Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Synthesis and Enzymatic photo-activity of O_2 tolerant hydrogenase/CdSe@CdS quantum rod bioconjugate

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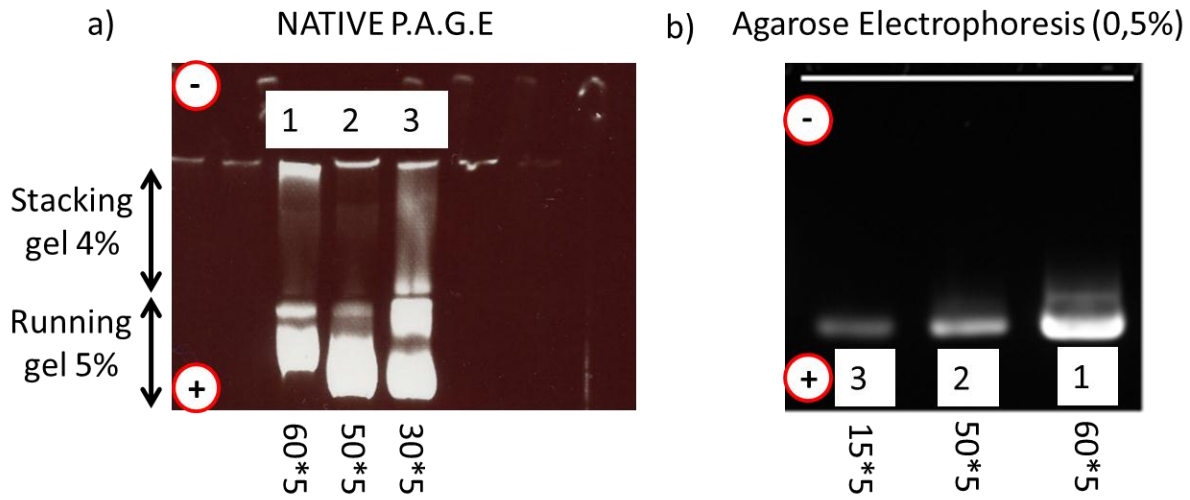

Figure **S1**: a) Native P.A.G.E of QRs with various aspect ratios functionalized in water. The concentration of QRs is fixed at 20 mg/ml for all lanes. Separation on native PAGE performed in Tris/Glycine buffer under 25mA/gel and photographed under UV illumination. Length*diameter of QRs from lane 1 to 3: 60*5 nm, 50*5 nm, 30*5 nm respectively. b) Agarose gel electrophoresis of QRs with various aspect ratios. Length*width of QRs from lane 3 to 1: 15*5 nm, 50*5 nm, 60*5 nm respectively. A 0.5% agarose gel was used and migration performed for 45 min in 20 mM borate buffer pH 8.5 under a 3.3 $V.cm^{-1}$ electric field.

