Supporting Information

Interaction of functionalized nanoparticles with serum proteins and its impact on colloidal stability and cargo leaching

*Kathrin Abstiens, Sara Maslanka Figueroa, Manuel Gregoritza and Achim M. Goepferich**

Department of Pharmaceutical Technology, Faculty of Chemistry and Pharmacy, University of Regensburg, 93040 Regensburg, Germany

*E-mail: achim.goepferich@ur.de

Nanoparticle quantification by an iodine assay[1]

Three batches of each NP formulation (carboxyl-NPs, amine-NPs, zwitterion-NPs and methoxy-NPs) were prepared in ultrapure water, purified and concentrated by ultracentrifugation. A colorimetric iodine complexation assay was used to quantify the exact amount of PEG. In brief, NP samples were diluted in ultrapure water to a final PEG concentration of approximately 2.5 to 50 µg mL^{-1}. Dilutions of methoxy poly(ethylene glycol) with a molecular mass of 5000 g mol^{-1} were used as calibration standards (0 – 50 µg mL^{-1}). Two parts of a solution containing 5% (m/v) barium chloride in 1 N HCl were mixed with 1 part of a 0.1 N iodine/ potassium iodide solution immediately before use. 140 µL of sample and standard dilutions were transferred into a 96-well plate, mixed with 60 µL of the iodine reagent und incubated for 15 min at room temperature. Absorbance was measured at 535 nm using a FluoStar Omega fluorescence microplate reader (BMG Labtech, Ortenberg, Germany). The exact polymer concentration was determined gravimetrically after lyophilization of the concentrated NP samples and plotted against the PEG content determined by the iodine assay. As the PEG content of each NP formulation was considered to be constant, from this point on, the PEG content of freshly prepared samples was determined against the mPEG5k standard and used to calculate the polymer concentration.

