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14/650,767	06/09/2015	Hikaru MIZUMURA	Q220395	1531
23373 7590 09/16/2016 SUGHRUE MION, PLLC 2100 PENNSYLVANIA AVENUE, N.W. SUITE 800 WASHINGTON, DC 20037			EXAMINER STEADMAN, DAVID J	
			ART UNIT 1656	PAPER NUMBER
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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Office Action Summary	Application No. 14/650,767	Applicant(s) MIZUMURA ET AL.	
	Examiner DAVID J. STEADMAN	Art Unit 1656	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 08/24/2016.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☒ Claim(s) 43-64 is/are pending in the application.
5a) Of the above claim(s) 51-53, 58-61 and 64 is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☒ Claim(s) 43-50, 54-57, 62 and 63 is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10) ☒ The specification is objected to by the Examiner.

11) ☒ The drawing(s) filed on 06/09/2015 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☒ All b) ☐ Some** c) ☐ None of the:

1. ☒ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. ____.

3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date ____.

3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____.

4) ☒ Other: Appendix A.

DETAILED ACTION

Status of the Application

1. The present application is being examined under the pre-AIA first to invent provisions.
2. Claims 43-64 are pending in the application.

Election/Restriction

3. Applicant's election without traverse of Group I, claims 43-50, 54-57, 62, and 63, and the species SEQ ID NO: 2 in the reply filed on 8/24/2016 is acknowledged.

Claims 51-53, 58-61, and 64 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on 8/24/2016.

Claims 43-50, 54-57, 62, and 63 are being examined on the merits with claim 49 being examined only to the extent the claim reads on the elected subject matter.

It is noted that claims 54, 62, and 63 depend from non-elected claim 51 and it is suggested that claim 54 be re-written in independent form, longer being dependent from a non-elected claim.

Claim for Priority

4. This application is a 35 U.S.C. 371 national stage filing of PCT/JP2013/083082, filed on 12/10/2013, which claims foreign priority under 35 USC § 119(a)-(d) to

Japanese application 2012-269840, filed on 12/10/2012. A certified copy of the foreign priority document has been filed in this application on 6/9/2015.

Information Disclosure Statement

5. The information disclosure statement (IDS) submitted on 6/9/2015, 7/16/2015, 8/5/2015, and 8/3/2016 was filed prior to the mailing date of the instant first Office action on the merits. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

The IDS filed on 6/9/2015 and 7/16/2015 have been lined through because the cited references are duplicates to those cited in the IDS filed on 8/5/2015.

Specification/Informalities

6. The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. See specification at p. 78, paragraph 115. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code; references to websites should be limited to the top-level domain name without any prefix such as http:// or other browser-executable code. See MPEP § 608.01.

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 43-50, 54-57, 62, and 63 are rejected under 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

MPEP 2163.II.A.2.(a).i) states, "Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention". For claims drawn to a genus, MPEP § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. MPEP § 2163 further states that a "representative number of species" means that the species which are adequately described are representative of the entire

genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

Claim 43 (claims 56 and 57 dependent therefrom) is drawn to a genus of horseshoe crab Factor C polypeptides having activity of Factor C. According to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28).

Claim 44 is drawn to the Factor C according to claim 43, which is a recombinant protein. According to the specification, 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 (paragraph 19).

Claim 45 is drawn to the Factor C according to claim 43, which contains (α -2,3) linked terminal sialic acid.

Claim 46 is drawn to the Factor C according to claim 45, which contains (α -2,3) linked terminal sialic acid in a greater amount, as compared with a native Factor C and/or a horseshoe crab Factor C expressed in Sf9 as a host cell.

Claim 47 is drawn to the Factor C according to claim 43, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Claim 48 is drawn to the Factor C according to claim 43, which has a molecular weight of 115 kDa to 140 kDa or a molecular weight of 127 kDa $\pm$ 5 kDa, as measured through SDS-PAGE under a non-reducing condition.

Claim 49 is drawn to (in relevant part) the Factor C according to claim 43, which is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2; and

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

As noted above, according to the specification, “the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B” (spec at p. 36, paragraph 28). The structure of the Factor C is unlimited.

Claim 50 is drawn to the Factor C according to claim 43, which is water-soluble.

Claim 54 (claims 62 and 63 dependent therefrom) is drawn to a horseshoe crab Factor C producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

Claim 55 is drawn to the Factor C according to claim 43, which is producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

In this case, the specification discloses an actual reduction to practice of the following representative species of the genus of claimed horseshoe crab Factor C polypeptides: Factor C isolated from *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* hemocyte lysate and *Tachypleus tridentatus* Factor C expressed in Sf9 insect cells, Chinese Hamster Ovary (CHO) cells, or human embryonic kidney (HEK)

cells, which Factor C polypeptides have Factor C activity. While one of skill in the art would expect a horseshoe crab Factor C polypeptide that is isolated from its natural source to have activity of Factor C, the specification supports a high level of unpredictability regarding the activity of a horseshoe crab Factor C that is recombinantly expressed in a heterologous host cell. For example, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in a *Drosophila* S2 cell does not have Factor C activity (paragraph 132). Also, regarding the terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, solubility, and expression in a mammalian cell, the specification supports a high level of unpredictability regarding the terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, solubility, and expression in a mammalian cell of a horseshoe crab Factor C that is recombinantly expressed. For example, regarding the terminal sialic acid, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in Sf9 cells does not have any (α -2,3) linked terminal sialic acid, while Factor C that is recombinantly expressed in CHO and HEK cells contains (α -2,3) linked terminal sialic acid (paragraph 154). Regarding residual activity in the presence of sodium citrate, Figure 7 shows that Factor C that is recombinantly expressed in Sf9 cells has almost no residual activity in the presence of 21 mM sodium citrate, Factor C that is recombinantly expressed in CHO and HEK cells retains about 25% residual activity in the presence of 21 mM sodium citrate. Regarding molecular weight, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in Sf9 cells has a molecular

weight of 109 kDa, while the same Factor C that is recombinantly expressed in CHO and HEK cells has a molecular weight of 128 or 124 kDa, respectively (Table 1). Regarding amino acid sequence, the specification fails to identify any alterations to the amino acid sequence of SEQ ID NO: 2 that can be made and still have the identity of a horseshoe crab Factor C polypeptide and as described above, the same horseshoe crab Factor C expressed in different host cells can have very different characteristics and even be non-functional. Regarding solubility, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in COS cells is insoluble (paragraph 11). In this case, the genus of claimed horseshoe crab Factor C polypeptides is widely variant, encompassing any horseshoe crab Factor C polypeptide expressed from any host cell and in view of the relative few disclosed representative species and the high level of unpredictability regarding the functionality, terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, and solubility, one of skill in the art would not accept the disclosure of Factor C isolated from *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* hemocyte lysate and *Tachypleus tridentatus* Factor C expressed in Sf9 insect cells, CHO cells, or HEK cells as being representative of all other horseshoe crab Factor C polypeptides as encompassed by the claims. As such, the specification, taken with the pre-existing knowledge in the art of amino acid substitution, fails to satisfy the written description requirement of 35 U.S.C. 112, first paragraph.

8. Claims 43-50, 54-57, 62, and 63 are rejected under 35 U.S.C. 112 (pre-AIA), first paragraph, because the specification, while being enabling for Factor C isolated from *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* hemocyte lysate and *Tachypleus tridentatus* Factor C expressed in Sf9 insect cells, CHO cells, or HEK cells, does not reasonably provide enablement for all horseshoe crab Factor C polypeptides as encompassed by the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

“The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue.” *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors considered to be most relevant to the instant rejection are addressed in detail below.

The nature of the invention: According to the specification, “there has never been known a case where functional Factor C is produced using mammalian cells as host cells” (paragraph 11) and “[a]n object of the present invention is to provide a novel

recombinant horseshoe crab Factor C...another object of the present invention is to provide a reconstituted cascade reaction system which is not susceptible to reaction inhibition even in the presence of a salt (ion)" (paragraphs 14 and 15).

The breadth of the claims: Claim 43 (claims 56 and 57 dependent therefrom) is drawn to a genus of horseshoe crab Factor C polypeptides having activity of Factor C. According to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28).

Claim 44 is drawn to the Factor C according to claim 43, which is a recombinant protein. According to the specification, 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 (paragraph 19).

Claim 45 is drawn to the Factor C according to claim 43, which contains (α -2,3) linked terminal sialic acid.

Claim 46 is drawn to the Factor C according to claim 45, which contains (α -2,3) linked terminal sialic acid in a greater amount, as compared with a native Factor C and/or a horseshoe crab Factor C expressed in Sf9 as a host cell.

Claim 47 is drawn to the Factor C according to claim 43, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Claim 48 is drawn to the Factor C according to claim 43, which has a molecular weight of 115 kDa to 140 kDa or a molecular weight of 127 kDa $\pm$ 5 kDa, as measured through SDS-PAGE under a non-reducing condition.

Claim 49 is drawn to (in relevant part) the Factor C according to claim 43, which is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2; and

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

As noted above, according to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28). The structure of the Factor C is unlimited.

Claim 50 is drawn to the Factor C according to claim 43, which is water-soluble.

Claim 54 (claims 62 and 63 dependent therefrom) is drawn to a horseshoe crab Factor C producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

The claims encompass non-functional horseshoe crab Factor C polypeptides.

Claim 55 is drawn to the Factor C according to claim 43, which is producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

(C) The state of the prior art; (D) The level of one of ordinary skill; and (E) The level of predictability in the art: While one of skill in the art would have expected a horseshoe crab Factor C polypeptide that is isolated from its natural source to have activity of Factor C, the specification supports a high level of unpredictability regarding

the activity of a horseshoe crab Factor C that is recombinantly expressed in a heterologous host cell. For example, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in a *Drosophila* S2 cell does not have Factor C activity (paragraph 132). Also, regarding the terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, solubility, and expression in a mammalian cell, the specification supports a high level of unpredictability regarding the terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, solubility, and expression in a mammalian cell of a horseshoe crab Factor C that is recombinantly expressed. For example, regarding the terminal sialic acid, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in Sf9 cells does not have any (α -2,3) linked terminal sialic acid, while Factor C that is recombinantly expressed in CHO and HEK cells contains (α -2,3) linked terminal sialic acid (paragraph 154). Regarding residual activity in the presence of sodium citrate, Figure 7 shows that Factor C that is recombinantly expressed in Sf9 cells has almost no residual activity in the presence of 21 mM sodium citrate, Factor C that is recombinantly expressed in CHO and HEK cells retains about 25% residual activity in the presence of 21 mM sodium citrate. Regarding molecular weight, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in Sf9 cells has a molecular weight of 109 kDa, while the same Factor C that is recombinantly expressed in CHO and HEK cells has a molecular weight of 128 or 124 kDa, respectively (Table 1). Regarding amino acid sequence, the specification fails to identify any alterations to the amino acid sequence of SEQ ID NO:

2 that can be made and still have the identity of a horseshoe crab Factor C polypeptide and as described above, the same horseshoe crab Factor C expressed in different host cells can have very different characteristics and even be non-functional. Regarding solubility, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in COS cells is insoluble (paragraph 11).

(F) The amount of direction provided by the inventor and (G) The existence of working examples: The specification discloses the following working examples of a horseshoe crab Factor C as encompassed by the claims, *i.e.*, Factor C isolated from *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* hemocyte lysate and *Tachypleus tridentatus* Factor C expressed in Sf9 insect cells, CHO cells, or HEK cells. Other than these working examples, the specification fails to disclose any other working examples of a horseshoe crab Factor C as encompassed by the claims and other than *Tachypleus tridentatus* Factor C expressed in Sf9 insect cells, CHO cells, or HEK cells, the specification fails to provide guidance regarding the combination of horseshoe crab Factor C and expression hosts, particularly mammalian expression hosts, that are useful in accordance with the invention. The specification further fails to disclose how to use non-functional horseshoe crab Factor C polypeptides as encompassed by claim 54.

In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, and the state of the prior art, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention. Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner

reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this

Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claim(s) 43, 44, 49, 50, 56, and 57 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on 6/9/2015; hereafter “Mizumura”).

Claims 43, 44, 49, and 50 are drawn to a horseshoe crab Factor C having activity of Factor C. According to the specification, “the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B” (spec at p. 36, paragraph 28).

Claim 56 is drawn to an endotoxin assay agent containing the Factor C according to claim 43.

Claim 57 is drawn to an endotoxin assay kit comprising the assay agent according to claim 56.

Regarding claims 43 and 44, Mizumura teaches recombinant production of a *Tachypleus tridentatus* factor C polypeptide using insect Sf9 cells as an expression host cell (Example 1, beginning at paragraph 81), which has activity to convert Factor B into activated Factor B as measured by proclotting enzyme-activating capacity (Example 2-2, beginning at paragraph 101).

Regarding claim 49, Mizumura teaches the amino acid sequence of *Tachypleus tridentatus* factor C is SEQ ID NO: 2, which has 100% sequence identity to the amino acid sequence of SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Regarding claim 50, Mizumura teaches the factor C is present in the culture broth (Example 1-2-2, paragraph 97) and thus the factor C is considered to be water-soluble.

Regarding claim 56, Mizumura teaches an endotoxin measuring agent comprising the *Tachypleus tridentatus* factor C polypeptide (paragraph 17).

Regarding claim 57, Mizumura teaches the endotoxin measuring agent may be provided as an endotoxin-measuring kit (paragraph 47).

This anticipates claims 43, 44, 49, 50, 56, and 57 as written.

10. Claim(s) 54, 62, and 63 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Koshiba et al. (*J. Biol. Chem.* 282:3962-3967, 2007; cited on the attached Form PTO-892; hereafter "Koshiba").

Claim 54 is drawn to a horseshoe crab Factor C producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

Claim 62 is drawn to an endotoxin assay agent containing the Factor C according to claim 54.

Claim 63 is drawn to an endotoxin assay kit comprising the assay agent according to claim 62.

Regarding claim 54, Koshiba teaches recombinant production of a *Tachypleus tridentatus* factor C polypeptide light chain and heavy chain using HEK293 cells (p. 3963, paragraph bridging columns 1 and 2).

Regarding claims 62 and 63, Koshiba teaches forming a complex of the factor C polypeptide heavy chain and LPS (p. 3964, Figure 2; p. 3966, paragraph bridging columns 1 and 2), which is considered to be an endotoxin assay agent and an endotoxin assay kit as encompassed by claims 62 and 63, respectively.

This anticipates claims 54, 62, and 63 as written.

Claim Rejections - 35 USC § 102/103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

11. Claim(s) 43-46, 48-50, 56, and 57 are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Nakamura et al. (*J. Biochem.* 99:847-857, 1986; cited on the IDS filed on 6/9/2015; hereafter “Nakamura”) as evidenced by Mizumura (*supra*). See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103. See MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 43-46 and 48-50 are drawn to a horseshoe crab Factor C having activity of Factor C. According to the specification, “the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B” (spec at p. 36, paragraph 28).

Claim 56 is drawn to an endotoxin assay agent containing the Factor C according to claim 43.

Claim 57 is drawn to an endotoxin assay kit comprising the assay agent according to claim 56.

Regarding claim 43, Nakamura teaches an activated factor C polypeptide purified from the hemocyte lysate of *Tachypleus tridentatus* (p. 848, sentence bridging columns 1 and 2). Nakamura teaches activation of factor B by the activated factor C (p. 851, column 2).

Regarding claim 44, according to the specification, 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 (paragraph 19). The recitation of "recombinant" is interpreted as a "product-by-process" limitation and because the claim does not limit the "host cell" from which the protein is heterologously expressed, the recitation of "recombinant" fails to structurally and/or functionally distinguish the claimed horseshoe crab factor C from Nakamura's factor C polypeptide.

Regarding claim 45, Nakamura does not teach the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* has (α -2,3) linked terminal sialic acid. However, the specification teaches that factor C polypeptide derived from *T. tridentatus* reacts with a (α -2,3)-linked terminal sialic acid-specific lectin (paragraph 153). As such, since Nakamura's activated factor C polypeptide is derived from *T. tridentatus*, it follows that Nakamura's activated factor C polypeptide necessarily has (α -2,3)-linked terminal sialic acid.

Regarding claim 46, Nakamura does not teach the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* has (α -2,3) linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell. However, as noted above, the specification teaches that factor C polypeptide

derived from *T. tridentatus* has (α -2,3)-linked terminal sialic acid (paragraph 153), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since factor C polypeptide derived from *T. tridentatus* has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Nakamura's activated factor C polypeptide purified from *T. tridentatus* necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 48, Nakamura does not teach the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* has a molecular weight as recited in the claim. However, the specification teaches that factor C polypeptide derived from *T. tridentatus* has a molecular weight of 124 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Nakamura's activated factor C polypeptide is derived from *T. tridentatus*, it follows that Nakamura's activated factor C polypeptide necessarily has a molecular weight of 124 kDa by SDS-PAGE under non-reducing conditions.

