Supporting information for

Enzyme encapsulation in zeolitic imidazolate frameworks: a comparison between controlled co-precipitation and biomimetic mineralisation

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Materials

Urease from *Canavalia ensiformis* (Jack bean), Polyvinylpyrrolidone (average mol wt 10 k, 29 k, 40 k, and 55 kDa), 2-methylimidazole were purchased from Sigma-Aldrich. Zinc acetate dihydrate was obtained from Alfa Aesar. All other reactants were purchased from Sigma-Aldrich and used without further modification.

Methods

Controlled co-precipitation of urease@ZIF-8. Urease (8 mg), 2-methylimidazole (26.3 mg) and PVP (16 mg) were combined and dissolved in deionised water (2 mL) in a 12 mL falcon tube. To another falcon tube Zinc acetate dihydrate (17.6 mg) was dissolved in deionised water (2 mL). The samples were then combined and the solution slowly turned cloudy. The reaction mixture was left at room temperature overnight. The sample was washed in EtOH:H_2O (50:50) three times and finally resuspended in 1 mL H_2O.

Biomimetic mineralisation of urease@ZIF-8. Urease (8 mg) and 2-methylimidazole (26.3 mg) were combined in deionised water (2 mL) in a 12 mL falcon tube. To another falcon tube Zinc acetate dihydrate (17.6 mg) was dissolved in deionised water (2 mL). The samples were then combined and the solution slowly turned cloudy. The reaction mixture was left at room

temperature overnight. The sample was washed in EtOH:H_2O (50:50) three times and finally resuspended in 1 mL H_2O.

The encapsulation efficiency of urease was determined using FITC-labelled urease.[1] A standard fluorescence emission vs. concentration of FITC-urease curve was determined using a HORIBA Jobin-Yvon Fluorolog-3 spectrofluorometer. To evaluate the encapsulation efficiency of urease, crystals of the ZIF-8 biocomposites were washed (3 times) with sodium dodecyl sulfate to remove surface bound enzyme. Then EDTA was added to completely digest the ZIF-8 crystals and release the encapsulated FITC-urease. The fluorescent emission intensity was measured of the FITC-enzyme solution and the total amount present was calculated using a known concentration (FITC-urease) vs. emission calibration curve (Fig S3). For the enzymatic assays, the volume of urease@ZIF-8 suspension added to the assay mixture was adjusted according to the urease encapsulation efficiency. As a consequence the encapsulation efficiency, and enzyme activity, is calculated independent of the amount of PVP and ZIF-8.

Urease Assay. A solution of urea (30 mg/mL) in PBS buffer (2.91 mL, pH 7.4) was added to a cuvette, to this phenol red (30 μL of 1 mg/mL) was added. The cuvette was placed in the UV spectrometer, and the absorbance set to zero. The sample was then allowed to incubate at the desired temperature for 2 minutes followed by 15 seconds of measurement to establish a baseline. Urease@ZIF-8 suspension (60 μL) was added and the cuvette was sealed. The increase in the absorbance at 560 nm versus time was used to measure the relative activity of the encapsulated urease.[2]

Characterisation techniques

Scanning electron microscope (SEM) images of samples were taken on a Zeiss MERLIN SEM at an accelerating voltage of 3.0 kV. Samples for SEM measurements were prepared by first suspending the composites in methanol and then 1-10 microliters of the sample solution was dropped onto a silica wafer. After all methanol was evaporated, the silica wafer was attached to a carbon paste and then sputter-coated with a thin conductive iridium layer to improve the electrical conductivity.

Synchrotron SAXS data were collected at the SAXS/WAXS beamline at the Australian Synchrotron. The scattered radiation was registered by the detector (Pilatus 1M).

UV-Vis absorption spectra were collected using a single beam spectrometer model SpectroVis Plus (Vernier Software & Technology, Beaverton OR, USA). Activity gradients were collected using a Varian Cary 500 UV-VIS spectrophotometer.

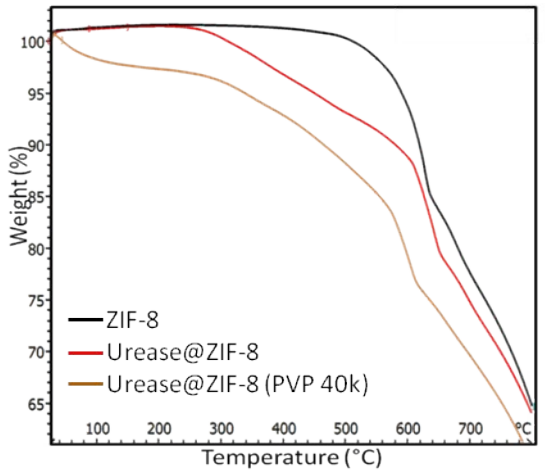

Figure S1. TGA analysis of urease@ZIF-8 composites pristine ZIF-8.

