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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 June 2008 (12.06.2008)

PCT

(10) International Publication Number
WO 2008/070152 A3

(51) International Patent Classification:
C12N 9/12 (2006.01)

(21) International Application Number:
PCT/US2007/024992

(22) International Filing Date:
6 December 2007 (06.12.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/873,753 6 December 2006 (06.12.2006) US

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(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, 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, 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:
18 December 2008

WO 2008/070152 A3

(54) Title: DEEPLY QUENCHED ENZYME SENSORS AND BINDING SENSORS

(57) Abstract: Sensors for detecting enzyme activity are provided that include a substrate module comprising a substrate for the enzyme of interest and a fluorescent label, a quencher, and a detection module. The detection module binds to the substrate module either before or after the enzyme acts on the substrate and sequesters the label from the quencher, resulting in an increased signal from the label. Sensors for detecting protein-protein interactions are also provided that include a quencher and a labeled first polypeptide. Binding of the first polypeptide to a second polypeptide sequesters the label from the quencher, resulting in an increased signal from the label. Methods using the sensors to detect enzyme activity and to screen for compounds affecting enzyme activity or to detect protein-protein interactions and to screen for compounds affecting protein-protein interactions, respectively, are also described.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/24992

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12N 9/12**(2006.01)

USPC: 435/194
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. : 435/194

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 7,056,683 (TING et a) 6 June 2006 (06.06.2006)	1-10 and 2-28

☐ 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 theart 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
25 July 2008 (25.07.2008)

Date of mailing of the international search report
20 OCT 2008

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/24992

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/24992

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.: 1-10 and 22-28

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/US07/24992

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) 1-30, drawn to a composition for assessing enzyme activity comprising a substrate, a detector, and a quencher.
Group I encompasses:
A. Comprising the enzyme
B. Not comprising the enzyme
Group I encompasses:
C. Not comprising a modulator of the enzyme
D. Comprising a modulator of the enzyme
Group I encompasses:
E. The substrate is a peptide
F. The substrate is not a peptide
Group I encompasses:
G. The detector comprises a peptide
H. The detector does not comprise a peptide
Group I encompasses:
I. The detectors recited in Claims 10 and 12
Group I encompasses:
J. The enzymes recited in Claims 9, 11, 13, 15, and 17
Group I encompasses:
K. The specific substrates recited in Claims 21 and 22 (SEQ ID NO: 1-13), and 23
Group I encompasses:
L. The substrate is not bound to the detector
M. The substrate is bound to the detector
Group I encompasses:
N. Covalent quencher
O. Non-covalent quencher
Group I encompasses:
P. The sensor does not comprise caging groups
Q. The sensor comprises caging groups

Group II, claim(s) 31-59, drawn to a method of assaying enzyme activity.
Group II encompasses:
R. The substrate is a peptide
S. The substrate is not a peptide
Group II encompasses:
T. The detector comprises a peptide
U. The detector does not comprise a peptide
Group II encompasses:
V. ~~The detectors recited in Claims 39 and 41~~

Group II encompasses:
W. The enzymes recited in Claims 38, 40, 44, 42, and 46
Group II encompasses:
X. The specific substrates recited in Claims 50, 51 (SEQ ID NO: 1-13), and 52
Group II encompasses:
Y. The substrate is bound to the detector
Z. The substrate is not bound to the detector
Group II encompasses:
AA. The substrate is not bound to the detector
BB. The substrate is bound to the detector
Group II encompasses:
CC. The sensor does not comprise caging groups
DD. The sensor comprises caging groups
Group II encompasses:
EE. Testing activity not in the presence of a test compound
FF. Testing activity in the presence of a test compound

Group III, claim(s) 60-74, drawn to a composition for assessing binding comprising a polypeptide, a binding partner, and a quencher.
Group III encompasses:
GG. The polypeptides of Claims 61-63 (SEQ ID NO: 1-13), 65
Group III encompasses:
HH. The binding partners of Claims 61 and 64
Group III encompasses:
II. Comprising a test compound
JJ. Comprising a test compound
Group III encompasses:
KK. The polypeptide does not comprise caging groups
LL. The polypeptide comprises caging groups

Group IV, claim(s) 75-88, drawn to a method for assessing binding.
Group IV encompasses:
MM. The polypeptides of Claims 76-78 (SEQ ID NO: 1-13), and 80
Group IV encompasses:
NN. The binding partners of Claims 76 and 79
Group IV encompasses:
OO. Testing binding in the presence of a test compound
PP. Testing binding in the presence of a test compound

Group V, claim(s) 89-95, drawn to a polypeptide.
Group V encompasses:
QQ. The polypeptides of Claims 89 and 90 (SEQ ID NO: 1-13), and 94

Invention I is directed to a composition for assessing enzyme activity comprising a substrate, a detector, and a quencher, Wherein the composition comprises the enzyme, does not comprise a modulator, the substrate is a peptide, the detector comprises a peptide, the detector is 14-3-3, the enzyme is a serine kinase, the specific substrate is SEQ ID NO: 13, the substrate is not bound to the detector, and the quencher is covalently bound to the substrate. Invention I encompasses Claims 1-10, 19-21, and 24-28.

The inventions listed as Groups I-V(A)-(UU) 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 listed as Groups I-V(A)-(UU) 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 feature for the following reasons: The technical feature linking Groups I-V(A)-(UU) appears to be that they all relate to fluorescent polypeptides. However, fluorescent polypeptides were well known in the art. Moreover, Ting et al, 2006 (published 30-DEC-2004) teach a composition comprising a fragment of histone linked to a fluorescent moiety and a quencher (col 12, pargs 2 & 5), wherein the histone fragment is a substrate for a serine kinase (parg bridging cols 37-38), the kinase, and a detector molecule comprising a fluorescent moiety (parg bridging cols 37-38), which anticipates Claim 1. Therefore Groups I-V(A)-(UU) share no special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art. Furthermore, the products of Groups I, III, and V do not share a special common structural and functional feature while, the methods of Groups II and IV do not use the same reagents or produce the same results. In addition, the methods of Groups II and IV do not comprise all of the methods for making or using the products of Groups I, III, and V. Accordingly, Groups I-V(A)-(UU) are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept.

US 7,056,683 Ting et al 06-JUN-2006; publised 30-DEC-2004 as US 2004/0265906.