Polymeric-Protein-MOF Nanoparticles with Stimuli-Responsive Disassembly and Highly Reproducible Synthesis
Electronic Supporting Information

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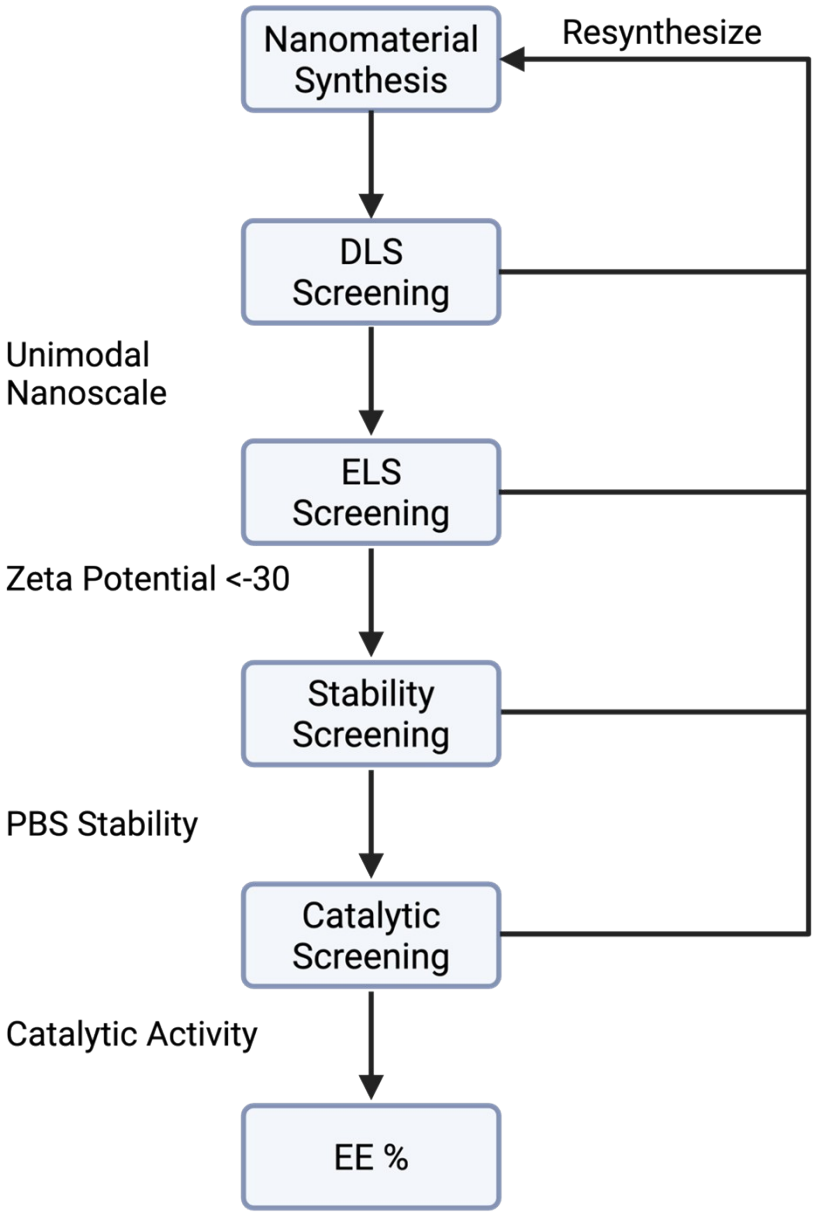
Standard operating procedure for the synthesis of doped and undoped PAA@BSA@cZIF-8 nanoparticles

Prepare stock solutions:

1. 20 mM ZnOAc solution (atleast 100 mL, dissolve 440 mg into 100 mL of water [<18.2 Ω MilliQ water])
2. Prepare a 1 M stock solution (10 mL) of $CuCl_2$ and $FeCl_3$
3. Prepare 100 mL of 20 mg/mL BSA (slowly dissolve with a small stir bar on 220 rpm if needed, this may take 1-2 hours, it is important to not rush this step.)
    a. If your final product has a PDI of >0.2, likely this step was rushed and there was BSA aggregation.
4. Pipette 9.8 mL of the 20 mg/mL BSA mixture into a scintillation vial.
5. Preassociate the BSA with Copper or Iron using the 1 M stock solution
    a. Pipette 5 µL of the 1 M Stock solution (Cu/Fe) into the scintillation vial
        i. The solution may become cloudy, simply stir slowly and this will dissipate.
    b. For Undoped, no cation is added
6. Add 1400 mM of 2-mi to the solution (1.15 g) and allow to dissolve.
7. Add requisite quantity of PAA to the solution, allow to dissolve
8. Measure pH of solution, adjust to 10.2-10.5 pH with NaOH
9. Add 10 mL of 20 mM ZnOAc solution and allow to react for 24 hours before working up the samples
10. Aliquot 1 mL of the sample and centrifuge at 14 K RPM for 20 minutes.
11. Redisperse the sample in water, wash once more.
12. Measure by DLS and ELS

[a] Check table, based on PAA concentration different NaOH stock should be used

PAA mg	NaOH Stock	Expected Drops
0	N/A	0
1	None or 1 N	0-2
2.5	1 N	1-2
5	1 N	2-4
10	1 N	4-5
25	5 N	2-3
50	5 N	3-5
100	10 N	2-3
250	10 N	3-4



Schematic 1. Screening process for nanoparticle optimization. Upon finding a catalytically active stable nanoparticle, the final structure is selected based on maximal BSA encapsulation efficiency.

