

Supporting Information for

Multi-Domain Short Peptide Molecules for in situ
Synthesis and Biofunctionalization of Gold
Nanoparticles for Integrin-Targeted Cell Uptake

Hilal Unal Gulsuner[‡], Hakan Ceylan[‡], Mustafa O. Guler^{}, Ayse B. Tekinay^{*}*

Institute of Materials Science and Nanotechnology, National Nanotechnology Research
Center (UNAM), Bilkent University, 06800, Ankara, Turkey.

Corresponding Author

^{*}E-mail: moguler@unam.bilkent.edu.tr (M.O.G.)

^{*}E-mail: atekinay@unam.bilkent.edu.tr (A.B.T.)

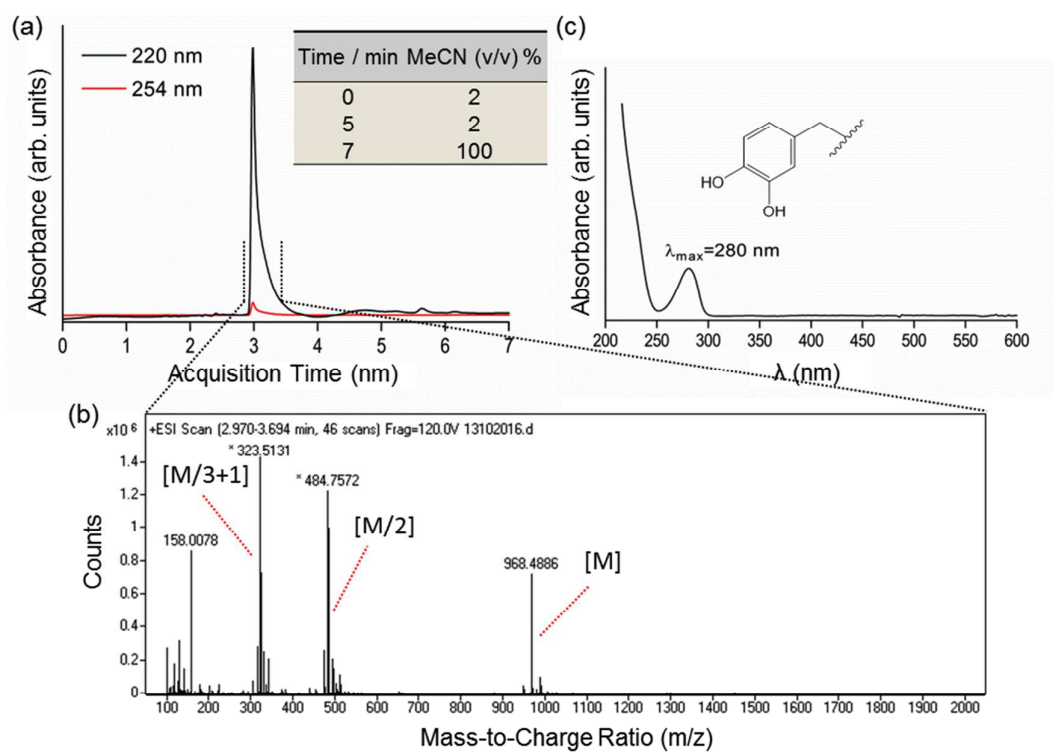


Figure S1. (a) Liquid chromatogram, (b) mass spectrum, and (c) UV-Vis analysis of the MDP.