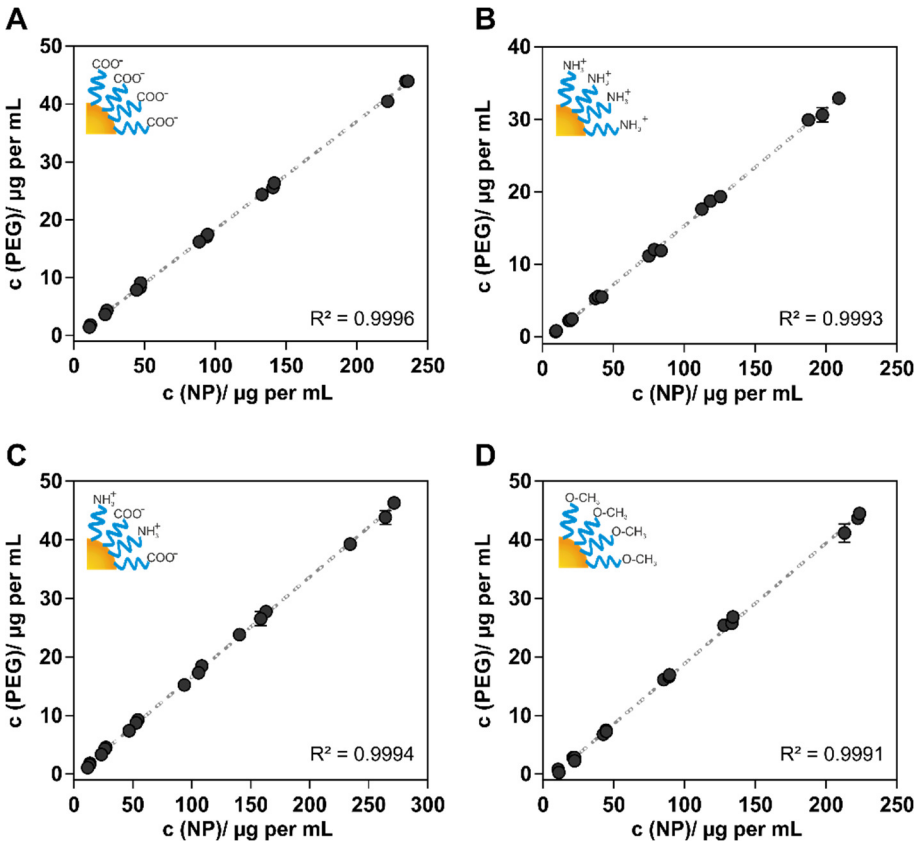

Figure S1. Determination of the NP concentration by an iodine assay. The linear correlation of the PEG content and the total polymer concentration of (**A**) carboxyl-NPs, (**B**) amine-NPs, (**C**) zwitterion-NPs and (**D**) methoxy_NPs allows for an absorbance based quantification of PLA-PEG/PLGA NPs.

1 C. E. Childs, *Microchem J*, 1975, **20**, 190–192.

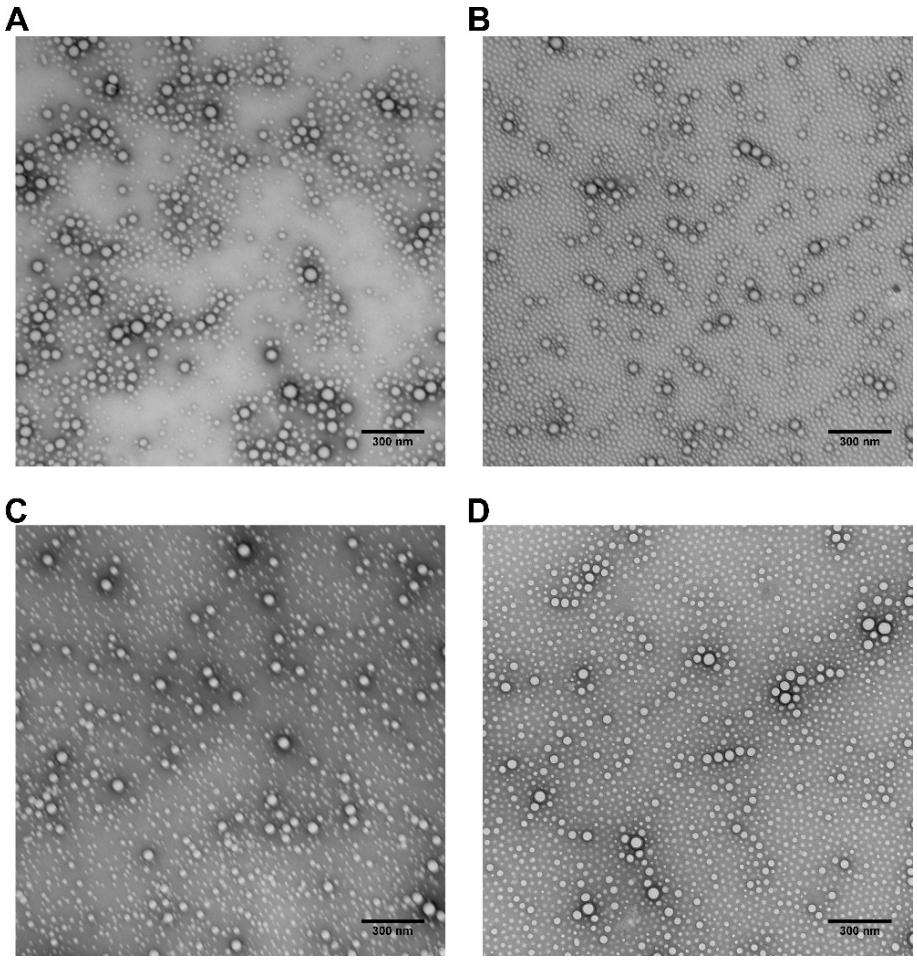

Figure S2. TEM images of (**A**) carboxyl-NPs, (**B**) amine-NPs, (**C**) zwitterion-NPs and (**D**) methoxy-NPs. TEM images were acquired at 50,000x magnification. Scale bars indicate 300 nm.

