

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CELLTRION, INC.,

Petitioner,

v.

REGENERON PHARMACEUTICALS, INC.,

Patent Owner.

Patent No. 10,464,992

Inter Partes Review No. IPR2023-00462

DECLARATION OF ALEXANDER M. KLIBANOV, Ph.D.

Celltrion Exhibit 1021

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I, Dr. Alexander M. Klibanov, make this declaration in connection with the proceeding identified above.

I. INTRODUCTION

1. I have been retained by counsel for Patent Owner, Regeneron Pharmaceuticals, Inc. (“Regeneron”), as a technical expert in connection with the proceeding identified above. I submit this declaration in support of Patent Owner’s Preliminary Response to the Petition for *Inter Partes* Review of United States Patent No. 10,464,992 (“the ’992 patent”).

2. I am being paid at my customary consulting rate of \$950 per hour for my time spent working on this matter. I have no personal or financial stake or interest in the outcome of the present proceeding.

II. BACKGROUND AND QUALIFICATIONS

3. I am currently a Professor Emeritus of Chemistry and Bioengineering at the Massachusetts Institute of Technology (“M.I.T.”), where I taught and conducted research for over 40 years. From 2014 to 2019 (and also from 2007 to 2012), I held the Novartis Endowed Chair Professorship at M.I.T. From 2012 to 2014, I held the Roger and Georges Firmenich Endowed Chair Professorship in Chemistry. Prior to that, I was a Professor of Chemistry and a Professor of Bioengineering at M.I.T., positions I held from 1988 and 2000, respectively. From 1979 to 1988, I was an Assistant Professor, then Associate Professor, and

thereafter a Full Professor of Applied Biochemistry in the Department of Applied Biological Sciences (formerly the Department of Nutrition and Food Science) at M.I.T.

4. I obtained my M.S. degree in Chemistry from Moscow University in Russia in 1971 and Ph.D. in Chemical Enzymology (a branch of medicinal chemistry) from the same University in 1974. Thereafter, I was a Research Chemist at Moscow University's Department of Chemistry for three years. From 1977 to 1979, following my immigration to the United States, I was a Post-Doctoral Associate at the Department of Chemistry, University of California in San Diego.

5. Over the last 50+ years as a practicing chemist, I have extensively researched, published, taught, and lectured in many areas of chemistry, including biological, protein, medicinal, and formulation chemistry.

6. I have earned numerous prestigious professional awards and honors. For example, I was elected to the U.S. National Academy of Sciences (considered among the highest honors that can be given to an American scientist) and also to the U.S. National Academy of Engineering (considered among the highest honors that can be given to an American engineer). I am also a Founding Fellow of the American Institute for Medical and Biological Engineering and a Corresponding Fellow of the Royal Society of Edinburgh (Scotland's National Academy of

Science and Letters). In addition, I have received the Arthur C. Cope Scholar Award, the Marvin J. Johnson Award, the Ipatieff Prize, and the Leo Friend Award, all from the American Chemical Society, as well as the International Enzyme Engineering Prize.

7. I currently serve on the Editorial Boards of over a dozen scientific journals, including “Open Journal of Pharmacology,” “Applied Biochemistry and Biotechnology,” “Nanocarriers,” “Open Access Academic Books in Chemistry,” “Biotechnology and Bioengineering,” “Journal of Biological Chemistry and Molecular Pharmacology,” “Recent Patents in Biotechnology,” “Archives of Medical Biotechnology,” “Current Pharmaceutical Biotechnology,” and “International Journal of Drug Design, Delivery, and Safety.”

8. I have published over 315 scientific papers in various areas of chemistry. Of particular relevance to this proceeding are my numerous publications specifically dealing with protein chemistry, including therapeutic proteins and their stability. I am a named inventor of 32 issued United States patents and of a number of pending ones. I have given over 370 invited lectures, including many distinguished named lectureships, at professional conferences, universities, and corporations all over the world, many dealing with formulation, stability, stabilization, and biological evaluation of pharmaceutically active proteins (including enzymes and their inhibitors, as well as antibodies and growth

factors). According to a recent Stanford University-led study, the overall impact of my published work places me in the top 0.01% of all scientists in the world.

9. In addition to my research and teaching activities at M.I.T., I have consulted widely for pharmaceutical, medical device, and biotechnology companies. Those consulting relationships have included both innovator and generic pharmaceutical companies.

10. I also have founded six pharmaceutical companies and have been on the scientific advisory boards and/or boards of directors of those companies and of many others. Of particular relevance to the present proceeding, one of the pharmaceutical companies founded by me focused on intravenous and subcutaneous delivery of therapeutic antibodies and fusion proteins.

11. A number of the aforementioned consulting, advisory, and directorship activities have dealt specifically with formulation (such as liquid), stabilization, administration (including parenteral), posttranslational modification, testing, and medical applications of therapeutically active proteins.

12. My *curriculum vitae* (Ex. 2002) summarizes my education and academic experience. Included in it is a list of my professional publications and patents.

13. My opinions and views set forth in this declaration are based on my education, training, and professional experience, as well as the materials I

reviewed (see the next paragraph) in preparing this declaration and the state of scientific knowledge in the art pertaining to the subject matter of the '992 patent at the time of its earliest priority date of June 16, 2006.

14. In forming my opinions expressed herein, I have reviewed the following materials: (a) the Petition for *Inter Partes* review of the '992 patent, IPR2023-00462 Paper 2 (the "Petition" or "Pet." for short); (b) the Declaration of Dr. Ralph Tarantino submitted in support of the Petition (the "Tarantino Declaration"), Ex. 1002; (c) the Institution Decision, IPR2023-00462 Paper 11; (d) the transcript of the deposition of Dr. Tarantino ("Tarantino Transcript"), Ex. 2054; (e) all exhibits cited in these documents; and (f) all articles, papers, and patent documents cited herein.

III. LEGAL STANDARDS

15. I am not an attorney or an expert in U.S. patent law. The following legal standards I have applied in forming my opinions expressed in this declaration were explained to me by counsel for Patent Owner ("counsel"). Thus, each description of legal standards in this section III is based on the instructions of counsel regarding the applicable law.

A. Claim Construction

16. A patent claim term is normally given its ordinary and customary meaning, which is the meaning that the term would have to a person of ordinary

skill in the art (a “POSA”) at the time of the invention (June 16, 2006, in the case of the ’992 patent), when read in the context of the so-called “intrinsic evidence” of the patent claims, specification, and file history. So-called extrinsic evidence outside of the specification and file history, such as dictionaries or treatises, also can be considered in assessing what meaning a claim term has in the context of the patent. Extrinsic evidence is less significant than intrinsic evidence in construing claim terms.

B. Anticipation

17. For a patent to be valid, the claimed invention must be novel (i.e., new) at the time of the invention. A claimed invention that is not novel is said to be “anticipated.”

18. For a patent claim to be deemed anticipated, every limitation of the claimed invention must be found in a single prior art reference, either expressly or inherently. The words of a prior art reference do not have to be the same as in the claim, but all claim limitations must be present.

19. A prior art reference may inherently disclose a claim limitation if the limitation is necessarily present or is the natural result of the combination of expressly disclosed prior art elements. But inherency cannot be established by probabilities or possibilities—the mere fact that a certain thing may result from a given set of circumstances is not sufficient to establish inherency.

20. Evidence beyond an asserted prior art reference, including data that are not prior art, may be relied on in the inherency analysis to demonstrate what characteristics were “necessarily present” given the information provided in the prior art reference about a product. However, post-priority-date information may not be relied upon to supply the identity of a prior art product that is not known or determinable based on the prior art disclosures of its characteristics.

C. Obviousness

21. For a patent to be valid, the claimed invention must be non-obvious to a POSA at the time of the invention. A patent claim is obvious in view of the prior art if the differences between the claim and the prior art are such that, at the time of the invention, the claimed subject matter as a whole would be obvious to a POSA.

22. The obviousness inquiry entails consideration of whether a POSA would be motivated to combine the teachings of the prior art references to achieve the claimed invention and would have a reasonable expectation of success in doing so.

23. Obviousness cannot be based on what was unknown to a POSA at the time an invention was made. Thus, inherency may not be relied upon to supply a missing claim limitation in the obviousness context unless the missing limitation is both inherent and expected, even if inherency is later established.

D. Level of a Person of Ordinary Skill in the Art (“POSA”)

24. Celltrion and Dr. Tarantino have relied on the following definition of a POSA for the ’992 patent:

[T]he POSA at the time of the invention would have had a Ph.D. in pharmaceutical sciences or a similar field, with at least several years of experience in the development, manufacture and characterization of formulations of therapeutic proteins, including, for example, fusion proteins or antibodies. The POSA may also have had less education but substantially more practical relevant work experience. This individual would have understood how to combine proteins with compatible excipients such as surfactants, stabilizers, salts and buffers of various pH values, and how to adjust these combinations in order to optimize their stability in liquid or solid form. This individual also would have been able to use state-of-the-art analytical methods, such as SEC, to assess stability and compatibility.

This POSA also would have had access to other individuals typically employed in developing protein active pharmaceutical ingredients and products, including those involved in upstream and downstream manufacturing, analytical chemistry, pharmacokinetics, clinical testing, pharmaceutical packaging, and regulatory affairs. These diversely-qualified individuals would have worked together as needed during development.

25. Counsel has asked me to adopt the same POSA definition for purposes of this declaration. Based on my experience with protein formulation and

the biopharmaceutical industry, I believe this definition generally reflects level of skill in the art.

26. I agree that a POSA in 2006 would have the ability to “combine proteins with compatible excipients such as surfactants, stabilizers, salts and buffers of various pH values, and [] to adjust these combinations in order to optimize their stability in liquid or solid form.” However, as discussed below in ¶¶ 51-63, 153-158, and 167-179, I do not believe that a POSA would turn a blind eye to other formulation constraints and objectives in order to single-mindedly optimize the stability of a protein. And not every pharmaceutical protein could be made stable while concurrently meeting other formulation constraints. I address these issues in the context of my opinions below addressing the validity of the challenged claims of the ’992 patent, as such issues are more appropriately addressed there, rather than in the context of the definition of a POSA.

IV. RELEVANT TECHNICAL BACKGROUND

A. Formulation of Protein Drugs

1. Types of chemical and physical degradation of proteins

27. Proteins can deteriorate or “degrade” by a number of different pathways. These are often grouped at a high level into “chemical” and “physical” degradation pathways. “Chemical degradation” of proteins refers to their covalent modifications and includes deamidation, polypeptide chain cleavage, and

oxidation. “Physical degradation” refers to a change in protein molecules which is not accompanied by breaking or formation of covalent bonds and includes aggregation, denaturation, and surface adsorption. Ex. 2015¹, 33-34.

28. One common way in which proteins degrade or deteriorate is through their aggregation. Aggregation is the association or agglomeration of protein molecules into larger aggregate structures which can be water-insoluble, and it can occur through chemical and/or physical processes. Aggregation can occur due to hydrophobic interactions among proteins, for example, when protein molecules first partially unfold and then clump together predominantly due to hydrophobic interactions. Ex. 2015, 62-64; Ex. 1047², 21. Protein “unfolding is primarily caused by the [aqueous] solvent environment (pH, salts, co-solvents, etc.), temperature, or surface interactions.” Ex. 2015, 63. Partial unfolding can also

¹ Frokjaer, S., & Hovgaard, L. (Eds.). (2000). *Pharmaceutical Formulation Development of Peptides and Proteins*. Taylor & Francis.

² Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. (1999). *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*. Food and Drug Administration, HHS. <https://www.fda.gov/media/71510/download>.

occur as a result of mechanical stresses on the protein, such as shear stress during handling. Ex. 2015, 69. The initial stages of aggregation are specific to the protein “in the sense that they involve the interaction of specific structural subunits of one [protein] molecule with ‘matching’ hydrophobic surface areas of structural subunits of a neighboring molecule.” Ex. 2015, 64. Many factors thus determine whether aggregates will form in a given pharmaceutical formulation, including the nature of the protein itself and the additional inactive ingredients typically present in the formulation (often referred to as “excipients”).

29. Another way proteins degrade in aqueous (i.e., water-based) solutions is through polypeptide chain cleavage (hydrolysis), which is the process through which they are broken down or cleaved into smaller peptide fragments. Ex. 1047, 21. Protein cleavage reactions can be catalyzed by either enzymes or certain chemicals. Enzymes that can cleave peptide bonds are called proteases. In cells, proteases regulate many important biological pathways, such as apoptosis or programmed cell death. Proteases are also responsible for breaking down excess or unusable proteins into shorter peptides, and ultimately constituent amino acids, that can be re-combined into useful proteins.

30. Other protein cleavage pathways are non-enzymatic and catalyzed by certain chemical agents. One such example is protein splicing, or intein splicing. Protein splicing occurs when a protein editor, or an intein, cuts itself out of a host

protein and creates a new peptide bond between its two flanking regions. Some steps of the protein splicing are not externally catalyzed. Ex. 2048³, 2. However, some other steps of the process—namely, conversion of an aspartic acid (“Asp”) amino acid residue into a succinimide intermediate—will not occur appreciably under physiological conditions without a catalyst. Ex. 2049⁴, 2. For example, phosphate ions can catalyze the formation of the succinimide intermediate (which I annotated by a red oval in the scheme below; Fig. 1). *Id.*, 1.

³ Vila-Perelló, M., & Muir, T. W. (2010). Biological applications of protein splicing. *Cell*, 143(2), 191-200.

⁴ Kirikoshi, R., Manabe, N., & Takahashi, O. (2018). Phosphate-catalyzed succinimide formation from Asp residues: A computational study of the mechanism. *International Journal of Molecular Sciences*, 19(2), 637.

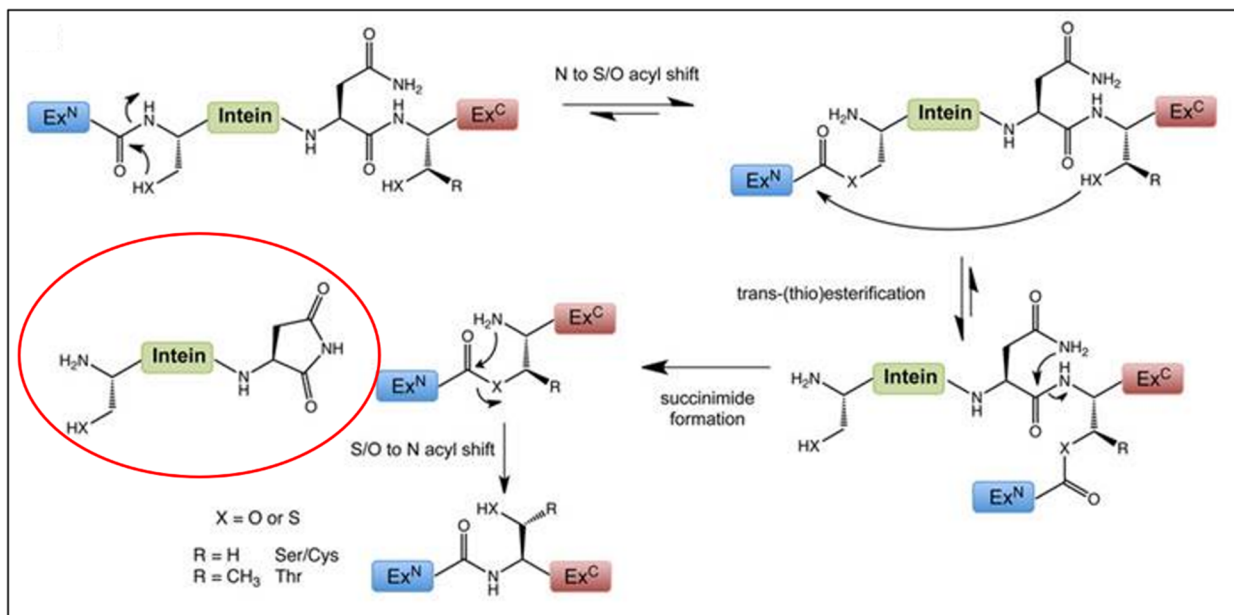


Figure 1: Protein splicing reaction.⁵

31. Deamidation is a chemical reaction in which an amide functional group in the side chain of the asparagine (“Asn”) or glutamine (“Gln”) amino acid residues is hydrolyzed to release ammonia and form a carboxyl functional group (thereby resulting in aspartic acid (“Asp”) or glutamic acid (“Glu”) residues, respectively). The non-enzymatic deamidation reactions of Asn and, to a lesser extent, Gln residues in proteins are associated with protein turnover and age-

⁵ Adapted from Vila-Perelló, M., & Muir, T. W. (2010). Biological applications of protein splicing. *Cell*, 143(2), 191-200.

related diseases. Ex. 2064⁶, 5, 40-42. The Asn deamidation in proteins begins with conversion of the Asn to a succinimide intermediate, and formation of this intermediate can be catalyzed by phosphate ions. Ex. 2049, 1.

32. The mechanism of the Asn deamidation reaction is depicted below:

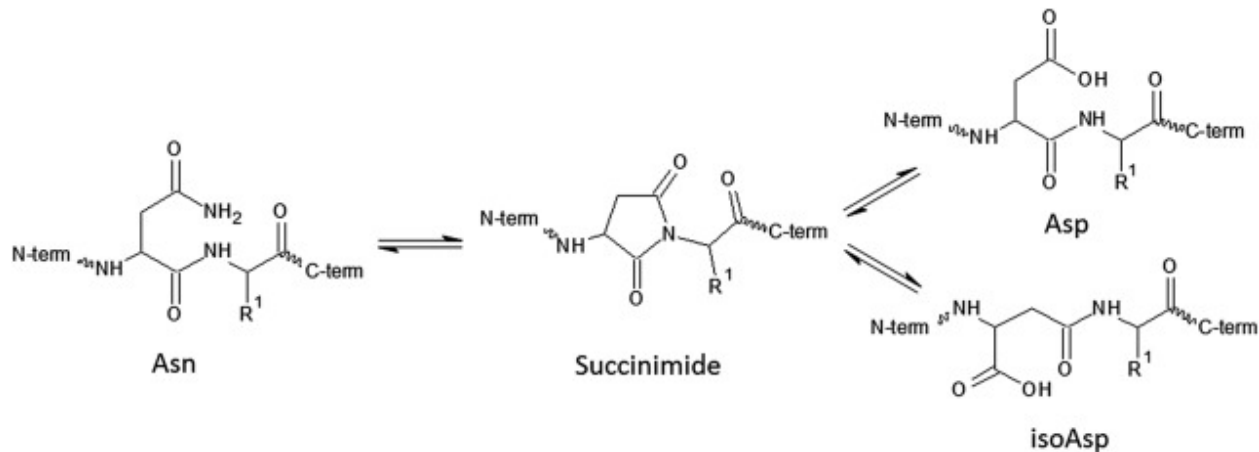


Figure 2: Deamidation of an asparagine residue in proteins.⁷

33. Protein degradation products, including aggregates and fragments, may arise during production and/or may form upon storage over a period of days,

⁶ Robinson, N. E., & Robinson, A. (2004). *Molecular Clocks: Deamidation of Asparaginyl and Glutaminyl Residues in Peptides and Proteins*. Althouse Press.

⁷ Adapted from Robotham, A. C., & Kelly, J. F. (2020). LC-MS characterization of antibody-based therapeutics: recent highlights and future prospects. In *Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics* (pp. 1-33). Elsevier.

weeks, or months. As Frokjaer 2000 (Ex. 2015) and Frokjaer 2005 (Ex. 2020) explain, aggregation generally occurs in phases. Ex. 2015, 63-64; Ex. 2020, 5. The first “lag” phase is characterized by very little visible aggregation. For many proteins, the lag phase is a nucleation phase where stable agglomerates (the “nuclei”) consisting of just a few protein molecules form. Then visible aggregation accelerates (and turbidity and precipitation are typically observed) as larger protein aggregates form around the nuclei. Ex. 2015, 63-64; Ex. 2020, 5.

2. Determinants of the native conformation

34. Various factors influence whether a protein remains in its native conformation in a liquid formulation. These factors include (1) the nature of the protein and the formulation, (2) post-translational modifications imparted by the host cell, and (3) storage conditions, including the container and temperature of storage.

b. Amino Acid Sequence

35. Notably, “[e]very protein has unique physicochemical characteristics and the potential to behave differently stability-wise in the presence of a particular excipient.” Ex. 1013⁸, 145. An amino acid sequence can impact protein stability. Dr. Tarantino acknowledged this well-known fact when he commented that

⁸ McNally, E. J. (Ed.). (2000). *Protein Formulation and Delivery*.

“stability is generally indicated by the amino acid sequence.” Ex. 2054, 17:13-20.

Even relatively small changes in the amino acid sequence of proteins can have large effects on their stability, including their propensity to aggregate or to undergo cleavage.

36. The foregoing has been well known for decades. As an illustration of how amino acid sequence can affect aggregation, Brange (Ex. 2063) reported that bovine insulin, which differs from human and porcine insulins by just two amino acid residues, was nonetheless significantly more prone to aggregate. *Id.*⁹, 2.

