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Microperoxidase-11

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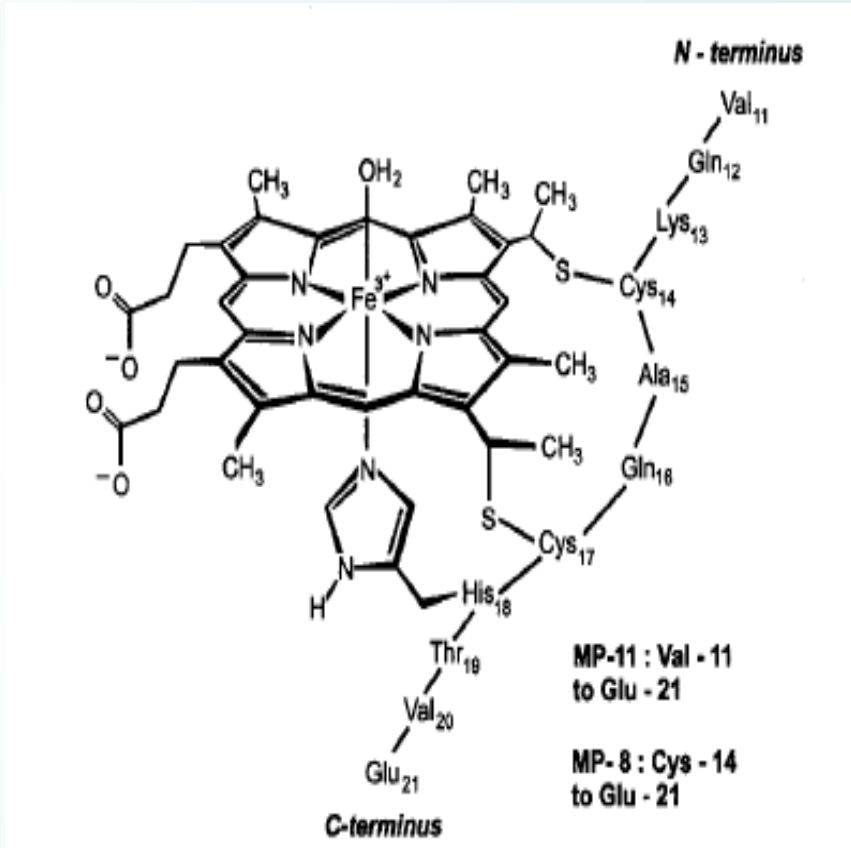
This Presentation

- Introduction
- Detection
- Redox Activity
- Biochemistry
- Applications
- Summary
- References

Microperoxidases (MP)

- A group of heme peptides derived from proteolysis of cytochrome c
- Intact iron heme-center connected to polypeptide by two thioether bridges
- MP-8 and MP-11 retain their iron at pH > 3.4
- Reduce H_2O_2 and other compounds
- MP-8 studied extensively, but study is often complicated by self-dimerization
- MP-11 aggregates less than MP-8 and holds promise for future study

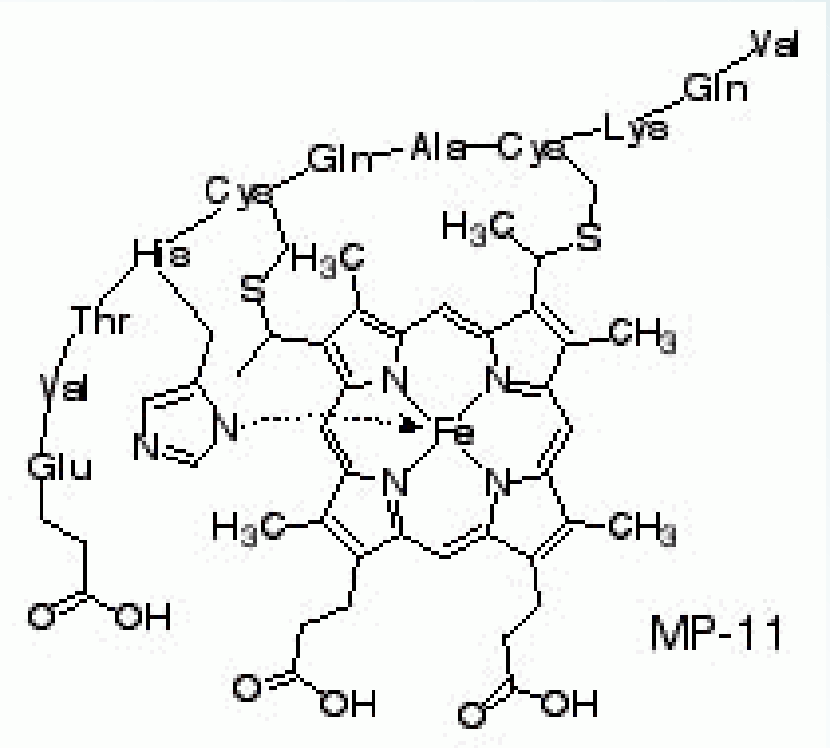


Microperoxidase	K_D
MP-8	11.7 x10^4 M^{-1}
MP-11 (pH 6.5)	5.2 x 10^4 M^{-1}

Marques HM, Perry CB. (1999) *J Inorg Biochem.***75** (4): 281-291.

