Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information

Enzymes hosted in redox-active ionically cross-linked polyelectrolyte networks enable more efficient biofuel cells

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Experimental

Reagents

$(NH_4)_2OsCl_6$, Pyridine aldehyde (PyCOH), poly(ethylene glycol) diglycidyl ether (PEDGE, average MW 500) and branched polyethyleneimine (BPEI) (cat # 408727) were purchased from Aldrich; 2,2'-Bipyridine (bpy) was purchased from Fluka, glucose oxidase, grade I, was from Roche. All other reagents were of analytical grade. All solutions used in this work have been prepared using milliQ water.

Synthesis of the redox polyelectrolyte (OsBPEI).

32 mg BPEI was dissolved in 25 mL methanol. This solution was added dropwise to the methanolic osmium complex solution (5 mg/mL). The mixture was left overnight under stirring at room temperature. Then an excess of sodium borohydride was added after cooling in an ice bath for 30 minutes and then stirring for 1 hour at room temperature. The non-reacting osmium complex is removed by dialysis using a 3 kDa cut-off membrane against water acidified at pH 3.0 with HCl. Osmium complex concentration was determined by UV-vis spectrophotometry at 490 nm (absorption coefficient, 8500 M^{-1} cm^{-1}). The amine:osmium ratio was determined by ^{1}H-NMR yielding 16:1 (Fig. S1).

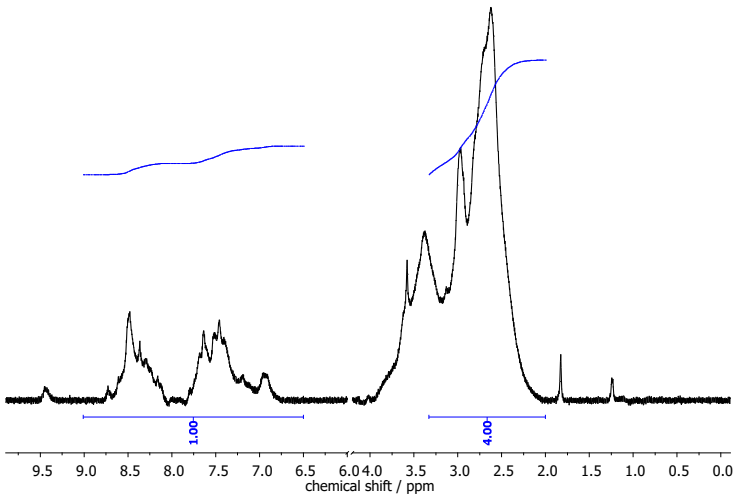

Fig. S1 ^{1}H-NMR of synthetized OsBPEI.

Quartz crystal balance measurements (QCM-D).

The QCM-D experiments were performed using a Q-Sense instrument (QCM-D, Q-Sense E1, Sweden) equipped with Q-Sense Flow Module (QFM 401). For all measurements, QSX 301 gold sensors were used. Samples were perfused using a peristaltic microflow system (ISMATEC, ISM 596D Glattbrugg, Switzerland). Gold sensors were activated with O_3 and UV for 15 min immediately before use. All experiments were performed at a flow rate of 50 µL/min at 25.0 °C. Solutions were passed for 4 minutes, then the flow was stopped, and each solution was left in contact with the gold sensor for 20 minutes. Then, the solution was removed by fluxing a solution with the same ion composition without OsBPEI or GOx, until signal stabilization was achieved.

Electrochemical experiments.

All cyclic voltammetry experiments were carried out in a solution containing 50 mM HEPES + 100 mM NaCl buffer solution at pH 7.0 or in a 100 mM glucose in 50 mM HEPES + 100 mM NaCl using a Gamry potentiostat (Gamry Interface 1000, Gamry Instruments, USA).
Compartment-less biofuel cells were constructed using the OPDC modified electrodes as described above and a high platinum loading gas diffusion electrode (HLGDE, 4 mg cm^{-2} Platinum Black – Cloth, FuelCellsEtc, College Station, Tx, USA). This electrode was chosen to ensure that the anode would be the limiting electrode in the biofuel cell. Both electrodes were immersed in a solution of 0.1 M glucose at pH 7.0, and oxygen was bubbled through the solution. Scan polarization at 2 mV/s was used to obtain polarization and power curves by monitoring the current as a function of potential (from the open circuit potential to 0 mV).

