RUHR-UNIVERSITÄT BOCHUM

Center for Electrochemical Sciences

RUB

Electron Transfer in Biophotoelectrochemical Devices

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COST Training School "Phototech for Biosensors and Energy"
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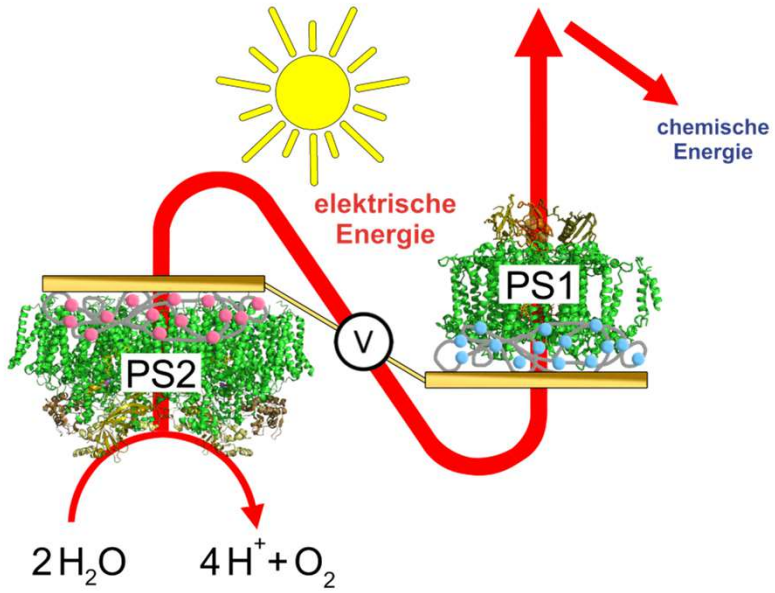
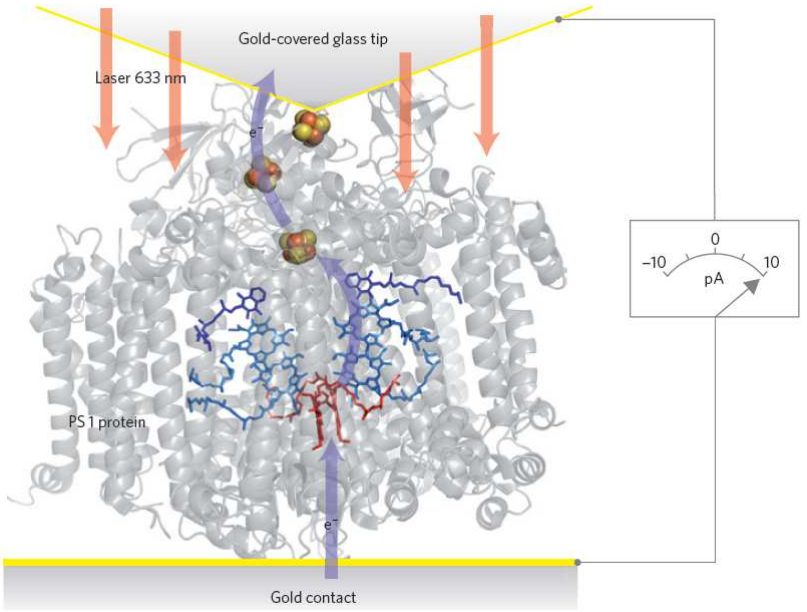
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

Photosystem based-photovoltaics





T. Kothe, N. Plumeré, A. Badura, M. M. Nowaczyk, D. A. Gushin, M. Rögner, W. Schuhmann, *Angewandte Chemie*, **2013**, accepted,

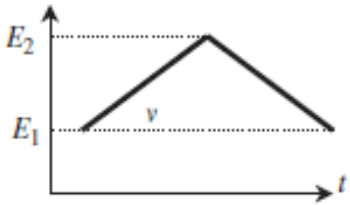
N. Plumeré, *Nature Nanotechnology*, **2012**, *7*(10), 616-617.

Gerster, D., Reichert, J., Bi, H., Barth, J. V., Kaniber, S. M., Holleitner, A. W., Visoly-Fisher, I., Sergani, S., Carmeli, I. *Nature Nanotech* **2012,** *7*, 673–676.

Heering, H. A., Hirst, J., Armstrong, F. A. *J. Phys. Chem. B* **1998**, *102*, 6889–6902.

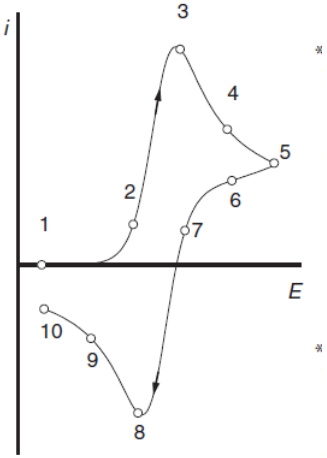
Nicholson, R. S., Shain, I. *Anal. Chem.* **1964**, *36*, 706–723.

Advanced Electroanalytical Methods



• Electrochemical methods offer efficient alternatives (to laser-flash experiments) for the study of electron transfer in biophotoelectrochemical systems.

• Kinetic measurements using, for example, cyclic voltammetry are based on the coupling between one heterogeneous electrochemical reaction and the catalytic reaction to be studied.

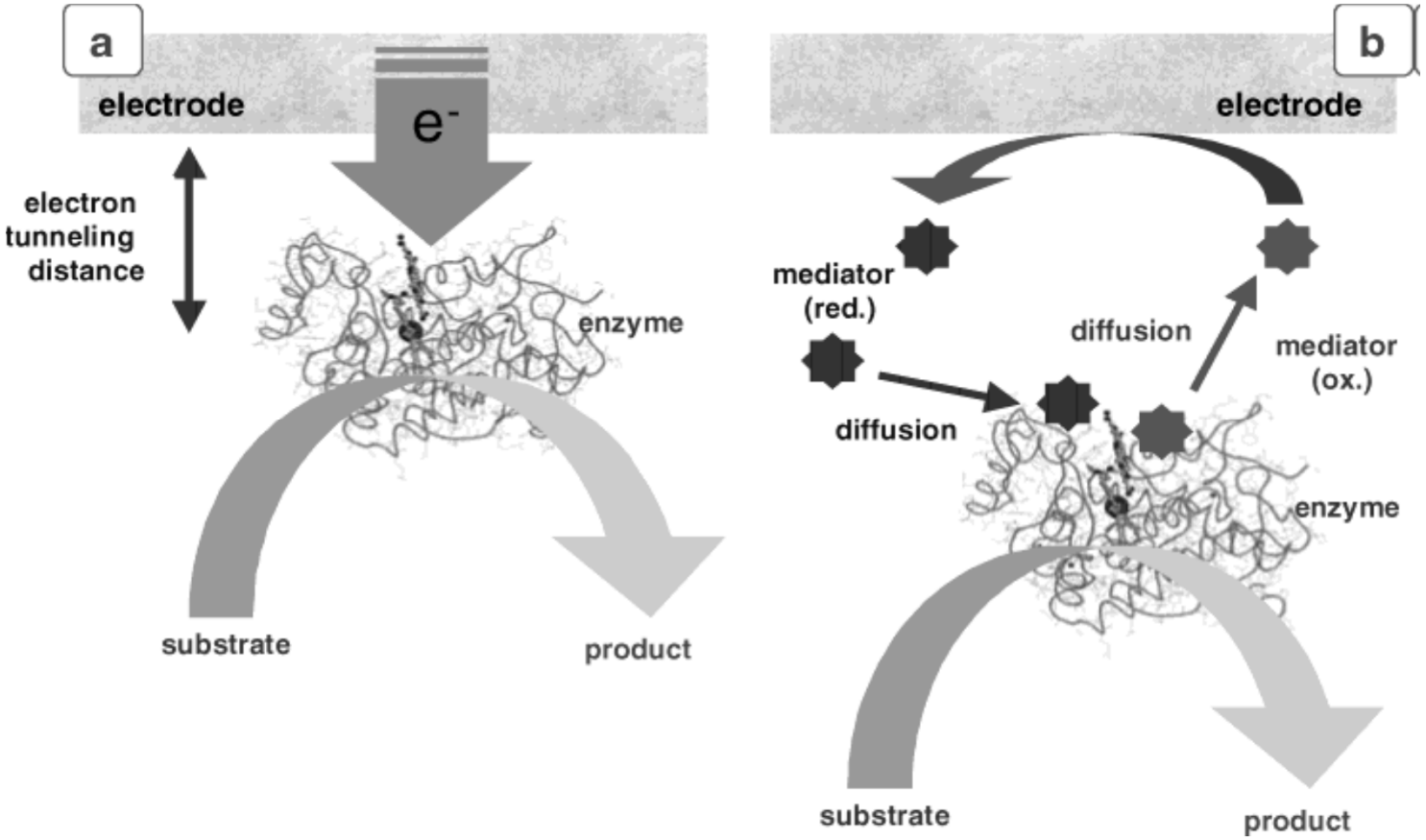


• The resulting “catalytic current” contains the kinetic information.

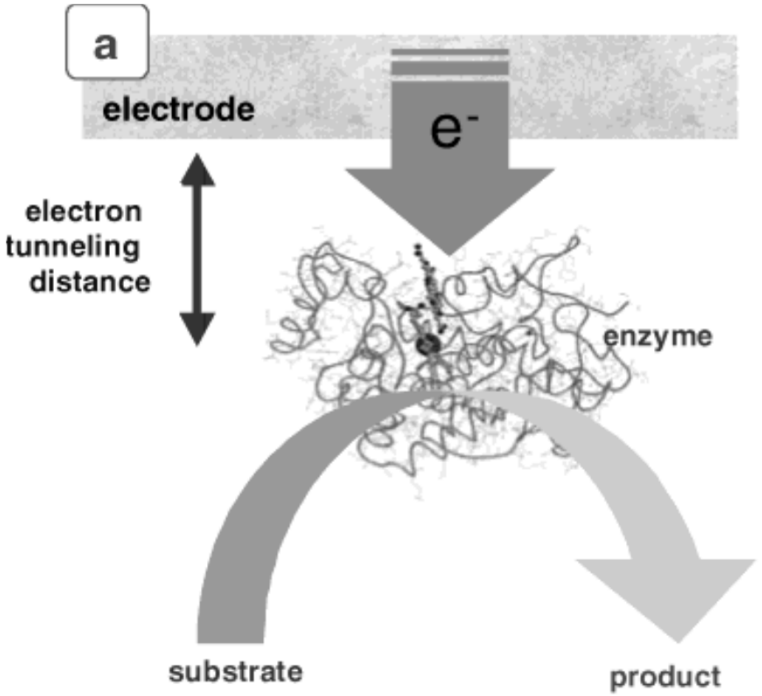
• The extraction of rate constants from (light driven) enzymatic electrocatalysis is well established.

More than measuring photocurrents: electroanalytical methods allow to identify rate limiting steps in bio(photo)electrochemical systems.

Direct vs Mediated Electron Transfer



Direct Electron Transfer

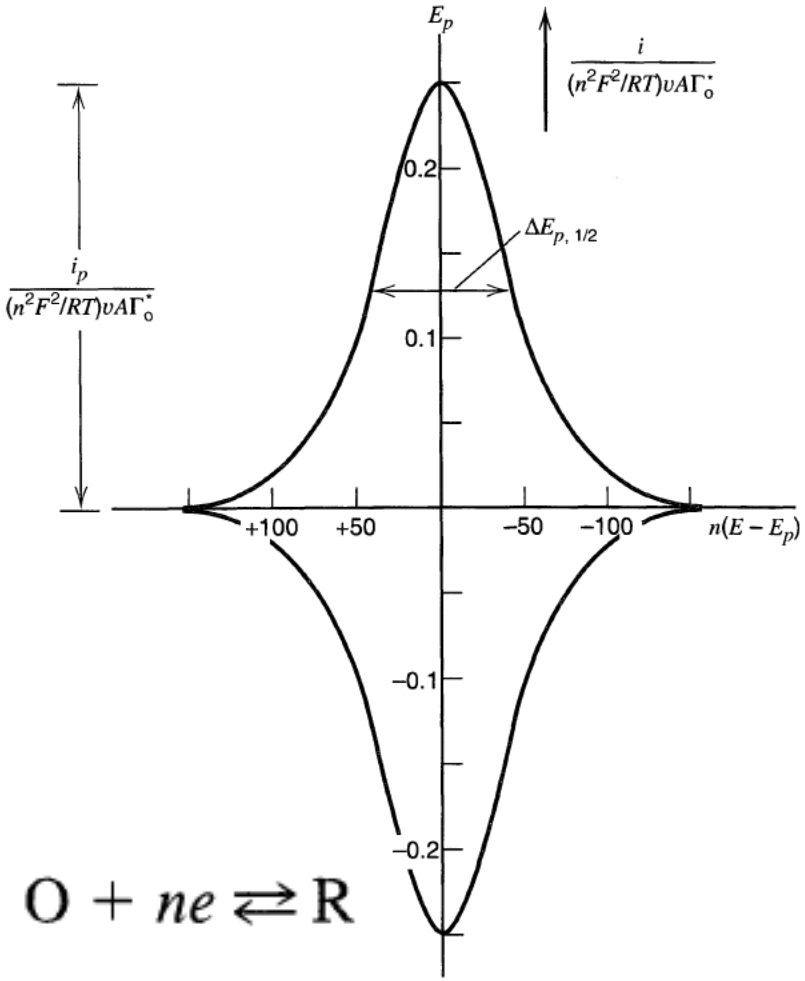


- **Determination of the heterogeneous electron transfer rates.**
- Determination of the kinetics of reactions with substrate or charge carrier.

J. Electroanal. Chem., 100 (1979) 263–270

Voltammetry of adsorbed monolayers

Reversible system - adsorbed species



The oxidation and reduction signal are symmetrical (ideal case).

$\Delta E_p = 0$ and $E_p = E^{0'}$

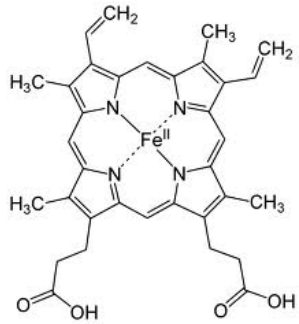
Peak current is given by:

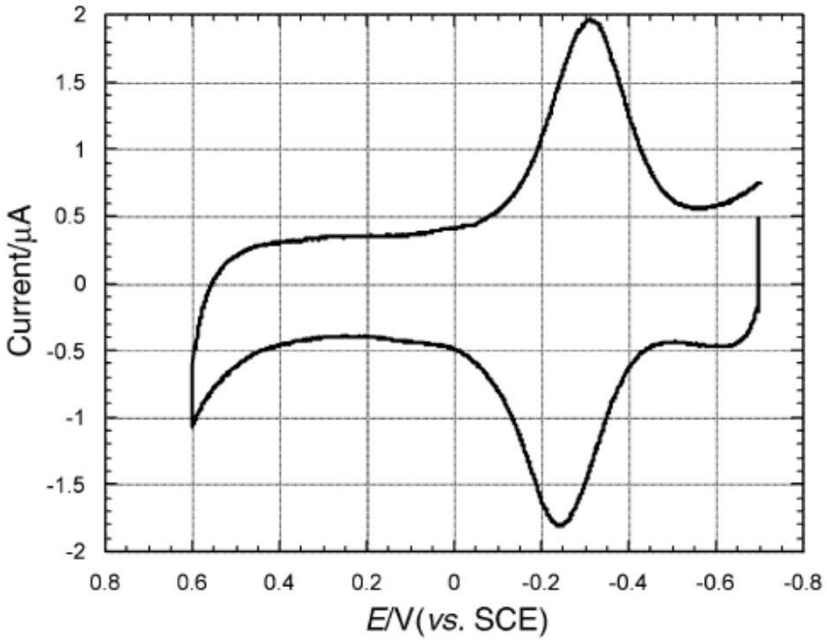
$$i_p = \frac{n^2F^2}{4RT}\upsilon A\Gamma_O^*$$

$$Q = nFA\Gamma_O$$

Protein Film Voltammetry
Direct electron transfer

Peroxidase with Heme





Chem. Commun., 2001, 177–178

Deviation from ideal protein film voltammetry:

$\Delta E_p = 55\ \mathrm{mV} > 0$

Reason for $\Delta E_p > 0$: kinetic limitation often because of slow electron transfer between electrode and redox sites.

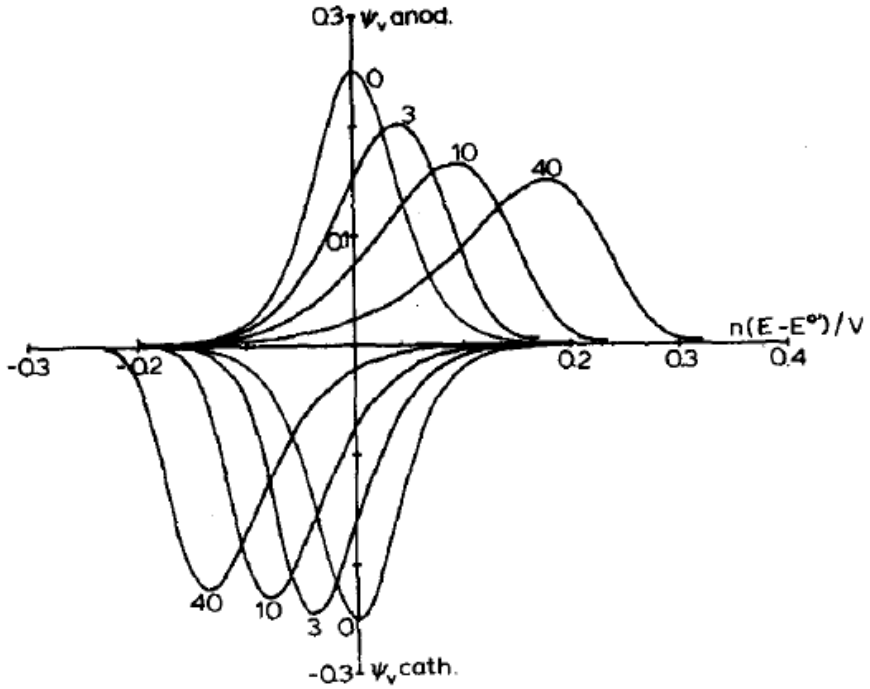
The electron transfer is not reversible but quasireversible: it is defined by the heterogeneous electron transfer rate (k_s) and the transfer coefficient (α).

J. Electroanal. Chem., 101 (1979) 19—28

Electron transfer rate constant

Determination of k_s and α based on ΔE_p by varying the scan rate υ.

If ΔE_p ($E_{p,c}$-$E_{p,a}$) value larger than 200 mV can be obtained experimentally by varying the scan rate υ, α can easily be obtained by plotting E_p vs log υ.



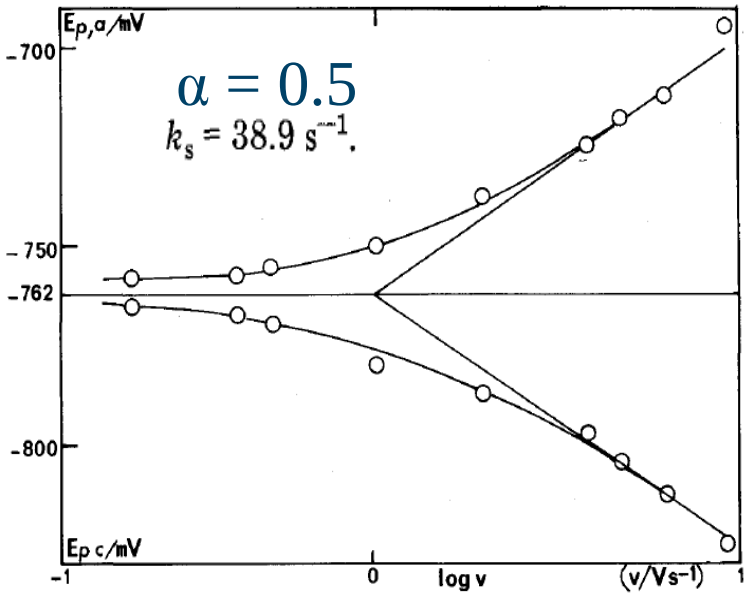


Fig. 6. ($E_p - E^{\frac{1}{2}}$) = f(log v) for benzo(c)cinnoline.

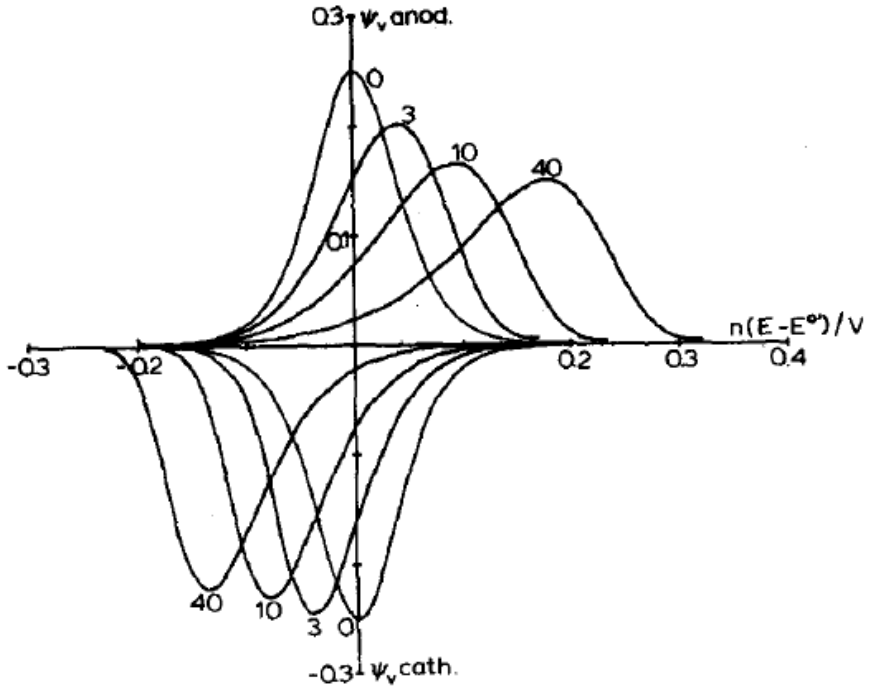
J. Electroanal. Chem., 101 (1979) 19—28

Electron transfer rate constant

Determination of k_s and α based on ΔE_p by varying the scan rate υ.

k_s can be calculated with the help of the equation:

$$\log k = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log(RT/nF\upsilon) - \alpha(1-\alpha)nF\,\Delta E_p / 2.3RT$$



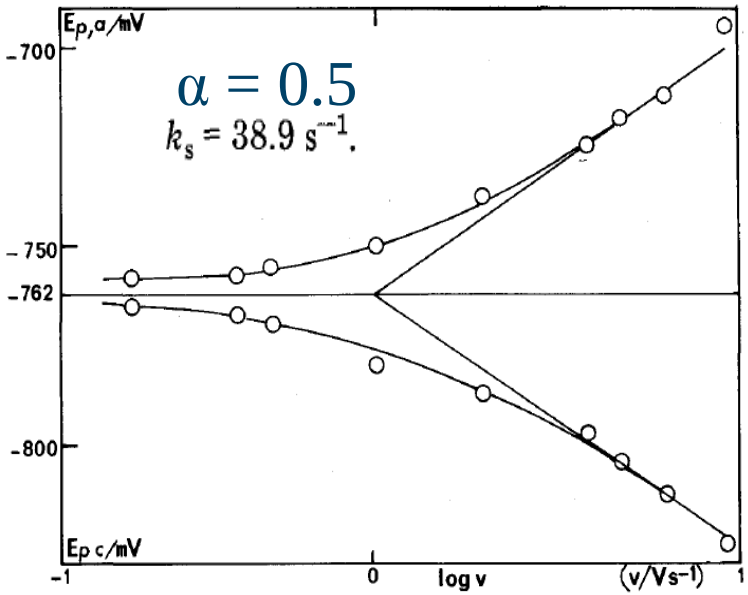
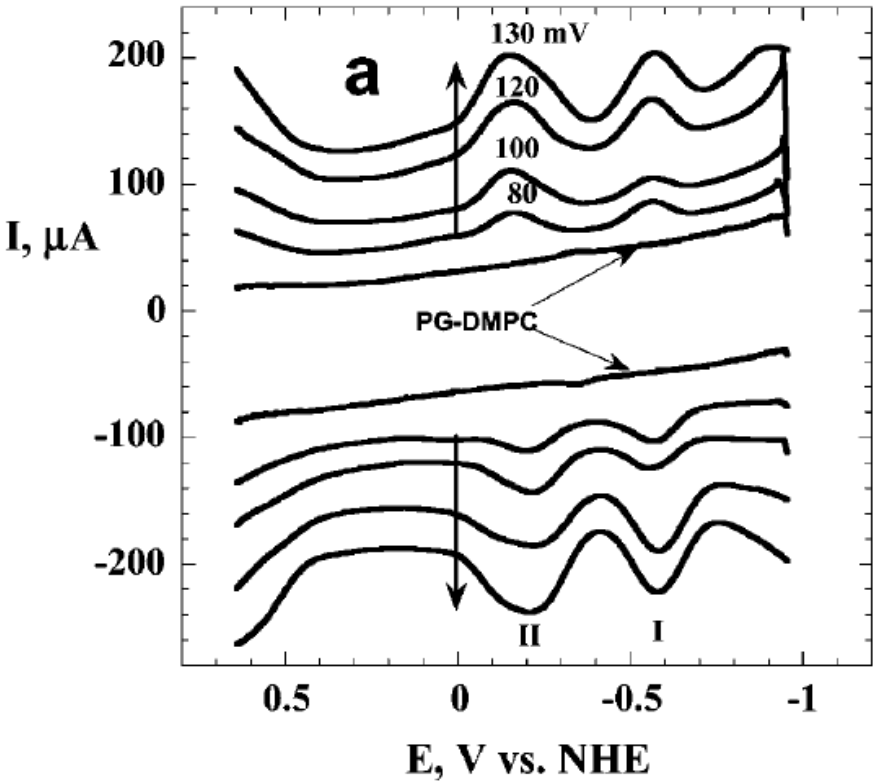


Fig. 6. $(E_p - E^{\frac{1}{2}}) = f(\log \upsilon)$ for benzo(*c*)cinnoline.

