No. 18-1933

In the United States Court of Appeals for the Federal Circuit

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GENENTECH, INC.,
APPELLANT

v.

HOSPIRA, INC.,
APPELLEE

ON APPEAL FROM THE UNITED STATES PATENT AND TRADEMARK OFFICE PATENT TRIAL AND APPEAL BOARD IN NO. IPR2016-01837

BRIEF OF APPELLANT GENENTECH, INC.

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CERTIFICATE OF INTEREST

Pursuant to Federal Circuit Rule 47.4, undersigned counsel for appellant certifies the following:

1. The full name of the party represented by me is Genentech, Inc.

2. The name of the real party in interest represented by me is the same.

3. Genentech, Inc. is a wholly-owned subsidiary of Roche Holdings Inc. Roche Holdings Inc.'s ultimate parent, Roche Holdings Ltd, is a publicly held Swiss corporation traded on the Swiss Stock Exchange. Upon information and belief, more than 10% of Roche Holdings Ltd's voting shares are held either directly or indirectly by Novartis AG, a publicly held Swiss corporation.

4. The following attorneys appeared for Genentech, Inc. in proceedings below or are expected to appear in this Court and are not already listed on the docket for the current case: Adam Perlman, Christopher Suarez, and Teagan Gregory of Williams & Connolly LLP, 725 Twelfth Street, N.W., Washington, D.C. 20005.

5. The title and number of any case known to counsel to be pending in this or any other court or agency that will directly affect or be directly affected by this court's decision in this pending appeal are *Genentech, Inc. and City of Hope v. Amgen, Inc.*, No. 17-1407 (D. Del.); *Genentech, Inc. and City of Hope v. Amgen, Inc.*, No. 17-1471 (D. Del.); *Genentech, Inc. et al. v. Pfizer, Inc.*, No. 17-1672 (D. Del.); *Genentech, Inc., et al. v. Sandoz, Inc., et al.*, No. 17-13507 (D.N.J.); *Genentech, Inc. et al. v. Celltrion, Inc., et al.*, No. 18-574 (D.N.J.); *Genentech, Inc. et al. v. Celltrion, Inc., et al.*, No. 18-00095 (D. Del.); *Genentech, Inc. et al. v. Celltrion, Inc. et al.*, No. 18-01025 (D. Del.); *Genentech, Inc. et al. v. Celltrion, Inc. et al.*, No. 18-11553 (D.N.J.).

AUGUST 17, 2018

/s/ Paul B. Gaffney
PAUL B. GAFFNEY

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STATEMENT OF RELATED CASES

No other appeal in or from the same inter partes review proceeding at the Patent Trial and Appeal Board ("PTAB" or the "Board") was previously before this Court or any other appellate court. The PTAB proceedings from which these appeals arise concern U.S. Patent No. 7,807,799 ("the '799 patent"). Genentech, Inc. has asserted the '799 patent in eight pending cases that will be affected by this Court's decision in the pending appeal: *Genentech, Inc. and City of Hope v. Amgen, Inc.*, No. 17-1407 (D. Del.); *Genentech, Inc. and City of Hope v. Amgen, Inc.*, No. 17-1471 (D. Del.); *Genentech, Inc. et al. v. Pfizer, Inc.*, No. 17-1672 (D. Del.); *Genentech, Inc., et al. v. Sandoz, Inc., et al.*, No. 17-13507 (D.N.J.); *Genentech, Inc. et al. v. Celltrion, Inc., et al.*, No. 18-574 (D.N.J.); *Genentech, Inc. et al. v. Celltrion, Inc., et al.*, No. 18-00095 (D. Del.); *Genentech, Inc. et al. v. Celltrion, Inc. et al.*, No. 18- 01025 (D. Del.); *Genentech, Inc. et al. v. Celltrion, Inc. et al.*, No. 18-11553 (D.N.J.).

JURISDICTIONAL STATEMENT

Hospira, Inc. filed an inter partes review petition, Case No. IPR2016-01837, challenging claims 1–3 and 5–11 of U.S. Patent No. 7,807,799 ("the '799 patent"). The PTAB had jurisdiction over the IPR proceeding pursuant to 35 U.S.C. § 6. On March 6, 2018, the PTAB issued a Final Written Decision in the IPR. Appx1–52. Genentech timely filed its notice of appeal on May 7, 2018. Appx349–354; 37 C.F.R. § 90.3(a)(1). This Court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(4)(A).

STATEMENT OF THE ISSUES

1. Whether the Board erred in construing the claim language "about 18° C."

2. Whether the Board erred in applying the law of anticipation and made findings not supported by substantial evidence.

3. Whether the Board erred in applying the law governing obviousness and in its ultimate obviousness conclusion.

4. Whether it violates the U.S. Constitution to subject patents issued prior to the America Invents Act, Pub. L. No. 112-29, 125 Stat. 284 (2011), to inter partes review.

STATEMENT OF THE CASE

The '799 patent claims methods of purifying certain proteins, and in particular, therapeutic antibodies. The claimed methods perform a purification technique known as "protein A chromatography" on solutions that have been chilled below room temperature to the range of "about 10° C to about 18° C." Patent Owner-Appellant Genentech, Inc. uses this process to manufacture each of the humanized antibodies that it has developed and marketed. Appx1428, Appx1438–1439.

Petitioner-appellee Hospira, Inc. is developing so-called "biosimilar" copies of Genentech's therapeutic antibodies. Ruling on Hospira's petition for inter partes review, the Patent Trial and Appeal Board found various claims of the '799 patent invalid as anticipated and other claims of the patent invalid for obviousness over various grounds, including one combination of no fewer than four references.

A. Factual Background

Over the course of the 1990s, genetically engineered antibodies emerged as an important treatment for a variety of human diseases. Such antibodies typically were (and still are) expressed recombinantly in mammalian cells cultured in massive bioreactors. The antibodies are secreted by the cells into the supernatant, which is then harvested. Appx1325.

One challenge in manufacturing therapeutic antibodies is purifying the antibody from this harvested cell culture fluid ("HCCF") in a manner that will satisfy the "extreme" purity requirements imposed by regulators. Appx1325. The '799 patent relates to this challenge.

1. Protein A Chromatography

Protein A chromatography is the typical first step in purifying a therapeutic antibody. Protein A is a bacterial cell wall protein[1] capable of binding specifically to antibodies, in particular to a conserved structural feature of antibodies known as the "C_H2/C_H3 region." Protein A can be attached covalently to a chromatography column. Solutions containing antibodies and other contaminants can then be pumped through the column; the antibody binds to the protein A ligand while the contaminants flow through. In a subsequent step, the binding between protein A and the antibody is disrupted, typically by using a low pH solution, and the purified antibody recovered. As explained in a review paper from the early 2000s, this "straightforward" protocol featuring "simple bind/elute chemistry" was highly efficient and produced "extreme purification." Appx1326–1329.

Protein A chromatography comes with a downside. Small amounts of protein A, on the order of nanograms per milligram of antibody, would "leach" from the column and contaminate the

[1] "Protein A" is also made recombinantly and may differ from the wild-type protein. Appx61.

otherwise-purified antibody solution. For therapeutic uses of antibodies, protein A is an unacceptable contaminant, even if present at the nanogram per milligram level. (By contrast, for non-clinical uses it is irrelevant if antibodies are contaminated with protein A.) Thus, the typical commercial manufacturing process for purifying antibodies involved one or more additional chromatography steps intended to remove, among other residual contaminants, leached protein A. Such "downstream" processes were described in several references, each of which demonstrated successful protocols for clearing leached protein A to undetectable levels. Appx1327, Appx1331–1334, Appx1338, Appx1375.

2. The Prior Art

Several references published before July 2003 are at issue on appeal.

Van Sommeren (1992).[2] Van Sommeren is a journal article from 1992, more than a decade before the priority date. As of 1992,

[2] van Sommeren *et al.*, "Effects of Temperature, Flow Rate and Composition of Binding Buffer on Adsorption of Mouse Monoclonal IgG_1 Antibodies to Protein A Sepharose 4 Fast Flow," 22 *Preparative Biochemistry* 135 (1992) ("van Sommeren").

commercial vendors marketed protein A media (the solid phase for the chromatography column with protein A ligands covalently bound to it), and van Sommeren describes experiments characterizing the "binding capacity" of one such media under various conditions. Appx555–574, Appx1326, Appx1354–1365.

"Binding capacity" refers to the amount of antibody that the column can bind before additional protein would simply flow through (for example, if all of the protein A ligand binding sites are occupied, the column cannot bind any more antibody). Van Sommeren tested whether the column's binding capacity was affected by: the composition of the solution containing the antibody; the pH of the solution; the ionic strength of the solution; the flow rate of the solution through the column; and whether the experiments were performed in a cold room (4°C) or at "ambient temperature," described as varying between 20°C and 25°C. Appx570–573, Appx1362.

WO 95/22389 ("the '389 Application," 1995).[3] Whereas van Sommeren focused on characterizing the properties of a particular protein A media, the '389 Application discloses an actual industrial

[3] WO 95/22389, published Aug. 24, 1995 ("WO '389").

process for purifying a therapeutic antibody. The process includes a protein A chromatography step, followed by additional, downstream purification steps. These processes were performed in a facility at "room temperature," which was permitted to vary between 18°C to 25°C. Appx522, Appx1349–1355.

The final downstream step taught in the '389 Application, known as "hydrophobic interaction chromatography," was characterized for its ability to remove leached protein A. The data demonstrate "significant" reductions in leached protein A, and the '389 Application commends that hydrophobic interaction chromatography "can be usefully employed to remove contaminating Protein A." Appx513, Appx1353–1355.

The Fahrner Paper (1999).[4] As the 1990s progressed, researchers continued to optimize the protein A chromatography process. Like van Sommeren, the 1999 Fahrner Paper addressed optimization of binding capacity. It explained that dynamic binding capacity "depends on many factors, including the type of protein A affinity chromatography media, the antibody concentration in the load, the column temperature and

[4] Fahrner *et al.*, *The optimal flow rate and column length for maximum production rate of protein A affinity chromatography*, 21 Bioprocess Engineering 287–292 (1999) (The "Fahrner Paper").

column length, the buffer, conductivity, and pH of the load, and the flow rate." Appx1312. Of those many factors, the Fahrner Paper "focuses on flow rate and column length" because those factors "would have little impact on the antibody itself." *Id.* Temperature, on the other hand, "could denature, precipitate, or otherwise affect the antibody." *Id.* The Fahrner Paper concluded that a "simple equation" and "small amount of empirical information" could be used to achieve the maximum production rate from a protein A chromatography step by optimizing only the flow rate and column length. Appx1316, Appx1329–1330.

The Fahrner Review (2001).[5] By 2001, after the field had developed substantial expertise in purifying antibodies intended for therapeutic use, several Genentech scientists collaborated to publish a book chapter to review "the methods used to purify these antibodies at industrial scale, focusing on chromatography processes, and with particular reference to recent work at Genentech." Appx1285, Appx1326–1331.

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[5] Fahrner *et al.*, *Industrial Purification of Pharmaceutical Antibodies: Development, Operation, and Validation of Chromatography Processes*, 18 Biotechnology and Genetic Engineering Reviews 301–327 (2001) (The "Fahrner Review").

The Fahrner Review explained that the "basic protocol of a protein A affinity column is straightforward: bind at neutral pH and elute at acid pH." Appx1293–1294. This "simple" process did not "leave much room" for optimization, but this was not a problem because the process provides "extreme purification in a single step" such that "even an unoptimized process can produce a highly purified antibody." *Id.* Thus, "the optimization effort typically focuses not on purity but on throughput." Appx1293–1294, Appx1328. As to optimizing throughput, the Fahrner Review's literature summary concluded that, while many factors *could* influence binding capacity, "the simplest to control for production and the ones that will have the most significant impact on capacity are the column length, the flow rate, and the chromatography media." Appx1294.

With respect to purity, while the process described in the '389 Application used hydrophobic interaction chromatography to clear leached protein A, the process outlined in the Fahrner Review used a technique called cation exchange chromatography. Regardless, the result was similar—the protein A that leached in the first step was

removed by the second such that its concentration was below the limit of detection. Appx1296, Appx1331–1333.

* * *

As these references demonstrate, the field of protein A chromatography developed rapidly between 1992 and 2003. Based on the work of Fahrner and others, process engineers as of 2003 had a "well-defined strategy for optimizing the protein A chromatography step" focusing on flow rate and column length. Appx1328–1331. The experiments described by van Sommeren a decade earlier were simply "archaic." Appx1374.

3. Experiments Not Reported in the Prior Art

While the references discussed above demonstrate extensive efforts to optimize the efficiency of the protein A step by varying flow rates and column lengths and to develop downstream efforts to remove leached protein A, no reference discussed in this appeal discloses efforts to optimize protein A chromatography to reduce leaching.

Nor does any reference characterize the protein A chromatography step at a series of intermediate temperatures, in order to test the effect of that variable on binding capacity. As Hospira's expert testified:

> Q. Are you aware of anyone prior to 24 July of 2003 doing protein A chromatography at a reduced temperature using a jacketed column and/or a chilling tank?
>
> A. I have not seen that.

Appx1667–1668. Musing as to why such experiments are reported nowhere, Hospira's expert speculated in his declaration that "skilled artisans may have chosen not to use reduced temperatures, because it requires additional lab equipment such as jacketed columns, or raises operating costs, or may simply be uncomfortable or inconvenient to laboratory personnel." Appx424.

Genentech's expert, Dr. Steven Cramer of Rensselaer Polytechnic Institute, explained that process engineers did not experiment with the effect of temperature on chromatography processes. The widely-used laboratory-scale apparatus for conducting chromatography research—the ÄKTAexplorer automated liquid handler—simply "did not have the capability to control the temperature of the solutions" it handled. Appx1334–1336.

4. The Claimed Invention

a. In the early 2000s, Genentech scientists were developing an improved "version 1.1" process to manufacture trastuzumab, the

antibody that is the active ingredient in Herceptin, Genentech's breast cancer therapy. They experienced a "curve ball," Appx1697, levels of leached protein A so high that their downstream purification steps could not successfully remove it. Appx1698, Appx1433–1434.

The Genentech research team spent months investigating the cause of the leaching, proposing hypotheses, and trying to solve this problem. Appx1434–1437, Appx1754. Eventually, they tried chilling the HCCF—that is, the cell culture fluid itself—below room temperature to see whether doing so would reduce leaching. Because the standard laboratory equipment for chromatography optimization had no mechanism to control temperature, Appx69, Appx1335, Appx1442, Appx1446, Appx1596–1597, the researchers improvised and modified their ÄKTAexplorer by submerging the inlet line and immersing the column itself in a water bath. Appx69.

The successful results of these investigations are described in Example 1 and Figure 1 of the '799 patent. Further experiments adding protease inhibitors to the HCCF discussed in Example 2 supported the hypothesis that the cause of the increased leaching was a contaminating enzyme capable of cleaving protein A from the column.

In addition to these laboratory experiments, the inventors also tested the claimed method at pilot scale (i.e., chilling 400 liters of HCCF) and then again at full scale (chilling 12,000 liters of HCCF). At all three scales the experiments demonstrated that chilling the HCCF reduced protein A leaching.

b. Genentech filed a priority application regarding this method in July 2003. One of the inventors, Dr. Amy Laverdiere, presented the results of this research at the 229th National Meeting of the American Chemical Society in San Diego, California on March 13–17, 2005. Appx2111, Appx1721, Appx1733, Appx1754. The ACS National Meeting is "one of the most important conferences" in the field of bioprocessing, and only research selected by conference organizers gets presented. Appx1391–1393, Appx1397, Appx1436–1437. Dr. Cramer, one of the organizers of the session in question, explained:

> [M]y colleagues and I attend symposia like the BIOT division of the American Chemical Society to learn about cutting edge developments in our field. It is not a forum for the presentation of "obvious" results or the product of "routine" development work.

Appx1392; *see also* Appx1437–1438.

5. The '799 Patent

The '799 patent claims the methods described above. Claim 1 involves conducting protein A chromatography on a composition (*e.g.*, the HCCF) chilled to "a temperature in the range from about 10°C to about 18°C." Appx77. Dependent claims 2 and 3 involve adding protease inhibitors to the composition being purified. Dependent claims 5–9 are directed to using claim 1's method to purify particular antibodies and claims 10–11 are directed to purifying other proteins.

B. Proceedings Before the Board

1. In its IPR petition, Hospira sought cancellation of claims 1 to 3 and 5 to 11 of the '799 patent on eight different grounds. It supported its petition with the testimony of Dr. Todd M. Przybycien from Carnegie Mellon University. The Board instituted review on all grounds on March 15, 2017.

Genentech filed its response on June 28, 2017, supported by the testimony of Dr. Cramer, one of the leaders in the field of protein purification according to Hospira's expert, Appx1496, and Dr. Christopher Dowd, Genentech's Senior Director and Head of Purification Development. Hospira filed its reply on September 11, 2017 supported by a second declaration from Dr. Przybycien.

2. In its final written decision on March 6, 2018, the Board construed claim 1 in two ways pertinent to this appeal.

First, the Board agreed with Genentech that the temperature range recited in claim 1 describes the temperature of the composition being purified, and not the ambient temperature of the facility in which the process is being performed. Appx16.

Second, the Board adopted Hospira's construction regarding the breadth of the range "about 10°C to about 18°C," construing its upper end point to mean "18°C ± 3°C," i.e., "21°C." The Board cited Example 1's full-scale experiment in which the 12,000 liters of cell culture fluid was held at 15°C ± 3°C, i.e., entirely within the claimed range of "about 10°C to about 18°C." In another portion of the specification, the '799 patent actually discusses the claimed temperature range, emphasizing that "the temperature of the composition is reduced below room temperature, . . . e.g. from about 10° C. to about 18° C." Appx68. The Board concluded that although its construction of "about 18°C" indisputably embraces room temperature, it was nevertheless proper because "the Specification teaches both the reduction of temperature

and 'below room temperature' as a [sic] merely preferred embodiments." Appx12.

Having construed the claimed temperature range in a manner that nearly doubled its breadth, the Board held all of the challenged claims invalid.

The Board concluded that van Sommeren anticipated claims 1, 2, and 5 because its disclosure of performing protein A chromatography at "ambient temperature" (20–25°C) overlapped the claimed temperature range, which it had construed as extending to 21°C. Appx25–28.

The Board concluded that the '389 Application also anticipated claims 1 and 5. Appx17–25. The Board's anticipation analysis here rested on the '389 Application's disclosure that "all steps are carried out at room temperature (18–25°C)." Appx19–22. Both experts testified that this disclosure referred to the temperature of the laboratory, not the composition being purified. Appx1547, Appx1556, Appx1350–1352. Both experts also agreed that the harvested cell culture fluid comes out of the production bioreactor at around 37°C and that the POSA would worry that holding it at room temperature would cause the protein of interest to degrade. Appx1531, Appx1350–1352. Despite the Board's

agreement with Genentech that the '389 Application "does not expressly call out the temperature of the HCCF," Appx20, the Board "infer[red]" that the composition being purified had "equilibrated" to the 18–25°C temperature range, and therefore concluded that the '389 Application's process anticipated claims 1 and 5.

The remaining six grounds concerned the obviousness of the dependent claims over van Sommeren or the '389 Application alone and in combination with various references. The Board found that the POSA would have understood temperature to be a "result effective variable" for two reasons. Appx38. First, the Board found that the POSA would have known that reducing the temperature of a solution would reduce proteolysis. Appx38. Second, the Board found that the POSA would have known that temperature could affect binding capacity. Appx38, Appx46–47.

Next, the Board found that it was "well known to regulate chromatography column temperature," and thus concluded that the dependent claims would have been obvious because they were "not more than routine experimentation." Appx39. With respect to the selection of the inventors' research for presentation at National Meeting of the

American Chemical Society, the Board minimized the significance of this evidence because, among other things, Genentech had not shown "why the meeting was particularly prestigious." Appx40.

This appeal followed.

SUMMARY OF THE ARGUMENT

The Board's final written decision misconstrued the claims, misapprehended the facts, and misapplied the law.

I. The Board's construction of "about 18°C" to embrace temperatures up to 21°C is unreasonable and incorrect. The specification makes clear that one embodiment of the invention involves the temperature of the composition being "reduced below room temperature, for instance in the range from about 3°C to about 20°C, e.g. from about 10° C. to about 18° C." This disclosure makes clear that the claim language "about 10°C to about 18°C" is a temperature range *below room temperature*. The Board's dismissal of this evidence as merely relating to a "preferred embodiment" elided the issue. Whether preferred or not, the patent's discussion of the temperatures relevant to this appeal makes clear they are "below room temperature." The Board's construction cannot be reconciled with this disclosure.

Correctly construed, "about 18°C" means "approximately 18°C" or 18°C ± 1°C. Under that construction, van Sommeren's disclosure of performing protein A chromatography at "ambient temperature" (20-25°C) cannot anticipate any claim.

II. The Board's determination that the '389 Application anticipates claims 1 and 5 also should be reversed. Although it agreed that this reference does not disclose the temperature of the composition being purified, the Board nevertheless concluded, relying on *Kennametal, Inc. v. Ingersoll Cutting Tool Co.*,[6] that it could "infer" that the composition being purified had equilibrated to "room temperature" (described broadly in the '389 Application as 18–25°C). This conclusion reflects yet another misapplication of *Kennametal* by the Board, *see Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 851 F.3d 1270 (Fed. Cir. 2017), and should be reversed.

III. The Board's obviousness conclusions all follow from a pair of findings: (1) that temperature would have been understood to be a "result-effective variable;" and (2) that varying temperature would have involved nothing more than "routine experimentation." Because there

[6] 780 F.3d 1376, 1381 (Fed. Cir. 2015).

is no support for combining these findings, the ultimate conclusion of obviousness drawn from them is mistaken.

A. The Board first found that temperature was a "result-effective variable" because the proteolytic cleavage of protein A would have been understood by the POSA to decrease with temperature. This finding is germane *only* to the performance of protein A chromatography for the manufacture of antibodies for human use, because it is only in that context where the presence of a trace contaminant (nanograms of protein A) is relevant. Performing protein A chromatography in this context—the manufacture of therapeutic antibodies—involves thousands of liters of aqueous solution.

On the other hand, the Board's finding that temperature could be controlled "with ease" was premised on evidence about the control of temperature at *laboratory scale*. The Board disregarded the only evidence of record about controlling temperature at the scale relevant to manufacturing therapeutic antibodies, evidence establishing that there is nothing "easy" about chilling thousands of liters of water weighing several tons.

Because the two findings made by the Board address different scales, they cannot be combined to support the Board's ultimate conclusions of obviousness.

B. The Board also found temperature to be a "result-effective variable" based on the disclosure in van Sommeren that temperature could affect the dynamic binding capacity of a column. It was undisputed, however, that in the decade between that publication and the priority date, the intervening art demonstrated how to "routinely" optimize dynamic binding capacity quickly and efficiently by focusing on parameters other than temperature. Neither Hospira nor the Board cited a single reference subsequent to van Sommeren disclosing allegedly "routine" experiments that varied the temperature at which protein A chromatography was conducted. By focusing on the POSA as of 1992, rather than the POSA as of 2003, the Board flouted section 103's requirement that obviousness be assessed "at the time the invention was made."

IV. Finally, the Board's decision should be vacated because it purports to cancel in an inter partes review a patent that issued prior to

the enactment of the America Invents Act. The retroactive application of inter partes review to such a patent is unconstitutional.

STANDARD OF REVIEW

A patent claim is anticipated "only if each and every element is found within a single prior art reference, arranged as claimed." *In re Smith Int'l, Inc.*, 871 F.3d 1375, 1381 (Fed. Cir. 2017) (internal quotation marks omitted). Anticipation is a question of fact the Court reviews for substantial evidence, *id.*, although claim constructions on which the Board relied, where based on intrinsic evidence, are reviewed *de novo*, *In re Power Integrations, Inc.*, 884 F.3d 1370, 1375 (Fed. Cir. 2018), as are the Board's interpretation and application of controlling law, *Arendi S.A.R.L. v. Apple Inc.*, 832 F.3d 1355, 1357, 1366–67 (Fed. Cir. 2016).

Accordingly, reversal is required where a finding of anticipation rests on an erroneous claim construction or misapplication of the law. *In re Power Integrations,* 884 F.3d at 1378 (reversing anticipation finding based on erroneous construction); *Nidec Motor*, 851 F.3d at 1274–75 (reversing anticipation finding premised on misapplication of precedent).

This Court reviews the Board's "ultimate determination of obviousness de novo and its underlying factual determinations for substantial evidence." *Pers. Web Techs., LLC v. Apple, Inc.*, 848 F.3d 987, 991 (Fed. Cir. 2017). Under "substantial evidence" review, this Court reverses Board determinations of obviousness that rely on an erroneous construction of the claim language, *see, e.g.*, *In re Smith Int'l, Inc.*, 871 F.3d at 1384, or a misapplication of the law, *see Arendi S.A.R.L.*, 832 F.3d at 1357, 1366–67, or where the Board credited conclusory expert testimony, *see*, *e.g.*, *DSS Tech. Mgmt., Inc. v. Apple Inc.*, 885 F.3d 1367, 1376 (Fed. Cir. 2018) (reversing obviousness finding based on conclusory paragraphs of expert declaration), or rejected argument with insufficient analysis, *see, e.g.*, *In re Nuvasive, Inc.*, 842 F.3d 1376, 1383 (Fed. Cir. 2016) ("[I]t is not adequate to summarize and reject arguments without explaining why the PTAB accepts the prevailing argument."). "Substantial evidence is something less than the weight of the evidence but more than a mere scintilla of evidence. In reviewing the record for substantial evidence, [the Court] must take into account evidence that both justifies and detracts from

the factual determinations." *In re Kotzab*, 217 F.3d 1365, 1369 (Fed. Cir. 2000) (citations omitted).

ARGUMENT

I. THE BOARD RELIED ON AN ERRONEOUS CONSTRUCTION OF "ABOUT 18°C" TO FIND THAT VAN SOMMEREN ANTICIPATES CERTAIN CLAIMS.

It is undisputed that van Sommeren disclosed protein A chromatography conducted in a laboratory at "ambient temperature (AT) (20-25°C)." Appx570. Claim 1, on the other hand, requires purifying a chilled composition having a temperature in the claimed range of "about 10°C to about 18°C."

The Board's anticipation finding rests on its erroneous construction that "about 18°C" means "18 ± 3°C, such that the upper bound of 'a temperature in the range from about 10°C to about 18°C' is 21°C." Appx15. Because the Board erroneously construed this claim language, the anticipation finding should be reversed. *In re Power Integrations*, 884 F.3d at 1378.

A. There Is No Basis to Depart from the Plain and Ordinary Meaning of "About."

Claim construction necessarily begins with the language of the claims. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005) (*en*

banc). The pertinent claim term here, "about," is ubiquitous in patent claims. As this court has repeatedly held, its ordinary meaning of "approximately" should be applied unless the "technologic and stylistic context" requires otherwise. *Pall Corp. v. Micron Separations, Inc.*, 66 F.3d 1211, 1217 (Fed. Cir. 1995) (range of "about 5:1 to about 7:1" means "approximately" within those endpoints); *Merck & Co., Inc. v. Teva Pharms. USA, Inc.*, 395 F.3d 1364, 1369-70 (Fed. Cir. 2005). Put another way, "about" should be construed as "approximately" unless it is "defined either explicitly or by implication by the specification." *Ferring B.V. v. Watson Labs., Inc.*, 764 F.3d 1382, 1389 (Fed. Cir. 2014).

