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Supporting Information

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Programmable Cell Adhesion Encoded by DNA Hybridization**

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Materials and Methods for experiments described in the main text.

Supplementary Data from experiments described in the main text.

Supplementary Figures S1-S2 (individually numbered and integrated into the above).

Supplementary Scheme S1 (individually numbered and integrated into the above).

Materials and Methods

General experimental methods

All reagents were obtained from commercial sources and used without further purification unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica Gel 60-F_{254} plates with visualization by ultraviolet (UV) irradiation at 254 nm and/or staining with potassium permanganate. Purifications by flash chromatography were performed using EM silica gel 60 (230-400 mesh). The eluting system for each purification was determined by TLC analysis. Chromatography solvents were used without distillation. All reactions were carried out under an argon atmosphere in flame-dried glassware unless otherwise noted. All organic solvents were removed under reduced pressure using a rotary evaporator. Tetrahydrofuran and triethylamine were distilled under an inert atmosphere from Na°/benzophenone and calcium hydride respectively. Water (mpH_2O) used in all procedures was purified using a NANOpure™ purification system (Barnstead, USA).

All oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). PolyPakII™ cartridges (Glen Research Corp., Sterling, VA) were used to purify phosphine-modified oligonucleotides. NAP-5™ gel filtration columns (Amersham Biosciences, Piscataway, NJ) were used for purifying thiolated oligonucleotides following reduction. G-25 Microspin Columns™ (Amersham) were used for buffer exchange procedures. Avidin-FITC (A-2901) was purchased from Sigma (St. Louis, MO). 7-amino-4-chloromethylcoumarin (CellTrackerBlue™), 5-chloromethylfluorescein diacetate (CellTrackerGreen™), and RedoxSensor™ Red CC-1 dyes were obtained from Molecular Probes (Eugene, OR).

All photolithographic and microfabrication techniques were performed at the UC-Berkeley Microfabrication Laboratory. For microfluidic device experiments, an Eclipse E800 epifluorescence microscope with digital camera (Nikon, Melville, NY) and a syringe pump from Bioanalytical Systems (West Lafayette, IN) were used.

All cell culture reagents were obtained from Gibco/Invitrogen Corp. (Carlsbad, CA) unless otherwise noted. Cell culture was accomplished using standard techniques. Briefly, Jurkat cells were grown in T-25 culture flasks (Corning, USA) in RPMI Medium 1640 supplemented with 10% (v/v) fetal bovine serum (FBS, HyClone, USA) and 1% (v/v) penicillin/streptomycin (P/S, Sigma, USA). Chinese Hamster Ovary (CHO) cells were cultured on 10 cm plates in F-12 (HAM) Nutrient Mixture supplemented with 10% FBS and 1% P/S. Human Embryonic Kidney cells (HEK) were grown on 10 cm plates in Minimum Essential Medium containing Earle's Salts and lacking L-glutamine. The media was supplemented with 10% FBS, 1% P/S, 2 mM L-glutamine, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate. Adherent cells were detached from their growth plates *via* treatment with trypsin-EDTA.

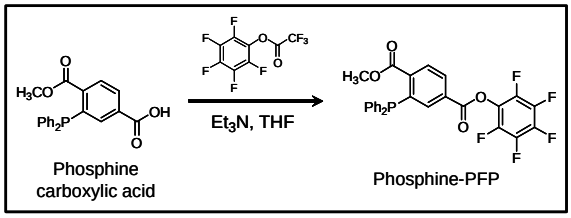
Matrix assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a Voyager-DE™ system (PerSeptive Biosystems, USA). All DNA-containing samples were co-crystallized using a 2',4',6'-trihydroxyacetophenone (Aldrich, USA) solution (11.5 mg in 2:1 acetonitrile:H_2O, with 2 mg of ammonium citrate). High performance liquid chromatography (HPLC) was performed on an Agilent 1100 Series HPLC System (Agilent Technologies, USA). Sample analysis for all HPLC experiments was achieved with an inline diode array detector (DAD). Both analytical and preparative reverse-phase HPLC of oligonucleotides was accomplished using a C18 stationary phase and an acetonitrile/100 mM triethylammonium acetate (TEAA) (pH = 6.9) gradient. Proton (^{1}H), carbon (^{13}C), phosphorous (^{31}P), and fluorine (^{19}F) spectra were collected with a Bruker AVB-300 (300 MHz) spectrometer. Ultraviolet absorption of the different oligonucleotides was

obtained at 260 nm on a UVIKON 933 double beam UV/Vis spectrophotometer (Kontron Instruments, United Kingdom). Flow cytometry analysis was carried out on a FACSCalibur machine (BD Biosciences, USA).

