

Endocytosis

tom kirchhausen

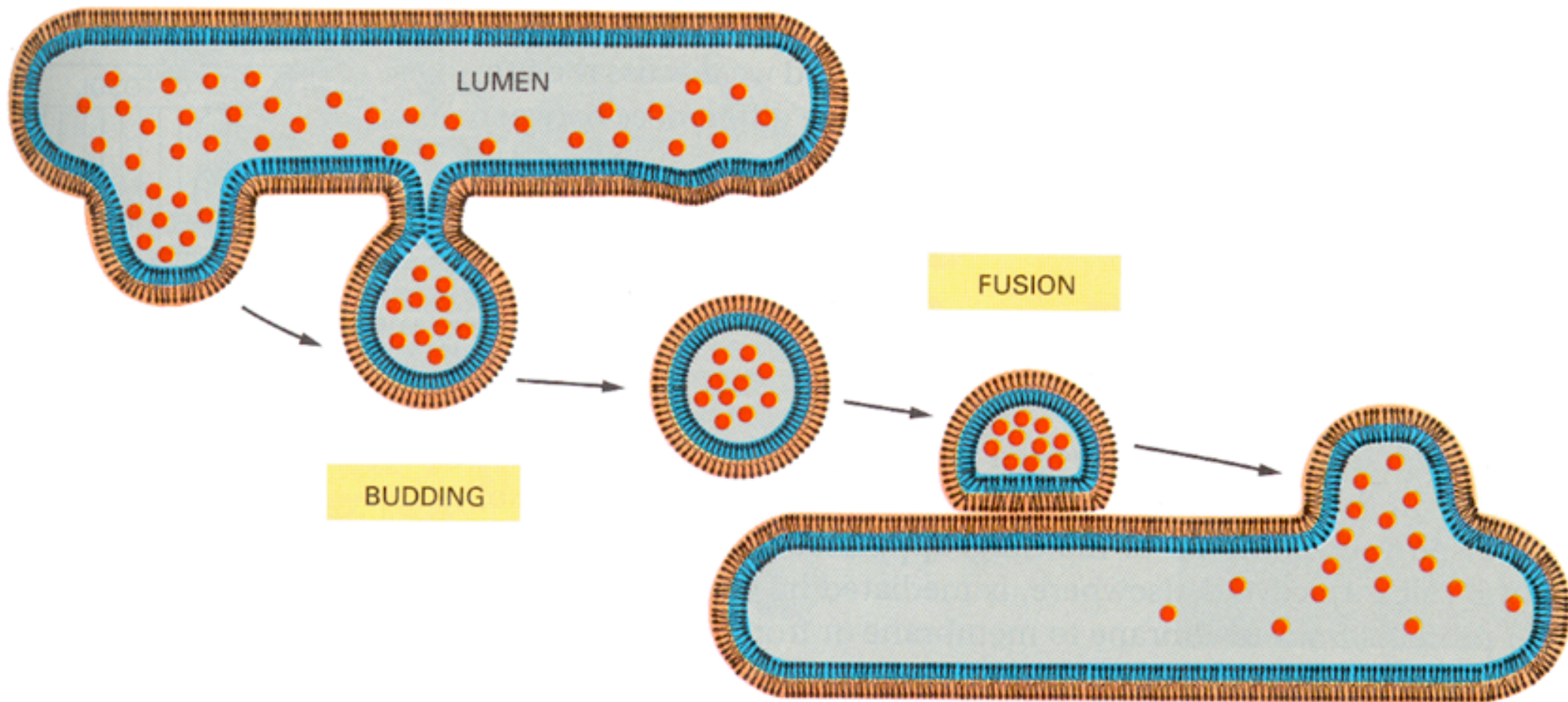
kirchhausen@crystal.harvard.edu

MIT 2006

Vesicular-based transport of proteins and lipid

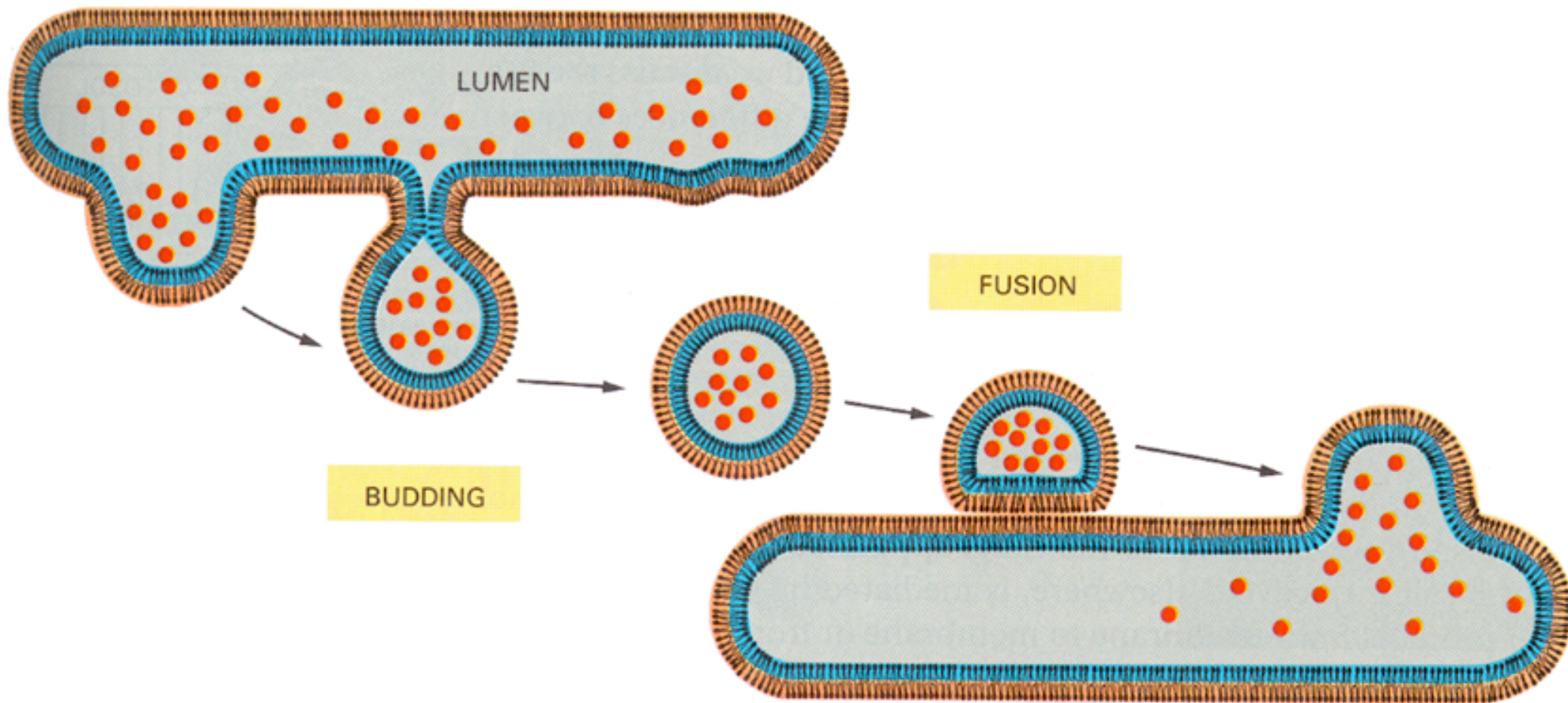
- Hallmark of eukaryotic cells
- Extraordinarily large & fast membrane fluxes along endocytic and secretory pathways
- Traffic is selective
- Preserves distinct identities of membranous organelles throughout the life of the cell

1a. basic considerations in vesicular transport



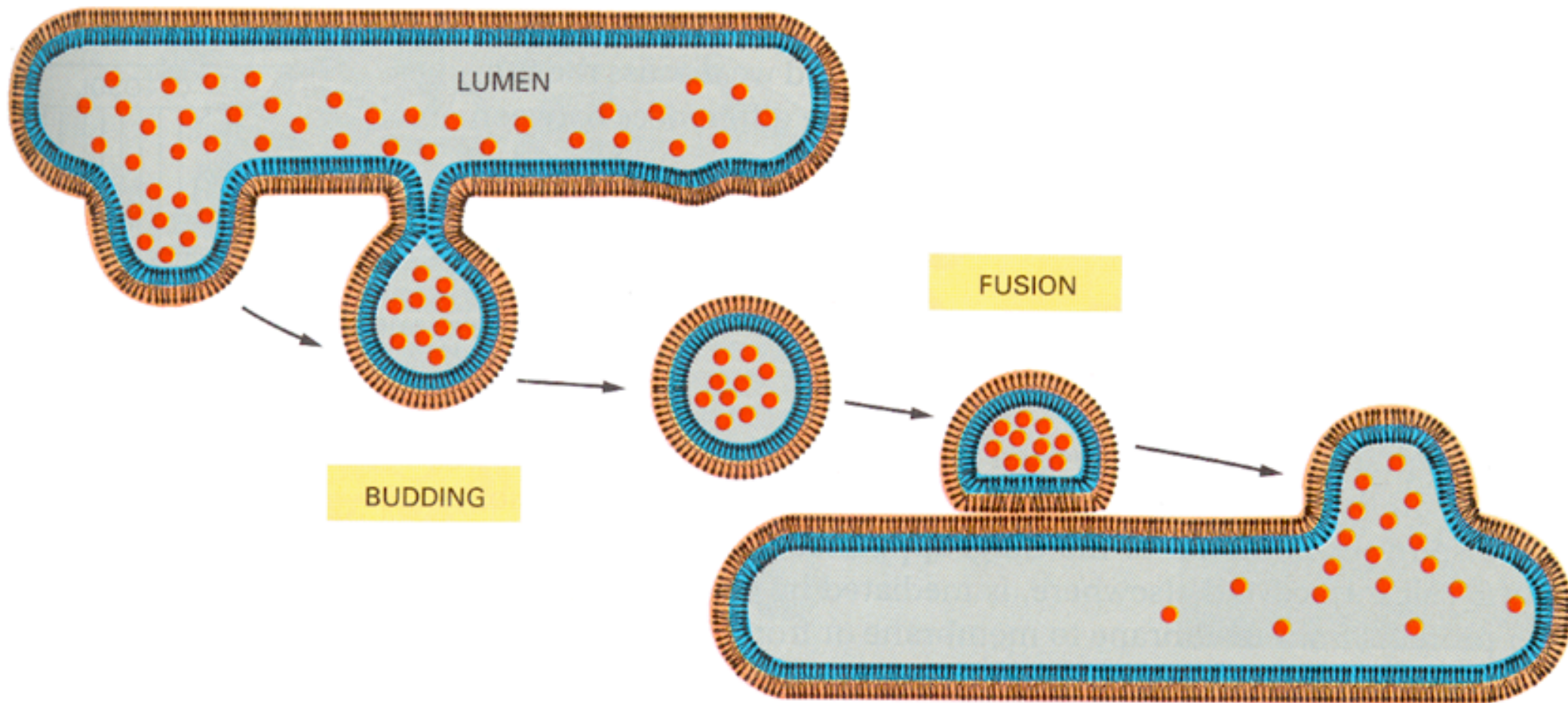
1a. basic considerations in vesicular transport

- selection: cargo sorting before budding



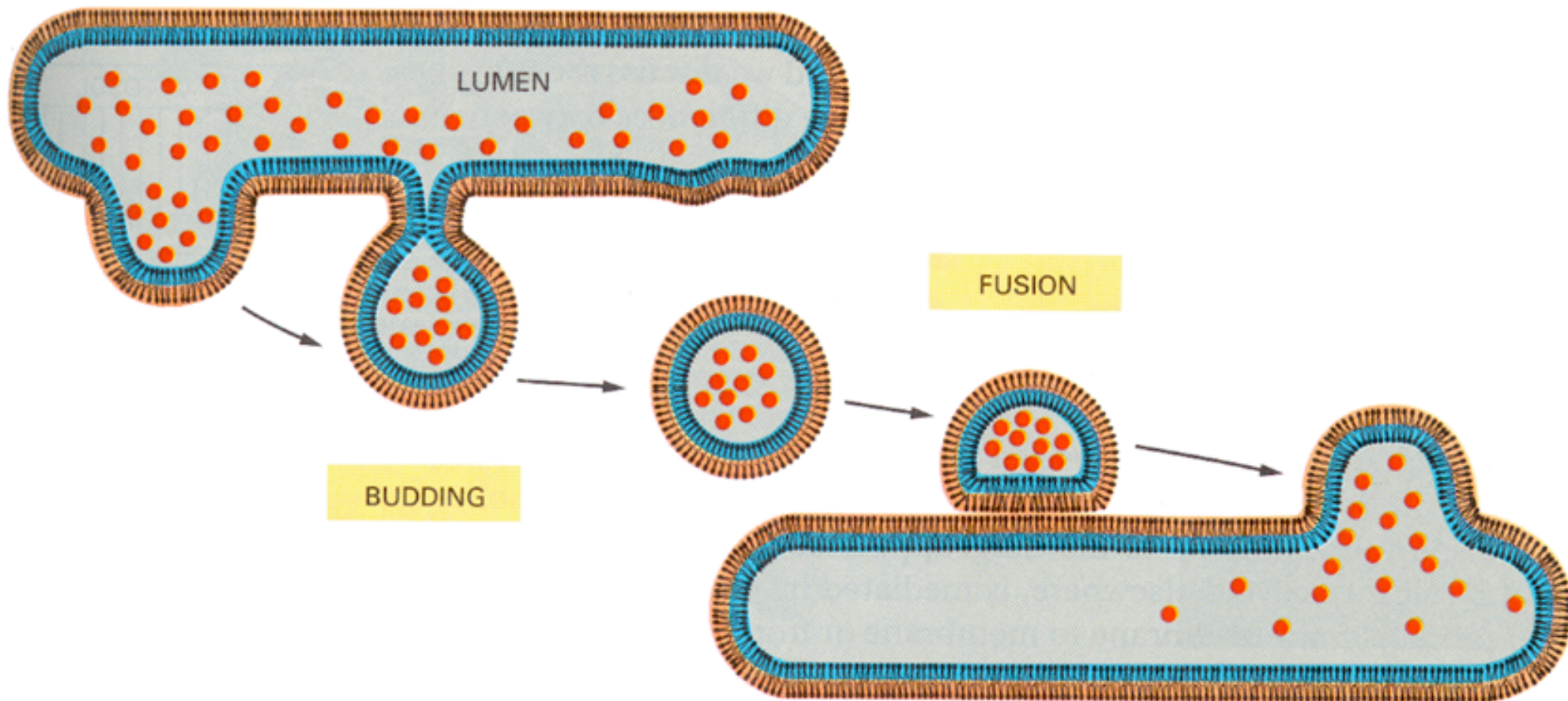
1a. basic considerations in vesicular transport

- selection: cargo sorting before budding
- budding: assembly of a cytosolic coat



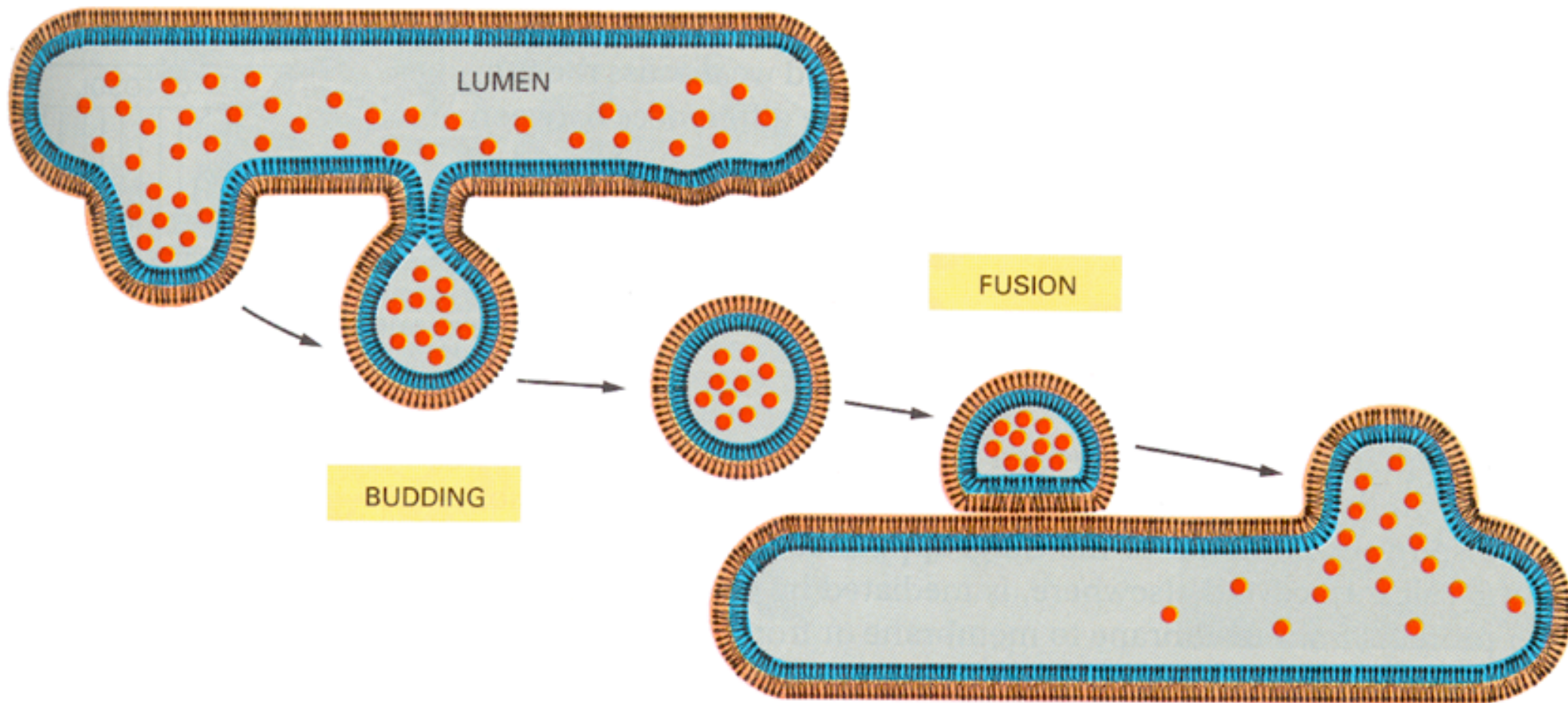
1a. basic considerations in vesicular transport

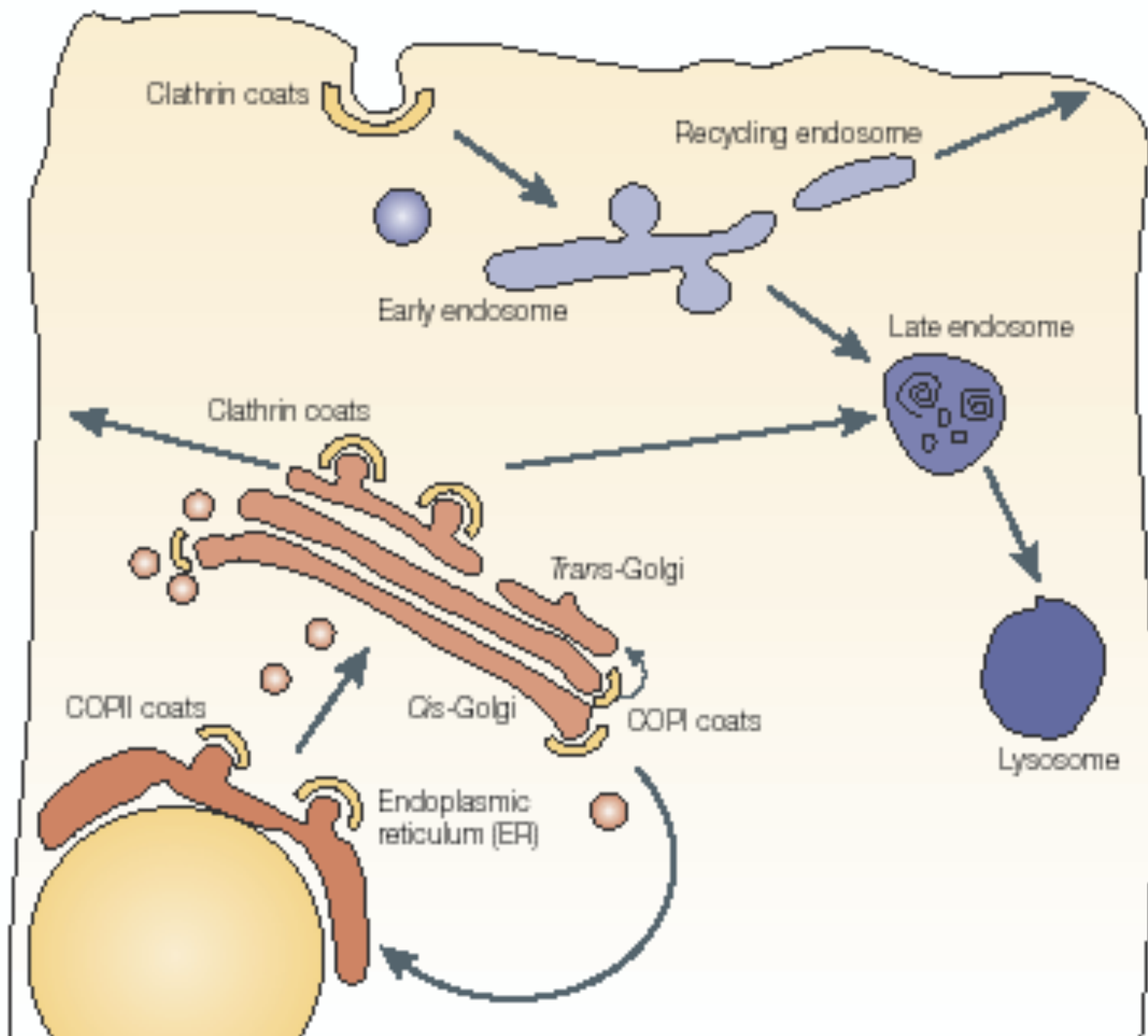
- selection: cargo sorting before budding
- budding: assembly of a cytosolic coat
- traffic: find destination



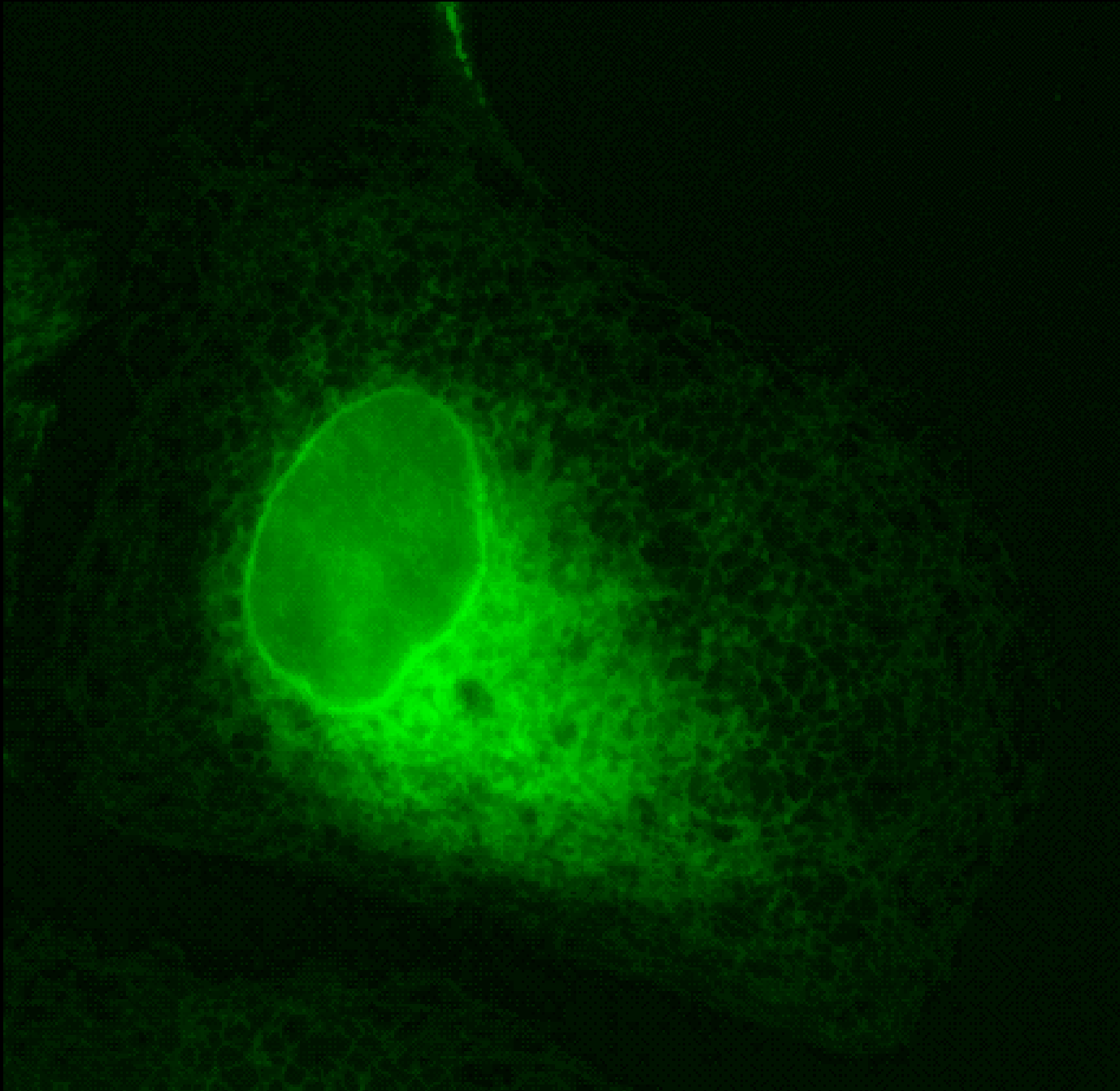
1a. basic considerations in vesicular transport

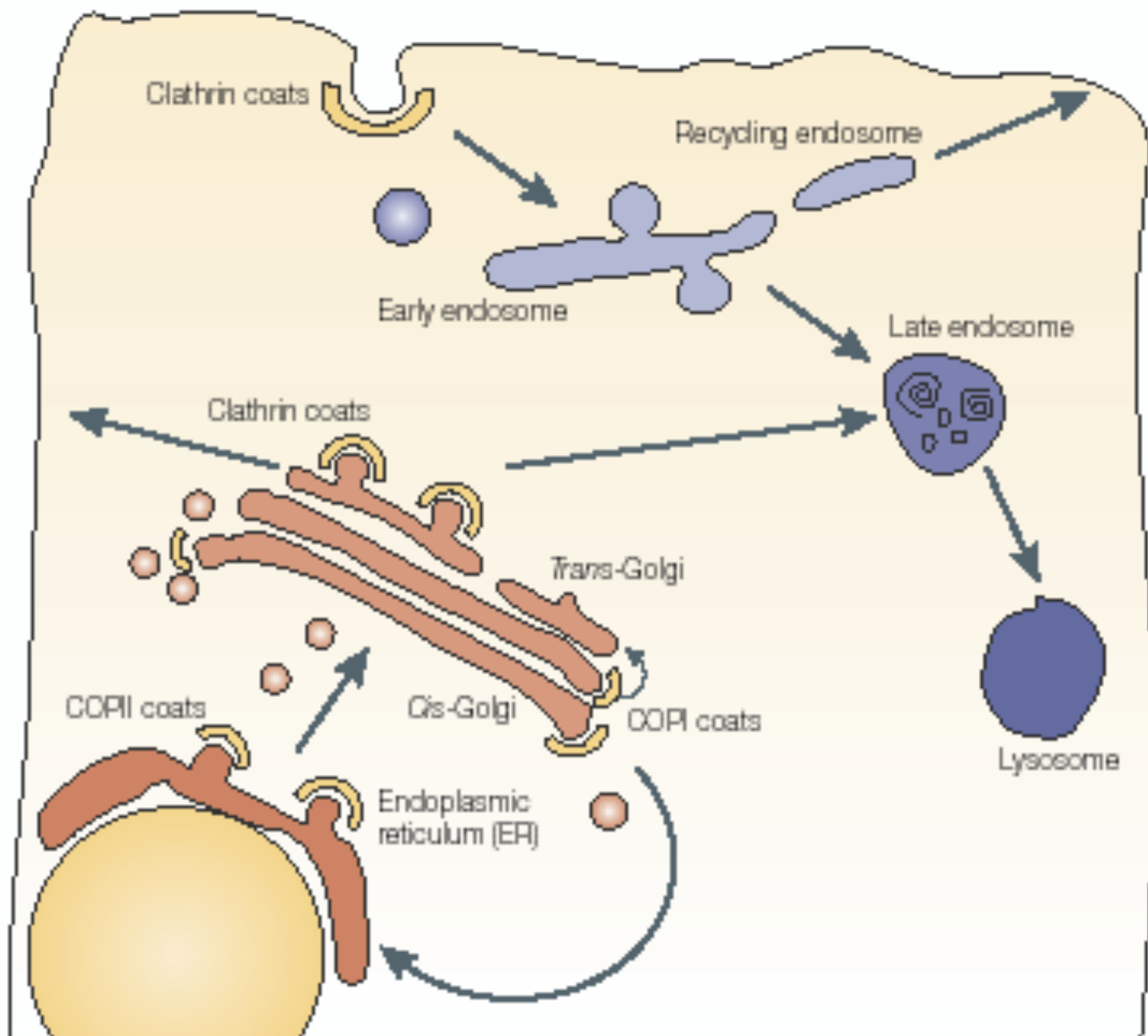
- selection: cargo sorting before budding
- budding: assembly of a cytosolic coat
- traffic: find destination
- fusion: dock and release cargo



