UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CHARLES RIVER LABORATORIES, INC.,

Petitioner,

v.

SEIKAGAKU CORPORATION,

Patent Owner.

Case No. IPR2025-00440
Patent No. 11,236,318

PATENT OWNER'S PRELIMINARY RESPONSE TO PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 11,236,318

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37 C.F.R. § 42.107(a).....1

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Exhibit No.	Description
2001	Excerpt from U.S. Application No. 14/650,767 File History – Claims dated June 9, 2015
2002	Excerpt from U.S. Application No. 14/650,767 File History – Non-Final Rejection dated September 16, 2016
2003	FAQs for Interim Processes for PTAB Workload Management, *available at* https://www.uspto.gov/patents/ptab/faqs/interim-processes-workload-management
2004	Declaration of Michael A. Frohman, M.D., Ph.D. and exhibits from Opposition for European Patent No. 3530670
2005	Notice of Opposition to European Patent No. 3441466
2006	Notice of Opposition to European Patent No. 3530670
2007	Extract from the Register of European Patents for European Patent No. 4291648
2008	Ding, et al., "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*," Molecular Marine Biology and Biotechnology (1995) 4(1), 90-103, submitted in Opposition to European Patent No. 3441466
2009	Ding, et al., "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*, Molecular Marine Biology and Biotechnology (1995) 4(1), 90-103, submitted in U.S. Patent Application No. 17/650,803
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2011	Ding, et al., "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*," Molecular Marine Biology and Biotechnology (1995) 4(1), 90-103, submitted in U.S. Patent Application No. 18/953,525
2012	Ding, et al., "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*," Molecular Marine Biology and Biotechnology (1995) 4(1), 90-103, submitted in Opposition to European Patent No. 3530670
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2017	Hossler, et al., "Optimal and consistent protein glycosylation in mammalian cell culture," Glycobiology (2009), 19(9): 936-949, submitted in Opposition to European Patent No. 3441466
2018	Hossler, et al., "Optimal and consistent protein glycosylation in mammalian cell culture," Glycobiology (2009), 19(9): 936-949, submitted in U.S. Patent Application No. 17/650,803
2019	Hossler, et al., "Optimal and consistent protein glycosylation in mammalian cell culture," Glycobiology (2009), 19(9): 936-949, submitted in U.S. Patent Application No. 18/275,777

2020	Hossler, et al., "Optimal and consistent protein glycosylation in mammalian cell culture," Glycobiology (2009), 19(9): 936-949, submitted in U.S. Patent Application No. 18/953,525
2021	Koshiba, et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria," (2007) J. Biological Chemistry, 282(6): 3962-3967, submitted in Opposition to European Patent No. 3441466
2022	Koshiba, et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria," (2007) J. Biological Chemistry, 282(6): 3962-3967, submitted in U.S. Patent Application No. 17/650,803
2023	Koshiba, et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria," (2007) J. Biological Chemistry, 282(6): 3962-3967, submitted in U.S. Patent Application No. 18/275,777
2024	Koshiba, et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria," (2007) J. Biological Chemistry, 282(6): 3962-3967, submitted in U.S. Patent Application No. 18/953,525
2025	English Translation of Hashiguchi, et al., "Expression of Recombinant Proteins in Cultured Human Cells Standard Protocol with 293 Strain Cells," (2008) Protein Science Society Archive 1, e017, submitted in Opposition to European Patent No. 3441466
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2027	English Translation of Hashiguchi, et al., "Expression of Recombinant Proteins in Cultured Human Cells Standard Protocol with 293 Strain Cells," (2008) Protein Science Society Archive 1, e017, submitted in U.S. Patent Application No. 18/275,777

2028	English Translation of Hashiguchi, et al., "Expression of Recombinant Proteins in Cultured Human Cells Standard Protocol with 293 Strain Cells," (2008) Protein Science Society Archive 1, e017, submitted in U.S. Patent Application No. 18/953,525
2029	Tokunaga, et al., "Further Studies on Lipopolysaccharide-Sensitive Serine Protease Zymogen (Factor C): Its Isolation from *Limulus polyphemus* Hemocytes and Identification as an Intracellular Zymogen Activated by α-Chymotrypsin, Not by Trypsin," (1991) J. Biochem, 109: 150-157, submitted in Opposition to European Patent No. 3441466
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2031	Tokunaga, et al., "Further Studies on Lipopolysaccharide-Sensitive Serine Protease Zymogen (Factor C): Its Isolation from *Limulus polyphemus* Hemocytes and Identification as an Intracellular Zymogen Activated by α-Chymotrypsin, Not by Trypsin," (1991) J. Biochem, 109: 150-157, submitted in U.S. Patent Application No. 18/275,777
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2033	D19 submitted in Opposition to European Patent No. 3441466
2034	U.S. Publication No. 2023/0258647
2035	U.S. Publication No. 2003/0054432
2036	Alwis, K.U. & Milton, D.K., "Recombinant Factor C Assay for Mea suring Endotoxin in House Dust: Comparison with LAL, and (1→3)-β-D-Glucans," Am. J. of Indus. Med. 49:296-300 (2006)

2037	D'Amato A., et al., "In Depth Exploration of the Hemolymph of Limulus polyphemus via Combinatorial Peptide Ligand Libraries," J. Proteome Rsch. (2010), 9:3260-3269

I. INTRODUCTION

Pursuant to 37 C.F.R. § 42.107(a), Patent Owner Seikagaku Corporation ("Seikagaku" or "PO") respectfully submits this Preliminary Response to the Petition for *Inter Partes* Review of claim 21 ("Claim 21") of U.S. Patent No. 11,236,318 ("'318 Patent"). Institution should be denied.

Petitioner Charles River Laboratories, Inc. ("Petitioner" or "CRL") asserts that Claim 21 is "entitled to an effective filing date no earlier than the April 30, 2019 actual filing date of the '318 patent." Pet. at 1. Specifically, it argues that Application No. 14/650,767 ("'767 Application") (Ex. 1009)—the earliest U.S. application in the priority chain for the '318 Patent—does not describe or enable Claim 21 such that the '318 Patent is not entitled to an earlier priority date.

This argument fails on numerous fronts. *First,* Petitioner glosses over the narrow scope of Claim 21, ignoring it recites a ***single species*** of horseshoe crab Factor C protein—*Limulus polyphemus*—recombinantly expressed from a ***specific*** host cell—HEK 293—that exhibits a ***specific*** minimum residual activity in ***specifically*** claimed salt solutions. Petitioner also ignores the detailed disclosures in the '767 Application that describe the invention, provide examples of recombinant expression of Factor C in HEK293 cells, and measure the residual activity of such recombinantly expressed Factor C in the claimed salt solution concentrations.

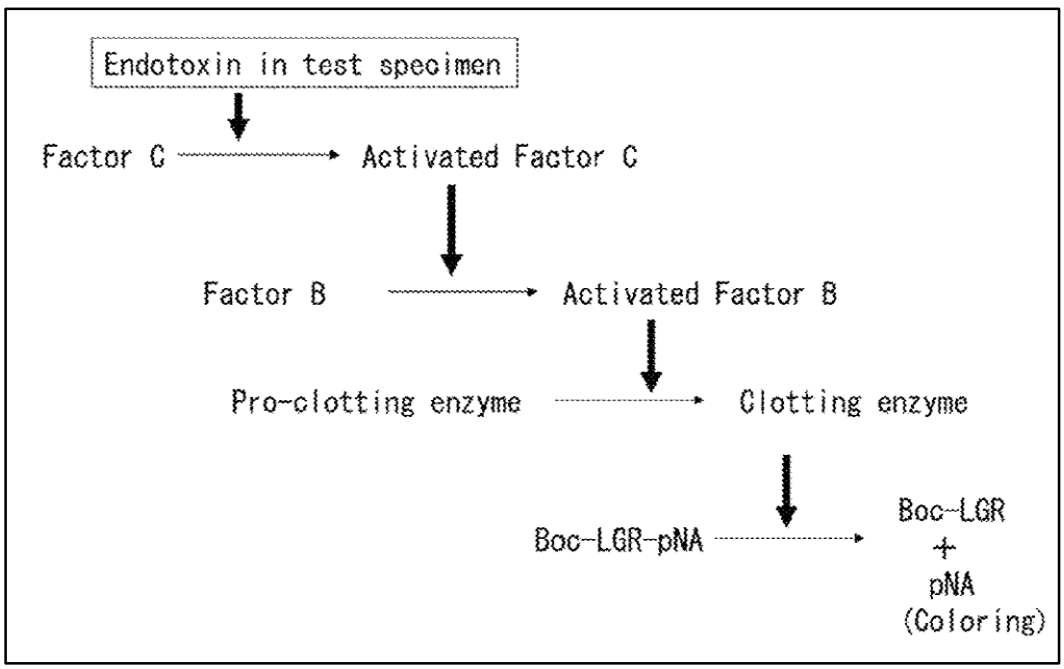
Second, Petitioner ignores that, years before the '767 Application, skilled artisans had isolated and characterized Factor C protein and knew that the *Limulus polyphemus* Factor C sequence had nearly 90% identity with its Asian counterpart. This knowledge, coupled with the admitted high level of skill, would have allowed a person of skill in the art ("POSA" or "skilled artisan") to (i) determine that the inventors possessed the full scope of Claim 21 and (ii) make and use its full scope without undue experimentation. Thus, the '767 Application provides ample written description support and enables Claim 21. Given the deficiencies in its priority date arguments, Petitioner's two obviousness grounds also fail because each relies on non-prior art references.

