

Supporting Information

Zwitterionic supramolecular nanoparticles: Self-assembly and responsive properties**

Carmen Stoffelen, Jurriaan Huskens*

C. Stoffelen, Prof. Dr. J. Huskens*

Molecular Nanofabrication group,

MESA⁺ Institute for Nanotechnology, University of Twente

P. O. Box 217, 7500 AE, Enschede (The Netherlands)

Fax: (+):+31 53489 4645

E-mail: j.huskens@utwente.nl

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1. Experimental section

1.1 Materials and Equipment

Starting materials for organic synthesis were obtained from Sigma-Aldrich and ABCR and used as received. Methyl-4,4-bipyridinium (methyl viologen, MV)-substituted poly(ethylene imine) (MV-PEI, degree of substitution: 4.5 MV units per polymer chain),¹ azobenzene (Azo)-terminated poly(amido amine) dendrimer of generation 1 (8 end groups),² and azobenzene-tri(ethylene glycol)³ were prepared according to literature procedures. Cucurbit[8]uril (CB[8]) was purchased from Strem Chemicals and the concentration of a stock solution was assessed by microcalorimetric titration (Microcal VP-ITC) against paraquat. For the preparation of SNPs, distilled water was purified by MilliQ Advantage A10, Millipore R=18.2 M Ω , before usage. The synthesized products were analyzed by ¹H-NMR and ¹³C-NMR on a Bruker 400 MHz and 600 MHz system. The samples were dissolved in deuterated solvent purchased from Cambridge Isotope Laboratories Inc. Mass analysis was done by electrospray ionization using a micromass LCT from Waters. Dynamic light scattering (DLS) analyses were carried out on a Nanotrac from Anaspec, operating with the Microtrac FLEX Operating Software, and on a Zetasizer from Malvern. Furthermore, the supramolecular SNPs were analyzed by a Carl-Zeiss 1500 high resolution scanning electron microscope (HR-SEM). UV and visible light irradiation experiments were carried out with a Hönle bluepoint 2 easycure using a shortpass filter $\lambda \leq 400$ nm. UV/Vis measurements were carried out on a Perkin Elmer UV/Vis spectrometer Lambda 850.

1. 2 Synthetic procedures

Synthesis of the azobenzene-substituted carboxybetaine analog (Azo-Zwit)

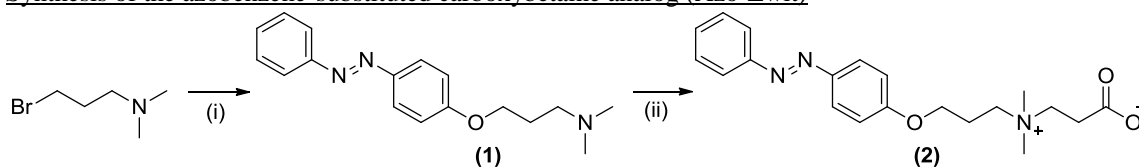
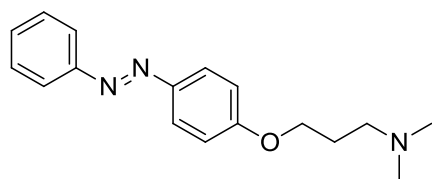


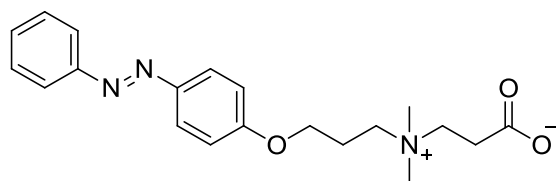
Figure S1: Synthesis of Azo-Zwit. Conditions: (i) 4-phenylazobenzene, K_2CO_3 , acetonitrile, 80°C, 12 h, 62 %; (ii) β -propiolactone, acetone, 2 h, 80°C, 92 %.

N,N-Dimethyl-3-(4-(phenyldiazenyl)phenoxy)propan-1-amine (1)



To a solution of 1-bromo-3-(*N,N*-diethyl)propane hydrobromide (200 mg, 0.405 mmol, 1 eq) in 10 mL acetonitrile, K_2CO_3 (170 g, 0.61 mmol, 1.5 eq) was added, and the suspension was stirred for 30 min at 70°C. After that time 4-phenylazobenzene (160 mg, 0.405 mmol, 1 eq) was added and stirring was continued at 80 °C for 12 h, followed by removal of the solvent. The residue was dissolved in 25 mL of $CHCl_3$ and extracted three times with distilled water (10 mL). The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated and the residue was subjected to silica gel column chromatography ($CH_2Cl_2/MeOH$ gradient elution 100:1 to 98:2). The product **1** was obtained as a yellow solid. **Yield:** 143 mg, 62%. **1H -NMR (400 MHz, $CDCl_3$):** δ = 7.86-7.79 (m, 4H, 4CH); 7.45-7.41 (m, 2H, 2CH); 7.39-7.35 (m, 1H, CH); 6.96-6.92 (m, 2H, 2CH); 4.07-4.04 (m, 2H, CH_2); 2.56 (t, 2H, CH_2); 2.32-2.27 (s, 6H, 2 CH_3); 2.05-1.99 (q, 2H, CH_2). **^{13}C -NMR (100 MHz, CD_2Cl_2):** δ = 163.5, 154.6, 148.8, 132.3, 130.9, 126.5, 124.3, 116.6, 68.2, 57.8, 46.8, 31.6, 28.8. **ESI-MS (m/z):** calculated for $[C_{17}H_{21}N_3OH]^+$: 284.17, found: 284.20

3-(Dimethyl(3-(4-(phenyldiazenyl)phenoxy)propyl)ammonio)propanoate (2, Azo-Zwit)



