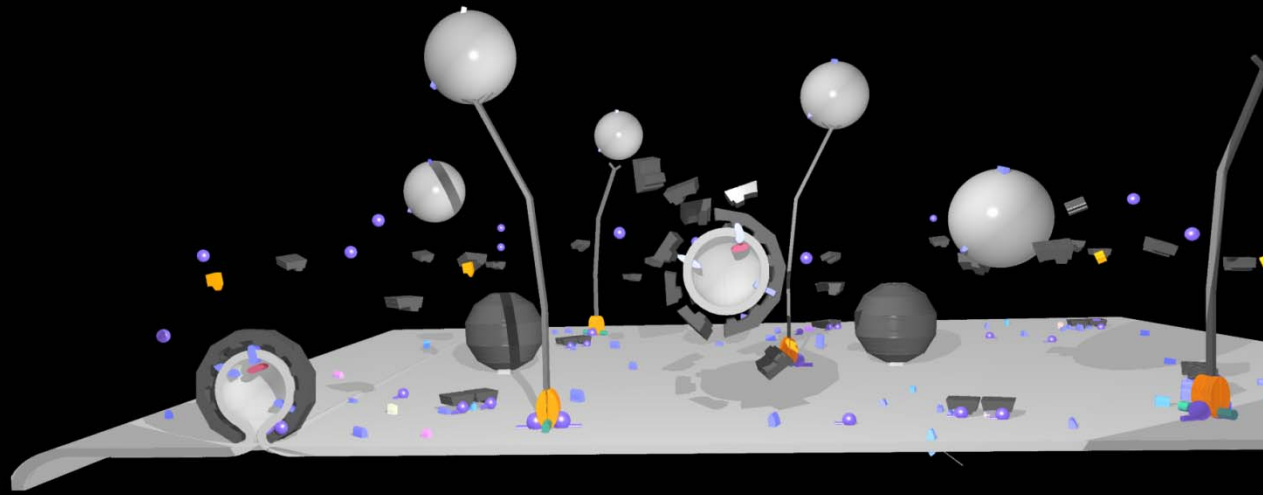


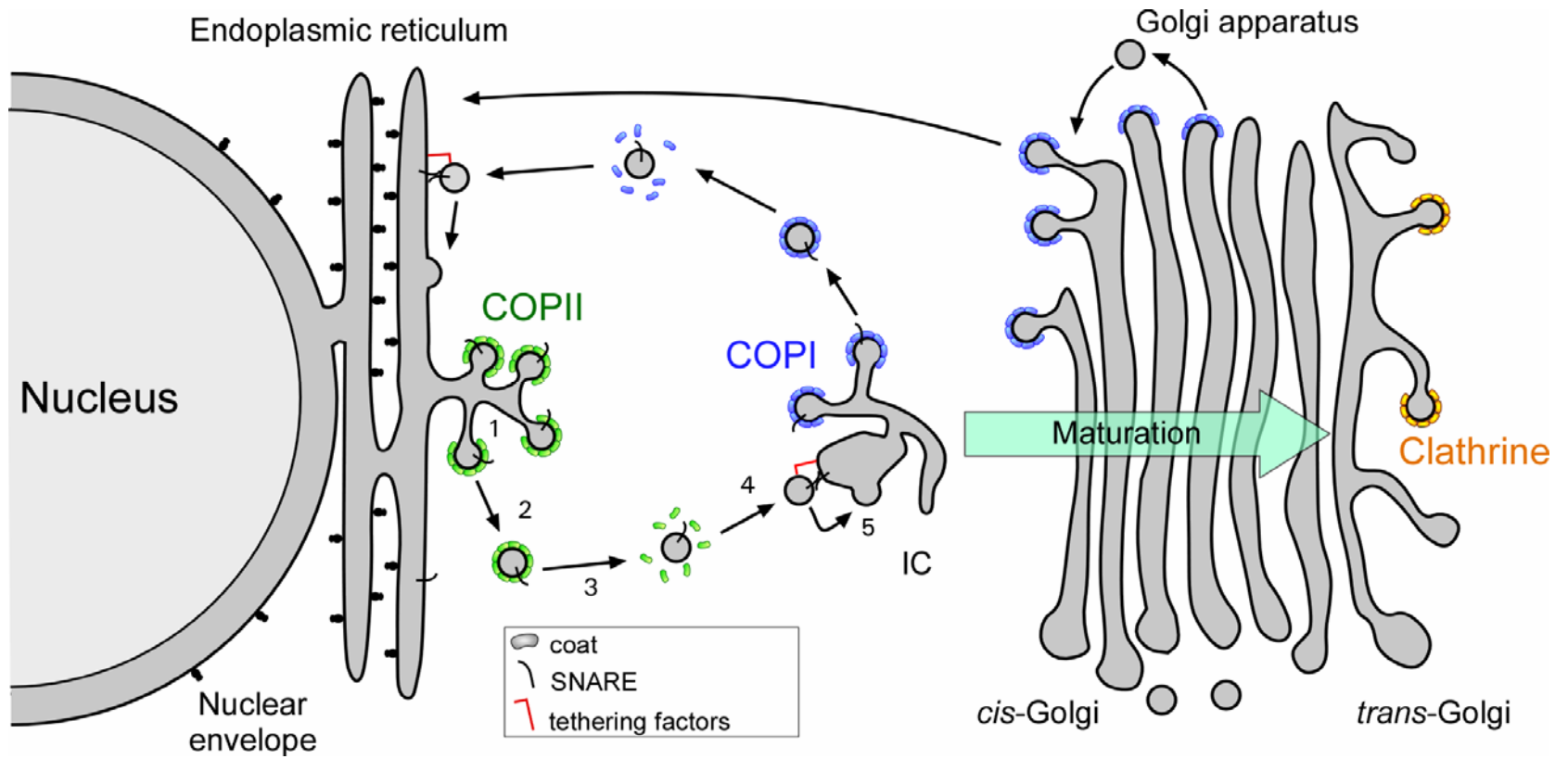
Targeting 2 distinct membranes: the case of tethering factors and lipid transporters

Guillaume Drin, CNRS

KITP Evo Cell, Feb 2, 2010

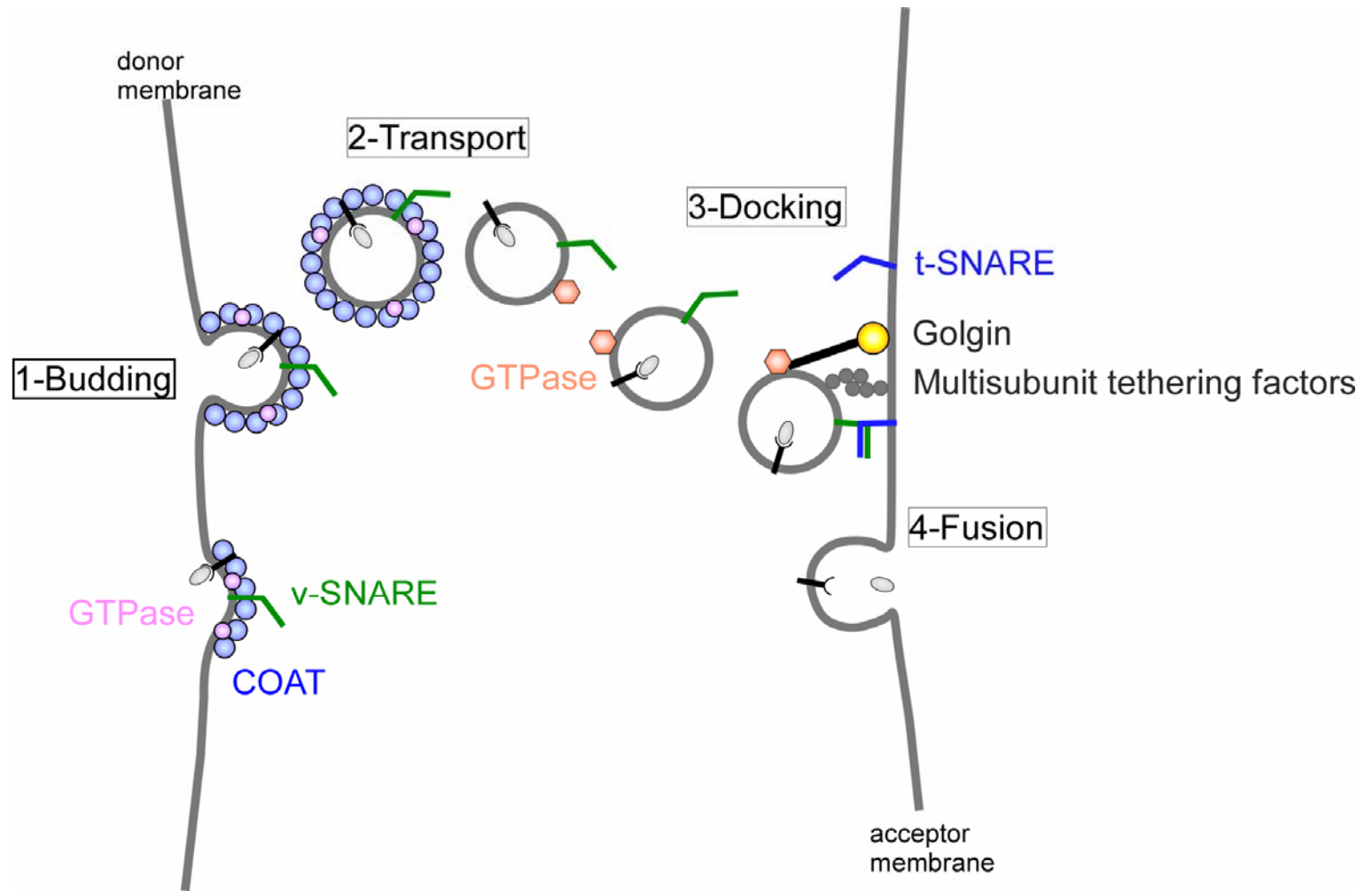


Vesicular transport



adapted from Bonifacino JS & Glick BS. Cell. 2004

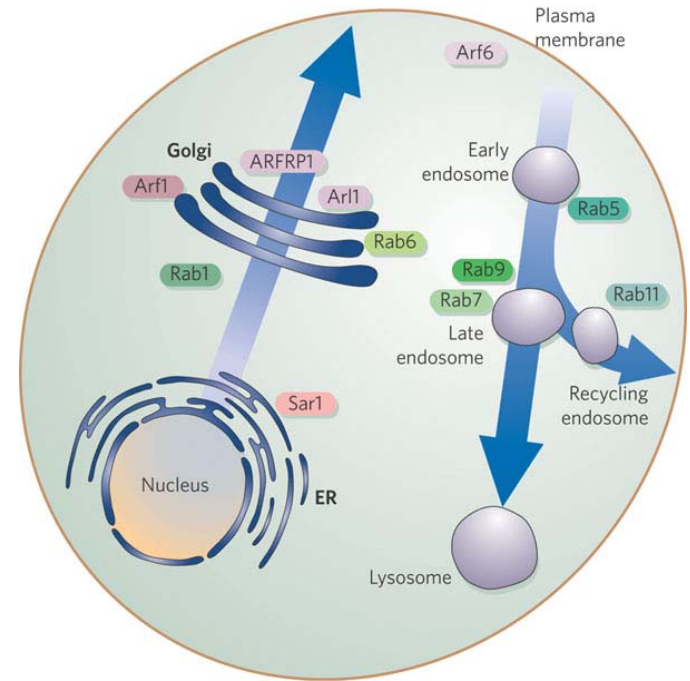
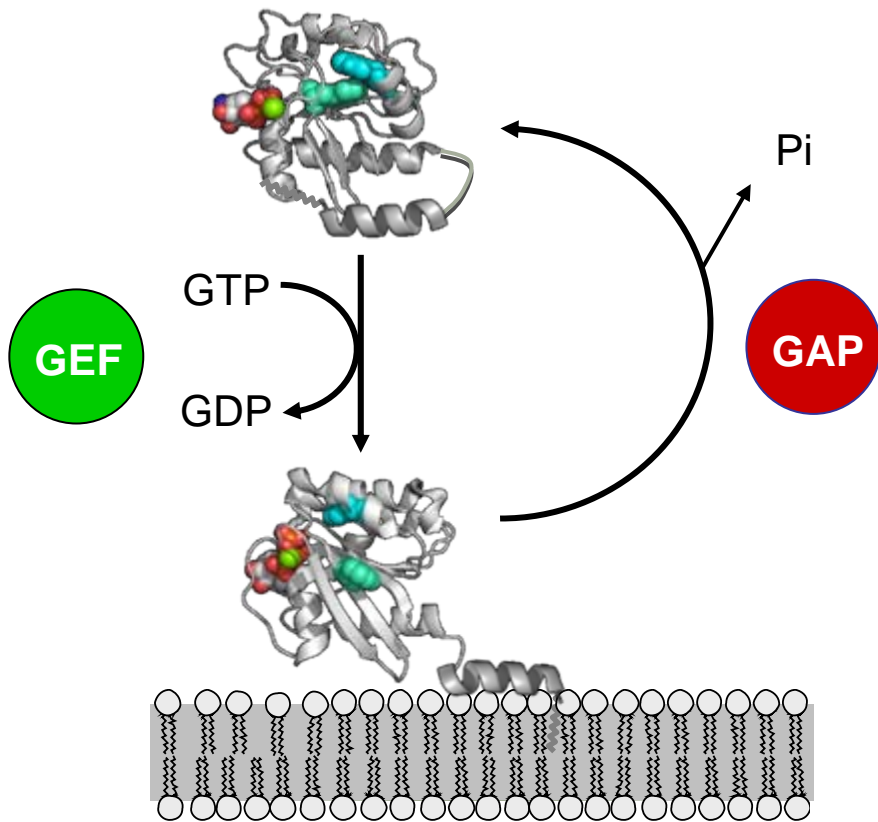
Vesicular transport



adapted from Behnia R, Munro S Nature. 2005 438:597-604

An important concept : membrane identity

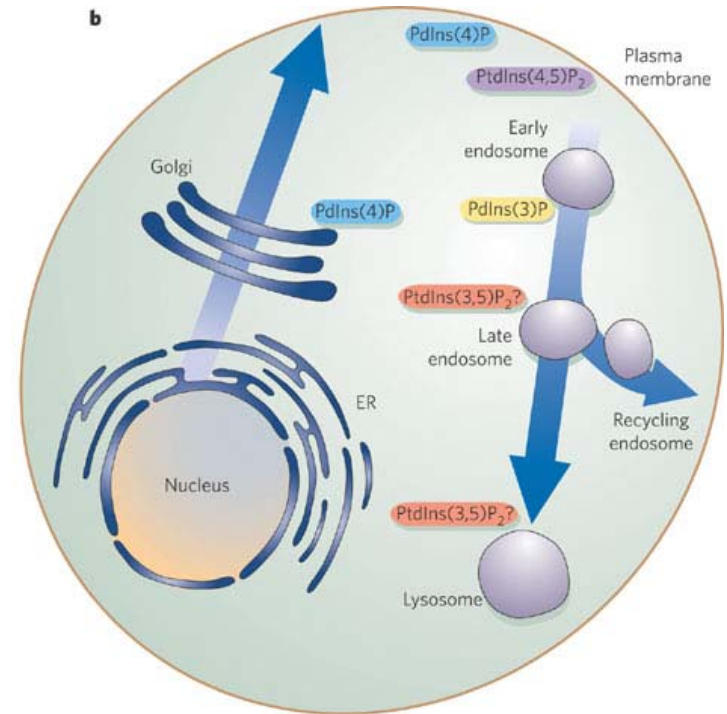
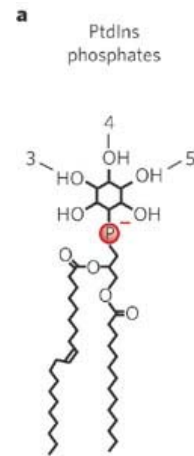
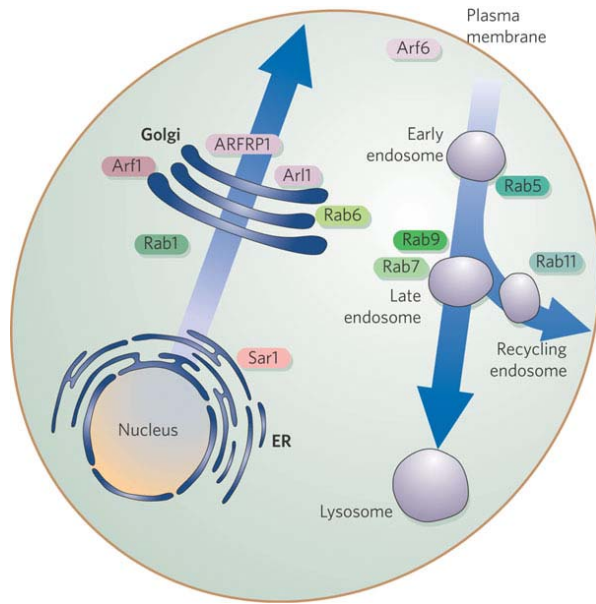
1st landmark : small GTPase



