# Spontaneous Lipid Nanodiscs Fomation by Amphiphilic Polymethacrylate Copolymers

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## Supporting information

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## EXPERIMENTAL SECTION

### *Materials*

1,2-dimyristoyl-*sn*-glycero-3-phosphochonine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DMPG) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Methacroylcholine chloride and butyl methacrylate were purchased from TCI Co., Ltd. (Tokyo, Japan). Human-IAPP was purchased from AnaSpec, Inc. (Fremont, CA, USA). Hydrolyzed Styrene maleic acid polymer (lipodisq) and Thioflavin- T were purchased from Sigma Aldrich USA. All other chemicals were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan) or Fischer Scientific Ltd (Pittsburgh, PA, USA) and were used without further purification.

### *Synthesis of polymers*

Amphiphilic polymethacrylates were synthesized by free radical polymerization initiated by azobisisobutyronitrile (AIBN) as shown in Scheme 1. Appropriate amount of methacryloylcholine chloride was dissolved in isopropyl alcohol. After the addition of 3-mercaptopropionate (MMP) and AIBN, the solution was bubbled with nitrogen gas to remove the dissolved oxygen. After the addition of butyl methacrylate, the solution was stirred at 65 °C overnight. After the polymerization, solvent was removed in vacuo and dissolved in a small amount (approx. 300 μL) of methanol. The resultant polymer solution was added to ice cold diethyl ether. Then, the resultant precipitate was collected by centrifugation and lyophilized from aqueous solutions to give cationic copolymers as a white powder. $^1$H NMR analysis was used to determine the degree of polymerization (DP) and the mole percentage of butyl methacrylate, *f*, of polymers. The integration of signals from the following chemical groups was used for the characterization of polymers: methoxy terminal of the polymethacrylate backbone ($CH_3OCO$, 4.0 ppm, 3H), methylene group in choline sidechains ($(CH_3)_3N^+CH_2$, 2.9 ppm, 2H), and the melthylene in both monomer units ($COOCH_2$, 2.2 ppm, 2H). A representative $^1$H NMR spectrum is shown in Figure S2.

### *Preparation of polymer based lipid nanodiscs*

Large unilamellar vesicles (LUVs) were first prepared by hydrating the lipid thin films as follows. Appropriate amounts of desired phospholipids were dissolved in chloroform. The solvent was evaporated under a stream of nitrogen gas and the residual trace solvent was completely removed in vacuo for 3 h to give a thin lipid film on the wall of a glass vial. The resultant lipid film was hydrated by vertex mixing with 10 mM HEPES buffer (pH=7.4, 150 mM NaCl) at 40 °C. To homogenize the size of LUVs, the liposome dispersion was subjected to five freeze-and-thaw cycles at −196 and 50 °C, followed by extrusion (11 times) through stacked 100 nm polycarbonate membrane filters installed in the LiposoFast miniextruder from Avestin. To test the solubilization of the vesicles, an appropriate amount of DMSO stock solution (2 μL) of the polymer was added to 198 μL of the vesicle solution and mixed using a mechanical pipette. The final concentration of the phospholipids in the polymer-vesicle mixture was set to 100 μM and incubated for 30 minutes at room temperature prior to measurement.

*Dynamic light scattering*

Light scattering and hydrodynamic diameter of nanodiscs were measured using a dynamic light scattering spectrometer equipped with a laser diode illuminating at 665 nm (ELS-Z1000ZS, Otsuka Electronics Co., Ltd., Osaka, Japan). Size distribution of nanodiscs in an aqueous solution was obtained by analyzing a time course of scattered light intensity at an angle of 165$^o$ from the incident light with the Contin method. The sample temperature was maintained at 25 °C by the thermostat temperature controller installed in the equipment. The sample dispersion was filtrated using commercially available hydrophilic syringe filter with 450 nm pores to remove any dust from the sample prior to measurement.

*Electron microscopy*

The specimens for transmission electron microscopy (TEM) was prepared by negative-stain and cryogenic (Cryo) techniques. For the negative-stain TEM, a copper grid with 200 mesh with a thin support film was pre-treated with glow-discharger (HDT-400, JEOL) to obtain hydrophilic surface. An aliquot (2 µL) of nanodisc solution was placed on the mesh. After 1 minute of sample deposition, excess solution was removed using a filter paper, then the residual salt on the grid was washed with 2 µL of Milli-Q water. 2 µL of 2 wt% phosphotungstic acid solution was subsequently placed on a grid and left for 1 minute. Excess amount of phosphotungstic acid solution was removed using a filter paper. Finally, the specimen was dried gently in a desiccator containing silica gel.

