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Supporting Material

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Running Title: BMP is not required for GM2AP to extract lipids

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CD Spectroscopy. CD spectra were measured with an Avis-400 Spectropolarimeter at 25 ºC. The cell path length was 1 mm. Spectra for each sample were collected from 250 nm to 190 nm every 1 nm with a 1 nm bandwidth**,** measured 2 times and averaged. For CD measurements as a function of pH, the eluate from the size column was dialyzed against an appropriate buffer. Sample concentration for measurements was 20 μM GM2AP.

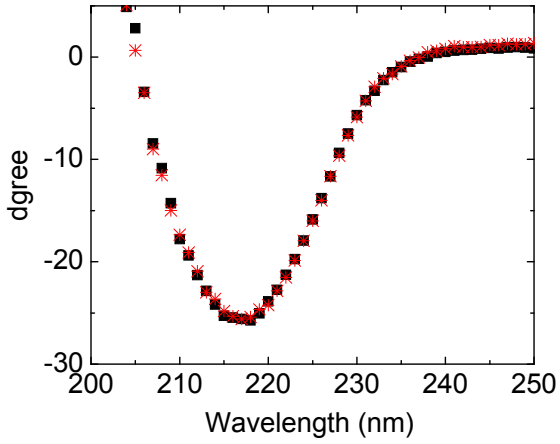

Figure S1: Circular dichroism spectra of 20μM GM2AP (stars) and GM2AP/His-tag (squares) in the buffer of 100 mM NaCl, 50 mM phosphate, pH 7.0. The spectra were recorded by using a 1 mm light path cuvette at 25 ºC. CD data were analyzed using CDPro program. (1) From the CD spectra of GM2AP at pH 4.5, the composition of α-helix and β-sheet are determined to be 5.3% and 50.5% respectively. These numbers agree very well with percentages determined from X-ray structures (5.6% α-helix and 60% β-sheet).

Sedimentation Assays for membrane partitioning and D-DHPE extraction

The sedimentation procedure is similar to that reported by Buser (2). To allow for sedimentation, the density on the outside of the vesicles was lowered by 5x dilution with 100 mM potassium chloride, 50 mM NaOAc buffer. The fraction of protein bound was determined from a series of samples that contained the same final concentration of protein (10 μM GM2AP in 200 μL total volume) with varied concentrations of sucrose loaded vesicles. Samples were allowed to incubate for 20 minutes at RT before separating soluble GM2AP from that bound to vesicles with an Air-Driven Ultracentrifuge (Beckman, Fullerton, CA) at a speed of 100,000 *g* for 1 hour. Protein quantification in both the supernatant and pelleted fractions proceeded by fluoresceamine labeling (2,3). As a control experiment, the membrane partitioning of melittin was obtained with this method and results agreed well with those published in the literature. To test for sedimentation efficiency, assays were performed that utilized sucrose loaded POPC:D-DHPE (2:1) vesicles in the absence of protein, where the residual D-DHPE fluorescence in the supernatant was measured. It was determined from these control experiments that between 5-9% of the lipids did not pellet as the total lipid concentration increased. Hence, with the Air-Driven Ultracentrifuge, only up to 1 mM total lipid concentration could be assayed, where above this value, the pelleting efficiency was less than 90%.

The ability of GM2AP to extract D-DHPE was determined by quantification of the fluorescence intensity of D-DHPE in the supernatant and pellet fractions of samples containing the same total amount of sucrose loaded vesicles with varying concentrations of GM2AP. After incubating for 20 minutes at room temperature, the vesicles were pelleted as described above. The amount of D-DHPE in both the pellet and the supernatant was quantified via fluorescence spectroscopy as described previously (4). A control sample containing 100 μM POPC:D-DHPE (2:1) (no protein) was use to determine the sedimentation efficiency. The fluorescence intensities of D-DHPE at 518 nm were acquired with an excitation wavelength of 340 nm. The measured fluorescence intensity of each sample was corrected for the appropriate dilution factor to give I_{sup} (signal in the supernatant) and I_{pel} (signal of the pellet). Given a 200 μL volume of 100 μM lipid (POPC:D-DHPE 2:1) the total concentration of D-DHPE in the sample was known to be 33.3 μM. Therefore, the concentration of D-DHPE in the supernatant C_{sup} (in μM) was calculated by:

$$C_{sup}=33.3\ I_{sup}/(I_{sup}+I_{pel}) \quad (1)$$

The residual lipid that remained in the supernatant from unpelleted vesicles was determined from control experiments and these values were set as C_{ctr}. Therefore, we define the change in D-DHPE concentration in the supernatant, $\Delta_{D\text{-}DHPE}$ as:

$$\Delta_{D\text{-}DHPE}=C_{sup}-C_{ctr} \quad (2)$$

Quantification of GM2 concentration via resorcinol assay.

