Our laboratory is interested in the supramolecular organization, motional dynamics and trafficking of neuronal and muscle-type nicotinic acetylcholine receptors (nAChRs). We study these neurotransmitter receptor proteins using a combination of ensemble averaging methods and single-molecule experimental techniques. STED and STORM/GSDIM superresolution microscopies (two forms of nanoscopy) have been applied to fixed and live specimens of a clonal cell line expressing muscle-type nAChR and to hippocampal neurons in culture. In the former case, STED disclosed ~55 nm cell-surface particles ("nano-clusters") which increase in size upon antibody ligation. Binding of the competitive antagonist α-bungarotoxin, or a monoclonal antibody against the receptor α-subunit, induces a clathrin- and dynamin-independent internalization of AChRs via surface-connected tubular compartments, eventually reaching lysosomes for degradation. Cholesterol levels modulate this process: under cholesterol deprivation the cell re-routes nAChRs to a ligand-independent, Arf-6 endocytic pathway. Cholesterol also affects the surface architecture and dynamics of the AChR assemblies, as revealed by other superresolution microscopies, and single-particle tracking (SPT) discloses the 2-D motional dynamics of the nAChR nanoclusters. In conclusion, the neutral lipid cholesterol modulates the supramolecular organization and dynamics of nAChR at the cell surface.

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