Nanoscale organization of multiple GPI-anchored proteins in living cell membranes

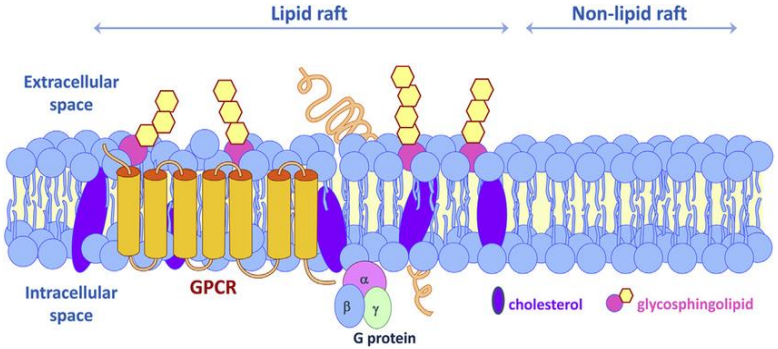
Charpié Coralie
Dias Gomes Vera
Richard Jonas
Reuse Coralie

Plan of the presentation:

- Introduction and definitions
- Aim of the study
- Methods used
- Explanation of the results
- Conclusion
- Key takeaways from the study

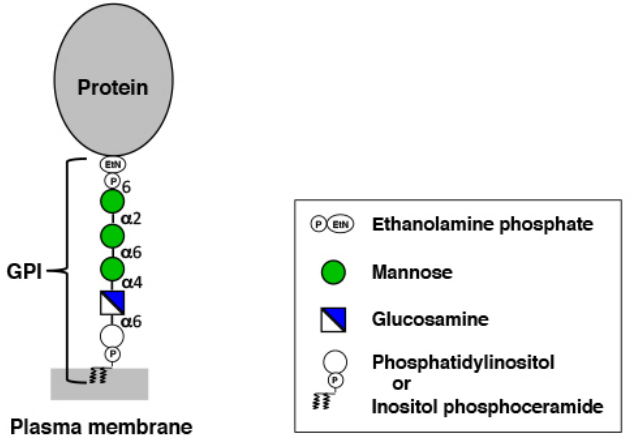
Rafts

- **Definition** : lipid microdomains within the cell membrane
- **Role** : organizing membranes components
- **Structure** : enriched in lipids (glycosphingolipids and cholesterol) and proteins (as GPI-APs)
- **Functions** : transmit signal, facilitate transport, membrane stability and resistance, cell communication



GPI-APs (glycosylphosphatidylinositol-anchored proteins)

- Protein attached to the outer surface of the cell membrane
- **Structure of GPI group**: lipid and sugar chain
- **Application** : can be used as markers for membrane rafts
- **Interest** : form small clusters that could represent a form of raft



Evolution of the concept of "rafts"

- **Initially** : large and stable structures acting as plateform for gathering proteins and lipids

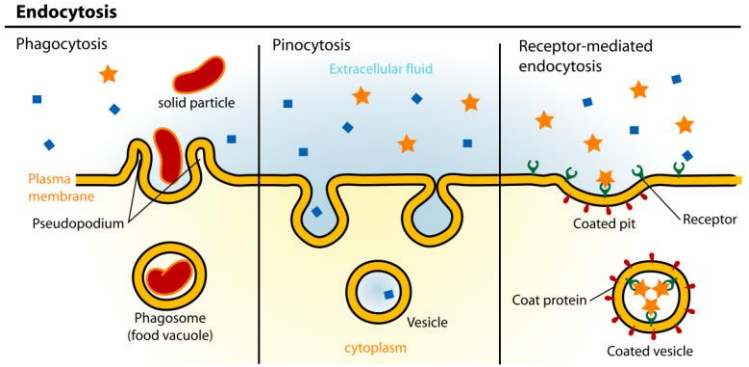
- **New hypothesis** : small and dynamic structure able to change shape and size

Aim of the study

- Investigate the nanoscale organization of GPI-APs.
- Provide direct evidence of lipid raft-like structures in living membranes.
- Clarify raft's role in cellular functions.

Endocytosis

- Process by which a cell absorbs substances by surrounding them with its membrane to form a vesicle that enters the interior of the cell.

