Non Specific Binding (NSB) in Antigen-Antibody Assays

Chem 395
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Instructor : Dr. James Rusling

Presenter : Bhaskara V. Chikkaveeraiah

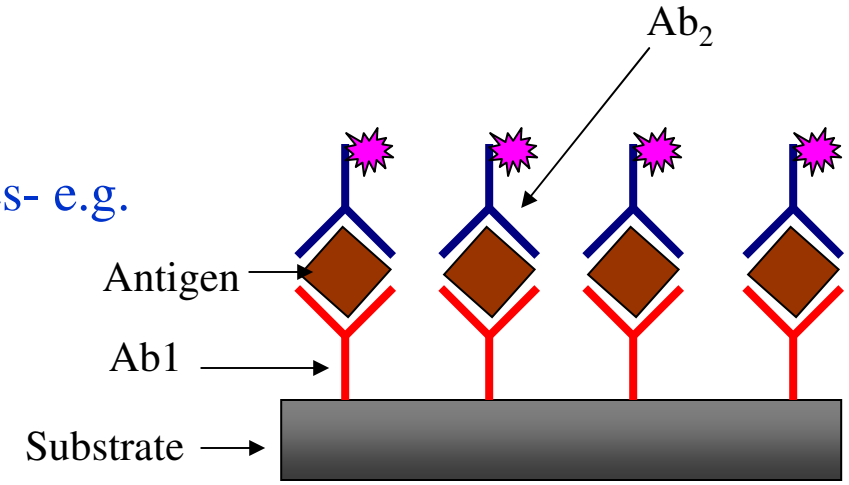
OUTLINE

- Immunoassays Introduction
- Factors contributing to Non-specific binding (NSB)
- NSB Blocking agents
- Minimizing NSB
- NSB in Immunoassay.

Immunoassay

- **Immunoassay**

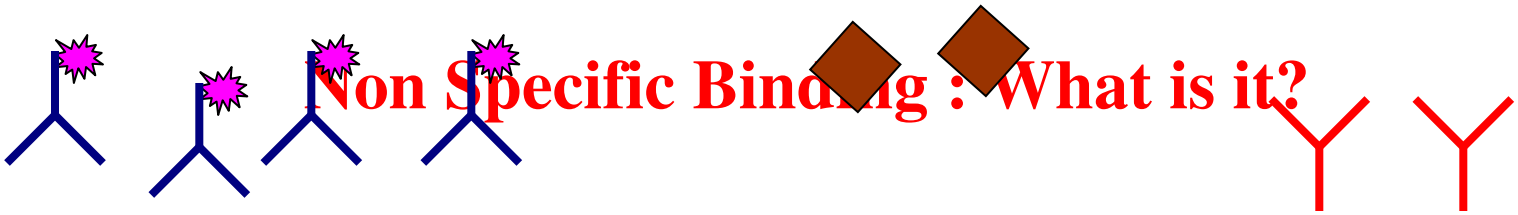
  A biochemical test-measures levels of a particular molecule in biological samples- e.g. serum – uses antibody binding to its antigen (specific binding).



- **Importance**
  - Detecting a disease at very early stage, at lowest antigen concentration.
  - Accuracy of the detection.

