Supporting Online Material for

Asymmetric Tethering of Flat and Curved Lipid Membranes by a Golgin

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Asymmetric Tethering of Flat and Curved Membranes by a Golgin

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**Material and Methods**

**Protein expression and purification**

Myristoylated Arf1 was purified from *E. coli* coexpressing N-Myristoyl Transferase and bovine Arf1 by ammonium sulfate precipitation, followed by DEAE and Mono S chromatography (S1). Full-length ArfGAP1 was purified from Sf9 cells (S2) by Nickel and Mono Q chromatography. The N-terminal fragment of GMAP-210 (GMAP_N = amino acids 1-375) and the “4K,” mutant of ArfGAP1 with 4 lysine residues in the ALPS1 motif have been described previously (S3).

The C-terminal fragments (GMAP_C-short = amino acids 1597-1830, GMAP_C-long = amino acids 1597-1843) were cloned via BamHI-EcoRI restriction sites into a pGEX-2T vector, which adds a GST tag and a thrombin cleavage site at the N-terminus. Point mutations were introduced by the QuikChange kit (Stratagene). MiniGMAP consists of amino acids 1-375 and 1597-1843 of GMAP-210 separated by a short linker with the following sequence: PGSTRAAAS. It was constructed as follows: the N-terminal fragment of GMAP-210 (aa 1-375) followed by the short linker and cloned into pGEX-4T was amplified by PCR and inserted into the pGEX-2T vector containing GMAP_C-long via BamHI-HindIII restriction sites. MiniGMAP lacking the N-terminal ALPS motif (the first 38 residues) was constructed with the same strategy using a construct, GMAP_N-ΔALPS (amino acids 39 to 375 of GMAP-210) described previously (S3). Constructs with a C-terminal hexahistidine tag, deriving from GMAP_N and GMAP_N-ΔALPS, were prepared by the QuikChange kit.

The GMAP constructs were expressed in *E. coli* at 17°C in the presence of 0.2 mM IPTG (at O.D._600 nm = 1.0) overnight. All purification steps were conducted in buffer A (50 mM Tris, pH 7.4, 120 mM NaCl, 1 mM MgCl$_2$, 1 mM DTT), which was supplemented during the first purification steps with PMSF (0.2 mM), bestatine (1 µM), pepstatine (10 µM), phosphoramidon (10 µM) and protease inhibitor tablets (Roche). Cells were lysed by a French press and the lysate was centrifuged at 200,000 g for 1 hour. The supernatant was applied to Glutathione Sepharose 4B beads. After 3 washing steps, the beads were incubated with thrombin to cleave the GST fusion and allow the release of the protein of interest. The eluate was further purified by gel filtration chromatography (Sephacryl S-300 for miniGMAP or Sephacryl S-200 for GMAP_C-short and GMAP_C-long). The [L1783A]GMAP_C-long mutant shows less solubility compared to the wild-
type form when expressed in *E. coli*. The yield of protein recovery in the soluble fraction was therefore lower. For this mutant, we omitted the last gel-filtration step. As a result this mutant was not as pure as the wild-type form (see Fig. S1). Proteins were stored at -80°C with 10% glycerol. To check the identity of the purified proteins, we analyzed them by mass spectroscopy (see Table S1). The exact sequence of the N-terminus of miniGMAP was also checked by N-terminal sequencing. The presence of a functional tag at the C-terminus of GMAPₜ-His₆ and GMAPₜΔALPS-His₆ was checked by Western-Blot using a monoclonal anti-polyhistidine antibody (Sigma). Because all GMAP constructs dimerize through their long coiled-coil regions, the concentration of dimer is used to express protein amount.

Analytical gel filtration

The various GMAP-210 constructs were analyzed by gel filtration on a Superose 12 column equilibrated in buffer A (see Table S1). Calibration was performed using the following standards: apoferritin (molecular weight 443 kDa, Stokes radius 6.1 nm), alcohol dehydrogenase (150 kDa, 4.5 nm), bovine serum albumin (67 kDa, 3.6 nm), carbonic anhydrase (31 kDa, 2.4 nm) and cytochrome c (12.6 kDa, 1.6 nm).

