SUPPLEMENTARY MATERIAL

A novel, proof-of-concept electrochemical impedimetric biosensor based on extracellular matrix protein-adhesin interaction

J. Leva-Bueno[a], I. Meuskens[b], D. Linke[c], P. A. Millner[d] & S. A. Peyman[e*]

[a]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, UK; E-mail address: bsjlev@leeds.ac.uk; Phone number: +44 (0) 1133433343; ORCID: 0000-0002-3859-986X

[b]Department of Biosciences, Section for Genetics and Evolutionary Biology, University of Oslo, Oslo, Norway; E-mail address: ina.meuskens@ibv.uio.no; ORCID: 0000-0002-5103-1566

[c]Department of Biosciences, Section for Genetics and Evolutionary Biology, University of Oslo, Oslo, Norway; E-mail address: dirk.linke@ibv.uio.no; Phone number: +47 22857654; ORCID: 0000-0003-3150-6752

[d]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, UK; E-mail address: P.A.Millner@leeds.ac.uk; Phone number: +44 (0) 1133433149; ORCID: 0000-0002-4813-4302

[e*]Corresponding author. Molecular and Nanoscale Physics Group, Department of Physics and Astronomy, University of Leeds, E-mail address: S.Peyman@leeds.ac.uk; Phone number: +44 (0) 1133433747; ORCID: 0000-0002-1600-5100

[e*]Corresponding author. Leeds Institute for Medical Research, Wellcome Trust Brenner Building, St James' Teaching Hospital, Leeds. LS9 7TF.

Each step of the biosensor construction was characterised by CV and EIS, giving information about the step-by-step layer construction.

Collagen-POc matrix electropolymerisation characterisation

Electropolymerisation was achieved when a characteristic oxidation peak around 0.6 V was observed for polyoctopamine (POct) and the matrix system.[1] In the second CV cycle, no oxidative peak at the mentioned potential was observed. This is indicative that the non-conductive polymer is electropolymerised in the first cycle, impeding the electron flow in the second one (Fig S1 a). This is observed for the collagen-POc matrix electropolymerisation profile (Fig S1 b).

