Supplementary Material (ESI) for *PCCP*

Effect of Peptide Orientation on Electron Transfer

Bushra R. Chaudhry, James D. E. T. Wilton-Ely, Alethea B. Tabor,* Daren J. Caruana*

Electronic Supporting Information

General experimental methods and materials

Chemical and reagents were purchased from Sigma-Aldrich Co. Ltd, Acros, Avocado, AGTC Bioproducts Ltd, BDH laboratory supplies, Fisher Chemical Co., Fluka, Lancaster Chemical Co., NovaBiochem, Senn Chemicals and unless stated these were used without further purification. Eicosanethiol was purchased from Robinsons Brothers Ltd. $Ru(Bipy)_2Cl_2$ was a kind gift from Dr James Wilton-Ely.

Solvents were used without further purification unless otherwise stated; anhydrous THF was distilled under nitrogen from sodium and benzophenone. Anhydrous dichloromethane was distilled under nitrogen and calcium hydride. Anhydrous triethylamine was distilled under nitrogen and calcium hydride. Ethyl acetate was dried over molecular sieves (4 Å, 1.6mm pellets) under an argon blanket. Absolute ethanol was purchased from BDH and was 99.7% pure. Anhydrous DMF was purchased from, Lancaster Chemical Co. Deionised water was Milli-Q Gradient 18.2 MΩ cm (at 25°C) water system. The term *in vacuo* refers to the removal of solvent(s) by evaporation under reduced pressure using a Buchi rotary evaporator.

Gold electrodes (1.6mm diameter), Glassy carbon electrode (3.0mm diameter) and silver/silver chloride (Ag/AgCl) reference electrodes were purchased from BAS, UK. Alumina polishing slurries and polishing microcloth were purchased from Buehler, USA.

Instrumentation

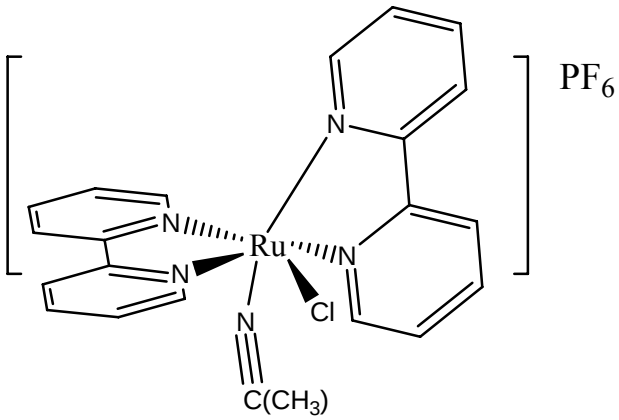
Proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded at 300 MHz using a Bruker AMZ-300 MHz machine. Splitting patterns have been abbreviated as: singlet (s), doublet (d), triplet (t), double doublet (dd) and multiplet (m). Coupling constants (*J*) are quoted in Hertz (Hz), chemical shifts (δ) are quoted as parts per million (ppm) relative to tetramethylsilane (TMS) as the reference. Mass Spectra (MS) was recorded on a Micro mass Quatro LC electrospray mass spectrometer. IR spectrographs were recoded on a Shimadzu 8700.

Automated Peptide synthesis was carried out on a MultiSynTech Syro Peptide Synthesiser (model MP-60). Analytical HPLC was carried out using Varian model 210 pumps with an autosampler model 410, a Supelco C18-10 column with a flow rate of 1.5ml/min, using Star 5.51 software. Preparatory HPLC was carried out using Varian model 210 pumps, a Supelco wide pore C18-10 column with a flow rate of 15ml/min using Galaxy software. In both cases the A and B solvents were HPLC grade water and acetonitrile respectively, both containing 0.1% TFA.

The vacuum oven used was a Precision vacuum oven, weighing scales used were Precision 125A and 180A. The pH meter was a Kent EIL 7045/45 with pH standards 3.2, 7.0 and 9.4 created using tablets obtained from BDH. Other instruments included a Vortex Genie 2, Sonicator UW, Dessicator (Secador).

Cyclic Voltammetry (CV) experiments were carried out a 3 electrode system. In all electrochemical measurements a background electrolyte was always present. The electrochemical cell consisted of a silver/silver chloride (Ag/AgCl) reference electrode (BAS, UK), a platinum flag (Johnson Matthey 99.9% pure) as the counter electrode and either a gold or carbon working electrode (BAS, UK) with a 1.6mm and 3.0mm diameter respectively. All three electrodes were connected to a potentiostat (Autolab; Windsor Scientific, UK) which was controlled using its PC software GPES 6.0.

