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Synergistic Integration of Experimental and Simulation Approaches for the *de Novo* Design of Silk-Based Materials

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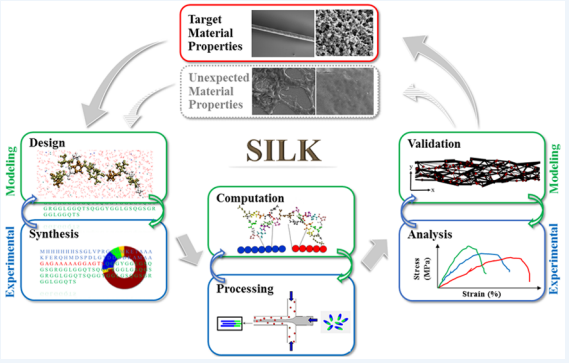
CONSPECTUS: Tailored biomaterials with tunable functional properties are crucial for a variety of task-specific applications ranging from healthcare to sustainable, novel bio−nanodevices. To generate polymeric materials with predictive functional outcomes, exploiting designs from nature while morphing them toward non-natural systems offers an important strategy. Silks are Nature's building blocks and are produced by arthropods for a variety of uses that are essential for their survival. Due to the genetic control of encoded protein sequence, mechanical properties, biocompatibility, and biodegradability, silk proteins have been selected as prototype models to emulate for the tunable designs of biomaterial systems.



The bottom up strategy of material design opens important opportunities to create predictive functional outcomes, following the exquisite polymeric templates inspired by silks. *Recombinant DNA technology* provides a systematic approach to recapitulate, vary, and evaluate the core structure peptide motifs in silks and then biosynthesize silk-based polymers by design. *Post-biosynthesis processing* allows for another dimension of material design by controlled or assisted assembly. *Multiscale modeling*, from the theoretical prospective, provides strategies to explore interactions at different length scales, leading to selective material properties. Synergy among experimental and modeling approaches can provide new and more rapid insights into the most appropriate structure−function relationships to pursue while also furthering our understanding in terms of the range of silk-based systems that can be generated. This approach utilizes nature as a blueprint for initial polymer designs with useful functions (e.g., silk fibers) but also employs modeling-guided experiments to expand the initial polymer designs into new domains of functional materials that do not exist in nature. The overall path to these new functional outcomes is greatly accelerated via the integration of modeling with experiment.

In this Account, we summarize recent advances in understanding and functionalization of silk-based protein systems, with a focus on the integration of simulation and experiment for biopolymer design. Spider silk was selected as an exemplary protein to address the fundamental challenges in polymer designs, including specific insights into the role of molecular weight, hydrophobic/hydrophilic partitioning, and shear stress for silk fiber formation. To expand current silk designs toward biointerfaces and stimuli responsive materials, peptide modules from other natural proteins were added to silk designs to introduce new functions, exploiting the modular nature of silk proteins and fibrous proteins in general. The integrated approaches explored suggest that protein folding, silk volume fraction, and protein amino acid sequence changes (e.g., mutations) are critical factors for functional biomaterial designs.

In summary, the integrated modeling−experimental approach described in this Account suggests a more rationally directed and more rapid method for the design of polymeric materials. It is expected that this combined use of experimental and computational approaches has a broad applicability not only for silk-based systems, but also for other polymer and composite materials.

1. INTRODUCTION

The design and synthesis of new polymeric materials is a key for future needs in science and technology. To generate polymeric materials with predictive functional outcomes, exploiting designs from nature while morphing them toward non-natural systems offers an important strategy. Silks are exquisite polymeric material systems that have been optimized in nature through evolution, resulting in a balance of chain length, sequence chemistry and aqueous processing. Despite the relatively simple amino acid building blocks, materials generated from silk proteins exhibit remarkable mechanical