Metabolic engineering of cells

Acetylated ManNAz (Ac_4ManNAz) was synthesized according to previously published procedures[1]. A 10 mM ethanolic stock solution of the sugar was sterilized prior to use using 0.2 µm mesh Acrodisc® 13 mm filters (Pall Life Sciences, USA). Cells were grown in the desired amount of azido sugar for three days under the conditions described in the General Experimental Methods section. The appropriate quantity of Ac_4ManNAz stock was pipetted using sterile technique into the culture flask or plate and the solvent was allowed to evaporate. Media was added to dissolve the sugar prior to addition of cells. CHO and HEK cells were grown in 30 µM Ac_4ManNAz, and Jurkat cells were grown in 25 µM Ac_4ManNAz.

Synthesis of phosphine-DNA conjugates



Supplementary Scheme S1. Synthesis of phosphine-PFP from phosphine carboxylic acid.

To synthesize phosphine-DNA conjugates, 5'-amine-modified oligonucleotides of the desired sequence were reacted with phosphine-PFP as outlined below. Phosphine-PFP was synthesized from phosphine carboxylic acid (the synthesis of which was previously reported[2]) as shown in Supplementary Scheme S1. Phosphine carboxylic acid (300 mg, 0.82 mmol) was dissolved in THF (6.4 mL) under argon. Triethylamine (167 mg, 1.65 mmol) was added, followed by a drop-wise addition of pentafluorophenyl trifluoroacetate (277 mg, 0.99 mmol) over a period of 2 minutes. The reaction was stirred at room temperature for 30 minutes and then concentrated. Purification by silica gel chromatography (1:1 ethyl acetate:hexanes) afforded phosphine-PFP (300 mg, 69% yield) as a solid: mp: 105-106 °C; IR (thin film): 1766, 1725, 1521, 1052 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): *d* 3.68 (s, 3H), 7.71 (app t, 1H, *J* = 3.9), 8.08 (app t, 2H, *J* = 3.0); ^{13}C NMR (75 MHz, $CDCl_3$): *d* 15.29, 30.02, 52.76, 66.26, 125.24, 129.05, 129.50, 129.86, 130.29, 131.26, 132.18, 134.18, 136.71, 136.84, 138.23, 139.78, 141.60, 142.87, 142.94, 161.99, 166.75; ^{31}P NMR (160 MHz, $CDCl_3$): *d* -3.96; HRMS (ESI): Calculated for $C_{27}H_{17}O_4PF_5$ $[M+H]^+$ 531.0779, found 531.0774.

For cell adhesion studies, two oligonucleotide sequence pairs (A/A' and B/B') were designed. The sequences were identical in composition and differed only in sequence. Therefore, both A and B were 20-mer ssDNAs comprised of 25% of each base randomized such that neither would bear appreciable affinity for the other's complement. Each sequence was also calculated to possess comparable melting temperatures and minimal secondary structures. The sequence identities were as follows:

[1] S. J. Luchansky, H. C. Hang, E. Saxon, J. R. Grunwell, C. Yu, D. H. Dube, C. R. Bertozzi, *Methods Enzymol.* **2003**, *362*, 249.
[2] K. L. Kiick, E. Saxon, D. A. Tirrell, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 19.

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A:   5'-GTA ACG ATC CAG CTG TCA CT-3'
A':  5'-AGT GAC AGC TGG ATC GTT AC-3'
B:   5'-TCA TAC GAC TCA CTC TAG GG-3'
B':  5'-CCC TAG AGT GAG TCG TAT GA-3'
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Generally, 5'-amine-modified oligonucleotides (2.5 mg, 400 nmol) were reacted with phosphine-PFP (6.3 mg, 12 µmol) in 2.5 mL of a 1:1:23 H_2O : *N*,*N*-diisopropylethylamine : *N*,*N*-dimethylformamide solution overnight. Three volumes of 0.1 M triethylammonium acetate (TEAA) buffer (pH 7.0) were then added and the entire mixture was extracted three times with chloroform. The aqueous layer was purified using PolyPakII^{TM} cartridges, eluting with a gradient of 5% to 50% acetonitrile in 0.1 M TEAA buffer. The eluent was then lyophilized, redissolved in mpH_2O, buffer exchanged into degassed phosphate-buffered saline (PBS, pH 7.4), and quantified by UV-Vis spectroscopy. The purity of the obtained samples was ascertained *via* analytical HPLC. Oligonucleotides were characterized by MALDI-TOF MS. Observed masses were within 0.090% of expected masses. See, for example, Supplementary Figure S1.