Non-specific binding (NSB) affects these two factors

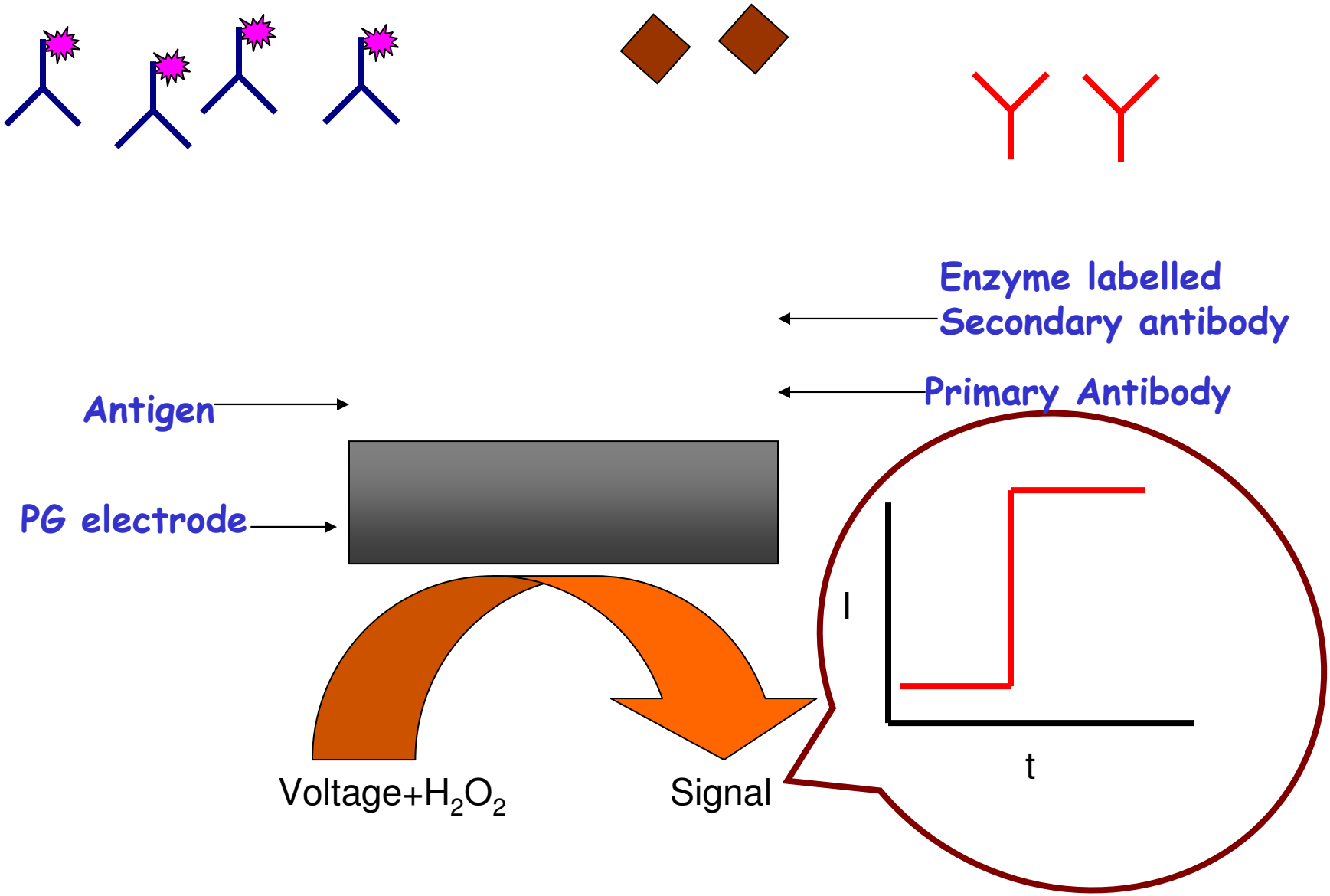
Non Specific Binding : What is it?



