Electronic Supplementary Material (ESI) for Journal of Materials Chemistry A.

Supplementary Information

The role of protein folding in prenucleation clusters on the activity of Enzyme@Metal-Organic Frameworks

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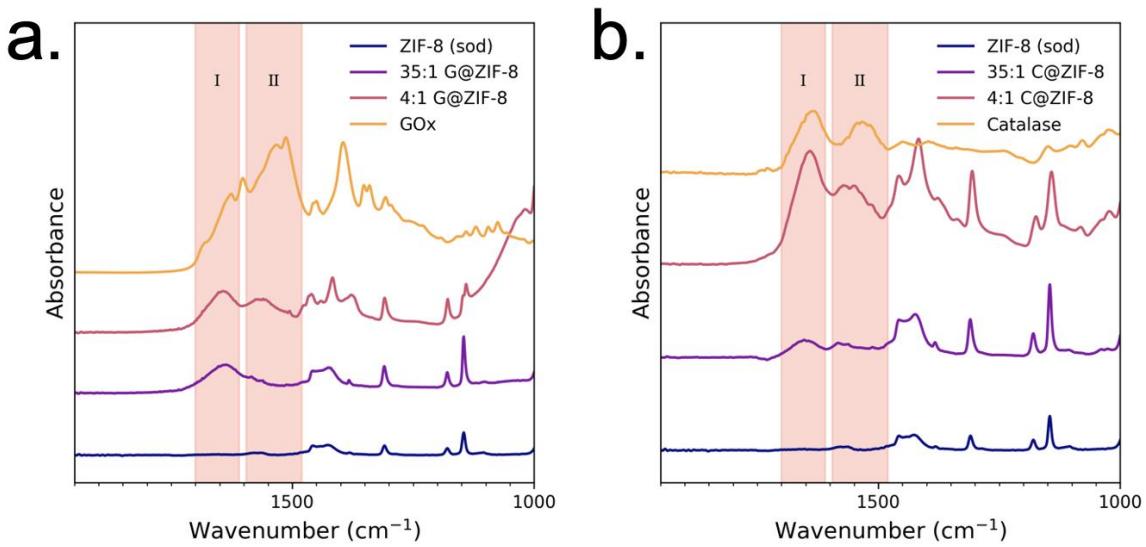
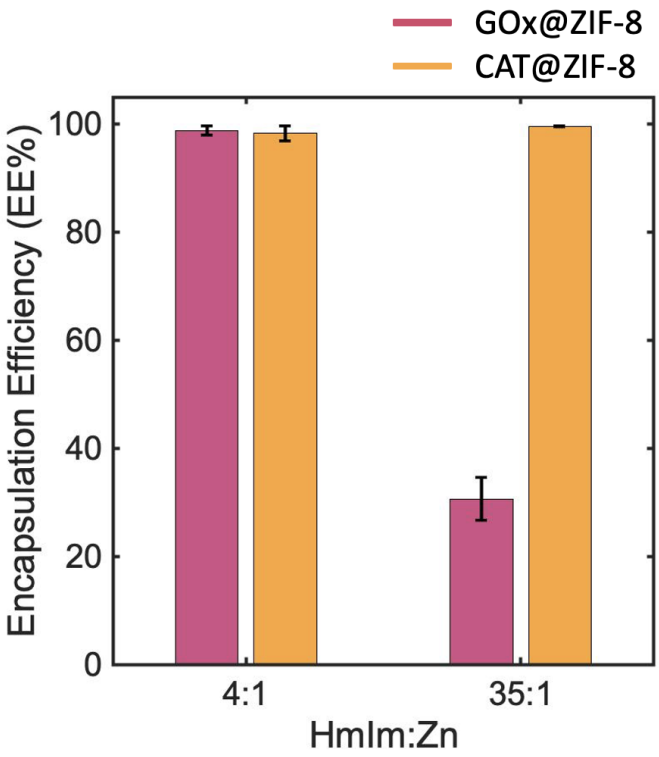
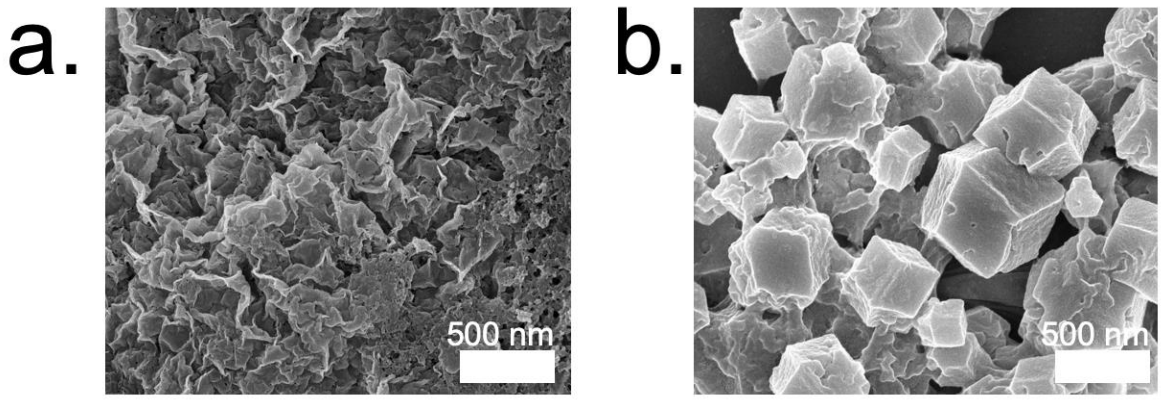


