, where the claims are given 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.

98. I understand that a patent claim is unpatentable for *obviousness* if any subject matter within the scope of the claim would have been obvious to a POSA

as of the effective priority date. I understand this entails considering (a) the scope and content of the prior art, (b) the differences between the prior art and the claimed invention, and (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.

99. 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 in the way the claimed invention does. I also understand that a POSA must have reasonably expected that the combination will work. That is, obviousness requires a “reasonable expectation of success” in achieving the claimed subject matter.

IX. Claims 1-8 and 11-13 of the '951 Patent Would Have Been Obvious over Deeter in view of Tanaka

A. Claim 1

100. All the challenged claims ultimately depend from claim 1, which recites:

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;
- ii) has less than 1 EU endotoxin/mg albumin; and
- iii) less than 2% aggregated albumin.

101. In my opinion, independent claim 1 would have been obvious over Deeter (EX1003) and Tanaka (EX1053), which collectively taught a cell culture media supplement comprising rHSA produced in a transgenic plant having less than 1 EU endotoxin per mg albumin and less than 2% aggregated albumin.

1. “A cell culture media supplement or complete media composition”

102. Deeter discloses “cell culture media supplement,” i.e., “a combination of one or multiple cell culture media components ... for addition to cell culture media.” EX1003 (Deeter), [0068]. For example, Deeter disclosed using rHSA in cell culture media and determined that it is functionally equivalent to plasma-derived HSA. EX1003 (Deeter), [0118]–[0120] (“Example 8: Cell culture media supplemented with recombinant human serum albumin promotes hybridoma cell growth in reduced -serum media,” “Example 9: Cell culture media supplemented with recombinant human serum albumin promotes hybridoma cell growth in serum-free media”).

103. A POSA would have understood that Deeter’s rHSA purified using the guidance of Tanaka would continue to be a cell culture media component, because

removing endotoxins from Deeter's rHSA would have been reasonably expected to improve (or at a minimum not impair) the effectiveness of Deeter's rHSA in cell culture given the toxic nature of endotoxins. *See supra*, ¶¶ 28-30 (discussing endotoxins).

2. “a recombinant mammalian albumin”

104. Deeter discloses rHSA, which is a recombinant mammalian albumin, i.e., “a recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it) which has the amino acid sequence of a native mammalian albumin.”¹ For example, Deeter's rHSA is foreign to the transgenic rice producing it and has the amino acid sequence of a native mammalian (human) albumin. *See* EX1003 (Table 3), [0103], [0117]; *see also* EX1018, 3 (disclosing in 2007 that the amino acid sequence of Cellastim™ was identical to native HSA).

¹ Under the adopted construction, recombinant mammalian albumin” means “recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it) [1] which has the amino acid sequence of a native mammalian albumin *or* [2] which is a variant, derivative, fusion protein, or fragment of native mammalian albumin (provided the fragment retains the biological or therapeutically beneficial activity of the native mammalian albumin).” As Deeter's rHSA meets the first part of the construction, I do not address the second option.

105. Deeter's rHSA also satisfies Ventria's proposed construction of recombinant mammalian albumin, i.e., it is "mammalian albumin having a molecular weight between 66-69 kDa that is exogenous (i.e., heterologous or foreign) to the transgenic plant and retains the biological or therapeutically beneficial activity of its corresponding native mammalian albumin." For example, Deeter's rHSA has a molecular weight of 66.8-67.9 kDa and has the same physicochemical properties as the rHSA of the '951 patent. *Compare* EX1003, Table 3 (page 31); Patent, Table E2 (col. 66). Deeter also discloses that its rHSA promotes cell growth "equivalently or better" than pHSA, indicating that it retains the biological or therapeutically beneficial activity of pHSA.

106. A POSA would have understood that Deeter's rHSA purified using the guidance of Tanaka would continue to be a recombinant mammalian albumin.

3. "wherein said albumin is produced in a transgenic plant"

107. Deeter discloses rHSA produced in a transgenic plant, specifically, transgenic rice. EX1003, [0103]-[0105]. In fact, Deeter's process for producing rHSA is, in all relevant respects, identical to the process disclosed in the '951 patent. In the figure below, the text on the left provides Deeter's process for producing rHSA, and the text on the right provides the process in the '951 patent, with highlighting to show the matching text.

Deeter (2005)	'951 Patent (2010)
Example 3: Expression of recombinant human serum albumin in rice grain [0103] Protein sequences of human serum albumin (HSA) from various data bases were compared. The consensus sequence represented by accession number P02768 (Swiss-Prot) was used as a base for gene codon-optimization for suitable expression of human serum albumin in rice grain (Figure 17). Gene synthesis was carried out by Blue Heron (Seattle, WA) and the synthetic fragment was inserted into pUC-based vector to create pUC-HSA. After confirmation of correct DNA and protein sequences, pUC-HSA was digested with MlviI and XhoI. The fragment containing codon-optimized HSA gene was inserted into pAPI405, which had been pre-cut with NaeI and XhoI. Plasmid API405 was a derivative of pAPI141 which included Gt1 promoter, Gt1 signal sequence and a nos terminator. [0104] Insertion of MlviI/XhoI fragment into pAPI405 resulted in pAPI504 (Figure 18). Plasmid API504 was then cleaved with HindIII and EcoRI. The HindIII/EcoRI fragment containing the entire expression cassette, Gt1 promoter, Gt1 signal sequence, codon-optimized HSA gene and nos terminator, was cloned into pJH2600 predigested with the same enzyme resulting in pAPI508 (Figure 19). Plasmid JH2600 was a shuttle vector between E coli and Agrobacterium. After DNA sequence verification, pAPI508 was moved into Agrobacterium AGL-1 for rice transformation. Plasmid API504 was also used via bombardment-based transformation following the procedure described previously. Upon transformation, transgenic plants were generated and were sent to greenhouse where transgenic RO plants grew to maturity and set R1 seeds. [0105] To monitor the expression of HSA in rice seeds, 10 R1 seeds from each RO plant were extracted using 10 ml of extraction buffer. (50 mM Tris-HCl pH 8.0, 150 mM NaCl). The supernatant was collected and the expression level from rice extracts was monitored by an ELISA (Bethyl Laboratories, Montgomery, TX). The results showed that the HSA expression level in rice transgenic seeds ranges from 0.01 to 0.85% of brown flour weight (0.1 to 8.5 grams/kg flour). The results of the eight events with highest expression levels are shown in the following table (Table 1).	Example 3: Expression of Recombinant Human Albumin in Rice Grain Protein sequences of human albumin (HSA) from various data bases were compared. The consensus sequence represented by accession number P02768 (Swiss-Prot) was used as a base for gene codon-optimization for suitable expression of human albumin in rice grain (FIG. 5A-FIG. 5D). Gene synthesis was carried out by Blue Heron (Seattle, Wash.) and the synthetic fragment was inserted into pUC-based vector to create pUC-HSA. After confirmation of correct DNA and protein sequences, pUC-HSA was digested with MlviI and XhoI. The fragment containing codon-optimized HSA gene was inserted into pAPI405, which had been pre-cut with NaeI and XhoI. Plasmid API405 was a derivative of pAPI141 which included Gt1 promoter, Gt1 signal sequence and a nos terminator. Insertion of MlviI/XhoI fragment into pAPI405 resulted in pAPI504 (FIG. 6). Plasmid API504 was then cleaved with HindIII and EcoRI. The HindIII/EcoRI fragment containing the entire expression cassette, Gt1 promoter, Gt1 signal sequence, codon-optimized HSA gene and nos terminator, was cloned into pJH2600 predigested with the same enzyme resulting in pAPI508 (FIG. 7). Plasmid JH2600 was a shuttle vector between E coli and Agrobacterium. After DNA sequence verification, pAPI508 was moved into Agrobacterium AGL-1 for rice transformation. Plasmid API504 was also used via bombardment-based transformation following the procedure described previously. Upon transformation, transgenic plants were generated and were sent to greenhouse where transgenic RO plants grew to maturity and set R1 seeds. To monitor the expression of HSA in rice seeds, 10 R1 seeds from each RO plant were extracted using 10 mL of extraction buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl). The supernatant was collected and the expression level from rice extracts was monitored by an ELISA (Bethyl Laboratories, Montgomery, Tex.). The results showed that the HSA expression level in rice transgenic seeds ranges from 0.01 to 0.85% of brown flour weight (0.1 to 8.5 grams/kg flour). The results of the eight events with highest expression levels are shown in the following table (Table E1).

Figure 7. Comparison of Deeter's process for producing rHSA from transgenic rice (left, EX1003, [0103]-[0105]) to the process in the '951 patent (col. 64:9-37).

108. Deeter's rHSA "produced in a transgenic plant" satisfies the parties' and the adopted construction for that term. For example, Deeter's rHSA is "expressed within a host plant cell" (Healthgen's proposal) and "expressed within a host plant cell into which a recombinant or heterologous nucleic acid molecule has been introduced" (adopted construction). It is also "expressed within a plant cell that has been engineered to stably express recombinant albumin" (Ventria's proposal), at least to the extent that the plant cells of the '951 patent were "engineering to stably express recombinant albumin" (because the plant cells and process are the same in Deeter and the '951 patent).

4. “has less than 1 EU endotoxin/mg albumin”

109. Deeter’s rHSA purified using the guidance of Tanaka would have been reasonably expected to contain less than 1 EU endotoxin/mg albumin, as recited in claim 1 of the ’951 patent.

110. As noted above, Tanaka’s purification process provides albumin that is “pyrogen-free,” which a POSA would have understood means free of pyrogens including endotoxins. EX1053, 5. Thus, Deeter’s rHSA purified using the guidance of Tanaka would c have been expected to contain less than 1 EU endotoxin/mg albumin.

5. “less than 2% aggregated albumin”

111. Deeter’s rHSA purified using the guidance of Tanaka would contain less than 2% aggregated albumin, as recited in claim 1 of the ’951 patent.

112. As noted above, Tanaka’s purification process provides 99.0-99.3% monomeric albumin, i.e., less than 0.7-1.0% aggregated albumin. EX1053, 4. Thus, Deeter’s rHSA purified using the guidance of Tanaka would have been reasonably expected to contain less than 2% aggregated albumin.

B. Claim 2

113. Deeter’s rHSA necessarily contained “at least 0.01% wt/wt of a heat shock protein,” as discussed below, and a POSA would have reasonably expected that purifying Deeter’s rHSA using the guidance of Tanaka would retain heat shock proteins at levels well above 0.01% wt/wt, as I discuss in Section IX.M.

1. Deeter's rHSA Necessarily Contained More than 0.01% wt/wt of a heat shock protein

114. Deeter's rHSA contained "at least 0.01% wt/wt of a heat shock protein," as recited in claim 2 of the '951 patent.

115. Compared to the rHSA of Deeter, the rHSA of the '951 patent was further purified to remove endotoxins using additional steps in a process the specification refers to as a "new process." *E.g.*, Patent, 71:57, 72:1-2, 73:40. As I explain below, if anything, the additional purification steps would have reduced the level of Hsp70 proteins in the rHSA of the '951 patent relative to Deeter. Deeter's rHSA would thus necessarily have contained substantially more than 0.01% wt/wt of a heat shock protein, *e.g.*, the same level of Hsp70 proteins found to occur in Cellastim.

