Supporting Information for

Photocleavage Control of Nucleated DNA Nanosystems -
The Influence of Surface Strand Sterics

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1. Methods and techniques

Sources and purification of oligonucleotides.

<table>
<thead>
<tr>
<th>Oligo</th>
<th>Supplier/Purification</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA - photocleavable</td>
<td>Trilink-Biotechnology / RP-HPLC - 1 µmol scale.</td>
<td>Samples 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 $A_{260}$.</td>
</tr>
<tr>
<td>DNA nonphotocleavable</td>
<td>IDT denaturing PAGE 0.25 µmol scale.</td>
<td></td>
</tr>
</tbody>
</table>

Unless stated otherwise, DNA was dissolved at 10 µM (non-photocleavable) or 150 µM (photocleavable) in water and stored frozen at -20°C.

Water was purified through a Barnstead EasyPure II system to at least 18 MΩ. Unless stated otherwise, all reagents were from Fisher and used as received. A Hoefer SE600 gel system was used for all acrylamide gels. The sonicator was a VWR Symphony 2.8L, used at room temperature on its only intensity setting.

**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; samples were dissolved in this buffer and heated to 90°C for 4 minutes before loading. Gels were run on a Hoefer SE 600 electrophoresis unit (~31 V/cm, constant voltage).

**Nondenaturing Polyacrylamide Gel Electrophoresis.** Gels contained 6-15% 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 Gel Logic imaging system (Kodak) - for this article the “invert” function was used on Photoshop to invert the black/white scale.
**Assembly of monomers and oligomerization.** Procedure described in Lubrich\(^1\) was followed.

Oligomerization reactions were carried out in 100 mM NaCl, 5 mM MgCl\(_2\), 20 mM Tris·HCl and 1 mM EDTA at pH 8.0 (“Na Buffer”). The two monomers were prepared separately by heating (to 90°C) and rapidly cooling (in ice-water) mixtures of loop strands (0.5 μM) and closing strands (0.67 μM, to ensure that all loop strands are closed). Assembled monomers were mixed stoichiometrically to give final loop strand concentrations of 0.25 μM. Rubbish collectors were added to final concentrations of 0.37 μM.

Before every reaction, Monomers were freshly annealed, and rubbish collectors added just before the start of reaction. Polymerization was initiated within one hour of mixing. All samples on the same gel derive from the same batch of monomer/rubbish collectors.

**Solution reactions:** Different amounts of seed were then added: oligomerization was allowed to proceed for one hour at Room Temperature (measured between 22+/− 4°C over the period of experimentation)

**Photocleavable Surface preparation** All of the below processes were conducted on the same day as the monomer solutions were prepared.

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 seed-strand (4 µL, 100 µM, in phosphate buffer: 1.5 M Na, pH 8.5). A freshly-made solution of Sodium Cyanoborohydride (1 µL, 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 “Na Buffer”.

**Surface PC reactions** Monomer/rubbish collector dissolved in “Na Buffer” (7µL) as described above 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 (365 nm UV light Sylvania H44-GS 100W Mercury lamp in UVP B100 holder, standard exposure time 5 minutes - photocleavage reaction is complete during this time period as shown in the previous photocleavage study\(^2\)): the wells that were not to be exposed to UV were carefully covered with a mask made of aluminum foil.

Reactions were run for 1h after photocleavage unless stated otherwise; reactions under solution conditions were found to reach completion during this time. The entire well was pipetted and run on an analytical nondenaturing gel (for polymerization reactions) or denaturing gel (for quantitation of seed cleavage reactions).

Quantities of solution present in the well were always checked against the initial amount loaded into the well. With a well-sealed slide using a cover slip, losses were usually <5% by volume - if >10% by volume was lost, the well was considered invalid.
2. DNA Sequences

Solution strand sequences

As reported in Lubrich \(^1\) **Identical nomenclature to Lubrich is used as shown below.**

Seed
S ACTGGAACTAGTTGATGAAGCTG

Monomer 1
L1 GTGTGCCTATTATGTCTCCTCCTCCAGCTTCATCAACTAGTTCCAGT
C1 CTAGTTGATGAAGCTGGACATAATACGCACAC

Monomer 2
L2 AGGAGGAGACATAATACGCACACACTGGAACTAGTTGATGAAGCTG
C2 CAGCTTCATCAACTAGTTGATGAAGCTG

Rubbish Collectors
Rubbish collectors are strands that drive the formation of polymer by hybridizing to newly revealed closing strands, forming Waste1 and Waste2 complexes.

