ELECTROCHEMICAL BIOSENSORS

Modern and future approaches to medical diagnostics

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F. A. Armstrong, H. A. Heering, and J. Hirst, Reactions of complex metalloproteins studies by protein film voltammetry. *J. Chem. Soc. Rev.* **26**, 169-179 (1997).

J. F. Rusling, Z. Zhang, Designing functional biomolecular films on electrodes. in J. F. Rusling, Ed., *Biomolecular Films*, Marcel Dekker, N. Y., **2003**, pp. 1-64.

Medical Diagnostics

- **Doctors increasingly rely on testing**
- **Needs: rapid, cheap, and “low tech”**
- **Done by technicians or patients**
- **Some needs for *in-vivo* operation, with feedback**

Principle of Electrochemical Biosensors

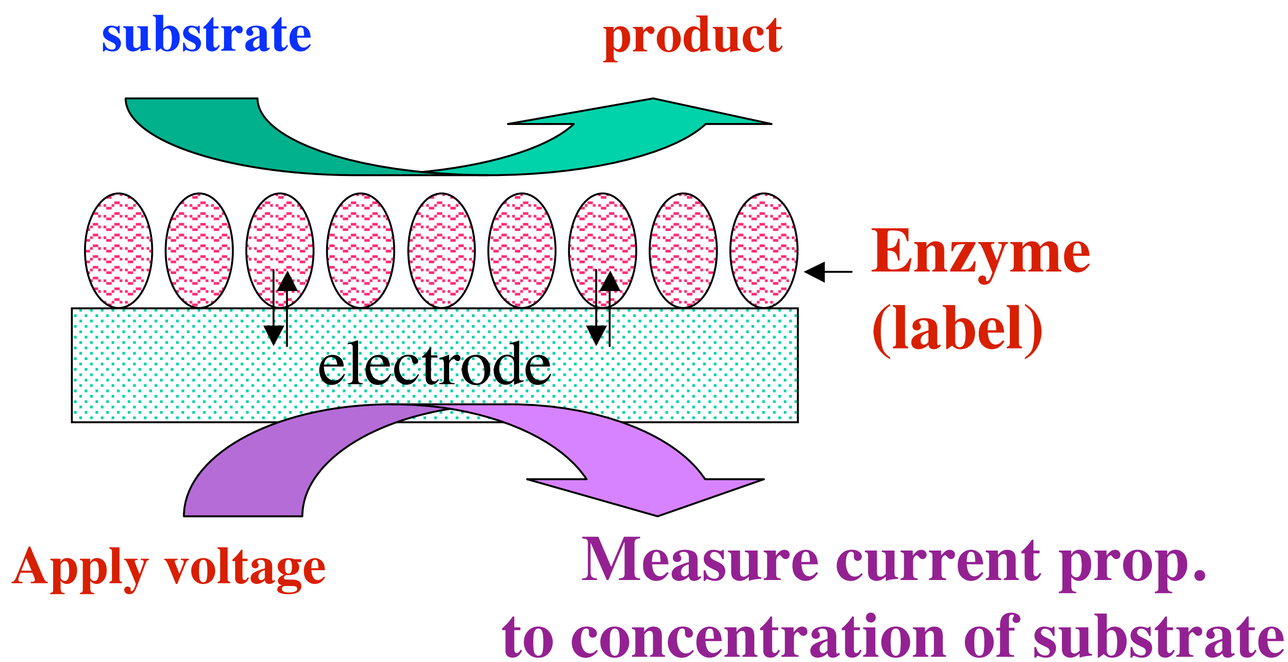
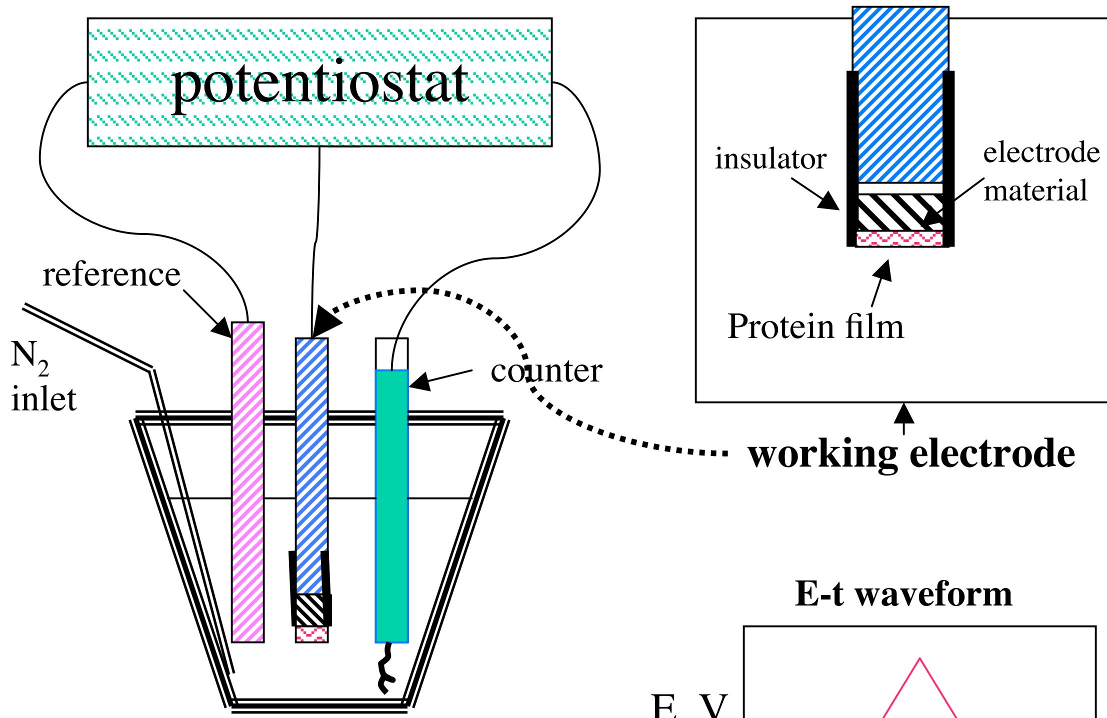
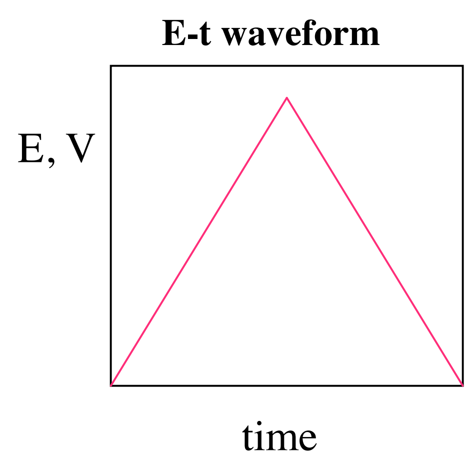


