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Measurement of coiled-coil stability by fluorescence resonance energy transfer JAMES PINO, BOMI KIM, MANUEL SANTIAGO, STEFANIE WHITSON, KRISTIN WHITSON, Departments of Chemistry and Physics, University of Tennessee at Chattanooga — Alpha helical coiled-coils are common structural motifs by which proteins assemble into larger complexes. The leucine zipper-like oligomerization domain of heterogeneous nuclear ribonucleoprotein C (CLZ) assembles into an anti-parallel tetramer with a coiled-coil fold. Fluorescently-tagged CLZ peptides were designed and characterized as appropriate to perform measurements using fluorescence resonance energy transfer (FRET) to study the overall stability of the assembly. Experiments revealed that the appearance of FRET in a sample was time dependent and thus reflective of the kinetics of the tetramer's formation. Furthermore, FRET assays using varying concentrations of labeled peptides have allowed the first measurement of the equilibrium dissociation constant (K_D) of the CLZ tetramer, which is representative of the strength of specific molecular interactions within the oligomer. The method can be used to study effects of mutations in this coiled coil on the thermodynamic stability of the complex.

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