The GM2 concentration in each fraction (with or without vesicles) was measured by the resorcinol assay where the total volume of the fraction was mixed with equal volume (45 μl) of freshly prepared resorcinol reagent in a 250 μl centrifuge tube. 10 ml resorcinol reagent contains: 8 ml concentrated hydrochloric acid, 1 ml 2% resorcinol, 25 μl of 100 mM copper sulphate and 975 μl water. The mixture was incubated in a boiling water bath for 15 minutes. After heating, the tubes were cooled in

running water. 60 µl of n-butyl acetate:n-butanol (85:15 by volume) was added to each tube. The tubes were shaken vigorously and placed in ice water for 15 minutes. The mixture was then spun at 4000rpm 2 minutes on benchtop centrifuge (Beckman, Fullerton, CA) to separate solvent phase. The OD580 nm of the organic solvent phase was measured for concentration determination. The extinction coefficient of the complex formed by GM2 with resorcinol was acquired from two methods. First, the extinction coefficient was directly measured with purchased GM2 (1 mg per vial), which gives an extinction coefficient of 5700 $mol^{-1}cm^{-}1$. In addition, a standard curve was generated using N-acetylneuraminic instead of GM2 due to its relative expense. From the best fit line, an extinction coefficient for the N-acetylneuraminic acid-resorcinol complex was obtained. According to references, this value can be divided by 0.77 to give an estimated extinction coefficient for resorcinol complexes with gangliosides (5). Following this method, we obtained a value of 5600 $mol^{-1}cm^{-1}$, which is very close to the value obtained directly for a solution of GM2.

The partitioning coefficient of GM2AP was independently determined from the gel filtration separation experiments. Because fractions containing vesicles scatter light and induce a high OD280 value, protein concentration could not readily be determined for those fractions containing vesicles. Attempts at solublizing the vesicles with detergents also resulted in protein denaturation. Hence, because the total protein concentration is known for each experiment, the amount bound is calculated as the difference between the total protein concentration and the concentration measured from fractions that do not contain vesicles. From control experiments that contained no vesicles, it was determined that only 1-3% of protein was lost on the column. In addition, a second control experiment (no protein only vesicles) showed that <5% lipid was lost by comparing the amount of GM2 that was collected to the known amount originally loaded. Vesicle containing fractions were determined from light scattering (OD550).

Fluorescence emission spectra and quenching

Fluorescence spectra were acquired on a FluoroMax-3 fluorimeter (Jobin Yvon Horiba, NJ) with a temperature controlled cell holder. All experiments were performed at 22^{o}C by using a HAAKE K20 water bath circulator (Thermo Electron Corporation, Waltham, MA). Measurements were made using a 4x4 mm light path quartz cuvette (Starna, Atascadero, CA). To reduce light scattering due to high concentrations of lipid vesicles, the excitation and emission polarizers were set to 90 and 0 degree orientations, respectively (6). The emission spectra 0.5 µM GM2AP in 50 mM NaOAc (pH 4.5) was recorded with excitation of 295 nm. Then POPC vesicles were added to a final concentration of 25 µM and incubate 10 minutes before spectra acquiring. The control experiment was performed as described but at pH 7.

For fluorescence quenching experiments, stock solutions of 4.0 M KI and 4.0 M acrylamide were freshly prepared and titrated into GM2AP solutions. The KI solution was supplemented with 1 mM of sodium thiosulfate to prevent the formation of triiodide (I_3^-). Emission spectra of 1 µM GM2AP in 50 mM NaOAc (pH 4.5) (excitation wavelength of 295 nm) were recorded in the absence of quencher and as titrant was added. The ratio of fluorescence intensity at 344 nm in the absence of quencher (F_0) to that in the presence of quencher (F, corrected for dilution) was plotted to the quencher concentration ([Q]). For the quenching in the presence of vesicle, 1 µM GM2AP was incubated with 50 µM POPC vesicles in 50 mM NaOAc (pH 4.5) for 30 minutes at room temperature and then subjected to the titration as described. The data was shown in Figure 1 B. The data was shown in Figure 1 B. As described by Posokhov et al (7), if the TRP really interacted with membrane, the TRP

fluorescence emission suppose to have a pronounced reduction in quenching. In the presence of 100μM POPC LUV, the Stern-Volmer constant of GM2AP for acrylamide/KI quenching is reduced from 5.4/3.3 M^{-1} to 4.4/1.7 M^{-1} respectively. The results show the TRP residues only get partial protection by membrane and probably can be explained by only 15% GM2AP really bound on membrane in the equilibrium status.

