Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
This journal is © the Owner Societies 2017

ELECTRONIC SUPPLEMENTARY INFORMATION

Structure, Electrocatalysis and Dynamics of Immobilized Cytochrome PccH and its Microperoxidase

Célia M. Silveira,[a,b,*] María A. Castro,[c] Joana M. Dantas,[b] Carlos Salgueiro,[b] Daniel H. Murgida,[c] Smilja Todorovic[a,*]

[a] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

[b] UCIBIO, REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Monte de Caparica, Portugal

[c] Departamento de Química Inorgánica, Analítica y Química Física and INQUIMAE (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

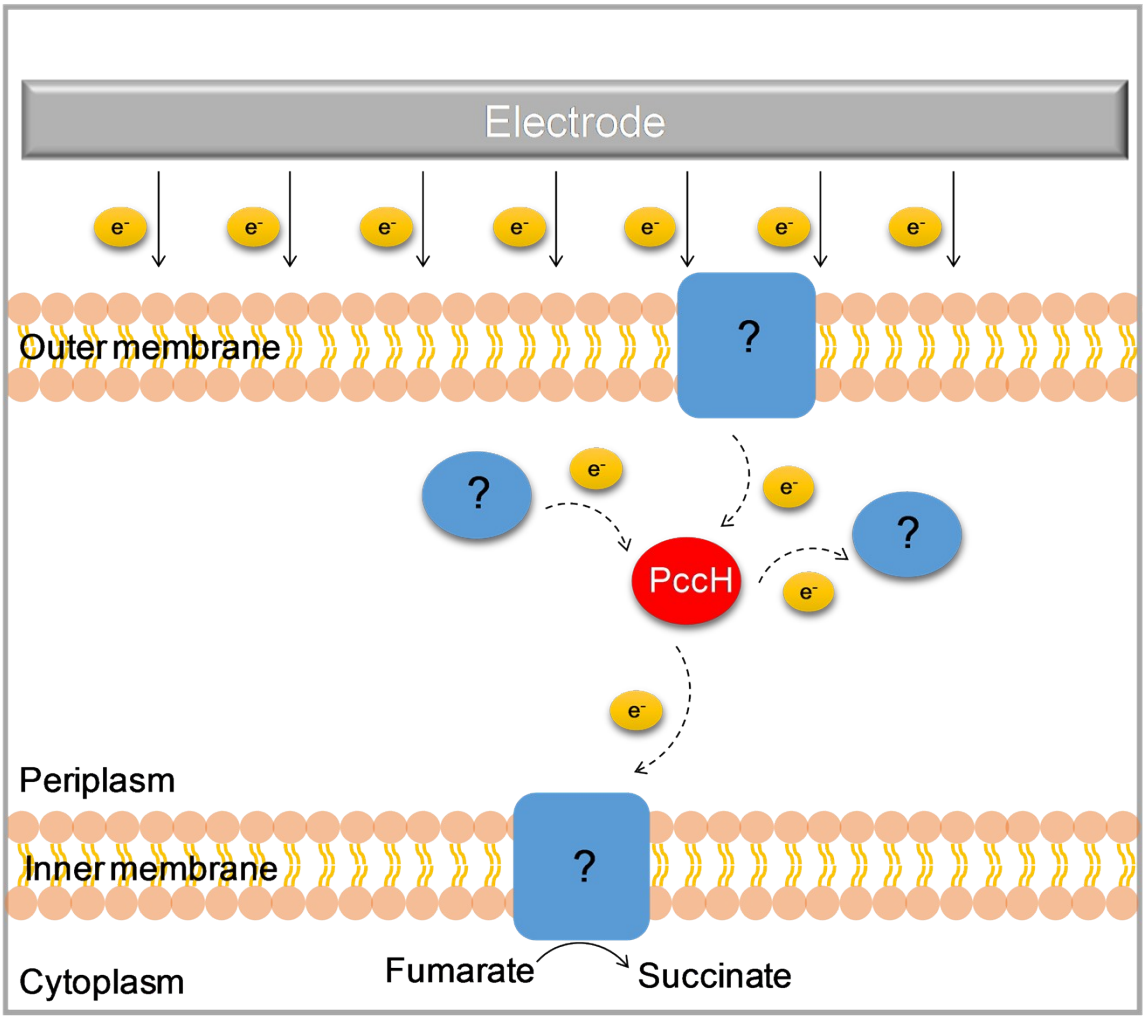


Figure S1- Schematic representation of possible electron transfer pathways that involve PccH in *G. sulfurreducens* cells grown using graphite cathode (poised at -293 mV) as a sole electron donor and fumarate (E^0 = 30 mV) as terminal electron acceptor.[3] The dashed arrows indicate possible electron flow from / to still unknown redox partners of PccH (E^0 = - 24 mV).[4] The values of reduction potential are referred to the NHE.