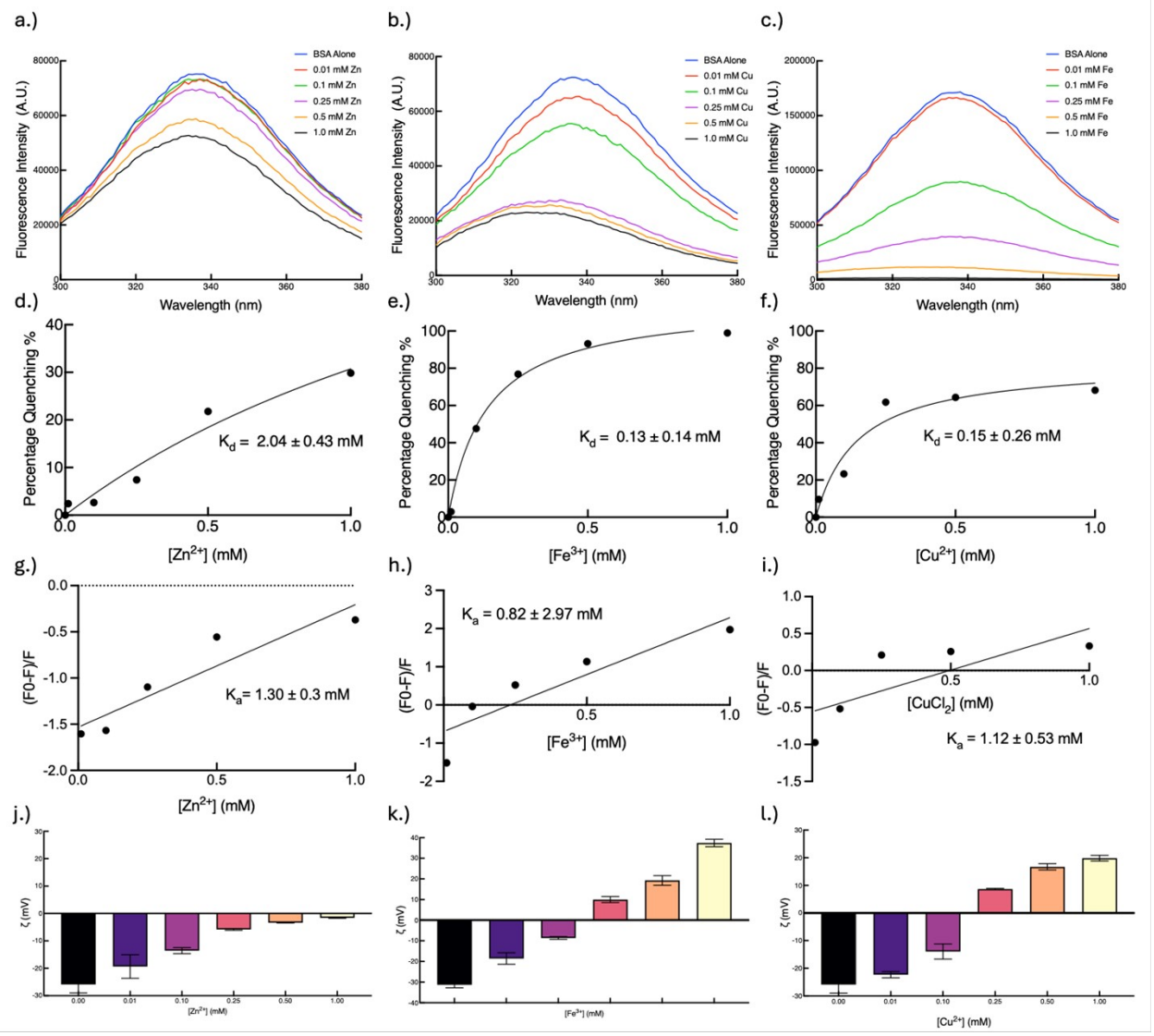


Figure S1. Data relating to fluorescence quenching and surface charge screening for the BSA-metal absorption experiments. a-c.) shows the fluorescence quenching upon successive addition of zinc, copper and iron. d-f.) shows the Langmuir adsorption isotherms for BSA when interacting with zinc, iron and copper, respectively. g-i.) shows the Stern Volmer plots for BSA interacting with zinc, iron and copper, respectively. For d.-i.) kinetic data is provided in each plot. j-l.) shows the effect of binding on surface charge of the BSA, measured by electrophoretic light scattering

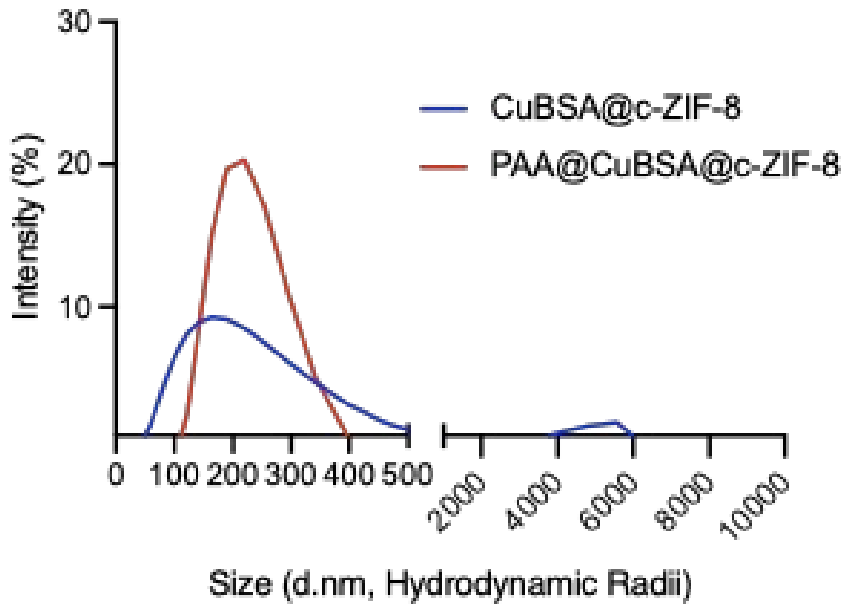


Figure S2. Stability of the copper doped PAA@BSA@c-ZIF-8 nanoparticles variant after 24 hours incubated in 1 x PBS pH 6.0. PAA@CuBSA@c-ZIF-8 PDI was 0.1 ± 0.1 after 24 hours, while CuBSA@c-ZIF-8 was 0.5 ± 0.2 after 24 hours.

Table S1. Summary of all 30 nanosystems synthesized in this work along with various important qualities which were used to screen for colloidally stable catalytically active particles. Green represents a property which is matches our pre-determined characteristics, light green is a property which matches our characteristics, but other nanoparticles have improved properties, while red shows a when a characteristic was not obtained for a nanoparticle

Construct	[PAA]	Size (nm)	Mono-modal?	Zeta Potential (mV)	PDI_{24}	Catalytic?	EE %
BSA@c-ZIF-8		110 ± 20	Yes	-30 ± 10	0.1 ± 0.1	No	
CuBSA@c-ZIF-8		90 ± 30	Yes	-10 ± 10	0.5 ± 0.2		
FeBSA@c-ZIF-8		120 ± 20	Yes	-0 ± 10	0.3 ± 0.2		
$PAA_{25K-45k}$@CuBSA@c-ZIF-8	50 mg	130 ± 20 & 1400 ± 400	No				
$PAA_{2k-unadj}$@CuBSA@c-ZIF-8	50 mg	120 ± 20	Yes	-10 ± 10			
$PAA_{2k-unadj}$@FeBSA@c-ZIF-8	50 mg	150 ± 60	Yes	-10 ± 10			
PAA@BSA@c-ZIF-8*	1 mg	160 ± 10	Yes	-30 ± 10		No	
PAA@BSA@c-ZIF-8	2.5 mg	170 ± 10	Yes	-40 ± 10		No	
PAA@BSA@c-ZIF-8	10 mg	210 ± 10	Yes	-50 ± 10	0.2 ± 0.1	No	
PAA@BSA@c-ZIF-8	25 mg	200 ± 20	Yes	-50 ± 10	0.1 ±	No	60 ± 20

