

## PROJECT SUMMARY (See instructions):

Synapses are integrated units that exhibit precise molecular organization leading to efficient and reliable synaptic transmission while the same time accommodating processes such as short- and long-term plasticity. Importantly, imbalances in synaptic function are postulated to underlie a variety of synaptic pathologies, including neuropsychiatric / developmental disorders like schizophrenia and autism. The protein machinery at synapses has been reasonably well studied and while gaps remain, the basic proteome of the synapse is largely established. What is entirely missing is an understanding of the lipid composition of synapses, how this composition is established and regulated, how it influences protein function, and whether lipid disturbances lead to synaptic dysfunction. Our team is in a unique position to begin filling this gap in the understanding of protein/lipid interactions in synaptic function and have generated novel insights that allow us to explore this complex problem. The anatomical structure responsible for organizing receptors and signaling molecules on the postsynaptic side of excitatory synapses is called the postsynaptic density (PSD). The PSD has a fundamental role in maintaining the structural/functional stability of the synapse while serving the demands of synaptic plasticity, most notably in the activity-dependent recruitment of AMPA-type glutamate receptors. However, there remains no consensus on the molecular mechanisms mediating this recruitment. Moreover, despite its physiological importance, the mechanisms of assembly and organization of the PSD are unknown, nor are the consequences of PSD interactions with the post-synaptic plasma membrane. We have discovered a role for the PSD in organizing the lipid composition of the overlying synaptic plasma membrane (PSD-PM). Lipidomic analysis of synaptic membranes and the PSD-PM revealed dramatic compositional remodeling during early post-natal development, with both becoming enriched in lipids associated with membrane rafts. These results were confirmed by electron cryotomography. Lipid rearrangements were concomitant with enhanced expression and post-translational lipidation of the key membrane associating structural scaffold PSD-95, and with structural maturation of the PSD. ***We hypothesize that the PSD recruits specific membrane lipids that facilitate the formation of membrane microdomains, which in turn recruit membrane proteins modified by palmitoylation. We propose that palmitoylation levels of signal transduction proteins are regulated by synaptic stimulation, comprising a positive feedback between synapse stimulation and synaptic remodeling, leading to reinforcement of the synaptic response and signal potentiation.*** In **Aim 1**, we will quantify the detailed, holistic lipid composition of the PSD-associated PM, and determine whether it facilitates membrane domain formation. By reconstitution of synaptic membranes, we will assay how isolated PSDs organize the lipid-driven membrane domains. In **Aim 2**, we will determine if proteins are selectively recruited to the PSD-associated plasma membrane by the dynamic regulation of palmitoylation levels by pharmacological and chemical interference with palmitoylation both in hippocampal slices and in reconstituted model membranes. Further, we will quantify the effect of synaptic stimulation on the palmitoylation levels of both PSD scaffold proteins (PSD-95) and neuronal signal transducers (GluR1 and NR2a) to determine whether palmitoylation levels determine synaptic recruitment. Overall, our studies will provide a blueprint to understand how modifications of the PSD and the post-synaptic membrane can support structural plasticity and provide mechanistic insights into how protein modifications in the PSD regulate synaptic localization and function. Our long-term goal is to establish an integrated view of how proteins and lipids in the synaptic membrane are coordinated to support synaptic function and plasticity.

## RELEVANCE (See instructions):

This project is ideally suited for support under the UT Brain seed grant program that has as its goal to foster new collaborations in the fields of neuroscience and neurotechnology with investigators of diverse disciplines. Ours is a unique, synergistic collaboration, capitalizing on Dr. Levental's extensive experience in lipid biophysics and membrane biology and Dr. Waxham's expertise in the molecular mechanisms of neuronal signal transduction and synapse development. The combined expertise of the two laboratories has enormous potential to contribute novel understanding to the function of synaptic membranes by employing state-of-the-art technology. Detailed characterization of the lipid composition and lateral organization of the synaptic membrane, and how these are regulated by developmental and synaptic stimulation, has the potential to revolutionize our understanding of synaptic function and provide novel potential insights into how to treat, cure and prevent brain disorders. The timing of this initiative is ideal to launch our efforts. By borrowing resources from other projects, we have established initial feasibility and generated preliminary results that seed the aims described in the present proposal. The only issue slowing progress at the moment is limited resources (personnel and supplies). With one year of funding from the UT Brain seed grant program, we will be in an ideal position to secure long-term NIH funding for this project.