37. As Dr. Tarantino commented, sulfhydryl (-SH) groups in proteins are one driver of chemical aggregation. Since these groups reside only in cysteine (“Cys”) amino acid residues, changing the amino acid sequence by deleting or introducing these residues can affect the propensity of a protein to aggregate by this degradation pathway. Ex. 2054, 115:22-116:7.

38. Notably, changing even a single amino acid residue in a protein can sometimes significantly alter the stability. For example, my own pre-2006

⁹ Brange, J., Andersen, L., Laursen, E. D., Meyn, G., & Rasmussen, E. (1997). Toward understanding insulin fibrillation. *Journal of Pharmaceutical Sciences*, 86(5), 517-525.

publication (Ex. 2041)¹⁰ reported that replacing asparagine (“N”) with aspartic acid (“D”) at position 78 of the enzyme triosephosphate isomerase markedly altered, namely reduced, this protein’s stability. In particular, the mutant protein was less resistant than its wild-type predecessor to denaturation and inactivation caused by elevated temperature, denaturants, alkaline pH, and proteolysis.

39. Likewise, Sanfelice (Ex. 2019) describes a protein that typically undergoes cold denaturation at 16.8° C, but changing only one amino acid residue resulted in it undergoing cold denaturation only at 3-4° C. *Id.*¹¹, 2-3, Table 1. In other words, a change to only one amino acid residue allows a protein to be stored

¹⁰ Casal, J. I., Ahern, T. J., Davenport, R. C., Petsko, G. A., & Klibanov, A. M. (1987). Subunit interface of triosephosphate isomerase: site-directed mutagenesis and characterization of the altered enzyme. *Biochemistry*, 26(5), 1258-1264.

¹¹ Sanfelice, D., Morandi, E., Pastore, A., Niccolai, N., & Temussi, P. A. (2015). Cold denaturation unveiled: molecular mechanism of the asymmetric unfolding of yeast frataxin. *ChemPhysChem*, 16(17), 3599-3602.

at 5° C when the wild-type predecessor could not be. Also, Yang (Ex. 2042¹²) describes a COVID-19 spike protein where a single mutation increased stability when compared to the wild-type counterpart.

40. The amino acid sequence also can impact the susceptibility to peptide-bond hydrolysis in proteins. As discussed by Robinson (Ex. 2065) and by Robinson & Robinson (Ex. 2064), such cleavage usually occurs following Asp, Asn, and Glu residues. Ex. 2065¹³, 8; Ex. 2064, 49-50. Enzymatic hydrolysis of peptide bonds in proteins is also sequence-dependent because proteases recognize cleavage sites with specific amino acid sequences. For these reasons, I agree with

¹² Yang, T. J., Yu, P. Y., Chang, Y. C., & Hsu, S. T. D. (2021). D614G mutation in the SARS-CoV-2 spike protein enhances viral fitness by desensitizing it to temperature-dependent denaturation. *Journal of Biological Chemistry*, 297(4), 101238.

¹³ Robinson, N. E., Robinson, Z. W., Robinson, B. R., Robinson, A. L., Robinson, J. A., Robinson, M. L., & Robinson, A. B. (2004). Structure-dependent nonenzymatic deamidation of glutaminyl and asparaginyl pentapeptides. *Journal of Peptide Research*, 63(5), 426-436.

Dr. Tarantino that cleavage is a sequence-dependent degradation pathway. Ex. 2054, 21:15–22:5.

41. Linker sequences between protein domains can affect stability, too. For example, Robinson (Ex. 2038) constructed a library of linkers, varying the linker length and composition, and found that protein stability changed substantially as a function of both, noting that “[a]lterations in linker regions have been found to affect the stability, oligometric state, proteolytic resistance, and solubility of single-chain proteins.” *Id.*¹⁴, 1.

42. The Tong reference (Ex. 2039¹⁵) examined the effect of varying the inter-domain linker’s amino acid sequence between various domains of the human protein titin and reported significant differences in stability among the different resulting protein variants.

¹⁴ Robinson, C. R., & Sauer, R. T. (1998). Optimizing the stability of single-chain proteins by linker length and composition mutagenesis. *Proceedings of the National Academy of Sciences of the U.S.A.*, 95(11), 5929-5934.

¹⁵ Tong, B., Tian, F., & Zheng, P. (2022). Interdomain linker effect on the mechanical stability of Ig domains in titin. *International Journal of Molecular Sciences*, 23(17), 9836.

43. The Chen reference (Ex. 2040¹⁶) further reviewed examples of different linker amino acid sequences that can improve protein stability or intrinsic biological activity that otherwise have the same functional domains. *See id.*¹⁶, Table 3.

44. Because amino acid sequence can impact protein stability, multiple literature sources recommend manipulating the amino acid sequence as a potential way to enhance physical stability. Ex. 2015, 72; Ex. 1006¹⁷, 14. Wang observes, though, that this approach is “seldom used” because the effects of a change to the amino acid sequence are difficult to predict. Ex. 2053¹⁸, 23.

¹⁶ Chen, X., Zaro, J. L., & Shen, W. C. (2013). Fusion protein linkers: property, design and functionality. *Advanced Drug Delivery Reviews*, 65(10), 1357-1369.

¹⁷ Chang, B. S., & Hershenson, S. (2002). Practical approaches to protein formulation development. In *Rational Design of Stable Protein Formulations: Theory and Practice*, 1-25.

¹⁸ Wang, W. (2005). Protein aggregation and its inhibition in biopharmaceutics. *International Journal of Pharmaceutics*, 289(1-2), 1-30.

c. Glycosylation pattern

45. Its glycosylation pattern may affect protein's propensity to aggregate. Glycosylation is the process by which eukaryotic cells, after producing the polypeptide chain, covalently attach carbohydrates to certain positions therein. These carbohydrates, or "glycans," are typically hydrophilic. The sites of glycan attachment, as well as the structure of the attached glycan, can vary depending on the host cell line and other conditions. Depending on their nature, size, and location, glycans can block hydrophobic residues on the protein surface from interacting with hydrophobic residues on the surfaces of a neighboring protein molecule. As Solá and Griebenow (Ex. 2066) summarized in their review of the effects of glycosylation on protein stability, changes to glycosylation patterns had been shown to reduce or even prevent aggregation in such therapeutic proteins as insulin, α -galactosidase, thyroid-stimulating hormone, and erythropoietin. *Id.*¹⁹, 11.

d. Formulation

46. Excipients also can impact protein stability. The unfolding that oftentimes precedes aggregation, for example, is driven by factors including "the

¹⁹ Solá, R. J., & Griebenow, K. (2009). Effects of glycosylation on the stability of protein pharmaceuticals. *Journal of Pharmaceutical Sciences*, 98(4), 1223-1245.

solvent environment (pH, salt, co-solvents, etc.).” Ex. 2015, 63-64. Cleavage of the polypeptide chain also can be driven by excipients. Phosphate, in particular, can catalyze protein cleavage, as discussed above in ¶¶ 30-32 and below in ¶¶ 190-197.

47. Higher protein concentration generally leads to more aggregation. Ex. 1002, ¶ 102 (“formulations with lower protein concentrations tend to exhibit *less* aggregation than ones with higher concentrations.”) (citing Ex. 1006, Table 6); *see* Ex. 1013, 146 (“rates of degradation typically vary as a function of protein concentration.”); Ex. 2053, 7, 9; Ex. 2050, 470:8-14. While this is a general trend, the relationship between protein concentration and protein aggregation is neither uniform nor linear. At low concentrations in solution, for example, a greater proportion of the protein will be present at air-water and container surface-water interfaces, so aggregation may remain high at those locations. For some oligomeric proteins, such as insulin (which is typically a hexamer in the presence of Zn^{2+}), low concentrations result in a higher concentration of less-stable monomers, thereby increasing the propensity to aggregate. Ex. 2015, 64; Ex. 2063, 1, 3.

e. Freeze-thaw cycles

48. Freeze-thaw cycles can also impact whether a protein remains in the native conformation. Arakawa (Ex. 2060) discloses that “[m]ost proteins are only

marginally stable at neutral pH and room temperature and hence are readily denature by...freeze-thawing and freeze-drying procedures.” *Id.*²⁰, 2.

49. Further, Tang (Ex. 2061) teaches that “[f]reezing often induces many destabilizing stresses, particularly for protein drugs. These stresses include increase of protein concentration that enhances the protein–protein interaction leading to aggregation, pH change arising from crystallization of buffer salts, reduced hydrophobic interactions caused by the ‘dehydration’ effect of ice formation that removes bulk water from the protein phase, formation of large ice–aqueous interfaces, and an enormous increase in ionic strength.” *Id.*²¹, 3. In the second step of a freeze-thaw cycle, the thawing itself causes additional damage by “exert[ing] additional interfacial tension or shear on the entrapped proteins and

²⁰ Arakawa, T., Prestrelski, S. J., Kenney, W. C., & Carpenter, J. F. (2001). Factors affecting short-term and long-term stabilities of proteins. *Advanced Drug Delivery Reviews*, 46(1-3), 307-326.

²¹ Tang, X., & Pikal, M. J. (2004). Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharmaceutical Research*, 21, 191-200.

hence causes additional damage to the latter.” Ex. 2062²², 1. Shear stress can contribute to denaturation of a protein and thus create the potential for aggregation. Ex. 2015, 69; Ex. 2053, 20.

50. All the factors laid out above have the potential to affect the conformation and stability of a protein. In 2006, these general principles were understood—indeed, it was known that even a minor change in any of the aforementioned variables could affect whether a protein remains in its native conformation over time. However, the magnitude of the effect would be impossible to predict based on theory alone.

3. Formulation objectives and constraints

51. Development of liquid protein formulations for therapeutic use is a balancing act involving many objectives and constraints that often conflict with each other. These objectives and constraints include, for example, (1) maintaining adequate stability over the shelf-life of a product, (2) providing sufficient concentration for the preferred route of administration, (3) avoiding formulations

²² Cao, E., Chen, Y., Cui, Z., & Foster, P. R. (2003). Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. *Biotechnology & Bioengineering*, 82(6), 684-690.

so viscous as to impede administration to a patient, and (4) avoiding pH and tonicity levels that might cause patient discomfort.

52. The first aforementioned objective is attaining adequate stability of the protein-based product over its shelf-life. In doing so, various types of physical and chemical instability must be considered. What does or does not constitute an acceptable stability profile for a protein formulation is also specific to that protein, its formulation, and its intended use. Tolerance for various impurities is set on a product-by-product basis, with around 95% retention of the native protein conformation being accepted for some protein products as of 2006.

53. The route of administration may impose constraints on the volume of a therapeutic dose. For subcutaneous injections, for example, the volume of injection is ideally less than 0.5 mL, as it has been shown that increasing the injection volume from 0.5 to 1.0 mL increases the pain experienced by a patient significantly. Ex. 2052²³, 2. For intravitreal injections, the volume is even more limited because a medication is injected into the small space in the back of the eye

²³ Jørgensen, J. T., Rømsing, J., Rasmussen, M., Møller-Sonnergaard, J., Vang, L., & Musæus, L. (1996). Pain assessment of subcutaneous injections. *Annals of Pharmacotherapy*, 30(7-8), 729-732.

called the vitreous cavity. Ex. 2018²⁴, 4 (finding elevated intraocular pressure even with a dose volume of just 0.05 mL).

54. The syringeability and injectability of a formulation also must be taken into account. Very viscous formulations are physically difficult to inject.

55. Patient comfort and acceptance is another important factor. Injection site pain may result from administering a formulation with extreme (i.e., very distinct from physiological) tonicity or pH. “Tonicity” is related to the concentration of solutes that do not permeate cell membranes (like salts, such as NaCl) at the delivery site. “Hypertonic” solutions are those that have a higher concentration of non-permeating solutes than the plasma or other physiological fluids at the delivery site. Very high tonicity of an injectable solution can contribute to injection site pain. Ex. 2083²⁵, 1 (“[h]ypertonic sodium chloride or

²⁴ Hollands, H., Wong, J., Bruen, R., Campbell, R. J., Sharma, S., & Gale, J. (2007). Short-term intraocular pressure changes after intravitreal injection of bevacizumab. *Canadian Journal of Ophthalmology*, 42(6), 807-811.

²⁵ Svendsen, O., Edwards, C. N., Lauritzen, B., & Rasmussen, A. D. (2005). Intramuscular injection of hypertonic saline: *in vitro* and *in vivo* muscle tissue

potassium chloride causes pain when injected into human muscles or other deep tissues”).

56. The various conflicting objectives and constraints must be balanced against one another, and often improving a formulation along one dimension requires sacrifices in another.

57. In particular, different stability requirements may be in tension with each other. For example, it may be desirable to include a preservative in the pharmaceutical formulation to prevent microbial contamination and growth. Ex. 1006, 10. But this extra excipient introduces additional stability concerns because “[i]n general, the addition of preservative(s), regardless of the preservative used, significantly changes the stability profiles of proteins,” thus sometimes leading to aggregation and other modes of protein degradation. Ex. 1006, 10.

58. As noted above in ¶ 47 above, raising the concentration of a protein in a liquid formulation typically increases the likelihood of aggregation. In other words, having a higher protein concentration (*i.e.*, a more “crowded” aqueous formulation) can adversely impact long-term stability because, depending on the

toxicity and spinal neurone c-fos expression. *Basic & Clinical Pharmacology & Toxicology*, 97(1), 52-57.

formulation, a more concentrated protein solution generally tends to exhibit more aggregation over time. Ex. 1002, ¶ 102 (“formulations with lower protein concentrations tend to exhibit *less* aggregation than ones with higher concentrations.”) (citing Ex. 1006, Table 6); *see also* Ex. 1013, 146 (“rates of degradation typically vary as a function of protein concentration.”).

59. Furthermore, while protein stabilizing agents, such as sucrose and glycerol, can be used in formulations to help maintain the native secondary and tertiary structure of proteins, thereby potentially preventing degradation processes like aggregation, addition of these stabilizers also has the undesirable side effect of increasing viscosity and tonicity of the aqueous solution.

60. Protein aggregation kinetics are also associated with the pH of aqueous solutions. For example, in certain proteins, aggregation accelerates when the pH is lowered from pH 5.5 to 4.0. Ex. 2092²⁶, 3. As a higher buffer concentration affords greater buffering capacity, increasing buffer concentration

²⁶ Pfefferkorn, C. M., McGlinchey, R. P., & Lee, J. C. (2010). Effects of pH on aggregation kinetics of the repeat domain of a functional amyloid, Pmel17. *Proceedings of the National Academy of Sciences of the U.S.A.*, 107(50), 21447-21452.

could mitigate pH changes that lead to aggregation. However, high buffer concentrations can lead to other forms of instability, resulting in protein degradation, particularly in phosphate buffers, which are known to accelerate deamidation and peptide bond hydrolysis. Ex. 2064, 22, 24, 50.

61. Developing a formulation to achieve sufficient protein stability in a liquid form for a pharmaceutically relevant period of time is not a simple task, and a POSA would expect that some degradation will occur regardless of the formulation. For example, this may occur as a result of an unexpected freezing of a protein aqueous solution during distribution and storage or of an excessive agitation during product shipping. Ex. 1006, 9.

62. Sometimes, a suitably stable aqueous formulation that meets the other constraints cannot be developed for a particular protein, and adequate properties can only be achieved by using a more complex and less convenient type of formulation, such as a lyophilized formulation, or by changing the protein itself. Ex. 1013, 139; *see* ¶ 44 above.

63. Notably, “[e]very protein has unique physicochemical characteristics and the potential to behave differently stability-wise in the presence of a particular excipient.” Ex. 1013, 145.

B. Protein Structure Generally

64. Amino acids are the building blocks of all proteins. There are some 20 “standard” amino acids (whose names can be referred to by their respective unique three-letter or one-letter abbreviations), and proteins are formed by covalently linking amino acids to each other by means of peptide bonds to form a polypeptide chain. Ex. 2043²⁷, 4-5. The sequence of amino acids defining a protein is referred to as the protein’s primary structure. But the overall protein structure is much more complex than the primary structure alone. That is because the polypeptide chain defining a protein exists not in linear two-dimensional form but instead is folded into a complex three-dimensional shape. For example, portions of the polypeptide chain will spontaneously fold into defined regions (such as helices), which are referred to as the secondary structure of the protein. And these primary and secondary structures spontaneously combine to form the overall three-dimensional folded form of the protein, called its tertiary structure. Within a given protein tertiary structure, there may be structural segments or “domains,” which can be thought of as the portions of the protein that can fold up

²⁷ Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistry* (5th edn.). W. H. Freeman and Company.

and function almost independently of their neighboring regions. Ex. 2027²⁸, 2. As an example, the diagram below shows the three domains of the protein argininosuccinate lyase (ASL), numbered consecutively:

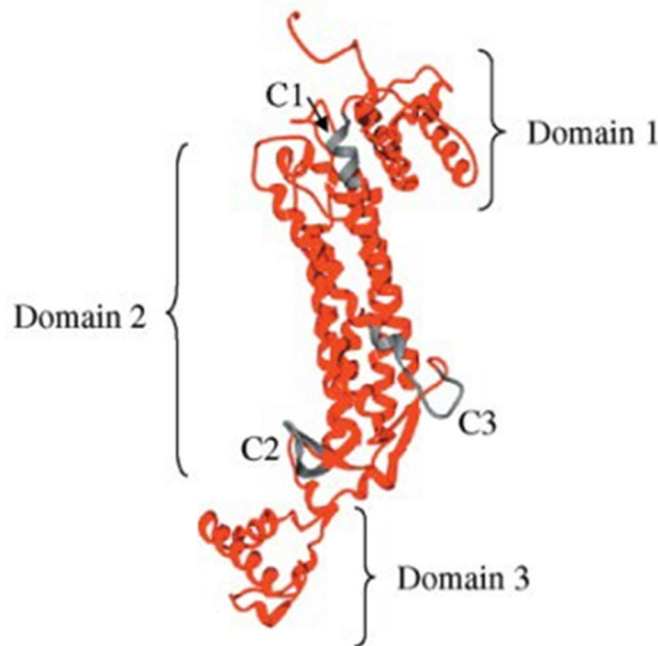


Figure 3: Three structural domains of ASL protein.²⁹

²⁸ Doolittle, R. F. (1995). The multiplicity of domains in proteins. *Annual Review of Biochemistry*, 64(1), 287-314.

²⁹ Adapted from Sampaleanu, L. M., Vallée, F., Thompson, G. D., & Howell, P. L. (2001). Three-dimensional structure of the argininosuccinate lyase frequently complementing allele Q286R. *Biochemistry*, 40(51), 15570-15580. Ex. 2028, 5.

C. VEGF Receptor Proteins

65. Vascular endothelial growth factor (“VEGF”) is a type of protein that has been implicated in various diseases ranging from vascular disease in the retina of the eye to cancer. The human VEGF family consists of five related proteins that occur naturally in the human body: VEGFA, VEGFB, VEGFC, VEGFD, and PlGF (placental growth factor). Ex. 2029³⁰, 1. These VEGF proteins interact with the corresponding portions of one of three distinct “VEGF receptor” (“VEGFR”) proteins (*see* Fig. 4 below): VEGFR1, VEGFR2, and VEGFR3 (VEGF receptors 1, 2, and 3, respectively). *Id.* VEGFR1 is also known as Flt1 (Fms-like tyrosine kinase 1, where Fms is yet another protein), and VEGFR2 is also known as KDR (kinase insert domain receptor) or Flk1 (fetal liver kinase 1). *Id.*

³⁰ Pandey, A. K., Singhi, E. K., ... & Moslehi, J. (2018). Mechanisms of VEGF (vascular endothelial growth factor) inhibitor-associated hypertension and vascular disease. *Hypertension*, 71(2), e1-e8.

