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Electrochemiluminescence platform for the detection of C-Reactive Protein : Application of recombinant antibody technology to cardiac biomarker detection

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Materials

Human CRP (1401) was purchased from Sigma-Aldrich (Ireland). All other general reagents, of the highest available grade, were purchased from Sigma-Aldrich (Ireland). For electrochemical measurements all solutions were made up to volume with MilliQ water (18 MΩcm). All solvents used were of spectroscopic grade and were stored over activated molecular sieves.

The $[Ru(bpy)_2PICH_2]^{2+}$ label was synthesised as previously described.[1] Recombinant scFv antibodies with high affinity (KD) for monomeric C-Reactive Protein (mCRP - 3.53 x 10^{-10} M) were purified according to previously published procedures.[2] The affinity of the scFV fragments was established using a Biacore A100 platform. Competitive binding studies with comparative antigens were also performed.[3]

Instrumentation

Electrochemical experiments were performed in a standard electrochemical cell using a CH instruments (Memphis TN.) model 660 potentiostat. Cyclic Voltammetry experiments were carried out using a 3 mm diameter platinum carbon working electrode in a conventional three electrode assembly using a platinum flag as the counter electrode. Potentials were measured versus a standard Ag/AgCl aqueous reference electrode (3M KCL). Measurements involving simultaneous detection of light and current utilized a CH instrument model 760B connected to an Oriel 70680 photomultiplier tube (PMT). The PMT was biased at -850 V by a high voltage power supply (Oriel, model 70705) and an amplifier/recorder (Oriel, model 70701) was used in all the experiments. During the experiments, the cell was kept in a light-tight box in a specially designed holder where the working electrode was positioned directly opposite to the fibre optic bundle, the other end of which was coupled to the PMT. An Oriel model IS520 gated intensified CCD operated at −20 ºC, coupled to an Oriel model MS125 spectrograph, was used to acquire ECL spectra. Where necessary, thin film emission spectra were smoothed using an eight-point Savitsky–Golay algorithm.

All measurements were made at room temperature (20°C). For all ECL experiments 0.5 mM $Na_2C_2O_4$ (pH 6) was used as the co-reactant. All other reagents used were of analytical grade, and all solutions were prepared in milli-Q water (18 mΩ cm).

Absorbance and photoluminescence spectra were recorded using a Shimadzu UV-240 spectrophotometer and a PerkinElmer LS-50 luminescence spectrometer, respectively.

Labelling of ScFv Antibody

Labelling of the CRP ScFv antibody with $[Ru(bpy)_2PICH_2]^{2+}$ was achieved by dissolving $[Ru(bpy)_2PICH_2]^{2+}$ with NHS (N-hydroxysuccinimide) in anhydrous DMF at 0^oC. DCC was then added and the reaction mixture was kept under blanket of argon, gradually warmed to room temperature and allowed to stir for 4 hrs. Following this, the activated ester was added directly to an eppendorf containing a PBS solution (pH = 8.0) of polyclonal IgG and allowed to slowly stir at room temperature for 4 hrs. After that, the reaction mixture was placed in a refrigerator overnight to allow the unreacted DCC to hydrolyze and then the reaction was worked up by size-exclusion chromatography.

The amount of molecules attached to IgG was subsequently determined by the UV-vis spectroscopy. The average number of conjugated molecules was 20; this number was decreased when a PBS buffer with lower pH than 7 was used.