116. The rHSA of the '951 patent (Cellastim) contained at least 0.22% wt/wt rice Hsp70 proteins—more than twenty times the minimal level of 0.01% wt/wt recited in claim 1. In particular, the '951 patent reports that three rice heat shock protein 70 family members were "found to occur in recombinant Cellastim"

at 0.07% wt/wt (Patent, 78:47–51), 0.09% wt/wt (Patent, 79:8-11), and 0.06% wt/wt (Patent, 79:34–37).²

117. Based on my analysis below, the rHSA of Deeter (which is also Cellastim) would have also contained rice Hsp70 proteins at levels substantially above 0.01% wt/wt.

118. First, Deeter's rHSA is remarkably similar to the rHSA of the '951 patent. For example, Deeter's rHSA and the rHSA of the '951 patent were produced using the same process in the same transgenic host (rice). *Supra*, Figure 7 (page 44). Moreover, Deeter's rHSA and the rHSA of the '951 patent are both identified as Cellastim™. EX1003 (Deeter), [0119]; Patent, 69:30-34.

119. In fact, the only known difference between the Cellastim/rHSA of the '951 patent and Deeter is the level of endotoxin. *See also* EX1024 (declaration averring that Deeter's rHSA contained more than 1 EU endotoxin/mg albumin).

² In my opinion, a POSA would read the claims of the '951 patent as requiring at least 0.01% wt/wt total heat shock protein. However, even if the claims are interpreted as requiring 0.01% wt/wt of a single heat shock protein, the rHSA of Deeter meets this limitation based on the analysis herein. Indeed, the amounts of the individual Hsp70 family members in the '951 patent are 7, 9, and 6 times the lower limit of the claim (0.07%, 0.09%, and 0.06% by weight).

Notably, during prosecution of the '951 patent, the Examiner rejected the pending claims as inherently anticipated over Deeter's Cellastim, arguing that Deeter's Cellastim would "inherently lack any endotoxin" and that heat shock proteins "would be inherent to the recombinant human serum albumin produced by Deeter et al." EX1042, 560–61; EX1008. In response, Ventria asserted that Deeter's Cellastim would not have inherently contained less than 1 EU endotoxin—while remaining silent on the heat shock proteins, which the Examiner noted were necessarily present. EX1042, 652.

120. The lower level of endotoxin in the rHSA of the '951 patent resulted from additional purification steps used in the '951 patent and absent in Deeter, discussed below.

121. A comparison of the different purification processes shows that Deeter's rHSA necessarily contained at least the level of rice Hsp70 protein in the rHSA of the '951 patent. The flow chart below shows that the "new" purification process described in the '951 patent to the process in Deeter.

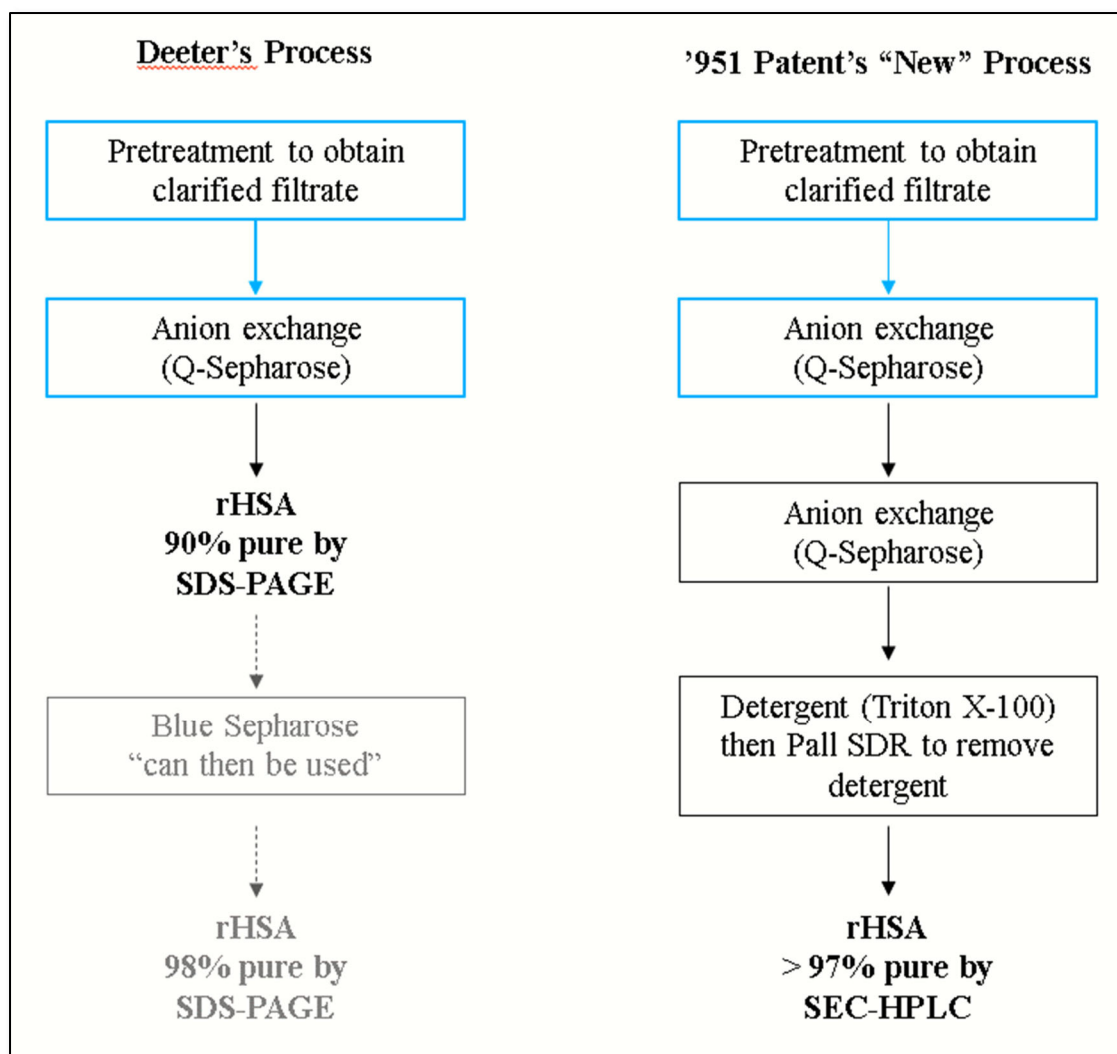


Figure 8. Comparison of Deeter's purification process (EX1003, [0116]-[0117] to the "new" process of the '951 patent (68:47-69:22).

122. As shown above, the "new" process of the '951 patent and the process of Deeter are quite similar, e.g., both employ ion exchange chromatography as their main separation steps. In particular, Deeter used Q-Sepharose (and suggested

Blue Sepharose could also be used)³ (EX1003 (Deeter), [0116]), and the '951 patent also used Q-Sepharose (twice), along with a detergent wash with Triton X-100, and hydrophobic chromatography (Pall SDR⁴) to remove the detergent. Patent, 68:47-69:22.

123. Because the purification process was more rigorous in the '951 patent, the rHSA prepared using the “new” process may have contained even less heat shock protein than what was naturally present in Deeter’s rHSA.

124. In my view, the most notable differences the addition and removal of detergent in the '951 patent and Deeter’s proposal to use Blue Sepharose.

125. First, the use of Triton X detergent in the '951 patent, followed by detergent removal, would not have *increased* the level of rice Hsp70 proteins in

³ Deeter states that “Blue Sepharose *can then be used* to increase the purity from 90% to approximately 98% based on scanning analysis of SDS-PAGE gels.”

EX1003 (Deeter), [0116]. I am informed that this prophetic language indicates that Deeter did not actually perform the Blue Sepharose chromatography step. By contrast, Deeter uses the past tense when disclosing the steps actually performed. EX1003 (Deeter), [0116] (“After clarification by filtration, the filtrate *was loaded* onto an anion exchange column (Q-Sepharose; GE Healthcare) . . .”).

⁴ “SDR” refers to solvent-detergent removal.

rHSA of Cellastim. This detergent treatment would have been expected to assist in the removal of endotoxins bound to proteins including albumin. *See* EX1060 (Aida), 1-3 (describing a similar process for removing endotoxin from albumin using Triton X-114). If anything, the added detergent wash and subsequent detergent removal may have reduced the level of heat shock protein bound to albumin, which indicates that the rHSA of the '951 patent may have contained less heat shock protein than Deeter's rHSA.

126. By contrast, none of the purification steps in Deeter would have removed Hsp70 proteins to below the very low threshold of 0.01% wt/wt. For example, Deeter suggests using Blue Sepharose to further purify its rHSA, although nothing in Deeter indicates that Deeter in fact, performed that step. EX1003 (Deeter), [0116] ("Blue Sepharose (GE Healthcare) *can then be used* to increase the purity from 90% to approximately 98% based on scanning analysis of SDS-PAGE gels."). Even if that step had been used, Blue Sepharose would not function to remove rice Hsp70 proteins from rHSA. Blue Sepharose columns may function as either ion exchange or affinity columns depending on the particular protein and environmental conditions, but the blue chromophore has a broad specificity with "nonspecific" ion-exchange effects that would not distinguish Hsp70 from rHSA. EX1038, 173; EX1037, 199.

127. Indeed, the '951 patent acknowledges that ion exchange chromatography *enhances co-purification* of Hsp70 proteins with rHSA and further recommends using Blue Sepharose as a suitable ion exchange chromatography resin. Patent, 50:39-55, 67:37-42. These disclosures confirm that Deeter's process resulted in the co-purification of rice Hsp70 proteins with its rHSA.

128. Similarly, Deeter's Q-Sepharose would not have removed rice Hsp70 proteins from its rHSA. As noted above, the '951 patent also uses Q-Sepharose (twice) and reports that it provides rHSA "enriched" in rice Hsp70 proteins, as recited below:

Due to the similar electronegativity of albumin and hsp70, anion exchange chromatography is the preferred method to prepare albumin enriched in Hsps. For example, both albumin and Hsp70 bind to anion exchange columns with resins consisting of either quaternary amine [e.g., Q Sepharose] or diethylaminoethyl Examples of such resins are General Electric (GE) Q Sepharose and GE DEAE Sepharose.

Patent, 50:39-48. Moreover, Q-Sepharose separates proteins based on net charge and thus would not have separated rHSA from any heat shock proteins having similar isoelectric points, e.g., Hsp70 (pI of about 5 versus about 5.3 for Deeter's rHSA, Cellastim).

129. Deeter also described that after purification, its rHSA was “concentrated and desalted via ultrafiltration prior to freeze drying.” EX1003 (Deeter), [0116]. It was known in the art that ultrafiltration separates molecules based on size, and accordingly, these processing steps would not have removed Hsp70 from rHSA, as rHSA and Hsp70 are approximately the same size. Indeed, the ’951 patent discloses multiple ultrafiltration and diafiltration steps as part of its purification process, and still obtained rHSA comprising three Hsp70 family members, Hsp70 (0.07%), BiP (0.09%), and stromal Hsp70 (0.06%) totaling 0.22% rice Hsp70 proteins. *See supra*, ¶ 53; Patent, 68:67, 69:14-16.

