Supporting Information for:

Gold Nanoparticles Functionalized with a Fragment of the Neural Cell Adhesion Molecule L1 Stimulate L1-Mediated Functions

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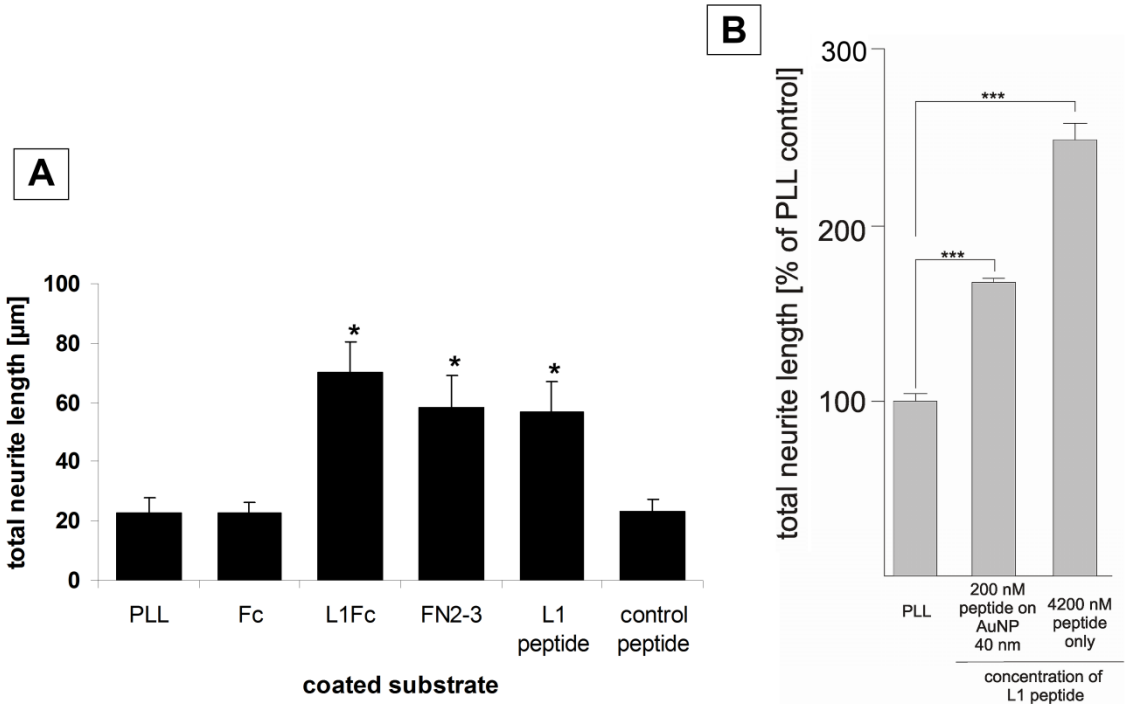
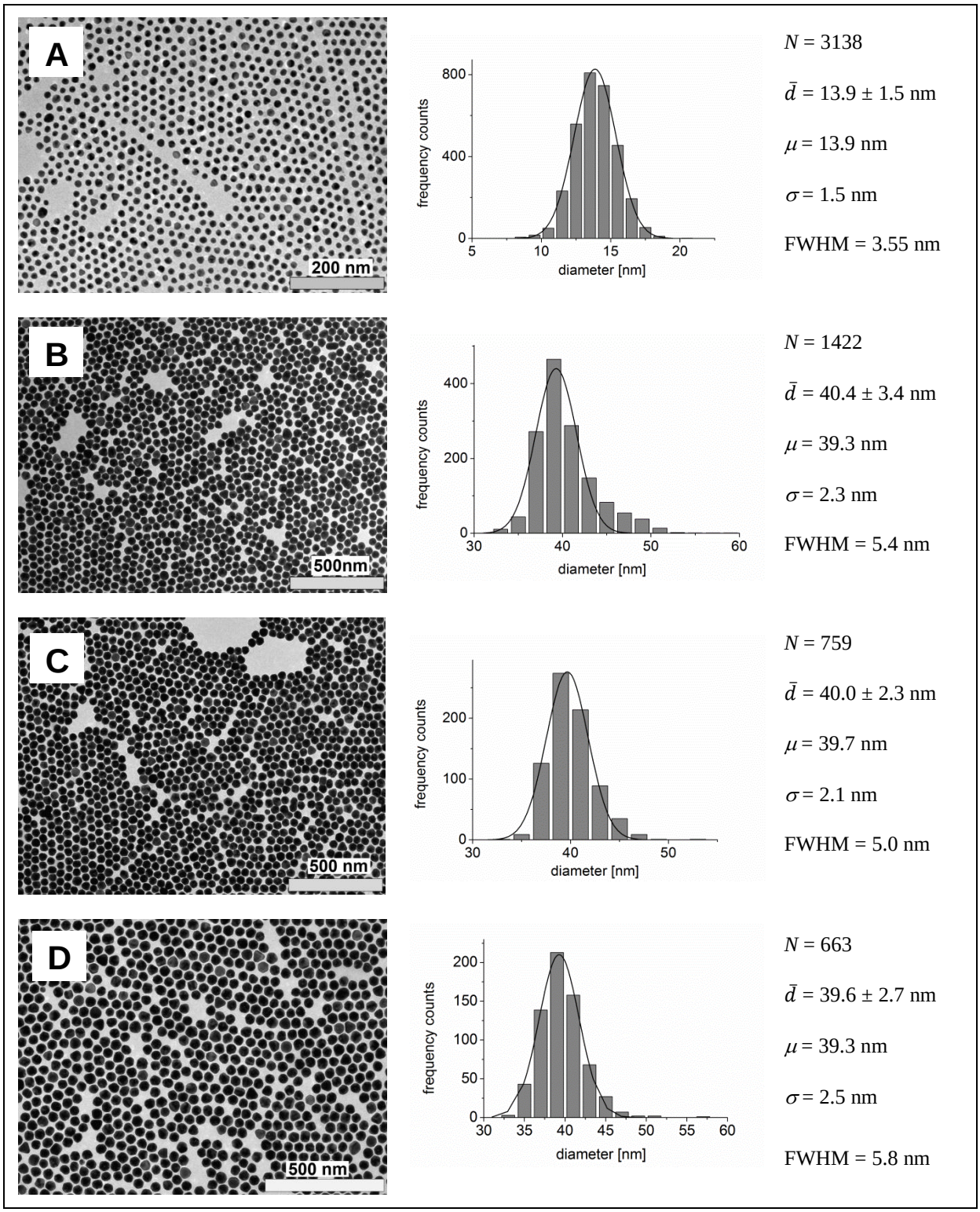
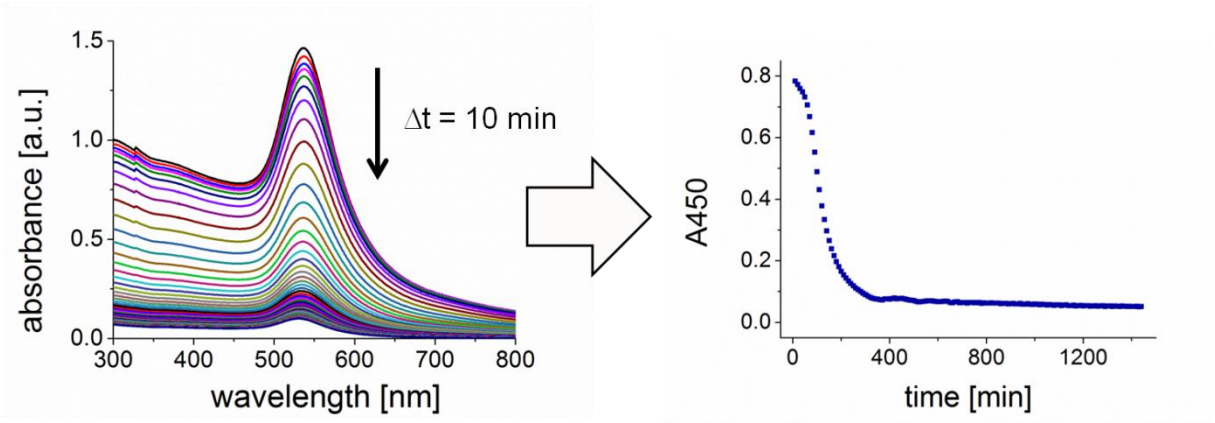