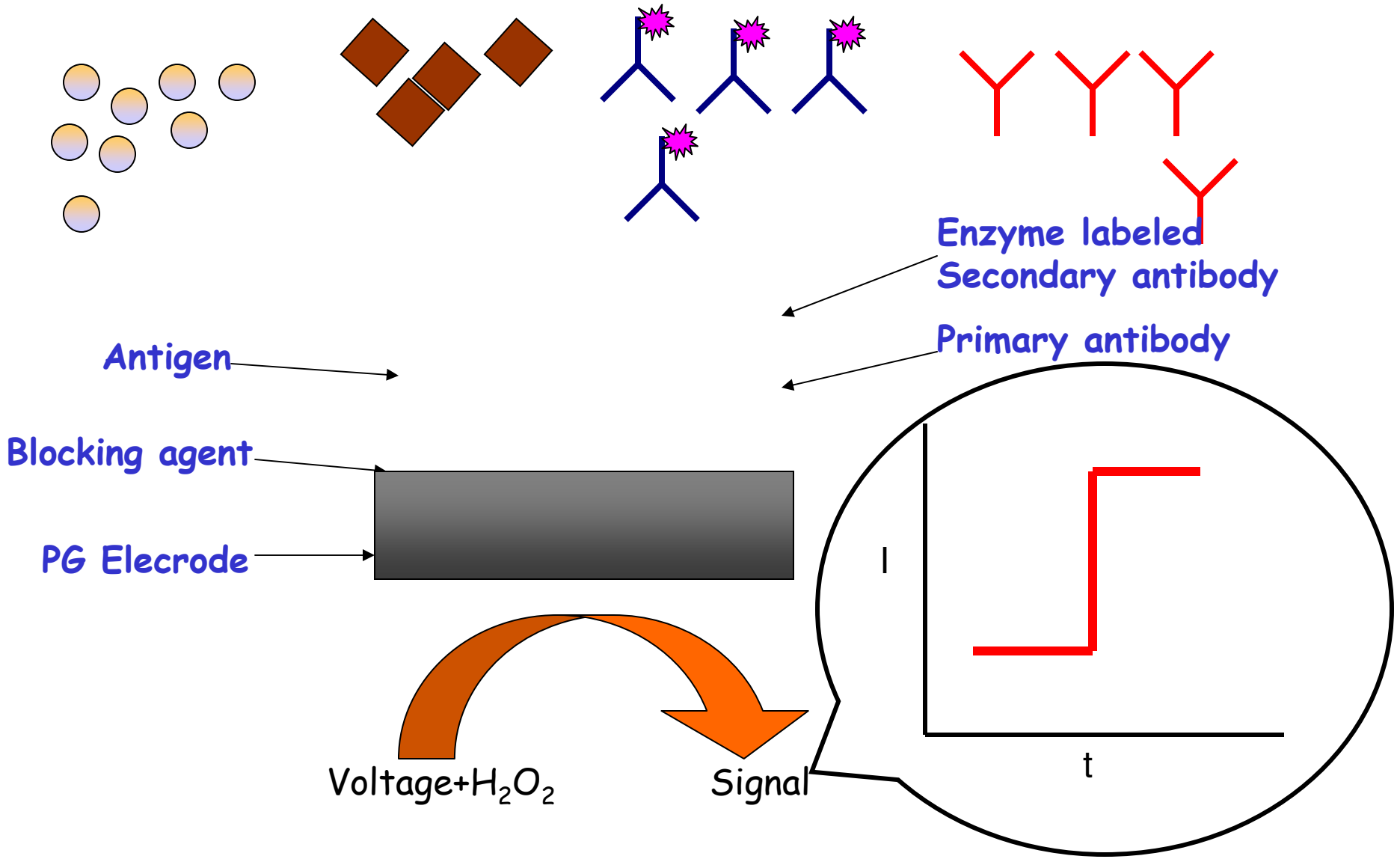
In addition to binding to receptors of interest, sec. antibody may also bind to other sites. Binding to the receptor of interest is called ***specific binding***, while binding to the other sites is called ***nonspecific binding (NSB)***.

NSB can be minimized by saturating the unoccupied binding sites with a blocking reagent (NSB agent) without taking active part in specific assay reaction.

Immunoassay without NSB blocking agent



Immunoassay with NSB Blocking agent



Properties of the blocking agent.

- Inhibit NSB (passive or covalent) of assay components to the surface,
- Inhibit non-specific protein - protein interaction.
- Exhibit no cross reactivity with subsequent assay components (antibodies, protein)
- Not disrupt the bonds that immobilize the specific protein or biomolecule to the surface.
- Exhibit consistent, reproducible performance with every lot.

Blocking agents

- Detergent Blockers
  - Tween-20, Triton X-100
- Protein Blockers
  - Bovine serum albumin, Casein, Fish Gelatin, Whole Sera,
- Polymer based Blockers
  - Polyethylene glycol (PEG), Polyvinyl alcohol (PVA), Polyvinylpyrrolidone (PVP), Polyacrylic acid (PAA), Polyacrylic maleic acid (PAMA).

Detergent Blockers

- Disrupt ionic and hydrophobic biomolecule-surface bonds.
- Inhibits enzyme-substrate reactions.