Microperoxidase-11

- Microperoxidase-11 (MP-11) is 1.9 kDa oligopeptide obtained by proteolysis of cytochrome c by trypsin.
- MP-11 contains the active-site (heme undecapeptide) of cytochrome c and although they are similar structure, they have different redox properties.*



	E^o
Cytochrome C	+12 mV
MP-11 (pH 6.5)	-400 mV

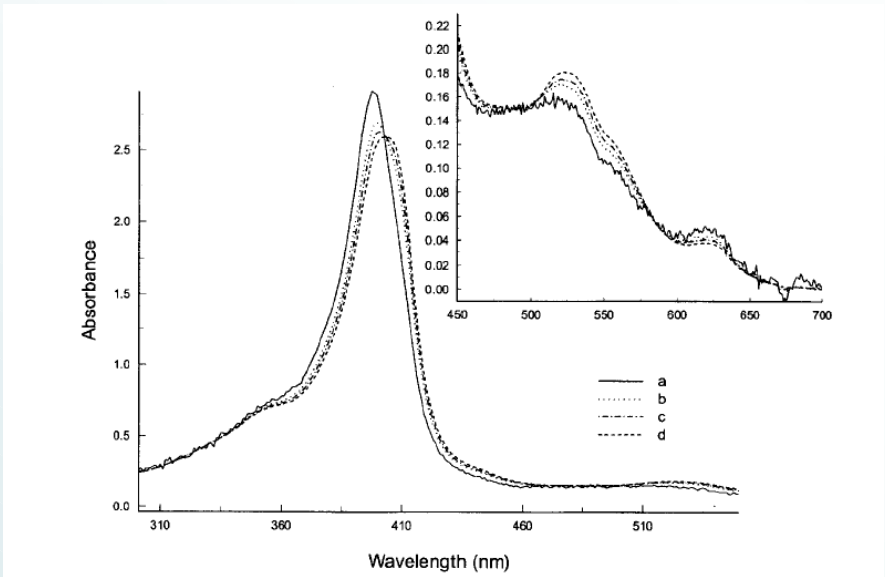
*Hill HAO, Walton NJ, Higgins IJ. (1981) *FEBS Lett.* **126:** 282-284.

Detection

(Marques *et al.* 1999)

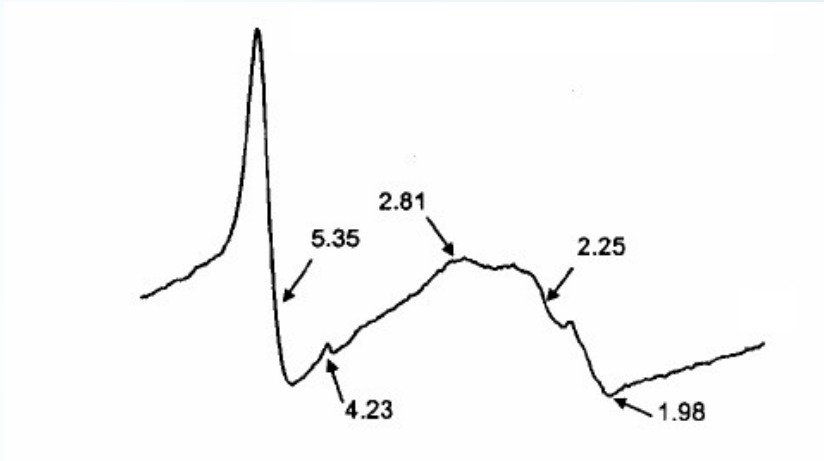
UV-Visible Spectroscopy

Property	Value
Monomer Absorbtivity, ε_M	1.48×10^5 $M^{-1}cm^{-1}$
Dimer Absorbtivity, ε_D	1.21×10^5 $M^{-1}cm^{-1}$
Dimerization Constant, K_D	5.2×10^4 M^{-1}



At pH 6.5 the absorbance spectrum for MP-11 varies with MP-11 concentration: (a) 0.55 μM, (b)4.4 μM, (c) 9.3 μM, and (d) 18.9 μM.

EPR



EPR spectra of N-Acetyl-MP-11 at pH 7.6. This spectrum also varies with pH.

Redox Activity

Moore *et al*. 1996 and Lotzbeyer *et al.* 1994

The redox potential of MP-11 differs depending upon solvent.

Solvent	E^o (mV)
H_2O	- 398
Acetonitrile	- 290
Ethanol	- 286
DMSO	- 270

MP-11 is so small that it can easily assemble as a monolayer on surfaces such as gold electrodes. Electron reduction potentials can be measured from these monolayer attachments.

The MP-11 redox process includes transition of the heme-iron between Fe(III) and Fe (II). In aqueous solution, H_2O_2 is reduced via Fe(IV) intermediate species. This intermediate does not exist in non-aqueous conditions.

