Electronic Supplementary Material (ESI) for Chemical Communications

Surface induced growth of dipeptide hydrogel layers above the pK_a of the terminal amino acid.

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Experimental: The synthesis of Fmoc-LG has been described previously (D. J. Adams, M. F. Butler, W. J. Frith, M. Kirkland, L. Mullen and P. Sanderson, Soft Matter, 2009, 5, 1856-1862), the purity was calculated to be >97% as judged by 1H NMR. A thin gel layer (thickness ~80 nm; see Figure 1S) was grown on the surface of a gold electrode by electrochemically generating a surface localised pH drop following protocols described previously (Hydroquinone, Sigma-Aldrich >99%, NaCl, Sigma-Aldrich >99.5%, E. K. Johnson, D. J. Adams and P. J. Cameron, J. Am. Chem. Soc., 2010, 132, 5130-5136.). The thin film was gently rinsed with NaCl (0.098 mol dm^{-3}, adjusted to pH 7) and then left in contact with ~ 0.5 cm^{-3} of Fmoc-LG (2.4 mmol dm^{-3} in 0.098 mol dm^{-3} NaCl adjusted to pH 7) for 48 hours without the application of any current. No further electrochemical experiments of any kind were carried out, rather the thin Fmoc-LG layer was left in the growth solution for up to 48 hours while the film density was monitored by SPR. Thicker layers also formed spontaneously on top of an electrochemically seeded layer placed into 2.4 mmoldm^{-3} Fmoc-LG-OH in phosphate buffered saline (PBS, tablets, Fluka) at pH 7. Figure 2S and Table 1S show SPR data for the growth of gel layers.

Control experiments were carried out in two ways: (i) an electrode without a seeding layer was placed in Fmoc-LG-OH solution for 48 hours (2.4 mmol dm^{-3} Fmoc-LG in 0.098 mol dm^{-3} NaCl adjusted to pH 7). (ii) All experimental steps were carried out in the absence of hydroquinone. Without the seeding layer no thicker gel layer nucleated on the surface. The apparent pK_a of Fmoc-LG was measured by pH titration and increases slightly in 0.1 M NaCl to approximately 6.1 as compared to 5.8 in the absence of salt (Figure 3S).

Figures S3 and T2 show SPR data for the growth of gel layers in the presence of Horse Radish peroxidase (HRP, 50-150 U/mg, Sigma-Aldrich). The electrochemical seeding layer was grown under identical conditions to those outlined above. HRP enzyme was added to the solution during the second nucleated growth phase. The second layer was grown from a solution containing 1 x 10^{-2} mol dm^{-3} PBS buffer at pH 7, 2.4 x 10^{-3} mol dm^{-3} FmocLG and 5.35 x 10^{-8} mol dm^{-3} HRP. After 48 hours, the growth solution was removed from the cell and a solution of 7.4 x 10^{-5} mol dm^{-3} o-phenylenediamine (OPD, >99%, Fluka) and 7.4 x 10^{-5} mol dm^{-3} H_2O_2 (30% solution in H_2O, Sigma Aldrich) were introduced to the cell in fresh PBS buffer. The conversion of OPD to 2,3-diaminophenazine (DAP) was monitored at 450 nm using a UV-Vis spectrometer with a reflectance probe (Ocean Optics DT mini light source with USB2000 fibre optic spectrometer) where the light passed through the gel and was reflected back from the underlying gold film. Controls were carried out by doing an identical experiment with a gel that did not contain HRP. The conversion of OPD to DAP in the gel was compared to turnover in a solution containing 5.35 x 10^{-8} mol dm^{-3} HRP (Figure S4 shows a calibration plot). The reaction reached equilibrium in less than 10 minutes in solution compared to > 60 minutes in the gel. Assuming that the HRP in the gel turns over OPD at the same rate as HRP in solution, calibration curves (Figure S3) showed that the total amount of DAP in the gel was 60% of that in solution suggesting 3.21 x 10^{-8} mol dm^{-3} HRP had been incorporated.

