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Viologen-Functionalized Monolayers in Nanopores of Anodic Aluminum Oxide for Reagentless Multiplexed Biosensing

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OUTLINE

I. **Formation of Viologen-Functionalized Monolayers on Bulky Gold for Reagentless Biosensing**

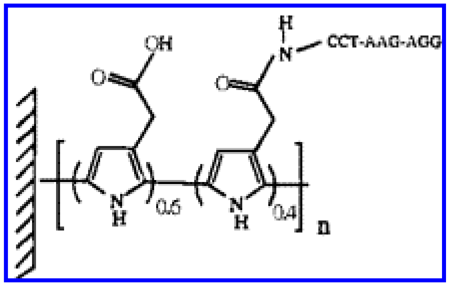
II. **Fabrication of Anodic Aluminum Oxide (AAO)**

III. **Formation of Viologen-Functionalized Monolayers in AAO for Reagentless Multiplexed Biosensing**

ibe

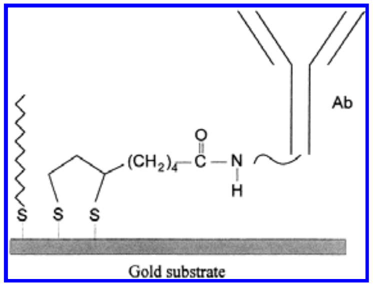
Direct Transduction of Biorecognition on Surfaces

- **Direct Transduction Modes**
  - ♦ **mechano-acoustic**
  - ♦ **surface plasmon resonance & fluorescence**
  - ♦ **piezoelectric & electrochemical**
- **Electrochemical Transduction**
  - ♦ **excellent for interfacing, at the molecular level, biorecognition events & electronic signal transduction processes.**
  - ♦ **uniquely qualified for meeting the size, cost, low-volume, and power requirements of decentralized testing.**



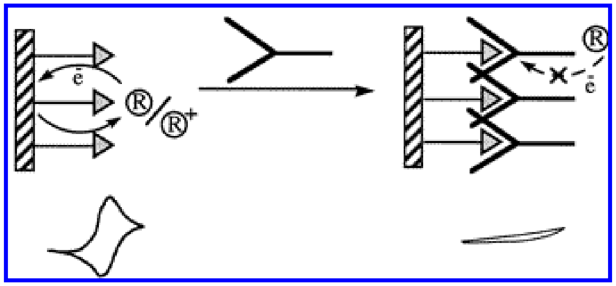
J. Am. Chem. Soc. **1997, 119, 7386.**

Low interaction efficiency



Anal. Chem. **1997, 69, 3651.**

Small signal



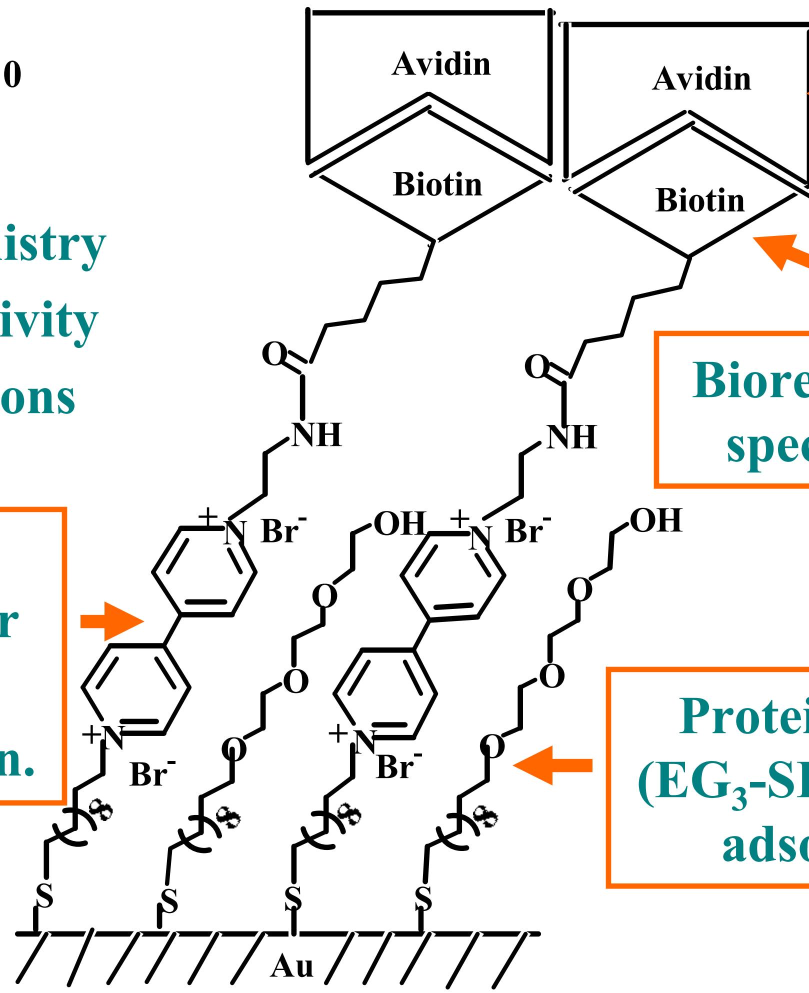
Angew. Chem. Int. Ed. **2000, 39, 1180.**

Low reproducibility

Modular Molecular Assemblies

$$V^{2+} \underset{E_1^\circ}{\overset{e^{-1}}{\Longleftrightarrow}} V^{+\bullet} \underset{E_2^\circ}{\overset{e^{-1}}{\Longleftrightarrow}} V^{0}$$

