

# Formation of Biotinylated Alkylthiolate Self-Assembled Monolayers on Gold

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## ABSTRACT

The formation of mixed self-assembled monolayers (SAMs) from novel biotinylated alkylthiols (BAT) and diluent triethyleneglycol (TEG) alkylthiols on gold has been accomplished using aqueous solvents. We compare the effects of aqueous and ethanolic solvents on the binding performance of mixed SAMs and the integrity of monolayers formed. We use cyclic voltammetry (CV) and fluorescence microscopy to characterize the films. Comparison of fluorescence data between solution mixtures of alkylthiols in various concentrations of ethanol and water solvent indicates that functional SAMs may be formed at ethanolic solvent compositions as low as 2% in water. In addition, CV data indicate that SAMs adsorbed from aqueous solution form faster and have fewer defects than those formed using ethanol.

**Keywords:** SAM, monolayer, streptavidin, biotin, gold

## 1 INTRODUCTION

Understanding of the interactions of proteins and other biological molecules with surfaces is vital for the development of detection systems and assay platforms. These relationships are frequently quite complex, involving hydrophobic interactions, electrostatic interactions, van der Waals forces and covalent chemical bonds [1]. We can utilize these interactions in a surface capture device by modifying the surface substrate with thin films and monolayers. Employing self-assembled monolayers (SAMs), in particular, enables substrates to exhibit a variety of chemical properties and reactivities. In a SAM, alkyl thiols and disulfides form stable film structures on Au via a Au-S interaction. In literature, SAMs employing biotinylated thiolate molecules in gold and streptavidin sandwiches have been studied and utilized for the attachment of DNA and other biological molecules [2]. The development of such surfaces is a contribution towards bioassay technologies such as DNA chips, protein chips and small molecule biosensors.

We are building a new family of biofunctionalized nanoelectromechanical systems (BioNEMS) that will carry the analysis of biological processes to the stochastic limit. To carry out these experiments, we are building a device with a large array of NEMS cantilevers in a small liquid

volume and have designed and synthesized alkylthiol molecules that are specifically attached in the form of self-assembled monolayers to Au pads located at the cantilever tips. The nature of our BioNEMS system requires more stringent conditions than previously demonstrated in literature. As biological assay platforms evolve into integrated microfluidics-packaged chips, the in situ formation of SAMs in devices needs to be addressed. Traditionally, SAMs are adsorbed from ethanol, DMSO, or other organic solvent systems. Many of these solvents will swell polydimethylsiloxane (PDMS) polymers or evaporate from microfluidic channels before well assembled monolayers can be formed [3]. Aqueous media, however, is compatible in PDMS and does not evaporate out of the polymer as quickly. Precedence in literature demonstrates the use of surfactants in water to aid in solvation of longer chain alkane thiols and their adsorption onto gold surfaces [4]. Additional studies by Yang, et al. indicate that SAMs are more stable when stored in water, as opposed to organic solvent [5]. Submersing SAMs on gold in water after a short thiolate adsorption time has also been shown to promote more crystalline packing of alkanethiolate chains. The hydrophobic interactions between the long alkyl chain and water are believed to promote organization of the monolayer on the surface.

## 2 MATERIALS AND METHODS

Silicon wafers were purchased from Wafer World. Chromium was purchased from R. D. Mathis Company and gold shots from Refining Systems, Inc. Phosphate buffered saline (PBS) was prepared as 0.139 M NaCl, 2.68 mM KCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub> and 1.1 mM K<sub>2</sub>HPO<sub>4</sub> (Malinckroft) in Nanopure water. Potassium ferrocyanide and potassium ferricyanide were purchased from Aldrich. Absolute ethanol was purchased from Aaper Alcohol and Chemical Company (Kentucky.) Cy3 labeled streptavidin was purchased from Zymed, Inc.

## 2.1 Alkylthiol Reagents

Reagents BAT and TEG were synthesized in house with reagents purchased from Sigma and Aldrich (Figure 1) [6].

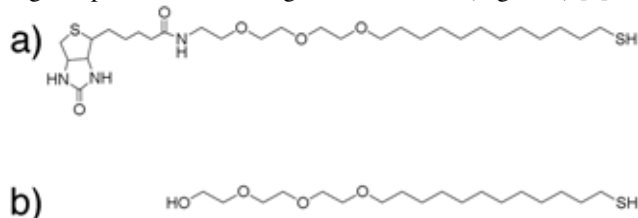


Figure 1. a) biotinylated tri(ethylene glycol) dodecylthiol (BAT). b) tri(ethylene glycol) dodecylthiol (TEG).