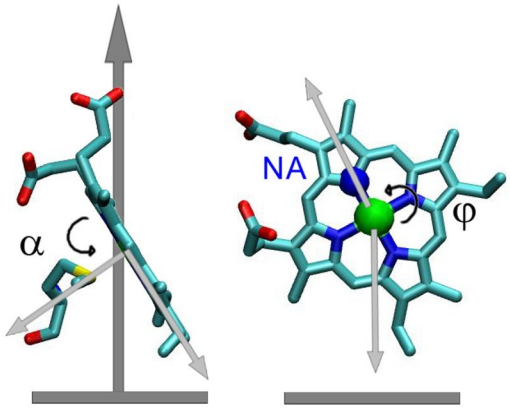


Figure S2- Representation of the rotational angles of the heme group that define the orientation of the protein with respect to the SAM surface. Left: α is the angle between the Fe–S (Met84) bond, which is perpendicular to the heme plane, and the Z-axis. For α values close to 0◦ or 180◦ the heme lies parallel to the SAM, whereas α ~ 90◦ means that it is orthogonal. Right: φ is defined by the Fe–N (pyrrole A) bond and the vector pointing towards the SAM that is contained by the heme plane. This angle describes the rotational orientation of the heme.

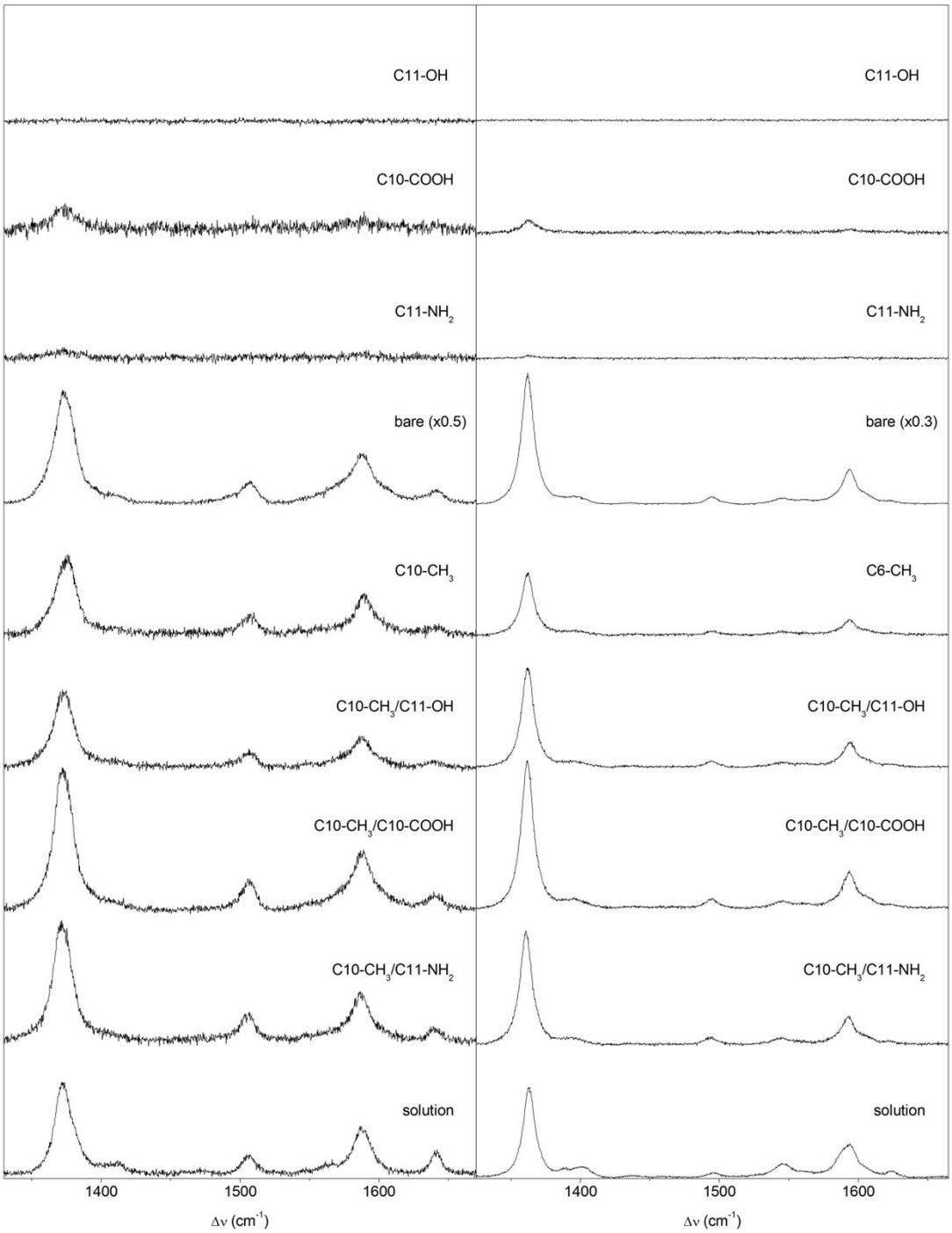


Figure S3 – SERR spectra of PccH immobilized on SAM coated or ´bare´ electrodes and RR spectra of PccH in solution. Ag electrodes are coated with (from top to bottom) C11-OH, C10-COOH, C11-NH_2, bare Ag, C10-COOH, C10-CH_3 (C5-CH_3 in the right panel, due to absence of SERR signals of ferrous PccH at C10), C10-CH_3/C11-OH, C10-CH_3/C10-COOH