Supplementary Figure S1: A peptide derived from the third fibronectin type III-like (FNIII-like) domain of L1 stimulates L1-mediated neurite outgrowth and can be used as surrogate for L1. (**A**) Cerebellar neurons were cultured on the neutral substrate PLL and in the absence or presence of L1-Fc (extracellular domain of murine L1 fused to human Fc), Fc (control), the FNIII-like domains 2 and 3 of murine L1 (FN2-3), L1 peptide and scrambled L1 peptide (control peptide). (**B**) To compare the effect of L1 peptides and AuNPs with hybrid shell containing PEGMUA and L1 peptide cells were either treated with buffer (PLL), L1 peptide (4,200 nM) or AuNP40@L1-C-Lys/PEGMUA(1:3) (c(AuNP) = 0.14 nM, with 1,400 L1-C-Lys molecules per AuNP as a rough estimate the resulting concentration of coupled peptide is ~200 nM). When coupled to AuNPs, a much lesser extent of L1 peptides was needed to stimulate neuritogenesis in comparison to uncoupled L1 peptides in solution. (**A, B**) Neurite outgrowth was determined after 24 h in culture. For each treatment at least 100 cells were counted per experiment. Data represent mean with standard deviation of three independent experiments. * $p < 0.001$, *** $p < 0.0005$ Student´s t-test.

