Bioengineering 6003

Cellular Electrophysiology and Biophysics

Cardiac cell-cell Communication

Part 2

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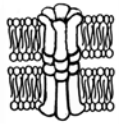


Regulation of intercellular communication

- It is simple

  Electrically we evaluate gj or junction conductance

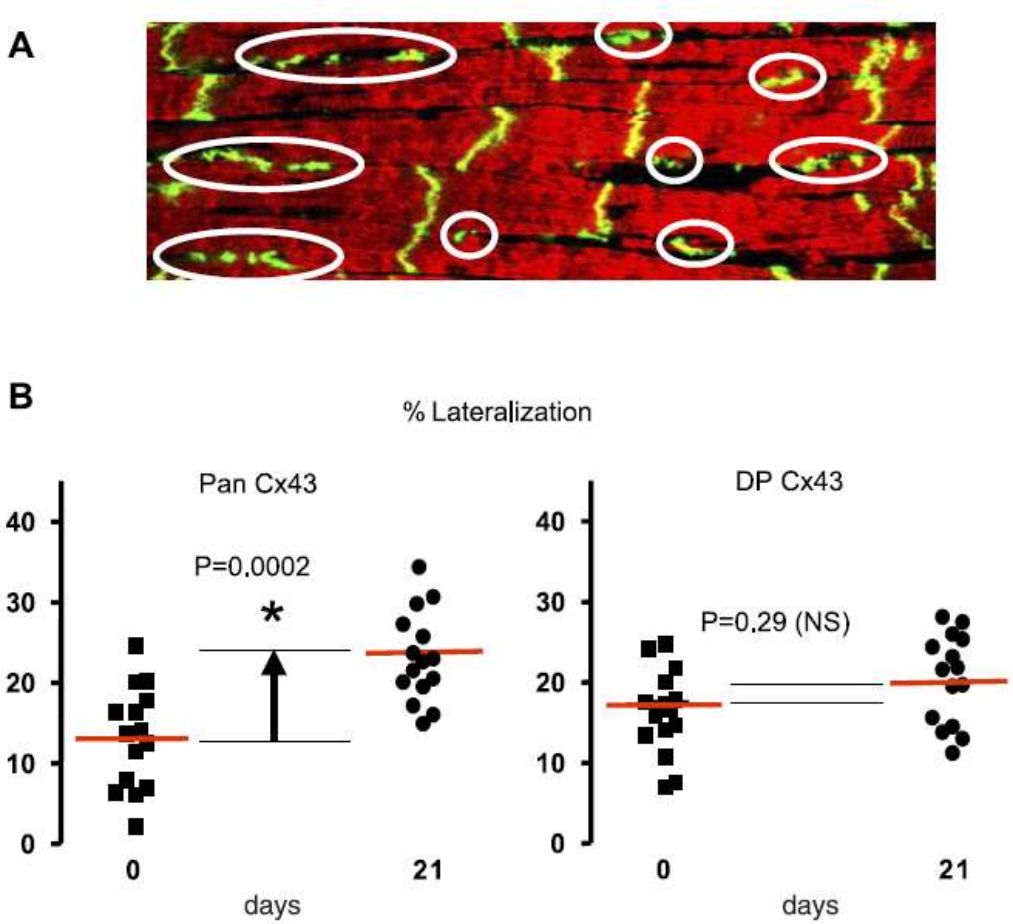
$$g_j = n * \gamma_j * Po$$

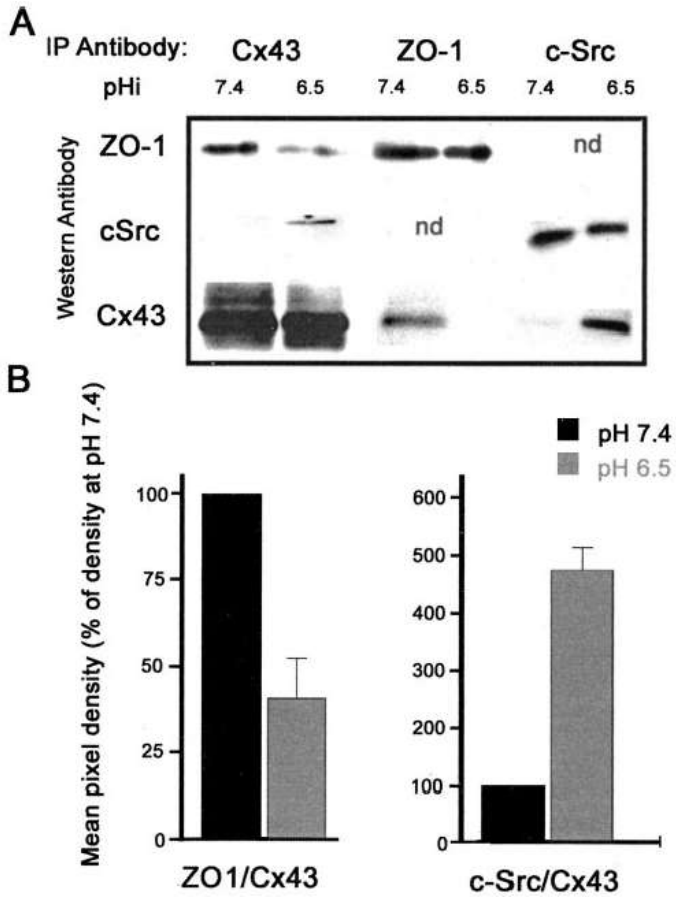
n = number of channels

γ_j = unitary conductance

Po = open probability

Remodeling (long term ischemia or heart failure)

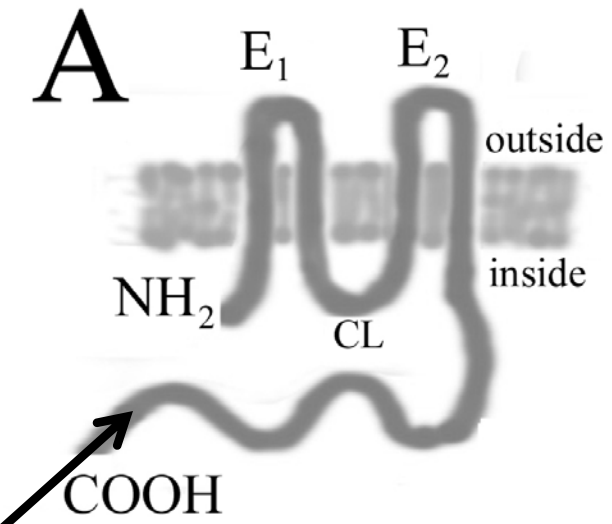




Gating of gap junction channels

- Gating by voltage
  - Transjunctional and transmembrane
- Gating by intracellular pH
- Gating by protein phosphorylation