The Board did not identify any such explicit definition in the specification (there is none), nor did it find an "implicit" definition in the only portion of the specification that uses the phrase "about 18°C." Despite having this Court's controlling case law cited to it by Genentech, Appx195–196, the Board simply ignored it. The Board's final written decision does not cite or discuss any of *Pall*, *Merck*, or *Ferring*. Appx11–15. Under the controlling rubric announced in these decisions, the correct construction of "about" is "approximately," and

"approximately 10°C to approximately 18°C" cannot be reasonably construed to add 3°C to each end of the claimed range.

B. The Board's Construction Is Not Reasonable in View of the Specification.

To the extent that "about 18°C" could mean something other than "approximately 18°C" based upon the specification, *Ferring*, 764 F.3d at 1389, it would mean no more than "18 ±1°C." This conclusion necessarily flows from how the specification uses the phrase "about 18°C" in the only place it appears:

> Preferably, the method comprises reducing the temperature of the composition subjected to the protein A affinity chromatography, e.g. where the temperature of the composition is *reduced below room temperature*, *for instance* in the range from about 3° C. to *about 20° C.*, e.g. from about 10° C. to *about 18° C.*

Appx68 (emphasis added). In this context, the temperature "about 20°C" is described as being a temperature that is "below room temperature." "Room temperature" is commonly understood to span 21°C to 25°C, i.e., 69–77°F. Appx1346–1350, Appx1600. Thus, to the extent that "about" is being defined by implication here, it must mean no more than ±1°C.

The Board's consideration of this passage of the specification—the only one that uses the phrase "about 18°C"—misapplies basic principles

of claim construction. The Board noted that this passage describes a preferred embodiment and, on that basis, ignored it because "claims are not necessarily and not usually limited in scope simply to the preferred embodiment." Appx12.

That was error. Whether a claim is limited to a preferred embodiment is a different question from whether the language used in describing that embodiment sheds light on the meaning of a claim term. Whether this passage describes a preferred embodiment or not, it states clearly that "about 20°C" and "about 18°C" are temperatures "below room temperature." Under *Ferring*, the only reason to consult the specification in construing "about" is to see whether it has been defined by implication different from its ordinary meaning. To the extent the specification could include such a definition-by-implication, it must be in this passage, and the guidance it provides contradicts the construction the Board announced.

As for the Board's reliance on the principle that claims should not be limited to a preferred embodiment, that makes no sense in this particular case where claim 1 is explicitly limited to the preferred embodiment's temperature range of "about 10°C to about 18°C." Even

Hospira's expert acknowledged that the claimed invention was directed to the preferred embodiment of purifying a composition that was "below room temperature." Appx1583. Surely the specification's description of an embodiment is relevant evidence of the construction to be given to that embodiment when it is claimed. The Board was simply wrong in concluding that including the phrase "preferred embodiment" in a passage of the specification renders it irrelevant to claim construction. *Astrazeneca AB, Aktiebolaget Hassle, KBI-E, Inc. v. Mut. Pharm. Co.*, 384 F.3d 1333, 1339–41 (Fed. Cir. 2004).

Disregarding this passage of the specification, the Board relied instead on the disclosure that the inventors' full-scale experiments applying the claimed method to 12,000 liters of cell culture fluid could only hold such a mass of liquid at "15 ± 3°C." Appx14, Appx70. The specification does not refer to this as "about" 15°C, and the context of this passage belies the Board's reliance on it. A composition chilled to "15 ± 3°C" falls completely within the claimed range of "about 10°C to about 18°C." The results of the full-scale experiments do not purport to demonstrate that the invention works at 21°C—they demonstrate that

the invention works when the composition's temperature is maintained within the range of "about 10°C to about 18°C."

The Board also disregarded that this passage uses ± 3°C to define the variance in temperature around a single point (15°C), not the endpoints of a range. In this context, ± 3°C means that the temperature range of the 12,000 liters of liquid was 12°C to 18°C, not that either 12°C or 18°C is "about" 15°C.

Simply put, to the extent the specification illuminates the disputed claim language, it establishes that "about 18°C" must be "below room temperature." It is inconsistent with the specification and this Court's many precedents on the construing claim language to find "about 18°C" to capture a temperature—21°C—that is within the consensus definition of room temperature.

C. The Board's Reliance on the Prosecution History Was Erroneous.

The Board also concluded that its "broad construction" of "about 18°C" was supported by a series of exchanges between Genentech and the Patent Office during the prosecution of a parent to the '799 patent. Appx14–15.

As an initial matter, because the ordinary meaning of "about" and the specification's use of the phrase "about 18°C" are both *narrower* than the construction the Board derived from the prosecution history, the Board's reasoning necessarily fails. The prosecution history "cannot trump the plain language of the claims and the direct teaching of the specification." *Telcordia Techs., Inc. v. Cisco Sys., Inc.*, 612 F.3d 1365, 1375 (Fed. Cir. 2010). As the *en banc* Court explained in *Phillips*, the file history should be consulted to see "whether the inventor limited the invention in the course of prosecution, making the claim scope *narrower* than it would otherwise be." *Phillips*, 415 F.3d at 1317 (emphasis added). There is no basis for resorting to the file history to *broaden* the meaning of a term beyond what it would otherwise be in view of its ordinary meaning and the specification.

Factually, the Board's analysis is equally unsound. The Board described Genentech as amending the upper limits of its claim from "20 °C" to "about 20 °C" and then to "about 18 °C" to overcome art "at 22 °C," Appx14, indicating (in the Board's view) that "about" must mean at least ±2 °C but less than ±4 °C. Appx14. In fact, the upper bound of the proposed claim was amended to "about 18 °C" to overcome art

understood to be at "*about* 22 °C," *see* Appx716, Appx725, Appx733–734, Appx745–746 (emphasis added), not (as the Board stated) "at 22 °C." Appx14. "About" therefore cannot be ± 3 °C, otherwise "about 22 °C" would overlap with "about 18 °C." Genentech during prosecution also made it clear that it disagreed with the Examiner's rejections. Appx197–198. The file history has a role in claim construction because it "can often inform the meaning of the claim language by demonstrating *how the inventor understood the invention*[.]" *Phillips*, 415 F.3d at 1317 (emphasis added). Genentech made clear that it did *not* understand the invention in the manner suggested by the Examiner. While the Board took the position that "self-serving statements in the prosecution history" are "accord[ed] little weight," Appx15, it cited no precedent authorizing it to simply disregard what an applicant says during prosecution.

D. Under the Correct Construction of "About 18°C," van Sommeren Does Not Anticipate.

The Board held that van Sommeren anticipated claims 1, 2, and 5 because it disclosed purifying HCCF "at ambient temperature, defined therein as from 20°C to 25°C, and which overlaps with our construction of 'about 18°C' as having an upper bound of 21°C." Appx26–27. It is

undisputed that, were "about 18°C" construed as Genentech has urged, van Sommeren would not anticipate. *See* Appx570. Because Genentech's construction is the correct construction, the Board's ruling with respect to anticipation by van Sommeren should be reversed.

II. THE BOARD ERRED BY "INFERRING" THAT THE '389 APPLICATION DISCLOSED THE TEMPERATURE OF THE COMPOSITION BEING PURIFIED.

The '389 Application disclosed an industrial process for purifying a therapeutic antibody in which "[a]ll steps are carried out at room temperature (18-25°C)." Appx522. Both parties' experts agreed that this statement expressly disclosed only the temperature of the *laboratory* where the experiments were conducted, not the temperature of the *composition* being purified. *See* Appx1350–1353, Appx1547–1548. And the Board agreed that the '389 Application "does not expressly call out the temperature of the [harvested cell culture fluid]," i.e., the composition being purified. Appx20.

The Board nevertheless concluded that it could "infer" that the temperature of the composition being purified had equilibrated to "room temperature (18-25°C)," and on that basis held claims 1 and 5 anticipated. Appx20–22. The Board cannot base its anticipation

determination on "improperly assuming disclosure of a claim element." *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd*, 851 F.3d 1270, 1274 (Fed. Cir. 2017); *see also Eli Lilly & Co. v. Los Angeles Biomedical Research Inst. at Harbor-UCLA Med. Ctr.*, 849 F.3d 1073, 1074 (Fed. Cir. 2017) ("[T]he question is not whether a prior art reference 'suggests' the claimed subject matter."). Because the Board's ruling is based on a misapplication of law, it should be reversed.

A. The '389 Application Does Not Disclose the Temperature of the Composition Being Purified.

Both experts agreed that the cell culture fluid described in the '389 Application would have been approximately 37°C when harvested from the bioreactor. Appx1531, Appx1350–1353. Both experts also agreed that the '389 Application does not disclose how long the cell culture fluid was held before being subjected to protein A chromatography or under what conditions. Appx1554–1555, Appx1350–1353. And both experts agreed again that the POSA would not leave harvested cell culture fluid sitting out because doing so could affect product quality. Appx1352, Appx2296. The import of this undisputed testimony is that even if the laboratory was at room temperature, the cell culture fluid need not have been, any more than

the fact that a judge's chambers is at room temperature means that the cup of coffee on her desk necessarily is as well. Not surprisingly, when pressed in deposition as to whether the cell culture fluid in the '389 Application would inevitably have equilibrated to "room temperature," Hospira's expert conceded the point, and did so unambiguously:

> Q. It's not inevitable that it's at room temperature, correct?
>
> A. That's correct.

Appx1555.

Despite this record, the Board nevertheless credited the rebuttal declaration testimony of Hospira's expert that "a POSA would understand that experiments are being conducted at ambient temperature with all materials equilibrated," Appx21, notwithstanding his prior deposition testimony to the contrary. Quoting *Kennametal, Inc. v. Ingersoll Cutting Tool Co.*, the Board reasoned that a reference can anticipate "even if it does not expressly spell out" the claim's limitations so long as the POSA could "at once envisage" the claimed invention. Appx17. As a result, the Board found that temperature range claimed in the '799 patent and the range disclosed in the '389 Application overlapped and that claims 1 and 5 were anticipated.

The Board has, once again, misinterpreted the distinction between what a reference discloses and what the POSA could "envision" based upon a disclosure. *See*, *e.g.*, *Nidec Motor Corp.*, 851 F.3d at 1274–75; *Goeddel v. Sugano*, 617 F.3d 1350, 1356 (Fed. Cir. 2010). Simply put, "*Kennametal* does not permit the Board to fill in missing limitations simply because a skilled artisan would immediately envision them." *Nidec Motor*, 851 F.3d at 1274–75.

As explained in *Nidec Motor*, *Kennametal* concerned a "prior art reference [that] disclosed five binding agents (one of which was ruthenium) and three coating agents (one of which was PVD)." *Id.* at 1274. There was substantial evidence to support the anticipation finding in *Kennametal* because the patent at issue claimed ruthenium and PVD, one of the fifteen combinations "effectively taught" in the prior art. *Id.*

But the Court went on to warn against reading the quoted language in *Kennametal* too broadly:

> *Kennametal* does not stand for the proposition that a reference missing a limitation can anticipate a claim if a skilled artisan viewing the reference would "at once envisage" the missing limitation. Rather, *Kennametal* addresses whether the disclosure of a limited number

> of combination possibilities discloses one of the possible combinations.

Id.

Here the Board repeated the same mistake. This is not a case involving "a limited number of combination possibilities." It is a case in which the Board concluded that the reference "does not expressly call out the temperature of the HCCF," Appx20, where both experts agreed that the composition was not necessarily at "room temperature," yet nevertheless decided that it could "infer that during the washing step, the entrained composition is also at 18-25°C, and, thus, within the temperature range of claim 1[.]" Appx22. That is a legally inappropriate analysis of the issue of anticipation premised on a misreading of *Kennametal*. It should be reversed.

B. The Board's Reliance on Dr. Przybycien's Rebuttal Declaration Testimony over His Deposition Testimony Is Not "Substantial Evidence."

To the extent that this Court agrees that claims 1 and 5 may be anticipated by the '389 Application if the POSA would "envisage" that the cell culture fluid being purified had equilibrated to room temperature, the Board's finding that the POSA would have interpreted

the '389 Application in that manner is not supported by substantial evidence.

As explained above in section II.A, the Board cited Hospira's expert's rebuttal declaration to support this finding, despite his having testified in deposition that it was not inevitable that the cell culture fluid would have equilibrated to room temperature. The Board nowhere attempted to explain how it could reconcile Dr. Przybycien's contradictory testimony, *see* Appx21, acting instead as though he were never deposed.

The Board's failure to explain how it resolves this contradiction precludes the conclusion that its finding is supported by "substantial evidence." As this court held in *Kotzab*, "[i]n reviewing the record for substantial evidence, [the Court] must take into account evidence that both justifies and detracts from the factual determinations." 217 F.3d at 1369. Because the Board ignored the expert's contradictory deposition testimony, its finding is not supported by substantial evidence and must be reversed.

III. THE BOARD ERRED IN ITS OBVIOUSNESS ANALYSIS.

Grounds 3-8 of Hospira's Petition alleged obviousness over the '389 Application alone (ground 3), van Sommeren alone (ground 7), and in combinations with various references (grounds 4–6, 8). The Board discussed grounds 3 (Appx38–39) and 7 (Appx46–47), and then relied on that analysis in discussing the other grounds. *See* Appx42 (ground 4), Appx43 (ground 5), Appx44 (ground 6), Appx48 (ground 8).

With respect to obviousness over the '389 Application alone, the Board concluded that temperature was a "result-effective variable" based on (1) the finding that leaching is caused by proteolysis of protein A and that the rate of proteolysis depends upon temperature; and (2) that temperature could be "routinely optimized." Appx38–39.

With respect to obviousness over van Sommeren alone, the Board concluded that temperature was a "result effective variable" because van Sommeren had shown it could impact dynamic binding capacity. Appx46–47. The Board then referenced its prior analysis regarding the '389 Application, presumably meaning to reference its conclusion that temperature was a variable that could be "routinely optimized." Appx47.

In short, the Board's rulings of obviousness on each of these six grounds all distill to its conclusion that the challenged claims would have been the obvious result of "routine optimization." The invocation of "routine optimization" alone is not sufficient to hold claims obvious. The Board must explain *why* that conclusion follows from the record. *In re Stepan Co.*, 868 F.3d 1342, 1346 (Fed. Cir. 2017). The Board's conclusions of obviousness based on "routine optimization" here were mistaken and should be reversed.

A. The Board's Conclusion that the POSA Would Have "Routinely Optimized" Temperature to Reduce Proteolysis is Not Supported by Substantial Evidence.

The Board's conclusion of obviousness based on "routine optimization" of the process in the '389 Application proceeded in two steps. First, the Board determined it was known that protein A leaching was caused by proteolysis and therefore could be affected by the temperature of the solution containing the proteolytic enzyme. Appx38. Second, the Board concluded that "given the ease with which temperature can be varied, it would have been obvious to try conducting protein A temperature at the claimed range," such that "exploring the temperature dependence of protein A leaching is not more than routine

experimentation." Appx39 (internal quotation marks and alterations omitted).

The Board's ultimate conclusion of obviousness, which the Court reviews de novo, *Arendi S.A.R.L.*, 832 F.3d at 1360–61, should be reversed because it rests on an improper combination of findings made at two different scales: (1) that temperature is a "result-effective variable" because it might reduce leaching in an industrial purification process; and (2) that temperature is "easy" to control in a lab-scale process.

1. The amount of protein A that leaches from a protein A column is literally measured in nanograms. Appx54, Appx60, Appx61, Appx70, Appx71, Appx1453–1454. Given this miniscule amount, its presence matters *only* in the industrial production of therapeutic antibodies, where concerns about product purity require the elimination of leached protein A. Appx1337–1338, Appx1375, Appx1607. As Genentech's expert Dr. Cramer explained, for non-clinical uses it is unnecessary to remove the protein A that leaches during this chromatography step. Appx1375. Hospira's expert, Dr. Przybycien,

agreed that the POSA would have been focused on developing a protein A chromatography process "for commercial scales." Appx1607.

2. In finding that controlling temperature was "eas[y]," the Board cited Dr. Przybycien's discussion of laboratory-scale experiments. But the record is undisputed that controlling temperature at industrial/commercial scales is anything but easy. Appx220, Appx1375–1377. As the patent makes clear and as Dr. Cramer explained, at commercial scale the volume of liquid involved is staggering, on the order of 12,000 liters. Appx70, Appx1375–1376. As a matter of basic physics, chilling that volume of liquid by about 20°C[7] is challenging. Appx1375–1377.

The Board ignored this explanation from Dr. Cramer. Appx39. To support the "ease with which temperature can be varied," the Board (quoting from Hospira's brief) cited a single paragraph of Dr. Przybycien's declaration, of which a single, conclusory sentence addresses temperature:

> In addition, proteolytic degradation and leaching of protein A were known problems. (Ex. 1003, WO '389 at

[7] Antibodies are made using mammalian cells grown in culture. It was undisputed that the cell culture fluid that comes out of the bioreactor is warm, approximately 37°C. Appx1531, Appx1350–1353.

> 14-15). In my opinion, a POSA would have known, based on the general knowledge available to those skilled in the art, that reactions such as proteolysis are temperature dependent, and that decreasing the temperature would decrease proteolysis. *Temperature is one of the easiest conditions to modulate in the context of protein A chromatography, for example, by using conventional refrigeration.* It would have been obvious to try temperatures at the claimed range, and observe whether varying the temperature affected protein A leaching and/or proteolysis.

Appx461 (emphasis added). Whatever "conventional refrigeration" is intended to mean, it cannot support a finding that it would have been "easy" to control the temperature of thousands of liters of cell culture fluid at an industrial scale.

The Board then cited two further paragraphs of Hospira's expert's declaration testimony in support of its "routine optimization" conclusion. The first speaks to the difficulty (and undesirability) of controlling temperature at laboratory scale only, and actually emphasizes the difficulty of controlling temperature:

> It is rare for *a lab room* to be maintained at about 10°C to about 18°C, because *laboratory spaces* are commonly designed for either room temperature operation, or refrigeration temperature operation. Maintaining an intermediate temperature during chromatography, such as about 10° C to about 18° C, could be accomplished by refrigerating the harvested cell culture fluid (HCCF) and buffers used during

> chromatography, or using a jacketed column *in a room temperature laboratory*. Both of these technologies are old, and were commercially available well before 2003. Persons of ordinary skill in the art have known for decades how to control the temperature of chromatography columns using conventional technology, which is why the '799 Patent provides no instructions for reducing temperature. But, skilled artisans may have chosen not to use reduced temperatures, because it requires additional lab equipment such as jacketed columns, or raises operating costs, or may simply be uncomfortable or inconvenient to laboratory personnel.

Appx424 (emphasis added).

The other paragraph the Board cited came from Dr. Przybycien's reply declaration. Appx39. Because Genentech's response had emphasized that temperature was not a variable that could be controlled easily at industrial scale, and thus would not have been the target of "routine optimization" efforts, Hospira's expert provided the following reply:

> Dr. Cramer seems to argue that because temperature control in the industrial context involves some expense and effort, that practitioners would never have attempted it. Again, Dr. Cramer has addressed only *industrial* purification, even though the '799 Patent and its claims are not so limited. Furthermore, his argument suggests that experimentation must be as trivial as using one type of off-the-shelf equipment in order to be considered routine. (Ex. 2008, Cramer Decl. at ¶ 138.) However, Dr. Cramer admitted that

> *laboratory tools for controlling temperature would have been available to a POSA* before 2003. (Ex. 1022, Cramer Dep. at 200:5-12.) For example, the temperature of the chromatography column or other implements could be readily controlled using commercially available refrigerated spaces, water baths and jacketed columns—tools designed for the sole purpose of facilitating temperature control by POSAs who desired this.

Appx977 (emphasis added). Dr. Przybycien notably did not dispute Dr. Cramer's testimony regarding the difficulty of controlling temperature at industrial scale; he merely reiterated his position that temperature could be controlled in the laboratory setting.

None of this testimony from Hospira's expert supports a finding that the temperature of the cell culture fluid could be routinely controlled at industrial scales. But that is the finding the Board needed to make to justify its conclusion of obviousness. Because the POSA's desire to reduce protein A leaching applies only to the industrial manufacture of therapeutic antibodies, the finding that temperature is a variable that can be "routinely optimized" must also be made at the scale. After all, it would be pointless to develop a process at lab scale that could not be implemented at the industrial scale for which it was intended. Appx1375–1377.

Elsewhere in its decision, the Board suggested that the challenged claims are not "limited to the large scale industrial processes envisioned by Dr. Cramer," Appx20 suggesting the Board's indifference to the relevance of scale. That misses the point. The only reason the Board gave for the POSA to experiment with temperature—to reduce protein A leaching—is valid only in an industrial setting where antibodies are produced for therapeutic use. But the manner in which the Board held the POSA would have conducted such "routine optimization" experiments is valid only in the laboratory setting. *Cf. Institut Pasteur & Universite Pierre Et Marie Curie v. Focarino*, 738 F.3d 1337, 1349 (Fed. Cir. 2013). While the Board's findings might individually be owed deference, these two findings cannot be combined, and the conclusion of obviousness the Board drew from them should be reviewed *de novo* and reversed.

B. The Board's Conclusion that the POSA Would Have "Routinely Optimized" Temperature to Improve "Dynamic Binding Capacity" Disregarded the Art "at the Time the Invention Was Made."

The Board's analysis of obviousness in view of van Sommeren (Grounds 7 and 8, Appx44–48) added a second rationale for why the claims would have been the obvious result of "routine optimization":

the POSA, familiar with this 1992 publication, would have been motivated to improve the "dynamic binding capacity" of the protein A column.

But the Board's finding here completely ignored Genentech's evidence that as of the time of the invention—the provisional application was filed in July 2003—advances in the art provided the POSA a framework for optimizing dynamic binding capacity, and that framework did not include varying temperature. Indeed it discouraged doing so. Appx1328–1331, Appx1362–1363, Appx1379, Appx215–216.

The Fahrner Paper published in 1999 demonstrated that dynamic binding capacity was easily optimized by varying the column's length and flow-rate. Appx1329–1330. Both of those variables, but not temperature, could be studied easily using lab-scale chromatography equipment. Appx1334–1336.

The Fahrner Review published in 2001 surveyed the field's literature and concluded that, with the respect to protein A chromatography parameters, "the simplest to control for production and the ones that will have the most significant impact on capacity are the column length, the flow rate, and the chromatography media," not the

temperature of the composition being purified. Appx1294, Appx1329–1330. A contemporaneous paper from 2003 comparing and contrasting fifteen different types of protein A media chose to optimize the protein A chromatography procedure based only on flow rate, though it acknowledged that column geometry (i.e., length) could also have been pertinent. Appx1330–1331.

Given these teachings, it was not surprising that Hospira's expert testified as follows:

> Q. Are you aware of anyone prior to 24 July of 2003 doing protein A chromatography at a reduced temperature using a jacketed column and/or a chilling tank?
>
> A. I have not seen that.

Appx1666–1668. Dr. Przybycien could not identify anyone having performed such allegedly "routine" work as of the priority date because, as all of the literature cited above shows, varying temperature was not a routine method for optimizing dynamic binding capacity as of the priority date.

The Board's conclusion of obviousness based on van Sommeren, and without discussing any of the literature above, was legally erroneous for failing to consider the obviousness of the claimed methods

"at the time the invention was made." 35 U.S.C. § 103(a); *Institut Pasteur*, 738 F.3d at 1349. The undisputed record that the POSA in 2003 had no motivation to make temperature adjustments to improve binding capacity plainly trumps any suggestion to the contrary that might be gleaned from a publication eleven years earlier. *See* Appx572.

The Court in *Leo Pharmaceutical Products, Ltd. v. Rea*, 726 F.3d 1346 (Fed. Cir. 2013), addressed essentially the same issue. In *Leo*, the Board concluded that the claimed compositions would have been "obvious to try" based on articles published several years before the priority date. *Id.* at 1356–57. Reversing the Board's conclusion of obviousness, the Court explained that the claimed invention "was not obvious to try. Indeed this considerable time lapse [between the art and the time of the invention] suggests instead that the Board only traverses the obstacles to this inventive enterprise with a resort to hindsight." *Id.* at 1356.

The Board made the same mistake here. It concluded that the claimed methods would have been discovered through "routine optimization," even though (1) the art taught different ways to optimize the variable at issue and actually discouraged temperature

adjustments; and (2) the laboratory equipment for performing automated optimization of protein A chromatography processes could not optimize on this variable. Appx1312, Appx1293–1294, Appx1335, Appx1446, Appx1596–1597. Presumably, a "routine" development process is one that has been performed dozens of times. It defies the English language for the Board to call "routine" something the cited art discloses was done only once (van Sommeren), more than a decade before the priority date and never again in the intervening years. The fact that the Board supported its conclusion about "routine" efforts with a reference predating the invention by more than a decade in this fast-moving field suggests that, just as in *Leo*, the Board's conclusion is grounded in hindsight, not an objective review of the record. It should be reversed.

C. The Board's "Routine Optimization" Conclusions Cannot Be Reconciled with the Objective Evidence Concerning Genentech's Research.

As this Court has observed, "evidence of secondary considerations may often be the most probative and cogent evidence in the record. It may often establish that an invention appearing to have been obvious in

light of the prior art was not." *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538 (Fed. Cir. 1983).

After Genentech applied for the '799 patent, it shared the results of its research with the world, in the form of being selected to present the research at the American Chemical Society's National Meeting in 2005. Appx1391–1393, Appx1397, Appx1436–1438, Appx1754, Appx2111. As Dr. Cramer, one of the session chairs of the meeting, explained, the process of being selected to present at ACS is competitive, and the purpose of the meeting is "to learn about cutting edge developments in our field." Appx1391–1392.

Against that backdrop, the undisputed fact that the research embodied by the '799 patent was selected "as worthy of the time of the other attendees at the conference" is powerful, objective, contemporaneous evidence of the non-obviousness of the claimed methods. Yet despite this Court's time-honored guidance from *Stratoflex*, the Board gave this evidence short shrift. It reasoned that "Patent Owner does not establish *why* the presentation was selected." Appx40. Respectfully, Genentech made precisely that showing by proffering the testimony of one of the two people involved in selecting it.

