# Investigating the Structural Complexity of the UCH Knot Protein using Trapped Ion Mobility Spectrometry-Mass Spectrometry

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Knot proteins are rare proteins whose backbones adopt knotted configurations with a wide range of topologically interesting features [Lim, 2015]. They were regarded as structural impossibilities, until they were observed embedded in naturally-occurring proteins in the early 2000s [Taylor, 2000; Lim, 2003]. Ubiquitin carboxyl-terminal hydrolase, or UCH, is one of the most complex knot proteins studied to-date. UCH contains a knot structure composed of 5 crossings around the polypeptide chain ($5_2$ knot). Humans have four UCHs: UCH-L1, UCH-L3, UCH-L5 and BAP1. The UCH-L1 in particular is found in abundance in the nerve cells of the brain and is thought to be involved in the degradation of proteins. Mutations of UCH-L1 have been implicated in Parkinson's disease, a neurological condition involving poor movement and balance [Virnau, 2011]. UCH contains approximately 230 amino acids, which forms a pretzel-shaped (Gordian) knot with a core spanning from amino acid number 10 to 216 (meaning only 10 amino acids from the N-terminus must be cleaved for the knot to completely unravel). Whereas most experimental studies on knot proteins use chemical agents for following the overall in-solution denaturing and refolding by e.g. fluorescence or circular dichroism, the aim of the present study is to analyze the kinetically-trapped conformations of UCH in its different native and non-native states in one experiment. We use trapped ion mobility spectrometry-mass spectrometry (TIMS-MS) equipped with a nano-electrospray source to investigate the structural stability of UCH and the changes in its conformations according to the charge state while varying the solution conditions. The unfolding intermediates and the conformational space of UCH are probed as a function of the organic solvent content (methanol, acetonitrile) and using collisional gas-phase activation in front of the ion mobility measurement.