## 2.2 Preparation of Substrates and Monolayers

Silicon substrates were photo-patterned with  $\sim 7$  nm Cr and  $\sim 100$  nm Au by thermal evaporation. Au patterned substrates underwent plasma treatment at an oxygen flow rate of 0.8 L/minute in a UV ozone cleaner (SAMCO UV & Ozone Dry Stripper, Model UV-1) at  $100^\circ\text{C}$  for 30 minutes followed by a 2 minute nitrogen purge. Au substrates were then either submerged in thiol solution or underwent oxidative CV scans out to 1.2 V (vs Ag/AgCl, sat. KCl) in a solution 30 mM ferrocyanide/PBS of to remove any remaining surface contaminants. These electro-cleaned samples were then rinsed in copious amounts of water and ethanol, dried under a stream of Argon and immediately placed in thiol solution. Thiol solution compositions (BAT and TEG) were varied for a total disulfide concentration of 0.2 mM. Varied solvent compositions were attained by diluting an ethanolic stock solution of thiols in Nanopure water. After adsorption, samples were rinsed in ethanol and dried with Argon.

## 2.3 Characterization with Electrochemical Methods

The conductive properties of the Au substrate allow us to exploit electrochemical measurements in order to probe the properties of alkylthiolate assemblies on the surface. The structural integrity of the adsorbed monolayer may be characterized using CV methods [7]. During a CV scan, a tightly assembled monolayer will insulate the Au surface against electron transfer with a redox-active molecule in solution. Any defects in the thin film will be detected by CV and characterized by current flow. Electrochemistry measurements were carried out with a CH Instruments Model 600B potentiostat (Austin, TX.) A conventional three-electrode electrochemical cell was constructed with a platinum wire/mesh counter electrode and Ag/AgCl reference electrode in saturated KCl. The Au substrate served as the working electrode. CV measurements were taken in an electrolyte solution of 30 mM potassium

ferrocyanide at a scan rate of 100 mV/s from  $-0.2\text{V}$  to  $1.2\text{V}$ .

## 2.4 Protein-Binding Assay

Au substrates hosting BAT/TEG SAMs were submersed in a 200 nM solution of Cy3-labeled streptavidin in PBS for 30 minutes at room temperature. The samples were removed and washed five times with 1 ml of PBS, then stored in PBS for immediate analysis by fluorescence microscopy.

## 2.5 Fluorescence Microscopy

Images were acquired on an upright Zeiss Axioplan2 infinity corrected microscope (Zeiss, Germany) and acquired with a monochrome CCD Zeiss Axiocam HRm camera. Zeiss Plan-Neofluar objectives 10x/NA 0.3 and 20x/NA 0.5 were used in conjunction with a Chroma (Rockingham, VT) Cy3 filter set. A mercury arc lamp served as the excitation source. Images were acquired in 8-bit monochrome resolution and 1030 x 1300 pixel resolution.

# 3 RESULTS

## 3.1 Effect of Adsorption Solvent on SAM Formation Time

The adsorption of self-assembled monolayers is a versatile method for altering the chemical and physical properties of a surface. For Au substrates, SAMs are typically adsorbed from organic solutions. However, commonly used organics such as DMF and DMSO have several deleterious effects on our BioNEMS devices including swelling and solubilizing the PDMS microfluidics layers [3]. We have found that SAMs formed from BAT or TEG reagents may be adsorbed from ethanolic and aqueous solutions. Thus, we tested several different concentrations of aqueous ethanol solvents for thiol solubility and the ability of BAT and TEG to successfully form SAMs on Au substrates.

Alkylthiolates are believed to adsorb immediately onto the Au surface in a disordered fashion [8-11]. During the first few minutes, chemisorption of alkane thiolates results in 80-90% coverage of the substrate. In a longer subsequent adsorption stage, the alkane thiolates self-assemble into a more organized and insulating film [12]. Variables such as temperature, thiol concentration and solvent composition can affect the rate of monolayer formation [13].

CV techniques were used to evaluate surface coverage of Au substrates after adsorption of thiolates. Adsorption of BAT and TEG reagents after 12 hours at room temperature

and in 0.2 mM thiol results in comparable insulation of Au substrates when using either ethanolic or aqueous solvents. In order to compare the effects of solvent composition on SAM quality, we terminate monolayer formation after 1-2 hours and observe insulating properties of the monolayer at this intermediate timepoint. Comparison of CV traces for SAMs formed in water and ethanol reveals that the samples treated in aqueous solution result have more diminished cathodic and anodic peaks, indicating greater monolayer coverage on the Au substrate. In particular, TEG SAMs form at a faster rate than mixed BAT/TEG SAMs or pure BAT SAMs. (Figure 2).