Figure 9

Equipment for developing electrochemical biosensors

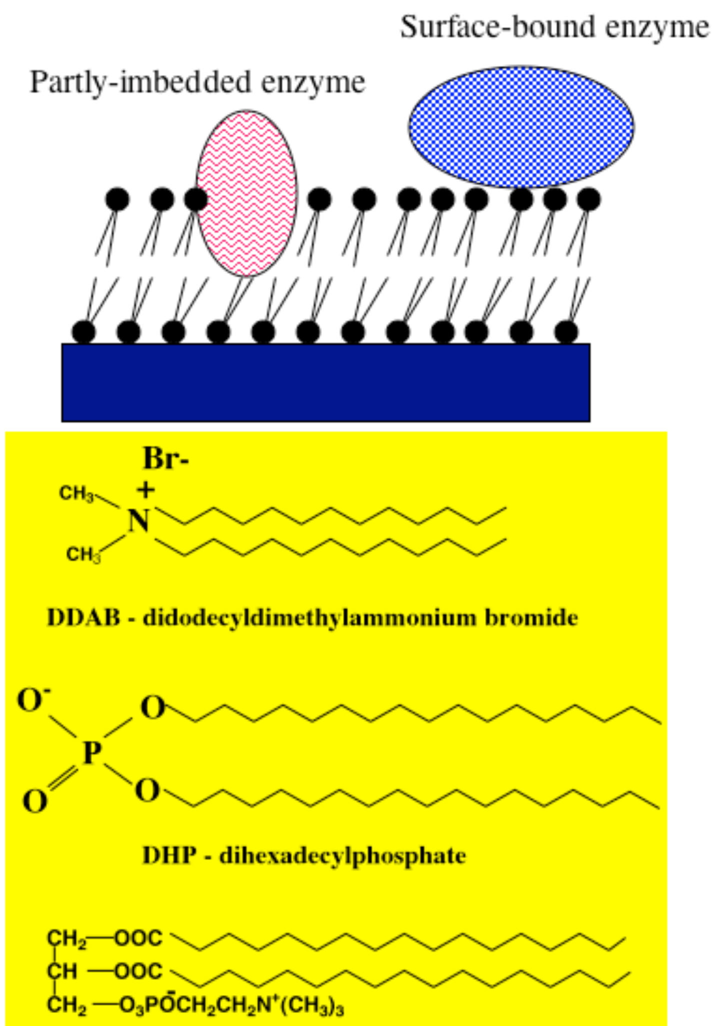


Electrochemical cell

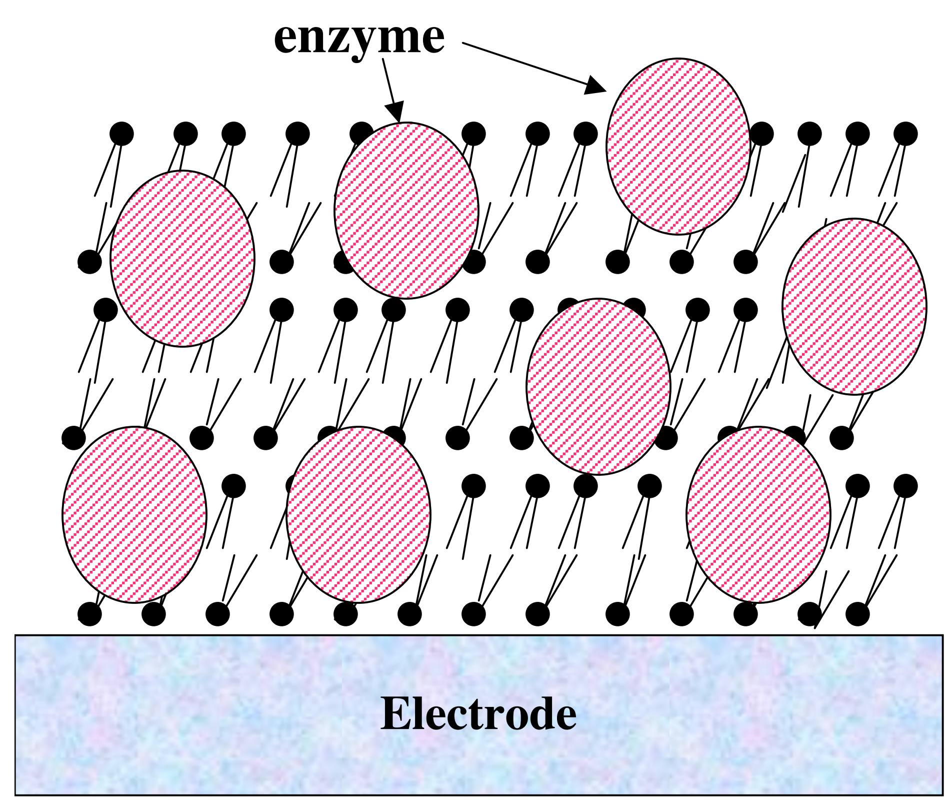


Cyclic voltammetry

A lipid-enzyme film

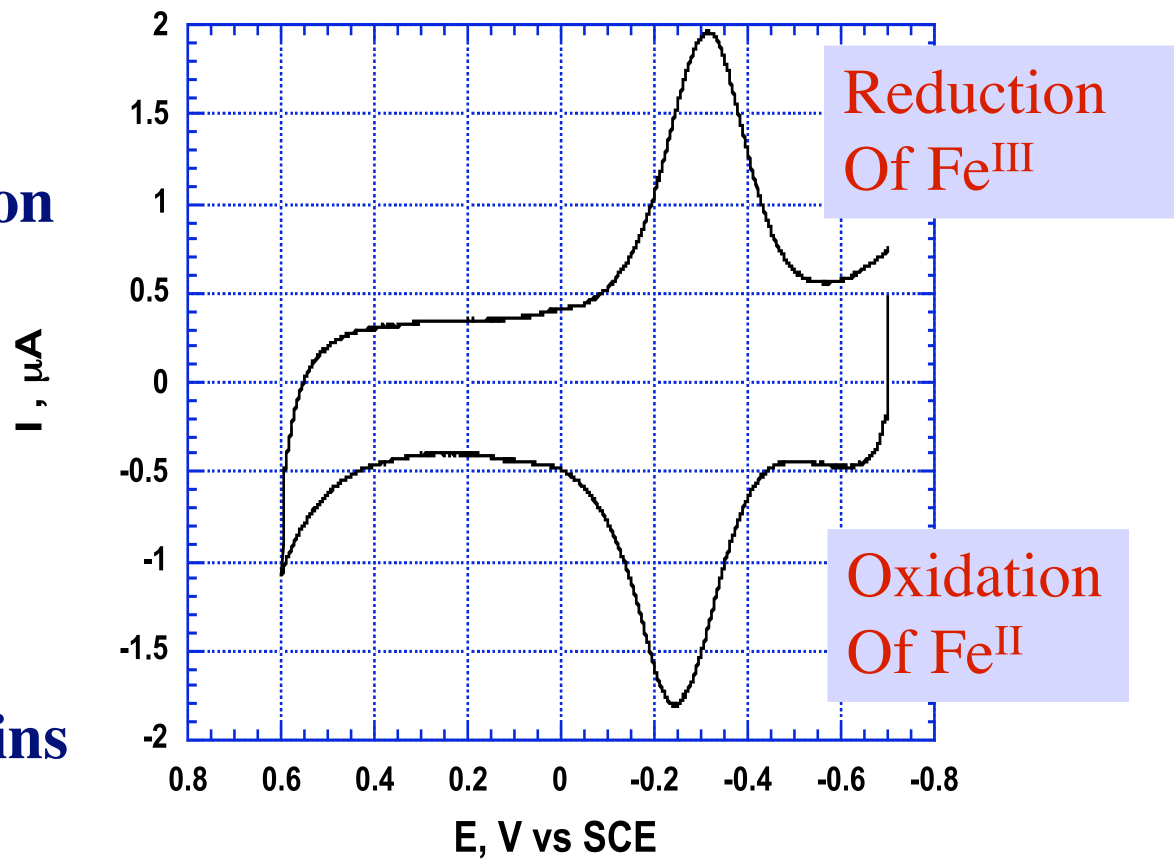


Dihexadecylphosphatidyl choline



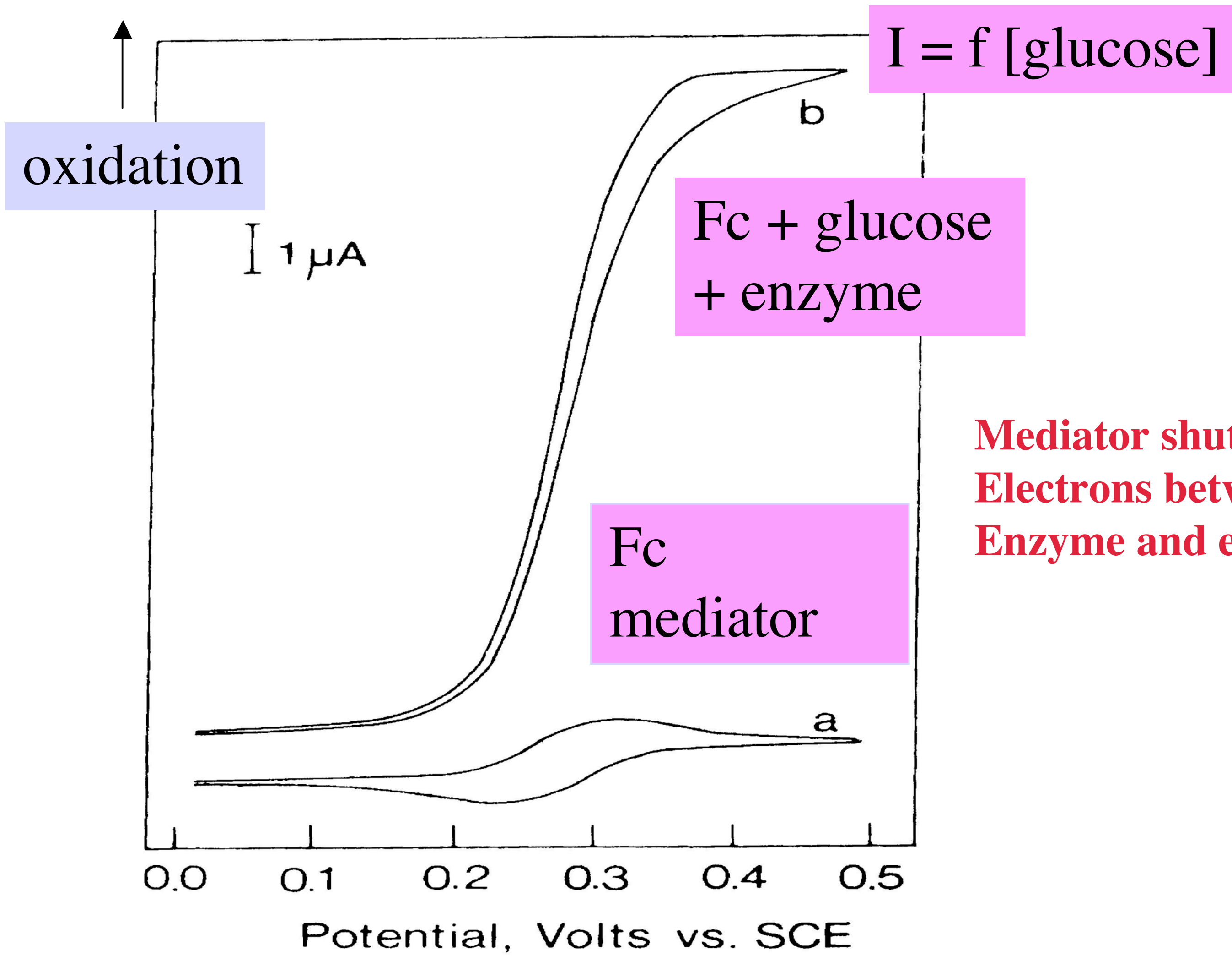
Cyclic voltammogram (CV) at 100 mV s^{-1} and 25 °C of *Mycobacterium Tuberculosis* KatG catalase-peroxidase in a thin film of dimyristoylphosphatidylcholine on basal plane PG electrode, in anaerobic pH 6.0 buffer.

Reversible Peaks for Direct electron Transfer



(not all proteins do this)

Catalytic enzyme electrochemistry
a basis for biosensor - glucose oxidase



Mediator shuttles
Electrons between
Enzyme and electrode

A. Cass, G. Davis, G. D. Francis, H. O. A. Hill, W. J. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott, A. P. F. Turner, *Anal. Chem.* **56,** 667-671 (1984).

Mechanism for catalytic oxidation of glucose With Glucose oxidase (GO) and Fc mediator

Scheme 2

Glucose + GO(FAD) + 2 H^+ → gluconolactone + GO($FADH_2$) (1)

GO($FADH_2$) + 2 Fc^+ → GO(FAD) + 2 Fc + 2 H^+ (4)

Fc → Fc^+ + e^- (at electrode) (5)

Fc = ferrocenecarboxylate

Signal can also be measured by amperometry:
Hold const. E where oxidation occurs, measure I vs time

Commercial Glucose Sensors

- **Biggest biosensor success story!**
- **Diabetic patients monitor blood glucose at home**
- **First made by Medisense (early 1990s), now 5 or more commercial test systems**
- **Rapid analysis from single drop of blood**
- **Enzyme-electrochemical device on a slide**

Patient Diabetes Management

- **Insulin secretion by pancreas regulated by blood glucose, 4.4 to 6.6 mM normal**
- **In diabetes, regulation breaks down**
- **Wide swings of glucose levels**
- **Glucose tests tell patient what action to take (e.g. administer insulin)**

- **Most sensors use enzyme called glucose oxidase (GO)**
- **Most sensors are constructed on electrodes, and use a <u>mediator</u> to carry electrons from GO to electrode**

Fc = mediator, ferrocene, an iron complex

These reactions occur in the sensor:

$$Fc \longleftrightarrow Fc^{+} + e^{-} \quad \text{(measured)}$$

$$GO_{R} + 2\,Fc^{+} \dashrightarrow GO_{ox} + 2\,Fc$$

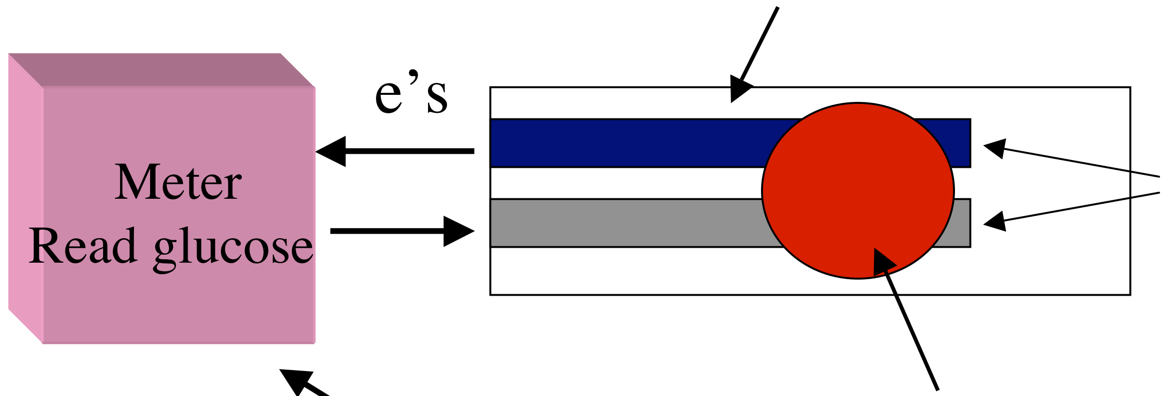
$$GO_{ox} + \text{glucose} \dashrightarrow GO_{R} + \text{gluconolactone}$$

Reach and Wilson, *Anal. Chem.* 64, 381A (1992)

G. Ramsay, *Commercial Biosensors*, J. Wiley, 1998.

Glucose biosensor test strips (~$0.50-1.00 ea.)