- excellent redox chemistry
- environmental sensitivity
- widespread applications



Targets (avidin)

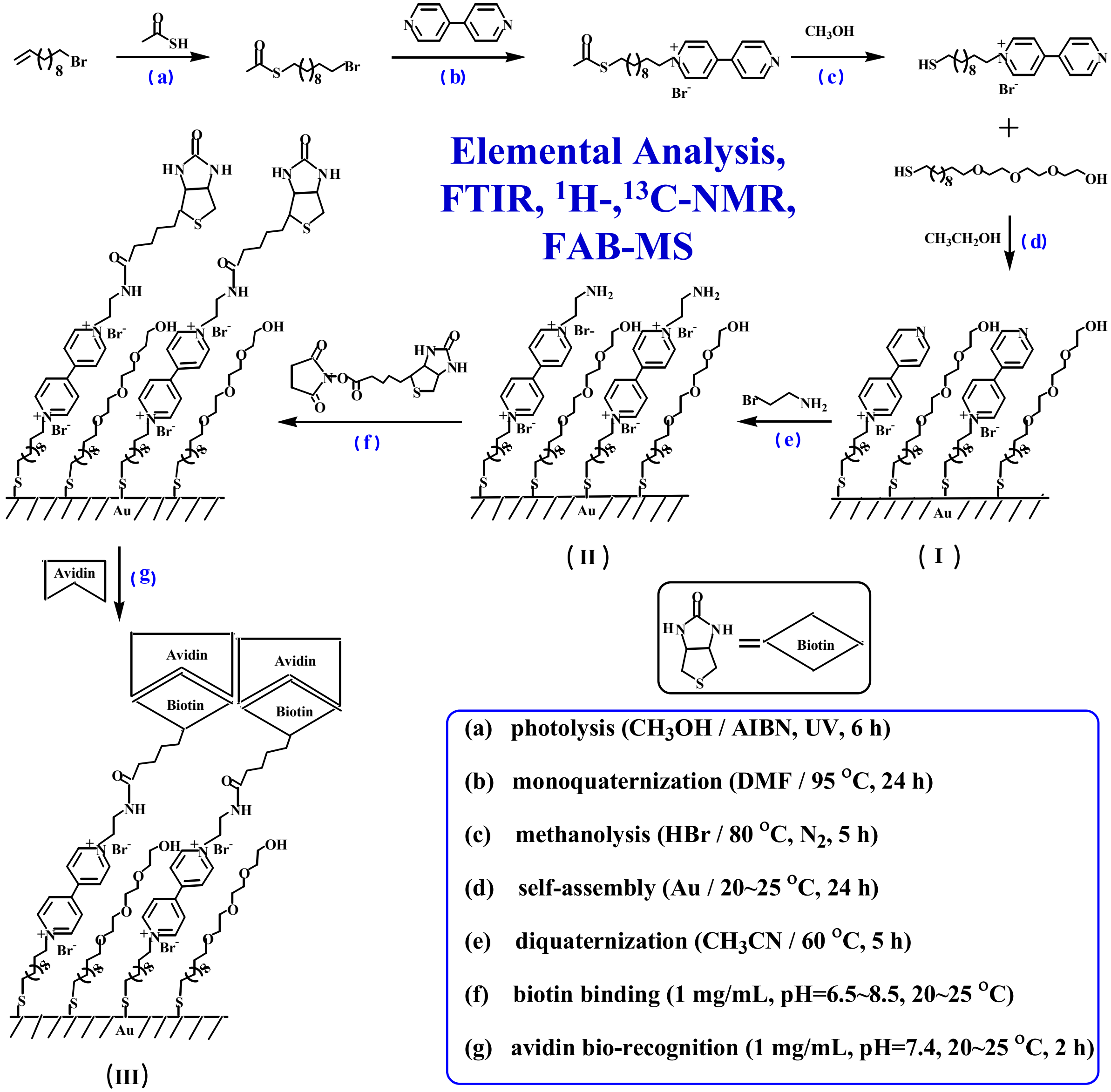
Bioreceptors (biotin), bind specifically with avidin.

Electroactive species (viologen), change their redox properties as avidin is bound to biotin.

Protein-resistant moieties (EG_3-SH), inhibit nonspecific adsorption of protein.

Solution-phase organic synthesis of the transducer molecule with sulfhydryl, viologen and biotin groups is nearly impossible.

Scheme 1. Synthetic Path, Surface Reactions and Binding of Avidin to the Surface.



(a) photolysis (CH_3OH / AIBN, UV, 6 h)

(b) monoquaternization (DMF / 95 °C, 24 h)

(c) methanolysis (HBr / 80 °C, N_2, 5 h)