Accordingly, the Board should deny institution and not waste its limited resources reviewing only a single claim (Claim 21) of the '318 Patent.

II. TECHNICAL BACKGROUND

At least as early as the 1960s, researchers discovered that the American horseshoe crab species (*Limulus polyphemus*) underwent blood clotting when infected with bacterial endotoxin. Ex. 1023 at 265; Ex. 1001 at 1:41-45. Because of the ubiquitousness of bacterial endotoxins, and the potential for harm to patients if these endotoxins end up in pharmaceutical and medical products, researchers began exploring this clotting phenomenon in horseshoe crabs as a means to detect the presence of endotoxins. Ex. 1028 at 277; Ex. 1001 at 1:27-31.

This research led to the development of a limulus amoebocyte lysate ("LAL"), which has "been used widely in the detection of endotoxin in pharmaceuticals, surgical implants, water and food." Ex. 1028 at 277. LAL relies on various proteins in the blood of horseshoe crabs that undergoes a coagulation cascade in the presence of an endotoxin. *Id.*; Ex. 1001 at 1:46-54. Figure 1 from the '318 Patent (reproduced below) illustrates this cascade.



While effective, LAL comes with significant drawbacks, including the need to catch and then bleed horseshoe crabs to obtain the lysate, which negatively impacts the environment by appreciably diminishing the population of horseshoe crabs. Ex. 1001 at 2:14-17; Ex. 1028 at 277. Due to these concerns, researchers started exploring alternative options, including recombinant technology.

By the 1990s, researchers had recombinantly expressed Factor C in *Escherichia coli*, yeast and mammalian cells. Ex. 1028 at 277; *see also* Ex. 1002 ¶ 39; Ex. 1001 at 2:32-41. Researchers also identified the "gene and amino acid sequences for Factor C and related proteins for *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* species of horseshoe crab." Ex. 1002 ¶ 40 (citing Ex. 1012, Ex. 1007).

As early as 1991, Dr. F. Tokunaga and his colleagues at Kyushu University also successfully isolated and characterized Factor C protein from the *Limulus polyphemus* species of horseshoe crab and compared its properties to Factor C from the *Tachypleus tridentatus* horseshoe crab. Ex. 1022 at 150. As Dr. Tokunaga noted, "the immunological and biochemical properties of the purified factor C [in *Limulus polyphemus*] are ***indistinguishable*** from those of factor C isolated from *Tachypleus* hemocytes." *Id*. at 150-151.[1] He also compared the amino acid compositions of the intact *Limulus polyphemus* Factor C with *Tachypleus tridentatus* Factor C and determined that "the individual amino acid compositions of the two proteins and of their chain subunits were ***indistinguishable***." *Id*. at 151.

In the new Millennium, the scientific literature reported additional discoveries. In 2003, US 2003/0054432 ("'432 Publication") disclosed that the

[1] Emphasis in text and annotations in figures added unless otherwise noted.

applicants had successfully "isolated the factor C from Limulus" and that "[t]ryptic peptide sequencing revealed that the Limulus factor C sequence closely matched the Asian species, ***with >90% sequence identity***." Ex. 2035 at [0134]. In 2006, Drs. Alwis and Milton reported that "the Factor C of *Carcinoscorpius rotundicauda* has 89.3% homology with that of *Limulus polyphemus,* North American horse shoe [*sic*] crab Factor C." Ex. 2036 at 4. In 2007, Takumi Koshiba and his group used HEK293 cells to express *Tachypleus tridentatus* Factor C partial peptides. Ex. 2021 at 2, 5. And in 2010, the literature reported that peptides obtained from *L. polyphemus* hemolymph were homologous to proteins from other crab species, including "Limulus clotting Factor C" from *T. tridentatus*. Ex. 2037 at 7.

Despite these breakthroughs, drawbacks persisted, including the suppression of Factor C activity in the presence of salt ions. Ex. 1001 at 2:24-31; Ex. 1002 ¶ 42. As explained below, the invention of the '318 Patent addressed this problem, unexpectedly demonstrating that Factor C expressed from a mammalian cell has less susceptibility to inhibition of activity in the presence of salt solutions. *See, e.g.*, Ex. 1001 at 3:13-19.

III. THE '318 PATENT

Filed on April 30, 2019, the application that led to the '318 Patent is a 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 **December 10, 2013**, now Pat. No. 10,144,923. The '318 Patent also claims foreign priority to JP 2012-269840, filed December 10, 2012.

A. Claim 21

Claim 21 recites:

> 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.

Ex. 1001 at cl. 21.

This claim captures a focal point of the inventors' contribution to the field: successful recombinant production of Factor C in a mammalian cell (e.g., a HEK293 cell) that exhibits less susceptibility to inhibition in the presence of ions.

B. Specification of the '318 Patent

The specification of the '318 Patent describes the "Factor C of the present invention" as having "low susceptibility to inhibition of activity by an ion," which 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." Ex. 1001 at 17:20-25. The specification provides ***specific means*** to quantify whether the recombinant Factor C demonstrates this inventive "low susceptibility to inhibition of activity by an ion" by (i) explaining how to determine the residual activity and (ii) providing minimum threshold values in specific salt concentrations for the residual activity. Ex. 1001 at 17:53-18:15. Specifically, it states:

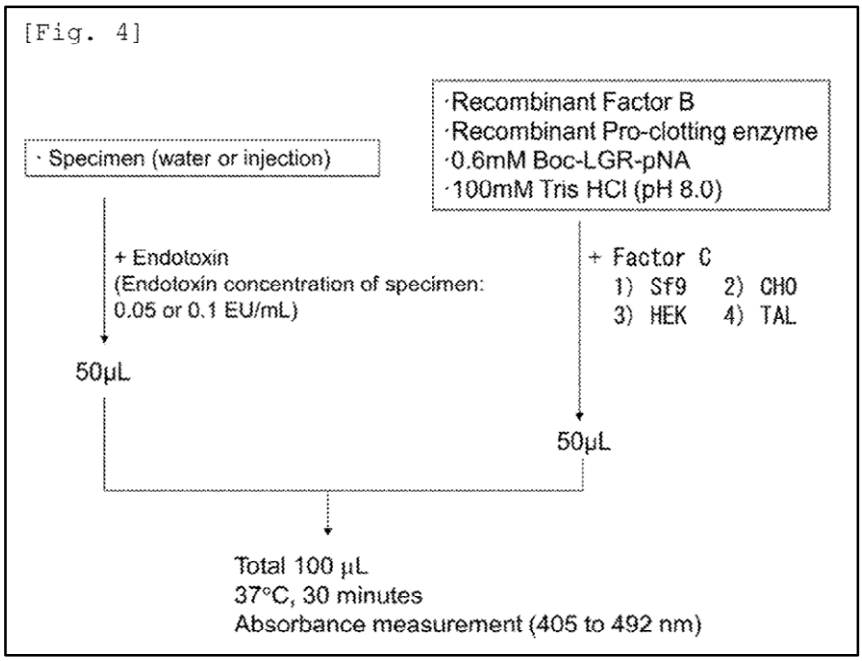
> ***Specific examples*** of "low susceptibility to inhibition of activity by an ion" further include such a property that the Factor C of the present invention exhibits a ***residual activity of 10% or higher*** or 20% or higher ***in the presence of 21 mM sodium citrate***, as compared with the control Factor C . . . ; such a 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. . . . The Factor C of the present invention may have any one of the above properties singly or two or more of the above properties in combination. The **"*residual activity*" *is defined as a relative reactivity (%)*** observed for a measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to an ion-containing test specimen, with respect to the reactivity observed for another measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to injection water as 100%, in the assay system shown in FIG. 4.