Regarding claim 49, Nakamura does not teach the amino acid sequence of the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *Tachypleus tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Regarding claim 50, Nakamura teaches the factor C has catalytic activity in an aqueous buffer (p. 852, Figure 6) and thus the factor C is considered to be water-soluble.

Regarding claims 56 and 57, Nakamura teaches reconstitution of the LPS-mediated clotting system, including the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus*, which is considered to be an endotoxin assay agent and an endotoxin assay kit as encompassed by claims 56 and 57, respectively.

This anticipates claims 43-46, 48-50, 56, and 57 as written.

12. Claim(s) 43-50, 54-57, 62, and 63 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Ding et al. (US 5,716,834; cited on the attached Form PTO-892; hereafter “Ding”) as evidenced by Mizumura (*supra*). See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103. See MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 43-50 and 55 are drawn to a horseshoe crab Factor C having activity of Factor C. According to the specification, “the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B” (spec at p. 36, paragraph 28).

Claim 54 is drawn to a horseshoe crab Factor C producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

Claims 56 and 62 are drawn to an endotoxin assay agent containing the Factor C according to claim 43 and 54, respectively.

Claims 57 and 63 are drawn to an endotoxin assay kit comprising the assay agent according to claims 56 and 62, respectively.

Regarding claims 43, 44, 54, and 55, the reference of Ding teaches a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-55). Ding teaches a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding teaches mammalian host cells include Chinese Hamster Ovary cells (column 19, lines 39-43). Ding teaches there are four species of horseshoe crab, including *T. tridentatus* (column 1, lines 18-20). Ding teaches activation of the Factor C by endotoxin (column 2, lines 7-9).

Regarding claim 45, Ding does not teach the activated factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid. However, the specification teaches that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid (paragraph 154). As such, since Ding teaches the embodiment of recombinantly expressing and recovering *T. tridentatus* factor C from CHO cells and activating the factor C by endotoxin, it follows that Ding's activated factor C polypeptide necessarily has (α -2,3)-linked terminal sialic acid.

Regarding claim 46, Ding does not teach the activated *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell. However, the specification teaches that factor C

polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph 154), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding's activated factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 47, Ding does not teach the activated *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. However, the Figure 7 of this application shows that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 10% sodium citrate with a dilution factor of 8, which, according to the specification is about 21 mM sodium citrate (p. 87). As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate, it follows that Ding's activated factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Regarding claim 48, Ding does not teach the activated *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight as recited in the claim. However, the specification teaches that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Ding's activated *T. tridentatus* factor C polypeptide is recombinantly expressed and recovered from CHO cells, it follows that Ding's activated factor C polypeptide necessarily has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions.

Regarding claim 49, Ding does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Regarding claim 50, Ding teaches the factor C can be secreted into the culture medium (column 3, lines 47-48) and thus the factor C is considered to be water-soluble.

Regarding claims 56, 57, 62, and 63, Ding teaches the recombinant Factor C may be employed in a chromogenic assay, wherein upon activation by endotoxin, the Factor C converts the substrate to a colored product, thereby detecting and quantifying endotoxin (column 2, lines 7-11), which is considered to be an endotoxin assay agent and an endotoxin assay kit as encompassed by claims 56, 57, 62, and 63 respectively.

This anticipates claims 43-46, 48-50, 56, and 57 as written.

13. Claim(s) 43-50, 54-57, 62, and 63 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Ding et al. (US 5,858,706; cited on the IDS filed on 6/9/2015; hereafter “Ding2”) as evidenced by Mizumura (*supra*). See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103. See MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 43-50 and 55 are drawn to a horseshoe crab Factor C having activity of Factor C. According to the specification, “the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B” (spec at p. 36, paragraph 28).

Claim 54 is drawn to a horseshoe crab Factor C producible by a method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

Claims 56 and 62 are drawn to an endotoxin assay agent containing the Factor C according to claim 43 and 54, respectively.

Claims 57 and 63 are drawn to an endotoxin assay kit comprising the assay agent according to claims 56 and 62, respectively.

Regarding claims 43, 44, 54, and 55, the reference of Ding2 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding2 teaches a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Ding2 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding2 teaches the cDNA for expression encodes a T.

tridentatus horseshoe crab Factor C (column 3, lines 14-20). Ding2 teaches activation of the Factor C by endotoxin (column 1, lines 44-47).

Regarding claim 45, Ding2 does not teach the activated factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid. However, the specification teaches that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid (paragraph 154). As such, since Ding2 teaches the embodiment of recombinantly expressing and recovering *T. tridentatus* factor C from CHO cells and activating the factor C by endotoxin, it follows that Ding2's activated factor C polypeptide necessarily has (α -2,3)-linked terminal sialic acid.

Regarding claim 46, Ding2 does not teach the activated *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell. However, the specification teaches that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph 154), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding2's activated factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily has a greater amount of (α -2,3)-

linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 47, Ding2 does not teach the activated *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. However, the Figure 7 of this application shows that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 10% sodium citrate with a dilution factor of 8, which, according to the specification is about 21 mM sodium citrate (p. 87). As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate, it follows that Ding2's activated factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Regarding claim 48, Ding2 does not teach the activated *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight as recited in the claim. However, the specification teaches that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Ding's activated *T. tridentatus* factor C polypeptide is recombinantly expressed and recovered from CHO cells, it follows that Ding2's

activated factor C polypeptide necessarily has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions.

Regarding claim 49, Ding2 does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Regarding claim 50, Ding2 teaches solubilization of the factor C (column 7, lines 456-53) and thus the factor C is considered to be water-soluble.

Regarding claims 56, 57, 62, and 63, Ding2 teaches the recombinant Factor C for use in an assay for endotoxin detection and teaches elements for conducting such an assay (column 8, lines 50-60), which is considered to be an endotoxin assay agent and an endotoxin assay kit as encompassed by claims 56, 57, 62, and 63 respectively.

This anticipates claims 43-46, 48-50, 56, and 57 as written.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

14. Claims 43-46, 48-50, 56, and 57 are rejected under 35 U.S.C. 101 because the claimed invention is directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more.

Claims 43-46 and 48-50 are drawn to a horseshoe crab Factor C having activity of Factor C. According to the specification, “the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B (spec at p. 36, paragraph 28).

Claim 56 is drawn to an endotoxin assay agent containing the Factor C according to claim 43.

Claim 57 is drawn to an endotoxin assay kit comprising the assay agent according to claim 56.

Given a broadest reasonable interpretation, the horseshoe crab Factor C of claims 43-46 and 48-50 encompasses a naturally occurring horseshoe crab Factor C, *e.g.*, activated factor C polypeptide present in the hemocytes of *T. tridentatus*. Also, the endotoxin assay agent and endotoxin assay kit of claims 56 and 57, which do not recite any additional element(s) of the endotoxin assay agent and endotoxin assay kit other than the horseshoe crab Factor C itself, encompass naturally occurring horseshoe crab hemocytes comprising an activated Factor C. Thus, the claims are considered to encompass naturally occurring products. Because there is no difference in characteristics (structural, functional, or otherwise) between the claimed and naturally occurring products, the claimed products do not have markedly different characteristics from what exists in nature, and thus are a “product of nature” exception. Accordingly, the claims are directed to a judicial exception. Because the claims do not include any additional features that could add significantly more to the exception, the claims do not qualify as eligible subject matter.

Conclusion

Status of the claims:

Claims 43-64 are pending.

Claims 51-53, 58-61, and 64 are withdrawn from consideration.

Claims 43-50, 54-57, 62, and 63 are rejected.

No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is 571-272-0942. The examiner can normally be reached on Mon to Fri, 7:30 am to 4:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David J. Steadman/
Primary Examiner, Art Unit 1656

APPENDIX A

AZZ33066
ID AZZ33066 standard; protein; 1019 AA.
XX
AC AZZ33066;
XX
DT 25-OCT-2012 (first entry)
XX
DE Tachypleus tridentatus factor C, SEQ ID 2.
XX
KW factor C; protein production; protein quantitation; BOND_PC;
KW limulus factor C precursor; limulus factor C; G04252; G05215; G05515;
KW G05529; G06508; G06810; G07155; G08233; G016020; G016021; G016787;
KW G042381.
XX
OS Tachypleus tridentatus.
XX
CC PN WO2012118226-A1.
XX
CC PD 07-SEP-2012.
XX
CC PF 28-FEB-2012; 2012WO-JP055728.
XX
PR 28-FEB-2011; 2011US-0447556P.
XX
CC PA (SEKG) SEIKAGAKU CORP.
XX
CC PI Mizumura H, Aizawa M, Oda T;
XX
DR WPI; 2012-L75079/61.
DR N-PSDB; AZZ33065, AZZ33071.
DR PC:NCBI; gi585398.
DR PC:SWISSPROT; P28175.
XX
CC PT Agent useful for measuring endotoxin, comprises factor C derived from
CC PT Tachypleus tridentatus, factor B of horseshoe crab and proclotting enzyme
CC PT of horseshoe crab.
XX
CC PS Claim 3; SEQ ID NO 2; 72pp; English.
XX
CC The present invention relates to a novel agent, useful for measuring
CC endotoxin. The agent comprises: (a) a factor C derived from Tachypleus
CC tridentatus, wherein factor C does not have his-tag sequence at the C-
CC terminal region; (b) a factor B derived from horseshoe crab; and (c) a
CC proclotting enzyme derived from horseshoe crab. The invention also
CC provides: a method for producing the endotoxin measuring agent by (a)
CC incorporating a DNA sequence encoding the factor C, factor B and
CC proclotting enzyme, into a viral DNA, (b) infecting insect cells with the
CC virus incorporated with the DNA, (c) allowing the infected insect cells
CC to express the protein; and a method for measuring endotoxin in a test
CC sample. The present sequence represents a specifically claimed Tachypleus
CC tridentatus factor C, which can be produced by the above mentioned
CC endotoxin measuring agent production method, for measuring endotoxin.
CC
CC Revised record issued on 18-OCT-2012 : Enhanced with precomputed
CC information from BOND.
XX
SQ Sequence 1019 AA;

Query Match 100.0%; Score 5619; DB 19; Length 1019;
Best Local Similarity 100.0%;
Matches 1019; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 MVLASFLVSGLVGLGILAQQMRPVQSRGVDLGLCDETRFECKCGDPGYVFNVPKQCTYFY 60
|||||

Art Unit: 1656

Db		1	MVLASFLVSGLVGLGILAQQMRPVQSRGVDLGLCDETRFECKCGDPPGYVFNVPMKQCTYFY	60
Qy		61	RWRPYCKPCDDLEAKDICPKYKRCQECKAGLDSCVTCPPNKYGTWCSGECQCKNGGICDQ	120
Db		61	RWRPYCKPCDDLEAKDICPKYKRCQECKAGLDSCVTCPPNKYGTWCSGECQCKNGGICDQ	120
Qy		121	RTGACTCRDRYEGAHCEILKGCPLLPDSQVQEVNRNPPDNPQTIDYSCSPGFKLKGVAR	180
Db		121	RTGACTCRDRYEGAHCEILKGCPLLPDSQVQEVNRNPPDNPQTIDYSCSPGFKLKGVAR	180
Qy		181	SCLPNGQWSSFPKCIKRECAKVSSPEHGKVNAPSGNMIEGATLRFSCDSPYYLIQETLT	240
Db		181	SCLPNGQWSSFPKCIKRECAKVSSPEHGKVNAPSGNMIEGATLRFSCDSPYYLIQETLT	240
Qy		241	CQGNQWQSGQIPQCKKLVFPCPLDPVNHAEHQVKIGVEQKYGQFPQGTETVYTCSGNYFL	300
Db		241	CQGNQWQSGQIPQCKKLVFPCPLDPVNHAEHQVKIGVEQKYGQFPQGTETVYTCSGNYFL	300
Qy		301	MGFNTLKCNPDGWSGSGQSPSCVKVADREVDCCSKAVDFLDDVGEPVRIHCPAGCSLTAGT	360
Db		301	MGFNTLKCNPDGWSGSGQSPSCVKVADREVDCCSKAVDFLDDVGEPVRIHCPAGCSLTAGT	360
Qy		361	VWGTAIYHELSSVCRAAIHAGKLPNSGGAVHVNNNGPYSDFLGSDNLNGIKSEELKSLARS	420
Db		361	VWGTAIYHELSSVCRAAIHAGKLPNSGGAVHVNNNGPYSDFLGSDNLNGIKSEELKSLARS	420
Qy		421	FRFDYVSSSTAGRSGCPDGWFEVEENCYVVTISKQRAWERAQGVCTNMAARLAVLDDKDLIP	480
Db		421	FRFDYVSSSTAGRSGCPDGWFEVEENCYVVTISKQRAWERAQGVCTNMAARLAVLDDKDLIP	480
Qy		481	SSLTETLRGKGLTTTWIGLHRLDAEKPFVWELMDRSNVVLDNLTFWASGEPGNETNCVY	540
Db		481	SSLTETLRGKGLTTTWIGLHRLDAEKPFVWELMDRSNVVLDNLTFWASGEPGNETNCVY	540
Qy		541	LDIRDQLQPWVKTKSCFQPSFACMMDLSDRNKAKCDDPGPLENGHATLHGQSIDGFYAG	600
Db		541	LDIRDQLQPWVKTKSCFQPSFACMMDLSDRNKAKCDDPGPLENGHATLHGQSIDGFYAG	600
Qy		601	SSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKIVITCQNPVPVPSYGSVEIKPPSRTN	660
Db		601	SSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKIVITCQNPVPVPSYGSVEIKPPSRTN	660
Qy		661	SISRVGSPFLRLPRLPLPLARAAKPPPKPRSSQPSSTDVLASKVKLPEGHYRVGSRAIYTC	720
Db		661	SISRVGSPFLRLPRLPLPLARAAKPPPKPRSSQPSSTDVLASKVKLPEGHYRVGSRAIYTC	720
Qy		721	ESRYEYELLSQGRRCDNSNGNSGRPASCPVCGRSDSPRSPFIWNGNSTEIGQWPWQAGI	780
Db		721	ESRYEYELLSQGRRCDNSNGNSGRPASCPVCGRSDSPRSPFIWNGNSTEIGQWPWQAGI	780
Qy		781	SRWLADHNMWFLQCGGSLNLEKWIIVTAAHCVTYSATAEIIDPSQFKIYLGKYYRDDSDD	840
Db		781	SRWLADHNMWFLQCGGSLNLEKWIIVTAAHCVTYSATAEIIDPSQFKIYLGKYYRDDSDD	840
Qy		841	DYVQVREALEIHVNPNDYDGNLNFIDIALIQLKTPVTLTTRVQPICLPTDITTREHLKEGT	900
Db		841	DYVQVREALEIHVNPNDYDGNLNFIDIALIQLKTPVTLTTRVQPICLPTDITTREHLKEGT	900
Qy		901	LAVVTGWGLNENNTYSEMIQAVLPVVAASTCEEYKEADLPLTVTENMFCAGYKKGGRYD	960
Db		901	LAVVTGWGLNENNTYSEMIQAVLPVVAASTCEEYKEADLPLTVTENMFCAGYKKGGRYD	960
Qy		961	ACSGDGGGLPVFADDSRTERRVWLEGIVSWGSPSGCGKANQYGGFTKVNVLFSWIRQFI	1019
Db		961	ACSGDGGGLPVFADDSRTERRVWLEGIVSWGSPSGCGKANQYGGFTKVNVLFSWIRQFI	1019

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Docket No: Q220395
Hikaru MIZUMURA et al.	Confirmation No.: 1531
Appln. No.: 14/650,767	Group Art Unit: 1656
Filed: June 9, 2015	Examiner: Steadman, David
For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN	

AMENDMENT UNDER 37 C.F.R. § 1.111

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Action dated September 16, 2016, please amend the
above-identified Application as follows on the accompanying pages.

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AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Page 78, paragraph [0115] is amended as follows:

[0115]

The Factor C expression plasmid was incorporated into cultured HEK293 cells through transfection using polyethylenimine (product of Sigma) (Hashiguti *et al.*, Expression of Recombinant Protein Using Cultured Human Cells -Standard Protocol by 293-type cells-, PSSJ Archives, 1, e017 (2008), www.pssj.jp/archives/Protocol/Expression/293_01/293_01_01.html~~http://www.pssj.jp/archives/Protocol/Expression/293_01/293_01_01.html~~). HEK293 cells were statically cultured in a DMEM medium (containing 10% fetal bovine serum and 2mM L-glutamine) (product of Sigma) at 37°C under feeding of 5% CO₂ without replacement of the medium. Four days after transfection, the culture broth was recovered, and was centrifuged (3,300×g, 30 minutes, 4°C), to thereby recover a culture supernatant. The supernatant was filtered through a 0.22-μm filter, and the thus-recovered fraction (i.e. filtrate) was employed as recombinant Factor C (HEK).

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1-42. (Canceled).

43. (Currently Amended): A horseshoe crab Factor C having activity of Factor C₁ which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*.

