Electronic Supplementary Material (ESI) for Analyst.
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Supplementary Information for

Direct bioelectrocatalysis by redox enzymes immobilized in electrostatically condensed oppositely charged polyelectrolyte electrode coatings

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1. Characterizations

1.1 GPMA monomer

1.1.1 HPLC analysis

HPLC analysis was performed on an Agilent 1260 Infinity LC system using ZORBAX Eclipse Plus C18 column (4.6 x 100 mm, 3.5 µm), with a guard column. As a mobile phase, we used Buffer A: H_2O + 0.1% TFA and Buffer B: Acetonitrile + 0.1% TFA, the flow rate at 1mL/min, and UV at 220nm.

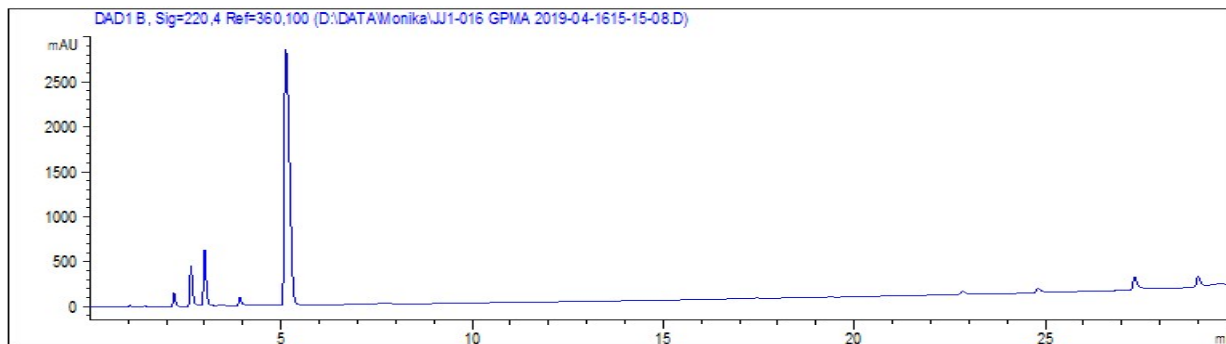

Figure S1. The HPLC analysis of GPMA monomer – the x-axis represents time, minutes, and the y-axis represents UV intensity, mAU.

1.1.2 ^{1}H NMR Analysis of GPMA

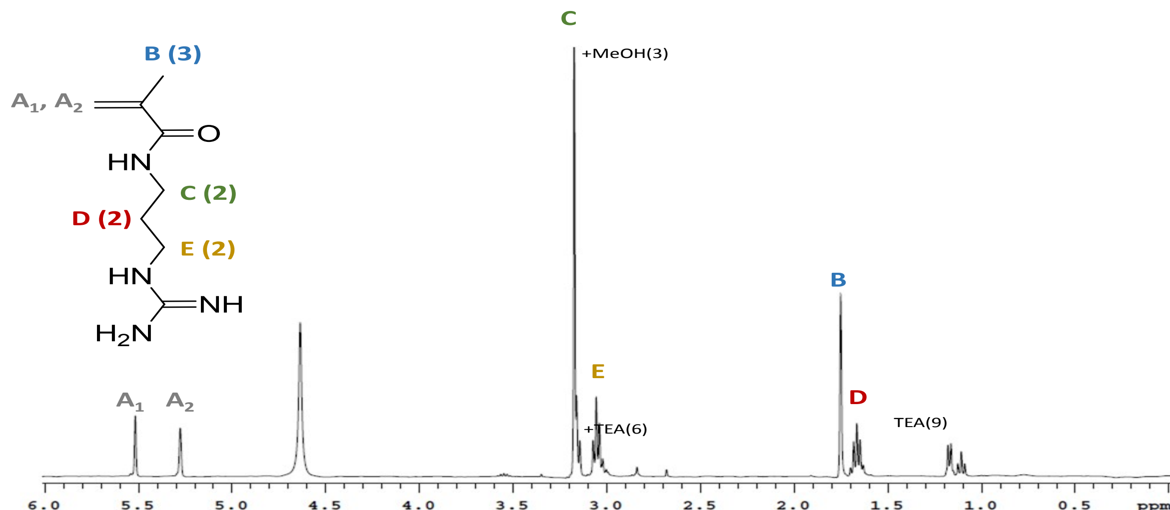

Figure S2. The ^{1}H NMR spectra of GPMA – the x-axis represents the frequency, ppm

The monomer was analyzed by ^{1}H NMR, high performance liquid chromatography (HPLC) and gel permeation chromatography (GPC). The analysis data is shown in Supplementary information. Based on the NMR analysis, the weight% and the mol% of GPMA in the final product was calculated and was used to calculate any concentration of GPMA used for further synthesis.

