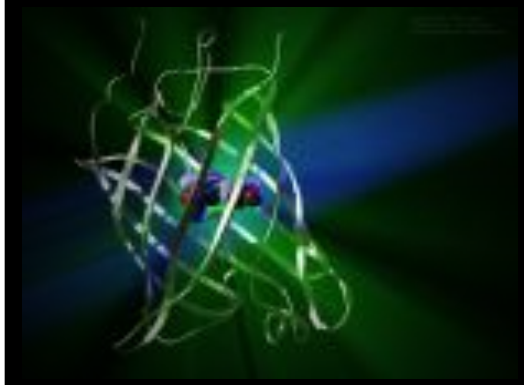
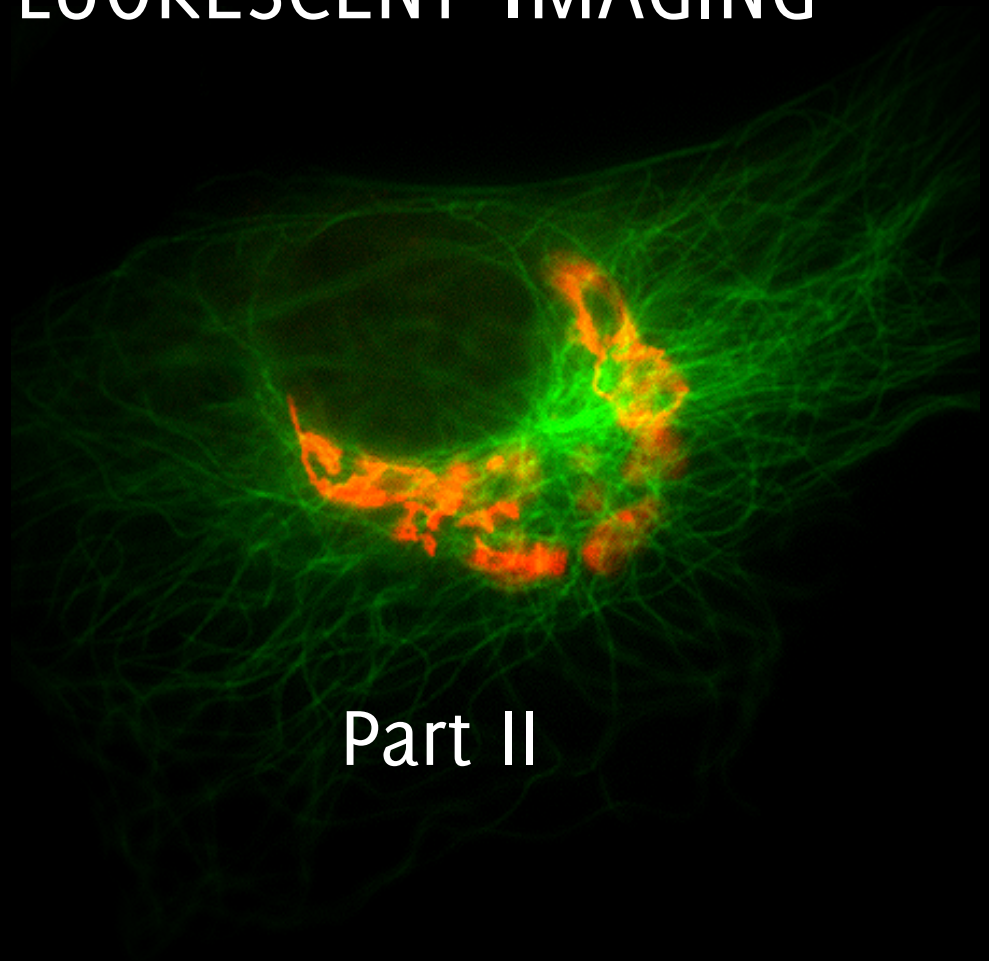
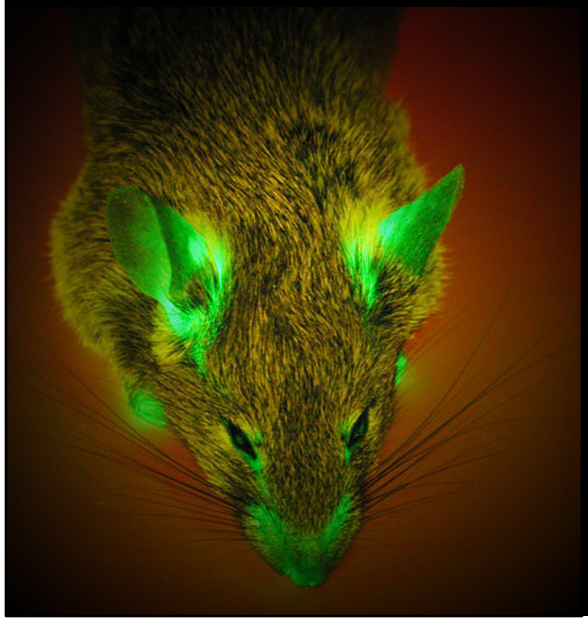


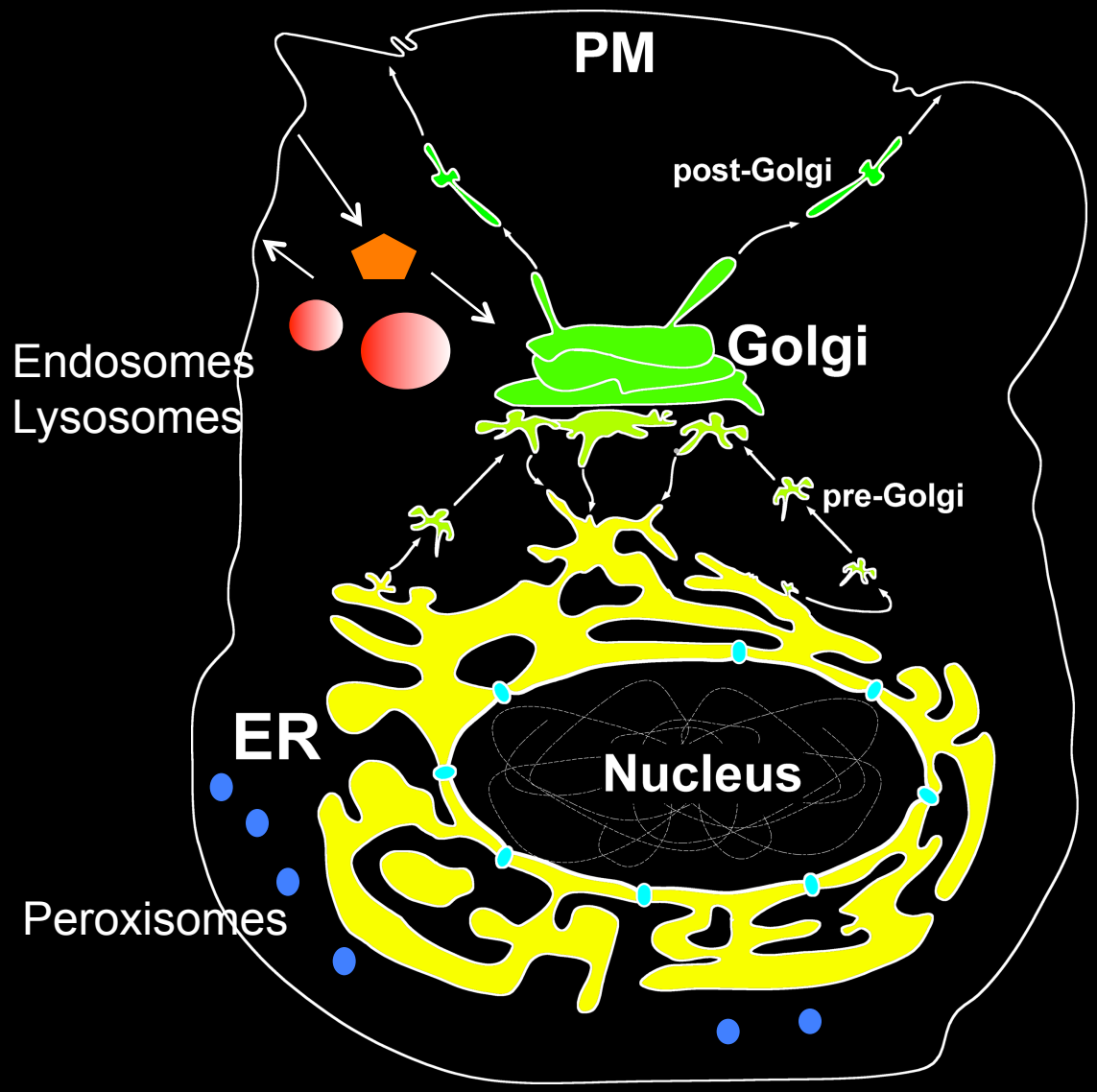
Breakthroughs in Intracellular Fluorescent Imaging



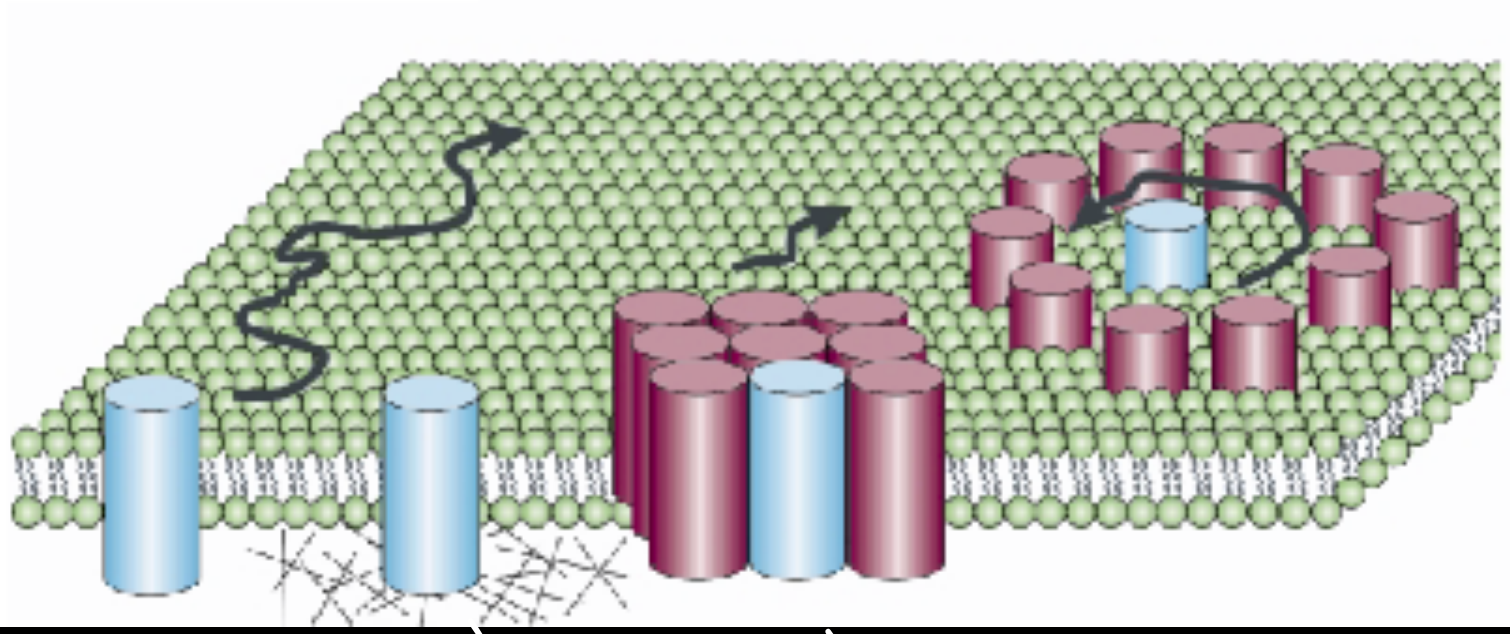


Part II

The Endomembrane System



Properties that affect a protein's lateral diffusion in a bilayer



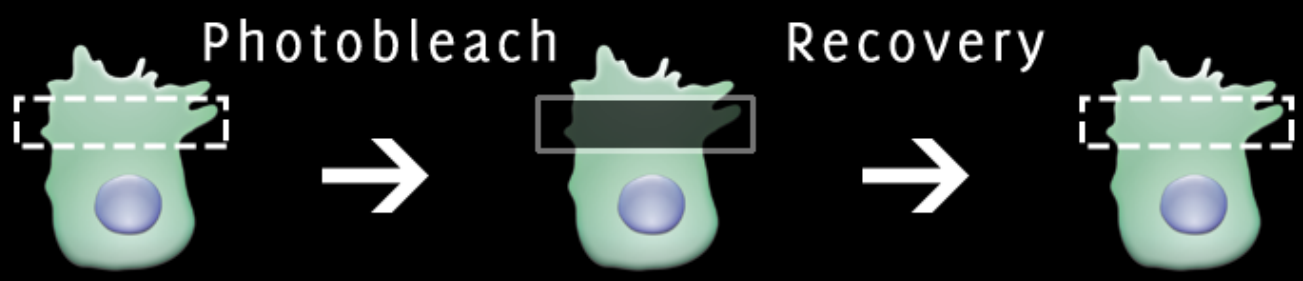
Free

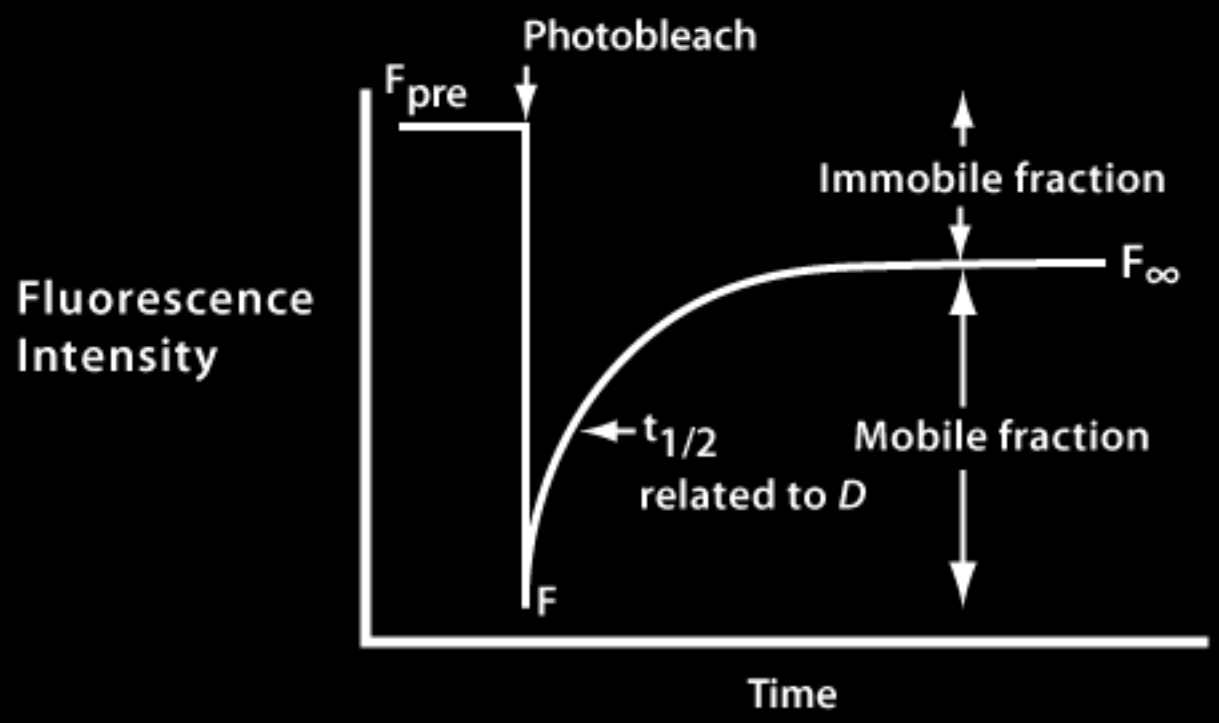
Immobilized

Assembled

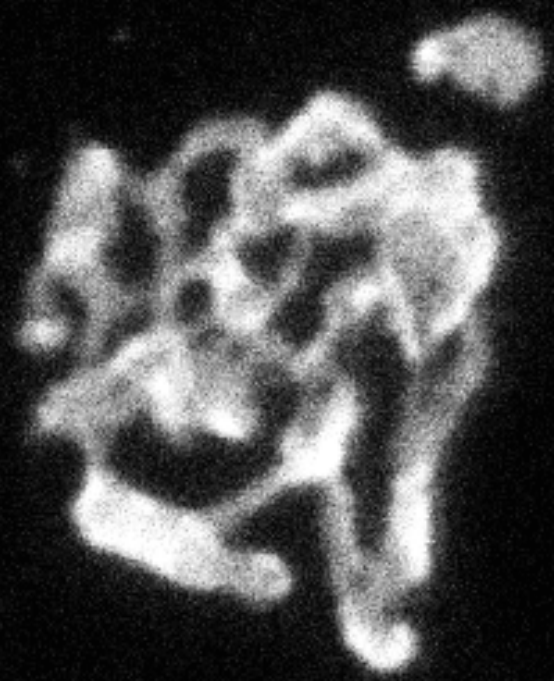
Confined

Fluorescence Recovery after Photobleaching (FRAP)

