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(71) Applicant: ISIS INNOVATION LTD. [GB/GB]; Ewert House, Ewert Place, Summertown, Oxford, Oxfordshire OX2 7SG (GB).

(72) Inventor: DUNN, Katherine; c/o ISIS Innovation Ltd, Ewert House, Ewert Place, Summertown, Oxford, Oxfordshire OX2 7SG (GB).

(74) Agent: FRESH IP; St. John's Innovation Centre, Cowley Road, Cambridge CB4 0WS (GB).

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(54) Title: ROTARY DNA MOTOR

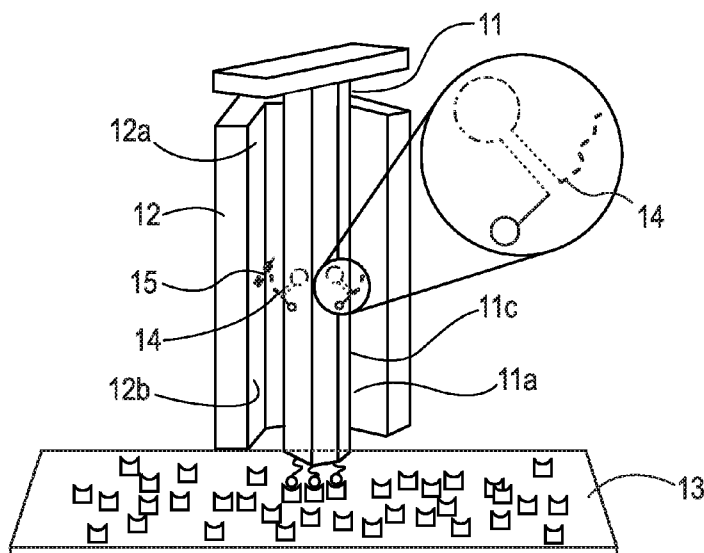


FIG. 1B

(57) Abstract: A rotary DNA motor comprising a static element, a rotary element rotatably movable relative to the static element, a binding domain provided on one of the static element and the rotary element, and a binding component provided on the other

[Continued on next page]



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Title: Rotary DNA Motor**Field of the Invention**

[0001] The present application relates to a rotary DNA motor, a method for forming a DNA motor, and a method of operating a rotary DNA motor

Background to the Invention

[0002] It is known to construct active devices from deoxyribonucleic acid (DNA), in which the active device is able to move or operate in some way. The devices offer the prospect of extremely small actuators or motors for micro or nano-scale devices.

[0003] Originally, linear DNA motors were described which travel along a track constructed from DNA duplexes or tiles, stepping from one site to the next. Various two-footed "walkers" have been developed, and generally inspired by the naturally occurring molecular walkers myosin and kinesin. Early examples of bipedal walkers were non-autonomous, in that the manual addition of 'set' and 'unset' DNA strands was required to cause the motor to progress. It has been shown that a single strand of DNA can be caused to step from one "state" to the next by using an enzyme to cut the stator-motor duplex such that a short piece of the stator dissociates, leaving a single-stranded toehold on the motor, which can then step to the next stator.

[0004] A particular example of a linear motor is that of an autonomous bipedal walker fuelled by DNA hairpins. DNA hairpins are loops of DNA formed by a strand of DNA with complementary domains at or towards each end. The complementary domains combine, forming a neck of the hairpin, and defining a loop from the portion of the strand between the two complementary domains. When a hairpin encounters a longer strand of DNA which is complementary to at least one of the neck domains, the hairpin can be opened, revealing the domains within the loop. This occurs because the longer bound domains will have a lower energy configuration than that of the closed loop. This phenomenon can be made more effective by providing an external domain, or 'toehold', extending beyond the neck and which is complementary to a domain on the opening strand, such that the neck domain and toehold domain corresponds to a continuous domain in the displacing strand.

[0005] In one example of a linear motor, a track was formed from a single linear strand of DNA, and the walker was initially in a configuration where both feet of the walker were bound. A short domain at the back of the front foot competed with an identical domain at the front of the rear foot, and

ultimately the domain from the rear foot was displaced, opening a loop on the rear foot and revealing the binding component for the toehold of a fuel hairpin. As the hairpin bound, it displaced the track and caused the foot to unbind. This revealed a binding component on the first fuel hairpin for a toehold of a second fuel hairpin and this provided a mechanism for removing the fuel from the foot to enable the foot to rebind.

[0006] A desirable modification of DNA motors would be to provide a rotary motor, analogous to conventional macro-scale motors. Attempts have been made to form DNA-based motors which provide some rotary motion. It is known for example to form a DNA tile which undergoes a confirmation change involving rotation on the addition of 'fuel' strands and 'set' strands of DNA. However, this simply results in the component flipping and then returning to the original state in a reverse direction, such that there is no complete rotation. A rotary motor is also known based on a DNA catenane. A catenane is two interlinked rings of DNA, and in the example, was selectively driven between three different configurations by the addition of mercury or cysteine, and changes in pH. The device could be cycled from state to state but does not correspond to a completely autonomous rotary motor.

[0007] It will be clear that known DNA rotary motors are not satisfactory for a number of reasons. Ideally, a rotary motor must be able to step continuously between states and ultimately, return to a starting state, to allow continual operation. In linear DNA motors, directionality is frequently assured using a "burnt-bridges" mechanism in which the track is destroyed by the motor as it steps to a next binding component. Clearly, this approach would not be appropriate for a rotary motor. It is desirable that a motor will operate continually, in the same direction, and autonomously, requiring no more input than an appropriate supply of fuel.

Summary of the Invention

[0008] According to the first aspect of the invention there is provided a rotary DNA motor comprising a static element, a rotary element rotatably movable relative to the static element, a binding domain provided on one of the static element and the rotary element, and a binding component provided on the other of the static element and the rotary element, the binding component may comprise a first DNA hairpin, the binding domain being connectable to the binding component to cause relative rotation of the rotary element and static element, the binding domain being releasable from the binding component by a release component.

[0009] The binding domain may be directly connectable to the binding component, and the release component may bind to the binding component to release the binding domain.

[0010] The release component may comprise a second DNA hairpin which binds to the binding component to release the binding domain.

[0011] The release component may be releasable from the binding component by a second release component such that the binding component may be available for subsequent adhesion to a binding domain.

[0012] The second release component may comprise a third DNA hairpin.

[0013] The rotary DNA motor may comprise a plurality of binding components, and preferably at least 3 binding components

[0014] The binding domain may be connectable to the binding component by a separate linker.

[0015] The release component may bind to the linker to detach the linker from the binding domain and binding component.

[0016] The rotary DNA motor may comprise a fuel element, comprising the release element and a further, different, linker to bind to a different binding domain and binding component.

[0017] The binding component may comprise a plurality of hairpins and defining a plurality of binding sites.

[0018] The rotary DNA motor may comprise a plurality of binding domains, each binding domain having a different capture domain.

[0019] Each linker may bind to a corresponding binding site on the binding component and a different binding domain.

[0020] At least one of the rotary element and the static element may be made from DNA origami.

[0021] One of the static element and the rotary element may comprise a hollow tube and the other of the static element and the rotary element may comprise an elongate rod received within the hollow tube.

[0022] The hollow tube may have a suitable diameter to admit the release component.

[0023] The static element may comprise the elongate rod and the rotary element may comprise the hollow tube.

[0024] The rotary element may rotate relative to the static element when the rotary DNA motor is supplied with release components.

[0025] According to a second aspect of the invention there is provided a method of forming a rotary DNA motor, comprising providing a static element and a rotary element, engaging the static element and rotary element such that the rotary element may be rotatably movable relative to the static element, providing a binding domain on one of the static element and the rotary element, and providing a binding component on the other of the static element and the rotary element, the binding component comprising a first DNA hairpin, the binding domain being connectable to the binding component to cause relative rotation of the rotary element and static element, the binding domain being releasable from the binding component by a release component.

[0026] The method may comprise the step of attaching the static element to a support element.

[0027] The method may comprise attaching the static element to the rotary element with an initial anchor, and, after assembly of the motor, breaking the initial anchor.

[0028] The initial anchor may be a double-stranded DNA linker where one strand may be connected to the rotary element and the other end may be connected to the static element.

[0029] The method may comprise blocking the binding domain during assembly, and, after assembly, unblocking the binding domain.

[0030] The method may comprise forming at least one of the static element and the rotary element using DNA origami.

[0031] The method may comprise forming one of the static element and the rotary element as a hollow tube and forming the other of the static element and the rotary element as an elongate rod received within the hollow tube.

[0032] The static element may comprise the elongate rod and the rotary element may comprise the hollow tube.

[0033] The method may comprise providing a plurality of binding components, and preferably at least 3 binding components.

[0034] The method may comprise providing the binding component on the static element and providing the binding domain on the rotary element.

[0035] According to a third aspect of the invention there is provided a method of operating a rotary DNA motor, comprising providing a rotary DNA motor according to the first aspect of the invention or forming a rotary DNA motor according the method of the second aspect of the invention, and supplying release components to the rotary DNA motor.

Brief Description of the Drawings

[0036] Embodiments of the invention are described by way of example only with reference to the accompanying drawings, wherein;

[0037] Figure 1a is a diagrammatic illustration of the components of a rotary DNA motor embodying the present invention,

[0038] figure 1b is a partially cutaway view of the assembled components,

[0039] figure 2a is an illustration of a binding domain for use with the motor of figure 1b,

[0040] figure 2b is an illustration of a binding component for use with the motor of figure 1b,

[0041] figure 2c is a diagrammatic illustration of a first release component to use with the motor of figure 1b,

[0042] figure 2d is a diagrammatic illustration of a second release component to use with the motor of figure 1b,

[0043] figure 3a is an illustration of the motor of figure 1b in a first operating condition,

[0044] figure 3b is a diagrammatic illustration of the motor of figure 1b in a second operating condition,

[0045] figure 3c is a diagrammatic illustration of the motor of figure 1b in a third operating condition,

[0046] figure 3d is a diagrammatic illustration of the waste product from the operation steps of figures 3c,

[0047] figure 4 is an illustration of the operating states of figures 3a to 3c,

[0048] figure 5a is a cross-sectional view of the rotor showing the rotor design,

[0049] figure 5b is an illustration of a hexagonal unit of DNA helices used to assemble the rotor and axle,

[0050] figure 5c is a top view of the cross bar of the axle of the motor of figure 1b,

[0051] figure 6a is an end view of a part of the rotor of figure 5a,

[0052] figure 6b is a perspective view of the part of figure 6a,

[0053] figure 7a is a view of part of the axle of the motor figure 1b,

[0054] figure 7b is a perspective view of the part of figure 7a,

[0055] figure 8 is a partially cutaway perspective view of the DNA motor, similar to figure 1b but during assembly of the motor,

[0056] figure 9a is a diagrammatic illustration of the components of a further rotary DNA motor embodying the present invention,

[0057] figure 9b is a diagrammatic illustration of a binding component of the motor of figure 9a,

[0058] figure 10 is a diagrammatic illustration of a binding component and binding domain of the further rotary DNA motor of figure 9a in a first operating condition,

[0059] figure 11 is a diagrammatic illustration of a binding component and binding domain of the further rotary DNA motor of figure 9a in a second operating condition,

[0060] figure 12 is a diagrammatic illustration of a binding component and binding domain of the further rotary DNA motor of figure 9a in a third operating condition,

[0061] figure 13 is a diagrammatic illustration of a first stage of the interaction of a fuel component with the rotary DNA motor to cause the motor to move between the first operating condition and second operating condition,

[0062] figure 14 is a diagrammatic illustration of a second stage of the interaction of a fuel component with the rotary DNA motor to cause the motor to move between the first operating condition and second operating condition,

[0063] figure 15 is a diagrammatic illustration of a first stage of the interaction of a further fuel component with the rotary DNA motor to cause the motor to move between the second operating condition and third operating condition, and

[0064] figure 16 is a diagrammatic illustration of a first stage of the interaction of a yet further fuel component with the rotary DNA motor to cause the motor to move between the third operating condition and first operating condition.

Detailed Description of the Preferred Embodiments

[0065] With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0066] Before explaining the embodiments of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is applicable to other embodiments or of being practised or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[0067] In the figures, equivalent DNA domains or sections, and their complements, are shown with consistent line styles.

[0068] Referring to figures 1a and 1b, a first embodiment of a rotary DNA motor embodying the present invention is generally illustrated at 10. The motor comprises a static element, in this example axle 11, and a rotary element, this example comprising rotor 12 in the form of an elongate tube. The axle 11 has a pin 11a and a crossbar 11b at a free end thereof to retain the rotor 12 in engagement with the axle. As shown in figure 1b, the axle 11 is mounted on a surface component 13, such that the axle 11 passes through the bore 12a of the tubular rotor 12. The rotor 12 is able to rotate about its longitudinal axis about axle 11 as will be described in more detail below.

[0069] A binding component 14 is located on an outer surface 11c of the pin 11a of the axle 11, and a binding domain 15 is provided on an interior surface 12b of the rotor 12. As will be described in more detail below, the rotor 11 has a plurality of binding components 14 located thereon, and the interaction of the binding domain 15 and the various binding components 14, mediated by first and second release components, will cause the rotor 12 to rotate about the axle 11. The axle 11 and rotor 12 are assembled using standard DNA origami synthesis techniques as described in more detail below.

[0070] In this embodiment, the binding component 14 defines a binding site, corresponding to those domains of the DNA hairpin which bind to the binding domain 15. As discussed with reference to the second embodiment below, the binding component may have a plurality of DNA hairpins, and the binding site may be defined by domains across more than one DNA hairpin.

