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(71) **Applicant**: **MASSACHUSETTS INSTITUTE OF TECHNOLOGY** [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) **Inventors**: **LIPPARD, Stephen, J.**; 975 Memorial Drive, Apt. 602, Cambridge, MA 02138 (US). **RADFORD, Robert, J.**; 33 Gloucester Street Apt. 2, Boston, MA 02135 (US).

(74) **Agents**: **GORDON, Dana, M.** et al.; Foley Hoag LLP, 155 Seaport Blvd, Boston, MA 02210-2600 (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.

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(88) **Date of publication of the international search report**:
18 June 2015

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(54) **Title**: ZINC-RESPONSIVE PEPTIDES, AND METHODS OF USE THEREOF

(57) **Abstract**: Described herein are compounds and methods useful in the detection of, e.g., Zn^{2+}, in vitro and in vivo. The compounds include amino acids and peptides functionalized with a moiety that binds, e.g., Zn^{2+}. The peptides may be further functionalized with a detectable moiety. The peptides may also comprise amino acid sequences known to localize extracellularly or in specific areas within a cell.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/21424

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 1/30; G01N 21/76; G01N 33/20 (2013.01)
USPC - 435/40.5
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)
USPC: 435/40.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 436/172, 436/81, 436/74 (keywords below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents/Scholar
Search Terms Used: Amino acid, fluorophore, linker, resorufin, phenoxazin, zinc imaging, pyridyl, lewis base

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0182971 A1 (McAuliffe, et al.) 31 July 2008 (31.07.2008) para [0019], [0057], Fig. 1B, SEQ ID NO: 7	1-2, 11-12
X -- Y	US 6,013,802 A (Hoyland, et al.) 11 January 2000 (11.01.2000) col 4, ln 1-7, col 8, ln 18-30, col 9, ln 22-67, col 11, ln 1-23, col 13, ln 21-29, ln 56-67	1-2 ---- 3
Y	US 2011/0086341 A1 (Batchelor, et al.) 14 April 2011 (14.04.2011) para [0091]-[0094], [0099], [0139]-[0140], claim 8	3
A	WO 2003/091689 A2 (Ebright, et al.) 06 November 2003 (06.11.2003) whole doc.	1-3, 11-12
A	WO 2011/019864 A2 (Lippard, et al.) 17 February 2011 (17.02.2011) whole doc.	1-3, 11-12
A, P	US 2012/0271100 A1 (Woodruff, et al.) 25 October 2012 (25.10.2012) whole doc.	1-3, 11-12

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

* 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 but 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
05 June 2013 (05.06.2013)	19 JUN 2013
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: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/21424

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.: 14-15, 22-80
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:

Group I+: claims 1-13 and 16-21, drawn to an amino acid compound, comprising a sensor moiety, a first linker, and a first amino acid, wherein the first amino acid is a natural amino acid or a non-natural amino acid wherein the sensor moiety comprises Formulas 1A, 2A and 3 . The first invention is restricted to Formula 1A wherein A is a diradical having one or more carbon atoms, Z is hydrogen, Q is O, K is optional, V is a Lewis base, Y is O, Z2 is N; and R1 is H; and the first amino acid, arginine, of claim 12 (Claims 1-3, 11-12) and will be searched without additional fee. Should an additional fee be paid, Applicant is invited to elect an additional sensor compound and amino acid. The exact claims searched will depend on Applicant's election. An exemplary election comprises the first sensor compound of claim 6 (Formula 2A) and the first amino acid, arginine, of claim 12 (Claims 1-6, 11-13, 16-18, 21). Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched/examined.

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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 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.:
1-3, 11-12, restricted to Formula IA as specified

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/US 13/21424

********** Supplemental Sheet **********

Box NO III. Observations where unity of invention is lacking

The inventions listed as Group I+ 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 of Group I+ share the technical feature of an amino acid compound, comprising a sensor moiety, a first linker, and a first amino acid, wherein the first amino acid is a natural amino acid or a non-natural amino acid. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2008/0182971 A1 to McAuliffe et al. (hereinafter 'McAuliffe'). McAuliffe discloses an amino acid compound, comprising a sensor moiety, a first linker, and a first amino acid, wherein the first amino acid is a natural amino acid or a non-natural amino acid (para [0018], [0057], The N-terminal of P5 peptide (SEQ ID NO: 7) with an Ahx (2-aminohexanoic acid) linker was labeled with fluorescein (Fig. 1B)). As said technical feature was known in the art at the time of the invention, these cannot be considered special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Groups I+ is the specific formulas recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained between formula III and formula 1A and 2A. Further, Ebright et al. (WO 03/091689) teaches claimed formula 2A (pg 10, ln 15-26, Formula (I), wherein X is formula (XX) on pg 20) wherein A is a diradical having one or more carbon atoms (pg 10, ln 15-26, Formula (I), wherein linker R2 and R2' further defined on pg 11, ln 14-24 and exemplified in formulas (XXIII) and (XXIV) on page 22-23, and X is formula (XX) shown on pg 20);
Z is hydrogen (pg 20, Formula (XX));
Q is O (pg 20, Formula (XX));
K is optionally one or more substituents of the indicated aromatic ring (pg 20, Formula (XX), R6 and R6');
V is a Lewis base (pg 10, ln 15-26, Formula (I), R1-N- or R1'-N, pg 11, ln 8-12, when R1 or R1' is absent or when R1 or R1' is CH(COO-);
Y is O (pg 20, Formula (XX));
Z2 is N (pg 20, Formula (XX)).
Although, Ebright does not expressly provide a specific embodiment for claimed Formula I, however, it would have been obvious to one of ordinary skill in the art to have obtained claimed Formula I, because Ebright teaches Formula I (pg 10), wherein X is Formula (XX) (pg 20) which is a probe comprising a sensor molecule for chelating transition metal (pg 10, ln 15-18) including exemplary formulas (XX), (XXII), (XXIII) and (XXIV), having a linker with amide bond (Formulas (XXIII) and (XXIV)), immobilization of the probe (pg 37, ln 28-30), target peptide sequences (pg 38, ln 10-14), and exemplary preparation methods (pg 39 to pg 48). Further, in view of the teachings of McAuliffe of a peptide-linker-fluorescein (para [0057]) that can be used as a template in the formation of silicon-based hybrid material (para [0006]), one of ordinary skill in the art would have been motivated to have prepared a fluorescein based sensor for chelating metal as taught by Ebright.

As the common technical features were known znd obvious in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, invenitons of Group I+ lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.