Long-Range Electron Transfer in Recombinant Peroxidases Anisotropically Oriented on Gold

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Supplementary Information

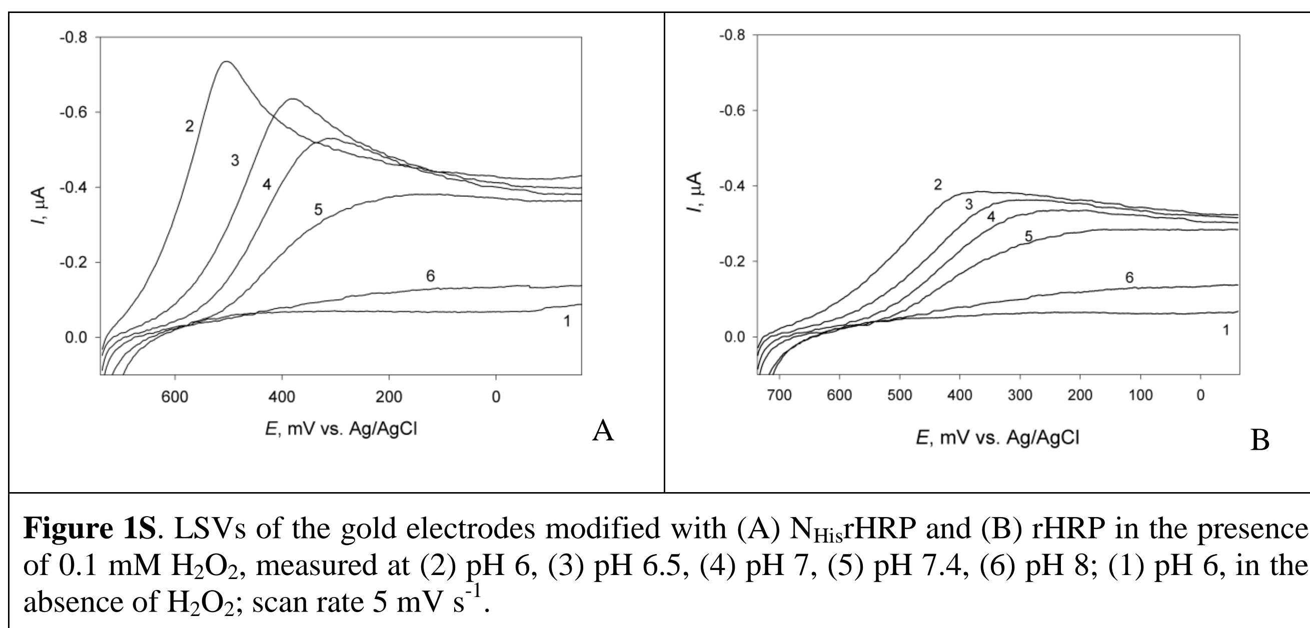
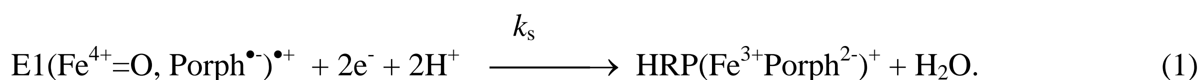


Figure 1S. LSVs of the gold electrodes modified with (A) N_{His}rHRP and (B) rHRP in the presence of 0.1 mM H_2O_2, measured at (2) pH 6, (3) pH 6.5, (4) pH 7, (5) pH 7.4, (6) pH 8; (1) pH 6, in the absence of H_2O_2; scan rate 5 mV s^{-1}.

Analysis of kinetic data obtained with HRP-modified gold electrodes in the RDE system

Peroxidase immobilised at the electrode surface can display bioelectrocatalytic properties in the cathodic reduction of H_2O_2 to H_2O both due to direct non-mediated ET between the electrode and the active site of HRP and a mediated one, when small redox-active molecules (mediators) are used to shuttle electrons between the electrode and the active site of HRP. The bioelectrocatalytic reduction of H_2O_2 catalysed by peroxidase involves direct electroreduction of E1 at the electrode surface to the initial ferric HRP, the electrode acting as an electron donor (Figure 2):



$$\mathrm{E1}(\mathrm{Fe}^{4+}{=}\mathrm{O},\ \mathrm{Porph}^{\bullet -})^{\bullet +} + 2e^- + 2H^+ \xrightarrow{k_s} \mathrm{HRP}(\mathrm{Fe}^{3+}\mathrm{Porph}^{2-})^+ + H_2O. \qquad (1)$$