Appx1391–1392. The Board next complained that, "Patent Owner does not establish . . . why the meeting was particularly prestigious." Appx40. Again, Genentech submitted the testimony of both Dr. Cramer and Genentech's head of purification, Dr. Dowd, explaining that the American Chemical Society's national meeting is one of the most prestigious meetings in the field. Appx1392, Appx1437–1438. The Board then faulted Genentech for not having established "how the presentation was received by attendees, or whether the disclosure has been relied on by others in the field, or been the subject of recognition or praise since the 2005 presentation." Appx40. While it is true that Genentech was not able to find an attendance record for the conference and survey the recipients, such evidence is not required to recognize the point. The point is that Hospira alleged, and the Board found, that Genentech's research was nothing more than product of "routine optimization." The objective, contemporaneous fact that such research was selected for presentation at ACS's national meeting belies that it was "routine" work. Appx1392, Appx1437–1438. This objective fact must be considered in the obviousness analysis, *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1334 (Fed. Cir. 2016); *see also Apple Inc. v.*

Samsung Elecs. Co., 839 F.3d 1034, 1052–53 (Fed. Cir. 2016), and it completely undermines the Board's conclusion that the claimed methods would have been the obvious result of "routine optimization." *In re Stepan Co.*, 868 F.3d at 1346.

IV. THE RETROACTIVE APPLICATION OF INTER PARTES REVIEW IS UNCONSTITUTIONAL.

The '799 patent issued on October 15, 2010, prior to the enactment of the Leahy-Smith America Invents Act ("AIA"), Pub. L. No. 112-29, 125 Stat. 284 (2011), establishing the inter partes review procedure. The retroactive application of inter partes review to a patent issued before that procedure existed is unconstitutional, a taking without just compensation and a denial of due process, and for this additional reason the Board's findings should be reversed.

Oil States Energy Servs., LLC v. Greene's Energy Grp., LLC, 138 S. Ct. 1365 (2018), the Supreme Court's recent decision upholding the constitutionality of inter partes review, preserved the retroactivity argument presented here. The Supreme Court "emphasize[d] the narrowness of [its] holding," *id.* at 1379, made it clear that *Oil States* "address[ed] only the precise constitutional challenges . . . raised [in that case]," *id.*, and expressly reserved the question Genentech now

raises, "challeng[ing] the retroactive application of *inter partes* review" to patents that issued prior to the AIA's enactment, *id.*

The Fifth Amendment prohibits "private property . . . taken for public use, without just compensation." U.S. Const. amend. V. Patents have long been considered property for purposes of the Takings Clause. *See Fla. Prepaid Postsecondary Educ. Expense Bd. v. Coll. Sav. Bank*, 527 U.S. 627, 642 (1999). "A patent confers upon the patentee an exclusive property in the patented invention which cannot be appropriated or used by the government itself, without just compensation[.]" *Horne v. Dep't of Agric.*, 135 S. Ct. 2419, 2427 (2015) (citations, brackets, and internal quotation marks omitted)). In evaluating whether a taking occurred, Courts consider factors such as whether a regulation "interfere[d] with reasonable investment-backed expectations." *Id.*

The Supreme Court has previously recognized that passage of a law that retroactively eliminated a claim for patent infringement "would seem to raise a serious question as to the constitutionality of the act . . . under the Fifth Amendment to the Federal Constitution." *Richmond Screw Anchor Co. v. United States*, 275 U.S. 331, 345 (1928);

see also E. Enterprises v. Apfel, 524 U.S. 498, 528–29 (1998) (explaining that for takings analysis, "legislation might be unconstitutional if it imposes severe retroactive liability on a limited class of parties that could not have anticipated the liability").

Here, the termination of Genentech's patent rights, based on legislation enacted after issuance, and without compensation, interferes with its investment-backed expectations and is an unconstitutional taking. *See Horne*, 135 S. Ct. at 2427; *see also Richmond Screw Anchor Co.*, 275 U.S. at 345. Genentech pursued the '799 patent based upon settled expectations at the time that did not include being subject to the subsequently enacted inter partes review process. Issuance of a patent comes at a cost to the patentee, most importantly the public disclosure of a discovery the patentee might otherwise have kept secret. "The disclosure required by the Patent Act is the *quid pro quo* of the right to exclude." *J.E.M. Ag Supply, Inc. v. Pioneer Hi–Bred Int'l, Inc.*, 534 U.S. 124, 142 (2001) (internal quotation marks omitted). "Fundamental alterations in [the patent rules] risk destroying the legitimate expectations of inventors in their property." *Festo Corp. v.*

Shoketsu Kinzoku Kogyo Kabushiki Co., 535 U.S. 722, 739 (2002).[8] By cancelling Genentech's earlier issued patent and putting that technology into the public domain, these AIA procedures constitute an unconstitutional taking.

This Court's previous opinion upholding the constitutionality of ex parte reexamination retroactively, *Patlex Corp. v. Mossinghoff*, 758 F.2d 594 (Fed. Cir. 1985), does not foreclose this question. Not only is inter partes review critically different from ex parte reexaminations, but *Oil States* explicitly recognized and left open this issue.[9] *See Oil States Energy Servs.,* 138 S. Ct. at 1379.

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[8] In *Oil States*, the Supreme Court drew on the 18th-century English tradition of petitioning the Privy Council to cancel a patent, which it explained "closely resembles inter partes review." 138 S. Ct. at 1377. Based on this practice the Court concluded that "it was well understood at the founding that a patent system could include a practice of granting patents subject to potential cancellation in the executive proceeding of the Privy Council." *Id.* Notably, however, patents subject to Privy Council review contained a "standard revocation clause" that permitted the Privy Council to declare the patent void, *id.*, eliminating any question when the patent was issued that the Privy Council could cancel it. *See* Davies, *The Early History of the Patent Specification*, 50 L.Q. Rev. 86, 103 (1934) (cited at *Oil States Energy Servs.*, 138 S. Ct. at 1377). Nothing similar exists in U.S. patent law to justify retroactive inter partes review.

[9] Even though the inter partes *reexamination* process existed at the time this patent issued, the inter partes *review* process, which replaced

CONCLUSION

For the foregoing reasons, Genentech respectfully requests that the Board's determination be reversed.

AUGUST 17, 2018

Respectfully submitted,

/s/ Paul B. Gaffney

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it, is considerably different. *See SAS Inst., Inc. v. Iancu*, 138 S. Ct. 1348, 1355 (2018) (comparing inter partes reexamination's "inquisitorial process for reconsidering patents" with inter partes review's "party-directed, adversarial process"). Inter partes review has "many of the usual trappings of litigation," as parties conduct discovery, brief issues, and argue at an oral hearing. *Id.* at 1354. Unlike civil litigation, however, the inter partes review process lacks certain procedural safeguards; for example, a petitioner only need prove her case that a patent is invalid by a "preponderance of the evidence." 35 U.S.C. § 316(e).

PROOF OF SERVICE

I, Paul B. Gaffney, counsel for appellant and a member of the Bar of this Court, certify that, on August 17, 2018, a copy of the attached Brief of Appellant was filed with the Clerk and served on the parties through the Court's electronic filing system. I further certify that all parties required to be served have been served.

/s/ Paul B. Gaffney
PAUL B. GAFFNEY

CERTIFICATE OF COMPLIANCE WITH TYPEFACE AND WORD-COUNT LIMITATIONS

I, Paul B. Gaffney, counsel for appellant and a member of the Bar of this Court, certify, pursuant to Federal Rule of Appellate Procedure 32(a)(7)(B), that the attached Brief of Appellant is proportionately spaced, has a typeface of 14 points or more, and contains 10,239 words.

/s/ Paul B. Gaffney
PAUL B. GAFFNEY

AUGUST 17, 2018

ADDENDUM

Trials@uspto.gov Paper No. 40
571-272-7822 Entered: March 6, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HOSPIRA, INC.,
Petitioner,

v.

GENENTECH, INC.,
Patent Owner.

Case IPR2016-01837
Patent 7,807,799 B2

Before SHERIDAN K. SNEDDEN, ZHENYU YANG, and ROBERT A. POLLOCK, *Administrative Patent Judges*.

POLLOCK, *Administrative Patent Judge*.

FINAL WRITTEN DECISION
Claims 1–3, and 5–11 Shown to Be Unpatentable
35 U.S.C. § 318(a); 37 C.F.R. § 42.73

I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review challenging the patentability of claims 1–3, and 5–11 (collectively, "the challenged claims") of U.S. Patent No. 7,807,799 B2 (Ex. 1001, "the '799 patent"). We have jurisdiction under 35 U.S.C. § 6. Petitioner bears the burden of proving

unpatentability of the challenged claims, and the burden of persuasion never shifts to Patent Owner. *Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015). For the reasons that follow, we determine that Petitioner has shown, by a preponderance of the evidence, that claims 1–3 and 5–11 of the '799 patent are unpatentable.

A. Procedural History

Hospira, Inc. ("Petitioner") filed a Petition requesting an *inter partes* review of claims 1–3 and 5–11 of the '799 Patent. Paper 1 ("Pet."). Genentech, Inc. ("Patent Owner") expressly waived its opportunity to file a Preliminary Response to the Petition. Paper 6.

Petitioner asserted eight grounds of invalidity based on the following references:

WO 95/22389, published Aug. 24, 1995. Ex. 1003. ("WO '389" or "Shadle").

Van Sommeren et al., "*Effects of Temperature, Flow Rate and Composition of Binding Buffer on Adsorption of Mouse Monoclonal IgG_1 Antibodies To Protein A Sepharose 4 Fast Flow*," 22 Preparative Biochemistry 135 (1992). Ex. 1004. ("van Sommeren").

Joseph P. Balint, Jr. and Frank R. Jones, "*Evidence for Proteolytic Cleavage of Covalently Bound Protein A from a Silica Based Extracorporeal Immunoadsorbent and Lack of Relationship to Treatment Effects*," 16 Transfus. Sci. 85 (1995). Ex. 1005. ("Balint").

Potier et al., "*Temperature-dependent changes in proteolytic activities and protein composition in the psychrotrophic bacterium Arthrobacter globiformis S_155*," 136 J. Gen. Microbiol. 283 (1990). Ex. 1006. ("Potier").

US 6,127,526, issued Oct. 3, 2000. Ex. 1007. ("the '526 Patent").

In view of Petitioner's submission, we instituted an *inter partes* review of the challenged claims on the following grounds:

Ground	Reference(s)	Basis	Claims
1	WO '389	§ 102(b)	1 and 5
2	van Sommeren	§ 102(b)	1, 2, and 5
3	WO '389	§ 103(a)	1 and 5
4	WO '389, Balint, and Potier	§ 103(a)	1–3 and 5
5	WO '389 and the '526 Patent	§ 103(a)	2, 3 and 6–11
6	WO '389, Balint, and Potier, and the '526 Patent	§ 103(a)	2, 3 and 6–11
7	van Sommeren	§ 103(a)	1, 2, and 5
8	van Sommeren and the '526 Patent	§ 103(a)	3 and 6–11

Paper 19, 20–21.

After institution of trial, Patent Owner filed a Patent Owner Response (Paper 22, "PO Resp."), to which Petitioner filed a Reply (Paper 28, "Pet. Reply").

In support of its challenges, Petitioner relies on the Declarations of Todd M. Przybycien, Ph.D. Exs. 1002, 1020. Patent Owner relies on the Declarations of Steven M. Cramer, Ph.D. (Ex. 2008) and Christopher J. Dowd, Ph.D. (Ex. 2009).

Patent Owner filed a motion for observations on the second deposition of Petitioner's expert, Dr. Przybycien (Paper 32) and Petitioner filed a response to that motion (Paper 36).

Oral argument was conducted on November 29, 2017. A transcript is entered as Paper 39 ("Tr.").

B. Related Applications and Proceedings

In the Petition, Petitioner stated that "[t]here are no judicial or administrative matters that would affect, or be affected by, a decision in the proceeding." Pet. 4. Patent Owner subsequently identified the following matters: *Genentech, Inc. v. Sandoz, Inc.*, No. 17-13507 (D.N.J.); *Genentech, Inc. v. Celltrion, Inc.*, No. 18-574 (D.N.J.); *Genentech, Inc. v. Pfizer, Inc.*, No. 17-1672 (D. Del.); *Genentech, Inc. v. Celltrion, Inc.*, No. 18-00095 (D. Del.); *Celltrion, Inc. v. Genentech, Inc.*, No. 18-274 (N.D. Cal.); and *Celltrion, Inc. v. Genentech, Inc.*, No. 18-276 (N.D. Cal.). Paper 38, 2.

C. The '799 Patent

The '799 Patent relates to improved methods for purifying antibodies and other proteins containing a C_H2/C_H3 region by protein A affinity chromatography. *See* Ex. 1001, 7:50–53. The methods involve "separation or purification of substances and/or particles using protein A, where the protein A is generally immobilized on a solid phase" glass, silica, polystyrene, or agarose matrix, such as a chromatography column resin. *Id.* at 4:27–47.

Protein A is a cell wall component of *Staphylococcus aureus* that reversibly binds with high affinity to the amino acids of a C_H2/C_H3 region in an antibody Fc domain. *Id*. at 2:6–11, 2:21–27, 4:20–26, 5:17–28. Although "[p]rotein A affinity chromatography is a powerful and widely-used tool for purifying antibodies," elution of antibodies from the solid phase matrix "leache[s] protein A into the product pool." *Id.* at 20:6–13. Because "protein A ligand is immunogenic . . . it must be cleared from the product pool by downstream processing." *Id.* at 20:13–15.

According to the Specification, "'leaching' refers to the detachment or washing of protein A (including fragments thereof) from a solid phase to

which it is bound." *Id*. at 4:48–50. The invention "concerns a method for reducing leaching of protein A during protein A affinity chromatography by reducing temperature or pH of, or by adding one or more protease inhibitors to, a composition that is subjected to protein A affinity chromatography." *Id*. at 1:15–21. "Preferably, the method comprises reducing the temperature of the composition subjected to the protein A affinity chromatography, e.g. where the temperature of the composition is reduced below room temperature, for instance in the range from about 3° C. to about 20° C., e.g. from about 10 °C. to about 18 °C." *Id.* at 18:4–9. "The temperature of the composition may be reduced prior to and/or during protein A affinity chromatography" and, in a preferred embodiment, involves "lowering the temperature of the harvested cell culture fluid (HCCF) which is subjected to chromatography." *Id.* at 18:9–16.

Example 1 discloses a series of experiments to characterize the temperature dependence of protein A leaching when purifying various proteins from HCCF at different reaction scales. *See id*. at 20:1–24:50. In "small," or "lab scale" experiments, the monoclonal antibody trastuzumab was purified from HCCF protein A affinity columns "at 7 temperature settings (10[], 12, 15, 18, 20, 25, and 30° C.)"; three other antibodies were purified at 10, 20, and 30° C. *Id*. at 20:16–58. In "pilot" scale experiments, trastuzumab HCCF was applied to protein A affinity columns at 10, 12, 15, 18, 20, 25, and 30° C. *Id*. at 20:59–21:3. "The HCCF was stored and chilled in a 400 L-jacketed tank. The temperature of the HCCF was controlled to within 1° C. of the desired temperature," measured prior to application to the protein A column and at the column outlet. *Id*. at 20:60–64. In "full scale" experiments (12,000 liter cell culture), "HCCF was collected and held at 15+/-3° C. for the duration of loading." *Id*. at 21:4–8.

For further context, column diameters ranged from 0.66 cm for small or lab scale columns, to 9 cm for pilot scale columns, and 80 cm for full scale columns. *See, e.g., id.* at 3:15–60, 21:4–8, 23:1–25, 24:1–20.

The Specification concludes that "[t]emperature affects protein A leaching during protein A affinity chromatography of antibodies to varying degrees. Some antibodies are more affected than others; HER2 antibodies Trastuzumab and humanized 2C4 were greatly affected." *Id*. at 24:24–28. "At large scale, Trastuzumab HCCF was chilled to 15+/-3° C. and protein A leaching was controlled to less than or equal to 10 ng/mg." *Id.* at 24:43–45. "At all scales, controlling the temperature of the HCCF during loading could control protein A leaching. Increasing HCCF temperature has an exponentially increasing effect on Protein A leaching." *Id*. at 24:46–50.

Example 2 addresses the use of various protease inhibitors in reducing leaching during protein A affinity chromatography. *Id. at* 24:52–26:66. Of the protease inhibitors tested, EDTA or PEFABLOC were effective in decreasing leaching and increasing concentrations of these compounds resulted in decreasing protein A leaching. *See id*. at 25:56–67.

D. The Challenged Claims of the '799 Patent

Claim 1, the sole independent claim at issue, recites:

> 1. A method of purifying a protein which comprises C_H2/C_H3 region, comprising subjecting a composition comprising said protein to protein A affinity chromatography at a temperature in the range from about 10 ° C. to about 18 ° C.

Id. at 35:44–47.

Dependent claims 2 and 3 further recite "exposing the composition subjected to protein A affinity chromatography to a protease inhibitor" (*id.* at 35:48–50) (claim 2), and in particular, protease inhibitors EDTA or AEBSF (*id.* at 35:51–53) (claim 3). Claims 5–11 define the "protein which

comprises a C_H2/C_H3 region" as either an antibody (claim 5) having a defined identity, substrate specificity, or other property (claims 6–9), or an immunoadhesin (claims 10 and 11). *Id*. at 35:57–36:49.

Because Patent Owner does not specifically address Petitioner's challenge to any dependent claim, we focus our analysis on independent claim 1.

E. Prosecution History Leading to the Issuance of the '799 Patent

The '799 Patent issued from Application No. 12/269,752, filed on November 12, 2008, which is a continuation of application No. 10/877,532, filed on June 24, 2004, now US Patent No. 7,485,704 ("the '704 patent" (Ex. 1008)). The '799 and '704 Patents, as well as related European Patent, EP 1 648 940 B1 ("EP '940" (Ex. 1009)), claim priority benefit of US Provisional Application No. 60/490,500, filed on July 28, 2003. Pet. 7.

A summary of relevant prosecution history is set forth at pages 11–17 of the Petition, which we adopt.

II. ANALYSIS

A. Person of Ordinary Skill in the Art.

Petitioner contends that a person of ordinary skill in the art would have "at least a graduate degree, such as a Ph.D., and several years of postgraduate training or practical experience in a relevant discipline such as biochemistry, process chemistry, protein chemistry, chemical engineering and/or biochemical engineering, among others." Pet. 22 (citing Ex. 1002 ¶ 32). "Such a person would also understand that protein purification is a multidisciplinary field, and could take advantage of the specialized skills of others using a collaborative approach." *Id*. Patent Owner does not contest this definition. *See* Ex. 2008 ¶¶ 46–47; Ex. 2009 ¶ 10. Petitioner's proposed

interpretation is consistent with the level of ordinary skill reflected in the prior art of record and we adopt it for the purpose of this proceeding. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001)*; In re GPAC Inc*., 57 F.3d 1573, 1579 (Fed. Cir. 1995).

B. Claim Construction

In an *inter partes* review, claim terms in an unexpired patent are interpreted according to their broadest reasonable construction in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); *Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 2131, 2144–46 (2016) (upholding the use of the broadest reasonable interpretation standard). "Under a broadest reasonable interpretation, words of the claim must be given their plain meaning, unless such meaning is inconsistent with the specification and prosecution history." *Trivascular, Inc. v. Samuels*, 812 F.3d 1056, 1062 (Fed. Cir. 2016). Any special definition for a claim term must be set forth in the specification with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

i. "Method of Purifying a Protein"

Petitioner proposes, in part, that we construe claim 1 "as a method of purifying a protein, which does not require reduction of protein A leaching." Pet. 17–18 (citing Ex. 1002 ¶ 88). We agree with this portion of Petitioner's construction, as does Patent Owner. *See* PO Resp. 13.

Although the Specification relates to "a method for reducing leaching of protein A during protein A affinity chromatography" (Ex. 1001, 1:15–21), claim 1, on its face, does not require a reduction of protein A leaching. And while "understanding the claim language may be aided by the explanations contained in the written description," our reviewing court cautions that "it is

important not to import into a claim limitations that are not a part of the claim," and we find no reason to do so on the present record. *See SuperGuide Corp. v. DirecTV Enters., Inc.*, 358 F.3d 870, 875 (Fed. Cir. 2004); *see also Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d 898, 906 (Fed. Cir. 2004) ("Even when the specification describes only a single embodiment, the claims of the patent will not be read restrictively unless the patentee has demonstrated a clear intention to limit the claim scope using 'words or expressions of manifest exclusion or restriction.'" (quoting *Teleflex, Inc. v. Ficosa N. Am. Corp.*, 299 F.3d 1313, 1327 (Fed. Cir. 2002)).

Petitioner further proposes, however, that we interpret claim 1 to mean "a method of separating the protein of interest from the other proteins produced by the cell," which could be read to *exclude* a reduction in protein A leaching or the purification of the protein of interest from non-cellular components. Pet. 17–18. For the reasons set forth on pages 11–13 of the Patent Owner Response, we decline to read claim 1 in this manner. *See also Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1583 (Fed.Cir.1996) (reasoning that an interpretation that excludes a preferred embodiment is unlikely to be correct).

Further, as noted at page 18 of the Petition, during prosecution leading to the issuance of the '799 Patent, Applicants deleted the phrase "such that protein A leaching is reduced" in order to overcome a rejection under §112, second paragraph. Ex. 1011, 10–11, 15, 18–19. *See Vitronics*, 90 F.3d at 1582 (stating that "the record before the Patent and Trademark Office is often of critical significance in determining the meaning of the claims"). On the present record, we see no reason to interpret the claims to exclude (or require) a limitation expressly deleted during prosecution. Rather, as Patent Owner argues, deleting this requirement broadens the scope such that the

method of claim 1 may, but need not, encompass a reduction in protein A leaching. *See* PO Resp. 12.

Petitioner also appears to argue that claim 1 excludes a reduction in protein A leaching because protein A is not a contaminant of HCCF, but is a by-product of the purification process itself. *See* Pet. Reply 6–7. As an initial matter, we note that claim 1 is directed to "subjecting a composition comprising said protein to protein A affinity chromatography," and is, thus, not limited to purifying proteins from HCCF. Moreover, the '799 Patent is directed to "purifying a C_H2/C_H3region-containing protein from impurities by protein A affinity chromatography" where those impurities are broadly defined as "material[s] different from the desired protein product," and expressly including "leached protein A." Ex. 1001, 4:53–59, 7:50–53; *see also* Ex. 2008 ¶¶ 20, 50–52. Accordingly, we do not find Petitioner's argument persuasive.

Our interpretation with respect to protein A leaching is further supported by the doctrine of claim differentiation. Claim differentiation

> stems from the common sense notion that different words or phrases used in separate claims are presumed to indicate that the claims have different meanings and scope. Although the doctrine is at its strongest where the limitation sought to be read into an independent claim already appears in a dependent claim, there is still a presumption that two independent claims have different scope when different words or phrases are used in those claims.

Seachange Int'l, Inc. v. C-COR, Inc., 413 F.3d 1361, 1368–69 (Fed. Cir. 2005) (internal citations and quotations omitted).

In the present case, claim 12 of the '799 Patent, directed to "[a] method of purifying a protein which comprises a C_H2/C_H3 region," expressly sets forth steps to "reduce leaching of protein A." Ex. 1001, 36:50–65. Similarly, claim 1 of the earlier-issued '704 Patent expressly

recites the limitation "such that protein A leaching is reduced." Ex. 1008, 35:46–59. As claim 12 of the '799 patent and claim 1 of the related '704 patent not only admit, but require, a reduction of protein A leaching, we find no evidence tending to rebut the presumption that a reduction in protein A leaching is encompassed by claim 1 of the '799 patent.

Accordingly, for the reasons set forth above, we interpret a "method of purifying a protein" to mean a method of separating a protein of interest from one or more impurities.

ii. "subjecting a composition comprising said protein to protein A affinity chromatography at a temperature in the range from about 10° C. to about 18° C."

Further with respect to claim 1, Petitioner proposes that we construe "about 18° C" in the upper bound of "a temperature in the range from about 10° C. to about 18° C." as encompassing ±3° C. Pet. 17–20; Pet. Reply 3–5. Patent Owner responds that "about 18 °C" encompasses no more than ±1° C, and "refer[s] to the temperature of the HCCF subjected to purification, not of the room in which the method is performed." PO Resp. 13–21. We address separately, the two parameters raised in Patent Owner Response.

1. "about 18 °C"

In support of its position that "about 18° C" encompasses ±3° C, Petitioner argues that the Specification indicates that this range reflects typical temperature fluctuations during protein A chromatography. Pet. 19. In particular, Petitioner relies on the inventor's representation that in the "full scale" experiments involving 12,000 liter volumes of cell culture, the "HCCF was collected and held at 15+/-3°C. for the duration of loading." *See* Ex. 1001, 21:7–8; *see also id.* 23:61–63, 24:43–45; Ex. 1002 ¶¶ 81–82. Petitioner further relies on Dr. Przybycien's testimony that a person of

ordinary skill in the art would have considered ±3° C to be a normal temperature fluctuation in the context of protein A affinity chromatography. Pet. 19–20 (citing Ex. 1002 ¶ 82).

In response, Patent Owner argues that one of ordinary skill in the art would understand the "about 18° C." limitation as directed to conducting protein A chromatography at "below room temperature." PO Resp. 18. Citing column 18, lines 4–9 of the Specification, Patent Owner reasons that because "the [S]pecification makes it clear that 'about 20° C' means 'below room temperature' . . . [*a*] *fortiori* so does "about 18° C." *Id.*

As an initial matter, we note that the challenged claims do not recite "below room temperature," but a defined range with an upper bound of "about 18 ° C." Moreover, with respect to reducing the temperature of a composition to, for example, "below room temperature," the Specification teaches both the reduction of temperature and "below room temperature" as a merely preferred embodiments. *See* Ex. 1001, 18:4–9 ("*Preferably*, the method comprises reducing the temperature of the composition subjected to protein A affinity chromatography in which the temperature of the composition is reduced *e.g*. . . . below room temperature.") (emphasis added). But "[c]laims are not necessarily and not usually limited in scope simply to the preferred embodiment." *Akamai Techs., Inc. v. Limelight Networks, Inc.*, 805 F.3d 1368, 1375 (Fed. Cir. 2015) (citation omitted). And on the record before us, we decline to rewrite claim 1 to include the term "below room temperature." *See SuperGuide Corp. v. DirecTV Enters., Inc.*, 358 F.3d 870, 875 (Fed. Cir. 2004). ("Though understanding the claim language may be aided by the explanations contained in the written description, it is important not to import into a claim limitations that are not a part of the claim.")

As we understand Patent Owner's argument, we should construe "'about' as no[t] more than ±1° C" because the Specification teaches that 20 ℃ is below room temperature, and "every reasonable scientist" would consider 21 ℃ to be room temperature. PO Resp. 18, 21 (citing Ex. 2010, 135:10–14; 2008 ¶¶ 66–67); *see also* Tr. 23:13–14 ("Where 'about' is not defined it should be construed as approximately or alternatively plus or minus 1 degree celsius."), 24:6–22.

Patent Owner's proposed construction is inconsistent with its own logic, however, because if "about" means "no[t] more than ±1 ℃," the upper limit of "about 20 ℃" is 21 ℃—which Patent Owner equates with room temperature. Thus, contrary to its position that claim 1 requires the method to be conducted at below room temperature, Patent Owner's construction would require 21 ℃ to be both room temperature *and* below room temperature.