- Inexpensive,
- stable, can be stored at room temperature (wash buffers)
- strips off loosely bound molecules in wash steps.
- 0.01 – 0.10% is commonly used.

Protein Blockers

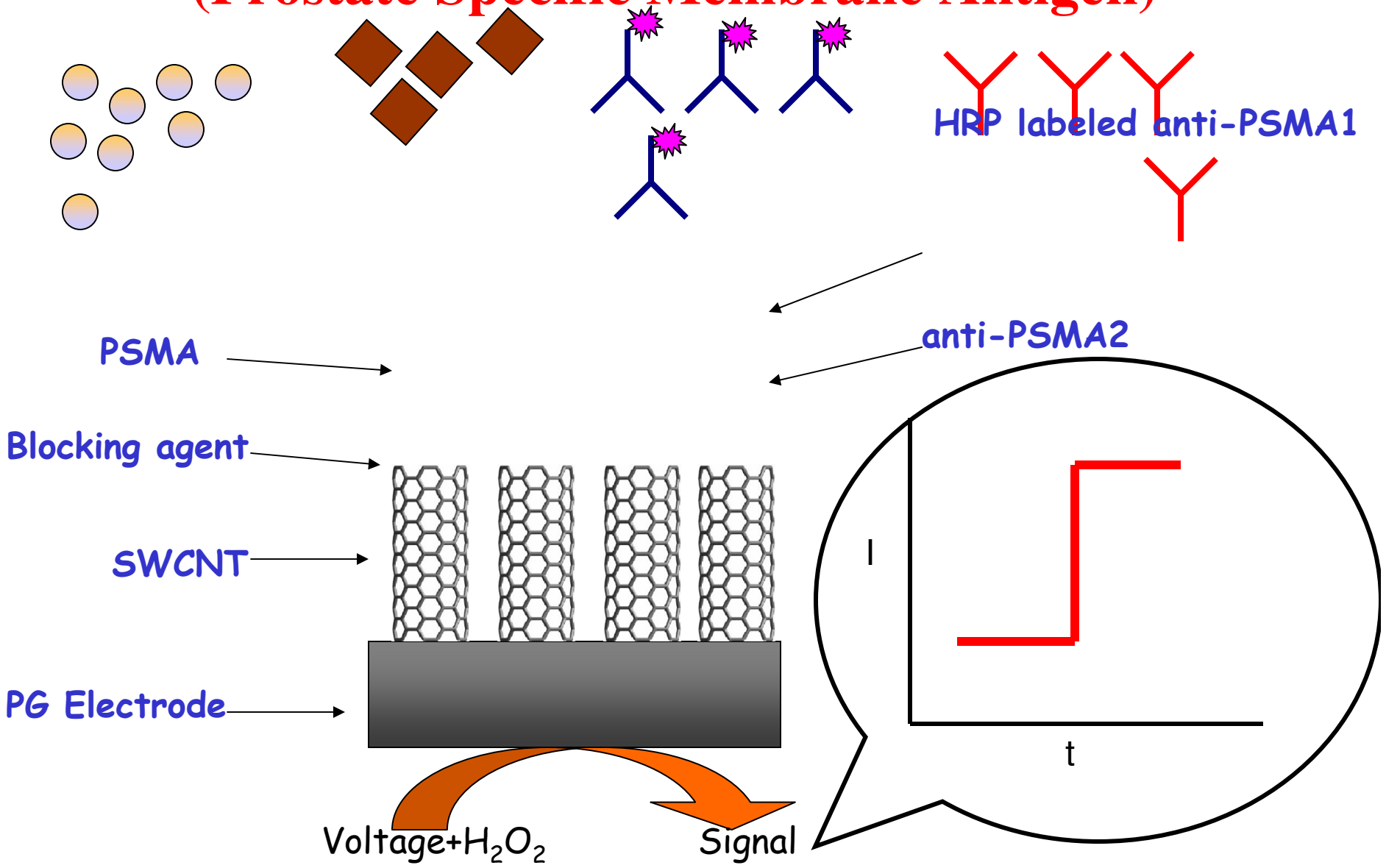
- Blocks the non occupied sites on the surface.
- Space out and stabilize biomolecules bound to the surface to reduce the steric hindrance.

