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(54) Title: SINGLE-DNA MOLECULE NANOMOTOR REGULATED BY PHOTONS

(57) Abstract: This invention provides photon-driven single-molecule nanomotors with biologically- based components, and methods of making the same. The nanomotors of the invention comprise a DNA hairpin-structured molecule incorporated with at least one azobenzene moiety (such as azobenzene phosphoramidite). The azobenzene moiety (or moieties) facilitates reversible photocontrollable conversion of the DNA molecule from the hairpin structure to an open state. This reversible extension-contraction behavior is considered a molecular motor function.



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DESCRIPTION

SINGLE-DNA MOLECULE NANOMOTOR REGULATED BY PHOTONS

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit of U.S. Provisional Application Serial No. 61/269,153, filed June 19, 2009, which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, and drawings.

GOVERNMENT SUPPORT

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BACKGROUND OF THE INVENTION

Biological machines, and biomolecular motors in particular, have been refined through eons of evolution. For example, nanomotors consisting of single protein molecules are abundant in living systems. Individual (or a very small number of) motors can transport cellular components within a cell, while ensembles of very large numbers of motors are arranged to move the largest creatures on earth. Nanoscale engineering by humans can be greatly enhanced by assimilation of biological specialization already achieved through natural evolution, and by envisioning additional modifications through molecular genetics.

Nucleic acid (DNA) self-assembly based on spontaneous hybridization between complementary strands is an effective way to construct multi-dimensional nano-objects such as macromolecular architecture (Seeman, N. C. *J. Biomol. Struc. Dyn.*, 8:573–581 (1990); Seeman, N. C. *J. Theor. Biol.*, 99:237–247 (1982); Chen, J. H.; Seeman, N. C. *Nature*, 350:631–633 (1991); Zhang, Y. W.; Seeman, N. C. *J. Am. Chem. Soc.*, 116:1661–1669 (1994); Shih, W. M. *et al.*, *Nature*, 427:618–621 (2004); and Goodman, R. P. *et al.*, *Science*, 310:1661–1665 (2005)), biochips (Robinson, B. H.; Seeman, N. C. *Protein Eng.*, 1:295–300 (1987)), photonic wires (Heilemann, M. *et al.*, *J. Am. Chem. Soc.*, 126:6514–6515 (2004)),

enzyme assemblies (Niemeyer, C. M. *et al.*, *ChemBioChem*, 3:242–245 (2002)) and functional DNA probes (Yang, X. *et al.*, *Biopolymers*, 45:69–83 (1998) and Shin, J. S.; Pierce, N. A. *J. Am. Chem. Soc.*, 126:10834–10835 (2004)). Similar to RNA or protein based nanostructures, DNA based nanomachines have been developed with the ability to change
5 their conformation upon external stimuli. See Higashi-Fujime, S. *et al.*, *FEBS Lett.*, 375:151–154 (1995); Cate, J. H. *et al.*, *Science*, 273:1678–1685 (1996); DeGrado, W. F. *et al. Annu. Rev. Biochem.*, 68:779–819 (1999); Yurke, B. *et al.*, *Nature*, 406:605–608 (2000); and Yan, H. *et al.*, *Nature*, 415:62–65 (2002).

One such development is a single-molecule nanomotor that can switch between two
10 conformations and perform extending-shrinking motion using at least two pieces of DNA to trigger the movement (Li, J. W.; Tan, W. H. *Nano Letters*, 2:315–318 (2002)). This single-DNA nanomotor requires addition and removal of fuel and waste strands for motor function (Turberfield, A. J. *et al. Phys. Rev. Lett.*, 90:118102–118109 (2003)). Such bimolecular or multimolecular interactions are often complex, difficult to manipulate and reproduce, and
15 concentration-dependent (*i.e.*, since movement is governed by intermolecular interactions of the DNA strands, the concentration of each DNA strand contributes to the overall intermolecular reaction rate and motor efficiency). Although some artificial nanomotors can utilize alternative energy sources, including hydrolysis of the DNA backbone (Tian, Y. *et al. Angew. Chem. Int. Edn*, 44:4355–4358 (2005)) and ATP (Yin, P. *et al. Angew. Chem. Int.*
20 *Edn*, 43:4906–4911 (2004)), applying an electromagnetic field as a convenient energy source is highly desired. To date, a photon-driven single-DNA nanomotor has not been contemplated or created. Such a nanomotor would be of great scientific interest as well as contribute to applications in the nanosciences.

Azobenzene molecules have been extensively studied due to their reversible
25 isomerization between planar *trans*- form and non-planar *cis*- form under UV and visible light irradiation. See Sudesh, G.; Neckers, D. C. *Chem. Rev.*, 89:1915–1925 (1989); Berg, R. H. *et al. Nature*, 383, 505–508 (1996); and Guang Diao, E. W. *J. Phys. Chem. A*, 108:950–956 (2004). The first light-powered molecular device using an azobenzene polymer has successfully proved the concept of optomechanical energy conversion in a single device
30 (Hugel, T. *et al. Science*, 296:1103–1106 (2002)). Several DNA-based nanostructures incorporated with azobenzene moiety have also been constructed and investigated with photoregulated capability. See Asanuma, H. *et al. Angew. Chem. Int. Ed.*, 40:2671–2673

(2001); Liang, X. *et al. Tetrahedron Lett.*, 42:6723–6725 (2001); Asanuma, H. *et al. M.Chembiochem*, 2:39–44 (2001); Liang, X. *et al. J. Am. Chem. Soc.*, 124:1877–1883 (2002); Asanuma, H. *et al. Nucleic Acids Symp. Ser.*, 49:35–36 (2005); Liang, X. *et al. ChemBioChem*, 9:702–705 (2008); and Takahashi, K. *et al. LNCS*, 3892:336–346 (2006).

5 Unfortunately, all of these structures require several DNA sequences to perform motor movement.

For example, a model of light driving an azobenzene-DNA molecular motor based on exchange of multiple DNA strands was been recently demonstrated (Liang, X. *et al. ChemBioChem*, 9:702–705 (2008)). The drawback to this nanomotor is that it
10 requires addition and removal of multiple DNA strands for motor function.

Thus, there exists a need in the art for a photoregulated single DNA molecule nanomotor that does not require the use of several components (such as several DNA sequences) for functional operation.

15 BRIEF SUMMARY OF THE INVENTION

The present invention provides a novel photon-driven single-DNA nanomotor. The nanomotor is constructed as a fully functionalized DNA molecule driven by harvesting photon energy. The subject nanomotor is highly advantageous due to its simplicity, high sensitivity, and significant energy conversion efficiency.

20 In one embodiment of the present invention, a photoregulated single-molecule DNA motor (PSMM) is provided, wherein the PSMM comprises a DNA backbone and at least one azobenzene moiety (such as azobenzene phosphoramidite). The DNA backbone preferably has a stable hairpin structure including a stem duplex and a single-strand base loop, wherein the azobenzene moiety (or moieties) is inserted to an arm of the stem duplex of the hairpin
25 DNA backbone.

Under UV and visible irradiations, the PSMM can adopt two distinct conformations or states. Exposure to UV and visible irradiations reversibly switches PSMM conformation between open (dehybridization) and close (hybridization) states, respectively, and enables the PSMM to perform an inchworm-like extending and shrinking motion. The regulation of the
30 open and close states of the hairpin DNA backbone structure is controlled by the photoresponsive azobenzene moiety (or moieties) integrated on the DNA bases in the hairpin's duplex stem segment. Specifically, the azobenzene moiety (or moieties) controls

the association and dissociation of the stem duplex to “open” or “close” the DNA backbone. The azobenzene moiety controls the hybridization (close) and dehybridization (open) of polynucleotides along the stem duplex of the DNA backbone, depending on UV and visible irradiation.

5 The nature of the PSMM of the invention is determined by predominant intramolecular interaction within the molecule instead of disjunctive DNA strand exchange. Compared with previous DNA motors, in which motor cycles involve bimolecular or multimolecular interactions among several independent DNA strands, the subject PSMM is a single-molecule nanomotor that can facilitate the open-close cycling by virtue of its unique
10 structural architecture. In the subject PSMM, the motor can act as a simple, yet precisely functionalized molecular motor. Because of its simplicity and intramolecular interaction, a nanomotor of the invention with reversible photoswitching function possesses novel properties superior to those consisting of multiple-component DNA nanostructures. For example, because of its structural architecture and photoregulated motor function, the
15 workings of the subject PSMM are concentration-independent and thus ideal for developing high-density molecular motors.

 In a related embodiment, the invention provides combinations of at least two PSMMs, wherein the PSMMs function in concert. In another embodiment, a plurality of PSMMs is associated with each other to form a space figure structure, such as a cube or cylinder. In yet
20 another embodiment, space figure structures comprising a plurality of PSMMs are associated with one another to form a multiplicity of synchronized PSMMs, such as in the form of a film.

 In another embodiment, a microarray of a plurality of PSMMs of the invention is provided. The microarray can be present in solution or attached to a substrate (*e.g.*,
25 immobilized on a gel substrate).

 In another embodiment, the PSMM further comprises at least one fluorophore/quencher (F/Q) pair for signaling motor movement. In a related embodiment, a fluorophore and quencher are attached to both ends of the DNA backbone. The fluorescence signal intensity generated by the F/Q pair is related to the distance between the fluorophore
30 and quencher. In the close (hybridized) state, the DNA forms a hairpin structure and fluorescence signaling is quenched due to the proximity of the fluorophore and quencher. In

the open (dehybridized) state, maximum fluorescence signaling is generated due to the separation between the fluorophore and quencher.

Yet another aspect of the invention provides a process for making a PSMM, which method comprises identifying, isolating and/or preparing a DNA backbone having a hairpin structure with a stem and loop, wherein at least one azo compound is integrated into an arm (of the base pair stem) of the backbone.

Advantageously, the compounds and methods of the present invention can provide a nanomotor of very simple structure that is easily obtained and functionalized. Further, the PSMM of the invention is driven by photons and operated by intramolecular interactions, thus circumventing the complicity introduced by intermolecular reactions as seen in previous nanomotors. The subject invention is further directed to a device that includes a PSMM of the invention, a structure composed of PSMMs, or a microarray of PSMMs.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawings(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1 shows an example of a PSMM of the invention comprising three azobenzene moieties inserted to the stem duplex.

Figure 2A shows the available positions for Azo- incorporation in stem moiety on each end of the DNA backbone.

Figure 2B shows the sequences of the six types of PSMM (PSMMs 1-6) in accordance with the subject invention.

Figures 3A-C are graphical illustrations of fluorescence spectra of PSMMs 1-3 after irradiation, where the blue curve represents pure DNA in buffer solution (100 nM); the green line represents five times of cDNA; and red line represents UV irradiation.

Figure 4 is a graphical illustration of the cycling of close-open of a PSMM of the invention following repeated Vis/UV radiations.

Figure 5 shows the synthesis of azobenzene-tethered phosphoramidite monomers.

Figure 6 shows the incorporation of azobenzene moiety to DNA sequences by DNA synthesizer.

Figure 7A is a graphical illustration of the melting temperature profiles of PSMMs 1-6 under the same conditions, wherein PSMM1 is represented by the orange curve, PSMM2 is represented by the sky blue curve, PSMM3 is represented by the purple curve, PSMM4 is represented by the blue curve, PSMM5 is represented by the green curve, and PSMM6 is represented by the brown curve.

Figure 7B shows the suggested mechanisms of photoresponse of PSMMs of the invention.

Figure 8 is an illustration of a photoregulation cycle of azobenzene-incorporated hairpin nanomotors of the invention.

Figures 9A-B show the sequences of various azo-incorporated linear DNA sequences.

Figures 10A-E are illustrations of fluorescence spectra of various azo-tethered linear DNA sequences following irradiation, where the red curve represents pure DNA in buffer solution (100 nM); the blue line represents five times of cDNA; and green line represents UV irradiation.

Figure 11 shows fluorescence spectra of an embodiment of the invention under different irradiations, where the red curve represents pure DNA in buffer solution (100 nM); the blue line represents five times of cDNA; and green line represents UV irradiation.

Figure 12 is a table illustrating the comparison of all DNA sequences with and without Azo-incorporation.

Figure 13 is a table illustrating the conversion efficiency of PSMM3 and L10-1 in different concentrations.

Figure 14A is an illustration of the sequences of a PSMM DNA hairpin structure with potential azobenzene insertion positions and the underlined sequences that form the stem structure.

Figure 14B is an illustration of the available positions for azobenzene insertions, as indicated by arrows 1-6, into the stem structure of a PSMM DNA hairpin structure of the invention when in the close and open state.

Figures 15A-D are illustrations of the steps involved in bottom up assembly of a space figure comprising a plurality of PSMM DNA structures associated with each other.

Figures 16A-C are illustrations of the various forms in which a space figure of **Figure 15D** can take. **Figure 16A** is an illustration of a cubic figure comprising a plurality of

PSMM DNA structures associated with each other, wherein the PSMM DNA structures are in a close state. **Figure 16B** is an illustration of a cubic figure comprising a plurality of PSMM DNA structures associated with each other, wherein the PSMM DNA structures are in an open state. **Figure 16C** is an illustration of several cubic figures associated with each other to form a multi-layer film.

Figure 17 is an illustration of a schematic device structure for the demonstration of solar-to-mechanical energy conversion.

Figure 18 is an illustration of a scheme of volume change measurement of PSMM DNA nanomotor operation in aqueous solution.

Figure 19 is an illustration of a scheme of volume change of PSMM DNA nanomotors attached to a substrate.

Figure 20 is an illustration of a schematic device for the demonstration of solar-to-mechanical energy conversion via volume change measurement of PSMM DNA nanomotor operation when attached to a hydrogel substrate.

BRIEF DESCRIPTION OF SEQUENCE LISTING

SEQ ID NO:1 is a nucleotide sequence of a hairpin DNA backbone that is a component of a nanomotor of the invention.

SEQ ID NOs:2-5 are nucleotide sequences of linear, single-stranded DNA.

SEQ ID NO:6 is a nucleotide sequence having tethered azobenzene moieties within a hairpin DNA backbone that is a component of a nanomotor of the invention.

SEQ ID NOs:7-12 are nucleotide sequences having tethered azobenzene moieties within hairpin DNA backbones that are components of nanomotors of the invention.

SEQ ID NOs:13-17 are nucleotide sequences of linear, single-stranded DNA with tethered azobenzene moieties therein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel photon-driven single-DNA nanomotor. The nanomotor is constructed as a fully functionalized DNA molecule driven by harvesting photon energy. The subject nanomotor is highly advantageous due to its simplicity, high sensitivity, and significant energy conversion efficiency.

In one embodiment of the present invention, a photoregulated single-molecule DNA motor (PSMM) is provided, wherein the PSMM comprises a DNA backbone and at least one azobenzene moiety (such as azobenzene phosphoramidite). The DNA backbone preferably has a stable hairpin structure including a stem duplex and a single-strand base loop, wherein the azobenzene moiety (or moieties) is inserted to an arm of the stem duplex of the hairpin DNA backbone.

According to the subject invention, the terms azobenzene and azo are interchangeable and refer to a wide class of molecules that share the core azobenzene structure. The core azobenzene structure is composed of two phenyl rings linked by a N=N double bond. Azobenzene molecules of the subject invention are those molecules that reversibly isomerizes between *trans*- and *cis*-form under particular wavelengths of light. In one embodiment of the invention, azobenzene isomerizes between *trans*- and *cis*-form under UV and visible light irradiation.

The present invention provides novel and advantageous single-molecule DNA nanomotors regulated by photons. The DNA nanomotor comprises a DNA backbone composed of polynucleotides and at least one azobenzene moiety. The DNA backbone has a hairpin structure, wherein the DNA backbone is composed of at least three segments of polynucleotides. Polynucleotides from the first segment and second segment are separated by polynucleotides of at least the third segment of the DNA backbone. The polynucleotides from the first and second segment associate with one another to form a stem duplex and the third segment forms a single-strand base loop of polynucleotides, thus forming the hairpin structure of the DNA nanomotor. To at least one segment of polynucleotides that form the duplex stem segment, at least one azobenzene moiety is attached.

Dehybridization (open state) and hybridization (close state) of a hairpin DNA structure is controlled by azobenzene moieties integrated on the DNA bases in the hairpin's duplex stem segment. The association (hybridization or close state) of the duplex stem segment occurs when tethered azobenzene moiety (or moieties) have *trans*-conformation under visible light irradiation. The dissociation (dehybridization or open state) of the duplex stem occurs when azobenzene moiety (or moieties) take on *cis*-conformation under UV light irradiation. Because of its reversible extension-contraction behavior, the open-close cycle of the hairpin DNA backbone is considered a molecular motor.

The nanomotor of the invention comprises a movable DNA backbone that moves substantially in an inchworm fashion as a result of biomolecular interaction of DNA base pairs and azobenzene moiety response to irradiation. The nanomotors of the invention will permit the construction of a wide variety of devices. Examples of devices that might incorporate the nanomotors of the invention include valves of microfluidics, gates for controlled release of substances, movement of a shutter for control of optical pathways, or synthetic chromatophore. A nanomotor of the invention will generally be less than 20 nanometers in length, depending on the size of the DNA backbone and design of the nanomotor.

The nanomotor of the subject invention is particularly advantageous in that it is driven by photons without any additional DNA strands as fuel. The incorporation of photosensitive Azo-moiety successfully produces a reversible photoregulated DNA nanomotor. This clean, photon-fueled nanomotor holds promise for applications that require the conversion of photonic energy into other forms of energy, such as mechanical movement. In addition, photoregulated DNA nanomotors can be easily manipulated and reproduced, as the synthesis and operational strategy for application of the nanomotors are simple. Thus, the intramolecular interaction that occurs in this system circumvents the complexity introduced by intermolecular reactions that occurred in previous DNA nanomotors. Compared to concentration-dependent linear DNAs, the concentration-independence of the subject PSMM allows high efficiency at any concentrations and situations that would otherwise be difficult to achieve as a result of interference by multi-molecular interactions. Continued investigation of the relationship between hairpin structure and energy conversion efficiency will further elucidate the mechanisms underlying the energy conversion process. Meanwhile, the coupling of nanomotors to useful applications for force production on the nanoscale remains a significant challenge.

In a preferred embodiment, a single-DNA nanomotor driven by photons is provided. The single-molecule nanomotor was constructed as a fully functionalized DNA molecule driven by harvesting photon energy. An illustration of the photoswitchable single-molecule DNA motor (PSMM) is shown in **Figure 1**. In **Figure 1**, a PSMM with three azobenzene moieties inserted to the stem duplex on the arm labelled by quencher is shown. The structural change of DNA displayed a contraction (CLOSE) state when tethered azobenzene moiety takes *trans*-conformation under visible light irradiation and an extension (OPEN) state when

azobenzene takes *cis*- conformation under UV light irradiation. The L1 is the average size of hairpin structure based on the distance between F/Q pair, and L2 is the average size of extended molecules based on persistence length of a single DNA strand.

The main components of the PSMM in **Figure 1** include the hairpin backbone, azobenzene moiety (such as azobenzene phosphoramidite) and fluorophore/quencher (F/Q) pair for signaling motor movement. Under UV and visible irradiations, the PSMM can reversibly switch conformation between open (dehybridization) and close (hybridization) states, respectively. The reversible extension (open) and contraction (close) cycle of the “arms” (forming stem duplex on close state) functions as a molecular motor because of the cycling and stretching actions by the DNA.

