

## Supplementary information for:

# In situ synthesis of stable mixed ligand Fe<sup>2+</sup> complexes on functionalized carbon nanotube templates

Raoudha<sup>a</sup> Haddad, Karine Gorgy<sup>a</sup>, Michael Holzinger<sup>a</sup>, and Serge Cosnier<sup>a\*</sup>

*Département de Chimie Moléculaire, UMR-5250, ICMG FR-2607, CNRS, Université Joseph Fourier BP 53,  
38041 Grenoble Cédex 9, France,*

## Materials and methods

Acetonitrile (HPLC grade) used for electrochemistry measurements was obtained from Rathburn and used without further modification. Tetrabutylammonium perchlorate (Fluka) [Bu<sub>4</sub>N]ClO<sub>4</sub> (TBAP) was used as the supporting electrolyte in organic media. Dimethylformamide (DMF, Sigma-Aldrich) was used as received. Avidin, biotin-labeled glucose oxidase (GOX, 120 U mg<sup>-1</sup>) and glucose were purchased from Sigma. LiClO<sub>4</sub> was obtained from Acros Organics. All reagents and chemicals products purchased from Aldrich were of reagent grade quality and used as received unless it is mentioned. Single walled carbon nanotubes, produced by the HiPco® process (Purified, Unidym Grade/Lot # PO346), were purchased from Unidym Inc. and dispersed as described <sup>1</sup>. The electrode surfaces were modified by successively casting and drying five times 20 µl of the CNT-THF dispersion on Pt-electrodes (5mm diameter) polished with 2 µm diamond paste (MECAPREX Press PM).

<sup>1</sup>H NMR spectra were recorded on a Bruker AVANCE 300.12 operating at 300.0 MHz.

## Electrochemistry measurements

Electropolymerization and cyclic voltammetric experiments were performed with an autolab potentiostat 100 (Ecochemie, Utrecht, the Netherlands). All electrochemical experiments were carried out in a conventional three-electrode cell under argon atmosphere in a glove box. The amperometric measurements were performed with a Tacussel PRG-DL potentiostat in phosphate buffer solution (0.1M, pH 7). All enzymatic electrodes were prepared either on bare platinum electrodes, glassy carbon electrodes, or on carbon nanotubes covered electrodes, deposit as previously mentioned <sup>2</sup>. An aqueous saturated calomel electrode (SCE) was used as reference electrode while a Pt wire placed in a separate compartment containing the aqueous electrolyte served as counter electrode. An Ag<sup>+</sup>/Ag (10<sup>-2</sup> molL<sup>-1</sup>) electrodes was used as reference in organic media.

## Synthesis

4,4' bipyridine-2,2' dibiotin ester (bipy-biotin, L1) and 4,4' bipyridine-2,2' dipyrene (bipy pyrene, L2) were synthesized using identical procedures. Biotin (488mg, 2 mmol) and pyrene butyric acid (577 mg, 2mM) were each dissolved in 50 mL DMF together with 216 mg of 2,2' dihydroxy - 4,4' bipyridine (1 mM), dicyclohehylcarbodiimid (DCC, 560 mg, 2.2 mmol), and dimethylaminopyridine (DMAP, 100 mg, 0.8 mmol). The reaction mixtures were stirred at room temperature for 3 days. Both products were isolated by recrystallization in DMF, followed by filtration over a membrane filter (PTFE, 0.45 µm pore size) and washing with cyclohexane and diethylether.

L<sub>1</sub> (bipy-biotin): <sup>1</sup>H-NMR (MeOD<sub>d4</sub>, 300MHz): δ (ppm) = 8.67 (d, 2H), 8.36 (d, 2H), 8.20 (s, 2H), 6.47 (s, 4H), 5.52 (s, 2H), 5.00 (s, 2H), 4.51 (t, 2H) 4.43 (t, 4H), 4.05 (d, 2H), 3.35 (t, 4H), 2.92 (d, 2H), 2.72 (dd, 4H), 2.27 (t, 4H), 1.90-1.23 (m, 16H). <sup>13</sup>C NMR (DMSO<sub>d6</sub>, 300 MHz): δ (ppm) = 172.5 (2C), 162.5 (2C), 156.4 (2C), 155.2 (2C), 149.3 (2C), 148.7 (2C), 144.4 (2C), 64.2 (2C), 61.7 (2C), 60.0 (2C), 55.2 (2C), 33.9 (4C), 33.0 (4C), 28.6 (4C), 24.7 (4C).

