Supporting info for

Positional Photocleavage Control of DNA-based nanoswitches

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DNA Synthesis. Integrated DNA Technologies (Coralville, IA) supplied DNA oligos. Photocleavable DNA oligos were synthesized at the 1 µmol scale using standard phosphoramidite protocols and reagents (Glen Research) on an Applied Biosystems 394 automatic DNA synthesizer. UltraMild Bases/supports and the 5’-PC-Amino-phosphoramidite (Glen Research) were used. Oligos were cleaved from the support and deprotected using 30% aqueous ammonia for 8 h at RT, evaporated, dissolved in water and desalted using G-10 columns (GE Healthcare, MiniTrap) before purification. All water used was distilled & deionized water (Millipore) with a minimum resistivity of 16.5 megaohm.

Purification. Non-Photocleavable DNA oligos were purified by denaturing gel electrophoresis; after ethidium bromide staining, bands were cut out of 12-20% denaturing gels (run as described below) and eluted for 24 hours in 1 ml of elution buffer (500 mM ammonium acetate, 10 mM magnesium acetate, and 1 mM EDTA). These solutions were extracted with 3 x 0.5ml of butan-1-ol, precipitated in 80% ethanol at -40°C, the pellet rinsed with ice cold 70% ethanol. The pellet was dissolved and solution concentration quantified using A₂₆₀.

Photocleavable DNA oligos were HPLC purified as described below, desalted carefully (making sure not to exceed stated elution volumes: ammonium ions from HPLC buffer appear to interfere with surface functionalization) using G-10 (GE Healthcare, MiniTrap) columns and then quantified using A₂₆₀.

Formation of actuators. Complexes were formed by mixing a stochiometric quantity (10 pmol) of each strand as estimated by OD₂₆₀, in TAEMg buffer (40mM Tris, 40mM Acetic Acid, 12.5 mM Magnesium Acetate, and 2 mM EDTA) at a final concentration of 50 nM. This mixture was annealed from 90 °C (5mins), 65, 45, 37 and 20 °C (20 mins each). Identity and stochiometry were verified by nondenaturing gel.
Strand diagram

S1, S2, S4 = black
S3-set-closed = red
S3-fuel-closed = green
S3-set-open = blue
S3-fuel-open = orange
**Strand sequences**

The strands S1+S2+S4 form the ‘frame’. Frame+S3-Set-closed is the ‘closed’ state. Frame+S3-Set-open is the ‘open state’. The fuel strands remove the set strands from these complexes and return them to the frame conformation.

The ‘photocleavable’ S3 strands are identical in sequence to their non-PC counterparts, but with a 5’ photocleavable-NH₂ nucleotide on the 5’ end.

**Strand Name (Length)**

**Sequence**

<table>
<thead>
<tr>
<th>Strand Name (Length)</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>S1 (79)</td>
<td>CTGATAGGACGCTGAGATGCTCAGCTACGAGAACTGTCTGCTGTTTTTTACGGACATTGTGTTAGATCGTCACTGGACGTATCC</td>
</tr>
<tr>
<td>S2 (17)</td>
<td>GGATACGTCCAGTGACG</td>
</tr>
<tr>
<td>S4 (20)</td>
<td>CATCTCAGCCGTCTATCA</td>
</tr>
<tr>
<td>S3-SET-OPEN (75)</td>
<td>CTCTAACAATGTCCGTAATACAGGACAGTTCTCGTAGCTACGAGAATCTTTTTTTACGGACATTGTGTTAGATCGTCACTGGACGTATCC</td>
</tr>
<tr>
<td>S3-SET-CLOSED (54)</td>
<td>CTCTAACGTTCTCGTAGCTACGAGAATCTTTTTTTACGGACATTGTGTTAGATCGTCACTGGACGTATCC</td>
</tr>
<tr>
<td>S3-FUEL-OPEN (75)</td>
<td>ATCCAAGGCTACGCTACGAGAATCTTTTTTTAATTACGGACATTGTGTTAGAGCGAAGTGCTTTTTGCACTTCGGT</td>
</tr>
<tr>
<td>S3-FUEL-CLOSED (54)</td>
<td>TAAGGAACCTCGCTACGAGAATCTTTTTTTACGTAGTTGTTTTTACCTACTCGC</td>
</tr>
<tr>
<td>T15</td>
<td>5’NH₂-TTTTTTTTTTTTTTT</td>
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The frame sequences (S1,S2, S4) are identical to those published by Yan et al² - the S3 strands have the same sequence as this paper but have a 10 bp hairpin appended to the 3’ end with a T₅ loop. The hairpin’s sequences were designed using SEQUIN³ to minimize unwanted interactions with other strands. The hairpin is designed to do two things i) sterically block the approach of the actuator to the surface. The surface is where the 5’ end of strand contains unpaired DNA that is complementary to the actuator state resides, thus the hairpin is referred to the ‘steric block’. The sequence in the already-folded hairpin are designed to be unable to hybridize successfully with any other sequences present in solution.
**Solution phase operation of actuator.** 1.2 equivalents of strand (in TAEMg) were added to either the actuator in the 'frame', 'closed' or 'open state' (dissolved in TAEMg at 50 nM) and let sit for a minimum of 10 minutes. An aliquot was taken and run on a 10% non-denaturing gel as shown below. The lanes are annotated from Left to Right in the order that the experiment was run (i.e., in the first gel, lane 1 is frame, lane 2 is a solution of frame that set open was added to, lane 3 is a solution of open, lane 4 is a solution of open that ‘fuel open’ has been added to etc).