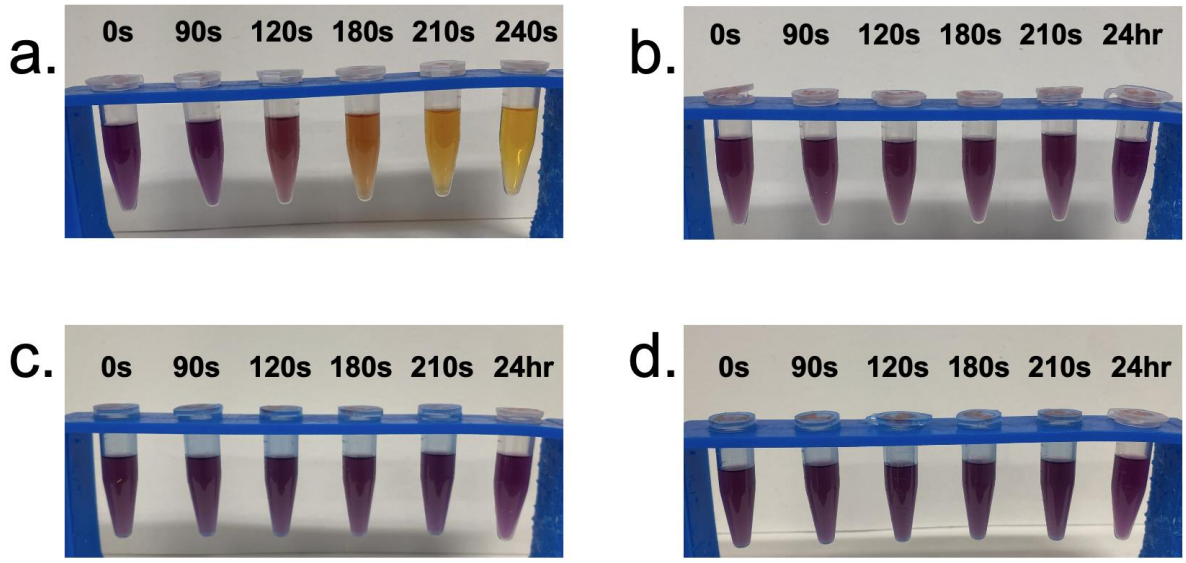
Supplemental Figure S1: FTIR spectra of enzymes (yellow) and enzyme MOFs at 4:1 (pink) and 35:1 (purple) synthetic conditions for a.) GOx and b.) CAT. FTIR of ZIF-8 without enzymes (blue) is also included. Enzyme@ZIF-8 samples were washed 3x with water and the precipitant was analyzed with a Jasco V-670 spectrometer. Data was plotted using the pandas and matplotlib pyplot python libraries, used in an author-written python script. Amide I and amide II peaks are highlighted indicating incorporation of enzymes in each biocomposite.



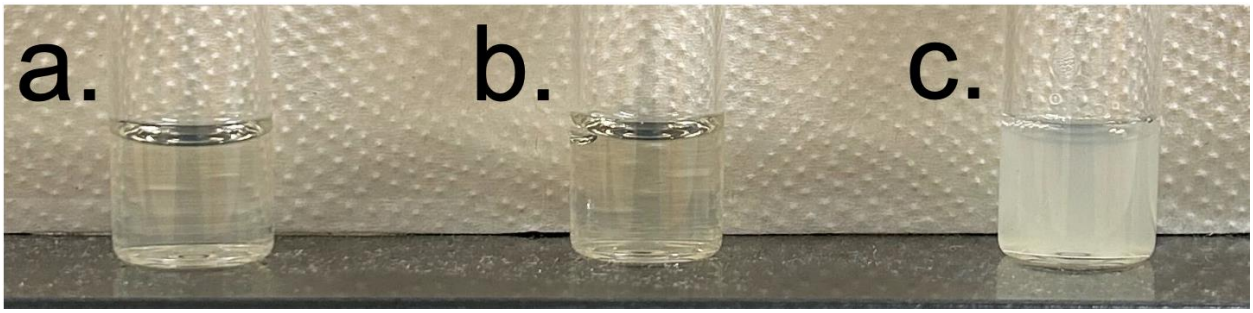
Supplemental Figure S2: Encapsulation efficiency of a.) GOx@ZIF-8 and b.) CAT@ZIF-8 at 4:1 and 35:1 ratios of HmIM:Zn. Calculations were determined using a standard calibration curve of each protein concentration on a fluorescence spectrometer while following a previously published manuscript.



