



**Charles River Laboratories, Inc.
Petitioner,
v.
Seikagaku Corporation
Patent Owner.**

**Case No. IPR2025-00440
U.S. Patent No. 11,236,318
Seikagaku Ex. 2109**

**Seikagaku Trial Demonstratives
June 9, 2026**

Petitioner Has Not Established Any Ground of Unpatentability

I. Claim 21 is Entitled to a December 10, 2013 Priority Date

II. Petitioner Does Not Meet its Burden to Demonstrate Unpatentability

III. Unexpected Results of Non-Obviousness Exist

IV. Contingent Motion to Amend

I. Claim 21 is Entitled to a December 10, 2013 Priority Date

la.

The '767 Application Provides Written Description Support for Claim 21

“The ‘767 Application **does not disclose any example** (prophetic or experimental) of any recombinant protein from *Limulus polyphemus* (i.e., the American horseshoe crab), let alone any such protein with claim 21’s functional requirement.”



“[T]he absence of examples . . . **does not** render the written description inadequate” and “actual reduction to practice is **not required** for written description

Falko-Gunter Falkner v. Inglis, 448 F.3d 1357, 1366 (Fed. Cir. 2006) (emphasis added)



“[A] patentee ***may rely on information that is ‘well-known in the art’*** for purposes of meeting the written description requirement” because “the specification is viewed from the perspective of one of skill” in the relevant art.

Bos. Sci. Corp. v. Johnson & Johnson, 647 F.3d 1353, 1366 (Fed. Cir. 2011) (emphasis added)

Challenged Claim 21 of the '318 Patent



US011236318B2

(12) **United States Patent**
Mizumura et al.

(10) **Patent No.:** US 11,236,318 B2
(45) **Date of Patent:** *Feb. 1, 2022

(54) **RECOMBINANT FACTOR C AND METHOD
FOR PRODUCING THE SAME, AND
METHOD FOR MEASURING ENDOTOXIN**

10,144,923	B2 *	12/2018	Mizumura		C12N 9/6408
2002/0068325	A1 *	6/2002	Ng		C12Y 302/01096
					435/69.1
2003/0054432	A1	3/2003	Chen et al.		
2004/0235080	A1	11/2004	Chen et al.		
2008/0131929	A1	6/2008	Prentice et al.		
2009/0208995	A1	8/2009	Tamura et al.		

(71) Applicant: **SEIKAGAKU CORPORATION,**
Tokyo (JP)

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 16/399,386

(22) Filed: Apr. 30, 2019

(65) **Prior Publication Data**

US 2019/0270977 A1 Sep. 5, 2019

SG 94673 A1 3/2003
WO 9915676 A1 4/1999
WO 2008/004674 A1 1/2008
WO 2012091124 A1 7/2012
WO 2012/118226 A1 9/2012

OTHER PUBLICATIONS

Bram Blomme et al., "Alteration of protein glycosylation in liver diseases", Journal of Hepatology, 2009, vol. 50, pp. 592-603 (12 pages total).

Callaghan et al., "Effect of protein glycosylation on the stability of protein-ligand complexes", J. Biol. Chem., 2009, vol. 284, pp. 16201-16208 (8 pages total).

pages total).

Office Action dated Feb. 12, 2020 issued in U.S. Appl. No. 15/983,725. Stephanie Holst et al., "Profiling of different pancreatic cancer cells used as models for metastatic behaviour shows large variation in their N-glycosylation" Scientific Reports, Nov. 30, 2017, vol. 7, No. 16623, pp. 1-15 (15 pages total). Communication dated Apr. 19, 2017, from the State Intellectual Property Office of the P.R.C., in counterpart Chinese application No. 201380064649.3.

Related U.S. Application Data

(63) Continuation of application No. 15/983,725, filed on May 18, 2018, now Pat. No. 10,982,202, which is a continuation of application No. 14/650,767, filed as application No. PCT/JP2013/083082 on Dec. 10, 2013, now Pat. No. 10,144,923.

C12N 15/85 (2006.01)
G01N 33/579 (2006.01)

in counterpart European Application No. 13862466.3.
(Continued)

(52) **U.S. CL.**
CPC: C12N 9/6408 (2013.01); C12Y 302/01096 (2013.01); C12Y 304/21069 (2013.01); G01N 33/56911 (2013.01); G01N 33/579 (2013.01); G01N 2333/96411 (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,712,144 A 1/1998 Ding et al.
5,716,834 A 2/1998 Ding et al.
5,795,962 A 8/1998 Iwanaga et al.
5,840,510 A 11/1998 Tanaka et al.
5,858,706 A 1/1999 Ding et al.
6,077,946 A 1/2000 Iwanaga et al.
6,645,724 B1 11/2003 Ding et al.

(57) **ABSTRACT**

A horseshoe crab Factor C protein having activity of Factor C, wherein the horseshoe crab is selected from *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary (CHO) DG44 cell or HEK cell.

21 Claims, 10 Drawing Sheets
Specification includes a Sequence Listing.

21. A horseshoe crab Factor C protein having activity of Factor C,
wherein the horseshoe crab is *Limulus polyphemus*, and
wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a **Human Embryonic Kidney (HEK) 293 cell**, and
wherein the Factor C protein exhibits a **residual activity of at least one** selected from the group consisting of:
(1) 10% or higher in the presence of 21 mM sodium citrate; and
(2) 15% or higher in the presence of 16 mM magnesium sulfate.

The '767 Application Provides Written Description Support for Claim 21

The '767 Application identifies the claimed *Limulus polyphemus* species and expression in HEK293 cells:

Modes for Carrying Out the Invention

[0019]

(1) Factor C of the present invention

The Factor C of the present invention is a Factor C derived from a horseshoe crab. The Factor C of the present invention is a recombinant protein. The term "recombinant protein" refers to a protein obtained through introduction of a gene encoding the protein into a host cell and heterologous expression of the gene. The Factor C of the present invention has activity of Factor C.

[0020]

Examples of the horseshoe crab include *Tachypleus tridentatus* (Japanese horseshoe crab), *Limulus polyphemus* (American horseshoe crab), *Carcinoscorpius rotundicauda*

(3) Expression of Factor C in mammalian cells (HEK293)

In this section, Factor C was prepared by use of HEK293, which is a human embryo kidney cell line, as a host through the following procedure.

