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UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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*Ex parte* KAZUAKI OKUNO and MASAYUKI YABUTA

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Appeal 2014-003834
Application 10/573,821
Technology Center 1600

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Before DONALD E. ADAMS, TAWEN CHANG, and
RACHEL H. TOWNSEND, *Administrative Patent Judges*.

ADAMS, *Administrative Patent Judge*.

DECISION ON APPEAL[1]

This appeal under 35 U.S.C. § 134(a) involves claims 49, 51–56, 59–61, 67, and 68 (App. Br. 2). Examiner entered rejections under 35 U.S.C. § 103(a). We have jurisdiction under 35 U.S.C. § 6(b).

We REVERSE.

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[1] Appellants identify "[t]he real party in interest [as] Asubio Pharma Co., Ltd." (App. Br. 2.)

STATEMENT OF THE CASE

Appellants disclose "a method for directly cleaving a physiologically active peptide, protein or its derivative from a fusion protein utilizing *E. coli* OmpT protease or a variant thereof" (Spec. 1:6–9). Claims 49, 54, 67, and 68 are representative and reproduced below:

> 49. A process for cleaving a polypeptide comprising cleaving the polypeptide with an *E coli* OmpT protease variant consisting of an amino acid substitution at the 97th position of the amino acid sequence of SEQ ID NO: 41,
>
> wherein the 97th amino acid from the N-terminus of the *E coli* OmpT protease variant is leucine, methionine, or histidine,
>
> wherein the polypeptide comprises a cleavage site that is a peptide bond between the Pl position and the Pl' position, and
>
> wherein the Pl position is arginine or lysine and the Pl' position is:
>
> (1) serine or alanine when the 97th amino acid from the N-terminus of the *E. coli* OmpT protease variant is leucine;
>
> (2) phenylalanine, alanine, serine, cysteine, or tyrosine when the 97th amino acid from the N-terminus of the *E. coli* OmpT protease variant is methionine; or
>
> (3) alanine, valine, isoleucine, methionine, serine, threonine, cysteine, or asparagine when the 97th amino acid from the N-terminus of the *E. coli* OmpT protease variant is histidine.

(App. Br. 14.)

> 54. The process of claim 49,
>
> wherein the polypeptide is a fusion protein comprising a protecting peptide and a target peptide,

> wherein the C-terminus of the protecting peptide is the Pl position and the N-terminus of the target peptide is the Pl' position,
>
> wherein the fusion protein is produced by expressing a gene encoding the fusion protein in a host cell, and
>
> wherein cleavage of the fusion protein liberates the target peptide.

(App. Br. 15.)

> 67. The process of claim 54, wherein the target peptide consists of between 22 and 45 amino acid residues.

(App. Br. 17.)

> 68. The process of claim 67, wherein the target peptide is adrenocorticotropic hormone (1-24), motilin, or calcitonin precursor.

(App. Br. 17.)

The claims stand rejected as follows:

Claims 49 and 51–53 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Kramer,[2] Bott,[3] and Okuno.[4]

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[2] R. Arjen Kramer et al., *Identification of essential acidic residues of outer membrane protease OmpT supports a novel active site*, 505 FEBS Letters 426–430 (2001).

[3] Bott et al., US 5,700,676, issued Dec. 23, 1997.

[4] Kazuaki Okuno et al., *Substrate Specificity at the P1' Site of Escherichia coli OmpT under Denaturing Conditions*, 66 Biosci. Biotechnol. Biochem. 127–134 (2002).

Claims 54–56, 59–61, and 67 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Kramer, Bott, Okuno, Stumpe,[5] Suzuki,[6] Dekker,[7] and Darnell.[8]

Claim 68 stands rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Yamamoto,[9] Kramer, Bott, Okuno, Stumpe, Suzuki, Dekker, and Darnell.