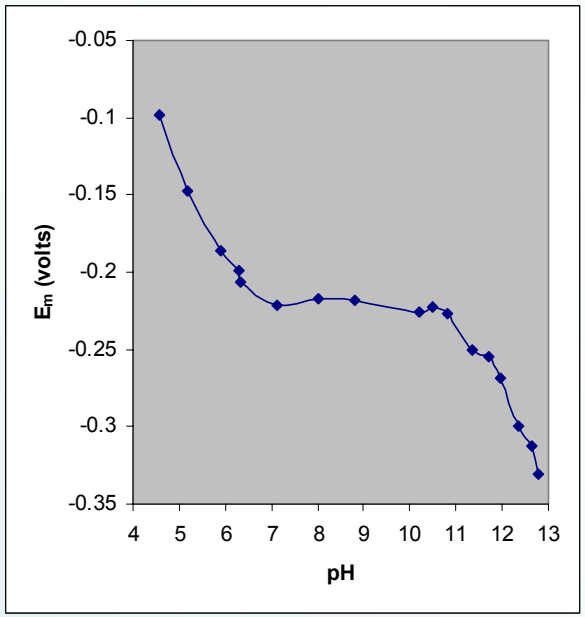
(1) [heme-Fe(III)] + $H_2O_2 \rightarrow$ [heme-Fe(IV)=O]$^{\cdot +}$ + H_2O

(2) [heme-Fe(IV)=O]$^{\cdot +}$ + e^- + $H^+ \rightarrow$ [heme-Fe(IV)-OH]

(3) [heme-Fe(IV)-OH] + e^- + $H^+ \rightarrow$ [heme-Fe(III)]

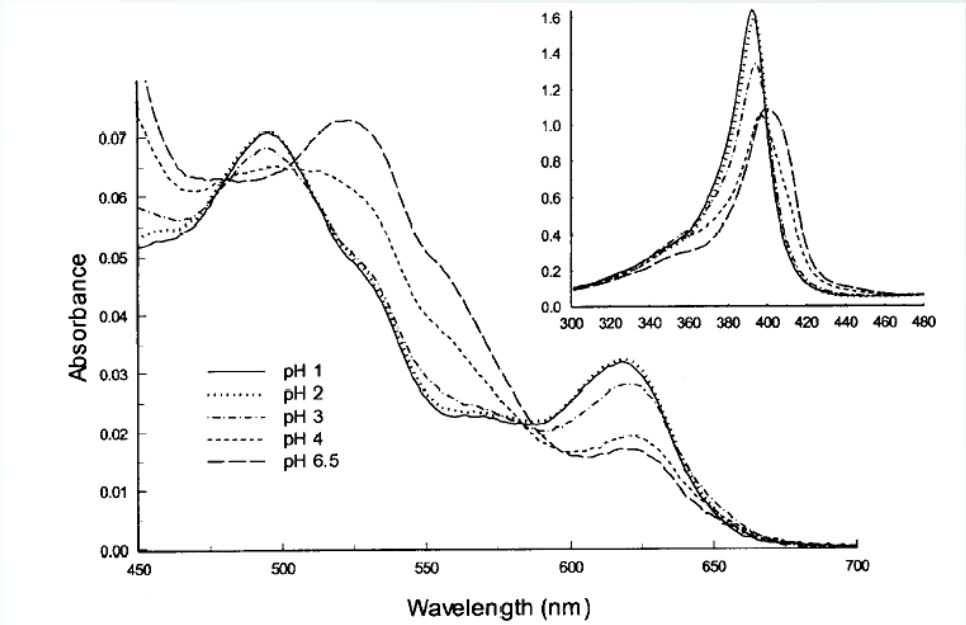
pH Dependence

The reduction potential of MP-11 is dependent on pH.

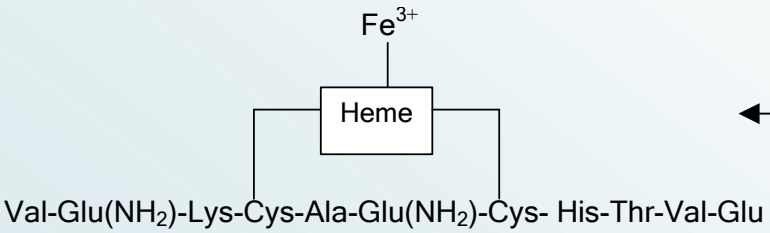


Harbury HA, Loach PA.(1960) *J Biol Chem.* **235:**3640-3645.

The UV-visible absorbance of MP-11 is dependent on pH.



Marques *et al.* (1999) *J Inorg Biochem.* **75:** 281-291.



The pKa values for the peptide are 3.4, 5.8 and 7.6 which are related to protonation the imidazole group of histidine, and the α- and β- amino groups of lysine.

Wilson MT, Ranson RJ. (1977) *Eur J Biochem.* **77:**193-199.