IV. Structure function relationship

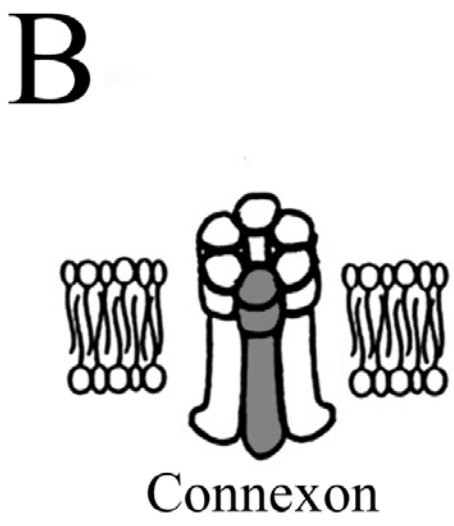


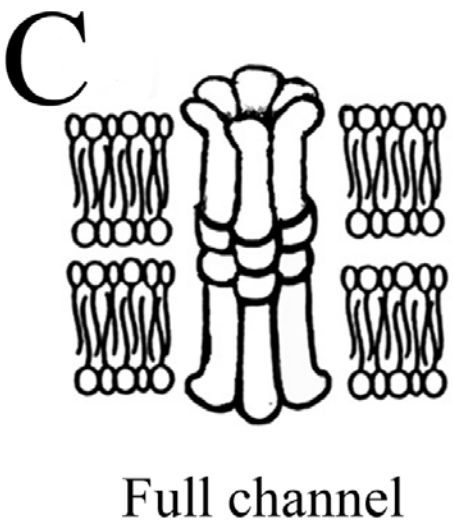
Connexin26

↓

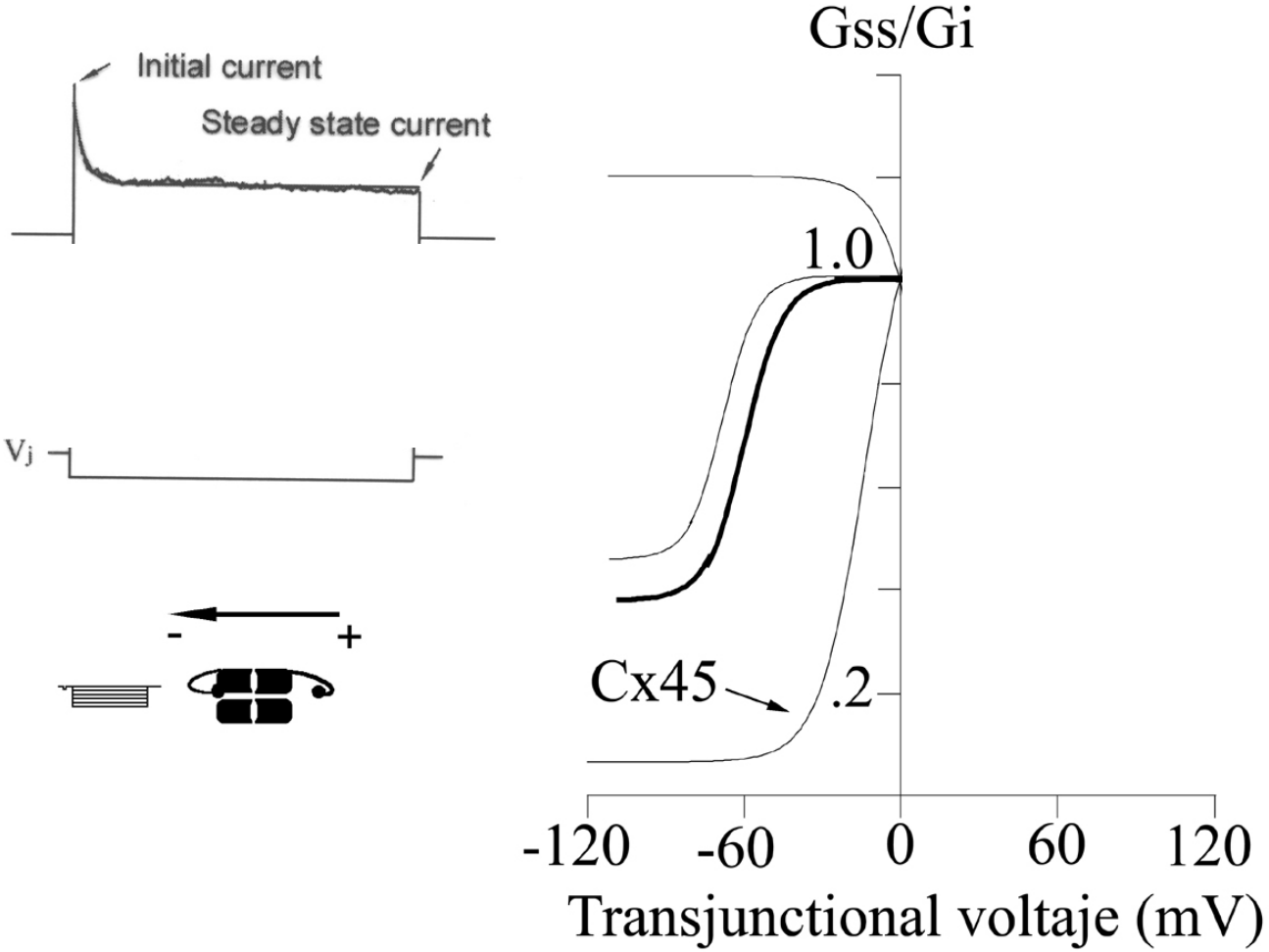
Connexin56

1. pH gating
2. Fast voltage gating
3. Phosphorylation





Transjunctional voltage dependence



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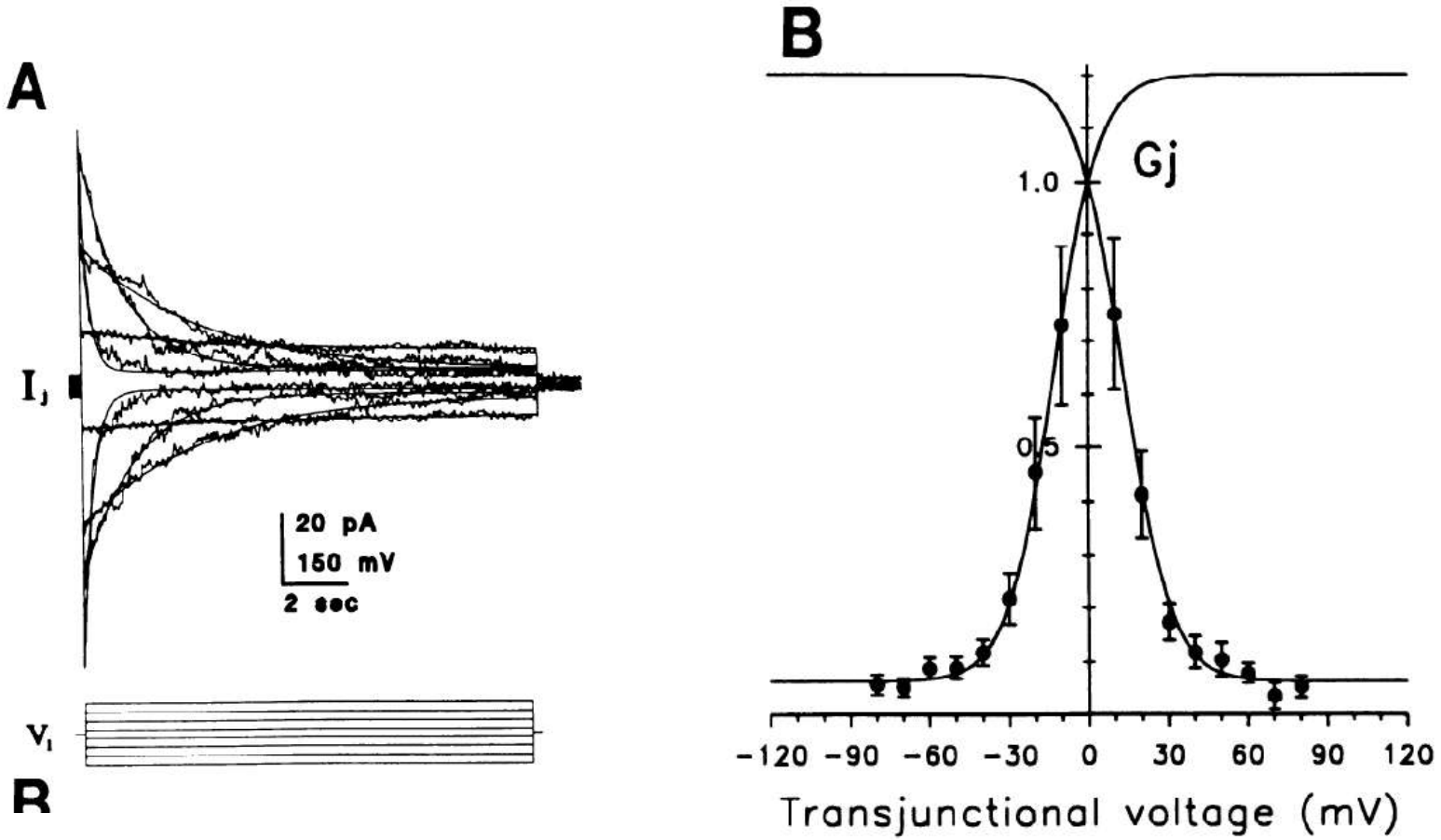
Voltage gating of Cx45



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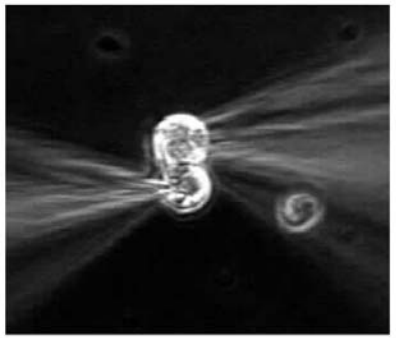
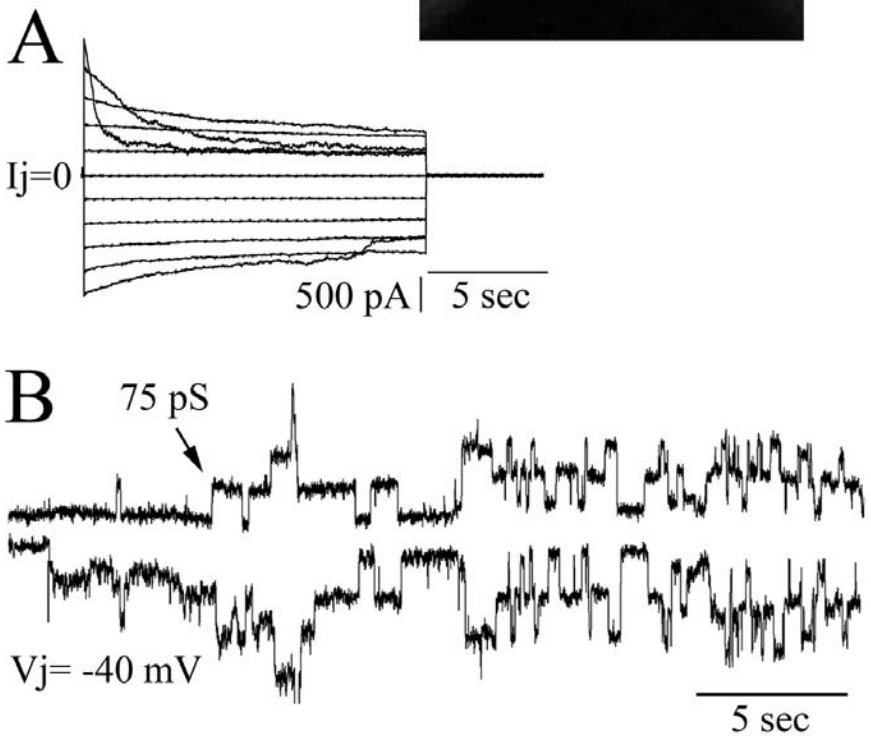
Rectifying channels from murine embryonic isolated ventricle cells

The formation of heterotypic channels can be demonstrated in this preparation, where gap junctions show gating rectification.

Isolated cardyocytes from 18-day murine embryo





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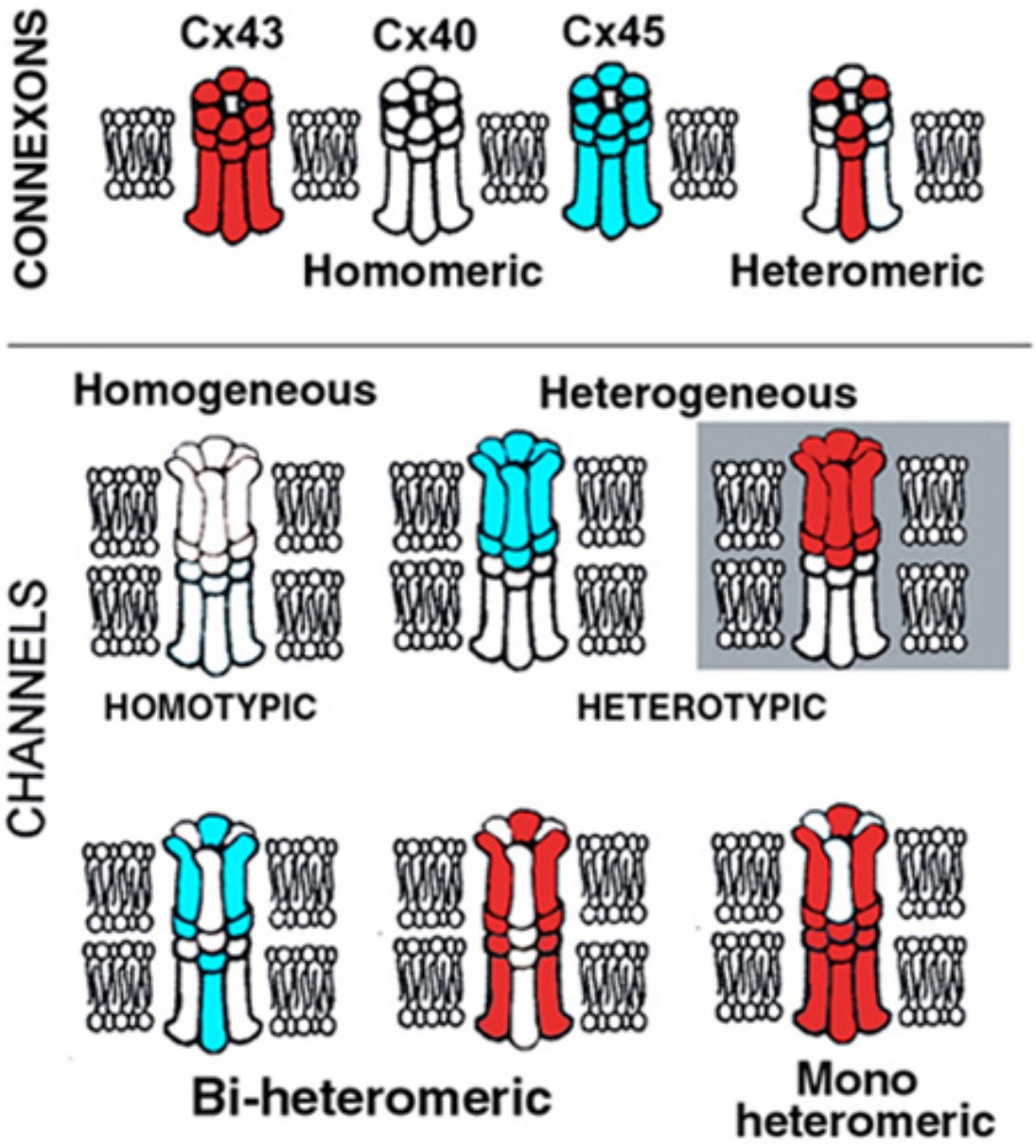
Gap Junction Channels Multiple Configurations