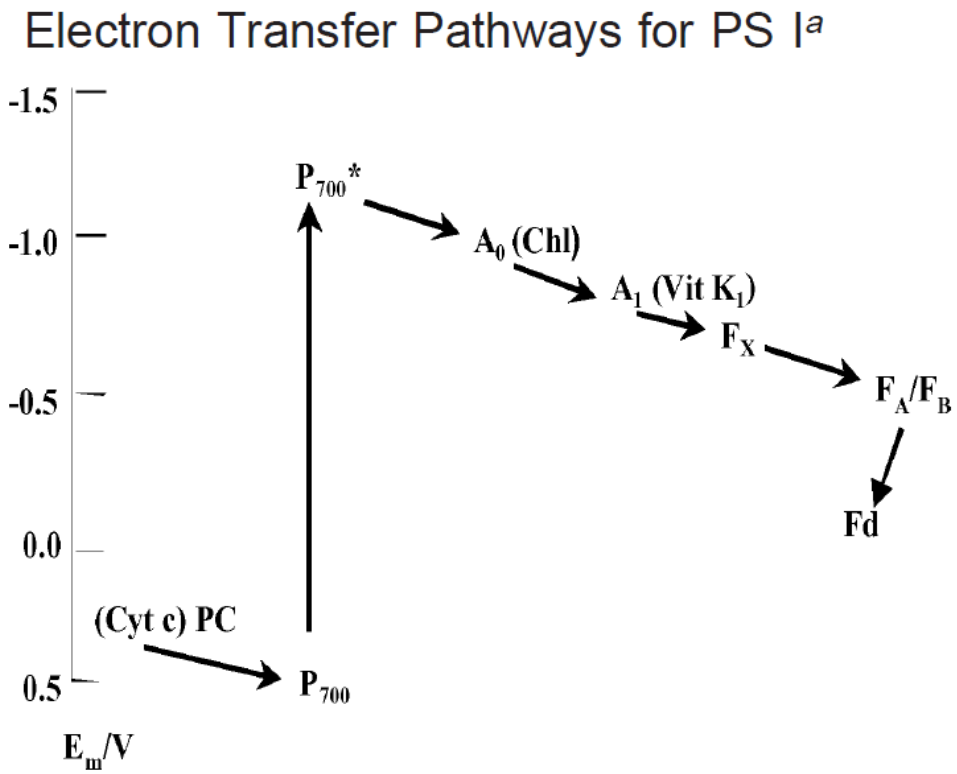
Munge, B., Das, S. K., Ilagan, R., Pendon, Z., Yang, J., Frank, H. A., Rusling, J. F. *J. Am. Chem. Soc.* **2003**, *125*, 12457–12463.

Direct electrochemistry of PS1

PS1 immobilized on pyrolytic graphite electrodes



Square Wave Voltammetry



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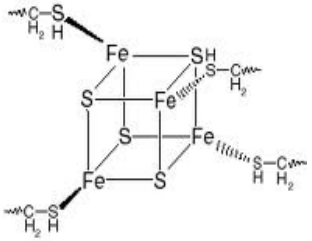
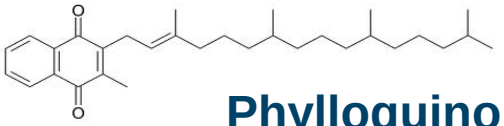
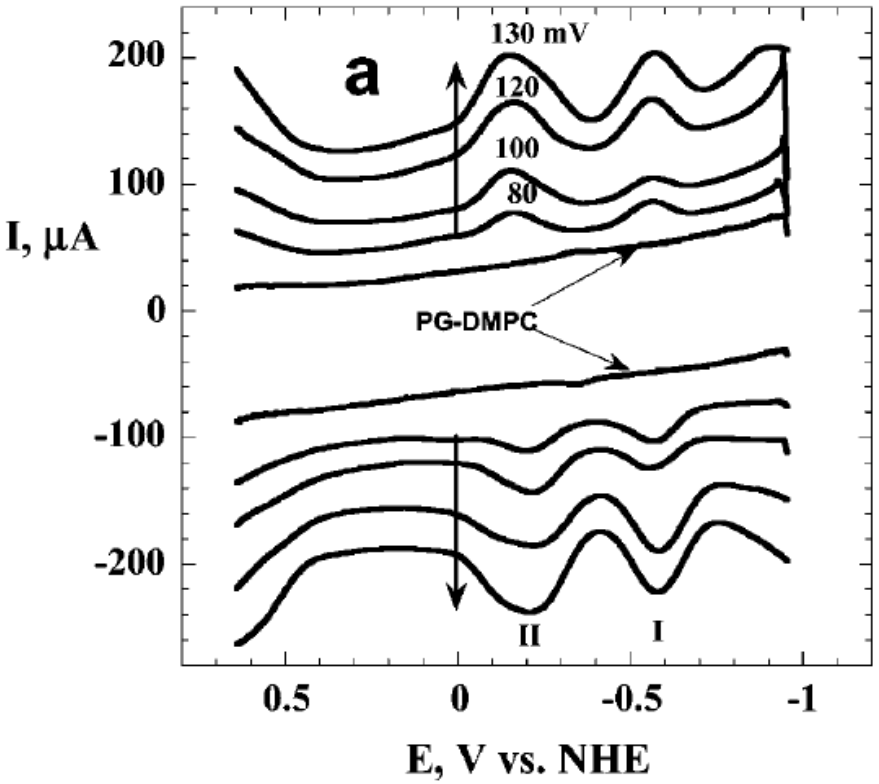


4Fe4S (F_A/F_B)



Phylloquinone (A1)



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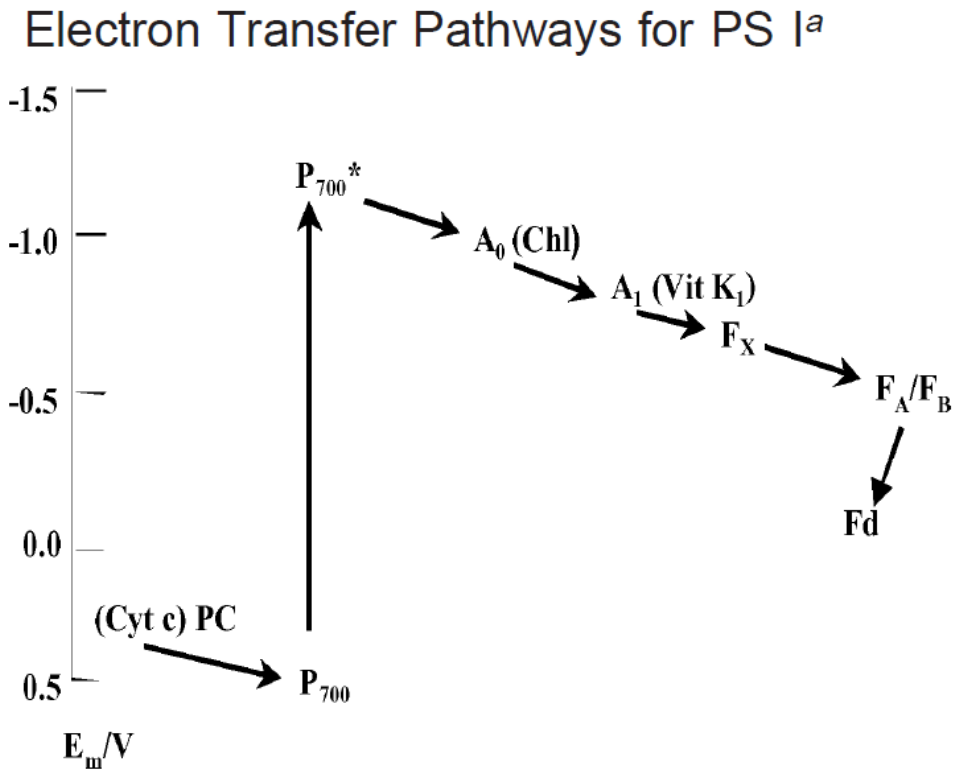

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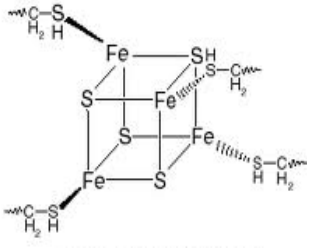

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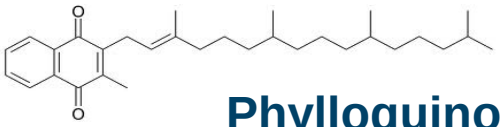

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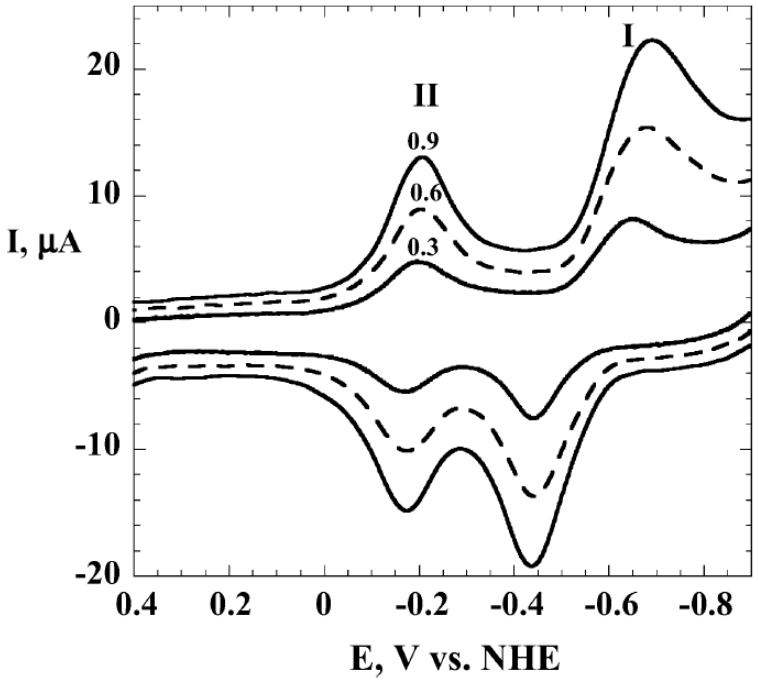
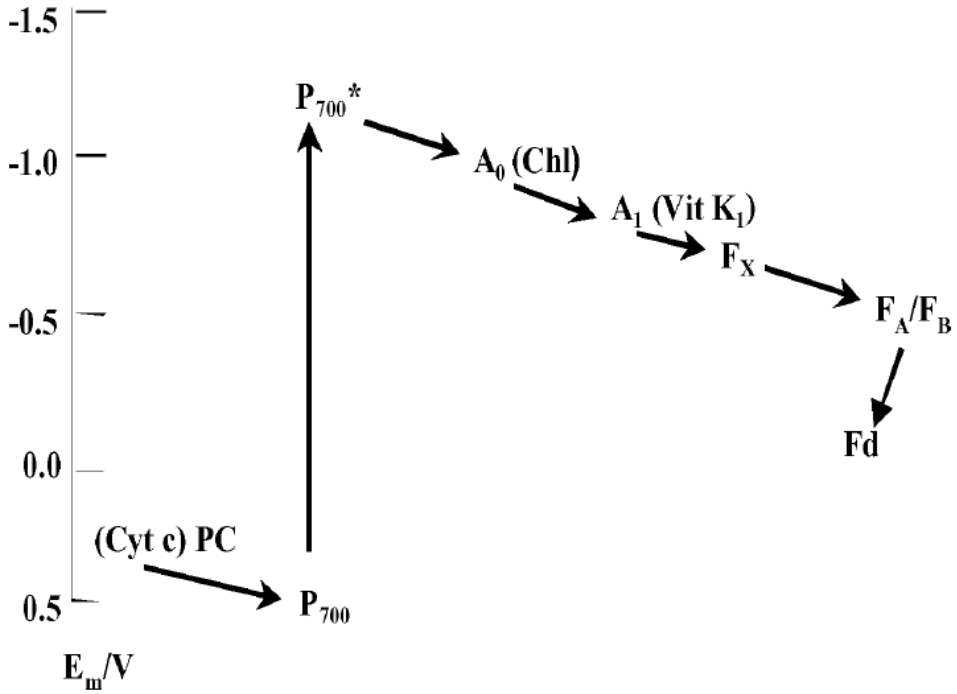


Cyclic Voltammetry

Electron Transfer Pathways for PS I[a]



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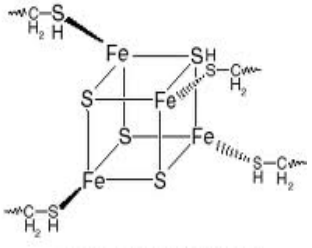
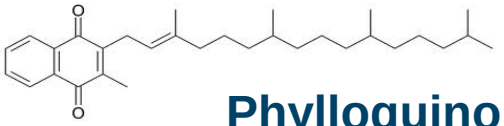
Direct electrochemistry of PS1



4Fe4S (F_A/F_B)



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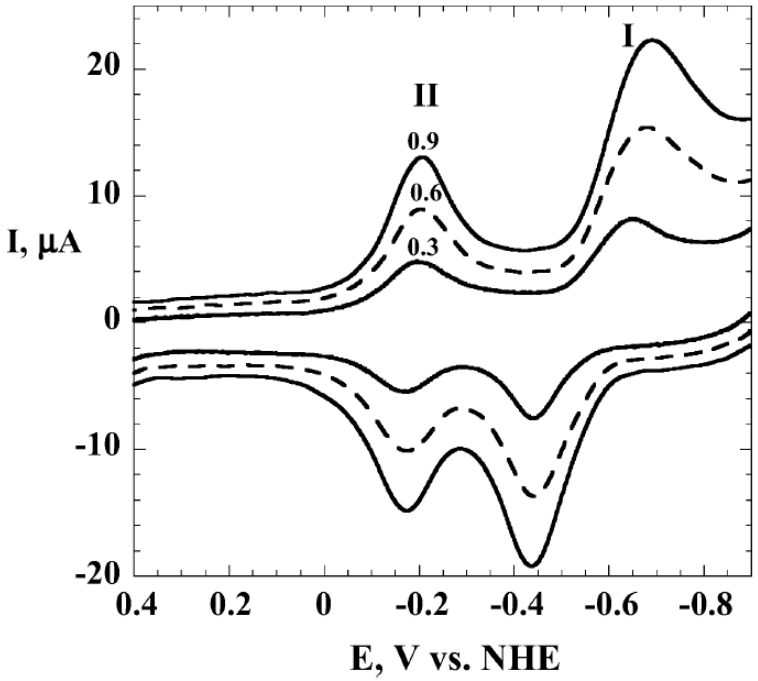
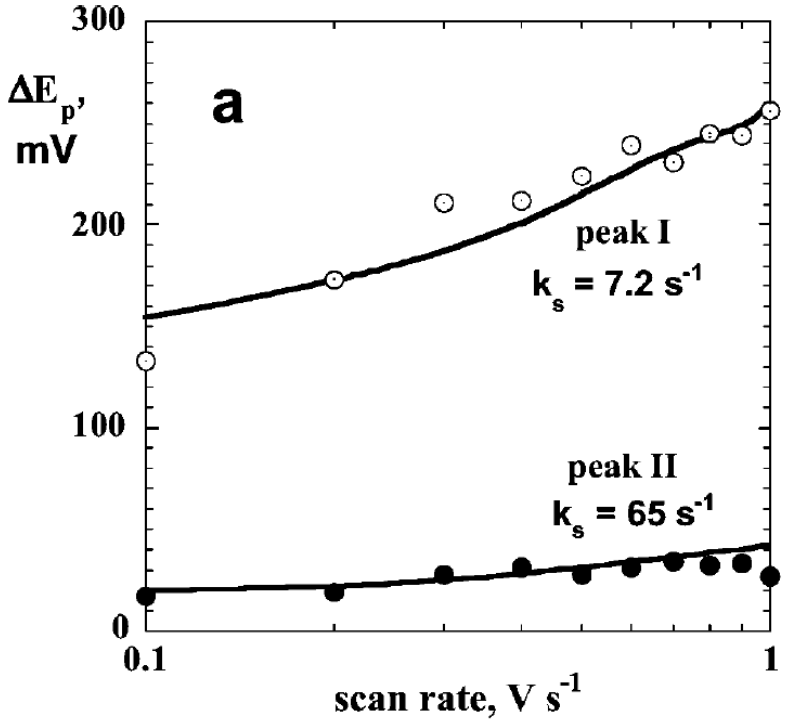


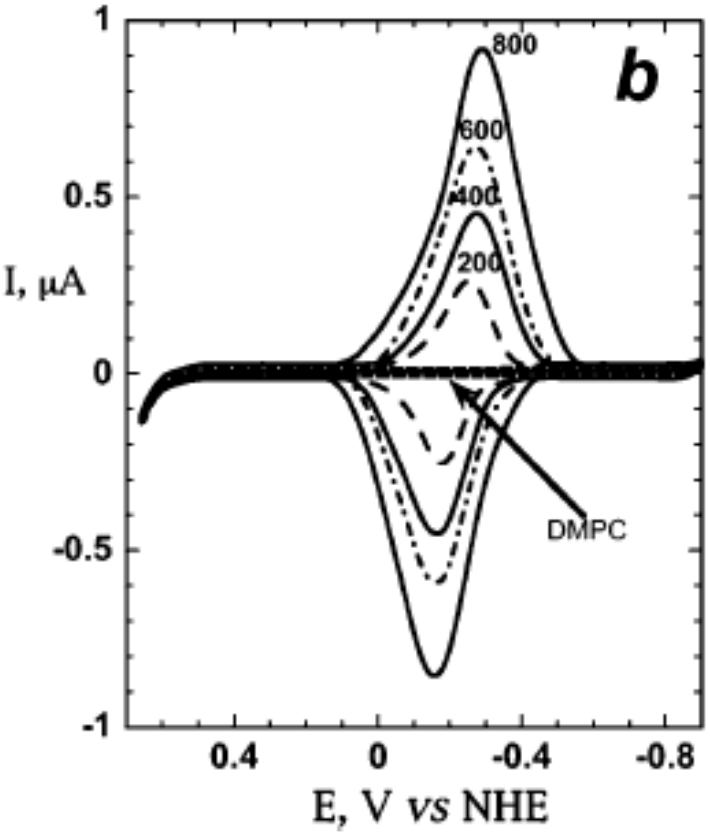


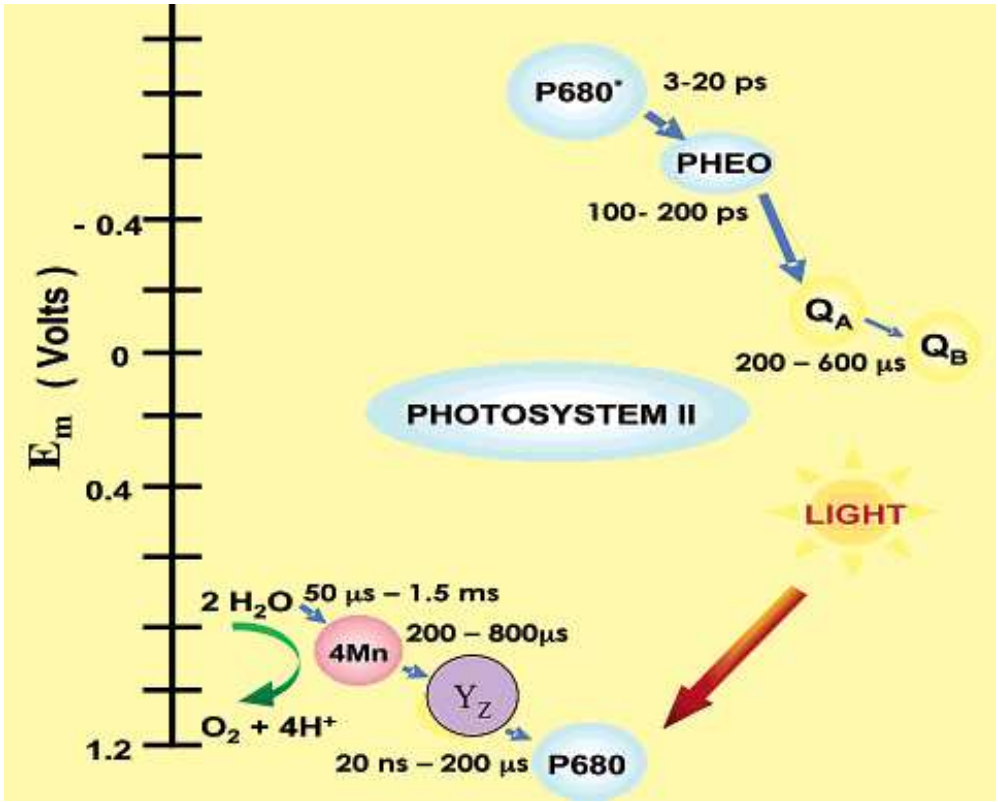
Cyclic Voltammetry

Alcantara, K., Munge, B., Pendon, Z., Frank, H. A., Rusling, J. F. *J. Am. Chem. Soc.* **2006**, *128*, 14930–14937.

Direct electrochemistry of PS2

PS2 immobilized on pyrolytic graphite electrodes





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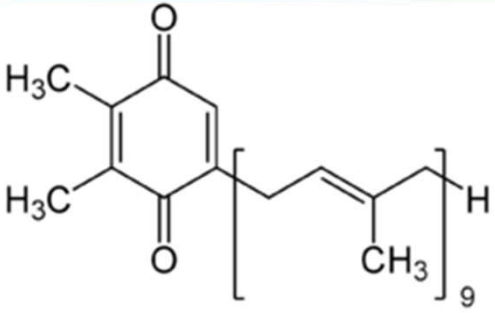
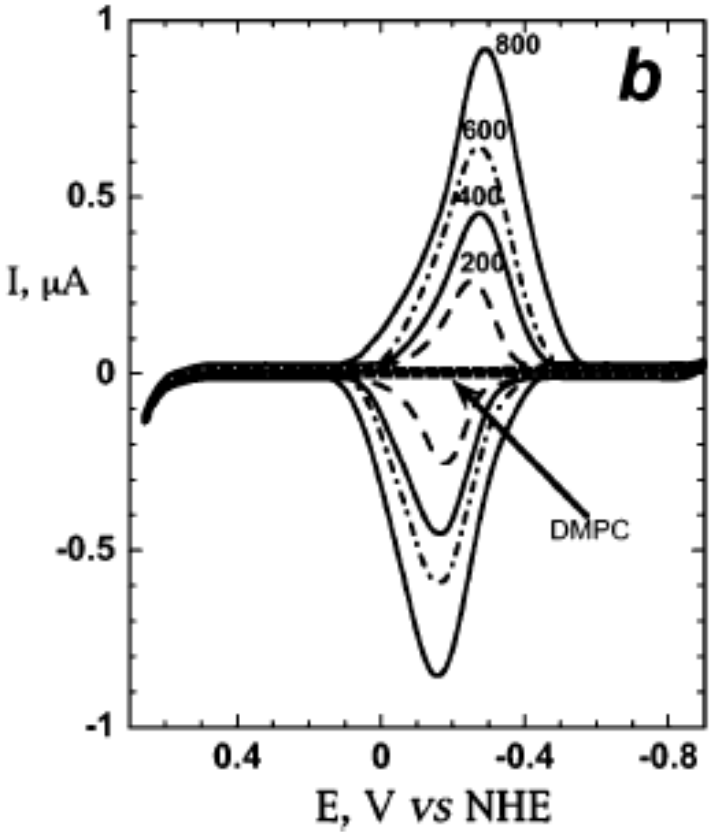
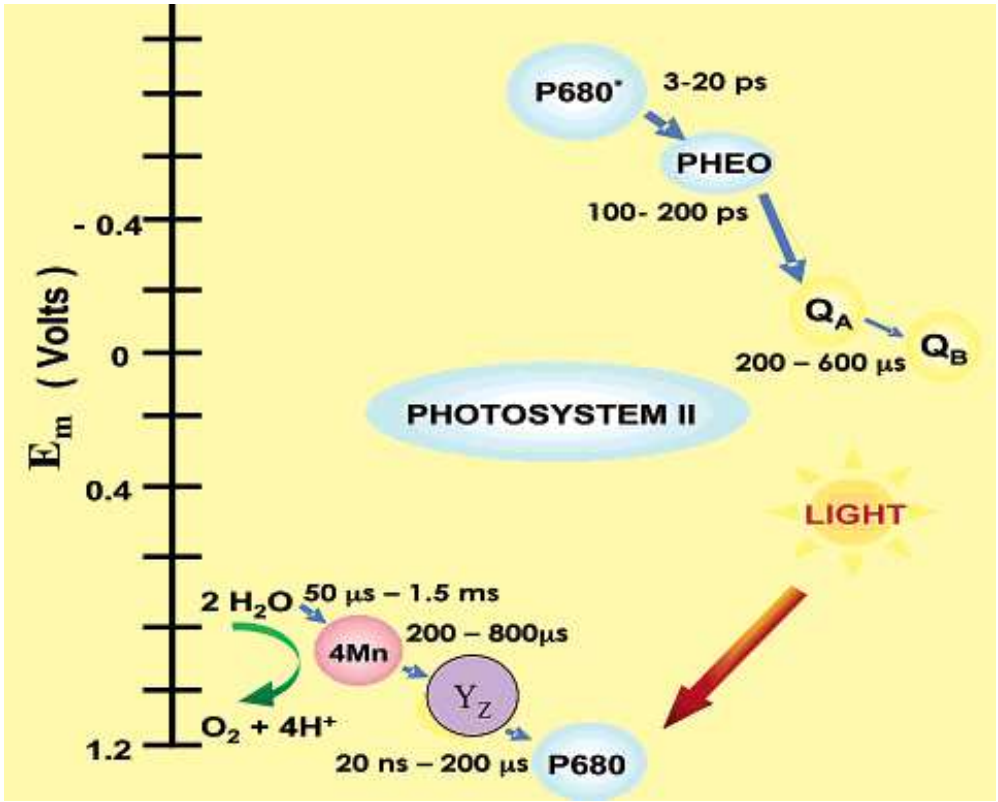
Direct electrochemistry of PS2



