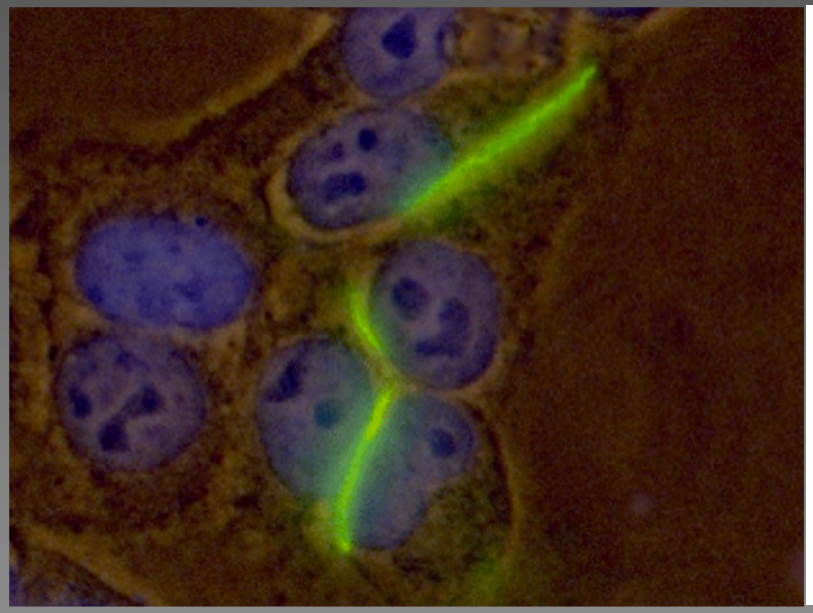
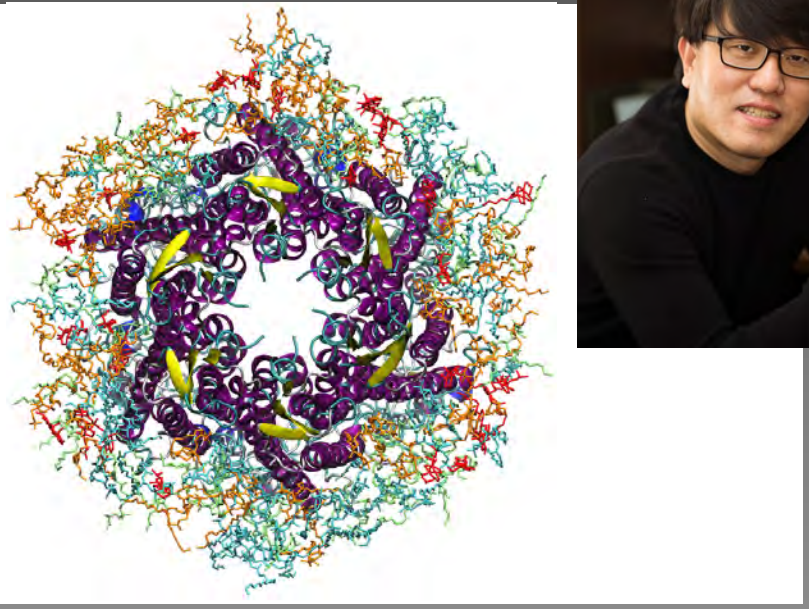
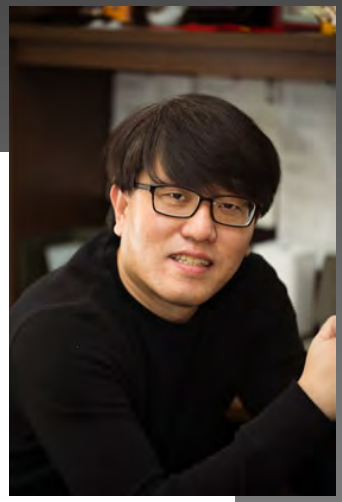
Modeling Gap Junctions and Direct Cell-to-Cell Communication: A few Examples

Matthias M. Falk & Wonpil Im







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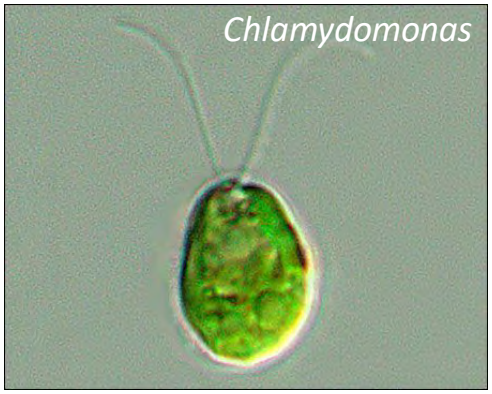
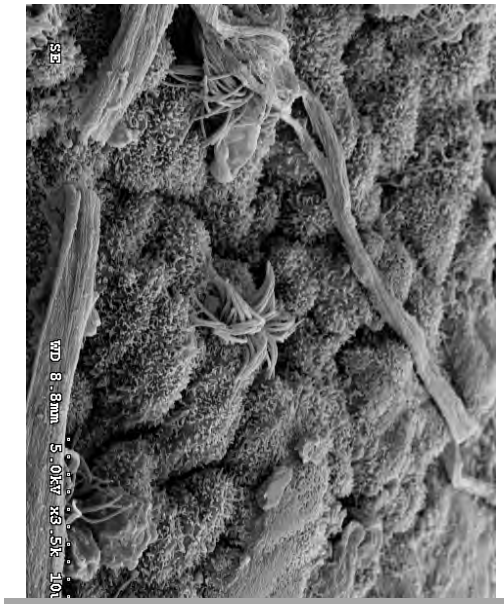
Modeling Gap Junctions and Direct Cell-to-Cell Communication: A few Examples

A story about:

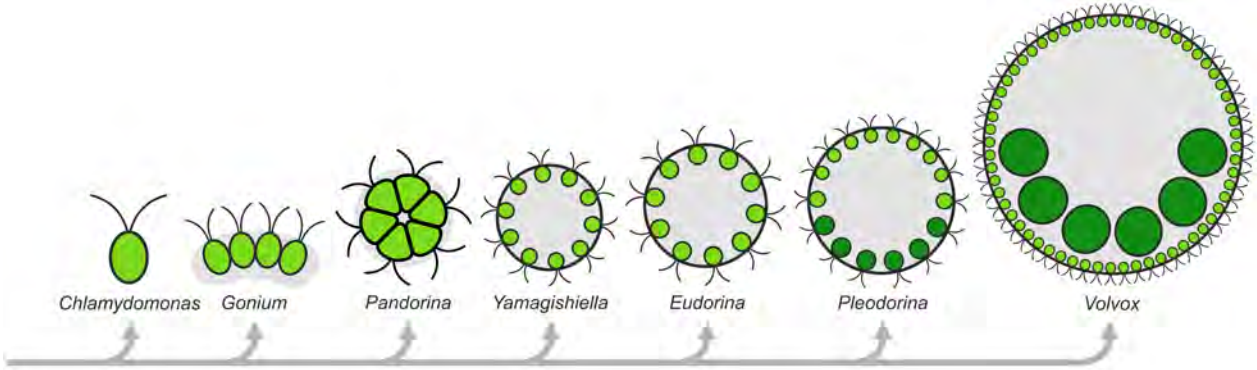
- Cell-to-Cell Communication
- Gap Junctions (GJs) and GJ turnover
- (Molecular) Modeling!



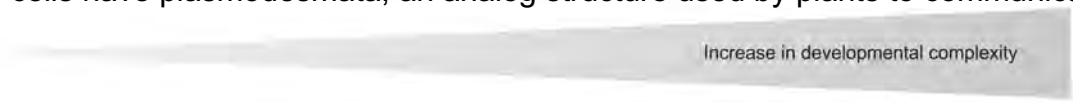
Gap Junctions: Who has them and why?

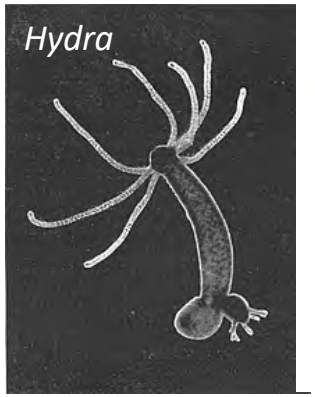


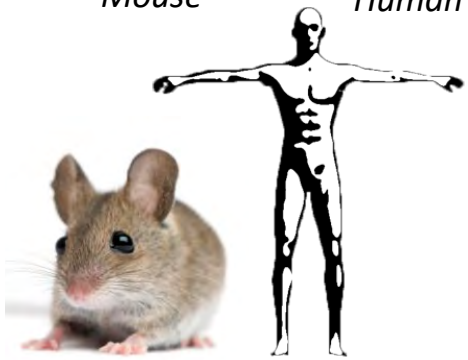
Human ovarian tube epithelium

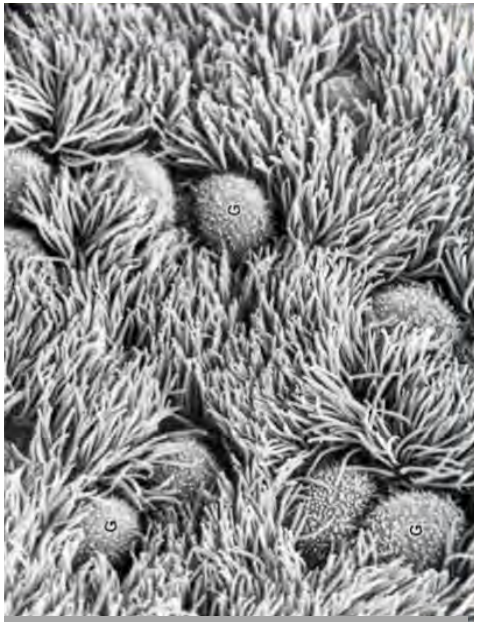


(These cells have plasmodesmata, an analog structure used by plants to communicate)



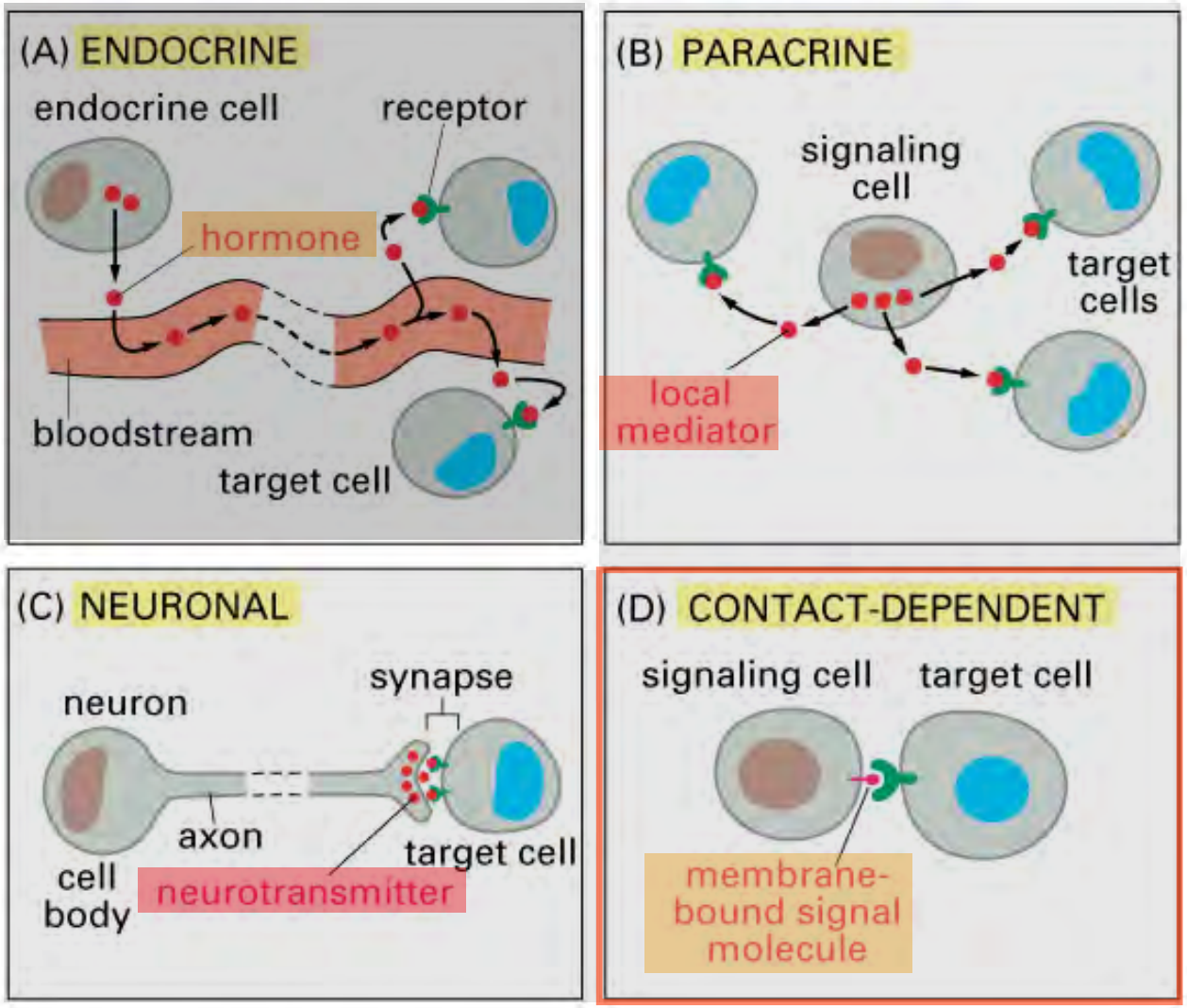






...Human airway epithelium

Cell signaling occurs over long (A) and short (B, C, D) ranges:
-- Four principle mechanisms to signal from one cell to another --