44-46. (Cancelled).

47. (Previously Presented): The Factor C according to claim 43, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

48. (Currently Amended): The Factor C according to claim 43, which has a molecular weight of 115 kDa to 140 kDa ~~or a molecular weight of 127 kDa $\pm$ 5 kDa~~, as measured through SDS-PAGE under a non-reducing condition.

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(Attorney Docket No. Q220395)

49. (Previously Presented): The Factor C according to claim 43, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

50. (Previously Presented): The Factor C according to claim 43, which is water-soluble.

51. (Withdrawn): A method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

52. (Withdrawn): The method according to claim 51, wherein the mammalian cell is selected from the group consisting of:

- (A) a mammalian cell other than COS-1;
- (B) a cell of a mammal selected from the group consisting of a primate and a rodent, other than monkey;

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(Attorney Docket No. Q220395)

- (C) a Chinese hamster cell or a human cell;
- (D) CHO or HEK; and
- (E) CHO DG44 or HEK293.

53. (Withdrawn): The method according to claim 51, wherein the Factor C is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

54. (Currently Amended): A horseshoe crab Factor C having activity of Factor C producible by ~~the method according to claim 51~~ a method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C using a mammalian cell as a host cell,

wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*,

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(Attorney Docket No. Q220395)

wherein the mammalian cell is selected from the group consisting of a Chinese hamster cell and a human cell.

55-56. (Cancelled).

57. (Currently Amended): An endotoxin assay kit comprising ~~the~~ an endotoxin assay agent containing the Factor C according to claim ~~56~~43 and one or more elements.

58. (Withdrawn): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the assay agent according to claim 56 and the test specimen, and a step of measuring the progress of a cascade reaction.

59. (Withdrawn): The method according to claim 58, wherein the test specimen contains at least one of:

- (A) an ion;
- (B) a cation;
- (C) a metal ion;
- (D) an alkali metal ion or an alkaline earth metal ion; and
- (E) one or more kinds of ions selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

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60. (Withdrawn): The method according to claim 59, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 1 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

61. (Withdrawn): The method according to claim 58, wherein the test specimen is an injection.

62. (Cancelled).

63. (Currently Amended): An endotoxin assay kit comprising ~~the~~ an endotoxin assay agent containing the Factor C according to claim ~~62~~54 and one or more elements.

64. (Withdrawn): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the assay agent according to claim 62 and the test specimen, and a step of measuring the progress of a cascade reaction.

65. (New): The Factor C according to claim 48, which has a molecular weight of 127 kDa $\pm$ 5 kDa.

66. (New): The Factor C according to claim 43, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

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67. (New): The Factor C according to claim 43, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

68. (New): The Factor C according to claim 43, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

69. (New): The Factor C according to claim 43, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

70. (New): The Factor C according to claim 54, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

71. (New): The Factor C according to claim 54, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

72. (New): The Factor C according to claim 54, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

73. (New): The Factor C according to claim 54, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

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74. (New): The Factor C according to claim 54, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

75. (New): The Factor C according to claim 54, which has a molecular weight of 115 kDa to 140 kDa as measured through SDS-PAGE under a non-reducing condition.

76. (New): The Factor C according to claim 75, which has a molecular weight of 127 kDa $\pm$ 5 kDa.

77. (New): The Factor C according to claim 54, which is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

78. (New): The Factor C according to claim 54, which is water soluble.

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REMARKS

The specification has been amended to remove a hyperlink. Claims 43, 47-54, 57-61, 63-64 and 65-78 are all the claims pending in the application. Claims 43, 48, 54, 57 and 63 are amended; claims 1-42, 44-46, 55-56 and 62 are cancelled; claims 51-53, 58-61 and 64 are withdrawn; and claims 65-78 are newly added. Support for the amendments to claim 43 can be found, *inter alia*, in original claim 46 and at paragraph [0020] of the specification as filed. Claim 48 is amended to remove a preferred embodiment. Support for the amendments to claim 54 can be found, *inter alia*, in original claim 51 and at paragraphs [0020], [0016] and [0038] of the specification as filed. Support for the amendments to claim 57 can be found, *inter alia*, at paragraph [0077] of the specification as filed. Support for the amendments to claim 63 can be found, *inter alia*, in original claim 53 and at paragraph [0077] of the specification as filed. Support for new claims 65-78 can be found, *inter alia*, at claim 48; [1.2.3] in [0017]; [1.2.5] in [0017]; [1.2.7] in [0017]; [1.2.9] in [0017]; [3.3.1] in [0017]; [3.3.3] in [0017]; [3.3.5] in [0017]; [3.3.7] in [0017]; [3.3.9] in [0017]; [3.5.1] in [0017]; [3.5.5] in [0017]; [2.2.1] in [0017]; and [3.6] in [0017], respectively. No new matter is added. Entry and consideration of this Amendment are respectfully requested.

I. Specification

In paragraph 6, on page 3 of the Office Action, the disclosure is objected to because it contains an embedded hyperlink.

The hyperlink has been deleted.

II. Rejections under 35 U.S.C. §112

A. In paragraph 7, on page 4 of the Office Action, claims 43-50, 54-57, 62, and 63 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

According to the Examiner, the specification does not have a sufficient written description of all possible horseshoe crab factor C proteins that fall within the claims. In particular, the Examiner asserts that the claims do not require the factor C proteins to have any particular structure, and to the extent that specific factor C proteins are disclosed in the specification, there is insufficient information to define a common structure. To the extent other characteristics of the claimed factor C are specified (terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, solubility, and expression in a mammalian cell), the Examiner asserts that the characteristics are not sufficient to predict that a protein falling within the claim will have factor C activity.

Without agreeing with or acquiescing to the merits of the rejection, the rejection is overcome by amending independent claims 43 and 54 to recite that the horseshoe crab is *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, or *Tachypleus gigas*. Furthermore, claim 43 is amended to recite that the Factor C has (α -2,3)-linked terminal sialic acid, and claim 54 is amended to recite that the mammalian cell is a Chinese hamster cell (superordinate concept of CHO) or a human cell (superordinate concept of HEK).

Accordingly, the Examiner is requested, respectfully, to reconsider and remove this rejection.

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B. In paragraph 8, on page 9 of the Office Action, claims 43-50, 54-57, 62 and 63 are rejected under 35 U.S.C. §112, first paragraph, as lacking enablement in the specification. While the Examiner admits that the specification in enabling for factor C isolated from *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* hemocyte lysate and *Tachypleus tridentatus* factor C expressed in Sf9 insect cells, CHO cells, or HEK cells, the Examiner asserts that the specification does not reasonably provide enablement for all horseshoe crab factor C polypeptides as encompassed by the claims.

Without agreeing with or acquiescing to the merits of this rejection, Applicant submits that the amendments to claims 43 and 54, as discussed above, overcome this rejection.

Reconsideration is requested, respectfully.

III. Rejections under 35 U.S.C. §102/103(a)

A. In paragraph 9, on page 14 of the Office Action, claims 43, 44, 49, 50, 56 and 57 are rejected under 35 U.S.C. §102(b) as being anticipated by Mizumura et al. (WO 2012/118226; hereafter "Mizumura").

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Mizumura discloses Factor C derived from *Tachypleus tridentatus* "expressed using insect cells." In paragraph [0049], Mizumura teaches that the insect cells are preferably Sf9. Mizumura does not disclose any Factor C expressed using a mammalian cell, as recited in amended claim 54.

Paragraph [0154] of the present application states as follows: "The above analysis indicates that the N-type sugar chain of recombinant Factor C expressed in mammalian cells

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(CHO and HEK) contains (α -2,3)-linked terminal sialic acid, and that the N-type sugar chain of recombinant Factor C expressed in insect cells (Sf9) contains no (α -2,3)-linked terminal sialic acid."

Therefore, the Factor C in independent claim 43 is different from the Factor C disclosed in Mizumura, and is novel there over.

B. In paragraph 10, on page 16 of the Office Action, claims 54, 62, and 63 are rejected under 35 U.S.C. §102(b) as being anticipated by Koshiba et al. (J. Biol. Chem., 282:3962-3967 (2007); hereafter "Koshiba").

Regarding claim 54, the Examiner asserts that Koshiba teaches recombinant production of a *Tachypleus tridentatus* factor C polypeptide light chain and heavy chain using HEK293 cells.

Regarding claims 62 (now cancelled) and 63, the Examiner asserts that Koshiba teaches forming a complex of the factor C polypeptide heavy chain and LPS (page 3964, Figure 2; page 3966, paragraph bridging columns 1 and 2), which the Examiner considers to be an endotoxin assay agent and an endotoxin assay kit.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Koshiba discloses recombinant production of a *Tachypleus tridentatus* Factor C polypeptide light chain and heavy chain using HEK293 cells (page 3963, paragraph bridging columns 1 and 2).

Paragraph [0005] of the present specification describes that "When endotoxin comes into contact with the lysate, Factor C, which is a serine protease zymogen present in the lysate, is

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activated to thereby form activated Factor C." Accordingly, Factor C having activity of Factor C inevitably has both of endotoxin-binding domain and protease domain.

Koshiba describes several Factor C variants, all of which are a "truncated form of Factor C." (page 3963, *Cloning and Mutagenesis*). Koshiba discloses that protease domain of *Tachypleus tridentatus* Factor C consists of the amino acid residues from 738 to 994, which exists in its C-terminal (page 3963, *Cloning and Mutagenesis*). Koshiba discloses that endotoxin-binding domain of *Tachypleus tridentatus* Factor C exists in its N-terminal (page 3967, *Implications for Innate Immunity*). None of Factor C disclosed in Koshiba has both of an endotoxin-binding domain and a protease domain. Accordingly, Factor C disclosed in Koshiba does not have "activity of Factor C" as claimed.¹

Therefore, the Factor C in independent claim 54 is different from the Factor C disclosed in Koshiba, and is novel there over.

C. In paragraph 11, on page 17 of the Office Action, claims 43-46, 48-50, 56, and 57 are rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Nakamura et al. (J. Biochem., 99:847-857 (1986); hereafter "Nakamura") as evidenced by Mizumura (*supra*).

¹ Paragraph [0028] of the specification states that "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B." In other words, the activity of Factor C of the present invention is the "protease activity." Note that the "activity of Factor C" in the present invention is not equal to "the ability to bind LPS (Lipopolysaccharide, also known as endotoxin)" as defined by Koshiba (infra).

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According to the Examiner, Nakamura teaches an activated factor C polypeptide purified from the hemocyte lysate of *Tachypleus tridentatus*. With regard to claim 44 (now cancelled), the Examiner states that claim 44 is a product by process claim and, because the host cell is not specified, claim 44 does not further limit the factor C of claim 43. Regarding the remaining dependent claims, the Examiner asserts that because the factor C is purified from *Tachypleus tridentatus*, it has all of the characteristics of the proteins described in the present specification.

Mizumura is cited to allegedly show that the amino acid sequence of *Tachypleus tridentatus* factor C is identical to SEQ ID NO: 2.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Nakamura discloses purified "natural" Factor C of *Tachypleus tridentatus*, and does not disclose a recombinantly expressed Factor C of *Tachypleus tridentatus* using a mammalian cell as a host cell.

Paragraph [0153] of the present specification discloses that "MAM ((α -2,3)-linked terminal sialic acid-specific lectin) was strongly bonded to CHO and less strongly bonded to HEK. TAL and fetuin have almost the same weak reactivity, but did not react with Sf9." In paragraph [0018] in the specification, it is described that TAL is a native Factor C derived from *Tachypleus tridentatus*. Accordingly, a horseshoe crab Factor C expressed using a mammalian cell as a host cell contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with TAL, which is the same protein as the Factor C disclosed in Nakamura.

Therefore, the Factor C in independent claim 43 is different from the Factor C disclosed in Nakamura, and thereby novel and unobvious thereover.

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D. In paragraph 12, on page 20 of the Office Action claims 43-50, 54-57, 62 and 63 are rejected under 35 U.S.C. §102(b) as being anticipated by Ding et al. (U.S. Patent 5,716,834; hereafter "Ding") as evidenced by Mizumura (*supra*).

Regarding claims 43, 44 (now cancelled), 54, and 55 (now cancelled), the Examiner asserts that Ding teaches a mammalian host cell transformed with a recombinant vector for expressing a factor C. The Examiner further asserts that Ding teaches that the host cell can be CHO cells and the factor C can be derived from *Tachypleus tridentatus*.

Regarding claim 45 (now cancelled), the Examiner admits that Ding does not teach the activated factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid. However, according to the Examiner, this characteristic would be inherent in any factor C from *T. tridentatus* expressed in CHO cells. The Examiner takes a similar position with regard to (now cancelled) claim 46 (the factor C has (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab factor C expressed in Sf9 as a host cell), claim 47 (the factor C exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate), and claim 48 (the factor C has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions).

Regarding claim 49, The Examiner admits that Ding does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, Mizumura is cited as evidence to show that the amino acid sequence of *T. tridentatus* factor C is allegedly identical to SEQ ID NO: 2.

Regarding claim 50, the Examiner asserts that Ding teaches that the factor C can be secreted into the culture medium and thus the factor C is considered to be water-soluble.

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Regarding claims 56 (cancelled), 57, 62 (cancelled), and 63, the Examiner asserts that Ding teaches the recombinant factor C may be employed in a chromogenic assay, wherein upon activation by endotoxin, the factor C converts the substrate to a colored product, thereby detecting and quantifying endotoxin, which the Examiner considers to be an endotoxin assay agent and an endotoxin assay kit.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Ding discloses a mammalian cell transformed with a recombinant vector comprising DNA encoding an amino acid sequence of *Carcinoscorpius rotundicauda* Factor C. However, Ding neither discloses nor suggests any Factor C "having activity of Factor C," as claimed.

For example, Ding prepared a recombinant vector comprising DNA encoding an amino acid sequence of *Carcinoscorpius rotundicauda* Factor C in working example 8. However, Ding does not practically prepare a recombinant cell having ability for expressing Factor C having activity of Factor C. Moreover, Ding does not show any scientific evidence for presuming that any Factor C in Ding has activity of Factor C, rather than endotoxin-binding activity. For example, in working example 12, endotoxin binding activity was evaluated. However, activity of Factor C was not evaluated.

Therefore, the Factor C in independent claims 43 and 54 is different from the Factor C disclosed in Ding, and claims 43 and 54 are novel there over.

E. In paragraph 13 on page 24 of the Office Action, claims 43-50, 54-57, 62 and 63 are rejected under 35 U.S.C. §102(b) as being anticipated by Ding et al. (U.S. Patent 5,858,706; hereafter "Ding2") as evidenced by Mizumura (*supra*).

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Regarding claims 43, 44 (cancelled), 54, and 55 (cancelled), the Examiner asserts that Ding2 teaches a mammalian host cell transformed with a recombinant vector for expressing a factor C. The Examiner further asserts that Ding teaches that the host cell can be CHO cells and the factor C can be derived from *Tachypleus tridentatus*.

Regarding claims 45 - 48 (45 and 46 are cancelled), the Examiner admits that Ding2 does not teach the additional recitations. However, according to the Examiner, these characteristics would be inherent in any factor C from *T. tridentatus* expressed in CHO cells.

Regarding claim 49, the Examiner relies on Mizumura as allegedly teaching the amino acid sequence of *T. tridentatus*.

Regarding claim 50, the Examiner asserts that Ding2 teaches solubilization of the factor C and the Examiner considers this to mean that the factor C is water-soluble.

Regarding claims 56 (cancelled), 57, 62 (cancelled) and 63, the Examiner asserts that Ding2 teaches the recombinant factor C for use in an assay for endotoxin detection and teaches elements for conducting such an assay, which is considered to be an endotoxin assay agent and an endotoxin assay kit.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Ding2 discloses a mammalian cell transformed with a recombinant vector comprising DNA encoding an amino acid sequence of *Carcinoscorpius rotundicauda* Factor C, as does Ding (supra). In Ding2, a recombinant Factor C "having endotoxin-binding activity" is disclosed. However, Ding 2 never discloses nor suggests a recombinant Factor C "having activity of Factor C," as claimed.

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In Ding2, cDNA of *Carcinoscorpius rotundicauda* Factor C (CrFC21) was cloned into mammalian expression vector. However, production of a recombinant Factor C was conducted using a yeast cell, and not conducted using a mammalian cell.

The subject of Ding 2 is to offer a Factor C "having endotoxin-binding activity." Thus, the subject of Ding 2 is not to offer a Factor C "having activity of Factor C."

That is, in Ding2, other substantial applications than endotoxin-binding are not disclosed as an application of recombinant Factor C. Moreover, Ding2 argues an advantage of his invention by comparison with a clotting reaction using LAL, which is equal to the cascade reaction in the present application. Furthermore, in paragraph 3 of the Detailed Description of the Invention in Ding2, it is described that the "rCrFC can be purified by affinity methods using gram-negative LPS as the affinity reagent."