and C10-CH_3/C11-NH_2 terminated SAMs. SERR spectra are measured at poised potential of 300 mV (left panel) and -200 mV (right panel) in 12.5 mM phosphate buffer, pH 7 and 12.5 mM K_2SO_4. The RR spectra of PccH (100 μM solution) are acquired in the resting state (left panel) and upon sodium dithionite reduction (right panel) in 20 mM phosphate buffer pH 7.2. Laser (413 nm) power and accumulation time were 1.5 mW and 30 s (SERR) or 3 mW and 40 s (RR). Left (and right) panel spectra are represented with the same intensity axis scaling except for bare electrode spectra.

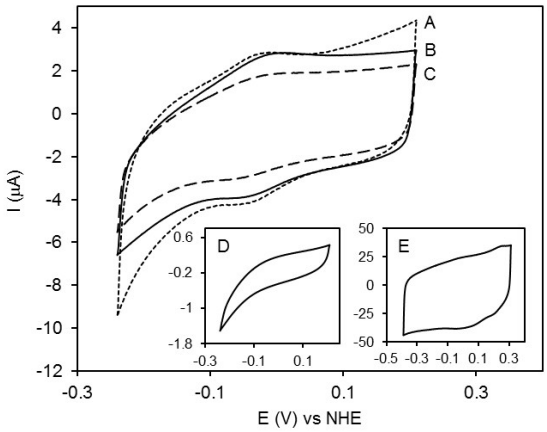


Figure S4 – Cyclic voltammograms of PccH adsorbed on SAM coated and bare Ag surfaces. Electrodes are coated with **A)** C2-CH_3; **B)** C10-CH_3/C11-OH; **C)** C10-CH_3/C10-COOH and **D)** C10-CH_3 terminated SAMs; **E)** bare electrode. All measurements are performed in 12.5 mM phosphate buffer, pH 7 and 12.5 mM K_2SO_4 at 50 mV/s sweep rate.