from Behnia R, Munro S Nature. 2005 438:597-604

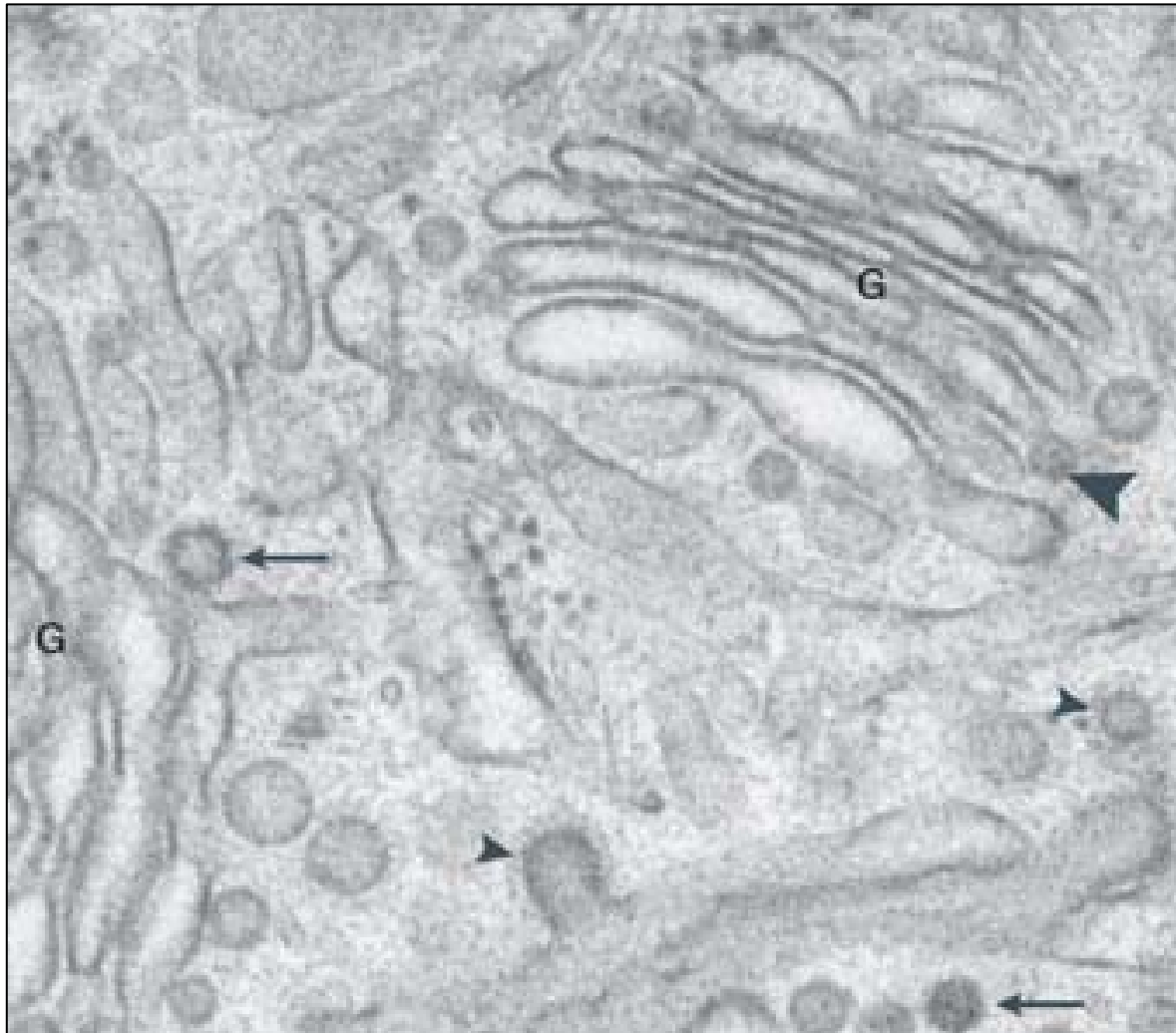
An important concept : membrane identity

2nd landmark : membrane composition (phosphoinositide)



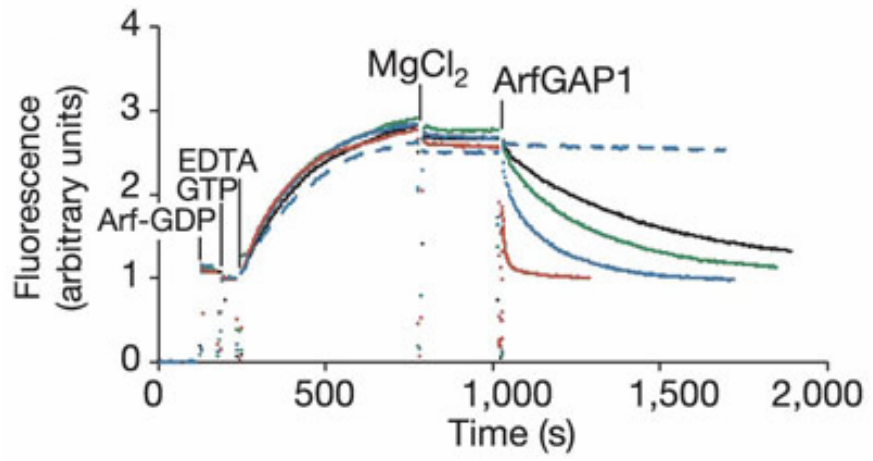
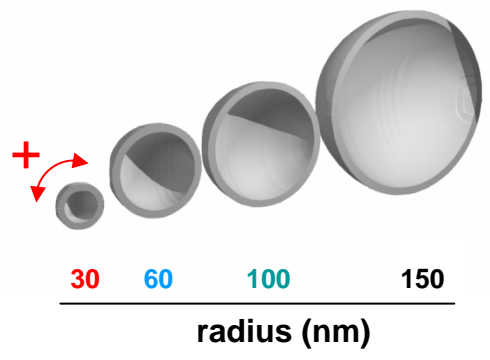
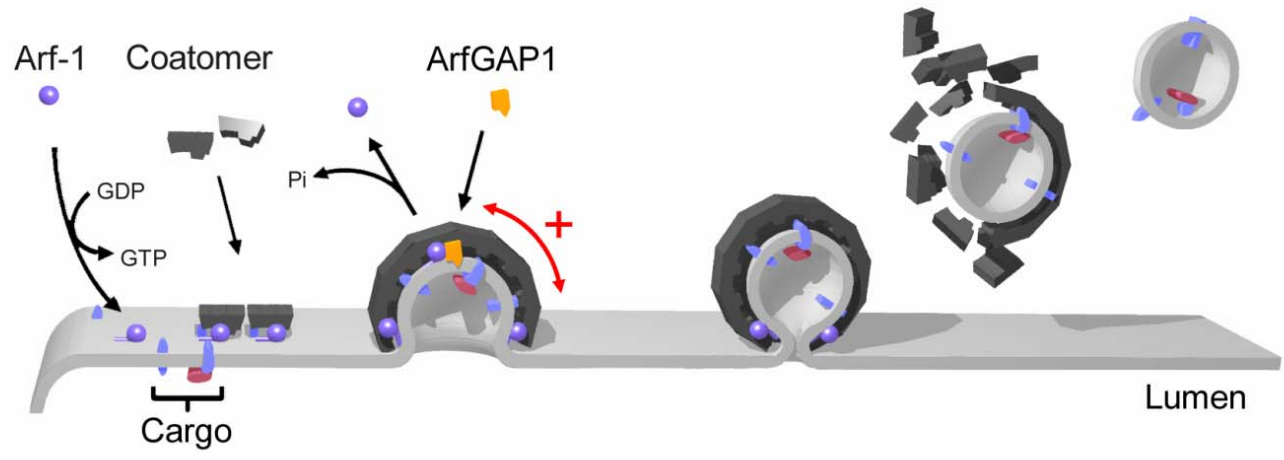
from Behnia R, Munro S Nature. 2005 438:597-604

A third landmark : membrane shape (**curvature**)

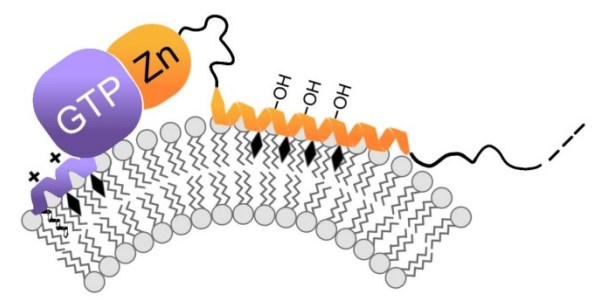
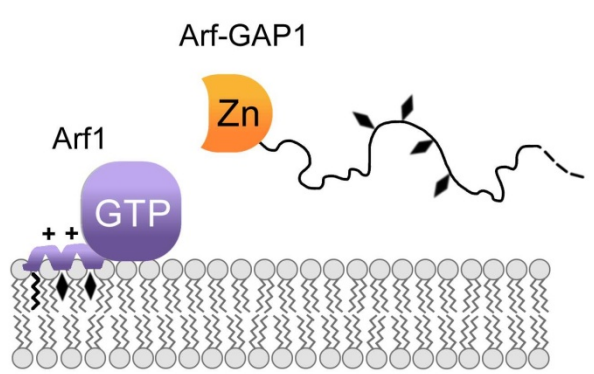
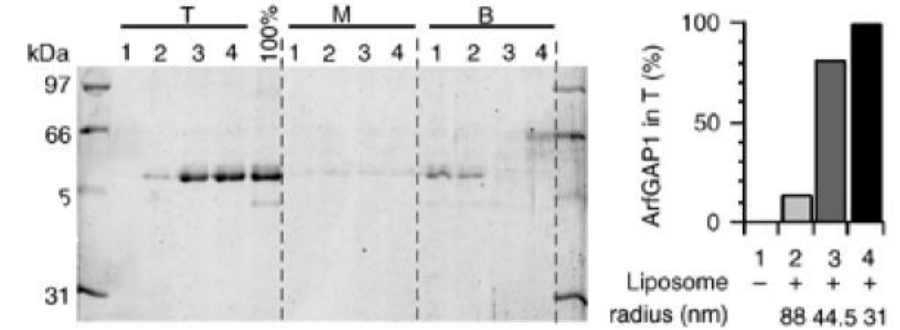
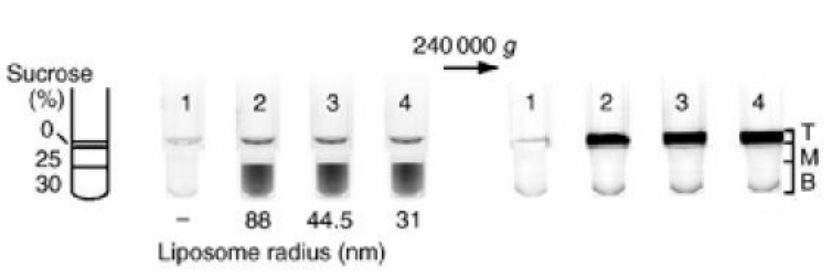



200 nm

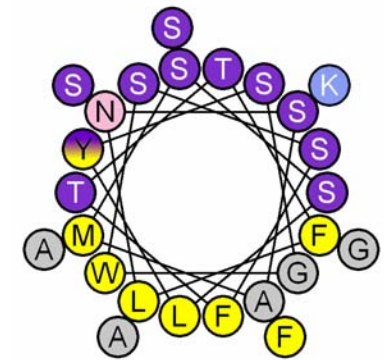
Curvature programs COPII depolymerisation by **ArfGAP1**



Detection of membrane curvature by ALPS motif

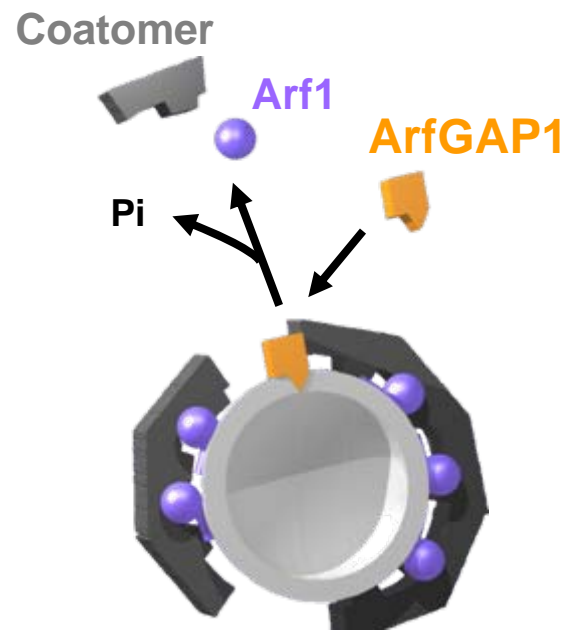
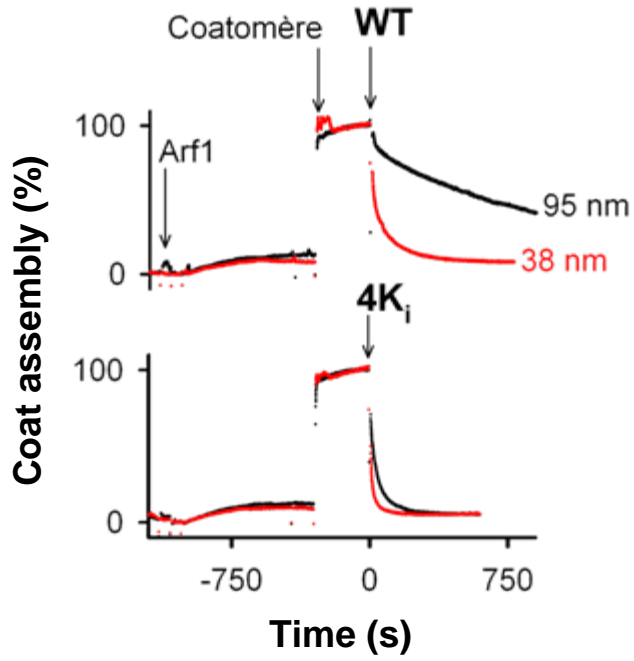
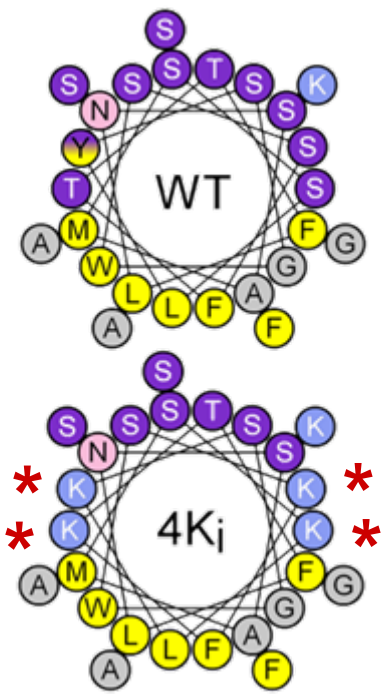