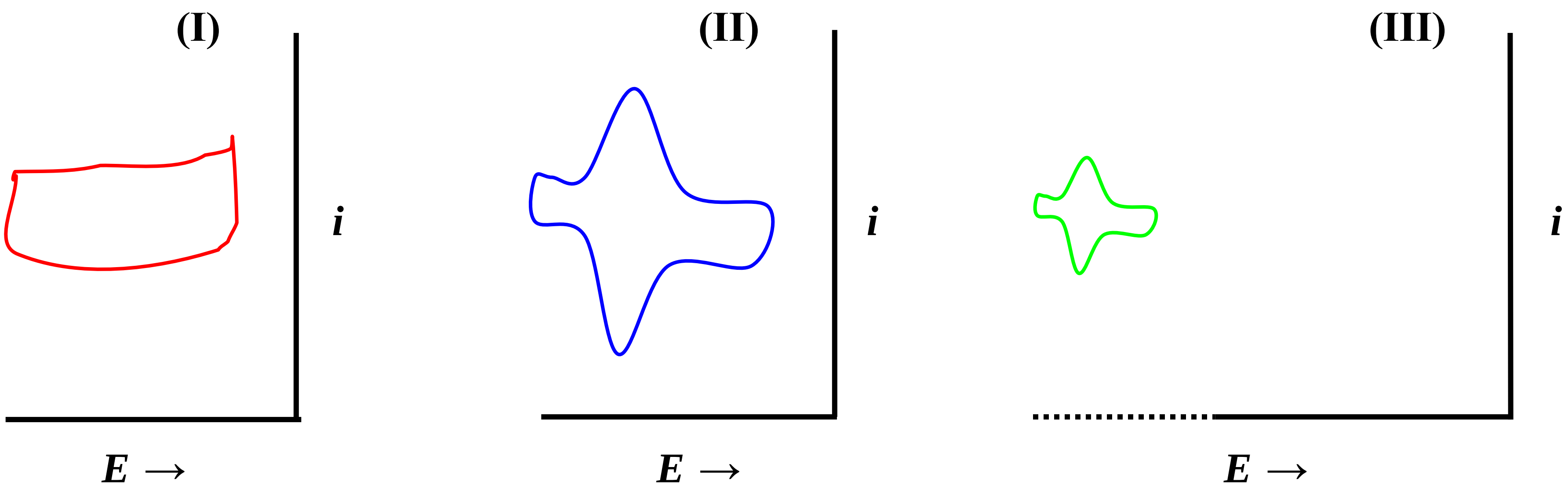
(d) self-assembly (Au / 20~25 °C, 24 h)

(e) diquaternization (CH_3CN / 60 °C, 5 h)

(f) biotin binding (1 mg/mL, pH=6.5~8.5, 20~25 °C)

(g) avidin bio-recognition (1 mg/mL, pH=7.4, 20~25 °C, 2 h)

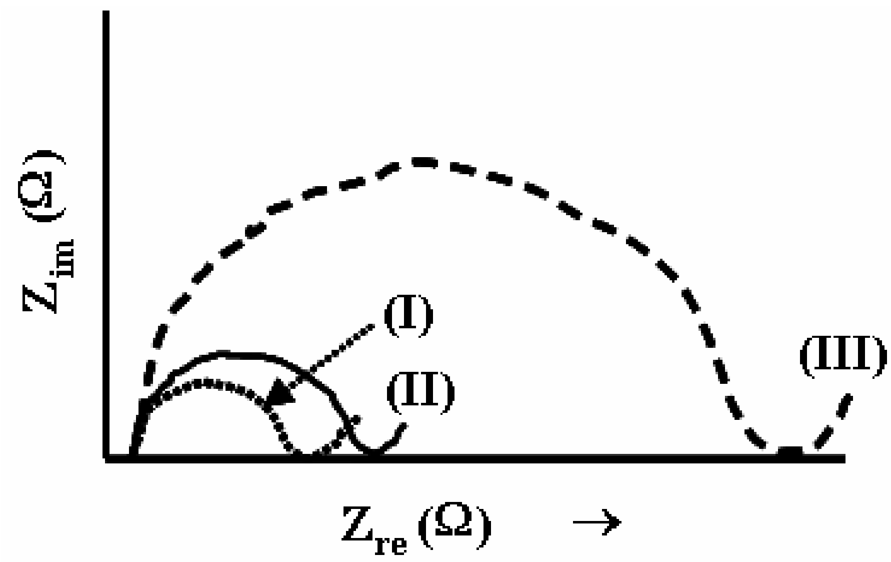
Cyclic Voltammetric Transduction



Scheme 2. Schematic Cyclic Voltammograms of the SAMs of **(I), (II)** *and* **(III)** *in a Supporting Electrolyte.*

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

Electrochemical Impedance Spectroscopic Transduction



Scheme 3. Schematic Nyquist plots of the Faraday impedance spectra of the SAMs of **(I)**, **(II)** *and* **(III)**.

Transduction is based on the change in the electron transfer resistance (R_{ct}) between the electrode and the redox markers in solution.

If more than one ligands are attached to the surface, it is possible to detect and quantify multi-analytes simultaneously.

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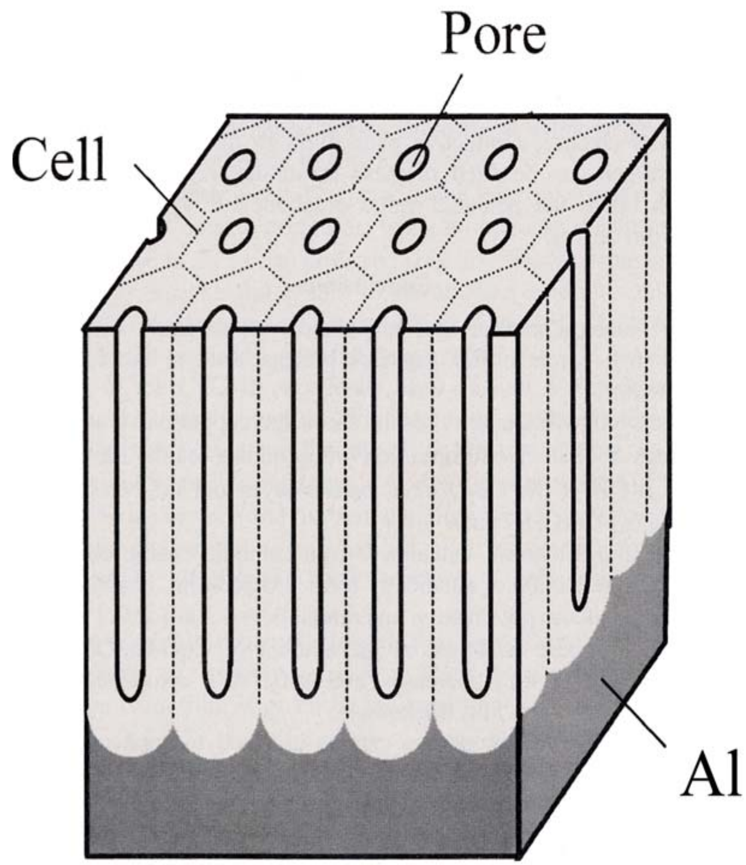
Idealized Structure of AAO