130. In addition, it was known that Hsp70 can bind substrates, including rHSA, in their unfolded or fully folded or near-native state, provided that their binding sites are accessible. EX1019, 5. As such, ion exchange chromatography, which separates proteins based on their net charge, would not separate rHSA from heat shock proteins such as Hsp70 in any configuration, folded or unfolded.

131. For all the reasons above, I conclude that Deeter’s rHSA necessarily contained more than 0.01% wt/wt of a heat shock protein, e.g., around 0.22% wt/wt heat shock protein.

132. Notably, consistent with my analysis, both the US Patent & Trademark Office and the Japan Patent Office acknowledged that Deeter’s rHSA contained the heat shock proteins recited in the claims of the ’951 patent. EX1042,

560-61; EX1055 (JPO), 2. In particular, the Japan Patent Office rejected the claims of the Japanese counterpart to the '951 patent over Deeter, noting that in Deeter's rHSA, "heat shock proteins are included in the same degree as the present invention, and the kinds thereof are not different from each other." EX1055 (JPO), 2. The Japan Patent Office explained that "the heat shock proteins are derived from the genes inherent in rice," and the purification steps of Deeter would not have removed them from rHSA, as set forth below:

According to the above, the heat shock proteins are derived from the genes inherent in rice, and methods for purification of heat shock proteins including anion exchange chromatography and ATP agarose affinity chromatography are well-known in the art. It is considered that the isoelectric point is simultaneously purified with albumin by the anion exchange chromatography because the isoelectric point is similar to the albumin. In the working example of cited document 1 [Deeter], a rice of the same breed as the Invention is used (the description of the present application : [0330], cited document 1 [Deeter] : [0101]), and albumin is purified by using anion exchange chromatography, Blue-Sepharose, and the like in the same manner as the Invention (the description of the present application : [034] 3. Cited Document 1 [Deeter] : [0116] In the albumin of the invention described in Cited Document 1 [Deeter], heat shock proteins are included in the same degree as the present Invention, and the kinds thereof are not different from each other.

133. In the next section, I discuss additional evidence supporting my conclusion that Deeter's rHSA necessarily contained substantially more than 0.01% wt/wt of a heat shock protein.

2. The Activity of Deeter's rHSA Further Supports the Conclusion that It Contained More than 0.01% wt/wt Heat Shock Protein

134. The activity of Deeter's rHSA in cell culture media confirms the presence of at least 0.01% wt/wt heat shock proteins. Heat shock proteins were known to promote cell viability. *E.g.*, EX1033; EX1032, 10; EX1051 (Lasunskiaia). Although the '951 patent states that the co-purified rice heat shock proteins in the "new" Cellastim contribute to improved cell viability compared to "old" versions of Cellastim (Patent, 73:44-59), there are no data in the '951 patent to support that claim. In fact, the data refute that statement.

135. For example, the data demonstrate that "old" versions of Cellastim (including rHSA that Ventria called "Deeter et al.-produced" rHSA) performed just as well as the rHSA prepared using the "new" process described in the '951 patent (0.1 to 2 mg/ml, *see* Table 1, below).⁵ Because heat shock proteins contribute to

⁵ I understand that Ventria represented during prosecution of the '951 patent that the data in Tables E3 and E4 of the '951 patent reflect the activity of "Deeter et al.-produced albumin" and the rHSA prepared in the '951 patent. EX1024, 2.

activity in cell culture media, the comparable viabilities of Deeter et al.-produced “old” rHSA to the rHSA of the '951 patent support the conclusion that the rHSA of Deeter contained comparable levels of Hsp70. *See* Table 1 below. Patent, 73:1–35 (Tables E3, E4).

Table 1. Viable cell density (10⁵ cells/ml) representative of “old”/“Deeter et al. produced” rHSA and the “new” rHSA of the '951 patent					
Neg Control	pHSA	“old” rHSA	'951 “new” rHSA	'951 “new” rHSA	'951 “new” rHSA
1 mg/ml				0.5 mg/ml	2 mg/ml
~ 3 Patent, Fig. 10A.	~6 Patent, Fig. 10A.	14-17.5 Patent, Table E3.	~ 14.5 Patent, Fig. 10A.	11.1-18.4 Patent, Table E4.	14.4-17.5 Patent, Table E4.

136. As shown in Table 1, the Deeter et al.-produced “old” rHSA performed just as well as the “new” rHSA of the '951 patent in improving cell viability at low concentrations, e.g., 1 mg/ml, and was far superior to pHSA and untreated (negative control) media.⁶ Patent, Fig. 10A, Tables E3 and E4.

⁶ Table E4 of the '951 patent does not contain data for “new” rHSA at 1 mg/ml, although those data are shown in Figure 10A. This table thus compares the viability with “old” rHSA at 1 mg/ml to the “new” rHSA at 0.5, 1, and 2 mg/ml.

137. The data in Deeter further reflect the performance of its rHSA, support, showing that Deeter's rHSA provided cell viabilities of $12\text{--}13 \times 10^5$ (at 0.1 mg/ml), well above the control data in the '951 patent. EX1003 (Deeter), Fig. 23. Because Hsp70 contributes to cell viability, these data support the conclusion that Deeter's rHSA contained about the same level of rice Hsp70 proteins as the "new" rHSA of the '951 patent.

138. At higher concentrations, the "new" rHSA resulted in greater cell viabilities versus the "old" rHSA (e.g., average viability of 15.52×10^5 for "new" versus 7.29×10^5 for "old" at 5 mg/ml). This difference, however, is attributable to the endotoxins in the "old" rHSA—not the amount of the heat shock proteins—as the activity of the "old" rHSA decreases at high concentrations, while the activity of the "new" rHSA remains about the same, as shown in Figure 9.

Since Tables E3 and E4 do not make a comparison to a control, the negative control of Fig. 10A is used as a basis for the cell viability data.

TABLE E3								
Cellastim - Old Process B000								
Production	Batch	Grams	EU/mg Dry	Detergent	Viability (number of viable cells/ml/10 ⁵)			
Date	Number	product	Material	(ppm)	1 mg/ml	2 mg/ml	5 mg/ml	10/mg/ml
Feb. 27, 2008	B202	70.6	25.3	815.3	15	7.9	4.1	0.9
Feb. 27, 2008	B203	58.8	23.1	267.7	15.8	12.4	6.5	4.1
Feb. 28, 2008	B204	59.9	29.2	589.2	16.3	11.1	5.2	2.9
Feb. 28, 2008	B205	63	64.3	133.4	14	11.6	6.9	3.8
Mar. 3, 2008	B206	41.1	35.1	1182.2	14.9	2.3	0.0	0.0
Mar. 3, 2008	B207	94.2	28.7	388	16.5	12.7	7.6	4
Mar. 4, 2008	B208	24.5	>80	467.2	15.3	11.4	7.1	2.7
Mar. 4, 2008	B209	66.1	91.8	406.4	16.7	15.2	6.9	4.1
Mar. 4, 2008	B210	87.3	67.8	76.4	17.5	19.1	15.4	8.5
Mar. 11, 2008	B217	62.09	4.4	127.3	16.7	18.7	13.2	6.6
Averages			44.97	445.31	15.87	12.24	7.29	3.76

TABLE E4								
Cellastim - New Process B0000C								
Production	Batch	Grams	EU/mg Dry	Detergent	Viability (number of viable cells/ml/10 ⁵)			
Date	Number	product	Material	(ppm)	0.5 mg/ml	2 mg/ml	5 mg/ml	10/mg/ml
Feb. 2, 2009	B0032C	209.4	0.35	3.1	13.1	15.7	13.4	11.2
Feb. 2, 2009	B0033C	271.4	0.26	7.9	18.4	17.4	15.1	14.3
Feb. 10, 2009	B0041C	247.1	0.18	5.4	15.4	17.2	14.1	12.1
Apr. 27, 2009	B0118C	617.4	0.11	Less than 1	15.7	15.4	19.2	16.8
May 14, 2009	B0138C	610.6	0.48	Less than 1	12.5	15.8	15.1	14.6
Jun. 9, 2009	B0158C	598.0	0.13	3.9	13.7	17.1	13.2	11
Jun. 15, 2009	B0162C	618.6	0.20	2.0	15.5	17.5	17.2	14.7
Jul. 28, 2009	B0196C	507.0	0.36	3.9	17.4	17.3	18.8	14.8
Aug. 26, 2009	B0219C	851.4	0.86	8.2	17.9	16.3	16.6	13.7
Aug. 31, 2009	B0220C	897.8	0.93	Less than 1	13.5	16	15.5	15.1
Sep. 9, 2009	B0227C	929.9	0.13	Less than 1	11.1	14.4	12.5	11.4
Averages			0.36	3.45	14.92	16.37	15.52	13.6

Data representative of “Deeter et. al- produced” Cellastim show decreasing activity at higher concentration attributable to toxic contaminants such as endotoxin

Cellastim prepared by the “new” process show comparable activity at all concentrations.

Figure 9. Tables E3 of the '951 patent (annotated, top left), showing decreasing activity at higher concentrations for “old” rHSA and Table E4 of the '951 patent (annotated, bottom left) showing that activity is about the same at higher concentrations.

139. Indeed, the '951 patent shows that removing Hsp70 from rHSA decreases cell viability, e.g., from about 13.5 to 10 x 10⁵ viable cells (0.5 mg/ml). Patent, Fig. 12. Accordingly, the comparable activity of Deeter’s rHSA and the rHSA prepared in the '951 patent, and the fact that they were prepared using the same recombinant production system, confirms that Deeter’s rHSA contained Hsp70 family members at levels comparable to those disclosed in the '951 patent.

This explains why both Deeter's rHSA and the rHSA of the '951 patent provide comparable cell growth and viability at low concentrations (e.g., 1 mg/ml).

140. I note that in a separate patent, Ventria admits compositions comprising rHSA and heat shock proteins *discovered*, further supporting the conclusion that heat shock proteins were inherently present in Deeter. In particular, when describing the "invention" of the '416 patent, Ventria stated that the claimed compositions were based on "the *discovery* that heat shock proteins co-purified, or co-mixed in solution with recombinant cell culture component proteins, result in the formation of protein complexes which are capable of significantly enhancing the cell culturing properties of the recombinant cell culture component proteins." Patent, 3:11–16. Thus, the presence of a heat shock proteins appears to have been merely a "discovery" of an intrinsic property in Deeter. Patent, 3:12–17. This echoes the language in the '951 patent, which repeatedly states that Hsp 70 family members were "found to occur" in Cellastim. Patent, 78:47-50, 79:34-36, 79:7-11.

141. These additional considerations support my conclusion that Deeter's rice-derived rHSA necessarily contained "at least 0.01% wt/wt of a heat shock protein," as required by claim 2 of the '951 patent.

