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Isolating Different Pools of Tau in the Brain

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Isolating Different Pools of Tau in the Brain

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Alzheimer disease (AD) is a debilitating neurodegenerative disease. One of the proteins implicated in this disease is Tau, a microtubule associated protein. It normally binds to and stabilizes the microtubule but, in AD, it becomes hyper-phosphorylated, breaks off from the microtubule, and forms aggregates. While the general mechanism is known, it has not been quantitatively analyzed. Using a tandem Liquid Chromatography/ Mass Spectrometry procedure, along with the highly sensitive MS instruments, the phosphorylation of Tau can be more thoroughly quantified. This is done by comparing the ratio of signal strengths of phosphorylated residues to non-phosphorylated residues of Tau, giving us a phosphorylation probability for each residue. In the brain, Tau can be separated into two pools: Non-aggregate and Aggregate. Thus, by comparing phosphorylation ratios, different quantitative profiles of the different pools of Tau can be established. In order to obtain an accurate quantitation, it is necessary to find a procedure to separate the pools of Tau in the brain; that is the goal of this portion of the project. In particular, this project seeks to find the optimal method for separating Aggregate Tau from Non-Aggregate Tau. This relies on the fact that the different pools of Tau have enough of a difference in solubility in certain reagents that, under ultracentrifugation, aggregate Tau may successfully pellet from the *solution.* Based on protocols in the literature, we tested a series of different protocols using an MES buffer or the detergent Sarkosyl to separate the dissociate and aggregate Tau. The aggregate Tau is then re-solubilized and purified through a second series of buffers and centrifugation steps. Finally, Tau is Immuno-precipitated, trypsinized, and run through an LC/MS procedure. The signal strengths of the peptides and the differences in phosphorylation ratios are used to compare protocols. From this, a quantitative profile of Tau can be established.