Patent Owner quotes *Modine Mfg. Co. v. U.S. Int'l Trade Comm'n*, 75 F.3d 1545, 1555[1] (Fed. Cir. 1996), abrogated on other grounds by *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 234 F.3d 558 (Fed. Cir. 2000), for the proposition: "Although it is rarely feasible to attach a precise limit to 'about,' the usage can usually be understood in light of the technology embodied in the invention." PO Resp. 21. We apply that proposition here. Although the Specification provides no express definition of "about," the scope of "about 18° C." is informed by the variations in temperature noted in the supporting examples. We note, in particular, that

[1] We assume that Patent Owner meant to cite here to page 1555, instead of 155. We regard this as a clerical error, and, in any event, it does not change our analysis.

although the Specification discloses that "pilot scale" experiments were conducted "within 1° C. of the desired temperature" (Ex. 1001, 20:61–64), it repeatedly asserts that HCCF used in the "full scale" experiments was subject to ±3° C. variation around the target temperature, which suggests a broad meaning of the term "about." *See* Ex. 1001, 21:7–8, 23:61–63, 24:43–45; *see also* Ex. 1002 ¶ 82.

Our reading of claim 1 in light of the Specification, thus, supports a construction of "about 18° C." to mean "18 ±3° C.", such that the upper bound of "a temperature in the range from about 10° C to about 18° C" is 21° C.

A broad construction of this term is further supported by the prosecution history of the earlier-issued '704 Patent, which shows that Applicants avoided a rejection over prior art disclosing protein A chromatography at 22° C by amending the upper limit of then-pending claims from "20° C" to "about 20° C" and, subsequently, to "about 18° C," thereby indicating that "about" must mean at least ±2° C., but less than ±4° C. *See* Pet. 12–13, 20; Ex. 1010, 38, 50, 55, 59, 74–75, 79; Ex. 1002 ¶ 82.

Patent Owner attempts to avoid this conclusion by asserting that Applicants did not acquiesce to the rejection in amending the claims. PO Resp. 20. In support, Patent Owner points to Applicants' statements in the prosecution history that:

> Without acquiescing to the rejection, claims 1 and 12 have been amended to recite '20°C' as the upper limit of the temperature range for conducting protein A affinity chromatography, and therefore Horenstein et al. clearly does not anticipate these claims, as currently amended, or the claims dependent therefrom.

and

> All amendments and cancellations were made without prejudice or disclaimer. Applicants explicitly reserve the right to pursue any removed subject matter in one or more continuing applications.

Id. (referencing Ex. 1010, 59, 77, respectively). But as Petitioner notes, "Applicant *did* acquiesce by narrowing the claimed range; and it never again pursued a broader temperature range." *See* Pet. Reply 5. Based on the record before us, we accord little weight to the above-cited self-serving statements in the prosecution history.

For the reasons set forth above, we find that during prosecution, Applicants limited the meaning of "about" in the term "about 18 °C" to at least ±2 °C, but less than ±4° C. Consistent with this conclusion, we note that prior to allowing the instant claims to issue, the Examiner pointed out that Stahl[2] and Horenstein[3] taught protein A affinity chromatography at 4°C and 22°C, respectively. Ex. 1011, 11. The Examiner did not base a rejection on Stahl and/or Horenstein, however, because 4°C and 22°C as taught in those references were "not in the temperature range required by claim 20"—now claim 1 of the '799 Patent. *See id*.

Accordingly, in light of the intrinsic record as a whole, we conclude that "about 18° C" means "18 ±3° C," such that the upper bound of "a temperature in the range from about 10° C to about 18° C" is 21° C.

> *2. "subjecting a composition . . . to protein A affinity chromatography at a temperature in the range from about 10 °C to about 18 °C"*

Patent Owner contends that the temperature range set forth in claim 1 refers to the temperature of the composition being purified. PO Resp. 13–

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[2] Stahl et al., US 6,927,044 B2.
[3] Horenstein et al., 275 J. Immunol. Meth. 99–112 (2003).

17. Patent Owner states, for example, that "the only reasonable construction of the claims is that they refer to the temperature of the HCCF subjected to purification, not of the room in which the method is performed." *Id.* at 14. We agree with Patent Owner's construction with two caveats.

First, the claims do not require the "composition" subjected to protein A affinity chromatography to be HCCF. To the contrary, the Specification indicates that antibodies and other proteins having a C_H2/C_H3 region may be purified from a variety of compositions including whole animal serum, proteolytic digests, and the products of chemical cross-linking reactions. *See* Ex. 1001, 7:50–55, 9:43–10:5, 10:61–67, 12:47–64, 12:65–14:36.

Second, Patent Owner appears to imply that the claims require actively cooling the composition (e.g. HCCF) to a range of about 10° C. to about 18° C. prior to the chromatography step. *See* PO Resp. 14–16 & n.7. But the language of the challenged claims requires neither an express cooling step nor that the target temperature is reached prior to applying the composition to a protein A chromatography matrix. *See, e.g.,* Ex. 1020 ¶¶ 32–36. Moreover, the Specification makes clear that the target temperature may be reached "prior to and/or during protein A affinity chromatography." Ex. 1001, 18:9–11.

With those caveats, we construe "subjecting a composition . . . to protein A affinity chromatography at a temperature in the range from about 10 °C to about 18 °C" as referring to the temperature of the composition prior to and/or during protein A affinity chromatography.

For purposes of this decision, we determine that no further construction is necessary. *See Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999) (only those terms that are in controversy

need be construed, and only to the extent necessary to resolve the controversy).

C. Anticipation

i. Legal Principles

To anticipate a claim under 35 U.S.C. § 102, "a single prior art reference must expressly or inherently disclose each claim limitation." *Finisar Corp. v. DirecTV Grp., Inc.*, 523 F.3d 1323, 1334 (Fed. Cir. 2008). That "single reference must describe the claimed invention with sufficient precision and detail to establish that the subject matter existed in the prior art." *Verve, LLC v. Crane Cams, Inc.*, 311 F.3d 1116, 1120 (Fed. Cir. 2002). While the elements must be arranged in the same way as is recited in the claim, "the reference need not satisfy an *ipsissimis verbis* test." *In re Gleave*, 560 F.3d 1331, 1334 (Fed. Cir. 2009). Moreover, "it is proper to take into account not only specific teachings of the reference but also the inferences which one skilled in the art would reasonably be expected to draw therefrom." *In re Preda*, 401 F.2d 825, 826 (CCPA 1968). Accordingly, "a reference can anticipate a claim even if it 'd[oes] not expressly spell out' all the limitations arranged or combined as in the claim, if a person of skill in the art, reading the reference, would 'at once envisage' the claimed arrangement or combination." *Kennametal, Inc. v. Ingersoll Cutting Tool Co.*, 780 F.3d 1376, 1381 (Fed. Cir. 2015) (alteration in original) (quoting *In re Petering*, 301 F.2d 676, 681 (CCPA 1962)).

ii. Anticipation by WO '389 (Ground 1)

Petitioner asserts that claims 1 and 5 are anticipated by WO '389 under 35 U.S.C. § 102(b). Pet. 6, 28–33; Pet. Reply 7–16. Patent Owner opposes. PO Resp. 22–34. Having considered the record as whole, we

determine that Petitioner has shown by a preponderance of evidence that claims 1 and 5 are anticipated by WO '389. We begin with an overview of the asserted reference.

1. Overview of WO '389 (Ex. 1003)

WO '389 states that "[a]lthough Protein A affinity column chromatography is widely used, it is also appreciated that elution of antibody from such columns can result in leaching of residual Protein A from the support." Ex. 1003 at 4:1–3.[4] The reference teaches that size exclusion chromatography or hydrophobic interaction chromatography (HIC) can be used to remove the residual protein A that leaches from the column during elution. *Id.* at 4:7–9, 13:30–33; *see also* Ex. 1002 ¶¶ 68–69; Ex. 2008 ¶¶ 37, 81.

WO '389 discloses "the purification of an IgG antibody from conditioned cell culture medium containing same comprising sequentially subjecting the medium to (a) Protein A, (b) ion exchange chromatography, and (c) hydrophobic interaction chromatography." Ex. 1003 at 4:20–24; *see id*. at 40:23–26 (claim 9), 41:21–34 (claim 20). "The process in its most preferred embodiment consists of three purification steps (Protein A affinity, cation exchange, and hydrophobic interaction chromatography)." *Id*. at 13:9–13. "All steps are carried out at room temperature (18 - 25 °C)." *Id*. at 13:13.

In Example 1, WO '389 discloses that HCCF harvested by microfiltration or centrifugation is applied to a protein A chromatography

[4] Where possible, we refer to the native pagination of the cited references rather than to that supplied by the parties.

column. *Id.* at 14:10–17. "After loading the column, it is washed with at least 3 column volumes of PBS containing 0.1 M glycine" and eluted with a low pH buffer. *Id.* at 14:20–23; *see also, id.* at 19:1–10 (stating that HCCF was applied to a 5.0 liter affinity column, after which "approximately 15 liters of PBS/glycine was applied to the column at the same flow rate."), 29:1–14 (stating that HCCF was applied to a 5.5 liter affinity column, after which "approximately 17 liters of PBS/glycine was applied to the column at the same flow rate.").

2. Analysis of Ground 1

a) Whether the HCCF in WO '389 is within the claimed range

WO '389 teaches a method for purifying antibodies, including a step wherein HCCF is subject to protein A affinity chromatography. WO '389 teaches that "[a]ll steps are carried out at room temperature (18 - 25 °C)," which overlaps with the temperature range of "about 10 ° C. to about 18 ° C." recited in claim 1.[5] Patent Owner contends, however, that WO '389 "nowhere discloses or suggests chilling the harvested cell culture fluid prior to protein A chromatography" and, thus, fails to disclose "subjecting a composition . . . to protein A affinity chromatography at a temperature in the range from about 10 ° C. to about 18 ° C." required by independent claim 1. PO Resp. 22.

Patent Owner further contends WO '389's statement that "[a]ll steps are carried out at room temperature (18 - 25 °C)," "refers to the temperature of the laboratory where each 'step' in process was performed," and not to

[5] Because the range set forth in WO '389 overlaps with the "18 ° C." recited in claim 1, our anticipation analysis in view of this reference does not necessarily depend on the construction of "about."

the temperature of the HCCF applied to the protein A column. PO Resp. 23. With respect to the latter, Patent Owner argues that WO '389 is "completely silent" with respect to the temperature of the HCCF. *Id.* at 24.

We do not find this argument persuasive. While we agree with Patent Owner that WO '389 does not expressly call out the temperature of the HCCF, such specificity would be redundant in light of its blanket teaching to carry out "all steps . . . at room temperature (18 - 25 °C)." Consistent with this view, WO '389 *does* specify temperatures that fall outside of this range. *See* Ex. 1003, 14–15 (disclosing that after the viral inactivation step "[t]he resulting solution is . . . held in sterile containers at 4 ℃, or frozen and held at -70 ℃").

Patent Owner relies on the opinions of its expert, Dr. Cramer, which appear predicated on a view that the '799 Patent and relevant art are directed to large-scale, industrial purification. *See, e.g.,* Ex. 2008 ¶¶ 47, 141 (arguing that the '799 Patent is directed to "industrial purification"). According to Dr. Cramer:

> Efficiency is typically a goal of industrial processes, and absent an instruction to wait to allow the harvested cell culture fluid to cool to room temperature, the POSA would have interpreted [WO '389] as allowing the disclosed process to be performed with harvested cell culture fluid that was potentially warmer than room temperature.

Id. ¶ 78; *see id.* ¶ 98 (same argument with respect to van Sommeren). But neither the challenged claims, nor the disclosure of WO '389 are limited to the large scale industrial processes envisioned by Dr. Cramer. *See* Ex. 1003, 14:1–4 (indicating that the process may be "normalized for any scale"); Ex. 1001, 3:15–60, 20:35–58, 23:1–25 (exemplifying "small scale" and "lab scale" processes); Ex. 1020 ¶ 68. We further weigh Dr. Cramer's opinion

against his testimony that even for commercial scale systems he was not aware of any process where HCCF was filtered and applied directly into a protein A column. Ex. 1022, 85:6–15.

We instead credit the testimony of Dr. Przybycien in this matter. *See* Ex. 1020 ¶¶ 25–28. According to Dr. Przybycien,

> absent contrary language, a POSA would understand that experiments are being conducted at ambient temperature with all materials equilibrated, in order to obtain robust scientific data.
>
> * * *
>
> No POSA would understand WO '389 as teaching a practitioner to use HCCF having a temperature above 18º C – 25º C, after being explicitly directed to conduct "all steps" at 18º C – 25º C. In addition, no reasonable POSA would contact 37 º C HCCF to the chromatography column, and report having performed the step at 18º C – 25º C. In this case, the relatively warmer HCCF would raise the temperature of the entire system. A POSA would understand that the disclosure of 18º C – 25º C in WO '389 must refer to the temperature of all of the components involved in the experiment, including the composition being purified.

Id. ¶¶ 27–28; *see also* Ex. 2045, 255:6–19.

For at least the reasons set forth above, we find that WO '389 discloses all elements of claims 1 and 5 of the '799 patent.

b) Whether WO '389 discloses a composition subjected to protein A affinity chromatography within the claimed range

Further, to the extent Patent Owner argues that the HCCF must have been within the range of 18º C – 25º C at the time it was applied to the protein A affinity column in WO '389, we note that this is not a requirement of our claim construction. As set forth in section II(B)(ii)(2), above, we construe "subjecting a composition . . . to protein A affinity chromatography at a temperature in the range from about 10 ° C. to about 18 ° C." as

referring to the temperature of the composition prior to and/*or during* protein A affinity chromatography.

WO ’389 Example 1 discloses application of HCCF to a protein A affinity column, whereupon the entrained composition is washed with at least three column volumes of buffer before the antibody is eluted. *See* section II(C)(ii)(1), above. Insofar as WO ’389 teaches that “[a]ll steps are carried out at room temperature (18 - 25 °C),” we understand that the apparatus and column buffers are all within that temperature range. Accordingly, we infer that during the washing step, the entrained composition is also at 18–25 °C and, thus, within the temperature range of claim 1 as construed in section II(B)(ii), above. For this additional reason, we find that WO ’389 discloses all elements of claims 1 and 5 of the ’799 patent.

c) Whether the claimed range is critical

As discussed above, WO ’389 discloses a process carried out at temperature range of “18–25 °C,” which overlaps the “temperature in the range from about 10 ° C to about 18 ° C,” recited in independent claim 1, most particularly in light of our construction of that term. Where the patent claims a range, it is anticipated by prior art disclosing a point within the range, *see Titanium Metals Corp. v. Banner*, 778 F.2d 775, 782 (Fed. Cir. 1985), *unless* there is evidence establishing that the claimed range is “critical to the operability of the claimed invention.” *Ineos USA LLC v. Berry Plastics Corp.*, 783 F.3d 865, 871 (Fed. Cir. 2015); *see also ClearValue, Inc. v. Pearl River Polymers, Inc*., 668 F.3d 1340, 1344–45 (Fed. Cir. 2012) (finding the patented range anticipated by a broader range in the prior art because there was no allegation of criticality and no considerable difference between the claimed range and the broader range in the prior art).

Relying on the declarations of Drs. Dowd and Cramer, Patent Owner argues that claims 1 and 5 are not anticipated because the claimed range is critical to the operability of the invention. PO Resp. 22, 30–34 (citing Ex. 2008 ¶¶ 112–21; Ex. 2009 ¶¶ 31–71.[6] In this respect, Patent Owner focuses on the shape of the curve in plots of protein A leaching over a range of temperatures. *Id.* As summarized by Dr. Cramer, "the extent of protein A leaching is relatively flat within the claimed range of about 10℃ to about 18℃, whereas the extent of protein A leaching in the ranges of 18–25℃ and 20–25℃ tends to increase more sharply per degree relative to the claimed range." Ex. 2008 ¶ 115. We do not find Patent Owner's argument persuasive for the reasons set forth on pages 13 through 16 of Petitioner's Reply brief, and further detailed in paragraphs 37–45 of Dr. Przybycien's second declaration (Ex. 1020).

Criticality has been found where only a narrow range of temperature enabled a process to operate as claimed, and problems occurred in practicing the invention below or above the claimed range. *See Atofina v. Great Lakes Chem. Corp.*, 441 F.3d 991 (Fed. Cir. 2006). In the present case, however, we credit Dr. Przybycien's testimony that "[t]he claimed range in the '799 Patent is not critical, because protein A chromatography works in the same way at the prior art temperatures of 4° C, 18-25° C and 20-25° C as it does at the claimed range." Ex. 1020 ¶ 38; *see also id.* ¶¶ 37–45, 65; Ex. 1002 ¶¶ 85–89. With respect to the plots referenced by Patent Owner, we agree that protein A leaching shows an exponential or Arrhenius-type dependence

[6] We note that paragraphs 117 and 121 of Exhibit 2008, and paragraphs 51, 69, and 70 of Exhibit 2009 are among the paragraphs at issue in Patent Owner's presumptive motion to seal. *See* section III, below.

with respect to temperature, with a greater increase in leaching for each unit increase in temperature. *See* Ex. 1002 ¶¶ 49, 89, 93; Ex. 1020 ¶¶ 41–42. But, following this logic, one of ordinary skill in the art would expect protein A chromatography to work better—at least with respect to minimizing leaching—at temperatures *outside* the claimed range (e.g., at 4°C).

We also find convincing Petitioner's argument that the observed exponential temperature dependence profiles would have been expected because protein A leaching is driven by proteolysis, which has a well-known exponential temperature dependence. Pet. 33 (citing Ex. 1002, ¶¶ 49, 87, 93, 104); Ex. 1020, ¶¶41–42; *see also* Ex. 1005, 88–89 (concluding that protein A leaching is due to proteolytic activity) (discussed in section II(D)(ii), below). Although Patent Owner characterizes the leaching levels observed in the claimed temperature range of this curve, as "'relatively flat," we credit Dr. Przybycien's testimony that this does not render the relationship "special or optimal, it is simply the middle range of an exponential trend line." Ex. 1020 ¶ 42. As Dr. Przybycien explains, it is well known to conduct protein A chromatography at temperatures below the claimed range, and so doing would reveal "a continuation of the 'relatively flat' leaching trend observed at the claimed and prior art temperature ranges." *Id.* ¶¶ 43–44; *see also* Ex. 2045, 268:5–269:4. Again, because leaching varies inversely with temperature, conducting protein A chromatography at temperatures below the claimed range would be expected to further reduce leaching.

We, therefore, agree with Petitioner that "[t]he claimed range of "'about 10° C to about 18° C' cannot be critical to practicing the alleged invention if the sole alleged benefit is also achieved below the range, at temperatures disclosed in the prior art." Pet. Reply 15 (citing Ex. 1020

¶ 43). For the same reasons, we do not find persuasive Patent Owner's argument that performing protein A chromatography at the claimed temperature range produces unexpected results as compared to performing the process at other temperatures known in the art. *See* PO Resp. 53.

d) Conclusion

Based on the record before us, we conclude that Petitioner has demonstrated by a preponderance of evidence that claims 1 and 5 of the '799 patent are anticipated by WO '389.

iii. Anticipation by van Sommeren (Ground 2)

Petitioner asserts that claims 1, 2, and 5 are anticipated by van Sommeren under 35 U.S.C. § 102(b). Pet. 6, 33–37; Pet. Reply 7–16. Patent Owner opposes. PO Resp. 22, 26–34. Having considered the full trial record, we determine that Petitioner has shown by a preponderance of evidence that claims 1, 2, and 5 are anticipated by van Sommeren. We begin with an overview of the asserted reference.

1. Overview of van Sommeren (Ex. 1004)

Van Sommeren explores the effects of temperature, flow rate, and buffer composition on protein A affinity chromatography purification of IgG_1 monoclonal antibodies. Ex. 1004, Abstract, 135. In each of these studies:

> A protein A Sepharose 4 Fast Flow column (⌀10, h 13 mm) was equilibrated with binding buffer. The cell culture supernatant was diluted with an equal volume of binding buffer and filtered through a 0.2 *μm* pore size membrane filter. Subsequently a volume containing a fixed amount of [monoclonal antibody] was loaded onto the column. The non-bound fraction was washed from the column with binding buffer. The fraction bound to the column was desorbed with 0.1 M citric acid (pH 5.0).

Id. at 138.

With respect to temperature, van Sommeren compares the results of protein A chromatography conducted at "4 °C versus ambient temperature (AT) (20-25 °C)." *Id*. at 145. Van Sommeren notes that other researchers "reported a five times higher binding capacity of protein A Sepharose for mouse monoclonal IgG_1 antibodies at 4 °C in comparison with 20-26 °C, using a 0.1 M sodium phosphate binding buffer (pH 8.2)". *Id*. at 146. In comparison, van Sommeren reports that "[r]esults from the present study show that the temperature effect on the IgG_1 binding capacity becomes of minor importance, if adsorption is performed in a high ionic strength (1.5 M glycine, 3.0 M NaCl) buffer pH 8.9." *Id*. at 147. In particular, Table V of the reference shows that the binding capacity of protein A for various IgG_1 antibodies under these buffer conditions could decrease, stay the same, or increase by as much as 30 or 40% when run at 4°C as compared to ambient temperature (20–25°C). *Id.* at 144, 145.

Van Sommeren also notes that Cathepsin D protease activity in both the starting material and in the purified IgG is undesirable and suggests the addition of the protease inhibitor, pepstatin A to minimize proteolytic degradation of the IgG. *Id*. at 147–48; *see also* Ex. 1022, 127:24–129:18.

2. Analysis of Ground 2

In the Patent Owner Response and Petitioner's Reply brief, the parties largely address WO '389 and van Sommeren together. Accordingly, we refer to our discussion in section II(C)(ii), above, including our discussion regarding the criticality of the claimed range set forth in section II(C)(ii)(2)(c).

Van Sommeron discloses protein A chromatography of HCCF at ambient temperature, defined therein as from 20°C to 25°C, and which overlaps with our construction of "about 18° C." as having an upper bound

of 21 °C. *See* section II(B)(ii), above. Patent Owner contends, however, that the reference "never discloses cooling [the HCCF]" to that temperature. PO Resp. 28. Rather, Patent Owner argues, "van Sommeren discloses the temperature of the *lab space* where the experiments were conducted, not the temperature of the HCCF subjected to purification" and accordingly, "[t]here is no way to know from van Sommeren what temperature the composition was when it was loaded on the column." PO Resp. 27, 28 (citing Ex. 2009 ¶¶ 94–99).

We do not find Patent Owner's argument persuasive for the reasons set forth on pages 10–14 of the Petition.[7] Most particularly, we credit Dr. Przybycien's explanation that because van Sommeren studies binding behavior as a function of temperature, i.e., at 4 °C versus 20–25 °C, all of the starting materials must have been equilibrated to those temperatures in order to obtain valid experimental results. *See* Ex. 1020 ¶¶ 29–30. In contrast, "using HCCF of another temperature would render the experimental results meaningless." *Id.* ¶ 30 (citing Ex. 1022, 126:18–175:5). Thus, one of ordinary skill in the art "would not have interpreted van Sommeren as

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[7] We further note that Dr. Cramer states that van Sommeren "does not disclose any intermediate step between the harvest of cell culture fluid from the bioreactor and the harvested cell culture fluid being subjected to protein A affinity chromatography," which, in the context of his report, implies that the HCCF applied to the column would be at 37°C—the temperature at which the antibodies are grown. Ex. 2008 ¶ 97. This is not correct. Van Sommeren discloses intermediate steps between the harvesting of HCCF and application of the composition to protein A affinity chromatography, including the addition of binding buffer, presumably at ambient temperature. *See* Ex. 1004, 138 ("The cell culture supernatant was diluted with an equal volume of binding buffer and filtered through a 0.2 μm pore size membrane filter.")

suggesting or allowing the disclosed process to be performed with 'warm' cell culture fluid, given that doing so would guarantee invalid experimental data." *Id.* ¶ 30.

Accordingly, we find that van Sommeren discloses all elements of challenged claims 1, 2, and 5. For the reasons set forth in Section II(C)(ii)(2)(c), above, the overlap between the claimed range and that disclosed in van Sommeren is not critical to the practice of the invention. Based on the record before us, we conclude that Petitioner has demonstrated by a preponderance of evidence that claims 1, 2, and 5 of the '799 patent are anticipated by van Sommeren.

D. Obviousness

i. Legal Principles

A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which that subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007).

> Rejecting a blinkered focus on individual documents, the [*KSR*] Court required an analysis that reads the prior art in context, taking account of "demands known to the design community," "the background knowledge possessed by a person having ordinary skill in the art," and "the inferences and creative steps that a person of ordinary skill in the art would employ."

Randall Mfg. v. Rea, 733 F.3d 1355, 1362 (Fed. Cir. 2013) (citing *KSR*, 550 U.S. at 418).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill

in the art "to combine . . . known elements in the fashion claimed by the patent at issue." *KSR*, 550 U.S. at 418. Although evidence pertaining to secondary considerations must be taken into account whenever present, it does not necessarily control the obviousness conclusion. *See, e.g., Pfizer, Inc. v. Apotex, Inc.* 480 F.3d 1348, 1372 (Fed. Cir. 2007).

A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *KSR*, 550 U.S. at 418. Rather, "any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed." *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that "a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so." *In re Magnum Oil Tools Int'l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted); *see also Belden Inc. v. Berk–Tek LLC,* 805 F.3d 1064, 1073 (Fed. Cir. 2015) ("[O]bviousness concerns whether a skilled artisan not only *could have made* but *would have been motivated to make* the combinations or modifications of prior art to arrive at the claimed invention.").

ii. Analysis

Petitioner asserts that claims 1 and 5 would have been obvious in view of WO '389 (Ground 3); claims 1–3 and 5 would have been obvious in view of WO '389, Balint, and Potier (Ground 4); claims 2, 3, and 6–11 would have been obvious in view of WO '389 and the '526 Patent (Ground 5); claims 2, 3, and 6–11 would have been obvious in view of WO '389, Balint, Potier, and the '526 Patent (Ground 6); claims 1, 2, and 5 would have been

obvious in view of van Sommeren (Ground 7); and claims 3 and 6–11 would have been obvious in view of van Sommeren and the '526 Patent (Ground 8). Pet. 6–7. Patent Owner generally responds to Grounds 3–8 collectively. *See* PO Resp. 34–55.

With respect the above grounds, we credit Dr. Przybycien's testimony that

> In the early days of protein A chromatography, researchers relied on chromatographic substrates that were unable to support fast flow rates, resulting in long processing times. In order to ensure that the binding, washing and elution of the target protein was not outpaced by proteolytic degradation, chromatography was often run in the cold room. Newer resins with faster flow properties[8]—for example, PROSEP-A® and SEPHAROSE® Fast Flow—became available before July 2003. Using these improved resins allowed researchers to step out of the cold room, and conduct protein A chromatography at ambient temperatures when they preferred to do so. As a result, studies involving protein A chromatography, such as those disclosed in van Sommeren and WO '389, would often use either cold room temperature (~4° C), or ambient temperature.

