Supporting Information

Development of a universal conductive platform for anchoring photo- and electroactive proteins using organometallic terpyridine molecular wires

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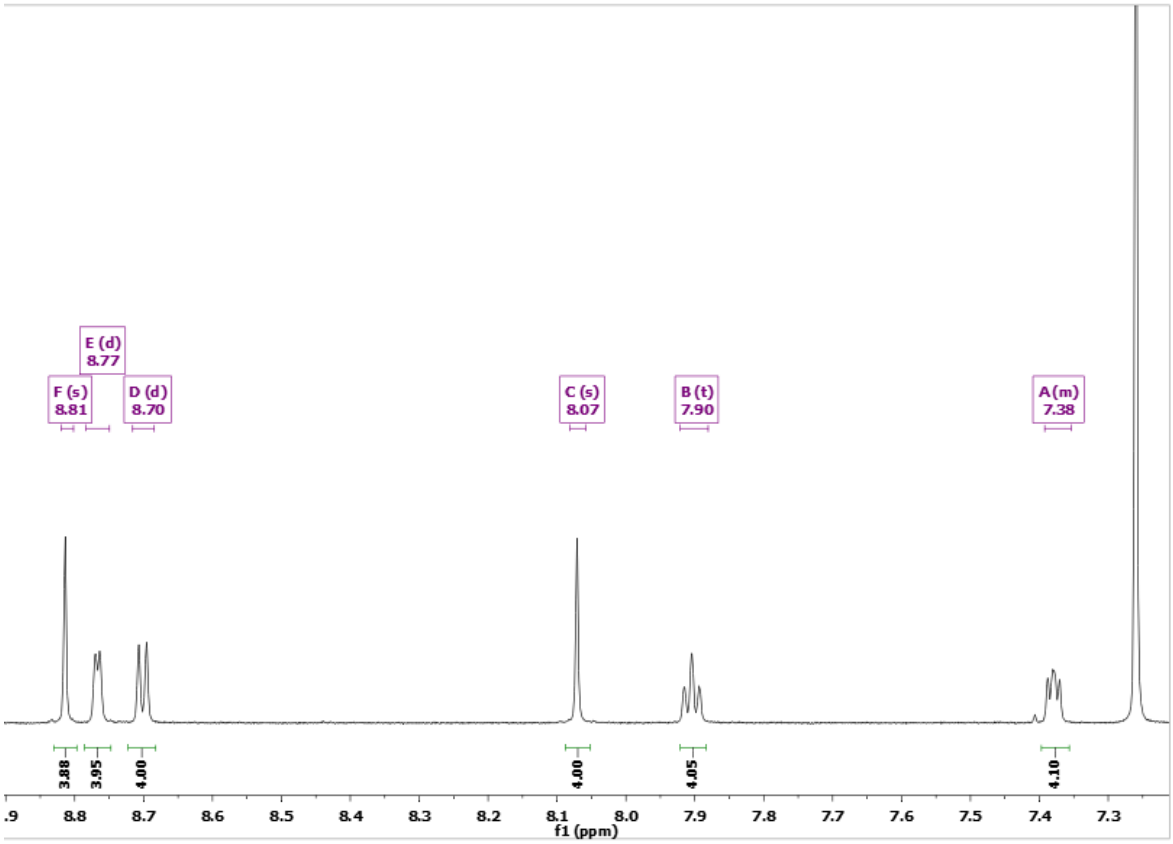

Fig. S1. ^{1}H NMR in $CDCl_3$ of the ditopic ligand **Lφ**.