Circular Dichroism

CD measurements were performed on a Jasco J-815 spectrometer at room temperature using a thin quartz cell (path length 0.05 cm). Before measurement, the proteins were dialyzed against 10 mM Tris, pH 7.5, 150 mM KCl to remove glycerol. Potential aggregates were removed by ultracentrifugation. Each spectrum is the average of several scans recorded from 195 to 260 nm with a bandwidth of 1 nm, a step size of 0.5 nm and a scan speed of 50 nm.min⁻¹. The buffer contribution was subtracted and the corrected spectra were analyzed in the 195-240 nm range with the CDPro software (S4).

Liposomes

The standard liposome composition, also named “Golgi-mix” (S5), consists of egg phosphatidylcholine (50 mol%), liver phosphatidylethanolamine (19%), liver phosphatidylinositol (10%), brain phosphatidylserine (5%) and cholesterol (16%). Liposomes used in flotation experiments as well as in aggregation measurements contained 0.2 % NBD-PE.
Liposomes used in light microscopy experiments contained 1% rhodamine DHPE (1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine) or 1% Oregon-Green 488 DHPE (Molecular Probes). When indicated the liposome formulation also contains 2% DOGS-Ni\(^{2+}\)-NTA (1,2-dioleoyl-sn-glycero-3-\{N(5-amino-1-carboxypentyl)iminodiaceticacid)succinyl\}) or 4% brain PI(4,5)P\(_2\). Liposomes used to follow the recruitment of NBD-labeled GMAP\(_{\text{C-long}}\) contained no fluorescent probe.

Liposomes of defined size were obtained by the extrusion technique. Lipids as stock solutions in chloroform (Avanti Polar Lipids) were mixed and the solvent was removed in a rotary evaporator. The lipid film was hydrated in 50 mM Hepes, pH 7.2, 120 mM K-acetate (HK buffer) giving a suspension of large multilamellar liposomes (lipid concentration, 1 to 4 mM). The suspension was then frozen and thawed 5 times (using liquid nitrogen and a water bath) and then extruded sequentially through polycarbonate filters of decreasing pore size (0.4, 0.2, 0.1, 0.05 and 0.03 µm) using a mini-extruder (Avanti Polar Lipids). At each step, we assessed the size distribution of the liposomes by dynamic light scattering.

The large liposomes obtained by extrusion through 0.4 µm polycarbonate filters have a relatively broad size distribution. To improve size homogeneity and eliminate the smallest liposomes that could interfere in the aggregation measurements, the suspension was centrifuged at 25,000 rpm for 15 min in a swing rotor (TLS-55). The pellet containing the largest liposomes was resuspended in HK buffer. NBD fluorescence was used to estimate the amount of lipid recovered after centrifugation. In addition, because these large liposomes are not strictly unilamellar, we determined the fraction of accessible lipids (in the outer leaflet) by measuring at 533 nm (bandwidth 20 nm) upon excitation at 470 nm (bandwidth 1.5 nm), the fast decline in NBD fluorescence induced by the addition of sodium dithionite (10 mM), a non-permeable chemical that quenches NBD fluorescence (S6). Typically, sodium dithionite eliminates 30-32 % of the NBD fluorescence of the large liposomes (extrusion 0.4 µm and centrifugation step) and 50 % of the small liposomes (extrusion 0.03 µm).

Giant liposomes were prepared by the gentle hydration method derived from Akashi et al. (S7). The Golgi-mix formulation contains more than 10 % (mol/mol) of negatively charged lipids, which facilitate the spontaneous formation of giant liposomes. A lipid film was prepared in a 50-ml round flask, sealed under argon, and then hydrated overnight at 37°C in 20 mM
Hepes, pH=7.2, 210 mM sucrose. “Clouds” formed by giant liposomes in the supernatant were harvested and lipids concentration was estimated by fluorescence.

**Preparation of liposomes loaded with Arf1GTP**

We used a standard procedure that consists of incubating myristoylated Arf1GDP with liposomes and with an excess of GTP at low Mg$^{2+}$ concentration (S8). Typically the sample containing proteins and liposomes was prepared in HKM buffer (50 mM Hepes, pH 7.2, 120 mM K-acetate, 1 mM MgCl$_2$, 1 mM DTT). At time zero GTP and 2 mM EDTA were added. This lowers the concentration of free Mg$^{2+}$ in the micromolar range. Under these conditions, GDP to GTP exchange on myristoylated Arf1, which is correlated to its translocation to the liposomes, occurs with a half-time of about 2 min at 37°C and can be followed by tryptophan fluorescence (S8). After 15 min incubation, the concentration of free Mg$^{2+}$ was raised back to 1 mM by adding 2 mM MgCl$_2$.