To an ice-cooled solution of **1** (60 mg, 0.21 mmol 1 eq) in 10 mL dry acetone, β -propiolactone (18 mg, 0.252 mmol, 1.2 eq) in 1 mL acetone was added slowly. The reaction mixture was stirred 2 h at 5°C and slowly warmed up to RT. The yellow precipitate was filtered and washed with anhydrous acetone and anhydrous ether. The product was dried under reduced pressure to obtain the final (E)-3-(dimethyl(3-(4-(phenyldiazenyl)phenoxy)propyl)ammonio) propanoate (**2**, Azo-Zwit). **Yield:** 27 mg, 36%. **1H -NMR (400 MHz, D_2O):** δ = 7.75-7.69 (dd, 4H, 4CH); 7.50-7.48 (d, 3H, 3CH); 7.03-7.01 (d, 2H, 2CH); 4.11 (t, 2H, CH_2); 3.54-3.50 (t, 2H, CH_2); 3.43 (t, 2H, CH_2); 3.02 (s, 6H, 2 CH_3); 2.70-2.66 (t, 2H, CH_2); 2.18-2.12 (t, 2H, CH_2). **^{13}C -NMR (150 MHz, $DMSO-d_6$):** δ = 170.98, 161.43, 152.46, 146.95, 131.40, 129.89, 129.57, 125.08, 123.44, 122.74, 119.78, 115.63, 114.88, 65.69, 61.25, 60.81, 50.64, 30.28, 20.68. **ESI-MS (m/z):** calculated for $[C_{20}H_{25}N_3O_3H]^+$: 356.19, found: 356.19.

1.3 Supramolecular nanoparticle assembly

Supporting Information: Zwitterionic supramolecular nanoparticles: Self-assembly and responsive properties

For the preparation of the zwitterionic supramolecular nanoparticles (ZSNPs), aqueous or PBS solutions of CB[8], MV-PEI and different concentrations of Azo-Zwit were prepared before mixing. DMSO was used to dissolve different concentrations of Azo₈-PAMAM. For example, for preparing a solution having 30% Azo entities derived from the aliphatic Azo₈-PAMAM dendrimer, 1000 μL MV-PEI (0.31 μM) was added to a previously prepared solution of 500 μL Azo-Zwit (1.96 μM), 20 μL Azo₈-PAMAM (2.65 μM) and 500 μL CB[8] (2.8 μM). The sample was mixed and kept at room temperature for 2 days before DLS and SEM analysis.

1.4 Analysis of ZSNPs

Dynamic Light Scattering (DLS)

The hydrodynamic diameters of the self-assembled zwitterionic particles were investigated by dynamic light scattering. The observed sizes and standard deviations of the SNPs are based on the average number distributions of minimum 5 individual measurements per sample. Three samples were measured for each reported ZSNP formulation.

Scanning Electron Microscopy (SEM)

Sizes and shapes of SNPs were analyzed by SEM. Aqueous solutions of the prepared SNPs were dropcast on a Formvar coated copper TEM grid. The solution was dried and analyzed without further treatment.

1.5. Control experiments for ZSNP formation

To evaluate whether ZSNP formation is triggered by the formation of the ternary complex between Azo, MV, and CB[8], different control experiments were carried out. ZSNP formation using 30% Azo from Azo₈-PAMAM was carried out without CB[8] or in presence of the cucurbit[n]uril homolog CB[7]. Hereto, the ZSNP solutions were prepared with 500 μL water or 500 μL aqueous CB[7] (2.8 μM) instead of CB[8]. Additionally, ZSNP formation was evaluated in the absence of MV-PEI.

1.6 SNP formation with Azo-tri(ethylene glycol)

To get a better understanding of the stabilizing role of Azo-Zwit in the formation of ZSNPs, SNP assembly was attempted in PBS using Azo-tri(ethylene glycol) as the shell-forming component. Different formulations, containing 10%, 20% and 30% Azo from Azo₈-PAMAM, were tested. As for the ZSNPs and for the Azo-PEG stabilized SNPs reported before,² SNP formation was evaluated by DLS after 2 days and after an additional 5 days upon mixing. SEM images are shown in Figure 1d and Figure S6.

1.7 Formation and stability of ZSNPs in PBS with different salt concentrations

In order to investigate the ZSNP formation in PBS at different ionic strengths, solutions of MV-PEI (0.31 μM), Azo-Zwit (1.96 μM) and CB[8] (2.8 μM) were prepared in PBS (pH 7.4) containing 50 mM, 100 mM, 140 mM, 300 mM and 700 mM KCl. These solutions were mixed according the recipe given for the preparation of the ZSNPs in water (containing 30% Azo from Azo₈-PAMAM). ZSNP formation was analyzed by DLS after 2 days (Figure S7) at RT, and by SEM (Figure 2) 7 days after preparation.

1.8 Stability of ZSNPs in PBS containing BSA to reflect protein concentration in human body

To verify the stability of the ZSNPs in the presence of proteins, ZSNPs were formed in PBS using an overall concentration of 14 μM of the supramolecular host-guest binding partners and 30% Azo from Azo₈-PAMAM. This mixture was kept 2 d at room temperature to ensure stable ZSNP formation. In addition, a 5 wt% dispersion of BSA in water was prepared. Stability of the ZSNPs was controlled after mixing 500 μL of the BSA solution with 250 μL of the ZSNP solution. DLS results of the formed ZSNPs, the BSA dispersion and the mixture of both are shown in Figure S8. As the ZSNPs were not observable by DLS after mixing with the BSA solution, SEM was carried out 1 d after mixing the two solutions. As visible in the SEM images, ZSNPs are clearly observable in between a thick layer of protein (Figure 2f) and at the edge of the SEM preparation drying spot (Figure S8d).

1.9 Photoisomerization of Azo-Zwit

Supramolecular encapsulation of Azo-Zwit in presence of CB[8] and in presence of paraquat (MV) and CB[8] was evaluated by UV/Vis spectroscopy at room temperature. Stock solutions of Azo-Zwit, CB[8] and paraquat were prepared in water. The stock solutions were mixed to result in solutions with Azo-Zwit (12.5 μM), Azo-Zwit/paraquat (12.5 μM /12.5 μM), Azo-Zwit/CB[8] (12.5 μM /6.25 μM), Azo-Zwit/paraquat/CB[8] (12.5 μM /12.5 μM /6.25 μM), Azo-Zwit/CB[8] (12.5 μM /12.5 μM), Azo-Zwit/paraquat/CB[8] (12.5 μM /12.5 μM /12.5 μM). The UV/Vis absorbance spectra of these solutions were measured at visible light conditions (Figure S11) and after irradiation with UV light (Figure S12) to evaluate the encapsulation efficiencies of the two photoisomers of Azo-Zwit by CB[8] in the absence and presence of paraquat.

