

Inter Partes Review No.: IPR2022-01531

U.S. Patent No. 10,131,682

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SYNAFFIX B.V.

Petitioner

v.

HANGZHOU DAC BIOTECH CO., LTD.,

Patent Owner

Patent No. 10,131,682

Ex Parte Reexamination Certificate Issued: January 4, 2021

Title: HYDROPHILIC LINKERS AND THEIR USES FOR CONJUGATION OF
DRUGS TO A CELL BINDING MOLECULES

Inter Partes Review No. IPR2022-01531

DECLARATION OF DR. SHEILA BAKER

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I, Dr. Sheila Baker, declare and state as follows:

I. INTRODUCTION

1. I am over the age of eighteen (18) and otherwise competent to make this declaration.

2. I have been retained by Foley & Lardner, LLP, counsel for Petitioner, as an expert declarant in the above captioned *inter partes* review (“IPR”), to provide my testimony and opinions relating to certain issues arising in the proceeding.

3. I have no financial stake in the outcome of this proceeding. While I am being compensated for my time at my normal hourly rate for such services, my compensation does not depend on the content or specifics of my testimony or opinions provided herein. The testimony and opinions expressed herein are my own.

II. BACKGROUND AND QUALIFICATIONS

4. My qualifications are summarized in an abbreviated version of my CV, which I understand is being submitted as an exhibit in this proceeding. In summary, I am currently an Associate Professor in the Department of Biochemistry and Molecular Biology, Carver College of Medicine, at the University of Iowa. I have held this position since 2017, and I was an Assistant Professor at the same institution from 2010 to 2017.

5. I have extensive research experience related to photoreceptor cells and their molecular biology. However, I understand that my overall research is not the subject of my relevant expertise here. Instead, I have been asked to opine general about various techniques used in relation to cell-binding research and studies. In regards to this topic, as part of my overall research, I have been conducting and overseeing others conduct cell-binding experiments for over two decades. Such experiments are regularly conducted in my lab, and I am very familiar with the various techniques used to measure cell-binding in various settings.

6. I understand that in this proceeding, a “person of ordinary skill in the art” (“POSA”) has been defined as follows:

[A POSA] would have had a PhD degree in organic chemistry, biochemistry, medicinal chemistry and/or pharmacology, along with 1-2 years of relevant applied research and/or industry experience in the field of linkers and conjugates for biologically active molecules. Alternatively, a POSA would have had a Master’s or Bachelor’s degree in one of these same fields with, respectively, at least 3-5 or 5-7 years work experience. Such a person would have been familiar with protein/drug conjugates, including antibody-drug conjugates, the use of antibodies to selectively target cells and tissues associated with various diseases and conditions, linker design and selective attachment of the linker to proteins/antibodies and drugs, including targeted release of the drugs, as well as the need for water soluble conjugates for use with

hydrophobic drugs, and methods for enhancing the potency of the protein/antibody-drug conjugates.

7. My Ph.D. is in cell biology, neurobiology, and anatomy which is consider to be comparable to biochemistry. I also have extensive experience studying cell properties and function, including the binding of proteins to cell surfaces, both *in vivo* and *in vitro*. Thus, my experience would far exceed the definition above with respect to the binding of molecules or other agents to cell surfaces, including in regarding to my level of education, my work/research experience, and my background knowledge regarding both cell binding experiments and the cell fixation techniques performed as a part of such experiments.

8. This topic is also covered in the courses I regularly teach at the University of Iowa, including Biochemistry and Molecular Biology I, Biochemistry and Molecular Biology II, and Independent Study.

III. DOCUMENTS AND INFORMATION CONSIDERED

9. In providing the testimony and opinions in this declaration, I have reviewed the additional documents listed below:

Ex. No.	Prior Art Citation
EX1006	Straus & Keller, "Unusual Binding Sites for Horseradish Peroxidase on the Surface of Cultured and Isolated Mammalian Cells," <i>Histochemistry</i> 85, July 1986, pages 277-285 ("Straus")
EX1074	Maria Célia Jamur and Constance Oliver,

	Immunocytochemical Methods and Protocols, Methods in Molecular Biology, Vol. 588, 2010
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IV. DISCUSSION

A. A POSA Would Understand Straus to Teach that HRP Binds to Cell Surfaces

10. I understand that the Straus reference (Ex. 1006) was relied upon by Petitioner to demonstrate that the protein known as “HRP” (which is an abbreviation for horseradish peroxidase) binds to the surfaces of certain types of cells. I agree that Straus teaches this as a matter of fact, and that a POSA would have this understanding upon reviewing Straus.