Ex. 1002 ¶ 34. For the reasons set forth below, and having considered the record as a whole, we agree with Petitioner that because it was well known to conduct protein A chromatography at 4 °C and at ambient temperature, doing so in the claimed intermediate temperature range would have been an obvious design choice that balances the cost and effort of using reduced temperatures against the benefit of reducing proteolysis of the antibody target and/or selection of a protein A column matrix. *See id.* ¶¶ 103–104;

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[8] Although the '799 Specification exemplifies PROSEP-A and SEPHAROSE column matrices, Patent Owner does not argue that the claims are limited to column matrices with such properties. *See* Ex. 1001, 4:28–47 (discussing a range of solid phase supports within the scope of the invention).

Ex. 1020 ¶ 72. Moreover, as summarized by Dr. Przybycien: "There is nothing unexpected or unique about the intermediate level of protein A leaching achieved using an intermediate temperature, because protein A leaching was known to be temperature-dependent." Ex. 1025 ¶ 38.

Because, at a minimum, Balint and Potier provide background with respect to reductions in proteolysis, we begin with a discussion of those references. *See Randall*, 733 F.3d at 1362 ("By narrowly focusing on the four prior-art references cited by the Examiner and ignoring the additional record evidence Randall cited to demonstrate the knowledge and perspective of one of ordinary skill in the art, the Board failed to account for critical background information that could easily explain why an ordinarily skilled artisan would have been motivated to combine or modify the cited references to arrive at the claimed inventions.").

iii. Balint (Ex. 1005) and the role of proteolysis in Protein A Leaching

Balint investigates potential causes of protein A leaching during affinity column chromatography of IgG from blood plasma or serum. Ex. 1005, 85. Balint explores properties relevant to "an extracorporeal immunoadsorbent column (PROSORBA® column) containing purified Staphylococcal protein A (SpA) covalently bound to a silica matrix." *Id*. According to Balint, "[p]rior to the development of this column, there was concern about the potential for [protein A] to 'leach' from the immunoadsorbent matrix into patient plasma." *Id*. at 86. To investigate these concerns, Balint conducted studies using "[p]ooled human plasma, serum, and chicken serum," "to evaluate the potential cause for release of covalently bound Staphylococcal protein A (SpA) from a silica based extracorporeal immunoadsorbent matrix." *Id.* at 85–86; *see id.* at 86 (detailing the protein A–matrix coupling process).

Balint reports that protein A was released from the protein A affinity matrix "in a linear fashion with time . . . indicat[ing] that mere binding of mammalian IgG to the immunoadsorbent is not required for the release of [protein A]." *Id*. at 88. Based on studies involving the addition of either (1) formalin (as a general stabilizer and protease inhibitor) or (2) a cocktail of protease inhibitors to the serum samples, Balint concludes that the protein A leaching was due to inherent endogenous proteolytic activity, which cleaved protein fragments from the chromatography matrix. *Id*. at 88–89.

Patent Owner does not dispute that Balint teaches that protein A leaching is caused by proteolytic cleavage, but argues that Balint is not analogous art and, thus, should not be considered prior art with respect to the claimed invention. PO Resp. 44–49; Ex. 1022 at 147:4–23. "Two separate tests define the scope of analogous prior art: (1) whether the art is from the same field of endeavor, regardless of the problem addressed and, (2) if the reference is not within the field of the inventor's endeavor, whether the reference still is reasonably pertinent to the particular problem with which the inventor is involved." *In re Bigio*, 381 F.3d 1320, 1325 (Fed. Cir. 2004) (citations omitted).

With respect to the first of these tests, Patent Owner argues that Balint is not within the same field of endeavor because it was published in the journal *Transfusion Science* and concerned therapeutic applications in the "field of apheresis" rather than "protein purification," "bioprocessing" or, as described by Dr. Cramer, "the industrial purification of therapeutic proteins." PO Resp. 45–46; Ex. 2008 ¶¶ 47, 160. With respect to the second test, Patent Owner argues that Balint is not reasonably pertinent to the particular problem with which the inventor is involved insofar as Balint used protein A bound to a silica-based matrix. PO Resp. 47 (citing Ex. 2008

¶ 160; Ex. 2010, 158:9–160:24). According to Patent Owner, such composition "would be unthinkable in the field of bioprocessing because . . . a silica-based matrix would be destroyed by the harsh (very basic) washing conditions used to regenerate protein A columns." *Id*.

For the reasons set forth at pages 21–24 of Petitioner's Reply we do not find Patent Owner's arguments with respect to Balint persuasive. We note, for example, that Balint was repeatedly cited by the Examiner during prosecution. Ex. 1010, 50–52, 70–71 (rejections involving Balint). Applicants did not argue that Balint was nonanalogous, but responded to the rejections with the apparent understanding that Balint was prior art. *See id.* at 54–62 (cancelling claims in view of the Examiner's rejections), 73–81 (arguing rejection on the merits); Pet. Reply 22. Accordingly, we infer that the Examiner—as well as the inventors—considered Balint at least reasonably pertinent to the particular problem addressed in the '799 patent.

We also agree with Petitioner that protein A chromatography is not limited to protein purification, and the challenged claims are not limited to industrial purification of therapeutic proteins. *See* Pet. 22–23. Nor does our understanding of the challenged claims demand a column matrix be capable of regeneration or prohibit the use of silica-based matrices. To the contrary, the Specification expressly provides that the solid phase matrix "may comprise . . . silica." *See* Ex. 1001, 4:41–47.

Nor, as we have discussed, above, in section II(B)(i), are the challenged claims limited to the use of HCCF. Rather, the Specification provides that antibodies may be separated from the "culture medium, ascites fluid, *or serum* by . . . for example, protein A-Sepharose . . . . Preferably the protein A affinity chromatography procedure described herein is used." *Id.* at 10:61–67 (emphasis added); *see also id*. at 7:50–55, 9:43–10:5, 12:47–64,

12:65–14:36 (indicating that the invention is applicable to purification from a variety of compositions including whole animal serum, proteolytic digests, and chemical cross-linking reactions).

Patent Owner further argues that Balint is not reasonably pertinent because it "report[s] on clinical testing of an immunoadsorbent column marketed as a medical device called 'PROSORBA' . . . . [used for] extracting unwanted antibodies from a patient's blood as it was removed and then returning it to the body by way of intravenous tubing." PO Resp. 45 (citing Ex. 2008 ¶ 150); *see* also, *id.* at 47–48 ("[t]here would have been no reason for the POSA to think that what happened when blood was poured on a silica-based column would have any pertinence to what would happen when HCCF was poured on a protein A column made of different material"); Tr. 29:9–26 (arguing that "the material being purified [in Balint] is human blood").

Patent Owner's attempts to distinguish Balint as limited to the purification of blood are inapposite because Balint described experiments using not blood, but "[p]ooled human plasma, serum, and chicken serum." Ex. 1005, 86. Nor, as Patent Owner appears to suggest, is Balint directed to the analysis of clinical trials, but to the results of *in vitro* testing on the effect of protease inhibitors in reducing the leaching from protein A coupled to a silica matrix.

Thus, based on the record before us, we agree with Petitioner that Balint is within the field of the invention and reasonably pertinent to the particular problem addressed by the inventors. Accordingly, because Balint was published more than one year before the priority date of the '799 patent, Balint qualifies as prior art under 35 U.S.C § 102(b).

Further, although Petitioner relies on Balint as disclosing proteolysis as a cause of protein A leaching in protein A chromatography, we find that this was otherwise known in the prior art. Dr. Cramer, for example, conceded at his deposition that two references, dated prior to the critical date of the '799 patent, suggested proteolysis as the cause of protein A leaching from Protein A affinity columns. *See* Pet. Reply 21; Ex. 1020 ¶¶ 60–61; Ex. 1022, 213:2–8, 220:1–24, 221:7–23, 224:15–23; 225:3–15; Ex. 1017, 212; Ex. 1018, 172. Dr. Cramer's testimony is confirmed by our own reading of those references. Gagnon asserts that protein A chromatography columns are "notorious for leaching" and, in a section titled "leaching by proteolysis," discloses that:

> Leaching occurs by 3 different pathways: breakdown of the support matrix, breakdown of the immobilization linkage, and proteolytic cleavage of the interdomain sequences of protein A. . . . The occurrence of leakage with even commercially purified polyclonal IgG preparations probably reflects their ubiquitous contamination with proteases.
>
> * * *
>
> Other indications that proteolysis is the primary leakage pathway include the fact that leaching is often highly elevated in the first run after storage of used media. . . . Elevated leakage is likewise seen when feedstreams carry high protease loads, such as when there has been a large amount of cell lysis.

Ex. 1018, 172–173.[9]

Guerrier similarly notes the link between proteolysis and protein A leaching. Guerrier discusses hydrophobic charge induction chromatography as an alternative to protein A affinity chromatography. Ex. 1017, Abstract,

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[9] Gagnon, P. Chapter 9, "Protein A Affinity Chromatography," in *Purification Tools for Monoclonal Antibodies*. © Validated Biosystems (1996).

211–212.[10] With respect to the latter, Guerrier notes that "as chromatographers have been called upon to design schemes for process-scale purification of antibodies, various practical complications associated with Protein-A chromatography have come under increasing scrutiny." *Id.* at 211. For example, "Protein A is subject to degradation by proteases present in the feedstocks" and "[l]eaching of Protein A (or fragments) must be addressed in the overall scheme." *Id.* at 212.

For the above reasons, we conclude that one of ordinary skill in the art, as of the filing date of the '799 patent, understood that proteolysis is a known cause of protein A leaching in protein A chromatography.

iv. Potier (Ex. 1006) and the Relationship between Temperature and Proteolytic Cleavage

Potier investigates temperature-dependent changes in proteolytic activities in the bacterium *Arthrobacter globiformis* $S_1$55. Ex. 1006, 283. In one set of experiments, the authors determined that with increasing temperature, insulin– and casein–degrading protease activities showed "similar and expected increases in activity," up to 30° C. *Id*. at 286, Fig. 1a.

According to Patent Owner, "Potier adds nothing" to Petitioner's case. PO Resp. 49–50; *see id.* at 49 ("[Petitioner cites] Potier, for the unremarkable proposition that the POSA would have known that proteolytic activity increases with temperature."). To the contrary, we find that it underscores and exemplifies Dr. Przybycien's opinion that as of the filing date of the '799 patent, "a POSA would have known, based on the general knowledge available to those skilled in the art, that reactions such as

[10] Guerrier et al. *New method for selective capture of antibodies under physiological conditions*. 9 Bioseparation 211 (2000).

proteolysis are temperature dependent, and that decreasing the temperature would decrease proteolysis." *See* Ex. 1002 ¶ 103; *see also id*. ¶ 49; Ex. 1020 ¶ 62; Ex. 2045 at 289:5–20.

Accordingly, we conclude that one of ordinary skill in the art, as of the filing date of the '799 patent, understood that proteolysis was temperature dependent such that decreasing temperature would decrease proteolysis.

v. Obviousness in view of WO '389 (Ground 3)

Petitioner asserts that claims 1 and 5 would have been obvious under 35 U.S.C. § 103(a) over WO '389 in view of the background knowledge of one of ordinary skill in the art. Pet. 6, 37–39; Pet. Reply 17–21. Patent Owner opposes. *See* PO Resp. 34–55.

Petitioner argues that WO '389 teaches that protein A chromatography may be used to purify antibodies at "about 18° C.," which overlaps with the claimed range of "about 10° C. to about 18° C." and, thus, absent evidence that the claimed range is critical (*see* Ex. 1002 ¶¶ 87–89), renders claims 1 and 5 obvious. Pet. 37–38. Petitioner further argues that one of ordinary skill in the art would have understood that protein A chromatography could be carried out at 18° C or lower, and that proteolysis is reduced at lower temperatures. *Id*. at 38–39 (citing Ex. 1002 ¶¶ 102–104). Accordingly, Petitioner contends that it would have been obvious to conduct protein A chromatography at the lower temperatures set forth in claim 1 in order to reduce proteolysis. *See id*; *see also id*. at 39 (arguing that "it would have been obvious to try conducting protein A chromatography at the claimed range in order to observe whether lower temperatures could affect unwanted leaching of protein A").

Patent Owner responds that one of ordinary skill in the art would not have sought to modify WO '389 because the reference already teaches a downstream process to remove leached protein A that avoids the expense and inconvenience of conducting chromatography at reduced temperatures. PO Resp. 35–36, 50–51. Patent Owner makes a similar argument with respect to Balint's suggestion to reduce protein A leaching by adding protease inhibitors. *Id.* at 51. Quoting Dr. Cramer, Patent Owner argues that "it 'would not make sense to the POSA, to consider modifying these processes *further* as part of 'routine optimization.'" *Id.* at 36 (quoting Ex. 2008 ¶ 129). To the contrary, we agree with Petitioner that:

> The fact that means for reducing leached protein A—such as additional purification steps, or employing protease inhibitors—were available, would not prevent a POSA from seeking additional solutions to the problem. (Ex. 1020, ¶¶69–71). Even today, increasing purity is the focus of protein A chromatography optimization. (Ex. 1021 at 48:2–5).

Pet. Reply 19.

Because one of ordinary skill in the art would have recognized that proteolysis resulted in the degradation of matrix-bound protein A (as illustrated in Balint, Gagnon, and Guerrier), and that proteolysis is inherently temperature dependent (as illustrated in Potier), the skilled artisan would have recognized that the temperature for conducting protein A chromatography was a result effective variable. *See* Pet. 39; Pet. Reply 18 (citing Ex. 1002 ¶¶ 83–84; Ex. 1020 ¶ 67); *see also*, Ex. 2006, 310 (binding capacity of protein A affinity columns are "affected by many variables, including . . . column temperature"); Ex. 1004, 146–147 (temperature a result effective variable with respect to binding capacity for some antibodies, and under some buffer conditions). That WO '389 suggests removing

leached protein A by subjecting the eluate of a protein A column to hydrophobic interaction chromatography does not negate the motivation to develop other, possibly faster, simpler, or less expensive solutions to the problem.

In light of the above, and "[g]iven the ease with which temperature can be varied, it would have been obvious to try conducting protein A chromatography at the claimed range in order to observe whether lower temperatures could affect unwanted leaching of protein A" or the degradation of the desired antibody product. *See id*. (citing Ex. 1002 ¶ 103). In this respect, we do not find persuasive Patent Owner's argument that the inventors developed a system of temperature adjustment in order to precisely control the temperature of their chromatography experiments. *See* PO Resp. 40–41; Ex. 2009 ¶ 71 (estimating that it took four weeks and 150 man hours to set up and conduct lab-scale experiment similar to those disclosed in the '799 patent). Considering the record as a whole, we conclude that exploring the temperature dependence of protein A leaching is not more than routine experimentation. *See, e.g.*, Ex. 1002 ¶ 35; Ex. 1020 ¶ 68 (well known to regulate chromatography column temperature by using refrigerated HCCF and chromatography buffers, and/or conducting the procedure in jacket-cooled chromatography columns, refrigerated spaces, or temperature-controlled water baths).

Patent Owner also argues the secondary considerations of unexpected results and recognition by others in the field. PO Resp. 53–55. We do not find these arguments persuasive. With respect to the temperature range set forth in WO '389, even a slight overlap in range may establish obviousness unless there is evidence of unexpected results to show criticality in the claimed range. *See In re Peterson*, 315 F.3d 1325, 1329, 1330 (Fed. Cir.

2003). But where the general conditions of a claim are disclosed in the art, "it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Applied Materials, Inc.*, 692 F.3d 1289, 1295 (Fed. Cir. 2012) (citing *In re Aller*, 220 F.2d 454, 456 (C.C.P.A. 1955)). As discussed in section II(C)(ii)(2)(c), above, we do not find the evidence to support that the claimed temperature range achieves unexpected results or is critical to the claimed purification method.

We also find unpersuasive Patent Owner's remaining evidence of secondary considerations. In this respect, Patent Owner relies on the selection of a presentation relating to the claimed method for oral presentation at the 2005 National Meeting of the Division of Biochemical Technology of the American Chemical Society. PO Resp. 53–55; Ex. 2012. According to Patent Owner, this reflects "[i]ndustry praise" and "[r]recognition by one's peers." *Id.* at 54–55. But Patent Owner does not establish *why* the presentation was selected; why the meeting was particularly prestigious, how the presentation was received by the attendees, or whether the disclosure has been relied on by others in the field, or been the subject of recognition or praise since the 2005 presentation. *See* Pet. Reply 24–25. For these reasons we accord little weight to Patent Owner's evidence of secondary considerations.

Considering the record as a whole, we conclude that Petitioner has demonstrated by a preponderance of evidence that claims 1 and 5 of the '799 patent would have been obvious in view of WO '389.

vi. Obviousness in view of WO '389, Balint, and Potier (Ground 4)

Petitioner asserts that claims 1–3 and 5 would have been obvious under 35 U.S.C. § 103(a) in view of WO '389, Balint, and Potier (Ground

4). Pet. 7, 40–44; Pet. Reply 17–21. Patent Owner opposes. PO Resp. 34–55.

Petitioner's arguments with respect to Ground 4 are largely the same as for Ground 3, except that Petitioner relies expressly on Balint and Potier. In this respect, Petitioner contends that "Balint teaches that protein A leaching following affinity chromatography 'is due to inherent endogenous proteolytic activity which cleaves protein fragments from the matrix'" (Pet. 41–42 (citing Ex. 1005, 4)); it was known in the art that lower temperatures tend to reduce protease activity (*id*. at 42 (citing Ex. 1002 ¶¶ 87, 105)); and Potier expressly demonstrates increasing proteolytic activity with increasing temperature (*id*. (citing Ex. 1006, 7, 9; Ex. 1002 ¶ 105)). One of ordinary skill in the art would, therefore, have understood that lowering temperature reduces the activity of proteases and consequently reduces "protein A leaching." *Id*. (citing Ex. 1002 ¶ 105). As with Ground 3, the skilled artisan

> would have been motivated to practice the protein A chromatography at intermediate temperatures such as the claimed range, rather than the coldest available range. The predictable temperature dependence of protein A leaching follows an exponential Arrhenius curve, which means that relatively small changes in protein A reduction are observed at lower temperatures. In view of these diminishing returns, and the higher cost and effort required to maintain very cold temperatures, finding an optimal middle range would have been nothing more than routine experimentation.

Id. at 42–43 (internal citations to Ex. 1002 ¶ 104 omitted).

With respect to claims 2 and 3, Petitioner argues that one of ordinary skill in the art would have been motivated to include the protease inhibitor EDTA as taught by Balint "to further reduce the leakage of protein A—thereby preserving costly column materials while obtaining effective purification of the target antibody." *Id*. at 43 (citing Ex. 1002 ¶ 108).

Patent Owner's arguments with respect to Ground 3 apply equally with respect to Ground 4, as does our analysis. *See, e.g.*, PO Resp. 55. For the reasons set forth in section II(D)(v), above, we conclude that Petitioner has demonstrated by a preponderance of evidence that claims 1, 2, and 5 of the '799 patent are obvious in view of WO '389, Balint, and Potier.

vii. Obviousness in view of WO '389 and the '526 Patent (Ground 5)

Petitioner asserts that claims 2, 3, and 6–11 would have been obvious under 35 U.S.C. § 103(a) in view of WO '389 and the '526 Patent. Pet. 7, 44–49; Pet. Reply 17–21. Patent Owner opposes. PO Resp. 34–55. As the teachings of WO '389 and the knowledge of those of ordinary skill in the art regarding the links between protein A leaching, proteolysis, and temperature have been discussed above, we begin with an overview of the '526 Patent.

1. Overview of the '526 Patent

The '526 Patent discloses "a method for purifying C_H2/C_H3 region-containing proteins, such as antibodies and immunoadhesins, by Protein A affinity chromatography." Ex. 1007, 1:9–14. The invention comprises the steps of (a) adsorbing the protein to protein A immobilized on a solid phase comprising silica or glass; (b) removing contaminants bound to the solid phase by washing the solid phase with a hydrophobic electrolyte solvent; and (c) recovering the protein from the solid phase. *Id*. at 2:28–37. Buffers used in the practice of the method may include the protease inhibitor EDTA. *See id*. at 3:33–39, 14:27–30.

"In preferred embodiments, the protein is an antibody (e.g. an anti-HER2, anti-IgE or anti-CD20 antibody) or an immunoadhesin (e.g. a TNF receptor immunoadhesin)." *Id*. at 2:38–40; *see* 13:67–14:6.

> Preferred molecular targets for antibodies encompassed by the present invention include . . . members of the ErbB receptor

> family such as the EGF receptor, HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-1, Mac1, p150,95, VLA-4, ICAM-1, VCAM and αv/β3 integrin including either α or β subunits thereof (e.g. anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors such as VEGF; IgE . . . .

Id. at 6:13–20. Example 1 of the '526 Patent involves protein A chromatography of the C_H2/C_H3 region containing protein; humanized anti-HER2 antibody (humAb4D5-8). *Id*. at 15:22–24.

2. Analysis of Ground 5 Under 35 U.S.C. 103(a)

Petitioner's arguments with respect to Ground 5 are largely the same as for Ground 3, except regarding dependent claims 2, 3, and 6–11. With respect to dependent claims 2 and 3, Petitioner argues that:

> The '526 Patent additionally discloses including EDTA in the buffer used to equilibrate the solid phase for the protein A chromatography. ([Ex. 1007] at 3:34–35; 14:27–30.) A POSA, knowing EDTA to be a commonly used chelator and protease inhibitor, would immediately have appreciated the benefits of including EDTA in the buffer for the purpose of reducing impurities. (Ex. 1002, Przybycien Decl. at ¶ 110.) Therefore, it would have been obvious to combine the teachings of WO '389 and the '526 Patent as discussed here, in order to optimize the chromatography process while using only common excipients widely known in the prior art. (*Id*.)

Pet. 45. With respect to dependent claims 6–11, Petitioner further points to the '526 Patent's disclosure of specific C_H2/C_H3 region-containing antibodies and immunoadhesins that may be purified using protein A affinity chromatography. *See* Pet. 45–49.

Patent Owner does not address the '526 Patent with any degree of specificity, and its arguments with respect to Grounds 3 and 4 apply equally with respect to Ground 5, as does our analysis. *See, e.g.*, PO Resp. 56. Considering the record as a whole, and for the reasons set forth with

particularity in section II(D)(v), above, we conclude that Petitioner has demonstrated by a preponderance of evidence claims 2, 3, and 6–11 would have been obvious under 35 U.S.C. § 103(a) in view of WO '389 and the '526 Patent.

viii. Obviousness in view of WO '389, Balint, Potier, and the '526 Patent (Ground 6)

Petitioner asserts that claims 2, 3, and 6–11 are obvious under 35 U.S.C. § 103(a) in view of WO '389, Balint, Potier, and the '526 Patent. Pet. 7, 49–51; Pet. Reply 17–21. Patent Owner opposes. PO Resp. 34–55.

Patent Owner does not address Ground 6 separately from Grounds 3–5, and its arguments with respect to those grounds apply equally with respect to Ground 6, as does our analysis. *See, e.g.*, PO Resp. 56. Considering the record as a whole, and for the reasons set forth with particularity in section II(D)(v), above, we conclude that Petitioner has demonstrated by a preponderance of evidence claims 2, 3, and 6–11 would have been obvious under 35 U.S.C. § 103(a) in view of WO '389, Balint, Potier, and the '526 Patent.

ix. Obviousness in view of van Sommeren (Ground 7)

Petitioner asserts that claims 1, 2, and 5 would have been obvious under 35 U.S.C. § 103(a) in view of van Sommeren. Pet. 7, 51–53; Pet. Reply 17–21. Patent Owner opposes. *See* PO Resp. 34–55.

Petitioner's arguments with respect to Ground 7 (based on van Sommeren) are essentially the same as those with respect to Ground 3 (based on WO '589) but add an additional reason that one of ordinary skill in the art would be motivated to practice protein A chromatography at the claimed range, which Patent Owner addresses on pages 37–38 of its Response.

Our analysis of van Sommeren is set forth in section II(C)(iii), above, in the context of anticipation. Because the bulk of Patent Owner's arguments regarding obviousness address van Sommeren and WO '589 together, we further rely on the analysis set forth in section II(C)(ii), above, and address Petitioner's additional argument below.

In short, Petitioner argues that "van Sommeren anticipates claims 1 and 5 because it discloses purifying an antibody using protein A chromatography at temperatures that overlap with the claimed range of about 10° C to about 18° C." Pet. 51. Petitioner argues that there is nothing critical about the claimed temperature range. *Id.* at 51–52, (citing Ex. 1002 ¶¶ 34–35, 121) (indicating that the 4°C and 20–25° C disclosed in van Sommeren are merely convenient temperatures found in laboratory settings, and there is no evidence that researchers actively sought to avoid intermediate temperatures). Petitioner argues that, to the extent temperature ranges disclosed in van Sommeren "were not deemed anticipatory, other disclosures in van Sommeren render the claimed range of about 10° C to about 18° C obvious." *Id.*

Similar to its argument in Ground 3 with respect to WO '589, Petitioner argues that because van Sommeren's disclosure that contamination due to proteolysis was a known problem (*see* Ex. 1004, 147–148), it would have been obvious "to try temperatures within the claimed range, since temperature is an easily varied condition, in order to see if lower temperature could affect contamination caused by proteolysis." Pet. 52 (citing Ex. 1002 ¶ 120); *see also* sections II(D)(iii) and (iv), above (finding that one of ordinary skill in the art would have understood that proteolysis is temperature dependent and a well-known cause of protein A leaching). For the reasons discussed in section II(D)(v), above, we find Petitioner's

argument sufficient to establish a reason to practice protein A chromatography in the claimed range.

Petitioner also argues that in light of van Sommeren's teaching that conducting protein A chromatography at 4° C improves the binding of certain antibodies as compared to room temperature, one of ordinary skill in the art "would have appreciated that lowering the temperature of the process below ambient temperature could enhance its performance, and would have been motivated to determine a more optimal range using routine experimentation." Pet. 51–52 (citing Ex. 1002, ¶ 119); *see* Ex. 1004, 145–147.

Responding to the latter argument, Patent Owner states that "[w]hile binding capacity may well have been a reasonable target for optimization efforts," one of ordinary skill in the art "'would have understood . . . that temperature had an unpredictable, typically relatively minor effect on dynamic binding capacity' and that it 'was not an important or reasonable parameter to investigate if the POSA were trying to improve dynamic binding capacity.'" PO Resp. 37–38 (quoting Ex. 2008 ¶¶ 101–02).

We do not find Patent Owner's arguments persuasive in light of van Sommeren's teaching that for IgG_1–class antibodies, binding may be as much as 5 fold higher at 4°C as compared to 20–26°C under some buffer conditions. *See* Ex. 1004, 146–147; *see also*, Ex. 2006, 310 (indicating that temperature is a result effective variable with respect to protein A capacity). In particular, referencing other prior art, van Sommeren states: "When adso[r]ption buffers of relatively low ionic strength are used, improvement of the binding of IgG_1 antibodies to protein A can also be obtained by lowering the temperature." Ex. 1004, 136; *see id.* at 146 ("For the IgG_1 mabs however, [a prior art reference] reported a five times higher binding

capacity of protein A Sepharose for mouse monoclonal lgG1 antibodies at 4 ℃ in comparison with 20-26 ℃, using a 0.1 M sodium phosphate binding buffer (pH 8.2).").