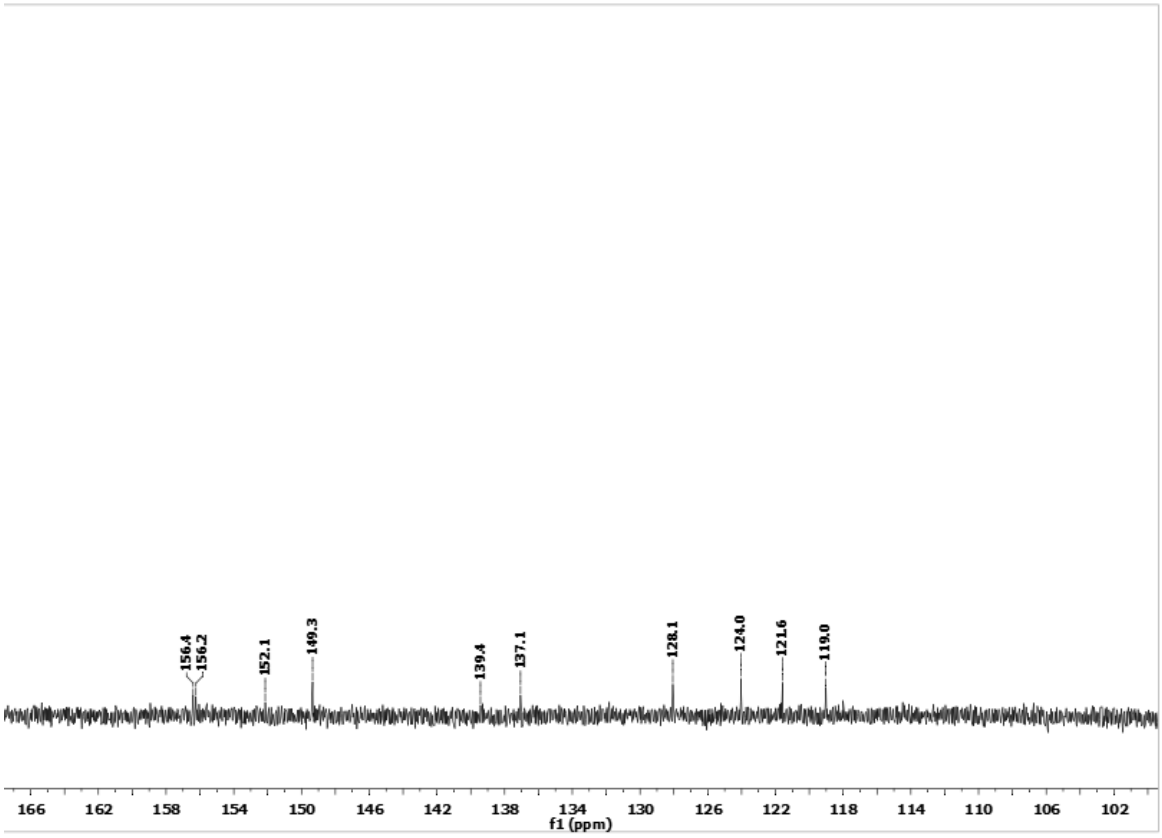

Fig. S2. ^{13}C NMR in $CDCl_3$ of the ditopic ligand **Lφ**.

