Purification and Properties of Homo-Oligomeric Human CCT subunits
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Numerous proteins, including essential proteins such as tubulin and actin, are unable to fold to their native state in the cell without assistance from chaperones. 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. Each CCT subunit may have distinct substrate recognition and ATP-binding properties. Additionally, mutations in CCT4 (C450Y) and CCT5 (H147R) have been identified as causing hereditary sensory neuropathies in a stock of Sprague-Dawley rats and in a Moroccan family, respectively. Our aim was to express each human CCT subunit individually in *E. coli* to investigate whether they form chaperonin-like double rings complexes. This gives us the opportunity to study the specificity and redundancy of each CCT subunit in a chaperonin context.

We have expressed and purified both human CCT4 and CCT5. They form two back-to-back rings of eight subunits each as seen by negative stain and cryo electron microscopy. This morphology is consistent with that of the hetero-oligomeric TRiC. 3D reconstructions of CCT5 in various ATP states are ongoing. Both CCT4 and CCT5 are active as assayed by the luciferase refolding assay and the human gammaD crystallin aggregation suppression assay. This activity is ATP-dependent, consistent with what is known of TRiC refolding activity. We are continuing our efforts to purify the other six CCT subunits to investigate their morphologies and activities, in addition to studying the structure and function of the neuropathy mutations of CCT4 and CCT5.