Novel phase transition peptide polymers with LCST and UCST behavior

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Statement of Purpose: Polymers that undergo a pronounced soluble to insoluble phase transition in response to temperature and other stimuli (e.g., pNIPAM) have enabled the development "smart" material systems for biotechnology and medicine. Although this phase transition may occur upon heating above a lower critical solution temperature (LCST) or upon cooling below an upper critical solution temperature (UCST), most applications involve LCST polymers [1], as UCST polymers with tunable phase behavior in physiologically-relevant buffers are rare. Despite the enormity of sequence space encompassed by proteins, examples of phase transition polymers composed of peptide repeats are limited. Elastin-like polypeptides (ELPs) built from repeats of a simple pentapeptide (VPGXG) remain virtually the sole scaffold for the design of LCST peptide polymers [2] and UCST peptide polymers are essentially nonexistent. This talk will show our recent success in uncovering the sequence space of amino acid motifs that upon polymerization exhibit either LCST or UCST phase behavior under physiologically relevant conditions.

Methods: To identify a generalizable, protein-based, unstructured scaffold for the design of LCST and UCST peptide polymers, we implemented in-house scripts to study amino acid sequence patterns in Pro and Gly-rich, intrinsically-disordered proteins that are known to exhibit LCST and UCST phase behavior. We used insights from these studies to rationally design more than 40 unique amino acid motifs (5-10 residues in length) that we hypothesized to exhibit one of the two behaviors upon polymerization. Recombinant peptide polymers were produced in E. coli and purified by exploiting their stimulus-responsive behavior, which we further characterized by temperature-dependent turbidimetry, circular dichroism and dynamic light scattering.

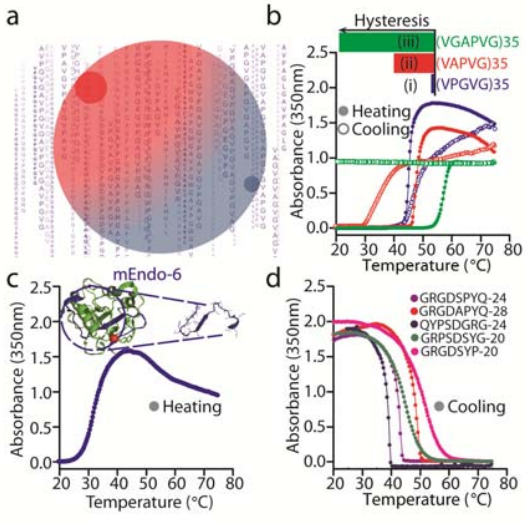

Fig 1. (a) Depiction of the (Pro-X_n-Gly) sequence space of amino acid motifs that exhibit LCST (red) or UCST (blue) behavior upon polymerization. Small circles represent the known sequence space previous to this work. (b) Phase behavior of hysteretic LCST peptide polymers in PBS. (c) LCST behavior of polymers built from the 27-residue bioactive peptide from murine endostatin [3]. (d) Examples of UCST peptide polymers with tunable phase behavior.

Results: Bioinformatics suggested and experiments demonstrated that (Pro-X_n-Gly)-containing motifs, where X is any amino acid and n varies from 0 to 4 residues, constitute a universal scaffold (i.e., sequence space) for the synthesis of unstructured peptide polymers with programmable phase behavior [4]. These findings significantly expand the set of amino acid building blocks available for the synthesis of LCST peptide polymers (Fig. 1a). We further observed that the introduction of key residue interactions (e.g, hydrophobic, polar charged or uncharged) into this scaffold, at the X position or in residues surrounding the (Pro-X_n-Gly) unit, defines the phase balance of the polymer to occur above an LCST or below an UCST. Unlike ELPs, these peptide polymers may exhibit three types of LCST phase behavior with zero (ELP-like), intermediate or large hysteresis (Fig. 1b). Circular dichroism shows that the emergence of secondary structure on aggregation is responsible for the observed hysteresis (not shown). Within this sequence space lie biologically active peptides that we can use for the synthesis of drug-like "smart" polymers, as we show for polymers of the antio-angiogenic protein endostatin (Fig. 1c) or for polymers of the canonical cell adhesion motif GRGDSP from fibronectin (Fig. 1d). Finally, we demonstrate the ability to rationally tune the phase behavior of UCST peptide polymers under physiologically relevant conditions by a number of parameters that are readily controlled in our genetically encoded polymers.

Conclusions: This work presents a radically new picture (Fig. 1a) of the sequence space of amino acid motifs that exhibit "smart" phase behavior upon polymerization. This picture captures the unexpected vastness of the sequence space that exhibit LCST and UCST phase behavior and reveals that this space is a continuum that encompasses amino acid motifs with opposing phase behaviors upon polymerization, namely LCST and UCST. We are currently exploring the use of hysteretic LCST peptide polymers for the design of materials with thermally-triggered shape memory and the use of UCST peptide polymers to control nanoparticle disassembly upon hyperthermia. We hope that the biomaterials community will rapidly incorporate these new phase transition peptide polymers into novel "smart" biomaterials, particularly those with intrinsic biological function.

References: [1] D. Roy, et al. *Chemical Society Reviews*, 42, 7214-7243, 2013. **[2]** F. Garcia Quiroz, et al. *Nature Materials*, 10:141–148, 2011. **[3]** RMTT Sijin, et al. *Cancer research* 65, 3656, 2005. **[4]** A. Chilkoti, et al. US Patent No. 8,470,967. June 25, 2013.