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

[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR ANALYTE DETECTION USING NANOSWITCHES

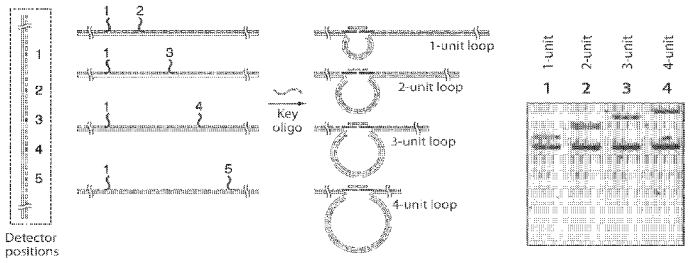

FIG. 1C

(57) Abstract: Provided herein are nucleic acid-based nanoswitches that can detect specific nucleic acids and other analytes types by for example a simple gel electrophoresis readout. Binding of the target to the nanoswitches induces a conformation change from a linear, open conformation to a looped, closed conformation. These nanoswitches may be used in diagnostic assays such as nucleic acid- based diagnostic assays, to detect, measure and/or purify a variety of targets including low abundance targets.

WO 2017/003950 A3

— *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:
23 March 2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/39654

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; G01N 33/53, 27/447; C12P 19/34 (2016.01)
CPC - C12Q 1/6816, 1/6813, 1/6804; G01N 33/5308, 27/447; C12P 19/34
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; G01N 33/53, 27/447; C12P 19/34 (2016.01)
CPC: C12Q 1/6816, 1/6813, 1/6804; G01N 33/5308, 27/447; C12P 19/34

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)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; PubMed; EBSCO Discovery Service; scaffold, Nucleic acid, DNA, RNA, Hybridized, Oligonucleotides, 3', 5', Overhang

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	(KOUSSA, MA et al.) DNA Nanoswitches: A Quantitative Platform for Gel-Based Biomolecular Interaction Analysis. Nature Methods. 08 December 2014, Vol. 12, No. 2, pages 1-17; abstract; page 8, second paragraph; Figures 1a-c; 2d, 2f; DOI: 10.1038/nmeth.3209	1, 2, 6/1-2, 7/1-2 --------- 3/1-2, 8/7/1, 26/1-2, 27/26/1-2
Y	US 2014/0255939 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE, et al.) September 11, 2014; figure 2b; paragraphs [0009]-[0013], [0029], [0036], [0039], [0050], [0053], [0099], [0107], [0108], [0125]	26/1-2, 27/26/1-2
Y	WO 2014/011800 A1 (PIVOT BIO, INC.) January 16, 2014; paragraph [0029]	3/1-2
Y	XhoI [online]. Wikipedia. 14 May 2014 [retrieved on 2016-10-18]. Retrieved from the Internet: <URL: https://en.wikipedia.org/w/index.php?title=XhoI&oldid=608536958>; page 1.	6/1-2
Y	(CONDE, J et al.) Implantable Hydrogel Embedded Dark-Gold Nanoswitch as a Theranostic Probe to Sense and Overcome Cancer Multidrug Resistance. PNAS. 02 March 2015, Vol. 112, No. 11, E1278-E1287; page E1279, first column, second paragraph; figure 1A; DOI: 10.1073/pnas.1421229112	8/7/1-2
A	WO 2015/006626 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE, et al.) January 15, 2015; page 10, lines 8-23; figure 7	25/1-2, 27/25/1-2
A	(HALVORSEN, K et al.) Nanoengineering a Single-Molecule Mechanical Switch Using DNA Self-Assembly. Nanotechnology. 21 November 2011, Vol. 22, pages 1-14; supplemental figure 2; DOI: 10.1088/0957-4484/22/49/494005.	25/1-2, 27/25/1-2, 92-95

☐ 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 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
19 October 2016 (19.10.2016)	07 FEB 2017

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/39654

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.: 4, 5, 9-24, 28-91
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 Supplemental Page-***-

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 1-3, 6-8, 25-27 and 92-95

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
Information on patent family members

International application No.
PCT/US16/39654

-***-Continuation of 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 1-3, 6-8, 25-27 and 92-95 are directed toward nucleic acid complex comprising scaffold nucleic acid hybridized to a plurality of oligonucleotides and methods for detecting a target nucleic acid therewith.

Group II, Claims 96-105 are directed toward a method for stabilizing a first binding interaction in a nanoswitch, comprising providing a nucleic acid-based nanoswitch in a looped conformation as a result of a first binding interaction of the nanoswitch with a target, and inducing a latch binding interaction in the nanoswitch.

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 special technical features of Group I include a scaffold nucleic acid, not present in Group II; the special technical features of Group II include a nanoswitch, not present in Group I.

Groups I and II share the technical features including: a nucleic acid in a looped conformation; and binding with a target.

However, these shared technical features are previously disclosed by the article 'Nanoengineering a single-molecule mechanical switch using DNA self-assembly' by Halvorsen et al. (hereinafter 'Halvorsen').

Halvorsen discloses a nucleic acid in a looped conformation (looped DNA (a nucleic acid in a looped conformation; Figure 3, page 3, second column, second paragraph); and binding with a target (a receptor-ligand pair (binding with a target); Figure 3, page 3, second column, second paragraph).

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