Petitioner's Admissions Before the European Patent Office

Petitioner admitted the '767 Application discloses recombinant Factor C protein derived from *Limulus polyphemus*:

51. In that regard, we refer to US Application No. 14/650,767, which corresponds to D1 and contains the same description as this disclosure. In the US case, the Proprietor sought to obtain a claim directed to a recombinant Factor C which is a protein having an identity of 80% or higher with respect to the *Tachypleus tridentatus* Factor C, or the *Carcinoscorpius rotundicauda* Factor C (therein SEQ ID NO 2 and 4, respectively) (see claim 77 corresponding to the remarks submitted in **D3**, submitted herewith as **D3b**). It is noted that this percent identity encompasses the Factor

155. However, this technical effect should be disregarded because it is not based on the true difference. D1 already discloses "a recombinant Factor C protein derived from Limulus polyphemus (LFC)". The only difference compared to this disclosure is the explicit sequence of LFC, but not recombinant LFC itself. In that regard it should also be noted that the applicant

Petitioner's Admissions Before the European Patent Office

Petitioner admitted the '767 Application directs a POSA to recombinantly produce *Limulus polyphemus* Factor C:

30. In particular, each one of D1, D5, and D6 discloses methods for recombinantly producing Factor C, comprising a step of artificially expressing the protein and collecting the expressed recombinant protein as a solution fraction which is a culture solution or culture supernatant; and directly points the skilled person to carry out this method with a Factor C derived from *Limulus polyphemus* species. Each of these documents could equally serve as closest prior art.

Petitioner's Argument Contradicts Positions it Took Before the European Patent Office

Petitioner admitted that determining the sequence of a known protein is “routine practice”:

to carry out its recombinant production. However, and as noted by the Examiner during prosecution, this was a well-known, publicly available protein, the specific amino acid sequence (as defined in items (A)-(C)) “being an inherent property of the protein” (cf., item 1.2.1 of the ESO, submitted herewith as D7). Accordingly, it would have merely been a matter of routine analysis to obtain the sequences recited in feature (ii.b) of claim 1. Thus, to the extent that reciting the

56. Moreover, it was common general knowledge that the *Limulus* Factor C had been used for decades as the first component of the LAL assay for testing pharmaceuticals, owing to its extreme sensitivity to bacterial endotoxin. In that regard, we refer to the following disclosure from D4:

59. Deriving the solution to this problem (i.e., one of the sequences recited in claim 1) does not require any inventive skill. As we have already noted above, it is a matter of routine practice for a person skilled in the art to determine the sequence of a known protein.

University of Connecticut sequenced and deposited a 188 Mb Limulus polyphemus cDNA amoebocyte transcriptome:

Limulus polyphemus (Atlantic horseshoe crab)

Accession: PRJNA178588

Limulus polyphemus Transcriptome or Gene expression

Normalized 454 cDNA amoebocyte transcriptome. An individual sampled from coastal waters near Cape Cod (in Woods Hole, MA).

Accession	PRJNA178588
Data Type	Transcriptome or Gene expression
Scope	Monoisolate
Organism	Limulus polyphemus [Taxonomy ID: 6850] Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Merostomata; Xiphosura; Limulidae; Limulus; Limulus polyphemus
Submission	Registration date: 31-Oct-2012 - University of Connecticut - University of Connecticut Center for Applied Genetics and Technology

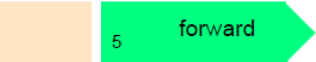
Design: Normalized polyA selected RNA using Evrogen Trimmer Kit and used broken T primer approach for cDNA construction and amplification.

Submitted by: UNIVERSITY OF CONNECTICUT

Study: **Limulus polyphemus** Transcriptome or Gene expression
[PRJNA178588](#) • [SRP016911](#) • [All experiments](#) • [All runs](#)
[hide Abstract](#)
Normalized 454 cDNA amoebocyte transcriptome. An individual sampled from coastal waters near Cape Cod (in Woods Hole, MA).

Sample: Horseshoe Crab Transcriptome
[SAMN01797697](#) • SRS373647 • [All experiments](#) • [All runs](#)
Organism: [Limulus polyphemus](#)

Library:
Instrument: 454 GS FLX Titanium
Strategy: WGS
Source: TRANSCRIPTOMIC
Selection: cDNA
Layout: SINGLE

Spot descriptor:


Runs: 1 run, 772,309 spots, 188.2M bases, [131.6Mb](#)

Run	# of Spots	# of Bases	Size	Published
SRR610305	772,309	188.2M	131.6Mb	2013-10-31

University of Connecticut sequenced and deposited a 4Gb shotgun sequence of *Limulus polyphemus* genome:

Limulus polyphemus (Atlantic horseshoe crab)
Limulus polyphemus Genome sequencing
Shotgun and Pair-End 454 sequencing of genomic dna. Individual samples sampled from Long Island Sound, USA.

Accession	PRJNA178587
Data Type	Genome sequencing
Scope	Monoisolate
Organism	Limulus polyphemus [Taxonomy ID: 6850] Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Merostomata; Xiphosura; Limulidae; Limulus; Limulus polyphemus
Submission	Registration date: 31-Oct-2012 - University of Connecticut - University of Connecticut Center for Applied Genetics and Technology

Design: Published protocols for 454 paired-end 3KB sequencing
Submitted by: UNIVERSITY OF CONNECTICUT
Study: **Limulus polyphemus Genome sequencing**
[PRJNA178587](#) • [SRP016906](#) • [All experiments](#) • [All runs](#)
[show Abstract](#)
Sample: Genome Reads
[SAMN01797690](#) • SRS373640 • [All experiments](#) • [All runs](#)
Organism: [Limulus polyphemus](#)
Library:
Instrument: 454 GS FLX Titanium
Strategy: WGS
Source: GENOMIC
Selection: RANDOM
Layout: PAIRED
Spot descriptor:

Runs: 1 run, 2.6M spots, 1.1G bases, [2.6Gb](#)

Run	# of Spots	# of Bases	Size	Published
SRR610297	2,581,149	1.1G	2.6Gb	2013-10-31

A POSA Could Have Obtained the cDNA and Amino Acid Sequences of *L. Polyphemus* Factor C in 2013



Patent Owner's Expert
Dr. Brian Schaefer

reads, using the full protein sequences from *Tachypleus tridentatus* Factor C. I confirmed that 97.2% of the full Factor C coding sequence could be assembled from the transcriptome deposit (a segment of 29 amino acids from residues 516-543 was not covered by any sequence reads in the BLAST alignment). However, this sequence could be easily recovered by standard PCR of *Limulus polyphemus* cDNA using primers complementary to sequences from the reads on each side of this gap; specifically, using reads SRR610305.117350.2 at the 5' end of the gap and SRR610305.219525.2 at the 3' end of the gap. Also, final validation of the assembled complete sequences for Factor C would be performed by PCR and sequencing of the entire ORF for Factor C. Design of the required primers would be readily enabled by analysis of the BioProject sequencing data, as described above.