**Flotation experiments**

Flotation assays similar to that described previously (S9) were used to determine protein binding to liposomes. Proteins and liposomes were first mixed in HKM buffer (sample volume 150 µl). When indicated, GDP to GTP exchange on Arf1 was performed using the protocol described above. Thereafter the sample was adjusted to 30% sucrose (total volume 250 µl) and covered with two cushions (200 µl of 25% sucrose in HKM and 50 µl of HKM buffer). After 1 hour centrifugation at 240,000 g and 20°C in a TLS-55 swing rotor, the top 100 µl fraction, which contains the liposomes, was collected and analyzed by SDS-PAGE using Sypro Orange staining (Invitrogen) and a fluorescence imaging system (FUJI LAS-3000).

**NBD Labeling of GMAP$_{C-long}$**

NBD labeling of GMAP$_{C-long}$ was performed on a double mutant lacking the single endogenous cysteine (C1722S) and displaying a cysteine in the C-terminal amphipathic helix (T1829C) (Fig. S2A). This mutant was purified using Glutathione Sepharose 4B beads and gel filtration chromatography as for the wild-type form. NBD labeling was performed between these two chromatographic steps using a 5-fold molar excess of N,N’-dimethyl-N-(iodoacetyl)-N’-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD-amide, FluoProbes). After 5 minutes
at room temperature the reaction was stopped by the addition of 1 mM cysteine. The excess probe was removed during the gel-filtration step. The purified protein was analyzed by SDS-PAGE and UV-visible spectroscopy (see Fig. S2B-C). The gel was directly visualized in a fluorescence imaging system (FUJI LAS-3000) to detect NBD-labeled GMAP_{C-long} and then stained with Sypro Orange to determine the purity ($p$) of GMAP_{C-long}. The percentage of labeling was then estimated from the optical density (OD) at 280 and 495 nm:

$$\text{Labeling (\%)} = 100 \left[ \frac{\text{OD}_{495}/\varepsilon_{495}}{[p(\text{OD}_{280}/\varepsilon_{280})]} \right],$$

where $\varepsilon_{280}$ is the calculated extinction coefficient of GMAP_{C-long} at 280 nm (19,750 M$^{-1}$ cm$^{-1}$) and $\varepsilon_{495}$ is the extinction coefficient of NBD at 495 nm (25,000 M$^{-1}$ cm$^{-1}$). From the data shown in figure S2 (panels B and C) this gives a value of 106\%, suggesting a complete and stoichiometric labeling of the protein.

**NBD-fluorescence**

For kinetics measurements, NBD fluorescence was continuously measured at 537 nm (bandwidth 10 nm) upon excitation at 495 nm (bandwidth 3 nm) in a Shimadzu RF 5301-PC fluorimeter equipped with stirring, injections and temperature control facilities. The sample initially contained 0.2 mM Golgi-mix liposomes in HKM buffer (total volume 600 µl) and was placed in a cylindrical quartz cell, which was continuously stirred with a small magnetic bar and thermostated at 37°C. At the indicated times, NBD-labeled GMAP_{C-long}, Arf1GDP, GTP, EDTA, MgCl$_2$ and ArfGAP1 were injected from stock solutions through a guide in the cover of the fluorimeter adapted to Hamilton syringes, such as to not interrupt the fluorescence recording (see Fig. 1D and Fig. S3A). Emission spectra (see Fig. S3C) were recorded upon excitation at 495 nm (excitation and emission bandwidths = 3 nm).

**Dynamic light scattering measurements of liposome aggregation induced by miniGMAP**

All experiments were performed at 25°C in a Dynapro apparatus (Protein Solutions). The sample initially contained extruded liposomes (loaded or not with Arf1GTP) in HKM buffer in a small quartz cell (volume 20 µl). A first set of about 12 autocorrelation curves was acquired to assess the size distribution of the initial liposome suspension. Then proteins and additional liposomes were added manually, mixed thoroughly and the kinetics of aggregation was followed by acquiring one autocorrelation curve every 10 seconds. At the end of the experiment, when aggregation reached a plateau, a final set of 12 autocorrelation functions was acquired. The data
were analyzed using two different algorithms provided by the Dynamics v6.1 software (Protein Solutions). During the aggregation process, the autocorrelation functions were fitted assuming that the size distribution is a simple Gaussian function. This mode, referred as the monomodal or cumulant algorithm, gives a mean radius, R, and the width (or polydispersity). The polydispersity is represented in the kinetics measurements by the shaded area and can reach very large values because of the simultaneous presence of free liposomes and of liposome aggregates of various sizes. Before and after the aggregation process, the autocorrelation functions were fitted using a more refined algorithm, referred as a regularization algorithm. This algorithm is capable of resolving several populations of different size, such as free liposomes and liposome aggregates. Therefore, it gives a better view of the size distribution in the sample. All calculations take into account the percentage of glycerol from the protein stock solutions.