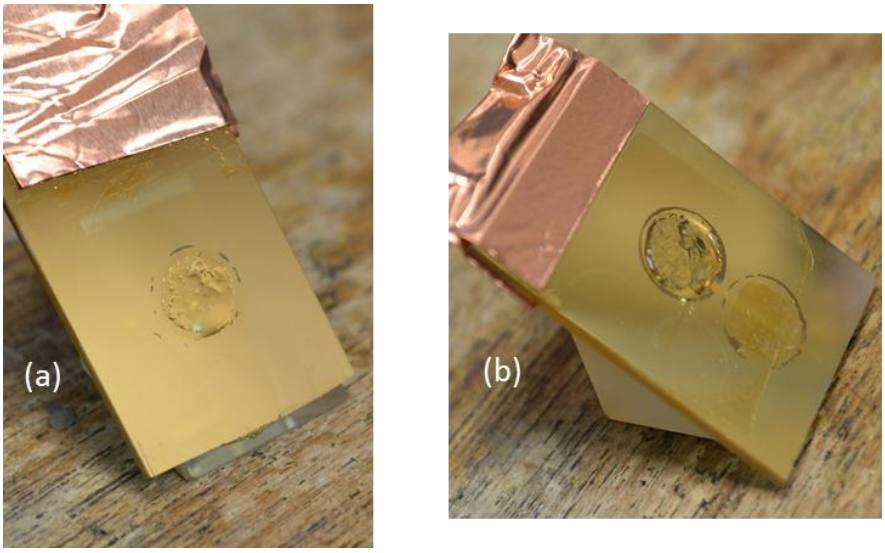

Figure 1S. Photographs of the gold electrodes after the electrochemical SPR cell (which defines an active area by a 0.5cm diameter o-ring) has been removed. A thicker than average seeding layer is shown in (a) so that it is clearly visible to the naked eye. S1(b) shows the film that develops after a seeding layer is placed in contact with 2.4 mmol

dm^{-3} Fmoc-LG in 0.098 mol dm^{-3} NaCl adjusted to pH 7 for 48 hours. The seeding layer used in (b) was grown using the same conditions as the one shown in (a). (Difficulties in replacing the SPR cell accurately mean that it is difficult to photograph a seeding layer and then replace the film to watch the second layer grow).

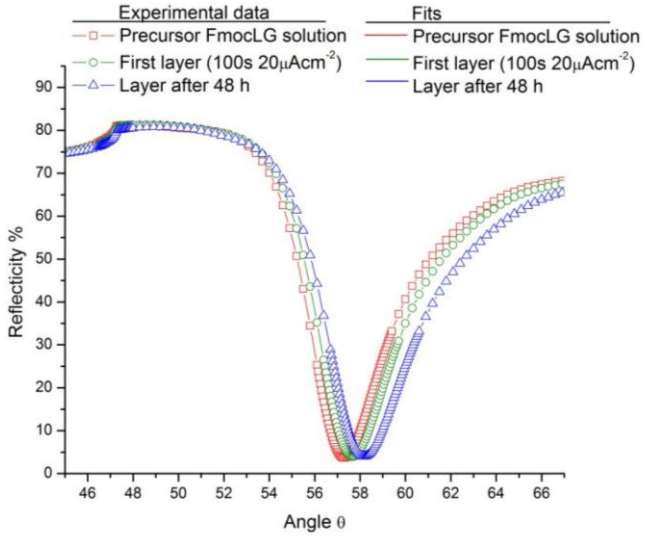

Figure 2S. Angle scans showing the minimum shift associated with the growth of the first seeding layer (circles) and the second thicker layer (triangles). The solid line shows the fit to the multi-layer Fresnel model outlined in Table 1S.

Layer	Thickness / nm	ε(real)	ε(imaginary)
Prism	∞	3.4117	0
Gold	55.6	-12.3326	1.2257
Air	∞	1.0016	0
Precursor solution	∞	1.7816	0
Gel layer (100s 20μAcm^{-2})	80	1.789	0.002
Gel layer (48 h)	∞	1.817	0.004

Table 1S. Fresnel fitting of the SPR curves shown in Fig. S1. Initially, a very thin film (~80nm) film is formed on the gold surface with a refractive index close to that of the precursor solution. In the second step, the refractive index increases over 48 hours. Even though the refractive index of a pure gel is unknown, it is possible to use effective medium theory to calculate that over 48 hours the density of the gel increases fivefold.

