Electronic Supplementary Material (ESI) for Materials Advances.
This journal is © The Royal Society of Chemistry 2024

Improving Colloidal Stability of Protein@ZIF-8 Nanoparticles in Biologically Relevant Buffers

Justin Van Houten[a†], Ruben Castillo Barberi[a†], Jared King[a], Alana Ogata[a*]

[†]Denotes equal contribution

[a]Department of Chemistry, University of Toronto, UTM 1867 Inner Circle Road, Mississauga, ON L5L 1C6, Canada

Corresponding Author: alana.ogata@utoronto.ca

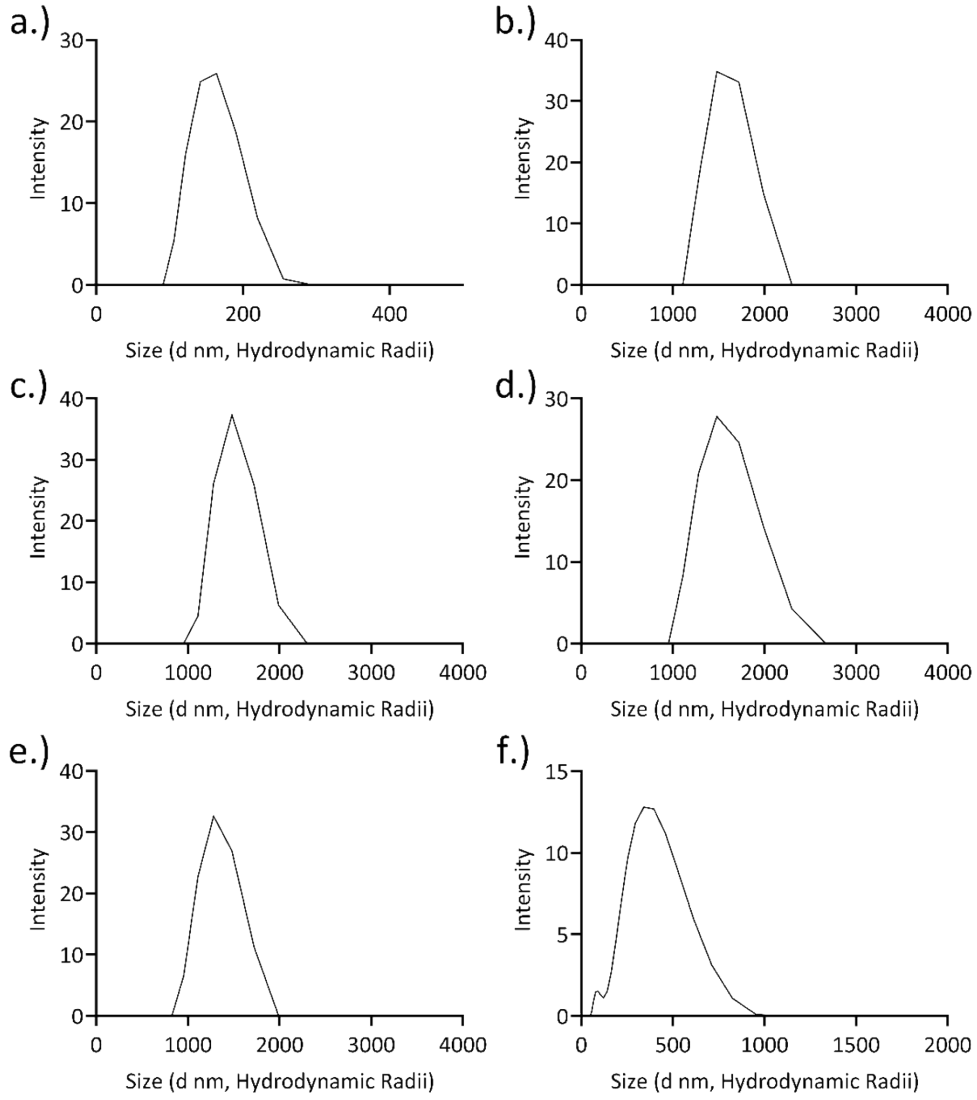


Figure S1: Contin plots of ZIF-8 following 5-hour incubation in a.) Water, b.) PBS (pH 6.1), c.) PBS (pH 6.6), d.) PBS (pH 7.1), e.) PBS (pH 7.6), f.) PBS (pH 8.1).

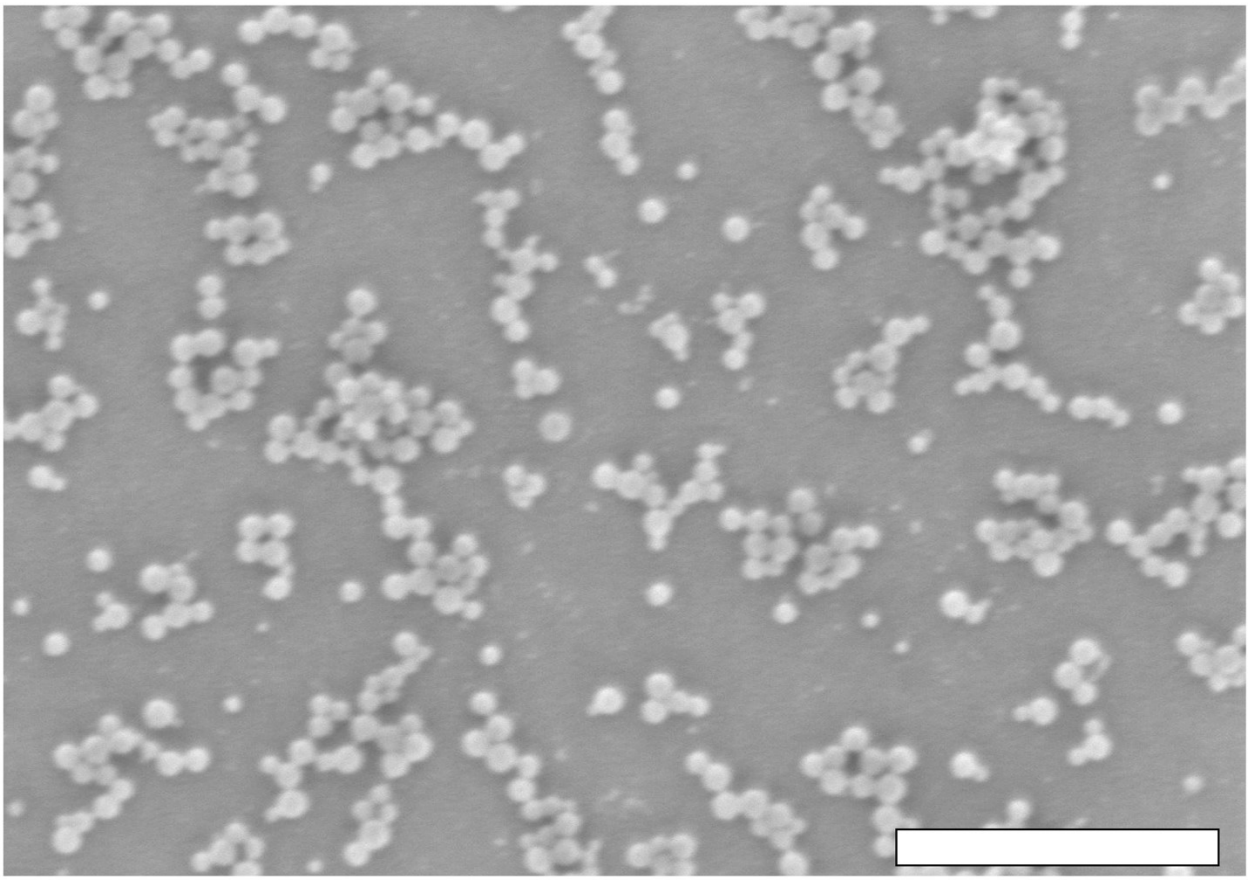


Figure S2: Scanning Electron Micrographs of 1:100 ZIF8 after 6h incubation in water. Scale bar set to 1μm.

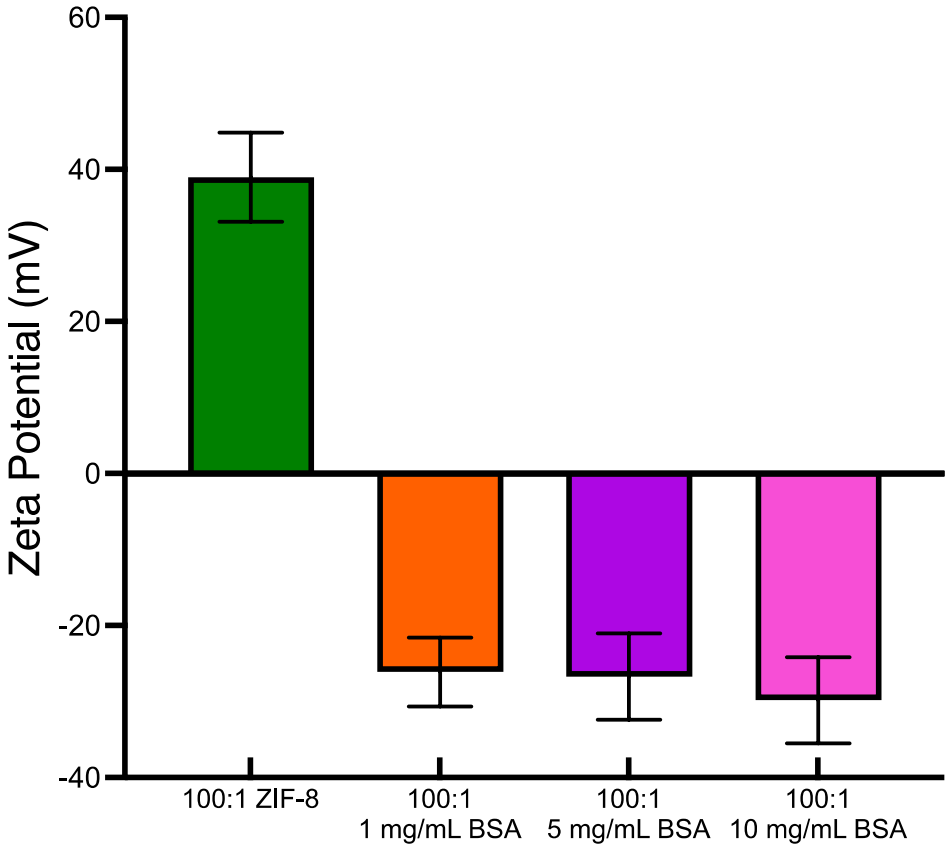


Figure S3: Zeta potential of ZIF-8 with varying concentrations of BSA (1 mg/mL, 5mg/mL, and 10 mg/mL). Each bar and error are the average and standard deviation of three batches measured in triplicate. (n = 9).

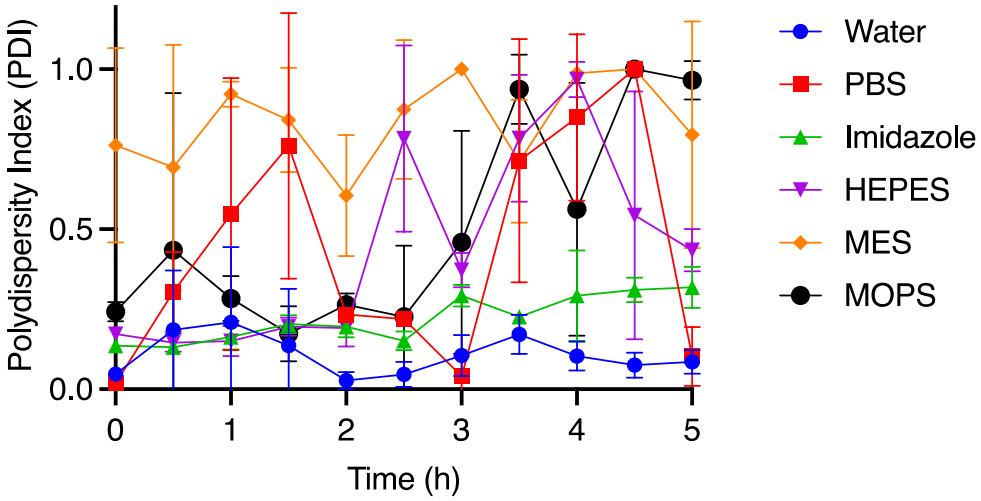


Figure S4: Polydispersity Index (PDI) over 5-hour temporal study of ZIF-8 in Water, PBS (pH 7.1), Imidazole (pH 7.5), HEPES (pH 7.2), MES (pH 6.0), and MOPS (pH 7.4) Each point, and error bar is the average and standard deviation of each point (n = 3).

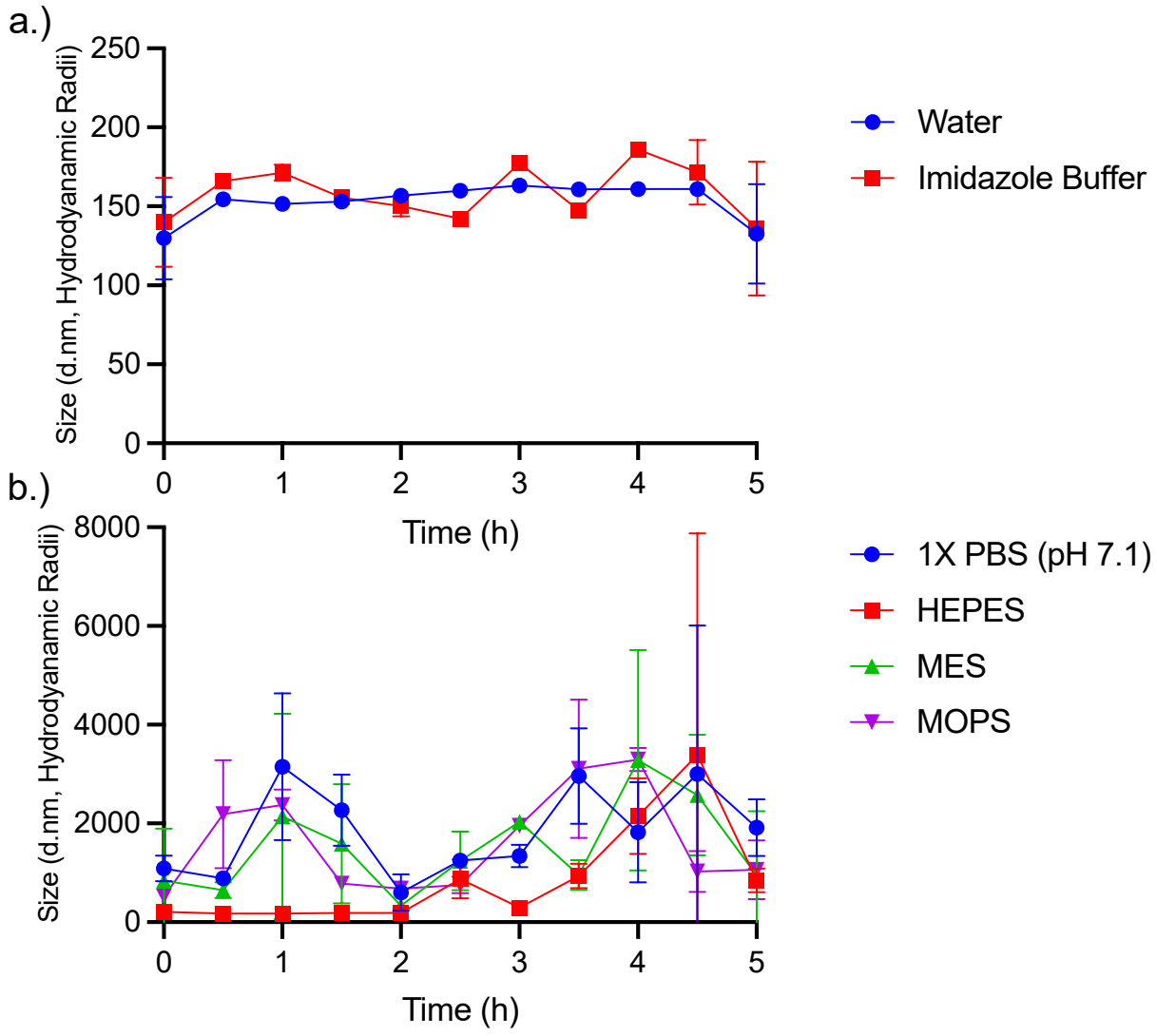


Figure S5: Hydrodynamic radius (nm) ZIF-8 across 5-hour temporal study in a.) water and Imidazole (pH 7.5), b.) PBS (pH 7.1), HEPES (pH 7.4), MES (pH 6.0), and MOPS (pH 7.2). Each point represents the average and standard deviation across three batches measured thrice (n = 9).