The specimen for cryo-TEM was prepared by rapid freezing of vesicular dispersion. A copper microgrid with 200 mesh treated with a glow-discharger was used. An aliquot (3 µL) of nanodisc dispersion was placed on the mesh and immediately plunged into liquid propane using a specimen preparation machine (EM CPC, Leica). The temperature of the specimen was maintained by a cryotransfer holder (Model 626.DH, Gatan) at lower than -140 °C during the observation. All TEM measurements were carried out using a transmission electron microscope (JEM-3100FEF, JEOL) at an acceleration voltage of 300 kV. The microscopic image was recorded using a CCD camera (ORIUS SC-200, Gatan) installed in the microscope.

*NMR experiments on polymer solution*

2 mg of polymer was dissolved in 600 µl of 100% $D_2O$ to make a final concentration of 0.8 mM. 2D $^1$H-$^1$H NOESY experiment was carried out on a Bruker Avance II 600 MHz NMR spectrometer equipped with a cryoprobe. The spectrum was acquired at 25 °C using the following parameters: 8.2 µs 90° pulse, 64 scans and 64 $t_1$ increments, 60 ms of mixing time, 0.25 s acquisition time and 1 s recycle delay.

*NMR measurement of nanodiscs*

2D $^1$H-$^1$H NOESY NMR experiment was carried out on a Bruker 500 MHz spectrometer equipped with a 4 mm CMP HXY probe under magic angle spinning condition. All samples were prepared for a final concentration of 10 mg/ml in deuterated buffer. The NOESY spectra were acquired using the following parameters under 8 kHz at 20 °C: 3.15 µs 90° pulse, 60 ms NOESY mixing time, 30 ms acquisition time, 128 scans, 128 $t_1$ increments and 4 s recycling delay. 1H chemical shifts in both dimensions of the 2D spectrum are referenced to the terminal methyl group of DMPC which was set at 0.89 ppm.

$^{31}P$ NMR spectra were acquired using an Agilent/Varian 400 MHz solid-state NMR spectrometer using 5 mm triple-resonance and double-resonance probes. 5 µs 90° pulse, 30 kHz $^{1}H$ continuous wave decoupling, 512 scans, and 6 s recycle delay were used in experiments. $^{31}P$ NMR spectra were referenced by setting the $^{31}P$ chemical shift of 100 % $H_3PO_4$ sample to 0 ppm.

*Fluorescence experiments*

9:1 molar ratio of DMPC:DMPG was dissolved in CHCl3, dried under nitrogen gas, and the residual solvent was removed under high vacuum overnight. The resulting lipid film was rehydrated in 30 mM acetic acid buffer (pH 5.3) to make a stock solution of the lipid (10 mg/ml). 450 µl of polymer (10 mg/ml in water) was added to 300 µl lipid solution and incubated overnight. Excess polymer was removed by washing with buffer using Amicon filter using a 30 kDa cutoff membrane. The resulting solution was used for experimental measurements reported in this study. All samples were prepared freshly before the start of each experiment.

Aggregation of naturally-occurring C-amidated form of human islet amyloid polypeptide (hIAPP, also known as human amylin) was followed using florescence of ThT dye that binds to amyloid fibers. ThT was added to cold acetic acid buffer to make a final concertation of 20 µM. A required amount of hIAPP monomer solution was added to the polymer nanodisc solution. Time dependence of the fluorescence emission was measured using a BioTek Synergy 2 microplate spectrofluorometer with excitation and emission wavelengths of 440 and 480 nm, respectively.

The fluorescence spectra of ThT were acquired using 2 ml quartz cuvette in a FluroMax fluorimeter. All spectra were acquired using 440 nm excitation wavelength with a 2 nm slit width. 2 ml of a 5 µM ThT stock solution prepared in 10 mM acetic acid buffer (pH 5.3) added to a required amount of polymer or DMPC nanodiscs solution.

