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Supporting Information

Formation of pH-Resistant Monodispersed Polymer–Lipid Nanodiscs

*Thirupathi Ravula[+], Nathaniel Z. Hardin[+], Sudheer Kumar Ramadugu, Sarah J. Cox, and Ayyalusamy Ramamoorthy**

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Supporting Information

Materials and Methods

Poly(Styrene-co- Maleic Anhydride) cumene terminated ~1.3:1 (SMA Mn ~1600 g/mol), (2-Aminoethyl)trimethylammonium chloride hydrochloride, Triethylamine (Et_3N), phosphoric acid (H_3PO_4), sodium phosphate, sodium chloride, hydrochloric acid (HCl), sodium hydroxide (NaOH), trifluoracetic acid (TFA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), diethyl ether (Ether), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), Dimethylformamide (DMF), Acetic Anhydride, and Sodium Acetate were purchased from Sigma-Aldrich®. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Lipids Polar, Inc®.

Synthesis of SMA-QA:

1 g of SMA was dissolved in 30 ml of anhydrous DMF dried over molecular sieves. 1.3 g of (2-aminoethyl)trimethylammonium chloride hydrochloride was then added to the solution and to this mixture 5 ml of trimethylamine was added causing the solution to turn dark yellow. The reaction mixture was then stirred at 100°C for 12 hours. The solution was cooled to room temperature and precipitated with diethyl ether. The precipitate was washed 3 times with diethyl ether and dried under vacuum. The dried intermediate was then added to 30 ml acetic anhydride. 660 mg of sodium acetate and 200 mg of triethyl amine were then added. The reaction mixture was heated at 100 °C overnight and precipitated in ether. The precipitate was washed 3 times with ether and dried under vacuum. The product was then dissolved in water and passed through a saphadex LH-20 column. The product was collected and then lyophilized to give a 850 mg brown powder.

Formation of nanodiscs:

Nanodiscs of differing sizes were prepared using DMPC (10 mg/ml) in 20 mM sodium phosphate buffer containing 50 mM NaCl at pH 7.4. 10 mg/ml of polymer stock solutions were made in the same buffer solution. The required amount of polymer solution was added to the DMPC mixture and incubated for 4 hr at 35 ^{o}C. The samples made using DMPC:SMA-QA weight ratios of 1:0.25 and 1:0.5 were prepared using three freeze thaw cycles alternating between liquid nitrogen temperature and 35 °C. After the freeze thaw cycles the samples were further incubated at 35 ^{o}C for 4 hrs.

SLS experiments:

The pH stability and metal ion stability experiments were carried out by measuring the intensity of scattered light at a 90° angle using a Fluro Fluorimeter under identical DMPC concentrations. 500 ul of nanodiscs were dispensed into a 2 ml cuvette under stirring. Then the solutions were diluted to 2 ml with buffer. pH titrations were performed using 1M HCl and NaOH. Metal ion titrations were performed using 5M $MgCl_2$, 5M NaCl, and 3.3M $CaCl_2$. The excitation and emission wavelengths were set at 400 nm and 404 nm respectively. The slit opening was set to 2 nm. All SLS experimental measurements were carried out using a FluoroMax 4® from Horiba

Scientific®. Time Dependent solubilization studies were run using the same fluorimeter settings as noted above using indicated polymer to lipid ratios.

Solid state NMR Spectroscopy: Phosphorous-31 NMR spectra were acquired using an Agilent/Varian 400 MHz solid-state NMR spectrometer and a 5 mm triple-resonance probe with ^{31}P and ^{1}H resonance frequencies of 161.974 MHz and 400.114 MHz, respectively. 5μs 90^0 pulse, 30 kHz ^{1}H continuous-wave decoupling, 2,000 scans, and a 4 s recycle delay were used to acquire ^{31}P NMR spectra. ^{31}P chemical shifts were referenced by setting the ^{31}P chemical shift of 100 % H_3PO_4 sample to 0 ppm.