In paragraph [0005] of the present specification, it is explained that "when endotoxin comes into contact with the lysate, Factor C, which is a serine protease zymogen present in the lysate, is activated to thereby form activated Factor C. The thus-formed activated Factor C activates Factor B present in the lysate, to thereby form activated Factor B." That is, Factor C is a protease zymogen which matures into activated Factor C when contact with endotoxin. Accordingly, if rCrFC in Ding2 was Factor C having activity of Factor C, rCrFC purified by affinity methods using gram-negative LPS as the affinity reagent should be activated Factor C, rather than Factor C (protease zymogen).

Activated Factor C already expresses activity for transforming Factor B into activated Factor B without depending on the presence or absence of endotoxin. Accordingly, activated Factor C cannot be used for endotoxin detection based on protease activity. That is, if rCrFC in

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Ding2 was Factor C having activity of Factor C, rCrFC could not be used for endotoxin detection based on protease activity. Therefore, at the time of the invention, one of ordinary skill in the art would readily recognize that a recombinant Factor C in Ding2 has endotoxin-binding activity, but does not have activity of Factor C.

In addition, a subsequent application of Ding (US 6,645,724) describes in paragraph 3 of the Related Art that "also, it was found that LPS could not activate the enzymatic activity of yeast rFC, thus, a direct enzyme-based LPS detection is not possible using rFC produced in yeast." That is, the inventor (Ding) himself admits that a recombinant Factor C disclosed in Ding2 does not have activity of Factor C, and thereby cannot be used for endotoxin detection based on enzymatic activity.

Therefore, in view of the description in Ding2 and also based on scientific facts, Factor C having activity of Factor C is not disclosed in Ding2. Moreover, specific means for producing Factor C having activity of Factor C is neither disclosed nor suggested in Ding2.

In conclusion, the Factor C in independent claims 43 and 54 is different from the Factor C disclosed in Ding2, and claims 43 and 54 are novel there over.

IV. Rejection under 35 U.S.C. §101

In paragraph 14 at page 27 of the Office Action, claims 43-46, 48-50, 56, and 57 are rejected under 35 U.S.C. §101 because the claimed invention allegedly is directed to a judicial exception without significantly more.

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According to the Examiner, given a broadest reasonable interpretation, the horseshoe crab factor C of claims 43-46 and 48-50 encompasses a naturally occurring horseshoe crab factor C.

Applicant submits that the amended claims 45 and 54 do not encompass naturally occurring Factor C. Claim 43 recites that the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a native horseshoe crab Factor C and claim 54 recites a Factor C expressed in a mammalian host cell.

Accordingly, the Examiner is requested, respectfully, to reconsider and remove this rejection.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The U.S. Patent and Trademark Office is hereby directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/Alan C. Townsley/ Reg. No. 64,740 *for*

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/650,767	06/09/2015	Hikaru MIZUMURA	Q220395	1531
23373 7590 04/13/2017 SUGHRUE MION, PLLC 2100 PENNSYLVANIA AVENUE, N.W. SUITE 800 WASHINGTON, DC 20037			EXAMINER STEADMAN, DAVID J	
			ART UNIT 1656	PAPER NUMBER
			NOTIFICATION DATE 04/13/2017	DELIVERY MODE ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No. 14/650,767	Applicant(s) MIZUMURA ET AL.	
	Examiner DAVID J. STEADMAN	Art Unit 1656	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 03/16/2017.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 43,47-54,57-61 and 63-78 is/are pending in the application.
5a) Of the above claim(s) 51-53,58-61 and 64 is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☒ Claim(s) 43,47-50,54,57,63 and 65-78 is/are rejected.
- 8) ☐ Claim(s) ____ is/are objected to.
- 9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☒ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|--|
| 1) ☐ Notice of References Cited (PTO-892) | 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____ |
| 2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date ____ | 4) ☐ Other: ____ |

DETAILED ACTION

Status of the Application

1. The present application is being examined under the pre-AIA first to invent provisions.
2. Claims 43, 47-54, 57-61, and 63-78 are pending in the application.
3. Applicant's amendment to the claims, filed on 3/16/2017, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.
4. Applicant's amendment to the specification, filed on 3/16/2017, is acknowledged.
5. Applicant's remarks filed on 3/16/2017 in response to the non-final rejection mailed on 9/16/2016 have been fully considered.
6. Rejections previously applied to claims 44-46, 55, 56, and 62 are withdrawn solely in view of the instant amendment to cancel these claims.
7. The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Election/Restriction

8. Claims 51-53, 58-61, and 64 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on 8/24/2016.

9. Claims 43-50, 54-57, 62, and 63 are being examined on the merits with claim 49 being examined only to the extent the claim reads on the elected subject matter of the species of SEQ ID NO: 2.

Information Disclosure Statement

10. The information disclosure statement (IDS) submitted on 10/26/2016 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Specification/Informalities

11. The objection to the disclosure because it contains an embedded hyperlink and/or other form of browser-executable code is maintained for the reasons of record and the reasons set forth below. Applicant's amendment to the specification to remove "http://" is acknowledged, however, the disclosure of "www.pssj.jp..." is still considered to be browser executable code. In the interest of compact prosecution, applicant may consider amending the "www.pssj.jp..." to "available on the world wide web at pssj.jp..."

Claim Objection

12. Claim 54 is objected to in the recitation of "Chinese hamster cell" and in the interest of improving claim form, it is suggested that the noted phrase be amended to recite, *e.g.*, "Chinese hamster ovary cell".

Claim Rejections - 35 USC § 112, Fourth Paragraph

The following is a quotation of pre-AIA 35 U.S.C. 112, fourth paragraph:

Subject to the following paragraph [i.e., the fifth paragraph of pre-AIA 35 U.S.C. 112], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

13. Claims 49 and 77 are rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. This rejection is necessitated by applicant's amendment to claims 43 and 54 to recite "wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*".

As amended, the horseshoe crab Factor C of claims 43 and 54 is limited to a *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, or *Tachypleus gigas* Factor C. In view of the recitation of "but which includes substitution, deletion, insertion, or addition of one or several amino acid residues", the amino acid sequences encompassed by claims 49 and 77 are unlimited and thus do not further limit claims 43 and 54, respectively. Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements. Applicant may consider amending claims 49 and 77 to delete parts (B) and (D).

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

14. Claims 49 and 77 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention. This rejection is necessitated by applicant's amendment to claims 43 and 54 to recite "wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*".

As amended, the horseshoe crab Factor C of claims 43 and 54 is limited to a *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, or *Tachypleus gigas* Factor C. However, in view of the recitation of "but which includes substitution, deletion, insertion, or addition of one or several amino acid residues" in claims 49 and 77, the amino acid sequences encompassed by claims 49 and 77 (which depend from claims 43 and 54, respectively) are unlimited and thus it is unclear as to the scope of Factor C polypeptides that are intended as being encompassed by claims 49 and 77. It is suggested that applicant clarify claims 49 and 77. In the interest of compact prosecution, applicant may consider amending claims 49 and 77 to delete parts (B) and (D).

Claim Rejections - 35 USC § 112, First Paragraph

15. The written description and scope of enablement rejections of claims 43, 47-50, 54, 57, and 63 under 35 U.S.C. 112 (pre-AIA), first paragraph, are withdrawn in view of applicant's amendment to claims 43 and 54 to recite "A horseshoe crab Factor C...wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*". Recombinant expression of Factor C polypeptides of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas* were well-known at the time of the invention. Also, it was well-known in the prior art at the time of the invention that different cell types add a sialic acid to an expressed polypeptide using only a particular linkage, *e.g.*, Chinese Hamster Ovary (CHO) cells are only able to add sialic acid using an α -2,3 linkage (see, *e.g.*, paragraph 8 of Cottingham et al., US 2014/0249082, which has priority to 2010, cited on the IDS filed on 10/26/2016). As such, one of skill in the art would recognize that the specification in view of the state of the art at the time of the invention adequately describes and enables the full scope of the claimed horseshoe crab Factor C polypeptides.

Claim Rejections - 35 USC § 102

16. The rejection of claims 43, 49, 50, and 57 under pre-AIA 35 U.S.C. 102(b) as being anticipated by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on 6/9/2015; hereafter "Mizumura") is withdrawn in view of applicant's amendment to claim 43 to recite "which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C

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expressed using Sf9 as a host cell". According to the specification, a *Tachypleus tridentatus* Factor C polypeptide recombinantly produced using insect Sf9 cells as an expression host does not react with a (α -2,3)-linked terminal sialic acid-specific lectin (specification at paragraph 153), indicating that (α -2,3)-linked terminal sialic acid is not present on the Sf9 cell-produced *T. tridentatus* Factor C polypeptide. Further according to the specification, a native *Tachypleus tridentatus* Factor C polypeptide reacts with a (α -2,3)-linked terminal sialic acid-specific lectin (specification at paragraph 153), indicating that (α -2,3)-linked terminal sialic acid is present on the native *T. tridentatus* Factor C polypeptide. As such, Mizumura's Sf9 cell-produced *T. tridentatus* Factor C polypeptide *does not* contain (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, and thus does not satisfy all claim limitations.

17. The rejection of claims 54, 62, and 63 under pre-AIA 35 U.S.C. 102(b) as being anticipated by Koshiba et al. (*J. Biol. Chem.* 282:3962-3967, 2007; cited on Form PTO-892; hereafter "Koshiba") is withdrawn in view of the amendment to claim 54 to recite "having activity of Factor C". According to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (specification at p. 36, paragraph 28), which as noted by applicant, is considered to be the "protease activity" of Factor C, and does not refer to the ability to bind to LPS (remarks at p. 14, footnote). Koshiba does not teach a

horseshoe crab Factor C having "Factor C activity" as defined according to the specification.

Claim Rejections - 35 USC § 102/103

18. The rejection of claims 43, 48-50, and 57 under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Nakamura et al. (*J. Biochem.* 99:847-857, 1986; cited on the IDS filed on 6/9/2015; hereafter "Nakamura") as evidenced by Mizumura (*supra*) is maintained for the reasons of record and the reasons set forth below. The rejection has been modified to address applicant's amendment to the claims.

Claims 43, 48-50, 57, and 65 are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Nakamura (*supra*) as evidenced by Mizumura (*supra*).

Claims 43, 48-50, and 65 are drawn to a horseshoe crab Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*.

Claim 57 is drawn to an endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 43 and one or more elements.

Regarding claim 43, Nakamura teaches an activated Factor C polypeptide purified from the hemocyte lysate of *Tachypleus tridentatus* (p. 848, sentence bridging columns 1 and 2). Nakamura teaches activation of factor B by the activated Factor C (p. 851, column 2), and thus Nakamura's purified Factor C has "activity of Factor C".

Nakamura does not teach the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell. However, according to the specification, Factor C polypeptide derived from *T. tridentatus* has (α -2,3)-linked terminal sialic acid (paragraph 153), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since factor C polypeptide derived from *T. tridentatus* has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Nakamura's activated Factor C polypeptide purified from *T. tridentatus* necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claims 48 and 65, Nakamura does not teach the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* has a molecular weight as recited in the claims. However, the specification teaches that factor C polypeptide derived from *T. tridentatus* has a molecular weight of 124 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Nakamura's

activated factor C polypeptide is derived from *T. tridentatus*, it follows that Nakamura's activated factor C polypeptide necessarily has a molecular weight of 124 kDa by SDS-PAGE under non-reducing conditions.

Regarding claim 49, Nakamura does not teach the amino acid sequence of the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus* has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *Tachypleus tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A at pp. 30 and 31 of the Office action mailed on 9/16/2016).

Regarding claim 50, Nakamura teaches the Factor C has catalytic activity in an aqueous buffer (p. 852, Figure 6) and thus Nakamura's Factor C is considered to be water-soluble.

Regarding claim 57, Nakamura teaches reconstitution of the LPS-mediated clotting system, including the activated factor C polypeptide purified from the hemocyte lysate of *T. tridentatus*, which is considered to be an endotoxin assay kit as encompassed by claim 57.

19. RESPONSE TO REMARKS: Beginning at p. 14 of the remarks, applicant argues that Nakamura discloses native *T. tridentatus* Factor C and does not disclose a recombinantly expressed Factor C of *T. tridentatus* using a mammalian cell as a host cell. Applicant argues that the specification at paragraph 153 discloses that a recombinantly expressed Factor C of *T. tridentatus* using a mammalian cell as a host

cell contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with native *T. tridentatus* Factor C, which native *T. tridentatus* Factor C is the same as Nakamura's Factor C and thus the Factor C of claim 43 is different from Nakamura's Factor C.

Applicant's argument is not found persuasive. First, claims 43, 48-50, 57, and 65 do not require a recombinantly expressed Factor C of *T. tridentatus* using a mammalian cell as a host cell. Second, claim 43 recites the alternative of containing (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. As noted above, according to the specification, Factor C polypeptide derived from *T. tridentatus* has (α -2,3)-linked terminal sialic acid (paragraph 153), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). Thus, Factor C polypeptide derived from *T. tridentatus* contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. For these reasons, Nakamura's native *T. tridentatus* Factor C anticipates the claims.

20. The rejection of claims 43, 47-50, 54, 57, and 63 under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Ding et al. (US 5,716,834; cited on Form PTO-892 mailed on 9/16/2016; hereafter "Ding") as evidenced by Mizumura (*supra*) is maintained for the reasons of record and

the reasons set forth below. The rejection has been modified to address applicant's amendment to the claims.

Claims 43, 47-50, 54, 57, 63, and 65-78 are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Ding (*supra*) as evidenced by Mizumura (*supra*). See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103 and see MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 43, 47-50, and 65-69 are drawn to a horseshoe crab Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*.

Claims 54 and 70-78 are drawn to a horseshoe crab Factor C having activity of Factor C producible by a method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C using a mammalian cell as a host cell,

wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*,

wherein the mammalian cell is selected from the group consisting of a Chinese hamster cell and a human cell.

Claims 57 and 63 are drawn to an endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 43 or 54, respectively, and one or more elements.

Regarding claims 43 and 54, the reference of Ding teaches a mammalian host cell transformed with a recombinant vector for expressing a horseshoe crab Factor C (column 2, lines 46-55). Ding teaches a method for producing the horseshoe crab Factor C by culturing the host cell and recovering the horseshoe crab Factor C protein (column 2, lines 58-63). Ding teaches the mammalian host cell includes a Chinese Hamster Ovary cell (column 19, lines 39-43). Ding teaches four species of horseshoe crab, including *T. tridentatus* and *C. rotundicauda* (column 1, lines 18-20) and polynucleotides encoding each of the *T. tridentatus* and *C. rotundicauda* Factor C polypeptides (Figure 11). Ding teaches activation of the horseshoe crab Factor C by endotoxin (column 2, lines 7-9).

Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell. However, the specification teaches that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph 154), while a native horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and a native horseshoe

crab Factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell.

Regarding claims 47 and 70, Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 10% sodium citrate with a dilution factor of 8, which, according to the specification is about 21 mM sodium citrate (p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate, it follows that Ding's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Regarding claims 48, 65, 75, and 76, Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight as recited in the claims. However, the specification teaches that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered

from CHO cells has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Ding's activated *T. tridentatus* factor C polypeptide is recombinantly expressed and recovered from CHO cells, it follows that Ding's activated factor C polypeptide necessarily has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions.

Regarding claims 49 and 77, Ding does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A at pp. 30 and 31 of the Office action mailed on 9/16/2016).

Regarding claims 50 and 78, Ding teaches the horseshoe crab factor C can be secreted into the culture medium (column 3, lines 47-48) and thus the horseshoe crab factor C is considered to be water-soluble.

Regarding claims 57 and 63, Ding teaches the recombinant horseshoe crab Factor C may be employed in a chromogenic assay, wherein upon activation by endotoxin, the horseshoe crab Factor C converts the substrate to a colored product, thereby detecting and quantifying endotoxin (column 2, lines 7-11), which is considered to be an endotoxin assay kit as encompassed by claims 57 and 63.

Regarding claims 66 and 71, Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide

recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of about 7% sodium citrate with a dilution factor of 8, which, according to the specification is about 52 mM sodium hydrogencarbonate (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate, it follows that Ding's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogenate.

Regarding claims 67 and 72, Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of about 10% sodium citrate with a dilution factor of 4, which, according to the specification is about 214 mM sodium chloride (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride, it follows that Ding's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

Regarding claims 68 and 73, Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of about 0.5 M magnesium sulfate with a dilution factor of 16, which, according to the specification is about 16 mM magnesium sulfate (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate, it follows that Ding's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

Regarding claims 69 and 74, Ding does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 0.5 M calcium chloride with a dilution factor of 100, which, according to the specification is about 2.5 mM calcium chloride (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a

residual activity of 35% or higher in the presence of 2.5 mM calcium chloride, it follows that Ding's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

21. RESPONSE TO REMARKS: Beginning at p. 16 of the remarks, applicant argues that Ding does not teach or suggest a Factor C having Factor C activity as claimed. According to applicant, Ding "does not practically prepare a recombinant cell having ability for expressing Factor C having activity of Factor C" and "does not show any scientific evidence for presuming that Factor C in Ding has activity of Factor C", rather than just endotoxin binding activity. As evidence, applicant points to Example 12 of Ding, which evaluated endotoxin binding but according to applicant, did not evaluate Factor C activity.