Plastoquinone A or B





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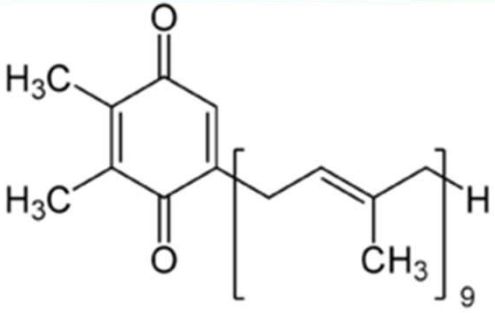
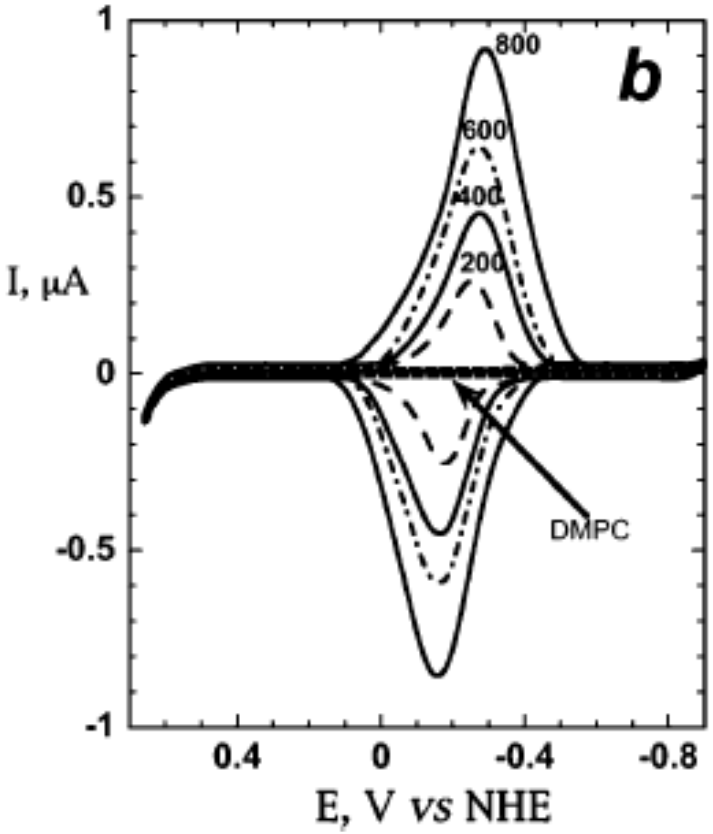
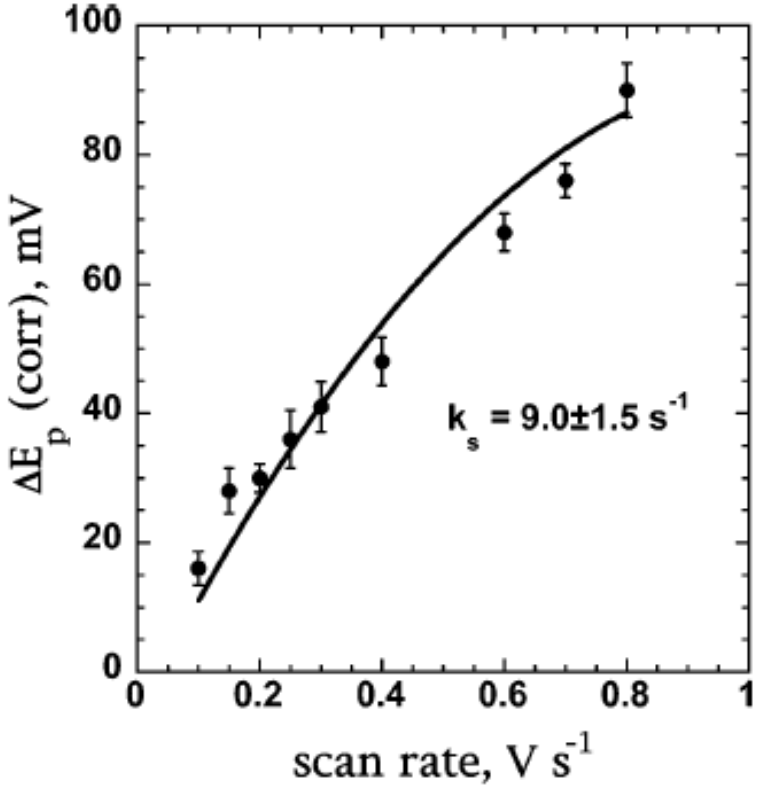
Direct electrochemistry of PS2

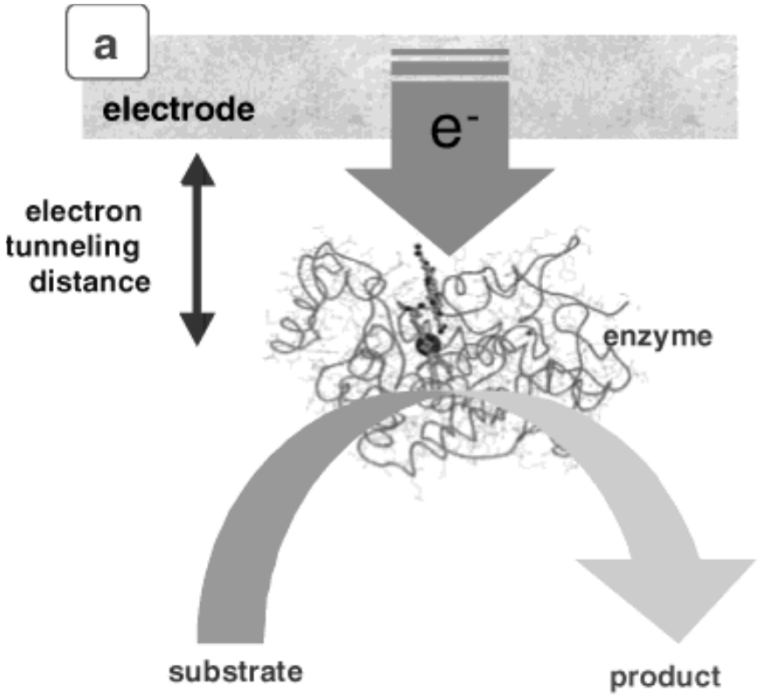


Plastoquinone A or B





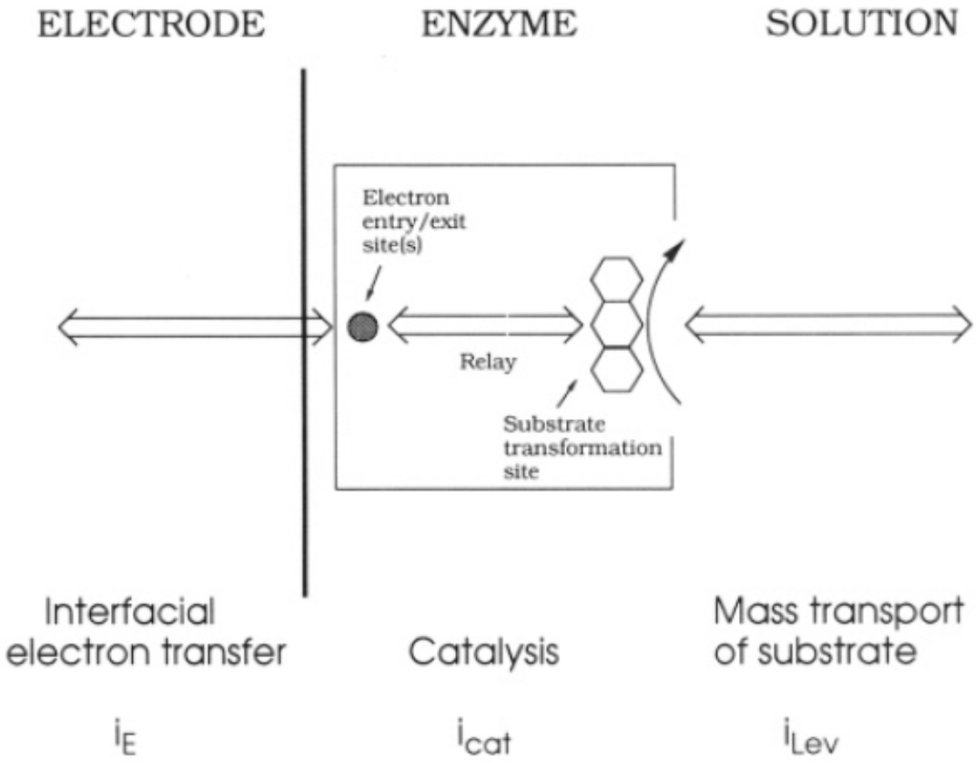
Direct Electron Transfer

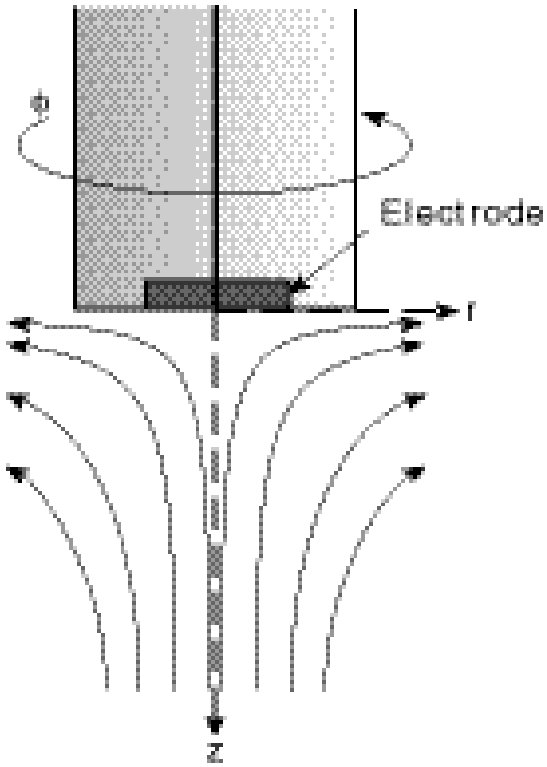


- Determination of the heterogeneous electron transfer rates.
- **Determination of the kinetics of reactions with substrate or charge carrier.**

Kinetics of reactions with charge carrier or substrate

In presence of substrate - Rotating disk electrodes (RDE)





Sucheta, A., Cammack, R., Weiner, J., Armstrong, F. A. *Biochemistry* **1993**, *32*, 5455–5465

Kinetics of reactions with charge carrier or substrate

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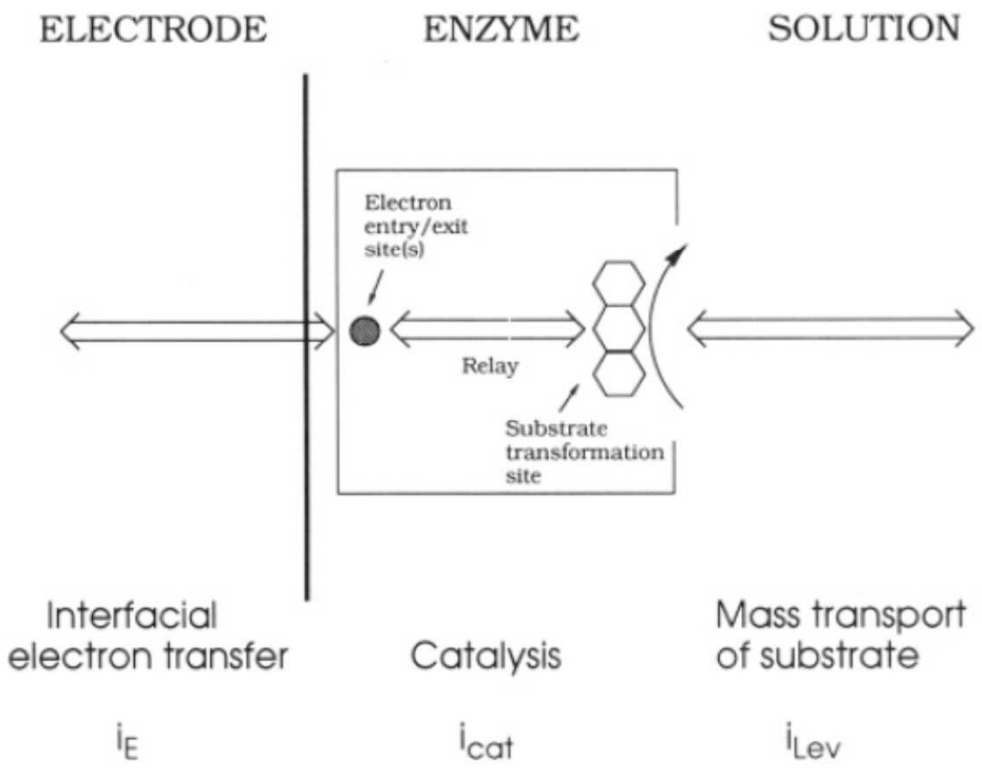


Under steady state conditions:

$$\frac{1}{i} = \frac{1}{i_{Lev}} + \frac{1}{i_E} + \frac{1}{i_{cat}}$$

The 1st term deals with transport of substrate molecules between bulk of solution and enzyme, which is described by the levich equation:

$$i_{Lev} = 0.62nFAD^{2/3}C\nu^{-1/6}\omega^{1/2}$$

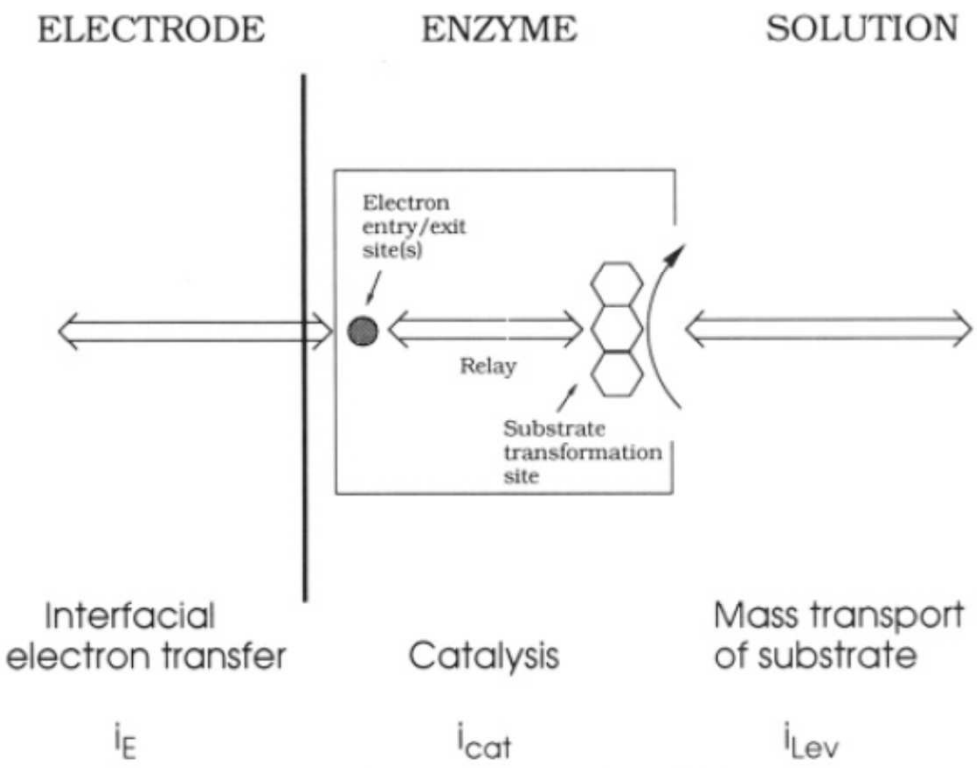
With :

- ***A*: electrode surface area**
- ***C*: bulk concentration of substrate**
- ***D*: diffusion coefficient of substrate**
- **v: kinematic viscosity of the solution**
- **ω: electrode rotation rate**

Sucheta, A., Cammack, R., Weiner, J., Armstrong, F. A. *Biochemistry* **1993**, *32*, 5455–5465

Kinetics of reactions with charge carrier or substrate

In presence of substrate - Rotating disk electrodes (RDE)



The 2nd term is the current contribution due to interfacial electron transfer between the electrode and the primary electron entry/exit site on the enzyme. The potential dependence of i_E is given by marcus theory or Butler-Volmer model:

$$i_E = nFAk_s[\Gamma_O exp\left\{-\frac{\alpha nF(E-E^{0'})}{RT}\right\} - \Gamma_R exp\left\{\frac{(1-\alpha)nF(E-E^{0'})}{RT}\right\}$$

With :
- Γ: surface concentration
- α: transfer coefficient
- *n*: number of electron
- *E*: applied electrode potential
- $E^{0'}$: apparent standard potential

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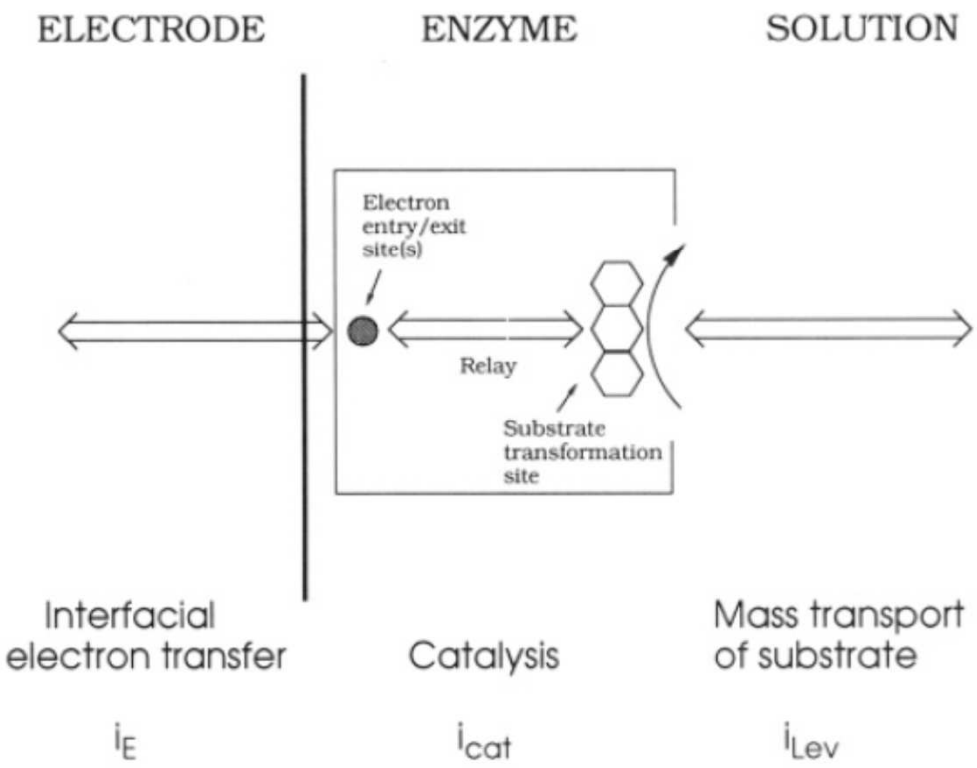
Kinetics of reactions with charge carrier or substrate

In presence of substrate - Rotating disk electrodes (RDE)



The 3rd term describes the catalytic properties of the enzyme and is assumed to be independent of electrode rotation. It can be expressed as the electrochemical form of the Michaelis Menten equation:

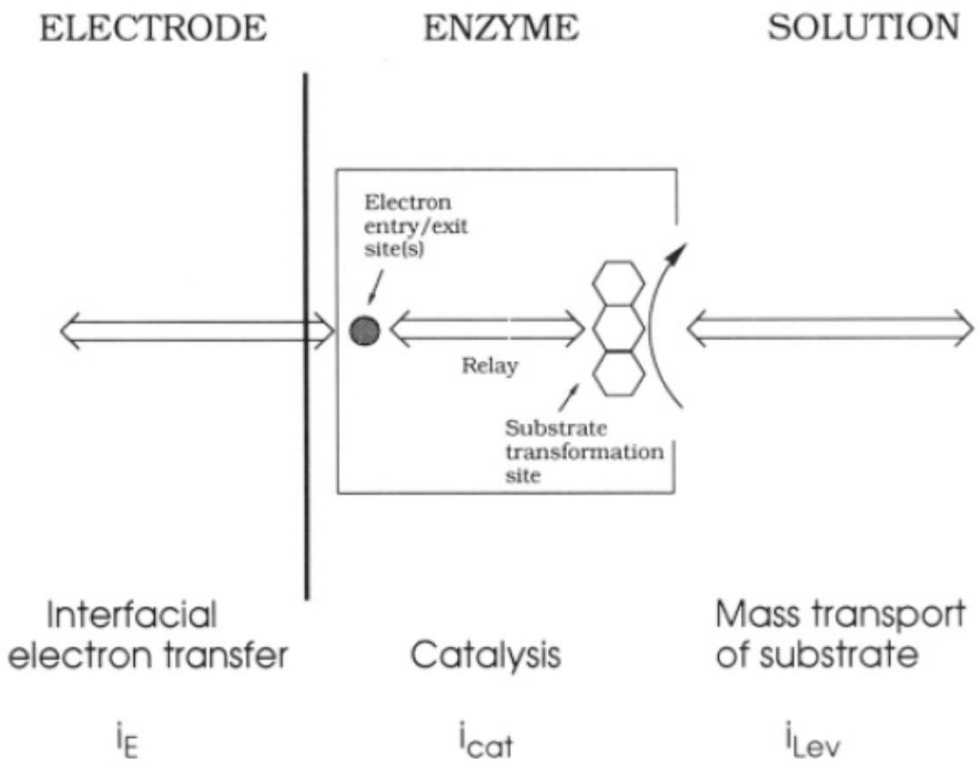
$$i_{cat} = \frac{nFA\Gamma k_{cat} C}{C + K_M}$$

K_M and k_{cat} are the apparent michaelis-Menten parameters, which are assumed to be independent of applied potential. For $C >> K_M$, i_{cat} becomes independant of C.

Sucheta, A., Cammack, R., Weiner, J., Armstrong, F. A. *Biochemistry* **1993**, *32*, 5455–5465

Kinetics of reactions with charge carrier or substrate

In presence of substrate - Rotating disk electrodes (RDE)



$$\frac{1}{i} = \frac{1}{i_{Lev}} + \frac{1}{i_E} + \frac{1}{i_{cat}},$$

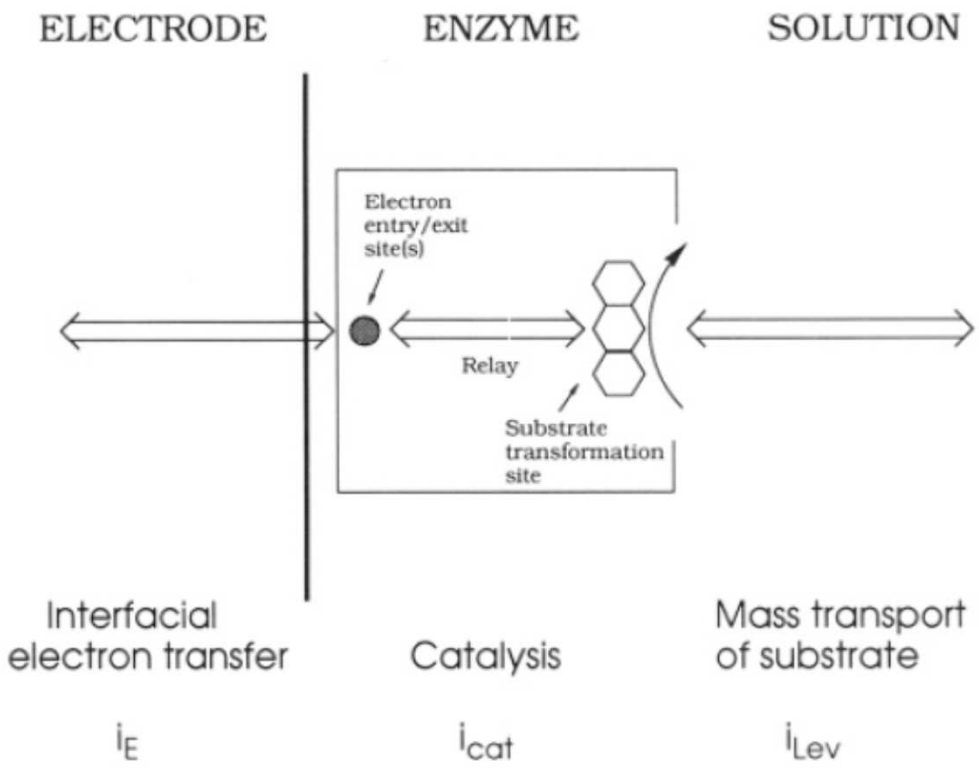
$1/i_E$ vanishes for $i_E >> i_{cat}$ and i_{Lev} .

