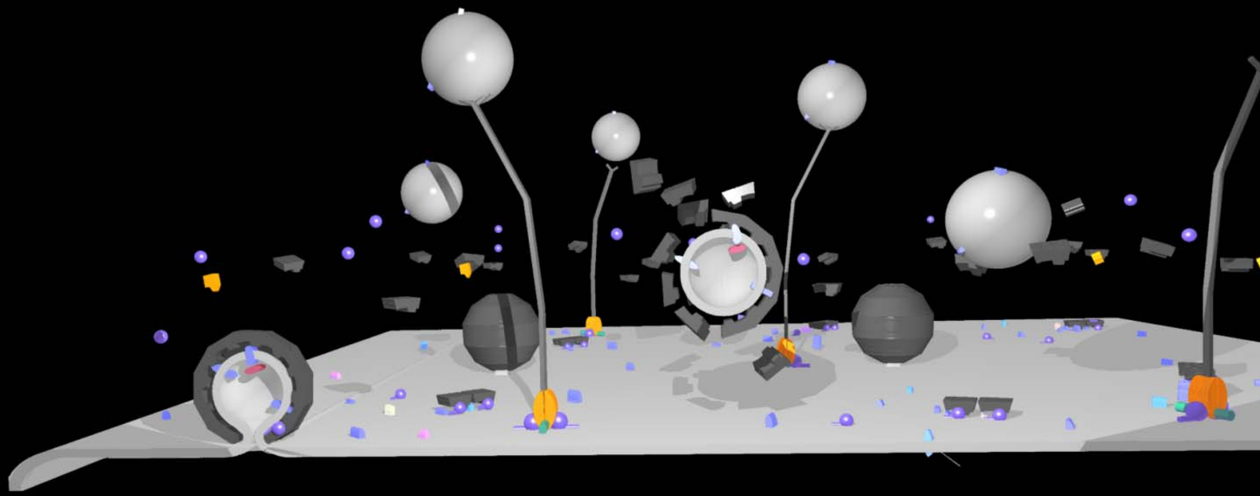
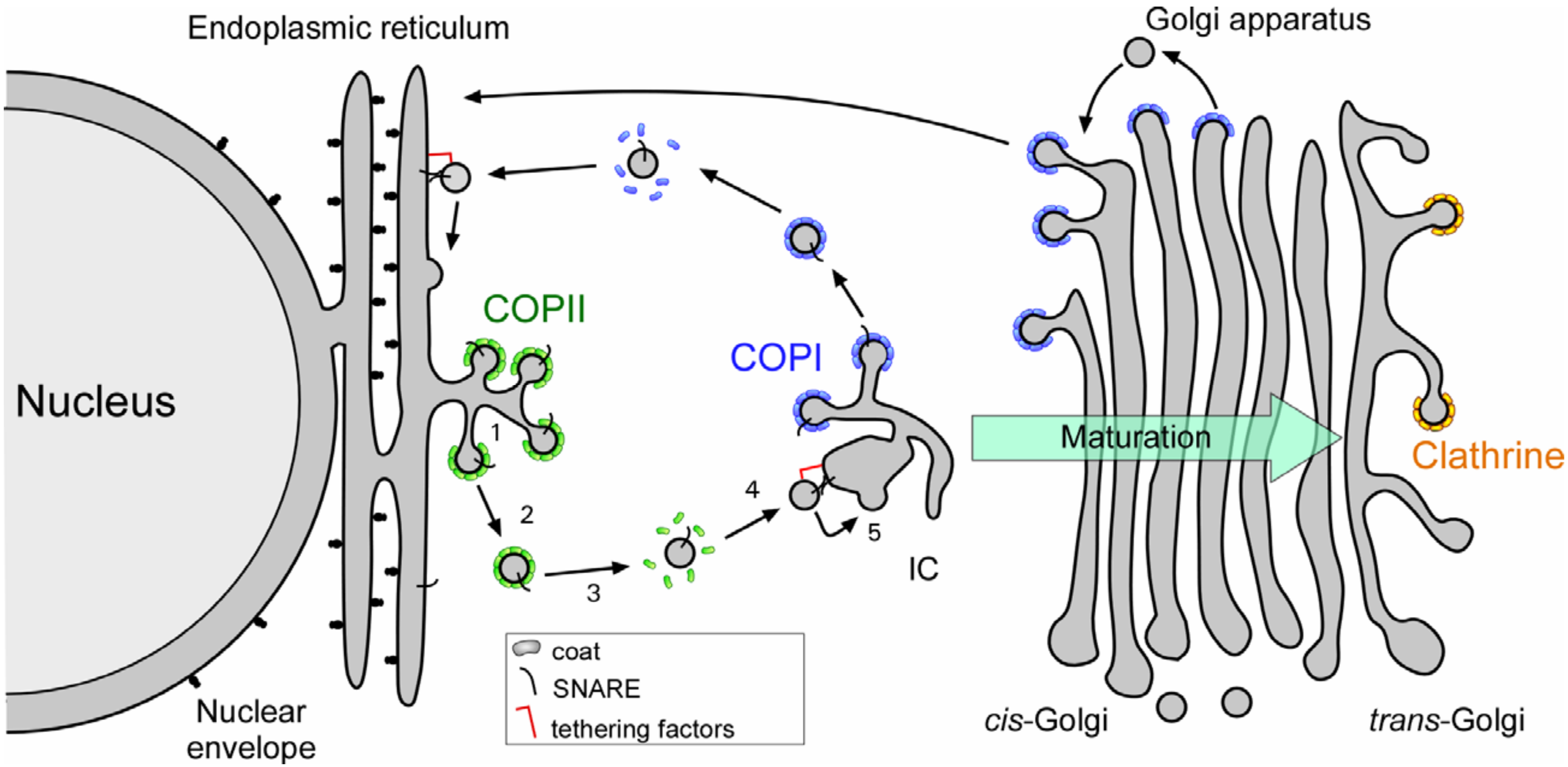
Targeting 2 distinct membranes: the case of tethering factors and lipid transporters