Reaction with Nitric Oxide

(Sharma *et al.* 1983)

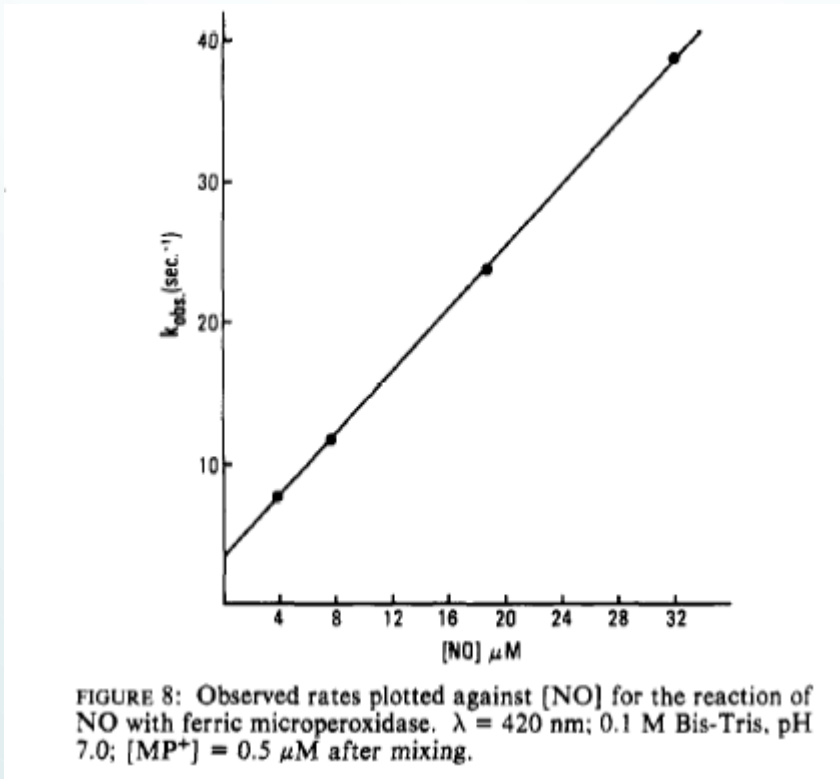


FIGURE 8: Observed rates plotted against [NO] for the reaction of NO with ferric microperoxidase. λ = 420 nm; 0.1 M Bis-Tris, pH 7.0; [MP$^+$] = 0.5 μM after mixing.

Microperoxidase reacts with nitric oxide with similar rate constants to those of other heme containing proteins like myoglobin (Mb+) and hemoglobin (Hb+).

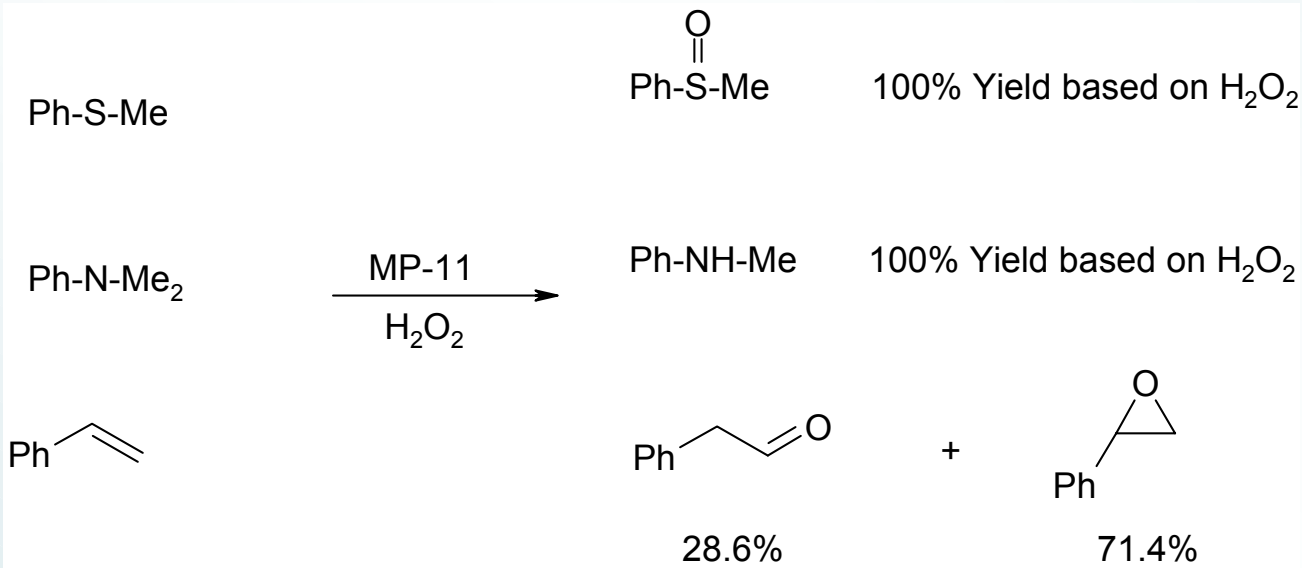
$$MP^+ + NO \underset{k' = 3.4 s^{-1}}{\overset{k = 1.1x10^6 M^{-1}s^{-1}}{\longleftrightarrow}} \left(MP^+\right)NO$$

Sample	On [k ($M^{-1}s^{-1}$)]	Off [k' (s^{-1})]
Mb^+	5.3 x 10^4	14
Hb^+ OP α–chains	1.3 x 10^6	13
Hb^+ OP β-chains	1.68 x 10^4	2.9
MP^+	1.1 x 10^6	3.4

Oxidation of Phenolics

(Mashino *et al.* 1990)

MP-11 oxidizes phenolic compounds in the presence of hydrogen peroxide: Methylphenyl sulfide, N,N-dimethyl-aniline, and olefins.