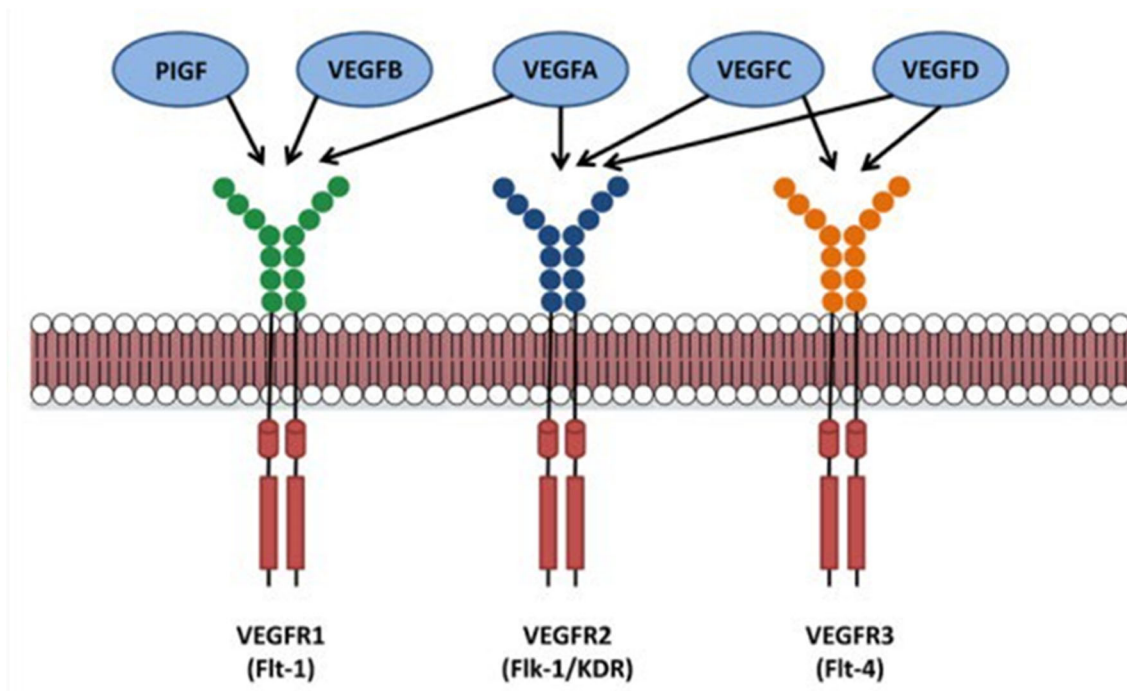


Figure 4: VEGF ligands and receptors.³¹

66. Structurally, the VEGFR1, VEGFR2, and VEGFR3 receptor proteins each has 7 distinct domains that extend from the surface of the cell (depicted in the figure above as individual green, blue, and orange circles) that are referred to numerically (*e.g.*, “VEGFR1 domain 1” or “VEGFR2 domain 2”).

³¹ Adapted from Ex. 2029, 2.

D. Fusion Proteins

67. As their name suggests, fusion proteins are engineered in the laboratory by chemically combining different parts of distinct proteins together to obtain a “hybrid” protein that does not naturally occur as such. Ex. 2017³², 6.

68. In 2006, fusion proteins were an emerging class of therapeutic proteins. Only three fusion proteins were approved by the U.S. Food and Drug Administration (“FDA”) by then: Enbrel (in 1998), Amevive (in 2003), and Orencia (in 2005). Ex. 2030³³, 2, Table 4.1. In 2011, Regeneron obtained its first FDA approval for aflibercept (or EYLEA[®]), which is a fusion protein now approved in the United States and in the European Union to treat certain diseases, like wet age-related macular degeneration and metastatic colorectal cancer. A schematic representation of the general architecture of the aflibercept protein is shown below:

³² Chamow, S. M., & Ashkenazi, A. (1999). *Antibody Fusion Proteins*. John Wiley & Sons.

³³ Shukla, A. A., & Gottschalk, U. (2014). Downstream processing of Fc-fusion proteins. In *Therapeutic Fc-Fusion Proteins*, 97-114.

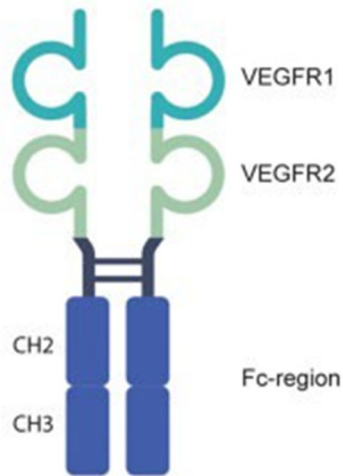


Figure 5: Schematic depiction of aflibercept.³⁴

69. As seen above, aflibercept is formed via a fusion of parts from VEGF receptor proteins (specifically, domain 2 from VEGFR1 (“VEGFR1”) and domain 3 from VEGFR2 (“VEGFR2”)) combined with parts from an antibody protein (specifically, the CH2 and CH3 domains of the “Fc region” of the antibody). But this generalized structural description of aflibercept is simplistic and incomplete, for example, because typically fusion proteins contain bridging (or “hinge”) regions, which are amino acid sequences that hold protein chains together, and

³⁴ Adapted from Lode, H. E., Gjøllberg, T. T., ... & Andersen, J. T. (2019). A new method for pharmaceutical compounding and storage of anti-VEGF biologics for intravitreal use in silicone oil-free prefilled plastic syringes. *Scientific Reports*, 9(1), 18021.

“signal” sequences, which are short chains of amino acid residues found at the end of a polypeptide sequence that act like a “flag” to signal to the transport mechanism of the cell where the protein should go. Ex. 2093³⁵, 4. Thus, importantly, even if they comprise the same structural domains, two fusion proteins may still have quite different amino acid sequences overall.

70. Whereas the first therapeutic antibody (muromonab-CD3) was successfully developed in 1986, fusion proteins were still a relatively nascent biotechnology in 2006, with the first fusion protein, etanercept being approved in 1998. Ex. 2009³⁶, 3, Table 1. Given the lack of experience and knowledge with developing formulations for fusion products, the exact challenges and conditions for creating a stable fusion protein formulation were not well-described at the time.

³⁵ Hansson, K., & Stenflo, J. (2005). Post-translational modifications in proteins involved in blood coagulation. *Journal of Thrombosis and Haemostasis*, 3(12), 2633-2648.

³⁶ Rodgers, K. R., & Chou, R. C. (2016). Therapeutic monoclonal antibodies and derivatives: Historical perspectives and future directions. *Biotechnology Advances*, 34(6), 1149-1158.

Achieving a stable protein formulation would have required many rounds of testing and relied on an empirical trial-and-error approach.

V. THE CITED REFERENCES

71. Dr. Tarantino primarily relies on two prior art references as disclosures of the VEGF antagonist formulated as required by the claims of the '992 patent: Fraser (Ex. 1009³⁷) and Wulff (Ex. 1016³⁸). Both of these

³⁷ Fraser, H. M., Wilson, H., Rudge, J. S., & Wiegand, S. J. (2005). Single injections of vascular endothelial growth factor trap block ovulation in the macaque and produce a prolonged, dose-related suppression of ovarian function. *Journal of Clinical Endocrinology & Metabolism*, 90(2), 1114-1122. (“Fraser”).

³⁸ Wulff, C., Wilson, H., Wiegand, S. J., Rudge, J. S., & Fraser, H. M. (2002). Prevention of thecal angiogenesis, antral follicular growth, and ovulation in the primate by treatment with vascular endothelial growth factor Trap R1R2. *Endocrinology*, 143(7), 2797-2807. (“Wulff”).

references, in turn, cite the Holash reference (Ex. 1010³⁹), and Wulff additionally cites the '319 Publication (Ex. 1029⁴⁰). Dr. Tarantino also relies on the '309 publication (Ex. 2017⁴¹), although it is not cited in the other four references. The relevant disclosures in these five prior art references are summarized below.

A. Fraser

72. Fraser studied the effects on ovarian function in macaques resulting from doses of a VEGF antagonist that the paper refers to variously as “VEGF Trap” or “VEGFTrap_{R1R2}.” Ex. 1009, 1114-15, 1121-22. According to Fraser, the protein was provided by “Regeneron Pharmaceuticals, Inc., Tarrytown, NY.” *Id.*, 1115. Apart from these names, Fraser states that the fusion protein used “compris[es] Ig [*i.e.*, immunoglobulin] domain 2 of human VEGF-R1 and Ig

³⁹ Holash, J., Davis, S., Papadopoulos, N., ... & Rudge, J. S. (2002). VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proceedings of the National Academy of Sciences of the U.S.A.*, 99(17), 11393-11398. (“Holash”).

⁴⁰ International Publication No. WO 00/75319 to Papadopoulos et al. (“’319 Publication”).

⁴¹ U.S. Patent Publication No. 20040265309A1 to Kandel et al. (“’309 Publication”).

domain 3 of human VEGF-R2, expressed in sequence with the human Fc.” *Id.*

Fraser does not explicitly state either the amino acid sequence of its VEGF Trap or what host cell line was used to produce its VEGF Trap.

73. Fraser also provides information about the formulation of its VEGF Trap. The VEGF Trap was formulated “at a concentration of 24.3 mg/ml in 2-ml aliquots in buffer composed of 5 mM phosphate, 5 mM citrate, 100 mM NaCl (pH 6.0), and 0.1% wt/vol Tween 20, with either 20% glycerol or 20% sucrose.” Ex. 1009, 1115. Fraser further discloses how the formulation was handled: all formulations “were stored at –20° C until required, at which time they were thawed. Any compound remaining was stored at 4° C and used within 2 [weeks].” *Id.* Fraser does not explicitly disclose any native conformation data, or stability data of any kind, for these formulations.

B. Wulff

74. Wulff studied the effects of administering VEGF antagonist subcutaneously on ovarian function in marmoset monkeys. Ex. 1016, 2797-98. Wulff, like Fraser, variably describes the VEGF antagonist as “VEGF Trap” (*id.*, 2797), “VEGF Trap R1R2” (*id.*, 2797-98), “VEGF R1R2” (*id.*, 2806) or just “R1R2” (*id.*, 2800-05). Wulff states that its VEGF Trap was produced in CHO cells. *Id.*, 2798. Wulff does not expressly state the amino acid sequence of its VEGF Trap but provides a functional description of the protein as “compris[ing]

portions of the extracellular, ligand binding domains of the human VEGF receptors Flt-1 (VEGF-R1, Ig domain 2) and KDR (VEGF-R2, Ig domain 3) expressed in sequence with the Fc portion of human IgG.” *Id.* Wulff illustrates the fusion protein used therein with the following cartoon drawing, that I have annotated with green arrows, showing the fusion protein on the right-hand side:

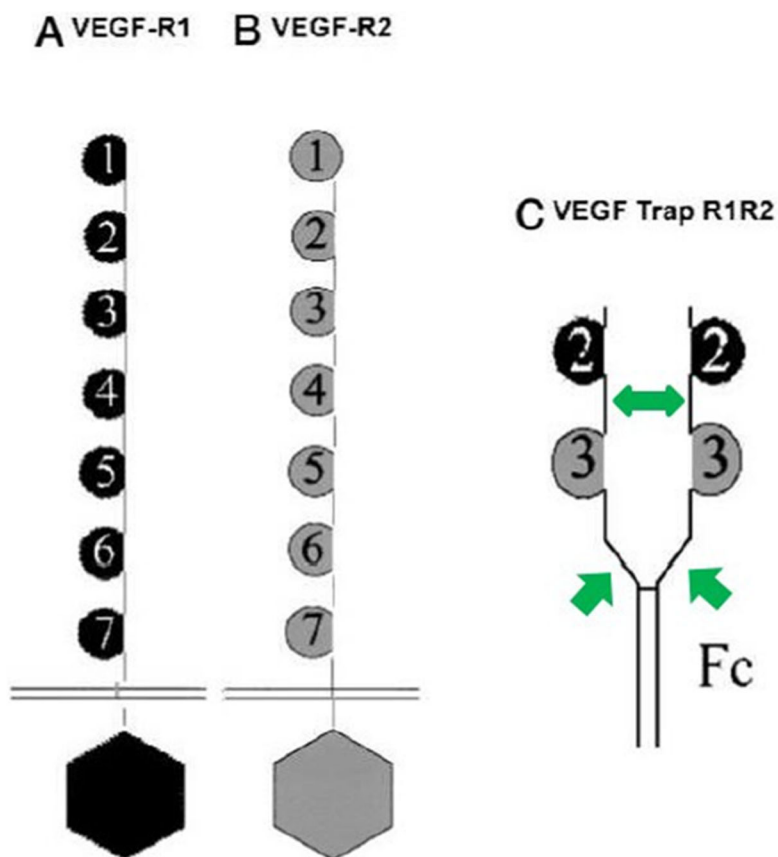


Figure 6: Structures of VEGF-R1 (A) and VEGF-R2 (B), along with VEGF Trap R1R2 (C).⁴²

⁴² As shown in Ex. 1016 (Wulff), Fig. 1.

75. A POSA would recognize that Fig. 6 above (which is Fig. 1 in Wulff) is a schematic diagram, of a type common in the scientific literature, and it shows VEGF receptors 1 and 2, as well as their respective binding domains 2 and 3, that form the VEGF Trap R1R2 molecule. Unlike an amino acid sequence, where one particular amino acid unambiguously follows a certain other amino acid along the polypeptide chain, a schematic diagram like Fig. 6 is inevitably imprecise. For example, Wulff's pictorial diagram connects the binding domains (depicted as equally sized, incomplete, black and grey numbered circles) and the Fc fragment of human IgG (depicted as a long three-sided column) with straight lines, representing some sort of peptide linkers between the domains and the Fc fragment, which I have marked with green arrows. The amino acid residues that encompass these linkers are not specified in any way.

76. Like Fraser, Wulff presents no stability data for any protein formulation.

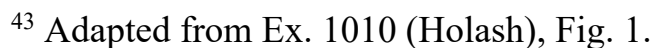
C. Holash

77. Holash (Ex. 1010) is a Regeneron-authored article published in 2002. Ex. 1010, 11393. Holash discloses results of pre-clinical trials that assessed the pharmacokinetic and pharmacodynamic properties of a genus of VEGF antagonists known as "VEGF Traps." *Id.*, 11393-94. Specifically, in a section entitled "Engineering VEGF-Traps," Holash describes modifying a "parental VEGF-Trap"

to create “VEGF-Trap_{ΔB1},” “VEGF-Trap_{ΔB2},” and other fusion proteins, yielding various “VEGF-Trap variants.” *Id.* Holash then reports on the performance of these various “VEGF-Traps” (*i.e.*, plural). *Id.*, 11394-95 (referring to “test[ing] these various VEGF-Traps” and “[u]sing blockade of VEGF-induced acute hypotension to pharmacodynamically compare VEGF-Traps”). Therefore, Holash makes clear that the term “VEGF Traps” refer to a **group** of proteins.

78. Holash does not expressly state the sequences of any of the VEGF Trap proteins it describes. Instead, it states the VEGF receptor domains that each comprises. *Id.*, 11393-94. Holash discusses properties of a VEGF trap protein that it refers to as “R1R2” (Ex. 1010, 11395, Fig. 2) or VEGF-Trap_{R1R2} (*id.*, 11395). Holash explains that this fusion protein “possesses the second Ig domain of VEGFR1 and the third Ig domain of VEGFR2 fused to the Fc portion of human IgG1.” *Id.*, 11394.

79. Like Wulff above, Holash provides what it calls “[s]chematics” of various VEGF Traps, including the R1R2 trap. I have excerpted and annotated one such schematic (Fig. 1A from Holash) here:



45

81. Holash does not disclose any VEGF Trap formulations. And, like Fraser and Wulff, Holash presents no stability data for the proteins it describes.

D. '319 Publication

82. The '319 Publication (Ex. 1029) is a PCT patent publication referenced in Wulff. Ex. 1016, 2798 at n. 1. The '319 Publication discloses

several different VEGF antagonist proteins, including two denoted **Flt1.D2.Flk1D3.FcΔC1(a)** and **VEGFR1R2-FcΔC1(a)**. These two proteins both include Fc regions from human antibody fused to domain 2 from VEGFR1 and domain 3 from VEGFR2. Ex. 1029, 58:22-59:10. Both of these proteins are generally referred to in the '319 Publication as “R1R2” proteins because they include the same general structural domains. Despite these similarities, the '319 Publication also confirms that these two proteins have different amino acid sequences and are, in fact, distinct proteins (as discussed in further detail below in ¶¶ 104-126).

83. The '319 publication names “suitable host cells” for producing the fusion proteins described in the publication, including “a bacterial cell, yeast cell, insect cell, or mammalian cell,” and the more specific examples of *E. coli*, COS, and CHO cells. Ex. 1029, 12:19-26. The '319 publication discloses experimental data relating to VEGF Traps expressed in CHO cells. Ex. 1029, 77:6-80:18. It also discloses experimental data for VEGF Traps expressed in COS cells. Ex. 1029, 55:23-56:6.

84. Like Fraser, Wulff, and Holash, the '319 Publication presents no stability data for the protein formulations it describes.

E. '309 Publication

85. The '309 Publication (Ex. 1027) is a United States patent application published in December of 2004. The publication “relates to methods of promoting regression of tumors and metastases by inhibiting vascular endothelial growth factor (VEGF) activity” (Ex. 1027, ¶ 2) and discloses experiments testing the effect of VEGF inhibitors on tumors in mice.

86. The '309 Publication discloses and claims multiple VEGF Trap proteins. Ex. 1027, ¶ 5, claim 4. Among these, it names **VEGFR1R2.FcΔC1(a)** as one. Ex. 1027, ¶ 22. The '309 publication provides an amino acid sequence for **VEGFR1R2.FcΔC1(a)** and incorporates by reference the '319 publication in its discussion of **VEGFR1R2.FcΔC1(a)**. *Id.* The '309 Publication states that **VEGFR1R2.FcΔC1(a)** is “also termed VEGF trap_{R1R2},” (*id.*, ¶ 5), but it does not state that this is the only amino acid sequence that is “termed VEGF trap_{R1R2}.” In fact, the '309 Publication also describes VEGF Traps in terms of their constituent domains, explaining that VEGF-specific fusion proteins may be constructed using VEGFR1, VEGFR2, and Fc as components. *Id.*, ¶ 24. It further discloses the other VEGF Trap_{R1R2} protein that is described in the '319 Publication, **Flt1.D2.Flk1D3.FcΔC1(a)**. Ex. 1027, ¶ 5, claim 4.

87. The '309 Publication discloses a number of “suitable host cell[s] for producing VEGF Trap proteins, including “a bacterial cell such as *E. coli*, a yeast

cell, such as *Pichia pastoris*, an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS, CHO, 293, BHK, or NS0 cell.” Ex. 1027, ¶ 26.

88. The ’309 Publication neither describes any specific VEGF Trap formulations nor provides any stability data.

VI. CLAIM CONSTRUCTION

A. “Native Conformation”

89. The claims of the ’992 patent include limitations on the minimal percent of the protein present in its “native conformation following storage at 5° C. for two months as measured by size exclusion chromatography.”⁴⁴ I have been asked by counsel to apply Petitioner’s and Dr. Tarantino’s construction of the claim terms “native” and “native conformation.” Petitioner and Dr. Tarantino state that the claim term “native” refers to “the fully-intact and functional conformation of the VEGF-antagonist protein referred to in the claims.” Ex. 1002, ¶ 22.

90. Petitioner and Dr. Tarantino further note, and I agree, that certain degradation products have similar molecular size and shape (conformation) to those of the native protein and thus would not be detectable by size exclusion chromatography (“SEC”). *Id.*, ¶ 80. Thus, they clarify that the percent of the

⁴⁴ As discussed by Dr. Tarantino, size exclusion chromatography is a method of separating proteins by their size. Ex. 1002, ¶ 79.

protein present in its “native conformation” specifically refers to the fraction of the protein “in [its] native conformation *as measurable by SEC.*” *Id.* (emphasis added).

B. “Vial”

91. The claim term “vial” appears in the preamble of independent claim 1 of the ’992 patent. Counsel has asked me to assume the preamble of claim 1 is a claim limitation. Even without that assumption, however, a POSA in 2006 would understand that the “vial” in claim 1 gives meaning to the claim because it specifies the precise container in which the VEGF antagonist formulation must be held, unlike the other independent claim of the patent (claim 10) which does not recite a container.

92. The ’992 patent relates to pharmaceutical formulations and uses the term “vial” in that technical context. For example, under the heading “Field of the Invention,” in column 1, the ’992 patent states, “The present invention is directed to pharmaceutical formulations.” Claim 1 requires “[a] vial comprising” a formulation of a VEGF antagonist with various excipients. Examples 1, 2, and 5 of the ’992 patent in columns 8-10 describe testing a “Liquid Formulation...in 3 ml Glass Vials.”

93. Based on my multi-decade experience with pharmaceutical formulation development, the ordinary and customary meaning of the term “vial”

in the context of pharmaceutical formulations is a small bottle. This definition matches the use of the term in the specification of the '992 patent. Examples 1, 2, and 5 describe testing a “Liquid Formulation Stored at 5° C. in 3 ml Glass Vials,” and Examples 7 and 8 describe testing “Lyophilized . . . Formulation Stored at 5° C. in 3 ml Glass Vials.” Small, 3 mL glass bottles can be used to store liquid protein formulations, as well to store and reconstitute lyophilized protein formulations. The “small bottle” definition is also consistent with the clear distinction the patent specification makes between a vial and a pre-filled syringe. For example, the '992 patent discloses that “[t]he invention further features ophthalmic formulations provided in a pre-filled syringe *or* vial...” (emphasis added), which indicates that a pre-filled syringe is a container not encompassed by the inventors’ intended definition of a “vial.” Ex. 1001, 5:7-9. Additionally, the Examples present stability studies of VEGF Trap stored at 5° C, and both the titles and contents of the Examples clearly distinguish between stability studies that were performed in glass vials (Examples 1-3, 5, 7-8; “in 3 mL Glass Vials”), and those performed in pre-filled glass syringes (Examples 4 and 6; “in Prefilled Glass Syringe”). *Id.*, 8:8-9:25, 9:56-10:19, 9:27-53, 10:22-50. A pre-filled syringe is not a small bottle, but rather a tube with a plunger on one end and a needle on the other (although the plunger rod and needle may be provided separately).

94. Relevant technical literature, including specialized dictionaries, also supports defining a vial to mean “a small bottle.”