For an RDE system an exact mathematical description for the analyte flows at the electrode surface exists, enabling quantitative estimation of the results. The current I registered in the system is

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usually presented as a superposition of diffusion, I_{lim}, and kinetically, I_{kin}, limited currents according to the Koutecky-Levich theory [1]:

$$1/I = 1/I_k + 1/I_{lim} \quad (2)$$

In the case of the HRP modified electrodes, I_{kin} could be limited by the heterogeneous ET reaction (shown to be kinetically slow for a majority of electrode materials) or the enzymatic reaction of a substrate (hydrogen peroxide) transformation [2]. I_{lim} is determined by the mass-transfer of H_2O_2 to the electrode surface. The diffusion limited current for the RDE depends on the angular velocity, ω, and the bulk concentration of H_2O_2, c_{H2O2}, according to the Levich equation [1]:

$$I_{lim} = 0.620nF\, c_{H2O2}D^{2/3}S\nu^{-1/6}\omega^{-1/2}\,, \quad (3)$$

where n (= 2 in the present case) represents the number of electrons transferred upon reduction of H_2O_2 to H_2O. F is the Faraday constant, c_{H2O2} is the bulk concentration of H_2O_2; D is the diffusion coefficient of H_2O_2 ($1.6\ 10^{-5}$ cm^2 s^{-1}), A is the geometrical area of the electrode (0.03142 cm^2), and ν is the kinematic viscosity of water (0.01 cm^2 s^{-1}).

The kinetic and diffusion components of the measured current, I, were separated using the Koutecky-Levich (KL) approach: the values of the current measured at different rotation speeds, ω, were linearised in $I^{-1} - \omega^{-1/2}$ co-ordinates (Eq. 2,3, Figure 2S-A).

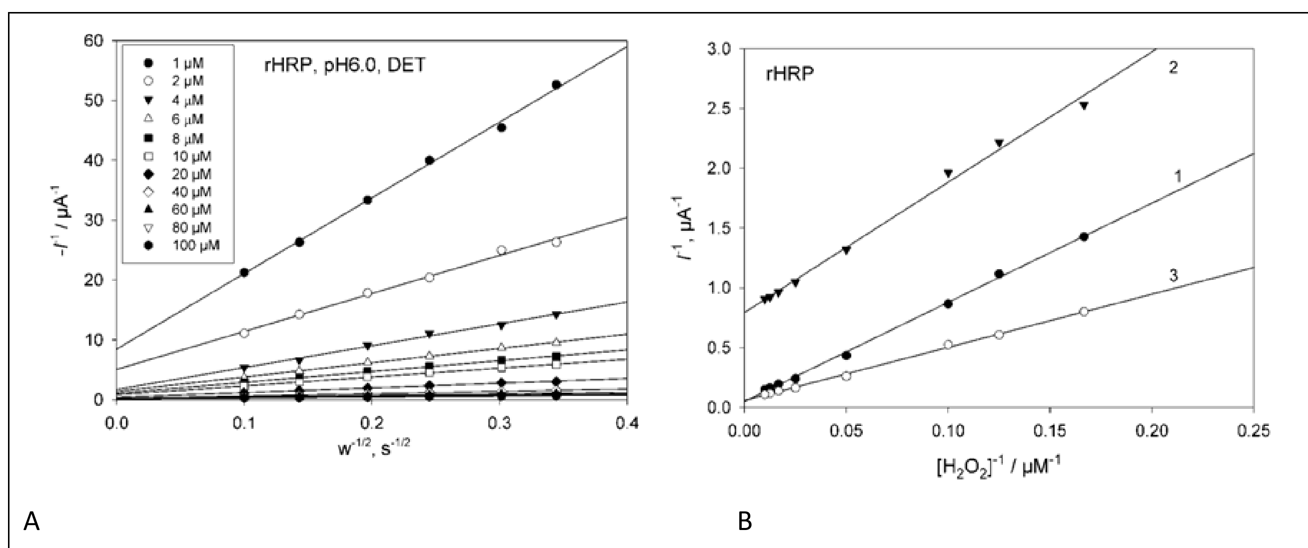

Figure 2S. (A) Representative Koutecky-Levich plots for rHRP-modified electrodes constructed for different concentrations of H_2O_2. (B) The kinetically limited currents (from the intercepts of Koutecky-Levich plots) plotted *vs*. the concentration of H_2O_2 in inverted co-ordinates for rHRP-modified electrodes (1,2) in the absence and (3) in the presence of $5\ 10^{-4}$ M catechol, (1) pH 6, (2) pH 7.4. The ET parameters k_s and percentage of the enzyme molecules active in direct ET are, respectively: 57% (0.54 pmoles), k_s^{pH6} of 168 s^{-1}, $k_s^{pH\ 7.4}$ of 13 s^{-1}.