					0.1		
PAA@BSA@c-ZIF-8	50 mg	200 ± 20	Yes	-50 ± 10	0.1 ± 0.1	No	80 ± 10
PAA@BSA@c-ZIF-8	100 mg	190 ± 20	Yes	-40 ± 10	0.1 ± 0.1	No	70 ± 10
PAA@BSA@c-ZIF-8	250 mg	190 ± 20	Yes	-30 ± 10	0.1 ± 0.1	No	60 ± 10
PAA@CuBSA@c-ZIF-8*	1 mg	120 ± 10	Yes	-10 ± 10		No	
PAA@CuBSA@c-ZIF-8	2.5 mg	140 ± 20	Yes	-20 ± 10		No	
PAA@CuBSA@c-ZIF-8	10 mg	210 ± 10	Yes	-40 ± 10	0.1 ± 0.1	Yes	50 ± 10
PAA@CuBSA@c-ZIF-8	25 mg	220 ± 20	Yes	-40 ± 10	0.1 ± 0.1	Yes	70 ± 10
PAA@CuBSA@c-ZIF-8	50 mg	180 ± 20	Yes	-50 ± 10	0.1 ± 0.1	Yes	90 ± 10
PAA@CuBSA@c-ZIF-8	100 mg	190 ± 10	Yes	-40 ± 10	0.1 ± 0.1	Yes	80 ± 10
PAA@CuBSA@c-ZIF-8	250 mg	160 ± 10	Yes	-30 ± 10	0.1 ± 0.1	Yes	60 ± 10
PAA@FeBSA@c-ZIF-8	1 mg	130 ± 10	Yes	-10 ± 10		No	
PAA@FeBSA@c-ZIF-8	2.5 mg	150 ± 20	Yes	-20 ± 10		Yes	
PAA@FeBSA@c-ZIF-8	10 mg	230 ± 20	Yes	-40 ± 10	0.1 ± 0.1	Yes	50 ± 20
PAA@FeBSA@c-ZIF-8	25 mg	240 ± 10	Yes	-40 ± 10	0.1 ± 0.1	Yes	60 ± 20
PAA@FeBSA@c-ZIF-8	50 mg	210 ± 20	Yes	-50 ± 10	0.1 ± 0.1	Yes	80 ± 10
PAA@FeBSA@c-ZIF-8	100 mg	180 ± 10	Yes	-40 ± 10	0.1 ± 0.1	Yes	50 ± 20
PAA@FeBSA@c-ZIF-8	250 mg	180 ± 30	Yes	-30 ± 10	0.1 ± 0.1	Yes	60 ± 10

*PAA@BSA@c-ZIF-8, PAA@CuBSA@c-ZIF-8, PAA@FeBSA@c-ZIF-8 are synthesized with 2 k M_n PAA and pH adjusted, but these details are omitted from the names for clarity.

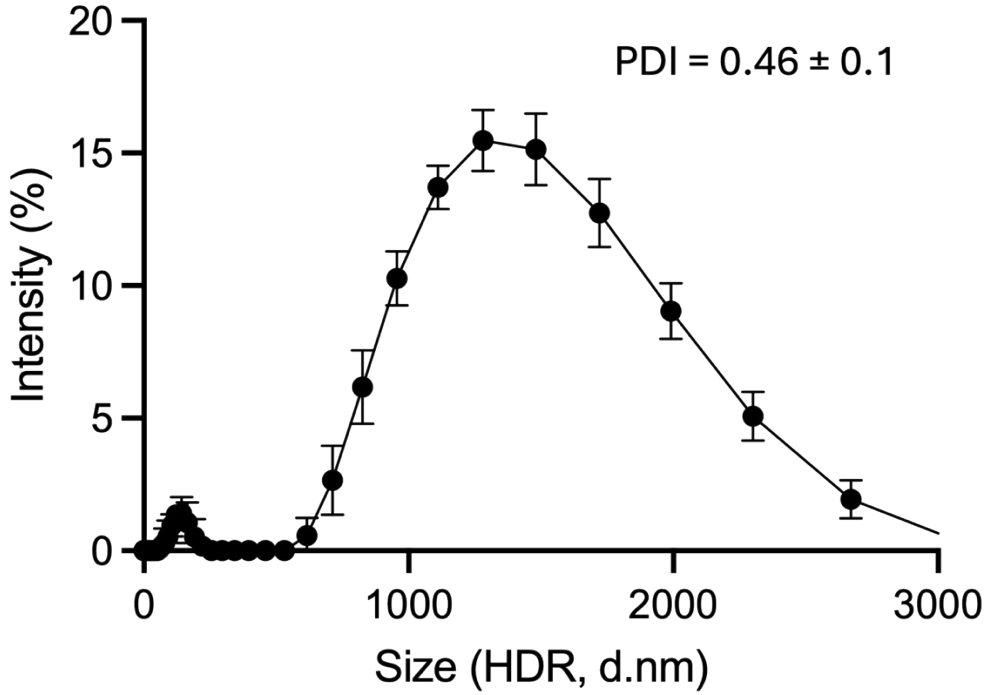


Figure S3 – Continuing distribution analysis plot for PAA@BSA@c-ZIF-8 synthesis when using 25k – 45k M_n PAA polymer.

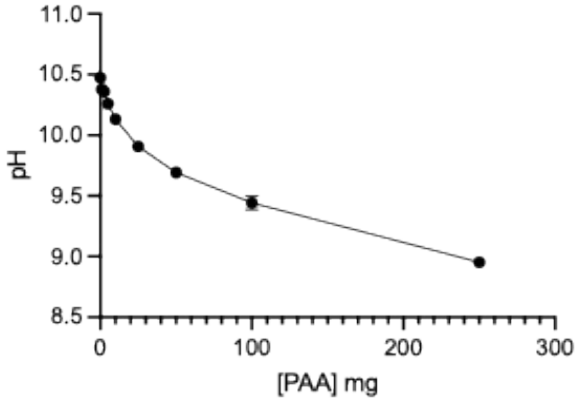


Figure S4. pH compared to ~2000 M_n [PAA] addition to the 2-mi/BSA mother solution, prior to ZnOAc addition.

