Electronic Supplementary Information for

Covalent immobilization of glucose oxidase on amino MOFs *via* post synthetic modification approach

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Materials

Glucose oxidase (GOx) from *Aspergillus niger*, peroxidase from horseradish peroxidase (reagent grade) (HRP), *o*-Dianisidine, 2-aminoterephthalic acid, Chromium(III) nitrate nonahydrate ($Cr(NO_3)_3{\cdot}9H_2O$), 2-nitroterephtalic acid, Hydrofluoric acid (HF), Ammonium fluoride (NH_4F), Tin(II) chloride dihydrate ($SnCl_2{\cdot}2H_2O$), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (DCC), acetonitrile, dimethylformamide, 1,4-dioxane were purchased from Sigma-Aldrich and used without further purification.

Methods

Synthesis of NO_2-MIL101(Cr)

NH_2-MIL101(Cr) was synthesised according to the protocol published previously and employed in our group.[i] ($Cr(NO_3)_3{\cdot}9H_2O$ (1.63 g, 4 mmol) and 2-nitroterephtalic acid (0.89 g, 0.4 mmol) were dispersed in 20 mL of distilled water in Teflon-lined autoclave container and stirred for 30 min. Then, HF (150 mg) was added and reaction mixture submitted for 8 h at 220 °C in a static oven. Then, it was cooled to room temperature, filtered and subsequently solvothermally treated with 30 mL of ethanol for 24 h at 80 °C in the static oven. The product was recovered by centrifugation and further solvothermally treated in a Teflon-lined autoclave container in 30 mL of 1 M NH_4F aqueous solution for 24 h at 70 °C. The product was recovered by centrifugation and dried under vacuum at room temperature overnight.

Synthesis of NH_2-MIL101(Cr)

NH_2-MIL101(Cr) was synthesised according to slightly modified previously established procedure.[ii] NO_2-MIL101(Cr) (350 mg) was suspended in ethanol and sonicated for 15 min. Then, $SnCl_2{\cdot}2H_2O$ (2.32 g, 10.2 mmol) was added and reaction was heated at reflux under stirring. The product was isolated by centrifugation, washed with concentrated hydrochloric acid, water (three times) and ethanol. The obtained green powder was dried under vacuum at room temperature.

Synthesis of NH_2-MIL53(Al)

2-aminoterephthalic acid (1.90 g, 10.4 mmol) was dissolved in 10.5 mL of 2.0 M aq. NaOH solution at room temperature. $Al(NO_3)_3.9H_2O$ (3.93 g, 10.4 mmol) was added to a separate tube. Both volumes are increased to a total of 75 ml by addition of H_2O. Both solutions are added in a flask. The flask was heated under reflux – atmospheric pressure at 100 °C for 3 days. The sample was washed with DMF (130 °C) and MeOH (65 °C). The washing with DMF and MeOH happens in the reflux setup overnight. After this, the sample was washed twice with EtOH. The sample was dried in the vacuum oven at 100 °C (overnight).

Synthesis of COOH-MOFs

NH_2-MOFs (100 mg) was suspended in acetonitrile (25 mL) and glutaric anhydride (200 mg, 1.75 mmol) was added. The reaction mixture sonicated for 5 min and heated in reflux setup overnight under stirring. The product was recovered by centrifugation, washed with acetonitrile (three times), and dried under vacuum at room temperature overnight. COOH-MIL53(Al) was isolated as slightly yellowish powder and COOH-MIL101(Cr) as green powder.

Synthesis of NHS-MOFs

COOH-MOF (25 mg), *N*-hydroxysuccinimide (500 mg, 43 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (50 mg) were dissolved in dimethylformamide (6 mL) sonicated for 5 minutes and stirred at room temperature for 1 h (2 h in case of COOH-MIL53(Al)). The product was recovered by centrifugation, washed with fresh portions of dimethylformamide (two times) and ethanol (one time), and dried under

vacuum under ambient temperature overnight. NHS-MIL53(Al) was isolated as slightly yellowish powder and NHS-MIL101(Cr) as green powder.

Synthesis of MOF@GOx

NHS-MOF (20 mg) was dispersed in 1,4-dioxane/PBS (50 mM in water, pH = 7.4) 99/1 (1.5 mL) solvent mixture and sonicated for 5 min. Then, chilled to 10-12 °C in water bath and the enzyme GOx (5 mg) was added. Reaction mixture was stirred for 2 h maintaining 10-12 °C temperature. Product was recovered by centrifugation and washed with fresh portions of solvent until UV/Vis showed no characteristic absorption bands in the washing liquid. Then, the product was divided in two portions. One portion was dried under vacuum at room temperature overnight for the characterization. The second portion was suspended in the above-mentioned reaction solvent mixture and kept at -20 °C for further uses.