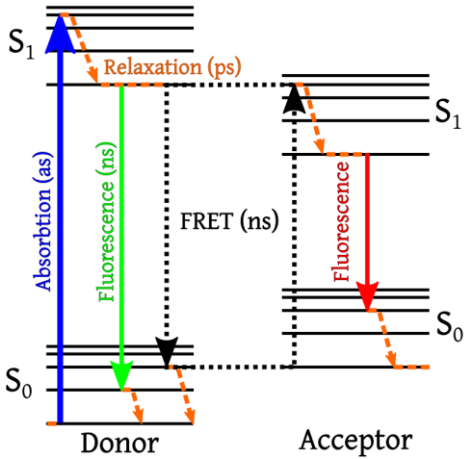


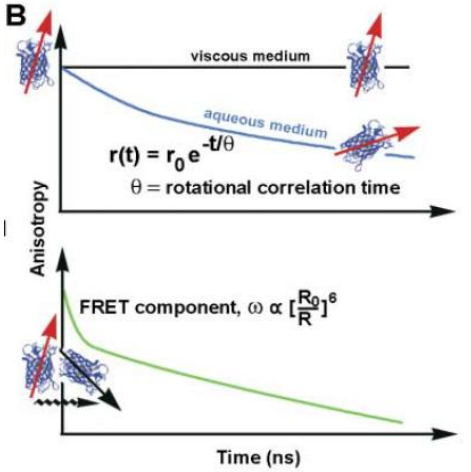
Aim of the study

- Investigate the nanoscale organization of GPI-APs and their connection to the concept of lipid rafts.
- Determine informations about clusters.
- Seeks to provide direct evidence of lipid raft-like structures in living membranes.
- Clarify raft's role in cellular functions.

Methods used

- Fluorescence and electron **microscopy**
- homo-Fluorescence Resonance Energy Transfer (**FRET**) microscopy: to deduce protein clustering.
- **time-resolved anisotropy decay experiments**: to measure intermolecular distances.
- **photobleaching** experiments combined with **theoretical modeling**: to analyze cluster size and density.

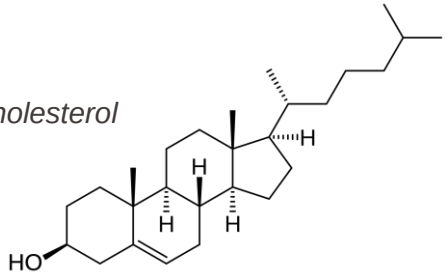




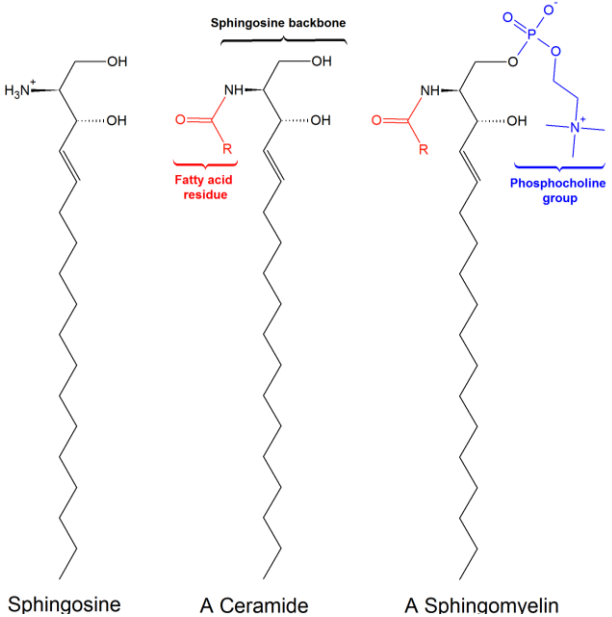
Methods used

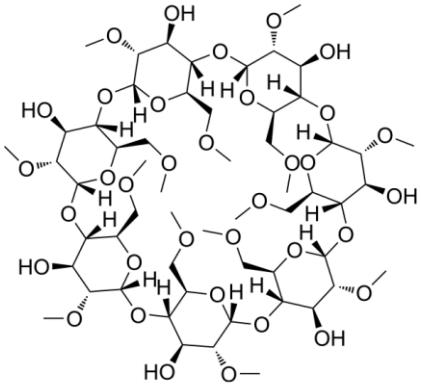
- **Cholesterol and Sphingolipids depletion**: role of lipids in maintaining GPI-AP organization.

