

Supporting Informarion

Water-soluble polymer bound biomimetic analogs of cytochrome c oxidase catalyze 4e Reduction of O₂ to water

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Experimental details:

Chemicals: Solvents for the polymerizations were dried and air was removed by several freeze pump thaw cycles. The porphyrins^{1,2} and the ligand 1-methyl-5-imidazolecarboxylic acid³ were synthesized according to previously described protocols. Ruthenium(III)acetylacetonate, AIBN and 4,4'-Azobis(4-cyanovaleric acid) were purchased from Aldrich.

Quantitative Analysis: The amount of iron in the polymers was determined by an Iris Advantage/1000 Radial ICAP spectrometer.

UV/Vis-spectroscopy: Spectra were recorded on a Hewlett-Packard 8453 Instrument. Reduction and oxygenation experiments were carried out in sealed cuvettes under nitrogen atmosphere using gastight syringes. Kinetic experiments were performed with a resolution up to 0.4 s/spectrum. 10⁻³-10⁻⁴ M solutions of **RuIm** were added with a gastight syringe to quartz cuvettes, containing the polymer bound porphyrins in a solvent that was saturated with air or mixtures of nitrogen and oxygen. The spectra were recorded after a mixing dead time of 1 s was passed. Concentrations were calculated by $c = (A - \epsilon_{Ox} \cdot c_0) / (\epsilon_{Red} - \epsilon_{Ox})$, where A is the absorbance at 468 nm for **RuIm** after the absorption of the porphyrins were subtracted, c₀ the initial concentration of the reduced form and ϵ_{Ox} and ϵ_{Red} are the extinction coefficients of the oxidized and reduced form respectively.

Electrochemistry: Experiments were carried out using an edge plan graphite (EPG) rotating ring disk electrode (pine instruments) in 100 mL cells with a tightly fitting teflon lid. A BAS CV-50W potentiostat (Bioanalytical systems) and an ASR speed controller were used. The reference and auxiliary electrodes were a low flow rate calomel electrode and a Pt mesh., respectively. The EPG disks (1.925 cm²) were cleaned with 600 grit SiC paper and sonicated for 1 min in methanol. The following polymer solutions were prepared: Samples of 20 mg **Fe-1-PAA** were dissolved in 100 mL buffered aqueous solution at pH 4, 7, 8 and 10. 50 mg **Fe-1-PVP** and 30 mg **Fe-2-PVP** were each dissolved in 100 mL buffered aqueous solution at pH 4. The latter two copolymers precipitated at higher pH. KNO₃ (0.1 M) was used as the supporting electrolyte for each polymer solution. Under anaerobic conditions all polymers exhibited surface irreversible waves in cyclic voltammetry (CV) when they were dissolved in aqueous solution, suggesting that the polymers tend to adsorb at the electrode surface and that they slowly diffuse in solution. However, it was necessary to maintain a minimum concentration of polymers in solution in order to avoid a migration of electrode-adsorbed polymers into the solution. Higher concentrations did not effect a remarkable increase of current.

Synthetic procedures:

Diacetonitrile bis-(acetylacetonato)ruthenium

200 mg ruthenium(III)acetylacetonate was dissolved in 50 ml acetonitrile and 5 ml water. Nitrogen was blown through the solution for 10 min and 7 pieces of freshly prepared zinc amalgam were added to the solution. The mixture was refluxed for 40 min whereby the color of the solution changed from red to light orange. After the mixture was cooled to rt the mixture was passed through a frit and the filtrate evaporated. The product was crystallized from methanol giving 150 mg yield.

Bisacetylacetonato-bis-(1-Methyl-5-carboxylimidazole)ruthenium (RuIm)

100 mg Ru(acac)₂(CH₃CN)₂ (0.26 mmol) and 132 mg 1-methyl-5-imidazolecarboxylic acid were refluxed in 100 ml ethanol under air for 2 hrs. A violet solution was obtained. After the solvent was removed by evaporation the remaining solid was precipitated from methanol by addition of acetone and the product isolated by filtration. 50 mg violet solid were obtained and subsequently transferred into a dry box with a nitrogen atmosphere. The solid was dissolved in N₂-saturated ethanol and refluxed in the presence of a few pieces of zinc amalgam. After filtration the solution containing **RuIm** was removed by evaporation affording an orange/brown residue.

