Supplementary Material of

# Microfluidic-Based Cationic Cholesterol Lipid siRNA Delivery Nanosystem: Highly Efficient In Vitro Gene Silencing and the Intracellular Behavior

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Figure S1. Synthesis routes and molecular structures of Cho-lys-es lipids

Figure S2. Nuclear magnetic resonance spectrum of Chol-es-Lys

Figure S3. Luciferase gene silencing efficiencies of CEL/siRNA nanocomplexes with different concentrations

Table S1. The hydrodynamic sizes of the particles in the media of 10% FBS

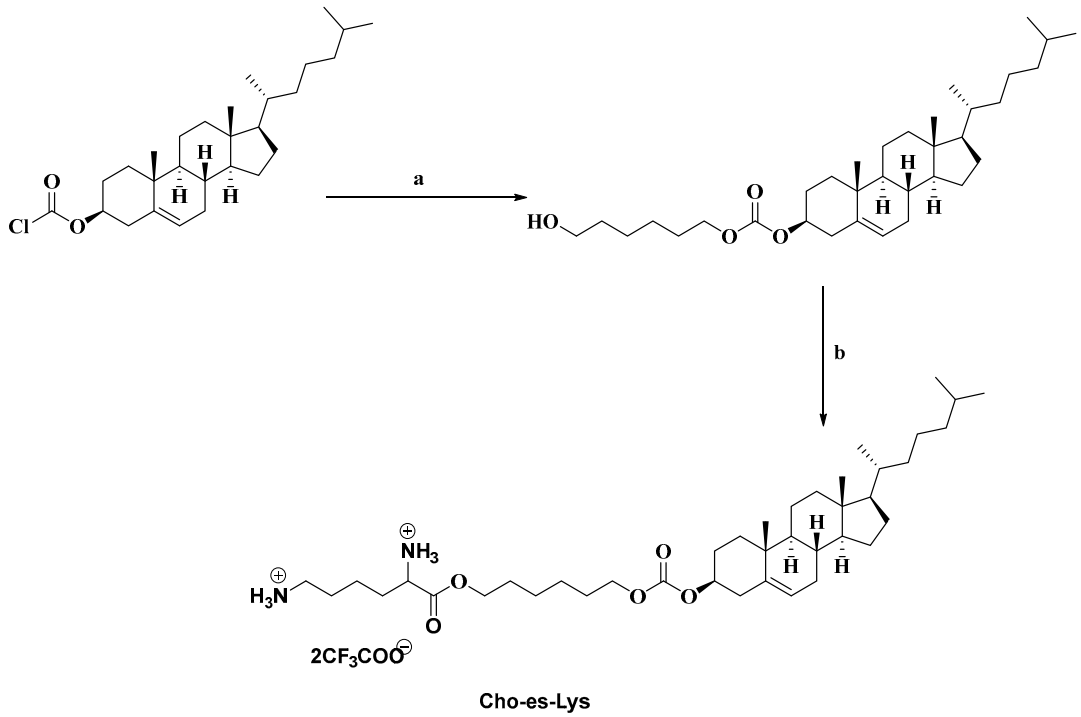


Figure S1. Synthesis routes and molecular structures of Cho-lys-es lipids

(a) 1,6-hexanediol, $CH_2Cl_2$/pyridine, 12h, r.t. (b) 1. Nα-,Nε-bis-Boc-L-lysine, DCC/DMAP, $CH_2Cl_2$, 24 h, r.t.; 2. trifluoroacetic acid, 0°C, $CH_2Cl_2$, 0.5h.

The synthesis of Cho-es-Lys lipid could be accomplished within several simple and facile steps.[1] First, cholesteryl chloroformate was reacted with excess of 1,6-hexanediol to prepare Cholest-5-en-3-yloxycarbonyloxyhexan-6-ol (Cho-es-OH) as a precursor in 74% yield, which was then acylated with L-2,6-bis((tert-butoxycarbonyl)amino) hexanoic acid (Nα-,Nε-bis-Boc-L-lysine). After that, the Boc groups were deprotected in TFA at 0°C to produce two carbon-ate ester linkage bearing cholesterol cationic lipids Cho-es-Lys (yield: 62%)

1.R. Sheng, T. Luo, H. Li, J. Sun, Z. Wang and A. Cao, *Colloids And Surfaces B-Biointerfaces*, **2014**, *116*, 32-40.

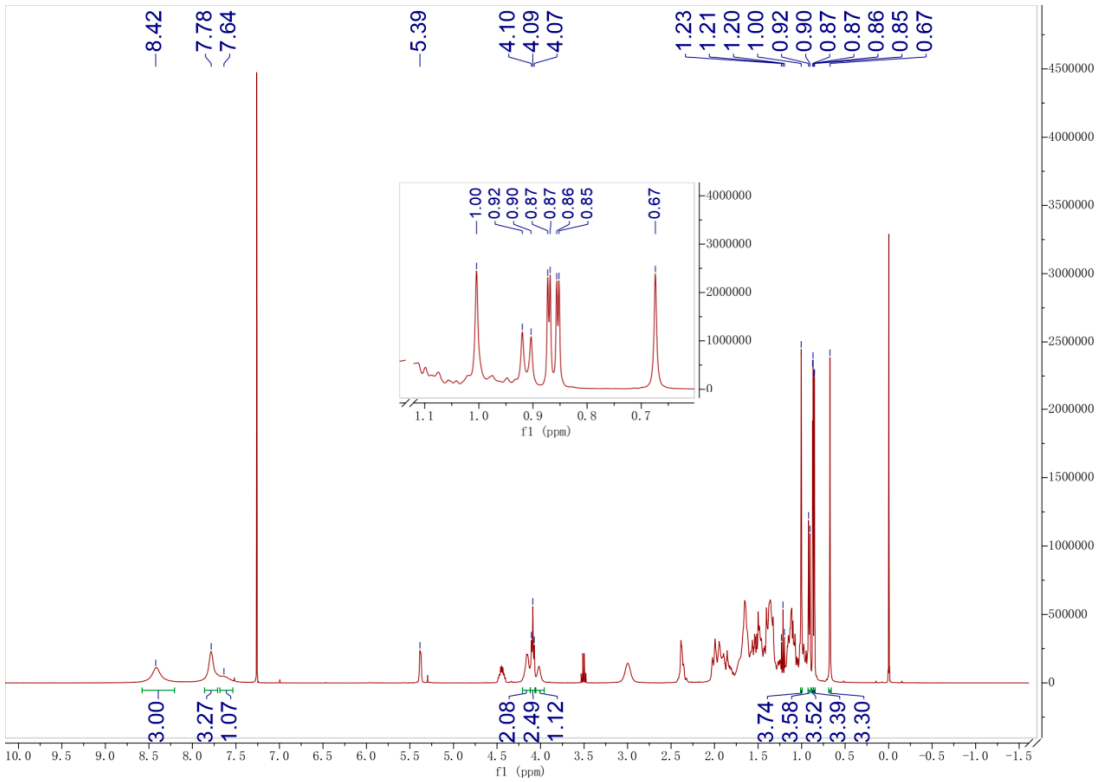


Figure S2. nuclear magnetic resonance spectrum of Chol-es-Lys

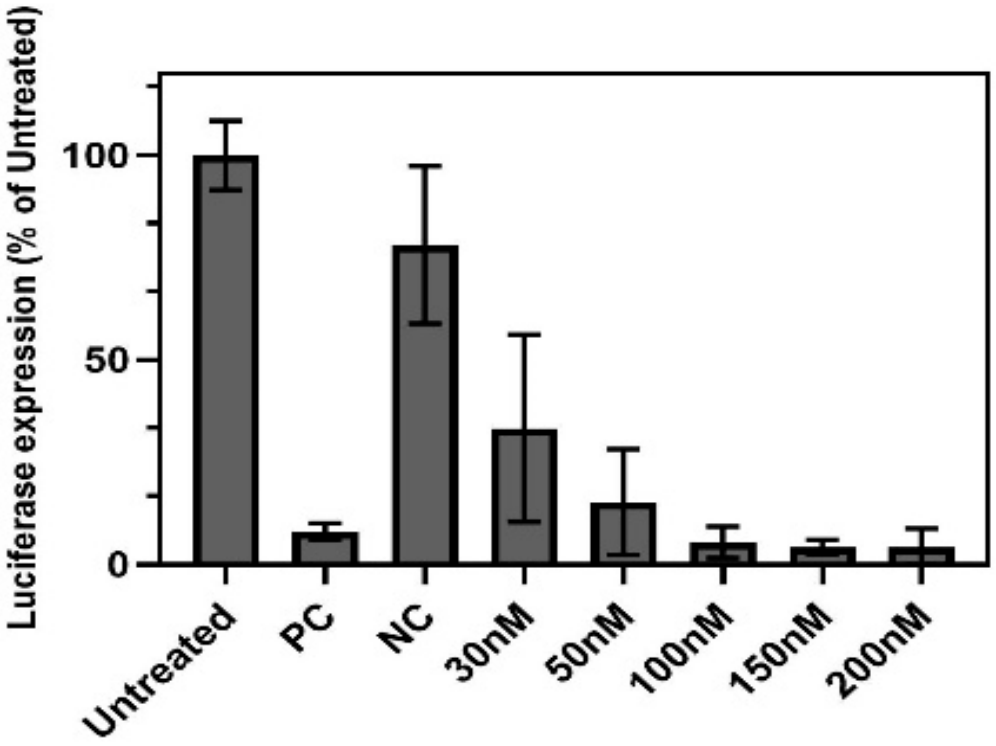


Figure S3. Luciferase gene silencing efficiencies of CEL/siRNA nanocomplexes with different concentrations

PC, lipofectamine 2000 as positive control in 100nM; NC, CEL/NC-siRNA nanocomplexes as negative control. CEL, Chol-es-Lys.

Table S1. The hydrodynamic sizes of the particles in the media of 10% FBS

| Time | Hydrodynamic sizes of the particles (nm) | PDI |
|---|---|---|
| 0h | 125.4 ± 4.4 | 0.213 |
| 4h | 129.8 ± 14.5 | 0.252 |
| 24h | 375.0 ± 37.6 | 0.338 |
| 72h | 812.8 ± 102.1 | 0.359 |