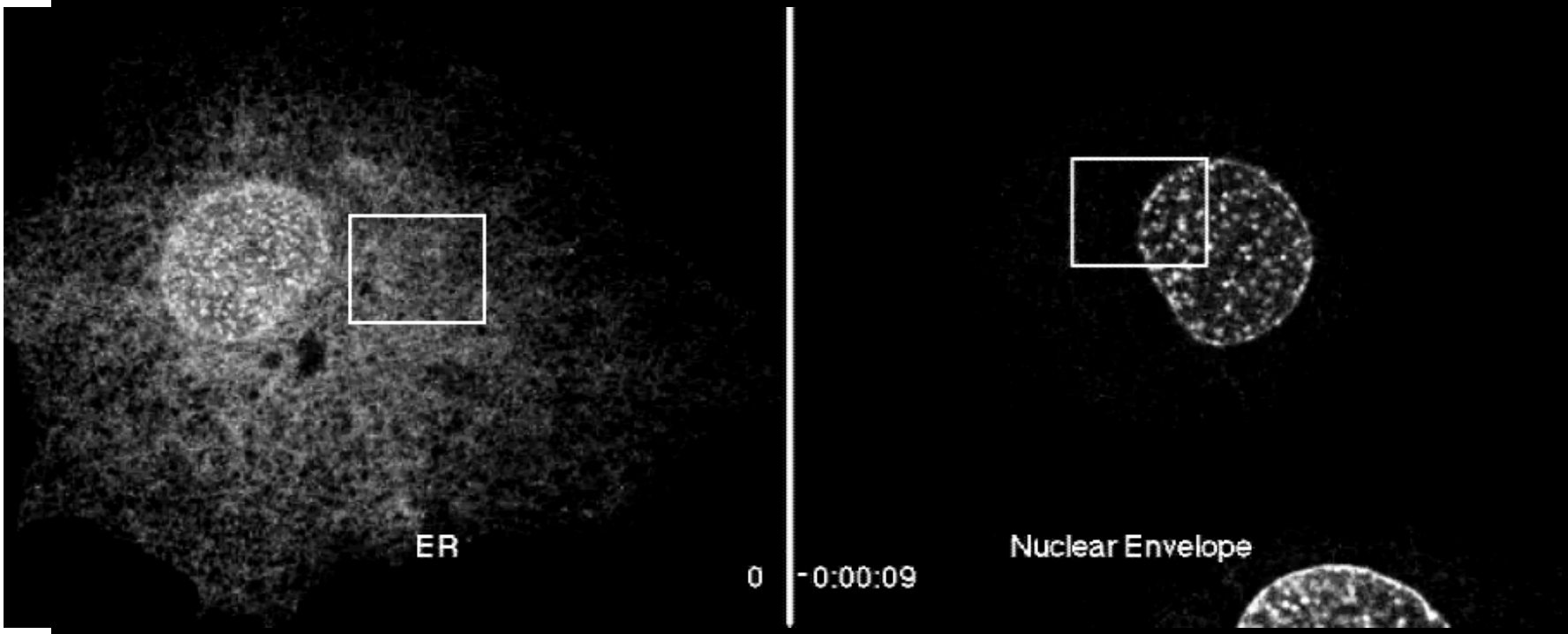




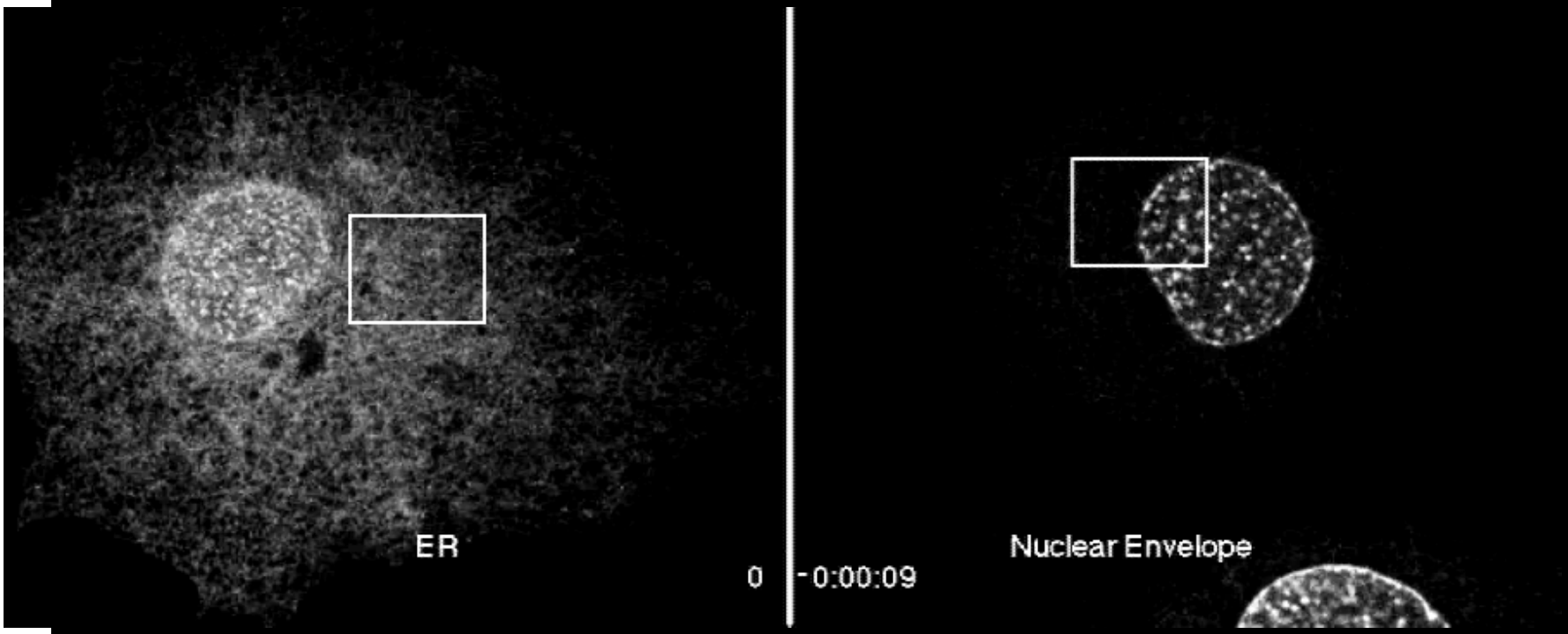
FRAP of Golgi apparatus



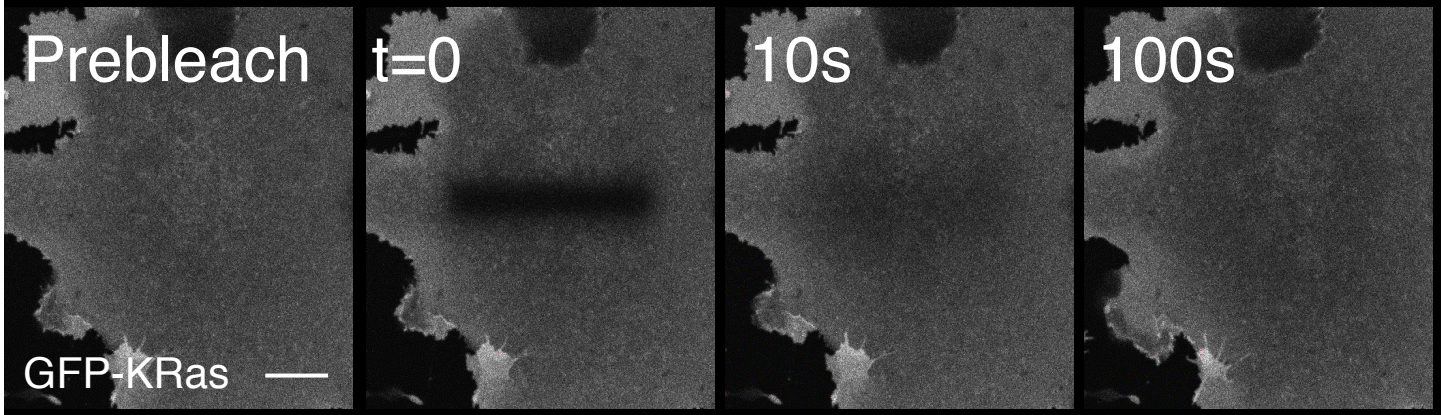
FRAP of ER and Nuclear Envelope



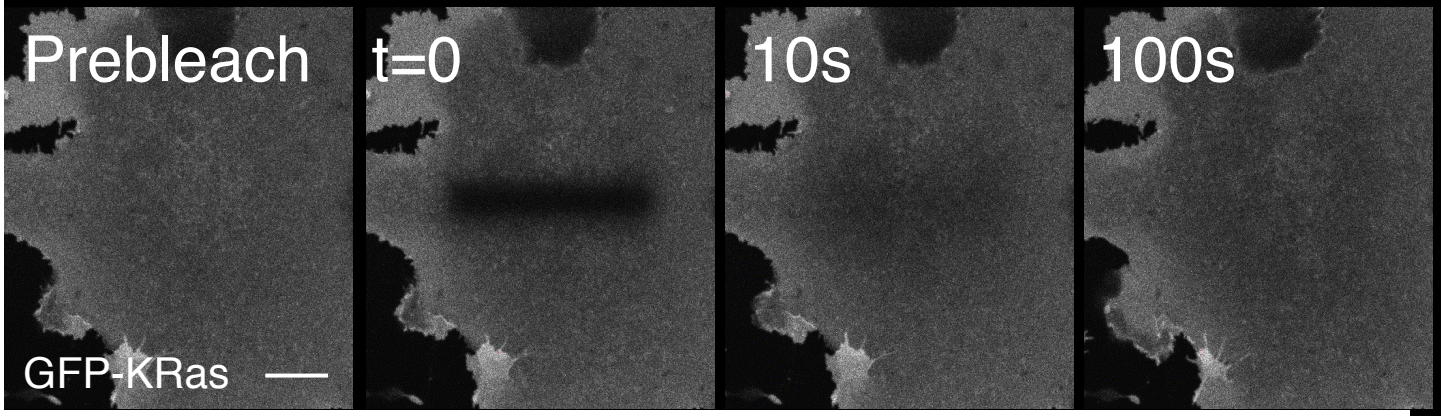
FRAP of ER and Nuclear Envelope

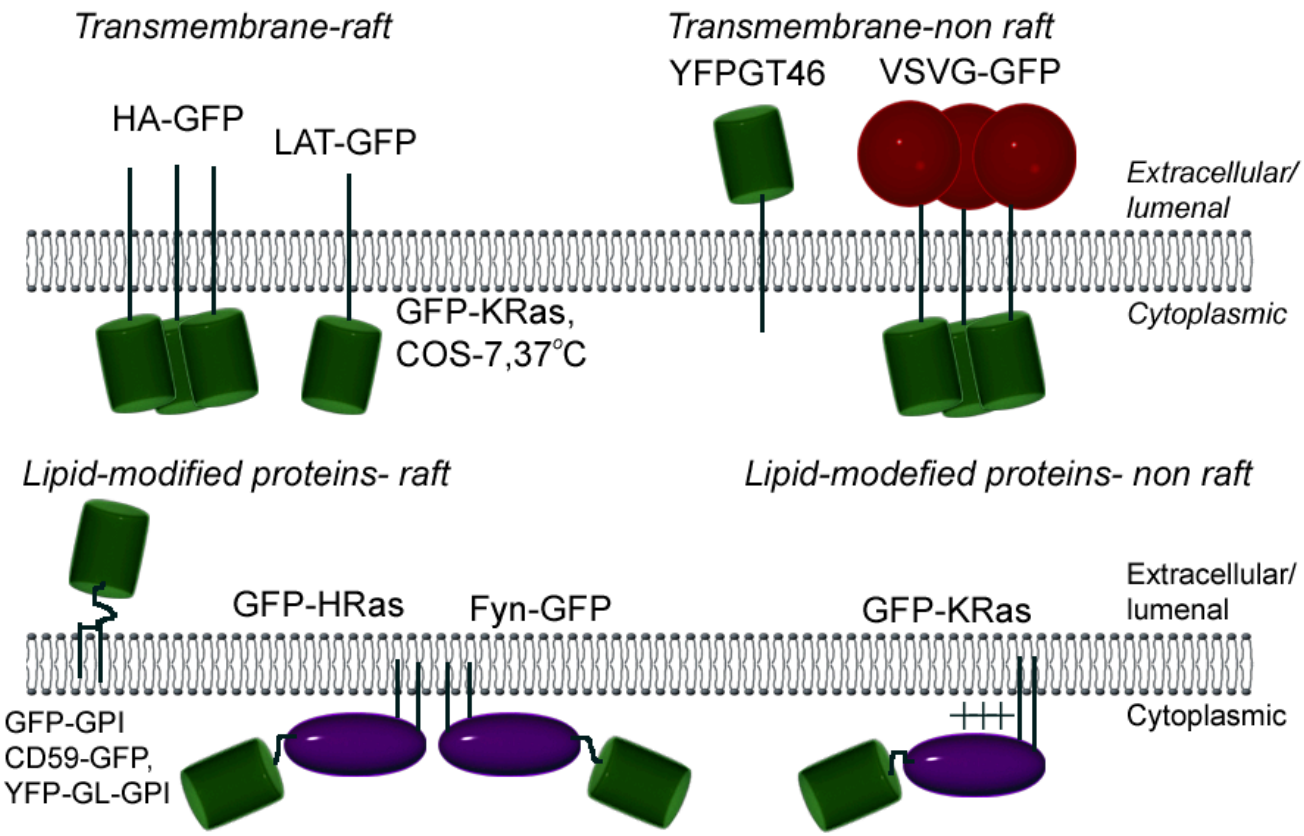


FRAP of Plasma Membrane

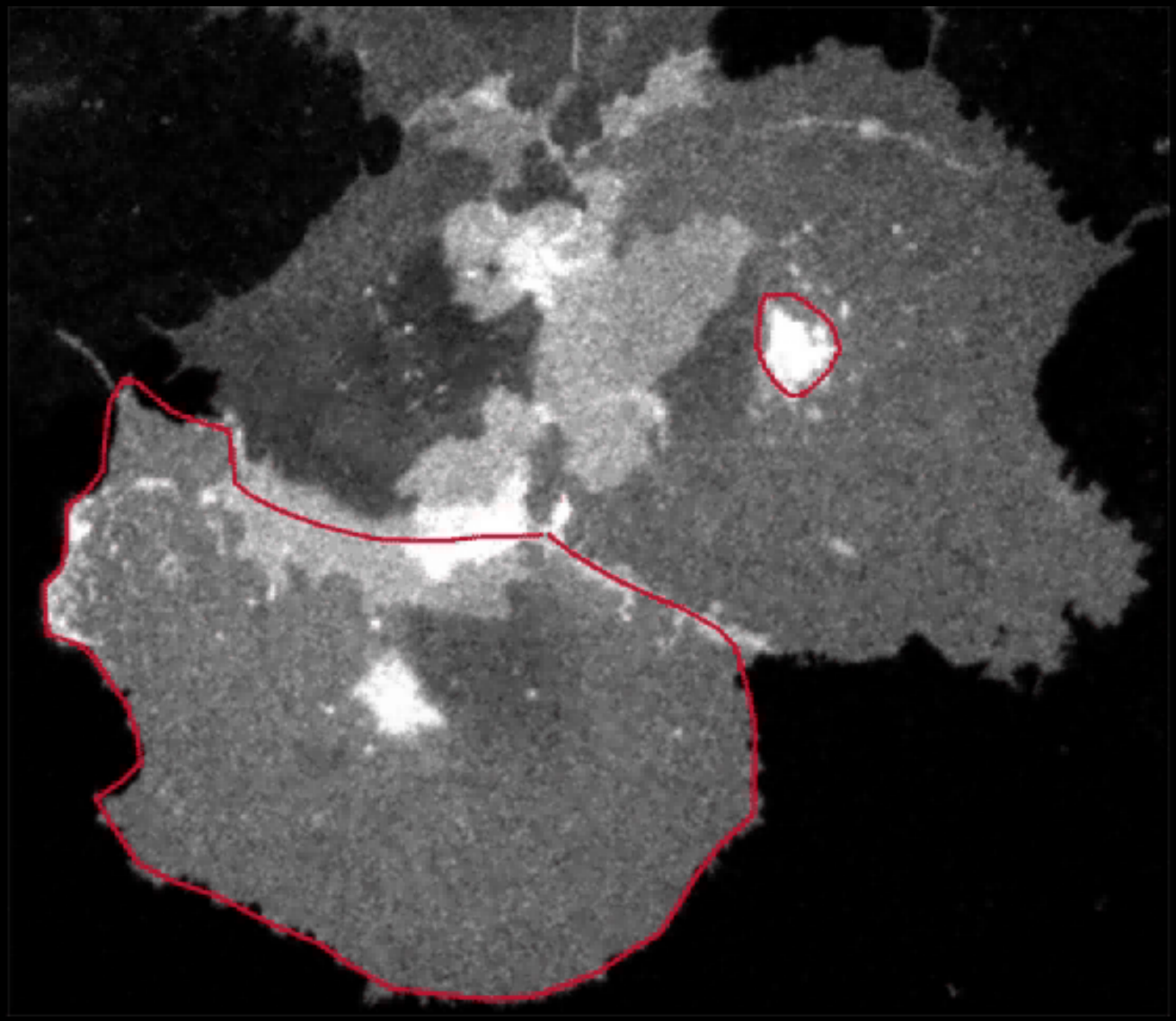