¹H-NMR (methanol-d₄): δ /ppm 7.1-7.3 (m, 2H, Im), 3.95 (s, 3H, N-CH₃); 1.76 (2s, 12H, CH₃); UV/vis Ru(II) (waster pH10) λ_{max}/nm (ε): 465 (5300)

Polymerization: It was found advantageous to metalate the porphyrins before the polymerization was carried out in order to avoid the presence of any excess free metal ions in the polymer network, which could not be removed quantitatively.

Copolymerization of porphyrin Fe-1 with 4-vinylpyridine (Fe-1-PVP)

3.5 mg of **Fe-1** (4 μmol) and 300 μl (2.76 mmol) freshly distilled 4-vinylpyridine were dissolved in 1 ml methanol. After addition of 10 mg AIBN all oxygen was removed by three freeze pump thaw cycles and the flask was sealed. The polymerization started at 80° C as observed by a gradual increase of the viscosity and a change of the color from violet to brown. If the heating period exceeded 2 hrs an insoluble product was formed. Heating was therefore discontinued after 90 min and the vessel was opened to allow the contact to air. The solvent was removed in vacuum and the residue redissolved in 1 ml methanol. A mixture of ether/hexane (5:1) was added dropwise to the solution until the polymer precipitated. The precipitate was collected and washed with benzene on a small frit. A solution of the polymer in methanol was passed through a column filled with Sephadex G75 and the main fraction was collected. Volatile solvent was subsequently removed in high vacuum at 50 °C. Yield: 270 mg

Copolymerization of Fe-1 with acrylic acid (Fe-1-PAA)

750 μl freshly distilled acrylic acid was added dropwise with a syringe to a solution of 17 mg (14.6 μmol) porphyrin **Fe-1** in 900 μl acetone in a dry box under nitrogen. The solution was alkalized with 900 μl 1 N KOH. 12 mg 4,4'-Azobis(4-cyanovaleric acid) was added and the evacuated flask was sealed. The flask was heated in an oil bath at 90° C for 24 hrs (WARNING: heating a liquid in a closed container represents an explosion hazard. Adequate precautions must be taken) and then allowed to cool down. In air 15 ml ethanol were added. The polymer precipitated after aqueous KOH was added and was collected in a frit. An aqueous solution of the

polymer was passed through a column filled with Sephadex 400 HR. Only the main fraction was collected. After water was removed by evaporation 440 mg polymer was obtained.

Copolymerization of Fe-2 with vinylpyridine (Fe-2-PVP)

20 mg Fe-2 and 500 ml freshly distilled 4-vinylpyridine were dissolved in 1 ml methanol. After addition of 13 mg AIBN all oxygen was removed by three freeze pump thaw cycles and the flask was sealed. Polymerization was carried out at 80° C for 24 hrs (WARNING: heating a liquid in a closed container represents an explosion hazard. Adequate precautions must be taken) giving a highly viscous liquid. After the solution was diluted to 5 ml it was slowly given to a mixture of hexane/ether (5:1) where the product precipitated. Filtration and drying afforded 385 mg solid. A solution of the polymer in methanol was passed through a column filled with Sephadex G75 and the main fraction was collected.

Supporting Table 1: Select physicochemical parameters of the copolymers.

Entry	Porphyrin (mol)	Co-monomer (mol)	Porphyrin content (mol%) ^a	MW distribution of the copolymer ^b	molecules of Fe porphyrin per one molecule of polymer
Fe-1-PAA	Fe-1(1.46×10^{-5})	acrylic acid (1.05×10^{-2})	0.072	50,000-1,000,000	0.5-10
Fe-1-PVP	Fe-1(0.3×10^{-5})	4-vinylpyridine (2.8×10^{-3})	0.070	10,000-80,000	0.07-0.5
Fe-2-PVP	Fe-2(1.69×10^{-5})	4-vinylpyridine (4.7×10^{-3})	0.326	10,000-80,000	0.3-2.5

^a Porphyrin/co-monomer ratio by ICP spectrometry.