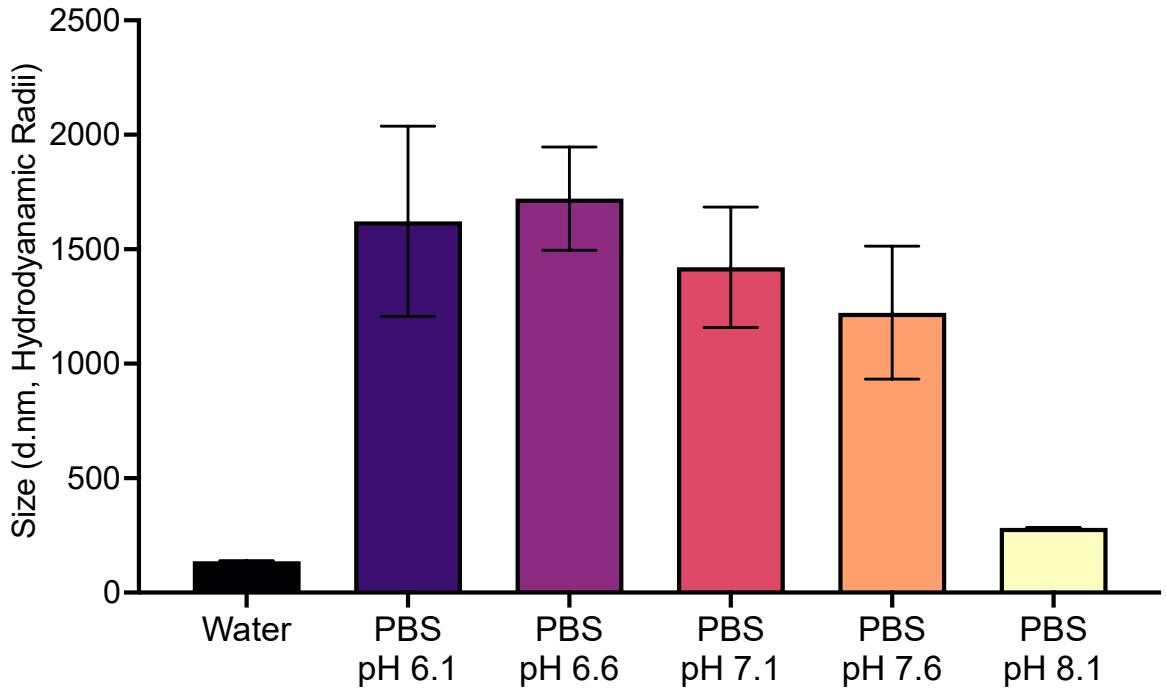


Figure S6: Hydrodynamic radius (nm) of ZIF-8 following 5-hour incubation in a.) Water, b.) PBS (pH 6.1), c.) PBS (pH 6.6), d.) PBS (pH 7.1), e.) PBS (pH 7.6), f.) PBS (pH 8.1). Each point represents the average and standard deviation across three batches measured thrice (n = 9)

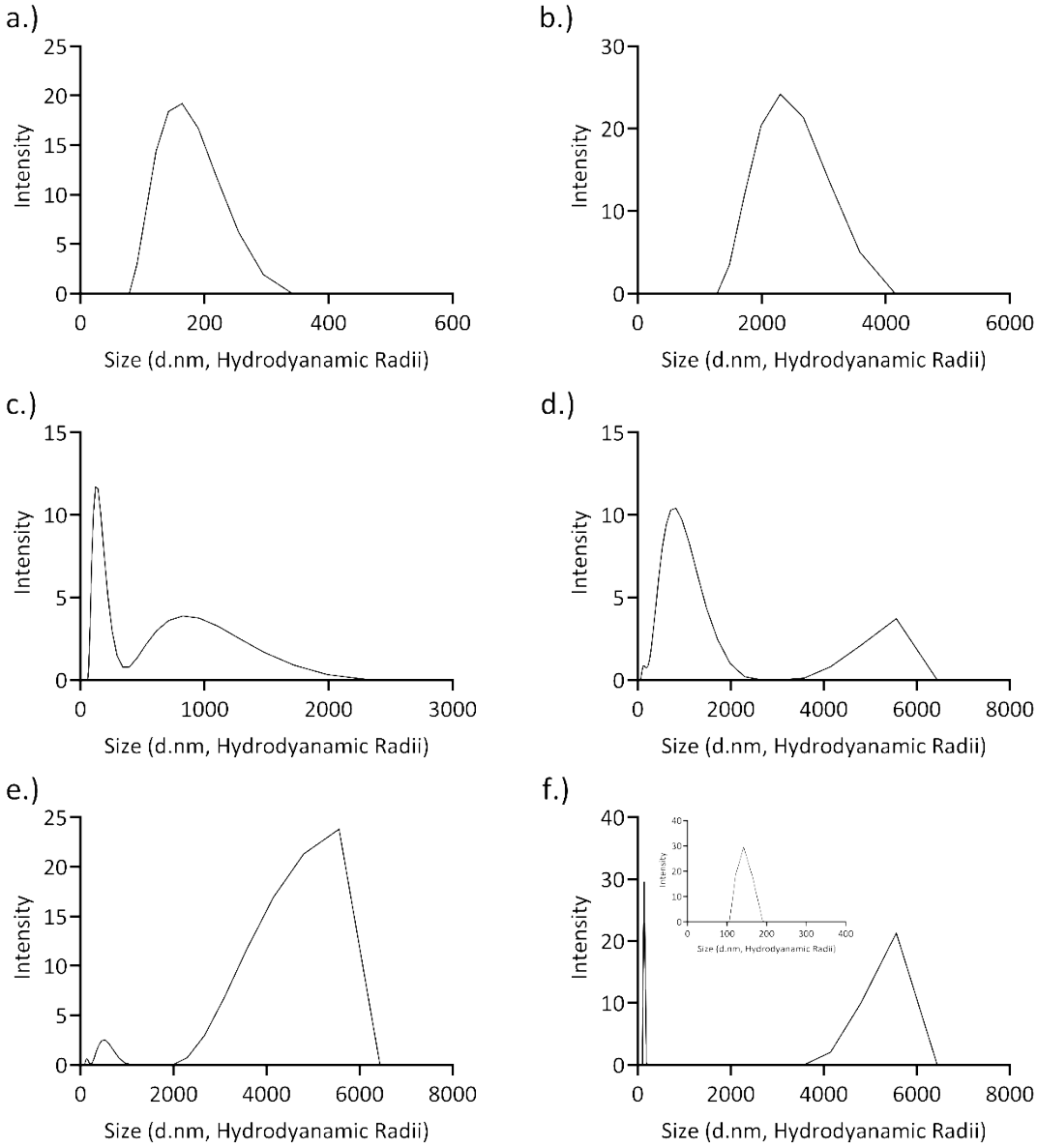


Figure S7: CONTIN plots of ZIF-8 at after 5h incubation in. a.) Water, b.) PBS (pH 7.1), c.) Imidazole (pH 7.5), d.) HEPES (pH 7.4), e.) MES (pH 6.0), f.) MOPS (pH 7.2).

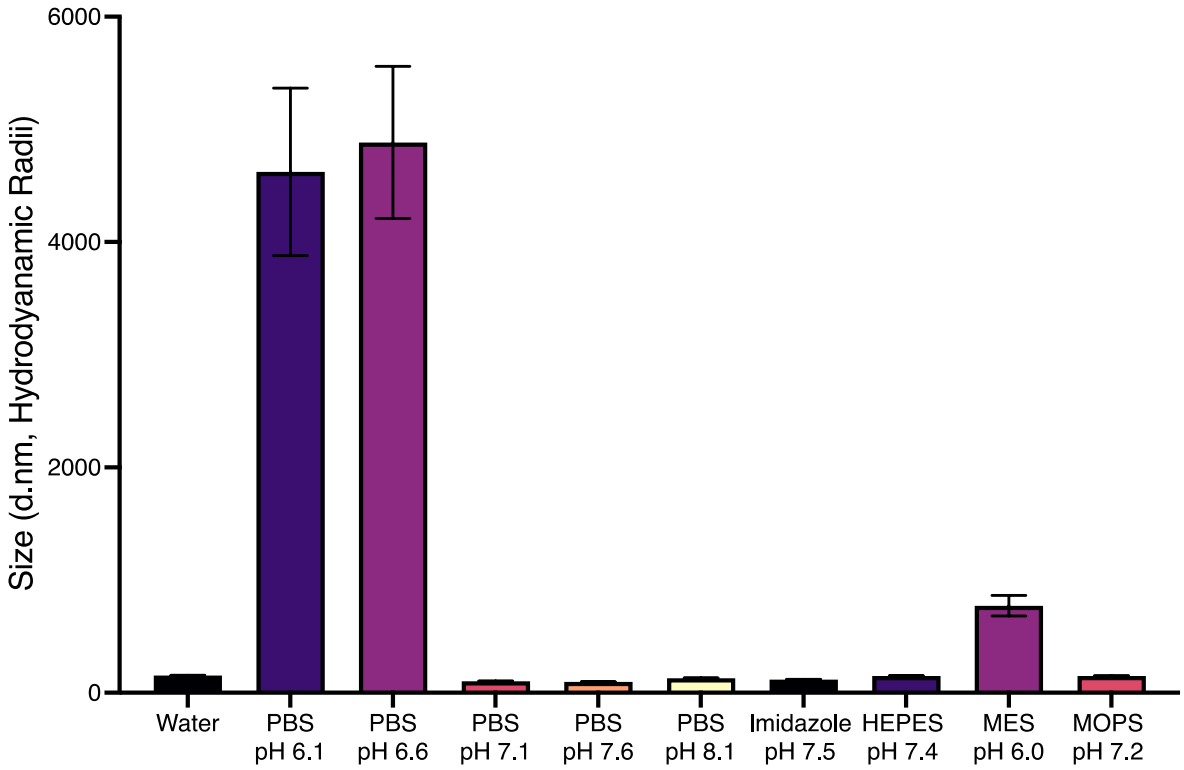


Figure S8: Hydrodynamic radii (d.nm) of ZIF8 spiked with 10 mg/mL BSA following 1 day incubation in different buffer conditions. Each point represents the average and standard deviation across three batches measured thrice (n = 9)

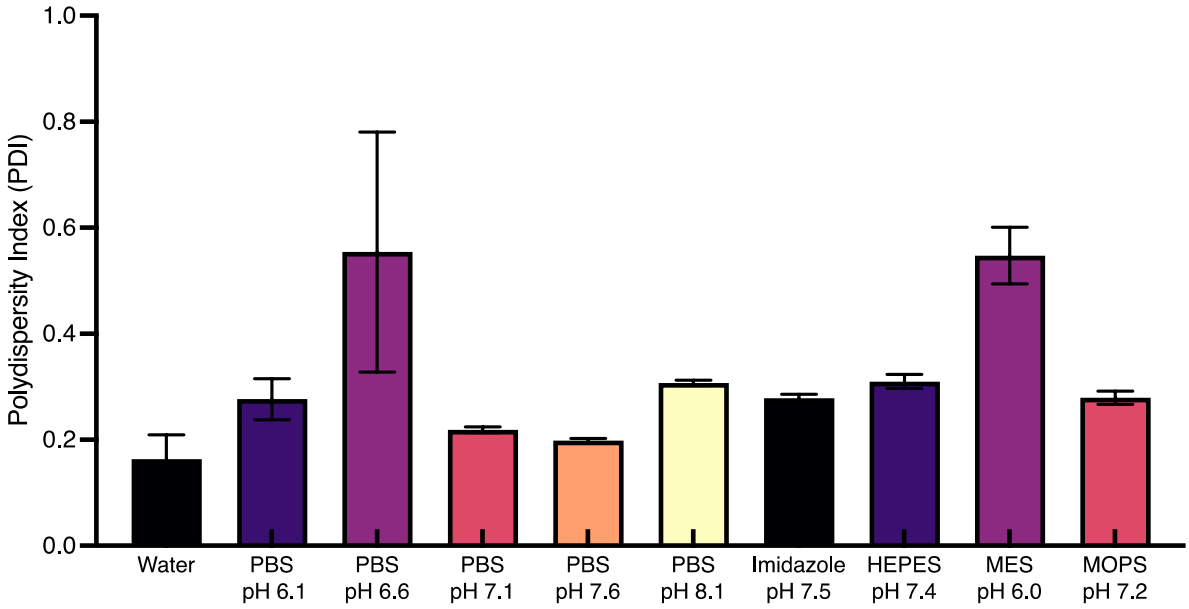


Figure S9: Polydispersity Index (PDI) of ZIF8 spiked with 10 mg/mL BSA following 1 day incubation in different buffer conditions. Each point represents the average and standard deviation across three batches measured thrice (n = 9).