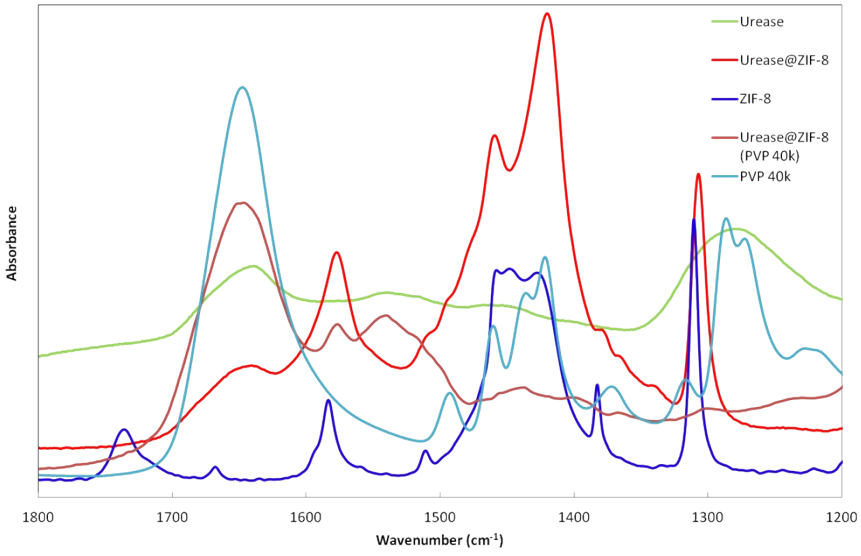

Figure S2. FT-IR measurements of free urease, urease@ZIF-8 biocomposites, and ZIF-8 crystals.

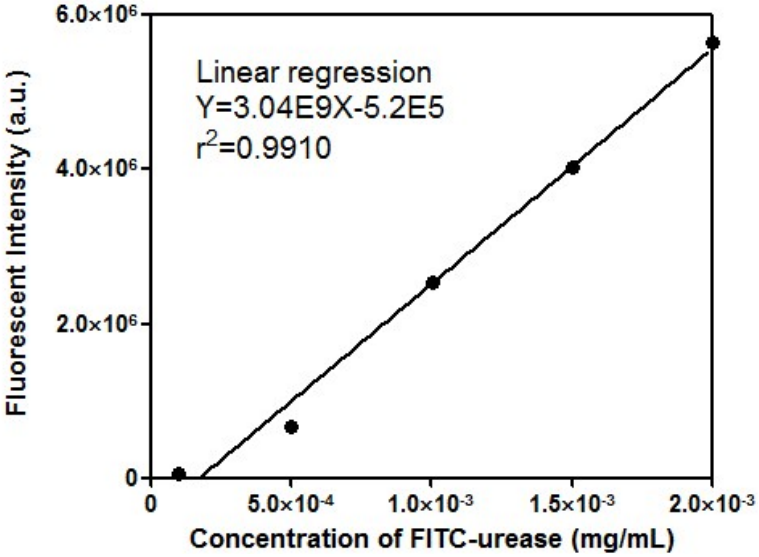

Figure S3. Standard fluorescent emission vs. concentration of FITC-urease calibration curve, assessed using fluorescent spectrophotometry.

