Characterization of the substrate recognition and subunit assembly properties of homo-oligomeric human CCT subunits of TRiC. Oksana A. Sergeeva, Cameron Haase-Pettingell, Jonathan A. King. Department of Biology, Massachusetts Institute of Technology. jaking@mit.edu

Chaperonins are a family of chaperones that encapsulate their substrates and assist their folding in an ATP-dependent manner. The eukaryotic chaperonin, TCP-1 Ring Complex (TRiC) is a hetero-oligomeric complex composed of eight different Chaperonin Containing TCP-1 (CCT) subunits that is needed to fold actin and tubulin in the cell. Each CCT subunit may have distinct substrate recognition properties, and mutations in CCT4 (C450Y) and CCT5 (H147R) have been identified as causing hereditary sensory neuropathies. Our aim was to express each human CCT subunit individually in *E. coli* to investigate the specificity and redundancy of each subunit in a chaperonin context.

We found that both CCT4 and CCT5, but not the other six CCT subunits, have the property of forming functional eight-fold double rings absent the other subunits. Using these homo-oligomeric chaperonins and human TRiC as a control, we are studying the recognition and refolding of more stringent substrates such as mutant huntingtin, tubulin, and actin. To understand the regulation of TRiC assembly, we are assaying the formation of hetero-oligomeric rings with all eight CCT subunits and either CCT4 or CCT5. We are also studying the structure and function of the neuropathy mutations of CCT4 and CCT5 by purifying these mutants and assaying their structure and function *in vitro*, and making these mutations in *Saccharomyces cerevisiae.*