In some instances, model Staudinger ligations on samples of phosphine-DNA conjugation reactions were conducted prior to purification. One volume of neat benzyl azide was added to 14 volumes of the reaction solution detailed above and allowed to react at room temperature for 30 minutes. Oligonucleotide masses were immediately obtained using MALDI-TOF.

Quantification of cell-surface DNA labeling

Both HEK and Jurkat cells bearing azides were utilized. HEK cells were detached from their growth plates *via* treatment with trypsin-EDTA. Cells were allowed to react for 1-2 hours at room temperature with up to 430 µM solutions of phosphine-DNA that had been prehybridized with a 5'-biotinylated complement (in PBS containing 1% FBS). The cells were then rinsed to remove unreacted DNA and treated with two aliquots of 5.6 µg/mL of avidin-FITC on ice (10 minutes each). The cells were then analyzed by flow cytometry. From measurements obtained using biotinylated beads of known biotin density and a similar diameter to that of the cells, we were able to correlate the fluorescence intensities observed by flow cytometry with the number of dye molecules on each particle or cell. The number of bound dye molecules was equated to the number of bound DNAs since only one dye molecule is expected per bound DNA. Relevant control conditions were run to confirm the azide- and phosphine-dependence of the binding.

Construction and validation of microfluidic device

Microfluidic devices consisted of a (poly)-dimethylsiloxane (PDMS) channel layer bonded to a glass substrate. 40 nm-thick Au pads were patterned on top of a 20 nm-thick Cr adhesion layer on cleaned 4 in-diameter borofloat glass wafers utilizing a liftoff protocol. Bare glass wafers were treated with hexamethyldisilizane (HMDS) for 5 min to promote photoresist adhesion, and Shipley 1818 photoresist was spun at 2500 rpm for 30 sec and subsequently cured at 120 ºC for 90 sec. The pads were patterned in a Karl Suss MA6 Mask Aligner through a chrome mask. Exposed positive photoresist was removed in 1:1 diluted microposit developer. A 20 nm seed layer of Cr and 40 nm Au film were evaporated onto the patterned glass substrate. The wafer was then treated with photoresist stripping solution, PRS-3000 (Mallinckrodt Baker, Phillipsburg, NJ), which removed the deposited Cr and Au metal from unexposed regions.

Fluidic channels were formed by placing SU-8 molded PDMS over the patterned wafer. Standard photolithography techniques were used to create 6 cm long channel molds that were 200 µm wide and 32 µm deep. Channels were formed by pouring PDMS (10:1 Sylgard 184 silicone elastomer base to curing agent, Dow Corning, Midland, MI) over the SU-8 mold and curing for 48 hr at 37 ºC. Fluidic access ports were created by punching 1 mm holes at each end of the SU-8 defined PDMS

channel. The device was formed by bonding the PDMS channels to the patterned glass substrate. The PDMS substrate was removed from the SU-8 mold and cleaned in a UV ozone oven for 8 min to promote glass-PDMS irreversible bonding. The PDMS substrate was aligned to the glass wafer with alignment marks, contacted, and the substrate sandwich was heated at 100 ºC for 15 min to promote permanent bonding. An external syringe pump was used to control flow rates as indicated here and in the manuscript text.

For coating of the Au pads with ssDNA, 20 µM solutions of thiolated DNA in PBS were flowed into the channel and allowed to stand for 90 minutes. The channels were subsequently rinsed with PBS for nine minutes at 100 µL/min prior to use. Solutions were generally introduced into the channels at flow rates of 1-4 µL/min.

For device validation/DNA segregation experiments, a mixed solution with 10 µM each of Texas Red-A and FITC-B in PBS was flowed into channels with pads bearing A' or B' strands and incubated for 30 minutes. The pads were visualized after washing (nine minutes, 100 µL/min, two times).

For experiments conducted without the PDMS channel layer, only the Au-patterned glass substrate was used. Solutions were delivered manually, without an extemal syringe pump. Identical concentrations of thiolated DNA were used to coat the Au pads.

