Supporting Information

Selective Biomolecular Nanoarrays for Parallel Single-Molecule Investigations

Matteo Palma[#], Justin A. Abramson, Alon A. Gorodetsky, Erika Penzo, Ruben L. Gonzalez, Michael P. Sheetz, Colin Nuckolls, James Hone* and Shalom J. Wind*

Departments of Applied Physics & Applied Mathematics, Mechanical Engineering, Chemistry, and Biological Sciences, Columbia University, New York, New York, 10027.

RECEIVED DATE (automatically inserted by publisher) **E-mail: jh2228@columbia,edu; sw2128@columbia.edu**

MATERIALS AND METHODS

Nanodot fabrication: Electron beam lithography is performed in an FEI eletron beam microscope, which has been retrofitted with automated stage and beam control, operating at 30kV. Glass coverslips of thickness #1.5 (Corning) are cleaned by sonicaton in acetone for 2 minutes, sonication in IPA for 2 minutes, and then rinsed for 5 minutes with DI water. Coverslips are placed wet into a fresh piranha solution of 3:1 sulfuric acid, hydrogen peroxide for 10 minutes, rinsed for 5 minutes in running DI water stream, and blown dry with nitrogen or argon. Samples are dehydration baked on a hotplate at 180^{o}C for 1 minute immediately prior to resist application. A layer of 495k MW PMMA (microchem) at A2 concentration is spun on at 4000rpm for 45 seconds, with a ramp rate of 1000rpm/second, and baked for 5 minutes at 180^{o}C. A second layer of 950k MW PMMA (microchem) at A2 concentration is spun on at 4000rpms for 45 seconds, with a ramp rate of 1000rpm/second, and baked for 5 minutes at 180C. After allowing to cool, a thin layer of Aquasave is applied on the resist spinner at a speed of 800rpms for 35 seconds. Samples are then written in the FEI EBL system. Spot size 1 is used for nano-scale features and spot size 5 for larger alignment marks. Dosage must be empirically determined for each application through testing. Post writing and rinsing of the Aquasave with DI water, development is performed cold at 4C in 3:1 IPA to DI water, with sonication. Metal deposition is performed in the Semicore electron beam evaporator tool, and consists of a ca. 1nm Ti adhesion (~4.2% power, .1A/s) layer followed by ca. 2nm of AuPd (~4.5% power, .1A/s). Samples are lifted off overnight in Acetone at room temperature and are then ready for subsequent characterization and functionalization.

Nanoimprint lithography samples were fabricated according to published procedures.[1,2] In brief, nanoimprint was done either on Si substrates or glass cover slips covered with a 60 nm film of PMMA (35K, Microresist Technology GmbH) using a Nanonex BX-200 nanoimprinter. Typical imprint parameters used were: an imprint temperature of 180 ^{o}C, a pressure of 500 psi and an imprint time of 5 min. A hard mask, 12 nm of Ti was deposited in a Semicore SC2000 e-beam evaporator, at an evaporation rate of 0.2 Angstrom/sec. During this evaporation the substrates were tilted 30 degrees to the vertical direction. After Ti mask evaporation, the residual PMMA layer was removed by etching in an oxygen plasma asher (Technics 800, 200mTorr, 50 W, 30s). 3 nm of AuPd with a 1 nm Ti adhesion layer were deposited by e-beam evaporation after the etching. The areas, patterned with the arrays of nanodots, are clearly visible in the optical microscope at this stage. These patterned areas were marked with a wafer scriber (RA 120, Carl Suss) by making scribes near the pattern borders, in order to assist location of the patterns after liftoff. Finally, the liftoff was done by dipping the substrates for a few minutes in boiling acetone, followed by an additional rinse with acetone and isopropanol, and nitrogen blow drying.The thermal annealing, whose purpose is to provide to the fabricated nanodots with a uniform spherical shape along with a reduced diameter was carried out in a nitrogen atmosphere, at temperature of 450^{o}C for 1 hr.