Synthesis of $[Ru(bpy)_2(MeCN)Cl]PF_6$



$Ru(bpy)_2Cl_2$ (0.200g, 0.4mmol) along with 12 ml of acetonitrile (0.22 mol) was refluxed for 25 min. Potassium hexaflurophosphate (molar excess) dissolved in 50/50 water/EtOH was

added. The mixture then was refluxed for a further 30 min. The reaction was then cooled to room temperature and the acetonitrile solution was then evaporated to dryness. The reddish-orange solid was then recrystallised from acetonitrile and ether and dried under vacuum overnight to give a reddish-orange solid (233 mg, 91.6%). ^{1}H NMR δ 2.37 (s, -CC<u>H</u>$_3$-, 3H), 7.08-7.12 (t, *J* = 6.0 Hz, bpy 1H), 7.18- 7.23 (t, *J* = 7 Hz, bpy, 1H), 7.51-7.52 (d, *J* = 5.5 Hz, bpy, 1H), 7.68- 7.80 (m, bpy, 5H), 8.00-8.05 (t, *J* = 7.7 Hz, bpy, 1H), 8.08-8.14 (bpy, 2H), 8.21-8.27 (bpy, 2H), 8.36-8.39 (d, *J* = 8 Hz, bpy, 1H), 9.46-9.48(d, *J* = 5 Hz, bpy, 1H), 10.04-10.06 (d, *J* = 5 Hz, bpy, 1H); IR ν_{max} (cm^{-1}); 2260 (C≡N), 1604 (C=C); *m/z* (ES +) 490 ((M+1) - PF_6 salt) in water/acetonitrile.

Peptide Synthesis

The peptides were synthesised on a MultiSynTech Syro Peptide Synthesiser – model MP-60 using Fmoc-protected amino acids (Novobiochem). Rink amide- MBHA PS resin crosslinked with 1% DVB 100-200 mesh was supplied by Senn Chemicals; resin loading 0.81mmol/g, 0.86mmol/g.

HBTU Coupling

To each syringe from the amino acid rack, amino acids dissolved in DMF (600 µl, 0.4M) were added, followed by the addition of HBTU in DMF (600 µl, 0.4M) and DIPEA in NMP (300 µl, 1.7M). The mixture was then reacted for 40 min with a fast vortex every 20 seconds every 4 min. The solution were then drained and the resin washed with DMF (4 x 1600 µl).

Fmoc Deprotection

To each syringe (1500 µl) of piperidine (40% v/v) in DMF was added and left to react for 3 min with a fast vortex of the reaction for 10 seconds every 1 min, and then drained. This was then followed by the addition of (750 µl) DMF and (750 µl) of piperidine (40% v/v) in DMF and reacted for 10 min with a fast vortex of the reaction for 20 seconds every 2 min and then drained. The resin was then washed and drained with DMF (6 x 1600µl) with a 10 second vortex.

Acetyl capping

The peptides were then capped using a cocktail solution ratio of 1:1:8 of acetic anhydride, DIPEA and DMF. This was then added to each syringe and reacted for 1hr (vortex for 30

seconds every 4 min). After completion the solution was drained from the syringe and the resin agitated and washed with DMF (6 x 1ml).

Cleavage, deprotection and HPLC of peptides

The resin was then washed (no vortex) with CH_2Cl_2 (3 x 3ml per syringe), MeOH (3 x 3ml per syringe) and ether (3 x 3ml per syringe). After the ether wash the resin was vacuum pumped several times more, to ensure all the ether had been removed. The resin was then treated to cleavage and deprotection mixture (94% TFA, 2.5% water, 2.5% ethanedithiol, 1% triisopropylsilane) for 3 hr to cleave the peptide from the resin and side-chain protecting groups. The cleaved peptide solutions were collected and the syringe rinsed with TFA (2 x 0.5 ml). The combined solutions were then concentrated *in vacuo* so that the volume was halved. Precipitation of the peptide was induced by adding cold ether to the solution. This mixture was then placed in the freezer for 30 min to induce further precipitation, and then centrifuged (10 min at 3500 rpm at 0°C). The supernatant ethereal layer was then decanted. The peptide pellet was then resuspended in cold ether and the centrifugation procedure was then repeated twice more. The peptide pellet was then dissolved in the minimum amount of ddw water and then freeze-dried to produce a white fluffy solid.