- Bovine serum albumin (BSA)
  - Widely used, Inexpensive, blocks non specific protein-surface binding. ~1 – 3% ic commonly used.
- Fish Gelatin
  - Mainly blocks protein-protein(Ab1-Ab2) interactions
- Whole sera
  - Blocks biomolecule-surface passive, covalent interactions, protein-protein interactions, and acts as protein stabilizer.

Factors affecting NSB

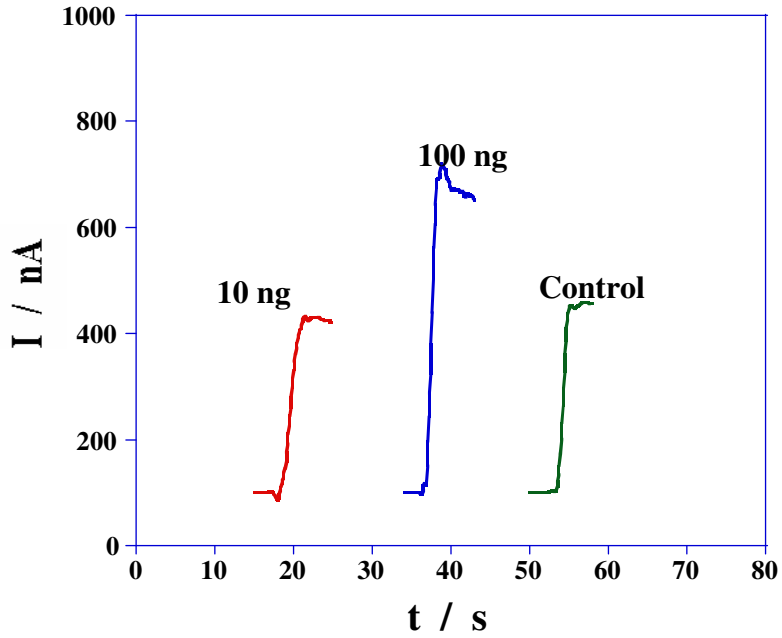
- Ab1 / Ab2 ratio (~ 500:1)
- Type of blocking agent used.
  - Bovine serum albumin, Casein, etc.
- Washing steps
  - Consistent and vigorous.

PSMA Immunoassay
(Prostate Specific Membrane Antigen)



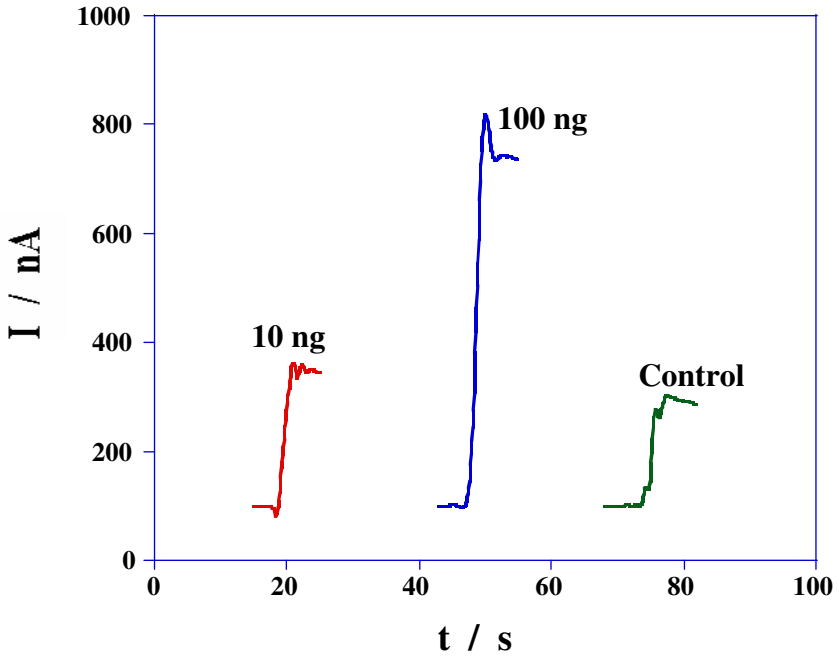
Effect of blocking agents in PSMA Immunoassay

