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Electronic Supporting Information for Manuscript:

Glutamate detection at the cellular level by means of polymer/enzyme multilayer modified carbon nanoelectrodes

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Electrode characterization and controls

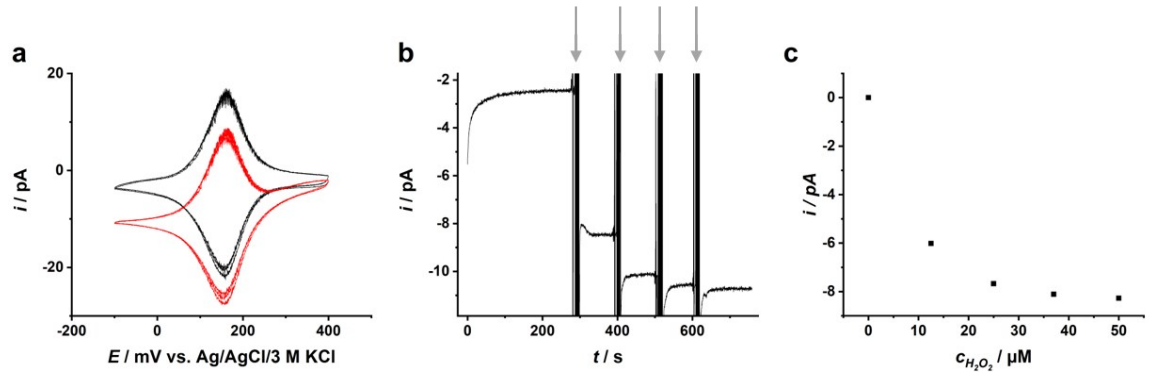


Figure S1: Electrochemical characterization of a P(VI-AA_{NH2})-Os/HRP-modified HF-etched CNE in PBS (pH 7.28). **a:** cyclic voltammogram in the absence (black trace) and presence (red trace) of H_2O_2 (50 µM); scan rate = 10 mV s^{-1}. **b:** chronoamperogram at an applied potential of E_{appl} = -50 mV vs. Ag/AgCl/3 M KCl measured with increasing H_2O_2 concentration (additions are indicated by grey arrows); final H_2O_2 concentration was 50 µM. **c:** plot of the steady state currents (background corrected) extracted from (**b**) vs. H_2O_2 concentrations.

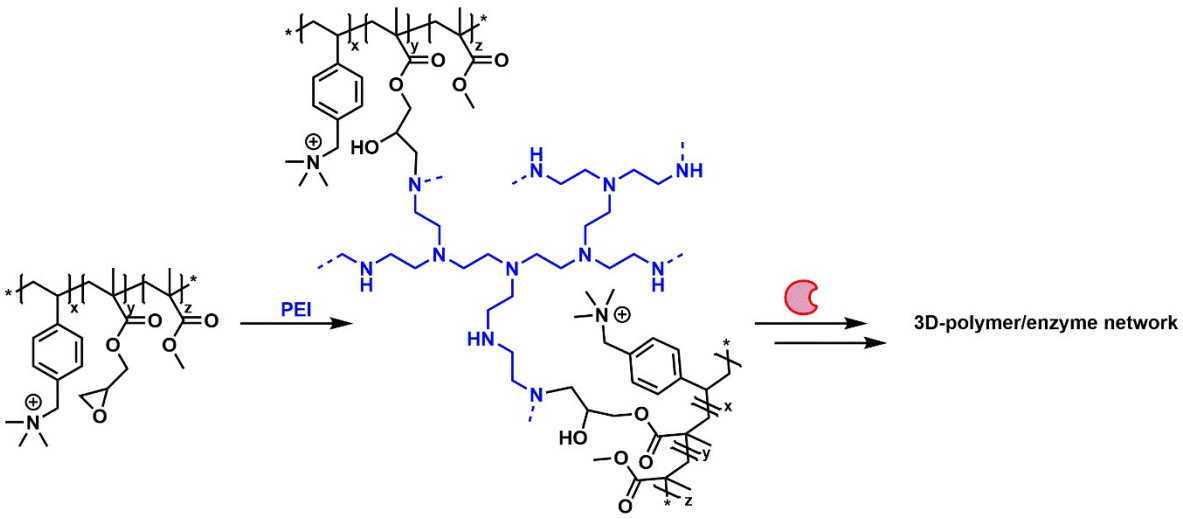


Figure S2: Scheme of the reaction of the positively charged redox-silent polymer P(VBTMA-MMA-GMA) with the polyamine-based cross-linker poly(ethyleneimine) and entrapment of the enzyme GlutOx. Note that primary amino groups will form secondary amino functions after reaction with an epoxide which are more nucleophilic than primary amines and may thus react with a second epoxide function.

