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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte KE-HE RUAN

Appeal 2019-004812
Application 14/885,561
Technology Center 1600

Before DONALD E. ADAMS, ERIC B. GRIMES, and JENNIFER MEYER CHAGNON, *Administrative Patent Judges*.

ADAMS, *Administrative Patent Judge*.

DECISION ON APPEAL

Pursuant to 35 U.S.C. § 134(a), Appellant[1] appeals from Examiner's decision to reject claims 1–3, 5, 16, 19, and 20 (Appeal Br. 5). We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

[1] We use the word "Appellant" to refer to "applicant" as defined in 37 C.F.R. § 1.42. Appellant identifies the real party in interest as "University of Houston System" (Appellant's December 11, 2018 Appeal Brief (Appeal Br.) 3).

STATEMENT OF THE CASE

Appellant's disclosure "relates to the construction and expression of a single linked protein molecule that possesses both the enzyme functions of the native . . . [cyclooxygenase (COX)] and that of . . . [prostacyclin synthase (PGIS)]" (Spec. ¶ 3). Claim 1 is representative and reproduced below:

> 1. An isolated hybrid protein molecule comprising an enzymatically active human cyclooxygenase isoform-1 (COX-1) enzyme and a human prostacyclin synthase (PGIS) enzyme wherein said COX-1 enzyme and said PGIS enzyme are connected with a linker, wherein said linker connects C-terminus of the COX-1 enzyme with N-terminus of the PGIS enzyme; and wherein said hybrid protein molecule converts AA (arachidonic acid) to PGI_2 (prostacyclin).

(Appeal Br. 17.)

Ground of rejection before this Panel for review:[2]

Claims 1–3, 5, 16, 19, and 20 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Ruan '05,[3] Ruan '04,[4] Sakaki,[5] Lindbladh,[6] and Hargrave.[7]

ISSUE

Does the preponderance of evidence relied upon by Examiner support a conclusion of obviousness?

FACTUAL FINDINGS (FF)

FF 1. Ruan '05 discloses a "cyclooxygenase (COX)/ prostaglandin I_2 synthase (PGIS) coupling reaction system . . . used to determine the

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[2] Appellant's claim 1 stands objected on this record (*see* Examiner's September 20, 2018 Non-Final Action (Non-Final Act.) 2). For the reasons set forth by Examiner, we find this objection to be a Petitionable rather than Appealable issue and, therefore, decline to discuss this objection further (Examiner's April 2, 2019 Answer (Ans.) 8).

[3] Ke-He Ruan et al., *The N-terminal membrane anchor domain of the membrane-bound prostacyclin synthase involved in the substrate presentation of the coupling reaction with cyclooxygenase*, 435 Archives of Biochemistry and Biophysics 372–81 (2005).

[4] Ke-He Ruan, *Advance in Understanding the Biosynthesis of Prostacyclin and Thromboxane A_2 in the Endoplasmic Reticulum Membrane via the Cyclooxygenase Pathway*, 4 Mini-Reviews in Medicinal Chemistry 639-47 (2004).

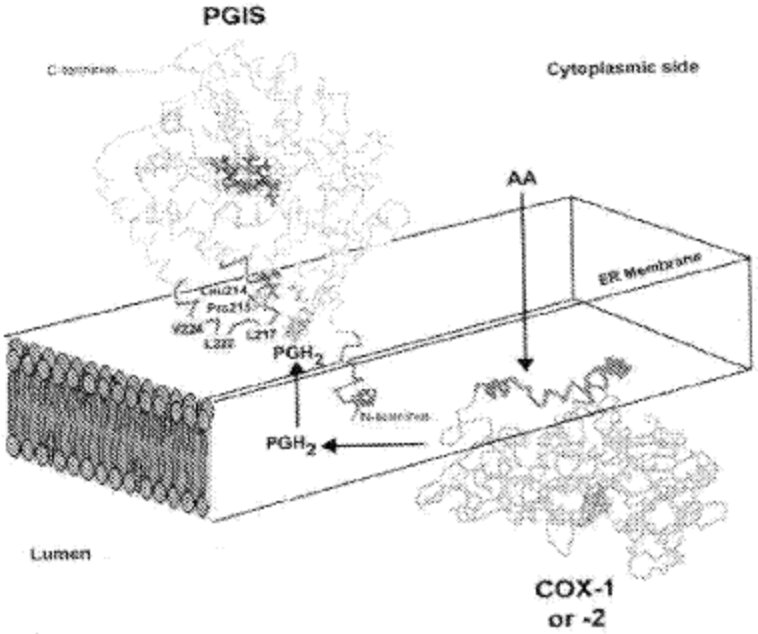
[5] Toshiyuki Sakaki et al., *Kinetic Studies on a Genetically Engineered Fused Enzyme between Rat Cytochrome P4501A1 and Yeast NADPT-P450 Reductase*, 33 Biochemistry 4933–39 (1994).