Cholesterol

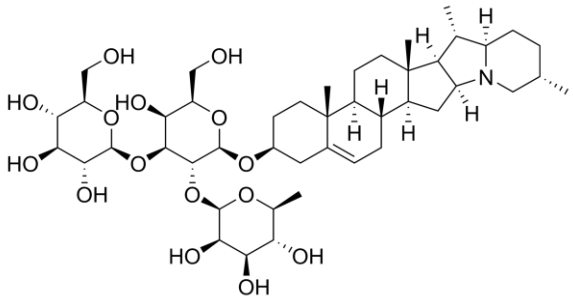


Sphingolipids





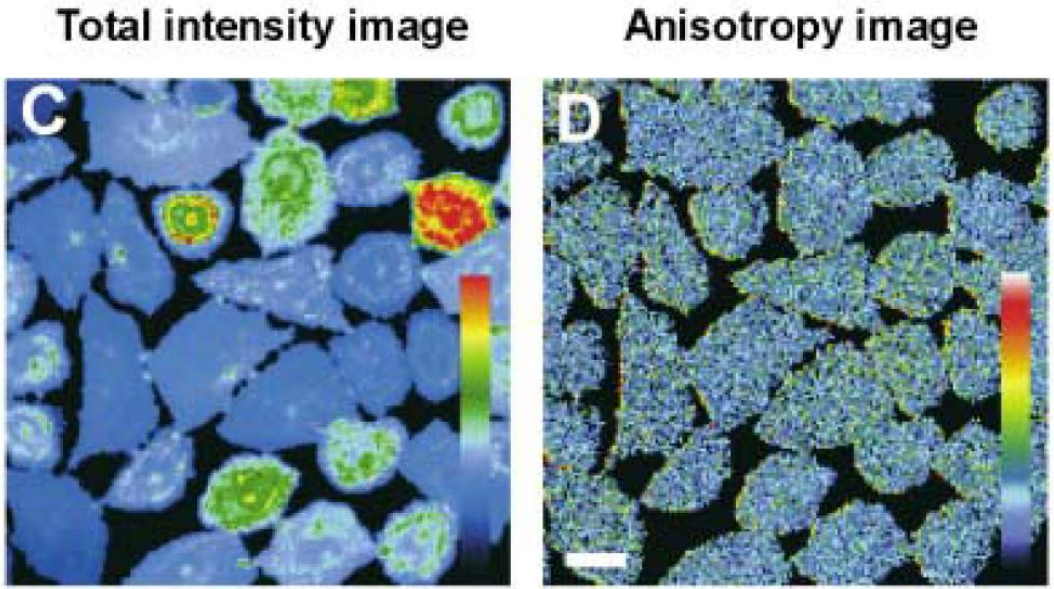
methyl-β-cyclodextrin



saponin

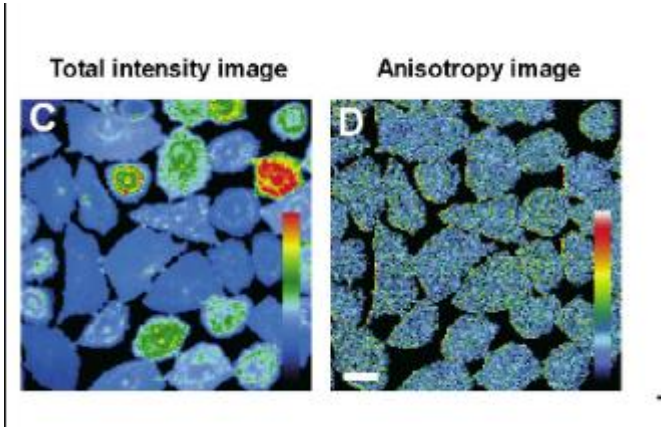
Methods used

- **Hetero-FRET**: to detect clustering between distinct GPI-AP species.
- **Antibody-mediated crosslinking**: to reorganize clusters.