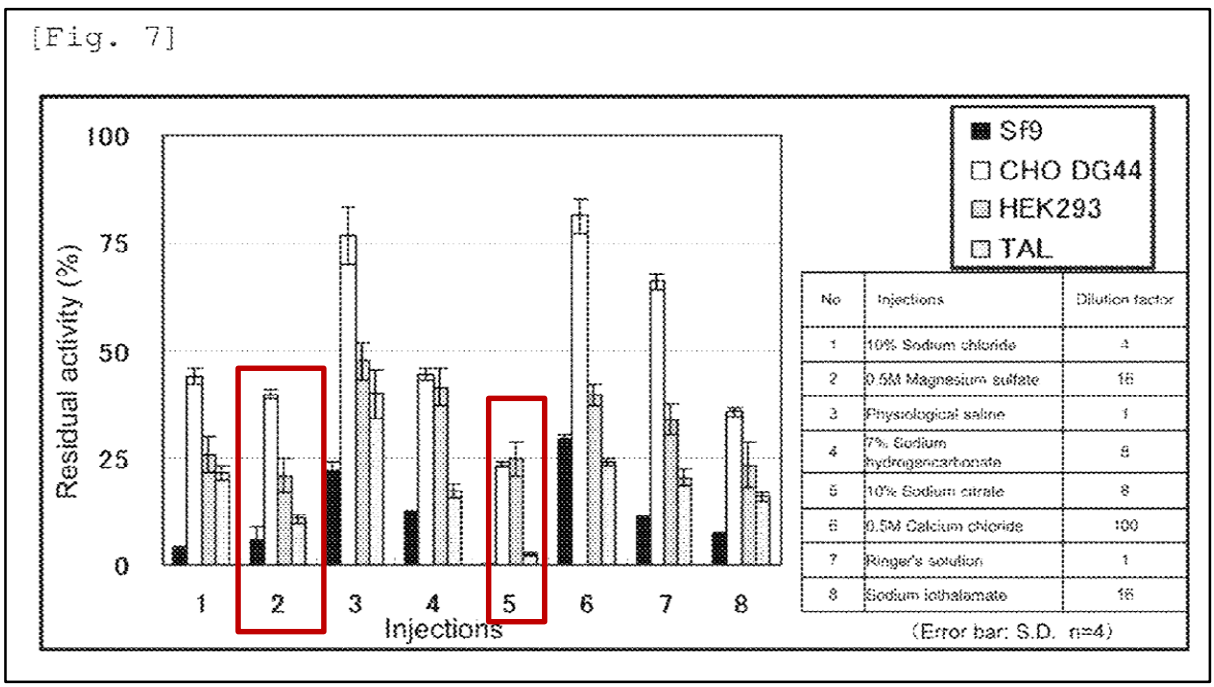
Id.

Claim 21 thus recites specific examples from the specification that demonstrate the inventive feature of recombinant Factor C having low susceptibility to inhibition of activity by an ion. Indeed, Petitioner's expert concedes that "residual activity" captures the concept of mitigating reaction inhibition in the presence of an ion. Ex. 1002 ¶ 43.

The specification also describes the preparation of Factor C using HEK293 cells as host cells and measuring the inhibition of activity of the Factor C in the presence of salt ions. Ex. 1001 at 31:14-43, 34:39-35:43. Figure 4 shows the procedure by which activity of Factor C was measured, and Figure 7 shows that expressing Factor C in HEK293 cells leads to low susceptibility to inhibition of activity by an ion, including in the presence of the claimed salt solutions (*i.e.*, 21 mM sodium citrate and 16 mM magnesium sulfate).[2] Ex. 1001 at 35:20-21, 35:26-43, Figs. 4, 7.

[2] As the specification explains, an 8-fold dilute of 10 w/v% sodium citrate injection yields a final sodium concentration of about 21 mM, and a 16-fold dilute of 0.5 M magnesium sulfate injection yields a final magnesium sulfate concentration of about 16 mM. Ex. 1001 at 35:4-7, 15-17.





No	Injections	Dilution factor
1	10% Sodium chloride	4
2	0.5M Magnesium sulfate	16
3	Physiological saline	1
4	7% Sodium hydrogencarbonate	8
5	10% Sodium citrate	8
6	0.5M Calcium chloride	100
7	Ringer's solution	1
8	Sodium isothalamate	16

Ex. 1001 at Figs. 4, 7 (annotated).

C. Relevant File History of the '318 Patent

On June 30, 2020, the United States Patent and Trademark Office ("PTO") issued a Non-Final Rejection under various 35 U.S.C. § 112 grounds, including

rejecting then-pending claims 2-9, 12, and 13 for lack of written description. Ex. 1004 at 371-376.

Then-pending independent claim 2 and dependent claim 8 recited:

> A Factor C protein having activity of Factor C,
>
> wherein the Factor C protein comprises an animo acid sequence selected from the group consisting of:
>
> (A) the amino acid sequence of SEQ ID NO: 2;
>
> (B) an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2, and having activity of Factor C;
>
> (C) the amino acid sequence of SEQ ID NO: 4; and
>
> (D) an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4, and having activity of Factor C,
>
> wherein the Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary (CHO) DG44 cell or HEK cell, and
>
> wherein the Factor C protein exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.
>
> 8. The Factor C protein according to claim 2, which exhibits a residual Factor C activity of 15% or higher in the presence of 16 mM magnesium sulfate.

Ex. 1004 at 515-516. SEQ ID NO: 2 corresponds to the amino acid sequence of Factor C derived from the *Tachypleus tridentatus* horseshoe crab, and SEQ ID NO:

4 corresponds to the amino acid sequence of Factor C derived from the *Carcinoscorpius rotundicauda* horseshoe crab. Ex. 1001 at 13:39-41, 43-45.

The PTO asserted that claim 2 (i) lacked ***insufficient representative species***; and (ii) "[o]ther than a recombinant *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* Factor C protein expressed in [CHO] or [HEK] cells, which Factor C protein has Factor C activity and contains (α-2, 3)-linked terminal sialic acid in a greater amount as compared with corresponding native horseshoe crab Factor C protein and/or a corresponding horseshoe crab Factor C protein, there is ***no disclosed or art-recognized correlation between the structure of the recited Factor C protein and the characteristic of residual activity of 10% or higher in the presence of 21 mM sodium citrate and the additional characteristics recited in claims 3-9***." Ex. 1004 at 374-376.

On September 17, 2020, Applicant responded to the written description rejection by submitting a declaration from Dr. Hikaru Mizumura, one of the inventors of the '318 Patent. Ex. 1004 at 243-248. Among other things, Dr. Mizumura included the below table listing the amino acid sequence identity of Factor C derived from each of (i) *Tachypleus tridentatus (Tt)*, (ii) *Carcinoscorpius rotundicauda (Cr)*, and (iii) *Limulus polyphemus (Lp)*:

(Table 1) Amino acid sequence identity of three types of Factor Cs

Factor C between	Identity (%)
Tt and Cr	990 / 1019 *100 = 97.2
Tt and Lp	936 / 1020 *100= 91.8
Lp and Cr	926 / 1020 *100= 90.8

Ex. 1004 at 245.

On October 8, 2020, the PTO issued a Final Rejection, which withdrew the Section 112 grounds and only raised obviousness grounds. Ex. 1004 at 183. The Applicant and PTO went back-and-forth, including the PTO issuing a Non-Final Rejection on March 9, 2021. Ex. 1004 at 99-116. In response, Applicant submitted an agenda for a phone interview with the PTO, which included new claim 24 that ultimately issued as Claim 21. Ex. 1004 at 96-98. After the interview, Applicant amended its claims to include claim 24 and presented another declaration from Dr. Mizumura demonstrating the superior salt tolerance results for *Limulus polyphemus* Factor C expressed by HEK293 cells. Ex. 1004 at 67-87. The PTO subsequently allowed the claims, noting that unexpected results existed to rebut the *prima facie* case of obviousness. Ex. 1004 at 21.

IV. PERSON OF ORDINARY SKILL IN THE ART

Petitioner proposes the following definition of a POSA:

> A POSA in the technical field relating to the '318 patent would have a Ph.D. degree in biological, chemical or biochemical engineering, molecular biology, or biochemistry and at least two years of experience utilizing or assessing horseshoe crab Factor C (lysate, purified native and/or recombinantly produced) for endotoxin detection and quantitation. More education could substitute for experience, and vice versa.

Pet. at 19.

Solely for purposes of this Preliminary Response, PO does not dispute Petitioner's proposed definition. PO reserves the right to present a different definition in the unlikely event that the Board grants institution.

V. CLAIM CONSTRUCTION

The Petition does not assert that any terms require construction. Pet. at 19-20. For purposes of this Preliminary Response, PO agrees that the terms in Claim 21 can be afforded their plain and ordinary meaning.