Dry coating of GO + Fc

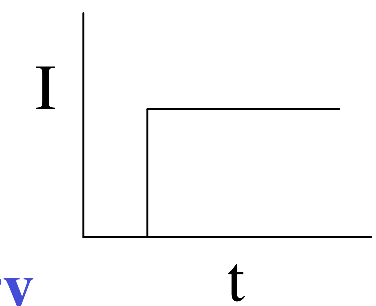


electrodes

Patient adds drop of blood,
then inserts slide into meter

Patient reads glucose level on meter

*(**B.B. King*** http://www.bbking.com/)



Output:
Amperometry
Constant E

Research on new glucose sensors

- **Non-invasive biosensors - skin, saliva**
- **Implantable glucose sensors to accompany artificial pancreas - feedback control of insulin supply**
- **Record is ~4 weeks for implantable sensor in humans**

Other biosensors

- **Cholesterol - based on cholesterol oxidase**
- **Immunosensors - pathogenic bacteria, disease detection (biomarkers)**
- **Small molecules and ions in living things: H^+, K^+, Na^+, CO_2, H_2O_2**
- **DNA hybridization and damage**
- **Micro or nanoarrays, optical abs or fluor.**

Layer by layer Film construction:

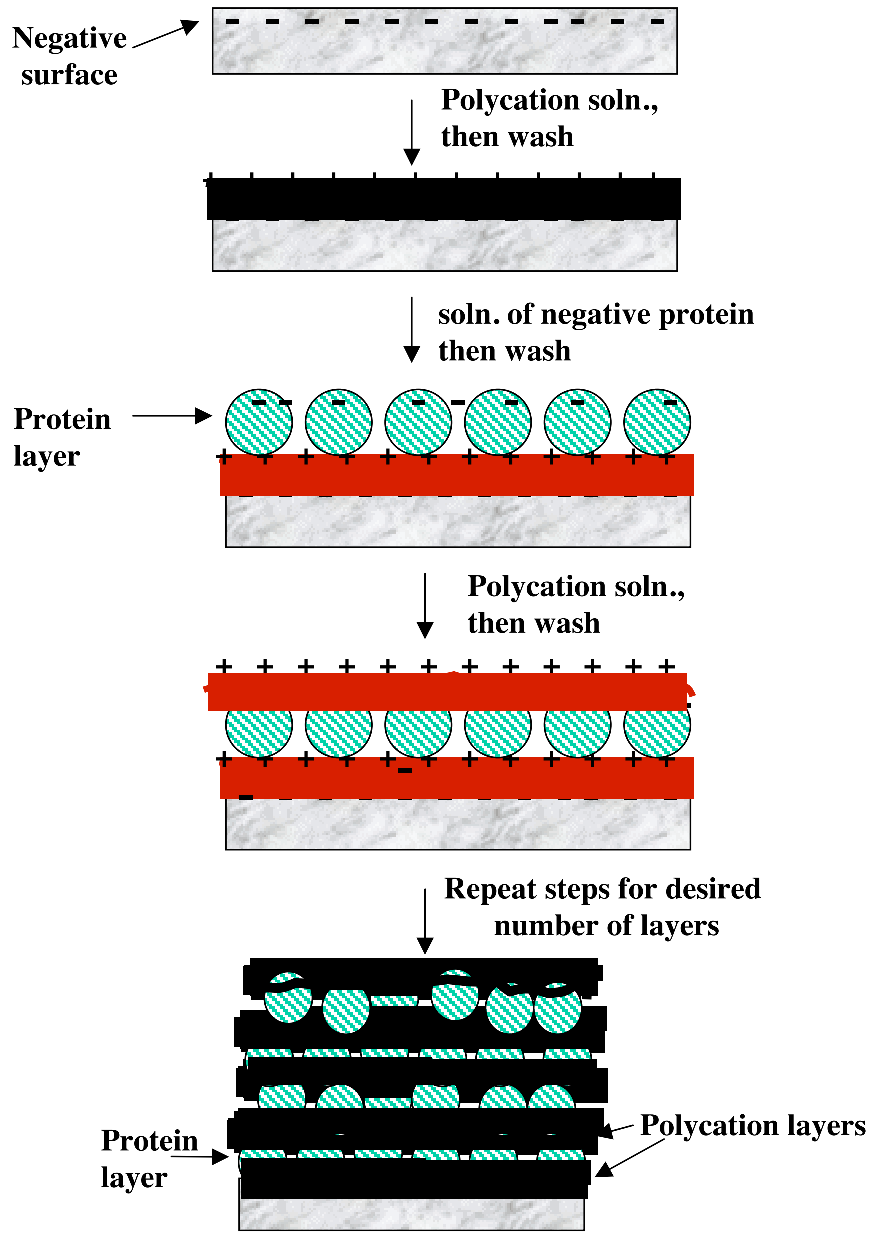
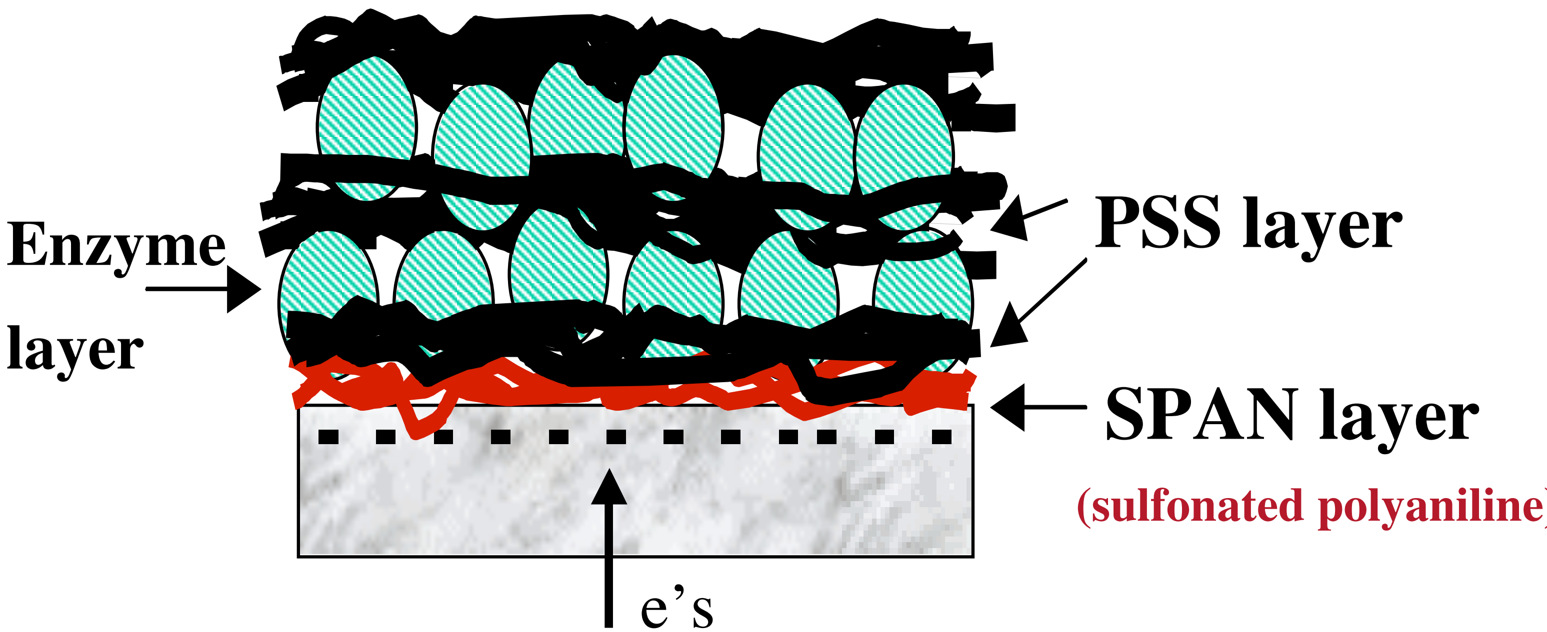


Figure 19

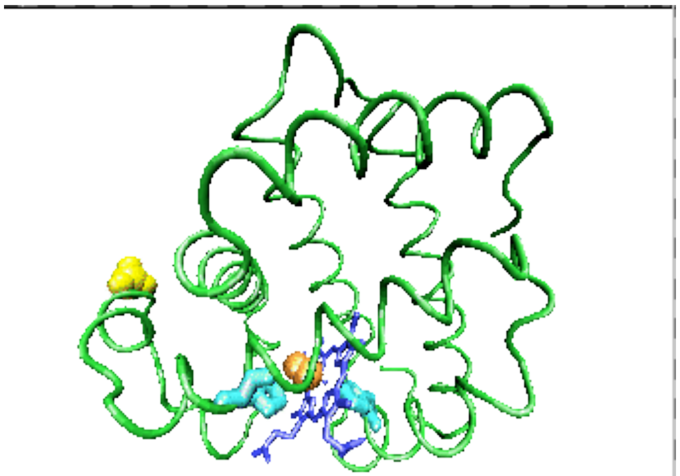
Detection of hydrogen peroxide

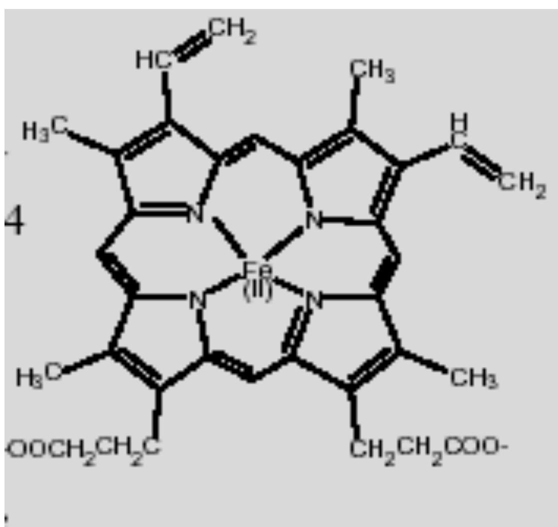
Conductive polymers efficiently wire peroxidase enzymes to graphite

