Supporting Information

Polymeric Nanocapsules for Enzyme Stabilization in Organic Solvents

Fumi Ishizuka,[†] Robert Chapman,[‡, ‖] Rhiannon P. Kuchel,[§] Marion Coureault,[‡] Per B. Zetterlund[†,*] and Martina H. Stenzel[‡,*]

[†]Centre for Advanced Macromolecular Design, School of Chemical Engineering, The University of New South Wales, Sydney, NSW, 2052, Australia.

[‡]Centre for Advanced Macromolecular Design, School of Chemistry, The University of New South Wales, Sydney, NSW, 2052, Australia

[‖]Australian Centre for Nanomedicine, School of Chemistry, The University of New South Wales, Sydney, NSW, 2052, Australia

[§]Electron Microscope Unit, Mark Wainwright Analytical Centre, The University of New South Wales, Sydney, NSW, 2052, Australia.

*Corresponding authors: p.zetterlund@unsw.edu.au; m.stenzel@unsw.edu.au

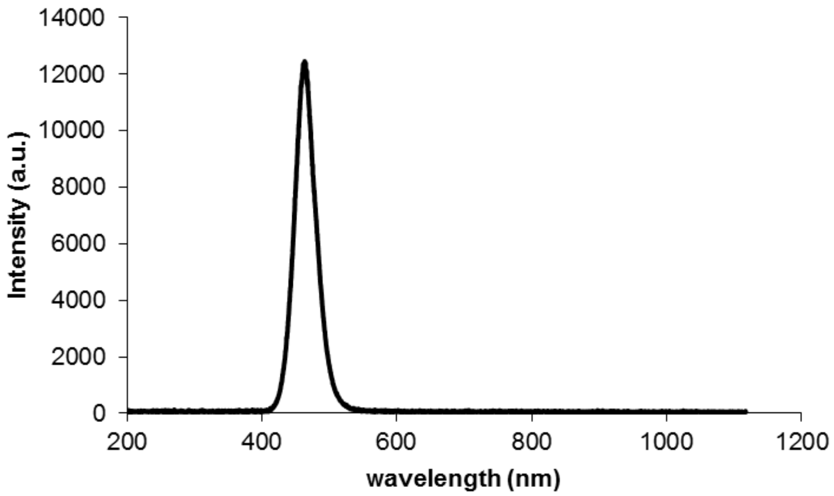

Figure S1. The emission spectrum of the blue LED bulb.

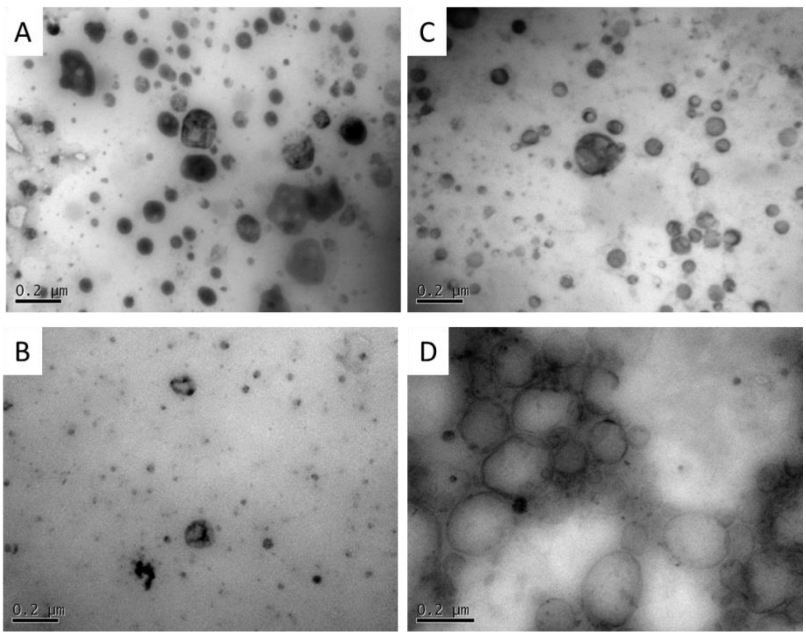

Figure S2. TEM micrographs of GOx loaded polymeric nanocapsules from reaction (A) R1, (B) R2, (C) R3 and (D) R4.