This is the case when :
k_s is large,
k_{cat} is small,
K_M is large,
***C* is large,**
ω is low or
the electrochemical driving force (*E*-$E^{0'}$) is sufficiently high.
In the resulting *i-E* profilr the current reaches a constant value i_L once the electrochemical driving force ceases to be a controlling factor

Sucheta, A., Cammack, R., Weiner, J., Armstrong, F. A. *Biochemistry* **1993**, *32*, 5455–5465

Kinetics of reactions with charge carrier or substrate

In presence of substrate - Rotating disk electrodes (RDE)



Therefore,

$$\frac{1}{i_L} = \frac{1}{i_{Lev}} + \frac{1}{i_{cat}}$$

or:

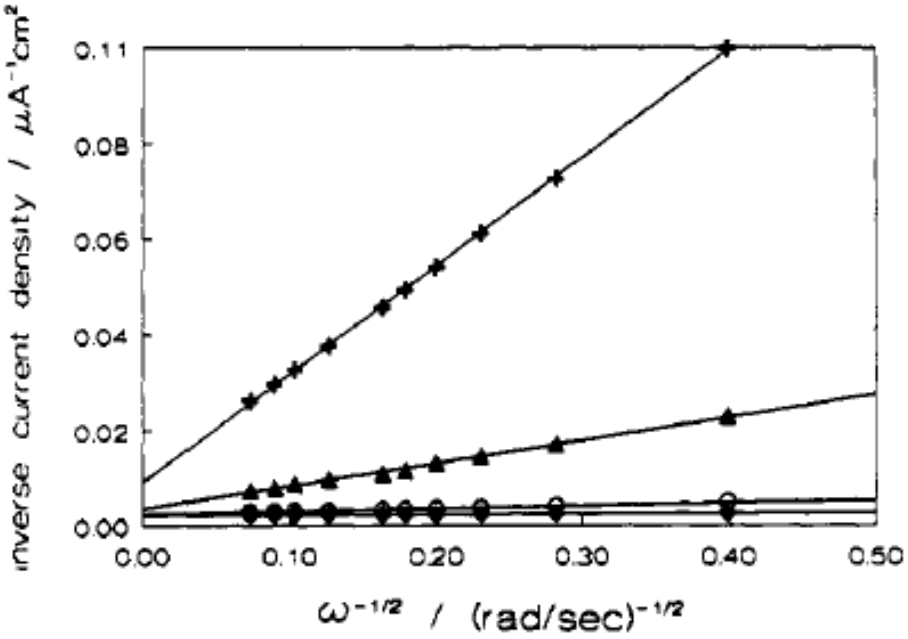
$$\frac{1}{i_L} = 0.62nFAD^{2/3}C\nu^{-1/6}\omega^{1/2} + \frac{nFA\Gamma k_{cat}C}{C + K_M}$$

i_L is measured for various ω, and $1/i_L$ is plotted vs $\omega^{1/2}$. The intercept on the $1/i_L$ axis yields $1/i_{cat}$. (*D* can be obtained from the slope).

Sucheta, A., Cammack, R., Weiner, J., Armstrong, F. A. *Biochemistry* **1993**, *32*, 5455–5465

Voltammetry of enzymes

In presence of substrate - Rotating disk electrodes (RDE)



Therefore,

$$\frac{1}{i_L} = \frac{1}{i_{Lev}} + \frac{1}{i_{cat}}$$

or:

$$\frac{1}{i_L} = \frac{1}{0.62nFAD^{\frac{2}{3}}C\nu^{-\frac{1}{6}}\omega^{\frac{1}{2}}} + \frac{C + K_M}{nFA\Gamma k_{cat}C}$$

i_L is measured for various ω, and $1/i_L$ is plotted vs $\omega^{1/2}$. The intercept on the $1/i_L$ axis yields $1/i_{cat}$.
(*D* can be obtained from the slope).

Sucheta, A., Cammack, R., Weiner, J., Armstrong, F. A. *Biochemistry* **1993**, *32*, 5455–5465

Kinetics of reactions with charge carrier or substrate

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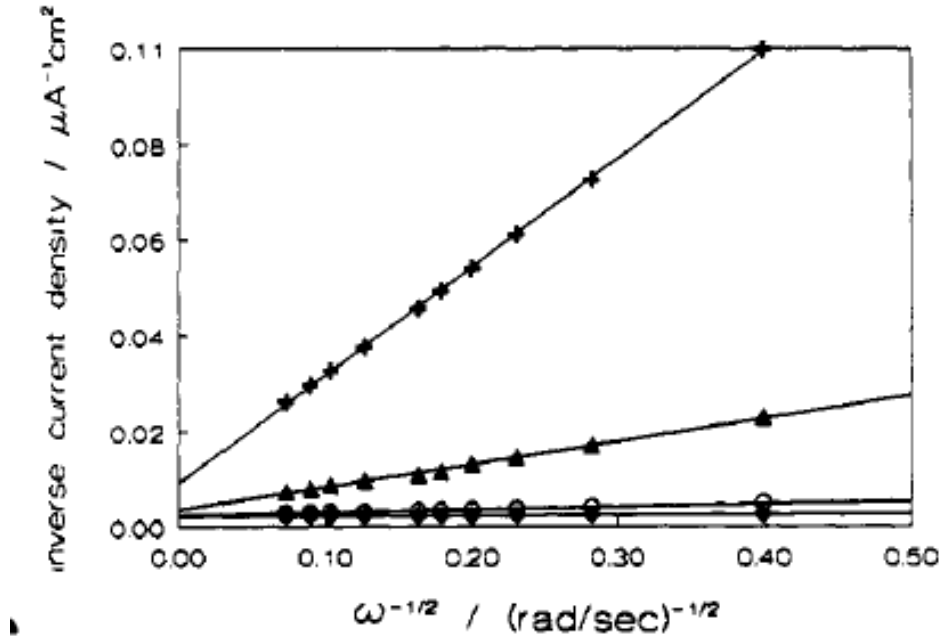

$$\frac{1}{i_L} = \frac{1}{0.62nFAD^{\frac{2}{3}}C\nu^{-\frac{1}{6}}\omega^{\frac{1}{2}}} + \frac{C + K_M}{nFA\Gamma k_{cat}C}$$

The measurements are repeated for increasing substrate concentration.

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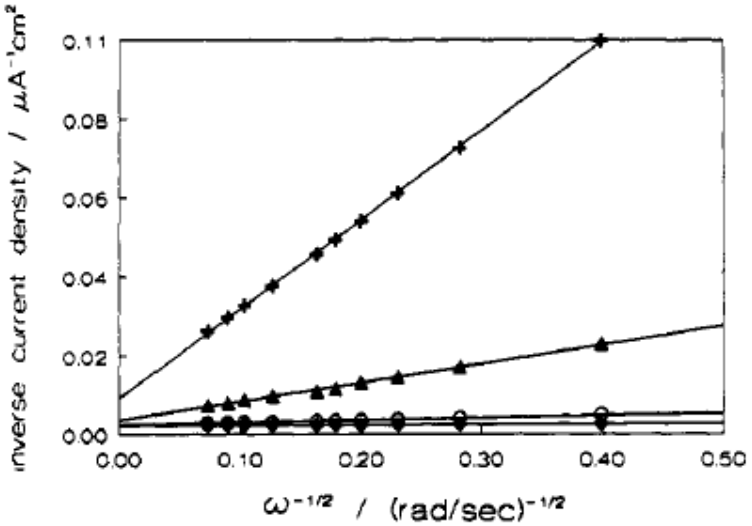
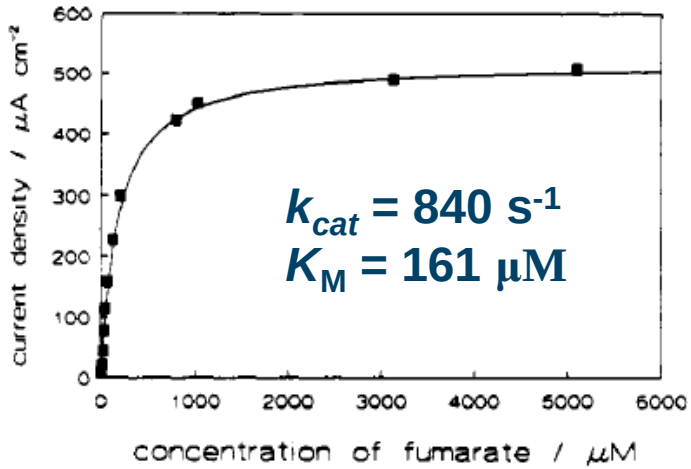
Kinetics of reactions with charge carrier or substrate

In presence of substrate - Rotating disk electrodes (RDE)





$$\frac{1}{i_L} = \frac{1}{0.62nFAD^{\frac{2}{3}}C\nu^{-\frac{1}{6}}\omega^{\frac{1}{2}}} + \frac{C + K_M}{nFA\Gamma k_{cat}C}$$

The measurements are repeated for increasing substrate concentration.

And the i_{cat} values are plotted vs the corresponding C values.

k_{cat} and K_M are obtain from this Michaelis-Menten curve by inserting numerical values for n, F, A and Γ (obtained from CV) in:

$$i_{cat} = \frac{nFA\Gamma k_{cat}C}{C + K_M}$$

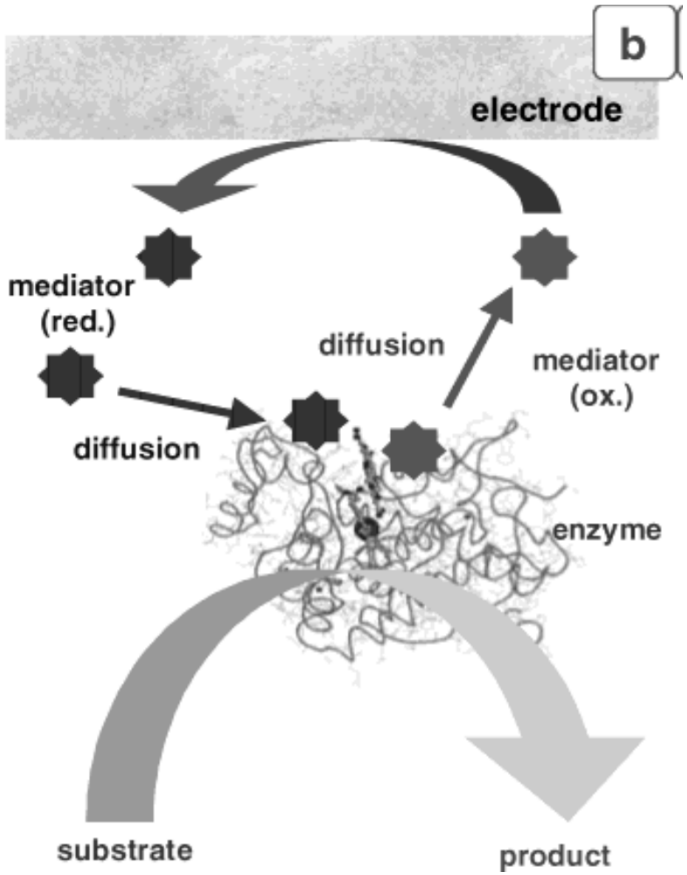
Mediated Electron Transfer (MET)

3 common cases:

- **Both enzyme and mediator are freely diffusing in solution.**

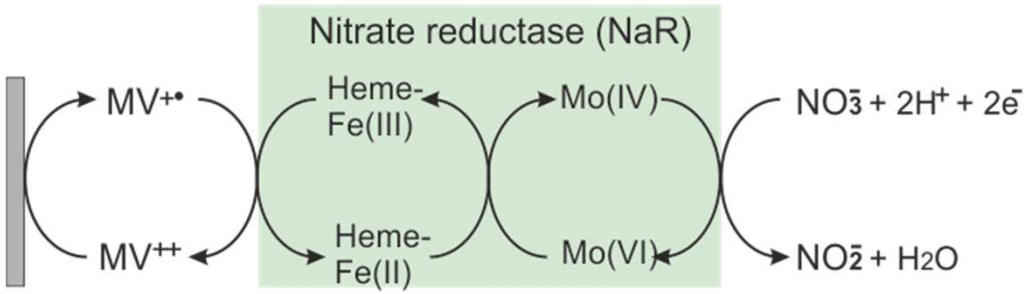
- Enzyme is adsorbed on electrode surface and mediator is freely diffusing in solution.

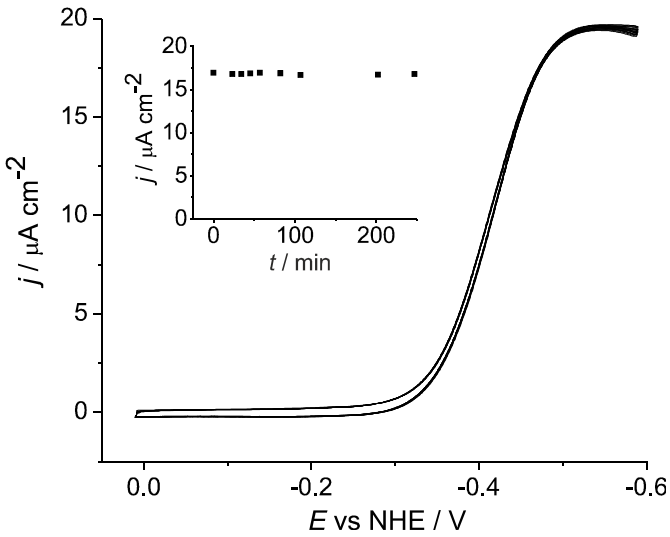
- Both enzyme and mediator are adsorbed on electrode surface.

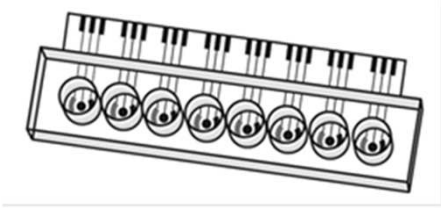


Mediated Bioelectrochemistry

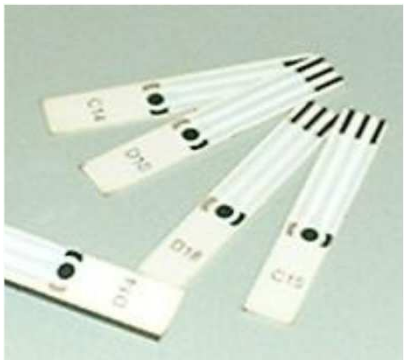
Enzyme and mediator freely diffusing in solution







V_{tot} = 200 µL



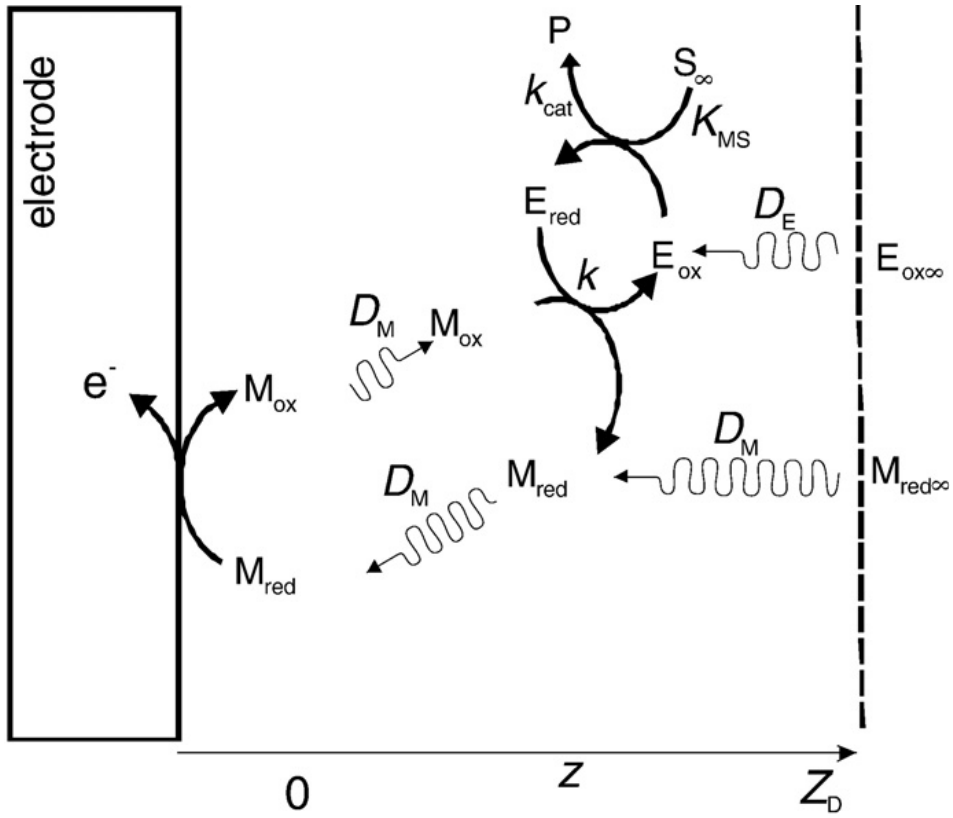
Plumeré, Henig, Campbell,
Anal. Chem., **2012**, 84, 2141-2146

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Mediated Bioelectrochemistry

Enzyme and mediator freely diffusing in solution

- M_{ox} and M_{red}: oxidized and reduced forms of the mediator
- E_{ox} and E_{red}: oxidized and reduced enzyme.
- S and P: substrate and product of the enzymatic reaction.
- D_E and D_M: diffusion coefficients of enzyme and mediator.



Determination of k and k_{cat} ?

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Mediated Bioelectrochemistry

Enzyme and mediator freely diffusing in solution

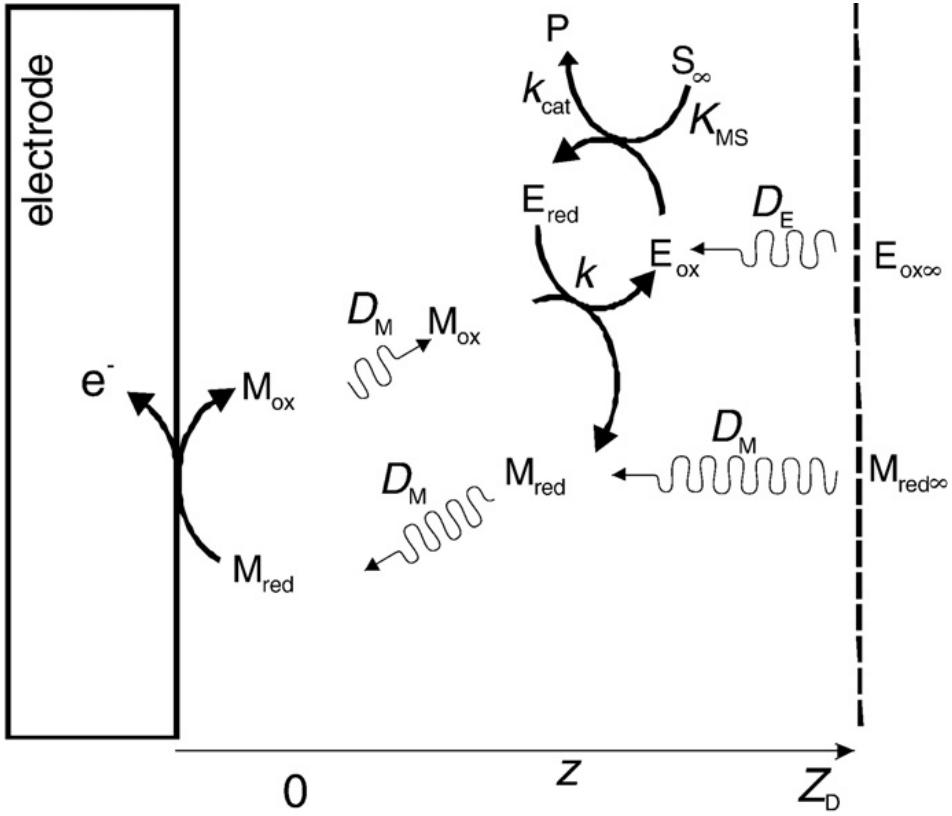
• The reactions occuring in solutions are:

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

$$M_{ox} + E_{red} \xrightarrow{k} M_{red} + E_{ox}$$

with

$$K_{MS} = (k_{-1} + k_{cat})/k_1$$



• At the electrode surface:

$$M_{red} \rightarrow M_{ox} + e^-$$

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Determination of *k*

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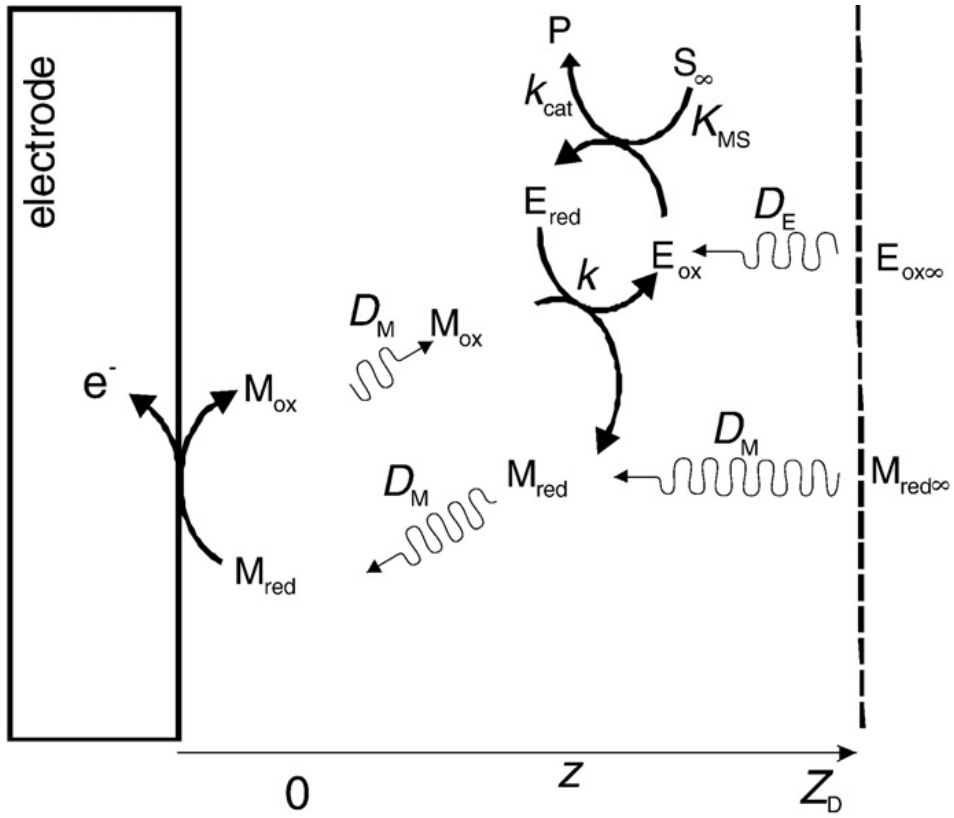

Enzyme - Mediator limited kinetics

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

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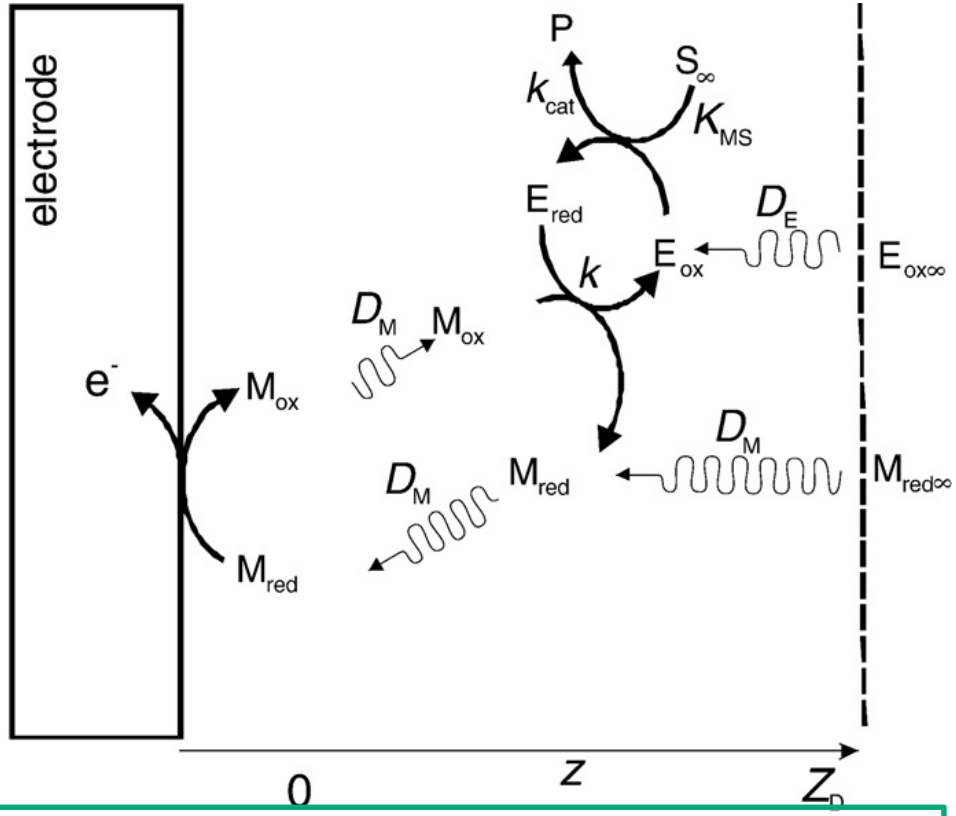


Catalytic reaction E_rC_i' $\quad M_{red} \rightarrow M_{ox} + e^-$

$k\,c_M << k_{cat}$ $\quad M_{ox} + E_{red} \xrightarrow{k} M_{red} + E_{ox}$

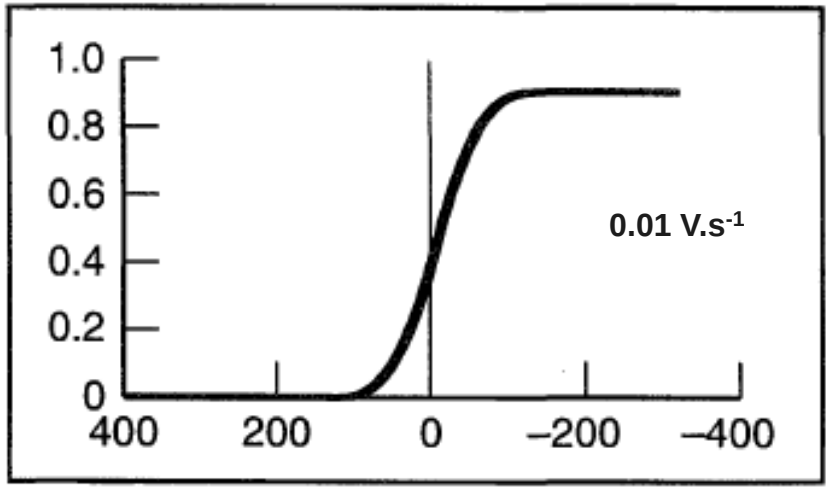
Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Determination of *k*

$$M_{red} \rightarrow M_{ox} + e^-$$

$$M_{ox} + E_{red} \xrightarrow{k} M_{red} + E_{ox}$$

Enzyme - Mediator limited kinetics



i / µA

E / mV

Assumptions:
1. pseudo-first order conditions,
i.e: $k\,c_M << k_{cat}$
2. substrate concentration (c_S) remains constant,
i.e: $c_S >> c_P$
3. long time scale (low scan rates):

$$i_p = nFAc_M(D_M k c_E)^{1/2}$$

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
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Determination of k_{cat} (and K_{MS})