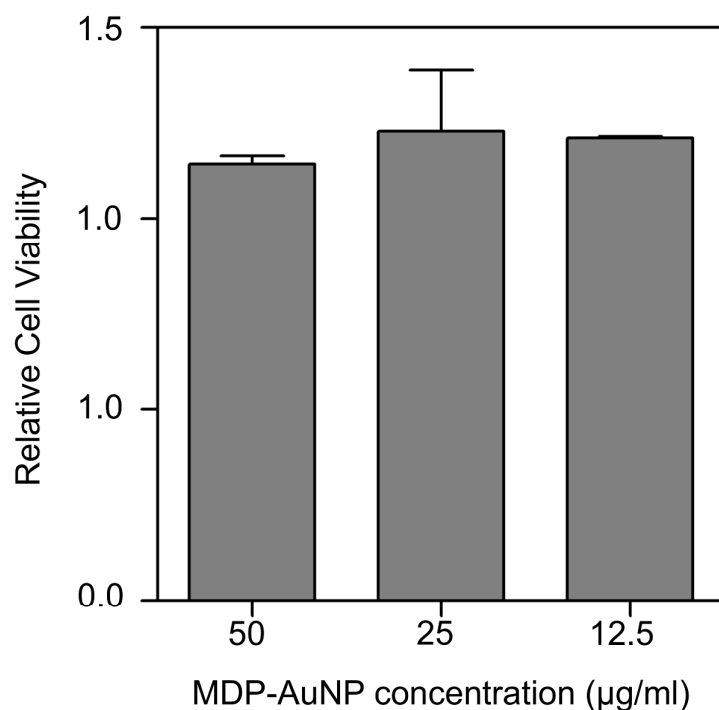


Figure S2. Cytotoxicity of Gold Nanoparticles. MCF7 cells were seeded on 24-well plates in complete growth medium until cells reached 80-90% confluency. Cells were treated with varying concentrations of peptide-capped AuNPs ($50 \mu\text{g mL}^{-1}$, $25 \mu\text{g mL}^{-1}$, $12.5 \mu\text{g mL}^{-1}$). Viability experiments of cells after 3 h of incubation with nanoparticles were performed by using MTT in vitro toxicity assay kit (Sigma) according to manufacturer's protocol. Cell numbers were normalized to untreated MCF7 cells. Absorbance of dye was measured at a wavelength of 570 nm with background subtraction at 690 nm by SpectraMax M5 Multi-Mode Microplate Reader.

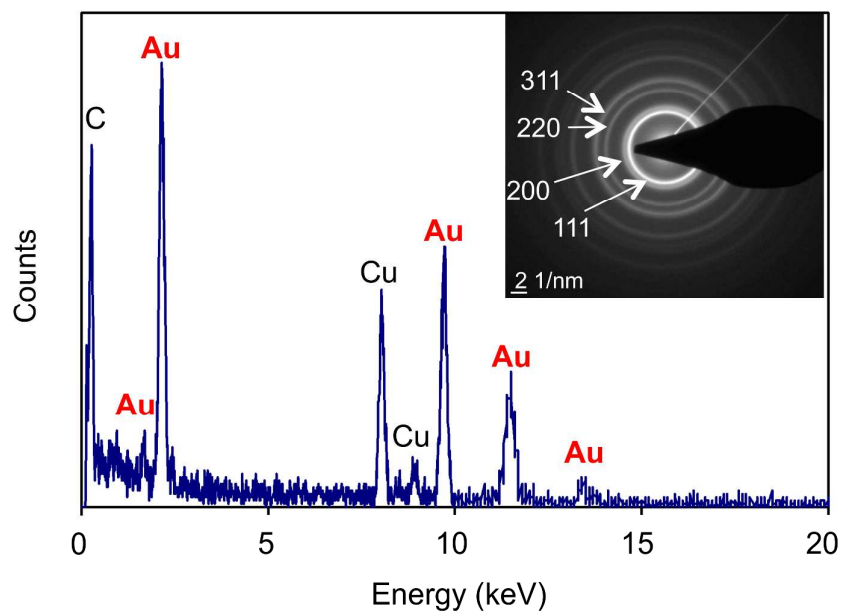


Figure S3. Energy-dispersive X-ray spectrum. The inset shows the selected area electron diffraction of AuNPs. AuNPs are in polycrystalline nature, with plane directions similar to those of synthesized with different methods.¹⁻³