^b Isolated fractions, by GPC.

Supporting Table 2: Electrochemical characteristics of the copolymers; data refer to pH 4 unless noted otherwise.

Copolymer	n_{av} ^a	$E_{1/2}$, V vs. NHE ^b	E^0 , V vs. NHE ^c	TOF _{max} , s ⁻¹ ^d
Fe-1-PVP	3.3	0.180	0.180	0.1
Fe-2-PVP	4.0	0.210	0.170	>300
Fe-1-PAA	(pH 2) 3.6 4.0 (pH 7) (pH 10)	0.200 0.060 0.0 -0.100	 0.030 0.0	1 ^e 10 >10 ^e 20 ^e

^a The average number of electrons exchanged per one O₂ molecule in a catalytic turnover; calculated from the ratio of disk and ring currents in RRDE experiments.

^b The potential of the catalytic wave at half height.

^c The peak potential of the reduction wave in cyclic voltammetry under anaerobic conditions

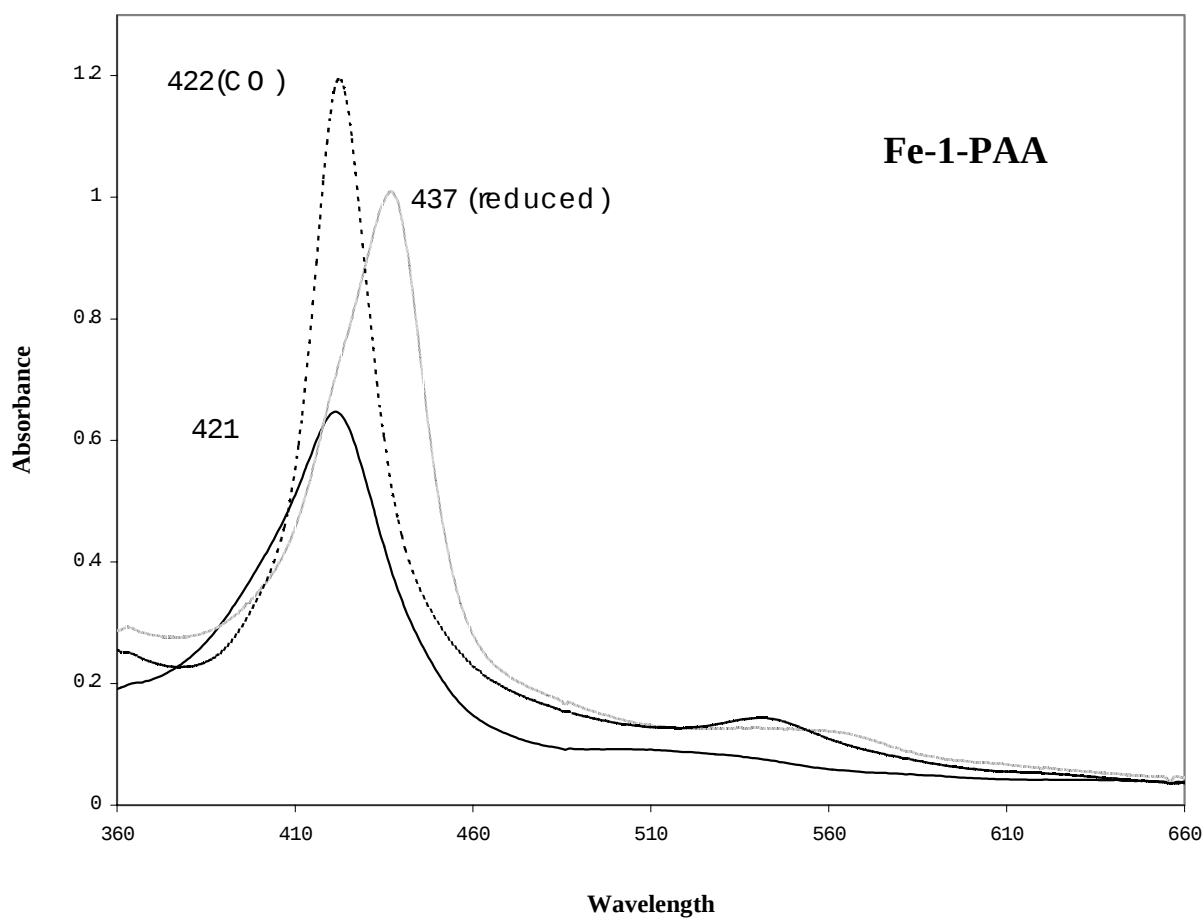
^d Maximum turnover frequency in air-saturated solution determined from the intercept of the Koutecky-Levich graph using limiting currents; the amount of the electrode-adsorbed catalyst was estimated at 10⁻¹⁰ mol by cyclic voltammetry.