11. Straus is entitled “Unusual Binding Sites for Horseradish Peroxidase on the Surface of Cultured and Isolated Mammalian Cells.” In its Summary, Straus reports that:

Binding sites for horseradish peroxidase (HRP), with unusual properties, were detected on the surface of cultured and isolated cells after the cells (on cover slips) had been quickly dried, fixed in cold methanol, and postfixed in a paraformaldehyde solution.

Ex. 1006, 1.¹ Straus' Summary further reports the types and concentrations of various reagents that were found to suppress the binding of HRP to cell surfaces.

Id. In addition, Straus reports that the addition of calcium (Ca^{2+}) was not required for the binding of HRP to the cell surface. *Id.*

12. Straus' Introduction acknowledges that prior studies had shown that HRP binds to "macrophages, fibroblasts, mast cells and endothelial cells of rat tissues" in the presence of calcium. *Id.* I note that calcium is present in all living organisms, and in mammalian cells the availability of free calcium is tightly controlled to prevent artifacts from non-specific binding of proteins to cells or cells to other cells; quality control experiments were conducted with low and high calcium to demonstrate the HRP binding was specific. The Summary further provides that in its study, "binding sites for HRP with unusual properties were detected at the surface of cultured and isolated cells."² *Id.* The Summary also indicates that the reported results had been presented at a recent meeting associated

¹ For my citations to Straus in this declaration, I will refer to the page numbers that appear to have been added to the document, in which the first page is numbered page 1.

² In a note apparently added after the original proof of the article had been submitted, Straus indicates that "relatively high concentrations of added CaCl_2 , intensify the reaction for surface-bound HRP." *Id.*, 9.

with the Journal of Histochemistry and Cytochemistry. *Id.*, 1 (citing Straus and Keller, 1986), 9.

13. The “Results” section of Straus contains various reports on and references to the binding of HRP to cell surfaces, too numerous to call out. Figures 1-14 show reactions for cell surface-bound HRP under various conditions and for various types of cells. And in the Results subsection labeled “*Comparative observations on the reaction for surface-bound HRP in various cell lines*,” Straus reports that:

The reaction for surface-bound HRP showed the highest intensity in sub-confluent cultures of the following cell lines: GH; cells; HeLa cells; SV40-3T3 cells; SV40-1-3T3 cells; and L cells. The reaction was of intermediate intensity in the following cell lines: Swiss mouse 3T3 cells; Chinese hamster ovary cells; WI-38 cells; Rat 1 cells; and mouse myeloma cells. The following cell lines showed only a weak surface reaction: HSWP cells and GM 0337 cells.

Ex. 1006, 5. The above listing includes cells from different species (human, rat, mouse, hamster), that form parts of the immune, reproductive, or connective tissue systems. This represents very good experimental design because, while showing HRP binds to many types of cells, it also shows that some cell types have little to no binding, thus demonstrating that Straus’ experiments could distinguish between the two circumstances. This section also notes that “the reaction for surface-

bound HRP was much decreased or abolished when cells became confluent.” *Id.*

This means that when adjacent cells began to come together and touch each other, HRP adsorption on the surfaces was not observed in those areas.

14. Taking all of Straus’ reported results together, or even considering them individually, a POSA would have understood that Straus reports that HRP binds to the surfaces of the cell types described, with HRP binding to some of the listed cell types more strongly than others. This is typical of cell binding agents, where the agent binds more strongly to some types of cells and less strongly to other types of cells.

15. This conclusion is further bolstered by Straus’ observation that binding did not occur when the cells became confluent, where the cell surfaces shield each other from the HRP. The conclusion is bolstered even further by Straus’ observations regarding various conditions that reversed or prevented HRP binding to the cell surfaces (*e.g., id., 4 (“Inhibition of the surface binding of HRP by various carbohydrates, or compounds containing carbohydrates”)*)), which shows that there are conditions in which HRP’s cell-surface binding can be inhibited or reversed.

B. A POSA's Understanding from Straus that HRP Binds to Cell Surfaces Would Not Have Been Undermined by Straus' Cell Fixation Techniques

16. I understand that DAC has argued that because Straus uses a cell fixation technique whereby the cells are treated with cold methanol and paraformaldehyde that kills the cells, this undermines Straus' teaching that HRP binds to cells. I understand the implication to be that because Straus' binding experiments are carried out on cells that have been killed first by the fixation technique, they therefore are not representative of HRP binding to living cells. This is not the understanding a POSA would take away from Straus.