TV/Radio broadcast

Flyer posted on selected message boards

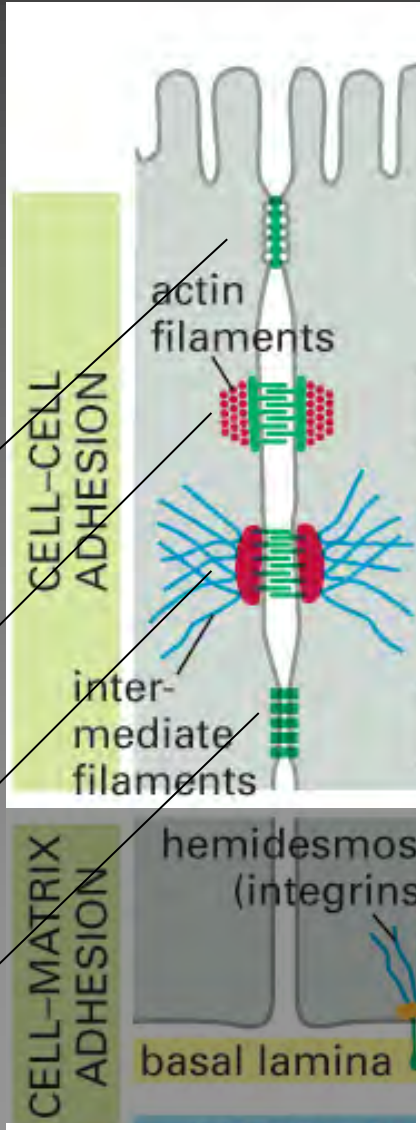
Long-distance call; email

Face-to-face communication

Cell-Cell Junctions Play a Key Role for Complex, Multi-Cellular Life

- Development and Morphogenesis
- Differentiation
- Cell and Tissue Function

- **Tight Junctions**
  Barrier Function and Cell Polarization
- **Adherens Junctions**
  Cell-Cell Adhesion and Cell Migration
- **Desmosomes**
  Tissue Strength
- **Gap Junctions**
  Direct Cell-to-Cell Communication

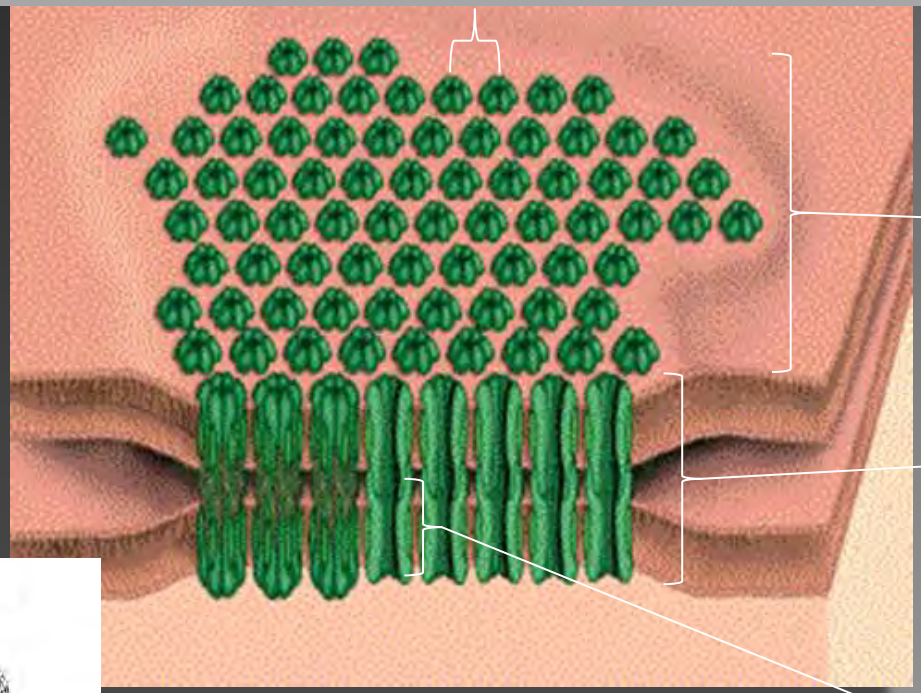


- <u>Gap Junction Functions Include:</u>
- Coordination of heart beat
- Onset of labor
- Conduct neuronal signals through electrical synapses
- Insulin secretion by pancreatic β-cells
- Maintenance and modulation of cell-cell contacts
- many more!

- <u>Known diseases include:</u>
- Heart diseases
- Neuropathies
- Deafness
- Lens Cataracts
- Skin Disorders
- Bone Malformations

Gap Junctions (GJs) mediate direct cell-to-cell communication

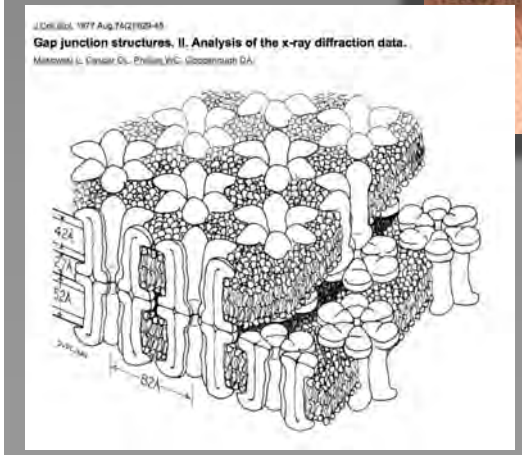
GJ channels are small and DENSELY packed! Only 10 nm center-to-center spacing



GJ Plaque

GJ Channel

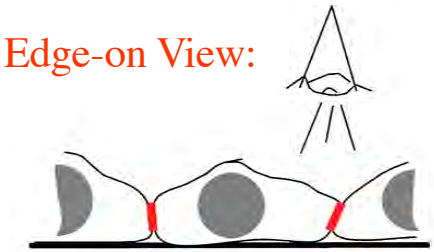
GJ Hemi-Channel or Connexon made of 6 Connexin (Cx) proteins



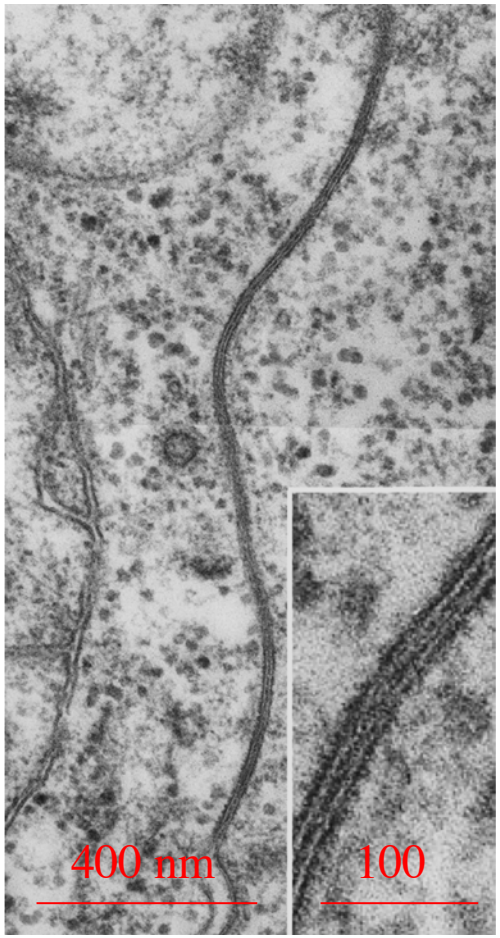
Gap junction structures. II. Analysis of the x-ray diffraction data.

Electron Microscopic (EM) Structure of Gap Junctions

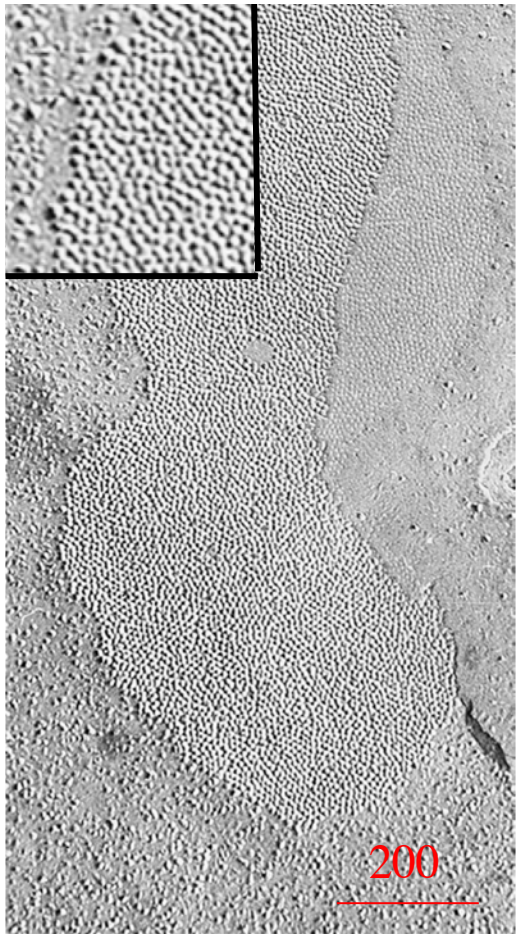
Edge-on View:



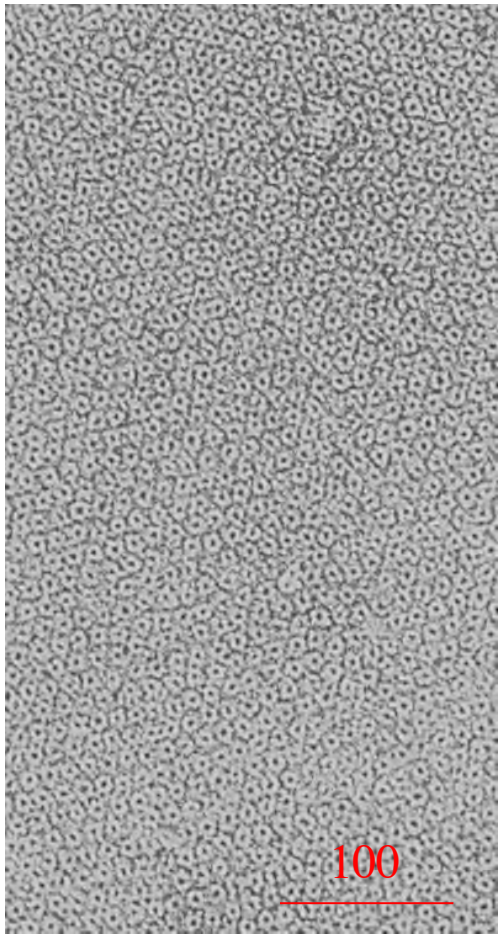
Surface-Views:



Thin Section

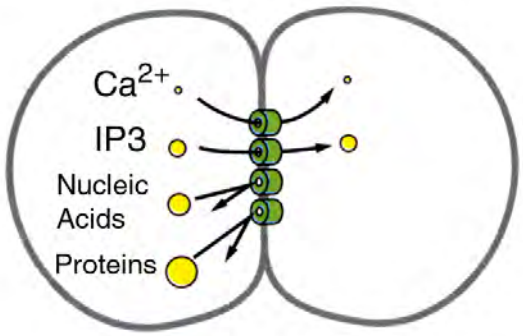


Freeze Fracture

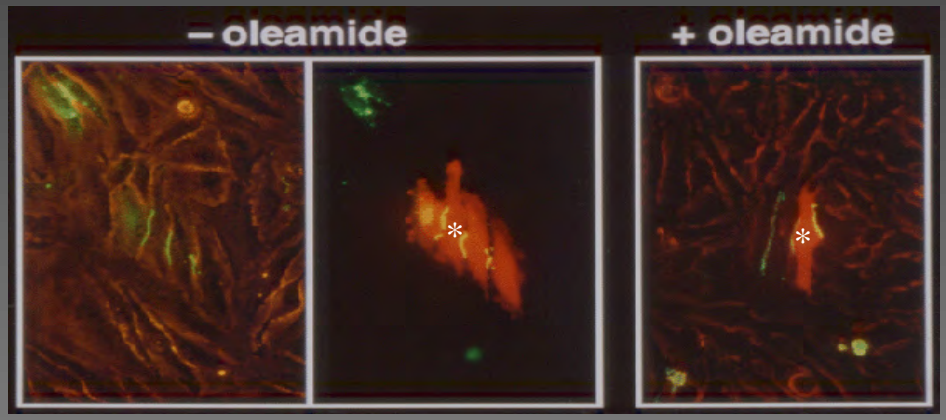


Negative Stain

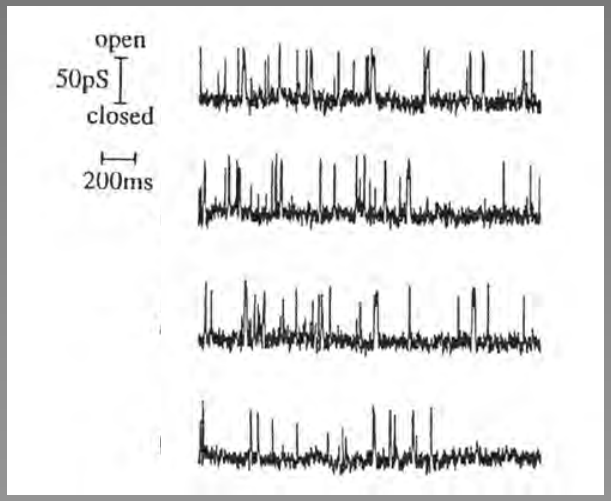
Gap Junction Channel Function Demonstrated by Dye-Transfer Assays and Electrophysiological Measurements



GJ channel pore diameter = 1.5 nm



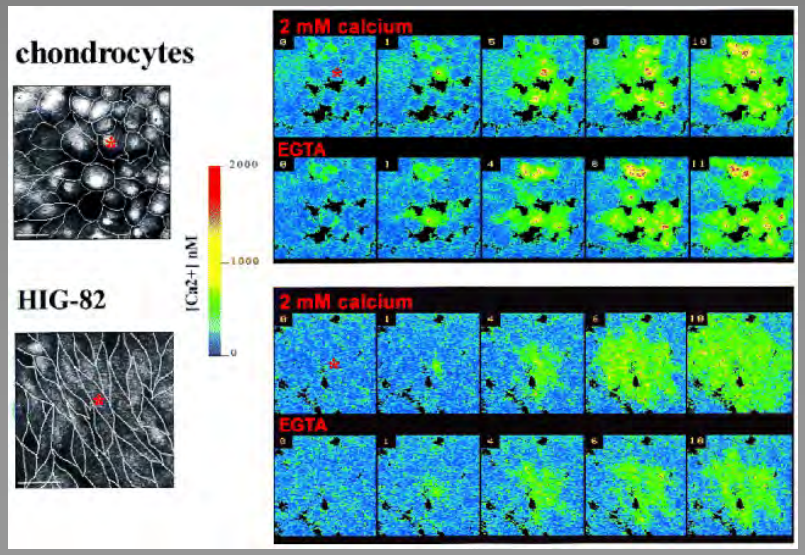
Sulpho-Rhodamine B (MW: 558 Da)

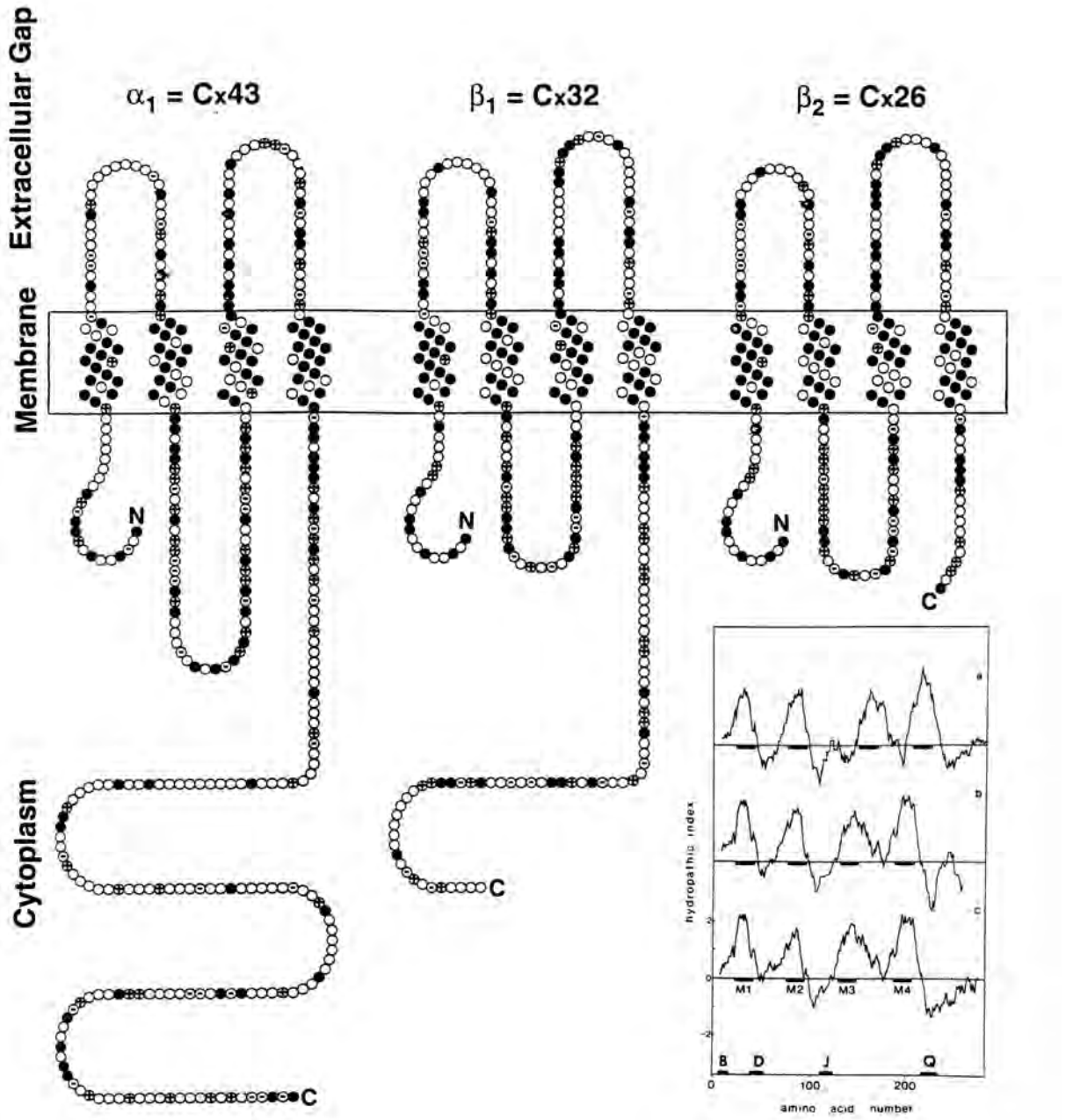


Recombinant Cx26 connexons expressed and reconstituted from baculovirus-infected insect cells.

(From Falk, Buehler et al. EMBO J. 16:2703-16, 1997)

Fura-2 Ca^{2+}-wave





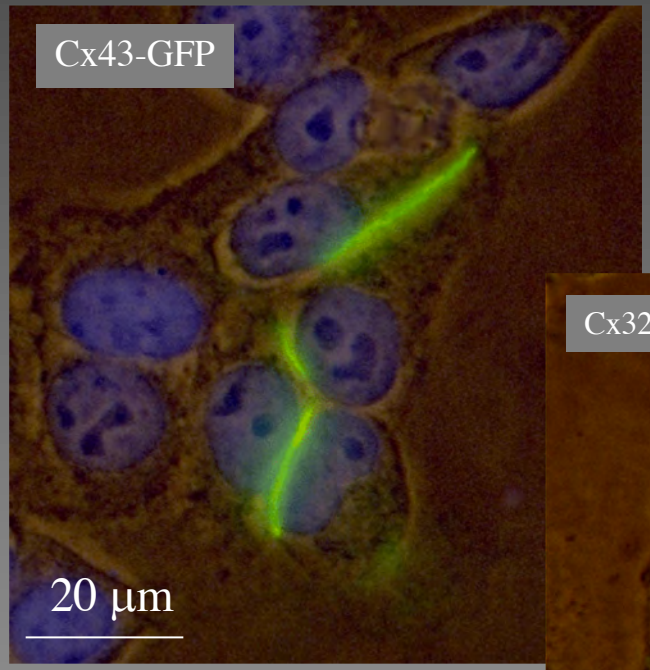
Connexins (Cxs) are polytopic trans-membrane proteins

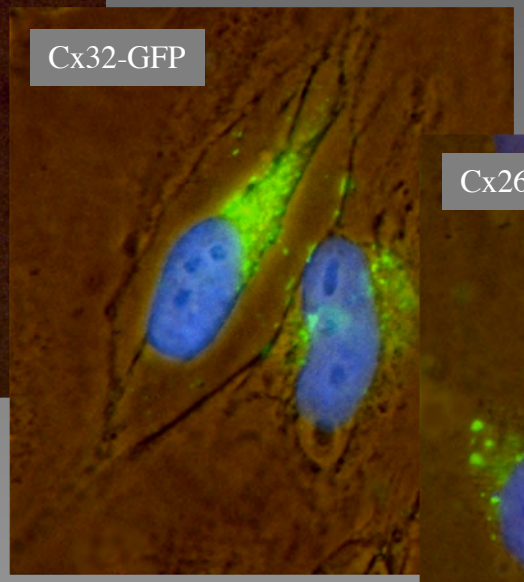
(21 different Cxs in Humans)

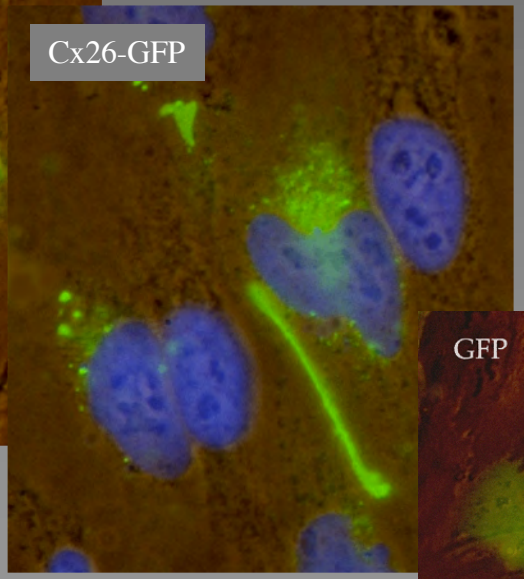
- Hydrophobicity Plots
- Anti-Peptide Antibody Mapping
- Protease Protection Assays