[0071] Referring now to figures 2a to 2d, the binding domain, binding component, first release component and second release component are shown in figures 2a to 2d respectively. To illustrate the relationship between the various domains of each element, corresponding domains are indicated by the same letter, while their complements are shown by the same letter marked with an asterisk. As shown in figure 2a, the binding domain 15 has a first domain 15a and a second domain 15b. As illustrated in figure 2b, the binding component 14 is a first DNA hairpin and comprises a first domain 14a, a second domain 14b, a third domain 14c, a fourth domain 14d, a fifth domain 14e and a sixth domain 14f. The first domain 14a is the complement of the first domain 15a of the binding domain. The second and fifth domains 14b, 14e are complementary, and form a neck 14g of the binding domain. Domains 14c to 14d form a loop 14h of the DNA hairpin. Second domain 14b is complementary to second domain 15b of the binding component 15. Accordingly, it will be apparent that the entire length of first and second domains 14a, 14b together is complementary to the entire length of binding domain 15. Domain 14f, here represented by a domain E, is a single-stranded linker which should have no secondary structure, but should be both short and flexible, such as TTTT. The sixth domain 14f serves to connect the binding component 14 to a surface 11c of the axle 11.

[0072] As shown in figure 2c, the first release component comprises a second DNA hairpin generally shown at 16. The DNA hairpin comprises a first domain 16a, a second domain 16b, a third domain 16c, a fourth domain 16d, a fifth domain 16e, and a sixth domain 16f. As can be seen from the domain labels, first domain 16a is the same as third domain 14c of the binding component 14, and is complementary to the fifth domain 16e, shown by labels C, C*, which together form a neck 16g of the second DNA hairpin 16. Sixth domain 16f provides a toehold and is the complement of the fourth

domain 14d of binding component 14, as shown by labels D, D*. Domains 16b, 16c and 16d form loop 16h of the DNA hairpin.

[0073] Finally, as shown in figure 2d, second release component 17 in this example comprises a third DNA hairpin. The third DNA hairpin 17 comprises a first domain 17a, a second domain 17b, a third domain 17c, a fourth domain 17d, a fifth domain 17e and a sixth domain 17f. Domains 17a and 17e are complementary, as shown by labels F, F* and form the neck 17g of the third DNA hairpin 17. Domains 17b to 17d form a loop 17h of the third DNA hairpin 17. Second domain 17b is complementary to the sixth domain 16f of the second DNA hairpin 16 as shown by labels D, D*. Third domain 17c is complementary to fifth domain 16e of the second DNA hairpin 16. Fourth domain 17d is complementary to the fourth domain 16d of the second DNA hairpin 16. Fifth and sixth domains 17e, 17f are complementary to second and third domains 16c, 16b of the second DNA hairpin 16. It will thus be apparent that there are substantial proportions of the second and third DNA hairpins 16, 17 which are complementary, that is the second to sixth domains 16b to 16f of the second DNA hairpin are complementary to the sixth to second domains 17f to 17b of the third DNA hairpin 17. Although the second release component used here is a DNA hairpin, it may comprise a different structure as appropriate, such as a duplex with an unpaired overhang to provide a toehold to permit binding to the first release component.

[0074] The binding domain 15, binding component 14 and first and second release components 16, 17 interact as illustrated in figures 3a to 3d. Referring to figures 3a to c, these diagrams each show a general cross-sectional view through the axle 11 and rotor 12. In these diagrams, binding components 14 are provided at three locations on the axle 11, each comprising a first DNA hairpin in an initial closed state. In figure 3a, binding domain 15 is free, that is unattached to any of the binding components 14.

[0075] As illustrated in figure 3b, once the binding domain 15 is in a position close to a binding component 14, the binding domain 15 is then able to hybridise with the binding component 14. As the first and second domains 15a, 15b of the binding component 15 are complements of the first and second domains 14a, 14b of the binding component 14, the first and second domains of the binding domain 15 and binding component 14 bind, displacing the fifth domain 14e from the second domain 14b and opening the loop 14h of the binding component 14.

[0076] As the loop 14h is opened, it exposes the third and fourth domains 14c, 14d. As will be apparent, these are complementary to the fifth and six domains 16e, 16f of the second DNA hairpin of the first release component 16. This reveals a toehold for the first release component 16. As

shown in figure 3c, the first release component 16 thus binds to the binding component 14, such that the sixth, fifth and fourth domains 16f, 16e, 16d bind with the complementary domains 14d, 14c, 14b on the binding component 14, displacing the rotor domain 15 and freeing the binding component 14 from the rotor domain 15. Accordingly, the connection between the axle 11 and rotor 12 is broken, freeing the binding domain 15 to rotate into proximity to another binding component 14.

[0077] Movement of the axle 11 and rotor 12 when the binding domain is unbound is caused by Brownian motion. Thermal fluctuations cause the rotor to move sufficiently to bring the binding domain 15 close to the next binding component 14, where the binding and release steps described above occur. Advantageously, it will be apparent that the previous binding component 14 is blocked for immediate rebinding to the binding domain 15, due to the adhesion between the binding component 14 and first release component 16. However, once the binding domain 15 has moved on a subsequent binding component, it is necessary to free the binding component 14 for subsequent reuse. To do so, a second release component comprising third DNA hairpin 17 is provided. As illustrated in figure 3c, as the loop 16g of the first release component 16 is open, domains 16b, 16c of the first release component are exposed to provide a toehold for binding to the second release component 17. First and second release components 16, 17 are substantially complementary along the whole of their length, with the exception of end domains 16a, 17f and accordingly first and second release components bind to one another, displacing the binding component 14 from the first release component 16. As illustrated in figure 3d, this results in a waste component generally shown at 18, which comprises one strand of a first release component 16 and a strand from a second release component 17 where the strands have been opened and are bound substantially along their entire length. As there is no more advantageous binding available at (i.e. all available possible binding lengths are shorter) this waste product will not substantially interact with any of the components or elements of the motor and can be removed. The released binding component 14 is then able to revert to its hairpin configuration where domains B, B*, i.e. domains 14b, 14e, bind to form the neck 14g.

[0078] Accordingly, it will be apparent that this sequence of operations enables the binding domain 15 to move to successive binding components 14. The binding domain is then released from that binding component and the binding component is subsequently made available for subsequent connection to the binding domain. The provision of a succession of binding components around the axle 11, preferably at least three sites to allow the three-step operation described above, allows the binding domain to successively connect to subsequent binding components, and when the rotor has

a completed a circuit, the initial binding component will be available to bind to the binding domain. The rotor 12 will thus move around the axle 11 in a continuous manner, providing only that there is an adequate supply of first and second release component 16, 17 to allow the process illustrated in figures 3a to 3c to continue.

[0079] It is known that the speed at which strands of DNA bind to one another is dependent on the number of complementary nucleotides in the respective strands. This phenomenon can be used to control the relative rates of the three reaction process steps illustrated in figures 3a to 3c. In particular it is the length of domains A, D* and G that control the rates of the processes in figures 3a, 3b and 3c respectively. Ideally the binding domain 15 should bind to the binding component 14 quickly. The reason for this is that it is desirable that the binding domain 15 and binding component 14 bind as quickly as possible to maximise the likelihood that the two components will bind when they are physically close, which may be a comparatively short time. The first release component 16 should displace the binding domain 15 from the binding component 14 at a relatively slow speed, and the second release component 17 should displace the first release component 16 from the binding component 14 in a still slower manner. The speed of these steps are preferably relatively slow so that the binding domain 15 has continued on to the next binding component before the previous binding component is available for rebinding. The rate of strand displacement as a function of toehold length varies by approximately an order of magnitude for low nucleotide numbers, and in the present example domains A, D and G and their complementary domains have lengths of five, four and two nucleotides respectively. The relative lengths of these strands thus drive the rotation of the rotor.

[0080] Example sequences for the domains are illustrated in table 1. Sequences in this table were selected by randomly generating 30 twelve-base components and preserving those with 50 percent GC content. 30 five-base sequences were also generated without constraints on the GC content. Three non-interacting strands were identified, where sequences were regarded as orthogonal and independent if fewer than 2% interacted incorrectly.

Domain	Length	Sequence
A	5	AGCGT
B	12	GGGTGTGATAAG
C	12	GTCTTTGTTGGG
D	4	TTCA
E	4	TTTT
F	12	ACAGGCATTGTC
G	2	AG

Table 1: example sequences for domains

[0081] It is desirable that substantially the only reactions that occur are those described above, and the reverse reactions occur at a negligible rate, and also that the rate of leak reactions is negligible. Leak reactions may occur if DNA hairpins are opened as a result of strands binding to toeholds within the loop, but these reactions should be 10 to 100 times slower than the intended reaction. If this is not sufficiently slow, the efficiency of the internal toehold could be reduced by moving two of the bases into the neck of the hairpin. Kissing interactions between the loops of hairpins may also interfere with successful operation of the motor and it might prove necessary to suppress these by adding short splints inside the hairpin. Other strategies may be adopted to minimise unwanted interactions between strands or hairpins such as including weak secondary structures within the loops of the DNA hairpins or the inclusion of mismatches to disrupt the formation of unwanted complexes.

[0082] The relative timing of the operations of the binding components in the rotation of DNA motor 10 is illustrated in figure 4. The state of each of three binding components is illustrated in the triangular diagram at the top of the figure, where a solid circle represents the binding component being bound to the binding domain, a circle and cross indicates that the binding component is

unbound from the binding domain but unavailable (i.e. is in the configuration shown in figure 3c), and where an open circle shows that the binding component is not bound to the binding domain and is available, i.e. after the first release component has been unbound from the binding component by the second release component. The bars show the relative state of each binding component throughout the cycle. As illustrated by the lowest graph, there is a short transition time between the binding domain and binding component being unbound, and the binding domain binding to the next available unbound binding component. The relatively long third process step shown in figure 3c thus prevents the binding domain binding to an unwanted binding component, as it ensures that only the desired binding component is available.

[0083] The overall geometry of the motor 10 and the various components is shown in figures 5a to 7b. Figures 6a to 7b in particular show the assembly of the rotor 12 and axle through DNA origami synthesis, where each circle or rod 30 is a DNA helix. In the rotor 12, as illustrated in figure 5a, the largest dimension of the bore 12a in the centre is $13H - 2r \approx 50$ nm, where H is the distance between the centres of two helices diametrically opposite each other in a hexagonal unit as illustrated at 31 in figure 5b, ≈ 4 nm, and where r is the radius of a helix (≈ 1 nm). The cross-bar 11b of the axle 11 is designed to be $10H + 18r \approx 58$ nm as illustrated in figure 5c; for the rotor to escape, the two components would have to tilt at a relative angle of around 37° . The size of the hole 12a is determined with reference to the maximum size of the complex formed by the release component hairpins 16, 17 with the binding components 14. This will by definition be smaller than the waste product, which consists of one double-stranded 2 base-pair domain, three double-stranded 12 base-pair domains, one double-stranded 4 base-pair domain, and two single-stranded 12 base domains (as shown in figure 3d). The double stranded section would be approximately 14 nm long, and this can be used as an estimate for the space required within the rotor cavity on either side of the axle pin. The axle 11 itself measures $3H/2 + 2r \approx 8$ nm across, and hence the internal cavity is designed to be slightly larger than $2 \times 14 + 8 = 36$ nm in size.

[0084] Both parts of the motor - rotor 12 and axle 11- would be based on a hexagonal lattice. Assembly of the rotor could be accomplished most effectively by connection of three identical units 32 as shown in figures 6a and 6b. A scaffold is routed along 54 helices, to be connected by linkers between helix 32a and helix 32b. Staples on helix 32a would be extended with a sequence A, while staples on helix 32b would carry the sequence A*. Hybridization of these two domains would join together the units of the rotor. The length of scaffold needed to make a single unit is around 3200 bases, which suggests that a customized plasmid should be used.

[0085] A schematic diagram of the axle 11 is shown in figures 7a and 7b, illustrating the overall shape. The required scaffold length is around 2700 bases, again requiring a custom plasmid. The axle pin 11a is designed to project below the cross-bar 11b, exceeding the height of the rotor 12. The staple layout of both rotor 12 and axle 11 would be determined as necessary.

[0086] The assembly of the axle 11 and rotor 12 would then proceed as follows, with reference to figure 8. The binding domain 15 would be blocked so it could not bind to binding components 14 on the axle 11. The rotor 12 is attached to the axle 11 by an anchor 40 which would prevent relative rotation. In this example, the anchor 40 is a double-stranded DNA link consisting of one strand connected to the rotor 12 and its complement connected to the axle 11. The strand on the axle is terminated with a few bases which are not connected to the strand on the rotor 12 to provide a toehold to enable the initial anchor 40 to be released. With the rotor 12 and axle 11 locked together in this manner, the axle would be mounted on surface 13 and connected thereto by suitable linkers 41, in this case streptavidin-biotin linkers, or by sulphur bonds to a gold layer on surface 13. The initial anchor 40 is then released, for example by using a DNA-cutting enzyme or by using DNA strand displacement using a suitable strand.

[0087] Finally, to enable the motor to rotate, a trigger strand is added to the system to unblock the binding domain 15, thus allowing the binding domain to hybridise with binding components 14 on the axle 11 and enabling the motor to operate as discussed above.

[0088] A second embodiment of a rotary DNA motor is shown in figures 9a to 16. The second embodiment is more complex than the embodiment of figures 1a to 8, but ensures that the motor will rotate in a selected direction.

[0089] Referring now to figures 9a and 9b, an axle is shown at 111 and a rotor is shown at 112. The axle and rotor 112 may be formed in like manner to axle 11 and rotor 12 described above. The axle 111 has a pin 111a and a crossbar 116 at an end thereof to retain the rotor 112 on the axle 111. In place of binding domain 15, the rotor 112 has a plurality of binding domains or capture units 115. Each capture unit comprises a unique capture domain 115a, 115'a, 115''a, connected to the rotor 112 by a connector 115b. In the present example, three binding domains or capture units 115 are provided on the rotor 111, with respective capture domains 115a, 115'a, 115''a denoted by d_1 , d_2 , d_3 .