*Circular dichroism*

A freshly prepared human-IAPP monomer solution was added to a solution of polymer-based nanodiscs (in 30 mM acetic acid buffer, pH 5.3) to prepare a final concentration of 20 µm (1:100 molar ratio of hIAPP and DMPC). All CD measurements were performed on a JASCO J-815 spectropolarimeter using a 0.1 cm path length quartz cell.

*Direct fragmentation of E. coli*

The overnight culture of *Escherichia coli* ATCC 25922 in Muller-Hinton (MH) broth at pH 7.3 was diluted to OD of 0.4. Prior to the addition of the nanodisc-forming polymer, *E. coli* cells were washed by three cycles of centrifugation and re-dispersion in HEPES buffer (pH 7.3, [NaCl] = 150 mM) whose osmolality was adjusted to same as the MH broth. To the 100 µL of *E. coli* suspension, 100 µL of the N-C4-60-4.7 (10 mM) was added followed with an incubation for 10 minutes at room temperature. Fragmentation of *E. coli* membrane was confirmed by means DLS as well as negative-stain TEM as described above (Figure S1).

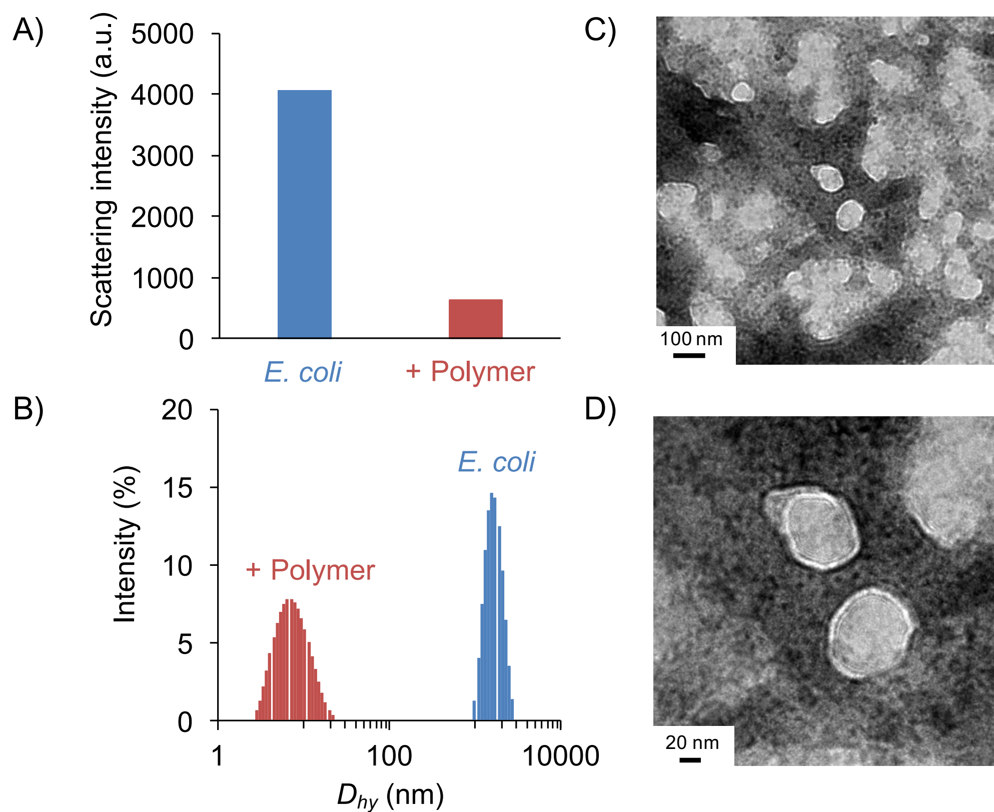


**Figure S1. Direct fragmentation of intact *E. coli* cells by polymer.** A) Effect of the nanodisc-forming polymer (C4-60-4.7) on the scattering intensity of the *E. coli* suspension. B) DLS data of *E. coli* suspension in the absence (blue) and presence (red) of the C4-60-4.7 polymer. C) Wide-field negative-stain TEM image of the polymer-*E. coli* mixture. D) Magnified negative-stain TEM image of the complex formed by the polymer.