R1 TGCGTATTATGTCCAGCTT
R2 GCACACCTAGTTGATGAAG

<table>
<thead>
<tr>
<th>Seed (S)</th>
<th>Rubbish collector 1 (R1)</th>
<th>Monomer 1 (M1)</th>
<th>Waste 1 (W1)</th>
<th>Rubbish collector 2 (R2)</th>
<th>Monomer 2 (M2)</th>
<th>Waste 2 (W2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Diagram" /></td>
<td><img src="image.png" alt="Diagram" /></td>
<td><img src="image.png" alt="Diagram" /></td>
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<td><img src="image.png" alt="Diagram" /></td>
<td><img src="image.png" alt="Diagram" /></td>
<td><img src="image.png" alt="Diagram" /></td>
</tr>
</tbody>
</table>
This reaction is sub-stoichiometric because 1 molecule of seed generates polymers that utilize many molecules of monomers: see the enclosed diagram b) from Lubrich that shows the polymerization cycle: c) shows the polymer formed.
Surface-attached strand sequences

HP-Seed
[5'PC-Amino-Linker]-ACTGGAACTAGTTGATGAAGCTGGGCATTCGGTCTTTTTTACCGAATGCC

5'-Seed
[5'PC-Amino-Linker]-ACTGGAACTAGTTGATGAAGCTG

3'-Seed
ACTGGAACTAGTTGATGAAGCTG-[PC-Linker]-[3'AminoC6]

T15
[Amino-C6]-TTTTTTTTTTTTTTTTT

Initially for comparison we synthesized the 3’seed with reverse amidites (glen research) and the
5’ PC-Amino linker, but did not obtain enough of this material to use in investigations.

Structures of linker units - as can be seen there are differences between the 5’-PC-Amino
linker and the PC-Linker-3’Amino C6 pair; the different steric environment around the 5’ and 3
end of the seed strand is also noted.
3. Demonstration of purity/activity of PC NHS strands

We used the same process as in our previously published paper\(^2\) to ensure activity/purity of our photocleavable strands.

**Purity, identity, amino activity and photocleavage operation of the NH2-PC seed-strands.**

HPLC-purified and desalted seed-strands (20 pmol) were dissolved in neat DMF (10 µL), and a large excess of 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 acylation conditions are as recommended by the manufacturer of PEG NHS ester

The gel on the left shows:

i) (Strand Lanes) That the HPLC-purified strands are mostly homogenous and the expected length. We note that the hairpin strand has a small amount of “dimer” in it, and the 5'/3' strands have some strand missing the PC amino group - this gel shows that impurities do not react with the NHS esters - and thus will not stick to the surface in surface functionalizations.

ii) (Strand+PEG lanes) That the PC-amino functionalized strands react essentially quantitatively with NHS esters to give a single higher molecular weight product

iii) (Strand+PEG+UV lanes) That upon exposure to UV 365nm light the PEG is quantitatively cleaved (~95% yield in the case of this sample of HPPC) and the original strand is returned (HPPC/5’ missing the PC amino group, with a 5’ phosphate . 3’: missing the PC aminolinker with a 3’ C2 alcohol)

Notes: 5’amino functionalized strands - (i.e. strands without the PC unit, but with a 5’ C6 amine) do not cleave as shown in this gel. Non-primary amino functionalized strands do not react with PEGylation reagent\(^2\)

We also note the HP-seed purity is much higher than casual inspection of the gel intensity would suggest - the unmodified hairpin strand main band has significant “internal quenching” of the SYBR dye (evidenced by the dark ring around the main band) , meaning that there is a large amount of the material in that band. Note that this self-quenching only occurs when single bands have >8 pmol of strand in them like in these lanes; no self quenching is observed in the later nondenaturing gels. We also note that monitoring relative quantities of DNA using SYBR fluorescently stained gels has significant precedent in the literature\(^3,4\).
4. Demonstration of solution-initiated reaction by 5’/3’/HP PC strands

Samples of photocleaved 5’/3’/HPPC were added to monomer as described in “Assembly of monomers and oligomerization”. This gel replicates the original polymerization reaction performed by Lubrich with our seeds and is the solution-based parallel to the experiment shown in Figure 3 in the text.
5. Surface Photocleavage Yield Demonstration

Here we compare the yield of seed release from the surface with known quantities of seeds on a gel. Denaturing gels below show 6 photocleavage experiments for each of the 3 strands. We perform these experiments to make sure that our other observations are consistent with quantities of seed being released from surfaces.