ALPS



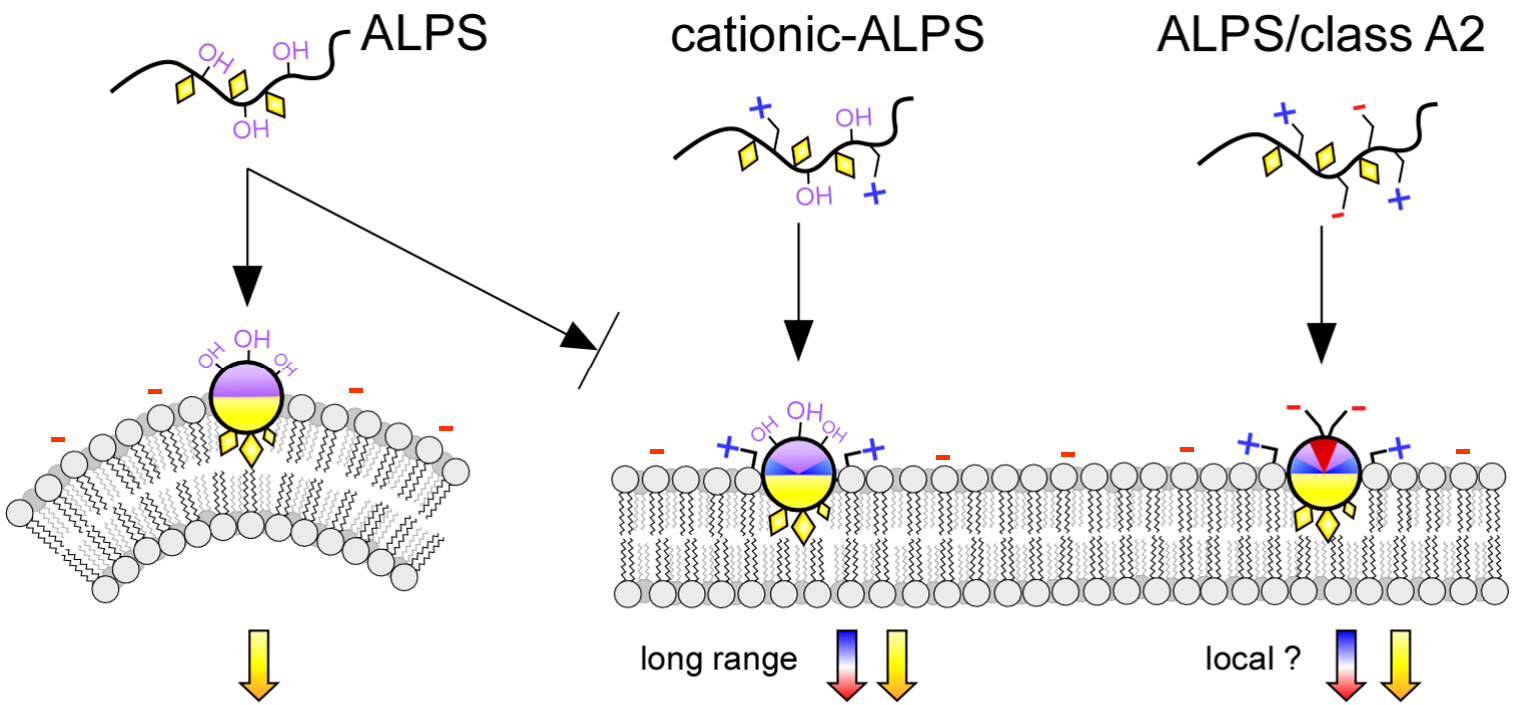
➔ The **unusual polar face** of the ALPS helix could explain its ability to recognize membrane curvature

Detection of membrane curvature by ALPS motif



➔ The **absence of positively charged residues** in the ALPS motif explain its ability to sense membrane curvature.

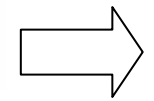
Detection of membrane curvature by ALPS motif



Is the motif ALPS present in other proteins ?

ALPS

FLNNA**MSS**LY**SGW**SS**F**TT

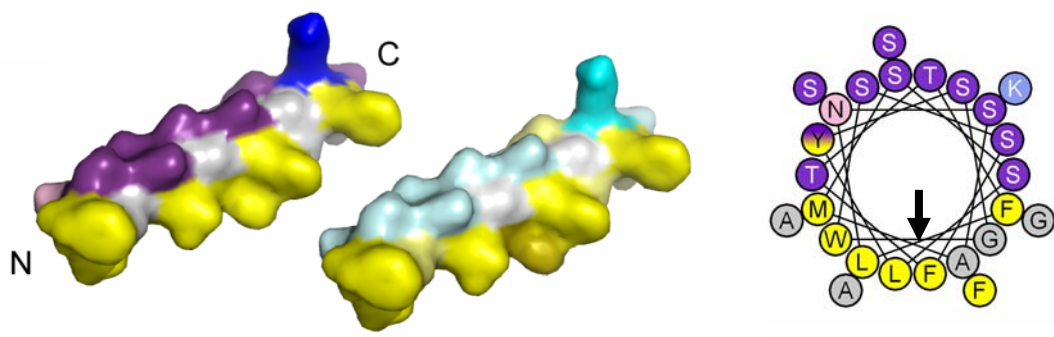


PSI-BLAST

Sequence	Score	E Value
gi 121587732.1 ADP-ribosylation factor GTPase activating prot...	80.5	2e-14
gi 1AAM7095.1 ArfGp1 protein (Rattus norvegicus)	80.5	2e-14
gi 1AAG2182.1 ADP-ribosylation factor GTPase activating prot...	80.5	2e-14
gi 1AAG2183.1 ADP-ribosylation factor GTPase activating prot...	80.5	2e-14
ref NP_635558.1 ADP-ribosylation factor GTPase-activating pr...	80.5	2e-14
ref NP_001054527.1 PREDICTED: similar to ADP-ribosylation fa...	77.8	1e-13
gi 1E5107371.1 ADP-ribosylation factor GTPase activating prot...	77.8	1e-13
gi 1E5107370.1 ADP-ribosylation factor GTPase activating prot...	77.8	1e-13
gi 1E5107366.1 ADP-ribosylation factor GTPase activating prot...	77.8	1e-13
gi 1E5107367.1 ADP-ribosylation factor GTPase activating prot...	77.8	1e-13
gi 1E5107369.1 ADP-ribosylation factor GTPase activating prot...	77.8	1e-13
ref NP_001368072.1 PREDICTED: hypothetical protein (Homo sapi...	77.8	1e-13
gi 1AAM16228.1 ArfGp1 protein (Mus musculus) >gi 1E5107366.1...	77.8	1e-13
gi 1AAM52022.1 ArfGp1 protein (Mus musculus)	77.8	1e-13
gi 1AAM52017.1 ArfGp1 protein (Mus musculus)	77.8	1e-13
gi 1BAE22120.1 unnamed protein product (Mus musculus) >dbj B...	77.8	1e-13
gi 1BAE22096.1 unnamed protein product (Mus musculus) >emb C...	77.8	1e-13
ref NP_665703.2 ADP-ribosylation factor GTPase activating pr...	77.8	1e-13
emb CAC18731.1 ADP-ribosylation factor 1 GTPase activating p...	77.8	1e-13
gi 1BAM13964.1 unnamed protein product (Homo sapiens)	74.9	1e-12
gi 1BAM13924.1 unnamed protein product (Homo sapiens)	74.9	1e-12
gi 1EAM71302.1 ADP-ribosylation factor GTPase activating prot...	74.9	1e-12
gi 1EAM71300.1 ADP-ribosylation factor GTPase activating prot...	74.9	1e-12
gi 1EAM71303.1 ADP-ribosylation factor GTPase activating prot...	74.9	1e-12
ref NP_214925.1 PREDICTED: hypothetical protein isoform 9 (P...	74.9	1e-12
ref NP_001149011.1 PREDICTED: hypothetical protein isoform 6...	74.9	1e-12
ref NP_001149021.1 PREDICTED: ADP-ribosylation factor GTPase...	74.9	1e-12
ref NP_001149029.1 PREDICTED: hypothetical protein isoform 3...	74.9	1e-12
ref NP_001149023.1 PREDICTED: similar to ADP-ribosylation fa...	74.9	1e-12
gi 1AAM57445.1 ADP-ribosylation factor GTPase activating pr...	74.9	1e-12
gi 1AAM50055.1 ARFGAP1 protein (Homo sapiens) >gi 1AAM1876.1...	74.9	1e-12
ref NP_763202.1 ADP-ribosylation factor GTPase activating pr...	74.9	1e-12

.....we found **nothing else than ArfGAP1**

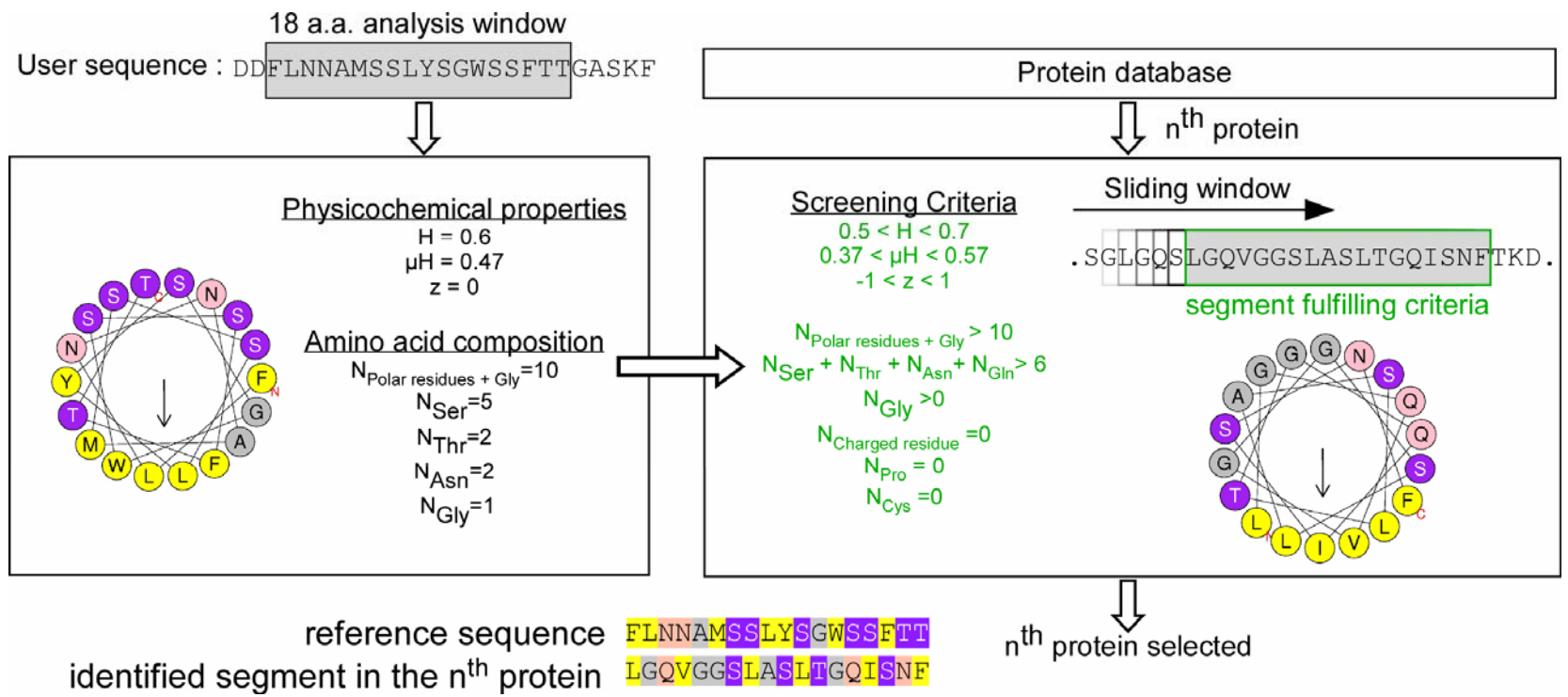
Physico-chemical properties are important, not key residues



Global parameters

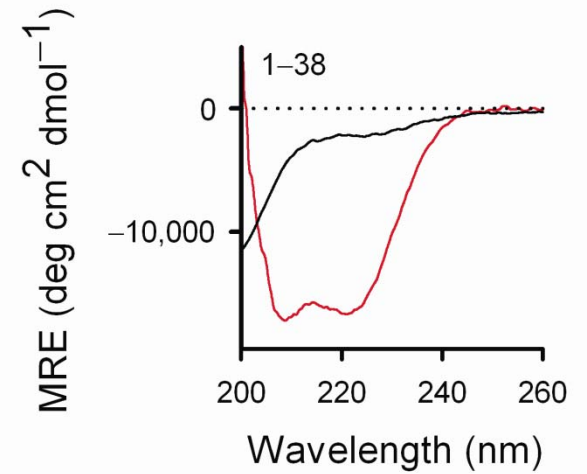
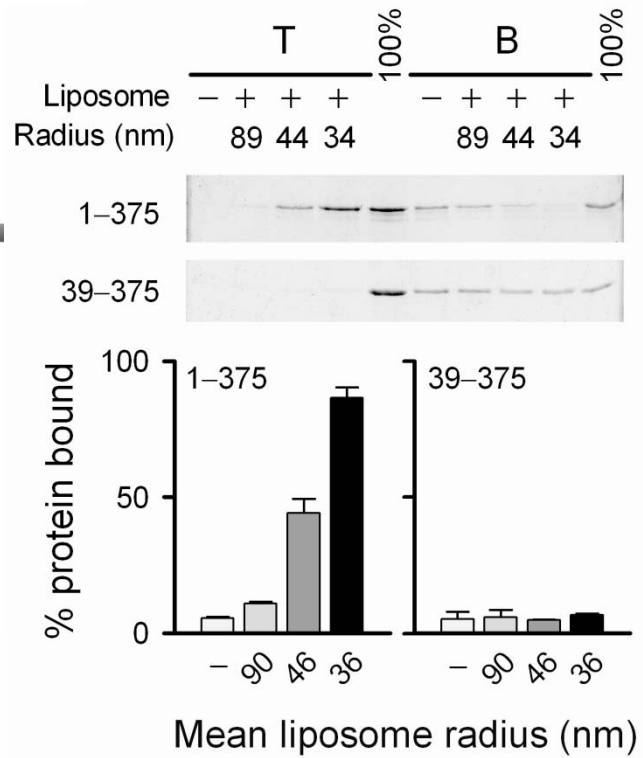
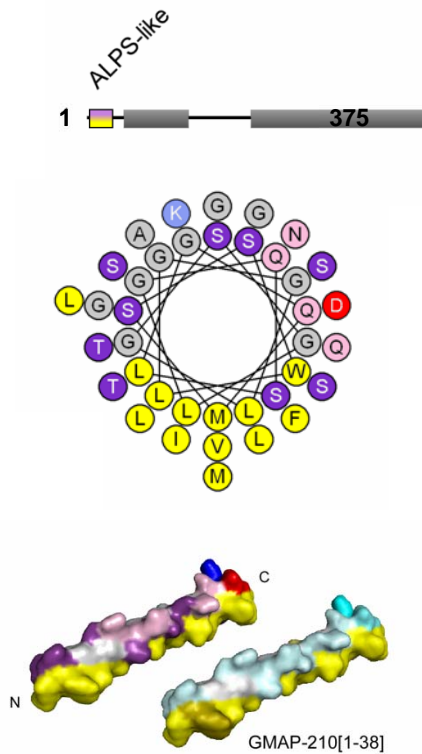
- Hydrophobicity
- Charge
- Hydrophobic moment