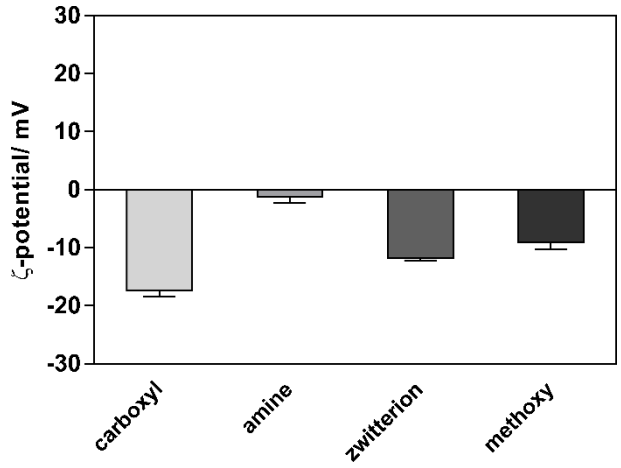

Figure S3. ζ-potential of (**A**) carboxyl-NPs, (**B**) amine-NPs, (**C**) zwitterion-NPs and (**D**) methoxy-NPs in 10 mM NaCl (pH 7.3) after incubation with FCS and subsequent purification by centrifugation. In contrast to native NPs, all NPs incubated with FCS possessed a negative ζ-potential.

