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David W. Highet, VP & Chief IP Counsel
Becton, Dickinson and Company
(Morgan, Lewis & Bockius, LLP)
1 Becton Drive, MC 110
Franklin Lakes, NJ 07417-1880

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YANG, NELSON C

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte JAVIER ALARCON, HELEN V. HSIEH,
JON A. ROWLEY, ROSS W. JACOBSON,
J. BRUCE PITNER, and DOUGLAS B. SHERMAN

Appeal 2011-002974
Application 10/428,295
Technology Center 1600

Before FRANCISCO C. PRATS, STEPHEN WALSH, and
JOHN A. EVANS, *Administrative Patent Judges*.

PRATS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims to a matrix for use in a glucose biosensor. The Examiner entered two rejections for obviousness.

We have jurisdiction under 35 U.S.C. § 6(b). We affirm one of the Examiner's rejections, but reverse the other.

STATEMENT OF THE CASE

Claims 1, 3-6, 14, 15, and 21 stand rejected and appealed (App. Br. 1-2). Claim 1, the only independent claim, is representative and reads as follows (paragraph separation and spacing added):

> 1. A matrix for use in a glucose biosensor, comprising:
>
> a core, said core comprising a hydrogel and a periplasmic glucose/galactose binding protein (GGBP) that is covalently bound to said hydrogel, and
>
> an outer layer comprising a hydrogel or a sol-gel,
>
> wherein said outer layer of hydrogel or sol-gel is free of said GGBP and surrounds said core and is selectively permeable to glucose.

The following rejections are before us for review:

(1) Claims 1, 3-6, 14, 15, and 21, under 35 U.S.C. § 103(a) as obvious over Van Antwerp[1] and Daunert[2] (Ans. 3-6); and

(2) Claims 1, 3-6, 14, 15, and 21, under 35 U.S.C. § 103(a) as obvious over Polak[3] and Daunert (Ans. 6-7).

Appellants have not argued the patentability of any of the claims separately, so the claims stand or fall together. *See* 37 C.F.R. § 41.37(c)(1)(vii).

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[1] U.S. Patent No. 6,002,954 (issued December 14, 1999).
[2] U.S. Patent App. Pub. No. 2003/0232383 A1 (filed May 6, 2002).
[3] U.S. Patent App. Pub. No. 2002/0182658 A1 (published December 5, 2002).

OBVIOUSNESS – VAN ANTWERP AND DAUNERT

The Examiner cited Van Antwerp as disclosing a glucose biosensor with inner and outer layers, both taught as suitably being hydrogels, and thus encompassed by claim 1 (*see* Ans. 4). The Examiner noted Van Antwerp's disclosure that fluorescently labeled lectin proteins bound to the inner layer were taught as being suitable reporter moieties, the lectins showing "a change in fluorescence when bound to glucose, presumably due to a change in molecular configuration" (*id.* (citing Van Antwerp, col. 7, ll. 4-30)).

The Examiner conceded that Van Antwerp differed from claim 1 in that Van Antwerp did not teach using a periplasmic glucose/galactose binding protein (GBP) as the glucose-binding reporter moiety (*id.*).

To remedy that deficiency, the Examiner cited Daunert as disclosing the use of GBPs, described as being suitably bound to hydrogels, as reporter proteins in glucose biosensors, the GBPs being labeled "such that a conformational change that the protein undergoes upon binding to glucose will induce a change in the signal" (*id.*). The Examiner also noted Daunert's teaching that "the use of GBPs allows for the development of biosensing systems that are sensitive to submicromolar concentrations of glucose" (*id.* at 4-5 (citing Daunert [0045]).

Based on the references' teachings, the Examiner reasoned that an ordinary artisan would have considered it obvious to "entrap periplasmic binding proteins such as mutated glucose/galactose binding proteins in the polymer matrix of the glucose biosensor of Van Antwerp et al, as suggested by Daunert et al., so that the development of biosensing systems that are sensitive to submicromolar concentrations of glucose is possible" (*id.* at 5). The Examiner further reasoned that an ordinary artisan would have had a

reasonable expectation of success in using a GBP as the reporter protein in Van Antwerp's device, because Van Antwerp taught that proteins "may produce changes in detectable signals by changes in conformation, and Daunert et al. teach that the GBPs produce changes in signal upon binding to glucose due to a change in conformation, thus indicating that the mechanism of signal detection would function similarly in the invention of Van Antwerp" (*id.*).

As stated in *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992):

> [T]he examiner bears the initial burden . . . of presenting a *prima facie* case of unpatentability. . . .
>
> After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of argument.