1.10 Photoswitching of ZSNPs

To evaluate the photoresponsive properties of the particles, ZSNPs containing 30% Azo from Azo₈-PAMAM were prepared. Samples were irradiated with UV light at $\lambda < 400$ nm for different times. DLS analysis was performed immediately after exposure, while keeping the SNP samples in the dark. Representative DLS data are shown in Figure S13 for different irradiation times and after subsequent visible light treatment. It has to be noted that the obtained hydrodynamic diameters of the UV-treated ZSNP samples are not absolute values since the polydispersity of the samples was too high.

1.11 pH-induced aggregation of the ZSNPs

In order to investigate the ZSNP formation in PBS, solutions of MV-PEI (0.31 μM), Azo-Zwit (1.96 μM) and CB[8] (2.8 μM) were prepared in PBS at pH 5.6, 6.2, 6.8 and 7.4. ZSNP formation (30% Azo from Azo₈-PAMAM) at these pH values was carried out as described for ZSNP preparation in water by mixing the aqueous solutions with Azo₈-PAMAM in DMSO (2.62 μM). ZSNP formation was analyzed by DLS after 2 days at RT (Figure S14a and b) and by SEM 5 days after preparation (Figure 3 and Figure S14c and d).

2. **Supporting figures**

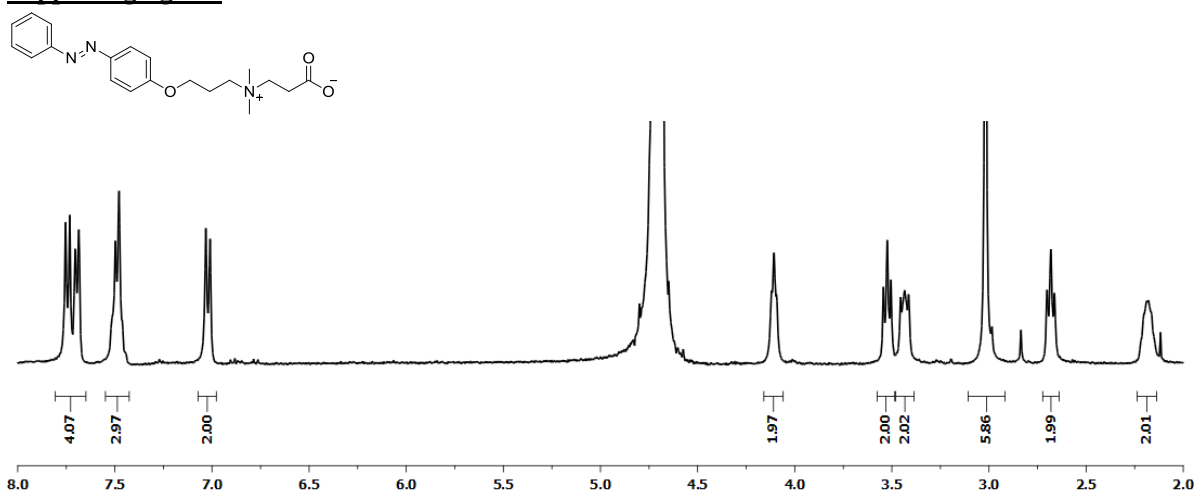


Figure S2: ¹H-NMR of compound (2, Azo-Zwit) (400 MHz, D₂O, solvent peak at 4.7 ppm).

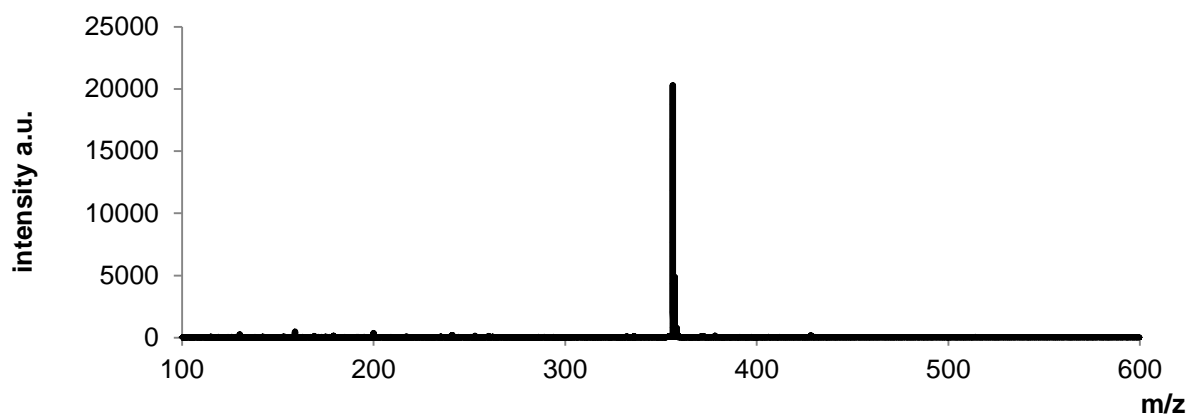


Figure S3: ESI-MS spectrum of (2, Azo-Zwit).