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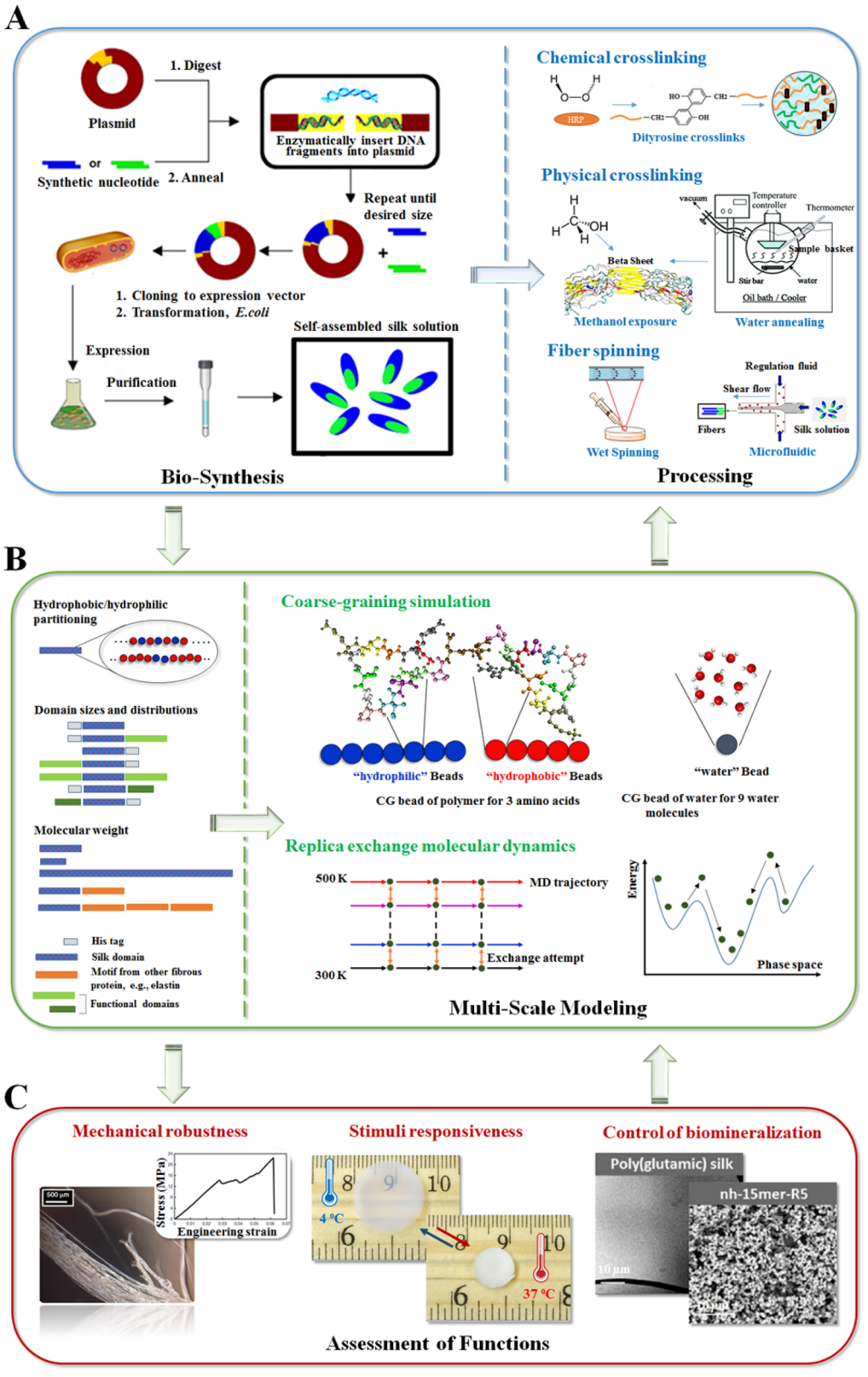

Figure 1. Synergetic integration of genetic engineering, polymer processing, multiscale modeling, and functional assessment toward the *de novo* design of new functional biomaterials. (A) General biosynthesis scheme of genetically engineered proteins using recombinant DNA technology and common polymer processing methods of protein-based biomaterials using chemical/physical cross-linking approaches and shear flow spinning process. (B) Multiscale modeling, illustrated by coarse-grained simulation and replica exchange molecular dynamics modeling, bridges the gap between molecular design, processing conditions, and target macroscopic physical properties. (C) Assessment of functions provides a feedback loop to revise the simulation models during iteration. Adapted with permission from refs 11, 24, 26, and 27. Copyright 2015 Nature Publishing Group, 2016 WILEY-VCH, 2016 American Chemical Society, and 2011 American Chemical Society.

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properties.[1−4] Silk based biomaterials are also useful in a wide range of applications.[2,5−8] Therefore, silks have been selected as a prototype polymer model to emulate for future material designs.[9−12]

Silks are highly modular protein polymers, with large internal repetitive sequences flanked by short nonrepetitive N- and C-terminal domains.[13,14] Within the internal repetitive sequences, highly conserved poly(Gly-Ala) and poly-Ala motifs form hydrophobic β-sheet crystalline domains, and the glycine-rich motifs form hydrophilic noncrystalline domains (e.g., random coil, helices, β-turn).[3,5] These motifs self-assemble into hierarchical architecture, where stiff orderly cross-linked β-sheet nanocrystals are confined within an elastic semi-amorphous protein matrix, making silk fibers one of the toughest and most versatile materials known.[15,16]