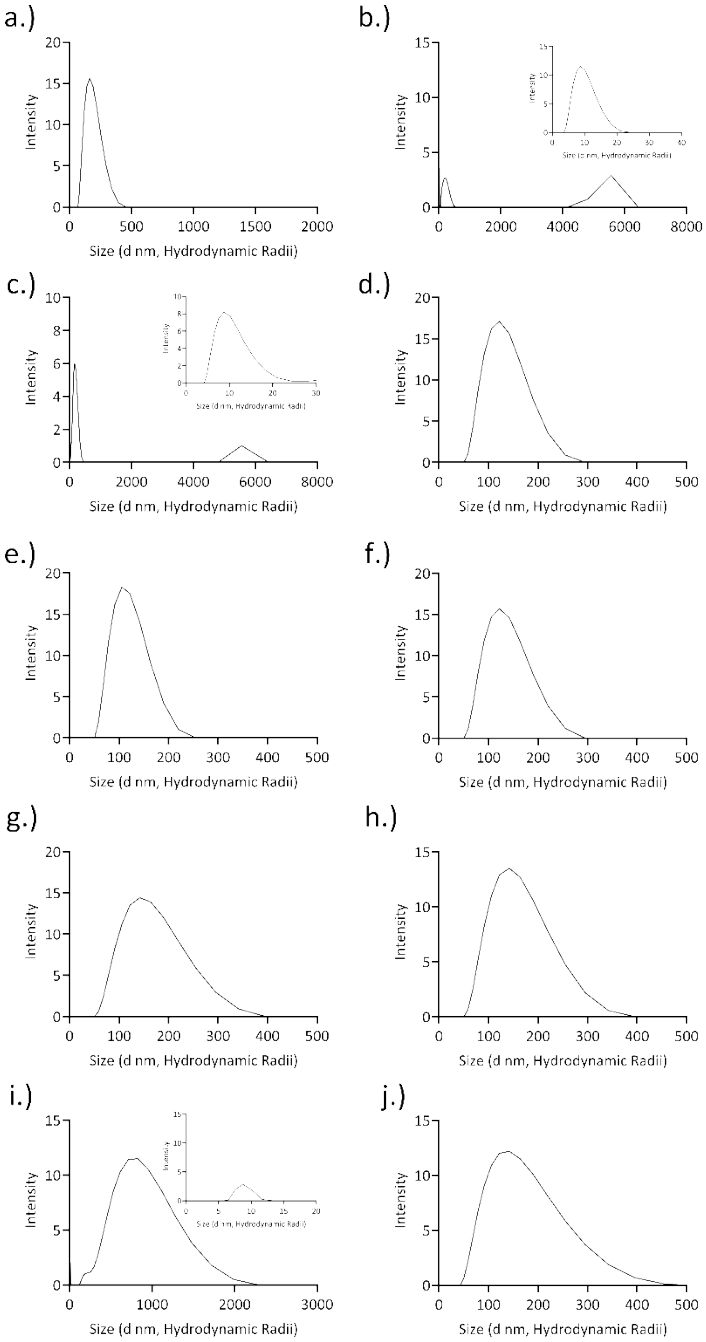


Figure S10: CONTIN plots of 10 mg/mL ZIF-8 spiked with 10 mg/mL BSA, following 1 day incubation in a.) Water, b.) PBS (pH 6.1), c.) PBS (pH 6.6), d.) PBS (pH 7.1), e.) PBS (pH 7.6), f.) PBS (pH 8.1), g.) Imidazole (pH 7.5), h.) HEPES (pH 7.4), i.) MES (pH 6.0), and j.) MOPS (pH 7.2)

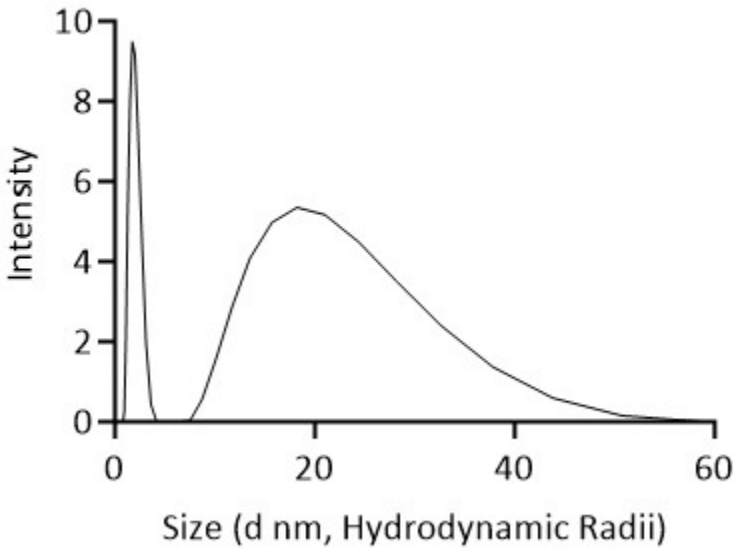


Figure S11: CONTIN plot of 10 mg/mL BSA dissolved in water.

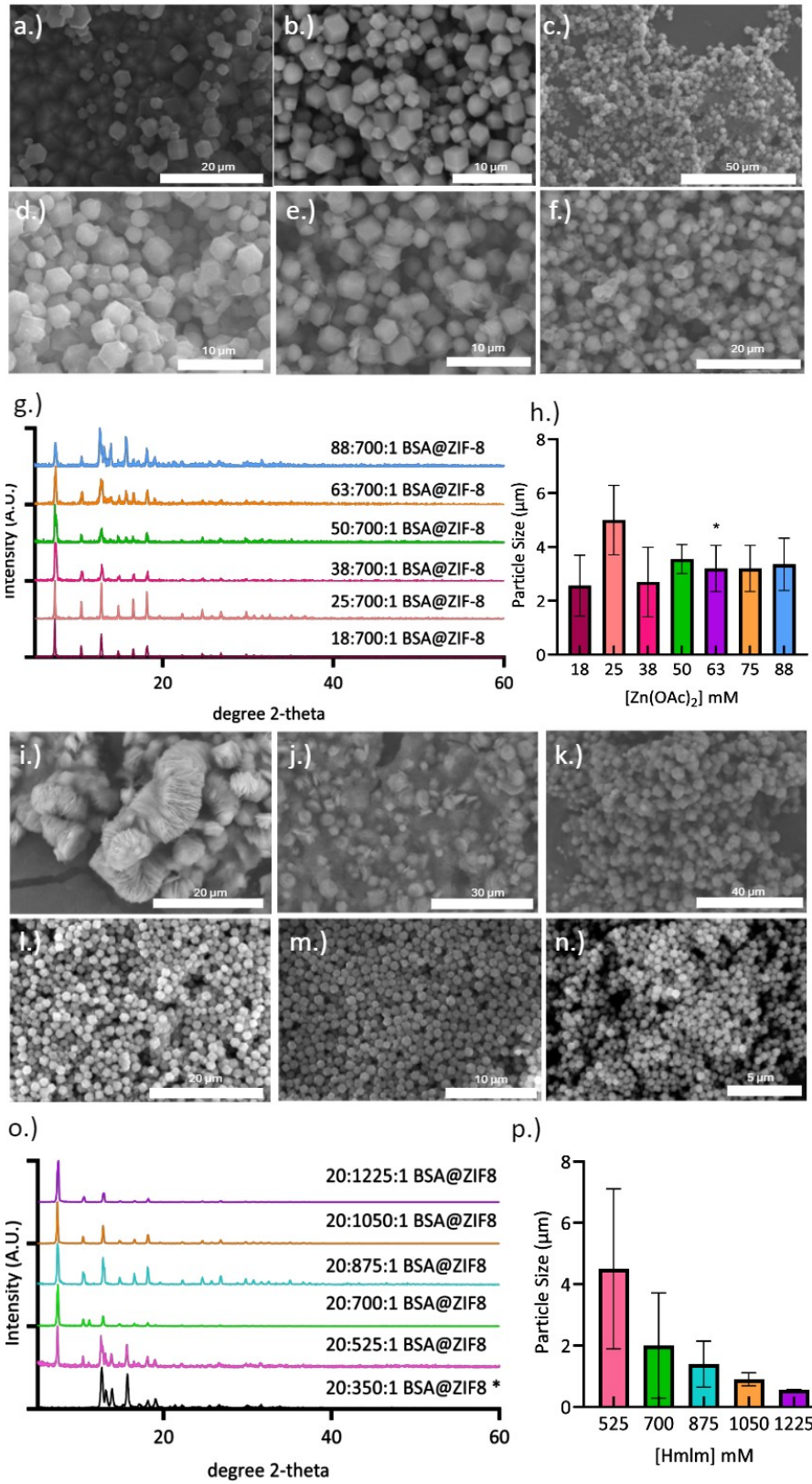


Figure S12: Scanning electron micrographs, diffractograms, and particle size distribution plots of BSA@ZIF-8 MOF particles with varying precursor concentrations**.** a) SEM image of 1

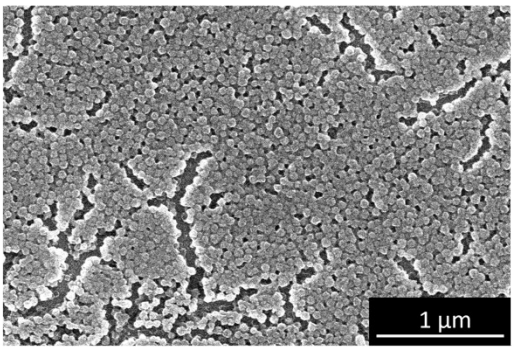
mg/mL BSA@ZIF-8 MOF particles with fixed [Hmlm] at 700 mM and varying [$Zn(OAc)_2$] at 25 mM, b) 38 mM, c) 50 mM, d) 63 mM, e) 75 mM, f) 88 mM, g) of BSA@ZIF-8 particles with varying $Zn(OAc)_2$ concentration between 50-175 mM, while maintaining concentration of Hmlm at 700 mM. h) Particle size distribution bar graph of BSA@ZIF-8 particles with varying $Zn(OAc)_2$ concentration between 25-88 mM, with fixed concentration of Hmlm at 700 mM, i) SEM images of BSA@ZIF-8 MOF particles fixed [$Zn(OAc)_2$] at 20 mM and [Hmlm] at 350 mM, j) 525 mM, k) 700 mM, l) 875 mM, m) 1050 mM, n) 1225 mM, o) Diffractogram of BSA@ZIF-8 particles with varying Hmlm concentration between 350-1225 mM, while maintaining concentration of $Zn(OAc)_2$ at 20 mM. p) Particle size distribution bar graph of BSA@ZIF-8 particles with varying Hmlm concentration between 350-1225 mM, and fixed concentration of $Zn(OAc)_2$ at 20 mM. All conditions include BSA concentration of 1 mg/mL.

Table S1: Description of the synthetic ratios trialed to produce a colloidal dispersion by varying $Zn(OAc)_2$ concentration and maintaining fixed Hmlm concentration at 700 mM. Each measurement consists of three batches measured thrice for a replicate count of n = 9.

$Zn(OAc)_2$	Hmlm	BSA	Hydrodynamic Radii (d nm)	Coefficient of Variation HDR (CV%)	PDI	Coefficient of Variation PDI (CV%)
18	700	1	2877.0 ± 765.7	26.6	0.83 ± 0.25	29.9
25	700	1	2678.3 ± 845.3	31.6	1 ± 0.0	0.0
38	700	1	1643.3 ± 624.6	38.0	0.67 ± 0.30	44.9
50	700	1	1259.6 ± 595.7	47.3	0.63 ± 0.30	44.9
63	700	1	1081.5 ± 332.6	30.8	0.73 ± 0.46	62.9
88	700	1	786.3 ± 281.9	35.8	0.47 ± 0.19	40.9

Table S2: Description of the synthetic ratios trialed to produce a colloidal dispersion by varying Hmlm concentration and maintaining fixed $Zn(OAc)_2$ concentration at 20 mM. Each measurement consists of three batches measured thrice for a replicate count of n = 9.

$Zn(OAc)_2$	Hmlm	BSA	Hydrodynamic Radii (d nm)	Coefficient of Variation HDR (CV%)	PDI	Coefficient of Variation PDI (CV%)
20	350	1	4854.7 ± 3485.2	71.8	0.43 ± 0.11	25.1
20	525	1	1499.0 ± 411.2	27.4	0.22 ± 0.09	39.8
20	750	1	2438.3 ± 1948.4	79.9	0.71 ± 0.36	50.4
20	875	1	755.8 ± 395.3	52.3	0.27 ± 0.13	48.8
20	1050	1	667.9 ± 6.7	1.0	0.99 ± 0.01	0.6
20	1225	1	547.2 ± 12.7	2.3	0.36 ±0.29	80.4



Sample ID	Zn	HMIM	BSA
BSA@c-ZIF-8_1	20 mM	700 mM	5 mg/mL
BSA@c-ZIF-8_2	20mM	700 mM	20 mg/mL
BSA@c-ZIF-8_3	20 mM	700 mM	10 mg/mL
BSA@c-ZIF-8_4	5 mM	700 mM	10 mg/mL
BSA@c-ZIF-8_5	10 mM	350 mM	10 mg/mL
BSA@c-ZIF-8_6	10 mM	700 mM	10 mg/mL

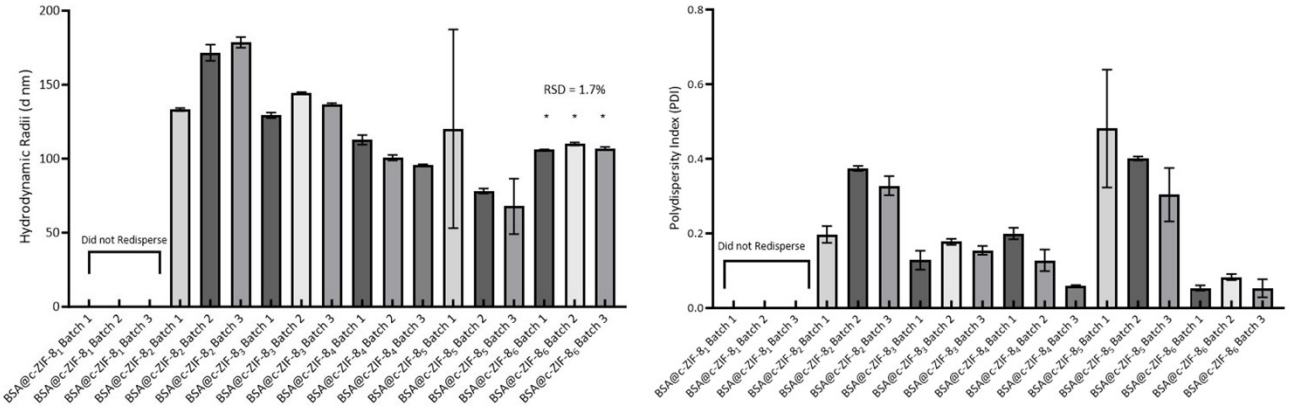


Figure S13: Electron micrograph, synthesis route, and reproducibility of BSA@c-ZIF-8, a) Electron micrograph of BSA@c-ZIF-8, b) table of different synthetic conditions attempted to obtain colloidally stable ZIF8 while varying concentrations of Hmlm, $Zn(OAc)_2$, and BSA, c) DLS data of ZIF8 batches based on hydrodynamic radius (d nm), d) DLS data of ZIF8 batches (Original, RJ1-RJ5) based on polydispersity index (PDI).