C. Claim 3

142. Claim 3 of the '951 patent depends from claim 2 and requires that the heat shock protein "is a rice heat shock protein." Patent, claim 3.

143. As explained above for claim 2, the heat shock proteins naturally present in Deeter's rHSA are rice heat shock proteins, and these rice heat shock proteins would have reasonably been expected to remain at levels above 0.01% wt/wt in Deeter's rHSA after purification following the guidance of Tanaka.

D. Claim 4

144. Claim 4 of the '951 patent depends from claim 2 and further requires that the heat shock protein is selected from the group consisting of "Rice HSP70 genes, and rice endosperm luminal binding proteins." Patent, claim 4.

145. Claim 4 encompasses the rice heat shock proteins "found to occur" in Cellastim. Patent, 78:47-50, 79:34-36, 79:7-11. For example, the '951 patent notes that Cellastim contains "3 rice heat shock protein super family members that co-purify with albumin, 2 Rice HSP70 genes, (gblACJ54890.11), EEC69073, and AAB63469—a BiP homolog from rice endosperm tissue (endosperm luminal binding protein)." Patent, 78:40-44. The rice HSP70 genes were present at 0.07% wt/wt and 0.06% wt/wt, while the rice "endosperm luminal binding protein" was present at 0.09%. Patent, 78:47-50, 79:34-36, 79:7-11.

146. As detailed for claim 2, these Hsp70 family members would necessarily have been present in Deeter's Cellastim and would have reasonably been expected to remain at levels above 0.01% wt/wt after purification following the guidance of Tanaka. *Supra*, ¶¶ 113-141; *infra*, ¶¶ 193-99.

E. Claim 5

147. Claim 5 depends from claim 2 and further requires that the heat shock protein be selected from the group consisting of Rice (gb1ACJ54890.11), EEC69073/OsI_3 7938, and AAB63469. Again, these are the same Hsp 70 family members “found to occur” in Cellastim. Patent, 78:47-50, 79:34-36, 79:7-11. As discussed for claim 2, these heat shock proteins would have been necessarily present in Deeter’s Cellastim would have reasonably been expected to remain at levels above 0.01% wt/wt after purification following the guidance of Tanaka.

F. Claim 6

148. Claim 5 depends from claim 2 and requires that “said supplement comprises at least 0.04% wt/wt HSP70.”

149. The ’951 patent acknowledges that the three heat shock proteins “found to occur” in Cellastim are “Hsp70 proteins.” Patent, 75:46-48 (referring to “three Hsp70 proteins”); Patent, 78:47-50, 79:34-36, 79:7-11 (identifying Hsp 70 family members “found to occur” in Cellastim).

150. As discussed for claim 2, these heat shock proteins would have been necessarily present in Deeter’s Cellastim would have reasonably been expected to remain at levels above 0.04% wt/wt (e.g., the amounts “found to occur” in the rHSA in Cellastim) after purification following the guidance of Tanaka.

G. Claim 7

151. Claim 7 depends from claim 2 and further requires that the composition comprise “a recombinant transferrin related protein, wherein said transferrin related protein is lactoferrin.”

152. A POSA would have been motivated to include recombinant human lactoferrin in the cell culture media composition of claim 2, as taught by the combination of Deeter and Tanaka.

153. For example, Deeter suggests using “*at least* one plant-produced heterologous protein as a cell culture component” and thus specifically suggests combining more than one recombinant protein in cell culture media. EX1003 (Deeter), [0081].

154. Deeter describes preparing both rHSA and recombinant human holo-lactoferrin in transgenic rice for use as cell culture media supplements. EX1003 (Deeter), [0100]-[0102] (Example 2), [0103]-[0105] (Example 3).

155. Deeter demonstrates that plant-derived rHSA promotes cell growth “equivalently or better” than pHSA. EX1003, [0118]. The base media used by Deeter in its cell culture media experiments comprised transferrin, a common component of cell culture media. EX1003, [0118], [0119].

156. Deeter discloses that plant-derived proteins provide advantages over animal-derived proteins as cell culture media supplements, including reduced risk

of contamination with pathogens of animal and human origin. EX1003, [0006]. A POSA would have thus recognized advantages in replacing the transferrin used in Deeter's cell culture media with a plant-produced recombinant transferrin related protein.

157. In this regard, Deeter discloses that plant-produced recombinant human lactoferrin "can replace animal-derived transferrin" in cell culture media, specifically demonstrating that recombinant human holo-lactoferrin promotes cell growth and productivity in cell culture media as well as transferrin, while providing greater productivity when compared to transferrin. EX1003, [0114]-[0115] ("plant-derived recombinant human hololactoferrin can promote hybridoma cell growth and replace animal derived transferrin."); *id.*, [0115] ("In medium containing LACROMIN™ [recombinant human holo-lactoferrin], more IgG is produced than in medium containing transferrin.").

158. In view of Deeter's teachings, it would have been a routine matter for a POSA to replace transferrin with recombinant human lactoferrin in cell culture media comprising rHSA. A POSA would have been motivated to make this modification to achieve the benefits of plant-derived recombinant proteins over animal-derived proteins and would have had a reasonable expectation of success in doing so in view of the results in Deeter.

159. I conclude that claim 7 would have been obvious over Deeter in view of Tanaka.

H. Claim 8

160. Claim 8 depends from claim 2 and further requires “a recombinant transferrin related protein, wherein said transferrin related protein is transferrin.”

161. Deeter in combination with Tanaka satisfy the limitations of claim 2, as discussed above. In addition, a POSA would have been motivated to combine recombinant transferrin with the cell culture media supplement of claim 2.

162. Deeter discloses that rHSA promoted the growth of cells in culture when used in DMEM base medium supplemented with transferrin, among other components. EX1003, [0118]. Deeter also discloses that components of cell culture media such as transferrin are extracted from animal sources “have several undesirable features,” including the risk of contamination with pathogens. EX1003 (Deeter), [0006].

163. In view of the well-established disadvantages of animal-sourced proteins such as transferrin, a POSA would have been motivated to replace transferrin with recombinant human transferrin. A POSA would have considered recombinant transferrin a natural replacement for animal-derived transferrin, as the recombinant protein would have been expected to perform the same function as its natural counterpart.

164. A POSA would have had a reasonable expectation of success in making this simple substitution, as recombinant human transferrin was commercially available by 2008. *See* EX1059 (Grosvenor), 4.

I. Claim 11

165. Claim 11 depends from claim 1 and requires that the albumin “is recombinant human serum albumin.” As discussed above for claim 1, Deeter’s rHSA purified following the guidance of Tanaka is recombinant human serum albumin satisfying the requirements of claim 1.

J. Claim 12

166. Claim 12 depends from claim 1 and requires that the transgenic plant is a transgenic grain. As discussed above for claim 1, Deeter’s rHSA is produced in transgenic rice, which is a grain. I thus conclude that Deeter in view of Tanaka renders claim 12 obvious for the reasons stated above for claim 1.

K. Claim 13

167. Claim 13 depends from claim 1 and requires that the transgenic grain is transgenic rice. As discussed above for claim 1, Deeter’s rHSA is produced in transgenic rice, which is a grain. I thus conclude that Deeter in view of Tanaka renders claim 13 obvious for the reasons stated above for claim 1.

L. Motivation to Combine

168. As discussed above, Deeter disclosed a cell culture media composition comprising rHSA produced in a transgenic plant. EX1003, [0103]-[0105], [0116]-[0121]; *supra*, ¶¶ 100-108.

169. A POSA would have considered Deeter's rHSA a beneficial alternative to mammalian-produced albumin. As Deeter explains, components of cell culture media such as albumin extracted from animal sources "have several undesirable features," e.g., "there is no way to ensure that extraneous animal products are completely excluded from the [albumin]," and animal-derived components "carry the risk of contamination of pathogens or animal and human origin such as BSE, HIV and hepatitis virus." EX1003, [0006]. Moreover, "small proteinaceous particles, such as prions, could be present and current scientific evidence suggests that even a very small number could result in disease." EX1003, [0007].

170. As Deeter explains, given the concerns about the safety of animal-derived cell culture media components, regulators encouraged "replacing animal components with recombinant proteins in vaccine production and other applications of cell culture techniques." EX1003, [0007].

171. While Deeter's rHSA would have been recognized as a beneficial alternative to traditional, animal-sourced albumin, it contained undesirable levels

of endotoxin, which would have been readily detected and known to a skilled artisan. As acknowledged by Ventria during prosecution of the '951 patent, “[t]he average endotoxin level in Deeter et al.-produced albumin was **44.97 EU/mg**.” EX1042, pp. 693–94 (emphasis in original); EX1042, p. 693 (identifying Deeter et al. as WO 2007/002762).

172. Given Deeter’s lack of any description for use of sterile techniques, a person of ordinary skill would have recognized that endotoxins would be present in Deeter’s rHSA. Endotoxins are ubiquitous and would expectedly be present in the absence of steps to remove them. Moreover, endotoxins in Deeter’s rHSA would have been readily detectable using routine methodology. *See, e.g.*, EX1030. Indeed, endotoxin was known to be toxic to cells, and thus researchers had developed techniques for detecting and removing endotoxins. EX1026, 292; EX1027, 1:49–52; EX1028, 261; EX1029, 3.

173. Accordingly, a person skilled in the art would have sought to reduce the endotoxin level in Deeter’s rHSA and would have looked to Tanaka for guidance, as Tanaka discloses a simplified process for purifying human albumin to provide albumin free of endotoxins (non-pyrogenic). EX1053, 5.

174. A POSA would have understood that Tanaka’s process could be beneficially applied to purify Deeter’s rHSA. For example, a POSA would have understood that Tanaka’s process could be used to purify the rHSA in Deeter’s

clarified filtrate or the eluent of Deeter's Q-Sepharose or Blue Sepharose chromatography. *See* EX1003 (Deeter), [0116]. With Deeter's rHSA in-hand, a POSA would have been motivated to use the guidance of Tanaka to further purify the product using DEAE-Sepharose chromatography, fatty acid removal, Sephacryl S-200 HR gel permeation, and viral inactivation.

175. Tanaka's process further provides albumin beneficially containing 99.0-99.3% monomeric albumin, e.g., less than 1% aggregated albumin, as detailed above. *Supra*, ¶¶ 49-59. Tanaka explains that its purification process is both time- and cost-effective. EX1053, 5. These added benefits would have further motivated a POSA to use to guidance of Tanaka to remove endotoxins from Deeter's rHSA.

M. Reasonable Expectation that Deeter's rHSA Purified Following the Guidance of Tanaka Would Have the Low Endotoxin and Aggregated Albumin Levels of Tanaka's pHSA

176. A POSA would have reasonably expected that Deeter's rHSA purified following the guidance of Tanaka would contain "less than 1 EU endotoxin/mg albumin" and "less than 2% aggregated albumin," as recited in claim 1.

177. For example, a POSA would have reasonably expected that Deeter's rHSA purified using the guidance of Tanaka would be "pyrogen-free," i.e., free of pyrogens including endotoxin. Such a product would contain well below 1 EU endotoxin per mg albumin.