A POSA Could Have Obtained the cDNA Sequences of *L. Polyphemus* Factor C in 2013



Patent Owner's Expert
Dr. Brian Schaefer

73. It is further my opinion that the sequence of *Limulus polyphemus* Factor C could also have been obtained by at least December 10, 2013 using a RACE method based on the sequence information in the '767 Application or other publications describing the *Tachypleus tridentatus* sequences, in combination with the amino acid sequences from *Limulus polyphemus*. EX1022; EX2077; EX2078.

I agree with Prof. Frohman that success does not depend on the specific protocol or kit used. EX2004 ¶¶34-37.

Challenged Claim 21 of the '318 Patent



US011236318B2

(12) **United States Patent**
Mizumura et al.

(10) **Patent No.:** US 11,236,318 B2
(45) **Date of Patent:** *Feb. 1, 2022

(54) **RECOMBINANT FACTOR C AND METHOD
FOR PRODUCING THE SAME, AND
METHOD FOR MEASURING ENDOTOXIN**

10,144,923	B2 *	12/2018	Mizumura		C12N 9/6408
2002/0068325	A1 *	6/2002	Ng		C12Y 302/01096
					435/69.1
2003/0054432	A1	3/2003	Chen et al.		
2004/0235080	A1	11/2004	Chen et al.		
2008/0131929	A1	6/2008	Prentice et al.		
2009/0208995	A1	8/2009	Tamura et al.		

(71) Applicant: **SEIKAGAKU CORPORATION,**
Tokyo (JP)

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 16/399,386

(22) Filed: Apr. 30, 2019

(65) **Prior Publication Data**

US 2019/0270977 A1 Sep. 5, 2019

SG 94673 A1 3/2003
WO 9915676 A1 4/1999
WO 2008/004674 A1 1/2008
WO 2012/091124 A1 7/2012
WO 2012/118226 A1 9/2012

OTHER PUBLICATIONS

Bram Blomme et al., "Alteration of protein glycosylation in liver diseases", Journal of Hepatology, 2009, vol. 50, pp. 592-603 (12 pages total).

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Office Action dated Feb. 12, 2020 issued in U.S. Appl. No. 15/983,725.

Stephanie Holst et al., "Profiling of different pancreatic cancer cells used as models for metastatic behaviour shows large variation in their N-glycosylation" Scientific Reports, Nov. 30, 2017, vol. 7, No. 16623, pp. 1-15 (15 pages total).

Communication dated Apr. 19, 2017, from the State Intellectual Property Office of the P.R.C., in counterpart Chinese application No. 201380064649.3.

in counterpart European Application No. 13862466.3.
(Continued)

Related U.S. Application Data

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C12N 15/85 (2006.01)
G01N 33/579 (2006.01)

(52) **U.S. CL.**
CPC: C12N 9/6408 (2013.01); C12Y 302/01096 (2013.01); C12Y 302/01096 (2013.01); G01N 33/56911 (2013.01); G01N 33/579 (2013.01); G01N 2333/96411 (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,712,144 A 1/1998 Ding et al.
5,716,834 A 2/1998 Ding et al.
5,795,962 A 8/1998 Iwanaga et al.
5,840,510 A 11/1998 Tanaka et al.
5,858,706 A 1/1999 Ding et al.
6,077,946 A 1/2000 Iwanaga et al.
6,645,724 B1 11/2003 Ding et al.

Primary Examiner — David Stendman
(74) Attorney, Agent, or Firm — Sughrue Mion, PLLC

(57) ABSTRACT

A horseshoe crab Factor C protein having activity of Factor C, wherein the horseshoe crab is selected from *Tachyplesus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary (CHO) DG44 cell or HEK cell.

21 Claims, 10 Drawing Sheets
Specification includes a Sequence Listing.

21. A horseshoe crab Factor C protein having activity of Factor C,

wherein the horseshoe crab is *Limulus polyphemus*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell, and wherein the Factor C protein exhibits a residual activity of at least one selected from the group consisting of:

- (1) 10% or higher in the presence of 21 mM sodium citrate; and
- (2) 15% or higher in the presence of 16 mM magnesium sulfate.

The '767 Application Provides Written Description Support for Claim 21

The '767 Application describes the claimed threshold residual activity values:

[0035]

The Factor C of the present invention may have low susceptibility to inhibition of activity by an ion. The

as compared with the control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" further include such a property that the Factor C of the present invention exhibits a residual activity of 10% or higher or 20% or higher in the presence of 21 mM sodium citrate, as compared with the control Factor C; such a

chloride, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a residual activity of 15% or higher or 25% or higher in the presence of 16 mM magnesium sulfate, as compared with the control Factor C; and such a property that the Factor C of