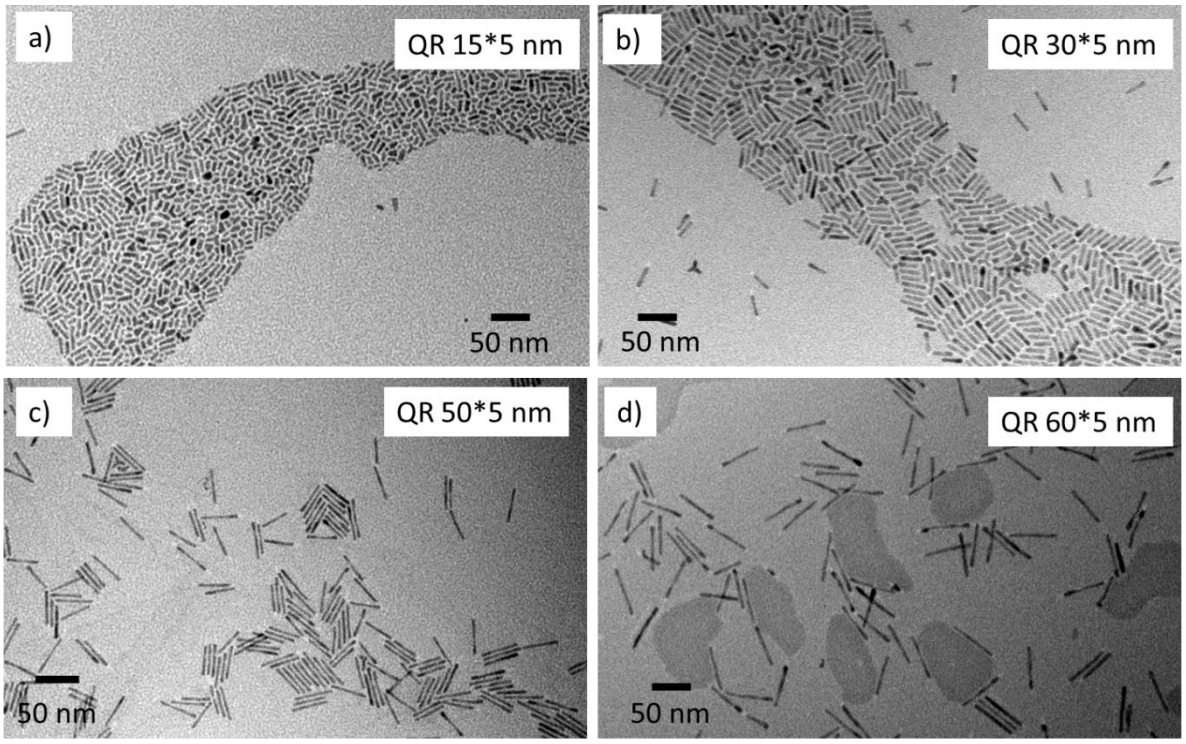

Figure **S2**: a, b, c, d) TEM images of the QRs used for gels of electrophoresis in Figures S1 and S3. The length*diameter of QRs is written in the upper right corner of each TEM image.

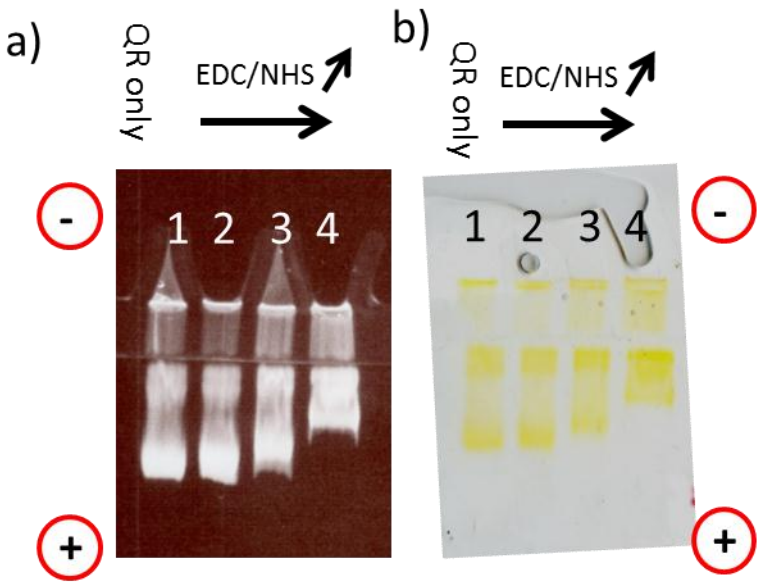

Figure **S3**: a-b) Images of native P.A.G.E of QRs with an increasing amount of coupling agents in solution. The concentration of QRs was fixed at 20 mg/ml for all lanes: a) under UV illumination and b) under visible light. Lane 1: QRs alone, lane 2-4: QRs suspensions containing increasing concentrations of EDC/NHS: 4/1 mM, 40/10 mM, 400/100 mM respectively.

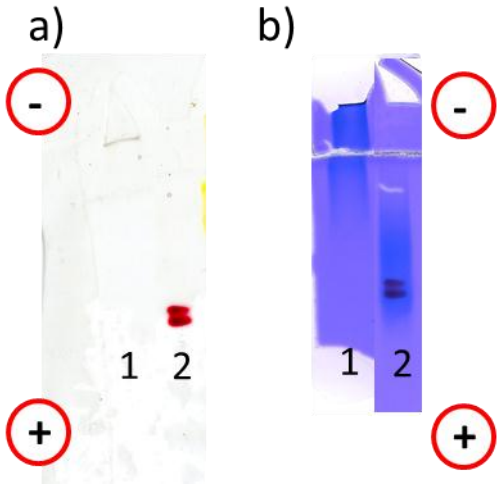

Figure **S4**: Native P.A.G.E of MbH1 (12.5 µM) incubated in the presence of 400 mM/100 mM EDC/NHS (lane 1) or not (lane 2). a) Detection of Hase activity by "in gel assay" characterized by a red color. b) Gel photographed after a further Coomassie blue staining.
The Hase is thus not stable in presence of 400 mM/100 mM EDC/NHS as no migration was observed (lane 1). Therefore the excess of coupling agents in the QR suspension was removed by size exclusion chromatography before the incubation with MbH1 to form the QR-MbH1 complex (see synthetic procedure).

