Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B
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Supplementary Information

Protein triggered fluorescence switching of near-infrared emitting nanoparticles for contrast-enhanced imaging

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Experimental

Calculation of grafting densities:

The molar extinction coefficient of the free dye in the dilute regime and wavelength range of 615 nm to 685 nm is used to estimate the number of fluorophores attached to the particles. First, the molar extinction coefficient for the dye in the dilute regime is measured. The concentration of the modified particles solution is estimated using Beer's law ($A = \varepsilon bc$ where A is absorbance, ε is molar extinction coefficient, in $M^{-1}cm^{-1}$, b is path length in cm, and c is concentration in M). 500 μL of the modified particles solution is dried out to measure the mass. The diameter of the unmodified particle is measured using the DLS (Dynamic Light Scattering) and the surface area, mass and volume of a particle are calculated. Using the mass of a particle and the mass of 500 μL of solution, the number of modified particles per mL is calculated by assuming that the mass of the dye is negligible compared to the mass of the particle. Finally, the concentration and number of particles is used to determine the grafting density and distance between two chromophores.

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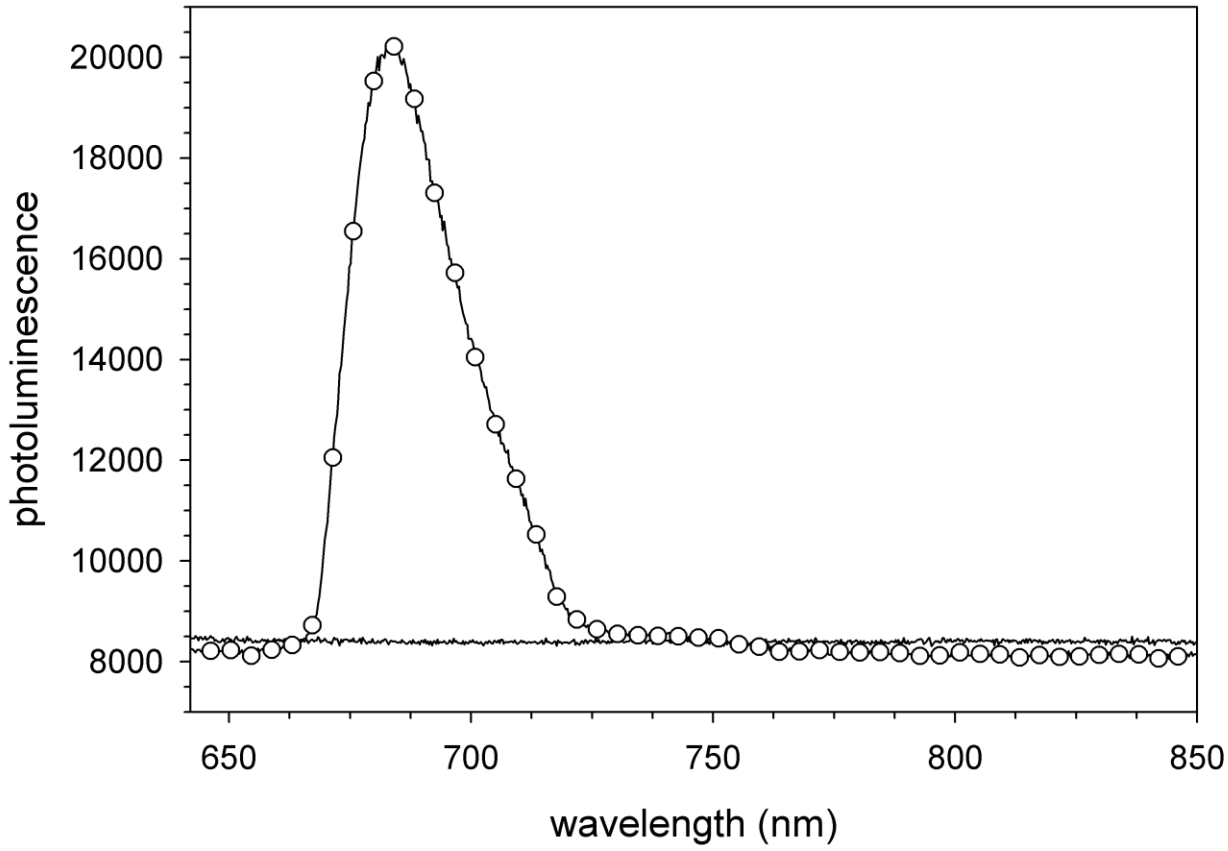
Results & Discussion

Squaraine/ Protein Complexation

Fluorescence response spectra of FP_5 nanoparticles before and after addition of the surfactant, sodium dodecyl sulfate (SDS):



Fluorescence spectra of FP_5 nanoparticles (6.53 µM) in water before (—) and 18 hours after addition of 50 mg of SDS (○). Excitation at 630 nm.