While this is arguably a close case, Appellants' arguments do not persuade us that a preponderance of the evidence fails to support the Examiner's conclusion that claim 1 would have been obvious to an ordinary artisan.

Claim 1 recites a matrix for use in a glucose biosensor. The matrix contains a core, which includes a hydrogel. The matrix also has an outer layer which surrounds the core. The outer layer includes either a hydrogel or a sol-gel.

Claim 1 requires the core to contain a periplasmic glucose/galactose binding protein (GGBP) covalently bound to the hydrogel. Claim 1 requires the outer layer to be free of the GGBP, and to be selectively permeable to glucose.

As the Examiner pointed out, Van Antwerp discloses implantable glucose biosensors that contain "[a]mplification [c]omponents" that allow optical detection of glucose (Van Antwerp, col. 6, l. 1). Van Antwerp discloses that the amplification components may be enzymes, such as glucose oxidase, or glucose binding proteins, such as the lectin protein, concanavalin A (*id.* at col. 6, l. 1, through col. 7, l. 29).

Van Antwerp explains that fluorescently labeled lectins can be used to detect and quantitate glucose entering the biosensor, due to a detectable change in the fluorescent signal emitted by the labeled lectin, the change in the fluorescent signal presumed to be caused by a change in the lectin's molecular conformation which occurs upon glucose binding (*see id.* at col 7, ll. 4-24):

> The mechanism of action of the lectin fluorescence quenching is presumably due to changes in the molecular conformation of the glucose containing lectin to that without the glucose present. In the case of lectins, fluorescence quenching of a fluorescein or rhodamine label occurs via an unknown mechanism, but possibly due to the conformational change.

Van Antwerp discloses embodiments in which its biosensor includes layers corresponding to the core and outer layer recited in Appellants' claim 1, with the glucose-binding lectin concanavalin A disclosed as being in the inner layer corresponding to the core of claim 1 (*see id.* at Figure 14A). As required by claim 1, Van Antwerp explains that the biocompatible matrix forming the layer containing the amplification components can include "a solid substrate (e.g., polyurethanes/polyureas, silicon-containing polymers, *hydrogels*, solgels and the like)" (*id.* at col. 10, ll. 40-42 (emphasis added)).

As also required by claim 1, Van Antwerp discloses that the glucose-detecting protein can be covalently bound to the matrix constituting the inner layer, and that an outer layer composed of a hydrogel, which Appellants do not dispute is taught or suggested as being selectively permeable to glucose, suitably covers the inner layer:

> In some embodiments, the amplification components will be entrapped or encased via covalent attachment, within a matrix which is itself permeable to the analyte of interest and biocompatible (see FIG. **14**B). In these embodiments, a second permeable layer is unnecessary. *Nevertheless, the use of a permeable layer such as a hydrogel which further facilitates tissue implantation is preferred (see FIG. **14**C).*

(*Id.* at col. 11, ll. 7-13 (emphasis added).) As Van Antwerp explains, "[c]ovalent attachment of the components to a polymer matrix prevents leakage of the components to surrounding tissue, and other undesirable contact of the amplification components with non-target fluids" (*id.* at col. 8, ll. 1-4).

Thus, we agree with the Examiner's finding that Van Antwerp differs from claim 1 in that Van Antwerp does not describe the use of a periplasmic glucose/galactose binding protein as the glucose-binding/detecting protein bound to the inner layer of the biosensor.

As the Examiner also found, however, Daunert discloses biosensors that contain "a periplasmic binding protein-galactose/glucose binding protein (GBP)" (Daunert [0005]). As Daunert explains:

> When GBP is labeled to provide an analytical signal (thermal, mass, electrochemical, or optical), a change in the signal is seen when the glucose ligand binds GBP and induces a change in the conformation of the protein. This change in signal can then be related to the concentration of carbohydrate in the sample.

(*Id.* at [0032].) Daunert discloses that "[u]sing this system, the present inventors developed biosensing systems that are sensitive to submicromolar concentrations of glucose" (*id.* at [0045]).

As required by claim 1, Daunert discloses that its GBP can be covalently bound to its underlying substrate (*id.* at [0072] ("The GBP protein can be site-specifically immobilized on a solid surface. For that, a unique cysteine can be introduced at the C- or N-terminus of the native GBP, which does not contain any cysteine molecule, using molecular biology techniques.")).

As also required by claim 1, the GBP can be bound to a hydrogel (*id.* at [0076] ("The nature of the surface can be diverse, e.g. . . . a hydrogel . . . .")).