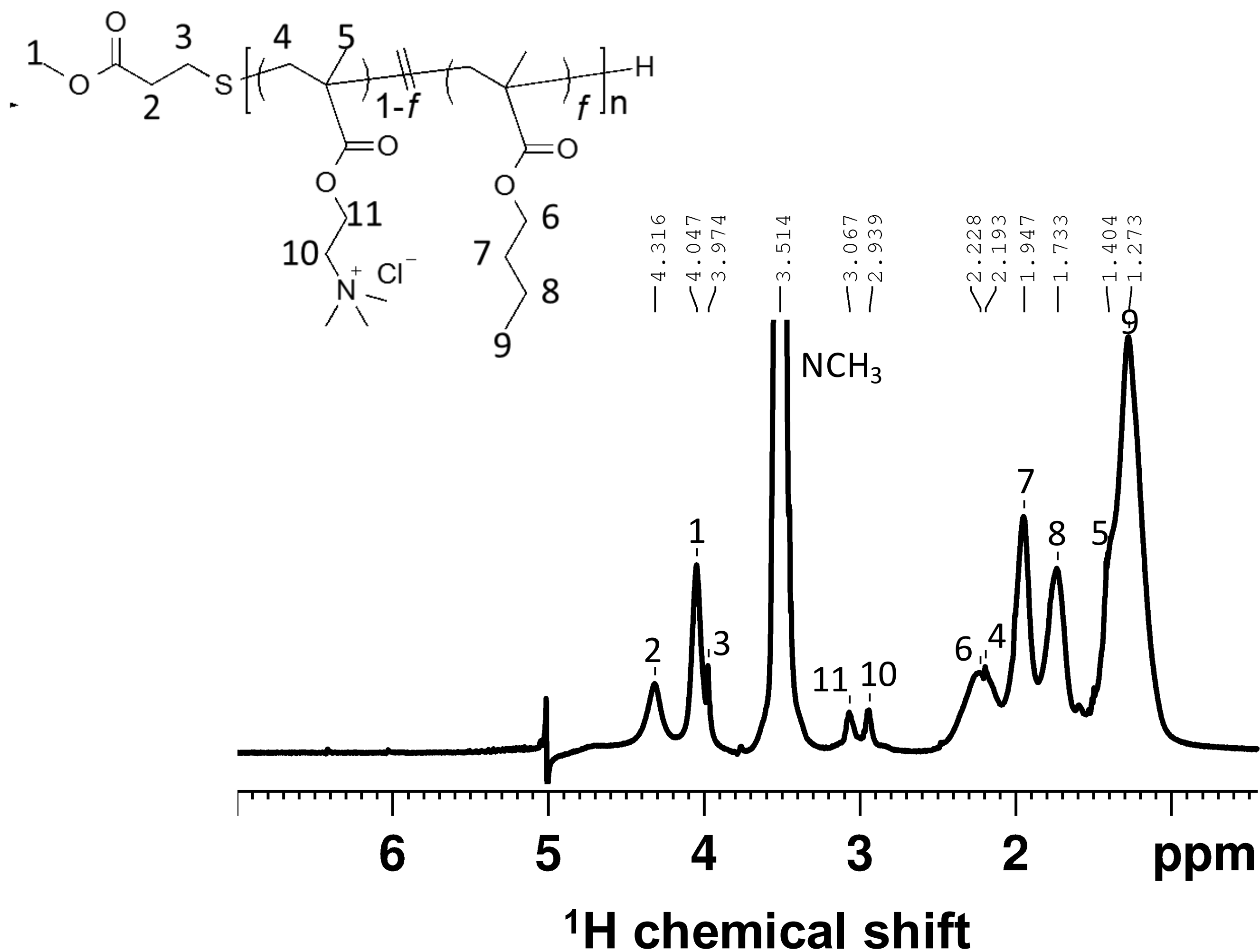


**Figure S2.** [1]H spectrum of the polymer in aqueous solution obtained at 25 °C. 2 mg of polymer sample was dissolved in 600 µl of 100% $D_2O$ to make a final concentration of 0.8 mM.