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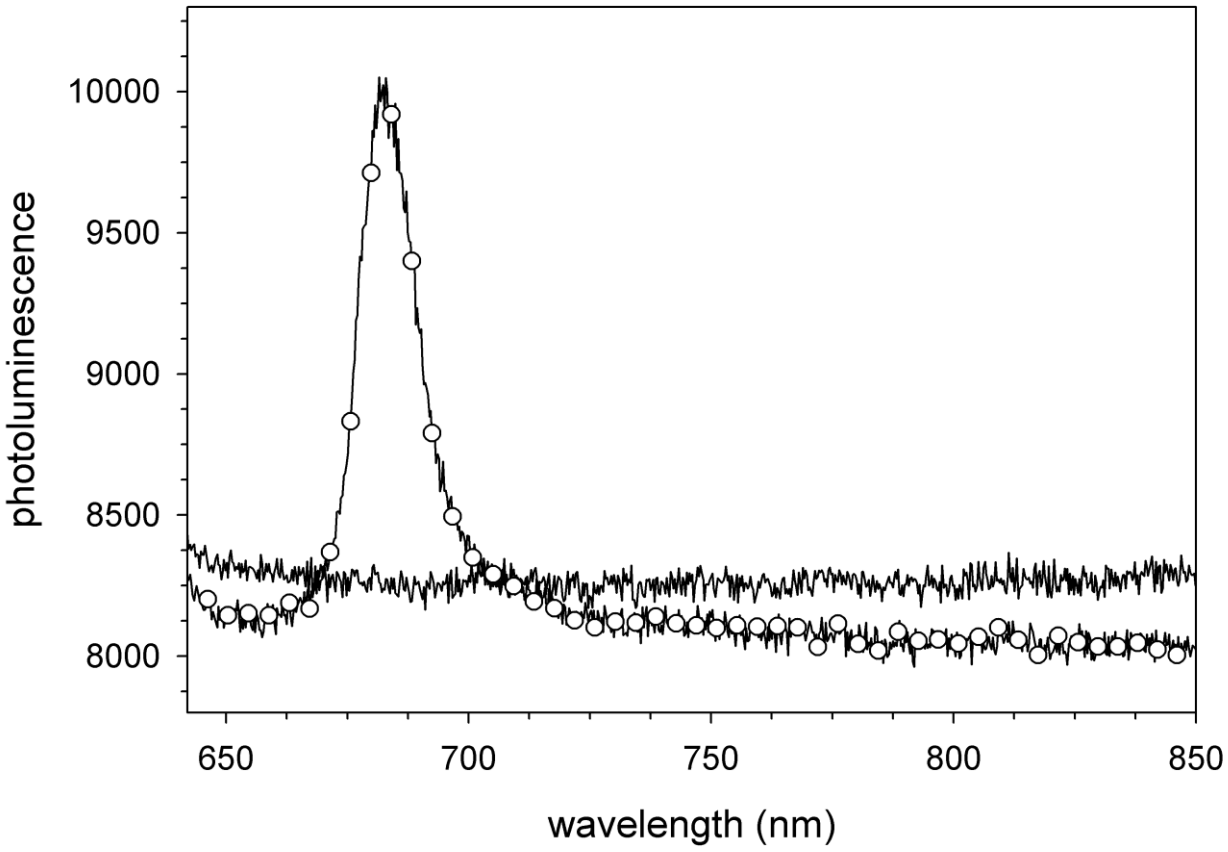
Fluorescence response spectra of FP_7 nanoparticles before and after addition of the surfactant, sodium dodecyl sulfate (SDS):



Fluorescence spectra of FP_7 nanoparticles (4.97 µM) in water before (—) and 18 hours after addition of 50 mg of SDS (○). Excitation at 630 nm.