Dynamic light scattering measurements

The hydrodynamic diameter and z-potential of the colloids were determined by Dynamic Light Scattering employing a Zetasizer Nano (ZEN3600, Malvern, UK) configured in the backscattered detection optic arrangement, that is the detector at 173° of the incident light beam, a 633 nm He-Ne laser and a temperature of 25 °C. For the zeta potential calculations, the Smoluchowski approximation of the Henry equation was employed. Measurements were performed in triplicate using disposable capillary cells (DTS 1061 1070, Malvern) with a drive cell voltage of 30 V.

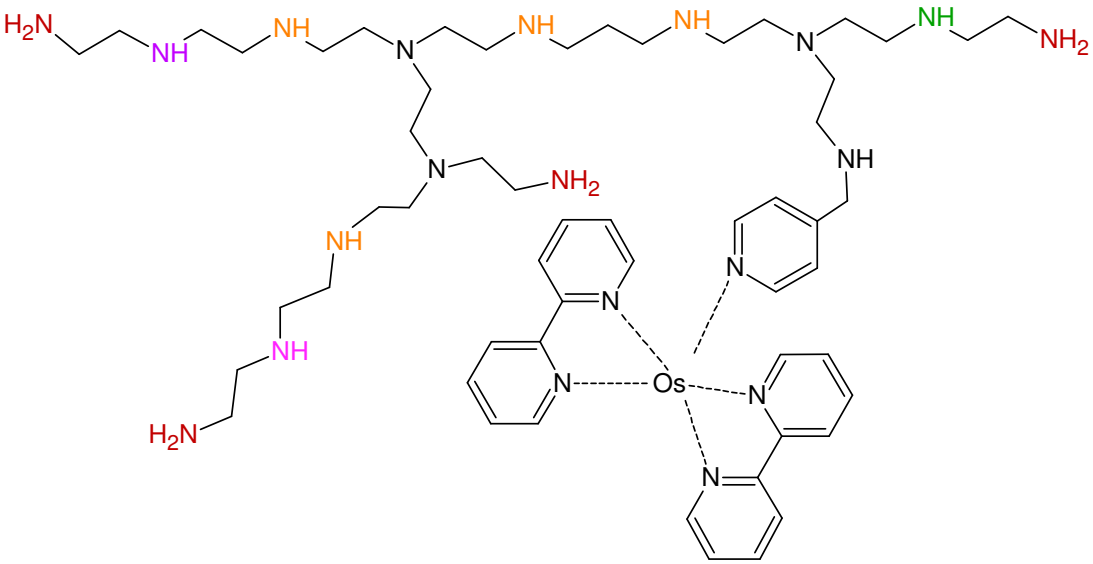

Fig. S2 – Structure of OsBPEI. The different amino types are identified by the different colors. Red, primary amino (pK´= 9.4); black; tertiary amino; orange, green, and purple correspond to secondary amino groups between secondary and tertiary (pK´= 8.6), primary and tertiary (pK´= 6.8), and primary and secondary (pK´= 4.4), respectively. pK´ correspond to those given in reference 13 (main text).

Potentiometric titration

10.0 mL of 10 mM PEI (in monomers) was titrated with a 10.0 mM solution of HCl. Taking into account the following charge equilibrium the degree of protonation at any pH can be calculated:

$$[HPEI^+] + [H^+] = [OH^-] + [Cl^-]$$

$$\frac{[HPEI^+]}{[PEI_T]} = \alpha = \frac{[Cl^-] - [H^+] + [OH^-]}{[PEI_T]}$$

$[PEI_T]$= total concentration of monomeric equivalents of PEI

$[HPEI^+]$ = protonated concentration of monomeric equivalents of PEI

α = degree of protonation

$[Cl^-]$= equivalent to added proton concentration as resulting from the added HCl.