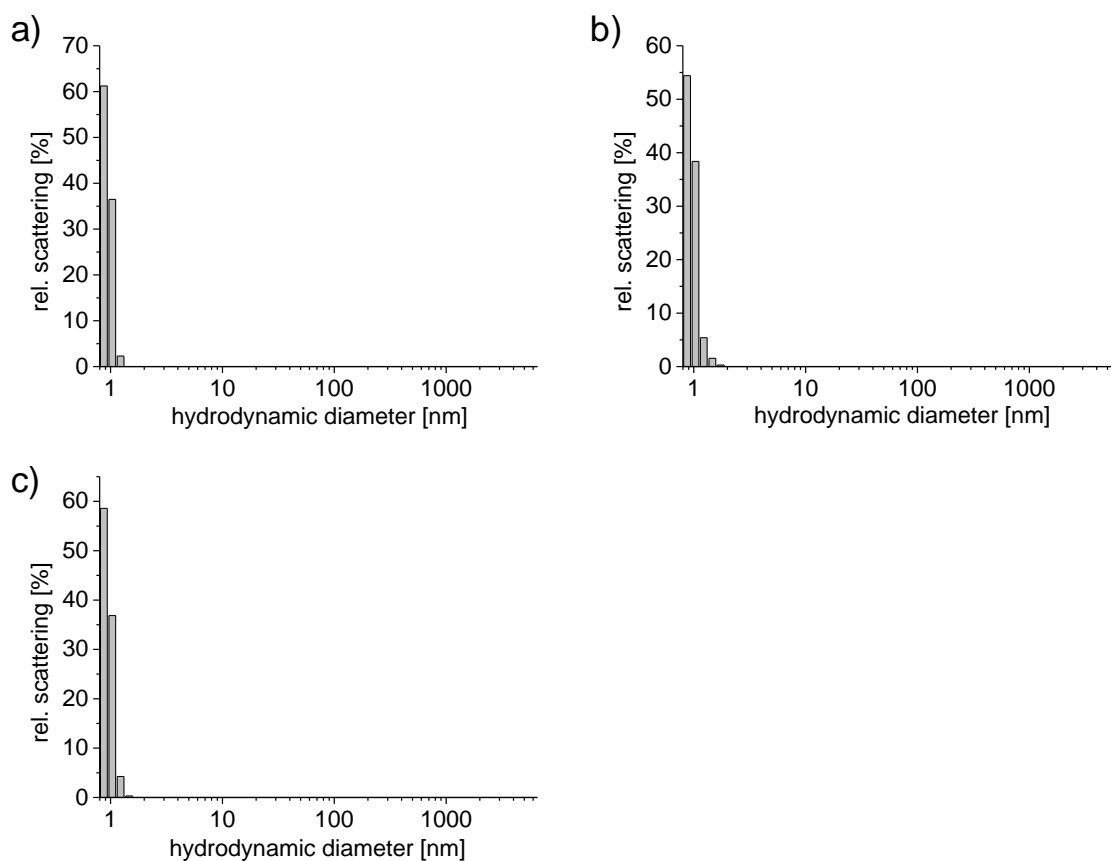


Figure S4: DLS analysis of ZSNP solutions (30% Azo from Azo₈-PAMAM, in water) a) in the absence of CB[8], b) in the presence of CB[7] instead of CB[8], c) in the absence of MV-PEI.

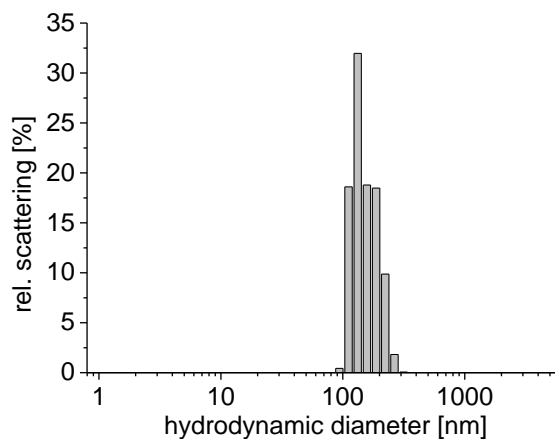


Figure S5: DLS analysis of a ZSNP solution prepared with 30% Azo from Azo₈-PAMAM in PBS, 140 mM KCl, pH 7.4 ($M_n = 137.2$, PDI = 0.3)

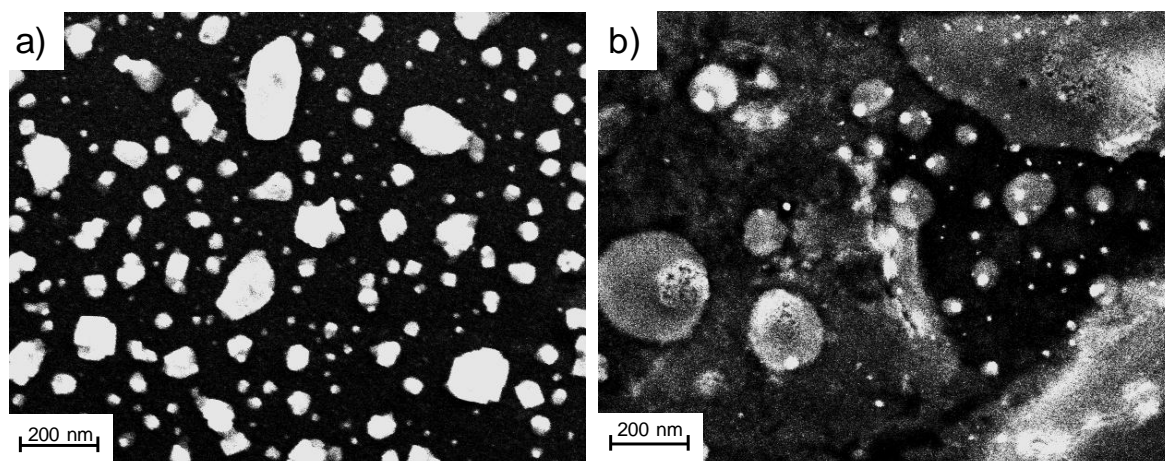


Figure S6: SEM images of different SNP formulations prepared with Azo-tri(ethylene glycol): a) 10% Azo from AzO₈-PAMAM b) 20% Azo from AzO₈-PAMAM.

