Elastin-like Polypeptides: Protein-based Polymers for Biopharmaceutical Development

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New formulations of engineered proteins designed to achieve targeted delivery to specific cell types and induce a biological effect are of high interest in the molecular engineering field, and in the biopharmaceutical industry. Elastin-like polypeptides (ELPs) are one chassis in biomolecular engineering that allow combining precise genetic programmability of protein sequences together with self-assembly and an ability to carry bioactive peptides, small molecules or antibodies. ELPs are engineered artificial proteins containing a repetitive sequence motif derived from the human extracellular matrix protein tropoelastin. Amongst their various properties, ELPs exhibit stimuli-responsive solubility that can be used to direct self-assembly of these proteins into nanoparticles, self-associative hydrogels, rod micelles, and phase separated coacervates and droplets. These environmentally sensitive self-associative interactions can be used to construct drug delivery vehicles and molecular sensors for a variety of applications. With this short column, I hope to introduce readers to ELP technology, explain the basics of their composition, physico-chemical properties and phase separation capabilities, and highlight recent technological and commercial developments in the area of ELPs for drug delivery.

ELPs are encoded through recombinant gene technology and produced in biological hosts, most commonly E. coli. They can serve as fusion partners for enhancing the stability and circulation half-life of therapeutic proteins (e.g., antibodies, bioactive peptides, therapeutic enzymes), or serve as delivery vehicles when complexed with active agents (e.g., small molecule drugs, therapeutic oligonucleotides). Depending on their sequence composition, ELPs are intrinsically disordered or can adopt a beta helical character. With a standard peptide backbone and a human parent sequence, engineered ELPs are biocompatible, biodegradable, and generally non-immunogenic. Based on these desirable properties, ELPs represent a valuable and modular platform for the development of therapeutic drug delivery vehicles to treat a wide range of diseases. Several high-quality review articles focusing on ELP technology have appeared in recent years[1–3].

ELPs are one class of the broader category of recombinant protein-based polymers. Specifically, ELPs comprise repeating pentapeptides of the sequence (VPGXG)n. The guest residue X can be engineered to contain any amino acid except proline. The subscript ‘n’ refers to the number of pentapeptide repeats in the sequence, which is analogous to the degree of polymerization of a synthetic polymer. ELPs exhibit stimuli-responsive phase transition behavior, which means they undergo hydrophilic to hydrophobic phase separation in response to small changes in salt, pH or temperature (Fig. 1). This behavior resembles that of several stimuli-responsive synthetic polymers that are important in the biomedical field, including poly(N-isopropylacrylamide), poly(oligoethylene glycol) methacrylate, and poly((2-dimethylamino)ethyl methacrylate)). The driving force behind the phase separation of all these polymers including ELPs is the endothermic chain dehydration reaction triggered by a significant gain in water entropy upon loss of water contact with the hydrophobic portions of the chain above the lower critical solution temperature (LCST). This behavior can be used to direct self-assembly of ELPs into nanoscale aggregates of tunable size and shape, and provides a direct route toward multivalent nanoparticles suitable for drug delivery.
constructed, ELP gene blocks can be used for expression of block polypeptides with precisely defined sequences and molecular weights. Different regions of the molecule can be encoded with varying degrees of hydrophobic character, which can significantly lower the LCST at one end of the molecule. This can be used to generate amphiphilic character along the length of the chain and drive assembly into nanoparticles or nanorods. If needed, the cores of these protein micelles can be covalently cross-linked, for example using dityrrosine cross-coupling reactions or bioorthogonal strategies\(^9\).

Chromatography-free purification and homogeneous immunoassay

ELPs have found many applications in biotechnology and biotherapeutics. Many works have demonstrated how ELPs can be genetically fused to a protein of interest and used as a phase-separating purification tag (Fig 2a). In these applications, the protein of interest fused with an ELP tag is overexpressed and purified from clarified fermentation broths by applying the stimuli (e.g., salt, pH, temperature or combinations thereof) that collapses and phase separates the ELP into a protein-rich coacervate phase. Bulk centrifugation, sedimentation and/or filtration can then be used to isolate the protein of interest, and the ELP tag is removed by protease treatment. This approach was used recently to enhance the purification and serum stability of an ELP fusion with interferon-alpha/gamma\(^9\). Affinity-based phase separation has also been developed where a binding domain (e.g., antibody binding domain) is fused with an ELP\(^9\). This reagent can then be used to fish out proteins from complex mixtures. Targets can include recombinant antibodies from bioreactors or protein biomarkers in clinical samples. By using affinity protein-ELPs, the protease digestion step is circumvented and replaced by simple elution of the target from the ELP purification reagent. This approach has diverse applications in scalable biomanufacturing of monoclonal antibody therapeutics, adeno-associated viruses (AAVs), or as analytical sensing platforms for performing homogeneous immunoassays.

Drug depots and delivery vehicles

For use inside the body, ELPs have been developed as injectable hydrogels that aggregate in subcutaneous tissue and serve as slow eluting drug depots (Fig 2b). This approach has been used to deliver bioactive peptides including glucagon-like peptide 1 for diabetes treatment, as well as antibiotics \([7,8]\). In the bloodstream, ELPs can be used to carry drug molecules or biologics to target tissues. In work from my lab, we demonstrated new formulations of ELPs containing a specific peptide sequence that is recognized by the clotting-associated transglutaminase FXIIIa. These hemo- static elastin-like polypeptides (hELPs) are recognized by host-derived clotting factor XIIIa and cross-linked into a protein hydrogel to support clotting and hemostasis\([8]\). Another developing area of ELP technology is in delivery of small interfering RNA (siRNAs). These ELP RNA carriers are typically designed with cationic charges in the form of Arg residues to drive formation of electrostatically assembled polyplexes of polycationic ELPs with polyanionic RNA molecules. These complexes can protect RNA therapies against nuclease degradation in vivo, boosting the efficacy and improving the immunogenicity and safety profiles of RNA-based therapeutics. A final example worth highlighting is the enhancement of stability, valency, and circulation half-life that can be achieved by fusing ELPs to antibodies, binding domains or therapeutic proteins more generally and directing their self-assembly into multivalent nanoparticles (Fig. 2c)\([10,11]\).

These research directions that I have highlighted demonstrate the potential of ELPs to address several urgent needs in the areas of medicinal chemistry and chemical biology. ELPs represent only one of several known protein polymers with intrinsic disorder and phase separation capabilities, with other significant examples including silk-like and resolin-like recombiners. What is now known is that there is likely a vast sequence space of protein polymer sequences possessing phase separation capabilities. A future frontier area is therefore the discovery of new disordered repetitive polypeptide sequences with favorable pharmacological and immunological properties, along with high recombinant expression levels that can be developed into new modular compositions for applications in drug delivery, tissue engineering, and molecular engineering more generally.

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**Figure 2. Overview of various architectures of molecular assemblies that are achievable with a modular ELP platform.**

(a) ELPs can be fused to binding proteins and enzymes to modulate properties such as serum stability, in vivo half life, and binding or catalytic activity. (b) Bulk ELP hydrogels can be assembled through covalent or non-covalent interactions. (c) Diblock ELPs containing hydrophobic and hydrophilic regions will spontaneously self-assemble into higher order structures such as multivalent nanoparticles, cylindrical protein micelles and protein vesicles.