Electron microscopy of liposomes.

The sample containing the liposomes and the proteins was prepared in HKM buffer and mixed in a 0.5 ml tube. At the indicated time, 20 µl was placed on a piece of parafilm and an EM grid with a carbon film was applied to the drop followed by staining with uranyl acetate (1%). The grid was examined with a Philips CM12 electron microscope at 80 kV.

Cell culture, immunofluorescence and electron microscopy

Full-length GMAP-210 and miniGMAP were cloned via EcoRI-NotI and BamH1-EcoRI restriction sites respectively into a pcDNA3 vector containing a Kozak sequence upstream the coding sequence. HeLa cells were cultured on collagen-coated coverslips in 6-well plates in DMEM containing 10% FCS, penicilline and streptomycin. Cells were transfected with 4 µg DNA/well with Lipofectamine2000 (Life Technologies) according to manufacturer's protocol for 22 hours. After transfection, cells were fixed in 3% paraformaldehyde for 30 min, permeabilized with 0.5% saponin for 10 minutes in PBS followed by treatment for 1 hour with 10% HS and 0.05% saponin in PBS to avoid non-specific labeling. Cells were then incubated for 1 hour with a polyclonal rabbit anti-GM130 (1:50, AbCam) and a mouse anti-GMAP-210 (1:100, BD Biosciences). Then, cells were treated with anti-mouse and anti-rabbit secondary antibodies (1:100) conjugated to fluorescence probes (FluoProbe) in presence of Hoechst for 1 hour before wash. Coverslips were mounted with Mowiol overnight on glass side in the dark. The ability of
the anti-GMAP-210 antibody to recognize full-length GMAP-210 as well as miniGMAP was assessed by Western-Blot. For electron microscopy, cells were fixed in situ with 1.6% glutaraldehyde in 0.1 M phosphate buffer at room temperature and then for several hours at 4°C. Samples were rinsed in the same buffer and post-fixed with 1% osmium tetroxide and 1% potassium ferrocyanide in a 0.1 M cacodylate buffer for 1h at room temperature to enhance the staining of cytoplasmic membranes (S10). Cells were rinsed with distilled water and embedded in epoxy resin. Embedded samples were then conventionally processed for thin sectioning and counterstaining. Thin sections were observed with a Philips CM12 transmission electron microscope equipped with an Olympus SIS CCD camera.

Tethering assay with giant liposomes

All experiments were done at room temperature in a final volume of 200 µl in a Lab-Tek 8-chamber coverglass (Nunc) coated with 5 mg/ml casein to avoid liposome adsorption. Small liposomes (Golgi-mix containing 4% PI(4,5)P₂ and 1% rhodamine DHPE) were diluted in a chamber in 20 mM Hepes, pH 7.5, 120 mM K-acetate, 1 mM MgCl₂ and 1 mM DTT (this buffer is iso-osmotic with the sucrose buffer used to prepare the giant liposomes) and mixed manually with Arf1 and Arno. Then, a small volume of giant liposomes (Golgi-mix containing 4% PI(4,5)P₂ and 1% Oregon-Green 488 DHPE) was added. To initiate the activation of Arf1, a drop of GTP (20 µl, 1.3 mM in HKM) was added. After 5 minutes, 5 µl of ArfGAP1 or of HKM buffer were added and mixed with the liposome suspension by gently rotating the chamber. At this stage, the sample volume was 150 µl and contained 500 nM Arf1, 25 nM Arno, 250 nM ArfGAP1 and 0.17 mM GTP. After 2.5 min incubation with ArfGAP1, 50 µl of miniGMAP in HKM buffer was added to give a final concentration of 62.5 nM. For experiments with the His-tagged GMAP₅ constructs, the small liposomes were first diluted in the chamber in HKM buffer (with no DTT) before the addition of giant liposomes doped with 2% DOGS-Ni²⁺-NTA. Then, the protein was added and gently mixed with the liposomes. The sample was observed before and 30-45 min after the addition of miniGMAP or of relative constructs.
**Fluorescence microscopy.**