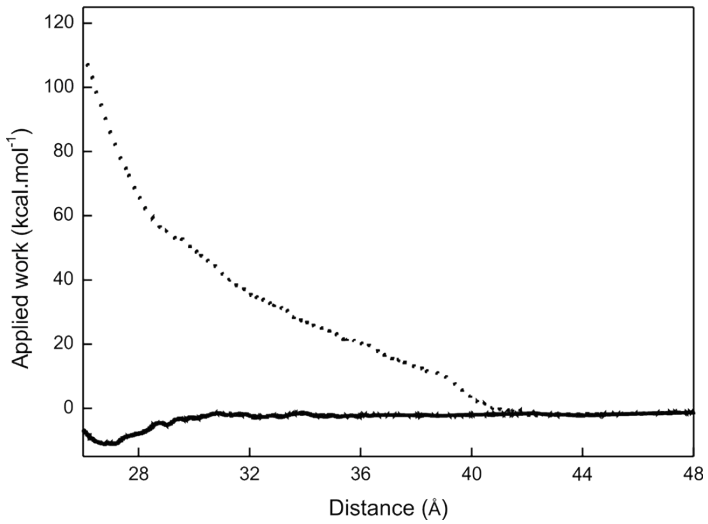


Figure S5 – Representative work profiles obtained by SMD for the binding of ferric PccH on COOH terminated SAMs starting from two different orientations. Solid line: binding orientation. Dotted line: non-binding orientation. The distance is defined from the center of mass of PccH to the SAM surface.

Table S1 – Structural parameters for the stable PccH/-COOH terminated SAM complexes as obtained by SMD (BO=binding orientation, MZ=main zone).

	α (°)	φ* (°)	Lys contacts	BO

Ferric PccH	36	42	119	
	132	224	36, 80	MZ
	118	280	33, 36, 38, 77, 80	MZ
	111	294	33, 36, 77, 80, 113	MZ
	138	210	80	MZ
	122	250	33, 77, 80	MZ
	136	217	36, 74, 77, 80	MZ
	99	291	33, 36, 38, 77, 113	MZ
	129	218	36, 80	MZ
	35	55	7, 119	
	74	176	42	
Ferrous PccH	174	205	74, 80	
	108	296	33, 36, 80, 113	MZ
	12	138	7, 10	
	125	211		MZ
	115	203	42, 77, 80	MZ
	141	220	74, 80	MZ
	121	291	36, 77, 80, 113	MZ
	33	150	7, 10	
	103	192	42	MZ
	113	87	7, 74, 113	
	33	317	119	
	146	200	74, 80	MZ
	35	153	7, 10	
	104	300	33, 36, 80, 113	MZ
	80	308	33, 113	MZ
	65	59	7, 113	
	105	301	33, 36, 77, 113	MZ

Table S2 – Structural parameters for the stable PccH/1:1 $–NH_2$ /-CH_3 terminated SAM complexes as obtained by SMD (BO=binding orientation, MZI=main zone).

	α (°)	φ* (°)	Glu/Asp contacts	zone
Ferr	109	202		MZI
	134	213	37, 92	MZI

	78	145	93	MZI
	38	155	24	MZI
	76	184	24	MZI
	133	221	37, 92	MZI
	134	216	37	MZI
	144	216	92, 93	MZI
	42	328	28	
	141	84	93, 120	
	120	87	93, 120	
H	128	227	37, 92	MZI
	119	205	92	MZI
	96	184	93	MZI
	70	145	14, 93	MZI
	60	147		MZI
	82	191	93	MZI
	96	189	93	MZI
	76	192	14, 24	MZI
	120	73	93, 120	
Ferrous PccH	139	220	37, 92	MZI
	131	233	37	MZI
	129	93	93	
	85	190	24	MZI
	136	221	92	MZI
	131	209	37, 92	MZI