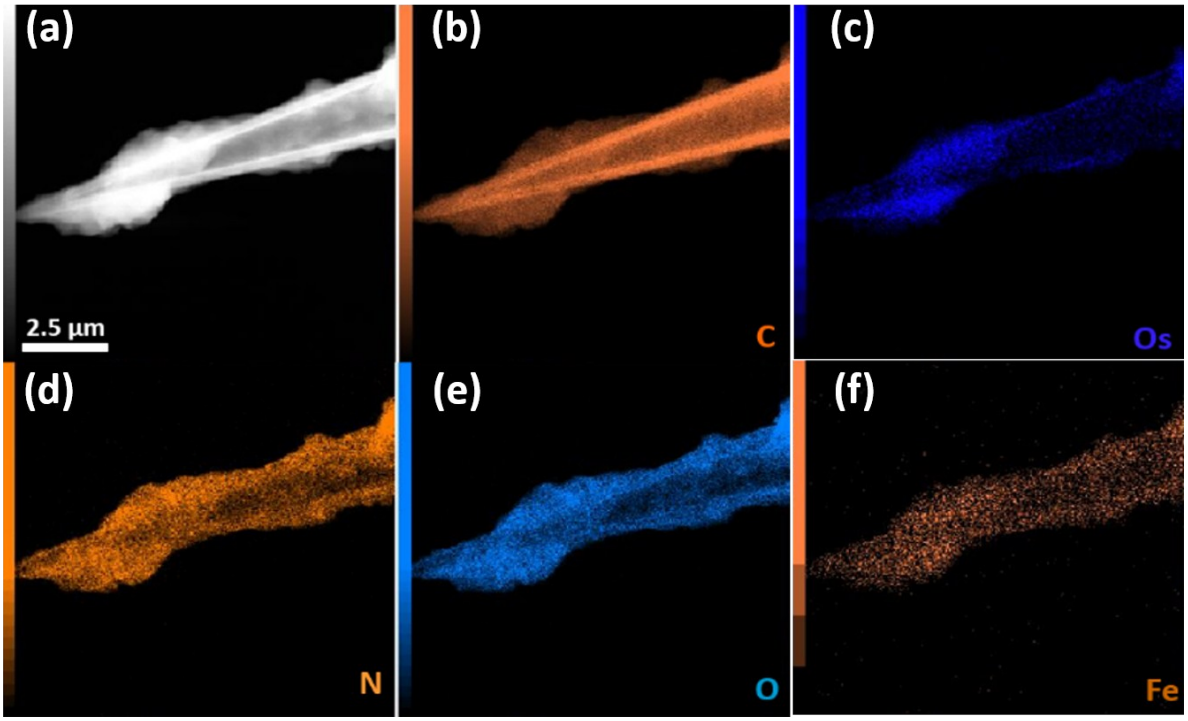


Figure S3: Transmission electron micrograph **(a)** and element sensitive electron dispersive spectroscopy (EDS) of the P(VBTMA-MMA-GMA)/GlutOx//P(VI-AA_{NH2})-Os-modified CNEs **(b-f)**. All characteristic elements (C, Os, N, O and Fe) are present as indicated by EDS.

