Supplemental Information for

Self-assembled Cationic Amphiphiles as Antimicrobial Peptides Mimics: Role of Hydrophobicity, Linkage Type, and Assembly State

Characterization

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) were recorded on a Varian 400 or 500 MHz spectrophotometer. Samples (~ 2 – 10 mg/mL) were dissolved in deuterated chloroform ($CDCl_3$) or deuterated CD_3OD with trimethylsilane (TMS) or deuterated solvent (CD_3OH) as an internal reference. Fourier transform infrared (FT-IR) spectra were acquired using a Thermo Scientific Nicolet iS10 spectrophotometer by solvent-casting onto sodium chloride (NaCl) plates; each spectrum was an average of 32 scans. Molecular weights were determined by a ThermoQuest Finnigan LCQ-DUO system (Thermo Scientific, Waltham, MA) equipped with an electrospray ionization (ESI) source, mass spectrometer (MS) detector, a syringe pump and the Xcalibur data system. Samples were dissolved in spectrophotometric grade methanol (MeOH) at a concentration of 10 μg/mL.

Synthesis of Ether-linked Cationic Amphiphiles (CAms)

*Synthesis of alkylated di-tert-butyl L-tartrate (**2**).*

The alkylation of di-*tert*-butyl L-tartrate with 1-bromooctane to prepare ***2a*** is presented as an example. Following a modified literature procedure[1], di-*tert*-butyl L-tartrate (600 mg, 2.29 mmol) was dissolved in 20 mL anhydrous DMF under argon, and the solution was then cooled to 0 ˚C using an ice bath. Sodium hydride (NaH, 192 mg, 4.80 mmol) was added and the reaction stirred

for 20 min. 1-Bromododecane (0.88 mL, 5.03 mmol) was added dropwise and the reaction mixture was allowed to stir overnight and warmed to room temperature. The reaction was quenched with 20 mL saturated ammonium chloride (NH_4Cl) solution and extracted with ethyl acetate (3x 20 mL). Organic layers were combined, washed with brine (1x 60 mL), and dried over magnesium sulfate ($MgSO_4$) before solvent was removed *in vacuo*. ***2a*** was purified on silica gel via column chromatography using a hexane: ethyl acetate gradient (100:0 to 98:2).

2a. Yield: 353 mg, 48% (colorless oil). ^{1}H-NMR (500 MHz, $CDCl_3$): 4.16 (s, 2H), 3.72 (m, 2H), 3.30 (m, 2H), 1.57 (m, 4H), 1.49 (s, 18H), 1.25 (br, 20H), 0.86 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): 169.17, 81.92, 80.67, 72.68, 32.04, 29.68, 29.43, 28.36, 26.24, 22.86, 14.29. IR (cm^{-1}, thin film from $CHCl_3$): 1748 (C=O, ester), 1109 (C-O). ESI-MS m/z: 509.2 $[M+Na]^+$.

2b. Yield: 435 mg, 60% (colorless oil). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.15 (s, 2H), 3.72 (m, 2H), 3.29 (m, 2H), 1.57 (m, 4H), 1.49 (t, 18H), 1.24 (br, 28H), 0.87 (t, 6H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 169.12, 81.87, 80.65, 72.65, 32.09, 29.85, 29.76, 29.71, 29.51, 28.33, 26.23, 22.87, 14.29. IR (cm^{-1}, thin film from $CHCl_3$): 1755 (C=O, ester), 1116 (C-O). ESI-MS m/z: 565.3 $[M+Na]^+$.

2c. Yield: 275 mg, 60 % (colorless oil). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.16 (s, 2H), 3.72 (m, 2H), 3.30 (m, 2H), 1.60 (br, 4H), 1.49 (s, 18H), 1.24 (b, 36H), 0.88 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ 169.18, 81.93, 80.69, 72.68, 32.15, 29.87, 29.57, 28.37, 26.25, 22.91, 14.34. IR (cm^{-1}, thin film from $CHCl_3$): 1751 (C=O, ester), 1112 (C-O). ESI-MS m/z: 621.4 $[M+Na]^+$.

*Synthesis of alkylated L-tartaric acid (**3**).*

The deprotection of ***2*** to afford ***3*** is presented using ***3a*** as an example. Following a modified literature procedure[2], ***2a*** (397 mg, 0.82 mmol) was dissolved in 13 mL anhydrous dichloromethane (DCM) under argon and the solution was cooled to 0 ˚C using an ice bath. Trifluoroacetic acid (TFA, 2.5 mL, 32.64 mmol) was added dropwise via a syringe, and the reaction mixture was allowed to stir overnight and warmed up to room temperature. The crude mixture was concentrated in *vacuo* to remove solvent and TFA, and then precipitated in chilled hexane (100 mL). The pure product was isolated via vacuum filtration.