In order to demonstrate PSMM regulation by photons, a 31 base DNA was selected for synthesizing a simple nanomotor. This DNA has a stable hairpin structure including a 6 base pair stem and a 19 base loop, with a Fluorescein (FAM) at the 5' end and a Dabcyl quencher at the 3' end: 5' FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC TAG G-Dabcyl 3' (SEQ ID NO. 1) (underlined bases represent stem moieties). In the close state, the DNA forms a hairpin structure, and fluorescence is quenched due to the proximity of fluorescein and Dabcyl. Importantly, the fluorescence intensity is related to the distance between the F/Q pair, which indicates the close or open state of the PSMM movement. Therefore, the completely open state with fully extended structure will occur when complementary DNA (cDNA) is bound to the molecule with strong binding affinity, thereby disrupting the hairpin motor structure. This naturally results in maximum fluorescent intensity due to the largest separation between fluorescein and Dabcyl. In this embodiment, the F/Q was incorporated on the molecule with the intention of applying fluorescence variation to monitor the molecular structural changes. Hence, the difference in separation distance between the close and fully open states provides a functional “open” operational range for the molecular motor when the PSMM is driven by light irradiations in a reversibly contracting and extending movement (see **Figures 2A and 2B**). In other words, the open state fluorescence intensity will vary between the ranges due to limitations of extension capability of a single DNA strand. In this manner, the hairpin-to-extension structure exchange can be regarded as a single molecule's motion where the push-and-pull dynamic is achieved by photons if photon-sensitive objects are tethered on the two ends of the DNA strand. Thus, a well-controlled photoswitchable DNA nanomotor can be produced based on this design.

Reversibility of the PSMM

In order to reversibly control the close-open process of the hairpin DNA, at least one azobenzene moiety was incorporated onto the hairpin backbone to regulate the molecular structure by azobenzene isomerization upon photo irradiation. The single-molecule motor is in close state in normal conditions, since the azobenzene moiety (or moieties) takes the more stable *trans*- state in visible light, and the hairpin structure is maintained. When UV light is applied, the photons will initiate the Azo-isomerization from *trans*- to *cis*- conformation. In consequence, this conformational change destabilizes the stem duplex structure, allowing the dissociation of base-pairs in the duplex and extending the hairpin structure. This phenomenon can be regarded as a stretching movement of the motor. A subsequent visible light irradiation will rapidly isomerize the Azo- back to *trans*- conformation and reform the hairpin structure. This step can be regarded as a contraction.

As indicated in **Figures 2A and 2B**, in the open or extended state, Azo- takes on *cis*- conformation, while, in close state, tethered Azo- takes on *trans*- conformation. Thus, by utilizing the hairpin-to-extension exchange, the responsive function of these molecules by otherwise ordinary target-response is converted into a reversible movement function, making a highly efficient molecular motor possible. Moreover, the whole reversible motor function can be characterized by fluorescence variation induced by structural conversion, a convenient way to monitor the movement of the motor at this stage. Accordingly, a four-step structure cycle (*S1-S4*) driven by photons is illustrated in **Figure 8**. Photophysical study and experimental results on Azo-incorporated DNAs have suggested the order of these steps for a full cycle.

Figure 8 shows a photoregulation cycle of azobenzene-incorporated hairpin nanomotor (PSMM). Under normal conditions, the azobenzene takes the stable *trans*- state, both before and after visible light irradiation, and the PSMM remains in hairpin structure. However, the *trans*- azobenzene can be converted to the unstable *cis*- state when irradiated by UV light to produce hairpin structure intermediate *I* (*S1*, *II*) where the structure is about to transform with *cis*- state azobenzene destabilize the stem duplex. The *cis*- azobenzene aids in the dissociation of the stem duplex of the hairpin structure, and an extended single-strand DNA is formed (*S2*). The linear state PSMM is comparably more stable than *II* and can stay in the dark state for minutes. Under visible light irradiation, the *cis*- azobenzene can be switched back to *trans*- state very fast (within several seconds to minutes), and a linear

intermediate (*I2*) is formed (*S3*). *I2* favors a hybridization of complementary strands so that the hairpin structure reforms (*S4*). Both of the *S3* and *S4* are much faster than *S1* and *S2* due to stability of azobenzene in different conformation. The cycle is reversible by repeated UV and visible light irradiation in turn.

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Number of Azo moieties in a PSMM and the Impact on the PSMM

In certain embodiments of the invention, the deliberate design of hairpin molecules with specific secondary loop structures leads to stem modification that might have a direct impact on stem duplex stability. According to the subject invention, several azobenzene moieties are incorporated to the PSMM stem in different amounts and at different positions. Six designed motors were named PSMM1 to PSMM6 (**Figures 2A and 2B**). In **Figure 2A**, the available positions for azobenzene moiety incorporation in stem moiety on each end is illustrated. **Figure 2B** illustrates the sequences of the six types of PSMM (PSMMs 1-6). PSMM1, PSMM2, and PSMM3 (or named PSMM1-3 for all three types), where PSMM 1-3 are hairpin structures with one to three azobenzene moieties on the 3' end, and PSMM4, PSMM5, and PSMM6 (or named PSMM4-6 for all three types) are hairpin structures with one to three azobenzene moieties on the 5' end. The blue bases are stem moiety; red are azobenzene unit, and black bases are on loop moiety. All of these PSMMs were then screened by fluorescence changes under light irradiations and the photoconversion of these structures were studied.

20

Since the azobenzene structure is similar to Dabcyl quencher, the quenching effect was expected when Azo-moieties were spatially proximal to the fluorophore. A simple fluorescence measurement revealed that PSMM 4-6 showed no fluorescence increase when cDNA was added, while PSMM 1-3 displayed the expected "on-off" fluorescence on the motor's open-close cycles. The melting curves displayed the difference of these nanomotors on fluorescence recovery in terms of stem duplex opening (see Example 6 below, **Figure 7A**). **Figure 7A** shows the melting temperature profiles of PSMM 1-6 (represented by the orange, sky blue, purple, blue, green and brown curve, respectively) under the same conditions (the melting temperatures of PSMM 1-3 were summarized in **Figure 12**). The measurements were repeated twice for each sample. The concentration was 100 mM in buffer solution (buffer: 20 mM Tris buffer pH 8.0, Na⁺: 20 mM, Mg⁺ mM).

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Without being constrained to any one theory, it is hypothesized that the lack of fluorescence response in these Azo-incorporated PSMMs arises from fluorescence quenching by azobenzene (with a chemical structure similar to Dabcyl, hence a similar quenching mechanism). The fluorescence and quenching mechanism is depicted in **Figure 7B** with possible energy transfers between fluorophore and quencher. **Figure 7B** shows suggested mechanisms of photoresponse of PSMM 1-3 (left) and PSMM 4-6 (right). Pre-treatment of visible light ensured the hairpin structure formation and the lowest fluorescence background at the starting point.

Significant fluorescence enhancement for PSMM1-3 was observed when cDNA was added, thus confirming the selective quenching from both Dabcyl and azo on FAM at close distance. Because of the difficulties of monitoring structural changes by fluorescence intensity variation, observation was limited to PSMM1-3 with Azo-incorporated from the quencher end. By this selection of positions of Azo- incorporation, the photoregulation cycle of the PSMMs was investigated by comparing fluorescence intensities from the close state to the fully open state. It may be recalled that the distance variations between the close and fully open states provides a necessary and functional “open” operational range for the subject molecular motors because the PSMM is driven by light in a reversible contracting and extending movement.

To accomplish this motor function with optimization, two main factors were investigated that were suspected to impact the efficiency and reversibility of PSMM1-3 nanomotors: buffer solution and light sources (see Examples 1 and 2 below). A buffer solution was chosen after optimizing the balance to favor both open and close states: 20 mM Tris-HCl pH 8.0, 20 mM NaCl, 2 mM MgCl₂. Additionally, a 60W table lamp with a 450 nm filter was chosen as the visible light source in all cases, and a portable 6W UV light source (irradiated at 350 nm) was chosen for the UV light source. Reversible photoregulation was carried out by repeated irradiations at 450 nm and 350 nm at fixed time periods, followed by emission scans (λ_{ex} = 488 nm). Although a higher power UV light source over the one selected would help to improve the *trans*- to *cis*- conversion, its use raises serious problems in terms of damaging the DNA structure and photobleaching the fluorophore, which have both occurred with these lamps after long-term irradiation.

With the selected light sources and buffer conditions, PSMM1-3 were tested as described in below. All three nanomotors displayed different fluorescence recovery after

visible irradiation followed by UV irradiation (**Figures 3A-C, Figure 12**). **Figures 3A-C** illustrate the fluorescence spectra of PSMM1-3 (λ_{ex} = 488 nm), respectively, from top to bottom after irradiation under a 6W UV lamp (350 nm) and a 60W desktop lamp with 450 nm filter at 25 °C. All other conditions are the same. The blue curve is pure DNA in buffer solution (100 nM); green line is with five times of cDNA; and red line is after UV irradiation. Buffer: 20 mM Tris buffer pH 8.0, Na⁺: 20 mM, Mg²⁺: 2 mM, [PSMM3] = 100 nM, [cDNA] = 500 nM.

As expected, more azo-incorporations, such as those incorporated in PSMM3, resulted in higher fluorescence recovery, indicating that increasing of the amount of Azo-moiety can introduce a higher impact from azobenzene isomerization to hairpin structure stability. Excess amount of cDNA by five-fold was added at the end for each type of nanomotor after photoregulation in order to compare the fluorescence intensity of all PSMMs at fully open state (induced by cDNA). By setting the fluorescence intensity when azobenzene takes the *trans*- form as a baseline (0%) and the intensity after addition of excess amount of cDNA as 100%, the number of PSMMs in the open state for each photoregulation process could be estimated. With these embodiments, the fluorescence intensity was set on close state at 488 nm as the baseline (blue curves) and the fully open state (green curves) as 100%. A fluorescence recovery parameter was then established, Recovery (%), based on the three states as defined below in order to evaluate the close-open conversion efficiency:

$$\text{Recovery (\%)} = (I_{uv} - I_0) / (I_t - I_0)$$

I_{uv} is the fluorescence intensity of DNA solution after UV irradiation; I_0 is the fluorescence intensity after visible irradiation; I_t is the fluorescence intensity of DNA solution after adding extra cDNA. The higher the recovery value, the higher the amount of molecules that will be found in open state driven by photonic energy, and the better the efficiency of the conversion from close to open state. Since the reversible open to close conversion step is very fast for each of the PSMMs disclosed in this embodiment, the Recovery percentage is focused on close to open conversion and the motor efficiency assessed accordingly.

The Recovery (%) was used to compare the efficiency of energy conversion from photonic energy to molecular motion for PSMM1-3 under the same conditions. Results showed approximately 14.2% of recovery from *trans*- to *cis*- for PSMM1, 26.3% for

PSMM2, and 54.7% for PSMM3. This result supported the assumption that multiple azobenzene incorporation will introduce higher impact to hairpin structure stability or photoregulation capability. The improvement of this Recovery (%) is, however, not proportional to the number of Azo- moiety, although it does gradually increase as the number of Azo- increases. Specifically, the triple Azo- nanomotor (PSMM3) displayed a much higher open-to-close ratio than PSMM1 and PSMM2, which supports this argument. Furthermore, the result is consistent with a previous study of the relationship between Azo-moiety and duplex association/dissociation conversion. Because the energy barrier of azobenzene isomerization from *trans*- to *cis*- is higher than *cis*- to *trans*- , the *trans*- to *cis*- conversion requires a long UV light irradiation time to drive this conversion. These results demonstrate that Recovery (%) can reach to about 60% ($\pm 3.0\%$) after 20 min of UV irradiation before photobleaching of FAM fluorophore appears to become a serious problem. Thus, for realistic usage, which balances input and output energy, a UV irradiation time from 2-10 min is satisfactory for the PSMM running in numerous cycles without losing apparent functionality and efficiency.

Theoretical calculation of the extension and contraction forces is based on free energy and extending capability of single- and double-stranded DNA. The force was estimated based on Gibbs free energy and the distance variation from close and open structures. The L_1 and L_2 are approximately 10.2 and 2.2 nm and give the estimated values of two forces of 1.5 and 3.1 pN (the average forces based on an irradiation timeline), respectively (see Example 7 below). Noticeably, the single-stranded DNA has the nature of forming random coil in solution instead of an extended structure. With this embodiment, the sizes were estimated by free energy and persistent length when applied. The actual size variation will determine the potential motor strength. The total input photon energy on extending the nanomotor can be calculated by UV lamp power (0.197 mW) and irradiation time (5 min) with $E_{input} = P s = 5.91 \times 10^{-2}$ J. Based on previously calculated extension force (1.5 pN) and distance (8 nm), each nanomotor has the extension work of 1.2×10^{-20} J ($w_{output} = Fs$). The extension work can be regarded as the output mechanical energy. Therefore, the total output work W_{output} for each type of nanomotor under the specified conditions can be calculated by $W_{output} = [(\text{Extended molecule})\%] \times [\text{Total molecule number } (100 \text{ nM} \times 120 \mu\text{L} \times N_A = 7.22 \times 10^{12})] \times [w_{output}]$: PSMM1 (14.2%): 1.23×10^{-8} J, PSMM2 (26.3%): 2.28×10^{-8} J, and

PSMM3(54.7%): 4.74×10^{-8} J. The energy conversion efficiencies (E_{input}/W_{output}) are: 2.09×10^{-7} , 3.85×10^{-7} and 8.02×10^{-7} , respectively.

Figure 4 shows the reversibility of the PSMM3 nanomotor for ten rounds of close-open cycles. Specifically, Figure 4 shows cycling of close-open from Vis/UV irradiations at 25 °C by repeated visible and UV irradiations. Vis (450 nm): 1min; UV (350 nm): 3 min. Fluorescence intensities at the maximum emission (525 nm) were recorded immediately after each irradiation. Buffer: 20 mM Tris buffer pH 8.0, Na^+ : 20 mM, Mg^{2+} : 2 mM, [PSMM3] = 100 nM, [cDNA] = 500 nM. For each cycle, 1 min visible irradiation and 3 min of UV irradiation were applied. Compared to an approximate 40% decrease in cycling recovery for the previous DNA-fueled nanomachines under more favorable conditions (higher temperatures, more Azo- ratio), the cycling of PSMM3 maintains its recovery consistency and has no tendency to decrease after ten cycles. Additionally, all the cycles were performed at room temperature (25 °C) where the close state is more favored. As such, it can be predicted that conversion efficiency of the subject nanomotor can be further improved at higher temperatures (close to T_m) as the stem is close to the transition point from duplex to single-strand state. This result demonstrates that the DNA hairpin nanomotor possesses high close-open conversion efficiency and molecular stability under current operating conditions. Repeated nanomotor cycles displayed no obvious decomposition of the motor (up to 20 cycles; data not shown). Overall, these results demonstrate a long-lasting molecular motor with high conversion efficiency using a clean energy input.

A comparably high fluorescence recovery seems very interesting from the perspective of energy application since it is related to energy conversion efficiency. That is, under the same operating conditions for similar molecular nanomotors labeled with F/Q, a higher fluorescence variation illustrates that higher ratios of molecules are driven from one state to another state. This comparison is applicable to hairpin structures as well as linear DNA strands as long as the conditions are normalized and correlated parameters are established. In this case, the previously defined Recovery percentage can be used as the indicator of energy conversion efficiency. Under these conditions, a higher Recovery percentage means that comparable DNA motor systems can convert more absorbed energy to drive the structure changes. This efficiency can therefore be related to the capability of fulfilling motor-like function. Although it is possible that different molecular structures might absorb different

amounts of photon energy under the same conditions, it can still be counted as part of the overall energy conversion capability for specific molecules.

In order to compare this conversion efficiency between the subject hairpin single-molecule nanomotors and previously investigated models, a series linear DNAs were designed, as well as another hairpin structure, using melting temperature (T_m) as the correlated parameter for comparison. The details of the rationale underlying this assumption are discussed below. The definition of T_m for short DNA duplex can be expressed by:

$$T_m (^{\circ}\text{C}) = \Delta H^{\circ} / [\Delta S^{\circ} + R \ln C_{\text{DNA}}] - 273.15$$

ΔH° and ΔS° are the melting parameters; R is the ideal gas constant; C_{DNA} is the molar concentration. For DNA sequences with the same concentrations, C_{DNA} is the same, while ΔS° varies slightly. Therefore, T_m is approximately proportional to ΔH° , which is the energy absorbed by the DNA molecule to dissociate duplex structures. Therefore, T_m value can be regarded as the capability of absorbing sufficient energy to dissociate DNA duplex structures. For DNA nanomotors involved in duplex dissociation, T_m can be used as the standard by which to evaluate the structure conversion or motor operation capability by absorbing external energy. For different duplex structures, such as hairpin and linear duplex with the same T_m value, they are expected to display the same Recovery percentage under the same photoregulation conditions.

Optimization of PSMM design and synthesis

In addition to identifying the optimal number of photoresponsive moieties (such as Azo moieties) in a PSMM, the subject application contemplates optimizing the design and synthesis of PSMMs via modification of the bases comprising the stem structure of the PSMM and of the position of the photoresponsive moieties to provide the highest efficiency of conversion from an “open” to “close” state (see **Figure 14B**).

Since the typical DNA base pairs (A-T and G-C) have different hybridization affinities (e.g. G-C > A-T), the relative number of G-C base pairs can be adjusted as well as the overall length of the DNA hairpin sequence (in particular, the stem structure of the PSMM hairpin sequence). Optimization of the number of G-C pair numbers in the stem structure of PSMMs (see, for example, **Figure 14A**) has the potential for high impact. The

stability of PSMM self-hybridizing DNA nanomotors can be determined by melting temperature (T_m) and free energy difference between the “close” and “open” states or conformation, ΔG_{closed} and ΔG_{open} . By adjusting the relative number of G-C base pairs and overall length of the DNA hairpin sequence, PSMMs can be designed with different ΔG_{closed} and ΔG_{open} . As understood by the skilled artisan, data for ΔG of both open and close states in a PSMM nanomotor of the invention can be easily established using a variety of known DNA analysis techniques .

The DNA sequences described herein (see **Figures 2B** and **7B**) contain 4 G-C base pairs, and the calculated $\Delta G_{closed} = -2.75 \text{ kcal/mole}$ and $\Delta G_{open} = -59.04 \text{ kcal/mole}$. Therefore, the $\Delta(\Delta G) = \Delta G_{closed} - \Delta G_{open} = 56.29 \text{ kcal/mole}$. Systematically altering the relative number of G-C base pair numbers in the hairpin stem and the length of the sequences will yield a PSMM nanomotor sequence with the largest $\Delta(\Delta G)$ between the two states and, hence, the largest tensile force and force displacement.

Similar to sequence optimization (e.g., altering relative number of G-C base pairs), PSMM nanomotor designs can be systematically optimized by varying the azobenzene position and number to obtain the most efficient energy conversion and structural change, (i.e., the largest force displacement). As shown in **Figure 14B**, the DNA hairpin stem contains six possible positions for the insertion of azobenzene. Once the optimized base sequence has been obtained as described above, the motor can be redesigned with the number and position of azobenzenes giving the largest structural disturbance to the closed state relative for a constant photon input. This design will give the highest efficiency for conversion of the nanomotor from the closed to the open state by photon absorption, thus improving the efficiency of a single PSMM DNA nanomotor and producing a larger force.

Linear DNA probe comparison

Two groups of linear DNAs bearing Azo- and Dabcyl and their cDNA bearing FAM have been synthesized (L12-1, L12-2 and L12-3/L12-cDNA; L10-1 and L10-2/L10-cDNA, **Figures 9A-B**). **Figure 9A** shows the sequences of 12 bases Azo-incorporated linear sequences and **Figure 9B** shows 10 bases azo-incorporated linear sequences and cDNA. The sequences of linear DNAs are designed in a manner similar to the truncation of the hairpin DNA from the 3' end and have a T_m comparable to that of PSMM1-3 in order to compare the fluorescence recovery and quality of motor function (**Figure 12**). For L12-1 to L12-3, all

three linear DNAs have almost the same T_m (53.7.2~55.2 °C) as PSMMs (55~57 °C). Therefore, they should be able to dissociate the duplex structure with the same recovery as PSMMs under the same conditions. As mentioned above, it is possible that the energy absorption capabilities between heat and light are different for hairpin structure and linear structures. If that is the case, then the subject hairpin-structured-based nanomotor has the advantage over linear DNAs of absorbing higher light energy that can be converted to molecular movement. L10-1 and L10-2 were intentionally selected with much lower T_m (47.5~48.6 °C) than PSMMs where they are expected to display higher recovery.