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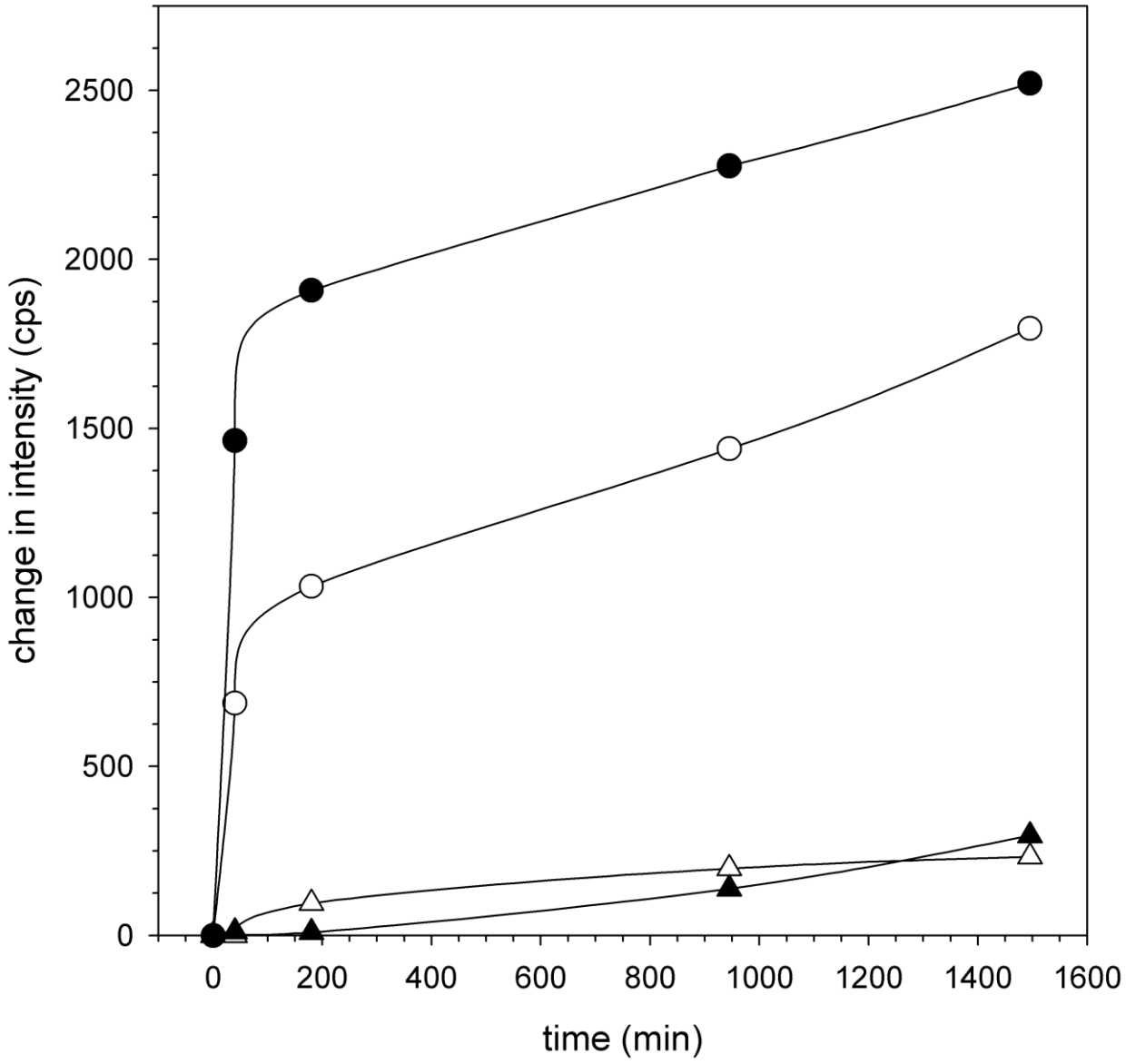
Change in intensity of FP_5 nanoparticles activated selectively by albumins:



Changes in photoluminescence intensity of FP_5 nanoparticles (0.03 mM) in phosphate buffered solution (PBS) with the addition of the four different proteins (0.04 mM) [Bovine serum albumin (○), human serum albumin (●), lysozyme (△), and trypsin (▼)]. The HSA and BSA curves typically merge after 4 days of incubation, though the lysozyme and trypsin never significantly increase the particle's emission. Excitation at 630 nm.

