Supporting Information for:

Ligand Layer Engineering to Control Stability and Interfacial Properties of Nanoparticles

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Content

TEM characterization

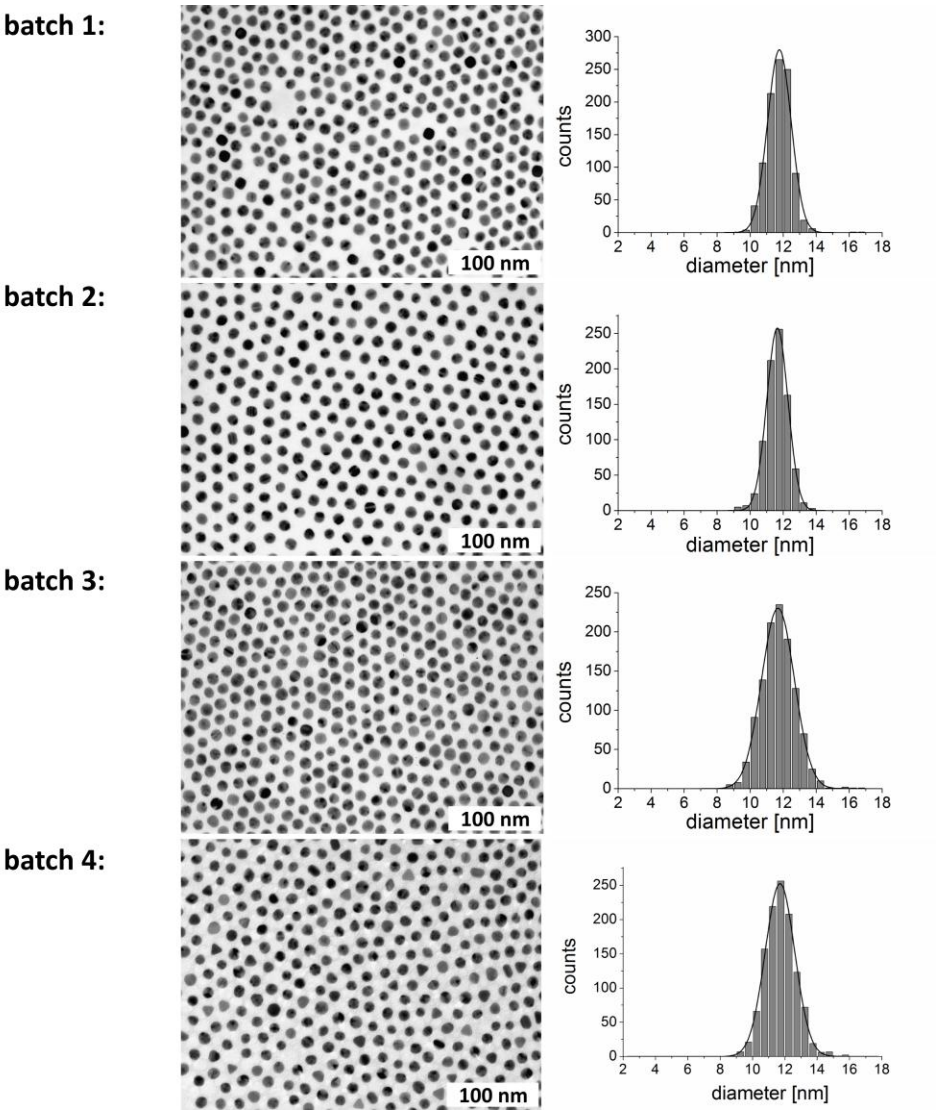

Figure S1. TEM characterization of AuNP used in this study. TEM images and size distribution histograms with Gauss fits obtained by analysis of several images are shown. The mean diameters from top were 11.7 ± 0.8 nm (N = 1000), 11.6 ± 0.7 nm (N = 838), 11.7 ± 1.0 nm (N = 1153) and 11.7 ± 0.9 nm (N = 1164). All AuNP were functionalized with PEGMUA before TEM sample preparation as described previously.[1] Batch 1 and 2 were used for the SAXS experiments (Figure 5, Figure S2), the stability tests in serum (Figure S4), the experiments testing the cellular uptake (Figure 9) and the toxicity tests (Figure S5), batch 3 was used to test the control of ligand layer composition by FTIR (Figure 2) and gel electrophoresis (Figure 3) and for the tests of colloidal (Figure 6) and chemical stability (Figure 8 and Figure S3), batch 