Supplementary information

ArfGAP1 generates an Arf1 gradient on continuous lipid membranes displaying flat and curved regions

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Supplementary Materials and Methods

Reagents

1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC), egg phosphatidylcholine (EPC), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine-N-(Cap Biotinyl) (BiotPE) and 1,2-Distearoyl-*sn*-Glycero-3-Phosphoethanolamine-N-[Biotinyl(Polyethylene Glycol)2000] (Biot-PEG-PE) were purchased from Avanti Polar Lipids. *N*-((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-yl)phenoxy)acetyl)sphingosine (BODIPY® TR ceramide, BodTRCer) and Alexa^{488} C5-Maleimide were from Invitrogen. ATP, GTP, GDP and GTPγS were purchased from Roche Molecular Biochemicals and streptavidin beads (3 μm) from Bangs Laboratories

(Carmel, IN). Biotinylated hemagglutinin-kinesin (a gift of F. Nédélec, European Molecular Biology Laboratory, Heidelberg) was purified as described (Surrey et al, 1998). Secondary fluorescent antibodies were from Jackson Immunoresearch and Molecular Probes. All other chemicals were purchased from Sigma Aldrich.

Protein expression, purification, and labeling

Arf1

The complete protocol has been published elsewhere (Manneville et al, 2008). In brief, myristoylated Arf1 with an extra C-terminal cysteine (C182) was purified from E. Coli coexpressing N-Myristoyl Transferase by ammonium sulfate precipitation and by DEAE and Mono S chromatography. Labeling was performed with a 10-fold excess of Oregon Green 488 Maleimide (Molecular Probes). Excess dye was removed by gel-filtration. The final dye/protein mole ratio was around 1 suggesting complete labeling. The labeled protein (named Arf1-OG) behaves similarly as wild-type Arf1 in biochemical assays. Notably, it binds in a GTP dependent manner to liposomes upon GDP to GTP exchange and dissociates from liposomes upon GTP hydrolysis catalyzed by ArfGAP1 (Manneville et al, 2008).

ALPS peptides

The ALPS1-ALPS2 peptide corresponding to the two ALPS motifs of ArfGAP1 (residues 192-304) and bearing the K297C mutation was purified according to previous protocols (Bigay et al, 2005; Mesmin et al, 2007) with some modifications. The fragment was cloned in a pGEX-2T expression vector leading to a GST fusion with a thrombin cleavage site. Transformed bacteria were resuspended in 50 mM Tris (pH 7.4) and 150 mM NaCl (TN buffer), supplemented with protease inhibitors (1 mM PMSF, 1 mM pepstatin, 10 mM bestatin, 10 mM phosphoramidon) and 1 mM DTT. Bacteria were lysed with a French press or by incubation with lysozyme (1 mg/ml) for 30 min on ice followed by sonication. The lysate was ultra-centrifuged at 160,000g for 30-60 min. The supernatant was incubated for 1 h with glutathione-Sepharose 4B beads (Amersham). The beads were washed three times with TN buffer and then incubated with thrombin overnight to cleave the peptide from the GST tag. After cleavage, 1 mM DTT was added and the supernatant was passed through a phase-reverse C18 column (Chromolite Performance RP-18 100-4.6, Merck). The separation was achieved with an acetonitrile gradient. The fractions were analyzed by SDS-PAGE and those corresponding to the peptide molecular weight were pooled, lyophilized and stored at -20°C. The K297C mutation in the peptide sequence allows labeling with the maleimide-derivative

of Alexa^{488}. Labeling was performed by resuspending the desired amount of lyophilized peptide in 2 ml HK buffer (HEPES 50mM, KAcetate 120 mM) supplemented with 5% (Vol/Vol) dimethyl formamide and then adding this volume to lyophilized Alexa^{488}-C5-maleimide (protein/dye ratio: 0.1 to 0.2). After 1-2 hours, the labeling reaction was stopped with 10 mM cysteine and the fluorescently-labeled peptide was separated from excess dye by reverse phase chromatography using an acetonitrile gradient. The dye:peptide ratio was around 1. The same protocol was used for the purification and labeling of ALPS 1 (rat ArfGAP1 192-257, A236C) and ALPS 2 (rat ArfGAP1 257-304, K297C).