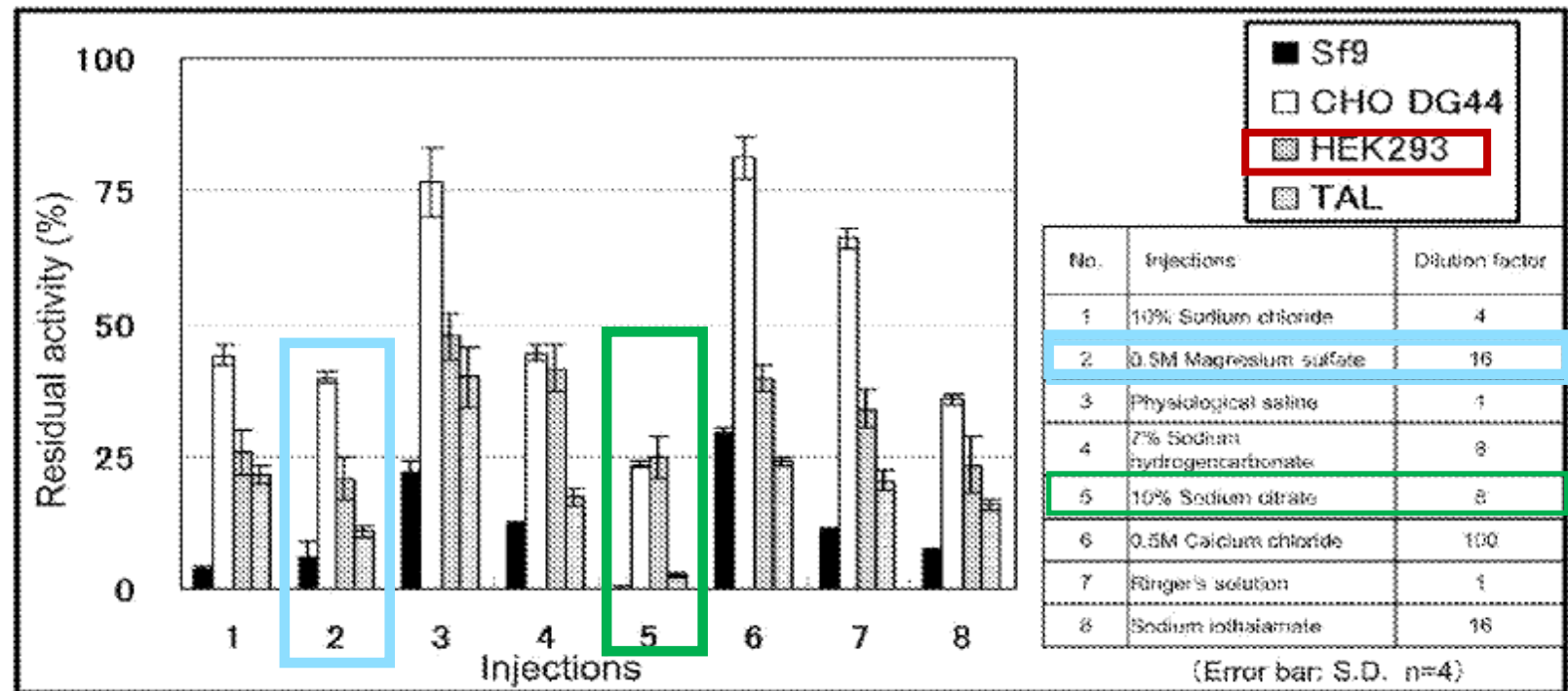
The '767 Application Provides Written Description Support for Claim 21

The '767 Application demonstrates the claimed residual activity in the claimed salt solutions for *Tachypleus tridentatus* Factor C:

[Fig. 7]

Claim 21 requires "at least one":

- 10% or higher in 21 mM sodium citrate;
- 15% or higher in 16 mM magnesium sulfate



“A POSA in 2013 would have understood that ***knowledge of a protein sequence giving rise to a particular function for one species*** (i.e., *Tachypleus tridentatus*) ***did not equate to possession of a protein sequence with a comparable function from a different, uncharacterized, species*** (i.e., *Limulus polyphemus*), let alone possession of such protein across an exceedingly wide range of residual activity function.”

Petitioner's Argument Contradicts Positions it Took Before the European Patent Office

Petitioner admitted that the functional property may be “expected” for recombinantly produced Factor C from any source, including *Limulus polyphemus*:

polyphemus). Given that Factor C sequences from the Genus *Tachypleus* are disclosed in **D1**, **D5** and **D6**, the skilled person would have had a high incentive to also obtain a recombinant Factor C of the only other genus, i.e., *Limulus*. Paragraph [0150]-[0151] of **D1** explicitly prompt the skilled person towards recombinant expression of Factor C, including from *Limulus*, and so the skilled person would naturally have been interested to obtain the *Limulus* Factor C and test its properties. In particular, **D1** also highlights that “Factor C expressed in mammalian cells can have low susceptibility to activity inhibition by ions” (paragraph [271]), and that this property may be expected for a recombinantly produced Factor C from any source including *Limulus*, see paragraphs [0166] and [0232] of **D1**, referring to “[t]he Factor C of the present invention” (see also paragraph 160 of our Reply).

A POSA Would Have Expected Functional Properties to be Highly Conserved Among Species



Patent Owner's Expert
Dr. Brian Schaefer

As discussed above, it was well-known that horseshoe crabs evolve slowly. See EX2044 at 5 (“unusually low divergence rates in horseshoe crabs may be the relatively long generation time... [which] are expected to produce slower evolutionary rates.”). The coagulation cascade is a key component of the horseshoe crab innate immune defense system, which is critical for survival. EX2070 at 11. It is widely understood that genes that are critical for survival are generally protected from functionally detrimental mutations by “purifying selection.” See, e.g., EX2078 at 6. In other words, the effect of purifying selection is to slow the rate of mutation of essential genes and functional regions, such as the genes of the horseshoe crab clotting cascade. The expectation would thus be that key regions encoding functional units of each enzyme and motifs for interactions between partner proteins would be highly conserved across species, due to their required function for survival of individuals and the species.

A POSA Would Have Expected Functional Properties to be Highly Conserved Among Species



Patent Owner's Expert
Dr. Brian Schaefer

92. As a POSA in December 2013 could have and would have done, I compared the amino acid sequences of *Limulus polyphemus* Factor C with the *Carcinoscorpius rotundicauda* sequence and the *Tachyplesus tridentatus* sequence disclosed in the '767 Application. My goal was to identify residues in the *Limulus polyphemus* sequence that are non-conserved with regard to both other species. I used the Clustal algorithm to identify fully conserved residues, conservation between groups of strongly similar properties, conservation between groups of weakly similar properties, and non-conserved residues.⁸

93. My analysis is found in EX2081. It indicates that out of 1020 amino acids, there were only 11 non-conservative substitutions found in neither *Carcinoscorpius rotundicauda* or *Tachyplesus tridentatus* Factor C. In other words, the *Limulus* Factor C is 98.9% similar to the other two Factor C proteins, when considered together. My analysis shows that these proteins are highly similar overall.

A POSA Would Have Expected Functional Properties to be Highly Conserved Among Species



Patent Owner's Expert
Dr. Brian Schaefer

97. Of the 11 non-conservative substitutions, only five are within identified conserved domains (1/159= 0.6% in the PHA02927/secreted complement-binding protein domain; 2/129= 1.6% in the CLECT domain; 2/255= 0.78% in the serine protease domain), meaning that the functional domains are very highly conserved between species. A graphical depiction of the Clustal alignment for the three Factor C proteins, with conserved domains and non-conservative substitutions is provided below and in EX2103.

“PO’s contention that sequencing *L. polyphemus* Factor C was ‘routine’ is undermined by its own arguments [in the ‘498 PCT].”