Supplemental Figure S3: SEM images of CAT@ZIF-8 samples when synthesized at HmIM:Zn ratios of a.) 4:1 and b.) 35:1.



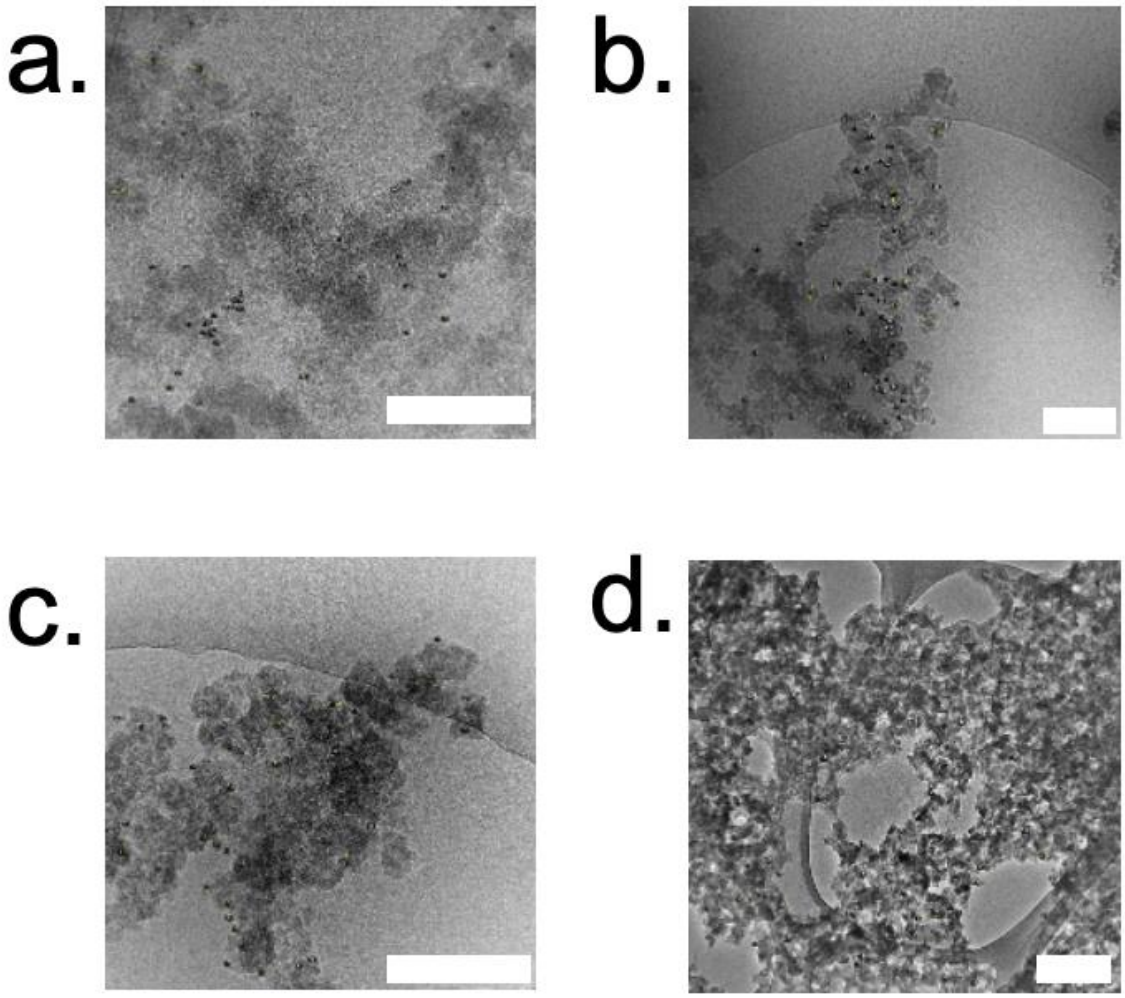
Supplemental Figure S4: Images from the reaction of a.) CAT, b.) 4:1 CAT@ZIF-8 after 3x washes with water and c.) 3x washes with methanol, and d.) 35:1 CAT@ZIF-8 with hydrogen peroxide once aliquots of the reaction were added to the FOX regent. Aliquots were taken at 0 s, 90 s, 120 s, 180 s, 210 s, and 240 s for CAT. Aliquots after 24 hrs were taken for CAT@ZIF-8 system; however, no change in regent could be observed.