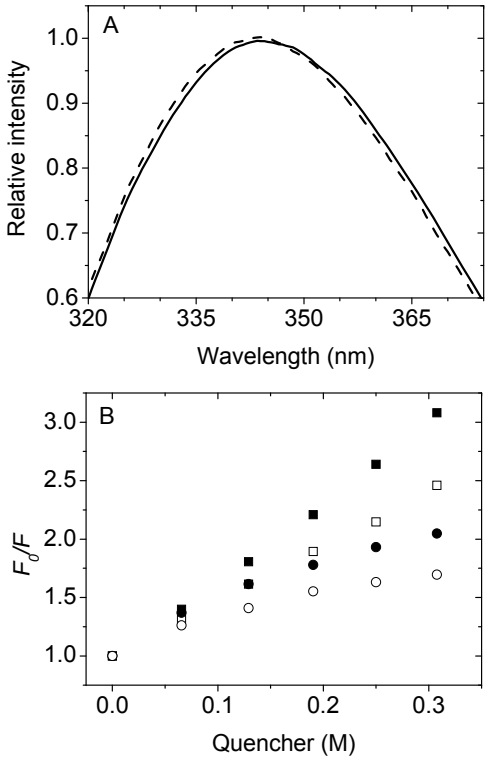

Figure S2. (A) Fluorescence emission of 0.5 μM GM2AP (50 NaOAc mM, pH 4.5) in the absence (solid) and presence (dash) of lipid vesicles; 25 μM POPC (incubated for 30 minutes prior to measurement) demonstrating the effects that membrane binding has upon tryptophan fluorescence emission spectra. (B) Quenching of 1 μM GM2AP by: acrylamide in the absence (■) and presence of lipid vesicles; 50 μM POPC (□); quenching by KI in the absence (●) and presence of lipid vesicles; 50 μM POPC (○). All experiments were performed at 22 ºC with an excitation wavelength of 295 nm and emission wavelength of 344 nm. Only minor protection from quenching is obtained in the presence of vesicles.

Thin-layer chromatography

The supernatants (2x190 μL) of the lipid extraction experiment were dried under nitrogen. 50 μL chloroform was added to dissolve the lipid in the solid mixture, transfer the sample to a new tube and concentrate to a suitable volume for loading onto a TLC plate (silica gel, 250 μm thick). The running solvent was chloroform-methanol-2.5 M aqueous ammonia (60:40:9 by volume). TLC plates were developed with iodine vapor (Figure S2). The presence of D-DHPE was located by its green fluorescence under UV irradiation.

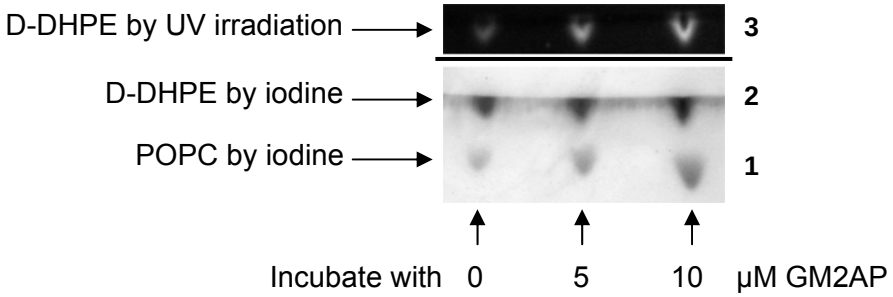

Figure S3. Thin-layer chromatography of POPC and D-DHPE in the supernatants after extraction and ultracentrifugation. Column left to right show 100 μM POPC:D-DHPE (2:1) vesicles incubated 0, 5 and 10 μM GM2AP in a total volume of 400 μl. Row 1 and 2, POPC and D-DHPE were developed by iodine. Row 3, D-DHPE as shown in row 2 was located by the dansyl group's fluorescence under irradiation of the UV light.

GM2AP extracts lipids and induces minor leakage of vesicle contents.

To test that the extraction of lipids by GM2AP did not cause vesicle rupture that could alter the efficiency of sedimentation by sucrose loaded vesicle experiments, we monitored the fluorescence intensity for a series of GM2AP:lipid compositions, where the liposomes were loaded with the fluorescence quenching pair ANTS/DPX. There is slight leakage from the vesicles for the LOWEST lipid concentration; which may add larger errors to the lowest Protein:lipid ratios. However for the end points of the binding assays, where the lipid concentration is higher, there is very little leakage, hence the leakage is not significantly affecting our results. In addition, given the relatively small volume of the 100 nm vesicles, if one were to do a calculation given a 20% leakage of the sucrose within the vesicle (176 mM), the change in the external sucrose concentration (3.5 mM) would increase only slightly (3.61 mM with the internal concentration decreasing to near 150 mM. This difference in density would still be enough to pellet the vesicles efficiently.