ArfGAP1

ArfGAP1 with a carboxy-terminal hexahistidine tag was purified from Sf9 cells by nickel and mono-Q chromatography. For antibody-labeling of ArfGAP1, 0.5 μL of the primary antibody against ArfGAP1 (gift from Dan Cassel) were incubated with 1 μL of the specific fluorescent secondary antibody (0.5-1 mg/mL) in 20 μL final (10 minutes at room temperature). This amount was split in two aliquots of 10 μL each and either and 43 pmol of ArfGAP1 (from 1 μL) or 1 μL of ArfGAP1 buffer (for control experiments) were added and incubated 10 minutes at room temperature. For the experiments, we used a dilution of this stock to a final 0.5-3 μM ArfGAP1 concentration.

Supplementary References

Bigay J, Casella JF, Drin G, Mesmin B, Antonny B (2005) ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. *EMBO J* **24**(13)**:** 2244-2253

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Mesmin B, Drin G, Levi S, Rawet M, Cassel D, Bigay J, Antonny B (2007) Two lipid-packing sensor motifs contribute to the sensitivity of ArfGAP1 to membrane curvature. *Biochemistry* **46**(7)**:** 1779-1790

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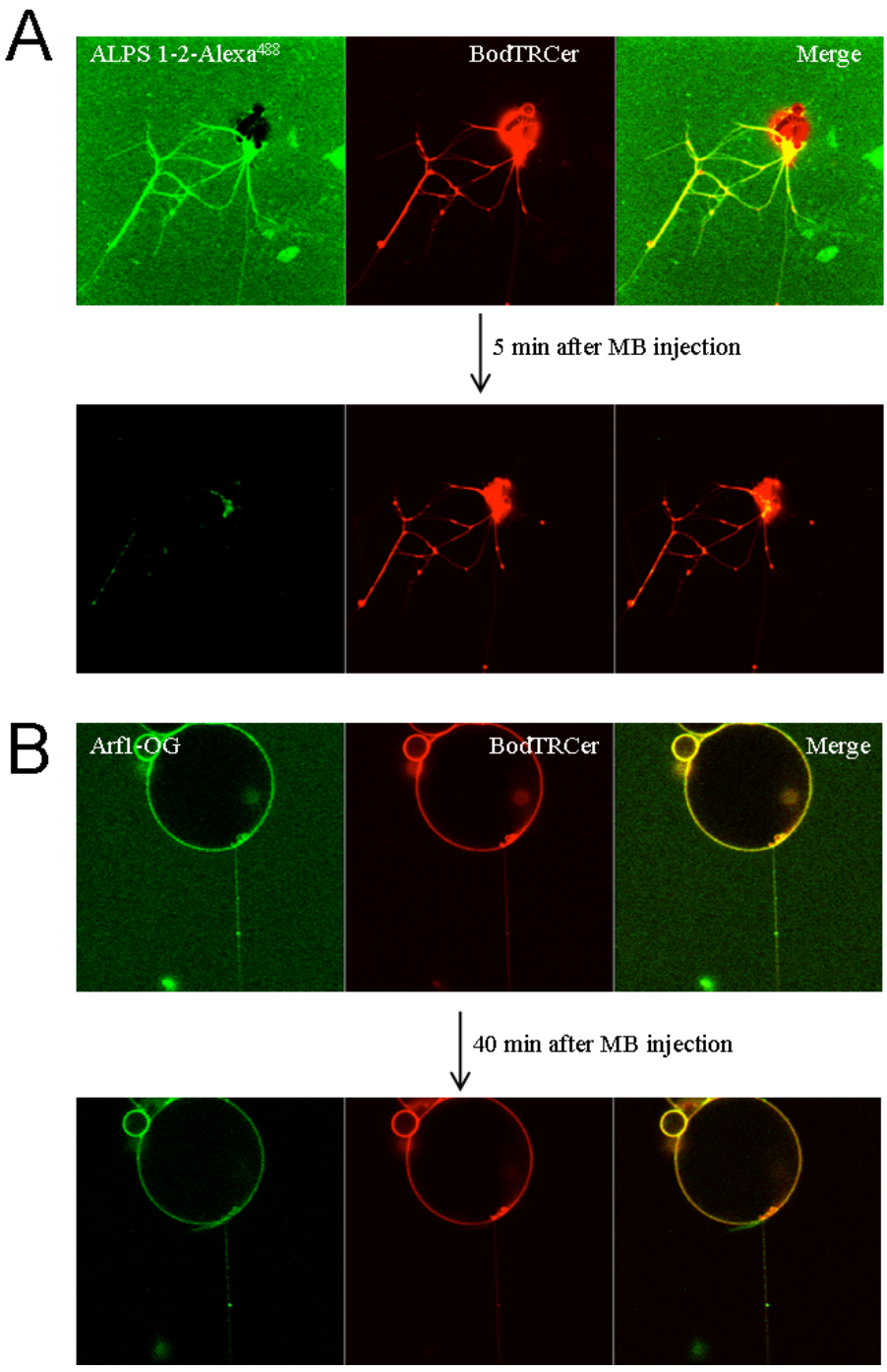
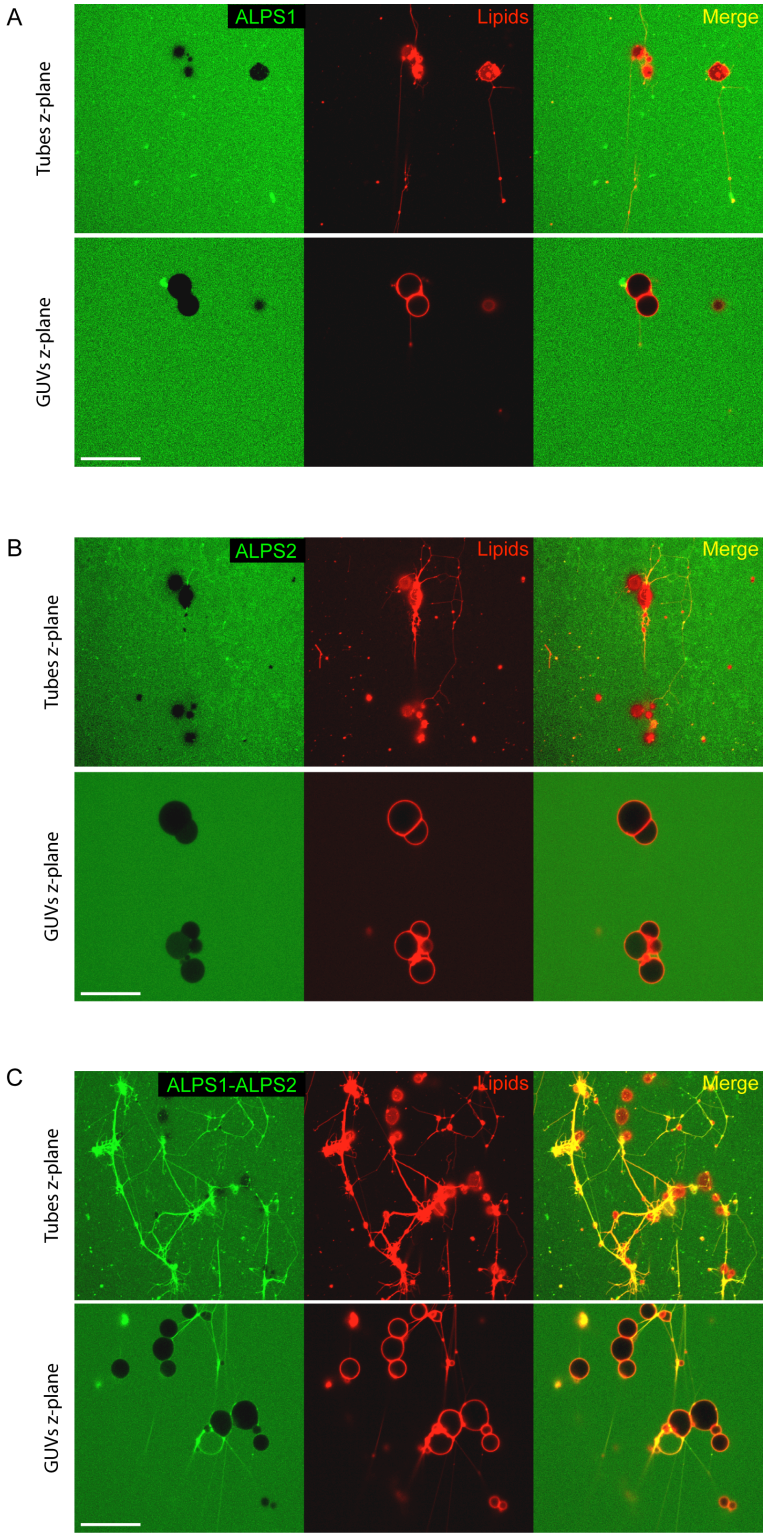
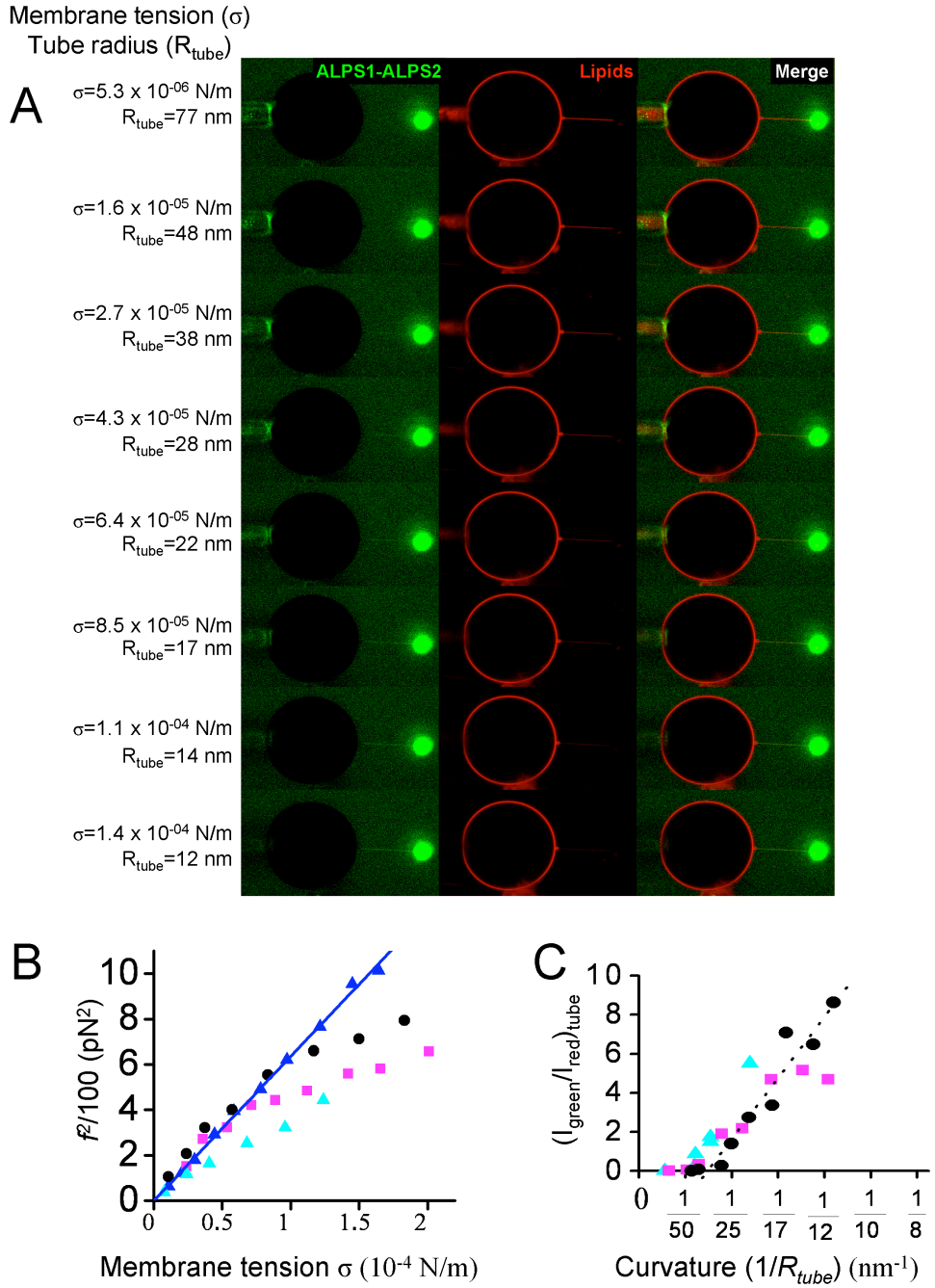