Interactions with Lipid Membrane

Huang *et al.* 2001

DDAB lipids bind to MP-11 causing a conformational change that can be seen through cyclic voltammetry (CV), UV-Visible spectrometry and far-UV circular dichroism (CD spectra).

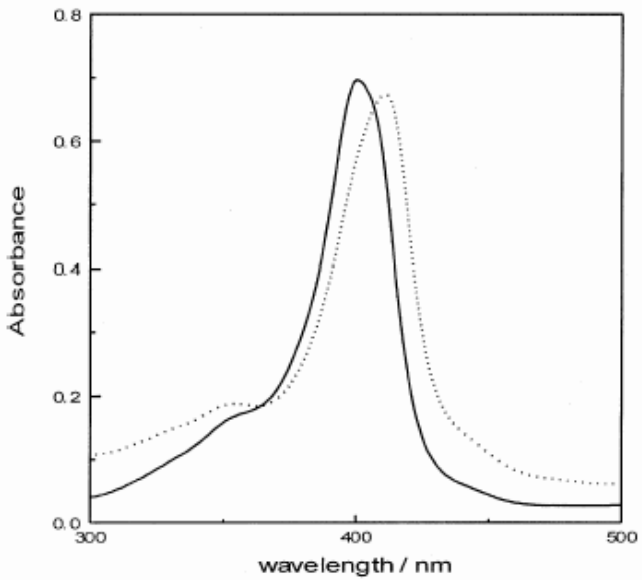


Fig. 4. UV-Vis absorption spectra of MP11 in pH 6.9 phosphate buffer (solid line) and when bound to DDAB vesicles (dotted line), MP11 4.3 μM, DDAB 44 μM. Spectra were obtained at room temperature (25°C), cell path lengths of 1 cm.

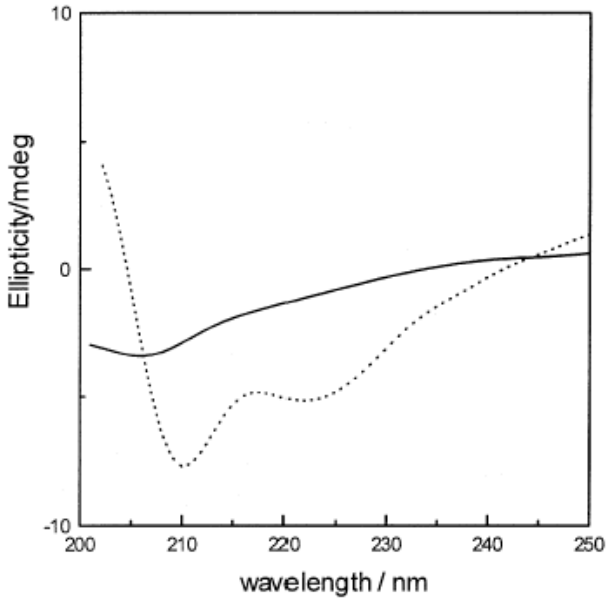
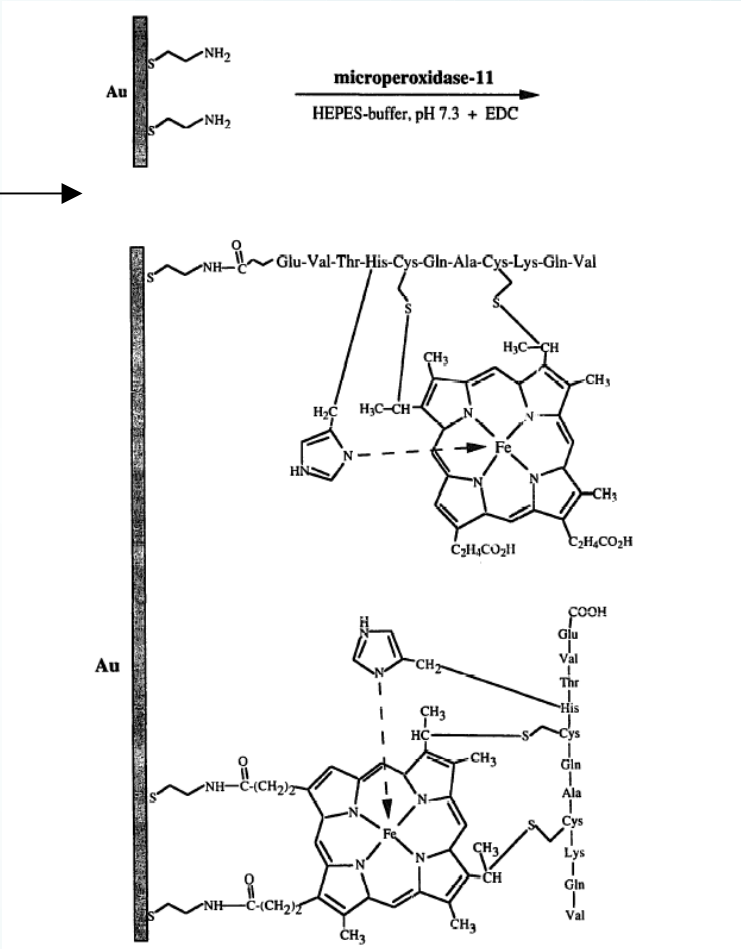


