Phase Behavior of Smart Peptide Polymers Shows Remarkable Tolerance to Structured Domains

Stefan Roberts, Felipe García Quioroz, Ashutosh Chilkoti
Duke University, Durham, NC

Purpose: The development of materials exhibiting conformational changes in response to external stimuli has greatly contributed to innovations in biotechnology and materials. One category of particular interest is that of biopolymers displaying lower critical solution temperature (LCST) phase behavior. These polymers can be tuned to respond to stimuli with sharp changes in solubility and have been utilized for a variety of purposes including nano-architecture control and drug delivery [1,2]. The intrinsic disordered nature of elastin-like polypeptides (ELPs) is known to be of importance for their sharp LCST behavior; however, the structural limit that these biopolymers can withstand without loss of their material properties has not been tested. If high degrees of structure can be tolerated, then researchers can move beyond polymer fusion to active domains and begin to directly encode interesting structured motifs into the material, allowing simultaneous programming of phase behavior and other material or bioactive properties. Using modified polyalanine motifs as helical domains, we show that ELPs can tolerate a remarkably high percentage of embedded structure without loss of material properties. By finely controlling the helical composition and percentage, we can exploit the structured domains to better control nanoparticle self-assembly and can eliminate it to incorporate helical bioactive domains.

Methods: Polyalanine domains $G(A)_{25}$ (Helix 1), $GK(A)_{25}K$ (Helix 2), and $GK(AAAAK)_5$ (Helix 3) were genetically encoded into the ELP domain $(VPGVG)_n$, with final molecular weights of 32-33kDa. Oligonucleotides were polymerized in a modified pet24 vector using Recursive Directional Ligation by Plasmid Reconstruction [3]. The plasmid with polymerized oligonucleotides was then transfected into E. coli and grown overnight in Terrific Broth. The polymers were purified by inverse transition cycling (ITC), a purification method that uses the LCST transition of the polymer. Phase behavior and hysteresis were determined by monitoring the optical density in solution at 350nm as a function of temperature. Structural content of the polymers was evaluated with circular dichroism (CD) over a range of 190-260nm.

Results: ELPs containing 7.25%, 12.5%, 25%, and 50% helical domains were successfully expressed and purified using ITC. Despite the inclusion of such a high degree of order, all of the polymers retained extremely sharp phase behavior. Upon cooling, the polymers display varying degrees of thermal hysteresis (Tt-cooling < Tt-heating). The concentration independence of Tt-cooling indicates that hysteresis is driven by inter-chain hydrogen bonding of the structured domains upon aggregation rather than hydrophobic dissociation. This property is further supported by CD data during phase transition and atomistic molecular modeling. The degree of hysteresis observed is directly correlated with the hydrophobicity of the helices, with polymers containing Helix 3, the most hydrophilic, displaying none. Circular dichroism confirms the presence of a high degree of structure, which scales appropriately with increased incorporation of helical domains. Polymers cooled to between their Tt-heating and Tt-cooling will maintain aggregates for many weeks, with subsequent cooling returning them to their fully solvated state.

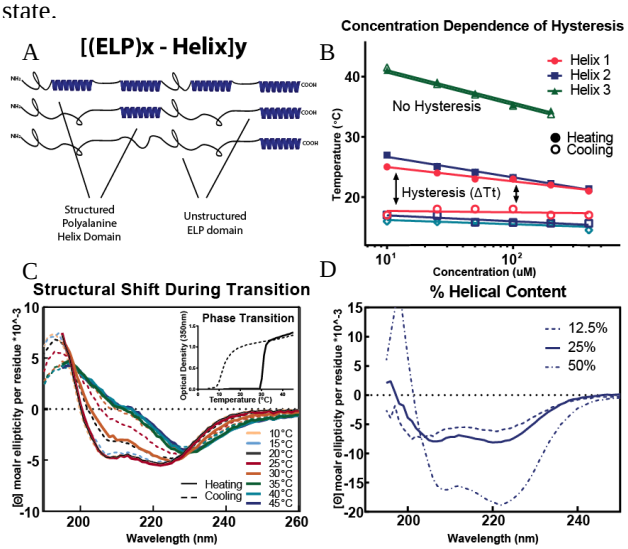

Figure 1: (A) Design scheme for structured biopolymers. (B) Controlling hysteresis with different helix domains and concentration. (C) Relationship of structure of phase behavior for Helix 1 (D) CD of Helix 2 with increasing helical content.

Conclusions: Elastin-like polypeptides depend on their disordered regions for their reversible phase behavior; however, we have shown that structured domains may comprise at least 50% of the polymers length without loss of material properties. In fact, the incorporation of structured domains adds additional variables that can be precisely programmed. Whereas the phase transition upon heating is controlled by the disordered ELP domains, solubilization upon cooling is controlled by the syntax of structured domain. This allows dual tuning of both phase transition temperatures using short motifs and without complete polymer redesign. The high degree of stability and reversibility of the structured domains make them ideal for incorporation into self-assembly nanoparticle systems including micelles with reversible, but exceptionally thermally stable cores. Additionally, hydrophilic helices represent a broad class of bioactive domains. The ability of ELPs to tolerate extensive structural components inspires the design of thermoresponsive biopolymers with embedded bioactive domains, allowing researchers to improve polymer loading efficiencies since they are no longer limited to simple terminal fusions.

References:

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3. McDaniel J. BioMacromol. 2010; 11:944-52