1.2 p(GPMA-co-MA)

Sample #228-3

Mw/Mn	1.1(0.4%)
Mz/Mn	1.2 (0.5%)

Molar mass moments (g/mol)

Mn	4.2 e+4(0.3%)
Mp	5.4 e+4(0.2%)
Mv	n/a
Mw	4.7 e+4(0.2%)
Mz	5.1 e+4(0.4%)

1.2.1 GPC analysis

The polymer was characterized by aqueous size exclusion chromatography (SEC) on an Agilent HPLC 1260 Infinity equipped with DAD, Refractive Index detector, and a Wyatt miniDAWN TREOS Light Scattering detector. An eluent of 1 wt% acetic acid in 0.1 M LiBr (pH 3.3) was run at 1 mL/min on an Eprogen CATSEC 300 column.

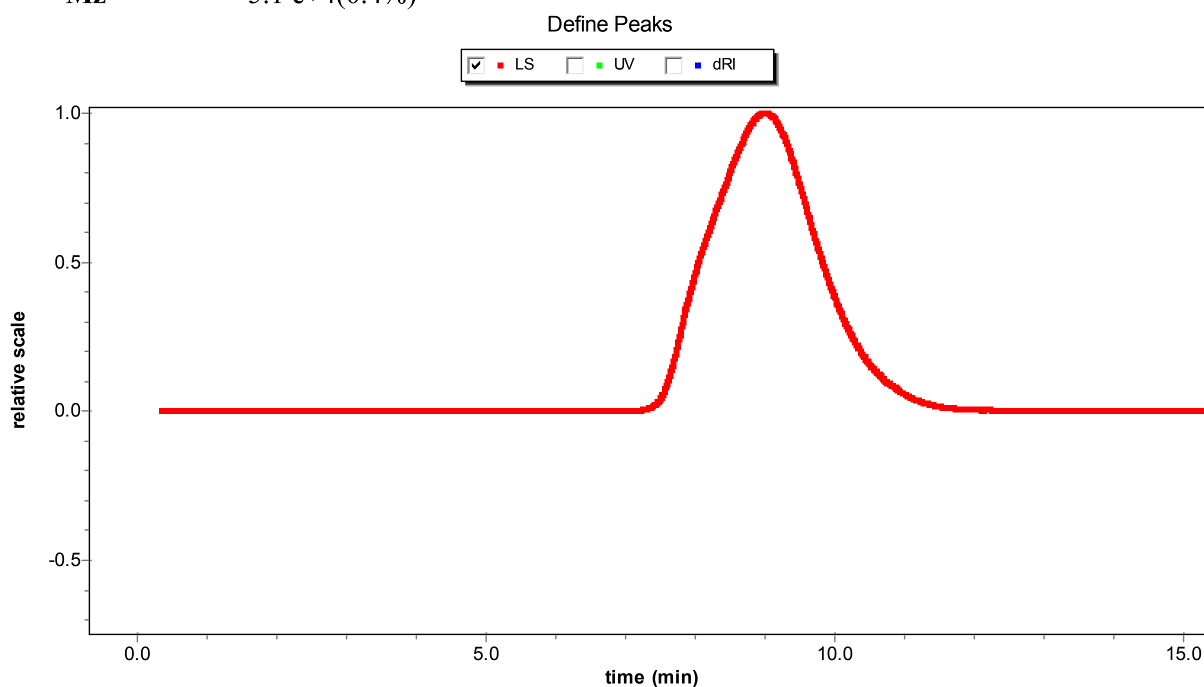

Figure S3. A GPC chromatogram of the synthesized p(GPMA-co-MA) – the x-axis represents time, minutes, and the y-axis represents a relative scale of light scatting intensity.