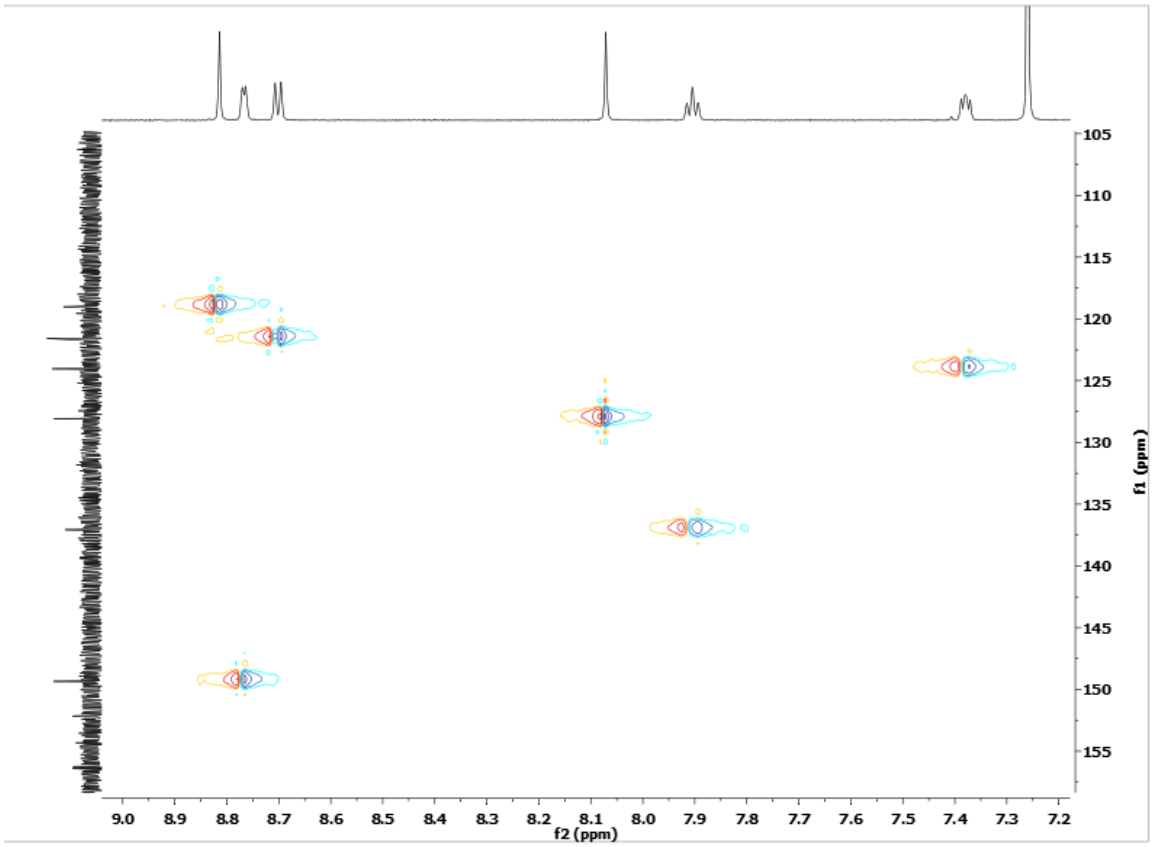

Fig. S3. HSQC NMR in $CDCl_3$ of the ditopic ligand **Lφ**.

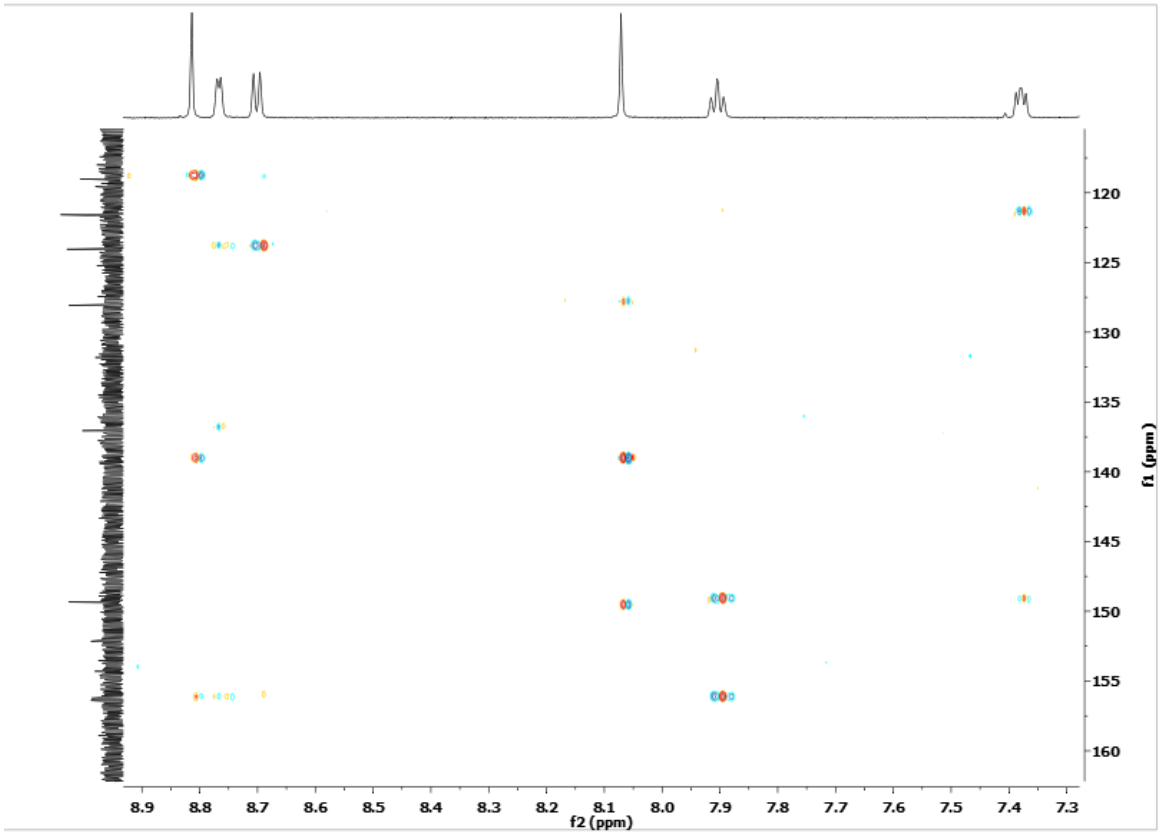

Fig. S4. HMBC NMR in $CDCl_3$ of the ditopic ligand **Lφ**.