(Masuda, H., et al,

***Science* 1995, 268, 1466)**

Properties and Applications of AAO

Tunable pore features

- diameter (10 - 300 nm)
- density (10^8 - 10^{12} cm^{-2})
- spacing (30 - 500 nm)
- depth (up to hundreds of μm)

Specific advantages

- structural variability
- optical transparency
- excellent stability
- excellent dielectric properties
- low cost and high throughput
- potential for large-scale production

Potential applications

- nanotemplates
- etch masks
- microfluidic devices
- base materials
- MEMS
- bio-supports
- 2D photonic crystals
- ultra-filtration membranes
- micro-engineered catalysts
- magnetic memory arrays

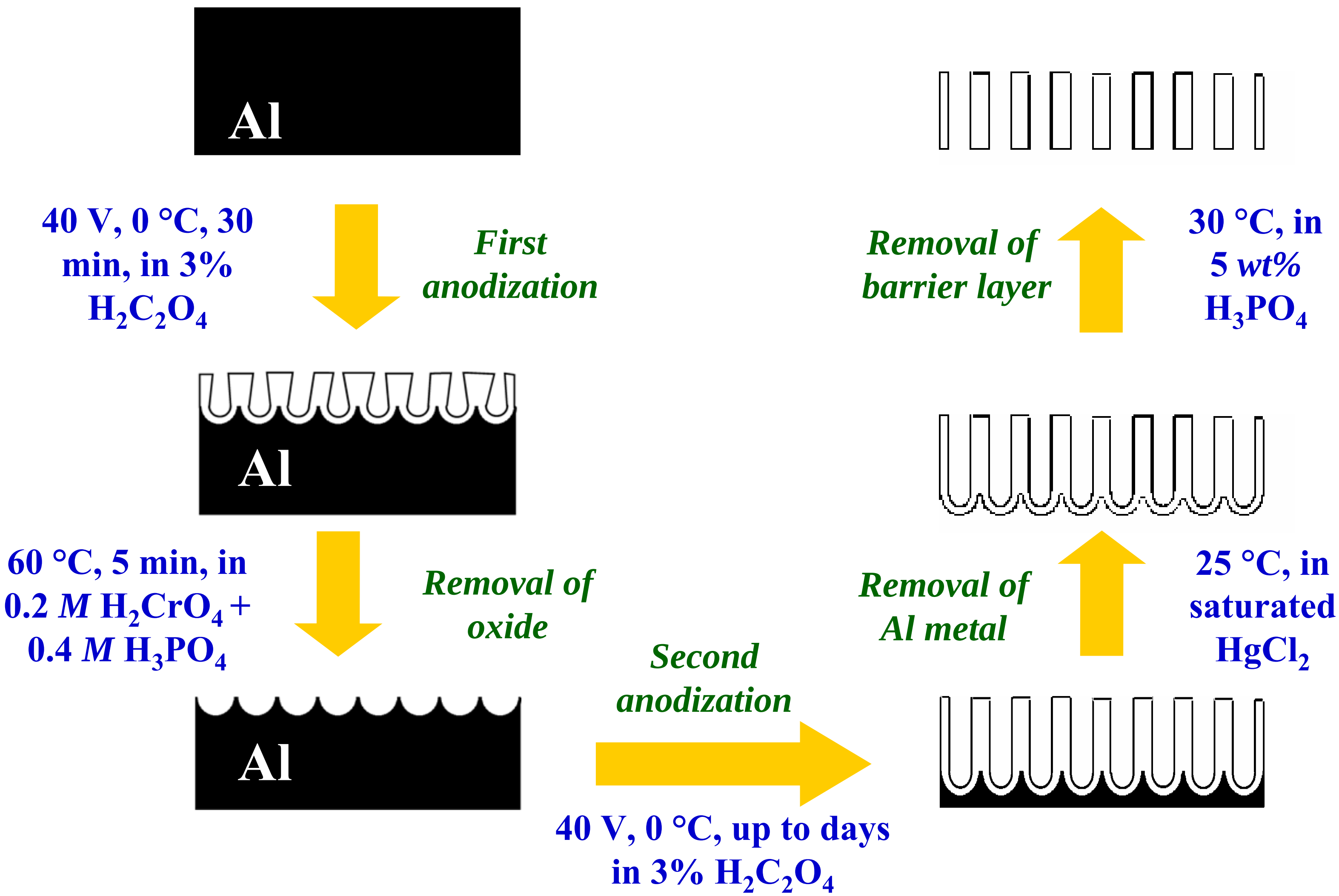
Fabrication of AAO

At a constant low temperature and in an appropriate acidic electrolyte, NAA grow through galvanostatic or potentiostatic anodization of aluminum (5*N*). This process is rapid, inexpensive, reproducible, and controllable.

