# SELECTIVE PROTEIN DEPOSITION VIA A XEROGRAPHY-LIKE PROCESS

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We recently presented an emulsion-based Xerography-like process for a localized modification of solid surfaces with biomolecules.[1] Arbitrary protein patterns are generated by a process combining the serial lithography characteristics of an atomic force microscope (AFM) with parallel deposition techniques based on electric fields. The attachment of gold-nanoparticles following the same reaction scheme has been shown previously.[2] Here, we report on applying this method to the fabrication of multi-layered protein structures using the avidin-biotin reaction.

The process scheme is shown in Figure 1: The AFM is used for locally depositing charges into the sample surface (Fig. 1A) by applying a voltage between tip and sample. The sample consists of a silicon wafer coated with a thin electret layer, usually PMMA (poly(methyl methacrylate)), having excellent charge storage properties. The deposited charge patterns create an electric field, which is subsequently used to attract the first layer of molecules. To this end, the substrate is immersed into a water-in-oil emulsion, consisting of aqueous-phase droplets containing the biomolecules (here: biotin-modified IgG, in buffer solution) and an insulating fluorocarbon oil to prevent the sample from discharging (Fig. 1B). The droplets are attracted to the charge patterns by Coulomb- and dipolar forces. Prior to further modification, the sample is rinsed to remove excess molecules that are not tightly bound to the surface. Afterwards, the sample is immersed into an aqueous buffer solution of the appropriate protein (here: Avidin, modified with a fluorescene marker (FITC)) to build up the second layer of molecules via the avidin-biotin reaction (Fig. 1C).

Results of this procedure are shown in Figure 2, and can be summarized as follows:

(1) The emulsion-based method can be used to create arbitrary patterns of biomolecules on electret surfaces with a sub-μm resolution (Fig. 2A).
(2) After deposition of molecules from emulsion (Fig. 1B), the samples can be immersed into aqueous buffer solution, without losing the pattern definition.
(3) The deposited IgG-biotin-molecules keep their ability to specifically bind the avidin, as can be seen in the fluorescence images (Fig. 2C,E), where the FITC-labeled Avidin can be detected.

Above results indicate that this method may find applications e.g. in the field of biosensor-fabrication, where one crucial step is the exact positioning of chemical or biological receptor molecules, or as an easy way to create interfaces of biological molecules with electronic microchips. Our process is particularly suited for handling biomolecules and does not require expensive cleanroom-equipment, as all steps are done either in liquid or under normal ambient conditions.

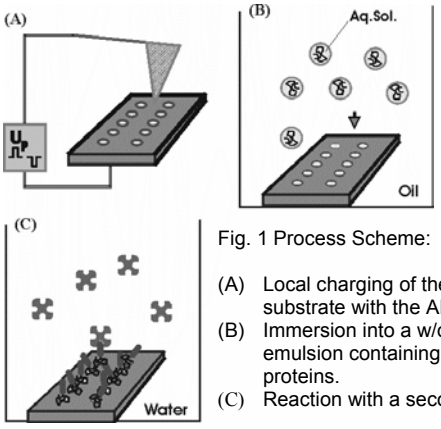


Fig. 1 Process Scheme:

(A) Local charging of the substrate with the AFM.
(B) Immersion into a w/o-emulsion containing proteins.
(C) Reaction with a second

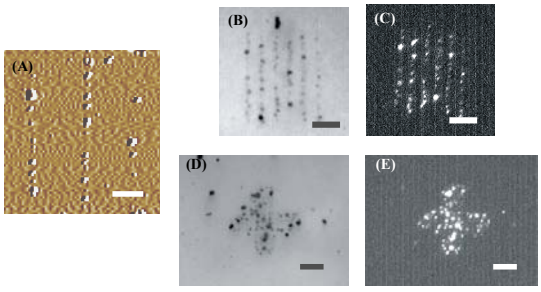


Fig. 2 Images of IgG-biotin deposited onto PMMA, modified with FITC-Avidin.
(A) AFM amplitude image of a line pattern (arbitrary units).
(B,D) Light microscopy images of various patterns (lines and cross). 400x
(C,E) Fluorescence microscopy images of the same structures. 400x

1) N. Naujoks, A. Stemmer, Microel. Eng. Vol. 67-68C, 734 (2003).
2) P. Mesquida, A. Stemmer, Microel. Eng. Vol. 61-66, 671 (2002).