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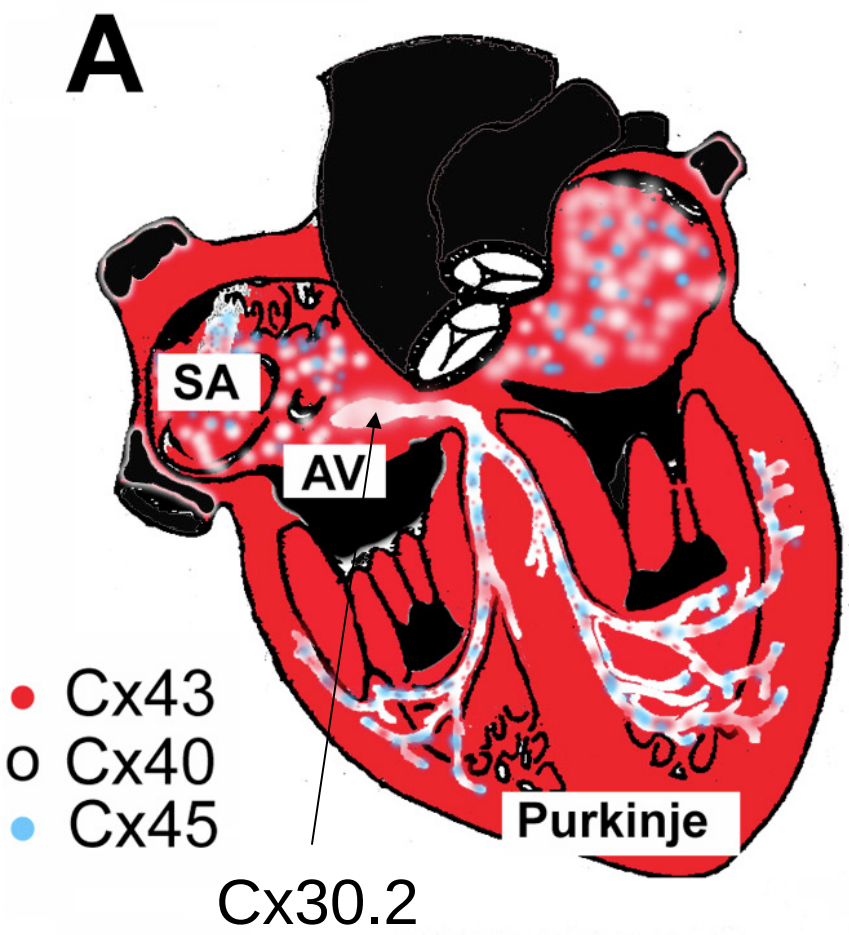
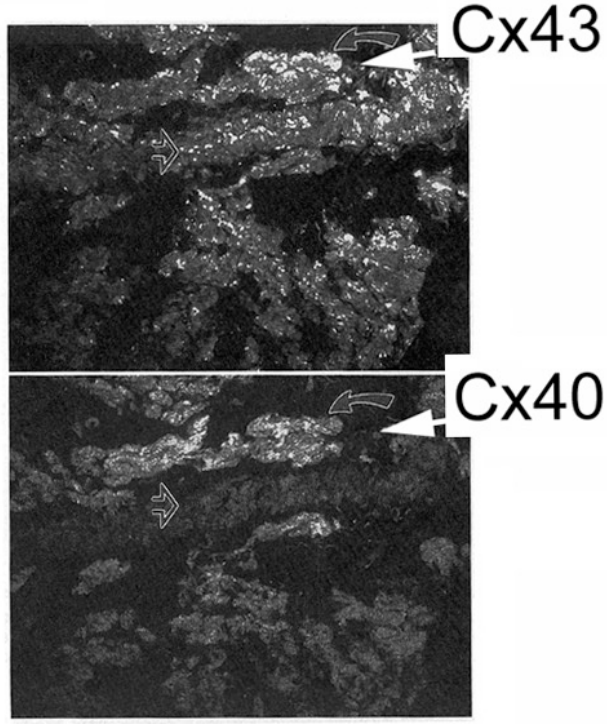
Multiple expression of connexins in a tissue

Connexins in the heart

Example of the co-expression of connexins





Canine sinus node
(Kwong et al, Circ Res. 1998)

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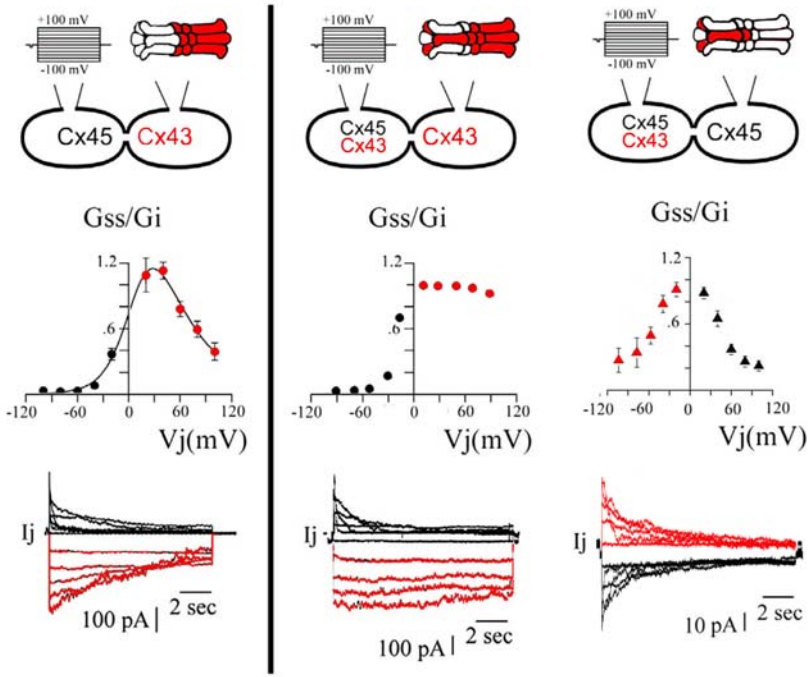
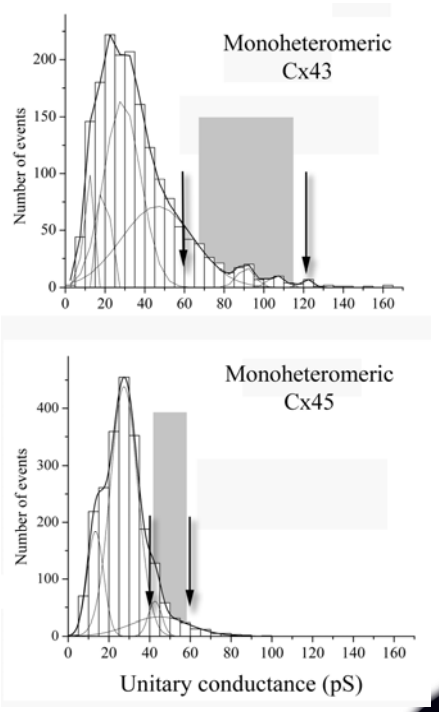
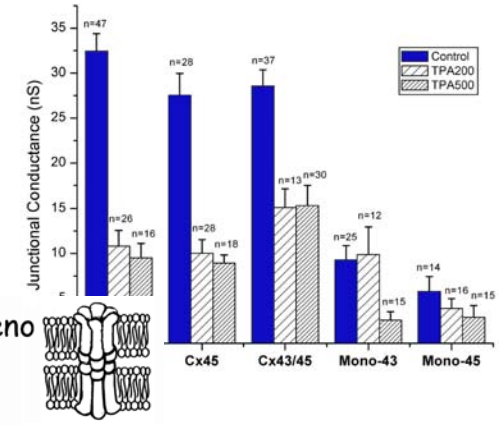
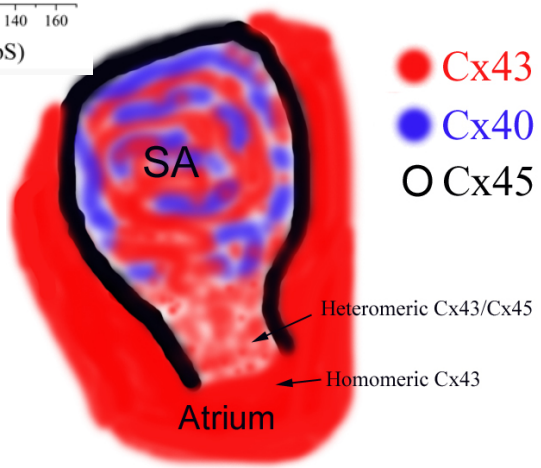
Heteromeric combinations modulate total and unitary conductances









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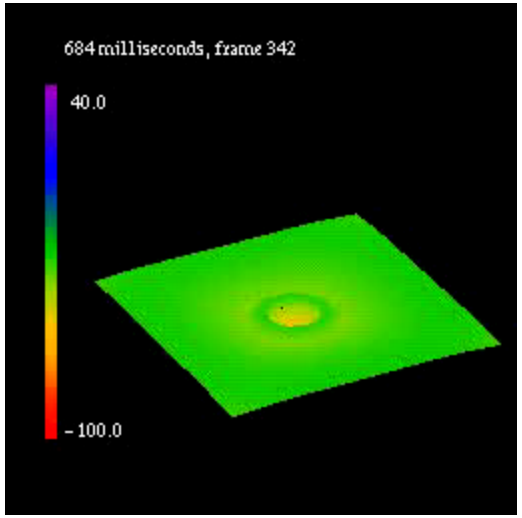
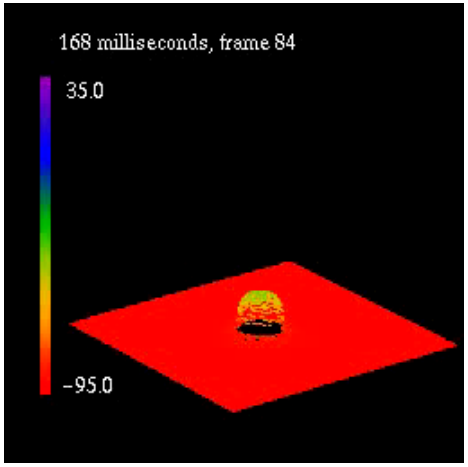
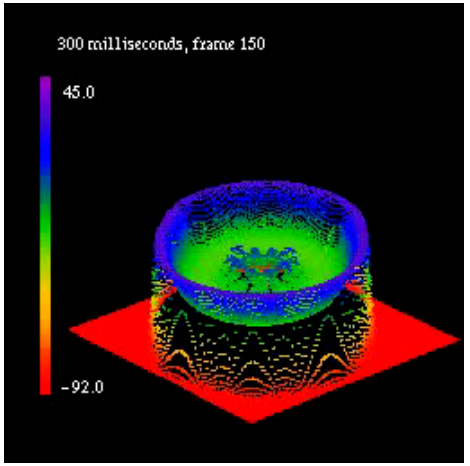
Electrical propagation from the SA node to the musculature



Control



Uncoupled



Adding connections

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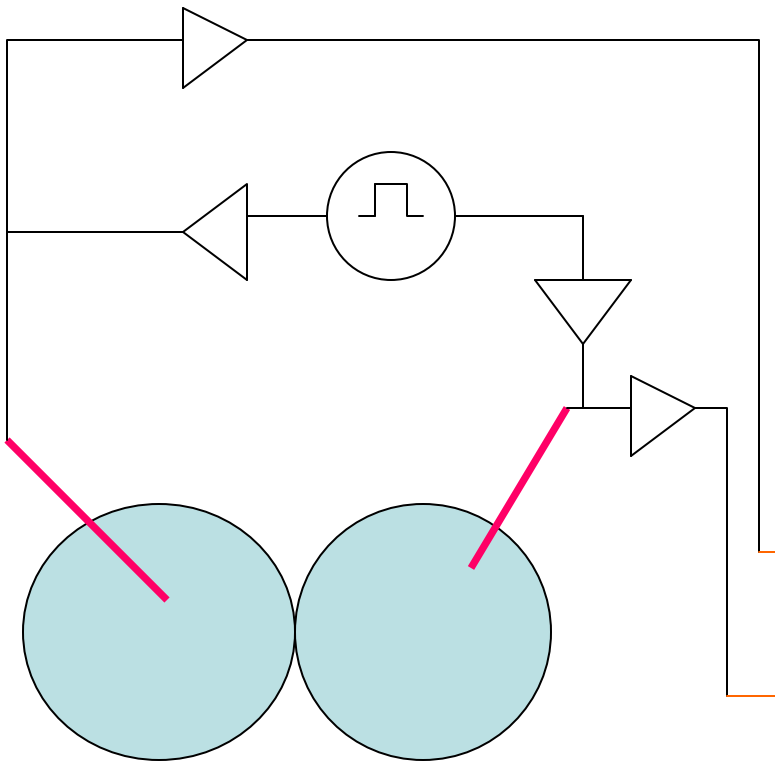
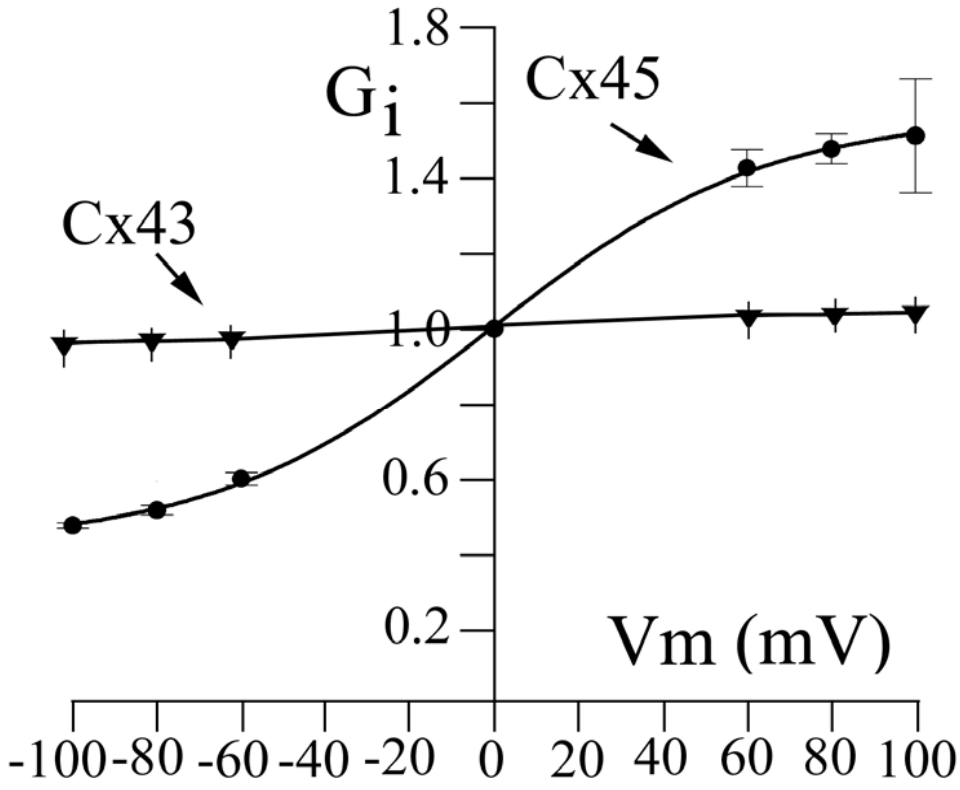
Gating by Transmembrane voltage