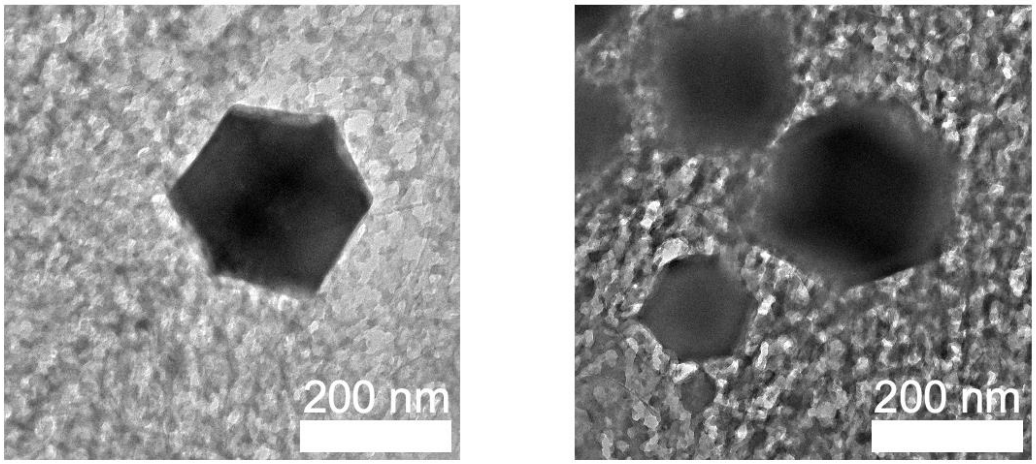
Supplemental Figure S5: Images of water solutions of a.) CAT, b.) CAT and HmIM, and c.) CAT and HmTz at final concentrations of CAT of 2.5 mg/mL and ligand of 120 mM.

Crystal Size Analysis

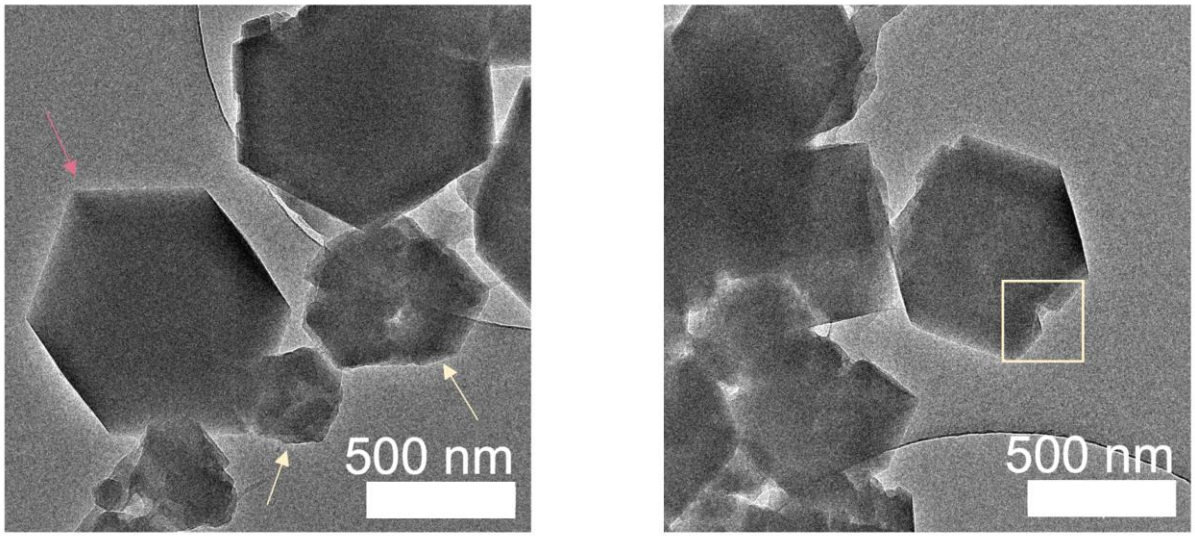
CryoTEM and TEM were used to determine crystal diameters by averaging the length of ~50 crystals per sample using Fiji, ImageJ (Figure S6). Crystal sizes were then binned into groups of 5 nm and plotted, using the matplotlib pyplot python library in an author-written python script.