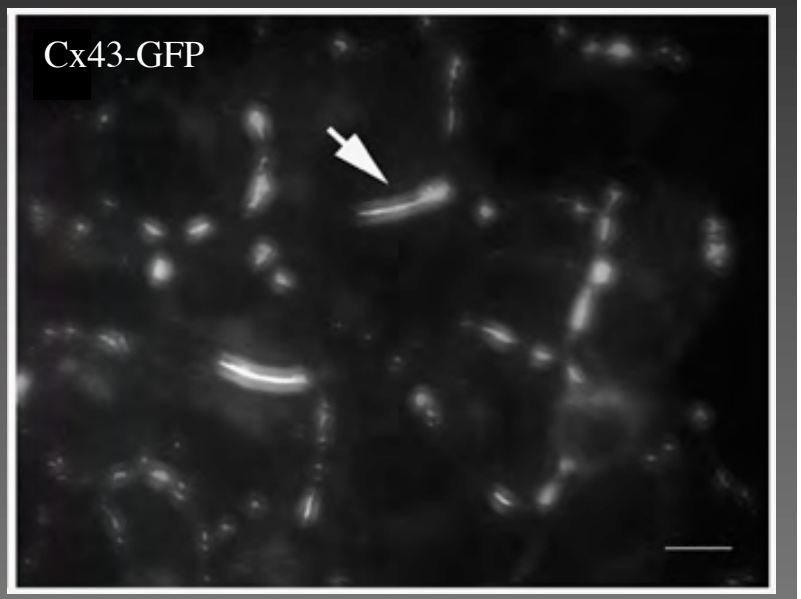
Gap Junction Plaques Assembled from Different GFP-tagged Connexins are Functional

(Transfected HeLa cells)





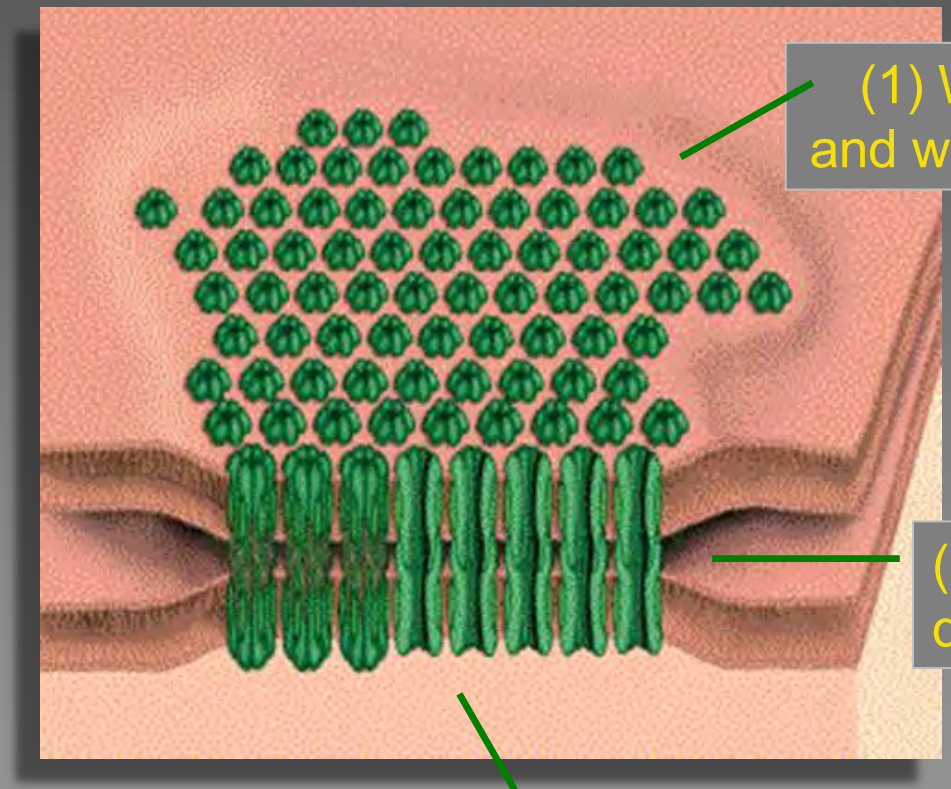




(From Falk, J. Cell Sci. 113:4109-20, 2000)

Gap Junctions: Many Interesting Questions for Modeling

(3 Examples)



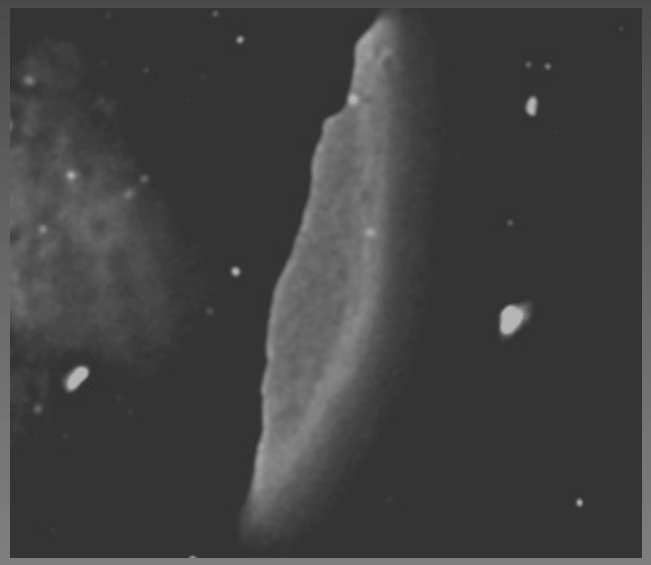
(1) Why do GJ channels cluster and what holds the cluster together?

(2) How and where do connexons dock?

(3) What happens on the cytoplasmic surface of a gap junction that is endocytosed?

Gap Junctions are dynamic assemblies of channels

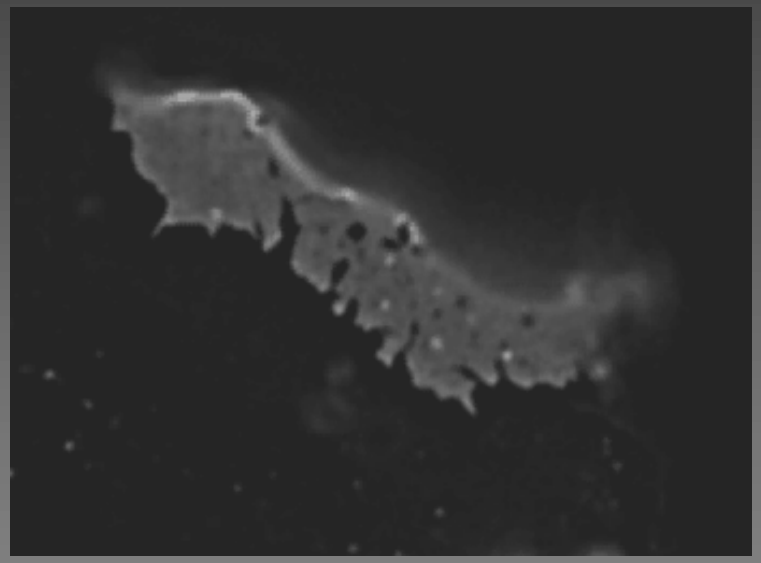
(Lateral Motion of Gap Junctions in the Plane of the Membrane; Surface Views)



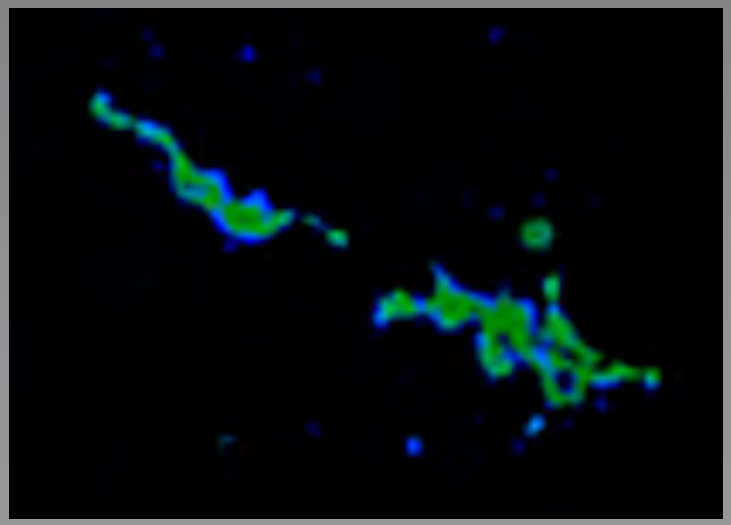
60 frames, 30 sec apart, 30 min total time, looped

Cx43-GFP

2 μm



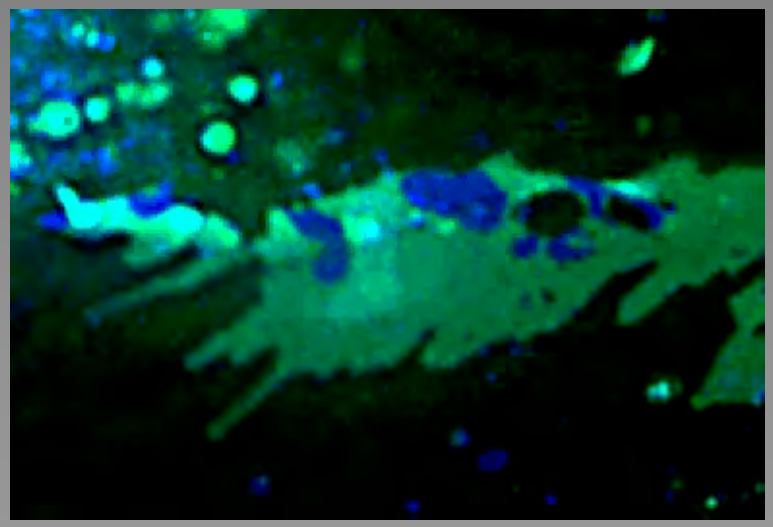
91 frames, 10 sec apart, 15 min total time, looped



75 frames, 30 sec apart, 37.5 min total time, looped

Cx43-CFP/
Cx26-YFP

2 μm

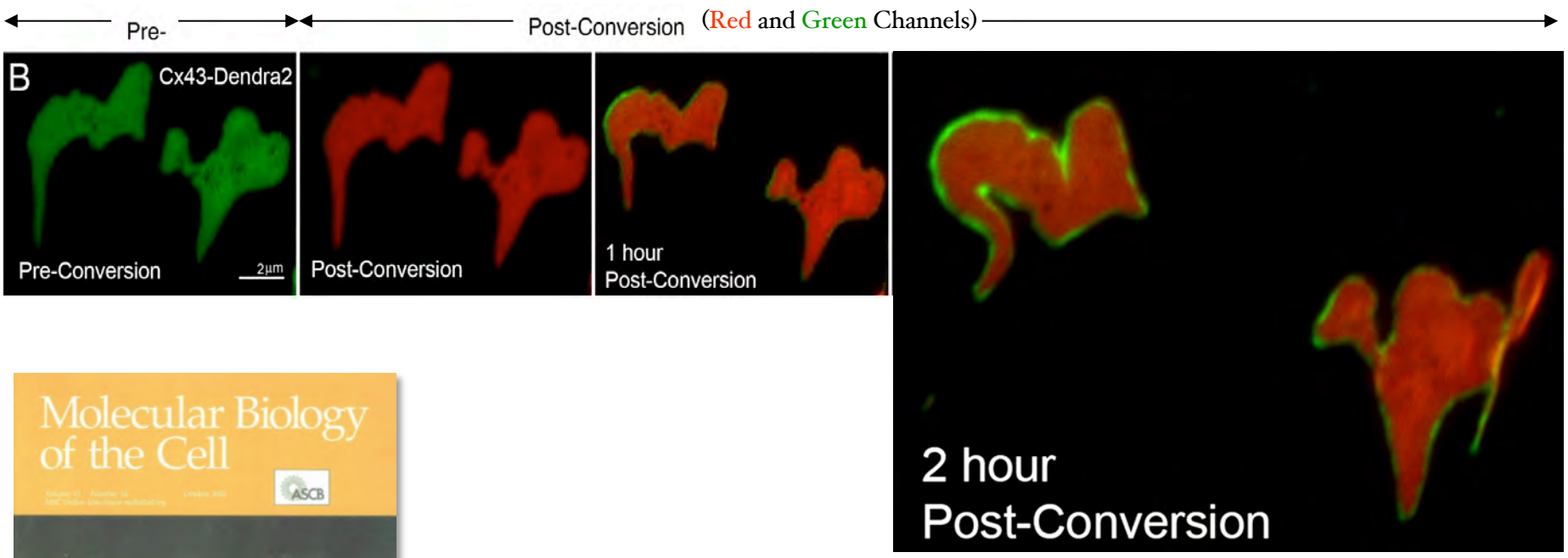


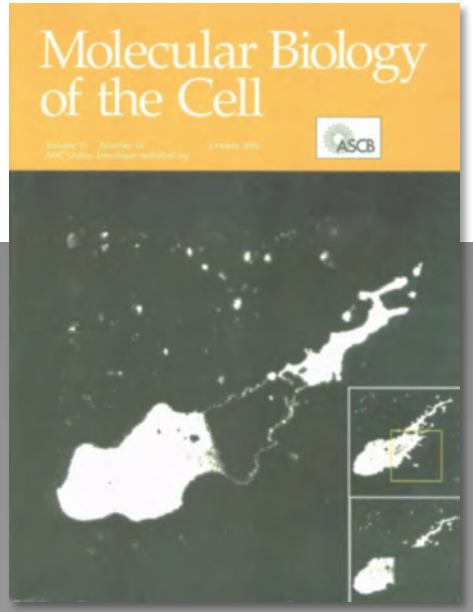
70 frames, 30 sec apart, 35 min total time, looped