[0090] A binding component 114 is provided on the axle 111a. Unlike the binding components 14 of the first embodiment, which comprise a DNA single hairpin, the binding component 114 comprises three distinct hairpins here shown at 120, 121, 122. Each hairpin 120, 121, 122 comprises four

domains, two complementary domains which form the neck of the hairpin, a domain that forms the loop of a hairpin and a fourth connection domain which is attached to a rigid connector 123a, 123b, 123c which joins the respective hairpin to the axle 111.

[0091] Accordingly, first hairpin 120 comprises first domain 120a, second domain 120b, third domain 120c and fourth domain 120d. First and third domains 120a, 120c are complementary, shown by the labels a_1 , a_1^* and form the neck of the hairpin 120. Second domain 120b, shown as domain a_2 forms a loop of the hairpin. Fourth domain 120d is shown as domain a_9 and is connected to axle connector 123a. Similarly, second hairpin 121 has first, second, third and fourth domains 121a, 121b, 121c, 121d, where the first and third domains 121a, 121c are complementary as shown by labels a_4 , a_4^* and form of the neck of hairpin 121. The second domain 121b, shown with label a_5 forms a loop of the second hairpin 121. Fourth domain 121d, labelled with a_3 is connected to axle connector 123b. Finally, in like manner, third hairpin 122 has first, second, third and fourth domains 122a, 122b, 122c, 122d, where the first and third domains 122a, 122c are complementary as shown by labels a_7 , a_7^* and form of the neck of hairpin 122. The second domain 122b, shown with label a_8 , forms a loop of the second hairpin 122. Fourth domain 122d, labelled with a_9 is connected to axle connector 123c.

[0092] To connect the capture units 115 and respective sites comprising parts of the binding component 114, three types of linkers are provided illustrated at 130, 130', 130'' in figures 10 to 12, the motor being in a first, second and third operating condition respectively. Each linker 130, 130', 130'' has a first portion 130a, 130'a, 130''a which is complementary to and binds to a corresponding capture domain 115a, 115'a, 115''a. An intermediate domain 130b, 130'b, 130''b serves to provide an elongate and flexible connection to the capture domain. A binding portion 130c, 130'c, 130''c is provided at an end part of the intermediate domain 130b, 130'b, 130''b to bind to a respective complementary binding site 140, 140', 140'' comprising part of the binding component 114. Each binding site 140, 140', 140'' extends between the neck domains of two of the hairpins 120, 121, 122. Hence, the binding portion 130c comprises a sequence of four domains a_2^* , a_1 , a_3^* , a_4^* which are complementary to the sequence of four domains a_2 , a_1^* , a_3 , a_4 on the binding component 114, and binding portions 130'c, 130''c similarly comprise a sequence of 4 domains. As apparent from figures 10 to 12, when linker 130 extends between capture unit 115 and binding component 114, hairpins 120, 121 are open, when linker 130' extends between capture unit 115' and binding component 114, hairpins 121, 122 are open, and when linker 130'' extends between capture unit 115'' and binding component 114, hairpins 122, 120 are open. Finally, a toehold portion 130d, 130'd, 130''d is

connected to the free end of binding portion 130c, 130'c, 130''c to facilitate detachment of the linker 130, 130', 130'' from the binding component as discussed below.

[0093] To cause rotation of the motor, it will be apparent that a connected linker 130, 130', 130'' must be detached from the respective capture unit 115, 115', 115'' and binding site 140, 140', 140'' of the binding component, and a further linker connected between a different capture unit and corresponding binding component to cause the motor to move between operating conditions. To drive the motor, the motor is supplied with a fuel unit comprising two elements, a linker 130, 130', 130'', and a fuel element which is complementary to the previous linker which is to be displaced.

[0094] Accordingly, as shown in figure 13, the replacement of a linker 130 with a linker 130' is shown at a first stage of the replacement process. A fuel unit 150 is shown, including the linker 130' and a fuel element 151. The fuel element 151 comprises a main portion 151a which is complementary to linker 130. The fuel element 151 also comprises first and second joining portions 151b, 151c, which are complementary to two adjacent domains a_4 , a_6 of the binding portion 130'c of linker 130'. The first and second joining portions join the fuel element 151 and linker 130' to form the fuel element which can be supplied to the motor. First joining portion 151b also comprises a further domain 151d complementary to the end domain of first joining portion 151b separated therefrom by spacer 151e.

[0095] As seen in figure 13, first portion 130'a has bound to capture domain 115'a and the end most domain a_5^* of the binding portion 130'c has bound to the corresponding domain of open hairpin 121 of the binding component 114. Then, as shown in figure 14, at a second stage the next domain a_4 binds to the corresponding domain of hairpin 121, at the same time displacing the endmost domain of joining portion 151b of fuel element 151. The first joining portion 151b then forms a closed hairpin, and exposes the whole of main portion 151a. The toehold portion 130d of the linker 130, t_z , binds to the corresponding domain t_z^* of the main portion 151a, and strand displacement subsequently occurs, in which the whole of linker 130 is displaced from binding attachment 114 and capture domain 115a and binds along its length to the main portion 151a of fuel element 151.

[0096] At the same time, binding portion 130'c of linker 130' binds to the corresponding domains of binding component 114. Hairpin 122 is opened, and hairpin 120, separated from linker 130, is able to close, and the motor will be in the configuration of figure 12, having moved through 1/3 of a complete rotation. The bound strands of linker 130 and fuel element 151 comprise a waste product of the operation of the motor.

[0097] The equivalent fuel units 150', 150'' are shown in figures 15 and 16, and it will be apparent that the sequence of connection, displacement and reconnection of linkers by fuel elements will cause rotation of the motor, in the specific direction selected by the combination of linkers and fuel elements making up the fuel units. The reaction rates may be controlled by selecting domain lengths as described above.

[0098] As the fuel units 150, 150', 150'' comprise relatively large hairpin loops, the domains hidden inside the loops may still be partly accessible before the reactions shown above occur. If necessary, additional secondary structures may be provided on one or both of the linker and fuel element to prevent premature reaction of the internal domains.

[0099] It will be apparent that the motors described herein may be made and assembled in any other manner as appropriate. Although the rotor and axle are described as being assembled from DNA origami, it will be clear that they may be manufactured in any desirable manner, such as the 'single-stranded DNA tile' approach, or from any other suitable material or substrate such as through nano-machining, silicon fabrication techniques or otherwise. The motor described herein has an external rotor moving around a fixed axle, in order to allow for a fluorescent molecule to be attached to the outside of the rotor and for the movement of the fluorescent molecule to be detected, for measurement purposes. However, it will be apparent that the apparatus may be reversed, with an elongate rotating element located within a substantially tubular fixed static element. The number of binding components may be selected as desired, depending on the size of the static or rotating element which they are attached, and any number of binding domains may be provided, providing the binding domains are suitably spaced from another to allow for correct binding of each domain to a binding component. It will be apparent that the binding domains and binding components may be placed on either the static element or the rotary element as desired, and, if desired, the binding and release process may have any number of steps, for example using additional release components where appropriate. Such a DNA motor may be used for any suitable application, such as a nano-scale actuator or winch, or for mismatch detection, where the speed of the motor depends on the quality of the match between control strands. The motor could be used as a gatekeeper for a pore, where rotation of the motor would open or block a pore to allow passage of molecules. This would be ineffective as a means of creating an artificial ion channel because the pore would not be sealed tightly enough to prevent the passage of ions, but it might be effective for larger molecules. The motor may also be applicable to chemical synthesis where the rotation of the motor could be coupled to chemical reactions. A modulated plasmonic antenna could be created by attaching a fluorophore to the rotor such that it regularly passes through a gap between a pair of

gold nanoparticles, where fluorescence would be enhanced. Such motors may also be used to assemble gates for DNA computing.

[0100] In the above description, an embodiment is an example or implementation of the invention. The various appearances of “one embodiment”, “an embodiment” or “some embodiments” do not necessarily all refer to the same embodiments.

[0101] Although various features of the invention may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention may be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment.

[0102] Furthermore, it is to be understood that the invention can be carried out or practiced in various ways and that the invention can be implemented in embodiments other than the ones outlined in the description above.

[0103] Meanings of technical and scientific terms used herein are to be commonly understood as by one of ordinary skill in the art to which the invention belongs, unless otherwise defined.

CLAIMS

1. A rotary DNA motor comprising;
a static element,
a rotary element rotatably movable relative to the static element,
a binding domain provided on one of the static element and the rotary element, and
a binding component provided on the other of the static element and the rotary element,
the binding component comprising a first DNA hairpin,
the binding domain being connectable to the binding component to cause relative rotation of the rotary element and static element,
the binding domain being releasable from the binding component by a release component.
2. A rotary DNA motor according to claim 1 wherein the binding domain is directly connectable to the binding component and the release component binds to the binding component to release the binding domain.
3. A rotary DNA motor according to claim 2 wherein the release component comprises a second DNA hairpin which binds to the binding component to release the binding domain.
4. A rotary DNA motor according to claim 3 wherein the release component is releasable from the binding component by a second release component such that the binding component is available for subsequent adhesion to a binding domain.
5. A rotary DNA motor according to claim 4 wherein the second release component comprises a third DNA hairpin.
6. A rotary DNA motor according to any one of the preceding claims comprising a plurality of binding components, and preferably at least 3 binding components.
7. A rotary DNA motor according to claim 1 wherein the binding domain is connectable to the binding component by a separate linker.
8. A rotary DNA motor according to claim 7 wherein the release component binds to the linker to detach the linker from the binding domain and binding component.

9. A rotary DNA motor according to claim 8 comprising a fuel element comprising the release element and a further, different, linker to bind to a different binding domain and binding component.
10. A rotary DNA motor according to any one claim 7 to 9 wherein the binding component comprises a plurality of hairpins and defines a plurality of binding sites.
11. A rotary DNA motor according to any one of claims 7 to 10 comprising a plurality of binding domains, each binding domain having a different capture domain.
12. A rotary DNA motor according to claim 11 where dependent on claim 10, wherein each linker binds to a corresponding binding site on the binding component and a different binding domain.
13. A rotary DNA motor according to any one of the preceding claims wherein at least one of the rotary element and the static element are made from DNA origami.
14. A rotary DNA motor according to any one of the preceding claims wherein one of the static element and the rotary element comprises a hollow tube and the other of the static element and the rotary element comprises an elongate rod received within the hollow tube.
15. A rotary DNA motor according to claim 14 wherein the hollow tube has a suitable diameter to admit the release component.
16. A rotary DNA motor according to claim 14 or claim 15 wherein the static element comprises the elongate rod and the rotary element comprises the hollow tube.
17. A rotary DNA motor according to one of the preceding claims wherein the rotary element rotates relative to the static element when the rotary DNA motor is supplied with release components.
18. A method of forming a rotary DNA motor comprising providing a static element and a rotary element,

engaging the static element and rotary element such that the rotary element is rotatably movable relative to the static element,

providing a binding domain on one of the static element and the rotary element, and

providing a binding component on the other of the static element and the rotary element,

the binding component comprising a first DNA hairpin,

the binding domain being connectable to the binding component to cause relative rotation of the rotary element and static element,

the binding domain being releasable from the binding component by a release component.

19. A method according to claim 18 comprising the step of attaching the static element to a support element.

20. A method according to claim 18 or claim 19 comprising attaching the static element to the rotary element with an initial anchor, and, after assembly of the motor, breaking the initial anchor.

21. A method according to claim 20 wherein the initial anchor is a double-stranded DNA linker where one strand is connected to the rotary element and the other end is connected to the static element.

22. A method according to any one of claims 18 to 21 comprising blocking the binding domain during assembly, and, after assembly, unblocking the binding domain.

23. A method according to any one of claims 18 to 22 comprising forming at least one of the static element and the rotary element using DNA origami.

24. A method according any one of claims 18 to 23 comprising forming one of the static element and the rotary element as a hollow tube and forming the other of the static element and the rotary element as an elongate rod received within the hollow tube.

25. A method according to claim 24 wherein the static element comprises the elongate rod and the rotary element comprises the hollow tube.

26. A method according any one of claims 18 to 25 comprising providing a plurality of binding components, and preferably at least 3 binding components.

27. A method according to any one of claims 18 to 26 comprising providing the or each binding component on the static element and providing the binding domain on the rotary element.

28. A method of operating a rotary DNA motor comprising providing a rotary DNA motor according to any one of claims 1 to 17 or forming a rotary DNA motor according to any one of claims 18 to 27, and supplying release components to the rotary DNA motor.

