

**CONFORMATIONAL DYNAMICS IN CONNEXON STRUCTURE AT MOLECULAR RESOLUTION USING COORDINATED ATOMIC FORCE (AFM) AND ELECTRON MICROSCOPY (EM)**

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We have isolated large, crystalline Cx26 gap junctions (GJs) from stably transfected HeLa cells that are recognizable with EM and AFM. While EM reveals internal structure at molecular resolution, AFM images provide a surface relief of macromolecules at molecular resolution ( $< 10 \text{ \AA}$  lateral,  $< 2 \text{ \AA}$  vertical resolution). EM studies are limited to static structures however; AFM can image samples under aqueous conditions and image conformational changes in time lapse on the same sample. Our AFM images show exquisite detail at the extracellular surface of force dissected GJs. The cytoplasmic domains are very flexible and reversibly collapse onto the surface thereby forming a superstructure. In both conformations, the cytoplasmic surface exhibits a pore opening wider than observed for the extracellular surface. This is the first time that the cytoplasmic pore has been seen by AFM. It is due to increased force sensitivity, resolution, optimization of imaging conditions, and the small cytoplasmic domain of Cx26. AFM topographs of the rigid extracellular surface confirm the hexagonality of connexons even in the "raw" images. We find an  $\sim 9 \text{ \AA}$  decrease in extracellular pore diameter during incubation with  $\text{Ca}^{2+}$ . A different effect due to  $\text{Ca}^{2+}$  occurs at the cytoplasmic surface.