Epifluorescence microscopy was performed using a Zeiss inverted microscope (Axiovert 200M) with a 63X oil immersion objective. Digital images were acquired by using a CoolSnap HQ CCD camera driven by the Metamorph software. Confocal microscopy observations were performed with a laser scanning confocal microscope (TCS SP5, Leica) equipped with a DMI6000 inverted microscope, using a Plan Apo 63X oil immersion objective. The fluorescent markers were excited by an argon ion laser and their fluorescence was detected through optimized spectral windows. Images were processed using the ImageJ 1.35 software (http://rsb.info.nih.gov/ij) and an in-house macro (from F. Braud) was used to extract intensity values from the green and red channels.
Supporting Figures

Figure S1. The L1783A mutation abolishes the binding of GMAPC-long to liposome-bound Arf1GTP. GMAPC-long or [L1783A]GMAPC-long (0.5µM) was incubated with Golgi-mix liposomes in the presence of Arf1GDP or Arf1GTP (1 µM) for 5 min at 20°C. Liposome-bound proteins were recovered by flotation on sucrose cushions and analyzed by SDS-PAGE using Sypro Orange staining.

Figure S2. NBD-labeling of GMAPC-long. (A) Domain organization of GMAPC-long. The predicted secondary structural elements of the GRAB domain as well as the C-terminal amphipathic helix are indicated (S11). Hydrophobic and basic residues in the helical wheel representation are shown in yellow and blue respectively. The position of the T1829C mutation used for NBD labeling is also shown. (B) SDS-PAGE of purified NBD-labeled GMAPC-long. The gel was directly visualized in a fluorescence imaging system to identify labeled proteins and then stained with Sypro Orange to visualize all proteins. (C) UV-visible absorption spectrum of NBD-labeled GMAPC-long. As judged from the peaks at 280 nm and 495 nm and the purity of the protein sample, GMAPC-long was labeled with the NBD probe in a 1:1 ratio.
Figure S3. Membrane translocation of NBD-labeled GMAPC-long. (A) Time course measurements. The fluorescence cuvette initially contains Golgi-mix liposomes (0.2 mM) in HKM buffer. Then, NBD-labeled GMAPC-long (0.125 µM), Arf1GDP (0.125 to 1.25 µM), GTP (40 µM) and EDTA (2 mM) were sequentially added. (B) Plot of the fluorescence change as a function of Arf1 concentration. (C) Emission fluorescence spectrum of NBD-labeled GMAPC-long before (red) and after (blue) recruitment by Arf1GTP. The experiment was conducted as in (A) with 0.75 µM Arf1 and the emission fluorescence spectrum of NBD was recorded before and after the GDP/GTP exchange reaction. Note the blue shift in NBD fluorescence. (D) Scheme of the interactions involved in the membrane recruitment of the C-terminal region of GMAP-210. The GRAB domain interacts with membrane-bound Arf1GTP and also directly with the lipid membrane surface through its amphipathic helix.
Figure S4. Overexpression of miniGMAP in HeLa cells. Confocal microscopy images of fixed HeLa cells after transfection with GMAP-210 or miniGMAP. The cells were co-stained and merged in green for endogenous GM130, a cis-Golgi marker, and in red for GMAP-210 or miniGMAP. Note that the overexpression of GMAP-210 or miniGMAP leads to an enlargement and scattering of the Golgi apparatus whereas untransfected cells exhibit a normal perinuclear Golgi.
Figure S5. Overexpression of miniGMAP in HeLa cells results in the apparition of clusters of small vesicles at the Golgi level. HeLa cells were transfected with miniGMAP under conditions similar to those used for immunofluorescence and were observed by thin-section EM. As shown in Fig. S4, the overexpression of miniGMAP leads to an enlargement and scattering of the Golgi apparatus. At the ultrastructural level, we observed instead of a normal Golgi apparatus, clusters of vesicles scattered in the cytoplasm that correspond very likely to the large fluorescent spots in Fig. S4. One example is shown at high magnification (top picture) where one can discern numerous vesicles of the size of transport vesicles (≈50 nm of diameter) and a complete absence of well-defined Golgi cisternae. Other cells showed intermediate phenotypes where the vesicle clusters surrounded remnants of stacked Golgi cisternae. The bottom picture, taken from untransfected cells, shows a typical stack of Golgi cisternae surrounded by vesicular/tubular profiles.
Figure S6. Analysis by negative stain electron microscopy of aggregates formed by small liposomes covered with Arf1GTP after incubation with miniGMAP. Golgi-mix liposomes (50 µM accessible lipids, R = 38 ± 9 nm) covered with Arf1GTP (0.25 µM) were incubated with miniGMAP at different concentrations for two min at room temperature. Note that liposome aggregation is detected with as low as 25 nM miniGMAP. Considering that a lipid occupies a surface area of $\approx 0.7 \text{ nm}^2$, a liposome with a radius of 38 nm has about $2 \pi (38)^2 / 0.7 = 50,000$ lipids. This gives for the molar concentration of liposomes a value of 1.9 nM. Therefore we estimate that before aggregation, the number of miniGMAP molecules per liposome is from 13 to 65 when the concentration of miniGMAP varies from 25 to 125 nM.
Figure S7. Arf1GTP is required for efficient aggregation of small liposomes by miniGMAP.
Golgi-mix liposomes (50 µM accessible lipids, R = 38 ± 9 nm) were loaded or not with Arf1GTP (0.25 µM) and then incubated with miniGMAP (125 nM) for 10 min at room temperature. The liposomes were visualized by negative staining. Slight liposome aggregation was observed in the absence of Arf1GTP in agreement with the DLS experiments shown in Fig. 2A. This may be due to some basal interaction of the amphipathic helix of the GRAB domain with the liposomes.
Figure S8. Effect of liposome size on miniGMAP-induced aggregation.

