(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 August 2013 (08.08.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/115897 A3

(51) **International Patent Classification**:
G01N 33/543 (2006.01) *G01N 33/553* (2006.01)
G01N 33/569 (2006.01)

(21) **International Application Number**:
PCT/US2012/065655

(22) **International Filing Date**:
16 November 2012 (16.11.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/560,501 16 November 2011 (16.11.2011) US

(71) **Applicant**: **UES, INC.** [US/US]; 4401 Dayton-Xenia Road, Dayton, OH 45432 (US).

(72) **Inventors**: **TOMCZAK, Melanie**; 5121 Oak Tree Drive, South, Kettering, OH 45440 (US). **LIPTAK, David, C.**; 40 Whipporwill Lane, Centerville, OH 45459 (US). **OSTENDORF, Melinda, A.**; 2152 Blanton Drive, Miamisburg, OH 45342 (US). **SMITH, Clint, B.**; 43304 Jerpoint Court, Chantilly, VA 20152 (US).

(74) **Agents**: **WHERLEY, Hallie, W.** et al.; Dinsmore & Shohl LLP, One South Main Street, Fifth Third Center - Suite 1300, Dayton, OH 45402 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) **Title**: PATHOGEN DETECTION USING METAL ENHANCED FLUORESCENCE

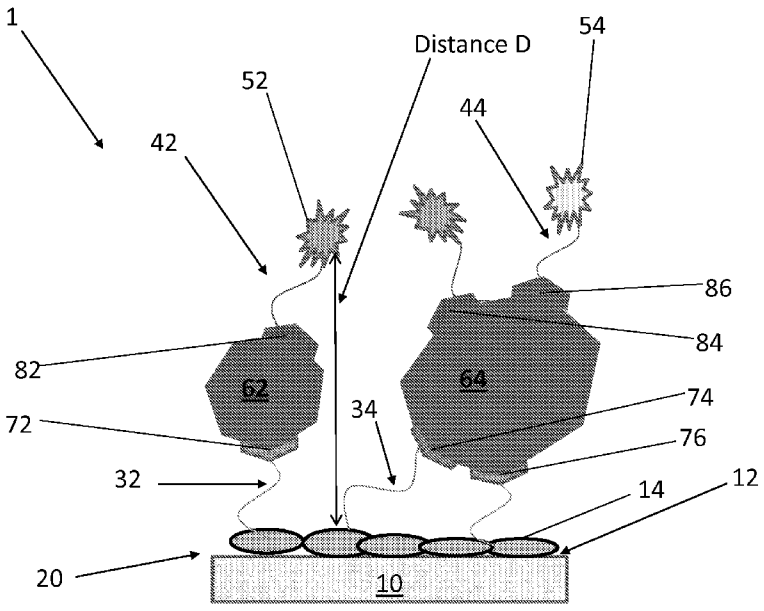

FIG. 1

(57) **Abstract**: Pathogen sensors, systems for pathogen detection, peptide ligands, and methods for detecting pathogens are disclosed. The pathogen sensors include silver particles attached to optically clear substrates, immobilized capture peptide or glycan ligands attached to the silver particles, and free labeling peptide or glycan ligands having fluorophores. Binding of the immobilized capture ligands and the free labeling ligands to the pathogen positions the fluorophore at a distance from the silver particles sufficient to initiate metal enhanced fluorescence.

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
28 November 2013

(15) Information about Correction:
Previous Correction:
see Notice of 26 September 2013

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/065655

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/543 G01N33/569 G01N33/553
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 2 248 912 A1 (UNIV MARYLAND BIOTECHNOLOGY [US] UNIV MARYLAND [US]) 10 November 2010 (2010-11-10) paragraph [0011]; figure 1	1-16, 20-48
X	WO 2007/084141 A2 (STANFORD RES INST INT [US]; FARIS GREGORY W [US]; HAUSHALTER JEANNE P) 26 July 2007 (2007-07-26)	1-16, 20-34
Y	paragraphs [0019], [0020], [0030], [0036]; examples 1-3	35-48
X,P	US 2012/238035 A1 (GEDDES CHRIS D [US]) 20 September 2012 (2012-09-20) paragraph [0046] - paragraph [0051]	1-4,7-9, 12-15, 20-34

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 October 2013	16/10/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Fleitmann, J