[6] C. Lindbladh et al., *Preparation and Kinetic Characterization of a Fusion Protein of Yeast Mitrochondrial Citrate Synthase and Malate Dehydrogenase*, 33 Biochemistry 11692–98 (1994).

[7] P.A. Hargrave et al., *The Structure of Bovine Rhodopsin*, 9 Biophys. Struct. Mech. 235–44 (1983).

coordination of PGIS with COX for the biosynthesis of prostacyclin (PGI_2) using arachidonic acid (AA) as a substrate in a membrane-bound environment" (Ruan '05, Abstract; *see id.* at 373 ("PGI_2 serves as one of the most important cardiovascular protectors in physiopathological conditions"); Ans. 3).

FF 2. Ruan '04's Figure 8 is reproduced below:



Ruan '04's Fig. 8 illustrates "[a] model of the coordination of PGIS and COX in the biosynthesis of PGI_2 in the ER membrane" (Ruan '04 646: Fig. 8, Legend).

FF 3. Ruan '05 discloses that "the two isoforms of the COX enzymes, cyclooxygenase-1 (COX-1) and -2 (COX-2) . . . [and] PGIS . . . are mainly localized in the endoplasmic reticulum (ER) membrane," wherein the catalytic domains of the COX "proteins lie on the luminal side of the ER, and are anchored to the ER membrane by hydrophobic side chains of the amphipathic helices A–D" and the catalytic domain of PGIS is "on the cytoplasmic [side of the ER membrane and] . . . is anchored to the ER

membrane by the first 20 residues and residues in the helix F/G Loop" (Ruan '05 373 (endnotes omitted); *see also* Ruan '04 646: § 8; *see* Ans. 3–4).

FF 4. Ruan '05 discloses that PGIS is topologically arranged opposite the orientation of COX-1 and -2 with respect to the ER membrane (Ruan '05 373; *see also* Ans. 4; Ans. 13 (Examiner finds that Ruan '04 and Ruan '05 disclose that the amino acid sequences of human COX-1 and human PGIS were known in the art at the time of Appellant's claimed invention)).

FF 5. Examiner finds that neither Ruan '04 nor Ruan '05 "teach a fusion protein comprising COX-1 and PGIS" (Ans. 4).

FF 6. Sakaki discloses a recombinant hybrid fusion protein comprising membrane-bound yeast NADPH-P450 reductase and rat cytochrome P4501A1 (*see* Sakaki 4933; Ans. 4).

FF 7. Sakaki discloses that its "fused enzyme is capable of rapidly transferring electrons from . . . the reductase part" of the fused enzyme to the cytochrome portion of the fused enzyme (*see* Sakaki 4938; Ans. 4).

FF 8. Examiner finds that Sakaki does "not teach a fusion protein comprising human COX-1 and human PGIS" (Ans. 4).

FF 9. Lindbladh discloses that "many enzymes that act within a metabolic pathway interact with each other to form organized enzyme complexes" and that "[a] considerable body of evidence . . . demonstrates these interactions and shows differences in kinetics of enzymes which catalyze sequential metabolic reactions when they are in close proximity compared to the kinetics of the same enzymes in random solution" (Lindbladh 11692; *see* Ans. 5).

FF 10. Lindbladh discloses that "[a] number of bienzymatic fusion proteins which catalyze sequential reactions have been constructed," which "have

shown kinetic benefits for the overall reaction" (Lindbladh 11692; *see* Ans. 5).

FF 11. Examiner finds that Lindbladh discloses "the preparation of a fusion protein comprising citrate dehydrogenase and malate dehydrogenase joined by a linker peptide" (Ans. 5 (citing Lindbladh, Abstract)).