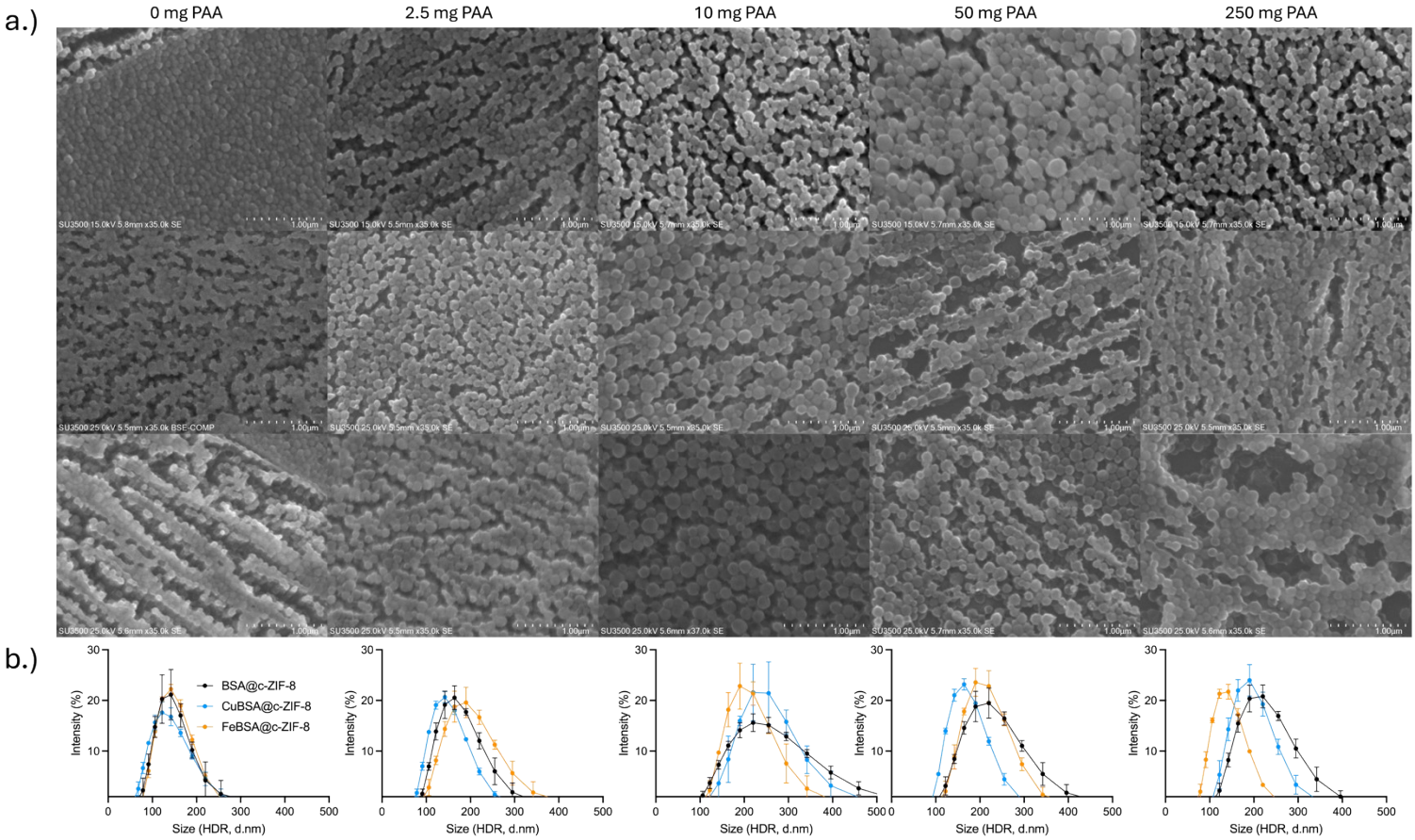


Figure S5. a.) scanning electron micrographs of PAA laden nanocomposites. The quantities of PAA are provided at the top of the SEMs. The top row is PAA@BSA@c-ZIF-8, the middle row is PAA@CuBSA@c-ZIF-8 and the bottom row is PAA@FeBSA@c-ZIF-8. b.) shows the continuing distribution analysis plots from DLS showing the size distributions of the resultant nanoparticles.

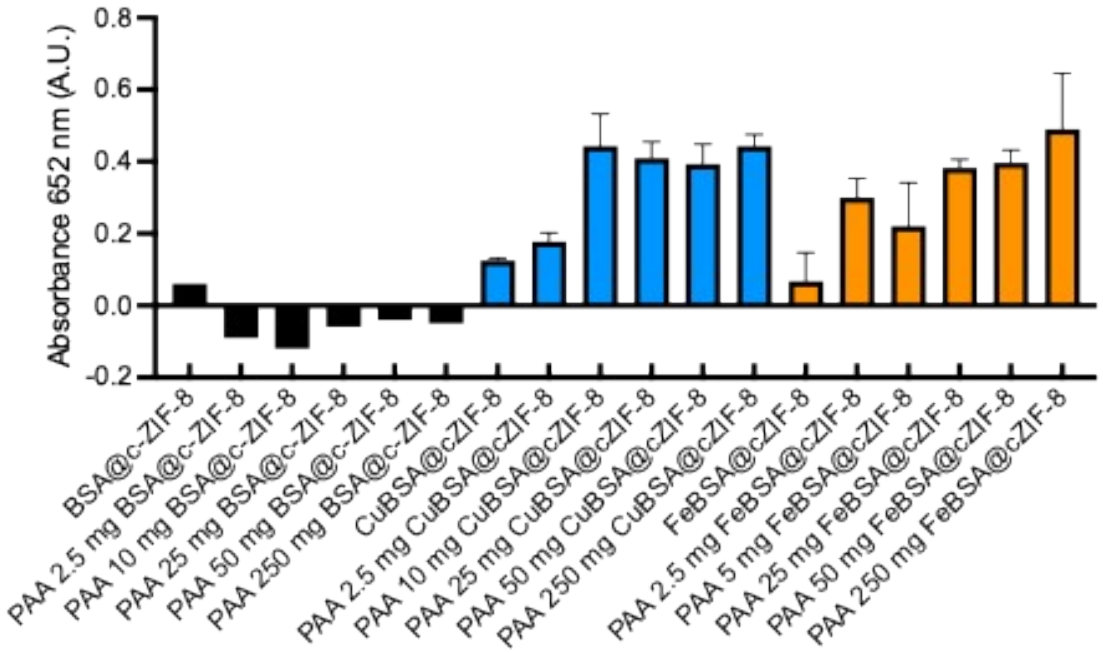


Figure S6. Catalytic activity of nanoparticles from this work toward peroxide, measured by the TMB assay.

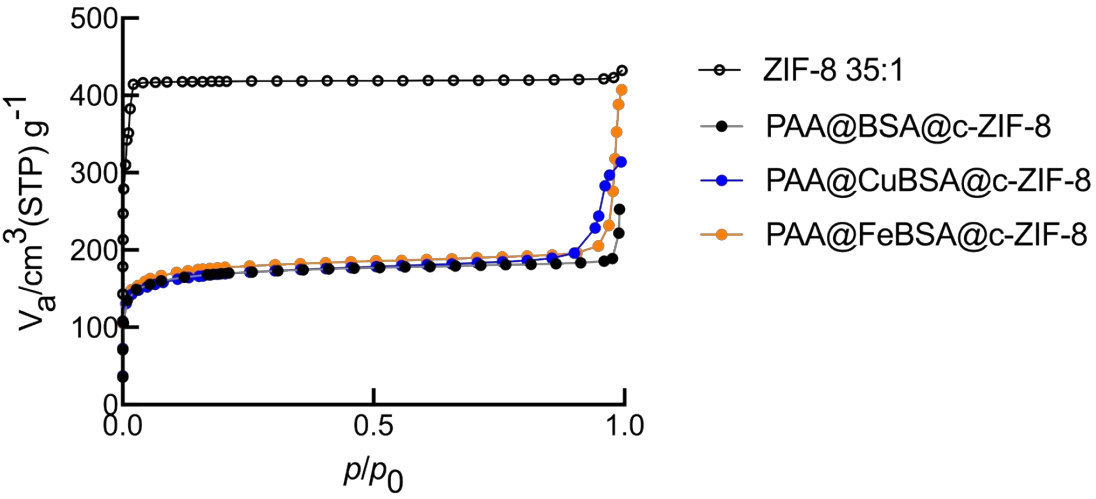


Figure S7. N_2 adsorption isotherms used to calculate surface area for ZIF-8, PAA@BSA@c-ZIF-8, PAA@CuBSA@c-ZIF-8 and PAA@FeBSA@c-ZIF-8.

