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(54) Title: CTRSD GATE AND PERFORMING CO-TRANSCRIPTIONAL ENCODING

(57) Abstract: A method for the production and use of scalable co-transcriptional RNA strand displacement (ctRSD) circuits using RNA toehold exchange gates is described. The ctRSD circuits described address the limitations of existing DNA-based strand displacement circuits by isothermally producing circuit components via transcription.

CTRSD GATE AND PERFORMING CO-TRANSCRIPTIONAL ENCODING

[0001] This invention was made with United States Government support from the National Institute of Standards and Technology (NIST), an agency of the United States Department of Commerce. The Government has certain rights in this invention.

[0002] The following description cannot be considered limiting in any way. Various objectives, features, and advantages of the disclosed subject matter can be more fully appreciated with reference to the following detailed description of the disclosed subject matter when considered in connection with the following drawings, in which like reference numerals identify like elements.

[0003] FIG. 1 shows an output strand 201, according to some embodiments.

[0004] FIG. 2 shows an output strand 201, according to some embodiments.

[0005] FIG. 3 shows an output strand 201, according to some embodiments.

[0006] FIG. 4 shows an output strand 201, according to some embodiments.

[0007] FIG. 5 shows (A) production of a strand displacement product 216 from a reaction between an input template strand 215.1 and a ctRSD gate 200, wherein strand displacement product 216 and a second input template strand 215.2 is produced; and (B) a serial reaction in which a series of input template strands 215 react with various ctRSD gates 200, wherein the serial reaction is propagated by reactant products (e.g., 215.2, …, 215.5) formed from prior parent ctRSD gates 200 (e.g., 200.1, 200.2, 200.3, 200.4). It should be appreciated that the serial reaction can propagate for an arbitrary number of layers although only four layers are shown.

[0008] A detailed description of one or more embodiments is presented herein and the accompanying parts of the specification by way of exemplification and not limitation.

[0009] Engineered molecular circuits process information in biological systems and can address emerging human health and biomanufacturing needs. A scalable co-transcriptional RNA strand displacement (ctRSD) circuit is rationally

programmed via base pairing interactions. Conventional DNA-based strand displacement circuits can be computationally powerful molecular circuits but are limited in biological systems due to difficulty in genetically encoding components. The ctRSD overcomes this limitation of such conventional technology by isothermally producing circuit components via transcription. The programmability of ctRSD *in vitro* occurs by designing logic and amplification elements and multi-layer signaling cascades. Further, kinetics of ctRSD are predicted by a model of coupled transcription and strand displacement. The ctRSD provides rational design of molecular circuits that operate in biological systems, including living cells.

[0010] It is contemplated that co-transcriptional RNA strand displacement (ctRSD) circuits are scalable and programmable. In ctRSD, circuit components isothermally self-assemble and execute programmed computations in a single transcription reaction. This is achieved through an HDV self-cleaving ribozyme to isothermally prepare kinetically trapped RNA strand displacement intermediates via transcription, and a set of nucleic acid sequence design rules that allow mutiple RNA strand displacement sequences with similar performance to be readily created. The ctRSD overcomes limitations of conventional DNA-based strand displacement such as degradation in biological environments and single-use operation. Moreover, ctRSD provides nucleic acid strand displacement circuits that are genetically encoded into living cells for cellular engineering applications.

[0011] Conventional DNA-based strand displacement circuits are a molecular computing paradigm. However, conventional DNA circuits are susceptible to degradation in biological systems. Further, conventional DNA-based circuits are only single-use, wherein they can only execute one computation unless their components are replenished via external perturbation. Finally, there is currently no mechanism to produce these state-of-the-art circuits in the same sample where they operate.

[0012] Advantageously and unexpectedly, co-transcriptional RNA strand displacement circuits provide powerful computing features of DNA-based circuits and can be genetically encoded to overcome limitations of conventional DNA-based circuits in biological systems. Co-transcriptional RNA strand displacement circuits can be encoded into living cells for the same programmability and functionality of DNA-

based circuits for cellular engineering applications. Co-transcriptional RNA strand displacement circuits provide real-time cell state monitoring through recognition of differential RNA expression patterns. Co-transcriptional RNA strand displacement circuits can provide real-time monitoring of cell-state to improve biomanufacturing processes or for real-time detection of cellular disease states. Nucleic acid pattern recognition has occurred with DNA-based circuits *in vitro* but has never been demonstrated in living cells, something which co-transcriptional RNA strand displacement circuits can provide for engineering cellular sensing and response.

[0013] Co-transcriptional RNA strand displacement circuits can be applied in *in vitro* environments. Co-transcriptional RNA strand displacement circuits can be used in an *in vitro* transcription-based biosensor for detecting water contaminants, wherien such biosensors provide more sophisticated computations to be executed than conventional technology.

