
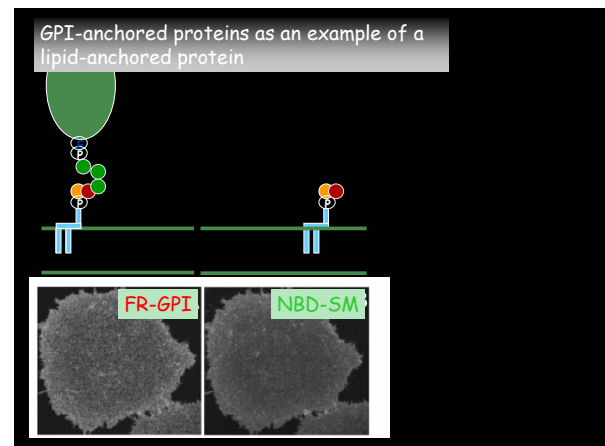
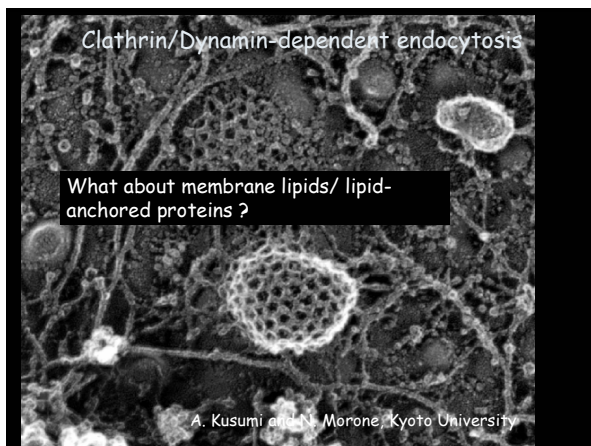
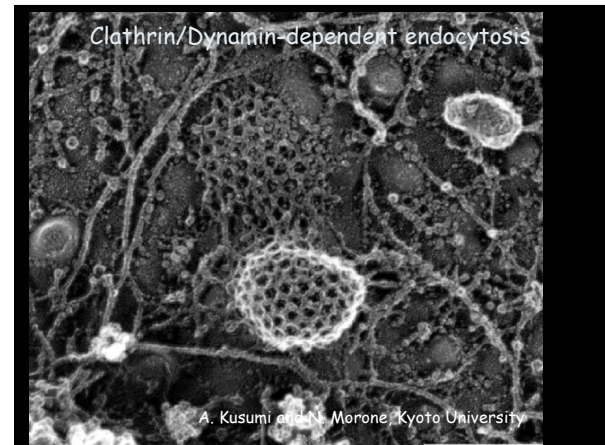
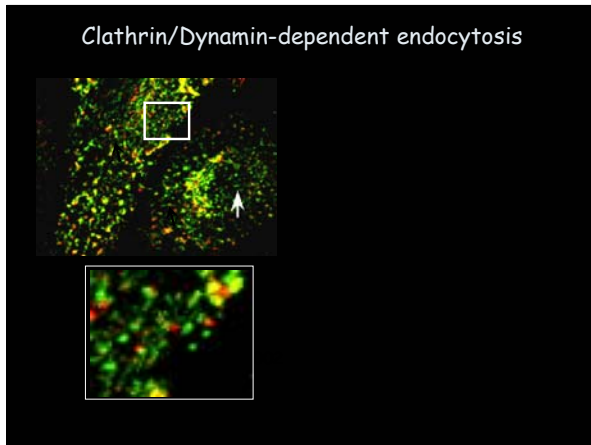
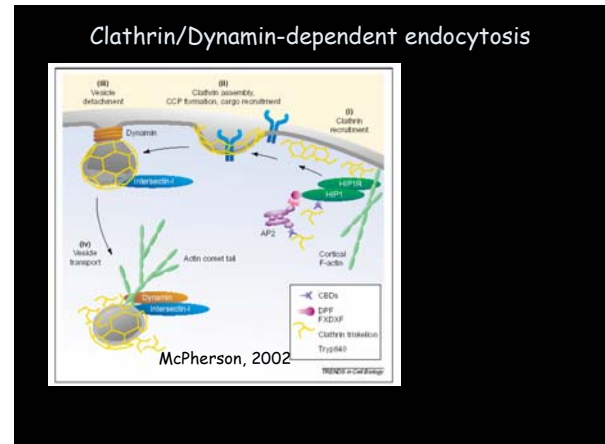