Evaluation of changes in total conductance due to synchronous stimulation in both cells

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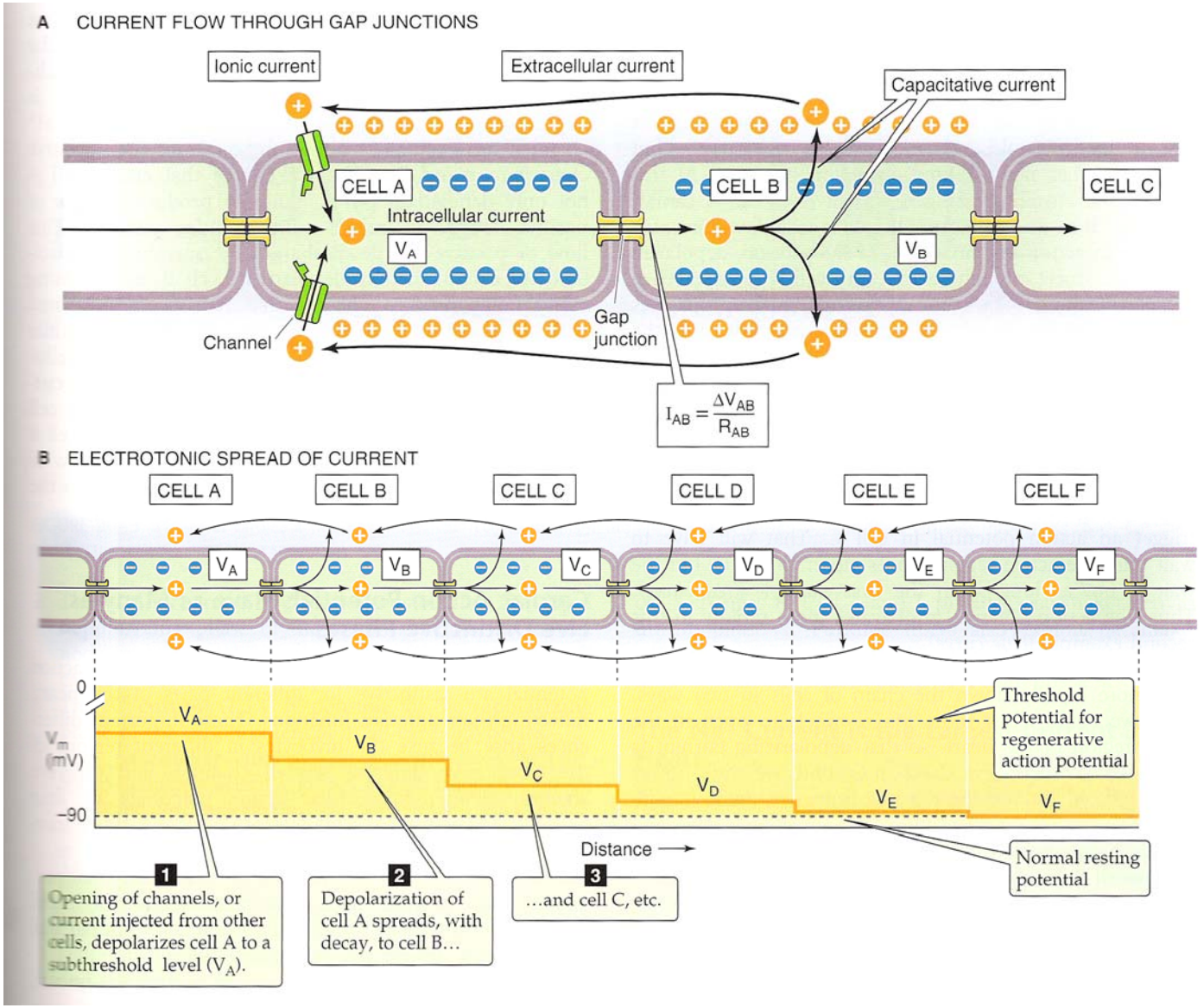
Electrotonic conduction in cardiac fibers

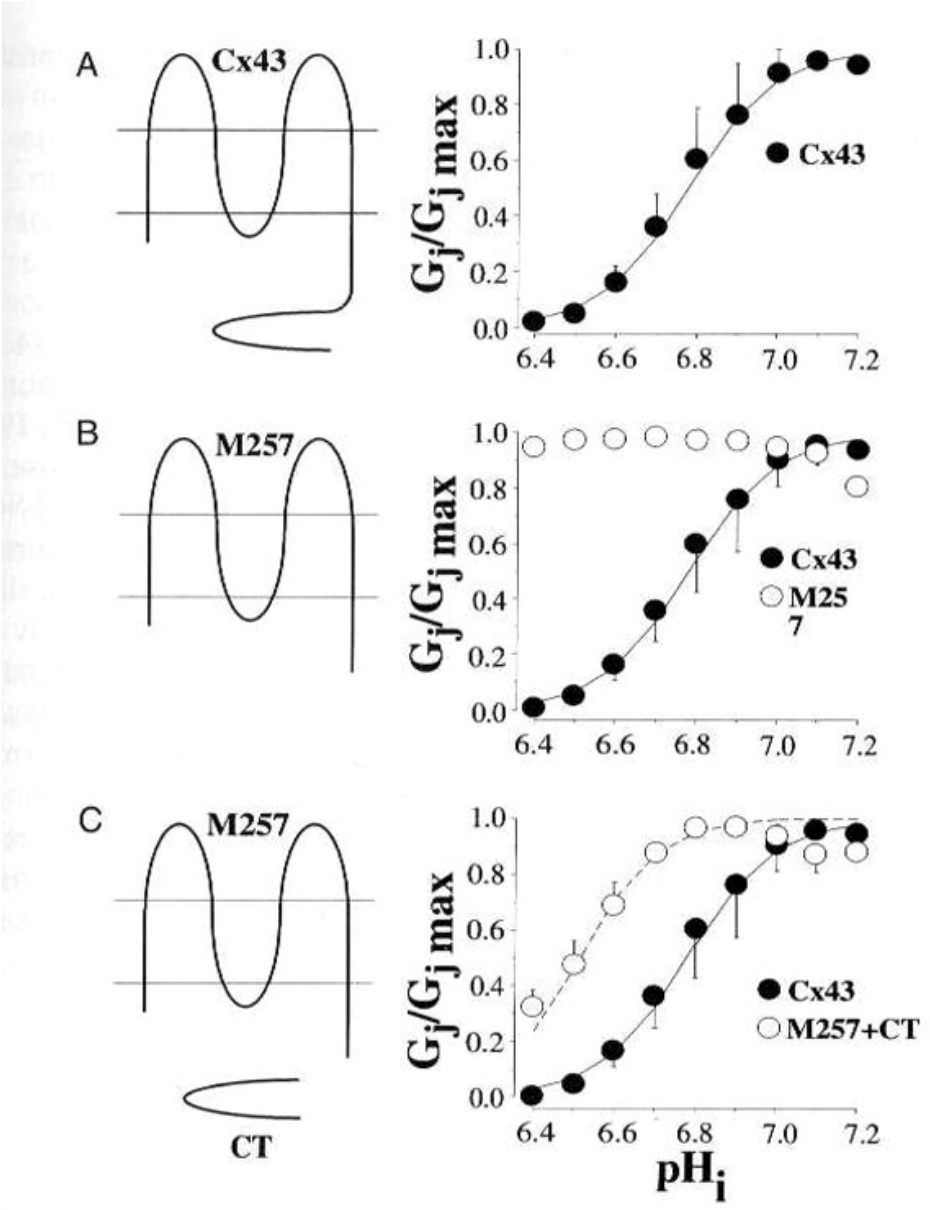


Gating by pH

The reduction of intracellular pH causes a reduction in the conductance of the junction (Gj/Gmax).

When the COOH tail is removed, there is no gating by pH.

If the COOH tail is co-expressed, the gating by pH is re-established.



M. Delmar et al. Current Topics in Membranes (2000)

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Intra-molecular interactions rule pH gating for acidosis

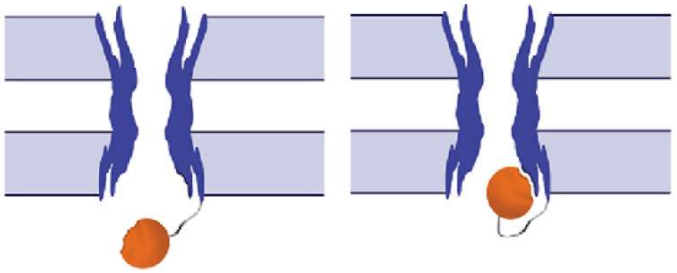

Fig. 1. Diagram illustrating the concept of a ball and chain model for chemical regulation of connexins. An intracellular flexible domain (the carboxyl terminal domain) acts as a gating particle. Under normal conditions, the gate is away from the pore. Under the appropriate stimulus, the gate would swing toward the mouth of the channel, bind to a "receptor" affiliated with the pore, and close (or modify) the channel.

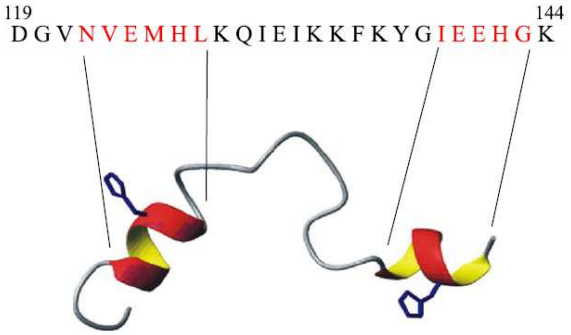

Fig. 6. Secondary structure of a peptide corresponding to amino acids 119–144 of Cx43, as solved by nuclear magnetic resonance. We propose that this structure acts as a receptor for the gating particle during chemical regulation of Cx43 channels.