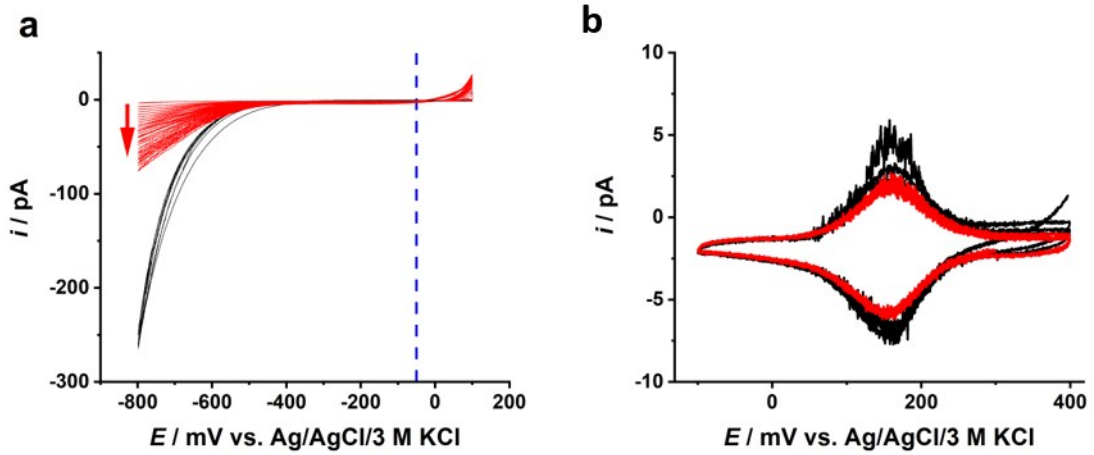


Figure S4: Cyclic voltammograms of a bare HF-etched CNE (**a**) and a P(VI-AA_{NH2})-Os/HRP//P(VBTMA-MMA-GMA) (**b**) recorded in PBS (pH 7.28). **a:** red trace: argon atmosphere; black trace: air-saturated buffer; scan rate = 20 mV s^{-1}. **b:** black trace: without glutamate; red trace: with glutamate (0.86 mM); scan rate = 10 mV s^{-1}; the electrode was modified with a P(VBTMA-MMA-GMA) layer; GlutOx was absent.

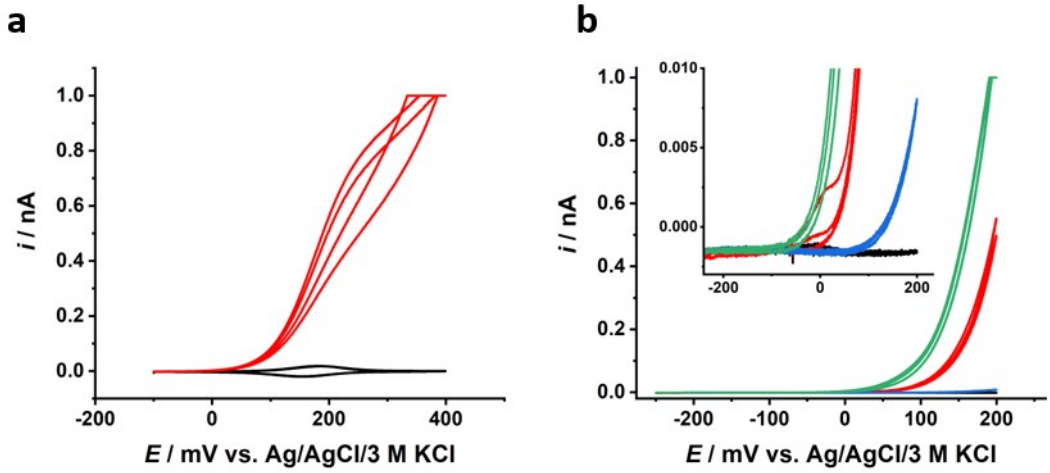


Figure S5: Electrochemical oxidation of the interference ascorbic acid at a P(VI-AA_{NH2})-Os-modified electrode (**a** and **b**, green trace) and at a bare CNE (**b**, red trace) as well as at a 1,7-DAH modified CNE (**b**, blue trace). Working electrolyte: PBS, pH 7.28; scan rate = 10 mV s^{-1}.

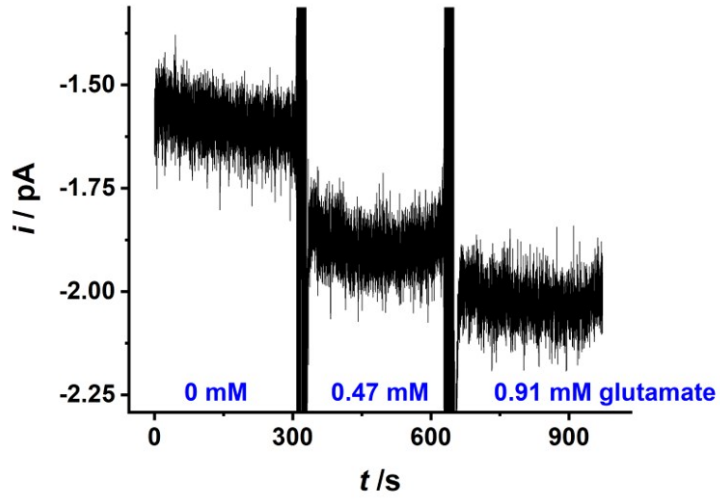


Figure S6: Three-point calibration of a glutamate sensor in 10 mM HEPES at pH 7.4 with $E_{appl.}$ = -150 mV vs. Ag/AgCl/3 M KCl and in presence of 400 µM ascorbic acid. Glutamate was stepwise added to the solution to reach concentrations of 0.47 and 0.91 mM.