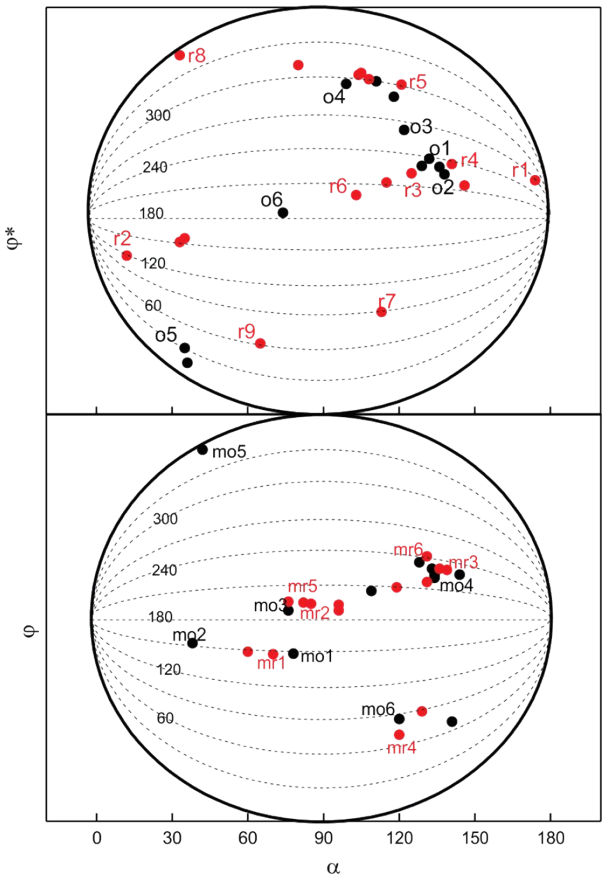


Figure S6 – α-vs-φ* plots as obtained by SMD for the initial adsorption complexes of PccH on –COOH terminated SAM (Top) and 1:1 $–NH_2$ /-CH_3 terminated SAM (Bottom). Black circles: ferric PccH. Red circles: ferrous PccH. Isolines indicate the φ* values.

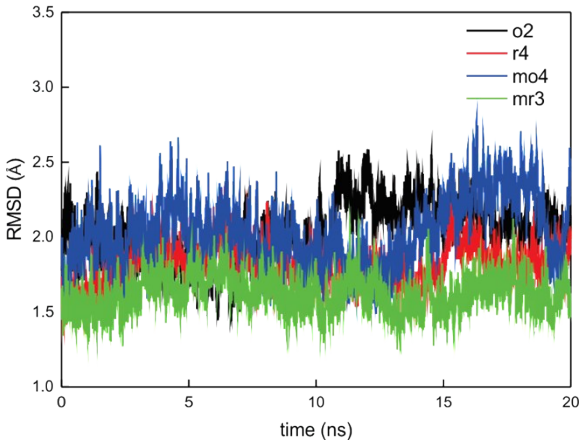


Figure S7 – Representative plots of RMSD values with respect to the initial PccH structure along the 20 ns MD simulations of PccH on –COOH terminated SAM (ferric: black, ferrous: red) and 1:1 $–NH_2$ /-CH_3 terminated SAM (ferric: blue, ferrous: green).

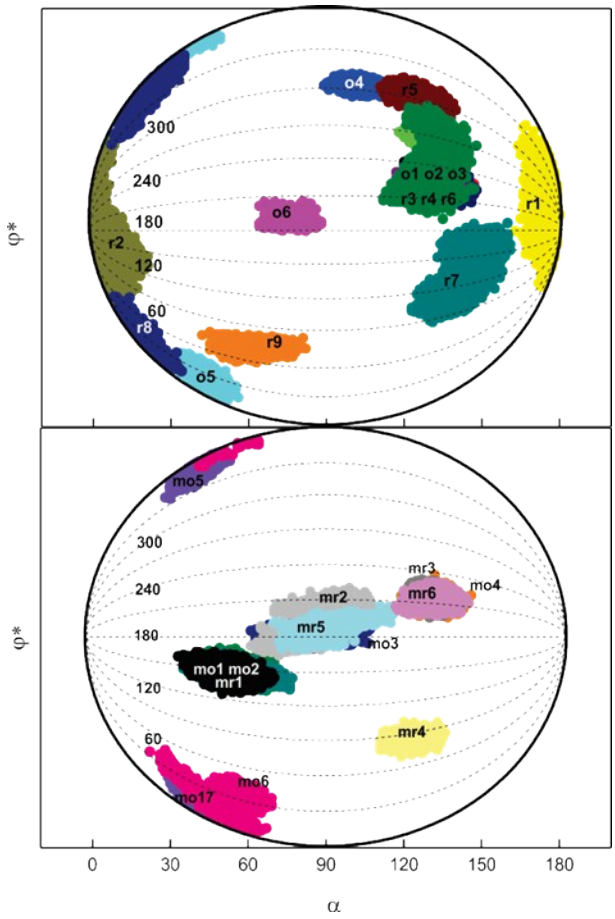


Figure S8 – α-vs-φ* plot as obtained after 20 ns MD simulation for PccH on –COOH terminated (Top) and the 1:1 $–NH_2$ /$-CH_3$ terminated (Bottom) SAMs. Each color represents one of the binding minima used as starting points. Isolines indicate the φ* values.

Table S3 - Mean values of the binding energies for PccH/SAM complexes obtained with MM/PBSA calculations.