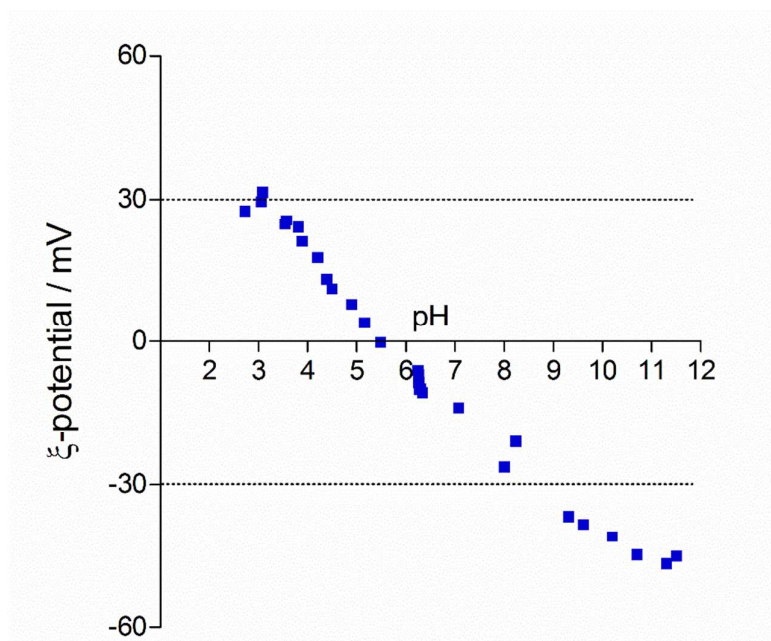


Figure S4. Zeta potential of AuNPs (MDP: 0.6 mM; H[AuCl₄]: 19.2 mM; [MDP]:[Au (III)] = 1:32) as a function of pH..

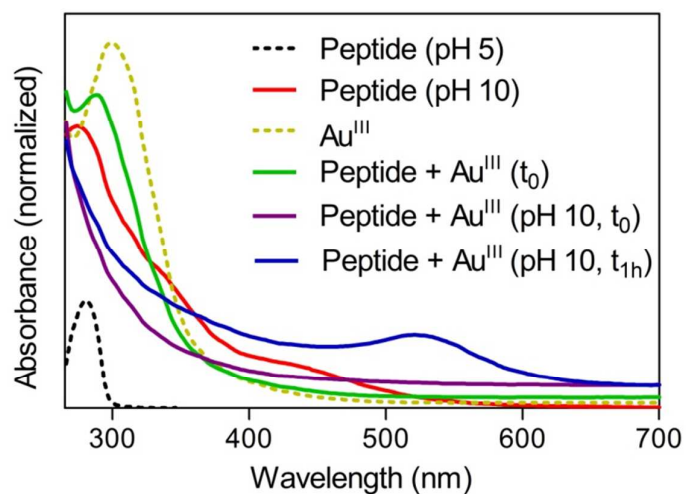


Figure S5. Synthesis mechanism of AuNPs by MDPs. UV-Vis spectra of the MDP and auric acid solution before and after mixing indicated that oxidation of the peptide couples with the reduction of gold.

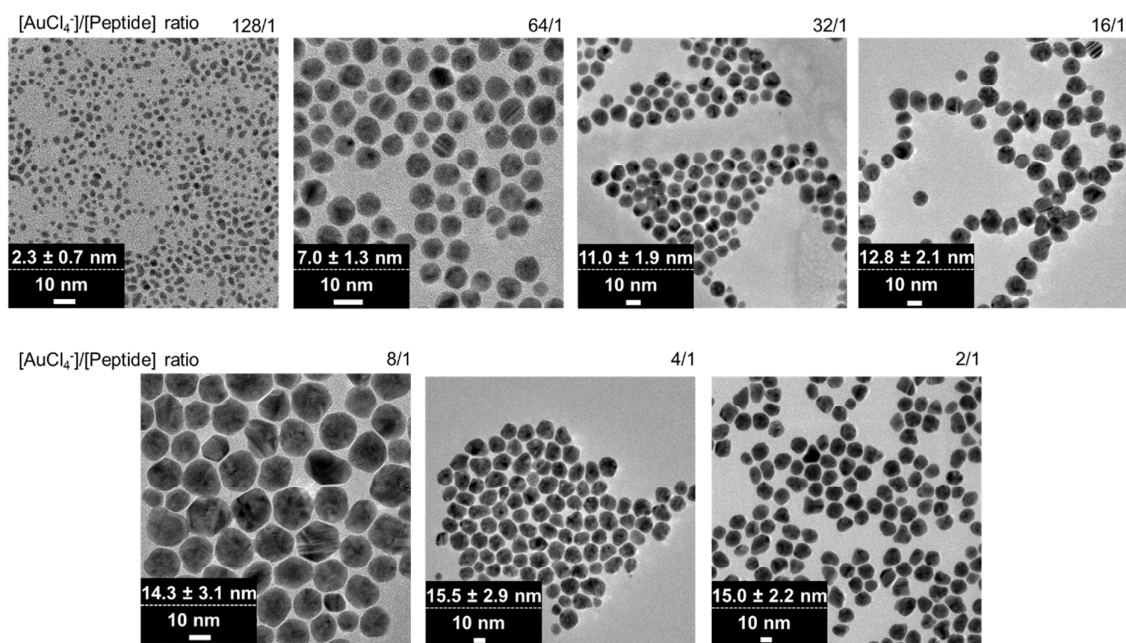


Figure S6. TEM images of AuNPs synthesized and stabilized with MDPs. Average size and standard deviations are presented for each image along with their $[\text{AuCl}_4^-]/[\text{Peptide}]$ molar ratios.