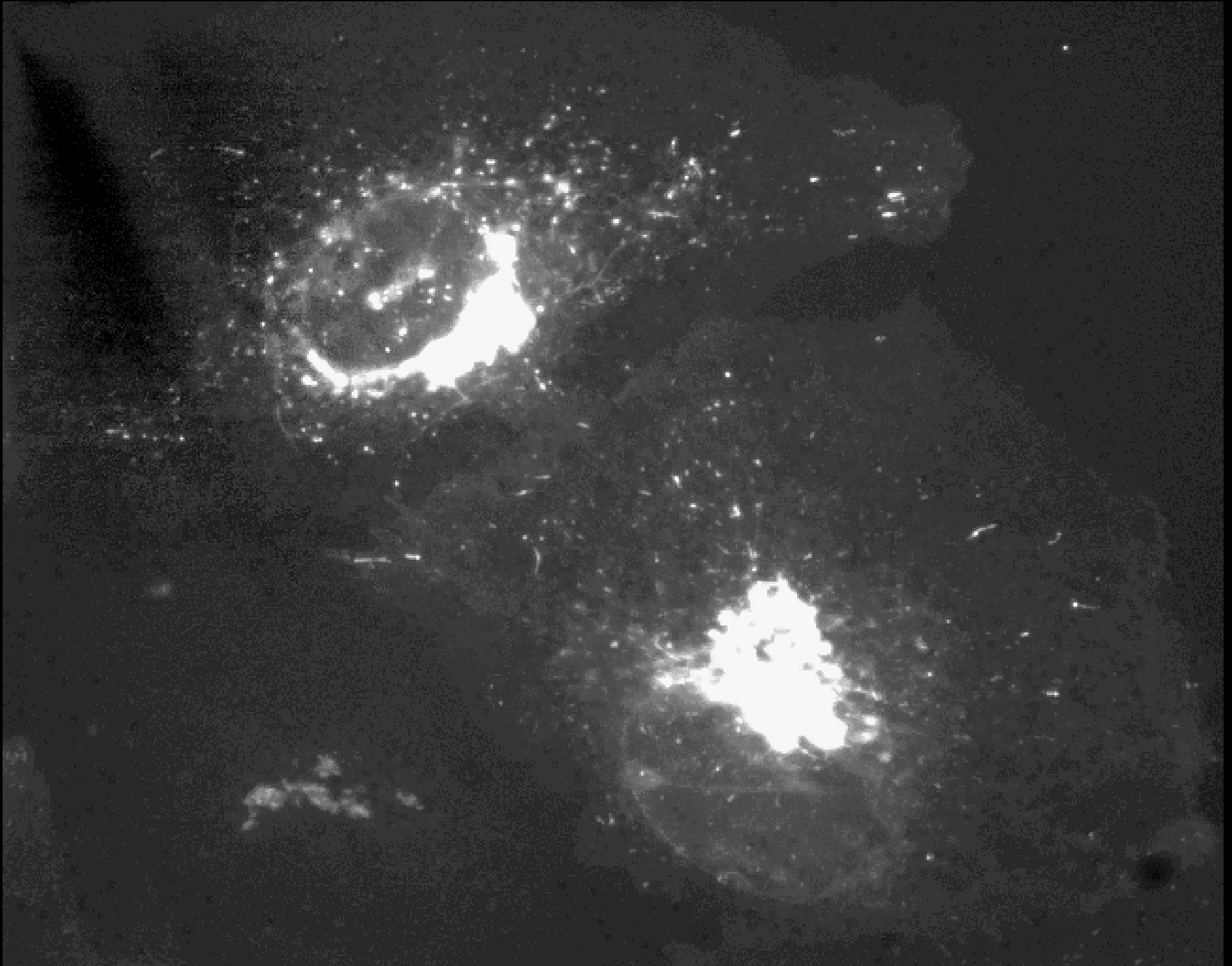


anterograde vesicular traffic: VSVG_{TS} GFP from the ER to the Golgi

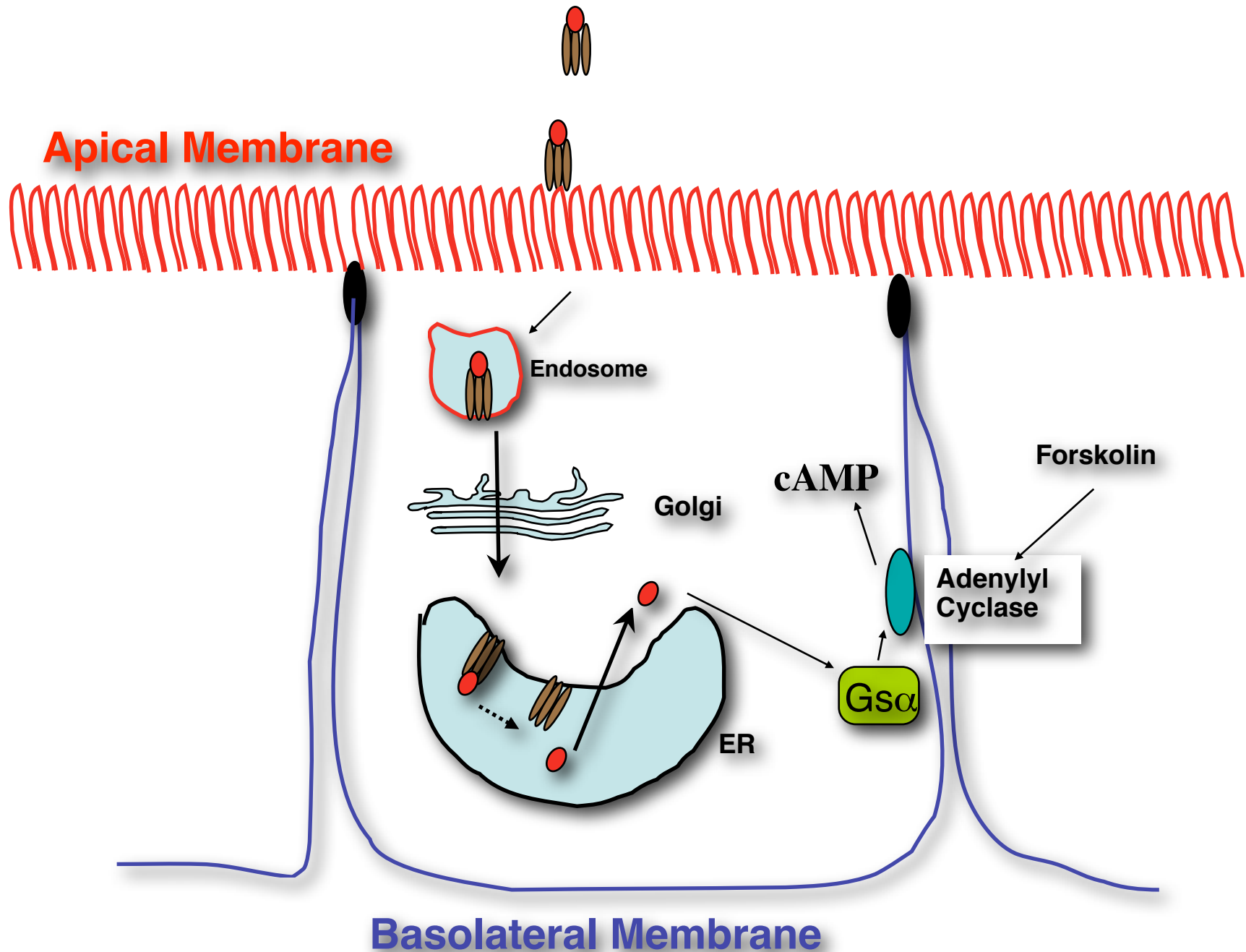




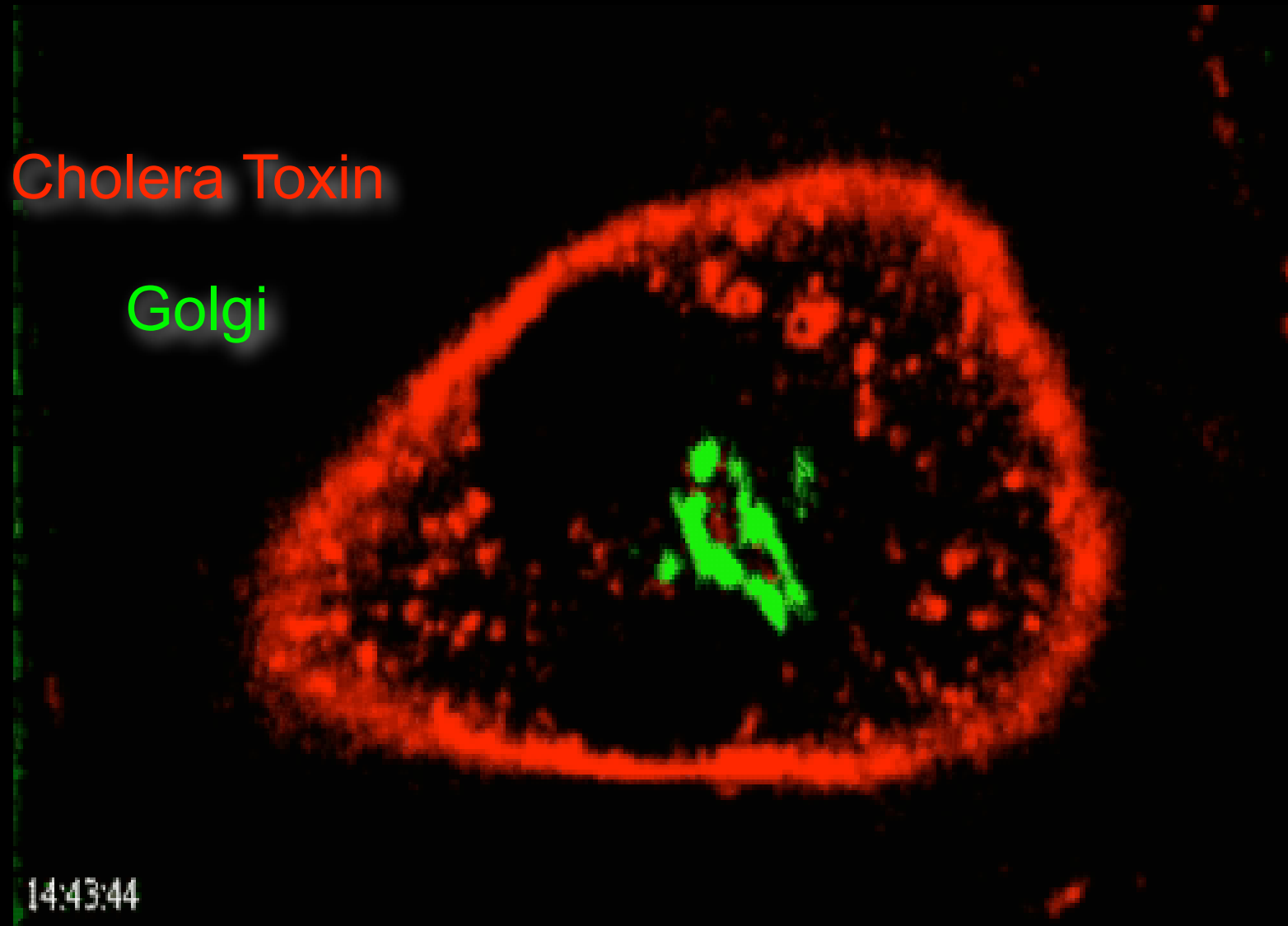
VSVG_{TS} - EGFP : GOLGI > PLASMA MEMBRANE



Cholera Toxin - Mechanism of Action

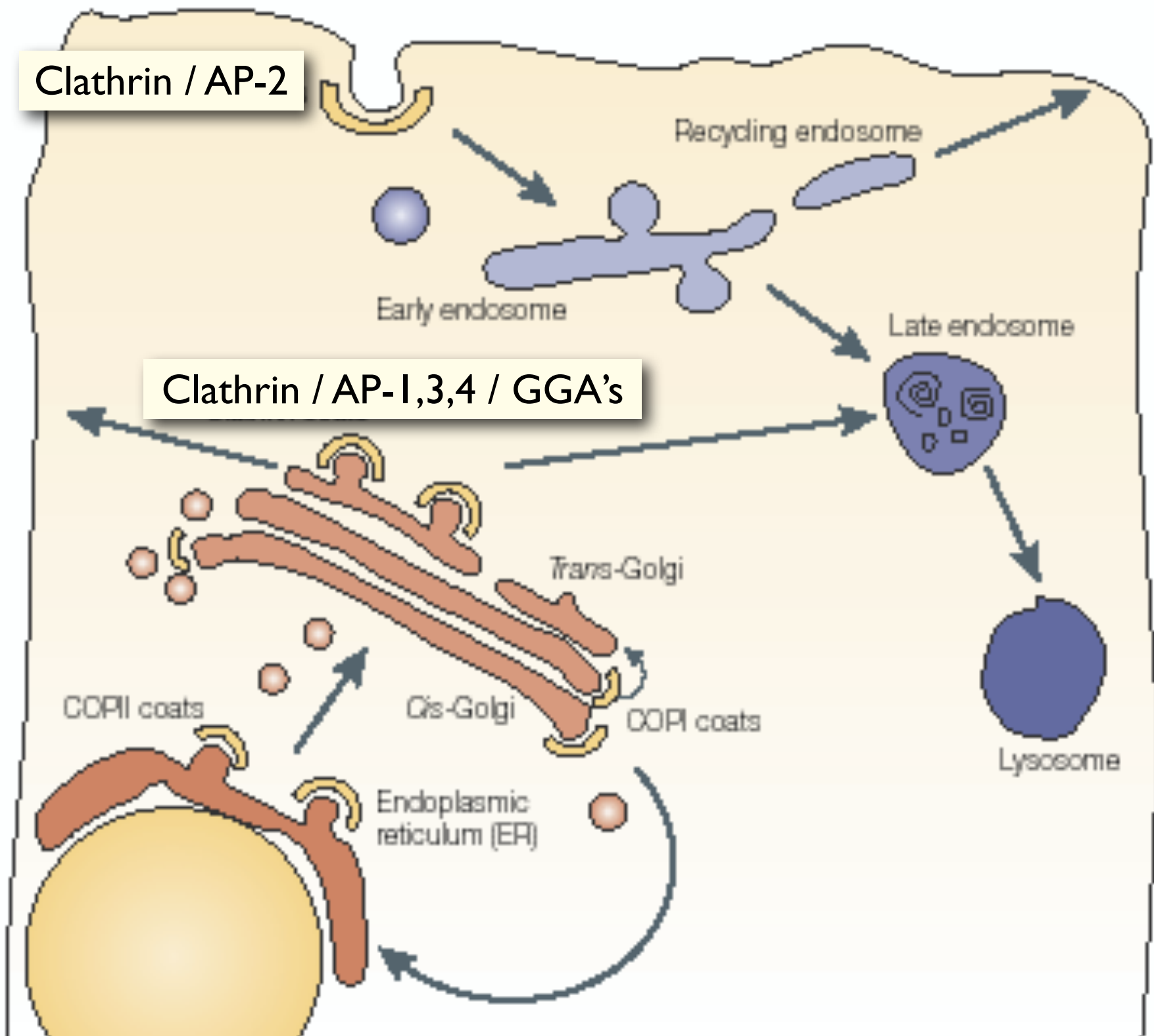


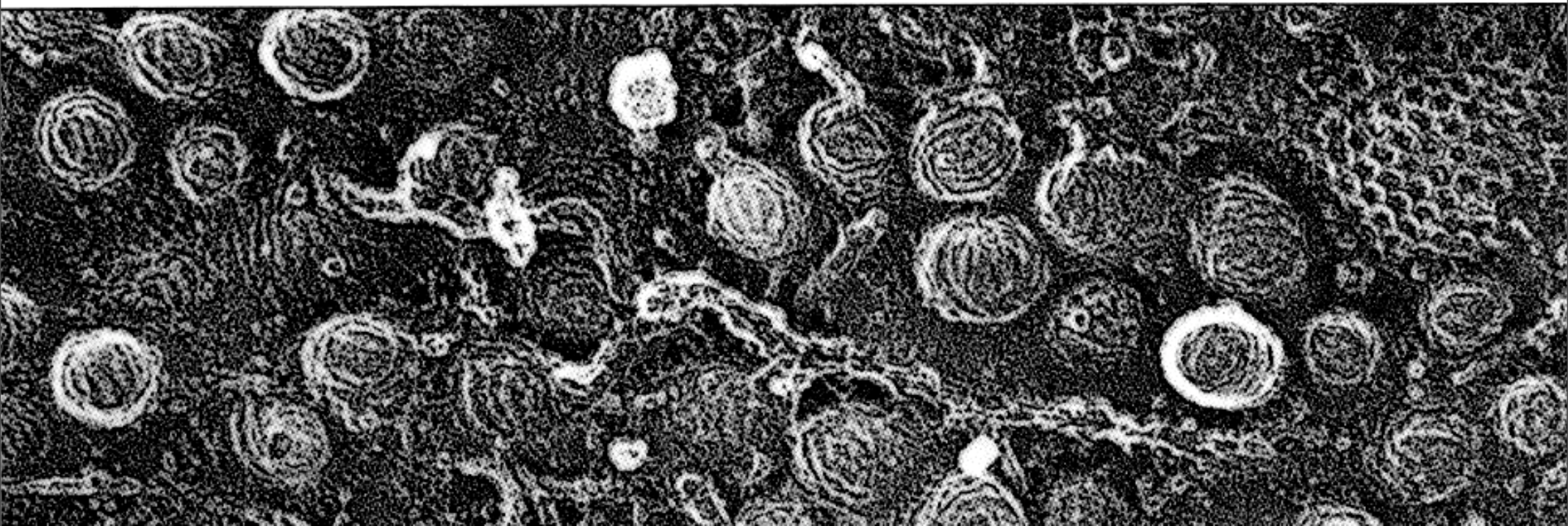
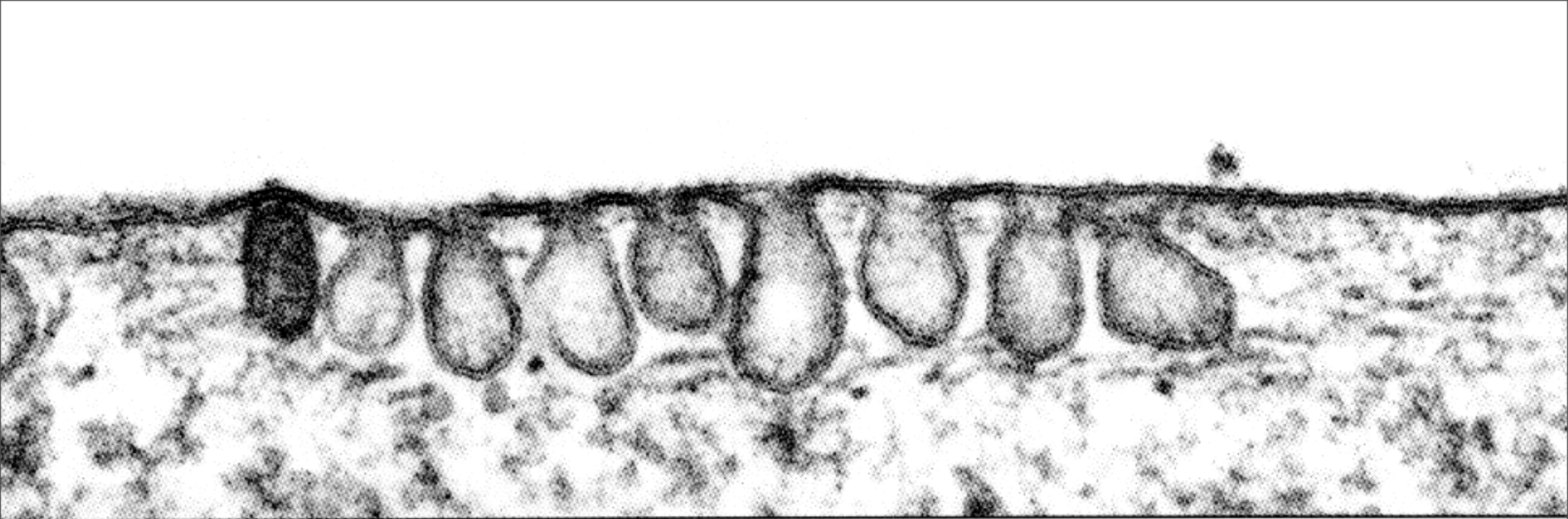
entry of Cholera Toxin followed by live-cell imaging