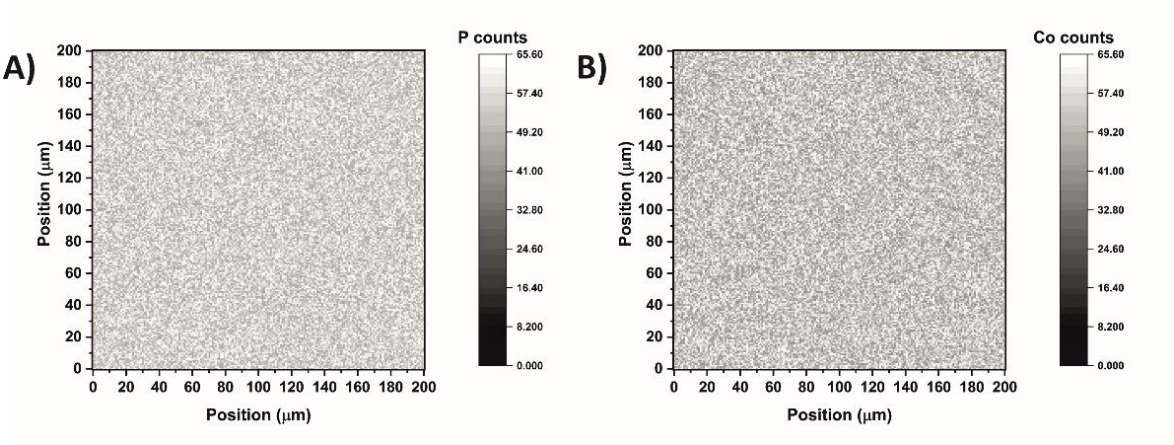

Fig. S5. Secondary Ion Mass Spectrometry analysis of ITO-TPY-Co-TPY electrodes with phosphorous (A) and cobalt (B) atoms.

Purification and biochemical characterisation of cytochrome *c*$_{553}$ 19 AA peptide linker variant

The *petJ* gene encoding for the cytochrome c_{553} was amplified *via* polymerase chain reaction (PCR) from the *Cyanidioschyzon merolae* genome and genetically modified by adding a 19 amino acid linker and a poly-histidine tag at the *C*-terminus. The procedure to engineer and express the recombinant cyt protein is described elsewhere[1]. The holoprotein was purified via Immobilized Metal Affinity Chromatography using a HisTrap column and applying a linear 50-500 mM imidazole gradient.[1]

The purity of the cyt c_{553} protein was confirmed by sodium-dodecyl phosphate gel electrophoresis (see **Fig. S6A**). The presence of the His_6- tag was confirmed by Western blot analysis using a His-Probe chemiluminescent detection system (see **Fig. S6B**). The redox activity of the purified holoprotein was confirmed with a redox difference absorption spectroscopy, using a UV-VIS Shimadzu UV 1800 spectrophotometer. The holoprotein was chemically reduced with sodium dithionite and then oxidized with ferrocyanide in a standard phosphate buffer (pH 7) at room temperature.[1] The spectra were measured for the reduced and oxidized cyt samples, as shown in **Fig. S6C**.

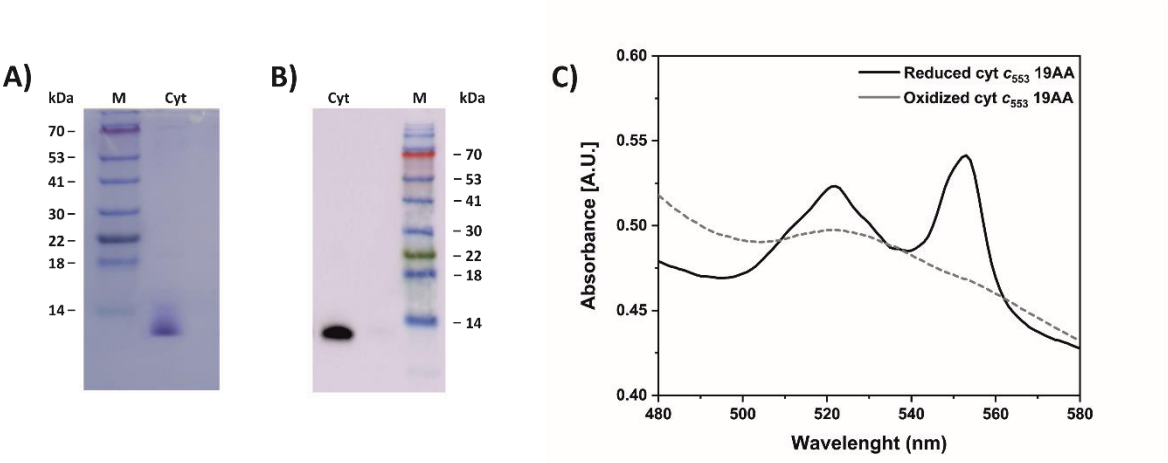

Fig. S6. Biochemical characterisation of purified His_6-tagged cyt c_{553} 19 amino acid variant. **A)** SDS-PAGE analysis shows the purified profile of the cyt *c*$_{553}$. **B)** Western Blot analysis on PVDF membrane. The His_6 -tag at the *C*-terminus of the holoprotein was detected using the HisProbe system. M, protein pre-stained size marker; cyt, cytochrome *c*$_{553}$ 19 AA variant (1.5 µg protein per lane). **C)** Redox difference adsorption spectroscopy analysis of the pure cyt *c*$_{553}$ sample. The UV-VIS spectrum of the reduced sample is shown in black (λ_{max} = 521 nm and 553 nm), while the spectrum of the oxidized cyt sample is depicted with a dashed grey line.