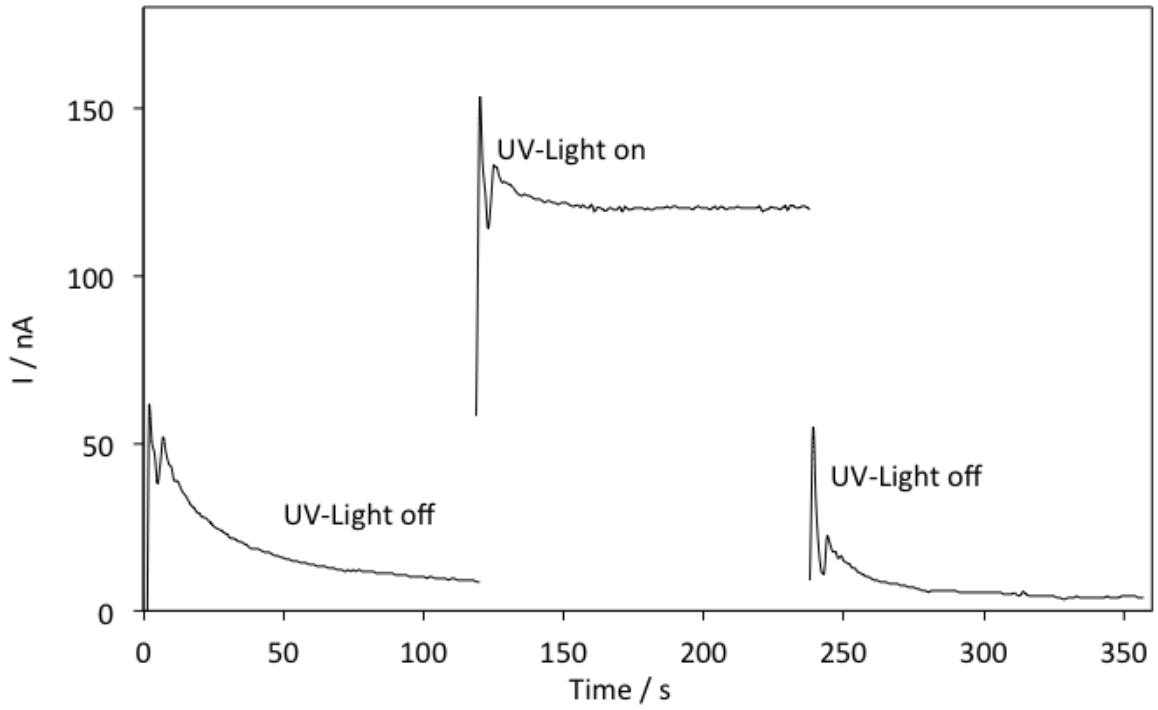

Figure **S5**: Chronoamperogram of QRs immobilized at the surface of a pyrolitic graphite (PG) electrode covered with a dialysis membrane and in the presence of 10 µM Methylene Blue (MB) recorded at -0.1 V vs Ag/AgCl and under H_2 atmosphere. Light pulses were applied every 120 s. The experiment was performed in 50 mM HEPES buffer solution at pH 6.8 and 60°C.

