Electronic Supplementary Material (ESI) for ChemComm.

Materials and methods

Switching an O_2 sensitive glucose oxidase bioelectrode into an almost insensitive one by cofactor redesign

All the experiments were performed in triplicate and all the errors correspond to standard deviation.

Material. Casamino acids, horseradish peroxidase from *Amoracia rusticana* (type II, EC 1.11.1.7) (HRP), 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS), ferrocenemethanol (FM_{red}), and the other chemicals were of analytical grade or higher, and were purchased from Sigma-Aldrich (Sigma-Aldrich, Saint-Louis, MO) or Euromedex (Souffelweyersheim, France). All solutions were made with deionized water passed through an AQ 10 Milli-Q purification system from Millipore (Molsheim, France). Ultra-pure oxygen (O_2) and argon (Ar) were purchased from Air Liquide (Paris, France). AKTA purifier 10 UV 900 system, Superdex 200 and anionic exchange column (DEAE) were from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Biogel PD 10 were from Biorad. Amicon Ultra ultrafiltration columns, Amicon Stiring Cell and YM filtration membranes were from Millipore (Molsheim, France). Spectroscopic UV-visible measurements were performed on a Cary 100 system from Varian, Inc (Palo Alto, CA), equipped with a peltier thermostable multicell holder. Details on the redox polymers, PVP-[Osmium-[(N,N'-dimethyl-1,1'-biimidazole)$_2$-(methylpyridyl-biimidazole)]$^{2+/3+}$ (polymer I) and PVP-[Osmium-[(N,N'-methylpyridyl-1,1'-imidazole)$_3$]$^{+/2+}$ (polymer II) can be found elsewhere. {Prévoteau, 2012 #1986} The synthesis of 7,8-dichloro-riboflavin (step **1**) has been described earlier. {Courjean, 2012 #1970} The production/purification of FAD-synthetase (step **2**), the preparation of : apo-FAD synthetase (step **3**); 7,8-dichloro-FAD (step **4**); apo-glucose oxidase (step **5**) and the reconstitution of glucose oxidase with 7,8-dichloro-FAD (step **6**) are described in supporting information.

Electrode preparation. All the proteins were diluted at 10 mg/ml in sodium phosphate buffer 100 mM pH 5.1 to keep the same ionic strength and salinity in all samples. Unless otherwise stated, electrodes were prepared as previously reported and consisted of 35 wt% enzyme, 55 wt% of polymer I and 10 wt% of PEGDGE for a total dry loading of 200 $\mu g.cm^{-2}$. The electrodes were cured for 18h at 4°C and left 40 min at ambient temperature before they were used.

Electrochemical Instruments. Electrochemical experiments were carried out on a potentiostat (CH Instruments, model CHI 660C, Austin, TX, USA) with a dedicated computer. The bulk electrolysis cell was purchased from BAS (West Lafayette, IN). A 5 mm diameter glassy carbon electrode (Pine Instrument, Raleigh, NC) was used as working electrode. A platinum spiral wire was used as counter electrode and all potentials were referred to an Ag/AgCl (3 M NaCl) electrode (BAS, West Lafayette, IN). All calibration curves with the modified electrodes were performed, following the methodology explained in the text, at +0.3 V *vs*. Ag/AgCl in a water-jacked electrochemical cell in a PBS buffer (20 mM phosphate buffer, 140 mM NaCl, pH 7) at 37 °C with the electrodes rotating at 1000 rpm. The temperature was controlled by an isothermal circulator (Jeio Tech, Seoul, Korea).

Enzyme assay and kinetic measurements. The preparation of ferrocenium-methanol (FMox) and the experiments were performed as previously described. {Courjean, 2011 #1951} For the determination of kinetics constant with various glucose concentrations, 50nM of GOx (or GOx-$FADCl_2$) solution was added to 2.5 ml reaction mix containing 1 mM FMox, 0 to 500 mM of glucose in 50 mM sodium phosphate, pH 7.5 buffer. For the determination of kinetics constant with various FMox concentration, 50 nM of GOx (or GOx-$FADCl_2$) solution was added to 2.5 ml reaction mix containing 0 to 1.5 mM FMox, 200 mM of glucose in 50 mM sodium phosphate, pH 7.5 buffer.

Bacterial strain and plasmids.

