Modifications of Planar and Nanostructured Surfaces for the Interrogation of new Sensor Designs

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Surfaces and the chemistry that defines them are critical parameters in the application of functional devices to the extended field of sensing in biotechnology.[1,2,3] More specifically, controlling how a surface interacts with biological solutions directly affects that surface's sensing capacity. Immunoassays and other sensing platforms that utilize a lock and key strategy to bind specific targets need to minimize the nonspecific binding of other biomolecules that could give false positives.[4,5] In these particular cases, the penultimate goal is minimizing the nonspecific binding while retaining a high affinity for the analyte of choice. These goals have direct applications for label free sensing motifs such as surface plasmon resonance (SPR). SPR has become a widely accepted method to detect binding events in real time due to the commercial availability of these systems.[6] However, the methods to functionalize the plasmonically active gold surface have focused mainly on self-assembled monolayers for the attachment of specific chemical moieties to the surface.[2]

As an alternative to using self assembled monolayers as the basis for surface modifications, we have designed a class of polymers that are both easily synthesized and inherently resistant to nonspecific adsorption of proteins when attached to a surface. This new class of materials for surface modifications is based around a polyacrylamide-co-n-acryloxysuccinimide linear chain polymer. The polyacrylamide backbone is functionalized via the n-hydroxysuccinimide (NHS) couplings with 3-methylthiopropylamine (MTP) and nitrilotriacetic acid (NTA). These films are engineered to specifically bind histidine tagged proteins (6His) via the NTA group in the presence of Ni^{2+} (Figure 1).[7] These bifunctional polymers readily self-assemble onto gold surfaces, both planar and nanostructured, and have been thoroughly characterized using a variety of analytical techniques (RAIRS, ellipsometry, SPR, and MALDI) to examine their structure and function. The relative amounts of the grafted side chains play an important role in determining the performance of these thin films and how they can be utilized in detection formats. As SPR becomes more popular within the scientific community, there exists a need for lower cost methods to harness the SPR phenomena.

It has been found that nanostructured grating arrays can support both local and propagating surface plasmons without the complex instrumentation and optics needed for more traditional prism-based SPR implementations.[8] Interest in utilizing nanostructured plasmonic architectures for sensing applications has flourished over the last decade. Advances in lithographic techniques coupled with a better understanding of how plasmons respond to changes in the dielectric at the interface has culminated in the development of sensors with increased sensitivity. We have developed a nanostructured plasmonic crystal fabricated by soft lithographic methods that is extremely sensitive to refractive index changes near the metal-dielectric interface.[9,10] Utilizing a responsive hydrogel coupled to the surface of a plasmonic crystal, we are able to optically detect small changes in the pH of a buffered solution even in the absence of bulk refractive index changes. Exposure to basic solution deprotonates acrylic acid groups within the hydrogen, leading to electrostatic repulsion of the acrylate groups and an expansion of the hydrogel. In order to enhance this sensitivity, gold nanoparticles were embedded in the hydrogel leading to an enhancement of the integrated spectral response due to coupling between the plasmonic modes

of the fabricated plasmonic crystal and electromagnetic fields supported on the gold nanoparticles. In addition to an overall increase in sensitivity, we also observe a significant enhancement at visible wavelengths, opening a possible route to a colorimetric sensor.

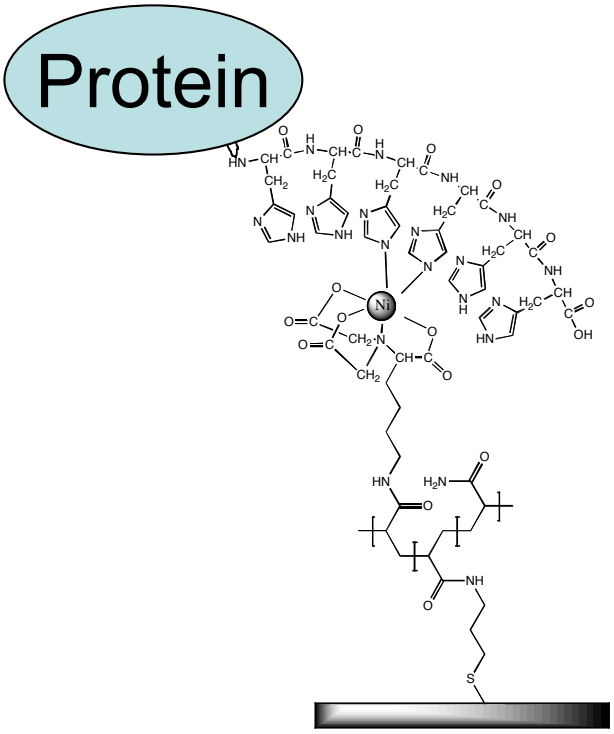

Figure 1: Schematic of the metal affinity capture used to bind hexahistidine tagged proteins from solution

These nanostructured surfaces also play a distinct role in a new method of optical imaging. The ability to image patterned thin films of small molecules and proteins is unique in that the plasmonic crystals enable imaging with nanometer resolution in the direction normal to the surface plane. We have adapted an easily fabricated plasmonic sensor to measure image intensity changes due to thin film adsorption over different wavelength regions using a simple reflection imaging approach. The spectral sensitivities of the supported plasmons were isolated using bandpass filters, which enables the direct imaging of changes in spectral responses to the presence of thin patterned films of protein and a 1-octadecanethiol self-assembled monolayer. These plasmonic crystals exhibited optimal sensitivity between 500-550 nm, although the wavelength range of 610-700 nm was only slightly less sensitive. It is noted that there is contrast inversion between these two wavelength ranges relative to the background intensities, and similar behavior was observed in the imaging of *Aplysia* bag cell neuronal cultures (Figure 2). Both experimental and finite difference time domain simulations support the dominant role of SPR effects as the source of these contrast inversions. Calibrations curves derived from the measurement of contrast changes of the patterned thin films were used to quantify the mass coverage changes in *Aplysia* neurons cultured and fixed on the plasmonic crystal surface.

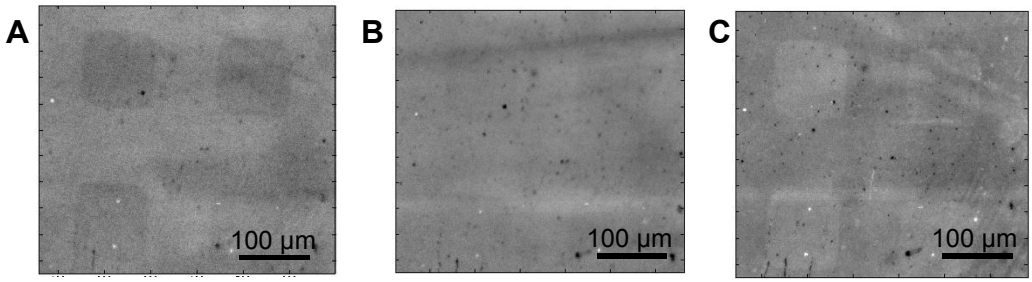

Figure 2: Reflection mode images of μCP ODT on plasmonic crystal using bandpass filters to isolate spectral responses showing contrast inversion A) 500-550 nm B) 540-590 nm and C) 610-700 nm

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