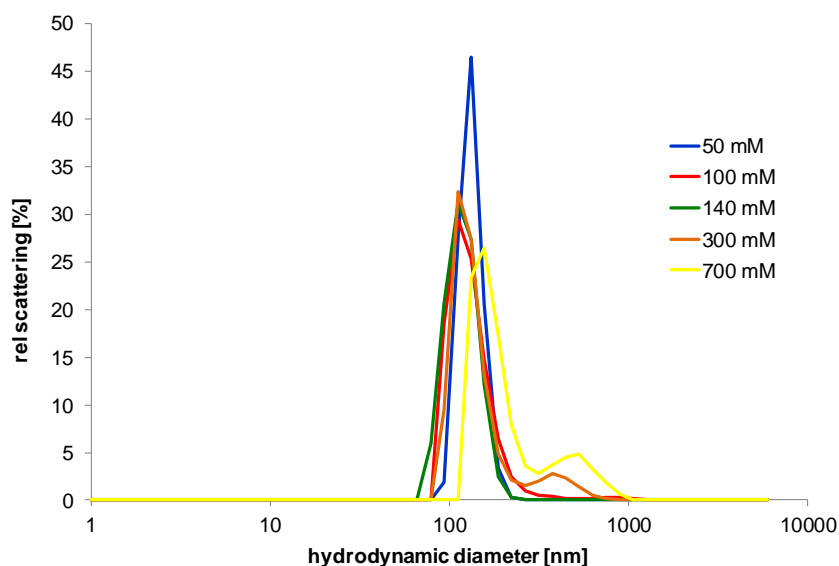


Figure S7: Representative DLS graphs observed for the ZSNPs (30% Azo from AzO₈-PAMAM) prepared in PBS (pH 7.4) with different KCl concentrations.

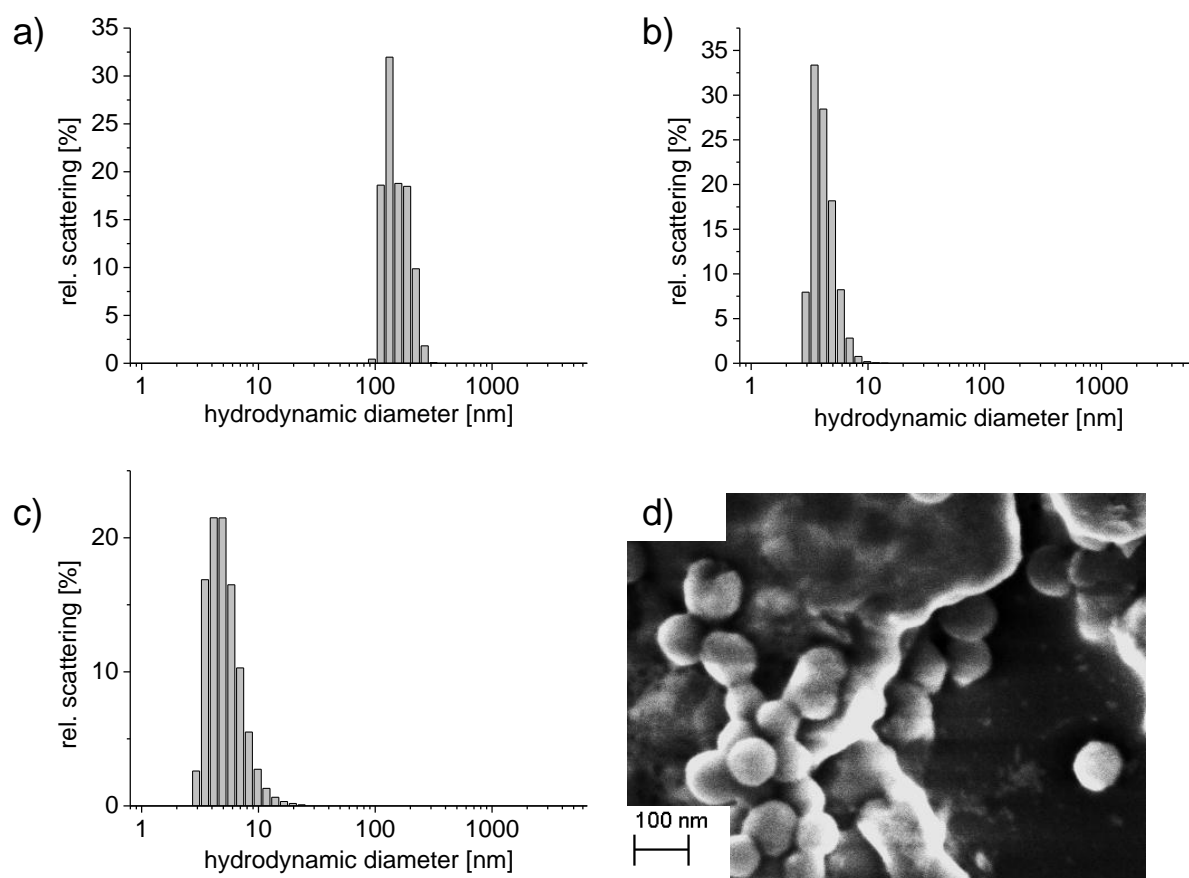


Figure S8: DLS and SEM analysis of ZSNPs with BSA in PBS: a) ZSNPs in PBS prepared with 14 μM concentrations of Azo, MV, and CB[8] moieties, b) 5 wt% BSA in PBS, c) 1:2 v/v mixture of ZSNP and BSA solutions, d) SEM image of ZSNPs prepared at 140 KCl and mixed subsequently with 5 wt% BSA (v:v 1:2), imaged at the center of the sample.