Enzyme and mediator freely diffusing in solution

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with

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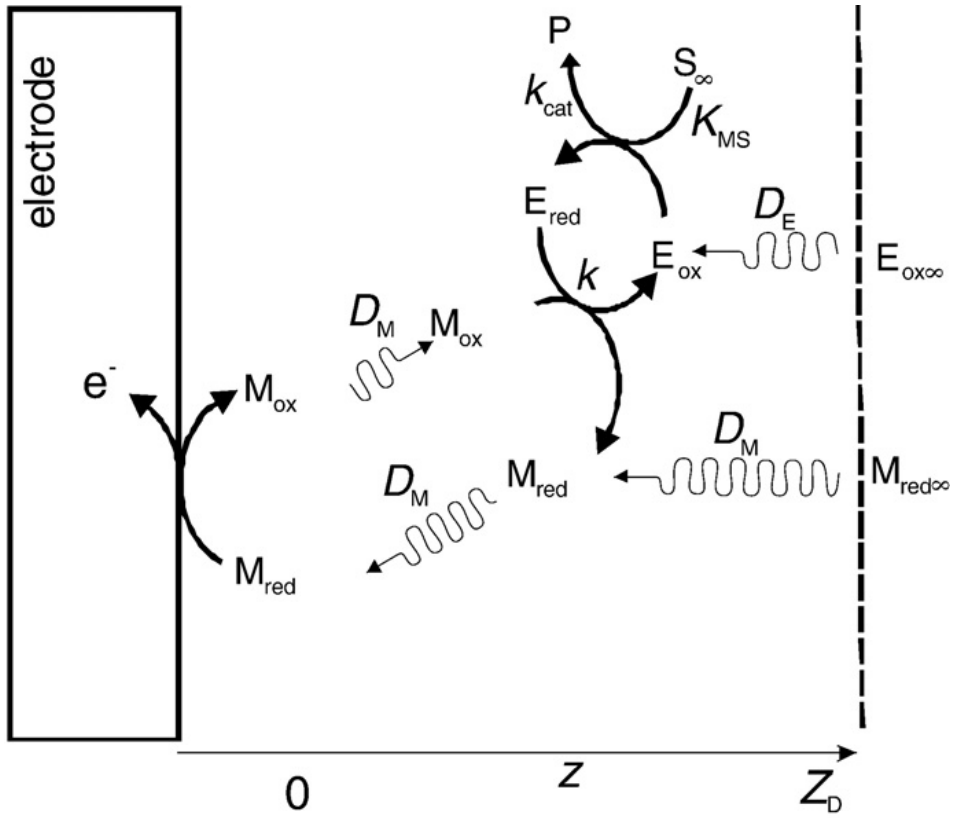


Enzyme - Substrate limited kinetics

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Determination of k_{cat} (and K_{MS})

Enzyme and mediator freely diffusing in solution

Enzyme - Substrate limited kinetics:

At high mediator concentration, the catalytic current is given by:

$$\boldsymbol{i = nFA}\left(\frac{D_{\mathrm{M}} k_{\mathrm{cat}} c_{\mathrm{E}} c_{\mathrm{M}} c_{\mathrm{S}}}{c_{\mathrm{S}} + K_{\mathrm{MS}}}\right)^{\mathbf{1}/\mathbf{2}}$$

For $c_S \ll K_{MS}$

$$\boldsymbol{i = nFA}\left(\frac{D_{\mathrm{M}} k_{\mathrm{cat}} c_{\mathrm{E}} c_{\mathrm{M}} c_{\mathrm{S}}}{K_{\mathrm{MS}}}\right)^{\mathbf{1}/\mathbf{2}}$$

Thus a plot of i against $c_S^{1/2}$ should give a straight line through the origin at low c_S. From the slope of this line a value for the ratio k_{cat}/K_{MS} can be obtained.

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Determination of k_{cat} (and K_{MS})

Enzyme and mediator freely diffusing in solution

Enzyme - Substrate limited kinetics:

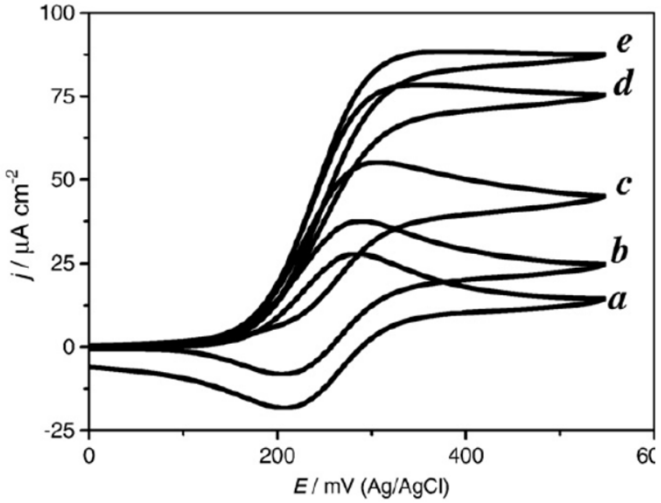
At high mediator concentration, the catalytic current is given by:

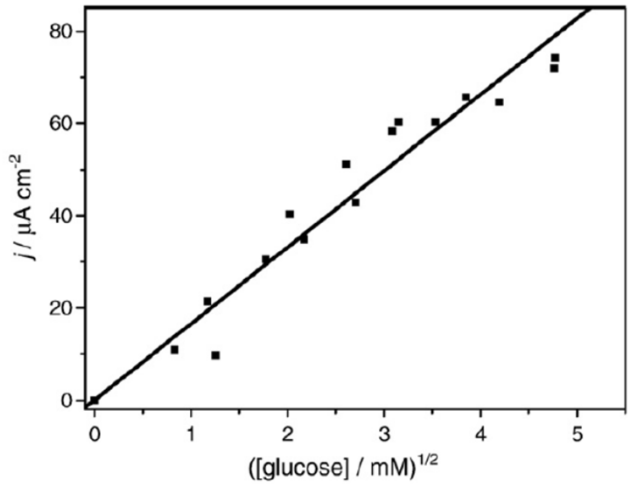
$$\boldsymbol{i = nFA}\left(\frac{D_{\mathrm{M}} k_{\mathrm{cat}} c_{\mathrm{E}} c_{\mathrm{M}} c_{\mathrm{S}}}{c_{\mathrm{S}} + K_{\mathrm{MS}}}\right)^{\frac{1}{2}}$$

For $c_S << K_{MS}$

$$\boldsymbol{i = nFA}\left(\frac{D_{\mathrm{M}} k_{\mathrm{cat}} c_{\mathrm{E}} c_{\mathrm{M}} c_{\mathrm{S}}}{K_{\mathrm{MS}}}\right)^{\frac{1}{2}}$$

Thus a plot of i against $c_S^{1/2}$ should give a straight line through the origin at low c_S. From the slope of this line a value for the ratio k_{cat}/K_{MS} can be obtained.





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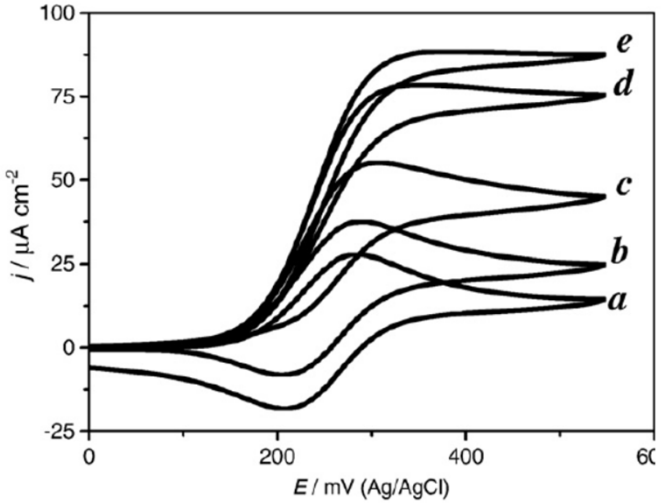
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Determination of k_{cat} (and K_{MS})

Enzyme and mediator freely diffusing in solution

$$i = nFA\left(\frac{D_M k_{cat} c_E c_M c_S}{c_S + K_{MS}}\right)^{\frac{1}{2}}$$



The value of the rate constant k_{cat} is calculated from the current in the saturated region, imax, i.e. when $c_S >> K_{MS}$

$$i_{max} = nFA(D_M k_{cat} c_E c_M)^{\frac{1}{2}}$$

To verify the assumptions based on K_{MS}, the value of the latter is then extracted from the value of k_{cat}/K_{MS}

Bourdillon, C., Demaille, C., Moiroux, J., Saveant, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2–10
Flexer, V., Ielmini, M., Calvo, E., Bartlett, P. *Bioelectrochemistry* **2008**, *74*, 201–209.

Determination of k, k_{cat} (and K_M)

Summary: Enzyme and mediator freely diffusing in solution

Enzyme - Mediator limited kinetics
(Determination of k)

$$k\, c_M << k_{cat}$$

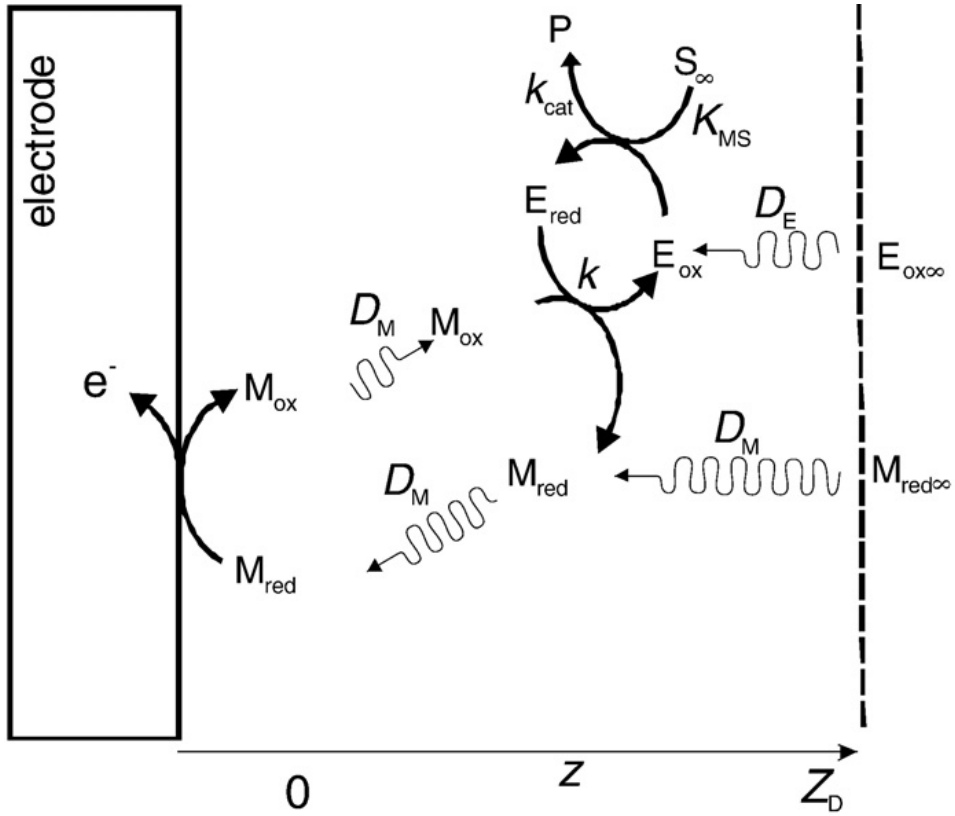
$$c_S << c_M$$

Enzyme - substrate limited kinetics
(determination of k_{cat})

$$c_S >> K_{MS}$$

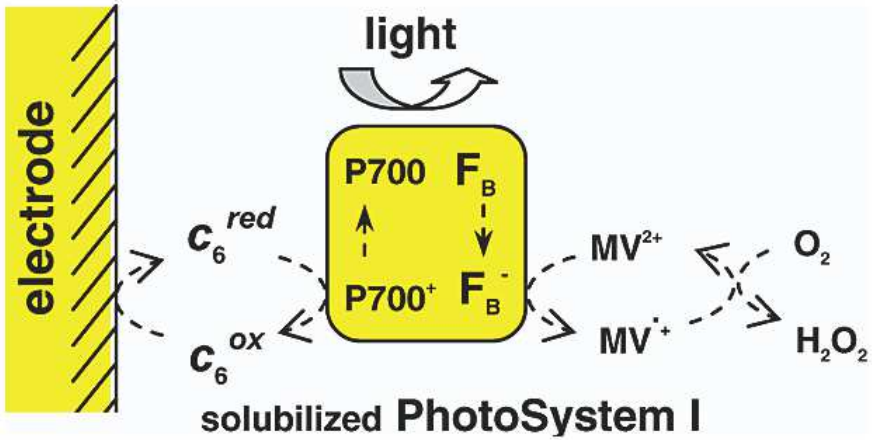
Enzyme - substrate limited kinetics
(determination of K_{MS} via k_{cat}/K_{MS})

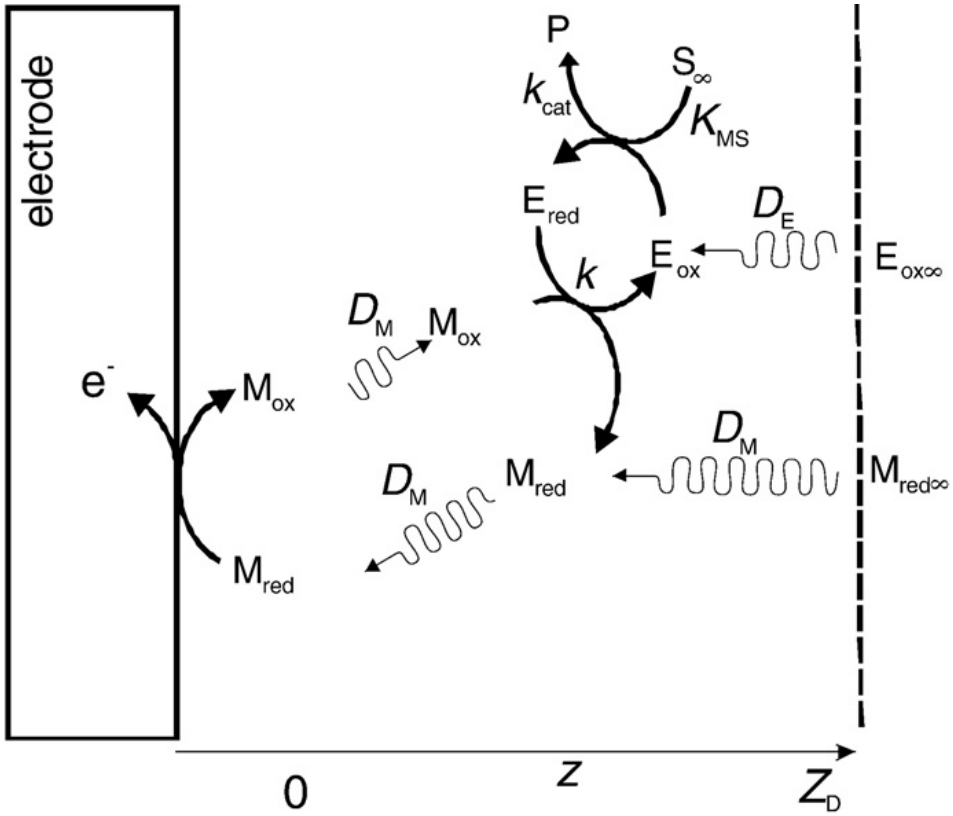
$$c_S << K_{MS}$$



Mediated Bioelectrochemistry – PS1

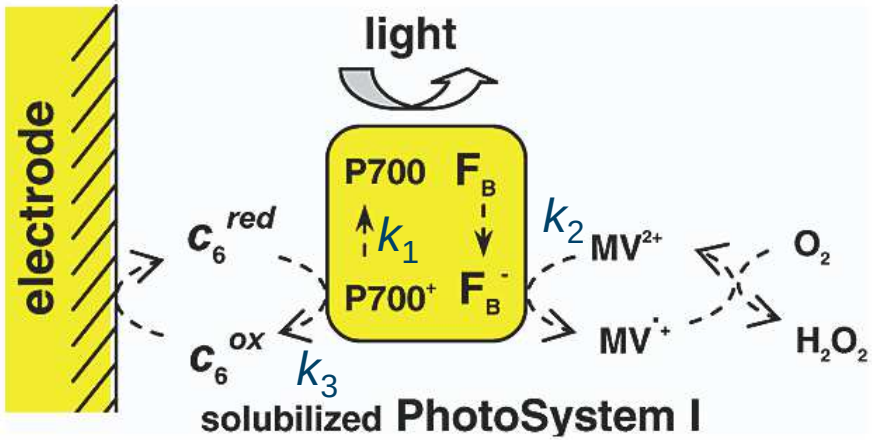
PS1 and mediators freely diffusing in solution

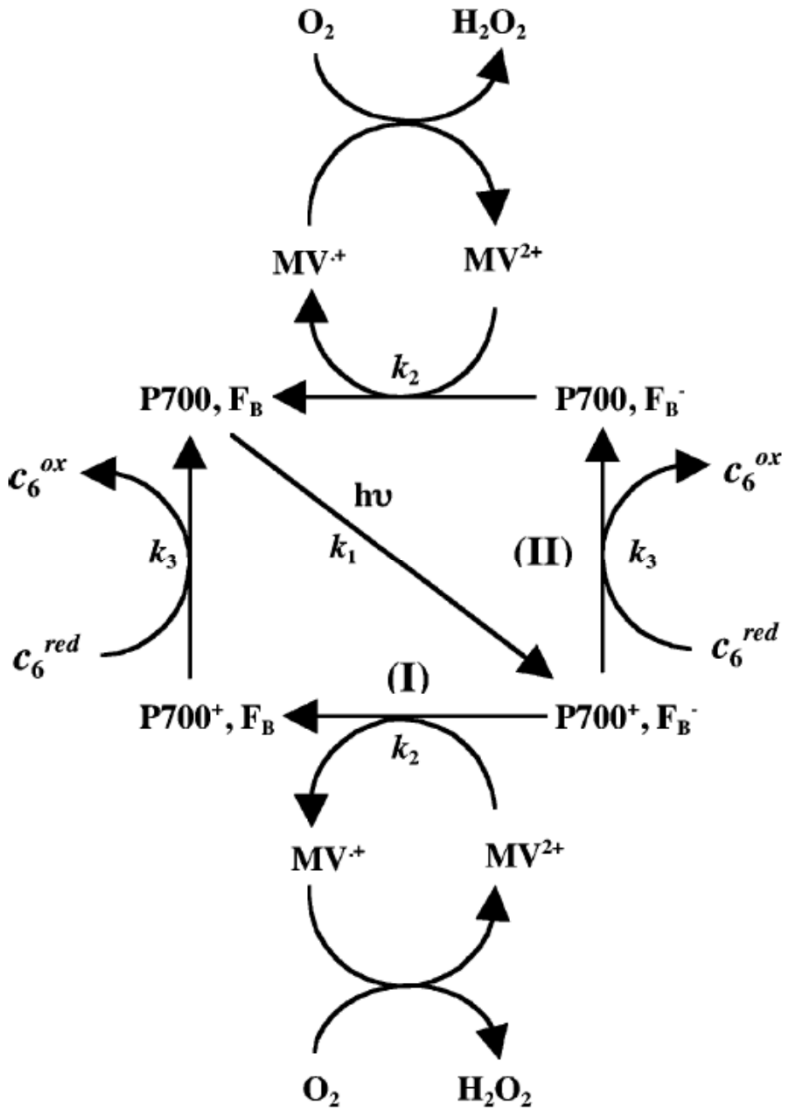




Proux-Delrouyre, V., Demaille, C., Leibl, W., Sétif, P., Bottin, H., Bourdillon, C.
J. Am. Chem. Soc. **2003**, *125*, 13686–13692.

Mediated Bioelectrochemistry – PS1





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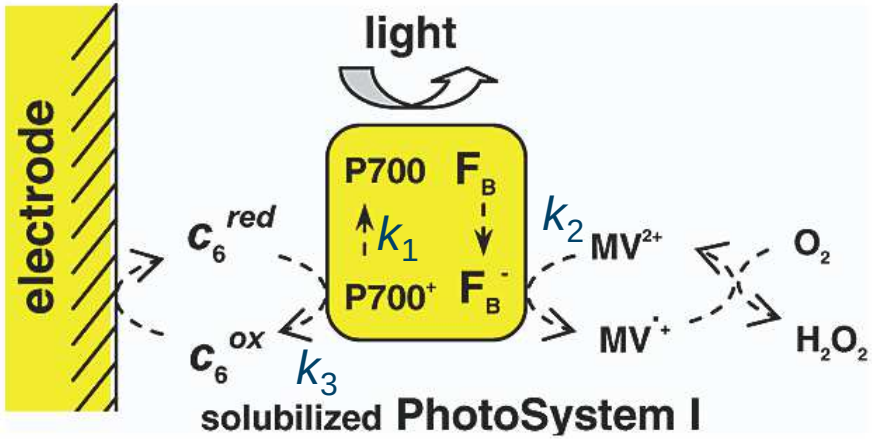
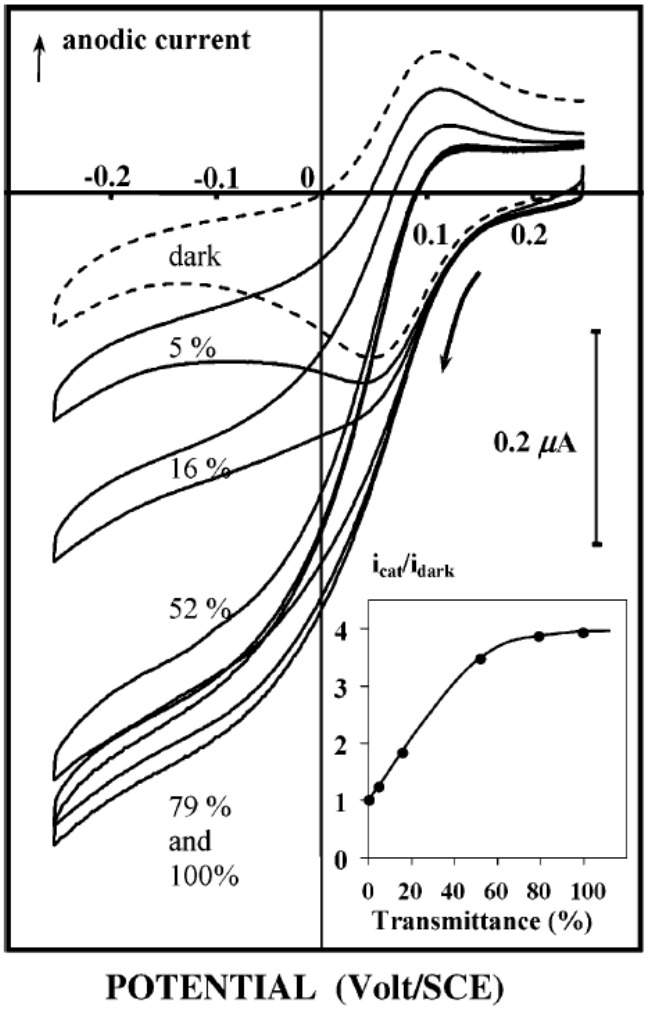
Mediated Bioelectrochemistry – PS1

PS1 and mediators freely diffusing in solution





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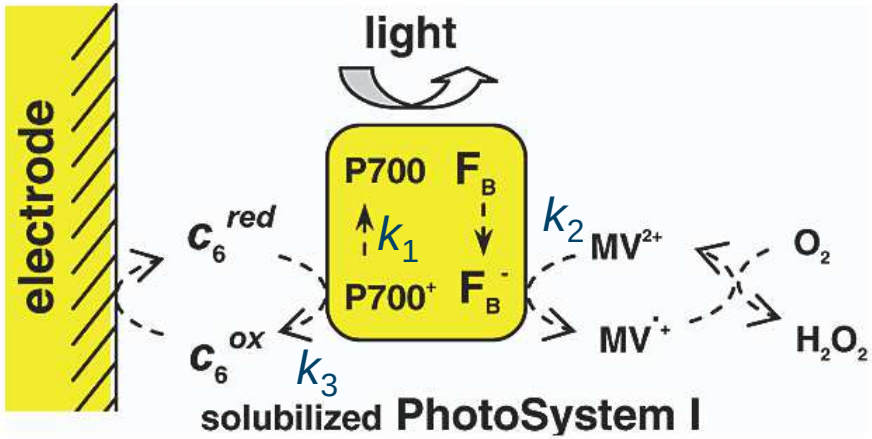
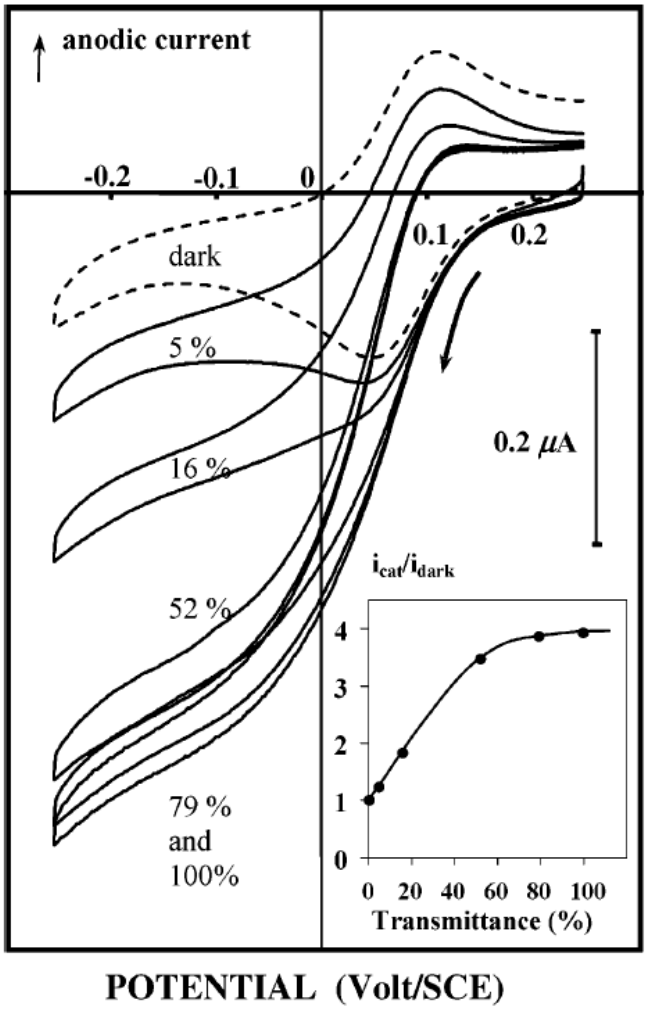
Mediated Bioelectrochemistry – PS1