VI. CLAIM 21 IS ENTITLED TO ITS DECEMBER 10, 2013 EFFECTIVE FILING DATE

Petitioner erroneously asserts that the '767 Application in the priority chain "fails to describe and enable" Claim 21. Pet. at 22-42. As described below, the '767 Application provides ample written description support and enables the full scope of Claim 21.

A. The '767 Application Provides Written Description Support for Claim 21

Petitioner's written description argument hinges on its assertion that Claim 21 "encompasses a ***genus*** of recombinantly produced *Limulus polyphemus* Factor C proteins satisfying either one of these exceedingly wide ranges for the functional characteristic of 'residual activity.'" Pet. at 23. In support, Petitioner argues (i) no examples using *Limulus polyphemus* exist in the '767 Application; (ii) differences in species would yield differences in protein function; (iii) no structural feature common to the claimed genus correlates with function; and (iv) Applicants' statements in a later application confirm the lack of written description support. Pet. at 23-31. Each argument fails.

1. The Law of Written Description Does Not Require Specific Examples

"There is ***no rigid requirement that the disclosure contain 'either examples or an actual reduction to practice'***; the proper inquiry is whether the patentee has provided an adequate description that 'in a definite way identifies the claimed invention' in sufficient detail such that a person of ordinary skill would understand that the inventor had made the invention at the time of filing." *Regeneron Pharms., Inc. v. Mylan Pharms. Inc*., 127 F.4th 896, 916 (Fed. Cir. 2025).

The '767 Application provides this requisite description such that a POSA would have understood the inventors had possession of the invention of Claim 21 as

of at least the 2013 filing date for the '767 Application.[3] In a section titled "***Modes for Carrying Out the Invention,***" the '767 Application states that the "Factor C of the present invention is a ***Factor C derived from a horseshoe crab***" and specifically identifies *Limulus polyphemus* as a known species. Ex. 1006 at 40.[4] It also describes a specific example titled "Expression of Factor C in mammalian cells (HEK 293)," which describes steps to produce Factor C using HEK 293 cells as the host cell—the same host cell Claim 21 recites. Ex. 1006 at 87.

Moreover, the '767 Application includes an example titled "Inhibition of activity by injection" that details measuring the residual activity of Factor C recombinantly expressed in HEK293 cells in the claimed salt solutions of 21 mM sodium citrate and 16 mM magnesium sulfate. Ex. 1006 at 96-99. The '767

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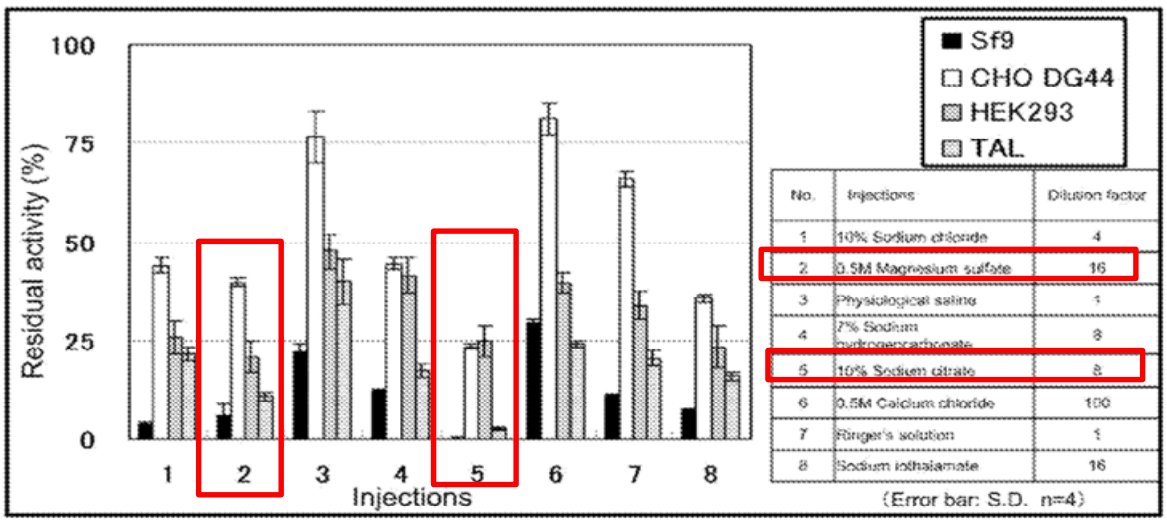
[3] The '318 Patent also claims foreign priority to JP App. No. 2012-269840, filed December 10, 2012. Petitioner does not separately assert that the '318 Patent is not entitled to that priority date but instead states that it analyzed the '767 Application "as representative of each disclosure (including the '840 JP Priority Application and the '082 PCT (Ex. 1035)) in the priority chain extending back to December 10, 2012." Pet. at 22.

[4] For all citations to Ex. 1006, PO cites to the page numbers reflected in the footnote exhibit stamp applied by Petitioner.

Application not only discloses the procedure used for this measurement in Figure 4, but it also provides results in Figure 7, which demonstrate that expression of Factor C in HEK293 cells resulted in the claimed residual activity of 10% or higher in the presence of 21 mM sodium citrate and 15% or higher in the presence of 16 mM magnesium sulfate. Ex. 1006 at 96-99, 119.

[Fig. 7]



Ex. 1006 at 122 (annotated).

Although the example relies on *Tachypleus tridentatus* horseshoe crab, not the claimed *Limulus polyphemus*, Petitioner's reliance on this point misses the mark. As discussed, "the 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); *see also Regeneron*, 127 F.4th at 916.

Petitioner also ignores that the '767 Application pointedly states that its "test results indicate that ***Factor C expressed in mammalian cells*** can have ***low susceptibility to activity inhibition by ions***." Ex. 1006 at 99. And, as discussed, the '767 Application identifies *Limulus polyphemus* as a known horseshoe crab species in a section specifically titled "Modes for Carrying Out the Invention." Ex. 1006 at 12, 40.

Petitioner's argument that written description support does not exist because Claim 21 requires a "wide range" of residual activities likewise fails. Pet. at 23-24. The '767 Application provides written description support for these claimed residual activity amounts, as it discloses these ***exact*** values in describing the invention. Specifically, it states that the "***Factor C of the present invention*** may have low susceptibility to inhibition of activity by an ion" and that "[s]pecific examples" include "a residual activity of ***10% or higher***" in the presence of 21 mM sodium citrate and "a residual activity of ***15% or higher***" in the presence of 16 mM magnesium sulfate. Ex. 1006 at 50-52. The specification thus expressly describes the same residual activity values recited in Claim 21.

Attempting to undercut this disclosure, Petitioner asserts that the '767 Application only includes one example of a specific numerical value for each of the claimed salt solutions. But "[t]he specification does not need to describe 'every conceivable and possible future embodiment of [the] invention.'" *Regeneron*, 127

F.4th at 916. Instead, written description looks at whether the specification "describe[s] an invention understandable to [a] skilled artisan and show[s] that the inventor actually invented the invention claimed." *Immunex Corp. v. Sandoz Inc*., 964 F.3d 1049, 1063 (Fed. Cir. 2020). The '767 Application does just that, thereby providing more than enough written description for Claim 21.

2. High Degrees of Similarity Exist Between the Species of Horseshoe Crabs

Petitioner asserts that differences in species would yield differences in protein function such that "the failure to provide any experimental example or verified structural link between species giving rise to such similar function only confirms lack of possession." Pet. at 26-27. Specifically, Petitioner asserts that "[w]ithout the full gene and amino acid sequence information for *Limulus polyphemus* Factor C in 2013 from which ***any comparison*** could even be made to any other species, any view about the resulting functional characteristics . . . would have been mere speculation." Pet. at 27 (emphasis in original).

Yet Petitioner ignores that skilled artisans had not only successfully isolated and characterized Factor C protein from the *Limulus polyphemus* species well before 2013 but also had compared it to Factor C from the *Tachypleus tridentatus* horseshoe crab—the species used in Figure 7 of the '767 Application.

Indeed, as early as 1991, Dr. Tokunaga and his colleagues isolated and characterized Factor C protein from the *Limulus polyphemus* species of horseshoe crab and compared its properties to Factor C from the *Tachypleus tridentatus* horseshoe crab. Ex. 1022 at 150, 155. Dr. Tokunaga determined that "the ***immunological and biochemical properties*** of the purified factor C [in *Limulus polyphemus*] are ***indistinguishable*** from those of factor C isolated from *Tachypleus* hemocytes." *Id*. at 150-151. He also compared the amino acid compositions of the intact *Limulus polyphemus* Factor C with *Tachypleus tridentatus* Factor C and determined that "the individual amino acid compositions of the two proteins and of their chain subunits were ***indistinguishable***." *Id*. at 151.

