

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 16

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

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Ex parte WIESLAW KUDLICKI and BOYD HARDESTY

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Appeal No. 2001-2500  
Application No. 08/590,729

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ON BRIEF

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Before WILLIAM F. SMITH, SCHEINER, and GRIMES, Administrative Patent Judges.

GRIMES, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1-9, all of the claims in the application. Claims 1 and 2 are representative and read as follows:

1. A method of synthesizing a fluorescently labeled protein in a prokaryotic cell-free protein synthesis system, comprising the steps of :
  - (a) incubating a sample of ribosomes obtained from a cell-free extract with plasmid DNA containing a coding sequence for a

protein of interest, said sample incubated in a coupled transcription/translation medium together with an aminoacyl tRNA having fluorescent label;

- (b) partially purifying said fluorescently labeled protein by separating newly synthesized, fluorescently-labeled protein from other fluorescent components within said sample;
  - (c) measuring the amount of protein synthesized;
  - (d) determining fluorescence of newly synthesized protein; and
  - (e) determining the biological activity of the newly synthesized protein.
2. The method of claim 1, wherein said fluorescent label is on the N-terminus of said protein.

The examiner relies on the following references:

Hildenbrand et al. (Hildenbrand), "Sugar transport by the bacterial phosphotransferase system," The Journal of Biological Chemistry, Vol. 257, No. 23, pp. 14518-14525 (1952)

Stryer, "Protein synthesis in bacteria is initiated by formylmethionyl transfer rna," Biochemistry, pp. 752-758 (1988)

Picking et al. (Picking), "Fluorescence characterization of the environment encountered by nascent polyalanine and polyserine as they exit Escherichia coli ribosomes during translation," Biochemistry, Vol. 31, pp. 2368-2375 (1992)

Kudlicki et al. (Kudlicki), "High efficiency cell-free synthesis of proteins : refinement of the coupled transcription/translation system," Analytical Biochemistry, Vol. 206, pp. 389-393 (1992)

Claims 1 and 5-9 stand rejected under 35 U.S.C. § 103 as obvious over the combined disclosures of Kudlicki, Picking, and Hildenbrand.

Claims 2-4 stand rejected under 35 U.S.C. § 103 as obvious over the combined disclosures of Kudlicki, Picking, Hildenbrand, and Stryer.

We affirm the rejection of claims 1 and 5-9 but reverse the rejection of claims 2-4.

### Background

The specification discloses a “method of synthesizing a fluorescently labeled protein in a cell-free protein synthesis system.” Page 1. Specifically, “coumarin-maleimidyl-SAcMet-tRNA<sub>f</sub> (CPM-SAcMet-tRNA<sub>f</sub>) was incorporated into the N-terminus of nascent polypeptides that were synthesized on ribosomes in a bacterial cell-free coupled transcription/translation system.” Page 2. Then “[f]luorescence techniques were used to monitor changes in the local environment and mobility of the N-terminal probe and in turn effects of the chaperones on folding, activation and release of the nascent or full-length polypeptides from the ribosomes.” Id.

### Discussion

#### 1. The rejection of claims 1 and 5-9.

The claims subject to each rejection stand or fall together. See the Appeal Brief, page 5. We therefore limit our consideration of this rejection to claim 1, the broadest claim subject to this rejection. Claim 1 is directed to a method of making a fluorescently labeled protein, comprising incubating plasmid DNA encoding the protein of interest with a sample of ribosomes in a prokaryotic coupled transcription/translation system, together with an aminoacyl tRNA having a fluorescent label. The method also includes steps of partially purifying the newly synthesized, fluorescently labeled protein away from other fluorescent

components of the mixture, measuring the amount of protein produced, and determining the fluorescence and biological activity of the labeled protein.

The examiner rejected claim 1, and dependent claims 5-9, under 35 U.S.C. § 103, on the basis that Kudlicki teaches a cell-free protein synthesis method meeting all of the limitations of claim 1 except that Kudlicki does not teach synthesis of fluorescently labeled proteins. Examiner's Answer, page 4. The examiner found this deficiency to be remedied by the secondary references. In particular, the examiner cites Picking as teaching "a synthetic alanyl-tRNA and a synthetic lysyl-tRNA, covalently linked to the fluorescent molecule coumarin," and production of labeled peptides incorporating the fluorescently labeled amino acids. See id.