1.2.2 ^{1}H NMR Analysis of p(GPMA-co-MA)

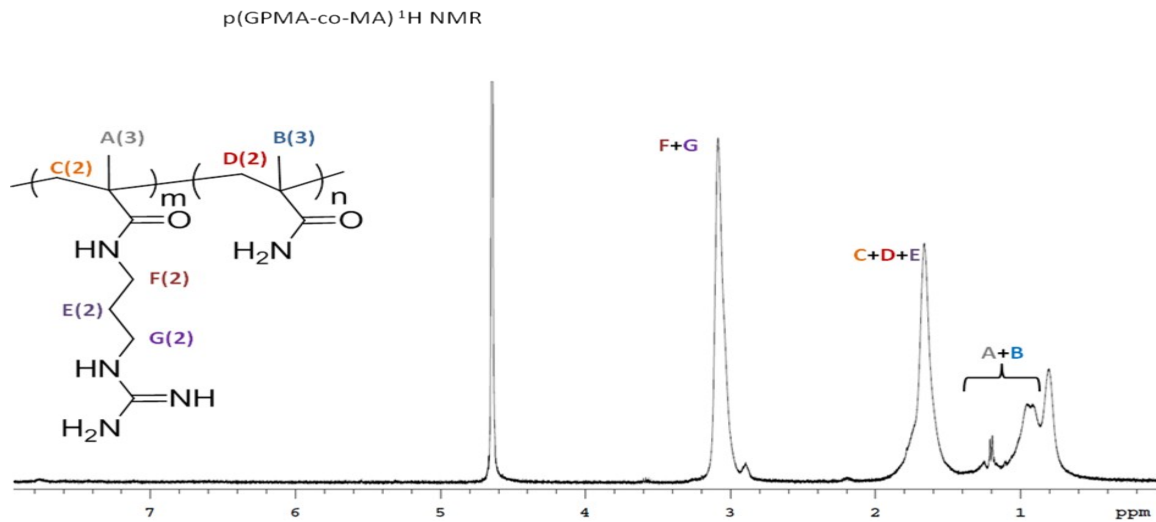

Figure S4. The ^{1}H NMR Analysis of p(GPMA-co-MA) – the x-axis represents the frequency, ppm.

PGP6 electrode coating optimization

2.1 Dithiothreitol (DTT) treatment for chloride inhibition of laccase

A PGP6-LAC mixture was made using 18 µL of PGP6, and 2 µL of 50 mg ml^{-1} laccase solution. Out of this mixture, 1.5 µL was deposited onto the pre-cut 0.25 cm^2 Avcarb electrodes. These electrodes were stored in water for 30 minutes and then, placed into a solution of 1 mM dithiothreitol (DTT) for 0, 10 minutes, and 35 minutes before the electrochemical testing. At 10 minutes of DTT treatment, it was able to sufficiently reverse the chloride inhibition of laccase without a significant loss of electrochemical activity due to denaturation.