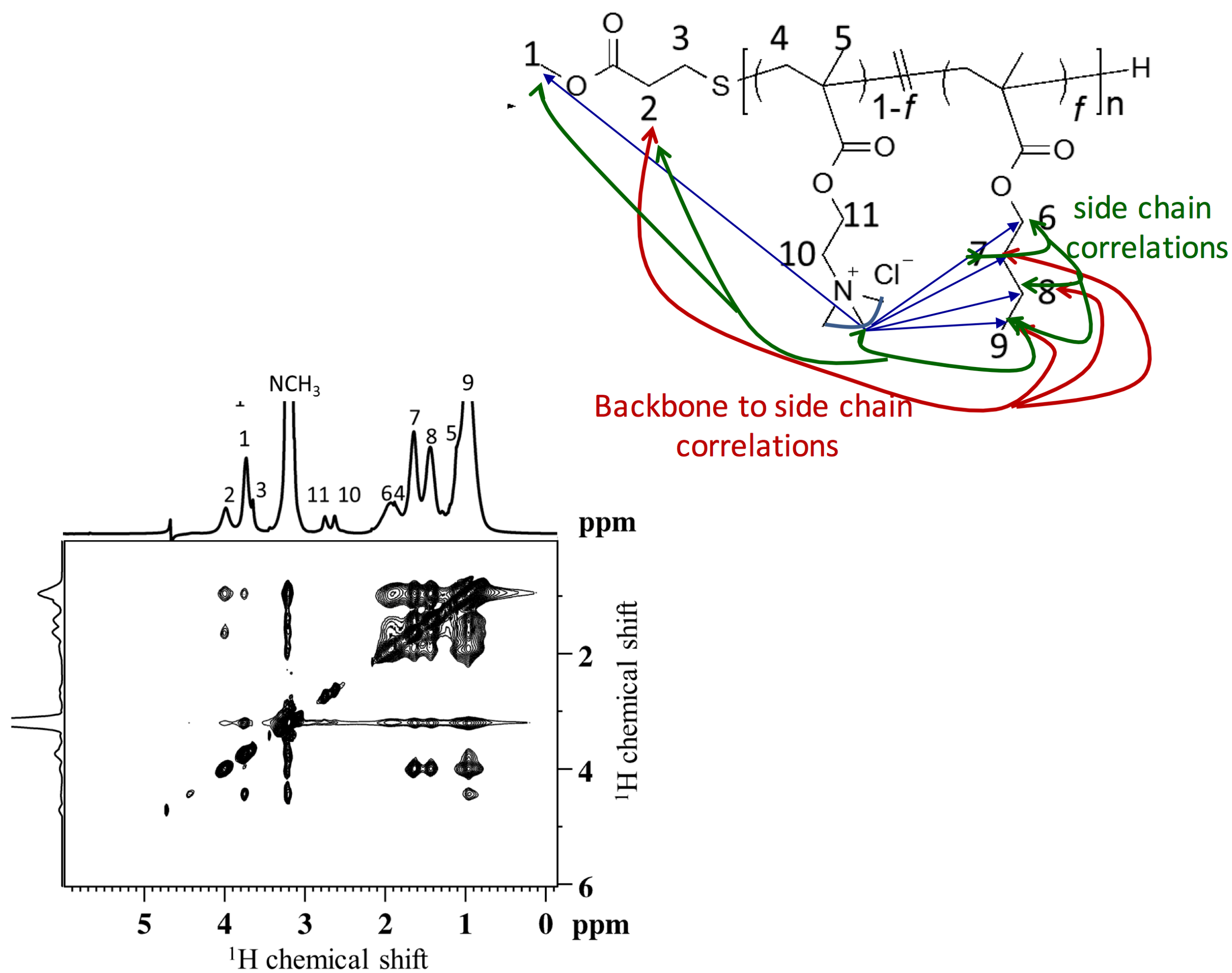


**Figure S3.** 2D $^1$H-$^1$H NOESY spectrum of the polymer in aqueous solution obtained at 25 °C. 2 mg of polymer sample was dissolved in 600 $\mu$l of 100% $D_2O$ to make a final concentration of 0.8 mM.