Moreover, the '432 Publication in 2003 described recombinant Factor C and noted that "[m]ethods of producing proteins recombinantly are well known in the art." Ex. 2035 at Abstract, [0030], [0032]. As the '432 Publication explained, researchers had successfully "isolated the Factor C from Limulus" and determined that "the Limulus factor C sequence ***closely matched the Asian species, with >90% sequence identity***." *Id*. at [0134]. And in 2006, Drs. Alwis and Milton reported that "the Factor C of *Carcinoscorpius rotundicauda* has 89.3% homology with that of *Limulus polyphemus,* North American horse shoe [*sic*] crab Factor C." Ex. 2036 at 4.

These publications (i) rebut Petitioner's assertion that "the resulting functional characteristics . . . of a recombinantly produced protein from *Limulus polyphemus* would have been mere speculation," Pet. at 27, and (ii) confirm what skilled artisans knew years before the filing date of the '767 Application. Since "a patentee can rely on information that is 'well-known in the art' to satisfy written description," this knowledge and the disclosures in the '767 Application demonstrate Claim 21 has ample written description support. *Streck, Inc. v. Rsch. & Diagnostic Sys., Inc.*, 665 F.3d 1269, 1285 (Fed. Cir. 2012); *see also Zoltek Corp. v. U.S.*, 815 F.3d 1302, 1308 (Fed. Cir. 2016) ("The written description 'need not include information that is already known and available to the experienced public.'").

3. Petitioner Improperly Asserts that a Structural Feature Common to the Claims is Required for Written Description Support

Petitioner also argues that Claim 21 lacks written description support because the inventors failed to provide a structural feature common to the claimed genus that correlates with function. Pet. at 29-31. But, as discussed in Sections VI.A.1-2, *supra*, the '767 Application provides sufficient written description support for the claimed invention, particularly when viewed from a skilled artisan's perspective.

Moreover, Petitioner's argument incorrectly analyzes Claim 21 as a broad genus claim that requires either a disclosure of representative species or structural features common to the members of the genus such that a POSA could visualize or

recognize the members of the genus. Pet. at 30-31. But Claim 21 does ***not*** recite a broad genus. To the contrary, it ***narrowly*** claims (i) Factor C from a specific horseshoe crab species—*Limulus polyphemus*—(ii) that is recombinantly expressed from a specific host cell—HEK 293—and (iii) that meets a minimum residual activity threshold in specifically claimed salt solution concentrations. Ex. 1001 at cl. 21.

According to the Federal Circuit, where, as here, the claim is narrow and the specification discloses its subject matter in terms of a broad genus, the specification must "'***provide sufficient 'blaze marks' to guide a reader through the forest of disclosed possibilities' toward the claimed invention***." *Pharmacyclics LLC v. Alvogen, Inc*., No. 2021-2270, 2022 WL 16943006, at *7 (Fed. Cir. Nov. 15, 2022).[5]

[5] The '767 Application, repeatedly notes the various attributes and characteristics of "***Factor C of the present invention***." *See, e.g.*, Ex. 1006 at 50-52 (describing that "Factor C of the present invention" exhibits the claimed residual activities in the claimed salt solutions); *id.* at 53-54 (describing that "Factor C of the present invention" can be expressed in HEK293 cells). While the '767 Application also provides specific examples that rely on the *Tachypleus tridentatus* species of horseshoe crab, those examples do not mean that the inventors ***only*** possessed Factor C from that particular species. Indeed, the inventors specifically cited

That is precisely what the '767 Application provides. As discussed, it explains that the "Factor C of the present invention may have ***low susceptibility to inhibition of activity by an ion***" and identifies the claimed residual activity values of 10% or higher in the presence of 21 mM sodium citrate and 15% or higher in the presence of 16 mM magnesium sulfate as *"**[s]pecific examples*** of 'low susceptibility to inhibition of activity by an ion.'" Ex. 1006 at 50-52. It also includes examples (i) utilizing HEK293 cells as the host cells to recombinantly produce Factor C and (ii) demonstrating that Factor C expressed in these cells meets the minimum threshold of the claimed residual activity. Ex. 1006 at 53-54, 87-88, 96-99, Fig. 4, Fig. 7. And it identifies *Limulus polyphemus* as one of ***four*** existing species of horseshoe crab in the "Modes for Carrying Out the Invention" section. Ex. 1006 at 40. Thus, a POSA would have understood that the '767 Application provides sufficient blaze marks towards the narrowly claimed invention of Claim 21.

4. PO's Statements in the '498 PCT Do Not Support Petitioner's Lack of Written Description Argument

Petitioner points to statements in the '498 PCT (Ex. 1005), which has two overlapping inventors with the '318 Patent, as supporting its lack of written description argument. Pet. at 31-32. Specifically, Petitioner asserts the statement

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Limulus polyphemus in its "Modes for Carrying Out the Invention" section. Ex. 1006 at 40.

that "in *Limulus polyphemus*, the proteins and their genes involved in the mechanism of coagulation caused by endotoxin ***have not been completely identified structurally or functionally***" is "fatal" to the question of written description support for Claim 21. Pet. at 32 (emphasis in original).

Not so. Although the complete amino acid sequence of *Limulus polyphemus* Factor C may not have been publicly available when the '767 Application was filed in 2013, skilled artisans by 2006 had (i) isolated Factor C from *Limulus polyphemus*; (ii) sufficiently identified its sequence to run a homology comparison with other species of horseshoe crabs; and (iii) documented the substantial homology among Factor C proteins from various horseshoe crabs, including *Limulus polyphemus*. *See, e.g.*, Ex. 2036 at 4; Ex. 1022 at 152; Ex. 2035 at [0134].

The '498 PCT also cannot change that, as early as 1991, skilled artisans knew that the "immunological and biochemical properties" of the purified Factor C from *Limulus polyphemus* are "***indistinguishable***" from those of Factor C isolated from *Tachypleus tridentatus.* Ex. 1022 at 150-151, 153.

Thus, the knowledge of a POSA at the time of the '767 Application and the disclosures in the application itself, provide adequate written description support for Claim 21.

B. The '767 Application Enables the Full Scope of Challenged Claim 21 of the '318 Patent

Petitioner also asserts that ethe '767 Application does not enable the full scope of Claim 21. Pet. at 35. Specifically, Petitioner contends that "the '767 Application does not teach a POSA how to make or use recombinant *Limulus polyphemus* Factor C proteins with any particular residual activity value" and "[t]o do so a POSA would have needed to engage in significant experimentation to make proteins exhibiting a residual activity across such a wide claimed range." Pet. at 37.

But the relevant inquiry is whether ***undue*** experimentation is required, as "a specification may call for a ***reasonable amount*** of experimentation to make and use a patented invention." *US Synthetic Corp. v. Int'l Trade Comm'n*, 128 F.4th 1272, 1285 (Fed. Cir. 2025) (quoting *Amgen Inc. v. Sanofi*, 598 U.S. 594 (2023)).

To assess whether undue experimentation is required, courts examine a number of factual considerations referred to as the *Wands* factors, which include (i) the quantity of experimentation necessary, (ii) the amount of direction or guidance presented, (iii) presence or absence of working examples, (iv) nature of the invention, (v) state of the prior art, (vi) relative skill of those in the art, (vii) predictability or unpredictability of the art, and (viii) breadth of the claims. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

Petitioner's assertion that undue experimentation would be required cannot be squared with the Federal Circuit's decision in *Wands*. *Wands* involved hybridoma technology and commercial screening assays whereby antibodies were produced from hybridomas and then screened for the desired characteristics. 858 F.2d at 740. The Federal Circuit held the claims were enabled "because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials using methods that are well known in the monoclonal antibody art." *Id*. at 736.

Similarly, the '767 Application teaches the skilled artisan (i) how to recombinantly produce Factor C proteins in HEK293 cells and (ii) how to measure the residual activity in the claimed salt solution concentrations. As in *Wands*, a skilled artisan carrying out the disclosed process in the '767 Application, particularly given the knowledge in the art, could have successfully made recombinant Factor C in HEK293 cells with the claimed residual activity without undue experimentation.

A proper analysis of the *Wands* factors further demonstrates that undue experimentation would ***not*** have been required, and the '767 Application enables the full scope of Claim 21.

1. Claim 21 is Not Broad (*Wands* Factor 8)

Petitioner asserts that Claim 21 of the '318 Patent "broadly encompasses Factor C proteins satisfying a wide range of values of residual activity in the

presence of certain ions." Pet. at 37. But Petitioner does not even attempt to quantify the size of this genus. Moreover, its argument that Claim 21 "encompasses Factor C proteins" glosses over that Claim 21 is ***narrowly*** confined to a specific species of horseshoe crab (*Limulus polyphemus*) recombinantly expressed from a specific host cell (HEK293) with minimum threshold residual activity values in specifically claimed concentrations of specific salt solutions. *Id.* Petitioner provides no support for its conclusion that "Claim 21 is Broad." *Id*.

