BIOSENSORS

Modern and future approaches to medical diagnostics

J. F. Rusling

Dept. of Chemistry

Dept. of Pharmacology, Univ. of CT Health Center

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

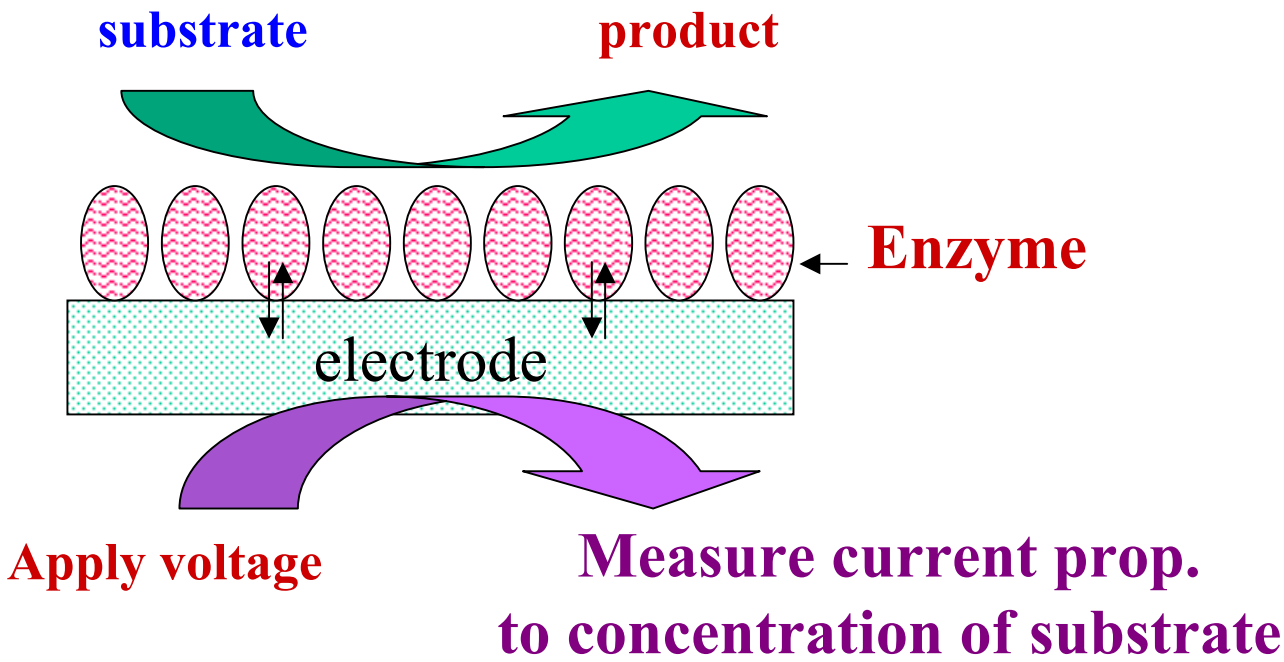
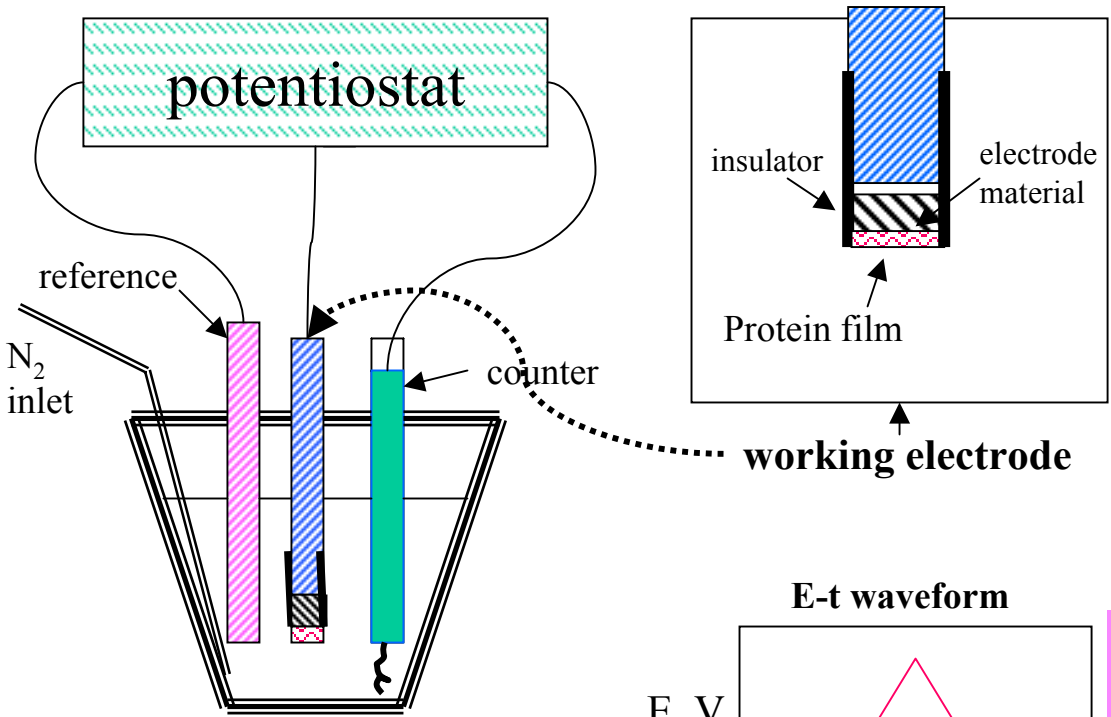
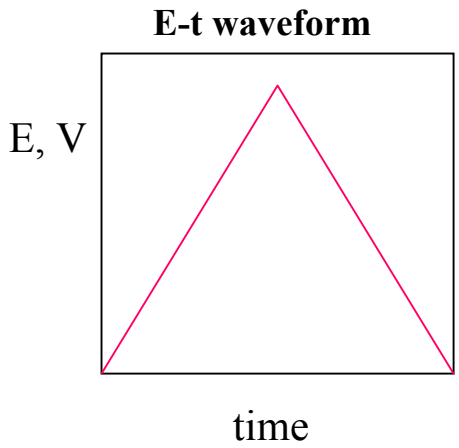