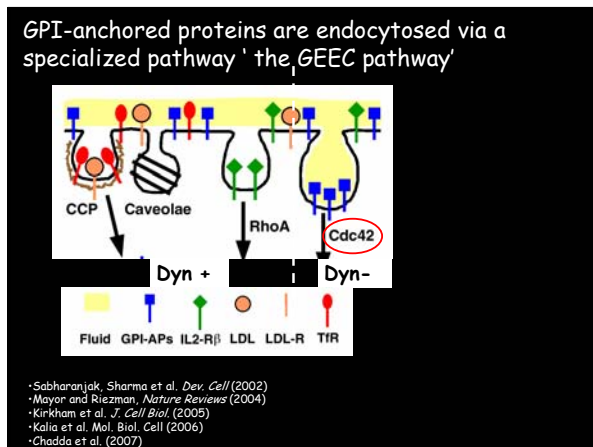
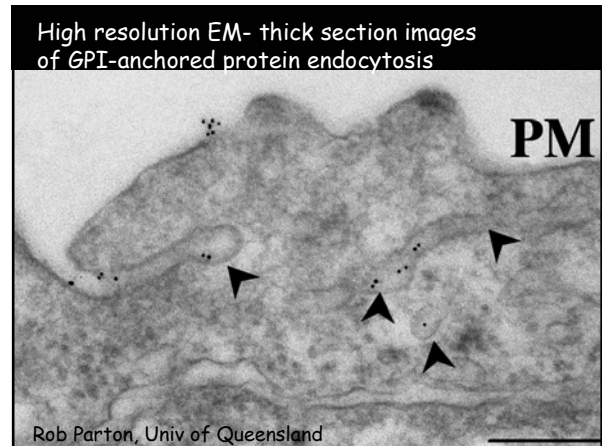
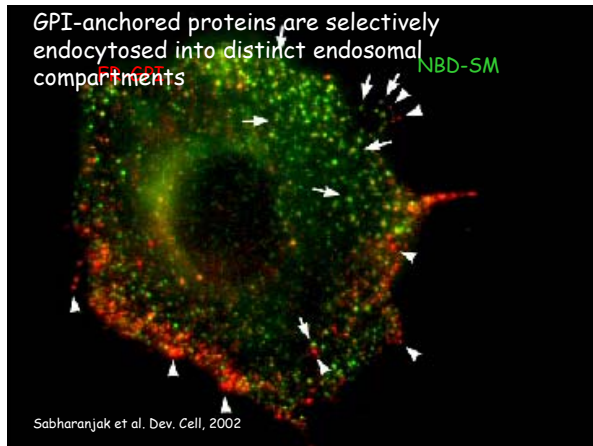
iBio Seminars Part II:
Looking for functional rafts in cell membranes

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GPI-anchored proteins are internalized via an pinocytic pathway that is

- Constitutive
- Lipid-selective
- Lipid-dependent
- Cdc42-regulated
- Actin-dependent

Does not utilize

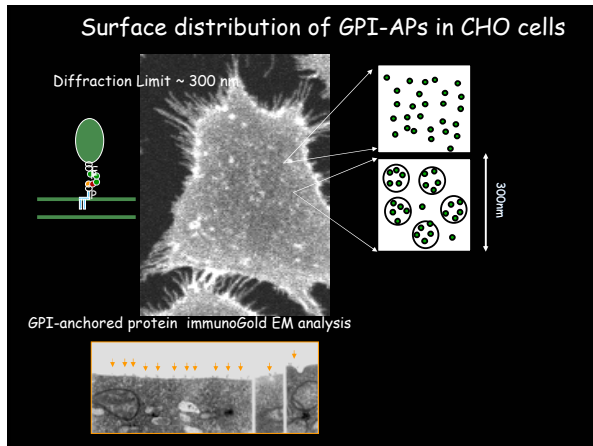
- Dynamin
- Clathrin/ Caveolin
- Arf6
- RhoA
- Rac1

Membrane Rafts?