(From Lopez, …, & Falk, Cell Com. & Adhes., 8:237, 2001)

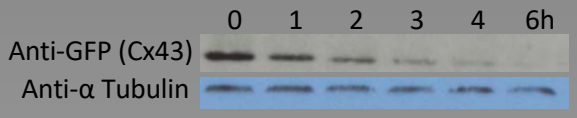
Weird, GJs have an unusually short half-life of only a view hours!

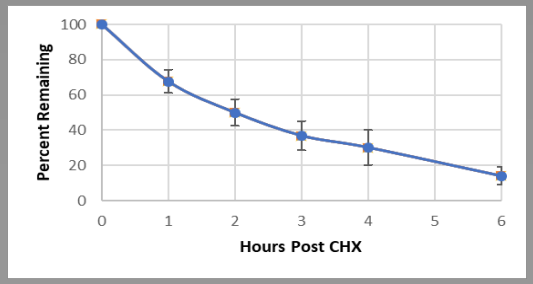
(Newly synthesized Connexons are Recruited to the Periphery of GJ Plaques while simultaneously older channels are removed from plaque centers (Dendra2 – Photoconversion))





FRAP



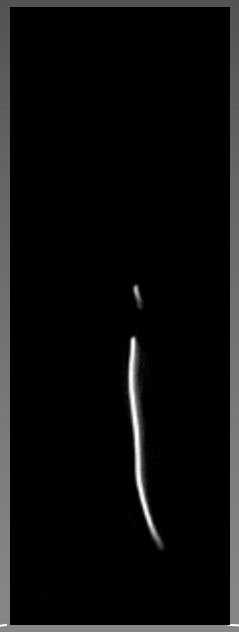


$T^1/_2$ = ~2h

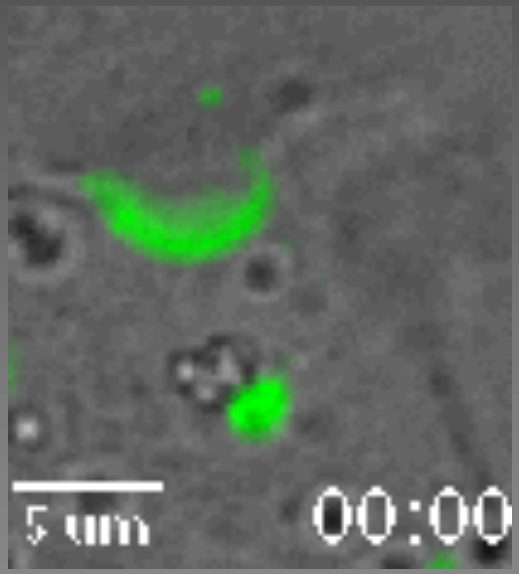
(Lauf et al., PNAS 2002;
Falk et al., MBoC 2009
Thevenin et al., MBoC 2018)

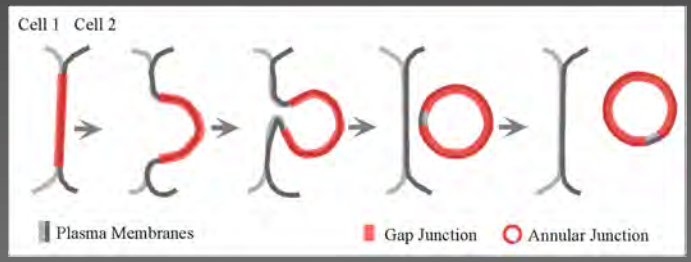
Gap Junctions internalize into one of the coupled cells to form cytoplasmic double-membrane vesicles termed Annular Gap Junctions (AGJs)

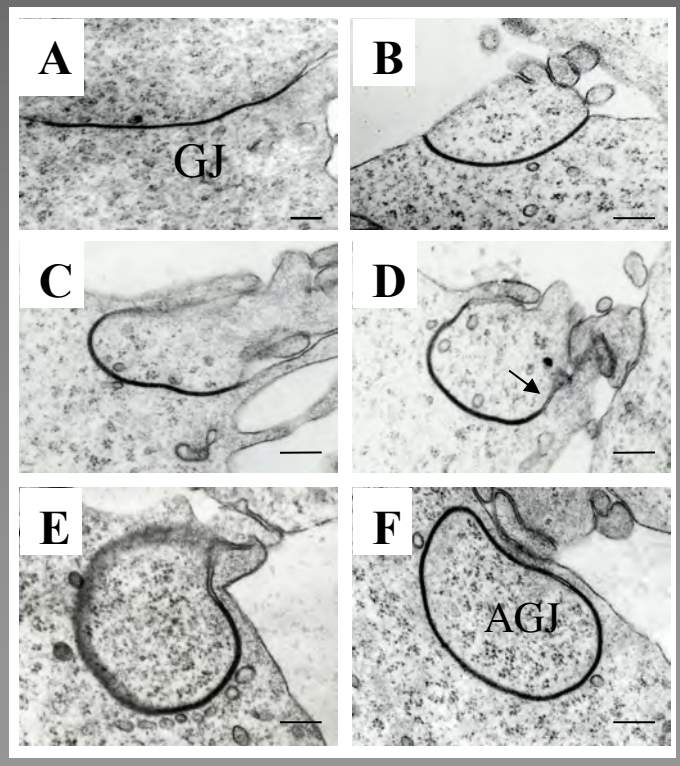
(Transfected HeLa cells)

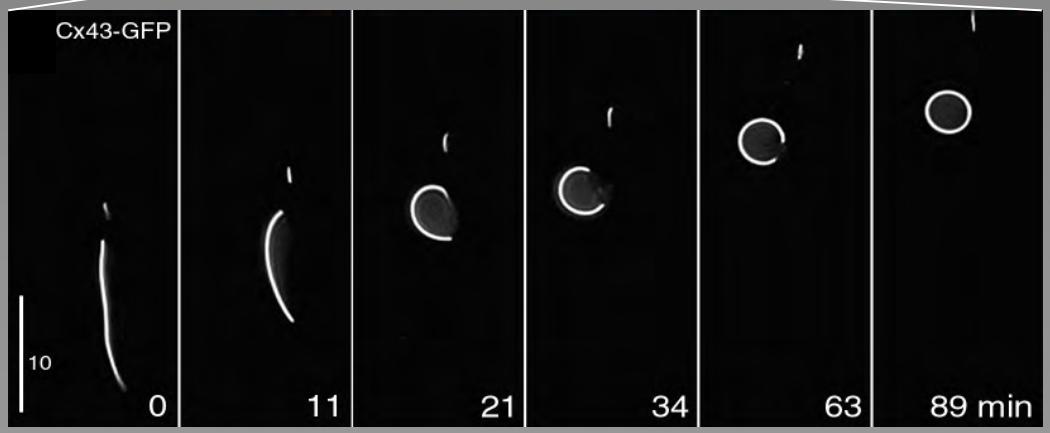


(Fluorescence and Phase Contrast)





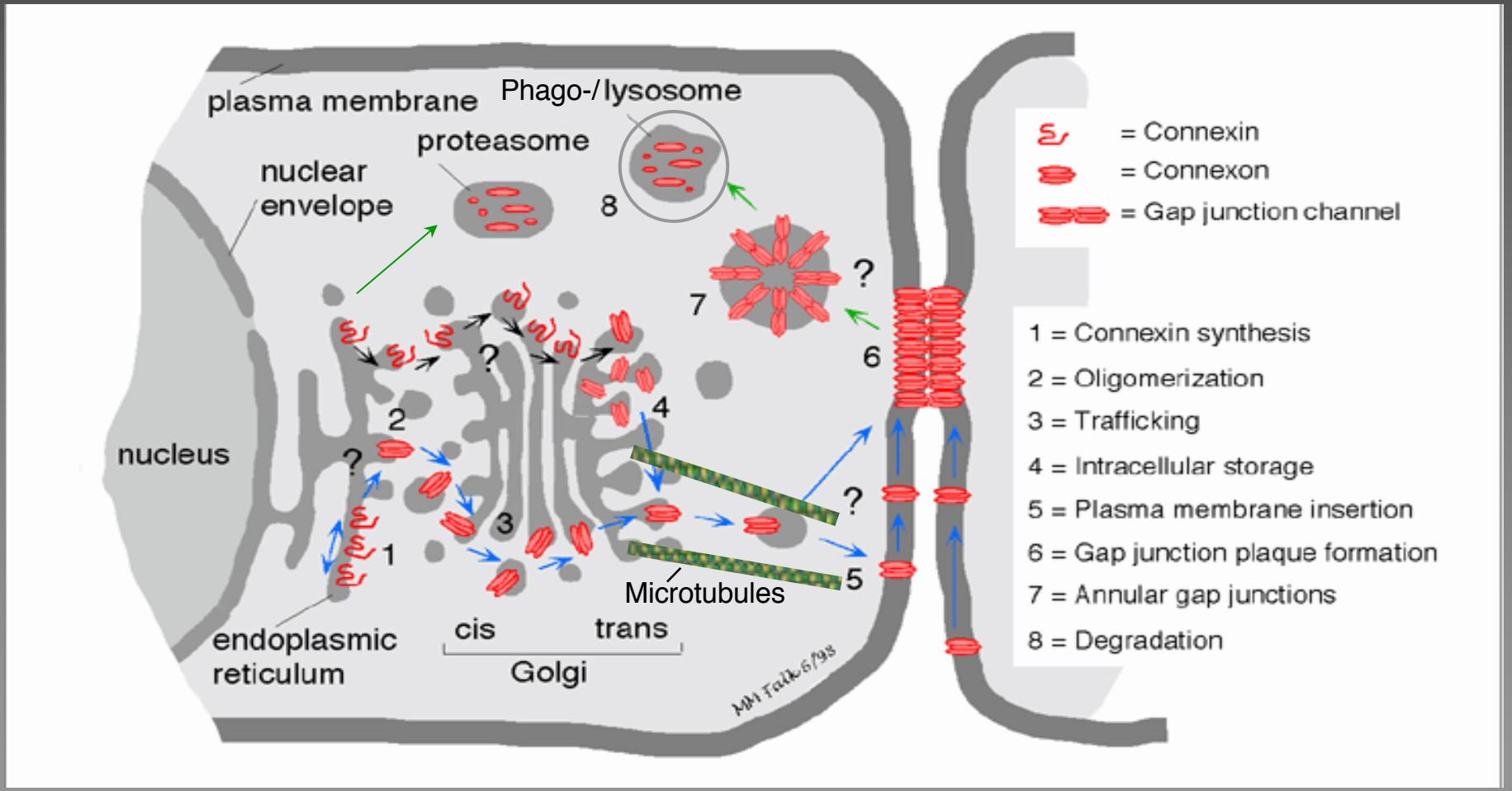




(From: Piehl et al., MBoC, 2007)

