Optimization of the GluCl/IVM Neuronal Silencing Tool via Protein Engineering

Thesis by

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In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2012

(Defended June 6, 2012)

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The following work is dedicated to Professor JM Tomich.

Thank you for my foundation.

Thank you for my fortitude.

Thank you for my future.

ACKNOWLEDGEMENTS

I would like to thank my Ph.D. advisor, Professor Henry Lester, for his support throughout this endeavor and co-advisor, Professor Dennis Dougherty, for his genuine interest in seeing me succeed. Thanks to my additional committee members, Professor David Anderson and Professor Shu-ou Shan, for perceptive scientific discussions. I would especially like to thank my first-year post-doc mentor, Dr. Fraser Moss, for providing me with extensive electrophysiology training and sage advice about everything from graduate school to life in general. And, I would like to thank Dr. Bruce Cohen for his electrophysiology expertise and worthwhile mentorship, as well as his tolerant company and daily chatter about food and travel. I would also like to acknowledge Dr. Chris Richards and Weston Nichols for their patience and friendly assistance with imaging experiments. Thanks to lab manager Purnima Deshpande for the supply ordering and equipment maintenance that kept my experiments going, to Sheri McKinney for providing my weekly neuronal cultures, and to other current and former Lester lab members for their constructive criticisms and lively conference room conversations. To our wonderful 'lab Momma' Eloisa Imel, thank you for fostering an enjoyable work environment and for your obliging resourcefulness with day-to-day tasks and formalities, but more importantly for being a great friend, keeping me encouraged, well-fed and caffeinated. Finally, I would like to give special thanks to fellow lab members Dr. Ying Wang, Crystal Dilworth, and Rell Parker for their friendship, tremendous support and counsel through the rollercoaster ride we call graduate school.

ABSTRACT

A variety of genetically encoded tools have been developed for deciphering the neural circuitry of the brain. Such tools allow physical manipulation of neuronal excitability in a reversible, cell-specific manner, enabling researchers to establish how electrical activity and connectivity facilitate the information processing that mediates perception and drives behavior. An expanding toolkit of engineered neuroreceptors, particularly those actuated by orthogonal pharmacological ligands, provide noninvasive manipulation of regional or disperse neuronal populations with adequate spatiotemporal precision and great potential for multiplexing. We previously engineered an invertebrate glutamate-gated chloride channel (GluCl αβ) that enabled pharmacologically induced silencing of electrical activity in targeted CNS neurons *in vivo* by the anthelmintic drug compound ivermectin (IVM; Lerchner et al., 2007). With this receptor, GluCl opt α-CFP + opt β-YFP Y182F, the concentration of IVM necessary to elicit a consistent silencing phenotype was higher than expected, raising concern about its potential side effects. Considerable variability in the extent of spike suppression was also apparent and was attributed to variable co-expression levels of α and β subunits. Thus, a rational protein engineering strategy was employed to optimize the GluCl/IVM tool. To increase agonist sensitivity, a gain-of-function gating mutation involving the highly conserved leucine 9’ residue of the α pore-lining M2 transmembrane domain was introduced. Various mutations at this position facilitate channel opening in the absence and presence of ligand. Analysis of side chain properties revealed that helix-destabilizing energy correlated with increases in agonist sensitivity. One mutation, L9’F, enhances β subunit incorporation to substantially

increase IVM sensitivity without permitting unliganded channel opening. Removal of an arginine-based ER retention motif (RSR_AAA) from the intracellular loop of β promoted plasma membrane expression of heteromeric GluCl αβ by preventing ER-associated degradation of the β subunit. An additional monomeric XFP mutation complements these effects. The newly engineered GluCl opt α-mXFP L9'F + opt β-mXFP Y182F RSR_AAA receptor significantly increases conductance and reduces variability in evoked spike generation *in vitro* using a lower concentration of IVM. This receptor, dubbed 'GluClv2.0', is an improved tool for IVM-induced silencing.

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