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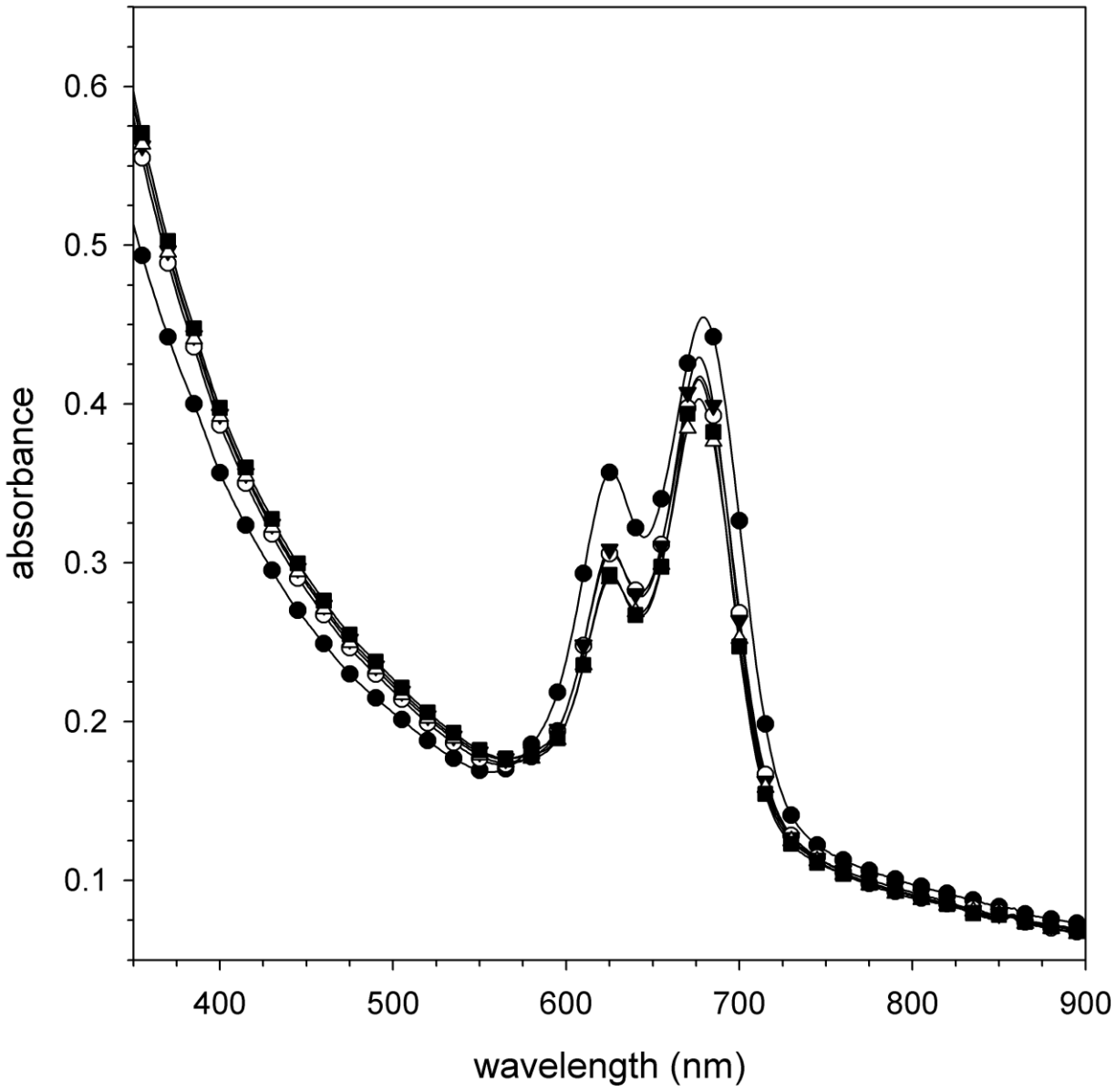
Changes in absorbance spectra during annealing of FP_5 particles with bovine serum albumin (BSA):



Changes in absorbance spectra of FP_5 nanoparticles in phosphate buffered solution (PBS) with 15 mg of BSA. [Initially at 20 ^{o}C (●), at 70 ^{o}C (○), after 70 ^{o}C anneal then cooled to 20 ^{o}C (▼), after 70 ^{o}C, cool to 20 ^{o}C, heated to 70 ^{o}C (△) and after 70 ^{o}C, cool to 20 ^{o}C, heated to 70 ^{o}C, then cooled to 20 ^{o}C (■)]