Clathrin / AP-2

Clathrin / AP-1,3,4 / GGA's





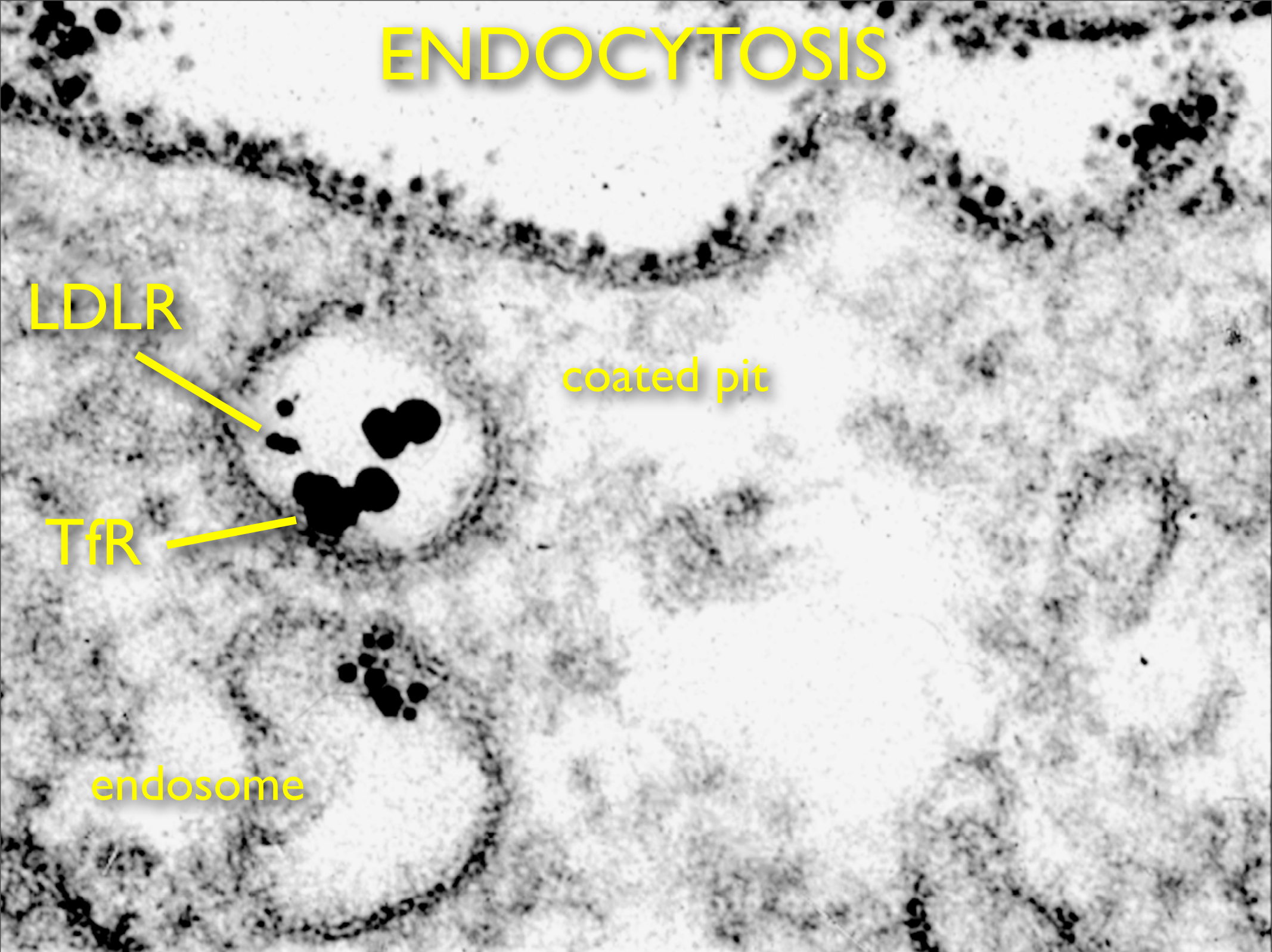
ENDOCYTOSIS

LDLR

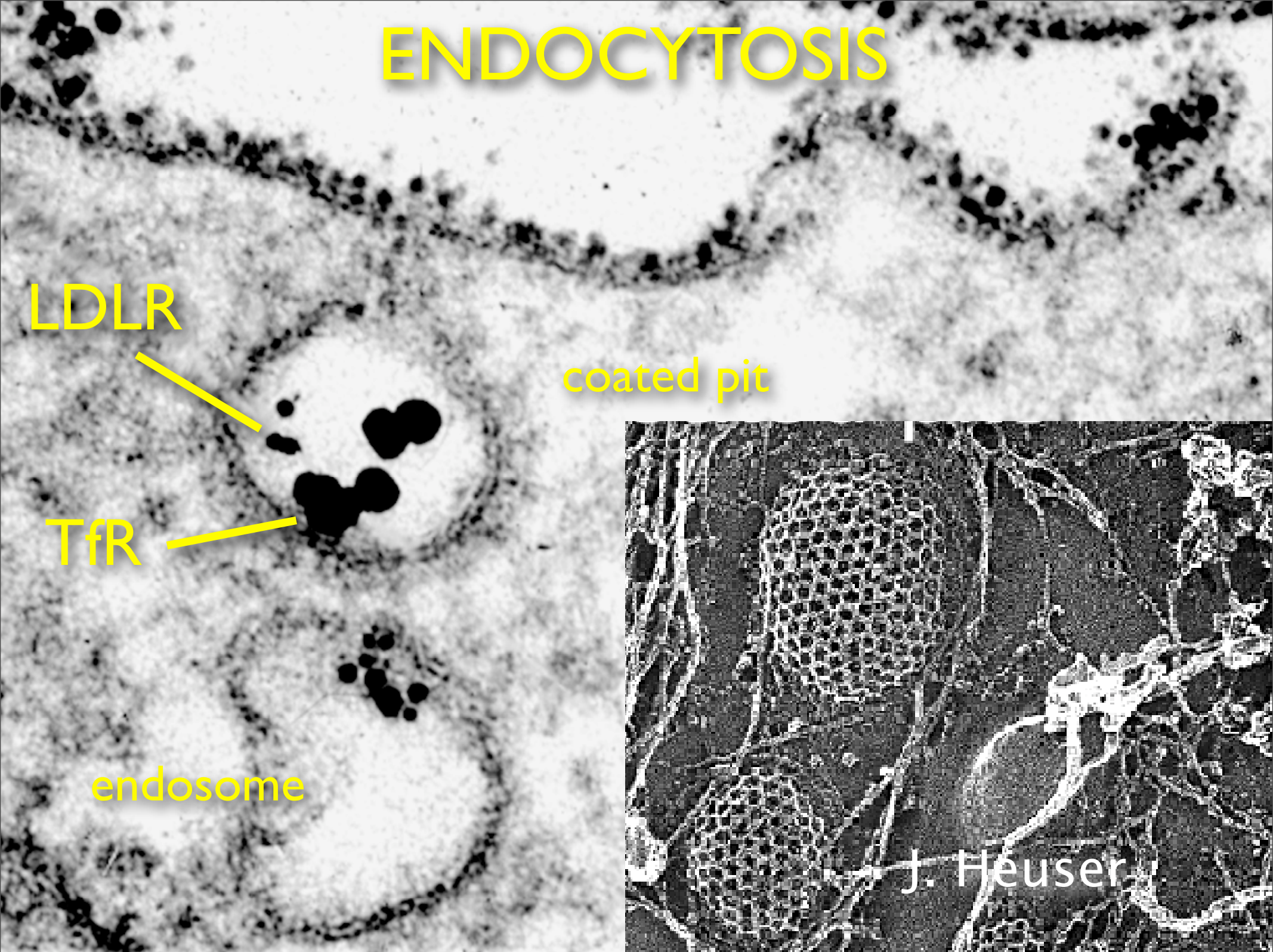
coated pit

TfR

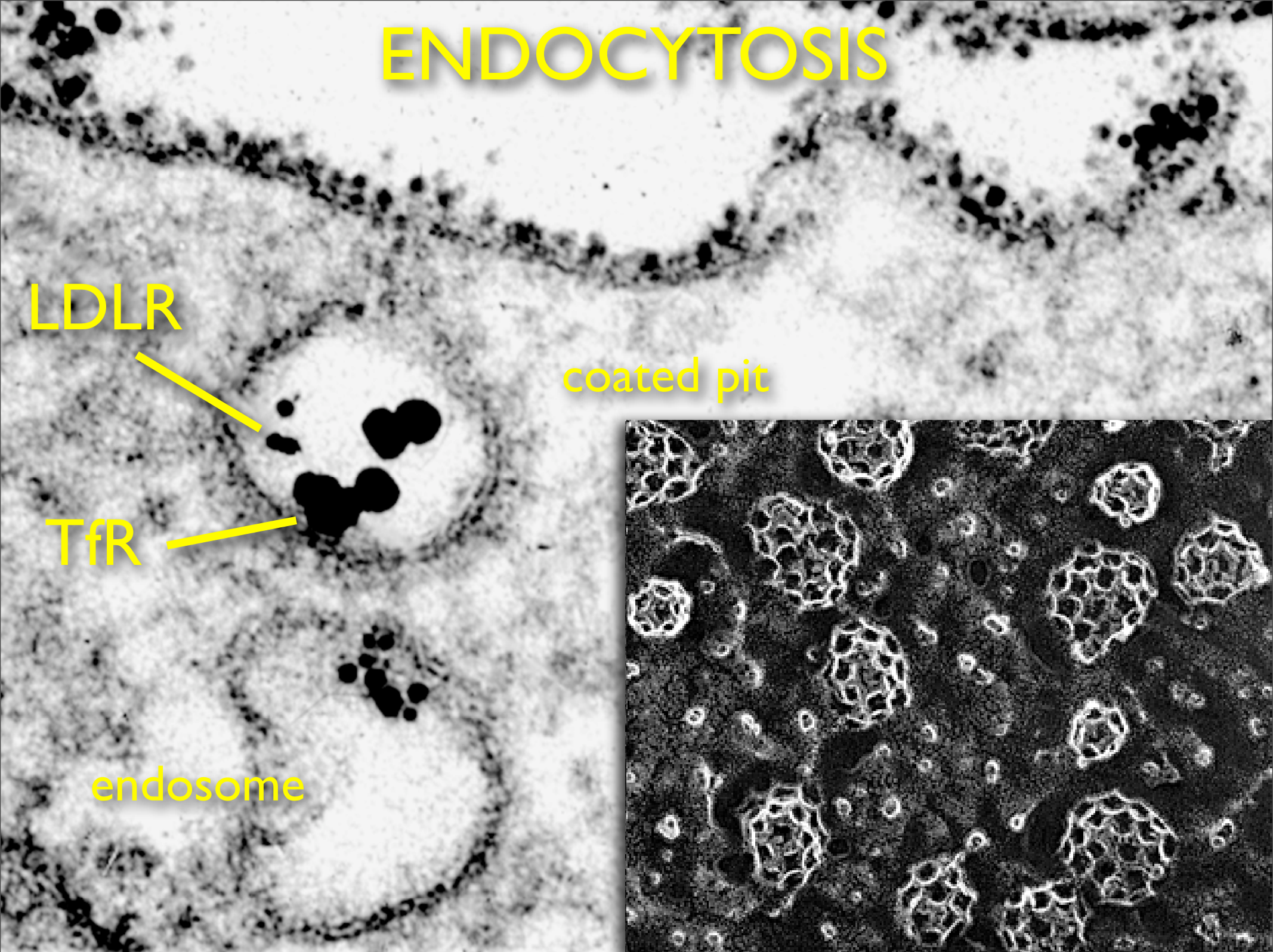
endosome



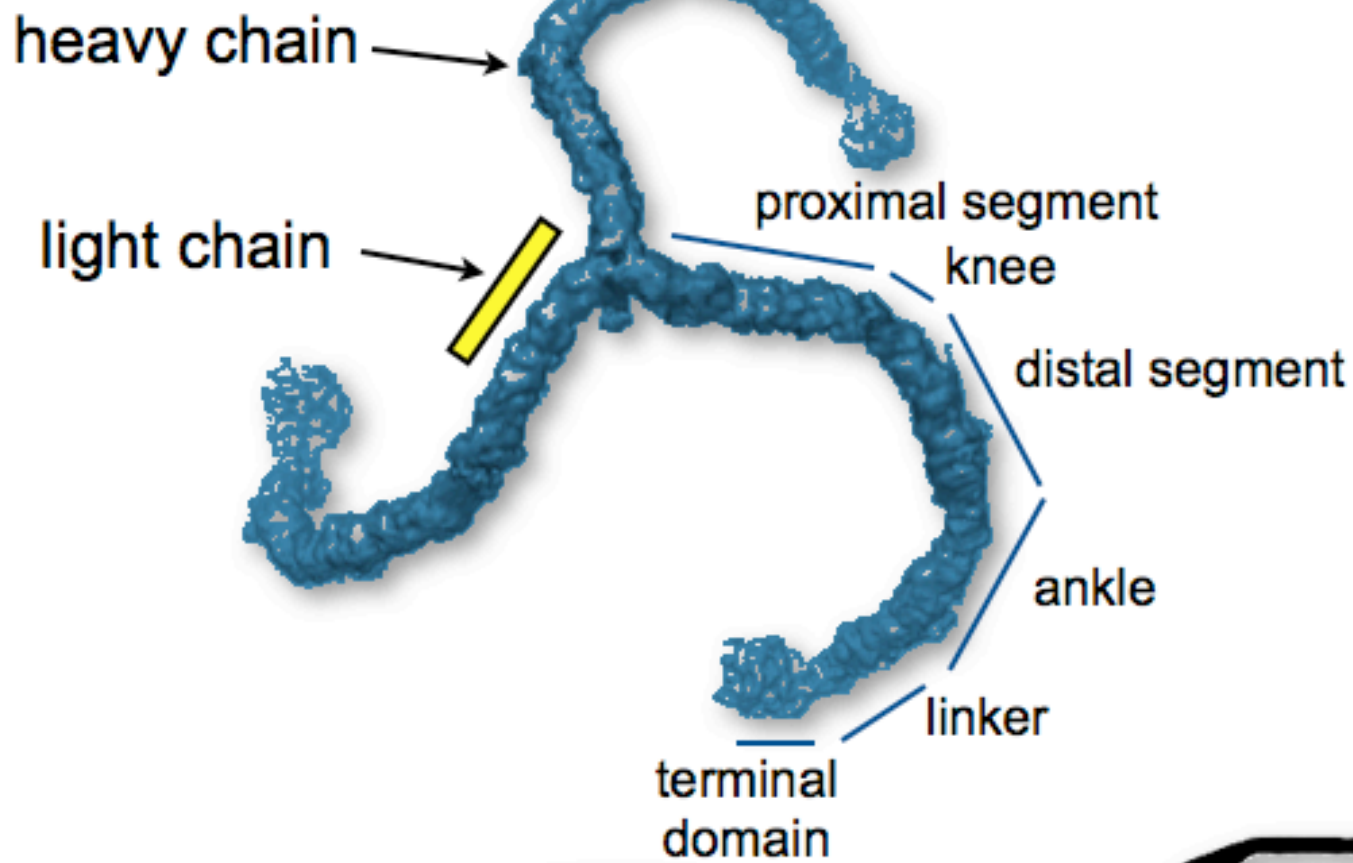
ENDOCYTOSIS



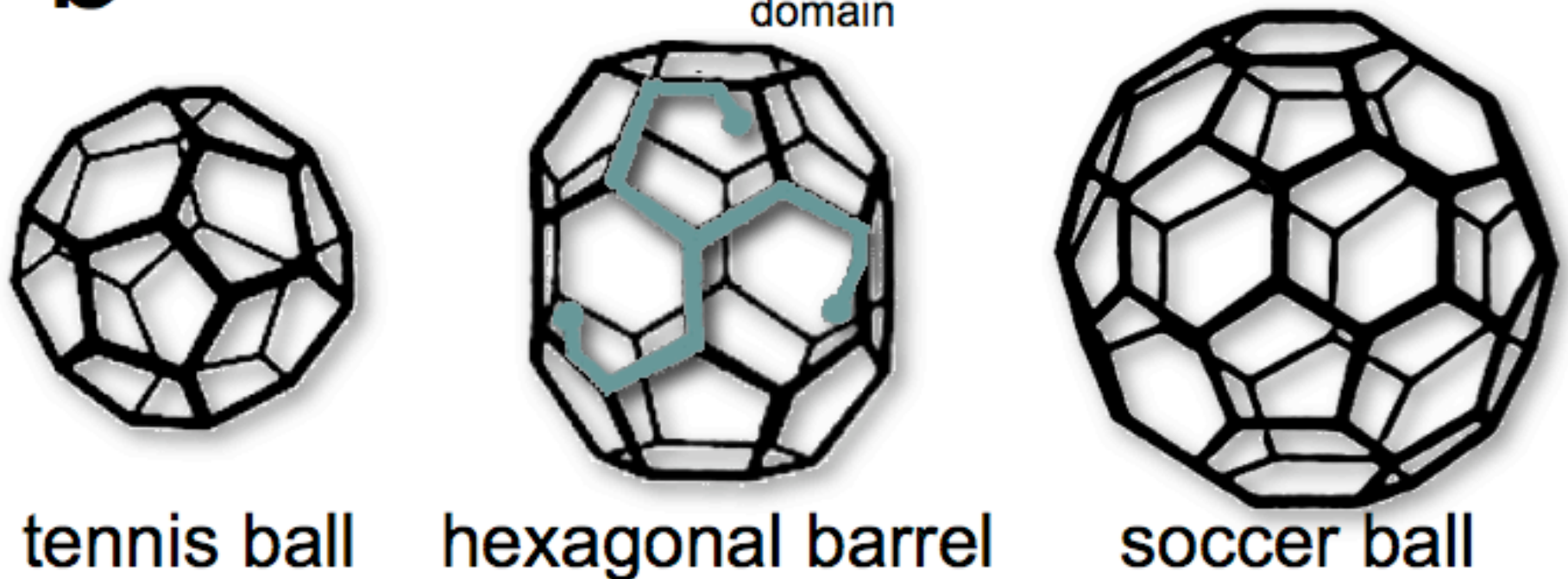
ENDOCYTOSIS

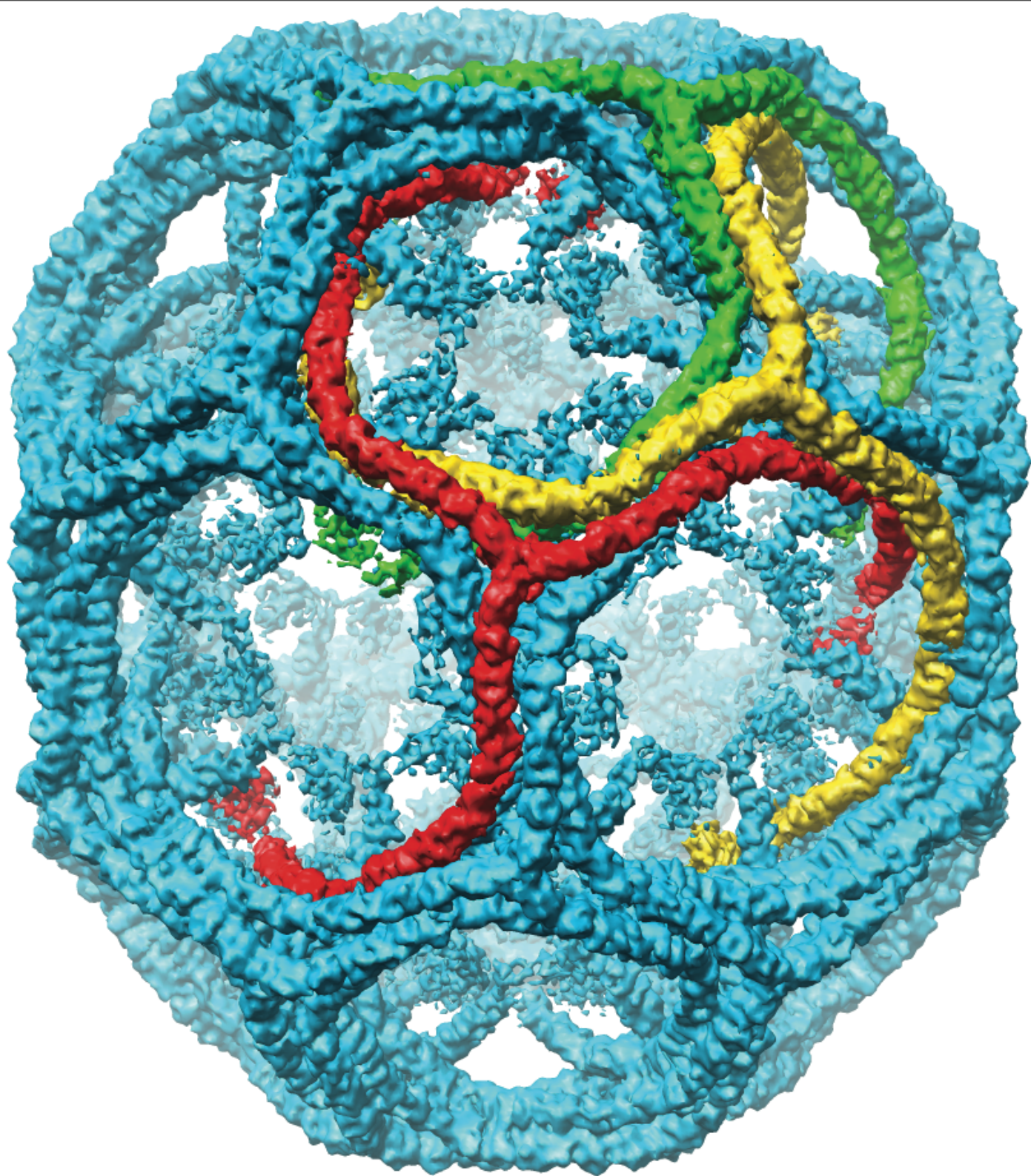


a

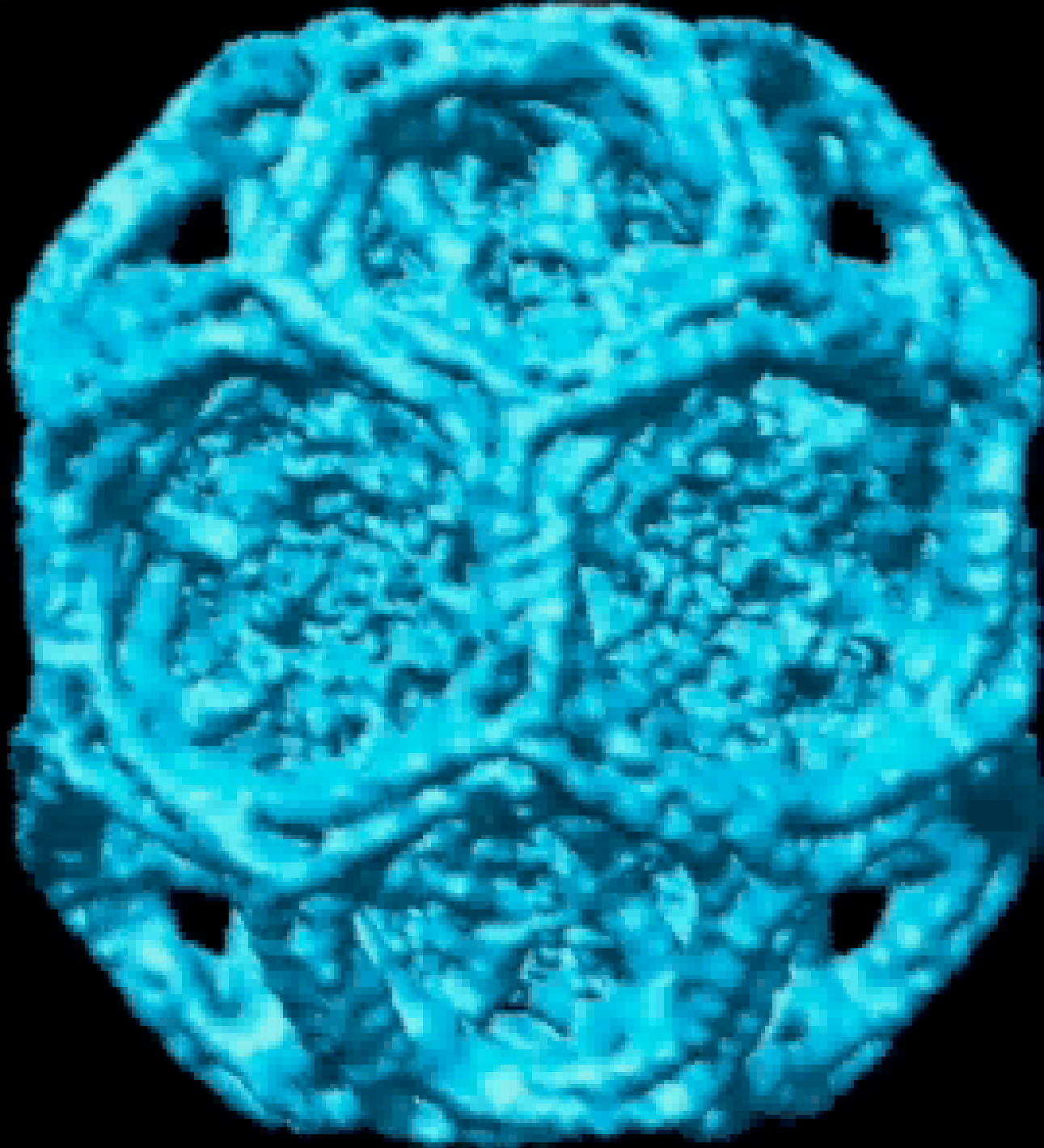


b





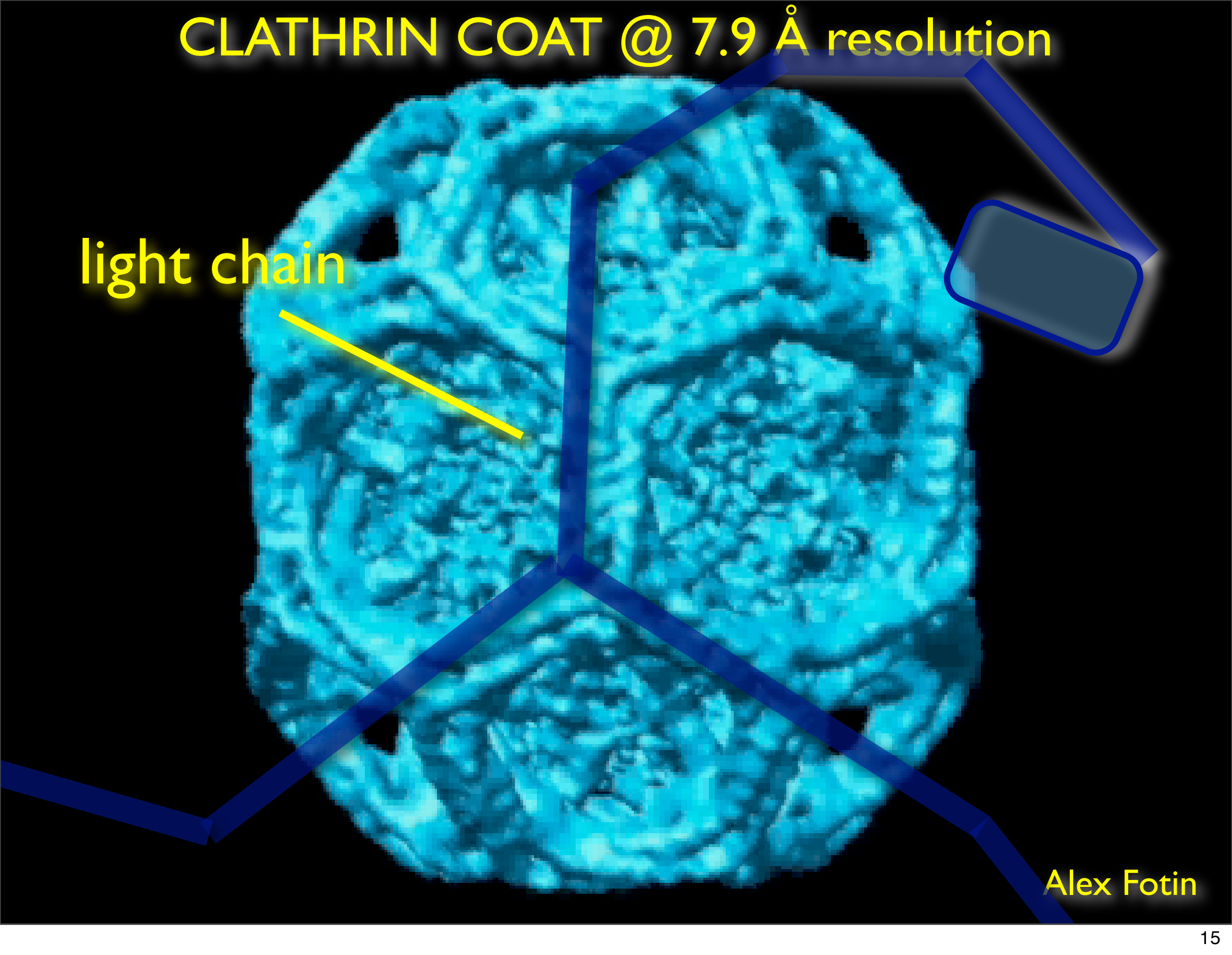
CLATHRIN COAT @ 7.9 Å resolution



Alex Fotin

CLATHRIN COAT @ 7.9 Å resolution

light chain



Alex Fotin



clathrin

Activation (TGN)

Arf6

GTP
GDP

AP-2

Activation (plasma membrane)

ATP
ADP+P_i

Cargo capture

Hsc70

Auxilin

clathrin

Coat assembly

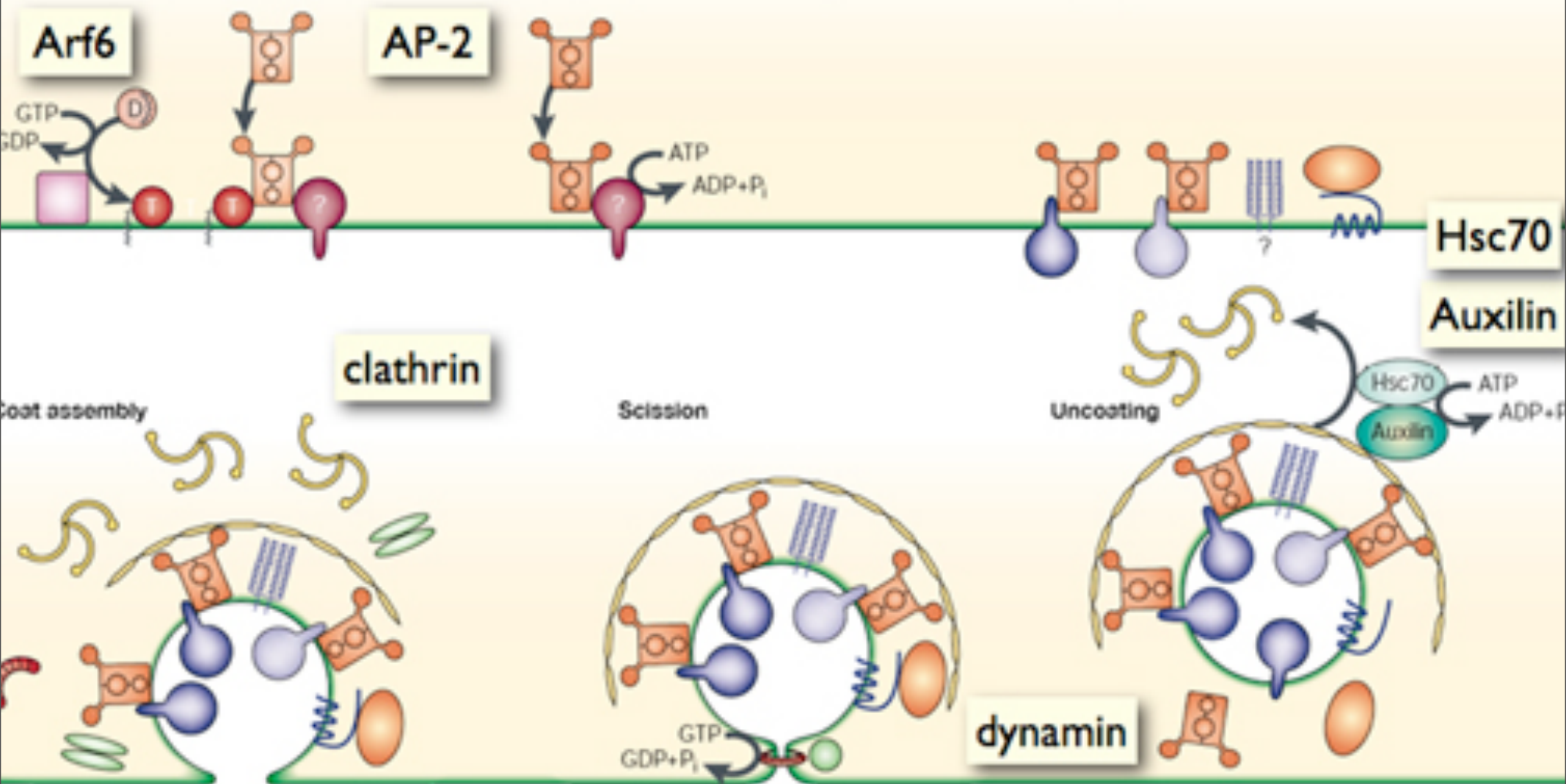
Scission

Uncoating

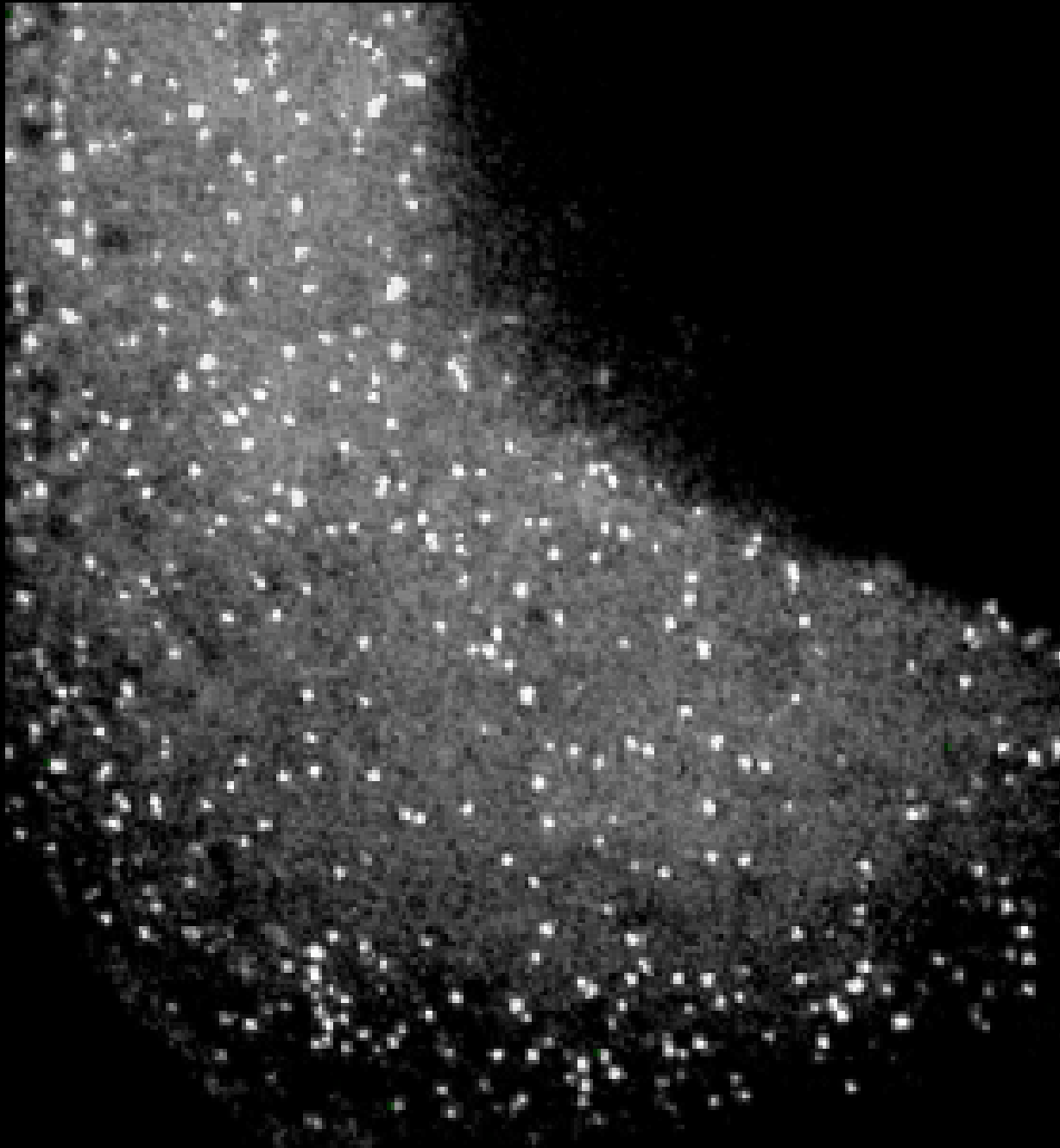
ATP
ADP+P_i

dynamin

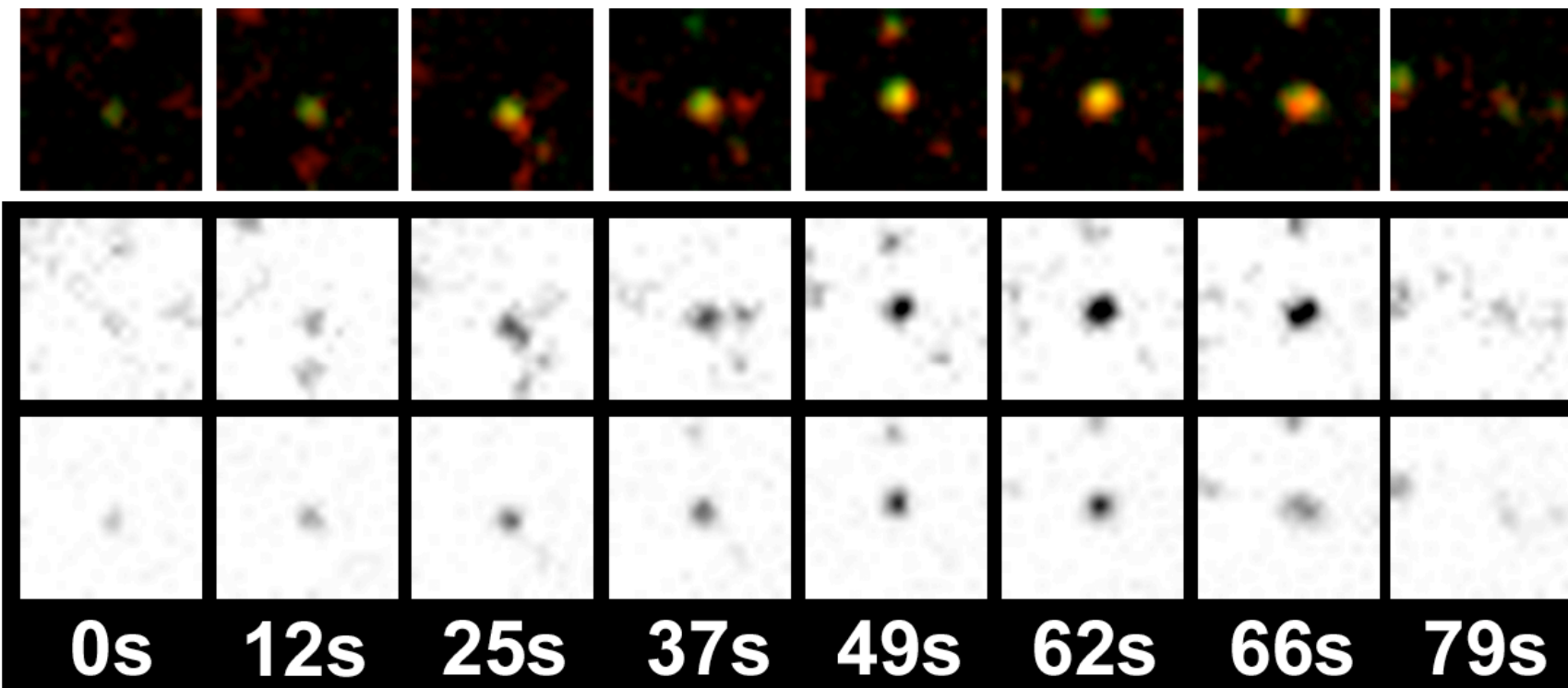
GTP
GDP+P_i



σ^2 - EGFP (AP-2)

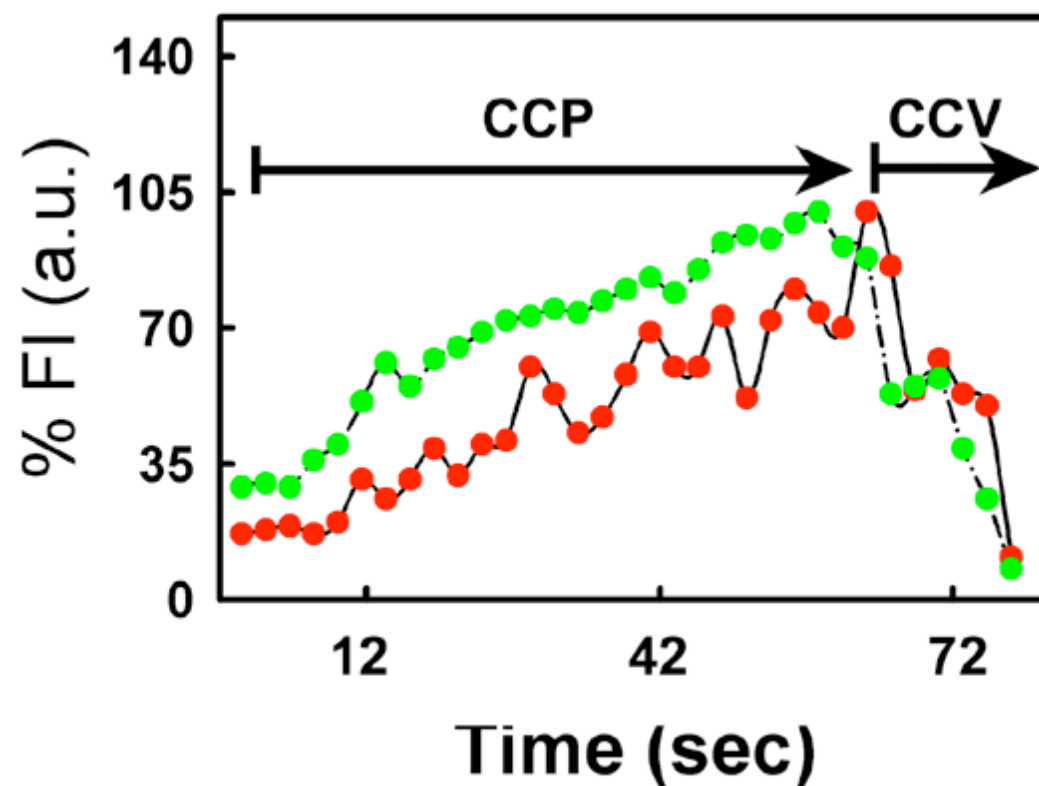


Ehrlich et al 2004

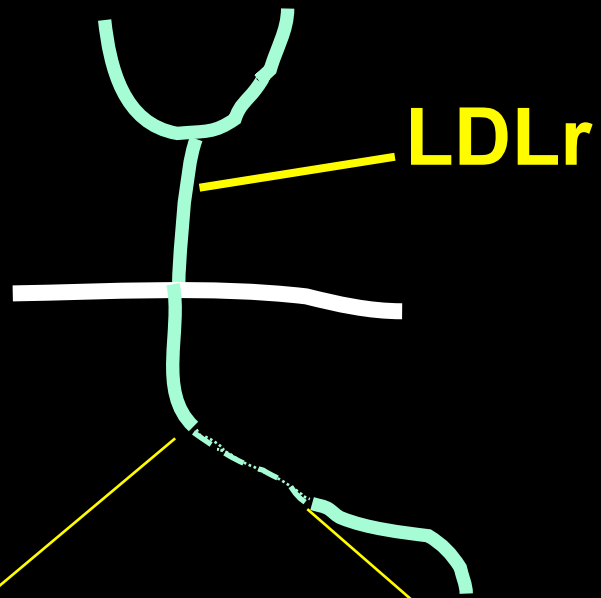


Clathrin

AP-2



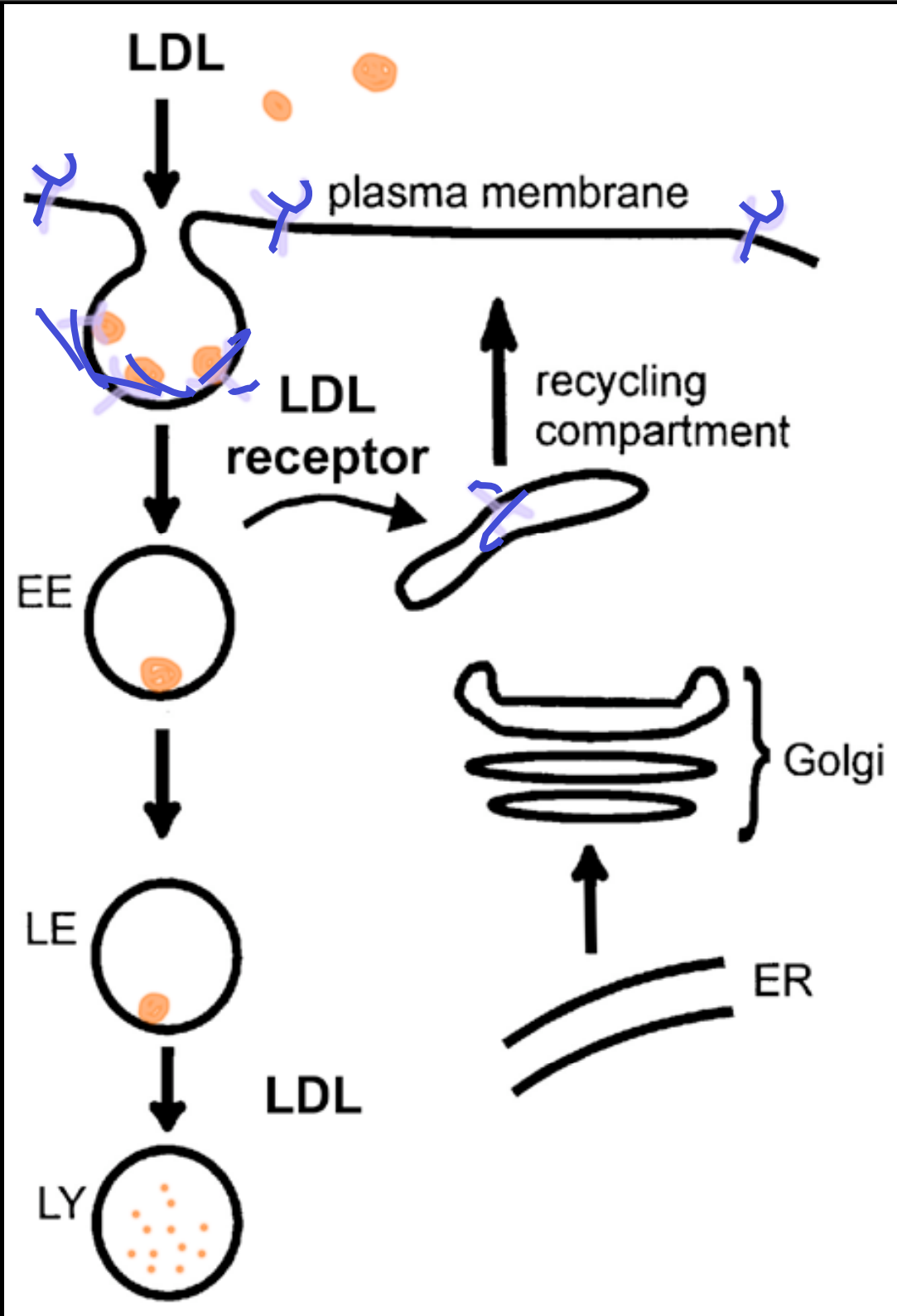
Ehrlich et al 2004

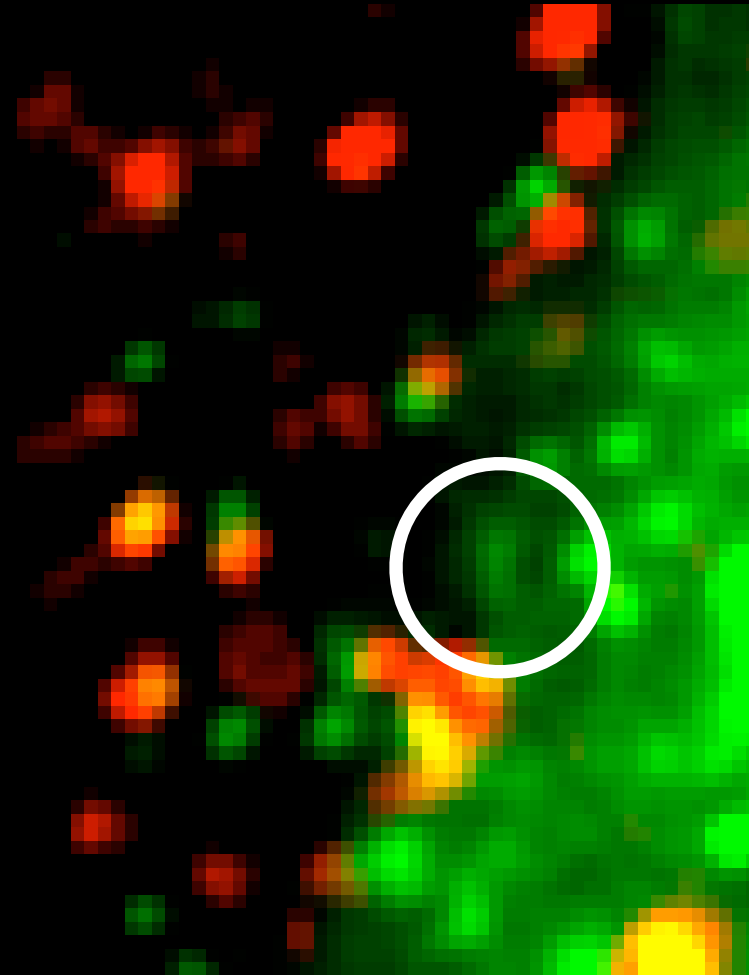


...FDNPVY...

...FDNPVC...