95. For example, *Dorland’s Illustrated Medical Dictionary* defines a “vial” as “a small bottle.” Ex. 2056⁴⁵. This medical dictionary definition matches my understanding of the term “vial” in pharmaceuticals. This definition is also fitting in the context of the ’992 patent because it excludes pre-filled syringes that the patent contrasts with a “vial.” Likewise, the FDA Container Closure guidance states that a small-volume parenteral “may be packaged in a disposable cartridge, a disposable syringe, a vial, an ampule or a flexible bag.” Ex. 1038, 24. This statement indicates, consistent with my own understanding, that a vial is a container different from a cartridge, a syringe, an ampule or a flexible bag.

96. Other technical dictionaries and sources from around the priority date of the ’992 patent provide “vial” definitions that are consistent with Dorland’s and further detail the typical materials or purpose of a vial: Ex. 2057⁴⁶ (“A small glass

⁴⁵ Gosling, P. (2003). *Dorland’s Illustrated Medical Dictionary* (28th edn.).

Saunders.

⁴⁶ Venes D. & Taber C. W. (2005). *Taber’s Cyclopedic Medical Dictionary* (20th edn.). F.A. Davis.

bottle for medicines or chemicals”); Ex. 2058⁴⁷ (“a glass container with a metal-enclosed rubber seal”); Ex. 2059⁴⁸ (“glass container closed with a rubber stopper and an aluminum band; removal of contents requires a needle; may be multidose (with a preservative included) or single dose (lacking a preservative); most often used for parenteral products”). These definitions illustrate the specific types of containers that would come to mind when a POSA sees the term “vial.” Dr. Tarantino likewise notes that glass or plastic “vials for injection” with synthetic rubber stoppers and aluminum caps were in common use in the early 2000s. Ex. 1002, ¶¶ 107-108. This evidence shows that Dorland’s and Taber’s medical dictionary definition is an accurate meaning of the term “vial,” as it would be understood by a POSA in 2006.

97. A POSA would not understand the term “vial” in the ’992 patent to have the Merriam-Webster (Ex. 1031) general-dictionary definition, “a small closed or closable vessel, especially for liquid,” that Petitioner and Dr. Tarantino use. Ex. 1002, ¶ 23. This non-technical definition is broader than what a POSA

⁴⁷ Anderson D. M. (2002). *Mosby's Medical Nursing & Allied Health Dictionary* (6th edn.). Elsevier.

⁴⁸ Worthen, D. (Ed.). (2017). *Dictionary of Pharmacy*. Routledge.

would understand the term “vial” to mean in pharmaceuticals. For example, pre-filled syringes are also “small closed or closable vessel[s]” for liquid, thus clearly making the general, non-technical definition directly at odds with the usage of the term “vial” within the ’992 patent specification. Since cartridges, syringes, ampules, and flexible bags are all “small closed or closeable vessel[s]” for liquid, Petitioner’s and Dr. Tarantino’s definition is also inconsistent with the aforementioned FDA Container Closure Guidance.

VII. DETAILED BASES FOR OPINIONS

A. Petitioner Fails to Show that Fraser Anticipates

1. Fraser does not disclose a “vial” (claims 1-9)

98. A POSA would not read Fraser as disclosing the aflibercept formulation to be contained within a “vial” according to the Patent Owner’s claim construction. Fraser discloses that VEGF TrapR1R2 “was provided...in 2-ml aliquots.” Ex. 1009, 1115. A POSA would understand “aliquot” in this context to refer to a sample, not to any particular container. The Petitioner contends that a specific type of a vial in Fraser, a “vial for injection,” must have been used in order to “prevent contamination of the aliquots during use and storage, to ensure the well-being of the macaques, and to avoid compromising the study data” pursuant to good laboratory practice and ethical conventions (Pet. 49). Dr. Tarantino notes in his declaration other features of the container: it must 1) be sealable to prevent spillage, evaporation/sublimation, and contamination; 2) bear a label; and 3) be

“designed to preclude the possibility of contamination, deterioration, or damage.”

Ex. 1002, ¶¶ 105-107. However, even in view of these specific criteria, a POSA in 2006 would be able to easily identify containers other than vials that fulfill these requirements. Such containers are described, *e.g.*, in the above-referenced FDA Container Closure Guidance, which discloses that a disposable cartridge, a disposable syringe, a vial, an ampule, or a flexible bag are all suitable containers that meet the regulatory standards at the time for a small-volume injectable drug product. Ex. 1038, 24.

99. For example, pre-filled syringes are easy for patients to use, because they are discreet, eliminate the step of extracting the drug from a vial or ampule into a syringe, and eliminate the need for overfill in vials.

100. Around the time of Fraser’s study, a POSA knew that pre-filled syringes could provide such benefits. By 2004, the FDA had approved protein drugs to be provided in pre-filled syringes, such as the fusion protein Enbrel (Ex.

2069⁴⁹), as well as cancer drug Neulasta (Ex. 2071)⁵⁰. Furthermore, it was shown that Avastin, a recombinant humanized monoclonal IgG1 VEGF inhibitor antibody, could be stored frozen or unfrozen in pre-filled syringes. Ex. 2072⁵¹, Table 1, 3. Immunex had also reported favorable results testing fusion protein etanercept in syringes under frozen conditions. Ex. 2051⁵², ¶¶ 71, 73.

101. A POSA would identify ampules as another suitable container for pharmaceutical products that must be protected from potential sources of degradation. An ampule is a sealed glass vessel used to hold a solution, typically for injection. The sealed, all-glass closure of an ampule eliminates potential

⁴⁹ Immunex Corp. (2004). *ENBREL® (etanercept): For Subcutaneous Injection*. Thousand Oaks, CA 91320: Immunex Corp.

⁵⁰ Amgen Inc. (2002). *Neulasta™ (pegfilgrastim)*. Thousand Oaks, CA 91320: Amgen, Inc.

⁵¹ Bakri, S. J., Snyder, M. R., Pulido, J. S., McCannel, C. A., Weiss, W. T., & Singh, R. J. (2006). Six-month stability of bevacizumab (Avastin) binding to vascular endothelial growth factor after withdrawal into a syringe and refrigeration or freezing. *Retina*, 26(5), 519-522.

⁵² U.S. Patent Publication No. 20030180287 to Wayne et al.

problems with contaminants, such as plasticizers, leaching from various rubber stoppers into the drug solution. McNally (Ex. 1013) discloses that “[s]everal publications have addressed the issue of extractables from the container closure systems and the associated problems of these leaching into a product,” as well as associated problems including “precipitation, discoloration, and contamination of products.” Ex. 1013, 155. Precipitation of the protein drug will diminish its concentration in solution, while discoloration and contamination suggest that its purity and stability are compromised. Therefore, closure-associated leaching was (and still is) a well-known problem in the field that may significantly and adversely affect the concentration, purity, and stability of the injectable liquid protein product. In fact, International Council for Harmonisation Q5C guidance (Ex. 2073⁵³) states that “[c]hanges in the quality of the product may occur due to the interactions between the formulated biotechnological/biological product and container/closure” in almost all liquid products and that “the lack of interactions

⁵³ Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. (1996). *Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products*. Food and Drug Administration, HHS. <https://www.fda.gov/media/71441/download>.

cannot be excluded in liquid products (*other than sealed ampules*),” thus highlighting the exceptional quality of ampules in eliminating the risk for this common source of contamination. *Id.*, 10 (emphasis added). Another benefit of all-glass ampules is that they provide an excellent air barrier, thus protecting O₂-sensitive drugs (as many protein drugs are).

102. Ampules were in use in the 2000s for both trials and commercial drug products. For example, in a clinical trial sponsored by Bayer starting in 2003, subjects received treatment injections of 1g of testosterone undecanoate supplied in an ampule. Ex. 2074⁵⁴, 4. In a clinical trial sponsored by Stanford University starting in 2006, one of the study drugs was “100 mg etoposide as 5 mL solution in clear ampules for injection.” Ex. 2075⁵⁵, 4. Lidocaine and fentanyl were also

⁵⁴ Bayer. (Last updated 30 December 2014), *Assessment of long term treatment with testosterone undecanoate in males with hypogonadism*. Identifier NCT00220298. <https://clinicaltrials.gov/study/NCT00220298?a=11&tab=table>.

⁵⁵ Arai, S. (Last updated 12 December 2017), *High-dose sequential therapy and single autologous transplantation for multiple myeloma*. Identifier NCT00349778. <https://clinicaltrials.gov/study/NCT00349778?tab=table>.

commercial products supplied in ampules Ex. 2076⁵⁶; Ex. 2077⁵⁷. Ampules were used for liquid protein formulations, too. For example, the protein drug Atgam (equine thymocyte immune globulin injection), initially approved in 1981, has long been supplied in ampules. Ex. 2078⁵⁸; Ex. 2079⁵⁹.

103. Fraser does not state that a vial was used, and a POSA would recognize that the formulations described in Fraser were not necessarily stored in a vial. Instead, a POSA would understand that Fraser's formulation could have been contained within, for example, pre-filled syringes or ampoules. Since Fraser does not expressly or inherently disclose that the formulation used in the study was contained within a "vial" under the Patent Owner's proposed claim construction,

⁵⁶ Hospira, Inc. (2006). *Lidocaine Hydrochloride and Dextrose*. Lake Forest, IL 60045: Hospira, Inc.

⁵⁷ Baxter Healthcare Corporation (2006). *Fentanyl Citrate*. Deerfield, IL 60015: Baxter Healthcare Corporation.

⁵⁸ Pharmacia & Upjohn Co. (2010). *ATGAM[®] Sterile Solution*. New York, NY 10017: Pharmacia & Upjohn Co Division of Pfizer, Inc.

⁵⁹ Pharmacia & Upjohn Co (2023). *ATGAM[®] Sterile Solution*. New York, NY 10017: Pharmacia & Upjohn Co. Division of Pfizer, Inc.

Fraser does not disclose every element of claims 1-9, and hence does not anticipate claims 1-9, of the '992 patent.

2. Fraser does not disclose “SEQ ID:4”

104. Claims 4 and 13 of the '992 patent recite, “wherein the fusion protein comprises amino acids 27-457 of SEQ ID NO:4.” Claims 5-7 and 14-16 depend from claims 4 and 13, respectively, reciting “wherein the VEGF antagonist is a dimer of the fusion protein” and aspects of the formulation.

105. Fraser does not disclose this claim requirement and instead identifies its protein by various terms like “VEGF Trap” and “VEGFTrap_{R1R2}.” As shown below, these terms refer to a group or a *genus* of fusion proteins with certain characteristics in common and not necessarily to a single protein, much less to the protein encoded by SEQ ID:4.

106. I have reviewed the Board’s Decision Granting Institution. I disagree that a POSA would understand Fraser to inherently disclose the claimed “SEQ ID:4” or aflibercept. Nor do I agree that a POSA would understand that Fraser’s protein is the same protein as in the Dix reference. Fraser does not state a sequence or use the word “aflibercept.” While Fraser does refer to “the VEGF Trap_{R1R2}” (*see* Paper 11 at 28), a POSA would not understand that phrase to mean that there was only a single VEGF Trap_{R1R2} protein. Rather, at most, a POSA would understand Fraser as simply referring to *the* VEGF Trap_{R1R2} protein that

was the subject of Fraser's study, as opposed to other proteins in the genus. There is nothing in Dix, Fraser, or the other references in the Petition that would suggest to a POSA in 2006 that Regeneron was using only a single VEGF Trap_{R1R2} protein. In fact, a POSA would recognize that a pharmaceutical company like Regeneron was likely working with various VEGF Trap_{R1R2} proteins. In 2006, pharmaceutical protein formulators would be trying multiple related proteins as part of their formulation efforts.

107. A POSA also would know that protein formulators often used varying and sometimes inconsistent naming conventions. Nor would a POSA expect publications associated with a particular company to disclose each and every protein formulation under development, as it would be understood in 2006 that many proteins and their formulations were kept confidential from competitors.

108. In my decades of experience, for example, I have worked on many proteins and formulations that have never been made public. Similarly, naming conventions for particular protein formulations can, and often do, change and can become inconsistent over time. Dr. Tarantino himself did not know Regeneron's naming procedures for the therapeutic proteins it investigates. Ex. 2054, 185:10-186:10. Accordingly, a POSA would not read Fraser or the other references in the Petition to be indicating that each referred to an identical amino acid sequence.

109. Rather, a POSA would believe that “the VEGF Trap R1R2” and similar phrases referred to the genus of fusion proteins that trap VEGF that contain particular domains. The linking sequences, however, could be changed, and the resulting protein would still be a fusion protein with domains to trap VEGF; in other words, distinct proteins could be VEGF Trap R1R2 proteins, as explained in detail below. A POSA would also know that multiple VEGF Trap proteins—both those reported in Regeneron publications and others, which Regeneron may not have described in its publications—could fall under the umbrella of a “VEGF Trap” or “VEGF Trap R1R2” protein.

110. As explained below, the '319 Publication describes multiple VEGF Trap proteins (called [Flt1.D2.Flk1D3.FcΔC1\(a\)](#) and [VEGFR1R2-FcΔC1\(a\)](#) in the '319 Publication), which include R1R2 domains, and are, in fact, referred to as R1R2 proteins. Ex. 1029, 58:22-59:10. The Daly reference (Ex. 2013)⁶⁰ also refers to proteins that differ in this manner as “R1R2” proteins. These proteins could be termed “VEGF Trap R1R2,” even though they have different amino acid sequences, as the '319 Publication confirms. The nucleotide and deduced amino acid sequences for [Flt1.D2.Flk1D3.FcΔC1\(a\)](#) are listed in Figs. 21A-21C (*id.*,

⁶⁰ U.S. Patent Publication No. 20060058234A1 to Daly et al.

21:16-17), while those of **VEGFR1R2-Fc Δ C1(a)** are listed in Figs. 24A-24C (*id.*, 22:1-2). Dr. Tarantino's deposition testimony is consistent with this view because he testified—and I agree—that molecules can be “termed” in accordance with a group name, e.g., trehalose is a type of carbohydrate. Thus, it would be perfectly reasonable so describe the trehalose in a formulation as “the carbohydrate.” Ex. 2054, 54:2-55:8. But it certainly does not follow from that description that trehalose is the only carbohydrate (with Fraser's sucrose, for example, being another common one). Similarly, it does not follow from a reference to “the VEGF Trap R1R2” that there is only a single protein that meets that description.

111. A POSA also would not consider material whether the “t” in “trap” is capitalized or whether R1R2 is in subscript. Instead, those differences could be ascribed to a journal's own editing standards or the author's preference. In either case, the phrase “VEGF Trap R1R2” refers to a genus of proteins that have a VEGF trap in the R1R2 domains but can have distinct linking amino acid sequences.

112. The '319 Publication is particularly relevant to the use of the term “R1R2” in Wulff (and other cited prior art references) because it is a Regeneron patent publication that is directly referenced in Wulff. Wulff states that “[t]he detailed molecular structure and how it was created are described in the patent

REG710-A-PCT, VEGF Trap Application published December 2000, Publication WO 00/75319 A1.” Ex. 1016, 2798 at n. 1.

113. As indicated above, the '319 Publication discloses two specific fusion proteins that are referred to as “R1R2” proteins: **Flt1.D2.Flk1D3.FcΔC1(a)** and **VEGFR1R2-FcΔC1(a)**, which I color-code herein for ease of reference. The '319 Publication provides the sequences for both **Flt1.D2.Flk1D3.FcΔC1(a)** and **VEGFR1R2-FcΔC1(a)** in Figs. 21A-21C (*id.*, 21:16-17) and Figs. 24A-24C (*id.*, 22:1-2), respectively. When these amino acid sequences are aligned, they demonstrate that the two VEGF Trap R1R2 proteins have distinct primary structures due to different amino acid residues in the linking sequence of each protein (the portion of the protein between two domains).

Score	Expect	Method	Identities	Positives	Gaps
930 bits(2404)	0.0	Compositional matrix adjust.	455/461(99%)	455/461(98%)	6/461(1%)
Query 1	MVSYWDTGVLLCALLSCLLLTGSSSG--	GRPFVEMYSEIPEIIHMTGRELVIPCRVTS	57		
Sbjct 1	MVSYWDTGVLLCALLSCLLLTGSSSG	GRPFVEMYSEIPEIIHMTGRELVIPCRVTS	60		
Query 58	PNITVTLKKFPLDTLIPDGKRIIWD	SRKGFIIISNATYKEIGLLTCEATVNGHLYKTN	117		
Sbjct 61	PNITVTLKKFPLDTLIPDGKRIIWD	SRKGFIIISNATYKEIGLLTCEATVNGHLYKTN	120		
Query 118	HRQTNTIIDVVLSPSHGIELSVGEKLV	LNCTARTELVGIDFNWEYPSSKHQHKLVNRD	177		
Sbjct 121	HRQTNTIIDVVLSPSHGIELSVGEKLV	LNCTARTELVGIDFNWEYPSSKHQHKLVNRD	180		
Query 178	LKTQSGSEMKKFLSTLTIDGVT	RSQGLYTCAASSGLMTKKNSTFVRVHEK	237		
Sbjct 181	LKTQSGSEMKKFLSTLTIDGVT	RSQGLYTCAASSGLMTKKNSTFVRVHEK--DKTHTC	237		
Query 238	PPCPAPELLGGPSVFLFPPKPKDTL	MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN	297		
Sbjct 238	PPCPAPELLGGPSVFLFPPKPKDTL	MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN	297		
Query 298	AKTKPREEQYNSTYRVVSVLTVLHQ	DWLNKEYCKVSNKALPAPIEKTISKAKGQPREP	357		
Sbjct 298	AKTKPREEQYNSTYRVVSVLTVLHQ	DWLNKEYCKVSNKALPAPIEKTISKAKGQPREP	357		
Query 358	QVYTLPPSRDELTKNQVSLTCLVKGF	YPSDIAVEWESNGQPENNYKTTPVLDS	417		
Sbjct 358	QVYTLPPSRDELTKNQVSLTCLVKGF	YPSDIAVEWESNGQPENNYKTTPVLDS	417		
Query 418	YSKLTVDKSRWQQGNVFSCSVMHEAL	HNHYTQKSLSLSPGK	458		
Sbjct 418	YSKLTVDKSRWQQGNVFSCSVMHEAL	HNHYTQKSLSLSPGK	458		

Figure 8: Amino acid sequence alignment of Flt1.D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a).⁶¹

⁶¹ The blastp suite of the BLAST® computer program with default alignment parameters was used to create the sequence comparison. It is available at <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins> (“Align two or more sequences”).

114. Specifically, as the yellow highlighting above indicates, **Flt1.D2.Flk1D3.FcΔC1(a)** (shown as the “Query” sequence above) (1) lacks three amino acid residues (serine-aspartic acid-threonine, denoted “SDT”) present in **VEGFR1R2-FcΔC1(a)** (shown as the “Sbjct” sequence above); and (2) contains three additional amino acid residues (glycine-proline-glycine, denoted “GPG”) that are not present in **VEGFR1R2-FcΔC1(a)**. Ex.1029, 67:717.

115. The sequence alignment presented above does not show the location of the different sequences in the VEGF antagonists described in the '319 Publication. This information can be obtained by identifying the different sequences above in the Figures of the '319 Publication, which include domain information. Fig. 24A, which gives the nucleic acid and amino acid sequences for **VEGFR1R2-FcΔC1(a)**, shows the additional “SDT” (grey) that is not found in **Flt1.D2.Flk1D3.FcΔC1(a)**. In the excerpt below, the end of the signal sequence⁶² is shown in pink, the beginning of the Flt1 (VEGR1) domain 2 in orange, and the additional “SDT” sequence is part of the linking sequence between the domains:

⁶² A signal sequence enables a protein to find its correct location outside the cell membrane by tagging the protein for transport through the cell membrane and out of the cell. Ex. 2015, 23.

```

      70      80      90      100      110      120
      *      *      *      *      *      *
ACA GGA TCT AGT TCC GGA AGT GAT ACC GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC
TGT CCT AGA TCA AGG CCT TCA CTA TGG CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG
T  G  S  S  S  G>
21_hFLT1 SIGNAL SEQ_26>
      S  D  T  G  R  P  F  V  E  M  Y  S  E  I>
      _27_____30_hFLT1 IG DOMAIN 2_____40>

```

Figure 9: Nucleic acid and amino acid sequences for **VEGFR1R2-FcΔC1(a)**.⁶³

116. Figure 21B from the '319 Publication shows the nucleic acid and amino acid sequences for **Flt1.D2.Flk1D3.FcΔC1(a)**, which include a GPG sequence not present in **VEGFR1R2-FcΔC1(a)**. The end of the Flk1 (VEGFR2) domain 3 is highlighted in violet, the beginning of the human Fc domain is highlighted in gold, and the GPG sequence highlighted in green bridges the two domains (hence the label “Srf_Bridge_”) without being a part of either:

```

      >Srf_Bridge_
      |
      730      740      750      760      770      780      790      800
AGAACAGCACATTTGTTCAGGGTCCATGAAAAGGGCCCGGGGCGACAAACTCACACATGCCACCGTGCCACAGCACCTGAA
TCTTGTCTGTGTAAACAGTCCAGGTACTTTTCCCGGGCCCGCTGTGTTTGAGTGTGTACGGGTGGCACGGGTCTGTGACTT
K  N  S  T  F  V  R  V  H  E  K>
_____HFLK1 D3_____>
      G  P  G>
      _____>
      D  K  T  H  T  C  P  P  C  P  A  P  E>
      _____FCΔC1 (A)_____>
      244

```

⁶³ Adapted from Ex. 1029, Fig. 24A (annotations added); *see also* Ex. 1029, 51:5-12.