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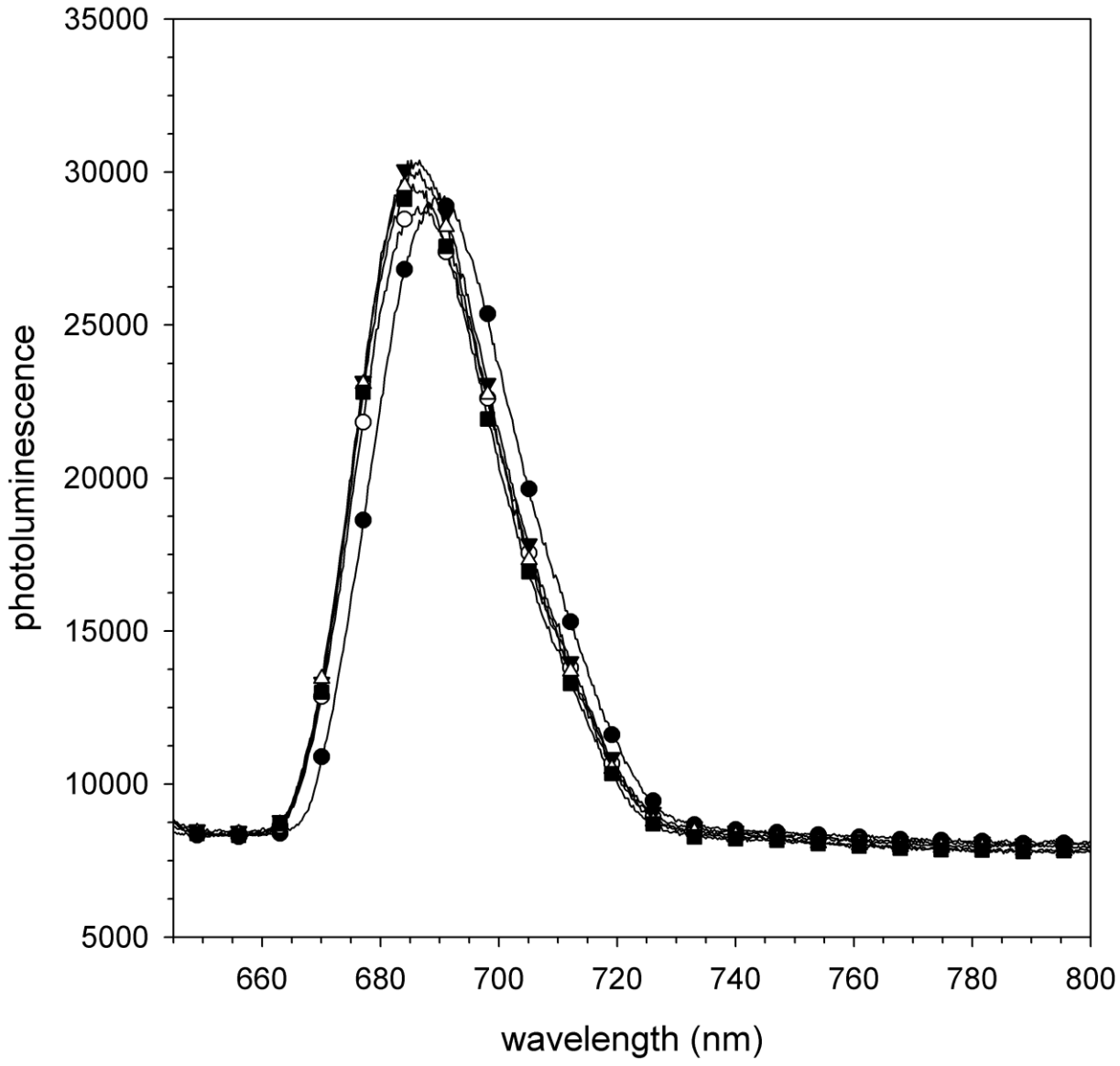
Changes in photoluminescence during annealing of FP_7 particles with bovine serum albumin (BSA):



Changes in photoluminescence of FP_7 nanoparticles in phosphate buffered solution (PBS) with 5 mg of BSA. [Initially at 20 ^{o}C (●), after heat and cool cycle 1 (○), after heat and cool cycle 2 (▼), after heat and cool cycle 3 (△) and after heat and cool cycle 4 (■)]. Excitation at 630 nm.

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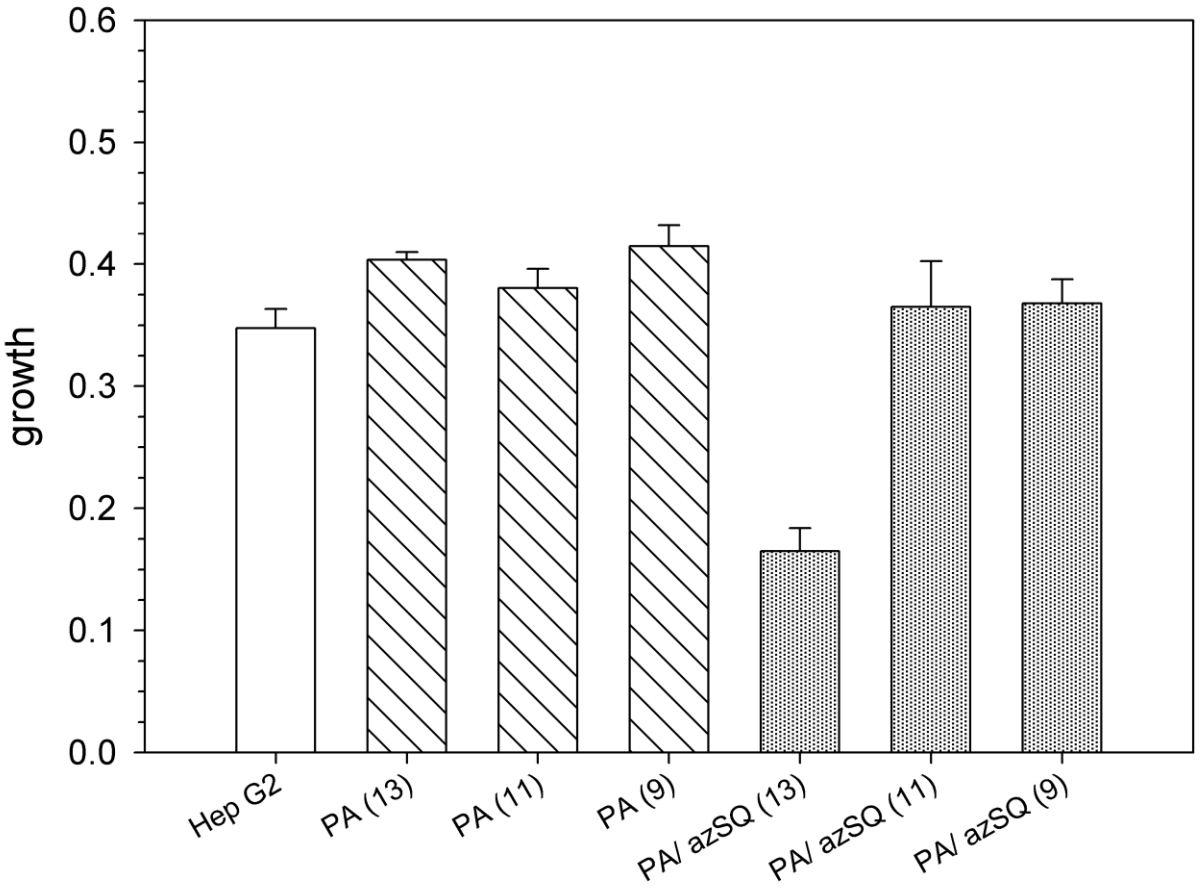
In Vitro Co-localization