$[H^+]$ = free proton concentration obtained from pH measurement

$[OH^-]$ = hydroxyl ions

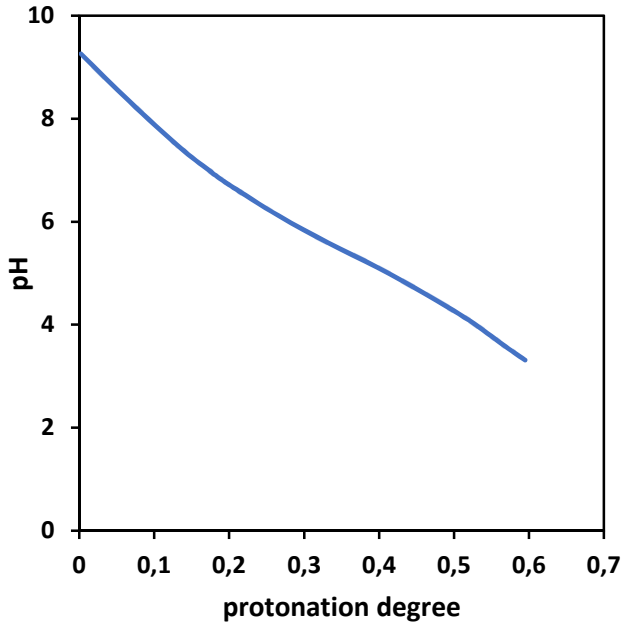

Fig. S3 pH vs protonation degree obtained by titration of 10 mM PEI (monomer equivalents) with HCl.

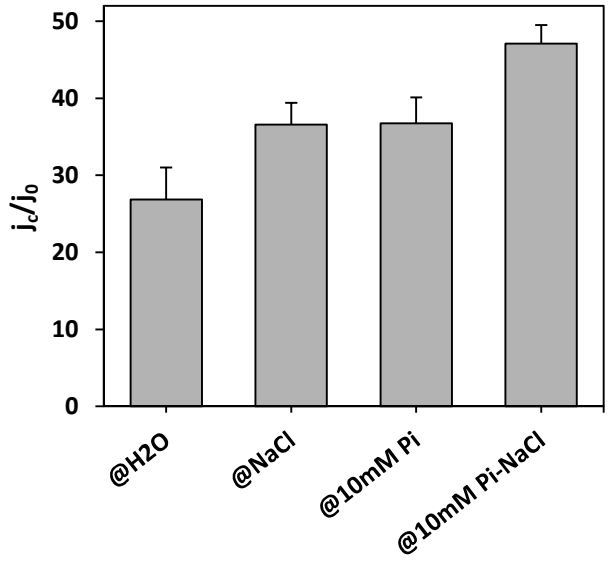

Fig. S4 j_c/j_0 current ratio for the different assemblies. j_c, corresponds to the catalytic current density in the presence of 100 mM of glucose, and j_0 represents the peak current density in the absence of glucose.