The use of *recombinant DNA technology* to emulate and expand the modular silk templates provides new opportunities to build materials from the molecular level. In addition to altering sequence, a second level of control of material assembly is achieved via the use of *post-biosynthesis processing*. Significant progress has been achieved over the past decade to address the fundamental challenges in biopolymer design and processing by using recombinant silk mimetic peptides, to provide specific insight into the roles of molecular weight,[11,17] domain sizes and distributions,[18−20] and hydrophobic/hydrophilic partitioning,[11] on protein self-assembly and the resulting mechanical properties.[14,21,22] Importantly, recombinant DNA technology has also been used to construct silk biomaterials *de novo* through the addition of key modules from other proteins or functional groups for chemical and physical processing to introduce new functions, exploiting the modular nature of silk proteins. For example, silk−elastin-like proteins (SELPs) were developed as new polymers for targeted drug delivery[23] and chemically modified into soft stimuli responsive hydrogel actuators.[24] Silk−silica fusion proteins were designed as biomaterials for bone regeneration.[25] However, conventional recombinant protein methods are limited by generally tedious and low-yielding cloning and biosynthesis methods because of the repetitive nature and length of the silk sequences. In addition, the serial design process, where new genetic/protein constructs are prepared and characterized in sequential fashion, also leads to a slower, trial-and-error process to reach specific functional goals for the materials. Therefore, there is an unmet need for developing new predictive tools, such as modeling, to accelerate the material development process.

Bottom-up *multiscale material simulations* provide information into the mechanisms behind changes in material properties starting with the interactions of atoms to the assembly of clusters of atoms based on primary sequence, chain folding and interactions, and processing conditions. Integrating computational modeling at early stages of material design suggests a time- and cost-efficient solution to generate function from the molecular building blocks: a synergistic approach, which starts from a specific functional goal, combines inputs from both simulations and experiments, provides new insights into structure−function relationships,[11,13,14] and guides functional material designs.[24,26] Such an approach is also amenable to combinatorial strategies and offers potential to serve as a roadmap for innovations in polymer, polymer alloy, and polymer composite systems.

This Account summarizes recent advances in the understanding and functionalization of silk-based systems, with an emphasis on the power of integrated simulation and experimental approaches for biopolymer design. This Account is based on previous work on silk-based systems that focused on the structure and biomedical applications of silks[2−8] and highlights more recent studies of novel silk designs achieved through a combined genetic engineering and computational modeling approach. Challenges and opportunities for this approach are also discussed in each section.

2. INTEGRATING EXPERIMENTAL APPROACHES AND MULTISCALE MODELING FOR BIOPOLYMER DESIGN

Developing fundamental tools and insight into biomaterial designs for predictive functional outcomes should be key to propelling polymer discovery forward. The integration of biosynthesis, processing, multiscale modeling, and experimental validation provides a path toward the *de novo* design of silk-based materials with tailored properties (Figure 1). Each aspect of this process informs further understanding of material properties and also feeds into the other steps, toward a more optimized or predictable material outcome.

2.1. Biosynthesis of Silks

As summarized in Figure 1A, the main steps to generate recombinant silks by design are gene design, cloning, expression, and protein purification. A variety of cloning strategies have been used toward this goal, including step by step directional ligation and recursive directional ligation for the production of new silk-related genes with control of size[28] and concatemerization for the construction of gene libraries.[29] The main purification method used for recombinant silk proteins has been Ni-NTA affinity chromatography,[11] while inverse temperature cycling is preferred for some silk constructs with elastin domains.[24] The key advantages of genetically engineered silk proteins include the tailorability of sequence, versatility in protein chemistry, and control of protein size. Recombinant DNA methods provide tools to study sequence−function relationships yet remain limiting when generating high molecular weight silk, when cloning the full-length silk genes, or when large-scale production of silk proteins is needed.[17]

2.2. Post-biosynthesis Processing

As summarized in Figure 1A, microfluidic[14] and wet spinning techniques,[11] which mimic the natural silk spinning process, have been exploited to fabricate recombinant silk fibers and further the understanding of the natural assembly process. Chemical and physical modification, including methanol vapor treatment and water annealing for the induction of β-sheet secondary structures and enzymatic cross-linking for the formation of dityrosine networks, have also been utilized to facilitate the assembly of the polymer chains into defined secondary structures or polymer networks for enhanced material properties.[24,26] Modifying silk processing parameters, together with protein sequence alterations, allows for the exploration of a large design space to develop new functional polymeric materials.