[0014] Although DNA-based strand displacement components can expand computational capabilities, such biosensors are often freeze-dried for long-term storage and transport, and a limitation of using DNA-based components in these sensors is that the DNA strand displacement components result in much shorter shelf-lives when freeze dried compared to longer transcription templates or plasmids. For example, the DNA-based components showed significant decrease in performance only one week after freeze drying. In contrast, long linear DNA templates have been shown to be stable for over a month and DNA plasmids containing transcription templates have been shown to be stable for 2 years after freeze drying. Thus, encoding co-transcriptional RNA strand displacement components in long linear templates or in plasmids offers the same functionality as existing DNA circuits but with improved stability in freeze dried samples.

[0015] Certain *in vitro* sensors for detecting viral infections and other diseases operate by detecting specific RNA sequences that then trigger the production of a fluorescent output. Co-transcriptional RNA strand displacement circuits can be an upstream information processing layer in such diagnostics.

[0016] The use of co-transcriptional RNA strand displacement circuits in these diagnostics could enable more complex computations, such as mathematical

operations or neural network pattern recognition. These capabilities could enable more robust and reliable diagnostics by integrating more input information before making a diagnosis.

[0017] Co-transcriptional RNA strand displacement provides sophisticated DNA-based diagnostics to be robustly operated in biological systems. Certain conventional DNA-based molecular neural networks recognize differential gene expression levels associated with cancers, but that circuit has been operated in a pure *in vitro* setting. Using co-transcriptional RNA strand displacement provides this sophisticated diagnostic circuit to robustly operate in blood or fecal samples where DNA-based circuits would be limited by degradation.

[0018] The ctRSD provides a predictive engineering of biology and programmable cellular engineering. Beneficially, modular RNA gates are isothermally produced in a kinetically trapped form in the same reaction vessel. This has not been achieved in conventional DNA-based systems.

[0019] Various embodiments are listed below.

[0020] Embodiment 1. An ctRSD gate 200 for performing co-transcriptional encoding, the ctRSD gate 200 comprising: an output strand 201 comprising: an input branch migration domain 206; an output branch migration domain 204 sequentially connected to the input branch migration domain 206; and an output toehold domain 205 sequentially interposed between the input branch migration domain 206 and the output branch migration domain 204; and a gate prime strand 202 electrostatically associated with the output strand 201 and comprising; a self-cleaving ribozyme 209; an output toehold sequester domain 213 sequentially connected to the self-cleaving ribozyme 209; a substrate domain 211 sequentially interposed between the self-cleaving ribozyme 209 and the output toehold sequester domain 213, such that a portion of the substrate domain 211 is sequentially complementary to a portion of the input branch migration domain 206 that results in the gate prime strand 202 being electrostatically associated with the output strand 201; and an input toehold domain 210 sequentially interposed between the self-cleaving ribozyme 209 and the substrate domain 211,wherein the output strand 201 and the gate prime strand 202 indepedently consist essentially of RNA.

[0021] Embodiment 2. The ctRSD gate 200 of Embodiment 1, wherein the output strand 201 further comprises: a hairpin-forming sequence 203 sequentially connected to the output branch migration domain 204 such that output branch migration domain 204 is sequentially interposed between the hairpin-forming sequence 203 and the output toehold domain 205.

[0022] Embodiment 3. The ctRSD gate 200 of Embodiment 1, wherein the output strand 201 further comprises: an output wobble domain 207 sequentially connected to the input branch migration domain 206 such that the output wobble domain 207 is sequentially interposed between a first portion of the input branch migration domain 206 and a second portion of the input branch migration domain 206.

[0023] Embodiment 4. The ctRSD gate 200 of Embodiment 1, wherein the output strand 201 further comprises: a linker sequence 208 sequentially connected to the input branch migration domain 206 such that input branch migration domain 206 is sequentially interposed between the linker sequence 208 and the linker sequence 208.

[0024] Embodiment 5. The ctRSD gate 200 of Embodiment 1, wherein the gate prime strand 202 further comprises: a transcription termination sequence 214 sequentially connected to the output toehold sequester domain 213 such that output toehold sequester domain 213 is sequentially interposed between the transcription termination sequence 214 and the substrate domain 211.

[0025] Embodiment 6. The ctRSD gate 200 of Embodiment 1, wherein the gate prime strand 202 further comprises: a gate prime wobble domain 212 sequentially connected to the substrate domain 211 such that the gate prime wobble domain 212 is sequentially interposed between a first portion of the substrate domain 211 and a second portion of the substrate domain 211.

[0026] Embodiment 7. The ctRSD gate 200 of Embodiment 1, wherein the output strand 201 produces a strand displacement product 216 in response to contact with an input template strand 215.

[0027] Embodiment 8. The ctRSD gate 200 of Embodiment 1, wherein the output strand 201 further comprises a second input branch migration domain 206.2 sequentially connected to the input branch migration domain 206.

[0028] Embodiment 9. The ctRSD gate 200 of Embodiment 1, wherein the gate prime strand 202 further comprises a second substrate domain 211.2 sequentially connected to the substrate domain 211.

[0029] Embodiment 10. A process for producing a strand displacement product 216, the process comprising: providing a ctRSD gate 200; contacting the ctRSD gate 200 with a input template strand 215; and producing the strand displacement product 216 from the ctRSD gate 200 in response to contacting the ctRSD gate 200 with the input template strand 215.