Figure 9

Equipment for developing electrochemical biosensors



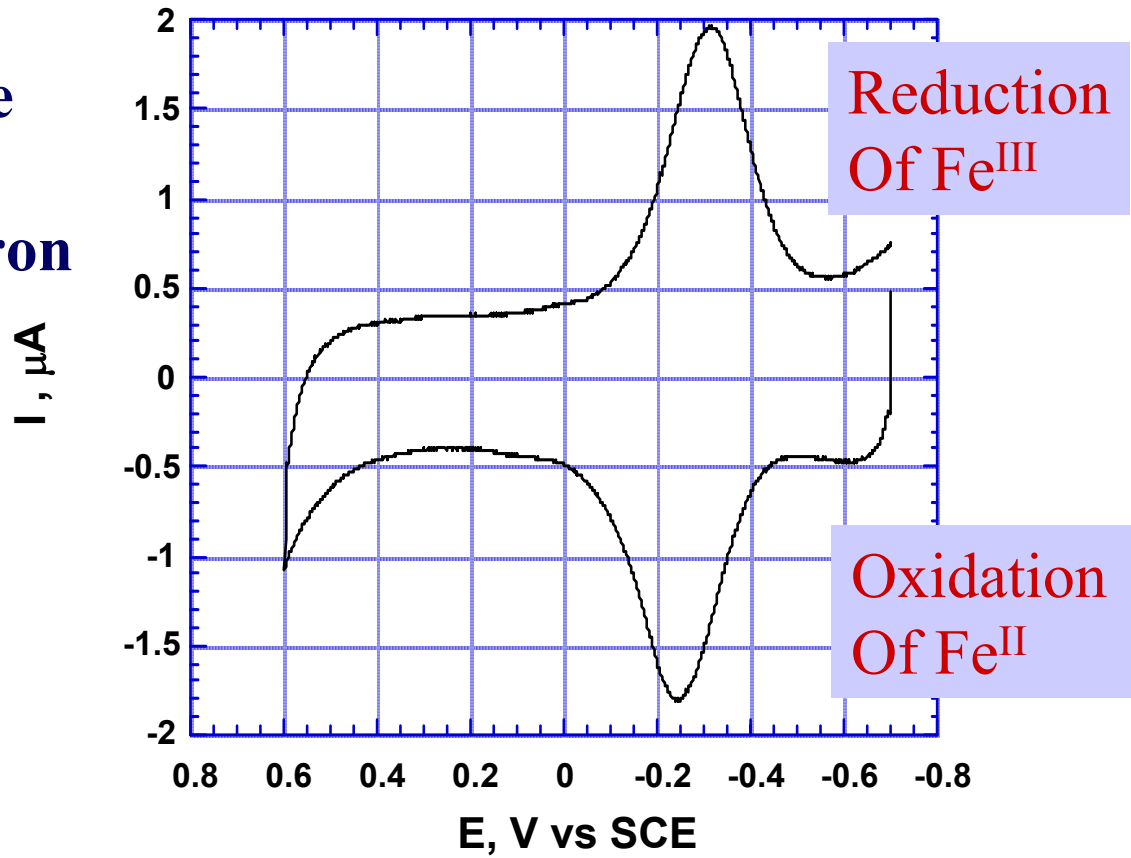
Electrochemical cell



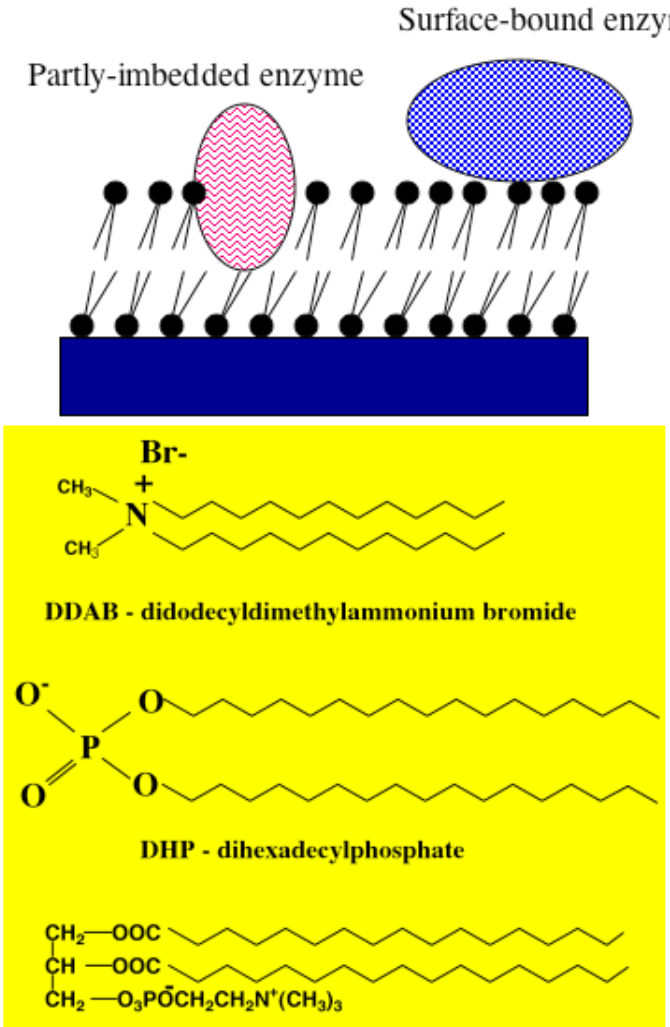
Cyclic voltammetry

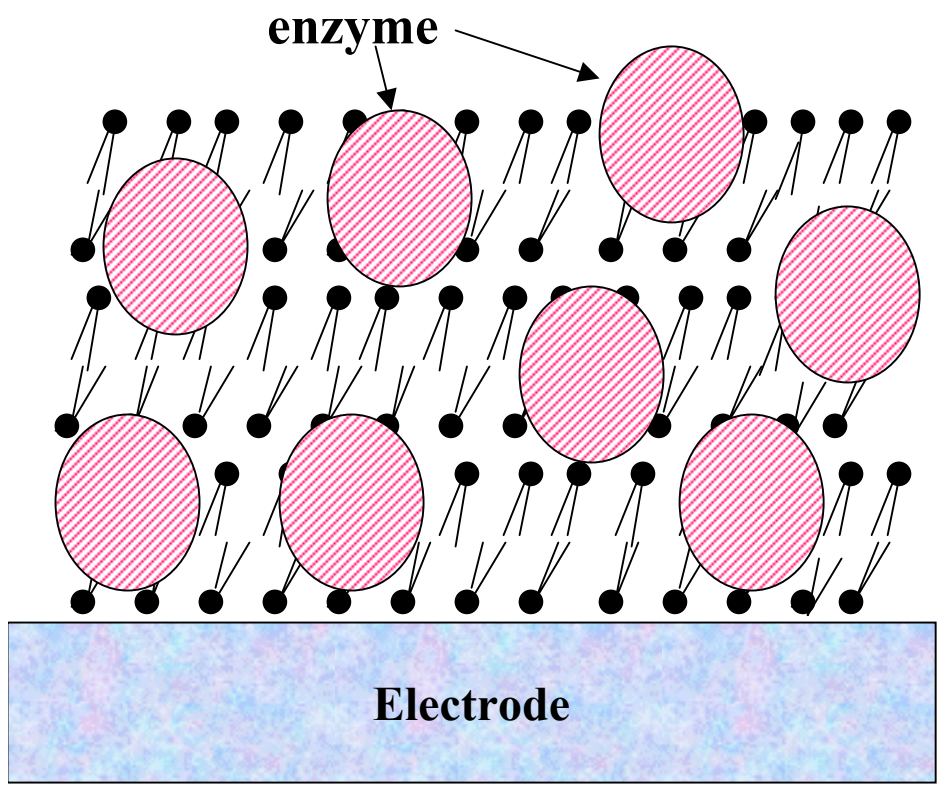
Cyclic voltammogram (CV) at 100 mVs^{-1} and 25 °C of *Mycobacterium Tuberculosis* KatG catalase-peroxidase in thin film of dimyristoylphosphatidylcholine on a pyrolytic graphite electrode in anaerobic pH 6.0 buffer.