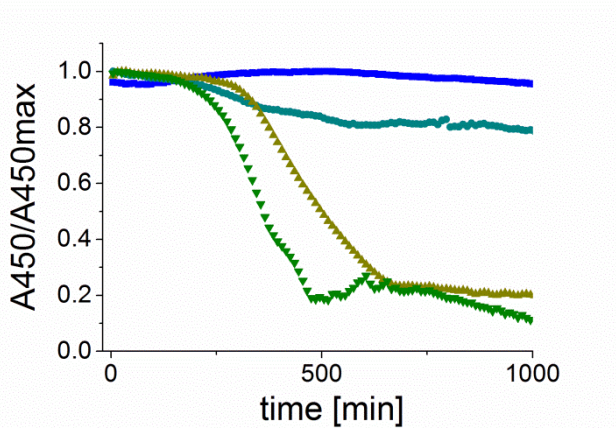


Supplementary Figure S2: Characterization of AuNP batches. TEM image, size distribution and statistical analysis of AuNP14 (**A**), AuNP40a (**B**), AuNP40b (**C**) and AuNP40c (**D**). $\bar{d}$ with standard deviation is the mean diameter of N counted particles on several TEM images. μ is the expectation (i.e. the peak position of the Gaussian distribution function), σ the standard deviation of the Gaussian fit (solid black line). The full width at half maximum (FWHM) of the fit is also given. The particles were functionalized with PEGMUA before TEM analysis, which results in large areas of well separated particles on the grid, which is a great benefit for statistical analysis. The preparation of these AuNP@PEGMUA TEM-samples is facile: 10 µl sample solution were placed on the copper grid and left drying for 24 h.

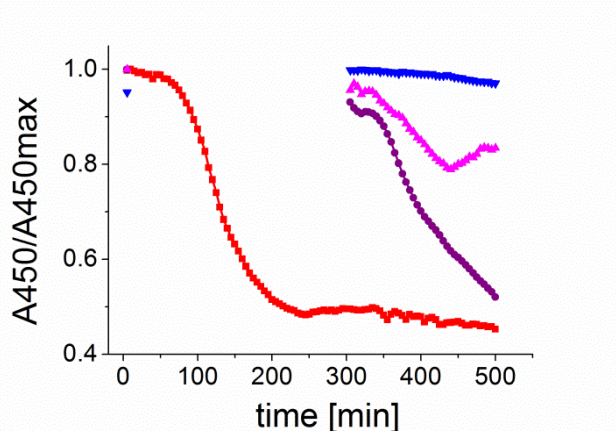


Supplementary Figure S3: Monitoring of AuNP-aggregation with time-resolved UV/vis spectroscopy. Absorbance spectra of AuNP40@L1-C-Lys/PEGMUA(3:1) collected directly after preparation of the sample in cycles of 10 minutes (left graph). Plotting of the absorbance at 450 nm (A450) versus the time visualizes the aggregation process (right graph).

