

Fluorescence Microscopy of Nicotinic
Acetylcholine Receptors

Thesis by
Crystal N. Dilworth

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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.

TABLE OF CONTENTS

Acknowledgements	iii
Abstract.....	iv
Table of Contents	v
Introduction	1
Chapter I: Construction of a Fluorescent $\alpha 5$ Subunit	4
Chapter II: Investigation into miR-346 Regulation of the nAChR $\alpha 5$ Subunit	15
Chapter III: Expression of $\alpha 5$ -mEGFP in Mouse Cortical Neurons	22
Chapter IV: Determination of nAChR stoichiometry using NFRET	31
Chapter V: TIRF-FLIM-FRET: Engineering a Technique for High Resolution Detection of nAChR of nAChR Composition and Stoichiometry.....	43
Chapter IV: Conclusions	57
Appendix i: Plasmid maps.....	61
PCR Protocols	62
Appendix ii: Transfection Protocols	63
N2a Cells for Fluorescence Imaging (Expressfект)	63
N2a Cells for Differentiation and HEK293-T (Lipofectamine 2000)....	64
Neuronal Transfection Non-optimized	65
Neuronal Transfection Optimized.....	66
Maintenance of Neuronal Cultures	67
Imaging Settings	69
Appendix iii: Image work-up for NFRET Experiments.....	70
Statistical Analysis: t-test $\alpha 4$ vs. $\alpha 4\beta 2$ Total NFRET Pixels.....	72
Statistical Analysis: t-test $\alpha 5\alpha 4$ vs. $\alpha 5\alpha 4\beta 2$ Total NFRET Pixels	73
Statistical Analysis: t-test $\alpha 5\alpha 4$ vs. $\alpha 5\alpha 4\beta 2$ Mean Cell Values	74
Bibliography	75