Sensor Fabrication

A platinum working electrode (Pt, 3 mm in diameter) was polished repeatedly with 0.3 and 0.05 mm alumina slurry, followed by successive sonication in acetone, ethanol and distilled water for 5 min and dried in air. The electrodes were then modified with a thiol C11 monolayer by immersion in a 1 mM ethanolic solution of mercaptoundecanodic acid overnight. Electrodes were then washed in phosphate buffered saline (PBS), pH 7.4, before being immersed in a mixture of PBS, *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride, EDC and N-hydroxysulfosuccinimide sodium salt, NHS solution for approximately 1 hour at room temperature. Following monolayer modification, the electrode was immersed in a CRP ScFv aqueous solution for approximately 1 hour at 37°C, and a blocked by incubation in 3% bovine serum albumin (BSA) for 1 hour at 37°C. Each electrode was then washed with PBS Tween (PBST 0.05% v/v), three times, and PBS three times. After coating with the CRP ScFV, the electrodes were incubated in various concentrations of CRP in 1% (w/v) PBST containing 1% BSA over night at 4°C followed by washing (PBST x 3 and PBS x 3). After incubation the electrodes were immersed in a PBST solution containing 1% BSA and a 1:1000 dilution of the soluble expressed labelled ScFv for 1 hour at 37°C and again washed, (PBST x 3 and PBS x 3), prior to ECL analysis.

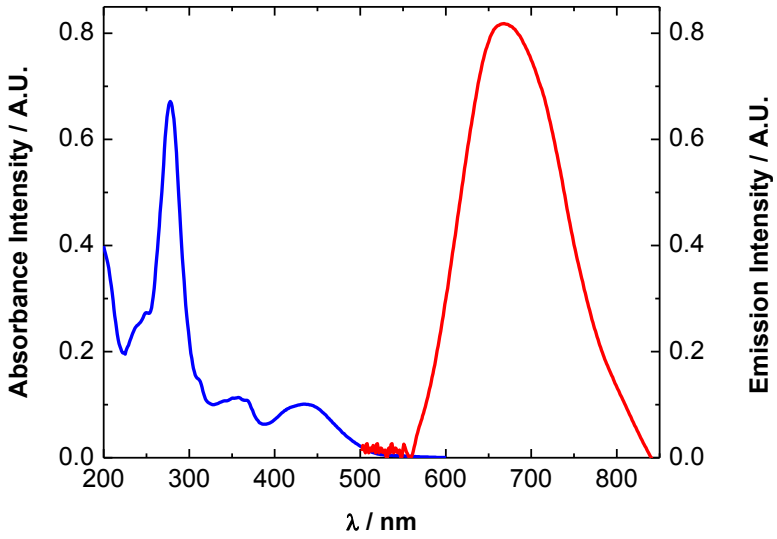

Figure S1: Typical absorbance profile (blue line) and photoluminescence (red line) of the $[Ru(bpy)_2PICH_2]^{2+}$ label in aqueous solution. An excitation wavelength of 355 nm was utilised for the photoluminescence and both intensities were normalised for clarity.

