Graduate Research Fellowship Project – Research Plan

Research Goals and Scientific Impacts:

Studying the structures and dynamics of Transient Receptor of Potential (TRP) ion channel complexes is important for understanding the relationship between physical, biological and chemical systems[1, 2]. The TRP ion channels are transmembrane proteins which are expressed throughout metazoan organisms, and are considered to be major players in cell signaling. There are 27 channels from the TRP super-family expressed in humans and they are gated (activated) by various different environmental stimuli[2].Whilst the structures of some TRP channels are known[3, 4], there is much to be studied regarding the interactions and dynamics between ion channels and ion channel modulating proteins. For my graduate research project, I will use solution nuclear magnetic resonance (NMR) spectroscopy to probe the structure of Transmembrane Protein 100 (TMEM100), a recently discovered modulator of TRP channels[9]. NMR will also be used to explore the effects of TMEM100 on TRP ion channel activity.

TRPV1, a human hot temperature sensor[5], is also gated by small molecules like capsaicin, pH, voltage[6] and other proteins[7]. A protein which has been shown to modulate the channel is the Phosphoinositide regulator of TRP channel (Pirt)[7, 8], a two-span transmembrane protein with a 21.7% sequence identity with TMEM100. Mice model studies have shown that TMEM100 interacts with a TRPV1-TRPA1 complex by directly modulating the activity of TRPA1[9] in vivo. Using an in vitro approach, NMR can reveal specific amino acids important in the molecular interactions. Changes in chemical shifts ($\Delta\delta$) can offer insight into thermodynamic information such as binding constants (k_D), enthalpies (ΔH) and Gibbs free energy (ΔG). The effects of TMEM100 on TRPV1 and TRPA1 (or other TRP channels) have not yet been studied using NMR spectroscopy and offer insight into the structure and thermodynamics of a biologically relevant molecular system. I hypothesize that the NMR analysis of TMEM100 will elucidate key structural features which reduce the activity of human TRPV1 and TRPA1.

Methods:

Isotopically labeled (^{15}N, ^{13}C) Human TMEM100 will be expressed in *E. coli* bacteria and purified using Nickel (Ni^{2+}-NTA) affinity, cation exchange (HiTrap™) and size exclusion (Superdex™) chromatography. The protein will then be screened using an NMR spectrometer with an experiments called Transverse Relaxation Optimization spectroscopy (TROSy) Heteronuclear Single Quantum Coherence (HSQC) for suitable hydrophobic environments such as micelles, bicelles, nanodiscs, reverse micelles for structural studies. In a suitable environment, TMEM100 will be probed for structural information with TROSy HSQC, sidechain, backbone and Nuclear Overhauser Effect spectroscopy (NOESy) NMR experiments.

Starting with human TRPV1, TRPA1 and TRPM8 will be expressed and purified using similar methods as those used for TMEM100. Proteins will also be verified using LC-MS/MS and western blot. TRPV1 and TMEM100 will be co-inserted into hydrophobic membrane mimics and probed using NMR for structural and thermodynamic features important in the protein interactions and the function of the channel. This will be done with amino acid mutations, and ligand and temperature titrations. This process will be repeated for with TRPA1.

Broader Impacts of the Proposed Work:

TRP channel studies are at the very root of what makes humans able to sense stimuli[2], and the phenomenon of sensation can be observed by youth. Making science an approachable subject for young students of all demographics is an important step to encouraging careers in STEM. My research of TRP channels will pave the way for new types of biochemical demonstrations for children by showing them that the human nervous system can react to physical and chemical changes in the environment. This will intertwine complex biochemical and physical concepts in a system which will be simplified for the students.

Exposure to the TRP agonists is inclusive to certain food products (wasabi[10]), chewing gums (menthol[11], cinnamaldehyde[10]) and riot control gases (pepper spray[6], tear gas[12]) and can be found throughout the world. Studies of this sort will contribute to the better use of the current TRP channel agonists in consumer and industrial products. The studies will also give insight towards the design of novel TRP channel agonists and antagonists which can be used with more targeted effects.

I, [redacted] certify that the above Graduate Research Plan is my own original work.

References and Works Cited:

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. [redacted]
7. [redacted]
8. [redacted]
9. [redacted]
10. [redacted]
11. [redacted]
12. [redacted]