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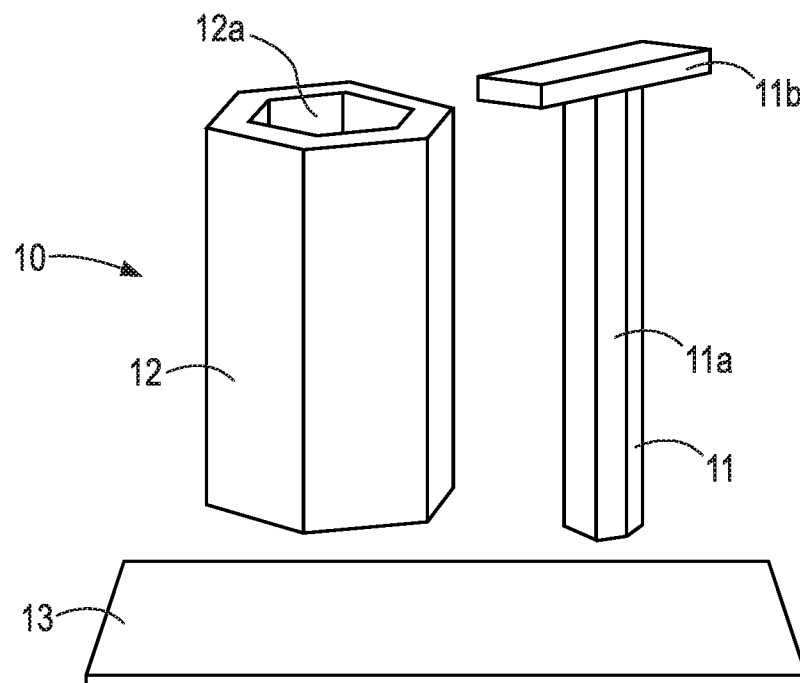


FIG. 1A

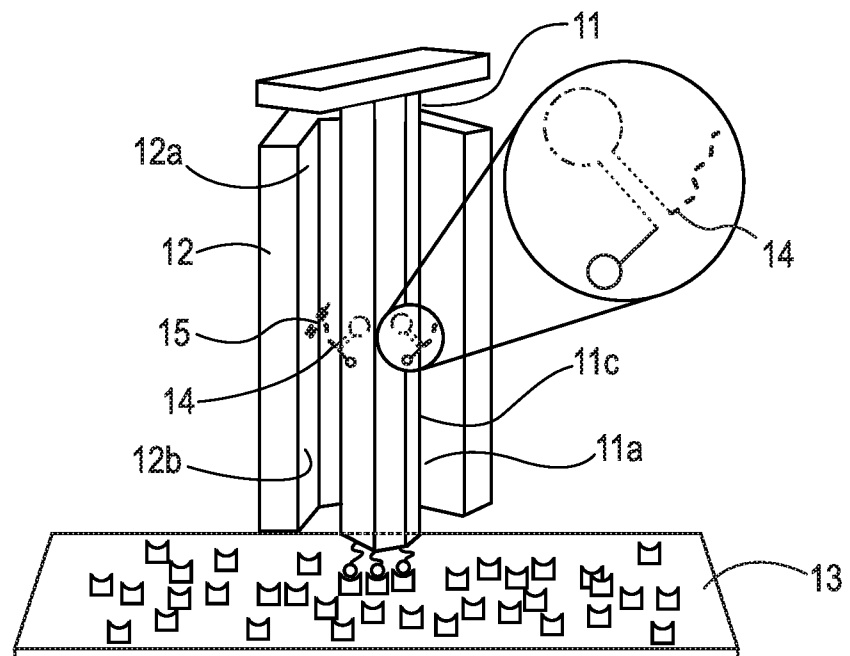


FIG. 1B

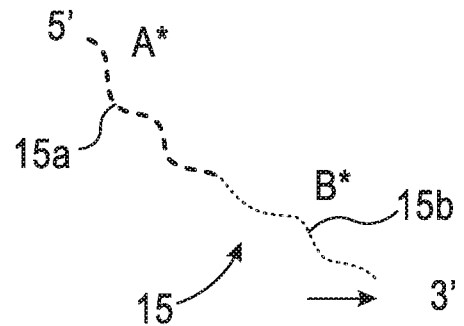


FIG. 2A

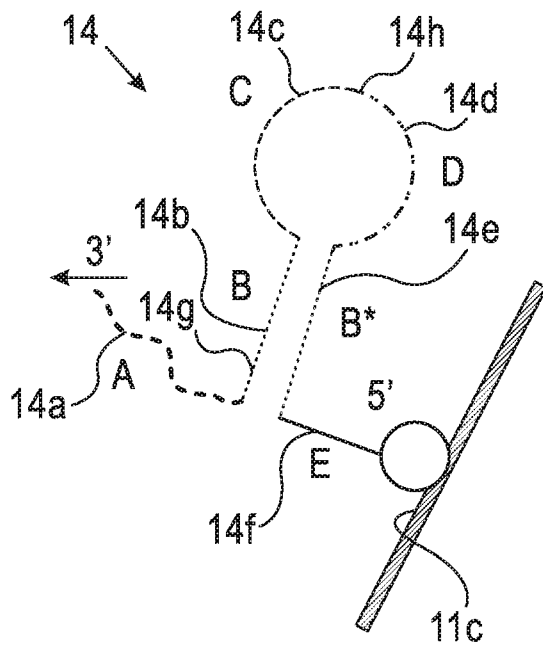


FIG. 2B

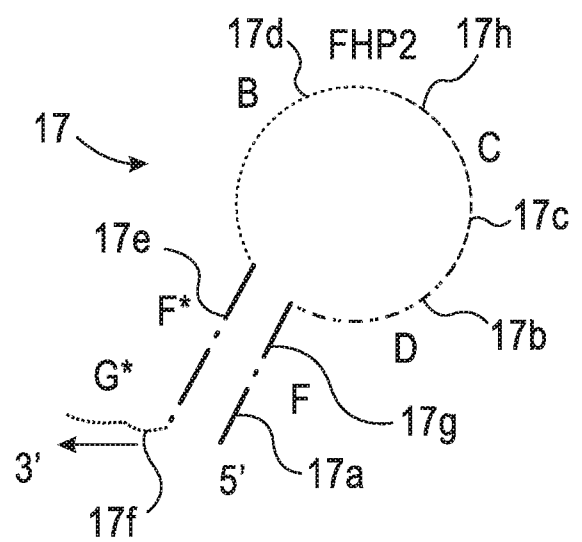


FIG. 2D

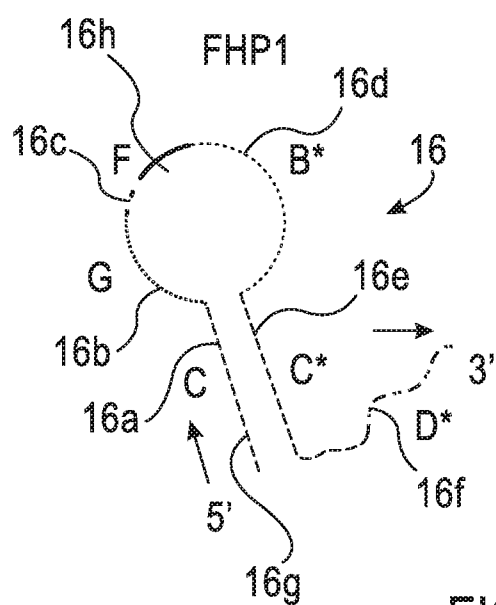


FIG. 2C

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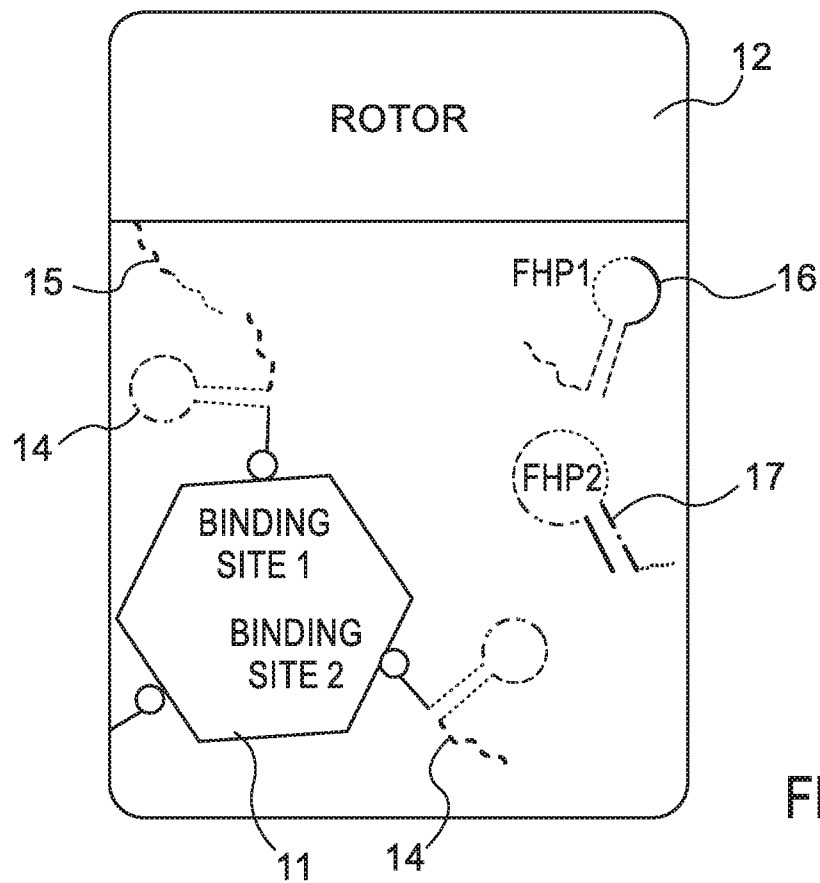


