

Optimized Expression and Temperature-Dependent Structural Dynamics of Recombinant Human Tropoelastin

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Elastin is one of the most hydrophobic proteins known. It is mostly found in tissues requiring elasticity such as skin, lungs and ligaments. The monomeric precursor, tropoelastin, is a ~70KDa intrinsically disordered protein whose hydrophobic residues make up 82% of its primary sequence. Because tropoelastin is quickly crosslinked into insoluble fibers in vivo, isolation of pure native protein is difficult and inefficient. Here, we present a strategy for the expression and purification of gram quantities of recombinant human tropoelastin based on codon optimization and tailored expression conditions. We showed through turbidity assays that tropoelastin can phase separate in vitro in a concentration and salt dependent. We also demonstrate through confocal microscopy that the recombinant protein can coalesce into droplets that mature over time, displaying early stages of material formation. By using Diffusion ordered NMR spectroscopy and Small Angle X-Ray Diffraction, we also show that monomeric human tropoelastin undergoes a temperature dependent compaction before coacervation. Ultimately, we obtained a well resolved 2D NMR spectrum, demonstrating the feasibility of future resonance assignments and thermodynamic characterization.