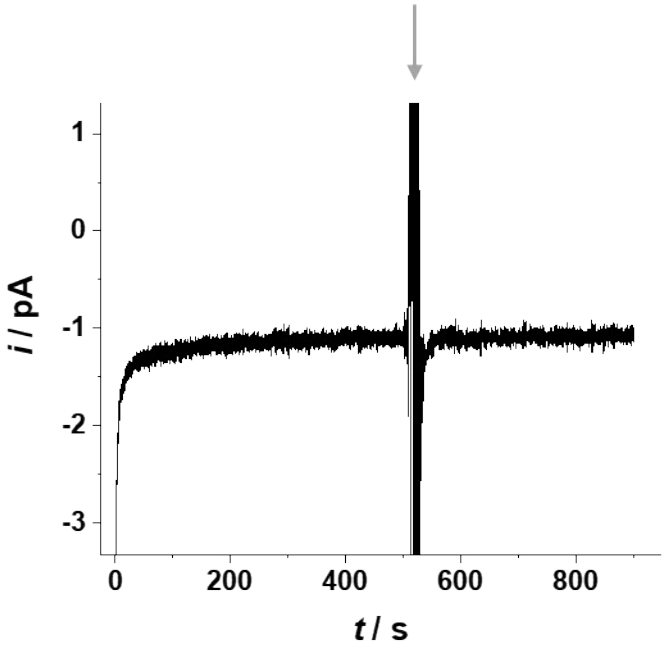


Figure S7: Chronoamperometry of a HF-etched CNE modified with P(VI-AA_{NH2})-Os/HRP//P(VBTMA-MMA-GMA)/GlutOx- in 10 mM HEPES, pH 7.4 at an applied potential of -50 mV vs. Ag/AgCl/3 M KCl. Grey arrow indicates KCl addition (111 mM).

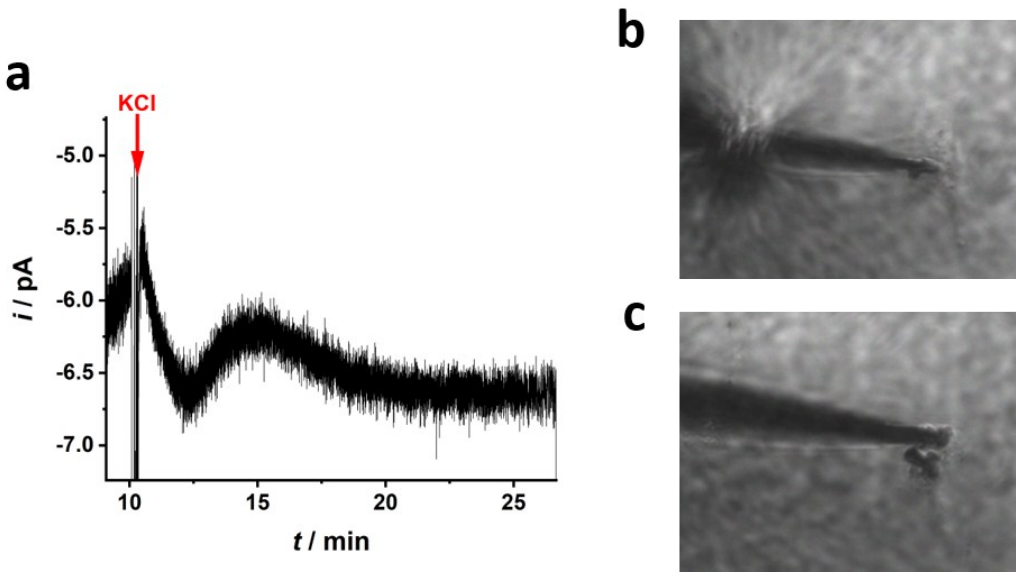
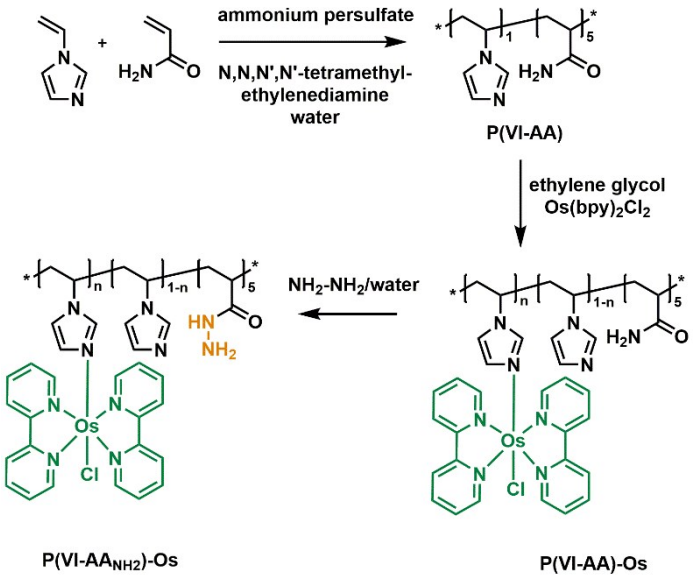