Guillaume Drin, CNRS

KITP Evo Cell, Feb 2, 2010

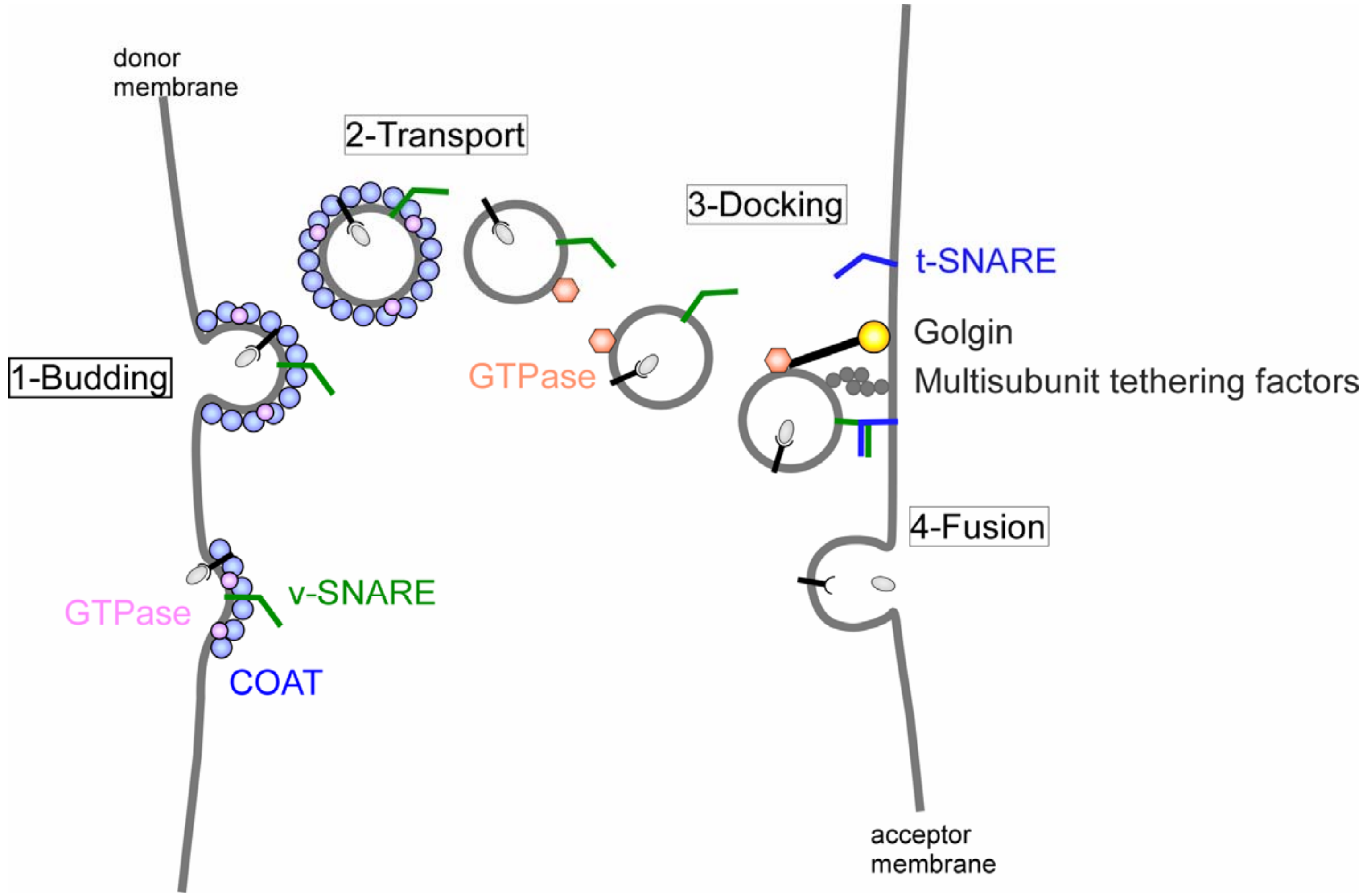


Vesicular transport



adapted from Bonifacino JS & Glick BS. Cell. 2004

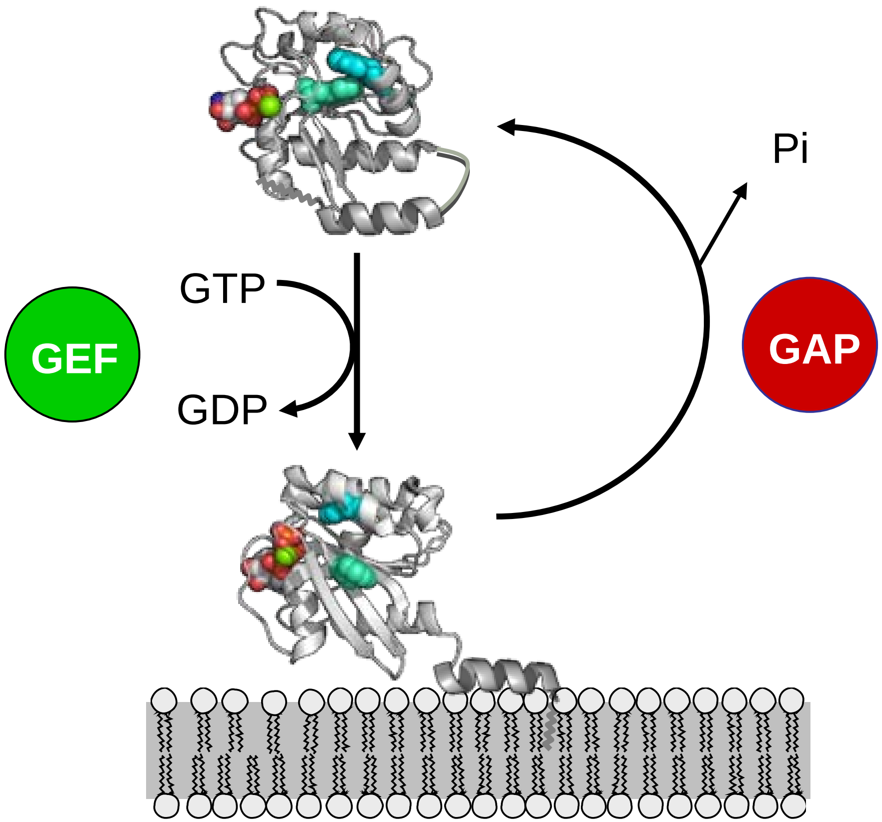
Vesicular transport

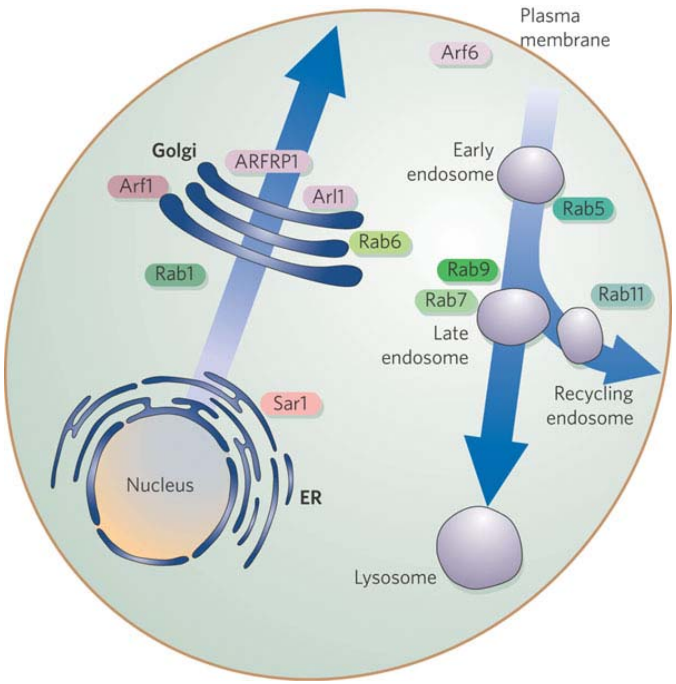


adapted from Behnia R, Munro S Nature. 2005 438:597-604

An important concept : membrane identity

1st landmark : small GTPase

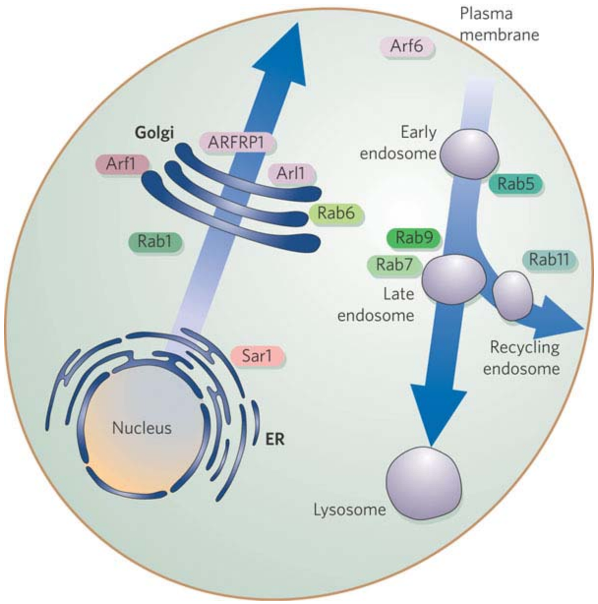


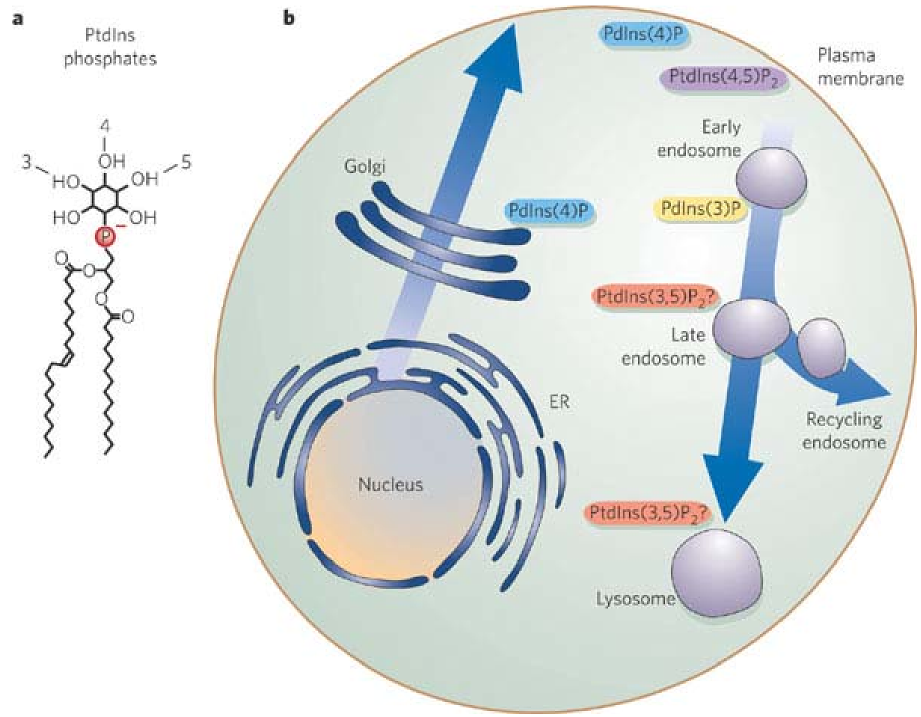


from Behnia R, Munro S Nature. 2005 438:597-604

An important concept : membrane identity

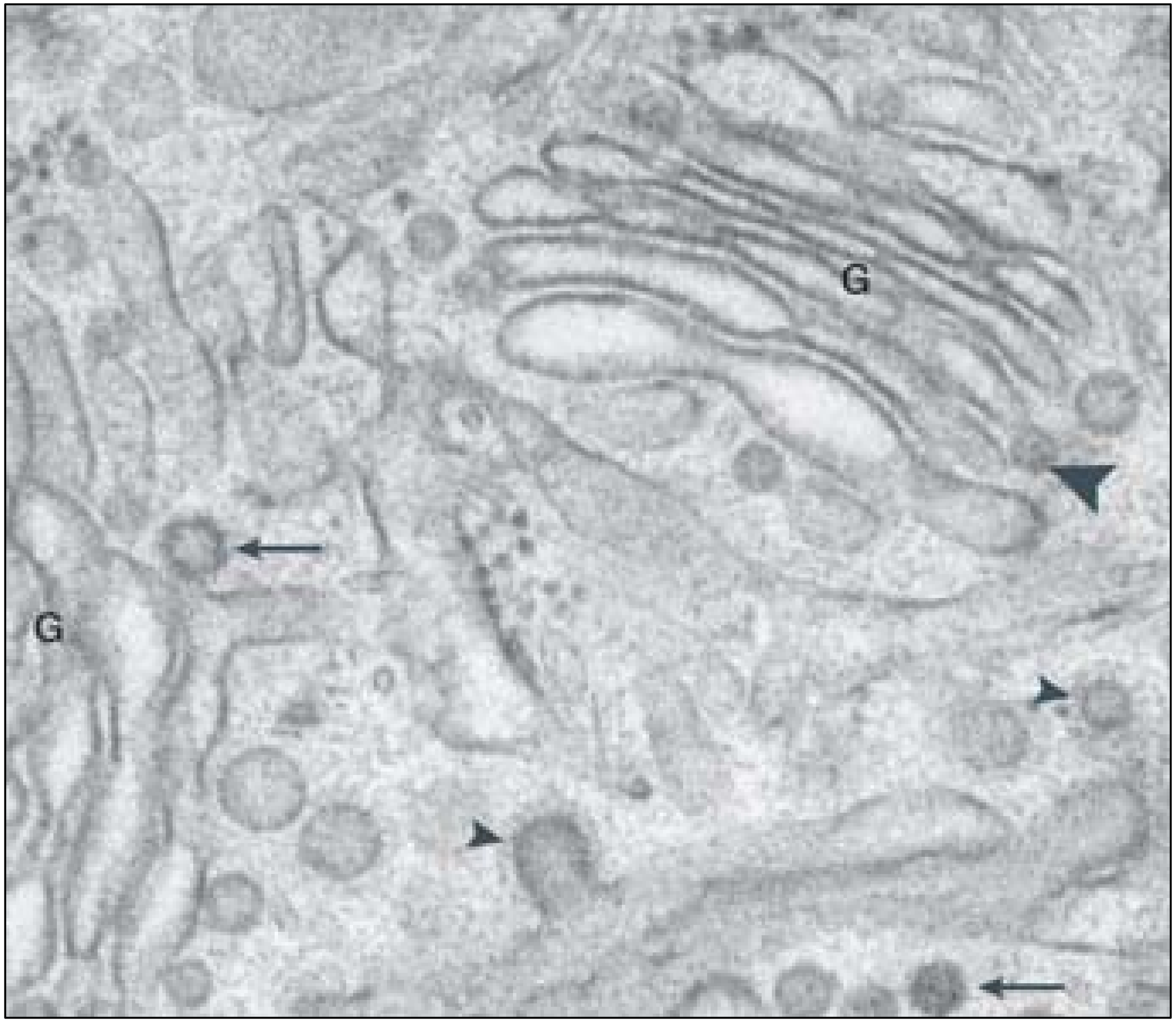
2nd landmark : membrane composition (phosphoinositide)





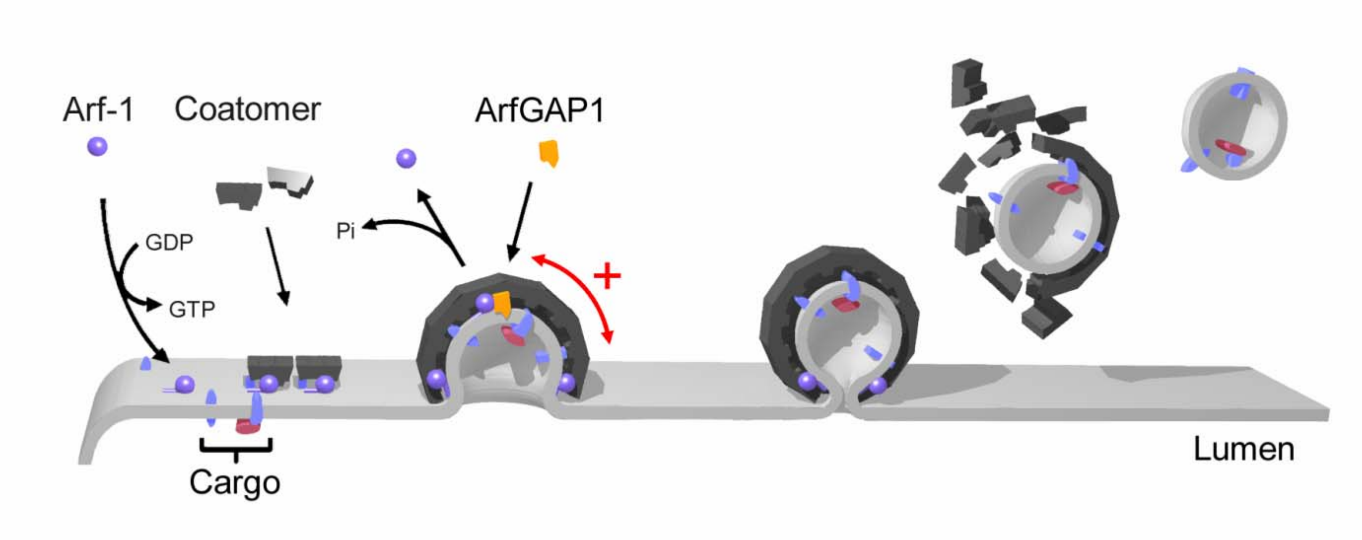
from Behnia R, Munro S Nature. 2005 438:597-604

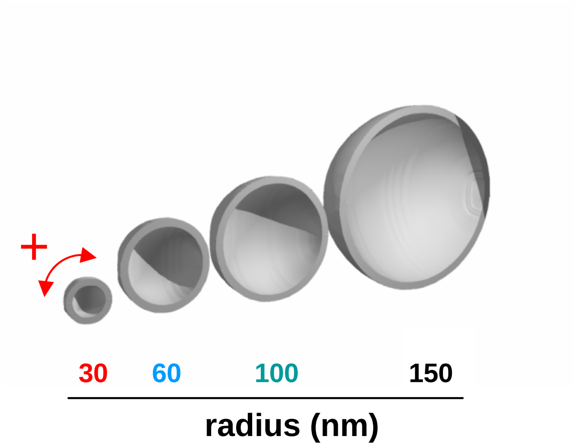
A third landmark : membrane shape (curvature)

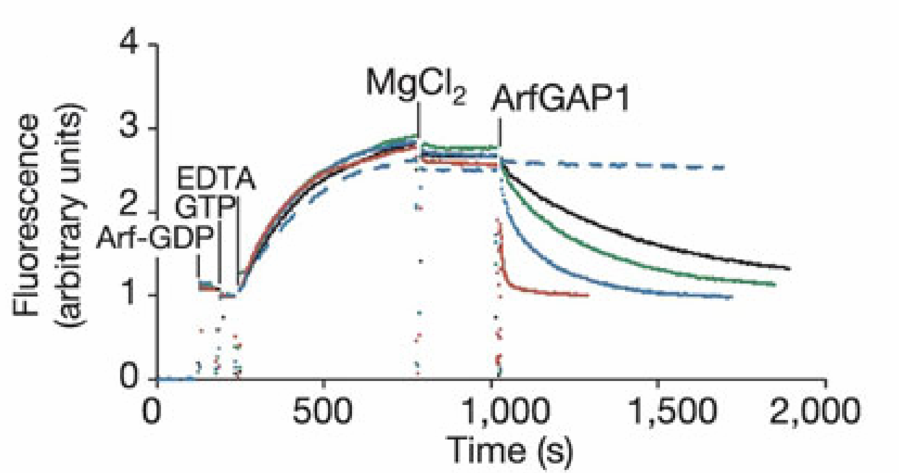


200 nm

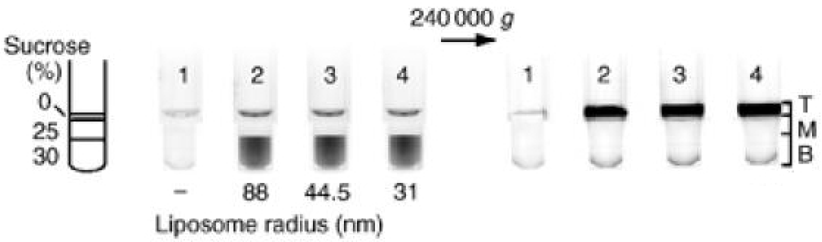
Curvature programs COPI depolymerisation by **ArfGAP1**

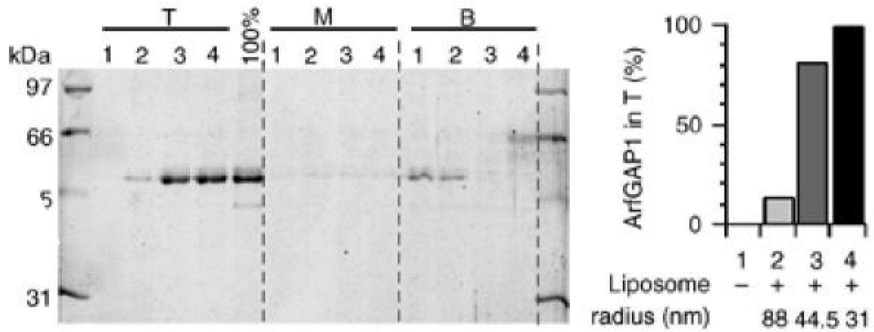


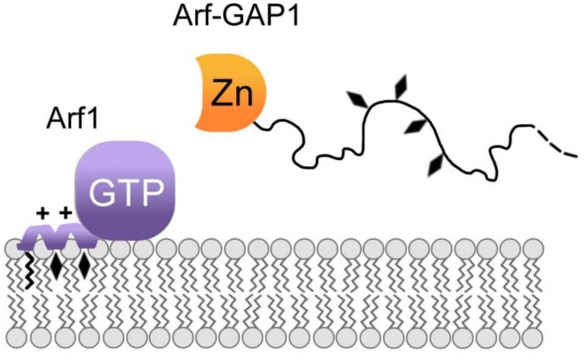


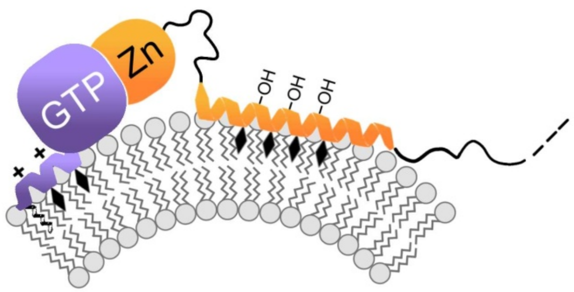


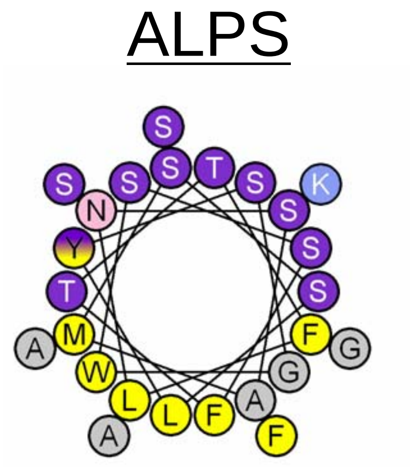
Detection of membrane curvature by ALPS motif





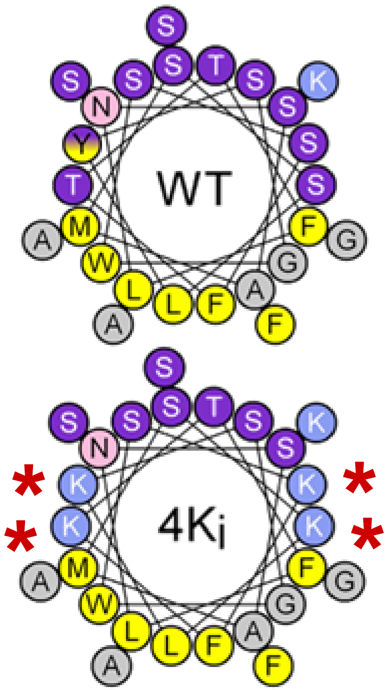


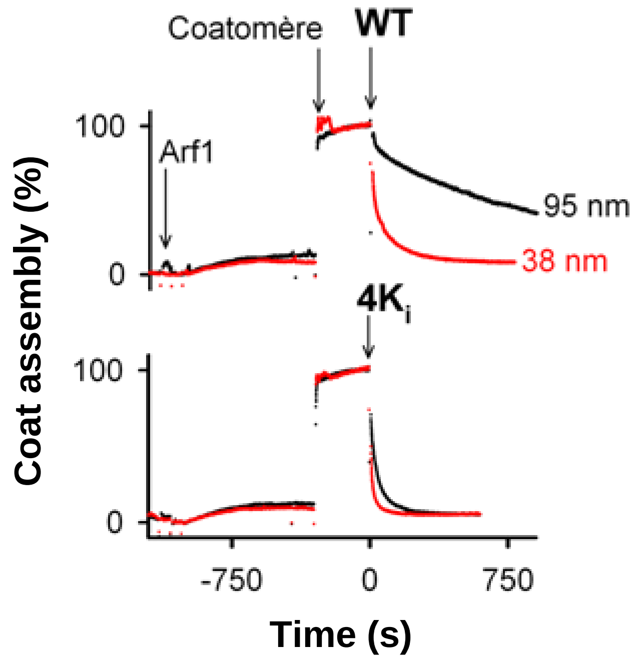


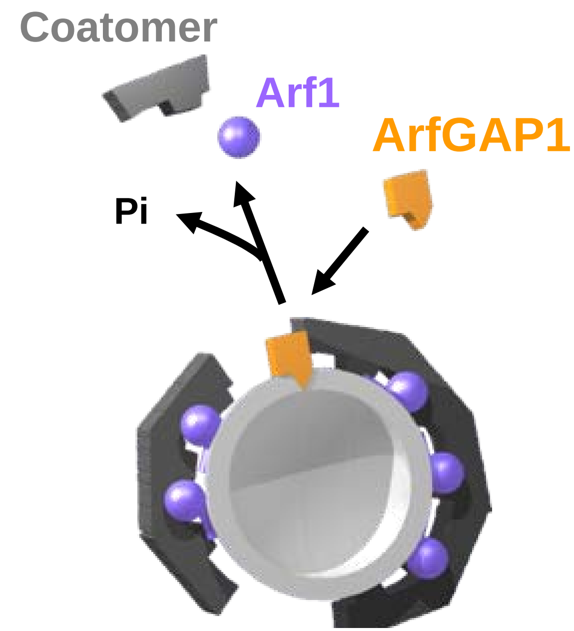


➡ The **unusual polar face** of the ALPS helix could explain its ability to recognize membrane curvature