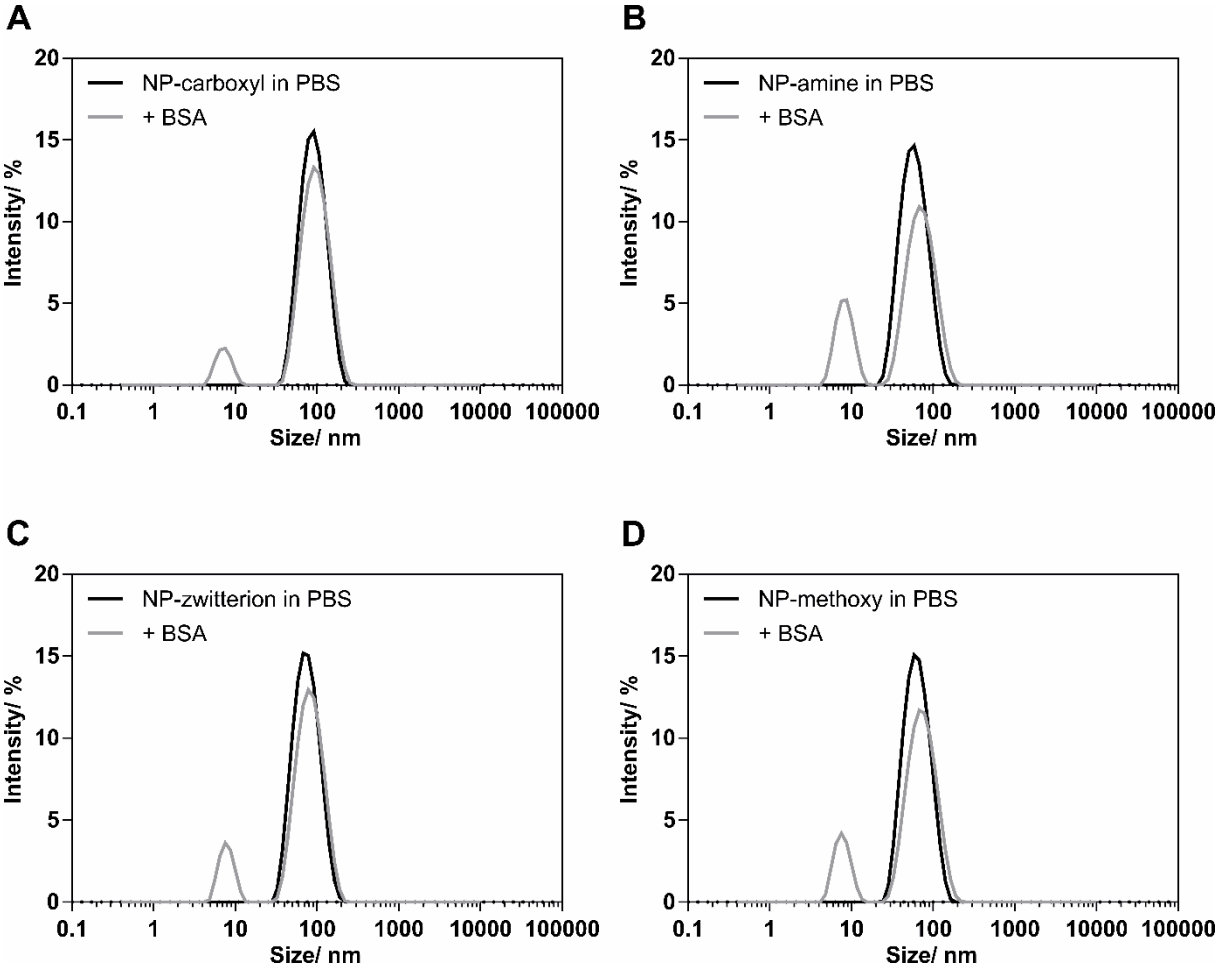

Figure S4. Unprocessed DLS data of (**A**) carboxyl-NPs, (**B**) amine-NPs, (**C**) zwitterion-NPs and (**D**) methoxy-NPs (corresponding to Figure 4).

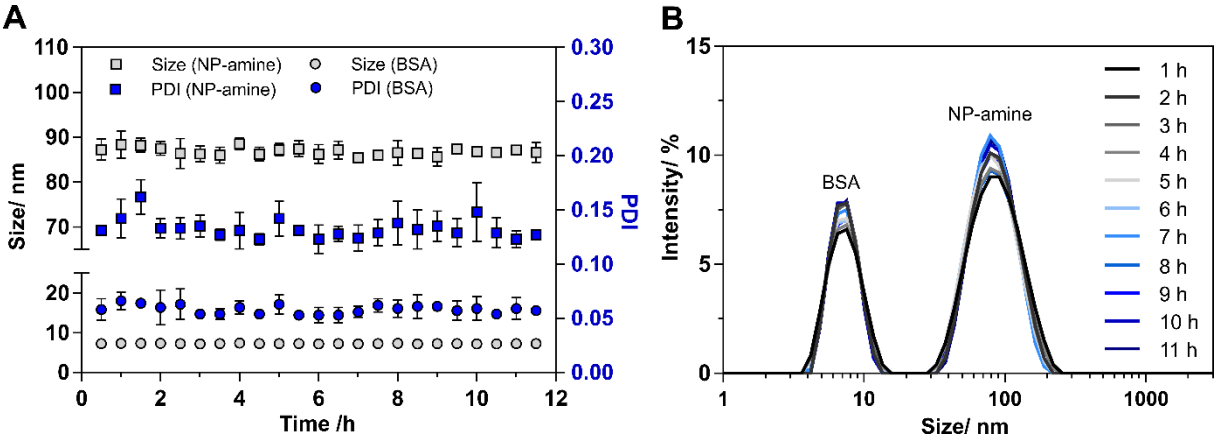

Figure S5. Size and size distribution of amine-NPs incubated with BSA at 37 °C. (**A**) Size[a] and PDI[b] of NP-amine (squares) and BSA (circles) measured in DPBS pH 7.4 as function of time. DLS measurements were conducted in 0.5 h intervals and respective peaks of NP-amine and BSA were evaluated for their mean size. (**B**) Corresponding intensity-weighed size distribution profiles as a function of incubation time. For reasons of clarity, only every second measurement is depicted.

[a] Size is expressed as mean peak size (intensity-weighed)

[b] PDI for each individual peak was calculated as:

$$\mathrm{PDI} = \left(\frac{\bar{x}}{\mathrm{SD}}\right)^2$$

with $\bar{x}$ being the mean peak size and SD being the standard deviation as obtained by the Malvern Zetasizer Software.