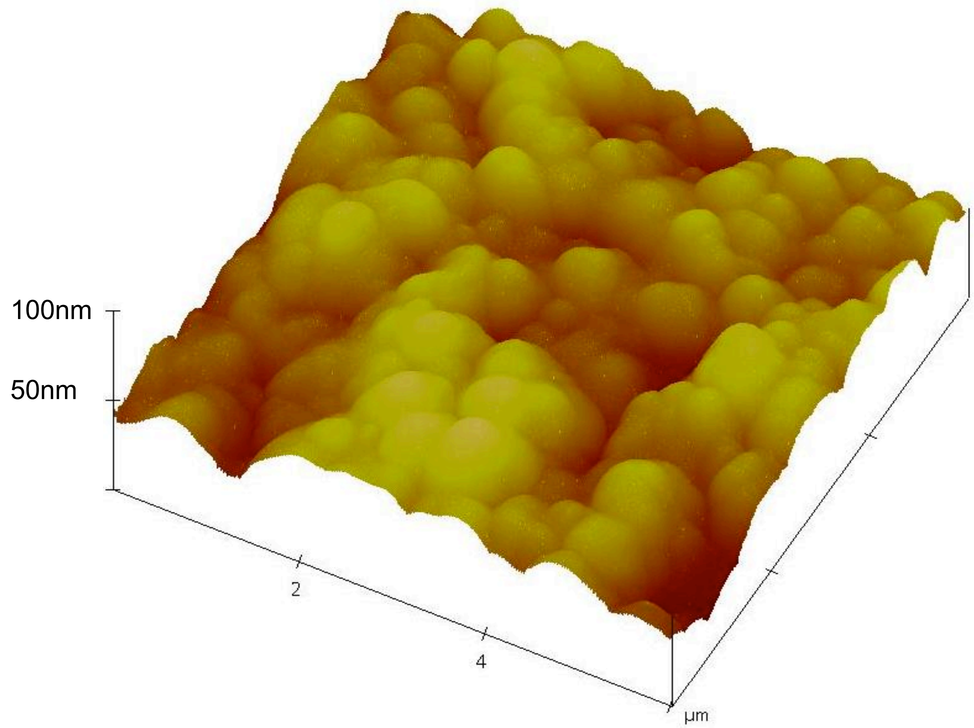


Xin Yu, G. A. Sotzing, F. Papadimitrakopoulos, J. F. Rusling, Highly Efficient Wiring of Enzymes to Electrodes by Ultrathin Conductive Polyion Underlayers: Enhanced Catalytic Response to Hydrogen Peroxide, *Anal. Chem.*, 2003, *75*, 4565-4571.

Horseradish Peroxidase (HRP)

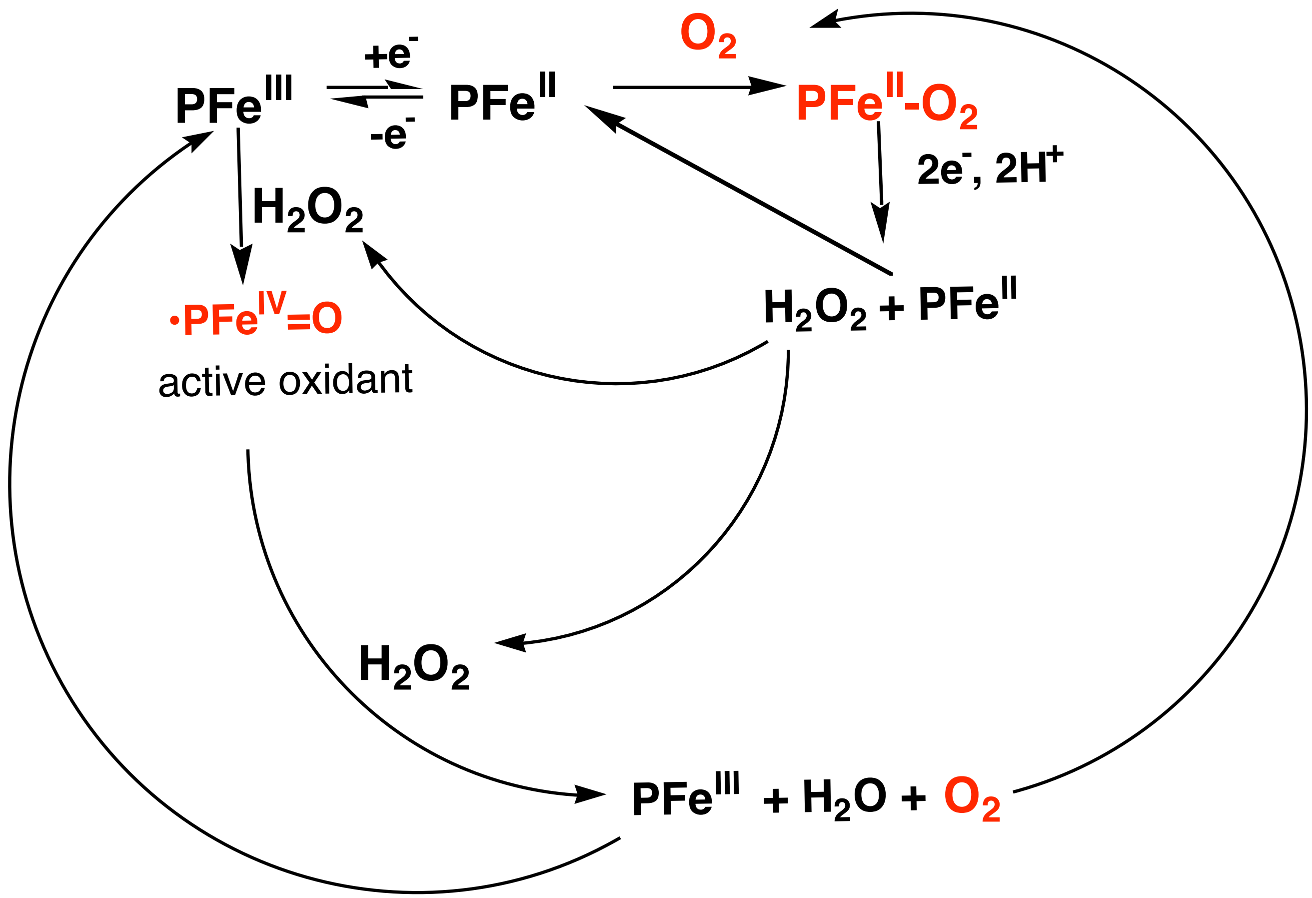






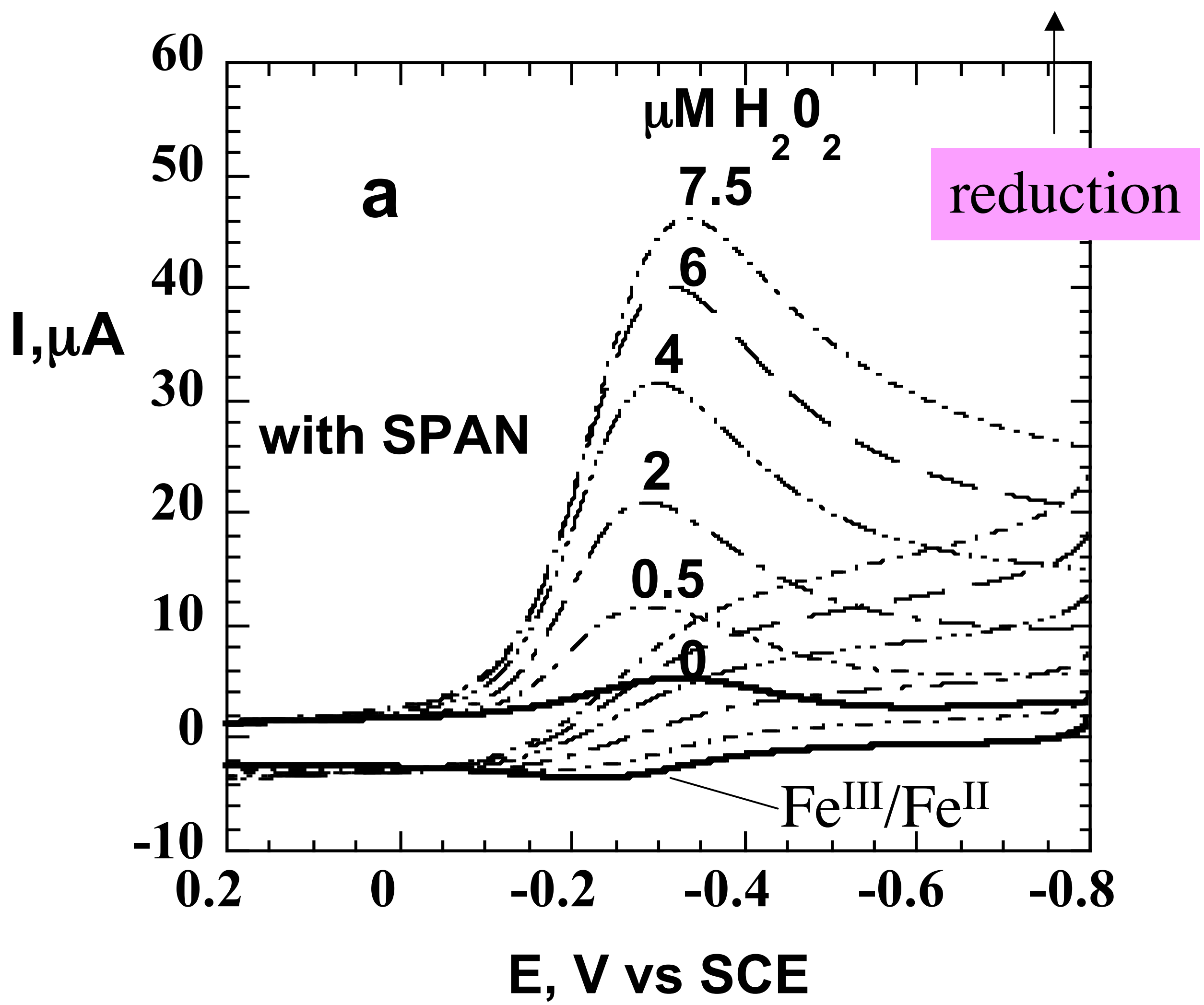
Tapping mode atomic force microscopy (AFM) image of HRP film