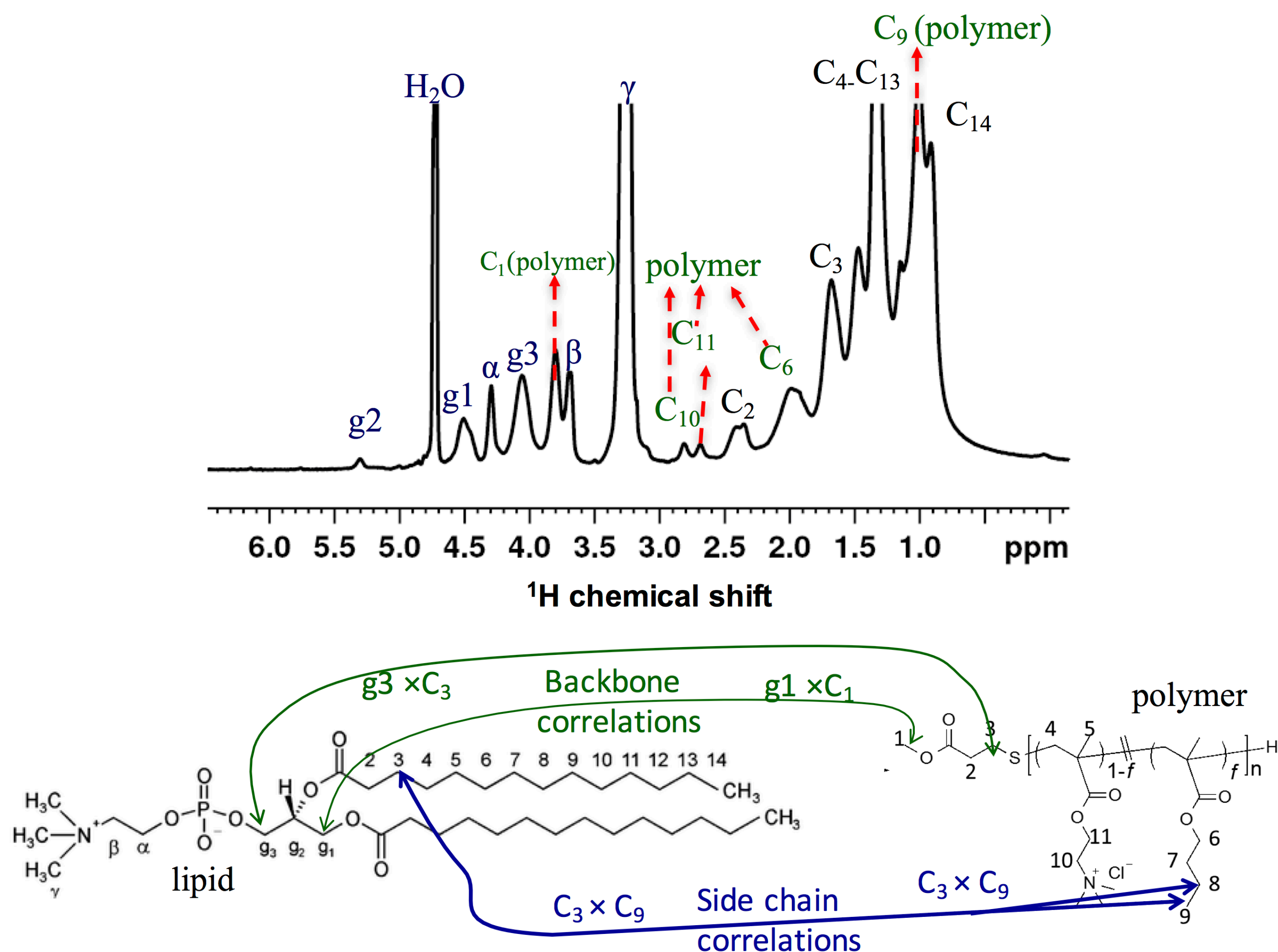


**Figure S4.** A) $^{1}H$ MAS spectrum of nanodiscs consisting of DMPC and the polymer obtained under 8 kHz MAS at 20 °C. 1 mg of DMPC lipid and 1.5 mg of polymer were dissolved in deuterated buffer to make a final concentration of 10 mg/ml. All other NMR experimental details are given in the main text. Based on the cross peaks observed in the 2D NOESY spectrum (Figure 5 B and C), intermolecular interactions are highlighted in the molecular structures of the polymer and lipid.