Figure S8: a: Chronoamperogram of the glutamate release from a KCl (115 mM) stimulated primary mouse astrocyte with a P(VI-AA_{NH2})-Os/HRP//P(VBTMA-MMA-GMA)/GlutOx-modified HF-etched CNE in 10 mM HEPES, pH 7.4; $E_{appl.}$ = -150 mV vs. Ag/AgCl/3 M KCl. **b, c:** The optical micrographs show the sensors positioned above the cells before (**b**) and after (**c**) the measurement.

Syntheses

Synthesis of the redox polymer P(VI-AA_{NH2})-Os



Scheme S1: Multistep synthesis of the redox polymer P(VI-AA_{NH2})-Os.

Synthesis of poly(1-vinylimidazole-co-acrylamide), P(VI-AA). The synthesis of the polymer backbone P(VI-AA) was conducted following procedures described in ref. [1]. In a 1 L round bottom flask, the monomers acrylamide (24 g, 0.33 mol) and 1-vinylimidazole (7.27 g, 0.077 mol) were dissolved in 150 mL of water. To this solution, an aqueous solution (50 mL) containing *N,N,N',N'*-tetramethylethylenediamine (0.7 mL, 4 mmol) and an aqueous solution (150 mL) containing the radical initiator ammonium persulfate

(0.6 g, 2.6 mmol) were added under stirring. The reaction mixture was stirred for 30 min in the closed flask. Then, 300 mL of methanol were slowly added to initiate the precipitation of the formed polymer. The solvent was decanted off and the crude polymer was re-dissolved in 400 mL of water. The polymer was precipitated again by slowly adding the aqueous solution to 2 L of methanol. The precipitate was separated and dried under vacuum to yield 25 g (80 %) of a colorless hard solid. ^{1}H-NMR (200.13 MHz, D_2O, Figure S9): δ/ppm 7.67 and 7.07 (s, broad, imidazole-H, integral = 3), 3.96 (broad, imidazole-C*H*, integral 1.16), 2.14, 1.93 and 1.62 (overlapping, broad, -CH_2- and -CH- of acrylamide, -CH_2- of 1-vinylimidazole, integral = 17.1). From the integrals, the ratio between VI and AA was calculated as 1:5.

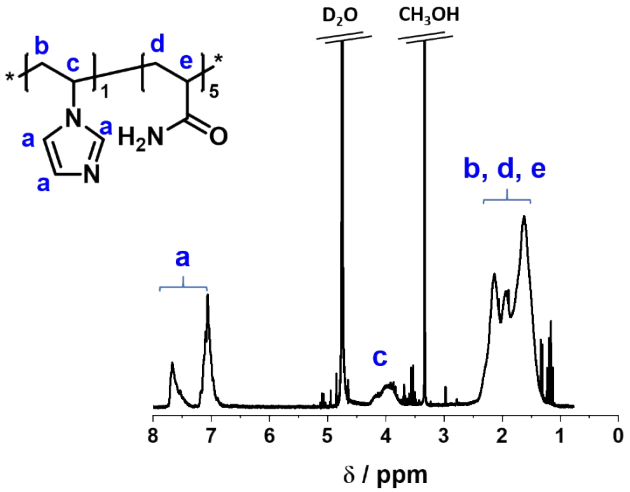


Figure S9: ^{1}H-NMR (200.13 MHz) spectrum of P(VI-AA) in D_2O. Signal assignment is attached to the graph.