Detection of membrane curvature by ALPS motif

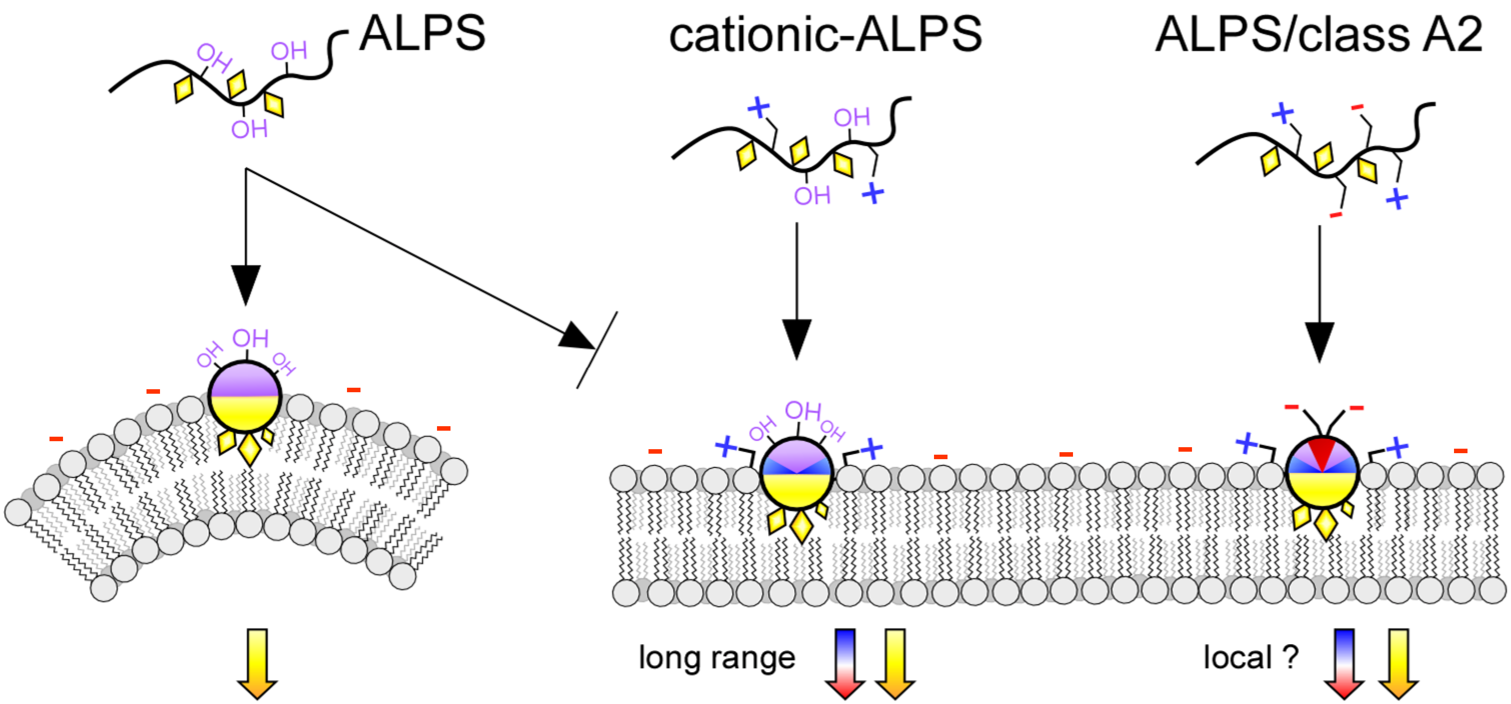






The **absence of positively charged residues** in the ALPS motif explain its ability to sense membrane curvature.

Detection of membrane curvature by ALPS motif

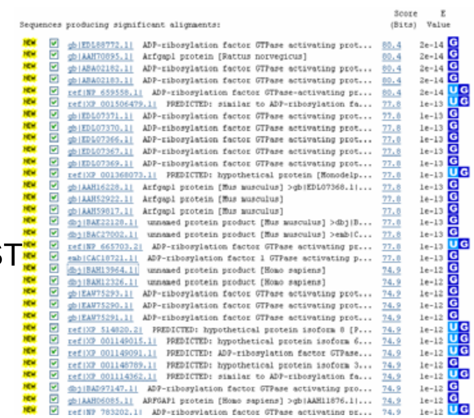


Is the motif ALPS present in other proteins ?

ALPS

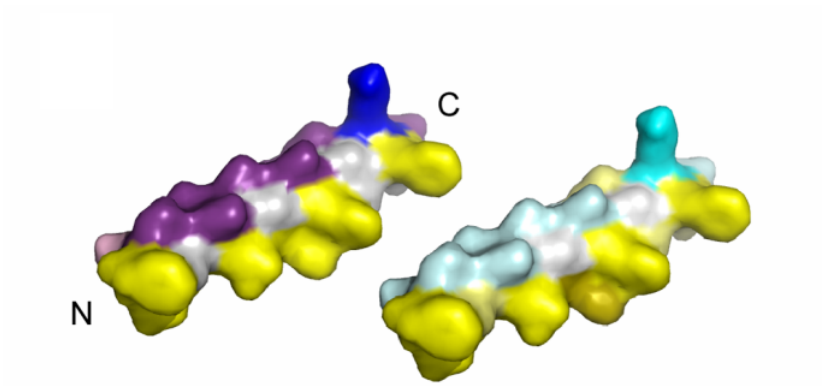
FLNNAMSSLYSGWSSFTT

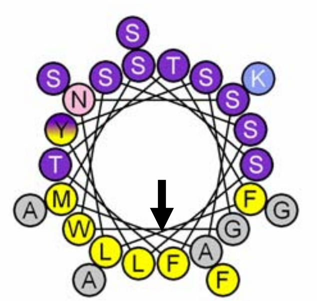
PSI-BLAST



……we found **nothing else than ArfGAP1**

Physico-chemical properties are important, not key residues

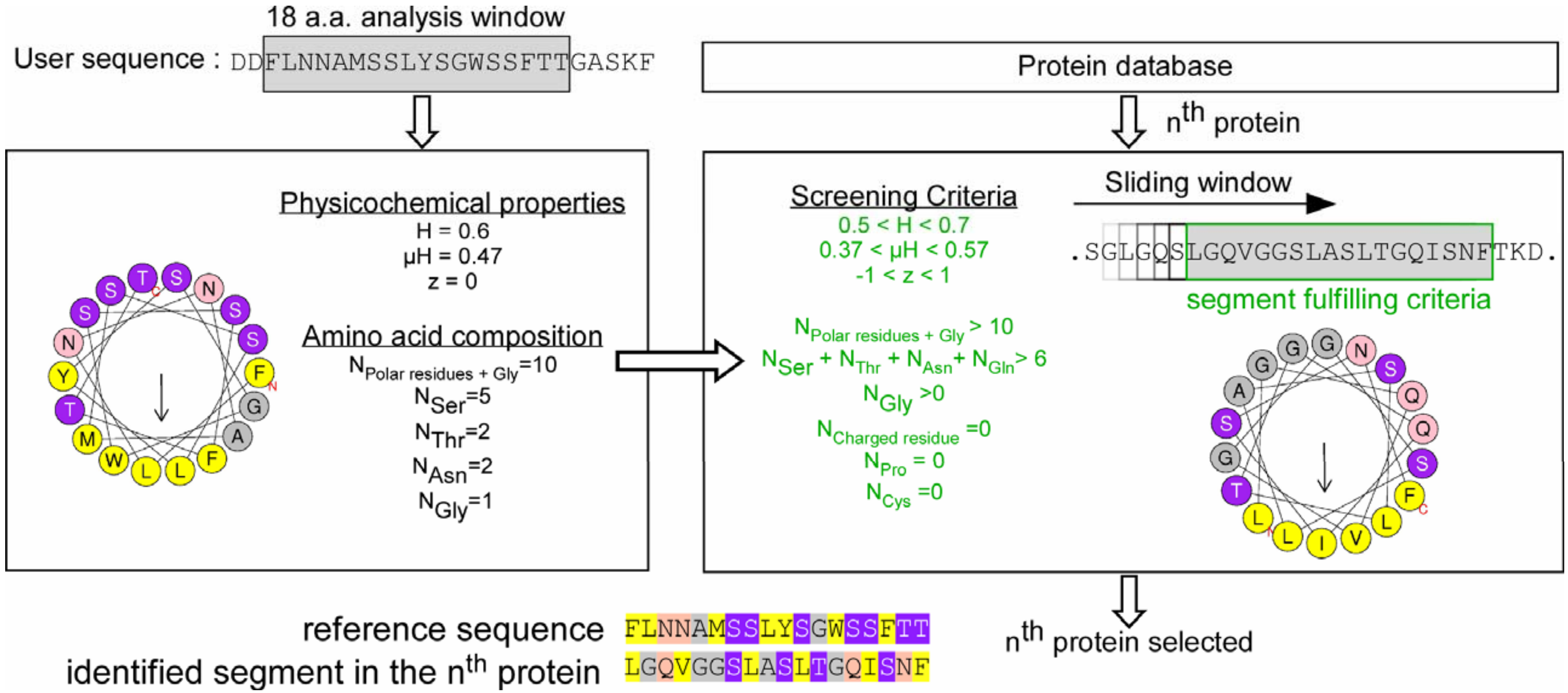




<u>Global parameters</u>

Hydrophobicity
Charge
Hydrophobic moment

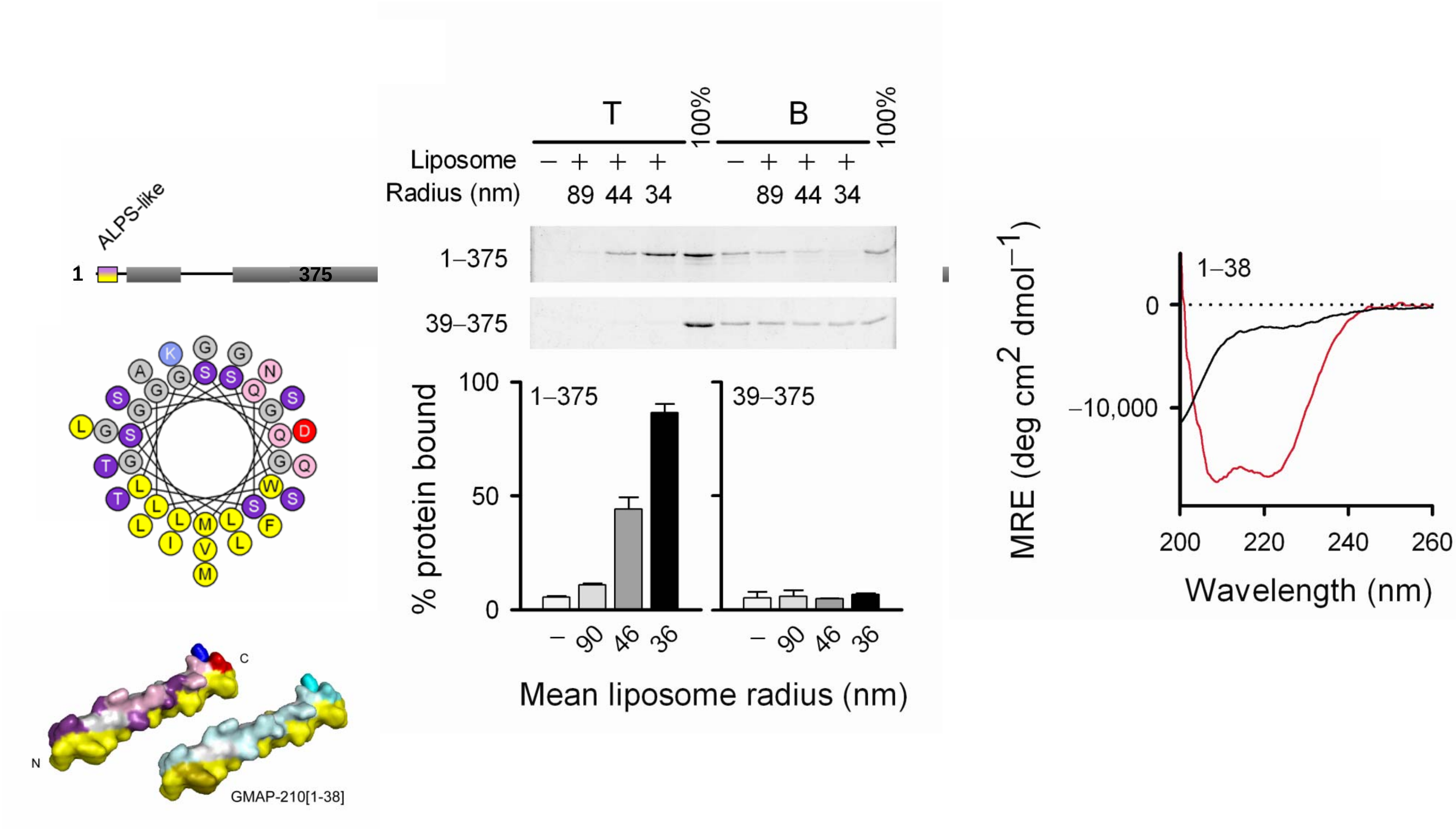
ALPS motif / Screening by bioinformatics



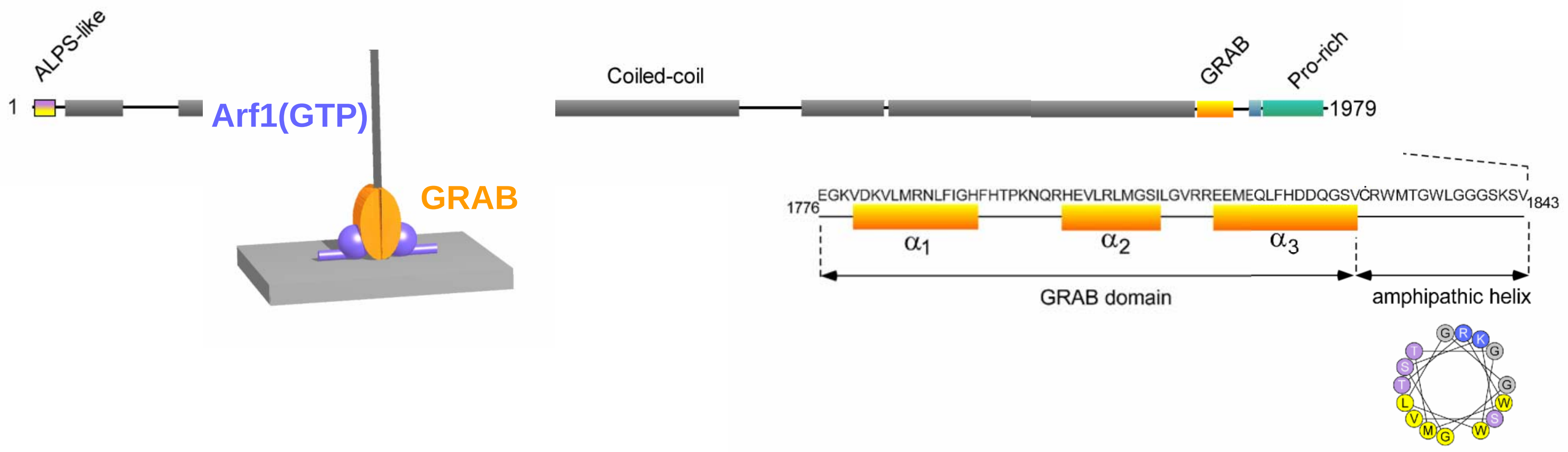
SwissProt ➡ **196 human sequences**
191 yest sequences ➡