Fig. 5. Far-UV CD spectra of MP11 in pH 6.9 phosphate buffer (solid line) and when bound to DDAB vesicles (dotted line), MP11 14 μM, DDAB 100 μM. Spectra were obtained at room temperature (25°C), cell path lengths of 1 cm; 4 scans were averaged per spectrum.

MP-11 and Au Electrodes

(Narvaez *et al.* 1997)

MP-11 attaches to a cystamine monolayer on a gold (Au) electrode in two ways: the peptide or the carboxylic end of the heme group can associate with the cystamine.



The voltammograms of the electrode are shown below.

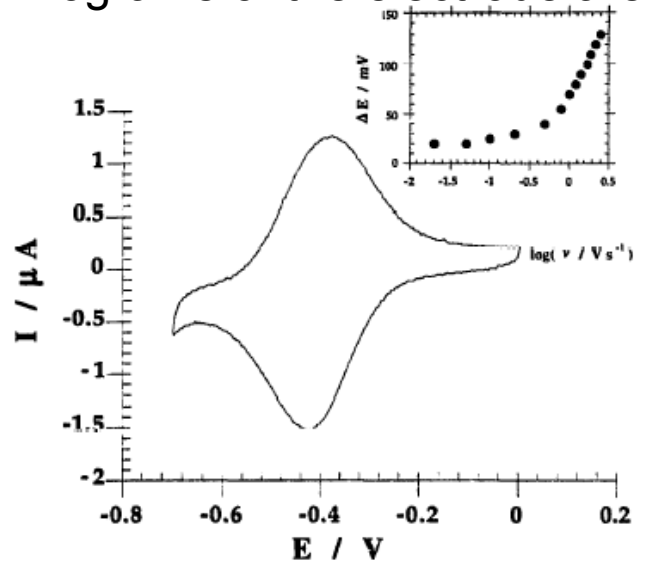


Fig. 1. Cyclic voltammograms of the microperoxidase-11 monolayer electrode, scan rate 100 mV s^{-1}; background electrolyte 0.05 M; phosphate buffer; pH = 7.0. Inset: Peak-to-peak separation of CV waves as a function of scan-rate.

MP-11 in non-aqueous environment

(Mabrouk *et al.* 1996)

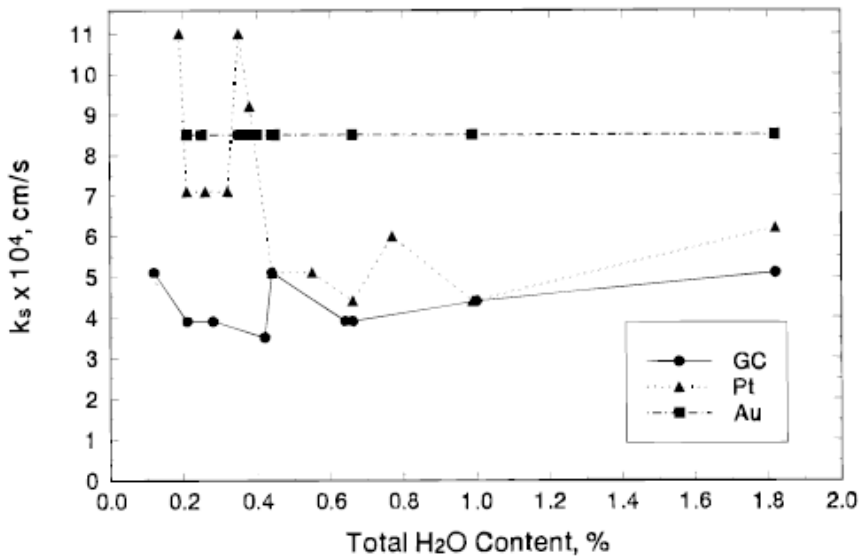


Figure 2. Heterogeneous electron transfer rate constant, k_s', versus the total water content represented as a percent for ~3 mM im-MP-11 in dimethyl sulfoxide solutions at Au (■), Pt (▲), and GC (●). k_s' was calculated using Nicholson's method[8] from background-subtracted data at 20 mV/s and assuming $n = 1$, $T = 25$ °C, and $\alpha = 0.5$. Each datum represents the analysis of a new sample after the electrode substrate was cleaned.