[1] J. D. J. Olmos, P. Becquet, D. Gront, J. Sar, A. Dąbrowski, G. Gawlik, M. Teodorczyk, D. Pawlak and J. Kargul, *RSC Adv.*, 2017, **7**, 47854–47866.

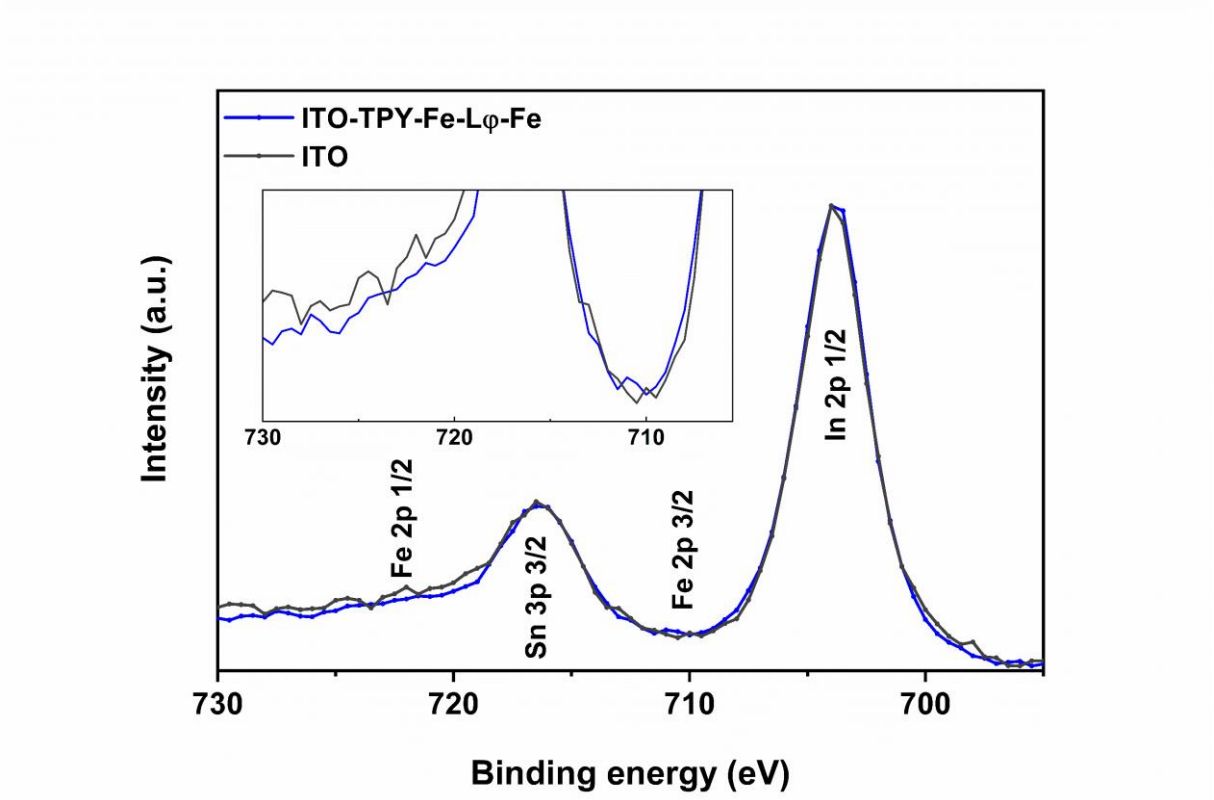

Fig. S7. Comparison of XP spectra registered for ITO and ITO-TPY-Fe-Lφ-Fe samples. Strong In $3p_{1/2}$ and Sn $3p_{3/2}$ signals mask the potential Fe 2*p* signals. Inset shows the enlargement of the region where the Fe 2*p* signals are expected.

