

AFM image of inositol 1,4,5-trisphosphate receptor (IP3R) tetramer structure in membrane

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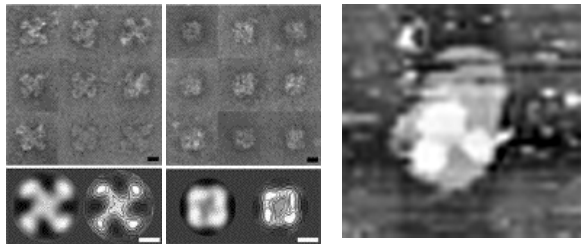
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The inositol 1,4,5-trisphosphate receptor (IP3R) is a ligand-gated ion channel, the opening of which is controlled by both IP₃ and Ca²⁺. This dual regulation is important in allowing IP3R to generate spatially and temporally complex intracellular Ca²⁺ signals. The channel structure exhibits the expected 4-fold symmetry and comprises two morphologically distinct regions: a large pinwheel and a smaller square [1]. The pinwheel region has four radial curved spokes interconnected by a central core. The IP₃-binding core domain has been localized within each spoke of the pinwheel region by fitting its x-ray structure into the reconstruction [2]. We purified



the membrane fraction from IP3R transfected cell lines. Biological samples were adsorbed to freshly cleaved mica. Contact mode AFM topography were recorded at room temperature after adjusting the electrolyte concentration of the buffer to allow

electrostatically balanced high-resolution imaging at a stylus loading force of <100 pN. The applied force was corrected manually to compensate for the thermal drift of the microscope. The AFM used was a SPI3800N equipped with a J-piezoscanner (scan size 0.02 mm) and a fluid cell. Calibration of the scanner in z-direction was performed on defects (large holes or steps) observed on layered solid-state crystals as described. The probe of cantilevers SN-AF01 was used, its nominal spring constant was 0.08 and 0.02 N/m.

[1] Hamada, K. Terauchi, A. Mikoshiba, K. “Three-dimensional rearrangements within inositol 1,4,5-trisphosphate receptor by calcium”, *J Biol Chem*, 278, 52881-9 (2003)

[2] Bosanac, I. Alattia, J. R. Mal, T. K. et al. “Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand”, *Nature*, 420, 696-700 (2002)