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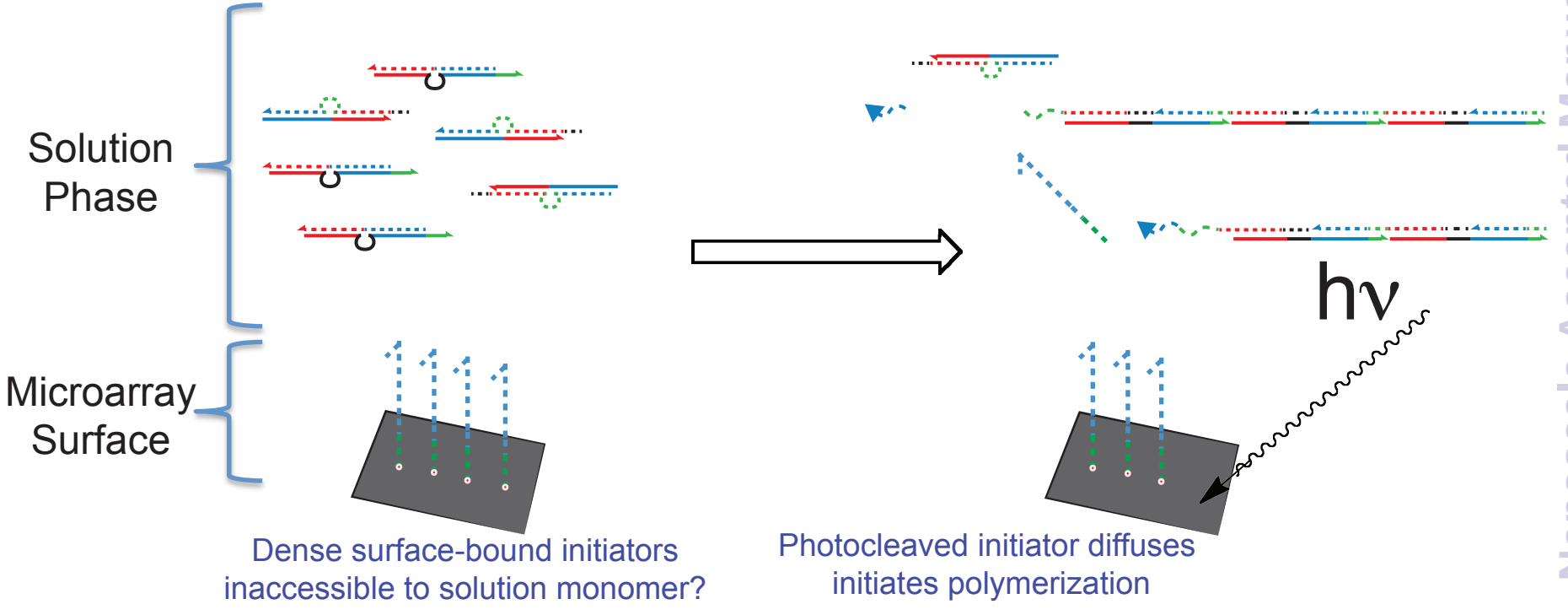
Text for graphical abstract

Surfaces that display sterically hindered, photocleavable strands that initiate a strand-displacement based polymerization are studied for their robustness to a 'leak' reaction and time dependence of photocleavage.

Model system for Surface-Operated Catalytic DNA Nanodevices

Orientation and steric protection of initiator affects surface usability.
Increased illumination time releases more initiator.



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ARTICLE TYPE

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

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We use sterically inaccessible 'seed' strands, released from a surface into solution by photocleavage to initiate a nucleated DNA polymerization reaction. We demonstrate control of the quantity of 'seed' release and that hairpin steric protection of the 'seed' leads to less 'leaky' surfaces. This polymerization is a model system for surface-photocleavage initiation of sub-stoichiometric reaction cascades; these cascades should find use as a component of labs-on-chips capable of bioanalytical and DNA-computing tasks.

Light can be used to cage and uncage proteins/oligonucleotides, as a traceless crosslinking 'reagent' for the generation of photosensitive nanomaterials[1] and for the control of gene circuits[2].

Dynamic DNA-based nanotechnology utilizes 'toehold mediated strand displacement'[3] (TMSD) to enable the control of circuits, catalytic amplifiers, autonomous molecular motors and reconfigurable nanostructures[4, 5]. The same photochemical tools that biochemists, nanomaterials scientists and gene-circuit engineers use has added a further dimension of control to TMSD systems. Photocleavage of nucleobase protecting groups has enabled control of a TMSD-based AND-gate[6], photocleavable hairpin linkers have controlled toehold formation[7], and photochemistry has been used in spatial-diffusion control of TMSD circuits in acrylamide gels[8].

Unlike in many surfaces displaying DNA for biosensing[9], our previously demonstrated light-controlled TMSD system[10] used photochemical cleavage of a microarray surface displaying **densely** packed regions of hairpin sterically-protected toehold-binding 'set' strands. When photocleaved, a particular 'set' strand stochiometrically reacted with a solution of a device layered above the surface, thus changing the device's state.