[0030] Embodiment 11. The process of Embodiment 8, wherein the ctRSD gate 200 comprises: an output strand 201 comprising: an input branch migration domain 206; an output branch migration domain 204 sequentially connected to the input branch migration domain 206; and an output toehold domain 205 sequentially interposed between the input branch migration domain 206 and the output branch migration domain 204; and a gate prime strand 202 electrostatically associated with the output strand 201 and comprising; a self-cleaving ribozyme 209; an output toehold sequester domain 213 sequentially connected to the self-cleaving ribozyme 209; a substrate domain 211 sequentially interposed between the self-cleaving ribozyme 209 and the output toehold sequester domain 213, such that a portion of the substrate domain 211 is sequentially complementary to a portion of the input branch migration domain 206 that results in the gate prime strand 202 being electrostatically associated with the output strand 201; and an input toehold domain 210 sequentially interposed between the self-cleaving ribozyme 209 and the substrate domain 211, wherein the output strand 201 and the gate prime strand 202 indepedently consist essentially of RNA.

[0031] Embodiment 12. The process of Embodiment 9, wherein the output strand 201 further comprises: a hairpin-forming sequence 203 sequentially connected to the output branch migration domain 204 such that output branch migration domain

204 is sequentially interposed between the hairpin-forming sequence 203 and the output toehold domain 205.

[0032] Embodiment 13. The process of Embodiment 9, wherein the output strand 201 further comprises: an output wobble domain 207 sequentially connected to the input branch migration domain 206 such that the output wobble domain 207 is sequentially interposed between a first portion of the input branch migration domain 206 and a second portion of the input branch migration domain 206.

[0033] Embodiment 14. The process of Embodiment 9, wherein the output strand 201 further comprises: a linker sequence 208 sequentially connected to the input branch migration domain 206 such that input branch migration domain 206 is sequentially interposed between the linker sequence 208 and the linker sequence 208.

[0034] Embodiment 15. The process of Embodiment 9, wherein the gate prime strand 202 further comprises: a transcription termination sequence 214 sequentially connected to the output toehold sequester domain 213 such that output toehold sequester domain 213 is sequentially interposed between the transcription termination sequence 214 and the substrate domain 211.

[0035] Embodiment 16. The process of Embodiment 9, wherein the gate prime strand 202 further comprises: a gate prime wobble domain 212 sequentially connected to the substrate domain 211 such that the gate prime wobble domain 212 is sequentially interposed between a first portion of the substrate domain 211 and a second portion of the substrate domain 211.

[0036] The following are incorporated by reference in their entirety.

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[0037] The processes and articles described herein may be embodied in, and fully automated via, software code modules executed by a computing system that includes one or more general purpose computers or processors. The code modules may be stored in any type of non-transitory computer-readable medium or other computer storage device. Some or all the methods may alternatively be embodied in specialized computer hardware. In addition, the components referred to herein may be implemented in hardware, software, firmware, or a combination thereof.

[0038] Many other variations than those described herein will be apparent from this disclosure. For example, depending on the embodiment, certain acts,

events, or functions of any of the algorithms described herein can be performed in a different sequence, can be added, merged, or left out altogether (e.g., not all described acts or events are necessary for the practice of the algorithms). Moreover, in certain embodiments, acts or events can be performed concurrently, e.g., through multi-threaded processing, interrupt processing, or multiple processors or processor cores or on other parallel architectures, rather than sequentially. In addition, different tasks or processes can be performed by different machines and/or computing systems that can function together.

[0039] Any logical blocks, modules, and algorithm elements described or used in connection with the embodiments disclosed herein can be implemented as electronic hardware, computer software, or combinations of both. To clearly illustrate this interchangeability of hardware and software, various illustrative components, blocks, modules, and elements have been described above generally in terms of their functionality. Whether such functionality is implemented as hardware or software depends upon the particular application and design constraints imposed on the overall system. The described functionality can be implemented in varying ways for each particular application, but such implementation decisions should not be interpreted as causing a departure from the scope of the disclosure.

[0040] The various illustrative logical blocks and modules described or used in connection with the embodiments disclosed herein can be implemented or performed by a machine, such as a processing unit or processor, a digital signal processor (DSP), an application specific integrated circuit (ASIC), a field programmable gate array (FPGA) or other programmable logic device, discrete gate or transistor logic, discrete hardware components, or any combination thereof designed to perform the functions described herein. A processor can be a microprocessor, but in the alternative, the processor can be a controller, microcontroller, or state machine, combinations of the same, or the like. A processor can include electrical circuitry configured to process computer-executable instructions. In another embodiment, a processor includes an FPGA or other programmable device that performs logic operations without processing computer-executable instructions. A processor can also be implemented as a combination of computing devices, e.g., a combination of a DSP and a microprocessor, a plurality of microprocessors, one or

more microprocessors in conjunction with a DSP core, or any other such configuration. Although described herein primarily with respect to digital technology, a processor may also include primarily analog components. For example, some or all of the signal processing algorithms described herein may be implemented in analog circuitry or mixed analog and digital circuitry. A computing environment can include any type of computer system, including, but not limited to, a computer system based on a microprocessor, a mainframe computer, a digital signal processor, a portable computing device, a device controller, or a computational engine within an appliance, to name a few.