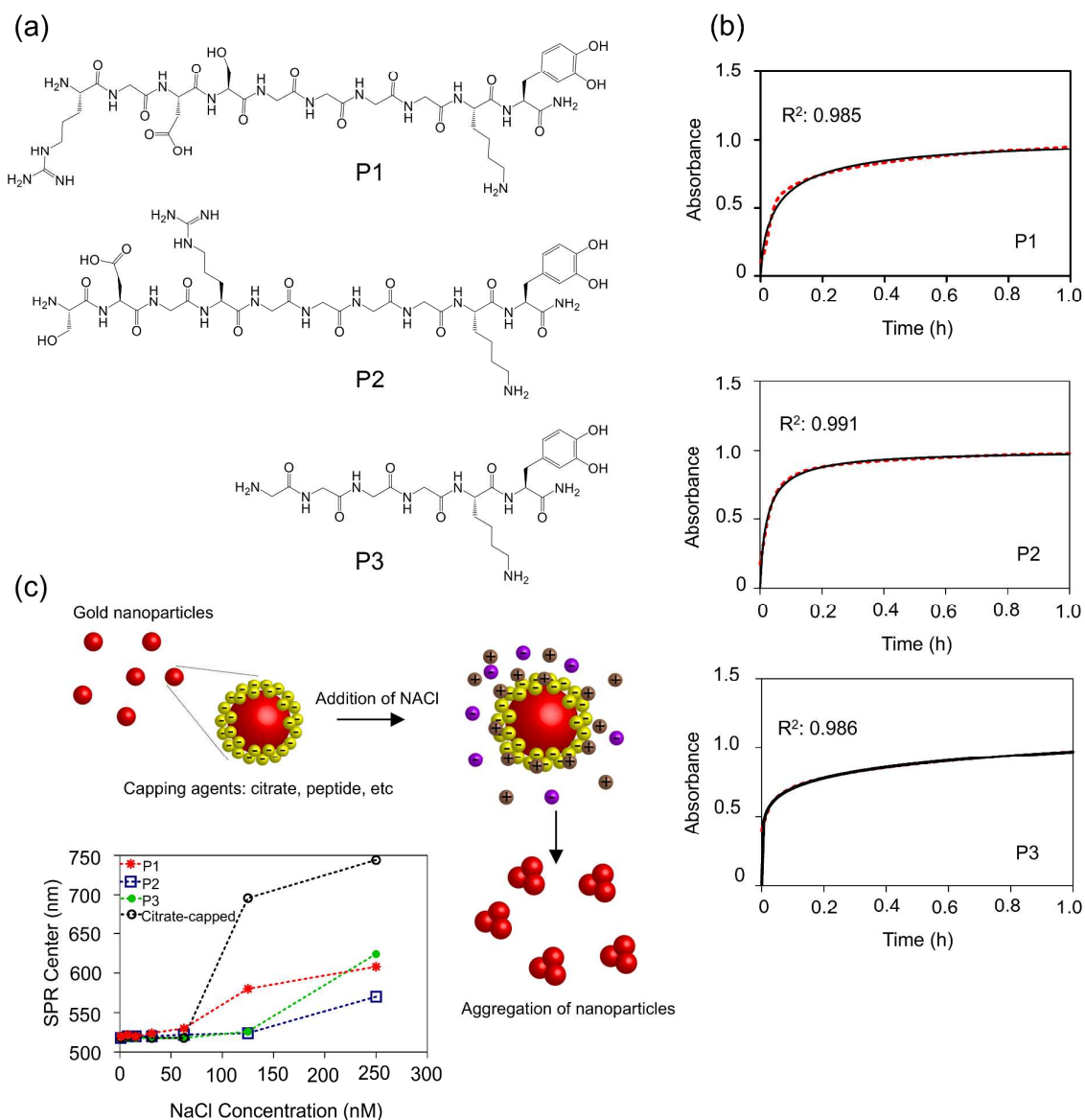


Figure S7. Multi-domain peptide approach with varying peptide sequences. (a) Kinetics of AuNP formation with capped with RGDSG₄KDopa-NH₂ (P1), SDGRG₄KDopa-NH₂ (P2) and G₄KDopa-NH₂ (P3) monitored by absorbance at 518 nm. (b) Chemical sketches of P1, P2 and P3. (c) Colloidal stability of peptide-capped nanoparticles under high salt concentrations.