PS1 and mediators freely diffusing in solution



$$i_{cat} = FS\sqrt{D}C_6^{red}\sqrt{k_{app}[PSI]}$$

$$\frac{i_{cat}}{i_{dark}} = 2.24\sqrt{\frac{RT[PSI]}{Fv}k_{app}}$$



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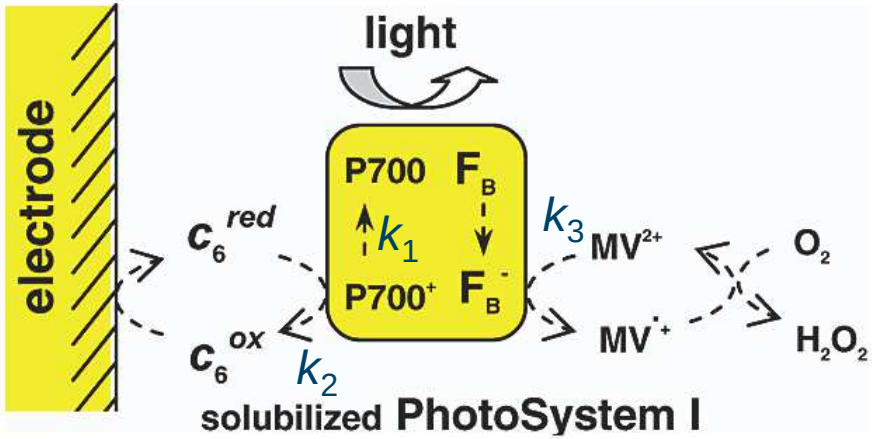
Mediated Bioelectrochemistry – PS1

PS1 and mediators freely diffusing in solution



$$i_{cat} = FS\sqrt{D}C_6^{red}\sqrt{k_{app}[PSI]}$$

$$\frac{i_{cat}}{i_{dark}} = 2.24\sqrt{\frac{RT[PSI]}{Fv}k_{app}}$$

Determination of k_3 ?

$$k_{app} = \frac{2k_3}{\rho+\sigma}\left\{1 - 2[(\rho+\sigma)(\rho-3\sigma)]^{-1/2}\left[\tan^{-1}\left((2\sigma+1)\sqrt{\frac{\rho+\sigma}{3\sigma-\rho}}\right) - \tan^{-1}\left(\sqrt{\frac{\rho+\sigma}{3\sigma-\rho}}\right)\right]\right\}$$

$$\sigma = k_3\, C_6^0/k_2\, C_{MV}^0 \qquad \rho = k_3\, C_6^0/k_1$$

For σ→0 and ρ→0, $k_{app} = k_3$

This is the case for $C_6 \rightarrow 0$

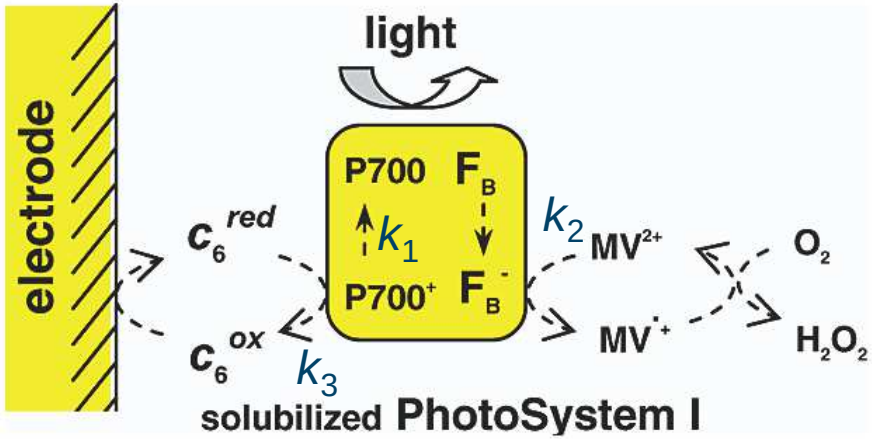
(reduction of $P700^+$ by C_6^{red} becomes rate limiting)

$k_3 = 6 \pm 0.5\ 10^6\ M^{-1}\ s^{-1}$

Proux-Delrouyre, V., Demaille, C., Leibl, W., Sétif, P., Bottin, H., Bourdillon, C. *J. Am. Chem. Soc.* **2003**, *125*, 13686–13692.

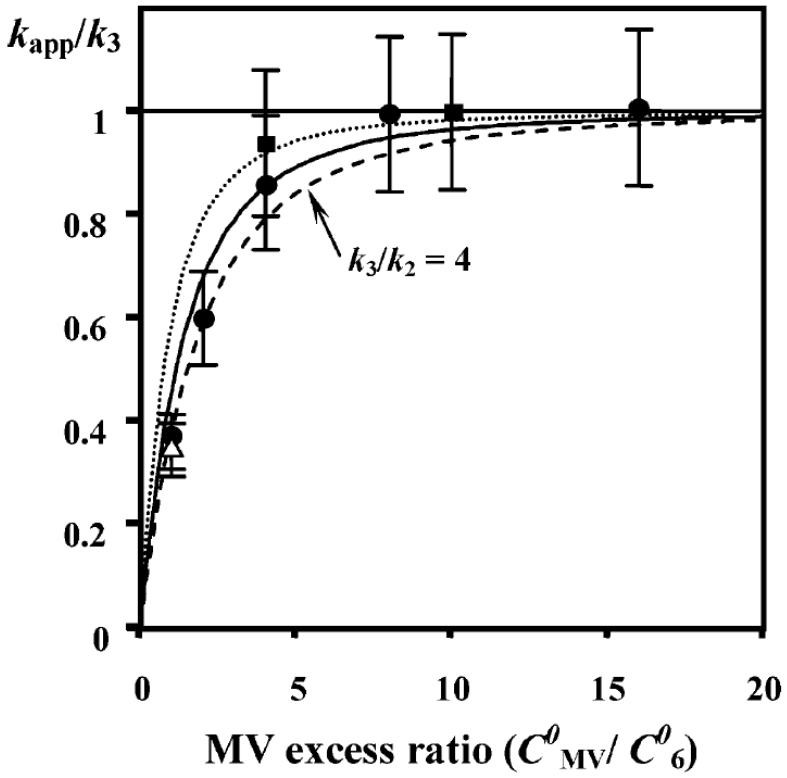
Mediated Bioelectrochemistry – PS1

PS1 and mediators freely diffusing in solution



$$i_{cat} = FS\sqrt{D}C_6^{red}\sqrt{k_{app}[PSI]}$$

$$\frac{i_{cat}}{i_{dark}} = 2.24\sqrt{\frac{RT[PSI]}{Fv}k_{app}}$$



Proux-Delrouyre, V., Demaille, C., Leibl, W., Sétif, P., Bottin, H., Bourdillon, C.
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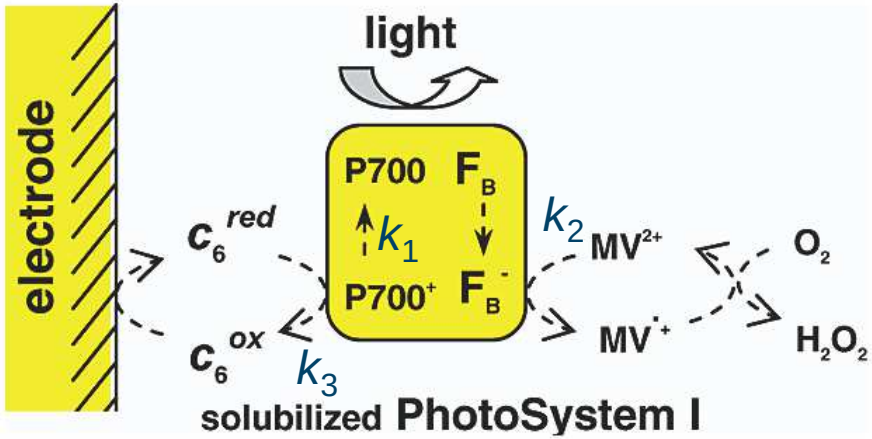


$$i_{cat} = FS\sqrt{D}C_6^{red}\sqrt{k_{app}[PSI]}$$

$$\frac{i_{cat}}{i_{dark}} = 2.24\sqrt{\frac{RT[PSI]}{Fv}k_{app}}$$

Determination of k_2 ?

$$\Psi_p = \frac{2k_3}{\rho+\sigma}\left\{1 - 2[(\rho+\sigma)(\rho-3\sigma)]^{-1/2}\left[\tan^{-1}\left((2\sigma+1)\sqrt{\frac{\rho+\sigma}{3\sigma-\rho}}\right) - \tan^{-1}\left(\sqrt{\frac{\rho+\sigma}{3\sigma-\rho}}\right)\right]\right\}$$

$$\sigma = k_3 C_6^0/k_2 C_{MV}^0 \qquad \rho = k_3 C_6^0/k_1$$

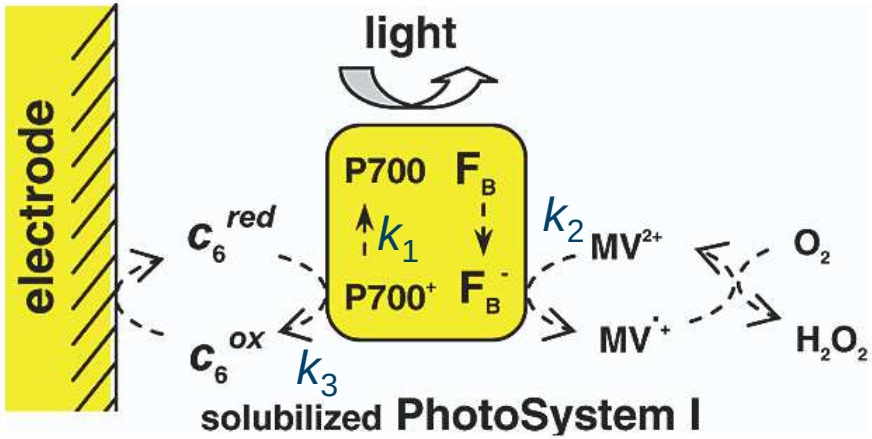
Decrease viologen concentration:
ncrease σ but ρ→0,

$$k_{app} = \frac{2k_2C_{MV}^0}{C_6^0}\left(1 - \frac{2k_2C_{MV}^0}{\sqrt{3}k_3C_6^0}\left[\tan^{-1}\left(\frac{1+2k_3C_6^0/k_2C_{MV}^0}{\sqrt{3}}\right) - \pi/6\right]\right)$$

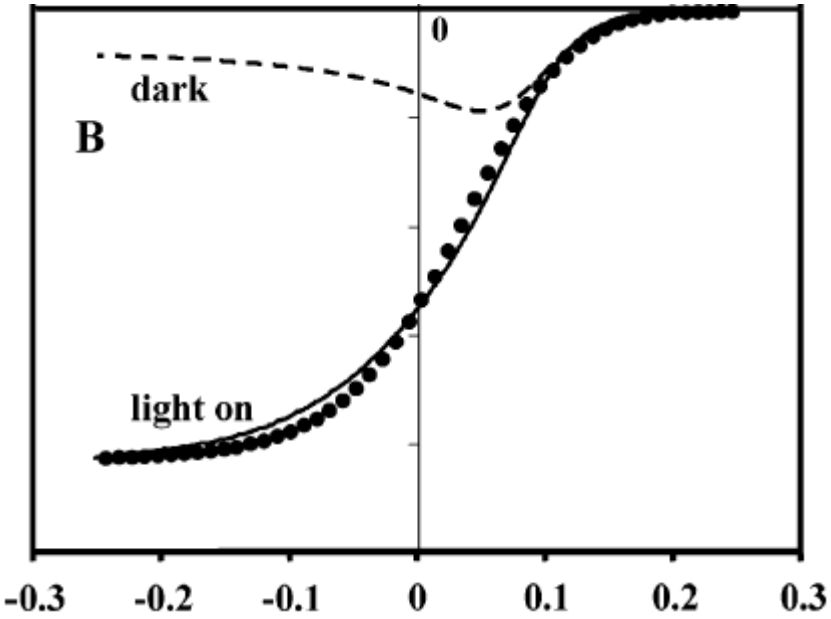
Proux-Delrouyre, V., Demaille, C., Leibl, W., Sétif, P., Bottin, H., Bourdillon, C.
J. Am. Chem. Soc. **2003**, *125*, 13686–13692.

Mediated Bioelectrochemistry – PS1

PS1 and mediators freely diffusing in solution



k_2 ?



$$i_{cat} = FS\sqrt{D}C_6^{red}\sqrt{k_{app}[PSI]}$$

$$\frac{i_{cat}}{i_{dark}} = 2.24\sqrt{\frac{RT[PSI]}{Fv}k_{app}}$$

$$k_{app} = \frac{2k_2C_{MV}^0}{C_6^0}\left(1 - \frac{2k_2C_{MV}^0}{\sqrt{3}k_3C_6^0}\left[\tan^{-1}\left(\frac{1 + 2k_3C_6^0/k_2C_{MV}^0}{\sqrt{3}}\right) - \pi/6\right]\right)$$

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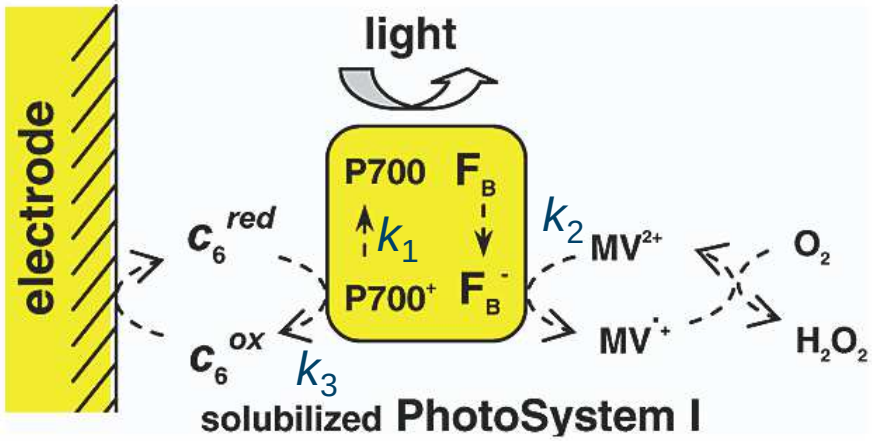
Mediated Bioelectrochemistry – PS1

PS1 and mediators freely diffusing in solution



→ increase concentration of the quencher (O_2) as qualitative test for charge recombination processes.

Charge recombination ?
- between $MV^{+\bullet}$ and electrode
- between $MV^{+\bullet}$ and $C6^{ox}$
- between $MV^{+\bullet}$ and $P700^+$

→ compare k_2 values from:
- Laser flash experiments (single turnover)
- Electrochemical experiments (steady-state conditions)

Proux-Delrouyre, V., Demaille, C., Leibl, W., Sétif, P., Bottin, H., Bourdillon, C.
J. Am. Chem. Soc. **2003**, *125*, 13686–13692.

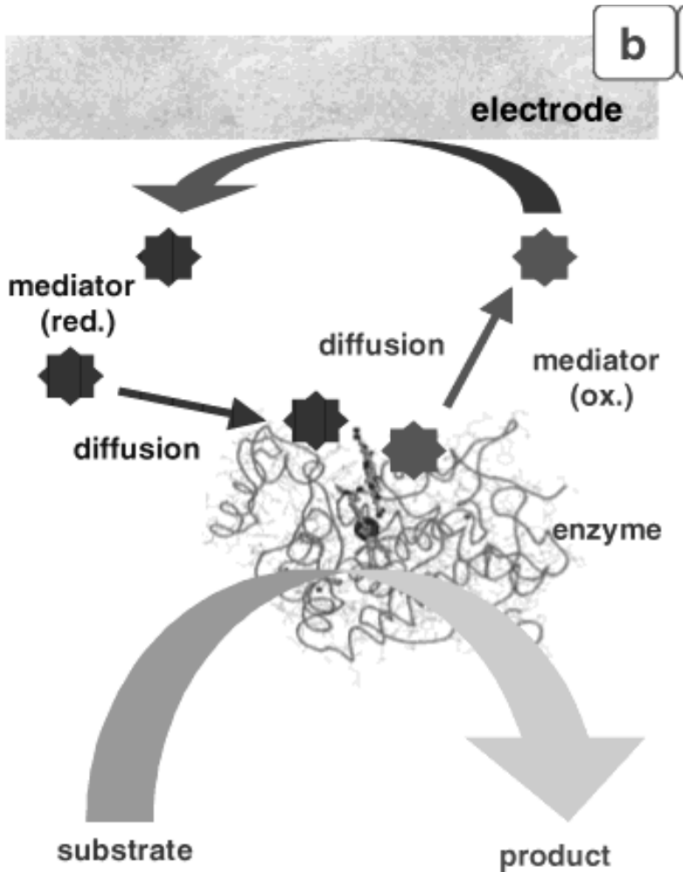
Mediated Bioelectrochemistry

3 common cases:

• Both enzyme and mediator are freely diffusing in solution

• **Enzyme adsorbed on electrode surface and mediator freely diffusing in solution**

• Both enzyme and mediator are adsorbed on electrode surface.



W. H. Campbell, J. Henig, N. Plumeré,
Bioelectrochemistry, **2013**, *93*, 46-50.

Balland, V., Hureau, C., Cusano, A., Liu, Y., Tron, T., Limoges, B.
Chem. Eur. J. **2008**, *14*, 7186–7192.

Mediated Bioelectrochemistry

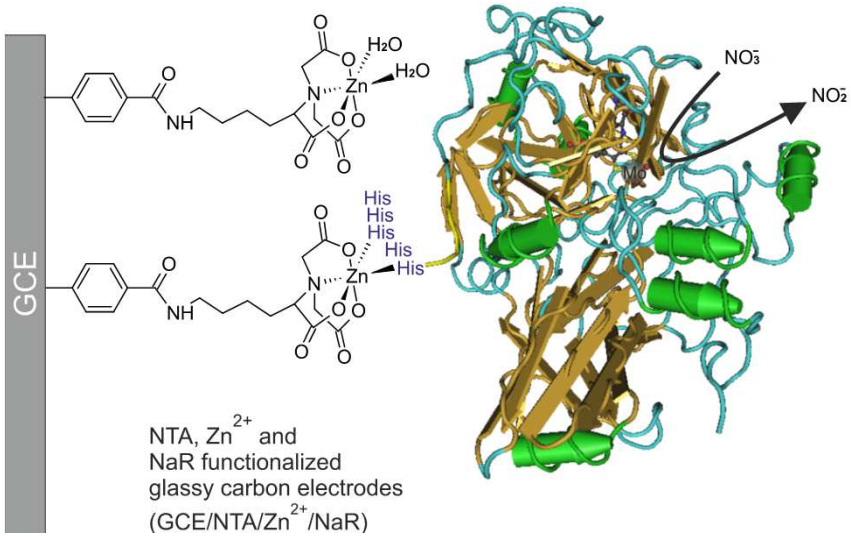
Enzyme adsorbed on electrode surface and mediator freely diffusing in solution

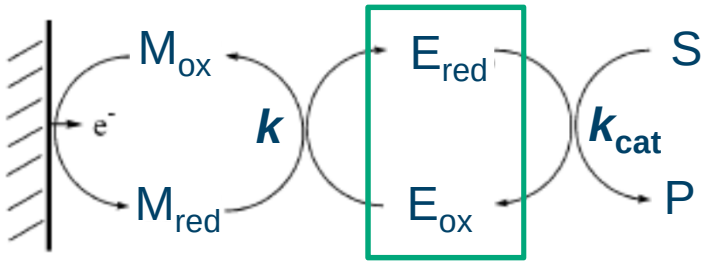
- M_{ox} and M_{red}: oxidized and reduced forms of the mediator

- E_{ox} and E_{red}: oxidized and reduced enzyme.

- S and P: substrate and product of the enzymatic reaction.

- D_M: diffusion coefficients of mediator.





Surface confined

Determination of *k* and k_{cat} ?

Balland, V., Hureau, C., Cusano, A., Liu, Y., Tron, T., Limoges, B.
Chem. Eur. J. **2008**, *14*, 7186–7192.

Determination of *k* and k_{cat}

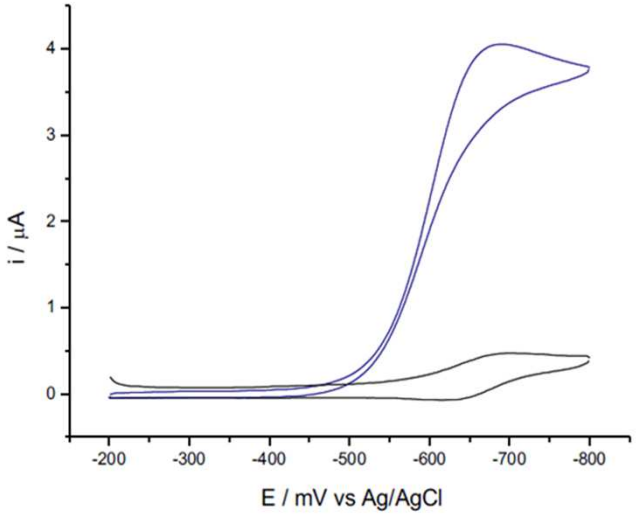
Enzyme adsorbed on electrode surface and mediator freely diffusing in solution

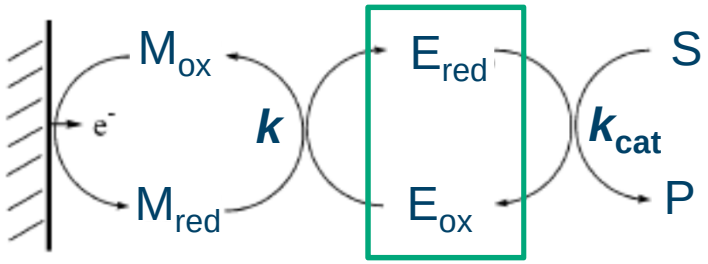
The current flowing through the electrode according to the reaction scheme below is given the following equation:

$$i = i_{\mathrm{D}} + i_{\mathrm{cat}}$$

$$i = FAD_{\mathrm{M}}\left(\frac{\partial c_{\mathrm{M}}}{\partial x}\right)_{x=0} + \frac{nFA\Gamma_{\mathrm{E}}}{\frac{1}{k_{\mathrm{cat}}} + \frac{1}{k\,c_{\mathrm{M}}}}$$

n: number of electrons involved in the reduction or oxidation of S to P.
F: Faraday constant
A: electrode surface area.
Γ_E: enzyme surface concentration.





Surface confined

Balland, V., Hureau, C., Cusano, A., Liu, Y., Tron, T., Limoges, B.
Chem. Eur. J. **2008**, *14*, 7186–7192.

Determination of k and k_{cat}

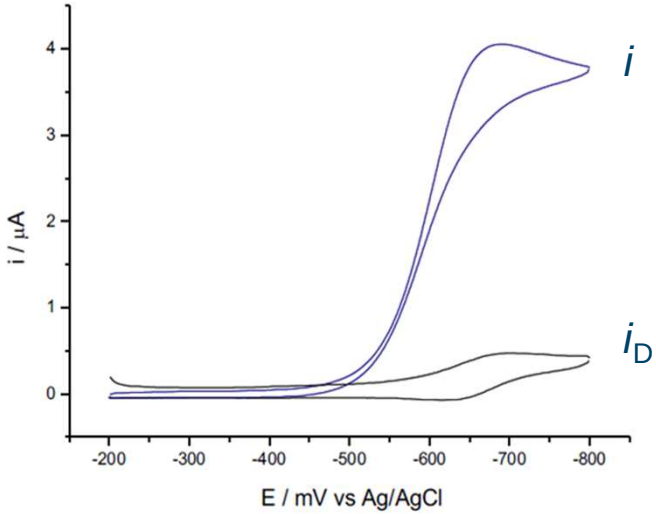
Enzyme adsorbed on electrode surface and mediator freely diffusing in solution

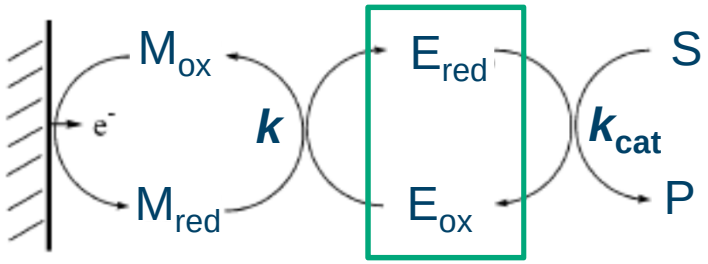
i_{cat} is obtained by subtracting the diffusion current (i_D) from the total current (i).