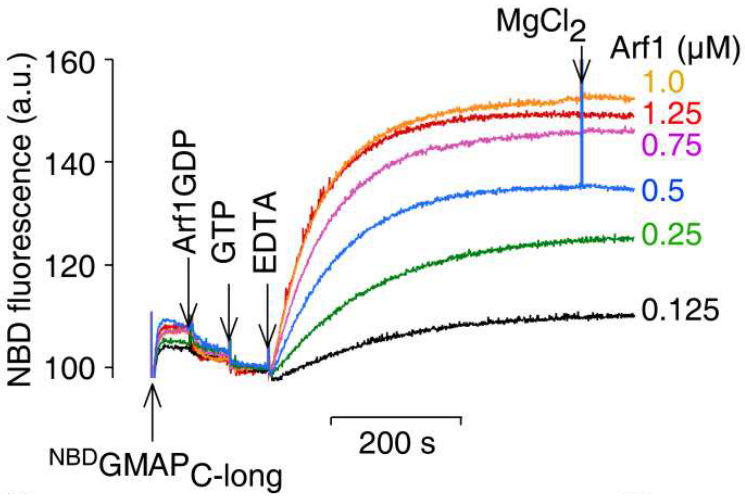
GMAP-210
Kes1p
Nup133

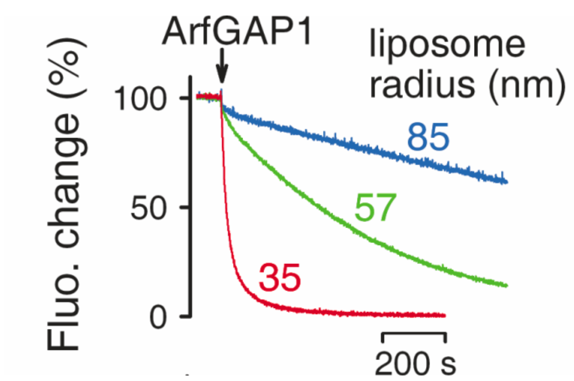
The N-terminus of GMAP-210 is an ALPS motif



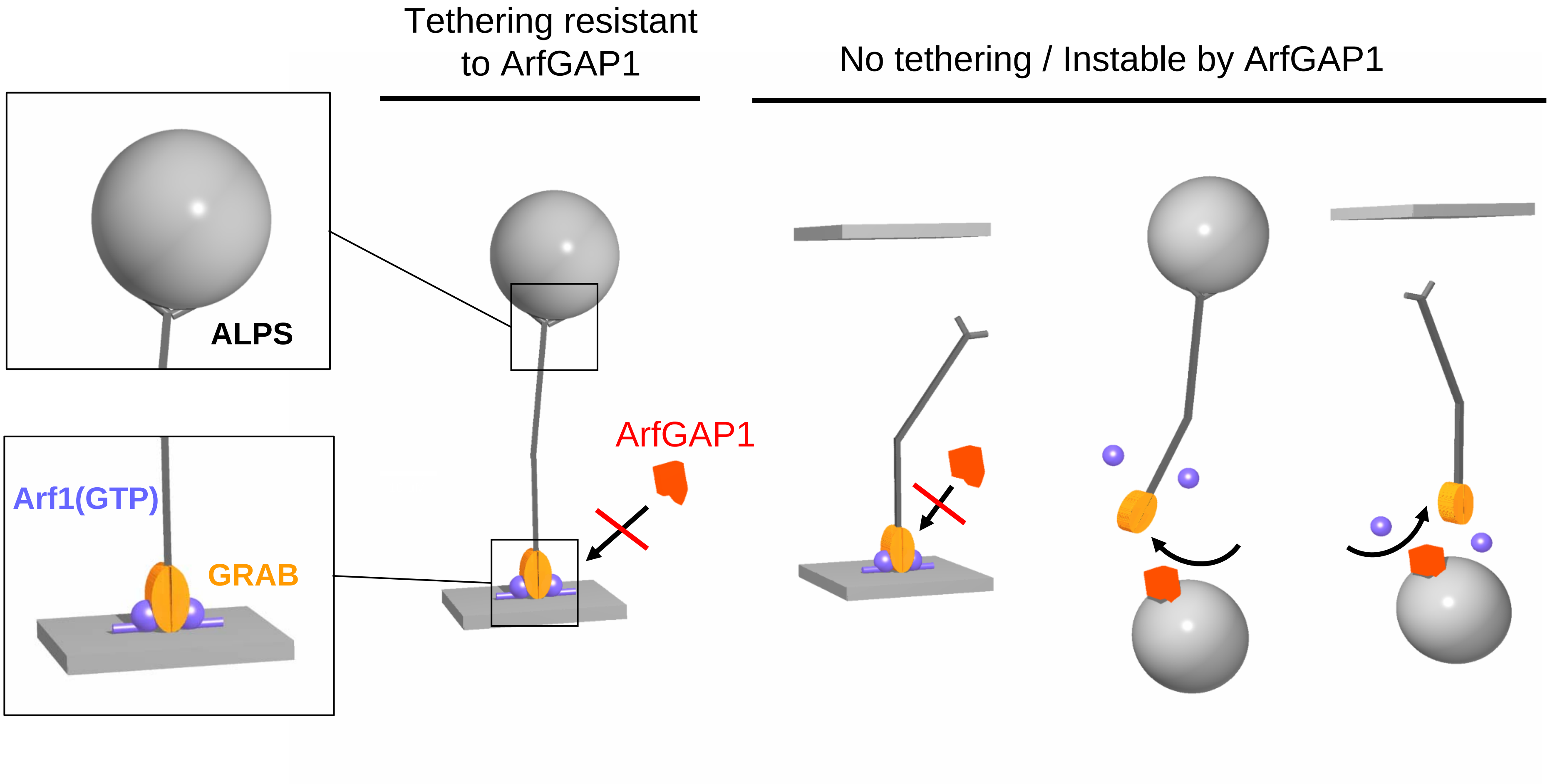
GMAP-210 binds to Arf1 with a C-terminal GRAB domain



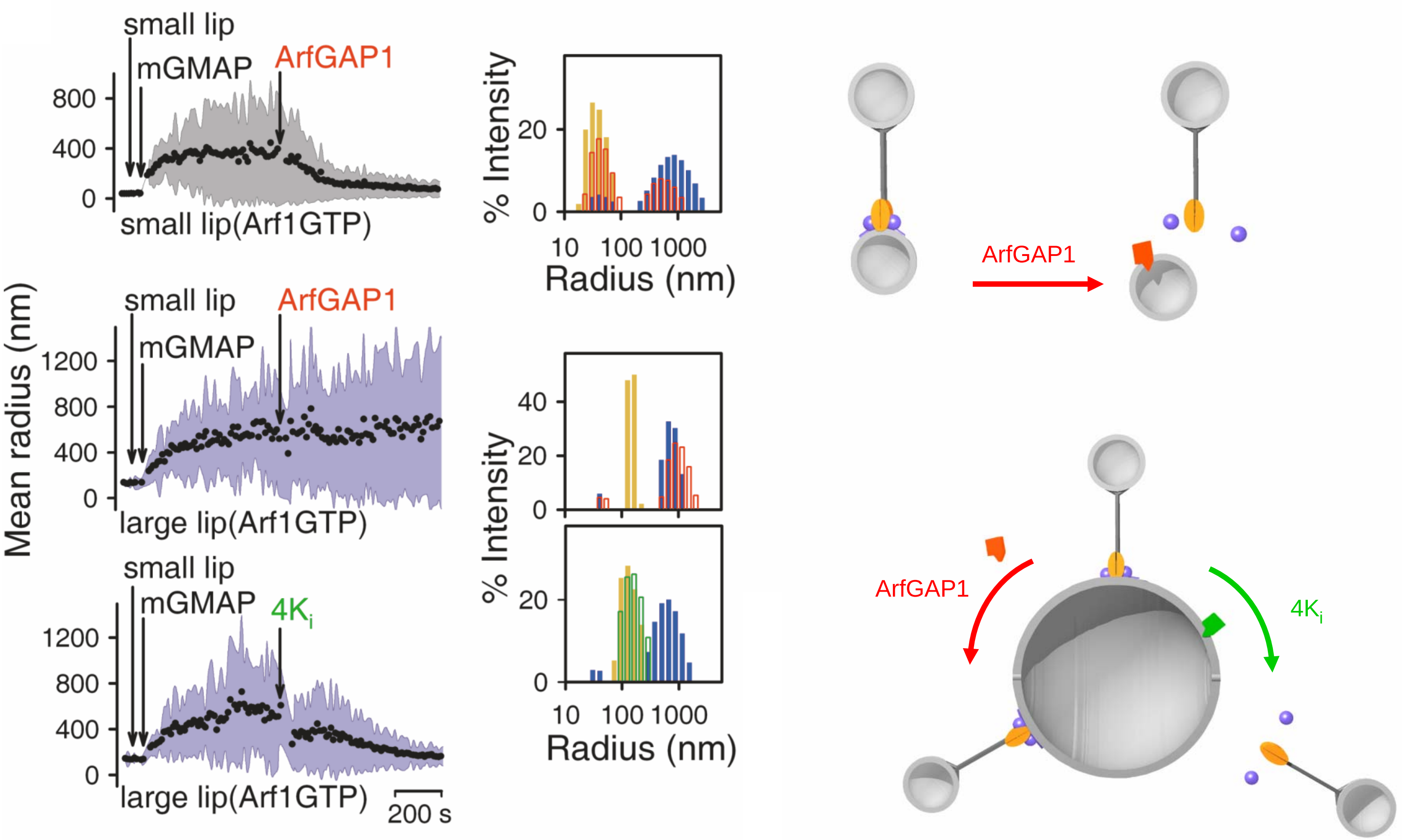




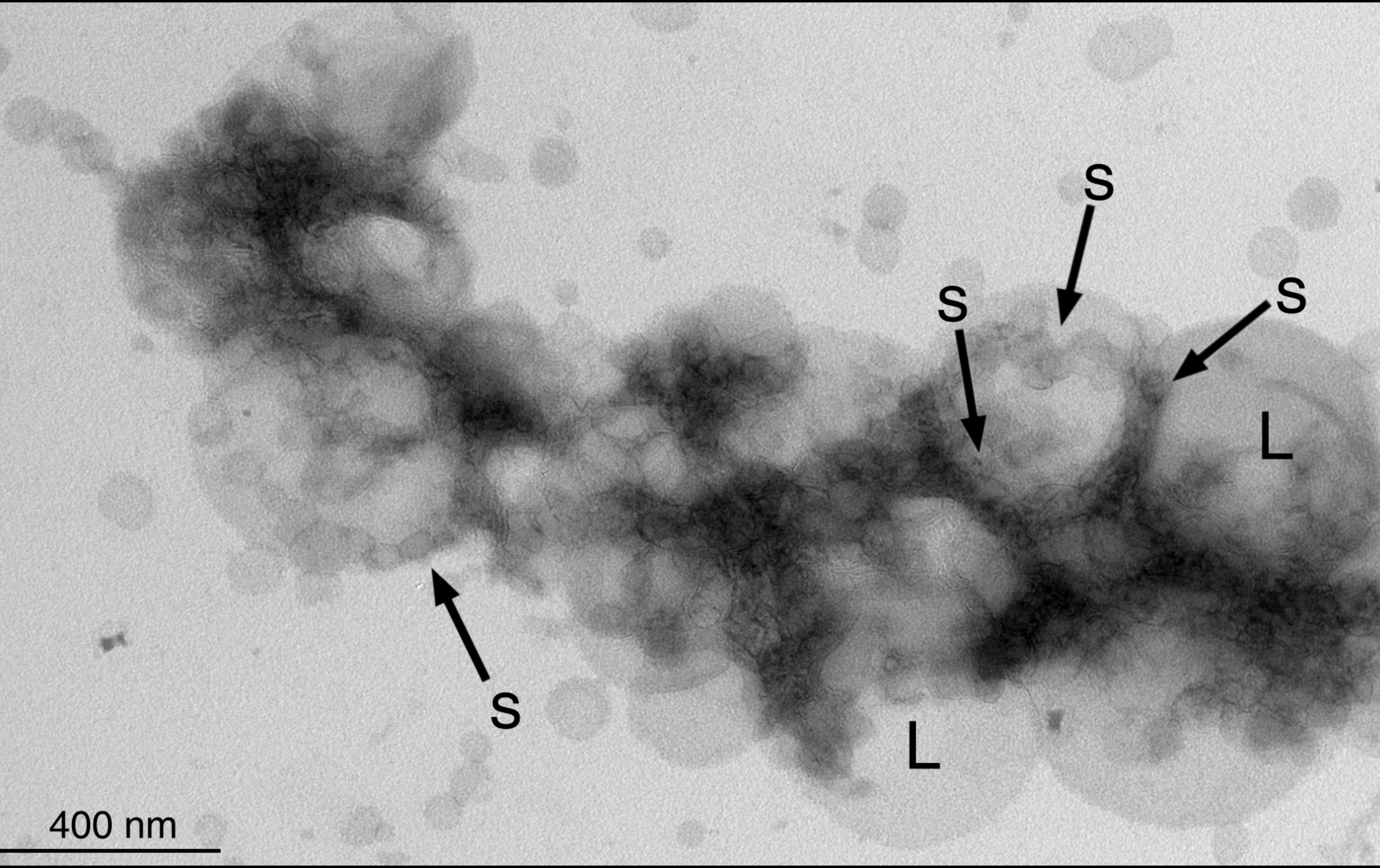
Model for asymetric tethering



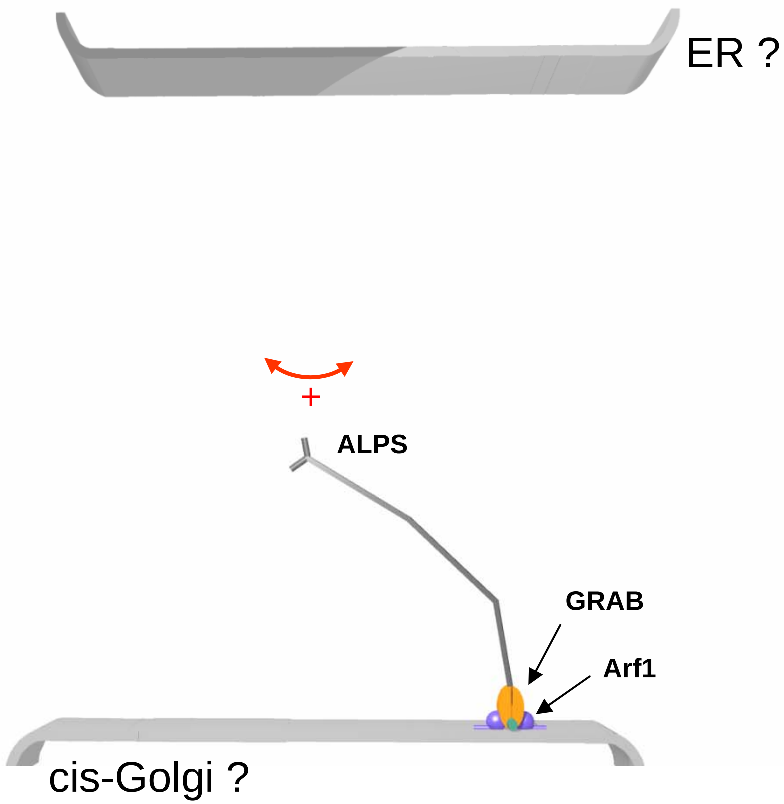
Asymetric tethering between a curved membrane and a flat one displaying Arf1(GTP)