[0041] The elements of a method, process, or algorithm described in connection with the embodiments disclosed herein can be embodied directly in hardware, in a software module stored in one or more memory devices and executed by one or more processors, or in a combination of the two. A software module can reside in RAM memory, flash memory, ROM memory, EPROM memory, EEPROM memory, registers, hard disk, a removable disk, a CD-ROM, or any other form of non-transitory computer-readable storage medium, media, or physical computer storage known in the art. An example storage medium can be coupled to the processor such that the processor can read information from, and write information to, the storage medium. In the alternative, the storage medium can be integral to the processor. The storage medium can be volatile or nonvolatile.

[0042] While one or more embodiments have been shown and described, modifications and substitutions may be made thereto without departing from the spirit and scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustrations and not limitation. Embodiments herein can be used independently or can be combined.

[0043] All ranges disclosed herein are inclusive of the endpoints, and the endpoints are independently combinable with each other. The ranges are continuous and thus contain every value and subset thereof in the range. Unless otherwise stated or contextually inapplicable, all percentages, when expressing a quantity, are weight percentages. The suffix (s) as used herein is intended to include both the singular and the plural of the term that it modifies, thereby including at least one of that term (e.g., the colorant(s) includes at least one colorants). Option, optional, or optionally means

that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event occurs and instances where it does not. As used herein, combination is inclusive of blends, mixtures, alloys, reaction products, collection of elements, and the like.

[0044] As used herein, a combination thereof refers to a combination comprising at least one of the named constituents, components, compounds, or elements, optionally together with one or more of the same class of constituents, components, compounds, or elements.

[0045] All references are incorporated herein by reference.

[0046] The use of the terms “a,” “an,” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. It can further be noted that the terms first, second, primary, secondary, and the like herein do not denote any order, quantity, or importance, but rather are used to distinguish one element from another. It will also be understood that, although the terms first, second, etc. are, in some instances, used herein to describe various elements, these elements should not be limited by these terms. For example, a first current could be termed a second current, and, similarly, a second current could be termed a first current, without departing from the scope of the various described embodiments. The first current and the second current are both currents, but they are not the same condition unless explicitly stated as such.

[0047] The modifier about used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (e.g., it includes the degree of error associated with measurement of the particular quantity). The conjunction or is used to link objects of a list or alternatives and is not disjunctive; rather the elements can be used separately or can be combined together under appropriate circumstances.

CLAIMS:

1. A method for the production of an RNA toehold exchange gate, that method comprising:

    a. encoding DNA for the production of an RNA toehold exchange gate;

    b. producing the RNA toehold exchange gate by transcription;

    wherein the RNA toehold exchange gate is co-transcriptionally folded into a kinetically trapped intermediate following transcription.

2. A method for using co-transcriptional RNA strand displacement (ctRSD) circuits for the execution of programmable logic, amplification, or cascades, that method comprising:

    a. encoding DNA for the production of a RNA toehold exchange gate;

    b. producing the RNA toehold exchange gate by transcription; and

    c. allowing the RNA toehold exchange gate to bind to a complementary single-stranded input

    wherein the RNA toehold exchange gate is co-transcriptionally folded into a kinetically trapped intermediate following transcription.

3. An RNA toehold exchange gate for use in co-transcriptional RNA strand displacement (ctRSD) circuits, the RNA toehold exchange gate comprising:

    a. a 5' hairpin;

    b. a 3' terminator;

    c. a self-cleaving ribozyme; and

    d. a gate output sequence limited to cystosine, adenine, or uracil bases.

4. A DNA sequence encoding an RNA toehold exchange gate for use in co-transcriptional RNA strand displacement (ctRSD) circuits, the DNA sequence comprising:

5’ hairpin: GGGAGATTCGTCTCCCA;

HDV ribozyme:
TTC
GGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCTACTTCGGTA
GGCTAAGGGAG; and

one T7 terminator selected from the group consisting of:

CTATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG and

ATATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG.