The experiment is repeated for several c_M values and $1/i_{cat}$ is plotted vs $1/c_M$

$$i_{cat} = \frac{nFA\Gamma_E}{\frac{1}{k_{cat}} + \frac{1}{k\,c_M}}$$

$$\frac{1}{i_{cat}} = \frac{1}{k_{cat}nFA\Gamma_E} + \frac{1}{kc_MnFA\Gamma_E}$$





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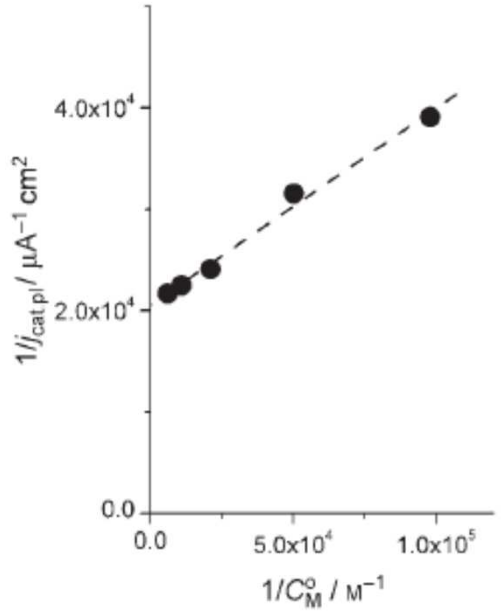
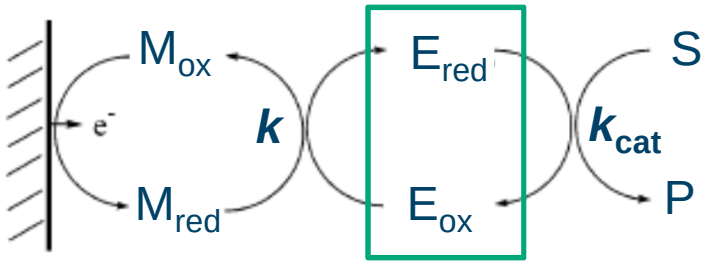
From intercept: $k_{cat}\Gamma_E$ value
From slope: $k\Gamma_E$ value





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Determination of k and k_{cat}

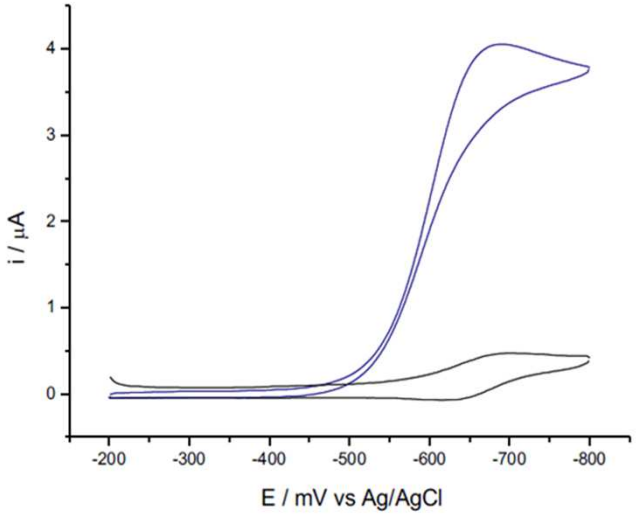
Enzyme adsorbed on electrode surface and mediator freely diffusing in solution

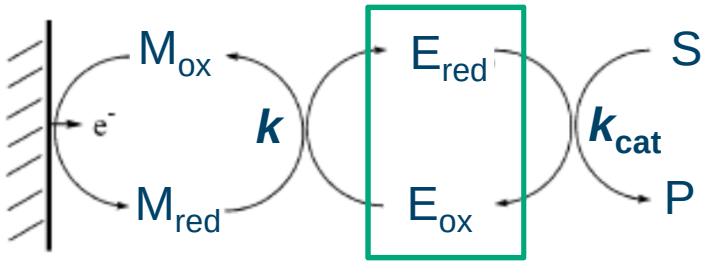
Alternative methods for k_{cat} determination based on equation:

$$i_{cat} = \frac{nFA\Gamma_E}{\frac{1}{k_{cat}} + \frac{1}{k\,c_M}} \qquad (1)$$

If $k_{cat} << kc_M$ (high c_M value), equation (1) simplifies to :

$$i_{cat} = nFA\Gamma_E k_{cat}$$





Surface confined

Determination of Γ_E

Enzyme adsorbed on electrode surface and mediator freely diffusing in solution

From intercept: $k_{cat}\Gamma_E$ value
From slope: $k\Gamma_E$ value

How to determine Γ_E value to obtain k and k_{cat} values ?

- **AFM**
- EQCM
- Desorption of the enzyme followed by quantification.
- SPR
- UV-Vis Absorbance

Manocchi, A. K., Baker, D. R., Pendley, S. S., Nguyen, K., Hurley, M. M., Bruce, B. D., Sumner, J. J., Lundgren, C. A. *Langmuir* **2013**, *29*, 2412–2419.

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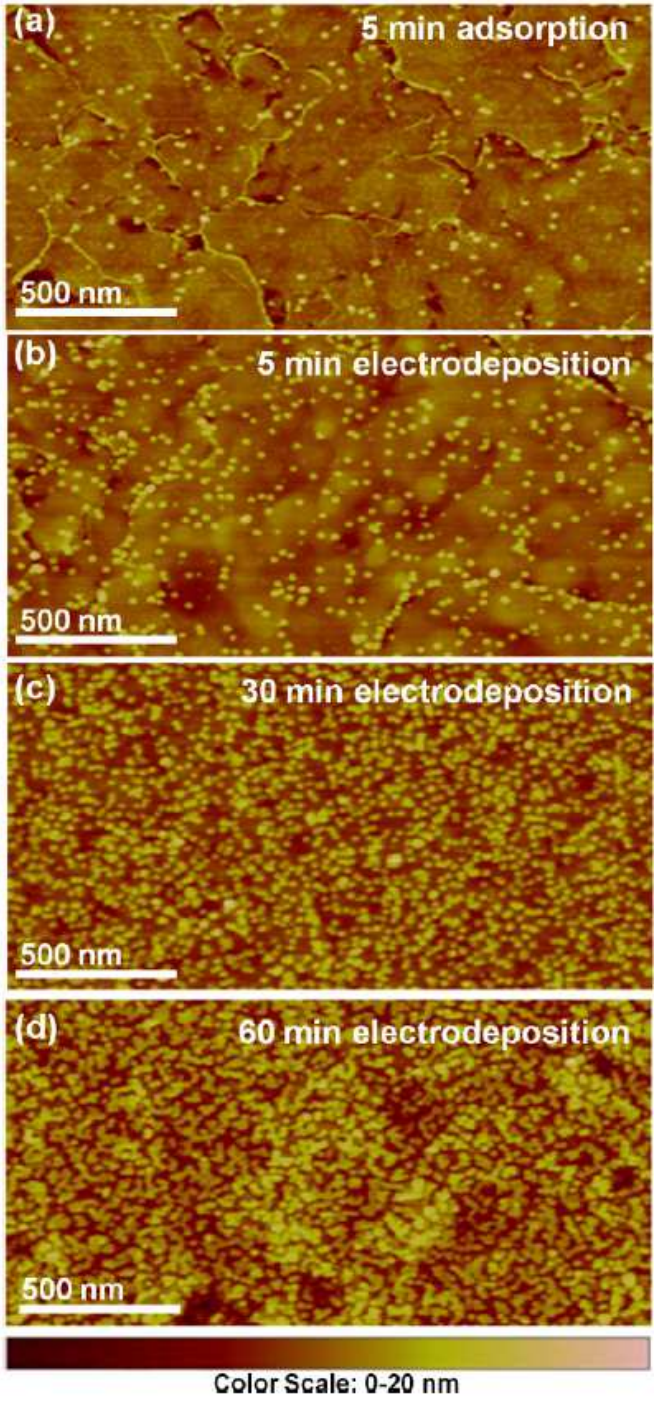

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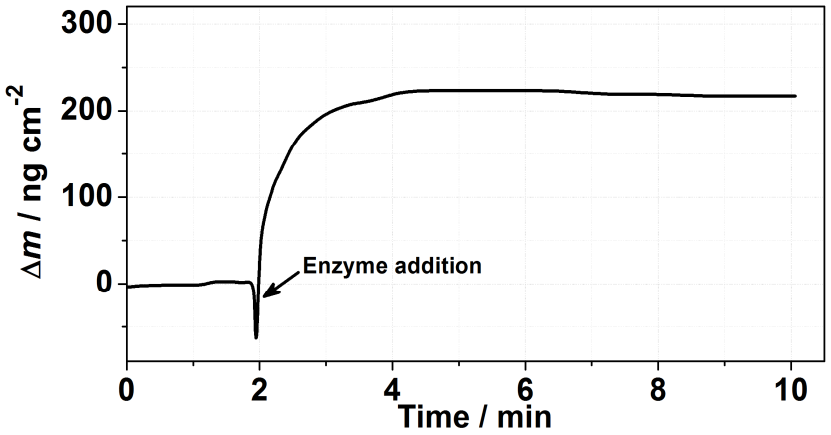

Determination of Γ_E

Enzyme adsorbed on electrode surface and mediator freely diffusing in solution

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From slope: $k\Gamma_E$ value

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- AFM
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- **Desorption of the enzyme followed by quantification.**
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- UV-Vis Absorbance

Balland, V., Hureau, C., Cusano, A., Liu, Y., Tron, T., Limoges, B. *Chem. Eur. J.* **2008**, *14*, 7186–7192.

Determination of Γ_E

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How to determine Γ_E value to obtain k and k_{cat} values ?

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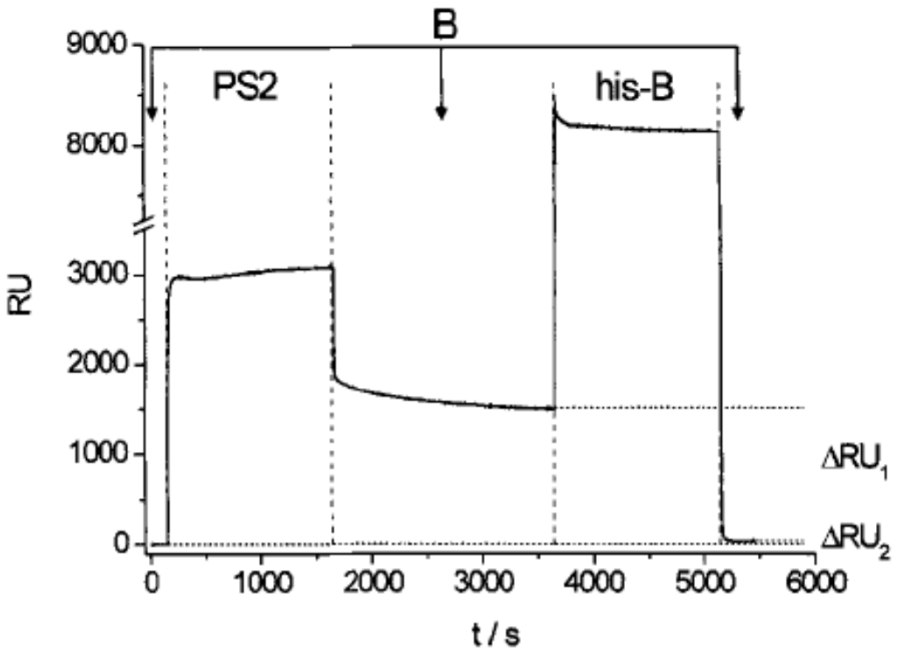
- EQCM
- Desorption of the enzyme followed by quantification.
- **SPR**
- UV-Vis Absorbance



Badura, A., Esper, B., Ataka, K., Grunwald, C., Wöll, C., Kuhlmann, J., Heberle, J., Rögner, M.
Photochem Photobiol **2006**, *82*, 1385.

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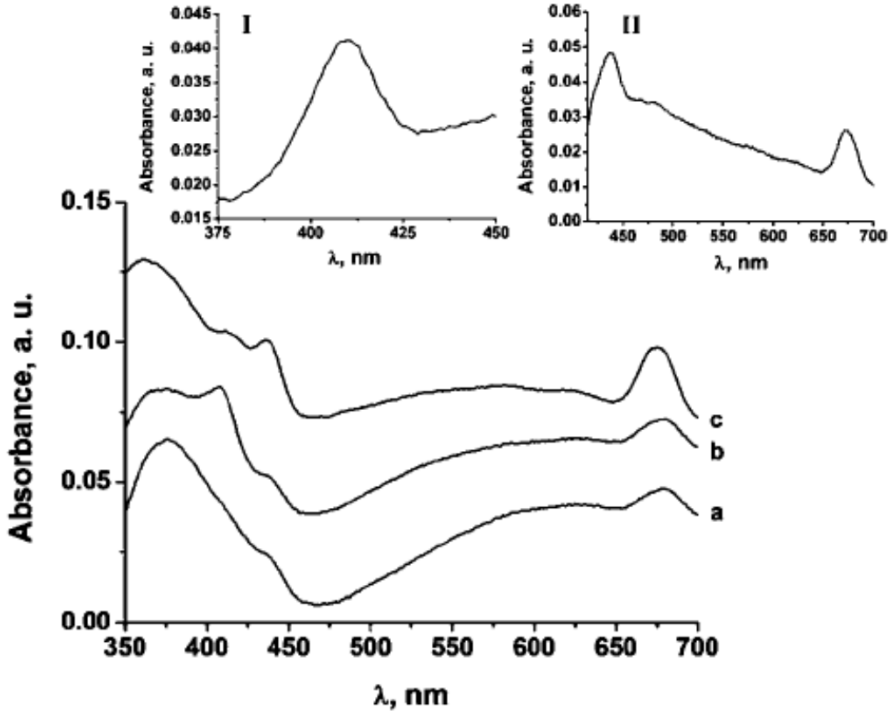


Efrati, A., Tel-Vered, R., Michaeli, D., Nechushtai, R., Willner, I. *Energy Environ. Sci.* **2013**, *6*, 2950.

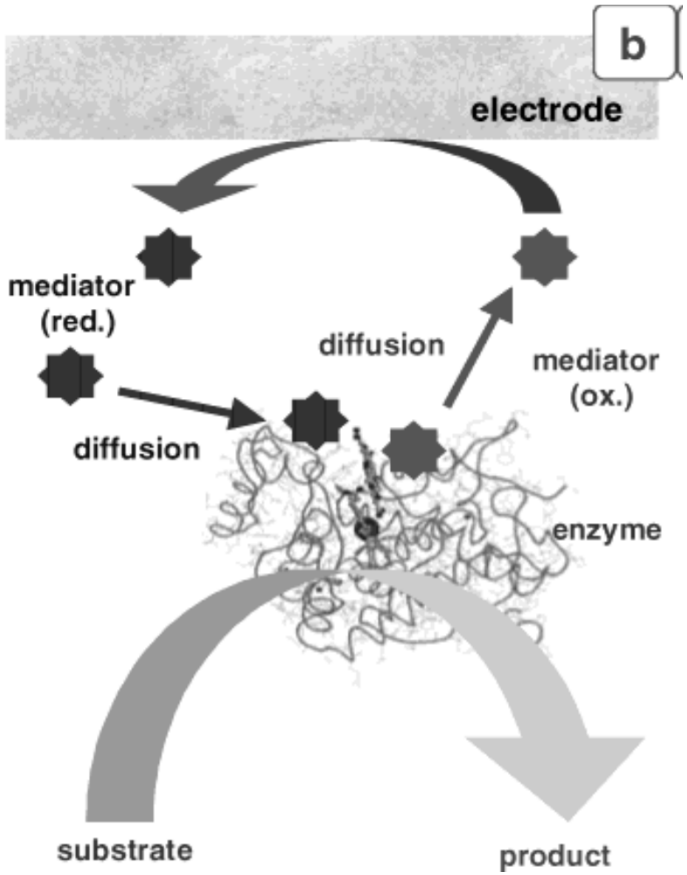
Mediated Bioelectrochemistry

3 cases:

• Both enzyme and mediator are freely diffusing in solution.

• Enzyme is adsorbed on electrode surface and mediator is freely diffusing in solution.

• **Both enzyme and mediator are adsorbed on electrode surface.**



Angew. Chem. Int. Ed. 2004, 43, 3292 –3300

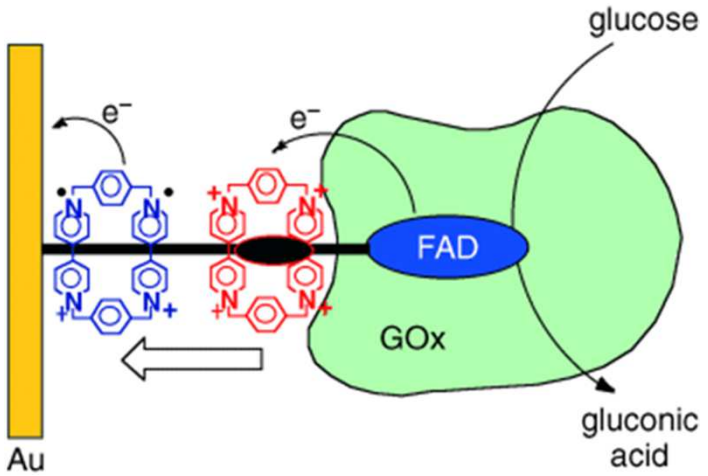
Mediated Bioelectrochemistry

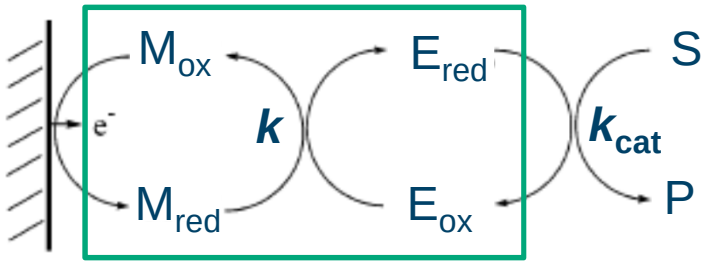
Enzyme and mediator adsorbed on electrode surface

- M_{ox} and M_{red}: oxidized and reduced forms of the mediator

- E_{ox} and E_{red}: oxidized and reduced enzyme.

- S and P: substrate and product of the enzymatic reaction.





Surface confined

Determination of *k* and k_{cat} ?

Mediated Bioelectrochemistry

Enzyme and mediator adsorbed on electrode surface

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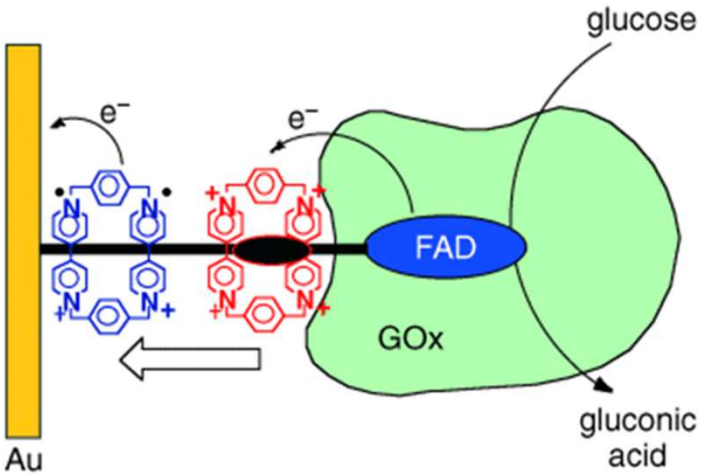
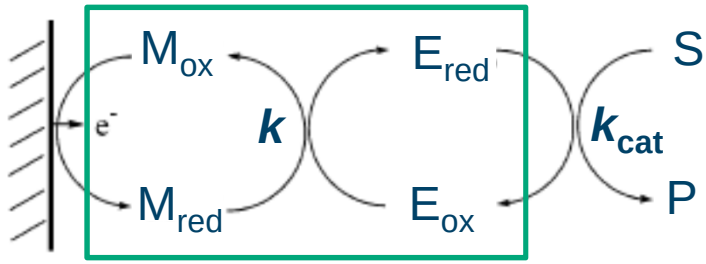


The determination of the individual values of *k* and k_{cat} is difficult since the concentration of M cannot be varied to reach pseudo first order conditions. Instead the global rate constant *k*‘ may be determined:

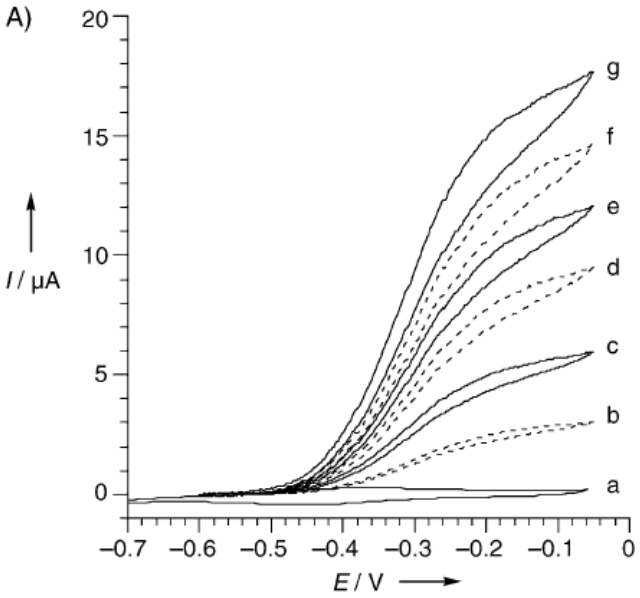
$$\frac{1}{k'} = \frac{1}{k_{cat}} + \frac{1}{k}$$

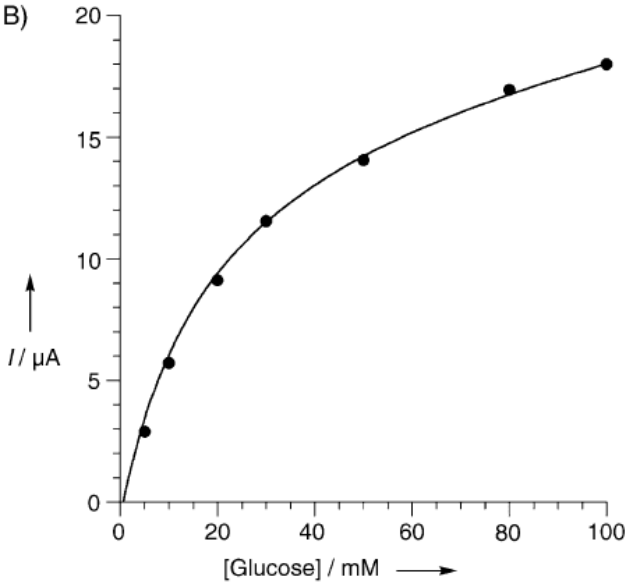


Surface confined

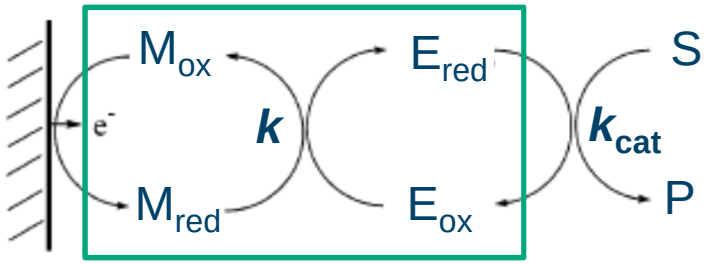
Determination of *k*‘

Enzyme and mediator adsorbed on electrode surface





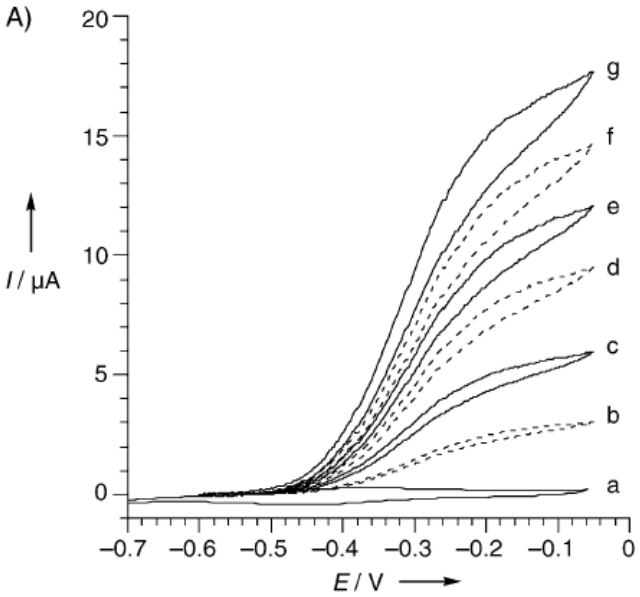
$$i = nFA\frac{k'\Gamma_E c_S}{c_S + K_{MS}}$$