Supplementary Figure S1: ALPS1-ALPS2-Alexa^{488} binds reversibly to curved membranes (A) DOPC membrane tubes were pulled by biotinylated-kinesin 1 from GUVs in the presence of ALPS1-ALPS2-Alexa^{488} (3 μM). After motility buffer (MB) injection, the ALPS1-ALPS2-Alexa^{488} signal from membrane tubes drops, indicating unbinding of the peptide. Green panel: ALPS1-ALPS2-Alexa^{488} fluorescence; red panel: lipid marker BodTRCer (1%) fluorescence. Scale bar: 15 μm. (**B**) Same as in panel A except that ALPS1-ALPS2-Alexa^{488} was replaced by GTP-bound Arf1-OG. Note the resistance of Arf-OG staining on the GUV to motility buffer (MB) injection.



Supplementary Figure S2: The two ALPS motifs of ArfGAP1 are necessary for binding to tubes pulled from DOPC GUVs. Fluorescent versions of the isolated ALPS motifs of ArfGAP1 (ALPS1-Alexa^{488}, panel A; ALPS2-Alexa^{488}, panel B) do not show any detectable binding at 3 μM to DOPC tubes pulled by biotinylated-kinesin 1 ('Tube z-plane') or to GUVs ('GUV z-plane'). In contrast, the sequence encompassing the two ALPS motifs (ALPS1-ALPS2-Alexa^{488}, panel C) strongly binds membrane tubes. Green panel: Alexa^{488} fluorescence; red panel: BodTRCer (1%) fluorescence. Scale bar: 15 μm.



Supplementary Figure S3: A critical radius for ALPS1-ALPS2-Alexa^{488} binding. **(A)** Complete sequence of images corresponding to Fig. 3A showing a membrane tube pulled from a DOPC GUV with a streptavidinated-bead in the presence of 0.9 μM ALPS1-ALPS2-Alexa^{488} at increasing membrane tension. Membrane tension was tuned by micropipette aspiration (on the left). The bead appeared as a bright green spot (on the right) due to non-specific peptide adsorption. Green panel: ALPS1-ALPS2-Alexa^{488} fluorescence; red panel: BodTRCer lipid marker fluorescence. **(B)** Force f exerted by the membrane tube on the bead at increasing membrane tension. The plot shows $f^2/100$ (pN^2) *vs* membrane tension in the absence (dark blue triangles) and in the presence (other symbols) of ALPS1-ALPS2-Alexa^{488}. In the absence of peptide, the slope of the linear fit is proportional to the bending rigidity of the membrane (κ=19.8 kT; kT is the thermal energy). **(C)** This plot is the same as in Figure 3A and is included here for clarity. It reports the I_{Green}/I_{Red} ratio as a function of the curvature of the tube from the same three independent experiments shown in panel B (same symbols). Scale bar: 15 μm.