The examiner concluded that

[i]t would have been obvious for one of ordinary skill in the art at the time of the invention to use the bacterial cell-free coupled transcription/translation system of Kudlicki et al. to synthesize fluorescently labeled proteins (as suggested on pg 393, last paragraph of Discussion) . . . by using aminoacyl-tRNAs linked to coumarin, especially like the fluorescent lysyl-tRNA of Picking et al.

Id., pages 4-5. He found motivation to be provided both by the express "suggest[ion] on pg 393, last paragraph of Discussion" in Kudlicki and by "the ability/utility to readily detect [the labeled proteins], over unlabeled proteins, in enzyme assays and their usefulness as markers." Id., page 5.

We agree with the examiner that the cited references would have rendered the claimed method obvious. Kudlicki discloses a "high efficiency

system for coupled transcription/translation of exogenously added genes.”

Abstract. Kudlicki’s disclosed method meets every limitation of the claimed method except for the inclusion of a fluorescently labeled aminoacyl tRNA. Instead, Kudlicki used an aminoacyl tRNA labeled with <sup>14</sup>C-leucine to produce radioactively labeled protein. See page 390, paragraph bridging the columns (“The system used to carry out coupled transcription/translation contained . . . 83 μM <sup>14</sup>C-leucine, 200 μM of each of the other 19 amino acids, 20 μg E. coli tRNA, . . . 1.2 A<sub>260</sub> units of the ribosome fraction;” i.e., the system apparently relies on enzymes in the ribosome fraction to couple the amino acids to the tRNA.).

In addition, Kudlicki expressly suggests modifying the disclosed cell-free synthesis system to produce fluorescently labeled proteins. See page 393, right-hand column: “Another advantage of an efficient in vitro protein-synthesizing system is that tRNAs with modified amino acids (for example, amino acids with covalently attached fluorophores) can be incorporated into nascent peptide chains.” Thus, Kudlicki might be viewed as an anticipatory disclosure, in that the fluorescent-label embodiment is expressly disclosed as an alternative to the exemplified radioactive-label embodiment. See Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc., 246 F.3d 1368, 1379, 58 USPQ2d 1508, (Fed. Cir. 2001) (“[A]nticipation does not require actual performance of suggestions in a disclosure. Rather, anticipation only requires that those suggestions be enabling to one of skill in the art.”); In re Donohue, 766 F.2d 531, 533, 226 USPQ 619, 621 (Fed. Cir. 1985) (“It is not, however, necessary that an invention disclosed in

a publication shall have actually been made in order to satisfy the enablement requirement.”).

Of course, to anticipate or render obvious a later-claimed invention, the prior art must provide an enabling disclosure. See In re Hoeksema, 399 F.2d 269, 274, 158 USPQ 596, 601 (CCPA 1968) (“[I]f the prior art of record fails to disclose or render obvious a method for making a claimed compound, at the time the invention was made, it may not be legally concluded that the compound itself is in the possession of the public.”). To the extent that it could be argued that Kudlicki does not provide an enabling disclosure of the instantly claimed method, we find that Picking provides whatever guidance might be missing from Kudlicki. Picking discloses production of alanyl-tRNA and lysyl-tRNA labeled with the fluorescent label CPM (3-(4-maleimidophenyl)-7-diethylamino-4-methylcoumarin). See page 2369, the paragraph bridging the columns and first paragraph, right-hand column.

Thus, it would have been obvious to a person of ordinary skill in the art to combine the CPM-labeled alanyl-tRNA or CPM-labeled lysyl-tRNA taught by Picking with the cell-free protein synthesis system taught by Kudlicki. The necessary suggestion to so modify Kudlicki’s system is provided by Kudlicki, who expressly suggests using fluorescently labeled aminoacyl tRNAs in the disclosed system. Kudlicki and Picking therefore render claim 1 prima facie obvious.<sup>1</sup>

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<sup>1</sup> Since Kudlicki and Picking are sufficient to establish prima facie obviousness, we see no need to discuss the teachings of Hildenbrand with respect to claim 1.