178. Notably, the '951 patent also acknowledges that techniques for purifying pHSA (the albumin of Tanaka) also work to purify the albumin of the invention produced in a transgenic plant. Patent, 50:1-12. For example, the inventors acknowledged that “recognized” processes for purifying pHSA were expected to work for rHSA produced in a transgenic plant, as reflected by the passage below:

A search of patents, published patent applications, and related publications will provide those skilled in the art reading this disclosure with *significant possible methods for preparing and purifying albumin*. For example, U.S. Pat. Nos. 4,075,197; 4,086,222; 4,093,612; 4,097,473; 4,136,094; **4,228,154**; **5,250,662**; 5,656,729; 5,677,424; 5,710,253; 5,728,553; 5,994,507; 6,001,974; 6,638,740; 6,617,133 and 7,423,124 disclose various processes for purifying albumin. In 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.

Patent, 50:1-12 (emphases added).

179. Notably, U.S. Patent 5,250,662 (“Chang”), listed above, provides a method for purifying pHSA by anion exchange resins such as DEAE-Sephadex. EX1062 (Chang), 10:18-11:2. As Chang explains: “Contaminants with a PI below the pH of the first aqueous solution, i.e., below the 4.5 to 4.7 pH of the solution,

will have a negative charge and will bind to the positively-charged resin during agitation.” EX1062 (Chang), 6:65-68.

180. Similarly, U.S. Patent 4,228,154 (“Fisher”), also listed in the passage above, describes a process for purifying pHSA to have sufficient purity for “therapeutic administration.” EX1063 (Fisher), 5:6-14. Fisher discloses that by using ion exchange chromatography, the pH may be adjusted to remove contaminants with isoelectric points below and above that of pHSA. *Id.*, 2:23-36, 3:38-67.

181. Thus, Tanaka, Matejtschuk, Chang, and Fisher all disclose that anion exchange chromatography on a DEAE-Sepharose column with an appropriate pH would bind contaminants with low isoelectric points (e.g., endotoxins) while eluting pHSA. And, as reflected in the passage above, the ’951 patent acknowledges that prior art processes for purifying pHSA were expected to work for rHSA.

182. In fact, a POSA would have considered the removal of endotoxin from rHSA a routine purification step and would have had several techniques at their disposal to do so. *See above*, ¶¶ 28-34.

183. For example, a POSA would have reasonably expected to replicate the successful results in Tanaka using rHSA, as the separation of rHSA from endotoxin does not depend on the source of the albumin (e.g., it does not depend on whether

the albumin is produced in a human as disclosed in Tanaka or transgenic rice as disclosed by Deeter).

184. On scientific grounds a POSA would have reasonably expected that albumin, including rHSA, would behave similarly on chromatography columns—regardless of the source or expression system used to produce the rHSA. Albumin is albumin, whether naturally expressed or recombinantly produced. A POSA would further have reasonably expected that endotoxin would behave similarly on the same chromatography columns. Indeed, endotoxin is not produced by the transgenic host—it is a ubiquitous contaminant produced by gram-negative bacteria such as *E. coli* (not rice or yeast). A skilled person would thus have reasonably expected the same types of chromatography that separated endotoxin from rHSA produced in a human (Tanaka’s albumin) would also work for rHSA produced in transgenic rice (Deeter).

185. A POSA’s reasonable expectation of success would have been bolstered by Matejtschuk. EX1005. Matejtschuk reported that DEAE-Sepharose chromatography “consistently lowered” endotoxin levels in serum-derived albumin. EX1005, 6. As Matejtschuk explains, on the DEAE-Sepharose column, impurities such as endotoxin are bound and removed (they stick to the column), while the albumin flows through. A schematic of this process is included below for reference.

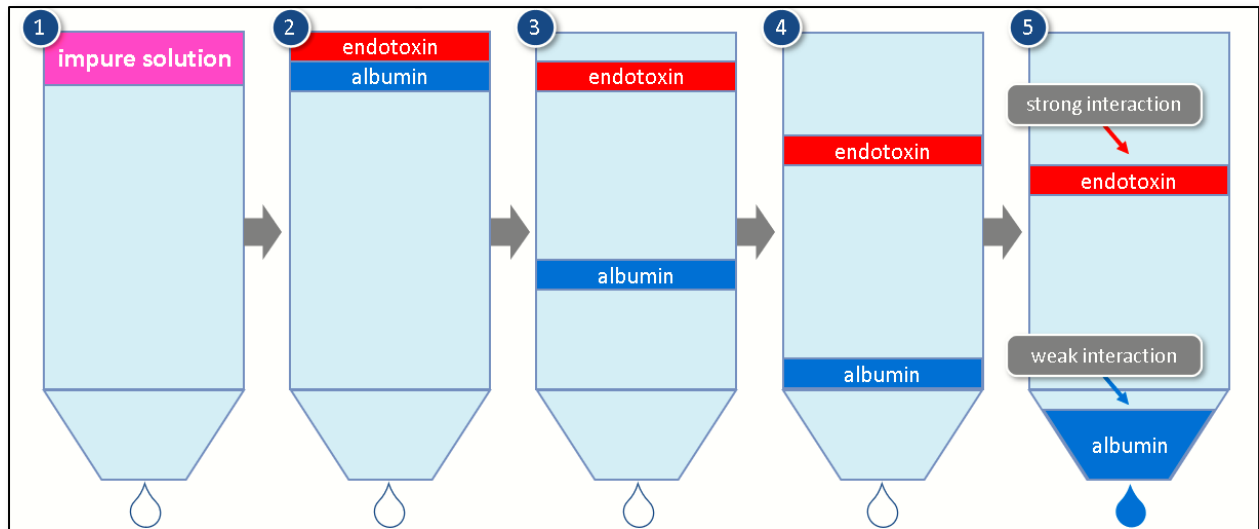


Figure 10. Schematic of Matejtschuk's Removal of Endotoxin from Albumin

186. This type of separation would have been expected using the conditions of Tanaka, which eluted albumin at a pH of 4.5, i.e., a pH above the isoelectric point of endotoxins (generally reported to be around 2) and below that of Deeter's rHSA (pI 5.3). EX1003 (Deeter), Table 3 (page 31).

187. Moreover, chromatography techniques were well-established and routine in the art, a thus a person skilled in the art would have recognized that minor variations could have been made to known processes, including the processes disclosed in Tanaka, to optimize working conditions for rHSA purification.

188. For instance, if more endotoxin was present in Deeter's rHSA compared to the albumin of Tanaka, a person skilled in the art would have understood that the rHSA sample could simply be run through either a fresh

column or a larger column until the level of endotoxin was lowered to desired levels.

189. In fact, Deeter's Q-Sepharose chromatography would have reasonably been expected to remove endotoxins from rHSA, *if performed under appropriate conditions*, e.g., using an appropriate pH and suitable loading conditions, including running the solution through a fresh column again to further reduce the level of endotoxin to desired levels.

190. In the unlikely event that routine chromatography and filtration steps did not reduce the level of endotoxins to the desired level (when performed under appropriate conditions), it would have been well within the general knowledge and skill of a POSA to use the simple modification reported by Aida to remove additional endotoxins. EX1060, 1, 4, 5 (reporting that Triton X-114 "aids in the dissociation of endotoxin from the protein"; that the detergent can be readily removed using chromatography; and that the process does not harm the proteins). In short, those of ordinary skill in the art had ample techniques at their disposal, and ample guidance from the literature, to achieve rHSA containing less than 1 EU endotoxin/mg albumin.

191. Researchers in the field often targeted levels at or below 1 EU endotoxin per mg protein, and a POSA would have reasonably expected to achieve rHSA containing less than 1 EU endotoxin/mg albumin using the guidance of

Tanaka and the general knowledge of common purification techniques. *Supra*, ¶ 22.

192. A POSA would have further expected Deeter's rHSA purified using the guidance of Tanaka would contain less than 2% aggregated albumin, as Tanaka's process provided at least 99% monomeric albumin in fifteen separate lots of albumin. EX1053, 4 (Table 1). Again, albumin is albumin, whether naturally expressed or recombinantly produced.

N. A POSA Would Have Reasonably Expected that Deeter's rHSA Purified by the Guidance of Tanaka Would Contain the Recited Heat Shock Protein

193. A POSA would have expected that Deeter's rHSA purified using the guidance of Tanaka would retain at least 0.04% wt/wt of the rice Hsp70 proteins found to occur in the rHSA of Cellastim, as none of the purification steps in Tanaka would have been expected to substantially reduce the levels of heat shock proteins in Deeter's rHSA.

194. First, Tanaka's DEAE-Sepharose would not have been expected to reduce the level of rice Hsp70 proteins. DEAE-Sepharose is an anion exchange resin, meaning it separates molecules based on their isoelectric points. The isoelectric point of Deeter's rHSA was 5.3, while the isoelectric point of Hsp70 is about 5. EX1003 (Deeter), [0117] (Table 3); EX1034, 4 (Fig. 1). Given the similar isoelectric points, a POSA would not expect ion exchange chromatography such as

DEAE-Sepharose would separate heat shock proteins from albumin, particularly at the pH of 4.5 employed by Tanaka. EX1053, 3 (Fig. 2 caption, reporting eluting albumin at 4.5).

195. Next, Tanaka discloses heating albumin at 55 °C for 3 hours to precipitate fatty acids. EX1053, 3. A POSA would not have expected that this step would remove rice Hsp70 proteins. Indeed, heat shock proteins “were originally identified as proteins whose expression is induced by heat stress.” EX1032 (Sharma), 1. As Sharma explains, “Hsps, primarily of the Hsp70 family, bind partially exposed hydrophobic surfaces, to prevent non-productive interactions that would lead to aggregation, and to promote protein refolding.” *Id.* A POSA would thus not expect that Tanaka’s heating step would remove heat shock proteins, which, if anything, may become more tightly bound to albumin under conditions of stress.

196. Tanaka further purifies albumin by chromatography with a Sephacryl S-200 HR filtration gel. EX1053, 2, 3. Sephacryl S-200 HR is (and was) a size-exclusion chromatography resin marketed for the separation of peptides and globular proteins with molecular weights ranging from about 5 kDa to about 150 kDa. EX1067 (BioProcess Product Guide 2007), 51. This technique would not have been expected to separate Deeter’s rHSA from the rice Hsp70 proteins found to occur in Cellastim in view of their properties, including similar molecular

weights. *See* EX1003 (Deeter), Table 1 (reporting rHSA molecular weight of 66.8-67.9 kDa); EX1035, 209 (explaining heat shock proteins are categorized by their molecular weights, e.g., Hsp70 (where “70” indicates approximate molecular weight), and noting that “HSP70 is a major, well-characterized family.”).⁷

197. Finally, Tanaka reports formulating albumin with sodium caprylate (0.16 mmol/g protein) to stabilize the product, then performing viral inactivation via pasteurization at 60 °C for 10 hours. EX1053, 4, 5. Heat shock proteins necessarily remain present during this step, as the solution is merely heated and no components are removed from the albumin solution. I further note that this heating step was a standard method of viral inactivation of human albumin, as reflected in regulations provided by the U.S. Food and Drug Administration, which require that HSA be heated to 60±0.5 °C for not less than 10, and not more than 11 hours, and stabilized with caprylate. EX1056 (FDA Regulations), § 640.81(e).

