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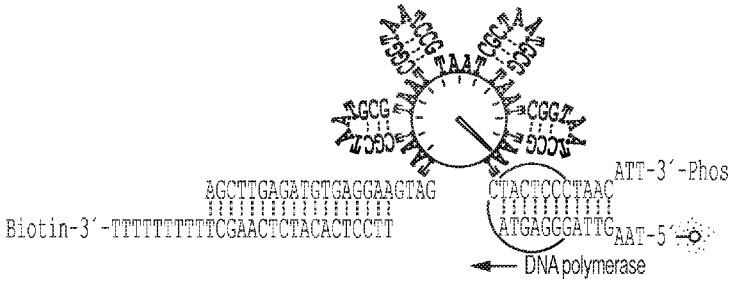
08 February 2018 (08.02.2018)

(54) Title: SINGLE MOLECULE TIMERS AND CLOCKS

Fig. 1



(57) Abstract: The present disclosure provides, in some aspects, single-molecule timers and clocks, systems and methods for kinetically encoded imaging.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/15057

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C12Q 1/68; C07H 21/04; G01N 21/62, 21/64 (2017.01)
CPC - C12Q 1/68, 1/6853, 1/6876, 1/6844, 1/686; C07H 21/04; G01N 21/62, 21/64

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0274135 A1 (ZHANG, D.Y et al.) 17 October 2013; figures 2A, 2B, 3A, 6, 12A, 14; paragraphs [0008], [0022], [0024], [0025], [0027], [0028], [0030], [0043]-[0044], [0051], [0052], [0064], [0073], [0075], [0082], [0084], [0089], [0092], [0107], [0149]-[0151], [0180], [0182], [0186], [0190], [0207], [0230], [0238], [0240], [0241], [0249], [0258].	1-5, 7, 11-24, 27-37, 42-45, 49, 51 ---------- 6, 8-10, 25-26, 38-41, 46-48, 50
Y	US 2014/0178878 A1 (MULLER, D. et al.) 26 June 2014; paragraph [0042].	6, 38
Y	US 2015/0292007 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 15 October 2015; paragraphs [0021], [0064]-[0065], [0067], [0105], [0116].	8-10, 39-41
Y	JOHNSON-BUCK, A. et al. Kinetic Fingerprinting To Identify And Count Single Nucleic Acids. Nature Biotechnology. July 2015, Vol. 33, pages 730-733; doi:10.1038/nbt.3246; figures 2b, 2e; page 733, first column, fourth paragraph; page 733, second column, third paragraph.	25-26
Y	US 2012/0058471 A1 (GRAHAM, D. et al.) 8 March 2012; paragraphs [0015], [0039], [0044], [0095], [0106], [0108], [0247], [0248].	46-48
Y	US 2009/0011956 A1 (YIN, P. et al.) 8 January 2009; figure 8b; paragraphs [0010], [0015], [0077], [0212].	50
A	US 2012/0263783 A1 (MESSMER, B.T.) 18 October 2012; paragraphs [0008], [0018], [0029], [0045], [0079], [0103], [0110]-[0111], [0208].	52-150

☒ 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)
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"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
28 November 2017 (28.11.2017)	14 DEC 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/15057

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2001/0010915 A1 (FRANK-KAMENETSKII, M.D. et al.) 2 August 2001; paragraphs [0015], [0019], [0021], [0079], [0081], [0155]-[0157].	52-150
A	US 2002/0076716 A1 (SABANAYAGAM, C.R. et al.) 20 June 2002; paragraphs [0012], [0033], [0038], [0093], [0111], [0114].	52-150
A	JUNGMANN, R. et al. Single-Molecule Kinetics And Super-Resolution Microscopy By Fluorescence Imaging Of Transient Binding On DNA Origami. Nano Letter. 19 October 2010, Vol. 10. Pages A-F, DOI: 10.1021/nl103427w; page C, first column, first paragraph.	52-106, 145-150
A	US 2013/0316917 A1 (STEMEERS, F. et al.) 28 November 2013; figure 9; paragraphs [0059], [0071], [0105], [0115].	52-150

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/15057

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 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.:

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 application No.
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-***-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 1-51 are directed toward a kinetically encoded imaging system and methods.

Group II, Claims 52-150 are directed toward compositions comprising a circular nucleic acid template comprising a primer binding sequence and interlocked with a circular nucleic acid leash; and methods associated therewith.

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 kinetically encoded imaging, not present in Group II; the special technical features of Group II include a circular nucleic acid leash, not present in Group I.

Groups I and II share the technical features including: nucleic acid imaging; combining a template; a primer; a labeled nucleic acid; a polymerase; dNTPs, thereby forming a reaction mixture; and incubating the reaction mixture under conditions that result in nucleic acid polymerization; and nucleic acid hybridization.

However, these shared technical features are previously disclosed by US 2015/0292007 A1 to President and Fellows of Harvard College (hereinafter 'Harvard').

Harvard discloses nucleic acid imaging (imaging a template nucleic acid; paragraph [0021]); a labeled or detectable nucleic acid (a labeled or detectable nucleic acid; paragraph [0021]); combining a template (combining a template: paragraph [0065]); a primer (primer; paragraph [0065]); a polymerase (a polymerase; paragraph [0065]); dNTPs (dNTPs; paragraph [0065]), thereby forming a reaction mixture (thereby forming a reaction mixture; paragraph [0065]); and incubating the reaction mixture under conditions that result in nucleic acid polymerization (and incubating the reaction mixture under conditions that result in nucleic acid polymerization; paragraph [0065]); and nucleic acid hybridization (nucleic acid hybridization; abstract).

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 Harvard reference, unity of invention is lacking.