ALPS motif / Screening by bioinformatics

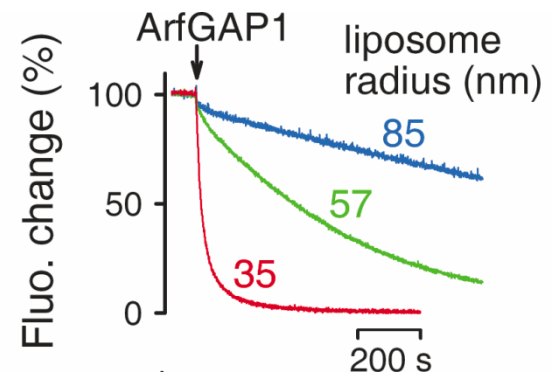
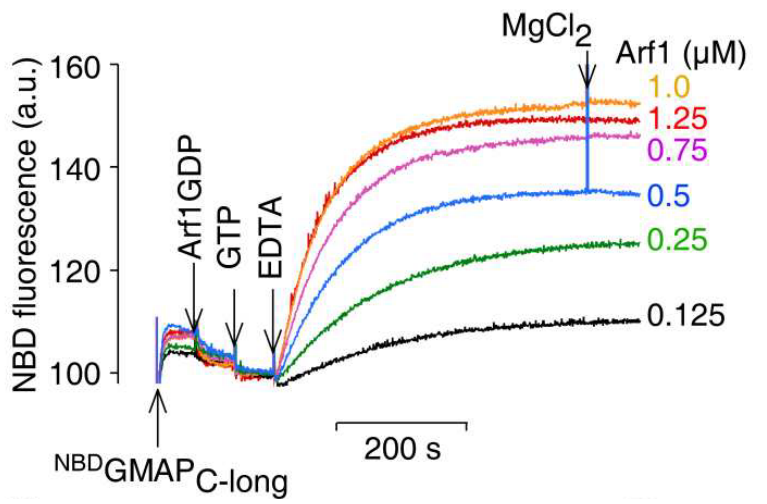
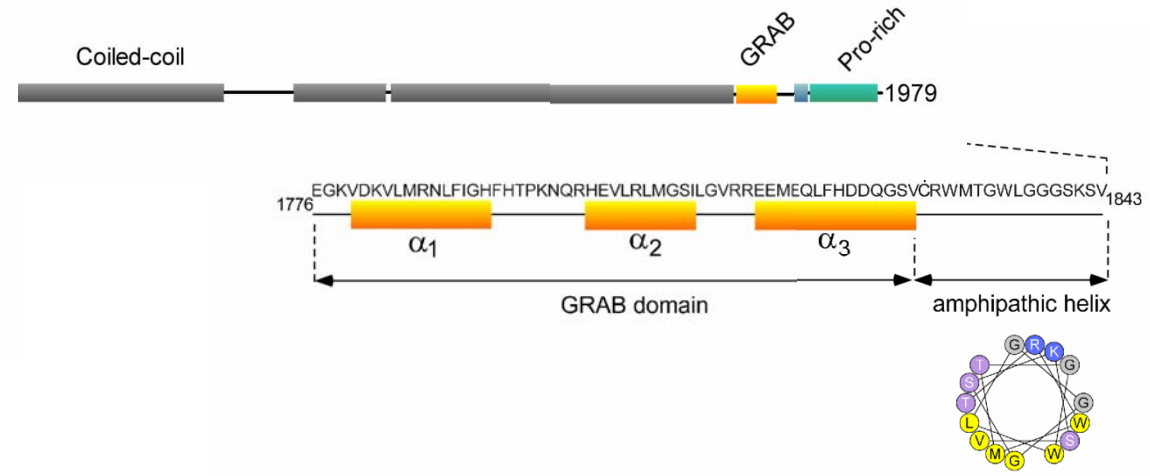
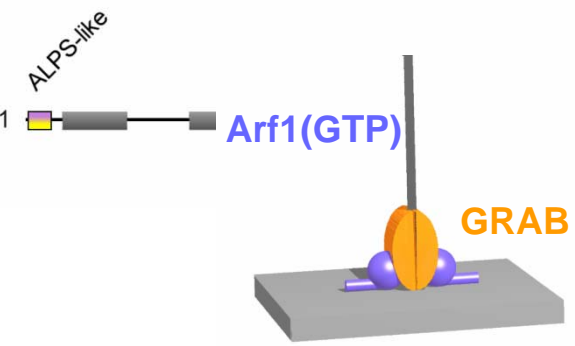


SwissProt → 196 human sequences
 191 yeast sequences → GMAP-210
 Kes1p
 Nup133

The N-terminus of GMAP-210 is an ALPS motif



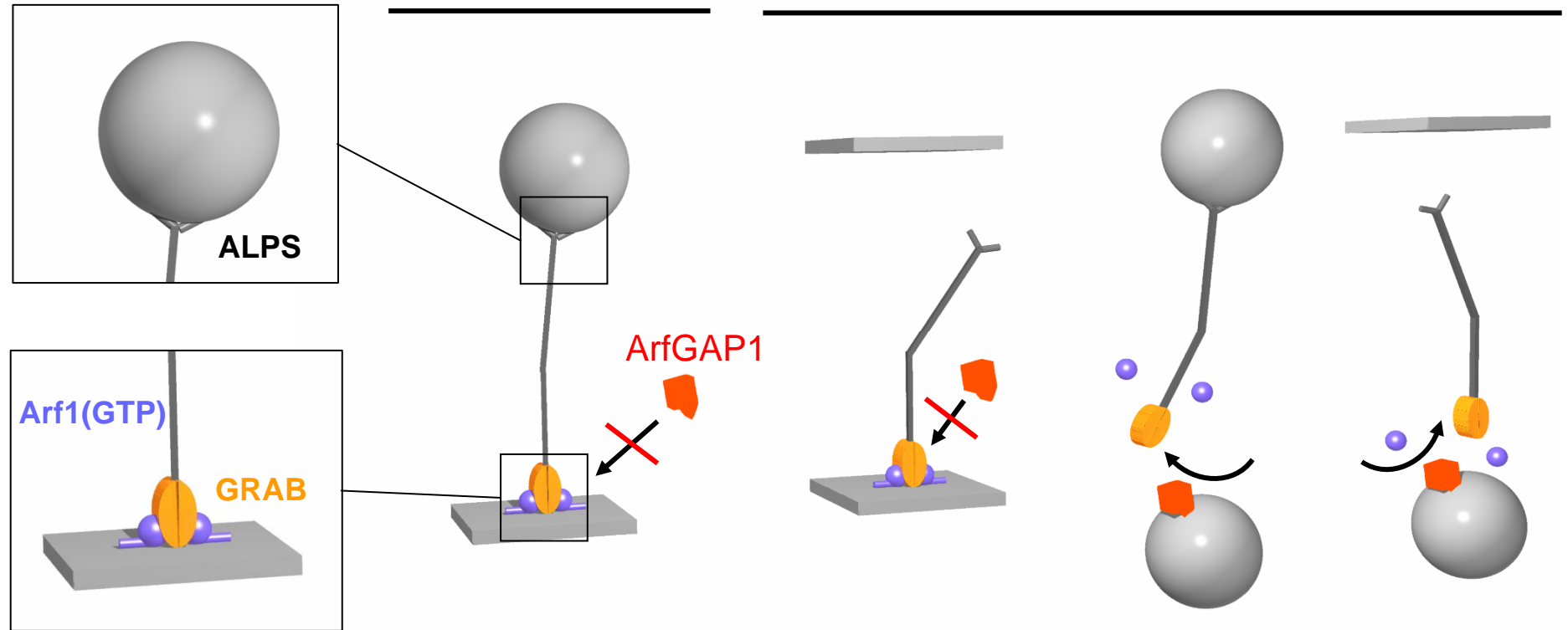
GMAP-210 binds to Arf1 with a C-terminal GRAB domain



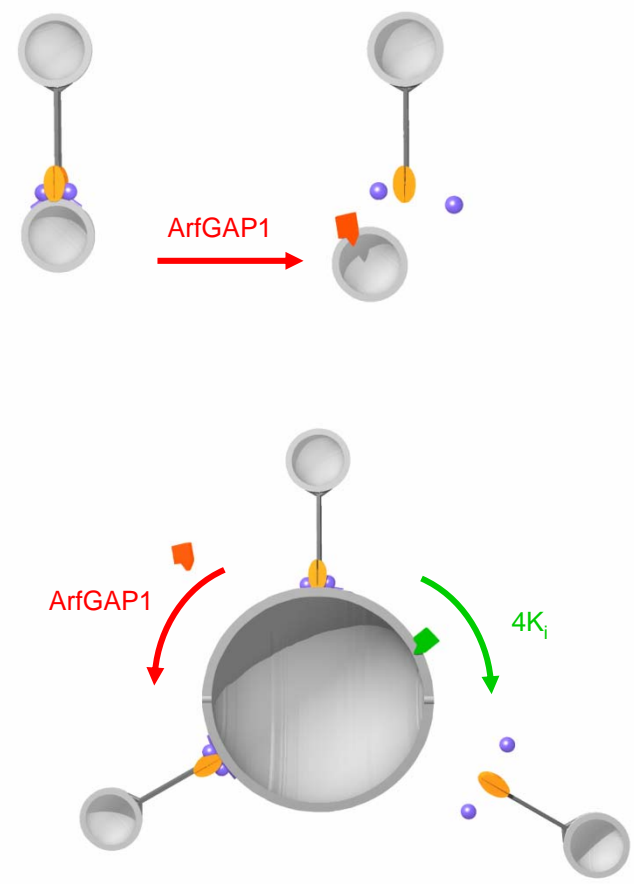
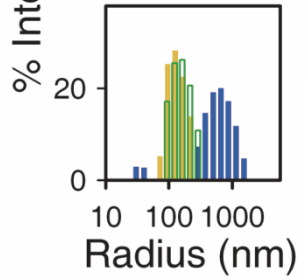
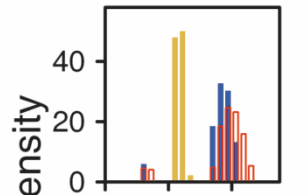
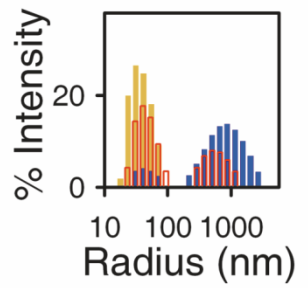
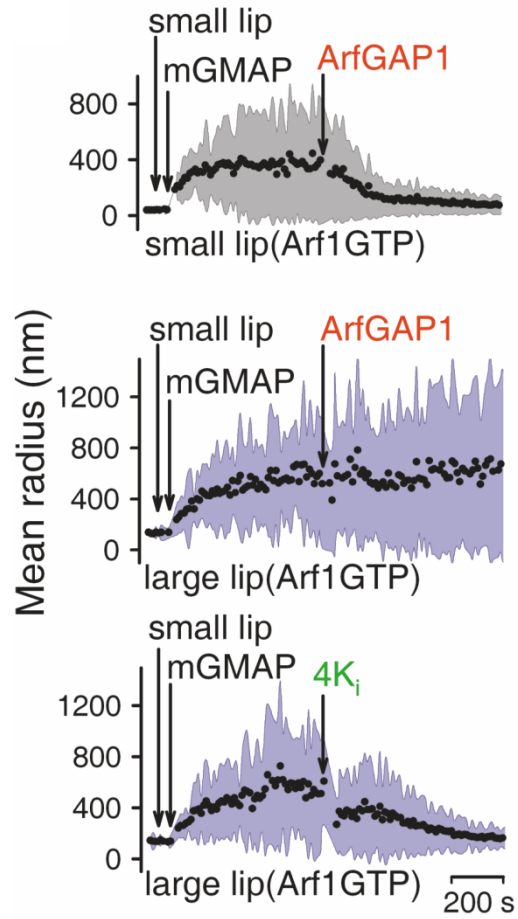
Model for asymmetric tethering