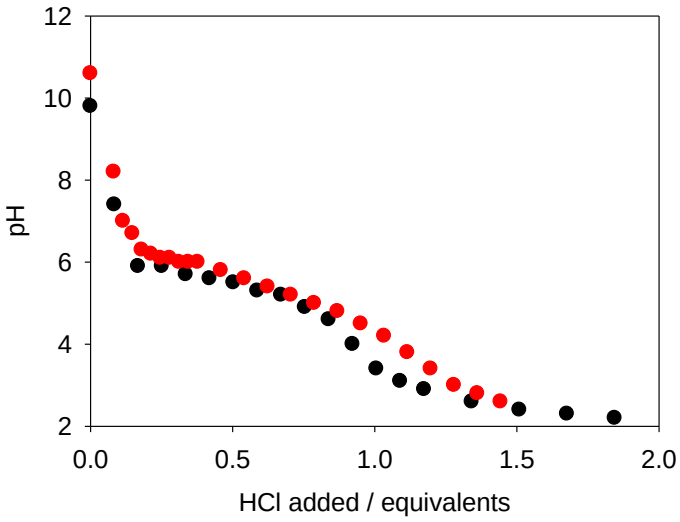

Figure 3S. "Titration" of Fmoc-LG-OH in the absence (black data) and presence (red data) of 0.1M NaCl. The apparent pK_a increases slightly in the presence of NaCl, but is still significantly below the pH of the solution.

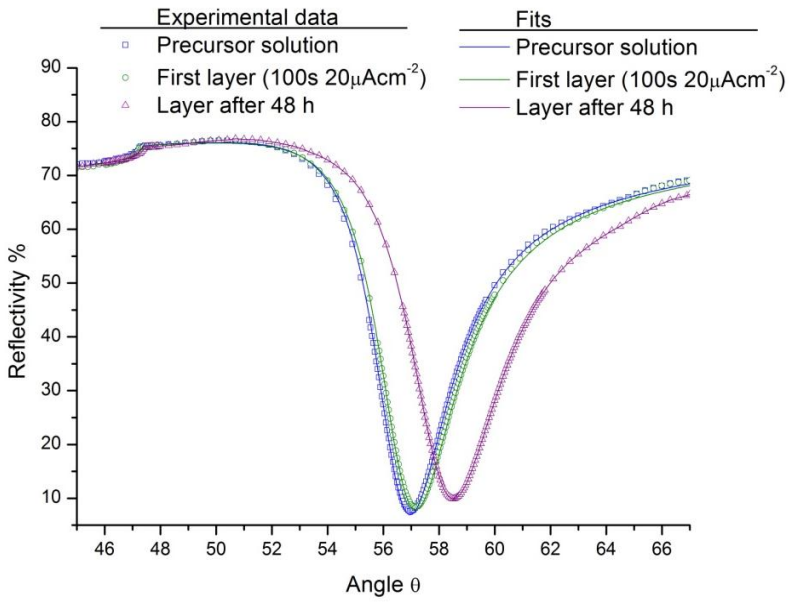

Figure 4S. Angle scans showing the formation of a seeding layer (green squares) and the subsequent growth of a thick hydrogel layer incorporating horse radish peroxidase (purple triangles). The solid lines give the multi-layered Fresnel fits outlined in Table 2S.

Layer	Thickness / nm	ε(real)	ε(imaginary)
Prism	∞	3.4117	0
Gold	52.46	-11.3187	1.5279
Air	∞	1.0016	0
Precursor solution	∞	1.7844	0
Gel layer (100s 20μAcm^{-2})	100	1.803	0.002
Gel layer (48 h)	∞	1.808	0.001

Table 2S. Fresnel fits for the SPR curves shown in Figure 2S.

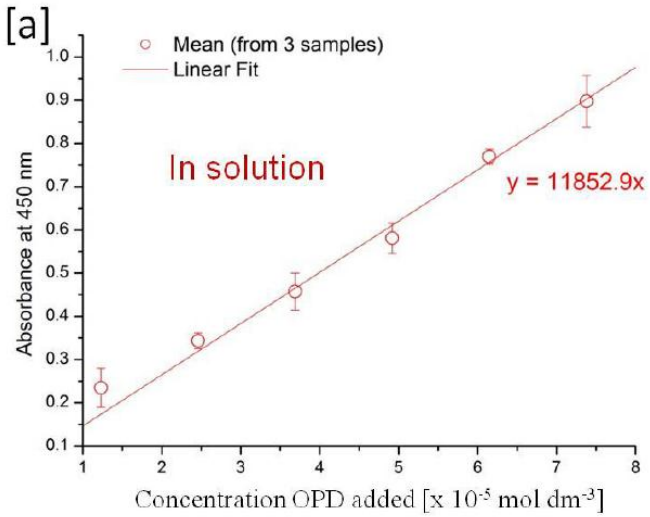

Figure 5S. Calibration plot showing the mean change in absorbance when OPD was converted to DAP by 5.35 x 10^{-8} mol dm^{-3} HRP in solution. Conversion was complete in under 10 minutes. The error bars show 1 standard deviation. Assuming a conversion of 100% in solution, the HRP in the gel converted 60% of the OPD within 60 minutes.