	Orientation	Binding energy (kcal.mol^{-1})
-COOH terminated SAM	o1	-26.87
	o2	-44.32
	o3	-19.54
	o4	-48.55
	o5	-12.44
	o6	-34.84
	r1	-18.16
	r2	-3.65
	r3	-42.59
	r4	-78.96
	r5	-19.83
	r6	-2.70
	r7	-11.79
	r8	-7.93
	r9	-4.22
1:1 –NH_2/-CH_3 terminated SAM	mo1	8.34
	mo2	8.76
	mo3	11.99
	mo4	-0.68
	mo5	7.58
	mo6	1.76
	mr1	7.14
	mr2	11.19
	mr3	-4.69
	mr4	-6.87
	mr5	14.95
	mr6	-3.65

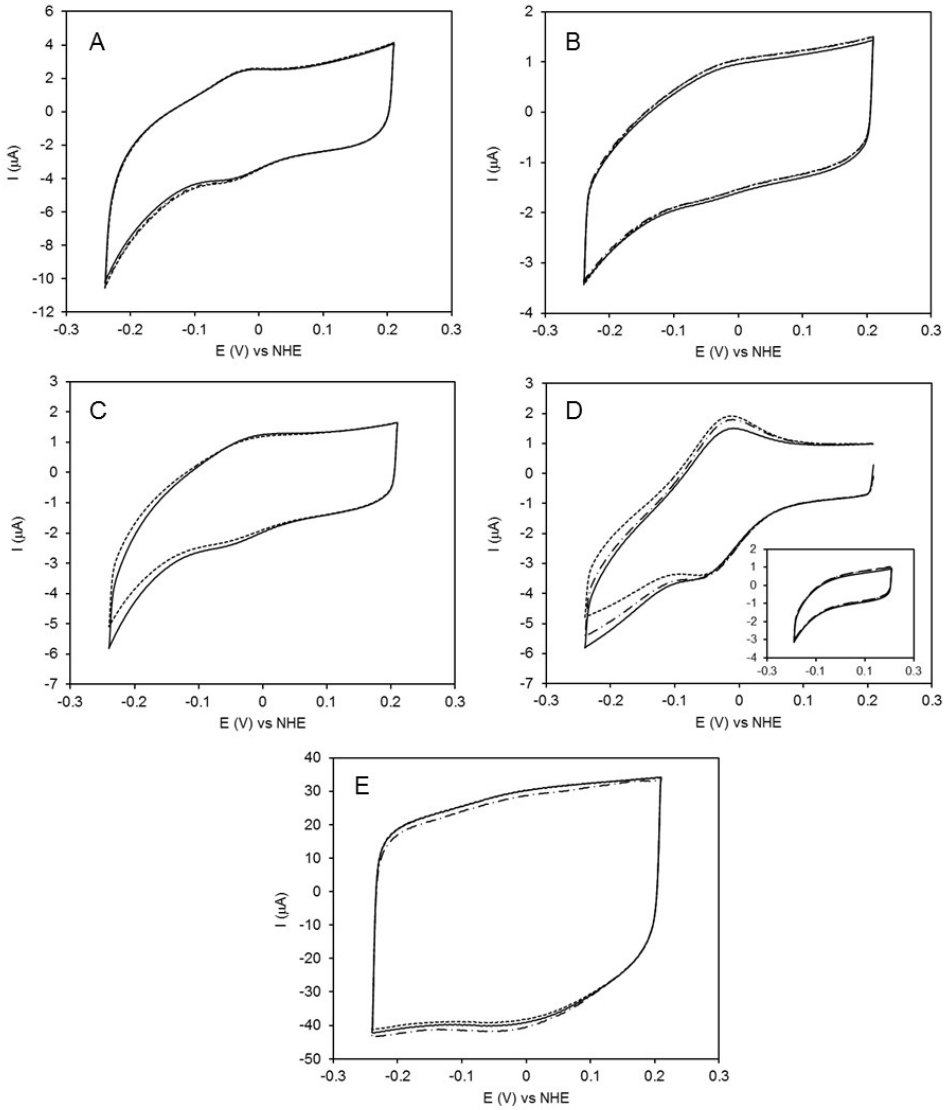
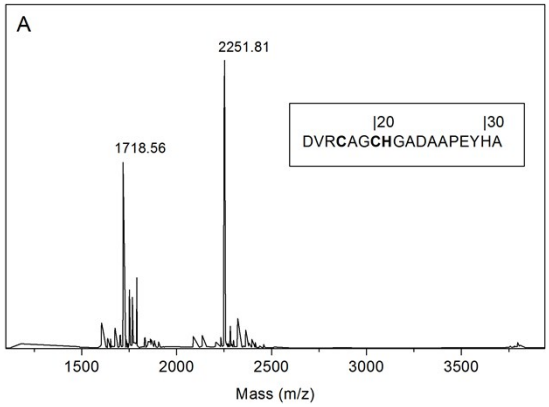