PBS buffer + 0.05% Tween-20 + 2% BSA



PSMA ng/mL	Current nA
10 ng/mL	345 ± 22
100 ng/mL	467 ± 83
Control 0 ng/mL	351 ± 16

PBS buffer + 0.05% Tween-20 + 2% Casein



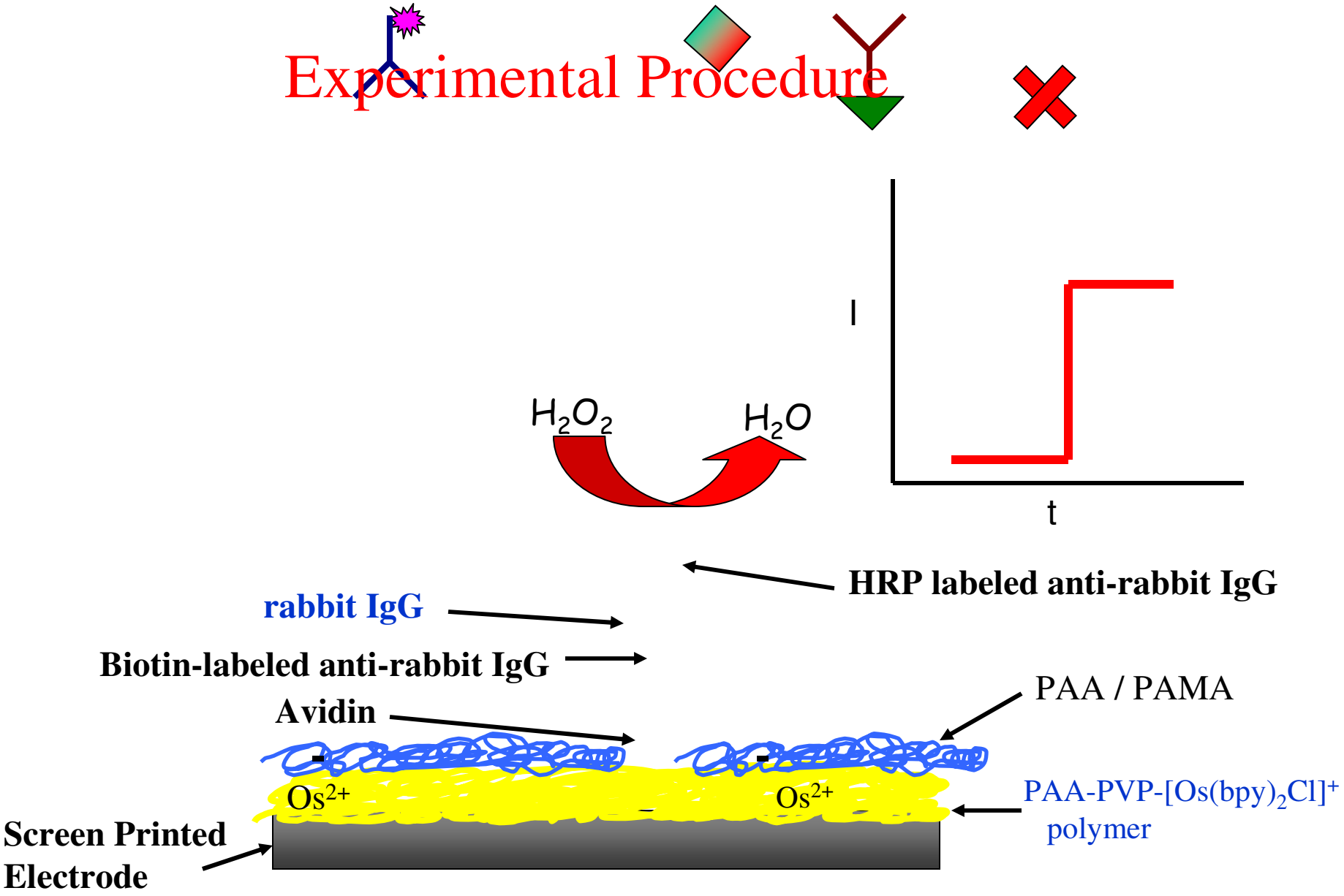
PSMA ng/mL	Current nA
10 ng/mL	325 ± 106
100 ng/mL	583 ± 97
Control 0 ng/mL	246 ± 53