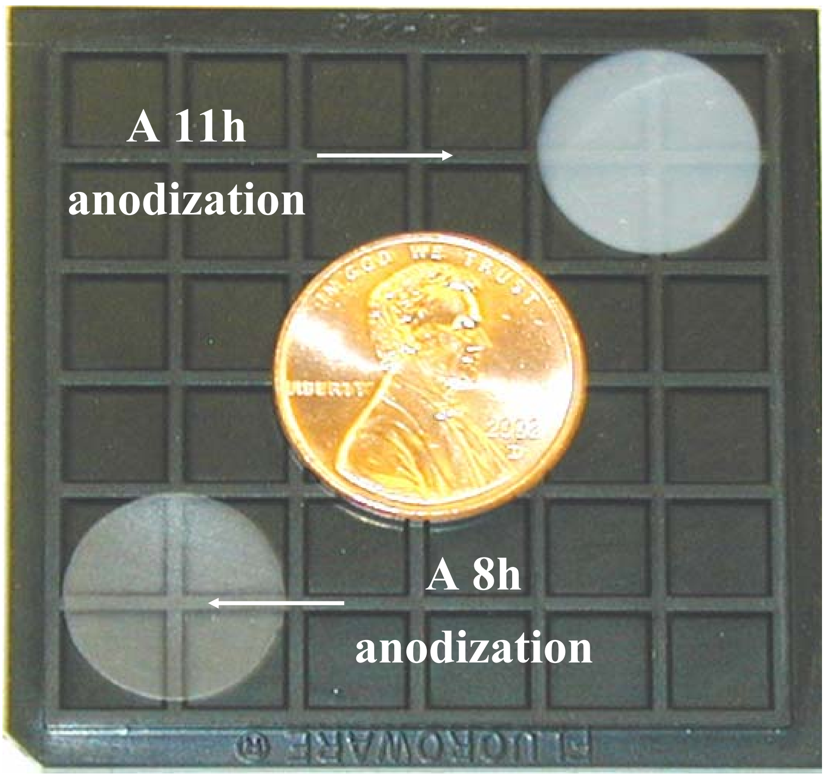
Optimized Anodization Parameters

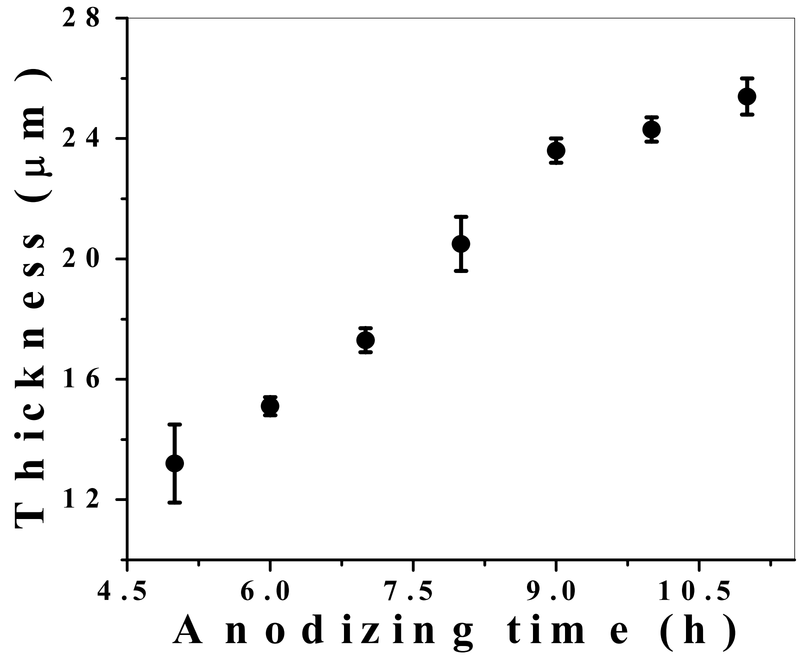
Electrolyte	Temp. (°C)	Voltage (V)	Diameter (nm)
1.2 M H_2SO_4	1	19	15
0.3 M H_2SO_4	1	26	20
0.5 M H_2SO_4	0	25	30
0.3 M $H_2C_2O_4$	14	40	40
0.3 M $H_2C_2O_4$	10	40	45
0.3 M $H_2C_2O_4$	14	60	60
1.0 M H_3PO_4	3	95	90
1.0 M H_3PO_4	0	160	400