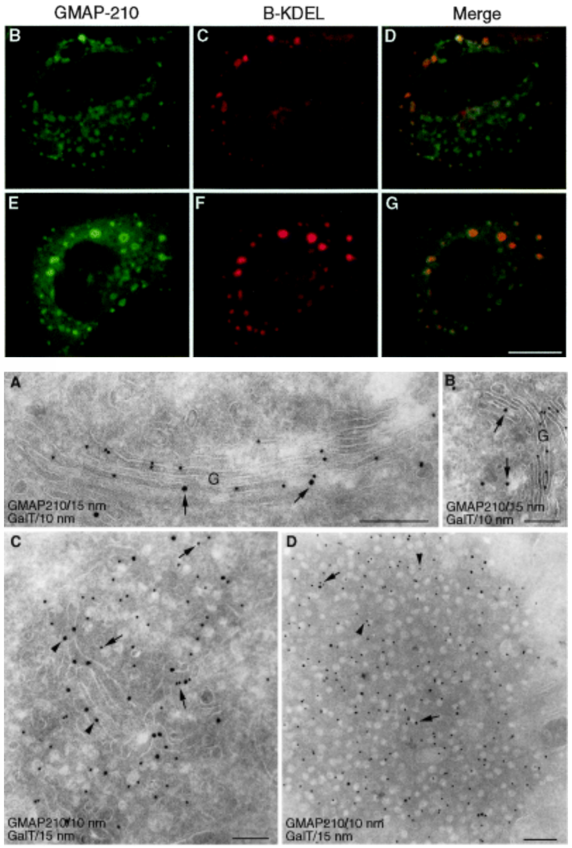


Asymetric tethering between a curved membrane and a flat one displaying Arf1(GTP)



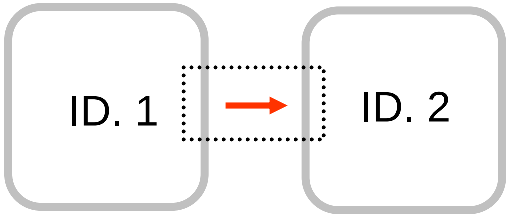
Model on the GMAP-210 function

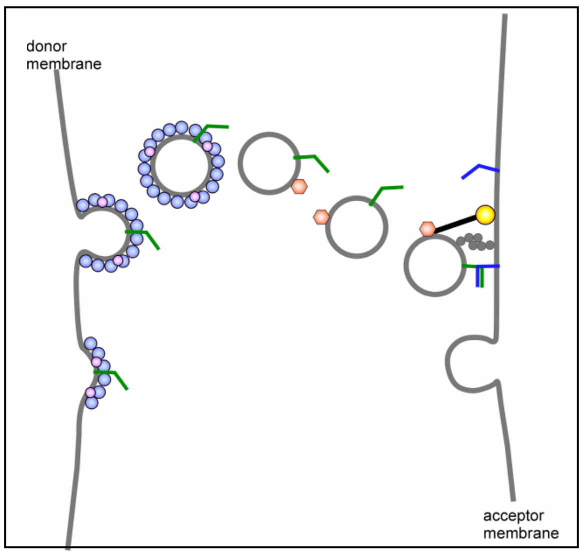


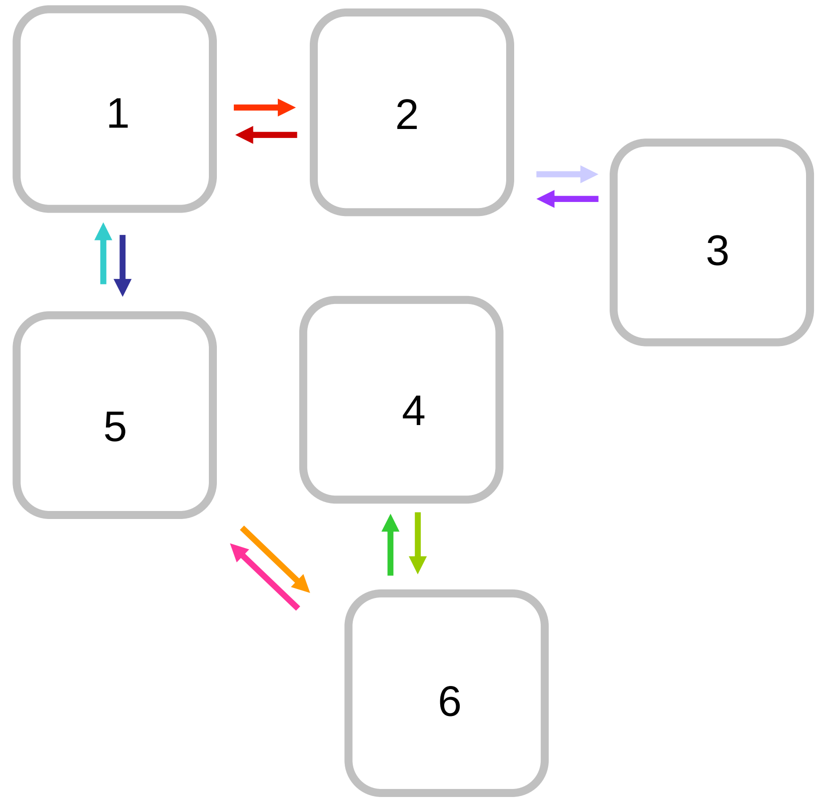


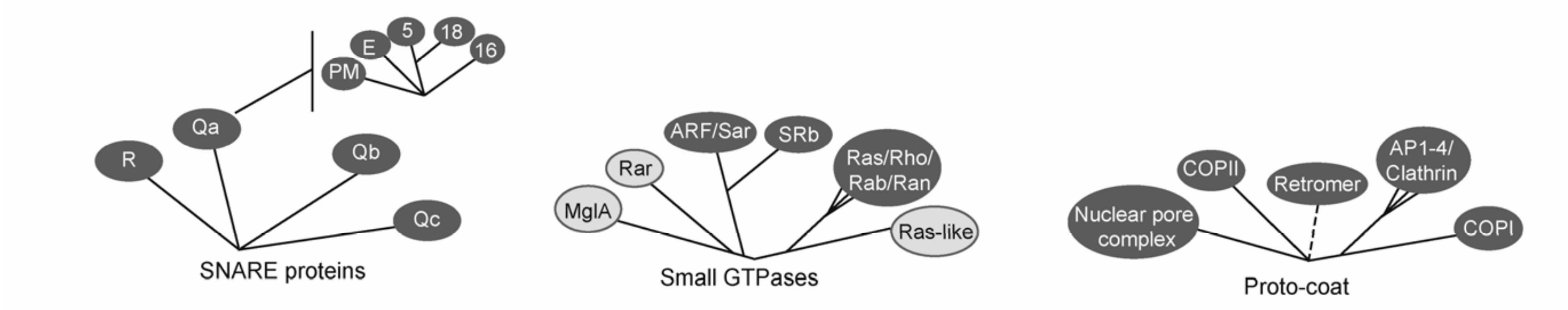
Pernet-Gallay K. *et coll*, 2002

Tethering factors / Evolution of endomembrane system



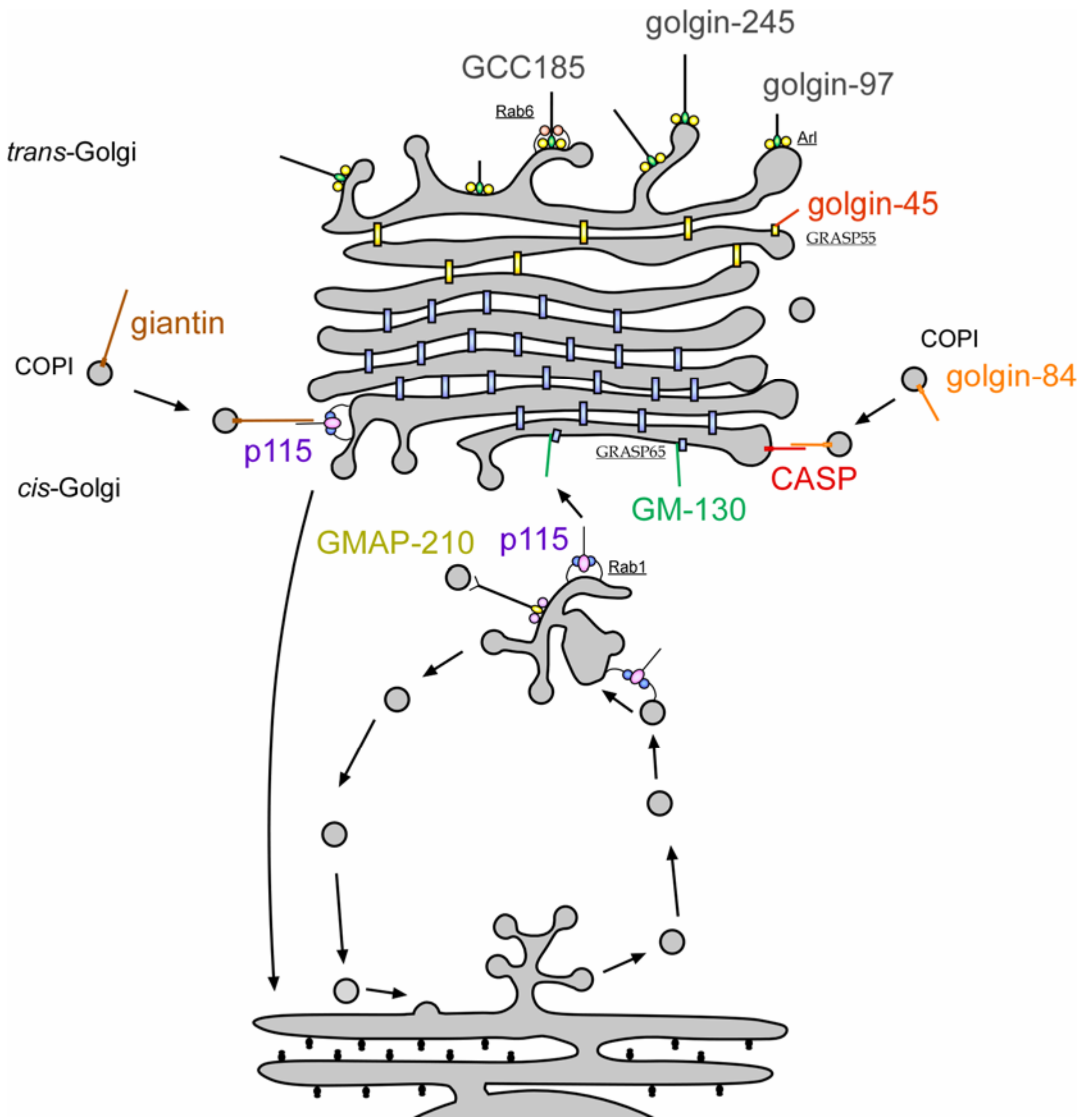






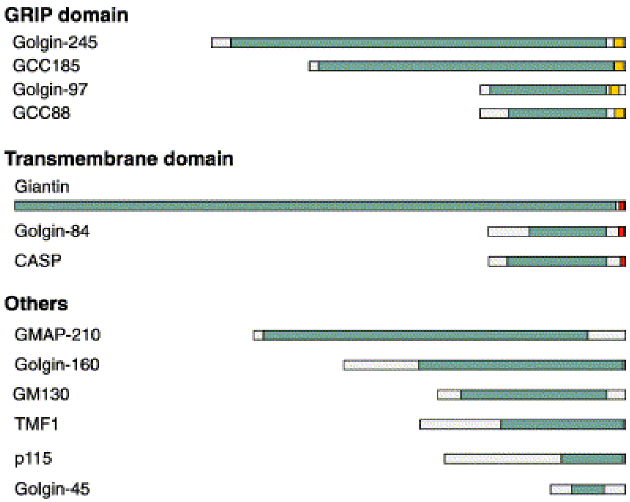
from Dacks JB, Peden AA, Field MC. Int J Biochem Cell Biol. 2009 41(2):330-40

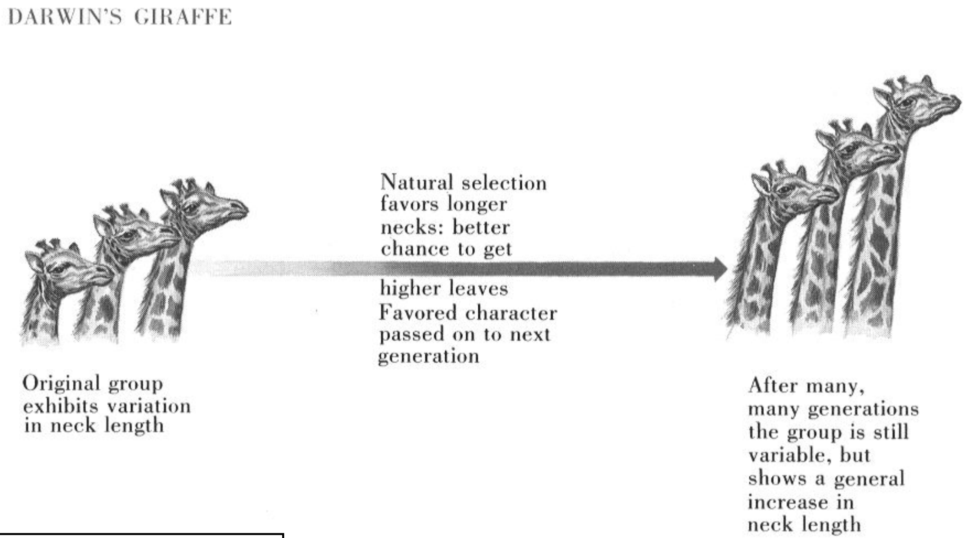
Tethering factors / Evolution of endomembrane system