^e Measured in 10% O₂/90% N₂ saturated electrolyte; to simplify the comparison the values are recalculated to air-saturated solution.

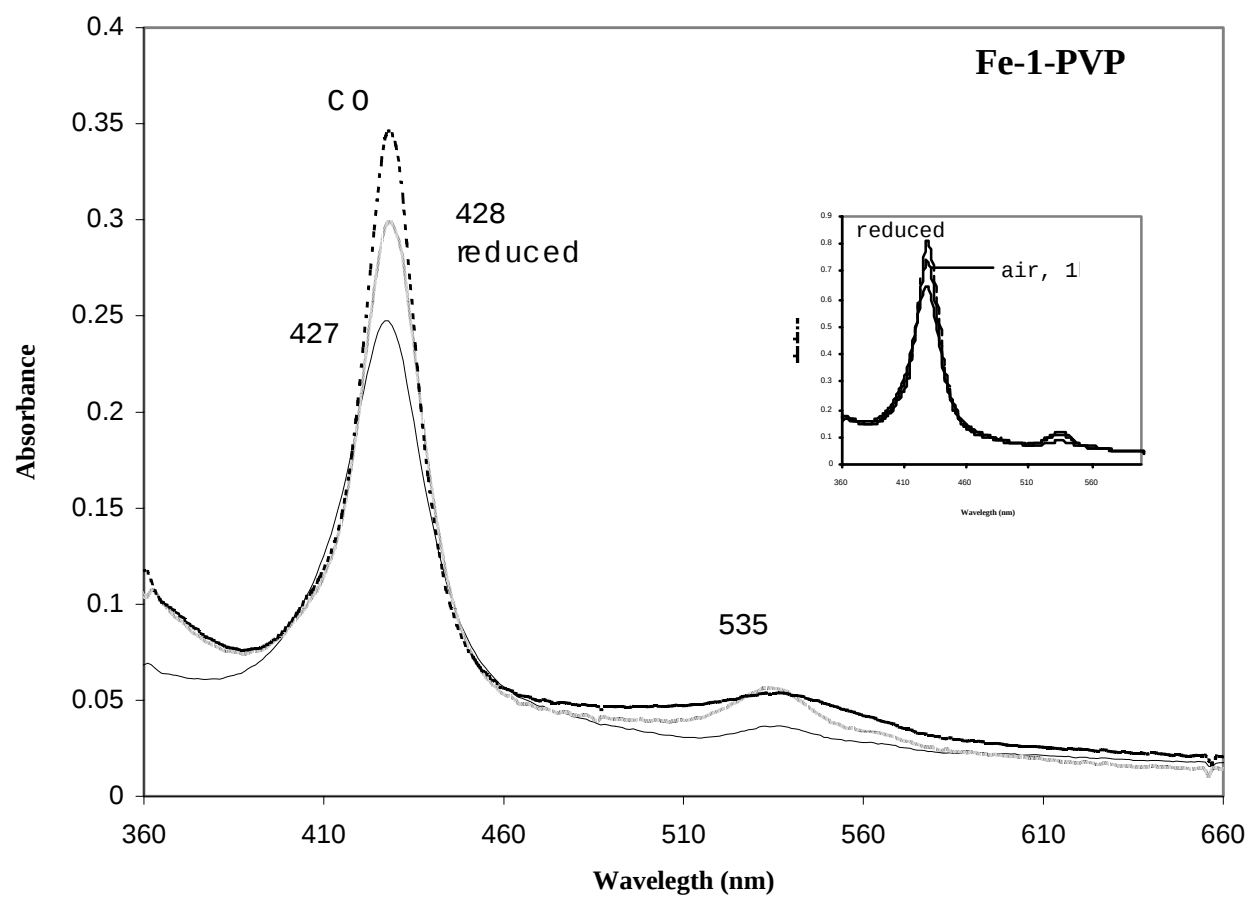
Supporting Figure 1

Electronic spectra of the copolymers (a) **Fe-1-PAA** (water), (b) **Fe-1PVP** (methanol) and (c) **Fe-2-PVP** (methanol). Starting with the oxidized form (solid) the porphyrins were reduced (gray) with **RuIm** (the