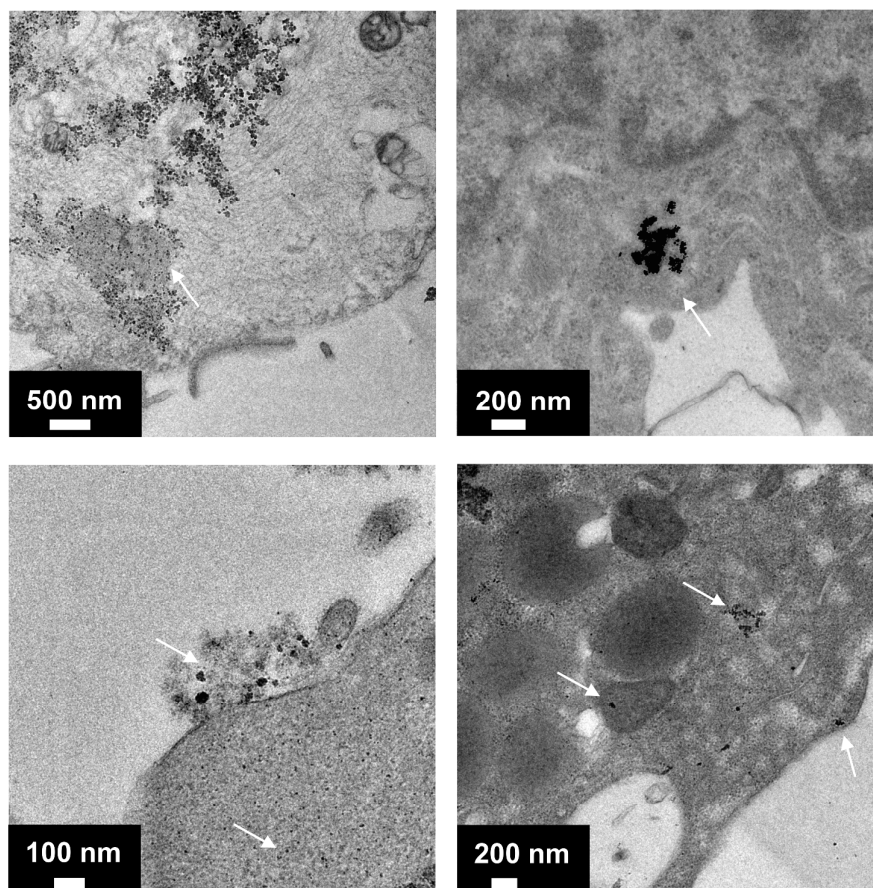


Figure S8. Representative TEM images of cross-sectional MCF7 cells incubated with 25 $\mu\text{g/mL}$ of MDP-AuNPs for 4 hours. MDP-AuNPs are present both in cytosol and in endosomes. In order to visualize cells via TEM imaging, AuNP uptake assay was repeated as described in Section 2.7, without using any inhibitors. Following the wash step, cells were re-suspended in PBS using trypsin/EDTA. Then a cell pellet was formed through centrifugation at 2,500 rpm for 10 min. This cell pellet was fixed in 2% gluteraldehyde solution overnight at 4 $^{\circ}\text{C}$. A secondary post-fixation was applied with 0.5% osmium tetroxide followed by infiltration and polymerization in an araldite 6005 resin from Electron Microscopy Sciences. Resin embedded samples were then sectioned by an ultramicrotome (Leica EMUC6 + EMFC6) and mounted on a copper grid. The sections were imaged under TEM from FEI Tecnai G2 F30 series operated at 100 kV.

References

- (1) Akamatsu, K.; Deki, S. TEM Investigation and Electron Diffraction Study on Dispersion of Gold Nanoparticles into a Nylon 11 Thin Film during Heat Treatment. *J. Colloid Interface Sci.* **1999**, 214, 353-361.
- (2) Long, N. N.; Vu, L. V.; Kiem, C. D.; Doanh, S. C.; Nguyet, C. T.; Hang, P. T.; Thien, N. D.; Quynh, L. M. Synthesis and Optical Properties of Colloidal Gold Nanoparticles. *J. Phys.: Conf. Ser.* **2009**, 187, 012026.
- (3) Xunjun, Y.; Shougang, C.; Aiguo, W. Green Chemistry Synthesis of Gold Nanoparticles Using Lactic Acid as a Reducing Agent. *Micro Nano Lett.* **2010**, 5, 270-273.