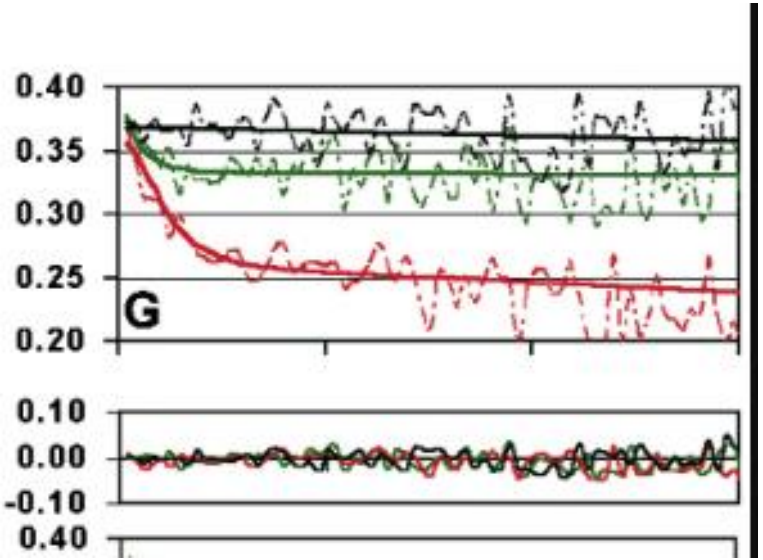
The presence of GPI-APs as clusters at the cell surface

- GFP-GPI shows fluorescence
- Reduced anisotropy values
- Homo-FRET vs rotational mobility



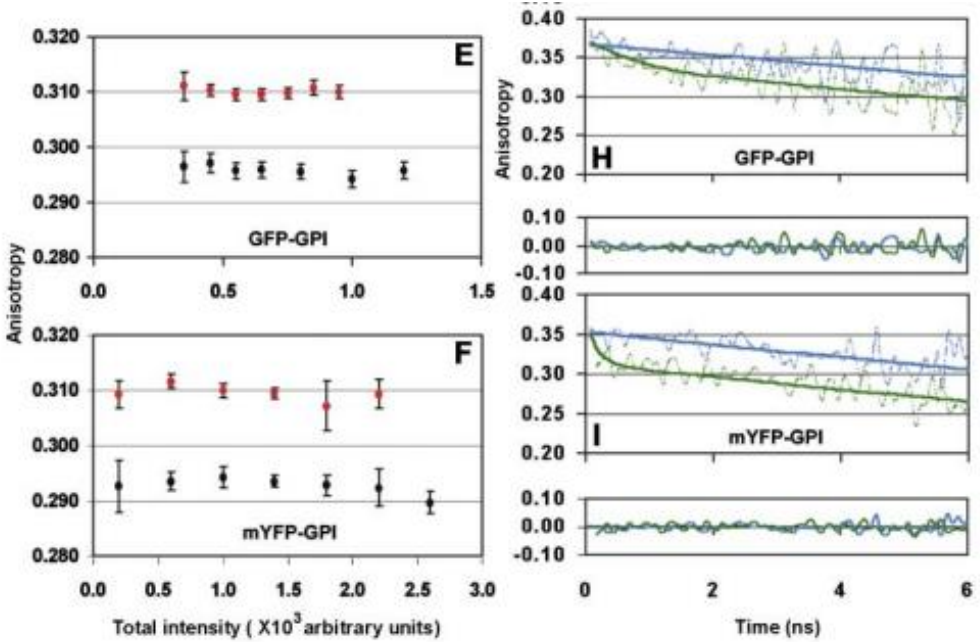
Decrease comes from FRET which proves clustering of GFP-GPI

- The decay is absent for GFP molecules in free solution (Black line)
- Fast decay is present upon cross linking with glutaraldheyde (Red line)
- Not sensitive to viscosity increase (Green line)
- Occurs at rate much faster than the lifetimes of fluorophores
- Reducing the fluorophores density increase the steady-state of anisotropy