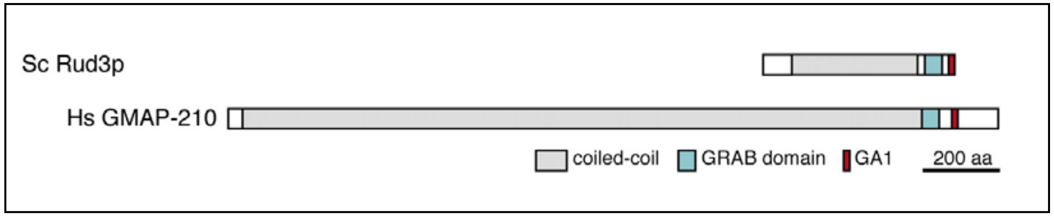


Tethering factors / Evolution of endomembrane system

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

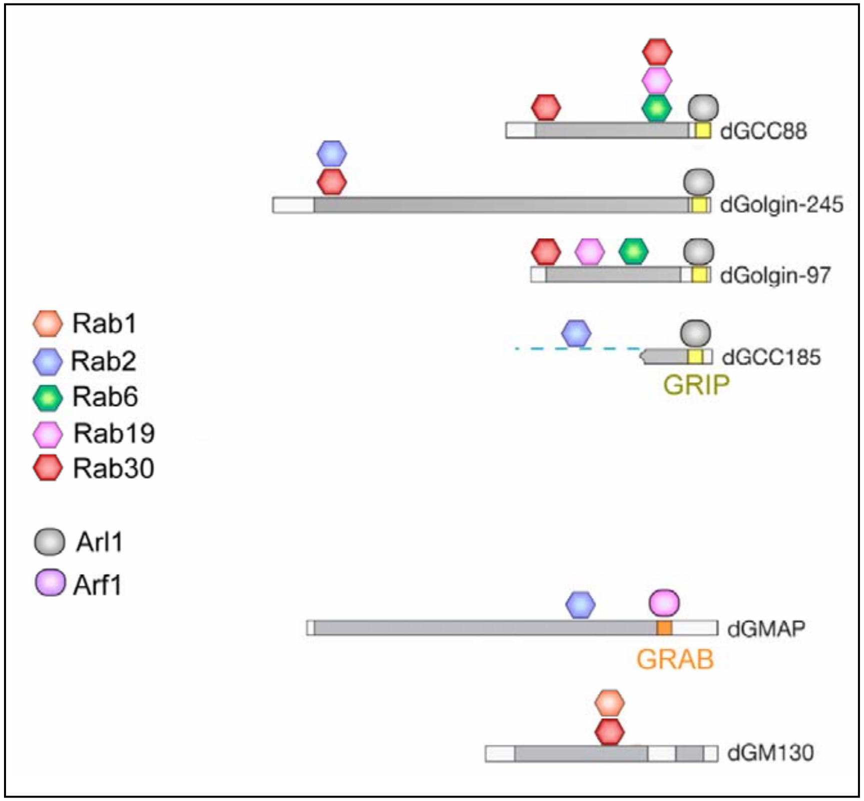


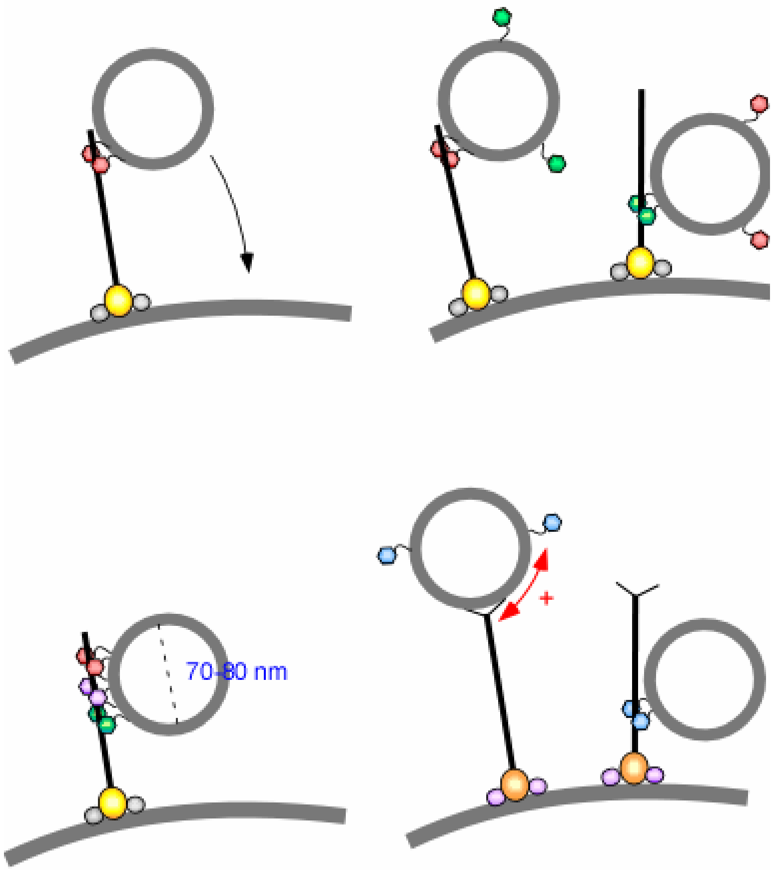




Evolving ideas about tethering factors

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

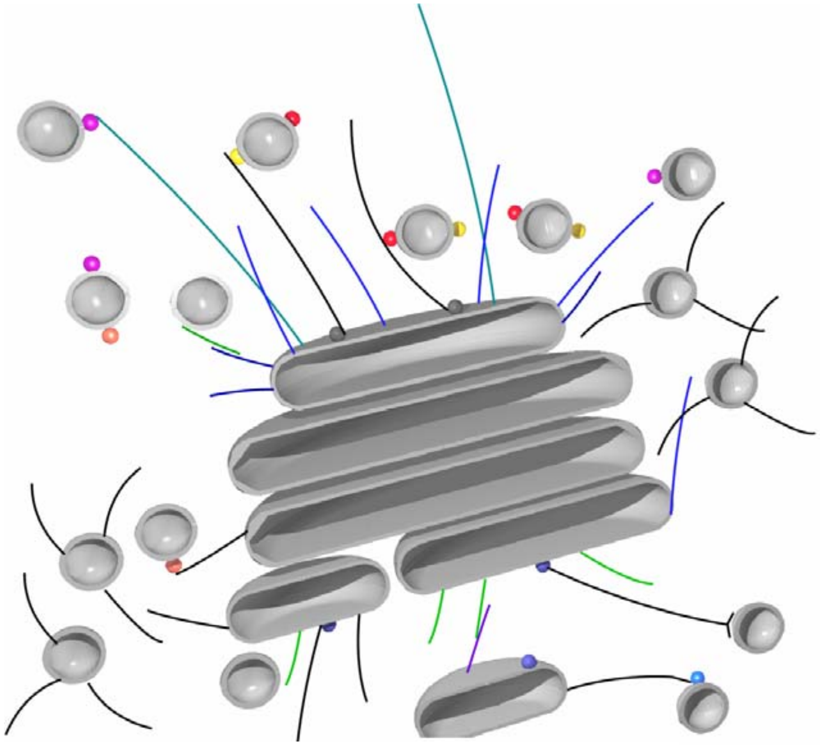




Tentacle model

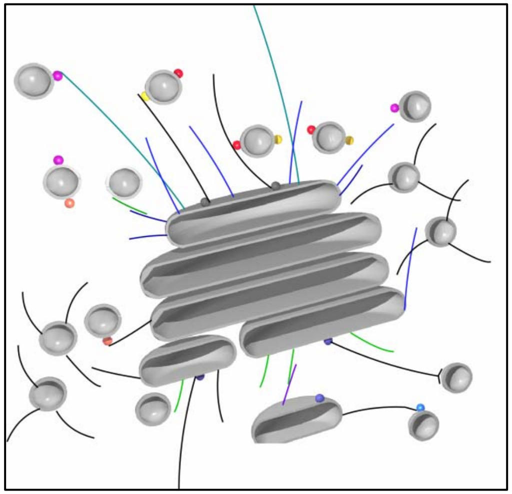
<u>Length + diverse binding-sites</u> : to create tethering factors able to deal with the increasing complexity of endomembrane system

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

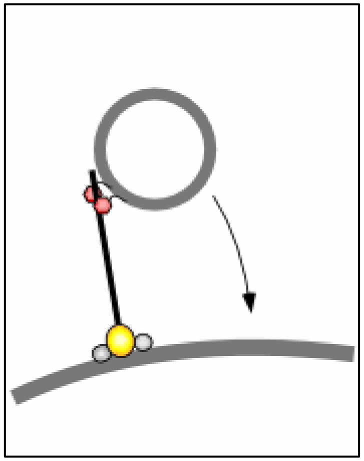


Reality

Lifetime, dynamic of tethering event

Strength of interaction

Number of tethers involved in a tethering event

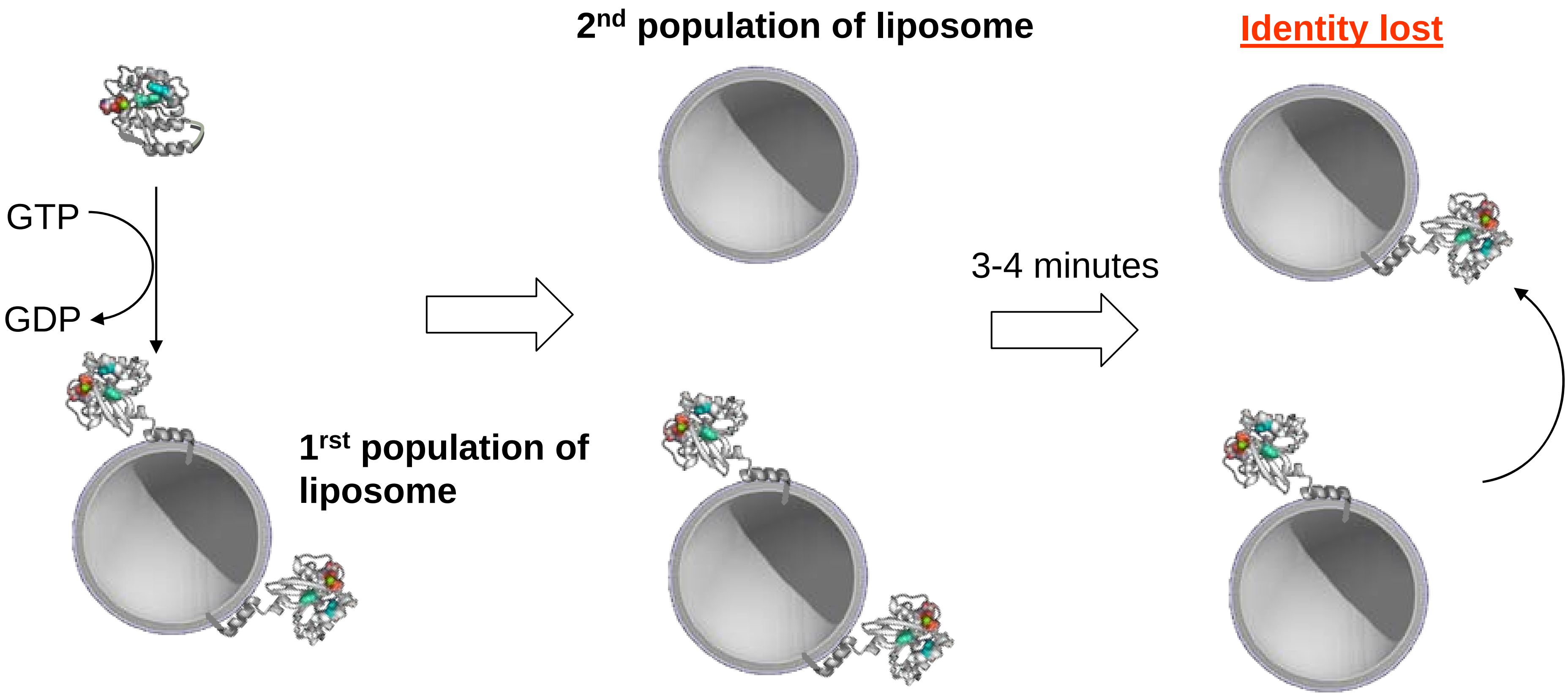


To monitor tethering *in vitro* with better accuracy **, we need to :**

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

Asymetric tethering / Controlling the membrane identity

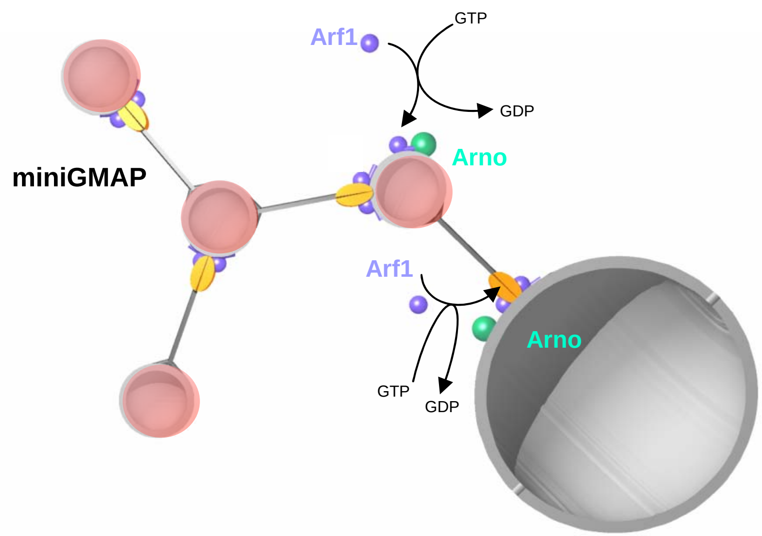
GTPase are **perimembranar** protein : binding is **dynamic**

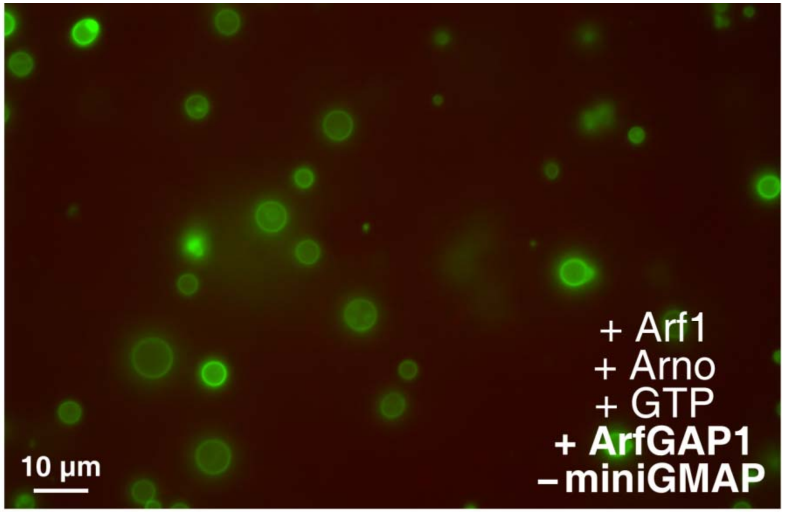


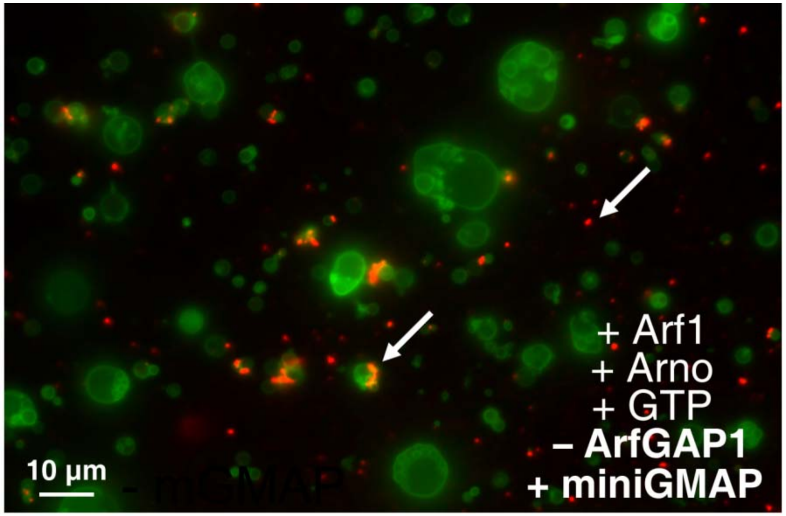
Control identity during tethering

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

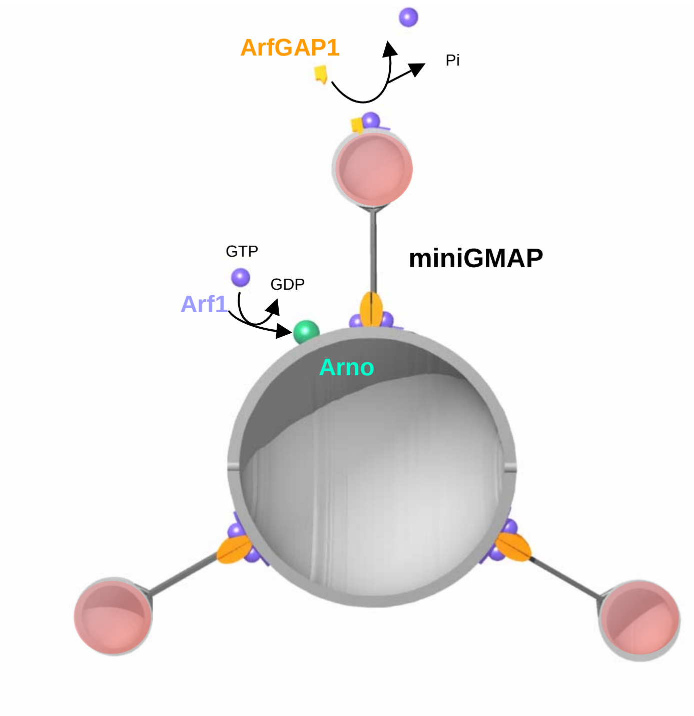
Asymetric tethering / Controlling the membrane identity