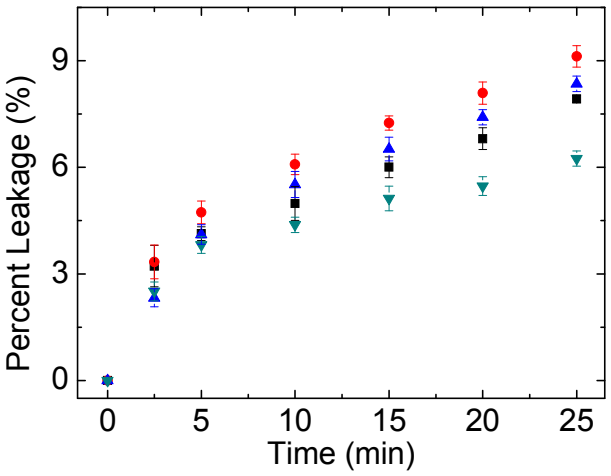

Figure S4. Results from leakage assays of POPC vesicles loaded with the fluorescence quenching pair ANTS/DPX. For each experiment, 10 μM GM2AP was incubated with 25 μM (black squares), 50 μM (red circles), 100 μM (blue up triangles) or 250 μM (green down triangles) POPC vesicles in buffer (100 mM NaCl, 20 mM NaOAc, pH 4.8 and at 22°C. The LUVs were loaded with 25 mM 8-aminonaphthalene-1,3,6-trisulfonic acid (disodium salt, ANTS), 90 mM p-xylene-bis-pyridinium bromide (DPX) as described in the literature (8,9). The fluorescence was excited at 360 nm and recorded emission at 530 nm. I_o, the initial fluorescence intensity was recorded in the absence of GM2AP. The fluorescence intensity after dissolving the vesicles completely with with 0.5% Triton-X100 was set as I_{max}, . Percentage leakage was determined by monitoring the fluorescence intensity as a functionof time, $I(t)$, after GM2AP was introduced and corrected accordingly by:

Percent leakage $=100 * (I(t) - I_0)/(I_{max} - I_0)$

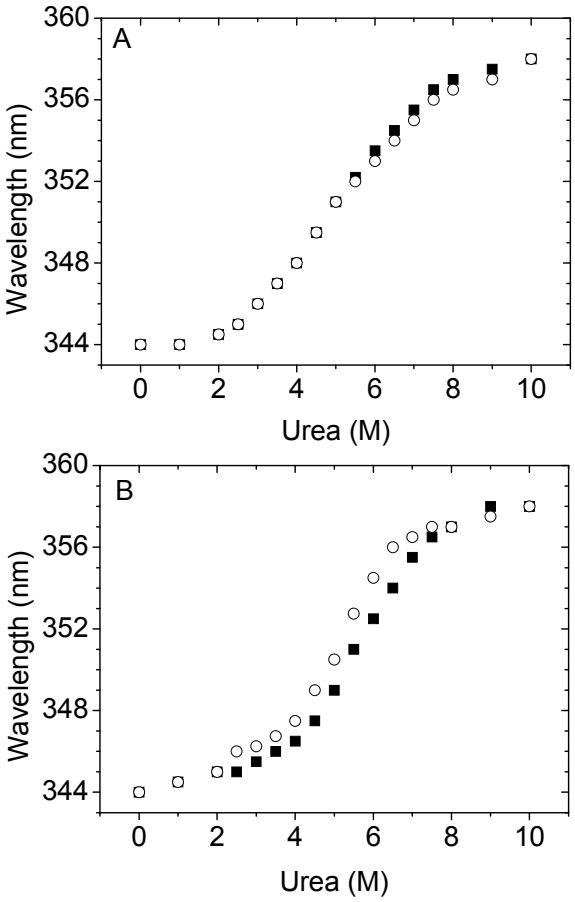

Figure S5. The dependence of fluorescence emission maximal wavelength on urea concentration for unfolding of (A) GM2AP and (B) GM2AP/His-tag at pH 4.5 (■) and pH 7.0 (○). For these experiments, a series of samples with final concentration of 1μM protein was prepared with varying urea concentration (0 to 10 M) in 50 mM NH_4Ac. Samples were allowed to incubate for 6 hours at room temperature prior to measurements, which were made at 22°C with an excitation wavelength of 295 nm.

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