Petitioner's Admissions Before the European Patent Office

Petitioner argued statements in the '498 PCT were “unsupported” and “factually incorrect”:

34. According to the opposed patent, the sequence of the *Limulus* Factor C had yet to be determined, allegedly “because accurate determination of the full-length sequence structures and elucidation of the expression and functions of these proteins and genes could not be achieved by general techniques due to various differences in biomolecules, diversities, etc. because of differences in genera or species of horseshoe crabs” (par. [0012]) (emphasis added).
35. However, this statement is very generic and entirely unsupported². Indeed, and as Professor Frohman has confirmed, the example in the opposed patent merely demonstrates the use of standard RACE techniques, which were available well before the priority date, to determine the claimed Factor C sequences (D8, section VII).

² In fact, Alwis & Milton (Am. J. Ind. Med., 2006 Apr, 49(4):296-30, submitted herewith as D32) discloses on page 299, right col., first par. that the factor C sequence of *Carcinoscorpius rotundicauda* was already determined by 2006 by the inventor named on D6, Lin Chen (although not published, perhaps for commercial reasons) and compared with the factor C sequence of *Limulus polyphemus*. D32 indicates a precise homology of 89.3% and therefore suggests that the entire sequence was accurately determined. This further supports our position that the patent's claim that the sequence could not previously be determined is factually incorrect and that there were no unforeseen technical difficulties in determining the sequence.

Ib. The '767 Application Enables the Full Scope of Claim 21

Claim 21 is Not Broad

21. A horseshoe crab Factor C protein having activity of Factor C,
wherein the horseshoe crab is *Limulus polyphemus*, and
wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell, and
wherein the Factor C protein exhibits a residual activity of at least one selected from the group consisting of:
(1) 10% or higher in the presence of 21 mM sodium citrate; and
(2) 15% or higher in the presence of 16 mM magnesium sulfate.

- **Specific** species of horseshoe crab (*Limulus polyphemus*)
- **Specific** host cell (HEK293)
- **Specific** salt solution concentrations for claimed residual activity

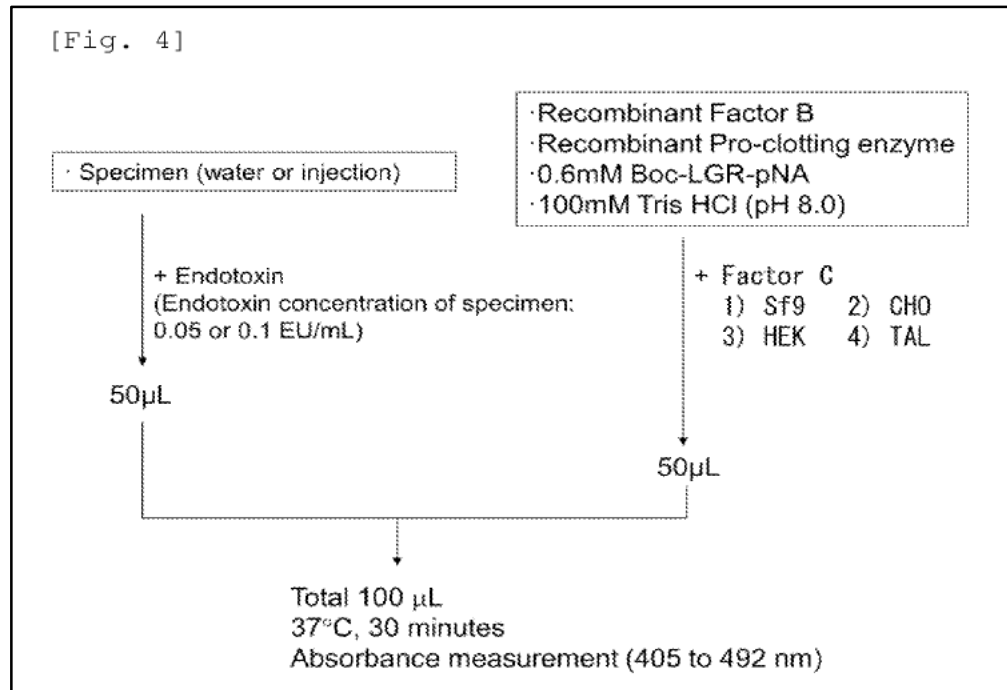
“[T]he '767 Application never describes or instructs a POSA how to make or use any recombinant proteins from this species of crab at all. The '767 Application provides no working examples of such proteins. And nothing in the application suggests any steps the inventors envisioned for ensuring that such *Limulus polyphemus* Factor C proteins (when they were eventually made) would satisfy the claimed residual activity requirement.”

The '767 Application Provides Examples that a POSA Could Use as a Roadmap

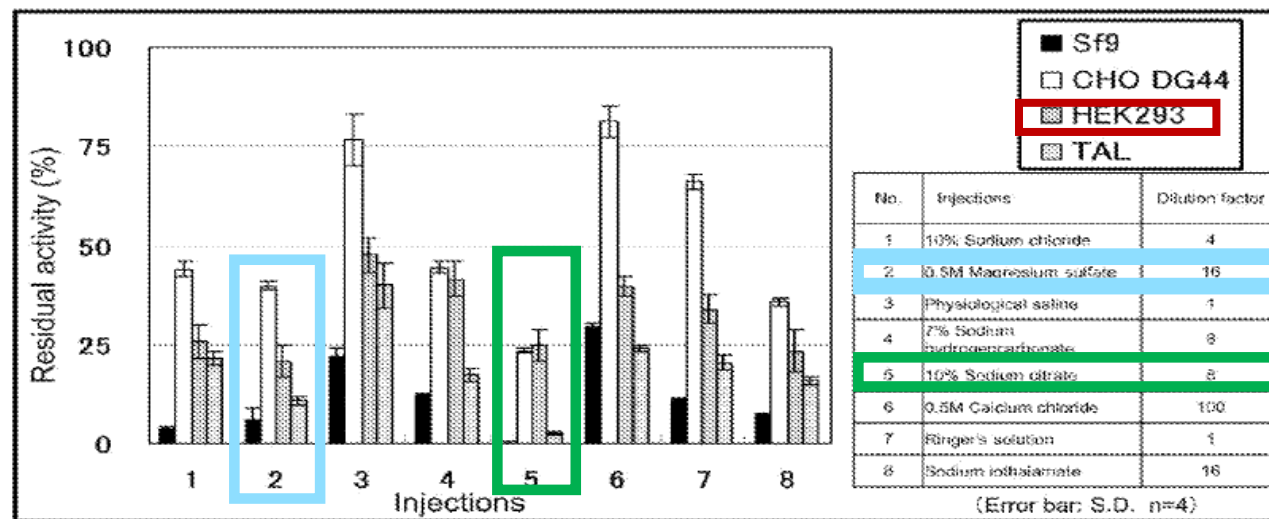
(3) Expression of Factor C in mammalian cells (HEK293)

In this section, Factor C was prepared by use of HEK293, which is a human embryo kidney cell line, as a host through the following procedure.