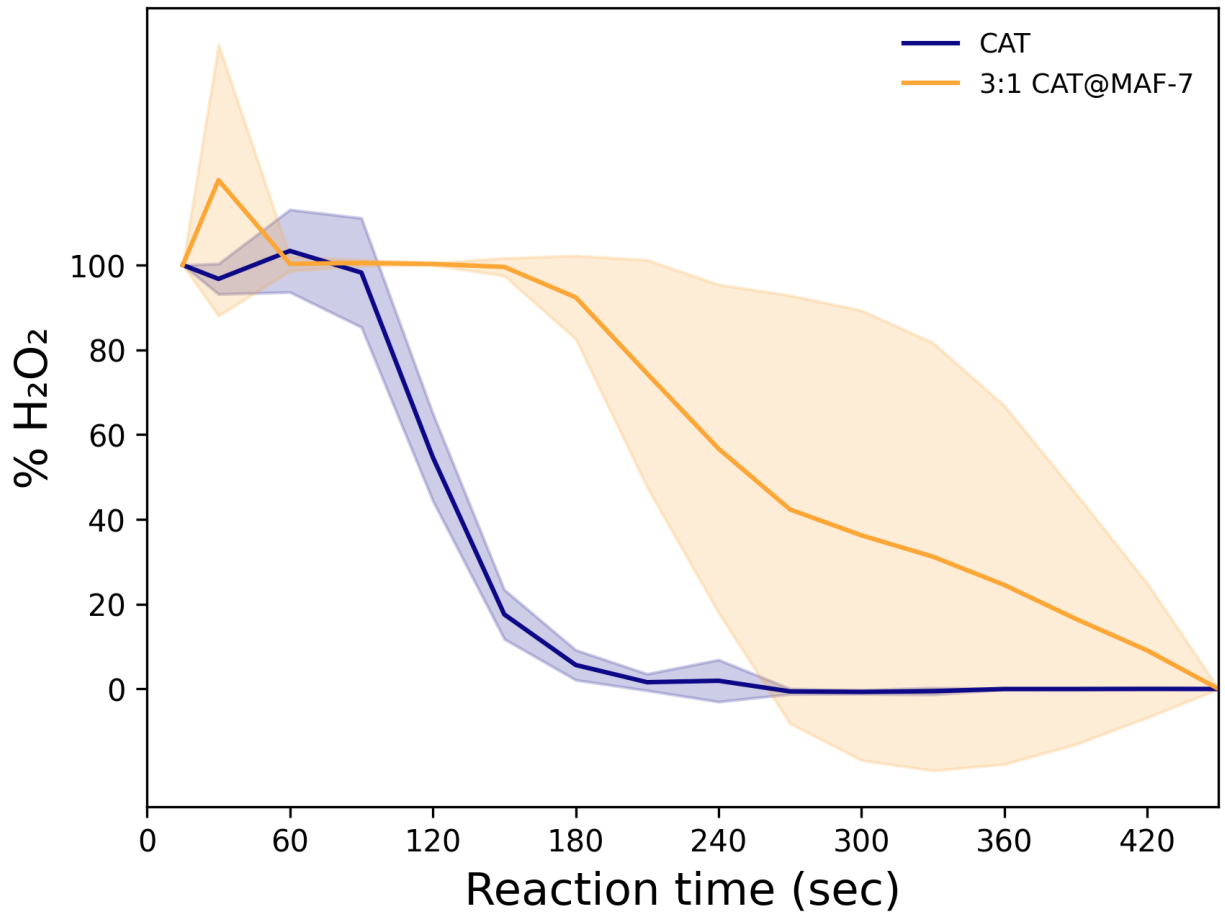
Supplemental Figure S6: CryoTEM (scale bar 200 nm) of 4:1 GOx@ZIF-8 at (a) 1 min (b) 30 min and (c.) 1 hr and dry-state TEM of 4:1 GOx@ZIF-8 at 24 hrs. The measured crystals are marked by numbered yellow lines.



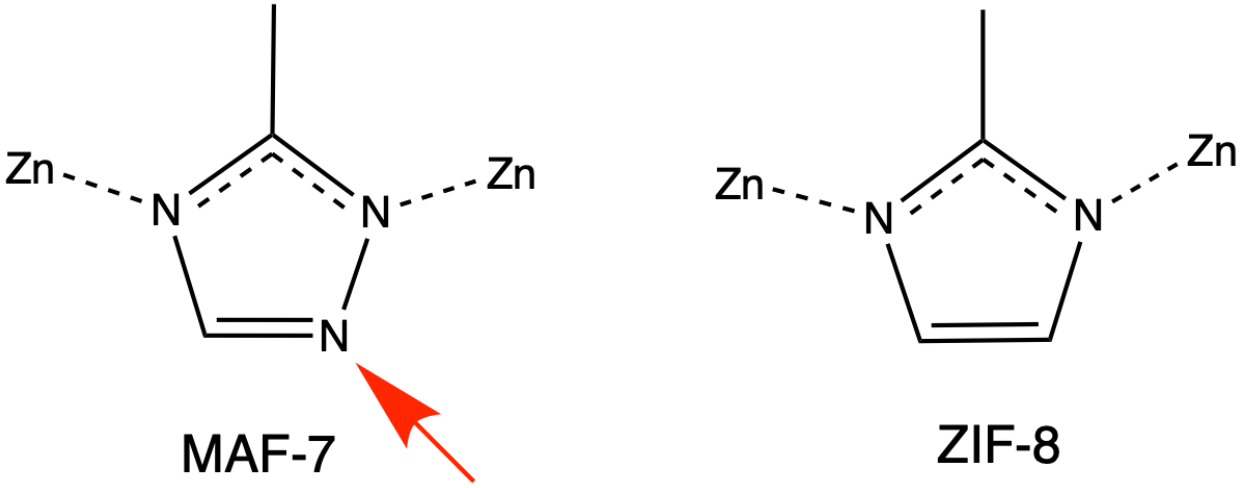
Supplemental Figure S7: Dry-state TEM images of 35:1 GOx@ZIF-8 at 1 hr. Images show classic rhombic dodecahedron crystals surrounded by dense particulate phase.