oxidized form of **RuIm** does not absorb in the region shown) and finally CO was bubbled into the solution (dotted). When air was bubbled into the solution the initial spectrum of the oxidized form (for **Fe-1-PAA** and **Fe-2-PVP**) was observed (not shown). In the case of **Fe-1-PVP** the spectrum in the presence of oxygen is shown in the inset of (b).

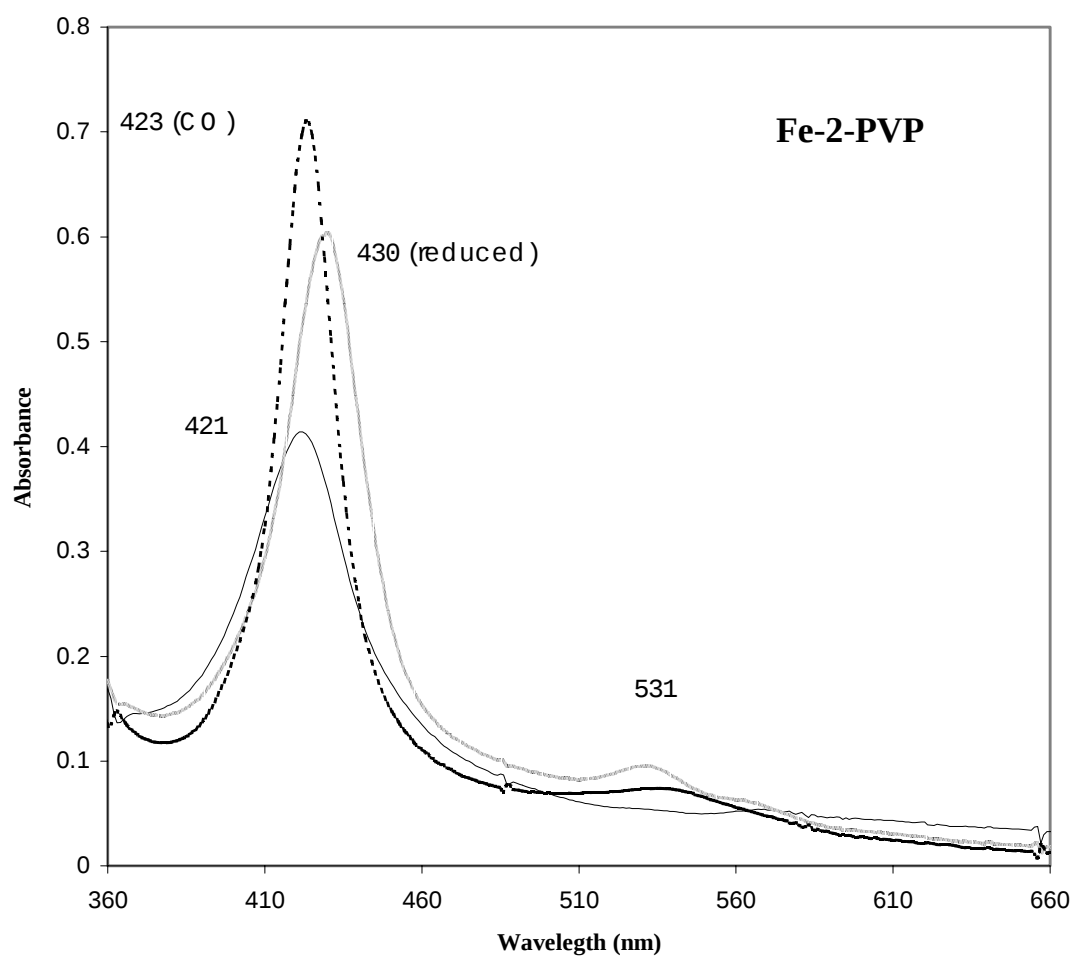
a)