Shear force tolerance of bound cells

Azide-engineered HEK cells were detached with trypsin-EDTA and divided into two subsets. The first portion was incubated with 430 µM phosphine-DNA (sequence A) in PBS with 1% FBS for 75 minutes, and the second portion kept on ice in the same buffer lacking DNA. The cells were rinsed following the incubation period. Each portion of cells was then introduced into microfluidic chambers with Au pads either lacking or bearing sequence A' (constructed as above), and incubated for 45 minutes. The chambers were initially washed with PBS at a flow rate of 2 µL/min for 5 minutes and observed. To test the shear force tolerance of the bound cells, the flow was varied from 2 µL/min to 50 µL/min over a period of 10 minutes, and the cells were observed. The flow rate and chamber volume were used to calculate the shear force in dyn/cm^2 using the Navier-Stokes equation.[3]

Analysis of sequence-specificity of cell immobilization

Azide-engineered Jurkat cells were divided into two subsets. To facilitate visual differentiation of the two populations, each subset was cytosol-labeled with either CellTracker Blue™ or CellTracker Green™ dyes. Each subset was then incubated with up to 612 µM phosphine-DNA (sequence A or B, respectively) in PBS with 1% FBS for 2 hours. The cells were rinsed, and equal amounts (volumes) of each population were mixed, introduced into microfluidic chambers with Au pads bearing either sequence A' or B' (constructed as above), and incubated for 35 minutes. Each chamber was then washed with PBS at a flow rate of 2-4 µL/min for 10-20 minutes. Chambers were observed before and after washing. Replicate data sets were collected in the following way: three device regions were selected at random before washing, photographed, and subsequently visualized after washing.

For the prehybridization study, a chamber with B'-coated pads was first incubated with 50 µM sequence B in PBS for 135 minutes. Following wash (nine minutes, 100 µL/min), green (B-bearing) cells were introduced and incubated as above. The chamber was washed with PBS and observed before and after washing.

[3] R. B. Bird, W. E. Stewart, E. N. Lightfoot, *Transport Phenomena*, Wiley, **1966**, 80-81.

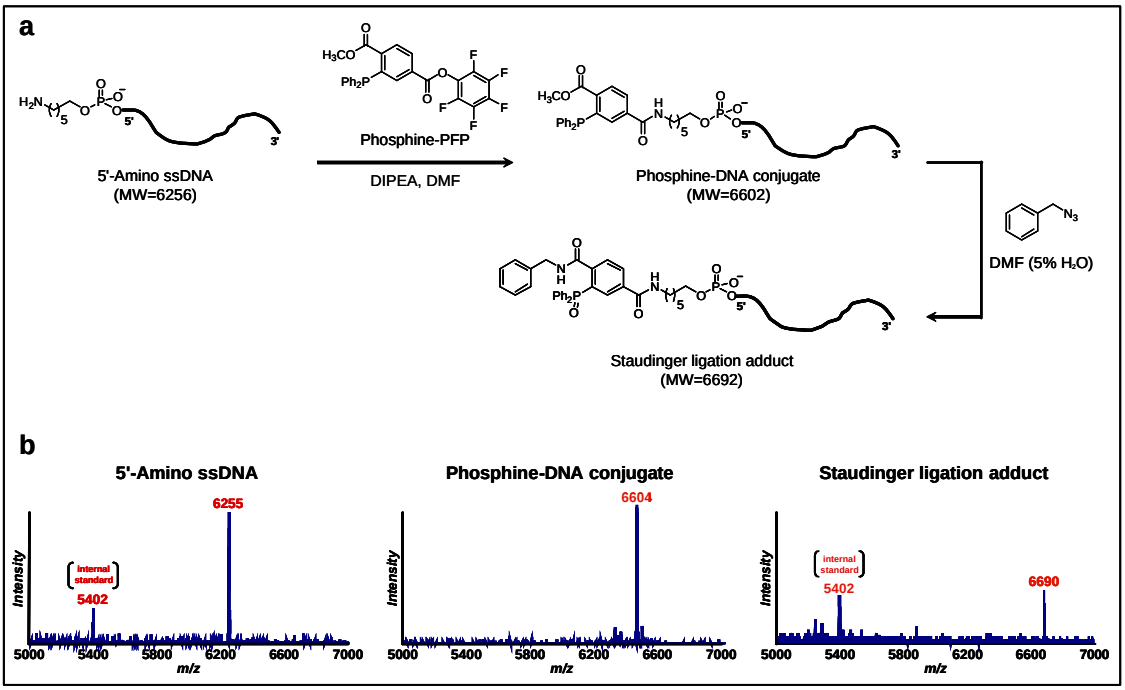
Cell position and viability studies

The two studies were conducted in parallel; azide-engineered Jurkat cells that were labeled with sequence A phosphine-DNA (as described above) were used for each study. For cell position studies, cells were introduced into microfluidic chambers with Au pads bearing sequence A' and incubated for 35 minutes. The chamber was washed with PBS (as above). Then, Jurkat culture medium was continuously flowed into the chamber at 0.4 µL/min for 25 hours. Chambers were observed using light microscopy at several time points to determine cell position.