Surface functionalization: Fabricated slides were first cleaned in an aged piranha solution (3:1 H_2SO_4:H_2O_2) to remove any residual material and to activate the glass surface for binding of PEG•silanes. Fresh piranha causes the metal nanopattern to delaminate, so piranha was aged 1.5 hours. Slides were then immersed in a 1mM mixed thiol solution containing a ratio of 3:1 EG•alkanethiol to biotinylated EG•alkanethiol (Prochimia) for ~18 hours (overnight) for the adsorption of a mixed SAM onto the gold dots. Slides were then immersed in a solution of 1.33 x 10^{-5}M mPEG•silane of molecular weight 5000 Daltons (Nektar) in anhydrous toluene (Sigma Aldrich) with 20µL of 17.6mM acetic acid (for catalytic purposes) for ~24 hours to passivate the glass surfaces against nonspecific protein adsorption. The slides were then rinsed with aceton then ethanol and finally blown dry with pressurized argon. Slides were then rinsed with phosphate•buffered saline (PBS; Dulbecco's Phosphate Buffered Saline 1X). They were then transferred to wells containing 20µL of 9.4 x10^{-6} M streptavidin•AlexaFluor488 (Invitrogen) diluted with 0.015 M chicken egg white albumin (Sigma Aldrich) in PBS to a final volume of 1.5 ml (final streptavidin concentration of 1.3 x 10^{-7}M) and incubated for 2 hours. Finally, the streptavidinated samples were rinsed in PBS and then immersed into a dsDNA solution. For dsDNA solution preparation we first hybridized in a ThermoScientific BupH™Phosphate Buffered Saline (pH=7.2) (TPBS) solution the following oligonucleotides (IDT): one 20-mer with a biotin functional group at the 5' position (5'- /52-Bio/GTC ACT TCA GCT GAG ACG CA -3') and the complementary strand with a RhodamineRed fluorophore at the 5'-end (5'- /5RhoR-XN/TGC GTC TCA GCT GAA GTG AC -3'); the samples were then immersed in a 1mm TPBS solution of the hybridized oligonulceotides for 2 hours, and then rinsed in TPBS. For the enzyme experiment we incubated the samples in a 7.7 x 10^{-9}M solution of PVUII-HF (New England Biolabs) in NEBuffer (New England Biolabs, 10mM magnesium acetate) and carried out the experiment at a temperature of 37°C.

Epi-Fluorescence Microscopy: Fluorescence measurements were performed using an Olympus IX81 (Olympus, Inc.) inverted microscope equipped with a 60x and 100x oil-objectives (Olympus) and a 512 x 512 pixel Cascade II CCD Camera (Photometrics, Inc.). Fluorescence images were analyzed with ImageJ, using the "analysis" macros for time-series of fluorescence intensities per pixel. The data were analyzed using both Microscoft Excel 2008 and MatLab.

Atomic Force Microscopy (AFM): AFM imaging experiments were performed with an XE-100 Advanced Scanning Probe Microscope (PSIA). For non-contact mode imaging, Mikromasch NSC16, (spring constant 40 N/m), n-type silicon (phosphorus doped) tips were utilized. The images were analyzed with XEI software, version 1.7.6.

