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Positional photocleavage control of DNA-based nanoswitches†

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DNA-based switches are currently operated by manual solution-phase addition of 'set-strands'. We demonstrate another operation method—sterically inaccessible 'set-strands', released from a surface into solution by spatially controlled photocleavage. This technique will enable microarrays of set-strands to operate many DNA-based switches in self-contained computational and diagnostic devices.

DNA-based nanotechnology[1] (DBN) allows the construction of nanoscale objects, lattices and machines. It utilizes the fruits of the biotechnological revolution: principally, the exquisite specificity and selectivity of Watson–Crick base-pairing to near-quantitatively self-assemble species often consisting of hundreds of components. Set-strand replacement[2] allows DBN species to act as *controllable nanoscale switches* that perform computations, act as sensors and control nanoscale motion.[3] In this context, a 'set-strand' is a DNA strand that binds to a molecular species containing non-base-paired DNA, either changing the DBN's conformation or removing a previous set-strand from the DBN. Set-strand controlled DBN switches are also used to activate and deactivate biologically relevant molecular species such as enzymes,[4] ribozymes[5] and aptamers.[6] Other synthetic systems capable of performing controlled molecular switching exist:[7] the strength of the DBN approach is that many different switches can be present in a system and be *operated with precise individual control* as they can all be *orthogonally* controlled by a vast set of non-overlapping DNA sequences.

Currently, manual (pipetting) addition of set-strand solutions is used to activate or deactivate DBN-modified biomolecules, sensors or reaction networks. When developing these model systems into applications for use in devices such as a lab-on-a-chip,[8] it will be necessary to operate a large number of switches in parallel, ideally with a stimulus that would allow many set-strands to be delivered into the system on demand, without the addition of external reagents.

One such approach involves transcriptional control of DNA switches,[9] however, the use of these biochemical circuits adds operational complexity due to the lability of RNA, the presence of multiple enzymes, and the necessity of balancing enzymatic activity for individual switches. Light-controlled DNA release techniques include melting of duplexes from nanoparticle surfaces[10] and non-specific reversible electrostatic surface desorption using photoisomerization:[11] neither appear suitable for control of multiple DBN switches.

We describe a light-controlled approach where a solution of DNA switches is placed above a microarray surface. On the microarray surface, there is a covalently immobilized array of densely packed, sterically blocked set-strands. The covalent linker is photocleavable; upon photocleavage of a particular array area, the desired strand diffuses away from the surface and operates the switch.

Fig. 1 shows the surface functionalization arrangement and chemistry. The sequence corresponding to the set-strand is blocked from interaction with the large solution-phase switch species by both the density of the strand coverage and a hairpin steric block.

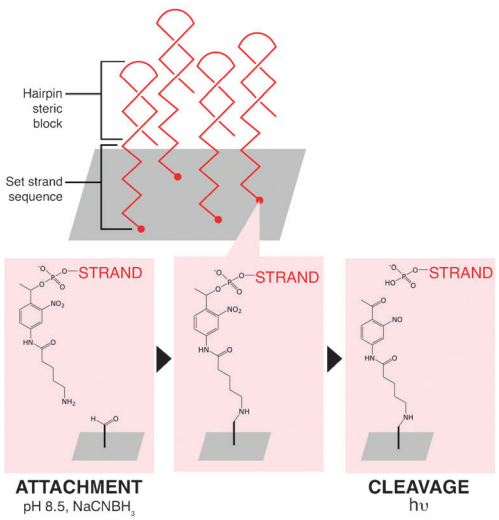

Fig. 1 The arrangement of set-strands on the surface (top) and attachment and cleavage chemistry (inset). (top) A 5′ photocleavable-NH_2 functionalized DNA strand[12] is attached to an aldehyde-functionalized glass microarray surface.[13] (inset) Reductive amination between a 1° amino group and a surface-bound aldehyde links the 5′ end of the DNA strand to the surface. Cleavage utilizes a modified 2-nitrobenzyl group, which upon irradiation with 365 nm light breaks a C–O bond releasing a 5′ phosphorylated set-strand. Note that photocleavage using this wavelength/linker combination does not damage the DNA strand.[14]

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† Electronic supplementary information (ESI) available: Experimental section describing actuator/set-strand sequences, synthesis, analysis and controls for their solution and surface behavior. See DOI: 10.1039/c1cc10292c