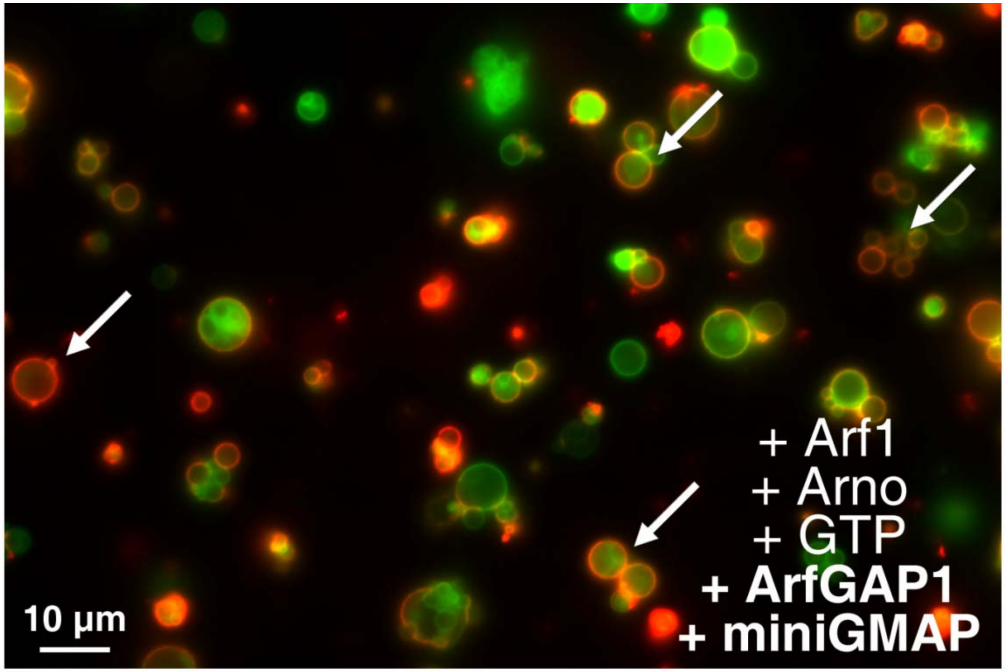


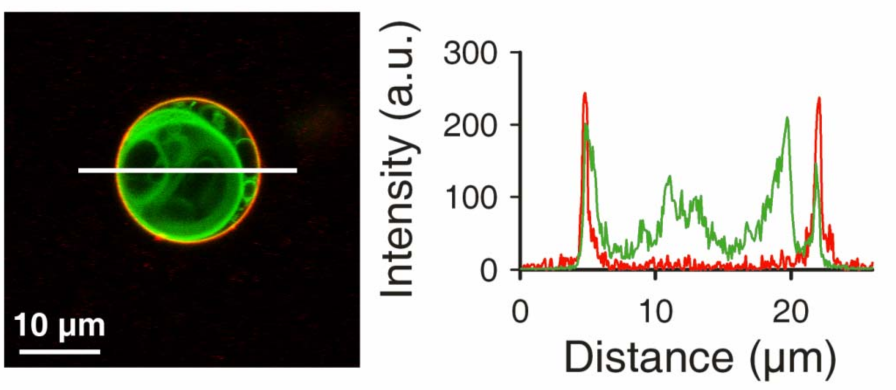




Asymetric tethering - Self-organisation by Arno, ArfGAP1 and membrane curvature

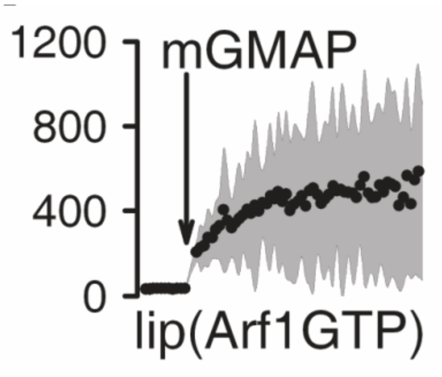






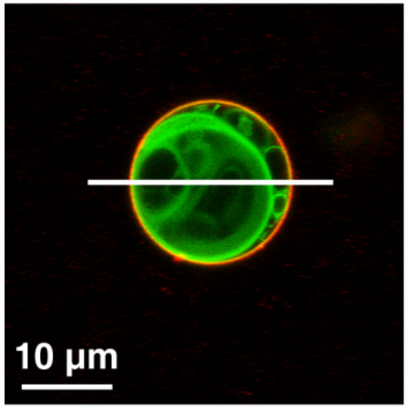
New technical approach : FCCS

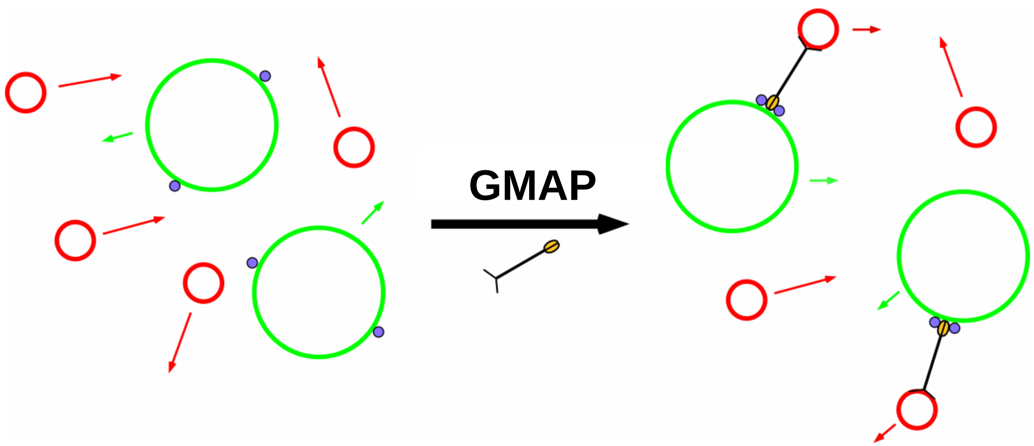
DLS



+

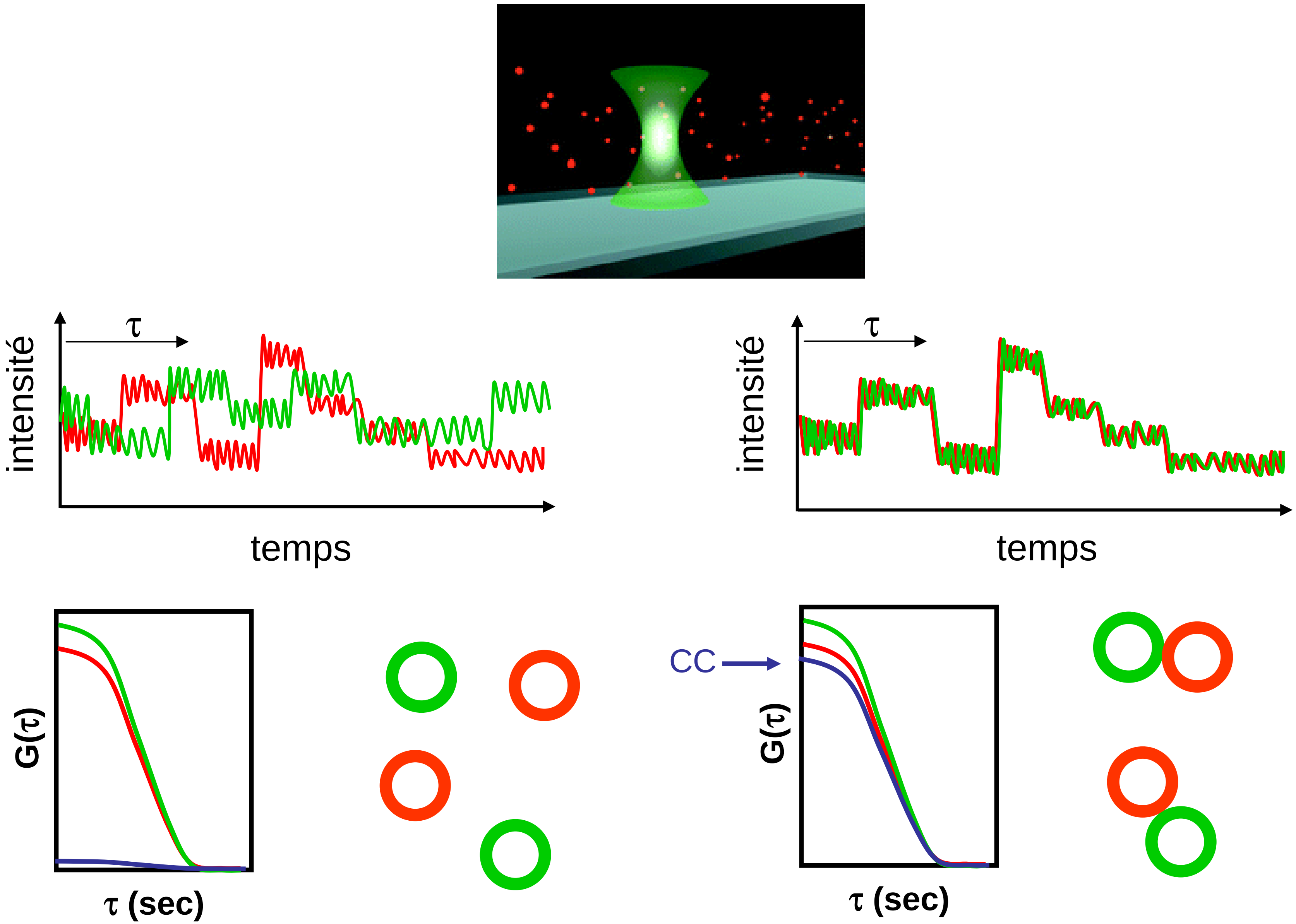
LM



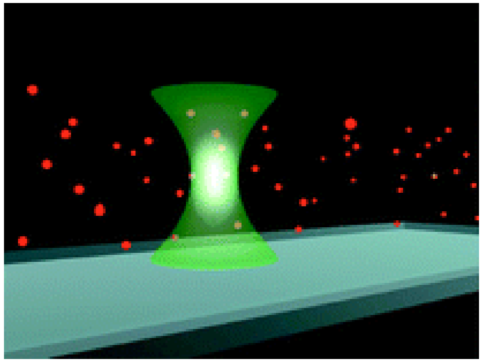


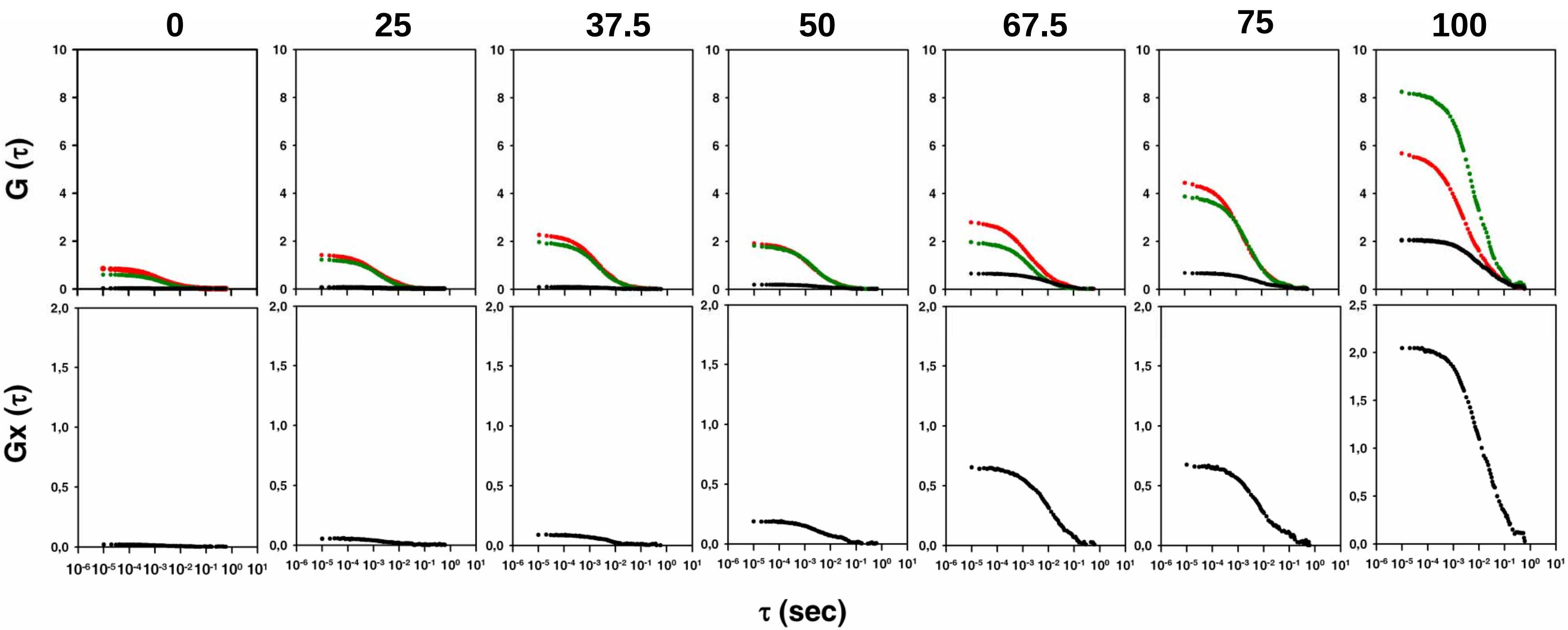
Quantify the connection of two distinct membranes