2. Quantity of Experimentation Necessary; Amount of Direction or Guidance Presented; Presence or Absence of Working Examples (*Wands* Factors 1-3)

Petitioner lumps *Wands* factors 1-3 together but then ignores factors 1 and 2, focusing instead only on the lack of a specific example of Factor C from *Limulus polyphemus* exhibiting the claimed residual activity. But, as discussed, the law does not require working examples to find enablement, *Alcon Rsch. Ltd. v. Barr Lab'ys, Inc*., 745 F.3d 1180, 1189-1190 (Fed. Cir. 2014), and Petitioner ignores that the '767 Application provides examples that a skilled artisan would have used as a roadmap to practice the full scope of the claimed invention without undue experimentation.

The '767 Application describes producing Factor C in mammalian cells, including HEK293 cells, and discusses a specific example detailing the production of recombinant Factor C in HEK293 cells. Ex. 1006 at 53-54, 87-88. It also provides a specific procedure in Figure 4 for measuring Factor C activity and discloses a

specific example where the residual activity of recombinantly produced Factor C, including Factor C expressed in HEK293 cells, was measured in the claimed salt solution concentrations and determined to meet the minimum residual activity threshold required by Claim 21. Ex. 1006 at 96-99, Figs. 4, 7.

With the specification's guidance, a skilled artisan would have known how to use HEK293 cells to recombinantly express Factor C and how to measure the residual activity of the Factor C in the claimed salt solution concentrations to achieve the minimum residual activity threshold claimed. The lack of the examples using *Limulus polyphemus* Factor C does not change the conclusion. Given what was well known in the prior art and the admitted high level of skill, a skilled artisan could have implemented the specification's teachings to make and use the claimed Factor C from *Limulus polyphemus* through routine experimentation.

In sum, the specification would have enabled a skilled artisan to make and use Claim 21 because (i) the experimentation required to practice the invention was routine, and (ii) the specification provides direction and examples regarding expression of Factor C in HEK293 cells and measuring residual activity in the claimed salt solutions.

3. Nature of the Invention; State of the Prior Art; and Predictability of the Art (*Wands* Factors 4, 5, 7)

Relying on its unsupported assertion that Claim 21 is broad, Petitioner asserts that "[r]eaching the full scope of claim 21 therefore requires an ***extensive amount of experimentation*** across multiple fronts: (1) to discover and characterize the gene and amino acid sequence for *Limulus polyphemus* Factor C itself, which was not known as of 2013 . . ., (2) to use this as-yet undiscovered genetic information to recombinantly generate Factor C across the full scope of residual activity function characteristic as defined by claim 21." Pet. at 38. As shown below, the record proves otherwise.

a. Undue Experimentation Would Not Have been Required to Characterize the Sequence of *Limulus Polyphemus* and Recombinantly Express it in HEK293 Cells Before 2013

As discussed, the '767 Application describes examples of recombinantly expressing Factor C in HEK293 cells. *See, e.g.*, Ex. 1006 at 53-54, 87-88. Additionally, as discussed in Section II, *supra*, more than ***two decades*** before the 2013 filing date of the '767 Application, Dr. Tokunaga and his group had already isolated Factor C from *Limulus polyphemus* and compared its properties and amino acid compositions with those of *Tachypleus tridentatus*—the same species of horseshoe crab used in the examples in the '767 Application. Ex. 1022 at 150-151, 155. As Dr. Tokunaga reported, the "immunological and biochemical properties"

of the purified Factor C from *Limulus polyphemus* are "***indistinguishable***" from those of Factor C isolated from *Tachypleus tridentatus*, and "the individual amino acid compositions of the two proteins and of their chain subunits were ***indistinguishable***." Ex. 1022 at 150-151, 153.

Moreover, in 2003, researchers had successfully isolated Factor C from *Limulus polyphemus* and determined that "the Limulus factor C sequence closely matched the ***Asian species, with >90% sequence identity***." Ex. 2035 at [0134]. Given the state of the prior art and the nature of the invention, a POSA would have had a ready, working knowledge of the sequence and the immunological and biochemical properties of *Limulus polyphemus* to practice the full scope of Claim 21 without undue experimentation.

Confirming this, in an Opposition proceeding filed against PO's European Patent No. 3530670 ("EP '670"), the challenger submitted a declaration from Michael A. Frohman, M.D., Ph.D.—an expert in "biochemistry, immunology, developmental and molecular biology, with ***38 years of experience*** in molecular cloning." Ex. 2004 ¶ 2.

As detailed in Section VI.B.5, *infra*, the evidence strongly suggests that Petitioner commissioned Professor Frohman's declaration. If true, Petitioner's arguments here should be considered, if at all, with extreme skepticism. In any event, Professor Frohman's declaration confirms that a skilled artisan would not

have had to unduly experiment to discover and characterize *Limulus polyphemus* Factor C and recombinantly express it in HEK293 cells.

As Professor Frohman notes, claim 1 in EP '670 "relates to a method for producing an endotoxin detecting agent comprising a step of artificially expressing a protein using a cDNA […], wherein the ***cDNA encodes [a limulus Factor C sequence***]." Ex. 2004 ¶ 4. In his declaration, Professor Frohman opines on a number of "questions from the perspective of a [POSA] charged with the determination of the full-length *Limulus* Factor C sequence **before October 18, 2016**," including whether it would have been difficult to obtain the cDNA sequence of *Limulus polyphemus* and express it after the sequence had been obtained. *Id*. ¶ 5 (emphasis in original). As Professor Frohman explains, a POSA would have been able to do these things with little difficulty.

To obtain the cDNA sequence of *Limulus polyphemus*, Professor Frohman explains a POSA "would have most likely used Rapid Amplification of cDNA Ends (RACE) to obtain the sequence of the cDNA encoding Factor C from *Limulus polyphemus*." Ex. 2004 ¶ 7. Although he focuses his analysis on what a POSA would have known in 2016, Professor Frohman points to publications and knowledge available well before 2013.

Specifically, Professor Frohman explains that he developed RACE in ***1988*** "as a quick approach to obtain full-length cDNA sequences in cases where a partial

stretch of the mRNA sequence was known" and that it "can be used to obtain a full-length cDNA sequence ***in a matter of days***." Ex. 2004 ¶ 8. He also states it would "have been entirely straightforward for the skilled person to design RACE primers based on the available *Limulus* sequence information listed in items (i)-(iii) above." *Id.* ¶ 10. Notably, item (iii) is the ***1991*** Tokunaga publication (Ex. 1022).

Professor Frohman also explains that a POSA could have also used protocols described in publications from the 1990s to obtain the cDNA sequence for *Limulus polyphemus*. Ex. 2004 ¶¶ 11-16. Relying on U.S. 5,716,384, which published in ***1998*** (Ex. 1012), and *Ding* in ***1995***, Professor Frohman opines that obtaining the sequence is "very straightforward" such that the "approach [used in these references] would have been successful to obtain the *Limulus* Factor C cDNA" with "no reason to expect any technical difficulties for repeating this protocol for *Limulus*." Ex. 2004 ¶ 14.

After obtaining the cDNA sequence of *Limulus polyphemus,* as Professor Frohman explains, expressing it also would have involved routine experimentation. Ex. 2004 ¶¶ 44-48. Professor Frohman opines that "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," pointing to "D1 ('241)." *Id.* ¶ 45. Notably, D1 refers to EP 2930241, filed December 10, 2013, which is in the same family as the '767 Application and the '318 Patent.

According to Professor Frohman, by following the "example of **D1** using the *Limulus* sequence," he would "not anticipate experiencing any difficulties." *Id*. ¶ 47.

As such, a skilled artisan would not have had to conduct undue experimentation to discover and characterize the gene and amino acid sequence for *Limulus polyphemus* Factor C and recombinantly express it in HEK293 cells, as Claim 21 requires.

b. Undue Experimentation Would Not Have Been Required to Practice the Full Scope of Residual Activity in Claim 21 Before 2013

Petitioner's assertion that a skilled artisan would have had to unduly experiment to "recombinantly generate Factor C protein across the full scope of residual activity function characteristic as defined by claim 21" likewise fails. Pet. at 38. As discussed, the '767 Application details how to measure residual activity of Factor C recombinantly expressed in HEK293 cells, including in the salt solution concentrations of Claim 21. Ex. 1006 at 96-99, Figs. 4, 7. It also discloses that these results "indicate that Factor C expressed in mammalian cells can have low susceptibility to activity inhibition by ions," noting that specific examples include the claimed minimum thresholds. Ex. 1006 at 99. It further identifies *Limulus polyphemus* in the "Modes for Carrying Out the Invention" section. Ex. 1006 at 40.