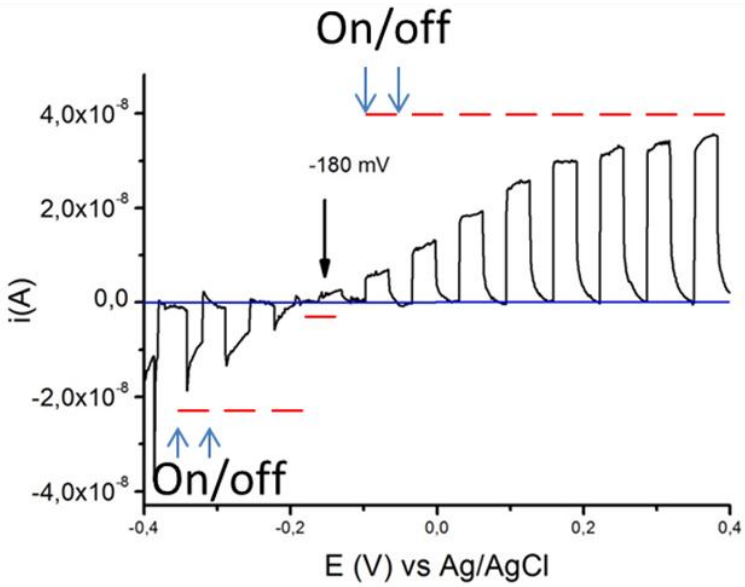

Figure **S6**: Linear sweep voltammogram of the quantum rods immobilized at the surface of a pyrolytic graphite (PG) electrode covered with a dialysis membrane. Light pulses were applied every 60 s. The experiment was performed in 50 mM HEPES buffer solution at pH 6.8 and 60°C (v = 1 mV/s, duration of the light pulses 30 s).

Synthetic Procedures:

Quantum rods CdSe/CdS synthesis:

The quantum rods are synthetized according to the Carbone et al. procedure.[1] The synthesis consists in a two steps protocol: first the synthesis of the CdSe seeds and seconds the synthesis of the CdS shell around the CdSe core. All chemicals were purchased from Sigma-Aldrich.

1/ To prepare the CdSe seeds, TOPO (TriOctylPhosphine oxide, 3.0 g), ODPA (OctaDecylPhosphonic Acid, 0.280 g) and CdO (Cadmium oxide, 0.060 g) were mixed in a 50 mL trinecked flask and heated to 150 °C under vacuum for 1 hour. Then, under argon, the mixture temperature was increased to 320 °C in order to dissociate CdO. When the reddish solution turned clear and colorless, 1.5 g of TOP (TriOctylPhosphine) was injected in the flask and the temperature is allowed to reach the temperature of 380°C. Then 0.058 g of Se dissolved in 0.360 g of TOP were added. The heating mantel was removed immediately to allow the flask to cool down to room temperature. The nanocrystals are then extensively washed with a mixture of toluene and methanol (ratio v/v 1:4). The size and the concentration of the seeds were determined according to a previously published protocol which determines their size and extinction coefficient by measuring the optical density at 350 nm as well as the position of their excitonic peak.[2] Thus the CdSe seeds have a mean diameter of 2.7 nm ($\lambda_{excitonic}$ = 535 nm) and are finally dispersed in TOP at a concentration of 400 µM.

2/ The CdO is mixed in a 50 mL trinecked flask together with TOPO, ODPA and HPA (HexylPhosphonic Acid) and heated to 150°C under vacuum. The synthesis of different length shell requires different amounts of reactants (see Carbone et al for precisions). Then, under argon, the mixture temperature was increased to 320 °C in order to dissociate CdO. When the reddish solution turned clear and colorless, 1.5 g of TOP (TriOctylPhosphine) was injected in the flask and the temperature is allowed to reach the temperature required for the injection of the solution containing the sulphur precursor and the CdSe seeds (at a concentration of 400 µM) dissolved in TOP (see Carbone et al for precision). After the precursor injection, the nanocrystals are allowed to grow for 8 minutes after a thermal quenching by immersing the trinecked flask in a water bath (60°C). The nanocrystals are then extensively washed with a mixture of toluene and methanol (ratio v/v 1:4) and finally dispersed in toluene and stored at 4°C.

Quantum rods functionalization in water:

Quantum rods are functionalized with peptidic derivatives in water inspired from our previous reported procedure on CdSe/ZnS QD.[3] The complete characterization of the ligand exchange will be published elsewhere. Peptidic ligands were synthetized by Polypeptide (Strasbourg, France). The quantum rods concentration is determined by extinction coefficient reported in the literature.[4]

The quantum rods suspension in toluene are precipitated with the addition of methanol and dissolved in chloroform at a concentration of 10 $mg.mL^{-1}$. A 10 times excess of peptidic

ligands is introduced depending on the accessible surface of the QRs. The mean area (s_{lig}) of one peptidic ligand at the surface of the QR is taken to be 0.5 nm^2. The total molar amount of ligand to be added (n_{lig}) is calculated according to the following formula:

$$n_{lig} = 10.n_{QR}.S_{QR} / s_{lig}$$

where n_{QR} is the molar amount of QRs. S_{QR} is the mean surface of one QR: $S_{QR}=2\pi R.L+4\pi R^2$ where R is QR radius and L is the QR length. After the addition of the aqueous peptidic ligands solution in the organic phase, a small amount (1-5 µL) of a phase transfer agent, the tetramethylammonium hydroxide (TMAOH, 25% w/w in methanol, Sigma) is introduced. After vigorous stirring, the nanoparticles are transferred from the organic to the aqueous phase. The organic phase is discarded and the chloroform traces in the aqueous phase are eliminated in a water bath (60°C) for at least 30 min. Until further use, the suspension of hydrophilic nanocrystals can be stored at 4°C.