FRAP of Plasma Membrane



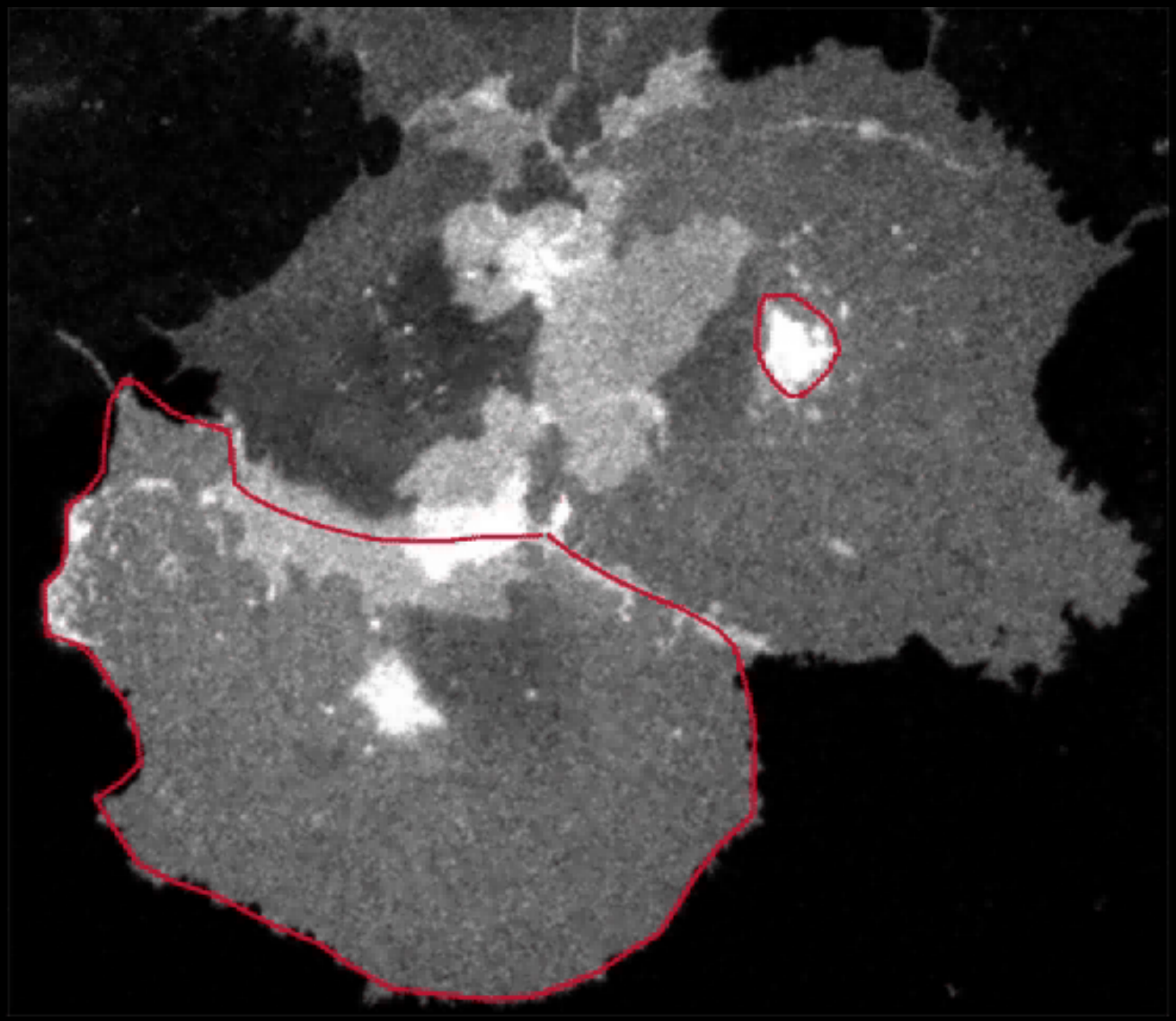


Protein cycling between plasma membrane and Golgi



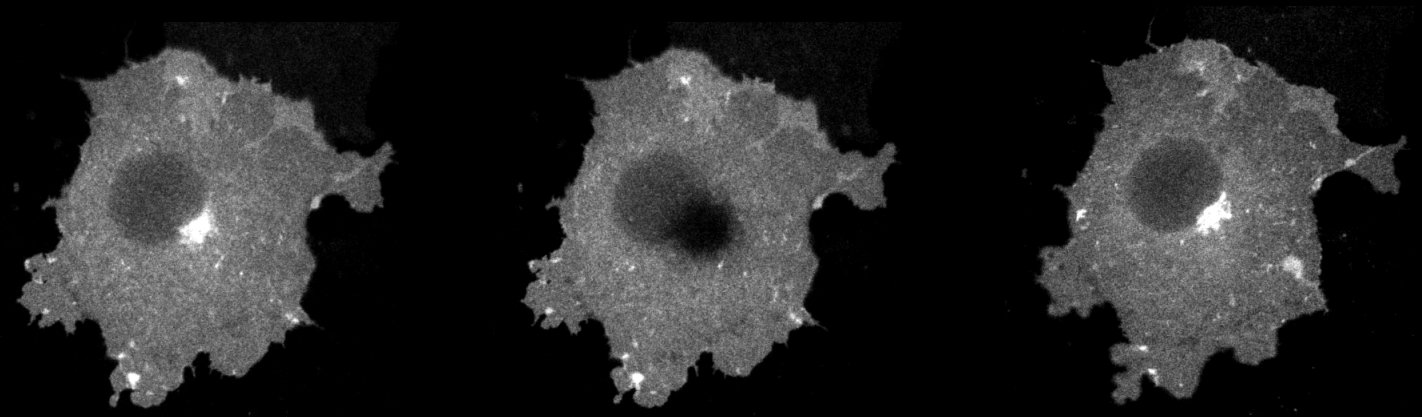
GPI-GFP

Protein cycling between plasma membrane and Golgi

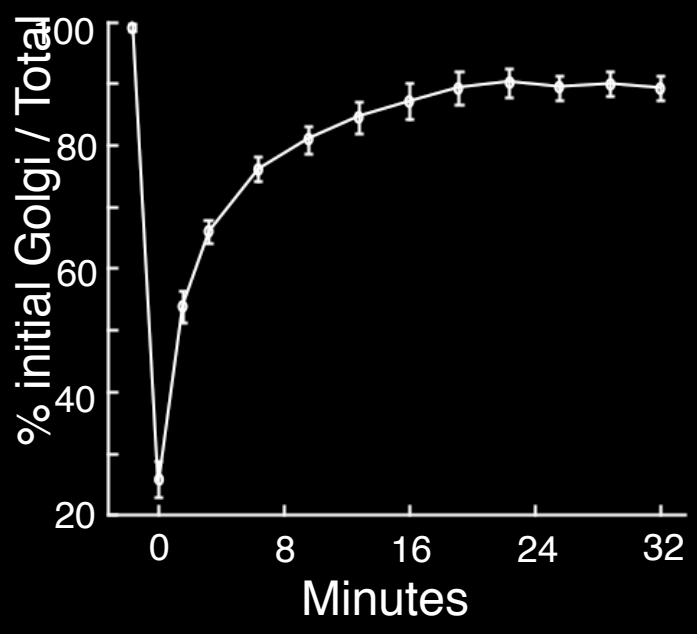


GPI-GFP

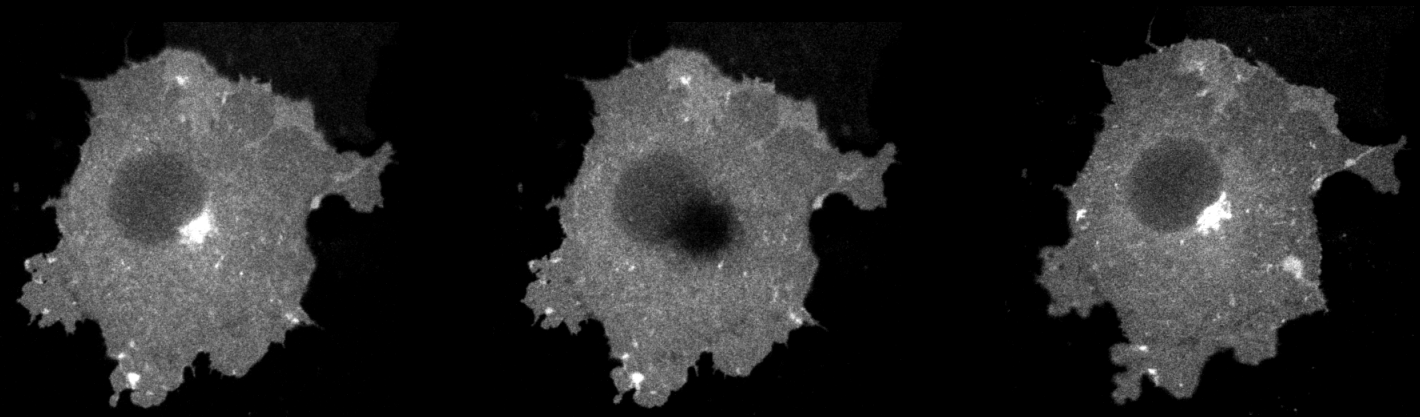
Kinetics of GPI-GFP cycling between PM and Golgi