The purity of the peptide was assessed by analytical hplc and mass spectra, and if required further purification was carried out using preparatory hplc.

Peptide sequences:

1 Ac-LysAlaAlaAlaAlaLysAlaAlaAlaAlaHisAlaAlaAlaAlaCys-NH_2

(RMM: 1408) *m/z*; (ES +) 470 $(M+3H)^{3+}$ R_T 6.743 ; gradient 90% - 0% water over 30 min

2 Ac-LysAlaAlaAlaAlaHisAlaAlaAlaAlaLysAlaAlaAlaAlaCys-NH_2

(RMM: 1408) *m/z*; (ES +) 470 $(M+3H)^{3+}$ R_T 6.487 ; gradient 90% - 0% water over 30 min

3 Ac-CysAlaAlaAlaAlaLysAlaAlaAlaAlaHisAlaAlaAlaAlaLys-NH_2

(RMM: 1408) *m/z*; (ES +) 470 $(M+3H)^{3+}$ R_T 6.283 ; gradient 90% - 0% water over 30 min

4 Ac-CysAlaAlaAlaAlaHisAlaAlaAlaAlaLysAlaAlaAlaAlaLys-NH_2

(RMM: 1408) *m/z*; (ES +) 470 $(M+3H)^{3+}$ R_T 6.667 ; gradient 90% - 0% water over 30 min

Analysis of results

By analysing the cyclic voltammograms of the ruthenium peptide complex **(2)** attached to different peptides at different scan rates, enabled the determination of the kinetics for electron transfer. The Laviron method [18] was used to calculate the kinetics for when $\Delta E_p < 200/n$ mV. By taking the anodic peak potentials for the second redox couple, shown in Figure S1, and normalising it against the $E_{1/2}$, characteristic, trumpet plots were obtained, shown below. The steepest part of the plot was analysed to provide *k* from the intercept on the x axis. As can be seen from the plots in figure S2, the anodic and cathodic legs of the plot are very symmetrical as the peak potentials from the anodic and cathodic CVs merge at low scan rates, v < 50 mV s^{-1}.