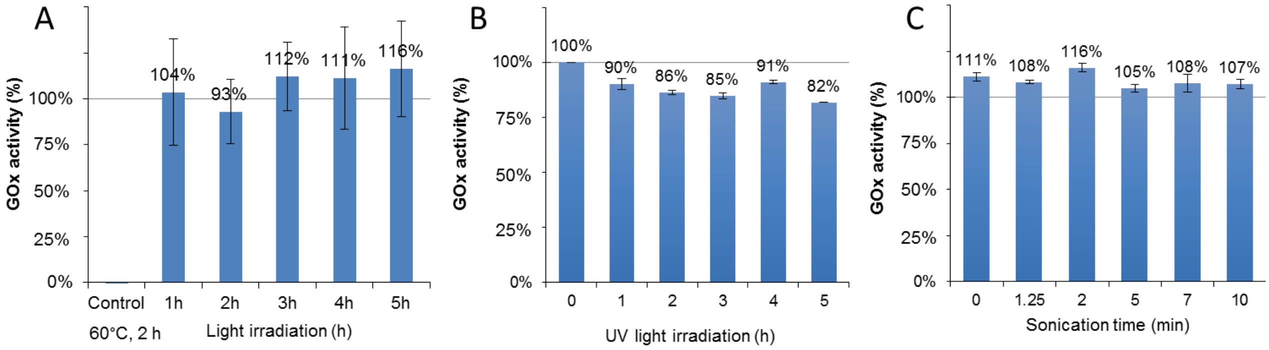

Figure S3. GOx activity after heating and visible light irradiation, respectively (A); UV light irradiation (B); sonication (C).

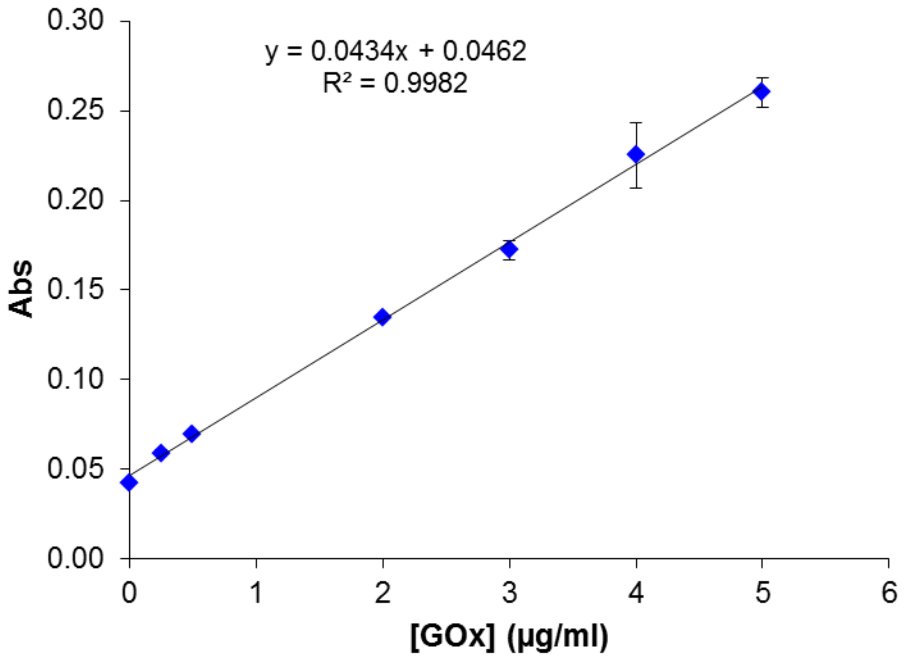

Figure S4. Standard curve of absorbance (at 450 nm) vs glucose oxidase (in PBS) after the HRP / TMB assay. This standard curve was used to determine the effective concentration (and from this the % activity) of the encapsulated GOx.

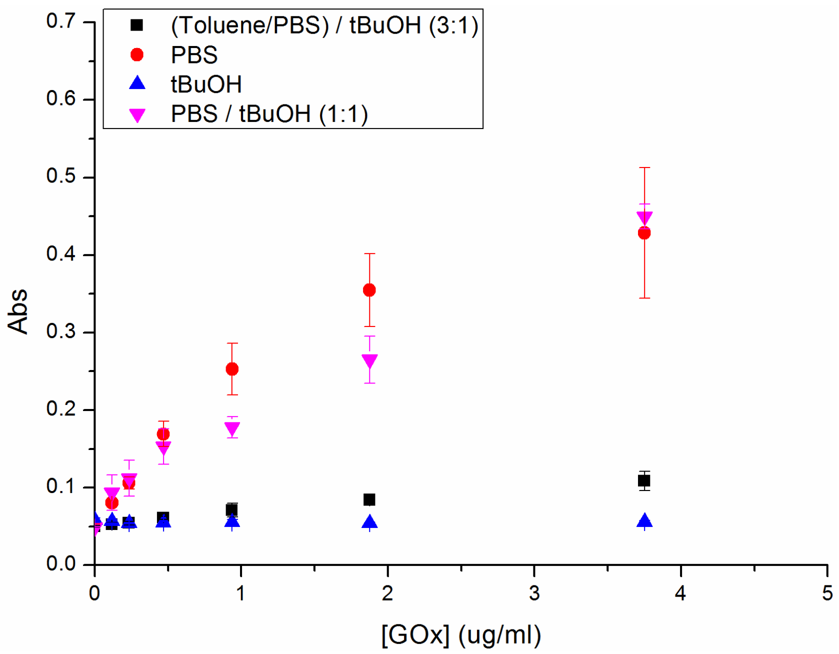

Figure S5. Standard curve of absorbance (at 450 nm) vs glucose oxidase concentration in various solvents from the HRP / TMB assay showing the denaturation of unprotected GOx in toluene and in pure *t*-BuOH