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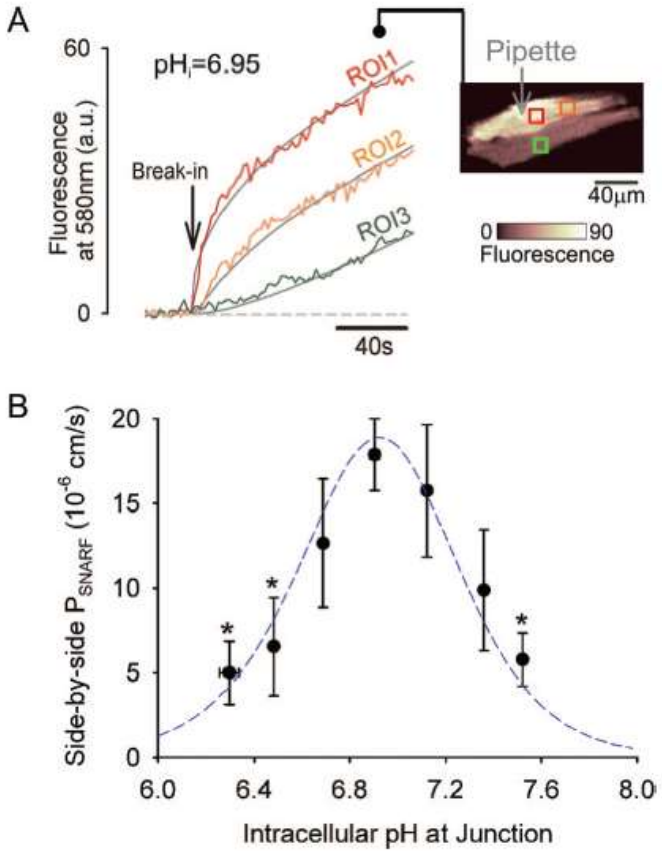
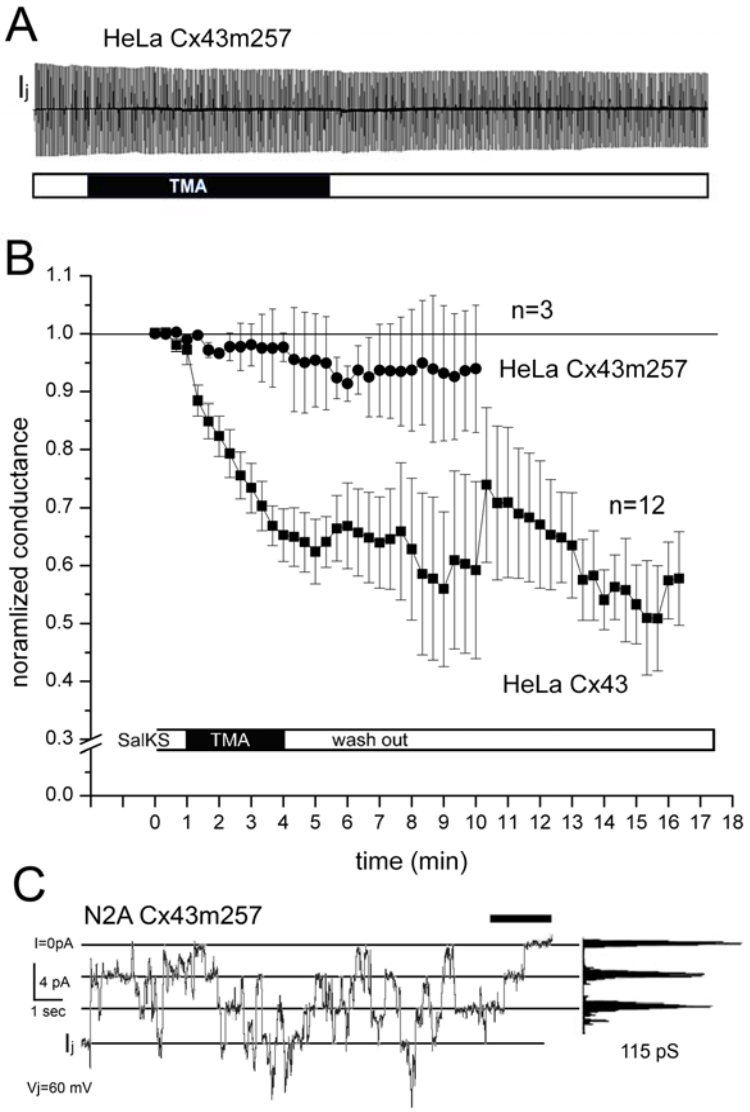
Connexins also gate for intracellular alkalosis





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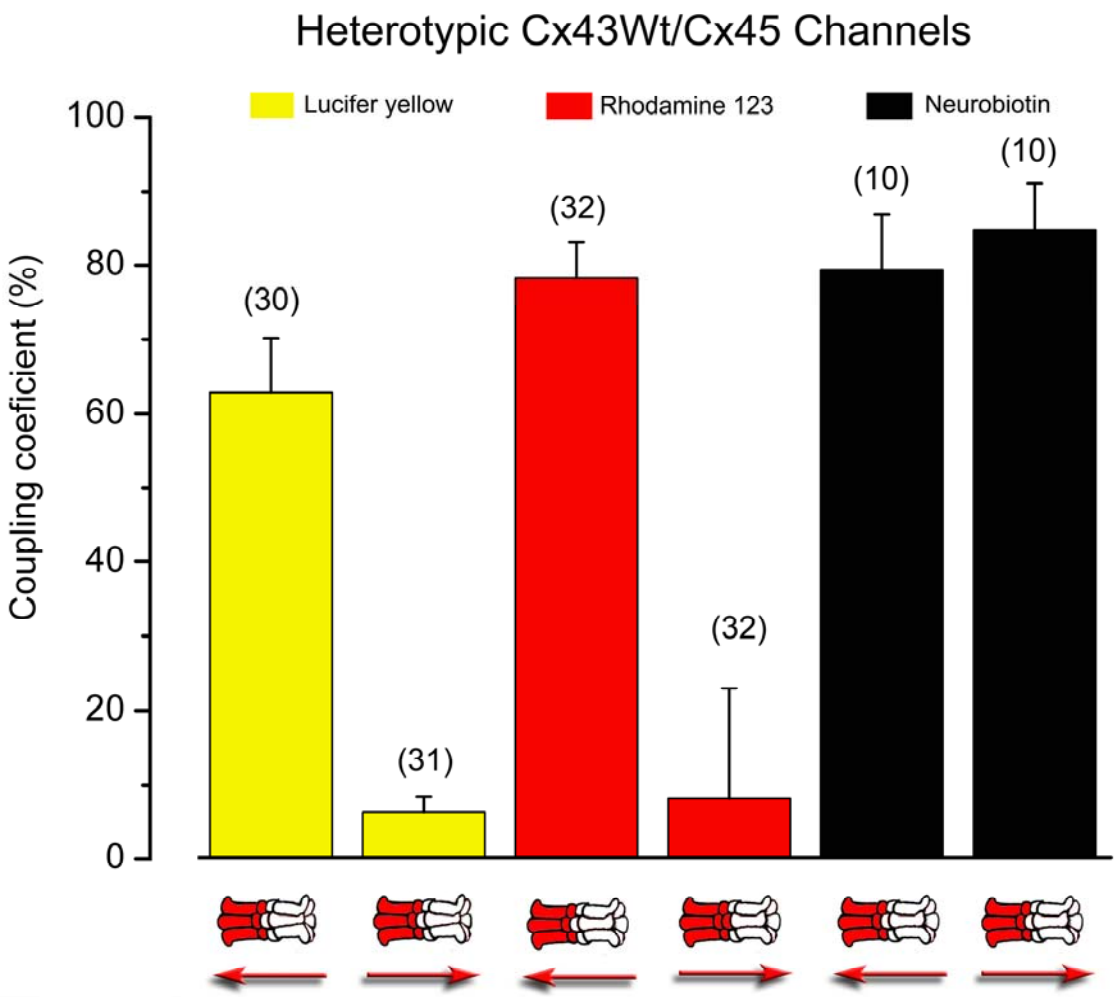
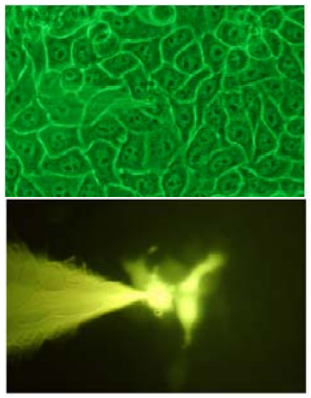
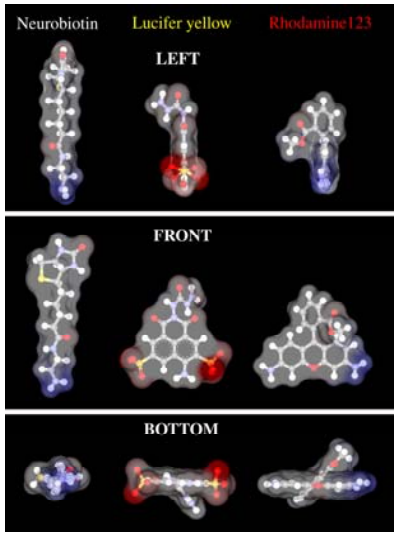
Heterotypic channel formation reverts permeance properties without changing charge selectivity







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Connexin26 channel at 3.5Å resolution
Details from the pore

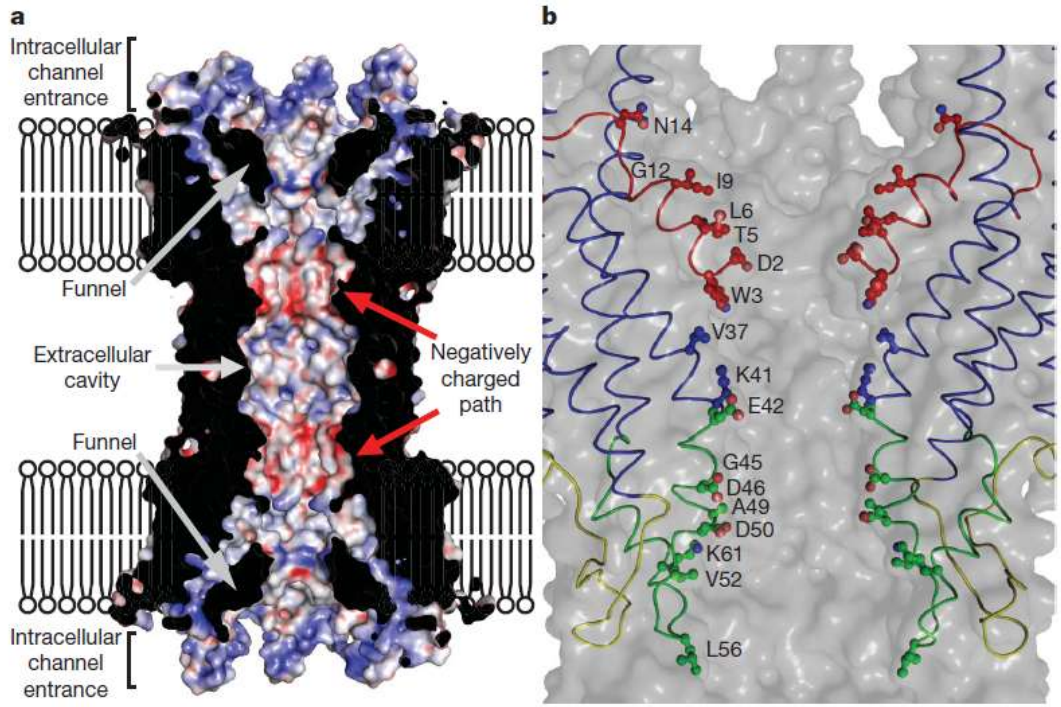
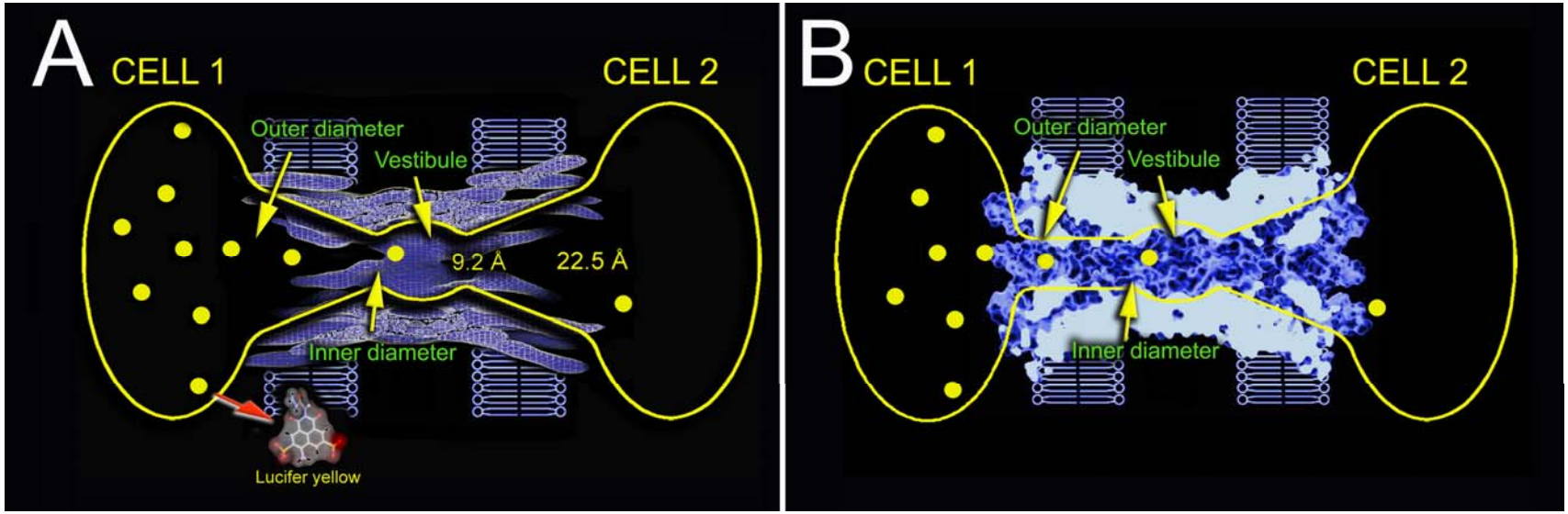


Figure 4 | Pore structure of the Cx26 gap junction channel. **a**, Vertical cross-section through the gap junction channel, showing the surface potential inside the channel. The channel features a wide cytoplasmic opening, which is restricted by the funnel structure, a negatively charged path and an extracellular cavity at the middle. Electrostatic surface potential of the Cx26 gap junction channel was calculated by the program APBS[43] as implemented in PyMOL under dielectric constants of 2.0 and 80.0 for protein and solvent regions, respectively. The displayed potentials range from −40 (red) to 40 (blue) kTe^{-1}. **b**, Pore-lining residues in a Cx26 gap junction channel. Side view of Cx26 gap junction channel pore; the main chain is depicted as a thin ribbon and side chains facing the pore as balls and sticks. For fine viewing, two subunits in the foreground are omitted in the surface representation and two further subunits in the background are omitted in the model depiction. The colouring is the same as in Fig. 3b.