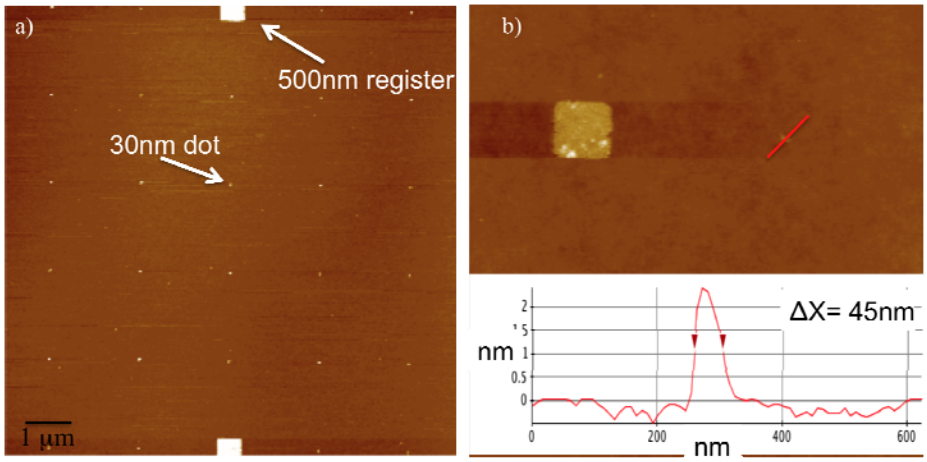

Figure SI-1: a)AFM image of Au/Pd nanodots arrays; b) AFM image of the array and height profile of a single nanodot showing a measured diameter of 45nm: because of AFM's tip-broadening effect,[3] the corrected diameter for the dot shown is 30nm.

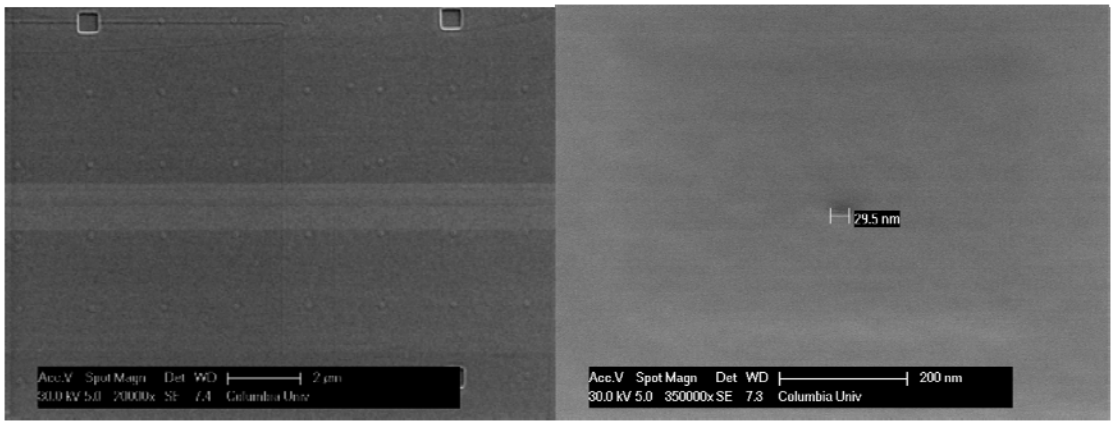

Figure SI-2. Scanning Electron Microscope (JEOL JSM-5600 LV) image of nanopattern holes prior to liftoff of the PMMA resist layer. The pattern has been written in the electron beam writer, developed, and the metal layers deposited, however the unpatterned glass surface is still covered with the PMMA bilayer, with metal on top. The large 1 µm square registers are visible, with ca 30nm holes in a square lattice with a 2 µm unit cell spacing in between.

Estimating the degree of non-specific adsorption of DNA: The average background fluorescence intensity per μm^2 measured on the glass surface in the absence of any fluorescently labeled moiety (in Arbitrary Units, AU, at 200 msec exposure) is 9654 (± 80) AU, while after exposure of the substrate to fluorescently labeled DNA, we measure an average fluorescence of 10389 (±464) AU per μm^2. From discrete step-like drops in the fluorescence intensity of physisorbed DNA (see figure SI-3), we can ascribe a fluorescence intensity of ca 2500 AU per fluorophore; this enables the determination of physisorbed DNA coverage on the glass surface of our bio-chip, to be between 0.1 and 0.5 μm^{-2} (i.e. less than one DNA every 2 μm^2).