Reversible Peaks for Direct electron transfer

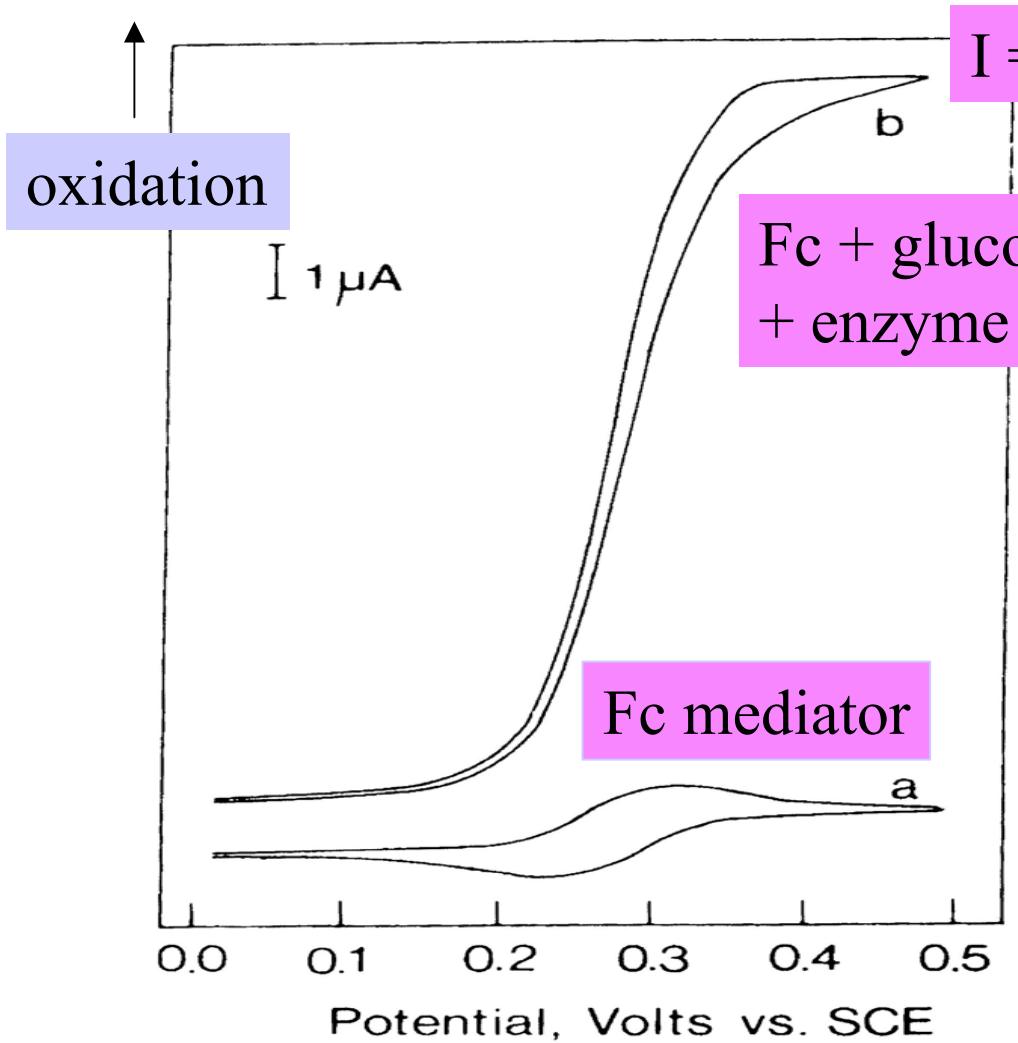


A lipid-enzyme film





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

$$\text{Glucose} + GO(FAD) \rightarrow \text{gluconolactone} + GO(FADH_2) \quad (1)$$

$$GO(FADH_2) + 2Fc^{+} \rightarrow GO(FAD) + 2Fc + 2H^{+} \quad (4)$$

$$Fc \rightarrow Fc^{+} + 2e^{-} \quad (\text{at electrode}) \quad (5)$$

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 how much insulin to administer**