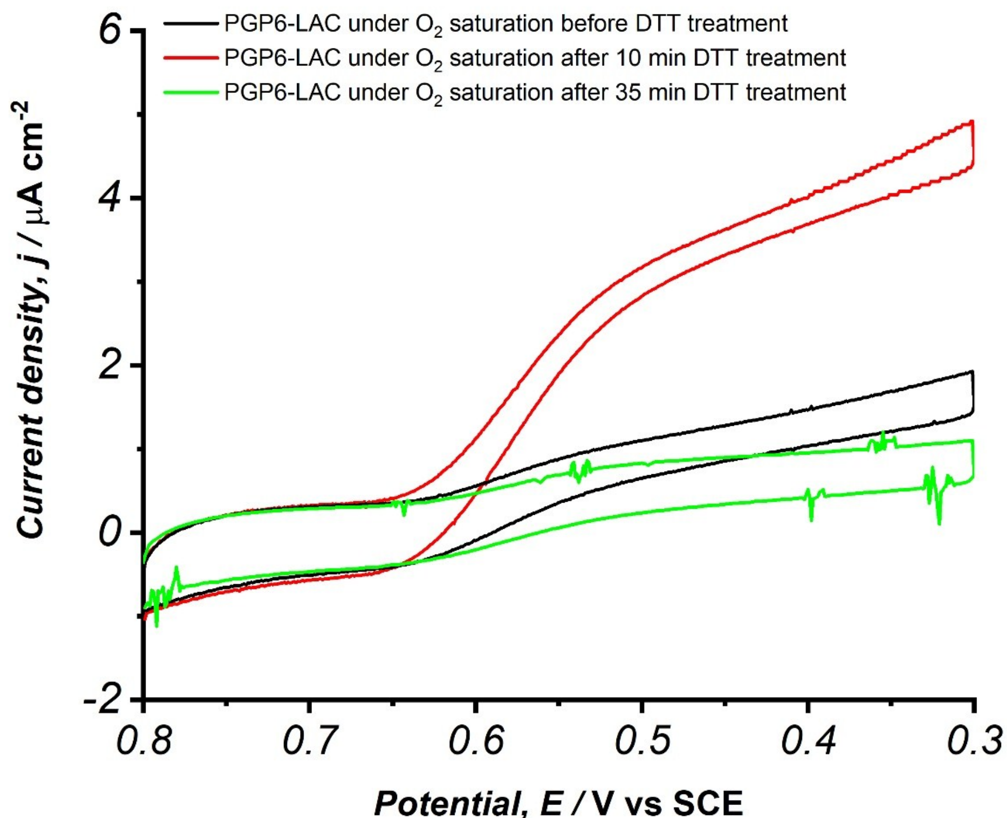

Figure S5. A representative CV of time-dependent DTT treatment of PGP6-LAC electrodes. All cyclic voltammograms were performed from 0.8 V_{SCE} to 0.3 V_{SCE} at 5 mv s^{-1} in 100mM acetate buffer at pH 4.5 under O_2 saturation.

2.2 Enzyme loading optimization using laccase

Different PGP6-LAC mixtures were made using 18 μL of PGP6, and 2 μL of 25, 50, 75 and 100 mg ml^{-1} laccase solution. Out of this mixture, 1.5 μL was deposited onto the pre-cut 0.25 cm^2 Avcarb electrodes. These electrodes were stored in water for 30 minutes and then, placed into a solution of 1 mM dithiothreitol (DTT) for 10 minutes before the electrochemical testing. During the ionic strength induced phase inversion of PGP6, it is hypothesized that the charge ratio of the complex coacervate influence the enzyme entrapment within the coating. At 50 mg ml^{-1}, the concentration of electrochemically available laccase on the electrode is maximized. Deviating from this concentration caused a shift in charge ratio due to the overall charge of laccase and thus, cause an insufficient entrapment of laccase.

