Abstract Title

Engineering Biosensor Surfaces at the Nanoscale using Automated Scanning Probe Lithography

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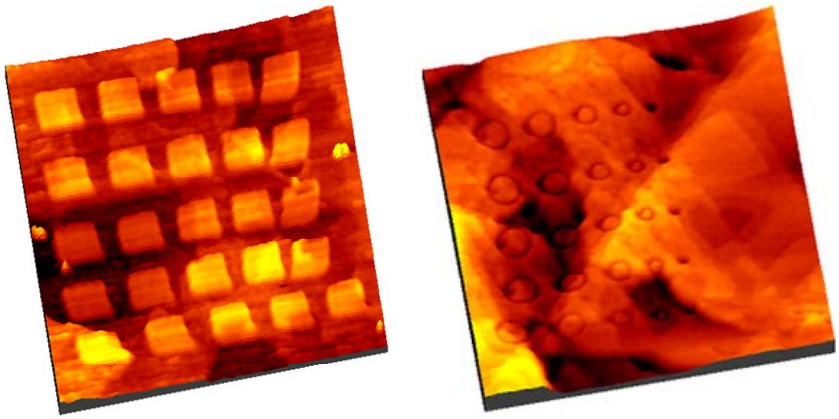
Abstract body

Nanostructured surfaces provide highly-controllable test environments for molecular scale investigations of protein binding. A number of researchers have begun to combine atomic force microscopy (AFM) and nanoscale lithography to develop protein assays with molecular level sensitivity. Studies using AFM can be conducted in ambient buffered environments to directly detect and visualize the binding of biomolecules on well-defined nanostructured surfaces with precise control of surface reaction conditions, spatial arrangement, ligand density, nanopattern geometry and chemical composition. Surfaces with regular arrays of protein nanopatterns of designated shapes and sizes can be used to compare immobilization chemistries and evaluate the specificity and selectivity of surface binding chemistries.

Protein patterning is essential for the integration of biological molecules into miniature bioelectronic and sensing devices. Nanopatterns of SAMs can be designed to present reactive groups (such as aldehyde or carboxylate) for binding proteins. After incubating the nanopatterns with desired proteins, the changes in height and surface morphology of the nanostructures provide insight regarding biochemical surface reactions and the activity of immobilized proteins. Height changes during *in situ* experiments furnish information regarding protein orientation and the selectivity and efficiency of SAMs for binding biomolecules to designed surfaces. Examples will be presented displaying nanostructures of model proteins immobilized on SAM nanopatterns through electrostatic and covalent interactions. Various immobilization strategies using SAMs will be compared at the nanoscale, such as the covalent attachment of proteins using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) activation of carboxylate-terminated SAMs. Several series of AFM images will be presented which display protein arrays produced by AFM-based lithography.

Automated scanning probe lithography, (SPL) offers tremendous advantages for the speed and reproducibility of nanopatterning and can generate highly sophisticated pattern arrangements and geometries. Commercial atomic force microscopes (AFM) typically include software with capabilities to control the length, direction, speed, bias pulse duration, residence time, and the applied force of the scanning motion of an AFM tip, analogous to a pen-plotter. Using SPL, the surface chemistry of nanopatterns of self-assembled monolayers (SAMs) can be chosen to present reactive groups such as aldehyde or carboxylate for binding biomolecules such as protein, DNA or peptides. Due to their stability, ease of preparation and well-ordered surface structures, SAMs of alkanethiols and alkylsilanes provide excellent models for studying protein binding, since layers of defined thickness and designed properties can be generated.[23, 24] Thiol endgroups of *n*-alkanethiols bond via chemisorption to metal

surfaces. SAM surface properties can be flexibly controlled by changing the functional (head) groups of the alkyl chain, also, these end groups can be used for further chemical reactions. The acidity, adhesion, wetting and structural properties of surfaces can be modified by choosing specific chemical headgroups (such as NH_2, OH, COOH, CH_3, glycol, etc.) The preparation, characterization, and properties of SAMs have been described and reviewed previously.[3-7]



Arrays of nanografted SAM patterns written by automated scanning probe lithography. (Left) Hexadecanethiol squares (100 nm) written in a glucosyldithiol matrix. (Right) Rings of glucosyldithiol written in a hexadecanethiol SAM. Both images display views of 1 x 1 μm^2 scan areas.

Force-induced SPL methods[1,2] such as nanografting and nanoshaving also provide control of spatial parameters such as ligand density for individual elements of nanopatterned arrays, ranging in size from tens to hundreds of nanometers. Example AFM topographs are shown for a 5 x 5 array of 100 nm squares of hexadecanethiol written in a glucosyldithiol matrix; and for a series of smaller rings of glucosyldithiol written within a hexadecanethiol monolayer. After writing designed geometries and chemistries, solutions of proteins are introduced during *in situ* studies of protein binding. A common feature of SPL methods is that an AFM tip is used as a tool for both nanofabrication and characterization of surfaces. A helpful analogy for describing SPL methods with SAMs is an AFM tip (*pen*) which writes with molecules (*ink*) on various surfaces (*paper*). SPL provides exquisite control of surface chemistry including parameters such as the spatial arrangement, chemical composition, and the written density of molecular ligands. The shape and dimensions of the tip dictate the detailed resolution of written nanostructures – SAM patterns as small as 5 nm have been reported, and it has become routine to achieve patterns of 20-50 nm (or larger). Given that the dimensions of proteins range from tens to hundreds of nanometers, SPL methods are ideally suited for surface studies of protein binding.

Since the reliability and sensitivity of protein biosensors and biochips depend on the affinity and viability of surface-bound biological components, molecular-level studies which combine nanoscale lithography with AFM characterization can be used to improve the selectivity and sensitivity of surface-bound protein assays for biosensors and biochips. Tools for nanofabrication have begun to provide important contributions for developing biochip and biosensing technologies, as well as in supplying basic research in protein-protein interactions and protein function. Scanning probe microscopes supply tools for visualization, physical measurements, and precise manipulation of atoms and molecules at the nanometer scale. Nanoscale studies which combine AFM characterizations and lithography can facilitate the development of new and better approaches for immobilization and bioconjugation chemistries, which are key technologies in manufacturing biochip and biosensing surfaces.

Keywords

Scanning probe lithography, nanopatterns, self-assembled monolayers, atomic force microscopy

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