FF 12. Examiner finds that Lindbladh does "not teach a fusion protein comprising human COX-1 and human PGIS" (Ans. 5).

FF 13. Examiner finds that Hargrave discloses "the structure of bovine rhodopsin," which "has seven transmembrane alpha helices" that "vary from 21-28 amino acids, and that this is a length that is quite adequate to span the distances of the fatty acid side chains in the lipid bilayer" (Ans. 5).

FF 14. Examiner finds that Hargrave does "not teach a fusion protein comprising human COX-1 and human PGIS" (Ans. 5).

FF 15. Appellant discloses Km values of a membrane-bound COX-2–10aa (linker)–PGIS fusion protein compared to those "previously characterized by several groups" for individual COX2 and PGIS enzymes as follows:

Table 1. Comparison of the Km values of COX2-10aa-PGIS and the individual enzymes

	COX2-10aa-PGIS	COX2	PGIS
Km	3.2μM (expressed in HEK cells)	6.5μM[1]	13.3μM[2]
	4 μM (expressed by BV system)	0.9μM[3]	30μM[4]
			9.5μM[5]

(Spec. ¶ 93; *see also id.* ¶ 68 (Appellant discloses a "COX-2–10aa–PGIS" fusion protein exhibiting a Km value of "~3.2 µM" (*see also* Ruan '06[8] 14009: Fig. 6(A) (disclosing a Km of 3.2 µM for a COX-2–10aa–PGIS fusion protein)))).

ANALYSIS

Examiner finds that the COX-1 and PGIS enzymes are topologically arranged in an ER membrane to act cooperatively as part of an extremely important pathway and, thus, those of ordinary skill in this art would have found it prima facie obvious to characterize the interaction of these enzymes (*see* Ans. 6; *see also id.* at 3–4; FF 1–4). In this regard, Examiner directs attention to Lindbladh's disclosure of proximity effects associated with organized enzyme complexes and, specifically to a fusion protein model designed to study such proximity effects (*see* Ans. 6; FF 9–11; *see also* FF 6–7 (disclosing the interaction of cooperating parts of a dual enzyme fusion protein)). Looking to the topological orientation of COX-1 and PGIS in the ER membrane, as disclosed by Ruan '05 and Ruan '04, Examiner reasons that a person of ordinary skill in this art would have found it prima facie obvious to utilize known methods, such as those disclosed by Sakaki and Lindbladh, to link the known sequences of the COX-1 and PGIS "proteins via a peptide linker that is part of a transmembrane domain, such as the Rhodopsin transmembrane helices," which are also known in the art, such that the COX-1 and PGIS "enzymes are in opposite directions at a distance similar to the distance between PGIS and COX-1 found in nature" (*see* Ans.

[8] Ke-He Ruan et al., *Engineering of a Protein with Cyclooxygenase and Prostacycline Synthase Activities That Converts Arachidonic Acid to Prostacyclin*, 45 Biochemistry 14003–11 (2006).

6; *see also* FF 1–4, 6, 7, 9–11, and 13). Thus, Examiner reasons, because such a fusion protein construct would be

> expected to locate to the ER membrane, placing the N-terminus of human PGIS next to the C-terminus of the linker (N_{ter}-COX-C_{ter}-linker-N_{ter}-PGIS-C_{ter}) is the *only possible configuration* that would allow the N-terminus of human PGIS to be in a position similar to that found in nature, namely closest to the ER membrane, *by virtue of its proximity to the linker*.

(Ans. 7.)

Therefore, based on the combination of Ruan '05, Ruan '04, Sakaki, Lindbladh, and Hargrave, Examiner concludes that, at the time Appellant's invention was made, it would have been prima facie obvious to have a fusion protein comprising human COX-1 and human PGIS, wherein the C-terminus of the human COX-1 protein is linked to the N-terminus of the human PGIS by an alpha-helix peptide from the bovine rhodopsin transmembrane domain (Ans. 6). According to Examiner, "[a] person of ordinary skill in the art [would have been] . . . motivated to make said fusion protein, purify it and place it in a buffer solution, for the benefit of placing together two enzymes of a well-known coupling reaction system . . . [to] further characterize the interaction of both proteins" (*id.*).