2.3. Multiscale Modeling and Validation

As summarized in Figure 1B, common approaches to model material features include molecular dynamics (MD) for small peptides and accelerated sampling methods such as replica exchange molecular dynamics (REMD) for protein polymers and multimolecular systems on the nanoscale,[30] coarse-grained modeling (CG) on the mesoscale,[11,24,26] and continuum methods on larger scales. Implicit solvent temperature replica

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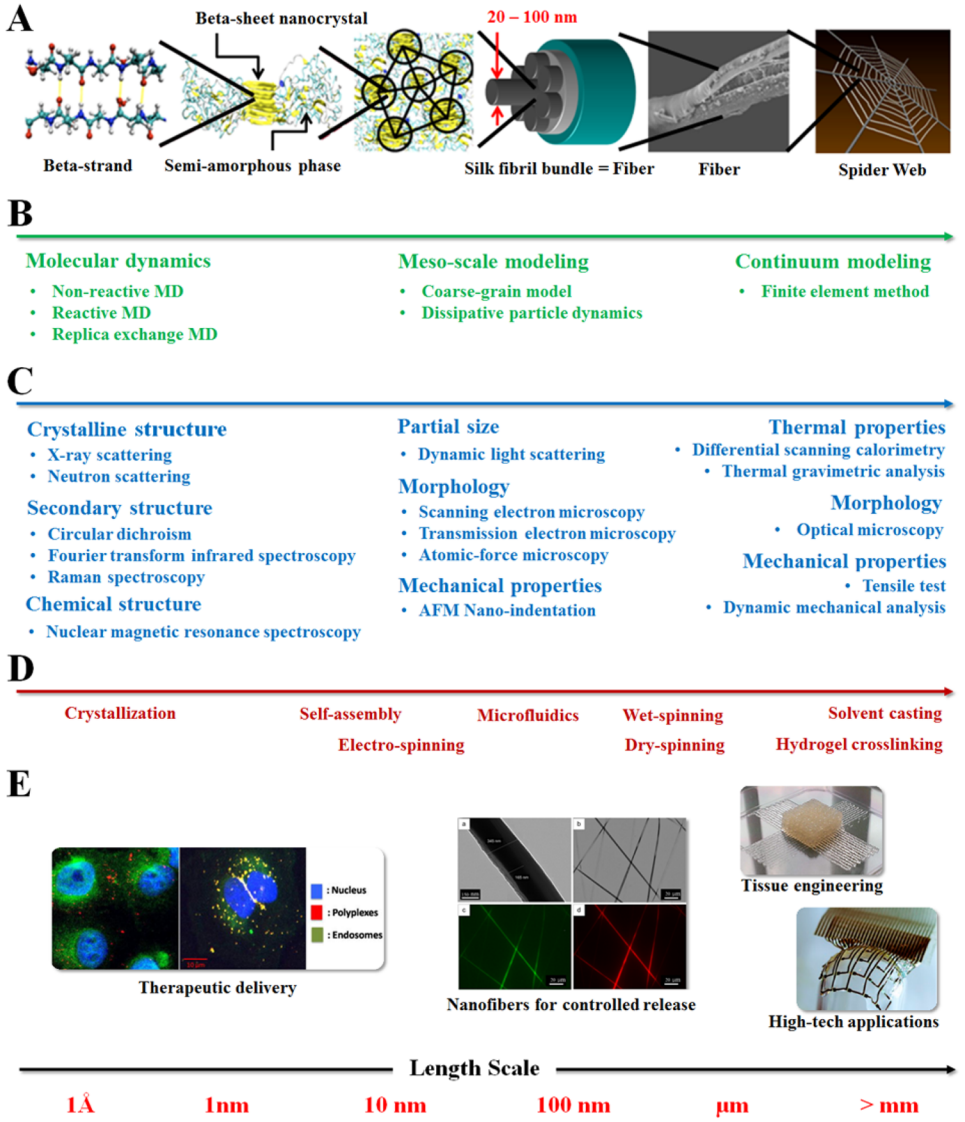

Figure 2. Experimental and computational methods to study the hierarchical structure and function of silk biopolymers at different length scales. (A) Schematic of the hierarchical spider silk structure that ranges from molecular to macroscopic scales. The hydrogen bonded β-strands at the angstrom level create a hetero-nanocomposite of β-sheet nanocrystals embedded in a soft semiamorphous matrix at the nanometer length scale, which self-assemble into silk fiber at the micrometer scale to create spider orb web structure. (B) Theoretical and simulation methods for self- and assisted-assembly mechanisms per the length scale they address. (C) Analytical characterization tools for resolving the physical properties of silk proteins at different length scales. (D) Processing methods for the formation of the hierarchical structure on different length scales. These methods control the resulting material structure and properties and lead to (E) various biomedical and technology applications of silk proteins from nano- to macroscopic scale. Adapted with permission from refs 33−37. Copyright 2010 Nature Publishing Group, 2014 WILEY-VCH, 2016 MDPI AG, 2015 American Chemical Society, and 2010 Nature Publishing Group.