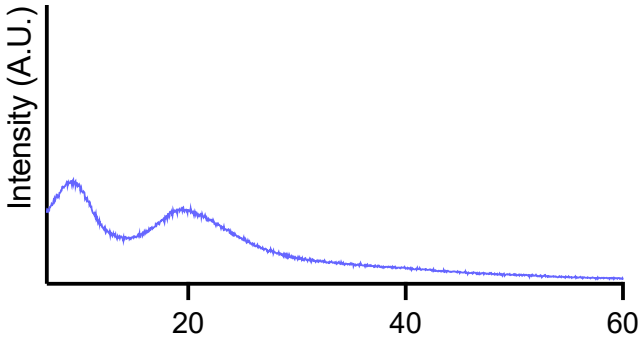


Figure S14. Powder x-ray diffractogram of BSA@ZIF-8 synthesized with 10 mg/mL BSA. We note the production of the **am** phase.

Figure S14 Discussion

We initially attempted to synthesize BSA@c-ZIF-8 with the original 1:100 ratio, but the resultant pXRD diffractogram revealed that this regime a diffractogram consistent with ***am*** topology (Figure S14). As a result, we excluded this precursor ratio from further analysis.

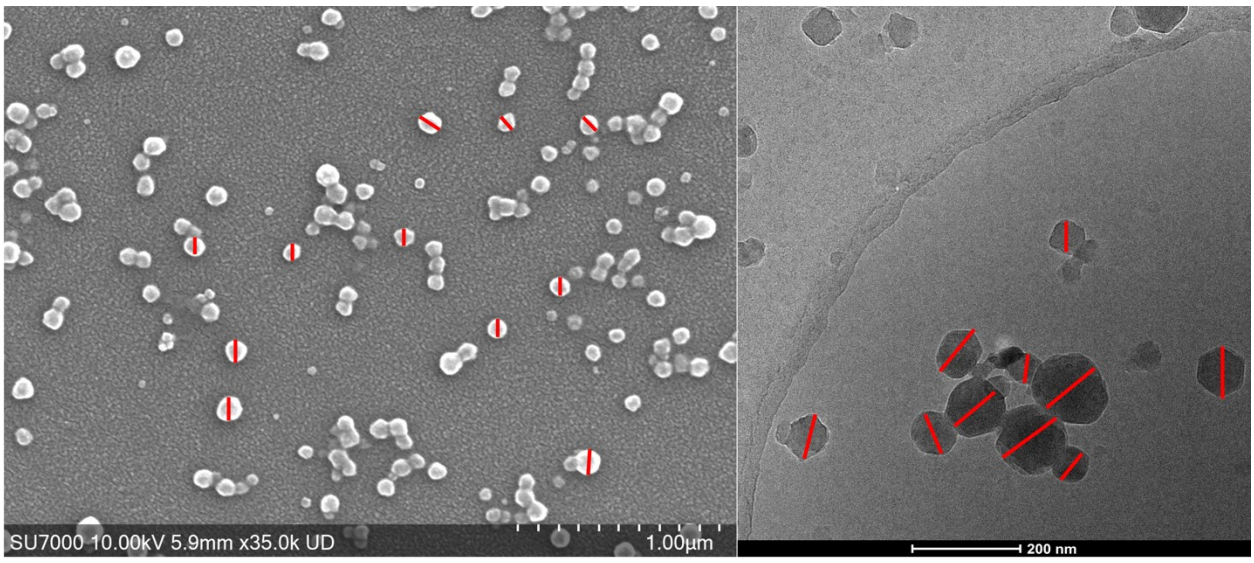


Figure S15: Size distribution analysis of BSA@c-ZIF-8 using a) Annotated Electron micrograph of BSA@c-ZIF-8, b.) annotated Cryo-EM image BSA@c-ZIF-8.

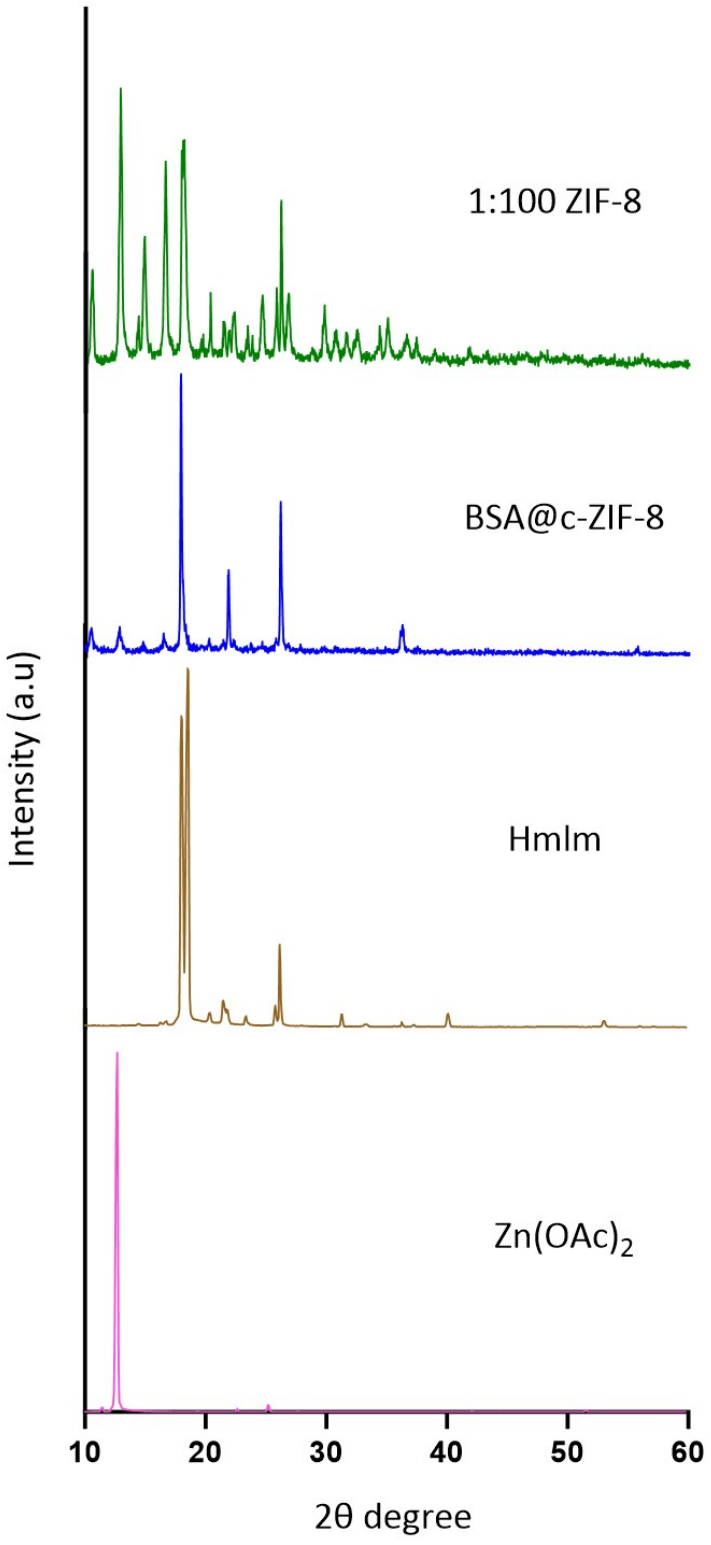


Figure S16: Powder x-ray diffractogram of Hmlm, $Zn(OAc)_2$, ZIF-8, and BSA@c-ZIF-8.

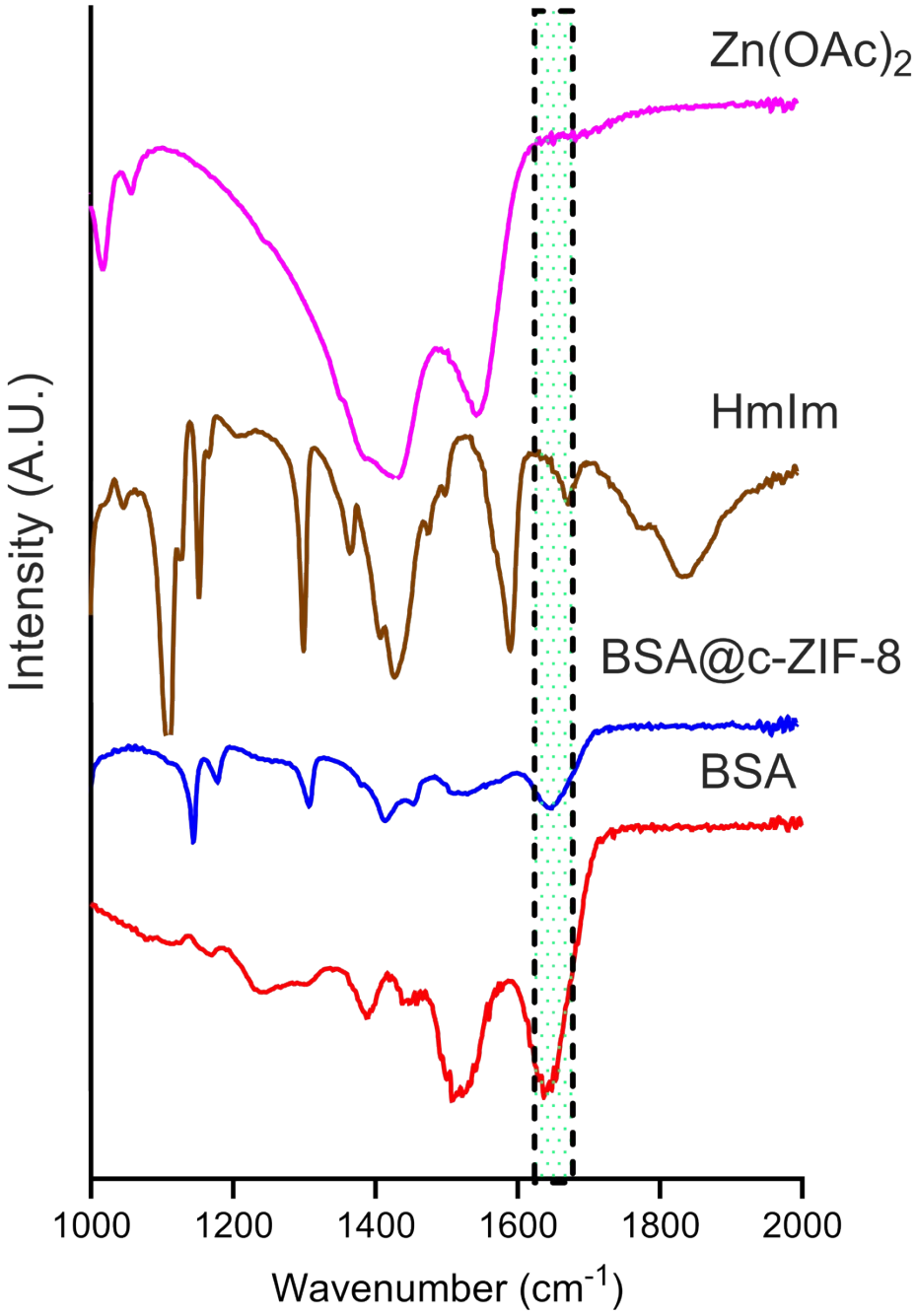


Figure S17: FTIR spectrum of Hmlm, $Zn(OAc)_2$, BSA, and BSA@c-ZIF-8

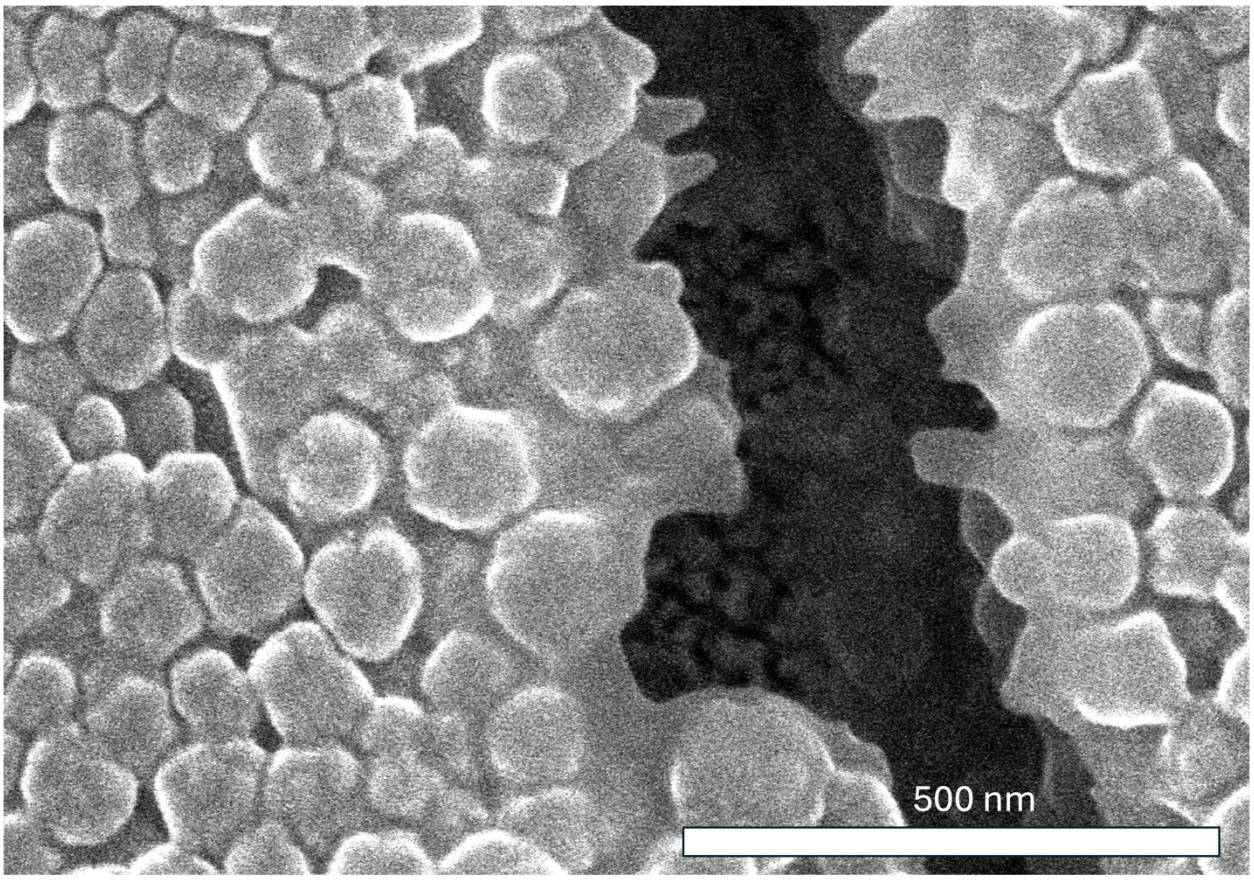


Figure S18. Scanning Electron Micrograph of BSA@c-ZIF-8 after incubation in PBS for 14 days.