Given this and the skilled artisan's knowledge (as Professor Frohman's declaration confirms), nothing more than routine experimentation would have been required to practice the full scope of Claim 21. *See, e.g.*, *Rimfrost AS v. Aker BioMarine Antarctic AS*, No. PGR2018-00033, Paper 9 at 10-14 (P.T.A.B. Aug. 29, 2018) (Petitioner failed to establish that the limitation "8% to 15% w/w ether phospholipid" was not enabled despite specification including only an example having 7.4%).

4. *Wands* Factor 6 Supports a Finding that Challenged Claim 21 is Enabled

Although Petitioner admits the level of skill would have been high, it still argues that making "a recombinant Factor C protein that actually has ***any*** desired activity. . . was known to be difficult." Pet. at 39. Yet, as even Petitioner's expert admits, recombinant technology was well known in 2013. *See, e.g.*, Ex. 1002 ¶ 5 (describing his work in 2006-2008 "evaluat[ing] the recombinant Factor C endotoxin kit").

Moreover, as discussed, the '767 Application describes the process for making recombinant Factor C using HEK293 cells and provides a procedure for assessing whether the recombinantly produced Factor C meets the claimed residual activity minimum thresholds in the claimed salt solution concentrations. Given the admitted high skill of a POSA, she would not have had to unduly experiment to

design, express, and test recombinant Factor C proteins. Instead, her experimentation would have been routine, particularly in view of the roadmap provided by the '767 Application.

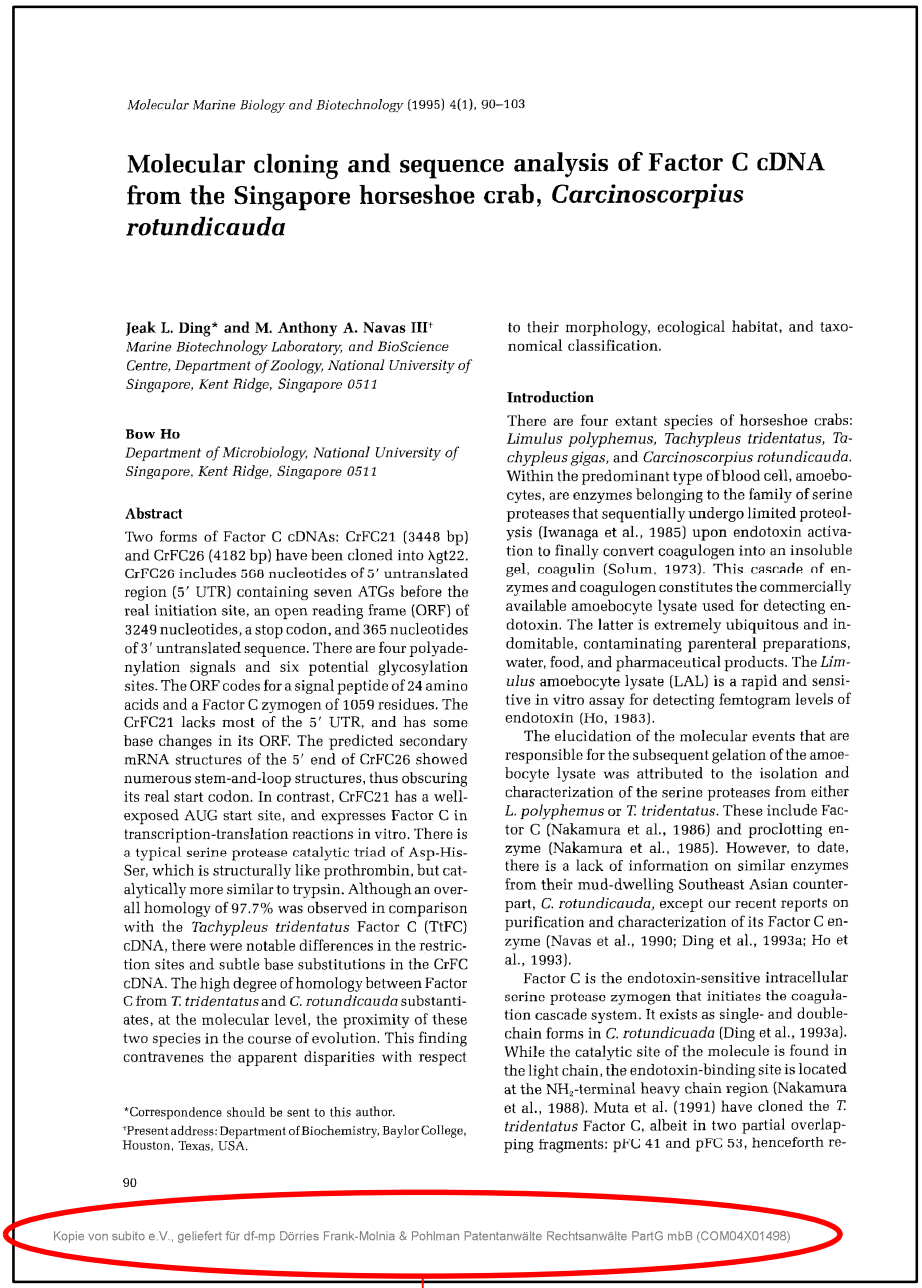
As such, under the *Wands* factors, the '767 Application teaches how to make and use the full scope of Claim 21 without undue experimentation and thus enables the claim.

5. Substantial Evidence Indicates that the Frohman Declaration in the European Opposition was Submitted on Petitioner's Behalf

As noted in Section VI.B.2, *supra*, in an Opposition proceeding filed against PO's EP '670, the party that filed the Opposition submitted a declaration from Professor Frohman. Ex. 2004 at 1. While the identity of that party is not disclosed, the evidence strongly indicates that Petitioner did so and sponsored Professor Frohman's opinions.

To begin with, Sandra Pohlman, co-founder of the law firm df-mp Dörries Frank-Molnia & Pohlman, submitted the Opposition in EP '670. Ex. 2006 at 1. Ms. Pohlman also submitted an Opposition against PO's European Patent No. EP3441***466*** ("EP '466"), which is in the same patent family as the '318 Patent. Ex. 2005. She also currently handles prosecution for Petitioner's European Patent Application EP4291648, as she is listed as the "Representative(s)." Ex. 2007.

As part of the Opposition in EP '670, Ms. Pohlman and her firm submitted document D24 by Jeak Ding and M. Anthony A. Navas III entitled "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*" ("*Ding*") (Ex. 2012), ***which included a watermark from her firm along with a unique identifier "COM04X01498***" (reproduced below).



Molecular Marine Biology and Biotechnology (1995) 4(1), 90–103

Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*

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Abstract

Two forms of Factor C cDNAs: CrFC21 (3448 bp) and CrFC26 (4182 bp) have been cloned into λgt22. CrFC26 includes 568 nucleotides of 5′ untranslated region (5′ UTR) containing seven ATGs before the real initiation site, an open reading frame (ORF) of 3249 nucleotides, a stop codon, and 365 nucleotides of 3′ untranslated sequence. There are four polyadenylation signals and six potential glycosylation sites. The ORF codes for a signal peptide of 24 amino acids and a Factor C zymogen of 1059 residues. The CrFC21 lacks most of the 5′ UTR, and has some base changes in its ORF. The predicted secondary mRNA structures of the 5′ end of CrFC26 showed numerous stem-and-loop structures, thus obscuring its real start codon. In contrast, CrFC21 has a well-exposed AUG start site, and expresses Factor C in transcription-translation reactions in vitro. There is a typical serine protease catalytic triad of Asp-His-Ser, which is structurally like prothrombin, but catalytically more similar to trypsin. Although an overall homology of 97.7% was observed in comparison with the *Tachypleus tridentatus* Factor C (TtFC) cDNA, there were notable differences in the restriction sites and subtle base substitutions in the CrFC cDNA. The high degree of homology between Factor C from *T. tridentatus* and *C. rotundicauda* substantiates, at the molecular level, the proximity of these two species in the course of evolution. This finding contravenes the apparent disparities with respect to their morphology, ecological habitat, and taxonomical classification.