Many TMSD-based devices use sub-stoichiometric (or catalytic) quantities of toehold-binding strand to initiate reaction cascades, usable in bioanalytical systems such as a lab-on-a-chip. These devices should be much more sensitive to 'leaks' from the surface; that is, an unwanted initiation of reaction from toehold-binding-strand before cleavage. If surface-based photocleavage is to be used to control reaction cascades, these leaks need to be minimized. These systems can also probe the likelihood of multiple stoichiometrically controlled devices functioning well above a surface: if one stoichiometric reaction has a small leak, it will be difficult to detect and have little consequence, but if hundreds of stoichiometric reactions each have a small leak, then crosstalk will be problematic.

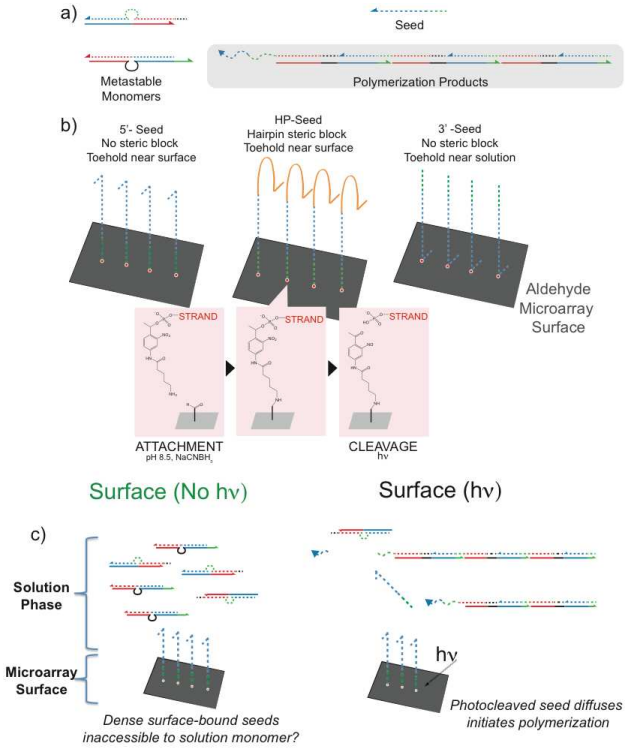

Figure 1. System design and experiment details a) The polymerization reaction developed by Lubrich. Polymerization of metastable monomers are initiated by a toehold-displaying 'seed' strand; complementary sequences are dashed and solid identically coloured lines. The 'toehold' is the green part of the seed. b) The seed sequence packed densely on an aldehyde-functionalized microarray slide, attached with a photocleavable linker. c) Illustration of the microarray surface overlaid with a solution of monomers before (left) and after (right) exposure to 365 nm UV light. Useful surfaces have minimal 'leakage' - that is, polymerization initiation by the surface bound-seed before photocleavage.

To explore these issues, we utilize as a model system a TMSD initiated polymerization[11], in which two toehold-displaying metastable monomers are polymerized by a 'seed' initiator strand. This reaction is sub-stochiometric - i.e. one seed molecule consumes tens to hundreds of monmers; thus this system should be ideal to test the leakiness of surfaces (See ESI for more details).

All surfaces are prepared under identical conditions with strands that contain the same seed sequence. However, as Figure 1b shows, the hairpin (HP) seed's toehold is both protected by its orientation (at the surface) and the hairpin. The 5' seed has the hairpin deleted so that only orientation should act as protection; and the 3' strand displays the green 'toehold' away from the surface near the solution phase. Figures 1a and 1c shows the solution and surface reaction scheme.

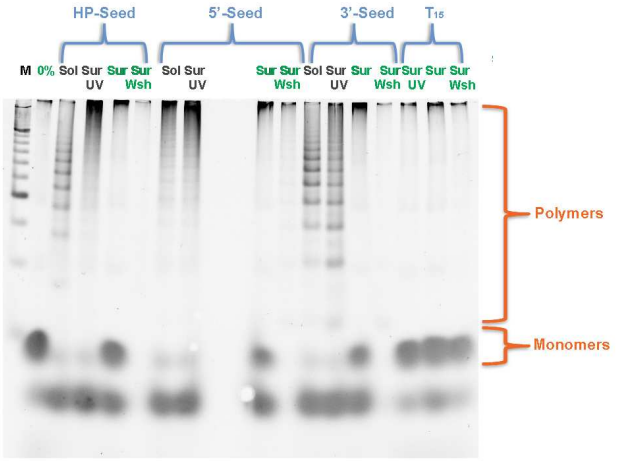

Figure 2. Gel showing polymerization initiation by surfaces displaying different photocleavable strands.
Key: M=50 bp Marker. 0%= No seed added in solution (control for 'background reaction'). Sol=20% seed added in solution (control for 'reaction going to completion'). Sur UV = seed cleaved from surface ('reaction'). Sur= Seed not cleaved from surface ('leak'). Sur Wsh=after Sur reaction, fresh buffer added, surface cleaved ('leak products stuck to surface'). T_{15}=surface with non-photocleavable T_{15} oligos attached (controls for 'the effect on the background reaction conducted in a microarray slide well displaying DNA on a surface rather than in a test tube'). Green lanes are controls where no polymer is meant to form because no seed is in solution. Black lanes are reactions where polymerization is meant to reach 100% The band below the monomers is waste duplex from polymerization. For details of solution/surface preparation and further controls/characterization see the SI.