CPMAS solid-state NMR experiments: Carbon-13 CPMAS experiments were carried on a Bruker 500 MHz solid-state NMR spectrometer under 12 kHz MAS using a 2.5 mm triple-resonance MAS probe operating at 500.112 MHz and 125.721 MHz for ^{1}H and ^{13}C, respectively. The reported CPMAS spectra (Figure 1 of the main text) were acquired using the following parameters: 3 μs 90° pulse, 2 ms CP contact time, 40 ms acquisition time, 4096 number of scans, 4s recycle delay and a 50 kHz radio-frequency decoupling of protons during the acquisition of ^{13}C magnetization. ^{13}C chemical shifts were calibrated by setting the chemical shift of CH_2 resonance of adamantane powder sample to 28.5 ppm.

^{14}N NMR Experiments: Nitrogen-14 NMR spectra were acquired using an Agilent/Varian 400 MHz solid-state NMR spectrometer and a 5 mm triple-resonance probe operating at the ^{14}N resonance frequency of 29.910 MHz. ^{14}N NMR spectra were recorded using the quadrupole-echo pulse sequence with a 90° pulse length of 5 μs and an echo-delay of 1.1 ms. ^{14}N magnetization was acquired using 25 ms acquisition time, 10000 scans and a recycle delay of 1.5 s with no ^{1}H decoupling.

Fourier - Transform Infrared (FT - IR) Spectroscopy: The FT-IR spectra from 4000 cm^{-1} to 800 cm^{-1} were recorded using a Thermos scientific ATR-FTIR instrument. Water was removed by lyophilization from each of the samples before recording the spectrum (Figure S1).

Dynamic Light Scattering (DLS): All DLS experiments were performed using Wyatt Technology® DynaPro® NanoStar® using a 1 μL quartz MicroCuvette.

Transmission Electron Microscopy (TEM): All TEM micrographs were obtained using a Technai® T - 20® machine (FEI®, Netherlands) with a 80 kV operating voltage. A dilute solution was dropped on a carbon-coated copper grid and dried overnight at room temperature in a desiccator before using in experiments.

Size exclusion chromatography: Polymer-lipid nanodiscs were purified by size exclusion chromatography (SEC), using Superdex 200 Increase 300/10 GL column operated on an AKTA purifier (GE Healthcare, Freiburg, Germany). The elution profiles of polymer nanodisc contained two peaks one corresponding to nanodisc and other to the free polymer. All nanodisc samples were subjected to SEC to remove the free polymer.

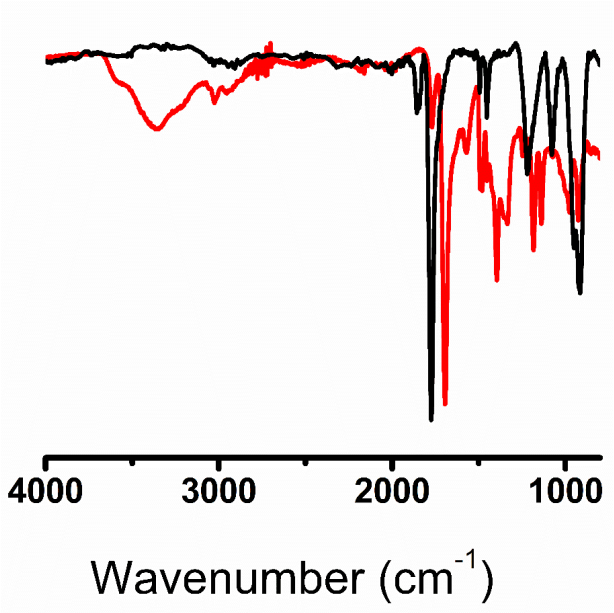

Figure S1: FTIR spectra of SMA (black) and the newly synthesized SMA-QA (red) polymers.

