

Fluorescence Microscopy of Nicotinic
Acetylcholine Receptors

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ABSTRACT

Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand gated ion channels abundantly expressed in the central nervous system. Changes in the assembly and trafficking of nAChRs are pertinent to disease states including nicotine dependence, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and Parkinson's disease (PD). Here we investigate the application of high resolution fluorescence techniques for the study of nAChR assembly and trafficking. We also describe the construction and validation of a fluorescent $\alpha 5$ subunit and subsequent experiments to elucidate the cellular mechanisms through which $\alpha 5$ subunits are expressed, assembled into mature receptors, and trafficked to the cell surface. The effects of a known single nucleotide polymorphism, D398N, in the intracellular loop of $\alpha 5$ are also examined

Additionally, this report describes the development of a combined total internal reflection fluorescence (TIRF) and lifetime imaging (FLIM) technique and the first application of this methodology for elucidation of stoichiometric composition of nAChRs. Many distinct subunit combinations can form functional receptors. Receptor composition and stoichiometry confers unique biophysical and pharmacological properties to each receptor sub-type. Understanding the nature of assembly and expression of each receptor subtype yields important information about the molecular processes that may underlie the mechanisms through which nAChR contribute to disease and addiction states.

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