Figure 10: nucleic acid and amino acid sequences for Flt1.D2.Flk1D3.FcΔC1(a).⁶⁴

117. As reflected in the above-quoted excerpts from the '319 Publication, a fusion protein that, like this one, is accurately described as being comprised of VEGFR1 domain 2 and VEGFR2 domain 3 expressed in sequence with a human Fc antibody constant region, may also contain additional amino acid sequences, such as a signal sequence, as well as bridging sequences that do not form part of these domains. Thus, the explicit disclosures of the '319 Publication confirm that a “R1R2” protein is not a single, particular, unique protein, but instead refers to a *group* of several proteins having different amino acid sequences.

118. Additional prior art references further confirm that the term “R1R2” is used as a general structural description for a group of proteins, not as a specific description of a particular protein's sequence. For example, Daly (Ex. 2013), a published Regeneron patent application, explains that there are a variety of fusion proteins comprising “a fusion polypeptide comprising receptor components R1-R2-F, wherein R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 (Flt1D2), R2 is VEGF receptor component Ig

⁶⁴ Adapted from Ex. 1029, Fig. 21B (annotations added).

domain 3 of Flk-1 (Flk1D3) (also known as KDR), and F is a fusion component.”

Id., ¶ 5.

119. Consistent with the '319 Publication, Daly (Ex. 2013) explains that “R1R2” proteins can differ in their bridging sequences. It describes a protein called “pTE115” that “w[as] constructed from a precursor of the parent VEGF trap, **VEGFR1R2.FcΔC1(a)** (SEQ ID Nos:7-8) [one of the two fusion proteins described in the '319 Publication], in which the three amino acid residues glycine-alanine-proline served as a [peptide] linker between the Flk1D3 and FcΔC1(a)” but which “[i]n all other respects” was “identical to that of the VEGF trap, **VEGFR1R2.FcΔC1(a)**.” This pTE115 protein was used to construct additional VEGF trap proteins, all of which satisfy the structural definition of a R1R2 protein. But as the Daly reference makes clear, pTE115 and **VEGFR1R2.FcΔC1(a)** (which are both R1R2 proteins) have distinct amino acid sequences due to differences between the linker/bridging sequences in each protein. *Id.*, ¶ 70.

120. When discussing these VEGF trap proteins, Daly (Ex. 2013) states that “[t]he term ‘Ig domain’ of Flt-1 or Flk-1 is intended to encompass not only the complete wild-type domain, but also insertional, deletional, and/or substitutional variants thereof which substantially retain the functional characteristics of the intact domain. The term ‘functional equivalents’ when used in reference to R1 and

R2, is intended to encompass an R1 and R2 domain with at least one alteration, *e.g.*, a deletion, addition, and/or substitution, which retains substantially the same functional characteristics as does the wild type R1 and R2, that is, a substantially equivalent binding to VEGF. It will be appreciated that various amino acid substitutions can be made in R1 and R2 without departing from the spirit of the invention with respect to the ability of these receptor components to bind and inactivate VEGF.” *Id.*, ¶¶ 29-30. Thus, multiple VEGF antagonist proteins may properly be characterized as “R1R2” despite their amino acid sequence differences.

121. Indeed, Daly (Ex. 2013) discloses multiple possible amino acid sequences for R1 and R2. For example, it discloses that “[i]n the specific embodiments, R1 is amino acids 27-126 of SEQ ID NO:6, or 1-126 of SEQ ID NO:6 (including the signal sequence 1-26); or amino acids 27-129 of SEQ ID NO:8, or 1-129 of SEQ ID NO:8 (including the signal sequence at 1-26).” *Id.*, ¶ 32.

122. Daly (Ex. 2013) goes on to disclose that “[i]n the most specific embodiments, R2 is amino acids 127-228 of SEQ ID NO:6, or amino acids 130-231 of SEQ ID NO:8.” *Id.*, ¶ 32. By specifying multiple sequences for R1 and R2, Daly teaches that there are distinct amino acid sequences that are nonetheless all R1R2 VEGF Trap fusion proteins.

123. Another reason for the difference among “R1R2” proteins stems from the fact that each of the components of an “R1R2” protein—VEGFR1 domain 2

and VEGFR2 domain 3—was not understood to correspond to a single amino acid sequence.

124. The prior art further confirms that more than one amino acid sequence may be generally characterized as VEGFR1 domain 2, *i.e.*, “R1.” Below is a chart summarizing various amino acid sequences of VEGFR1 domain 2, as defined by different prior art literature sources. I show how these sequences were derived in Appendix A.

Literature Source	VEGFR1 (Flt-1) domain 2		
	Starting Amino Acid	Ending Amino Acid	Domain Length
Davis-Smyth (Ex. 2013)	134 Pro (P)	226 Thr (T)	93 residues
UNIPROT P17948 (Ex. 2032 ⁶⁵) (Ex. 2033 ⁶⁶)	151 Gly (G)	214 His (H)	64 residues
'319 Publication (Ex. 1029)	27 Gly (G)	126 Asp (D)	100 residues

⁶⁵ P17948 VGFR1_Human, available at

<https://www.uniprot.org/uniprotkb/P17948/entry> (“UNIPROT P17948”).

⁶⁶ P17948 VGFR1_Human, Entry Version 140 (txt) (Jan. 11, 2011), available at

<https://rest.uniprot.org/unisave/P17948?format=txt&versions=140>

(“UNIPROT P37948 v140”).

125. Similarly, the prior art confirms that more than one amino acid sequence may be generally characterized as VEGFR2 domain 3, *i.e.*, “R2.” Below is a chart summarizing various amino acid sequences of VEGFR2 domain 3, as defined by different literature sources. I show how these sequences were derived in Appendix B.

Literature Source	VEGFR2 (KDR) domain 3		
	Starting Amino Acid	Ending Amino Acid	Domain Length
Davis-Smyth (Ex. 2031)	226 Val (V)	327 Lys (K)	102 residues
Lu et al., 2000 (Ex. 2082 ⁶⁷)	221 Tyr (Y)	334 Ser (S)	114 residues
Franklin et al., 2011 (Ex. 2035 ⁶⁸)	220 Gly (G)	328 Ser (S)	109 residues
UNIPROT P35968 (Ex. 2036 ⁶⁹)	224 Tyr (Y)	320 Thr (T)	97 residues

⁶⁷ Lu, D., Kussie, P., Pytowski, B., Persaud, K., Bohlen, P., Witte, L., & Zhu, Z. (2000). Identification of the residues in the extracellular region of KDR important for interaction with vascular endothelial growth factor and neutralizing anti-KDR antibodies. *Journal of Biological Chemistry*, 275(19), 14321-14330.

⁶⁸ Franklin, M. C., Navarro, E. C., ... & Kussie, P. (2011). The structural basis for the function of two anti-VEGF receptor 2 antibodies. *Structure*, 19(8), 1097-1107.

⁶⁹ P35968 VEGFR2_Human, available at <https://www.uniprot.org/uniprotkb/P35968/entry> (“UNIPROT P35968”).

(Ex. 2037 ⁷⁰)			
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126. Thus, the structural information from Fraser that the fusion protein used “compris[es] Ig domain 2 of human VEGF-R1 and Ig domain 3 of human VEGF-R2, expressed in sequence with the human Fc” (Ex. 1009, 1115) refers to a genus of potential proteins, not necessarily to any one, specific VEGF antagonist, namely the fusion protein encoded by SEQ ID NO:4. Therefore, Fraser does not expressly or inherently disclose a fusion protein comprising amino acids 27-457 of SEQ ID NO:4, never mind the unique combination of a fusion protein comprising amino acids 27-457 of SEQ ID NO:4 and the formulation disclosed by claims 5-7 and 14-16. Therefore, Fraser does not disclose every element of claims 4-7 and 13-16, and thus does not anticipate claims 4-7 and 13-16, of the ’992 patent.

3. Fraser does not disclose that its VEGF trap protein was created in “CHO” cells

127. Claim 1 and 10 of the ’992 patent recite “wherein the VEGF antagonist is a fusion protein produced in a Chinese Hamster Ovary (CHO) cell.” In contrast, Fraser (Ex. 1009) does not disclose the host cell(s) used to produce the VEGF Trap_{R1R2} protein described therein. A POSA, therefore, would have no

⁷⁰ P35968 VGFR2_Human, Entry Version 127 (txt) (Jan. 11, 2011), available at <https://rest.uniprot.org/unisave/P35968?format=txt&versions=127> (“UNIPROT P35968 v127”).

reason to read Fraser as necessarily disclosing a protein that was created in CHO cells.

128. I understand that Petitioner and Dr. Tarantino contend that Holash (Ex. 1010) supplied this missing information. Based upon my understanding of the applicable legal principles and my review of Holash and Fraser, however, I disagree. Holash, which is cited in Fraser, discloses that “the VEGF-Trap variants were produced and purified from Chinese hamster ovary cells.” Ex. 1010, 11394. However, Fraser does not disclose or suggest that it used Holash’s host cell line. Instead, Fraser cites Holash to describe other properties, namely “improved bioavailability and pharmacokinetic properties (21).” Ex. 1009, 1115 (emphasis added). Reference 21 in Fraser is Holash.

129. Furthermore, a POSA would know that in the two years between the publication of Holash in 2002 and the publication of Fraser in 2004, it was possible for a company such as Regeneron to switch cell lines for a variety of reasons, such as low production stability (which means that the cell line loses productivity during long-term culture) and low yield. Gellissen (Ex. 2094⁷¹) discusses the fact that

⁷¹ Gellissen, G. (Ed.). (2005). *Production of Recombinant Proteins: Novel Microbial and Eukaryotic Expression Systems*. John Wiley & Sons.

“[m]ammalian cells [*e.g.*, Chinese hamster ovary (CHO) cells and baby hamster kidney (BHK) cells] are capable of faithfully modifying heterologous compounds according to a mammalian pattern. However, the fermentation procedure is expensive and yields are much lower than those reported for various microbial systems.” *Id.*, 5. In the two years between Holash and Fraser, Regeneron was shifting from *in vitro* studies (Holash) to a late-stage animal trial (Fraser) and undoubtedly was considering scaling up for human clinical trials. A POSA would know that an expression system chosen for exploratory *in vitro* experiments would not necessarily be appropriate for production later on in the drug development cycle.

130. The prior art would inform a POSA that Regeneron had produced VEGF Traps in multiple cell lines. Specifically, the '319 Publication mentions some VEGF Traps produced in COS cells. Ex. 1029, 55:23-56:6. Daly discloses the production of “mini” variation of a VEGF Trap in *E. coli*. Ex. 2013, ¶ 43. And both the '309 and '319 Publications show that Regeneron viewed other cell lines as “suitable.” Ex. 1027, ¶ 26; Ex. 1029, 12:19-26, 94:5-19.

131. Therefore, a POSA would not necessarily understand that CHO cells were used in Fraser, despite Fraser’s reference to Holash and the fact that Fraser obtained the protein from Regeneron. Accordingly, Fraser does not disclose that the VEGF Trap_{R1R2} protein used therein was produced in CHO cells.

4. Petitioner fails to show that Fraser’s drug product necessarily met the “native conformation” claim limitation

132. Claims 1 and 10 of the ’992 patent include the limitation “wherein at least 98% of the VEGF antagonist is present in native conformation following storage at 5°C. for two months as measured by size exclusion chromatography.” Dependent claims 2 and 11 include the limitation “wherein about 99% or more of the weight of the fusion protein is in native conformation.”

133. As discussed above in ¶¶ 89-90, I have been asked by counsel to adopt Petitioner’s and Dr. Tarantino’s meaning of the “native conformation:” the percentage of the protein in its “fully-intact and functional conformation” “as measurable by SEC.” Ex. 1002, ¶¶ 22, 80.

134. To reiterate, at least the following types of protein degradation products are not in a “fully-intact and functional conformation” as “measurable by SEC.” Protein aggregates are not in their “fully-intact and functional conformation:” as discussed above in ¶¶ 28 and 33, they are often non-functional because critical binding domains may be blocked and large aggregates tend to precipitate. Protein aggregates are detectable as high-molecular weight (compared to individual protein molecules) impurities with SEC. Protein fragments are also not in a “fully-intact and functional conformation” because they may lack domains critical to binding their targets. Protein fragments are detectable as low-molecular

weight (compared to individual protein molecules) impurities with SEC.

Denatured proteins are by definition not in a “fully-intact and functional conformation.” Although denatured proteins typically have the same molecular weight as their natively-folded counterparts, they often can nevertheless be detected with SEC due to their significantly extended shape. While Dr. Tarantino appears to agree that each of these types of protein degradation products can be detected by SEC (Ex. 1002, ¶ 79), the portions of his declaration related to the “native conformation” claim limitation focus solely on aggregation. *Id.*, ¶¶ 72-75, 102, 154, 159.

a. VEGF TrapR1R2 is a genus of amino acid sequences, and variations in them can affect stability

135. In ¶¶ 104-126 above, I explain in detail the reasons why the VEGF Trap_{R1R2} disclosed by Fraser describes a genus of potential proteins, rather than any specific VEGF antagonist.

136. Furthermore, as detailed in ¶¶ 35-44 above, seemingly minor differences in amino acid sequences among proteins often can result in marked differences in stability, including whether a protein remains in its native conformation.

137. As such, two proteins which are similar but not identical would not necessarily have similar, let alone the same, stabilities. Because Fraser does not

identify a specific VEGF antagonist by amino acid sequence, a POSA would not know which of many VEGF Trap_{R1R2} amino acid sequences was being used, and it would be impossible to know whether the VEGF Trap of Fraser was identical to that of Dix. Therefore, the VEGF Trap protein of Fraser would not necessarily remain in its native conformation to the same degree as that of Dix.

b. Fraser does not disclose the post-translational modifications of the protein it used, and variations in them can affect stability

138. Fraser does not detail the glycosylation patterns or other post-translational modifications of the protein it used. In different cell lines, the sites of glycan attachment, as well as the structure of the attached glycan, can vary. As Dr. Yancopoulos explained in his recent trial testimony, the degree of glycosylation may be significantly different even between two CHO cell lines. Ex. 2050, 181:9-12.

139. As Petitioner correctly acknowledges, differences in “post-translational processing...could change the protein’s performance.” Pet. 56. As explained above, glycosylation can markedly affect the propensity of a protein to aggregate by blocking hydrophobic residues on the surface of a protein that could otherwise interact with hydrophobic residues on neighboring protein molecules and result in aggregation.

140. Because Fraser does not provide information about the post-translational modifications of the protein used, which can affect the extent to which a protein remains in its native conformation, it cannot be assumed that Fraser's VEGF Trap_{R1R2} formulation would exhibit the native conformation results reported in Dix.

c. Since Fraser's formulation was frozen and freezing-thawing can impact protein stability, the Dix formulation data shed no light on the native confirmation of Fraser's post-thaw formulation

141. Petitioner contends that the SEC test data of Dix “confirm that the Fraser formulation preserves greater than 99% of the aflibercept in native conformation (as measured by SEC) for over two years when stored at 5 °C.” Ex. 1002, ¶ 96. However, whereas the formulations of Fraser were frozen and then thawed (Ex. 1009, 1115: “The compounds were stored at -20 °C until required, at which time they were thawed.”), the Dix does not specify that its formulation underwent a freeze-thaw cycle.

142. A freeze-thaw cycle was known to affect whether a protein remains in its native conformation for a variety of reasons discussed above in ¶¶ 48-50. As ice crystals form, this generates ice-liquid interfaces and increases the concentration of the protein and other solutes in the remaining liquid, all of which can lead to protein denaturation and aggregation. Ex. 2061, 3. Freezing-thawing

also tends to create tension and shear stresses on proteins, which can further contribute to denaturation and aggregation. Ex. 2062, 1.

143. The foregoing destabilizing stresses associated with the freeze-thaw process make it impossible to deduce the stability of a protein that has undergone freezing-thawing from the stability of the protein that has not. Therefore, the stability data presented in Dix, which do not specify a freeze-thaw cycle, would not necessarily correctly inform a POSA about the stability of Fraser's post-freeze-thaw formulations.

5. Fraser's buffer is not "phosphate"

144. Claims 1 and 10 of the '992 patent require a "buffer," and dependent claims 9 and 18 further specify that "the buffer is phosphate."

145. Buffered aqueous solutions resist changes in pH upon the addition of an acidic or basic component. A buffer is typically prepared by mixing a weakly acidic species and a weakly basic species in aqueous solution. The CALBIOCHEM Buffers booklet, for example, lists several examples that illustrate this point. For example, an acetate buffer can be prepared by mixing acetic acid (a weak acid) with sodium acetate (a weak base). Ex. 2087⁷², 24. One might also

⁷² Mohan, C. (2006). *Buffers. A Guide for the Preparation and Use of Buffers in Biological Systems*. Calbiochem.

adjust the buffer to the desired pH using a strong acid or base, such as HCl or NaOH, respectively. *Id.*, 22.

146. A buffer may use a weak acid/base conjugate pair (e.g., acetate buffer made by combining of *acetic* acid and sodium *acetate*, as noted in the preceding paragraph) or may include multiple buffering species that, together, extend the buffering range of the aqueous solution. A buffer is effective within about $\text{pH} \pm 1.0$ of the pK_a value(s) of its constituent weak acid(s). Ex. 2087, 13. Phosphoric acid (H_3PO_4), being polyprotic, has three pK_a values of 2.12, 7.21, and 12.32; therefore, it can be effective as a buffer within about ± 1.0 pH unit from each of these pK_a values, but it is not very effective in a pH range of 3.1 to 6.2 or 8.2 to 11.3. *Id.*, 32. By adding another type of a buffering agent with different pK_a value(s), however, the pH range covered can be extended.

147. One type of buffer that has been known and commonly used for over 100 years is a citrate-phosphate buffer named after its original inventor: the McIlvaine buffer. Ex. 2080⁷³. Unlike a formulation in which the buffer is phosphate, a McIlvaine buffer includes a *combination* of citrate with phosphate.

⁷³ McIlvaine, T. C. (1921). A buffer solution for colorimetric comparison. *Journal of Biological Chemistry*, 49(1), 183-186.

Citric acid, being a polyprotic acid, has pK_a values of 3.13, 4.77, and 6.4; therefore, including a citrate species in the buffer makes the mixture effective even within the pH ranges where a phosphate alone would be less so. McIlvaine explained that one benefit of the citrate-phosphate buffer is that it can be created by combining just two stock solutions (*e.g.*, 0.2 M disodium phosphate and 0.1 M citric acid). *Id.*, 1. But, depending on the desired pH and buffering capacity, additional ingredients, such as sodium citrate or sodium hydroxide, could be included as well. In any case, a POSA would still understand the resulting buffer to be a citrate-phosphate or McIlvaine buffer.

148. The Fraser formulations used “buffer composed of **5 mM phosphate, 5 mM citrate**, 100 mM NaCl (pH 6.0).” Ex. 1009, 1115 (emphasis added). Wulff also used a buffer comprising both phosphate and citrate: “[c]ontrol animals were treated with vehicle containing **5 mM phosphate, 5 mM citrate**, 100 mM sodium chloride, 0.1% (wt/vol) Tween 20, and 20% (wt/vol) sucrose.” Ex. 1016, 2798 (emphasis added). A POSA would refer to these buffers as a citrate-phosphate or McIlvaine buffer. As explained above, a McIlvaine buffer is a distinct and well-known buffer with its own properties that are different from the properties of a phosphate buffer alone. A POSA would not understand that “the buffer is phosphate” in citrate-phosphate-buffered formulations.

149. The pharmaceutical art in 2006 (and now) clearly distinguished between a phosphate buffer and a citrate-phosphate buffer. For example, Zacchigna (Ex. 2081⁷⁴) studied the release of a drug tested in different buffer solutions, including in “*a phosphate/citrate buffer* (pH 5.5, 0.2 M), and *a phosphate buffer* (pH 7.4, 0.1 M).” *Id.*, 3 (emphasis added).

150. This unambiguous differentiation is to be expected because these two types of buffers are not always interchangeable. They have distinct chemical components and hence buffering capacities, and a POSA would likely use these two buffers at different pH values. Such is indeed the case in the Zacchigna study (Ex. 2081), wherein the phosphate buffer was used at a pH of 7.4, but the citrate-phosphate buffer was used at pH of 5.5, *i.e.*, almost two pH units lower, where the former buffer would have a far lower buffering capacity.⁷⁵

⁷⁴ Zacchigna, M., Di Luca, G., Cateni, F., & Maurich, V. (2004). Improvement of warfarin biopharmaceutics by conjugation with poly (ethylene glycol). *European Journal of Pharmaceutical Sciences*, 23(4-5), 379-384.