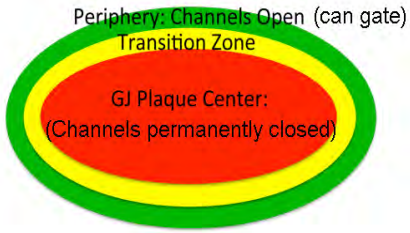
GJ turnover correlates with the short half-live of GJs and Cxs of 1-5 hours

Biosynthesis and Degradation of GJs: A Continuous Dynamic Process



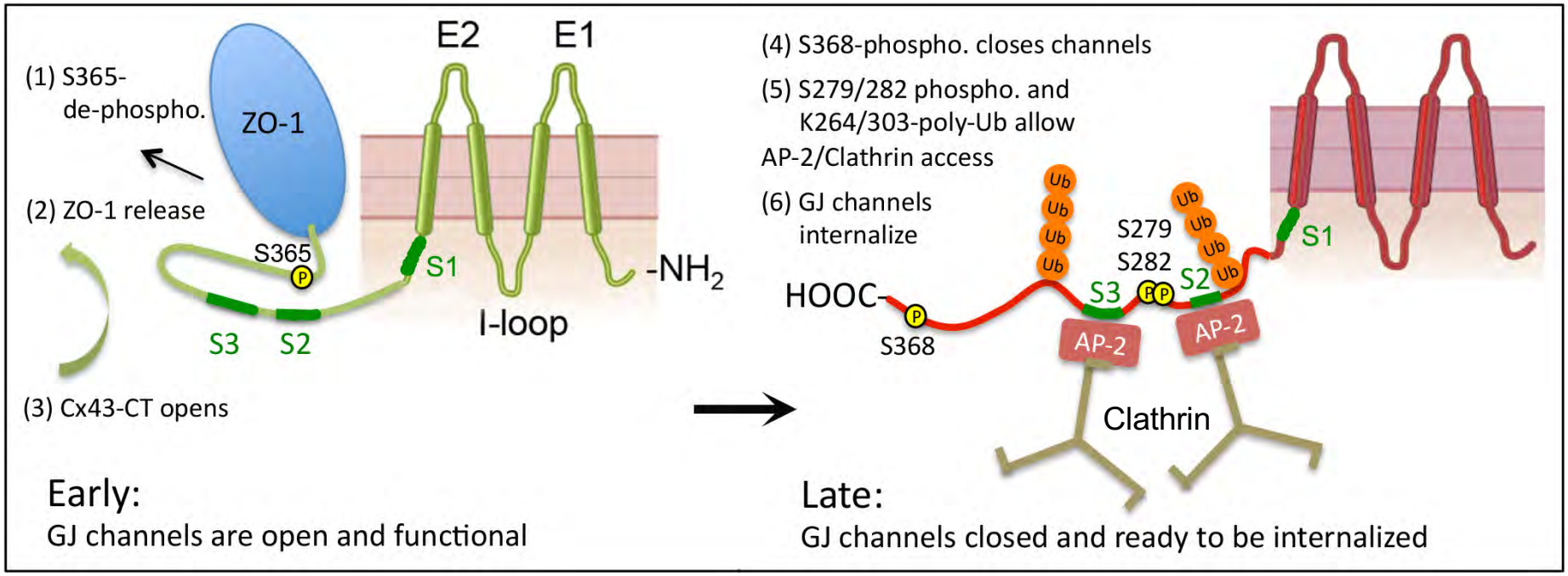
(From: Segretain & Falk, 2004, BBA, 1662:3-21, updated)

Qu.: Structurally, what is different between new (functional) and old (non-functional) GJ channels; or why do old channels interact with the endocytic machinery and are endocytosed while new ones do not?



Hypothesis: ZO-1 release, de-/phosphorylation and ubiquitination opens up the Cx43-CT to allow AP-2/clathrin to bind to Cxs and internalize GJs

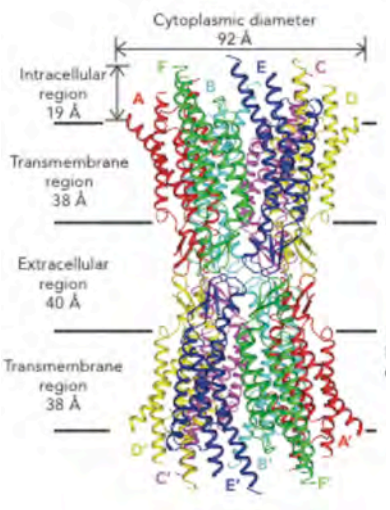
(In open, functional GJ channels the AP-2/clathrin binding sites are in-accessible/sterically blocked)



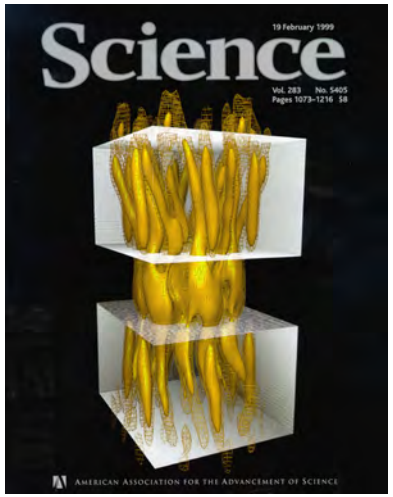
(from Falk et al., 2016, BMC Cell Bio 17:22)

Modeling Cx43 GJ Protein Structure

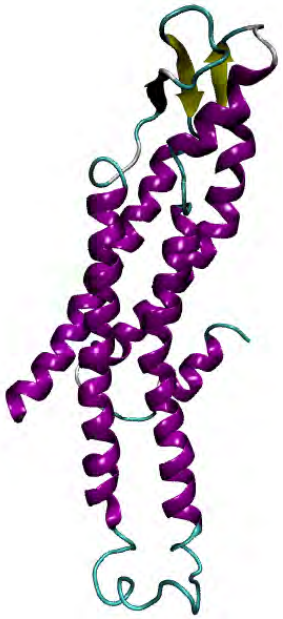
Cx26 atomic resolution structure (Zukihara Lab)



Cx43 7Å resolution structure (Yeager Lab)

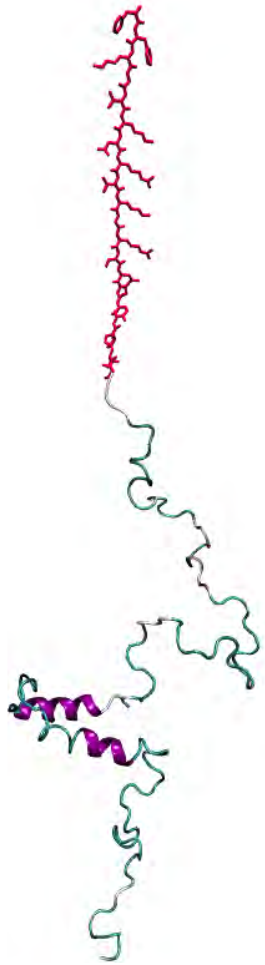


Cx26 structure (PDB: 2ZW5)



+

Cx43 C-Terminal Domain (lowest energy NMR-solution structure) (PDB: 1R5S)



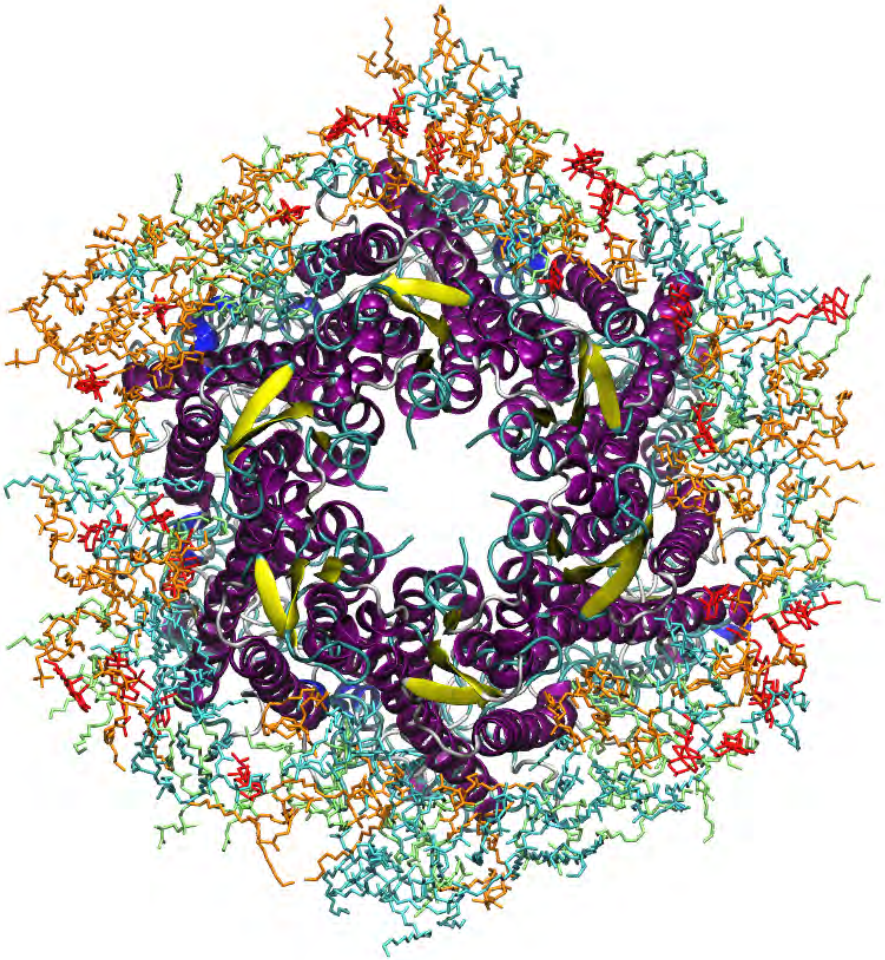
(Sorgen Lab)

=

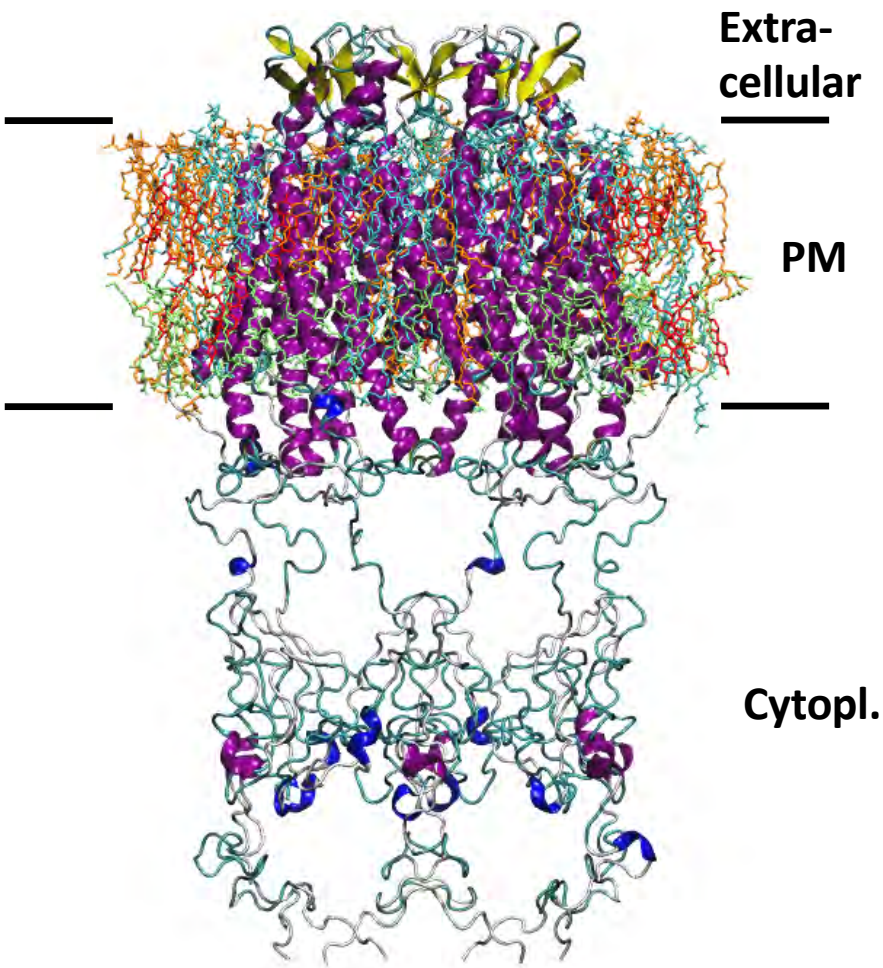
Combined Cx26/Cx43 hybrid structure after some minimization