Applicant's argument is not found persuasive. Ding discloses polynucleotides encoding each of the *T. tridentatus* and *C. rotundicauda* Factor C polypeptides (Figure 11), experimental methods for making a recombinant cell that expresses and produces a horseshoe crab Factor C polypeptide (*e.g.*, column 9, line 55 to column 21, line 35), a method for purifying the Factor C proenzyme (abstract; column 21, line 36 to column 24, line 16), and a method for activating Factor C by LPS endotoxin to activated Factor C that converts a fluorometric substrate (column 25, lines 25-27). Ding discloses that the recombinant horseshoe crab Factor C has "the same *enzymatic activity* as Factor C protein in assays for Gram negative bacterial endotoxin" (emphasis added; column 2,

lines 16-18) and notes that in the assay, when the Factor C is activated by endotoxin, it converts the substrate to a colored product (column 2, lines 7-11). At the time of the invention, the activation of Factor C to an enzymatically active form by endotoxin was well known in the prior art. For example, the reference of Iwanaga, S. (*Curr. Opin. Immun.* 5:74-82, 1993; cited on the IDS filed on 6/9/2015) teaches "In the presence of LPS, an LPS-sensitive serine protease zymogen, factor C, is autocatalytically activated. The active factor C then activates zymogen factor B" (p. 74, column 1). As such, given Ding's teachings that Factor C is activated to a substrate-cleaving form by endotoxin and that the recombinant horseshoe crab Factor C has the same *enzymatic activity* as Factor C protein in assays for Gram negative bacterial endotoxin, one would recognize that the activated recombinant horseshoe crab Factor C of Ding has "activity of Factor C" as defined in the specification, *i.e.*, Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B (specification at p. 36, paragraph 28) or as defined by applicant, *i.e.*, "protease activity" of Factor C (applicant's remarks at p. 14, footnote).

It should be noted that Ding's horseshoe crab Factor C and the horseshoe crab Factor C of the claims are made by identical or substantially identical processes and according to MPEP 2112.01.I, "[w]here the claimed and prior art products are...produced by identical or substantially identical processes, a *prima facie* case of either anticipation or obviousness has been established". Moreover, it is known in the prior art that CHO cells are only able to add sialic acid using the α -2,3 linkage (see, *e.g.*, paragraph 8 of Cottingham et al., US 2014/0249082, which has priority to 2010, cited on

the IDS filed on 10/26/2016). Since the Office does not have the facilities for examining and comparing applicant's recombinant Factor C with the Factor C of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the protein of the prior art does not possess the same material structural and functional characteristics of the claimed protein). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594.

Although applicant cites to Example 12 of Ding as allegedly disclosing only the evaluation of endotoxin (LPS) binding and not Factor C activity, Ding discloses that when Factor C was complexed to LPS strips and incubated with its fluorometric substrate, the Factor C activity remained detectable (column 25, lines 25-27). Thus, in view of Ding's teaching that Factor C is activated by endotoxin to have the activity of converting a substrate to a colored product (column 2, lines 7-11), one would recognize that the horseshoe crab Factor C of Ding's Example 12 has "activity of Factor C" as defined in the specification, i.e., Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B (specification at p. 36, paragraph 28) or as defined by applicant, i.e., "protease activity" of Factor C (applicant's remarks at p. 14, footnote).

In this case, it appears that applicant has discovered a new property of a prior art composition, e.g., a *T. tridentatus* recombinantly expressed by a CHO cell has increased (α -2,3)-linked terminal sialic acid as compared with native *T. tridentatus* Factor C and a horseshoe crab Factor C expressed using Sf9 as a host cell. However,

the discovery of a previously unappreciated property of a prior art composition does not render the old composition patentably new to the discoverer (MPEP 2112.I).

22. The rejection of claims 43, 47-50, 54, 57, and 63 under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Ding et al. (US 5,858,706; cited on the IDS filed on 6/9/2015; hereafter “Ding2”) as evidenced by Mizumura (*supra*) is maintained for the reasons of record and the reasons set forth below. The rejection has been modified to address applicant’s amendment to the claims.

Claims 43, 47-50, 54, 57, 63, and 65-78 are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Ding2 (*supra*) as evidenced by Mizumura (*supra*). See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103. See MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 43, 47-50, and 65-69 are drawn to a horseshoe crab Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*.

Claims 54 and 70-78 are drawn to a horseshoe crab Factor C having activity of Factor C producible by a method for producing a horseshoe crab Factor C, the method

comprising expressing the horseshoe crab Factor C using a mammalian cell as a host cell,

wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*,

wherein the mammalian cell is selected from the group consisting of a Chinese hamster cell and a human cell.

Claims 57 and 63 are drawn to an endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 43 or 54, respectively, and one or more elements.

Regarding claims 43 and 54, the reference of Ding2 teaches a mammalian host cell, including a CHO cell, transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding2 teaches cDNAs for expressing any horseshoe crab Factor C polypeptide, including the representative examples of *T. tridentatus* and *C. rotundicauda* Factor C polypeptides (column 3, lines 14-20). Ding2 teaches a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Ding2 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding2 teaches activation of the Factor C by endotoxin (column 1, lines 44-47).

Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell. However, the specification teaches that horseshoe crab Factor C

polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph 154), while a native horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and a native horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell.

Regarding claims 47 and 70, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 10% sodium citrate with a dilution factor of 8, which, according to the specification is about 21 mM sodium citrate (p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from

CHO cells necessarily exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Regarding claims 48, 65, 75, and 76, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight as recited in the claims. However, the specification teaches that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Ding2's activated *T. tridentatus* factor C polypeptide is recombinantly expressed and recovered from CHO cells, it follows that Ding's activated factor C polypeptide necessarily has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions.

Regarding claims 49 and 77, Ding2 does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A at pp. 30 and 31 of the Office action mailed on 9/16/2016).

Regarding claims 50 and 78, Ding2 teaches solubilization of the factor C (column 7, lines 456-53) and thus the factor C is considered to be water-soluble.

Regarding claims 57 and 63, Ding2 teaches the recombinant Factor C for use in an assay for endotoxin detection and teaches elements for conducting such an assay (column 8, lines 50-60), which is considered to be an endotoxin assay kit as encompassed by claims 57 and 63 respectively.

Regarding claims 66 and 71, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of about 7% sodium citrate with a dilution factor of 8, which, according to the specification is about 52 mM sodium hydrogencarbonate (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogenate.

Regarding claims 67 and 72, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of about 10% sodium citrate with a dilution factor of 4, which, according to the specification is about 214 mM sodium chloride (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered

from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

Regarding claims 68 and 73, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of about 0.5 M magnesium sulfate with a dilution factor of 16, which, according to the specification is about 16 mM magnesium sulfate (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

Regarding claims 69 and 74, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride. However,

Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 0.5 M calcium chloride with a dilution factor of 100, which, according to the specification is about 2.5 mM calcium chloride (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells necessarily exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

23. RESPONSE TO REMARKS: Beginning at p. 17 of the remarks, applicant argues that Ding2 teaches a recombinant Factor C having endotoxin binding activity and does not teach or suggest a Factor C "having activity of Factor C" as claimed. Applicant argues that although Ding2 discloses cloning a cDNA encoding a horseshoe Factor C into a mammalian expression vector, the production of a recombinant Factor C was conducted using a yeast cell, and not a mammalian cell. Applicant argues that the subject of Ding2 is to offer a recombinant Factor C having endotoxin-binding activity, but not "having activity of Factor C" and Ding2 does not disclose uses other than endotoxin binding for the recombinant Factor C. Applicant argues that since activated Factor C cannot be used for endotoxin detection based on protease activity, one of skill in the art

would recognize that Ding2's recombinant Factor C has endotoxin binding activity, but not activity of Factor C.

Applicant's argument is not found persuasive. As noted above, the reference of Ding2 discloses cDNAs for expressing any horseshoe crab Factor C polypeptide, including the representative examples of *T. tridentatus* and *C. rotundicauda* Factor C polypeptides (column 3, lines 14-20); teaches a mammalian host cell, including a CHO cell, transformed with a recombinant vector for expressing the cDNA encoding a Factor C (column 9, Example 1); and teaches a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Thus, contrary to applicant's position, the disclosure of Ding2 is not limited to producing a recombinant Factor C by a yeast cell.

The reference of Ding2 further discloses that upon binding to endotoxin, the serine protease activity of Factor C becomes activated (column 1, lines 44-47) and teaches assessing its endotoxin-triggered enzymatic activity by a method of removing detergent from a solubilized Factor C and assaying the endotoxin-activated serine protease activity of the recombinant Factor C by fluorometric or calorimetric methods (column 8, line 64 to column 9, line 11). As such, contrary to applicant's position, one of skill in the art would recognize the recombinant Factor C of Ding2 as having activity of Factor C.

Applicant further argues that in a subsequent publication of Ding (US Patent 6,645,724) discloses that the endotoxin LPS could not activate the enzymatic activity of yeast recombinant Factor C. According to applicant, this is an admission by Ding

himself that yeast recombinant Factor C does not have activity of Factor C, and cannot be used for endotoxin detection based on enzymatic activity.

Applicant's argument is not found persuasive. The rejection is not directed to the recombinant Factor C produced by a yeast cell. Rather, the rejection is directed to Ding2's teachings of producing a recombinant Factor C using a CHO cell and subsequently activating the recombinant Factor C, which, as noted above, activated Factor C is disclosed by Ding2 as having serine protease activity.

It should be noted that Ding2's CHO cell-expressed horseshoe crab Factor C and the horseshoe crab Factor C of the claims are made by identical or substantially identical processes and according to MPEP 2112.01.I, "[w]here the claimed and prior art products are...produced by identical or substantially identical processes, a *prima facie* case of either anticipation or obviousness has been established". Moreover, it is known in the prior art that CHO cells are only able to add sialic acid using the α -2,3 linkage (see, e.g., paragraph 8 of Cottingham et al., US 2014/0249082, which has priority to 2010, cited on the IDS filed on 10/26/2016). Since the Office does not have the facilities for examining and comparing applicant's recombinant Factor C with the Factor C of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the CHO cell-expressed horseshoe crab Factor C of the prior art does not possess the same material structural and functional characteristics of the claimed Factor C). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594.

In this case, it appears that applicant has discovered a new property of a prior art composition, *e.g.*, a *T. tridentatus* recombinantly expressed by a CHO cell has increased (α -2,3)-linked terminal sialic acid as compared with native *T. tridentatus* Factor C and a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the discovery of a previously unappreciated property of a prior art composition does not render the old composition patentably new to the discoverer (MPEP 2112.I).

Claim Rejections - 35 USC § 101

24. The rejection of claims 43, 48-50, and 57 under 35 U.S.C. 101 because the claimed invention is directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more is maintained for the reasons of record and the reasons set forth below. The rejection has been modified to address applicant's amendment to the claims.

Claims 43, 48-50, 57, and 65 are rejected under 35 U.S.C. 101 because the claimed invention is directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more.

Claims 43, 48-50, and 65 are drawn to a horseshoe crab Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*. Given a broadest reasonable interpretation, the

horseshoe crab Factor C of claims 43 and 48-50 encompasses a naturally occurring horseshoe crab Factor C, *e.g.*, activated factor C polypeptide present in the hemocytes of *T. tridentatus*. According to the specification, a native Factor C polypeptide derived from *T. tridentatus*, which has a molecular weight of 123 kDa, reacts with a (α -2,3)-linked terminal sialic acid-specific lectin, but Factor C recombinantly produced in Sf9 cells does not react (paragraphs 126 and 153).

Claim 57 is drawn to an endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 43 and one or more elements. Given a broadest reasonable interpretation, the endotoxin assay kit of claim 57 is considered to encompass naturally occurring horseshoe crab hemocytes, which comprise an activated Factor C.

Thus, the claims are considered to encompass naturally occurring products. Because there is no difference in characteristics (structural, functional, or otherwise) between the claimed and naturally occurring products, the claimed products do not have markedly different characteristics from what exists in nature, and thus are a “product of nature” exception. Accordingly, the claims are directed to judicial exceptions. Because the claims do not include any additional features that could add significantly more to the exception, the claims do not qualify as eligible subject matter.

25. RESPONSE TO REMARKS: Beginning at p. 20 of the remarks, applicant argues the claims do not encompass a naturally occurring product because the amended claim

43 recites that the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C.

Applicant's argument is not found persuasive because claim 43 recites the alternative of containing (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. According to the specification, Factor C polypeptide derived from *T. tridentatus*, which has a molecular weight of 123 kDa, has (α -2,3)-linked terminal sialic acid (paragraph 153), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraphs 126 and 154). Thus, Factor C polypeptide derived from *T. tridentatus*, which is a natural product, contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. As such, contrary to applicant's position, the claims encompass naturally occurring products.

Conclusion

26. Status of the claims:

Claims 43, 47-54, 57-61, and 63-78 are pending.

Claims 51-53, 58-61, and 64 are withdrawn from consideration.

Claims 43, 47-50, 54, 57, 63, and 65-78 are rejected.

No claim is in condition for allowance.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

Art Unit: 1656

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is 571-272-0942. The examiner can normally be reached on Mon to Fri, 7:30 am to 4:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David J. Steadman/
Primary Examiner, Art Unit 1656



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23373 7590 11/22/2017 SUGHRUE MION, PLLC 2100 PENNSYLVANIA AVENUE, N.W. SUITE 800 WASHINGTON, DC 20037			EXAMINER STEADMAN, DAVID J	
			ART UNIT 1656	PAPER NUMBER
			NOTIFICATION DATE 11/22/2017	DELIVERY MODE ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PPROCESSING@SUGHRUE.COM
sughrue@sughrue.com
USPTO@sughrue.com

DETAILED ACTION

Status of the Application

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on October 12, 2017 has been entered.

The present application is being examined under the pre-AIA first to invent provisions.

Claims 43, 47-54, 57-61, and 63-78 are pending in the application.

Applicant's amendment to the claims, filed on October 12, 2017, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Applicant's amendment to the specification, filed on October 12, 2017, is acknowledged.

Receipt of a declaration under 37 CFR 1.132, filed on October 12, 2017, is acknowledged.

Applicant's remarks filed on October 12, 2017 in response to the final rejection mailed on October 12, 2017 have been fully considered.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Election/Restriction

Claims 51-53, 58-61, and 64 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on 8/24/2016.

Claims 43-50, 54-57, 62, and 63 are being examined on the merits with claims 49 and 77 being examined only to the extent the claims read on the elected subject matter of the species of SEQ ID NO: 2.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on July 18, 2017 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Specification/Informalities

The objection to the disclosure because it contains an embedded hyperlink and/or other form of browser-executable code is withdrawn in view of applicant's amendment to the specification to replace the disclosure of "www.pssj.jp..." with "available on the world wide web at pssj.jp..."

Claim Objection

The objection to claim 54 in the recitation of "Chinese hamster cell" is withdrawn in view of applicant's amendment to delete the phrase at issue.

Claim 43 is objected to in the recitation of "CHO" and in the interest of improving claim form, it is suggested that the entire phrase for which the abbreviation is used, *i.e.*, "Chinese Hamster Ovary", be recited in the claim.

Claim Rejections - 35 USC § 112, Fourth Paragraph

The rejection of claims 49 and 77 under pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form is withdrawn in view of applicant's amendment to re-write claims 49 and 77 as independent claims.

Claim Rejections - 35 USC § 112, Second Paragraph

The rejection of claims 49 and 77 under 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite is withdrawn in view of applicant's amendment to re-write claims 49 and 77 as independent claims.

Claim 49 is rejected under 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 49 is indefinite in the recitation of "which is a protein shown..." because it is unclear as to whether the noted phrase refers to the Factor C having activity of Factor C, or to the native horseshoe crab Factor C. It is suggested that applicant clarify the meaning of the noted phrase.

Claim Rejections - 35 USC § 112, First Paragraph

Claims 49 and 77 are rejected under 35 U.S.C. 112 (pre-AIA), first paragraph, because the specification, while being enabling for *Tachypleus tridentatus* Factor C expressed in CHO DG44 cells, does not reasonably provide enablement for all Factor C polypeptides as encompassed by the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

“The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue.” *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors considered to be most relevant to the instant rejection are addressed in detail below.

The nature of the invention: According to the specification, “there has never been known a case where functional Factor C is produced using mammalian cells as host cells” (paragraph 11) and “[a]n object of the present invention is to provide a novel recombinant horseshoe crab Factor C...another object of the present invention is to

provide a reconstituted cascade reaction system which is not susceptible to reaction inhibition even in the presence of a salt (ion)" (paragraphs 14 and 15).