In contrast to the five-fold increase in binding in low ionic strength buffers shown by others, van Sommeren reports that where absorption is performed in a high ionic strength buffer (1.5 M glycine, 3.0 M NaCl at pH 8.9), "the temperature effect on the IgG_1 binding capacity becomes of minor importance." *Id.* at 147; *see id.* at 144, Table V (up to 30 or 40% increase in binding capacity at 4 ℃ for some IgG1 antibodies; no change, or decrease for others). Patent Owner does not, however, explain why one of ordinary skill in the art would choose to employ the buffer conditions used in van Sommeren, rather than, for example, the "0.1 M sodium phosphate binding buffer (pH 8.2)" reportedly associated with a five-fold increase in protein A binding capacity at lower temperatures. *See id.* at 146. Nor are we persuaded that one of ordinary skill in the art would not have been motivated by the more modest temperature-dependent increases reported by van Sommeren in a high ionic strength buffer. *See* Ex. 2008 ¶ 102, 141–43 (referencing development of "industrial purification process[es]").

Considering the record as a whole, and for the reasons set forth with particularity in section II(D)(v), above, with respect to WO '389, we conclude that Petitioner has demonstrated by a preponderance of evidence claims 1, 2, and 5 would have been obvious under 35 U.S.C. § 103(a) in view of van Sommeren.

x. Obviousness in view of van Sommeren and the '526 Patent (Ground 8)

Petitioner asserts that claims 3 and 6–11 would have been obvious under 35 U.S.C. § 103(a) in view of van Sommeren and the '526 Patent.

Pet. 7, 53–57. Patent Owner opposes. *See* PO Resp. 56. Petitioner asserts that "[i]t would have been obvious to use the protein A chromatography method of van Sommeren to purify the claimed C_H2/C_H3 region-containing antibodies and immunoadhesins as disclosed in the '526 Patent for the same reasons discussed above with regard to WO '389." Pet. 57 (citing Ex. 1002, ¶¶ 115, 126). We agree with Petitioner.

Considering the record as a whole, and for the reasons set forth with particularity in sections II(D)(v) and (ix), above, we conclude that Petitioner has demonstrated by a preponderance of evidence claims 3 and 6–11 would have been obvious under 35 U.S.C. § 103(a) in view of van Sommeren and the '526 Patent.

III. (PRESUMPTIVE) MOTIONS TO SEAL

The parties have filed Paper 22 (Patent Owner's Response), Paper 28 (Petitioner's Reply), and Exhibits 1020, 2008, 2009, 2011, 2016–2018, and 2029 under seal, along with redacted versions of Papers 22 and 28, and Exhibits 2008 and 2009. The Office Patent Trial Practice Guide states:

> 3. A party intending a document or thing to be sealed may file a motion to seal concurrent with the filing of the document or thing. § 42.14. The document or thing will be provisionally sealed on receipt of the motion and remain so pending the outcome of the decision on motion.
>
> 4. *Protective Orders:* A party may file a motion to seal where the motion contains a proposed protective order, such as the default protective order in Appendix B. § 42.54. Specifically, protective orders may be issued for good cause by the Board to protect a party from disclosing confidential information. § 42.54. Guidelines on proposing a protective order in a motion to seal, including a Standing Protective Order, are provided in Appendix B. The document or thing will be protected on receipt of the motion and remain so, pending the outcome of the decision on motion.

Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,760 (Aug. 14, 2012). Although the redacted material in Papers 22 and 28, and Exhibits 1020, 2008, 2009, 2011, 2016–2018, and 2029, appears to relate to Patent Owner's confidential information, none of these submissions are accompanied by a corresponding motion to seal, statement of good cause, or reference to any protective order. We, nonetheless, interpret the parties' sealed and redacted filings as presumptive motions to seal under our default Standing Protective Order.

"There is a strong public policy for making all information filed in a quasi-judicial administrative proceeding open to the public, especially in an inter partes review which determines the patentability of claims in an issued patent and therefore affects the rights of the public." *Garmin Int'l v. Cuozzo Speed Techs.*, LLC, IPR2012–00001, slip op. at 1–2 (PTAB Mar. 14, 2013) (Paper 34). For this reason, except as otherwise ordered, the record of an *inter partes* review trial shall be made available to the public. *See* 35 U.S.C. § 316(a)(1); 37 C.F.R. § 42.14. Motions to seal may be granted for good cause; until the motion is decided, documents filed with the motion shall be sealed provisionally. *See* 37 C.F.R. §§ 42.14, 42.54(a). The moving party bears the burden of showing that there is good cause to seal the record. *See* 37 C.F.R. § 42.20(c).

As set forth in the Board's Trial Practice Guide, confidential information that is sealed subject to a protective order ordinarily will become public 45 days after final judgment in a trial. Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,761 (Aug. 14, 2012). A party seeking to maintain confidentiality of information may file a motion to expunge the information before it becomes public; however, if the existence of the information is identified in a final written decision following trial,

there is an expectation that the information will be made public. *Id.* This rule "balances the needs of the parties to submit confidential information with the public interest in maintaining a complete and understandable file history for public notice purposes." *Id.*

Under the Board's procedures, there is an expectation that all exhibits, including those filed under seal here, will be made part of the public record. Furthermore, the public's interest in understanding the basis for our decision on patentability means that any good cause alleged in a motion to seal must overcome this heightened public interest. As neither party has formally filed a motion, no argument of record suggests good cause for sealing any document filed in this case. Because the Patent Owner Response and Petitioner's Reply are critical to our analysis, and to the public's understanding of the instant Opinion, the presumptive motions to seal are denied with respect to Papers 22 and 28.

We also deny the presumptive motions to seal with respect to Exhibits 1020, 2008, 2009, 2011, 2016–2018, and 2029. The normal consequence of a denial of a motion to seal would be to immediately unseal these documents. However, because the public release of documents would be irreversible, either party may file, within ten business days of this Decision, a motion to seal, addressing its justification for sealing one or more of these documents. Any such motion may be accompanied by narrowly redacted public versions of the exhibits sought to be sealed, which may be substituted for the redacted exhibits of record.

In the absence of any action on the part of a party, at the expiration of ten days from the date of this Decision, Exhibits 1020, 2008, 2009, 2011, 2016–2018, and 2029 will be made available to the public.

IV. CONCLUSION

Having weighed Petitioner's claim charts, arguments, and evidence as to those claims against Patent Owner's countervailing arguments and evidence, we determine that Petitioner has established by a preponderance of the evidence the unpatentability of claims 1–3 and 5–11 of the '799 Patent.

V. ORDER

For the above reasons, it is

ORDERED that claims 1 and 5 of the '799 Patent are unpatentable under 35 U.S.C. § 102(b) by WO '389;

FURTHER ORDERED that claims 1, 2, and 5 of the '799 Patent are unpatentable under 35 U.S.C. § 102(b) by van Sommeren;

FURTHER ORDERED that claims 1 and 5 of the '799 Patent are unpatentable under 35 U.S.C. § 103(a) in view of WO '389;

FURTHER ORDERED that claims 1–3, and 5 of the '799 Patent are unpatentable under 35 U.S.C. § 103(a) in view of WO '389, Balint, and Potier;

FURTHER ORDERED that claims 2, 3, and 6–11 of the '799 Patent are unpatentable under 35 U.S.C. § 103(a) in view of WO '389 and the '526 Patent;

FURTHER ORDERED that claims 2, 3, and 6–11 of the '799 Patent as unpatentable under 35 U.S.C. § 103(a) in view of WO '389, Balint, Potier, and the '526 Patent;

FURTHER ORDERED that claims 1, 2 and 5 of the '799 Patent are unpatentable under 35 U.S.C. § 103(a) in view of van Sommeren;

FURTHER ORDERED that claims 3 and 6–11 of the '799 Patent are unpatentable under 35 U.S.C. § 103(a) in view of van Sommeren and the

’526 Patent.

FURTHER ORDERED that, within ten business days of this Order, either party may file a renewed motion to seal Exhibits 1020, 2008, 2009, 2011, 2016–2018, and 2029.

FURTHER ORDERED that, because this is a final written decision, parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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(12) **United States Patent**
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(54) **REDUCING PROTEIN A LEACHING DURING PROTEIN A AFFINITY CHROMATOGRAPHY**

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Related U.S. Application Data

(63) Continuation of application No. 10/877,532, filed on Jun. 24, 2004, now Pat. No. 7,485,704.

(60) Provisional application No. 60/490,500, filed on Jul. 28, 2003.

(51) **Int. Cl.**
C07K 16/00 (2006.01)

(52) **U.S. Cl.** **530/413**; 530/412; 530/387.1

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

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(57) **ABSTRACT**

A method for reducing leaching of protein A during protein A affinity chromatography is described which involves reducing temperature or pH of, or by adding one or more protease inhibitors to, a composition that is subjected to protein A affinity chromatography.

12 Claims, 6 Drawing Sheets

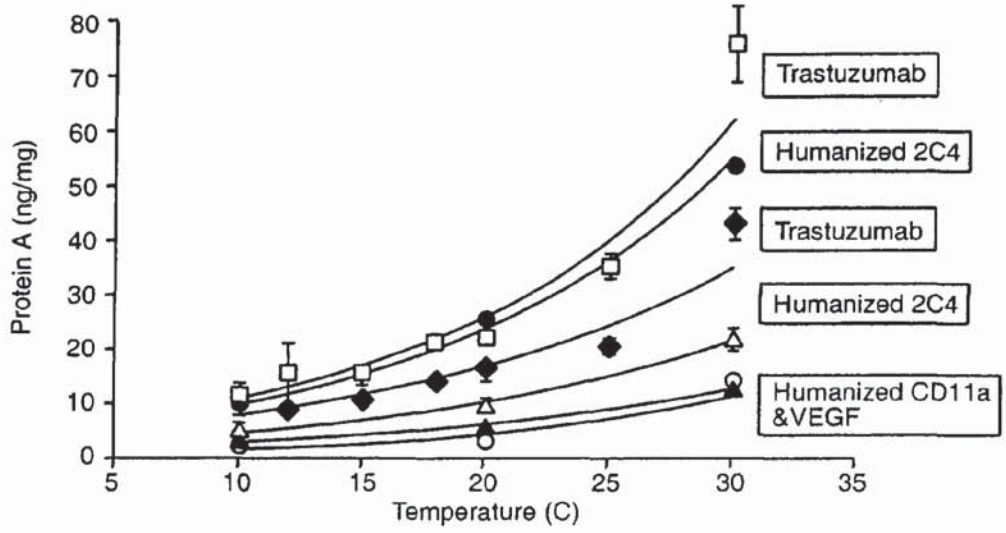
Protein A (ng/mg)
Temperature (C)
Trastuzumab
Humanized 2C4
Trastuzumab
Humanized 2C4
Humanized CD11a &VEGF

FIG._1

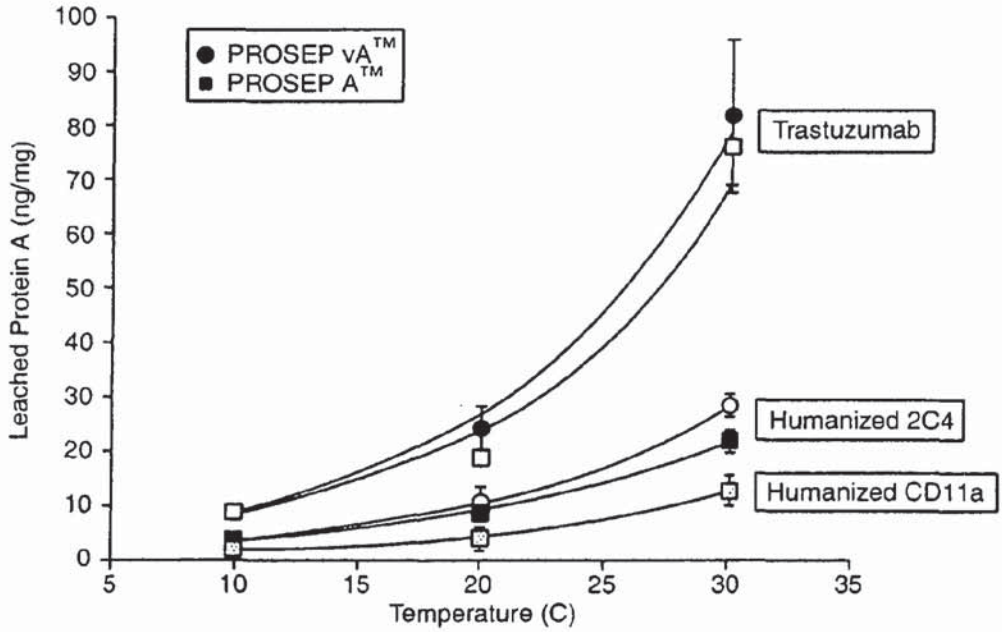
PROSEP vA™
PROSEP A™
Leached Protein A (ng/mg)
Temperature (C)
Trastuzumab
Humanized 2C4
Humanized CD11a

FIG._2

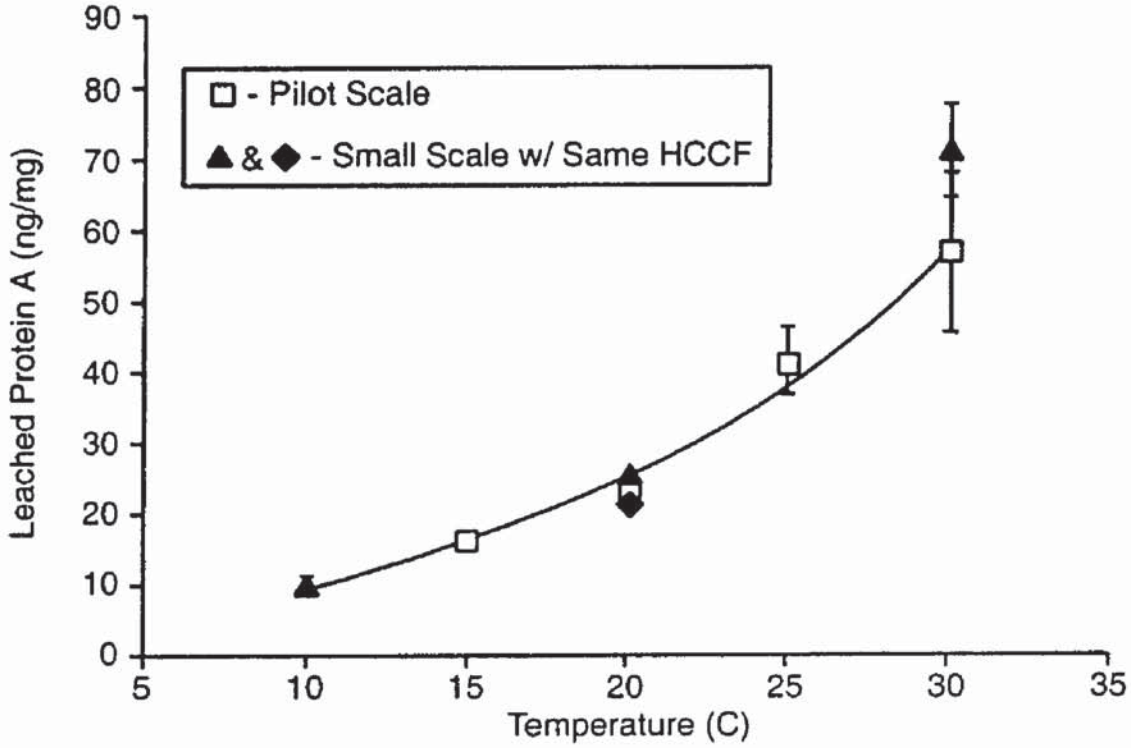
□ - Pilot Scale
▲ & ◆ - Small Scale w/ Same HCCF
Leached Protein A (ng/mg)
Temperature (C)
0
10
20
30
40
50
60
70
80
90
5
10
15
20
25
30
35

FIG._3

LIGHT CHAIN

```
1   D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K
46  L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q
91  H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L
136 L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T
181 L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
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(SEQ ID NO: 1)

FIG._4A

HEAVY CHAIN