- dynamic clustering of cholesterol and sphingolipids to form domains in membranes
- platforms for the segregation of specific proteins responsible for sorting and signaling function

Looking for rafts

- detergent insolubility
- diffusion measurements
- proximity-based methods

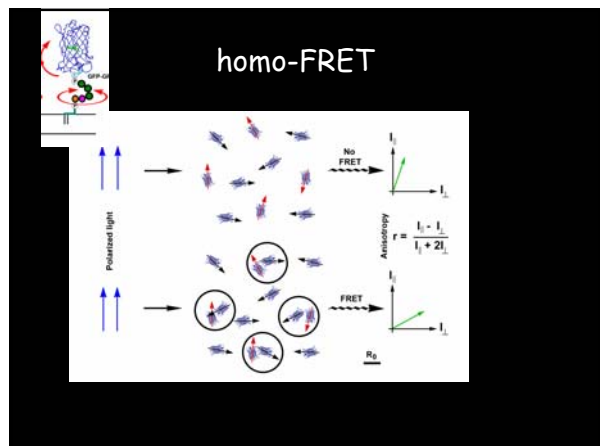
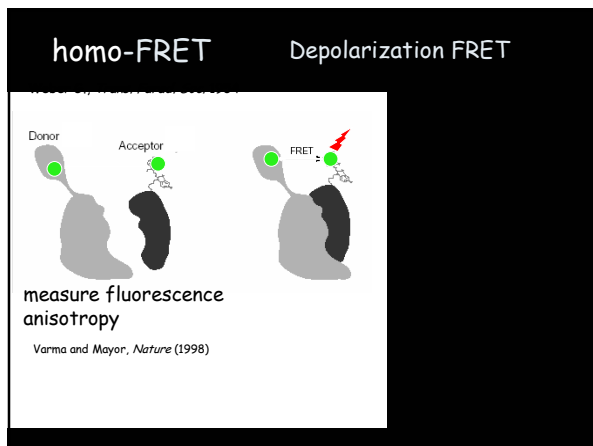
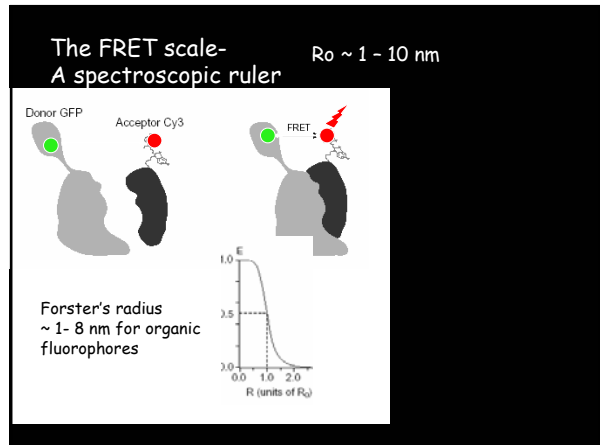
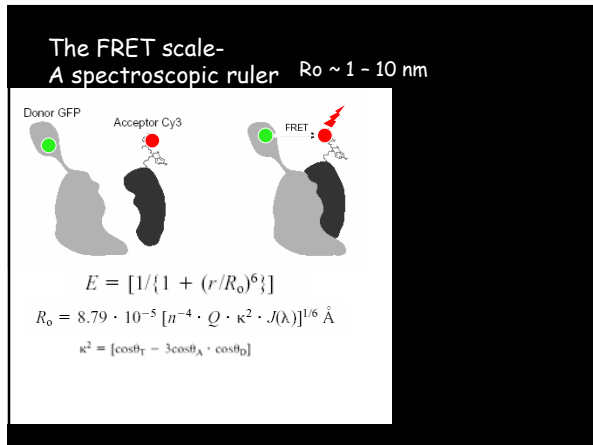