A mathematical model representing two cells coupled through a gap junction pore



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Permeation modeling equations in a particle collision model

External Forces

$$F_{Electrostatic} = \frac{1}{4\pi\varepsilon_0}.\frac{q_1.q_2}{r^2}\left(\hat{r}_{12}\right)$$

$$F_{Brownian} = K_{Br}.\hat{v}$$

$$F_{EF} = qE$$

$$F = F_{Electrostatic} + F_{Brownian} + F_{EF} + F_{Misc}$$

Particle-Particle Interactions

$$c = \hat{n}\cdot\left(u_1 - u_2\right)$$

$$v_1 = u_1 - \left(\frac{m_2 c}{m_1 + m_2}\right)(1+e)\hat{n}$$

$$v_2 = u_2 - \left(\frac{m_1 c}{m_1 + m_2}\right)(1+e)\hat{n}$$

Wall- Particle interactions

$$\vec{v} = \left|\vec{u}\right|\left(\hat{u} - 2\hat{n}\left(\hat{n}\cdot\hat{u}\right)\right)$$

Particle Dynamics

Velocity

$$v = u + a\Delta t$$

Acceleration

$$a = \frac{F}{m}$$

Position

$$s = s_0 + u\Delta t + \frac{1}{2}a\Delta t^2$$

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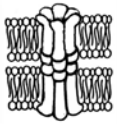
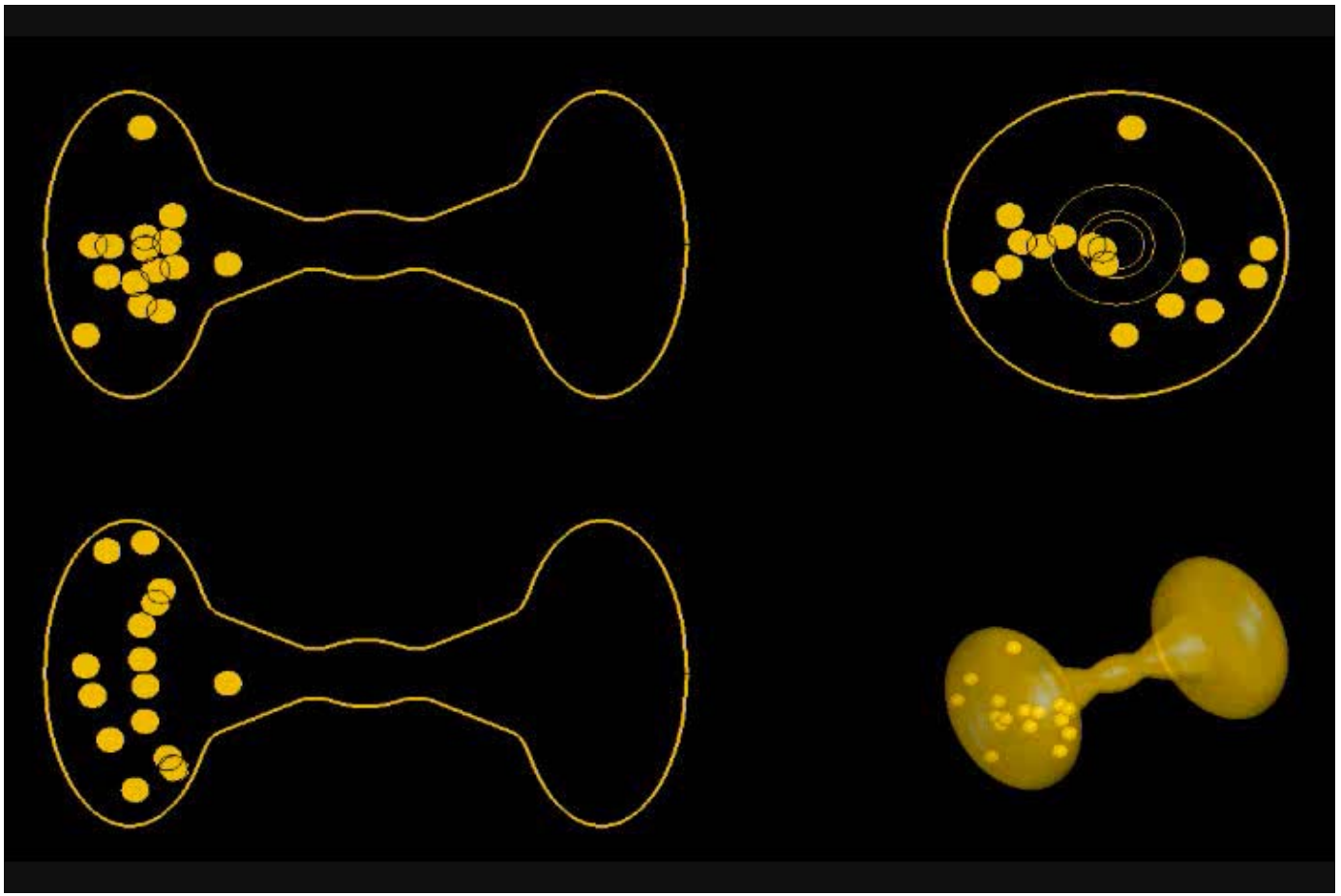


Collision model simulation of 16 Lucifer Yellow molecules in a two cell model connected through a gap junction pore.

Lateral view

Axial view



Top view

3D

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Mondal et al, Biophys. J. 2010a

Using Brownian Dynamics makes a more realistic model

$$x_{n+1} = x_n - \frac{1}{kT} D . \overrightarrow{F_{n+1}} \Delta t + \sqrt{2}\sqrt{D} . \Delta W_{n+1} + \frac{\partial}{\partial x} . D \Delta t$$

where, x is the position, n is the time step, k is the Boltzmann's constant, T is the temperature (in Kelvin), D is the diffusion matrix of the particle, F is the net force acting on the particle, Δt is the time step and ΔW is a random vector from Gaussian distribution with zero mean variance.

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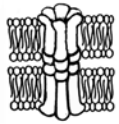