Electrochemical Response of Peroxidases

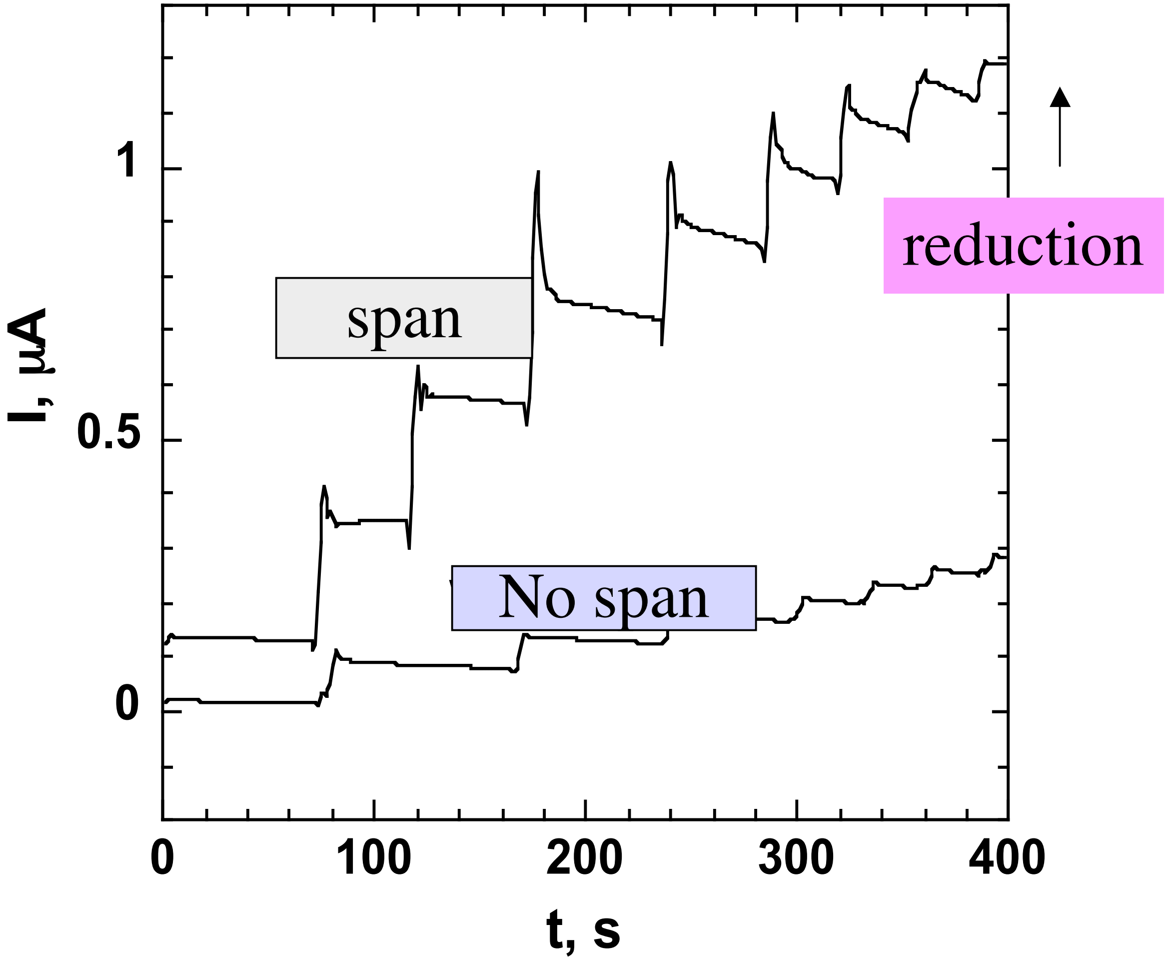


Possible reduced species in red

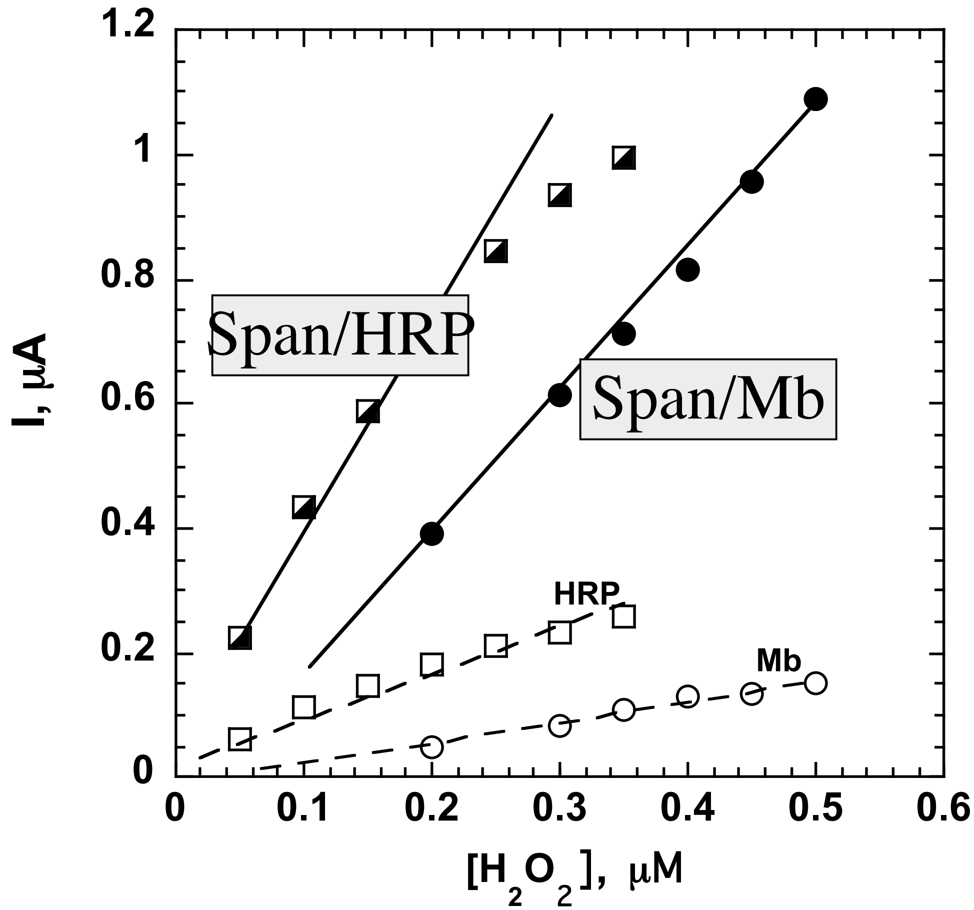
Catalytic reduction of H_2O_2 by peroxidase films
Catalytic cycles increase current



Rotating electrode amperometry at 0 V
HRP, 50 nmol H_2O_2 additions



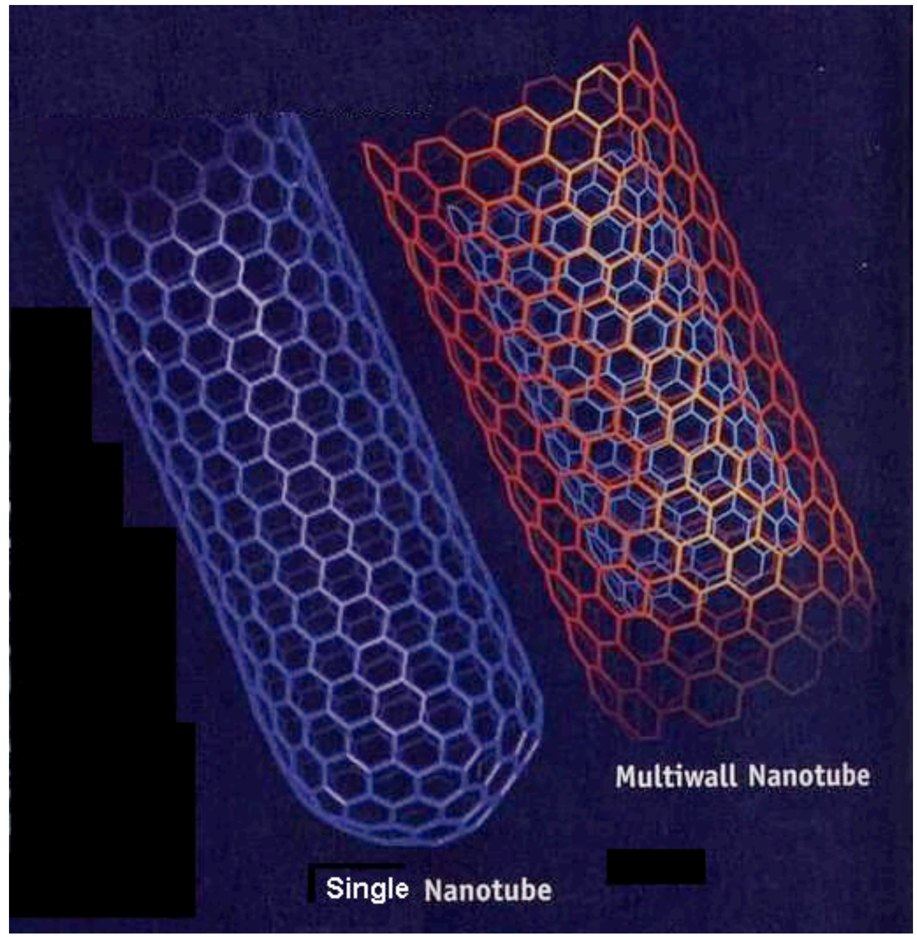
Rotating electrode amperometry at 0 V



Sensitivity much higher with conductive polymer (SPAN); Electrically wires all the protein to electrode