Purification of MbH1

Mbh1 from *Aquifex aeolicus* was purified as described by Luo and al. [5]

Formation of the QR/MbH1 complex:

The quantum rods suspension in water is purified from the excess of free ligands by a size exclusion chromatography (NAP-5, GE Healthcare), and finally dispersed in a HEPES buffer (50mM, pH=6.8) at a concentration of 20 $mg.mL^{-1}$. A large amount of N-(-3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/N-Hydroxysuccinimide (NHS) (400 mM/100 mM) is added for 0.5h. The excess of the coupling agents is removed by a size exclusion chromatography (NAP-5, GE Healthcare, eluent HEPES buffer 50 mM, pH=6.8). The nanocrystals are then concentrated with centrifugal filters (Amicon ULTRA, 0.5 mL, MWCO: 10-100k) at 3800g to reach a final QR concentration of 20 $mg.mL^{-1}$. 3 equivalents of MbH1 are then added and the resulting solution is left to incubate for at least 1h before gel characterization. The QR- MbH1 complex is stored at 4°C.

Analytical procedures.

Electrophoresis Quantum rods, Hydrogenase and QR-hydrogenase complexes were analysed by regular native gel (Tris/Glycine gels) using a mini-Protean III cell electrophoresis apparatus (Bio-Rad). After migration, gels were either submitted under UV light, or hydrogenase activity was revealed before analysis by mass spectrometry.

Separation on Gel Agarose Electrophoresis

Agarose gels (Agarose 'For Routine Use', Sigma) were prepared with 0.5% agarose (m/m water) and immersed in a Borate buffer (20 mM Borate, 20 mM KCl, pH=8.5). The gels were placed in a horizontal electrophoresis system (X_LUltra^{TM} V-2 Labnet International, USA) with electrode spacing of 15 cm. A small amount of a glycerol solution (30% Glycerol) was added to each sample of nanoparticle suspension in order to increase the viscosity in the well of the gel before loading.

In-gel Hydrogenase activity

Hydrogenase activity was revealed anaerobically in gel by incubating the native gel in 50mM Hepes buffer pH 7, 1 mM methyl viologen as electron acceptor saturated with argon then with H_2 as electron donor and finally by raising the temperature to 80°C for 15 min. The reduction of 2,3,5 Triphenyltetrazolium Chloride (TTC) fixed the red coloration of the bands corresponding to hydrogenase activity. Protein bands revealed with TTC assay were cut out from gels and stored at - 20°C for proteomic experiments. The Quantum Rods were detected by fluorescence under UV irradiation at 365 nm.

Protein identification by in-gel digestion and mass spectrometry

To unambigously identify the hydrogenase in the complex QR-hydrogenase, the protein bands were submitted to trypsin digestion and digested peptides were analysed by Peptide Mass Fingerprint (PMF) using either MALDI-ToF or LC-Q-ToF mass spectrometry. From a biological triplicate, hydrogenase was identified in both bands higher and lower from the complex QR-MbH1;[4] the best percentages of sequence coverage by MALDI-ToF-PMF were 52% (RMS 100 ppm) for the large subunit (found in n=4 mass spectrometry experiments) and 38 % (RMS 100 ppm) from the small subunit (found in n=3 mass spectrometry experiments). LC-MSMS performed by Q-ToF mass spectrometry allowed to strongly identify hydrogenase with 60% sequence coverage on the large subunit and 31% on the small subunit (n=3 mass spectrometry experiments). Bands coming from the hydrogenase alone or the QR alone corresponding to positive and negative controls were similarly analyzed by mass spectrometry and allowed to confirm the presence and absence of hydrogenase in the corresponding bands.

Tryptic digestion experiments

1D-bands were cut from gels, put into a 96-well microplate and treated by a liquid handling robotic workstation (freedom EVO 100, TECAN, Switzerland). Classical steps of washes (100 mM ammonium bicarbonate/acetonitrile, 50/50 v/v) were followed by reduction (10 mM dithiothreitol for 1h at 56°C), alkylation (55 mM iodoacetamide for 30 min at room temperature) and digestion by a trypsin solution (10 ng /µl, Sigma) containing ProteaseMax 0.01% (w/v) (Promega) in 50 mM ammonium bicarbonate for 8h at 37°C. Tryptic peptides were extracted by 0.1%TFA in water/acetonitrile (50/50, v/v) and dried into a speed vacuum.