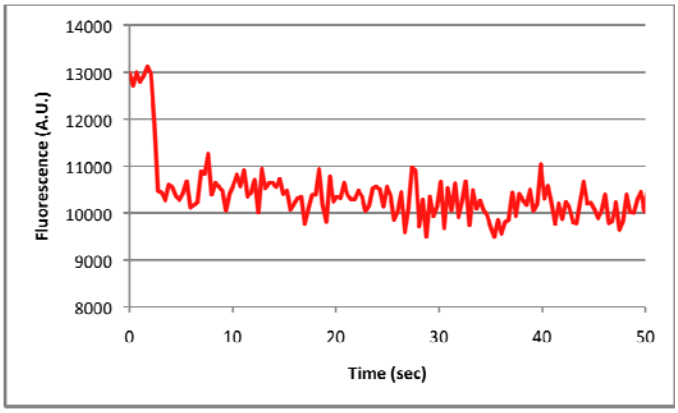

Figure SI-3. Representative single-step in the loss of DNA fluorescence due to enzyme-cleavage, monitored on non-specifically physisorbed DNA on the glass surface (200 msec exposure time)

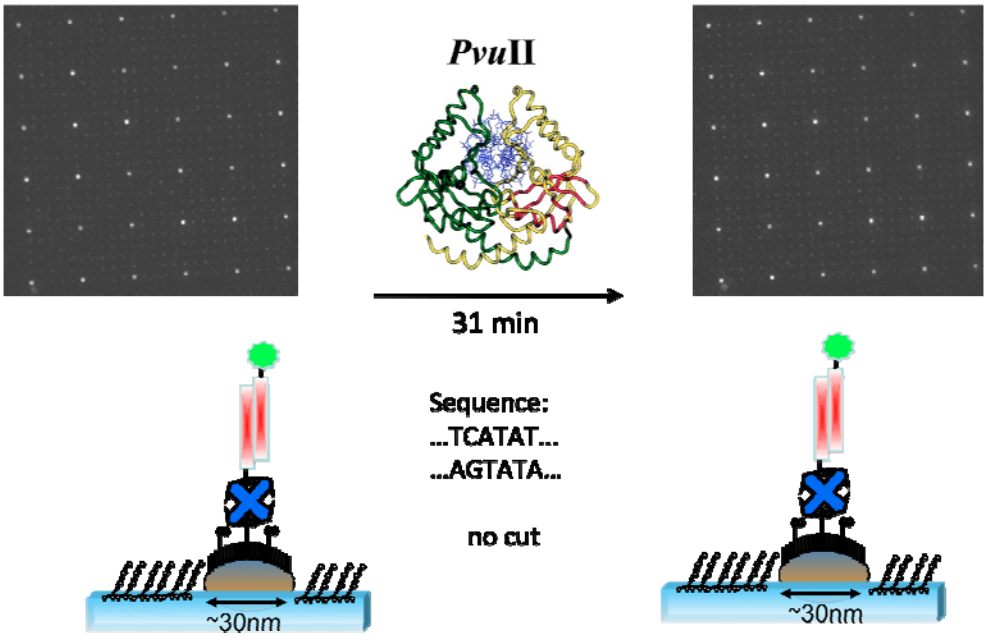

Figure SI-4. Scheme and Epi-fluorescence microscopy images of PVUII lack of recognition and cleavage of the nanodot-bound dsDNA, when this latter does not exhibit the right recognition sequence.