FIG. 3A

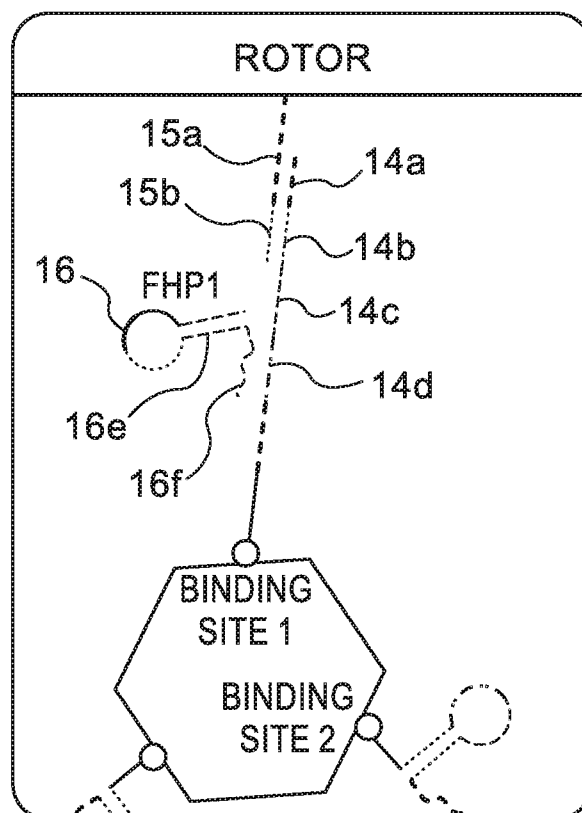


FIG. 3B

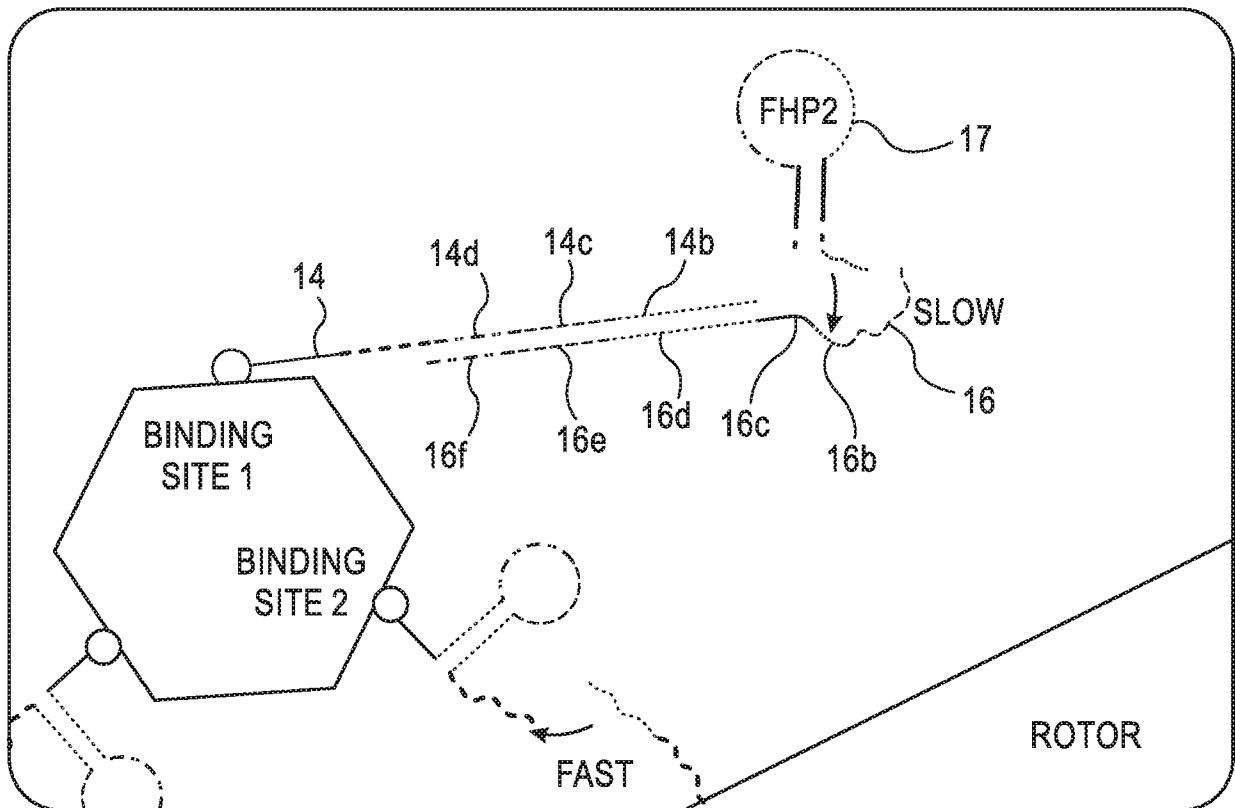


FIG. 3C

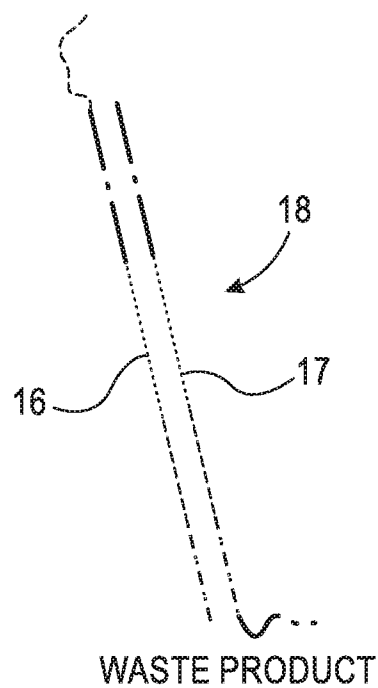


FIG. 3D

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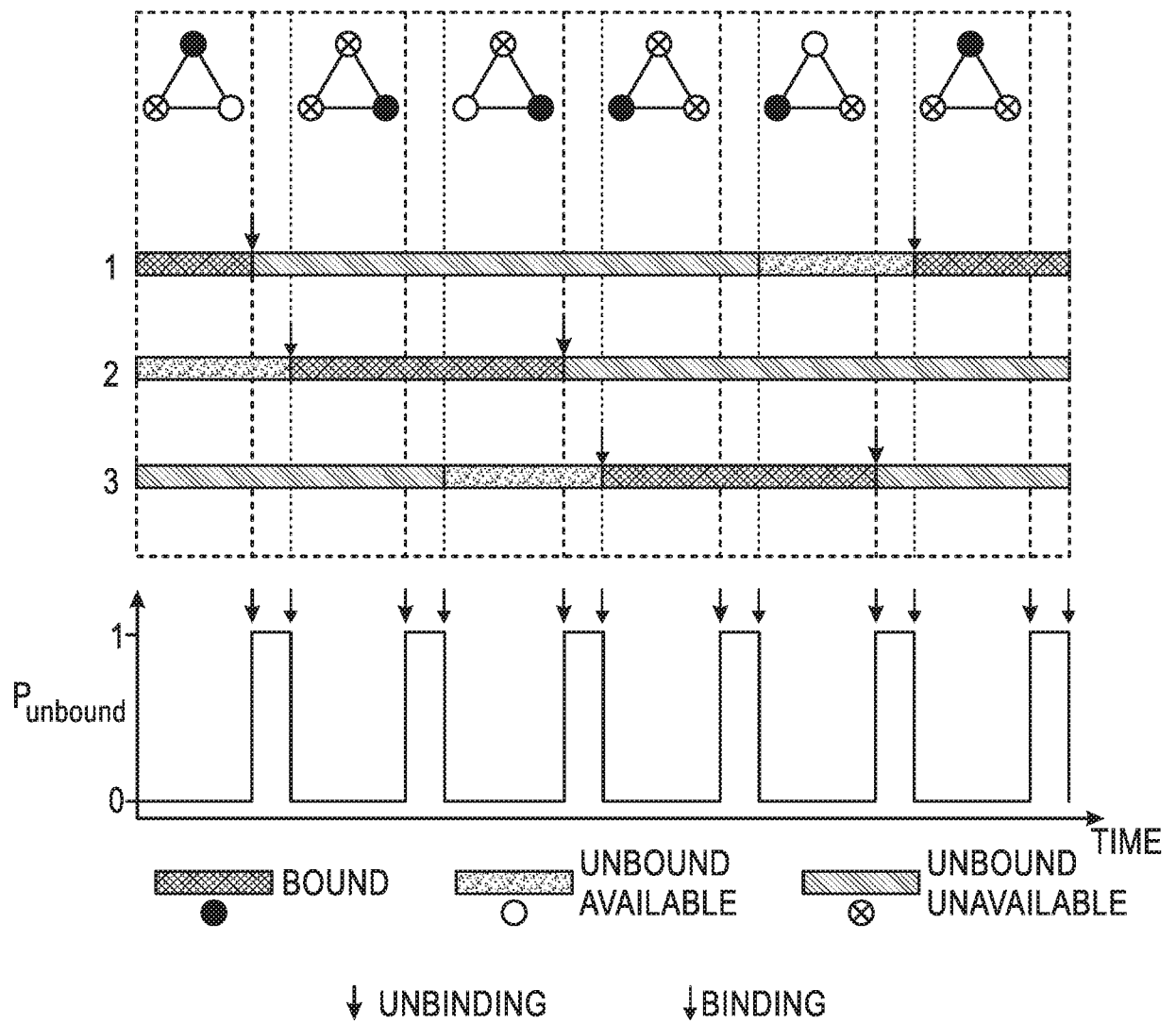


FIG. 4

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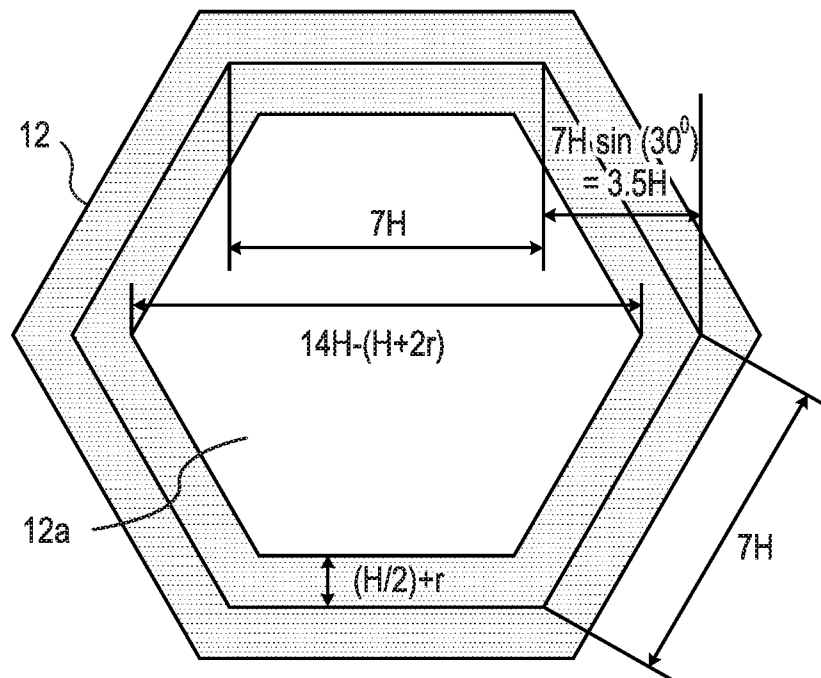


FIG. 5A

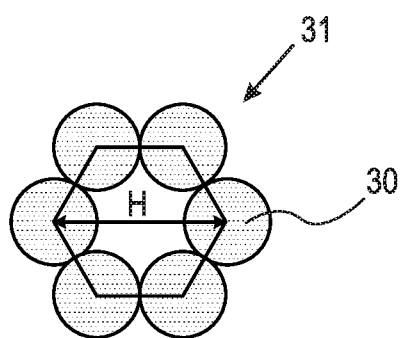


FIG. 5B

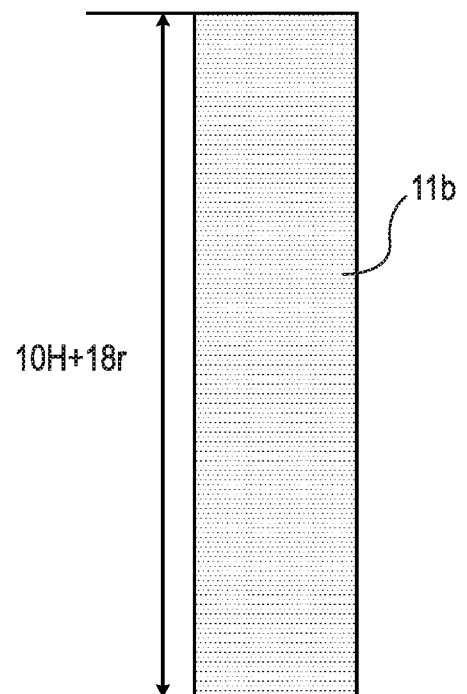


FIG. 5C

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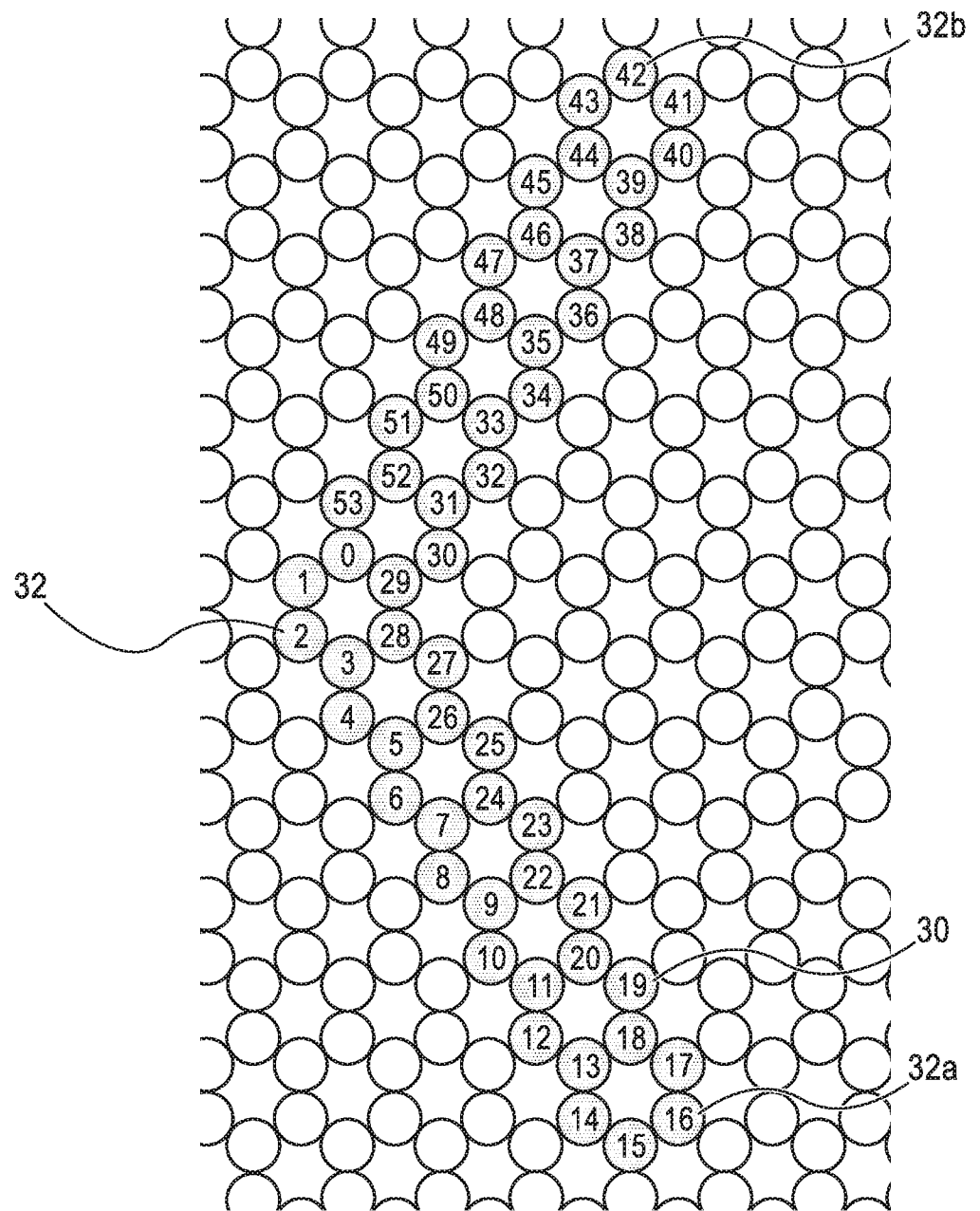


