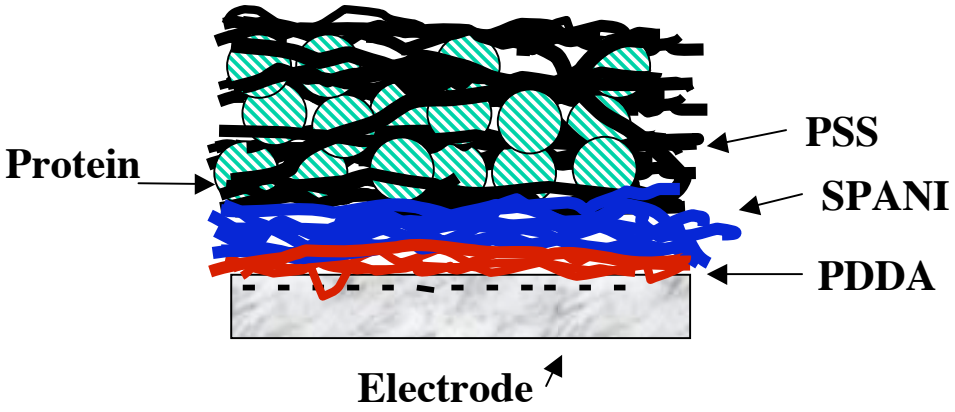
Thin film Protein Voltammetry

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

Film preparation