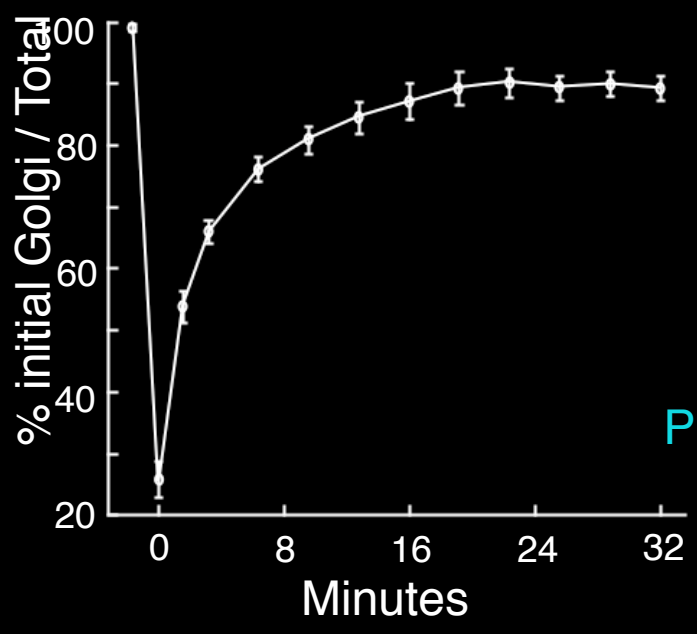
Pre-bleach Post-bleach Recovery, 20 min

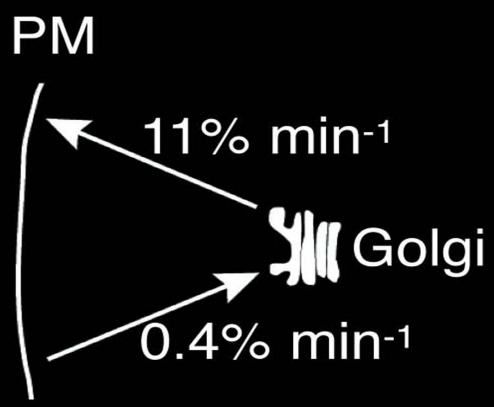


Kinetics of GPI-GFP cycling between PM and Golgi



Pre-bleach Post-bleach Recovery, 20 min

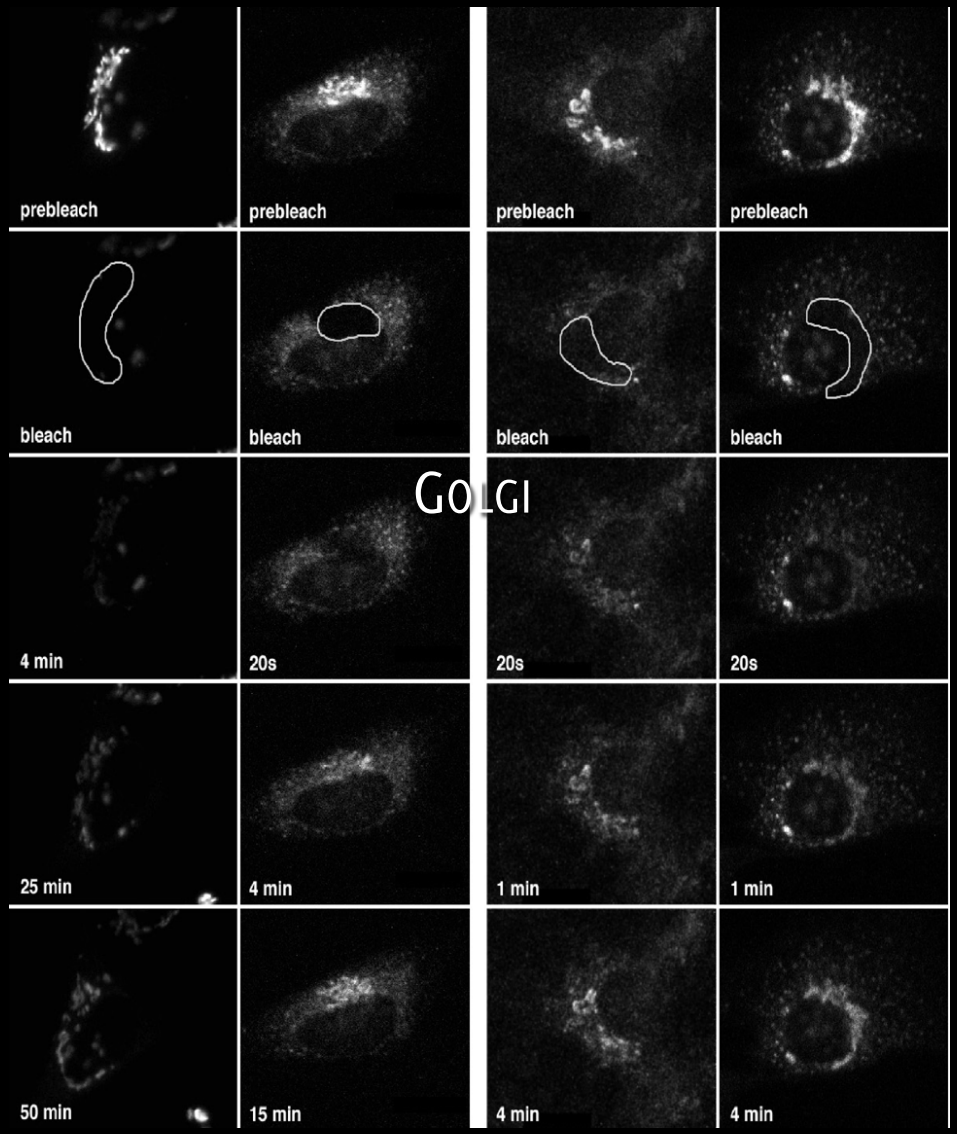




Residence times

PM (240 min) Golgi (9 min)

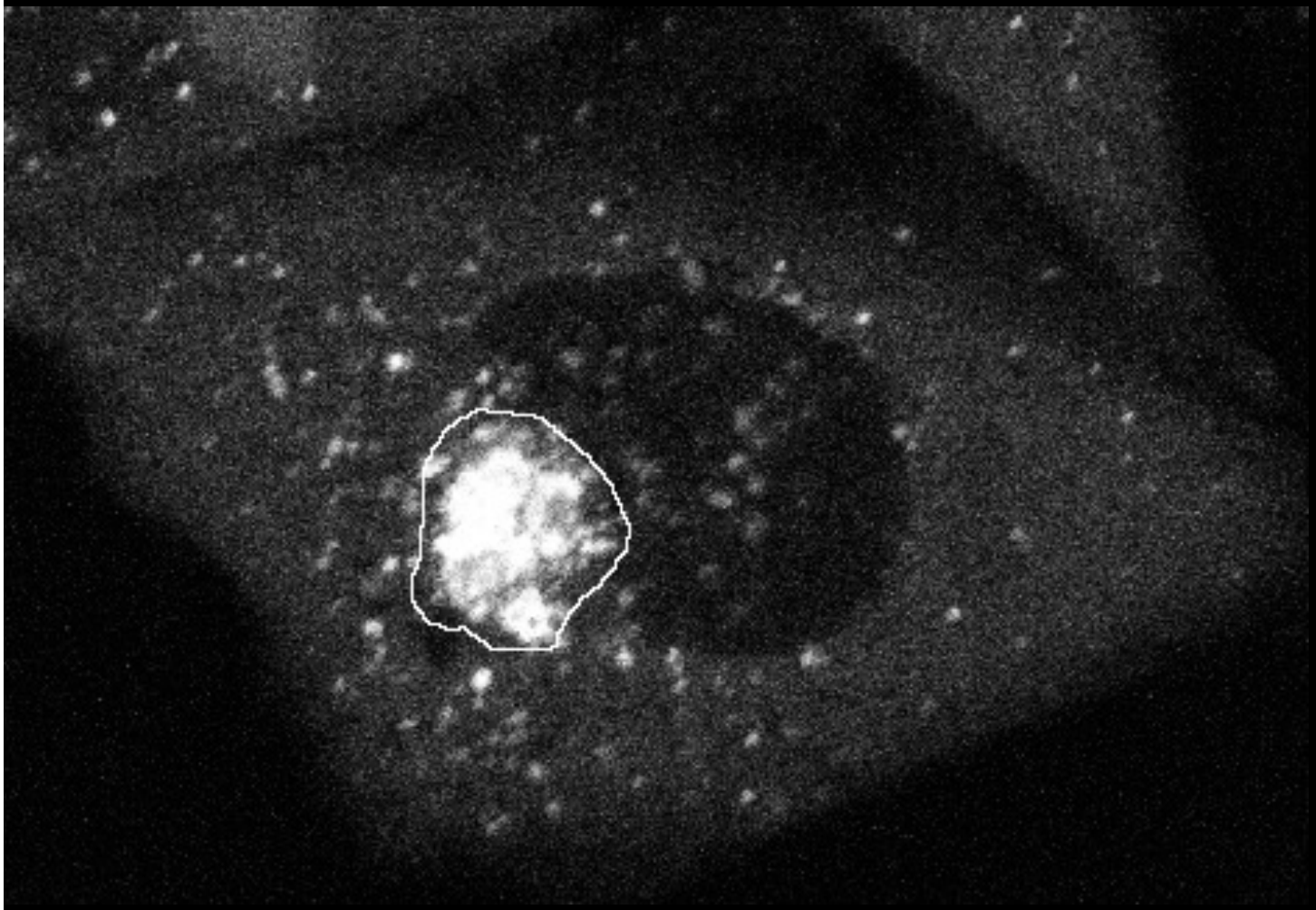
Golgi proteins all associate dynamically w/ Golgi