## ISSUE

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

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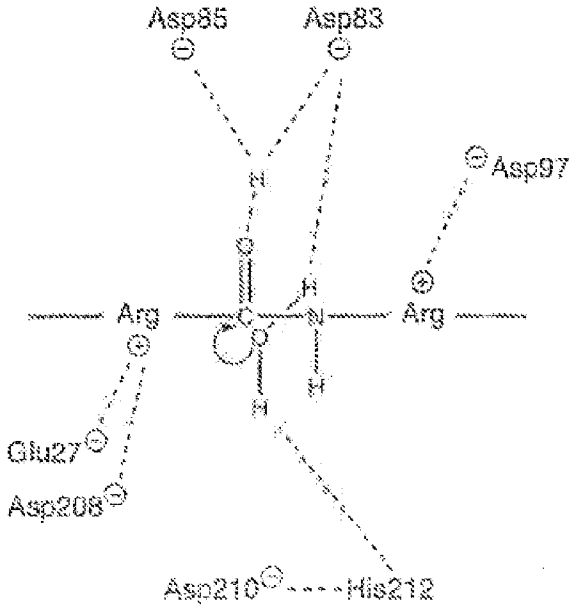
[5] Stefan Stumpe, *Identification of OmpT as the Protease That Hydrolyzes the Antimicrobial Peptide Protamine before It Enters Growing Cells of* Escherichia coli, 180 J. Bacteriol. 4002–4006 (1998).
[6] Koichi Suzuki and Toshio Ando, *Studies on Protamines XVI. The Complete Amino Acid Sequence of Clupeine YII*, 72 J. Biochem. 1419–1432 (1972).
[7] Niek Dekker et al., *Substrate Specificity of the Integral Membrane Protease OmpT Determined by Spatially Addressed Peptide Libraries*, 40 Biochemistry 1694–1701 (2001).
[8] James Darnell et al., *Molecular Cell Biology* 44–45 (2d ed. 1990).
[9] Yamamoto et al., US 5,506,120, issued Apr. 9, 1996.

FACTUAL FINDINGS (FF)

FF 1. Kramer's Figure 4 is reproduced below:




Kramer's Figure 4 represents a "[s]chematic two-dimensional model of a peptide in the active site of OmpT. Hydrogen bonds and ionic interactions are indicated by dashed lines. The curved arrow represents the nucleophilic attack on the carbonyl carbon of the scissile peptide bond" (Kramer 428: Fig. 4, Legend). The arginine (Arg) residue to the left of the scissile peptide bond represents the P1 position of the OmpT polypeptide substrate. The Arg residue to the right of the scissile peptide bond represents the P1' position of the OmpT polypeptide substrate. The Asp97 residue represents the amino acid at the 97th position of the OmpT protease. *See* Ans. 2–3.

FF 2. Kramer discloses OmpT protease variants, wherein the "six aspartates (at positions 43, 83, 85, 97, 208 and 210) and five glutamates (at positions 27, 111, 136, 193 and 250) . . . were replaced by alanines and the residual activities of the resulting variants were measured" (Kramer 426; *see generally* Ans. 2–3).

FF 3. Kramer reports that the activity of the OmpT protease variants is "in good agreement with" Kramer's earlier proposal, which was "based on the crystal structure of OmpT [], that $Glu^{27}$ and $Asp^{208}$ may define the high specificity of OmpT for Arg or Lys at position P1 in the substrate" and "[f]or the P1' position, the specificity was less exclusive, but a positively charged amino acid was preferred there as well [], likely due to interaction with an anionic residue of OmpT." Thus, Kramer reasons that "[a]ssuming [] the substrate has an extended conformation and that the P1 side chain points towards $Glu^{27}$ and $Asp^{208}$, the P1' chain would be located close to $Asp^{97}$" (Kramer 428–429 (endnotes omitted); *see generally* Ans. 2–3).

FF 4. Kramer reports that by replacing $Arg^{97}$ residue with an alanine, "only 6% residual activity" was observed; therefore, Kramer "propose[s] that $Asp^{97}$ is responsible for the observed P1' specificity" (Kramer 429; *see* Ans. 2).

FF 5. Examiner finds that Kramer fails to disclose "variants of OmpT protease having the single $Asp^{97}$ substituted with any amino acids other than Ala" (Ans. 3).

FF 6. Bott "relates to novel carbonyl hydrolase mutants derived from the amino acid sequence of naturally-occurring or recombinant non-human carbonyl hydrolases and to DNA sequences encoding the same" (Bott 1:22–25; Ans. 3–4).

FF 7. Bott discloses subtilisin mutants, wherein amino acids Gly166 and Gly169 were substituted with all 19 naturally occurring amino acids and finds that "the preferred replacement amino acids for Gly166 and/or Gly169 will depend on the specific amino acid occupying the P-1 position of a given substrate" (Bott 40:37–51).

FF 8. Okuno discloses modified OmpT substrates, wherein the "cleavage site of the substrate PRR was $Arg^{140}$-$Arg^{141}$ and the modified substrates PRX substituted all 19 natural amino acids at the P1' site instead of $Arg^{141}$" (Okuno, Abstract; *see* Ans. 3–4).

FF 9. Examiner relies on Stumpe to suggest that "OmpT protease cleaves protamines" (Ans. 4).

FF 10. Examiner relies on Suzuki to disclose "the sequence of the protamine component Clupein YII" (Ans. 4).