Layer-by-layer methods: versatile



Rusling and Lvov: peroxidases;
cyt P450s, myglobin

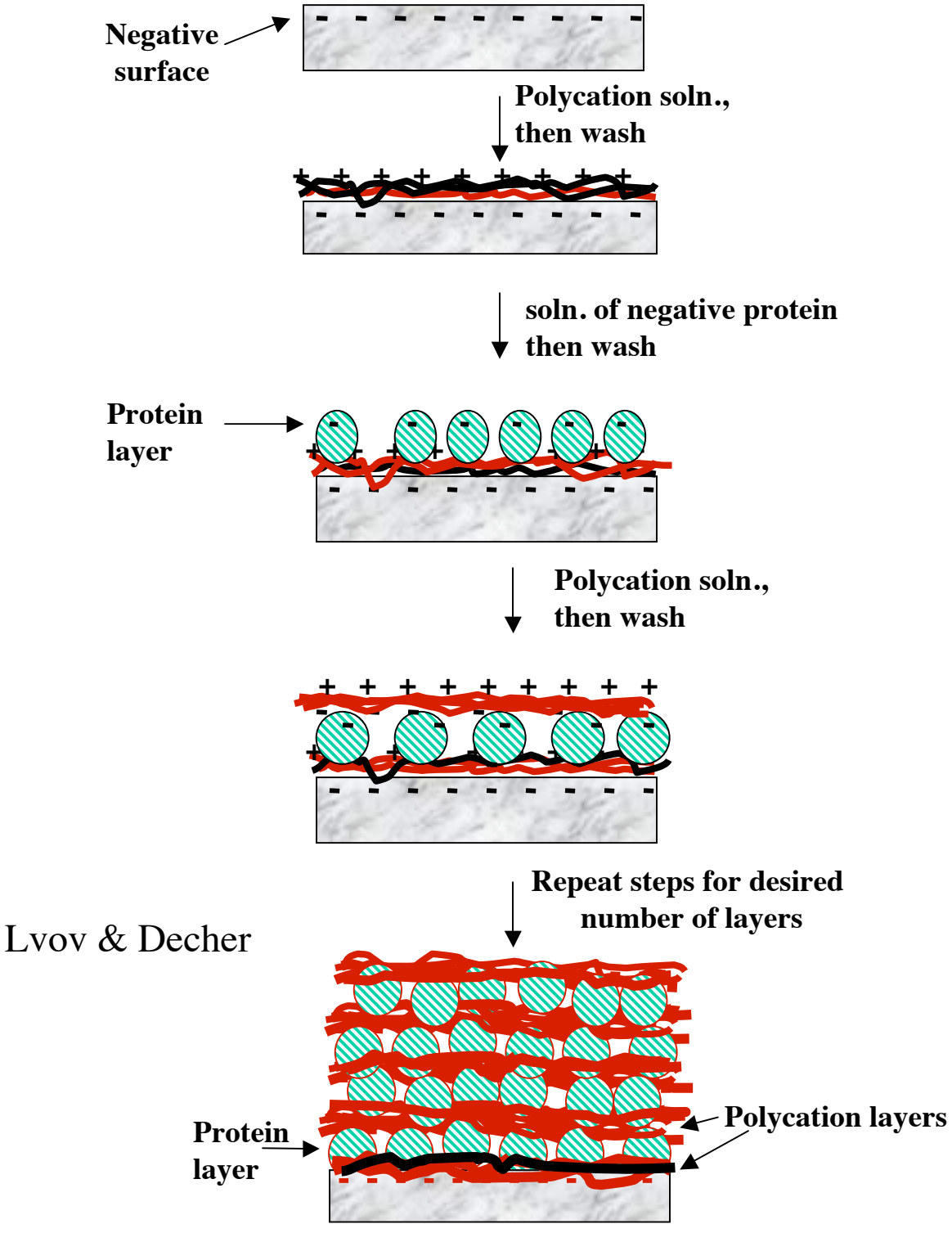
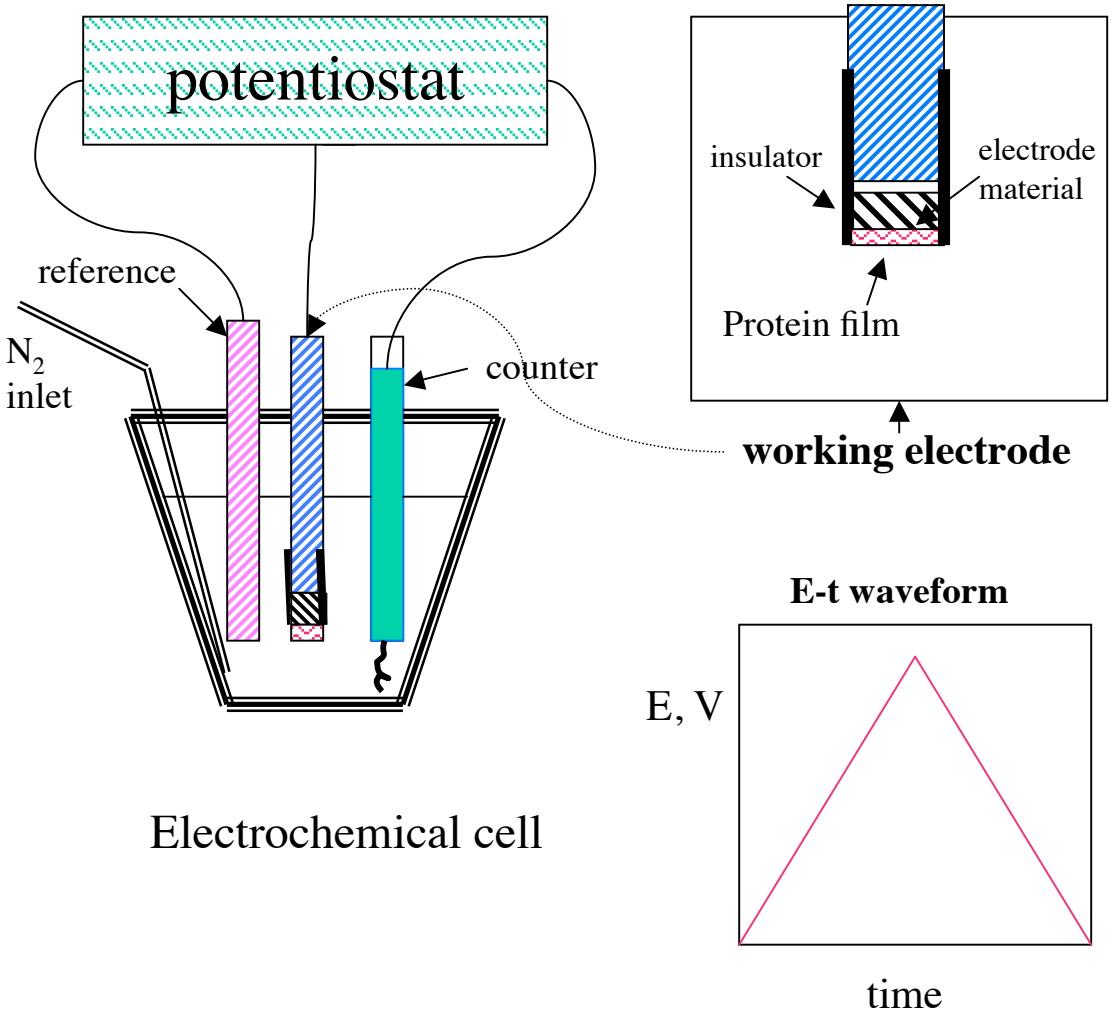