17. To begin with, it is true that treating (or "fixing") cells with methanol and paraformaldehyde will generally kill the cells. However, this misses the whole point of cell fixation. The purpose of cell fixation is to preserve the cells in as natural of a state as possible (*i.e.*, as close to their living state as possible) precisely so that cell binding experiments can then be conducted reliably and one can learn whether a given protein/agent will bind to those cells in their natural state.

18. Cell fixation is a technique I have commonly practiced and that is commonly practiced in my lab when conducting cell binding studies. The principles underlying cell fixation apply regardless of the cell type being tested. Moreover, this technique is taught in textbooks and in the classes I teach on the subject at the University of Iowa.

19. To illustrate, I can refer to a book chapter entitled “Cell Fixatives for Immunostaining” by Maria Célia Jamur and Constance Oliver, in the book *Immunocytochemical Methods and Protocols, Methods in Molecular Biology* (2010). The abstract of this chapter begins by explaining that:

Fixation is one of the most critical steps in immunostaining. The object of fixation is to achieve good morphological preservation, while at the same time preserving antigenicity.

Ex. 1074, 55. For reference, the cell binding experiments conducted by Straus are “immunostaining” type experiments. Jamur and Oliver go on to further explain that:

Fixation is also necessary to stabilize the sample and protect it from the deleterious effects of the immunostaining process. Fixation also helps to prevent artifactual diffusion of cell components, arrests enzymatic activity, and avoids decomposition of the tissue. Furthermore, the process of fixation helps to preserve the sample by inhibiting bacterial and fungal growth and by inhibiting lysosomal enzymes whose activity could lead to autolysis.... The most widely used fixative for immunostaining is formaldehyde [], either alone or in combination with a solvent such as methanol.

Id., 55-56. The chapter also makes specific reference to using cold methanol. *Id.*, 58 (Section 3.4 “Cold Methanol or Acetone”).

20. Thus, as confirmed by Jamur and Oliver (and many other similar references), a POSA understood that treatment with cold methanol and paraformaldehyde is a standard and necessary cell-fixation technique that is used to preserve the cell status quo so that binding studies using the immunostaining technique can be subsequently carried out. A POSA would understand that cell fixation is desired in order to obtain results that reliably determine the cell surface binding that will occur, or not, with living cells.

21. With this background knowledge understood by a POSA, Straus' reported HRP cell-surface binding results would have been viewed as reliable, especially given Straus' description of the efforts specifically taken to minimize background adsorption and other artifacts. *Id.*, 2-3 ("A systematic study was made in order to find conditions that resulted in a strong binding of HRP to the cell surface and only a slight "background" adsorption of HRP"; "The speed of drying also influenced the preservation of the binding sites at the cell surface."), 6 ("These observations suggest that the HRP-binding protein may be washed out of the cell surface during conventional types of fixation [i.e., absent treatment with cold methanol].").

22. Straus also identified a number of potential artifacts that might hypothetically have affected the results in some way, but Straus went on to explain why each such possibility could be ruled out as having a significant impact on the

result. *Id.*, 7-8 (“The possibility of artifacts must be considered when applying cytochemical methods.”). This indicates that the Straus researchers were thoughtful in conducting their experiments and developing their experimental methods, and lends further credibility to the reported results.

23. Consequently, the fact that Straus used well-known cell fixation techniques (even if this did kill the cells as part of the process) and addressed (and discounted) potential artifacts would not have led a POSA to question whether HRP binds to the surfaces of living cells of the identified types, but rather just the opposite. A POSA would take away from Straus an understanding that HRP does in fact bind to the surfaces of living cells of the identified types and would have been reasonable in believing that to be the case.³ This is my opinion based on my knowledge of and expertise with cell fixation techniques and cell binding experiments.

* * * * *

³ I also briefly reviewed the documents cited in reference to the first sentence of Straus’ Introduction (Ex. 1006, 1), i.e., “Straus 1981, 1983a, b, c.” These documents also report HRP cell binding, using varied methods for cell fixation. That the various methods of fixation used in those studies likewise show binding of HRP to cell surfaces in cultured cells and cells within intact tissue, lends further credibility to the conclusion that HRP binds to the surfaces of those cells in the living state, as reported in Straus.

I hereby declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct, and that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001).

Executed on 3/14/25, 2025

Sheila A Baker

Sheila Baker