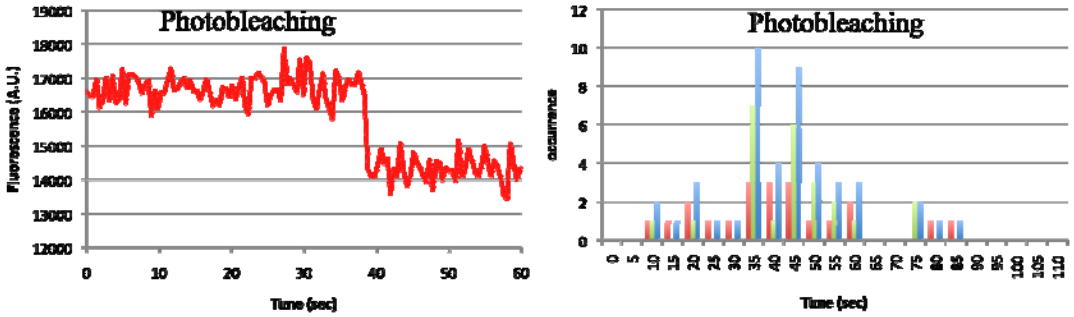

Figure SI-5 Representative single-step in the loss of DNA fluorescence due to photobleaching, monitored on an individual nanodot (300msec exposure times); and histogram of single-molecule photobleaching events per nanodots of nanoarrays

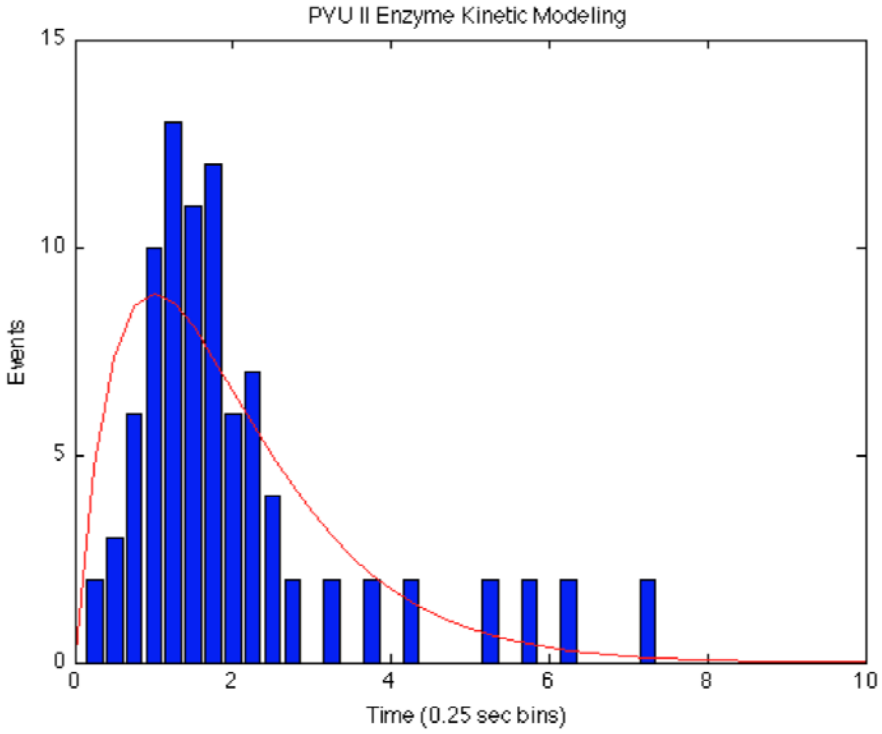

Figure SI-6 Representative histogram of single-molecule DNA cleavage events over an entire nanoarray. The red line is the fit obtained from a difference of two exponentials

$$f(t) = \frac{k_1 k}{k - k_1}\left(e^{-k_1 t} - e^{-k\ t}\right),$$ [4,5]

where the biomolecular rate constnat k_1 and the turnover rate constant k, derive form the kinetic equations for the Michaelis-Menten mechanism when the dissociation rate of the ES complex is negligible[5]:

$$E + S \xrightarrow{k1} ES \xrightarrow{k} E + P$$

where enzyme (E) and a substrate (S) form a complex (ES), that then forms the product (P).