Nitrogen-14 Quadrupole coupling: The ^{14}N quadrupole coupling is an axially symmetric tensor for the ^{14}N of choline group in DMPC lipid. Hence, the observed quadrupolar splitting is a direct measurement of the molecular order parameter of the C_β-N (CN) bond of the choline moiety. The frequency separation between the two perpendicular edges of the powder pattern spectrum is given by

$$\Delta\upsilon = \frac{3}{4}\frac{e^2qQ}{h}S_{mol}\frac{3\,cos^2\theta - 1}{2}$$

where $\frac{e^2qQ}{h}$ =135 kHz is the nitrogen quadrupole coupling constant, S_{mol} is the order parameter that accounts for motional averaging, and θ is the time and space averaged angle of the CN vector with respect to the external magnetic field direction (Figure S2).

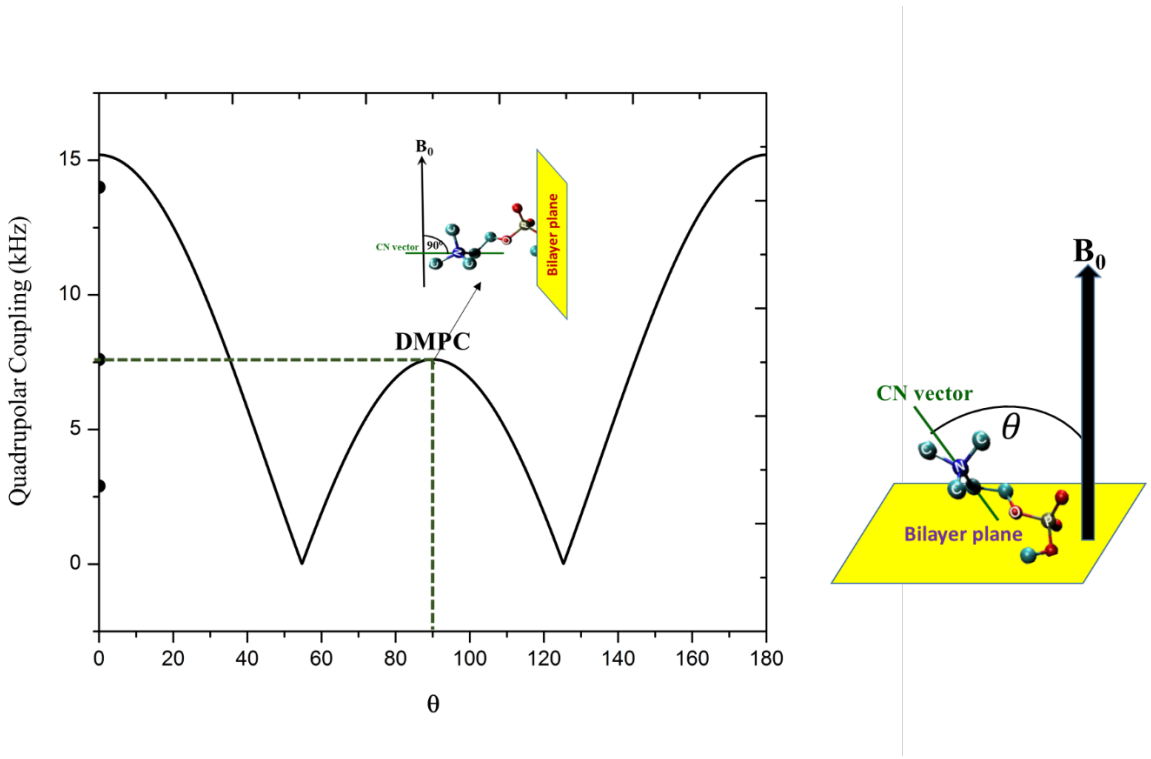

Figure S2. Plot of theta vs quadrupolar coupling which is scaled to reflect the experimentally determined value for DMPC. Schematic showing the definition of theta. The experimentally measured quadrupole coupling can be used determine the orientation of the choline group with respect to the direction of the external magnetic field (i.e., theta). The vertical dashed lines indicate the corresponding angles for the orientation of the quaternary group relative to the magnetic field direction.