Reverse FRET assay

BSA was labeled with fluorescein isothiocyanate (FITC) and purified by ultrafiltration using Microsep® Advance Centrifugal Devices with 3 kDa MWCO, followed by size exclusion chromatography using a Sephadex G-25 resin in a PD-10 column (GE Healthcare, Munich, Germany) with DPBS as the eluent. Fractions containing FITC-BSA were pooled and concentrated by ultrafiltration. The labeling efficiency was determined by absorbance measurements at 280 nm and 496 nm to determine the concentrations of BSA and FITC, respectively. For the reverse FRET experiments, unlabeled BSA was supplemented with 0.5 wt% FITC-labeled BSA. Amine-NPs were labeled with DiI (10 nmol mg^{-1}) that was physically entrapped in the hydrophobic core. DiI labeled amine-NPs were diluted in DPBS supplemented with 45 mg mL^{-1} of FITC-tagged BSA or DPBS to a final NP concentration of 0.1 mg mL^{-1} and incubated at 37 °C for different periods of time (0, 1, 2, 4, 7, 12, 24 h). FRET fluorescence was excited at 460 nm (FITC) and emission spectra (DiI) were recorded between 485 and 710 nm. FRET ratios were calculated as described in the methods section.

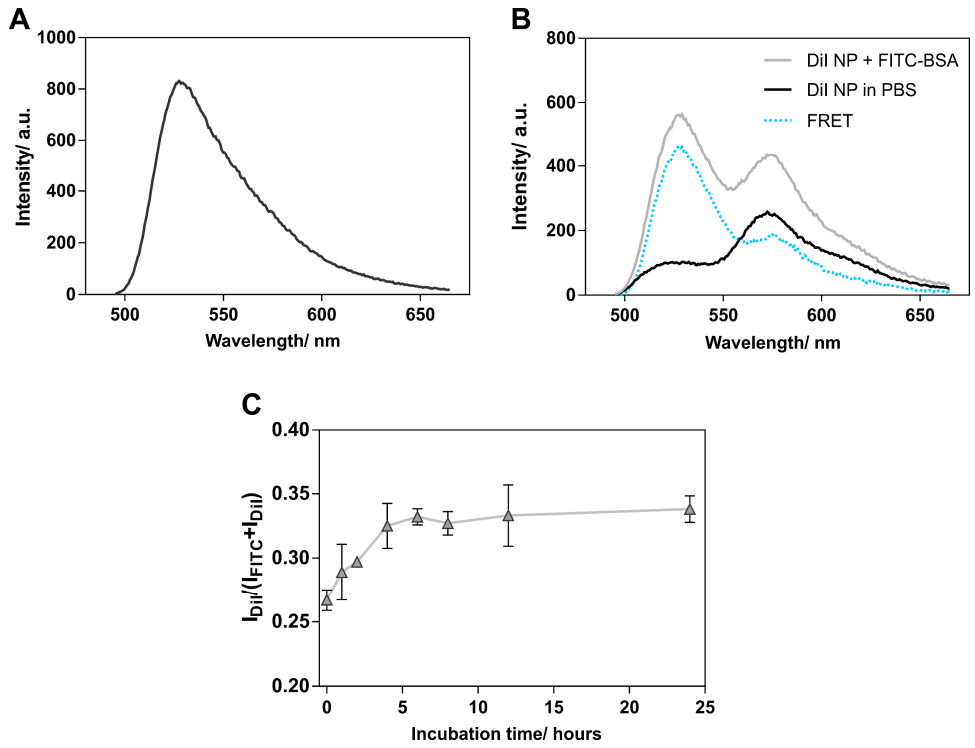

Figure S6. Cargo leaching assessed by reverse FRET. (A) Fluorescence spectrum of FITC-labeled BSA. (B) Fluorescence spectra of DiI-labeled amine-NPs in either DPBS pH 7.4 or after incubation with FITC-labeled BSA at 37 °C. The emission spectrum of DiI-labeled amine-NPs was subtracted from the measured emission spectrum of the samples (containing DiI-labeled NPs and 45 mg mL^{-1} BSA) to obtain FRET spectra. (C) FRET ratios of DiI-labeled amine-NPs and FITC-tagged BSA as a function of time.