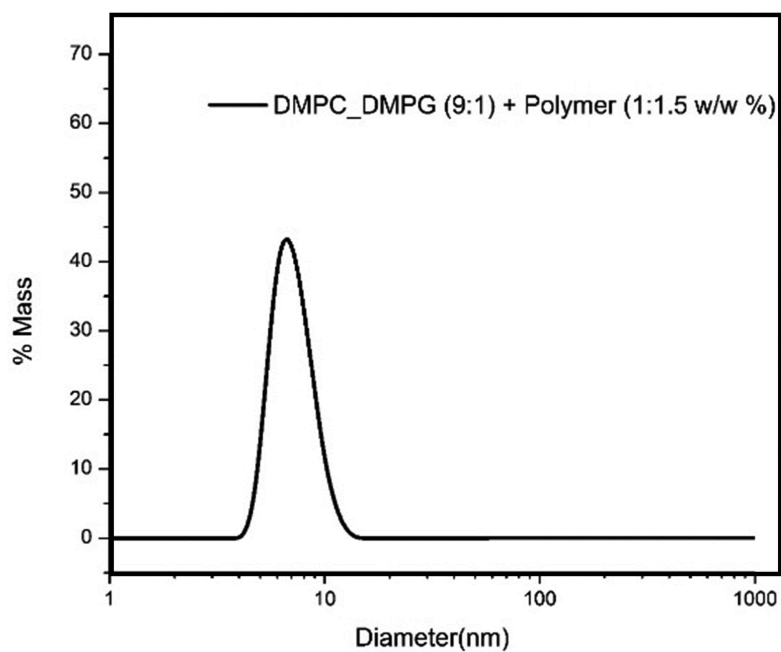


**Figure S5.** Size distribution of 9:1 DMPC:DMPG lipid bilayer containing polymer nanodiscs (1:1.5 w/w of lipid:polymer) in 10 mM acetic acid buffer pH 5.3.