Given these teachings, we agree with the Examiner that an ordinary artisan, advised by Daunert that the GBP's change in molecular conformation made it useful for detecting glucose in biosensors, would have been prompted to use Daunert's GBP as the glucose-binding/detecting protein in Van Antwerp's multi-layered biosensor, particularly given Van Antwerp's disclosure of the suitability in its biosensors of glucose-binding proteins, such as lectins, that provide glucose detection based on a change in molecular conformation upon glucose binding. Moreover, given Daunert's disclosure of the suitability of covalently immobilizing GBP to a hydrogel, we agree with the Examiner that an ordinary artisan would have been prompted to covalently bind the GBP to a hydrogel in the inner layer of Van Antwerp's multi-layer device.

As the Supreme Court explained in *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 417 (2007), "if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill."

Because Van Antwerp is directed to a reagentless biosensor that uses a protein to detect glucose based on a detectable change in the protein's molecular conformation that occurs upon glucose binding, similar to the situation that occurs when using GBP, we are not persuaded that Van Antwerp is irrelevant to the claimed invention, or to Daunert, as Appellants contend (*see* App. Br. 5-7).

Appellants also contend that, because Van Antwerp did not include a working example in which a functional protein was covalently immobilized in a hydrogel matrix, the Examiner erred in finding that Van Antwerp provided such a teaching (App. Br. 7-9; *see also* Reply Br. 2-4).[4] In particular, Appellants urge, the conditions described in Van Antwerp for immobilizing its amplification components were excessively harsh, to the extent that an ordinary artisan would have recognized that they would denature any protein (*see* App. Br. 9-10).

We do not find these arguments persuasive. It is well settled that, when evaluating obviousness, "[a]ll the disclosures in a reference must be evaluated, including nonpreferred embodiments, and a reference is not

[4] As the Reply Brief does not appear to include page numbers, we cite to it as if the first page was page 1, and the remaining pages were numbered consecutively.

limited to the disclosure of specific working examples." *In re Mills*, 470 F.2d 649, 651 (CCPA 1972) (citations omitted).

Thus, in the instant case, Van Antwerp might not provide a working example in which a functional protein was covalently immobilized in a hydrogel matrix, and then encased in a hydrogel outer layer. However, as noted above, Van Antwerp expressly describes an embodiment in which the lectin protein concanavalin A is disposed in an inner layer of its biosensor, with an outer layer including a hydrogel coating (*see* Van Antwerp, Figs. 14A, 14C). Given Van Antwerp's express teaching of a hydrogel as a suitable matrix for immobilizing its glucose-detecting components (*see id.* col. 10, ll. 39-42), and the desirability of covalently immobilizing its glucose detecting components (*see id.* at col. 11, ll. 7-13), as well as Daunert's disclosure of the desirability of covalently binding GBP to substrates such as hydrogels (*see* Daunert [0072], [0076]), we are not persuaded that the Examiner erred in finding that the combined teachings of the references would have suggested the combination of elements recited in claim 1, in the claimed configuration.

Moreover, as to Van Antwerp's alleged disclosure of conditions unsuitable for immobilizing functional proteins, Appellants point to no clear or specific evidence supporting the assertion that an ordinary artisan would have considered the conditions described in Van Antwerp unsuitable for immobilizing functional proteins. Indeed, Appellants concede that Van Antwerp's Example 3 uses room temperature conditions that would not denature proteins (*see* Reply Br. 3). While it might be true that Van Antwerp's Example 3 does not immobilize a lectin or GBP, Appellants provide no clear or specific evidence suggesting that an ordinary artisan,

expressly taught by Antwerp to immobilize its glucose-detecting components, would have been unable to covalently immobilize a functional GBP to a hydrogel, particularly given the explicit immobilizing techniques described in Daunert.

We acknowledge that glucose detection requires a change in the molecular conformation of the GBP to occur upon glucose binding, as Appellants urge (*see* App. Br. 6-7). We also note Appellants' argument that Daunert describes its GBP as being bound to a surface, and does not describe GBP as being encapsulated beneath a hydrogel layer, and the corollary argument that the Examiner therefore failed to establish that an ordinary artisan would have reasonably predicted that GBP would have been successfully incorporated into Van Antwerp's device (*see* App. Br. 10-12).