exchange molecular dynamics followed by explicit solvent conventional molecular dynamics have been used to investigate dynamics and structural properties of the systems with atomic resolution.[24,26] New mesoscale techniques have also been developed to access larger temporal and spatial scales, which currently exceed the computational capacity of full atomistic molecular dynamics simulations. Classical dissipative particle dynamics (DPD) was used, with two new terms added that describe the hydrogen bonds and covalent bonds in polymer chains. The LAMMPS code was modified to include the new terms in the integration of classical DPD simulations. This new coarse-grained model allows for the simulation of soft biopolymers such as silk proteins and captures the formation of micelles and polymer networks.[11] Multiscale modeling provides a useful guide to describe material features starting from fundamental laws of physics, yet it is currently not possible to study complex materials with a single computational method on all scales simultaneously. There are efforts to propose new modeling schemes with tunable resolution to balance accuracy and efficiency,[31] but these approaches are not yet in routine use and require further development and testing. New sampling methods, such as replica exchange with solute tempering,[32] software developments, and hardware to facilitate high performance computing and data storage help to improve the computational capacity and utility of simulations.

As summarized in Figure 1C, experimental characterization, coupled with simulation, is routinely used to validate outcomes and provide feedback to revise current simulation models at multiple scales. Common experimental methods that are used to assess the protein properties and validate the simulation

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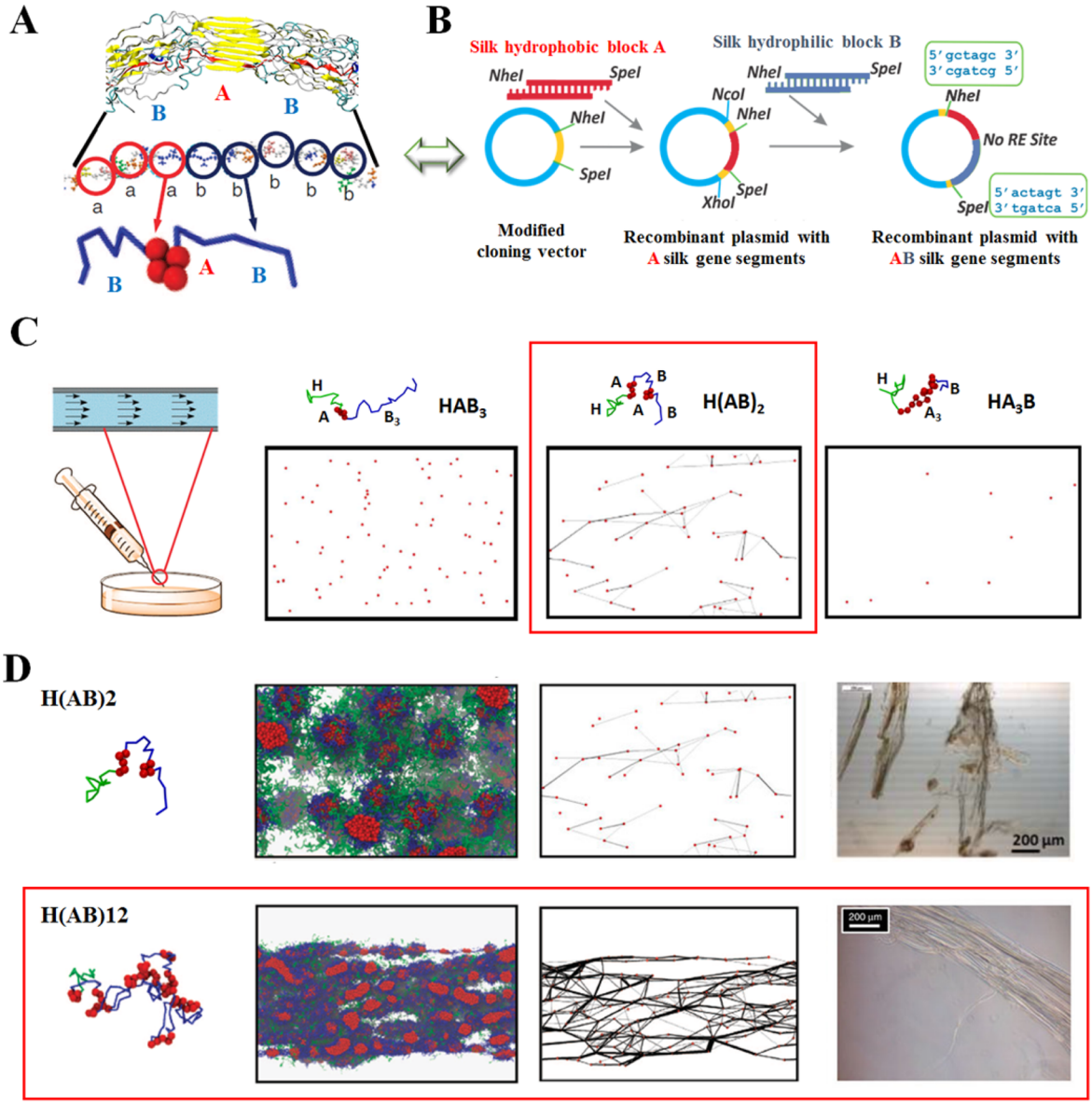