Appellants argue that the cited references do not support a prima facie case of obviousness.<sup>2</sup> Appeal Brief, pages 6-8. Specifically, Appellants argue that “Kudlicki et al. discloses a bacterial cell free coupled transcription/translation system but does not teach the synthesis of fluorescently labeled proteins.” Appeal Brief, page 7. The premise of this argument is questionable since, as we noted above, Kudlicki could well be viewed as teaching use of the disclosed system to produce fluorescently labeled proteins, in view of the statement that “tRNAs with modified amino acids (for example, amino acids with covalently attached fluorophores) can be incorporated into nascent peptide chains” (page 393). Regardless, the present rejection is based on 35 U.S.C. § 103, and “[t]he test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art.” In re Young, 927 F.2d 588, 591, 18 USPQ2d 1089, 1091 (Fed. Cir. 1991). See also In re Baird, 16 F.3d 380, 383, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994) (“[A] reference must be considered not only for what it expressly teaches, but also for what it fairly suggests.”).

Appellants also argue that

Picking et al. disclosed the use of a synthetic Ala-tRNA to initiate synthesis of a homopolymer. . . . With this particular tRNA, one cannot initiate protein synthesis at the AUG codon of naturally occurring mRNAs. In contrast, the procedure described in the present application uses a naturally occurring E. coli initiator tRNA which is . . . modified to carry the coumarin residue on the amino group instead of the formyl group as in a “natural” initiation.

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<sup>2</sup> Appellants direct some of their arguments on Hildenbrand but, as just noted, Hildenbrand is not necessary to the prima facie case with respect to claim 1. Therefore, Appellants’ arguments based on Hildenbrand do not persuade us of any defect in the case of obviousness fully supported by Kudlicki and Picking.

Appeal Brief, page 6. Appellants assert that this modified initiator tRNA gives as efficient initiation as the natural fMet-tRNA<sub>f</sub>. Id.

This argument is not persuasive. First, claim 1 reads on a method of producing fluorescently labeled proteins using any labeled aminoacyl tRNA, not just a labeled initiator tRNA. Thus, the fact that Picking's initiator tRNA would not initiate transcription of a naturally occurring mRNA is irrelevant, since Picking also teaches labeled elongator tRNAs (lysyl-tRNA and alanyl-tRNA) which would be expected to function in the claimed method to produce a fluorescently labeled product. In addition, to the extent that Appellants are relying on the asserted efficiency of their labeled initiator tRNA to show unexpected results, "it is well settled that unexpected results must be established by factual evidence." In re Geisler, 116 F.3d 1465, 1470, 43 USPQ2d 1362, 1365 (Fed. Cir. 1997). Appellants have provided no objective evidence to support the asserted efficiency.

Finally, Appellants argue that the references relied on by the examiner do not provide the required "teaching, suggestion, or incentive" to combine their respective teachings, nor do they provide a reasonable expectation of success. Appeal Brief, page 7. Appellants do not elaborate on why they believe the cited references are deficient in providing motivation to combine or an expectation of success.

These arguments are not persuasive. For the reasons discussed above, we disagree with Appellants' position. Specifically, Kudlicki states that an

advantage of the disclosed method is that “tRNAs with modified amino acids (for example, amino acids with covalently attached fluorophores) can be incorporated into nascent peptide chains.” Page 393. This statement would have provided those skilled in the art with both the motivation to modify the disclosed method and a reasonable expectation of success in doing so.

We conclude that Kudlicki and Picking render the method of claim 1 prima facie obvious. Appellants have neither rebutted the prima facie case nor shown objective evidence of nonobviousness. We therefore affirm the rejection of claim 1 under 35 U.S.C. § 103. Claims 5-9 fall with claim 1.

2. The rejection of claims 2-4.

Claims 2-4 depend from claim 1 and add the requirement that the fluorescent label is added to the N-terminus of the protein, i.e., the label is on the initiator tRNA. The examiner rejected claims 2-4 under 35 U.S.C. § 103, as obvious over the combined teachings of Kudlicki, Picking, Hildenbrand, and Stryer.

