divided into two types with respect to their biochemical characteristics and the nature of the process of their formation. The nanotubes of type I are shorter, more dynamic and contain actin filaments. They are formed when cells explore their surroundings in order to make contact with another cell. The nanotubes of type II are longer, more stable and have cytokeratin filaments. They are formed when two already connected cells start to move apart. On the nanotubes of both types small vesicles were found as an integral part of the nanotubes (i.e. dilatations of the nanotubes). The dilatations of type II nanotubes do not move along the nanotubes, while the nanotubes of type I have frequently dilatations (gondolas) that move along the nanotubes in both directions and are formed in different ways. We suggested theoretical models that may explain how these nanotubes are created and stabilised.

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1445-Pos Board B289

Simulation Of Particle Diffusion Across Gap Junction Channels Based On Their Pore Geometry Explains Unidirectional Fluxes

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Connexin proteins form gap junction channels that allow intercellular communication with distinct perm-selectivity properties. Mono-heteromeric and heterotypic combinations of cardiac connexin43 (Cx43) and Cx45 induce a preferential flux based on molecular size. For Lucifer Yellow or Rhodamine123, preferential flux was 3x larger from homomeric to heteromeric connexons. For heterotypic combinations, fluxes from homomeric Cx45 to homomeric Cx43 connexons were 4x larger. This favored direction was not expected. **Our objective was to use computational simulation of particle diffusion across gap junction channel pores to find if geometrical parameters can explain our in vitro permeability data.**

HeLa cells were stained with red or green dyes to differentiate expression and co-cultured on glass cover-slips for 8 hours. A Nikon epifluorescent Eclipse7000 microscope helped quantifying diffusion. Fluorescent dyes were iontophoretially injected into a single red cell surrounded by green cells. Coupling coefficient (cells touching the injected cell/touching cells with dye) was determined 3 min after.

3D geometric model of the pore was mathematically modeled combining cylinders, cones and an ellipsoid. Particle position, velocity, acceleration and force vectors were calculated after every time step (10^{-14} s), considering wall-particle and particle-particle elastic interactions, inter-particle electrostatic, Brownian and other forces. Particles' paths were recorded and those crossing the pore were counted. Without electric field, number of particles crossing increased linearly as mouth radius increased. With an electric field, number of particles crossing varied non-linearly with a maximum when radius was around 21.4 Å. **Our computer simulations predict that changes in phosphorylation, voltage or connexin recombination yield to changes in pore structure which in turn affects large molecules' permeability.**

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Diffusion-Sensing versus Quorum Sensing in a Model Biofilm

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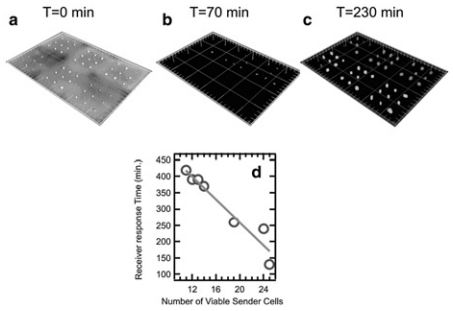
Cell interactions through soluble signaling molecules control differentiation, immune response and other physiologically vital processes in everything from tissue to biofilms. We tested a model of a bacterial biofilm, which uses an autoinducer (AI), N-acyl-L-homoserine lactone (AHL) for signaling, to discriminate quorum-sensing (QS) and diffusion-sensing (DS). The AI induces the transcription of a set of genes that includes the gene-encoding the AI-producing enzyme, promoting a positive feed-back. We creating a synthetic biofilm using a microfluidic network, to convey cells to an assembly area where multiple, time-shared optical tweezers are used to array them. The cells are encapsulated in a 30μm×30μm×45μm volume of hydrogel mimicking an extra-cellular matrix. To extend the size, shape and constituency of the array, we then step to an adjacent location while maintaining registration with the reference array, and repeat the process as illustrated in Figure 1(a). Using this step-and-repeat method, we formed arrays of *E. coli* engineered to produce AHL, which is functionally linked to a fluorescence reporter. As shown in Fig. 1(d), the threshold to induce AI production and fluorescence depends on the number of cells and the mass-transfer, indicating that QS is a side effect of DS.



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Identification of Transmembrane Helix 2 (M2) as the Main Pore-Lining Helix of Connexin 43 Gap-Junctional Hemichannels (GJH)

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GJH are connexin hexamers that dock head-to-head to form gap-junctional channels. Current GJH models are based on low-resolution structural data showing 4 transmembrane α helices per connexin monomer, with insufficient detail for helix assignment; helices have been named A, B, C and D, with B and C as pore-lining. Here, we used luminescence resonance energy transfer to calculate distances between same-position residues in diametrically-opposed monomers of purified GJH formed by functional single-cysteine Cx43 mutants. Mutants were labeled with one donor (chelated Tb^{3+})-labeled and one or more fluorescent acceptor-labeled monomers, and distances between donor-acceptor probes were calculated from sensitized acceptor emission lifetimes. The distances allowed for the assignment of M2 (~45 Å) and M3 (~41 Å) as pore-lining, and M1 (~57 Å) and M4 (~60 Å) as peripheral helices. On the extracellular side (narrower side of the pore), the distances were ~23 and ~40 Å, for M2 and M3 residues, respectively. The shorter M2 extracellular-side distance is evidence of tilting and suggests that M2 is helix C, the primary pore-lining helix. Single-Cys mutants of M1, M2 and M4 (8 per helix) were labeled with BADAN, a probe that displays emission maxima at longer wavelengths in hydrophilic environments. BADAN emission peaked at $\geq$ 480 nm in five of the M2 residues studied, none of the M1 and only one M4 residue position (probably exposed to the cytoplasm). From these and previous results, we built a new GJH model with the following helix assignment: M1 = A, M2 = C, M3 = B and M4 = D. This work was supported in part by NIH grants DC007150, GM068586 and GM79629, American Heart Association, Texas Affiliate grant 0755002Y, and Texas Advanced Research Program grant 010674-0046-2007.

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Bridging Cadherin-mediated Cell Adhesion to Multicellular Pattern Formation by Multiscale Modeling and Simulation

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The difficulty to study the cadherin-mediated cell adhesion lies in the complexity from cadherin binding specificity to multi-cellular pattern formation. Cadherins are the main adhesion molecules on the cell membrane to hold cells together. They play important roles in many biological processes, such as cell sorting during embryonic development, acting as tumor-suppressors and also mediating cell signaling. Cadherin binding specificities have been shown to be primarily homophilic, but can be more promiscuous. On the other hand, cell-cell adhesion specificity is not simply correlated with molecular-binding specificity within the cadherin family. It has been suggested that cellular binding specificity also arises from differences in overall cadherin cell surface concentration. In order to understand how these different factors lead to various multi-cellular pattern formation results, we present a computational strategy to model the relation between cadherin binding and cell adhesion in multiscale. Three levels of simulation schemes in different resolutions are constructed to model the multiple cellular system, cell membrane interface and cadherin molecular binding, respectively. Results generated from higher level of simulation are used as input parameters for the simulation in lower level, which combine different modeling into a comprehensive and hierarchic computational strategy. Results from different experimental methods can further be