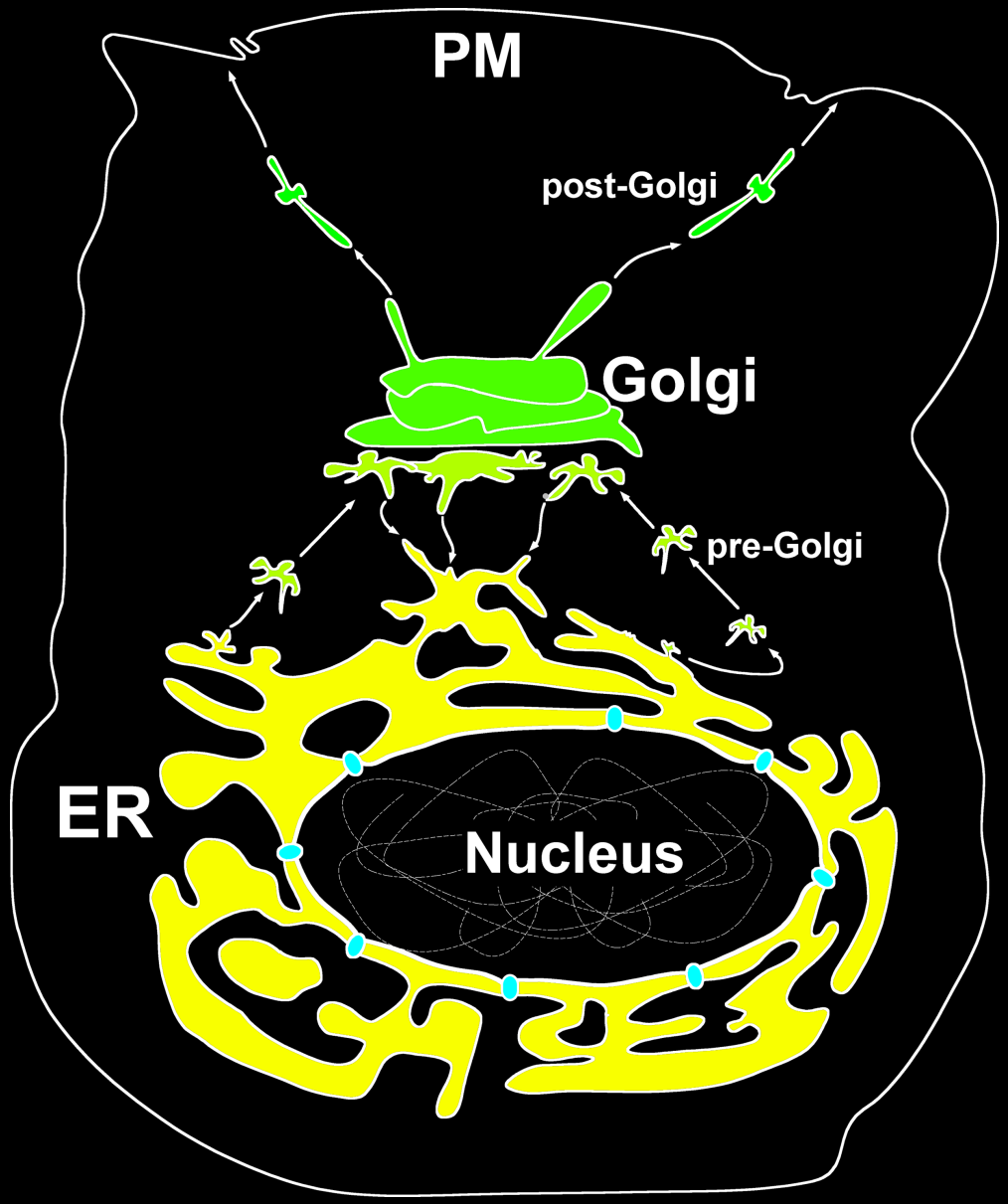
GalT-GFP p58-GFP GRASP-GFP ε-COP-GFP

FRAP of
Golgi ROI

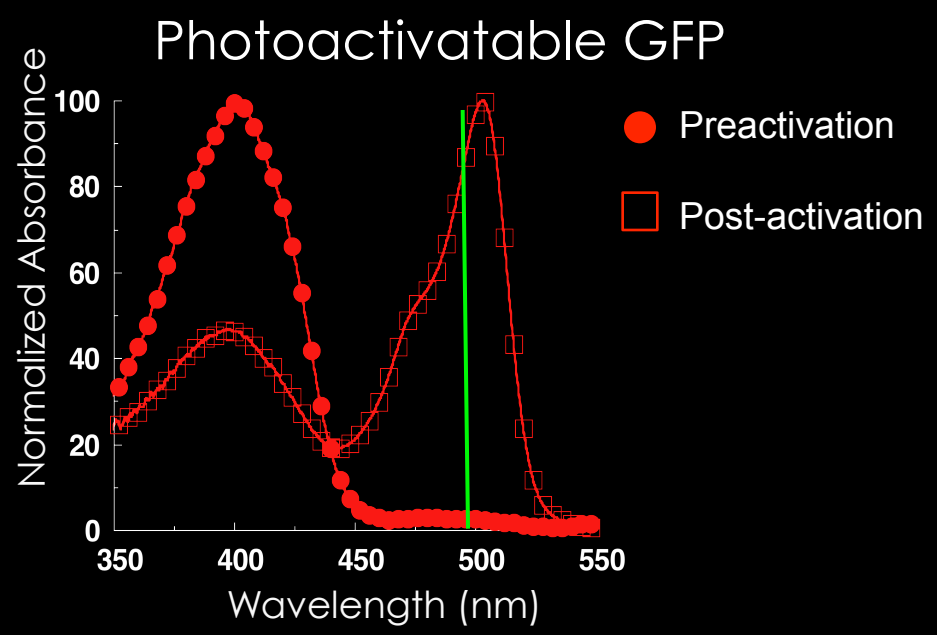
Coat cycling on & off Golgi membranes

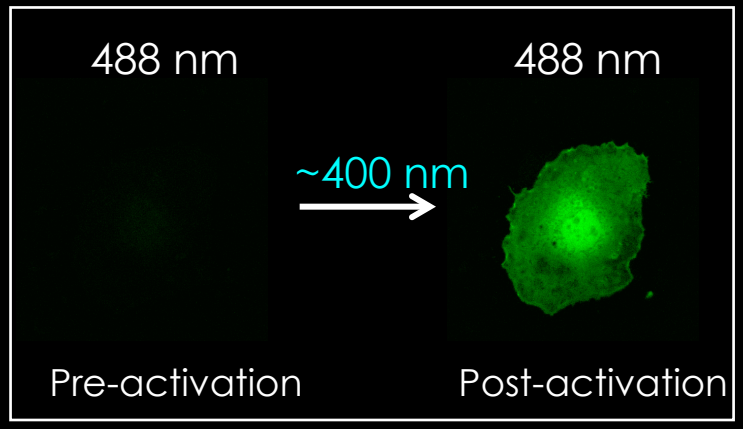


Golgi Apparatus as Steady-State Organelle

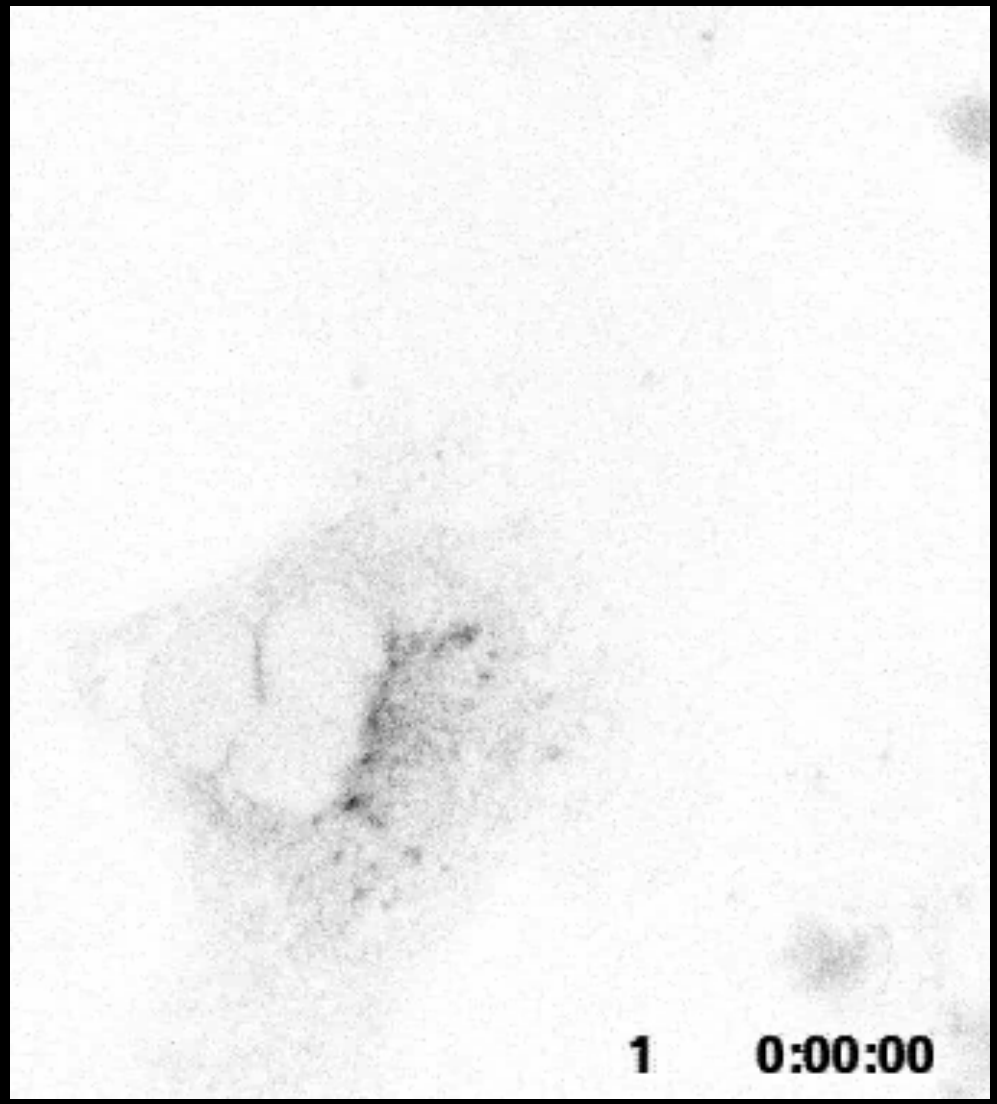


Highlighting using Photoactivation





Photoactivatable FP Applications



VSVG-PAGFP

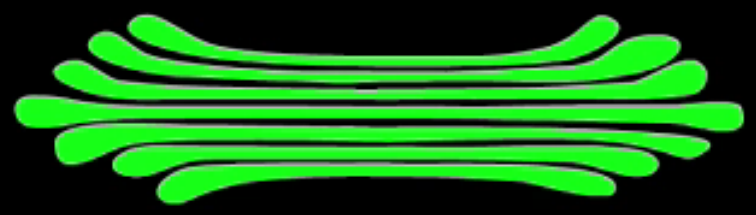
Quantifying transport from the Golgi

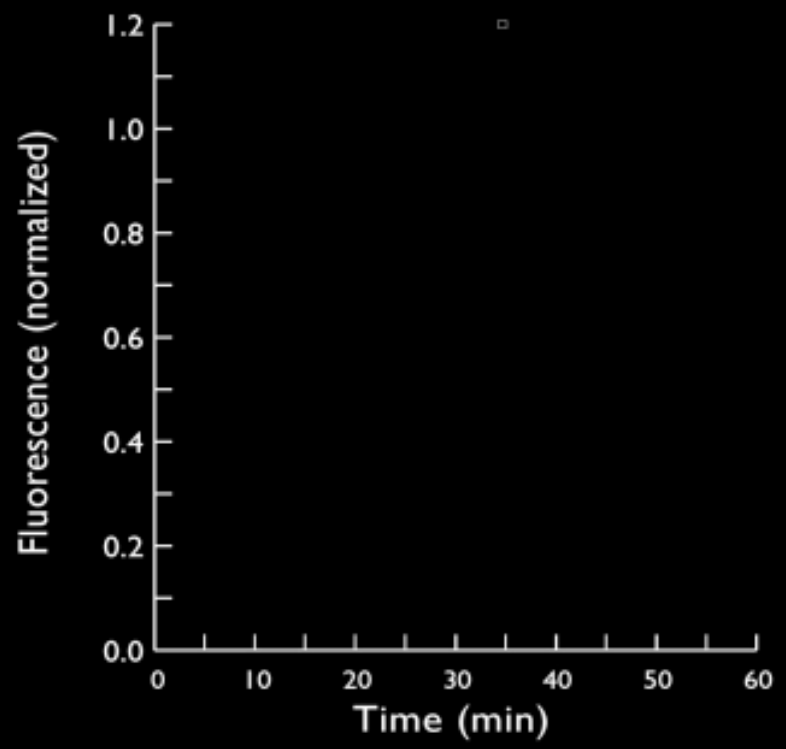
Cisternal Progression and its Predictions

cis (entry)

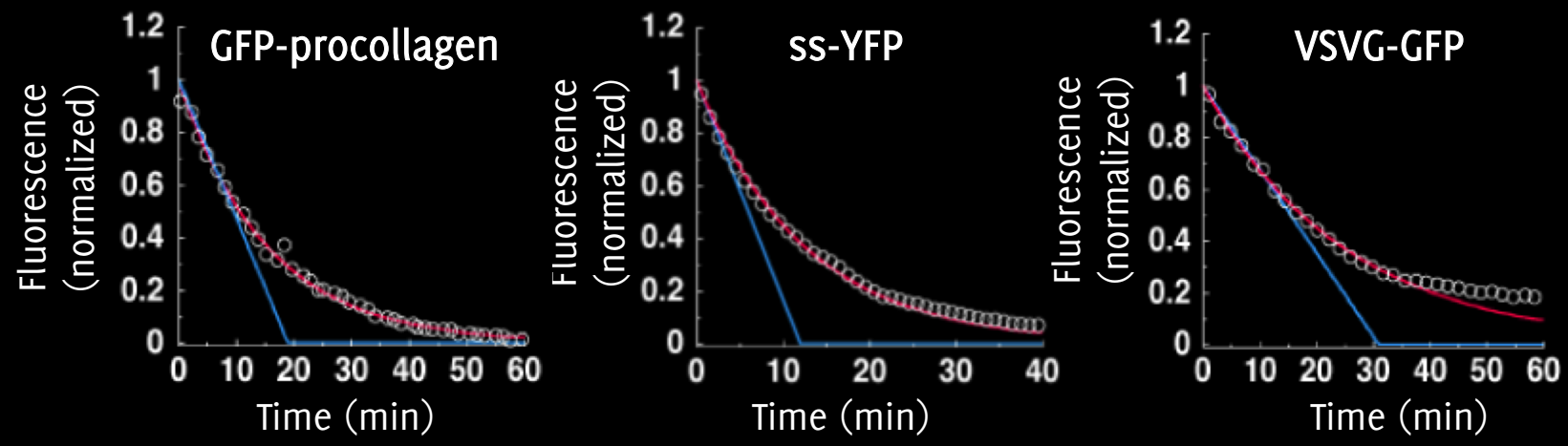
Golgi

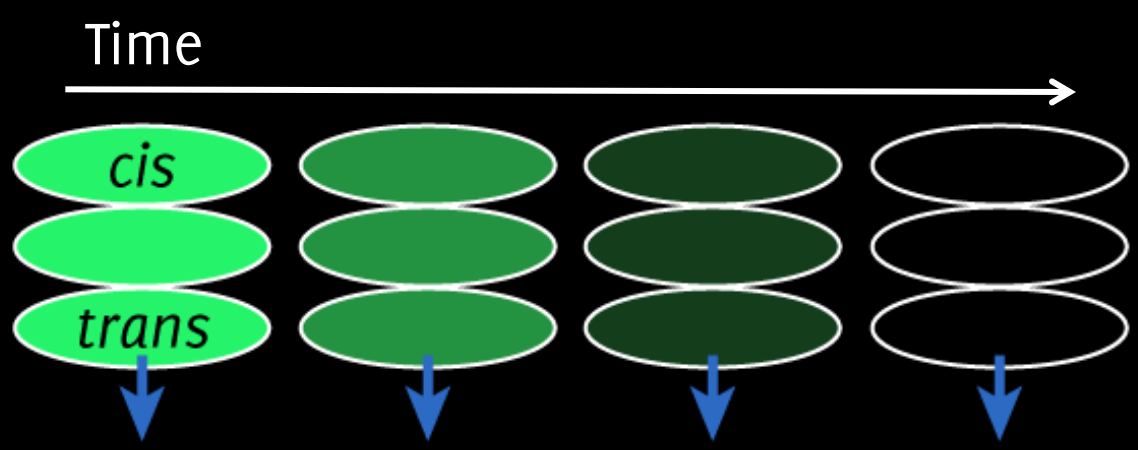
Trans (exit)





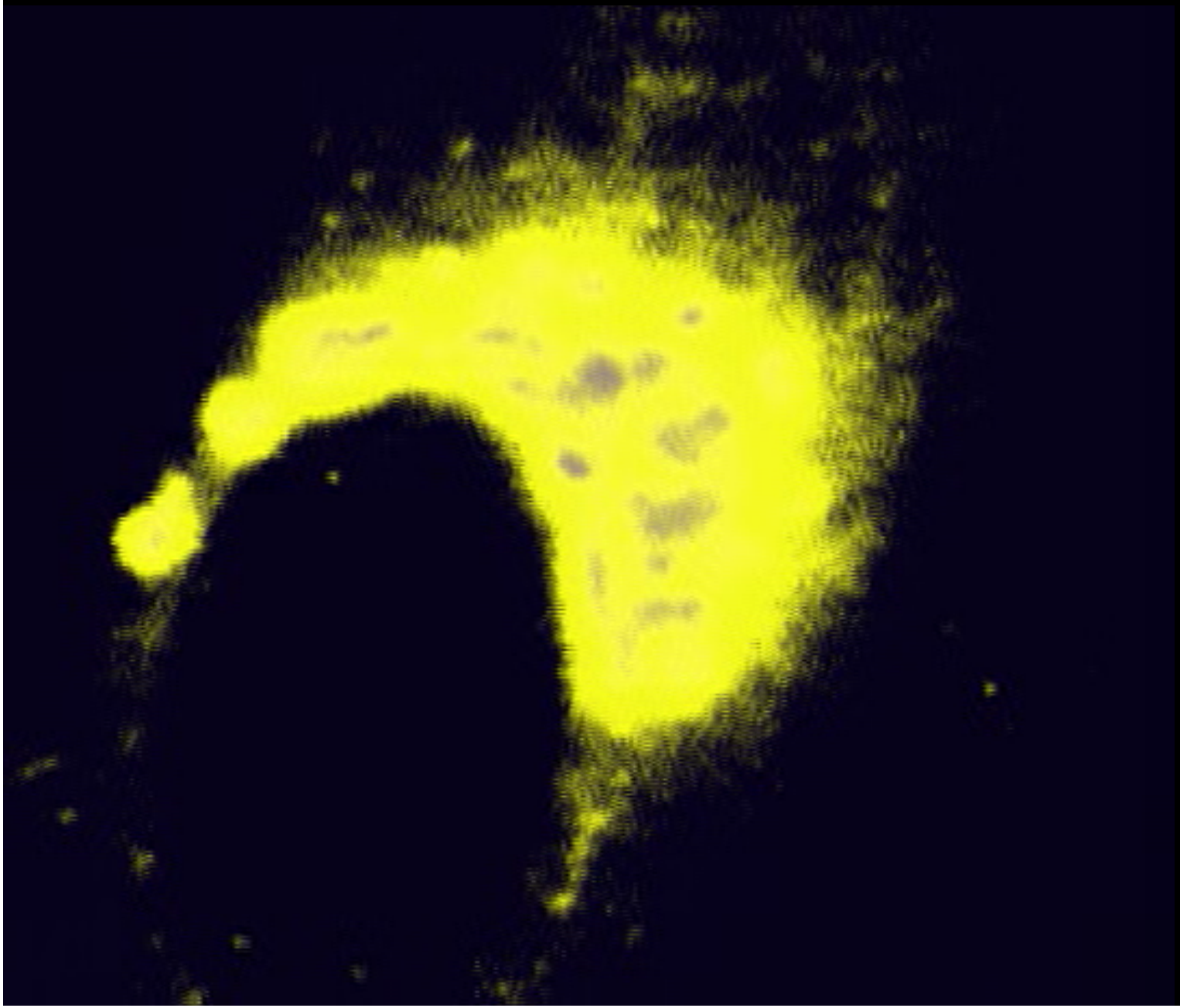
See Exponential, not linear, cargo export kinetics