J.D. PATIENT



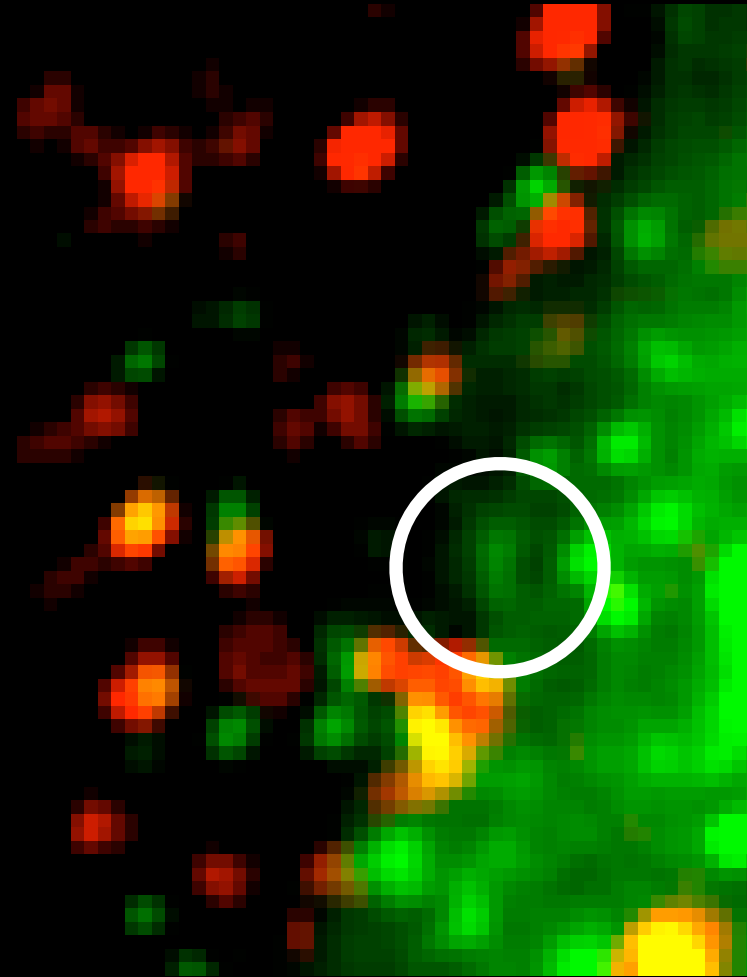


DiI - LDL

YFP - LCa Clathrin

Ehrlich et al 2004

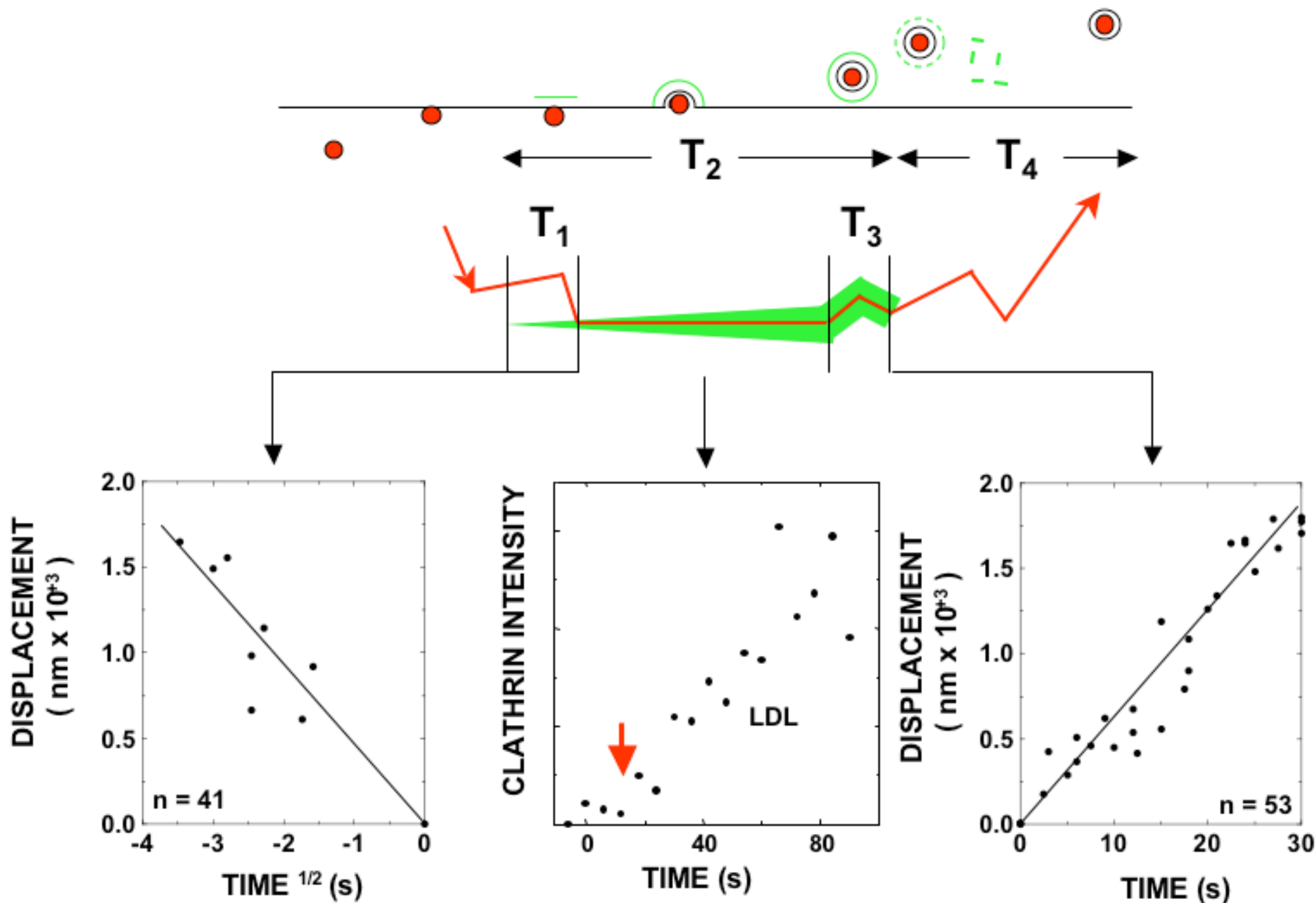
capture of LDL by a clathrin coated pit at the plasma membrane