The breadth of the claims: Claim 49 is drawn to a Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO: 2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO: 4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C, and

wherein the Factor C is produced through being expressed from a CHO DG44 cell.

According to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28). The structure of the Factor C is unlimited.

Claim 77 is drawn to a horseshoe crab Factor C having activity of Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C, and

wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell.

As noted above, according to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28). The structure of the horseshoe crab Factor C is unlimited.

The state of the prior art; The level of one of ordinary skill; and The level of predictability in the art: According to MPEP 2164.03, "...what is known in the art provides evidence as to the question of predictability."

Regarding the scope of the recited engineered enzyme, the structure of the recited engineered enzyme is interpreted as being unlimited. It is well-known in the prior art that the amino acid sequence of a polypeptide determines the polypeptide's structural and functional properties. Predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity/utility requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (*i.e.*, expectedly intolerant to

modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The positions within a protein's sequence where modifications can be made with a reasonable expectation of success in obtaining a polypeptide having the desired activity/utility are limited in any protein and the result of such modifications is highly unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, *e.g.*, multiple substitutions.

It is well-known in the art that even a single amino acid alteration can alter the folding of a polypeptide. See, *e.g.*, MPEP 2144.08.II.A.4.(c), which states, "[i]n the area of biotechnology, an exemplified species may differ from a claimed species by a conservative substitution ('the replacement in a protein of one amino acid by another, chemically similar, amino acid... [which] is generally expected to lead to either no change or only a small change in the properties of the protein.'" Dictionary of Biochemistry and Molecular Biology 97 (John Wiley & Sons, 2d ed. 1989)). The effect of a conservative substitution on protein function depends on the nature of the substitution and its location in the chain. Although at some locations a conservative substitution may be benign, in some proteins only one amino acid is allowed at a given position. For example, the gain or loss of even one methyl group can destabilize the structure if close packing is required in the interior of domains. James Darnell et al., Molecular Cell Biology 51 (2d ed. 1990)."

"[I]f one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is lack of predictability in the art." See MPEP § 2164.03.

The amount of direction provided by the inventor and The existence of working examples: The specification discloses a single working example of a Factor C polypeptide as encompassed by the claims, *i.e.*, *Tachypleus tridentatus* Factor C comprising the amino acid sequence of SEQ ID NO: 2 expressed in CHO DG44 cells. Other than this single working example, the specification fails to disclose any modifications to the amino acid sequence of a Factor C polypeptide with an expectation of maintaining Factor C activity and optionally containing (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C.

In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, and the state of the prior art, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention. Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 49 and 77 are rejected under 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably

convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

MPEP 2163.II.A.2.(a).i) states, "Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention". For claims drawn to a genus, MPEP § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. MPEP § 2163 further states that a "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

Claim 49 is drawn to a Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO: 2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO: 4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C, and

wherein the Factor C is produced through being expressed from a CHO DG44 cell.

According to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28). The structure of the Factor C is unlimited.

Claim 77 is drawn to a horseshoe crab Factor C having activity of Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO: 2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising the amino acid sequence shown in SEQ ID NO: 4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C, and

wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell.

As noted above, according to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (spec at p. 36, paragraph 28). The structure of the horseshoe crab Factor C is unlimited.

In this case, the specification discloses an actual reduction to practice of a single representative species of the genus of claimed horseshoe crab Factor C polypeptides, *i.e.*, *Tachypleus tridentatus* Factor C comprising the amino acid sequence of SEQ ID NO: 2 expressed in Chinese Hamster Ovary (CHO) DG44 cells. Regarding amino acid sequence, the specification fails to identify any alterations to the amino acid sequence of SEQ ID NO: 2 that can be made and still maintain Factor C activity and optionally contain (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C. As described in detail above, the effects of amino acid substitution on the activity of a polypeptide are highly unpredictable. In this case, the amino acid sequences of the respective genus of claimed Factor C polypeptides is widely variant, encompassing any Factor C or horseshoe Factor C polypeptide expressed from a CHO DG44 cell and the single disclosed representative species fails to reflect the wide variation among the vast number of species encompassed by the

claims. As such, one of skill in the art would not accept the disclosure of *Tachypleus tridentatus* Factor C comprising the amino acid sequence of SEQ ID NO: 2 expressed in CHO DG44 cells as being representative of all other Factor C polypeptides as encompassed by the claims. The specification, taken with the pre-existing knowledge in the art of amino acid substitution, fails to satisfy the written description requirement of 35 U.S.C. 112, first paragraph.

Claim Rejections - 35 USC § 102/103

The rejection of claims 43, 48-50, 57, and 65 under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Nakamura et al. (*J. Biochem.* 99:847-857, 1986; cited on the IDS filed on June 9, 2015; hereafter “Nakamura”) as evidenced by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on June 9, 2015; hereafter “Mizumura”) is withdrawn in view of applicant’s amendment to claim 43 to recite “...wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell” and applicant’s corresponding remarks at item V, beginning at p. 13. The reference of Nakamura does not teach a horseshoe crab Factor C produced through being expressed from a CHO DG44 cell.

The rejection of claims 43, 47-50, 54, 57, and 63 under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Ding et al. (US 5,716,834; cited on Form PTO-892 mailed on 9/16/2016; hereafter “Ding”) as evidenced by Mizumura (*supra*) is withdrawn in view of applicant’s amendment to claim 43 to recite “...wherein the horseshoe crab Factor C is produced

through being expressed from a CHO DG44 cell” and applicant’s corresponding remarks at item VI, beginning at p. 15. The reference of Ding does not teach a CHO DG44 cell.

The rejection of claims 43, 47-50, 54, 57, and 63 under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Ding et al. (US 5,858,706; cited on the IDS filed on 6/9/2015; hereafter “Ding2”) as evidenced by Mizumura (*supra*) is withdrawn in view of applicant’s amendment to claim 43 to recite “...wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell” and applicant’s corresponding remarks at item VII, beginning at p. 18. The reference of Ding2 does not teach a CHO DG44 cell.

Claim Rejections - 35 USC § 103

Claims 43, 47-50, 54, 57, 63, and 65-78 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Ding2 (*supra*) in view of *Current Protocols in Molecular Biology* (1993) 16.23.1-16.23.13, John Wiley and Sons, Inc., 2002 (cited on the attached Form PTO-892; hereafter “Current Protocols”) and as evidenced by Mizumura (*supra*).

Claims 43, 47, 48, 50, and 66-69 are drawn to a horseshoe crab Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus*

polyphemus, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*, and wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell.

Claim 49 is drawn to a Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C, and

wherein the Factor C is produced through being expressed from a CHO DG44 cell.

Claims 54, 70-76, and 78 are drawn to a horseshoe crab Factor C having activity of Factor C producible by a method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C using a mammalian cell as a host cell,

wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*, wherein the mammalian cell is a CHO DG44 cell.

Claim 77 is drawn to a horseshoe crab Factor C having activity of Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO: 2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO: 4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C, and

wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell.

Claims 57 and 63 are drawn to an endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 43 or 54, respectively, and one or more elements.

Regarding claims 43, 49, 54, and 77, the reference of Ding2 teaches a mammalian host cell, including a CHO cell, transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding2 teaches cDNAs for expressing a horseshoe crab Factor C polypeptide, including the representative examples of *T. tridentatus* and *C. rotundicauda* Factor C polypeptides (column 3, lines 14-20). Ding2 teaches a method for producing the Factor C polypeptide by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Ding2 teaches

mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1).

Ding2 teaches activation of the Factor C by endotoxin (column 1, lines 44-47).

Regarding claims 49 and 77, Ding2 does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A at pp. 30 and 31 of the Office action mailed on September 16, 2016).

Regarding claims 50 and 78, Ding2 teaches solubilization of the factor C (column 7, lines 456-53) and thus the factor C is considered to be water-soluble.

Regarding claims 57 and 63, Ding2 teaches the recombinant Factor C for use in an assay for endotoxin detection and teaches elements for conducting such an assay (column 8, lines 50-60), which is considered to be an endotoxin assay kit as encompassed by claims 57 and 63 respectively.

Ding does not teach or suggest CHO DG44 cells as an expression host.

The reference of Current Protocols generally teaches a method for using CHO cells as an expression host for amplifying and expressing a desired gene (see whole document). Current Protocols teaches CHO DG44 as a cell line that greatly increases the ease of gene amplification (p. 16.23.11).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ding2 and Current Protocols to recombinantly express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol as an expression host. One would have been motivated to do this because Ding2 teaches using CHO cells as an expression host and Current Protocol teaches a

method for expressing a desired gene using a CHO cell line, noting that CHO DG44 cell line greatly increases the ease of gene amplification. One would have had a reasonable expectation of success to recombinantly express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol as an expression host because Ding2 teaches cDNAs for expressing a horseshoe crab Factor C polypeptide using CHO cells and Current Protocol teaches a method for expressing a desired gene using a CHO DG44 cell line.

Regarding claim 43, the combination of Ding2 and Current Protocol does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell. However, the specification teaches that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph 154), while a native horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph 154). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and a native horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than horseshoe crab Factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 49, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a native horseshoe crab Factor C. However, the specification teaches that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has greater (α -2,3) linked terminal sialic acid than a native horseshoe crab Factor C polypeptide (paragraph 153). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells has greater (α -2,3)-linked terminal sialic acid than a native horseshoe crab Factor C polypeptide, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than a native horseshoe crab Factor C polypeptide.

Regarding claims 47 and 70, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 10% or higher in the presence of 10% sodium citrate with a dilution factor of 8, which, according to the specification is about 21 mM sodium citrate (p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate, it follows that Ding2's activated horseshoe crab

Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Regarding claims 48, 65, 75, and 76, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has a molecular weight as recited in the claims. However, the specification teaches that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Ding2's activated *T. tridentatus* factor C polypeptide is recombinantly expressed and recovered from CHO cells, it follows that Ding's activated factor C polypeptide expressed from CHO DG44 cells necessarily has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions.

Regarding claims 66 and 71, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 25% or higher in the presence of about 7% sodium citrate with a dilution factor of 8, which, according to the specification is about 52 mM sodium hydrogencarbonate (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual

activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogenate.

Regarding claims 67 and 72, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of about 10% sodium citrate with a dilution factor of 4, which, according to the specification is about 214 mM sodium chloride (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

Regarding claims 68 and 73, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed

and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of about 0.5 M magnesium sulfate with a dilution factor of 16, which, according to the specification is about 16 mM magnesium sulfate (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

Regarding claims 69 and 74, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 0.5 M calcium chloride with a dilution factor of 100, which, according to the specification is about 2.5 mM calcium chloride (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

Therefore, the invention of claims 43, 47-50, 54, 57, 63, and 65-78 would have been obvious to one of ordinary skill in the art at the time of the invention.

RESPONSE TO REMARKS: At item VII of the remarks, applicant argues that Ding2 does not teach or suggest the limitation of “wherein the Factor C is produced through being expressed from a CHO DG44 cell” as recited in the amended claims 43, 49, 54, and 77.

Applicant’s argument is not found persuasive. The rejection includes the reference of Current Protocols, which, as noted above, teaches recombinant expression of a desired gene using a CHO DG44 cell line.

Applicant further argues the claimed Factor C produced by expression in CHO DG44 cells has an unexpectedly superior property of being less susceptible to ion inhibition in an injection as compared to a Factor C produced by expression in CHO-S cells, referring to the accompanying declaration under 37 CFR 1.132 (hereafter “Mizumura declaration”), and is also structurally different by virtue of different molecular weights. According to applicant, one would have found it surprising that a Factor C expressed in CHO DG44 cells has a superior property of being less susceptible to inhibition by ions in an injection as compared to a Factor C expressed in CHO-S cells.

Applicant’s argument is not found persuasive. According to MPEP 716.02(d), the unexpected result must be commensurate in scope with the claimed invention. According to the Mizumura declaration at the paragraph bridging pp. 2 and 3, the result was obtained using a single Factor C polypeptide – recombinant *Tachypleus tridentatus* Factor C produced by CHO DG44 cells. However, claims 43 and 54 recite *Limulus*

polyphemus, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas* Factor C

polypeptides in addition to *Tachypleus tridentatus* Factor C and claims 49 and 77 are unlimited with respect to the amino acid sequences of the Factor C polypeptide. As such, applicant's result is not commensurate in scope with the claimed invention. While MPEP 716.02(d), I acknowledge that the nonobviousness of a broader claimed range can be supported by evidence based on unexpected results from testing a narrower range, in this case, there is no evidence of record that the genus of Factor C polypeptides as encompassed by the claims would also have the property of being less susceptible to ion inhibition in an injection as compared to a Factor C produced by expression in CHO-S cells. Moreover, it is noted that according to the Mizumura declaration at pp. 3 and 4, the result was obtained using various injection solutions comprising the CHO DG44-produced *Tachypleus giga* Factor C and ions. However, the claims do not recite the various injection solutions used to obtain the experimental result and there is no evidence of record that the genus of Factor C polypeptides as encompassed by the claims would also have increased residual activity in other solutions, e.g., non-ionic solutions or ionic solutions with very low concentration of ions. Applicant is reminded that it is applicant's burden to establish that a result is unexpected (MPEP 716.02(b)) and to show that the result is commensurate in scope with the claimed invention (MPEP 716.02(d)). Because the result is not commensurate in scope with the claimed invention, applicant's result fails to rebut the *prima facie* case of obviousness.

Claim Rejections - 35 USC § 101

The rejection of claims 43, 48-50, and 57 under 35 U.S.C. 101 because the claimed invention is directed to a judicial exception is withdrawn in view of applicant's amendment to claim 43 to recite "...wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell" and applicant's corresponding remarks at item VIII, beginning at p. 20.

Citation of Relevant Prior Art

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Slade et al., *J. Prot. Res.* 11:6175-6186, 2012.

Conclusion

Status of the claims:

Claims 43, 47-54, 57-61, and 63-78 are pending.

Claims 51-53, 58-61, and 64 are withdrawn from consideration.

Claims 43, 47-50, 54, 57, 63, and 65-78 are rejected.

No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached on Monday to Friday, 7:30 AM to 4:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on 571-272-0939939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656



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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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DETAILED ACTION

Status of the Application

The present application is being examined under the pre-AIA first to invent provisions.

Claims 43, 47-54, 57-61, and 63-78 are pending in the application.

Applicant's amendment to the claims, filed on February 22, 2018, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Receipt of a declaration under 37 CFR 1.132, filed on February 22, 2018, and is acknowledged.

Applicant's remarks and declaration filed on February 22, 2018 in response to the non-final rejection mailed on November 22, 2017 have been fully considered.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Election/Restriction

Claims 51-53, 58-61, and 64 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on 8/24/2016.

Claims 43, 47-50, 54, 57, 63, and 65-78 are being examined on the merits with claims 49 and 77 being examined only to the extent the claims read on the elected

subject matter of the species of SEQ ID NO: 2, which is the amino acid sequence of *Tachypleus tridentatus* Factor C polypeptide.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on February 22, 2018 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Objections

The objection to claim 43 in the recitation of "CHO" is withdrawn in view of applicant's amendment to recite "Chinese Hamster Ovary".

Claim 77 is objected to in the recitation of "Factor C, ," and in the interest of improving claim form, it is suggested that the second comma be deleted.

Claim Rejections - 35 USC § 112, Second Paragraph

The rejection of claim 49 under 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite in the recitation of "which is a protein shown..." is withdrawn in view of applicant's amendment to claim 49, line 4 to recite "wherein the Factor C is a protein shown..."

Claim Rejections - 35 USC § 112, First Paragraph

The scope of enablement rejection of claims 49 and 77 under 35 U.S.C. 112 (pre-AIA), first paragraph, is withdrawn in view of applicant's amendment to limit the protein of parts (B) and (D) to comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, respectively. Only routine experimentation would be required to make the full scope of claimed Factor C polypeptides.

The written description rejection of claims 49 and 77 under 35 U.S.C. 112 (pre-AIA), first paragraph, is maintained for the reasons of record and the reasons set forth below. The rejection has been modified to address applicant's amendment to the claims.

The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

MPEP 2163.II.A.2.(a).i) states, "Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention". For claims drawn to a genus, MPEP § 2163 states the written description

requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. MPEP § 2163 further states that a “representative number of species” means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

As amended, claim 49 is drawn to a Factor C having activity of Factor C, wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C; which wherein the Factor C is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4 and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4 and having activity of Factor C, and

wherein the Factor C is produced through being expressed from a CHO DG44 cell.

As amended, claim 77 is drawn to a horseshoe crab Factor C having activity of Factor C, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2 and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4 and having activity of Factor C, and

wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell.

As noted above, according to the specification, "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B" (specification at p. 36, paragraph 28). The claims encompass variants of SEQ ID NO: 2 having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2. The amino acid sequence of SEQ ID NO: 2 is 1019 amino acids and variants having 80% identity to SEQ ID NO: 2 would allow for 20% variance, which is approximately 204 amino acid variations relative to SEQ ID NO: 2.