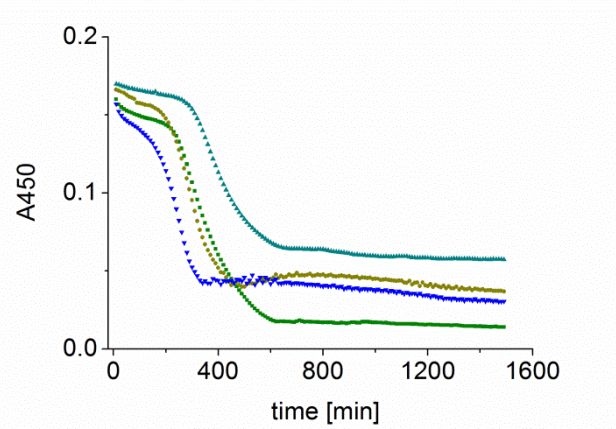
A



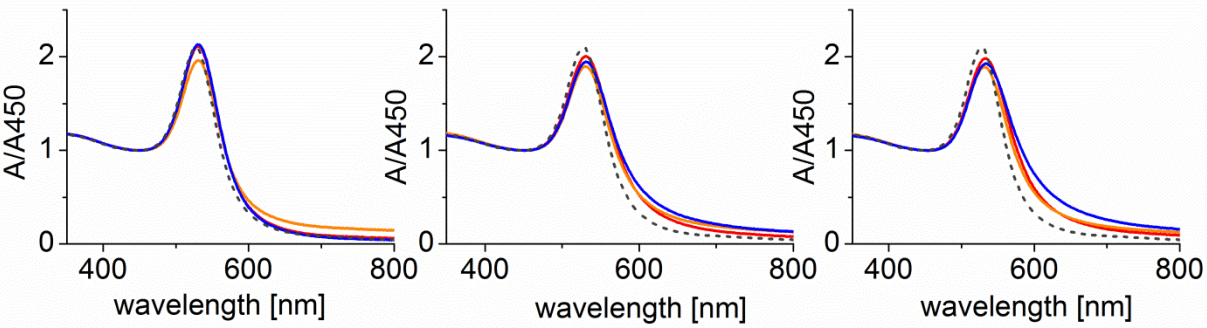
B



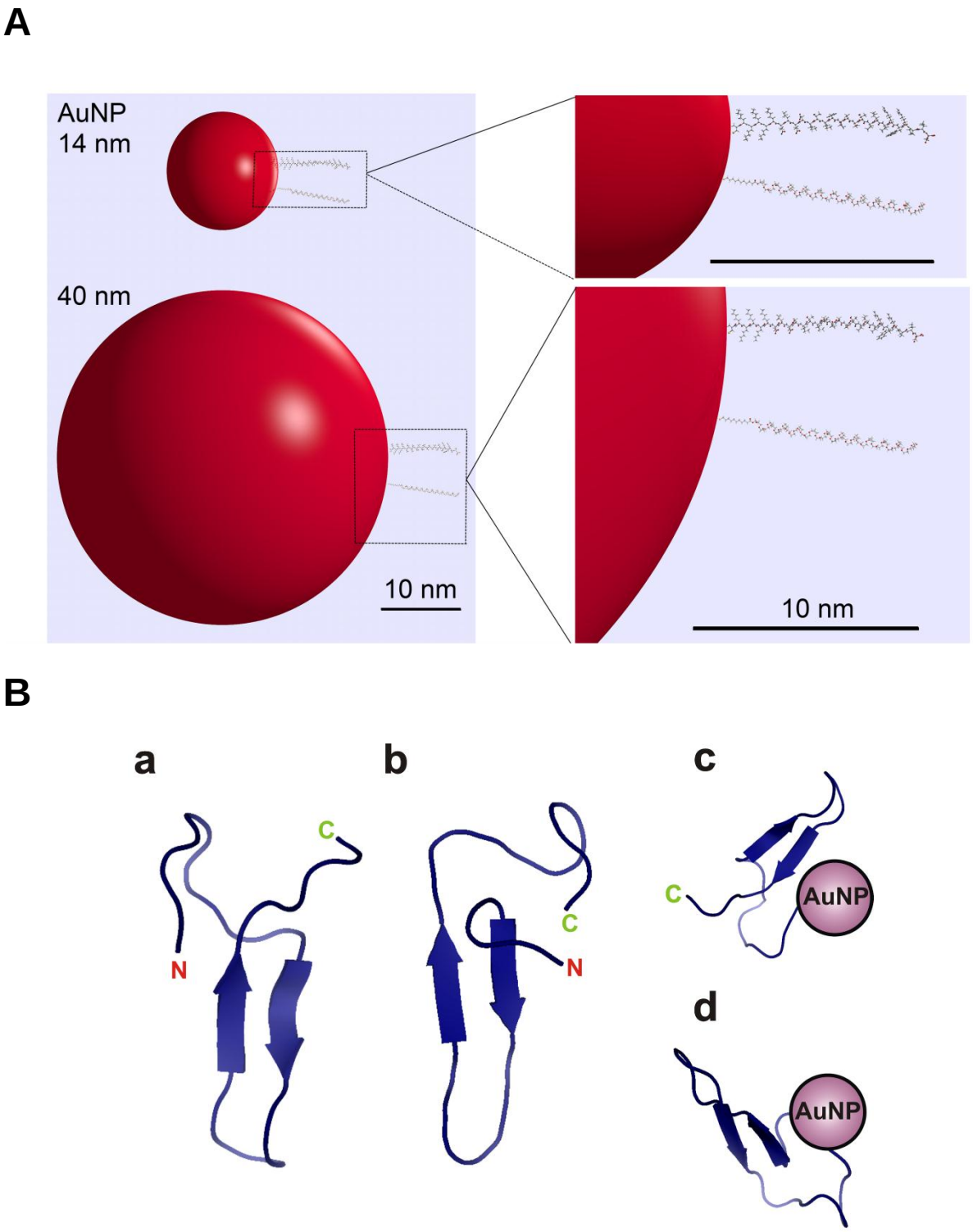
Supplementary Figure S4: Aggregation of AuNP14 conjugates. (**A**) Aggregation of AuNP14@L1-N-Lys/PEGMUA conjugates, with ratios L1-N-Lys:PEGMUA of 3:1 (dark yellow line), 1:1 (cyan line), 1:3 (blue line) and AuNP14 conjugates with L1-N-Lys pure (no PEGMUA addition, green line). The conjugates with peptide:PEGMUA ratio of 1:3 (blue line) did not show significant aggregation; the aggregation tendency correlates with the peptide ratio. (**B**) Aggregation of AuNP14@L1-C-Lys/PEGMUA conjugates, with ratios L1-C-Lys:PEGMUA of 3:1 (dark purple line), 1:1 (magenta line), 1:3 (blue line) and AuNP14 conjugates with L1-C-Lys pure (no PEGMUA addition, red line). The conjugates with peptide:PEGMUA ratio of 1:3 (blue line) did not show significant aggregation; the aggregation tendency correlates with the peptide ratio. To facilitate comparison, the absorbance at 450 nm normalized with the maximal absorbance at 450 nm (A450/A450max) is plotted versus the time.