3a. Yield: 296 mg, 97 % (white solid). ^{1}H-NMR (400 MHz, CDCl3): 4.39 (s, 2H), 3.73 (m, 2H), 3.49 (m, 2H), 1.60 (m, 4H), 1.26 (br, 20H), 0.88 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): 173.13, 79.65, 73.68, 32.00, 29.50, 29.47, 29.39, 25.94, 22.84, 14.28. IR (cm^{-1}, thin film from $CHCl_3$): 3350 – 3600 (COOH), 1731 (C=O), 1100 (C-O). ESI-MS m/z: 373.3 $[M-H]^-$.

3b. Yield: 205 mg, 97 % (white solid). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.38 (s, 2H), 3.71 (m, 2H), 3.50 (m, 2H), 1.60 (m, 4H), 1.26 (br, 28H), 0.88 (t, 6H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 172.09, 79.63, 73.81, 32.11, 29.77, 29.74, 29.56, 29.53, 25.96, 22.90, 14.33. IR (cm^{-1}, thin film from $CHCl_3$): 3300 – 3600 (COOH), 1735 (C=O), 1097 (C-O). ESI-MS m/z: 429.3 $[M-H]^-$.

3c. Yield: 200 mg, 89% (white solid). ^{1}H-NMR (400 MHz, $CDCl_3$): 4.38 (s, 2H), 3.69 (m, 2H), 3.53 (m, 2H), 1.60 (m, 4H), 1.25 (br, 36H), 0.88 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): 172.49, 79.40, 73.64, 34.20, 31.90, 29.63, 29.61, 29.57, 29.49, 29.35, 29.33,

29.27, 25.72, 22.67, 14.10. IR (cm^{-1}, thin film from $CHCl_3$): 3100 – 3600 (COOH), 1744 (C=O), 1109 (C-O). ESI-MS m/z: 485.7 $[M-1]^-$.

*Synthesis of N-Boc alkylated tartaric acid (**4**).*

The conjugation of N-Boc ethylendiamine to ***3*** to prepare ***4*** is presented using ***4a*** as an example. Following a previously published procedure[2], ***3a*** (296 mg, 0.79 mmol), EDC·HCl (637 mg, 3.32 mmol), and 4-dimethylaminopyridine (DMAP, 193 mg, 1.58 mmol) were dissolved in 7 mL anhydrous DCM under argon. Upon complete dissolution, N-Boc-ethylenediamine (0.31 mL, 1.98 mmol) was added via syringe and the reaction stirred overnight at room temperature. The reaction mixture was washed with 10% potassium bisulfate ($KHSO_4$, 2x 15mL) and brine (1x 15mL). The crude mixture was then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was then precipitated in chilled hexane (100 mL) and isolated via vacuum filtration.

4a. Yield: 316 mg, 61% (white powder). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.03 (s, 2H), 4.95 (s, 2H), 4.23 (s, 2H), 3.52 (m, 8H), 3.28 (m, 4H), 1.55 (m, 4H), 1.44 (s, 18H), 1.26 (br, 20H), 0.88 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ 171.11, 156.35, 81.44, 73.53, 40.73, 39.88, 32.03, 29.91, 29.58. 29.46, 28.60, 26.19, 22.84, 14.30. IR (cm^{-1}, thin film from $CHCl_3$): 3373 (NH), 1690 (C=O, carbamide), 1654 (C=O, amide). ESI-MS m/z: 681.2 $[M+Na]^+$.

4b. Yield: 300 mg, 68 % (white powder). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.05 (s, 2H), 4.97 (s, 2H), 4.22 (s, 2H), 3.52 (m, 8H), 3.28 (m, 4H), 1.54 (m, 4H), 1.43 (s, 18H), 1.25 (br, 28H), 0.87 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ 170.96, 156.45, 81.44, 79.75,

73.53, 40.73, 39.87, 32.11, 29.91, 29.52, 29.66, 29.81, 29.91, 28.61, 26.20, 22.89, 14.33. IR (cm^{-1}, thin film from $CHCl_3$): 3349 (NH), 1702 (C=O, carbamide), 1677 (C=O, amide). ESI-MS m/z: 738.1 $[M+Na]^+$.

4c. Yield: 300 mg, 70 % (white powder). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.04 (s, 2H), 4.96 (s, 2H), 4.22 (s, 2H), 3.52 (m, 8H), 3.28 (m, 4H), 1.54 (m, 4H), 1.44 (s, 36H), 1.25 (br, 28H), 0.87 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): ^{13}C-NMR (400 MHz, $CDCl_3$): 170.94, 98.92, 81.46, 77.97, 73.51, 32.12, 29.91, 29.85, 29.63, 29.55, 28.60, 26.20, 22.89, 14.31. IR (cm^{-1}, thin film from $CHCl_3$): 3340 (NH), 1693 (C=O, carbamide), 1655 (C=O, amide). ESI-MS m/z: 793.7 $[M+Na]^+$.

*Synthesis of of ether-linked cationic amphiphiles (CAm, **5**).*

The deprotection of ***4a*** to afford ***5a*** is presented as an example. Briefly, ***4a*** (305 mg, 0.463 mmol) was dissolved in 4.7 mL HCl (4M in dioxane, 18.52 mmol) and then cooled to 0 ˚C under argon using an ice bath. The reaction mixture was allowed to stir overnight and warmed to room temperature before work-up. The crude product was concentrated *in vacuo* and re-dissolved in minimal methanol (1 mL), followed by precipitation into a 50 mL centrifuge tube containing chilled diethyl ether (45 mL). ***5a*** was then isolated via centrifugation ((Hettich EBA 12, Beverly, MA; 3500 rpm, 3x 5 min) and the supernatant decanted.

5a. Yield: 255 mg, quantitative yield (off-white solid). ^{1}H-NMR (400 MHz, CD_3OD): δ 8.40 (br, 2H), 4.03 (s, 2H), 3.64 (m, 4H), 3.47 (m, 4H), 3.10 (m, 4H), 1.62 (m, 4H), 1.31 (br, 20H), 0.90 (t, 6H). ^{13}C-NMR (400 MHz, CD_3OD): δ 172.49, 82.77, 72.34, 36.56,

31.88, 29.56, 29.42, 29.29, 25.96, 22.56, 13.26. IR (cm^{-1}, thin film from $CHCl_3$): 3419 (NH), 1644 (C=O, amide). ESI-MS m/z: 460.5 $[M+H]^+$.

5b. Yield: 188 mg, 92 % (off-white solid). ^{1}H-NMR (400 MHz, CD_3OD): 8. 40 (br. 2H), 4.01 (s, 2H), 3.64 (m, 4H), 3.45 (m, 4H), 3.10 (t, 4H), 1.62 (m, 4H), 1.30 (s, 28H), 0.90 (t, 6H). ^{13}C-NMR (400 MHz, $CDCl_3$): 172.47, 82.84, 72.31, 39.93, 36.55, 31.91, 29.65, 29.61, 29.57, 29.47, 29.33, 25.98, 22.57, 13.27. IR (cm^{-1}, thin film from $CHCl_3$) 3424 (NH), 1648 (C=O, amide). ESI-MS m/z: 515.4 $[M+H]^+$.

5c. Yield: 220 mg, quantitative yield (off-white solid). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.42 (br, 2H), 4.00 (s, 2H), 3.66 (m, 4H), 3.46 (m, 4H), (s, 4H), 1.63 (m, 4H), 1.29 (s, 38H), 0.90 (t, 6H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ 172.47, 82.91, 72.29, 29.65, 29.62, 29.57, 29.49, 29.33, 25.99, 22.56, 13.27. IR (cm^{-1}, thin film from $CHCl_3$) 3424 (NH), 1656 (C=O, ester). ESI-MS m/z: 571.9 $[M+1]^+$.

Synthesis of Ester-linked CAms

*Synthesis of acylated di-2-bocaminoethyltartramide (**7**).*

The acylation of di-2-bocaminoethyltartramide (***6***) to prepare ***7*** is presented using ***7a*** as an example. Following a published procedure[2], octanoic acid (146 mg, 1.01 mmol), di-2-bocaminoethyltartramide (200 mg, 0.46 mmol), and DMAP (23 mg, 0.19 mmol) were dissolved in 16 mL anhydrous DCM and 7 mL anhydrous DMF under nitrogen. EDC·HCl (370 mg, 1.93 mmol) was added, and the reaction stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and then reconstituted in DCM, washed with aqueous solutions of 10%

$KHSO_4$ (3x 40 mL), saturated sodium bicarbonate ($NaHCO_3$, 3x 40 mL) solution, and brine (1x 50 mL). The combined organic layer was dried over $MgSO_4$, concentrated *in vacuo*, and then triturated in 100 mL hexanes for 4 h, and the pure product was isolated via vacuum filtration.

7a. Yield: 280 mg, 89% (white solid). ^{1}H-NMR (MHz, $CDCl_3$): δ 6.97 (s, 2H), 5.57 (s, 2H), 5.13 (s, 2H), 3.28 (m, 8H), 2.46 (m, 4H), 1.62 (m, 4H), 1.45 (s, 18H), 1.29 (br, 16H), 0.88 (t, 6H). ^{13}C-NMR (MHz, $CDCl_3$): δ 172.32, 167.01, 79.96, 72.42, 41.23, 39.94, 34.04, 31.88, 29.25, 28.60, 24.89, 22.81, 14.27. IR (cm^{-1}, thin film from $CHCl_3$): 3454 (NH), 1793 (C=O, ester), 1751 (C=O, carbamide), 1694 (C=O, amide). ESI-MS m/z: 709.5 $[M+Na]^+$.

7b. Yield: 290 mg, 85% (white solid). ^{1}H-NMR (MHz, $CDCl_3$): δ 7.04 (s, 2H), 5.58 (s, 2H), 5.17 (s, 2H), 3.29 (m, 8H), 2.44 (m, 4H), 1.63 (m, 4H), 1.45 (s, 18H), 1.26 (br, 24H), 0.88 (t, 6H). ^{13}C-NMR (MHz, $CDCl_3$): δ 172.33, 167.04, 79.96, 72.41, 41.23, 39.95, 34.04, 32.07, 29.66, 29.49, 29.31, 28.61, 24.89, 22.88, 14.31. IR (cm^{-1}, thin film from $CHCl_3$): 3454 (NH), 1794 (C=O, ester), 1752 (C=O, carbamide), 1694 (C=O, amide). ESI-MS m/z: 765.5 $[M+Na]^+$.

7c. Yield: 350 mg, 95% (white solid). ^{1}H-NMR (MHz, $CDCl_3$): δ 6.96 (s, 2H), 5.56 (s, 2H), 5.12 (s, 2H), 3.28 (m, 8H), 2.43 (m, 4H), 1.63 (m, 4H), 1.45 (s, 18H), 1.26 (br, 32H), 0.89 (t, 6H). ^{13}C-NMR (MHz, $CDCl_3$): δ 172.32, 167.02, 79.95, 72.44, 41.21, 39.94, 34.05, 32.12, 29.84, 29.72, 29.54, 29.32, 28.61, 24.90, 22.90, 14.33. IR (cm^{-1}, thin film from $CHCl_3$): 3454 (NH), 1794 (C=O, ester), 1752 (C=O, carbamide), 1694 (C=O, amide). ESI-MS m/z: 821.5 $[M+Na]^+$.

*Synthesis of ester-linked CAm (**8**)*.

The deprotection of ***7*** to afford ***8*** is presented using ***8a*** as an example. The deprotection was carried out in a similar manner as was the ether-linked CAms, using ***7a*** (275 mg, 0.40 mmol) and HCl (4M in dioxane, 16 mmol, 4 mL). If necessary, additional anhydrous dioxane (0.5 – 1mL) was added to improve stirring.

8a. Yield: 210 mg, 94% (off-white solid). ^{1}H-NMR (MHz, CD_3OD): δ 8.62 (s, 2H), 5.57 (s, 2H), 3.50 (m, 4H), 3.07 (m, 4H), 2.50 (m, 4H), 1.62 (m, 4H), 1.33 (br, 16H), 0.91 (t, 6H). ^{13}C-NMR (MHz, CD_3OD): δ 172.90, 168.96, 72.42, 39.44, 36.93, 33.35, 31.68, 28.93, 28.93, 24.59, 22.50, 13.22. IR (cm^{-1}, KBr): 3452 (NH), 1740 (C=O, ester), 1656 (C=O, amide). ESI-MS m/z: 487.4 $[M+H]^+$.

8b. Yield: 230 mg, 93% (white solid). ^{1}H-NMR (MHz, CD_3OD): δ 8.62 (s, 2H), 5.57 (s, 2H), 3.49 (m, 4H), 3.07 (m, 4H), 2.49 (m, 4H), 1.62 (m, 4H), 1.32 (br, 24H), 0.90 (t, 6H). ^{13}C-NMR (MHz, CD_3OD): δ 172.89, 168.96, 72.43, 39.45, 36.95, 33.37, 31.87, 29.42, 29.28, 29.26, 28.99, 24.60, 22.55, 13.25. IR (cm^{-1}, KBr): 3447 (NH), 1744 (C=O, ester), 1666 (C=O, amide). ESI-MS m/z: 543.3 $[M+H]^+$.

3c. Yield: 250 mg, 94% (white solid). ^{1}H-NMR (MHz, CD_3OD): δ δ 8.62 (s, 2H), 5.56 (s, 2H), 3.51 (m, 4H), 3.08 (m, 4H), 2.47 (m, 4H), 1.62 (m, 4H), 1.29 (br, 32H), 0.90 (t, 6H). ^{13}C-NMR (MHz, CD_3OD): δ 172.88, 168.95, 72.44, 39.46, 36.93, 33.39, 31.90, 29.60, 29.47, 29.30, 29.00, 24.62, 22.55, 13.25. IR (cm^{-1}, KBr): 3448 (NH), 1742 (C=O, ester), 1654 (C=O, amide). ESI-MS m/z: 599.5 $[M+H]^+$.

Molecular Dynamics (MD) refinement and equilibration

For refinement of molecular organization of constructed membranes and for modeling their interactions with CAms, we used molecular dynamics program suite Amber 14. Charge scheme and topology for lipid components POPC and POPE were obtained from Lipid14 force field and for POPG and POPS from Lipid11 force field. Membranes were solvated in a water box with at least 9 Å distance from the lipid heavy atoms and neutralized with sodium atoms as needed. Membrane structures obtained after 20 ns of MD simulations were used as a starting position to study behavior of CAms at the membrane surface.

CAm Parameterization

Structures of CAms were sketched, optimized by minimization with MMFF force field and saved in mol2 format in MOE program. For compatibility for MD simulation with Amber14 all minimized mol2 files were converted into topology files with antechamber subroutine from AmberTools 15 package using AM1-BCC charge method (-c bcc).

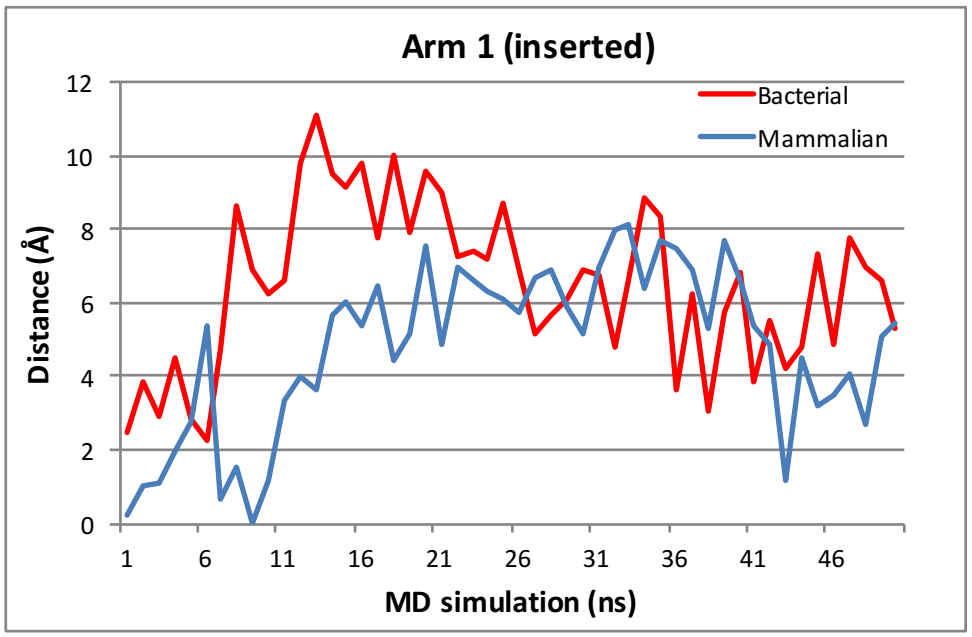

Figure S1: Distance of Arm 1 to bacterial and mammalian membranes determined via MD simulation. Positive values for penetration into membrane while negative values for above the membrane.