The fluorescence spectra of all the linear DNAs under the same conditions as PSMM are displayed in **Figures 10A-E**, and the recovery values are summarized in **Figure 12**. Under the same conditions, the recovery is from 14.2 to 54.7% for PSMM motors compared to 2.9-11.5% for L12 DNAs and 6.4% to 13.8% for L10 DNAs. These findings indicated that neither linear DNAs (L12s) with similar thermal response nor those with thermal response at lower temperature (L10) have the same response to light energy as PSMMs. With the assumption that these linear DNAs can do the same work as PSMM (actually less than PSMM due to smaller size changes), the energy conversion efficiencies based on Recovery percentage values for L12 DNAs (L12-1, L12-2, L12-3) are 4.26×10^{-8} , 8.38×10^{-8} and 1.69×10^{-7} , and for L10 DNAs (L10-1, L10-2) are 9.26×10^{-8} , and 2.03×10^{-7} . As a result, linear DNAs cannot produce a comparable recovery by absorbing light energy from the same input light energy and display less photon to mechanical energy conversion efficiency. The energy conversion efficiency of PSMM3 is 4.76 times over L12-3 and 3.96 times over L10-2. Moreover, the subject hairpin structure has the same or fewer Azo moieties tethered on the backbone than linear DNAs which theoretically absorb fewer photons per molecule, yet display a higher Recovery percentage.

These results strongly validate the expectation that the PSMMs have much higher energy conversion efficiency than linear DNA structures and that this directly results from the nanomotor structure. Additionally, the subject highly efficient nanomotors are operated under mild conditions of room temperature, illustrating that improvement of recovery can be further achieved by simply increasing the operational temperature. Although the recovery for PSMMs was not systematically examined at temperatures closer to their T_m , higher conversion recoveries were observed when the operational temperature was increased.

The main factor contributing to the high conversion efficiency is the hairpin structure of the motor and the intramolecular interaction arising from the single-stranded DNA. To investigate the influence of hairpin structure alone, another Azo- incorporated hairpin molecule was synthesized with a substitution of polyT loop from PSMM loop moiety. This hairpin structure also displays high response to photon energy, although it is lower than PSMM3 (see Example 8 below, **Figure 11**). This result suggests that carefully designed Azo-nanomotors with hairpin structure are intended to have high response to light energy.

Molecular interaction is also believed to play a key role in the significance of PSMMs. First, for linear DNA nanomotor systems, at least two pieces of DNA are needed to trigger a motor movement. Therefore, the hybridization process is based on an intermolecular interaction, and the concentration of each DNA strand contributes to the overall hybridization rate and, hence, the motor efficiency. For PSMMs, there is only one DNA strand that functions as a single-molecule motor. Thus, the hybridization within the PSMMs takes place by intramolecular interaction. The photoregulation of PSMM 3 and ten base linear DNAs (L10-1) were compared for their photocontrollability (**Figure 13**) at different concentrations. **Figure 13** is a table illustrating the conversion efficiency of PSMM3 and L10-1 in different concentrations. The constant distance of paired hybridizing moieties within a single hairpin-structured molecule produces a type of concentration-independent nanomotor which maintains a high conversion efficiency (from 54.7 to 44.7% on recovery), while, on the other hand, the linear L10-1 displays a significant concentration-dependence effect (6.3 to 0.7%). Therefore, the hairpin nanomotors are expected to overcome the low efficiency problem experienced by linear DNA nanomotors, especially in a situation where high density DNA motor packing is required.

Synthesis and Processing of Three-Dimensional PSMM Nanomotor Assembly

In aqueous solution, the random motion of individual PSMM DNA nanomotors will cancel the individual forces generated by each nanomotor. According to the subject invention, a plurality of PSMM DNA can be associated with one another to form a space figure of numerous nanomotors. Any known space figure can be derived from PSMM DNA nanomotors of the invention. Contemplated space figures to be derived from PSMM DNA nanomotors of the invention include, but are not limited to, polyhedrons (such as cubes,

prisms, and pyramids), spheres, cylinders, and cones. Such arrangements enable controllable collection and orientation of force from numerous nanomotors.

Figures 15A-D illustrate the bottom up self-assembly of a PSMM DNA multi-nanomotor cube. The diagram of such a design for a PSMM DNA space figure of a cube is depicted in **Figure 15D**. This structure can be the basic building block for a macro-size nanomotor assembly, as illustrated in **Figure 16C**. Such structures enable controlled collection and orientation of forces from each DNA nanomotor (for example, orientation of forces in the same direction).

As shown in **Figure 15A**, there are three main components needed to construct a PSMM DNA space figure of a cube via bottom-up self-assembly techniques under proper conditions. **Figure 15B** illustrates a how the individual DNA single strands illustrated in **Figure 15A** recognize and associate with each other to form three-point star tiles (see **Figure 15C**). The three-point-star tiles can recognize and associate with each other (for example, hybridization of complimentary strands of polynucleotides) to self-assemble into a multi-nanomotor space figure, such as a cube (**Figure 15D**).

Previous research has demonstrated that one-component star-shaped DNA motifs can assemble into a range of geometrically well-defined polyhedra including tetrahedra, dodecahedra, and buckyballs from 3-point-star motifs, and icosahedra and large nanocages from 5-point-star motifs. These structures are achieved by carefully balancing the flexibilities and the rigidities of the motifs and controlling the DNA concentrations. In this case, each vertex consists of a star tile and the separation between any two adjacent vertices is integral numbers of turns. With such a separation process, all tiles face to the same side and the tiles' intrinsic curvatures accumulate at the identical direction. This behavior can promote the formation of closed structures as illustrated instead of other plain structures such as extended sheets. Herein, polyhedral faces are restricted to consist of only even numbers of vertices and to assemble predominately DNA cubic structures.

PSMM DNA cube (**Figure 15D**) allows four incorporated PSMM DNA nanomotors to extend/contract simultaneously and in the vertical direction under UV/Vis light irradiation (see **Figure 16A** (close or contracted state) and **Figure 16B** (open or extended state)). The motion of the four nanomotors induces a corresponding movement of the DNA cube vertically. Therefore, alternate irradiation by UV and visible light will cause the cubic DNA nanostructure to perform reversible and synchronized extension and contraction.

Using the DNA cube unit (see **Figure 15D**), a three-dimensional film can be constructed by stacking these units regularly to develop multiple layers of cubic nanostructures (see **Figure 16C**). The conformational change of individual nanomotors can be oriented in one direction simultaneously to generate considerable force. At this scale, it is feasible to combine this thin film with pressure sensitive materials, such as piezoelectric materials, to convert the force to electrical energy.

Conversion of Solar Energy to Mechanical and/or Electrical Energy using the PSMM Nanomotors of the Invention

The schematic structure of a device for the conversion of solar-to-mechanical energy using PSMM DNA nanomotor films of the invention and piezoelectric materials is shown in **Figure 17**. The PSMM nanomotor assembly and the piezoelectric materials are tightly attached together and the two outer sides are confined by two immobilized substrates, one of which is a transparent window to allow light penetration. The principle of this conversion is to use the force and displacement generated from a nanomotor assembly under light irradiation to initiate a stress in the piezoelectric substrate, which, in turn, generates an electrical potential across the piezoelectric material. Due to the rapid response of the piezoelectric material, alternation of UV and visible light irradiation can trigger repetitive stresses for continuous electric current production.

In an alternate embodiment, the force and displacement from a nanomotor assembly could be transferred into a hydraulic system. According to the subject invention, "molecular excluded volume" is defined as the occupied space of evenly dispersed individual molecules in a system. The molecular excluded volume is determined by the molecular structure and the surrounding environment. Changes in the molecular structure will induce excluded volume changes, which will cause the overall system volume to vary. This characteristic can be used to set up a pump-like device to convert light energy absorbed by PSMM nanomotors of the invention into mechanical energy. The main advantage of this strategy is that it bypasses the difficulty of assembling large numbers of DNA nanomotors in a highly regular and dense manner. The random motion of nanomotors can still be efficiently collected by the system via volume changes with controllable orientation.

An apparatus for measuring volume change using concentrated DNA nanomotors in aqueous solution is depicted as **Figure 18**. PSMM DNA nanomotors were used in highly

concentrated aqueous solution. A device equipped with a capillary to measure a small volume change was designed for use with a confocal microscopy. A large portion of solution is sealed in a transparent container and connected to a long capillary tube. The whole system is sealed with one end of the capillary open for free movement when a volume change takes place. The large portion of solution can be irradiated with UV or visible light to initiate the volume change induced by the close and open states of the PSMM DNA nanomotors, and a confocal microscope is used to monitor the volume change by observing the solution level in the capillary.

In another embodiment, instead of using a solution of PSMM DNA nanomotors, a volume change can be maximized by using a polymer gel. As shown in **Figure 19**, by tethering PSMM DNA nanomotors to a hydrogel system, the sol-gel conversion can be controlled by nanomotor motion triggered by light irradiation. The volume change can be observed by using a capillary and confocal microscopy, as shown in **Figure 20**.

Materials and Methods

Chemicals and Reagents

The chemicals for synthesis of phosphoramidite monomer were purchased from Aldrich Chemical, Inc. The materials for DNA synthesis, including CPG columns and reagents for DNA modification and coupling, were purchased from Glen Research Co. All the chemicals were used without further purification, except as otherwise explained. In this example, a 31 base DNA probe was selected. These DNA probes were used to synthesize photoswitchable single molecular DNA motors (PSMM) of the invention. The PSMMs were synthesized by a DNA synthesizer from 3' end to 5' end, starting with CPG column labeled with Dabcyl quencher (Dabcyl CPG) and with FAM fluorophore coupled on 5'. The target cDNA for the PSMMs is a partially complementary strand, with the sequence complementary to the PSMMs from 3' end throughout the entire loop. The linear DNAs were synthesized with the same Dabcyl CPG on azobenzene-incorporated sequences, and general bases were CPG-coupled with FAM on 5' end for the complementary sequences.

Instruments

The purification of chemical compounds was performed by glass column for the silica gel chromatography and identified by thin layer chromatography (TLC) plate (silica gel

60F254; Merck) and NMR spectrometer (Mercury 300). An ABI3400 DNA/RNA synthesizer (Applied Biosystems) was used for all the DNA-related synthesis. The purifications were carried out on a ProStar HPLC system equipped with gradient unit (Varian) with 18 column (Econosil, 5U, 250 × 4.6 mm) (Alltech Associates). The characterizations of all DNAs on concentration were performed with a Cary Bio-300UV spectrometer (Varian) by calculating the absorbance of DNA at 260 nm. The melting properties were studied with MyiQ single-color RT-PCR system (Bio-Rad). A Fluorolog-Tau-3 Spectrofluorometer with a temperature controller (Jobin Yvon) was used for all steady-state fluorescence measurements. The samples were loaded with quartz cell for fluorescent spectrum (Starna Cells, Inc.). The UV light source was a portable 6 W UV-A fluorescent lamp (FL6BL-A; Toshiba), and the visible light source was a general table lamp with a 60 W lamp and optical filters (Asahi Technoglass).

Photoregulation of photoswitchable single molecular DNA motor (PSMM)

The phosphoramidite monomer was synthesized from protocol of Asanuma *et al* with minor modifications (see Example 3 below). The synthesis and purification of PSMMs and linear sequences followed usual procedures, except as otherwise mentioned (see Example 4 below). The concentration of each DNA was calculated by the absorption at 260 nm by UV spectrometer. The melting curves were measured with a PCR machine with the same buffer solution for all the measurements (see Example 6 below). For comparison, PSMMs were diluted to equal concentration for the entire experiment at room temperature (25 °C) using the same buffer solution. For the photoregulation of PSMMs, irradiations by UV light at 350 nm and visible light at 450 nm were applied for all experiments, although the irradiation times varied, as described. Fluorescence intensity was recorded under excitation at 488 nm. Once the temperature is set at 25 °C and stabilized, the quartz cell with samples is maintained at the set temperature for at least 5 minutes before each test. The steps for UV/Vis irradiation measurements are listed below:

The sample is diluted and added in quartz cell.

The quartz cell is set in a holder and maintained in place for 5 min.

Irradiation is performed with visible light at 450 nm for 1 min.

Fluorescence spectrum is measured (excited at 488 nm).

Irradiation is performed with UV lamp at 350 nm for 5 min.

Fluorescence spectrum is again measured (excited at 488 nm).

Steps 1 to 6 are repeated to confirm the reversibility of the photoregulation.

The intensity changes are compared after several rounds with a 5-fold addition of cDNA. For optimization experiments, the UV light varied from 1 min to 20 min. An efficiency of around 50% for PSMM3 was obtained at 10 min UV irradiation time. Longer periods can improve the efficiency slightly, but prolonged irradiation under current portable UV lamp over 30 min tends to result in photobleaching the fluorophore and decomposing the structure (data not shown). For the reversibility test, the UV irradiation time was set at 3 min for all the tests, except as otherwise noted.

Photoregulation of Linear DNAs

The 12 and 10 bases FAM DNAs were designed and synthesized as described in Example 5 below. They were first diluted in the same buffer solution as the hairpin molecules with a final concentration of 100 nM and set for 5 minutes until stable. An emission scan was performed with 488 nm excitation. 1.5-fold of each azobenzene-incorporated DNA was then added to the cell and stabilized for 5 minutes until the two strands were fully hybridized, followed by a second emission scan. The reversible irradiations with UV/Vis light were obtained by repeating steps 3-7 established for photoregulation of PSMMs.

The following examples illustrate materials and procedures for making and practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted. It will be apparent to those skilled in the art that the examples involve use of materials and reagents that are commercially available from known sources, e.g., chemical supply houses, so no details are given respecting them.

EXAMPLE 1—Buffer optimization

The buffer solution condition was optimized based on PSMM3 photoresponse under UV/Vis irradiation. Generally, a strong ion-strength buffer solution is able to stabilize the duplex structure (close state); it will also hinder the reverse process of destabilizing the duplex structure (open state). Therefore, proper ion strength is needed to balance this close-

open conversion by setting up conditions that favor both states equally. Salt concentration was used to adjust the ion strength. High salt concentration aids base pairing and improves the hybridization rate and stability of duplex (open to close), but it induces a uni-directional nanomotor with poor balance or reversible operation. On the other hand, a very low salt concentration of buffer solution will not be able to initiate a large amount of fully closed MB structure, although it does facilitate conversion between close and open states. Additionally, low salt concentration impedes the conversion rate kinetically. Therefore, a conventional MB buffer solution was used with main components of Tris-HCl (pH 8.0), NaCl and MgCl₂. The concentration of Na⁺ and Mg²⁺ from 1mM to 500 mM, and Tris-HCl concentration from 2 mM to 200 mM, was screened as a function of photoresponse of MB duplex with UV/Vis irradiation. The optimized buffer includes the following: 20 mM Tris-HCl (pH 8.0), 20 mM NaCl, and 2 mM MgCl₂. Under these conditions, PSMM3 nanomotor operation displayed a well-balanced rate of open and close states.

EXAMPLE 2—Light sources optimization

In order to examine the sensitivity of azobenzene isomerization to hairpin structure upon irradiation, two groups of light sources were selected. For both groups, a 60W table lamp with a 450 nm filter had enough power to trigger fast *cis*- to *trans*- conversion and was therefore chosen as the visible light source. A Fluorolog-Tau-3 Spectrofluorometer was chosen for group one as the UV light source, and a portable 6W UV light source (both irradiate at 350 nm) was chosen for group two. The power of the two UV light sources had been measured by power meter for group one with 0.028 mW (± 0.2) and for group two with 0.197mW (± 0.3) at the irradiated sample position. A ten-round test was performed with these two groups of light sources in the previously selected buffer solution. Reversible photoregulation was carried out by repeated irradiations at 450 nm and 350 nm, followed by emission scans ($\lambda_{\text{ex}} = 488$ nm). In all cases, the low power spectrofluorometer could not initiate a fluorescence variation over 5% with up to 20 minutes of irradiation. However, the high power portable light source was observed to drive the variation up to 60%, depending on buffer solutions and irradiation time. Therefore, the portable UV lamp was selected as the UV light source for the following experiments. In certain embodiments, an even stronger UV light source will help improve the *trans*- to *cis*- conversion, even though its use raises serious problems in terms of damaging the DNA structure and photobleaching the fluorophore.

Photobleaching was observed with the portable UV lamp for long-term irradiation over 30 minutes.

EXAMPLE 3—Synthesis of azobenzene phosphoramidite monomer (Azo-)

5 In order to obtain a photoregulated phosphoramidite monomer, azobenzene was selected for its reversible photoregulation property. D-Threoninol was chosen here as the linker for synthesizing optically pure diols. The synthesis routes of azobenzene-tethered phosphoramidite monomers are shown as **Figure 5**.¹ **Compound 1**. ¹H NMR (CDCl₃): δ 7.96-7.38 (m, 9H), δ 7.12 (d, 1H), δ 4.33 (m, 1H), δ 4.09 (m, 1H), δ 3.98 (d, 2H), δ 1.29 (d, 3H). **Compound 2**. ¹H NMR (CDCl₃): δ 8.00-6.78 (m, 23H), δ 4.25 (m, 1H), δ 4.17 (m, 1H),
10 δ 3.77 (s, 6H), δ 3.60 and 3.42 (dd, 2H), 1.23 (d, 3H). **Compound 3**. ¹H NMR (CDCl₃): δ 8.00-6.79 (m, 22H), δ 6.62 (d, 1H), δ 4.48 (m, 1H), δ 4.39 (m, 1H), δ 4.21-4.10 (m, 2H), δ 3.77 (s, 6H), δ 3.57-3.34 (m, 4H), δ 2.76-2.72 (m, 2H), δ 1.30-1.25 (m, 15H). ³¹P (CDCl₃): δ 149.

15

EXAMPLE 4—Synthesis and purification of hairpin molecules

Hairpin molecules were synthesized by using a DNA/RNA synthesizer ABI3400 (Applied Biosystems). A solid-phase synthesis method was used to couple FAM to the MBs' 5' ends. The synthesis started with a 3'-DabcyI controlled pore glass (CPG) column at 1
20 μmole scale. A routine coupling program was used to couple the normal bases from 3' end on DabcyI CPG. A proper amount of Azo- was dissolved in dry acetonitrile in a vial connected to the synthesizer (20 mg Azo- can make a single incorporation in the DNA at 1.0 μmole scale synthesis, generally Azo- coupling reagent can be prepared by dissolving in acetonitrile on 20 mg/200 μL). The coupling step can be performed in room temperature immediately
25 after the Azo- reagent preparation. See H. Asanuma *et al. Nature Protocols*, 2:203-213 (2007). It then can be regarded as a normal base for insertion in programming the synthesizer (**Figure 6**) with at least 600 second reaction time. **Figure 6** illustrates the incorporation of azobenzene to DNA sequences by DNA synthesizer. A coupling program of 900 second reaction time was applied to couple the 5' FAM fluorophore at the very end. After the
30 synthesis, the CPG substrate was transferred to a glass vial, and standard AMA (ammonium hydroxide: methylamine = 1:1) deprotection solution was added and incubated in water bath at 50 °C for 12 hours. After centrifuge to separate the solid beads from MB in the solution,

the clear supernatant was carefully collected. Then, the MBs were concentrated by ethanol precipitation. The precipitate was redissolved by TEAA solution (0.1 M) and delivered to reverse phase HPLC using a C18 column with a linear elution (with 30 min of gradient from 19% acetonitrile to 55%). The collected product was then vacuum dried, detritylated, and stored at -20 °C for future use.