Negative surface
Polycation soln., then wash
soln. of negative protein then wash
Protein layer
Polycation soln., then wash
Repeat steps for desired number of layers
Lvov & Decher
Protein layer
Polycation layers

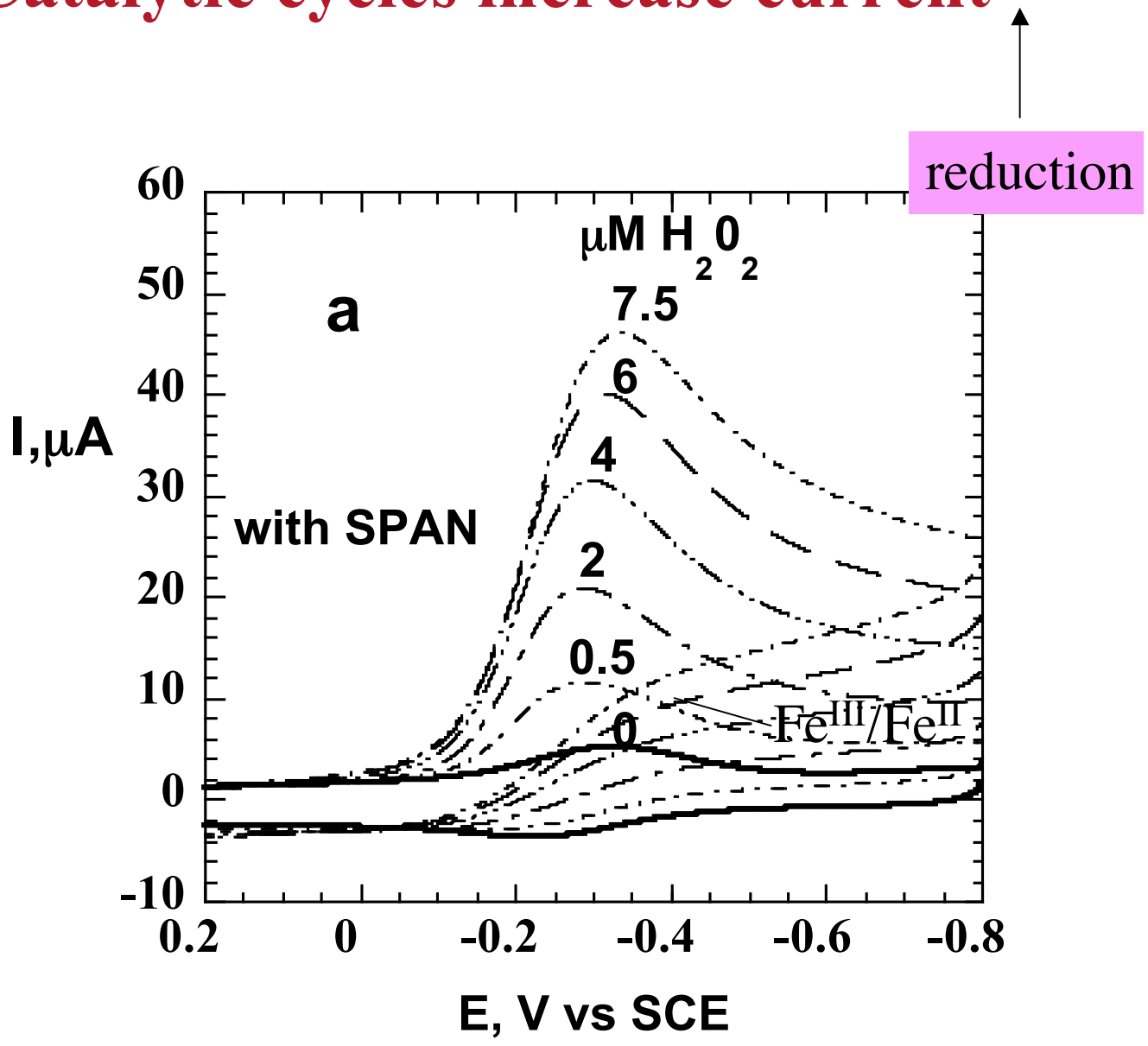
Figure 19



Electrochemical cell

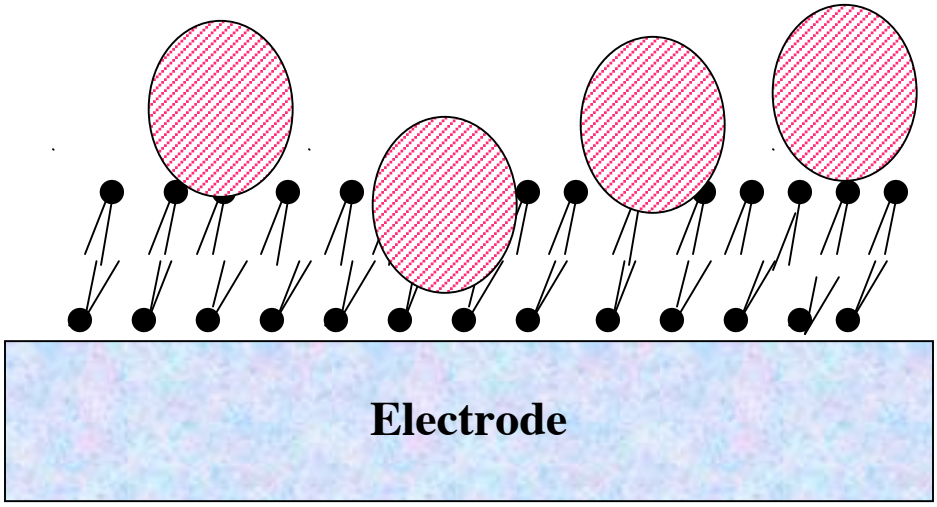
Figure1

Catalytic reduction of H_2O_2 by peroxidase films
Catalytic cycles increase current



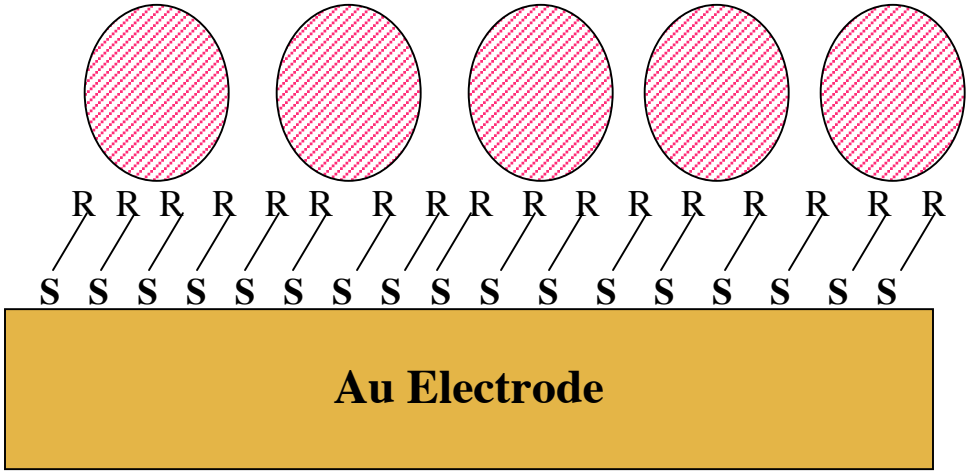
Other protein film methods:

Single bilayer on a surface



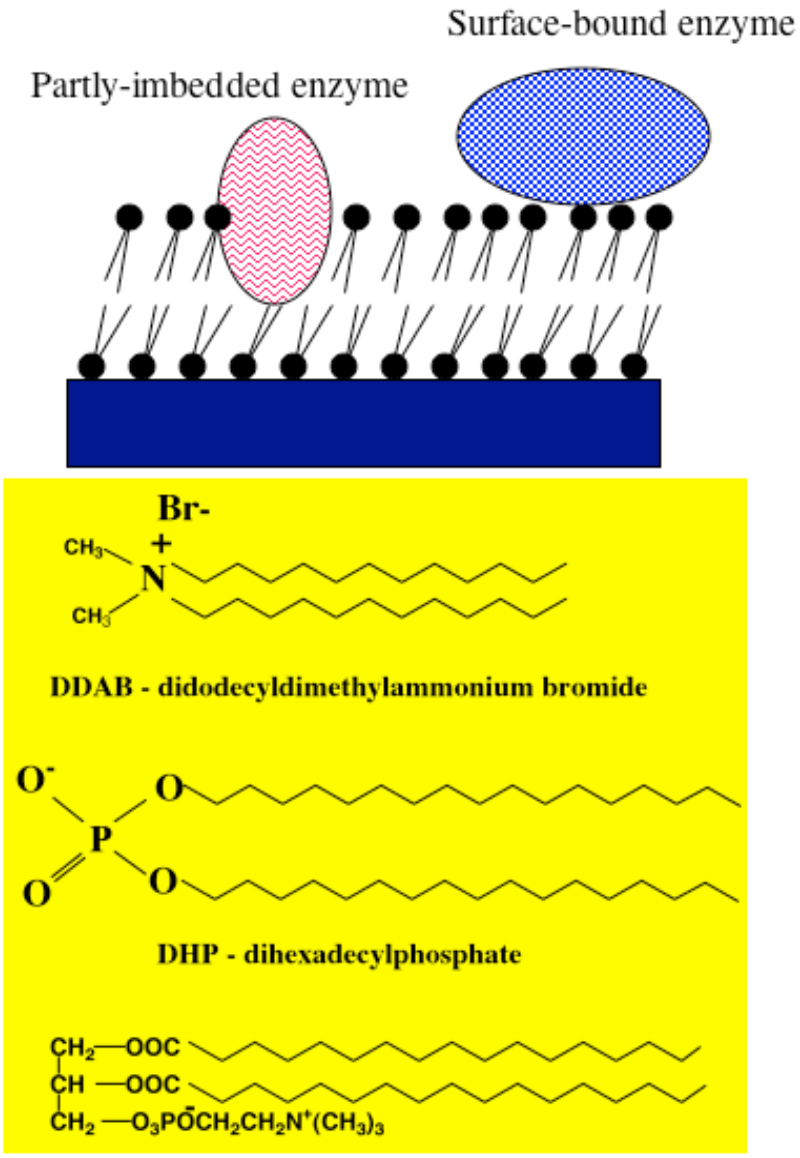
Hawkridge, Burgess…
Cyt c oxidase

SAM - self assembled monolayer

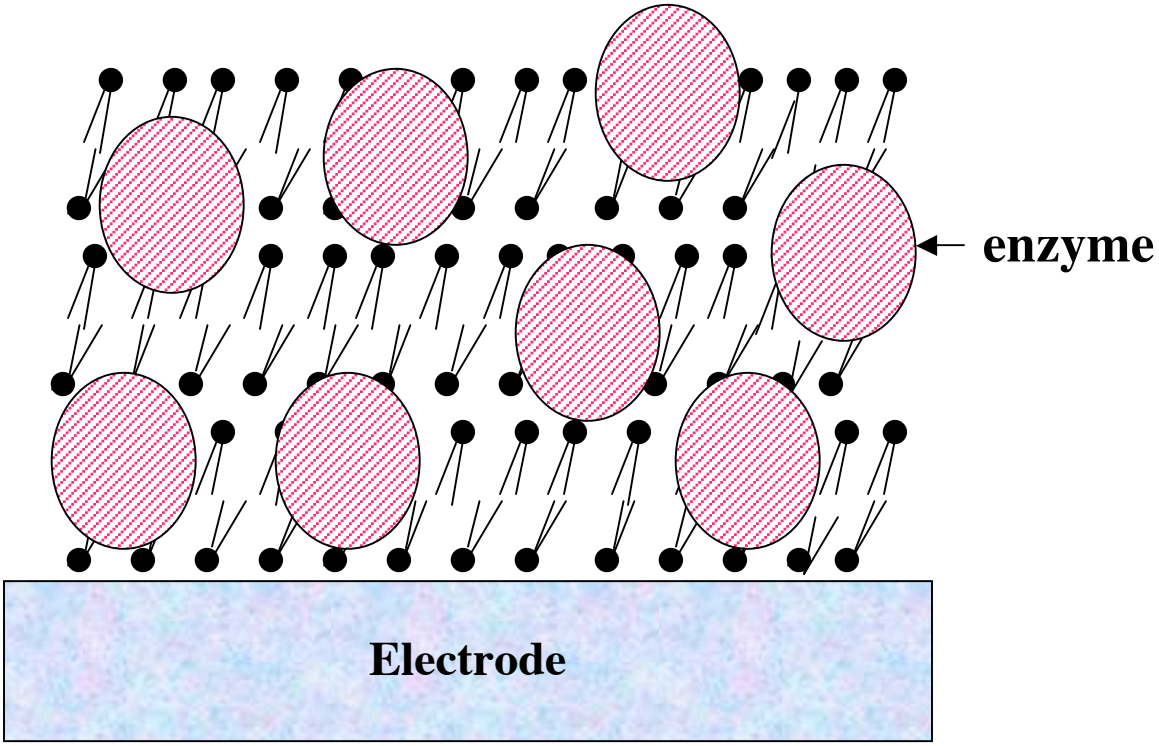


Example: R = COO-; protein = cyt c (+17)