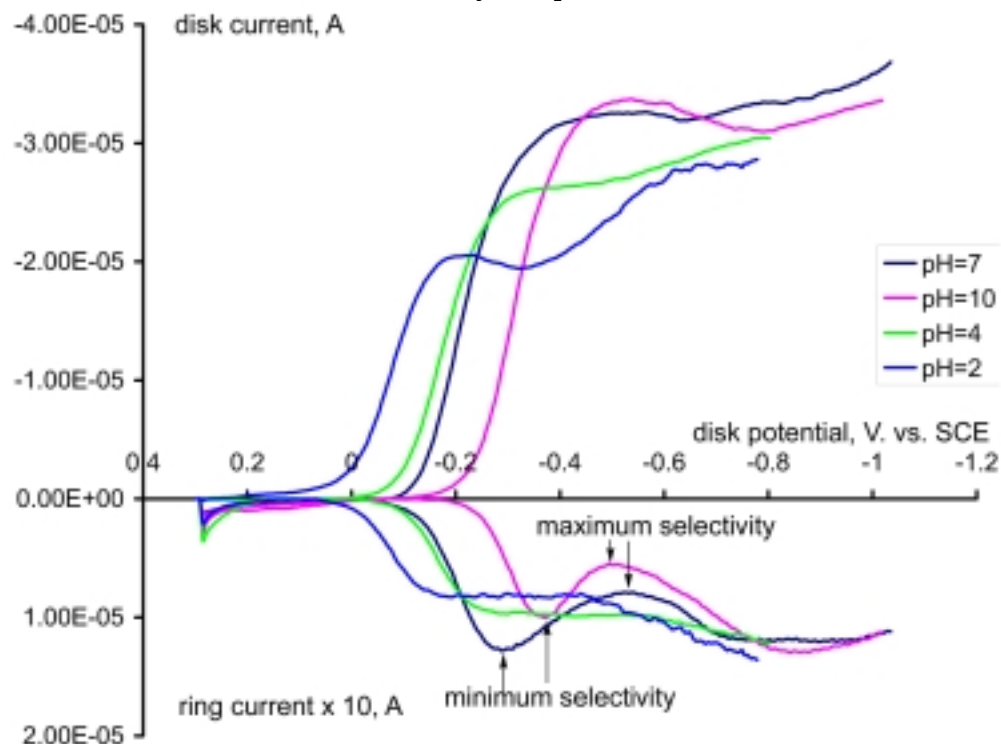
b)



c)



Supporting Figure 2. RRDE voltammograms in 10% O₂/90% N₂ saturated aqueous electrolyte at various pH using Fe-1-PAA as the catalyst. Experimental parameters are identical to those listed in the section “Electrochemistry” on p. S1.



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³ O'Connell, J. F.; Parquette, J.; Yelle, W. E.; Wang, W.; Rapoport, H *Synthesis* **1988**, 767-771