Tethering resistant
to ArfGAP1

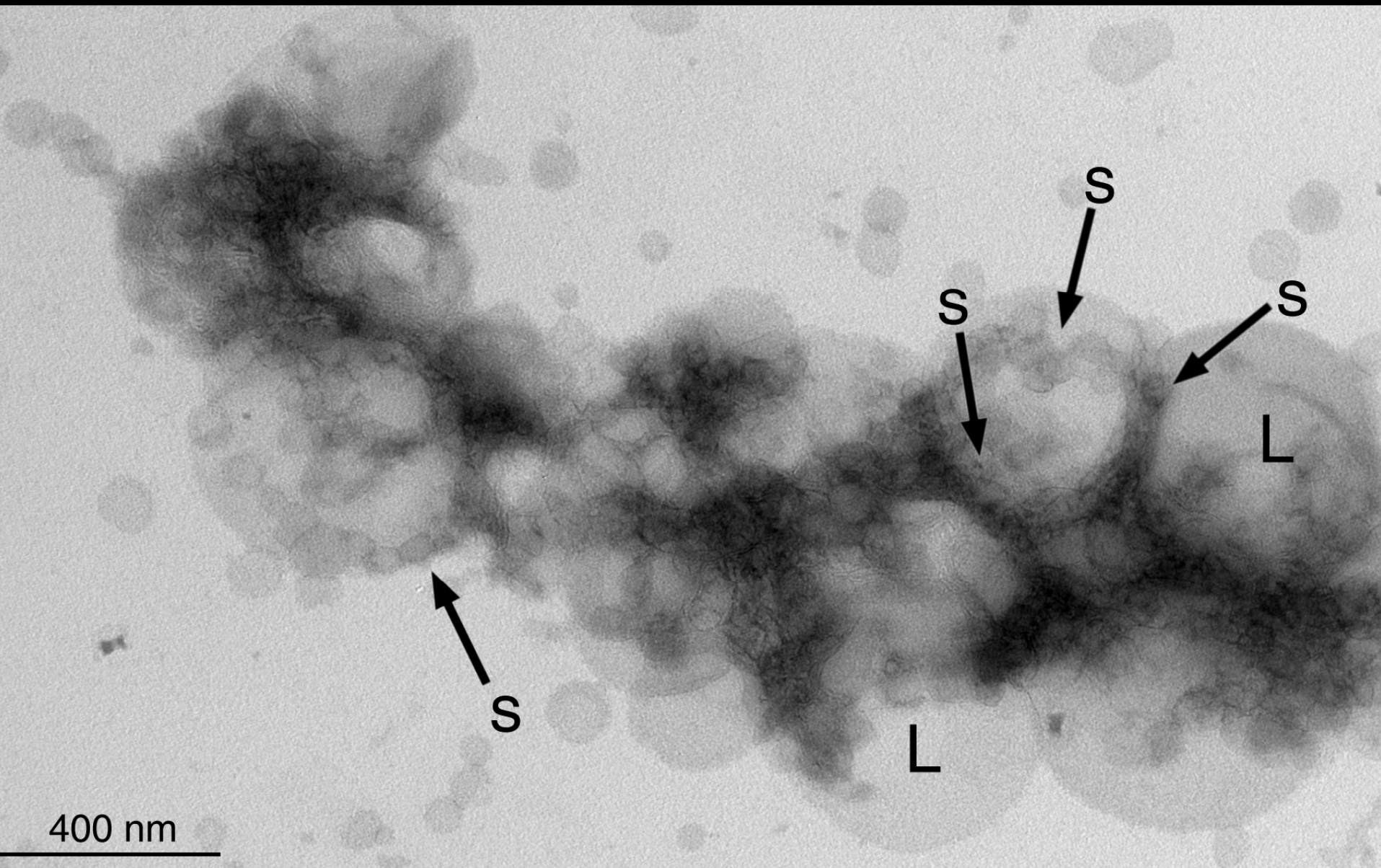
No tethering / Instable by ArfGAP1



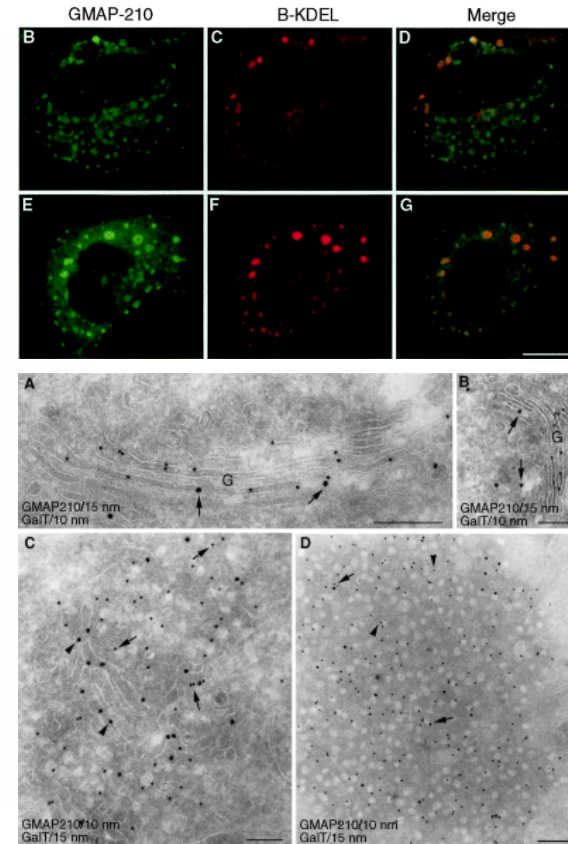
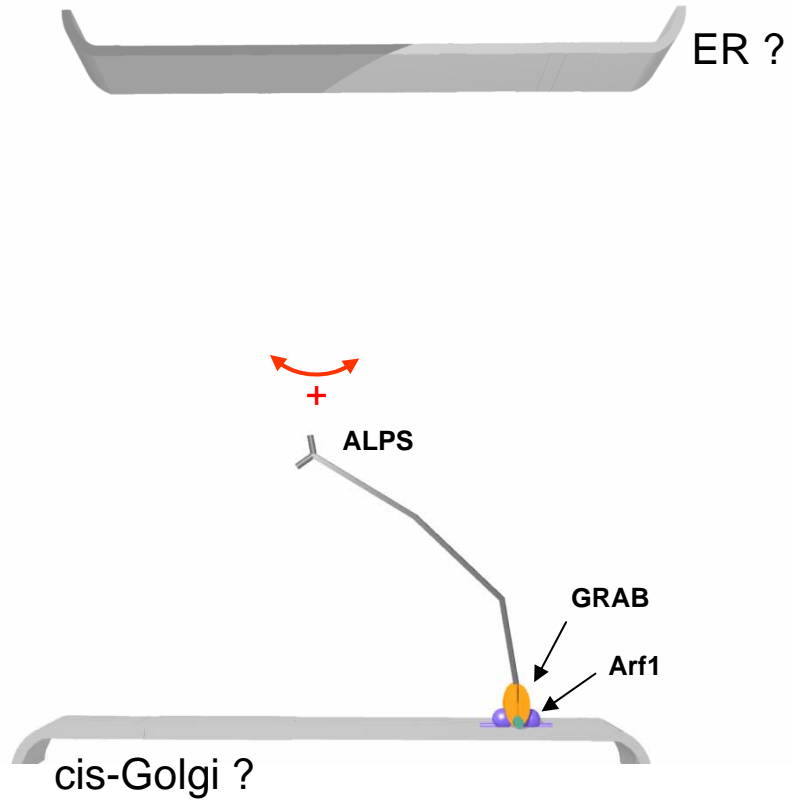
Asymmetric tethering between a curved membrane and a flat one displaying Arf1(GTP)



Asymmetric tethering between a curved membrane and a flat one displaying Arf1(GTP)

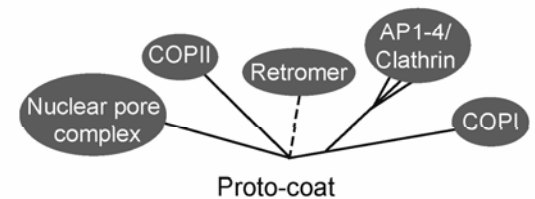
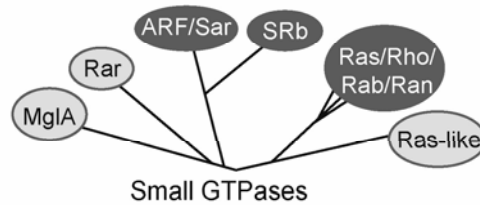
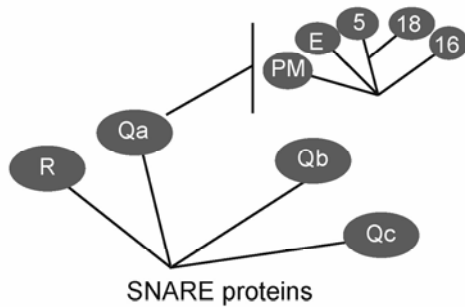
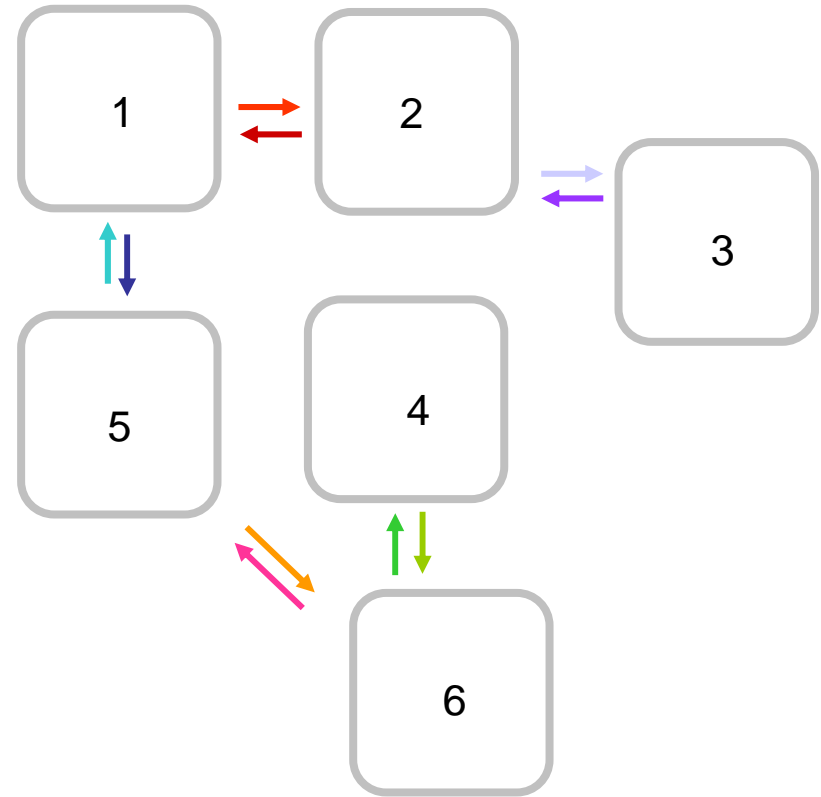
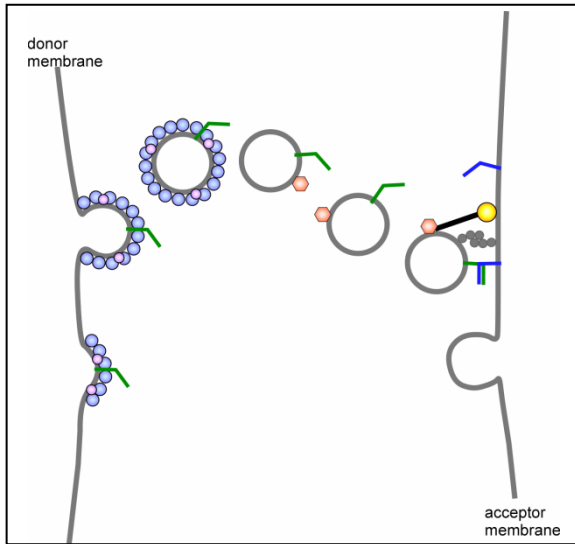
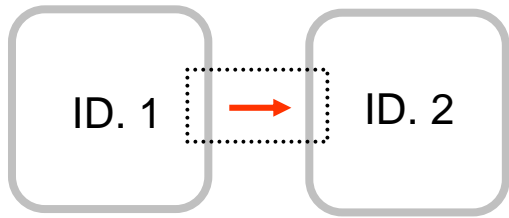


Model on the GMAP-210 function

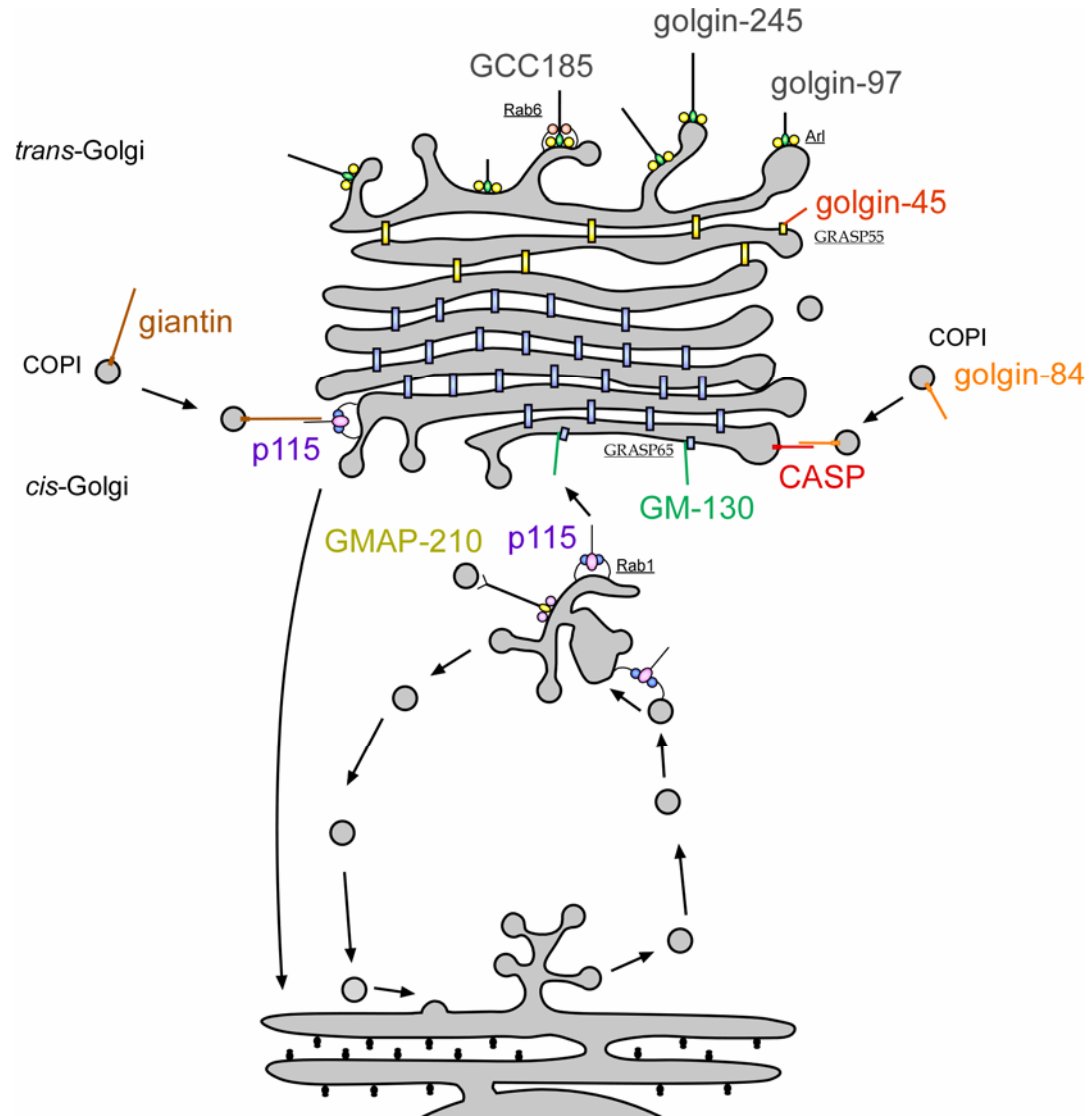


Pernet-Gallay K. *et coll*, 2002

Tethering factors / Evolution of endomembrane system



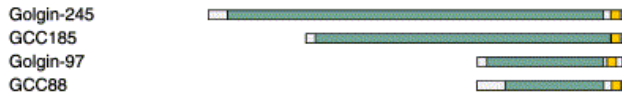
Tethering factors / Evolution of endomembrane system



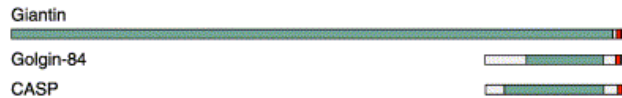
Tethering factors / Evolution of endomembrane system

A evident variation : length of the central coiled-coil region Various golgin appear not well-conserved

