

**Supplementary Information for:**

**The Label-Free Unambiguous Detection and Symbolic  
Display of Single Nucleotide Polymorphisms on DNA  
Origami**

Hari K. K. Subramanian, Banani Chakraborty, Ruojie Sha & Nadrian C. Seeman\*

Department of Chemistry, New York University, New York, NY 10003, USA

hks224@nyu.edu; bc517@nyu.edu; ruojie.sha@nyu.edu; ned.seeman@nyu.edu

**EXPERIMENTAL METHODS:**

**Synthesis and Purification of DNA.** The DNA molecules in this study were either synthesized on an Applied Biosystems 394 automatic DNA synthesizer, removed from the support and deprotected or were purchased from Integrated DNA Technologies and Bioneer Inc. All of the marker and marker-base strands were purified by using denaturing gel electrophoresis. Other staple strands were left unpurified. M13 genomic DNA was purchased from New England Biolabs. Photo-cleavable linkers (3-(4,4'-Dimethoxytrityl)-1-(2-nitrophenyl)-propan-1-yl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite) were purchased from Glen Research. While purifying strands with photo-cleavable linkers on denaturing gels, exposure of the gel to light was minimized. Since UV used to illuminate DNA bands containing ethidium to visualize bands for purification on denaturing gels can cleave the linker, only one reference band was illuminated while others were covered up. Using the position of the reference band all other bands were cut.

**Formation and Filtration of the DNA Origami Tiles.** Single-stranded M13 genomic DNA (5  $\mu\text{L}$  of 80 nM stock; New England Biolabs) was combined with the staple strands (1:10 molar ratio of plasmid to staple strands, 1:4 molar ratio of plasmid to purified marker-strands and marker-base strands) and added to a buffer solution containing 40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 2.5 mM EDTA and 12.5 mM magnesium acetate. The final volume of the system was 23.3  $\mu\text{L}$ . The system was cooled from 90  $^{\circ}\text{C}$  to 60  $^{\circ}\text{C}$  over 70 minutes, and then cooled further to 16  $^{\circ}\text{C}$  over 12 hours on a thermocycling device. After the self-assembly of the origami tiles, they were purified, 11.5  $\mu\text{L}$  at a time, using Microcon centrifugal filter devices (100,000 MWCO, 600 x g speed, 4 minutes) followed by washing four times (at 600 x g speed, 9 minutes) with above 1x TAE-Mg<sup>2+</sup> buffer (Tris, 40 mM; Acetic acid, 20 mM; EDTA, 2 mM; and magnesium acetate, 12.5 mM; pH 8.0) to get rid of the extra free staple strands. The sample was collected from the filter using 1x TAE-Mg<sup>2+</sup> buffer and the final volume was brought to 20  $\mu\text{L}$ . About 40% to 50% of the origami formed is lost during the filtration process, as estimated by the quantity of origami tiles present in a given area of a mica surface before and after filtration.

**Selective Marker Invasion on DNA Origami Tiles.** Photo-cleavable linkers (Glen Research, Sterling, VA) on markers were incorporated into the origami tile, and then cleaved by exposure to UV light (356 nm for 15 minutes at 4  $^{\circ}\text{C}$ ). The competitive inhibitor strand for 'T' and its complement, which are used as a controlling element to make all invasions work at a single condition, are annealed separately (in 1x TAE-Mg<sup>2+</sup>) from 90  $^{\circ}\text{C}$  to 16  $^{\circ}\text{C}$  over 2 hours to a final concentration of 5  $\mu\text{M}$ . For invasion, 2.2  $\mu\text{L}$  (~ 0.005  $\mu\text{M}$ ) of filtered, photo-cleaved, origami tiles are added with 1.25  $\mu\text{L}$  (10  $\mu\text{M}$  in 1x TAE-Mg<sup>2+</sup> buffer) invasion strand for one of the letters and 2.2  $\mu\text{L}$  (5  $\mu\text{M}$ ) of annealed competitor strands. The final volume was brought to 6.25  $\mu\text{L}$  using 1x TAE-Mg<sup>2+</sup> (Tris, 40 mM; Acetic acid, 20 mM; EDTA, 2 mM; and magnesium acetate, 12.5

mM; pH 8.0). The reaction mixture was incubated at 25 °C for 3 hours to ensure completion of the reaction.

**AFM Imaging by Tapping in Buffer.** A 6.25- $\mu$ L sample was spotted on freshly cleaved mica (Ted Pella), and the sample was left to adsorb for 30 seconds. Additional fresh 30  $\mu$ L of 1x TAE-Mg<sup>2+</sup> (12.5 mM) buffer was added to both the mica and to the liquid cell. The AFM imaging was performed on a NanoScope IV (Digital Instruments) in buffer in tapping mode, using Veeco's SNL (Sharp Nitride Lever) probe. Among the four tips in the SNL probe, the small-thick tip was found to give good images without more sample damage than the sharper small-thin tip. Best images were obtained at scan sizes of 1.5 - 1.9  $\mu$ m, below which sample damage increases.

**Alignment and Averaging of Origami Images from AFM.** A program, named PADO, was developed using MATLAB with which the user can select origami tiles from an AFM images and align them on top of each other and average the aligned images. The software finds the best alignment using correlation based pattern matching.<sup>16</sup> For this, 2D correlation for two images are found and the images are moved around till highest correlation position is identified, which would have the best alignment. Once aligned, the images are added together and divided by the number of images to get the average image.

**Subtracted Images.** To obtain the subtracted image, the first step is to align the average images for each case of invasion with an image of an origami tile that has not been invaded (termed a 'full' image). Then each of the aligned invaded images were subtracted from the full image. This results in the pixels of patterns present in both full and invaded images cancelling out each other and the invaded pattern is the only one visible in the resulting subtracted image.

**Pixel Mean Values.** The 'pixel mean' values reported in Figure 5 are the average of all pixel values around the area of each particular alphabet symbol. A lower value indicates the alphabet symbol has been invaded to a greater extent. A program with a user interface to compute these values is provided along with the PADO package.