Figure 3. Integrated modeling and experimental approaches for the production of robust recombinant spider silk fibers. Schematic of (A) coarse-graining simulation, (B) recombinant DNA strategy, and (C) processing method (e.g., wet spinning) used to study spider silk block copolymers. (C) Coarse-grained representations, and the mesoscopic dissipative particle dynamics (DPD) simulation node-bridge diagram under the shear flow of the recombinant spider silk peptides: HAB_3, $H(AB)_2$, and HA_3B. Simulation suggested that under shear flow spinning process, only the $H(AB)_2$ sequence (highlighted in red box) with alternating hydrophobic/hydrophilic domains at a 1:1 ratio promotes network formation over other domain distributions. (D) Coarse-grained representations, mesoscopic DPD simulation snapshots, and the node-bridge diagram under shear flow, and the bright-field microscopic images of spun fibers in bundles in the coagulation bath of the recombinant spider silk peptides $H(AB)_2$ and $H(AB)_{12}$. As length is increased from $H(AB)_2$ to $H(AB)_{12}$, mature networks for robust spider silk fibers formed in $H(AB)_{12}$ (highlighted in red box) under shear flow. Red beads, hydrophobic "A" beads in the "A" domain; blue lines, hydrophilic "B" beads in the "B" domain; green lines. hydrophilic "B" beads in the "H" domain. Water beads are not shown for clarity. Adapted with permission from refs 11 and 38. Copyright 2015 Nature Publishing Group and 2014 Elsevier Inc.

outcomes are listed in Figure 2. At atomic or nanoscale, experimental methods that are used to measure crystalline, secondary, or chemical structures provide information to identify atomic properties.[11,13] At the larger scales, experimental methods that measure thermal, optical, and mechanical properties provide information about the macroscopic physical properties.[13,24] These experimental results served as a feedback loop to revise the simulation models during the iteration.

As shown in the examples below, polymer synthesis, here in the form of *recombinant DNA technology*, is used to modulate the sequence chemistry on the molecular and nanoscale, which in turn has a major impact on higher level structures and final material properties. *Polymer processing* controls the environmental conditions to influence the assembly of polymer chains into defined secondary structures at the nanoscale and the formation of polymer networks at the macroscale and mesoscale. *Computational modeling*, together with experimental validation, guides the biopolymer design process by simulating structures and assembly at different length scales. Interactive steps involving experimental validation and modeling also provide a chance to revise the models during the iteration. This synergistic integration of genetic engineering, simulation, and

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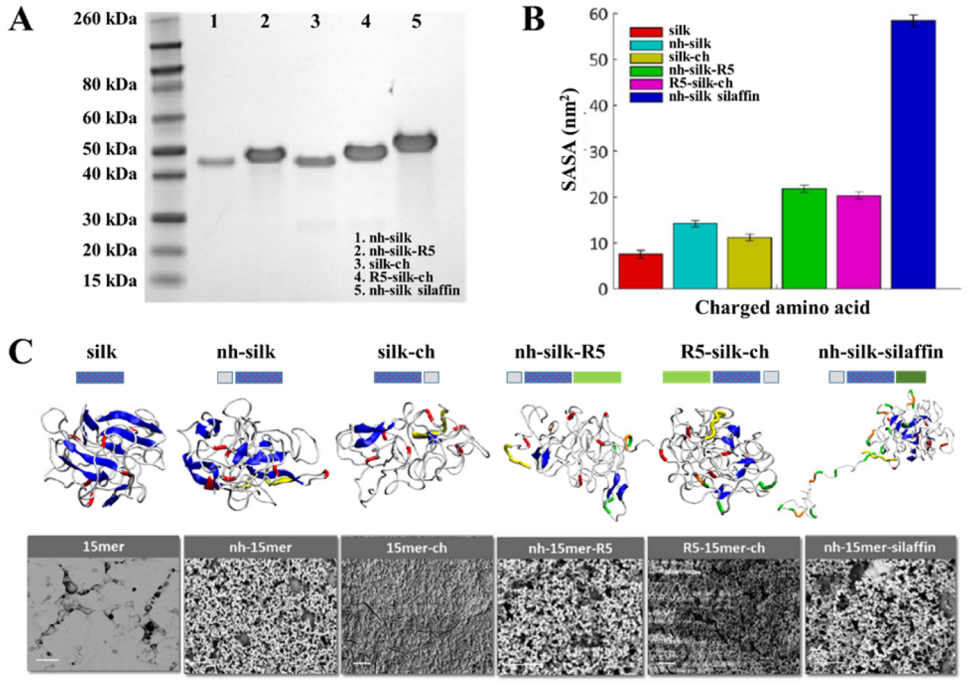