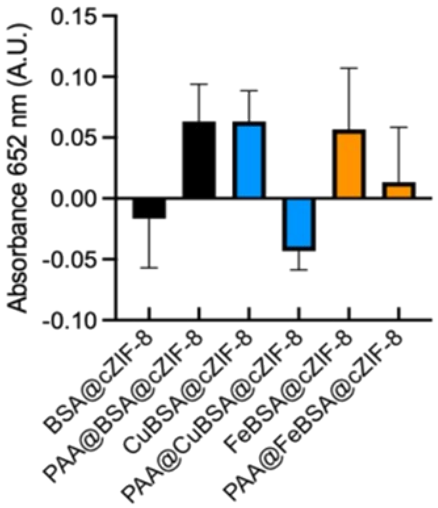


Figure S8. TMB response of supernatant after nanoparticle incubation were incubated for 20 minutes in 1 x PBS pH 6.0 .

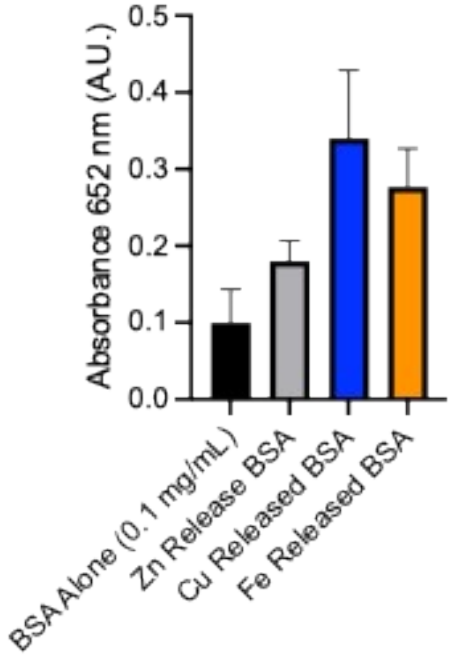


Figure S9 – Activity of BSA after isolation with molecular 30 kDa weight cut off filters toward the TMB assay.

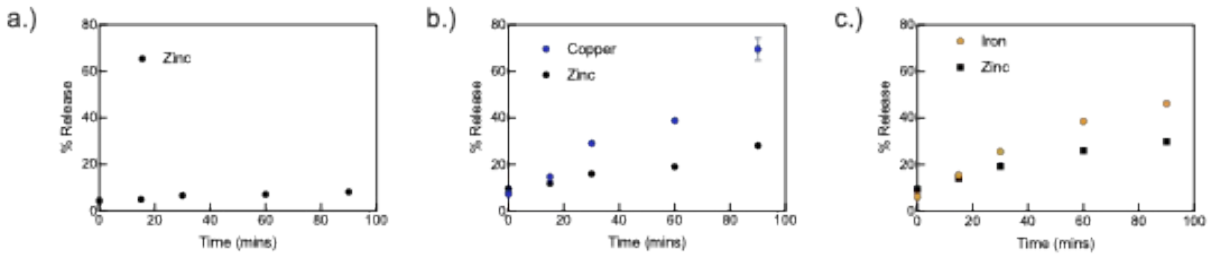


Figure S10. Release profiles of Zn from PAA@BSA@c-ZIF-8 (a.), Cu/Zn from PAA@CuBSA@c-ZIF-8 (b.), and Fe/Zn release from PAA@FeBSA@c-ZIF-8 in DMEM 10 % FBS with 40 µM H_2O_2.

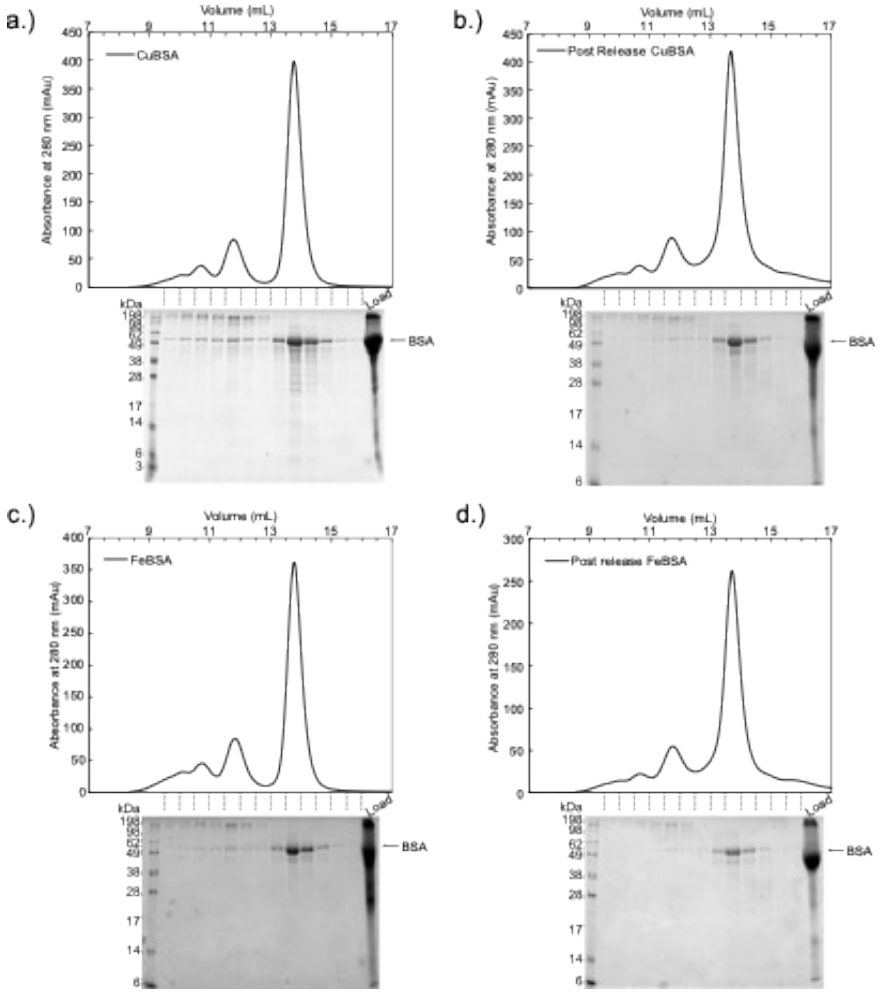


Figure S11. Size exclusion column trace and SDS-PAGE gel of the elution fractions for a.) CuBSA, b.) post release CuBSA, c.) FeBSA, and d.) post release FeBSA. 100 µL of 10 mg/mL samples were applied to S200 column at a flow rate of 0.5 mL/min. Elution was monitored by absorbance at 280 nm and analyzed using SDS-PAGE.