EXAMPLE 5—Synthesis and purification of linear sequences

The 12 bps linear sequence: 5' GGTCGCGCTAGG (SEQ ID NO:2)-Dabcyl 3', 10 bps: 5' TCGCGCTAGG (SEQ ID NO:3)-Dabcyl 3', and their cDNAs, 5' FAM-CCTAGCGCGACC 3' (SEQ ID NO:4) and 5' FAM-CCTAGCGCGA (SEQ ID NO:5), were prepared with the same protocol by means of corresponding fluorophore and quencher coupling. For Azo-incorporated sequences, Dabcyl CPG was used for synthesis, and FAM fluorophore was labeled to cDNA. The purifications of all linear sequences followed the same protocol as MBs with reverse phase HPLC on a C18 column.

EXAMPLE 6—Characterization of PSMM

The concentration of each DNA was calculated by the absorption at 260 nm by UV spectrometer. The concentration of the unmodified PSMM can be calculated by Beer's Law according to the following formula: $c = A_{260} / d(\epsilon_{\text{nat}})$, where c is the concentration of modified DNA (M), A_{260} is the absorbance at 260 nm, d is the thickness of the cell (cm), and ϵ_{nat} is the molar extinction coefficient of the native DNA. The concentration of the Azo- PSMMs can be calculated according to the following formula: $c = A_{260} / d(\epsilon_{\text{nat}} + n\epsilon_{\text{azo}})$, where n is the number of Azo- in the modified MBs and ϵ_{azo} is the extinction coefficient of the azobenzene moiety ($4,100 \text{ M}^{-1}\text{cm}^{-1}$). See H. Asanuma *et al. Nature Protocols*, 2:203-213 (2007).

Thermal denaturizing profiles of PSMMs and linear DNAs were measured with RT-PCR. To cover all the possible transition states, the temperature was slowly increased under the rate of 1 °C/min from 10 °C to 80 °C. The buffer solution for all the measurements is identical: 20 mM Tris buffer pH 8.0, Na^+ : 20 mM, Mg^{2+} : 2 mM.

EXAMPLE 7—Calculation of extension and contraction forces

In order to obtain the extension and contraction forces, Gibbs free energy and the distance traveled between the two ends of stem duplex were introduced for calculation. The

free energies of extension and contraction processes are the same due to base pairing and departing and can be calculated based on Mfold with molecular beacon hairpin structure giving $\Delta G = -3.56 \text{ kcal.mole}^{-1}$. See H.; Allawi, J. Jr. SantaLucia, *Biochemistry*, 36:10581-10594 (1997). However, the forces generated by each process differ by the stiffness of the structures during each cycle. The contraction process starts from soft single-strand coil and ends with a rigid hairpin structure, illustrating that most of the free energy is converted to mechanical motion. The distance between the two arms in open state can be calculated by the length of base pairs, which is 10.2 nm. It was presumed that the distance in close state is the same as the dsDNA helix diameter of 2.2 nm and that the net "contraction distance" is approximately 8 nm for the 31mer. Therefore, the contraction force is 3.1 pN. The extension force is limited by the softness of single-strand structure from hairpin structure. The effective distance that the single-strand DNA can reach is determined by persistence length and can be estimated by previous study. See B. Tinland *et al. Macromolecules*, 30:5763-5765 (1997). The distance is in the range of 4-5 nm. An extension force was derived around 1.5 pN.

EXAMPLE 8—Open-close conversion efficiency

The ratio of opened hairpin structure *versus* the total amount of molecules is set as conversion efficiency which is represented by recovery percentage. The ratios of Azo- to bases in a molecule for different PSMMs are significantly different. Given the same length of DNA sequences, if the smaller amount of Azo- incorporation yields the same photoregulation capacity, the motor will possess a higher efficiency property under same energy input. Thus, for the 31 bases MB hairpin backbone, the maximum Azo- incorporation number is 3 in the stem, which gives an Azo-/base ratio of 9.7% with around 50% regulation capability (the conversion efficiency is about 50%, as shown in **Figure 4**). In comparison, the reported linear DNA photoregulated nanomachines with Azo-/base ratio of 37.5% have around 60% regulation capability at elevated temperature. See Liang, X. *et al. ChemBioChem*, 9:702–705 (2008).

Other reported Azo- photoregulated linear DNA probes always maintain a ratio around 45% for highly responsive photoregulation. See Liang, X. *et al. Tetrahedron Lett.*, 42:6723–6725 (2001); Asanuma, H. *et al. Chembiochem*, 2:39–44 (2001); Liang, X. *et al. J. Am. Chem. Soc.*, 124:1877–1883 (2002); Asanuma, H. *et al. Nucleic Acids Symp. Ser.*, 49:35–36 (2005). It can easily be concluded that photoconversion efficiency of the hairpin

nanomotors is much higher than that of the linear probes. This is simply because the PSMM designed in this report is a single molecule DNA nanomotor, and intramolecular hybridization and dehybridization is much more efficient. To better quantitatively assess the two types of nanodevices, a series of linear DNAs were designed and synthesized for comparison.

In order to compare the efficiency of these DNA nanomotors as a function of sequences and length, melting temperature (T_m) was introduced as the correlated parameter to make this evaluation. Melting temperature is the temperature at which an oligonucleotide duplex is 50% in both single-stranded form and double-stranded form. A general method estimates T_m from the nearest-neighbor two-state model, which is applicable to short DNA duplexes:

$$T_m (^{\circ}\text{C}) = \Delta H^{\circ} / [\Delta S^{\circ} + R \ln C_{\text{DNA}}] - 273.15$$

where ΔH° (enthalpy) and ΔS° (entropy) are the melting parameters calculated from the sequence and the nearest-neighbor thermodynamic parameters (see Griffiths, *J. Chem. Soc. Rev.*, 1:481-493 (1972), R is the ideal gas constant ($1.987 \text{ cal} \cdot \text{K}^{-1} \cdot \text{mole}^{-1}$), and C_{DNA} is the molar concentration of a DNA.

Three 12 bases linear DNAs/cDNAs bearing Azo- and Dabcyl and their cDNA-bearing FAM have been designed and synthesized (L12-1-3, L12-cDNA, Scheme S4 A). Based on M-fold calculations, experimental results show the T_m s of the L12-1, L12-2, and L12-3 linear DNAs as 55.2°C , 54.8°C and $53.7^{\circ}\text{C} \pm 0.2$, respectively. These values are close to those of PSMMs 1-3, with the latter value very close to PSMM3 (55.0°C , **Figure 12**). **Figure 12** is a table illustrating the comparison of all DNA sequences with and without Azo-incorporation. Experimental measurements of all linear probes with their cDNA have been performed with variation within 1°C (data not shown).

Multiple Azo- moieties have been incorporated into the L12 sequences from 3' to 5' and at the same positions as PSMM1-3, with Azo-/base of 25%, 33.3%, and 41.7%, respectively. The L12-1 has triple Azo- incorporation, which is the same as PSMM3, but with a high Azo-/base ratio (50%). Based on the comparison standard of relationship between T_m and duplex stability set above, PSMM3 and L12-1 should have the same stability of duplex structure and should be able to absorb the same amount of photons (both have three Azo- for

each molecule). If the conversion efficiency is identical for both types of Azo- molecules, the same fluorescence recovery percentage was expected for L12-1 and PSMM3. L12-2 and L12-3 have slightly lower T_m , but since they have more Azo- than L12-1 and PSMM3, a higher recovery is expected for these two molecules. The photoregulation properties of all L12s are displayed in **Figures 10A-C** under the same conditions (buffer, temperature, DNA concentration, and UV-Vis irradiation) as those being used for hairpin PSMM3. **Figures 10A-C** show fluorescence spectra of L12-1, L12-2 and L12-3 (λ_{ex} = 488 nm) after UV/Vis irradiations. All conditions are the same as PSMM 1-3. All three linear DNAs display reversible photoregulation capability with different efficiencies: 2.9% for L12-1, 5.7% for L12-2, 11.5% for L12-3. Each linear DNA was photoregulated by UV/Vis for five cycles, and each displayed reversible photoregulation for all five cycles.

The fluorescence recovery of the L12 DNA, when compared with the PSMMs, demonstrates that the PSMMs have much higher efficiency response to photon energy. Since both PSMMs and linear DNAs have the same nuclear acid unit and Azo- components, the main factor contributing to their variable efficiencies is the difference between their respective structures. Specifically, the structure of the PSMM is folded hairpin where loop moiety affects the stability of stem duplex, while linear DNAs have extended duplex structure which is only affected by strand exchange. The short stem duplex of PSMM3 with triple Azo-incorporation is highly sensitive to conformational changes of Azo- isomerization, resulting in a 54.7% change in conformation. In contrast, the linear DNAs only seem to unzip from the end incorporated with multiple Azo- with partial duplex structure so that a complete departure of their complementary DNAs is unlikely (2.9% for L12-1). Moreover, even with the saturated Azo- loading of linear DNAs (L12-3), the duplex dissociation is still very low (11.5%) at room temperature. Therefore, based on the mechanism of fluorescence variation, there are fewer linear DNA duplexes dehybridized under these experimental conditions compared to PSMM molecules. Also, the low efficiency of linear DNAs is a function of the T_m s on both *trans*- and *cis*- conformations, which are much higher than room temperature $T_{(RT)}$: $T_{m(trans-)} > T_{m(cis-)} > T_{(RT)}$, whereas the T_m s of PSMMs, when azobenzene takes *cis*- conformations, are significantly lowered and less than room temperature: $T_{m(trans-)} > T_{(RT)} \geq T_{m(cis-)}$. This result demonstrates that hairpin-based nanomotors are energy efficient motors compared with motors based on linear DNAs.

Ten-base linear DNAs (L10-1, L10-2) incorporated with three and four Azo-, respectively (**Figure 12**) were synthesized. The T_m s of these two DNAs are nearly 8 degrees lower than PSMM3. It was expected that these linear DNAs would perform better under light cycling than L12 DNAs. Both L10-1 and L10-2 were photoregulated at the same conditions as PSMM3. The results displayed higher regulation efficiencies of 6.3% for L10-1 and 13.8% for L10-2 (**Figures 10D-E**). **Figures 10D-E** show fluorescence spectra of L10-1 and L10-2. The brown curve is DNA in buffer solution after visible irradiation; blue line is with five times of cDNA; green line is after UV irradiation: Vis(450nm): 1 min; UV(350 nm): 10min (buffer: 20 mM Tris buffer pH 8.0, Na^+ : 20 mM, Mg^{2+} : 2 mM). **Figures 10A-E** show the five cycles of UV/Vis irradiation. Although improvements in photo-responsiveness to UV/Vis irradiation are observed for these linear DNAs with shorter length and lower T_m , the efficiency of both L10-1 and L10-2 is still far below that of PSMM3. Taken together, these results indicate that the hairpin-based PSMMs are much more sensitive to photons than their linear DNA counterparts.

The specialized hairpin structure of PSMMs has been compared with another hairpin structure. While conventional DNA nanomotors involve only linear DNAs with single strand and duplex structures, PSMMs have a hairpin structure on the loop moiety that amplifies the impact of external stimuli (in this case, isomerization of azobenzene) on the open-close circulation, as determined by experimentation. The hairpin structure can stabilize the stem duplex for comparable T_m with shorter base pairs than linear DNAs, with and without azobenzene moieties. To further examine the impact of the special hairpin structure on nanomotor efficiency, a similar hairpin structure, PolyT(A3), was designed for comparison. The PolyT(A3) has 31 bases with the same stem duplex as PSMM3, but only a T base on the loop moiety: 5' FAM-CCT AGC TTT TTT TTT TTT TTT TTT T-Azo-GC-Azo-TA-Azo-G G (SEQ ID NO:6)-Dabcyl 3' (underlined bases represent stem moieties). Three Azo-moieties were incorporated at the same positions as those in PSMM3. The photoregulation of this nanomotor also displayed high efficiency and photoreversibility (**Figure 11**). **Figure 11** shows fluorescence spectra of PolyT(A3) (λ_{ex} =488 nm) under irradiation of 6W UV lamp (350 nm) and 60W desktop lamp with 450 nm filter at 25°C. The brown curve is the fluorescence intensity for pure DNA in buffer solution; blue line is the fluorescence intensity after five times of cDNA; green line is the fluorescence intensity after UV irradiation: Vis (450nm): 1 min; UV(350nm): 5 min (buffer: 20mM tris-HCl pH 8.0, 20mM Na^+ : 2mM Mg^{+}).

Moreover, the PolyT(A3) nanomotor had an average efficiency of 38.9% with at least five cycles of UV/Vis irradiation. As opposed to the structure of linear DNAs, these results illustrate that molecular motors based on hairpin structure do possess easier conversion structure for higher conversion efficiency. At the same time, their stability is not affected.

- 5 The PolyT(A3), which has a T base loop, does have a tendency to form a more regular and symmetric structure, while PSMM3 molecules have specific loop structure by their asymmetric base sequences. Nevertheless, both the PolyT(A3)- and PSMM3-based nanomotors displayed high nanomotor efficiency, which gives conclusive evidence that the hairpin structure enables DNA nanomotors to gain highly efficient conversion.

10

All patents, patent applications, provisional applications, and publications referred to or cited herein, *supra* or *infra*, are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

15

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

CLAIMS

We claim:

1. A nanomotor comprising: at least one DNA backbone having a hairpin structure composed of at least three segments of polynucleotides and at least one azobenzene moiety, wherein polynucleotides from the first segment and second segment are separated by polynucleotides of at least the third segment of the DNA backbone, wherein the polynucleotides from the first and second segment associate with one another to form a stem duplex and the third segment forms a single-strand base loop of polynucleotides, and wherein the azobenzene moiety(ies) is attached to at least one segment of polynucleotides at the stem duplex of the DNA backbone.
2. The nanomotor of claim 1, wherein a plurality of azobenzene moieties are attached at the stem duplex.
3. The nanomotor of claim 1, wherein the azobenzene moiety is azobenzene phosphoramidite.
4. The nanomotor of claim 1, further comprising at least one fluorophore/quencher pair of molecules.
5. The nanomotor of claim 4, wherein the fluorophore/quencher pair is fluorescein and dabcyl.
6. The nanomotor of claim 5, wherein the DNA backbone is selected from the group consisting of: SEQ ID NO:7 (PSMM1); SEQ ID NO:8 (PSMM2); SEQ ID NO:9 (PSMM3); SEQ ID NO:10 (PSMM4); SEQ ID NO:11 (PSMM5); and SEQ ID NO:12 (PSMM6).
7. The nanomotor of claim 1 comprising a plurality of DNA backbones associated with one another to form at least one space figure structure.

8. The nanomotor of claim 7, wherein the space figure structure is selected from the group consisting of: cube, prism, pyramid, cylinder, cone and sphere.

9. The nanomotor of claim 7, comprising a plurality of space figure structures of DNA backbones to form multiple layers of space figure structures.

10. A microarray comprising a plurality of nanomotors, wherein a nanomotor comprises: at least one DNA backbone having a hairpin structure composed of at least three segments of polynucleotides and at least one azobenzene moiety, wherein polynucleotides from the first segment and second segment are separated by polynucleotides of at least the third segment of the DNA backbone, wherein the polynucleotides from the first and second segment associate with one another to form a stem duplex and the third segment forms a single-strand base loop of polynucleotides, and wherein the azobenzene moiety(ies) is attached to at least one segment of polynucleotides at the stem duplex of the DNA backbone.

11. The microarray of claim 10, wherein the plurality of nanomotors are present in a solution.

12. The microarray of claim 10, wherein the plurality of nanomotors are attached to a substrate.

13. A device comprising at one nanomotor, wherein the nanomotor comprises: at least one DNA backbone having a hairpin structure composed of at least three segments of polynucleotides and at least one azobenzene moiety, wherein polynucleotides from the first segment and second segment are separated by polynucleotides of at least the third segment of the DNA backbone, wherein the polynucleotides from the first and second segment associate with one another to form a stem duplex and the third segment forms a single-strand base loop of polynucleotides, and wherein the azobenzene moiety(ies) is attached to at least one segment of polynucleotides at the stem duplex of the DNA backbone.

14. The device of claim 13, further comprising at least one piezoelectric substrate material attached to the at least one nanomotor.

15. The device of claim 13, further comprising a plurality of nanomotors in a microarray, wherein the nanomotors are present in a solution.

16. The device of claim 13, further comprising a plurality of nanomotors in a microarray, wherein the nanomotors are attached to a substrate.

17. A method for driving at least one DNA nanomotor comprising:

- a) providing the at least one DNA nanomotor, wherein the nanomotor comprises: at least one DNA backbone having a hairpin structure composed of at least three segments of polynucleotides and at least one azobenzene moiety, wherein polynucleotides from the first segment and second segment are separated by polynucleotides of at least the third segment of the DNA backbone, wherein the polynucleotides from the first and second segment associate with one another to form a stem duplex and the third segment forms a single-strand base loop of polynucleotides, and wherein the azobenzene moiety(ies) is attached to at least one segment of polynucleotides at the stem duplex to form a stem duplex with hybridized polynucleotides when in the presence of visible light and a stem duplex with dehybridized polynucleotides when in the presence of ultraviolet (UV) light; and
- b) exposing the at least one DNA nanomotor to visible or UV light to drive the at least one DNA nanomotor.