Reduction of the Nonspecific Binding of a Target Antibody and of Its Enzyme-Labeled Detection Probe Enabling Electrochemical Immunoassay of Antibody through the 7 pg/mL – 100 ng/mL (40 fM - 400 pM) Range

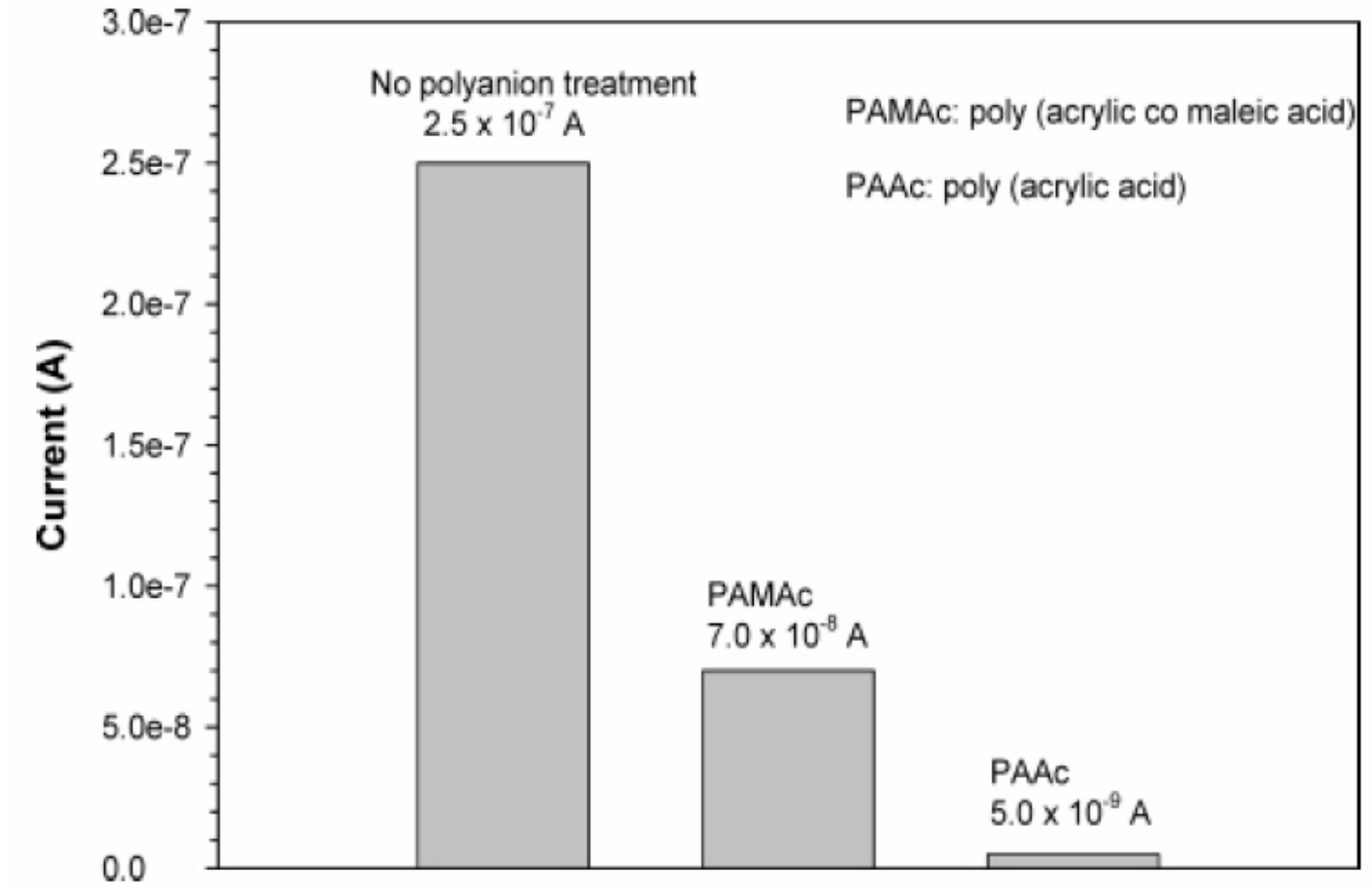
Yongchao Zhang and Adam Heller*
Department of Chemical Engineering and Texas Materials Institute
University of Texas at Austin.