Bowden, Niki - confirmed Marcus theory

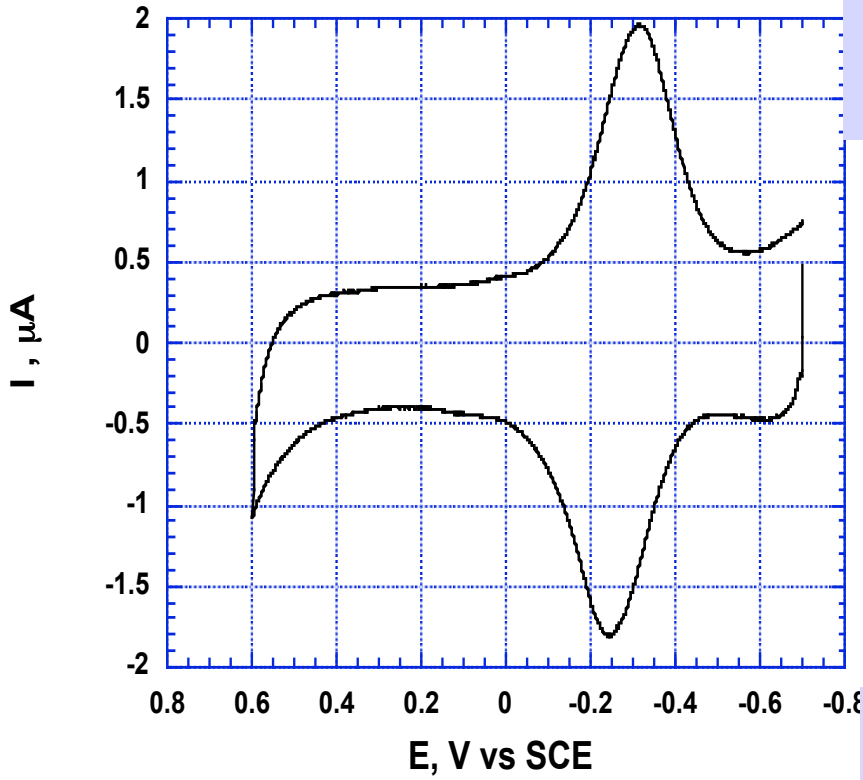


Dihexadecylphosphatidyl choline



Lipid films: Rusling, et al.
Myoglobin, peroxidases,
plant reaction center proteins - light harvesting

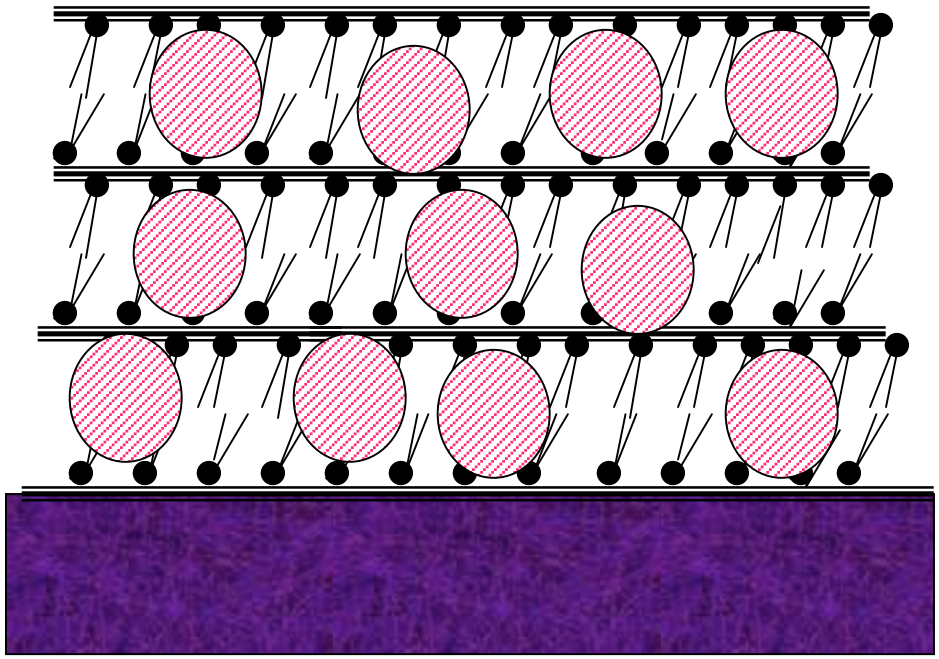
Cyclic voltammogram (CV) at 100 mV s^{-1} and 25 °C of *Mycobacterium Tuberculosis* KatG catalase-peroxidase in a thin film of dimyristoylphosphatidylcholine on basal plane PG electrode, in anaerobic pH 6.0 buffer.



Reduction Of Fe^{III}

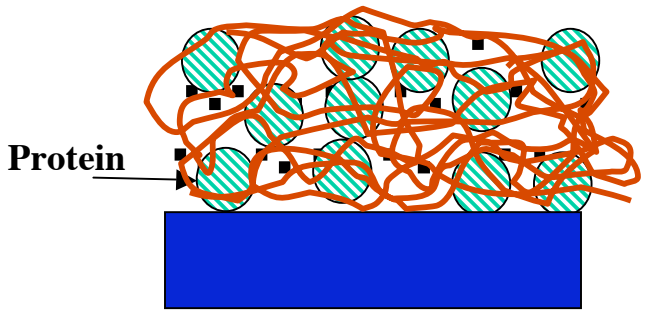
Oxidation Of Fe^{II}

Reversible Peaks for Direct electron Transfer



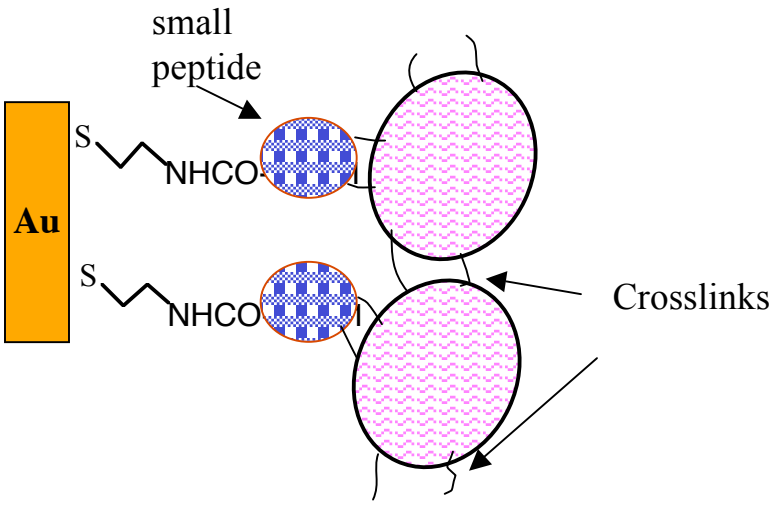
Lipid films with polyions as counterions: DDA+ and PSS

Hu, et al (Beijing)



Cast films of polyions and proteins; typically
Not very stable - leak!