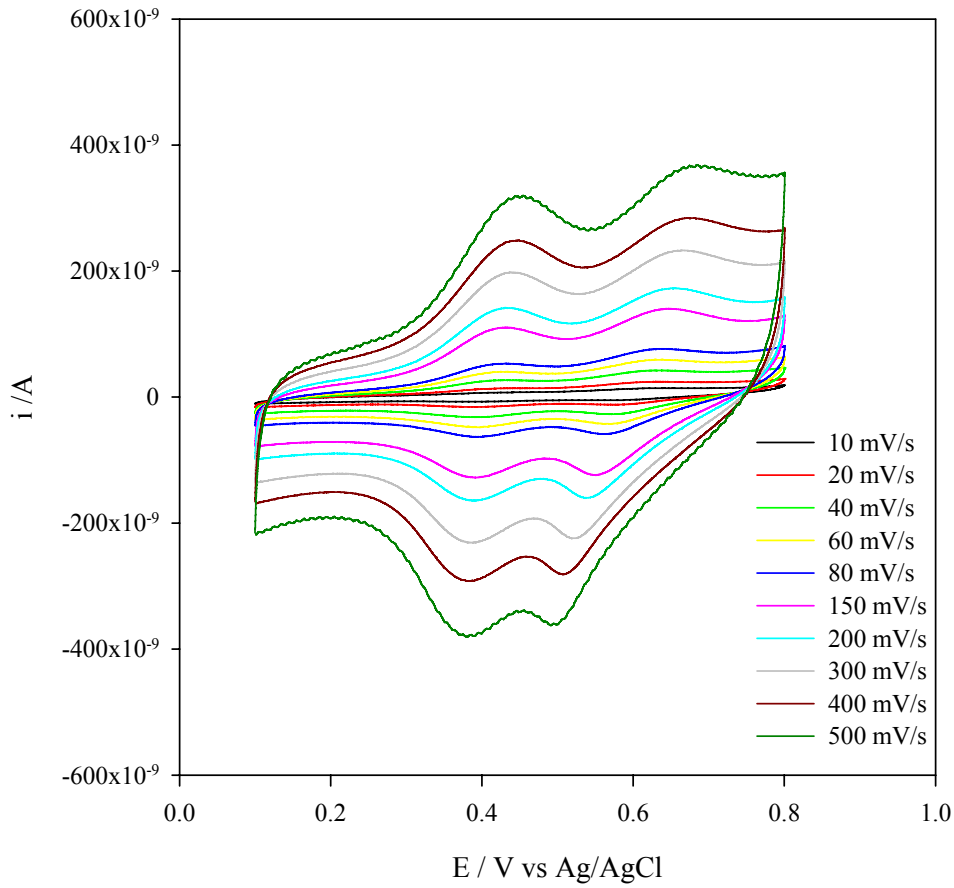


Figure S1: Plot showing multiple scan rates from 10 to 500 V s^{-1} of complex (2) attached to peptide 1 from SAM created using 10% of peptide 1 in thiol solution, in pH 7.0, 10 mM PBS at a 0.16 cm diameter gold electrode.

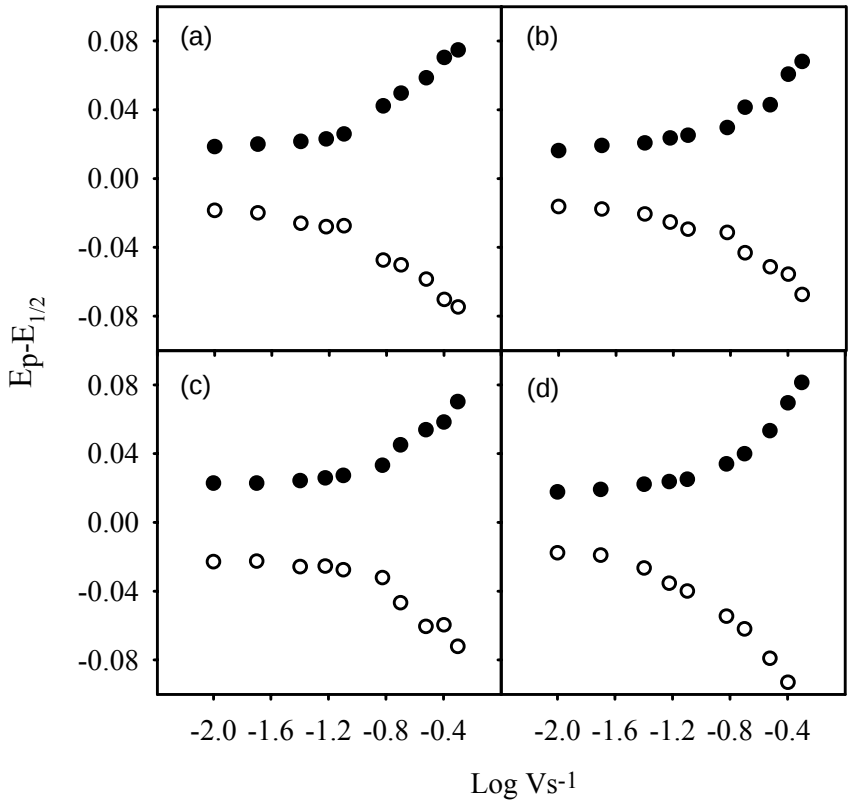


Table S2: Showing the trumpet plots, peak potential for anodic and cathodic peaks corrected for $E_{1/2}$ for the peak 2, from CVs of (a) 1, (b) 2, (c) 3 and (d) 4 from SAMs created using 10% peptide in mixed thiol solution, in pH 7.0, 10 mM PBS at a 0.16 cm diameter gold electrode. Scan rate analysis between 0.01 to 0.2 V s^{-1}.

Peptide	$E_{1/2}$ Peak 1 (mV)	$E_{1/2}$ Peak 2 (mV)	r^2	k (s^{-1})
1	$404 \pm 4x10^{-3}$	$604 \pm 4x10^{-3}$	0.99256	0.368 ± 0.024
2	$406 \pm 4x10^{-3}$	$599 \pm 4x10^{-3}$	0.99822	0.245 ± 0.022
3	$404 \pm 4x10^{-3}$	$594 \pm 4x10^{-3}$	0.99339	0.324 ± 0.026
4	$412 \pm 4x10^{-3}$	$601 \pm 4x10^{-3}$	0.98985	0.478 ± 0.035

Table S1: Data taken from scans comparing, $E_{1/2}$ for peak 1 and peak 2 for all four peptides and the kinetics for all four peptides. r^2 is given to reflect the 'goodness' of fit from the analysis for the plots in S2.

Table S1 lists the $E_{1/2}$ for both redox couples and the kinetics constants for all four peptides along with the r^2 showing the goodness of fit to the data in Figure S2.