⁷⁵ Since (as explained above in ¶¶ 145-146) a buffer is typically effective in a pH range within ± 1.0 pH units of its relevant pK_a value, in a roughly neutral pH

151. For the foregoing reasons, a POSA would understand the VEGF antagonist formulation of Fraser to use a citrate-phosphate buffer rather than a phosphate buffer. Therefore, claims 9 and 18 of the '992 patent are not anticipated by Fraser.

B. Petitioner Fails to Show that the Claims of the '992 Patent Are Obvious Over the Cited References

152. Petitioner and Dr. Tarantino assert that a POSA in 2006, being aware of Regeneron's promising *in vitro* and *in vivo* trials of VEGF Trap_{R1R2}, "would have been motivated to make formulations of" the same protein "that were sufficiently stable to be approved as a therapeutic product in humans and animals." Pet. at 56; Ex. 1002, ¶¶ 141-142. They further assert that a POSA would express VEGF Trap_{R1R2} in CHO cells, would use the formulation disclosed in Fraser, and would either achieve the native conformation result stated in the claims of the '992

region a phosphate buffer, due to phosphoric acid's middle pK_a value of 7.2, is effective in a pH range from 6.2 to 8.2. Ex. 2087, 13, 32. In contrast, a citrate-phosphate buffer in that region, with citric acid's highest pK_a value of 6.4, is effective in a pH range from 5.4 to 8.2. *Id.* Consequently, a POSA would recognize that while a citrate-phosphate buffer is very effective at pH 6.0 (the pH of the Fraser formulation), a phosphate buffer is not.

patent or adjust the formulation until this result was achieved. Ex. 1002, ¶¶ 143-160.

153. There are several flaws in this reasoning, as explained below in ¶¶ 154-189. A POSA would neither expect to achieve high native conformation results using Fraser's formulation nor maximize native conformation by neglecting other formulation constraints.

1. A POSA would have concerns about using Fraser's formulation to develop a commercial product

154. As an initial matter, a POSA would have justifiable doubts about developing a VEGF Trap formulation suitable for FDA's regulatory approval. In 2006, only three fusion proteins had been approved for commercial use, and none of them was a VEGF inhibitor. Ex. 2009, 3. While significant advances had been made regarding the development of therapeutic proteins, a POSA would recognize that the process of developing a therapeutic protein product was a process typically associated with failure, not with success. Ex. 2082⁷⁶, 1, 6, Table 1. Of the 775 biologics that entered human clinical trials between 2000 and 2010, only 105 (*i.e.*,

⁷⁶ Yamaguchi, S., Kaneko, M., & Narukawa, M. (2021). Approval success rates of drug candidates based on target, action, modality, application, and their combinations. *Clinical and Translational Science*, 14(3), 1113-1122.

well under 15%) were actually approved for commercial use, and the success rate for anticancer agents and ligand antagonists was particularly low. *Id.*, 1117, Fig. 2.

155. With respect to fusion proteins in particular, the POSA's misgivings would be further compounded by Fraser's disclosures. Fraser specifies that its VEGF Trap formulation was frozen, stored, and then thawed before use, and that "[a]ny compound remaining was stored at 4 ° C and used within 2 wk." Ex. 1009, 1115. Thus a POSA reading Fraser would assume that the formulations were not stable beyond two weeks even at 4° C because otherwise there was no reason for Fraser to state that cautionary detail in the publication.

156. Furthermore, Fraser's formulation used a relatively high concentration (20%) of sucrose or glycerol. Ex. 1009, 1115. Adding stabilizing agents in such a high concentration is one way to potentially reduce the likelihood of aggregation, but at the cost of producing a hypertonic solution that would be far from optimal for injection into a human. In Fraser's formulation, sucrose and sodium chloride are by far the largest contributors to the osmolarity of the solution. As the following calculations show, with 20% sucrose and 100 mM sodium chloride, the osmolarity of Fraser's formulation is over 785 mOsm/L.

$$20\% \text{ sucrose} = \frac{200 \text{ g sucrose}}{L} \times \frac{1 \text{ mol sucrose}}{342 \text{ g sucrose}} = \frac{0.585 \text{ mol sucrose}}{L} = 585 \frac{\text{mOsm}}{L}$$

$$100 \text{ mM NaCl} = \frac{1 \text{ mol NaCl}}{L} \times \frac{1 \text{ mol Na}^+ + 1 \text{ mol Cl}^- \text{ ions}}{1 \text{ mol NaCl}} = \frac{2 \text{ mol ions}}{L} = 200 \frac{\text{mOsm}}{L}$$

Total contribution of sucrose and NaCl to osmolarity:

$$585 \frac{\text{mOsm}}{\text{L}} + 200 \frac{\text{mOsm}}{\text{L}} = 785 \frac{\text{mOsm}}{\text{L}}$$

This is more than twice the 275 to 295 mOsm/L osmolality typical in human blood serum. Sucrose, sodium chloride, and the other ingredients in Fraser's formulation are generally considered non-permeable solutes, making Fraser's formulation very hypertonic.

157. Additionally, Fraser's formulation expressly used a citrate-phosphate buffer. Ex. 1009, 1115. This buffer is a common choice in the lab when the pH value has not been finalized yet because it is effective across a full range of physiologic pH values. But it has drawbacks for a final formulation—for example, as discussed further below in ¶¶ 193-200, the phosphate component therein tends to catalyze deamidation of Asn and Gln residues in proteins. Once the target pH was determined, therefore, a POSA would be inclined to select a buffer that was effective at that selected pH but did not negatively affect stability.

158. Petitioner argues that a POSA would use Fraser's formulation out of a belief that the POSA could thereby avoid “the “trial-and-error process of selecting and testing various combinations of commonly-used excipients to determine which were optimal for aflibercept.” Pet. 59. But because of the problems laid out here, a POSA would, in fact, doubt that Regeneron had determined which excipients were optimal for aflibercept. A POSA hence would not view Fraser's formulation as optimized and would have had reasonable doubts about using it.

2. A POSA would not be motivated to select a VEGF Trap protein with SEQ ID NO:4 (claims 4-7 and 13-16)

159. As discussed above in ¶¶ 104-126 and 135-137, “VEGF Trap_{R1R2}” is a term that could refer to any of a number of different proteins. There are different possibilities for the sequence of the R1 domain, of the R2 domain, and of bridging sequences between domains. Because Fraser, Wulff, and Holash use this and other such general terms to describe the protein that was used therein, a POSA would not know as of 2006 the exact amino acid sequence that Regeneron had used for those studies. Having reviewed the Petition and Dr. Tarantino’s declaration, I see neither of these documents as supplying a reason why a POSA in 2006, trying to replicate Regeneron’s success with VEGF Trap_{R1R2}, would choose one (much less which one) particular amino acid sequence.

160. Even if a POSA wanted to try one of the complete VEGF Trap_{R1R2} sequences that Regeneron disclosed in, for example, the ’319 Publication (Ex. 1029), the POSA would have no way of knowing which one was Regeneron’s preferred amino acid sequence. The ’319 Publication compares **Flt1.D2.Flk1D3.FcΔC1(a)** with **VEGFR1R2-FcΔC1(a)** and reports comparable binding stoichiometry (*id.*, 74:15-76:10, Fig. 28) and pharmacokinetic results (*id.*, 82:4-83:2, Fig. 37). The ’319 Publication discloses that Regeneron had stably expressed **Flt1.D2.Flk1D3.FcΔC1(a)** in CHO cells. *Id.*, 77:11-13. It also discloses

an analysis of the disulfide structures and peptide mapping of

[Flt1.D2.Flk1D3.FcΔC1\(a\)](#). *Id.*, 80:20-82:2. These disclosures suggest that

Regeneron was focused on [Flt1.D2.Flk1D3.FcΔC1\(a\)](#), which does ***not*** match SEQ ID NO:4 of the '992 patent.

161. Knowing the profound effects that even minor changes in the amino acid sequence might have on a protein's resistance to degradation and biological activity, a POSA could instead decide to modify the linker sequences of the VEGF TRAP_{R1R2} proteins disclosed in the '319 Publication further hoping to find an even more potent and suitable product candidate.

3. A POSA would not reasonably expect to achieve the native conformation claim limitations by either combining VEGF Trap_{R1R2} with Fraser's formulation or further optimization

a. A POSA would not expect the native conformation result

162. All claims of the '992 patent require “wherein at least 98% of the VEGF antagonist is present in native conformation following storage at 5° C. for two months as measured by size exclusion chromatography.” If a POSA in 2006 decided to combine a VEGF Trap_{R1R2} with the excipients disclosed in Fraser (Ex. 1009), the POSA would have no reason to expect this level of storage stability for several reasons.

163. First, a POSA would not read Fraser’s disclosure that “[a]ny compound remaining was stored at 4 C and used within 2 wk” (Ex. 1009, 1005) to suggest that the VEGF antagonist formulation would maintain “at least 98% of” its “native conformation following storage at 5° C. for two months.” Instead, as discussed above in ¶ 155, this statement would lead a POSA to assume that the formulations were not stable beyond two weeks.

164. Just because a formulation was used in animal or even early human trials does not indicate to a POSA that the formulation would achieve the level of storage stability recited in the claims of the ’992 patent. As discussed below in ¶¶ 168 and 170, a POSA would know that even FDA approval in 2006 did not require 98% of the native conformation and would be aware that some FDA-approved products did not meet this high standard. In other words, as of 2006, pharmaceutical protein formulations were not necessarily expected to achieve the level of storage stability required by claims 1 and 10, let alone by claims 2 and 11, of the ’992 patent, in order to be used in monkeys or even humans.

165. Adequate storage stability for two *weeks* does not imply adequate storage stability for two *months* (*i.e.*, over 4 times longer). Notably, Frokjaer (Ex. 2020) described protein aggregation as a “nucleation-dependent process, in which nuclei accumulate during the lag phase,” which is “typically very long,” “followed by the rapid accumulation” of aggregates. *Id.*, 5. In other words, due to the long

lag phase of aggregation, aggregates may not be observable in the early weeks of storage, but then accumulate quickly and exponentially thereafter, *e.g.*, by “two months” (as claimed in the ’992 patent).

166. Accordingly, Fraser’s disclosure of a preclinical VEGF antagonist formulation to be used within two weeks at 4 °C in a single-injection animal trial would not lead a POSA to expect that the formulation would have less than 2% (let alone less than 1%) aggregation after two months at 5 °C.

b. A POSA would not be motivated to optimize the formulation to achieve 98% native conformation with a reasonable expectation of success

i. Lack of motivation

167. As explained above in ¶¶ 51-63 and 153-158, development of liquid protein formulations is a balancing act involving many diverse objectives and constraints besides product stability, such as achieving acceptable protein concentration, as well as tonicity, viscosity, and patient’s acceptance of the formulation. As stated in Chang (Ex. 1006), “it would be ideal to have a pure protein in an absolutely stable formulation. In reality, however, scientists have to design formulation based on compromises that deal with several different potential problems.” *Id.*, 6.

168. A POSA would know that absolute stability was not required and that native conformation levels below those recited in the claims of the ’992 patent

were described as acceptable in the pre-2006 art, could be accepted by the FDA, and could be found in FDA-approved products.

169. In references cited by Petitioner, formulations with a much lower fraction of the native protein, e.g., between 90% and 95%, were described as stable. As noted above in ¶ 163, Andya (Ex. 1030) and Liu (Ex. 1024) disclosed that “a ‘stable’ formulation may be one wherein less than about 10% and preferably less than about 5% of the protein is present as an aggregate in the formulation.” Ex. 1030⁷⁷, 8:31-33; Ex. 1026⁷⁸, ¶ 63. And Kaisheva (Ex. 1025) taught the same criteria for stability, *i.e.*, wherein “[n]o more than 10%, preferably 5%, of the antibody monomer is degraded *as measured by SEC-HPLC.*” *Id.*⁷⁹, ¶ 25 (emphasis added).

⁷⁷ International Patent Publication No. 9704801 to Andya et al.

⁷⁸ U.S. Patent Publication No. 20040197324A1 to Liu et al.

⁷⁹ U.S. Patent Publication No. 20030138417A1 to Kaisheva et al.

170. The FDA guidance on stability testing of new drug substances (Ex. 2014⁸⁰) also echoes a 5% cut-off for a testable deterioration in a biologic product (such as a protein) by defining a “significant change for a drug product” as “[a] 5 percent change in assay from its initial value.” Ex. 2014, 11.

171. Shannon disclosed a 94% native conformation acceptance criterion for Enbrel® (Ex. 2085⁸¹, 3). Though the Shannon article was made public after the priority date, the maker of Enbrel, Immunex, had reported stability results in the US20030180287A1 pre-priority-date publication (Ex. 2051) showing lots of the product with 2.5% or more of high-molecular-weight “aggregates that formed” or low-molecular-weight degradants, as detected by size exclusion chromatography, at time 0 and after 3 months of storage at 2-8° C. Ex. 2051, Figs. 1, 3. The formulation of Example 2 of US20030180287A1 is identical to that of the Enbrel label (“a liquid formulation (25 mM phosphate, 25 mM L-arginine, 98 mM NaCl,

⁸⁰ Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. (2003). *Q1A(R2) Stability Testing of New Drug Substances and Products*. Food and Drug Administration, HHS.
<https://www.fda.gov/media/71707/download>.

1% sucrose, at pH 6.2)”) except that the patent does not specify the protein concentration used in that example (the concentration of the approved product is 50 mg/mL according to the Enbrel label). Since US20030180287A1 in ¶ 50 discloses that the formulation comprises about 25 mg/mL of the protein, which is half of that in the approved product, one would expect the formulation tested in the patent publication to be at least as good, if not better, than in the product itself. And yet, the formulation was not able to consistently maintain at least a 98% native conformation across the lots tested.

172. As another example, the USP monograph (Ex. 2021⁸²) for the protein hormone somatotropin permitted as high as 4% of high-molecular-weight degradants. *Id.*, 5.

173. In a study published in May of 2006, Bakri et al. (Ex. 2072) measured the stability of the anti-VEGF antibody drug Avastin. After three months’ storage at 4 °C, 8.8 to 9.6% of the protein had degraded. *Id.*, 3. The authors described this as a “minimal change in the concentration of bevacizumab in the samples at 3 months.” *Id.*, 4. Although the authors used an immunoassay to measure the

⁸² The United States Pharmacopeial Convention. (2005). *The United States Pharmacopeia* (29th edn.). The United States Pharmacopeial Convention.

concentration of functional bevacizumab present in the samples over time, rather than SEC (*id.*, 2), this study illustrates the extent of protein degradation that could be considered acceptable even in FDA-approved antibody-related products.

174. These examples speak to the generally accepted standards, as of 2006, that pharmaceutical formulations of therapeutic products with less than 98% of the native protein conformation could be considered stable.

175. A POSA would take into account a number of other priorities when developing a pharmaceutical formulation in addition to the percent of the protein present in its native conformation. Protein solution concentration, tonicity, and viscosity, as well as other forms of degradation, would be among the competing priorities a POSA would consider when using Fraser's formulation as a starting point to develop a formulation of VEGF Trap for use in treating cancer. These other competing priorities would discourage a POSA from making certain changes to Fraser's formulation, even if they could potentially reduce aggregation.

176. For example, a POSA would know of the general relationship between protein concentration and aggregation and that lowering protein formulation could help to reduce aggregation. But at a lower protein concentration, a larger dose volume would be required in order to deliver the same amount of the protein. In the formulation of Fraser, a lower protein concentration would limit the amount of protein that could be safely and comfortably

administered subcutaneously. Ex. 2052, 2. Too large of a dose volume would eliminate the possibility of a subcutaneous route of administration, which was one route researchers were investigating for VEGF Trap in treating cancer. Ex. 2084.

177. Increasing the concentration of stabilizing agents, such as sucrose or glycerol, would potentially inhibit aggregation. But at high concentrations they would do so at the cost of undesirably increasing the viscosity and/or tonicity of the protein solution. As discussed previously in ¶¶ 54-55, highly viscous formulations can be difficult to inject, and excessively hypertonic solutions can cause such side effects as injection-site pain. In the formulation of Fraser, the concentration of stabilizing agent was already high, so a POSA would not be motivated to increase the amount of stabilizing agent even further in order to prevent aggregation.

178. Since aggregation can be pH-dependent, adjusting the pH or increasing buffer concentration to minimize pH changes could potentially reduce aggregation. However, with the citrate-phosphate buffer of Fraser's formulation, a POSA would be disinclined to increase buffer concentration because phosphate ions accelerate such protein degradation reactions as Asn/Gln deamidation (as well as polypeptide chain hydrolysis at Asp/Glu residues) in a concentration-dependent

manner, as demonstrated, for example, by Tyler-Cross (Ex. 2089⁸³). *Id.*, 5, Table III. Further, adjusting the pH could potentially exacerbate degradation concerns, as “many degradative processes become more prevalent below pH 5 (acid hydrolysis) and above pH 7 (deamidation).” Ex. 1013, 141.

179. In view of the many conflicting objectives to take into account, a POSA would not focus exclusively on achieving a near-perfect storage stability of a therapeutic protein at the expense of other important considerations.

ii. No reasonable expectation of success

180. Though publications like Chang (Ex. 1006) and McNally (Ex. 1013) provided generalized approaches to formulation development, ultimately the appropriateness of a formulation and analytical tests to measure the stability thereof will depend on the protein’s “structural and functional characteristics.” Ex. 1013, 140. For example, McNally disclosed that protein concentration, pH, and temperature, as well as the nature and concentration of excipients and buffer, are all important considerations in protein formulation development, but offered

⁸³ Tyler-Cross, R., & Schirch, V. (1991). Effects of amino acid sequence, buffers, and ionic strength on the rate and mechanism of deamidation of asparagine residues in small peptides. *Journal of Biological Chemistry*, 266(33), 22549-22556.

limited advice to a POSA on how to troubleshoot problems that may arise from these factors. Ex. 1013, 141-156. While following such teachings may “allow the formulation scientist to determine *whether* a solution formulation will be acceptable for a given protein,” the formulator will not know the exact level of stability until the formulation is assayed using appropriate analytical tests, e.g., size exclusion chromatography. *Id.*, 139 (emphasis added).

181. As previously established in ¶¶ 67-70, VEGF Trap is a fusion protein, with which few POSAs would have any experience or expertise by 2006. A fusion protein is a distinct structural entity from an antibody (“Ab”), which typically comprises a receptor for the antigen of interest synthetically fused to the Fc region of human IgG. Ex. 2009, 3. In contrast, an antibody is a Fc fragment linked to two antigen-binding fragments and typically comprises four polypeptide chains linked to each other by disulfide bonds. As noted by Rodgers (Ex. 2009), fusion proteins are “a biologic of the second generation era” which are “distinct from [antibodies] due to their unique production process and the use of a receptor rather than a Fab region to bind the antigen of interest.” *Id.*, 3.

182. Table 1 of Rodgers (Ex. 2009) shows biologic agents by year of their FDA approval. *Id.*, 3. The numerous antibody-based therapeutics available by 2006 demonstrate that antibodies were a relatively mature technology. In stark contrast, there were only three FDA-approved fusion protein therapies: Enbrel

(1998), Alefacept (2003), and Abatacept (2005). Consequently, evidence cited by Petitioner of formulations of *antibodies*, *e.g.*, the trastuzumab, a humanized antibody of Andya (Ex. 1030), or the anti-IL-2 receptor antibody of Kaisheva (Ex. 1024), all achieving >98% protein native conformation, would not suggest to a POSA that a *fusion protein* formulation is likely to achieve the same result. Notably, in 2003, a formulation of fusion protein etanercept exhibited a total of about 2.5% of high- and low-molecular-weight degradants even at time equaled zero and after three months at 2-8° C and was described as an “aqueous formulation that *surprisingly* allows for stable long-term storage.” Ex. 2051, Figs. 1, 3, ¶ 23 (emphasis added). In other words, around the priority date of the ’992 patent, it was not expected that a fusion protein formulation would be able to contain even 97.5% of the protein in its native conformation.

183. POSA would believe, as Fast (Ex. 2016) explains, that in antibodies “interdomain interactions presumably have evolved to provide mutual stabilization.” Fusion proteins, by contrast, do not occur naturally and

consequently “may lack such stabilizing interdomain stabilization.” *Id.*⁸⁴, 11735; Ex. 2017, 44. This is a further reason for a POSA to question that very high levels of stability could be achieved for any given fusion protein.

184. Fraser itself would lead a POSA to believe that VEGF TrapR1R2 was likely to be a difficult protein to stabilize. In particular, a POSA could reasonably attribute both the high concentration of a stabilizing agent used and the protein solution disposal within two weeks after thawing to difficulties with stabilizing the protein.