1. Frame+SetOpen, 2. Open 3. Open+FuelOpen 5.10bp Marker (to 100bp)

1 2 3 4 5


6 7 8 9 10

These gels indicate that the actuator is switched essentially quantitatively from Open->Frame, Frame->Open, Closed->Frame, Frame->Closed by the addition of the appropriate set strands.
Purity, identity, amino activity and photocleavage operation of the 5’NH2-PC set-strands. 
HPLC-purified and desalted set-strands (20 pmol) were dissolved in DMF (10 µL), and PEG-NHS ester (0.5 mg, MW 2500, branched, Pierce Protein Reagents) was added. The strands were allowed to react for 1hr, water (90 µL) added and the sample desalted using a G-25 (GE Healthcare, microspin) column. An aliquot of this sample was dissolved in TAEMg, and exposed to 365 nm UV light (Sylvania H44-GS 100W Mercury lamp in UVP B100 holder, 5mins). The unreacted strand, PEG-NHS-Ester-reacted strand and photocleaved strand were run on a denaturing gel as below.

The gel above shows
i) (Strand Lanes) That the HPLC-purified strands (FO=S3 fuel open, SO=S3 set open, FC=S3 fuel closed, SC=Set Closed) are homogenous and the expected length.
ii) (Strand+PEG lanes) That these strands react essentially quantitatively with NHS esters to give a single higher molecular weight product (non-primary amino functionalized strands do not do this: data not shown)
iii) (Strand+PEG+UV lanes) That upon exposure to UV 365nm light the added moiety is quantitatively cleaved (80% yield in the case of this sample of FC) and the original strand is returned (- the PC amino group, with a 5’ phosphate) (non-photocleavable 5’amino functionalized strands do not do this- data not shown).
**Photocleavable Surface preparation** Adhesive wells (Silicone isolator, 2mm diameter circular wells, JTR16S-A-2.0, Grace Biolabs) were affixed to aldehyde-functionalized microarray Slides (Pierce, Aldehyde ES).

Individual wells were loaded with set-strand (4 µL, 400 µM, in phosphate buffer: 1.5 M Na, pH 8.5). A freshly-made solution of Sodium Cyanoborohydride (50 mM in water) was added to the wells, let react for 3 minutes and the slide placed in a vacuum chamber (speedvac) for 30 minutes until the surface was dry. The surface was thoroughly washed with 10x10 µL water and 10x10 µL TAEMg buffer before being placed in a sonicator filled with 1L water for 30 minutes, followed by another 5x10µL wash with water. Just before use, the surface was then washed/equilibrated with 20 x 10µL of TAEMg.

Separate experiments indicated that upon photocleavage these surfaces released ca. 1.4 +/- 0.2 pmol of strand (data not shown - in preparation). This corresponds to a density of 44 pmol/cm², which is a higher density than most microarrays - surface hybridization is inefficient or at least significantly slowed down at this surface density. We attribute this relatively dense packing to the high concentration of sample in the deposition and the high salt concentration (1.5 M Na+) used in the deposition chemistry.