Two-Step Anodization Process and Post-Treatments



Dependence of the Membrane Thickness of AAO on Anodization Time

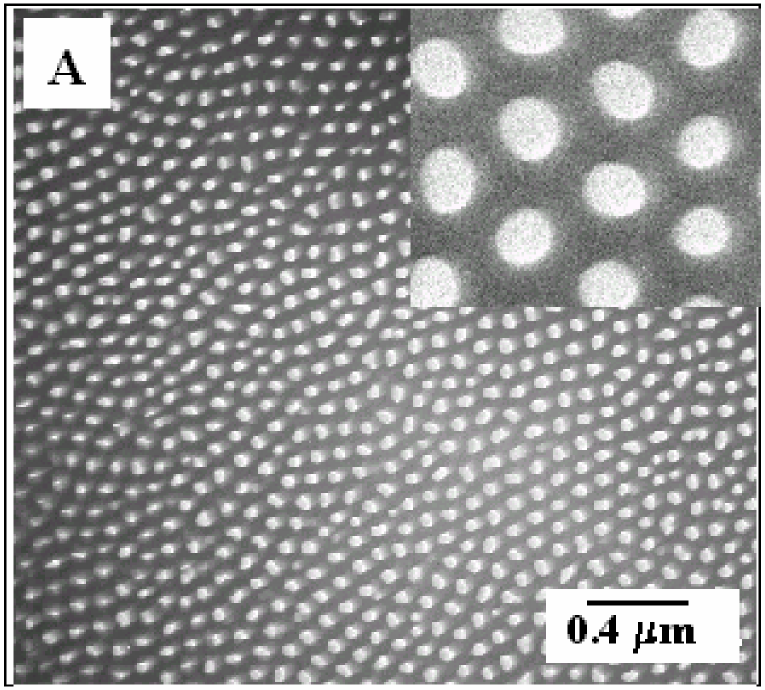




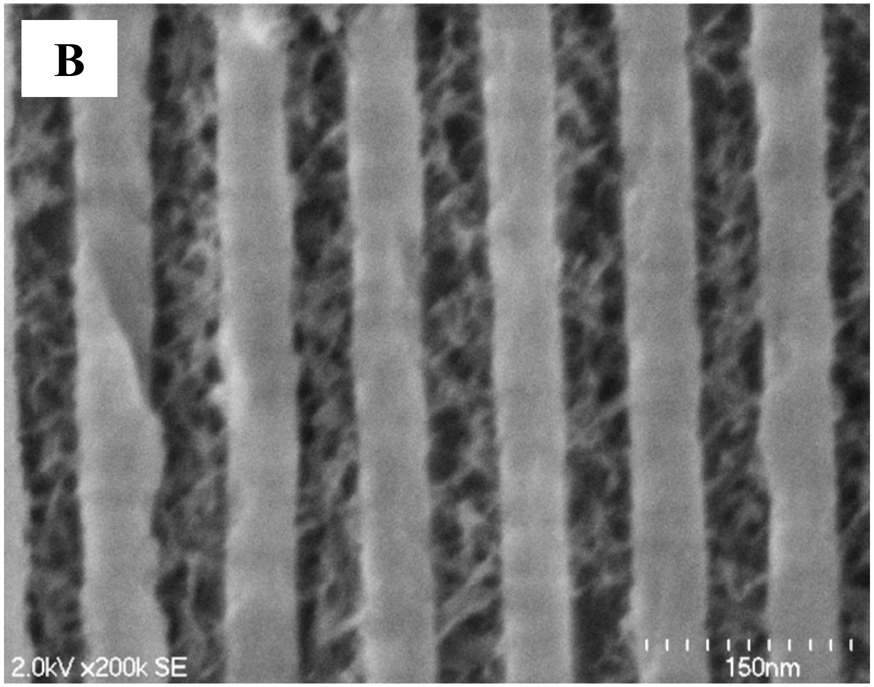
(Yan, J., et al,

***Adv. Mater*. 2003, 15, 2015)**

Electron Micrographs of the Freestanding, Through-Hole AAO Membranes



Plan view (TEM)



Cross-sectional view (SEM)

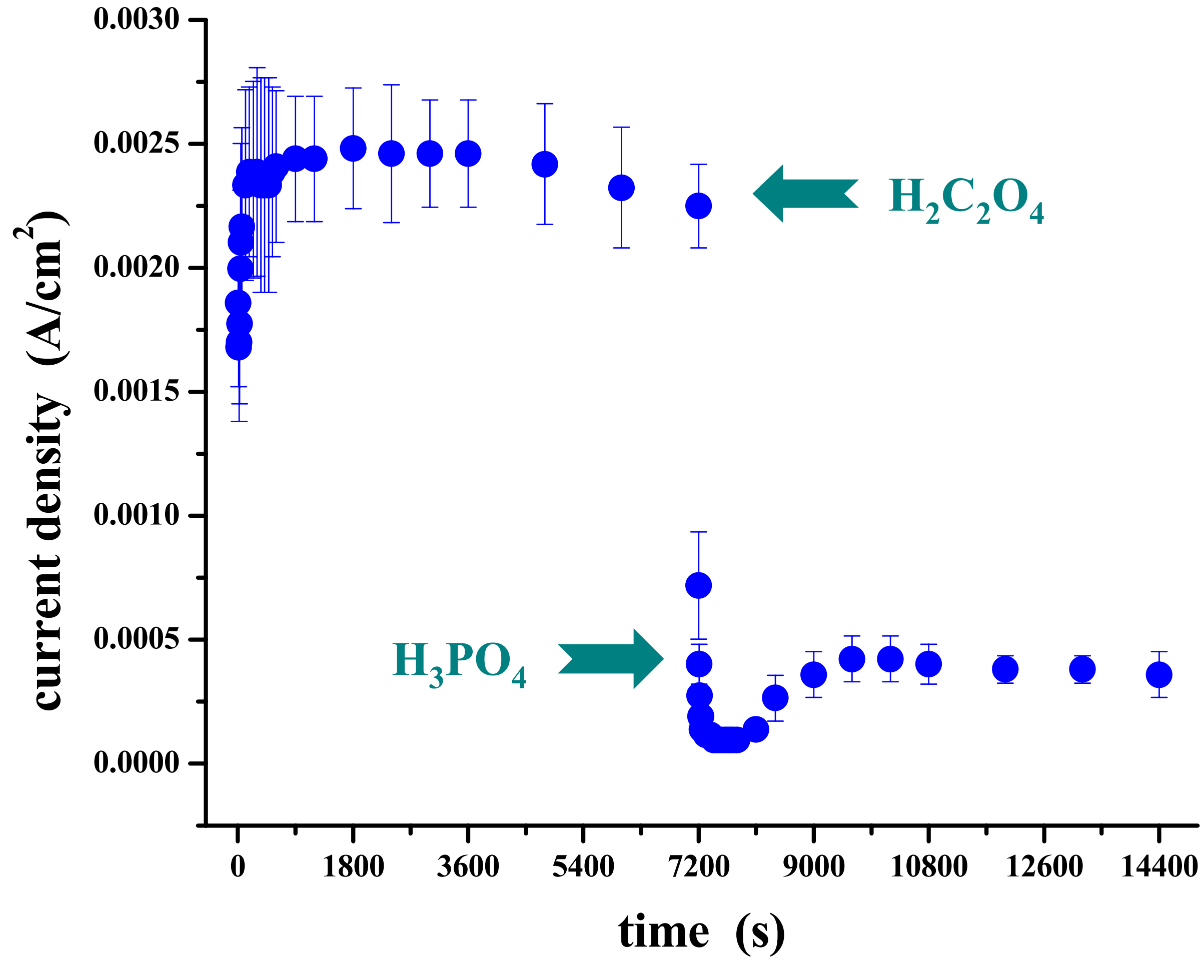
Pore Diameter: (45 ± 2) nm; Pore Depth: ~10 μm; Pore Density: 1.7×10^{10} cm^{-2}.