Results:

**Hairpin-Seed:** $0.5 \pm 0.1 \text{ pmol per well} - 15 \text{ pmol/cm}^2$

**5’ Seed:** $0.7 \pm 0.2 \text{ pmol per well} - 22 \text{ pmol/cm}^2$

**3’ Seed:** $1.5 \pm 0.2 \text{ pmol per well} - 45 \text{ pmol/cm}^2$

We are unsure of the source of this variation in seed density. The depositions were carried out in parallel on identical surfaces, in identical buffers, with identical concentrations of seed. We note the varying purities of the seeds - however, the depositions are carried out in large excess, the evaporation step in 1.5 M Na+ should drive formation of maximal surface density. Also, the impurities are inactive to covalent bond formation (see the solution based purity/activity gel in section 3); the gel above also shows only pure strands are released by photocleavage. We do not believe that other low MW amines that could confound the surface functionalization are present in any of our samples as they are carefully desalted.

We hypothesize that this variation could be a sequence effect (proximity on the surface of 5’ vs. 3’ end) or the nature of the linker.

Our confidence in these density trends is backed up by the polymerization data: when reactions initiated by surface cleavage reach completion (as seen in Fig 3 in the main text), the polymer length - (as evidenced by shorter polymer=more fast moving bands) follows the order $3’<5’<\text{HP-seed}$ which corresponds to quantity of seed released from the surface in order of $\text{HP}<5’<3’$. 
6. Notes on leak testing, quantitation and source of leak effect

Comparing seed leaks has to be done on the same slide, using the same monomer stock and the same gel, as we have found significant inter-slide variability. We calculate quantities by integrating the intensity of a fixed size gel section (using ImageJ image-processing software) corresponding to the polymer fraction*. We subtract background (an identically sized section of empty gel) and sum the corresponding leak values.

Below are some representative data after 1h:

<table>
<thead>
<tr>
<th>Seed</th>
<th>Sur</th>
<th>Sur Wash</th>
<th>Background</th>
<th>Total Leak</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>215659</td>
<td>124148</td>
<td>84809</td>
<td>170189</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>233979</td>
<td>176248</td>
<td>84809</td>
<td>240609</td>
<td>1.41</td>
</tr>
<tr>
<td>3</td>
<td>251282</td>
<td>118213</td>
<td>84809</td>
<td>199877</td>
<td>1.17</td>
</tr>
<tr>
<td>HP</td>
<td>520302</td>
<td>366726</td>
<td>311260</td>
<td>264508</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>541740</td>
<td>401533</td>
<td>311260</td>
<td>320753</td>
<td>1.21</td>
</tr>
<tr>
<td>3</td>
<td>532042</td>
<td>361281</td>
<td>311260</td>
<td>270803</td>
<td>1.02</td>
</tr>
</tbody>
</table>

After 4h on the surface, the leaks are more extensive, but the trend remains

<table>
<thead>
<tr>
<th>Seed</th>
<th>Sur</th>
<th>Sur Wash</th>
<th>Background</th>
<th>Total leak</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>509609</td>
<td>322629</td>
<td>274736</td>
<td>832238</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>523120</td>
<td>400046</td>
<td>274736</td>
<td>923166</td>
<td>1.11</td>
</tr>
<tr>
<td>3</td>
<td>538387</td>
<td>336945</td>
<td>274736</td>
<td>875332</td>
<td>1.05</td>
</tr>
</tbody>
</table>

We note there that the main effect observed is that the 5’ surface wash value is high - i.e. more polymer is left attached to the 5’ surface than the HP or 3’ surface. We hypothesize that low surface coverage of the HP seed doesn’t allow a lot of monomer to bind to the surface in the first place (less seed on surface); the Hairpin even at these densities stops polymer growth/binding (steric protection); while high density coverage of the 3’ doesn’t allow much monomer to bind to the surface (lots of seed on surface but unavailable)......the 5’ surface may hit an unfortunate ‘sweet spot’ spot of ‘density’ and ‘accessibility’, thus leaking more overall.

Given that the density of surface preparation in our hands is reproducible, (see section 5 in SI), we believe that hairpin surfaces at low (or high!) density offer the most robust preparation for low leak systems.

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*This can also be measured by the reduction of intensity of the monomer band compared to the control ‘0%’ lane; however, this tells us nothing about the surface leak (i.e. whether polymer was growing from the surface).

**We also note that the solution leak test quantitate whether polymer actually has the seed attached to it (i.e. the seed is coming off the surface via some undefined process - unlikely due to the strength of the covalent bond, and our extensive washing - see experimental section - but possible) or , as is more likely, the polymer forms initially on the surface then breaks off, leaving the seed behind.
Example leak test quantitation:

<table>
<thead>
<tr>
<th>Regions on gel</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1=</td>
<td>background</td>
</tr>
<tr>
<td>2,3</td>
<td>HP sur, sur wash</td>
</tr>
<tr>
<td>4,5</td>
<td>5’ sur, sur wash</td>
</tr>
<tr>
<td>6,7</td>
<td>3’ sur, sur wash</td>
</tr>
</tbody>
</table>

References