GRIP domain



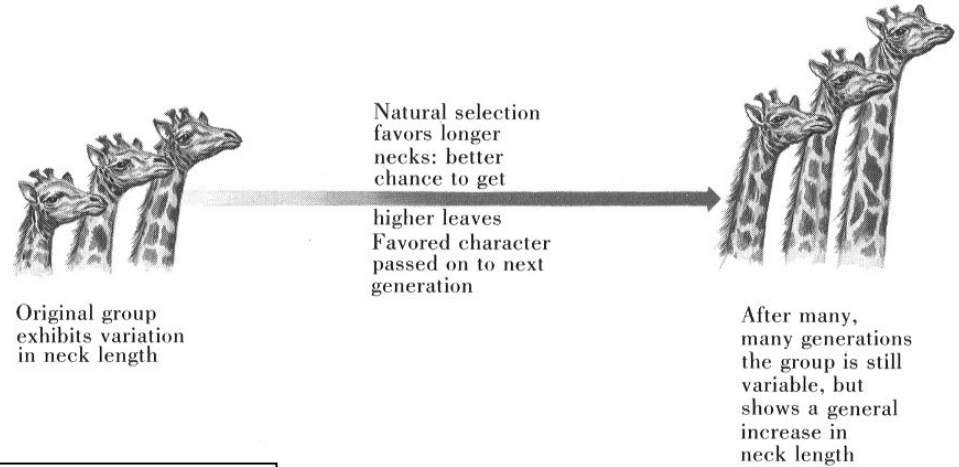
Transmembrane domain



Others

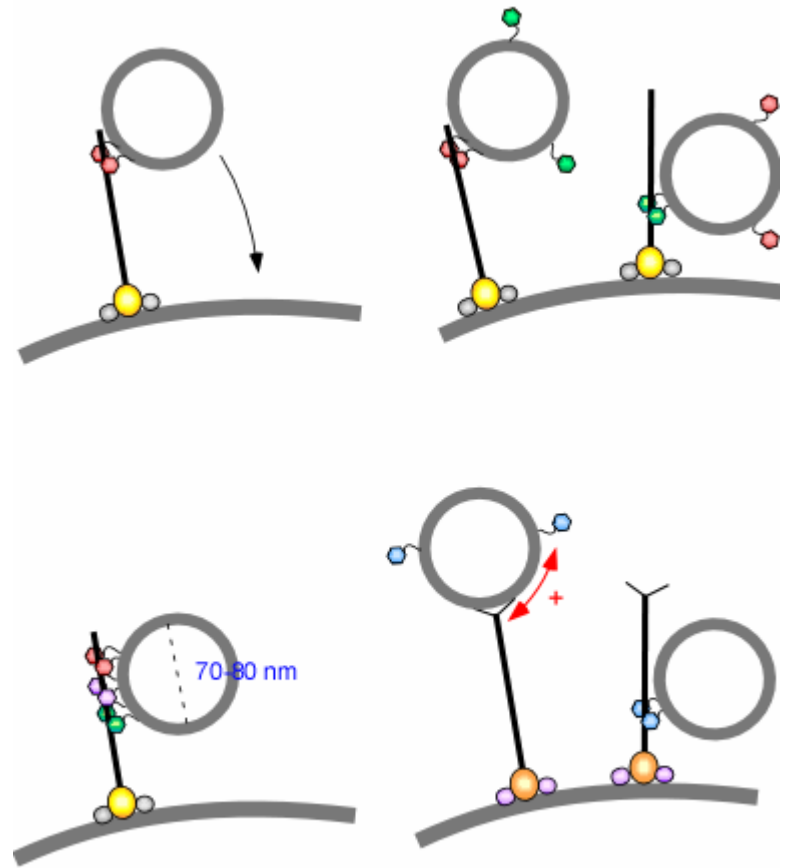
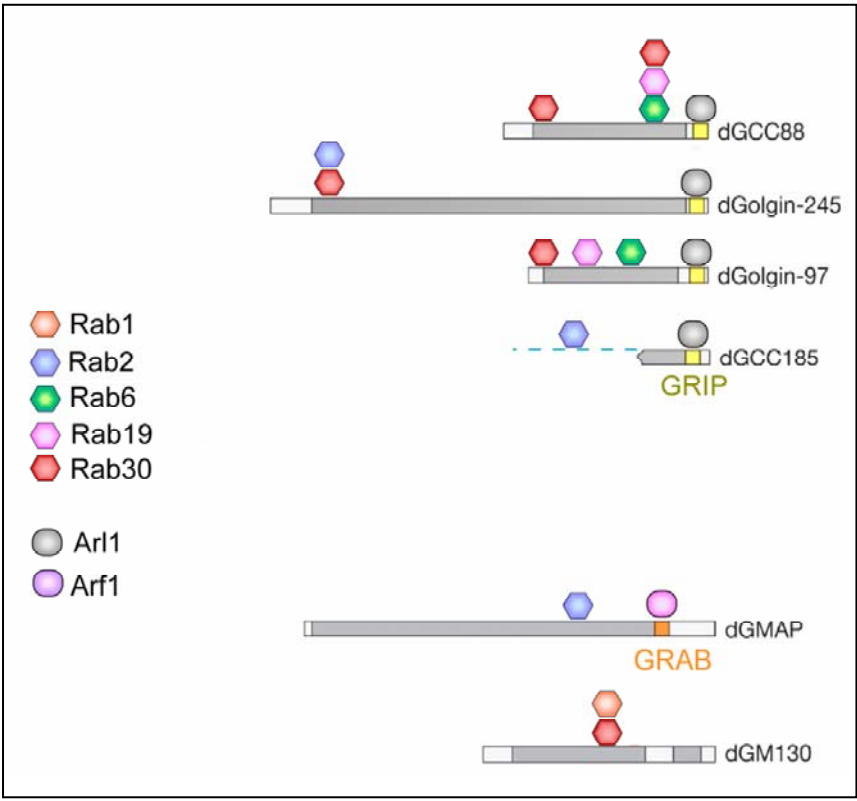


DARWIN'S GIRAFFE



Evolving ideas about tethering factors

A new feature : multiple binding-sites within golgin for small G proteins



Tentacle model

Length + diverse binding-sites : to create tethering factors able to deal with the increasing complexity of endomembrane system

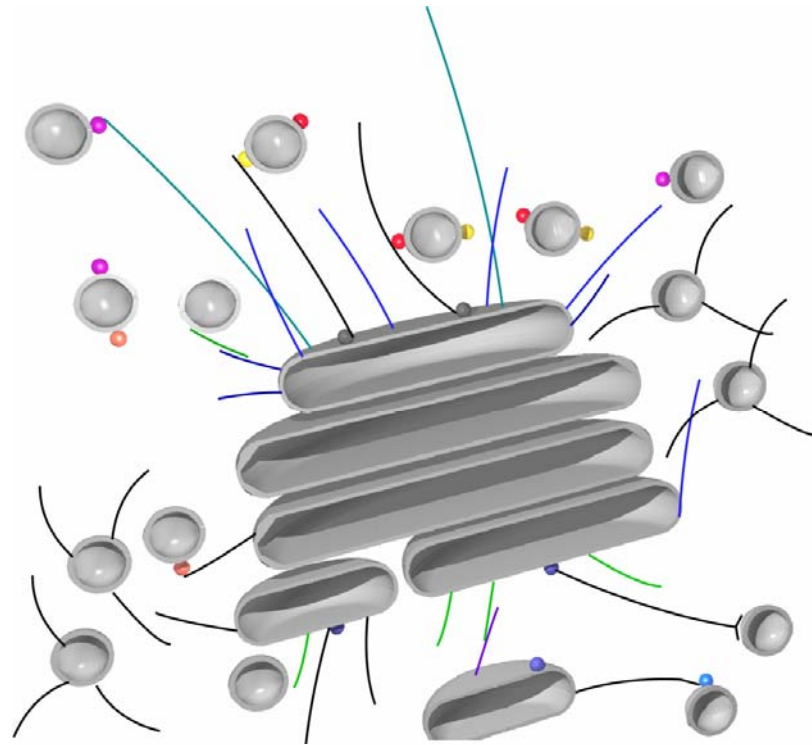
Distribution of various binding-sites (hook) in space

Organisation of vesicular trafic from and to Golgi apparatus in space and time

Sorting

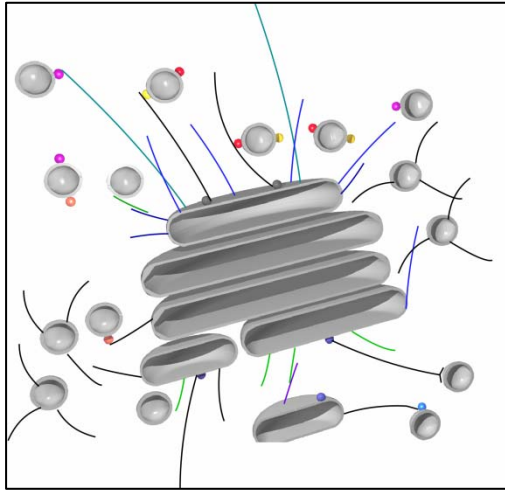
Processive transport

Control of membrane flux and of Golgi architecture



Biochemical evidences about tethering

→ Can we analyse this complex tethering system

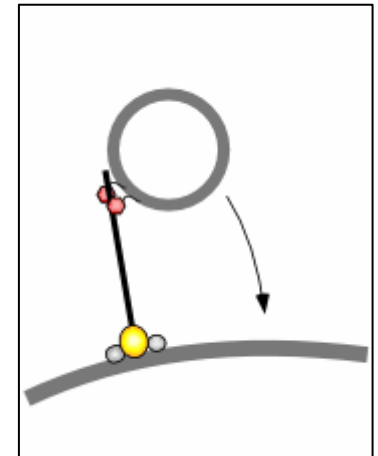


Reality

Lifetime, dynamic of tethering event

Strength of interaction

Number of tethers involved in a tethering event

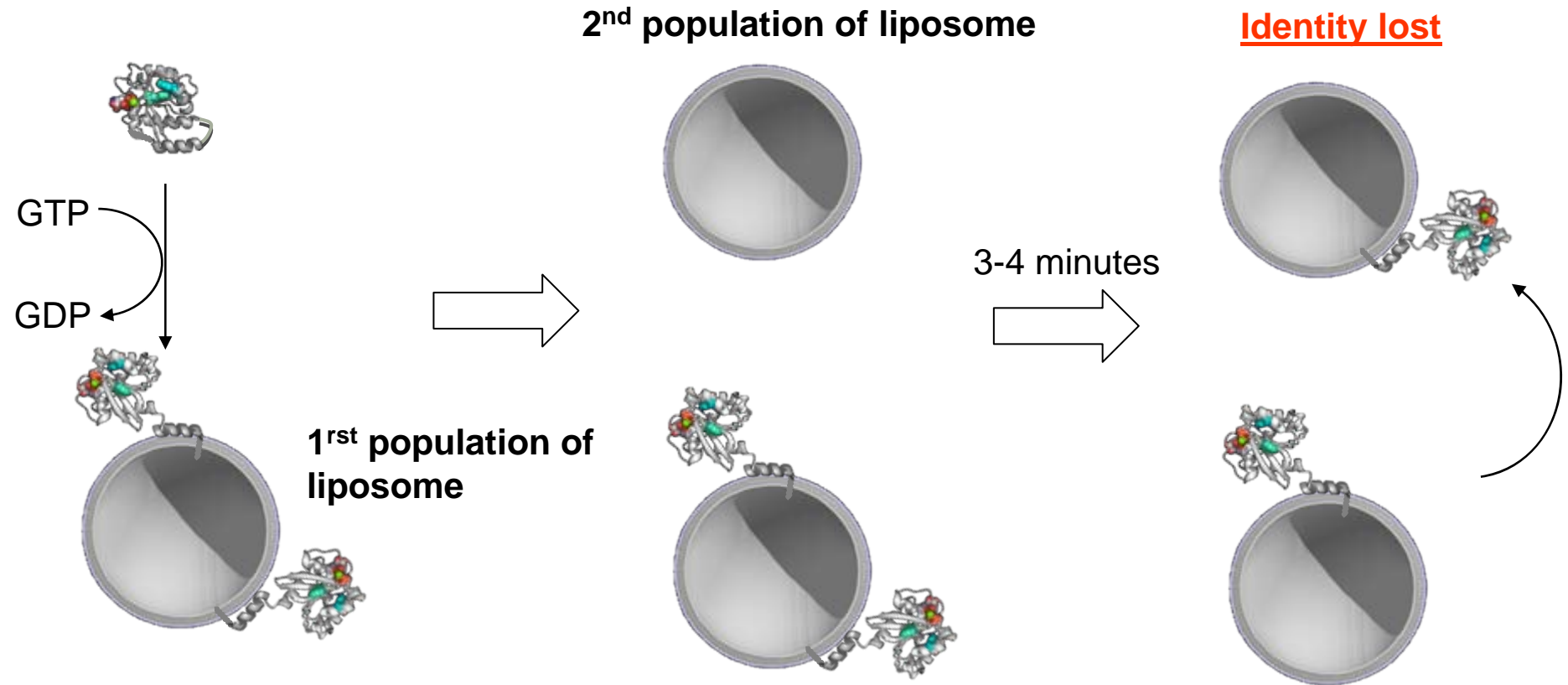


To monitor tethering *in vitro* with better accuracy , we need to :

- control membrane identity
- quantify the connection of two membranes
- control aggregation

Asymmetric tethering / Controlling the membrane identity

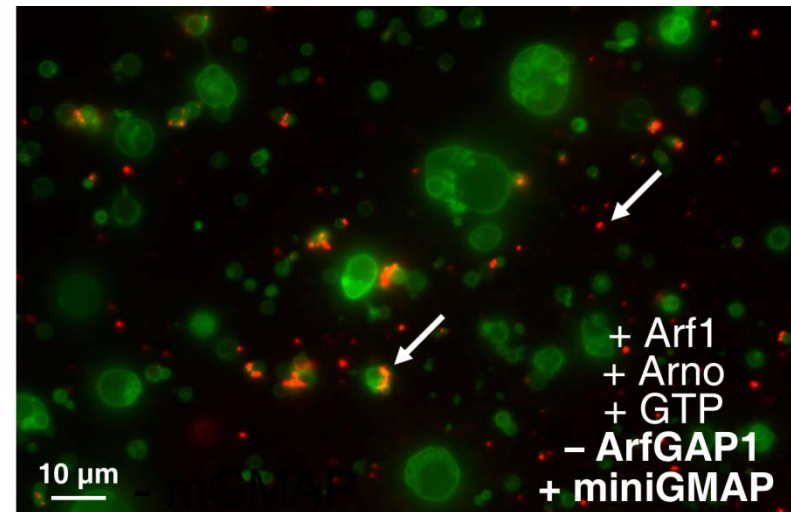
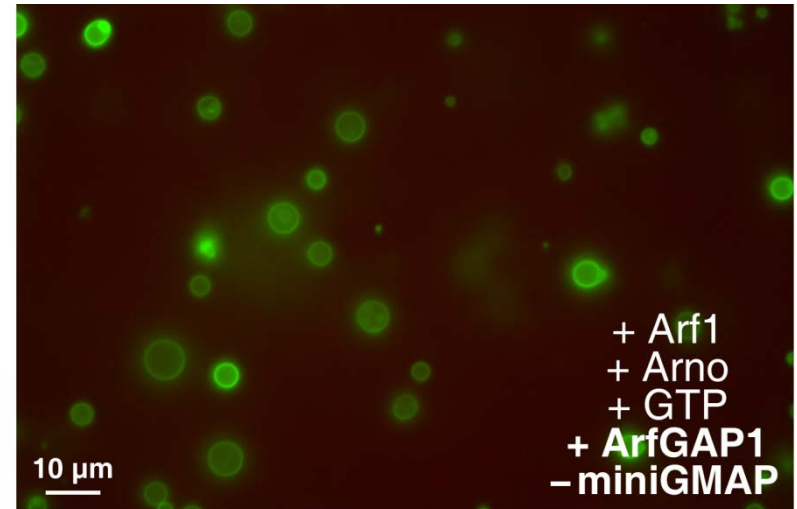
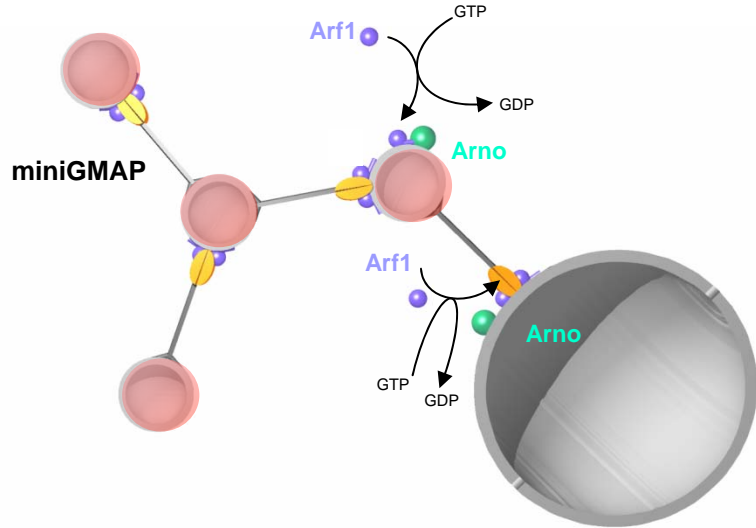
➔ GTPase are **perimembranar** protein : binding is **dynamic**