(Yan, J., et al, *Encyclopedia of Nanoscience and Nanotechnology, Schwarz, J.; et al, Eds.; 2004, 83*)

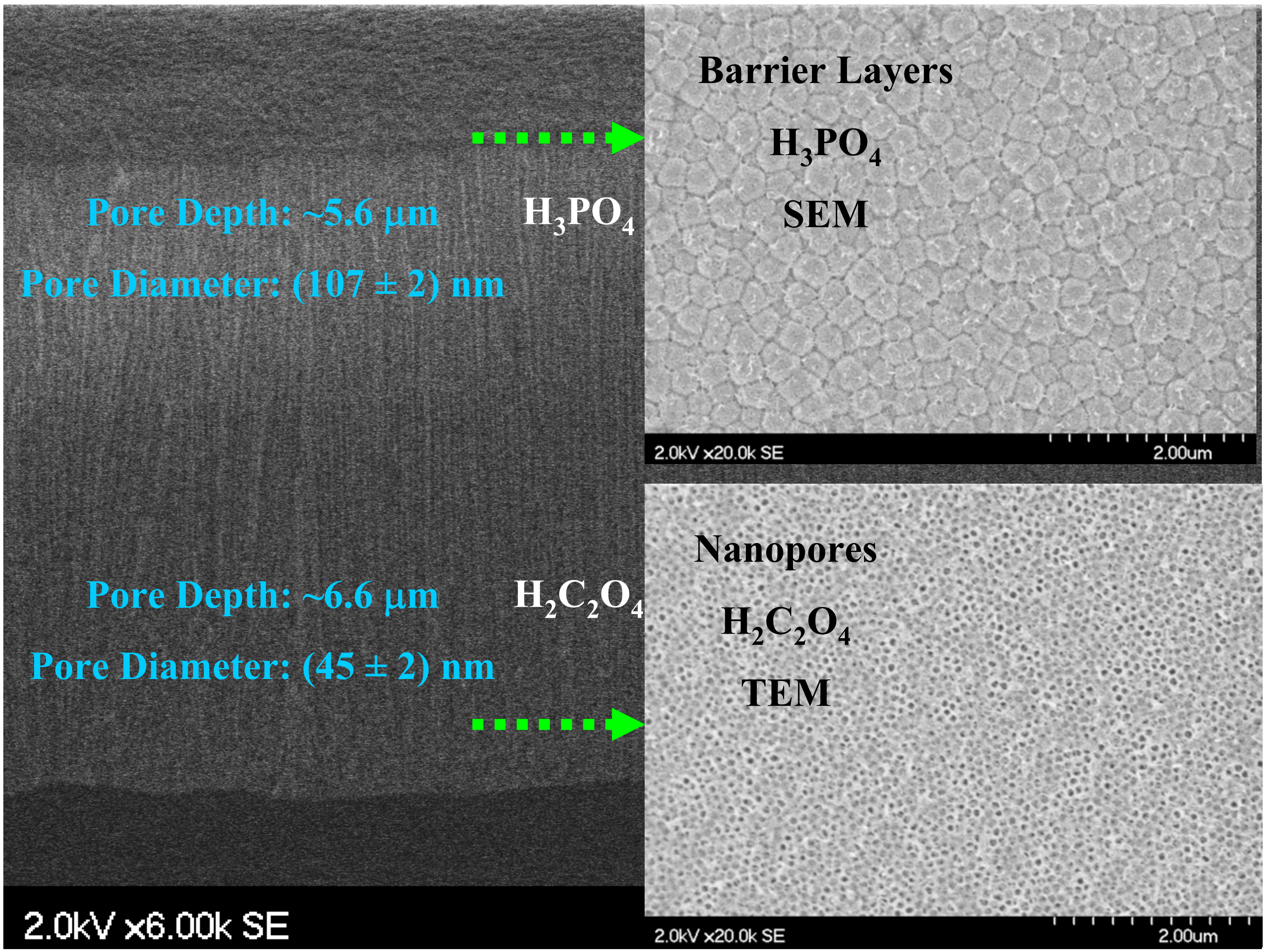
Motivations for Design of Interconnected AAO