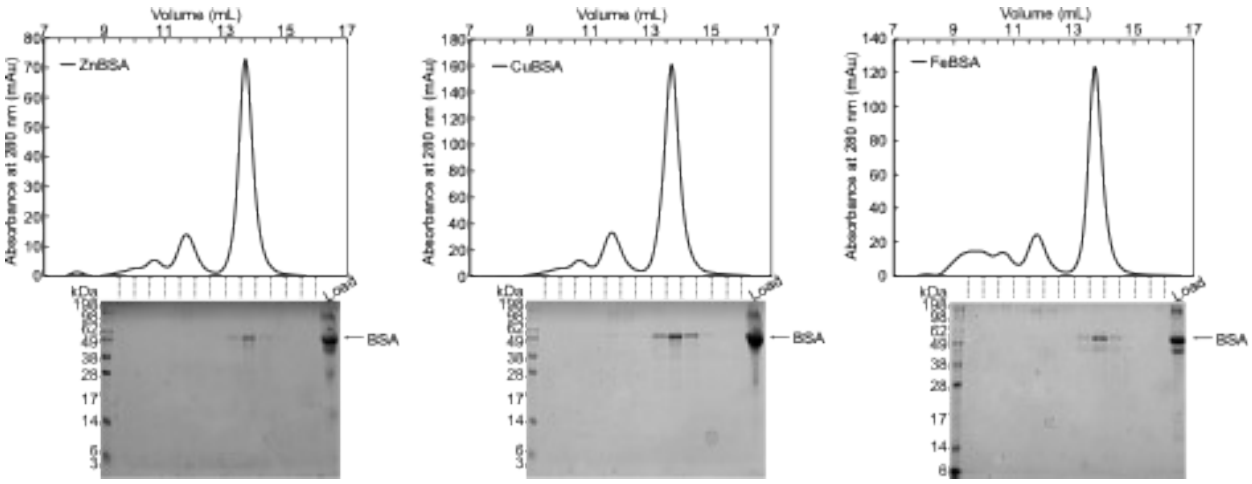


Figure S12. Size exclusion column trace and SDS-PAGE gel of the elution fractions for a.) ZnBSA, b.) CuBSA, and c.) FeBSA. 100 µL of 0.2 mg/mL samples were applied to S200 column at a flow rate of 0.5 mL/min. Elution was monitored by absorbance at 280 nm and analyzed using SDS-PAGE.

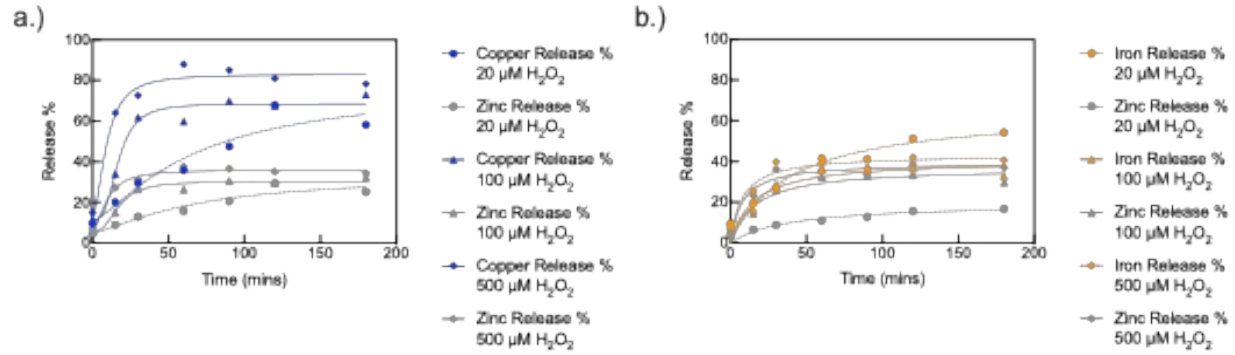


Figure S13. Release rates of (a.) copper and zinc from PAA@CuBSA@c-ZIF-8 and release rates of (b.) iron and zinc from PAA@FeBSA@c-ZIF-8 with different H_2O_2 concentrations, 20 µM, 100 µM and 500 µM.