4 was used for the experiments with different PEGMUA ligand lengths (Figure 7 in the main text). The TEM images in Figure 4 show another batch of AuNP with a mean diameter 12.6 ± 0.9 nm (N = 952).

Additional SAXS results

Table S1. Radii *r* and polydispersities *p* obtained by SAXS and TEM

sample	*r* (SAXS) [nm]	*r* (TEM) [nm]	*p* (SAXS) [%]	*p* (TEM) [%]
0 %, 25 % and 75 % MUA[a]	6.2	5.9	7.5	6.4
50 % MUA[b]	5.8	5.8	6.5	5.7

[a] these conjugates were prepared with the same batch of AuNP (batch 1 in Figure S1)
[b] prepared with batch 2 in Figure S1

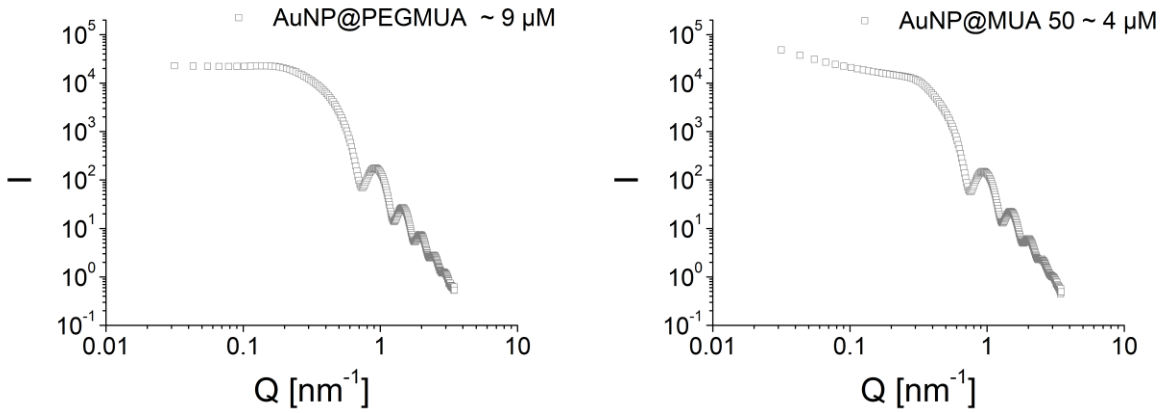

Figure S2. SAXS-Data of AuNP samples at high concentrations as indicated with 0 % (100 % PEGMUA) and 50 % MUA in the ligand layer. Left: for AuNP coated with just PEGMUA the colloidal integrity seems not to be affected, even at extremely high concentrations. Right: the increase of intensity I at low Q for AuNP with 50 % MUA in a mixed ligand layer indicates particle aggregation. However, the colloidal integrity of the main population seems not to be affected.