⁷ Based on the amino acid sequences for the Hsp70 family members identified in the '951 patent, which would also be present in Deeter's rHSA, their molecular weights range are about 77.1 kDa (Hsp70), 73.5 kDa (BiP homolog), and to 76.2 kDa (stromal Hsp70), as calculated at

https://www.bioinformatics.org/sms/prot_mw.html.

198. I further note that columns containing ATP Agarose affinity chromatography resin were well known systems for removing Hsp70 proteins. *See e.g.*, EX1039, 9; EX1040, 2-3; EX1041, 2; EX1032, 2 (“The ATP-bound form of Hsp70 has low substrate binding affinity.”); *see also* EX1031, 4 (“[c]o-purification of chaperonins is a common problem encountered during protein purification”). However, neither Deeter nor Tanaka employed a step using an ATP Agarose affinity column to remove heat shock proteins from albumin. EX1003 (Deeter), [0116]; EX1053 (Tanaka).

199. I conclude that a POSA would have reasonably expected that Deeter’s rHSA, purified using the guidance of Tanaka, would continue to comprise the rice Hsp70 proteins at levels comparable to those found to occur in Cellastim, which were well above the claimed levels of “at least 0.01% wt/wt of a heat shock protein” of claim 2 and “at least 0.04% wt/wt HSP70” of claim 6.

X. Claim 9 of the '951 Patent Would Have Been Obvious over Deeter in view of Tanaka and Meng

200. Claim 9 depends from claim 8 and further requires that the “ratio of said transferrin to said recombinant albumin is 1:50 to 1:5000.”

201. Claim 8 would have been obvious based on the combination of Deeter and Tanaka, as I discuss above.

202. Meng discloses media for stem cell culture containing recombinant human serum albumin and transferrin, among other components. EX1054 (Meng),

5:17-27 (disclosing stem cell culture media containing “insulin, transferrin, sodium selenite, ethanolamine, serum albumin, linoleic acid, linolenic acid, retinol and α -tocopherol, which medium does not contain blood serum,” where the serum albumin may be rHSA).

203. Meng further discloses that in its stem cell culture media, the concentration of transferrin is 0.1 mg/L to 10 mg/L, while the concentration of albumin is “not less than 0.1 g/L,” which is 100 mg/L. EX1054 (Meng), [0022]-[0023]. Thus, Meng teaches that the ratio of the concentrations of transferrin to rHSA should be at least 1:10 to 1:1000, which overlaps with the range of claim 9.

204. Meng specifically discloses stem cell culture media comprising, among other components, 5.5 mg/L transferrin and 2.5 g/L rHSA (2,500 mg/L rHSA), i.e., a ratio of concentrations of 1 transferrin to 455 rHSA (2,500 divided by 5.5), which is within the broad range of claim 9. EX1054, 13:11-24.

205. I note that even if claim 9 were interpreted to refer to the ratio of molecules of transferrin to molecules of rHSA, the ratio of Meng would still be within the broad range of claim 9.⁸

⁸ The molecular weight of transferrin is about 79.5 kDa, while the molecular weight of rHSA is about 67 kDa. The ratio of molecules in Meng may be readily obtained by adjusting the ratio of concentrations for this difference in molecular weight. In

206. A POSA would have been motivated to use the ratio of transferrin to rHSA in Meng, as Meng's cell culture media promoted stem cell growth.

207. A POSA would have had a reasonable expectation of success in using Meng's ratio of transferrin to rHSA in cell culture media, as a POSA would reasonably expect to achieve comparable results in a composition containing recombinant transferrin instead of transferrin; e.g., the composition of Deeter in view of Tanaka (see above for claim 8).

XI. Claims 1-8 and 11-13 of the '951 Patent Would Have Been Obvious over Deeter in view of Van Urk

A. Claim 1

208. In my opinion, independent claim 1 would have been obvious over Deeter (EX1003) and Van Urk (EX1004), which collectively taught cell culture media comprising rHSA produced in a transgenic plant having less than 1 EU endotoxin per mg albumin and less than 2% aggregated albumin.

particular, transferrin has a molecular weight that is about 1.2 times greater than that of rHSA (79.5 kDa/67 kDa). Taking the molecular weights into consideration, the ratio of transferrin molecules to rHSA molecules is about 1:540, which is well within the range of claim 9 (1:50 to 1:5000).

1. “A cell culture media supplement or complete media composition”

209. Deeter discloses “cell culture media supplement,” i.e., “a combination of one or multiple cell culture media components ... for addition to cell culture media.” EX1003 (Deeter), [0068]. For example, Deeter disclosed using rHSA in cell culture media and determined that it is functionally equivalent to plasma-derived HSA. EX1003 (Deeter), [0118]–[0120] (“Example 8: Cell culture media supplemented with recombinant human serum albumin promotes hybridoma cell growth in reduced -serum media,” “Example 9: Cell culture media supplemented with recombinant human serum albumin promotes hybridoma cell growth in serum-free media”).

210. Van Urk further discloses that its rHSA may be used “in cell culture.” EX1004, 23:26-24:4 (disclosing that the “albumin of the present invention” may be used “in cell culture”); *id.*, 1:8-9 (disclosing that the term “albumin” refers to HSA and/or recombinant human serum albumin, abbreviated “rHA” in Van Urk). Van Urk also acknowledges that albumin and rHSA are “used to supplement media used for growing higher eukaryotic cells.” EX1004, 1:13-15.

211. A POSA would have understood that Deeter’s rHSA purified using the guidance of Van Urk would continue to be a cell culture media component, because removing endotoxins from Deeter’s rHSA would have been reasonably expected to improve (or at a minimum not reduce) the effectiveness of Deeter’s rHSA in cell

culture given the toxic nature of endotoxins. *See supra*, ¶¶ 28-30 (discussing endotoxins).

2. “a recombinant mammalian albumin”

212. Deeter discloses rHSA, which is a recombinant mammalian albumin, i.e., “a recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it) which has the amino acid sequence of a native mammalian albumin.”⁹ For example, Deeter’s rHSA is foreign to the transgenic rice producing it and has the amino acid sequence of a native mammalian (human) albumin. *See* EX1003 (Table 3), [0103], [0117]; *see also* EX1018, 5 (disclosing in 2007 that the amino acid sequence of Cellastim™ was identical to native HSA).

213. Deeter’s rHSA also satisfies Ventria’s proposed construction of recombinant mammalian albumin, i.e., it is “mammalian albumin having a

⁹ Under the adopted construction, recombinant mammalian albumin” means “recombinant albumin (i.e., albumin heterologous or foreign to the transgenic plant producing it) [1] which has the amino acid sequence of a native mammalian albumin *or* [2] which is a variant, derivative, fusion protein, or fragment of native mammalian albumin (provided the fragment retains the biological or therapeutically beneficial activity of the native mammalian albumin).” As Deeter’s rHSA meets the first part of the construction, I do not address the second option.

molecular weight between 66-69 kDa that is exogenous (i.e., heterologous or foreign) to the transgenic plant and retains the biological or therapeutically beneficial activity of its corresponding native mammalian albumin.” For example, Deeter’s rHSA has a molecular weight of 66.8-67.9 kDa and has the same physicochemical properties as the rHSA of the ’951 patent. *Compare* EX1003, Table 3 (page 31); Patent, Table E2 (col. 66). Deeter also discloses that its rHSA promotes cell growth “equivalently or better” than pHSA, indicating that it retains the biological or therapeutically beneficial activity of pHSA.

214. A POSA would have understood that Deeter’s rHSA purified using the guidance of Van Urk would continue to be a recombinant mammalian albumin.

3. “wherein said albumin is produced in a transgenic plant”

215. Deeter discloses rHSA produced in a transgenic plant, specifically, transgenic rice. EX1003, [0103]-[0105]; *supra*, ¶¶ 107-108, Fig. 7.

216. Van Urk discloses processes for purifying rHSA, including recombinant human albumin produced using transgenic plants. EX1004, 1:3-9. For example, Van Urk discloses that its rHSA “may be obtained from a transgenic plant, such as tobacco, potato, or corn (maize). EX1004, 22:25-27.

4. “has less than 1 EU endotoxin/mg albumin”

217. Deeter’s rHSA purified using the guidance of Tanaka would have reasonably been expected to contain less than 1 EU endotoxin/mg albumin, as recited in claim 1 of the ’951 patent.

218. As noted above, Van Urk’s purification process provides albumin that has no detectable endotoxin by the stringent *Limulus* assay, rendering the product “non-pyrogenic.” i.e., free of pyrogens including endotoxins. EX1004, 65, 72-73. Thus, Deeter’s rHSA purified using the guidance of Van Urk would have been reasonably expected to contain less than 1 EU endotoxin/mg albumin. *See infra*, Section XI.M.

5. “less than 2% aggregated albumin”

219. Deeter’s rHSA purified using the guidance of Van Urk would have been expected to contain less than 2% aggregated albumin, as recited in claim 1 of the ’951 patent.

220. As noted above, Van Urk’s purification process provides rHSA containing up to 1% albumin dimers and no larger polymers or aggregates. EX1004, 65. Thus, Deeter’s rHSA purified using the guidance of Van Urk would have been reasonably expected contain less than 2% aggregated albumin. *See infra*, Section XI.M (e.g., ¶¶ 247-50, 254).

B. Claim 2

221. Claim 2 depends from claim 1 and requires that the recombinant albumin comprises “at least 0.01% wt/wt of a heat shock protein.”

222. As I discuss in Section IX.B., Deeter’s rHSA necessarily contained substantially more than 0.01% wt/wt of a heat shock protein.

223. A POSA would have reasonably expected that Deeter’s rHSA purified using the guidance of Van Urk would retain heat shock proteins at levels well above 0.01% wt/wt, as I discuss in Section XI.N.

224. I conclude that Deeter in combination with Van Urk satisfies all the limitations of claim 2 of the ’951 patent.

C. Claim 3

225. Claim 3 of the ’951 patent depends from claim 2 and requires that the heat shock protein “is a rice heat shock protein.” Patent, claim 3.

226. As explained above for claim 2, the heat shock proteins naturally present in Deeter’s rHSA are rice heat shock proteins, and these rice heat shock proteins would have been expected to remain at levels above 0.01% wt/wt in Deeter’s rHSA after purification following the guidance of Van Urk.

D. Claim 4

227. Claim 4 of the ’951 patent depends from claim 2 and further requires that the heat shock protein is selected from the group consisting of “Rice HSP70 genes, and rice endosperm luminal binding proteins.” Patent, claim 4. These are the

rice Hsp 70 family members “found to occur” in Cellastim. Patent, 78:40-44, 78:47-50, 79:34-36, 79:7-11.

228. As I discuss for claim 2, these Hsp70 family members would necessarily have been present in Deeter’s Cellastim and would have reasonably been expected to remain at levels above 0.01% wt/wt after purification following the guidance of Van Urk. *Supra*, Section IX.B; Section IX.N.