Electrical “wiring” using covalent links
Willner and Katz



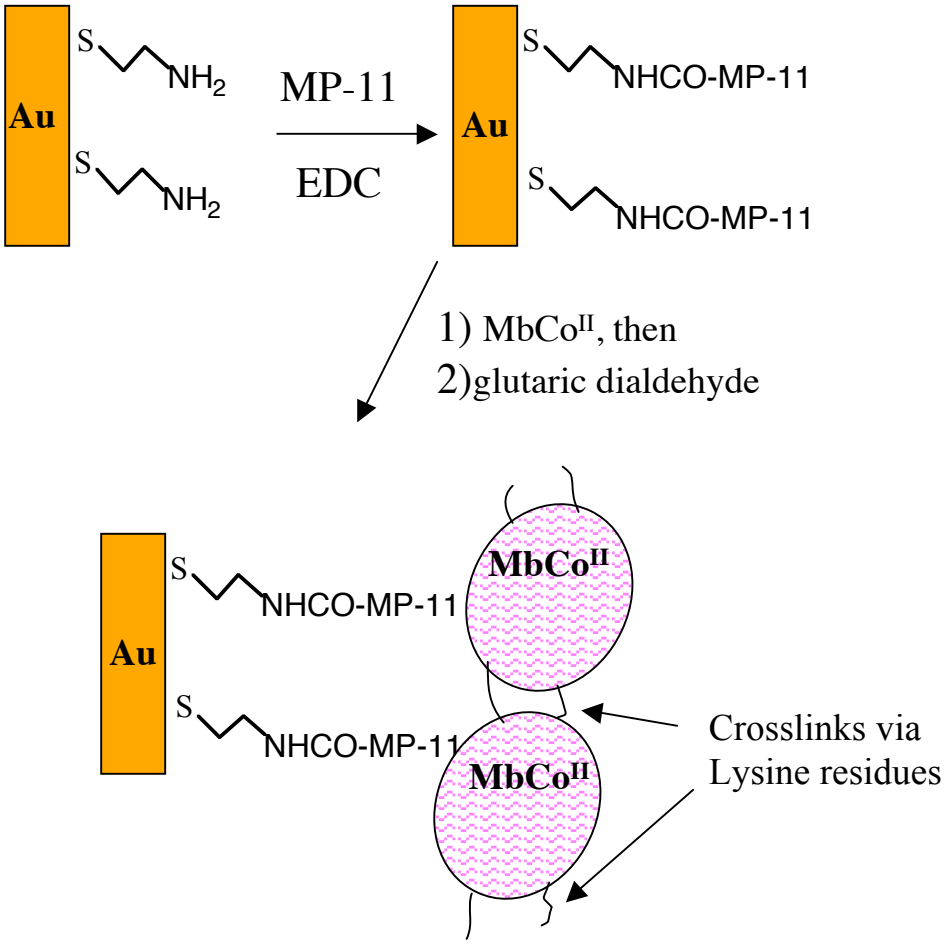

Au
S
NH2
S
NH2
MP-11
EDC
Au
S
NHCO-MP-11
S
NHCO-MP-11
1) MbCoII, then
2)glutaric dialdehyde
Au
S
NHCO-MP-11
S
NHCO-MP-11
MbCoII
MbCoII
Crosslinks via
Lysine residues

Figure 12

Enzyme Kinetics:
RDV and the Electrochemical Michaelis Eqn.

Under steady-state conditions, the RDV limiting current is given by:

$$\frac{1}{I_{Lim}} = \frac{1}{I_{Lev}} + \frac{1}{I_E} + \frac{1}{I_{cat}} \qquad (1)$$

where I_{Lim} is the limiting current, I_E is the exchange current due to interfacial electron transfer between the electrode and primary electron entry/exit site on the enzyme. I_{cat} is the catalytic current characteristic of the enzyme reaction, and I_{Lev} is the Levich current for the transport of substrate between the bulk solution and the enzyme film. Assuming that electron transfer is not limiting at high overpotential, equation 1 becomes

$$\frac{1}{I_{Lim}} = \frac{1}{I_{Lev}} + \frac{1}{I_{cat}} \qquad (2)$$

where I_{Lev} is the Levich current given by

$$I_{Lev} = 0.62nFAD^{2/3}Cv^{-1/6}\omega^{1/2}$$

So, a graph of $1/I_{lim}$ *vs.* $\omega^{-1/2}$ gives $1/I_{cat}$ as intercept

Michaelis-Menten Model

Scheme 1

$$E_{red} + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} E_{red}S \xrightarrow{k_{cat}} E_{ox} + P$$

give the protein-substrate complex ($E_{red}S$), which reacts to regenerate Mb (E_{ox}) in an oxidized form. If the electrochemistry of E_{ox} in the film is reversible and regenerates E_{red}, a catalytic current that can be used to measure reaction kinetics results from the electrochemical reduction of E_{ox} in the film. The catalytic current I_{cat} is given by the electrochemical Michaels-Menten equation[Error! Bookmark not defined.][Error! Bookmark not defined.]

$$I_{cat} = \frac{nF\mathrm{A}\Gamma k_{cat} C_s}{C_s + K_m} \qquad (3)$$

where *n* is the number of electrons in the electrochemical reaction, C_s is concentration of substrate in solution, Γ is the surface concentration of enzyme in the film (mol cm^{-2}), A is the electrode area (cm^2), *F* is Faraday's constant, k_{cat} is the catalytic rate constant (s^{-1}) and K_m is the Michaelis dissociation constant given by:

$$K_m = \frac{k_{-1} + k_{cat}}{k_1} = \frac{[E_{red}][S]}{[E_{red}S]} \qquad (4)$$