18. The method of claim 17, wherein a plurality of azobenzene moieties are attached at the stem duplex.

19. The method of claim 17, wherein the azobenzene moiety is azobenzene phosphoramidite.

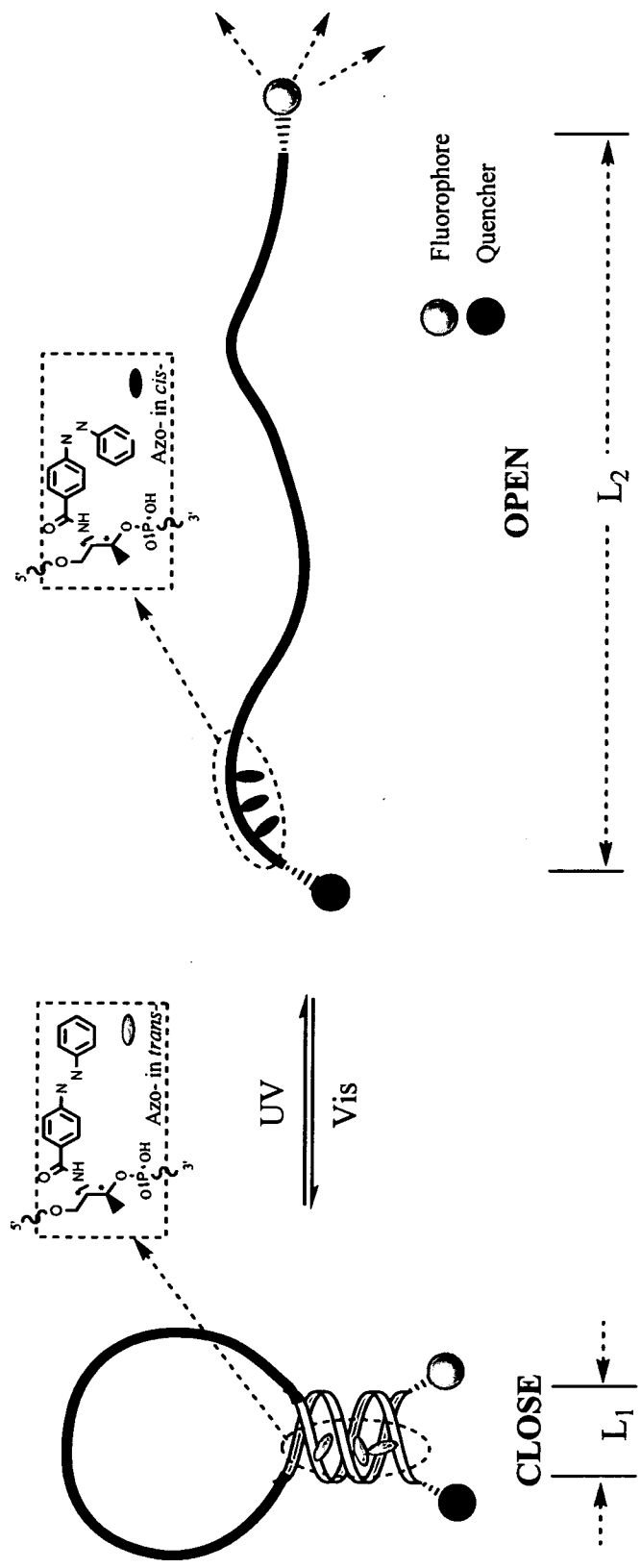


FIG. 1

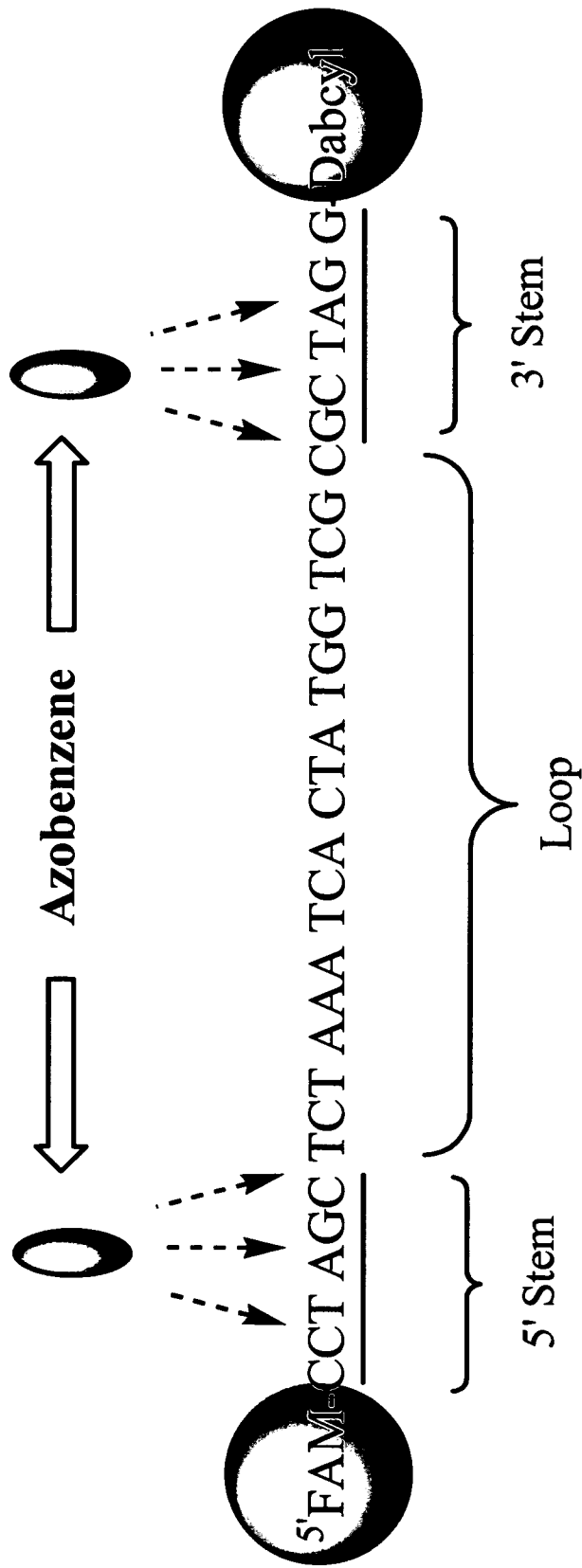


FIG. 2A

- PSMM1: 5'FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC TA-Azo-GG-Dabcyl3' SEQ ID NO: 7
- PSMM2: 5'FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC -Azo-TA-Azo-GG-Dabcyl3' SEQ ID NO: 8
- PSMM3: 5'FAM-CCT AGC TCT AAA TCA CTA TGG TCG C-Azo-GC -Azo-TA-Azo-GG-Dabcyl3' SEQ ID NO: 9
- PSMM4: 5'FAM-CC-Azo-TAGC TCT AAA TCA CTA TGG TCG CGC TAG G-Dabcyl3' SEQ ID NO: 10
- PSMM5: 5'FAM-CC-Azo-TA-Azo-GC TCT AAA TCA CTA TGG TCG CGC TAG G-Dabcyl3' SEQ ID NO: 11
- PSMM6: 5'FAM-CC-Azo-TA-Azo-GC-Azo-TCT AAA TCA CTA TGG TCG CGC TAG G-Dabcyl3' SEQ ID NO: 12