Determination of enzymatic activity

Glucose oxidase activity was monitored through a colorimetric assay, based on the oxidation of *o*-dianisidine by a peroxidase-coupled system.[iii]

$$\text{ß-D-glucose} + O_2 + H_2O \xrightarrow{\text{GOx}} \text{D-gluconolactone} + H_2O_2$$

$$H_2O_2 + \textit{o}\text{-dianiside (reduced)} \xrightarrow{\text{HRP}} \textit{o}\text{-dianiside (oxidised)}$$

The GOX activity assay comprised 2.40 mL of *o*-dianisidine solution (0.21 mM in 50 mM acetate buffer pH 5.10), 0.50 mL of D-glucose solution (100 mg/mL in 0.05 M phosphate buffer, pH 7.4), and 0.10 mL of peroxidase solution (60 purpurogallin U/mL in water). Then, 0.01 mL suspension of the MOF@GOx or free GOX solution was added into the mentioned mixture and the absorption of the mixture at 500 nm was immediately recorded for 6 min.

Detection of glucose oxidation by MOFs@GOx

0.5 mL of D-glucose solution at different concentrations (0-1 mM) was firstly mixed with MOF@GOx in a test tube at 37 °C. 2.4 mL o-dianisidine solution (0.21 mM) and 0.1 mL peroxidase solution (1 mg mL^{-1} in PBS buffer) were then added to the tube and incubate at room temperature for 10 min. Then, the reaction solution was centrifuged at 10 000 rpm for 1 min to remove the MOF@GOx and the absorbance at 450 nm was recorded.

Selectivity of MOFs@GOx

The selectivity of the MOFs@GOx for glucose detection was evaluated by monitoring the absorbance increase at 450 nm in the presence of various saccharides and other interferes. The experiments were taken by using 10 mM D-fructose, 10 mM D-mannose, 10 mM D-galactose, 10 mM D-sacharose and 1 mM D-glucose.

Characterization techniques

All spectroscopic measurements were carried out at room temperature. The absorption spectra were recorded with a double beam UV/Vis spectrophotometer. Dynamic light scattering and Z-Potential experiments were performed with a Nano Zetasizer (Malvern Instruments, Malvern, UK). Samples were analysed using a JEOL JSM-6010LA scanning electron microscope (SEM), an FEI Nova NanoSEM 450 (FE-SEM) and ImageJ software. Powder X-ray diffraction (PXRD) measurements were performed on the Bruker D8 Advance diffractometer with the goniometer radius 217.5 mm, 2° Sollers slits, and 0.3-mm receiving slit. DRIFT spectra were recorded on a Nicolet model 8700 spectrometer, equipped with a high-temperature DRIFT cell (KBr windows), with 100 scans collected per spectrum (scan range 600–4000 cm^{-1}, resolution 4 cm^{-1}). The PXRD patterns were recorded within an angle range 2θ of 5.0 – 80.0° at room temperature using CoKα radiation (λ = 1.790263 Å) with the following measurement conditions: Tube voltage of 40 kV, tube current of 40 mA, step- scan mode with a step size of 0.0092° 2θ , and counting time of 0.2 s/step. Thermogravimetric Analysis (TGA) was performed on a Mettler Toledo TGA/SDTA851[e] equipment, under air atmosphere with an heating rate of 10° min^{-1}.

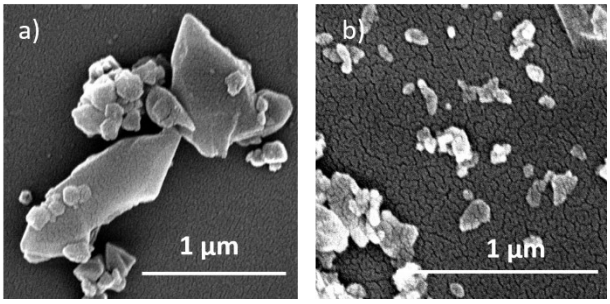

Figure S1. SEM images of a) MIL101(Cr)@GOx and b) MIL53(Al)@GOx.

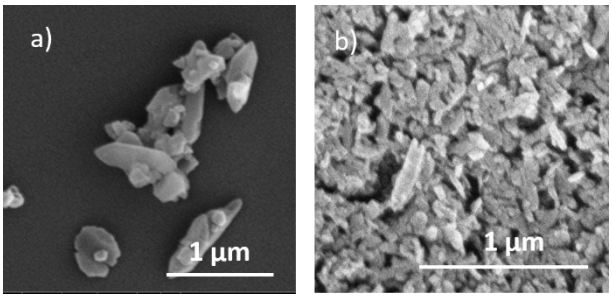

Figure S2. SEM images of a) NH_2-MIL101(Cr) and b) NH_2-MIL53(Al).