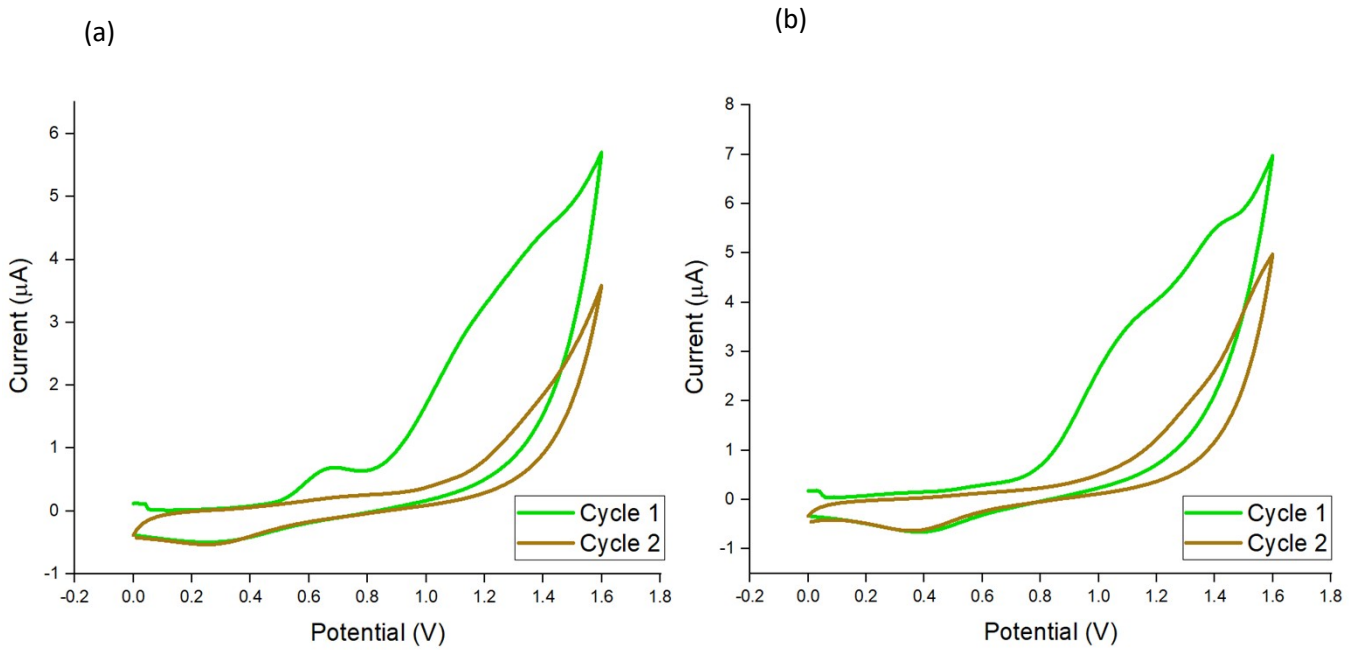

Fig. S1. Electropolymerisation profiles of POc and collagen-POc matrix. (a), electropolymerisation of 2.5 mM octopamine in 10 mM PB, pH 7.2. The electrode was cycled from +0.0 V to +1.6 V for 2 cycles at a scan rate of 100 mV/s; (b), electropolymerisation of 2.5 mM octopamine + 100 µg/ml of collagen in 10 mM PB, pH 7.2. The electrode was cycled from +0.0 V to +1.6 V for 2 cycles at a scan rate of 100 mV/s.

Collagen direct attachment biosensor

Different POc concentrations (1, 2.5, 5, 10, 25, 100, and 250 mM POc) were electropolymerised as the first step of the collagen direct attachment biosensor optimisation. As previously mentioned, electropolymerisation is achieved when a characteristic oxidation peak around 0.6 V is observed.[1] For clarity, the electropolymerisation profiles have been divided into 1st and 2nd cycles. For the first electropolymerisation cycle (Fig S2 a), the oxidation peaks for all respective concentrations can be observed. Then, from the second electropolymerisation cycle (Fig S2 b), no peak is observed for any of the concentrations, thus indicating the proper passivation of the surface.