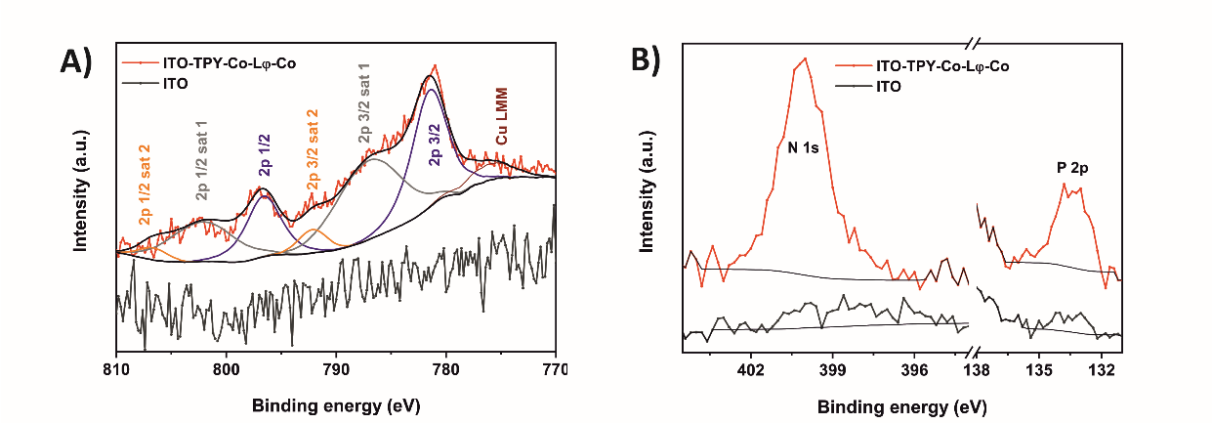

Fig. S8. A) XPS analysis of ITO and ITO-TPY-Co-Lφ-Co samples in the Co 2p region. For the Co-TPY spectrum Shirley background had been subtracted before linear background was applied. **B)** Comparison of N 1s and P 2p regions for ITO and ITO-TPY-Co-Lφ-Co samples.

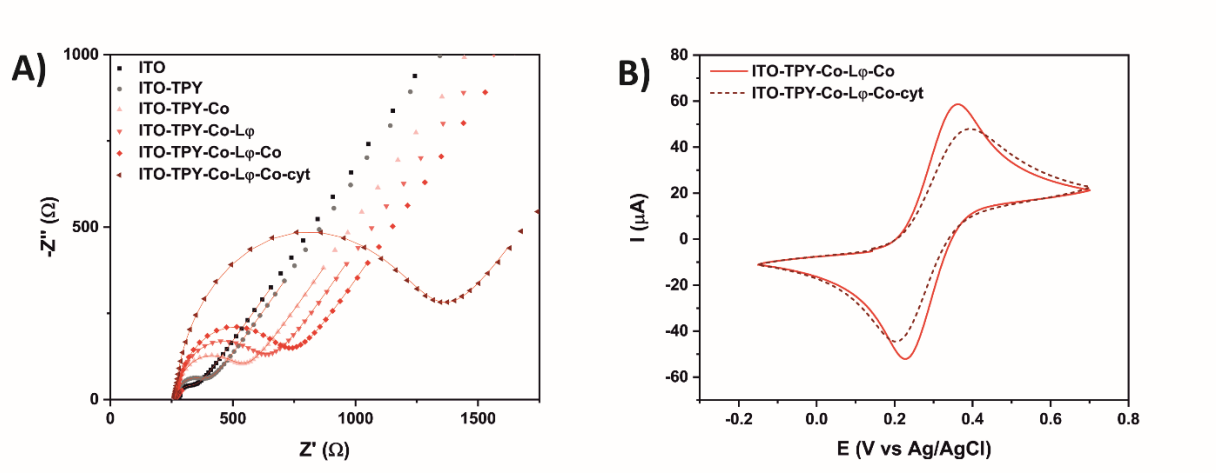

Fig. S9. (A) Nyquist plot with fitted curves of impedance spectra obtained for cobalt-based ITO electrodes at different stages of functionalisation. (B) Cyclic voltammetry of ITO-TPY-Co-Lφ-Co and ITO-TPY-Co-Lφ-Co-cyt at 100 mV/s. All measurements were performed with 1 mM 1,1'-ferrocenedimethanol in 0.1 M phosphate buffer (pH 7) under dark conditions.