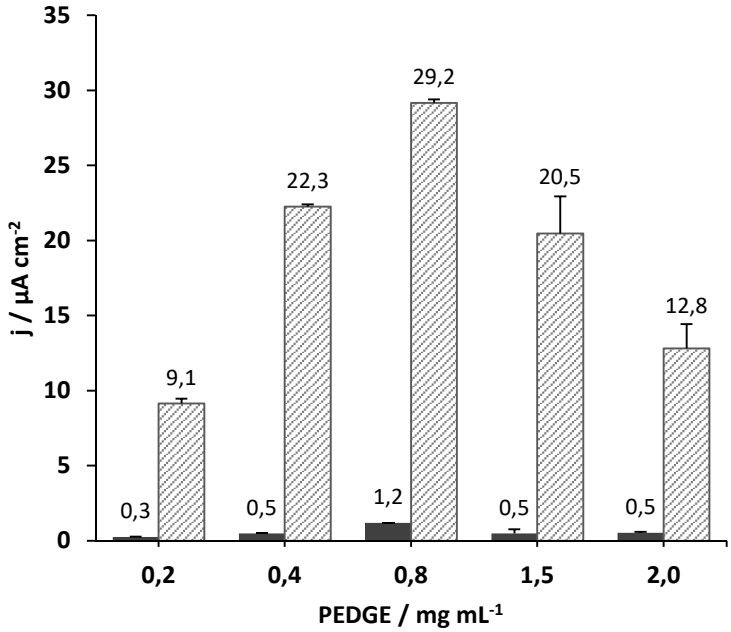

Fig. S5 Current densities for cyclic voltammetries carried out at 10 mV s^{-1} performed in 50 mM HEPES buffer + 0.1 M NaCl, pH = 7.0 (black) and in the presence of 100 mM Glucose in the same buffer (gray) for different concentration of PEDGE in the system OsBPEI@GOx,10mM Pi,NaCl,PEDGE.

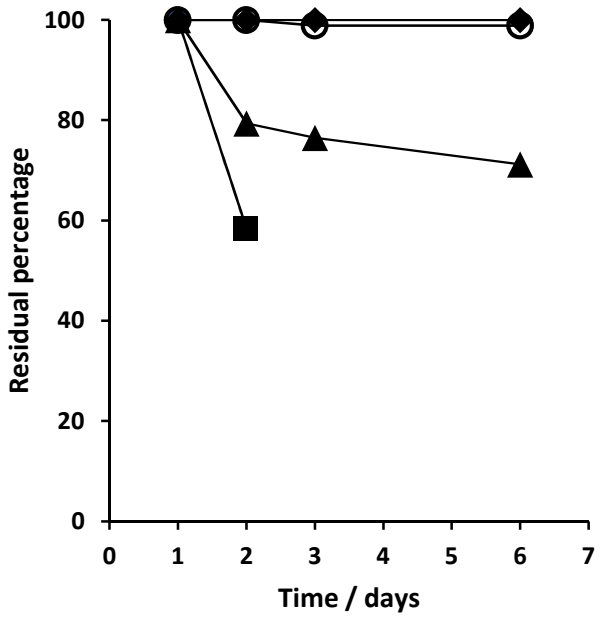

Fig. S6 Residual percentage of current densities for cyclic voltammetry carried out at 10 mV s^{-1} performed in 50 mM HEPES buffer + 0.1 M NaCl, pH = 7.0 with 0.0 mg mL^{-1} (black square), 0.2 mg mL^{-1} (black triangle), 0.4 mg mL^{-1} (open circle) and 0.8 mg mL^{-1} (black diamonds) of PEDGE in OsBPEI@GOx,10mM Pi, NaCl, PEDGE over several days.

Table S1. Comparison of power density per nmol of mediator

Bioanode	Redox mediator (R)	Power density μW $nmol^{-1}$ cm^{-2}	Reference
Inv/FDH/GOx/**R**-LPEI	$FcMe_4$-C_3-	0.42	[53]
GOx/**R**-LPEI	Fc-C_3	45	[10]
GOx/**R**-(EDA-GA-MWCNT)	FcCHO	7.8	[54]
FAD-GDH/**R**-PVI/MWCNT	$Os(dmbpy)_2Cl(im)$	8.8	[55]
GOx/**R**-(PVI-PAH)	$Os(bpy)_2Cl(im)$	6.1	[48]
R-BPEI@GOx,10mM Pi,NaCl,PEDGE	$Os(bpy)_2Cl(pyald)$	148	This work

Abbreviations: **Inv:** invertase; **FDH**: frutose dehydrogenase; **FcMe4-C_3**:3-(Tetramethylferrocenyl)propyl-ferrocene; **Fc-C_3**: propylferrocene; **EDA-GA**: ethylenediamine-glutaraldehyde crosslinked on MWCNT; **FcCHO**: ferrocene carboxyaldehyde; **FAD-GDH**: FAD glucose dehydrogenase, **PVI:** polyvynilimidazole; **dmbpy**: 4,4'-dimethyl-2,2´-bipyridine; **im:** imidazole; **PVI-PAH**: poly(1-vinylimidazole-co-allylamine).Other abbreviations have been previously used in the text. Reference numbers corresponds to those in the main text.