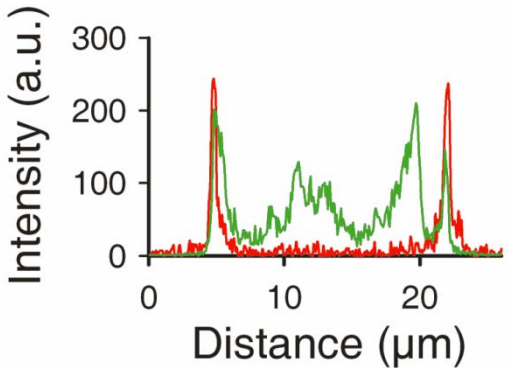
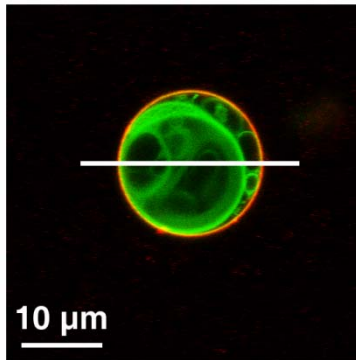
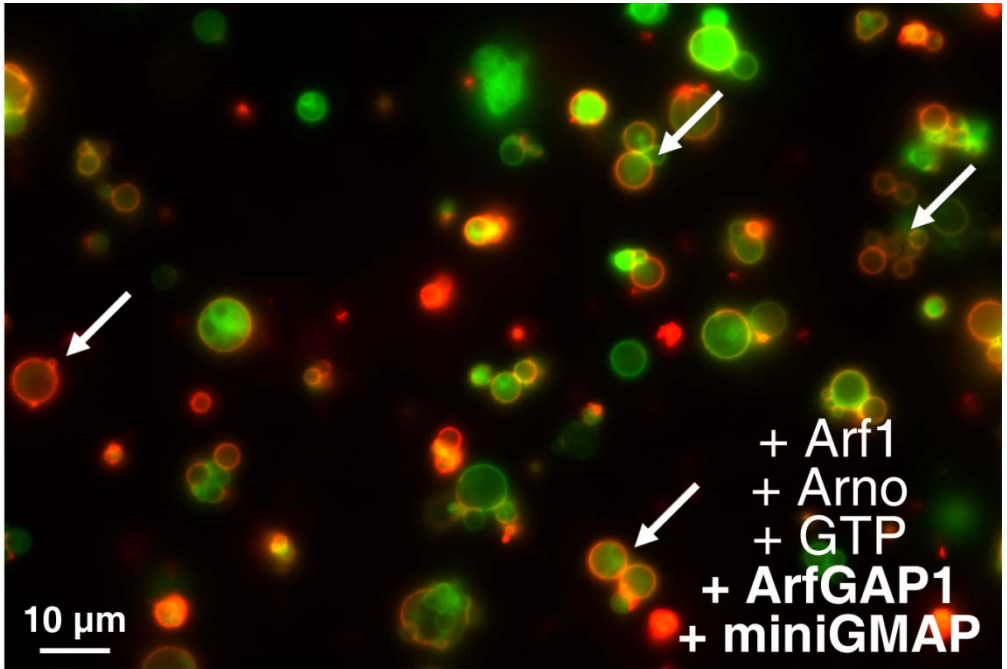
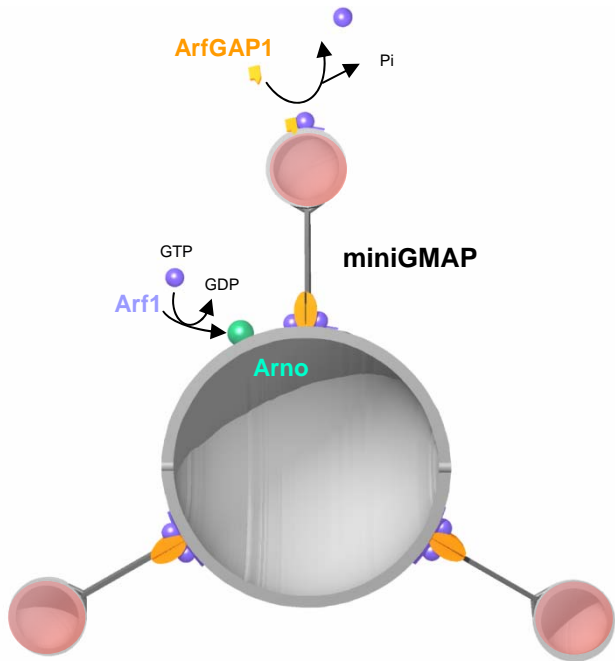
Control identity during tethering

- Quick mixing of liposomes with the tethering factor
- Chemical anchoring of GTPase to membrane
- Biochemical control of GTPase by **GEF/GAP**

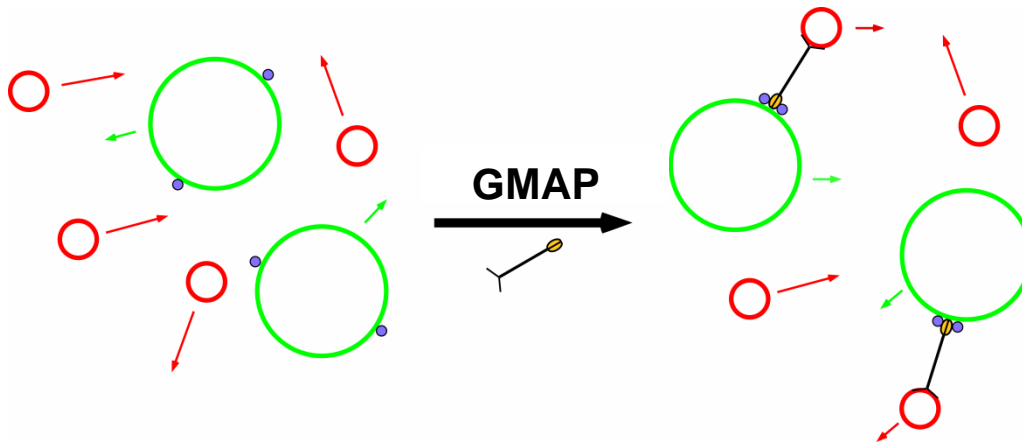
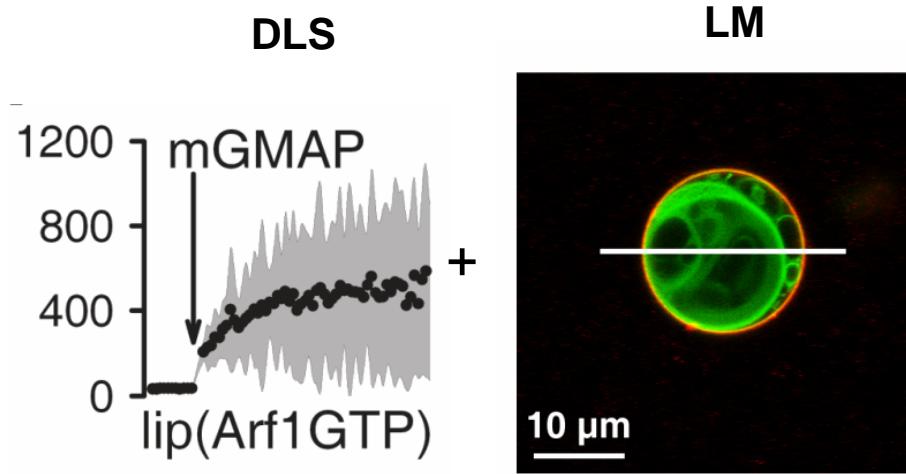
Asymmetric tethering / Controlling the membrane identity



Asymmetric tethering - Self-organisation by Arno, ArfGAP1 and membrane curvature

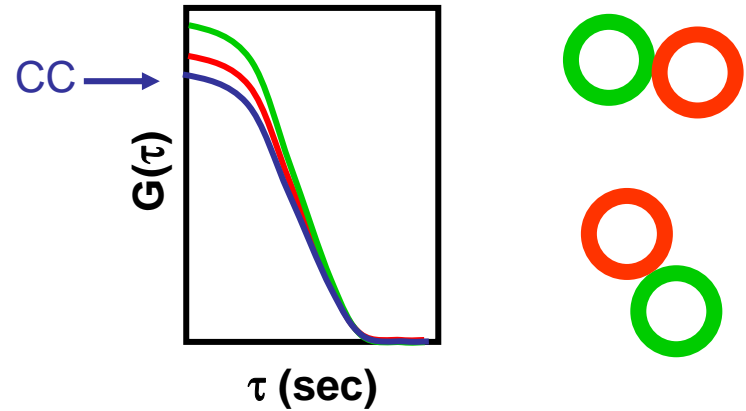
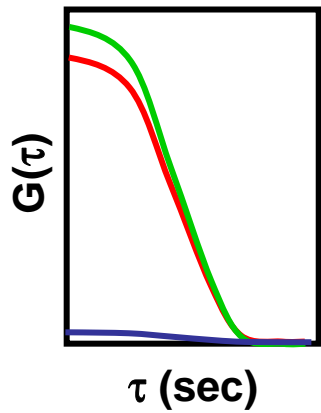
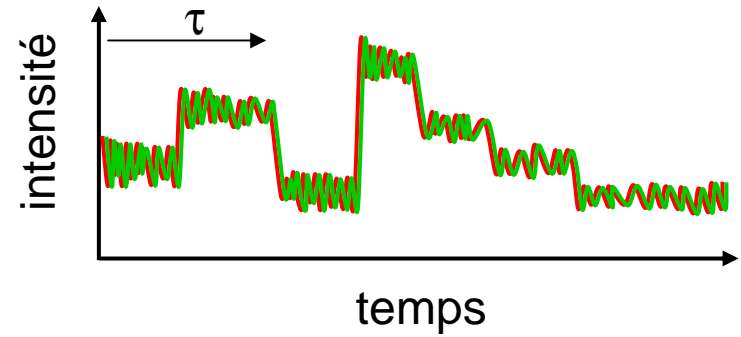
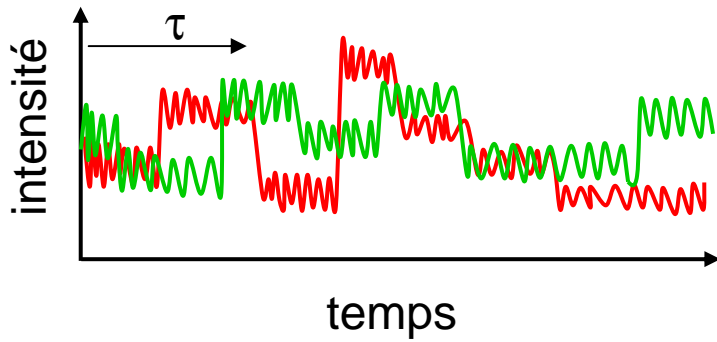
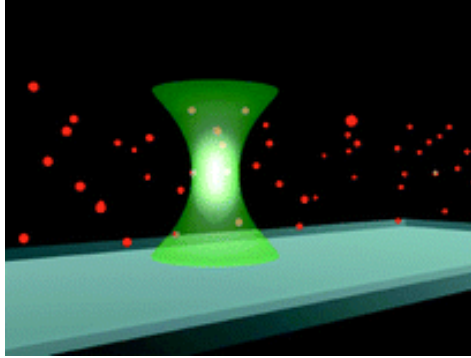


New technical approach : FCCS

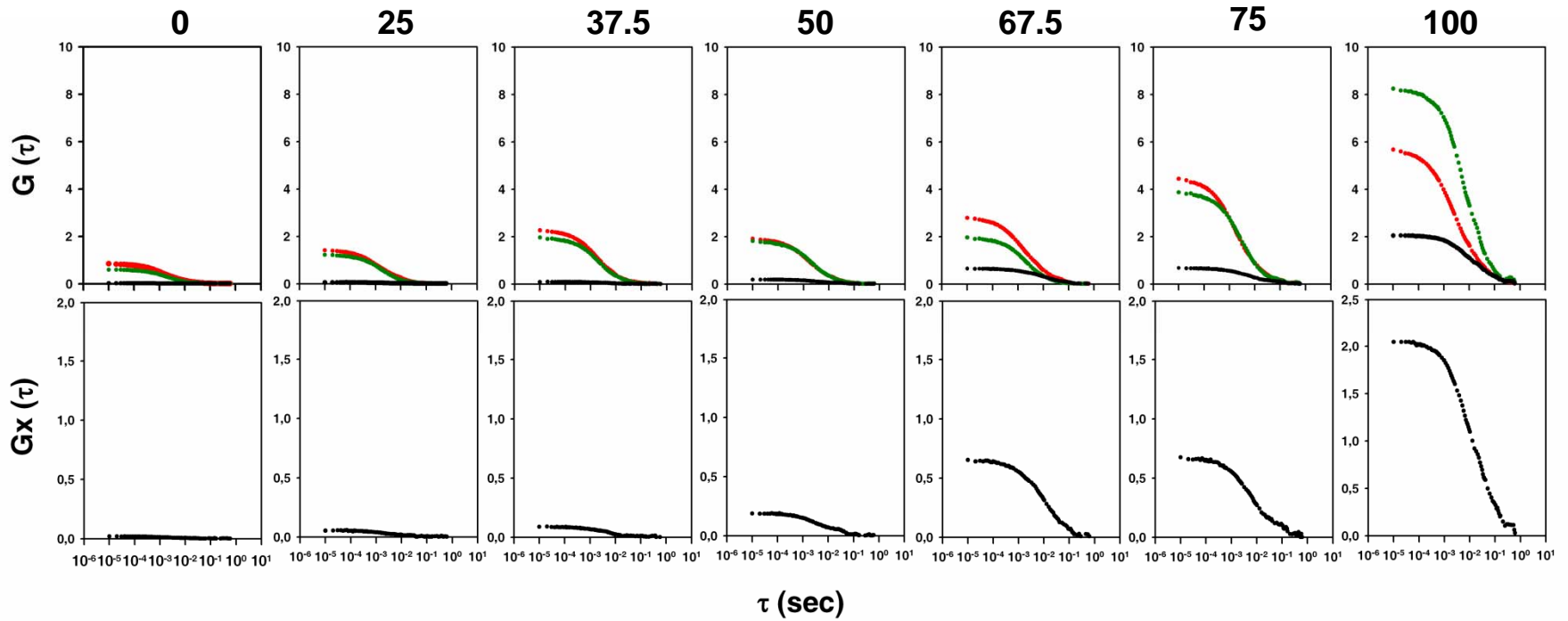
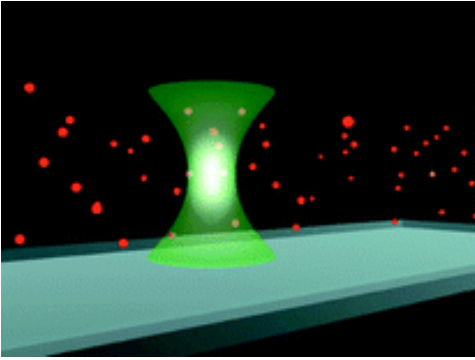