[Fig. 4]



[Fig. 7]



Undue Experimentation Not Required to Obtain *L. Polyphemus* Factor C cDNA

Petitioner admitted no undue experimentation required to obtain sequence:

59. Deriving the solution to this problem (i.e., one of the sequences recited in claim 1) does not require any inventive skill. As we have already noted above, it is a matter of routine practice for a person skilled in the art to determine the sequence of a known protein.

116. Deriving the solution to this problem (the sequences recited in claim 1) does not require any inventive skill. Indeed, and as we have already explained above, deriving the claimed *Limulus* sequence was merely a matter of routine analysis.

155. However, this technical effect should be disregarded because it is not based on the true difference. D1 already discloses "a recombinant Factor C protein derived from *Limulus polyphemus* (LFC)". The only difference compared to this disclosure is the explicit sequence of LFC, but not recombinant LFC itself. In that regard it should also be noted that the applicant

Undue Experimentation Not Required to Obtain *L. Polyphemus* Factor C cDNA

Petitioner's expert in the European Opposition admitted no undue experimentation required to obtain the sequence:

III.2 The skilled person could have followed the teaching described in example 1 of D23 (US 5,716,834), or equally D24 (Ding, 1995)

14. The protocol disclosed in D23/D24 is very straightforward as described above. There's every reason to believe that this approach would have been successful to obtain the *Limulus* Factor C cDNA. Given the success of obtaining the cDNA sequence of the other, closely related species, there would be no reason to expect any technical difficulties for repeating this protocol for *Limulus*.

Undue Experimentation Not Required to Recombinantly Express *L. Polyphemus* Factor C

Petitioner's expert in the European Opposition admitted no undue experimentation required to express the protein:

VIII. Is there any reason to believe that this protein would have been difficult to express after the cDNA sequence had been obtained?

44. No.

45. Again, the cDNA sequence of the *Limulus* Factor C is very similar to the other known species of horseshoe crabs, which had been successfully expressed in Sf9, CHO DG44 and HEK 293 cells. This has been shown in e.g., D1 ('241).

46. Given that these cells were able to incorporate vectors containing cDNA for *T. tridentatus* and/or *C. rotundicauda* and express the Factor C-encoding mRNAs and Factor C proteins, there's no reason to think that they would not be equally capable of handling the *Limulus* sequence.

47. If I were to follow the example of D1 using the *Limulus* sequence, I would start with the cells exemplified in this disclosure and would not anticipate experiencing any difficulties.

II.

Ground 1: Petitioner Fails to Show Obviousness Over Mizumura and '498 PCT

No Prior Art Discloses the Claimed Residual Activity in *L. Polyphemus* Factor C

Claim Term	Mizumura (EX1009)	'498 PCT (EX1005)
[21C1] (1) 10% or higher in the presence of 21 mM sodium citrate; and	"The above-described Factor C, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate." EX1009, ¶0099. "FIG. 7 A table and a graph showing the activity inhibition by injections of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (<i>Tachypleus tridentatus</i>)." EX1009, ¶0179, FIG. 7 (showing bar graphs for No. 5 "Sodium citrate" as around 25% (for HEK293 cells)).	
[21C2] (2) 15% or higher in the presence of 16 mM magnesium sulfate.	"The above-described Factor C, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate." EX1009, ¶0035. "FIG. 7 A table and a graph showing the activity inhibition by injections of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (<i>Tachypleus tridentatus</i>)." EX1009, ¶0179, FIG. 7 (showing bar graphs for No. 2 "Magnesium sulfate" as around 20% (for HEK293 cells)).	

Petitioner’s Reasonable Expectation of Success Arguments Contradict its 112 Arguments

Petitioner’s Section 112 Arguments	Petitioner’s Section 103 Arguments
“[B]ecause the inventors revealed that mammalian-expressed recombinant Factor C proteins exhibited residual activities that were significantly different than the native proteins from the same species of horseshoe crab with the same sequence (’767 Application, FIG. 7), <u>a POSA would have been reluctant to extrapolate similarities in gross function to more nuanced functional properties under particular assay conditions for recombinantly produced Factor C in mammalian cell lines across different species of horseshoe crabs.</u>” “PO <u>cannot</u> ‘make up for’ the absence of disclosure of recombinantly produced <i>Limulus polyphemus</i> Factor C and <u>extrapolate that protein’s claimed residual activity function based on information about other species</u> of horseshoe crab (where, as here, many variables are implicated).”	“It would have been within the skill and experience of a POSA as of 2019 to compare amino acid sequences from the then-known <i>Limulus polyphemus</i> horseshoe crab and the well-studied <i>Tachypleus tridentatus</i> horseshoe crab and, given similarities overall, <u>reasonably expect the two to be comparable in terms of residual activity.</u> Jain, ¶¶181-82.” “By 2019, a POSA would have had a reason to recombinantly express the then-elucidated full-length amino acid sequence of <i>Limulus polyphemus</i> horseshoe crabs via the HEK293 system as described in Mizumura and <u>would have reasonably expected the resulting proteins to exhibit similar residual activity as those reported in Mizumura for Tachypleus tridentatus.</u>”

III.