Competitive displacement with dithiothreitol

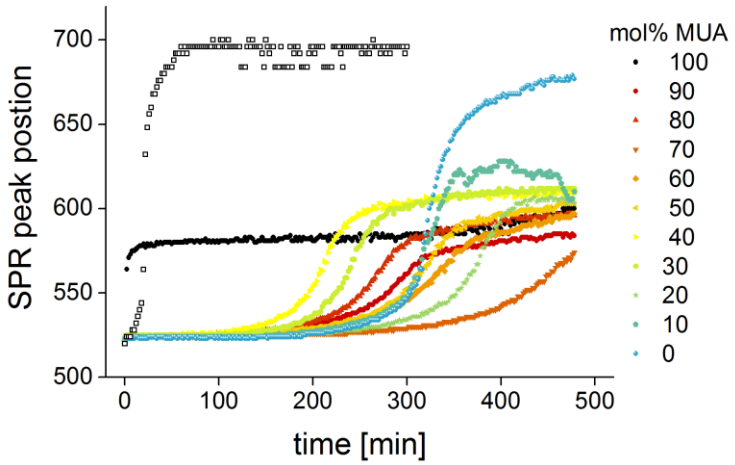

Figure S3. Electrolyte induced aggregation after competitive displacement with dithiothreitol (DTT). The aggregation of AuNP conjugates with mixed PEGMUA/MUA shells in the presence of DTT (1 M) and NaCl (0.4 M) was monitored by UV/Vis spectroscopy. The kinetics of the aggregation processes are analyzed by plotting the shifts of the localized surface plasmon resonance band (SPR peak position) versus the reaction time. A detailed discussion of the method and analysis can be found in previous work.[1] The theoretical mole percent MUA in PEGMUA/MUA mixed conjugates are indicated by the color code. AuNP coated with just MUA (100 % MUA, black spheres) aggregate very fast because of the high electrolyte concentration. All other samples are very stable with inflection points > 200 min. AuNP samples coated with PEG ligands of the same length but without C_{10}-spacer as in PEGMUA aggregate much faster under these conditions (AuNP@PEGSH, hollow squares, inflection point at 22 min). The magnitude of the SPR shifts and the differences in the aggregation kinetics of the AuNP@PEGMUA/MUA mixed conjugates cannot be explained at this point, because both, the aggregation of these samples and the optical response of the aggregates is complex. A clear correlation or trend of MUA ratio and stability was not observed.