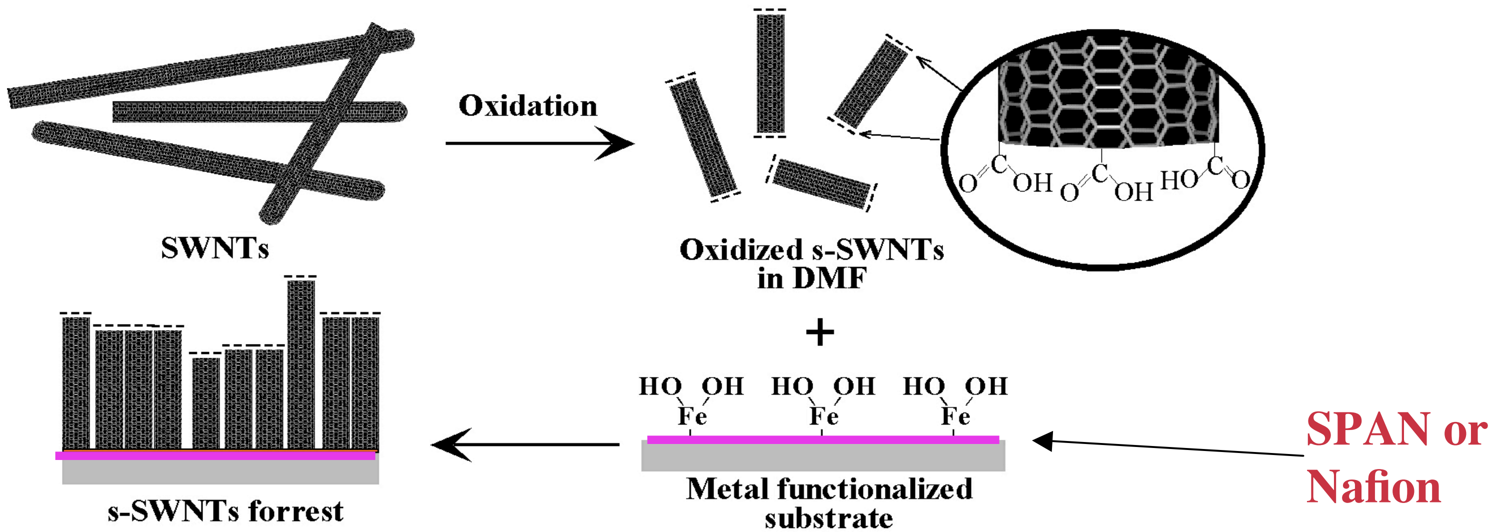
Carbon Nanotubes

- **Single walled (1.4 nm o.d.) and multi-walled**
- **Highly conductive, flexible, strong, patternable**
- **Commercially Available**



Single-Walled Carbon Nanotube Forests: Antigen-Antibody Sensing

~1.4 nm diameter, high conductivity

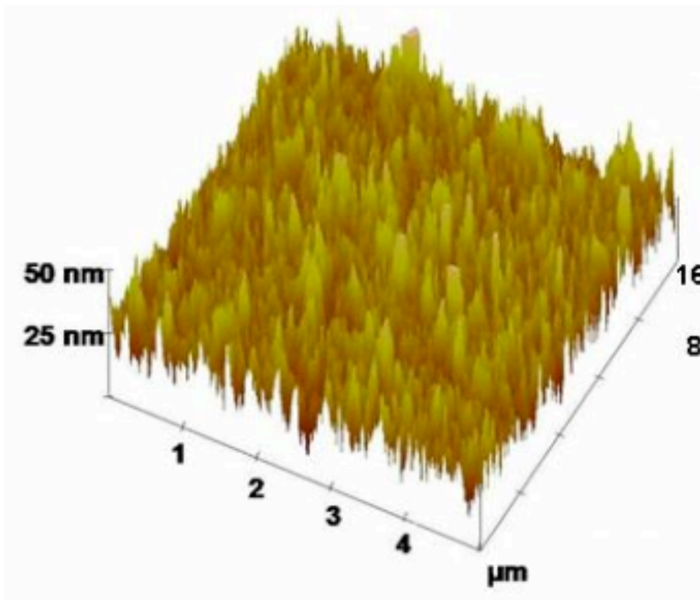


Chattopadhyay, Galeska, <u>Papadimitrakopoulos</u>, *J. Am. Chem. Soc.* 2001, *123*, 9451.

End COOH groups allow chemical attachment to proteins (antibodies)

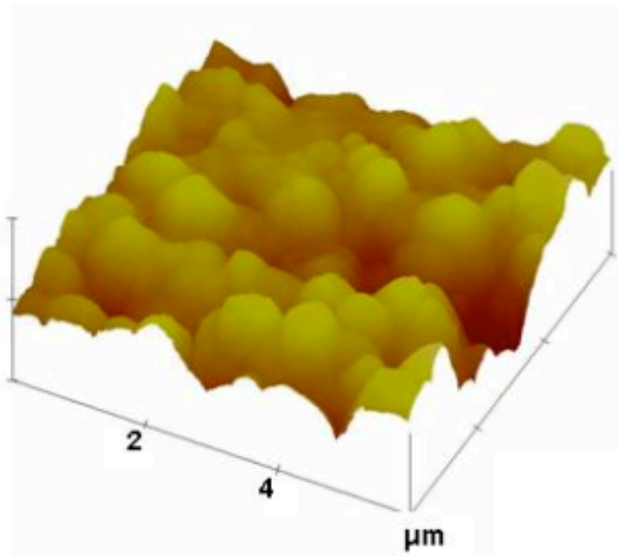
High conductivity to conduct signal (e's) from enzyme label to meas. circuit

AFM of SWNT forest with and without anti-HSA attached



(a)

SWNT forest on Si wafer

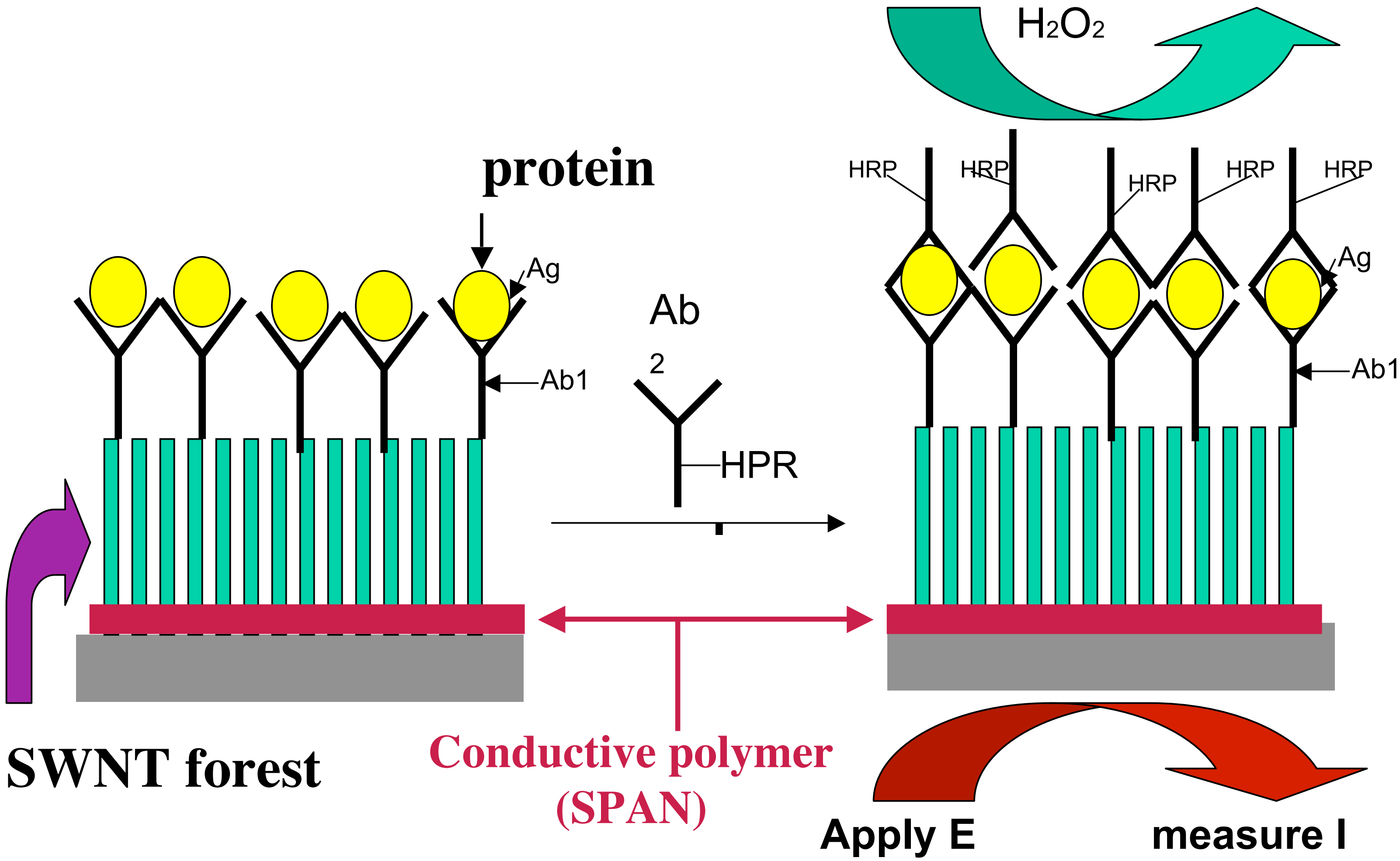


(b)

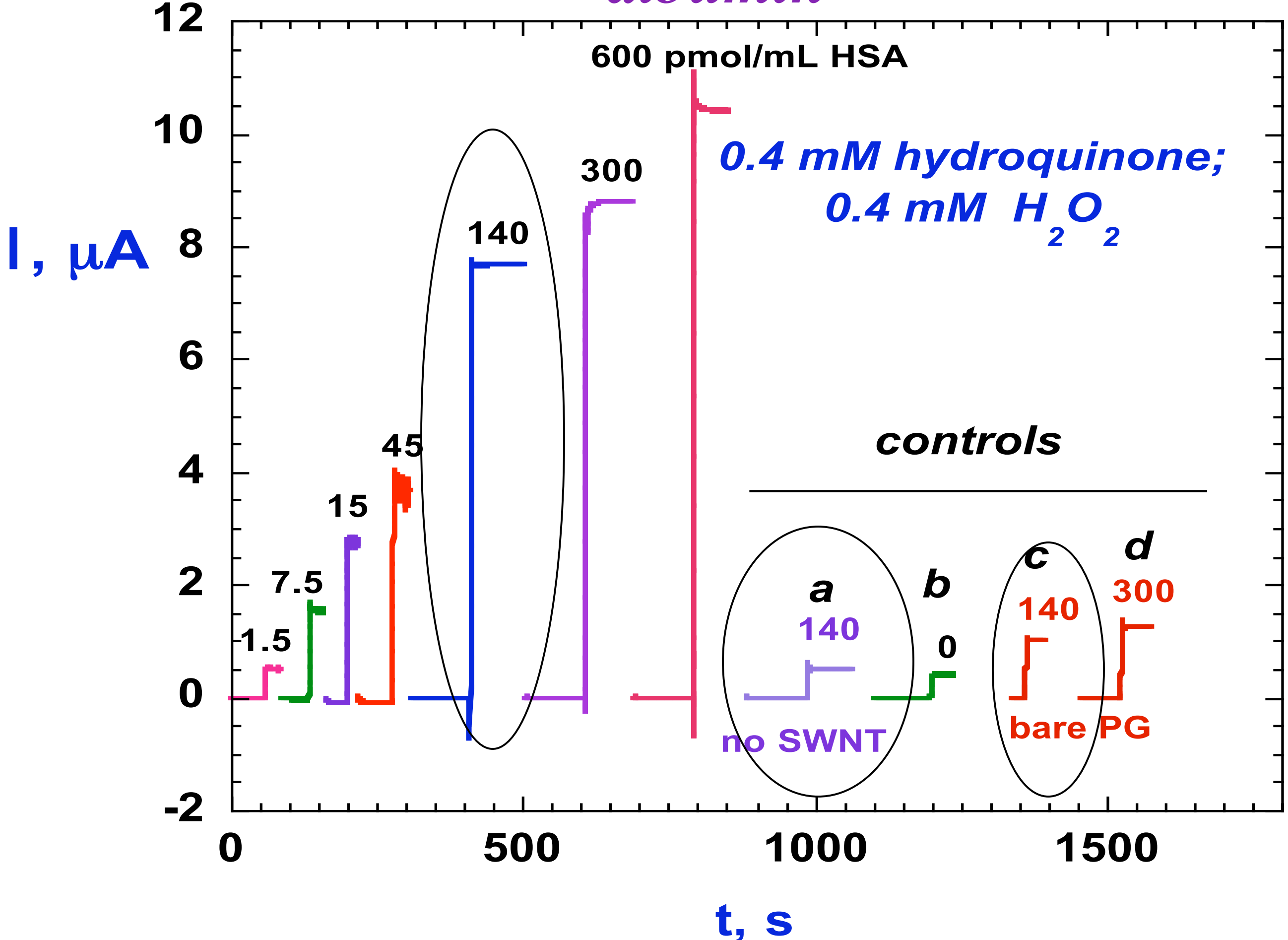
SWNT forest with anti-human serum albumin (HSA) attached by amide links