DiI - LDL

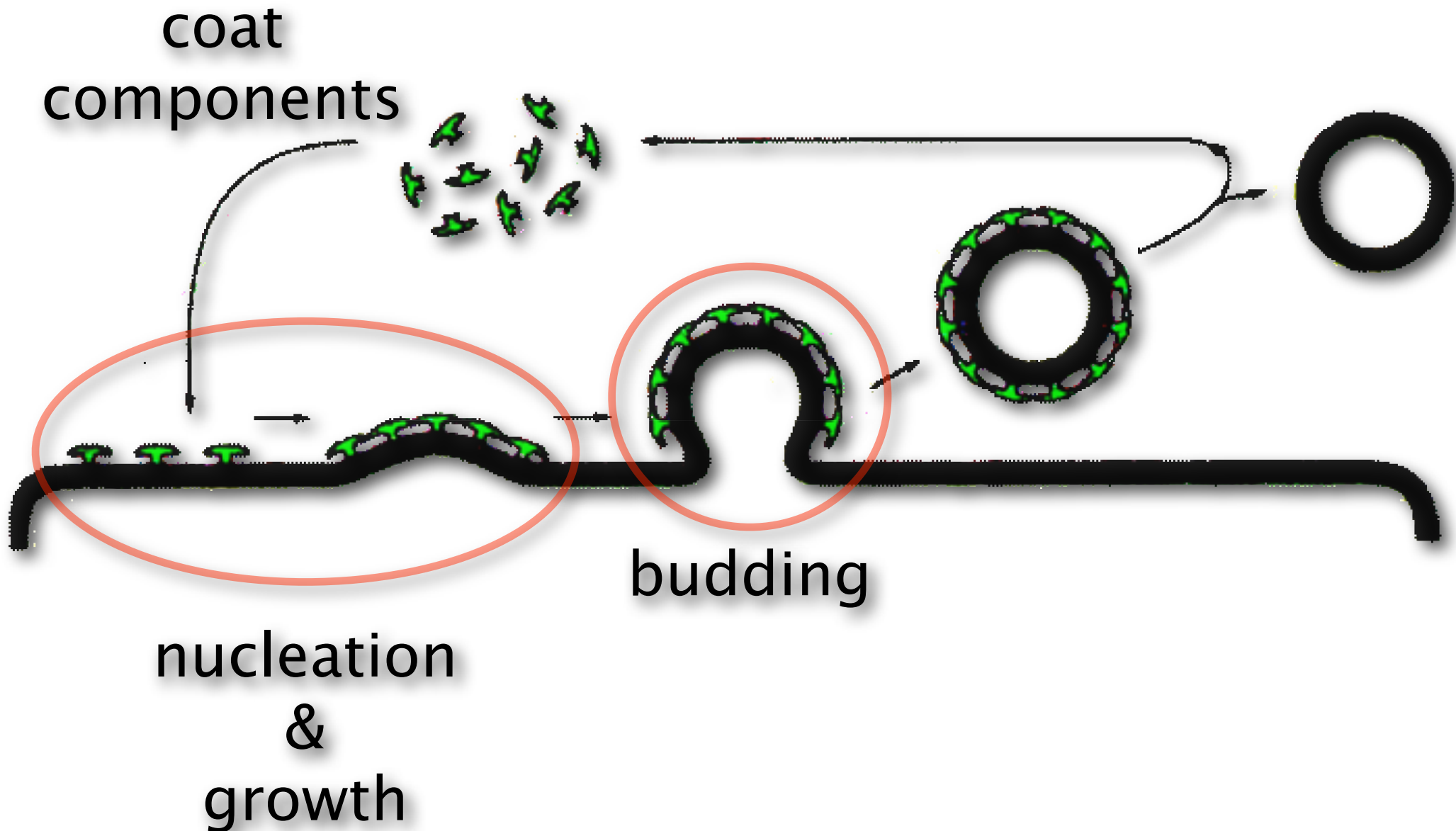
YFP - LCa Clathrin

Ehrlich et al 2004

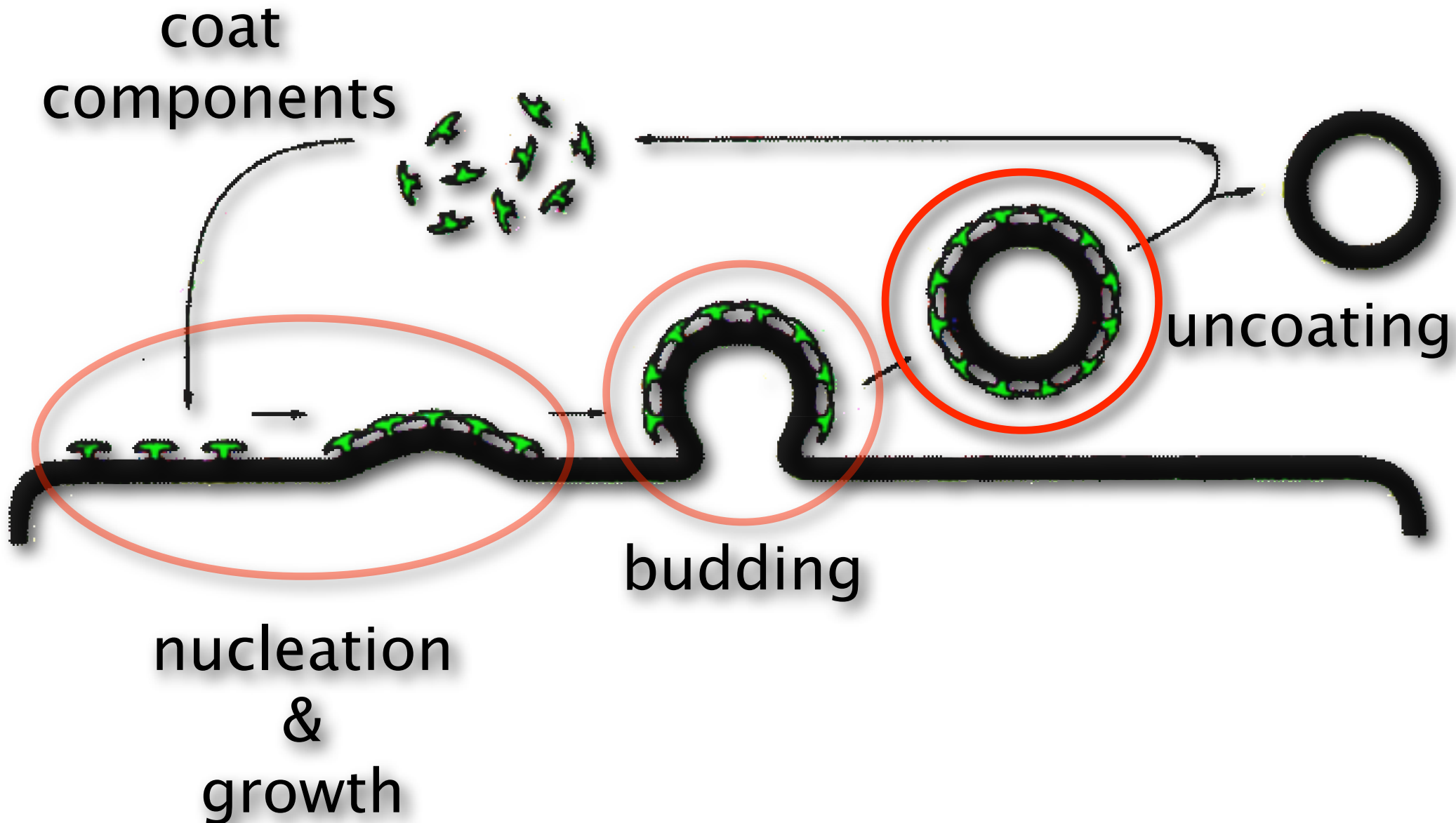


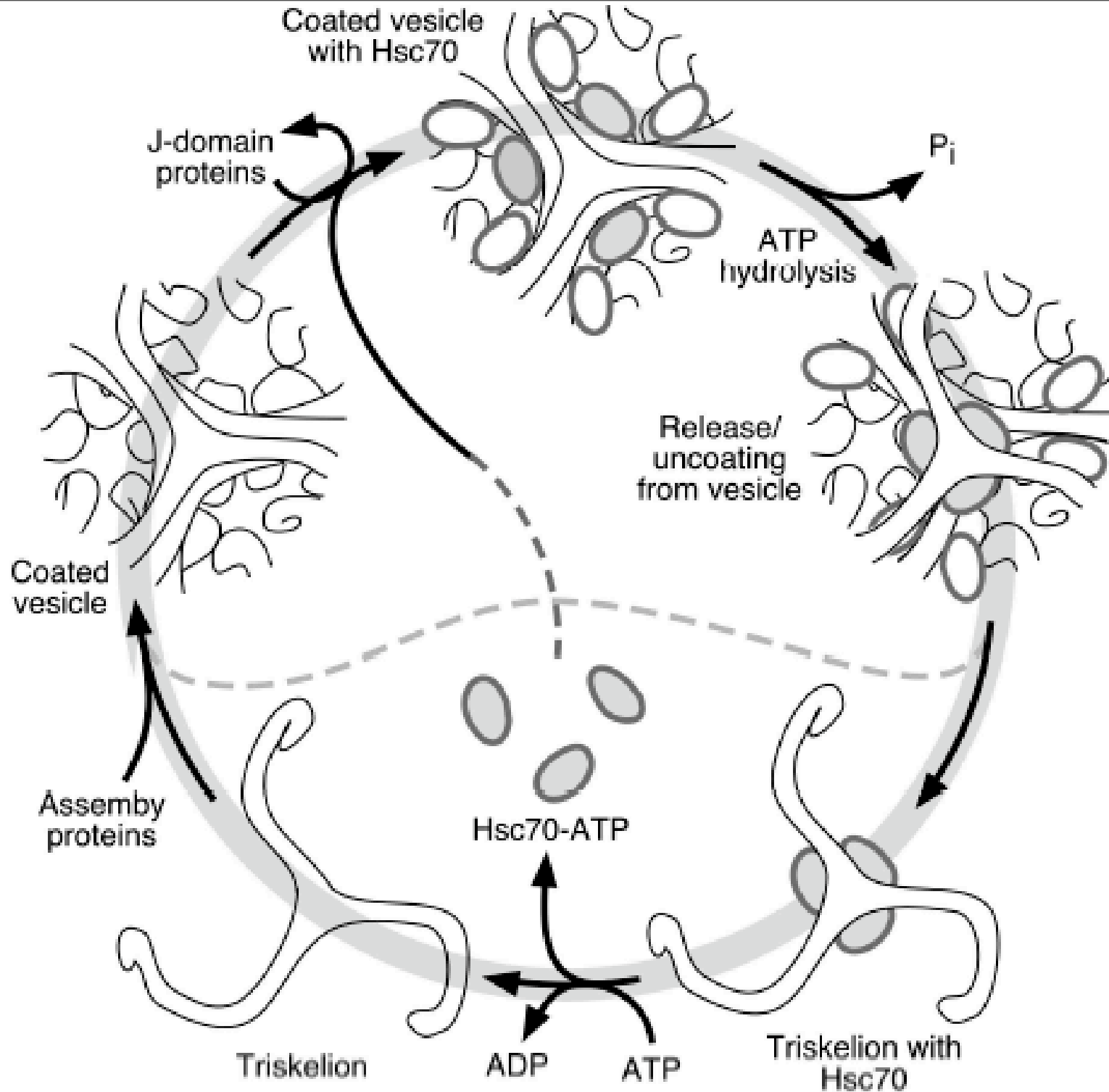
Ehrlich et al 2004

formation of clathrin coated pits and vesicles

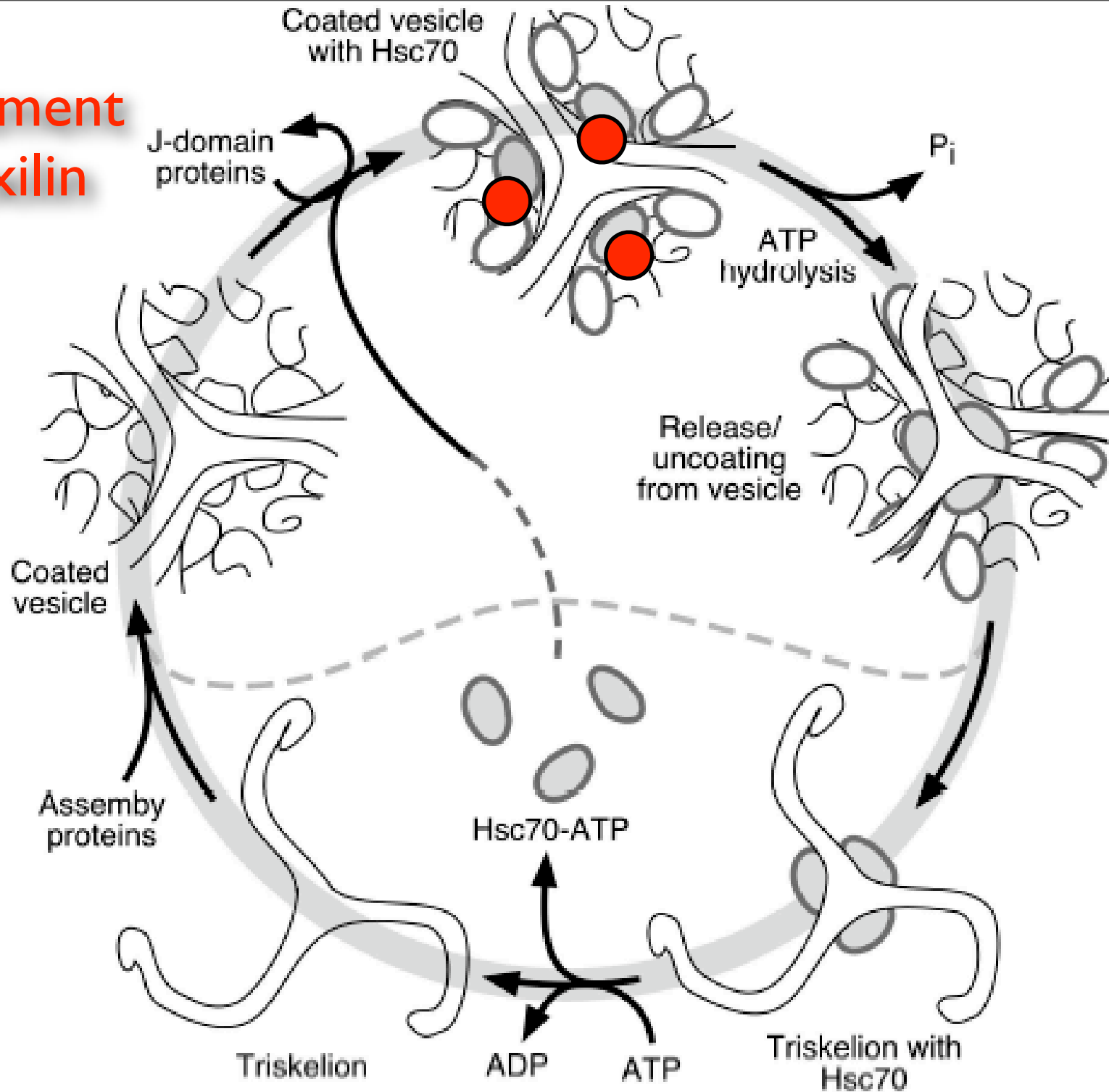


formation of clathrin coated pits and vesicles

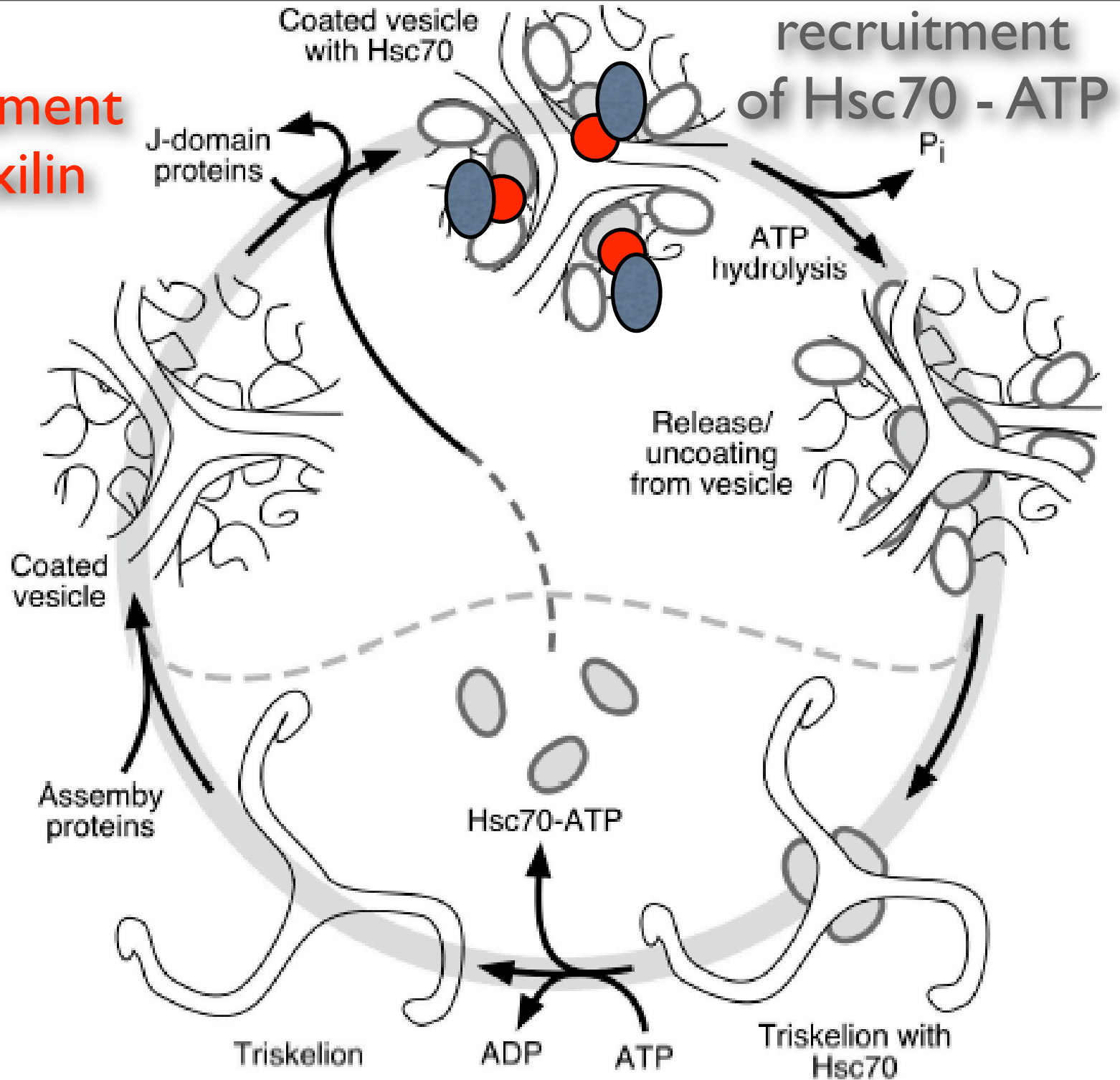




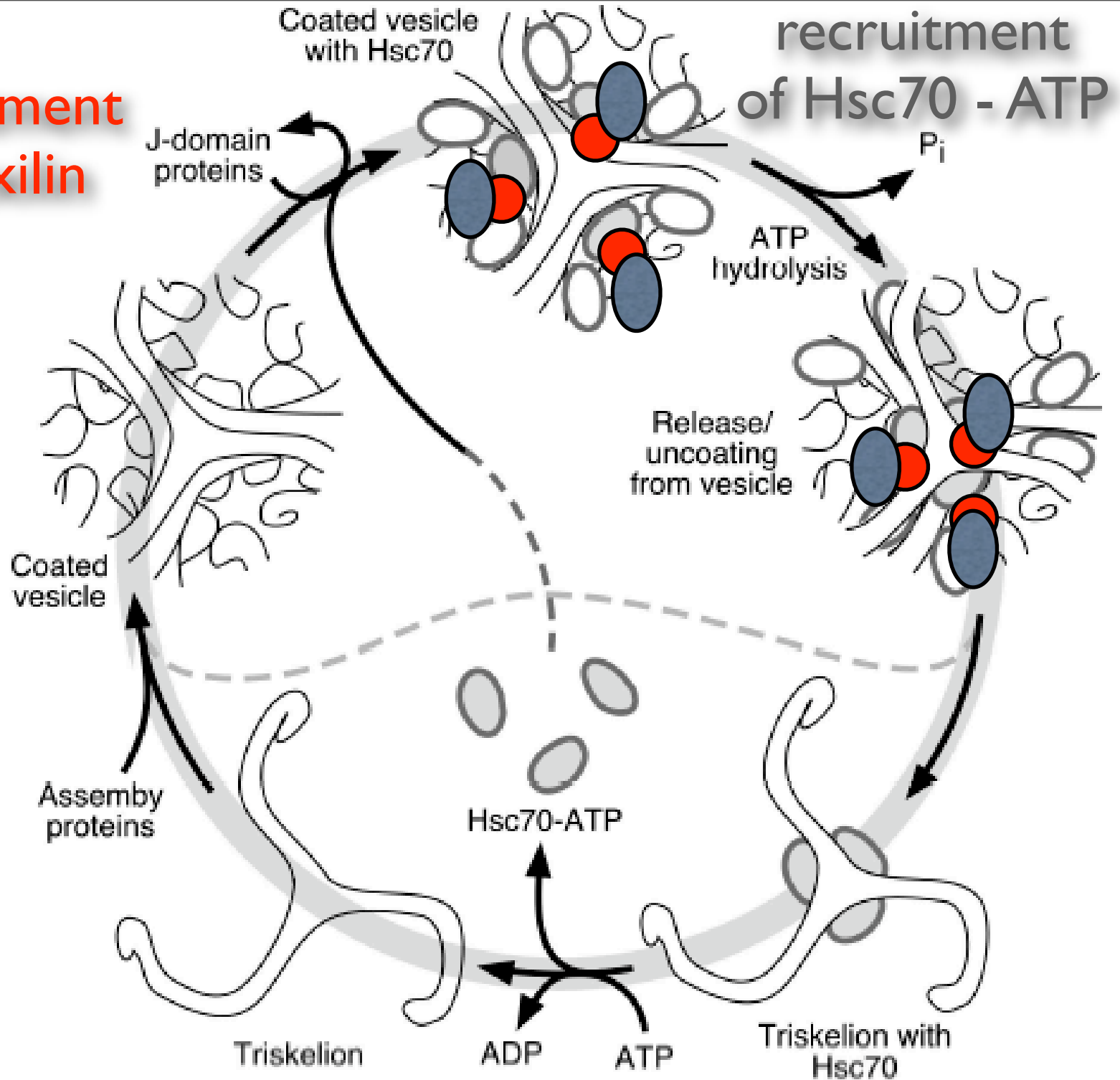
recruitment of auxilin



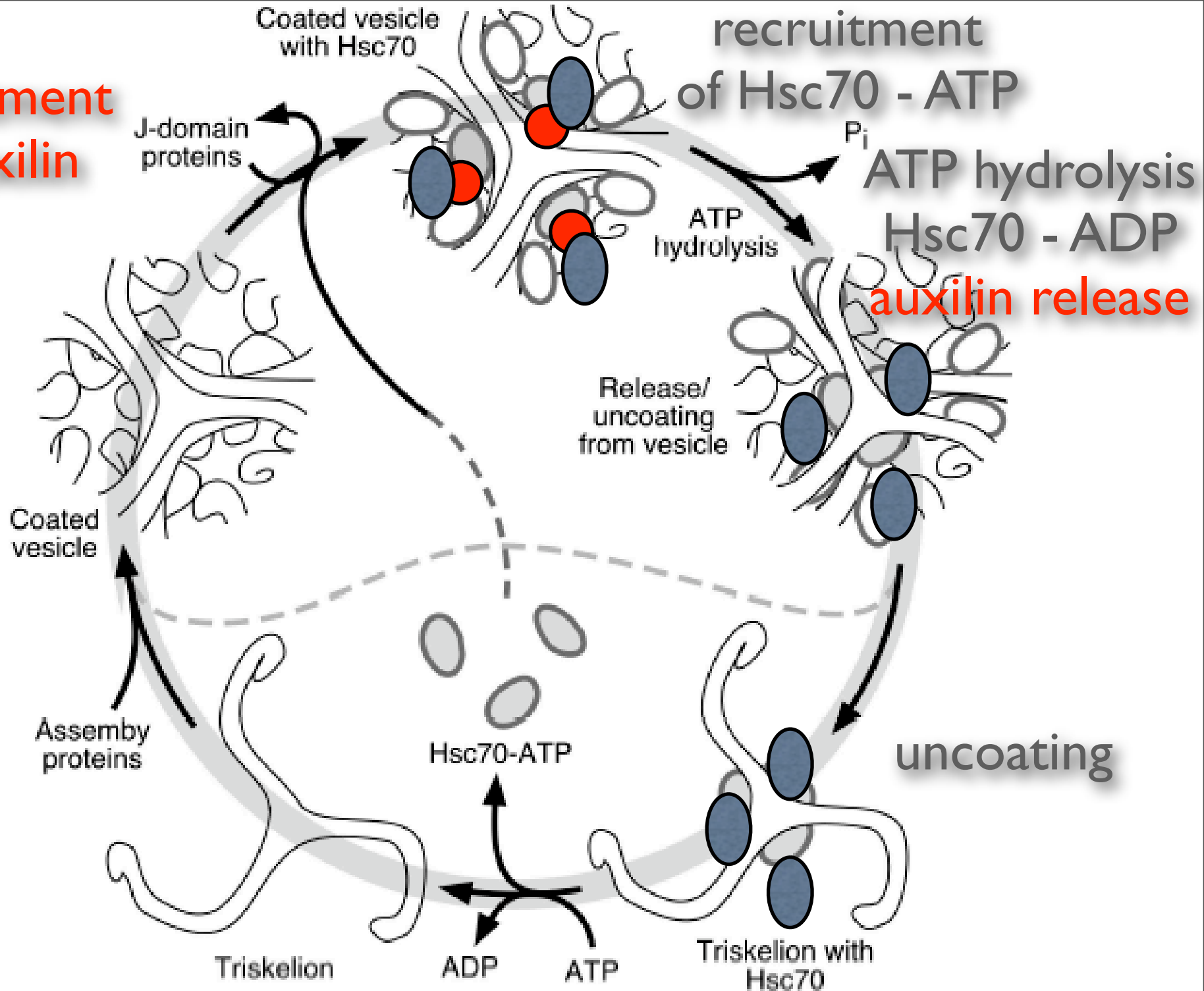
recruitment
of auxilin



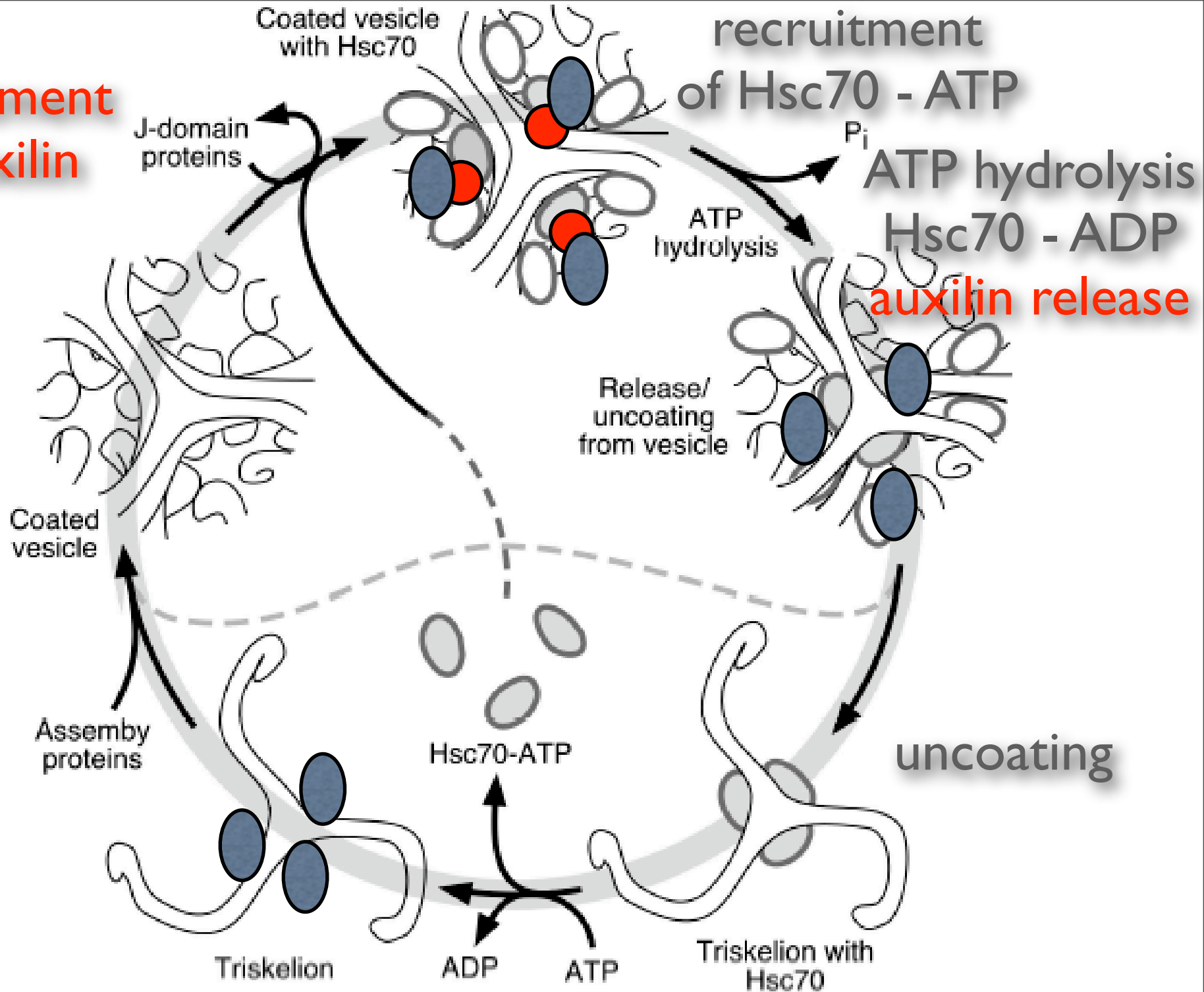
recruitment
of auxilin

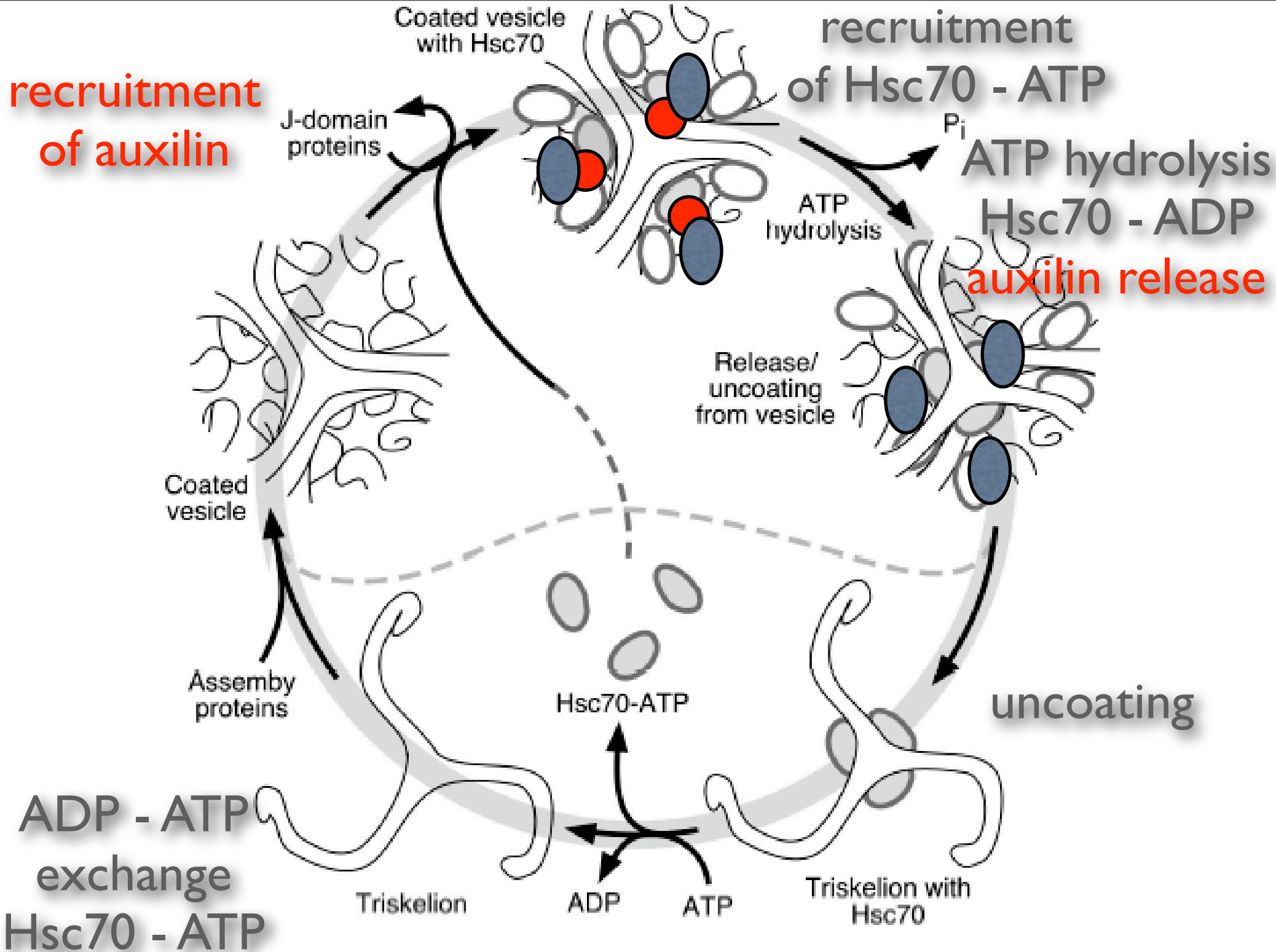


recruitment
of auxilin

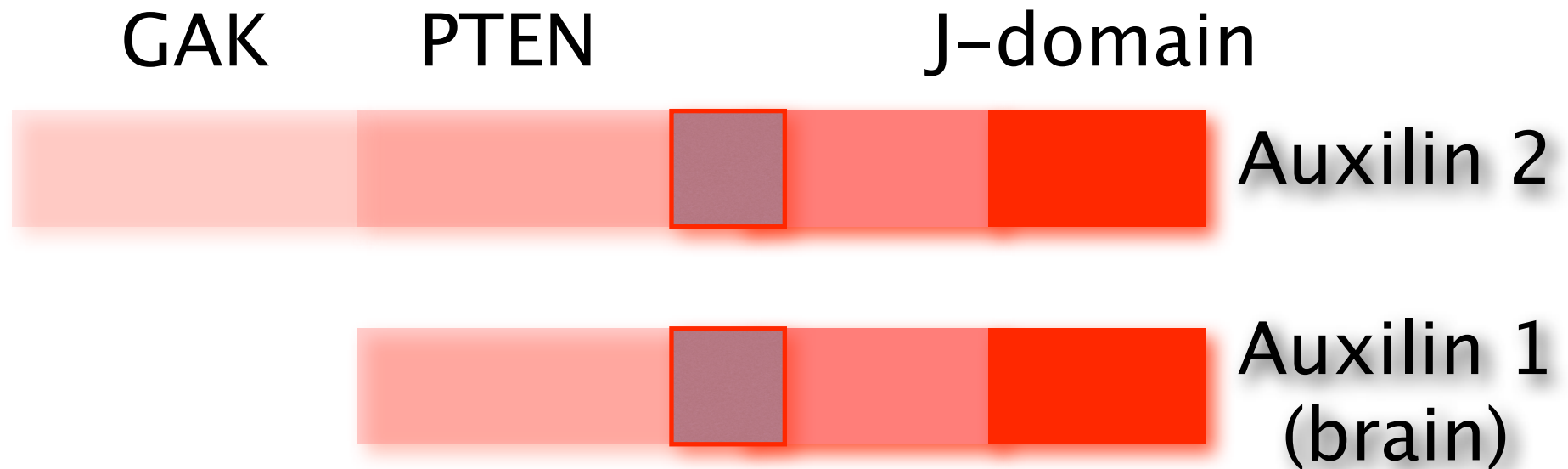


recruitment
of auxilin



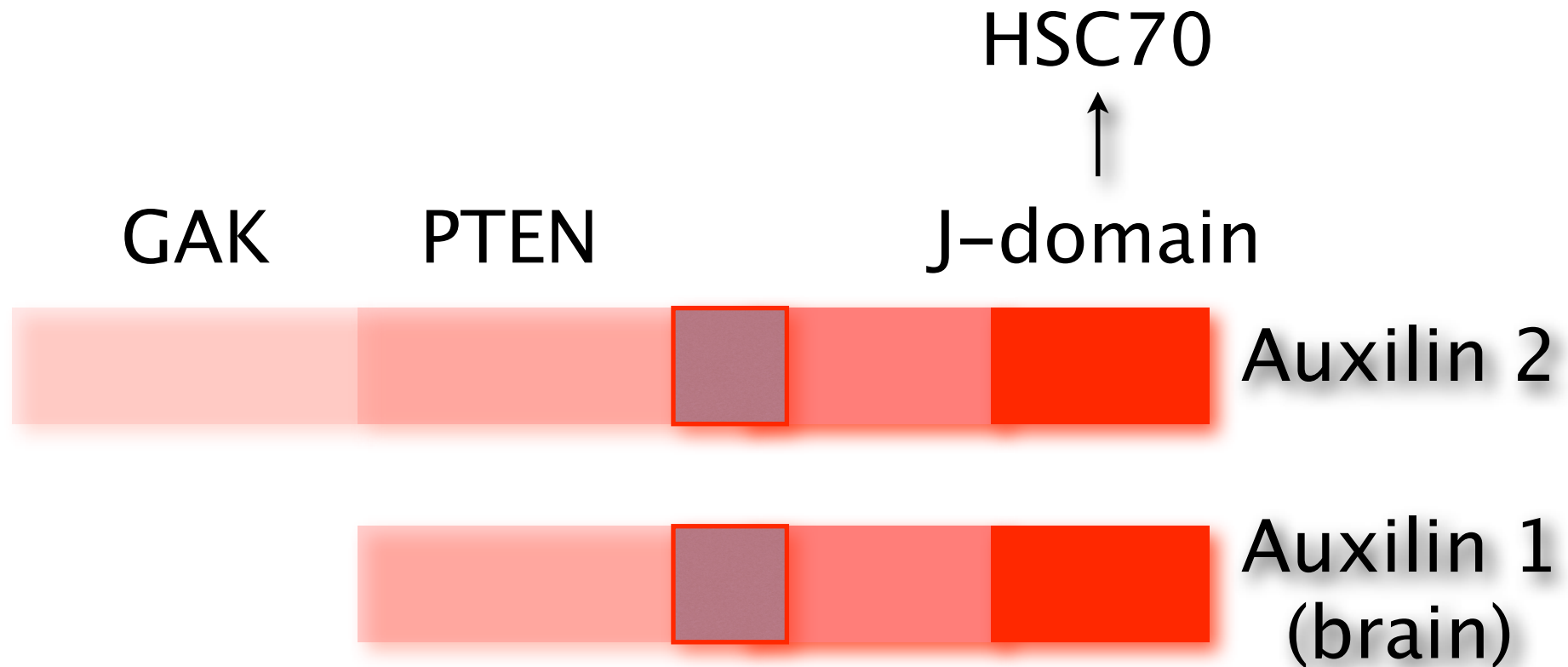


Auxilin -- domain organization



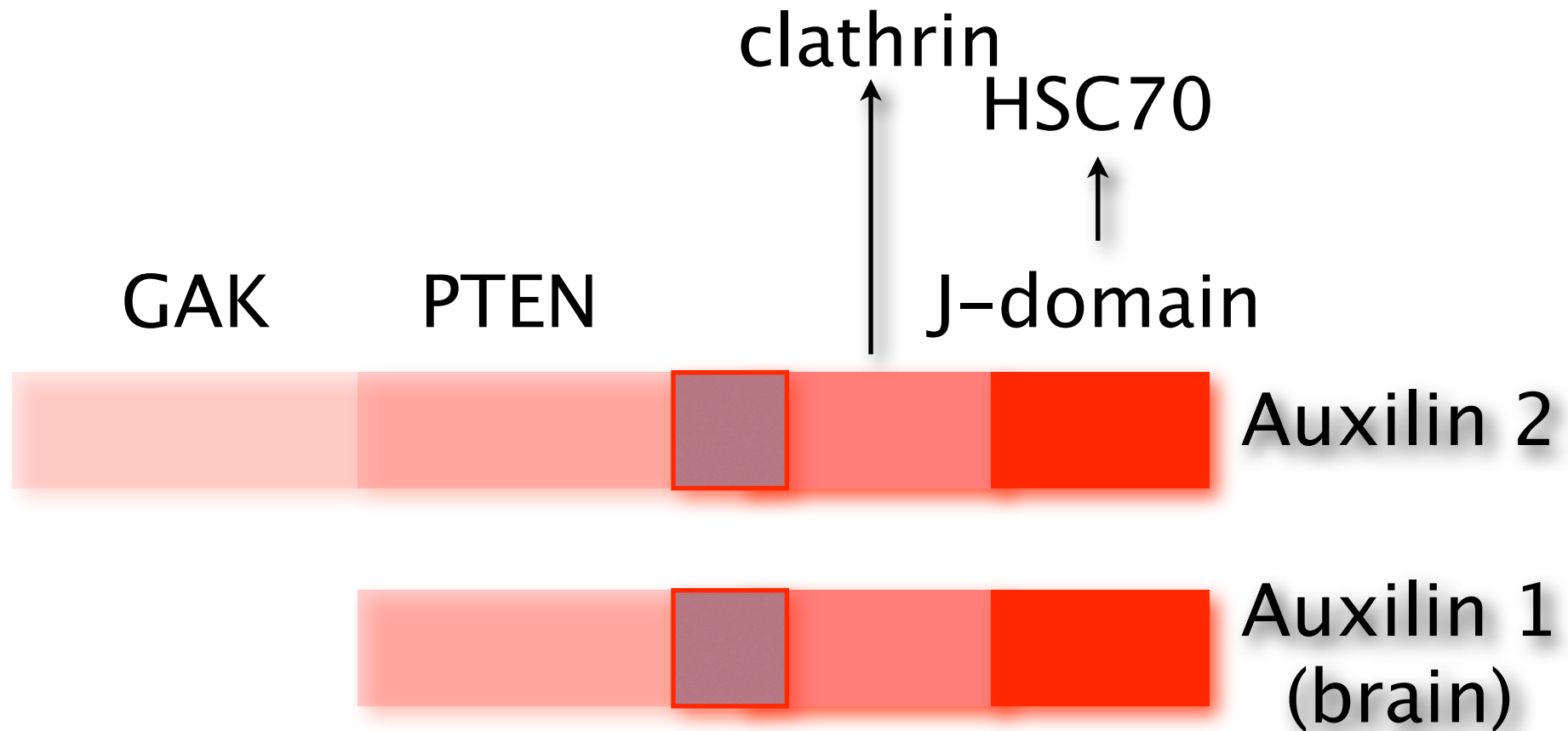
Massol et al 2006

Auxilin -- domain organization



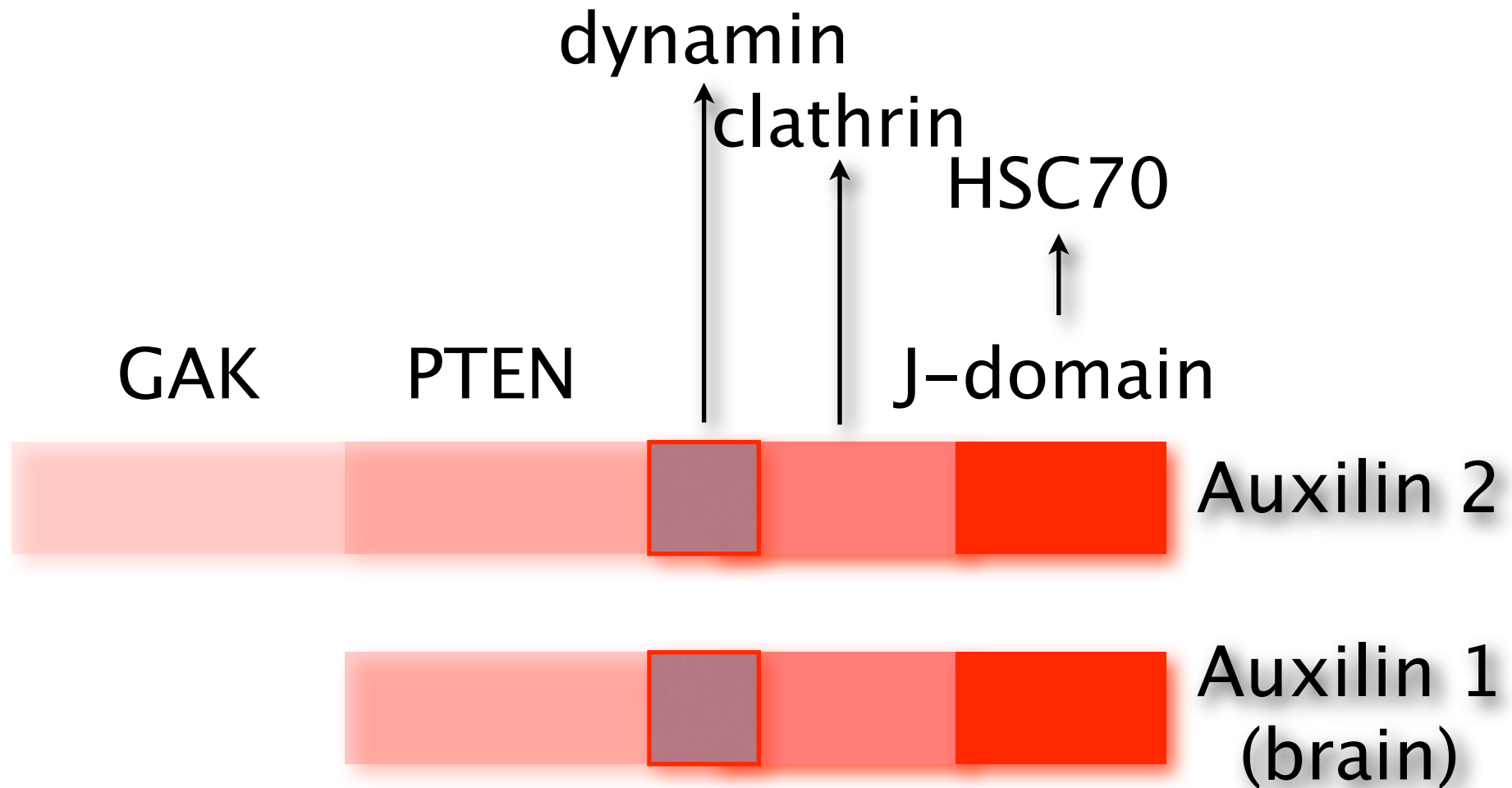
Massol et al 2006