MatLab version 7.8 has been used for the histogram fit shown in figure SI-6

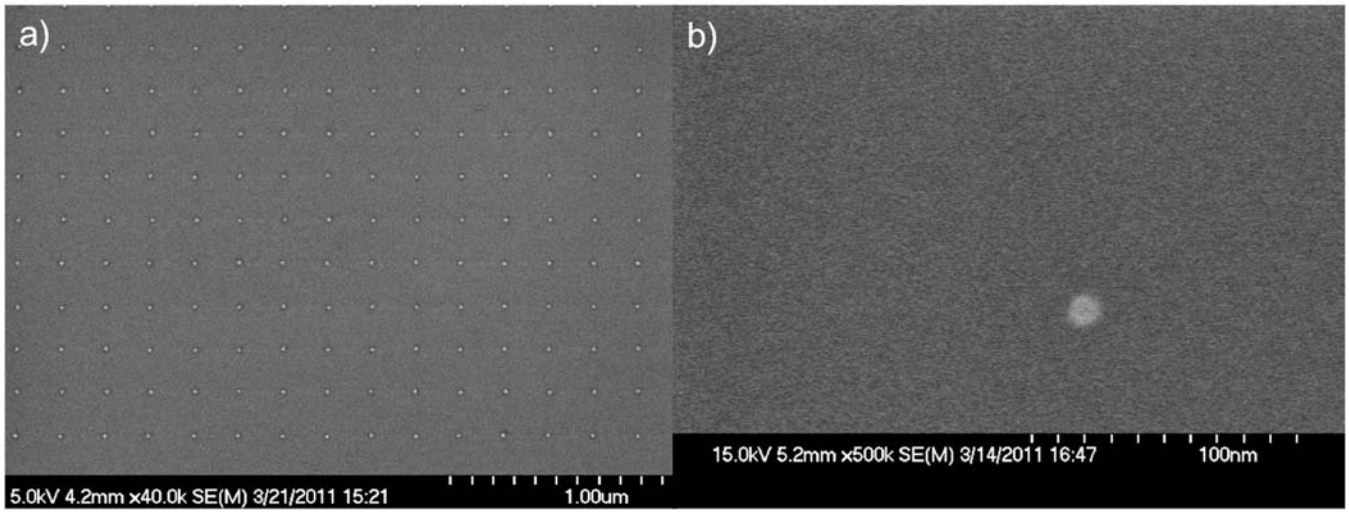

Figure SI-7 SEM images of the nanoarrays fabricated by nanoimprint lithography and employed for the experiments shown in figure 5 of the main manuscript: a) the nanodot array; b) a single dot showing the ~10nm diameter of the dot

Reference 18 of main text: Drmanac, R.; Sparks, A. B.; Callow, M. J.; Halpern, A. L.; Burns, N. L.; Kermani, B. G.; Carnevali, P.; Nazarenko, I.; Nilsen, G. B.; Yeung, G.; Dahl, F.; Fernandez, A.; Staker, B.; Pant, K. P.; Baccash, J.; Borcherding, A. P.; Brownley, A.; Cedeno, R.; Chen, L. S.; Chernikoff, D.; Cheung, A.; Chirita, R.; Curson, B.; Ebert, J. C.; Hacker, C. R.; Hartlage, R.; Hauser, B.; Huang, S.; Jiang, Y.; Karpinchyk, V.; Koenig, M.; Kong, C.; Landers, T.; Le, C.; Liu, J.; McBride, C. E.; Morenzoni, M.; Morey, R. E.; Mutch, K.; Perazich, H.; Perry, K.; Peters, B. A.; Peterson, J.; Pethiyagoda, C. L.; Pothuraju, K.; Richter, C.; Rosenbaum, A. M.; Roy, S.; Shafto, J.; Sharanhovich, U.; Shannon, K. W.; Sheppy, C. G.; Sun, M.; Thakuria, J. V.; Tran, A.; Vu, D.; Zaranek, A. W.; Wu, X. D.; Drmanac, S.; Oliphant, A. R.; Banyai, W. C.; Martin, B.; Ballinger, D. G.; Church, G. M.; Reid, C. A. *Science* **2010**, *327*, 78-81

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(2) Schvartzman, M.; Wind, S. J. *Nano Letters* **2009**, *9*, 3629-3634.

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