FIG. 6A

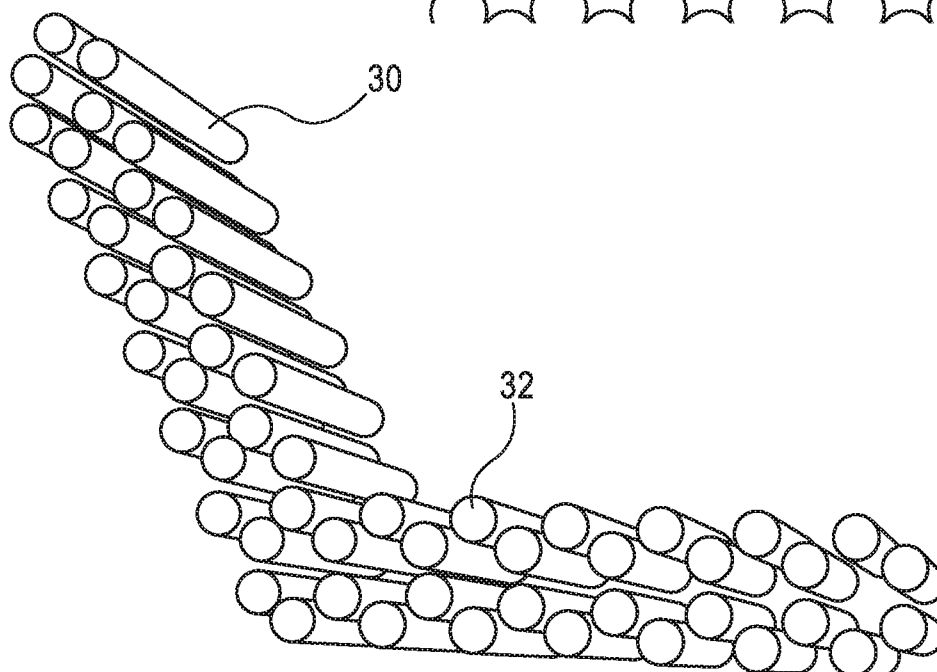


FIG. 6B

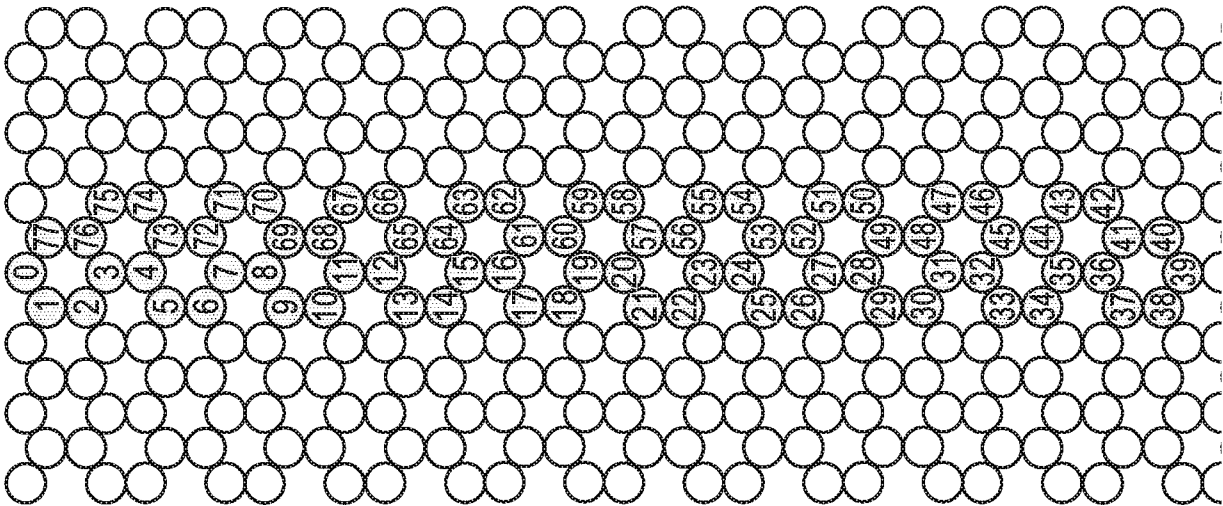


FIG. 7A

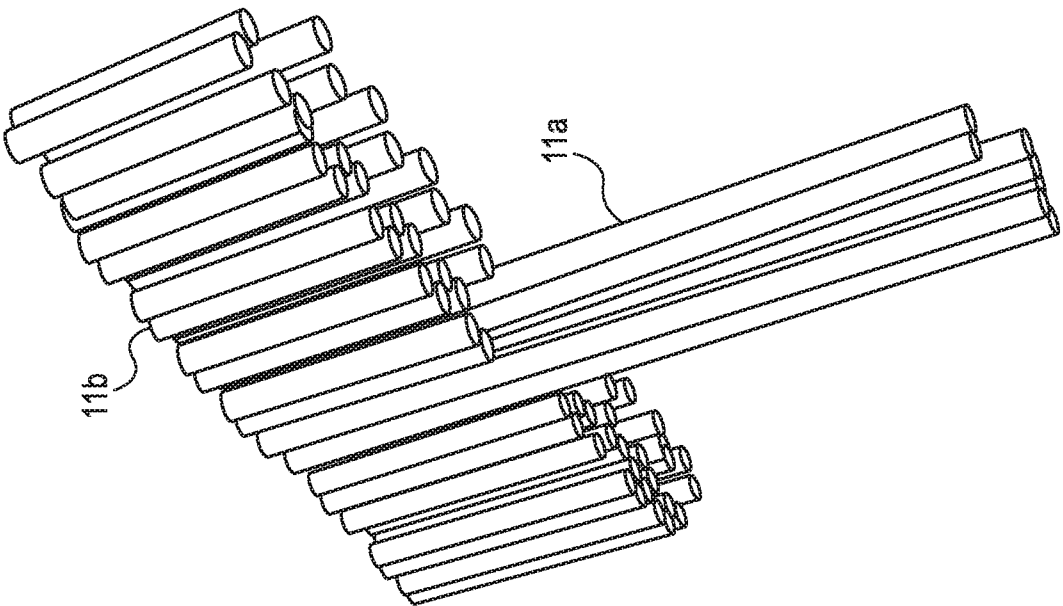


FIG. 7B

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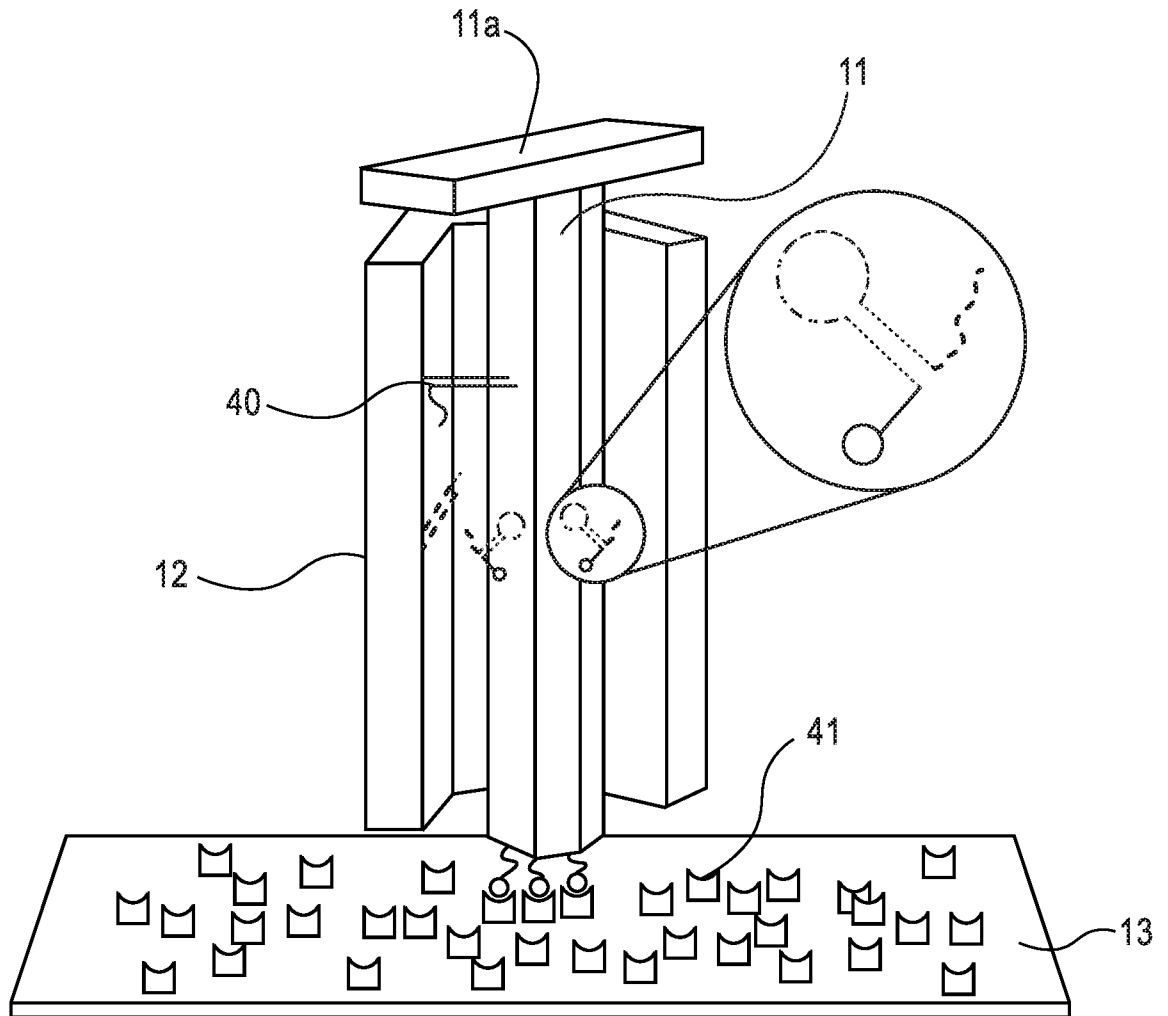


FIG. 8

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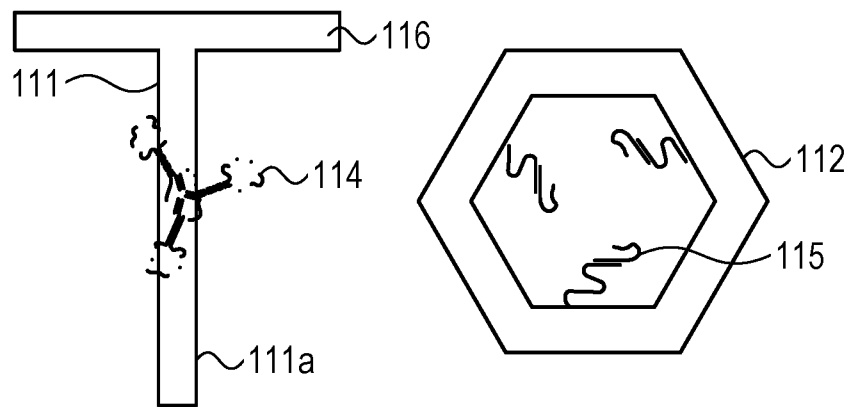