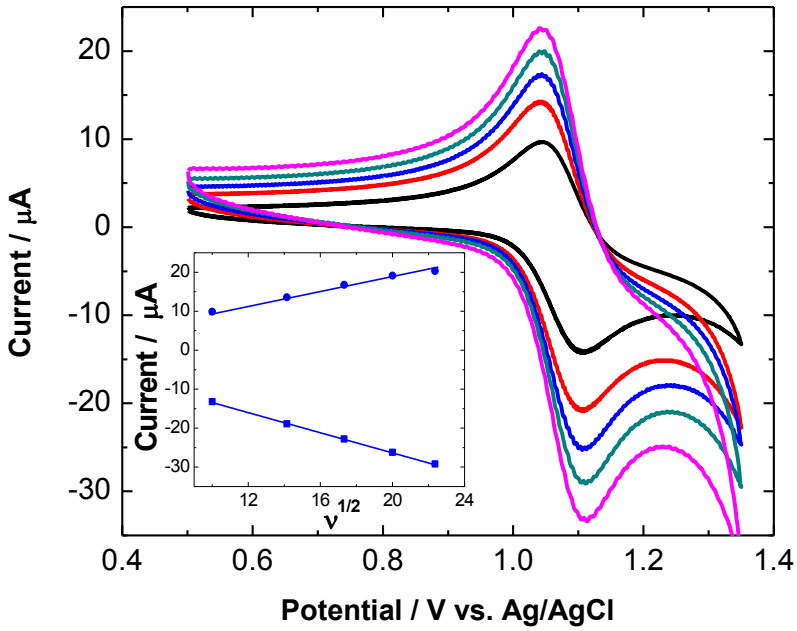

Figure S2: Scan rate dependency for $[Ru(bpy)_2PICH_2]^{2+}$ in 0.1 M PBS, $100<v<500 mVs^{-1}$. Analysis was performed at pH 6.5. Randles-Sevçik response for this data is given in the inset. The error bars are comparable to, or smaller than, the size of the symbols.

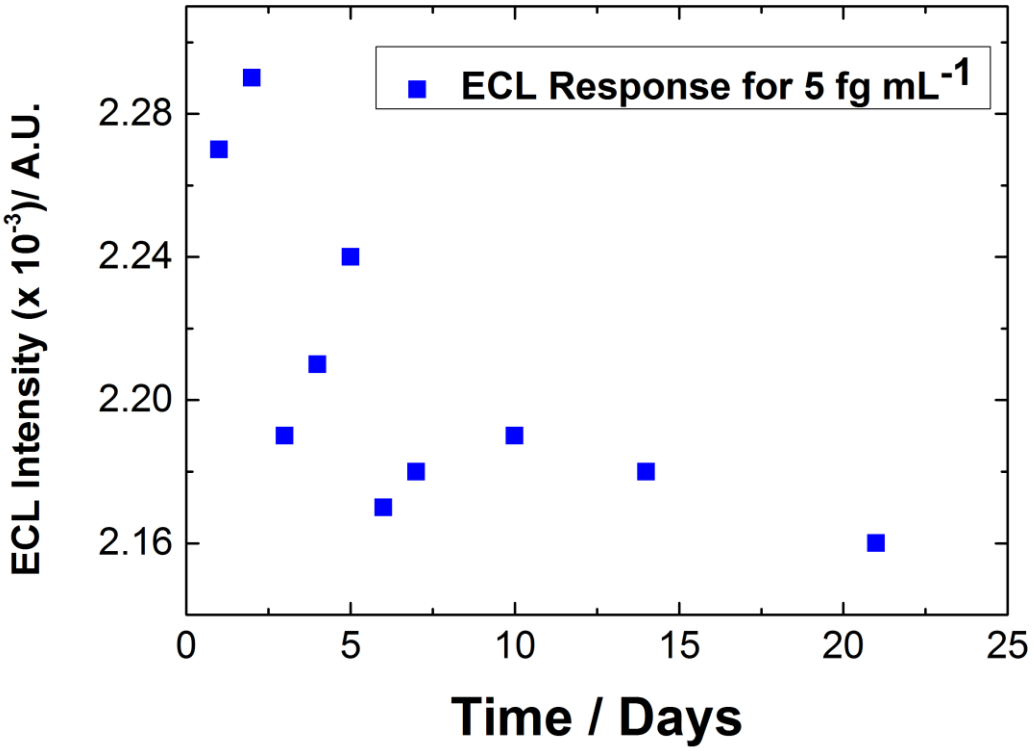

Figure S3: Stability of the sensor over 21 days. A decreases in ECL intensity of 4.8% is observed over the 21 day period.

1. Pellegrin, Y., R.J. Forster, and T.E. Keyes, *pH Dependent photophysics and role of medium on photoinduced electron transfer between ruthenium polypyridyl complex and anthraquinone.* Inorganica Chimica Acta, 2009. **362**(6): p. 1715-1722.
2. Leonard, P., et al., *High throughput ranking of recombinant avian scFv antibody fragments from crude lysates using the Biacore A100.* Journal of Immunological Methods, 2007. **323**(2): p. 172-179.
3. B, M., *Development of novel antibody-based diagnostics for the early and rapid detection of cardiac markers, Thesis Dissertation - Retrieved from* http://doras.dcu.ie/15710/.