DESIGN AND CHARACTERIZATION OF CIRCULARLY PERMUTED CASPASE-2 MUTANTS AND THEIR USE IN EVALUATION OF NOVEL CASPASE-2 INHIBITORS

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The exact cause of Alzheimer's disease (AD) has yet to be completely described despite the disease being defined over 100 years ago. A potential approach to better understand the pathogenesis of AD could be the development of selective caspase-2 (Casp2) probes, as we have shown that a Casp2-mediated cleavage product of tau (Δtau314) reversibly impairs cognitive and synaptic function in animal models of tauopathies. We have taken a multi-pronged approach to studying this target and are currently developing peptide inhibitors as well as characterizing both electrophilic and non-covalent fragments. Due to limitations with Casp2 protein production, we have expressed and characterized a recently published circularly permuted Casp2 (cpCasp2) to use as a surrogate for the wild type protein. cpCasp2 is both enzymatically and structurally similar to Casp2, but cpCasp2 does not appear to be conducive to the crystallographic studies needed to support our medicinal chemistry endeavors. The design of cpCasp2 involves linking loop 2 (L2) and L2', the N- and C-terminus, of Casp2 with a GS moiety, creating L2 of cpCasp2. This loop is not well ordered and appears to create challenges for crystal growth, stability, and data resolution. We have therefore designed six L2 mutants of cpCasp2 with the goal of eliminating structural clashes we have observed in its crystal packing. We expect that these mutations will stabilize crystal growth while preserving the enzymatic activity profile. Work from our recent publications and a closer look at the structural biology data generated to date on this project will be presented.