(A) For electron microscopy, two populations of liposomes of defined size (25 µM accessible lipids each) and either covered or not with Arf1GTP (0.125 µM) were mixed at time zero with miniGMAP (62.5 nM) as indicated. Liposomes radius: large, $R = 143 \pm 45$ nm; small, $R = 36 \pm 7$ nm. After incubation for 30 s at room temperature, the liposome suspension was stained with uranyl acetate. For dynamic light scattering, the protein concentration was twice as that used in EM (see also Fig. 2C). These EM and DLS experiments demonstrate that the presence of small naked liposomes is required to trigger efficient aggregation when Arf1GTP is present on large liposomes (compare “large(Arf1GTP)/small” with “large(Arf1GTP)/large”). Note also the difference between the second combination “large(Arf1GTP)/small” and the fourth combination “small(Arf1GTP)/large” as observed by EM. In the former case, the large liposomes are cemented by small liposomes, suggesting that both are involved in the tethering reaction. In the latter case, one sees clusters (c) of small liposomes and free large liposomes (L), suggesting that the large liposomes are excluded from the aggregation process. Note that it would be impossible to distinguish these two modes of aggregation by DLS. (B) To better compare the first combination “small(Arf1GTP)/small” and the third one “large(Arf1GTP)/large” shown in (A) the DLS aggregation curves had been normalized according to the initial liposome radius ($R_0$). This demonstrates that aggregation is strongly favored by membrane curvature.
Asymmetric aggregation between large liposomes covered with Arf1GTP and small naked liposomes in the presence of miniGMAP. All experiments were performed by quickly mixing large liposomes (L) covered with Arf1GTP with small naked liposomes (s) and miniGMAP.

(A and B) Large liposomes (R = 144 ± 55 nm, 25 µM accessible lipids) loaded with 0.125 µM Arf1GTP were incubated with small liposomes (R = 41 ± 16 nm, 50 µM accessible lipids) and miniGMAP (62.5 nM) for 30 s at room temperature before negative staining. These are the same experimental conditions as those used in Fig. 2D.

(C) Large liposomes (R = 145 ± 37 nm, 25 µM accessible lipids, loaded with 0.125 µM Arf1GTP) were incubated with small liposomes (R = 38 ± 10 nm, 50 µM accessible lipids) and miniGMAP (62.5 nM) for 30 s at 4°C before negative staining.

(D) Large liposomes (R = 145 ± 37 nm, 25 µM accessible lipids, loaded with 0.25 µM Arf1GTP) were incubated with small liposomes (R = 38 ± 10 nm, 25 µM accessible lipids) and miniGMAP (125 nM) for 10 min at room temperature before negative staining. These are the same experimental conditions as that used in the DLS experiments of Fig. 2C.