FIG. 2B

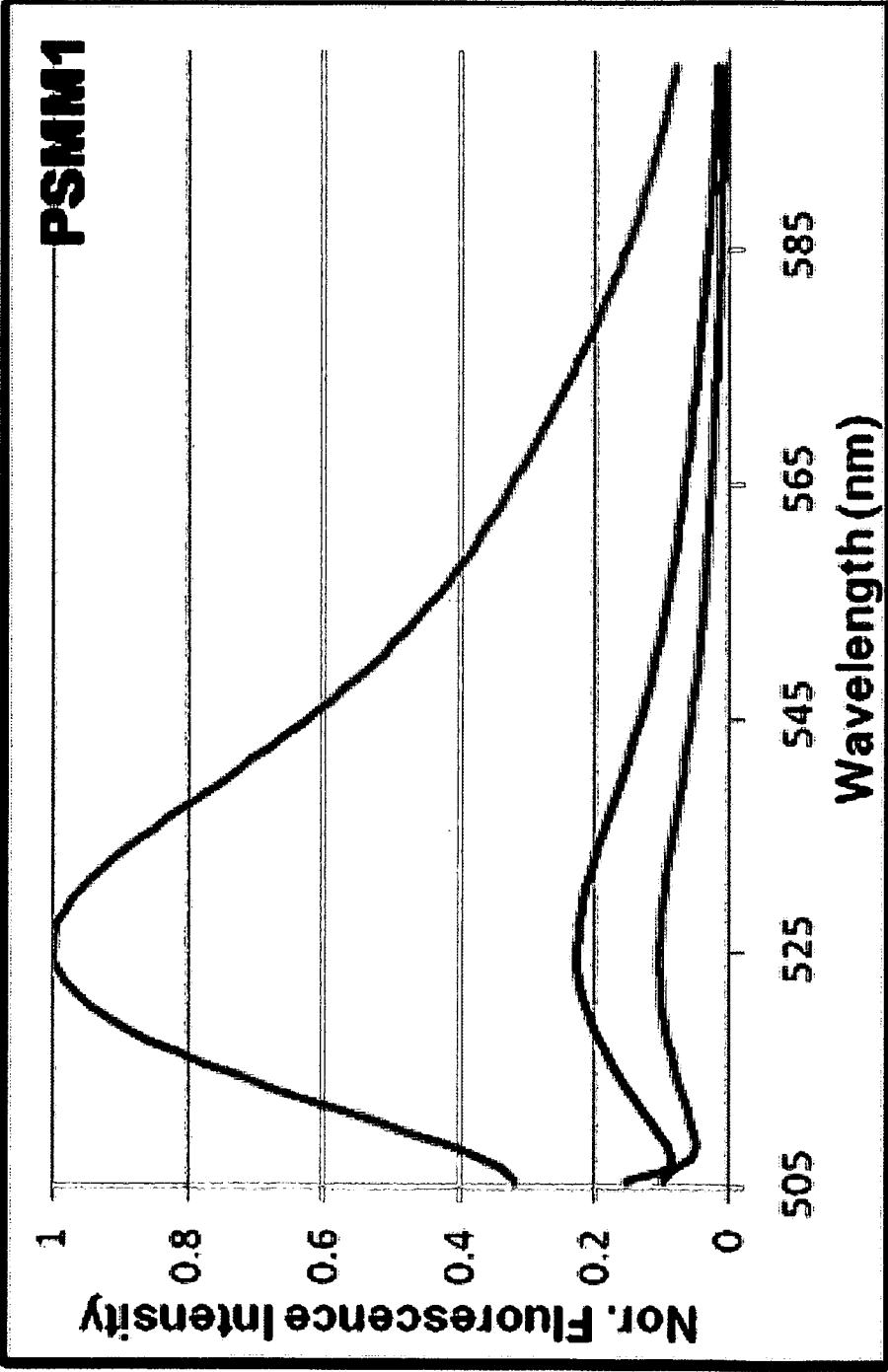


FIG. 3A

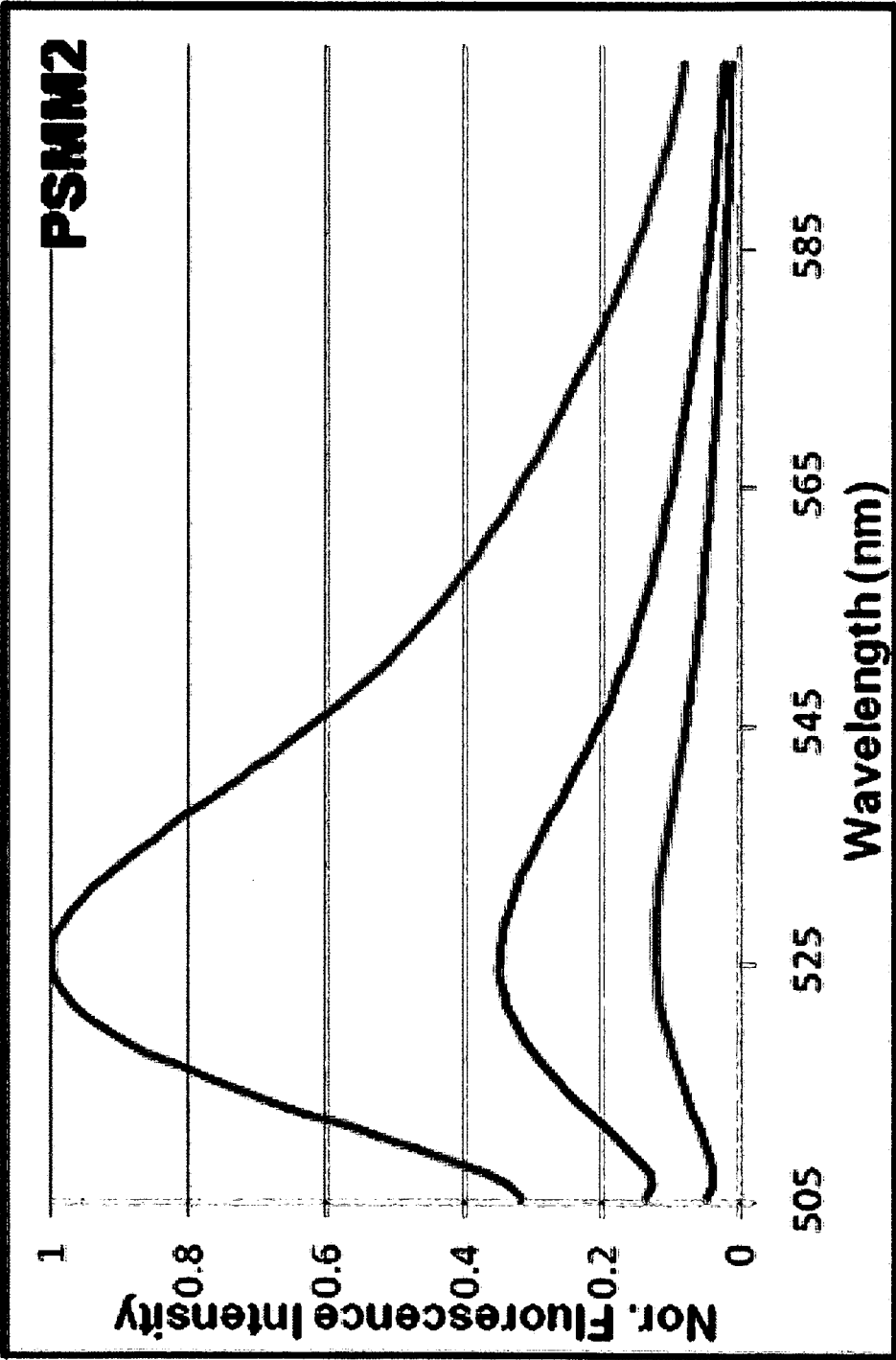


FIG. 3B

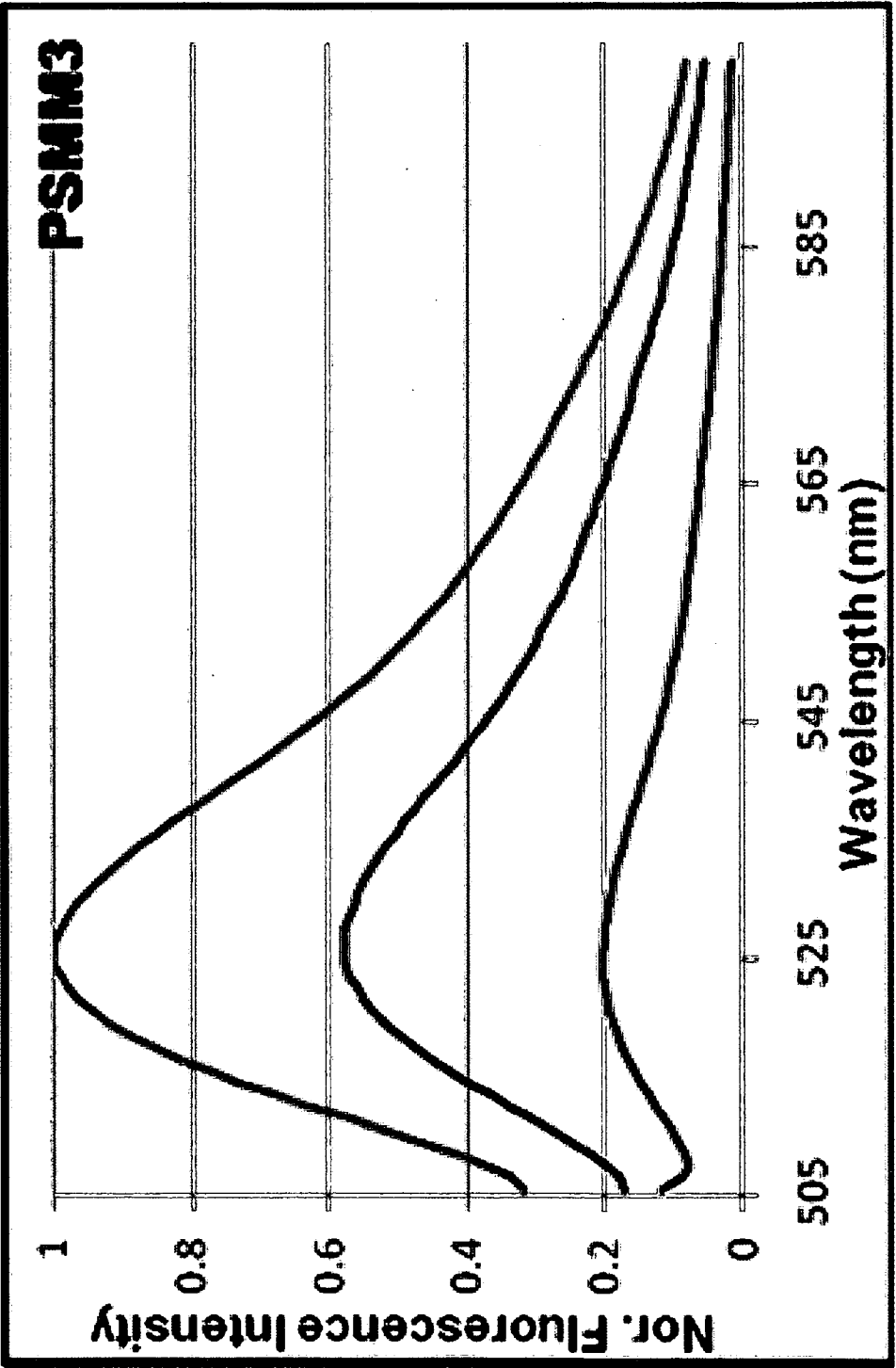


FIG. 3C

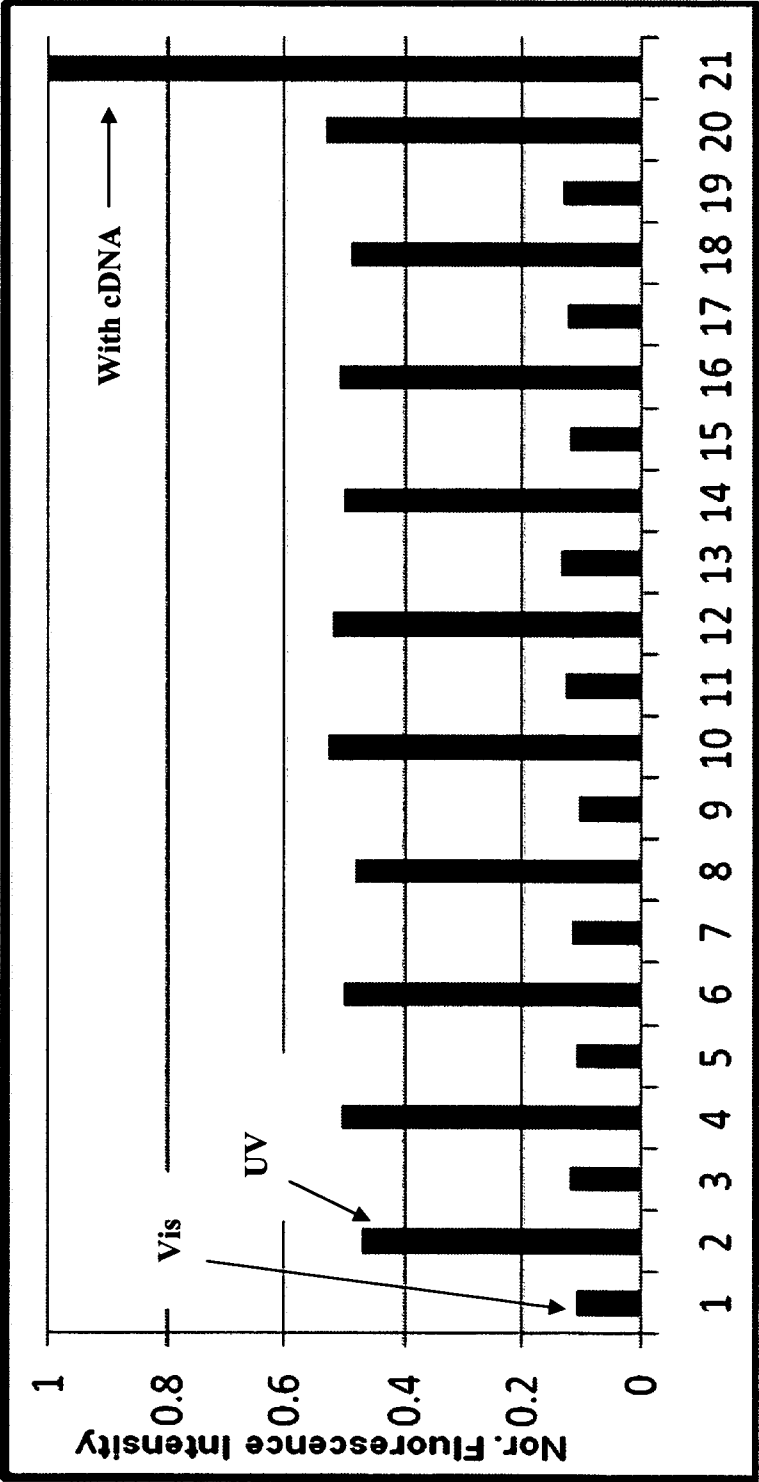


FIG. 4

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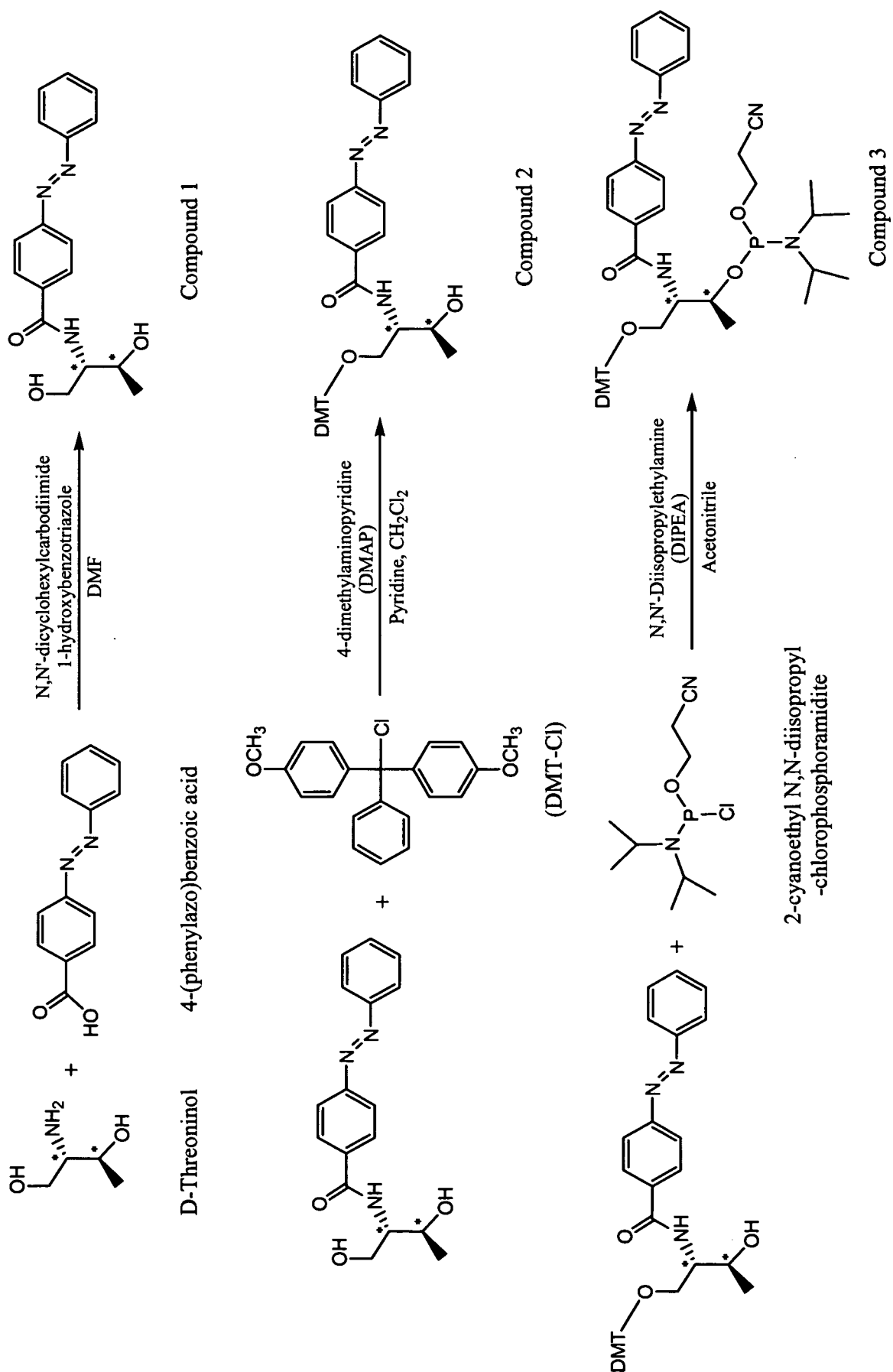


FIG. 5

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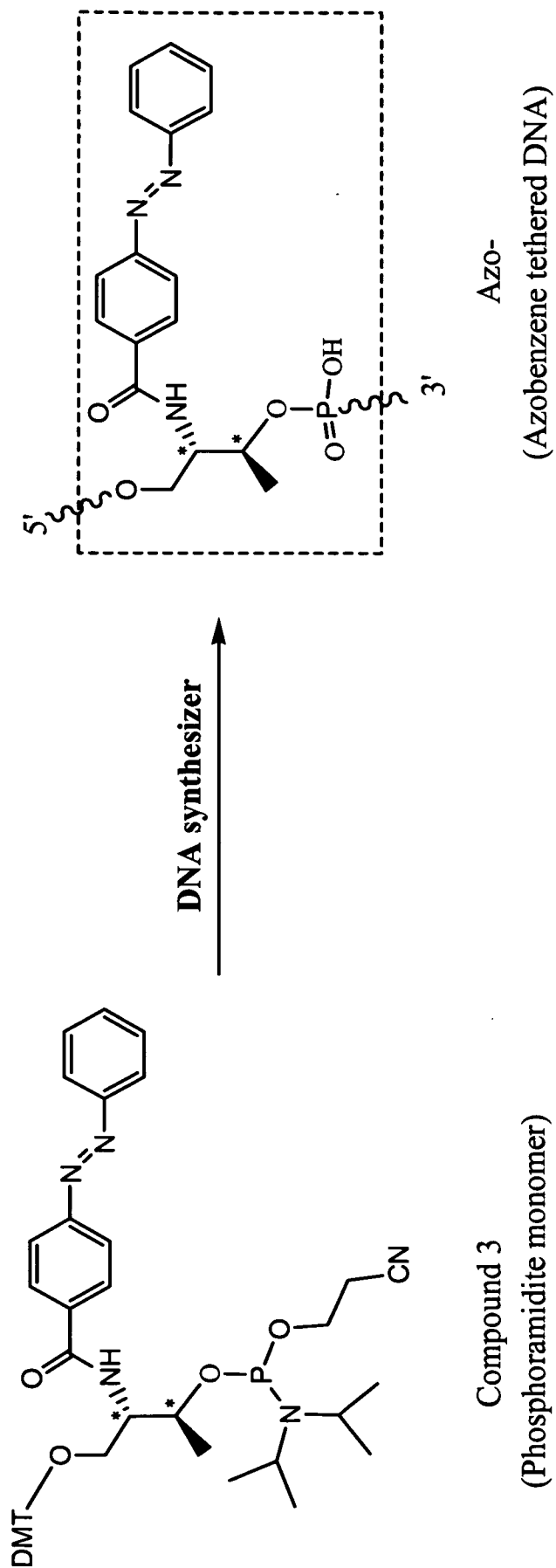


FIG. 6

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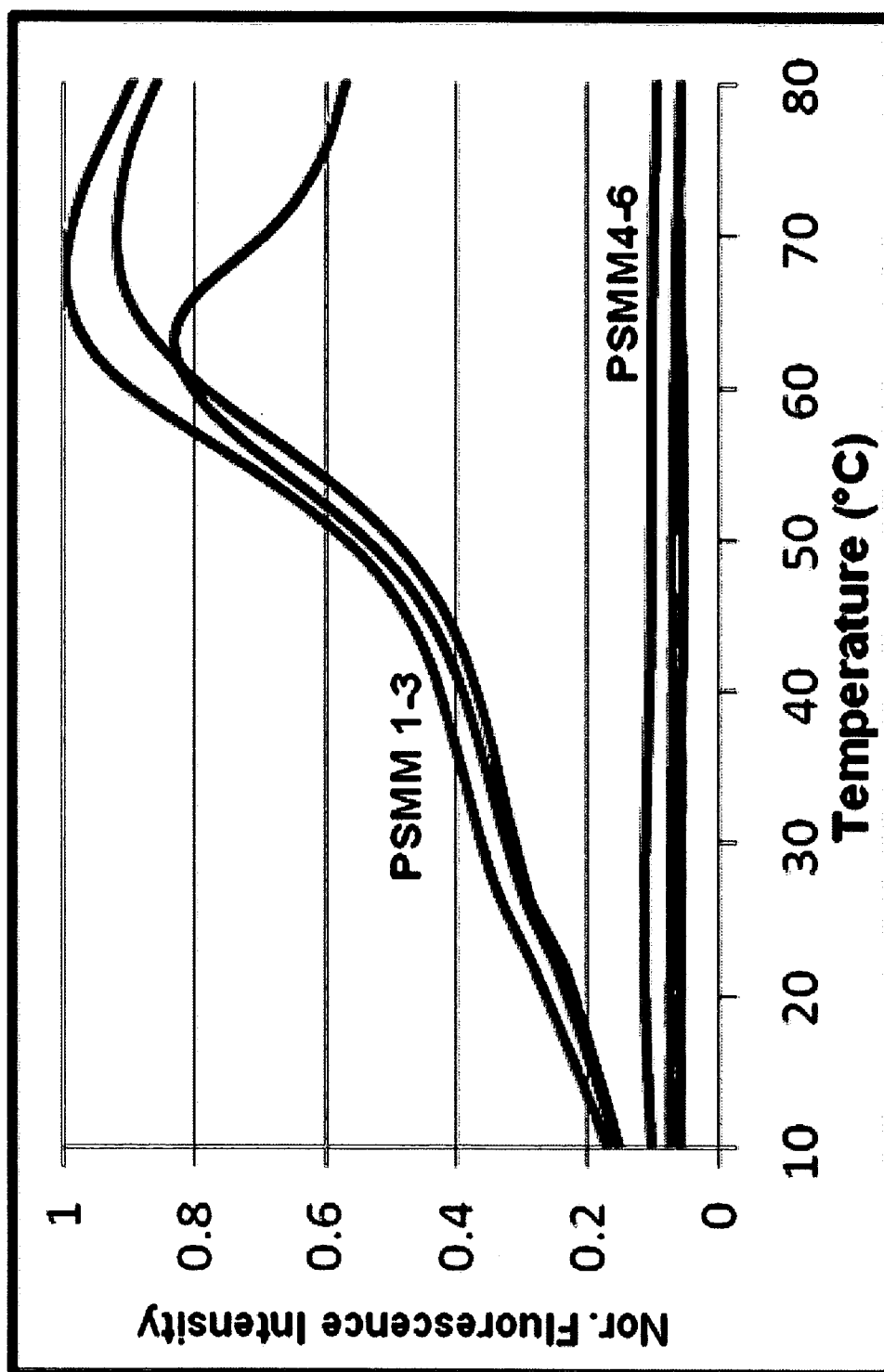


FIG. 7A

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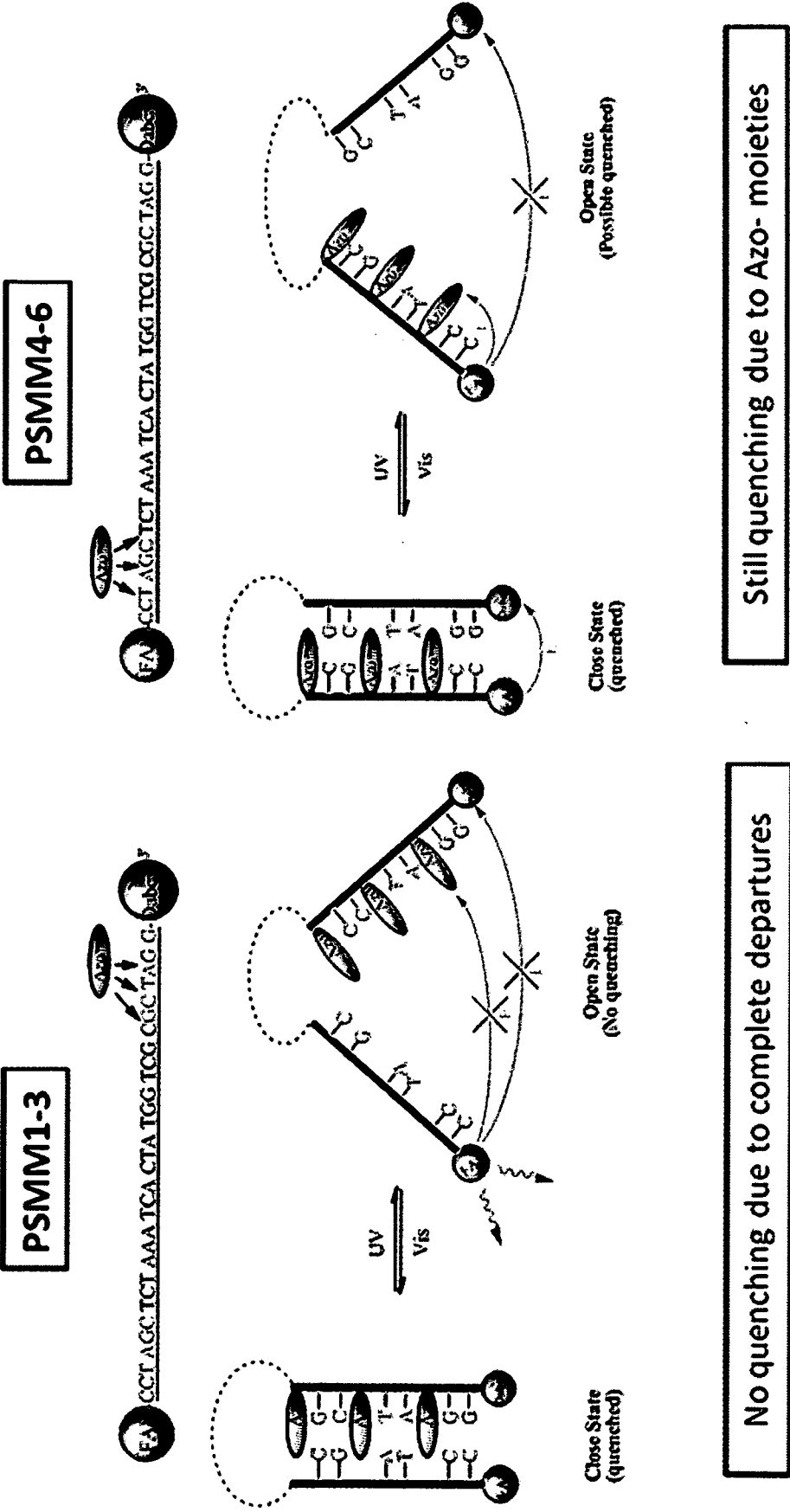


FIG. 7B

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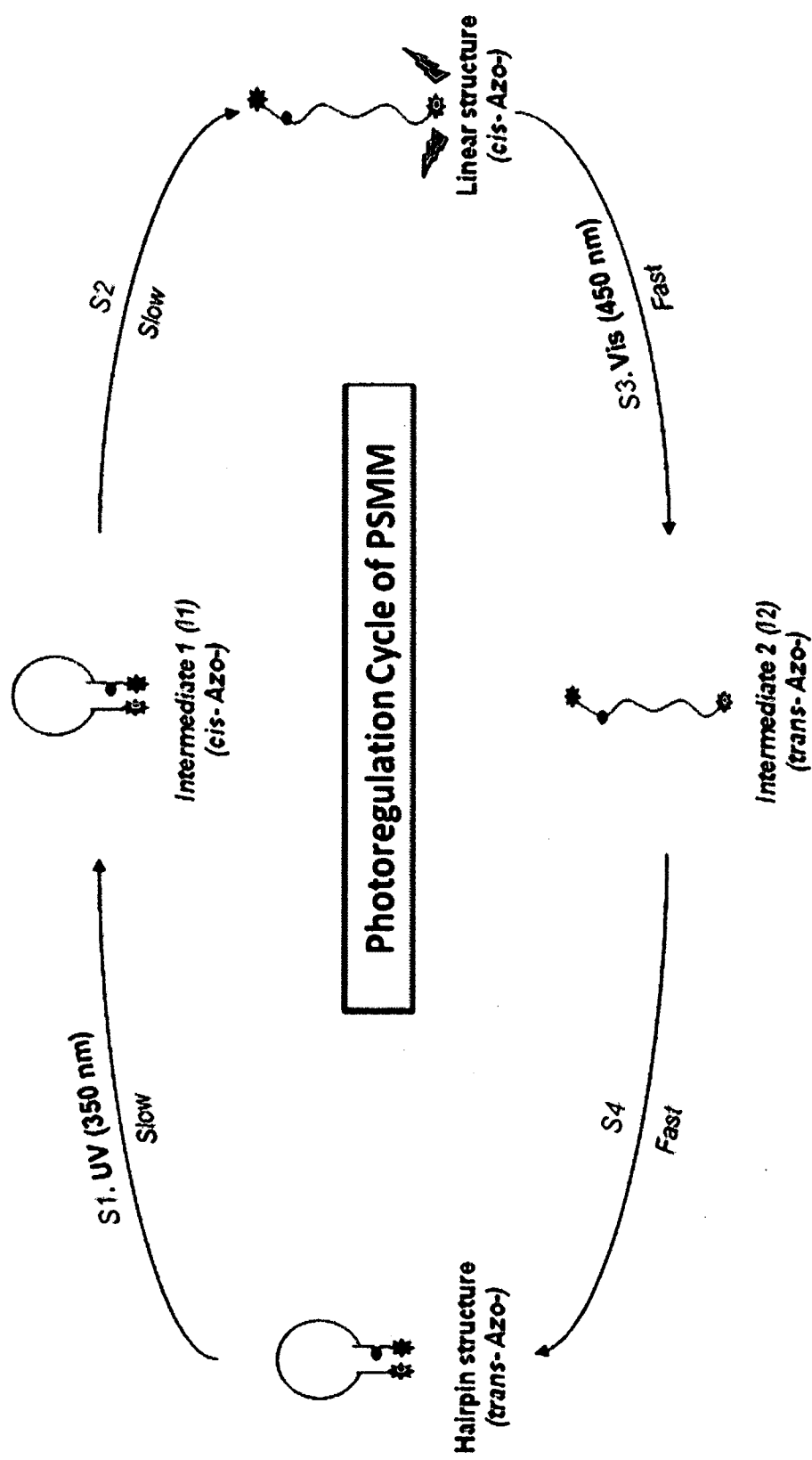


FIG. 8

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Linear sequences (12 base pairs): $53.7^{\circ}\text{C} \leq T_m \leq 55.2^{\circ}\text{C}$

L12-cDNA: 5'FAMCCTAGCGCGACCC SEQ ID NO: 4

L12-1: 5'GGTCGC-Azo-GC-Azo-TA-Azo-GG-DabcyI3' SEQ ID NO: 13

L12-2: 5'GGTC-Azo-GC-Azo-GC-Azo-TA-Azo-GG-DabcyI3' SEQ ID NO: 14

L12-3: 5'GG-Azo-TC-Azo-GC-Azo-GC-Azo-TA-Azo-GG-DabcyI3' SEQ ID NO: 15

FIG. 9A

Linear sequences (10 base pairs): $47.5^{\circ}\text{C} \leq T_m \leq 48.6^{\circ}\text{C}$