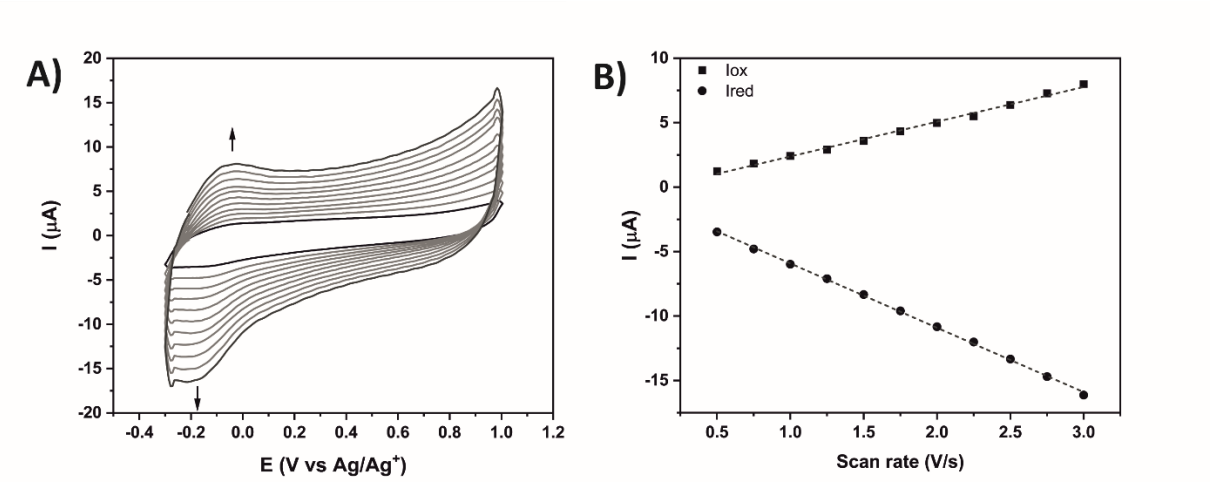

Fig. S10. (A) Cyclic voltammetry of ITO-TPY-Co-Lφ at different scan rates from 500 mV/s to 3 V/s in CH_3CN + 0.1 M HFPTBA. (B) Linear relation between current peak and scan rate obtained for ITO-TPY-Co-Lφ.