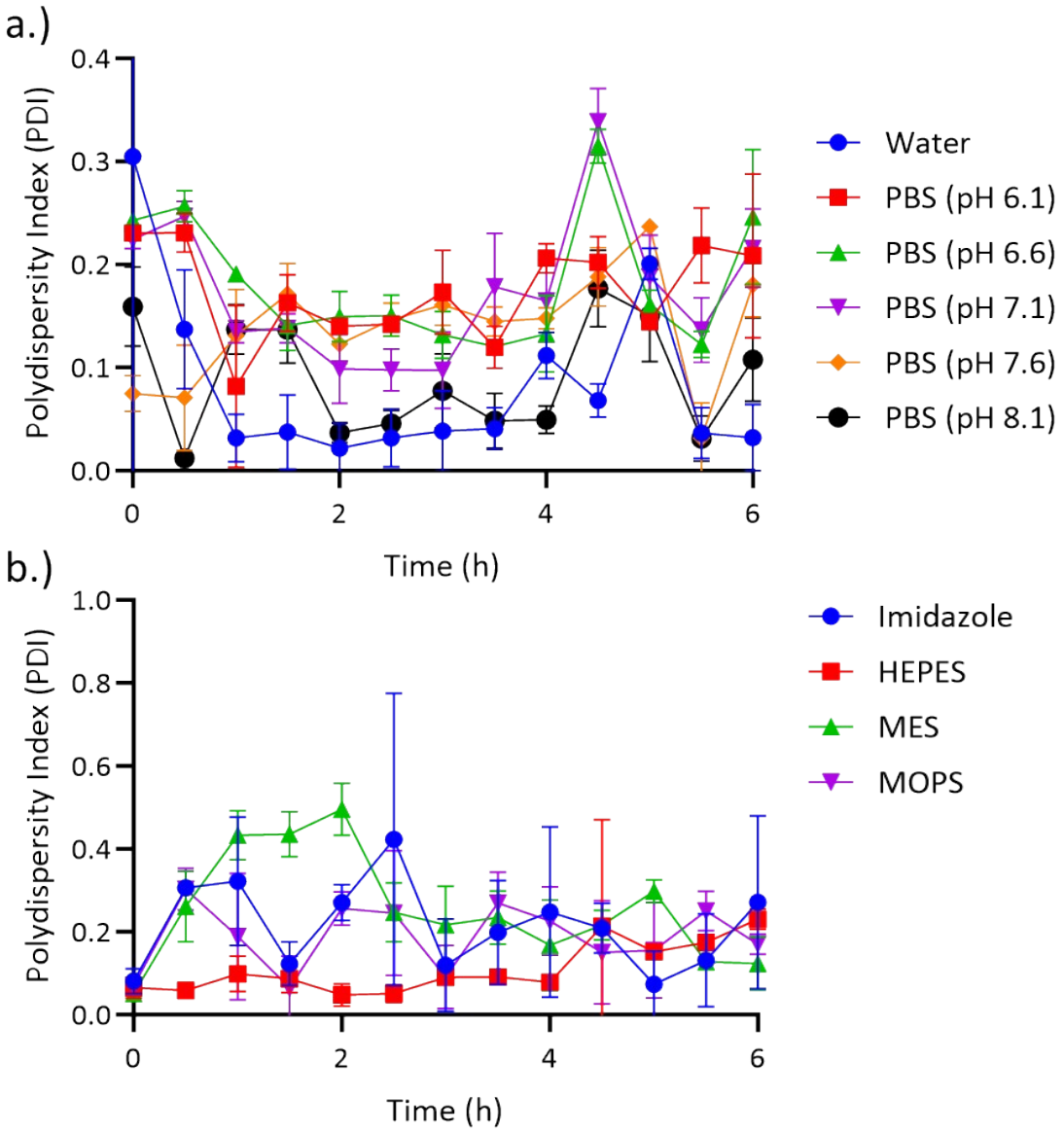


Figure S19: Polydispersity Index (PDI) over 6h temporal study of BSA@c-ZIF8 in a.) Water and PBS (pH 6.1-8.1), b.) Imidazole (pH 7.5), HEPES (pH 7.4), MES (pH 6.0), MOPS (pH 7.2). Each point represents the average and standard deviation across three batches measured thrice (n = 9)

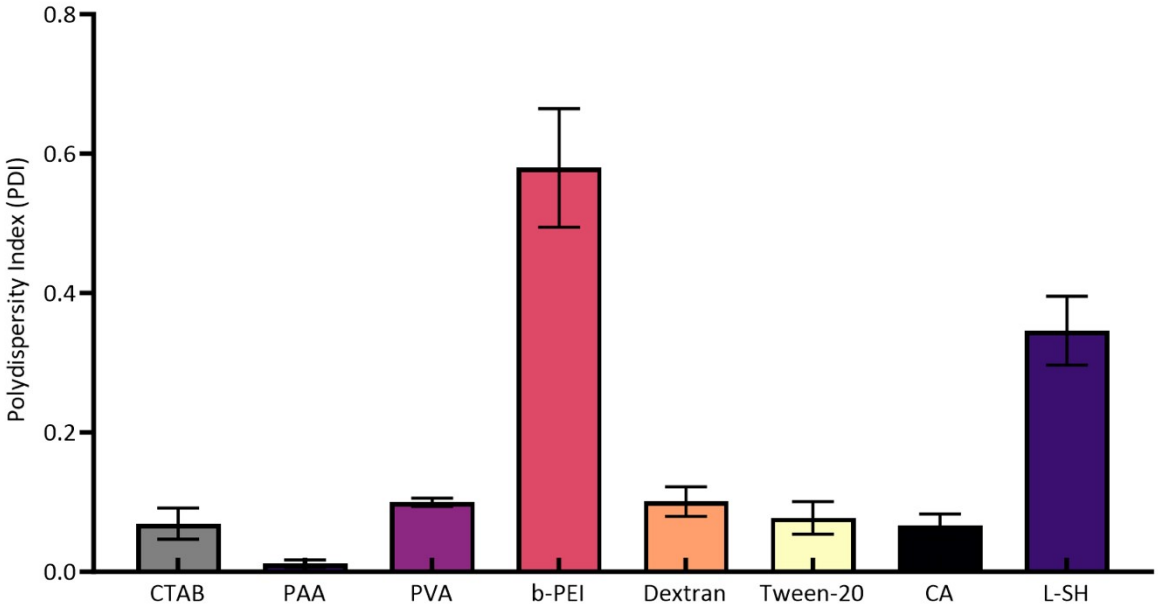


Figure S20: Polydispersity Index (PDI) of BSA@c-ZIF-8 coated with different polymers and incubated in water. Each point and error bar is the average and standard deviation of each point (n = 3).

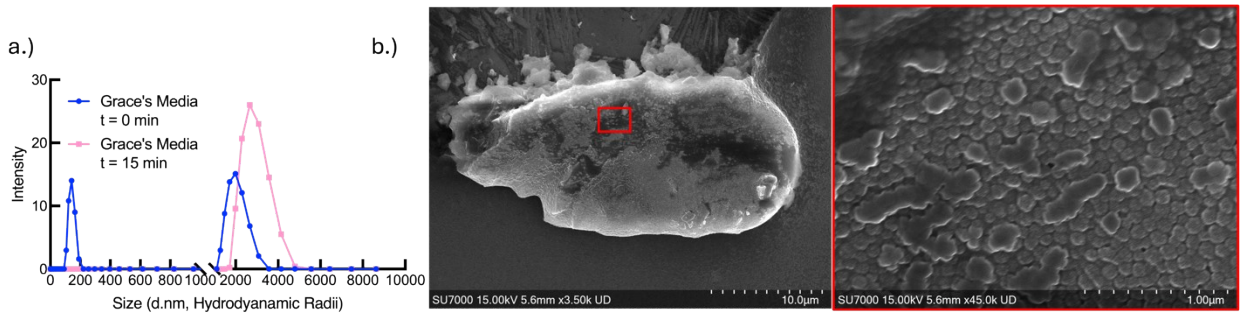


Figure S21: a.) Shows the CONTIN plots for BSA@c-ZIF-8 incubated in Grace's Media at t = 0 and t = 15. b.) the scanning electron micrograph of a large aggregate found on the Grace's Media stub. The red region is enlarged.

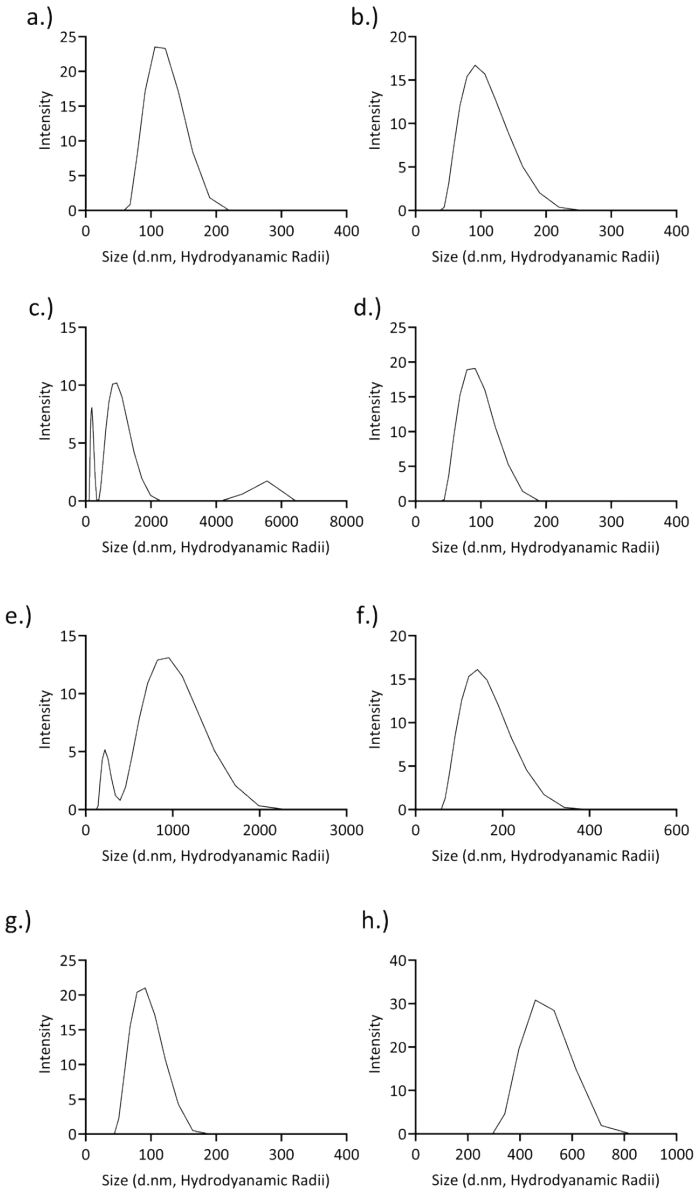


Figure S22: CONTIN plot of BSA@c-ZIF8 coated with a.) CTAB, b.) PAA, c.) b-PEI, d.) PVA, e.) LSH, f.) Dextran, g.) Tween-20, and h.) CA following 24-hour incubation.

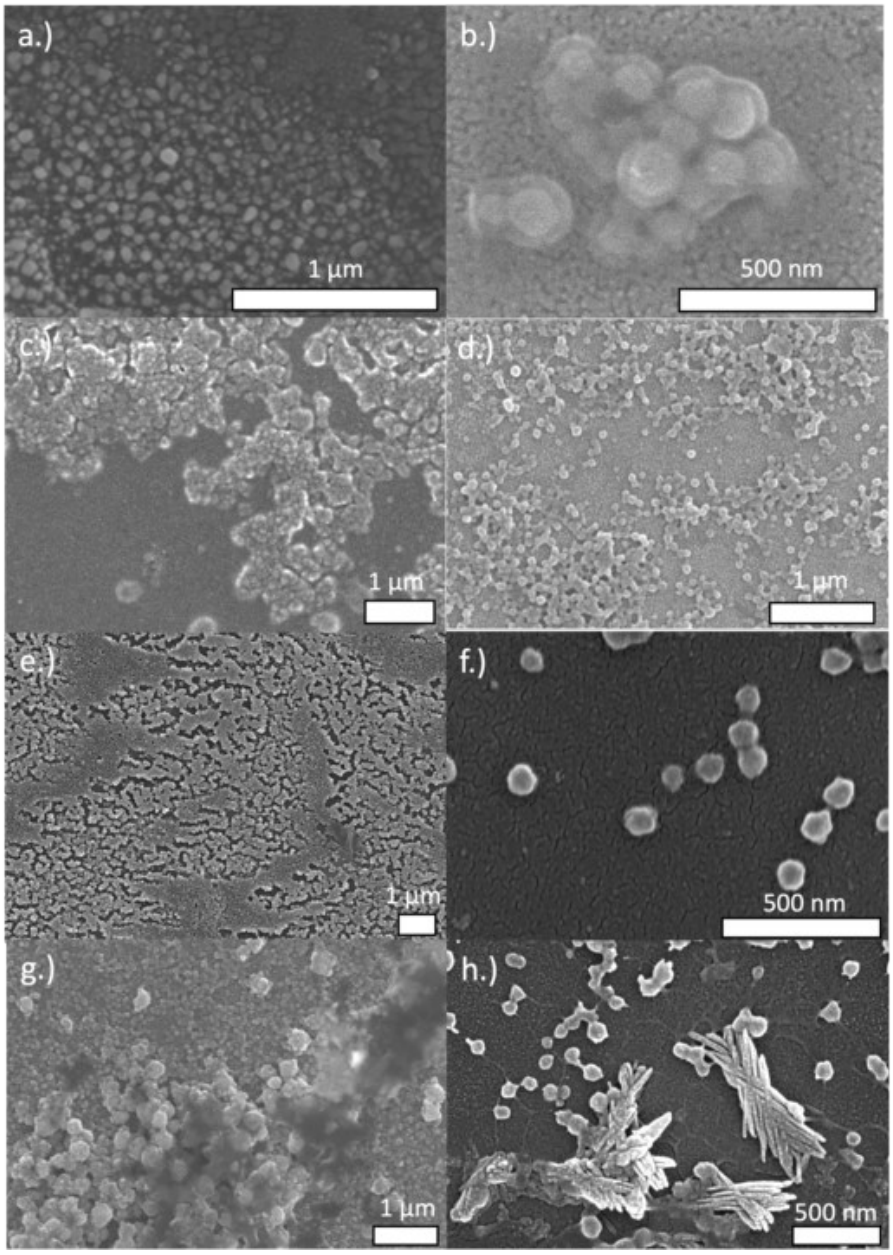


Figure S23. Electron micrographs of a.) CTAB-BSA@c-ZIF-8, b.) PAA-BSA@c-ZIF-8, c.) b-PEI-BSA@c-ZIF-8, d.) PVA-BSA@c-ZIF-8, e.) L-SH-BSA@c-ZIF-8, f.) Dextran-BSA@c-ZIF-8, g.) Tween-20-BSA@c-ZIF-8, h.) CA-BSA@c-ZIF-8.