L10-cDNA: 5'(FAM) CCTAGCGCGA SEQ ID NO: 5

L10-1: 5'TCGC-Azo-GC-Azo-TA-Azo-GG(DabcyI) SEQ ID NO: 16

L10-2: 5'TCGC-Azo-GC-Azo-TA-Azo-GG(DabcyI) SEQ ID NO: 17

FIG. 9B

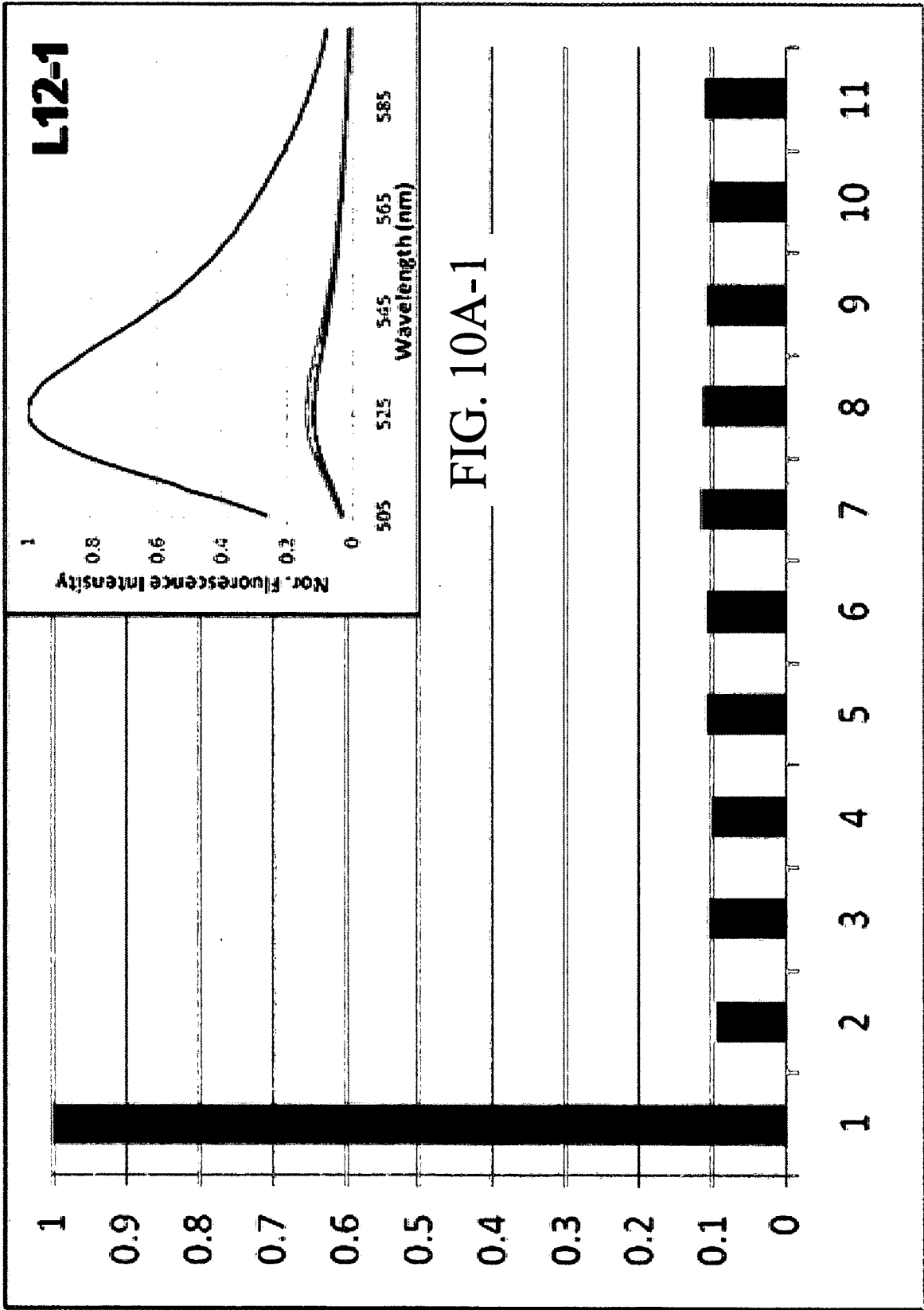
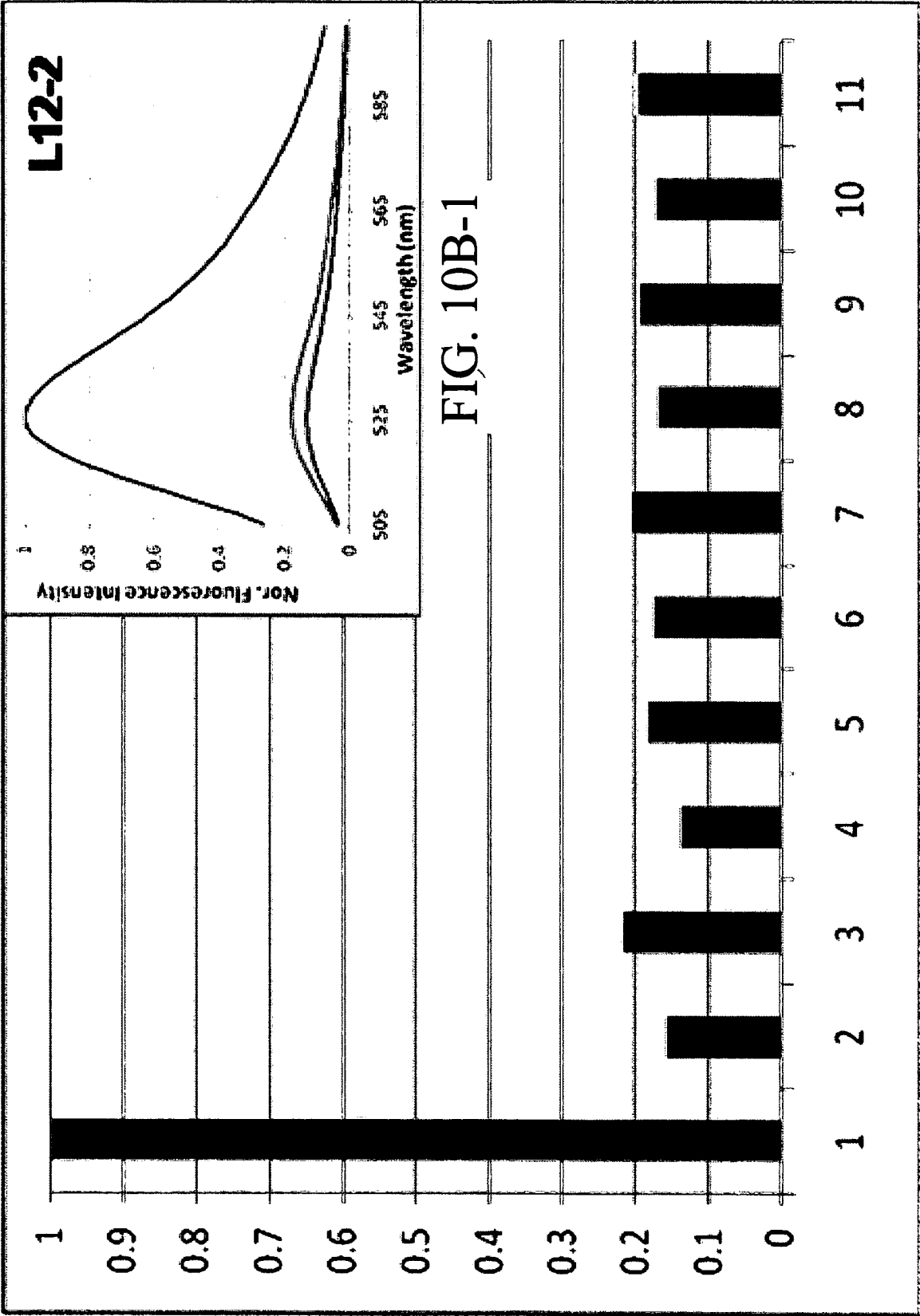
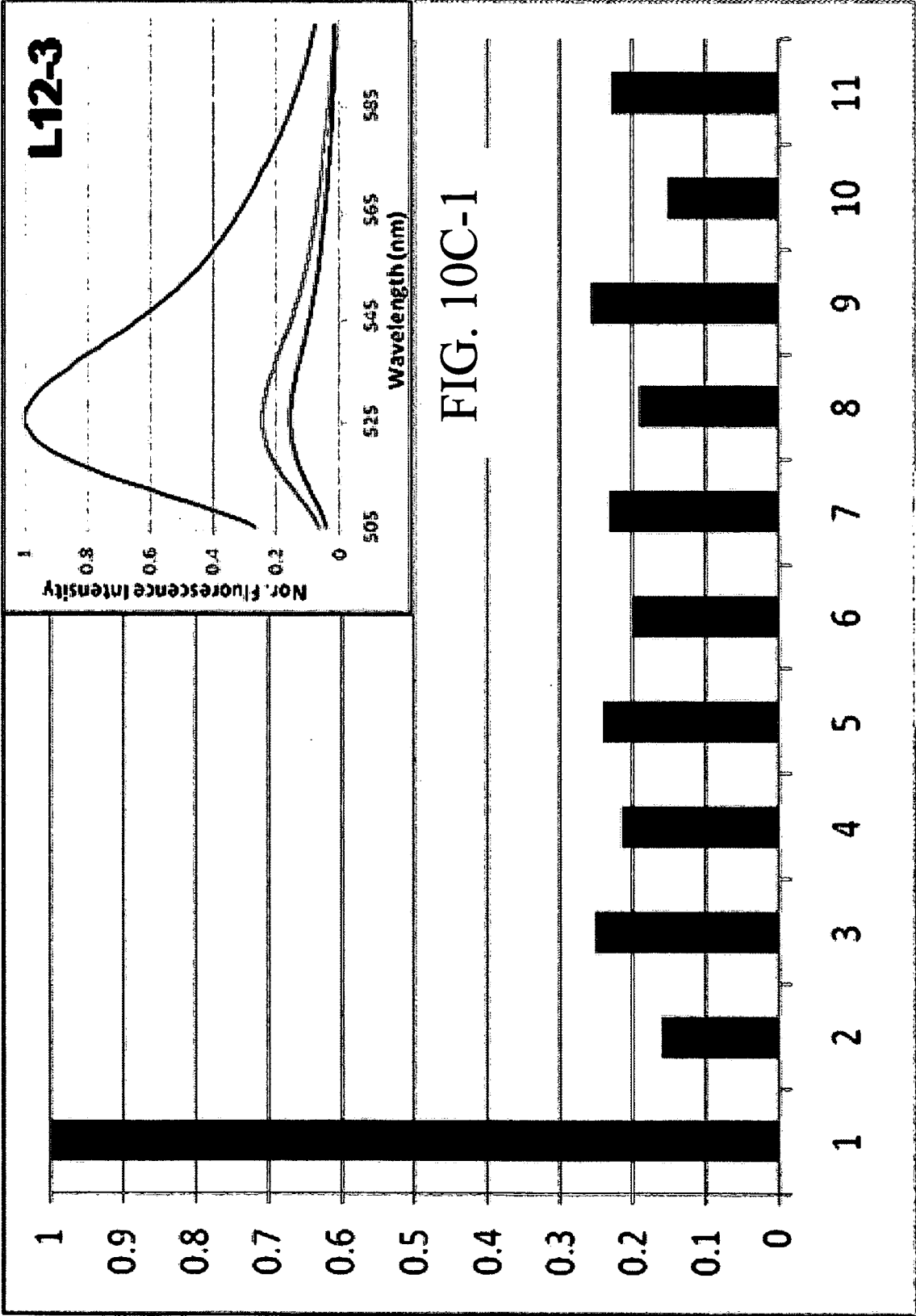