As discussed above, COX-1, PGIS, and transmembrane linker sequences were known in the art at the time of Appellant's claimed invention (*see* FF 4 and 13). Therefore, we are not persuaded by Appellant's intimation that the components necessary to produce Appellant's claimed fusion protein were not known in the art at the time of Appellant's claimed invention (*see* Appeal Br. 13).

As further discussed above, Sakaki and Lindbladh each disclose methods of producing bienzymatic fusion proteins which catalyze sequential

reactions (*see* FF 6, 7, and 9–11). Lindbladh further discloses that "[a] number of bienzymatic fusion proteins which catalyze sequential reactions have been constructed," which "have shown kinetic benefits for the overall reaction," suggesting the routine nature of producing such constructs (FF 10). Thus, at the time of Appellant's claimed invention, those of ordinary skill in this art understood how to construct an isolated hybrid protein molecule such as that required by Appellant's claimed invention without undue experimentation. Therefore, we are not persuaded by Appellant's contention that Examiner improperly relied upon Official Notice of facts to establish that methods of producing a fusion protein within the scope of Appellant's claimed invention were known in the art at the time of Appellant's claimed invention (Appeal Br. 14; *see* Reply Br. 2 (Appellant contends that "no prior art can be cited as providing a teaching as to a method of making the protein")).

Appellant failed to establish an evidentiary basis to support a conclusion that the evidence relied upon by Examiner would have been incapable of producing a functional fusion protein within the scope of Appellant's claim 1 (*see e.g.*, FF 4, 6, 7, 9–11, and 13). For the foregoing reasons, we are not persuaded by Appellant's unsupported contentions regarding "a plethora of parameters including," *inter alia*, "pH, temperature, concentration of reagents," etc., which Appellant contends are required "in order to achieve the correct folding of each of the protein subunits in which both enzymatic sites are so positioned that they are complementary to each other" (Appeal Br. 13; *see also* Reply Br. 3). For the foregoing reasons, we are not persuaded by Appellant's intimation that the combination of references relied upon by Examiner fails to provide guidance necessary to

produce the claimed fusion protein (*see* Appeal Br. 13; Reply Br. 2 (Appellant contends that "one of ordinary skill has no recourse other tha[n] to undertake the considerable burden of undue experimentation in order to arrive at the hybrid protein molecule of claim 1")). *See In re Pearson*, 494 F.2d 1399, 1405 (CCPA 1974) ("Attorney's argument in a brief cannot take the place of evidence.").

For the foregoing reasons, we are not persuaded by Appellant's contention that the combination of prior art relied upon by Examiner fails to enable the production of Appellant's claimed fusion protein (*see* Reply Br. 2).

Appellant contends that "[t]he inventors of the current application made a number of different fusion proteins before successfully achieving the claimed isolated fusion protein with the required Km of 3.2μM to 4 μM" (Appeal Br. 15 (citing Ruan '06); *see generally* Reply Br. 3). Appellant further contends that

> in order to obtain a hybrid protein with the same K[m] value as the hybrid protein molecule of claim 1, the prior art references must teach an identical hybrid protein sequence and one that is folded in an identical manner, which would further require the identical electrostatic environment as dictated by the specific experimental reagents used and as taught by the instant [S]pecification.

(Reply Br. 3.) We are not persuaded. Although Appellant discloses Km data for a COX-2–10aa–PGIS fusion protein, Appellant's claim 1 is not limited to this construct. To the contrary, Appellant's claim 1 relates to a fusion protein comprising COX-1, not COX-2, and a linker of undefined length (*see* Appeal Br. 17). Therefore, we are not persuaded by Appellant's

contentions relating to a COX-2–10aa–PGIS fusion protein (*see id.* at 15–16).

For the foregoing reasons we are not persuaded by Appellant's contention that Examiner relied upon impermissible hindsight (Appeal Br. 13).

CONCLUSION

The preponderance of evidence relied upon by Examiner supports a conclusion of obviousness. The rejection of claim 1 under 35 U.S.C. § 103(a) as unpatentable over the combination of Ruan '05, Ruan '04, Sakaki, Lindbladh, and Hargrave is affirmed. Claims 2, 3, 5, 16, 19, and 20 are not separately argued and fall with claim 1.

DECISION SUMMARY

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
1–3, 5, 16, 19, 20	103	Ruan '05, Ruan '04, Sakaki, Lindbladh, Hargrave	1–3, 5, 16, 19, 20	

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED