Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

The role of spacers on the self-assembly of DNA aptamer-amphiphiles into micelles and nanotapes

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Materials and Methods

Materials

Toluene, chloroform, acetone, methanol, and triethylamine were purchased from Fischer Chemical (Hanover Park, IL). Ammonium acetate, Nile red, sodium acetate, dimethylformimide, dichloromethane, aminododecanoic acid, succinic anhydride, dicyclohexylcarbodiimide, *N*-Hydroxysuccinimide, L-glutamic acid, p-toluene sulfonic acid monohydrate, hexadecanol, ethyl acetate, ethanol, and acetonitrile were purchased from Sigma-Aldrich (St Louis, MO). α-carboxy γ-amino heterobifunctional PEG_4 and PEG_8 spacers were purchased from Thermo Scientific (Rockford, IL), the aptamer from Integrated DNA Technologies (Coralville, IA), cetyl trimethylammonium bromide from Acros Organics (Morris Plains, NJ), hexafluroisopropanol (HFIP) from Oakwood Products Inc. (West Columbia, SC), lacey formvar/carbon, 200 mesh, copper grids from Ted Pella Inc. (Redding, CA), and 1.5 mm diameter quart capillaries from Charles Supper Company (Natick, MA).

Aptamer-amphiphile synthesis

Synthesis schemes are shown in Fig. S1 and the chemical structures of the aptamer-amphiphiles are shown in Fig. S2. Hydrophobic dialkyl tails previously synthesized by our group[1] were modified with *N*-hydroxysuccinimide (NHS) to make them amine reactive. To create the NHS activated tails, $(C_{16})_2$-Glu-C_2 tails were dissolved in ethyl acetate followed by the addition of 2x molar excess of both NHS and dicyclohexylcarbodiimide (DCC). The solution stirred at 40 °C for 2 h. The solution was filtered to remove the dicyclohexylurea byproduct and placed at -20 °C to precipitate the activated tails. The precipitate was collected and dried in a vacuum oven. Hydrophilic PEG_4 and PEG_8 spacers were added to $(C_{16})_2$-Glu-C_2-NHS tails by reacting the activated tails with the heterobifunctional PEG_4 or PEG_8 in dichloromethane at room temperature for 2 h. Unreacted PEG spacers were removed by washing the product with 1 mL aliquots of purified water. C_{12} spacers and $(C_{16})_2$-Glu-C_2-NHS tails were dissolved in 60 °C methanol, reacted for 2 h, and dried $(C_{16})_2$-Glu-C_2-C_{12} tails were redissolved with dichloromethane and filtered to remove unreacted C_{12} spacers. $(C_{16})_2$-Glu-C_2-PEG_x or $(C_{16})_2$-Glu-C_2-C_{12} tails were activated with NHS as described above. To create $(C_{16})_2$-Glu-C_2-C_{24} tails, C_{12} spacer was added to NHS-activated $(C_{16})_2$-Glu-C_2-C_{12} tails, followed by re-activation of the terminal carboxyl group with NHS.

To link the activated tails with the aptamer, 1.25x molar excess cetyl trimethylammonium bromide (CTAB) dissolved in water was added to Muc-1 aptamer. The NH_4^+ ammonium moiety of CTAB is electrostatically attracted to the PO_4^- of the aptamer backbone, forming a sheath of hydrocarbon chains that surround the hydrophilic DNA molecule, which renders the aptamer soluble in dimethylformamide (DMF). A 10x molar excess of activated tails were added to the CTAB-DNA complexes dissolved in DMF and the reaction was stirred at 50 °C for 24 h. After 24 h, the DMF was removed by evaporation and the aptamer-amphiphiles and any unreacted aptamers were purified by ethanol precipitation to remove unreacted tails and CTAB. Unreacted aptamer was separated from the aptamer-amphiphile using reverse-phase high performance liquid chromatography (RP-HPLC) after they were dissolved in water and filtered. HPLC information: Zorbax C18 300 Å SB column, 5-98 %B over 25 min, buffer A: H_2O+10 % methanol, 100 mM hexafluroisopropanol (HFIP), 14.4 mM triethylamine (TEA), buffer B: Methanol, 100 mM HFIP, 14.4 mM TEA. To confirm the success of the synthesis the molecular weights of the aptamer-amphiphiles were verified via liquid chromatography-mass spectroscopy (LC/MS) (Zorbax C18 300 Å SB column, 50-80 %B over 15 min, buffer A: H_2O+15 mM ammonium acetate, buffer B: Acetonitrile. Mass spectroscopy was acquired with an Agilent MSD ion trap). HPLC and LC/MS spectra are shown in Fig. S3.

Critical micelle concentration (CMC) evaluation

Concentrated solutions of each of the aptamer-amphiphiles were diluted with water to make amphiphile solutions ranging from 0.1-900 nM. 50 µL of these solutions were added to wells of a 96 well plate, followed by the addition of 1µL of a 0.05 mg/mL Nile red dye dissolved in acetone. An orbital shaker operating at 200 rpm was used to mix the solutions for 3 h prior to reading the Nile red fluorescence of

each well with a fluorescent plate reader (Ex: 540 nm, Em: 635 nm). Three experiments (n=3) for each amphiphile were performed in triplicate and averaged to produce a single fluorescence value for each (Fig. S4).

Cryogenic transmission electron microscopy (Cryo-TEM)

4 μL of 400 or 500 μM amphiphile samples dissolved in water or KCl were deposited onto lacey formvar/ carbon copper grids that were treated for 15 sec with glow discharge and vitrified in liquid ethane by Vitrobot (Vitrobot parameters: 5 sec blot time, -1 offset, 3 sec wait time, 3 sec relax time, 95 % humidity). Following vitrification, the grid was transferred to a Tecnai G2 Spirit TWIN 20-120 kV / LaB6 Transmission Electron Microscope. Images were captured using an Eagle 2k CCD camera with an accelerating voltage of 120 kV.

Small angle x-ray scattering (SAXS)

X-ray scattering experiments were conducted on the DND-CAT beamline at Argonne National Labs using a beam energy of 17 keV (λ=0.729 Å) and a sample to detector distance of 4.575 m, corresponding to a q-range of 0.0052 $Å^{-1}$ to 0.213 $Å^{-1}$. A low noise marCCD detector recorded the 2-D x-ray scattering data generated by 400 μM solutions of aptamer-amphiphiles sealed within quartz capillaries. Three exposures, each 4 sec in duration, were collected, averaged, and integrated over 120° to produce 1-D data of intensity versus q using FIT2D data reduction software. Scattering data were normalized to incident beam, exposure time, and transmission, and scaled to absolute units. Background scattering from a capillary containing only water was subtracted from the amphiphile scattering data prior to analysis.

Circular dichroism (CD)

9-13 μM solutions of aptamer and aptamer-amphiphiles in water or KCl were heated to 95 °C, quickly cooled to room temperature and transferred to 0.1 cm path length cuvettes immediately prior to collecting their CD spectra using a Jasco J-815 spectrapolarimeter. Data were collected from 320-200 nm at a read speed of 50 nm/min in 1 nm steps. 3 accumulations per amphiphile solution were obtained, the background spectra from the water were subtracted, and the data averaged. Raw ellipticity data were converted to molar ellipticity (Θ) and smoothed with a Matlab filter.

References

1 A. Mardilovich and E. Kokkoli. *Biomacromolecules*, 2004, **5**, 950-957.

Figures

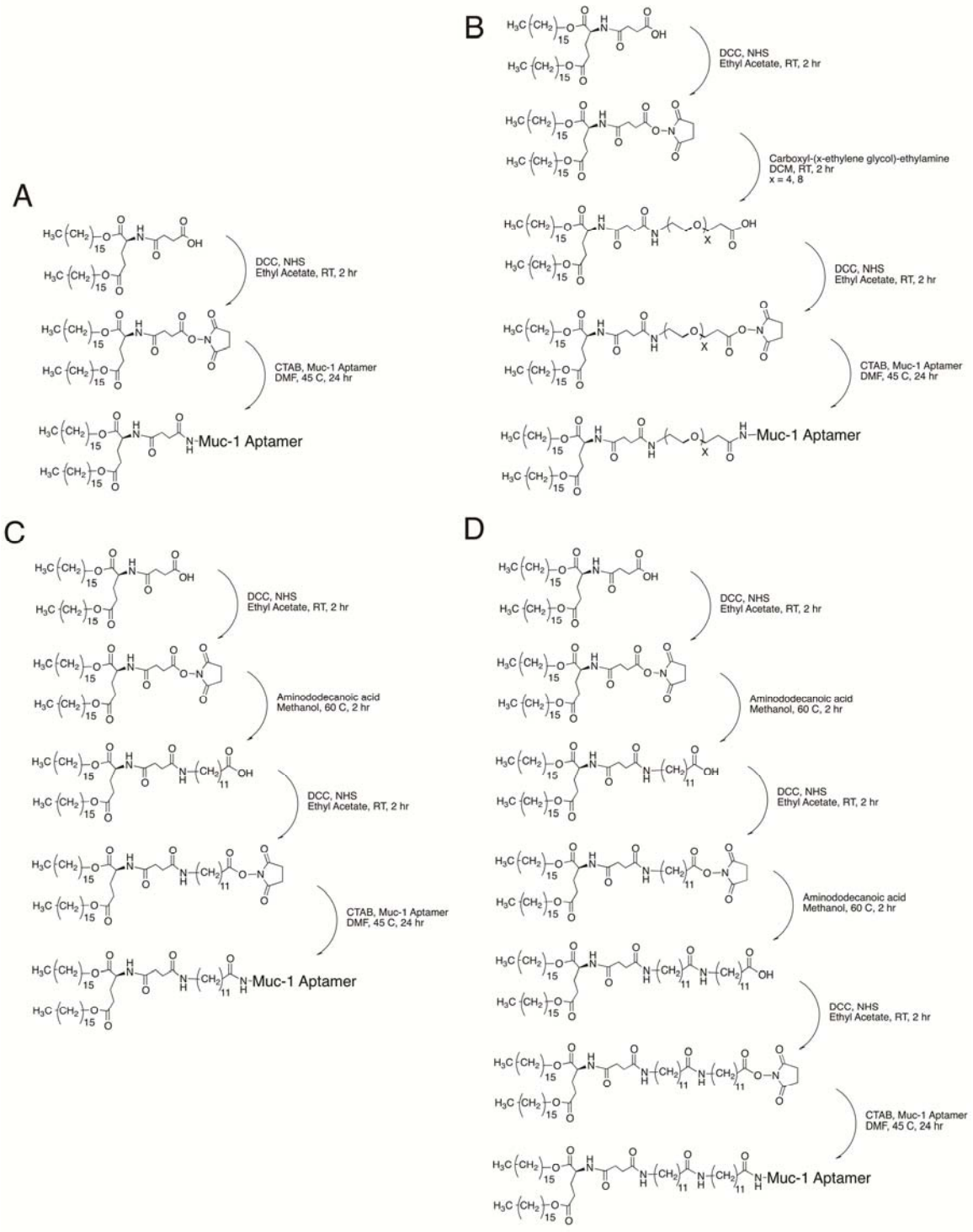

Fig. S1 Synthesis schemes for Muc-1 aptamer-amphiphiles with A) NoSPR, B) hydrophilic $PEG_{4,8}$ spacers, C) hydrophobic C_{12} spacer and D) hydrophobic C_{24} spacer.

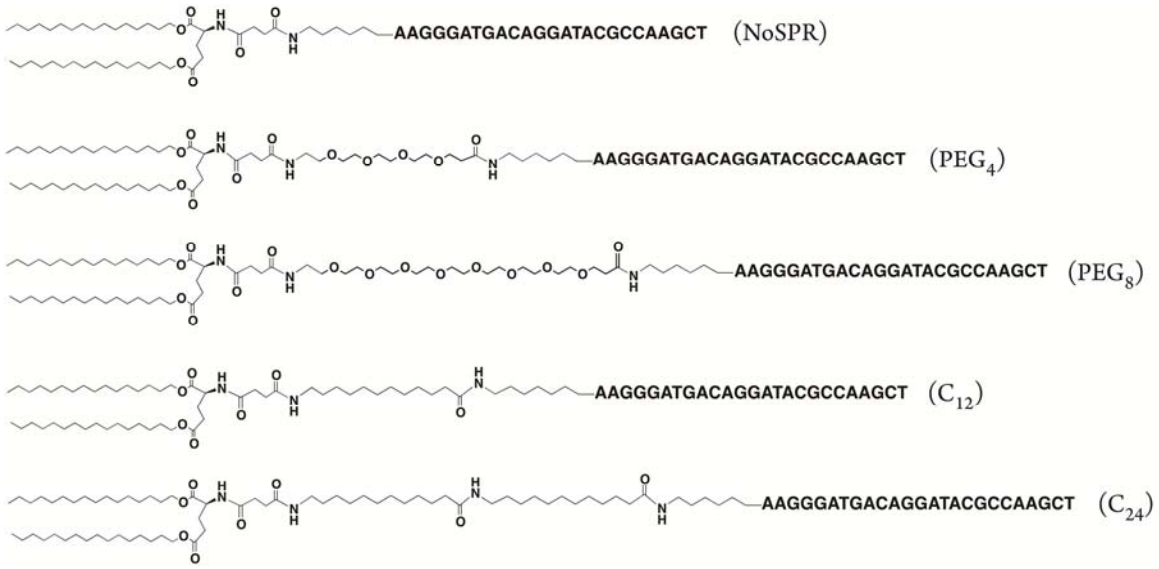

Fig. S2: Chemical structures of the five Muc-1 aptamer-amphiphiles synthesized.

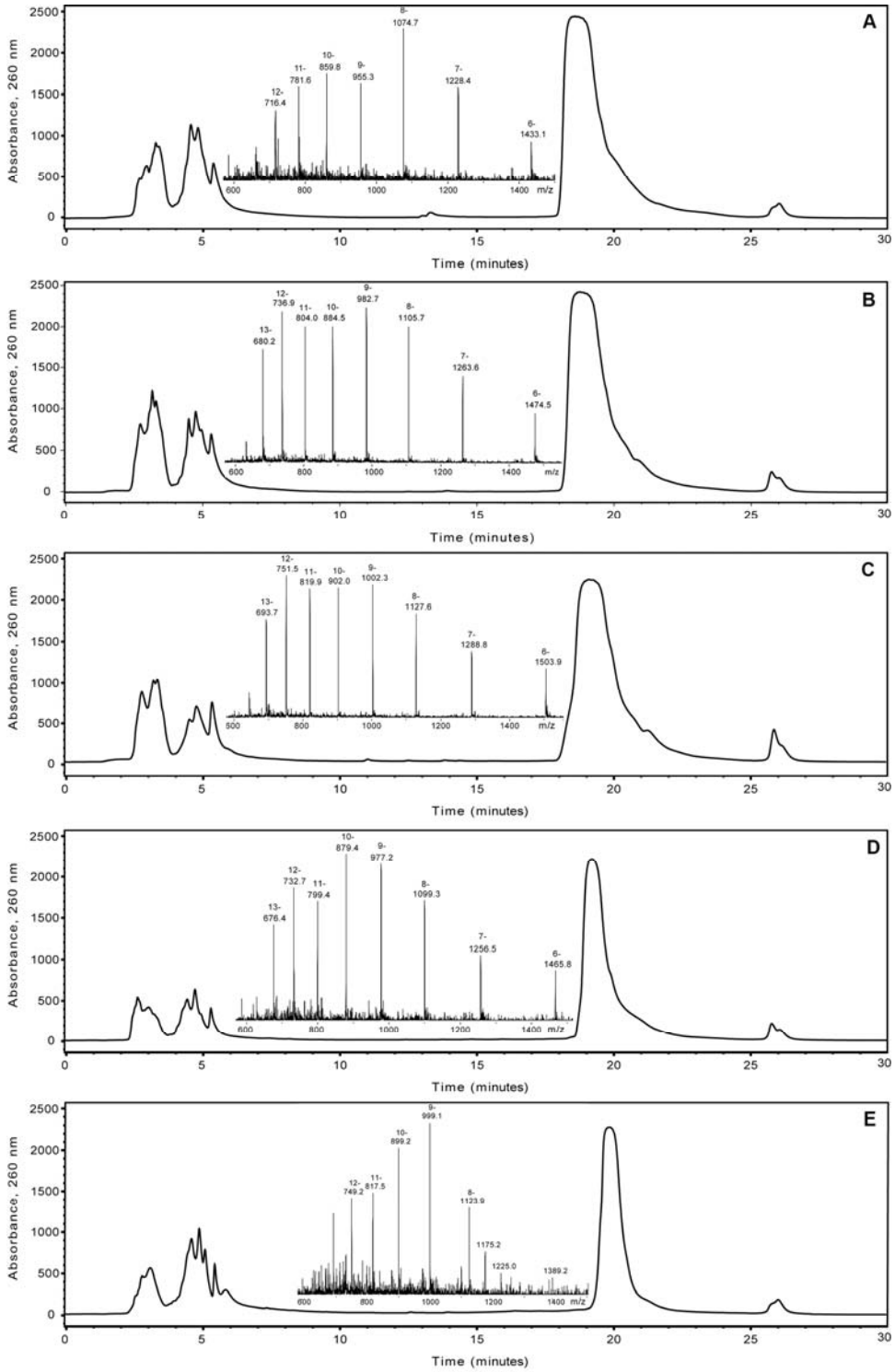

Fig. S3 HPLC chromatograms of the A) NoSPR, B) PEG_4, C) PEG_8, D) C_{12}, and E) C_{24} Muc-1 amphiphiles. The peaks from 3-6 min are from the aptamer while the peak near 20 min is the aptamer-amphiphile. The molecular masses of the aptamer-amphiphiles were determined by LC/MS (insets) and are in good agreement with the expected masses: A) expected mass: 8606.3; observed mass: 8606.9, B) expected mass: 8853.5; observed mass: 8853.4, C) expected mass: 9029.8; observed mass: 9029.1, D) expected mass: 8803.6; observed mass: 8803.3, E) expected mass: 9000.9; observed mass; 9000.9.

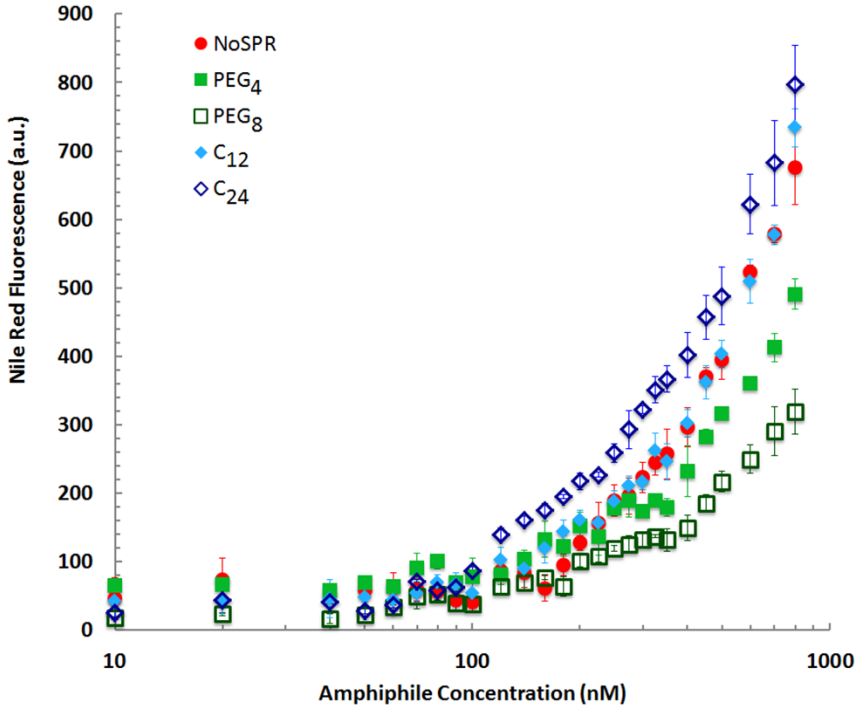

Fig. S4 Fluorescence intensity of the Nile red dye solubilized in aptamer-amphiphile assemblies versus the aptamer-amphiphile concentration.

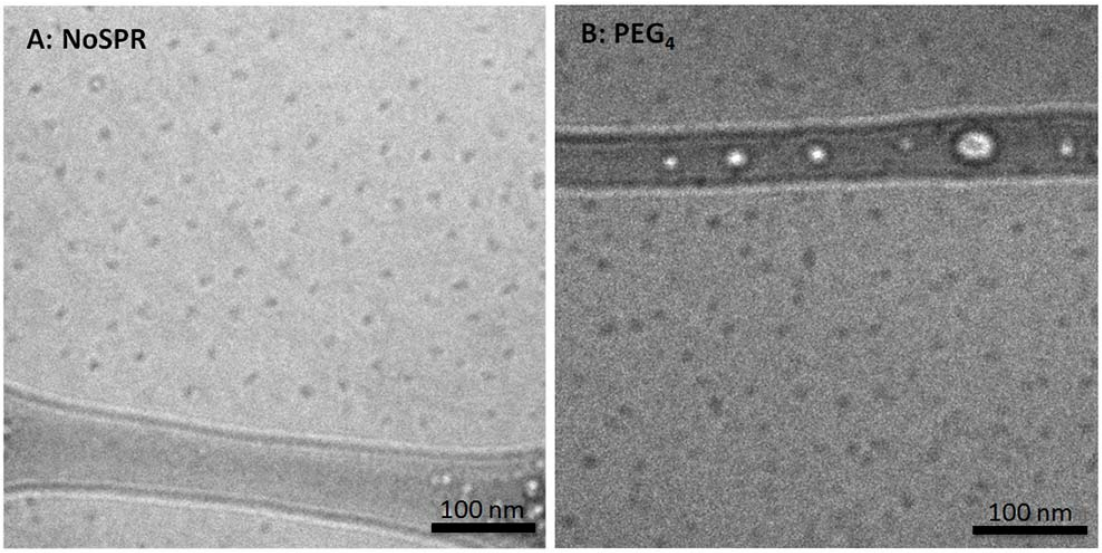

Fig. S5: Cryo-TEM images of 400 μM Muc-1 aptamer-amphiphile solutions in H_2O. Globular micelles formed by A) NoSPR amphiphiles and B) PEG_4 spacer amphiphiles.

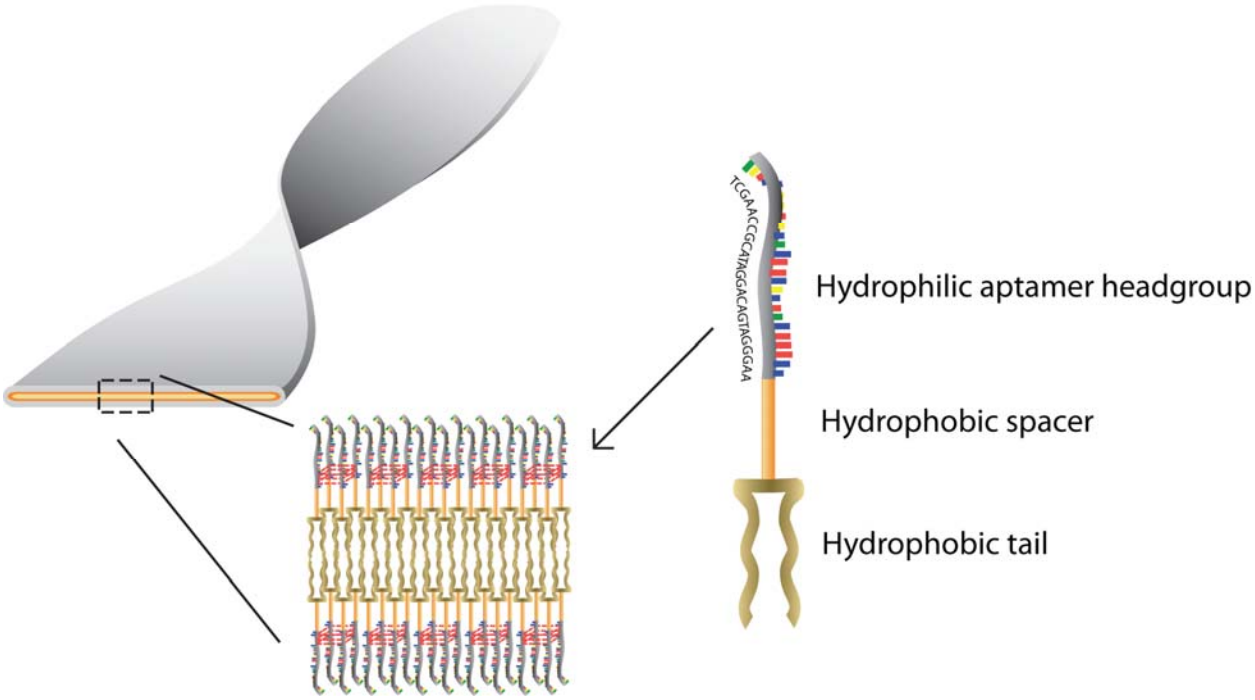

Fig. S6: Graphical representation of the nanotape structures formed from C_{12} and C_{24} spacer Muc-1 aptamer-amphiphiles. The red dotted lines show how we hypothesize that four aptamer headgroups may be forming G-quadruplexes.

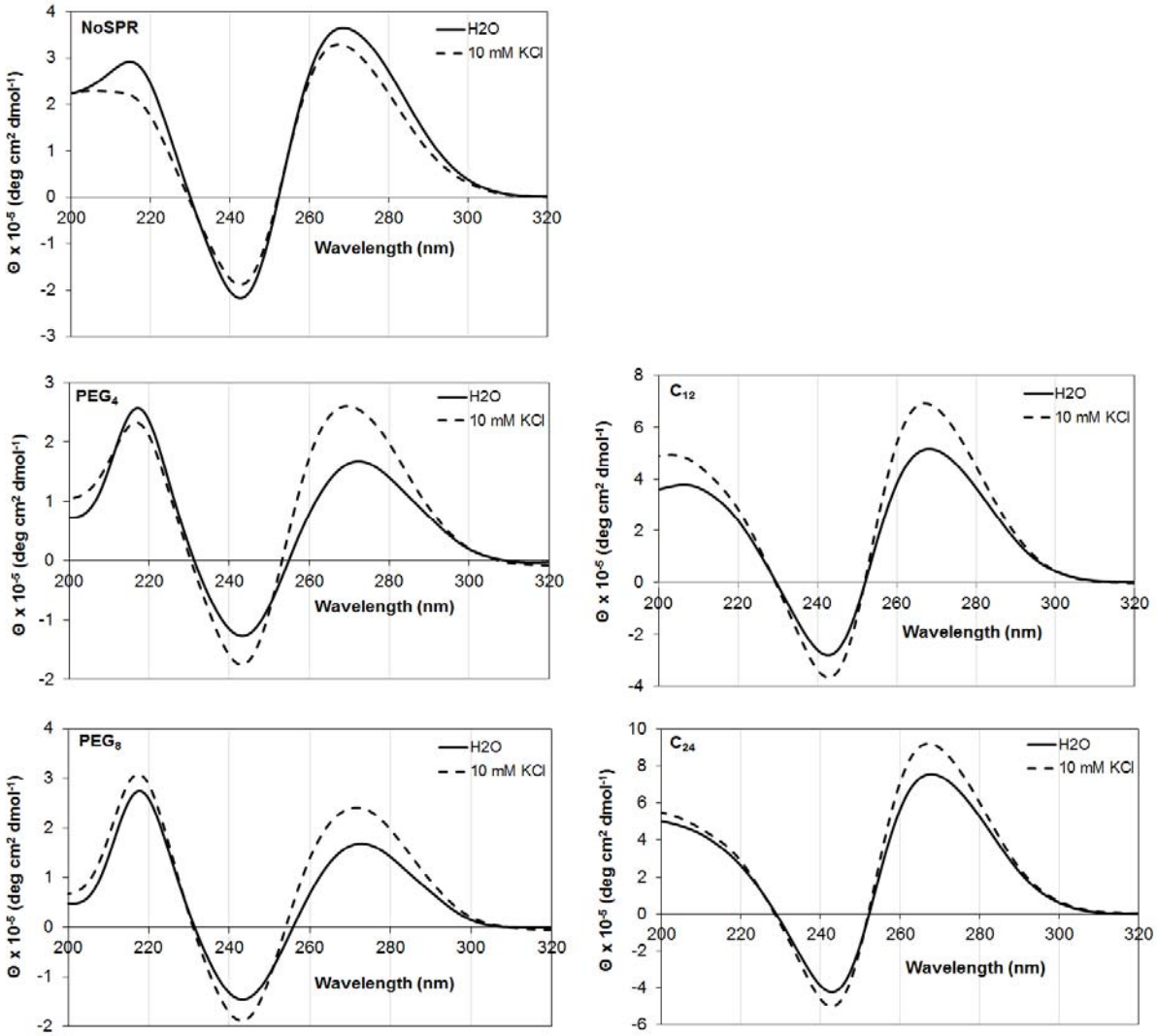

Fig. S7 CD spectra of Muc-1 aptamer-amphiphiles with different spacers (NoSPR, PEG_4, PEG_8, C_{12}, and C_{24}) dissolved in H_2O or 10 mM KCl. The CD spectra suggest that the addition of salt to the amphiphile solutions does not change the structure of the amphiphiles' headgroups significantly.

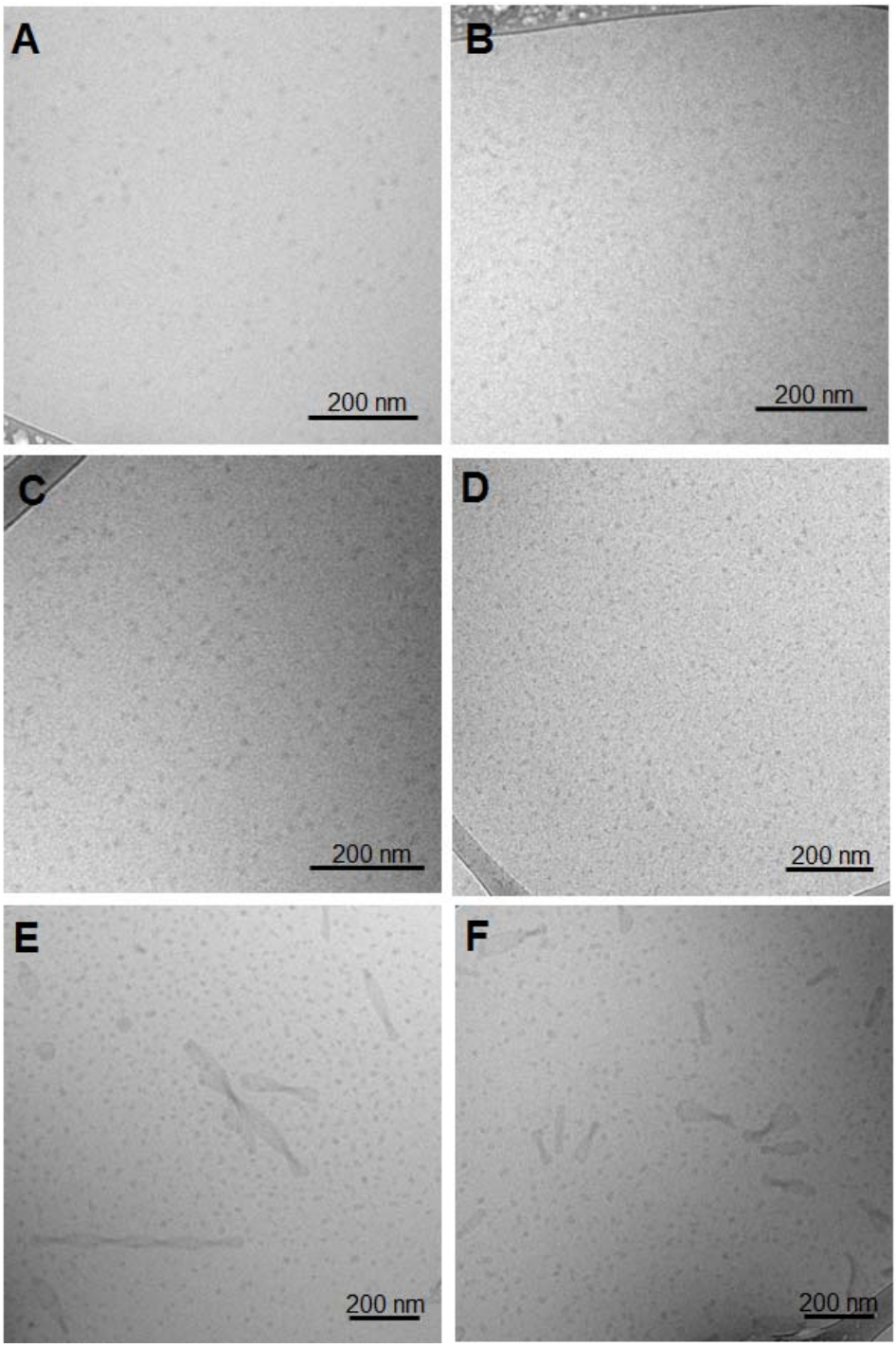

Fig. S8 Cryo-TEM images of 500 μM Muc-1 aptamer-amphiphile solutions in H_2O (A, C, E) or 10 mM KCl (B, D, F). Samples in H_2O were kept at room temperature for 28 days prior to freezing and imaging. The 10 mM KCl samples were frozen and imaged immediately after the amphiphiles were dissolved. Globular micelles remain the only structures present in the NoSPR (A, B) and PEG_8 (C, D) samples while the C_{24} (E, F) amphiphiles assemble into both micelles and nanotapes for both solutions.