185. Accordingly, a POSA would have no reasonable expectation of success in achieving at least 98% of the native protein conformation after two months in a fusion protein.

iii. No motivation or expectation of success for 99% native conformation (claims 2 and 11)

186. Achieving 99% native conformation or greater after months of storage is a very difficult bar for protein stability, as evidenced even by the references that Petitioner relies on to show high stability. For example, in the stability studies of

⁸⁴ Fast, J. L., Cordes, A. A., Carpenter, J. F., & Randolph, T. W. (2009). Physical instability of a therapeutic Fc fusion protein: domain contributions to conformational and colloidal stability. *Biochemistry*, 48(49), 11724-11736.

the Liu patent (Ex. 1026), when formulations of 80 mg/mL monoclonal antibody rhuMAb were stored at 5° C in a trehalose-containing (stabilizing sugar) formulation, 98.7% of the protein was in its native conformation after one month, as measured by size exclusion chromatography. *Id.*, ¶ 280. Additionally, in the stability studies of Kaisheva (Ex. 1025), when aqueous formulations of 100 mg/mL Daclizumab Ab were stored for 0, 2, 4, or 8 weeks at 5 °C, neither of the two formulations tested achieved greater than 99% of native protein conformation at any of the time points. *Id.*, 9, Table 5.

187. Petitioner relies on Dr. Tarantino’s assertion that that a POSA “would have been motivated to achieve as close to 100% native conformation as possible” in order to maximize the shelf-life and avoid immunogenicity from aggregates. Ex. 1002, ¶¶ 153-154, 165. As discussed above, however, the regulatory requirements for aggregates or fragments rarely, if ever, required less than 1% of these impurities either at the time of release or later in the shelf-life.

188. Dr. Tarantino’s evidence regarding immunogenicity also fails to supply a reason to strive for at least a 99% native conformation (even aside from the obvious fact that wanting to achieve a lofty goal does not mean that it will be

achieved). Dr. Tarantino cites Parkins (Ex. 1014⁸⁵, 131) for the proposition that protein aggregates may be immunogenic. Ex. 1002, ¶ 72. Parkins, in turn, relies on another reference, Rougeot (Ex. 2086⁸⁶), for the proposition that immunogenicity of aggregates is a concern. But Rougeot was not able to conclusively trace immunogenicity to the presence of protein aggregates in its formulations studies and, in fact, conceded that “[t]he difference to the various rhGH [*i.e.*, recombinant human growth hormone] preparations in the immunological tolerance cannot be explained by our data. All the preparations had similar chromatographic profiles.” Ex. 2086, 5-6. Rougeot merely *suggests* immunogenicity as a cause, noting that “[t]he presence of 2.5% [of] non-monomeric GH in the preparation which was most frequently induced antibodies.” *Id.*, 6. A POSA would not find this to be persuasive evidence for minimizing

⁸⁵ Parkins, D. A., & Lashmar, U. T. (2000). The formulation of biopharmaceutical products. *Pharmaceutical Science & Technology Today*, 3(4), 129-137.

⁸⁶ Rougeot, C., Marchand, P. M., Dray, F., Girard, F., Job, J. C., Pierson, M., ... & Rappaport, R. (1991). Comparative study of biosynthetic human growth hormone immunogenicity in growth hormone deficient children. *Hormone Research in Paediatrics*, 35(2), 76-81.

protein aggregates; if anything, it only supplies a reason to keep aggregates below about 2.5%, not to pursue a far more challenging 1% or less of protein aggregates and fragments.

189. In view of the stability data of other protein pharmaceutical formulations at the time, a POSA would have neither the motivation nor a reasonable expectation of success in achieving at least a 99% of protein in its native conformation.

4. Petitioner’s references do not render obvious the use of a “phosphate” buffer

190. Claims 9 and 18 of the ’992 patent require the buffer used in the formulation to be a “phosphate” buffer, which is the only specific buffer recited by the claims of the patent. Meanwhile, as discussed above in ¶¶ 147-148, Fraser uses a distinct buffer, namely a citrate-phosphate or McIlvaine buffer. As explained above in ¶¶ 147-151, a POSA would not understand a buffer comprising *both* citrate *and* phosphate to be viewed as a “phosphate” buffer. And a POSA would not be motivated to substitute the citrate-phosphate buffer of Fraser with a phosphate buffer at least because the latter buffer would perform suboptimally at the pH of Fraser’s formulations (pH 6.0).

191. A buffer enables an aqueous solution to maintain its pH because it is composed of a weak conjugate acid-base pair that can neutralize other added acids or bases. A buffer is most effective when it contains equal concentrations of each

species of the weak conjugate acid-base pair. Different buffers achieve this balance at different pH values depending on the pK_a value of the weak acid of the conjugate pair. Generally, as explained above in ¶¶ 145-146, a buffer is effective within about 1.0 pH unit from that pK_a value. Ex. 2087, 13. The target pH is thus a key driving factor in the selection of a buffer.

192. Fraser's formulations have a pH of 6.0: "VEGF TrapRIR2 (Regeneron Pharmaceuticals, Inc., Tarrytown, NY) was provided at a concentration of 24.3 mg/ml in 2-ml aliquots in buffer composed of 5 mM phosphate, 5 mM citrate, 100 mM NaCl (**pH 6.0**)" Ex. 1009, 1115 (emphasis added). However, as explained above, phosphate is a suboptimal buffer at pH 6.0 because the closest pK_a value of phosphoric acid (the weak acid of the conjugate pair) is more than one pH unit away. In contrast, a citrate-phosphate buffer is an excellent buffer at pH 6.0 owing to citric acid's relevant pK_a value of 6.4. Indeed, a POSA would understand that at pH 6.0, citrate is the main, and indeed dominant, buffering contributor in a citrate-phosphate buffer. Therefore, a POSA would have no motivation to substitute the citrate-phosphate buffer of the Fraser formulation for a phosphate buffer at least because the latter would be inferior at performing the primary function of a buffer—*i.e.*, stabilizing the pH of the aqueous solution—than a citrate-phosphate (or just citrate) buffer. In fact, a POSA would be taught away from such substitution.

193. Furthermore, with other things being equal, a POSA would be disinclined to use a buffer known to catalyze protein degradation. And, as described above in ¶¶ 30-32, a phosphate-containing buffer was known to catalyze protein degradation through at least two related but disparate mechanisms: deamidation of Asn residues and chemical cleavage of the polypeptide chain at Asp residues.

194. By 2006, in fact, deamidation was known to cause “undesirable impurities” in “peptides and proteins for pharmaceutical uses.” Ex. 2064, 46. Chelius (Ex. 2088) warned that “[d]eamidation of asparagine residues of biological pharmaceuticals is a major cause of chemical degradation if the compounds are not formulated and stored appropriately” Ex. 2088⁸⁷, 1. McNally (Ex. 1013) cautioned that “at a given target pH, where multiple buffer choices are available, there will be differences between specific buffer ions with respect to effect on certain degradative pathways or rates of degradation.” *Id.*, 143. Phosphate in particular

⁸⁷ Chelius, D., Rehder, D. S., & Bondarenko, P. V. (2005). Identification and characterization of deamidation sites in the conserved regions of human immunoglobulin gamma antibodies. *Analytical Chemistry*, 77(18), 6004-6011.

was known to accelerate deamidation compared to other common buffers, such as Tris. Ex. 2064, 22; Ex. 2089, 5, Table III.

195. Chemical (*i.e.*, non-enzymatic) hydrolysis of peptide bonds between amino acid residues in proteins is also accelerated by phosphate buffer. According to Robinson (Ex. 2064), for example, “cleavage of the peptide bonds following Asp [residues] also occurs in analogous fashion to that following Asn.... ***This cleavage is accelerated by phosphate buffer*** and by hydroxylamine and ***exhibits a sequence dependence similar to [that of] deamidation***. Glu undergoes the same reaction, but 20 to 40-fold more slowly.” Ex. 2064, 49-50 (emphases added). Moreover, Blodgett elucidated the mechanism by which peptides containing Asp residues are hydrolyzed and observed that they “are cleaved specifically at the carboxyl side of this residue at ***pH values ≥ 6*** The ***extent of cleavage is also increased significantly by phosphate buffers, but not by PIPES or imidazole buffers***.” Ex. 2095⁸⁸, 1 (emphases added). Also, it was known that proteolytic

⁸⁸ Blodgett, J. K., Loudon, G. M., & Collins, K. D. (1985). Specific cleavage of peptides containing an aspartic acid (beta-hydroxamic acid) residue. *Journal of the American Chemical Society*, 107(14), 4305-4313.

cleavage after Asn residues occurred via a protein-splicing mechanism, as depicted in Fig. 1 above. *See* Ex. 2064, 50.

196. The reason that phosphate is able to catalyze both deamidation and peptide chain cleavage at Asn residues is that both of these reactions occur through the formation of a succinimide intermediate. As explained in the Kirikoshi reference (Ex. 2049), these hydrolysis reactions “will not occur without a catalyst at room or physiological temperature because of high activation barriers.” *Id.*, 2. Kirikoshi demonstrates that the phosphate ion is a highly effective catalyst for driving the reaction toward the succinimide intermediate, which is a critical step in both the deamidation and protein splicing reactions.

197. Separately, Parkins (Ex. 1014) observed that “a 200 mg/mL antibody solution required the inclusion of an additional solubilizer” to reduce the extent of protein aggregation “if a phosphate buffer was used, whereas the selection of an acetate buffer obviated this need” for an extra solubilizer. *Id.*, 135. Buffers or salts generally may “interact[] with macromolecules via non-specific or specific binding,” which “may increase thermal stability, increase the solubility and reduce the extent of aggregation.” *Id.* As evidenced here, phosphate performs this role worse than, for example, citrate or acetate.

198. These degradation concerns would be further exacerbated if a POSA were to adjust the formulations in order to compensate for phosphate’s suboptimal

buffering capacity at pH 6.0. For example, increasing the pH to be closer to phosphoric acid's pK_a of 7.2 would accelerate deamidation, since McNally teaches that “many degradative processes become more prevalent below pH 5 (acid hydrolysis) and above **pH 7 (deamidation).**” Ex. 1013, 141 (emphasis added). This is confirmed in Figs. 1-3 of Ex. 2064, where it can be seen that the percent of Gln deamidation in 0.2 M phosphate at pH 7.2 is almost twice as high as at pH 6.0. Although in principle a POSA could compensate for the suboptimal buffering capacity of a phosphate buffer by using its higher concentration, the POSA would be disinclined to do so because, as previously discussed in ¶ 178, phosphate buffer accelerates these degradation reactions in a concentration-dependent manner.

199. If a POSA in 2006 were motivated to use some type of buffer other than Fraser's citrate-phosphate buffer, the POSA would be aware of the many other buffers suitable for use in injectable formulations and would turn to them. For example, Nema (Ex. 2090) catalogued a number of buffers used in injectable products by 1997. Ex. 2090⁸⁹, 5. Many of these, in contrast to phosphate, **both** are

⁸⁹ Nema, S., Washkuhn, R. J., & Brendel, R. J. (1997). Excipients and their use in injectable products. *PDA Journal of Pharmaceutical Science and Technology*, 51(4), 166-171.

effective at pH 6.0 *and* do not catalyze protein deamidation or cleavage. For example, citrate, carbonate, succinate, and histidine buffers all have pK_a values close to 6.0. Ex. 2091⁹⁰, 3.

200. Given phosphate's tendency to catalyze degradation reactions, such as deamidation and peptide bond cleavage, as well as its inferior buffering capacity at the pH of the Fraser formulations, a POSA would not be motivated—and indeed would be disinclined—to modify Fraser's disclosure and to use a phosphate buffer instead of a citrate buffer.

⁹⁰ Samuelsen, L., Holm, R., Lathuile, A., & Schönbeck, C. (2019). Buffer solutions in drug formulation and processing: how pK_a values depend on temperature, pressure and ionic strength. *International Journal of Pharmaceutics*, 560, 357-364.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code.

Dated: November 2, 2023



Alexander M. Klibanov, Ph.D.

Appendix A – Amino Acid Sequences for VEGFR1 Domain 2

201. This Appendix provides the details behind my determination of the first and last amino acid residues for VEGFR1 domain 2 in various prior art literature sources.

202. In determining the amino acid sequence comprising VEGFR1 domain 2 disclosed by Davis-Smyth (Ex. 2031), I reviewed the sequence information provided by Davis-Smyth's Fig. 1, which is an "[a]lignment of the E[xtracellular]C[ellular]D[omain]s of Flt-1, KDR and Flt-4" (where "Flt-1" refers to VEGFR1, "KDR" stands for kinase domain receptor and refers to VEGFR2, and "Flt-4" refers to VEGFR3). *Id.*, 2. An excerpt of Fig. 1 of Davis-Smyth is reproduced below starting at position 1:

```

Flt-1      1 MVS YWDTGVLLC-ALLSCLLLTGSS---SGSKLKDPELSLXGTQHMQAGQTLHLQCRGEAAHKWSLPENV-----SKESERLSITKSACGRNGKQFCS
KDR        1 M---ESKVLLAVALWLCVETRAASVGLPSVSLDLRLSLIQDKILTITANTTLQITCRGQRLDLWLPNNQ-----SGSEQRVEVTECSDG---LPCK
Flt-4      1 M---QRGAALCLRLWLCGLLDGLV--SGYSMTPTFLNITEESHVIDTGDSLISISCRGQHPLEWAWPGAQEPATGDKDSEDGTGVVRDCEGTDARPYCK

Flt-1      91 TLT LNTAQANHTGFYSCKYLAVPTSKKKETESAIYIFISDTGRSEVEMYSELPETIHM--TEGRELVIPCRVTSFNITVTL-KKFPDLTLIPDGKRIIWD
KDR        87 TLTIPKVIGNDTGAYKCFYR-----ETDLASVIYVYVQDYRSPPIASVSDQHGVDYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWD
Flt-4      95 VLLLEHVHANDTGSYVCYYKYIKARIEGTTAASSYVFVRDFEQPFI---NKPDTLLV--NRKDAMWVPCLVSIPLGNVTL--RSQSSVLWPDGQEVVWD

Flt-1      188 SRKGFTISNATYKEIGLLTCEATVNGHLYKTN-YLTHROGNTIIVQISTPRPVKLLRGHTLVNLCTATTPLNTRVQMTWSYPDEKNKRAS--VRRRIDQ
KDR        181 SKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVGYRIYDVVLSPSHGIELSVGEKLVNLCTARTELNVGIDFNWEYPPSKHQHKKLVNRDLKTQ
Flt-4      187 DRRGMLVSTPLLHDALYLQCEFTWGDQDFLSNPFVLVHITGNELYDIQLLPRKSLBLLVGEKLVNLCTWABFNQSVTFDWDYPGKQAERGKWPERRSQQ

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Figure 11: Alignment of the CDs of Flt1, KDR and Flt4.⁹¹

203. As expressly denoted in the figure caption, each of the "boxed areas" represents one of "seven Ig-like domains" of the three proteins aligned, with

⁹¹ Adapted from Ex. 2031, Fig. 1 (annotations added).

“Flt 1” representing VEGFR1. Therefore, the stretch of the amino acid sequence comprising VEGFR1 domain 2 is the “Flt-1” sequence contained in the second box (annotated by my addition of green highlight above). To determine where VEGFR1 domain 2 begins starting from Flt-1 position 91, a threonine (“T” using a one-letter code for amino acids henceforth unless stated otherwise), I counted the position of the first amino acid residue of Flt-1 contained in this second box. An enlarged and annotated excerpt below shows that the first amino acid residue of VEGFR1 domain 2, a proline (“P”), is located at position 134.

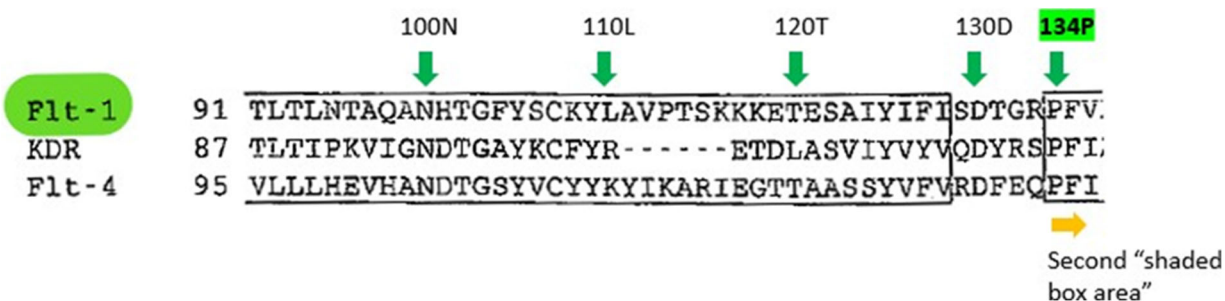


Figure 12: Inset showing first amino acid residue of VEGFR1 domain 2.⁹²

204. To determine where VEGFR1 domain 2 ends starting from Flt-1 position 188, a serine (“S”), I counted the position of the last amino acid residue contained in this second box. The “-” was not counted as an amino acid residue position. An enlarged and annotated excerpt below shows that the last amino acid residue of VEGFR1 domain 2, a threonine (“T”), is located at position 226.

⁹² Adapted from Ex. 2031, Fig. 1 (annotations added).



Figure 13: Inset showing last amino acid residue of VEGFR1 domain 2.⁹³

205. Based on the starting and ending positions extracted from Davis-Smyth, I calculated the length of the stretch of amino acid sequence comprising VEGFR1 domain 2 to be 93 amino acid residues (226 - 134 + 1).

206. In determining the amino acid sequence comprising VEGFR1 domain 2 disclosed by UNIPROT P17948 (Ex. 2032), I reviewed the "Family & Domains" section of the website. This section annotates the seven domains of VEGFR1 as "Ig-like C2-type" 1 through 7. When I expanded the description for VEGFR1 domain 2 ("Ig-like C2 type 2"), the annotation indicates that the domain spans amino acid positions "151-214" and provides the corresponding amino acid sequence of the domain. Taken together, this annotation indicates that the VEGFR1 domain 2 starts at position 151, a glycine ("G"), and ends at position

⁹³ Adapted from Ex. 2031, Fig. 1 (annotations added).

214, a histidine (“H”). An excerpt of the domain annotation information from UNIPROT P17948 is reproduced below.

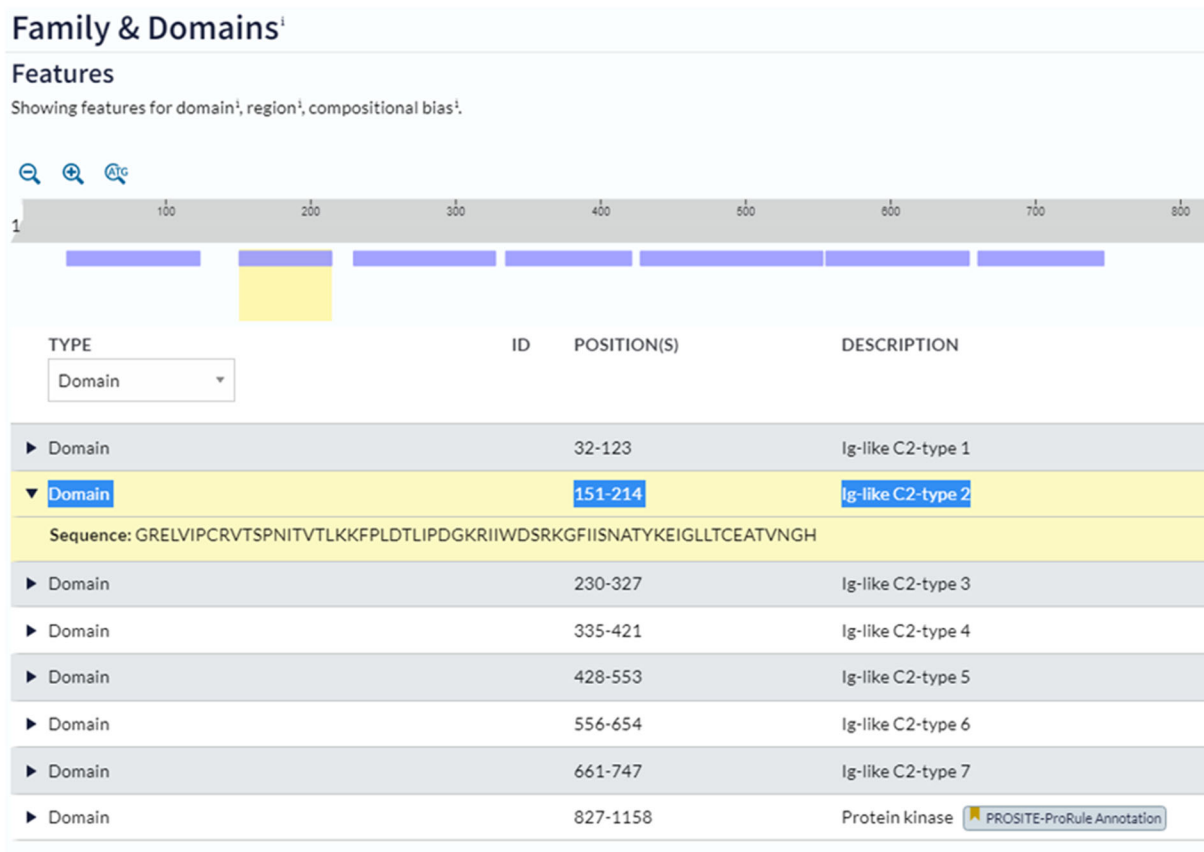


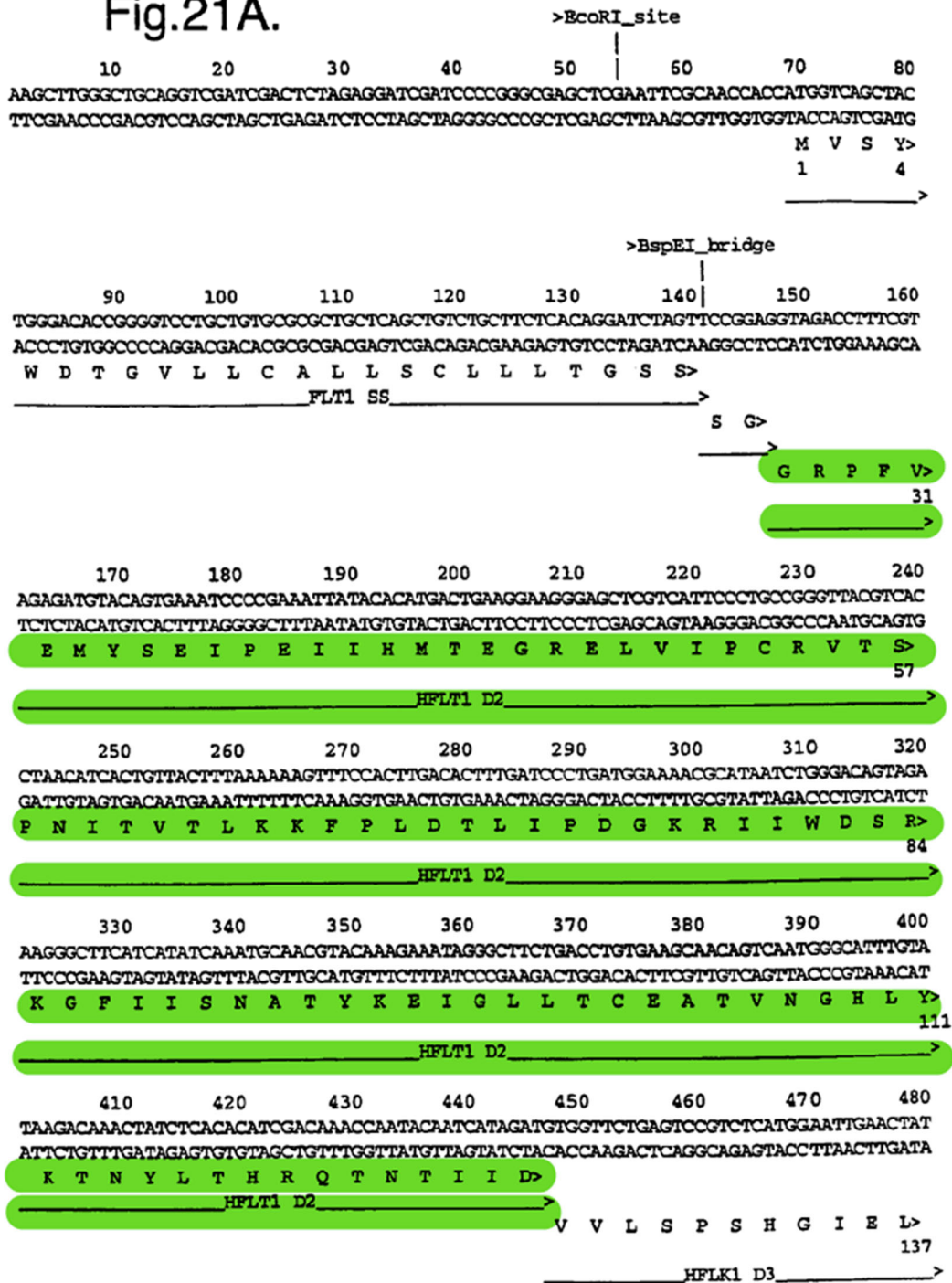
Figure 14: Domain annotation information for VEGFR1.⁹⁴

207. Based on the starting and ending positions extracted from UNIPROT P17948, I calculated the length of the stretch of amino acid sequence comprising VEGFR1 domain 2 to be 64 amino acid residues (214 - 151 + 1).

⁹⁴ Adapted from Ex. 2032, 9 (annotations added).

208. I further reviewed the amino acid sequence comprising VEGFR1 domain 2 disclosed by the '319 Publication (Ex. 1029). In the '319 Publication, the relevant amino acid sequence information is presented as a part of a fusion protein “[Flt1D2.Flk1D3.FcΔC1\(a\)](#)” with sequence information given in Fig. 21A-C. *Id.*, 21:16-17. I have annotated and reproduced this amino acid sequence below:

Fig.21A.

Figure 15: Amino acid sequence comprising VEGFR1 domain 2.⁹⁵⁹⁵ Adapted from Ex. 1029 ('319 Publication), Fig. 21 (annotations added).

209. In the excerpt depicted above, “HFLT1 D2” represents VEGFR1 domain 2. The corresponding stretch of amino acid sequence relevant to my analysis is highlighted in green above. To determine where VEGFR1 domain 2 begins starting from HFLT1 D2 position 31, a valine (“V”), I reverse-counted the position of the first amino acid residue in the domain. An enlarged and annotated excerpt below shows that the first amino acid residue of VEGFR1 domain 2, a glycine (“G”), is located at position 27.

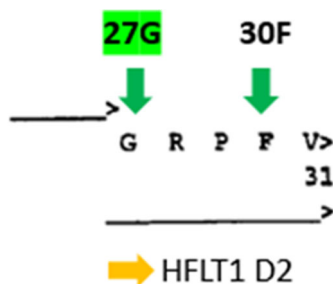


Figure 16: Detail of first amino acid residue of VEGFR1 domain 2.⁹⁶

210. To determine where VEGFR1 domain 2 ends starting from HFLT1 D2 position 112, a lysine (“K”), I counted the position of the last amino acid residue in the domain.⁹⁷ An enlarged and annotated excerpt below shows that the

⁹⁶ Adapted from Ex. 1029 (’319 Publication), Fig. 21 (annotations added).

⁹⁷ I understand that the above-starting lysine (“K”) is in position 112 because the immediately prior amino acid residue from the previous row is marked as position 111.

last amino acid residue of VEGFR1 domain 2, an aspartic acid (“D”), is located at position 126.

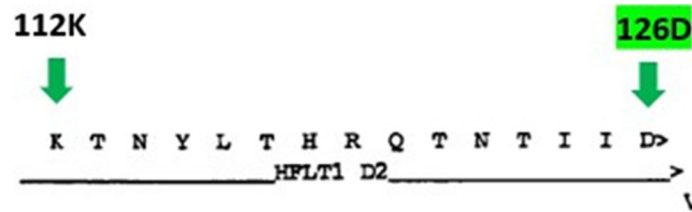


Figure 17: Detail of last amino acid residue of VEGFR1 domain 2.⁹⁸

211. Based on the starting and ending positions extracted from Papadopoulos, I calculated the length of the stretch of amino acid sequence disclosed as encoding VEGFR1 domain 2 to be 100 amino acid residues (126 - 27 + 1).

⁹⁸ Adapted from Ex. 1029 ('319 Publication), Fig. 21 (annotations added).

Appendix B – Amino Acid Sequences for VEGFR2 Domain 3

212. In determining the amino acid sequence comprising VEGFR2 domain 3 disclosed by Davis-Smyth (Ex. 2031), I reviewed the sequence information provided by the same Fig. 1 discussed above in ¶ 202, which is an “[a]lignment of the E[xtracellular]C[ellular]D[omain]s of Flt-1, KDR and Flt-4.” *Id.*, 2. An excerpt of Fig. 1 of Davis-Smyth is reproduced below starting at position 1:

```

---
KDR      1 M---ESKVLAVALLMLCVETRAASVGLPSVSLDLPRLSIQKDLTIKANTLQITCRGQRDLDLWLPNNQ-----SGSEQRVEVTECSDG---LFCK
Flt-4    1 M---QRGAALCLRLMLCLGLLDGLV--SGYSMTPTLTNITEESHVIDTGDLSLSISCRGQHPLEWAWPGACEAPATGDKDSEDGTGVVRDCEGTDARPYCK

Flt-1    91 TLTNLTAQANHTGPFYSCRYLAVPTSKKTESAIYIFPSDTGRPFVEMYSEIPEIHM--TEGRBLVIPCRVTSFNITVTL-KKFPDLTLIPDGKRIWD
KDR      87 TLTIPKVGINDTGAYKCFYR-----ETDLASVIYVYVQDYRSPFIASVSDQHGVDYITENKNTVVIPLGSIENLVSLCARYPEKRFVPDGNRISWD
Flt-4    95 VLLLEHVHANDTGSYVCYKYIKARIBGTTAASSYVFRDFEQFFI---NKPDTLLV--NRKDAMWVCLVSIPLGLNVTL--RSQSSVLWFDGQEVVND

Flt-1    188 SRKGFIIISNATYKEIGLLTCEATVNGHLYKTN-YLTHRQNTIIDVOISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNRAS--VRRRIDQ
KDR      181 SKKGFITIPSYMISYAGMVFCEAKINDESYQSIMYIVVVGYRIYDVLSPSHGIELSVGEKLVNCTARTELNVGIDFNWEYPSXKHQKKLVNRDLKTQ
Flt-4    187 DRRGMLVSTPLLDALYLQCEFTWGDQFLSNFFLVHITGNELYDIQLLPKSLBLVGEKLVNCTVWAEFNSGVTFPDWDYFGQAERGKWPERRSQQ

Flt-1    285 SNSHANIFYSVLTIDKMNKDKGLYTCVRVSCPSSEKSVNTEVHIYDRAFITVKHRKQVLETVAGKRSYRLSMKVKAFFSPPEVVNLKDGLFATEKSARYL
KDR      281 SGSEMKKPLSTLTIDGVTRSDQGLYTCASSGLMTKKNSTFVRVHENPFVAFGSGMESLVEATVGER-VRIPAKYLGYPPPEIKWYKNGIFLESN--HTI
Flt-4    287 THTFES---SILTIHNVSDHDLGSYVCKANNGIORPRESTRUTVHNRPTQVWLECDTLEATAGDSTSTQDPTAAVNDDEKAVVNDK---

```

Figure 18: Alignment of the Extracellular Cellular Domains of Flt-1, KDR and Flt-4.⁹⁹

213. Each of the “boxed areas” represents one of “seven Ig-like domains” of the three proteins aligned. In the excerpt above, the “KDR” (kinase domain receptor) represents VEGFR2. Therefore, the stretch of amino acid sequence comprising VEGFR2 domain 3 is the “KDR” amino acid sequence contained in the third box (annotated by my addition of green highlight above). *Id.*, Fig. 1. To determine where VEGFR2 domain 3 begins starting from KDR position 181, a

⁹⁹ Adapted from Ex. 2031, Fig. 1 (annotations added).

serine (“S”), I counted the position of the first amino acid residue of KDR contained in this third box. An enlarged and annotated excerpt below shows that the first amino acid residue of VEGFR2 domain 3, a valine (“V”), is located at position 226.

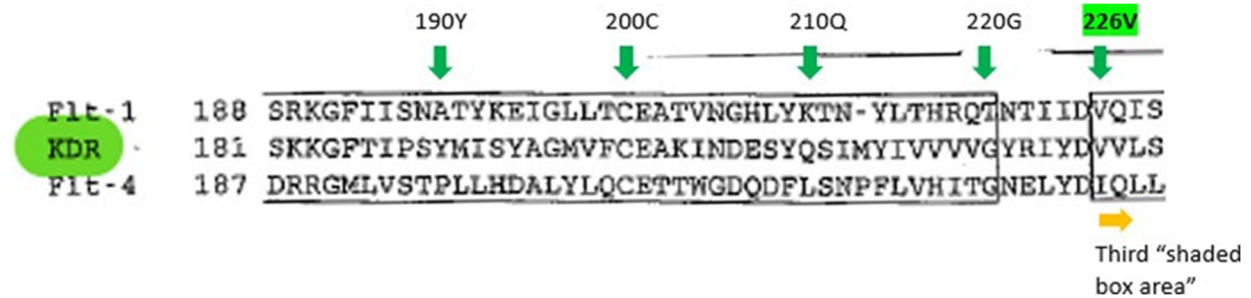


Figure 19: Detail of first amino acid residue of VEGFR2 domain 3.¹⁰⁰

214. To determine where VEGFR2 domain 3 ends starting from KDR position 281, a serine (“S”), I counted the position of the last amino residue contained in this third box. An enlarged and annotated excerpt below shows that the last amino acid residue of VEGFR2 domain 3, a lysine (“K”), is located at position 327.



¹⁰⁰ Adapted from Ex. 2031, Fig. 1 (annotations added).

Figure 20: Detail of last amino acid residue of VEGFR2 domain 3.¹⁰¹

215. Based on the starting and ending positions extracted from Davis-Smyth (Ex. 2013), I calculated the length of the stretch of amino acid sequence comprising VEGFR2 domain 3 to be 102 amino acid residues (327 - 226 + 1).

216. Lu (Ex. 2034) discloses a different set of starting and ending amino acid residues of VEGFR2 domain 3. Under the “experimental procedures” section, Lu provides a “KDR domain 3 (from Tyr²²¹ to Ser³³⁴).” *Id.*, 2. Based on this disclosure, the first amino acid residue of VEGFR2 domain 3 is a tyrosine (“T”) located at position 221 and the last amino acid residue is a serine (“S”) located at position 334. Therefore, Lu’s amino acid sequence comprising VEGFR2 domain 3 has a length of 114 amino acid residues (334 - 221 + 1).

217. Franklin (Ex. 2035) discloses yet another set of starting and ending amino acid residues of VEGFR2 domain 3. Under the “experimental procedures” section, Franklin provides a “KDR domain 3[] containing residues Gly220-Ser328 of human KDR.” *Id.*, 8. Based on this disclosure, the first amino acid residue of VEGFR2 domain 3 is a glycine (“G”) located at position 220 and the last amino acid residue is a serine (“S”) located at position 328. Therefore, Franklin’s amino

¹⁰¹ Adapted from Ex. 2031, Fig. 1 (annotations added).

acid sequence comprising VEGFR2 domain 3 has a length of 109 amino acid residues ($328 - 220 + 1$).

218. In determining the amino acid sequence comprising VEGFR2 domain 3 disclosed by UNIPROT P35968 (Ex. 2036), I reviewed the “Family & Domains” section of the website. This section annotates the seven domains of VEGFR2 as “Ig-like C2-type” 1 through 7. When I expanded the description for VEGFR2 domain 3 (“Ig-like C2 type 3”), the annotation indicates that the domain spans amino acid positions “224-320” and provides the corresponding amino acid sequence of the domain. Taken together, this annotation indicates that the VEGFR2 domain 3 starts at position 224, a tyrosine (“Y”), and ends at position 320, a threonine (“T”). An excerpt of the domain annotation information from UNIPROT P35968 is reproduced below.

Family & Domainsⁱ

Features

Showing features for domainⁱ, regionⁱ, compositional biasⁱ.

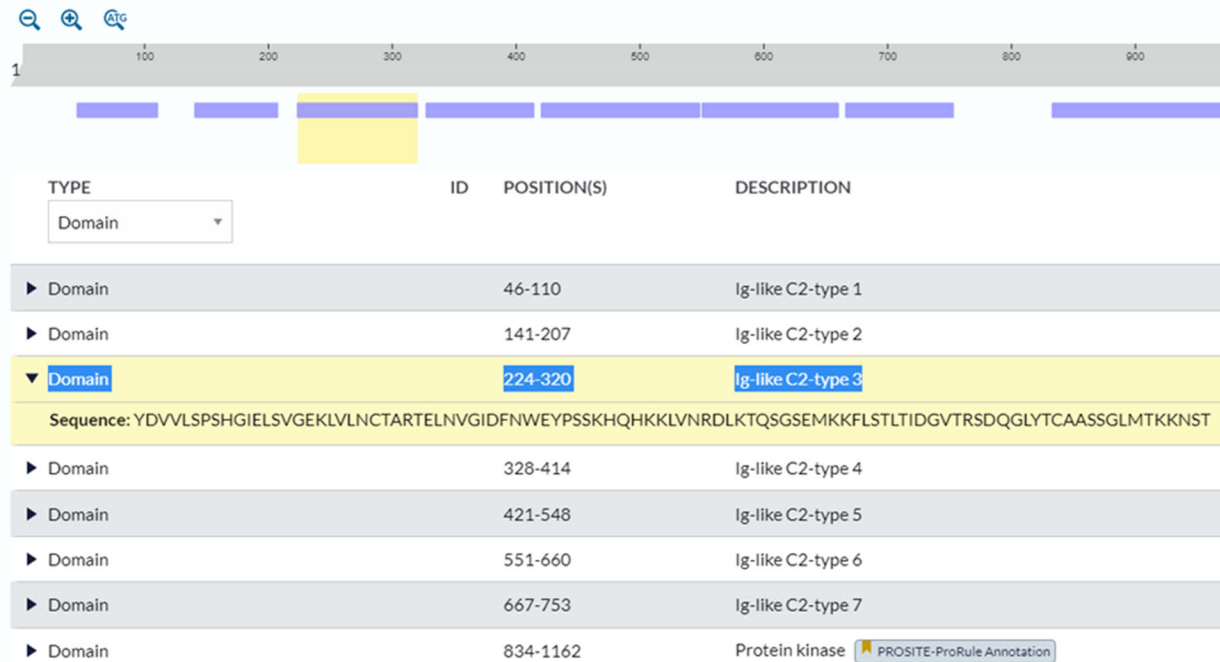


Figure 21: Domain annotation information for VEGFR2.¹⁰²

219. Based on the starting and ending positions extracted from UNIPROT P35968, I calculated the length of the stretch of amino acid sequence comprising VEGFR2 domain 3 to be 97 amino acid residues (320 - 224 + 1).

¹⁰² Adapted from Ex. 2036, 12 (annotations added).

Exhibit List for *Inter Partes* Review of U.S. Patent No. 10,464,992

Exhibit Description	Exhibit #
Declaration of Dr. Alexander M. Klibanov	2001
Dr. Alexander M. Klibanov <i>curriculum vitae</i>	2002
AVASTIN® Label (rev. Sept. 2011)	2003
Declaration of David M. Brown, M.D., <i>Mylan Pharms. Inc. v. Regeneron Pharms., Inc.</i> , IPR2021-00881, Ex. 2050 (Feb. 10, 2022)	2004
MACUGEN® Label (rev. Jul. 2011)	2005
MACUGEN® FDA Approval Letter (Dec. 17, 2004)	2006
AVASTIN® FDA Approval Letter (Feb. 26, 2004)	2007
LUCENTIS® Label (rev. June 2010)	2008
Kyla R. Rodgers & Richard C. Chou, <i>Therapeutic Monoclonal Antibodies and Derivatives: Historical Perspectives and Future Directions</i> , 34 BIOTECHNOLOGY ADVANCES 1149 (2016)	2009
Adis R&D Profile, <i>Aflibercept: AVE 0005, AVE 005, AVE0005, VEGF Trap – Regeneron, VEGF Trap (R1R2), VEGF Trap-Eye</i> , 9 DRUGS R&D 261 (2008)	2010
Quan Dong Nguyen et al., <i>A Phase I Trial of an IV-Administered Vascular Endothelial Growth Factor Trap for Treatment in Patients with Choroidal Neovascularization due to Age-Related Macular Degeneration</i> , 113 OPHTHAMOLOGY 1522 (2006).	2011
EYLEA® Label (rev. d Nov. 2011)	2012
U.S. Patent Publication No. 2006/0058234 to Daly et al.	2013
FDA, GUIDANCE FOR INDUSTRY: Q1A(R2) STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS (rev. 2, 2003)	2014
PHARMACEUTICAL FORMULATION DEVELOPMENT OF PEPTIDES AND PROTEINS (Sven Frokjaer & Lars Hovgaard, eds., 2000)	2015
Jonas L. Fast et al., <i>Physical Instability of a Therapeutic Fc Fusion Protein: Domain Contributions to Conformational and Colloidal Stability</i> , 48 BIOCHEMISTRY 11724 (2009)	2016

ANTIBODY FUSION PROTEINS (Steven M. Chamow & Avi Ashkenazi, eds. 1999)	2017
Hussein Hollands et al., <i>Short-Term Intraocular Pressure Changes After Intravitreal Injection of Bevacizumab</i> , 42 CAN. J. OPHTHAMOLOGY 807 (2007)	2018
Domenico Sanfelice et al., <i>Cold Denaturation Unveiled: Molecular Mechanism of the Asymmetric Unfolding of Yeast Frataxin</i> , 16 CHEMPHYSCHEM 3599 (2015)	2019
Sven Frokjaer & Daniel E. Otzen et al., <i>Protein Drug Stability: A Formulation Challenge</i> , 4 NATURE REVIEWS DRUG DISCOVERY 298 (2005)	2020
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