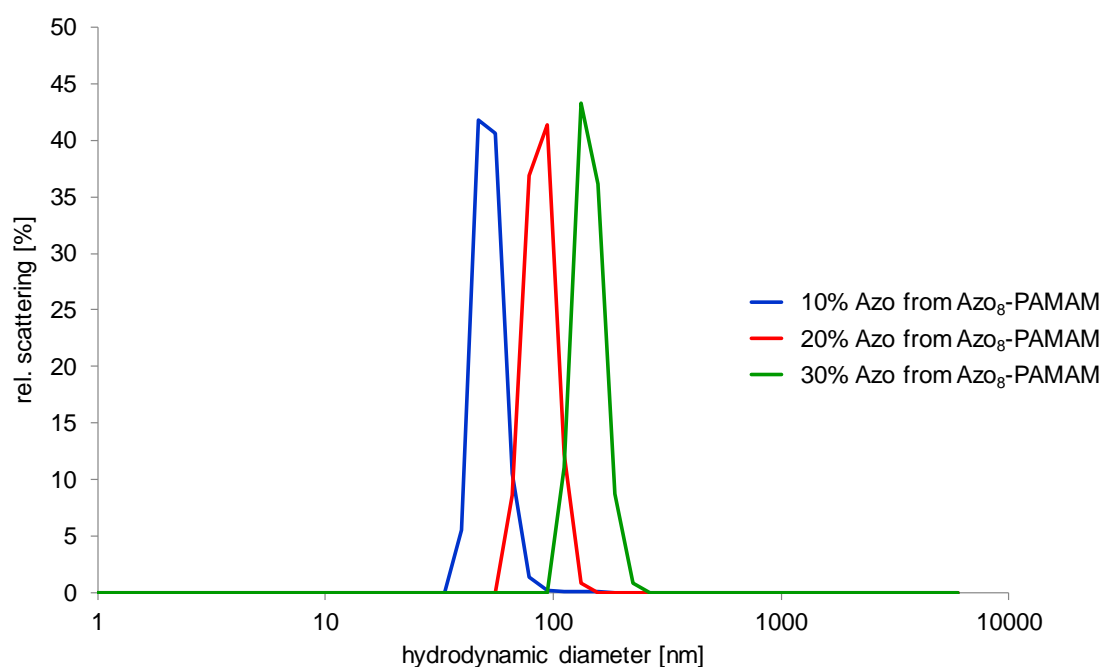


Figure S9: DLS graphs obtained for the ZSNP formulations prepared in water using different stoichiometries of the mono- and multivalent Azo components while maintaining Azo:MV:CB[8] at 1:1:1.

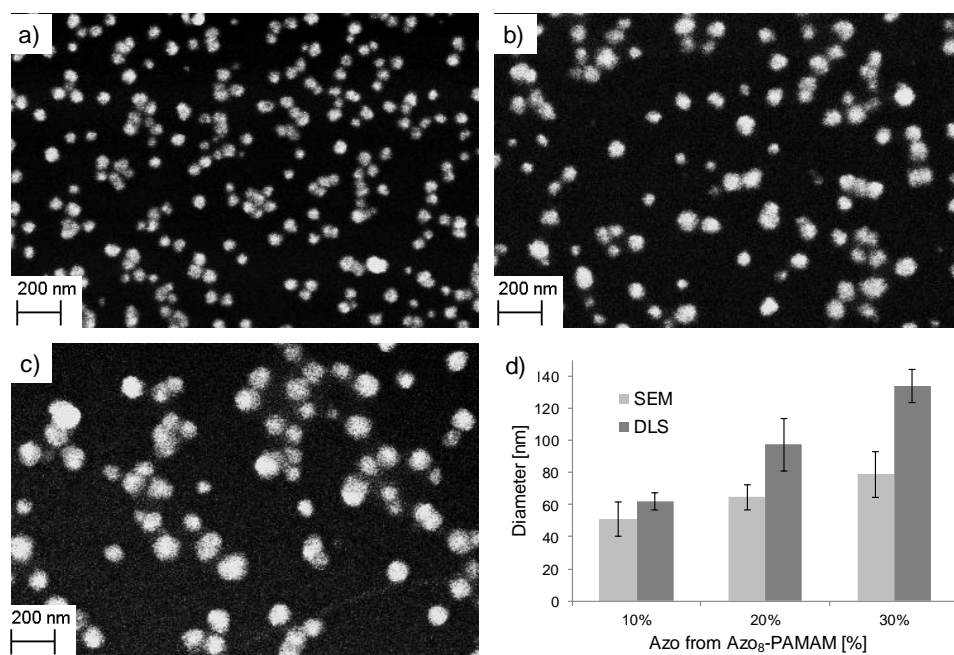


Figure S10: Size determination of ZSNPs prepared with different formulations: a-c) SEM images of ZSNPs prepared in water with a) 10%, b) 20%, and c) 30% Azo from Azo₈-PAMAM dendrimer; d) ZSNP diameters prepared in water measured by SEM (■) and DLS (■)

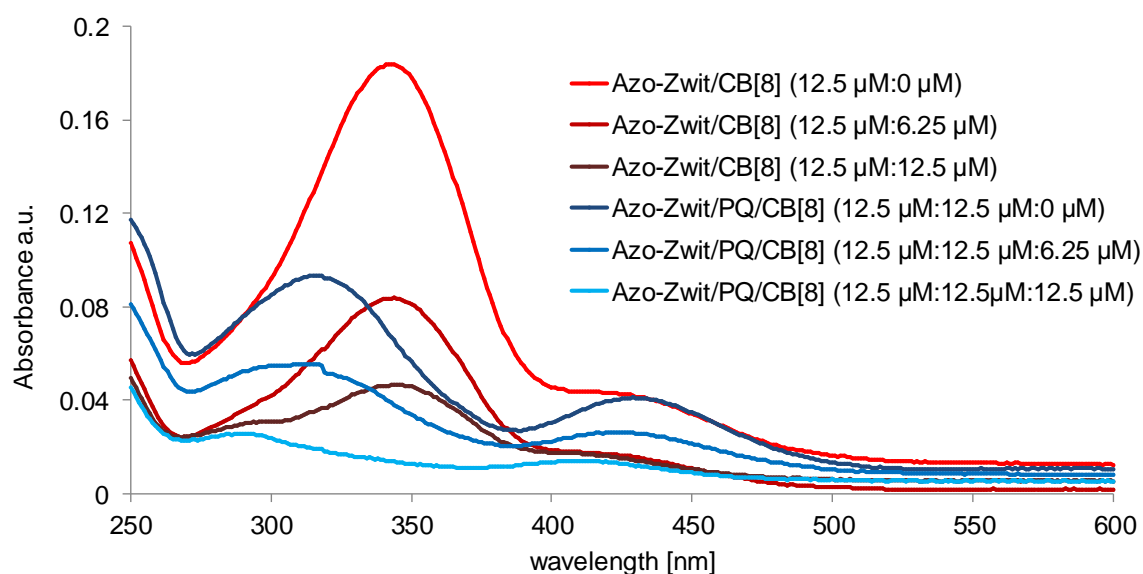


Figure S11: UV-Vis absorbance of Azo-Zwit and Azo-Zwit and paraquat (PQ) in presence of different CB[8] concentrations under ambient light conditions.

