

Biosensors Based on Biotin-linked Viologen SAM's on Gold in AAO

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OVERVIEW

Alkanethiols with an attached viologen precursor are synthesized in solution-phase, then assembled on gold as a monolayer (SAM). Subsequently the viologen is further modified by addition of a linker molecule and ligand (biotin) using solid-phase synthesis techniques (completing quarternization of the viologen in the process). The use of solid-phase synthesis for later stages avoids purification problems that would be expected in solution-phase synthesis.

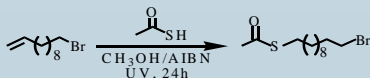
The monolayer assembly of viologens with attached biotin will allow testing as a redox biosensor for avidin. After initial proof of concept – other ligands can be attached to detect other analytes of interest.

By using gold at the bottom of AAO pores – we should be able to create arrays of (potentially) individually addressable biosensors. Due to the generality of this approach, we hope to be able to create arrays of individually addressable biosensors with a selection of different ligands attached for biosensing a variety of analytes.

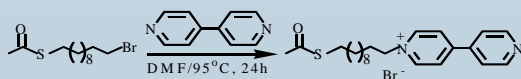
METHODOLOGY

Solution-Phase Synthesis of the Precursor

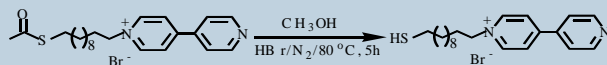
a. Photolysis



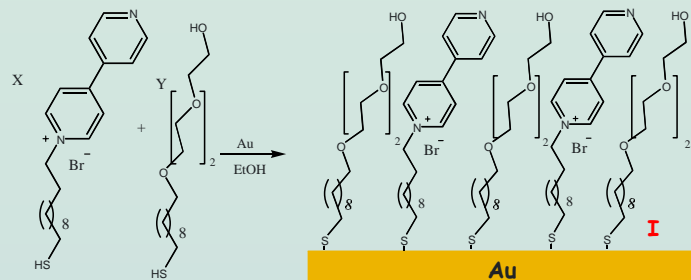
b. Monoquaternarization



c. Methanolysis



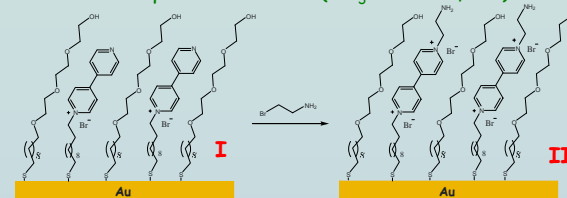
d. Formation of Self-Assembled Monolayer (SAM)



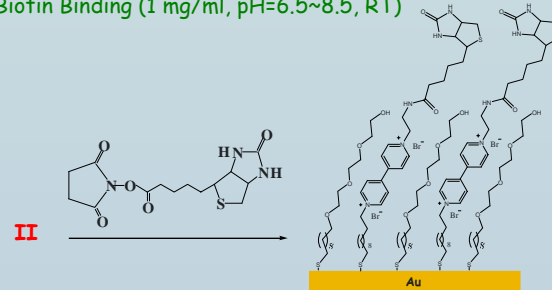
Note: Triethylene glycol terminated undecylthiol is included to minimize non-specific adsorption of analyte and/or matrix molecules (esp. proteins). Optimal ratio of X:Y to be determined by effect on transduction efficiency (signal/noise ratio).

Solid-Phase Synthesis of Redox Sensor

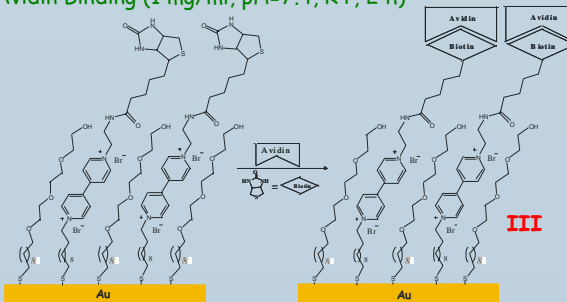
e. Surface Diquaternarization (CH₃CN/60°C, 5 h)



f. Biotin Binding (1 mg/ml, pH=6.5~8.5, RT)



g. Avidin Binding (1 mg/ml, pH=7.4, RT, 2 h)



Transduction of Biosensor Signal

Electrochemical transduction of analyte binding can be accomplished by cyclic voltmetry (CV) or AC impedance spectroscopy.

Transduction is based on the change in the electrochemical properties of the surface-confined viologen groups.

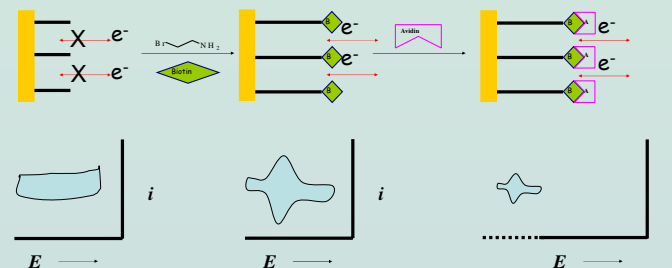


Figure 1. Schematic cyclic voltammograms of the SAMs of **I**, **II** and **III** in a supporting electrolyte.

Biosensor Arrays

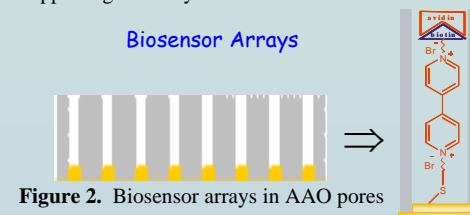


Figure 2. Biosensor arrays in AAO pores

After initial testing – the next stage is to perform the solid-phase synthesis (from step *e* on) on gold surfaces at the bottom of anodic aluminum oxide (AAO) pores. This may increase the sensitivity of analysis OR potentially be used to create individually addressable biosensors.

STATUS

We have completed solution-phase synthesis steps (through *c*) and are in the process of forming SAMs (step *d*) in order to complete the solid-phase synthesis needed. Once complete we will begin electrochemical testing and work to optimize surface chemistry (ratio of X:Y, etc.). As soon as we have demonstrated functional transduction, we will proceed to assemble biosensors on gold in AAO pores and begin investigation of alternate biosensing ligands in this system.

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