However, given Van Antwerp's express disclosure of the suitability of the presumed conformation-changing lectin concanavalin A in an inner layer of its biosensor, with an outer layer including a hydrogel coating (*see* Van Antwerp, Figs. 14A, 14C), we are not persuaded, absent clear and specific evidence to the contrary, that an ordinary artisan lacked a reasonable expectation that GBP would function suitably when covalently bound to an inner hydrogel layer of Van Antwerp's device.

In this regard, we note Brennan's[5] disclosure regarding entrapping proteins for use in reagentless biosensors like those described in Van Antwerp and Daunert:

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[5] John D. Brennan, *Preparation and Entrapment of Fluorescently Labeled Proteins for the Development of Reagentless Optical Biosensors*, 9 JOURNAL OF FLUORESCENCE 295-312 (1999).

> Given the strict requirements for orientational control inherent in the use of many of the proteins described above (particularly the PBPs [phosphate binding proteins] and membrane-associated proteins), physisorption and covalent attachment strategies are not likely to be successful. However, the entrapment of these species into polymeric matrixes is a promising method for interfacing proteins to inorganic devices.

(Brennan 307.)

Thus, it may be true that Brennan favored physical entrapment in polymeric matrices over covalent attachment. Brennan must, however, be viewed alongside all of the prior art of record, including Daunert, which was filed several years after Brennan's discussion of reagentless biosensors.

As noted above, Daunert expressly discloses a GBP immobilization technique involving the introduction of a cysteine residue into the C- or N-terminus of the native GBP protein, and the use of an amino acid spacer that allows immobilization while retaining adequate flexibility for glucose binding and detection (*see* Daunert [0072] ("The present inventors have demonstrated the feasibility of using this approach for the preparation of such an expression vector for the modified protein by employing the calcium-binding protein calmodulin . . . .")).

Appellants point to no instance where Brennan makes any clear or specific mention of GBP or its capacity for immobilization, nor do Appellants point to any discussion in Brennan of the technique Daunert used to solve the problem of immobilizing analyte-sensing proteins, like GBP, which require conformational flexibility. We are therefore not persuaded that an ordinary artisan would have viewed Brennan's teachings as adequate to undermine Daunert's express disclosure of the desirability of covalently immobilizing GBP.

We note Eggers'[6] disclosure that "not all proteins retain their native solution structure after encapsulation" in sol-gels, and that the lack of structure retention may be due to "(1) steric effects from molecular confinement; (2) adsorption to the silica matrix; and (3) the unusual physical properties of confined water" (Eggers 256). We also note Gonnelli's[7] disclosure that "[t]rapping of proteins in silica matrices may alter the native fold and in cases of marginally stable macromolecules it has led to loss of secondary structure and unfolding" (Gonnelli 165).

To the extent Eggers and Gonnelli reflect an ordinary artisan's understanding of this art at the time Appellants filed this application, these references support the position that an ordinary artisan would not have been able to absolutely predict whether GBP would retain its native structure and capacity to change molecular conformation when bound to the inner layer of Van Antwerp's multilayer device.

It is well settled, however, that "[o]bviousness does not require absolute predictability of success. . . . For obviousness under § 103, all that is required is a reasonable expectation of success." *In re O'Farrell*, 853 F.2d 894, 903-04 (Fed. Cir. 1988).

Thus, while Eggers may disclose that "not all" proteins retain their native structure when encapsulated in sol-gels (Eggers 256), Eggers also

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[6] Daryl K. Eggers and Joan S. Valentine, *Molecular confinement influences protein structure and enhances thermal protein stability*, 10 PROTEIN SCIENCE 250-261 (2001).
[7] Margherita Gonnelli and Giovanni B. Strambini, *Structure and dynamics of proteins encapsulated in silica hydrogels by Trp phosphorescence*, 104 BIOPHYSICAL CHEMISTRY 155-169 (2003).

discloses that a number of proteins do in fact retain their native conformations (*id.* at 250 (abstract) ("[S]ilica entrapment (1) is fully compatible with structure analysis by circular dichroism, (2) allows conformational studies in contact with solvents that would otherwise promote aggregation in solution . . . .")).

Similarly, while Gonnelli discloses that entrapment of proteins in sol-gels "*may* alter the native fold and in cases of marginally stable macromolecules it has led to loss of secondary structure and unfolding" (Gonnelli 165 (emphasis added)), Gonnelli also discloses that encapsulation in hydrogels did not inhibit conformational changes in the proteins within the gels (*see id.* at 155 (abstract) ("It was also noted that *large amplitude structural fluctuations*, as those involved in coenzyme binding to alcohol dehydrogenase or thermally activated in alkaline phosphatase, *were not restricted by gelation*." (Emphasis added.)).

