A. ABSTRACT

Neurons possess unique structural attributes that underlie their ability to respond to sensory stimuli, propagate electrical potentials across long distances, and transmit information through synaptic transmission. This functional diversity is enabled by the action of voltage and ligand-gated channels, trophic receptors, second messenger proteins, and transcription factors that exhibit precise temporal and spatial regulation. There are currently 1,194 FDA-approved small molecules and biologics that target 667 proteins. While 4,400 of the over 22,000 proteins expressed from the human genome exhibit druggable properties [1], most remain unexplored. Aside from critical housekeeping functions, the endoplasmic reticulum (ER) functions as both a sensor and an effector in the neuronal response to ischemic stress. Regulated subcellular trafficking of transcription factors, kinases, and other factors is a tactic neurons use to couple adaptive or maladaptive responses to oxidant stress [2]. We hypothesize that *live-cell localization proteomics can be used to identify relevant therapeutic targets activated by ischemia.* To test this, we mine the stress response proteome for tractable targets in neural cell lines and neural stem cultures using transposon-mediated random GFP-tagging and localization proteomics.

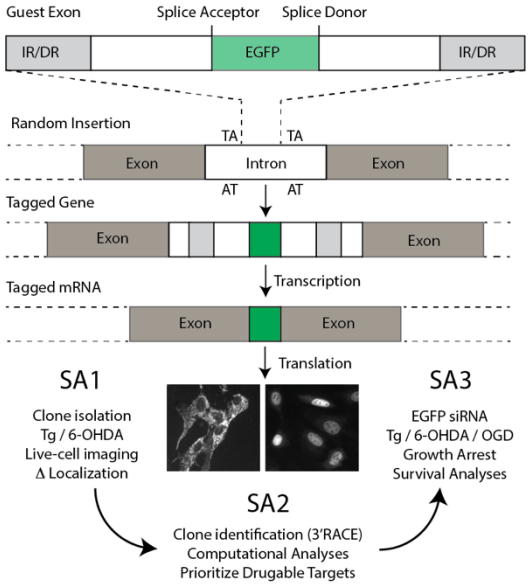

Figure 1. Overview of the EGFP-tagging strategy. The Sleeping Beauty system will insert the EGFP guest exon within TATA genomic sites. EGFP is incorporated into the endogenous mRNA and stable clones are isolated and studied using localization proteomics in response to oxidant stress to discern gene function.

SA1. Curate proteins exhibiting ischemia and ER stress-induced spatial redistribution. Preliminary studies indicate that in vitro localization proteomics effectively identifies physiologically responsive proteins in living cells. Using the Sleeping Beauty (SB) transposon system, we randomly tag single genes with an SD-EGFP-SA guest exon. Following FACS and clonal expansion, mirror cell arrays are exposed to thapsigargin (Tg), 6-hydroxy-dopamine (6-OHDA), or oxygen-glucose deprivation (OGD), to mimic ER, oxidant stress, and ischemia, respectively. We then utilize novel high-content imaging approaches and live-cell fluorescence imaging to identify proteins exhibiting spatial redistribution.

SA2. Define the set of priority FP-tagged proteins with druggable potential. We hypothesize that EGFP-tagged proteins exhibiting spatial redistribution function as regulators and reporters of cellular oxidant injury. cDNAs encoding these proteins will be isolated and sequenced by 3'RACE to determine protein identity. Using protein-protein and ligand-protein modeling tools developed by the Kozakov Lab, we will determine the subset of druggable targets. We will also conduct secondary screens to establish the subset of proteins that modulate stress-induced growth and survival in response to chemical oxidants or OGD. siRNA-dependent knockdown targeting either the EGFP tag or gene-specific mRNA sequences will test for the effect of haploinsufficiency or global gene knockdown on cell survival, respectively.

This project exceeds the capabilities of existing standard 'omics' approaches for drug target discovery, introducing a set of robust biological outcome measures to curate high-priority candidates. These experiments will create a functional map of the stress-induced ER proteome, facilitate the selection of druggable targets, and generate a repository of clonal reporter lines and online data resources for the neuroscience community.