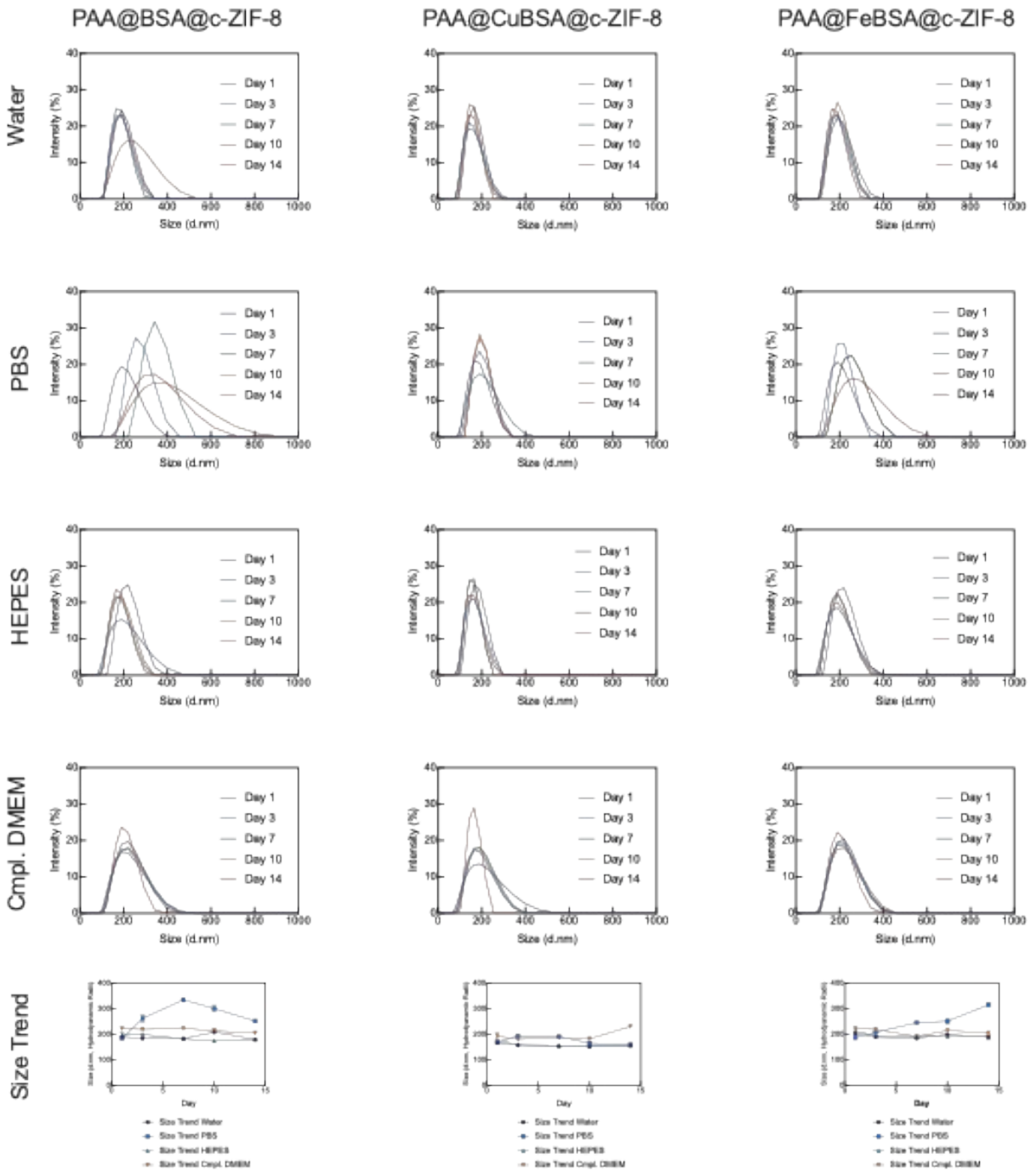


Figure S14. Stability of PAA@BSA@c-ZIF-8 (Left), PAA@CuBSA@c-ZIF-8 (Middle), PAA@FeBSA@c-ZIF-8 in various buffers. Buffers are labelled on the extreme left. The overall size trend is reported in the 5th row for each nanoparticle. For each sample, an entire batch of nanoparticles is used and measured in triplicate.

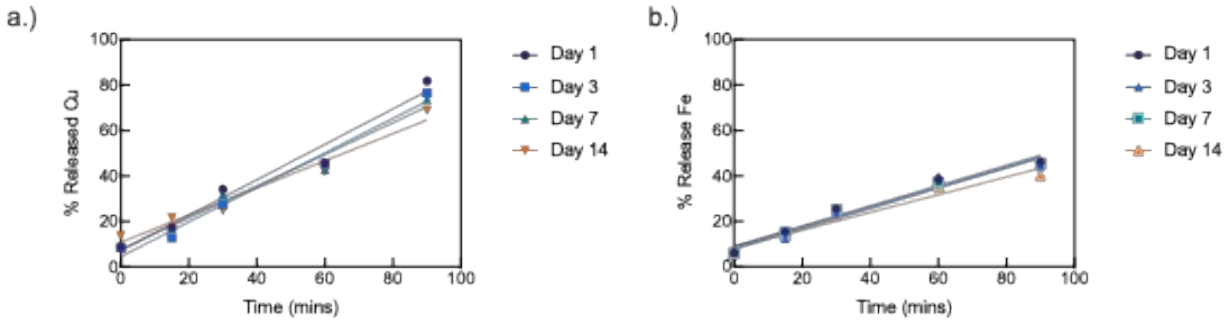


Figure S15. Release rate of (a.) copper from PAA@CuBSA@c-ZIF-8 for the first 90 minutes and release rate of (b.) iron from PAA@FeBSA@c-ZIF-8

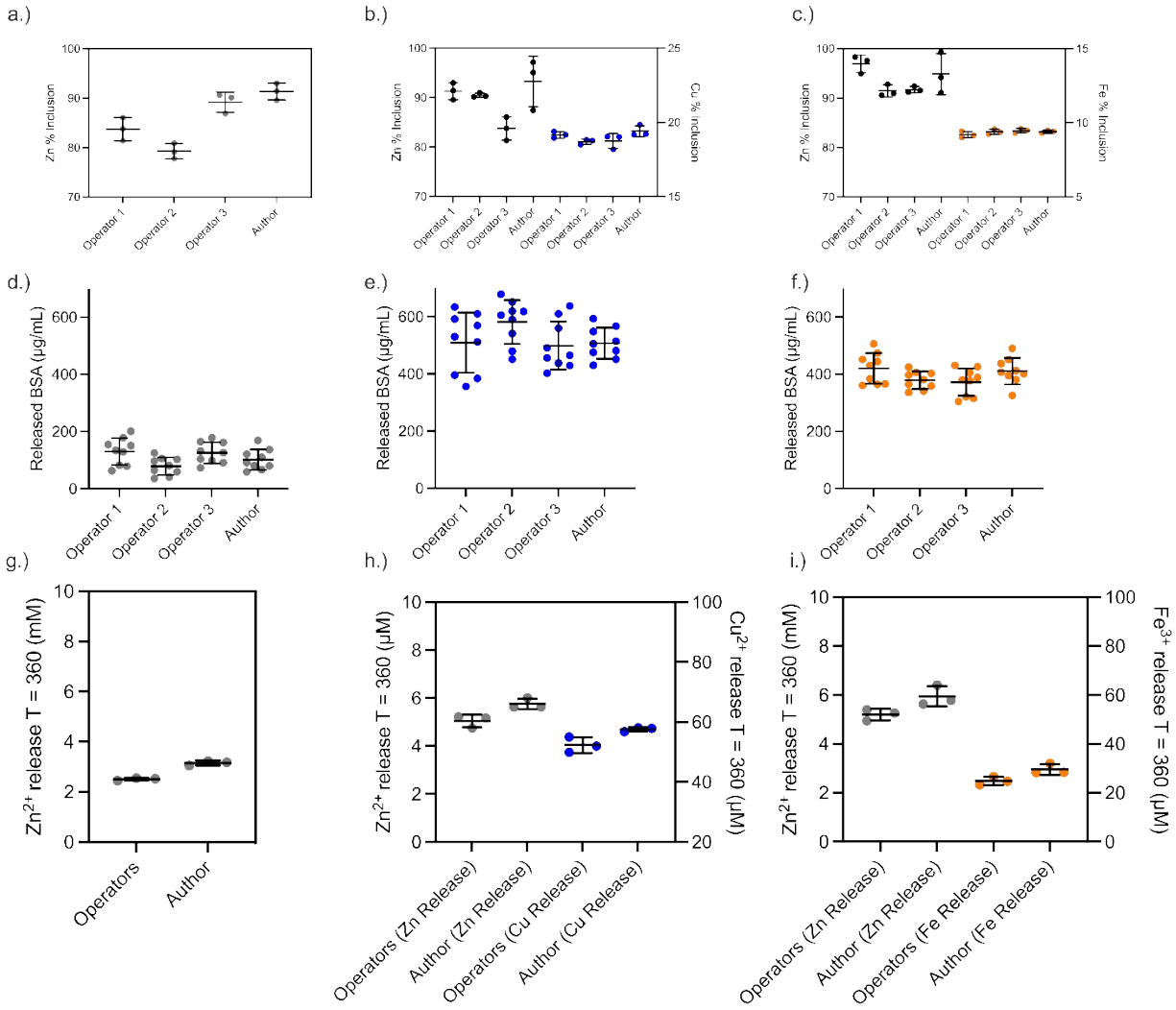


Figure S16. a-c.) shows the incorporation %s for each ion in each nanoparticle system as determined by ICP. For each operator one batch is tested thrice. d.-e.) Show the BSA release from the nanoparticles at t = 360 minutes incubation in 40 µM H_2O_2 and 1 x pH 6.0 PBS buffer. g-h.) show the ion release from one batch from each operator at t = 360 minutes.