```
1                15                30                45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L
46               60                75                90
E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D
91               105               120               135
T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S
136              150               165               180
K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S
181              195               210               225
G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K
226              240               255               270
T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S
271              285               300               315
H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D
316              330               345               360
W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E
361              375               390               405
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G
406              420               435               449
S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
                                                    (SEQ ID NO: 2)
```

FIG._4B

DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYR
YTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIK

(SEQ ID NO: 3)

FIG._5A

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPN
SGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDY
WGQGTLVTVSS

(SEQ ID NO: 4)

FIG._5B

DIQMTQSPSSLSASVGDRVTITCRASKTISKYLAWYQQKPGKAPKLLIYSGSTL
QSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNEYPLTFGQGTKVEIKR

(SEQ ID NO: 5)

FIG._6A

EVQLVESGGGLVQPGGSLRLSCAASGYSFTGHWMNWVRQAPGKGLEWVGMIHPS
DSETRYNQKFKDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARGIYFYGTTYF
DYWGQGTLVTVSS

(SEQ ID NO: 6)

FIG._6B

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSL
HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR

(SEQ ID NO: 7)

FIG._7A

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTY
TGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHW
YFDVWGQGTLVTVSS

(SEQ ID NO: 8)

FIG._7B

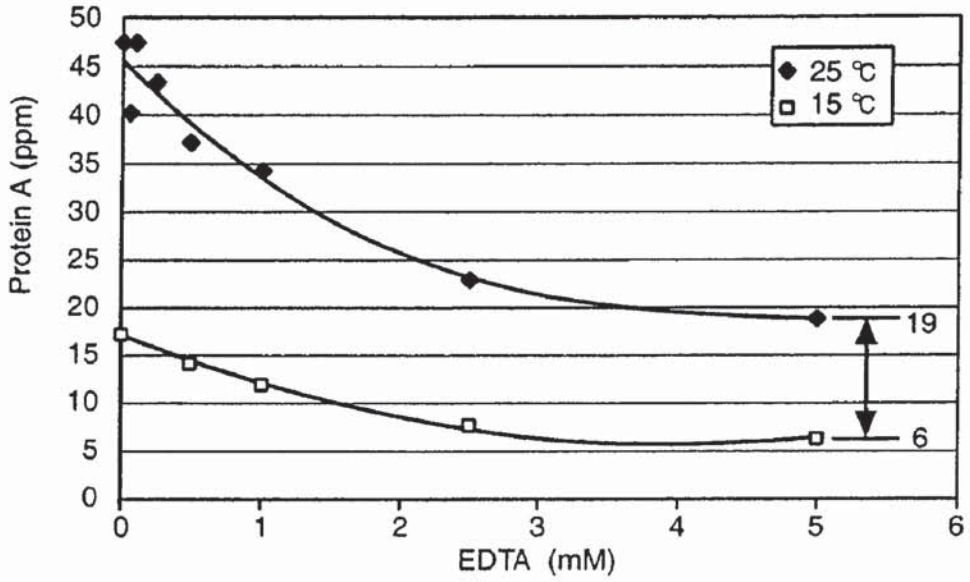
Protein A (ppm)
EDTA (mM)
25 °C
15 °C
19
6
0
5
10
15
20
25
30
35
40
45
50
0
1
2
3
4
5
6

FIG._8

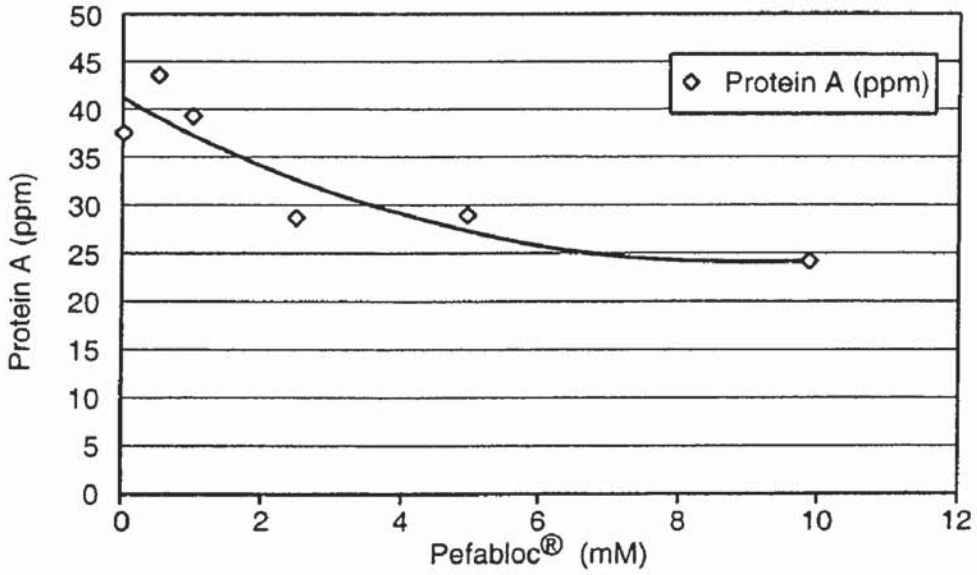
Protein A (ppm)
Pefabloc® (mM)
Protein A (ppm)
0
5
10
15
20
25
30
35
40
45
50
0
2
4
6
8
10
12

FIG._9

REDUCING PROTEIN A LEACHING DURING PROTEIN A AFFINITY CHROMATOGRAPHY

This application is a continuation under 37 C.F.R. §1.53(b) of U.S. patent application Ser. No. 10/877,532 filed Jun. 24, 2004, now U.S. Pat. No. 7,485,704, which is a non-provisional application claiming priority under 35 U.S.C. §119 to U.S. Provisional Patent Application Ser. No. 60/490,500 filed Jul. 28, 2003, the entire disclosures of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention concerns protein purification. In particular, the invention concerns a method for reducing leaching of protein A during protein A affinity chromatography by reducing temperature or pH of, or by adding one or more protease inhibitors to, a composition that is subjected to protein A affinity chromatography.

2. Description of Related Art

The large-scale, economic purification of proteins is increasingly an important problem for the biotechnology industry. Generally, proteins are produced by cell culture, using either mammalian or bacterial cell lines engineered to produce the protein of interest by insertion of a recombinant plasmid containing the gene for that protein. Since the cell lines used are living organisms, they must be fed with a complex growth medium, containing sugars, amino acids, and growth factors, usually supplied from preparations of animal serum. Separation of the desired protein from the mixture of compounds fed to the cells and from the by-products of the cells themselves to a purity sufficient for use as a human therapeutic poses a formidable challenge.

Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins can be caused to be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by differential centrifugation or by filtration. The same problem arises, although on a smaller scale, with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

Once a clarified solution containing the protein of interest has been obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when

the impurities specifically adhere to the column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through."

Affinity chromatography, which exploits a specific interaction between the protein to be purified and an immobilized capture agent, may also be an option for some proteins. Protein A is a useful adsorbent for affinity chromatography of proteins, such as antibodies, which contain an Fc region. Protein A is a 41kD cell wall protein from *Staphylococcus aureas* which binds with a high affinity (about 10^{-8}M to human IgG) to the Fc region of antibodies.

U.S. Pat. Nos. 6,127,526 and 6,333,398 (Blank, G.) describe an intermediate wash step during protein A affinity chromatography using hydrophobic electrolytes, e.g., tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC), to remove the impurities, but not the immobilized protein A or the protein of interest, bound to the protein A column.

SUMMARY OF THE INVENTION

The present invention concerns a method of purifying a protein which comprises a C_H2/C_H3 region, comprising reducing the temperature of a composition comprising the protein and one or more impurities subjected to protein A affinity chromatography in the range from about 3° C. to about 20° C., wherein protein A leaching is reduced.

Preferably the protein is an antibody, e.g. one which binds an antigen selected from the group consisting of HER2, vascular endothelial growth factor (VEGF), IgE, CD20, CD40, CD11a, tissue factor (TF), prostate stem cell antigen (PSCA), interleukin-8 (IL-8), epidermal growth factor receptor (EGFR), HER3, HER4, $\alpha4\beta7$ or $\alpha5\beta3$. In another embodiment, the protein is an immunoadhesin, such as a TNF receptor immunoadhesin.

The invention also concerns a method of purifying a protein which comprises a C_H2/C_H3 region by protein A affinity chromatography comprising:

(a) subjecting the protein to protein A affinity chromatography and measuring leached protein A in a composition comprising the protein which is recovered from the protein A affinity chromatography;

(b) if protein A leaching is detected in step (a), reducing the temperature of a composition comprising the protein and one or more impurities subjected to protein A affinity chromatography in the range from about 3° C. to about 20° C., such that protein A leaching is reduced.

The invention further provides a method for reducing leaching of protein A during protein A affinity chromatography comprising reducing protease activity in a composition subjected to protein A affinity chromatography, wherein the composition comprises a protein which comprises a C_H2/C_H3 region and one or more proteases.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts protein A leaching as a function of temperature for various antibody products on PROSEP A™. Leached protein A is shown in ng/mg (ng protein A per mg antibody). Temperature on the x-axis refers to the temperature of the water bath. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0 or 7.1, eluted with either 25 mM citrate pH 2.8, or 0.1 M acetic acid pH 2.9, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0. Trastuzumab was run on a bed height of 20 cm, loaded to 20

g Trastuzumab/L resin, washed with TMAC pH 5.0, eluted with 25 mM citrate pH 2.8, and pooled from 0.1 AU to 2 CV's. Humanized 2C4 was run on a 20 cm bed height column, loaded to 15 g humanized 2C4 per liter resin, washed with TMAC pH 7.1, eluted with 25 mM citrate pH 2.8, and pooled from 0.1 AU to 2 CV's pool volume. Humanized VEGF antibody was run on 14 cm bed height, loaded to 20 g humanized VEGF antibody per liter of resin, washed with TMAC pH 5.0, eluted with 0.1M acetic acid pH 2.9, and pooled from 0.2 AU to 2 CV's pool volume. Humanized CD11a antibody was run on a 14 cm bed height, loaded to 20 g humanized CD11a antibody per liter of resin, washed with TMAC pH 7.1, eluted with 0.1M acetic acid pH 2.9, and pooled from 0.2 AU to 2CV's.

FIG. **2** depicts a comparison of temperature dependent protein A leaching from PROSEP A™ and PROSEP vA™ with Trastuzumab, humanized 2C4, and humanized CD11a antibody. Leached protein A is shown in ng/mg (ng protein A per mg antibody). Temperature on the x-axis refers to the temperature of the water bath. All columns were 0.66 cm in diameter and either 14 cm or 20 cm in height. One lot of harvested cell culture fluid (HCCF) was used for each pair of runs. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0 or 7.1, eluted with either 25 mM citrate pH 2.8, or 0.1 M acetic acid pH 2.9, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0 at 40 CV/hr. Humanized CD11a antibody was run on a 14 cm bed height, loaded to 20 g humanized CD11a antibody per liter of resin, washed with TMAC pH 7.1, eluted with 0.1M acetic acid pH 2.9, and pooled from 0.2 AU to 2CV's. Humanized 2C4 was run on a 20 cm bed height column, loaded to 15 g humanized 2C4 per liter resin, washed with TMAC pH 7.1, eluted with 25 mM citrate pH 2.8, and pooled from 0.1 AU to 2 CV's pool volume. Trastuzumab (from pilot plant at 400 L scale at concentration of 0.57 mg/ml) was run on a bed height of 20 cm, loaded to 20 g Trastuzumab/L resin, washed with TMAC pH 5.0, eluted with 25 mM citrate pH 2.8, and pooled from 0.1 AU to 2 CV's.

FIG. **3** depicts protein A leaching at pilot scale versus temperature. Leached protein A is shown in ng/mg (ng protein A per mg antibody). Temperature on the x-axis refers to the set temperature of the HCCF tank. The column was packed with 1.26 L PROSEP vA™, 9 cm in diameter by 20 cm in height. Trastuzumab HCCF was at 0.59 mg/ml, and the temperature of the HCCF in the tank was maintained at 10, 15, 20, 25, or 30° C. The column was loaded to 20 g Trastuzumab per liter of resin. Temperature was measured in the HCCF tank, between the pump and the column, and at the outlet to the column. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0, eluted with either 25 mM citrate pH 2.8, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0. A sample of each HCCF was taken and run at lab scale on a 0.66 cm diameter by 20 cm high column packed with PROSEP vA™ using the same buffers as at pilot scale, represented on the graph by the circles.

FIGS. **4**A-B show the light chain amino acid sequence (SEQ ID NO:1) and heavy chain amino acid sequence (SEQ ID NO:2), respectively, of Trastuzumab (HERCEPTIN®).

FIGS. **5**A-B depict the amino acid sequences of the variable light (SEQ ID NO:3) and variable heavy (SEQ ID NO:4) domains, respectively, of a humanized 2C4.

FIGS. **6**A-B depict the amino acid sequences of the variable light (SEQ ID NO:5) and variable heavy (SEQ ID NO:6) domains, respectively, of a humanized CD11a antibody RAPTIVA™.

FIGS. **7**A-B depict the amino acid sequences of the variable light (SEQ ID NO:7) and variable heavy (SEQ ID NO:8) domains, respectively, of a humanized VEGF antibody AVASTIN™.

FIG. **8** depicts the effect of EDTA and temperature on Protein A leaching.

FIG. **9** depicts the effect of 4-(2-aminoethyl)-benzenesulfonyl-fluoride, hydrochloride (AEBSF) (PEFABLOC®), a serine protease inhibitor, on Protein A leaching

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

When used herein, the term "protein A" encompasses protein A recovered from a native source thereof, protein A produced synthetically (e.g. by peptide synthesis or by recombinant techniques), including variants or derivatives thereof which retain the ability to bind proteins which have a C_H2/C_H3 region. Protein A can be purchased commercially from Repligen, Pharmacia and Fermatech.

"Protein A affinity chromatography" refers to the separation or purification of substances and/or particles using protein A, where the protein A is generally immobilized on a solid phase. A protein comprising a C_H2/C_H3 region may be reversibly bound to, or adsorbed by, the protein A. Examples of protein A affinity chromatography columns for use in protein A affinity chromatography herein include protein A immobilized onto a controlled pore glass backbone, including the PROSEP A™ and PROSEP vA™ columns (Millipore Inc.); protein A immobilized on a polystyrene solid phase, e.g. the POROS 50A™ column (Applied BioSystems Inc.); or protein A immobilized on an agarose solid phase, for instance the rPROTEIN A SEPHAROSE FAST FLOW™ or MABSELECT™ columns (Amersham Biosciences Inc.).

By "solid phase" is meant a non-aqueous matrix to which the protein A can adhere or be covalently bound. The solid phase may comprise a glass, silica, polystyrene, or agarose surface for immobilizing the protein A, for instance. The solid phase may be a purification column, discontinuous phase of discrete particles, packed bed column, expanded bed column, membrane, etc.

Herein, "leaching" refers to the detachment or washing of protein A (including fragments thereof) from a solid phase to which it is bound. Leaching may result from various mechanisms such as mechanical shearing, low pH exposure, proteolytic activity etc.

An "impurity" is a material that is different from the desired protein product. The impurity may be a viral impurity, a variant of the desired protein or another protein, nucleic acid, endotoxin etc. Specific examples of impurities herein include proteins from the host cell producing the desired protein (e.g. Chinese Hamster Ovary proteins, CHOP, where the host cell is a CHO cell), protease(s), leached protein A etc.

"Proteases" are proteolytic enzymes including, but not limited to, serine, cysteine, metallo- and aspartic proteases. Proteases present in a composition comprising a protein of interest may be derived from a recombinant host producing the protein, or from a natural source of the protein. Examples of proteases include thermolysin, trypsin, chymotrypsin, plasmin, kallikrein, thrombin, papain, plasmin, cathepsin B, renin, chymosin etc.

"Protease activity" refers to the enzymatic activity of one or more proteases. Such activity may be measured indirectly by measuring leaching of protein A, for instance. The activity may be reduced by reducing temperature of a composition comprising the protease(s), and/or by adding one or more protease inhibitors to the composition etc.

A "protease inhibitor" is a compound or composition which reduces, to some extent, the enzymatic activity of protease(s). Examples of protease inhibitors include phenylmethylsulfonyl fluoride (PMSF), 4-(2-aminoethyl)-benzenesulfonyl-fluoride, hydrochloride (AEBSF) (PEFABLOC® SC), leupeptin, pepstatin, benzamidine, a metal ion chelator such as EDTA or imidazole for inhibiting metalloprotease activity etc. The preferred protease inhibitors inhibit metalloprotease activity (e.g. EDTA) and/or inhibit certain serine protease activities.

The protein of interest herein is one which comprises a C_H2/C_H3 region and therefore is amenable to purification by protein A affinity chromatography. The term "C_H2/C_H3 region" when used herein refers to those amino acid residues in the Fc region of an immunoglobulin molecule which interact with protein A. In preferred embodiments, the C_H2/C_H3 region comprises an intact C_H2 region followed by an intact C_H3 region, and most preferably comprises a Fc region of an immunoglobulin. Examples of C_H2/C_H3 region-containing proteins include antibodies, immunoadhesins and fusion proteins comprising a protein of interest fused to, or conjugated with, a C_H2/C_H3 region.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they retain, or are modified to comprise, a C_H2/C_H3 region as herein defined.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; single-chain antibody molecules; diabodies; linear antibodies; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al/, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature* 352:624-628 (1991) and Marks et al., *J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (i.e. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (i.e. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196: 901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332: 323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the "binding domain" of a heterologous "adhesin" protein (e.g. a receptor, ligand or enzyme) with the effector functions of an immunoglobulin constant domain. Structurally, the immunoadhesins comprise a fusion of the adhesin amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site (antigen combining site) of an antibody (i.e. is "heterologous") and an immunoglobulin constant domain sequence. The immunoglobulin constant domain sequence in the immunoadhesin is preferably derived from γ1, γ2, or γ4 heavy chains since immunoadhesins comprising these regions can be purified by protein A affinity chromatography (Lindmark et al., *J. Immunol. Meth.* 62:1-13 (1983)).

The term "ligand binding domain" as used herein refers to any native cell-surface receptor or any region or derivative thereof retaining at least a qualitative ligand binding of a corresponding native receptor. In a specific embodiment, the receptor is from a cell-surface polypeptide having an extracellular domain which is homologous to a member of the immunoglobulin supergenefamily. Other receptors, which are not members of the immunoglobulin supergenefamily but are nonetheless specifically covered by this definition, are receptors for cytokines, and in particular receptors with tyrosine kinase activity (receptor tyrosine kinases), members of the hematopoietin and nerve growth factor receptor superfamilies, and cell adhesion molecules, e.g. (E-, L- and P-) selectins.

The term "receptor binding domain" is used to designate any native ligand for a receptor, including cell adhesion molecules, or any region or derivative of such native ligand retaining at least a qualitative receptor binding ability of a corresponding native ligand. This definition, among others, specifically includes binding sequences from ligands for the above-mentioned receptors. An "antibody-immunoadhesin chimera" comprises a molecule which combines at least one binding domain of an antibody (as herein defined) with at least one immunoadhesin (as defined in this application). Exemplary antibody-immunoadhesin chimeras are the bispecific CD4-IgG chimeras described in Berg et al., *PNAS (USA)* 88:4723-4727 (1991) and Chamow et al., *J. Immunol.* 153: 4268 (1994).

The expression "HER2" refers to human HER2 protein described, for example, in Semba et al., *PNAS (USA)* 82:6497-6501 (1985) and Yamamoto et al. *Nature* 319:230-234 (1986) (Genebank accession number X03363).

"Trastuzumab" or "HERCEPTIN®" is a humanized HER2 antibody comprising the light chain amino acid sequence of SEQ ID NO:1 and the heavy chain amino acid sequence of SEQ ID NO:2, or amino acid sequence variants thereof which retain the ability to bind HER2 and inhibit growth of tumor cells which overexpress HER2 (see U.S. Pat. No. 5,677,171; expressly incorporated herein by reference).

"Humanized 2C4" is a humanized HER2 antibody comprising the variable light amino acid sequence of SEQ ID NO:3 and the variable heavy amino acid sequence of SEQ ID NO:4, or amino acid sequence variants thereof which retain the ability to bind HER2 and block ligand activation of HER2 (see WO01/00245; expressly incorporated herein by reference).

MODES FOR CARRYING OUT THE INVENTION

The process herein involves purifying a C_H2/C_H3 region-containing protein from impurities by protein A affinity chromatography. In preferred embodiments, the protein is an antibody, immunoadhesin or a protein fused to, or conjugated with, a C_H2/C_H3 region. Techniques for generating such molecules will be discussed below.

1. Antibodies

The preferred protein according to the present invention is an antibody. Antibodies within the scope of the present invention include, but are not limited to: anti-HER2 antibodies including Trastuzumab (HERCEPTIN®) (Carter et al., *Proc. Natl. Acad. Sci. USA,* 89:4285-4289 (1992), U.S. Pat. No. 5,725,856) and humanized 2C4 (WO01/00245, Adams et al.); anti-CD_{20} antibodies such as chimeric anti-CD_{20} "C2B8" as in U.S. Pat. No. 5,736,137 (RITUXAN®), a chimeric or humanized variant of the 2H7 antibody as in U.S. Pat. No. 5,721,108B1, or Tositumomab (BEXXAR®); anti-IL-8 antibodies (St John et al., *Chest,* 103:932 (1993), and International Publication No. WO 95/23865); anti-VEGF antibodies, including humanized and/or affinity matured anti-VEGF antibodies such as the humanized anti-VEGF antibody huA4.6.1 AVASTIN® (Kim et al., *Growth Factors,* 7:53-64 (1992), International Publication No. WO 96/30046, and WO 98/45331, published Oct. 15, 1998); anti-prostate stem cell antigen (PSCA) antibodies (WO01/40309); anti-CD40 antibodies, including S2C6 and humanized variants thereof (WO00/75348); anti-CD11a antibodies (U.S. Pat. No. 5,622,700, WO 98/23761, Steppe et al., *Transplant Intl.* 4:3-7 (1991), and Hourmant et al., *Transplantation* 58:377-380 (1994)); anti-CD18 (U.S. Pat. No. 5,622,700, issued Apr. 22, 1997, or as in WO 97/26912, published Jul. 31, 1997); anti-IgE antibodies (including E25, E26 and E27; U.S. Pat. No. 5,714,338, issued Feb. 3, 1998 or U.S. Pat. No. 5,091,313, issued Feb. 25, 1992, WO 93/04173 published Mar. 4, 1993, or International Application No. PCT/US98/13410 filed Jun. 30, 1998, U.S. Pat. No. 5,714,338, Presta et al., *J. Immunol.* 151:2623-2632 (1993), and International Publication No. WO 95/19181); anti-Apo-2 receptor antibodies (WO 98/51793 published Nov. 19, 1998); anti-TNF-α antibodies, including cA2 (REMICADE®), CDP571 and MAK-195 (See, U.S. Pat. No. 5,672,347 issued Sep. 30, 1997, Lorenz et al. *J. Immunol.* 156(4):1646-1653 (1996), and Dhainaut et al. *Crit. Care Med.* 23(9):1461-1469 (1995)); anti-Tissue Factor (TF) antibodies (European Patent No. 0 420 937 B1 granted Nov. 9, 1994); anti-human α4β7 integrin antibodies (WO 98/06248 published Feb. 19, 1998); anti-epidermal growth factor receptor (EGFR) antibodies (e.g. chimerized or humanized 225 antibody as in WO 96/40210 published Dec. 19, 1996); anti-CD3 antibodies such as OKT3 (U.S. Pat. No. 4,515,893 issued May 7, 1985); anti-CD25 or anti-Tac antibodies such as CHI-621 (SIMULECT®) and ZENAPAX® (See U.S. Pat. No. 5,693,762 issued Dec. 2, 1997); anti-CD4 antibodies such as the cM-7412 antibody (Choy et al. *Arthritis Rheum* 39(1):52-56 (1996)); anti-CD52 antibodies such as CAMPATH-1H (Riechmann et al. *Nature* 332:323-337 (1988)); anti-Fc receptor antibodies such as the M22 antibody directed against FcγRI as in Graziano et al. *J. Immunol.* 155 (10):4996-5002 (1995); anti-carcinoembryonic antigen (CEA) antibodies such as hMN-14 (Sharkey et al. *Cancer Res.* 55(23 Suppl): 5935s-5945s (1995); antibodies directed against breast epithelial cells including huBrE-3, hu-Mc 3 and CHL6 (Ceriani et al. *Cancer Res.* 55(23): 5852s-5856s (1995); and Richman et al. *Cancer Res.* 55(23 Supp): 5916s-5920s (1995)); antibodies that bind to colon carcinoma cells such as C242 (Litton et al. *Eur J. Immunol.* 26(1):1-9 (1996)); anti-CD38 antibodies, e.g. AT 13/5 (Ellis et al. *J. Immunol.* 155(2):925-937 (1995)); anti-CD33 antibodies such as Hu M195 (Jurcic et al. *Cancer Res* 55(23 Suppl):5908s-5910s (1995) and CMA-676 or CDP771; anti-CD22 antibodies such as LL2 or LymphoCide (Juweid et al. *Cancer Res* 55(23 Suppl):5899s-5907s (1995)); anti-EpCAM antibodies such as 17-1A (PANOREX®); anti-GpIIb/IIIa antibodies such as abciximab or c7E3 Fab (REOPRO®); anti-RSV antibodies such as MEDI-493 (SYNAGIS®); anti-CMV antibodies such as PROTOVIR®; anti-HIV antibodies such as PRO542; anti-hepatitis antibodies such as the anti-Hep B antibody OSTAVIR®; anti-CA 125 antibodies, such as OvaRex; anti-idiotypic GD3 epitope antibody BEC2; anti-αvβ3 antibodies, including VITAXIN®; anti-human renal cell carcinoma antibody such as ch-G250; ING-1; anti-human 17-1A antibody (3622W94); anti-human colorectal tumor antibody (A33); anti-human melanoma antibody R24 directed against GD3 ganglioside; anti-human squamous-cell carcinoma (SF-25); and anti-human leukocyte antigen (HLA) antibodies such as

Smart ID10 and the anti-HLA DR antibody Oncolym (Lym-1). The preferred target antigens for the antibody herein are: HER2 receptor, VEGF, IgE, CD_{20}, CD11a, and CD40.

Aside from the antibodies specifically identified above, the skilled practitioner could generate antibodies directed against an antigen of interest, e.g., using the techniques described below.

(i) Antigen Selection and Preparation

The antibody herein is directed against an antigen of interest. Preferably, the antigen is a biologically important polypeptide and administration of the antibody to a mammal suffering from a disease or disorder can result in a therapeutic benefit in that mammal. However, antibodies directed against nonpolypeptide antigens (such as tumor-associated glycolipid antigens; see U.S. Pat. No. 5,091,178) are also contemplated. Where the antigen is a polypeptide, it may be a transmembrane molecule (e.g. receptor) or ligand such as a growth factor. Exemplary antigens include those proteins described in section (3) below. Exemplary molecular targets for antibodies encompassed by the present invention include CD proteins such as CD3, CD4, CD8, CD19, CD_{20}, CD22 and CD34; members of the ErbB receptor family such as the EGFR, HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-1, Mac1, p150,95, VLA-4, ICAM-1, VCAM and $\alpha v/\beta 3$ integrin including either α or β subunits thereof (e.g. anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors such as VEGF; IgE; blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA-4; protein C, or any of the other antigens mentioned herein.

Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these (e.g. the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source (e.g. cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule.

Other antigens and forms thereof useful for preparing antibodies will be apparent to those in the art.

(ii) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to 1/10 the original amount of antigen or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(iii) Monoclonal Antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice, pp.* 59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.,* 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. Preferably the protein A affinity chromatography procedure described herein is used.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, et al., *Proc. Natl. Acad. Sci. USA,* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

In a further embodiment, monoclonal antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature,* 348:552-554 (1990). Clackson et al., *Nature,* 352:624-628 (1991) and Marks et al., *J. Mol. Biol.,* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *Bio/Technology,* 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids. Res.,* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional hybridoma techniques for isolation of monoclonal antibodies.

(iv) Humanized and Human Antibodies

A humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature,* 321:522-525 (1986); Riechmann et al., *Nature,* 332:323-327 (1988); Verhoeyen et al., *Science,* 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human FR for the humanized antibody (Sims et al., *J. Immunol.,* 151:2296 (1993)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci. USA,* 89:4285 (1992); Presta et al., *J. Immunol.,* 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA,* 90:2551 (1993); Jakobovits et al., *Nature,* 362:255-258 (1993); Bruggermann et al., *Year in Immuno.,* 7:33 (1993); and Duchosal et al. *Nature* 355:258 (1992). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.,* 227:381 (1991); Marks et al., *J. Mol. Biol.,* 222:581-597 (1991); Vaughan et al. *Nature Biotech* 14:309 (1996)).

(v) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan et al., *Science,* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185.

(vi) Multispecific Antibodies

Multispecific antibodies have binding specificities for at least two different antigens. While such molecules normally

will only bind two antigens (i.e. bispecific antibodies, BsAbs), antibodies with additional specificities such as trispecific antibodies are encompassed by this expression when used herein.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., *Nature,* 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., *EMBO J.,* 10:3655-3659 (1991).

According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science,* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E coli*, which can be chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.,* 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.,* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA,* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H, domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.,* 152:5368 (1994). Alternatively, the antibodies can be "linear antibodies" as described in Zapata et al. *Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al. *J. Immunol.* 147: 60 (1991).

2. Immunoadhesins

The simplest and most straightforward immunoadhesin design combines the binding domain(s) of the adhesin (e.g. the extracellular domain (ECD) of a receptor) with the hinge and Fc regions of an immunoglobulin heavy chain. Ordinarily, when preparing the immunoadhesins of the present invention, nucleic acid encoding the binding domain of the adhesin will be fused C-terminally to nucleic acid encoding the N-terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible.

Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally active hinge, C_H2 and C_H3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the C_H1 of the heavy chain or the corresponding region of the light chain. The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion, or binding characteristics of the immunoadhesin.

In a preferred embodiment, the adhesin sequence is fused to the N-terminus of the Fc domain of immunoglobulin G_1 (IgG_1). It is possible to fuse the entire heavy chain constant region to the adhesin sequence. However, more preferably, a sequence beginning in the hinge region just upstream of the papain cleavage site which defines IgG Fc chemically (i.e. residue 216, taking the first residue of heavy chain constant region to be 114), or analogous sites of other immunoglobulins is used in the fusion. In a particularly preferred embodiment, the adhesin amino acid sequence is fused to (a) the

hinge region and C_H2 and C_H3 or (b) the C_H1, hinge, C_H2 and C_H3 domains, of an IgG heavy chain.

For bispecific immunoadhesins, the immunoadhesins are assembled as multimers, and particularly as heterodimers or heterotetramers. Generally, these assembled immunoglobulins will have known unit structures. A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four chain unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of four basic units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in multimeric form in serum. In the case of multimer, each of the four units may be the same or different.

Various exemplary assembled immunoadhesins within the scope herein are schematically diagrammed below:

(a) AC_L-AC_L;

(b) AC_H-(AC_H, AC_L-AC_H, AC_L-V_HC_H, or V_LC_L-AC_H);

(c) AC_L-AC_H-(AC_L-AC_H, AC_L-V_HC_H, V_LC_L-AC_H, or V_LC_L-V_HC_H)

(d) AC_L-V_HC_H-(AC_H, or AC_L-V_HC_H, or V_LC_L-AC_H);

(e) V_LC_L-AC_H-(AC_L-V_HC_H, or V_LC_L-AC_H); and

(f) $(A\text{-}Y)_n$-$(V_LC_L$-$V_HC_H)_2$,

wherein each A represents identical or different adhesin amino acid sequences;

V_L is an immunoglobulin light chain variable domain;

V_H is an immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_H is an immunoglobulin heavy chain constant domain;

n is an integer greater than 1;

Y designates the residue of a covalent cross-linking agent.

In the interests of brevity, the foregoing structures only show key features; they do not indicate joining (J) or other domains of the immunoglobulins, nor are disulfide bonds shown. However, where such domains are required for binding activity, they shall be constructed to be present in the ordinary locations which they occupy in the immunoglobulin molecules.

Alternatively, the adhesin sequences can be inserted between immunoglobulin heavy chain and light chain sequences, such that an immunoglobulin comprising a chimeric heavy chain is obtained. In this embodiment, the adhesin sequences are fused to the 3' end of an immunoglobulin heavy chain in each arm of an immunoglobulin, either between the hinge and the C_H2 domain, or between the C_H2 and C_H3 domains. Similar constructs have been reported by Hoogenboom, et al., *Mol. Immunol.* 28:1027-1037 (1991).

Although the presence of an immunoglobulin light chain is not required in the immunoadhesins of the present invention, an immunoglobulin light chain might be present either covalently associated to an adhesin-immunoglobulin heavy chain fusion polypeptide, or directly fused to the adhesin. In the former case, DNA encoding an immunoglobulin light chain is typically coexpressed with the DNA encoding the adhesin-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain and the light chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chain-light chain pairs. Methods suitable for the preparation of such structures are, for example, disclosed in U.S. Pat. No. 4,816,567, issued 28 Mar. 1989.

Immunoadhesins are most conveniently constructed by fusing the cDNA sequence encoding the adhesin portion in-frame to an immunoglobulin cDNA sequence. However, fusion to genomic immunoglobulin fragments can also be used (see, e.g. Aruffo et al., *Cell* 61:1303-1313 (1990); and Stamenkovic et al., *Cell* 66:1133-1144 (1991)). The latter type of fusion requires the presence of 1 g regulatory sequences for expression. cDNAs encoding IgG heavy-chain constant regions can be isolated based on published sequences from cDNA libraries derived from spleen or peripheral blood lymphocytes, by hybridization or by polymerase chain reaction (PCR) techniques. The cDNAs encoding the "adhesin" and the immunoglobulin parts of the immunoadhesin are inserted in tandem into a plasmid vector that directs efficient expression in the chosen host cells.

3. Other C_H2/C_H3 Reqion-containing Proteins

In other embodiments, the protein to be purified is one which is fused to, or conjugated with, a C_H2/C_H3 region. Such fusion proteins may be produced so as to increase the serum half-life of the protein and/or to facilitate purification of the protein by protein A affinity chromatography. Examples of biologically important proteins which can be conjugated this way include renin; a growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA), such as CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; Protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD3, CD4, CD8, CD19 and CD_{20}; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs), e.g., IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4 and VCAM; a tumor associated antigen such as EGFR, HER2, HER3 or HER4 receptor; and fragments of any of the above-listed polypeptides.

4. Protein a Affinity Chromatography

The protein to be purified using the method described herein is generally produced using recombinant techniques or isolated from a native source thereof. Methods for producing

recombinant proteins are described, e.g., in U.S. Pat. Nos. 5,534,615 and 4,816,567, specifically incorporated herein by reference.

Preferably the C_H2/C_H3 region-containing protein or product of interest is an antibody, e.g. one which binds an antigen selected from the group consisting of HER2, vascular endothelial growth factor (VEGF), IgE, CD_{20}, CD40, CD11a, tissue factor (TF), prostate stem cell antigen (PSCA), interleukin-8 (IL-8), epidermal growth factor receptor (EGFR), HER3, HER4, $\alpha4\beta7$ or $\alpha5\beta3$. For instance, the antibody may bind the HER2 antigen as leaching of protein A during protein A affinity chromatography of such antibodies, was found to be particularly problematic. More specific examples of antibodies herein include Trastuzumab, humanized 2C4, humanized CD11a antibody, or humanized VEGF antibody. Other C_H2/C_H3 region-containing proteins of particular interest herein are immunoadhesins, e.g. TNF receptor immunoadhesin (e.g. etanercept, ENBREL®).

When using recombinant techniques, the protein may be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Where the protein is secreted into the medium, the recombinant host cells may also be separated from the cell culture medium by centrifugation or tangential flow filtration, for example.

The method herein reduces leaching of protein A which may, occur during protein A affinity chromatography of a composition comprising a C_H2/C_H3 region-containing protein and one or more impurities.

In one embodiment, the susceptibility of the protein to be associated with protein A leaching during protein A affinity chromatography is first assessed. Thus, the protein is subjected to protein A affinity chromatography and protein A leaching in the recovered composition is determined. For instance, where the recovered composition comprises greater than about 20 ng protein A per mg protein of interest (ng/mg), e.g. from about 20 ng/mg to about 500 ng/mg protein A, this may be considered unacceptable levels of leached protein A, in which case subsequent protein A chromatographic purification of the protein will include step(s) which reduce the amount of protein A in the recovered composition. Preferably, the amount of protein A in the recovered protein composition following the implementation of these step(s) is in the range from about 0 ng protein A per mg protein of interest (ng/mg) to about 15 ng/mg.

Protein A leaching can be measured using various techniques including enzyme linked immunosorbent assay (ELISA), SDS PAGE, Western blot, high pressure liquid chromatography (HPLC), mass spectrometry, etc.

The preferred assay for measuring leached protein A is ELISA. For example, a sandwich ELISA may be used. In this assay format, anti-protein A antibody may be coated onto a 96 well microtiter plate. Samples may be diluted to 0.2 mg/mL product antibody and applied onto the wells. The protein A in the samples binds to the coat antibody and the amount of bound protein A can be detected with anti-protein A coupled to Horseradish Peroxidase (HRP). To prevent product antibody inhibiting binding of protein A to the coat antibody and the HRP-conjugated antibody, one may match the inhibition exerted by product antibody in diluted samples using individual protein A standard curves that are spiked with 0.2 mg/mL homologous product antibody. Although this method is more time-consuming and costly, it provides a more accurate and precise determination of protein A levels. An exemplary protein A sandwich ELISA is described in more detail in the Example below.

Preferably, the method comprises reducing the temperature of the composition subjected to the protein A affinity chromatography, e.g. where the temperature of the composition is reduced below room temperature, for instance in the range from about 3° C. to about 20° C., e.g. from about 10° C. to about 18° C. The temperature of the composition may be reduced prior to and/or during protein A affinity chromatography thereof. However, according to the preferred embodiment of the invention, the method comprises lowering the temperature of the composition prior to subjecting the composition to protein A affinity chromatography, e.g. by lowering the temperature of harvested cell culture fluid (HCCF) which is subjected to chromatography.

In one embodiment, temperature reduction as disclosed above is combined with one or more other methods for reducing protein A leaching, e.g. by adding protease inhibitor(s) and/or lowering the pH of the composition that is subjected to protein A affinity chromatography.

Protease inhibitors (such as phenylmethylsulfonyl fluoride (PMSF), 4-(2-aminoethyl)-benzenesulfonyl-fluoride, hydrochloride (AEBSF) (PEFABLOC® SC), pepstatin, benzamidine, and/or a metal ion chelator such as EDTA or imidazole for inhibiting metalloprotease activity) may be added to the composition that is subjected to protein A affinity chromatography. The preferred protease inhibitors inhibit metalloprotease activity (e.g. EDTA) and/or inhibit certain serine protease activities. For instance, one may add the protease inhibitor(s) to the composition subjected to protein A affinity chromatography in an amount from about 0.001 μM to about 100 mM. The protease inhibitor(s) may be added to the composition before and/or during protein A affinity chromatography.

The present invention also contemplates lowering the pH of the composition prior to subjecting it to protein A affinity chromatography, e.g. to a pH in the range from about 2.5 to about 3.5, in order to reduce protein A leaching.

Various exemplary equilibration, loading, washing, and elution buffers and methods will now be described.

As an optional preliminary step, the solid phase for the protein A affinity chromatography may be equilibrated with a suitable buffer before chromatography of the protein of interest. For example, the equilibration buffer may be 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1.

The preparation comprising the protein of interest may then be loaded on the equilibrated solid phase using a loading buffer which may be the same as the equilibration buffer. As the contaminated preparation flows through the solid phase, the protein is adsorbed to the immobilized protein A.

Sometimes, certain impurities (such as Chinese Hamster Ovary Proteins, CHOP, where the protein is produced in a CHO cell) may bind nonspecifically to the solid phase, protein or protein A. If this occurs, an "intermediate wash step" may be used to remove such impurities prior to elution of the protein of interest. The solid phase may be equilibrated with equilibration buffer before beginning the intermediate wash step.

In one embodiment, the intermediate wash step is performed using a hydrophobic electrolyte solvent, e.g. where the hydrophobic electrolyte in the wash solvent is TMAC and/or TEAC. See U.S. Pat. Nos. 6,127,526 and 6,333,398 (Blank, G.). While a single hydrophobic electrolyte may be present in the wash solvent, in certain embodiments, two or more such electrolytes may be used. The hydrophobic electrolyte is preferably added to a pH buffered solution having a

pH in the range from about 4 to about 8, and preferably in the range from about 5 to about 7. Suitable buffers for this purpose include Tris, phosphate, MES, and MOPSO buffers. The preferred final concentration for the hydrophobic electrolyte in the wash solvent is in the range from about 0.1 to about 1.0M, and preferably in the range from about 0.25 to about 0.5M.

In an alternative embodiment, the intermediate wash buffer may comprise salt and a further compound, where the further compound is (a) detergent (preferably polysorbate, e.g. polysorbate 20 or polysorbate 80); (b) solvent (preferably hexylene glycol); and (c) polymer (preferably PEG).

The salt employed may be selected based on the protein of interest, but preferably is acetate (e.g. sodium acetate), especially where the antibody is an anti-HER2 antibody such as Trastuzumab; or citrate (e.g. sodium citrate), particularly where the antibody is an anti-IgE antibody such as E26.

The amounts of the salt and further compound in the composition are such that the combined amount elutes the impurity or impurities, without substantially removing the protein of interest. Preferred salt concentrations in such wash buffers are from about 0.1 to about 2M, and more preferably from about 0.2M to about 0.6M. Useful detergent concentrations are from about 0.01 to about 5%, more preferably from about 0.1 to 10%, and most preferably about 0.5%, e.g. where the detergent is polysorbate. Exemplary solvent concentrations are from about 1% to 40%, preferably from about 5 to about 25%. The preferred concentration of the solvent (hexylene glycol) for E26 is about 20%, whereas for Trastuzumab the preferred concentration of the solvent (again hexylene glycol) is about 10%. Where the further compound is a polymer (e.g. PEG 400 or PEG 8000), the concentration thereof may, for example, be from about 1% to about 20%, preferably from about 5% to about 15%.

In another embodiment, the intermediate wash step involves the use of a highly concentrated buffer solution, e.g. a buffer at a concentration of greater than about 0.8M, e.g. up to about 2M, and preferably in the range from about 0.8M to about 1.5M, most preferably about 1 M. In this embodiment, the buffer is preferably a Tris buffer, such as Tris acetate.

The pH of the intermediate wash buffer is preferably from about 4 to about 8, more preferably from about 4.5 to about 5.5, and most preferably about 5.0. In another preferred embodiment, the pH is about 7.0.

The protein of interest may be recovered from the column, using a suitable elution buffer. The protein may, for example, be eluted from the column using an elution buffer having a low pH, e.g. in the range from about 2 to about 5, and preferably in the range from about 2.5 to about 3.5. Examples of elution buffers for this purpose include citrate or acetate buffers. The eluted protein preparation may be subjected to additional purification steps either prior to, or after, the protein A affinity chromatography step. Exemplary further purification steps include, but are not limited to, filtration, hydroxylapatite chromatography; dialysis; affinity chromatography using an antibody to capture the protein; hydrophobic interaction chromatography (HIC); ammonium sulphate precipitation; anion or cation exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica; chromatofocusing; gel filtration, etc.

The protein thus recovered may be formulated in a pharmaceutically acceptable carrier and is used for various diagnostic, therapeutic or other uses known for such molecules.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLE 1

Temperature Reduction for Reducing Protein a Leaching During Protein a Affinity Chromatography

Protein A affinity chromatography is a powerful and widely-used tool for purifying antibodies. It efficiently removes host cell proteins, DNA, and small molecules from the product. Harvested cell culture fluid (HCCF) can be loaded directly onto the resin and the antibody binds to the protein A. Low pH elutes the bound antibody, but may carry leached protein A into the product pool. Since protein A ligand is immunogenic, derived from *Staphylococcus aureus*, it must be cleared from the product pool by downstream processing.

To characterize the temperature dependence of protein A leaching, the effect of temperature on protein A leaching was evaluated with respect to the following proteins:

1. Recombinant humanized HER2 antibody Trastuzumab (HERCEPTIN®); Carter et al., *Proc. Natl. Acad. Sci. USA,* 89:4285-4289 (1992), U.S. Pat. No. 5,725,856, U.S. Pat. No. 5,821,337, and FIGS. **4**A-B herein.
2. Humanized CD11a antibody MHM24, RAPTIVA™; Werther et al. *J Immunology* 157: 4986-4995 (1996), U.S. Pat. No. 5,622,700, WO 98/23761, and FIGS. **6**A-B herein.
3. Humanized VEGF antibody A4.6.1, F(ab)-12, AVASTIN®; Kim et al., *Growth Factors,* 7:53-64 (1992), Presta et al. *Cancer Research* 57: 4593-4599 (1997), International Publication No. WO 96/30046, WO 98/45331, published Oct. 15, 1998, and FIGS. **7**A-B herein.
4. Humanized 2C4; WO01/00245, and FIGS. **5**A-B herein.

Materials and Methods

Small-Scale: All small-scale experiments were performed using an AKTA EXPLORER 100™. The temperature was controlled by immersing the column and the 5 ml stainless-steel upstream line in a water bath controlled to the desired temperature of the run. The inlet line acted as a heat exchanger cooling or heating the HCCF prior to entering the protein A column, similar to the effect of chilling the HCCF in a tank at manufacturing scale. The outlet temperature was measured to be sure the desired temperature was achieved.

Several sets of protein A runs were performed to determine the temperature dependence of protein A leaching from PROSEP A® and PROSEP vA™ for various antibodies. Various lots of each type of resin were tested. Each condition was tested in triplicate. The column was pre-cycled with 3 column volumes (CV's) of elution buffer and 3 CV's of regeneration buffer prior to each use, and stored in 0.1 M sodium acetate, 2% benzyl alcohol pH 5.0 after each use. Trastuzumab was run at 7 temperature settings (10μ, 12, 15, 18, 20, 25, and 30° C.). The other antibodies were run at 3 temperature settings (10, 20, and 30° C.). The temperatures were run out of order to reduce systematic error. Trastuzumab HCCF from six 400 L runs were compared. Using one lot of Trastuzumab HCCF on one lot of resin at 20° C., the effect of bed height on protein A leaching was explored.

Pilot Scale: The pilot scale experiments were run with Trastuzumab HCCF. The HCCF was stored and chilled in a 400 L-jacketed tank. The temperature of the HCCF was controlled to within 1° C. of the desired temperature. The temperature was measured in the tank, after the pump but prior to the column, and at the outlet of the column. The column was pre-cycled with 3 CV's elution buffer and 3 CV's of regeneration buffer prior to each use, and stored in 0.1 M sodium acetate, 2% benzyl alcohol pH 5.0 after each use. Trastu-

zumab was run at 7 temperature settings (10, 12, 15, 18, 20, 25, and 30° C.). The temperatures were run out of order to reduce systematic error.

Full Scale (12,000 L cell culture): The column was 80 cm in diameter by 20 cm high for a total volume of 100.5 L PROSEP vA™. Five harvests were recovered through the protein A step. The HCCF was collected and held at 15+/−3° C. for the duration of loading.

Analysis: Each protein A pool was analyzed by OD at A280-A320/extinction coefficient for concentration. The extinction coefficients were 1.5 (mg/ml)-1 cm^{-1} for Trastuzumab and humanized 2C4, 1.46 (mg/ml)-1 cm^{-1} for humanized CD11a antibody, 1.7 (mg/ml)-1 cm^{-1} for humanized VEGF antibody. The yield of each run was calculated. If the yield was less than 85%, the run was repeated. Protein A leaching in each pool was measured using ELISA. Each sample was assayed in triplicate on separate plates to encompass as much of the assay and dilution variability as possible.

ELISA: Chicken anti-protein A is coated on a 96-well, polystyrene, microtiter plate and incubated at 2-8° C. for 12-72 hours. The plate is washed with a PBS/TWEEN 20™ Wash Buffer and Assay Diluent containing NaCl/$NaPO_4$/Fish Gelatin/TWEEN 20™ is added to the plate wells to block any unbound coat antibody. The plate is incubated at room temperature for 1-2 hours. During the plate incubation, protein A standard curve is prepared at a range of 0.39-50 ng/ml using Assay Diluent spiked with 0.2 mg/ml of product antibody homologous to the product antibody contained in the samples. Samples are diluted with unspiked Assay Diluent to 0.2 mg/ml of product antibody. An assay control prepared from the same product antibody is used. After the 1-2 hour incubation, the plate is washed with Wash Buffer to remove the Assay Diluent. The standard curve, assay control and samples are then applied onto the plate wells, and incubated at room temperature for 2 hours where the protein A in the standards, control and samples will bind to the coat antibody. After the 2 hour incubation, the plate is then washed with Wash Buffer to remove any unbound antibodies as well as the sample matrix. HRP-conjugated Chicken anti-protein A is then applied onto the wells and incubated at room temperature for 1 hour. The HRP-conjugated Chicken anti-protein A will bind to any bound protein A. After the 1 hour incubation, the plate is washed again with Wash Buffer to remove any unbound antibodies. The substrate solution, consisting of o-phenylenediamine tablet dissolved in H_2O_2 in phosphate buffered saline (PBS), is then added onto the plate wells and is processed by the HRP enzyme, causing the substrate solution to change color. Once the substrate color has reached a desired OD range, the enzyme reaction is stopped by the addition of sulfuric acid. The amount of bound protein A is determined by measuring the Optical Density at 490 nm using a microtiter plate reader.

Results and Discussion

Several antibodies were purified from HCCF by protein A affinity chromatography on PROSEP A™ or PROSEP vA™ at up to 7 temperatures at small scale to characterize the effect of temperature on protein A leaching. Protein A leaching is affected by temperature to varying degrees for the antibodies tested (FIG. **1**). Protein A leaching during elution of HER2 antibodies, Trastuzumab and humanized 2C4, is most significantly affected, while humanized VEGF and humanized CD11a antibodies were only slightly affected by temperature. The small error bars in conjunction with randomized run order ensure the effect of temperature on protein A leaching is real. The trend-lines on the graph represent an exponential fit for each set of data. This type of non-linear correlation would be consistent with temperature-activated proteolytic cleavage.

Several lots of Trastuzmab HCCF from 400 L pilot plant runs were run on PROSEP A™ at room temperature, to investigate the effect of HCCF lot-to-lot variability on Protein A leaching The results are shown in Table 1 below. Each lot of HCCF was run on PROSEP A™ in triplicate. The lots showed a range of protein A leaching from 4 to 13 ng/mg with a small standard deviation of 0.2 to 1.1 ng/mg. These numbers are low in comparison to previous protein A ELISA results using Trastuzumab. The positive control run in the ELISA on that day also ran low. Compared only with each other and not with samples assayed at other times, the results show some variability in leaching between lots of Trastusumab HCCF.

TABLE 1

Lot-to-Lot Variability
Runs were performed in triplicate on PROSEP A ™ resin packed in a 0.66 cm diameter by 20 cm high column. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0, eluted with 25 mM citrate pH 2.8, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0 at 40 CV/hr. Trastuzumab from the 400 L pilot plant runs was run on a bed height of 20 cm, loaded to 20 g Trastuzumab/L resin, eluted with 25 mM citrate pH 2.8, and pooled from 0.1 AU to 2 CV's.

Lot of Trastuzumab HCCF	Protein A (ng/mg)
1	7 +/− 0.3
2	4 +/− 0.4
3	5 +/− 0.2
4	7 +/− 0.8
5	13 +/− 1.1
6	7 +/− 0.7

FIG. **2** compares the effect of temperature on protein A leaching between PROSEP A™ and PROSEP vA™ for 3 antibodies. For humanized CD11a antibody, the PROSEP A™ and PROSEP vA™ results overlay exactly. In the cases of humanized 2C4 and Trastuzumab, the results do not overlay, but they are within the expected range for lot-to-lot variability of the resins (Table 1), and the results are probably not due to differences between PROSEP A™ and PROSEP vA™. The effect of temperature on protein A leaching from PROSEP A™ is equivalent to that from PROSEP vA™.

The product sequence of increasing leaching shown in FIG. **1** may have been related to inconsistencies in running each antibody, since we ran each at its pre-determined manufacturing conditions. Since the resin bed heights and elution buffers were not the same for each antibody tested initially, the possible dependence on bed height and elution buffer was also explored. Humanized 2C4 was tested previously using the acetate elution buffer, and the results are shown in Table 2. Humanized 2C4 was run at lab scale at room temperature and at pilot scale at 15° C. Within the variability between the runs and error in the assay, all the conditions produced similar leached protein A results. Citrate and acetate have approximately equivalent effects on protein A leaching. Bed height was the other potential contributor to the higher levels of protein A leaching seen with humanized 2C4 and Trastuzumab in comparison with the other antibodies tested. When one lot of Trastuzumab HCCF was run on three bed heights in triplicate, the leached protein A results were nearly identical as shown in Table 2. Bed height does not appear to affect the level of protein A leaching.

TABLE 2

Effect of Bed Height on Protein A Leaching
Runs were performed at 20° C. using Trastuzumab HCCF on PROSEP vA ™ resin packed in a 0.66 cm diameter by 20 cm high column. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0 or 7.1, eluted with either 25 mM citrate pH 2.8, or 0.1 M acetic acid pH 2.9, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0 at 40 CV/hr. The titer of Trastuzumab pilot plant 400 L HCCF was 0.7 mg/ml, and the column was loaded to 20 g Trastuzumab per liter of resin. The elution pool was collected from 0.2 AU to 2 CV's.

Bed Height cm	Protein A ng/mg
10	55 +/− 6
14	50 +/− 2
20	55 +/− 0

The effect of elution buffer on protein A leaching was also assessed. Citrate and acetate have approximately equivalent effects on protein A leaching as shown in Table 3 below.

TABLE 3

Effect of Elution Buffer on Leached Protein A
Leached protein A is shown in parts per million humanized 2C4 antibody was run on a 20 cm bed height column, loaded to 14 g humanized 2C4 per liter resin antibody. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 7.1, eluted with 0.1 M acetic acid pH 2.9, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0 at 40 CV/hr. Some runs were eluted with 25 mM citrate pH 2.8. The pool was collected from 0.5 AU to 2 CV's pool volume. The lab scale runs were performed on a 0.66 cm diameter column and the pilot scale runs were performed using a 10 cm diameter column containing PROSEP A ™. Two humanized 2C4 antibody runs were eluted with citrate and three humanized 2C4 antibody runs were eluted with acetate at pilot scale. Three humanized 2C4 antibody runs were performed with each elution buffer at lab scale.

MAb	Scale	Temperature ° C.	Protein A from Acetate (ng/mg)	Protein A from Citrate (ng/mg)
Humanized 2C4	Lab	room temp.	18 +/− 1	22 +/− 5
Humanized 2C4	Pilot	15	10 +/− 2	15 +/− 6

Protein A leaching with respect to temperature for 2 lots of Trastuzumab HCCF at pilot scale (1.26 L column) is shown in FIG. **3**. The same exponential trend at pilot scale observed at small scale was reproduced. Small-scale duplicate runs were performed using the lots of HCCF, which were used in the pilot plant. The pilot plant results line up exactly with lab scale results from runs performed with the same HCCF on the same lot of PROSEP vA™. Trastuzumab at full scale. The HCCF was chilled to 15+/−3° C. and run on PROSEP vA™ resin. Table 4 shows the level of protein A in the protein A pools for 5 runs. In all runs the leached protein A level was 10 ng/mg or less demonstrating that controlling the temperature of the HCCF controls protein A leaching.

TABLE 4

Leached Protein A In Protein A Pools 12,000 L Process
HCCF was chilled to 15 +/− 3° C., column was 100.5 L, 80 cm in diameter by 20 cm in height, and eluted with citrate. Temperature was measured in the HCCF tank, between the pump and the column, and at the outlet to the column. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0, eluted with either 25 mM citrate pH 2.8, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0.

Trastuzumab concentration (mg/mL)	Protein A in Pool (ng/mg)
0.69	8
0.69	7
0.67	10
0.72	8
0.68	7

CONCLUSIONS

Temperature affects protein A leaching during protein A affinity chromatography of antibodies to varying degrees. Some antibodies are more affected than others; HER2 antibodies Trastuzumab and humanized 2C4 were greatly affected. The lower leaching antibodies are all run on 14 cm bed height columns and are eluted with 0.1M acetic acid, while the higher-leaching ones are run on 20 cm bed height columns and eluted using 25 mM citric acid. The bed height correlation was investigated and found to have no influence on protein A leaching. Citrate or acetate elution had essentially equivalent effects on protein A leaching.

By controlling the HCCF temperature, the level of protein A in the protein A pool can be controlled, or reduced. A similar test was performed at pilot scale. Two lots of Trastuzumab HCCF were run on a 1.26 L PROSEP vA™ column at 5 temperatures and the level of protein A in the elution pools was measured. Protein A leaching depended on temperature identically to the same HCCF run at small scale, and to other lots of HCCF run at small scale. At large scale, Trastuzumab HCCF was chilled to 15+/−3° C. and protein A leaching was controlled to less than or equal to 10 ng/mg. All antibodies are affected by temperature, but to varying degrees. At all scales, controlling the temperature of the HCCF during loading could control protein A leaching. Increasing HCCF temperature has an exponentially increasing effect on Protein A leaching.

EXAMPLE 2

Protease Inhibitors for Reducing Protein a Leaching During Protein a Affinity Chromatography

Protein A chromatography may be used as an initial capture step in a recovery process for an antibody, such as an antibody recombinantly produced by a Chinese Hamster Ovary (CHO) cell. This step achieves a high degree of purity while maintaining a high yield. Leaching of the Protein A ligand into the elution pool is a disadvantage of this step, which may require subsequent chromatography steps to remove the leached Protein A. PROSEP A™ and PROSEP vA™ resins which can be used for Protein A chromatography, comprise the Protein A ligand immobilized onto a controlled pore glass (CPG) backbone.

Protein A can leach from the CPG backbone through several mechanisms, including, but not limited to, mechanical shearing, low pH exposure during the elution phase, and/or proteolytic activity. As shown in Example 1 above, Protein A leaching was shown to be dependent on temperature during loading.

Protein A leaching was also shown to be partially inhibited by pH treatment of the harvested cell culture fluid (HCCF). In particular, a 2 hour incubation of HCCF at pH 3 reduced leaching from approximately 30 ppm to 4 ppm.

Proteases can be organized into four major classes based on their mode of action. These are serine, cysteine, metallo- and aspartic proteases. Inhibitors that selectively inhibit these classes were tested over a range of concentrations (Table 5). These inhibitors were individually added to Trastuzumab HCCF, and the conditioned HCCF was purified across PROSEP VA™ resin at a fixed temperature of 25° C. If a reduction in leached Protein A was observed with a specific inhibitor, its effect was re-examined at 15° C., a temperature known to reduce leaching. This allowed an examination of the combined effect of temperature and inhibitor concentration on Protein A leaching. The inhibitors listed in Table 5 below have been tested, with the exception of Pepstatin.

TABLE 5

Inhibitors of the Four Major Classes of Proteases

Inhibitor	Class of Protease	Inhibits	Does not inhibit	Recommended Starting concentration
EDTA	Metallo-	thermolysin etc.		N/A
PEFABLOC ® SC	Serine	trypsin, chymotrypsin, plasmin, plasma kallikrein, and thrombin.		0.4-4.0 mM
Aprotinin	Serine	plasmin, kallikrein, trypsin, and chymotrypsin	thrombin or Factor X	0.01-0.3 mM
Leupeptin	Cysteine and serine with trypsin-like activity	trypsin, papain, plasmin, and cathepsin B.		1 mM
Pepstatin*	Aspartic	pepsin, renin, cathepsin D, chymosin, and many microbial acid proteases.		1 mM

*Pepstatin is not soluble in aqueous solutions; a water soluble aspartic protease inhibitor may be used instead.

RESULTS AND DISCUSSION

With increasing EDTA concentration, there was a decrease in Protein A leaching (FIG. **8**). There was further a combined effect of EDTA and temperature on the inhibition of Protein A leaching.

With increasing PEFABLOC® concentration, there was a decrease in Protein A leaching (FIG. **9**). This experiment shall be repeated at 15° C.

Aprotinin, another serine protease, did not have an effect on Protein A leaching (Table 6). Leupeptin, a protease inhibitor that can inhibit both serine and cysteine proteases, did not have an effect on Protein A leaching (Table 7).

TABLE 6

Effect of Aprotinin, a Serine Protease Inhibitor, on Protein A Leaching

Aprotinin (mg)	Aprotinin (uM)	Protein A (ppm)	1 Standard Deviation
0	0	37.4	5.2
14	12	35.6	0.1
28	25	31.1	0.4
54	47	34.53	0.0

TABLE 7

Effect of Leupeptin, a Serine and Cysteine Protease Inhibitor, on Protein A Leaching

Leupeptin (mM)	Protein A (ppm)	1 Standard Deviation
0	37.4	5.2
0.15	32.9	0.7
0.3	32.4	1.5
0.6	34.4	1.1

SEQUENCE LISTING

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Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 100                 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
               110                 115                 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
               125                 130                 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
               140                 145                 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
               155                 160                 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
               170                 175                 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
               185                 190                 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
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Arg Gly Glu Cys
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                50                  55                  60
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-continued

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Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 100                 105

Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
                125                 130                 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
                140                 145                 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
                155                 160                 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                170                 175                 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
                185                 190                 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
                200                 205                 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
                215                 220                 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
                230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                260                 265                 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
                275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
                290                 295                 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                305                 310                 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
                320                 325                 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
                335                 340                 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
                350                 355                 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                365                 370                 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
                380                 385                 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
                395                 400                 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                410                 415                 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
                425                 430                 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                440                 445
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Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser
20 25 30

Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr
20 25 30

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
50 55 60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
95 100 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

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1 5 10 15

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Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Thr Ile Ser
                20                  25                  30

Lys Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

His Asn Glu Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 100                 105

Ile Lys Arg


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  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

Gly His Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Tyr
                50                  55                  60

Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
                65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ile Tyr Phe Tyr Gly Thr
                95                 100                 105

Thr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
               110                 115                 120

Ser


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Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser
                20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
                50                  55                  60
```