- **Also linked enzymes to SWNT forests**: X. Yu, D. Chattopadhyay, I. Galeska, F. Papadimitrakopoulos, and J. F. Rusling, "Peroxidase activity of enzymes bound to the ends of single-wall carbon nanotubeforest electrodes", *Electrochem. Commun.*, 2003, *5*, 408-411.

Sandwich Electrochemical Immunosensor Proteins



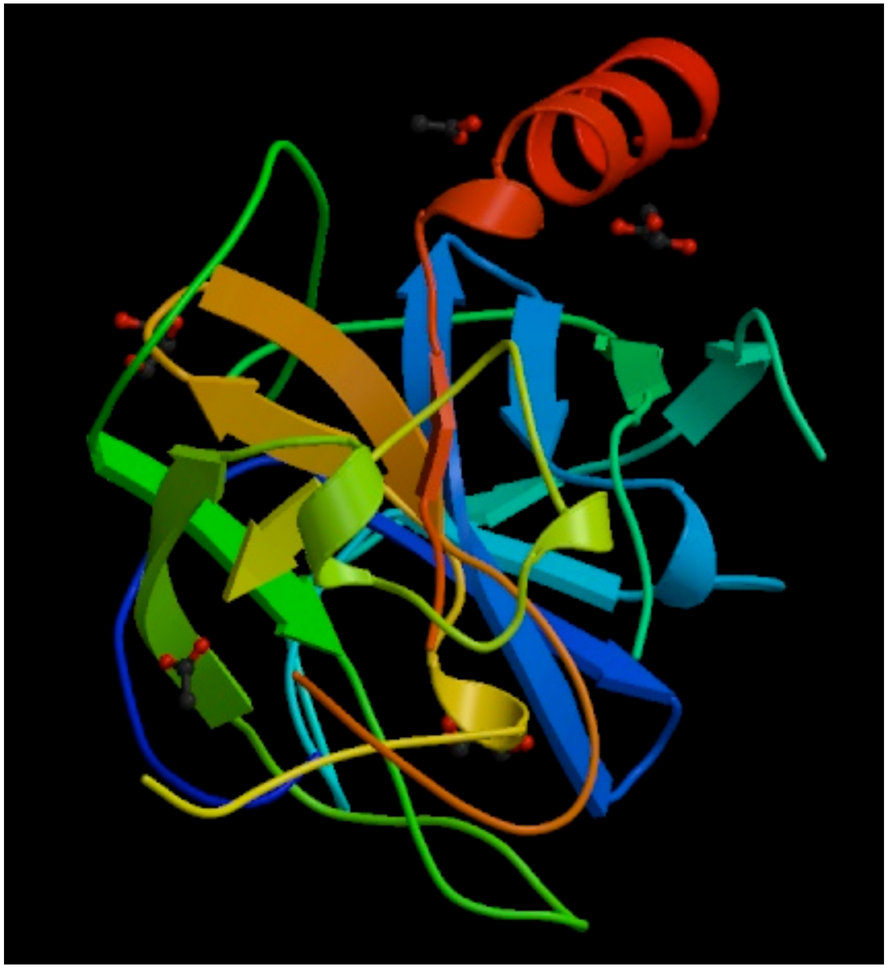
Amperometry Detection of Human Serum albumin



- SWNTs provide 10-20 fold signal enhancement
- Nanotubes aged in DMF ☛ fewer defects ☛ denser forests

. Xin Yu, Sang Nyon Kim, Fotios Papadimitrakopoulos and James F. Rusling, "Protein Immunosensor Using Single-Wall Carbon Nanotube Forests with Electrochemical Detection of Enzyme Labels", *Molecular Biosystems*, **2005**, *1*, 70-78.

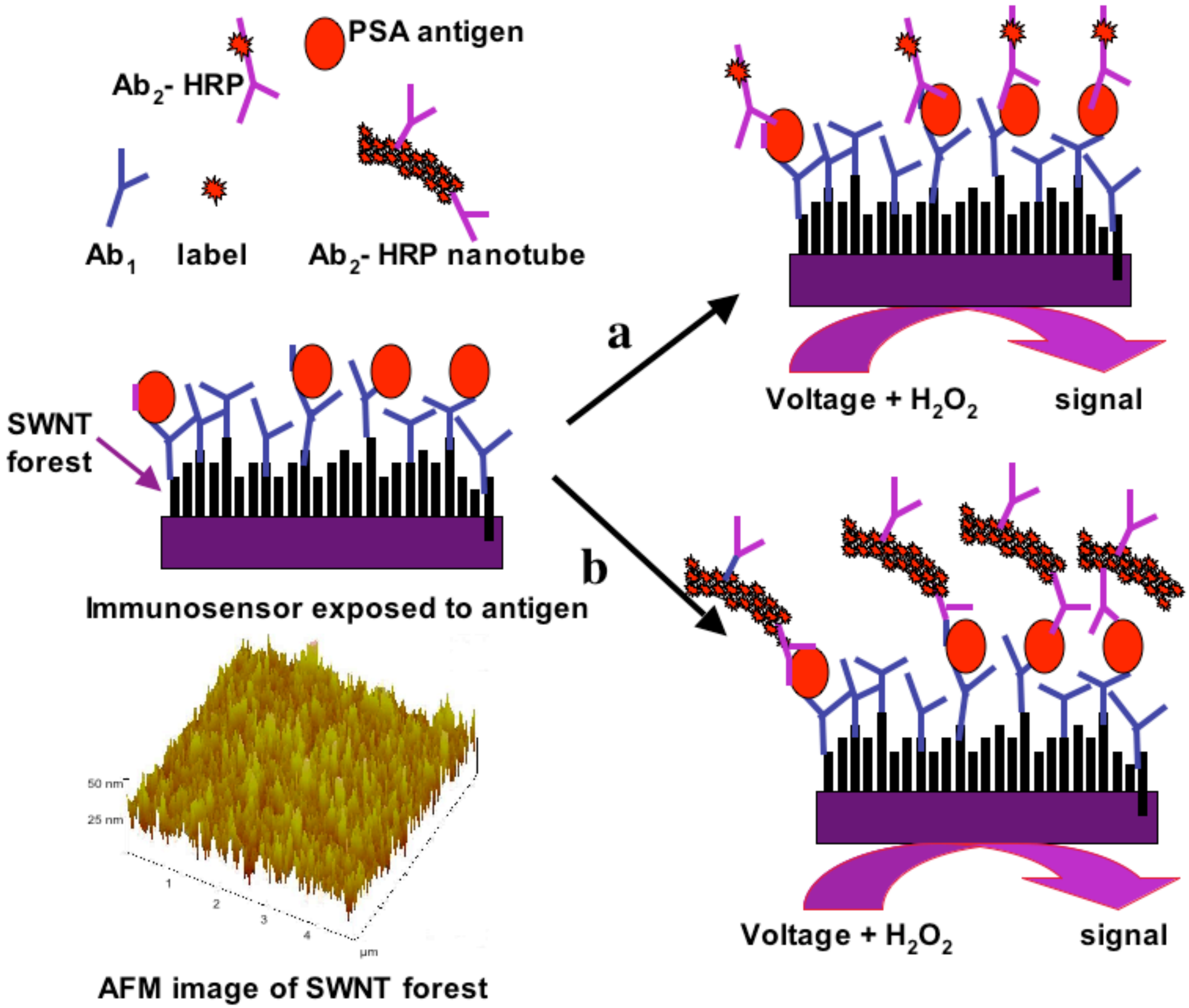
Initial Target: Prostate Specific Antigen



Adapted From Brookhaven Protein Databank

- PSA - Single chain glycoprotein , MW 33 kDa
- Sensitive, specific biomarker for detection of prostate cancer up to 5 years before clinical signs of disease
- Detection of PSA in serum: clinical method for detection of prostate cancer
- Led to less invasive treatment protocols, avoid surgery