Supplementary Figure S5: Aggregation of AuNP40@L1-N-Lys/PEGMUA conjugates. Aggregation of AuNP-conjugates with ratios L1-N-Lys:PEGMUA of 3:1 (dark yellow line), 1:1 (cyan line), 1:3 (blue line) and AuNP40 conjugates with L1-N-Lys pure (no PEGMUA addition, green line). The conjugates with peptide:PEGMUA ratio of 1:3 (blue line) do not follow the trend observed for the other conjugates, which aggregate stronger with increasing peptide ratio.



Supplementary Figure S6: Stability of AuNP40 conjugates in biologically relevant media. Absorbance spectra normalized with the absorbance at 450 nm (A/A450) of AuNP40@PEGMUA (left), AuNP40@L1-C-Lys/PEGMUA(1:3) (center) and AuNP40@L1-N-Lys/PEGMUA(1:3) (right) in different media. 4 weeks in PBS (blue lines), 1 minute in artificial cerebrospinal fluid (ACSF, red lines) and 24 hours in ACSF (orange lines). AuNP40@citrate (in aqueous solution of 2.2 mM sodium citrate) are shown for comparison (dashed lines). The ligands provide good colloidal stability in PBS and ACSF, while citrate stabilized particles are not stable in these media (data not shown).



Supplementary Figure S7: Structure modeling of L1 peptide-coupled AuNPs and the L1 peptides. (**A**) Dimension of the ligands (L1-N-Lys and PEGMUA) relative to the particle size of 14 nm and 40 nm AuNPs. For clarity, the ligands are not shown in a realistic conformation but with all ϕ = -120° and all ψ = 120° in case of the peptide L1-N-Lys and a stretched helical conformation in the case of PEGMUA. The actual conformation of the ligands and structure of the complete ligand shell is unknown. (**B**) The structures of the L1 peptide with the N-terminal cysteine and lysine linker (**a, c**) and the L1 peptide with the C-terminal cysteine and lysine linker (**b, d**) was modeled using the PEP-FOLD peptide structure prediction server.[1,2] The most probably adopted structures are shown (**a, b**) and the potential orientation of the

peptide at the AuNP surface (**c, d**). Note, that both peptides adopt a loop structure where the free terminus of the peptide is folded back and is in near vicinity of the coupled terminus and the AuNP surface. The middle part of the peptides is presented towards the surface of the AuNPs making it accessible for interactions.

References

1. Maupetit, J.; Derreumaux, P.; Tuffery, P. PEP-FOLD: An Online Resource for De Novo Peptide Structure Prediction. *Nucleic Acids Res.* **2009**, 37, W498–W503.
2. Maupetit, J.; Derreumaux, P.; Tufféry, P. A Fast Method for Large-scale De Novo Peptide and Miniprotein Structure Prediction. *J. Comput. Chem.* **2010**, 31, 726–738.