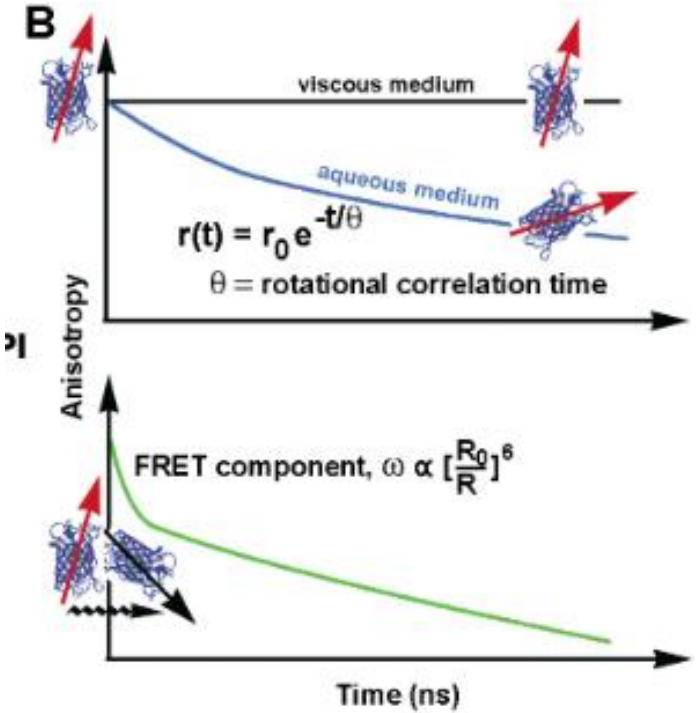
Origin of FRET between GPI-AP species

- Not from protein-protein interaction
- Loss of homo-FRET with cholesterol depletion (Figure H & I)
- Constant anisotropy (Figure E & F)
- Disparition of FRET dependance when changing the anchor



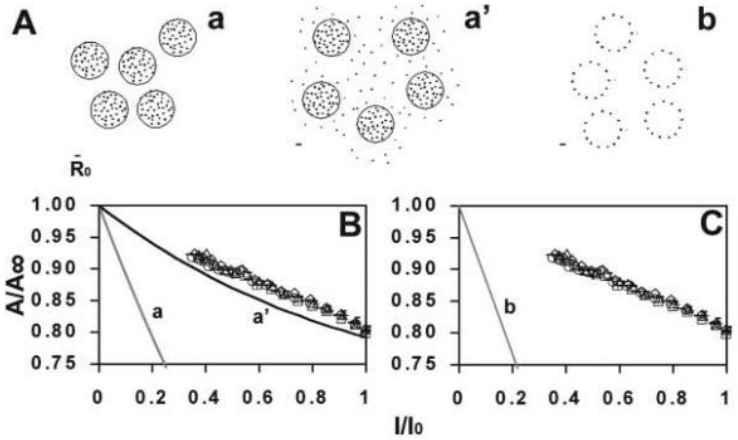
GPI-APs are present in extremely high-density structures

- Utility of the previous result
- More than 10% in high-density structure, 3.53 ± 0.455 nm
- Comparison with 0.3 nm crosslinker
- mYFP-GPI faster decay
- PFL-FR-GPI same
- So GPI-anchor are responsible of high density structure < 4nm



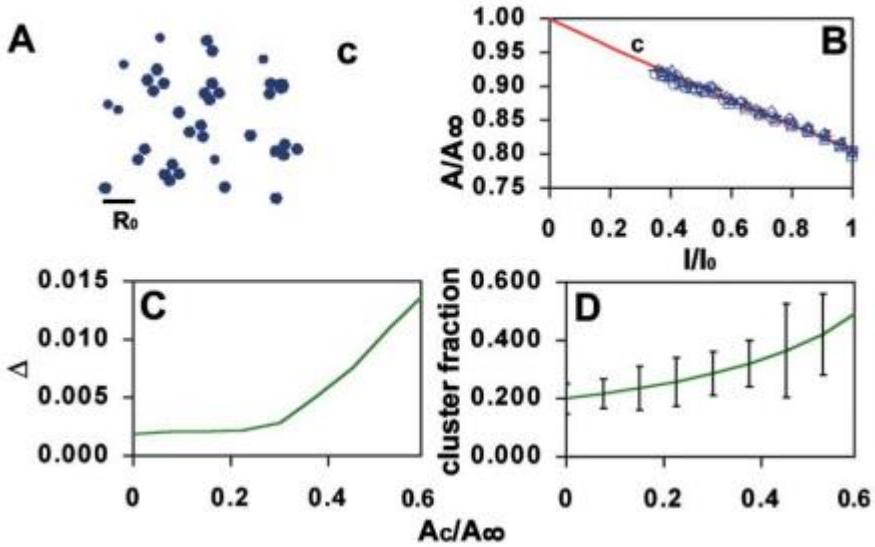
Three different models for describing spatial distribution of proteins

- Model A : GPI-APs are uniformly distributed in large domains (10 times larger than their molecular size, ~50 nm).
- Model A’ : a fraction of GPI-APs are in large domains, the rest are dispersed as monomers on the surface.
- Model B : GPI-APs are concentrated on the periphery of the domains.