Anal. Chem. 2005, 77, 7758 - 7762

Experimental Procedure

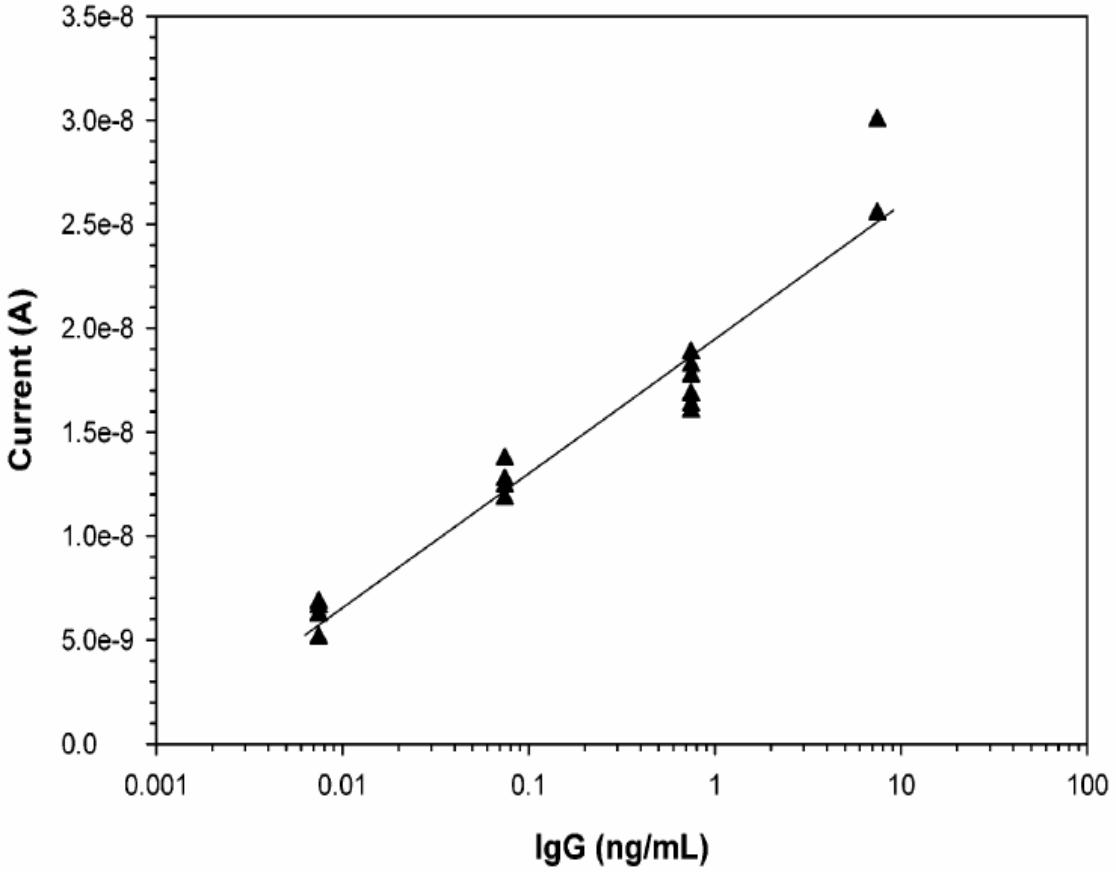


Results and Discussion



Suppression of the nonspecific binding-noise current by polyanions having terminal functions forming covalent bonds with amines and with thiols .

Results and Discussion



Dependance of H_2O_2 electroreduction current on the antigen concentration.

Summary

- Selection of appropriate blocking system is essential for the development of a specific and sensitive assay.

- Empirical testing is required to both choose and optimize the blocking procedure and is influenced by the surface chemistry.

Acknowledgements

- **Dr. Rusling**
- **Chem 395 Class**