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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 August 2008 (07.08.2008)

PCT

(10) International Publication Number
WO 2008/094273 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01) *G01N 33/533* (2006.01)
G01N 33/00 (2006.01) *C07H 21/04* (2006.01)
C12N 9/00 (2006.01)

(21) International Application Number:
PCT/US2007/067288

(22) International Filing Date: 24 April 2007 (24.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11/669,078 30 January 2007 (30.01.2007) US

(71) Applicants *(for all designated States except US)*: **CELSIS INTERNATIONAL PLC**; 400 W. Erie, Suite 300, Chicago, IL 60610 (US). **CORNELL UNIVERSITY** [—/US]; Ithaca, NY 14893 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BAEUMNER, Antje, J.** [DE/US]; 1040 Cayuga Heights Road, Ithaca, NY 14850 (US). **HEARN, Andrew** [GB/US]; 431 West Barry, Unit 329, Chicago, IL 60657 (US). **MADDEN, Judith** [CA/US]; 24415 Zermatt Lane, Valencia, CA 91355 (US). **SELLAPPAN, Subramani** [IN/US]; 2265 Cheshire Drive, Aurora, IL 60502 (US). **ZAYTSEVA, Natalya, V.** [RU/US]; 37 Uptown Road, Apt. 24E, Ithaca, NY 14850 (US).

(74) Agent: **LEITH, Nancy, J.**; Loeb & Loeb LLP, 321 North Clark Street, Suite 2300, Chicago, IL 60610 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
4 December 2008

(54) Title: DETECTION OF ANALYTES IN SAMPLES USING LIPOSOME-AMPLIFIED LUMINESCENCE AND MAGNETIC SEPARATION

(57) Abstract: The invention relates to the encapsulation of luminescence-related molecules, including but not limited to, adenosine triphosphate (ATP), adenylate kinase (AK), alkaline phosphatase (ALP), luminol and luciferin/luciferase cocktails, within liposomes. These liposomes can be employed to enhance the luminescence detection of microorganisms and compounds in various products and samples. The liposomes containing the luminescence-related molecules can bear a probe which has a specific sequence or structure that, in turn can be used to hybridize to, or couple with, a portion of the target analyte. Within the same assay, paramagnetic beads can bear a probe having a specific sequence or structure that, can hybridize to, or couple with, a second portion of the target analyte to create a complex of analyte bound to paramagnetic beads and liposomes. This type of assay can be often referred to as a 'sandwich' assay. Once the probes hybridize to, or couple with, their targets, a complex can be formed of the paramagnetic beads, the analyte, or portion thereof, and the liposomes. This complex can then be washed to remove those components that are non-hybridized or non-coupled. Then, the paramagnetic bead-analyte- liposome complexes can be isolated from the sample using magnetic separation techniques and can be treated so as to release their encapsulated ATP, AK or other luminescence-related compounds. The resulting luminescence can then be determined in a chemical assay. This determination can be qualitative (i.e., an absence/presence assay) or quantitative (i.e., which can measure a specific amount of analyte present). Through the use of a cocktail of probe types, the assay can also qualitatively or quantitatively measure the presence of more than one analyte simultaneously. This type of assay can be of commercial importance in clinical and forensic applications, the personal care, pharmaceutical, food and beverage markets, as well as in environmental sample assays.

WO 2008/094273 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/67288

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12Q 1/68**(2006.01);**G01N 33/00**(2006.01)
C12N 9/00(2006.01);G01N 33/533(2006.01);C07H 21/04(2006.01)

USPC: 435/6;436/94
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)
U.S. : 435/6, 183, 287.2; 436/94, 800; 536/23.1, 24.31

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)
EAST (USPAT, USPGP, EPO, JPO, DERWENT)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002/0058332 A1 (QUAKE et al.) 16 May 2002 (16.05.2002), see paragraph [0058].	1-3, 5, 7-22
Y	US 4,704,355 (BERNSTEIN) 03 November 1987 (03.11.1987), see abstract and columns 4-6.	1-3, 5, 7-22

☐ Further documents are listed in the continuation of Box C. ☐ 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

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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
27 August 2008 (27.08.2008)	09 OCT 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer /Bradley L. Sisson/ Telephone No. (571) 272-0600

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/67288

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:
Please See Continuation 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 additional fees, this Authority did not invite payment of any 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.:

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.: Please See Continuation Sheet

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/67288

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-22, drawn to a method for detecting an analyte.

Group II, claim(s) 23-37, drawn to a kit.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

In the above-identified Group I, the species are.

1. Luminescence-related amplificant
a. Adenosine triphosphate (ATP) (claim 3)
b. Adenylate kinase (AK) (claim 3)
c. LUMINOL (claim 3)
d. Alkaline phosphatase (ALP) (claim 3)
e. Luciferase/luciferin cocktail (claim 3)

2. Analyte comprises:
a. RNA (claim 8)
b. DNA (claim 8)
c. Antibody (claim 8)
d. Antigen (claim 8)

1. Liposomes are:
a. Unilamellar (claim 20)
b. Multilamellar (claim 20)

4. Reporter probe is specific for:
a. Target nucleic acid sequences (claim 22)
b. Antigen (claim 22)

In the above-identified Group II, the species are:

1. Encapsulated amplificant is
a. Adenosine triphosphate (claim 24)

b. Adenylate kinase (claim 24)
c. LUMINOL (claim 24)
d. Luciferase/luciferin cocktail (claim 24)
e.

2. Reporter probe is specific for
a. Target nucleic acid sequences (claim 26)
b. Antigen (claim 26)

3. Luminescence reagent is
a. Luciferase (claim 30)
b. Luciferin and adenosine diphosphate (claim 30)
c. Luciferase and luciferin (claim 30)
d. ATP (claim 30)

4. Liposomes are
a. Unilamellar (claim 31)
b. Multilamellar (claim 31)

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The inventions are linked through the presence or use of liposomes that comprise a probe on the surface of the liposome and an encapsulated amplificant. US Patent 4,704,355 (Bernstein; 03 November 1987), abstract, disclose using liposomes that comprise ATP and which have either antibodies or nucleic acids on their surface. Accordingly, the feature that links the inventions does not constitute a special technical feature and thusly, the inventions lack unity under PCT Article 13.2

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The inventions are linked through the presence or use of liposomes that comprise a probe on the surface of the liposome and an encapsulated amplificant. US Patent 4,704,355 (Bernstein; 03 November 1987), abstract, disclose using liposomes that comprise ATP and which have either antibodies or nucleic acids on their surface.

It is further noted that multiple species are found within a single claim. In accordance with PCT Article 13.3: "The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim." Accordingly, the feature that links the inventions does not constitute a special technical feature and thusly, the inventions lack unity under PCT Article 13.2.

Continuation of Box III Item 4:
1-3, 5, and 7-22 with species of claim 3 (ATP), Claim 8 (DNA), Claim 20 (unilamellar), and Claim 22 (target nucleic acid sequecnes)