New model

- ~20-40% of proteins are in cluster smaller than R0
- Protein close to each other which favors FRET ~5nm
- Remaining proteins are monomers distributed on the cell surface
- Membrane have clusters and monomers

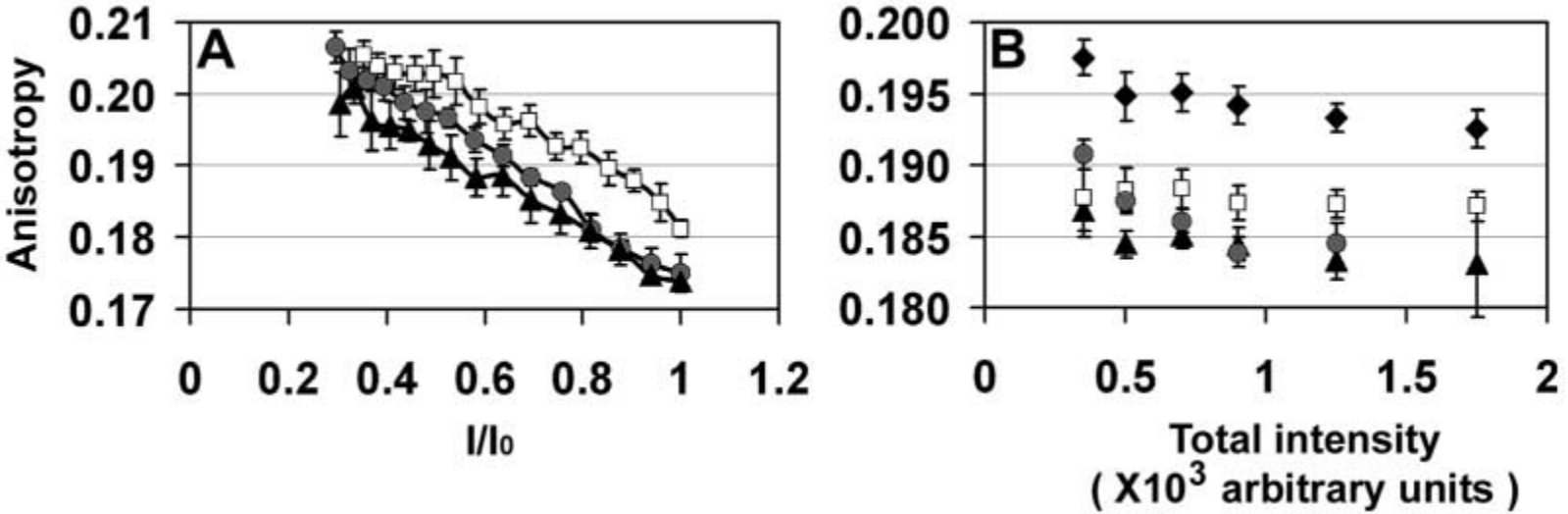


Organisation of GPI-APs into clusters

- Homo-FRET detected :
  - ~30% of GPI-APs are likely to be organized in very compact clusters
  - Small clusters and/or little proteins inside it
- Hetero-Fret undetected :
  - No signal between different fluorophore
  - No large cluster

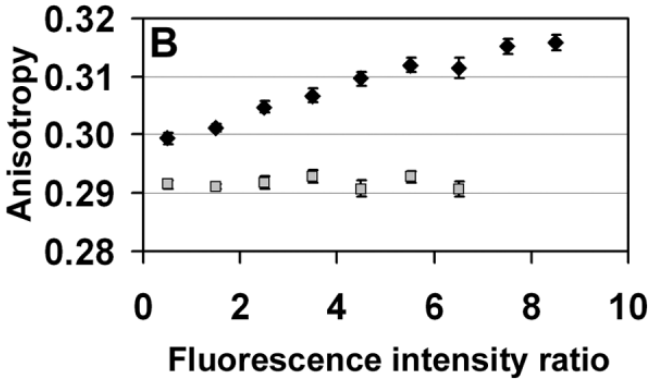
- Confirmation of the observation:
  - Big cluster forced with Aerolysine Y221G
  - Measurable Hetero-FRET
  - So absence of hetero-FRET was due to the size and fraction of the cluster
- Conclusion
  - ~20-40% of clusters
  - ≤ 4 molecules per cluster