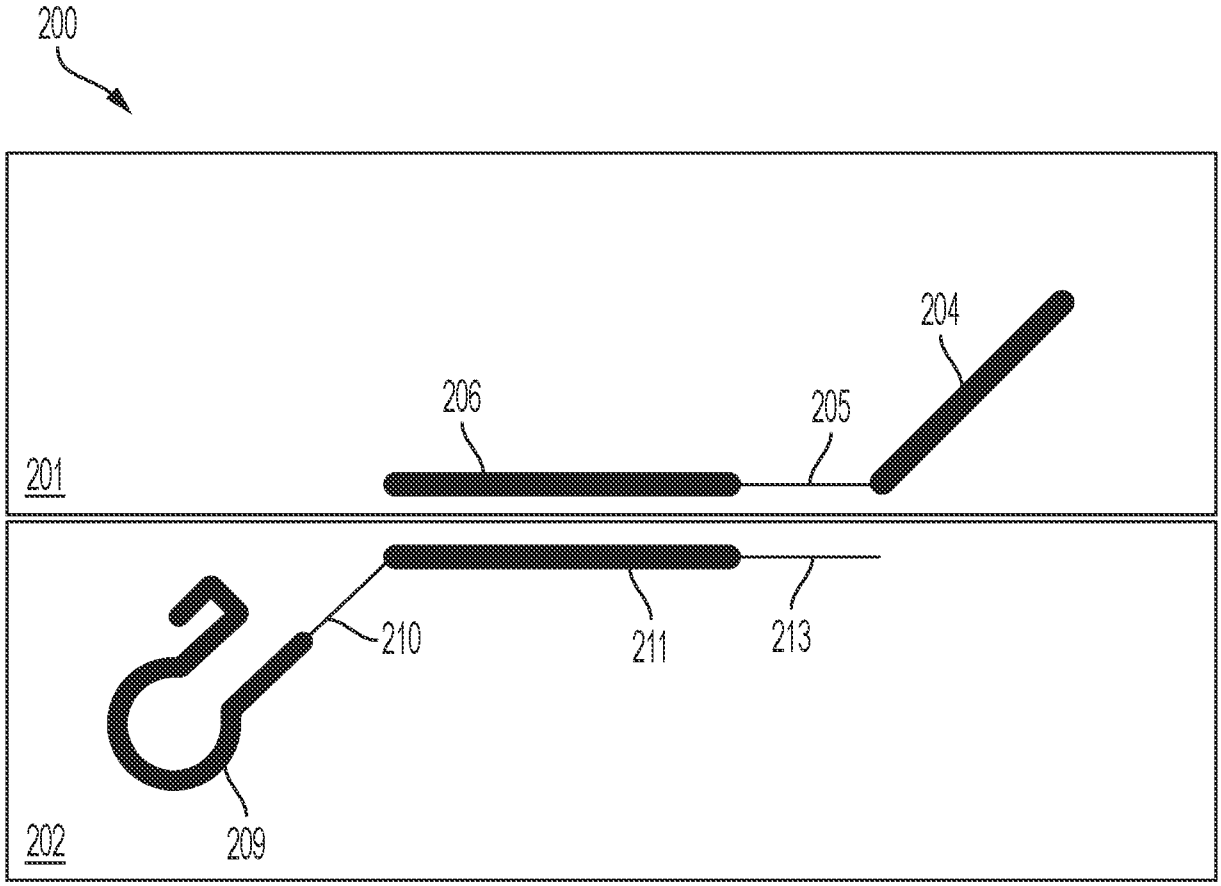

FIG. 1

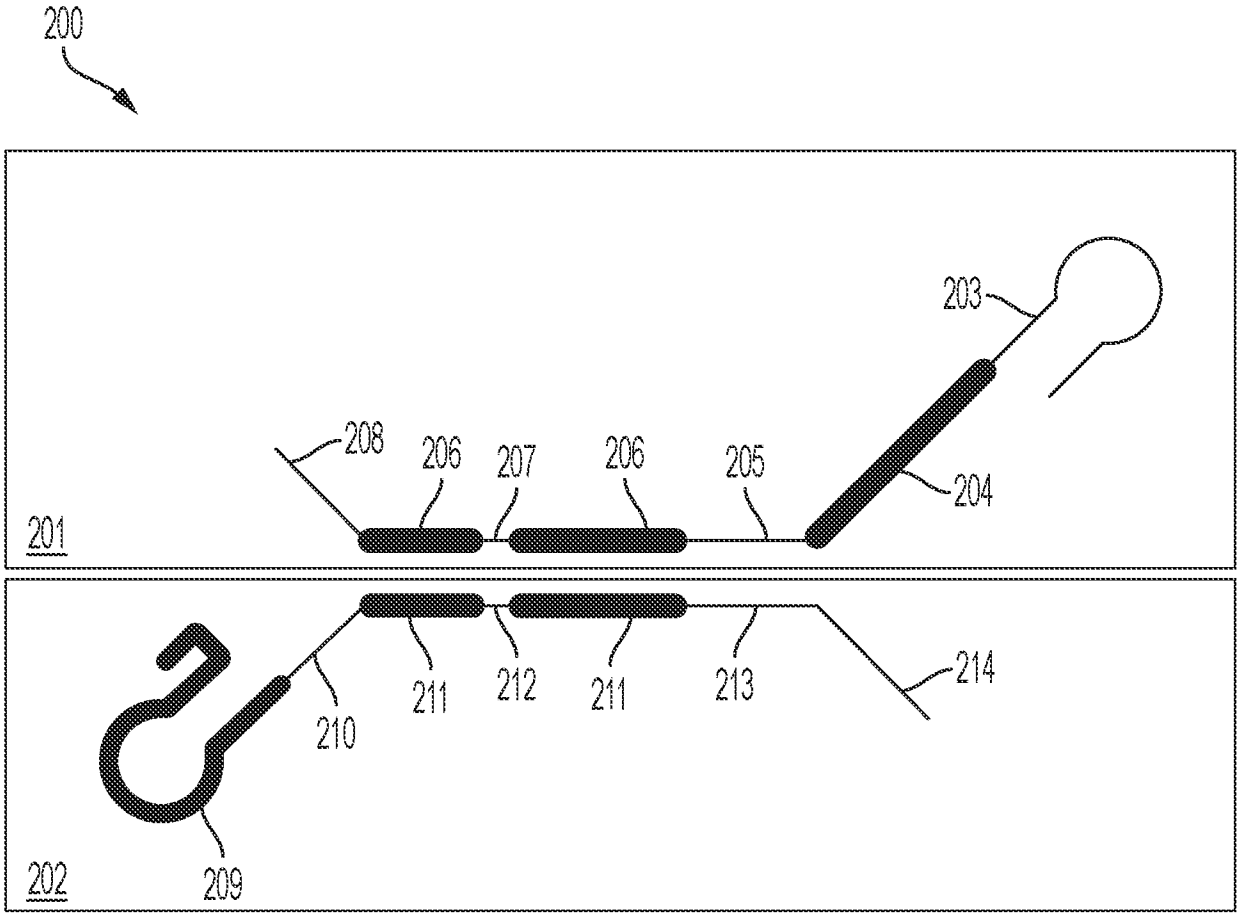
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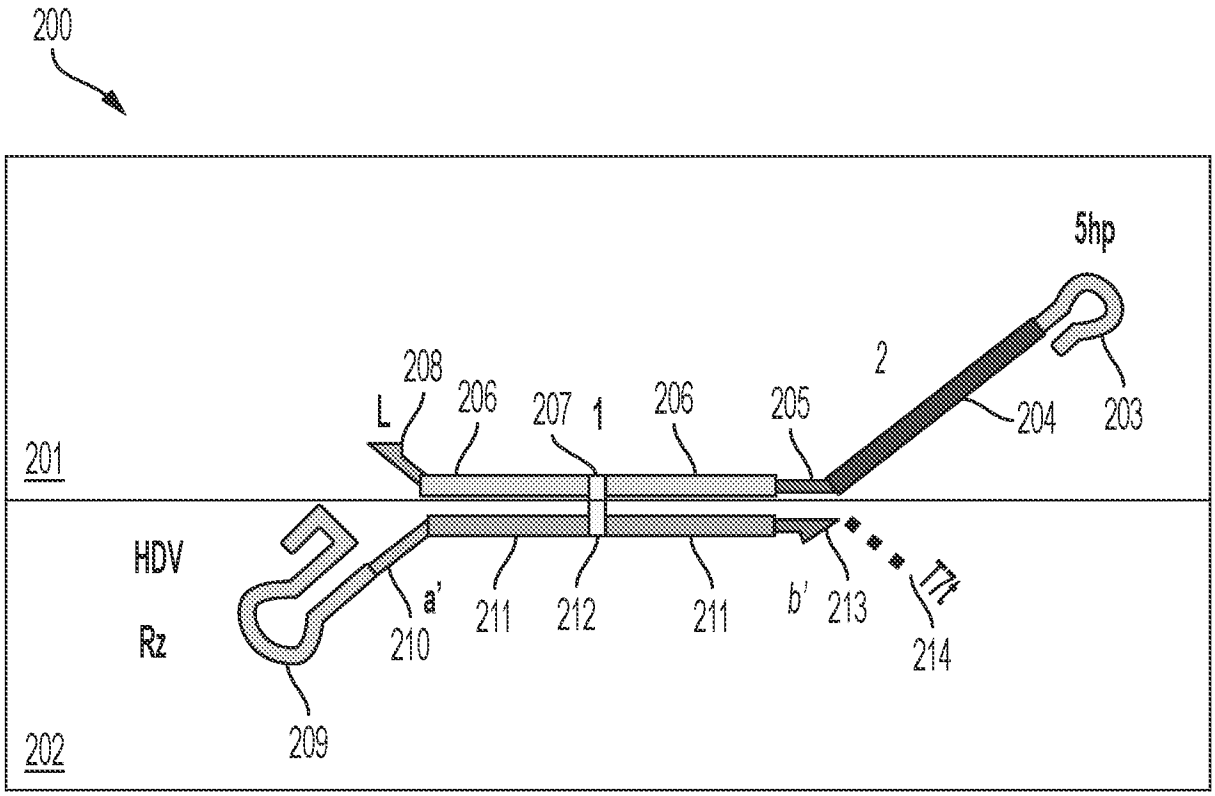