Figure S9 - Cyclic voltammograms of PccH adsorbed on SAM coated and bare Ag surfaces in the presence of 0 (dashed line), 0.1 (dashed-dotted line) and 0.5 mM H_2O_2 (solid line). Electrodes are coated with **A)** C2-CH_3; **B)** C10-CH_3/C11-OH; **C)** C10-CH_3/C10-COOH; **D)** C10-CH_3/C11-NH_2 terminated SAMs, **E)** bare electrode. **Inset:** C10-CH_3/C11-NH_2 functionalized electrode in the absence of immobilized PccH and in the presence of H_2O_2. All measurements are performed in 12.5 mM phosphate buffer, pH 7 and 12.5 mM K_2SO_4 at 50 mV/s.



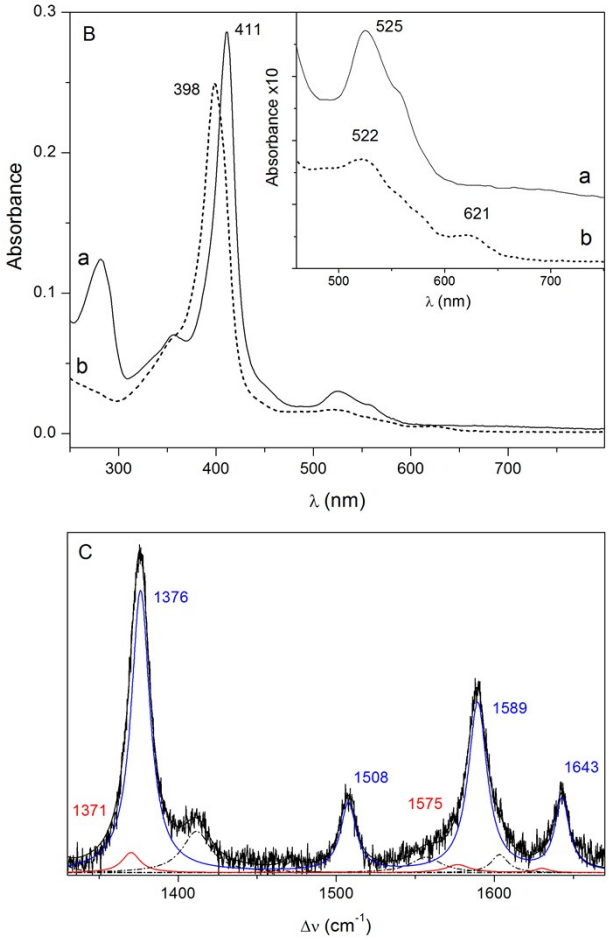


Figure S10 – Spectral characterization of ml-PccH. **A)** Mass spectrum of ml-PccH obtained by proteolysis of PccH, with designated heme containing (2.3 kDa) and heme depleted (1.7 kDa) fractions. **Inset:** Proposed sequence of the heme containing ml-PccH peptide. The residues in bold indicate the covalent attachment of the porphyrin and the axial iron coordination in PccH. **B)** Electronic absorption spectra of (**a**) PccH and (**b**) ml-PccH. Spectra of resting state samples ~2 µM in 20 mM phosphate buffer, pH 7 and 250 mM NaCl. **Inset:** Enlarged 450 - 750 nm region. **C)** RR spectrum of ferric ml-PccH in solution. The component spectra represent the overall fit (black line), His/His (blue line) and His/- (red line) species as well as non-assigned bands (dashed-dotted line). Spectrum is recorded with 413 nm excitation, 3 mW laser power and 40 s accumulation time, from 30 µM sample in 20 mM phosphate buffer, pH 7 and 250 mM NaCl.