Cholesterol and sphingolipid depletion differentially affect GPI-AP clustering

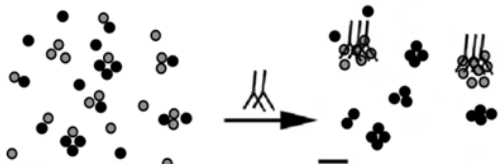


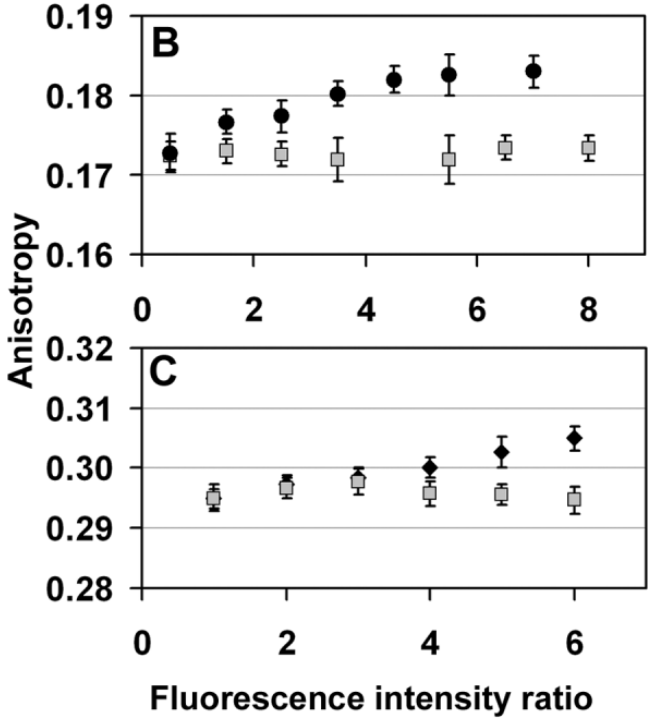
- Black triangle : untreated
- Gray circle : sphingolipid-depleted
- Open square : cholesterol-depleted
- Black diamond : depleted of both cholesterol and sphingolipid

Multiple GPI-APs inhabit the same nanometer-sized cluster



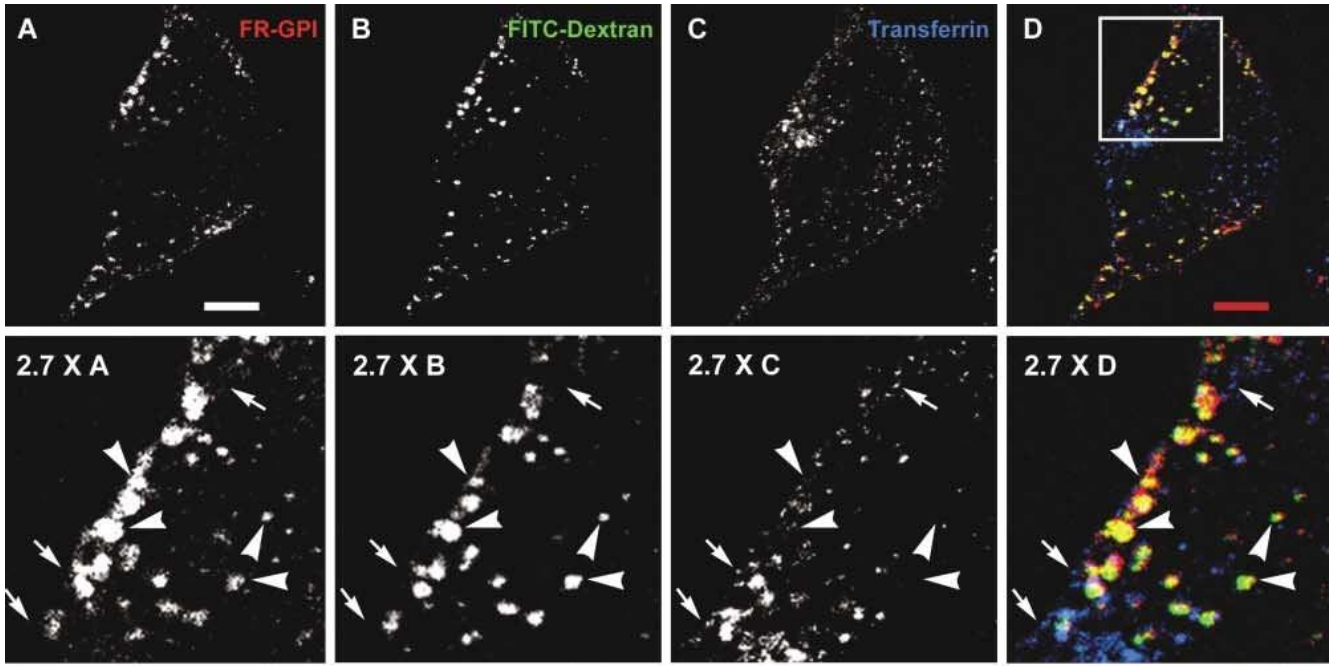
- Black diamond : FR-GPI
- Open square : FR-TM