To study viability and membrane integrity of bound cells qualitatively, the stain RedoxSensor^{TM} Red CC-1 was utilized. This stain passively enters live cells and is subsequently oxidized to a fluorescent product that is retained. A Au-patterned glass surface (coated with A' thiolated DNA) lacking a PDMS channel layer was used to mimic typical culture conditions more closely. Cells were introduced onto the surface and allowed to bind for 35 minutes. The surface was transferred to a Petri dish, and Jurkat culture medium was added. The dish was incubated at 37 °C for 25 hours. Following this period, excess culture medium was aspirated from the dish, and culture medium containing 5 µM of the dye was added. The dish was returned to 37 °C for an additional 30 minutes. The liquid was then aspirated, and the glass surface was manually rinsed with more culture medium. Both the rinse solution and cells that had remained bound were analyzed *via* light and fluorescence microscopy. Light microscopy was first used to determine the total number of cells bound or in solution. Fluorescence microscopy was then used to determine which of those cells had stained, and were therefore intact and viable.

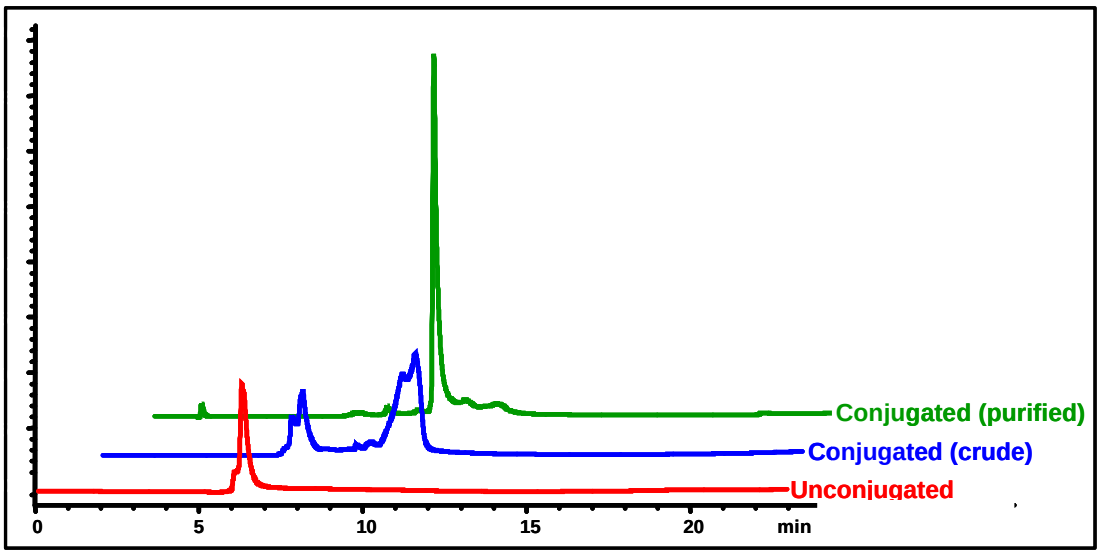
Supplementary Data

MALDI-TOF analysis of DNA conjugation and model Staudinger ligation



Supplementary Figure S1. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry of DNA oligonucleotide modification. a) Scheme for functionalization of 5'-amine-modified ssDNA (as in **Figure 1**) and model Staudinger ligation. b) MALDI-TOF spectra for each oligonucleotide species as indicated. In aggregate, these data support the conclusion that phosphine-ssDNA was successfully synthesized and was competent for Staudinger ligation.

HPLC analysis of phosphine-conjugated DNA



Supplementary Figure S2. Reverse phase HPLC data for phosphine conjugated ssDNA. The three traces are offset for clarity and note that the injection solution concentrations are unequal. The red trace corresponds to unconjugated DNA, the blue trace is for the crude phosphine-PFP labeling reaction (and therefore has modified and unmodified DNA), and the green trace is for the conjugated DNA after purification. The molecular weights of the purified products were confirmed using MALDI-TOF MS (see Supplementary Figure S1b). This technique indicated that little-to-no degradation (e.g. *via* phosphine oxidation) had occurred. Nevertheless, all DNAs were manipulated in degassed aqueous buffers and stored at -20 ºC until use.