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

Fc = mediator, ferrocene, an iron complex

These reactions occur in the sensor:

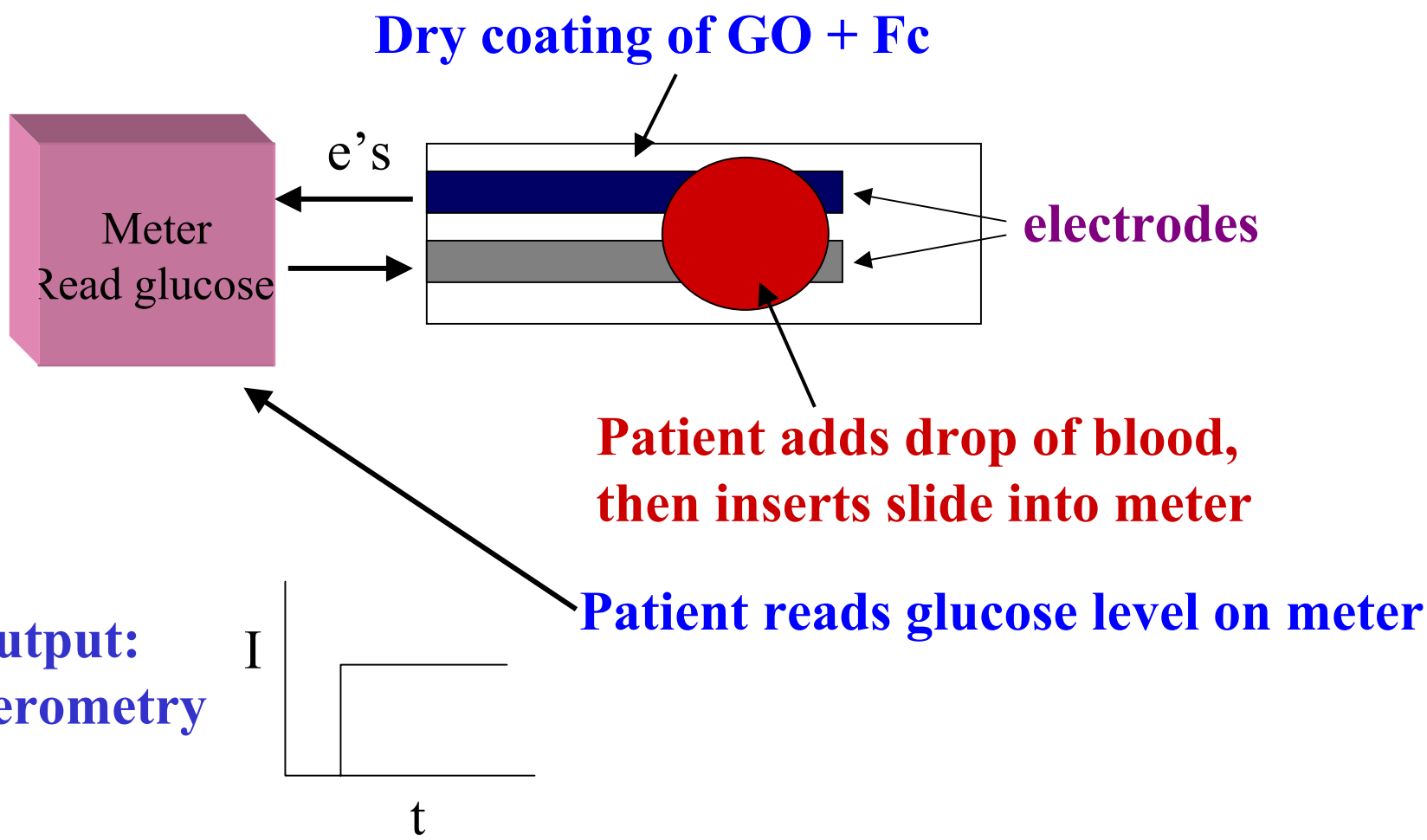
$$Fc \longleftrightarrow Fc^{+} + e^{-} \text{ (measured)}$$

$$GO_R + 2\,Fc^{+} \rightarrow GO_{ox} + 2\,Fc$$

$$GO_{ox} + \text{glucose} \rightarrow GO_R + \text{gluconolactone}$$

Reach and Wilson, *Anal. Chem.* 64, 381A (1992)
G. Ramsay, *Commercial Biosensors,* J. Wiley, 1998.

Glucose biosensor test strips (~$0.40-0.80 ea.)



Research on glucose sensors

- **Non-invasive biosensors - skin, saliva**
- **Implantable glucose sensors to accompany artificial pancreas - feedback control of insulin supply**
- **Record is 3-4 weeks for implantable sensor in humans**

Other biosensors

- **Cholesterol - based on cholesterol oxidase**
- **Antigen-antibody sensors - toxic substances, pathogenic bacteria**
- **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:

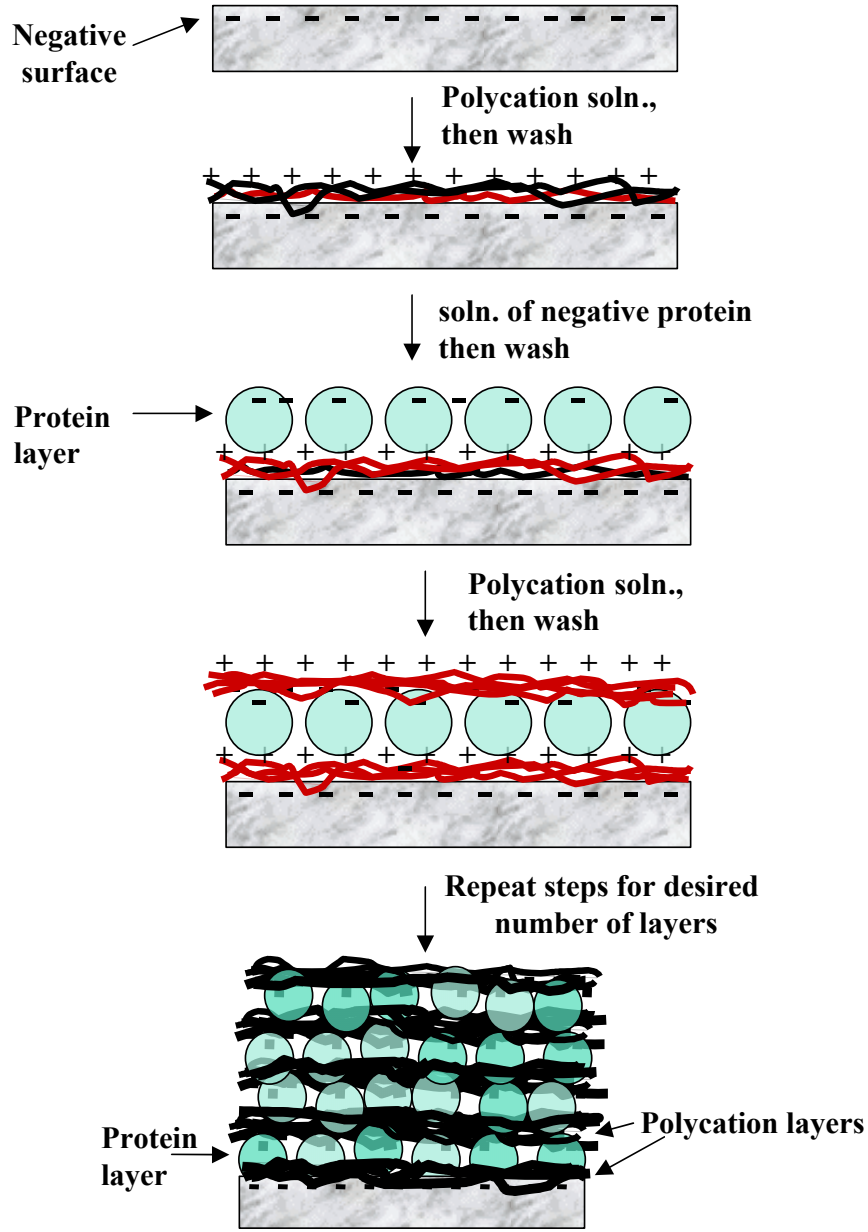
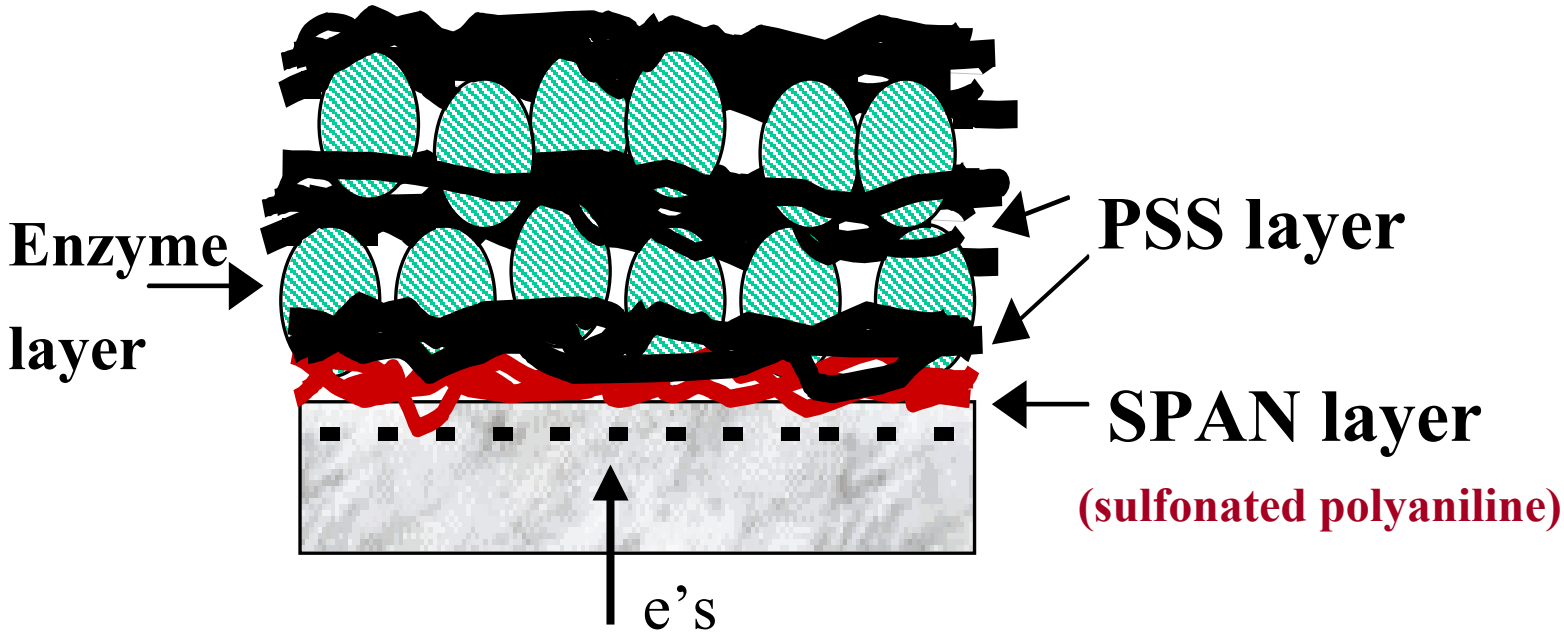