Figure S23 Discussion

CTAB-, PAA-, PVA- and Dextran-BSA@c-ZIF-8 show even size distributions of particles after surface passivation, in good agreement with the DLS measurements. PEI conjugated BSA@c-ZIF-8 shows a particulate network formed of multiple 100 nm sized BSA@c-ZIF-8 particles, explaining the large increase in size (DD_h = 930.9 ± 130.7 nm) after modification seen in the DLS plots, as well as the increased PDI of 0.58 ± 0.08 (Figure 4b.). Finally, Dextran, PAA and PVA modified nanoparticles show particles of similar size to unmodified particles, (Figure 5b,

5d, 5g.) and verify the sizes demonstrated by DLS. For CA incubated BSA@c-ZIF-8, there is an increase in the average size (DD_h = 712.6 ± 160.8 nm) shown by DLS, which is verified by the presence of a new material in the micrograph. L-SH modified BSA@c-ZIF-8 is polydisperse in nature (PDI = 0.21 ± 0.17) by DLS and verified by the aggregation of the particles into a film, while maintaining the original size of the nanoparticles. Tween-20 surfactant modified BSA@c-ZIF-8 shows two distinct particle populations, one which is the original BSA@c-ZIF-8 structure, and one with aggregated particles. VA conjugated BSA@c-ZIF-8 also shows two particle distributions.

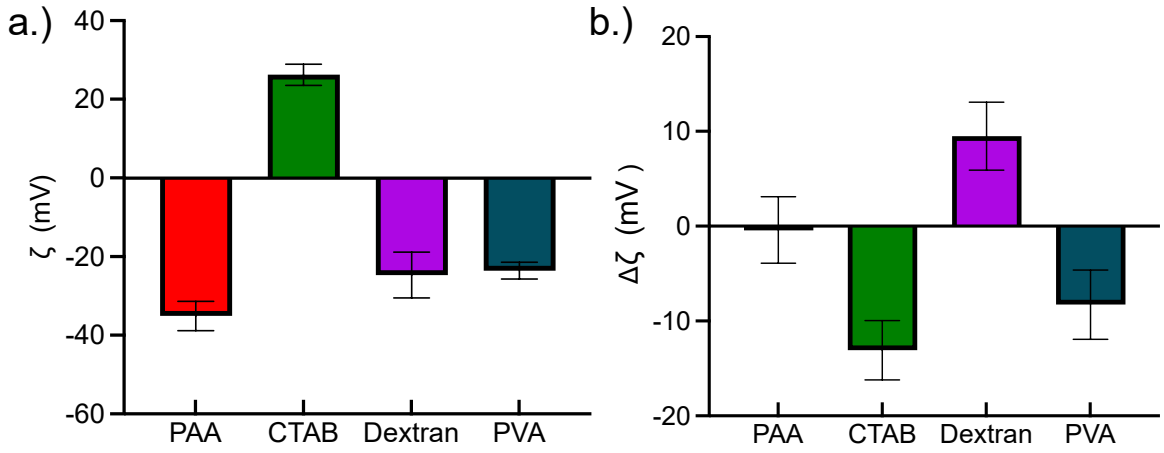


Figure S24: a.) Post wash Zeta Potential of BSA@c-ZIF-8 in Water after coating with PAA, CTAB, Dextran, and PVA. b.) ΔZeta Potential of BSA@c-ZIF-8 in water between pre and post wash after coating with PAA, CTAB, Dextran, and PVA. Each point represents the average and standard deviation across three batches measured thrice (n = 9)

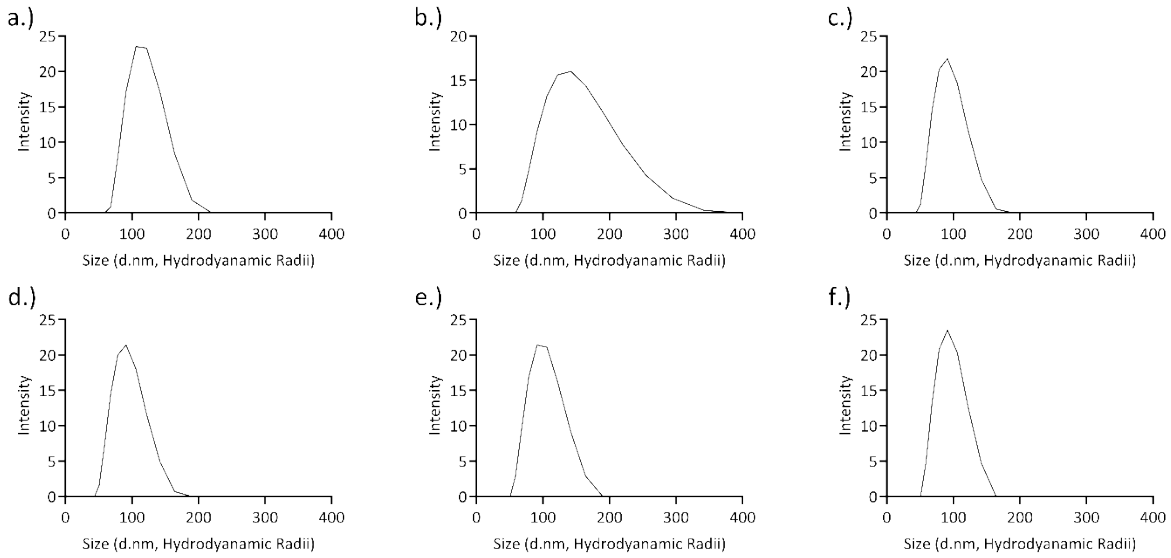


Figure S25: Pre-wash CONTIN plots of CTAB-BSA@c-ZIF8 following 24h incubation in a.) Water, b.) 1X PBS (pH 7.1), c.) Imidazole buffer (pH 7.5), d.) HEPES (pH 7.4), e.) MES (pH 6.0), f.) MOPS (pH 7.2).

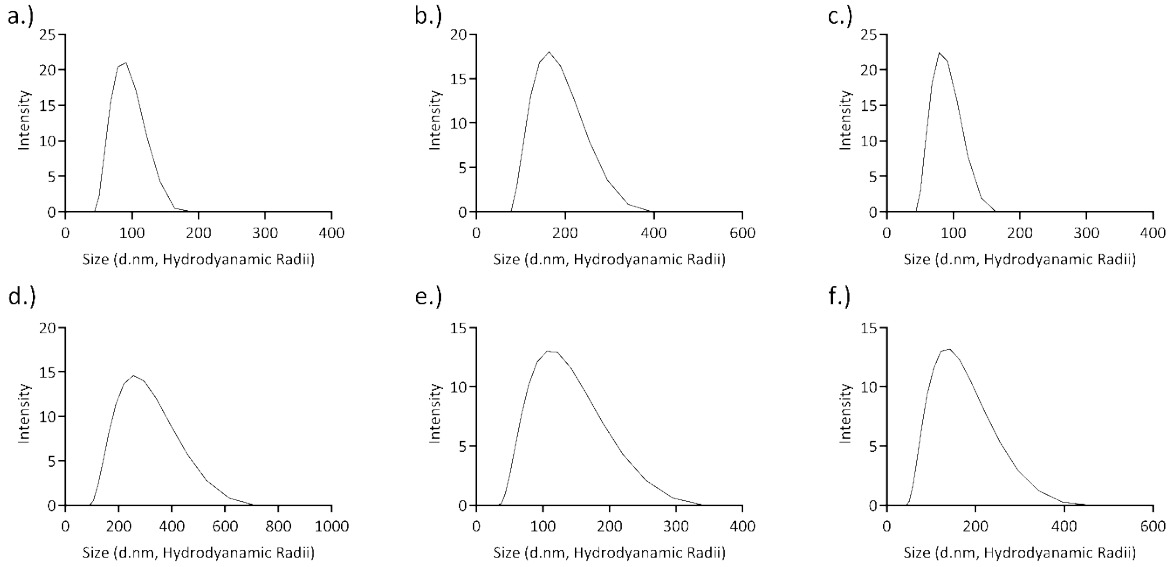


Figure S26: Pre-wash CONTIN plots of Dextran-BSA@c-ZIF8 following 24h incubation in a.) Water, b.) 1X PBS (pH 7.1), c.) Imidazole buffer (pH 7.5), d.) HEPES (pH 7.4), e.) MES (pH 6.0), f.) MOPS (pH 7.2).

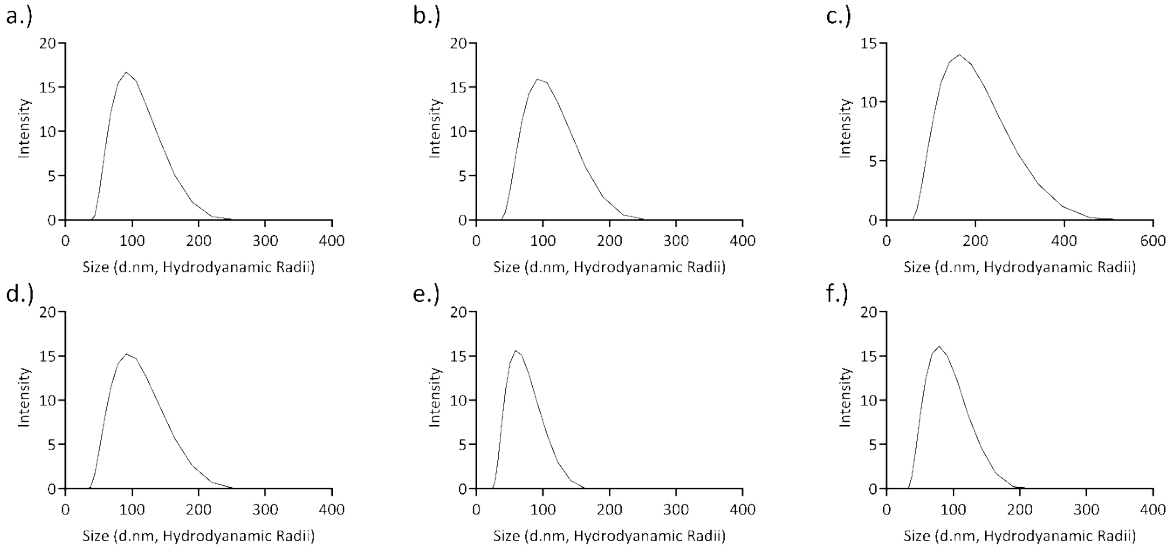


Figure S27: Pre-wash CONTIN plots of PAA-BSA@c-ZIF8 following 24h incubation in a.) Water, b.) 1X PBS (pH 7.1), c.) Imidazole buffer (pH 7.5), d.) HEPES (pH 7.4), e.) MES (pH 6.0), f.) MOPS (pH 7.2).

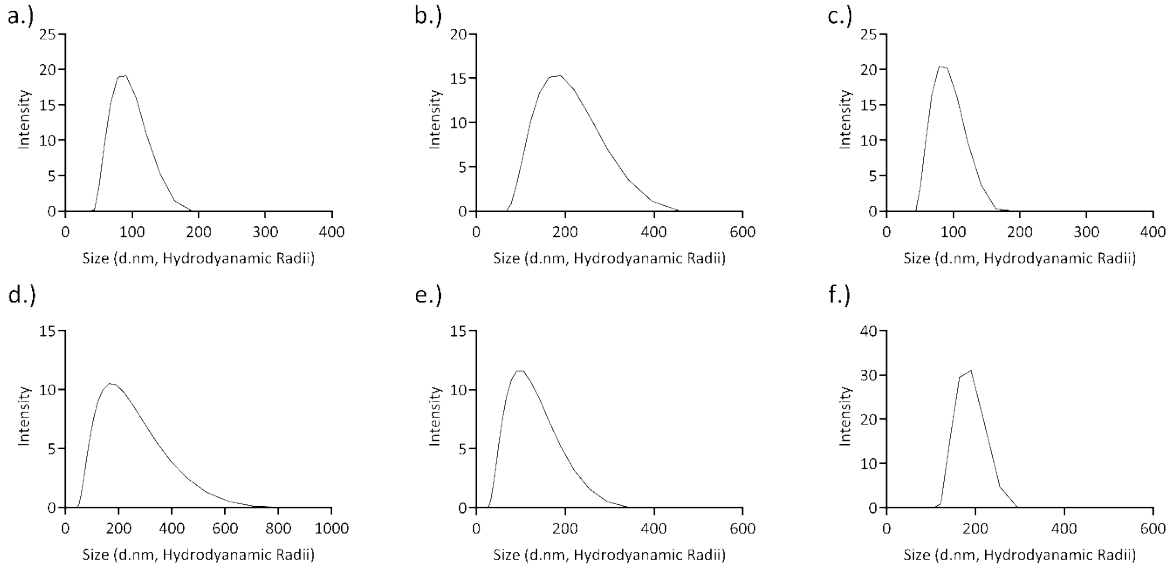


Figure S28: Pre-wash CONTIN plots of PVA-BSA@c-ZIF8 following 24h incubation in a.) Water, b.) 1X PBS (pH 7.1), c.) Imidazole buffer (pH 7.5), d.) HEPES (pH 7.4), e.) MES (pH 6.0), f.) MOPS (pH 7.2).

Figure S25-S28 Discussion

The CONTIN plots show unimodal size distribution which does not shift between 0 and 24 hours for PAA or PVA (Figure S21-24, PDIs found in **Table 2**.), indicating that the instability in MES and MOPS for BSA@c-ZIF-8 is halted by the presence of the surfactants, a summary of the PDIs and sizes is found in **Table S3** and **S4,** respectively**.** Further, we note that while PAA did not produce a statistically significant change in zeta potential or size after surface passivation, there is a significant improvement in stability, indicating that the PAA is causing surface changes to the particles which are improving the stability of the particles.