E. Claim 5

229. Claim 5 depends from claim 2 and further requires that the heat shock protein be selected from the group consisting of Rice (gb1ACJ54890. 11), EEC69073/OsI_3 7938, and AAB63469. Again, these are the same Hsp 70 family members “found to occur” in Cellastim. Patent, 78:47-50, 79:34-36, 79:7-11. As discussed for claim 2, these heat shock proteins would have been necessarily present in Deeter’s Cellastim and would have reasonably been expected to remain at levels above 0.01% wt/wt after purification following the guidance of Van Urk.

F. Claim 6

230. Claim 5 depends from claim 2 and requires that “said supplement comprises at least 0.04% wt/wt HSP70.” As noted above, the three heat shock proteins “found to occur” in Cellastim are “Hsp70 proteins.” Patent, 75:46-48 (referring to “three Hsp70 proteins”); Patent, 78:47-50, 79:34-36, 79:7-11 (identifying Hsp 70 family members “found to occur” in Cellastim).

231. As discussed for claim 2, these heat shock proteins would have been necessarily present in Deeter's Cellastim would have reasonably been expected to remain at levels above 0.04% wt/wt after purification following the guidance of Van Urk.

G. Claim 7

232. Claim 7 depends from claim 2 and further requires that the composition comprise "a recombinant transferrin related protein, wherein said transferrin related protein is lactoferrin."

233. A POSA would have been motivated to include recombinant human lactoferrin in the cell culture media composition of claim 2, as taught by the combination of Deeter and Van Urk.

234. I detail at paragraphs 152-158 the motivation for including recombinant human lactoferrin in the cell culture media comprising Deeter's rHSA. I incorporate that discussion herein for brevity. In short, a POSA would have considered the replacement of animal-derived transferrin (used in Deeter in combination with rHSA) with recombinant human lactoferrin a routine and advantageous modification. A person of ordinary skill in the art would have had a reasonable expectation of success in making this simple substitution in view of the results in Deeter.

235. I conclude that claim 7 would have been obvious over Deeter in view of Van Urk.

H. Claim 8

236. Claim 8 depends from claim 2 and further requires “a recombinant transferrin related protein, wherein said transferrin related protein is transferrin.”

237. Deeter in combination with Van Urk satisfy the limitations of claim 2, as discussed above. In addition, a POSA would have been motivated to combine recombinant transferrin with the cell culture media supplement of claim 2.

238. Deeter discloses that rHSA promoted the growth of cells in culture when used in DMEM base medium supplemented with transferrin, among other components. EX1003, [0118]. Deeter also discloses that components of cell culture media such as transferrin are extracted from animal sources “have several undesirable features,” including the risk of contamination with pathogens. EX1003 (Deeter), [0006].

239. In view of the well-established disadvantages of animal-sourced proteins such as transferrin, a POSA would have been motivated to replace transferrin with recombinant human transferrin. A POSA would have considered recombinant transferrin a natural replacement for animal-derived transferrin, as the recombinant protein would have been expected to perform the same function as its natural counterpart.

240. A POSA would have had a reasonable expectation of success in making this simple substitution, as recombinant human transferrin was commercially available by 2008. *See* EX1059 (Grosvenor), 4.

I. Claim 11

241. Claim 11 depends from claim 1 and requires that the albumin “is recombinant human serum albumin.” As discussed above for claim 1, Deeter’s rHSA purified following the guidance of Van Urk is recombinant human serum albumin satisfying the requirements of claim 1.

J. Claim 12

242. Claim 12 depends from claim 1 and requires that the transgenic plant is a transgenic grain. As discussed above for claim 1, Deeter’s rHSA is produced in transgenic rice, which is a grain. I thus conclude that Deeter in view of Van Urk renders claim 12 obvious for the reasons stated above for claim 1.

K. Claim 13

243. Claim 13 depends from claim 1 and requires that the transgenic grain is transgenic rice. As discussed above for claim 1, Deeter’s rHSA is produced in transgenic rice, which is a grain. I thus conclude that Deeter in view of Van Urk renders claim 13 obvious for the reasons stated above for claim 1.

L. Motivation to Combine

244. I have discussed pertinent disclosures of Deeter in detail above at paragraphs 168-172 and for the sake of brevity, I incorporate that discussion here.

In brief, Deeter discloses producing rHSA in transgenic rice at high yield, thereby providing an advantageous and effective cell culture media supplement that avoids the safety risks associated with animal-derived albumin. Deeter's rHSA, however, had an undesirably high level of endotoxin, which would have been detected by and known to a POSA.

245. Accordingly, a POSA would have been motivated to reduce the endotoxin level in Deeter's rHSA and would have naturally looked to Van Urk for guidance, as Van Urk details a process for purifying rHSA, including rHSA produced in transgenic plants, to achieve a product with no detectable endotoxins. EX1004, 1:3-9, page 73.

246. Van Urk's process further provides rHSA beneficially containing up to 1% aggregated albumin, as detailed above. *Supra*, ¶¶ 66, 220. This added benefit would have further motivated a POSA to use to guidance of Van Urk to remove endotoxins from Deeter's rHSA.

M. Reasonable Expectation that Deeter's rHSA Purified Following the Guidance of Van Urk Would Have the Low Endotoxin and Aggregated Albumin Levels of Van Urk's rHSA

247. A POSA would have reasonably expected that Deeter's rHSA, as purified using the guidance of Van Urk, would contain "less than 1 EU endotoxin/mg albumin" and "less than 2% aggregated albumin," as recited in claim 1. First, Van Urk specifically taught that its process applies to purifying rHSA

produced in transgenic plants, which alone would have provided a skilled artisan with a reasonable expectation of success. EX1004 (Van Urk), 1:3–9, 22:26-27 (disclosing that the albumin may be obtained from a transgenic plant, such as tobacco, potato or corn (maize).”).

248. The '951 patent also acknowledges that techniques for purifying albumin rHSA produced in transgenic yeast (the preferred rHSA of Van Urk) also work to purify the albumin of the invention produced in a transgenic plant. Patent, 50:1-12. For example, the inventors acknowledged that “recognized” processes for purifying rHSA were expected to work for rHSA produced in a transgenic plant, as reflected by the passage below:

A search of patents, published patent applications, and related publications will provide those skilled in the art reading this disclosure with *significant possible methods for preparing and purifying albumin*. For example, U.S. Pat. Nos. 4,075,197; 4,086,222; 4,093,612; 4,097,473; 4,136,094; 4,228,154; 5,250,662; 5,656,729; 5,677,424; 5,710,253; 5,728,553; 5,994,507; 6,001,974; **6,638,740**; 6,617,133 and 7,423,124 disclose various processes for purifying albumin. In 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.

Patent, 50:1-12 (emphases added).

249. Notably, U.S. Patent No. 6,638,740 (“Goodey”)—cited in the ’951 patent passage above—describes processes for purifying albumin, as exemplified by the purification of rHSA produced in yeast, to achieve albumin with no detectable endotoxin and about 0.1% aggregated albumin. EX1046, Abstract, 3:55–62 (purified albumin “essentially free” of endotoxin, meaning “below detectable levels”), 6:8–16:34 (Examples 1-3 exemplifying production, purification, and formulation of recombinant albumin expressed in yeast), 15:12-16 (“The eluate from the DE-FF column has been found to have less than 0.1% (w/w) albumin dimer and an undetectable level of albumin polymers or aggregates as analysed by GP HPLC . . .”). As such, the ’951 patent admits that processes exemplified in rHSA produced in yeast were expected to work for rHSA produced in a transgenic plant.

250. In fact, a POSA would have considered the removal of endotoxins and aggregates from rHSA routine purification steps and would have had several techniques at their disposal to do so.

251. For example, a POSA would have reasonably expected to replicate the successful results in Van Urk using rHSA produced in transgenic rice, as the separation of rHSA from endotoxin does not depend on the source of the albumin. Albumin is albumin, whether produced in transgenic yeast, transgenic rice, or naturally expressed. The same scientific principles discussed in Section IX.M.

apply here with equal force. In the interest of brevity, I do not repeat that discussion here. Suffice it to say that rHSA, endotoxins, and aggregated albumin would all have been expected to behave similarly during purification—regardless of how the rHSA was produced.

252. It is notable that like Tanaka, Chang, Fisher, and Matejtschuk, Van Urk describes purifying albumin using anion exchange chromatography (DEAE-Sepharose). EX1004, 49-50 (Table 3), 52. Indeed, Demmer reported that ion exchange resins were “the most common depyrogenation method for proteins.” EX1026 (Demmer), 10.

253. As I explain in Section IX.M, chromatography techniques were well-established and routine in the art, and thus a person skilled in the art would have recognized that minor variations could have been made to known processes, including the processes disclosed in Van Urk, to optimize working conditions for rHSA purification. *Supra*, ¶¶ 187-191.

254. A POSA would have further reasonably expected Deeter’s rHSA purified using the guidance of Van Urk would contain less than 2% aggregated albumin, as Van Urk’s process provided about 99% monomeric albumin. EX1004, 65, 71-72. Albumin is albumin, whether naturally expressed or recombinantly produced in yeast or rice.

N. A POSA Would Have Reasonably Expected that Deeter's rHSA Purified by the Guidance of Van Urk Would Contain the Recited Heat Shock Protein

255. A POSA would have reasonably expected that Deeter's rHSA purified following the guidance of Van Urk would have retained at least 0.04% wt/wt of the rice Hsp70 proteins found to occur in the rHSA of Cellastim, as none of the purification steps in Van Urk would have been expected to substantially reduce the levels of heat shock proteins in Deeter's rHSA.

256. Van Urk disclosed purifying rHSA using, e.g., (1) ion exchange chromatography columns, (2) chromatography columns containing DBA (Delta Blue Agarose, also called "Delta Blue" matrix), (3) a chromatography column containing aminophenylboronate ("PBA"), and (4) filtration (specifically, ultrafiltration and diafiltration). EX1004 (Van Urk), 4, 13, 43. As discussed below, these purification steps would not have been reasonably expected to remove the rice heat shock protein 70 family members naturally present in Deeter's rHSA.

257. First, the ion exchange chromatography would not have been expected to remove heat shock proteins. A person skilled in the art would have known that rHSA and heat shock proteins carry about the same net charge, such that ion exchange chromatography (which separates proteins based on charge) would not have effectively removed them. EX1003 (Deeter), 30, Table 3 (pI of rHSA is 5.3); EX1034, 4 (Fig. 1) (pI of rice Hsp70 is about 5); *see also* Patent, 50:39-67.

258. Similarly, blue-dye-containing columns (e.g., Blue Sepharose and DBA) were reported to function as ionic exchange columns. EX1037, 1; EX1038, 6. As such, DBA would not have been expected to separate rHSA from rice Hsp70 proteins, as they would have about the same net charge in solution.

259. Next, Van Urk's PBA column would not have been expected to separate heat shock proteins from rHSA. As an initial matter, Van Urk expressly taught that its PBA column was intended to remove glycoconjugates, not heat shock proteins. EX1004 (Van Urk), 44, 52. PBA columns were designed to bind molecules with two adjacent hydroxy groups (diols). *See* EX1043, 55; EX1044. As such, a PBA column would bind molecules such as carbohydrates and glycoproteins (proteins with a carbohydrate modification), but not albumin or heat shock proteins in their unglycosylated form (which are proteins, polypeptides).