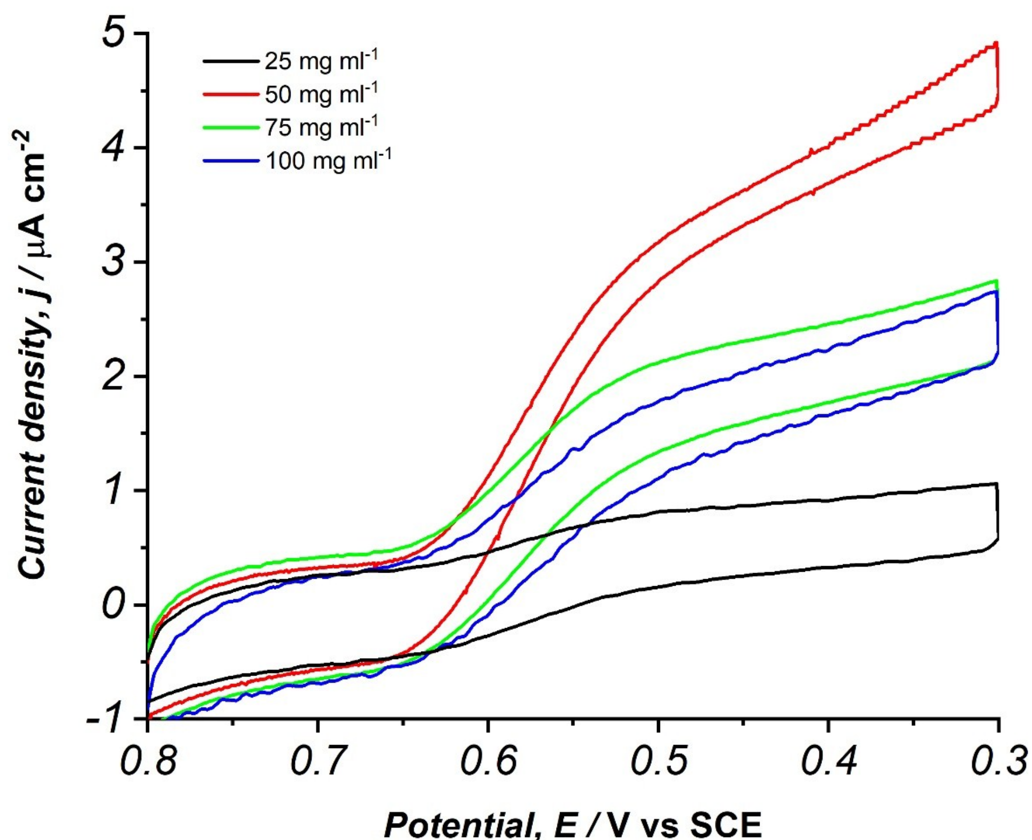

Figure S6. A representative CV of enzyme loading dependent PGP6-LAC electrodes. All cyclic voltammograms were performed from 0.8 V_{SCE} to 0.3 V_{SCE} at 5 mv s^{-1} in 100mM acetate buffer at pH 4.5 under O_2 saturation.

2.3 PGP6 coating thickness optimization

A PGP6-LAC mixture was made using 18 µL of PGP6 and 2 µL of 50 mg ml^{-1} laccase solution. Using a single PGP6-LAC solution, 1 µL, 1.5 µL, 3 µL of the mixture was spread across the electrode surface using an edge of a thick plastic spreader.