Figure 2 shows successful surface initiation of reactions with all 3 seeds. Lanes labeled with black text show that reactions reach completion (judged by dissappearance of monomer) whether initiated in solution or from the surface by photocleavage. The lanes labeled with green text show the 'leakage' reaction. We show two kinds of leakage per seed: a) 'Surface' : all of the solution is removed from above an uncleaved surface, showing 'solution leakage' - that is, reaction initiated on the surface, whose polymeric product diffuses into solution b) 'Surface Wash': the uncleaved surface well from a) is 'washed' by adding new buffer solution and photocleaved, showing if any polymer formed on the uncleaved surface remained attached.

After one hour, the 5' seed showed 20-40% more total leakage than the HP seed - as expected. The 3' seed, surprisingly, has a leak value 2-17% more than the hairpin (i.e. *less* than the 5' seed), not what would be expected based on toehold availability. Part of the reason for this appears to be the strand density of these surfaces. For reasons yet to be determined, despite preparing the surfaces under identical maximal-density forming condtions, the 3' seed surface has a higher density (~45 pmol/cm^3) than the 5' (~22 pmol/cm^3) and HP seed (~15 pmol/cm^3). The 3' seed is thus likely affording some crowding-based protection despite the solution accessibility of the toehold. This higher density is further evidenced by the shorter polymers formed in the surface clavage reaction with 3' Seed; since a larger quantity of Seed released gives shorter polymers. (See SI for data and further discussion on surface density and preparation).

All of these surface densities are, by design, considerably higher than standard microarray surfaces. The fact that the lowest density surface (HPPC) has the lowest total leak , confirms the advantage that this hairpin steric block has for robust surface preparation. All other things being equal, based on a surface crowding analysis we would expect *more* solution leak for a low density surface, since the seed sequence is more 'available'. The efficiency of different microarray surfaces blocking hybridization is an ongoing topic of study[12-14]; whatever the reasons for the HP-seed's lower leak, it is **effective** in this role. Investigations are under way to delineate the effect of seed/linker design, time, deposition and concentration on the behaviour of these surfaces.

It is worth noting that duplexes displaying a toehold (i.e. our monomers) are one of the more sterically sensitive designs we could test; thus the leak values that we obtain here are likely upper bounds for TMSD systems. We expect branched structures or origami based TMSD systems to be *more* robust to leaks. If steric protection of the toehold-displaying system in question does not help, less-leaky sequence designs[15] or photchemically protected toeholds[7] are alternate strategies which could be used.

It would be useful to be able to control the delivery of different amounts of seed from these surfaces, allowing light-control of the extent of TMSD reaction with seed/set strands. Figure 3 shows that this is possible: varying cleavage times results in delivery of different quantities of seed into solution.

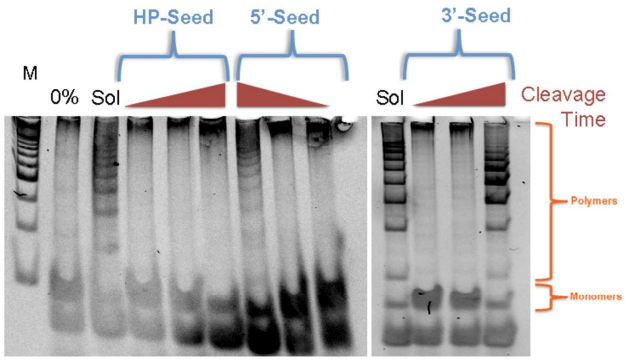

Figure 3. Demonstration of time controlled cleavage from surface. Lanes labelled with black text are solution controls or marker. As the cleavage time increases from 5 to 20 to 300 seconds (thus releasing more seed) the amount of monomer can be seen to decrease, yield of polymer increase, and the length of the polymer decrease (see SI for corresponding solution-based experiment). Note that all polymerization reactions took place over the same length of time, in *different* wells on the same slide; only the exposure time to UV light was changed.

In conclusion, we have demonstrated that photocleavable surfaces can be made more robust to leakage by using hairpin-protected strands and that we can release controlled amounts of these strands from surfaces. We expect that microarray surfaces displaying regions of hundreds to thousands of set/seed strands will be printable[16]; these surfaces should be addressable by a device similar to a maskless array synthesizer[17]; this experimental set-up should to be able to control and operate dozens of TMSD based-systems in parallel for computation or bioanalysis[18]. Investigations to achieve these ends are ongoing.

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Notes and references

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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/b000000x/

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