Note that although the size and morphology of the aggregates vary, the large liposomes (L) appeared always cemented by the small ones (as indicated by the curved lines “s”).
Figure S10. Recruitment of small red liposomes on giant green liposomes by a His-tagged construct of GMAP$_N$.

(A) Golgi-mix giant liposomes (2.1 µM lipid) containing 1% Oregon-Green 488 DHPE and 2% DOGS-Ni$^{2+}$-NTA were incubated with small liposomes (12.5 µM accessible lipid, R = 38 ± 16 nm) containing 1% rhodamine DHPE in the absence or in the presence of 62.5 nM GMAP$_N$-His$_6$ or GMAP-His$_6$-ΔALPS. Also shown is a control experiment performed with 50 mM imidazole to prevent protein recruitment. All images were obtained by confocal microscopy.

(B) Profil analysis of a giant liposome after incubation with GMAP$_N$-His$_6$ and with small liposomes. As expected, the small liposomes are recruited only on the outer membrane of this multilamellar liposome.
Figure S11. Recruitment of small liposomes on giant liposomes by miniGMAP as observed by confocal microscopy. (A) Schematic representation of the reference experiment. The sample contains giant liposomes (green) and small liposomes (red). All liposomes have the same lipid composition (Golgi-mix + 4% PI(4,5)P2) but the giant liposomes contain a green fluorophore and the small liposomes a red fluorophore. In (B) liposomes and proteins concentration were as in Fig. 3B. Homogenous recruitment of small liposomes (R = 36 ± 9 nm) at the surface of giant liposomes is observed only in the presence of ArfGAP1 and when GTP hydrolysis in Arf1 can occur. In contrast, in the absence of ArfGAP1 or in the presence a non-hydrolysable GTP analogue (GTPγS), red spots corresponding likely to clusters of small liposomes were observed (arrows). Some of them are connected to giant liposomes. In the absence of GTP, the red background stays homogenous and clean (no red spots) as in the absence of miniGMAP. This further demonstrates that Arf1GTP is required to promote tethering by miniGMAP (see Fig. 2A and Fig. S7).
**Supporting Tables**

### Table S1. Characterization of the GMAP-210 constructs

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1. by mass spectroscopy
2. as assessed by CD spectroscopy
3. predicted using Paircoil2 (S12)
4. as assessed by dynamic light scattering
5. as assessed by gel filtration chromatography
6. See also Drin et al. (2007), ref S3
7. ND, not determined

### Table S2. Ratio between proteins and liposomes in typical aggregation measurements

<table>
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<th>Experiments with small liposomes only</th>
<th>DLS (Fig. 2A)</th>
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<tr>
<td>number of Arf1GTP molecules / liposome</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>number of miniGMAP molecules / liposome</td>
<td>46</td>
<td>13 to 65</td>
</tr>
<tr>
<td>number of potential connections / liposome (5)</td>
<td>92</td>
<td>26 to 130</td>
</tr>
</tbody>
</table>

| Experiments with small and large liposomes (DLS experiments shown in Fig. 2C) |
|-----------------------------------|-------------------|
| large liposomes | liposome radius, R (1) | 143 nm |
| calculated number of lipids in the outer leaflet (3) | 367,100 |
| calculated molar concentration of the liposomes (4) | 0.007 nM |
| concentration of Arf1 | 250 nM |
| number of Arf1GTP molecules/large liposome | 3672 |
| small liposomes | liposome radius, R (1) | 36 nm |
| calculated number of lipids in the outer leaflet (3) | 23,300 |
| calculated molar concentration of liposomes (4) | 0.107 nM |
| concentration of miniGMAP | 125 nM |
| number of miniGMAP molecules / large liposome | 1836 |
| number of miniGMAP molecules / small liposome | 116 |
| number of potential connections / small liposome | 116 |
| small liposomes / large liposome | 16 |

1. as assessed by dynamic light scattering
2. as assessed by the dithionite assay
3. \( = 4\pi R^2/0.7 \), assuming that a lipid occupies a surface of 0.7 nm²
4. calculated by dividing the concentration of accessible lipids by the number of lipids in the outer leaflet: \( (4) = (2)/(3) \)
5. after tethering, the number of potential connections/liposome is twice the number of miniGMAP/liposome as a miniGMAP molecule can be connected to a liposome either via its ALPS motif or via its GRAB domain.
Supporting references