Auxilin -- domain organization



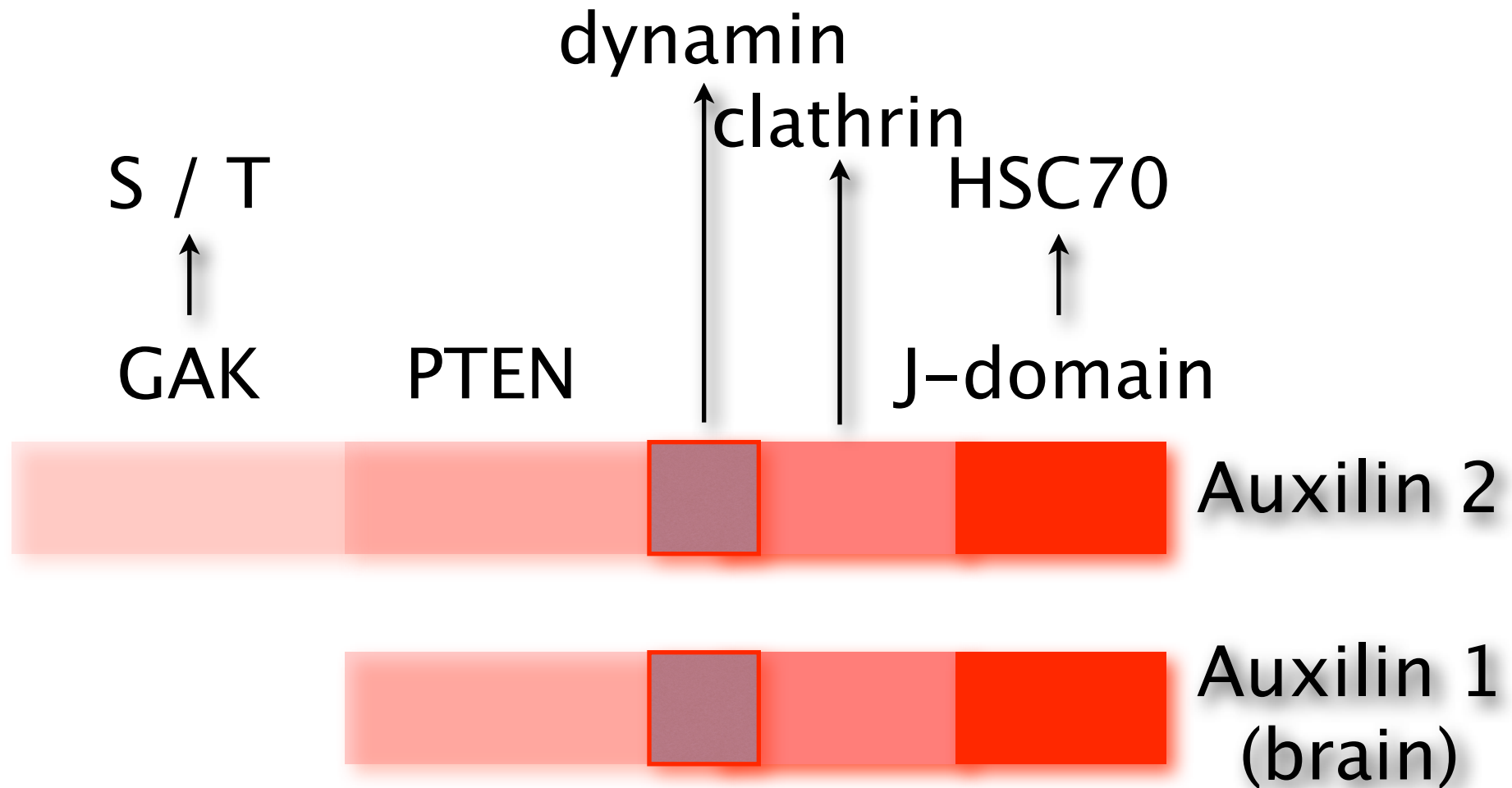
Massol et al 2006

Auxilin -- domain organization



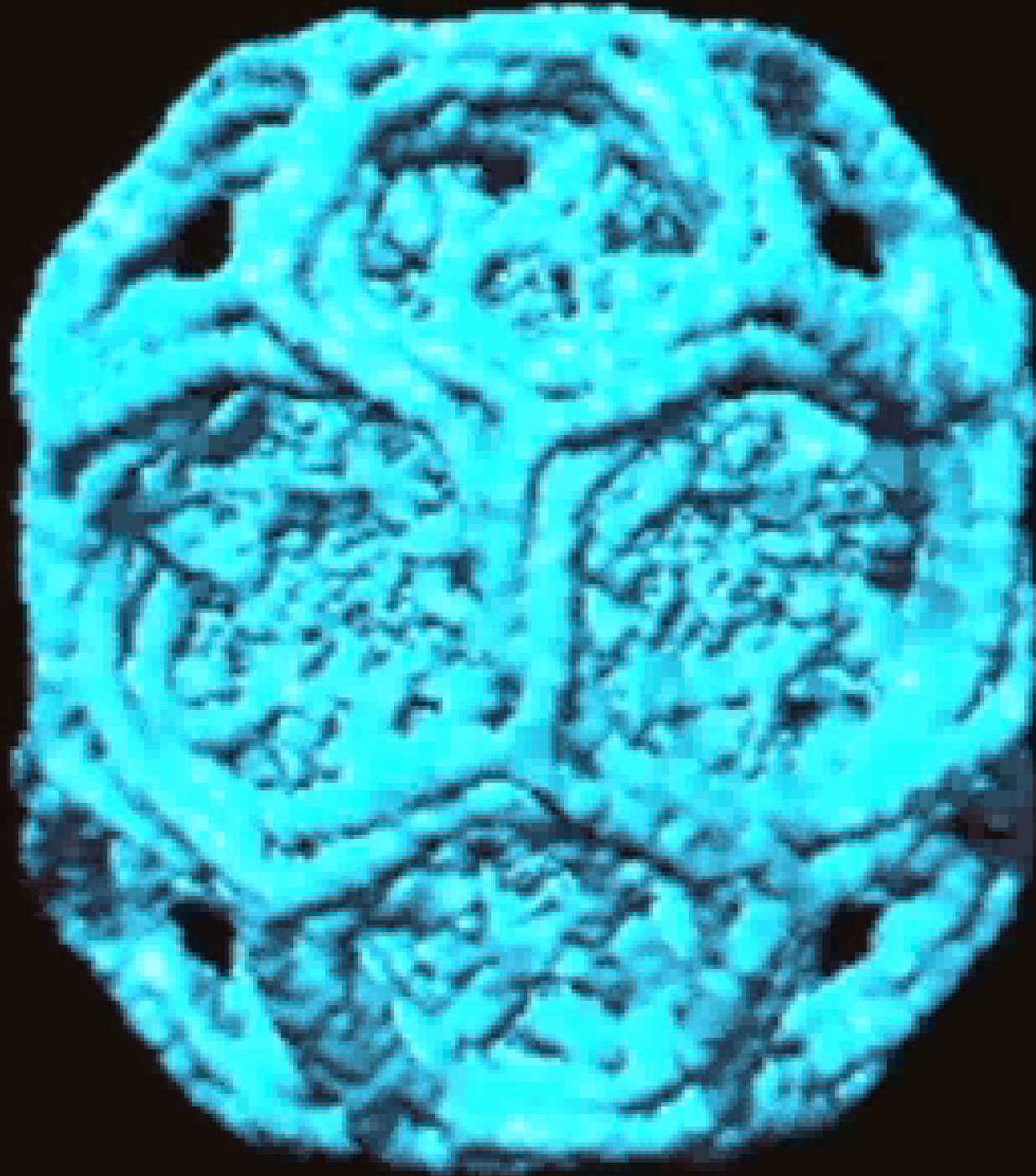
Massol et al 2006

Auxilin -- domain organization



Massol et al 2006

CLATHRIN COAT @ 12 Å resolution



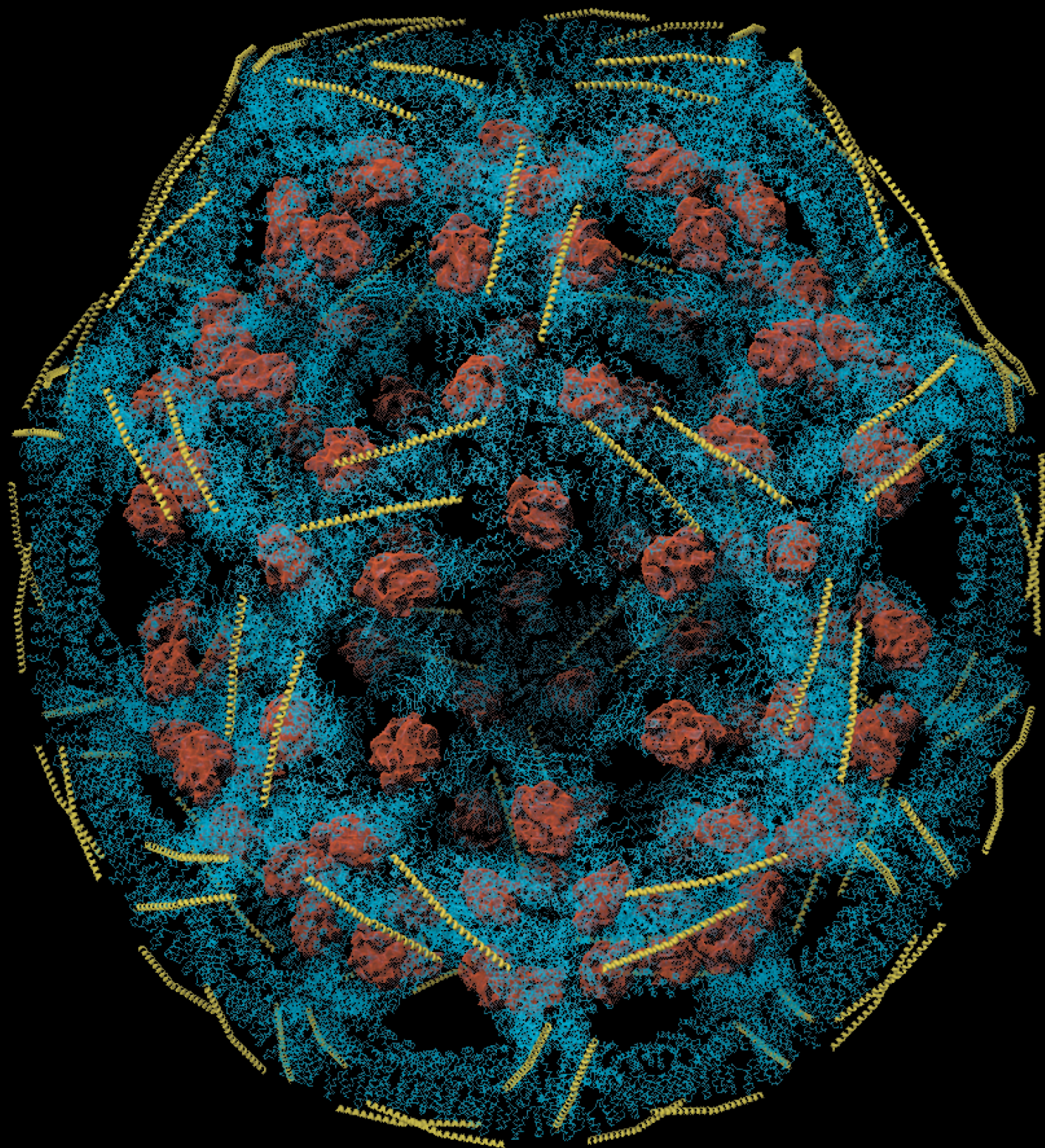
Alex Fotin

CLATHRIN COAT @ 12 Å resolution

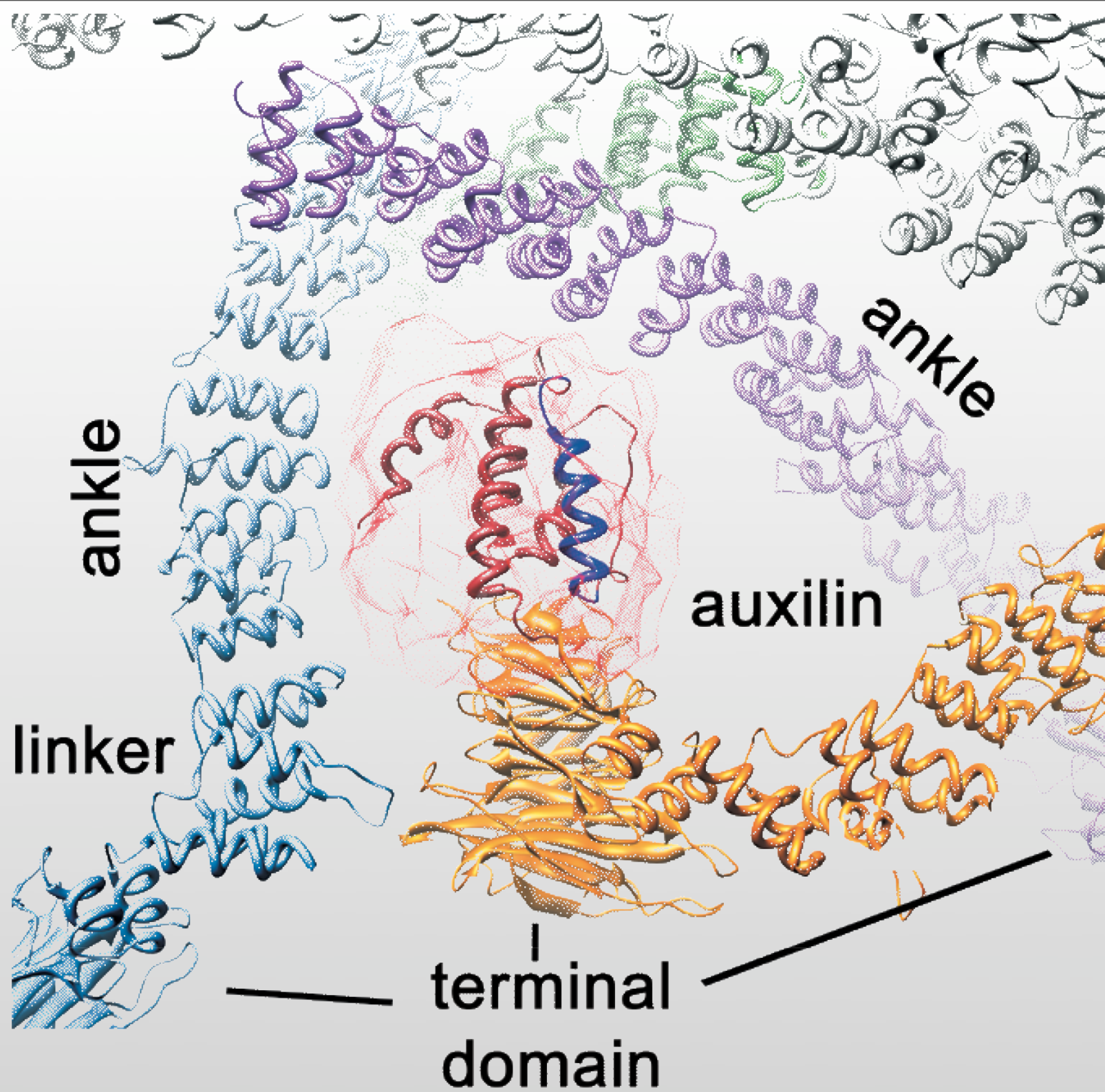
Auxilin

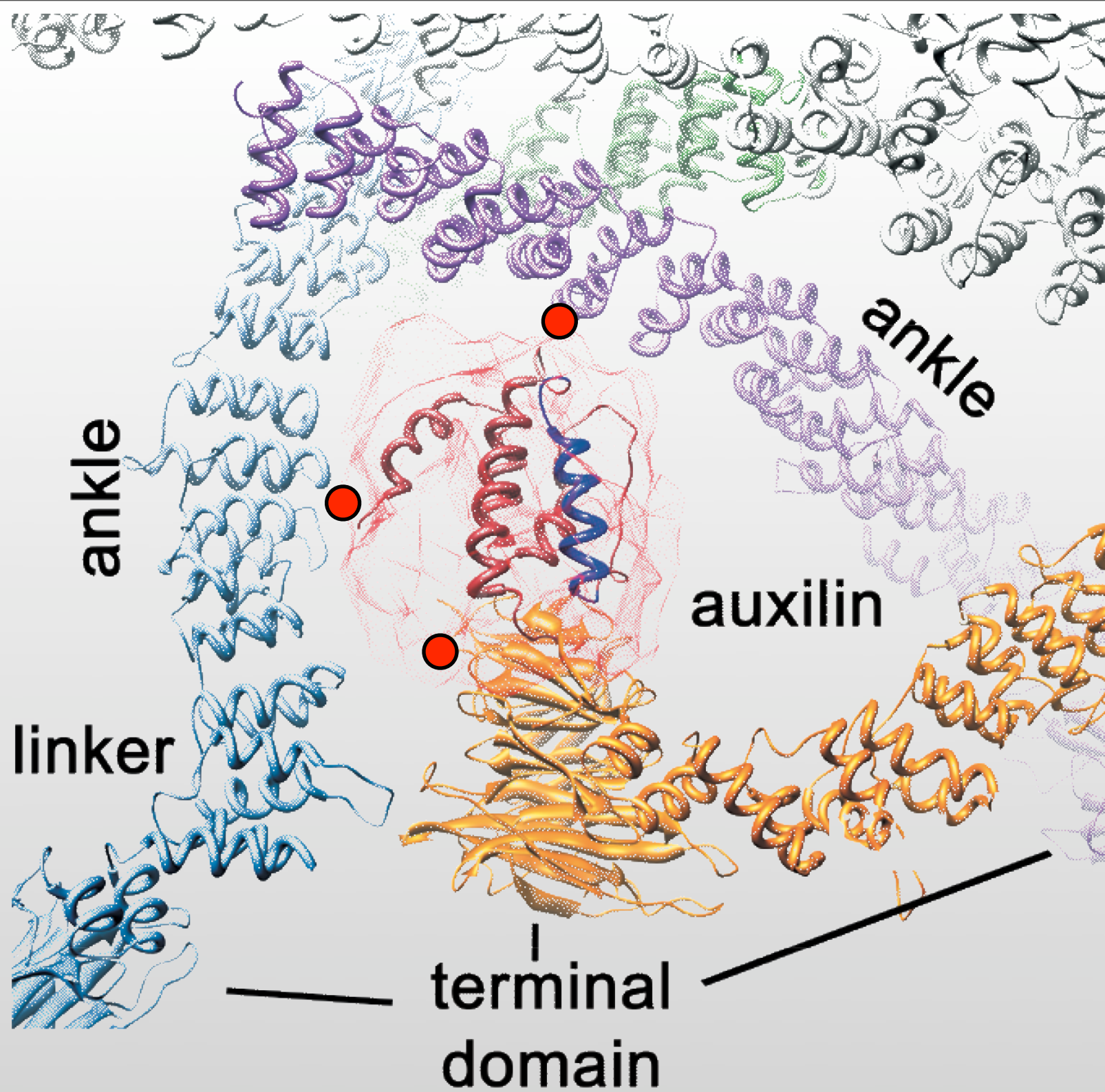
Alex Fotin

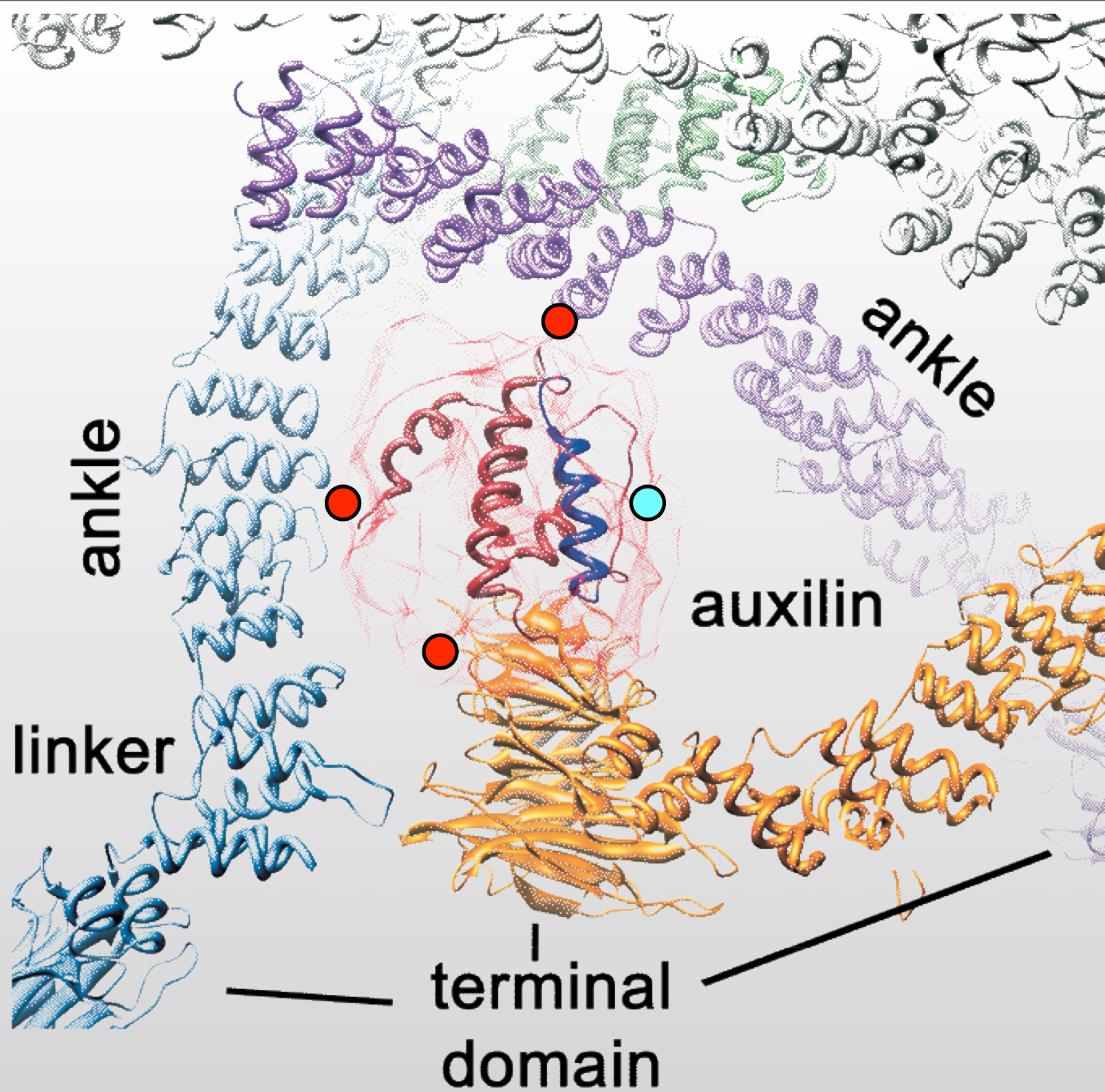
Alex Fotin

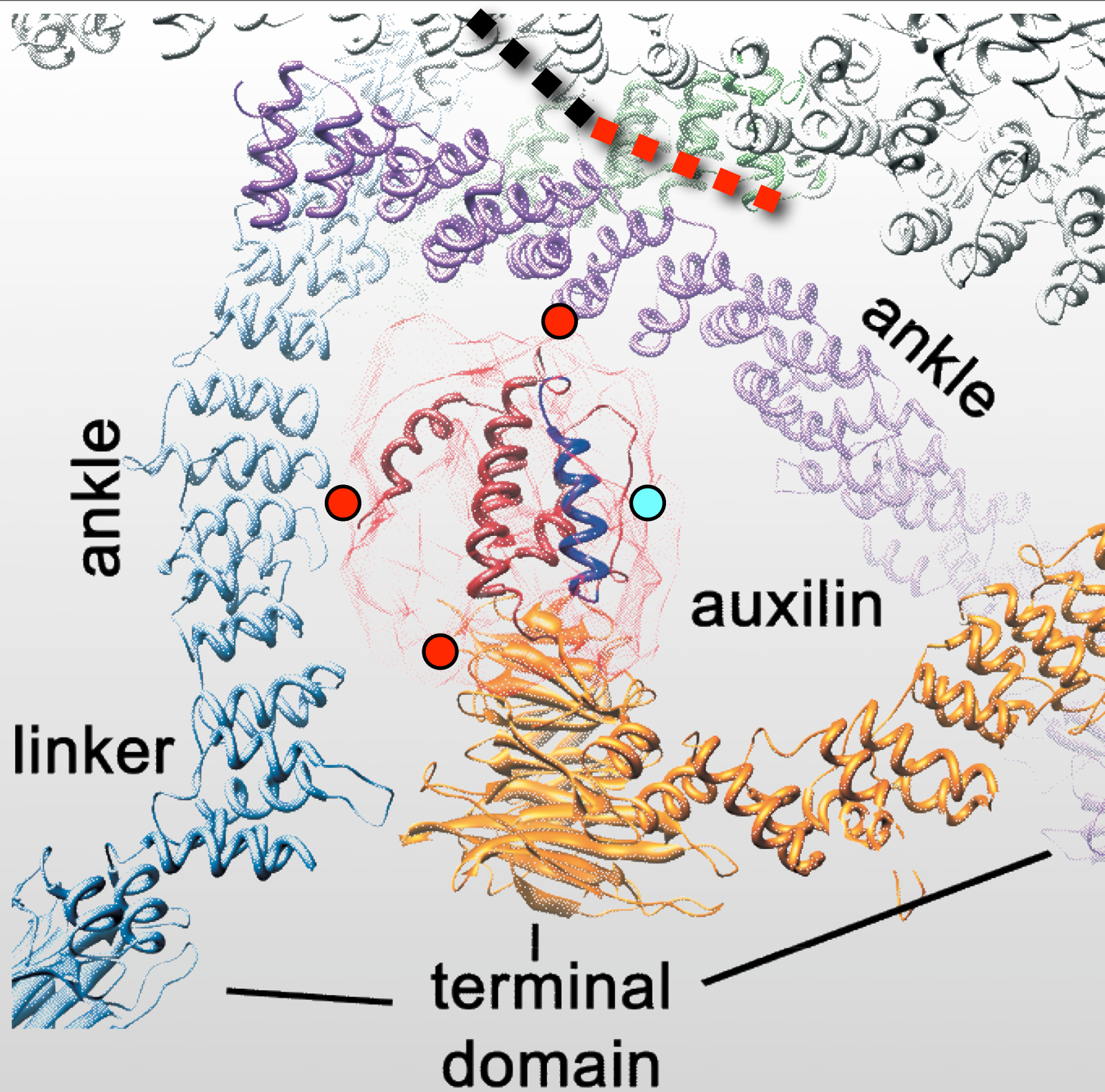


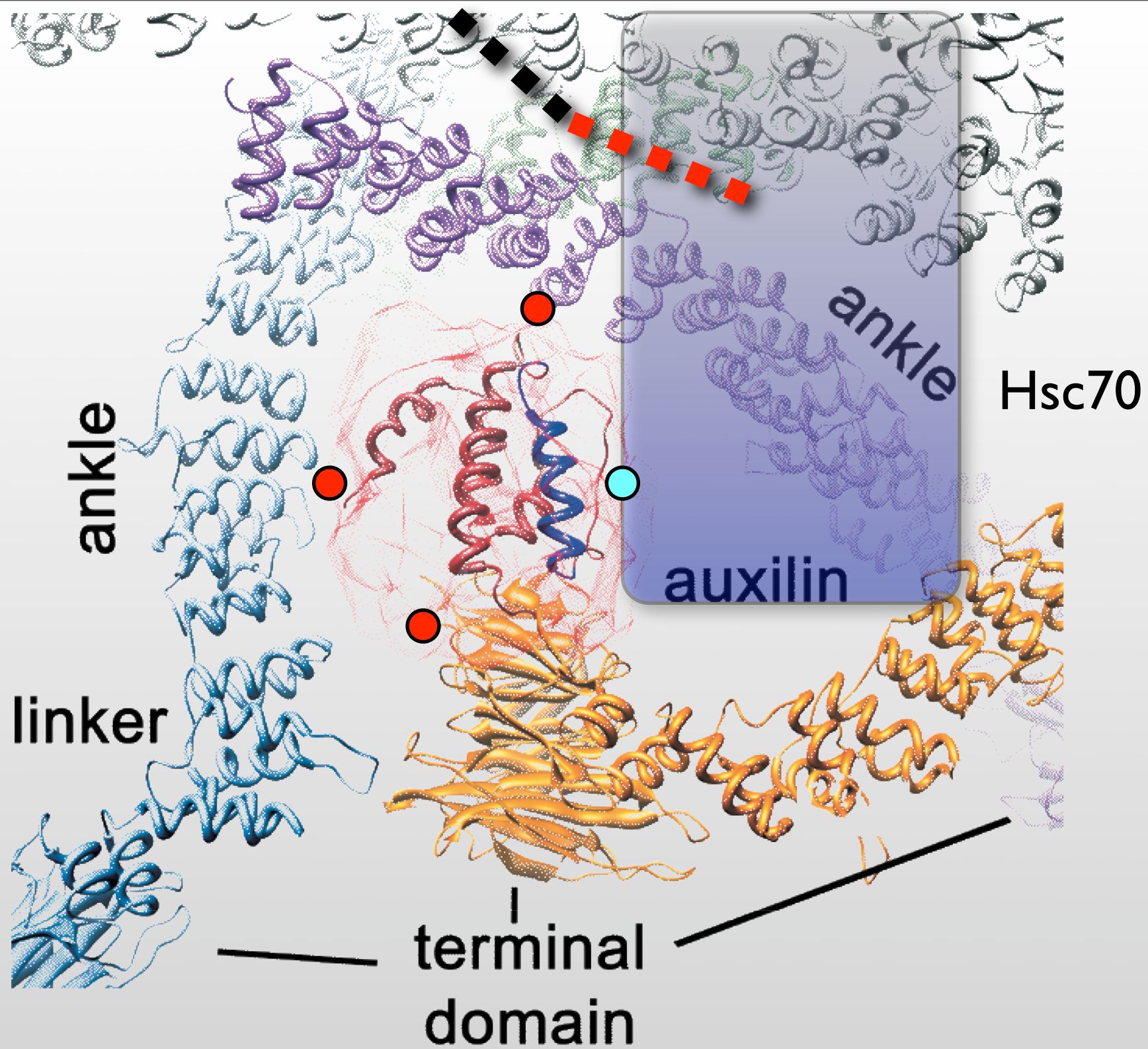
Alex Fotin



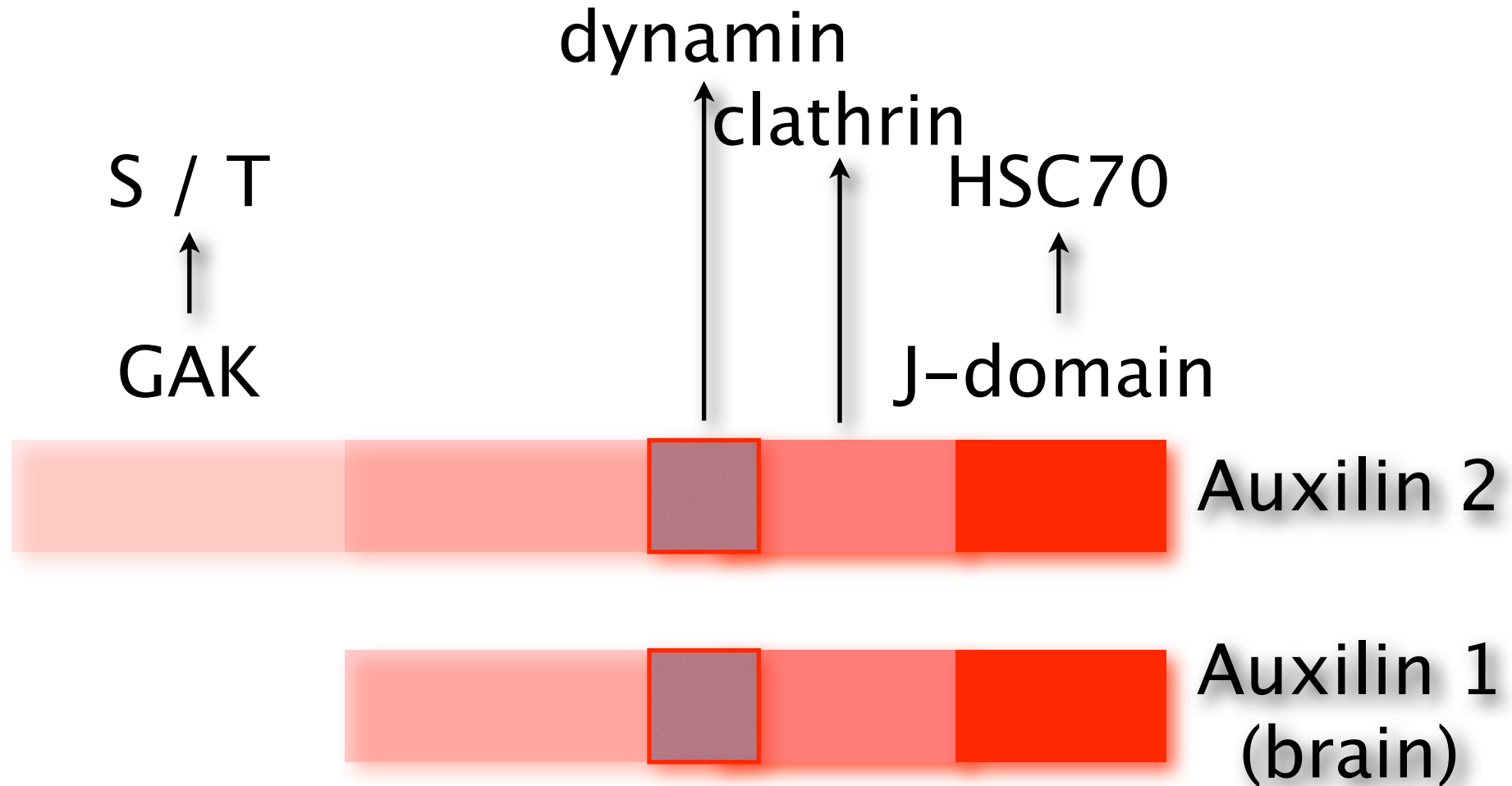






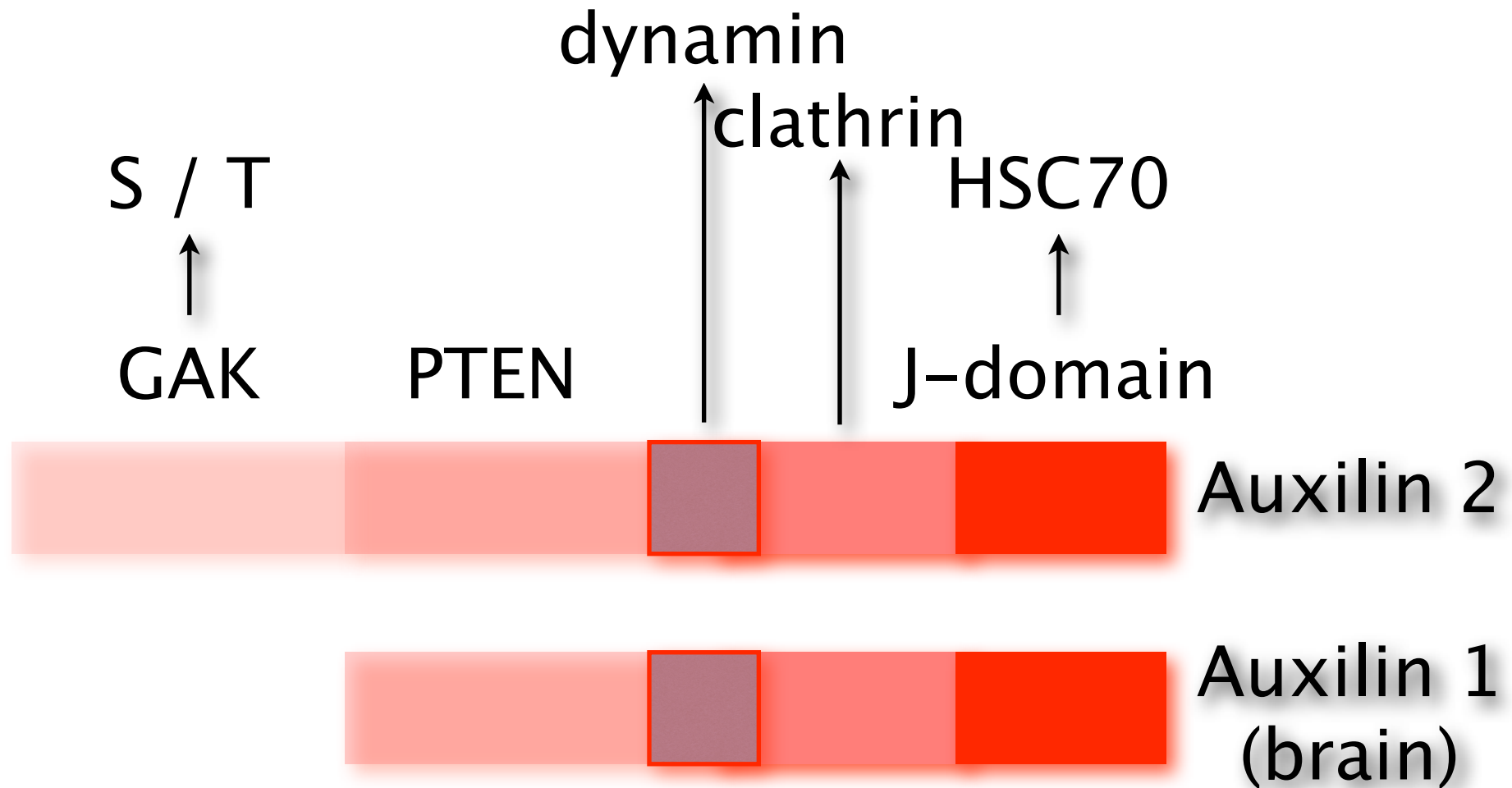


Auxilin -- domain organization



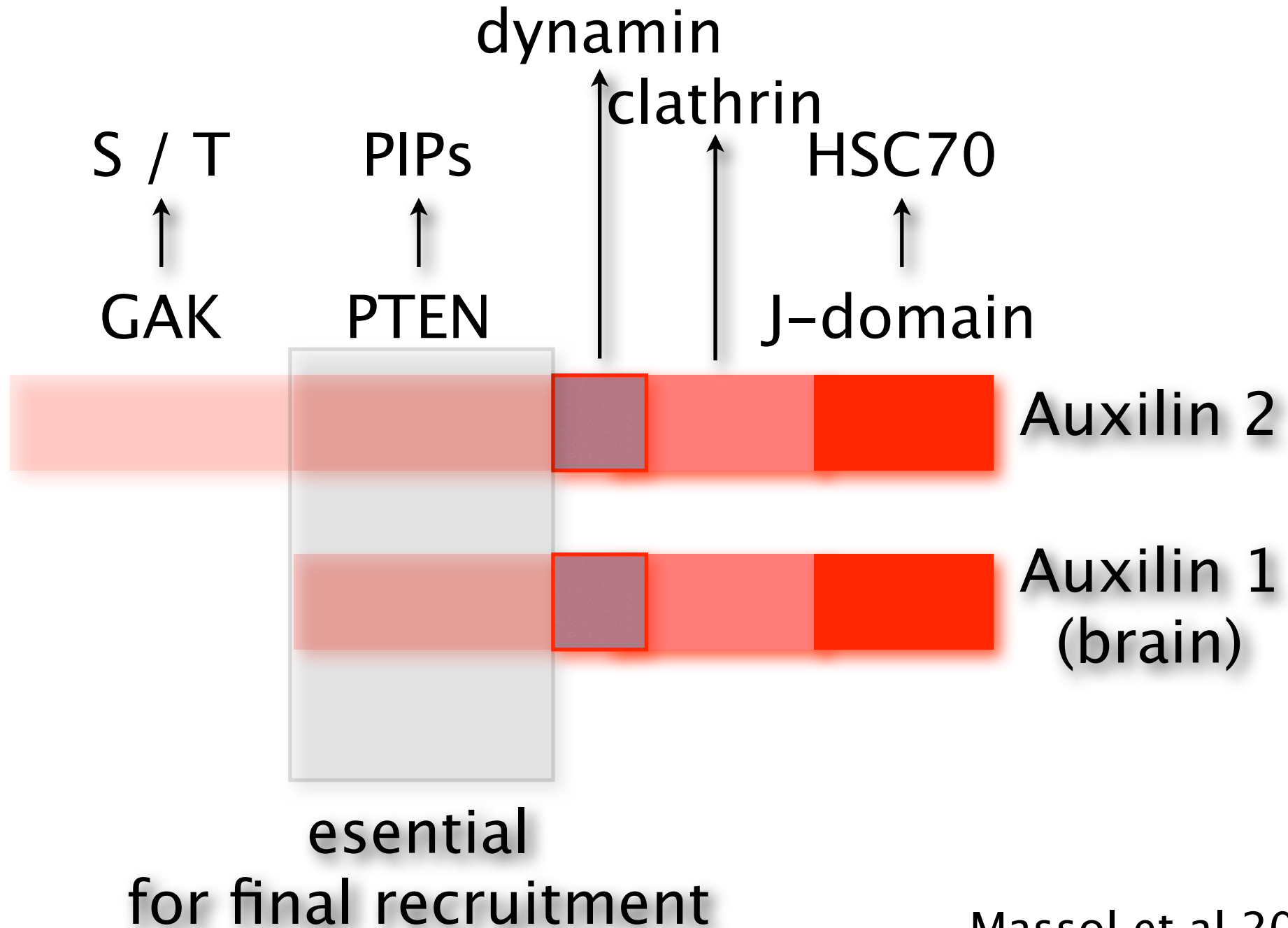
Massol et al 2006

Auxilin -- domain organization



Massol et al 2006

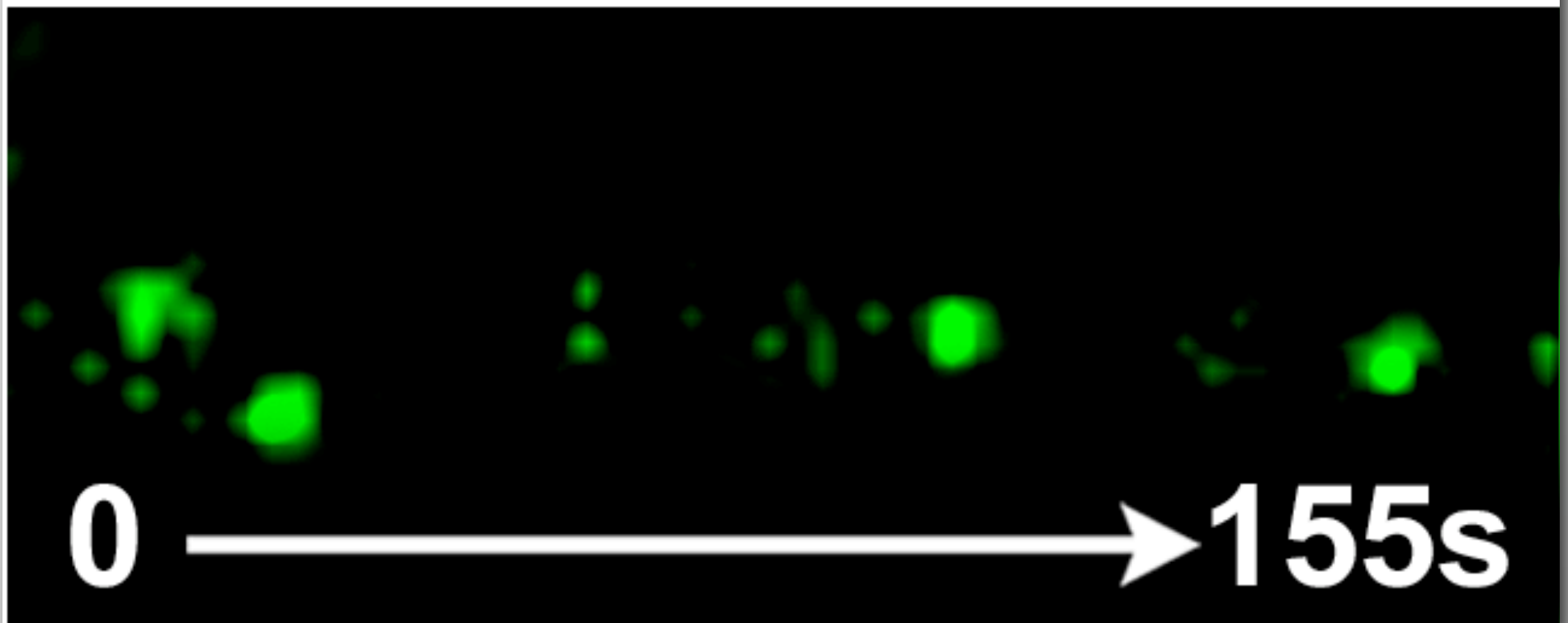
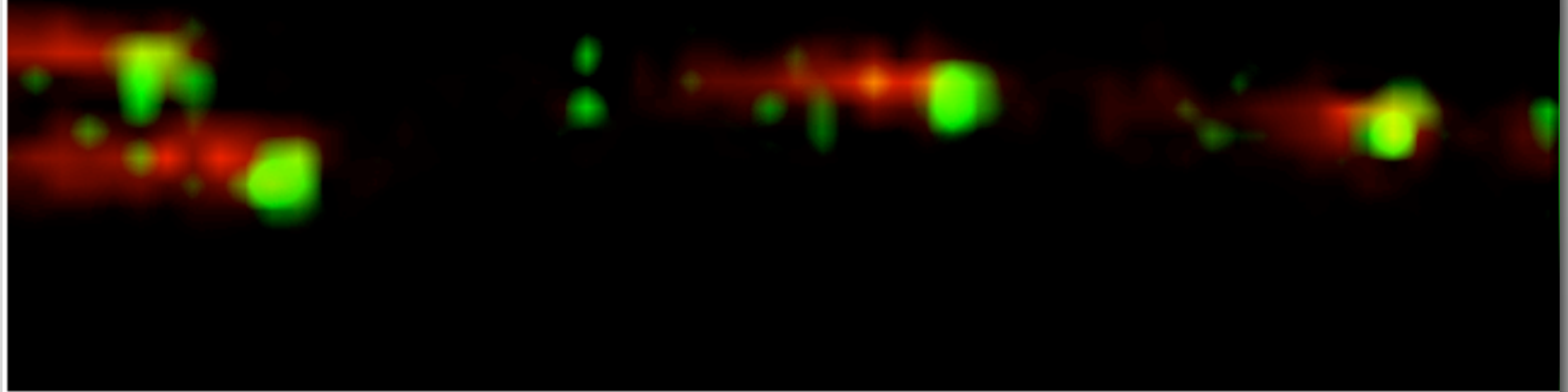
Auxilin -- domain organization