Connexon

Top View



Side View

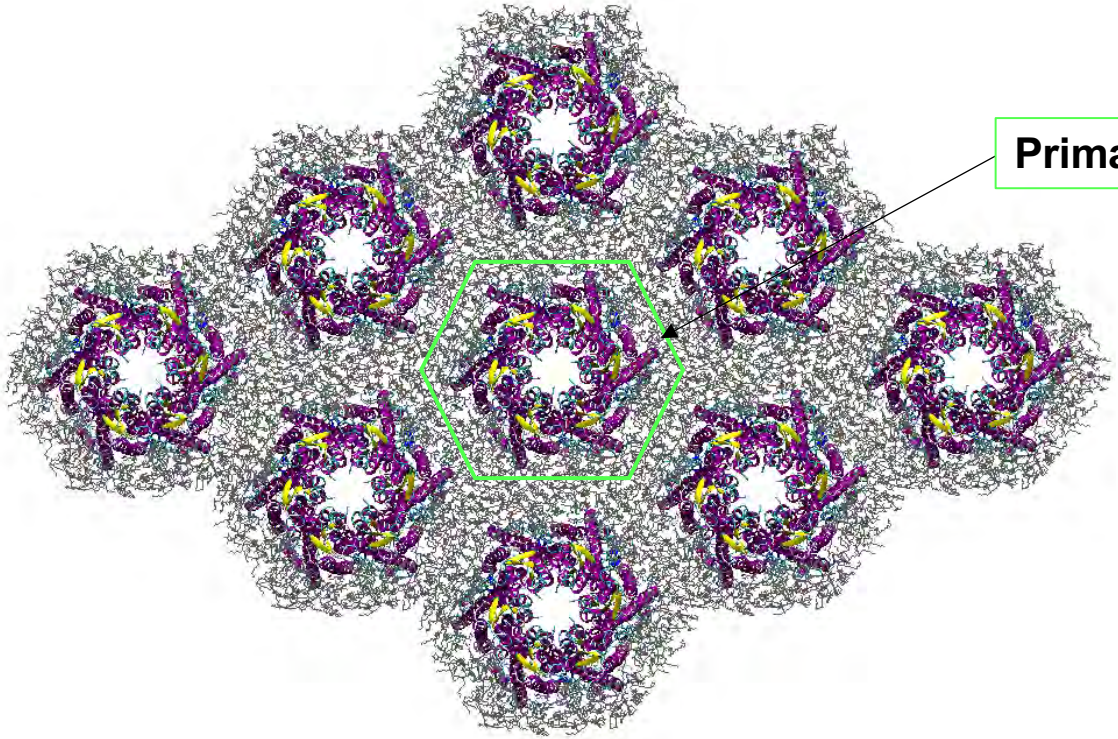


Membrane Lipid Composition:

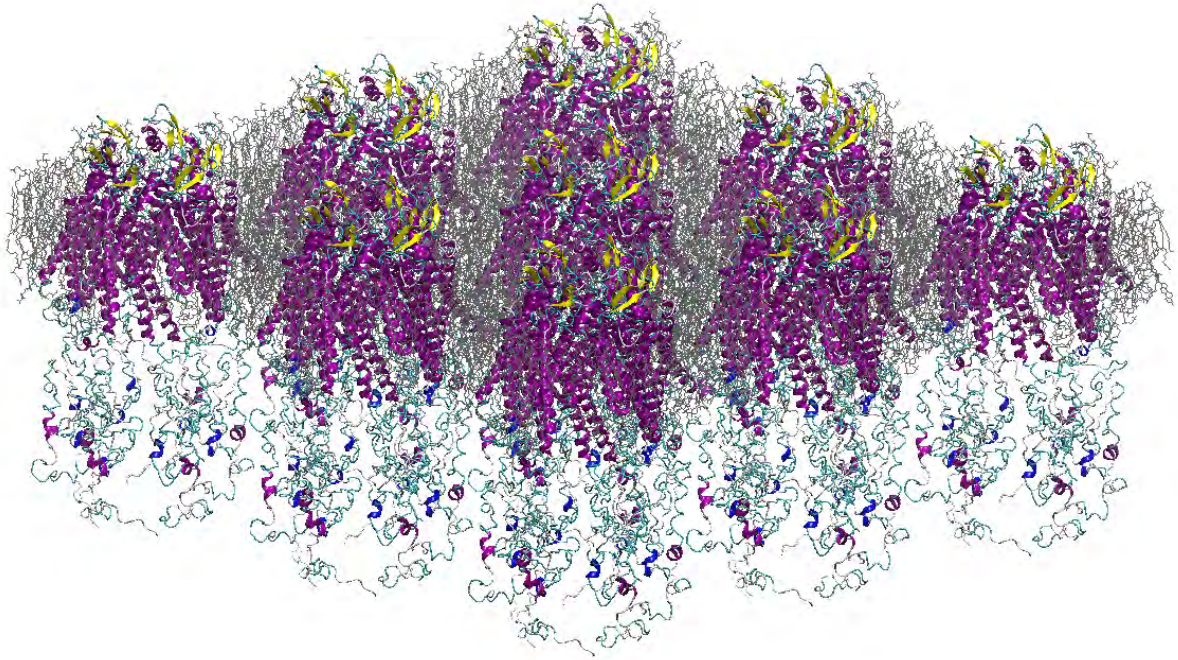
- Outer Leaflet: 18 CHL1, 36 POPC, 36 PSM
- Inner Leaflet: 9 CHL1, 12 POPC, 30 POPS, 9 PSM

One-half of a GJ plaque

Top view

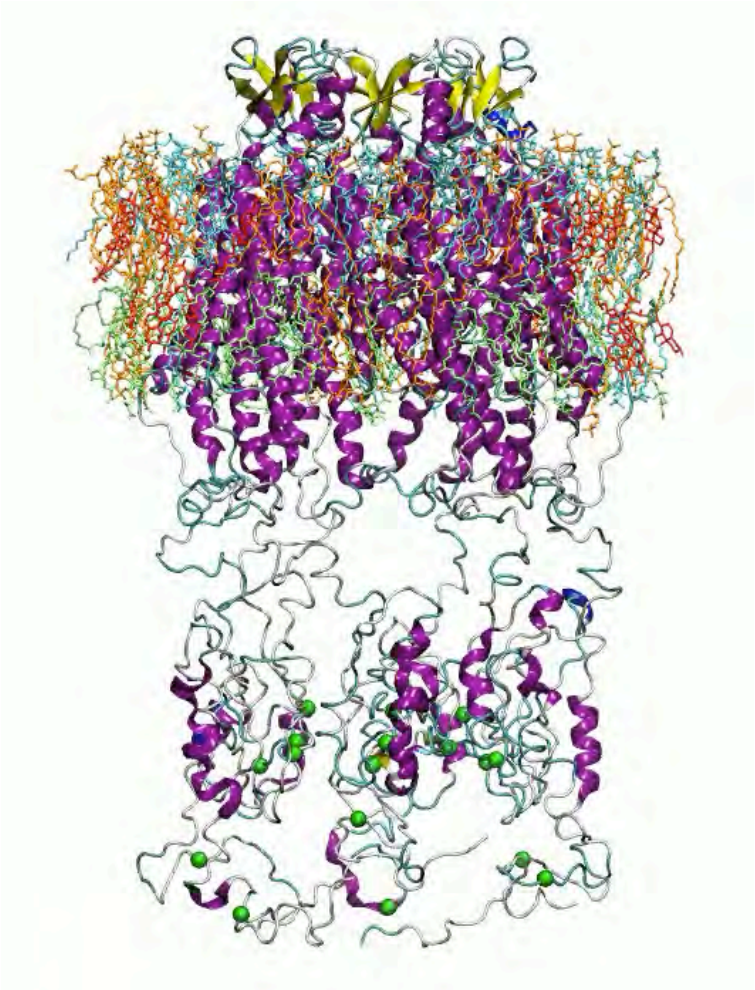


Side view

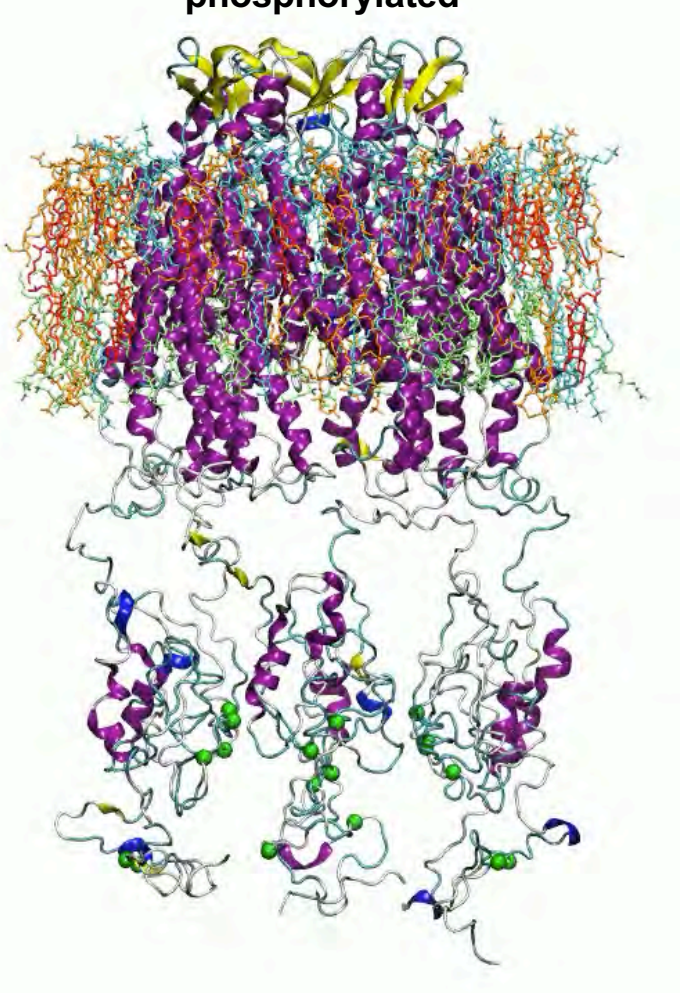


Molecular simulations using CHARMM-GUI

Animation of un-phosphorylated CT



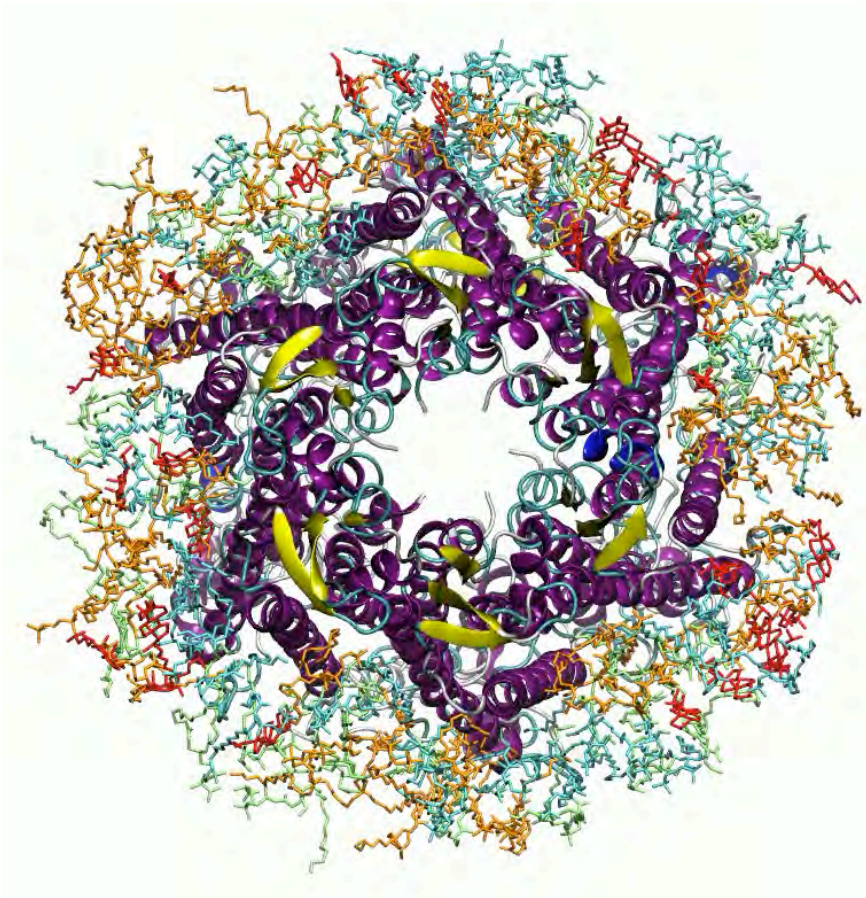
Animation with serines 279, 282, and 368 phosphorylated