Synthesis of poly(1-vinylimidazole-co-acrylamide)-[Os(bpy)$_2$Cl]$^+$, P(VI-AA)-Os. The synthesis of the Os-complex modified polymer P(VI-AA)-Os was conducted according to ref. [1]. In brief, the polymer backbone P(VI-AA) (83.3 mg, corresponding to 186 µmol VI units with 21 wt% VI in the backbone) was dissolved in ethylene glycol and deaerated by several vacuum/argon cycles. Then, $Os(bpy)_2Cl_2$ (105 mg, 183 µmol) in 1 mL ethylene glycol were added. The suspension was deaerated again by vacuum/argon cycles. The reaction mixture was stirred for 4 d at 90 °C and then allowed to cool down to room temperature. The red solution was poured slowly into a stirred solution of diethyl ether/acetone (320 mL/40 mL). The pale red solution was decanted off and the sticky highly viscous oil was dissolved in ≈20 mL methanol. The polymer was precipitated by adding ≈300 mL of diethyl ether. The solution was decanted off and the reddish solid was suspended in ≈250 mL of diethyl ether. The fine red solid was separated by centrifugation and air dried. Finally, the polymer was dried in an oven at 80 °C overnight to yield 154 mg of a red solid. The polymer was directly used for the next step without further purification. *E* = 0.19 V vs. Ag/AgCl/3 KCl in 0.1 M KCl/water, drop cast onto glassy carbon (Figure S10a). UV-Vis in DMSO (Figure S10b): λ/nm 297 (max), 361, 442, 538.

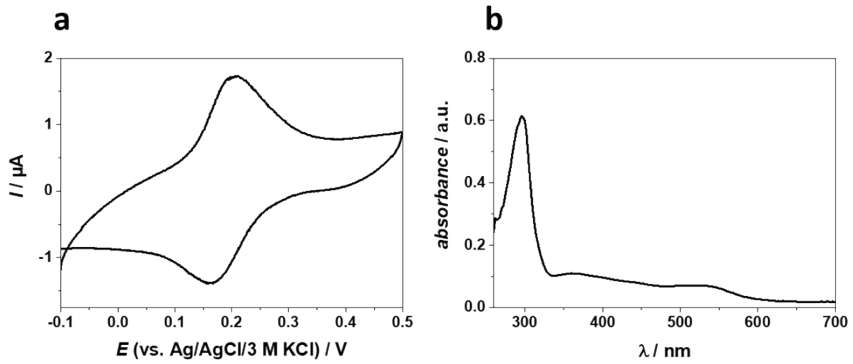


Figure S10: Electrochemical (**a**) and spectroscopic characterization (**b**) of P(VI-AA)-Os. **a)** Cyclic voltammogram of a glassy carbon electrode (*nominal diameter* = 3 mm) modified with P(VI-AA)-Os (drop cast process) in 0.1 M KCl/water; *scan rate* = 50 mV s^{-1}. **b)** UV-vis spectrum of P(VI-AA)-Os in DMSO.

Synthesis of poly(1-vinylimidazole-co-acrylhydrazide)-[Os(bpy)$_2$Cl]$^+$, P(VI-AA$_{NH2}$)-Os. Synthesis of the target polymer P(VI-AA$_{NH2}$)-Os was based on the procedure reported in ref. [1]. The Os-complex-modified polymer P(VI-AA)-Os (154 mg) was dissolved in ≈8 mL of water. Then, hydrazine hydrate (2 mL, N_2H_4 50-60 %) was added and the reaction mixture was stirred at 40 °C for ≈5 h (CAUTION: evolution of NH_3). The mixture was poured into a stirred solution of 200 mL of ethanol. The water/ethanol mixture was added to 400 mL of diethyl ether and the solvent was removed. The oily residue was dissolved in water again and ethanol was added. The solvent was removed again. The red residue was washed with diethyl ether, dissolved in ≈200 mL of ethanol and the solvent was evaporated again. Ethanol was added and evaporated again (extraction of water and remaining hydrazine/NH_3) until a red solid was obtained, which was dissolved in water to yield a stock solution of the polymer with a concentration of ≈10 mg mL^{-1}. The polymer was frozen in liquid nitrogen and stored at -20 °C. *E* = +0.19 V vs. Ag/AgCl/3 KCl in 0.1 M KCl/water, drop cast onto glassy carbon (Figure S11a). UV-Vis in DMSO (Figure S11b): λ/nm 296 (max), 371, 437, 539.