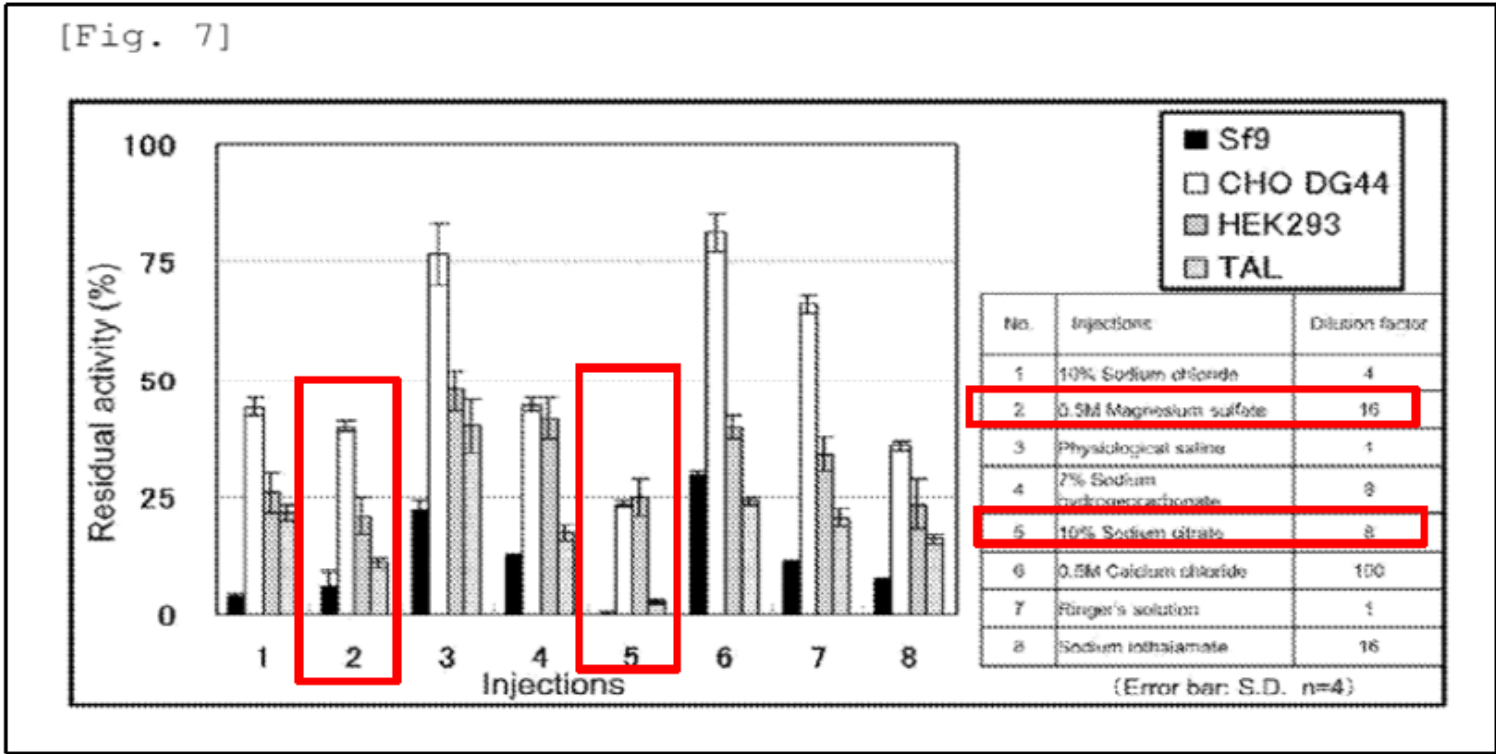
Ground 2: Petitioner Fails to Show Obviousness Over Mizumura and McClymont

“For the ***same reasons discussed in Section XI.A above, in Ground 1***, POSAs would have had reason to utilize the sequence information from McClymont to generate a recombinant Factor C protein of the *Limulus polyphemus* species in HEK293 cells as disclosed for *Tachypleus tridentatus* in Mizumura, and would have ***reasonably expected the resulting proteins to have residual activity described in Mizumura and falling within the claimed range.***”

IV. Unexpected Results of Non-Obviousness Exist

Mizumura Provides the Closest Prior Art

Mizumura shows residual activity of *Tachypleus tridentatus* Factor C in claimed salt solutions:

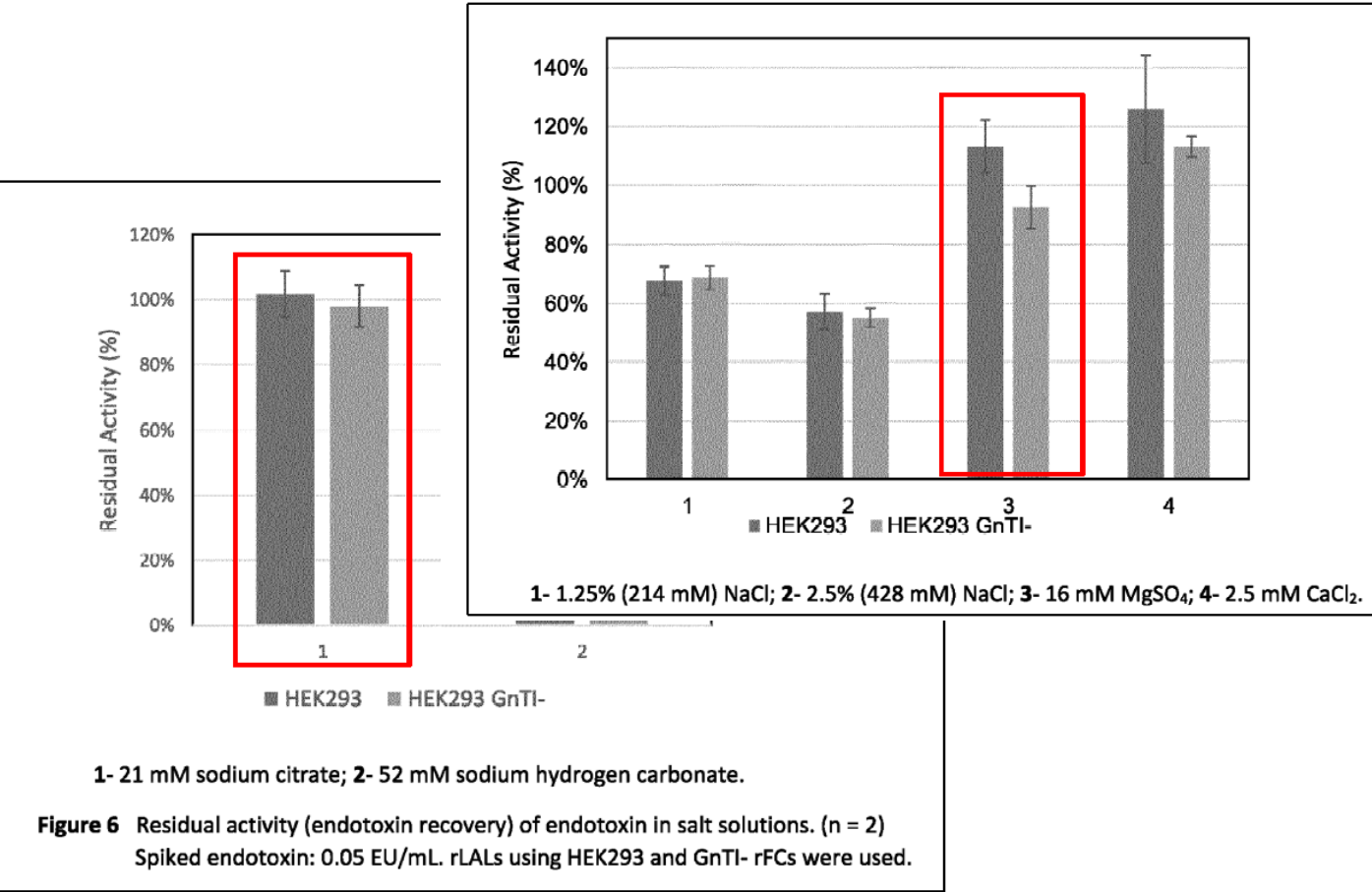


HEK293 Residual Activity

16 mM magnesium sulfate	21 mM sodium citrate
~20-25%	~20-30%

Opposition Proceeding Residual Activity Measurements

Data submitted in the Opposition showed unexpectedly superior residual activity for *L. polyphemus* Factor C:



HEK293 Residual Activity

16 mM magnesium sulfate	21 mM sodium citrate
~90-120%	~90-110%

A POSA Would Have Expected Functional Properties to be Highly Conserved Among Species



Patent Owner's Expert
Dr. Tina Morris

49. In published and documented history of international ring studies that include lysates derived from both *Limulus polyphemus* (LAL) and *Tachypleus tridentatus* (TAL), the species have been consistently considered functionally equivalent in terms of activity. See, e.g., Ex. 1022 at 153 (stating “[t]hese findings also lead to the idea that the hemocytes of *L. polyphemus* probably contain a coagulation system similar to that established for the *T. tridentatus* hemocytes.”).

50. A POSA would have expected this equivalence to carry forward into the recombinant expression systems and not find one species being unexpectedly superior over the other. See, e.g., Ex. 2062.

Unexpectedly Superior Residual Activity in *L. Polyphemus* Factor C

Data submitted in the Opposition showed unexpectedly superior residual activity in HEK293 cells for *L. polyphemus* Factor C as compared to *T. tridentatus* Factor C:

Species of Factor C	16 mM magnesium sulfate	21 mM sodium citrate
<i>Limulus polyphemus</i>	~90-120%	~90-110%
<i>Tachypleus tridentatus</i>	~20-25%	~20-30%

V. Revised Contingent Motion to Amend

Proposed Amended Claim 22 (Substitute for Claim 21)

A horseshoe crab Factor C protein having activity of Factor C,
wherein the horseshoe crab is *Limulus polyphemus*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell, and
wherein the Factor C protein exhibits a residual activity of at least one selected from the group consisting of:
(1) ~~[[10% or higher in the presence of 21 mM sodium citrate]]~~ a residual activity in the presence of 21 mM sodium citrate that is:
 (i) **higher than** the residual activity in the presence of 21 mM sodium citrate of a corresponding *Limulus polyphemus* Factor C protein expressed in Sf9 as a host cell, or
 (ii) **higher than** the residual activity in the presence of 21 mM sodium citrate of a native *Limulus polyphemus* Factor C protein, wherein the horseshoe crab Factor C protein is not a horseshoe crab Factor C protein having less than 10% residual activity in the presence of 21 mM sodium citrate; and
(2) ~~[[15% or higher in the presence of 16 mM magnesium sulfate]]~~ a residual activity in the presence of 16 mM magnesium sulfate that is:
 (i) **higher than** the residual activity in the presence of 16 mM magnesium sulfate of a corresponding *Limulus polyphemus* Factor C protein expressed in Sf9 as a host cell, or
 (ii) **higher than** the residual activity in the presence of 16 mM magnesium sulfate of a native *Limulus polyphemus* Factor C protein,
 wherein the horseshoe crab Factor C protein is not a horseshoe crab Factor C protein having less than 15% residual activity in the presence of 16 mM magnesium sulfate.

“While these inverted negative limitations *may resolve the broadened scope* of PO’s first amended claim 22, there is *no support in the ’767 Application for such additional negative claim limitations* and PO points to none.”

The '767 Application Provides Written Description Support for Revised Claim 22

The '767 Application specifically identifies the claimed residual activity values, including the claimed minimum:

[0017]

Accordingly, the present invention encompasses the following modes.

[1.2.1]

The above-described Factor C, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

[1.2.7]

The above-described Factor C, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

[0035]

The Factor C of the present invention may have low susceptibility to inhibition of activity by an ion. The

as compared with the control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" further include such a property that the Factor C of the present invention exhibits a residual activity of 10% or higher or 20% or higher in the presence of 21 mM sodium citrate, as compared with the control Factor C; such a

chloride, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a residual activity of 15% or higher or 25% or higher in the presence of 16 mM magnesium sulfate, as compared with the control Factor C; and such a property that the Factor C of

“In addition, the *introduction of these “higher than” limitations introduces an entirely different concept* into revised claim 22 relative to original claim 21 . . . The inventors undisputedly *did not describe such proteins or the claimed comparisons between such proteins* and so PO’s addition of this relationship between *Limulus polyphemus* proteins constitutes an *improper introduction of new matter.*”

Revised Claim 22 Does Not Introduce New Matter

The '767 Application provides support for the comparator limitations in revised claim 22:

The Factor C of the present invention may have low susceptibility to inhibition of activity by an ion. The expression "low susceptibility to inhibition of activity by an ion" refers to the state that the Factor C of the present invention exhibits a high residual activity in the presence of an ion, as compared with a control Factor C. Examples of the control Factor C include a recombinant Factor C expressed in insect cell Sf9 and a native Factor C. The meaning of the

control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" include such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 21 mM sodium citrate, as compared with the control Factor C; such a property that the

as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 16 mM magnesium sulfate, as compared with the control Factor C; and such a property

Revised Claim 22 is Entitled to a December 10, 2013 Priority Date

- **Revised Claim 22 Has Ample Written Description Support in the ‘767 Application:**
 - Written description support exists for recombinantly expressed *L. polyphemus* Factor C proteins for same reasons discussed for original claim 21.
 - Specification describes residual activity limitations, including comparison to residual activity in control Sf9 cells and native Factor C.
 - POSA would have understood functional domains highly conserved between species and expected residual activity in *T. tridentatus* to be maintained in *L. polyphemus* Factor C.
- **Revised Claim 22 is Enabled by the ‘767 Application:**
 - No undue experimentation to express *L. polyphemus* Factor C in HEK293 cells for reasons discussed for original claim 21.
 - Claim only requires residual activity in HEK293 cells be “higher” than control Factor C and then sets minimum floor.