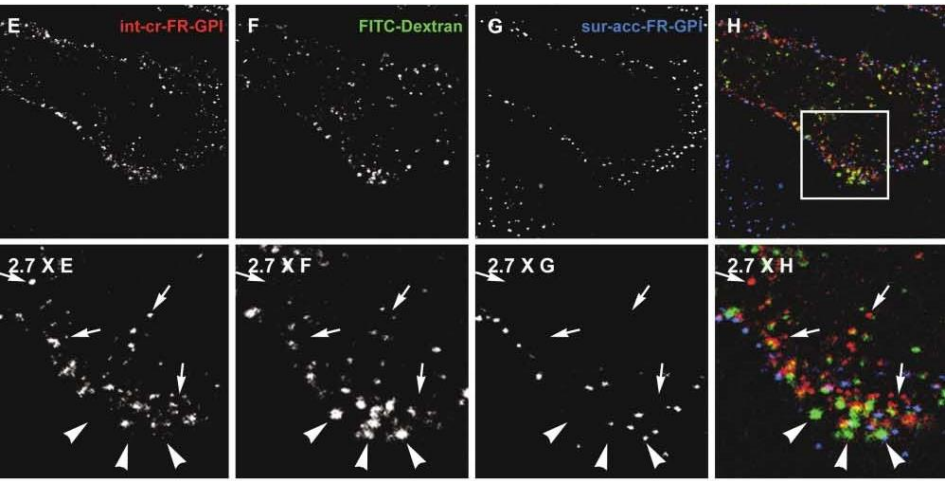
- Black circle : FR-GPI
- Black diamond : GFP-GPI
- Open square : crosslinking with DAF

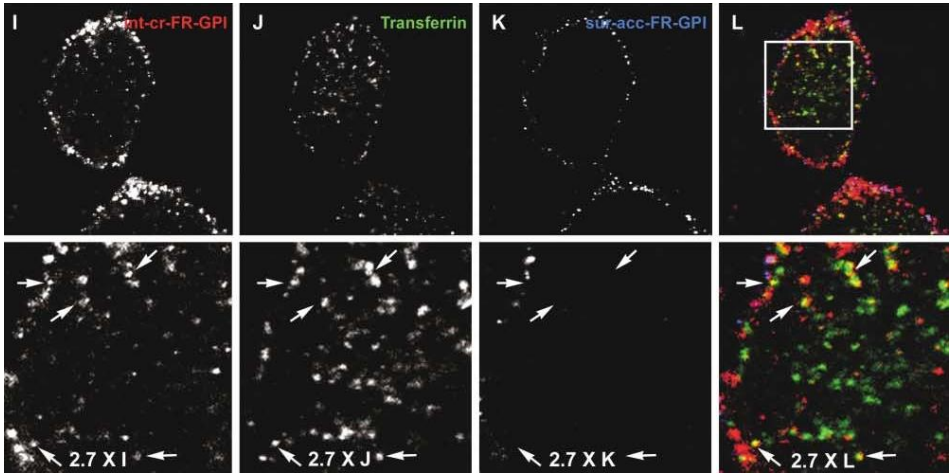
Antibody-crosslinking alters endocytic routing of crosslinked proteins



- FITC-dextran : GPI-AP-specific endocytic compartments, or GEECs
- Transferrin : clathrin-mediated pathway

Antibody-crosslinking alters endocytic routing of crosslinked proteins





- FITC-dextran : GPI-AP-specific endocytic compartments, or GEECs
- Transferrin : clathrin-mediated pathway
- Tertiary fluorescent antibody : to distinguish between internal and surface-bound FR-GPI

Conclusion:

- Possibility to measure the structures
- 20-40% of GPI-APs form small clusters of 3-4 molecules
- Remaining of GPI-APs are monomers
- Cholesterol is essential
- Sphingolipids play an indirect role
- Multiple GPI-Aps can be found in the same cluster
- Antibody crosslinking alters endocytic fate

Key Takeaways from the Study

Thank you for listening !