Cytotoxicity tests carried out in HepG2 carcinoma cells:



Proliferation of HepG2 cells after 2 days of incubation with neat PA and PA/ azSQ (FP_5) nanoparticles at concentrations of ca. 2 x 10^{13}, 2 x 10^{11} and 2 x 10^{9} particles/ mL. Each condition was tested in six replicates. Cell viability was determined via 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium inner salt (MTS) assay. 2 x 10^{13} particles/ mL is about 63 µM for PA/ azSQ (FP_5).

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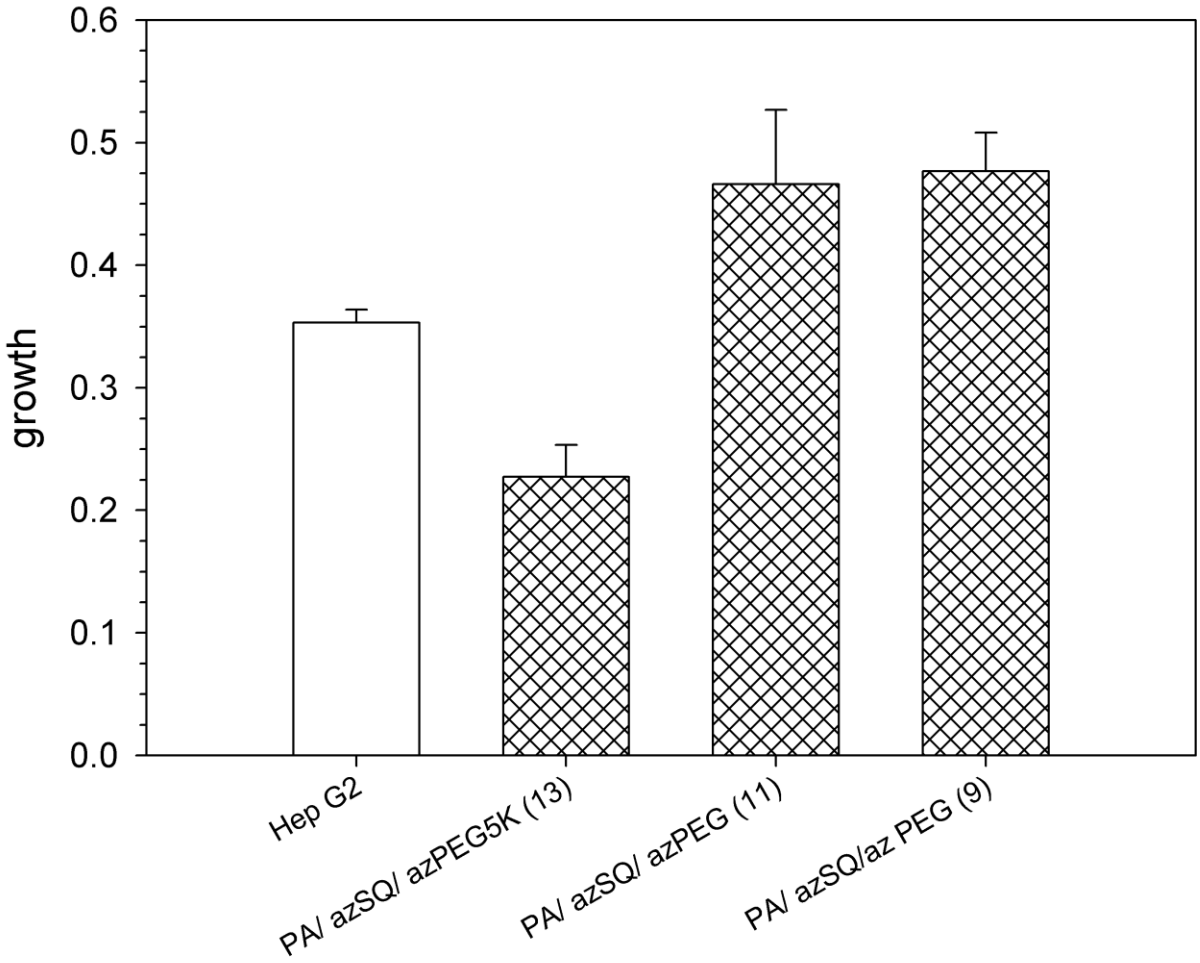