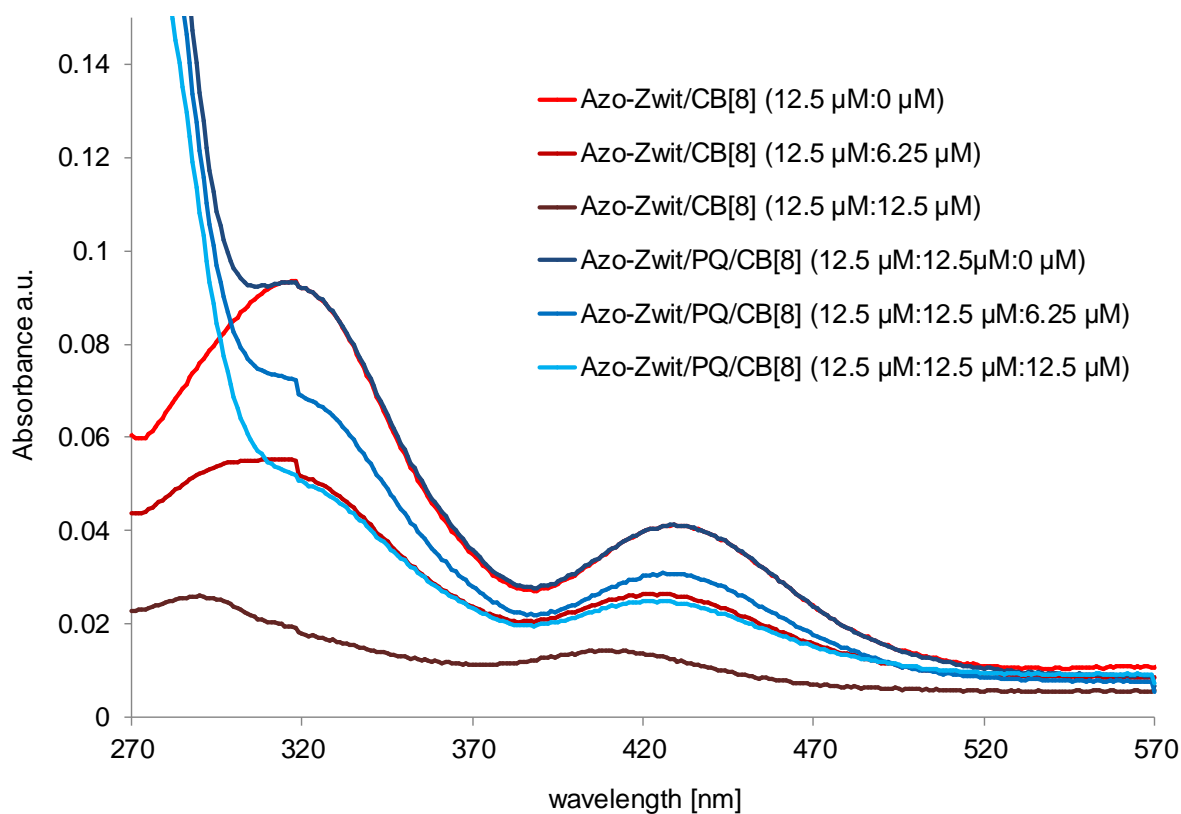


Figure S12: UV-Vis absorbance spectra of Azo-Zwit and Azo-Zwit and PQ in presence of different CB[8] concentrations in dark, after UV-light irradiation

Supporting Information: Zwitterionic supramolecular nanoparticles:
Self-assembly and responsive properties

