Efficient Gene Delivery Vectors by Tuning the Surface Charge Density of Amino Acid-Functionalized Gold Nanoparticles

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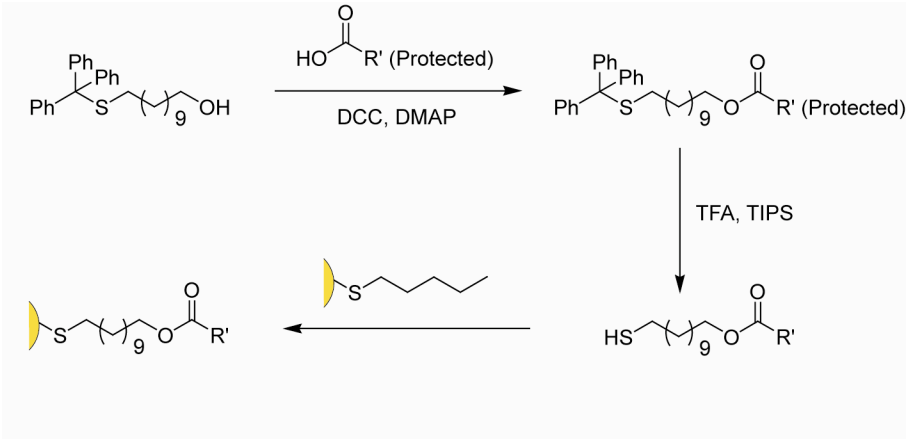
Scheme S1: General scheme for synthesis of amino acid-coated nanoparticles.



Synthesis of NP-Gly and NP-Lys: NP-Gly and NP-Lys were prepared according to literature procedures.[1,2]

Synthesis of Trit-C_{11}-LysG1-$(Boc)_4$

A dichloromethane solution of LysG1-$(Boc)_4$-OH[3] (2.50 g, 3.11 mmol), Trit-C_{11}-OH (1.00 g, 2.24 mmol), *N, N′*-dicyclohexylcarbodiimide (DCC, 0.69 g, 3.34 mmol), and 4-dimethylaminopyridine (DMAP, 0.08 g, 0.65 mmol) was stirred at room temperature for 12 h. The solution was poured into a mixture of dichloromethane and distilled water. The organic layer was collected and purified by column chromatography on silica gel using n-hexane/ethyl acetate (1:2, v/v) as an eluent, to give **Trit-C_{11}-LysG1-$(Boc)_4$** as a white solid (Yield 2.40 g, 87 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 6H, *Ph*-), 7.27 (m, 6H, *Ph*-), 7.20 (m, 3H, *Ph*-), 7.00 (brs, 1H) 5.93 (brs, 1H), 5.60 (brs, 1H), 4.92 (brs, 1H), 4.76 (brs, 1H), 4.35 (brs, 2H), 4.11 (brm, 3H, -C*H*$_2$-O- and COC*H*(R)NH-), 3.10 (brs, 6H, -C*H*$_2$NHBoc), 2.12 (t, J = 7.3 Hz, 2H, -S-C*H*$_2$-), 1.40 (m, 72H, -C*H*$_2$- and C*H*$_3$-); MS (ESI-MS) calcd for $C_{68}H_{106}N_6O_{12}S$ 1231.7, found 1254.0 $[M+Na]^+$.

Synthesis of HS-C_{11}-LysG1

A dichloromethane solution of **Trit-C_{11}-LysG1-$(Boc)_4$** (0.2 g, 0.16 mmol), trifluoroacetic acid (TFA, 0.6 ml), and triisopropylsilane (TIPS, 0.03 ml) was stirred at room temperature for 6 h under argon. After removal of the solvent at reduced pressure, the residue was purified by washing with diethyl ether (20 ml x 5). After drying residue under high vacuum, white solid of product was obtained (Yield 0.09 g, 94 %).

^{1}H NMR (400 MHz, CD_3OD) major peaks assigned δ 4.41 (brs, 1H), 4.13 (brs, 2H, -C*H*$_2$-O-), 4.00 (brs, 1H), 3.86 (brs, 1H), 3.50 (brs, 1H), 3.23 (brs, 2H), 2.95 (brs, 6H, -C*H*$_2$NHBoc), 2.51 (q, J=7.2 Hz, 2H, HS-C*H*$_2$-), 1.40 (m, 36H, -C*H*$_2$- and C*H*$_3$-); MS (ESI-MS) calcd for $C_{21}H_{47}N_7O_3S$ 588.89, found 589.6 $[M+H]^+$

Synthesis of NP-LysG1: 1-Pentanethiol-protected gold colloids were dissolved in dichloromethane (DCM) and ligands were dissolved in DCM-methanol (10:1, v/v) mixture. These solutions were separately purged with argon. After 30 min of purging, they were mixed together and stirred at room temperature for ~ 48 h. Then solvents were evaporated and excess ligands were removed by 5X washing with DCM-methanol (50:1, v/v) followed by dialysis (cut off 10 kDa, Pierce) for 2 days.

Ethidium bromide (EtBr) exclusion assay: Dye exclusion assay was tested with NP-Lys. A mixture of pDNA (1.2 nM), EtdBr (5 μM) and NP-Lys (0 μM, 0.24 μM and 2.4 μM) were incubated for 10 min in phosphate buffer saline (PBS, Sigma). Then fluorescence (λ_{em} = 595 nm, λ_{ex} = 545 nm) was monitored on a SpectroMax M5 micro-plate reader (Molecular Device). Nanoparticle absorbance was corrected using NP-TEG, a neutral particle. Addition of particles resulted in release of intercalated dyes and hence decreases fluorescence intensity.

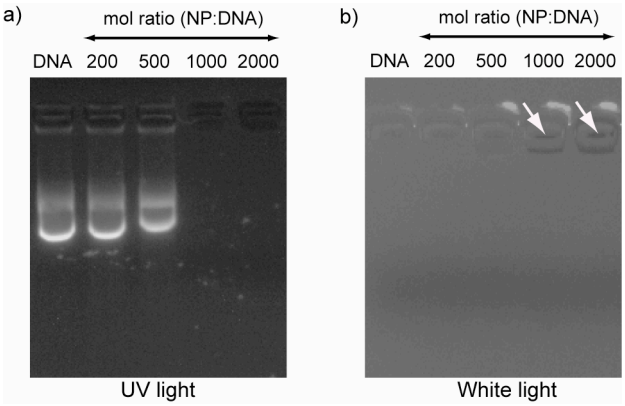


Figure S1: DNA and NP-Lys complexes were electrophoresed at different ratios and images were taken under ultraviolet (UV) light and white light. The nanoparticles (visible as black color) retained in wells at higher ratios (MR_{1000}, MR_{2000}) as indicated by arrows in white light image. The complete retardation at higher ratios probably arises from charge neutralization or the resultant complexes were too large to enter the gel.[4]

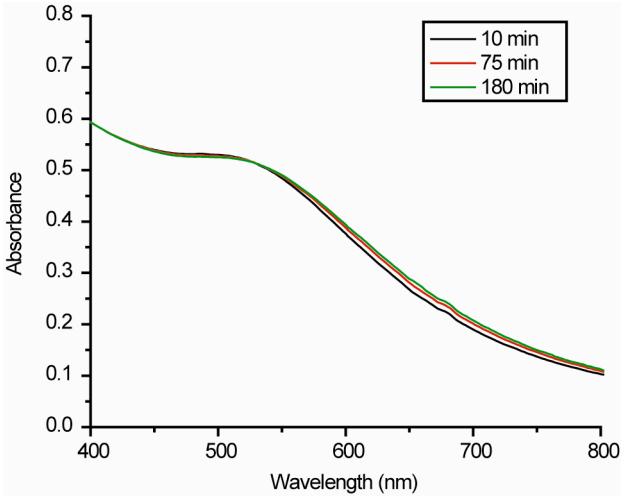


Figure S2: UV spectra of DNA:NP-Lys (MR_{2000}) complex at different incubation time. Small red shift of surface plasmon resonance (SPR) band indicates no large aggregation over the period of 3 h. The incubation time was long enough for endocytosis, which can occur within 2 h.[5]

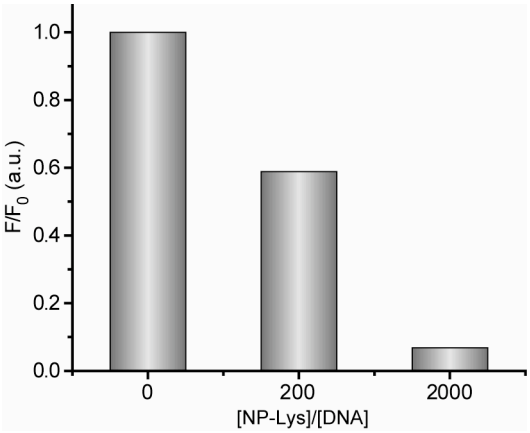


Figure S3: Addition of cationic nanoparticles (NP-Lys) displaced intercalated ethidium bromide within DNA and resulted decrease in fluorescence.

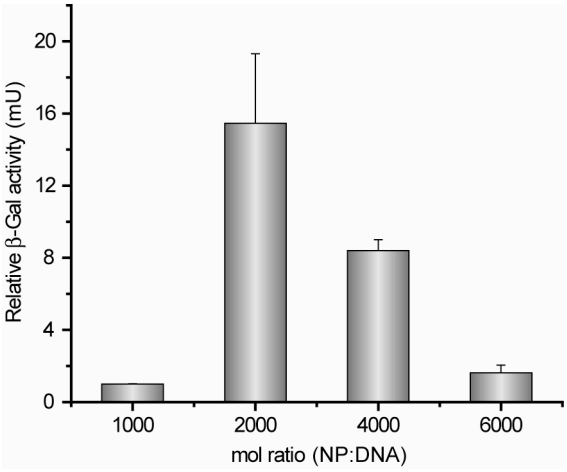


Figure S4. Transfection efficiency of NP-LysG1 at different mixing ratio. MR_{2000} was the optimal ratio.

Reference:

(1) Ghosh, P. S.; Han, G.; Erdogan, B.; Rosado, O.; Rotello, V. M. *J. Pept. Sci.* **2008**, *14*, 134-138.

(2) Ghosh, P. S.; Han, G.; Erdogan, B.; Rosado, O.; Krovi, S. A.; Rotello, V. M. *Chem. Biol. Drug Des.* **2007**, *70*, 13-18.

(3) Dykes, G. M.; Brierley, L. J.; Smith, D. K.; McGrail, P. T.; Seeley, G. J. *Chem. Eur. J.* **2001**, *7*, 4730-4739.

(4) Lynn, D. M.; Langer, R. *J. Am. Chem. Soc.* **2000**, *122*, 10761-10768.

(5) Lu¨hmann, T.; Rimann, M.; Bittermann, A. G.; Hall, H. *Bioconjugate Chem.* **2008**, *19,* 1907–1916.