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LOWER CRITICAL SOLUTION TEMPERATURE BEHAVIOR OF ELASTIN-COILED-COIL POLYMERS, A POTENTIAL PLATFORM FOR TREATMENT OF NON-HODGKIN'S LYMPHOMA

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Non-Hodgkin's lymphoma takes the lives of 20,000 Americans each year. When traditional therapies fail, clinicians have turned to a drug, rituximab, for treatment. However, rituximab is now curative for less than 50% of patients and new treatments are needed [1]. Drug-free macromolecular therapeutics (DFMTs) are an emerging form of therapeutics that seek to bridge the gap created by clinical drug resistance, specifically for Non-Hodgkin's Lymphoma. DFMTs function by clustering surface cell receptors to elicit a cellular response. Typically in DFMTs, a primary molecule targets cell-bound receptors, and a secondary crosslinker binds to the primary molecule causing supramolecular interactions, clustering cell receptors [2]. I hypothesize that elastin-like polypeptides (ELPs) with appropriate biorecognizable moieties can be used as well-defined polymeric backbones to cluster cell-surface receptors due to their lower critical solution temperature (LCST) behavior and to ultimately push development in non-Hodgkin's Lymphoma treatment. The LCST is the temperature above which the protein aggregates in an aqueous solution [3]. ELP fusion with complementary alpha-helical peptides capable of forming coiled coils (CCE and CCK) will produce novel, bifunctional biomaterials [4]. Four elastin-like fusion proteins were produced recombinantly using E. coli, purified by inverse transition cycling (ITC), and characterized (Figure 1).

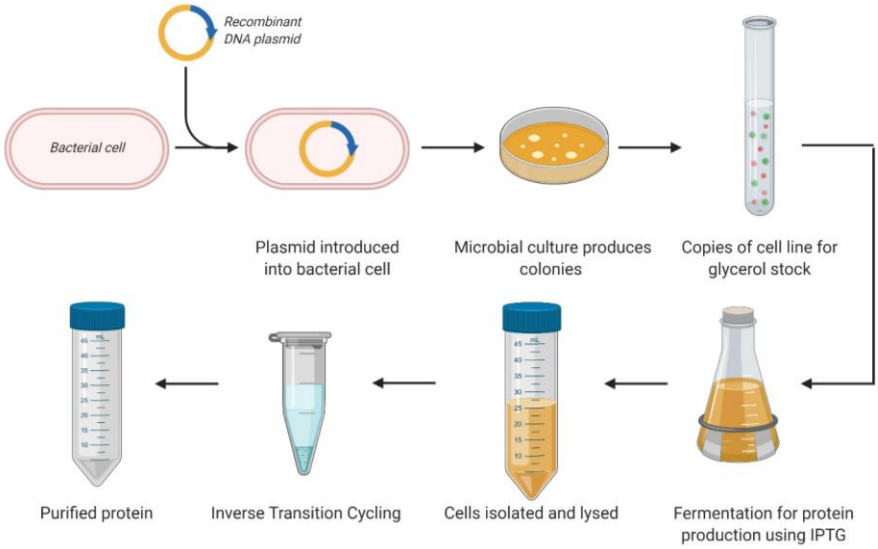

Figure 1 – Workflow for the synthesis, production, and purification of ELP fusion proteins.

The CCE-48ELP, CCK-48ELP, CCE-192ELP, and CCK-192ELP were confirmed via amino acid analysis and gel electrophoresis. The fusion proteins were then analyzed using dynamic light scattering (DLS), turbidimetry, and precipitation assays. Turbidimetry data show that at 10 µM the CCE-48ELP LCST is undetectable below 80°C. CCK-48ELP (10 µM) and mixing of CCE-48ELP/CCK-48ELP (5 µM/5 µM) have LCSTs of 54.8°C and 46.7°C, respectively. The CCE-192ELP (10 µM) has a higher LCST compared to the CCE/CCK-192ELP (5 µM/5 µM) mix at 29.7°C compared to 28.0°C (Figure 2). Additionally, CCE/CCK fusion protein mixtures generate increased aggregate size evidenced by dynamic light scattering by comparison at 23°C, 37°C, and 50°C. The CCE protein subunit also demonstrated capability as a solubility tag for proteins in solution.

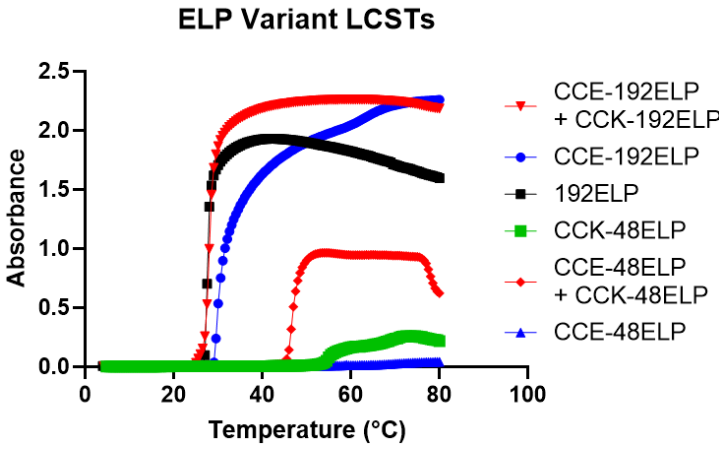

Figure 2 - Fusion protein turbidimetry and LCSTs for 48 and 192 ELP proteins.

With resistance to Non-Hodgkin's lymphoma claiming lives each year, new treatments and therapeutics are needed to better protect patients and improve survivability. Elastin-like polypeptides combined with alpha helical peptides form fusion proteins that show promise as therapeutics due to their biocompatibility and unique physiochemical responses. Characterization and quantification of their properties have shown the potential of these fusion proteins to serve as therapeutics paving the way for further studies into in-vivo efficacy. This study demonstrated the potential for controllability of physicochemical properties based on ELP chain length, concentration, and attached peptides.

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