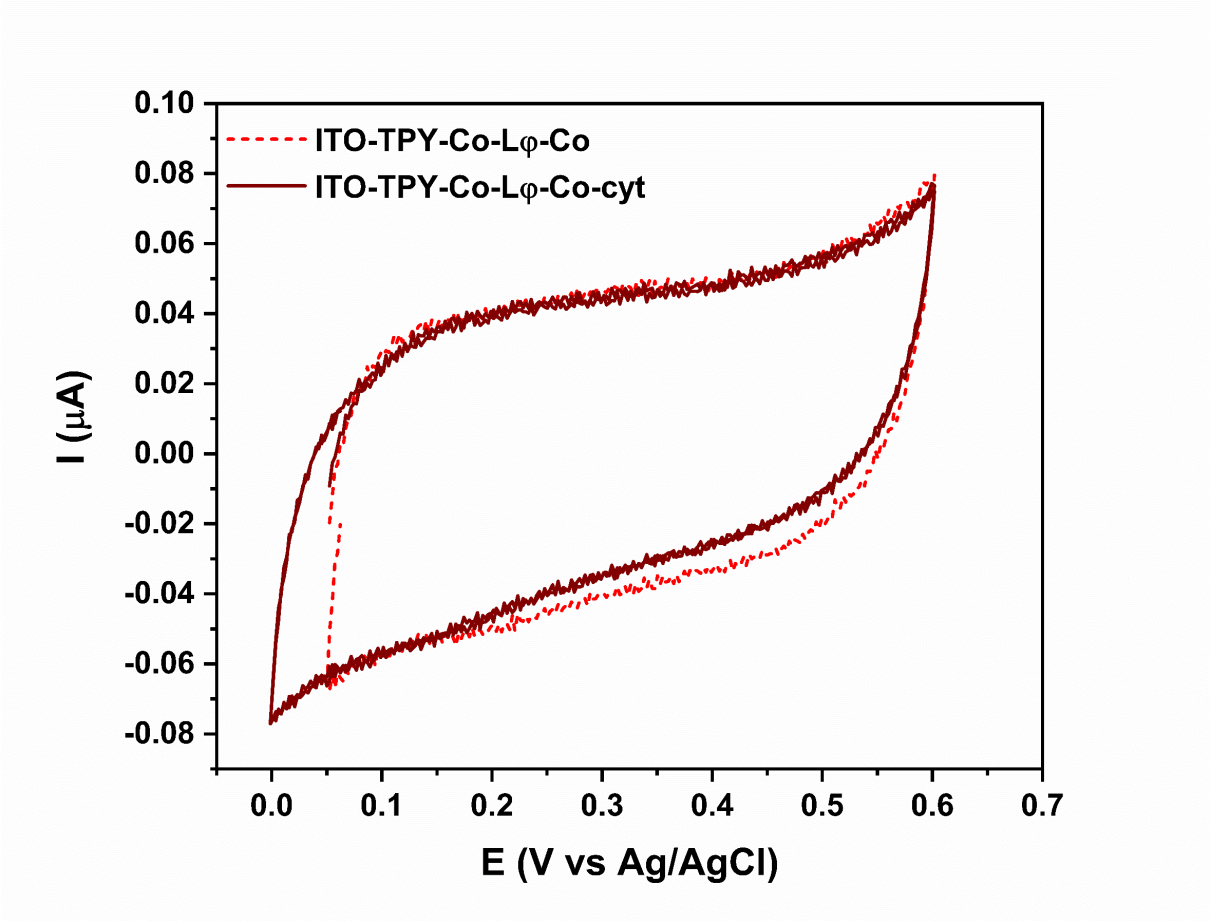

Fig. S11. Cyclic voltammetry at 5 mV/s in 0.1 M phosphate buffer (pH 7) of ITO Co-based electrodes without and with immobilised cytochrome.

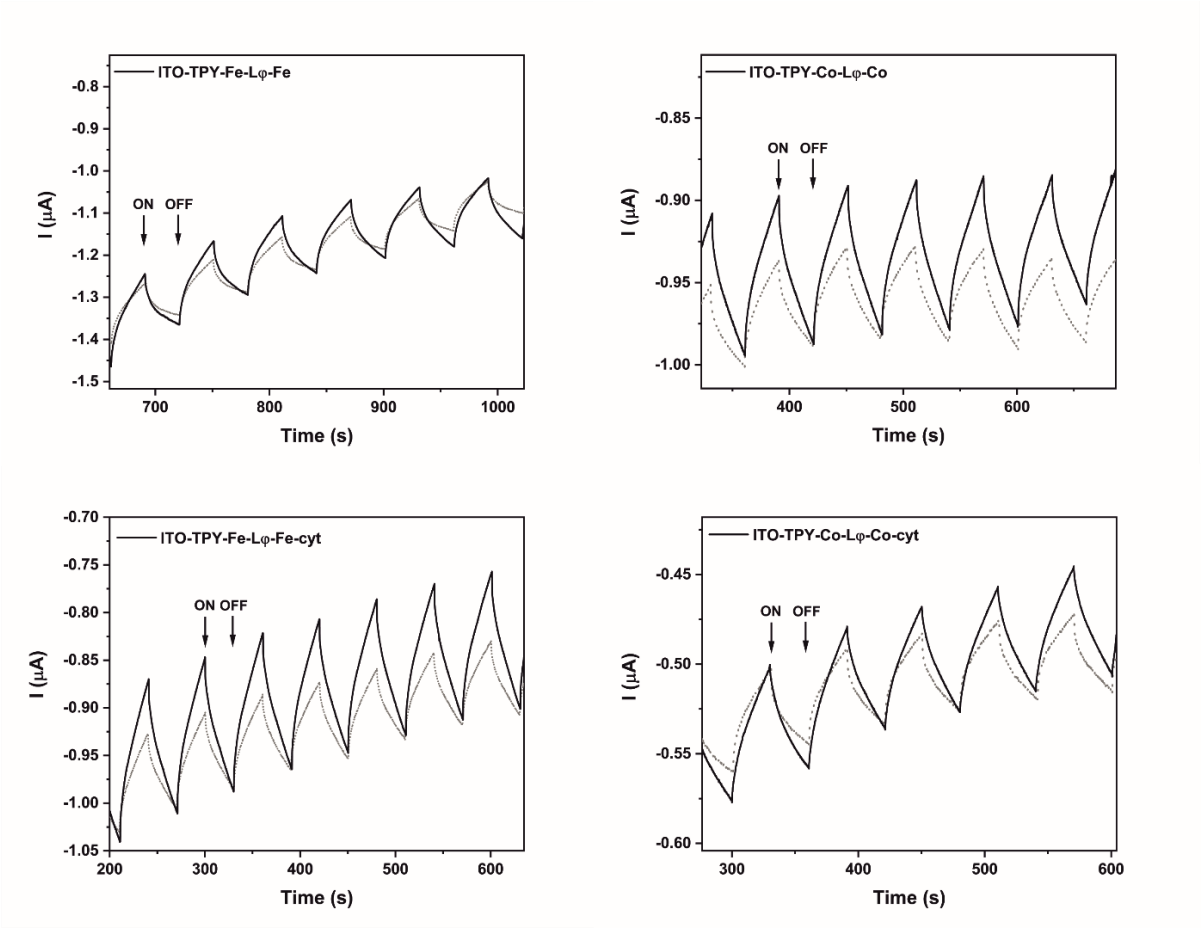

Fig. S12. Photochronoamperometry measurements of different TPY-based nanoassemblies in the presence (black) or absence (grey) of O_2. The experiments were performed at -300 mV vs Ag/AgCl with 30s. 'light ON/OFF' periods in 5 mM phosphate buffer (pH 7).