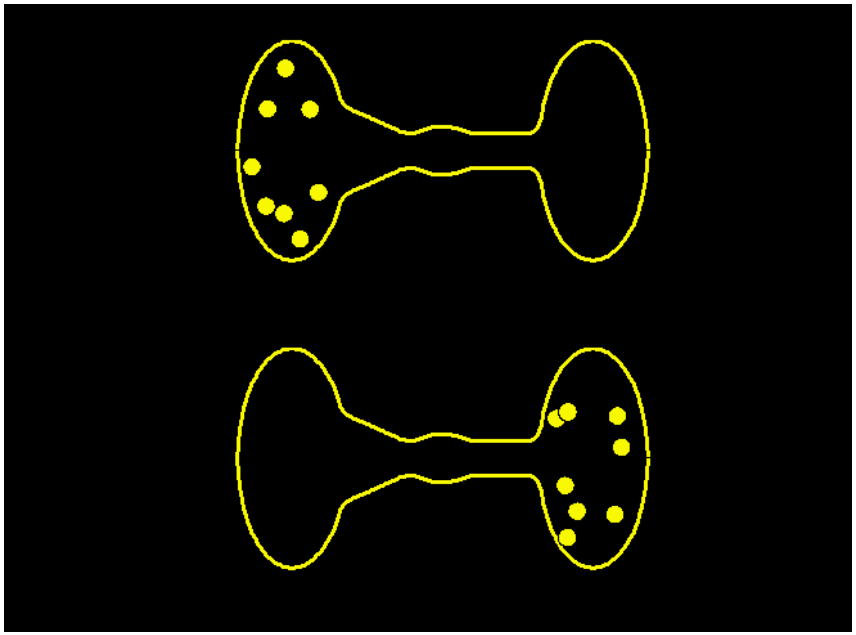

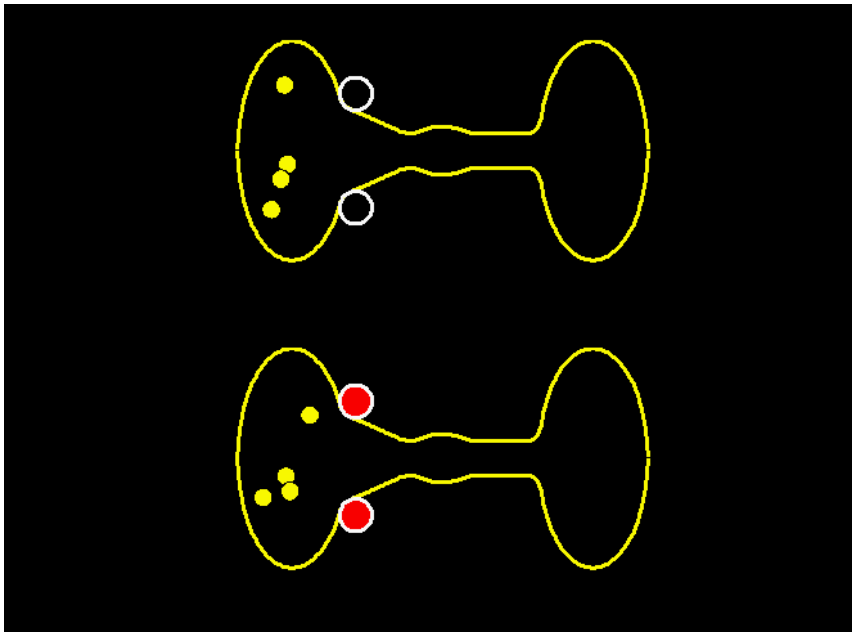

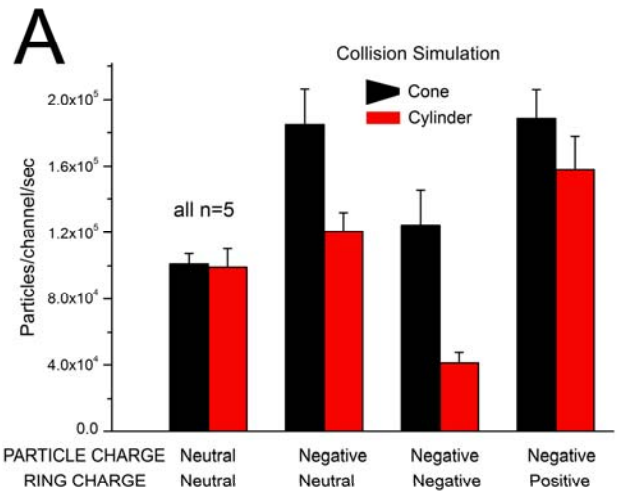

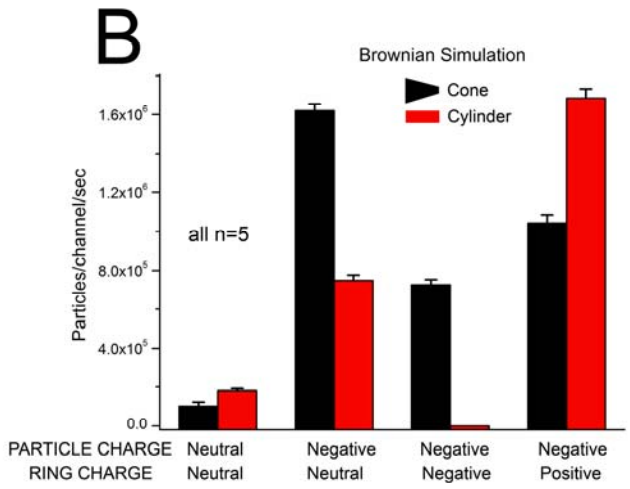

Combo Simulations V2

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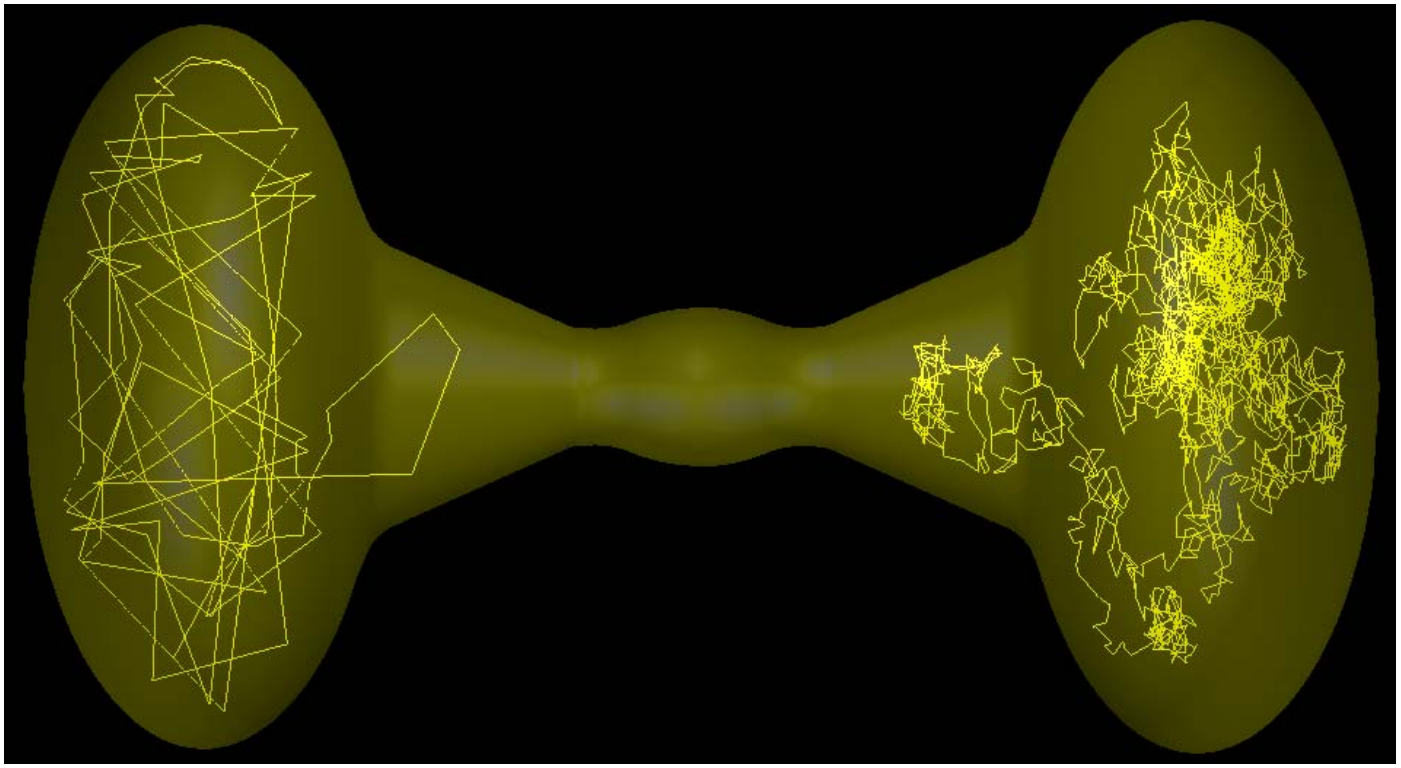
Comparison between simulated particle trajectories from two different mathematical models

Particle collision

Brownian dynamics



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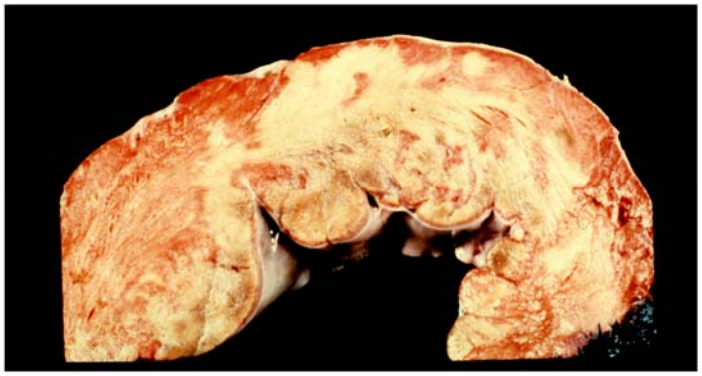
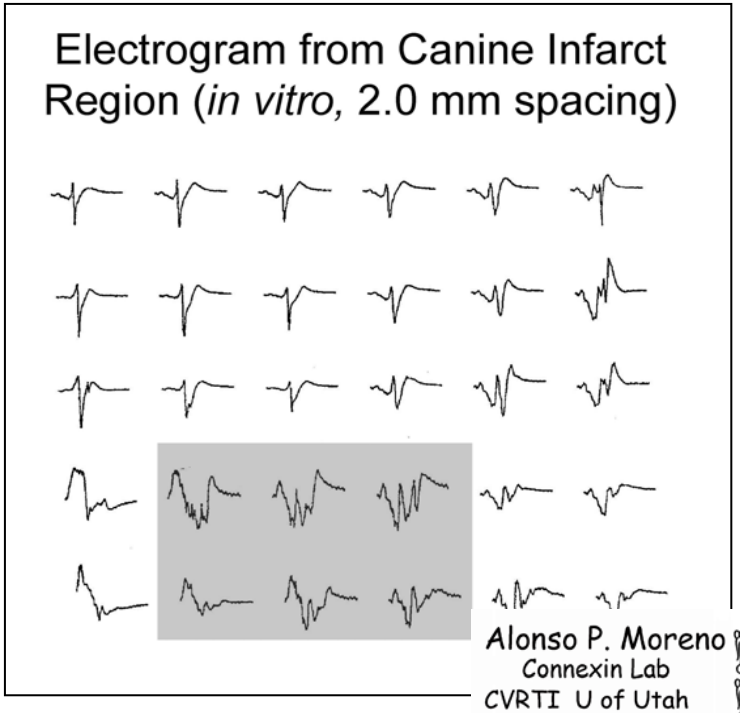
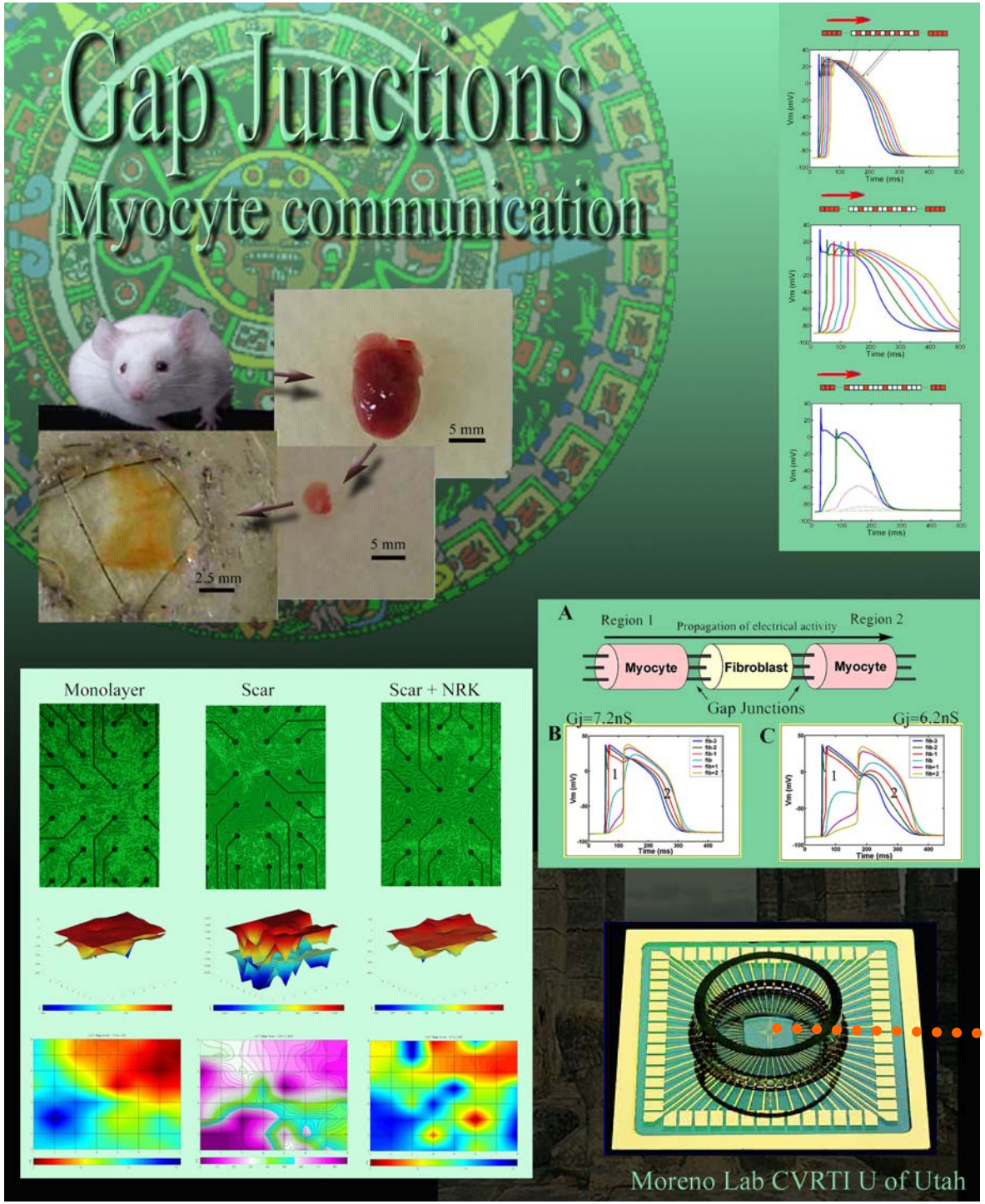
Conduction after infarction

- Myocardial infarction (MI) healing process includes revascularization and formation of scar tissue.
- Scar tissue is composed of fibroblast-like cells embedded in a collagen-fibrous matrix.
- Scar formation causes complex changes in the organization of cells in the infarcted area.