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/065655

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE Geneseq [Online] 21 October 2004 (2004-10-21), "GW182 peptide 106 for epitope mapping.", XP002703971, retrieved from EBI accession no. GSP:ADQ81249 Database accession no. ADQ81249 sequence -----	1-16, 20-34, 49,50
Y	DISNEY M D ET AL: "Detection of bacteria with carbohydrate-functionalized fluorescent polymers", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, ACS PUBLICATIONS, US, vol. 126, no. 41, 25 September 2004 (2004-09-25), pages 13343-13346, XP002366145, ISSN: 0002-7863, DOI: 10.1021/JA047936I abstract -----	1-16, 20-48
Y	DUANE M. HATCH ET AL: "Biotinylated Bi- and Tetra-antennary Glycoconjugates for Escherichia coli Detection", CHEMBIOCHEM, vol. 9, no. 15, 13 October 2008 (2008-10-13), pages 2433-2442, XP55081635, ISSN: 1439-4227, DOI: 10.1002/cbic.200800188 abstract -----	35-48
Y	WO 00/36419 A1 (EXIQON A S [DK]; JAKOBSEN MOGENS HAVSTEEN [DK]; BOAS ULRIK [DK]; JAUHO) 22 June 2000 (2000-06-22) example 28 -----	35-48

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/065655

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

35-48, 50(completely); 1-16, 20-34, 49(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 50(completely); 1-16, 20-34, 49(partially)

   Sensors and Systems for the detection of pathogens comprising optically clear substrate comprising silver particles deposited on a surface thereof, wherein the silver particles are immobilized on the surface; a plurality of immobilized capture peptide ligands comprising SEQ ID NO:1 attached to the silver particles, and a plurality of free labeling peptide ligands comprising fluorophores

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2. claims: 51(completely); 1-16, 20-34, 49(partially)

   Sensors and Systems for the detection of pathogens comprising optically clear substrate comprising silver particles deposited on a surface thereof, wherein the silver particles are immobilized on the surface; a plurality of immobilized capture peptide ligands comprising SEQ ID NO:1 attached to the silver particles, and a plurality of free labeling peptide ligands comprising fluorophores

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3. claims: 17, 52(completely); 1-15, 20-34, 49(partially)

   Sensors and Systems for the detection of pathogens comprising optically clear substrate comprising silver particles deposited on a surface thereof, wherein the silver particles are immobilized on the surface; a plurality of immobilized capture peptide ligands comprising SEQ ID NO:3 attached to the silver particles, and a plurality of free labeling peptide ligands comprising fluorophores

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4. claims: 18, 53(completely); 1-15, 20-34, 49(partially)

   Sensors and Systems for the detection of pathogens comprising optically clear substrate comprising silver particles deposited on a surface thereof, wherein the silver particles are immobilized on the surface; a plurality of immobilized capture peptide ligands comprising SEQ ID NO:4 attached to the silver particles, and a plurality of free labeling peptide ligands comprising fluorophores

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5. claims: 19, 54(completely); 1-15, 20-34, 49(partially)

   Sensors and Systems for the detection of pathogens comprising optically clear substrate comprising silver particles deposited on a surface thereof, wherein the silver particles are immobilized on the surface; a plurality of immobilized capture peptide ligands comprising SEQ ID NO:5 attached to the silver particles, and a plurality of free

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

labeling peptide ligands comprising fluorophores
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6. claims: 35-48

Sensors and Systems for the detection of pathogenic E. coli comprising optically clear substrate comprising silver particles deposited on a surface thereof, wherein the silver particles are immobilized on the surface; a plurality of immobilized capture glycan ligands attached to the silver particles, and a plurality of free labeling glycan ligands comprising fluorophores
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/065655

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2248912 A1	10-11-2010	AT 479775 T	15-09-2010
		AU 2003296014 A1	22-07-2004
		EP 1583948 A2	12-10-2005
		EP 2248912 A1	10-11-2010
		US 2006147927 A1	06-07-2006
		US 2012142552 A1	07-06-2012
		WO 2004059279 A2	15-07-2004
WO 2007084141 A2	26-07-2007	US 2008194044 A1	14-08-2008
		WO 2007084141 A2	26-07-2007
US 2012238035 A1	20-09-2012	NONE	
WO 0036419 A1	22-06-2000	AT 300048 T	15-08-2005
		AU 1650100 A	03-07-2000
		BR 9916330 A	11-09-2001
		CA 2355292 A1	22-06-2000
		CN 1335936 A	13-02-2002
		DE 69926261 D1	25-08-2005
		DE 69926261 T2	24-05-2006
		EP 1141718 A1	10-10-2001
		JP 2002532719 A	02-10-2002
		MX PA01006106 A	21-07-2003
		NZ 512295 A	25-07-2003
		WO 0036419 A1	22-06-2000