MALDI-ToF mass spectrometry

Matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) analyses were performed on a MicroFlex II mass spectrometer (Bruker Daltonics, Germany) in a positive reflectron mode. Range was set from 800 to 5000 Da and pulsed ion extraction was fixed to 150 ns. External mass calibration was done just before the acquisition of samples using peptide calibration standard (Bruker Daltonics, Germany). The dried digested peptides were dissolved in 10 μL of 0.1%TFA in water and desalted using the C18 ZipTip column (Millipore, Bedford, MA). A saturated solution of □-cyano-4-hydroxycinnamic acid in 70% acetonitrile/ water 0.1%TFA (v/v) was used as matrix. Deposits were made onto a MALDI Target plate according to the dried droplet method. A peak list was manually generated on the

FlexAnalysis software. Proteins were identified by using in house Mascot licence with NCBInr database restricted to *Aquifex aeolicus VF5* (1750 entries). The search parameters were set as followed: Trypsin enzyme, fixed modification of cysteine (carbamidomethylcysteine), optional oxidation of methionine, one miscleavage and a mass tolerance of 200 ppm. Proteins were considered as identified if the Mascot score was greater than 45 (p <0.05).

ESI-Q-TOF mass spectrometry

Electrospray quadrupole time of flight (ESI-Q-TOF) analyses were performed on a Synapt G1 mass spectrometer (Waters, Manchester) equipped with a NanoLockSpray ion source and coupled to a nano flow UPLC nanoAcquity (Waters, Manchester). Tryptic peptides were dissolved in the loading buffer (3% acetonitrile/0.1%TFA in water) and desalted on a C18 nano trap (Symmetry C18, 180µm x 2cm, 5µm, Waters) mounted on a 6-port valve, before on line elution onto a C18 column (BEH 130 C18, 100µm x 10 cm, 1.7 µm, Waters). Peptides were eluted with a linear gradient from 3% to 50 % of mobile phase B (100% acetonitrile/0.1% formic acid) in A (0.1% formic acid in water) for 30 min. The column was rinced with 85% of B for 6 min and then brought back in 1min to the initial condition (3 min). A blank (injection of loading buffer only) was done between each sample using the same chromatographic method.

The peptides were detected into the mass spectrometer in a positive ion mode using the MS^E mode (mass range: 100-2000 Da, time scan: 1s, ramp energy: 20-40V). The doubly charged ion of GluFibrinopeptide (785, 84 Da) was used as lock mass.

Processing of the spectra and protein search were made by Protein Lynx Global Server 2.5.2 (Waters) using the following parameters: *Aquifex aeolicus* database, Trypsin enzyme, one miscleavage, carbamidomethylation of cysteine as a fixed modification: oxidation of methionine as an optional modification and a mass tolerance set as automatic.

Proteins were considered as identified by passing these two filters: minimum 5 fragment consecutive ions from the b/y series matches per peptide and minimum 2 peptide matches per protein.

Electrochemistry.

CV and CA were done using a µAutolab type III potentiostat from Autolab. A conventional three-electrode system was used with an Ag/AgCl/NaCl (sat.) reference electrode, and a gold wire as auxiliary electrode. All potentials were quoted against the Ag/AgCl reference. Prior to each experiment, the solutions were deoxygenated by bubbling with high-purity nitrogen or hydrogen.

Double glass layer electrochemical cell was thermo-regulated with a water bath. The reference electrode was separated from the warmed electrolyte using a side junction maintained at room temperature. The current are the average of at least three replicates and have been normalized with the highest catalytic current.

The graphite working electrode was constructed from 3 mm rods of pyrolytic graphite (PG) inserted in Peek polymer casings from Bio-Logic SAS. The PG electrode was carefully polished with alumina 0.04µm (PRESI). 4µL of QR-MbH1 complex (purified as described[7]) were adsorbed on the PG electrode. A dialysis membrane was fit around the PG body to avoid sample leaching while allowing diffusion of small molecules.[8]This protocol allows to define a thin layer. Electrochemical experiments were performed in 50 mM Hepes buffer pH 7.2 at 60°C.

Illumination at 405 nm of the PG electrode was achieved by placing a LED (M405L2 from Thorlabs) triggered by a potentiometer (LEDD1B from Thorlabs) at 1 cm of the working PG electrode.

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