From the intercepts of the KL plots with the Y-axis the values of the kinetically limited currents of the bioelectrocatalytic reduction of H_2O_2 on HRP-modified electrodes, I_k, at different pHs were

obtained and from the KL-slopes the number of electrons transferred in the reaction was estimated being close to 2.To evaluate the kinetic characteristics of the overall process, the $1/I_k$ values were plotted vs. $[H_2O_2]^{-1}$ (Figure 2S-B) in accordance with Eq. 4 (the case of direct ET) and Eq. 5 (the case of mediated ET), corresponding to the description of bioelectrocatalytical kinetics [2] within the formal enzymatic kinetics model [3]:

$$1/I_k = 1/nFE_{DET}(1/(k_1 c_{H2O2})+1/k_s) , \quad (4)$$

$$1/I_k = 1/2n_1FE_{MET}(1/k_1\, c_{H2O2} + 1/k_3[S]) \quad (5)$$

Here, n_1 is the number of electrons transferred per mediator molecule and equals $0.5n$ for catechol; E_{DET} is the amount of the enzyme (in moles) participating in direct ET; E_{MET} is the total amount of active enzyme (in moles) on the electrode surface involved in the electrode reaction; k_s is the heterogeneous ET rate constant for direct ET; k_1 is the rate constant for H_2O_2 enzymatic reduction; [S] is the mediator concentration; k_3 is the rate constant of the mediated ET reaction, which represents the rate-determining step of E1 reduction by some electron donor S (other than the electrode) to the initial HRP state through the formation of E2, similar to enzymatic catalysis. The formed oxidised donor $S^{\bullet}$ is then electrochemically reduced by the electrode. From the experiments on mediated ET the part of the enzyme active in direct ET can be calculated as it is assumed that in the presence of a saturating concentration of a mediator ($5\ 10^{-4}$ M catechol) all HRP molecules adsorbed at the electrode participate in mediated ET, whereas in the absence of the mediator - only a fraction is available for direct ET [2]. The total amount of HRP adsorbed at the Au electrode, E_{MET}, was assumed to be 20 pmol cm^{-2} in the case of nHRP and 30 pmol cm^{-2} in the case of rHRPs, attributed to the Au surface area determined with the Cottrell equation, $0.032cm^2$.

As can be seen from Figure 2S-B, the obtained data show a linear dependence, from the slope of these plots k_1 was determined; from the intercepts with the Y- axis - the value of k_s in the case of direct ET and k_3 in the case of mediated ET. The ratio of the slopes in the presence and in the absence of a mediator gave the ratio between E_{DET} and E_{MET}.

Kinetics of bioelectrocatalysis with adsorbed on electrodes rHRPs was studied with the RDE system. However, RDE stirring affected the adsorption of HRP at the Au electrodes, so it was impossible to obtain any reliable data for native HRP-modified Au electrodes due to the intensive desorption of the enzyme from the electrode surface resulting in constantly decreasing amperometric response. Satisfactory stability of rHRPs at gold provided an opportunity to perform kinetic experiments at different pHs with the RDE system.

Table 1S. Enzymatic and bioelectrocatalytic activity of rHRPs immobilised on Au electrodes, determined from the amperometric response of the rHRPs-modified electrodes at -50 mV in the RDE system (data from Figure 4, main text)

Peroxidase	Activity (ABTS) U/mg	k_s, s^{-1}, pH 6.0
native HRP*	1200±10	12±4
rHRP	1400±25	178±52
C_{His}rHRP	1480±20	368±34
C_{His}rHRP57Cys	960±15	118±11
C_{His}rHRP189Cys	1700±10	45±7
N_{His}rHRP	900±20	454±33

*results obtained in a flow-through electrochemical system.