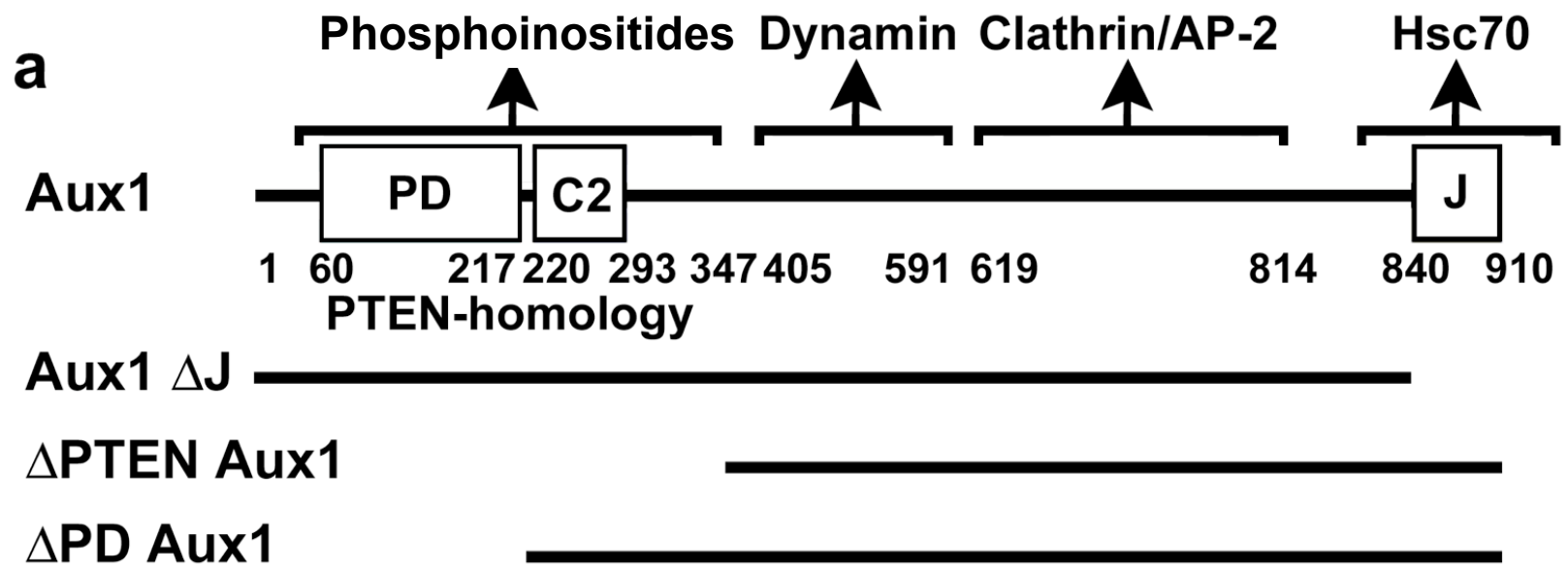


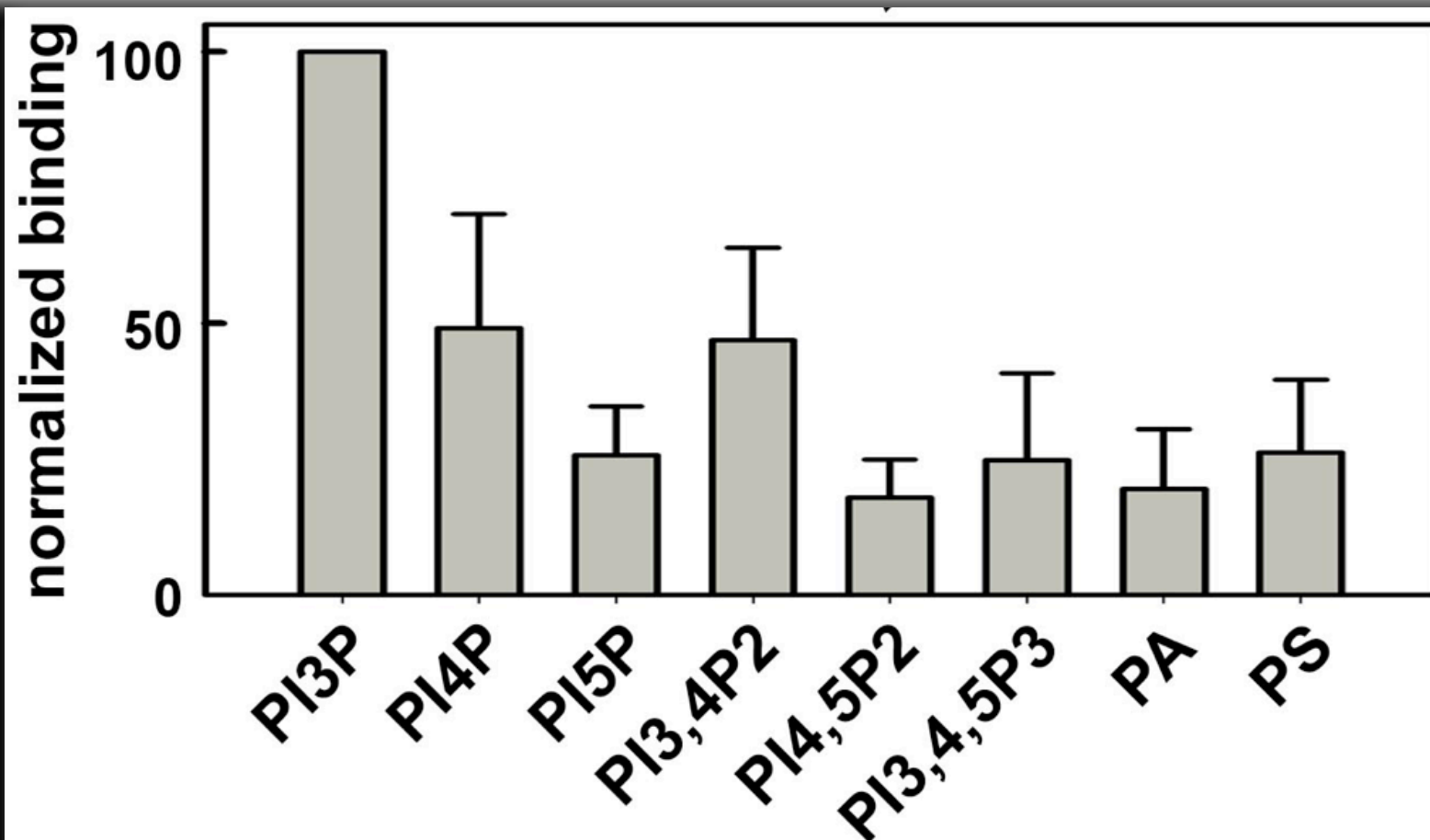
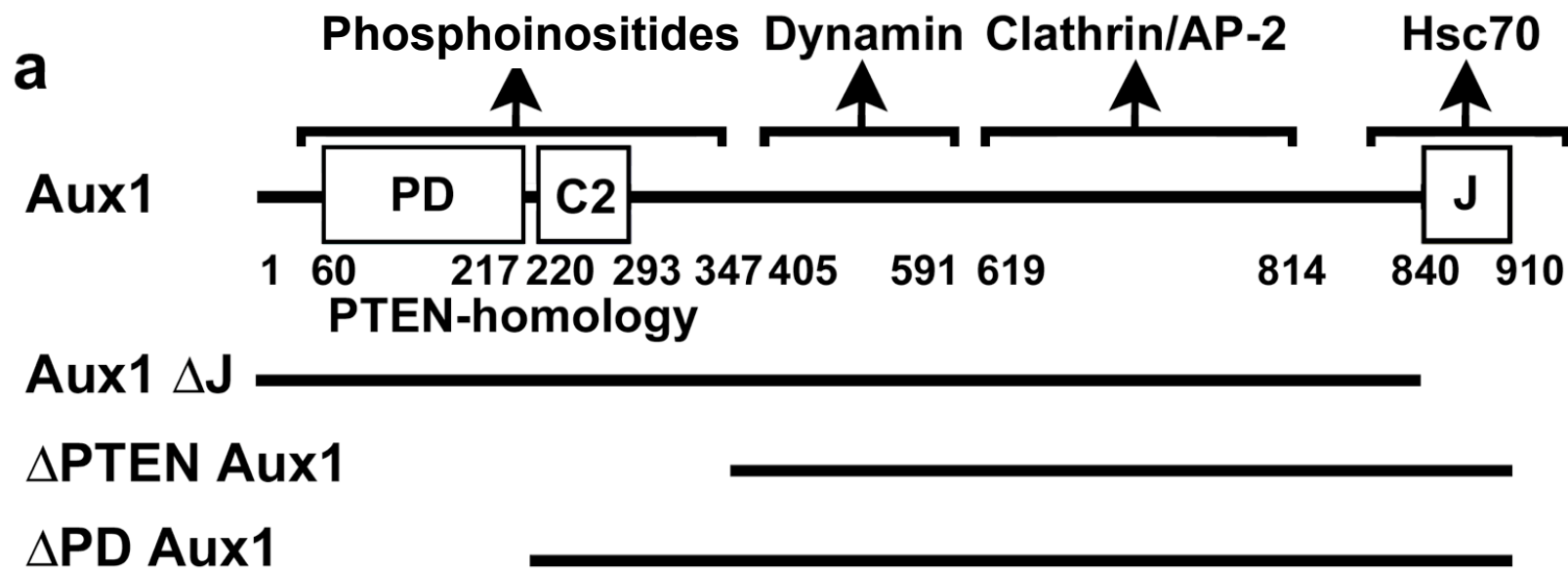
Massol et al 2006

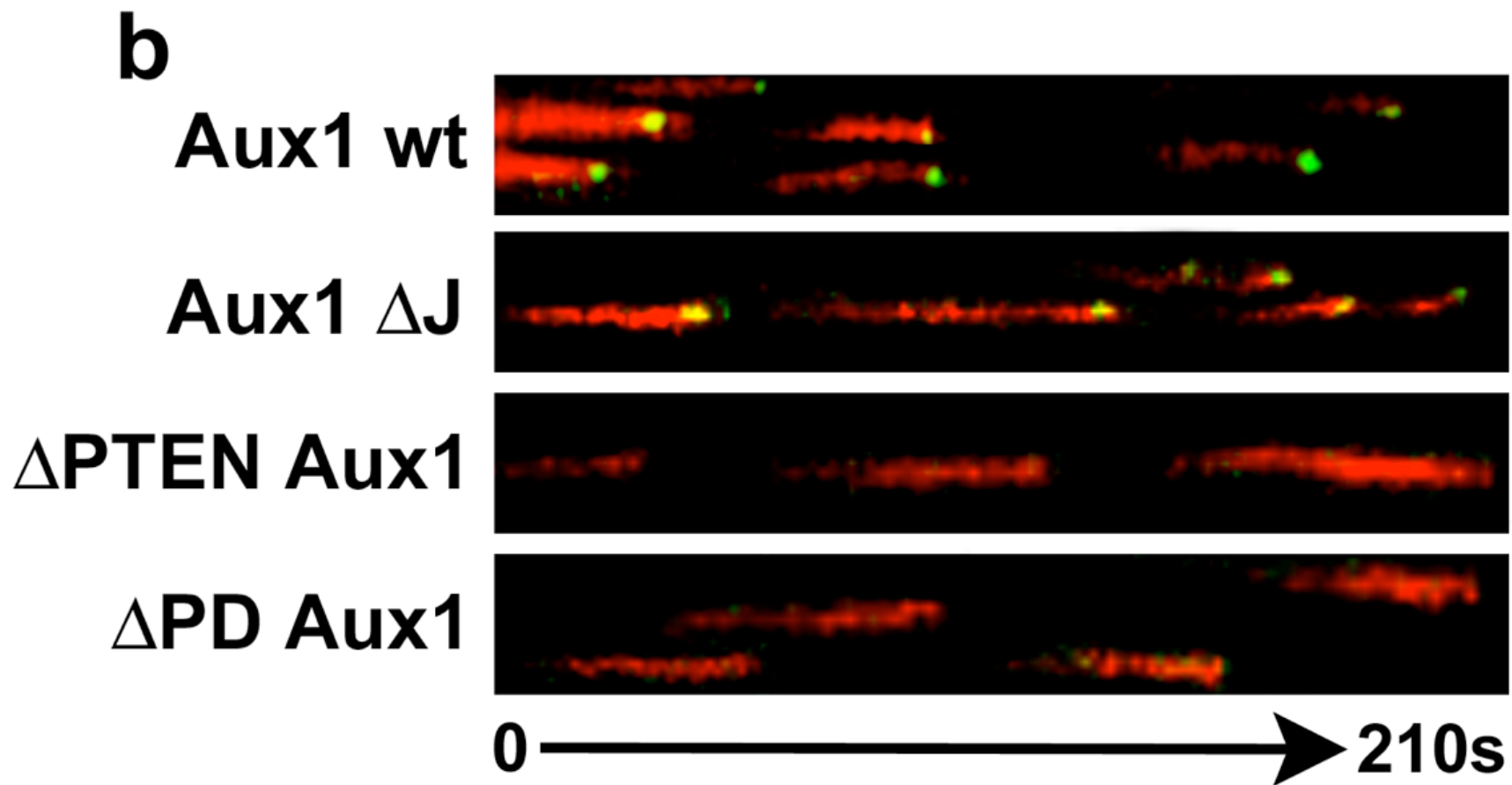
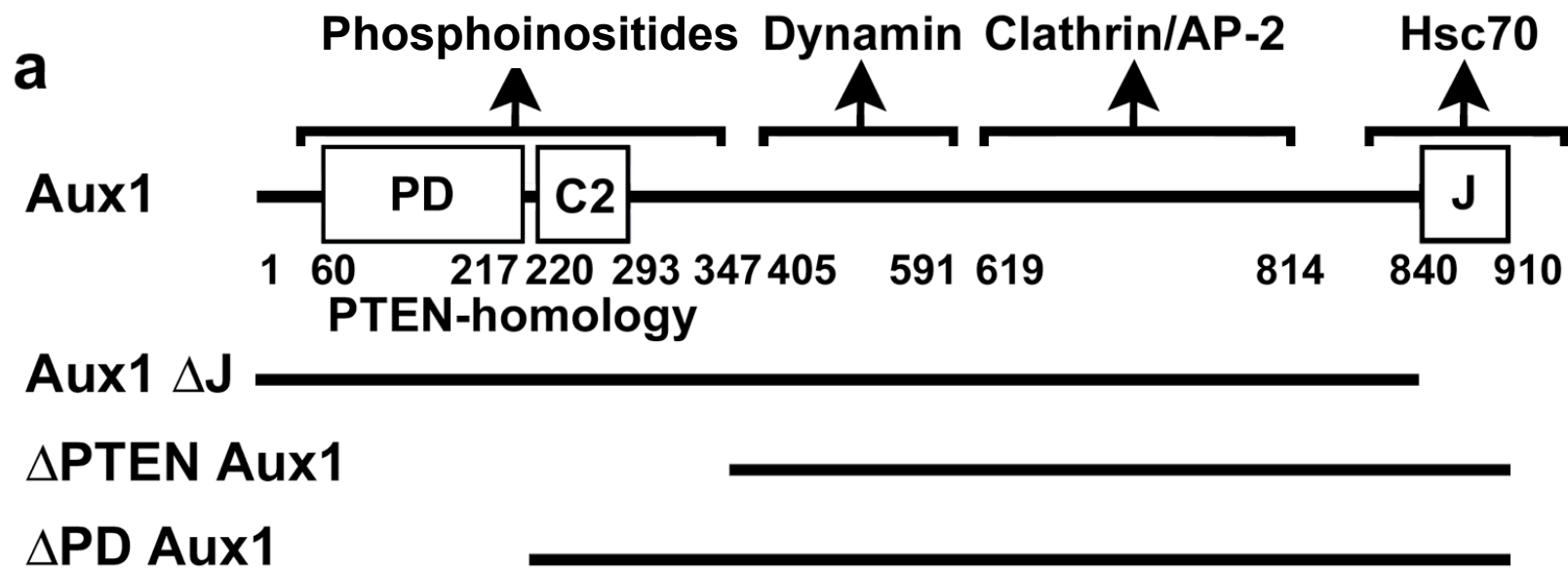
Clathrin

Auxilin



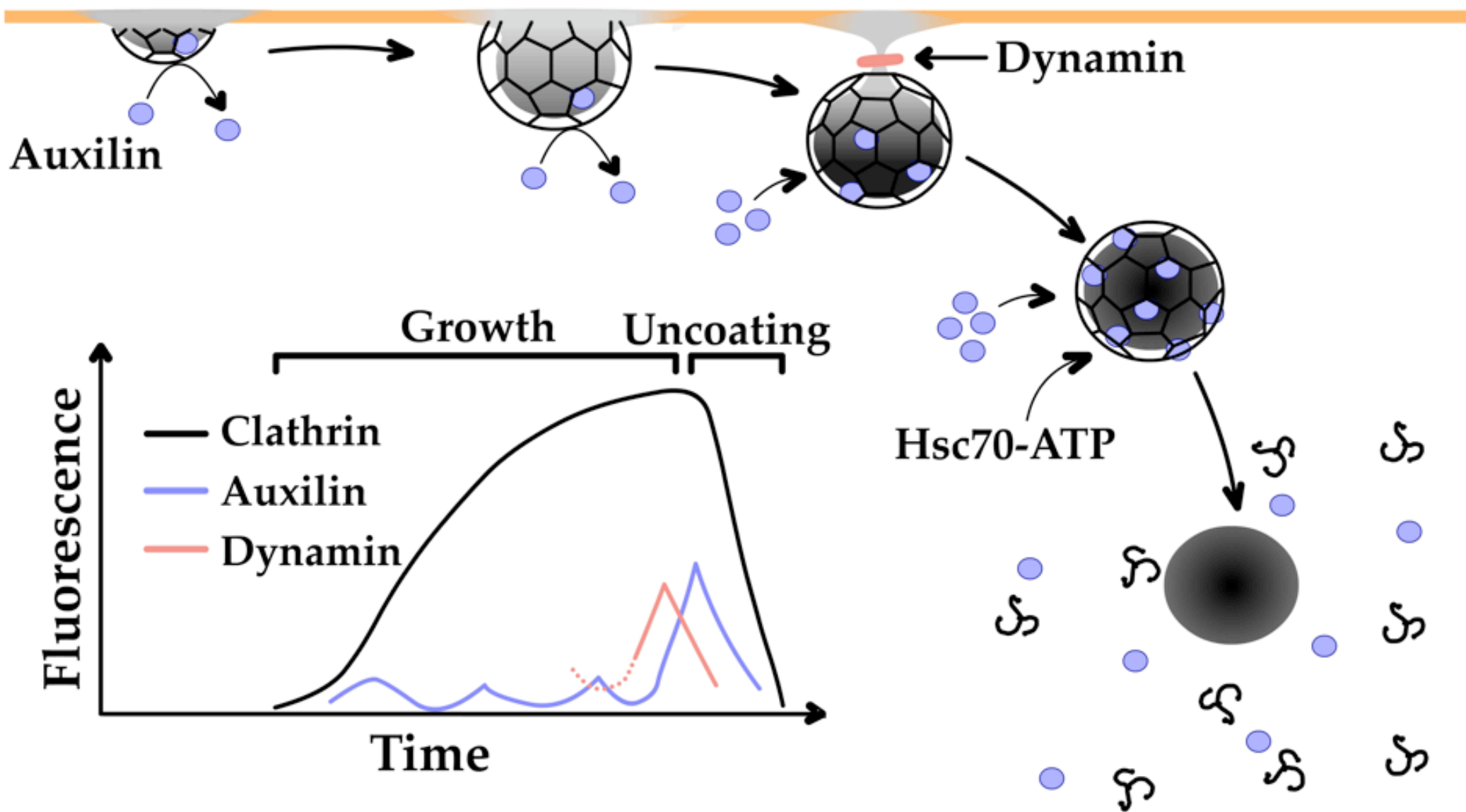




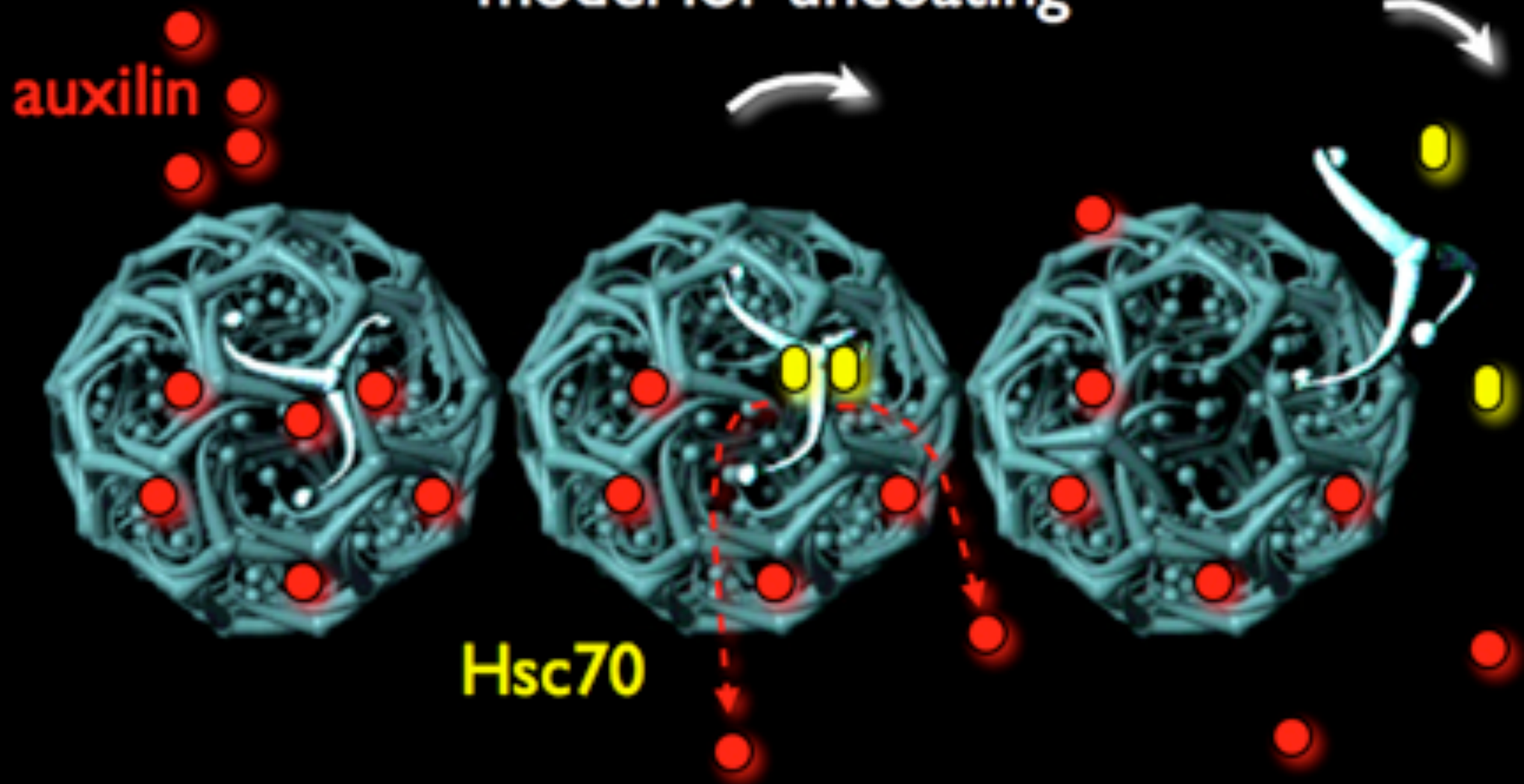


Growth

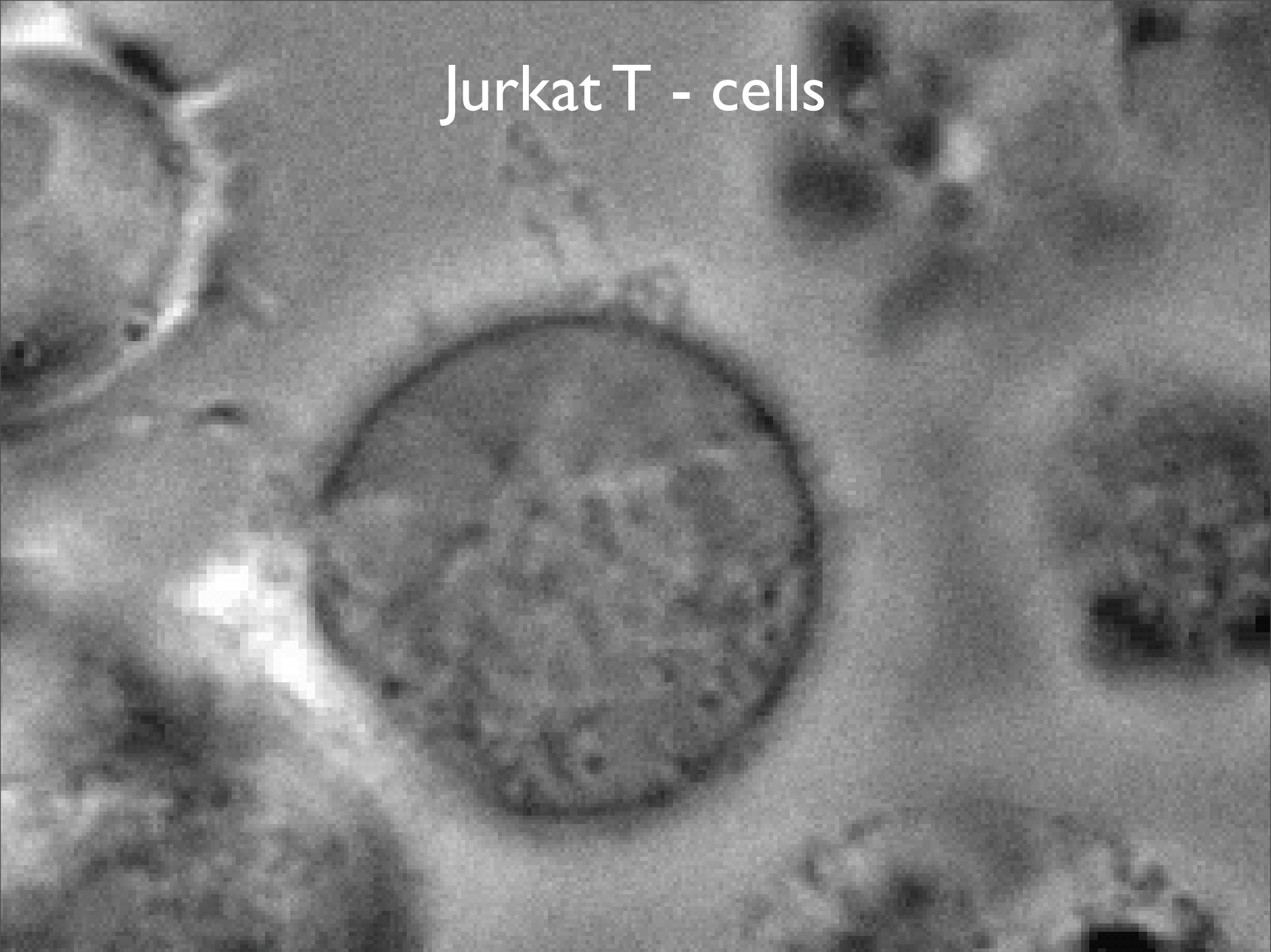
Uncoating

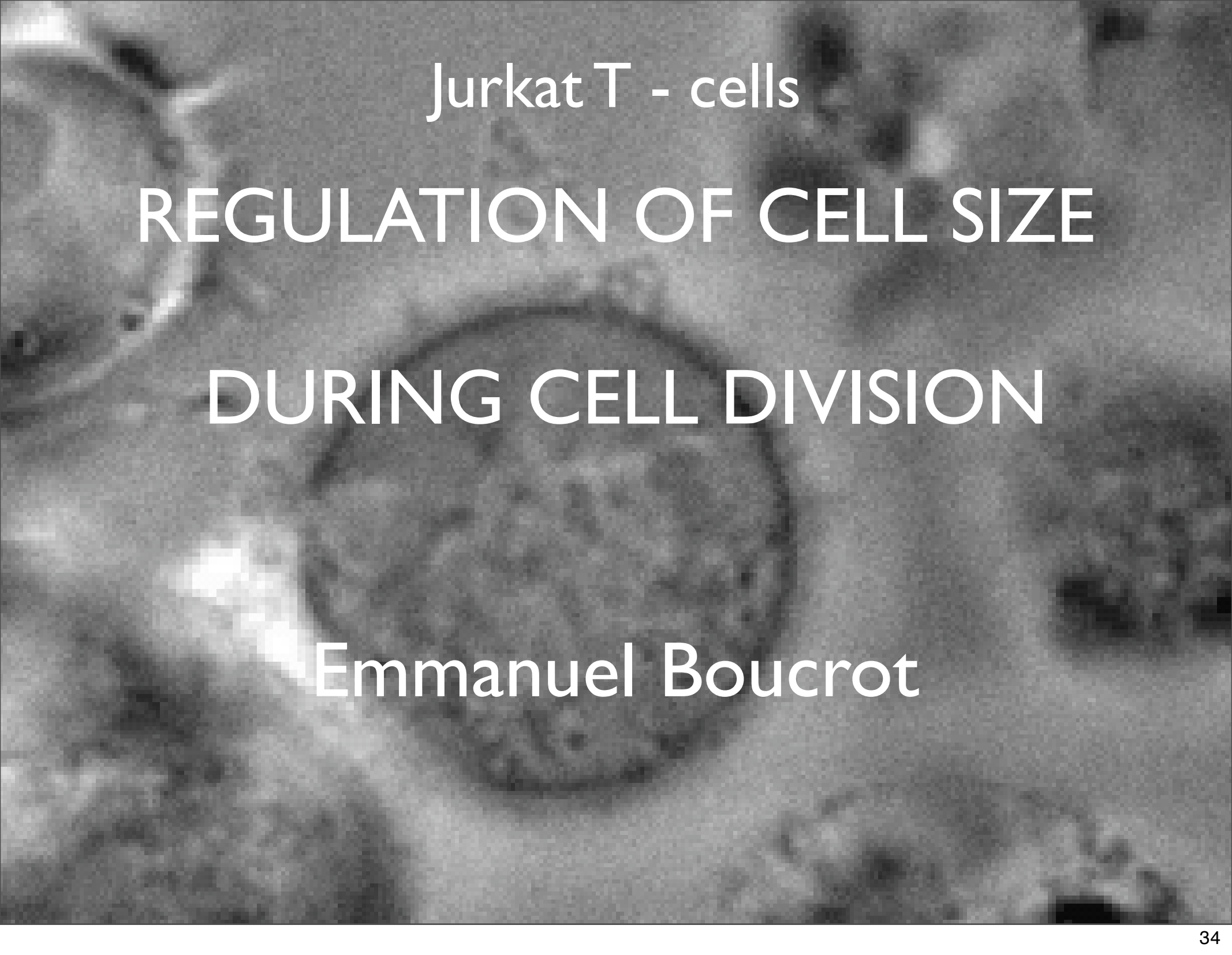


model for uncoating



Jurkat T - cells



A grayscale electron micrograph of a cell in the process of division. A large, dark, circular nucleus is visible in the center, with a prominent nucleolus. The surrounding cytoplasm is filled with various organelles and a network of filaments. The overall texture is grainy, typical of electron microscopy.

Jurkat T - cells

REGULATION OF CELL SIZE

DURING CELL DIVISION

Emmanuel Boucrot

cell division



► Interphase

Prophase

Prometaphase

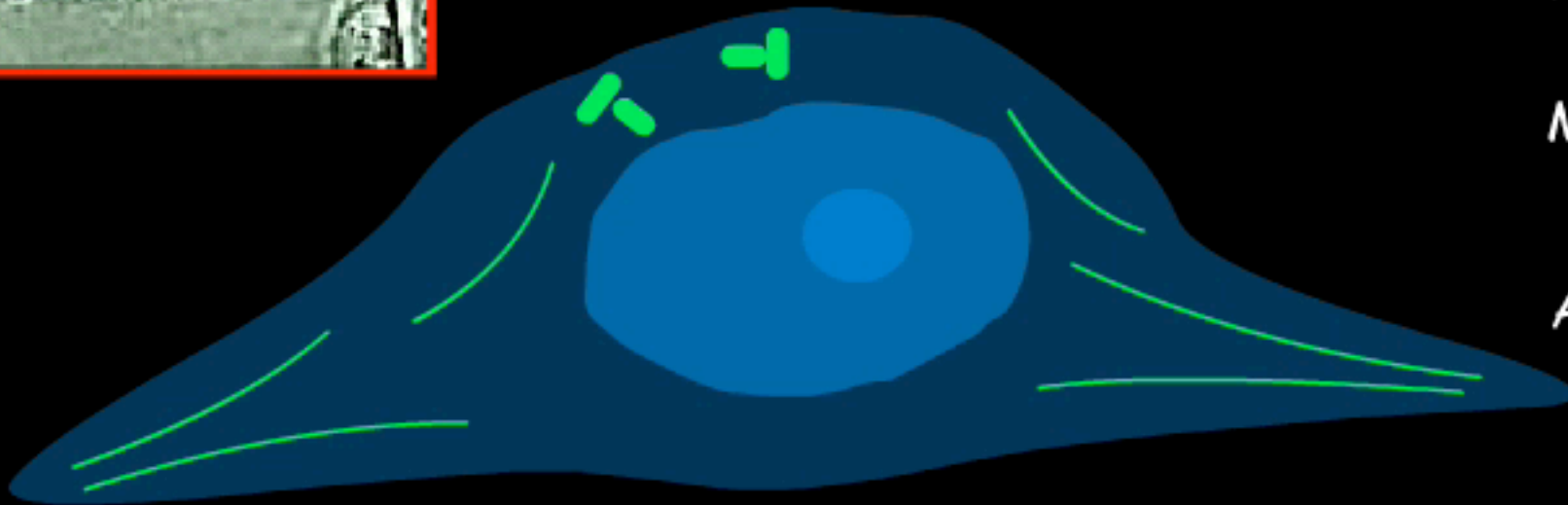
Metaphase

Anaphase

Telophase

Cytokinesis

Interphase

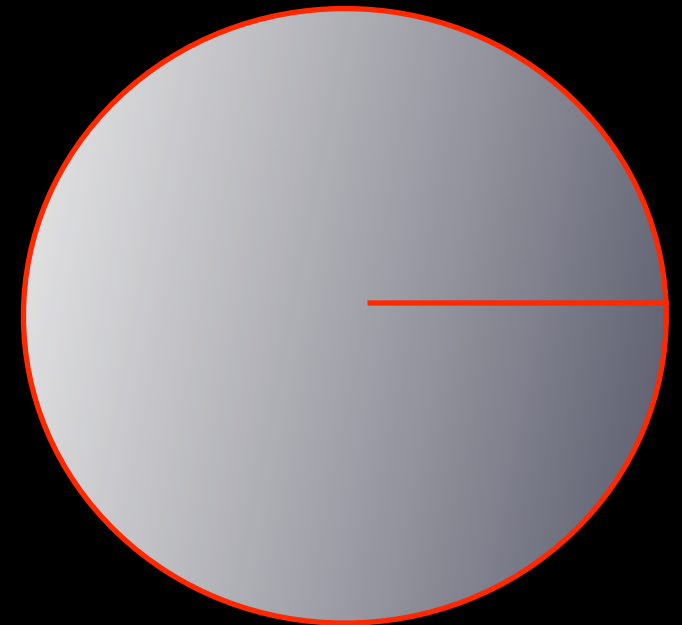
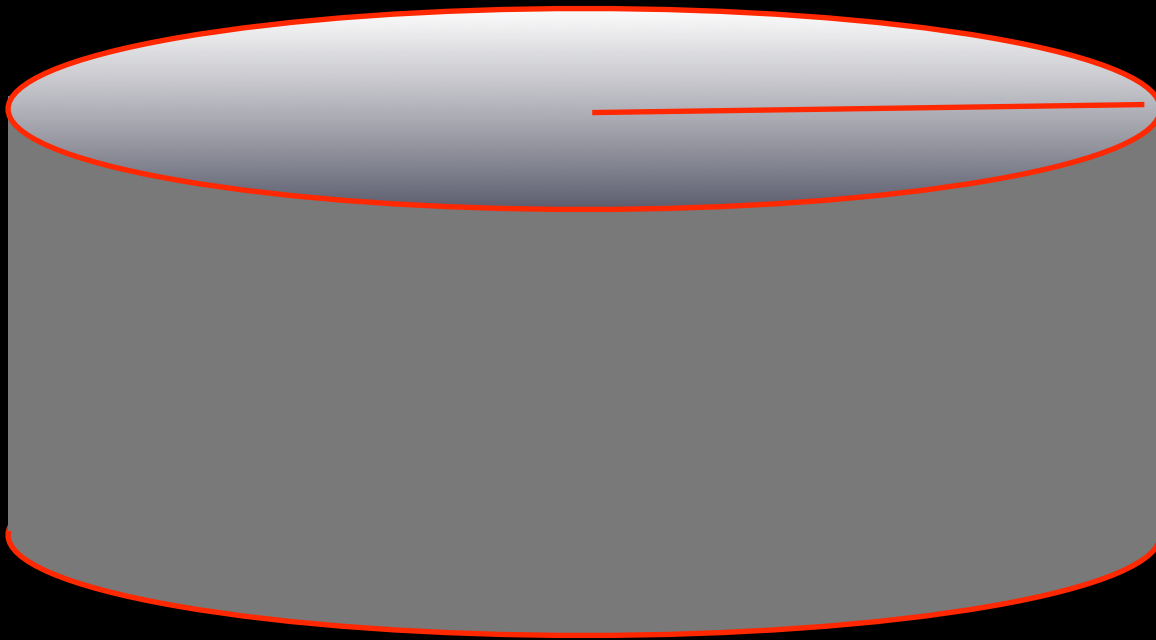