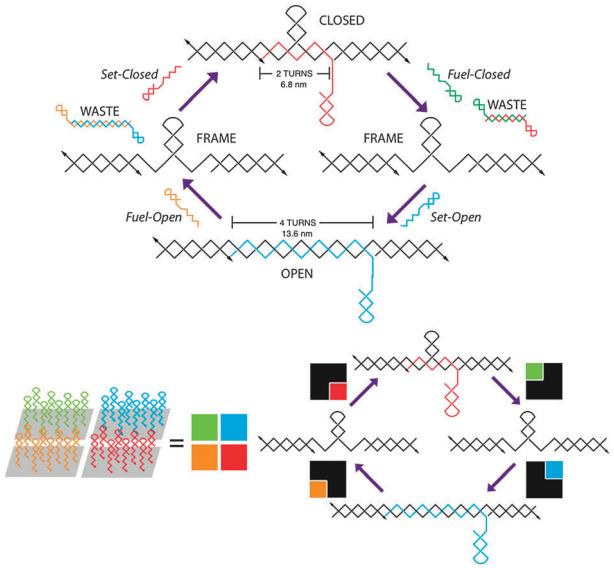

Fig. 2 (top) Solution-phase operation of a DBN actuator. Set-strands (represented by coloured strands—named in italic font) are added to a solution of the DNA actuator in a particular state (represented by black strands). The set-strands are either complementary to a particular actuator conformation (set: blue or red strand) or complementary to another set-strand (fuel: green or orange strand). Each *machine step* of the actuator is shown by a purple arrow: increases in base-pairing for each step drives a branch-migration equilibrium[2] that either removes a strand that is bound to the actuator or that changes the conformation of the actuator. The 'waste' strands are fully base-paired and do not interfere with the subsequent machine steps. Sequential addition of set-strands results in actuator's operation, which performs an extension-contraction motion of two turns of DNA (6.8 nm). Each actuator state ('CLOSED', 'FRAME', or 'OPEN') is distinguishable by nondenaturing gel electrophoresis. (bottom) The photocleavage experiment. Set strands are arrayed in wells on the glass slide. Solutions of the actuator in varying states are placed above the array. (Right) The well corresponding to the desired set-strand is illuminated: the other areas are protected from light by a mask. Sequential illumination of the red, green, blue and yellow set-strand areas operates a cycle of the actuator.

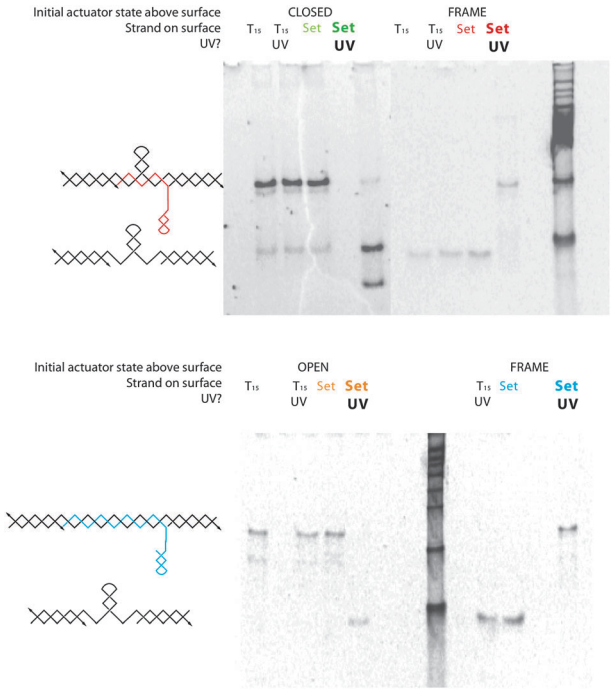

Fig. 3 Stained non-denaturing gels of the actuator being operated by photocleaved set-strands. The gel is annotated with set-strands that are identified by colour. The bold captions show lanes on the gel corresponding to UV exposure of a set-strand functionalized surface–this exposure causes an individual *machine step* of the device. Controls are T_{15} functionalized surface without UV, T_{15} functionalized surface + UV light, set-strand without UV. The DNA ladder is 50 bp. For details of solution/surface preparation and further controls/characterization see the ESI.

Fig. 2 shows the DBN 'Actuator'[15] system used to illustrate photocleavage operation of a set-strand controlled system.

Fig. 3 shows the operation of the actuator by photocleavage. Each actuator machine step is only activated by the appropriate combination of UV light and the presence on the surface of the corresponding photocleavable set-strand. The controls show that the actuator does not bind to DNA-functionalized surface—the intensity of the gel band corresponding to starting material would change if this were the case; the controls also show that the actuator is not operated in the absence of UV light or appropriate set-strand. The results of this experiment suggest that *any* system controlled by stoichiometric amounts of set-strands that is not affected by 365 nm UV light should be amenable to photocleavage control.

Catalytic networks have been developed[16–18] that operate DNA switches: these require minuscule amounts of set-strand, thus enabling the use of smaller array features, minimizing stoichiometry issues and allowing true multiplexing. Hundreds to thousands of micrometre-scale strand regions will be generated by the same techniques used to generate gene arrays; the parallel, time-controlled cleavage of these regions should be achievable by selective micrometre-scale illumination using a maskless array synthesizer[19] as UV source. Efforts to obtain photocleavage control of catalytic DNA switch systems to achieve these ends are ongoing.

To conclude, we have demonstrated that a branch-migration based DBN system can be operated by photocleavage of sterically protected set-strands from surfaces. We expect this technology to enable the development of DBN-based switches for use in self-contained labs on chips.

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