FIG. 10A





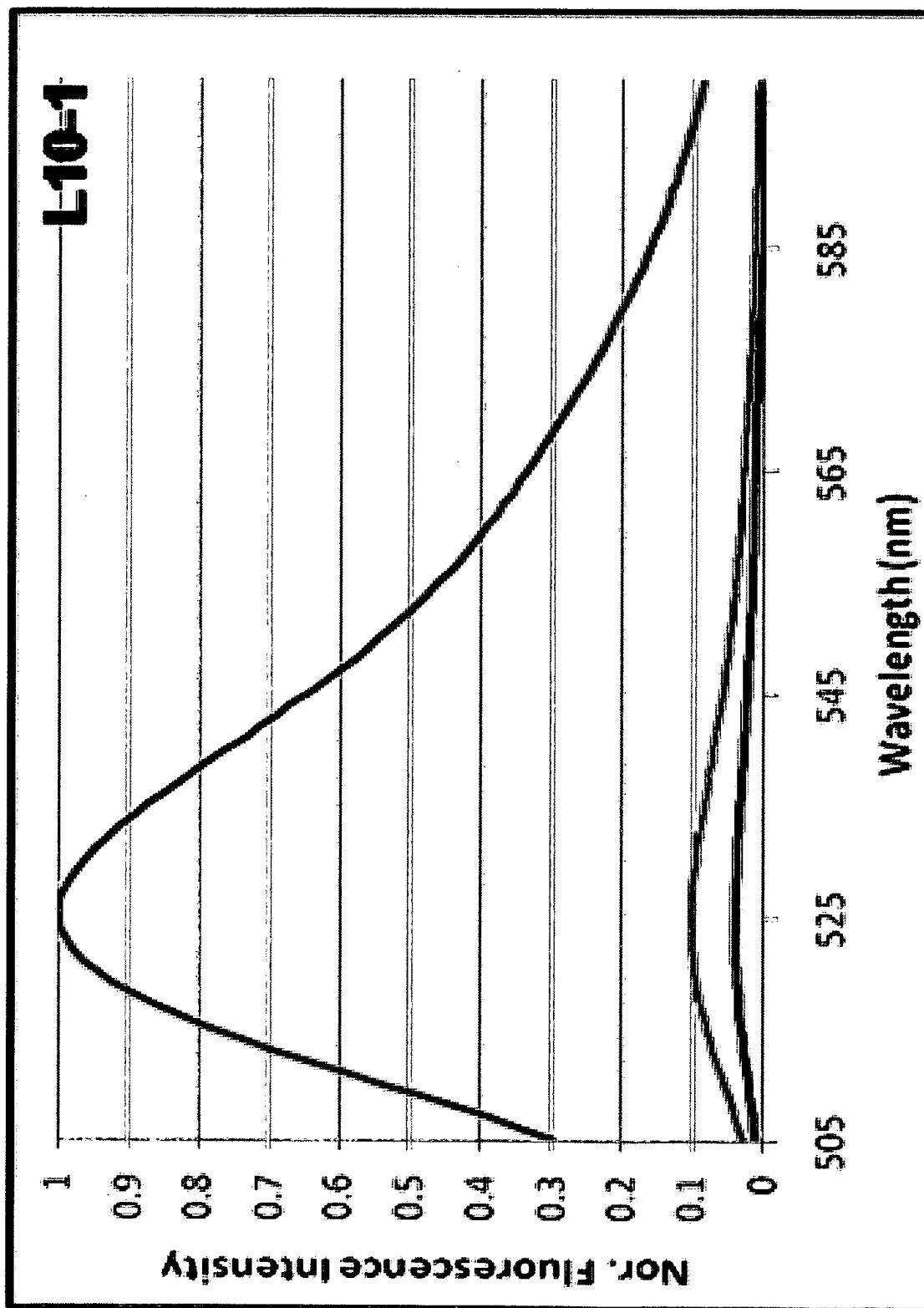


FIG. 10D

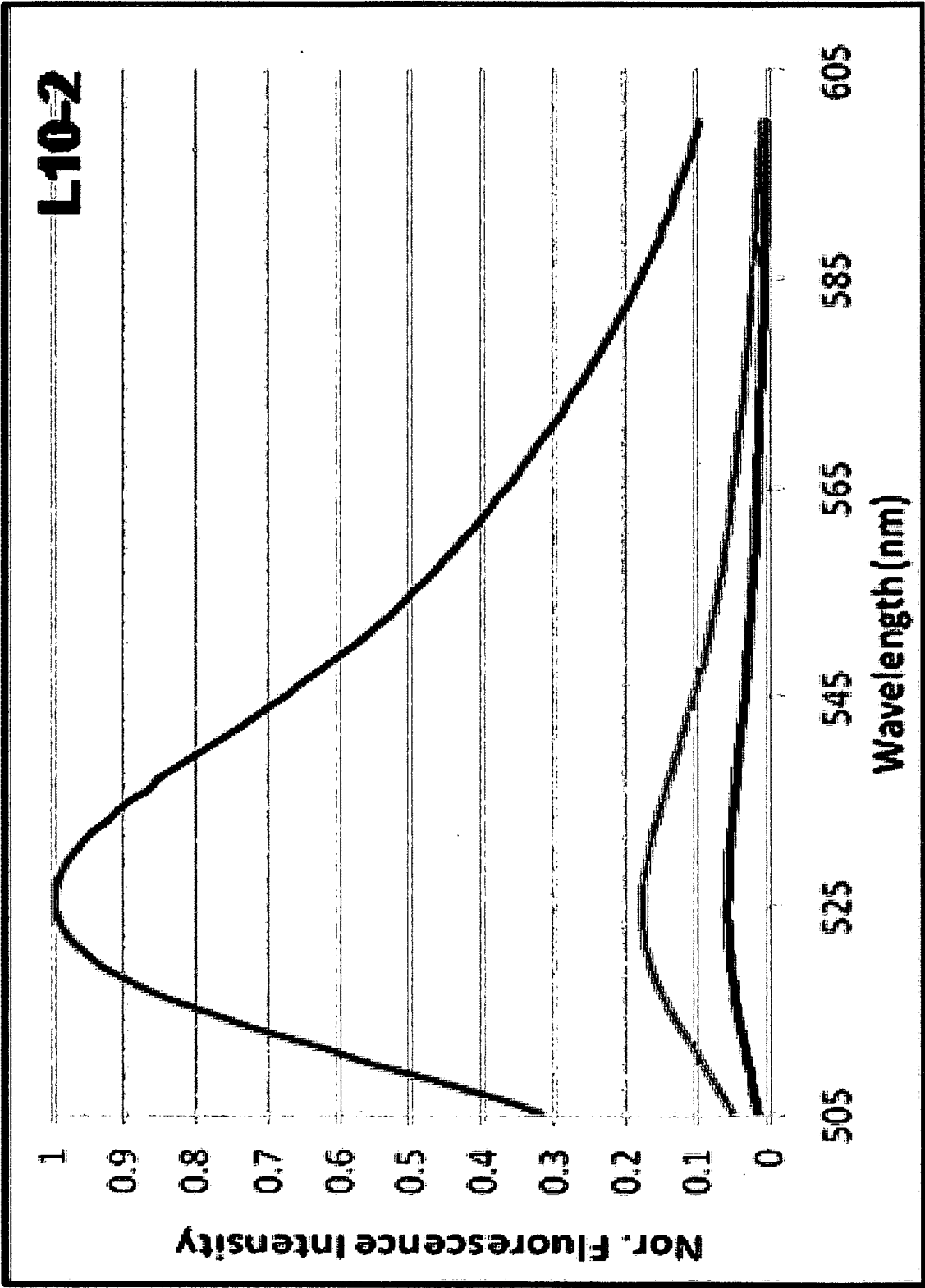


FIG. 10E

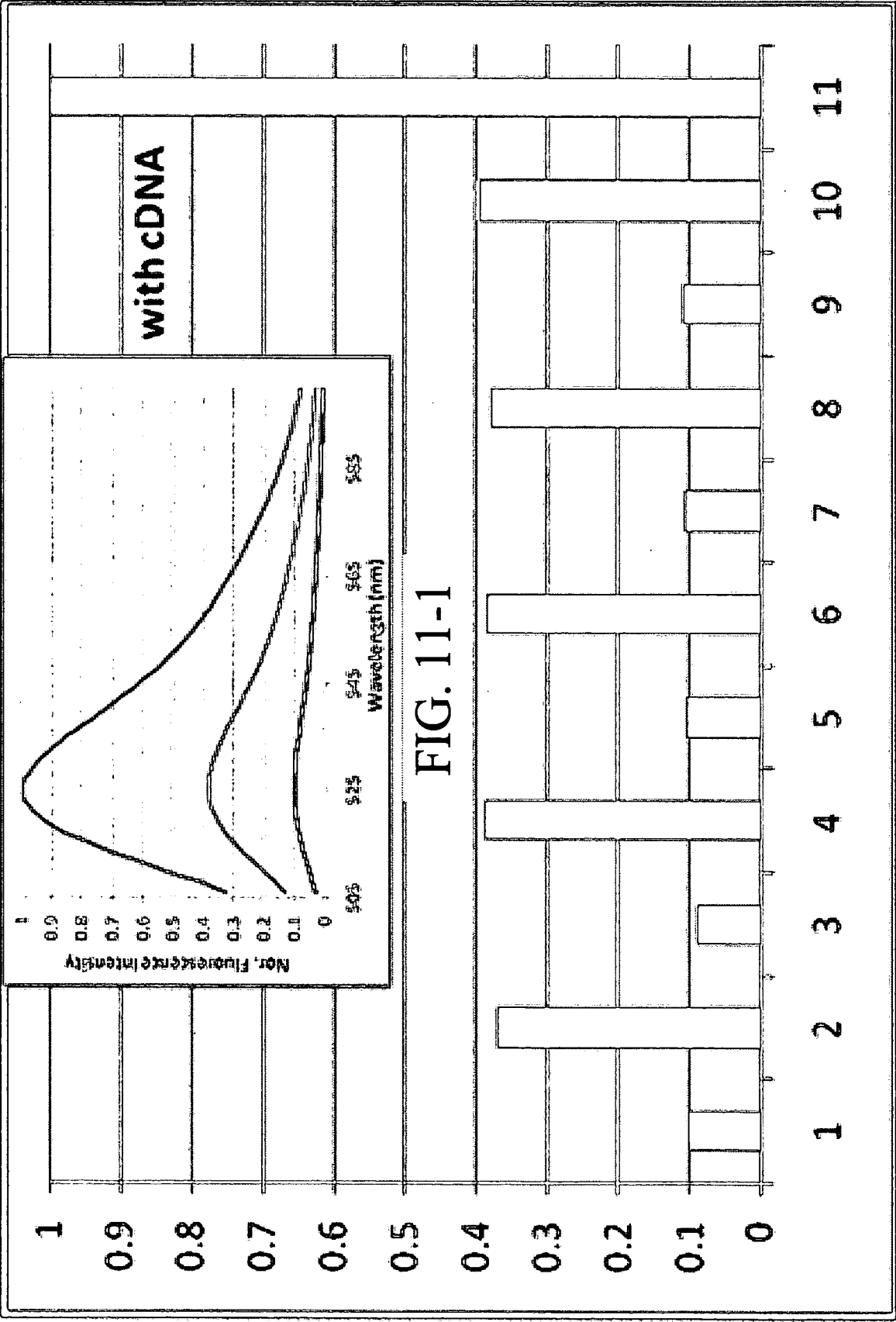


FIG. 11

Comparison of all DNA sequences with and without Azo- incorporation

Sequence Name	base number	Azo- number	Azo-/base ratio (%)	T _m (°C) ±0.5°C	Recovery (%)
MB	31	0	0	57.5	N/A
PSMM1	31	1	3.2	57.0	14.2 ±2.5
PSMM2	31	2	6.5	56.3	26.3 ±3.3
PSMM3	31	3	9.7	55.0	54.7 ±3.1
PolyT	31	3	9.7	54.9	31.3 ±2.5
L12-1	12	3	25	55.2	2.9 ±0.8
L12-2	12	4	33.3	54.8	5.7 ±1.1
L12-3	12	5	41.7	53.7	11.5 ±2.3
L10-1	10	3	30	47.5	6.3 ±1.6
L10-2	10	4	40	48.6	13.8 ±2.2

FIG. 12

Conversion efficiency of PSM3 and L10-1 in different concentrations

Sequence Name	Conversion efficiency by recovery (%)			
	100 nM	500 nM	5 μ M	50 μ M
PSM3	54.7 $\pm$ 3.1	44.6 $\pm$ 2.7	45.8 $\pm$ 3.1	44.7 $\pm$ 2.8
L10-1	6.3 $\pm$ 1.3	9.0 $\pm$ 1.2	4.4 $\pm$ 0.6	0.7 $\pm$ 0.2

FIG. 13

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FIG. 14A

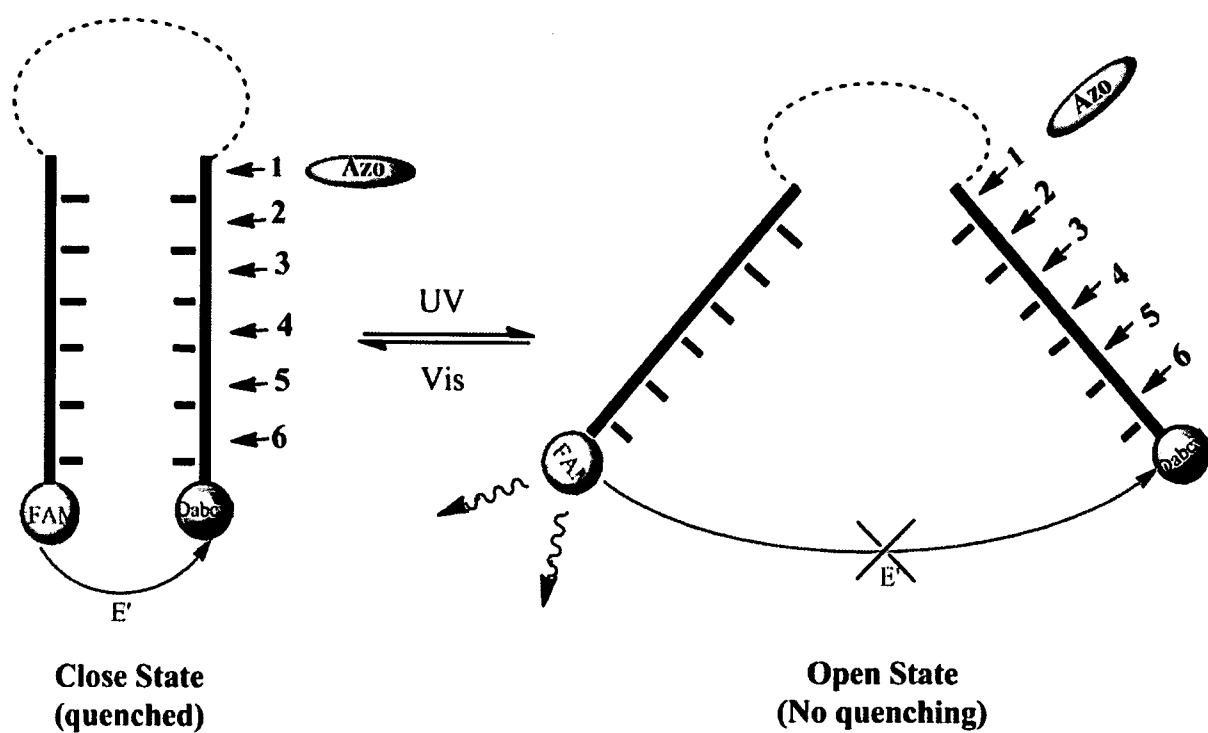


FIG. 14B

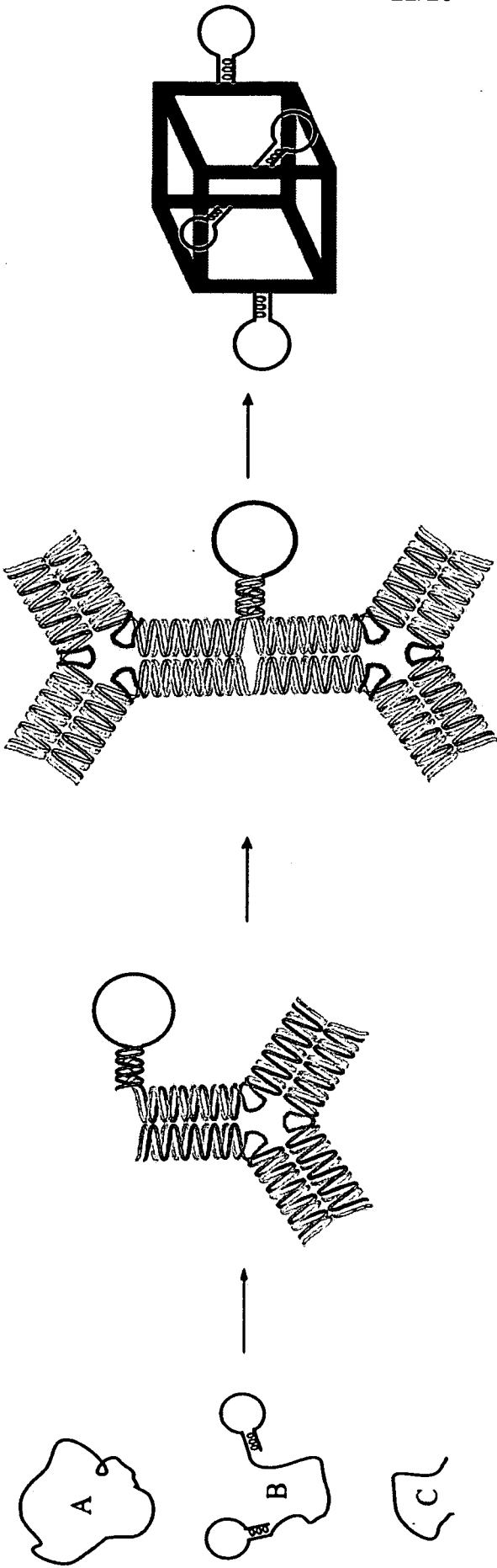


FIG. 15A

FIG. 15B

FIG. 15C

FIG. 15D

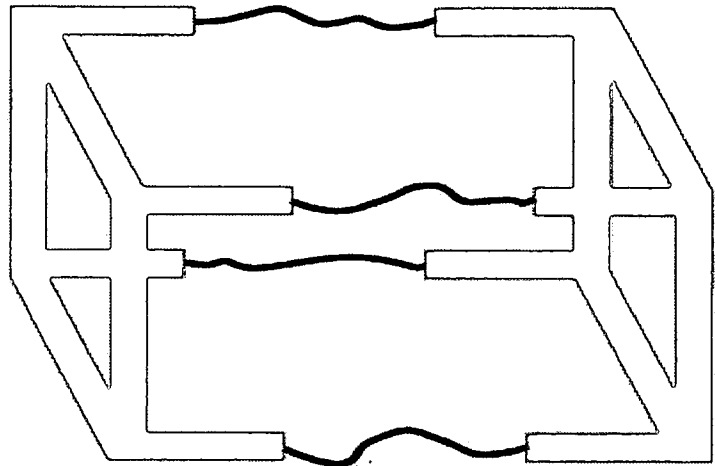


FIG. 16B

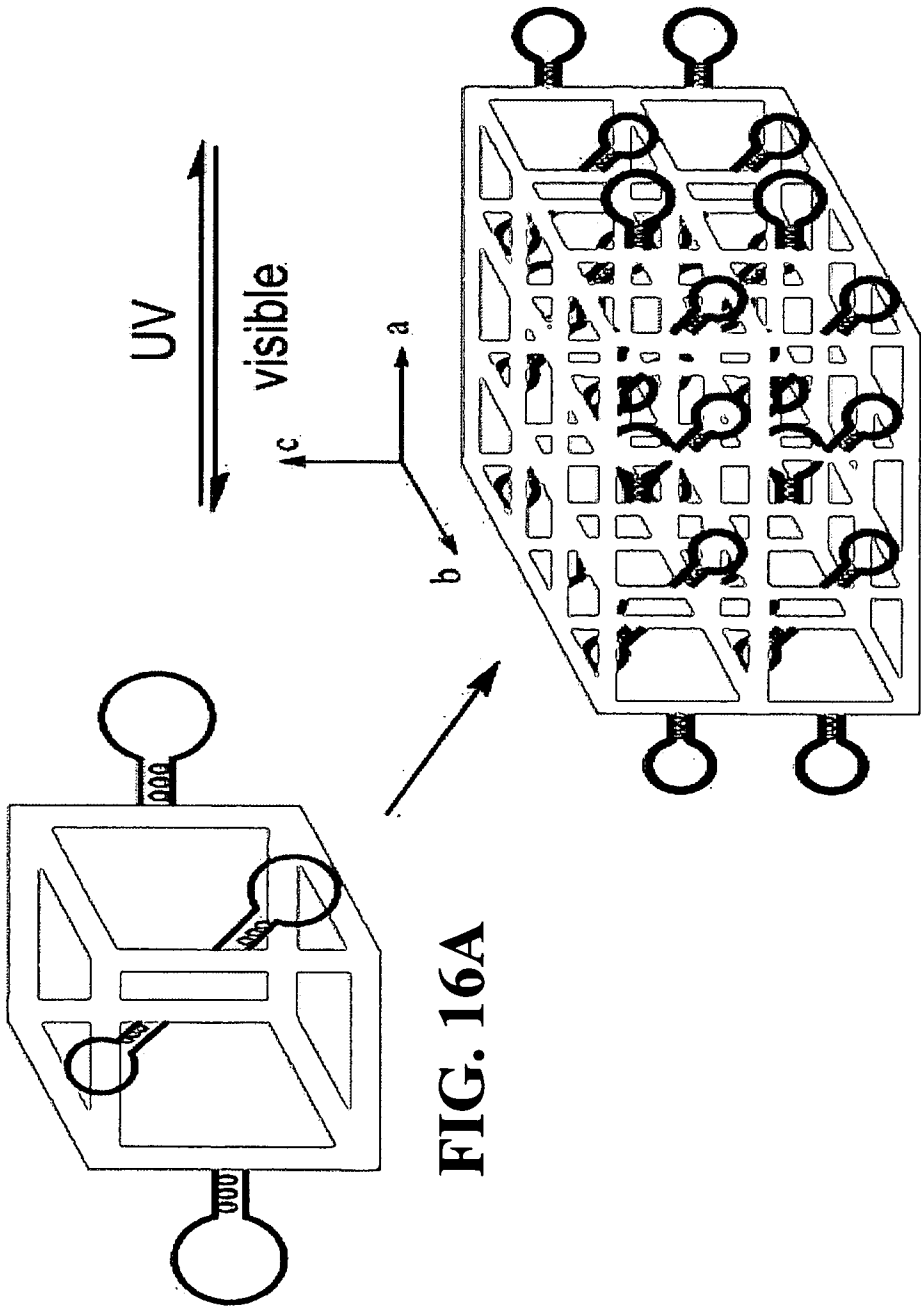
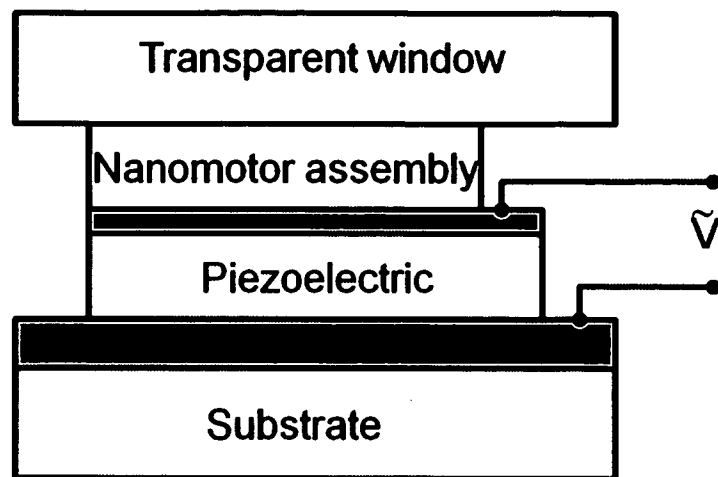
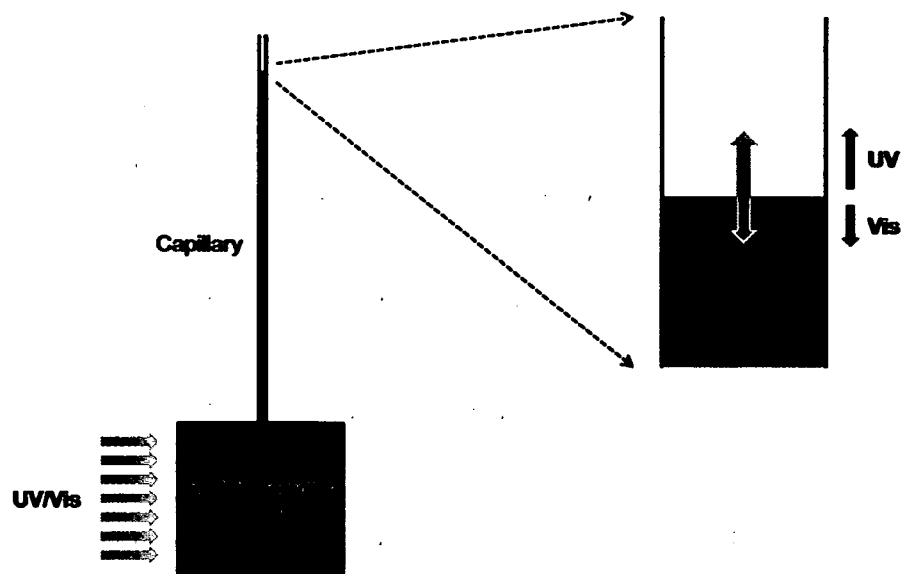


FIG. 16A

FIG. 16C

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**FIG. 17****FIG. 18**

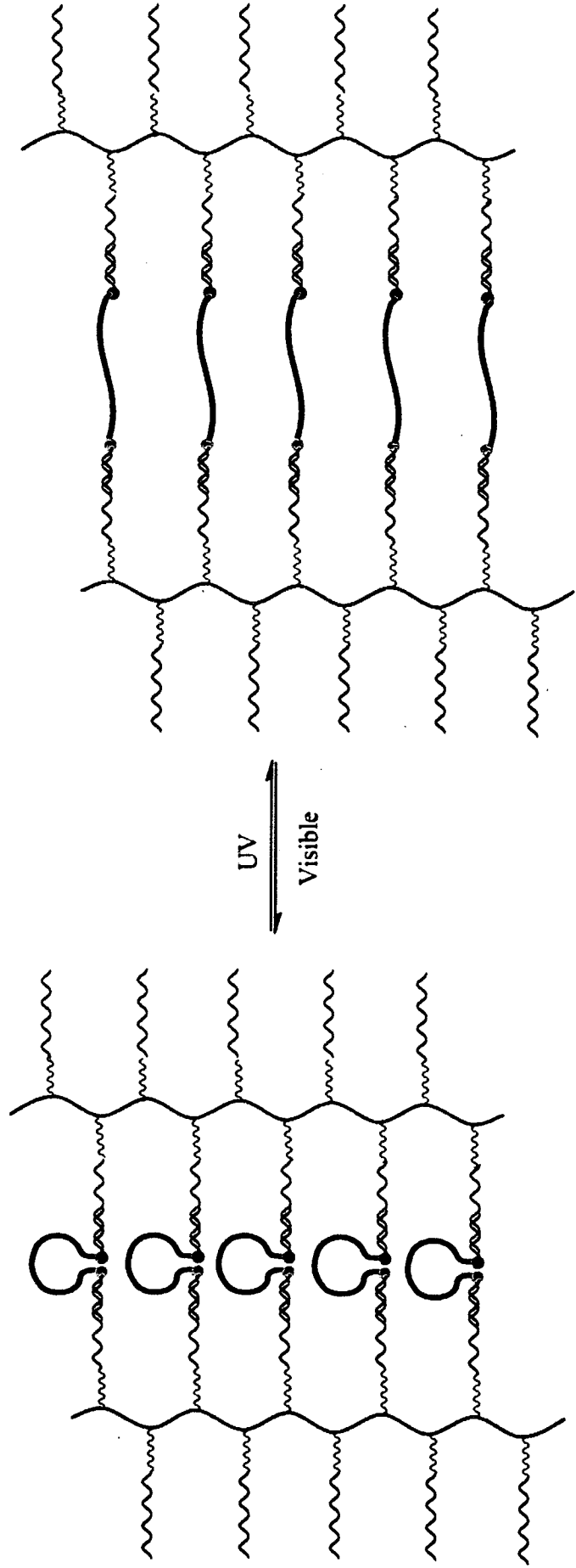


FIG. 19