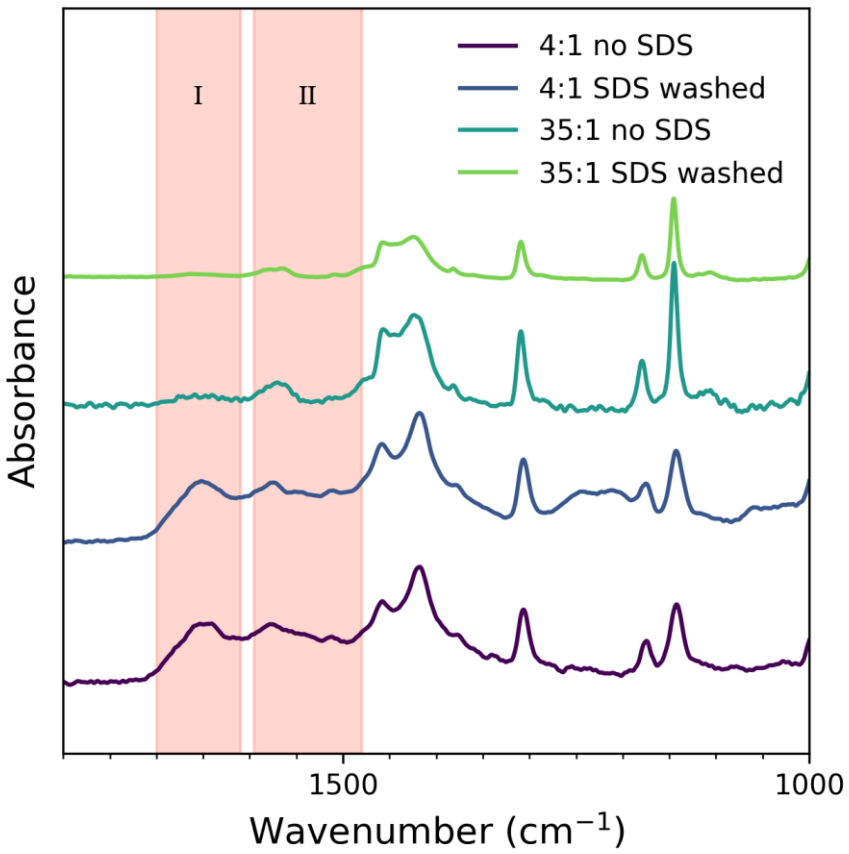
Supplemental Figure S8: Dry-state TEM images of 35:1 GOx@ZIF-8 after 24 hrs with water washes. Pink arrow indicates monocrystalline biocomposite. Yellow arrow points to disordered biocomposites that are hypothesized to be polycrystalline. Yellow box indicates a large defect in the crystal.