- **The fabrication of rationally designed, interconnected, nanotubes is vital to the development of nanotube electronics and biotechnologies. However, this is difficult to achieve using conventional processes (*e.g.*, *laser ablation, CVD, etc.*), which lack a high degree of control in the fabrication and in the properties of the products.**
- **Various postgrowth methods have been reported, but they have been hard to implement and have been subject to defects.**
- **Controlled anodization of ultra-pure aluminum has recently been demonstrated to have the potential of fabricating interconnected, ordered AAO.**

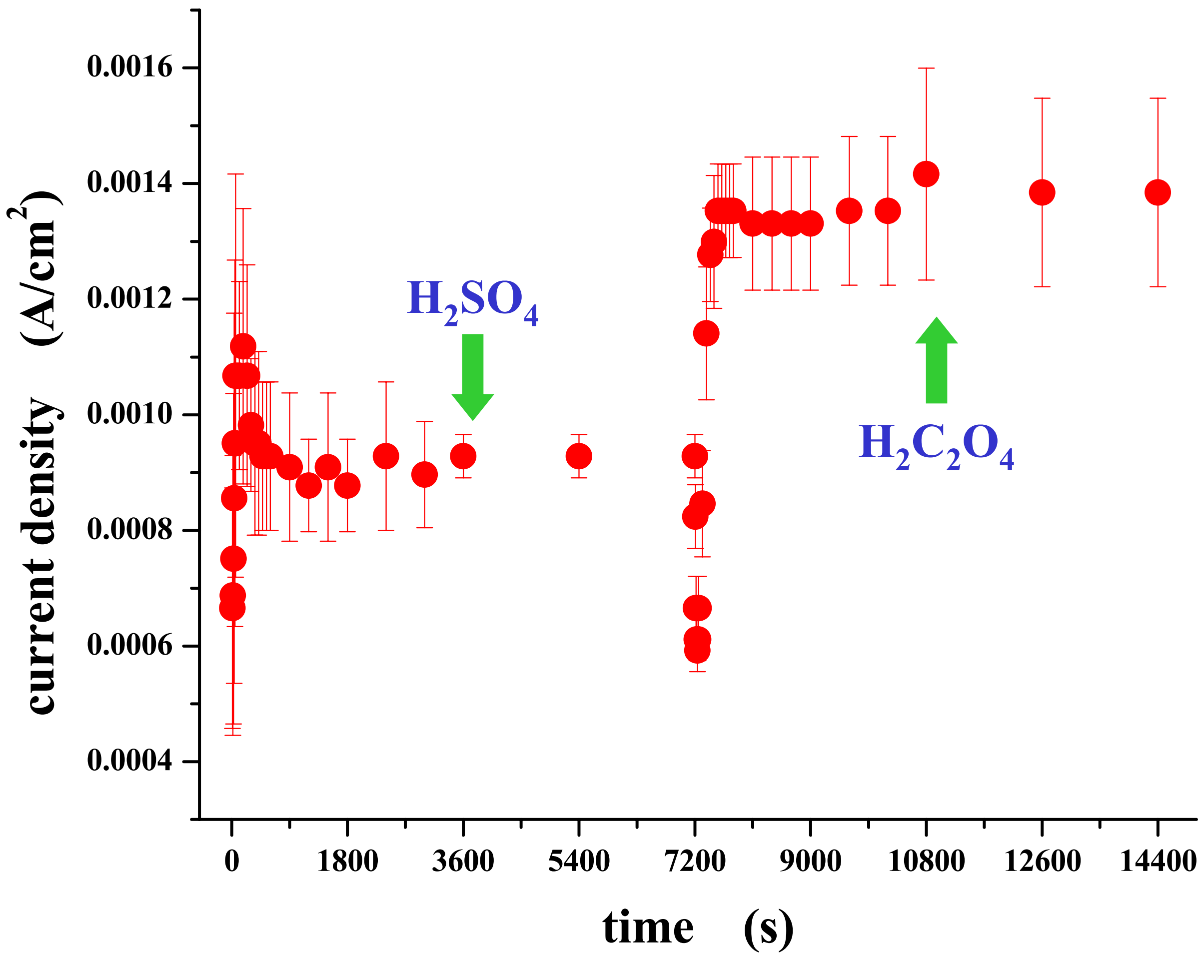
Current Density - Time Plot



Scanning Electron Micrographs of the Freestanding, Merged AAO



Switching Electrolytes Between Sulfuric and Oxalic Acids



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Motivations for Forming Viologen-Functionalized Monolayers in AAO

- **Identification & Quantitation of Biomolecules in Mixtures**
  - ♦ **Separation (e.g., gel electrophoresis & mass spectrometry)**

    — low throughput; lack of quantitation; high cost per assay.
  - ♦ **Through specific binding with a receptor molecule (e.g., commercially available DNA micro-arrays)**

    — not widely available for DNA-protein, protein-protein, & protein-ligand bindings; require fluorescent labels.
- **The modification of nanotubes with biorecognition elements imparts high-sensitivity and high-throughput onto devices based on 1-D nanostructured materials. This allows individual functionalizations, enables reagentless multiplexed binding assays for large mixtures of biomolecules, and offers novel opportunities of assembling nanosensors into functional integrated devices.**

Conclusions & Future Work

- **Combining a 3-step synthesis of a precursor with its subsequent surface derivatizations, we are expecting to produce the complex transducer molecule in higher purity and yield than would be possible with solution phase synthesis.**
- **Tunable, interconnected nanoporous AAO can be easily fabricated in large quantities by switching the electrolytes and adjusting the voltages accordingly.**
- **Our future work will be centered on: (1) attaching multiple ligands to the surface for simultaneous detection and quantitation of various affinity receptors; and (2) creating nanosensor arrays for parallel real-time monitoring of multiple analytes.**

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