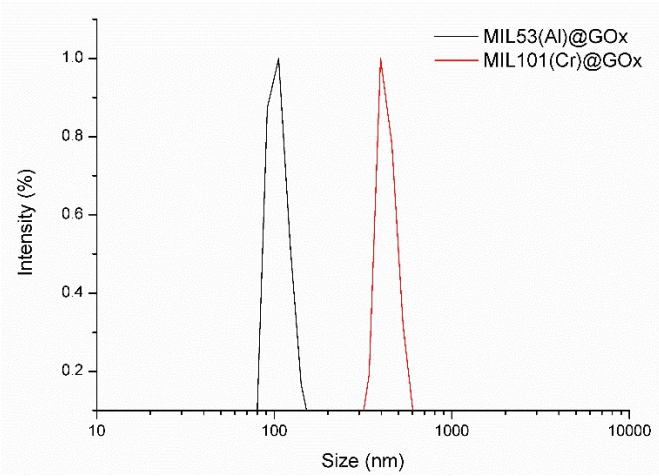

Figure S3. Size distribution in PBS of MIL101(Cr)@GOx (red line) and MIL53(Al)@GOx (black line).

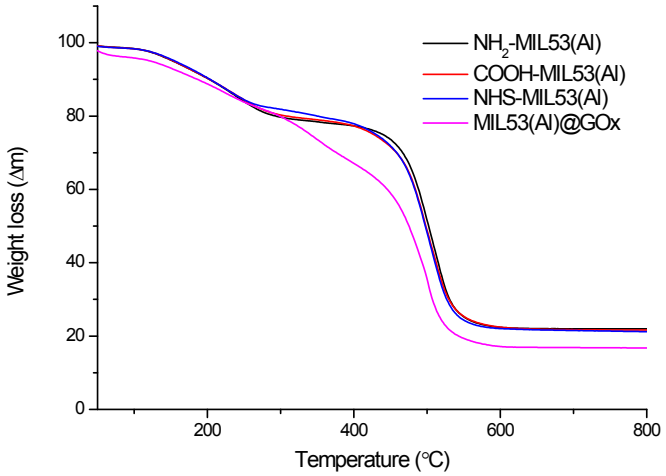

Figure S4. TGA curves comparison of NH_2-MIL53(Al) (black line), COOH-MIL53(Al) (red line), NHS-MIL53(Al) (blue line) and MIL53(Al)@GOx (magenta line).

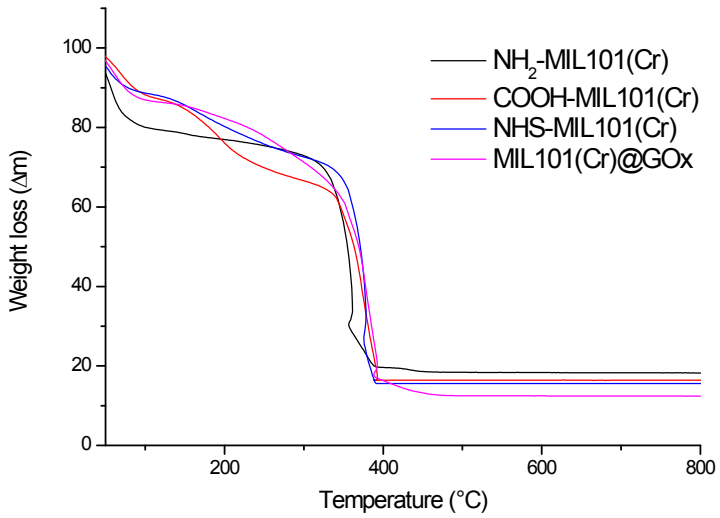

Figure S5. TGA curves comparison of NH_2-MIL101(Cr) (black line), COOH-MIL101(Cr) (red line), NHS-MIL101(Cr) (blue line) and MIL101(Cr)@GOx (magenta line).

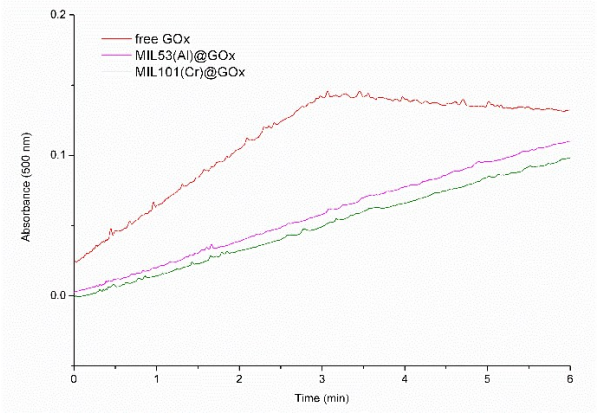

Figure S6. Time-dependent absorbance changes at 500 nm as the result of o-dianisidine oxidation with the different systems. MIL53(Al)@GOx (magenta line), MIL101(Cr)@GOx (green line) and free GOx (red line).