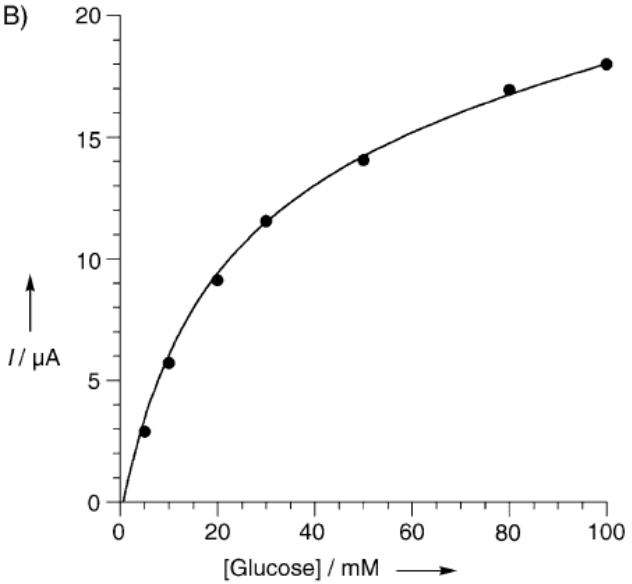


Surface confined

Determination of *k*‘

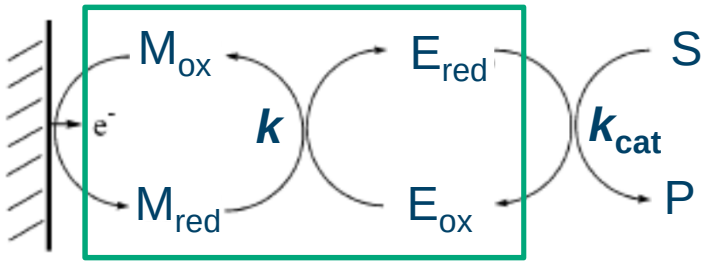
Enzyme and mediator adsorbed on electrode surface





For $c_S << K_{MS}$

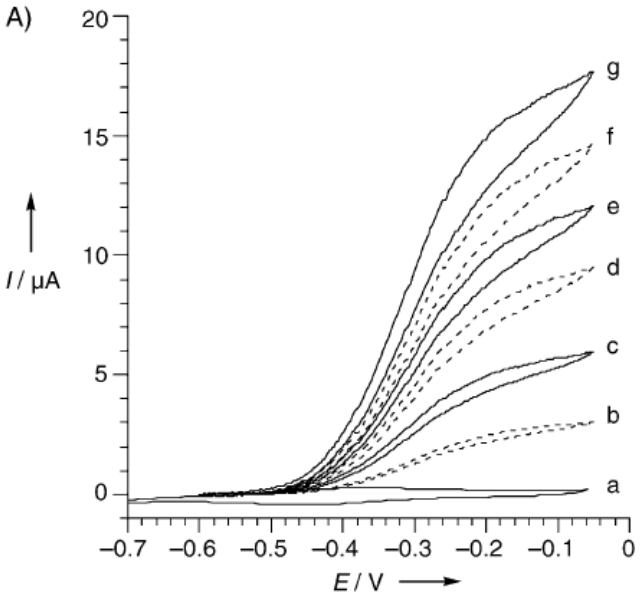
$$i = nFA\frac{k'\Gamma_E c_S}{K_{MS}}$$

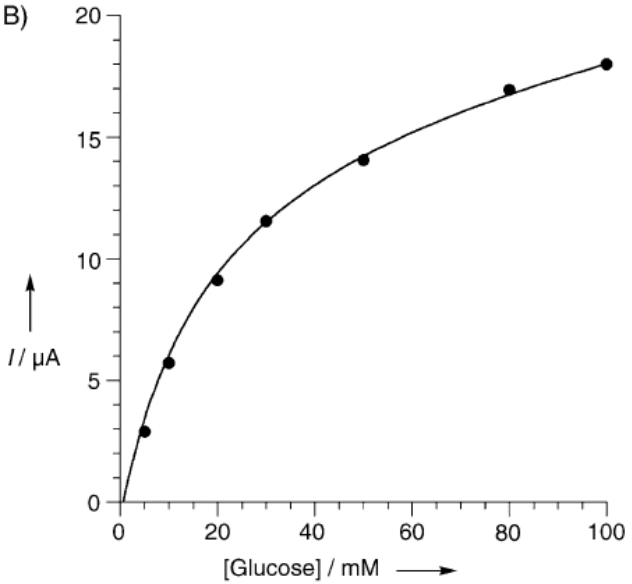


Surface confined

Determination of *k*‘

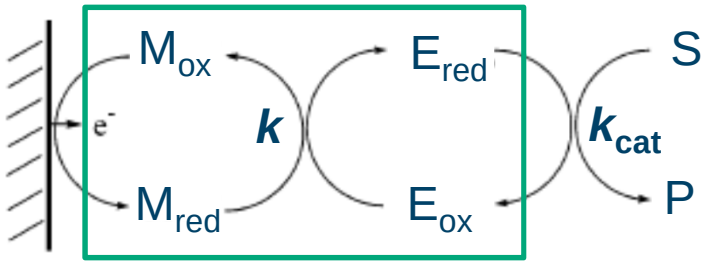
Enzyme and mediator adsorbed on electrode surface





For $c_S >> K_{MS}$

$$i = nFAk'\Gamma_E$$



Surface confined

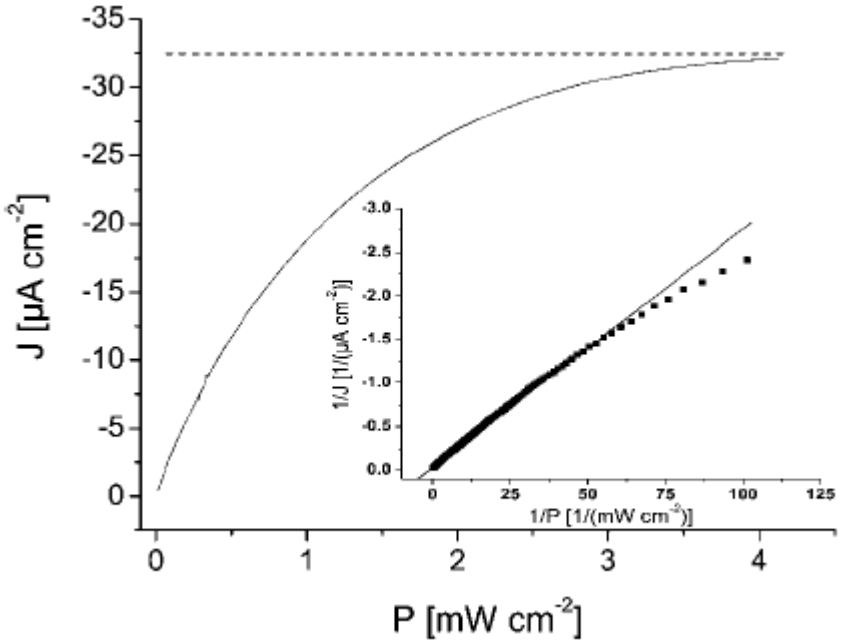
(RDE may be used here as well to ensure substrate mass transport is not limiting)

Mediated Bioelectrochemistry

PS1 and mediator adsorbed on electrode surface

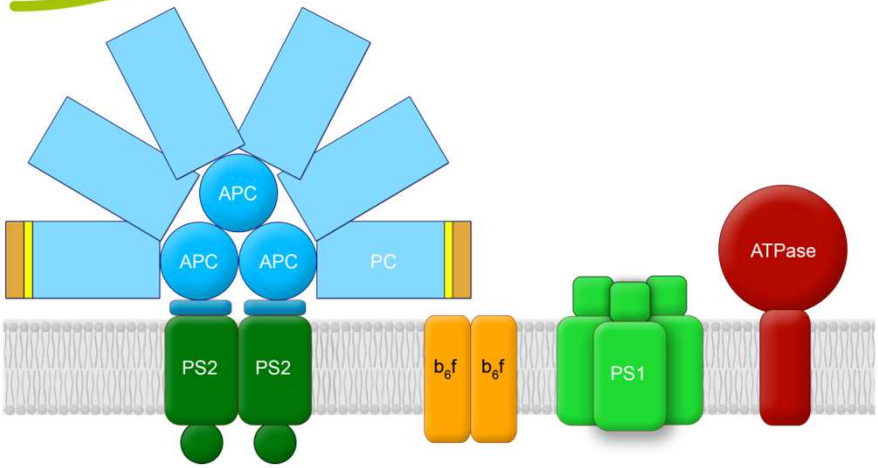
For enzymes, which can be excited by light, the light intensity can be considered as an equivalent to the substrate concentration and the Michaelis–Menten equation can be applied to yield $K_{M/app}$

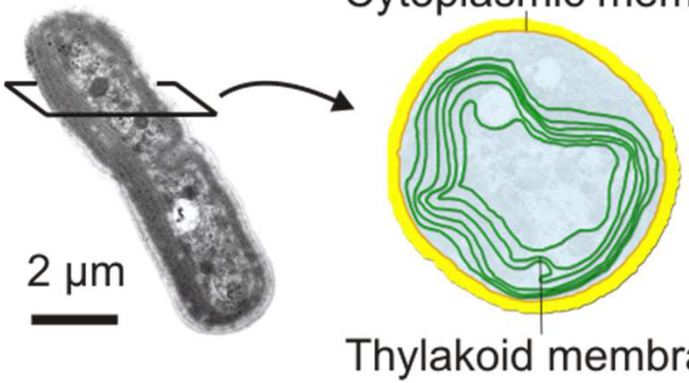
Light saturation curve of PS1 entrapped within a matrix of redox polymer on an electrode. The inset represents the reciprocal Lineweaver–Burk plot obtained from the saturation curve.



Badura, A., Guschin, D., Kothe, T., Kopczak, M. J., Schuhmann, W., Rögner, M. *Energy Environ. Sci.* **2011**, *4*, 2435–2440.

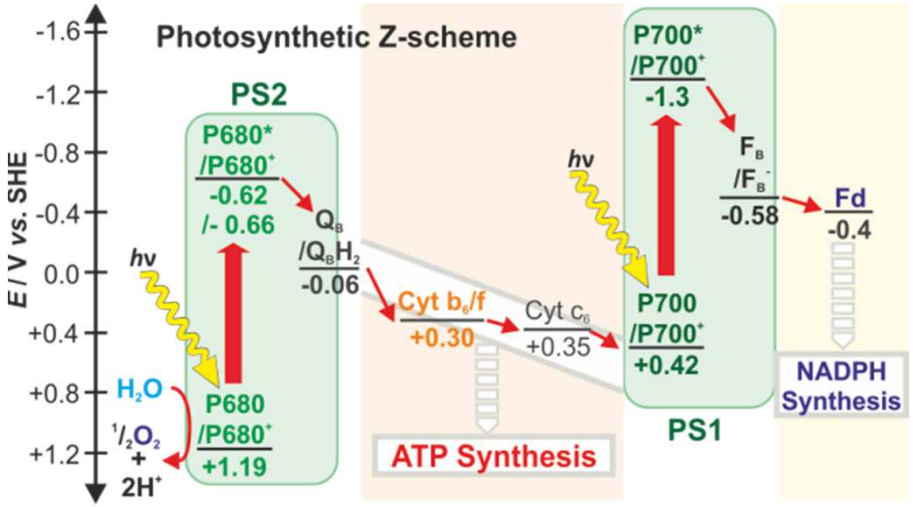
Photosynthesis





Cytoplasmic membrane

Thylakoid membrane

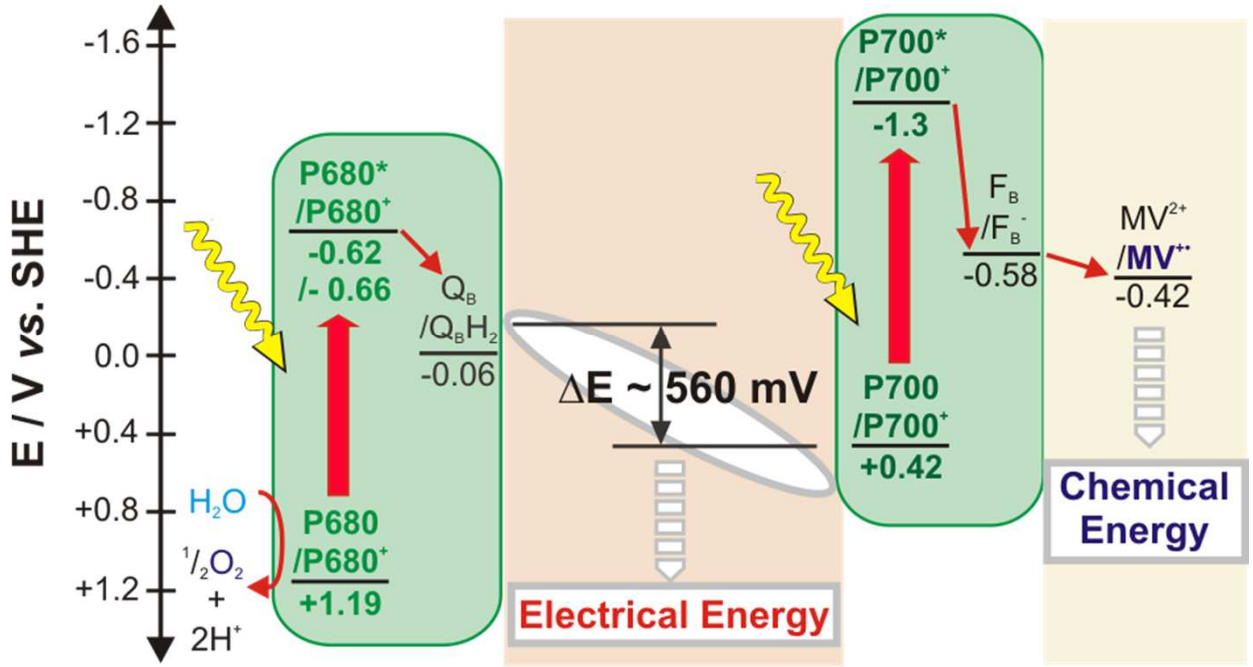


•*Thermosynechococcus elongatus*:

- unicellular, Gram-positive
- thermophilic organism (opt. 55℃)
- photoautotrophe
- completely sequenced genome

➔ modell organism for photosynthesis research

Semi-artificial Z-Scheme.



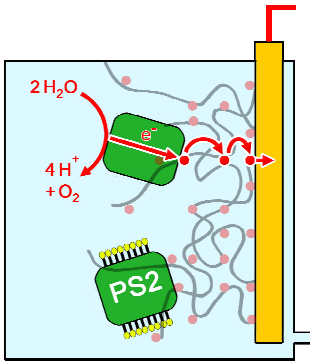
Combination of

- PS2: most oxidative force in nature
- PS1: most reductive force in nature

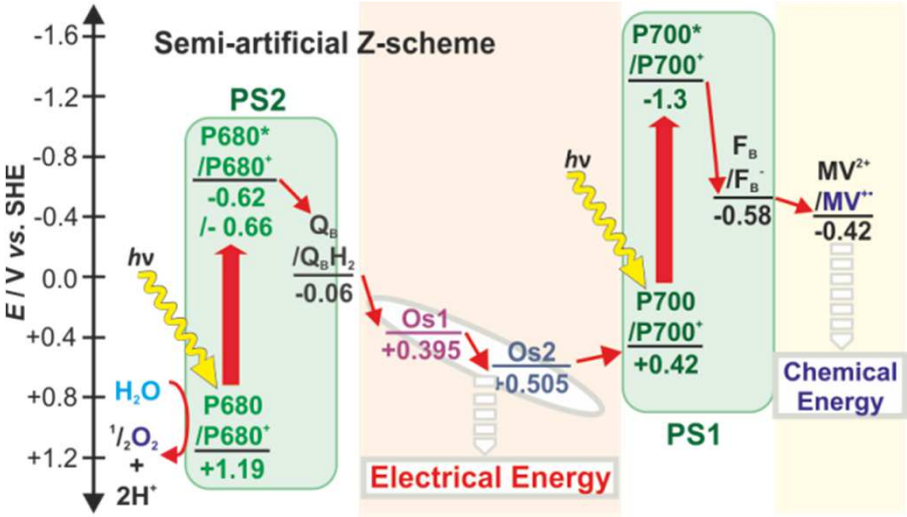
➔ ΔE of 560 mV

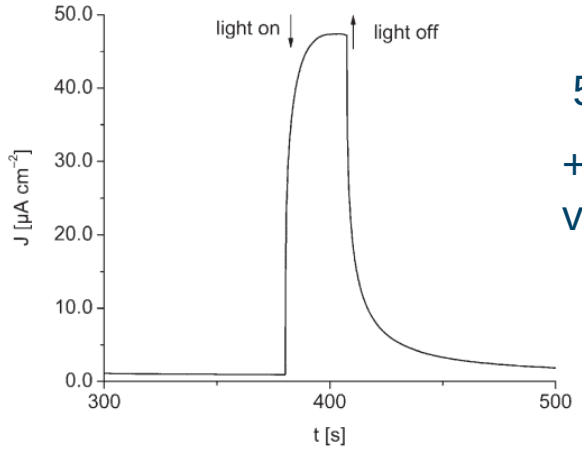
How to recover the energy from the potential difference between PS2 and PS1?

Semi-artificial photosynthesis - half cells



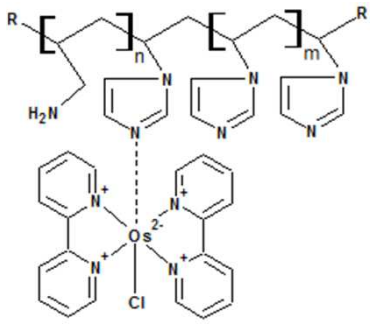
PS2
photoanode





50 µA cm^{-2}

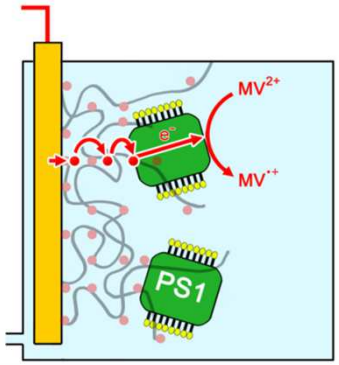
+300 mV
vs. Ag/AgCl



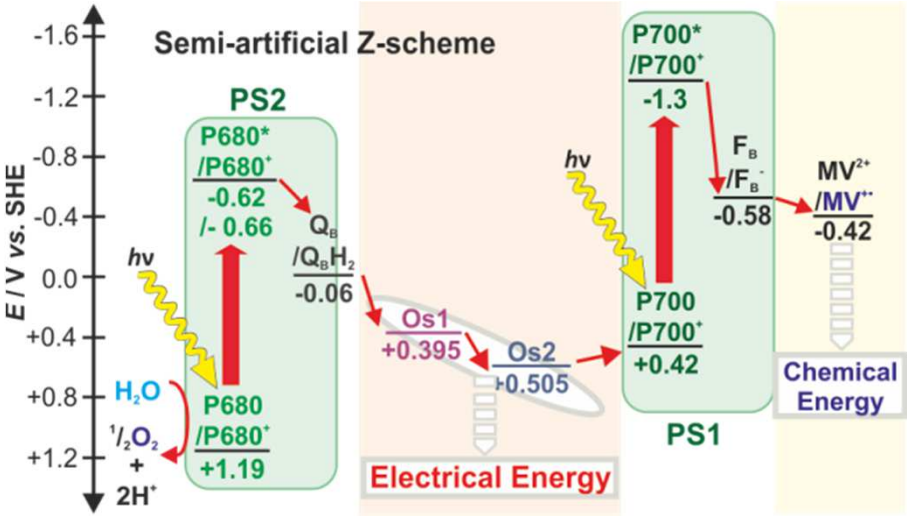
Os-redox polymer
➔ diffusion less electron mediator
➔ immobilization matrix

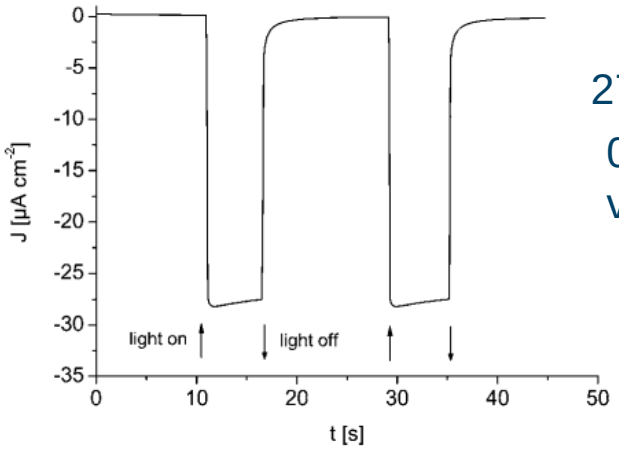
Badura, Guschin, Esper, Kothe, Neugebauer, Schuhmann, Rögner, Electroanalysis 20, **2008**, 10, 1043 – 1047

Semi-artificial photosynthesis - half cells

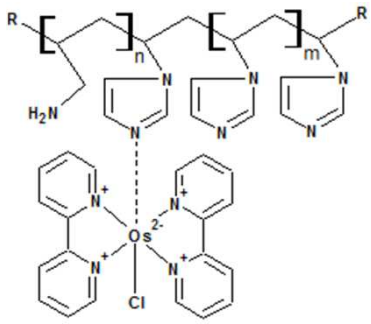


PS1
photocathode





27 μA cm^{-2}
0 mV
vs. Ag/AgCl

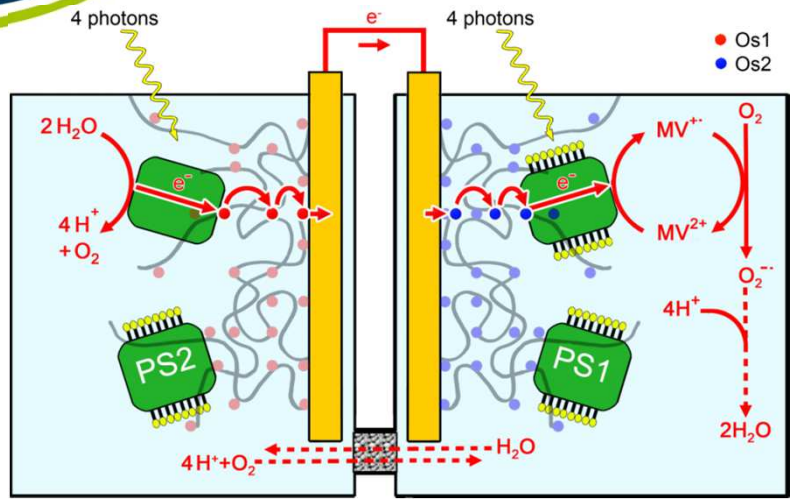


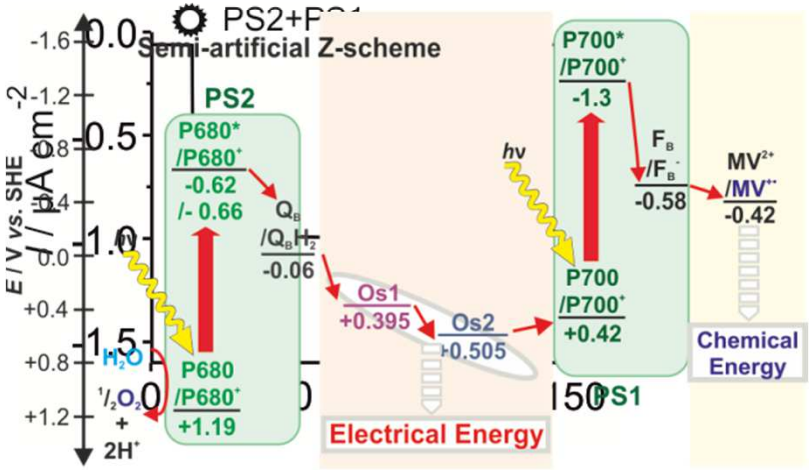
Os-redox polymer
➔ diffusion less electron mediator
➔ immobilization matrix

Badura, A., Guschin, D., Kothe, T., Kopczak, M. J., Schuhmann, W., Rögner, M. *Energy Environ. Sci.* **2011**, *4*, 2435–2440.

T. Kothe, N. Plumeré, A. Badura, M. M. Nowaczyk, D. A. Gushin, M. Rögner, W. Schuhmann, *Angewandte Chemie*, **2013**, accepted,

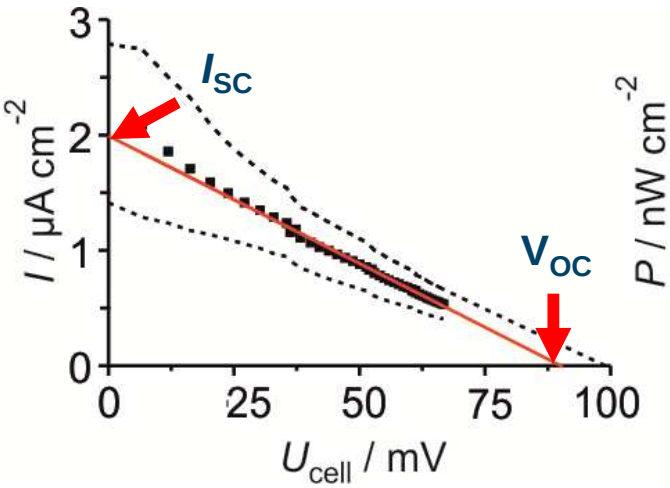
Semi-artificial photosynthesis - half cells

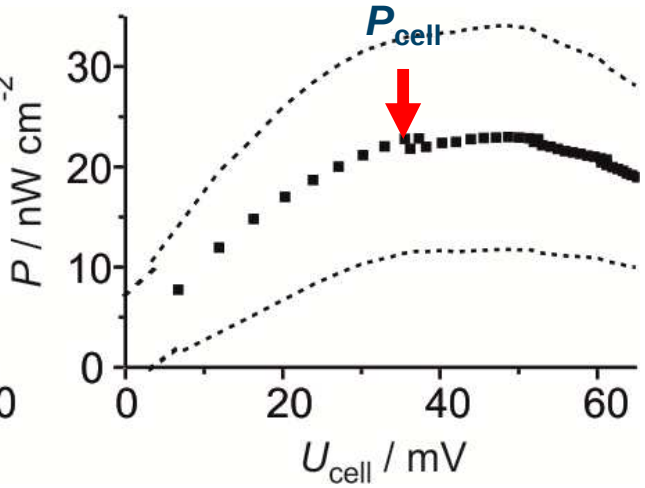




ΔE Os1 / Os2➔ 110 mV

➔ Autonomous photocurrent production





I_{SC} = 2.0 ± 0.7 µA cm^{-2} V_{OC} = 90 ± 20 mV P_{cell}= 23 ± 10 nW cm^{-2}