Biomaterials 127 (2017) 49–60

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Computational smart polymer design based on elastin protein mutability

Anna Tarakanova [a], Wenwen Huang [b], Anthony S. Weiss [c], David L. Kaplan [b], Markus J. Buehler [a, *]

[a] Laboratory for Atomistic and Molecular Mechanics, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
[b] Department of Biomedical Engineering, Tufts University, Medford, MA, USA
[c] School of Life and Environmental Sciences and Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia

ARTICLE INFO

Article history:
Received 6 September 2016
Received in revised form
13 January 2017
Accepted 28 January 2017
Available online 31 January 2017

Keywords:
Elastin-like peptide (ELP)
Inverse temperature transition
Peptide-water interaction
Single-molecule
Protein simulation library

ABSTRACT

Soluble elastin-like peptides (ELPs) can be engineered into a range of physical forms, from hydrogels and scaffolds to fibers and artificial tissues, finding numerous applications in medicine and engineering as "smart polymers". Elastin-like peptides are attractive candidates as a platform for novel biomaterial design because they exhibit a highly tunable response spectrum, with reversible phase transition capabilities. Here, we report the design of the first virtual library of elastin-like protein models using methods for enhanced sampling to study the effect of peptide chemistry, chain length, and salt concentration on the structural transitions of ELPs, exposing associated molecular mechanisms. We describe the behavior of the local molecular structure under increasing temperatures and the effect of peptide interactions with nearest hydration shell water molecules on peptide mobility and propensity to exhibit structural transitions. Shifts in the magnitude of structural transitions at the single-molecule scale are explained from the perspective of peptide-ion-water interactions in a library of four unique elastin-like peptide systems. Predictions of structural transitions are subsequently validated in experiment. This library is a valuable resource for recombinant protein design and synthesis as it elucidates mechanisms at the single-molecule level, paving a feedback path between simulation and experiment for smart material designs, with applications in biomedicine and diagnostic devices.

1. Introduction

Elastin is an extracellular matrix protein that imparts reversible elastic recoil and resilience to extensible tissues, including the lung, blood vessels, elastic ligaments and skin. Beyond its impressive mechanical elasticity, elastin and artificial elastin-like peptides (ELPs) have been demonstrated, through transmission electron microscopy, circular dichroism and nuclear magnetic resonance [1–5], to exhibit an inverse temperature transition, a reversible propensity to aggregate upon increasing temperature [2,6–12]. Above a specific temperature, highly dependent on the chemistry and environment of the ELP system, the formation of an aggregating coacervate phase takes place. This is similar to the natural coacervation process of tropoelastin, the precursor molecule to elastin, in the process of elastic fiber formation [13].

The stimuli-responsive quality confers controllable functionalities to ELPs, and in conjunction with their biocompatibility and biodegradability, makes them effective candidates as a platform for biomedical materials. ELPs have been designed into a range of material forms, including nanoparticle self-assemblies [24,25] and hydrogels [1,26,27]. The tunability of the ELP phase transitions has been used in biomedical applications from drug delivery [28–30] to tissue engineering [31]. Examples of such applications include elastin-based nanoparticles that have been designed to display cell-penetrating peptide motifs for controlled uptake into cells; thermal targeting of tumors by creation of a diffusion gradient of a drug into the tumor; tumor targeting with local hyperthermia; ELP conjugates for cancer therapy; and sustained release delivery systems for controlled release of peptide drugs to treat type 2 diabetes [32–36].

Underlying the application of ELPs in bioengineering and biomedicine is a need to understand fundamental design principles and molecular mechanisms yielding the stimuli-responsive quality

* Corresponding author.
E-mail address: mbuehler@mit.edu (M.J. Buehler).

http://dx.doi.org/10.1016/j.biomaterials.2017.01.041
0142-9612/

to ELPs. In the last half century, a series of pivotal studies have contributed to the understanding of transition effects in ELPs. Urry's laboratory conducted extensive studies to understand the effects of sequence modification on transition temperature by varying guest residue X in VPGXG repeat sequences, coining the "inverse temperature transition" term [14,15]. They found a correlation between hydrophobicity index of guest residues and the transition temperature T_t, such that hydrophobic residues reduced T_t, while polar and hydrophilic residues increased T_t [37].

Structural transitions in elastin have also been discussed in the context of elastin's elasticity by Tamburro and others, using the reductionist approach to study short synthesized glycine-rich peptides representative of elastin. In this description, a shifting set of sliding β-turns, polyproline II and unordered structures directs conformational flexibility in the protein, establishing a general equilibrium between folded and extended structures [38,39]. Tamburro's team studied the effect of sequence, chain length and environmental conditions on elastin's propensity to coacervate, suggesting particular functional roles for different domains based on these findings [39–41].

A number of experimental studies from a biomaterials design perspective have considered the possibility of developing polymeric ELP materials with introduced temperature-modulated switches. Stimuli include sequence chemistry modification [16–19], changes in salt concentration [17], molecular weight [19–21], pH [20,22], and light [23]. Beyond this, Chilkoti's team created a series of quantitative models for making specific predictions based on a set of sequence and solution parameters [11,22,42], with an emphasis on applications for drug delivery system development [43,44]. Several experimental groups have also considered the particular role of water and hydration on ELP transitions [45,46] and related elasticity [47].