Stability of AuNP conjugates in serum

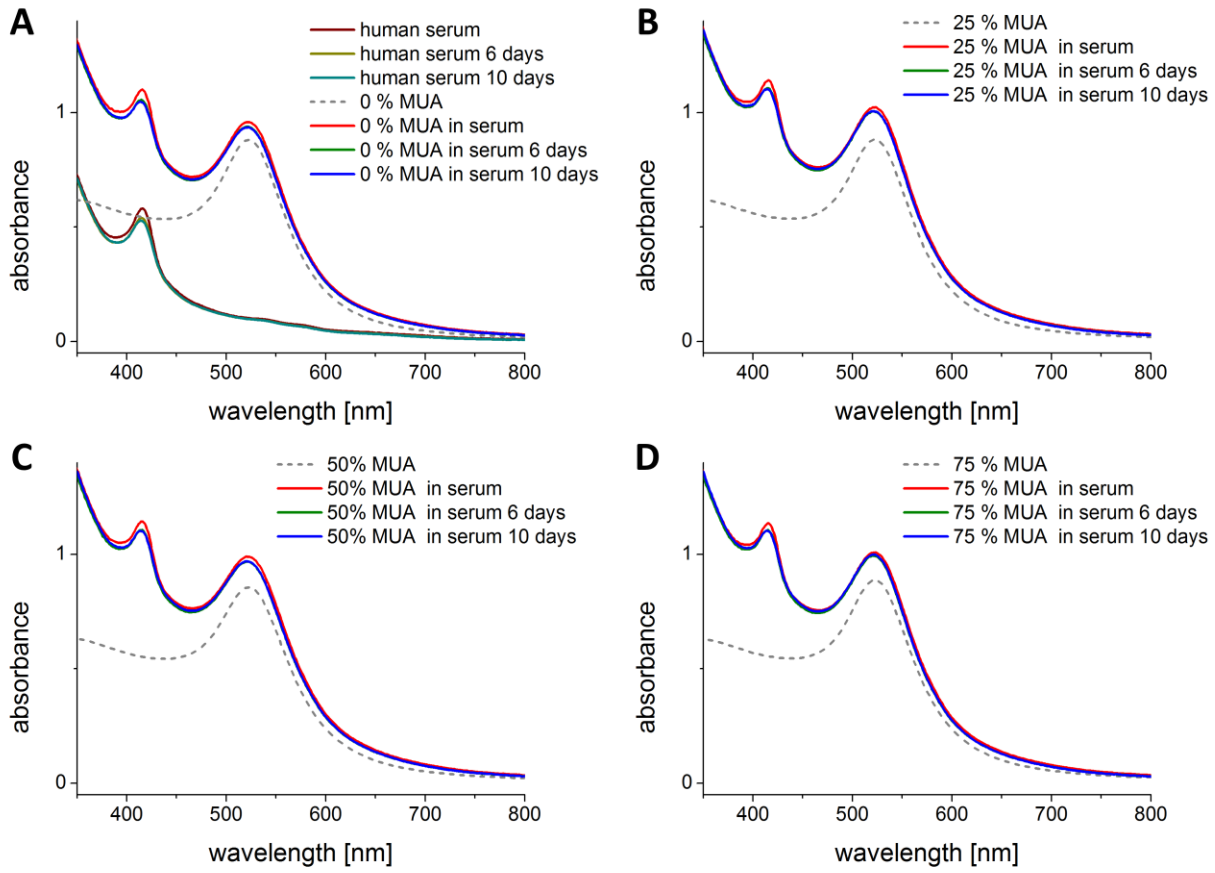

Figure S4. Absorbance spectra of AuNP after different times of incubation in human serum at 37 ° as indicated. AuNP with different mixed ligand layers containing 0 (**A**), 25 (**B**), 50 (**C**) or 75 (**D**) mole percent MUA were tested. Dashed lines are absorbance spectra of respective AuNP in water at the same concentration. Additionally, spectra of a control containing just serum are shown in A. The slight decrease of the absorbance at ~410 nm after 6 days is obviously due to changes in the serum and not to destabilization of the AuNP.

Toxicity of AuNP conjugates

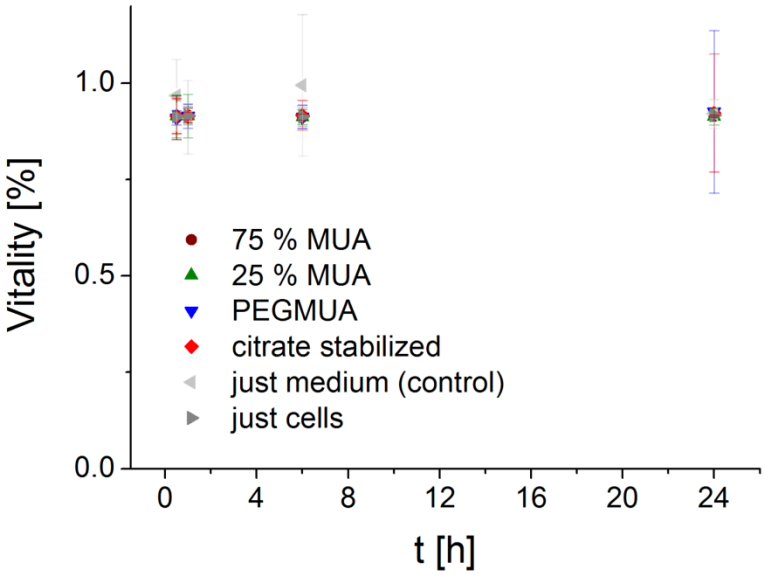

Figure S5. Toxicity tests of AuNP conjugates with mixed PEGMUA/MUA ligand layers (containing 75 and 25 % MUA), plain PEGMUA ligand layer, citrate ligand layer and controls as indicated. No toxicity was observed for any sample. Details are described in the experimental section of the main text. AuNP conjugates with 100 % MUA were not used in cell tests because their stability was not sufficient.

References

(1) Schulz, F.; Vossmeyer, T.; Bastús, N. G.; Weller, H. Effect of the Spacer Structure on the Stability of Gold Nanoparticles Functionalized with Monodentate Thiolated Poly(ethylene glycol) Ligands. *Langmuir* **2013**, *29*, 9897–9908.