- Cyclic voltammetry is tool that has been used to study the electron transfer between imidazole-MP-11 compounds and gold (Au), platinum (Pt), and Glassy Carbon (GC) in DMSO.

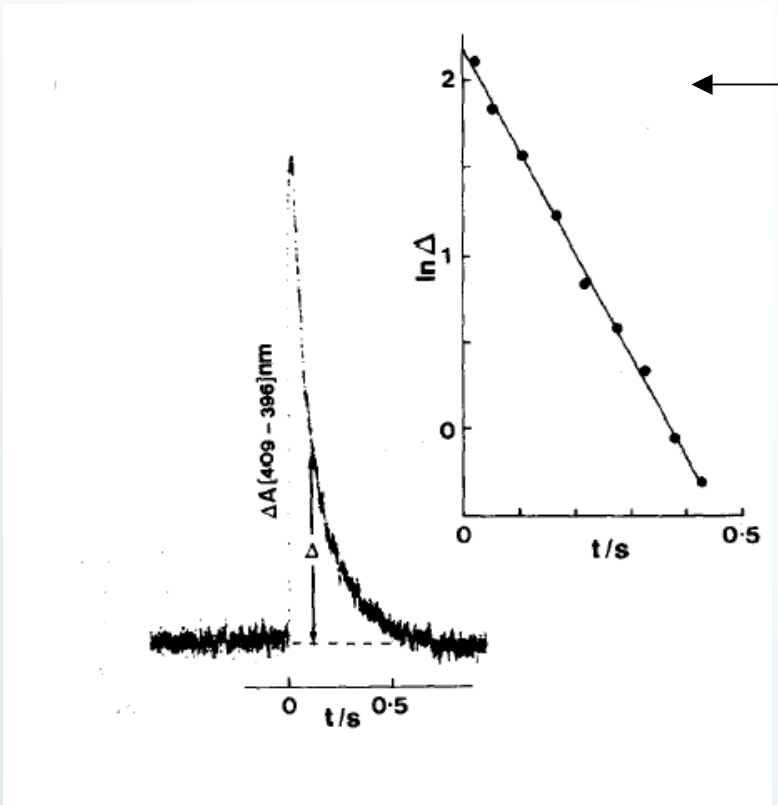
- Low concentrations of water have little effect on MP-11 electron transfer rates constant for Au and GC.

- These same concentrations have a large effect on the rate constant for electron transfer rates between MP-11 and Pt.

Interaction with Serum Albumin

(Adams *et al.* 1993)

$$Fe^{3+}MP11 + HSA \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} Fe^{3+}MP11HSA$$



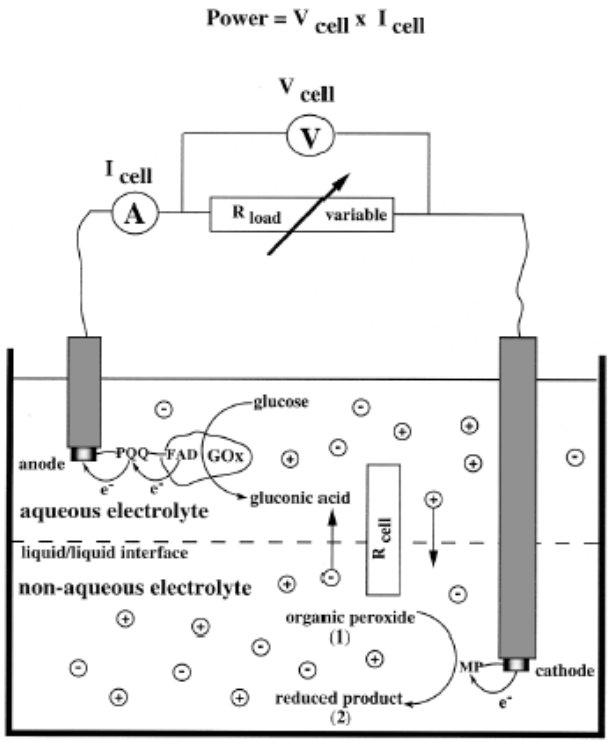
In 13 wt% aqueous methanol (MeOH) at pH 7.5, the pseudo first order reaction rate constant (k_{obs}) of of 2.5μM MP-11 and 100μM Human Serum Albumin (HSA) is determined to be 5.76 s^{-1}.