Quantify the connection of two distinct membranes

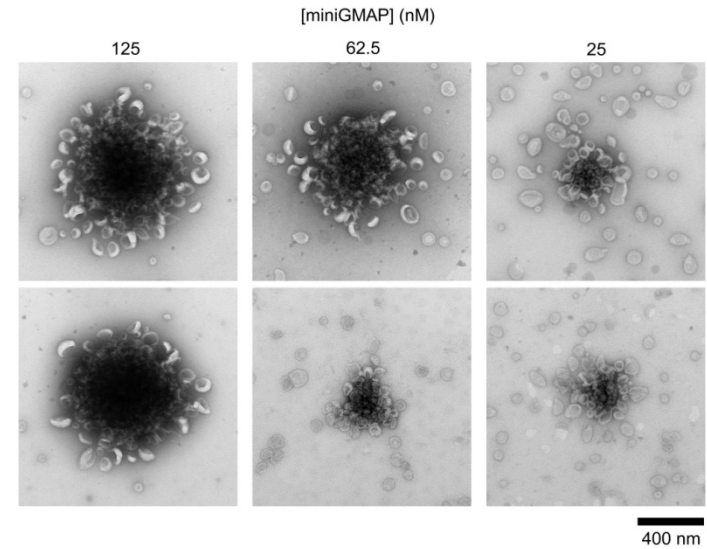
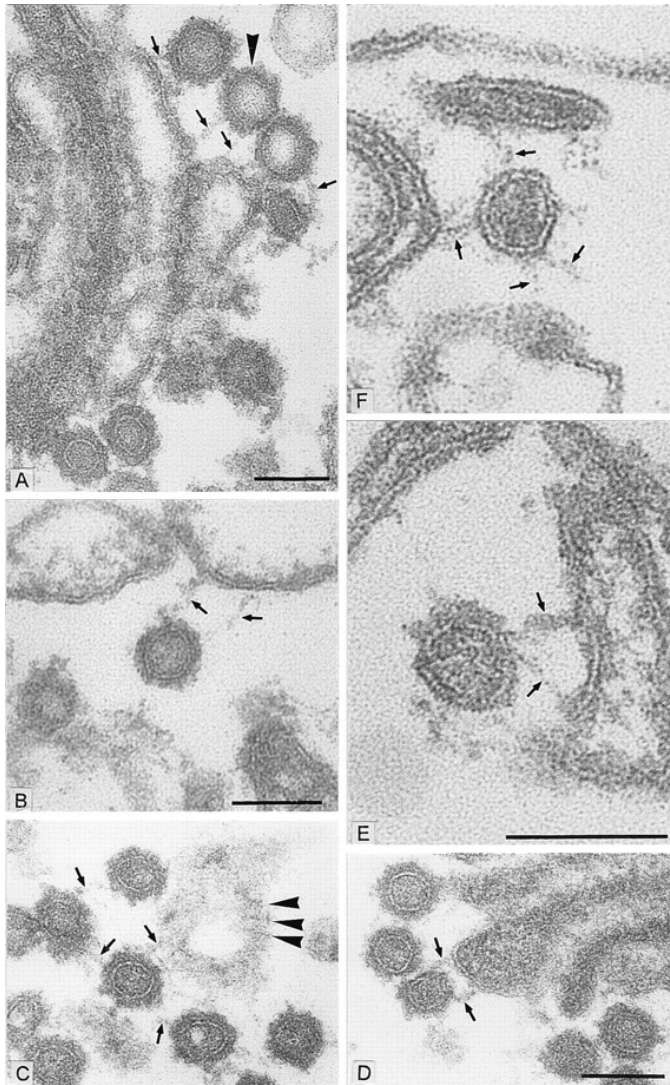
New technical approach : FCCS



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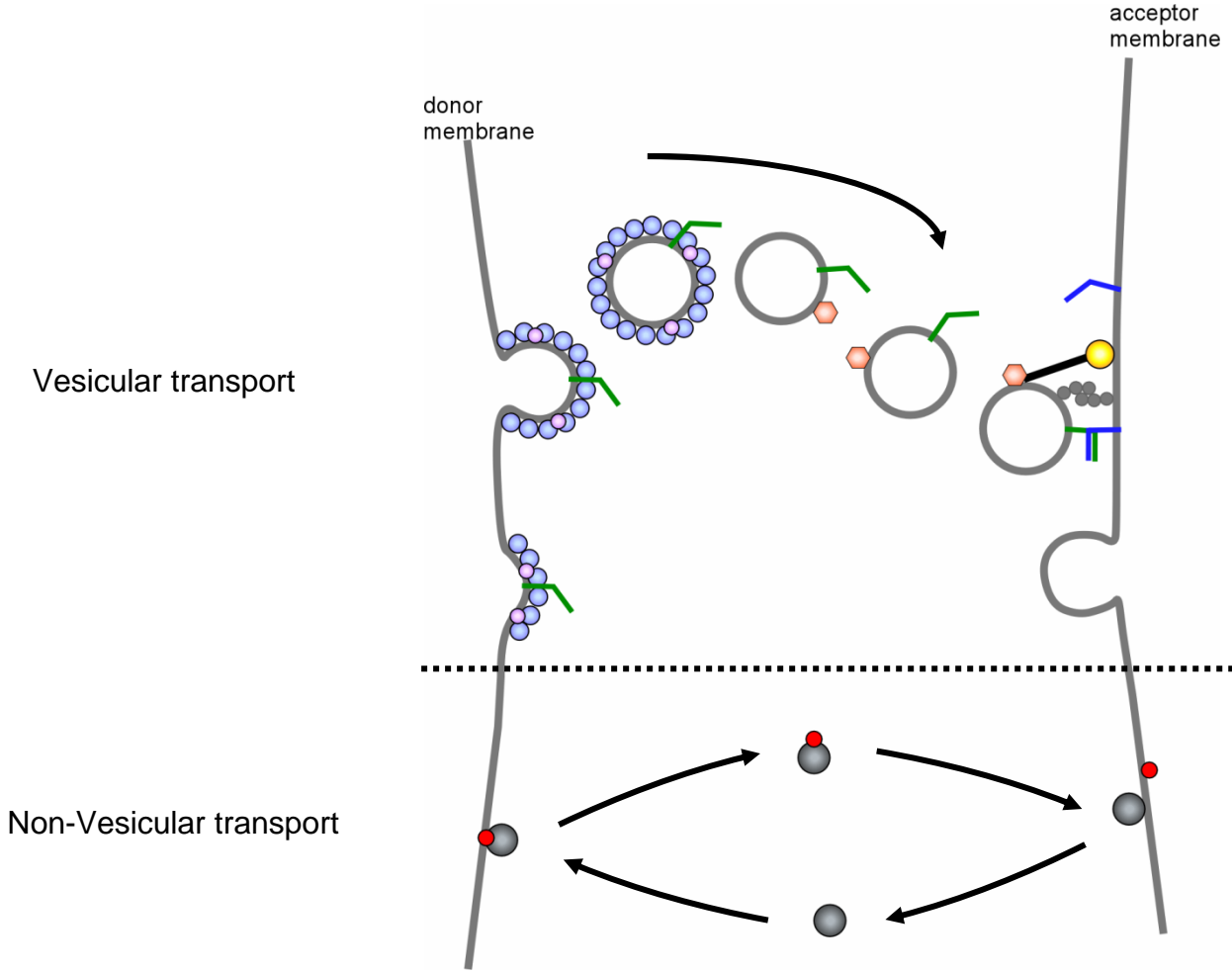


Controlling aggregation

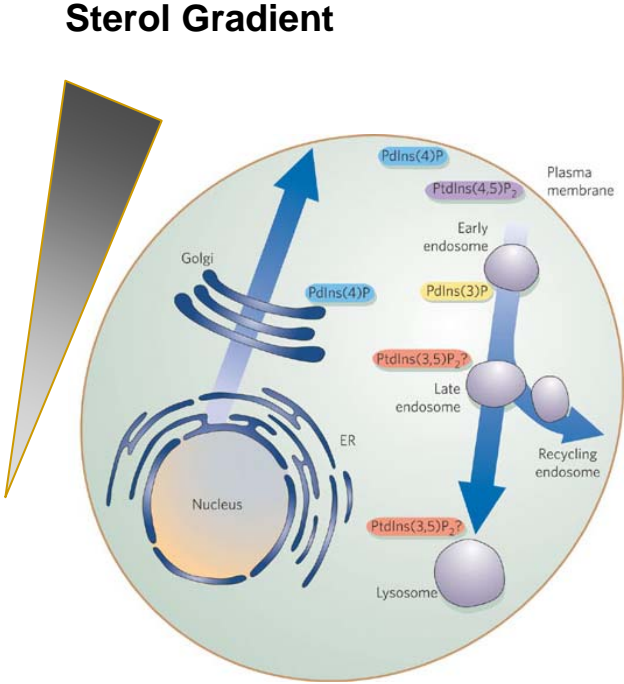
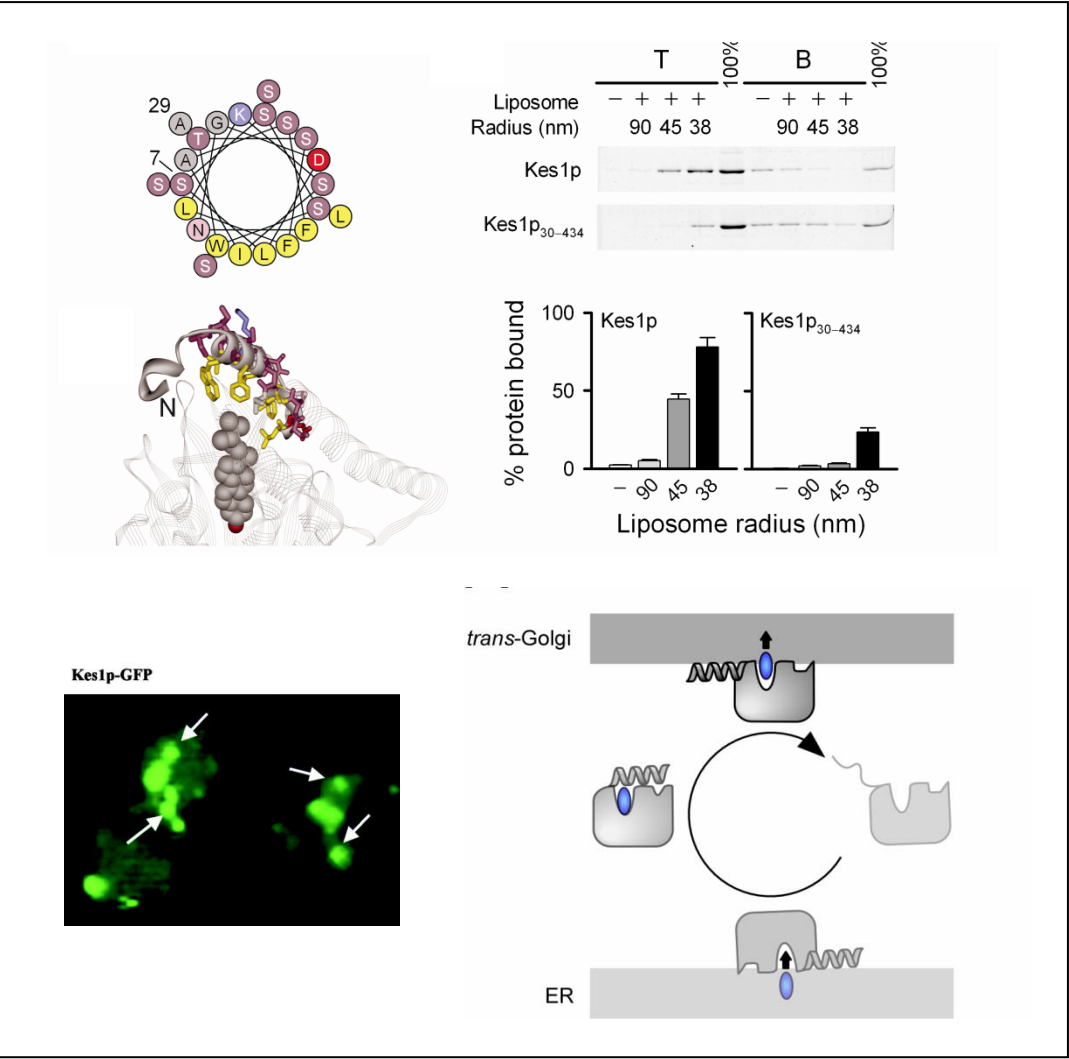


→ can we mimic what is done in cells ?

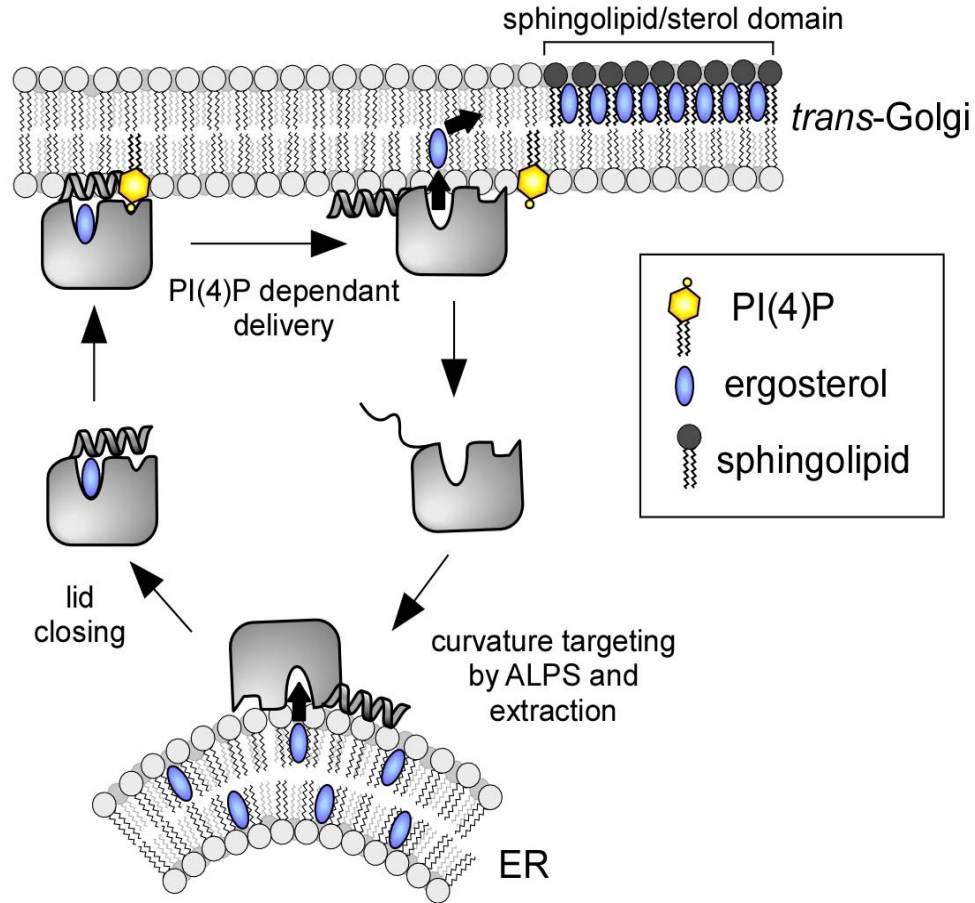
Vesicular transport / Non-vesicular transport



A sterol-transporter : Kes1p



Kes1p is likely designed to ensure **vectorial** transport



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