The specification discloses the reduction to practice of three representative species of the genus of recited Factor C polypeptides as encompassed by the claims, *i.e.*, *Tachypleus tridentatus*, *Limulus polyphemus*, or *Carcinoscorpius rotundicauda* Factor C. There are no other drawings or structural formulas disclosed of a polypeptide encompassed by the claims. However, with the aid of a computer, one of skill in the art could conceivably identify all of the amino acid sequences that have at least 80% identity with the amino acid sequence of SEQ ID NO: 2. However, there is no prior-art or disclosed teaching regarding which 20% (or 204 amino acids) of the amino acids can vary from SEQ ID NO: 2 and still result in a protein that retains activity of Factor C as required in claims 49 and 77, (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C as required in claim 49, and having the identity of being a horseshoe crab Factor C as recited in claim 77, and there is no disclosed or art-recognized correlation between any Factor C polypeptides other than *Tachypleus tridentatus*, *Limulus polyphemus*, or *Carcinoscorpius rotundicauda* Factor C and having Factor C activity, and optionally (α -2,3)-linked terminal sialic acid in a greater amount and having the identity of being a horseshoe crab Factor C. Given what is known in the art about the likely outcome of substitutions on structure, conservation of structure is not necessarily a surrogate for conservation of function (applicant's attention is directed to MPEP 2144.08.11.A.4.(c) for a description of the unpredictable effects of amino acid substitution on the function of a polypeptide). In this case, there is no disclosed correlation between structure and function. While general knowledge in the art may have allowed one of skill in the art to identify other proteins expected to have the same or similar tertiary structure of a polypeptide that has Factor C activity, and

optionally having (α -2,3)-linked terminal sialic acid in a greater amount and having the identity of being a horseshoe crab Factor C, there is no general knowledge in the art about Factor C activity, having (α -2,3)-linked terminal sialic acid in a greater amount and having the identity of being a horseshoe crab Factor C to suggest that general similarity of structure confers the activity. Accordingly, one of skill in the art would not accept the disclosure of *Tachypleus tridentatus*, *Limulus polyphemus*, or *Carcinoscorpius rotundicauda* Factor C polypeptides as being representative of other polypeptides having Factor C activity, and optionally having (α -2,3)-linked terminal sialic acid in a greater amount and having the identity of being a horseshoe crab Factor C as encompassed by the claims. As such, the specification, taken with the pre-existing knowledge in the art of amino acid substitution, fails to satisfy the written description requirement of 35 U.S.C. 112, first paragraph.

RESPONSE TO REMARKS: Applicant argues that possession can be shown by a number of factors in addition to the application's disclosure, including the method of making the claimed invention, and the level of skill in the art. Applicant argues that the method of making the claimed Factor C is well-established in view of the existing level of skill and knowledge in the art. Applicant argues that the specification discloses various distinguishing identifying characteristics of the genus of claimed Factor C polypeptides, including two representative sequences, their length, the molecular weight of *Tachypleus tridentatus* Factor C expressed in CHO-DG44 cells, and the structural features of amino acids that can be N-glycosylated.

Applicant's argument is not found persuasive. The genus of claimed Factor C polypeptides is not required to have or exhibit any of the distinguishing identifying characteristics as noted by the applicant. In this case, the features upon which applicant relies are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims.

Applicant points to Example 11B of the PTO's Guidance on Written Description, and further argues the specification has disclosed sequences of SEQ ID NO: 2 and 4 and the amino acid residues which can undergo N-type sugar chain modification and the declaration filed on February 22, 2018 shows consensus sequences of Factor C of *Limulus polyphemus* which can undergo N-type sugar chain.

Applicant's argument is not found persuasive. Claims 49 and 77 both require all members of the genus to have activity of Factor C, which as noted above, is described in the specification as activation of Factor C in the presence of endotoxin to convert Factor B into activated Factor B. In contrast to claim 2 of Example 11B, the claims require 80% identity, while claim 2 of Example 11B recites 85%, and as noted above, 20% (or 204 amino acids) variation is a substantial number of modifications. Also in contrast to claim 2 of Example 11B and as described in detail above, there is no apparent art-recognized or disclosed correlation between the structures of the genus of Factor C polypeptides and the activity of Factor C.

For these reasons, it is the examiner's position that the specification fails to adequately describe the genus of recited Factor C polypeptides.

Claim Rejections - 35 USC § 103

The rejection of claims 43, 47, 48, 50, 57, and 65-69 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Ding2 et al. (US 5,858,706; cited on the IDS filed on 6/9/2015; hereafter "Ding2") in view of *Current Protocols in Molecular Biology* (1993) 16.23.1-16.23.13, John Wiley and Sons, Inc., 2002 (cited on Form PTO-892 mailed on November 22, 2017; hereafter "Current Protocols") and as evidenced by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on June 9, 2015; hereafter "Mizumura") is withdrawn in view of applicant's evidence of an unexpected result as described in the declarations under 37 CFR 1.132 filed on October 12, 2017 and February 22, 2018, and applicant's corresponding remarks filed on October 12, 2017 at p. 19 and February 22, 2018 at p. 16.

The rejection of claims 49, 54, 63, and 70-78 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Ding2 (*supra*) in view of Current Protocols (*supra*) and as evidenced by Mizumura (*supra*) is maintained for the reasons of record and the reasons set forth below.

As amended, claim 49 is drawn to a Factor C having activity of Factor C, wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C; which wherein the Factor C is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4 and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
(D) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4 and having activity of Factor C, and

wherein the Factor C is produced through being expressed from a CHO DG44 cell.

As amended, claims 54, 70-76, and 78 are drawn to a horseshoe crab Factor C having activity of Factor C producible by a method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C using a mammalian cell as a host cell,

wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*,

wherein the mammalian cell is a CHO DG44 cell.

As amended, claim 77 is drawn to a horseshoe crab Factor C having activity of Factor C, which is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
(B) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2 and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
(D) a protein comprising an amino acid sequence having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 4 and having activity of Factor C, and

wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell.

Claim 63 is drawn to an endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 54, and one or more elements.

Regarding claims 49, 54, and 77, the reference of Ding2 teaches a mammalian host cell, including a CHO cell, transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding2 teaches cDNAs for expressing a horseshoe crab Factor C polypeptide, including the representative examples of *T. tridentatus* and *C. rotundicauda* Factor C polypeptides (column 3, lines 14-20). Ding2 teaches a method for producing the Factor C polypeptide by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Ding2 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding2 teaches activation of the Factor C by endotoxin (column 1, lines 44-47).

Further regarding claims 49 and 77, Ding2 does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A at pp. 30 and 31 of the Office action mailed on September 16, 2016).

Regarding claim 63, Ding2 teaches the recombinant Factor C for use in an assay for endotoxin detection and teaches elements for conducting such an assay (column 8, lines 50-60), which is considered to be an endotoxin assay kit as encompassed by claims 57 and 63 respectively.

Regarding claim 78, Ding2 teaches solubilization of the factor C (column 7, lines 456-53) and thus the factor C is considered to be water-soluble.

Ding does not teach or suggest CHO DG44 cells as an expression host.

The reference of Current Protocols generally teaches a method for using CHO cells as an expression host for amplifying and expressing a desired gene (see whole document). Current Protocols teaches CHO DG44 as a cell line that greatly increases the ease of gene amplification (p. 16.23.11).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ding2 and Current Protocols to recombinantly express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol as an expression host. One would have been motivated to do this because Ding2 teaches using CHO cells as an expression host and Current Protocol teaches a method for expressing a desired gene using a CHO cell line, noting that CHO DG44 cell line greatly increases the ease of gene amplification. One would have had a reasonable expectation of success to recombinantly express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol as an expression host because Ding2 teaches cDNAs for expressing a horseshoe crab Factor C polypeptide using CHO cells and Current Protocol teaches a method for expressing a desired gene using a CHO DG44 cell line.

Regarding claim 49, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has (α -2,3) linked terminal sialic acid in a greater amount as compared with a native horseshoe crab Factor C. However, the specification

teaches that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells has greater (α -2,3) linked terminal sialic acid than a native horseshoe crab Factor C polypeptide (paragraph 153). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells has greater (α -2,3)-linked terminal sialic acid than a native horseshoe crab Factor C polypeptide, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily has a greater amount of (α -2,3)-linked terminal sialic acid than a native horseshoe crab Factor C polypeptide.

Regarding claim 70, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 10% or higher in the presence of 10% sodium citrate with a dilution factor of 8, which, according to the specification is about 21 mM sodium citrate (p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Regarding claim 71, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and

recovered from CHO DG44 cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 25% or higher in the presence of about 7% sodium citrate with a dilution factor of 8, which, according to the specification is about 52 mM sodium hydrogencarbonate (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogenate.

Regarding claim 72, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of about 10% sodium citrate with a dilution factor of 4, which, according to the specification is about 214 mM sodium chloride (specification at p. 87). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride, it follows that Ding2's activated horseshoe crab

Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

Regarding claim 73, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of about 0.5 M magnesium sulfate with a dilution factor of 16, which, according to the specification is about 16 mM magnesium sulfate (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

Regarding claim 74, Ding2 does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride. However, Figure 7 of this application shows that horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 0.5 M calcium chloride with a dilution factor of 100,

which, according to the specification is about 2.5 mM calcium chloride (specification at p. 88). As such, since horseshoe crab Factor C recombinantly expressed and recovered from CHO cells recombinantly expressed and recovered from CHO cells exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride, it follows that Ding2's activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells necessarily exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

Regarding claims 75 and 76, the combination of Ding2 and Current Protocols does not teach the activated horseshoe crab Factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has a molecular weight as recited in the claims. However, the specification teaches that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO DG44 cells has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions (paragraph 144 and Table 1). As such, since Ding2's activated *T. tridentatus* factor C polypeptide is recombinantly expressed and recovered from CHO cells, it follows that Ding's activated factor C polypeptide expressed from CHO DG44 cells necessarily has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions.

Therefore, the invention of claims 49, 54, 63, and 70-78 would have been obvious to one of ordinary skill in the art at the time of the invention.

RESPONSE TO REMARKS: Applicant argues that CHO DG44 cells are deleted for the endogenous DHFR genes and require vectors containing a DHFR gene for amplification of a target gene. Applicant argues that Ding2 does not disclose or suggest

the use of a vector containing DHFR gene and does not teach or suggest using a DHFR-based gene amplification system as described in Current Protocols. Thus, according to applicant, one would not have been motivated to express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol since the expression system of Ding2 does not contain a DHFR gene and would not result in increased ease of gene amplification in CHO DG44 cells.

Applicant's argument is not found persuasive. Current Protocols provides detailed instructions for target gene amplification using CHO DG44 cells, including the appropriate expression vectors, which carry a DHFR gene for selection and gene amplification (see, *e.g.*, p. 16.23.1) and in view of the detailed instructions, it would have required only routine experimentation to modify the expression system of Ding2 according to Current Protocols in order to recombinantly express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol as an expression host. As noted above, one would have been motivated to do this because CHO DG44 cell line greatly increases the ease of gene amplification. Thus, contrary to applicant's position, one would have been motivated to and would have had a reasonable expectation of success to recombinantly express the Factor C polypeptide of Ding2 using the CHO DG44 cell line of Current Protocol as an expression host.

Applicant further argues that applicant's result shows that *Tachypleus tridentatus* Factor C polypeptide expressed in CHO DG44 cells and having a greater amount of (α 2,3)-linked sialic acid as compared to the corresponding native *Tachypleus tridentatus* Factor C polypeptide or *Tachypleus tridentatus* Factor C polypeptide expressed using Sf9 as a host cell has a superior property of being less susceptible to inhibition by ions

in an injection as compared to a *Tachypleus tridentatus* Factor C polypeptide expressed in CHO-S cells. Applicant argues that one of ordinary skill in the art would recognize this result can be extended to *Limulus polyphemus* and *Carcinoscorpius rotundicauda* Factor C polypeptides in view of the substantial similarity and conserved glycosylation residues between the sequences of *Tachypleus tridentatus* and *Limulus polyphemus* or *Carcinoscorpius rotundicauda* Factor C polypeptides.

According to MPEP 716.02(d), it is applicant's burden to show that the result is commensurate in scope with the claimed invention. It is acknowledged that an unexpected result is supported by the evidence presented in the declarations under 37 CFR 1.132 filed on October 12, 2017 and February 22, 2018, and applicant's corresponding remarks filed on October 12, 2017 at p. 19 and February 22, 2018 at p. 16. Accordingly, the rejection of claims 43, 47, 48, 50, 57, and 65-69, which are limited to *Tachypleus tridentatus*, *Limulus Polyphemus*, or *Carcinoscorpius rotundicauda* Factor C expressed in a CHO DG44 cell and having a greater amount of (α 2,3)-linked sialic acid as compared to the corresponding native Factor C polypeptide (defined in the specification at paragraph 24 as the corresponding Factor C isolated and purified from a horseshoe crab lysate) or the corresponding Factor C polypeptide expressed using Sf9 as a host cell, is withdrawn as noted above.

However, applicant's result is not found to be commensurate in scope with claims 49, 54, 63, and 70-78 for the reasons that follow. Regarding claim 77, in contrast to claim 43, claim 77 does not recite a greater amount of (α 2,3)-linked sialic acid as compared to the corresponding native Factor C polypeptide or the corresponding Factor C polypeptide expressed using Sf9 as a host cell and is not limited to *Tachypleus*

tridentatus, *Limulus Polyphemus*, or *Carcinoscorpius rotundicauda* Factor C. Claim 77

does not even require the presence of (α 2,3)-linked sialic acid and encompasses variants of SEQ ID NO: 2, which is the amino acid sequence of *Tachypleus tridentatus* Factor C polypeptide, having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2. The amino acid sequence of SEQ ID NO: 2 is 1019 amino acids and a variant having 80% identity to SEQ ID NO: 2 would allow for 20% variance, which is approximately 204 amino acid variations relative to SEQ ID NO: 2. Even though the claim requires expression from a CHO DG44 cell, given that the claim encompasses variants that have been modified to substitute the conserved glycosylation residues and does not even require the presence of (α 2,3)-linked sialic acid, one of ordinary skill in the art would recognize that applicant's result is not commensurate in scope with the claimed invention and thus fails to rebut the *prima facie* case of obviousness.

Regarding claims 54, 63, 70-76, and 78, in contrast to claim 43, claims 54, 63, 70-76, and 78 do not recite a greater amount of (α 2,3)-linked sialic acid as compared to the corresponding native Factor C polypeptide or the corresponding Factor C polypeptide expressed using Sf9 as a host cell. The claims do not even positively recite the presence of (α 2,3)-linked sialic acid. Claim 54 (and claims dependent therefrom) is a product-by-process claim and recites the transitional phrase "comprising", which, given a broadest reasonable interpretation includes additional method steps for producing the Factor C polypeptide, *e.g.*, treatment of the Factor C polypeptide expressed in a CHO DG44 cell with a glycosidase to remove (α 2,3)-linked sialic acid (see, *e.g.*, Example 9 of the specification beginning at p. 93). Even though claim 54 requires expression from a

CHO DG44 cell, given that the claim encompasses additional method steps that remove (α 2,3)-linked sialic acid and does not even require the presence of (α 2,3)-linked sialic acid, one of ordinary skill in the art would recognize that applicant's result is not commensurate in scope with the claimed invention and thus fails to rebut the *prima facie* case of obviousness.

Regarding claim 49, in contrast to claim 43, claim 49 is not limited to *Tachypleus tridentatus*, *Limulus Polyphemus*, or *Carcinoscorpius rotundicauda* Factor C. Rather, claim 49 encompasses variants of SEQ ID NO: 2 having 80% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2. The amino acid sequence of SEQ ID NO: 2 is 1019 amino acids and a variant having 80% identity to SEQ ID NO: 2 would allow for 20% variance, which is approximately 204 amino acid variations relative to SEQ ID NO: 2. These variants encompass amino acid sequences that do not maintain the conserved glycosylation residues and encompass variants with substitution(s) of the conserved glycosylation residues and insertion and/or addition of any other glycosylation residues that can be modified with the addition of (α 2,3)-linked sialic acid. According to Mizumura et al. (*Innate Immun.* 23:136-146, 2017; cited on the IDS filed on February 22, 2018), glycosylation is a typical post-translational protein modification (p. 141, column 2, bottom) and recombinant proteins are well known to exhibit different activity and stability depending on their different post-translational modifications (p. 143, column 1, bottom). While claim 49 requires a greater amount of (α 2,3)-linked sialic acid as compared to the corresponding native Factor C polypeptide and requires expression from a CHO DG44 cell, given that the claim encompasses variants that have been modified with respect to the glycosylation residue(s) and that recombinant proteins are

well known to exhibit different activity and stability depending on their different post-translational modifications (which includes glycosylation), one of ordinary skill in the art would recognize that applicant's result is not commensurate in scope with the claimed invention and thus fails to rebut the *prima facie* case of obviousness.