Figure 4. Integrated modeling and experimental approach for the design of biomineralization interfaces. (A) SDS-PAGE of recombinant silk−silica fusion proteins: nh-silk (40 kDa), nh-silk-R5 (43 kDa), silk-ch (40 kDa), R5-silk-ch (43 kDa), and nh-silk-sillafin (55 kDa). (B) Solvent accessible surface area (SASA) of positively charged amino acids in recombinant silk−silica fusion proteins. (C) Schematics of the silk−silica fusion protein designs, REMD simulation of protein folding, and SEM evaluation of the ability to induce silica formation. Correlation between REMD snapshot and SEM images suggested that the folding and exposure of charged functional groups played key roles in biomineralization. Peptide design: his-tag, gray box; spider silk polymer, dotted blue box; R5 domain, green box. REMD snapshot: β-sheets, blue; random coils, white; histidine, yellow; arginine (in silk domain), red; arginine (in R5 binding peptide), orange; lysine, green. SEM image scale bars are 10 μm. Adapted with permission from ref 26. Copyright 2016 American Chemical Society.

experiment provides a facile approach to modulate biopolymers at the nanoscale and control their function utility at the microscopic and macroscopic scales.

3. EXAMPLE 1: UNDERSTANDING SPIDER SILK DESIGNS FOR MECHANICALLY ROBUST BIOPOLYMERS

Considering the remarkable mechanical properties of silks, an important challenge remains: the mass production of spider silks and related biomimetic versions. Recombinant DNA approaches provide an alternative route to spider silk synthesis and give a starting point for developing the fundamental insights into spider silk sequence design, self-assembly mechanisms, and fiber spinning process. To understand sequence−function relationships and harness the self-assembly process for mechanically robust silk-based materials, recombinant spider silks with various molecular weight, domain sizes, domain distributions, and hydrophobic−hydrophilic domain ratios were biosynthesized via genetic engineering.[11,18−22,28,38] The sequence features of the recombinant spider silks were adapted from the representative sequence of MaSp1 in the spider dragline silk of *Nephila clavipes*, including hydrophobic A domains, GAGAAAAAGGAGTS, and hydrophilic B domains, QGGYGGLGSQGSGRGGLGGQTS.[11,18−22,28,38] The recombinant spider silk sequences followed generic design templates, for example, $(A_pB_q)_n$, and the secondary structure analysis of recombinant spider silks revealed that the hydrophobic A domains were responsible for the formation of β-sheet crystalline regions, and the hydrophilic B domains were responsible for the formation of the noncrystalline regions.[18,38]

Studies on the self-assembly of recombinant spider silks revealed that the hydrophobic−hydrophilic domain ratio impacted the formation of micelle structures in solution.[20,21,28] Studies of solution parameters suggested that environmental factors, such as pH and ion concentration (e.g., sulfate concentration), influenced the self-assembly of the recombinant spider silks.[22]

The integration of scalable modeling, biosynthesis, and processing also added new insight into the formation of robust silk fibers with a Young's modulus of 1−8 GPa, similar to native spider silk fibers. This combination approach suggested that a large volume fraction of the hydrophobic A domain in recombinant spider silks tended to have a higher β-sheet content for high mechanical stiffness and also tended to self-associate rather than forming intermolecular structures necessary for fiber formation.[14] Using modified DPD simulations, combined with biomimetic spinning and experimental validation, the results from these studies also suggested that (i) intermediate ratios of hydrophobic to hydrophilic domains at a 1:1 ratio resulted in well-connected networks, (ii) shear flow increased the connectivity of the polymer networks, which translated to improved silk fiber formation, and (iii) stronger polymer networks were formed when longer polymer chains (larger n value) were presented (Figure 3).[11] With the above examples of integrating genetic engineering, experiment, processing, and modeling, we demonstrate a synergistic path toward more predictable material outcomes, in this case mechanics.