Proliferation of HepG2 cells after 2 days of incubation with PA/ azSQ/ azPEG (FP_7) nanoparticles at concentrations of ca. 2 x 10^9, 2 x 10^{11} and 2 x 10^{13} particles/ mL. Each condition was tested in six replicates. Cell viability was determined via 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium inner salt (MTS) assay. 2 x 10^{13} particles/ mL is about 37 µM for PA/ azSQ/ azPEG (FP_7).

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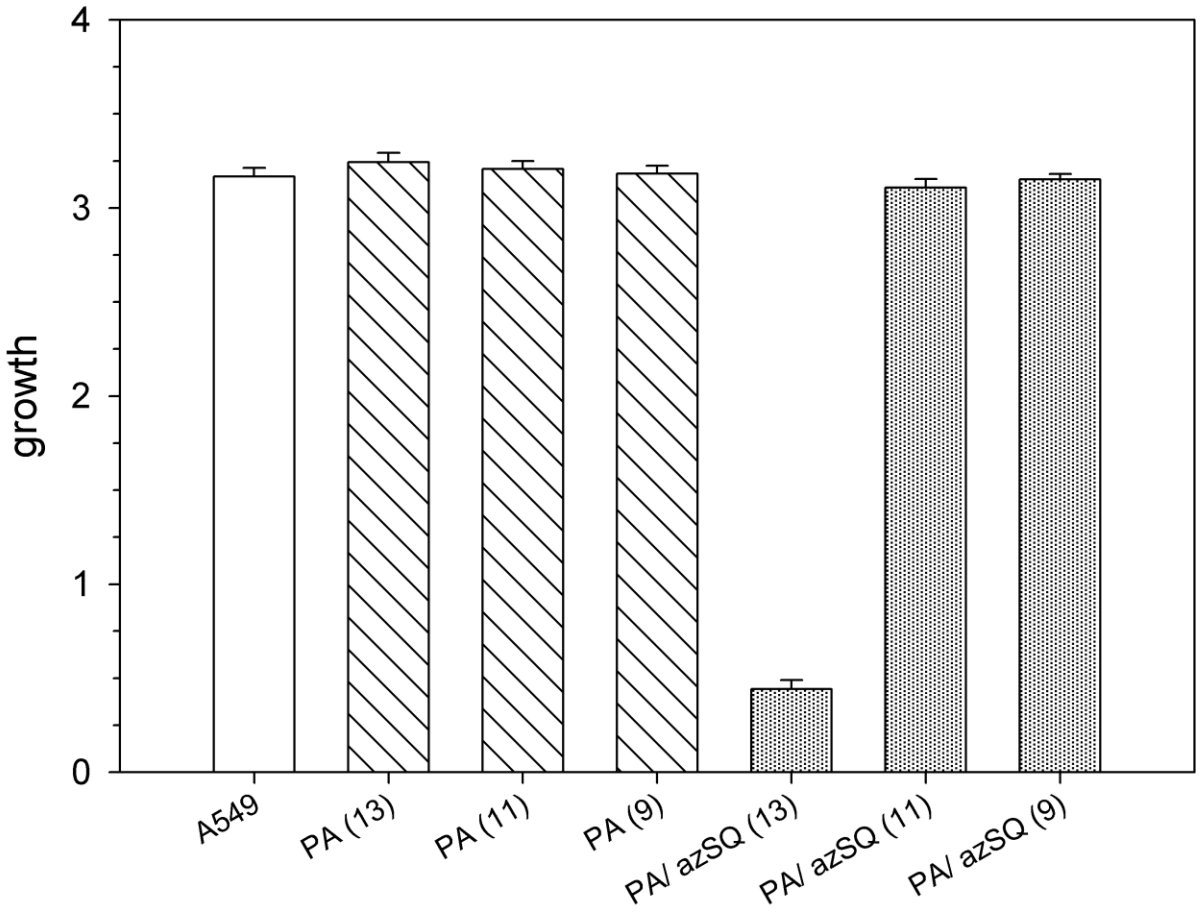
Cytotoxicity tests carried out in A549 carcinoma cells:



Proliferation of A549 cells after 2 days of incubation with neat PA and PA/ azSQ (FP_5) nanoparticles at concentrations of ca. 2 x 10^9, 2 x 10^{11} and 2 x 10^{13} particles/ mL. Each condition was tested in six replicates. Cell viability was determined via 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium inner salt (MTS) assay. 2 x 10^{13} particles/ mL is about 98 µM for PA/ azSQ (FP_5).

