Selective Biomolecular Nanoarrays for Parallel Single-Molecule Investigations

Matteo Palma,[1,2] Justin Abramson,[2] Alon Gorodetzky,[3] Colin Nuckolls,[3]
James Hone,[2] and Shalom J. Wind[1]
[1] *Department of Applied Physics and Applied Mathematics*
[2] *Department of Mechanical Engineering*
[3] *Department of Chemistry*
Columbia University, New York, NY

Control over the organization of biomolecules at solid substrates with nanometer-scale resolution is a powerful tool for addressing fundamental issues in many areas of biology. Nanoarrays of biomolecules offer unmatched sensitivity, smaller sample volumes in molecular diagnostics, and high throughput analysis. Furthermore, nanoscale control yields features at the same size-scale as individual biomolecules, opening up the opportunity to probe the activity of biomolecules at the single-molecule levels. We have developed a strategy to control the immobilization of biomolecules at surfaces in arrayed nanodomains, allowing for the simultaneous monitoring of specific protein/DNA binding events in parallel and at the single molecule level.

We fabricate biomolecular nanoarrays via selective self-assembly of single- and double-stranded DNA on surfaces patterned by electron beam lithography. Metallic nanodomains ~ 30 nm in size, are formed on glass or fused silica substrates. Each step of the biochemical functionalization is monitored by fluorescence microscopy and verified at the single nanodot level. Figure 1 shows the high selectivity achieved in the self-assembly of both streptavidin and DNA on our nanopatterned surface. This also enables us to quantify the minimized non-specific adsorption achieved: the physisorbed DNA coverage on our bio-chip was found to be less then one DNA molecule every 2 μm^2. In this presentation, we will further show how, by specific design of the biomolecular nanoarrays, it is possible to record *via* conventional epi-fluorescence microscopy imaging, hundreds of single-molecule events of biological interest simultaneously on a single 50x50 μm^2 biochip. Figure 2 shows the activity of PvuII, a DNA-binding enzyme, on our nanoarray. Fluorescently labeled DNA which contains a sequence targeted by PvuII is bound to the nanoarray pattern. Within seconds of introduction of the enzyme, the fluorescent signal is extinguished, indicating cleavage of the DNA oligomer by the PvuII. Noteworthy we have achieved single-molecule resolution in the parallel monitoring of such binding/cleavage events on the array's nanodots, as shown by the representative discrete step-like drop in fluorescence intensity displayed in figure 2. It is noteworthy that the histogram built form these single-molecule events is consistent with the existence of a Michaelis-Menten complex. In addition, our extrapolated value of the overall catalytic rate for PvuII is comparable to previously reported values from ensemble measurements. The discrete step-like drops in fluorescence intensity enable us to also determine the average number of DNA molecules immobilized to single nanodots: we find fewer than three DNAs on ~85% of the nanodots.

The strategy here presented is highly general and enables the parallel monitoring of biological activity in real-time and with single-molecule resolution. We envision that the high density and excellent resolution achievable with our platform can find general application in high throughput heterogeneous assays of a wide variety of biomolecular interactions.

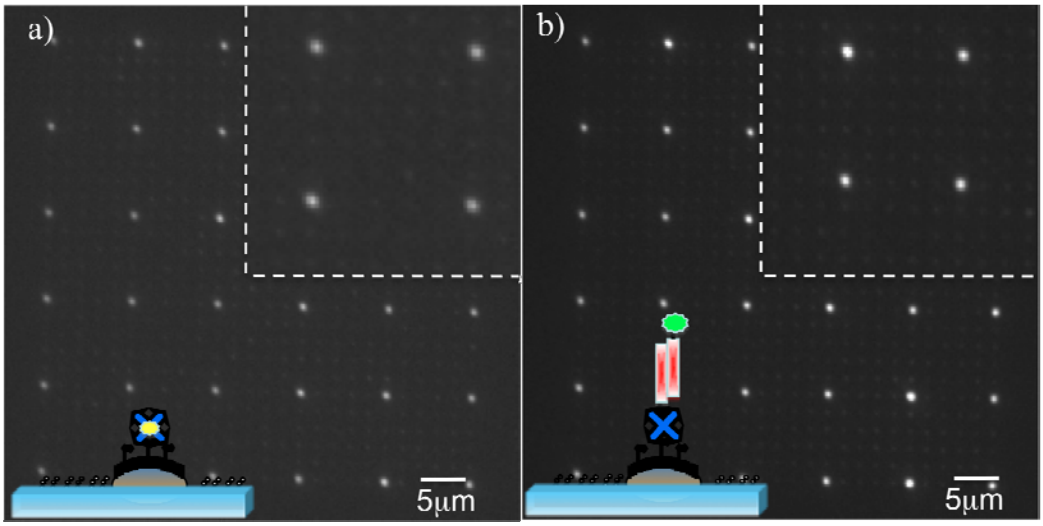

Figure 1*: a) Epi-fluorescence microscopy image of the nanoarray functionalized with Alexa488-labeled Streptavidin (100 ms exposure time); b) Epi-fluorescence microscopy image of the nanoarray functionalized with Rhodamine Red-labeled dsDNA (100 ms exposure time); the insets at the top right-hand corners of (a) and (b) show a zoomed fluorescence image of the array. The larger dots are 500nm registers, while the smaller dots displayed in the images are 30nm in size.*

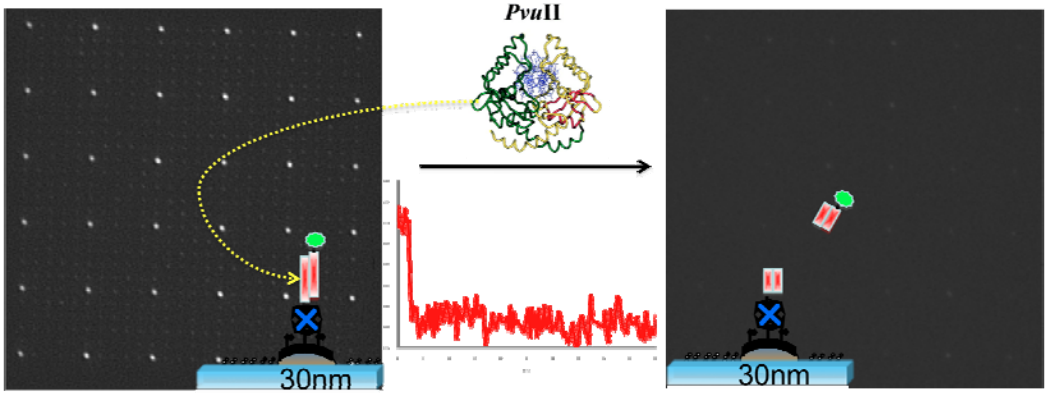

Figure 2 *: Epi-fluorescence images of the developed biomolecular nanoarray , and the specific PvuII/DNA binding event investigated: the loss of fluorescence due to DNA cleavage by the enzyme is observed within seconds of addition of the enzyme. The single-molecule resolution achieved is exemplified by the discrete step-like drop in fluorescence intensity at the single nanodot level.*