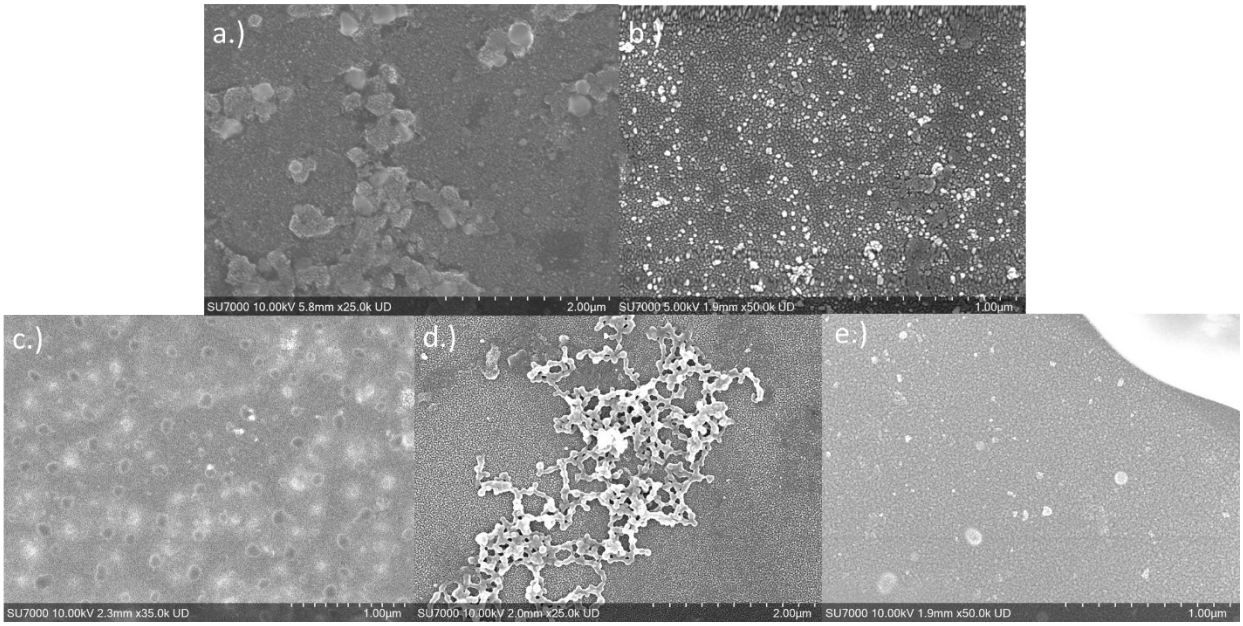


Figure S29: Scanning electron micrographs of PAA-coated BSA@c-ZIF8 following 6h incubation in a) PBS (pH 7.1), b) Imidazole (pH 7.5), c) HEPES (pH 7.4), d) MES (pH 6.0), e) MOPS (pH 7.2)

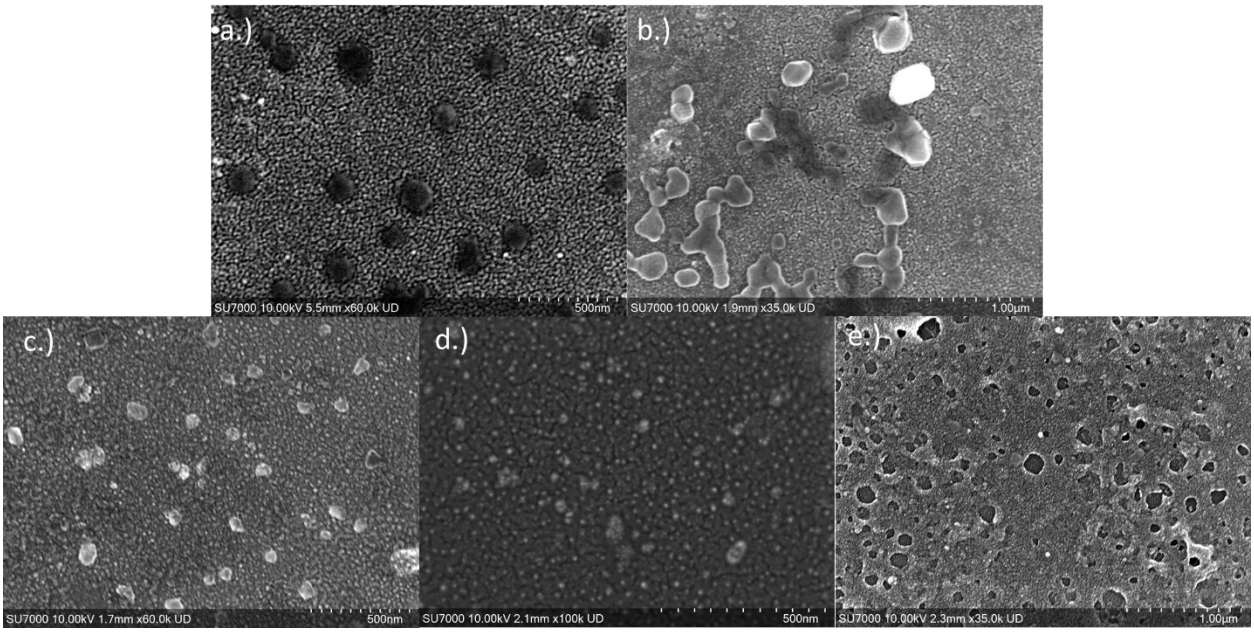


Figure S30: Scanning electron micrographs of CTAB-coated BSA@c-ZIF8 following 6h incubation in a) PBS (pH 7.1), b) Imidazole (pH 7.5), c) HEPES (pH 7.4), d) MES (pH 6.0), e) MOPS (pH 7.2)

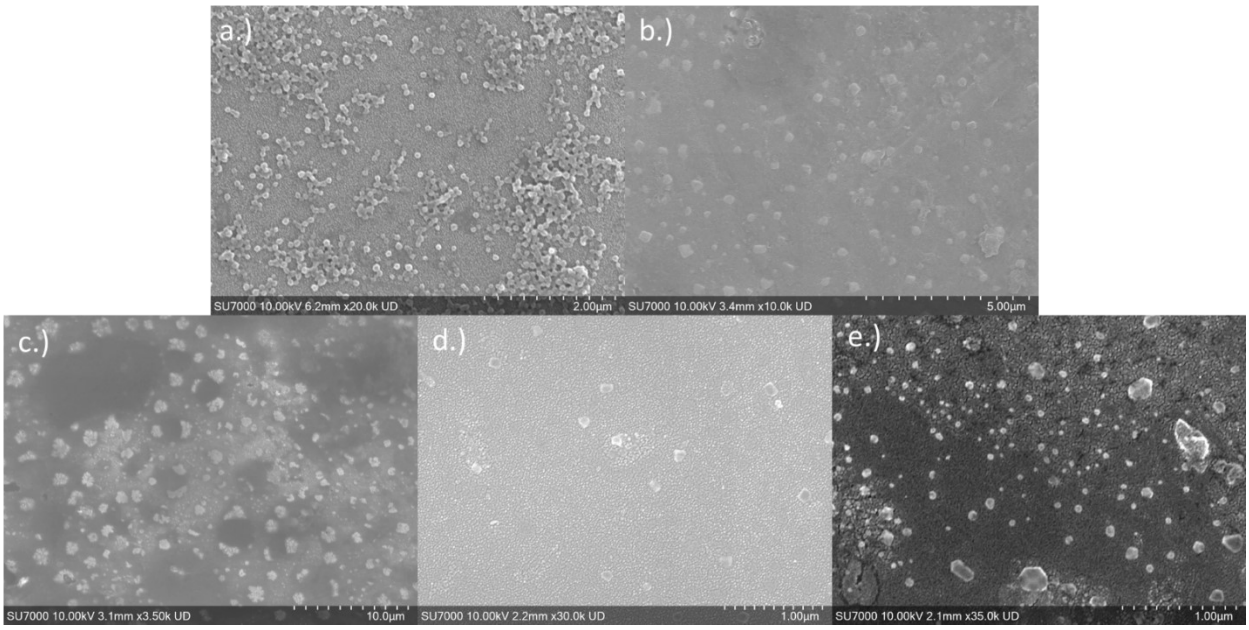


Figure S31: Scanning electron micrographs of Dextran-coated BSA@c-ZIF8 following 6h incubation in a) PBS (pH 7.1), b) Imidazole (pH 7.5), c) HEPES (pH 7.4), d) MES (pH 6.0), e) MOPS (pH 7.2)

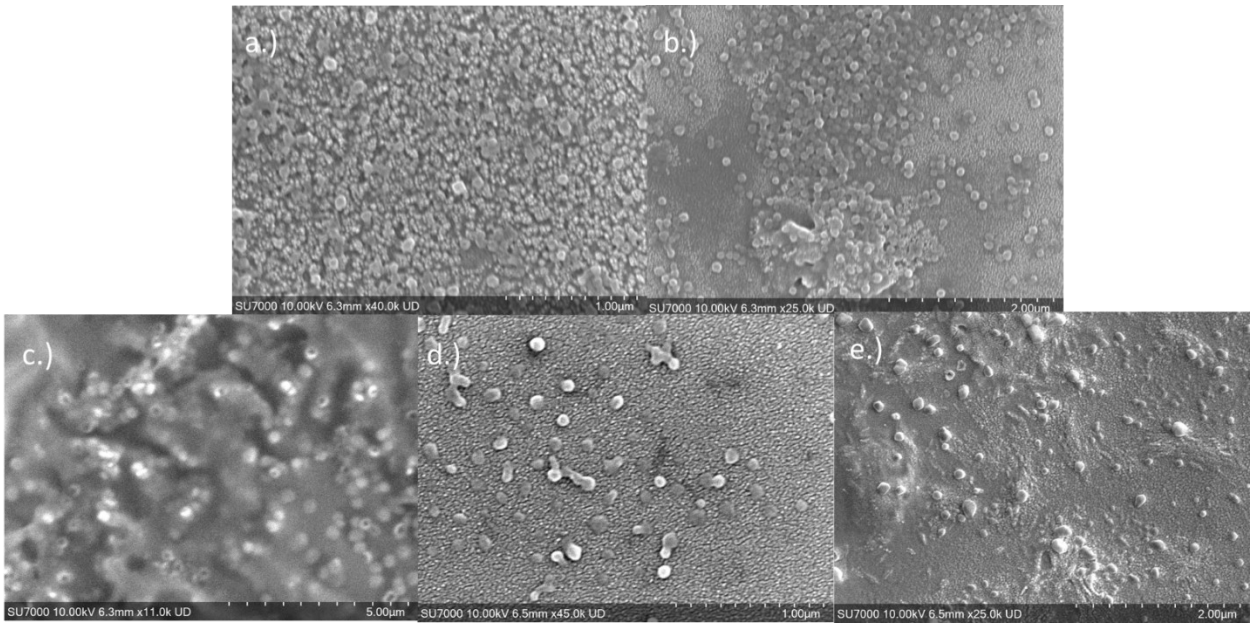


Figure S32: Scanning electron micrographs of PVA-coated BSA@c-ZIF8 following 6h incubation in a) PBS (pH 7.1), b) Imidazole (pH 7.5), c) HEPES (pH 7.4), d) MES (pH 6.0), e) MOPS (pH 7.2)

Figure S28-S32 discussion

We initially allowed the particles to incubate with the polymers for a day to ensure surface functionalization. We show that washing the surface passivated BSA@c-ZIF-8 (n = 9) has minimal effect on the zeta potential of our material shifting PAA to -35.1 ± 3.7 mV, CTAB to 26.2 ± 2.7 mV, Dextran to -24.7 ± 5.8 mV and finally PVA to -23.6 ± 2.2 mV (Figure S32). This indicates that surface functionalization was successful as the surfactant modified BSA@c-ZIF-8 still shows significantly different surface charge from that of BSA@c-ZIF-8 alone. PAA@BSA@c-ZIF-8 did not show a change in surface charge after washing, but a coating is clearly visible in the micrograph shown in Figure S23b.).

Table S3. Shows the pre and post wash PDI after 24 h incubation in selected the buffers (n = 9).

	PDI pre-wash 24 h					PDI post-was 24 h				
Buffer	PBS	IM	HEPES	MES	MOPS	PBS	IM	HEPES	MES	MOPS
PAA	0.09 ± 0.02	0.20 ± 0.01	0.13 ± 0.07	0.10 ± 0.01	0.12 ± 0.02	0.17 ± 0.04	0.03 ± 0.01	0.37 ± 0.17*†	0.14 ± 0.03	0.10 ± 0.01
CTAB	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.02	0.10 ± 0.02	0.59 ± 0.08*†	0.50 ± 0.02*†	0.12 ± 0.03	0.06 ± 0.01
Dextran	0.10 ± 0.03	0.03 ± 0.2	0.24 ± 0.02	0.19 ± 0.05	0.17 ± 0.02	0.20 ± 0.04	0.10 ± 0.02	0.71 ± 0.22*†	0.07 ± 0.02	0.16 ± 0.03

PVA	0.14 ± 0.13	0.06 ± 0.01	0.22 ± 0.01	0.18 ± 0.02	0.18 ± 0.01	0.13 ± 0.05	0.10 ± 0.03	0.64 ± 0.14*†	0.08 ± 0.02	0.23 ± 0.01

Note: <0.2 denotes monodispersed, while >0.4 indicates a polydisperse sample. Multimodal CONTIN plots are marked with an asterisk (*). PDIs which are significantly different p=0.95 from Surfactant@BSA@c-ZIF-8 in water are marked with †.

Table S4. Shows the pre and post wash HDR after 24 h incubation in selected the buffers (n = 9).

	HDR pre-wash 24 h (nm)					HDR post-was 24 h (nm)				
Buffer	PBS	IM	HEPES	MES	MOPS	PBS	IM	HEPES	MES	MOPS
PAA	101.0 ± 11.6	162.8 ± 52.3	101.6 ± 21.0	69.2 ± 11.9	81.7 ± 15.8	136.9 ± 5.6	149.5 ± 19.8	474.6 ± 337.8	104.0 ± 6.0	101.7 ± 7.3
CTAB	123.1 ± 18.1	96.1 ± 14.3	105.0 ± 28.9	115.7 ± 32.7	131.5 ± 45.3	120.4 ± 15.1	1832 ± 693	472.9 ± 301.9	132.3 ± 34.9	103.8 ± 5.7
Dextran	193.9 ± 42.4	94.8 ± 13.0	459.6 ± 331.6	265.3 ± 257.8	692.4 ± 664.0	132.3 ± 34.9	106.6 ± 9.4	1511 ± 2466	101.4 ± 8.5	124.2 ± 20.6
PVA	149.9 ± 25.8	92.3 ± 14.0	192.2 ± 58.0	201.7 ± 95.5	197.5 ± 62.8	125.4 ± 5.9	103.9 ± 9.2	897.3 ± 789.8	141.2 ± 12.3	142.0 ± 12.1

