Supplementary information for

From two-component enzyme complex to nanobiohybrid for energy-efficient water-gas shift reaction

Thomas Pichon, ^a Claudio Righetti, ^b Julien Pérard, ^a Alan Le Goff^{b*} and Christine Cavazza^{a*}

Material and methods

Materials and instruments

All reagents were purchased from Sigma Aldrich. All the reagents were used without further purification. All solvents were of analytical grade. Distilled water was passed through a Milli-Q water purification system. Commercial grade thin Multi-Walled Carbon Nanotubes (MWCNT, 9.5 nm diameter, purity > 99%). Carbon nanomaterials were used as received without any purification. 1-pyrenebutyric adamantyl amide acid and 4-carboxylatonaphtyldiazonium tetrafluoroborate were prepared as previously described.^{1,2} NiPNP^{Arg} was synthesized as previously described.³ RecRrCODH was co-produced in presence of the three Ni-chaperones (RrCooC, RrCooT and RrCooJ) and isolated as previously described.⁴ When not used, the enzymes were stored at 4 °C.

Electrochemical analysis

The electrochemical experiments in aqueous media were performed in 50 mM sodium citrate buffer pH 3, 50 mM sodium acetate buffer pH 4 to 5.5, or 50 mM Bis-Tris Propane buffer pH 6 to 9.5 in a three-electrode electrochemical cell, using a Biologic VMP3 Multi Potentiostat, inside an anaerobic glove box ($O_2 < 2$ ppm, Jacomex). A Pt wire placed was used as counter electrode, and the SCE or Ag/AgCl served as reference electrodes. All current densities are given considering the geometrical surface of the MWCNT-modified electrode (0.07 cm-2).

Gas phase chromatography analysis

Gas chromatography analyses were carried out using a PerkinElmer Clarus 500 on a 8 x 1/8 SS Porapak Q column using N₂ as vector gas. H₂ was detected using TCD detector and CO, CO₂ and CH₄ were detected using a FID detector. Instrument calibration was carried out using pure CO, a 80 % H₂ 20 % CO₂ gas mix, and a 30,22 \pm 0,60 % CO, QS H₂, 20,44 \pm 0,41 % CO₂, 10,25 \pm 0,20 % CH₄, 10,32 \pm 0,21 % N₂ gas mix.

Electrode preparation

Gas diffusion electrodes were modified with MWCNT. A dispersion of MWCNT in 50% H_2O and 50% ethanol solution was prepared by 4h sonication of 1mg mL⁻¹ MWCNT in solution. 2 mL of dispersion were drop casted on a 15mm diameter GDE, and the solvent was removed under vacuum. The GDE was rinsed with ethanol, and reduced to 3mm diameter before using.

Functionalization of MWCNT with 1-pyrenebutyric adamantyl amide acid, naphthoate function, NiPNP^{Arg} complex and RecRrCODH

MWCNT-modified electrodes were soaked for 30 minutes in DMF solution containing 5 mM 1pyrenebutyric acid adamantyl amide and after rinsed in DMF solution and two times in MilliQ water. MWCNT-modified electrodes were soaked for 30 minutes in DMF solution containing 1 mM 4carboxylatonaphtyldiazonium tetrafluoroborate and after rinsed in DMF solution and two times in MilliQ water.

The **Rec-RrCODH** modified electrodes were prepared by incubating MWCNT electrodes for 1 hour with 20 μ L of Rec-RrCODH solution in 50 mM TrisHCl buffer pH 8.5 (2 mg.mL⁻¹). The latter step was carried out inside an anaerobic glove box (O₂ <2 ppm, Jacomex). The electrodes were finally washed with 50

mM TrisHCl buffer pH 8.5. When not used the electrodes were kept in buffer 50 mM Tris-HCl, pH 8.5. The NiPNP^{Arg} modified electrodes were prepared by incubation with an aqueous 2mM NiPNP^{Arg} solution for 1h. The electrode was rinsed with water, and kept in water until use.

WGSR experiments

MWCNT were deposited on a hydrophilic PTFE filter (Sigma, 65 μ m thickness, 0.45 μ m pore size, 80% porosity). A dispersion of MWCNT in 50% H₂O and 50% ethanol solution was prepared by 4h sonication of 1mg.mL⁻¹ MWCNT in solution. 2mL of dispersion were drop casted on a 15mm diameter filter, and the solvent was removed under vacuum. The deposits were rinsed with ethanol, dried under vacuum, and stored in water until use. Deposits were functionalized with 1-pyrenebutyric acid adamantyl amide and 4-carboxylatonaphtyldiazonium tetrafluoroborate in the same way as the GDE electrodes.

In an anaerobic glove box, a deposit was placed on the bottom of a 8mL vial. NiPNP^{Arg} modification was carried out by incubating the deposit with 60 μ L of aqueous NiPNP^{Arg} solution for 12h at 4°C. The solution was then removed, and the deposit was rinsed with water. 60 μ L of **Rec-RrCODH** solution in 50 mM TrisHCl buffer pH 8.5 were then added for incubation. After 1h, the solution was removed and the deposit was rinsed with 50mM Bis-Tris propane buffer pH 7. 600 μ L of 50mM Bis-Tris propane buffer pH 7 were added to the vial, which was then closed using a rubber septa.

WGSR was started by adding CO or syngas mix using a gastight syringe. The vial was then put in a temperature-controlled chamber under agitation at 25°C. The gas phase in the vial was analyzed at regular interval by sampling 50μ L and injecting in gas chromatography.