260. Finally, Van Urk's filtration steps would not have been expected to remove most heat shock proteins from rHSA. Van Urk disclosed various ultrafiltration and diafiltration steps using membranes that separate out species smaller than 10,000 kDa or 30,000 kDa. EX1004 (Van Urk), 51, 53. These steps would not have removed any heat shock proteins larger than 10 kDa or 30 kDa, e.g., members of the Hsp70 family of proteins.

261. The expectation that Van Urk's purification process would not remove heat shock proteins would have been bolstered by their "sticky" nature. Hsp70 in

particular tends to bind “somewhat non-specifically to hydrophobic peptides,” which allows Hsp70 to act on a broad range of substrates, e.g., rHSA. EX1032, 1; EX1041, 2, 6. For example, Hsp70 prevents protein aggregation and promotes protein refolding. EX1032, 1. As Sharma explains, “Hsp70 is important for helping proteins adopt and maintain native conformations from the time they are synthesized until they are eventually degraded.” EX1032, 6. Heat shock proteins such as Hsp70 would have thus been expected to remain bound to rHSA after the routine purification steps employed by Van Urk.

262. Given their “sticky” nature, researchers in the field historically isolated heat shock proteins using columns containing ATP Agarose affinity chromatography resin, a resin that specifically binds to heat shock proteins. *See* EX1039, 9; EX1040, 2-3; EX1041, 2; EX1031, 4, 5. Nothing in Van Urk described using ATP Agarose affinity chromatography resin in its rHSA purification process. Accordingly, a skilled person’s general knowledge about difficulties with removing heat shock proteins would have reinforced the expectation that Van Urk’s process would not have removed heat shock proteins from Deeter’s rHSA preparation.

XII. Claim 9 of the ’951 Patent Would Have Been Obvious over Deeter in view of Van Urk and Meng

263. Claim 9 depends from claim 8 and further requires that the “ratio of said transferrin to said recombinant albumin is 1:50 to 1:5000.”

264. Claim 8 would have been obvious based on the combination of Deeter and Van Urk, as I discuss above.

265. Meng discloses that for stem cell culture media, the appropriate ratio of transferrin to rHSA should be at least 1:10 to 1:1000. EX1054 (Meng), [0022]-[0023] (disclosing that the concentration of transferrin is 0.1 mg/L to 10 mg/L, while the concentration of albumin is “not less than 0.1 g/L,” which is 100 mg/L). For example, Meng specifically discloses stem cell culture media in which the ratio of transferrin to rHSA is 1:455, which is within the range of claim 9. EX1054, 13:11-24 (disclosing media comprising 5.5 mg/L transferrin and 2.5 g/L rHSA (2,500 mg/L rHSA), where 2,500 divided by 5.5 is 455). As discussed above, even if claim 9 were interpreted to refer to the ratio of molecules of transferrin to molecules of rHSA, the ratio of Meng would still be within the broad range of claim 9.

266. A POSA would have been motivated to use the ratio of transferrin to rHSA in Meng, as Meng’s cell culture media promoted stem cell growth.

267. A POSA would have had a reasonable expectation of success in using Meng’s ratio of transferrin to rHSA in cell culture media, as a POSA would reasonably expect to achieve comparable results in a composition containing recombinant transferrin instead of transferrin; e.g., the composition of Deeter in view of Van Urk (see above for claim 8).

XIII. Secondary Considerations of Nonobviousness

A. Introduction

268. I am informed that secondary considerations refer to real-world evidence concerning obviousness or nonobviousness. 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.

269. 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.

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

271. Based on the '951 patent and the state of the prior art, I provide a few comments on secondary considerations below.

B. No Unexpected Results

272. In my opinion, the '951 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 2009 or 2010, which I believe would have been prior art albumin compositions, including Deeter, Tanaka, Van Urk, and Meng.

273. For example, a POSA would have reasonably expected to obtain cell culture media supplements comprising rHSA produced in transgenic rice having less than 1 EU endotoxin/mg albumin and less than 2% aggregated albumin, for all the reasons discussed above. *E.g., supra*, ¶¶ 28-34; *supra*, Sections IX.M, XI.M.

274. Additionally, I disagree with the statements in the '951 patent indicating that “surprising” results were achieved. For example, the '951 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.” Patent, 31:37-41.
- “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.” Patent, 31:41-44.
- “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.” Patent, 92:64-67.

- “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.” Patent, 94:35-38.

275. Combinations of proteins, including recombinant proteins, are routinely used in cell culture, along with other components such as insulin. 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.

276. Moreover, the prior art taught that it was advantageous to combine rHSA with other recombinantly produced proteins such as transferrin. EX1013, 27:19-21 (“it is advantageous to combine recombinantly produced albumin with some or all of the following compounds when they are produced recombinantly or naturally by microbial systems: insulin, transferrin, IGF1, EDF or other proteins, growth factors and metabolites.”). As a result, a POSA would have expected to obtain advantageous results using the claimed combinations of rHSA and recombinant transferrin-related proteins such as lactoferrin and transferrin.

277. As another example, Foghsgaard reports culturing cells in DMEM/F12 base media supplemented with (1) insulin, transferrin, and selenium (commonly abbreviated ITS) and (2) rHSA, bovine serum albumin (probumin), or

no albumin, and discloses that the media supplemented with rHSA had superior growth. EX1010, 29:14-20, Fig. 1 (page 34); *see also, e.g.*, EX1045, 2 (reporting results of cell culture media supplemented with rHSA, transferrin, and insulin); EX1003, [0118]-[0120] (reporting results of cell culture media supplemented with rHSA and transferrin); *see also* EX1006 (reporting that rHSA/Cellastim “outperforms” other chemically defined cell culture components and recombinant human lactoferrin, Lacromin, was a “strong growth factor”).

278. Moreover, the prior art taught that it was advantageous to combine rHSA with other recombinantly produced proteins such as transferrin. EX1013, 27:19-21 (“it is advantageous to combine recombinantly produced albumin with some or all of the following compounds when they are produced recombinantly or naturally by microbial systems: insulin, transferrin, IGF1, EDF or other proteins, growth factors and metabolites.”). As a result, a POSA would have expected to obtain advantageous results using the claimed combinations of rHSA and recombinant transferrin-related proteins such as lactoferrin and transferrin.

279. Next, the '951 patent states that “[s]urprisingly, despite the increased product purity, these changes in processing also resulted in enhanced co-purification of heat shock proteins, (see below) with the recombinant albumin.” Patent, 73:50-53. The patent does not provide any data to support this assertion,

and as discussed above, Deeter's prior art rHSA naturally contained the same Hsp70 proteins found to occur in Cellastim.

XIV. Conclusion

280. 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: 3/17/22

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

Hohenschuh 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, Castelvechio 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

1. P.A. Grieco, D.S. Clark, and G.P. Withers, "Direct Conversion of Carboxylic Acids into Amides", J. Org. Chem. **44**, 2945 (1979).

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).
13. E.J. Fernandez, F.B. Fernandez, R.B. Jagoda, and D.S. Clark, "EPR Spectroscopy Studies of Immobilized Monoclonal Antibody Structure and Function", in *Separation, Recovery, and Purification in Biotechnology: Recent Advances in Mathematical Modeling*, J.A. Asenjo and J. Hong, Eds., *ACS Symposium Series*, **314**, 208 (1986).
14. 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", *Ann. N.Y. Acad. Sci.*, **501**, 80 (1987).
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16. E.J. Fernandez and D.S. Clark, "NMR Spectroscopy: A Noninvasive Tool for Studying Intracellular Processes", *Enzyme Microb. Technol.*, **9**, 259 (1987).
17. P.S. Skerker, E.J. Fernandez, R.B. Jagoda, and D.S. Clark, "Spectroscopic Studies of Structure-Function Relationships in Free and Immobilized Alcohol Dehydrogenase", *Ann. N.Y. Acad. Sci.*, **506**, 117 (1987).
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26. J.F. Miller, N.N. Shah, C.M. Nelson, J.M. Ludlow, and D.S. Clark, "Pressure-Temperature Effects on the Growth and Methane Production of the Extreme Thermophile Methanococcus jannaschii," *Appl. Environ. Microbiol.*, **54**, 3039 (1988).
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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).
32. E.J. Fernandez, A. Mancuso, H.W. Blanch, and D.S. Clark, "Nuclear Magnetic Resonance Methods for Observing the Intracellular Environment of Mammalian Cells," Ann. N.Y. Acad. Sci., **589**, 458 (1990).
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).
39. D.S. Clark, "Noninvasive Techniques in Studies of Immobilized Cells," in *Physiology of Immobilized Cells* (Elsevier Science Publishing Co., New York, 1990).
40. R.M. Guinn, M. Fernandez, H.W. Blanch, and D.S. Clark, "Effect of a Water-Miscible Organic Solvent on the Kinetic and Structural Properties of Trypsin," Enzyme Microb. Technol., **13**, 320 (1991).

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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Complex Peptide Surfaces for Human Pluripotent Stem Cell Culture,” ACS Biomaterials Science & Engineering, **7**(4), 1344 (2021).

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285. P. Yang, D.S. Clark, O.M. Yaghi, “Envisioning the ‘Air Economy’ – Powered by Reticular Chemistry and Sunlight for Clean Air, Clean Energy, and Clean Water,” Molecular Frontiers Journal, **5** (1), 1 (2021). <https://doi.org/10.1142/S2529732521400046>

Submitted:

- S-1. A.J. Abel, J.M. Hilzinger, A.P Arkin, D.S. Clark, “Systems-informed genome mining for electroautotrophic microbial production,” Bioelectrochemistry (submitted).
- S-2. A.J. Abel, J.D. Adams, D.S. Clark, “A comparative life cycle analysis of electromicrobial production systems,” Energy & Environmental Science (submitted).

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).
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).

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," Castelveccchio 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. Khmelnitsky, 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

"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.

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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.

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Stauffer Chemical Company, Richmond, California, January 1987.

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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,"
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Appendix B

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Exhibit	Description
1001	U.S. Patent No. 10,618,951, issued April 14, 2020 (“Patent”).
1003	WO 2007/002762 A2 (“Deeter”), published Jan. 4, 2007.
1004	WO 2000/44772 A2 (“Van Urk”), published Aug. 3, 2000.
1005	P. Matejtschuk, et al., <i>Production of Human Albumin Solution: a Continually Developing Colloid</i> , 85 BR. J. ANAESTH., 887-95 (2000) (“Matejtschuk”).
1006	InVitria’s Cellastim Delivers Superior Results in Mammalian Cell Culture, 25 Sept. 2008, web archive (“InVitria 1 st Press Release”).
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1027	U.S. Patent No. 5,917,022 (“Davies”), issued Jun. 29, 2000.
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1064	T. Novitsky, <i>BET v. PT Non-Endotoxin Pyrogens</i> , LAL Update, Associates of Cape Cod, Incorporated, April 2002, Vol. 20, No. 2, 1-4
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