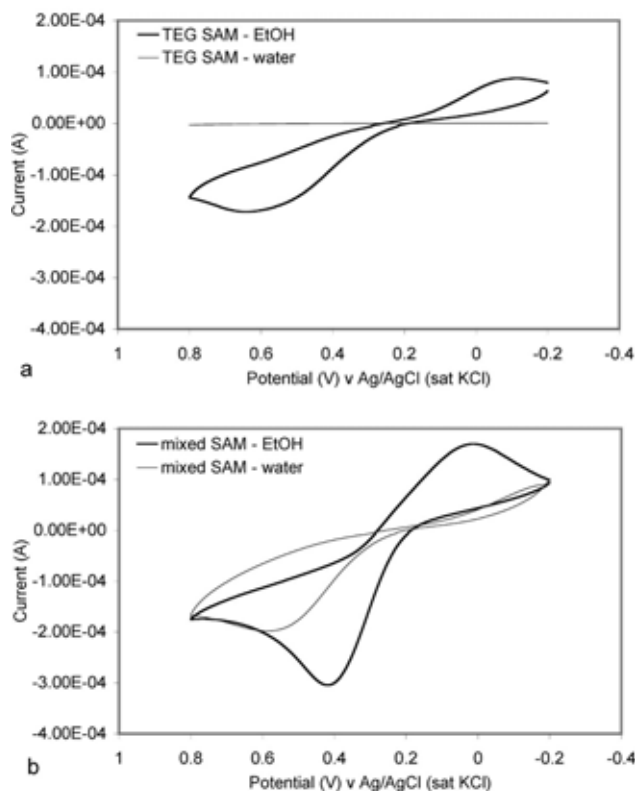


Figure 2. CV traces for Au electrodes covered with TEG and mixed biotin-TEG monolayers in Fe<sup>2+/3+</sup> and PBS. (a) TEG monolayers adsorbed from ethanol and water solutions. (b) Mixed biotin and TEG monolayers adsorbed from ethanol and water solutions.

### 3.2 Binding of Fluorescent Proteins to SAMs

In order to assay the functionality of the BAT SAMs prepared, we use fluorescence microscopy to qualitatively compare the relative fluorescence intensities of Cy3-labeled streptavidin adsorbed on SAM-covered Au substrates. Based on CV traces, monolayers adsorbed from water formed more complete monolayers than from ethanol. Au chip substrates were immersed in mixed thiol solutions ([BAT] + [TEG] = 0.1 mM) of varying solvent composition

(ethanol, aqueous ethanol, water) for 12 hours and subsequently incubated with Cy3-labeled streptavidin. Based on the fluorescent protein-binding assay, the monolayers formed from mixed water/ethanol solutions yielded consistently lower fluorescence intensities than those in water or even ethanol solutions (Figure 3). SAMs prepared at BAT/TEG ratios between 0.0625 to 0.25 behaved with similar trends. The fluorescence levels from adsorbed Cy3-streptavidin on the monolayers was 50-70% lower for SAMs adsorbed from aqueous ethanol than from ethanol or water solvents. At BAT/TEG ratios of 0.5 or 1, the trend breaks down, presumably due to the more disordered nature of these SAMs [14]. SAMs made from BAT reagent alone have been shown to be disordered, with biotin units buried in the monolayer, resulting in a loss of specific protein binding and an increase in non-specific binding.

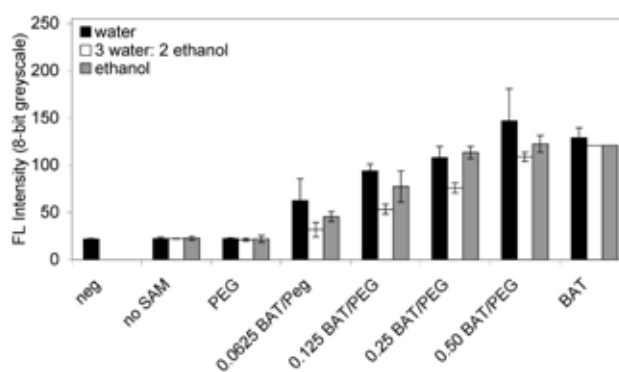


Figure 3. Fluorescence intensities plotted in bar graph displaying signal for each thiol adsorption condition.

However, these monolayers are still functional and are shown to specifically bind streptavidin. It is possible that the BAT and PEG reagents are most soluble in aqueous ethanol, making the solvation of the thiols more energetically favorable than adsorption onto the surface.

## 4 CONCLUSIONS

We demonstrate here that functional biotinylated monolayers may be adsorbed onto Au from aqueous solvents using methods that are compatible with a packaged BioNEMS device. These biotinylated monolayers are capable of binding fluorescently labeled streptavidin, as shown from fluorescence microscopy data. Electrochemical techniques confirm that monolayer coverage in different solvents is comparable, if not better in aqueous solvents.

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