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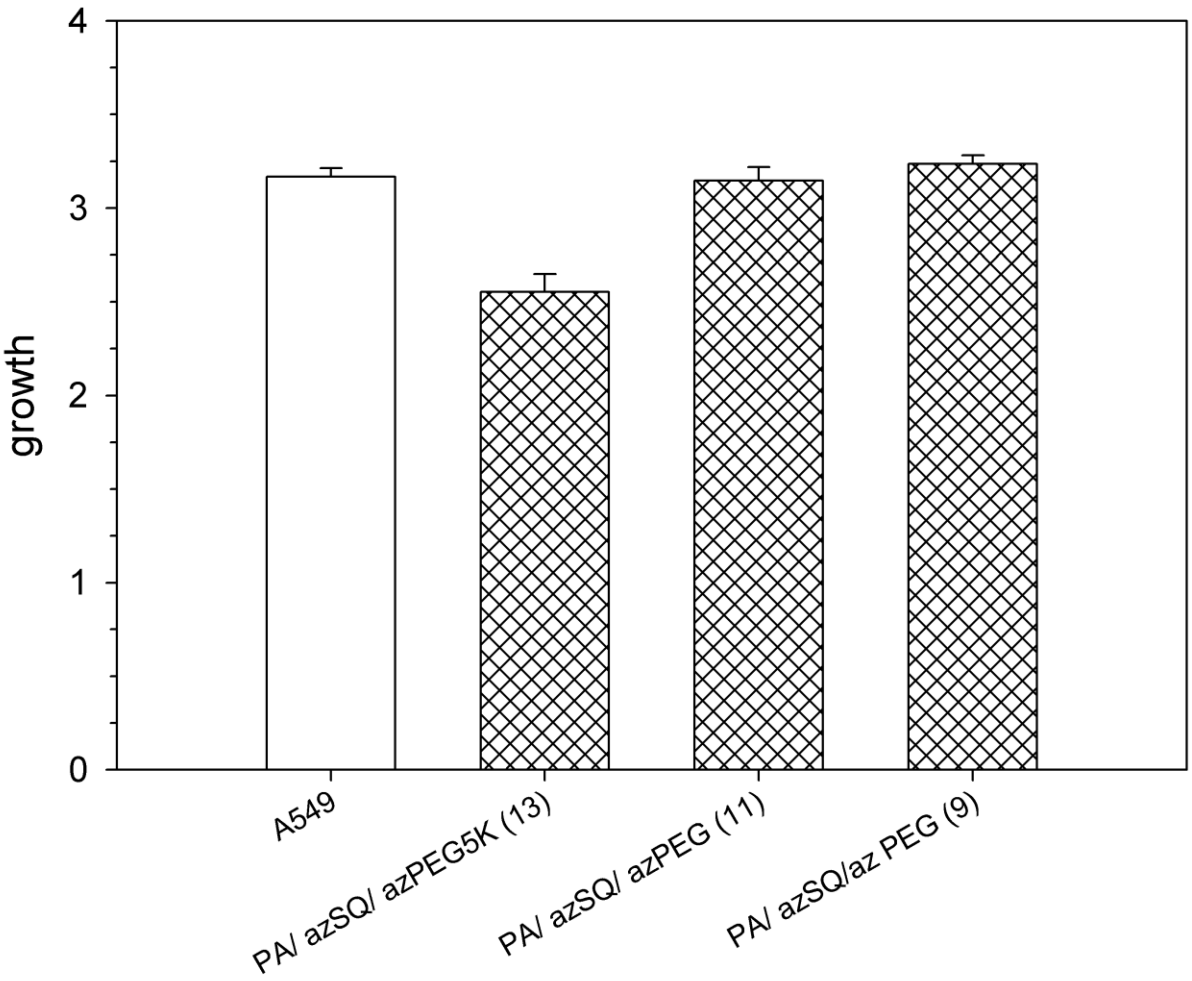

Proliferation of A549 cells after 2 days of incubation with PA/ azSQ/ azPEG (FP_7) nanoparticles at concentrations of ca. 2 x 10^9, 2 x 10^{11} and 2 x 10^{13} particles/ mL. Each condition was tested in six replicates. Cell viability was determined via 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium inner salt (MTS) assay. 2 x 10^{13} particles/ mL is about 75 µM for PA/ azSQ/ azPEG (FP_7).