New technical approach : FCCS

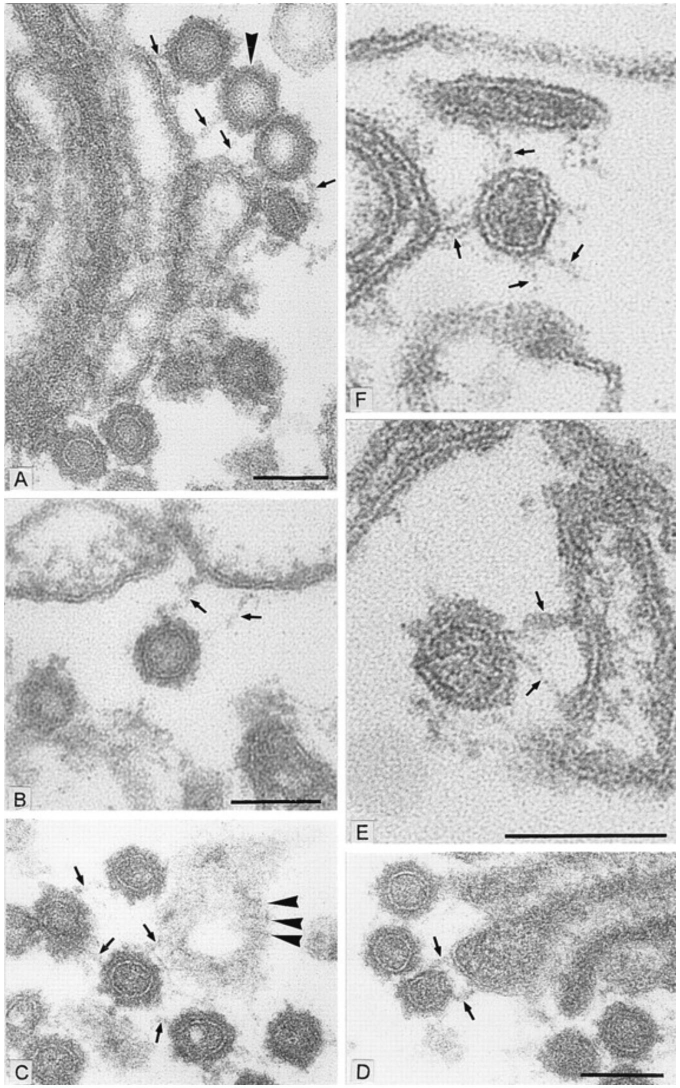


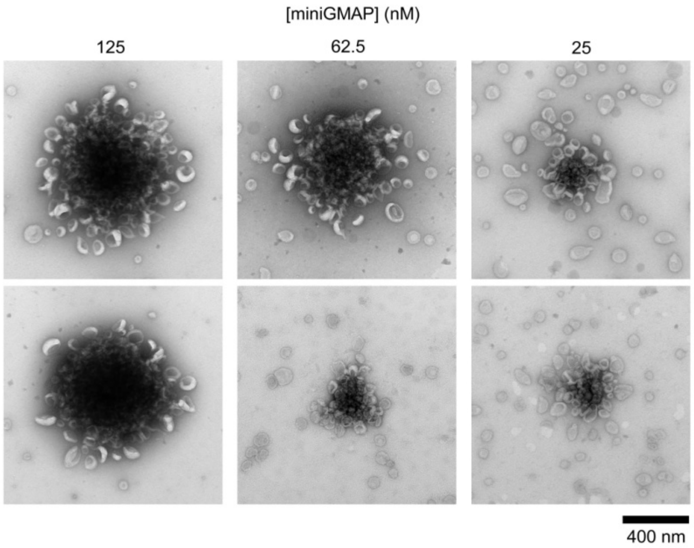
New technical approach : FCCS





Controling aggregation





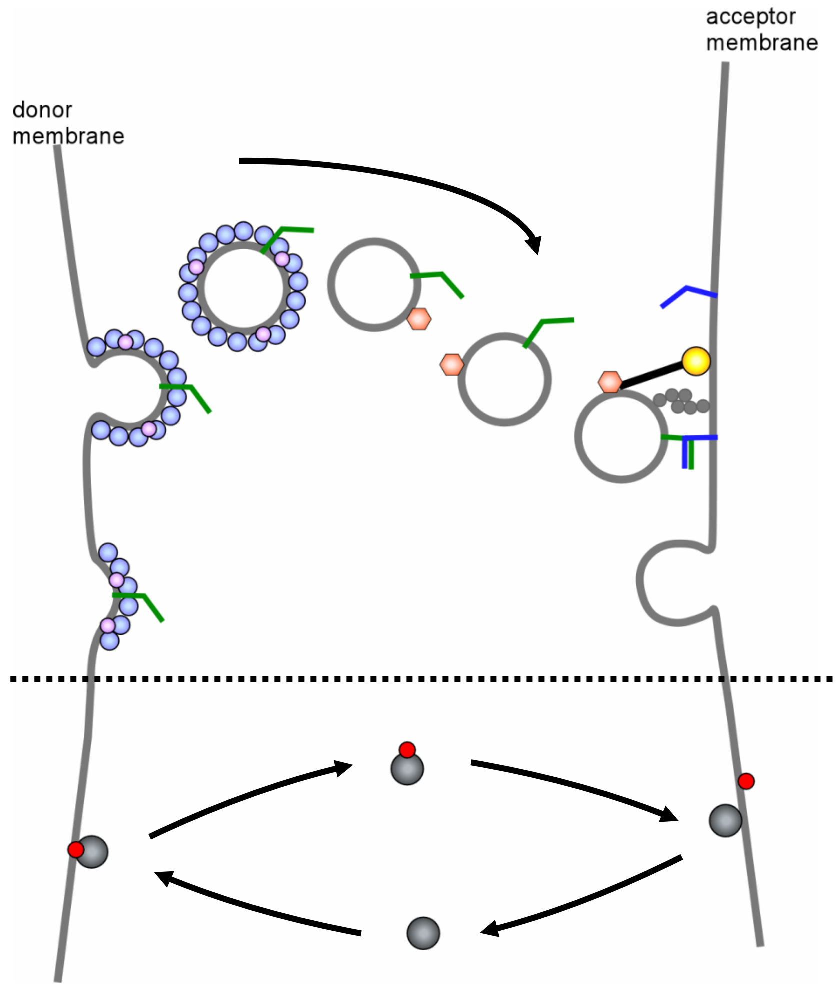
→ can we mimic what is done in cells ?

Orci L, Perrelet A, Rothman JE. Proc Natl Acad Sci U S A. 1998 95(5):2279-83.

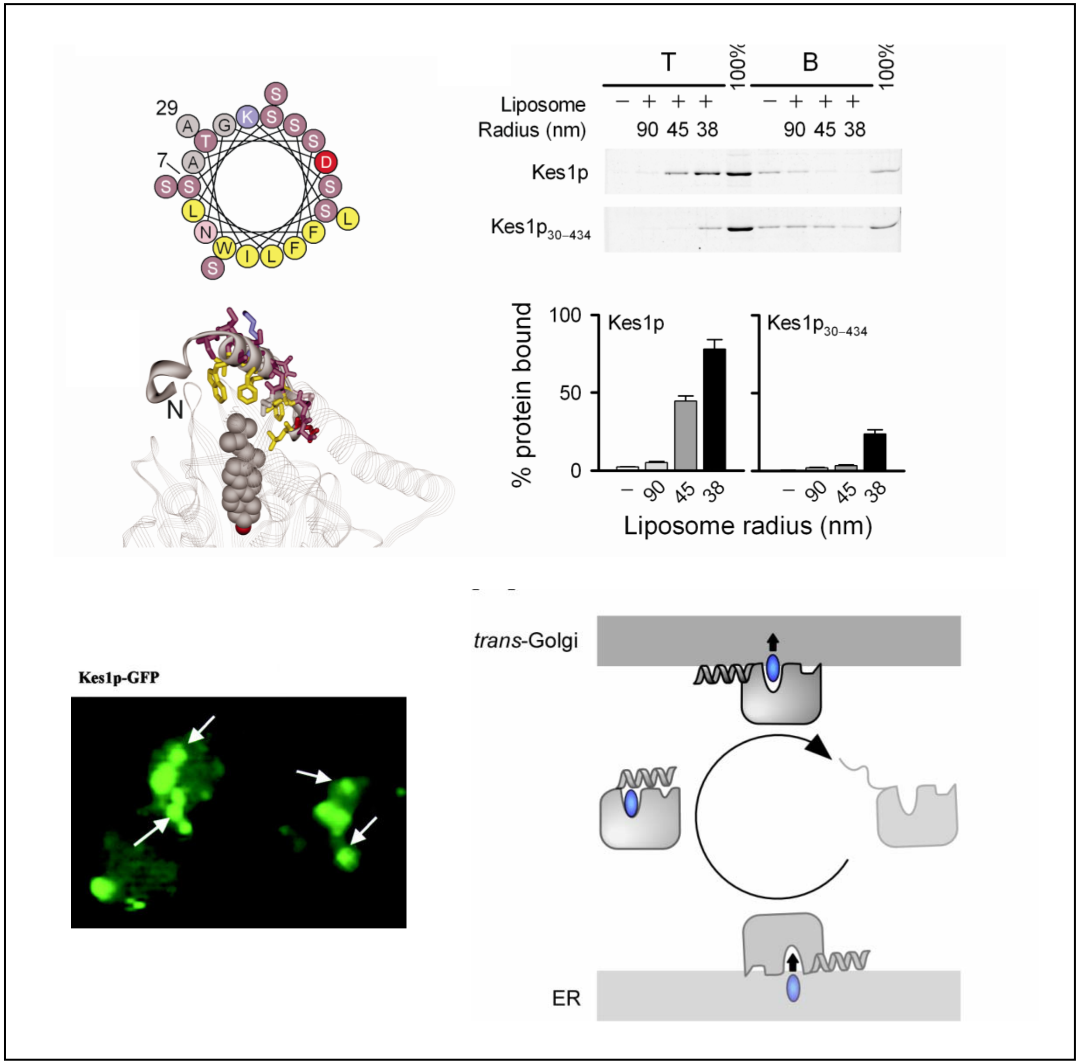
Vesicular transport / Non-vesicular transport

Vesicular transport

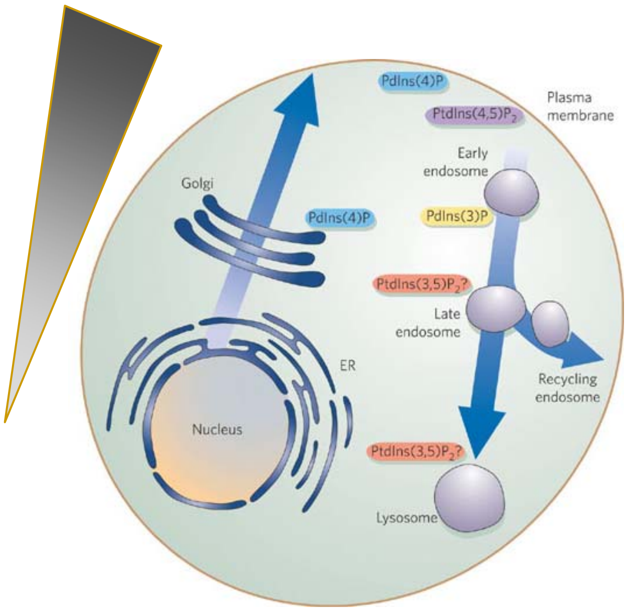
Non-Vesicular transport



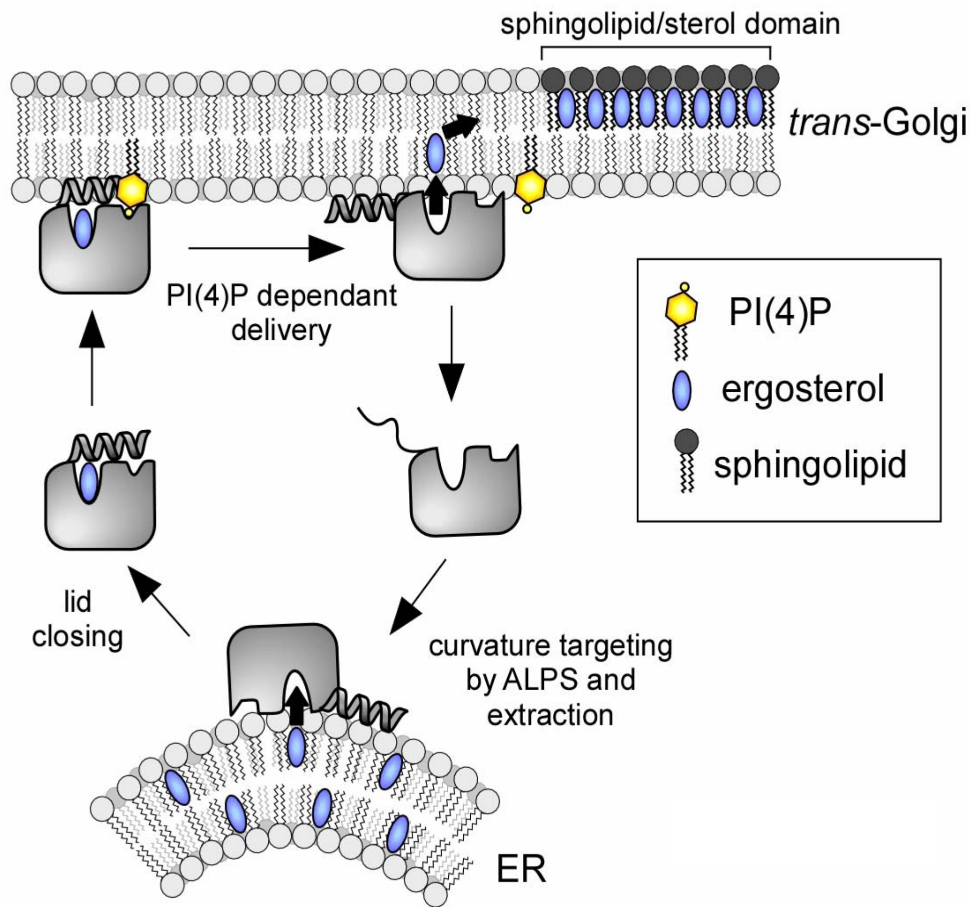
A sterol-transporter : Kes1p

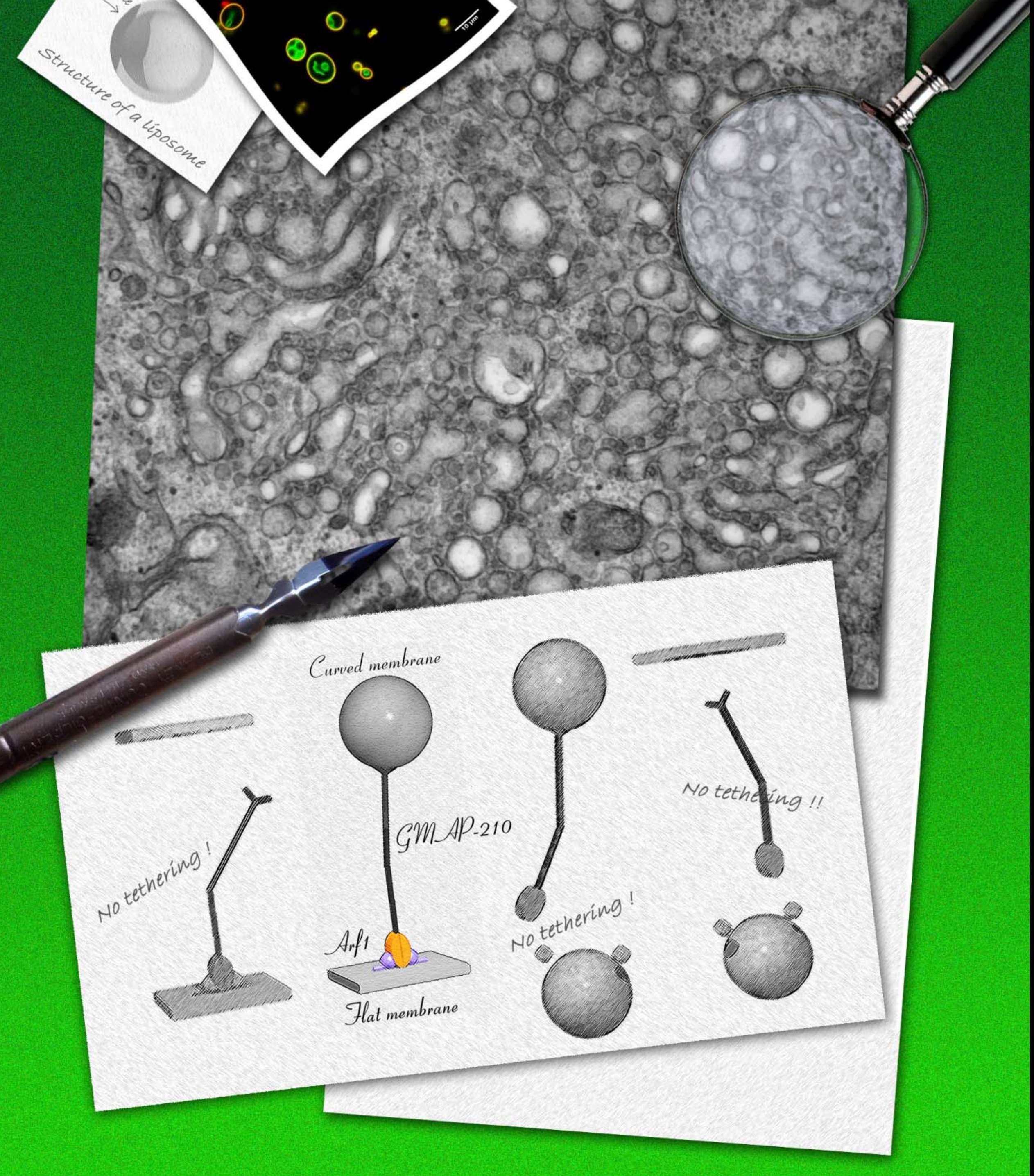


Sterol Gradient



Kes1p is likely designed to ensure vectorial transport





Acknowledgments

IPMC

Bruno Antonny
Vincent Morello
Jean-François Casella
Romain Gautier
Joëlle Bigay
Danièle Stalder
Hélène Barelli

CCMA – UNSA NICE

Pierre Gounon

ANR / RISC (CNRS)