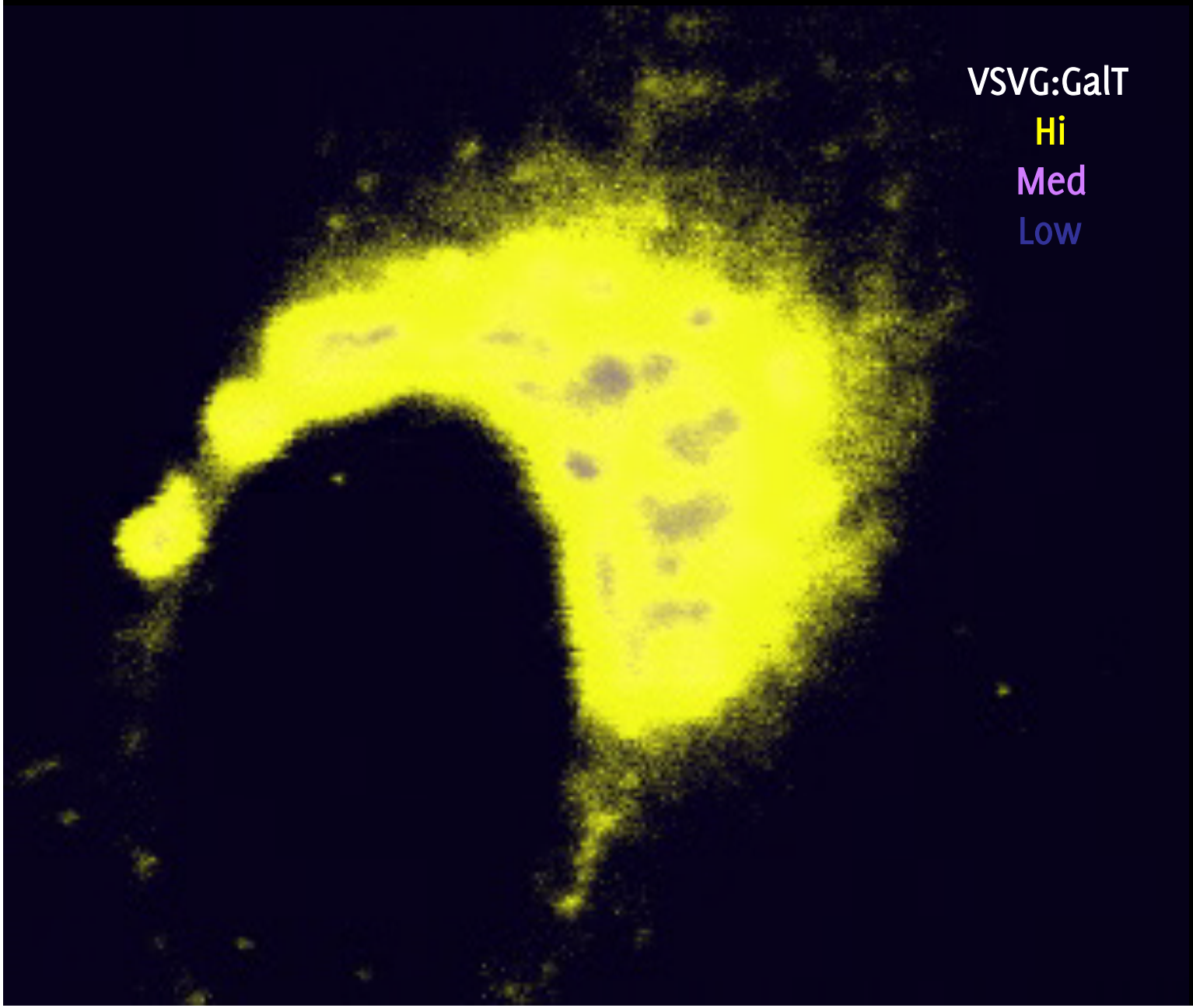


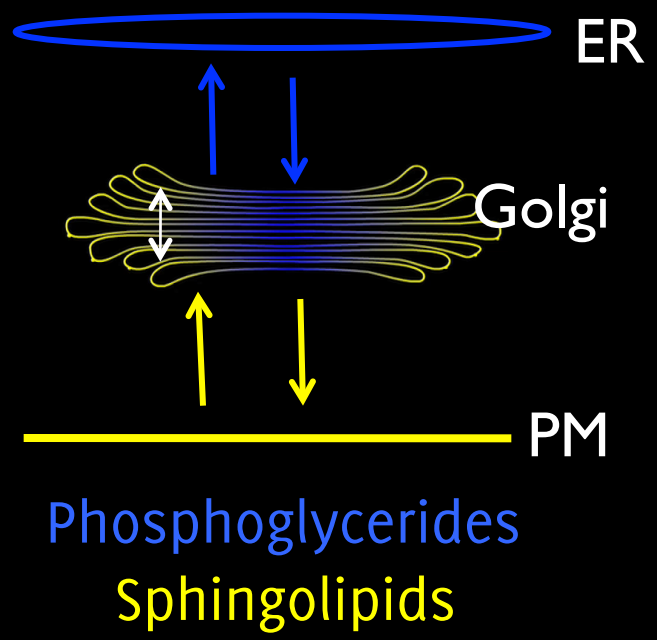
Rapid mixing

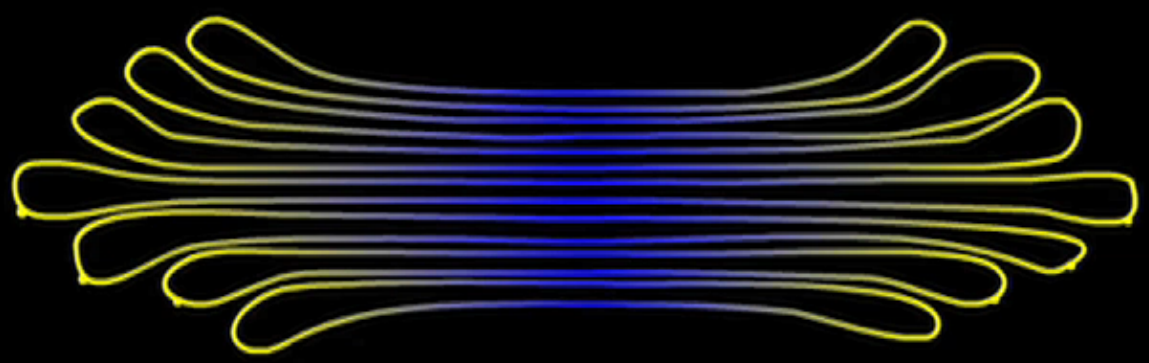
Protein Partitioning within the Golgi



Protein Partitioning within the Golgi

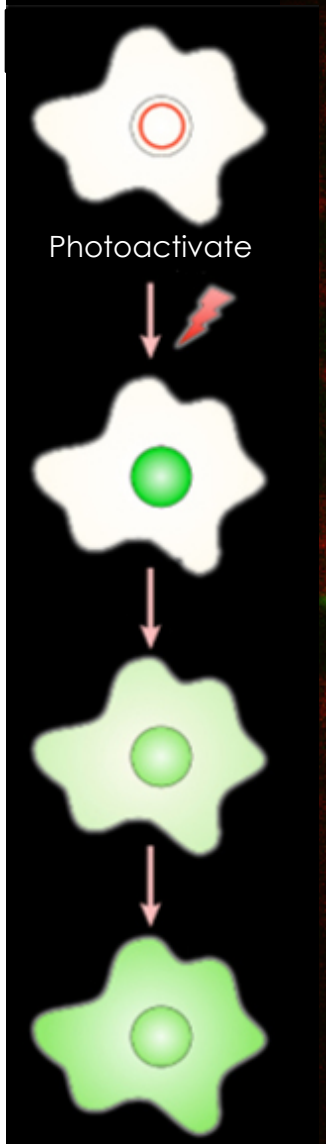


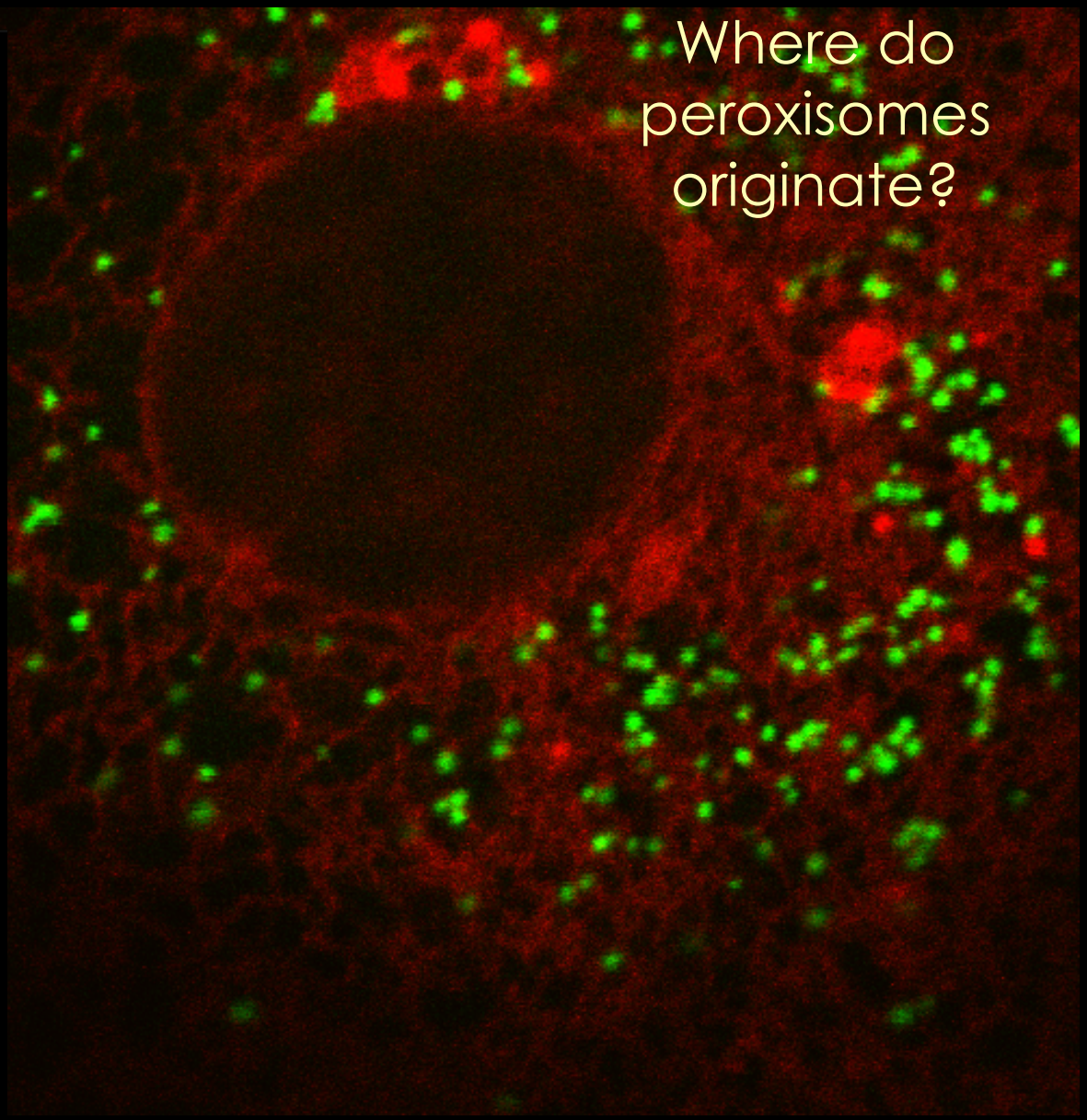




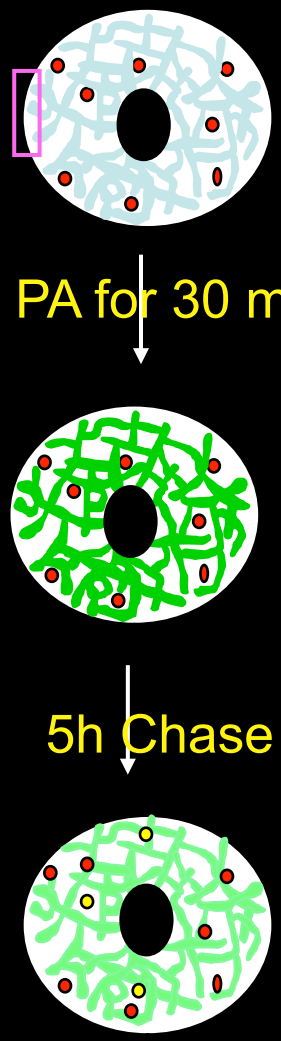
Intra-Golgi transport by rapid membrane partitioning

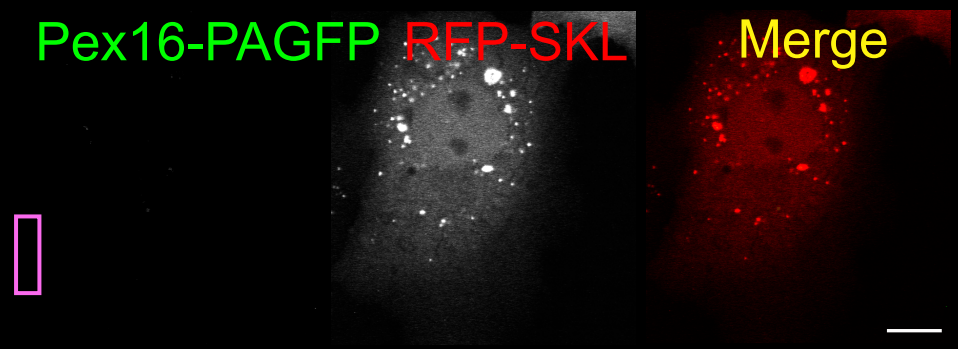
Peroxisome Biogenesis





In cellulo Pulse Chase Analysis





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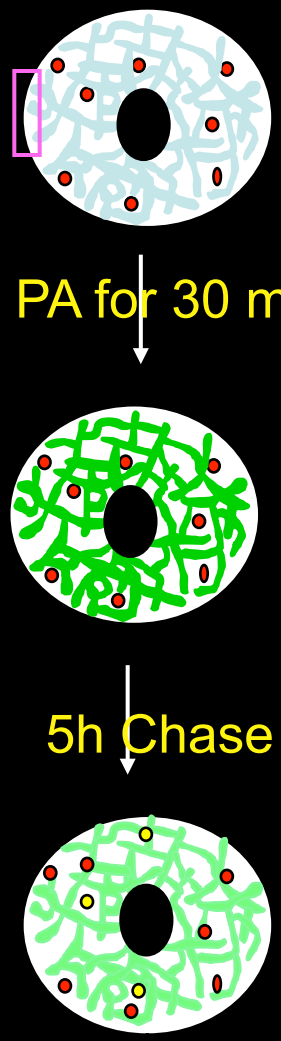
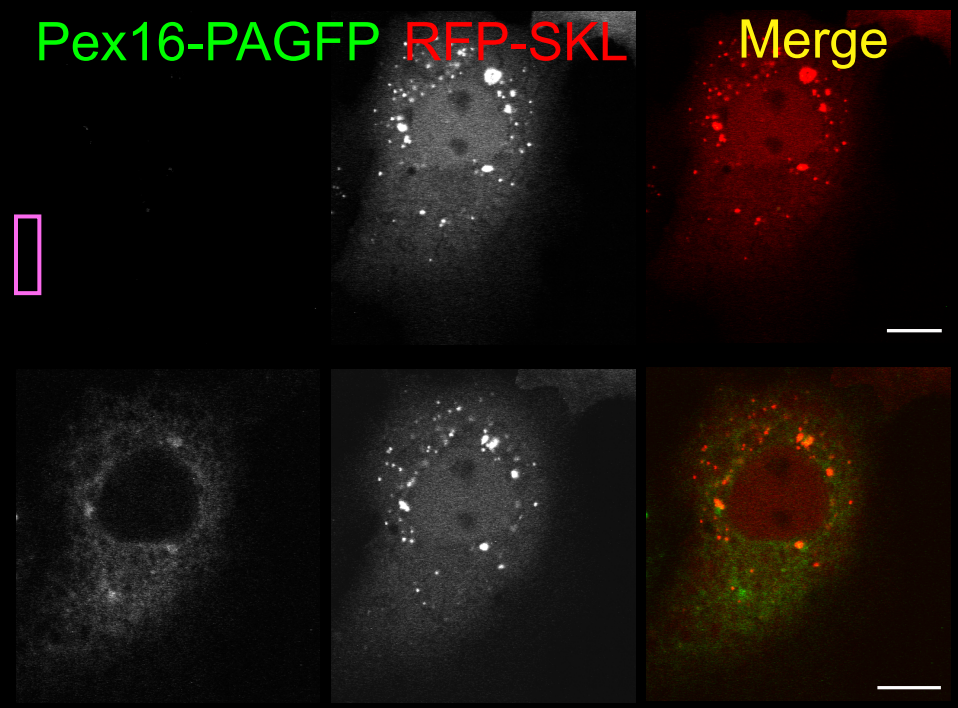
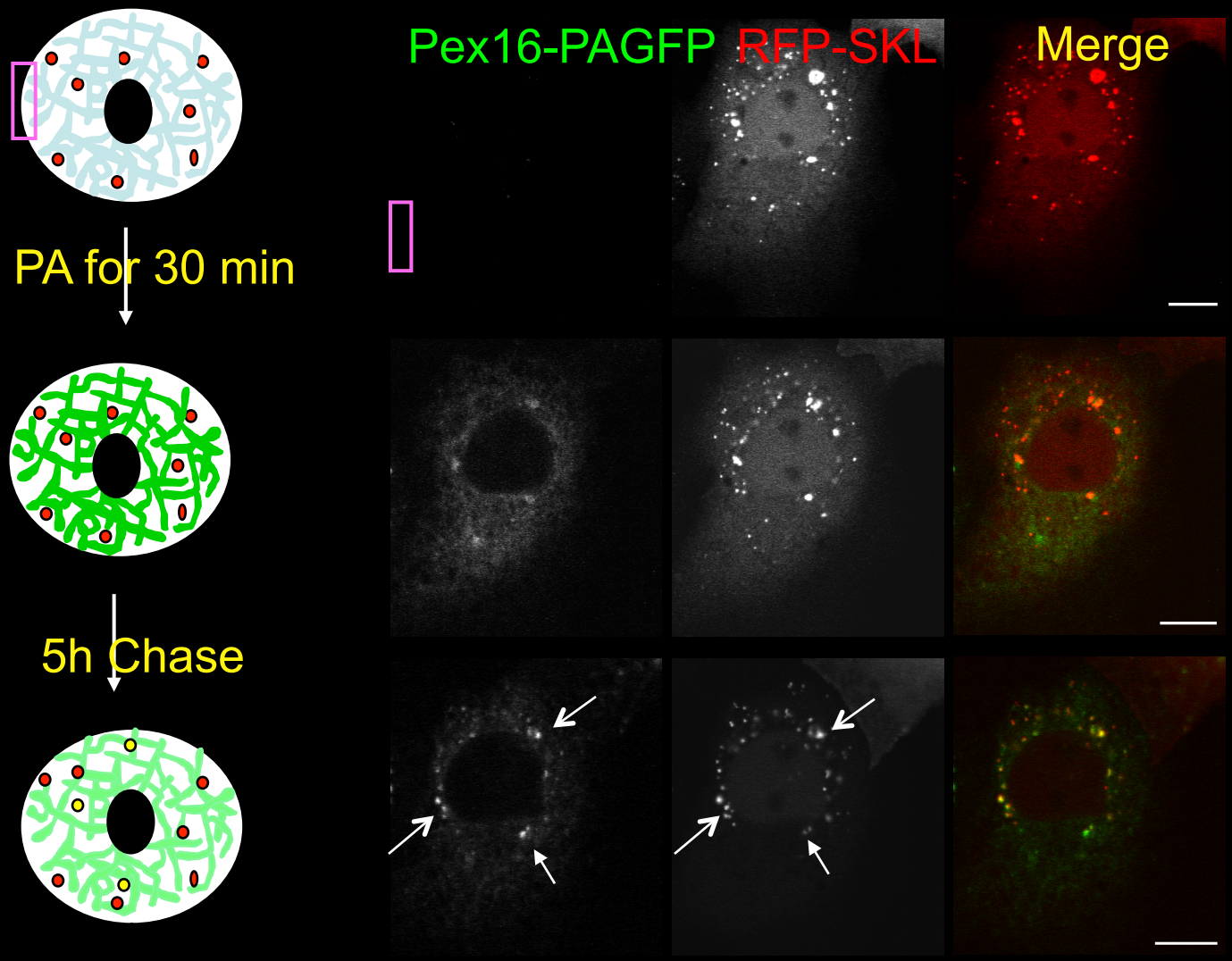
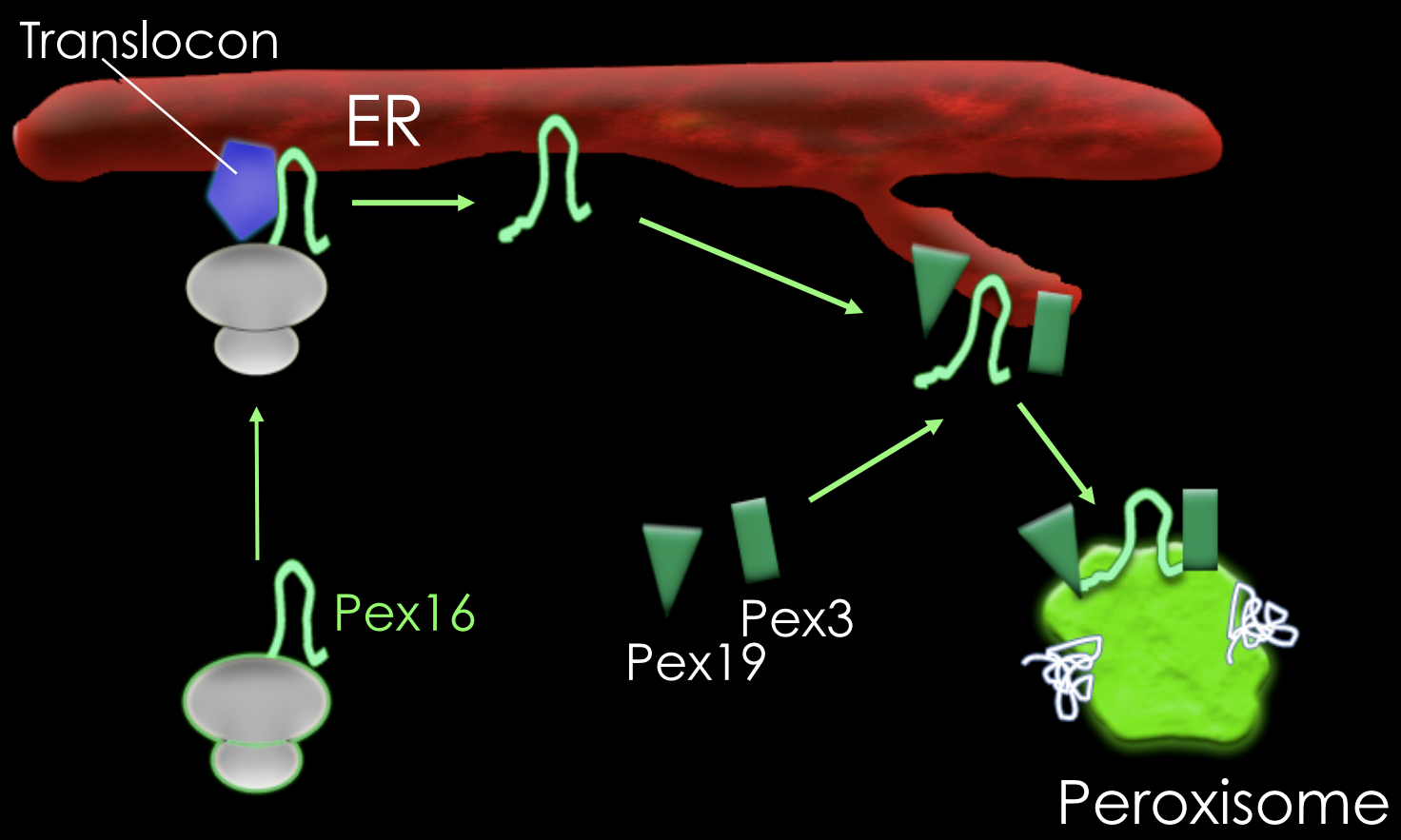