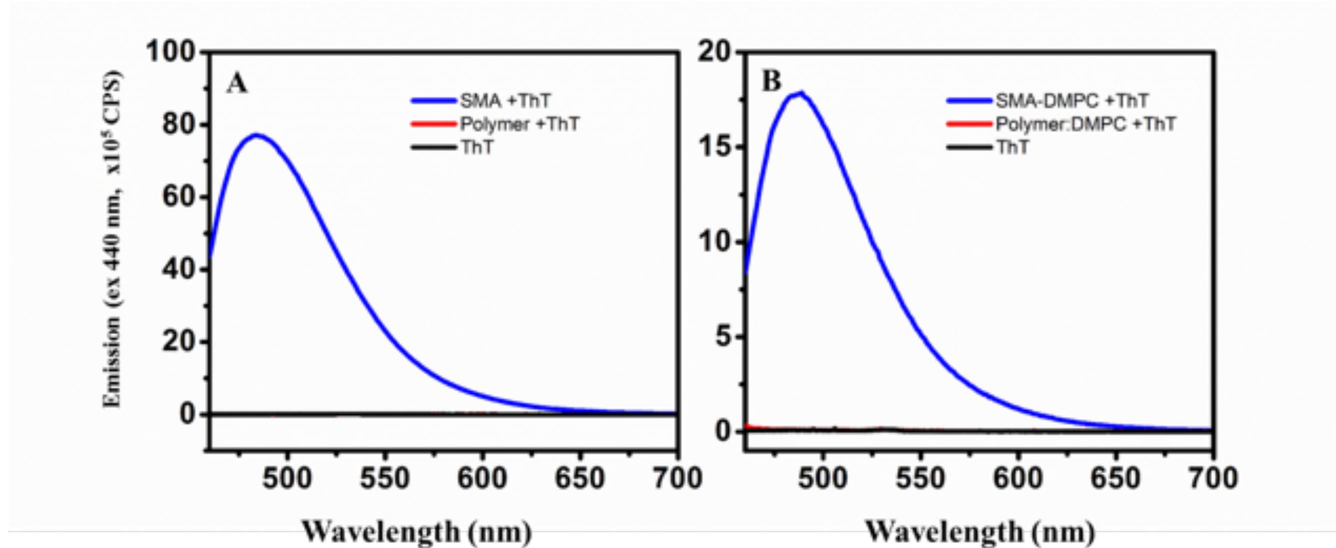


**Figure S6. Effect of polymers on ThT fluorescence.** A) Fluorescence spectra of ThT in the absence (black trace) and presence (blue trace) of SMA (~7.5 kDa) show an enhanced fluorescence of ThT in the presence of SMA polymer. On the other hand, the styrene-free methacrylate polymer does not influence the fluorescence of ThT (red trace). B) Fluorescence spectra of ThT in the presence of the DMPC nanodiscs made from SMA (blue trace) and new methacrylate polymer (red trace). These results show that the lipid nanodiscs made from SMA polymer interact with ThT dye, whereas the new methacrylate polymer nanodiscs do not interact with ThT. Therefore, the new methacrylate polymers reported in this study can be used to prepare lipid nanodiscs that can be used for ThT based fluorescence assays.

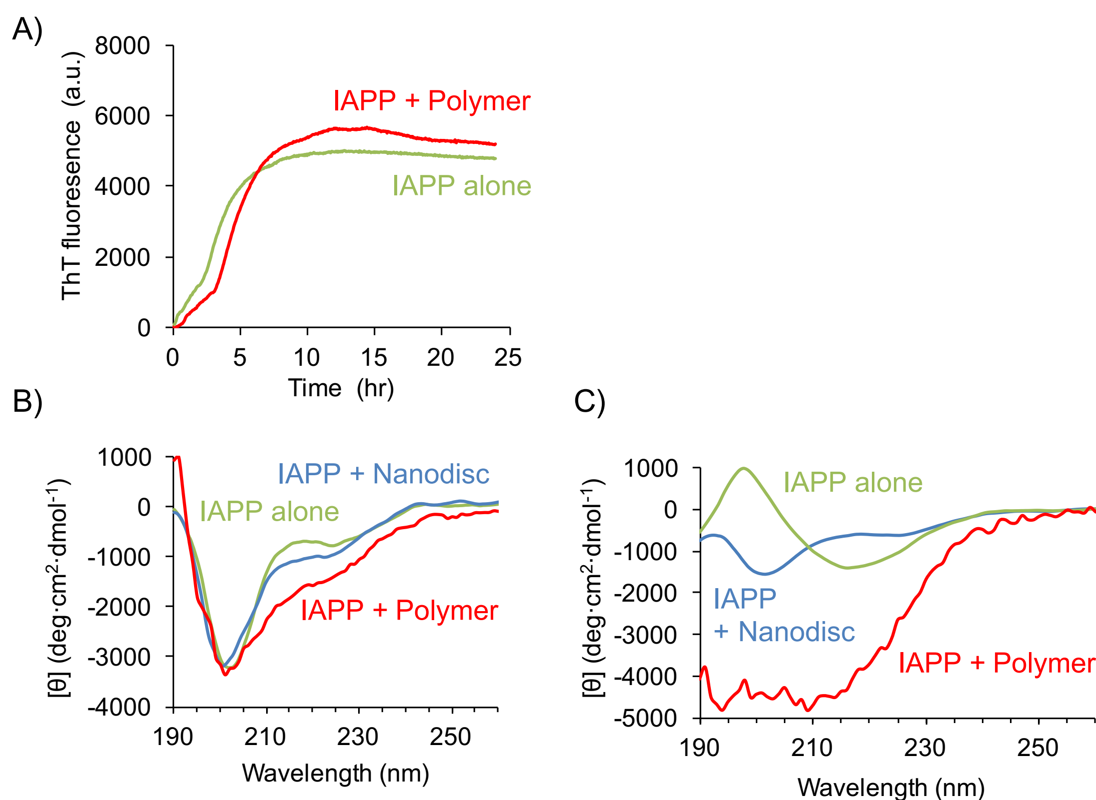


**Figure S7. Effect of polymer on the structure of IAPP.** A) Thioflavin T (ThT) fluorescence experiments showing the aggregation of human-IAPP (20 µM) in the absence (green) and in the presence of polymer (20 µM) (red). CD spectra of human-IAPP (20 µM), in the presence of nanodisc (2.0 mM of lipid, blue trace) and in the presence of polymer (20 µm, red trace; no lipids present) for an incubation time of 0hrs (B), and after 48 hrs of incubation (C). ThT experiment and CD spectra show that the polymer does not significantly affect the aggregation of human-IAPP.