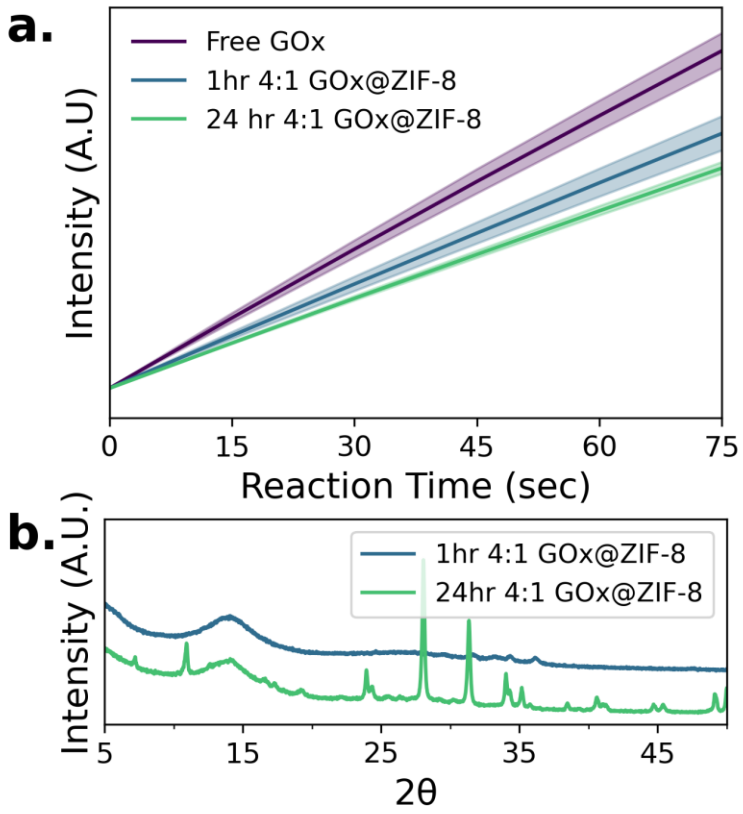
Supplemental Figure S9: Enzymatic assay of 3:1 CAT@MAF-7 composite, showing activity is present in the composite. Image was plotted without smoothing using pandas and pyplot libraries in an author-written python script.



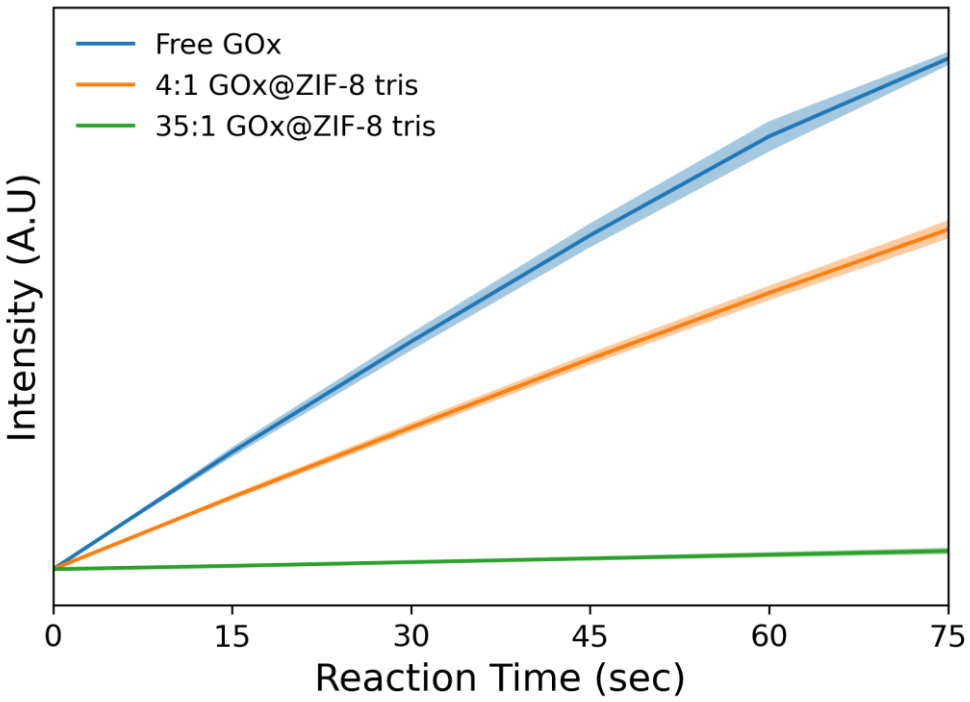
Supplemental Figure S10: Structural differences between MAF-7 and ZIF-8 linker when coordinated to zinc. The uncoordinated nitrogen on 3-methyl-1H-1,2,4 triazole is indicated with a red arrow.