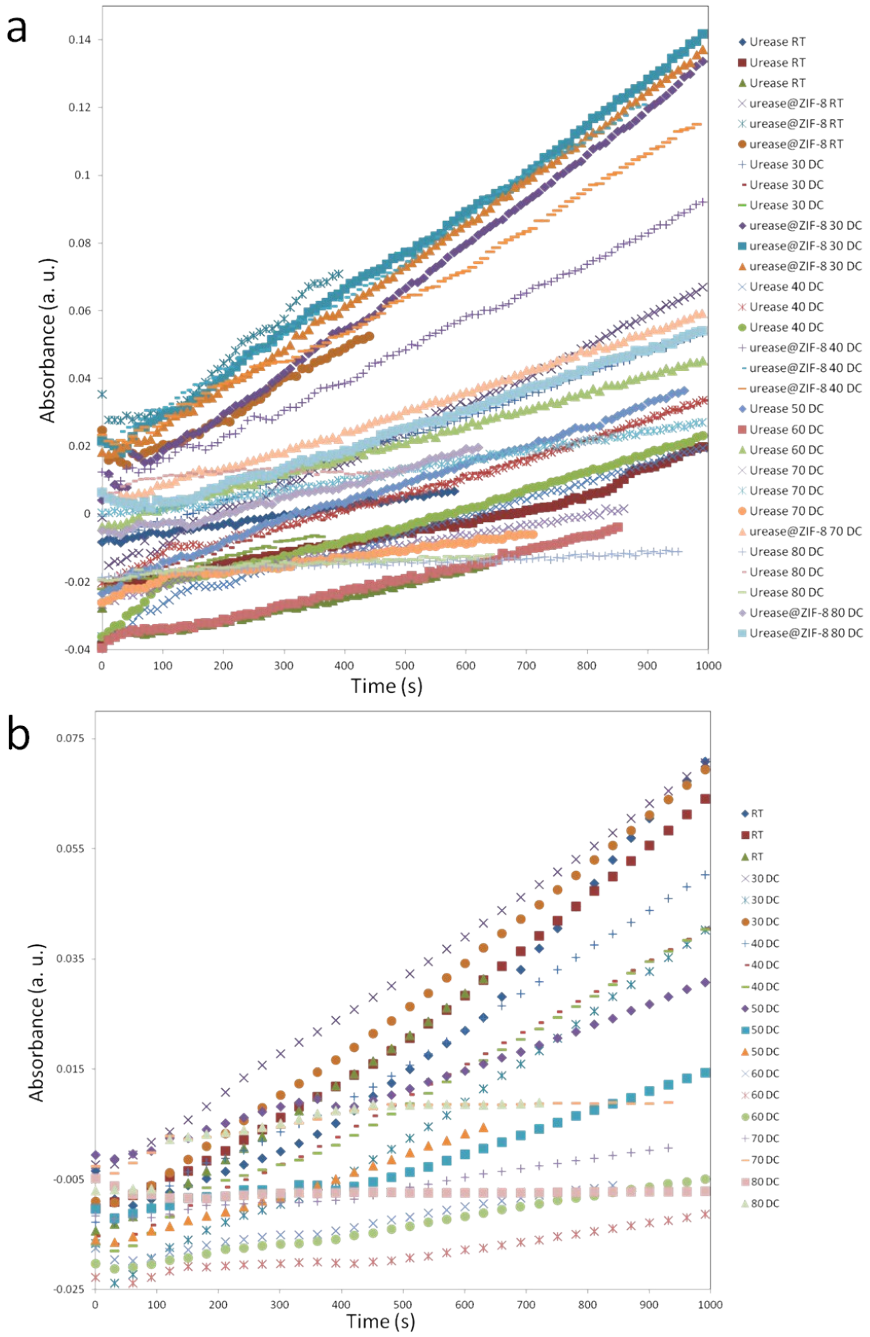

Figure S4. Activity curves of (a) free urease and biomimetically mineralised urease@ZIF-8 and (b) urease@ZIF-8 prepared by co-precipitation method after 30 min incubation at different temperatures.