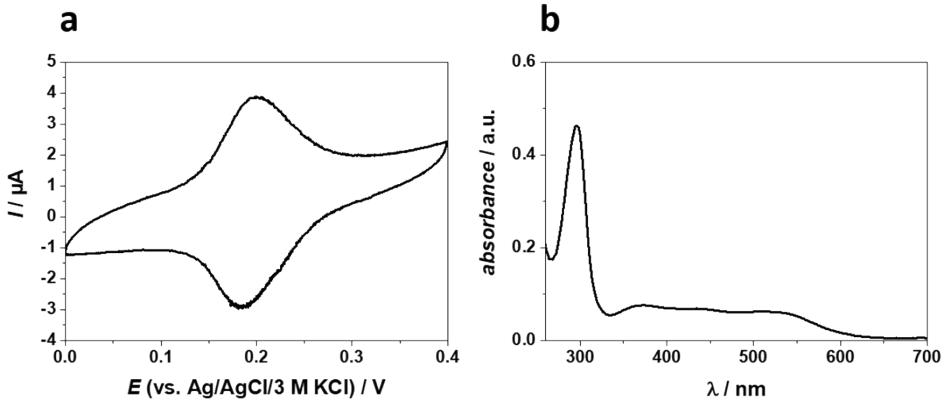
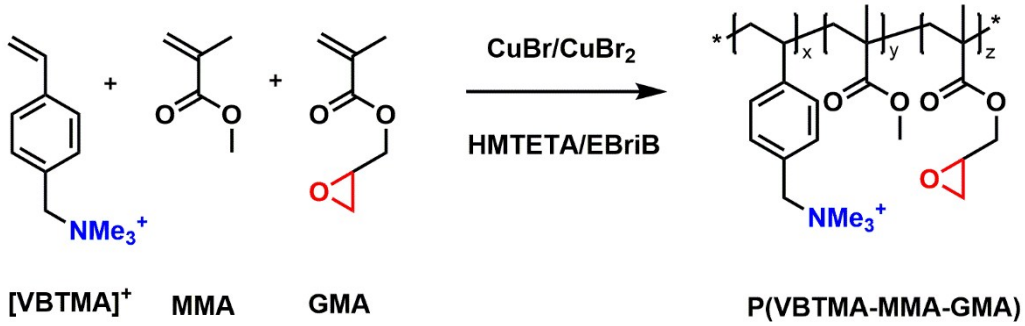


Figure S11: Electrochemical **(a)** and spectroscopic characterization **(b)** of P(VI-AA$_{NH2}$)-Os. **a)** Cyclic voltammogram of a glassy carbon electrode (*nominal diameter* = 3 mm) modified with a P(VI-AA)-Os/GOx film (drop cast process) in 0.1 M KCl/water; *scan rate* = 100 mV s^{-1}. b) UV-Vis spectrum of P(VI-AA$_{NH2}$)-Os in DMSO.

Synthesis of the positively charged polymer backbone for GlutOx immobilization



Scheme S2: Synthesis of the positively charged polymer P(VBTMA-MMA-GMA) via an ATRP process in MeCN.

Exchange of the Cl^- counterion in the monomer 4-(vinylbenzyltrimethyl)ammonium $[VBTMA]^+$ against BF_4^-. For the polymerization of the monomer $[VBTMA]^+$ the counterion Cl^- has to be exchanged by a less nucleophilic counterion since Cl^- might react with the epoxy groups in the final polymer. Moreover, the solubility in organic solvents is significantly enhanced by using the bulky BF_4^- anion.

Under an argon atmosphere, 2.2 g of 4-(vinylbenzyltrimethyl)ammonium·Cl ([VBTMA]Cl, 10.39 mmol) and 1.3 g $NaBF_4$ were suspended in 200 mL of acetonitrile. The turbid suspension was stirred overnight at room temperature. The mixture was filtered to remove insoluble parts and the organic phase was reduce to ≈50 mL total volume. The $[VBTMA]BF_4$ was then precipitated by adding 230 mL of diethyl ether and dried overnight in vacuo to yield 2.4 g (89 %) of colorless crystals. ^{1}H-NMR (200.13 MHz, acetonitrile-d_3): δ/ppm 7.39 – 7.23 (m, 4H, aromatic protons), 6.67 – 6.53 (dd, $J_{H,H}$ = 6.6 Hz, 1H, CH-aromat), 5.75 and 5.19 (dd, $J_{H,H}$ = 5.71 Hz, olefinic CH_2), 4.16 (s, 2H, -N-CH_2-), 2.78 (s, 9H, -N$(CH_3)_3$)