FIG. 2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Tft
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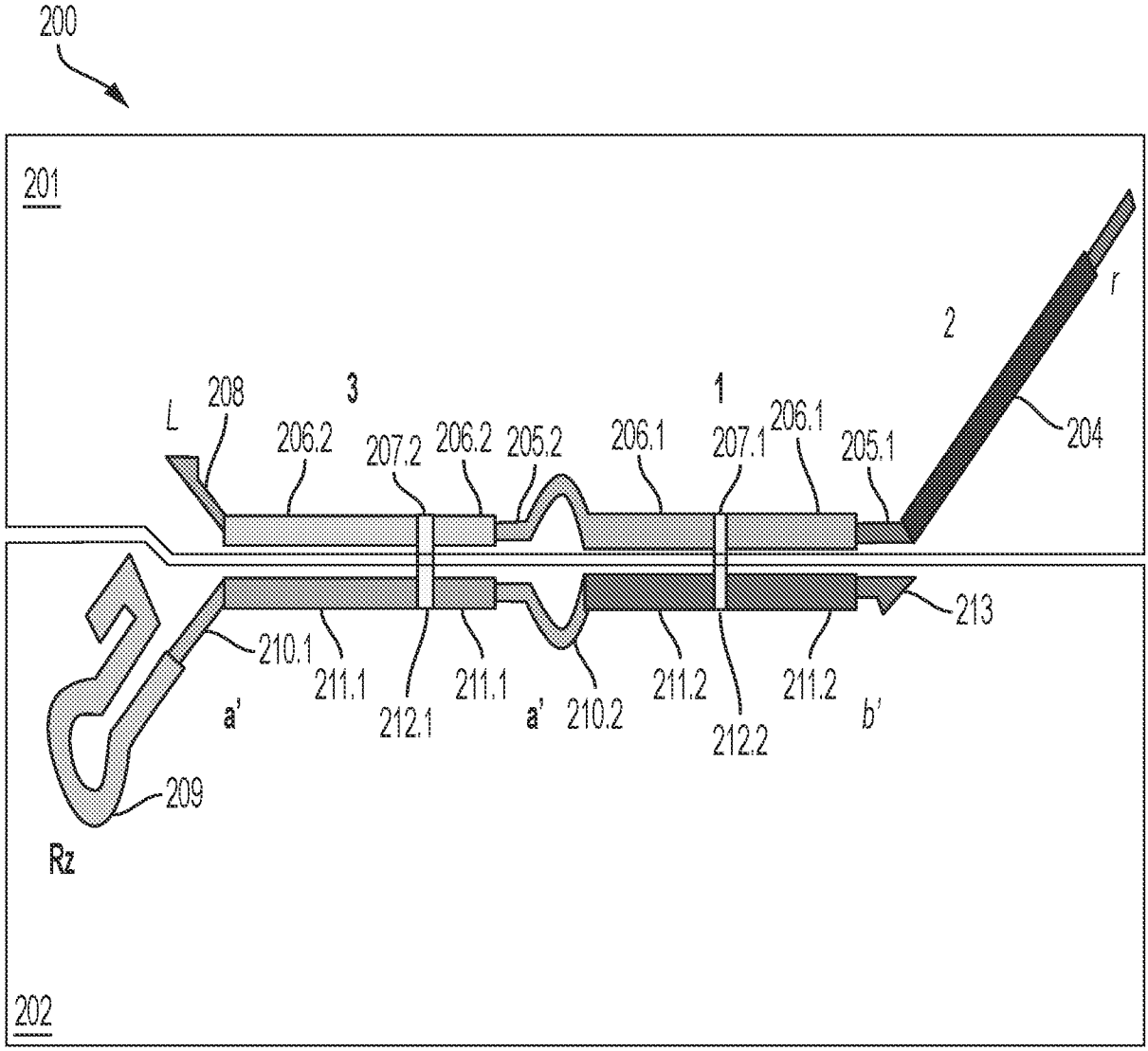
202