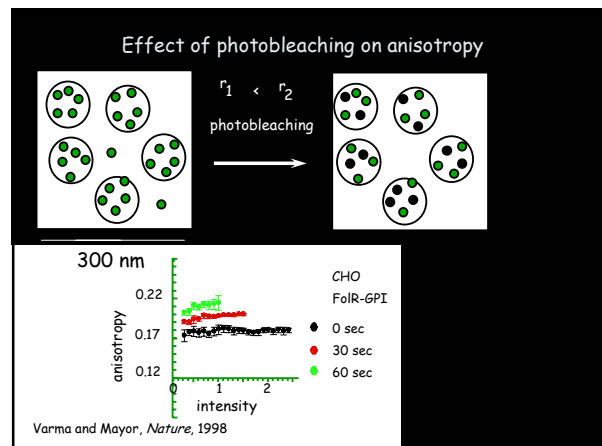
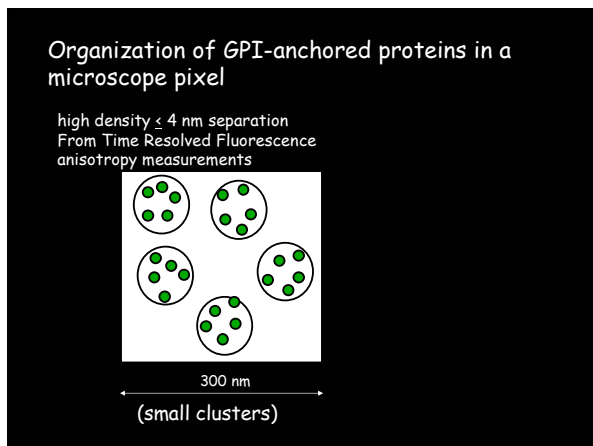
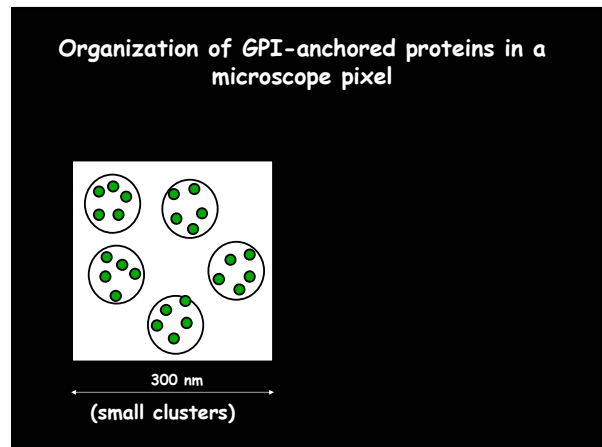
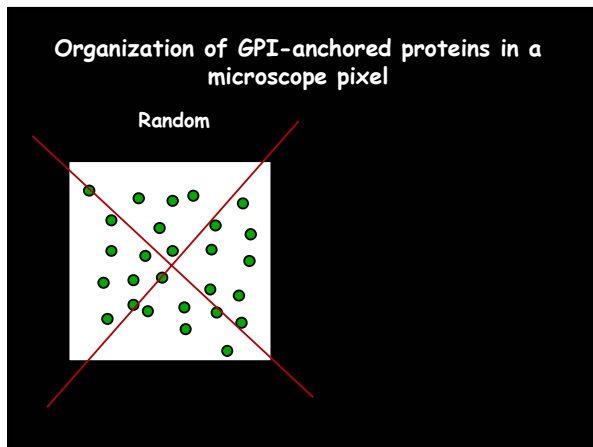
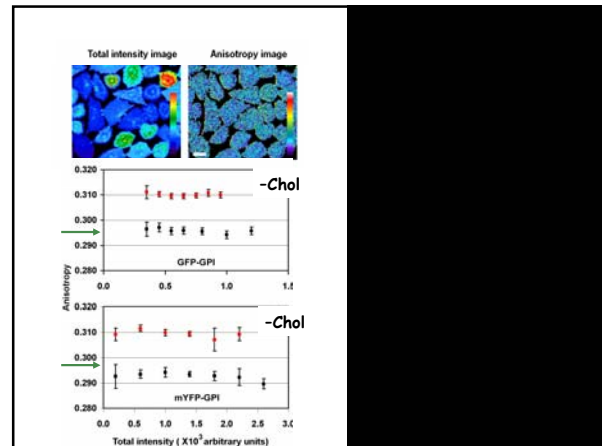
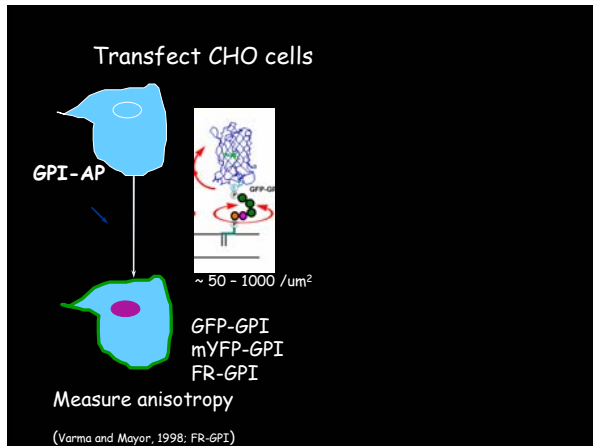
Proximity-based methods to examine segregation of GPI-anchored proteins