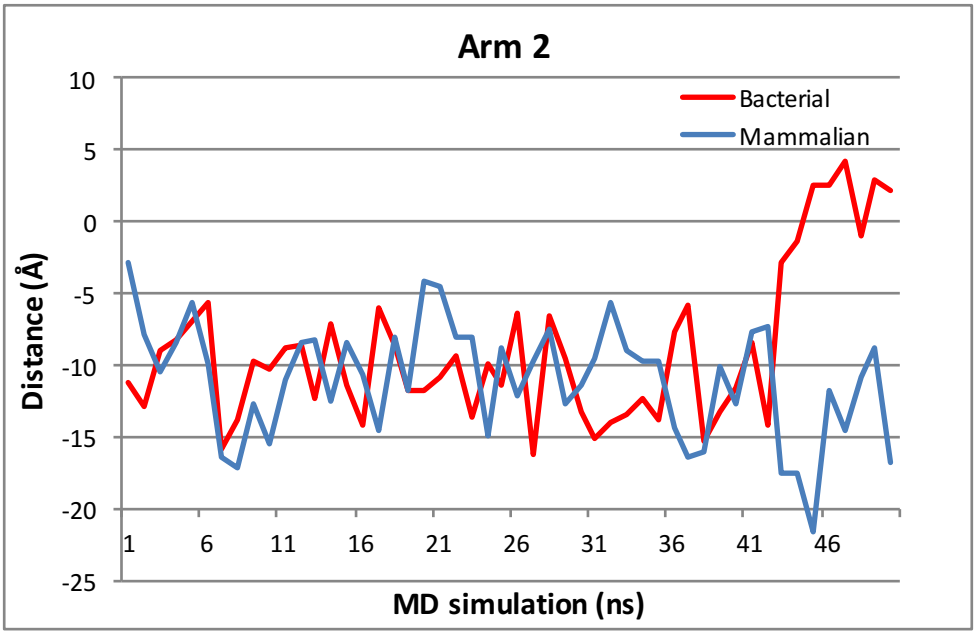

Figure S2: Distance of Arm 2 to bacterial and mammalian membranes determined via MD simulation. Positive values for penetration into membrane while negative values for above the membrane.

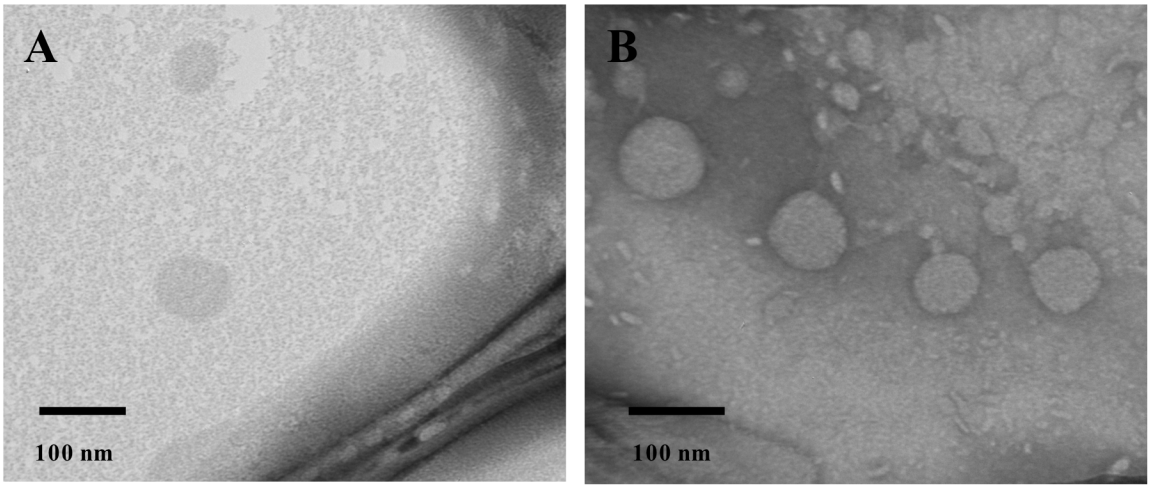

Figure S3: TEM images of ***3c*** (A) and ***5c*** (B).

Reference:

1. Abdelhamid DS, Zhang Y, Lewis DR, Moghe PV, Welsh WJ and Uhrich KE. Tartaric acid-based amphiphilic macromolecules with ether linkages exhibit enhanced repression of oxidized low density lipoprotein uptake. *Biomaterials*. 2015; 53: 32-9.

2. Faig A, Arthur TD, Fitzgerald PO, Chikindas M, Mintzer E and Uhrich KE. Biscationic Tartaric Acid-Based Amphiphiles: Charge Location Impacts Antimicrobial Activity. *Langmuir*. 2015; 31: 11875-85.