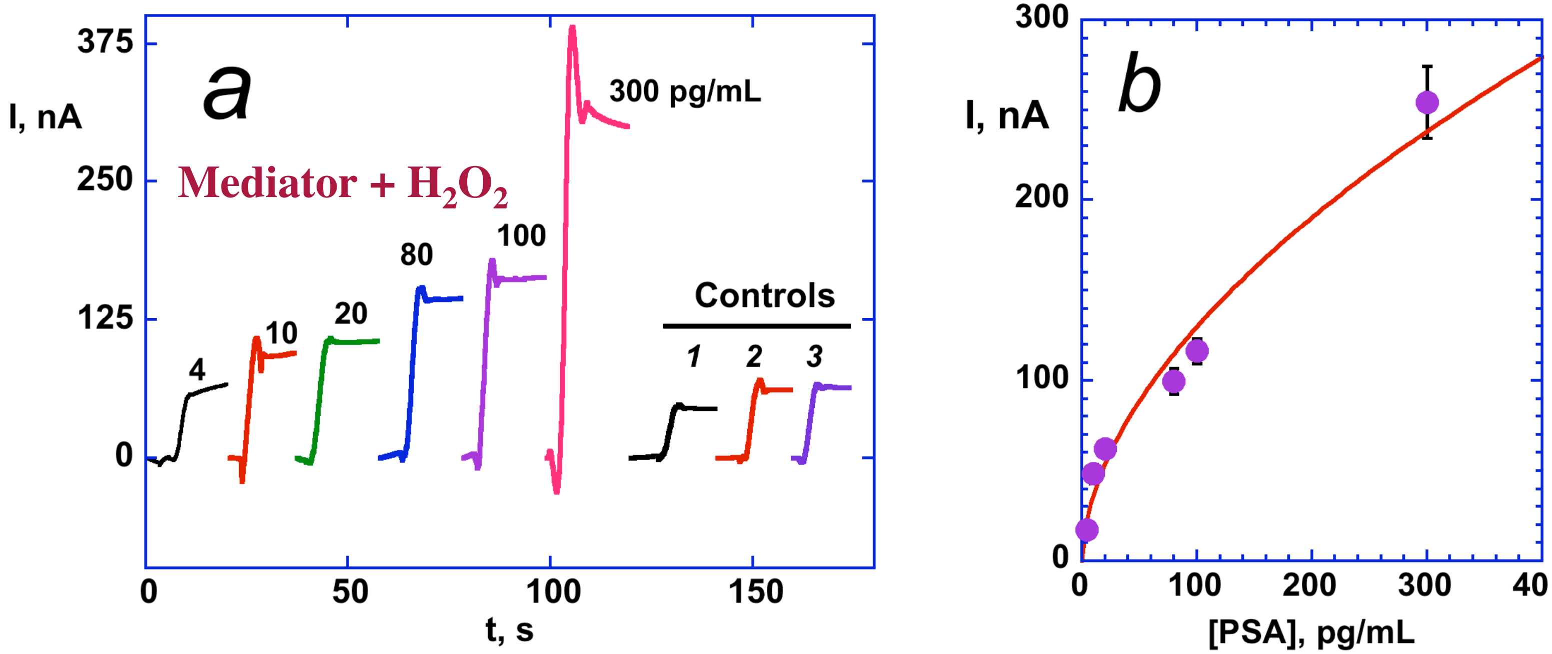
Nanotube Strategies for PSA detection



~170 labels per PSA

Using HRP-Ab_2-nanotube

Amperometric response at –0.3 V and 3000 rpm for SWNT immunosensors incubated with PSA in 10 µL undiluted newborn calf serum for 1.25 hr using the Ab_2-CNT-HRP bioconjugate



Washing with 2% BSA/0.05% Tween 20 to control non-specific binding

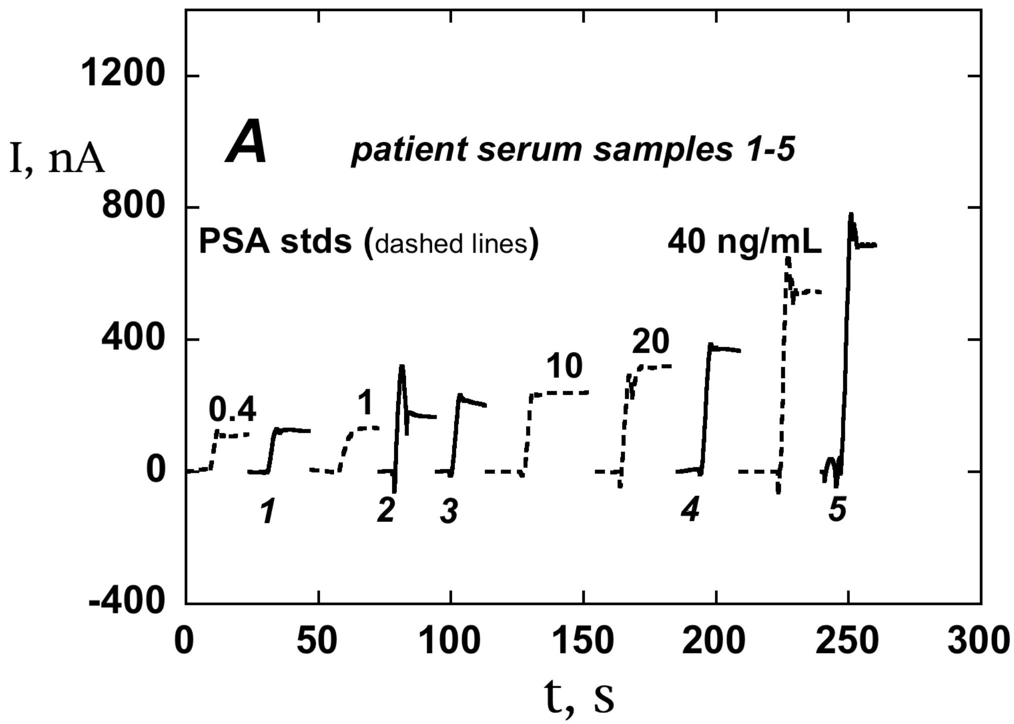
<u>LOD - 4 pg/mL</u>; 100-fold enhancement over HRP-Ab_2

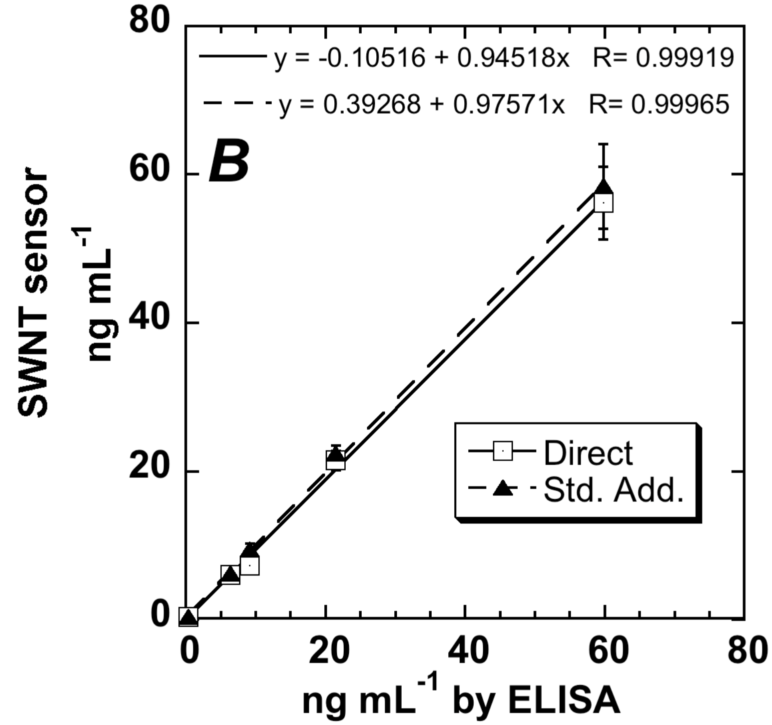
Xin Yu, Bernard Munge, Vyomesh Patel, Gary Jensen, Ashwin Bhirde, Joseph D. Gong, Sang-Nyon Kim, John Gillespie, J. Silvio Gutkind, Fotios Papadimitrakopoulos and James F. Rusling, "Carbon Nanotube Amplification Strategies for Highly Sensitive Immunosensing of Cancer Biomarkers in Serum and Tissue", *J. Am. Chem. Soc.*, **2006**, *128,* 11199-11205.

Accurate results obtained for cancer patient serum

Amperometric current at –0.3 V and 3000 rpm for human serum samples and PSA standards in calf serum

Using conventional HRP-Ab$_2$

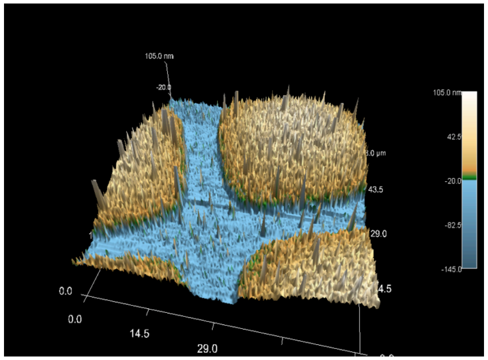




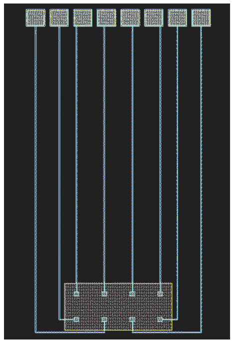
Good correlation with ELISA!

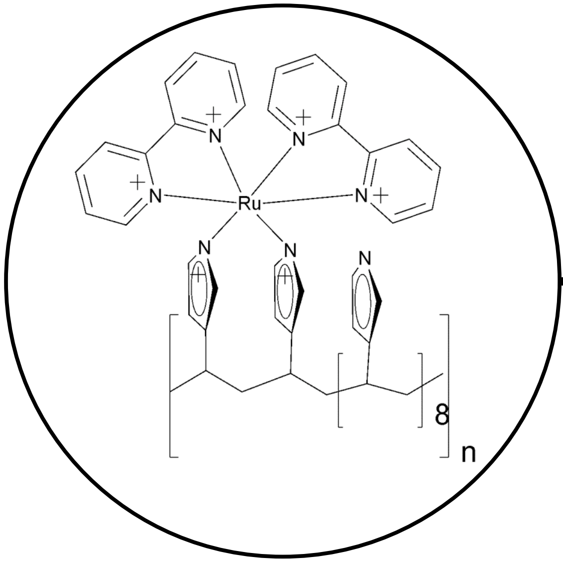
Future - arrays to detect many biomolecules at once

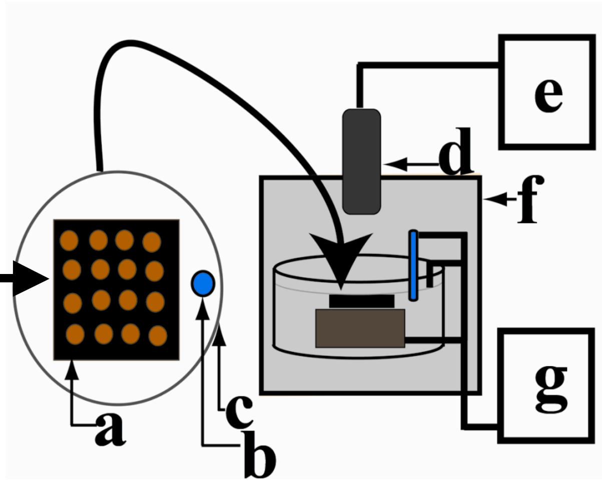
SWNT forest grown on 10 μm Au Array elements



Prototype 8-electrode Array, Univ. Edinburgh







Biosensors

- **Promising approach to medical diagnostics by patients or in doctors offices**
- **Other important applications: cancer biomarkers, DNA, peroxide, etc.**
- **Method of choice for blood glucose in diabetics**
- **Rapid diagnostics may lead to more timely and effective treatment**