FIG. 9A

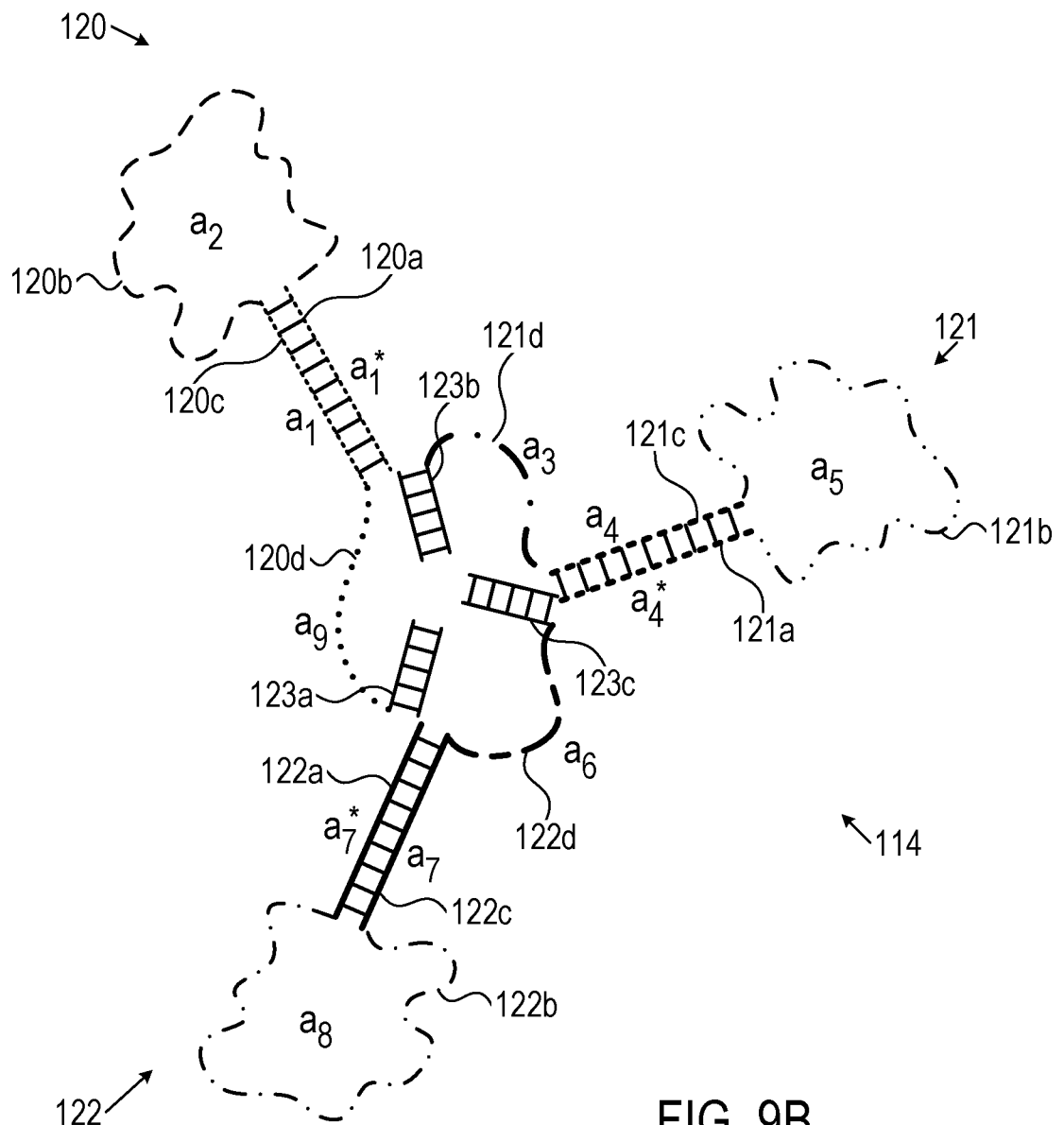


FIG. 9B

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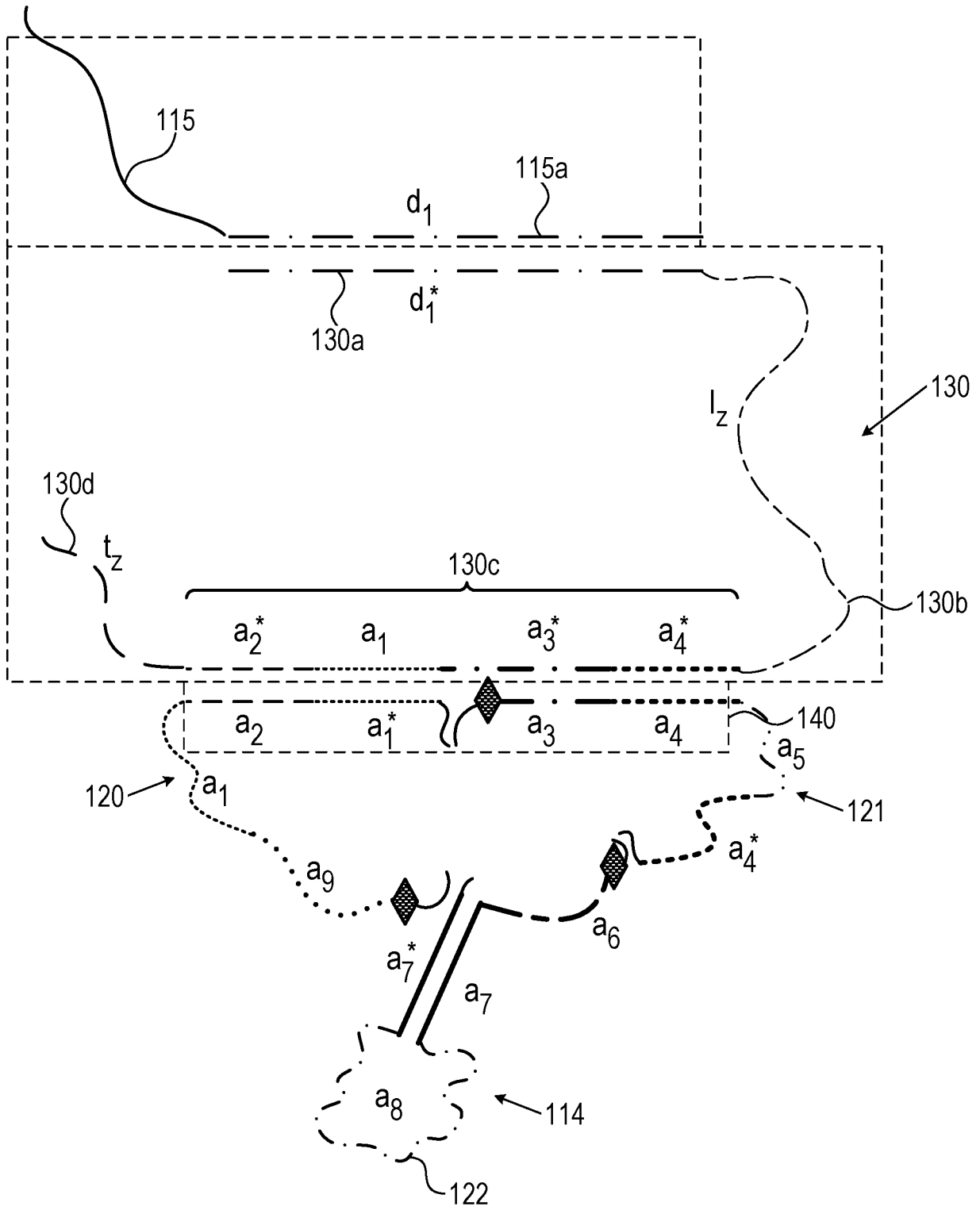


FIG. 10

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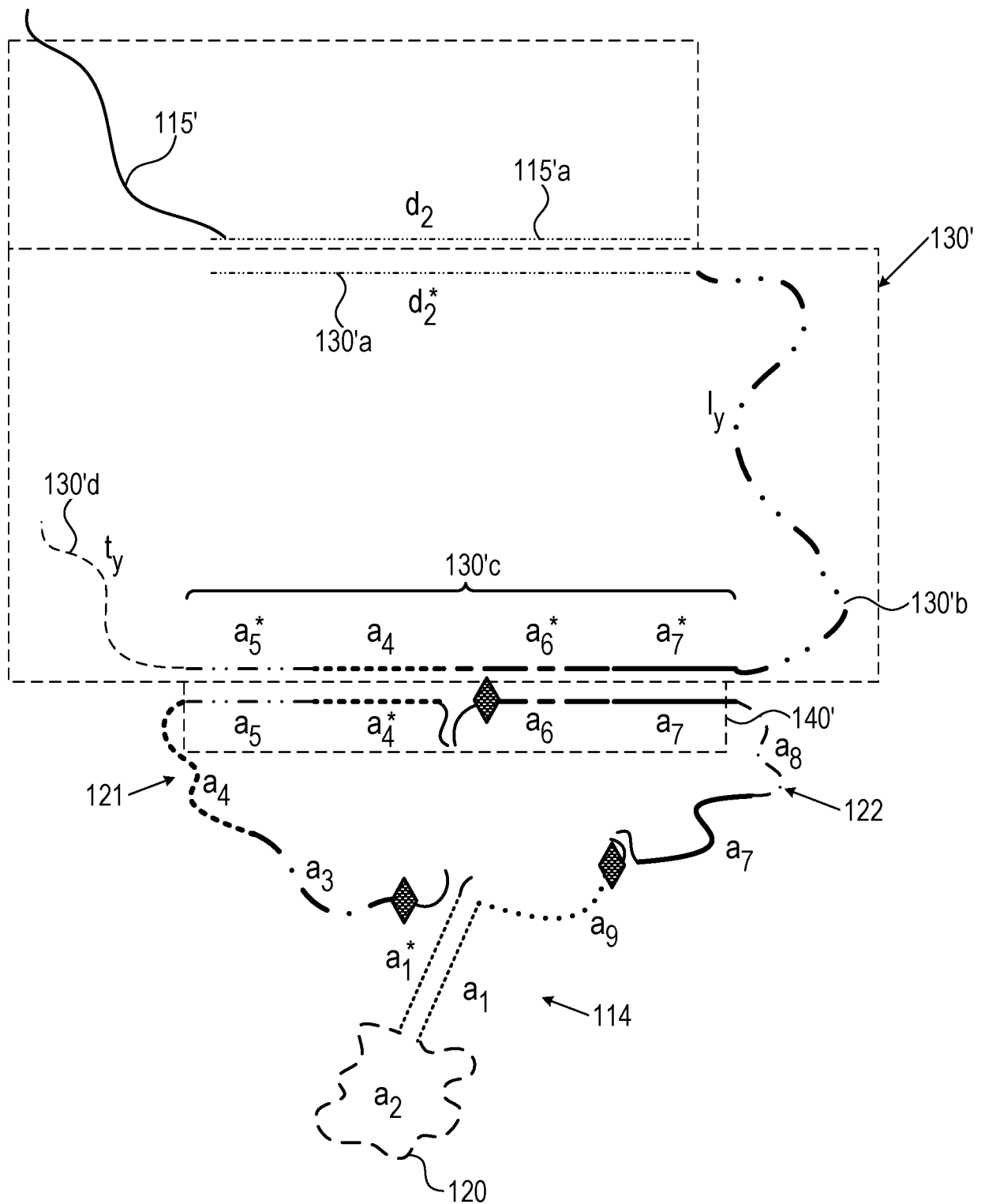