Supplementing experimental work, computational groups have developed ELP models. Several teams have used classical molecular dynamics to explore the molecular basis of elasticity and inverse temperature transition in ELPs [48–50]. The question of structure in ELPs has inspired studies that showed the sequence-dependent propensity of ELPs to assume amyloidogenic qualities [51,52]. Yet other studies have instead focused on ELP modeling to understand peptide receptor interactions and molecular implications in biological activity [53,54].

The present work builds on existing literature to expand the understanding of fundamentals of ELP transitions and factors that influence this behavior. Unlike earlier computational work where ELPs systems have been studied primarily using classical molecular dynamics for a sparsely distributed temperature range, this study introduces Replica Exchange Molecular Dynamics (REMD) to improve sampling and identify specific transition regions not previously captured in experiment nor simulation by considering 60 densely-distributed temperature systems. Using this method, we examine how varied conditions and triggers influence mechanisms involved in the presence and absence of transitions for different ELP sequences at the single-molecule scale.

We focus on intramolecular phenomena in order to understand basic events at the single-molecule and peptide domain level. A fundamental understanding at the sub-molecular scale can provide a foundation to extend this work for studying intermolecular events. The pentapeptide repeat unit VPGVG, and its permutation, GVGVP, are considered representative ELP polymer building blocks. Here we choose the GVGVP peptapeptide as proposed by Urry and used since by others as a model ELP [9,55,56]. Our work considers molecular compactness and secondary structure, hydrogen bonding organization, and distribution and arrangement of water in modulating ELP behavior based on changes in chemistry, salt concentration, and peptide chain length.

The approach introduced here can be extended to studying other synthetic or protein polymers for precise phase transition characterization at the molecular scale. For example, silk and silk-inspired sequences have shown a propensity to undergo temperature-induced phase transitions [57,58] and are popular candidate materials for biomedical applications [59]. A number of synthetic polymers also show temperature-dependent phase transition functionality [60] which can be further tuned for precise behavior and control with a modeling-experimental approach described in this work.

Based on our findings, we propose modeling as a complementary tool to provide fast, reliable predictions based on core molecular mechanisms, because current methods are inadequate and do not consider the contributions of fast and slow molecular dynamics on the one hand; and the balancing of interactions between peptide, ions and water on the other. Furthermore, understanding single molecule behavior mechanisms is a necessary prelude to studying intermolecular events such as coacervation. This approach can be used to quickly screen the sequence space from a library of candidate sequences, in order to define sequences that might be useful experimentally (Fig. 1).

2. Methods

2.1. Molecular simulation setup

Input structures for simulation are created based on elastin-like protein polymer (ELP) sequences of the form $[(GVGVP)(GXGVP)(GVGVP)]_n$ in single amino acid letter code (SI Fig. 1a). *X* is an interchangeable amino acid considered for shifting and modifying transition temperatures and *n* (equal to 1 or 2) determines sequence length. The systems considered in this study are described in SI Table 1. X-residue identity, chain length, and NaCl concentration are considered.

Extended conformations of the sequences are built using CHARMM version 24b1 [61]. The CHARMM force field is widely used for studying proteins [62]. It has been used to study other

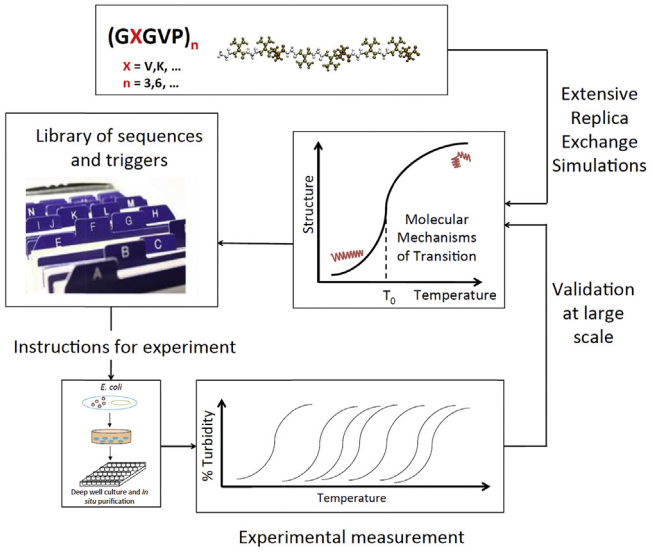

Fig. 1. Simulation-experiment material design paradigm flow-chart. Sequences of the form $[(GVGVP)(GXGVP)(GVGVP)]_n$ are simulated with extensive Replica Exchange Molecular Dynamics simulations to identify molecular mechanisms of structural transitions. This yields a basis for a library of available sequences to instruct experimental design. Recombinant DNA technology is used to synthesize proteins in vitro, feeding back into the simulation-experiment design loop.