Thus, given the teachings in Eggers and Gonnelli that a number of proteins encapsulated in hydrogels or sol-gels maintain not only their native structural conformations, but also their native capacity to change conformations, we agree with the Examiner that an ordinary artisan would have had a reasonable expectation that GBP would function suitably when bound to the inner layer of Van Antwerp's multilayer device, particularly in view of Van Antwerp's express teaching of encapsulating conformation-changing, glucose-binding proteins such as concanavalin A into its multilayer embodiment (*see, e.g.* Van Antwerp, Figs. 14A, 14C).

In sum, for the reasons discussed, Appellants' arguments do not persuade us that a preponderance of the evidence fails to support the Examiner's prima facie case as to claim 1. As Appellants point to no clear

or specific evidence of secondary considerations of non-obviousness that outweigh the evidence of prima facie obviousness advanced by the Examiner, we affirm the Examiner's rejection of claim 1 over Van Antwerp and Daunert.

As they were not argued separately, claims 3-6, 14, 15, and 21 fall with claim 1. *See* 37 C.F.R. § 41.37(c)(1)(vii).

OBVIOUSNESS – POLAK AND DAUNERT

In rejecting claim 1 as obvious over Polak and Daunert, the Examiner cited Polak as describing a glucose sensing device with "a core comprising a void volume and a binding substrate combined with a binder comprising a hydrogel with sufficient pores to allow labeled analogues to pass from the binding substrate to the void volume" (Ans. 6). The Examiner found that Polak taught that its device included "an analyte permeable membrane or shell enclosing the components of the device comprising hydrogels" as required by claim 1 (*id.*).

The Examiner conceded that Polak differed from claim 1 in that Polak did not teach that its "binding substrate comprises periplasmic glucose/galactose binding proteins that are covalently bound to a hydrogel" (*id.*).

To remedy that deficiency, the Examiner again cited Daunert's disclosure of using immobilized GBPs as glucose-detecting proteins (*id.* at 6-7). The Examiner thus reasoned that an ordinary artisan would have considered it obvious "to utilize the GGBP covalently attached hydrogel as the binding substrate and binder of Polak et al., as suggested by Daunert et al., so that the development of biosensing systems that are sensitive to

submicromolar concentrations of glucose is possible" (*id.* at 7 (citing Daunert [0076])).

While the Supreme Court in *KSR v. Teleflex* emphasized the importance of "an expansive and flexible approach" to evaluating obviousness, 550 U.S. at 415, the Court also reaffirmed the importance of determining "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *Id.* at 418.

Ultimately, therefore, "[i]n determining whether obviousness is established by combining the teachings of the prior art, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re GPAC Inc.*, 57 F.3d 1573, 1581 (Fed. Cir. 1995) (internal quotations omitted).

In this instance, we agree with Appellants that a preponderance of the evidence does not support the Examiner's finding that the cited references would have suggested using Daunert's GBP-bound hydrogel as Polak's binding substrate.

As Appellants point out, Polak's device is specifically configured to ascertain glucose concentrations through a competitive assay using a labeled analogue contained within the device's void (*see* Polak [0013] ("The devices and methods for monitoring an analyte in accord with the present invention are based on a competitive reaction for a binding site of the binding substrate between the analyte of interest and a fluorescently labeled analogue.")).

As Polak explains the "[l]abeled [a]nalogue" of its device refers to "one or a plurality of ligands that binds to the substrate at low analyte concentrations, and dissociates from the binding substrate as the

concentration of analyte increases. Suitable analogues include, but are not limited to dextran, lectins, Concanavalin-A . . ." (*id.* at [0024]-[0025]).

Thus, in addition to functioning in a significantly different manner than the direct detection of glucose binding employed in Daunert's device, in Polak's device the glucose binding moiety is soluble, rather than immobilized as in Daunert's device. Accordingly, because incorporating Daunert's hydrogel-GBP construct into Polak's device would not only require significant rearrangement of the various components of Polak's device, but would also require a significant change in how Polak's device functions, we are not persuaded that the cited references would have prompted the modification posited by the Examiner. We therefore reverse the Examiner's obviousness rejection of claim 1, and its dependents, over Polak and Daunert.

SUMMARY

We affirm the Examiner's obviousness rejection of claims 1, 3-6, 14, 15, and 21 over Van Antwerp and Daunert.

However, we reverse the Examiner's obviousness rejection of claims 1, 3-6, 14, 15, and 21 over Polak and Daunert.

TIME PERIOD

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

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