Figure 19

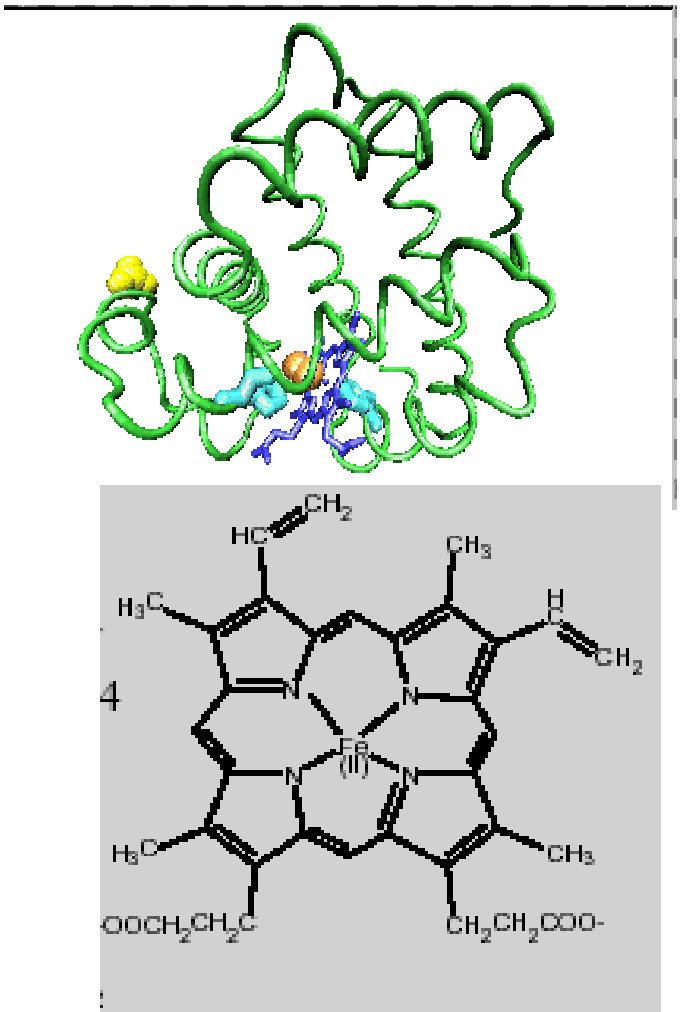
Detection of hydrogen peroxide

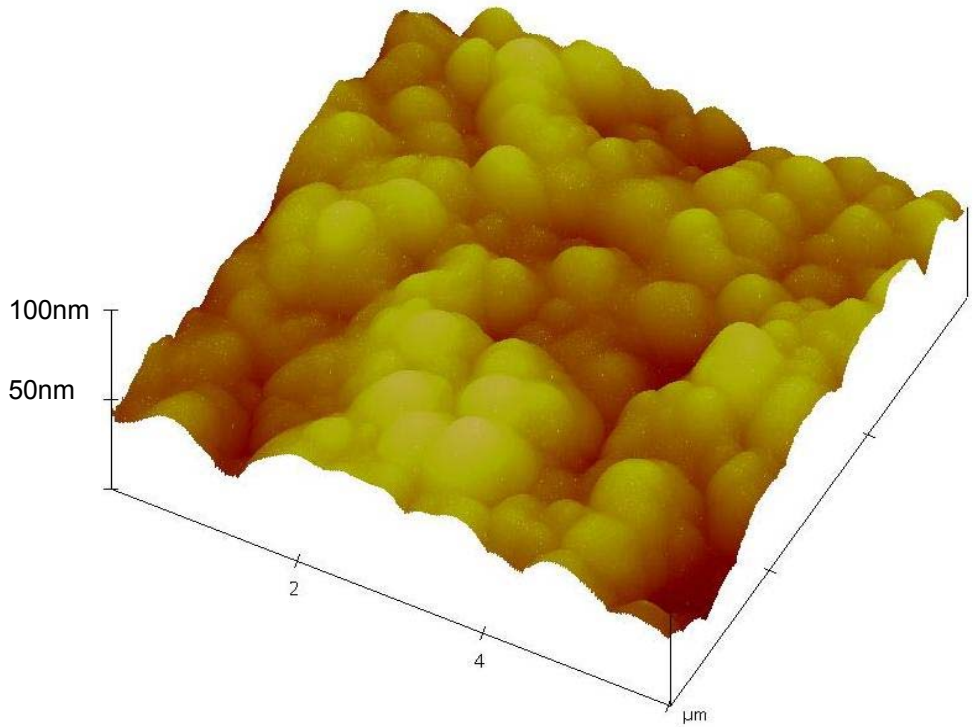
Conductive polymers efficiently wire peroxidase enzymes to graphite



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.

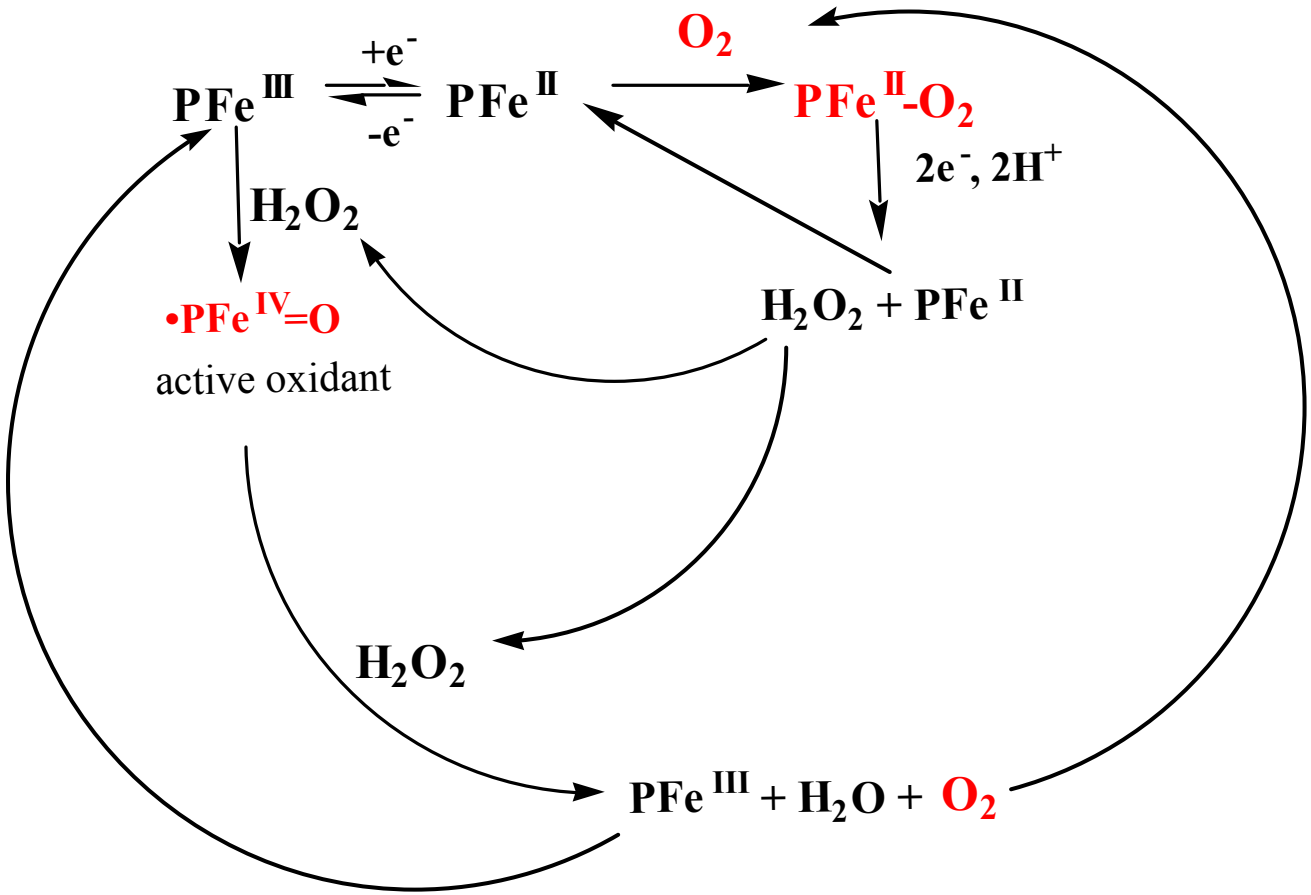
Horseradish Peroxidase (HRP)