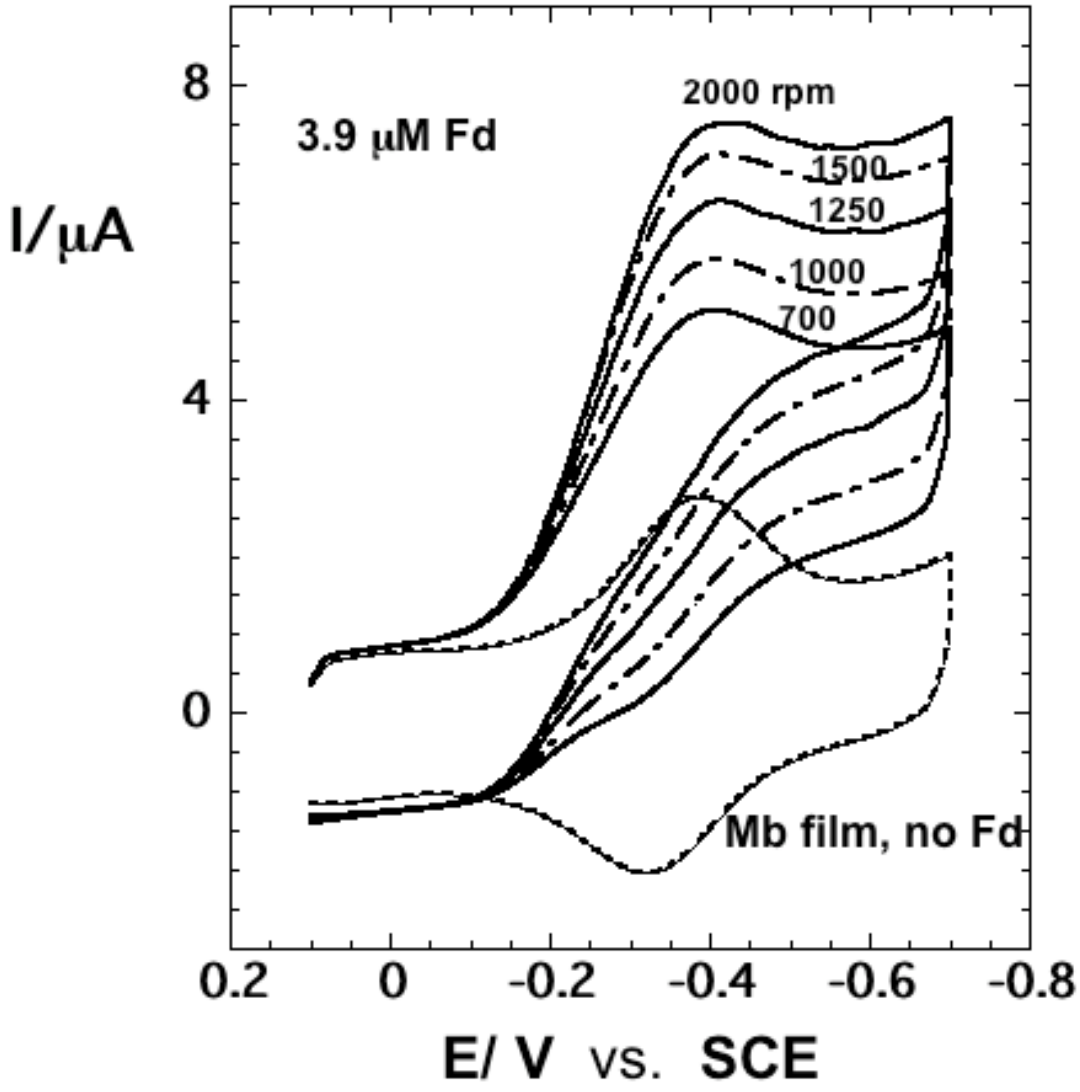
The ratio k_{cat}/K_m has units of a second order rate constant ($M^{-1}\ s^{-1}$) and provides a direct measure of catalytic efficiency.

Reaction of $MbFe^{III}$ with ferredoxin (Fd)