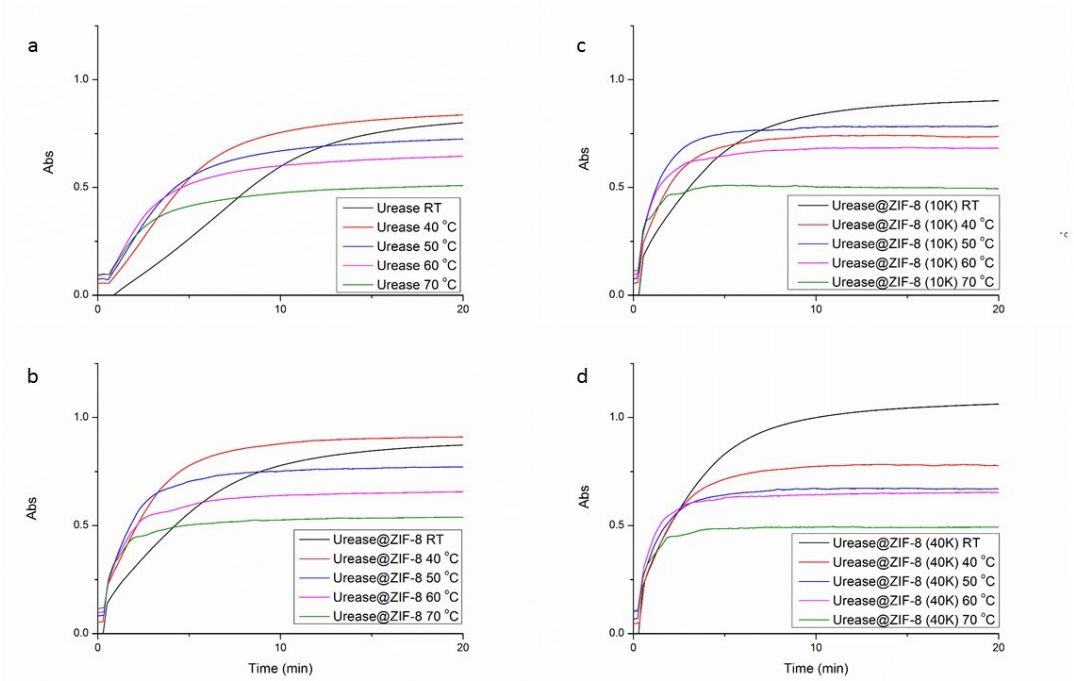

Figure S5. Activity curves for a) free urease b) biomimetically mineralised urease@ZIF-8 c) controlled co-precipitation of urease@ZIF-8 (PVP 10K) d) controlled co-precipitation urease@ZIF-8 (PVP 40K) assays run at different temperatures

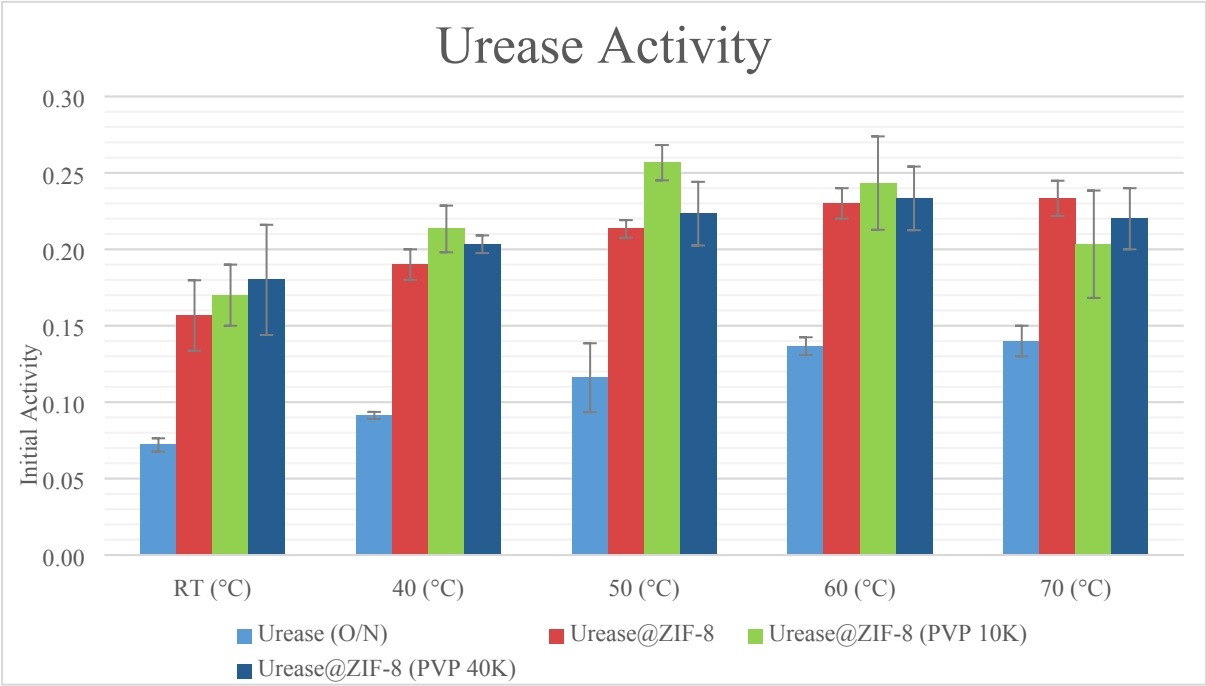

Figure S6. Relative activity for free urease and urease@ZIF-8 biocomposites prepared by the two methods at different assay temperatures.

References

1 K. Liang, R. Ricco, C. M. Doherty, M. J. Styles, S. Bell, N. Kirby, S. Mudie, D. Haylock, A. J. Hill, C. J. Doonan and P. Falcaro, *Nat. Commun.*, 2015, **6**, 7240.

2 Z. Štefanac, M. Tomašković and Z. Raković-tresić, *Anal. Lett.*, 1969, **2**, 197–210.