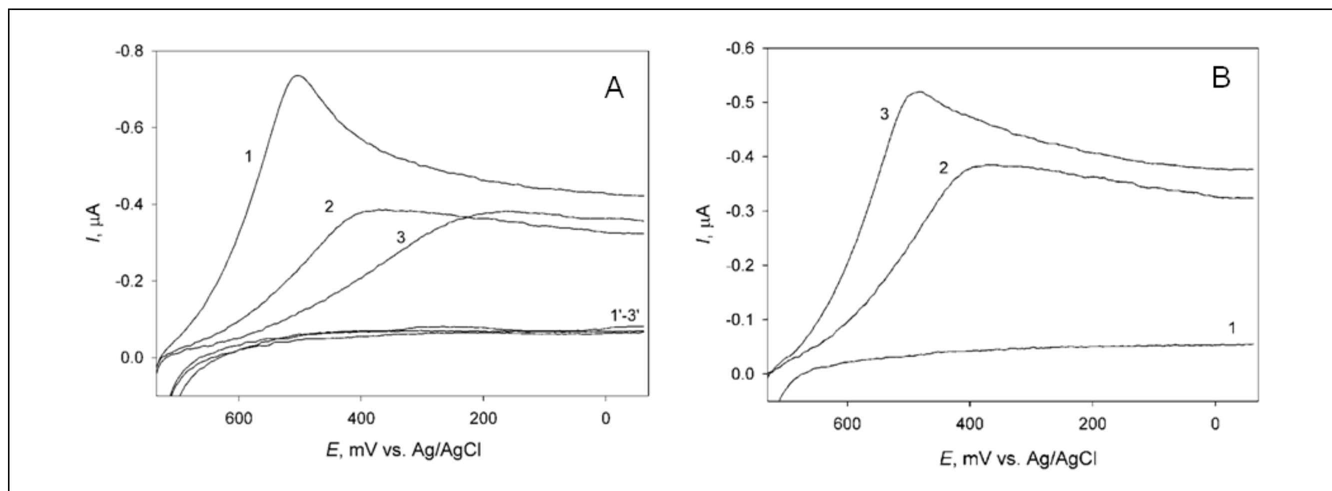

Figure 3S. Representative LSVs of the Au electrodes modified with (A) N_{His}rHRP (1100 U mg^{-1}), in the presence of 0.1 mM H_2O_2; (1´-3´) the corresponding background in the absence of H_2O_2; curves 1-3 correspond to θ (1) 1; (2) 0.84, and (3) 0.73 as determined by integration of the capacitive double-layer region of the background curves, in assumption that curve (1´) corresponds to the limiting monolayer coverage (θ=1); (B) rHRP, (2,3) in the presence of 0.1 mM H_2O_2, the enzyme activity is (2) 1200 and (3) 1600 U mg^{-1}.

Table 2S. Enzymatic and bioelectrocatalytic activity of rHRPs immobilised on Au electrodes (partially adapted from [4]), determined from the amperometric response of the rHRPs-modified electrodes at -50 mV in wall-jet flow-through electrochemical system.

Peroxidase	**Activity (ABTS) U/mg**	**% direct ET**	**k_s, s^{-1}, pH 7.4**	**k_s, s^{-1}, pH 6.0**	**k_1, 10^{-6} $M^{-1}s^{-1}$**	**k_3, 10^{-4} $M^{-1}s^{-1}$**
C_{Strep}rHRP	**438±15**	88±12	19±5	**115±17**	0.65 ±0.20	7.9±6.0
rHRP	**1400±25**	81±18	32±7	**260±31**		
rHRP	**565±15**	75 ±25	27±4	**202±27**	0.51 ±0.09	
C_{His}rHRP57Cys	**575±20**	80±20	31±9	**128±12**	0.34±0.04	1.7±0.3
C_{His}rHRP57Cys	**26±3**	68 ±17	2.5±0.1	**10±2**	0.12 ±0.01	0.9±0.2
C_{His}rHRP189Cys	**1710±178**	71±24	20±2	**87±20**	0.55±0.11	2.9±0.6
C_{His}rHRP189Cys	**1100±50**	75±20	10±3	**45±6**	0.35±0.05	2.5±0.5
C_{Strep}rHRP189Cys	**17±1**	58±20	1.9±0.3	**9±0.3**	0.08±0.01	0.5±0.1

References:

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