Synthesis of poly(4-(vinylbenzyl trimethyl)ammonium·BF_4-co-methyl methacrylate-co-glycidyl methacrylate), P(VBTMA-MMA-GMA). In 10 mL of acetonitrile, 162 µL of methyl methacrylate (1.52 mmol) were dissolved and the mixture was deaerated by five freeze/vacuum/thaw cycles. Then, 1 g of $[VBTMA]BF_4$ (3.8 mmol), 6.54 mg of CuBr (45.6 µmol) and 2.55 mg of $CuBr_2$ (114 µmol) were added. The green solution was again deaerated by applying several vacuum/argon cycles. To this solution, 15.5 µL HMTETA (57 µmol) were added and the blue mixture was heated to 75 °C. Then, the comonomer glycidyl methacrylate (GMA, 303 µL, 2.28 mmol) and the initiator EBriB (16.7 µL, 114 µmol) were added and the reaction mixture was stirred for 280 min at 75 °C. Afterwards, the reaction mixture was allowed to cool down in air to stop the reaction. The greenish solution was passed through a short column filled with alumina to remove inorganic material and remaining catalyst. The colorless solution was quenched with 200 mL of diethyl ether and the formed precipitate was separated by means of a centrifuge (4.000 rpm, 15 min, Baxter Megafuge 1.0). Residual solvent was removed under reduced pressure to yield 112 mg (7.6 %) of the crude polymer. The polymer was dissolved in water and dialyzed against 0.1 M KCl/water followed by pure water to remove remaining KCl by means of a centrifuge (4.000 rpm) using membrane

filters with a molecular weight cut-off of 5 kDa. ^{1}H-NMR (200.13 MHz, D_2O, Figure S12): δ/ppm 7.44 (broad, aromatic protons), 4.48 (s, benzylic H), 3 - 4 (multiple overlapping signals, weak, GMA and MMA), 3.09 (s, -N(CH_3)$_3$), 0.61, 1.03 and 1.88 (broad singlets, partially overlapping, -CH_2- and -CH_3 groups within backbone). A proper integration of the individual signal is not possible due to heavily overlapping signals. From the ratios of the signals it can be clearly seen that the VBTMA monomer is the major monomer within the backbone. Thus, the actual composition deviates from the nominal composition but remains unknown.

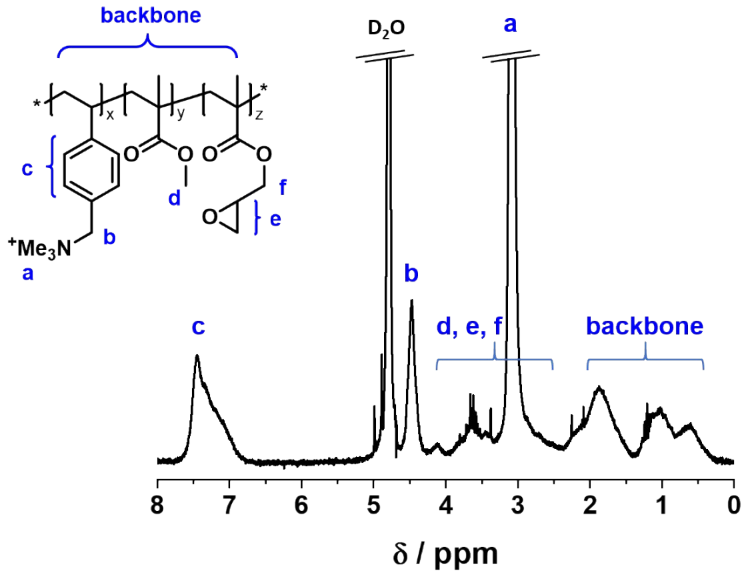


Figure S12: ^{1}H-NMR (200.13 MHz) spectrum of P(VBTMA-MMA-GMA) in D_2O. Signal assignment is attached to the graph.

References

1 T. de Lumley-Woodyear, P. Rocca, J. Lindsay, Y. Dror, A. Freeman and A. Heller, Polyacrylamide-based redox polymer for connecting redox centers of enzymes to electrodes, *Anal. Chem.*, 1995, **67**, 1332–1338. DOI: 10.1021/ac00104a006.