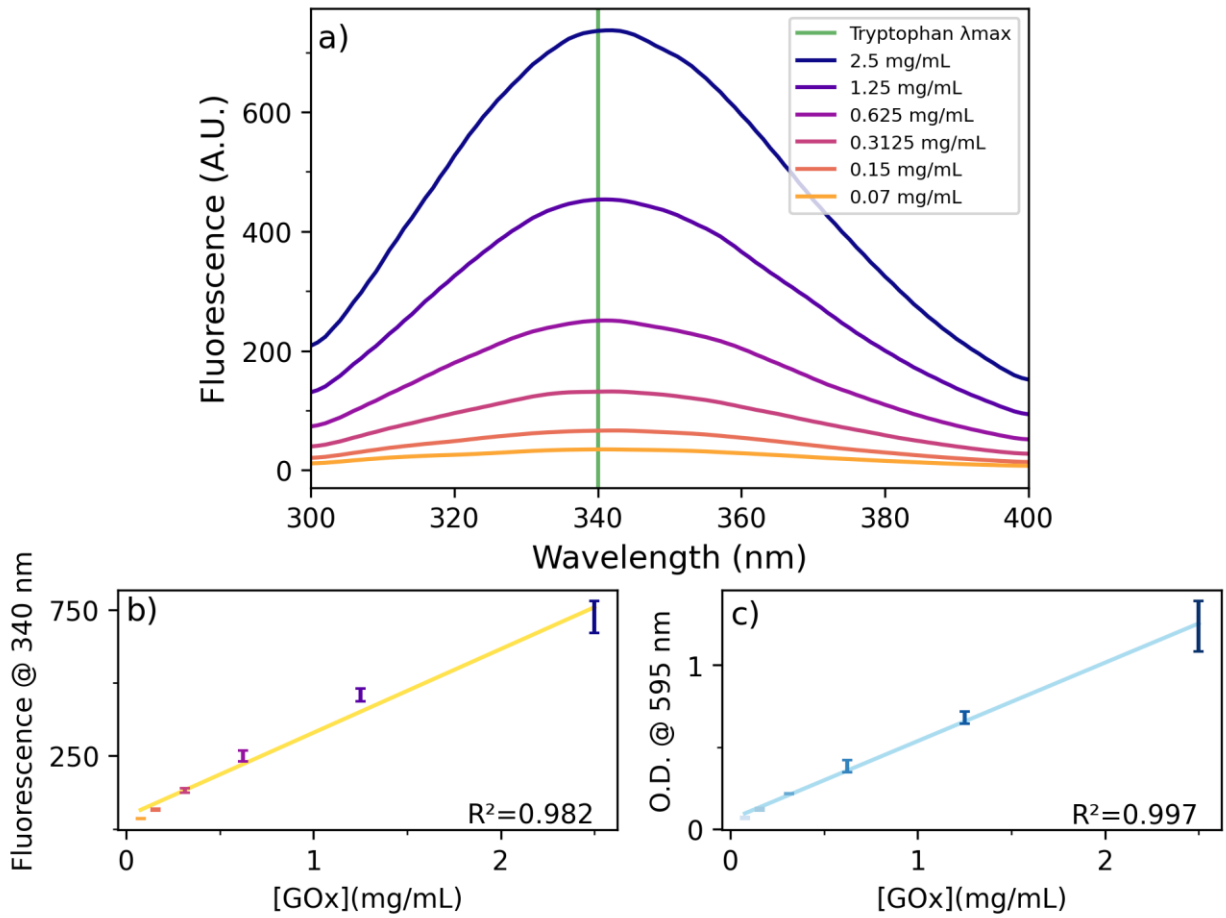
Supplemental Figure S11: FTIR spectra of GOx@ZIF-8 at HMIM:Zn of 4:1 (lime, teal) and 35:1 (blue, purple) before (purple, teal) and after (blue, lime) washing with 5% w/w Sodium dodecyl sulfate (SDS). MOFs were washed first with water 3x, then with SDS 1x, then water 1x. Amide I and II peaks are highlighted, the former indicating presence of protein.



Supplemental Figure S12: (a.) Enzymatic activity of free GOx (purple), 1 hr aged 4:1 GOx@ZIF-8 (blue), and 24 hr aged 4:1 GOx@ZIF-8 (green). (b.) Powder-XRD patterns of 1 hr aged 4:1 GOx@ZIF-8 (blue) and 24 hr aged 4:1 GOx@ZIF-8 (green)



Supplemental Figure S13: Enzymatic activity of free GOx (blue), 35:1 GOx@ZIF-8 (orange), and 4:1 GOx@ZIF-8 (green) in Tris-HCl buffer.



Supplemental Figure S14: Calibration curve validation for GOx. a) tryptophan fluorescence spectra of different concentrations of GOx, excited at 280 nm. b) Fluorescence calibration curve for GOx, compared to c) calibration curve of GOx obtained from Bradford assay. These results show that both the fluorescence and Bradford curves have high R^2 values.

Supplemental Table 1: Measured encapsulation efficiencies (EE%) and loading capacities (LC%) for GOx@ZIF-8 synthesized at both 4:1 and 35:1 L:M ratios. Loading capacity takes into account only enzymes immobilized within the interior of the MOF. To calculate loading capacity, MOFs were first synthesized following the standard protocol. After being aged for 24hrs, they were washed 2x with water and then 1x with SDS to remove surface-bound protein. The MOFs were then lysed with citric acid, and the protein concentration was measured using the standard Bradford assay. Enzyme weight percent (Enzyme wt %) was also calculated by dividing the LC% by the weight of the MOF.

Encapsulation and loading of GOx@ZIF-8

Sample	EE%	LC%	Enzyme wt%

4:1 GOx@ZIF-8	100.06	63.34	29.11
35:1 GOx@ZIF-8	23.36	0.536	0.162