FF 11. Examiner relies on Dekker to "rank[] the P2-P1' positions for cleavage by OmpT" (Ans. 4).

FF 12. Examiner relies on Darnell to "provide[] guidance as to the molecular basis for non-covalent bonding between amino acids" (Ans. 5).

FF 13. Examiner relies on Yamamoto to disclose "fusion proteins comprising a target peptide, including motilin or calcitonin, and cleaving such fusion protein using OmpT protease" (Ans. 6).

FF 14. Examiner finds that Yamamoto fails to suggest "cleaving a motilin- or calcitonin-comprising fusion protein using an OmpT variant having a single $Asp^{97}$Xaa substitution, wherein the target protein is released without any additional N-terminal amino acids" and relies on the combination of Kramer, Bott, Okuno, Stumpe, Suzuki, Dekker, and Darnell to make up for this deficiency in Yamamoto (Ans. 6).

## ANALYSIS

*The combination of Kramer, Bott, and Okuno*:

Based on the combination of Kramer, Bott, and Okuno, Examiner concludes that, at the time Appellants' invention was made, it would have

been prima facie obvious "to use the strategy of Bott [] to make all 19 $Asp^{97}$Xaa variants of OmpT protease, including [those required by Appellants' claimed invention] and test said variants for substrate specificity at the P1' position" using Okuno's fusion protein substrate library in order to arrive at Appellants' claimed invention (Ans. 3–4). We are not persuaded.

Initially, we note Examiner's concession that Kramer fails to disclose "variants of OmpT protease having the single $Asp^{97}$ substituted with any amino acids other than Ala" (FF 5). To make up for this deficiency in Kramer, Examiner directs attention to Bott's work with a protease, which is *not* OmpT, wherein glycine, *not* arginine, residues are substituted with various amino acids (FF 7). At best, Bott provides general guidance on the modification of a non-OmpT protease, that one may consider applying to Kramer to make additional OmpT protease variants. Nevertheless, even if a person of ordinary skill in this art would have combined Kramer with Bott's general guidance on the modification of a non-OmpT protease, a person of ordinary skill would have, at best, a collection of OmpT protease variants, with no knowledge of the variant's biological activity, or, if biologically active, what substrate cleavage motif any particular OmpT protease variant would recognize. To make up for the foregoing deficiency in the combination of Kramer and Bott, Examiner relies on Okuno to suggest that a collection of OmpT protease variant substrates was known in the art at the time of Appellants' claimed invention (FF 8).

In sum, Examiner concludes that a person of ordinary skill in this art could follow the general guidance set forth in the combination of Kramer, Bott, and Okuno and thereby arrive at Appellants claimed invention, which requires specific amino acids to be present in the P1 and P1' position of the

OmpT substrate when the 97th amino acid from the N-terminus of the OmpT protease contains a specifically defined amino acid (*see, e.g.*, App. Br. 14).

We conclude the Examiner's reasoning is precisely what the *O'Farrell* court articulated as an improper application of an "obvious to try" standard in an obviousness analysis *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). That is, Examiner reasons that a person of ordinary skill in this art would have "var[ied] all [the prior art] parameters or tr[ied] each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful" and/or "explore[d] a . . . general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of [Appellants'] claimed invention or how to achieve it." *Id.*; *see also* Appeal Br. 6–7. On this record, we find no evidence or reasoning from Examiner to support a conclusion that there were a finite number of identified and/or predictable solutions as to which specific modification(s) to the OmpT protease were necessary in order to define a specific OmpT substrate cleavage motif. *Cf. KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007).

*The combination of Kramer, Bott, Okuno, Stumpe, Suzuki, Dekker, and Darnell, with or without Yamamoto*:

Examiner failed to establish that any of Stumpe, Suzuki, Dekker, Darnell or Yamamoto make up for the foregoing deficiency in the combination of Kramer, Bott, and Okuno (*see* App. Br. 12 and 13; *cf.* FF 9–14).

## CONCLUSION OF LAW

The preponderance of evidence relied upon by Examiner fails to support a conclusion of obviousness.

The rejection of claims 49 and 51–53 under 35 U.S.C. § 103(a) as unpatentable over the combination of Kramer, Bott, and Okuno is reversed.

The rejection of claims 54–56, 59–61, and 67 under 35 U.S.C. § 103(a) as unpatentable over the combination of Kramer, Bott, Okuno, Stumpe, Suzuki, Dekker, and Darnell is reversed.

The rejection of claim 68 under 35 U.S.C. § 103(a) as unpatentable over the combination of Yamamoto, Kramer, Bott, Okuno, Stumpe, Suzuki, Dekker, and Darnell is reversed.

REVERSED