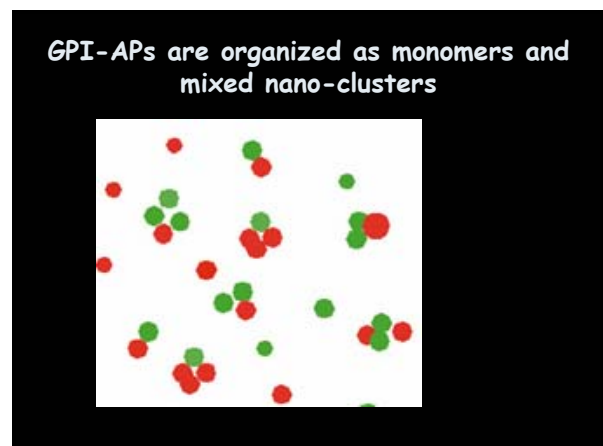
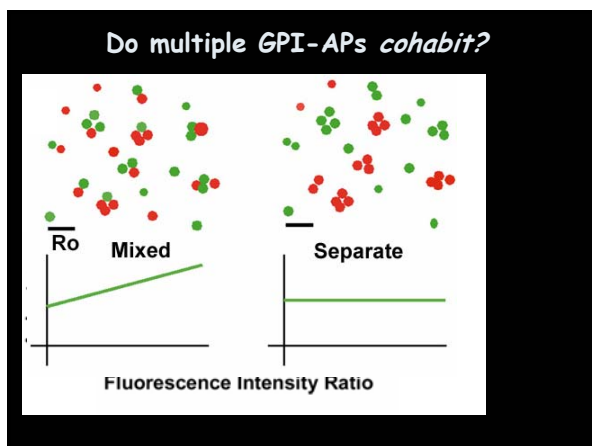
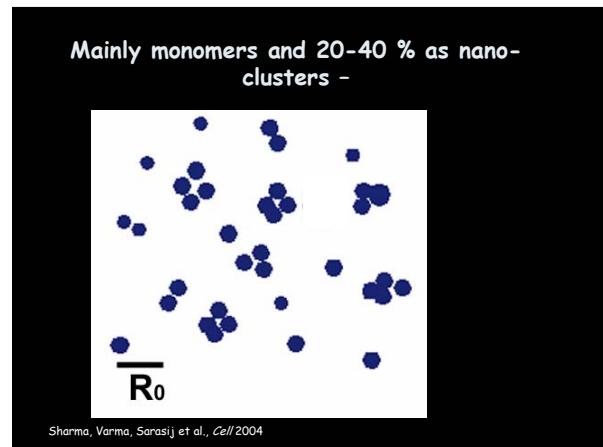
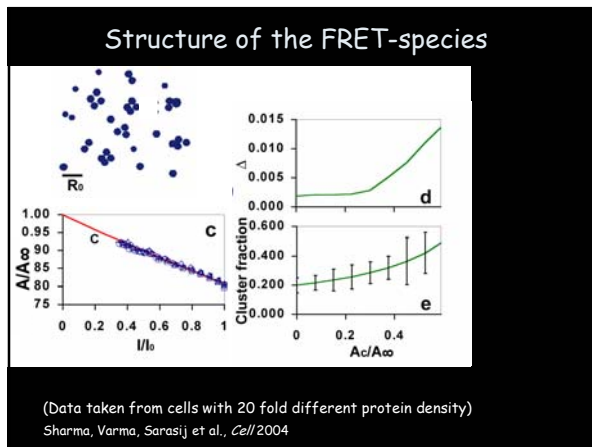
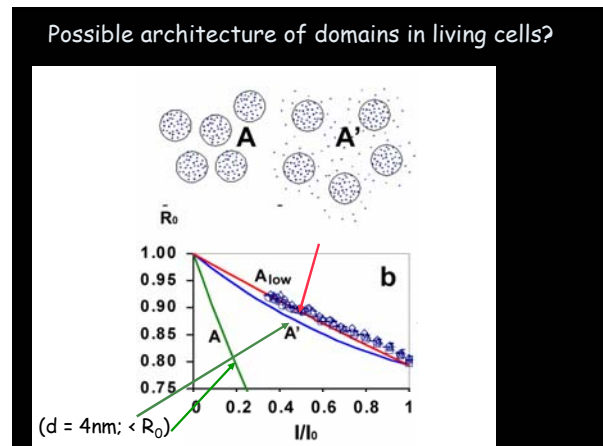
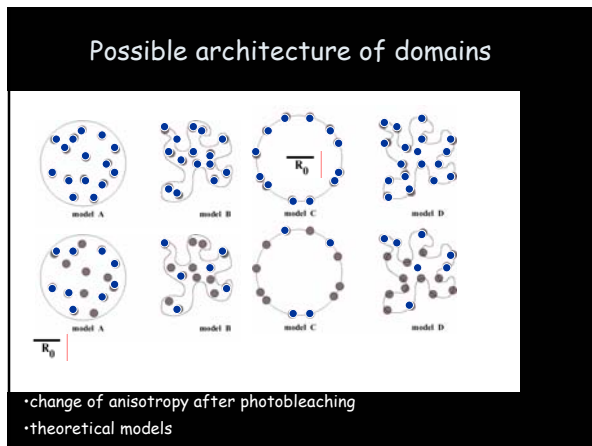
EM analysis (Mayor et al., *Science*, 1994; Prior et al., *JCB* 2003; Madore et al., *EMBO J* 2002)