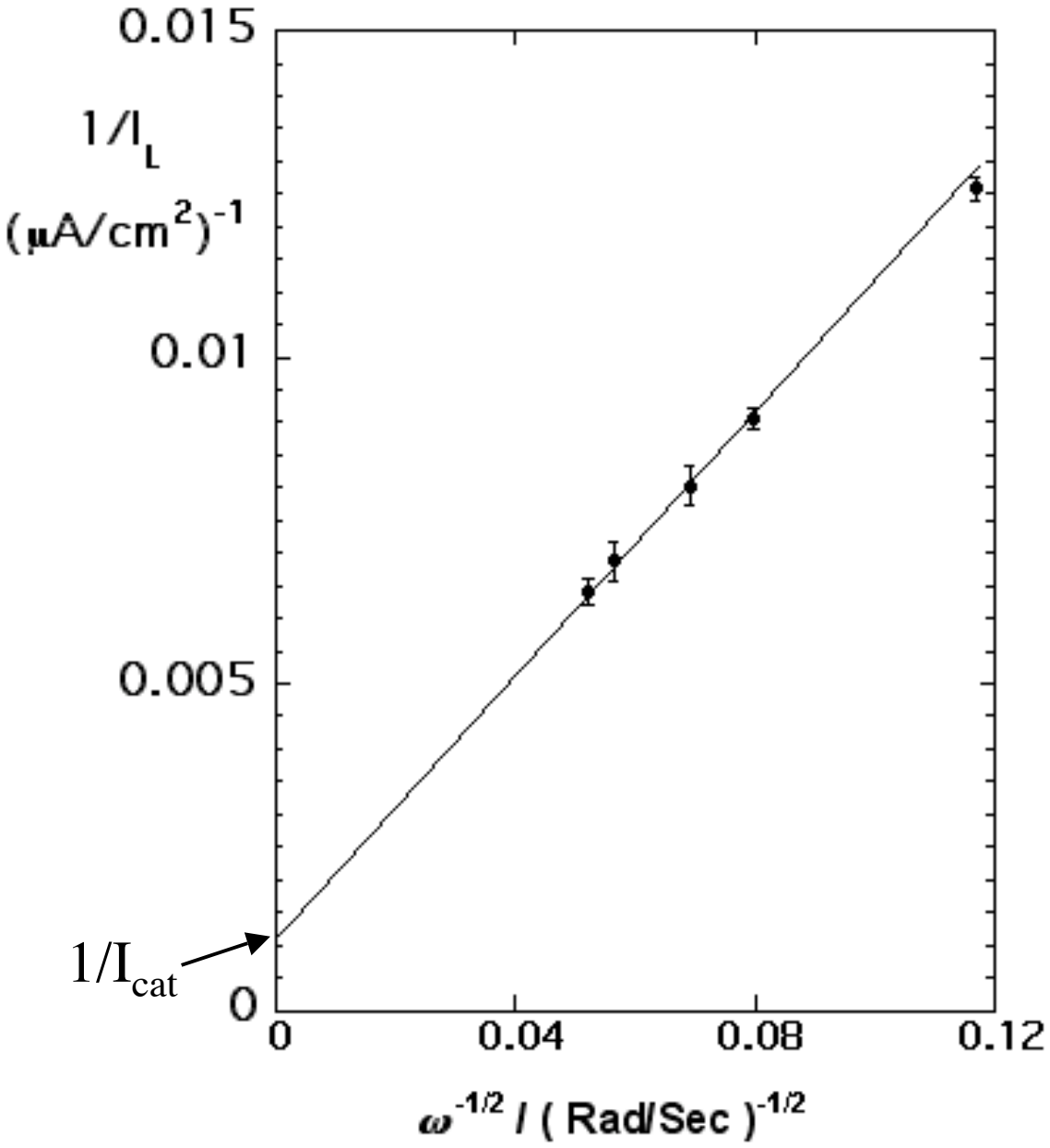
$$MbFe^{III} == MbFe^{II} + e-$$

$$MbFe^{II} + Fd_{ox} \rightleftharpoons [MbFe^{II}\bullet Fd_{ox}]$$

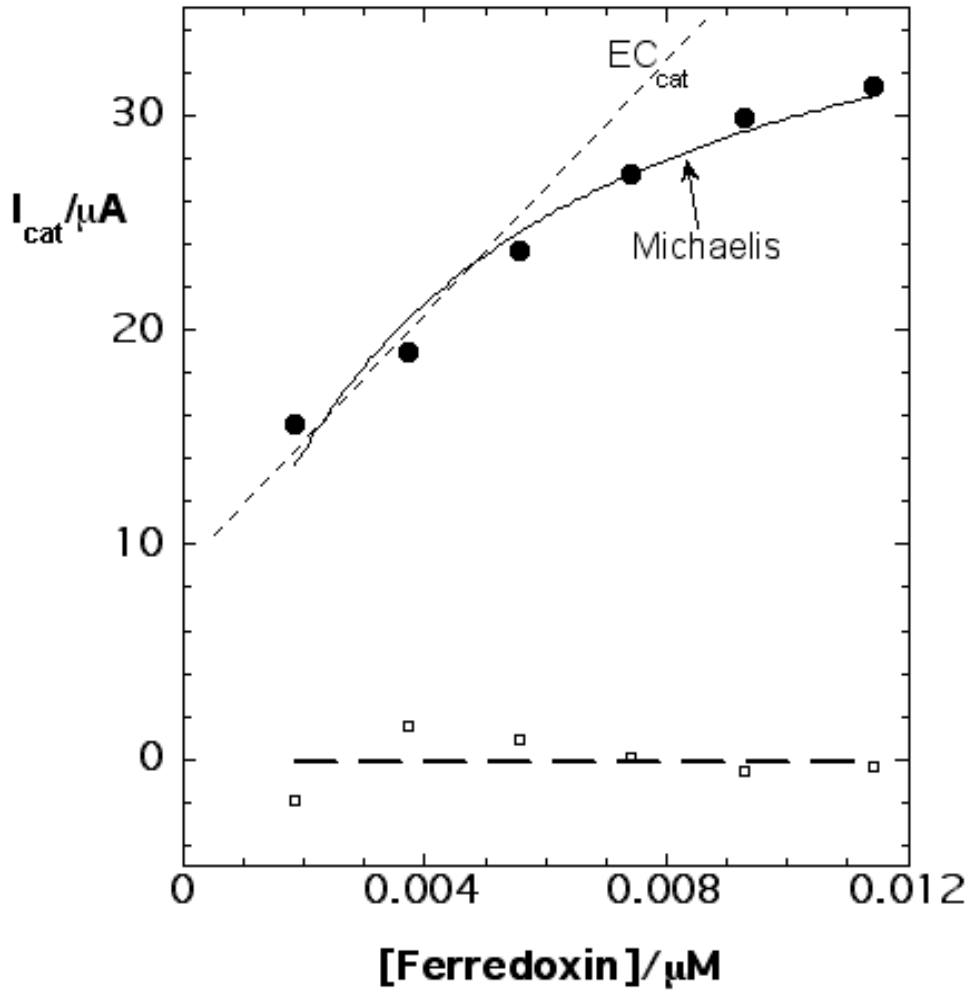
$$[MbFe^{II}\bullet Fd_{ox}] \longrightarrow MbFe^{III} + Fd_{red}$$



Extrapolation to get I_{cat}



Fit I_{cat} vs. [Fd] to obtain K_M and k_{cat}



$$k_{cat} = 102.2 \pm 0.2\ s^{-1} \text{ and } K_M = 112 \pm 3\ \mu M$$

k_{cat}/K_M with units of a second order rate constant was 9.1 x 10^4 M^{-1} s^{-1}.

Khrisna E. Alcantara and James F. Rusling, Voltammetric Measurement of Michaelis-Menten Kinetics for a Protein in a Lipid Film Reacting with a Protein in Solution, **Electrochem. Communications,** *2005*, **7, 223-226.**

Enzyme thin film voltammetry

- Many approaches available to make films
- avoids electrode fouling, slow diffusion
- often reversible voltammetry for cofactors
- method of choice for modern electrochemical studies of enzymes and redox proteins
- stable films for biosensors