A novel DNA chip for single molecule analysis

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The last two decades have seen the emergence of single-molecule experiments [1]. By avoiding the ensemble averaging inherent to traditional bulk-phase biochemistry, the study of molecular machineries at the single-molecule level permits a better understanding of the behavior of living systems. Indeed the dynamics of the machineries processes can be characterized and rare subpopulations can be identified [2].

In our laboratory, we implemented the Tethered Particle Motion (TPM) technique to monitor the conformational dynamics of single DNA molecules. The principle of a TPM experiment consists in tracking a bead tethered at the free end of a DNA molecule immobilized by the other end to a coverslip by means of optical videomicroscopy coupled to image analysis (Fig 1 – left panel). The amplitude of the Brownian motion of the bead is related to the effective length of the DNA molecule [3].

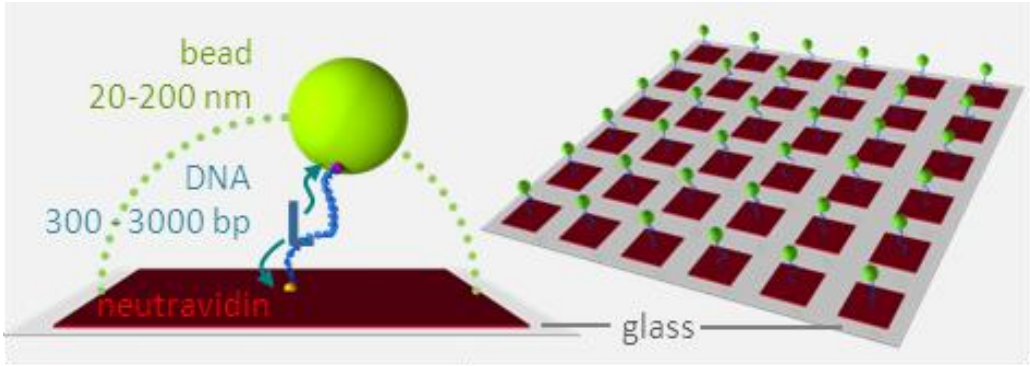

Figure 1: A nanoarray for the parallel analysis of DNA conformational changes by Tethered Particle Motion.

To increase the output of this powerful but time-consuming single-molecule assay, we have developed a novel single DNA chip allowing the simultaneous analysis of hundreds of single DNA molecules (Fig 1 – right panel). In our biochip, the controlled positioning of individual DNA molecules is achieved by self-assembly on nanoscale arrays fabricated through a standard microcontact printing method. Using this improved method, we currently analyze about 1000 single DNA molecules in parallel [4].

After the description of our technology, I will discuss the capacities of the single DNA biochip and the sensitivity of our methodology. As a demonstration of its informative potential, we will then examine the new details obtained on the enzymatic activity of the T7 bacteriophage exonuclease revealed by our high-throughput assay. Finally, I will present our on-going work on future industrial applications for the single-DNA chip.

[1] P.V. Cornish, T. Ha, Acs Chemical Biology 1 (2007) 53.
[2] A.M. Van Oijen, Nature Chemical Biology, 8 (2008) 440.
[3] H. Yin, R. Landick, J. Gelles, Biophys J, 6 (1994) 2468.
[4] T. Plénat, C. Tardin, P. Rousseau, L. Salomé, Nucleic Acid Res., 40 (2012) e89.