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NANOTECNOLOGIE

CROWDING EFFECTS ON BIOCHEMICAL REACTIONS WITH SURFACE-BOUND DNA

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Abstract

Next-generation DNA detection arrays are expected to achieve unprecedented sensitivity, reducing the minimum amount of genetic material that can be directly (PCR-free and label-free) and quantitatively detected, up to the single cell limit. To realize these goals, we propose a new method for the miniaturization of DNA arrays to the nano-scale, which has the unique capability of controlling the packing quality of the deposited bio- molecules.

We used *NanoGrafting*, a nano-lithography technique based on atomic force microscopy (AFM), to fabricate well ordered thiolated single stranded (ss)-DNA nanopatches within a self-assembled monolayer (SAM) of inert thiols on gold surfaces. By varying the "writing" parameters, in particular the number of scan lines, we were able to vary the density of the supported DNA molecules inside the nano-patches in a controlled manner. Our findings can be resumed in two parts:

1) Combining accurate height and compressibility measurements, before and after hybridization, we demonstrate that high-density ss-DNA nanografted patches hybridize with high efficiency, and that, contrary to current understanding, is not the density of probe molecules to be responsible for the lack of hybridization observed in high density ss-DNA SAMs, but the poor quality of their structure.

2) Dpn II enzymatic reactions were carried out over nanopatches with different molecular density and different geometries. Using nanopatch height measurements we

are able to show that the capability of the Dpn II enzyme to reach and react at the recognition site significantly depends on the molecular density in the nanopatches. In particular the inhibition of the reaction follows a step-wise fashion at relatively low DNA densities. These findings suggest that, due to the enzyme size, it is possible to tune the efficiency of an enzymatic reaction within surface-bound DNA nanostructures by changing only the crowding of DNA on the surface and without introducing any further physical or chemical variable.