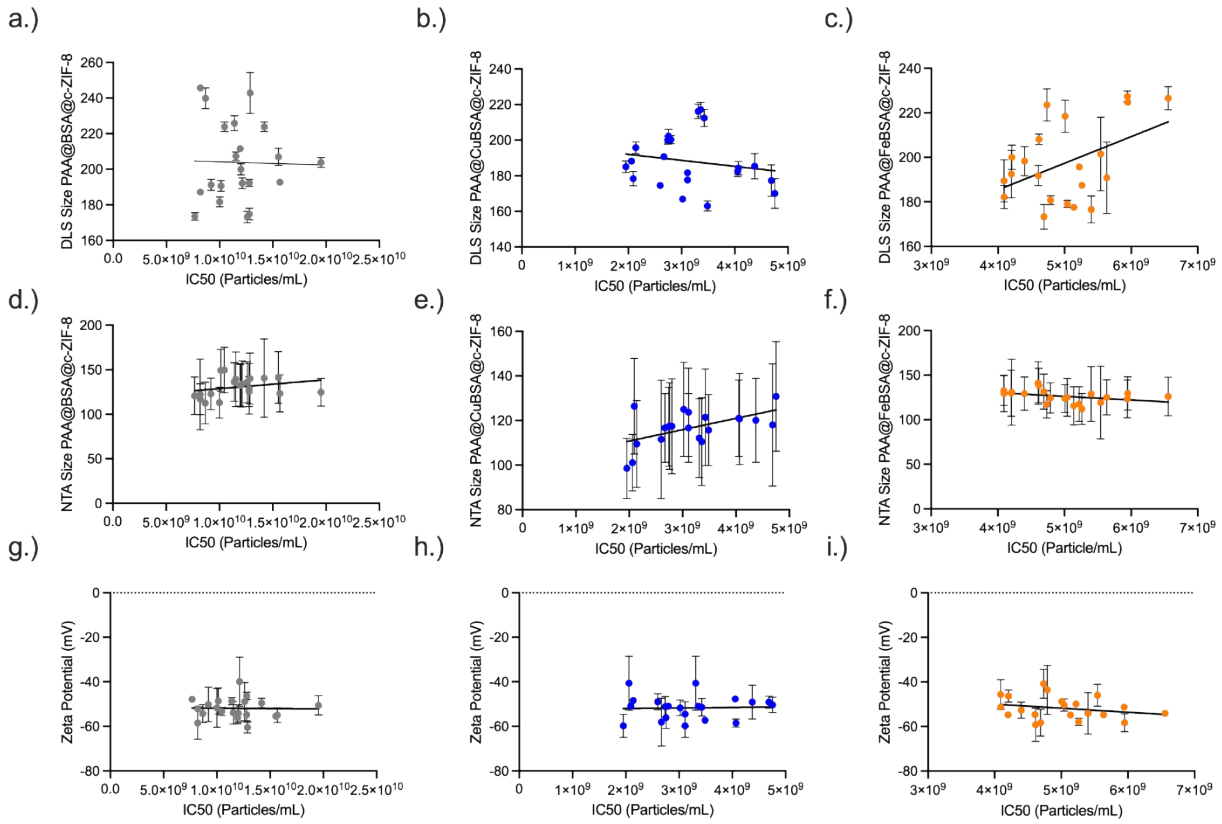


Figure S17. The correlation between Size/Zeta Potential and IC50 of each nanoparticle investigated in this work. Each dot and associated error represent the average and standard deviation of three measurements, respectively, n = 21. a.) shows the correlation for DLS PAA@BSA@c-ZIF-8 size and IC50, b.) shows the correlation for DLS PAA@CuBSA@c-ZIF-8 size and IC50 c.) shows the correlation for DLS PAA@FeBSA@c-ZIF-8 size and IC50. d.) shows the correlation for NTA PAA@BSA@c-ZIF-8 size and IC50, e.) shows the correlation for NTA PAA@CuBSA@c-ZIF-8 size and IC50 f.) shows the correlation for NTA PAA@FeBSA@c-ZIF-8 size and IC50 g.) shows the correlation for PAA@BSA@c-ZIF-8 zeta potential and IC50, h.) shows the correlation for PAA@CuBSA@c-ZIF-8 zeta potential and IC50, and i.) shows the correlation for PAA@FeBSA@c-ZIF-8 zeta potential and IC50.

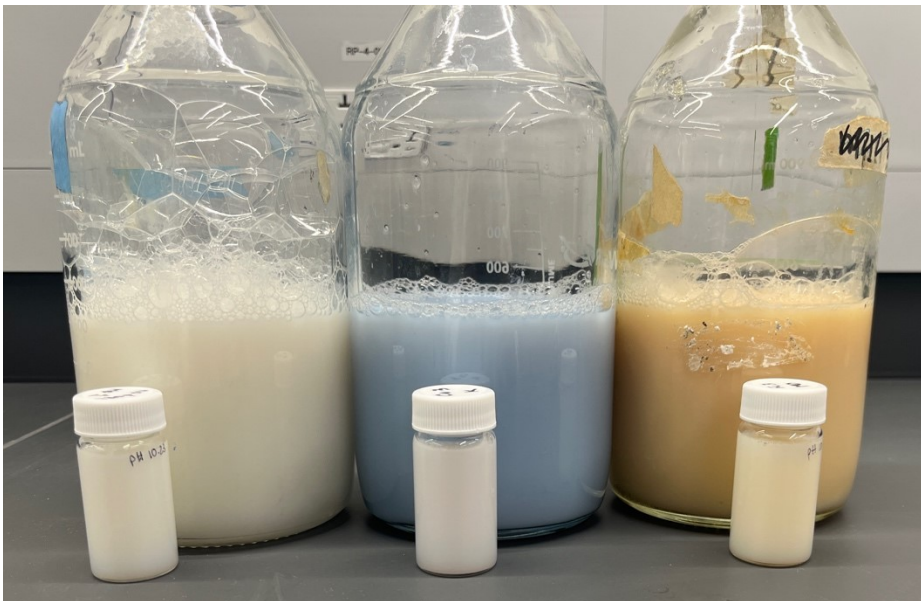


Figure S18: Picture of scaled material from 20 mL final volume to 500 mL final volume

Table S2. Data relating to the scaled synthesis of PAA@BSA@c-ZIF-8, PAA@CuBSA@c-ZIF-8, PAA@FeBSA@c-ZIF-8 compared to the "normal" synthesis. Values are from one batch measured thrice.						
	Scaled PAA@BSA@c-ZIF-8	Normal Batch PAA@BSA@c-ZIF-8	Scaled Batch PAA@CuBSA@c-ZIF-8	Normal PAA@CuBSA@c-ZIF-8	Scaled Batch PAA@FeBSA@c-ZIF-8	Normal PAA@FeBSA@c-ZIF-8
Size (nm)	200 ± 10	200 ± 20	170 ± 10	180 ± 20	200 ± 10	210 ± 20
PDI	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
ζp (mV)	-50 ± 2	-50 ± 10	-60 ± 1	-50 ± 10	-60 ± 5	-50 ± 10