FIG. 11

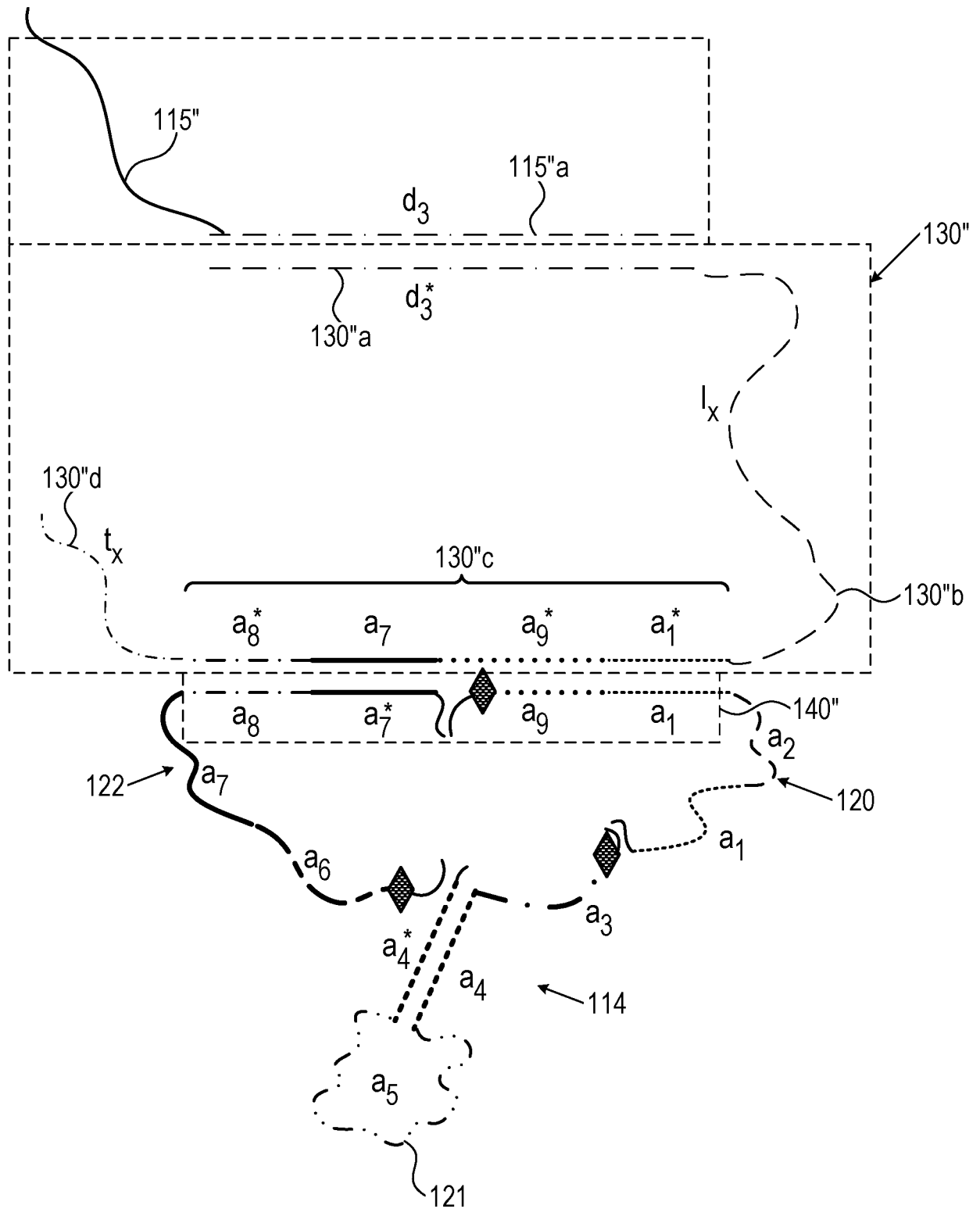


FIG. 12

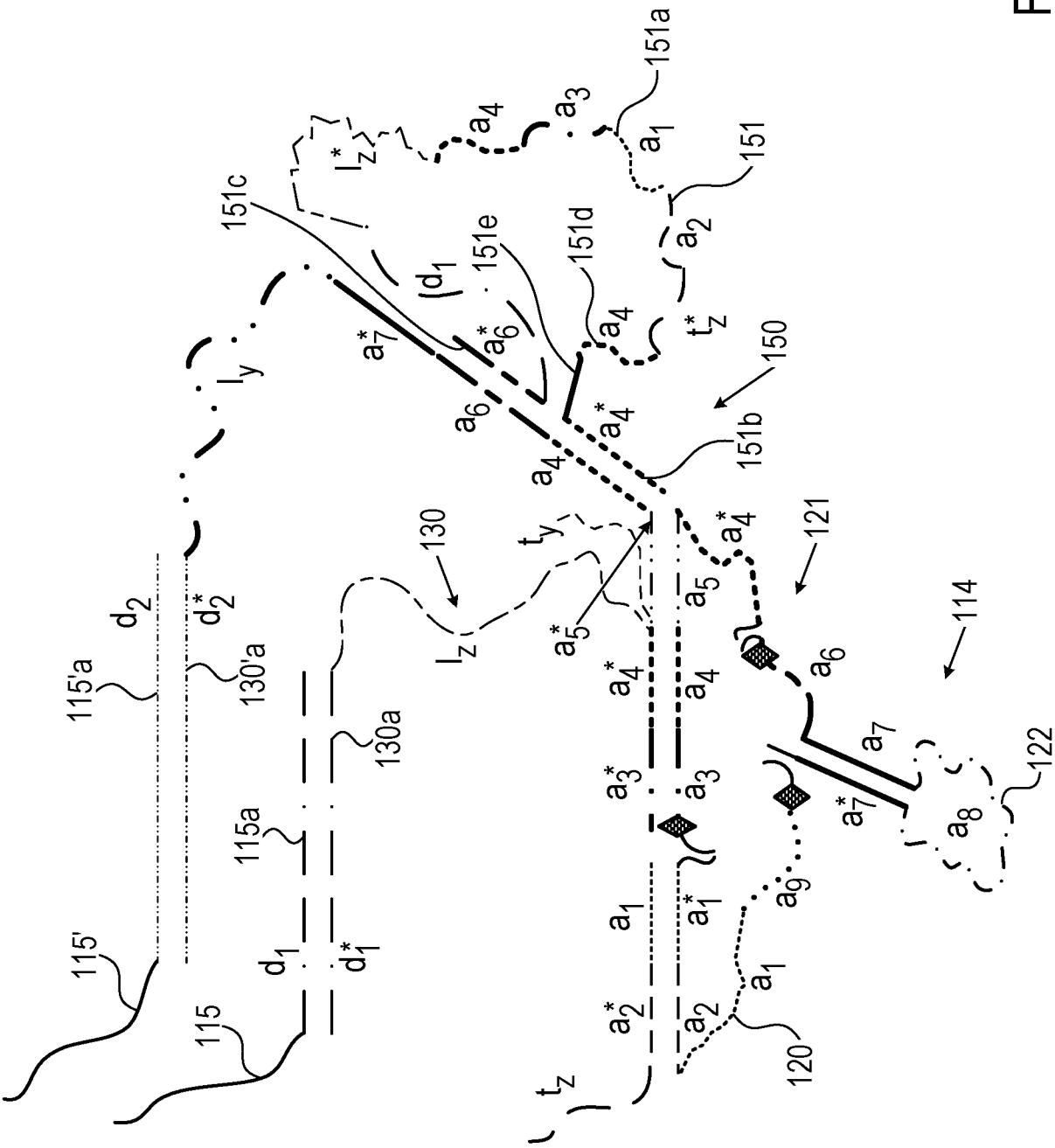


FIG. 13

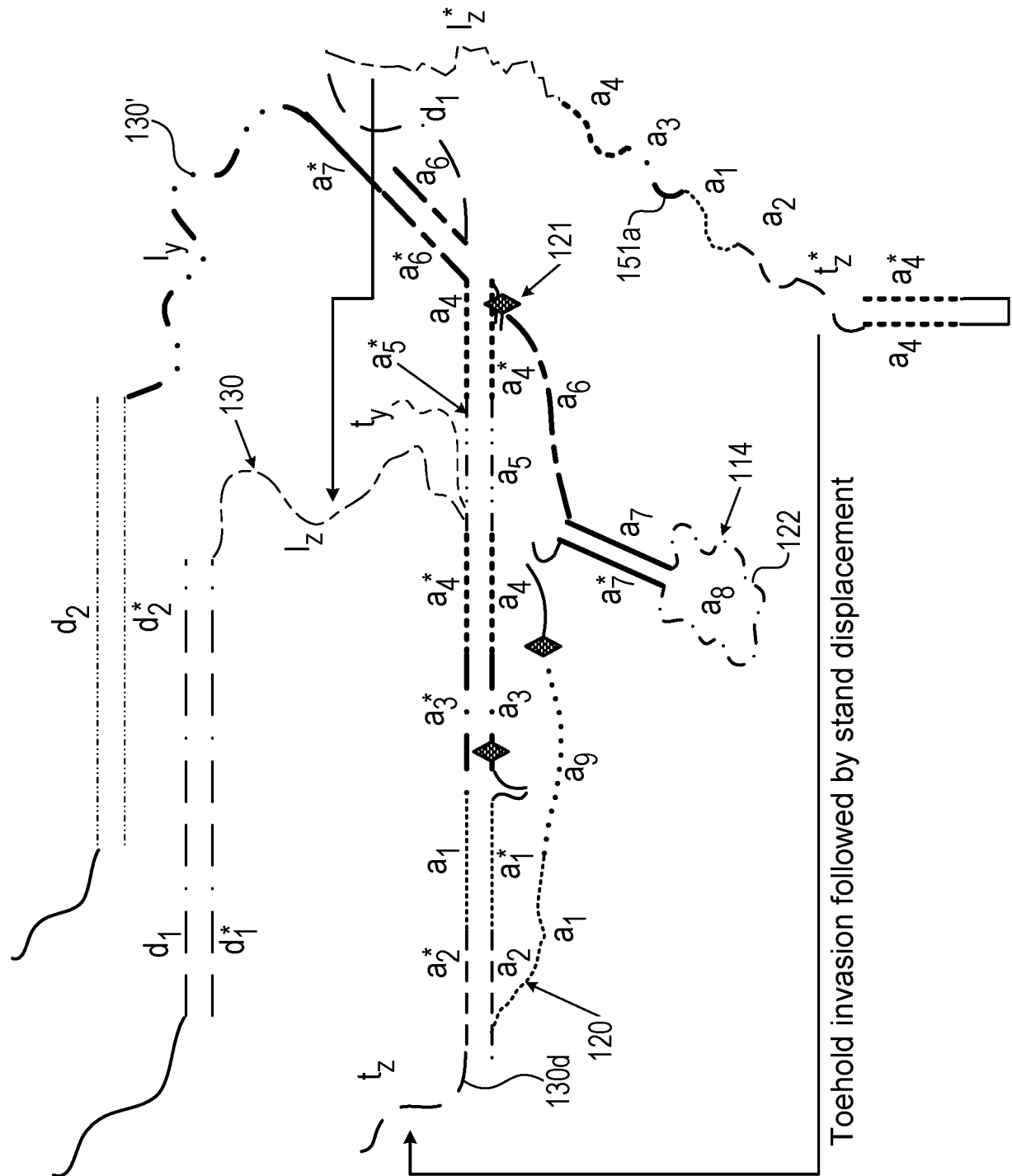


FIG. 14

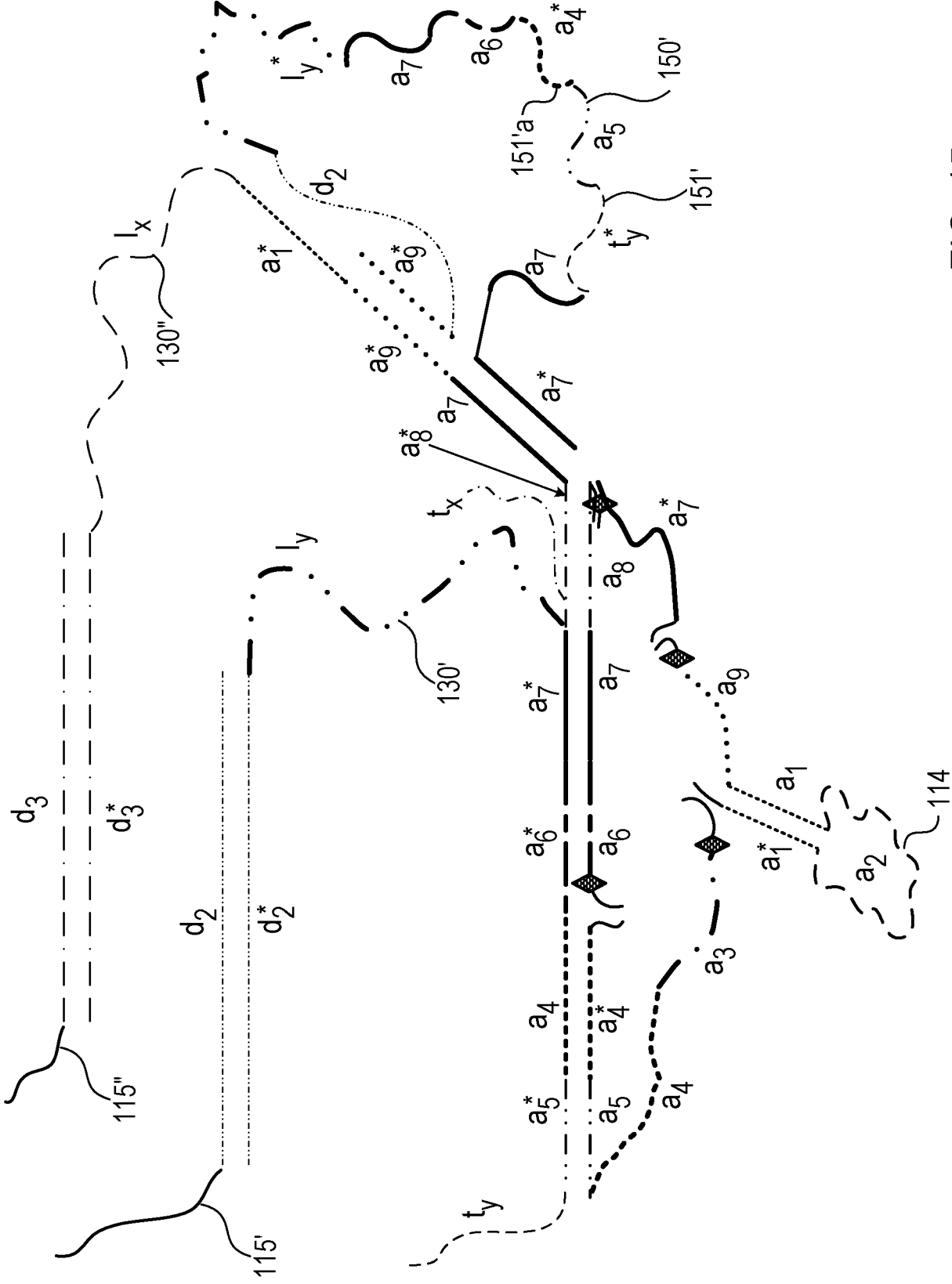


FIG. 15

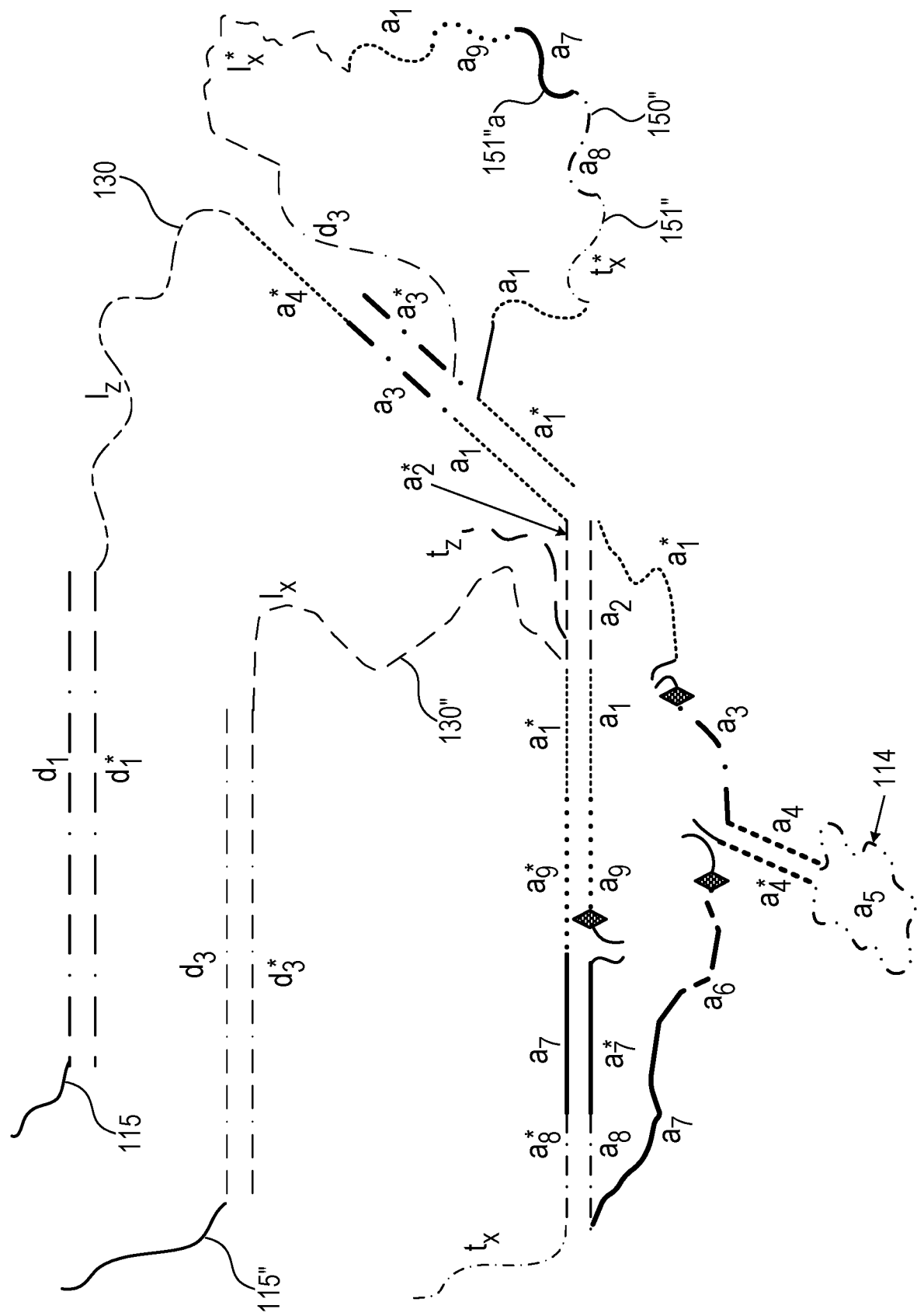


FIG. 16

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2015/053195

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12N15/09
 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

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/147673 A2 (UNIV FLORIDA [US]; TAN WEIHONG [US]; KANG HUAIZHI [CN]) 23 December 2010 (2010-12-23) the whole document	1-28
A	----- BRANDT KARSTEN ET AL: "Individual Interactions of the b Subunits within the Stator of the Escherichia coli ATP Synthase", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 288, no. 34, August 2013 (2013-08), pages 24465-24479, XP002742383, the whole document ----- -/--	1-28



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

17 July 2015

Date of mailing of the international search report

31/07/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040,
 Fax: (+31-70) 340-3016

Authorized officer

Young, Craig

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/053195

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ERNST STEFAN ET AL: "Elastic deformations of the rotary double motor of single FOF1-ATP synthases detected in real time by Forster resonance energy transfer", BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1817, no. 10, Sp. Iss. SI, October 2012 (2012-10), pages 1722-1731, XP002742384, the whole document -----	1-28
A	WAECHTER ANDRE ET AL: "Two rotary motors in F-ATP synthase are elastically coupled by a flexible rotor and a stiff stator stalk", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 108, no. 10, March 2011 (2011-03), pages 3924-3929, XP002742385, ISSN: 0027-8424 the whole document -----	1-28
A	SIELAFF HENDRIK ET AL: "Domain compliance and elastic power transmission in rotary FOF1-ATPase", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 105, no. 46, November 2008 (2008-11), pages 17760-17765, XP002742386, ISSN: 0027-8424 the whole document -----	1-28
A	US 2003/215844 A1 (CHAPSKY LARS [US] ET AL) 20 November 2003 (2003-11-20) the whole document -----	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2015/053195

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010147673 A2	23-12-2010	NONE	
US 2003215844 A1	20-11-2003	NONE	