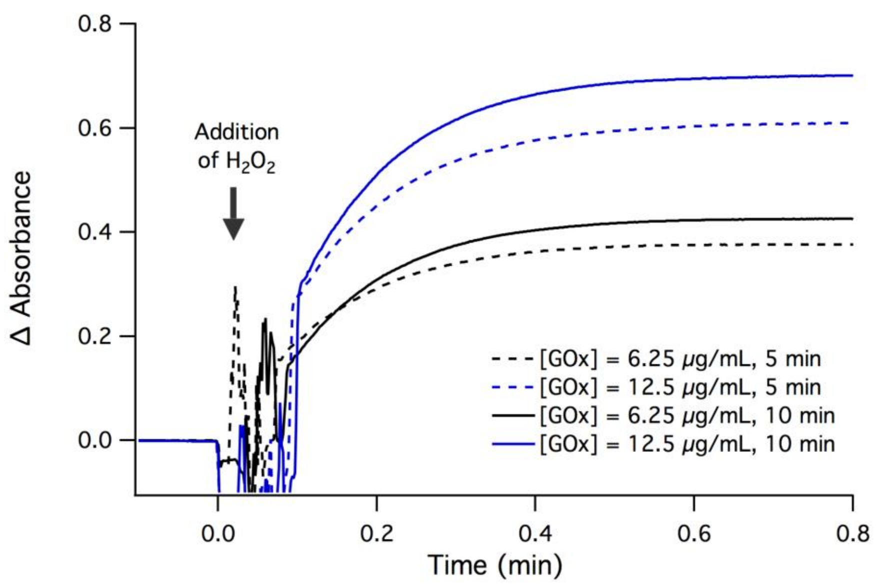

Figure S6. Kinetics of the reaction between the HRP / TMB solution and H_2O_2 generated by GOx. GOx (12.5 or 6.25 µg/mL in PBS, 75 µL) was mixed with a saturated solution of glucose (100 mM in *t*BuOH, 25 µL) and incubated at RT for 5 or 10 min. 50 µL of this solution was added to a mixture of HRP (5 µg/mL) / TMB (100 mM) (1800 µL) in citrate buffer (50 mM, pH 5.5) and the absorbance change at 650 nm was monitored. Complete consumption of H_2O_2 was observed in less than 0.5 min in all cases.

Theoretical calculation of shell thickness of nanocapsules

Theoretical shell thickness of nanocapsules was calculated based on the volume of monomer/crosslinker used for IMEPP.

The calculated droplet volume is given by the equation below, where d_d denotes the average diameter of the initial droplet:

$$V_{droplet} = \frac{4}{3} \times \pi \times \left(\frac{d_d}{2}\right)^3$$

The number of droplets in the system is based on the volume of dispersed phase and calculated as follows:

$$\text{Total number of droplets} = V_{dispersed\ phase} \div V_{droplet}$$

The volume of the polymeric shell comprising monomer/crosslinker consumed during IMEPP (V_s) was calculated based on the density of monomer/crosslinker (0.875, 0.868 and 1.05 g/ml for *t*-BMA, LMA and EGDMA, respectively).

The total volume of nanocapsules is given by:

$$V_{total} = V_s + V_{dispersed\ phase}$$

The average volume of nanocapsules (V_{NC}) can be calculated by:

$$V_{NC} = V_{total} \div Number\ of\ droplets$$

The shell thickness is calculated by:

$$Shell\ thickness = \frac{d_{NC} - d_d}{2}$$

where d_{NC} is the diameter corresponding to $V_{NC.}$

Theoretical calculation of number of GOx molecules in a nanocapsule

The theoretical number of GOx molecules in a nanocapsule was calculated based on the initial droplet diameter determined by DLS and the quantity of GOx added.

Based on the recipe, each sample contains 433 µg (2.71×10^{-9} mol) of GOx. Therefore, the number of GOx molecules in a sample is given by ($N_A = 6.022 \times 10^{23}$):

$$Number\ of\ GOx\ in\ a\ sample\ =\ 2.71\ \times\ 10^{-9}\ \times\ N_A\ =\ \ 1.63\ \times 10^{15}$$

The number of GOx in a nanocapsule (droplet) can be calculated by:

$$Number\ of\ GOx\ in\ a\ nanocapsule\ = 1.63\ \times 10^{15} \div\ total\ number\ of\ droplets$$

As discussed above, the total number of droplets can be calculated from the volume of dispersed phase (433 µL) and the initial droplet diameter (126- 175 nm).

For example, when the droplet diameter is 175 nm, the total number of droplets is calculated:

$$Number\ of\ droplets = 2.79 \times 10^{6} \times \frac{4}{3} \times \pi \times \left(\frac{175}{2}\right)^{3} = 7.77 \times 10^{13}$$

Finally, the number of GOx is calculated:

$$Number\ of\ GOx\ in\ a\ capsule = 1.63\ \times 10^{15} \div 7.77 \times 10^{13} = 21$$