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Introduction

There are four extant species of horseshoe crabs: *Limulus polyphemus, Tachypleus tridentatus, Tachypleus gigas,* and *Carcinoscorpius rotundicauda.* Within the predominant type of blood cell, amoebocytes, are enzymes belonging to the family of serine proteases that sequentially undergo limited proteolysis (Iwanaga et al., 1985) upon endotoxin activation to finally convert coagulogen into an insoluble gel, coagulin (Solum, 1973). This cascade of enzymes and coagulogen constitutes the commercially available amoebocyte lysate used for detecting endotoxin. The latter is extremely ubiquitous and indomitable, contaminating parenteral preparations, water, food, and pharmaceutical products. The *Limulus* amoebocyte lysate (LAL) is a rapid and sensitive in vitro assay for detecting femtogram levels of endotoxin (Ho, 1983).

The elucidation of the molecular events that are responsible for the subsequent gelation of the amoebocyte lysate was attributed to the isolation and characterization of the serine proteases from either *L. polyphemus* or *T. tridentatus*. These include Factor C (Nakamura et al., 1986) and proclotting enzyme (Nakamura et al., 1985). However, to date, there is a lack of information on similar enzymes from their mud-dwelling Southeast Asian counterpart, *C. rotundicauda,* except our recent reports on purification and characterization of its Factor C enzyme (Navas et al., 1990; Ding et al., 1993a; Ho et al., 1993).

Factor C is the endotoxin-sensitive intracellular serine protease zymogen that initiates the coagulation cascade system. It exists as single- and double-chain forms in *C. rotundicuada* (Ding et al., 1993a). While the catalytic site of the molecule is found in the light chain, the endotoxin-binding site is located at the NH_2-terminal heavy chain region (Nakamura et al., 1988). Muta et al. (1991) have cloned the *T. tridentatus* Factor C, albeit in two partial overlapping fragments: pFC 41 and pFC 53, henceforth re-

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Ex. 2012 at 1 (annotated).

The ***exact same watermark and unique identifier*** appear in (i) a copy of *Ding* submitted by Petitioner during prosecution of its U.S. Application Nos. 17/650,803 (Ex. 2009); 18/275,777 (Ex. 2010); and 18/953,525 (Ex. 2011), and (ii) the copy of

Ding submitted with the Opposition in EP '466 (Ex. 2008). That a document with the same watermark and unique identifier appears not only in the Opposition of EP '670 but also in the file histories of applications prosecuted by Petitioner, and the Opposition of EP '466, strongly suggests that Petitioner is involved in the EP '670 Opposition proceedings and commissioned Professor Frohman's declaration.

Further supporting this conclusion, as the table below shows, Ms. Pohlman and her firm not only submitted the same copy of *Ding* across these proceedings, but also submitted ***four other references*** in the Opposition in EP '466 that have ***identical markings*** (e.g., watermarks, translations, handwritten notations) to documents filed by Petitioner as part of prosecution for U.S. Application Nos. 17/650,803; 18/275,777; and 18/953,525:

Document	Matching characteristics	Filed in	Filed by
Wang et. al. 2002	Vertical watermark: "Downloaded from [website] by guest on March 28, 2020"	Opposition of EP '466 (Ex. 2013)	df-mp Dörries Frank-Molnia & Pohlman
		U.S. App. No. 17/650,803[6]	Petitioner

[6] Wang filed in U.S. Application No. 17/650,803 (Ex. 2014) also bears the same "D3" notation in the top right as Wang filed in the EP '466 Opposition (Ex. 2013).

		(Ex. 2014)	
		U.S. App. No. 18/275,777 (Ex. 2015)	Petitioner
		U.S. App. No. 18/953,525 (Ex. 2016)	Petitioner
Hossler et. al. 2009	Vertical watermark: "Downloaded from [website] by guest on March 30, 2020"	Opposition of EP '466 (Ex. 2017)	df-mp Dörries Frank-Molnia & Pohlman
		U.S. App. No. 17/650,803 (Ex. 2018)	Petitioner
		U.S. App. No. 18/275,777 (Ex. 2019)	Petitioner
		U.S. App. No. 18/953,525 (Ex. 2020)	Petitioner
Koshiba et al. 2007	Vertical watermark: "Downloaded from [website] by guest on December 18, 2014"	Opposition of EP '466 (Ex. 2021)	df-mp Dörries Frank-Molnia & Pohlman

		U.S. App. No. 17/650,803 (Ex. 2022)	Petitioner
		U.S. App. No. 18/275,777 (Ex. 2023)	Petitioner
		U.S. App. No. 18/953,525 (Ex. 2024)	Petitioner
Hashiguchi et al., 2008	English machine translation of the corresponding Japanese article	Opposition of EP '466 (Ex. 2025)	df-mp Dörries Frank-Molnia & Pohlman
		U.S. App. No. 17/650,803[7] (Ex. 2026)	Petitioner
		U.S. App. No. 18/275,777 (Ex. 2027)	Petitioner

[7] Hashiguchi filed in U.S. Application No. 17/650,803 (Ex. 2026) also bears the same "D51a" notation in the top right as Hashiguchi filed in the EP '466 Opposition (Ex. 2025).

		U.S. App. No. 18/953,525 (Ex. 2028)	Petitioner

Moreover, as part of the Opposition in EP '466, PO filed a reference to Tokunaga bearing a handwritten annotation "Reference (7) – Appendix B" in the upper right-hand corner. Ex. 2029. Notably, Petitioner filed the ***exact same*** document with the same handwritten annotation as part of the prosecution history for U.S. Patent Application Nos. 17/650,803 (Ex. 2030); 18/275,777 (Ex. 2031); and 18/953,525 (Ex. 2032).

Additionally, the Notice of Opposition to EP '466, dated November 5, 2020, relied upon D19: an unpublished document with no attribution that described experiments comparing the activity of *Limulus* Factor C in two HEK293 cell strains. Ex. 2005 at 1, 4; Ex. 2033. Petitioner later submitted figures and data from D19 to the PTO in Petitioner's U.S. Patent Application Number 17/650,803, filed February 11, 2022, and published as U.S. Patent Application Pub. No. US 2023/0268647 (Ex. 2034). *Compare* Ex. 2033 Figs. 1-4 *with* Ex. 2034 Figs. 11-13, 15.

Collectively, this evidence strongly indicates that Petitioner filed the Opposition in EP '670 and thus sponsored Professor Frohman's declaration. If so, Petitioner's positions in the EP '670 Opposition contradict what it asserts in its

Petition, and the Board should view its arguments here with extreme skepticism, if not disregard them entirely.[8]

VII. INSTITUTION SHOULD BE DENIED AS TO GROUND 1

Petitioner asserts that claim 21 is obvious over Mizumura (Ex. 1009) in view of the '498 PCT (Ex. 1005). Pet. at 45. As Petitioner concedes, however, Mizumura's disclosure "is identical to both the '767 Application and the '318 patent specification." Pet. at 45. Thus, Petitioner's obviousness argument hinges on its assertion that the '318 Patent is not entitled to its earliest priority date. As Petitioner failed to make this showing, as described in Section VI, *supra*, neither Mizumura nor '498 PCT is prior art to Claim 21. Ground 1 thus fails for this reason alone.

VIII. INSTITUTION SHOULD BE DENIED AS TO GROUND 2

Petitioner asserts that Claim 21 is obvious over Mizumura (Ex. 1009) in view of McClymont (Ex. 1010). Pet. at 60. As with Ground 1, Petitioner's obviousness argument hinges on its priority date argument. As Petitioner failed to make this showing, as described in Section VI, *supra*, neither Mizumura nor McClymont is prior art to Claim 21. Ground 2 thus fails for that reason alone.

[8] This would also amount to a violation of at least the routine-discovery obligations of 37 C.F.R. § 42.51(b)(1)(iii).

IX. SECONDARY CONSIDERATIONS OF NON-OBVIOUSNESS

PO reserves the right to rely on secondary considerations of non-obviousness should the Board decide to institute.

X. CONCLUSION

For reasons discussed above, the Board should deny institution and maintain the patentability of Claim 21 of the '318 Patent.

Respectfully Submitted,

Dated: June 11, 2025

/ Alexander B. Stein /
Alexander B. Stein, Reg. No. 71,397

CERTIFICATE OF COMPLIANCE

I, the undersigned, certify that the foregoing Patent Owner's Preliminary Response complies with the applicable type-volume limitations of 37 C.F.R. § 42.24(b)(1). Exclusive of the portions exempted by 37 C.F.R. § 42.24(a)(1), this Petition, including footnotes, contains 7,995 words, as counted by the word count function of Microsoft Word. This is less than the limit of 14,000 words as specified by 37 C.F.R. § 42.24(b)(1).

Respectfully Submitted,

Dated: June 11, 2025

/ Alexander B. Stein /
Alexander B. Stein, Reg. No. 71,397

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e)(4), lead counsel for Patent Owner hereby certifies that on June 11, 2025, a copy of this PATENT OWNER'S PRELIMINARY RESPONSE was served via the email correspondence address of record for Petitioner's counsel of record:

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