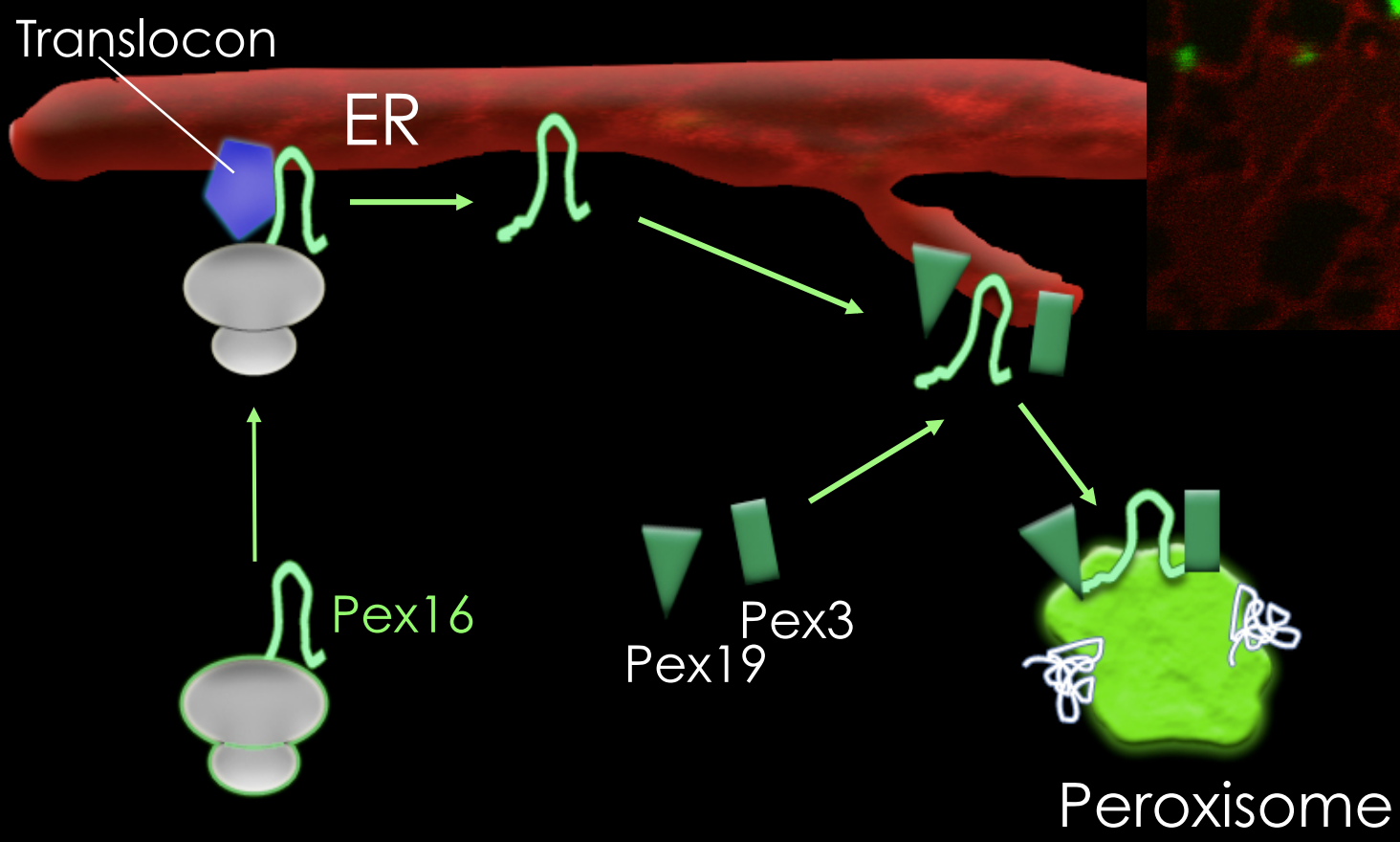
In cellulo Pulse Chase Analysis

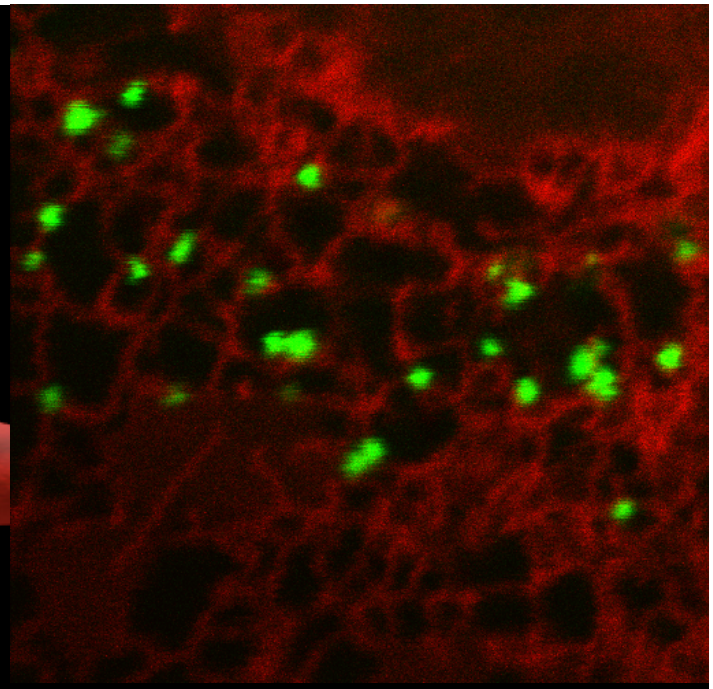


Biogenesis of peroxisomes from the ER

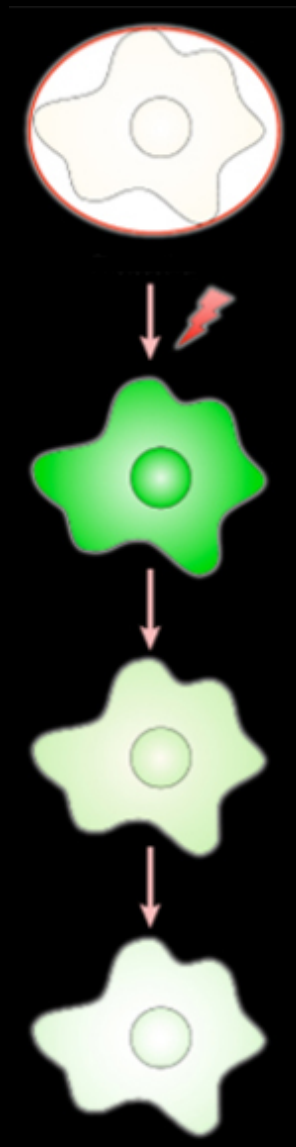


Biogenesis of peroxisomes from the ER



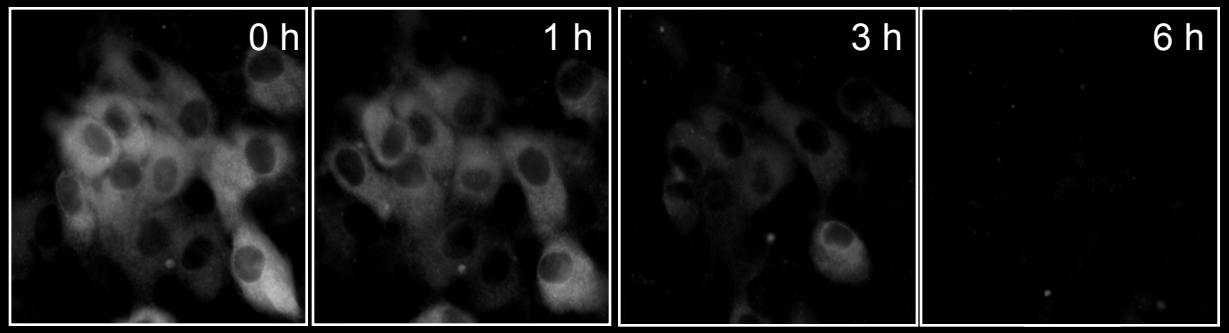


Photoactivatable FP Applications



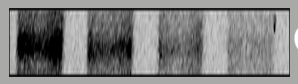
Turnover

CD3δ-PAGFP

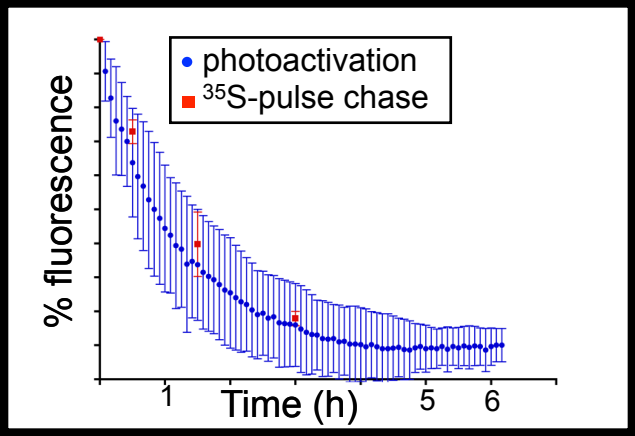


pulse chase:

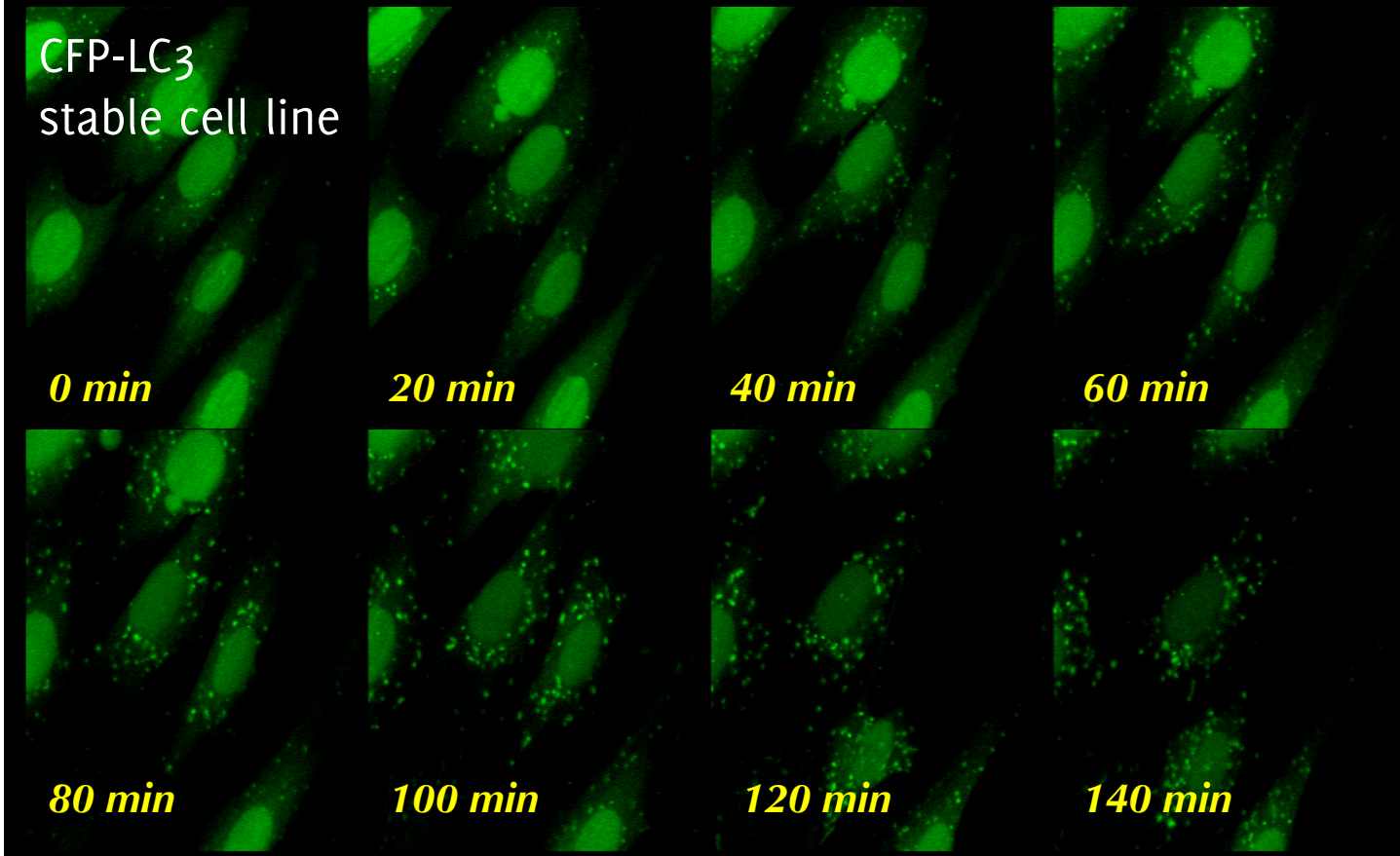
0 1 3 6 (h)



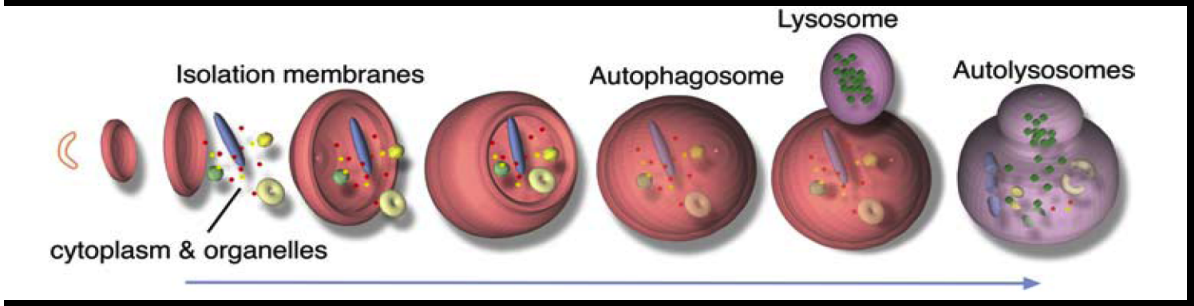
CD3δ-PAGFP

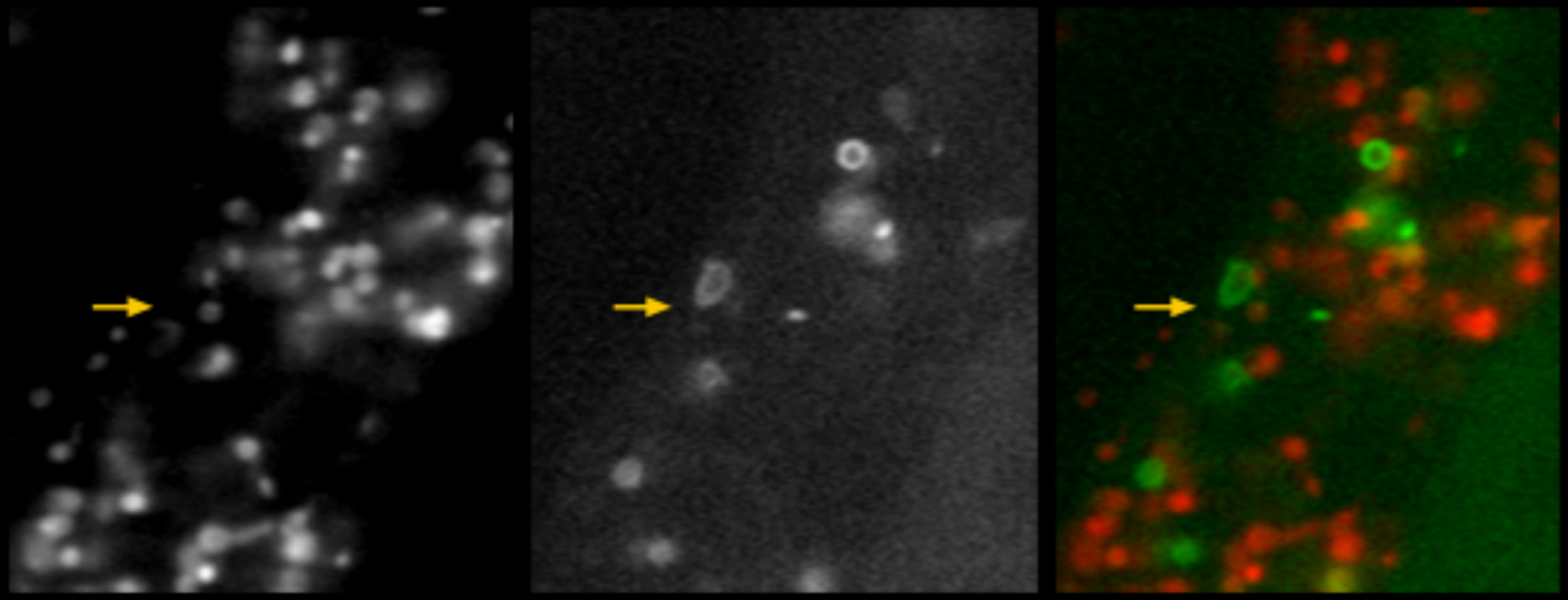


Starvation-induced Autophagy



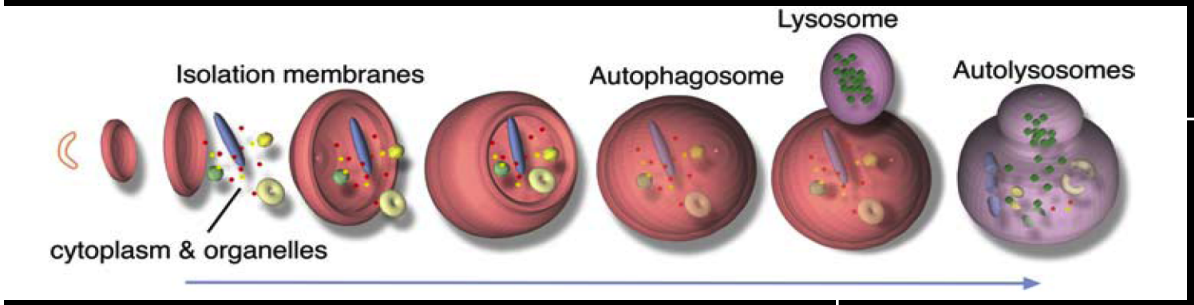
Starvation-induced Autophagy





Red= Lysotracker Green= GFP-LC3

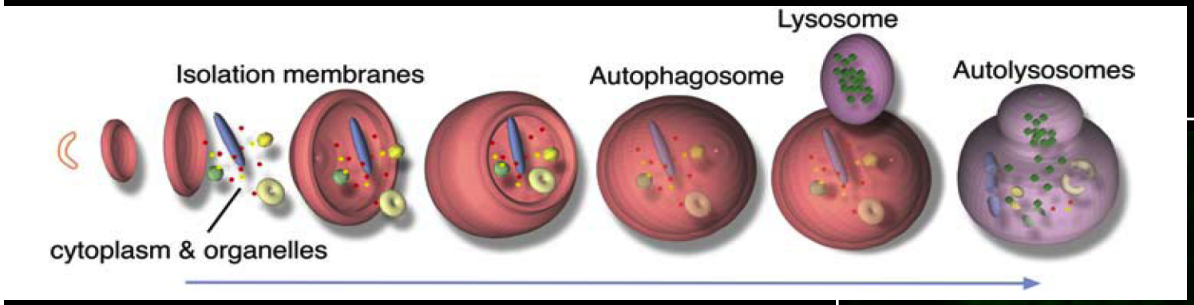
Starvation-induced Autophagy



PAGFP-LC3

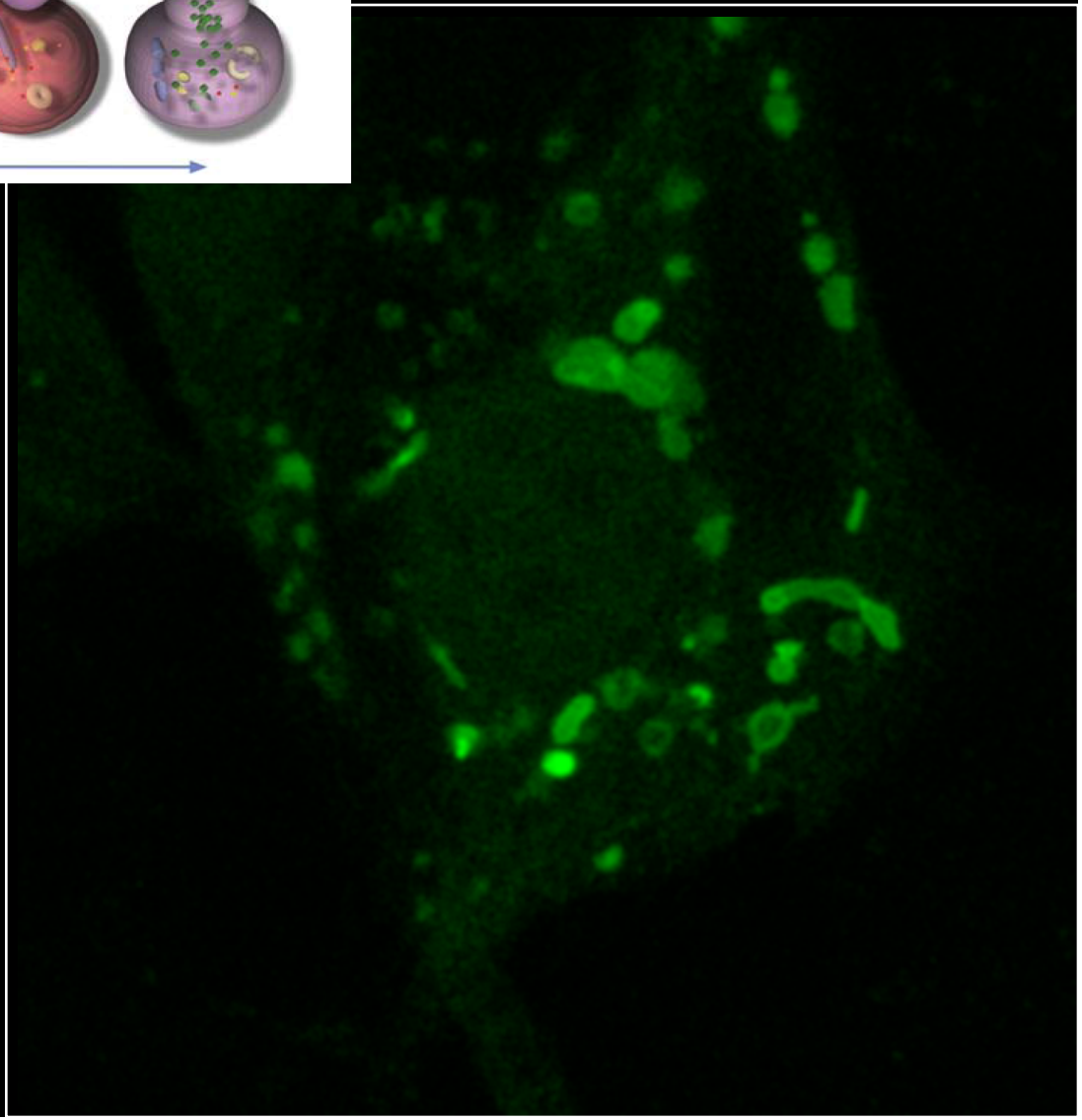
(5 min / frame)

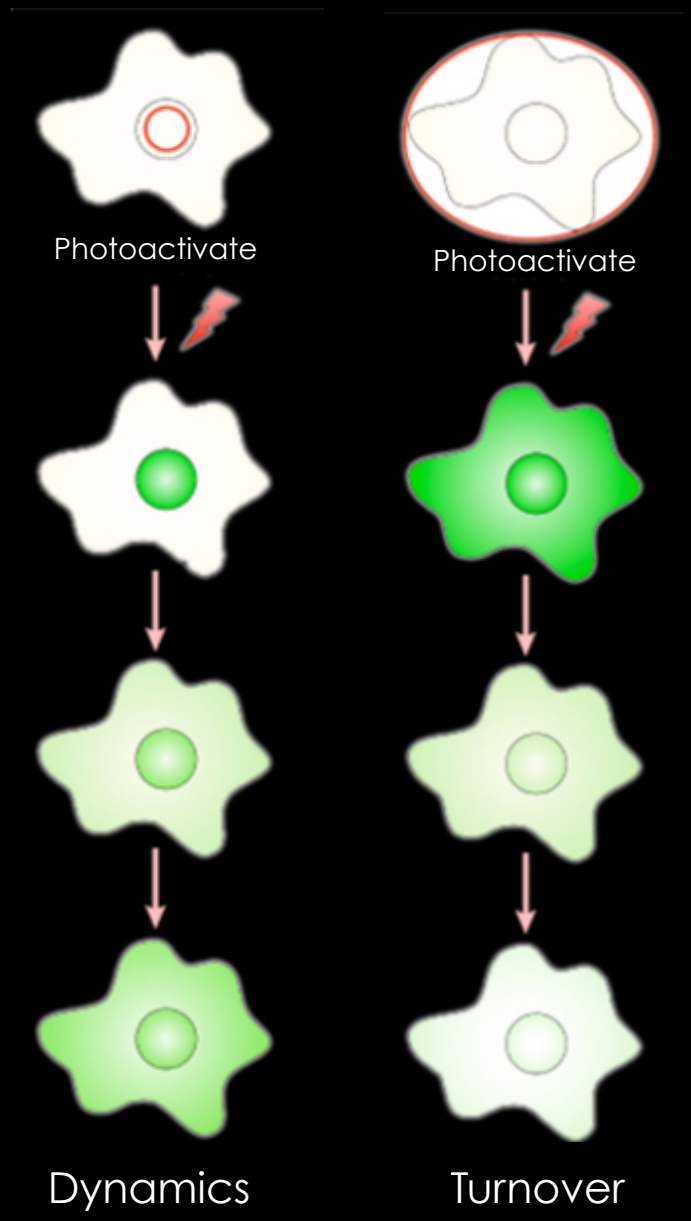
Starvation-induced Autophagy



PAGFP-LC3

(5 min / frame)



Autophagosome
Half-life ~ 18 min

Photoactivatable FP Applications