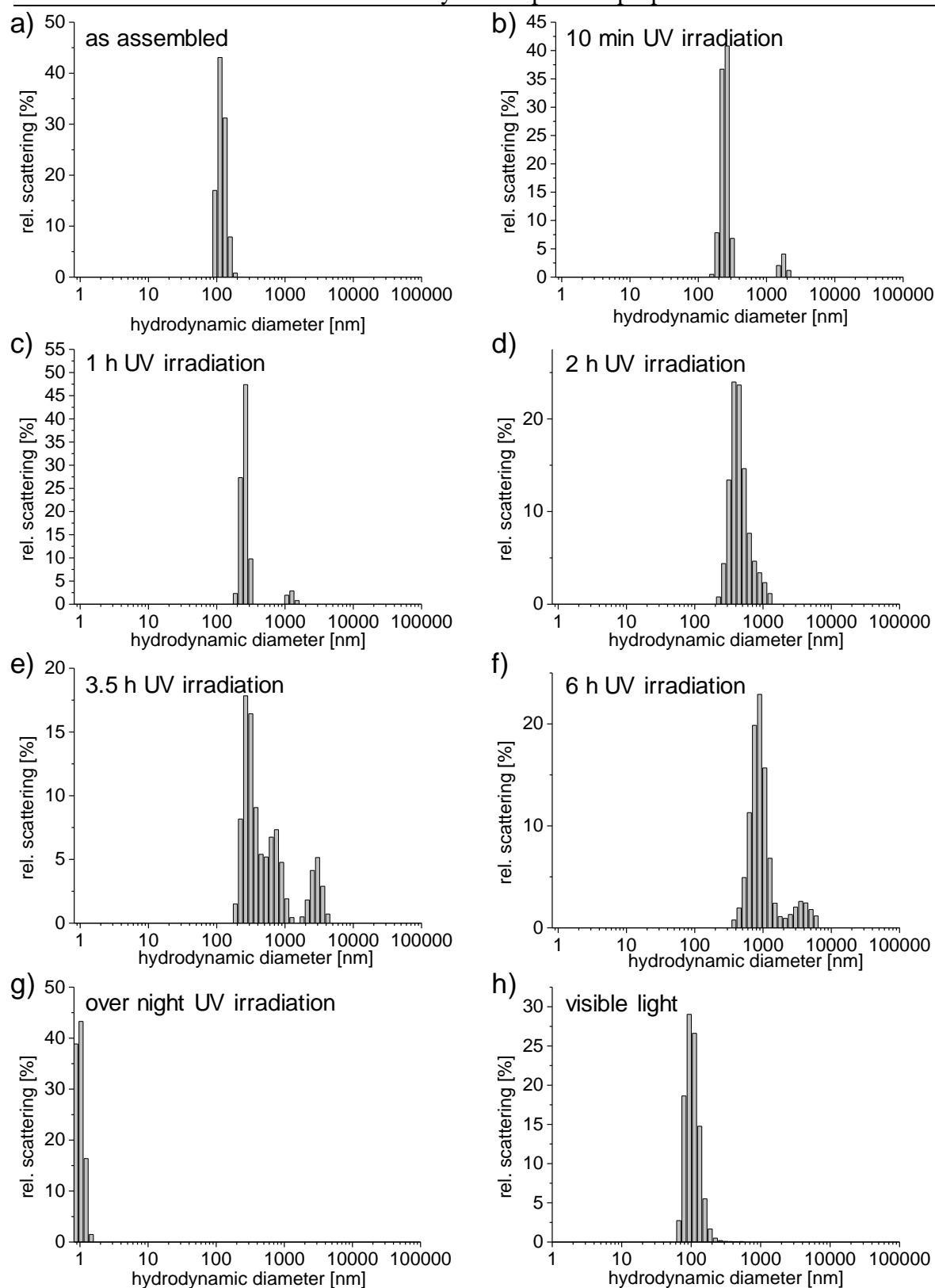


Figure S13: DLS analysis of a ZSNP solution: a) as assembled ($M_n = 132.5$ nm, PDI = 0.25), b) after 10 min UV irradiation, c) after 1 h UV irradiation, d) 2 h UV irradiation, e) 3.5 h UV irradiation, f) 6 h UV irradiation, g) 14 h UV irradiation, h) after 8 h visible light irradiation

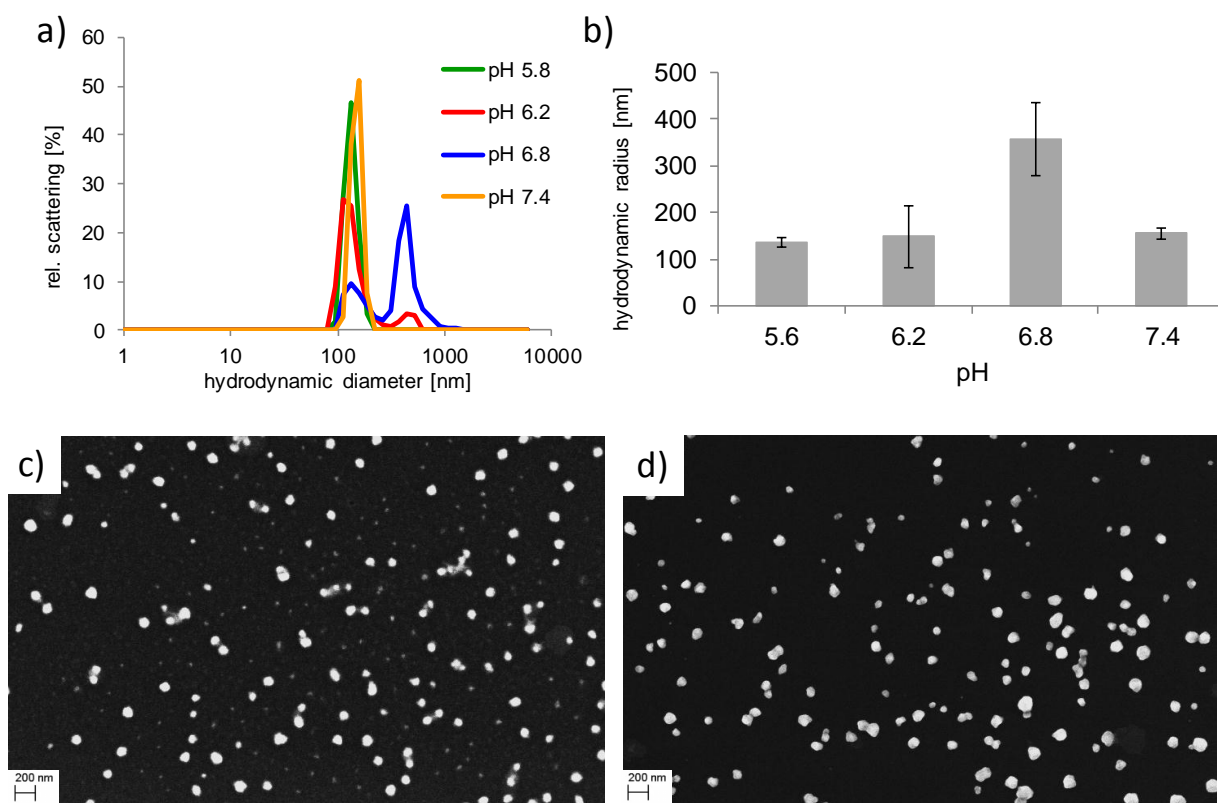


Figure S14: ZSNPs prepared in PBS at different pH values: a) representative DLS curves, b) average sizes of ZSNPs, c-d) SEM images obtained for ZSNPs prepared at c) pH 5.6 and d) pH 7.4.

3. References

- (1) Stoffelen, C.; Huskens, J. *Chem. Commun.* **2013**, *49*, 6740.
- (2) Stoffelen, C.; Voskuhl, J.; Jonkheijm, P.; Huskens, J. *Angew. Chem. Int. Ed.* **2014**, *53*, 3400.
- (3) Nalluri, S. K. M.; Voskuhl, J.; Bultema, J. B.; Boekema, E. J.; Ravoo, B. J. *Angew. Chem. Int. Ed.* **2011**, *50*, 9747.