FIG. 3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207.1
206.1
205.1
204
213
210.1
211.1
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a'
a'
210.2
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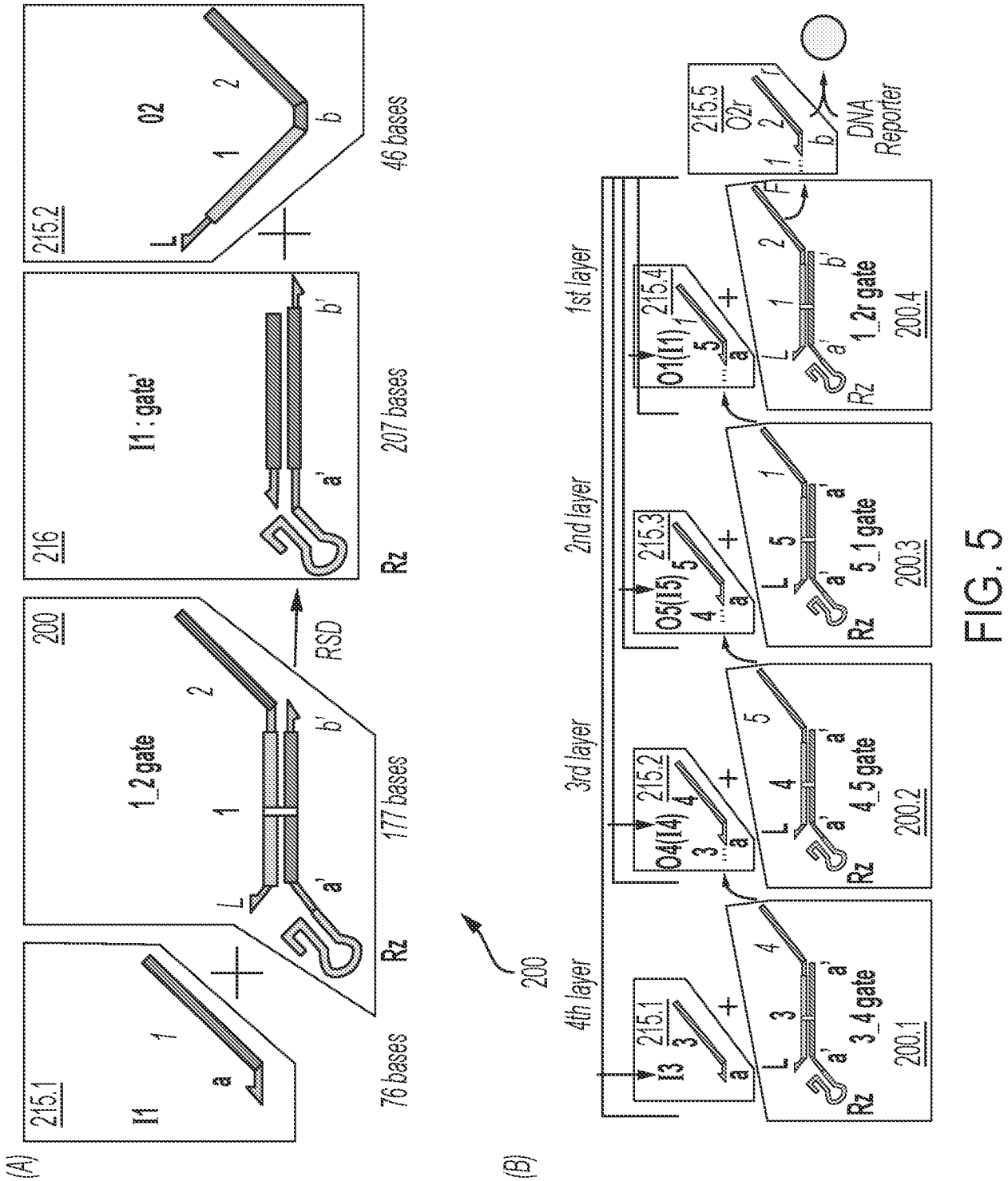
212.2
211.2
b'
209
Rz
202