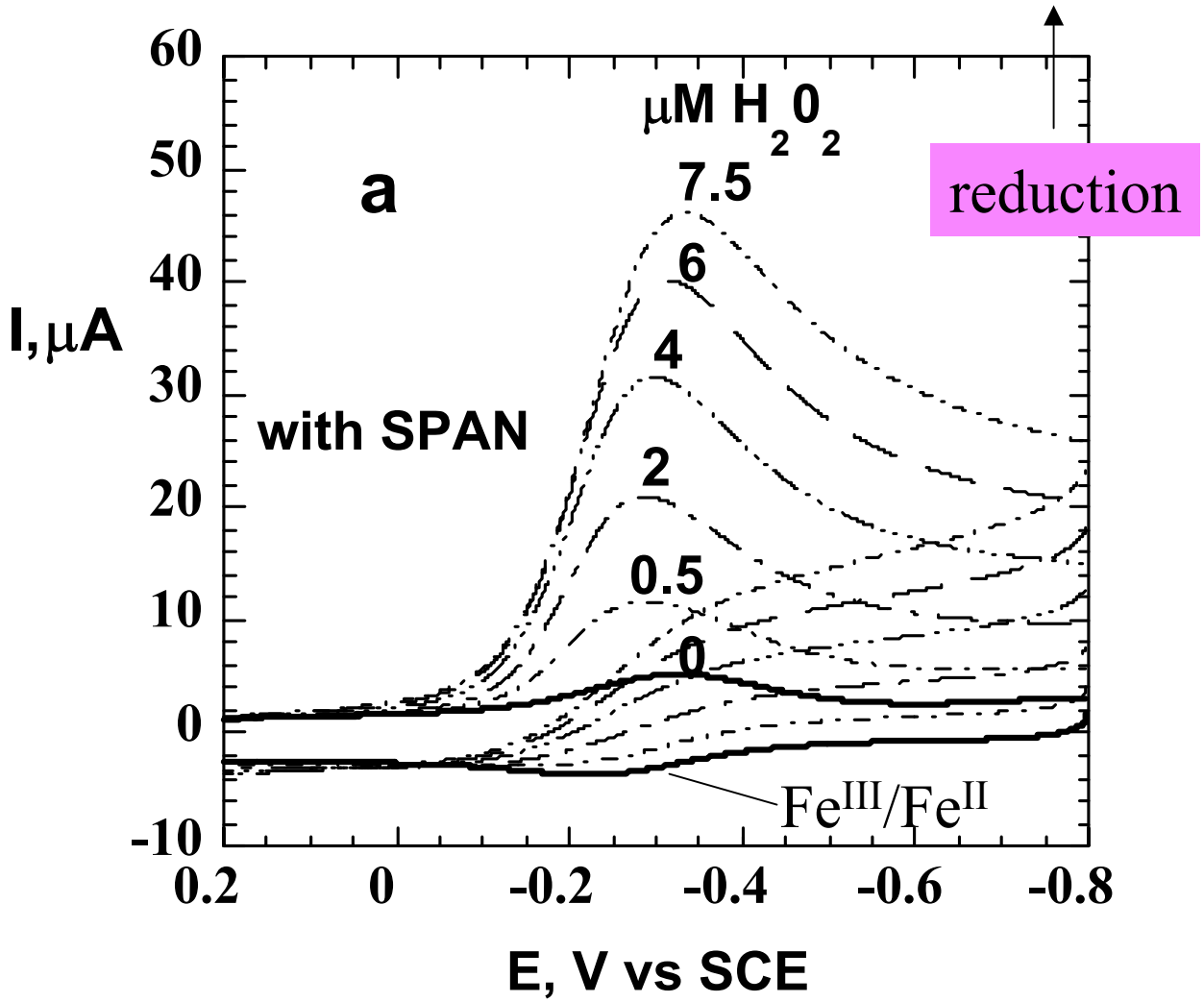
Tapping mode atomic force microscopy (AFM) image of HRP film