The examiner relied on Kudlicki and Picking for the same teachings discussed in detail above. In addition, the examiner cited Hildenbrand as teaching fluorescence labeling of the N-terminal methionine of a protein, and he cited Stryer as teaching that “protein synthesis in bacteria is only initiated by formylmethionyl-tRNA (fMet-tRNA<sub>f</sub>) and that the formylmethionine (fMet) is the N-terminal residue of a nascent peptide.” Examiner’s Answer, page 5. He also noted that Picking teaches that one of the coumarin-labeled synthetic alanyl-

tRNAs was structurally and functionally similar to initiator fMet-tRNA<sub>f</sub>. Id. He concluded that

[i]t would have been obvious to one of ordinary skill in the art at the time of the invention to synthesize fluorescently labeled proteins, like that of Hildenbrand et al., using the bacterial cell-free coupled transcription/translation system of Kudlicki et al., by substituting fMet-tRNA<sub>f</sub> linked to coumarin at the α-amino group of fMet in place of the fluorescent lysyl- and alanyl-tRNAs of Picking et al., with the expectation of producing proteins singly and uniquely labeled at the N-terminal residue. Motivation to produce N-terminal methionine labeled proteins is provided by their utility, as taught by Picking et al., in allowing the characterization of the environment around nascent proteins as they exit ribosomes during translation.

Id., page 6.

“It is well-established that before a conclusion of obviousness may be made based on a combination of references, there must have been a reason, suggestion, or motivation to lead an inventor to combine those references.” Pro-Mold and Tool Co. v. Great Lakes Plastics Inc., 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996). An adequate showing of motivation to combine requires “evidence that ‘a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.’” Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1075 (Fed. Cir. 2000) (quoting In re Rouffet, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1456 (Fed. Cir. 1998)).

Thus, the method of claims 2-4 would have been prima facie obvious only if the prior art would have led a person of ordinary skill in the art to select, from all

possible aminoacyl-tRNAs, fMet-tRNA<sub>f</sub> as the attachment site for a fluorescent label. We do not find the required “reason, suggestion, or motivation” to do so in the cited references. It is true that Hildenbrand’s fluorescent label was attached predominantly if not exclusively at the N-terminal methionine. See the abstract. However, Hildenbrand does not disclose that the label was deliberately localized to the N-terminus. In fact, Hildenbrand speculates that the reason the internal methionine was not also labeled is that it is buried within the folded protein. See page 14524, left-hand column. In addition, Hildenbrand does not disclose that limiting the location of the label to the N-terminus provides any advantages. Therefore, Hildenbrand cannot be said to suggest the method of claims 2-4.

Nor do any of the other references provide the required suggestion. Kudlicki contains no teaching regarding labeling the N-terminus of a synthesized protein. Picking discloses a fluorescently labeled “synthetic initiator alanyl-tRNA” for producing polyserine or polyalanine but does not disclose or suggest a fluorescently labeled fMet-tRNA<sub>f</sub>, such as would be necessary to initiate translation of a naturally occurring mRNA in Kudlicki’s protein synthesis system. Stryer discusses the translation process generally but does not discuss labeling of proteins.

Thus, the references relied on by the examiner fail to provide a reason, suggestion, or motivation that would have led a person of ordinary skill in the art to combine a fluorescently labeled initiator tRNA with a cell-free protein synthesis system. We therefore conclude that the references do not support a prima facie

case of obviousness with respect to claims 2-4. The rejection of these claims is reversed.

Summary

We affirm the rejection of claims 1 and 5-9 because the prior art would have rendered the claimed process obvious to a person of ordinary skill in the art. However, we reverse the rejection of claims 2-4 because the cited references do not provide an adequate reason, suggestion, or motivation to modify the prior art process so as to produce a protein having a fluorescent label attached to the N-terminal methionine.

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

AFFIRMED IN PART

WILLIAM F. SMITH	)	
Administrative Patent Judge	)	
	)	
	)	
	)	BOARD OF PATENT
TONI R. SCHEINER	)	
Administrative Patent Judge	)	APPEALS AND
	)	
	)	INTERFERENCES
	)	
ERIC GRIMES	)	
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