3D SURFACE TOPOGRAPGHY, LIGAND-INDUCED CONFORMATIONAL CHANGES AND AGGREGATION OF HEMICHANNELS: AFM STUDY OF RECONSTITUTED CONNEXONS

Julian Thimm, Hai Lin, Ratnesh Lal; University of California, Neuroscience Research Institute, Santa Barbara, California 93106

Hemichannels (connexons) from the opposing cells' plasma membranes form gap junctions. Non-junctional hemichannels modulate extracellular calcium($[Ca]_o$)-dependent cell volume. Each hemichannel consists of six connexins, each connexin has 4 trans-membrane domains. Molecular 3D architecture, especially of the extracellular region, their real-time conformations in response to external perturbants (e.g., $[Ca]_o$, free radicals), and the mechanism of connexon aggregation into gap junctions are poorly understood. We examined the surface topography of Cx43-connexons reconstituted in planar lipid membrane under physiological conditions using atomic force microscopy (AFM). At lower concentrations, individual and small patches of connexons are present. At higher concentrations, they form hexagonally-ordered clusters resembling in vivo gap junctions. High-resolution images of individual connexons show an outer diameter of 7-10 nm, a central pore of 1-1.5 nm, and extracellular subunit protrusions of 0.5-2 nm. Topographical features of individual connexins are consistent with the 4 trans-membrane domains. Structural conformations of both single connexons as well as the clusters of connexons are altered by $[Ca]_o$ and other divalent cations. Supported by NIH(GM-NIA) and Philip-Morris External Grant Program.