Electrochemical Response of Peroxidases

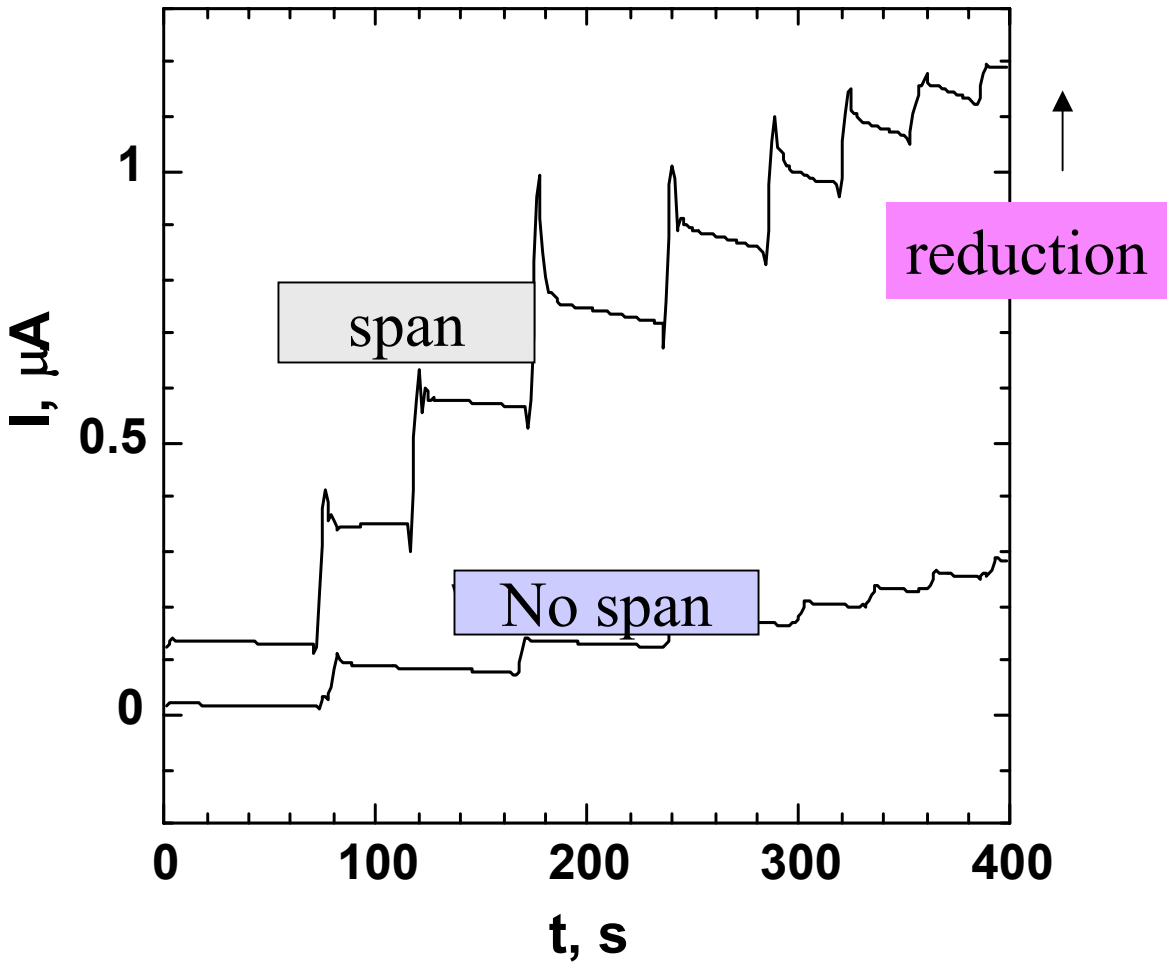


Possible reduced species in red

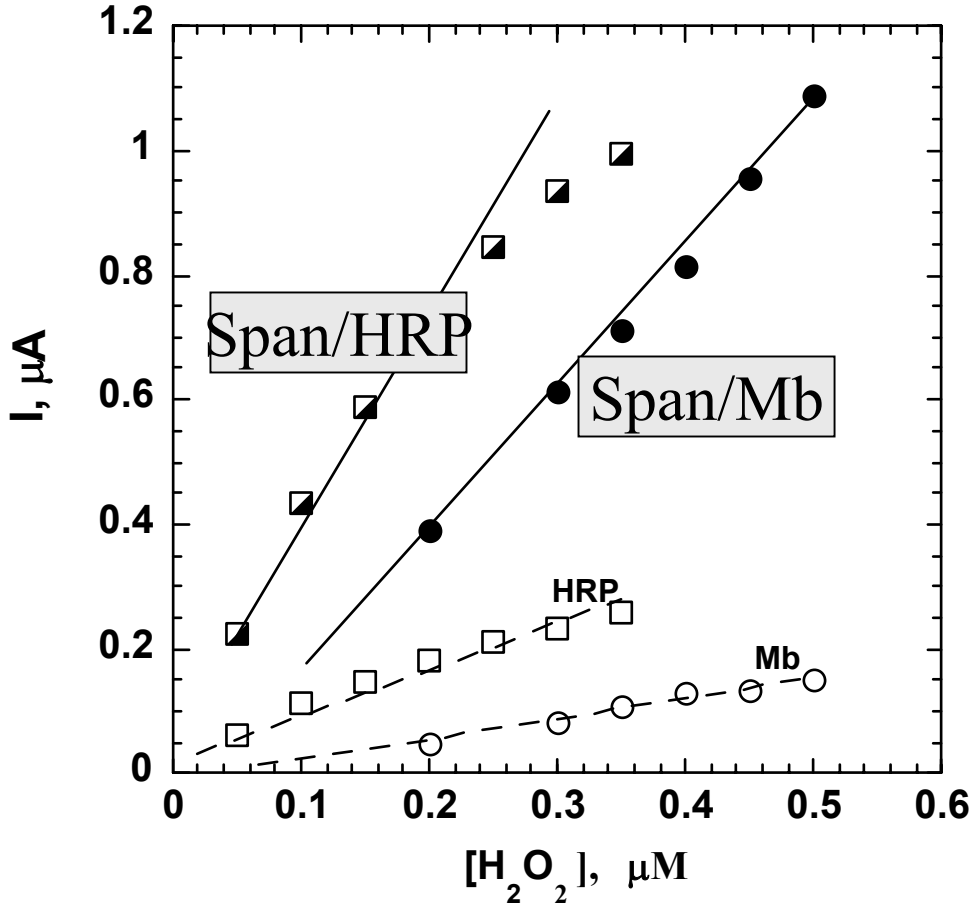
Catalytic reduction of H_2O_2 by peroxidase films
Catalytic cycles increase current



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

Biosensors

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