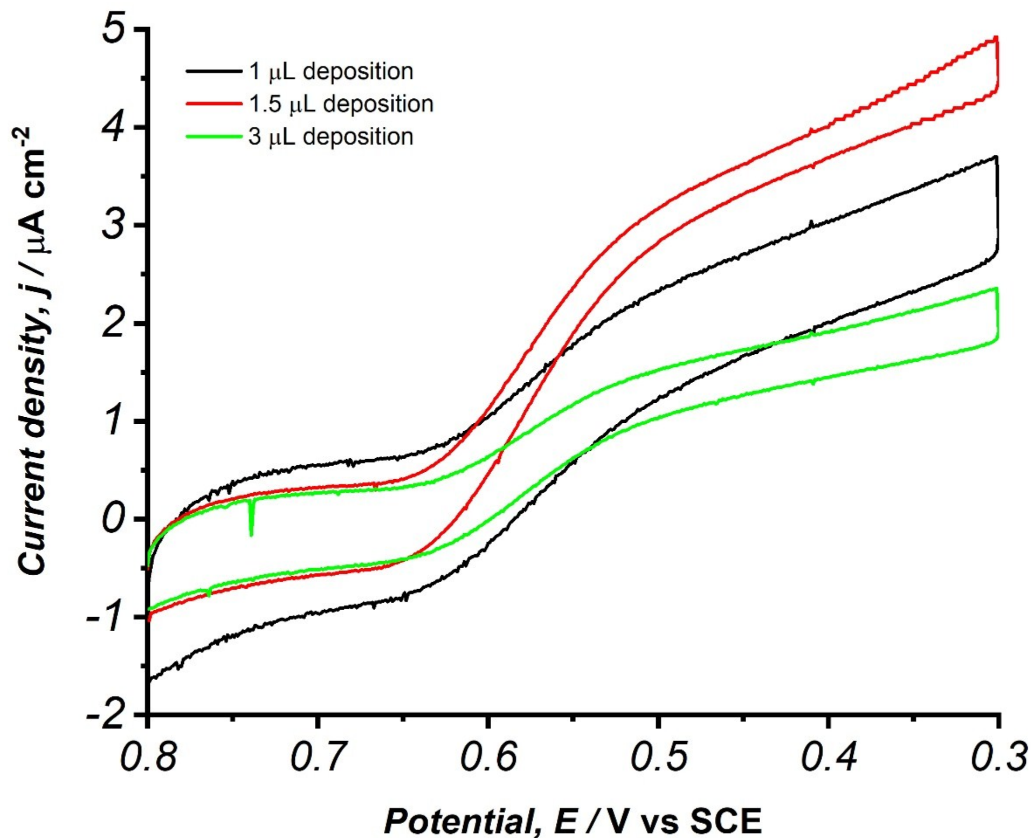

Figure S7. A representative CV of different thickness of PGP6-LAC coatings on the AvCarb electrodes. All cyclic voltammograms were performed from 0.8 V_{SCE} to 0.3 V_{SCE} at 5 mv s^{-1} in 100mM acetate buffer at pH 4.5 under O_2 saturation.

2.4 Blank CVs without any enzyme loading

A pre-cut 0.25 cm^2 AvCarb electrode was used as a blank electrode.

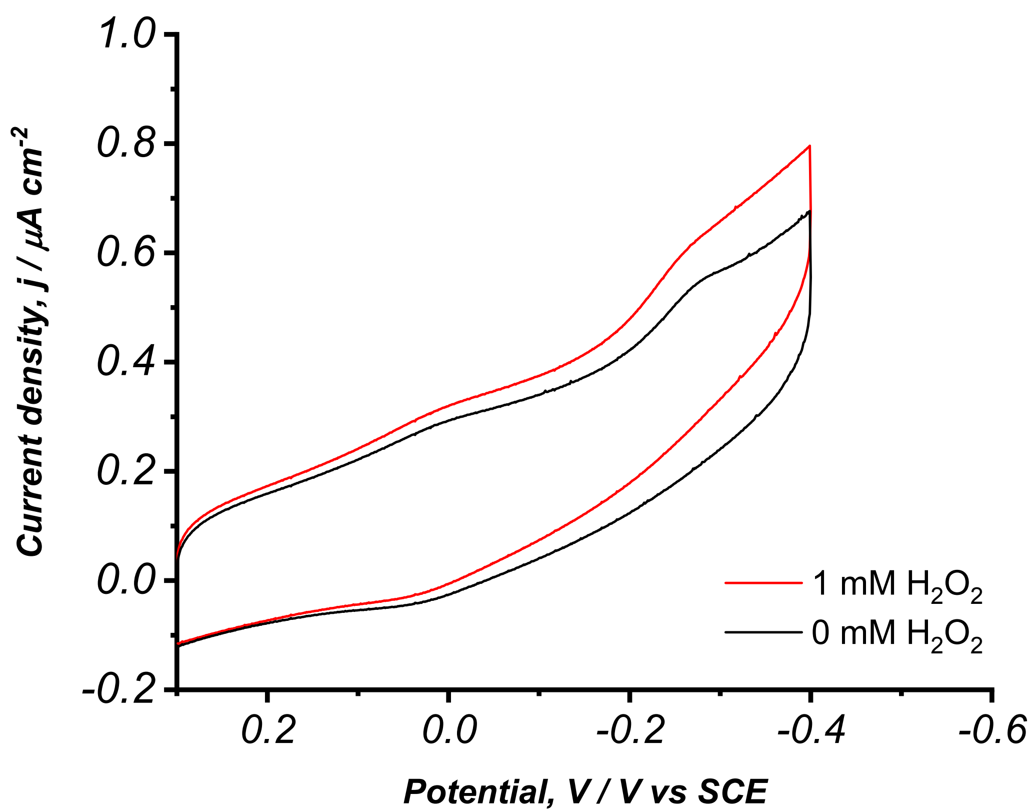

Figure S8. A representative CV of blank controls, AvCarb electrodes. All cyclic voltammograms were performed from 0.3 V_{SCE} to -0.4 V_{SCE} at 5 mV s^{-1} in 100mM MOPS buffer at pH 7 under anaerobic conditions.