ESI/MS: m/z = 669 [MH<sup>+</sup>], yield = 206 mg (44%).

L<sub>2</sub> (bipy-pyrene): <sup>1</sup>H NMR (DMSO<sub>d6</sub>, 300 MHz): δ (ppm) = 8.61 (d, 2H), 8.32 (d, 2H), 8.23 (1H, d, **9**), 8.17 (2H, dd, **4**), 8.15 (2H, d, **1**) 8.11 (4H, 2d, **2, 7**), 8.06 (s, 2H), 8.02 (4H, 2d, **3, 8**) 8.00 (2H, d, **6**), 7.85 (2H,d, **5**), 5.72 (s, 4H), 4.12 (t, 4H), 3.35 (t, 4H), 1.90-1.43 (m, 8H). <sup>13</sup>C NMR (DMSO<sub>d6</sub>, 300 MHz): δ (ppm) = 169.3 (2C) 136.3 (2C), 131.4 (2C), 130.8 (2C), 129.8 (2C), 128.5 (2C), 127.4 (2C), 127.3 (2C), 127.2 (2C), 126.6 (2C), 125.8 (2C), 125.1 (2C), 125.0 (2C), 124.9 (2C), 124.8 (2C) 124.7 (2C), 123.2 (2C), 52.4 (2C), 40.4 (2C), 28.2 (2C), 28.1 (2C), 28.1 (2C),

ESI/MS: m/z = 757 [MH<sup>+</sup>], yield = 189.8 mg (87.18%).

The asymmetric [Fe<sup>II</sup>(L<sub>1</sub>)<sub>2</sub>S<sub>2</sub>]<sup>2+</sup> precursor complex (S = CH<sub>3</sub>CN) was synthesized corresponding to the procedure in reference <sup>3</sup> with slight modifications. Fe<sup>III</sup>(ClO<sub>4</sub>)<sub>3</sub> (9.2 mg, 0.02 mmol) was dissolved with L<sub>1</sub> (26.7 mg, 0.04 mmol) together with Bu<sub>4</sub>NB(Ph)<sub>3</sub> (62 mg, 0.11mmol) in dry acetonitrile at -30°C under inert gas in a glove box. After stirring this mixture until quantitative reduction of Fe<sup>III</sup>, the formed complex precipitates as orange solid. The final product was obtained by filtering, followed by washing with cold CH<sub>3</sub>CN and a 1:1 mixture of CH<sub>3</sub>CN/ethylether and drying and stored at -23°C until use.

The reference complex [Fe<sup>II</sup>(L<sub>2</sub>)<sub>3</sub>]<sup>2+</sup> was synthesized by mixing 13.5 mg (0.03 mmol) of Fe<sup>II</sup>(ClO<sub>4</sub>)<sub>2</sub> and 88.5 mg (0.12 mmol) of L<sub>2</sub> in dry ethanol (50 mL). A red precipitate is formed after two day stirring at room temperature. The yield was optimized by further precipitation of

the complex with cold diethyl ether. After filtration and washing, the red product was obtained.

$^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 300 MHz):  $\delta$  (ppm) = 8.55 – 7.83 (m, 54H) 7.35 - 7.13 (m, 18H), 4.72 (t, 12H), 3.52 (t, 12H), 2.25 - 1.73 (m, 24H).  $^{13}\text{C}$  NMR ( $\text{DMSO-d}_6$ , 300 MHz):  $\delta$  (ppm) = 173.2 (6C) 135.6 (6C), 134.1 (6C), 130.7 (6C), 130.2 (6C), 130.1 (6C), 129.2 (6C), 127.9 (6C), 127.3 (6C), 127.2 (6C), 127.0 (6C), 126.4 (6C), 125.9 (6C), 124.8 (6C), 124.6 (6C) 123.9 (6C), 123.1 (6C), 42.7 (6C), 40.8 (6C), 35.2 (6C), 32.1 (6C), 27.3 (6C),

ESI/MS:  $m/z = 1162 [\text{M}(\text{ClO}_4)_2]^{2+}$ , yield = 11.6 mg (38.66%).