-continued

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Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 100                 105

Ile Lys Arg


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  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
                20                  25                  30

Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
                50                  55                  60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser
                65                  70                  75

Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser
                95                 100                 105

Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
               110                 115                 120

Val Ser Ser
```

The invention claimed is:

1. A method of purifying a protein which comprises a C_H2/C_H3 region, comprising subjecting a composition comprising said protein to protein A affinity chromatography at a temperature in the range from about 10 ° C. to about 18 ° C.

2. The method of claim **1** further comprising exposing the composition subjected to protein A affinity chromatography to a protease inhibitor.

3. The method of claim **2** wherein the protease inhibitor is EDTA or 4-(2-aminoethyl)-benzenesulfonyl-fluoride, hydrochloride (AEBSF).

4. The method of claim **2** comprising adjusting the pH of the composition prior to protein A affinity chromatography to a pH in the range from about 2.5 to about 3.5.

5. The method of claim **1** wherein the protein is an antibody.

6. The method of claim **5** wherein the antibody binds an antigen selected from the group consisting of HER2, vascular endothelial growth factor (VEGF), IgE, CD20, CD40, CD11a, tissue factor (TF), prostate stem cell antigen (PSCA), interleukin-8(IL-8), epidermal growth factor receptor (EGFR), HER3, HER4, $\alpha4\beta7$ and $\alpha5\beta3$.

7. The method of claim **5** wherein the antibody is selected from the group consisting of Trastuzumab, humanized 2C4, humanized CD11a antibody, and humanized VEGF antibody.

8. The method of claim **5** wherein the antibody binds HER2antigen.

9. The method of claim **8** wherein the antibody is Trastuzumab or humanized 2C4.

10. The method of claim **1** wherein the protein is an immunoadhesin.

11. The method of claim **10** wherein the immunoadhesin is a TNF receptor immunoadhesin.

12. A method of purifying a protein which comprises a C_H2/C_H3 region comprising:

a. subjecting a composition comprising said protein to protein A affinity chromatography to provide a recovered composition and measuring leached protein A in said recovered composition;

b. if greater than about 20 ng protein A per mg of said protein is measured in said recovered composition, then performing subsequent purification of compositions comprising said protein by protein A affinity chromatography at a temperature in the range from about 10° C. to about 18° C., such that protein A leaching is reduced; and

c. exposing the composition subjected to protein A affinity chromatography to a protease inhibitor in order to reduce the protease activity and further reduce leaching of protein A.

* * * * *