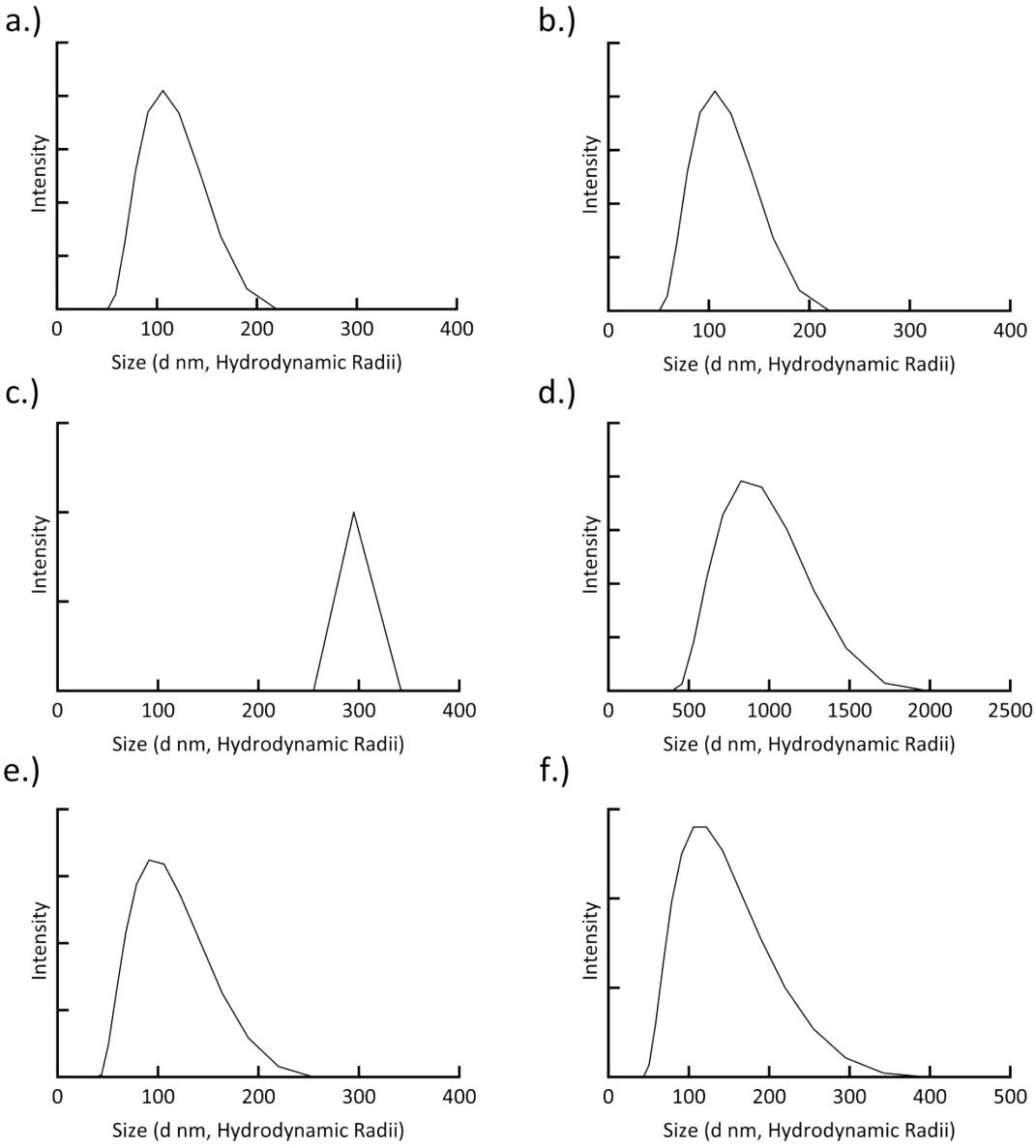


Figure S33: Post-wash CONTIN plots of CTAB-BSA@c-ZIF8 following 24h incubation in a.) 1X PBS (pH 7.1), b.) Imidazole buffer (pH 7.5), c.) HEPES (pH 7.4), d.) MES (pH 6.0), e.) MOPS (pH 7.2)

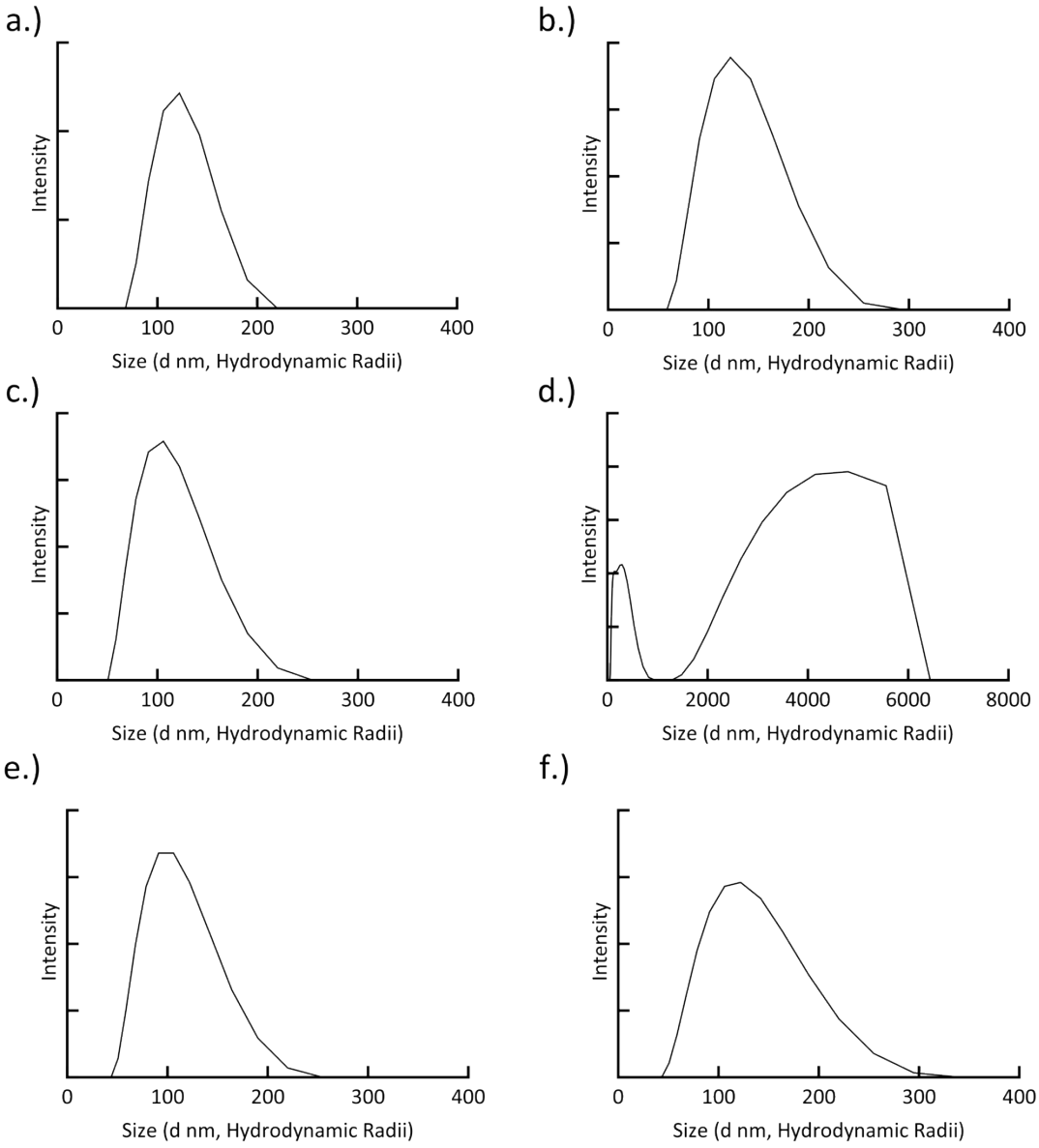


Figure S34*:* Post-wash CONTIN plots of Dextran-BSA@c-ZIF8 following 24h incubation in a.) 1X PBS (pH 7.1), b.) Imidazole buffer (pH 7.5), c.) HEPES (pH 7.4), d.) MES (pH 6.0), e.) MOPS (pH 7.2)

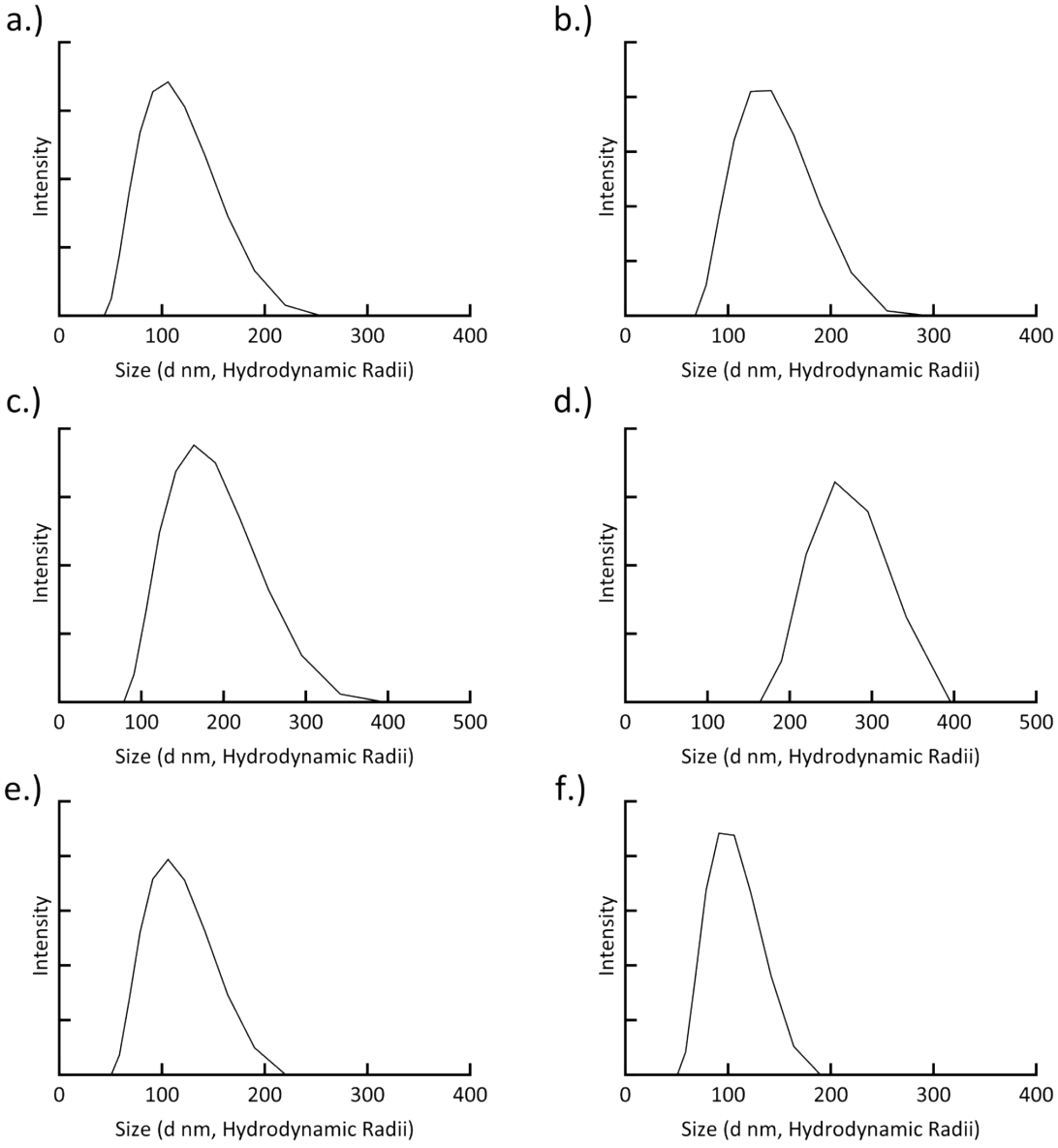


Figure S35: Post-wash CONTIN plots of PAA-BSA@c-ZIF8 following 24h incubation in a.) 1X PBS (pH 7.1), b.) Imidazole buffer (pH 7.5), c.) HEPES (pH 7.4), d.) MES (pH 6.0), e.) MOPS (pH 7.2)

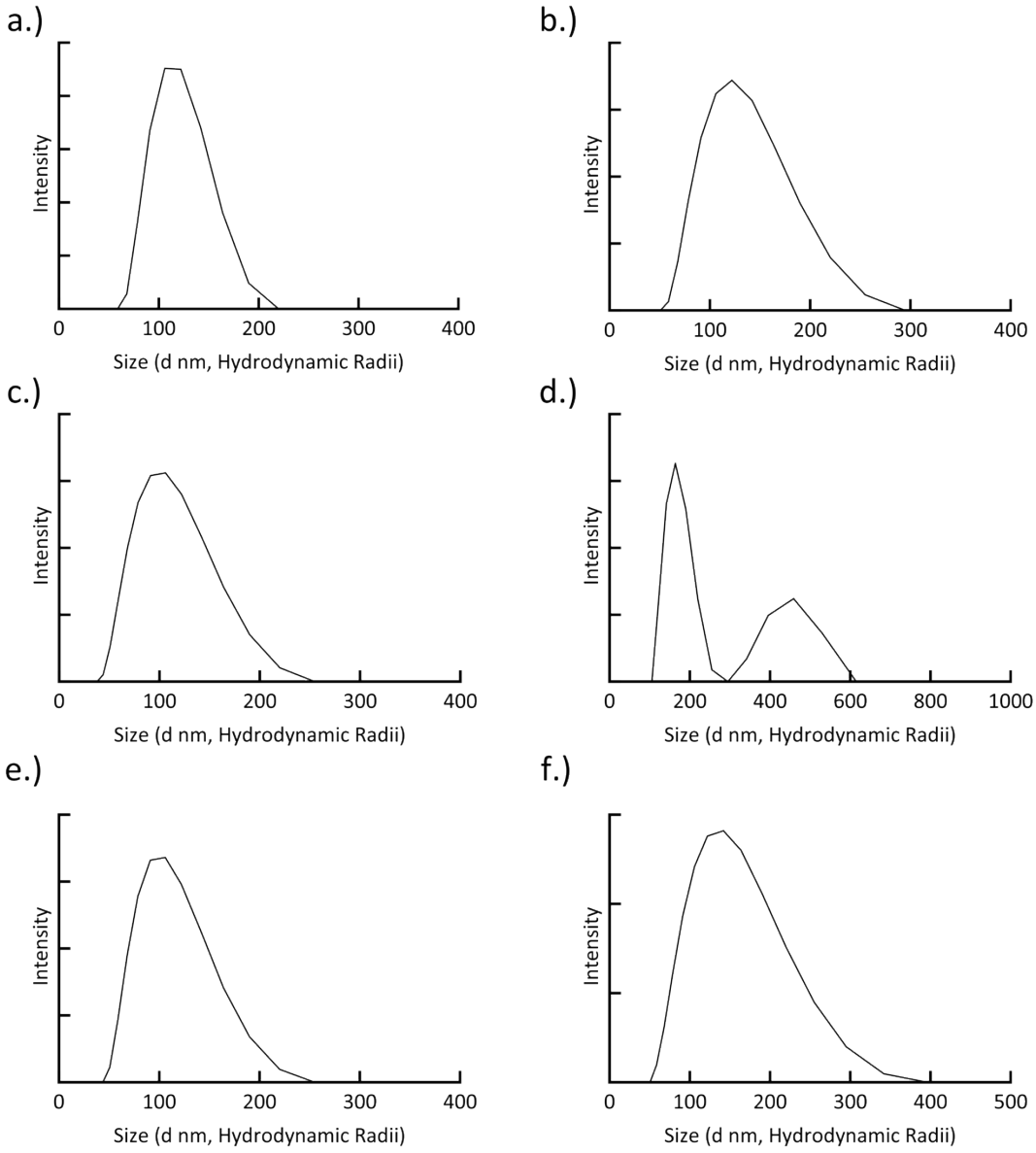


Figure S36: Post-wash CONTIN plots of PVA-BSA@c-ZIF8 following 24h incubation in a.) 1X PBS (pH 7.1), b.) Imidazole buffer (pH 7.5), c.) HEPES (pH 7.4), d.) MES (pH 6.0), e.) MOPS (pH 7.2)