FIG. 4


(A)
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I1
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a
200
1_2 gate
1
2
L
a'
b'
RSD
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Rz
177 bases
216
I1 ; gate'
L
a'
b'
Rz
207 bases
215.2
02
L
1
2
b
46 bases
(B)
200
4th layer
3rd layer
2nd layer
1st layer
215.1
I3
3
a
3_4 gate
200.1
215.2
O4(I4)
4
3
a
4_5 gate
200.2
215.3
O5(I5)
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a
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200.3
215.4
O1(I1)
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a
1_2r gate
200.4
215.5
O2r
1
2
b
DNA
Reporter

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/053229

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/11 G06N3/123
ADD.

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)
C12N G06N

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)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Schaffter Samuel W. ET AL: "Co-transcriptional RNA strand displacement circuits", bioRxiv, 20 July 2021 (2021-07-20), pages 1-18, XP93041052, DOI: 10.1101/2021.07.20.450530 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2021.07.20.450530v1 [retrieved on 2023-04-21] page 3, paragraph 2 - page 5, paragraph 2; figure 1 page 7, paragraph 4 - page 10, paragraph 1 -/--	1-4

[x] Further documents are listed in the continuation of Box C.

[x] 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

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"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

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Date of the actual completion of the international search	Date of mailing of the international search report
19 May 2023	26/05/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Salminen, Aaro

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& Schaffter Samuel W ET AL: "Co-transcriptional RNA strand displacement circuits (Supplementary Materials)", bioRxiv, 20 July 2021 (2021-07-20), pages 1-53, XP93044072, Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2021.07.20.450530v1 [retrieved on 2023-05-03] table 1	
A	US 2015/275203 A1 (GREEN ALEXANDER A [US] ET AL) 1 October 2015 (2015-10-01) the whole document	1-4
A	KIM JONGMIN ET AL: "De novo-designed translation-repressing riboregulators for multi-input cellular logic", NATURE CHEMICAL BIOLOGY, NATURE PUBLISHING GROUP US, NEW YORK, vol. 15, no. 12, 4 November 2019 (2019-11-04), pages 1173-1182, XP036927810, ISSN: 1552-4450, DOI: 10.1038/S41589-019-0388-1 [retrieved on 2019-11-04] the whole document	1-4
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A	FAN HONG ET AL: "Strand displacement: a fundamental mechanism in RNA biology?", ARXIV.ORG, CORNELL UNIVERSITY LIBRARY, 201 OLIN LIBRARY CORNELL UNIVERSITY ITHACA, NY 14853, 28 March 2019 (2019-03-28), XP081159441, the whole document	1-4

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/053229

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SULC PETR ET AL: "Modelling Toehold-Mediated RNA Strand Displacement", BIOPHYSICAL JOURNAL, ELSEVIER, AMSTERDAM, NL, vol. 108, no. 5, 10 March 2015 (2015-03-10), pages 1238-1247, XP029202035, ISSN: 0006-3495, DOI: 10.1016/J.BPJ.2015.01.023 the whole document	1-4

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/053229

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 sequence listing:

   a. ☐ forming part of the international application as filed.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      ☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/053229

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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