For these reasons, it is the examiner's position that the invention of claims 49, 54, 63, and 70-78 would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Conclusion

Status of the claims:

Claims 43, 47-54, 57-61, and 63-78 are pending.

Claims 51-53, 58-61, and 64 are withdrawn from consideration.

Claims 49, 54, 63, and 70-78 are rejected.

Claims 43, 47, 48, 50, 57, and 65-69 are allowable over the prior art of record.

Applicant is encouraged to amend claims 43, 47, 57, and 66-69 according to the proposed Examiner's amendment filed on April 3, 2018.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is

(571)272-0942. The examiner can normally be reached on Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Docket No: Q220395
Hikaru MIZUMURA, et al.	
Appln. No.: 14/650,767	Group Art Unit: 1656
Confirmation No.: 1531	Examiner: STEADMAN, DAVID J
Filed: June 9, 2015	
For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN	

SUPPLEMENTAL AMENDMENT UNDER 37 C.F.R. § 1.114(c)

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Further to the RCE submitted on July 16, 2018, please amend the above-identified application as follows on the accompanying pages.

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AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1-42. (Canceled).

43. (Previously Presented): A horseshoe crab Factor C having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*, and wherein the horseshoe crab Factor C is produced through being expressed from a Chinese Hamster Ovary (CHO) DG44 cell.

44-46. (Cancelled).

47. (Previously Presented): The Factor C according to claim 43, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

48. (previously presented): The Factor C according to claim 43, which has a molecular weight of 115 kDa to 140 kDa as measured through SDS-PAGE under a non-reducing condition.

49. (previously presented): A Factor C having activity of Factor C,
wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as
compared with a native horseshoe crab Factor C;
wherein the Factor C is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising 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) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising 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, ~~and~~
wherein the Factor C is produced through being expressed from a CHO DG44 cell, and
wherein the Factor C exhibits a residual activity of 10% or higher in the presence of 21
mM sodium citrate.

50. (Previously Presented): The Factor C according to claim 43, which is water-
soluble.

51. (Withdrawn): A method for producing a horseshoe crab Factor C, the method
comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

52. (Withdrawn): The method according to claim 51, wherein the mammalian cell is
selected from the group consisting of:

- (A) a mammalian cell other than COS-1;

(B) a cell of a mammal selected from the group consisting of a primate and a rodent,
other than monkey;

(C) a Chinese hamster cell or a human cell;

(D) CHO or HEK; and

(E) CHO DG44 or HEK293.

53. (Withdrawn): The method according to claim 51, wherein the Factor C is a
protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which
includes substitution, deletion, insertion, or addition of one or several amino acid residues, and
having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which
includes substitution, deletion, insertion, or addition of one or several amino acid residues, and
having activity of Factor C.

54. (Cancelled).

55-56. (Cancelled).

57. (previously presented): An endotoxin assay kit comprising an endotoxin assay
agent containing the Factor C according to claim 43 and one or more elements.

58. (Withdrawn): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the assay agent according to claim 56 and the test specimen, and a step of measuring the progress of a cascade reaction.

59. (Withdrawn): The method according to claim 58, wherein the test specimen contains at least one of:

- (A) an ion;
- (B) a cation;
- (C) a metal ion;
- (D) an alkali metal ion or an alkaline earth metal ion; and
- (E) one or more kinds of ions selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

60. (Withdrawn): The method according to claim 59, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 1 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

61. (Withdrawn): The method according to claim 58, wherein the test specimen is an injection.

62. (Cancelled).

63. (Cancelled).

64. (Withdrawn): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the assay agent according to claim 62 and the test specimen, and a step of measuring the progress of a cascade reaction.

65. (previously presented): The Factor C according to claim 48, which has a molecular weight of 127 kDa $\pm$ 5 kDa.

66. (previously presented): The Factor C according to claim 43, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

67. (previously presented): The Factor C according to claim 43, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

68. (previously presented): The Factor C according to claim 43, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

69. (previously presented): The Factor C according to claim 43, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

70-76. (Cancelled).

77. (Currently Amended): A horseshoe crab Factor C, having activity of Factor C, wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, which is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising 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) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising 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, ~~and~~ wherein the horseshoe crab Factor C is produced through being expressed from a CHO DG44 cell, and

wherein the Factor C exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

78. (Cancelled).

79. (previously presented): An endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 49 and one or more elements.

80. (previously presented): An endotoxin assay kit comprising an endotoxin assay agent containing the Factor C according to claim 77 and one or more elements.

REMARKS

Further to the RCE filed on July 16, 2018 entering the claims as amended in the Amendment under 37 C.F.R. §1.116, claim 49 and 77 are further amended herein to recite that Factor C exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate. Support for this amendment is found, for example, at paragraphs [0017] and [0035] of the specification as filed.

Additionally, claims 54, 63, 70-76 and 78 are canceled herein, without prejudice or disclaimer.

No new matter is presented.

Applicant submits the following in response to the Advisory Action dated June 21, 2018 to be considered in conjunction with the previously submitted arguments of record.

In the second paragraph of the Advisory Action, it is indicated that claims 43 and 54 raise the issue of duplicate claims. Since claim 54 and dependent claims therefrom are canceled in the present Supplemental Amendment, this rejection reason would be overcome.

In the fourth paragraph of the Advisory Action, it is indicated that “one of ordinary skill in the art would recognize that applicant's result of being less susceptible to inhibition by ions in an injection as compared to the same Factor C polypeptide expressed in CHO-S cells is not commensurate in scope with the claimed invention and thus fails to rebut the *prima facie* case of obviousness.”

In claims 49 and 77 in the present Supplemental Amendment, the feature “the Factor C exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate” is added.

As shown in the Declaration submitted on October 11, 2017, the residual activity of Factor C expressed in CHO-S cell for 21 mM sodium citrate is greatly lower than 10%, whereas

the residual activity of Factor C expressed in CHO DG-44 cell for 21 mM sodium citrate is greatly higher than 10% (Injection No. 5 in Figure S1 of the Declaration on October 12, 2017). Please note that when the measurement specimen was 8-fold dilute of 10 w/v% sodium citrate injection, the final sodium citrate concentration was about 21 mM (see, paragraph [0136] of the present English PCT specification).

In sum, it is submitted that the unexpected results presented in the Declaration Under 37 C.F.R. § 1.132 submitted October 12, 2017 are commensurate with the presently amended claims and that therefore, the presently claimed invention is non-obvious over the cited references.

Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/Jennifer M. Hayes/
Jennifer M. Hayes
Registration No. 40,641

SUGHRUE MION, PLLC
Telephone: 202.293.7060
Facsimile: 202.293.7860

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: July 24, 2018



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

23373 7590 08/28/2018
SUGHRUE MION, PLLC
2100 PENNSYLVANIA AVENUE, N.W.
SUITE 800
WASHINGTON, DC 20037

EXAMINER

STEADMAN, DAVID J

ART UNIT

PAPER NUMBER

1656

DATE MAILED: 08/28/2018

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/650,767	06/09/2015	Hikaru MIZUMURA	Q220395	1531

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	11/28/2018

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23373 7590 08/28/2018
SUGHRUE MION, PLLC
2100 PENNSYLVANIA AVENUE, N.W.
SUITE 800
WASHINGTON, DC 20037

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/650,767	06/09/2015	Hikaru MIZUMURA	Q220395	1531

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	11/28/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
STEADMAN, DAVID J	1656	435-219000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

☐ Issue Fee

☐ Publication Fee (No small entity discount permitted)

☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

☐ A check is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/650,767	06/09/2015	Hikaru MIZUMURA	Q220395	1531
23373	7590	08/28/2018	EXAMINER	
SUGHRUE MION, PLLC			STEADMAN, DAVID J	
2100 PENNSYLVANIA AVENUE, N.W.				
SUITE 800				
WASHINGTON, DC 20037				
			ART UNIT	PAPER NUMBER
			1656	
DATE MAILED: 08/28/2018				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/650,767	Applicant(s) MIZUMURA et al.	
	Examiner DAVID J STEADMAN	Art Unit 1656	AIA Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the application papers filed on June 14, 2018 and July 24, 2018.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.

4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
a) ☒ All b) ☐ Some *c) ☐ None of the:
1. ☒ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☒ Examiner's Amendment/Comment
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.	6. ☐ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.	7. ☐ Other _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.	

/David Steadman/ Primary Examiner, Art Unit 1656	
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Continuation of 3. The allowed claim(s) is/are: 43,47-51,53,57-61,65-69,77 and 79-80

ALLOWABILITY NOTICE

Status of the Application

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submissions filed on June 14, 2018 and July 24, 2018 have been entered.

The present application is being examined under the pre-AIA first to invent provisions.

Applicant's amendment to the claims, filed on July 24, 2018, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Applicant's remarks filed on June 14, 2018 and July 24, 2018 in response to the final rejection mailed on April 17, 2018 and the advisory action mailed on June 21, 2018 have been fully considered.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Examiner's Amendment to the Claims

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Mr. Jennifer M. Hayes on August 10, 2018.

1-42. (Canceled)

43. (Currently Amended) A horseshoe crab Factor C protein having activity of Factor C, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding native horseshoe crab Factor C protein and/or a corresponding horseshoe crab Factor C protein recombinantly expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscoryius rotundicauda*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary (CHO) DG44 cell.

44-46. (Canceled)

47. (Currently Amended) The Factor C protein according to claim 43, which exhibits a residual Factor C activity of 10% or higher in the presence of 21 mM sodium citrate.

48. (Currently Amended) The Factor C protein according to claim 43, which has a molecular weight of 115 kDa to 140 kDa as measured ~~by~~through SDS-PAGE under ~~[[a]]~~ non-reducing conditions.

49. (Currently Amended) A Factor C protein having activity of Factor C, wherein the Factor C protein contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding native horseshoe crab Factor C protein;

wherein the Factor C protein comprises an amino acid sequence selected from the group consisting of a protein shown in the following (A), (B), (C), or (D):

(A) ~~a protein comprising~~ the amino acid sequence of shown in SEQ ID NO: 2;

(B) ~~a protein comprising~~ 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) ~~a protein comprising~~ the amino acid sequence of shown in SEQ ID NO: 4; and

(D) ~~a protein comprising~~ 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 CHO DG44 cell, and

wherein the Factor C protein exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

50. (Currently Amended) The Factor C protein according to claim 43, which is water soluble.

51. (Currently Amended) A method for producing [[a]] the horseshoe crab Factor C protein of claim 43, the method comprising culturing a CHO DG44 cell recombinantly expressing said horseshoe crab Factor C protein under conditions suitable for the expression of the horseshoe crab Factor C to produce the horseshoe crab Factor C protein in a mammalian cell as a host cell.

52. (Canceled)

53. (Currently Amended) The method according to claim 51, wherein the Factor C protein comprises an amino acid sequence selected from the group consisting of ~~is a protein shown in the~~ following (A), (B), (C), or (D):

(A) ~~a protein comprising~~ the amino acid sequence of ~~shown in~~ SEQ ID NO: 2; and

(B) ~~a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which~~
~~includes substitution, deletion, insertion, or addition of one or several amino acid residues, and~~
~~having activity of Factor C;~~

(C) ~~a protein comprising~~ the amino acid sequence of ~~shown in~~ SEQ ID NO: 4[[:]]

(D) ~~a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which~~
~~includes substitution, deletion, insertion, or addition of one or several amino acid residues, and~~
~~having activity of Factor C.~~

54-56. (Canceled)

57. (Currently Amended) An endotoxin assay kit comprising ~~an endotoxin assay agent~~
~~containing~~ the Factor C protein according to claim 43 and one or more elements.

58. (Currently Amended) A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the horseshoe crab Factor C protein according to claim 43, Factor B, Pro-clotting enzyme, and t-butoxycarbonyl-leucyl-glycyl-arginyl-pNA (Boc-Leu-Gly-Arg-

~~pNA), assay agent according to claim 56 and the test specimen, and a step of measuring the release of pNA, thereby measuring endotoxin in the test specimen progress of a cascade reaction.~~

59. (Currently Amended) The method according to claim 58, wherein the test specimen ~~comprises~~contains at least one of:

- (A) an ion;
- (B) a cation;
- (C) a metal ion;
- (D) an alkali metal ion or an alkaline earth metal ion; and
- (E) one or more kinds of ions selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

60. (Currently Amended) The method according to claim 59, wherein the test specimen comprises a cation and the~~contained amount of the ion in the test specimen is such an amount that the final~~ concentration of the cation after mixing the test specimen with the assay agent~~is derived from the test specimen becomes 1 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.~~

61. (Previously Presented) The method according to claim 58, wherein the test specimen is an injection.

62-64. (Canceled)

65. (Currently Amended) The Factor C protein according to claim 48, which has a molecular weight of 127 kDa $\pm$ 5 kDa.

66. (Currently Amended) The Factor C protein according to claim 43, which exhibits a residual Factor C activity of 25% or higher in the presence of 52 mM sodium hydrogen carbonate.

67. (Currently Amended) The Factor C protein according to claim 43, which exhibits a residual Factor C activity of 25% or higher in the presence of 214 mM sodium chloride.

68. (Currently Amended) The Factor C protein according to claim 43, which exhibits a residual Factor C activity of 15% or higher in the presence of 16 mM magnesium sulfate.

69. (Currently Amended) The Factor C protein according to claim 43, which exhibits a residual Factor C activity of 35% or higher in the presence of 2.5 mM calcium chloride.

70-76. (Canceled)

77. (Currently Amended) A horseshoe crab Factor C[[,]] protein having activity of Factor C, wherein the horseshoe crab Factor C protein contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding native horseshoe crab Factor C protein and/or a horseshoe crab Factor C protein recombinantly expressed using Sf9 as a host cell,

wherein the horseshoe crab Factor C protein comprises an amino acid sequence selected from the group consisting of which is a protein shown in the following (A), (B), (C), or (D):

(A) ~~a protein comprising~~ the amino acid sequence of shown in SEQ ID NO: 2; and

(B) ~~a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;~~

(C) ~~a protein comprising~~ the amino acid sequence of shown in SEQ ID NO: 4;[[;]]

(D) ~~a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C~~

wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a CHO DG44 cell, and

wherein the horseshoe crab Factor C protein exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

78. (Canceled)

79. (Currently Amended) An endotoxin assay kit comprising ~~an endotoxin assay agent containing~~ the Factor C protein according to claim 49 and one or more elements.

80. (Currently Amended) An endotoxin assay kit comprising ~~an endotoxin assay agent containing~~ the horseshoe crab Factor C protein according to claim 77 and one or more elements.

Claim Objection

The objection to claim 77 in the recitation of "Factor C, ," is withdrawn in view of the amendment to the claim.

Claim Rejections - 35 USC § 112, First Paragraph

The written description rejection of claims 49 and 77 under 35 U.S.C. 112 (pre-AIA), first paragraph, is withdrawn in view of the amendment to claim 49 to limit the amino acid sequence of parts (B) and (D) to comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, respectively, and the amendment to claim 77 to limit the amino acid sequence of the horseshoe crab Factor C protein to comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4. Figures 8 and 9 show a sequence alignment between SEQ ID NO: 2 and 4 with an identification of the consensus sequences that undergo N-type sugar chain modification and the specification discloses a correlation between the presence of (α -2,3)-linked terminal sialic acid and being recombinantly expressed in a CHO DG44 cell and exhibiting a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

Claim Rejections - 35 USC § 103

The rejection of claims 49, 54, 63, and 70-78 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over (US 5,858,706; cited on the IDS filed on 6/9/2015; hereafter "Ding2") in view of *Current Protocols in Molecular Biology* (1993) 16.23.1-16.23.13, John Wiley and Sons, Inc., 2002 (cited on Form PTO-892 mailed on November 22,

2017; hereafter “Current Protocols”) and as evidenced by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on June 9, 2015; hereafter “Mizumura”) is withdrawn in view of applicant’s amendment to recite “wherein the horseshoe crab Factor C protein exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate” such that the unexpected result is commensurate in scope with the claimed invention, applicant’s evidence of an unexpected result as described in the declarations under 37 CFR 1.132 filed on October 12, 2017 and February 22, 2018, and applicant’s corresponding remarks filed on October 12, 2017 at p. 19 and February 22, 2018 at p. 16.

Election/Restriction

Claim 43 is directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(b), claims 51, 53, and 58-61, directed to the process of making or using the allowable product, previously withdrawn from consideration as a result of a restriction requirement, are hereby rejoined and fully examined for patentability under 37 CFR 1.104.

Because a claimed invention previously withdrawn from consideration under 37 CFR 1.142 has been rejoined, the restriction requirement among Groups I, II, and III as set forth in the Office action mailed on June 24, 2016 is hereby withdrawn. In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present

application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Claims 49 and 77 are directed to an allowable product and the requirement for an election of species between SEQ ID NO: 2 and SEQ ID NO: 4 as set forth in the Office action mailed on June 24, 2016 is hereby withdrawn. In view of the withdrawal of the requirement for an election of species, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached on Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656