Irrespective of the mechanism(s) of blockage of hybridization, likely a combination of (i) surface strand density, ii) the bulky hairpin attached to the 3’ end of the set strand blocking the actuator approaching the surface or iii) the size of the actuator; hybridization at the surface is shut down. These effects will be delineated in detail in a follow-up paper describing effects of surface chemistry and functionalization on more sterically challenging systems.
Control expt showing that strand is only released when there is both a PC linker and UV exposure. (i.e. that release from the surface is not just thermal or from nonspecifically adsorbed strand)

15% Denaturing gel of aliquots removed from surface. Surface prepared as shown above: surface functionalized with amino-terminated strand (S3-fuel-closed-NH$_2$) and photocleavable amino terminated strand (S3-fuel-closed-PC-NH$_2$). The non UV exposed wells were covered with a mask made of aluminum foil.

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<th>UV</th>
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<tbody>
<tr>
<td></td>
<td>PC-NH$_2$</td>
<td>+</td>
<td>-</td>
<td>+</td>
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**Photocleavable operation of the actuator** The ‘Frame’, ‘Closed’ or ‘Open’ actuator at 12 nM in TAEMg was deposited in a freshly functionalized and washed well as shown above. The silicone isolator was covered with a cover slip (held in place with a clip) and exposed to UV light for 5 minutes: the wells that were not to be exposed to UV were covered with a mask made of aluminum foil. An aliquot was removed from the well and loaded on a nondenaturing gel. This is the gel shown in Fig 3 of the article text.

**Denaturing Polyacrylamide Gel Electrophoresis.** Gels contained 12-20% acrylamide (19:1 acrylamide/bisacrylamide) and 8.3 M urea; they were run at 55 °C. The running buffer consisted of 89 mM Tris, 89 mM boric acid (pH 8.0), and 2 mM EDTA (TBE). The sample buffer consisted of 90% formamide, 10 mM NaOH and 1 mM EDTA, containing 0.1% xylene cyanol FF tracking dye. Gels were run on a Hoefer SE 600 electrophoresis unit (31 V/cm, constant voltage).

**Nondenaturing Polyacrylamide Gel electrophoresis.** Gels contained 10-15% polyacrylamide (19:1 acrylamide/bisacrylamide) and were run at room temperature. The running buffer consisted of 40mM Tris, 40mM Acetic Acid (pH 8.0), 12.5 mM Magnesium Acetate, and 2 mM EDTA (TAEMg). Just prior to running, 10% v/v of a 50% glycerol / 50% aqueous TAEMg solution of 0.1% bromophenol blue and 0.1% xylene cyanol FF tracking dye was added to the sample. Gels were run on a Hoefer SE 600 electrophoresis unit (10 V/cm, constant voltage).

Visualization of gels was performed with SYBR Gold stain (Invitrogen) according to instructions from the manufacturer and imaged using a GelDoc 2000 (Biorad). Sensitivity limits (per well) are ca 0.1 pmol of actuators on nondenaturing gels and 1 pmol of set strands on denaturing gels.

**HPLC.** Preparative HPLC was performed using an Agilent 1100 Series system, with in-line UV detection performed at 260 nm. Column used was a Waters XBridge C18 column (2.5 µm particle size, 4.6 mm ID, 75 mm length), with a pre-column. Elution was performed with a mixed solvent system run at 72 °C with an integrated column heater: Solvent A: 0.1M Triethylamine Acetate Solvent B: Acetonitrile. Typically, gradients running from A:B 95:5 to 80:20 over 45 minutes were used, with a wash of 5:95 for 5 minutes at the end of each run to remove hydrophobic impurities from the column. The major peaks from each run were collected and desalted/quantified as above. Approximately 10 HPLC runs from a 1 µmol scale synthesis yielded 20-50 nmol of pure strand. Purity and size, amine presence and photocleavable activity was established by denaturing gel electrophoresis, and switching function established by nondenaturing gel electrophoresis on actuator samples.
Implications of strand density for operating complex DNA systems

Using the surface set-strand densities described above, the surface of the microarray slide (18 cm³) would hold ca. 825 pmol of set-strand if entirely covered. Allowing ca 20% of the surface to be reserved for edges and spaces between surface features (this would allow for accurate printing of the surface using inkjets/pins and photocleavage using a maskless array synthesizer), this corresponds to ca. 660 pmol per slide.

The number of different areas that could be selectively, sequentially cleaved (corresponding to different or repeated sequences) would depend on the quantities of the solution species above the slide. If one took a generous estimate that required 1 pmol per device, this would allow >600 machine steps (of identical or different devices) - plenty for DNA systems as they are currently described.

Using ‘catalytic’ set strands (Lukeman et al, in preparation) would allow an increase in this number by at least an order of magnitude.

References