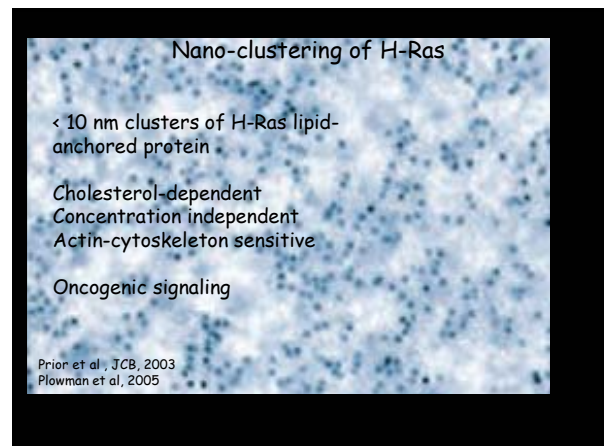
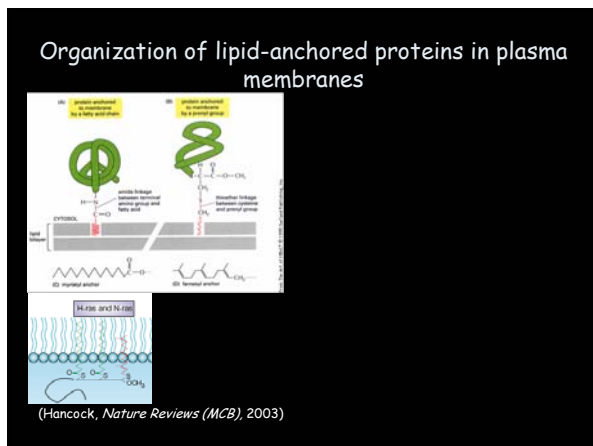
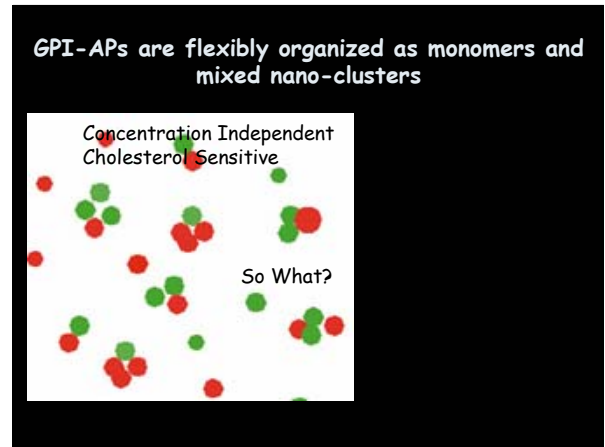
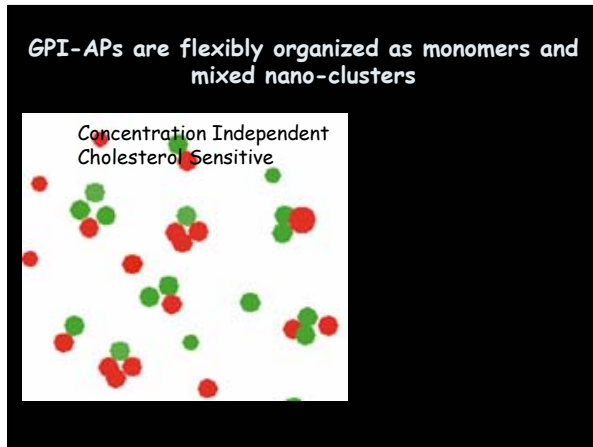
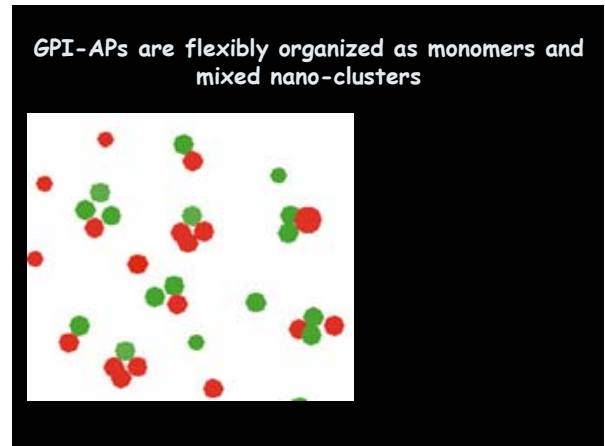
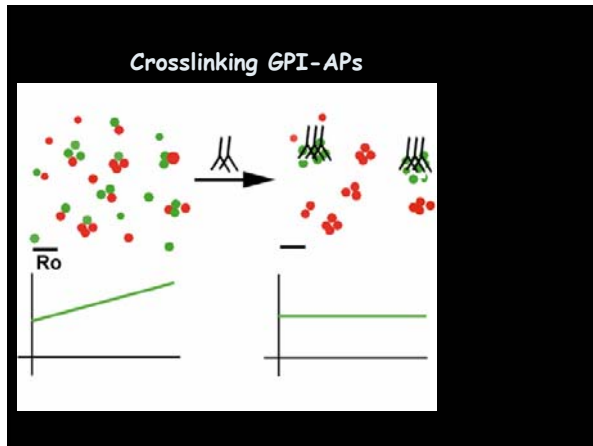
Chemical crosslinking *in situ* ~ microdomains: (Friedrichson and Kurzchalia, *Nature* 1998; Paladino et al., *JCB*, 2004)

Forster's Resonance Energy Transfer (FRET) (Varma and Mayor, *Nature* 1998; Kenworthy et al., *JCB*, 1998; Sharma et al., *Cell*, 2004)









Crosslinking GPI-anchored proteins induces H-Ras-domains to coalesce suggesting a trans-bilayer connection

GPI (2nm), tH (5nm)

patched

Prior et al., JCB, 2003

Other (glyco)lipids also show a clustered distribution

Gangliosides GM1 and GM3 exhibit a similar nano-clustered distribution

- sensitive to cholesterol depletion
- cold temperatures
- concentration independent

- Fujita, et al. MBC (2007)

Lipid-anchored proteins and lipids are flexibly organized as monomers and nano-clusters

Endocytosis of GPI-anchored proteins and nanoclusters?

CCP Caveolae RhoA Cdc42

Dyn + Dyn-

Fluid GPI-APs IL2-Rβ LDL LDL-R TIR

Crosslinked GPI-anchored proteins are not endocytosed via the GEEC-pathway

CCP Caveolae RhoA Cdc42

GPI-anchored protein 'rafts'

pre-existing organization induced rafts

Dyn - Cdc42

Mayor and Rao, Traffic (2004)