X_{MeOH} (wt%)	k_1	k_{-1}
0	590 $M^{-1}s^{-1}$	0.0095 s^{-1}
0.13	1370 $M^{-1}s^{-1}$	5.94 s^{-1}

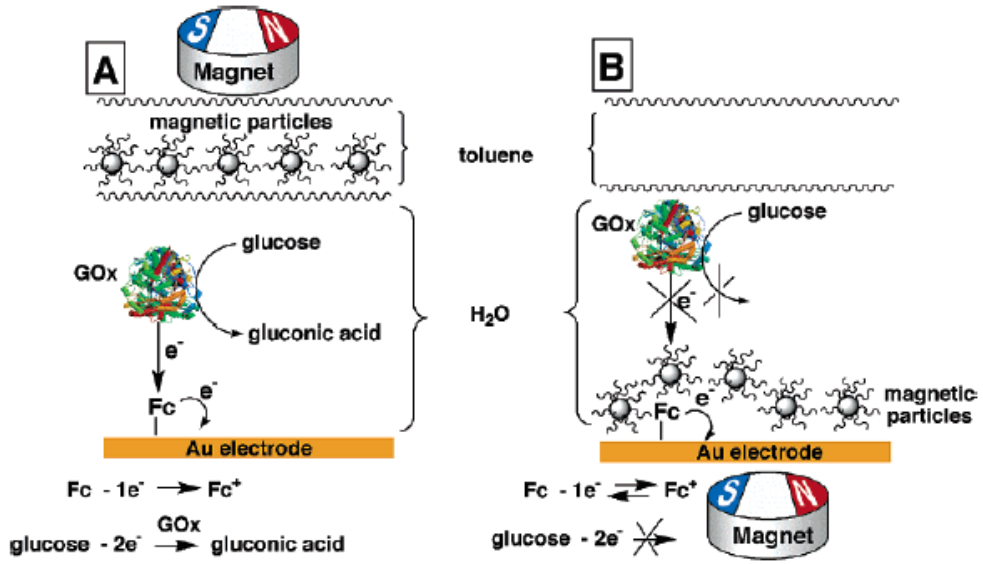
The rate constant for the formation of MP-11HSA is just more than doubled over the range of MeOH concentration while the rate constant for the dissociation of this complex is increased by nearly 600-fold over the same range of MeOH concentrations.

Applications: Biofuel

Glucose can be used as biofuel. MP-11 immobilized on a Au-electrode surface can catalyze the oxidation of glucose to gluconic acid. Below is the design a fuel cell utilizing this technology.



Katz *et al.* (1999) *New J Chem.* **23:** 481-487.



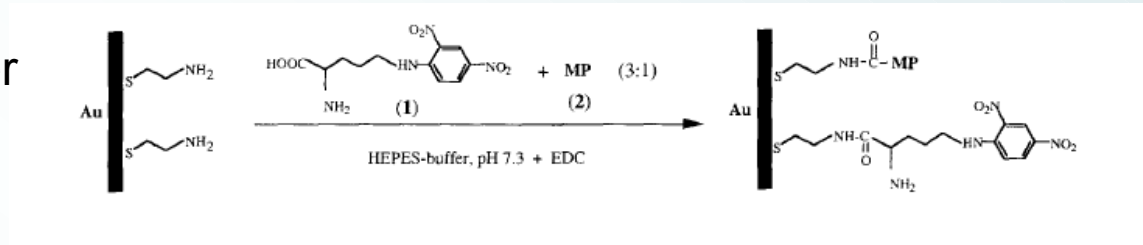
Biofuel consumption can be blocked by magnetic particles: A) The magnetic nanoparticles are retracted from the electrode surface, which is activated toward the ferrocene-mediated bioelectrocatalytic oxidation of glucose. (B) The electrode surface is blocked by the hydrophobic magnetic nanoparticles toward the diffusional bioelectrocatalytic process, while the surface-confined ferrocene is electrochemically active.

Katz *et al.* (2005) *J Am Chem Soc.* **127:** 4060-4070.

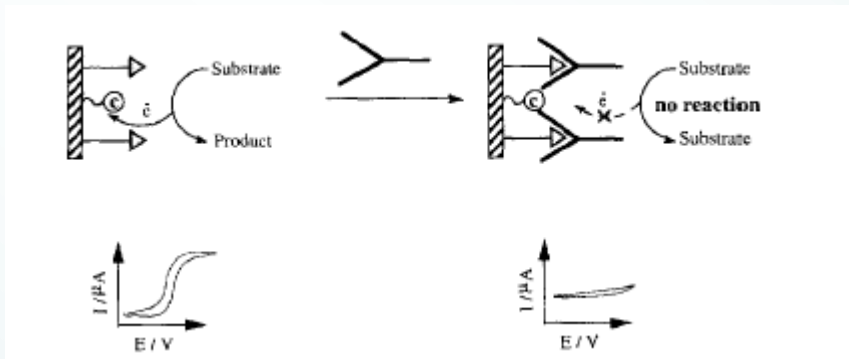
Applications: Immunosensors

(Katz *et al.* 1996)

• MP-11 and catalyst are arranged in a mixed monolayer on Au-electrode



•Electrochemical sensing of the antigen-antibody is shown. The voltammetry output is clearly different.



Summary

- Not an “enzyme” but catalyzes numerous reactions
- Can be detected by common spectroscopic methods
- Redox chemistry is pH and solvent dependent
- Reacts with peroxides, phenolic compounds, proteins and lipids
- Potential applications as biofuel and biosensor

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