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(71) Applicant: NATIONAL CENTRE FOR BIOLOGICAL SCIENCES (NCBS-TIFR) [IN/IN]; G.K.V.K Post, Bellary Road, Bangalore, Karnataka 560065 (IN).

(72) Inventors: KRISHNAN, Yamuna; 2, Mallige, National Centre for Biological Sciences, TIFR, GKVK Post, Bellary Road, Bangalore, Karnataka 560065 (IN). MODI, Souvik; 15/8/2 Laxmi Narayan Chakraborty Lane, Kadamtala, Howrah, West Bengal 711101 (IN). SURANA, Sunaina; 5/7 Buroshibtalla main road, Shailja Apartments, Tower 1, Flat 8C, Kolkata 700038 (IN).

(74) Agents: VIJAYAKRISHNAN, Sindhu et al.; K & S Partners, # 4121/B, 6th Cross, 19A Main, HAL II Stage (Extension), Bangalore 560 038 (IN).

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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, IR, IS, JP, KE, KG, 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, SA, 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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Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

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

(88) Date of publication of the international search report:

2 July 2015

(54) Title: METHODS OF MULTIPLEXING DNA SENSORS AND LOCALIZING DNA SENSOR

(57) Abstract: The present disclosure relates to a method of multiplexing DNA sensors and optionally measuring pH in cell, a method of localizing DNA sensor in Golgi Network of scFv-Furin expressing cell and a method of identifying optimal location of fluorophore pair on the DNA sensor for multiplexing DNA sensors. The DNA sensors of the present disclosure follow independent cellular pathways and do not interact with or compromise functionality of another DNA sensor.



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB14/59236

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68, 1/00; C12N 5/00 (2015.01)

CPC - C12Q 1/6883, 1/6827, 1/6816; A61K 38/00, 48/00; C07K 2319/00

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)

IPC(8): C12Q 1/68, 1/00; C12N 5/00

CPC: C12Q 1/6883, 1/6827, 1/6816; A61K 38/00, 48/00; C07K 2319/00; USPC: 435/6.1, 4, 325

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)

Pat Seer (US, EP, WO); Google; Google Scholar; Dialog ProQuest; Entrez Pubmed; 'DNA sensor,' multiplex, fluorophore, 'Golgi,' localization, endocytosis, 'scFv-Furin,' 'FRET anti-TGN46,' microscopy, conjugation

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                    | Relevant to claim No. |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | US 2010/0304370 A1 (KRISHNAN, Y et al.) December 02, 2010; abstract; figures 2, 4, 12C;                                                                                                                                               | 1, 3, 6-9             |
| ---       | paragraphs [0009], [0012], [0020], [0037], [0040], [0041], [0059], [0062], [0066], [0073], [0099],                                                                                                                                    | -----                 |
| Y         | [0103], [0115]-[0118]                                                                                                                                                                                                                 | 2, 4, 5, 10-12        |
| Y         | US 2004/0166553 A1 (NGUYEN, Q et al.) August 26, 2004; abstract; paragraphs [0038], [0295], [0324], [0327]                                                                                                                            | 2, 4, 5               |
| Y         | WO 2012/140274 A9 (KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN) October 18, 2012; page 22, lines 21-25; page 28, lines 10-16; page 31, lines 15-19; page 36, lines 9-12; page 39, lines 12-19; page 40, lines 18-19; figure 24 | 5, 10-12              |
| Y         | US 2010/0159468 A1 (HASLAM, DB) June 24, 2010; figure 3; paragraphs [0014], [0099]                                                                                                                                                    | 11                    |
| A         | US 2004/0219523 A1 (STANTON, M et al.) November 04, 2004; entire document                                                                                                                                                             | 1-12                  |

☐ 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

25 February 2015 (24.02.2015)

Date of mailing of the international search report

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Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
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Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300  
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB14/59236

## 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.: 13, 14, 20, 21  
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:

\*\*\*-Continued Within the Next Supplemental Box-\*\*\*

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.:  
Group I: Claims 1-12

### 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.

-\*\*\*-Continued from Box No. 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: Claims 1-12 are directed toward a method of multiplexing DNA sensors wherein said DNA sensor comprises Nucleic Acid Assembly and fluorophore, said method comprising acts of: a) adding the DNA sensors to the cell in labelling media for cellular uptake, to obtain a cell with the DNA sensors; b) incubating the cell obtained in step a) in the labelling media for multiplexing the DNA sensors.

Group II: Claims 15-19 are directed toward a method of identifying optimal location of fluorophore pair on DNA sensor for multiplexing DNA sensors,

The inventions listed as Groups I-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 special technical features of Group I include adding DNA sensor to a cell in labelling media, which is not present in Group II, the special technical features of Group II including determining spectral overlap between fluorophores and identifying fluorophore pair that displays maximum FRET.

Groups I-II share the technical features including multiplexing DNA sensors, wherein the DNA sensor comprises a Nucleic Acid Assembly and a fluorophore.

However, these shared technical features are previously disclosed by US 2010/0304370 A1 to Krishnan, et al. (hereinafter 'Krishnan'). Krishnan discloses multiplexing (a cell comprising at least a second DNA sensor with a distinct emission spectrum (multiplexing); paragraph [0087]) DNA sensors (paragraphs [0004], [0087]), wherein the DNA sensor (paragraph [0004]) comprises a Nucleic Acid Assembly (comprises a nucleic acid nanomachine (Nucleic Acid Assembly); paragraph [0004]) and a fluorophore (and donor and acceptor molecules including fluorescein ALEXA-488 and BODIPY-TMR (and fluorophore); paragraph [0009]).

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Krishnan reference, unity of invention is lacking.