$$\text{Area} = 27$$

$$\text{Vol} = 2$$

$$\text{Area} = 1$$

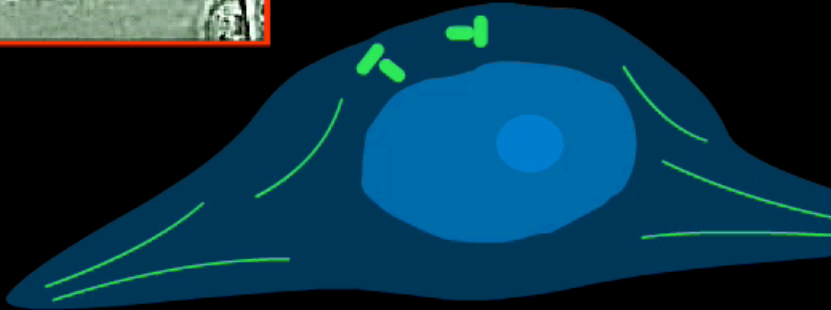
$$\text{Vol} = 2$$



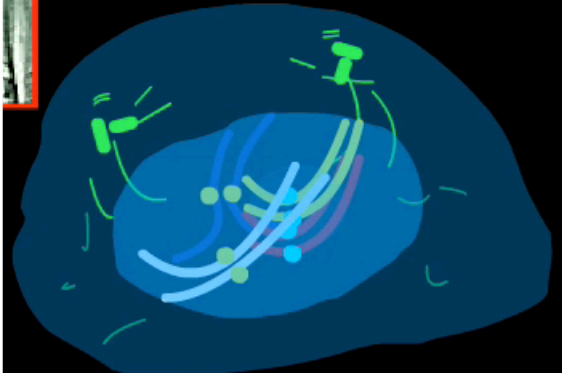
$$r_d = 3.0 r_s$$

$$h = 0.1 r_d$$

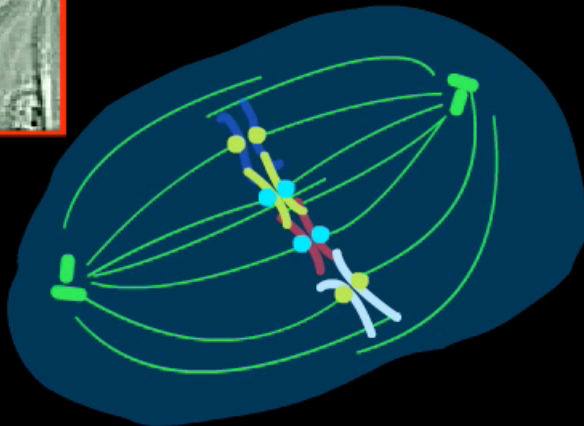
interphase



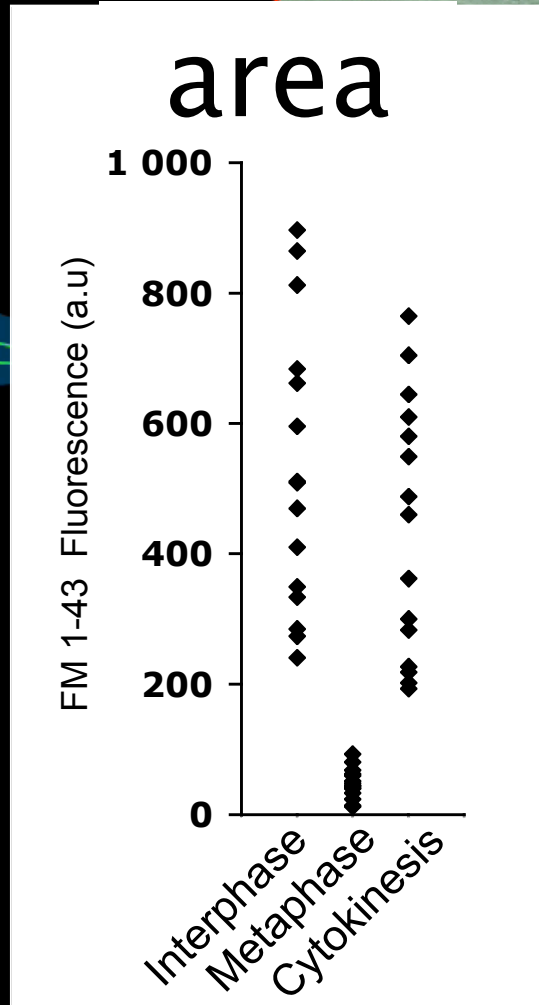
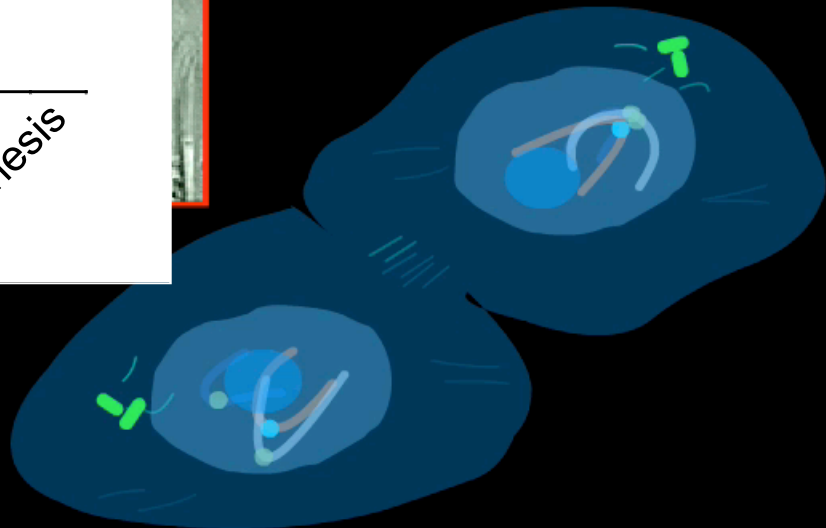
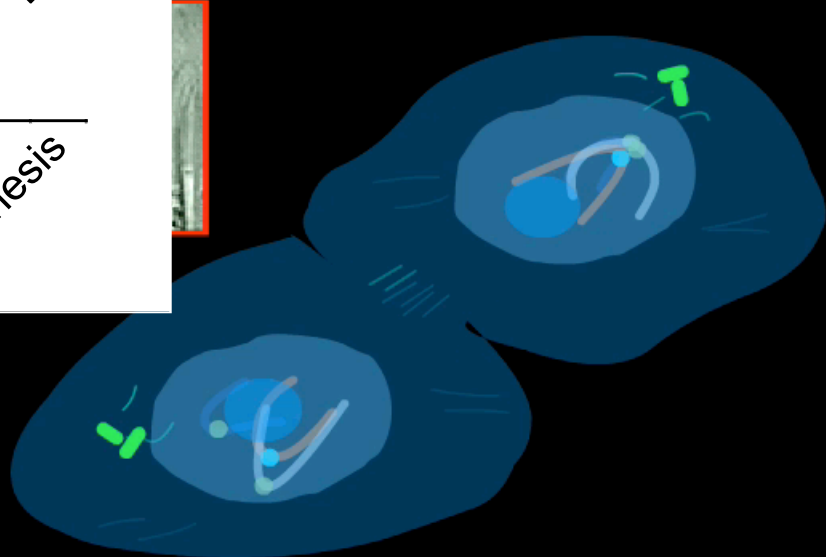
pro-metaphase



metaphase



cytokinesis

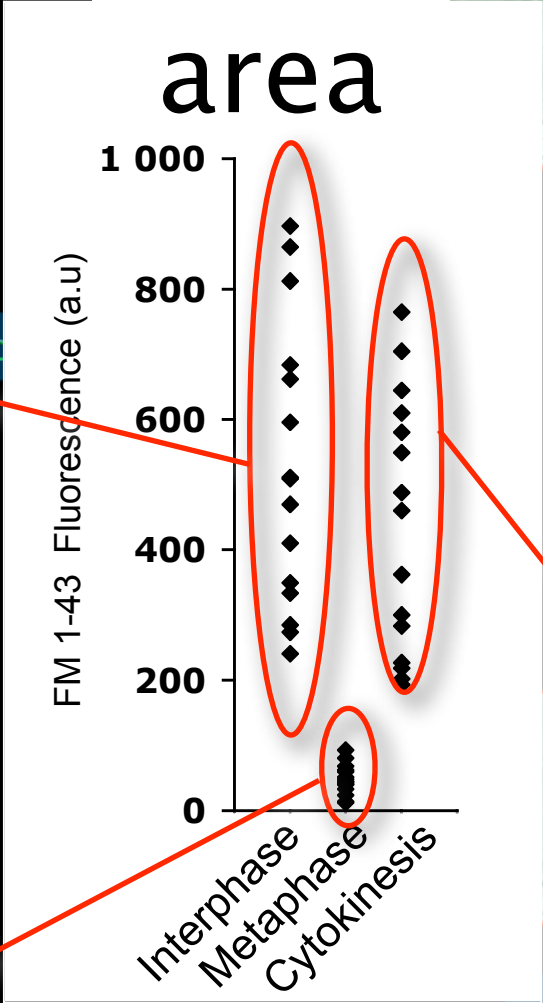


interphase

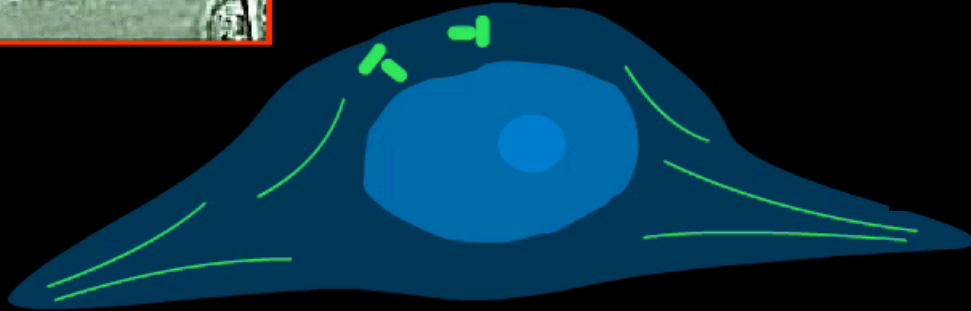
pro-metaphase

metaphase

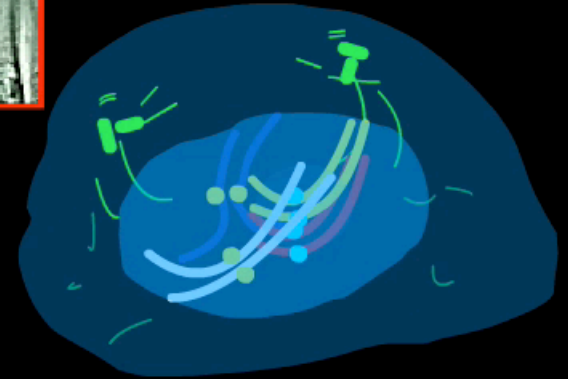
cytokinesis



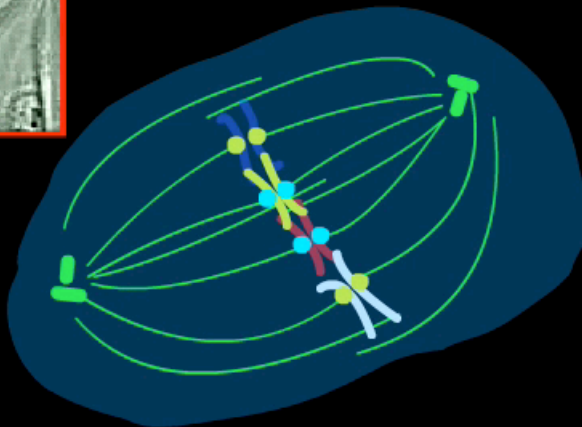
interphase



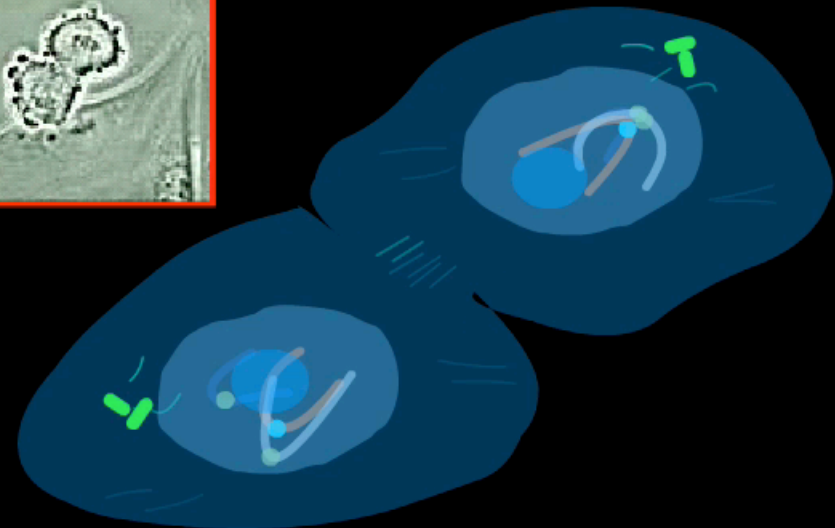
pro-metaphase



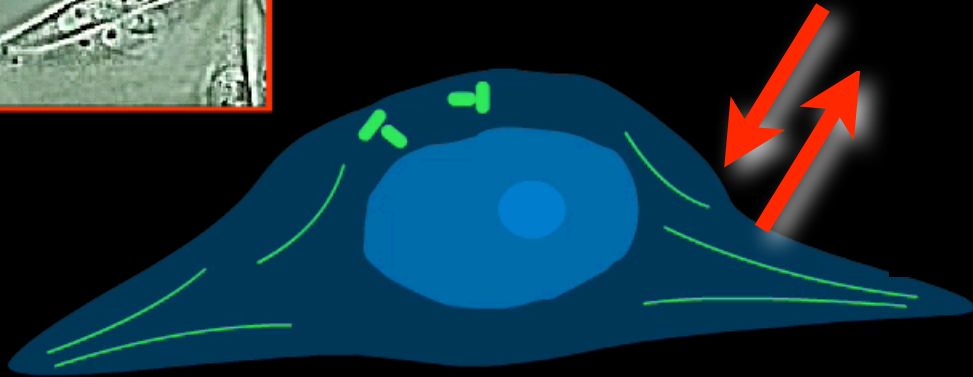
metaphase



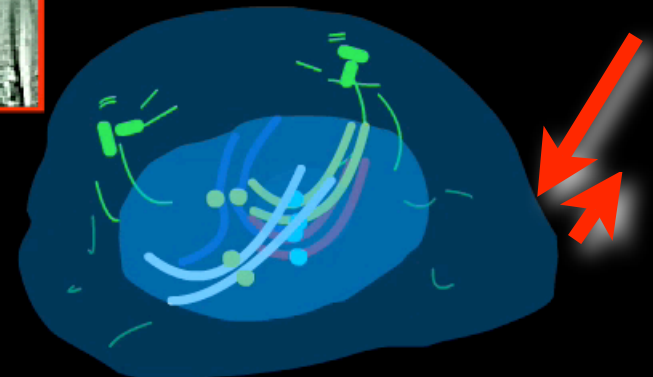
cytokinesis



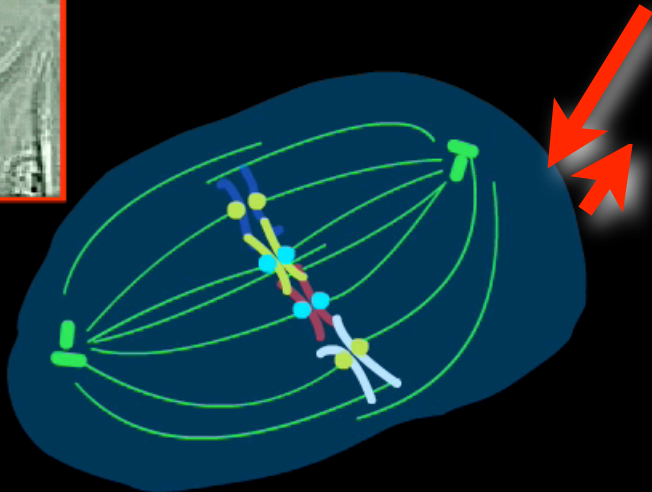
interphase



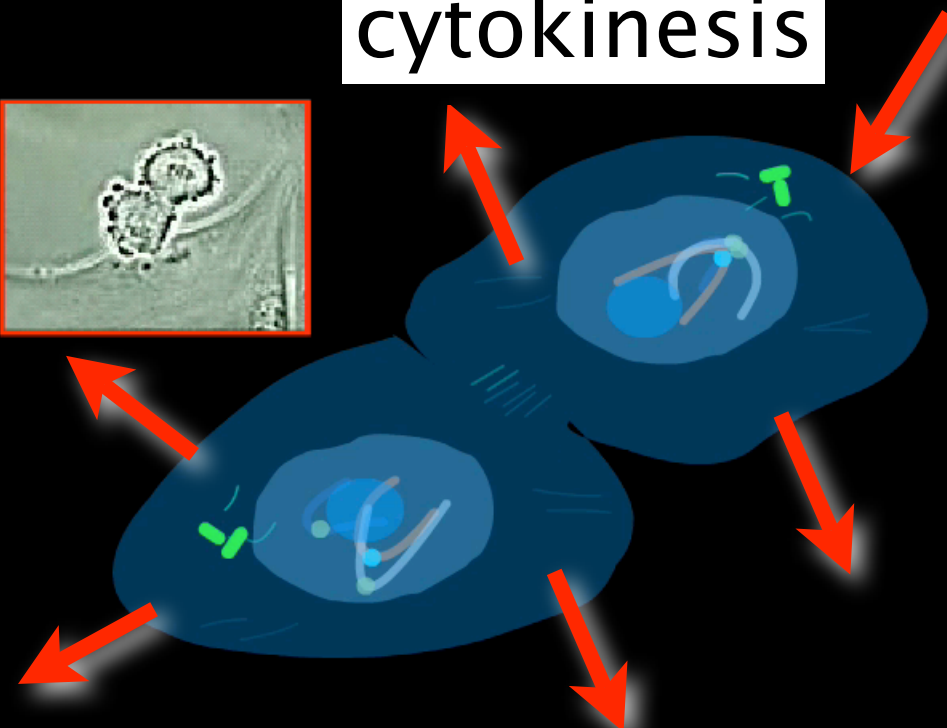
pro-metaphase

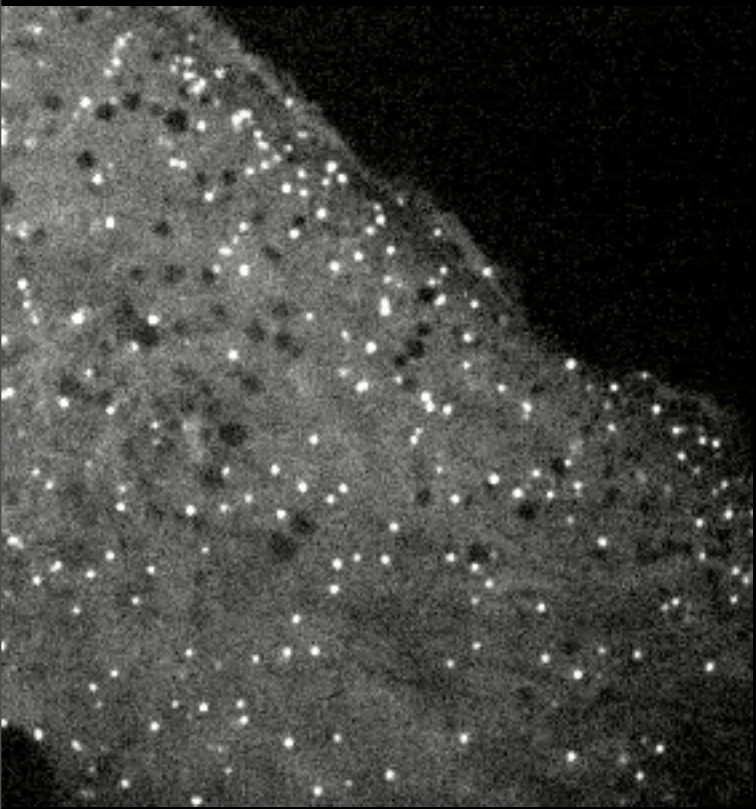


metaphase



cytokinesis

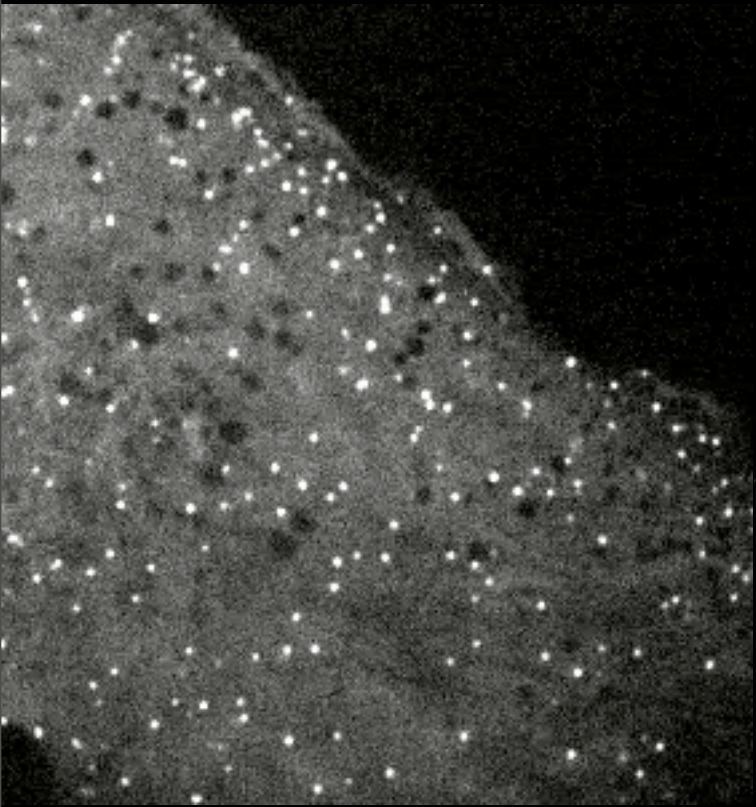




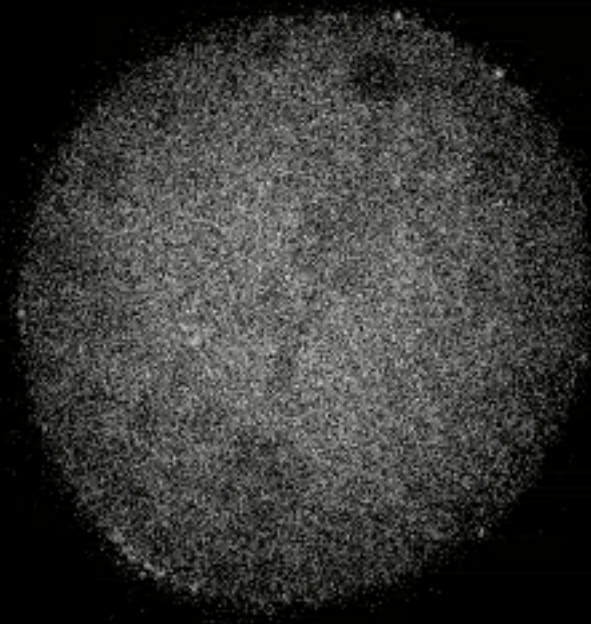
interphase

during mitosis:

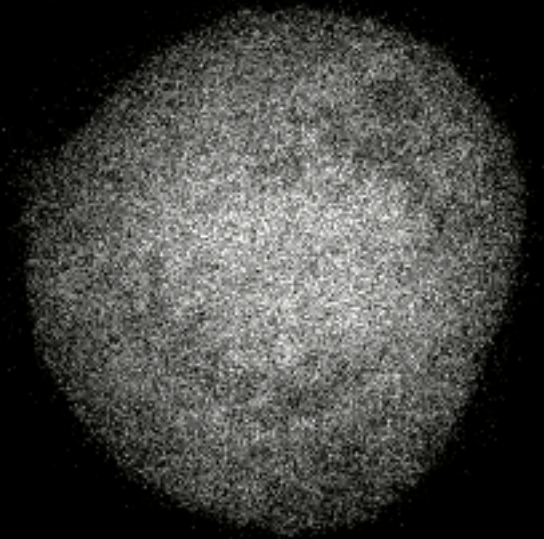
- clathrin endocytosis does not change



interphase



metaphase



anaphase >
cytokinesis

endocytosis of transferrin continues during cell division

interphase

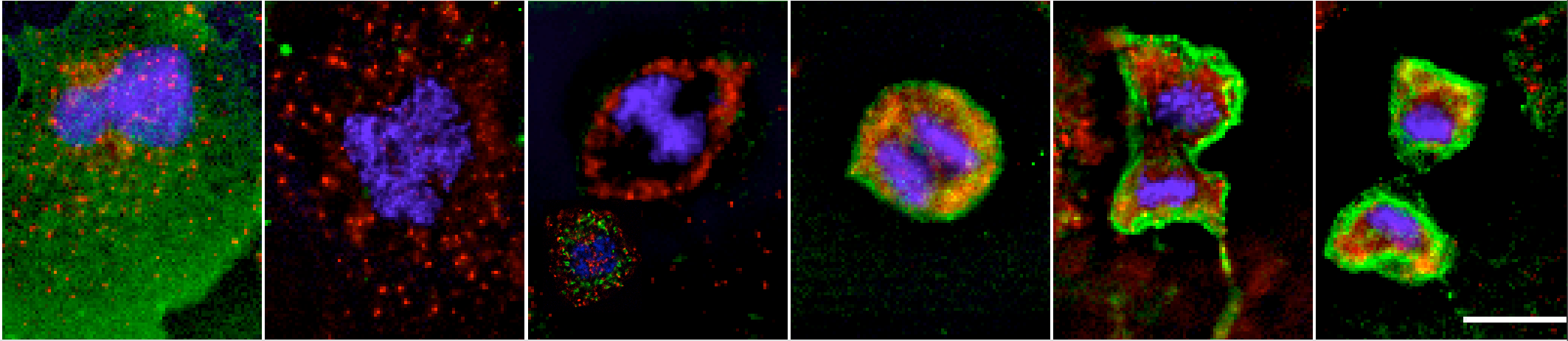
prophase

metaphase

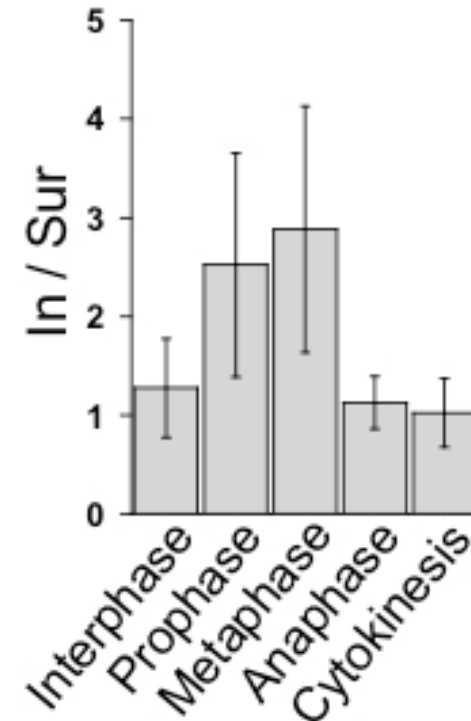
anaphase

telophase

cytokinesis

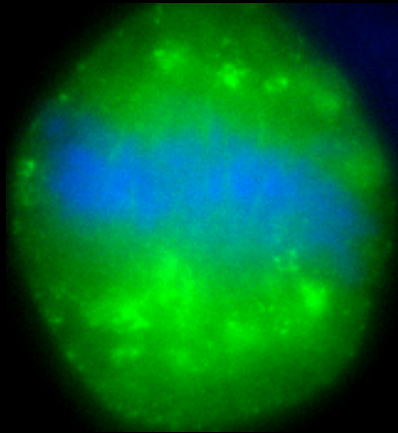


transferrin:
at the surface
internalized

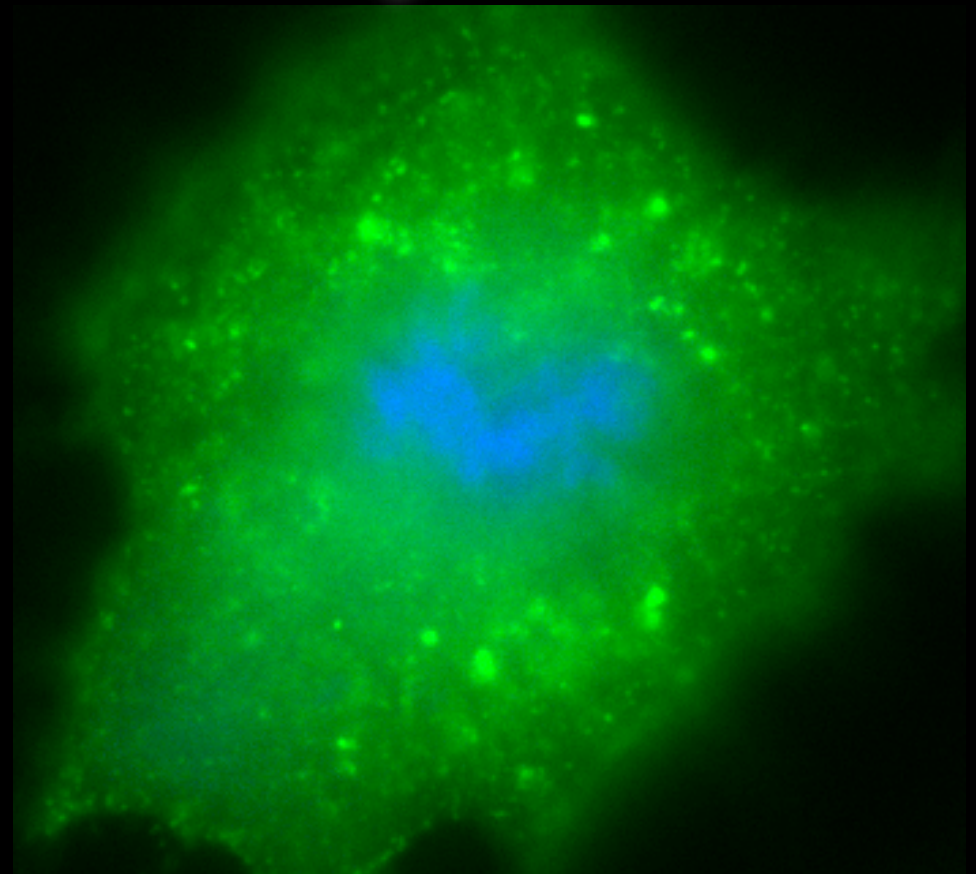


dynasore prevents mitotic roundup

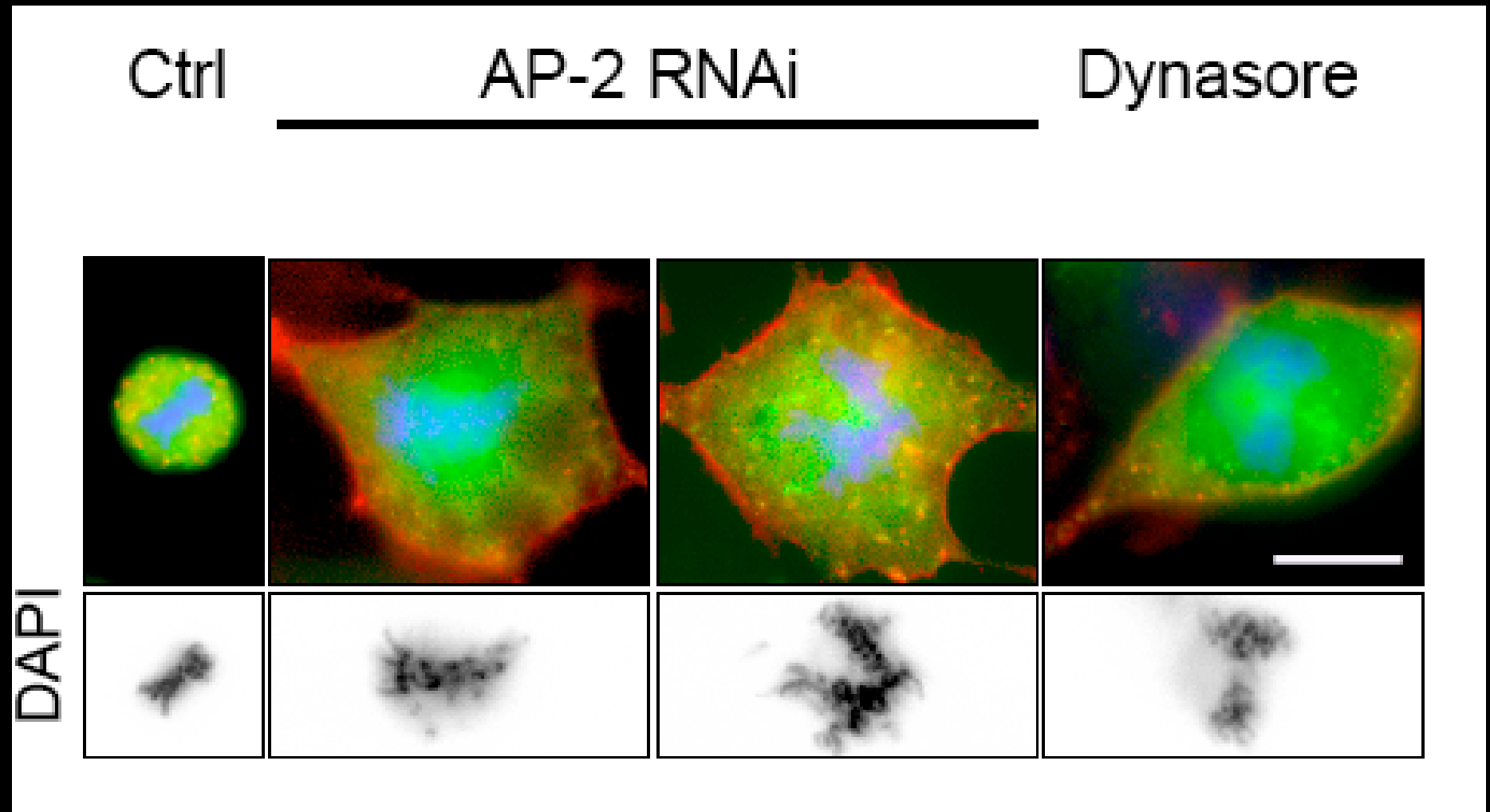
- dynasore



+ dynasore



endocytosis is required to reduce cell surface



endocytosis is required to reduce cell surface