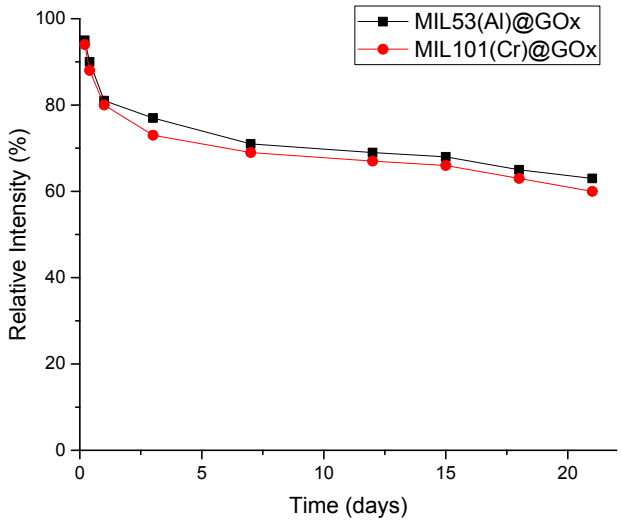

Figure S7. Long term stability of MIL53(Al)@GOx (black line) and MIL101(Cr)@GOx (red line).

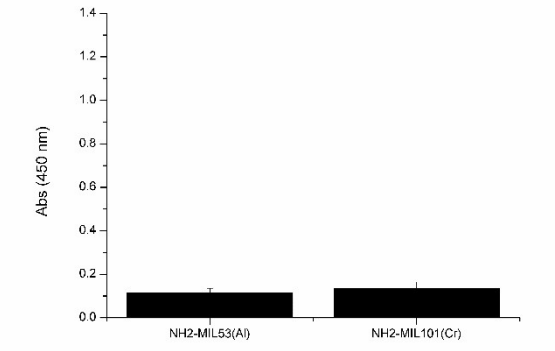

Figure S8. The glucose detection of NH_2-MIL53(Al) and NH_2-MIL101(Cr).

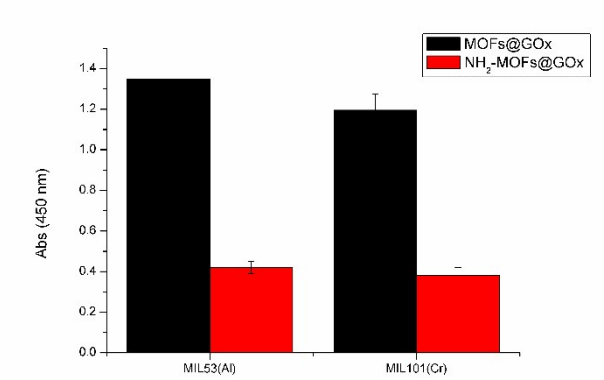

Figure S9. The glucose detection of MOFs@GOx (black bar) and NH_2-MOFs@GOx (red bar).

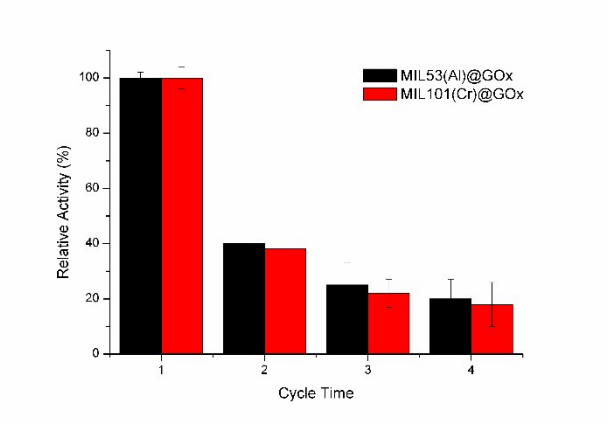

Figure S10. The glucose detection of MOFs@GOx after reusing for several cycles

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[i] G. Férey, C. Mellot-Draznieks, C. Serre, F. Millange, J. Dutour, S. Surblé, I. Margiolaki, *Science*, 2005, **308**, 2040.

[ii] M. Lammert, S. Bernt, F. Vermoortele, D. E. De Vos and N. Stock, *Inorg. Chem.,* 2013, **52**, 8521.

[iii] H. U. Bergmeyer, K. Gawehn and Grassl, *M. Methods of Enzymatic Analysis*, Volume I. (Academic Press, Inc., New York, NY, 1974).