XPS analysis

X-ray photoelectron spectroscopy (XPS) analyses were carried out with a Thermoelectron ESCALAB 250 device (ICGM, France). The X-ray excitation was provided by a monochromatic Al-K α (hu=1486.6 eV) source. The analyzed area was *ca.* 0.15 mm². The background signal was removed using the Shirley⁵ method. The surface atomic concentrations were determined from photoelectron peaks areas using the atomic sensitivity factors reported by Scofield. ⁶Binding energies (BE) of all core levels were referred to the C=C of C1s carbon at 284.4 eV. XPS spectra were simulated using OriginPro 12.0.



Figure S1. MWCNT film deposited by filtration on (A) carbon cloth, (B) PTFE membrane ; (C) Gas-tight vial for WGSR

experiments



Figure S2. Cyclic voltammetry of **NiPNP**^{Arg} modified pristine MWNCT electrode (black), ^{NA}MWCNT electrode (red) and ^{ADA}MWCNT electrode (blue) under Ar in 50mM BTP pH 7.° (recorded at 5mV.s⁻¹)



Figure S3. Quantity of H₂ in headspace of WGSR experiment determined by GC after 24h depending on (left) the surface loading of **Rec-RrCODH** for a **NiPNP**^{Arg} loading of 14 nmol cm⁻² and (right) **NiPNP**^{Arg} surface loading for a loading of RrCODH of (red) 0.7 nmol cm⁻² and (blue) 1.4 nmol cm⁻² in 50mM BTP pH 7.0 at 25°C (1mL CO injected at t0).



Figure S4. Typical UV-visible spectra of Rec-RrCODH soaking solution (a) before and (b) after immobilization on MWCNT electrode ; (inset) Absorbance at 280 nm (a) before and (b) after immobilization of Rec-RrCODH for solution of 0.2 and 2 nmol Rec-RrCODH (10-time dilution was performed for 2 nmol UV/Vis measurement)



Figure S5. Quantity of H_2 measured after 24 h after increasing CO injection in 50mM BTP pH 7 at 25°C (pristine MWCNT film, 0.1 nmol cm⁻² **Rec-RrCODH** and 14 nmol cm⁻² nmol **NiPNP**^{Arg})



Figure S6. TOF_{max} of WGSR depending on the surface loading of **Rec-RrCODH** in 50mM BTP pH 7.0 at 25°C, 1mL CO injected at t0 and 14 nmol cm⁻² **NiPNP**^{Arg}. The TOF was determined using the amount of H₂ produced after 1h of experiment and detected by GC.



Figure S7. (A) WGSR conversion yield *vs.* time. The vial headspace was flushed with 80 μ mol CO at 0, 25 and 50 hours; (B) Comparison between WGSR performed at (a) 0, (b) 50 μ L, (c) 250 μ L addition of air and (d) after 250 μ L addition of air at 3 hours (140 nmol cm⁻² **NiPNP**^{Arg}, 0.6 nmol cm⁻² **Rec-RrCODH** in 50 mM BTP pH 7.0, 2mM DTT, 80 umol CO)



Figure S8. XPS survey spectrum and XPS spectra of N 1s and P 2p core energy levels for MWCNT film modified with both **NiPNP**^{Arg}- and **Rec-RrCODH** before (black) and after (red) 24 h WGSR

References:

- Haddad, R.; Holzinger, M.; Villalonga, R.; Neumann, A.; Roots, J.; Maaref, A.; Cosnier, S. Pyrene-Adamantane-β-Cyclodextrin: An Efficient Host–Guest System for the Biofunctionalization of SWCNT Electrodes. *Carbon* **2011**, *49* (7), 2571–2578. https://doi.org/10.1016/j.carbon.2011.02.049.
- (2) Lalaoui, N.; Holzinger, M.; Le Goff, A.; Cosnier, S. Diazonium Functionalisation of Carbon Nanotubes for Specific Orientation of Multicopper Oxidases: Controlling Electron Entry Points and Oxygen Diffusion to the Enzyme. *Chemistry – A European Journal* **2016**, *22* (30), 10494– 10500. https://doi.org/10.1002/chem.201601377.
- (3) Dutta, A.; Roberts, J. A. S.; Shaw, W. J. Arginine-Containing Ligands Enhance H2 Oxidation Catalyst Performance. *Angewandte Chemie International Edition* **2014**, *53* (25), 6487–6491. https://doi.org/10.1002/anie.201402304.
- (4) Contaldo, U.; Guigliarelli, B.; Perard, J.; Rinaldi, C.; Le Goff, A.; Cavazza, C. Efficient Electrochemical CO2/CO Interconversion by an Engineered Carbon Monoxide Dehydrogenase on a Gas-Diffusion Carbon Nanotube-Based Bioelectrode. ACS Catal. 2021, 11 (9), 5808–5817. https://doi.org/10.1021/acscatal.0c05437.
- (5) Shirley, D. A. High-Resolution X-Ray Photoemission Spectrum of the Valence Bands of Gold. *Phys. Rev. B* **1972**, *5* (12), 4709–4714. https://doi.org/10.1103/PhysRevB.5.4709.
- (6) Scofield, J. H. Hartree-Slater Subshell Photoionization Cross-Sections at 1254 and 1487 eV. Journal of Electron Spectroscopy and Related Phenomena 1976, 8 (2), 129–137. https://doi.org/10.1016/0368-2048(76)80015-1.