Gap Junctions
Myocyte communication
5 mm
5 mm
2.5 mm
Vm (mV)
Time (ms)
A
Region 1
Propagation of electrical activity
Region 2
Myocyte
Fibroblast
Myocyte
Gap Junctions
B
Gj=7.2nS
C
Gj=6.2nS
Monolayer
Scar
Scar + NRK
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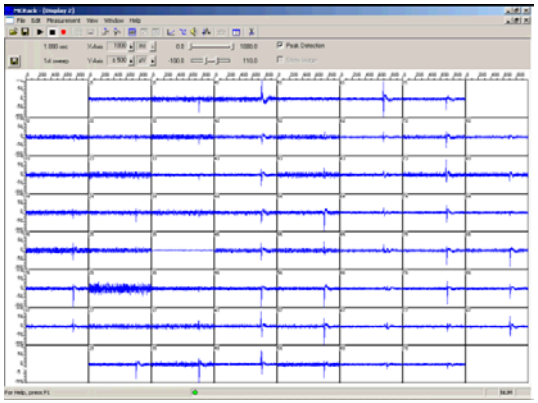


Modeling current interactions between myocytes and fibroblasts

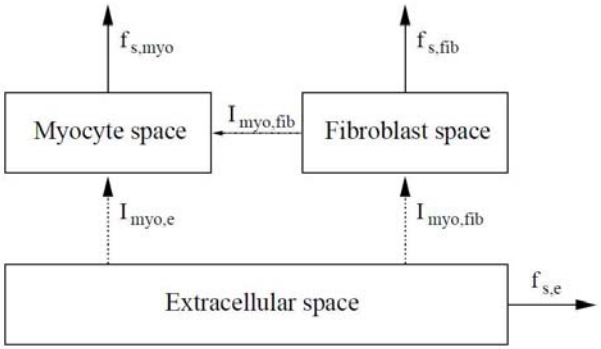
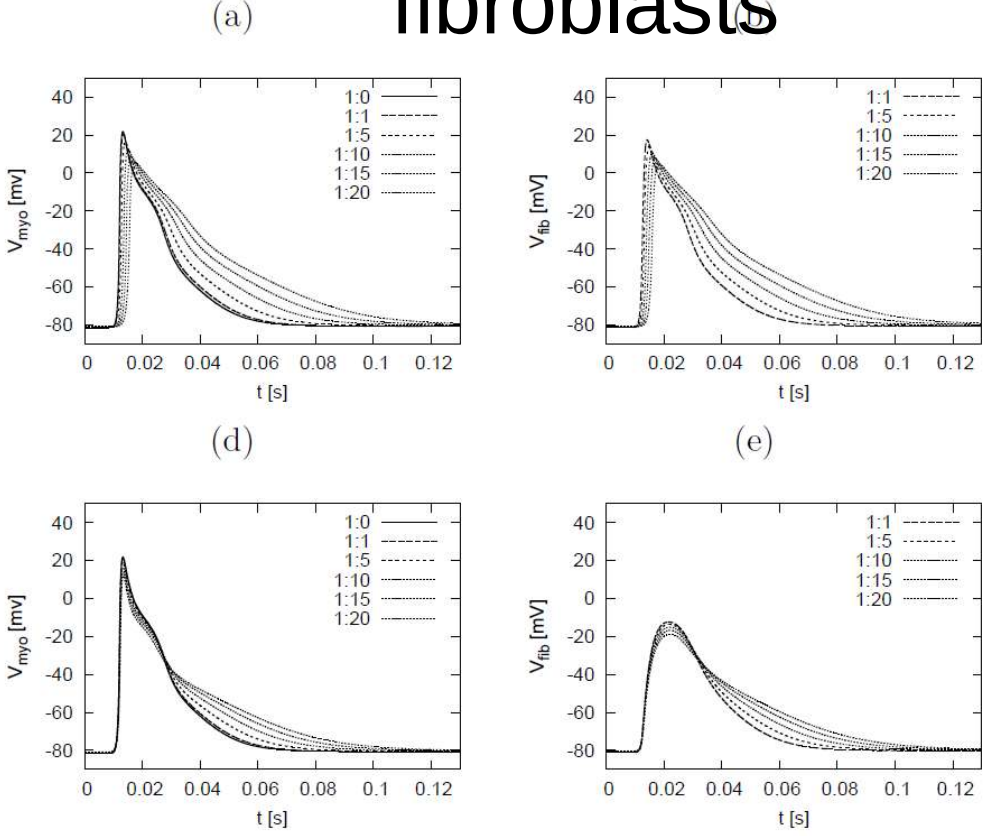
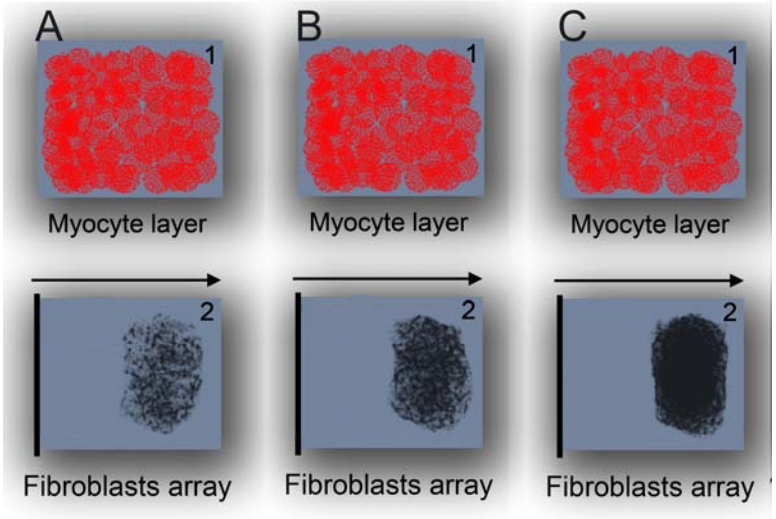


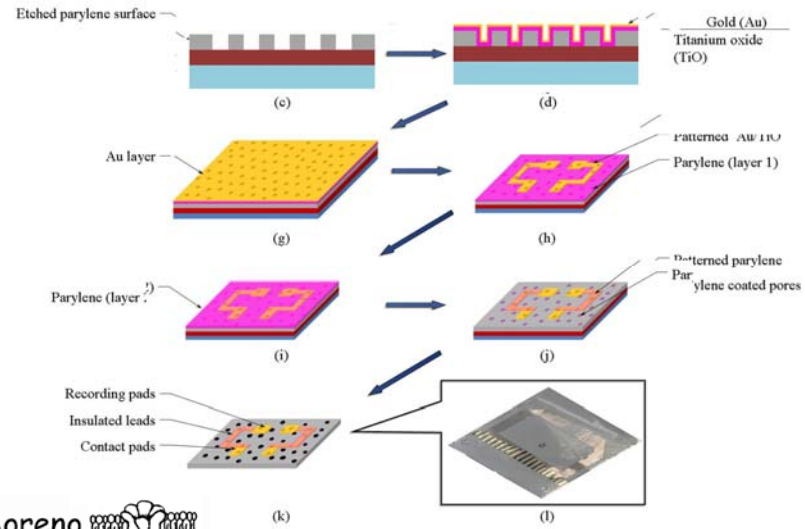
Figure 1: Sachse et al.

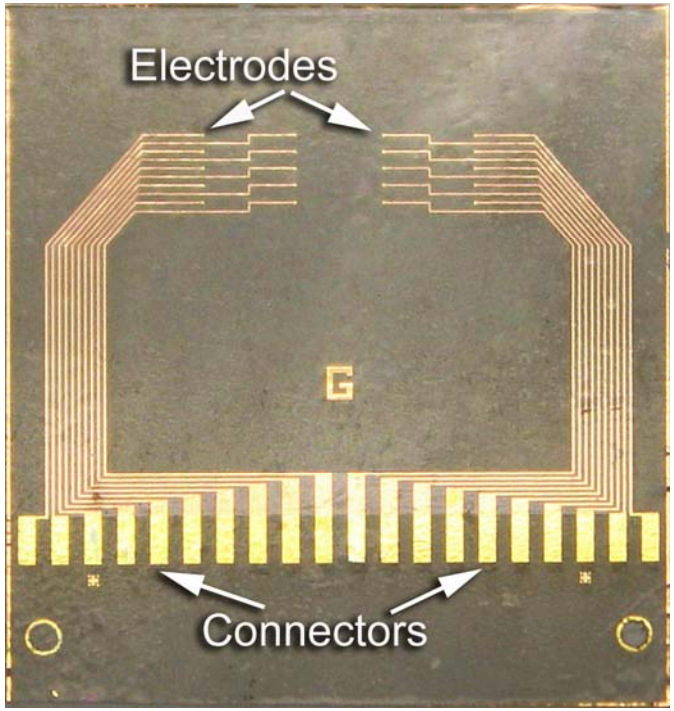


Sachse et al, Ann Biomed Eng, *37*; 2009

Building a FlexMEA as an alternative to regulate and study heterocellular communication







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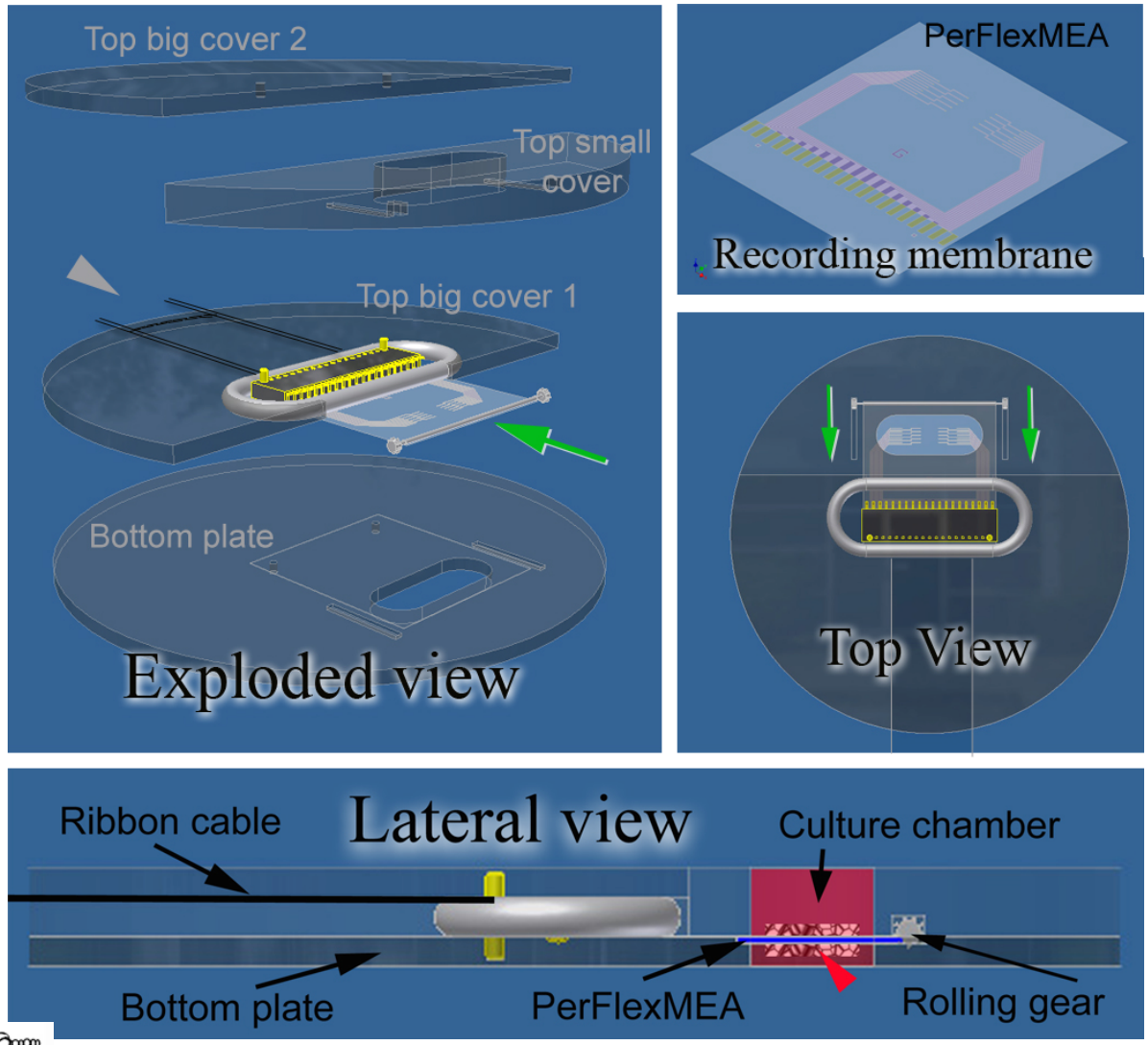
Recording system for the use of Perforated Flexible MEAs



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Summary

- Gap junction channels could be formed of distinct isomeric forms.
- Each isoform can form channels with unique gating for voltage and chemical agents.
- Permeability of gap junctions depends on the isoforms involved in the formation of channels and on the properties of the crossing-molecules.
- Heteromultimeric channels can become highly selective and are thought to be involved in various physiological and pathological processes.

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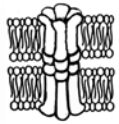

- Heterotypic communication can alter channel selectivity and create a preferential flux direction of metabolites across gap junction. One of the mechanisms to explain this preferential flux could be based in differences in pore mouth and charge.

- Long standing electrophysiological phenomena related to the complex nature of electrograms in the infarct border region could be explained by changes in cardiac conduction due to hetero-cellular coupling.

- Studies related to this mechanism of conduction could significantly impact the interpretation of cardiac electrograms, increase the understanding of arrhythmia substrates, and make a significant contribution on the development of more guided approaches to arrhythmia treatment.

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