The Flavin Adenine Dinucleotide Synthetase from *Streptomyces davawensis* was introduced in pUC57 vector and overexpressed in *E. coli* BL21 (pUC57FADSyn)

Elaboration of Glucose oxydase – $FADCl_2$

1. Production/purification of Apo FAD synthetase

1.1. Overexpression of FAD synthetase. (Step 2)

After checking by DNA sequencing that no mutations appears during DNA manipulations, pUC57FADSyn was transformed in an *E. coli* BL21 strain. A positive clone was then grown overnight in 50 ml of LB medium supplemented with kanamycine (1X) at 220 rpm and 37°C. When the optical density reached a value of 0.6; the overexpression of FAD synthetase was induced by the addition of 500 µM of IPTG. After 2h, the cells were harvested by centrifugation (4000 g, 4°C) and then resuspended in 30 ml of (Tris-HCl 100 mM, EDTA 10 mM, DTT 1 mM, pH 7.5) buffer. The solution was sonicated by pulse of 5 x 1min at 50 % of power. After 30 min of centrifugation (20 000 g, 4°C). The supernatant was dialysed in Tris 50 mM, pH 7.5 buffer, concentrated up to 5 ml and filtrated (0.2 µm) before loading on a Fast Protein Liquid Chromatography system.

1.2. Purification of Apo FAD synthetase. (Step 3)

The enzyme solution was purified on a Phenyl Sepharose column equilibrated with the binding buffer (50 mM Tris / HCl, 40 % ammonium sulfate, pH 7.5). FAD synthetase was eluted from the column at a flow rate of 2.5 $ml.min^{-1}$ with a linear gradient of 40 % to 0 % ammonium sulfate. Elution was continuously monitored at 280 nm and 460 nm to discriminate protein and FAD. Only the fraction containing the protein without FAD was collected, dialyzed in a 50 mM Tris/HCl pH 8 buffer, concentrated and loaded on an anionic exchange column (DEAE). The DEAE column was equilibrated with the binding buffer (50 mM Tris/HCl, pH 8). The purified enzyme was then eluted at a flow rate of 1 $ml.min^{-1}$ with a linear gradient of 0 to 100 % of 50 mM Tris/HCl, 500 mM sodium chloride, pH 8. Fractions were pooled, concentrated and equilibrated with a storage buffer 50 mM Na Phosphate pH 7.5.

1.3. Conversion of 7,8-dichloro-riboflavin into 7,8-dichloro-FAD ($FADCl_2$). (Step 4)

The synthesis of 7,8-dichloro-riboflavin **(Step 1)** has been described earlier. {Courjean, 2012 #1970} The conversion mix consists of 500 µM of 7,8-dichloro-riboflavin, 12.5 mM ATP, 1.25 mM $MgCl_2$ and 2.2 µM of apo FAD synthetase in a buffer 50 mM Na phosphate, pH 7.5. This mix is left for several hours at room temperature and protected from light. The conversion is followed by thin layer chromatography (eluant: isopropanol / water 90/10 v/v). At the end of the conversion, the solution is filtrated on a sartorius column (PES 10 kDa). The concentration of the $FADCl_2$ solution is checked by UV-visible spectroscopy (ε 450 nm = 11 $mM^{-1}.cm^{-1}$). This solution can be stored for several month à 4°C protected from light.

2. Preparation of Apo GOx. (Step 5)

120 mg of commercial GOx is solubilized in 2.5 ml of Buffer A (Na phosphate 25 mM, Glycerol 30 % w/v, pH 6), centrifugated 20 min at 5000 g and desalted on a PD 10 desalting column (GE Helthcare) using buffer A. 16 µL of H_2SO_4 (85%) is added to 4 ml of the enzyme solution and left for 3 h at 4°C under vigorous stirring and protected from light at 4°C. The FAD liberated from the partially unfolded GOx was removed by size exclusion chromatography on a Biogel P10 column (BioRad) pre-equilibrated with buffer B (Na phosphate 25 mM, Glycerol 30 % w/v, pH 1.4). The fractions containing the colorless apo-protein are instantly adjusted to pH 7 by addition of 10 ml of buffer C (Na phosphate 200 mM, pH 9). The concentration of the enzyme solution is checked by UV-spectroscopy (ε 280 = 260 $mM^{-1}.cm^{-1}$). This solution can be stored several month at -80°C protected from light.

3. Reconstitution/purification of GOx-$FADCl_2$. (Step 6)

3.1 Reconstitution of GOx-$FADCl_2$

5µM of $FADCl_2$ was added to 1 µM of Apo-GOx in Na phosphate 50 mM, pH 7.5 buffer and left 48 h at 4°C protected from light. The enzyme solution is then concentrated on

a YM 10 column, dialyzed on Na phosphate 50 mM, pH 6.5 buffer and concentrated before being loaded on the exclusion size column.

3.2. Purification of GOx-FADCl$_2$.

The Superdex 200 preparative column was equilibrated with the binding buffer (50 mM Na phosphate, pH 6.5). GOx-FADCL$_2$ was eluted from the column at a flow rate of 1 ml.min^{-1}. Elution was continuously monitored at 280 nm and 460 nm to discriminate protein and FAD. Only the fraction containing the protein with the FAD was collected, dialyzed in 100 mM Na Phosphate pH 5.1 buffer and concentrated. The enzyme can be stored for several weeks at 4°C or several months at -80 °C.