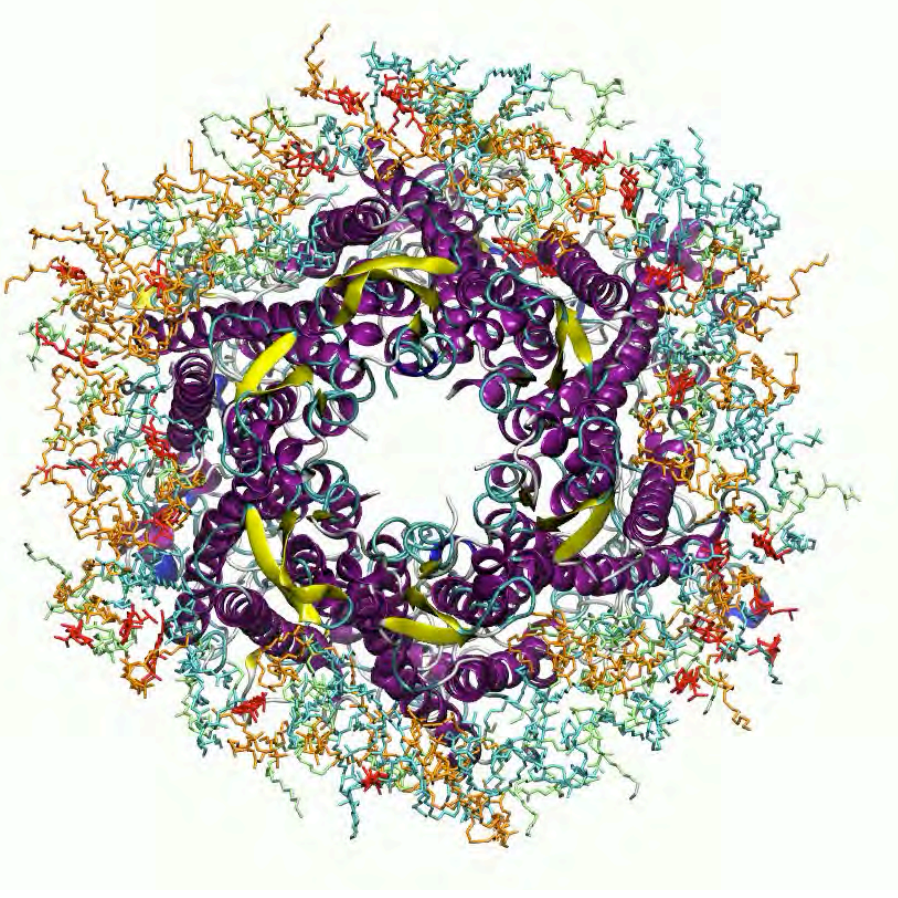
180 ns molecular dynamics simulation; 180 times repeated; ~ 28 sec total time
Green spheres depict the serine residues that can be phosphorylated

Molecular simulations

Animation of un-phosphorylated CT



Animation with serines 279, 282, and 368 phosphorylated



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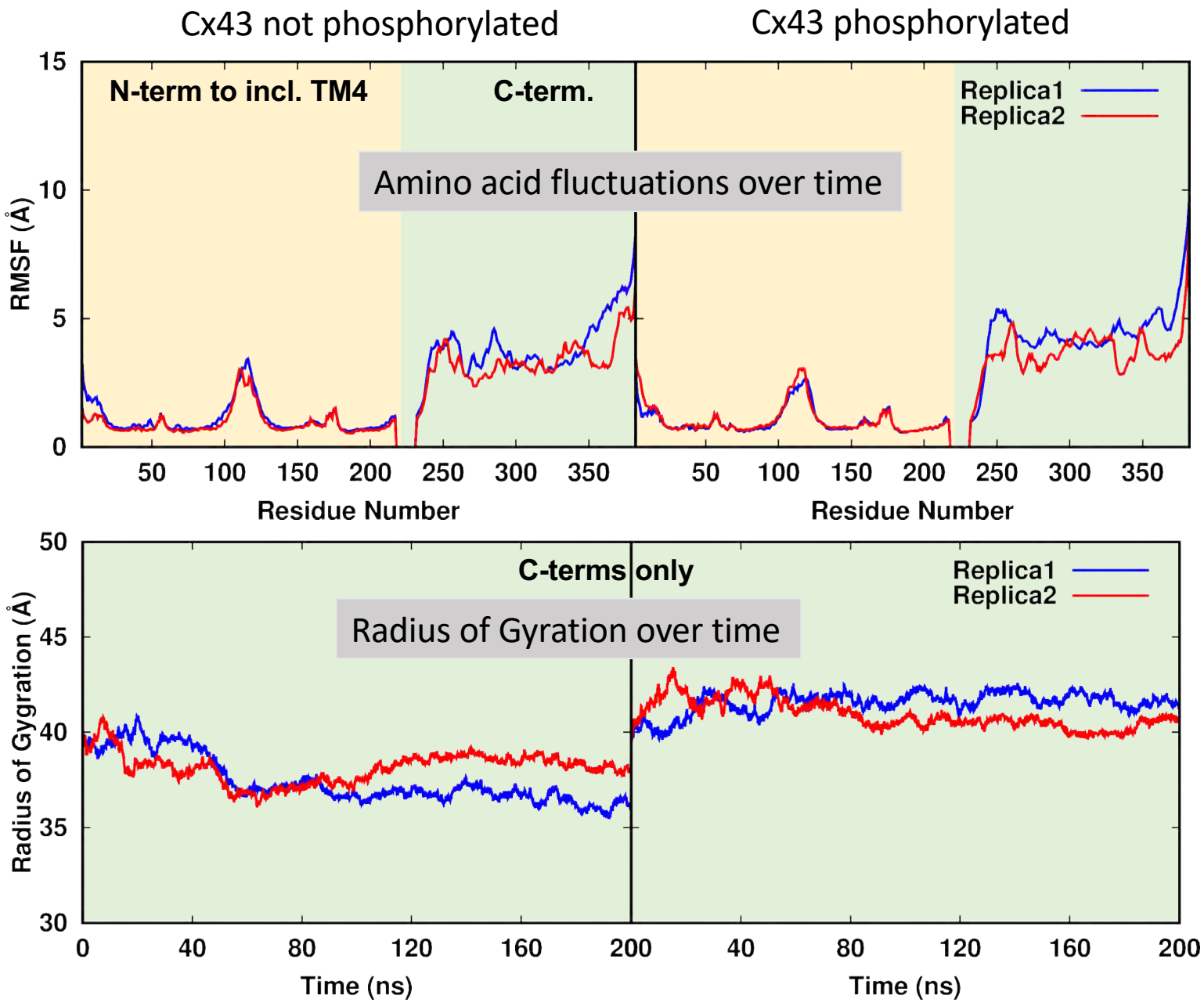

The Radius of Gyration indicates how compact a protein is; smaller is more compact!

Why does all this matter?

Preventing phosphorylation and other post-translational modifications on critical Cx43 C-terminal amino acid residues, interferes with balanced GJ turnover, and cause disease

Mol Biol Cell. 2004 Oct;15(10):4597-608. Epub 2004 Jul 28.

Defective epidermal barrier in neonatal mice lacking the C-terminal region of connexin43.

Maass K[1], Ghanem A, Kim JS, Saathoff M, Urschel S, Kirfel G, Grümmer R, Kretz M, Lewalter T, Tiemann K, Winterhager E, Herzog V, Willecke K.

Am J Med Genet A. 2007 Feb 15;143(4):360-3.

Skin changes in oculo-dento-digital dysplasia are correlated with C-terminal truncations of connexin Cx43

Vreeburg M[1], de Zwart-Storm EA, Schouten MI, Nellen RG, Marcus-Soekarman D, Devies M, van Geel M, van Steensel MA.

J Mol Cell Cardiol. 2014 Sep;74:330-9. doi: 10.1016/j.yjmcc.2014.06.010. Epub 2014 Jun 25.

Degradation of a connexin40 mutant linked to atrial fibrillation is accelerated.

Gemel J[1], Simon AR[1], Patel D[2], Xu Q[2], Matiukas A[2], Veenstra RD[3], Beyer EC[4].

Invest Ophthalmol Vis Sci. 2013 Nov 19;54(12):7614-22. doi: 10.1167/iovs.13-13188.

Connexin50D47A decreases levels of fiber cell connexins and impairs lens fiber cell differentiation.

Berthoud VM[1], Minogue PJ, Yu H, Schroeder R, Snabb JI, Beyer EC.

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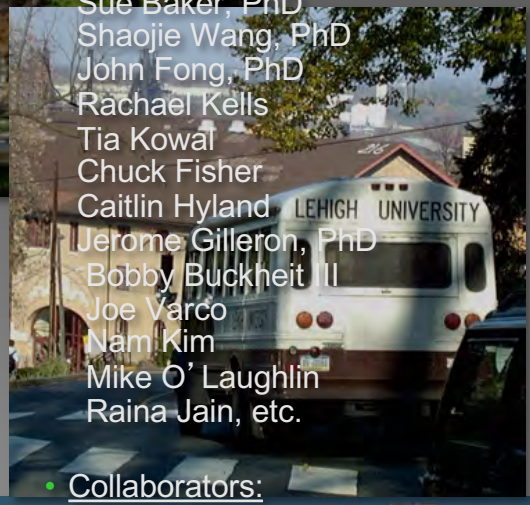

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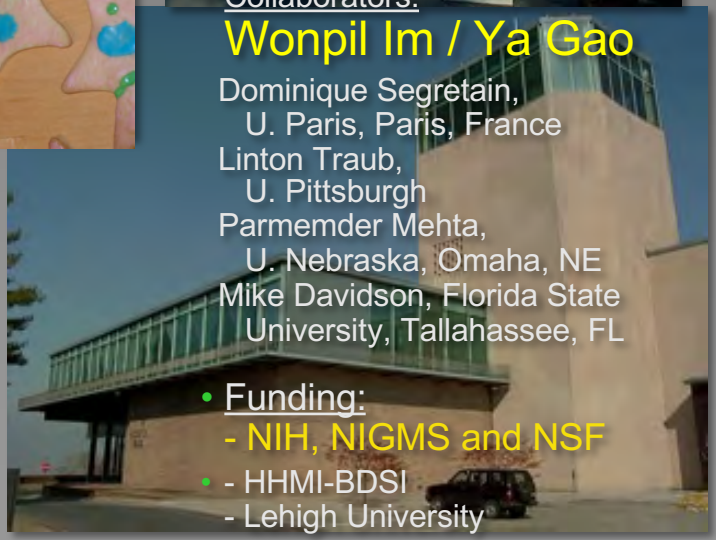

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  - Lehigh University

Lehigh University, Bethlehem, PA

5 Colleges:
Engineering, Arts & Sciences, Business & Economics, Education, Integrative Health

- Data X
- Biol. Sci., ~ 40 PhD Grads. ≥ 23 Faculty