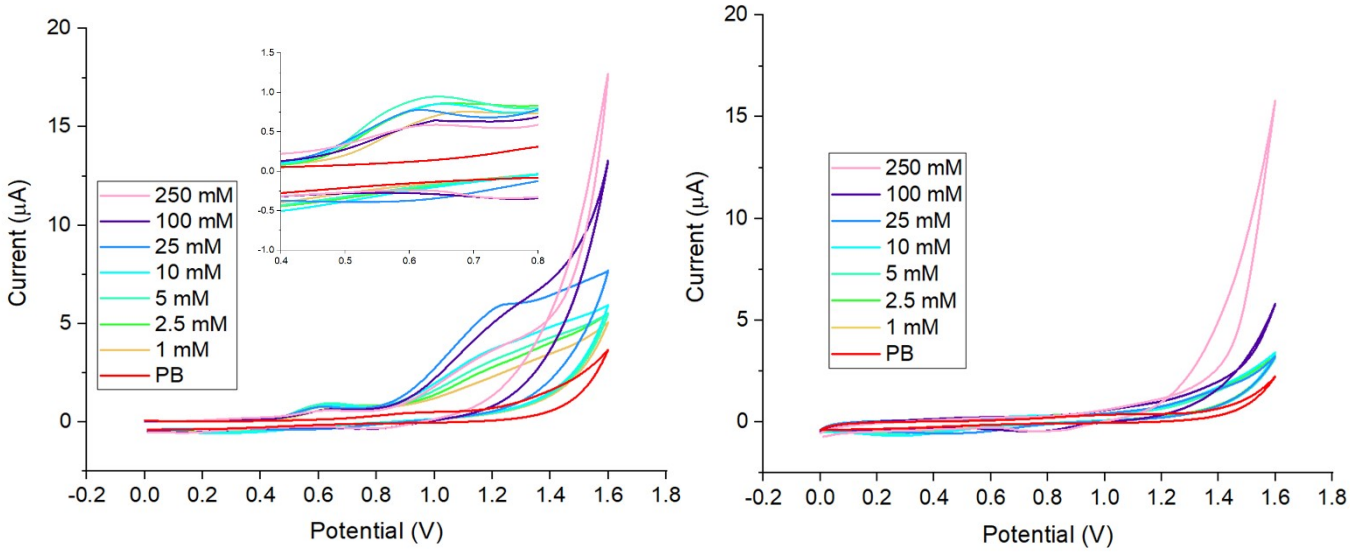

Fig. S2. **Electropolymerisation profiles for a range of POc concentrations.** Electropolymerisation of different concentrations of octopamine in 10 mM PB, pH 7.2 namely: 1, 2.5, 5, 10, 25, 100 and 250 mM. The electrode was cycled from +0.0 V to +1.6 V for 2 cycles at a scan rate of 100 mV/s. (a), only displays the first cycle of the electropolymerisation, whereas (b) only displays the second cycle of the electropolymerisation.

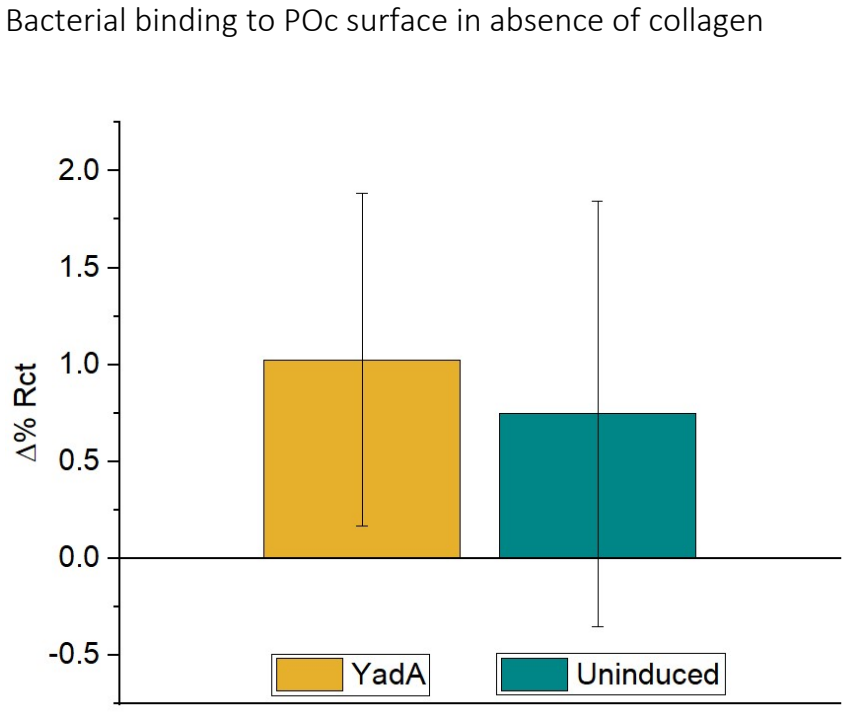

Fig. S3. Electrochemical binding measurements over POc surface in absence of collagen. 5 mM octopamine was electropolymerised over CX2220AT SPGEs from Metrohm DropSens employing Autolab type III Fra II potentiostat and NOVA (2.1.4.) software from Metrohm Autolab B.V. (The Netherlands)). Δ%Rct before and after analyte incubation with uninduced E. coli or E. coli expressing YadA for $8x10^7$ cfu in 10 µL. Incubation was for 30 min and Δ%Rct is calculated in accordance with Equation 1. Data are mean ± SD (n=4).

References

1. Shamsuddin, S. H. *et al.* Reagentless Affimer- and antibody-based impedimetric biosensors for CEA-detection using a novel non-conducting polymer. *Biosens. Bioelectron.* **178**, 113013 (2021).