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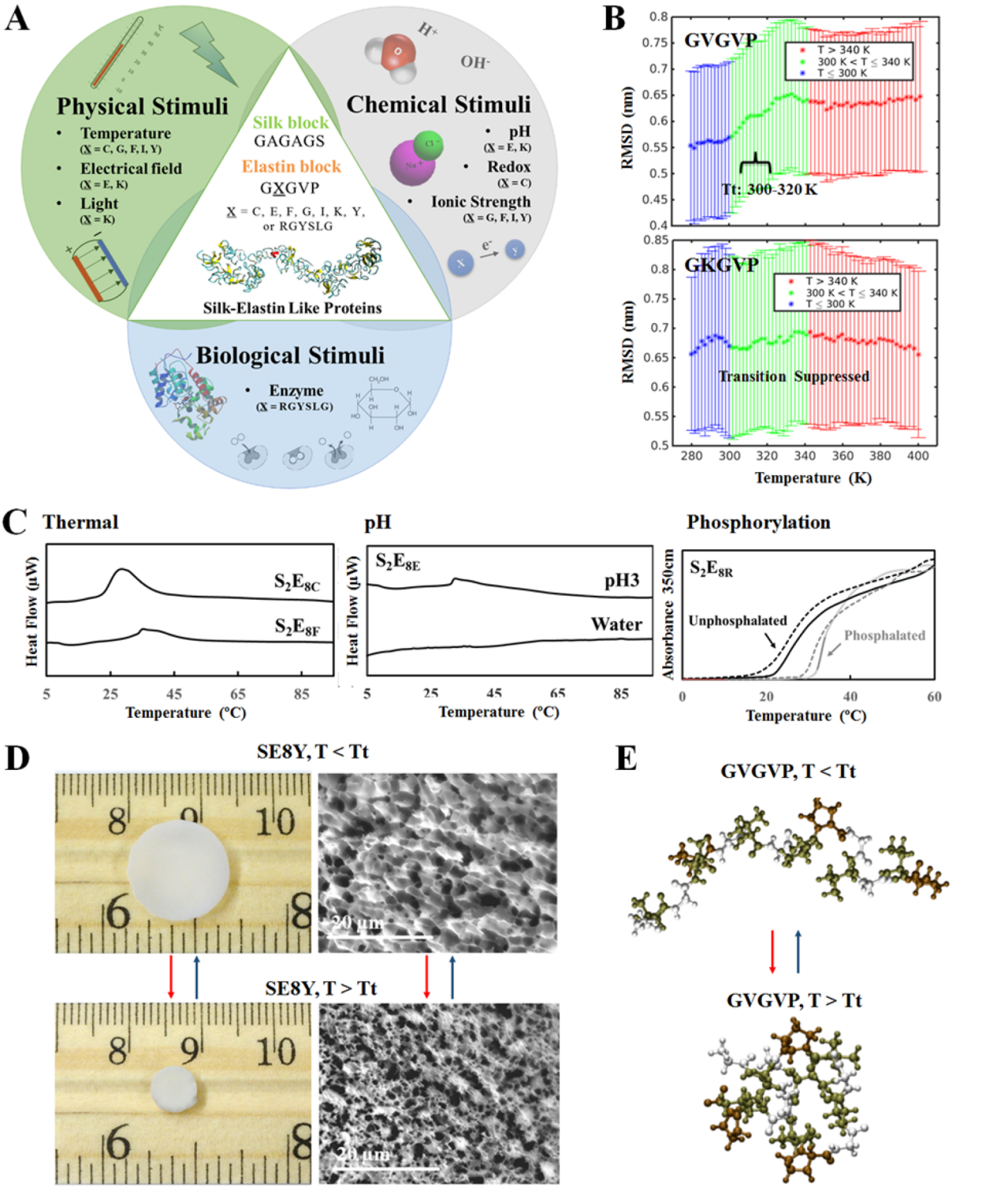

Figure 5. Integrated modeling and experimental approach for the design of stimuli-responsive biomaterials. (A) Schematic of the sequence dependent stimuli-responsive features of SELPs. By varying position "X" amino acid in the elastin domains, SELPs can be designed to predictably respond to physical stimuli (e.g., temperature, electric field, light), chemical stimuli (e.g., pH, redox state, ionic strength), and biological stimuli (e.g., enzymatic triggers). (B) Root mean square deviation (RMSD) values for the elastin domain (GVGVP)(GXGVP)(GVGVP) from REMD simulations. RMSD values for X = V indicate a transition from extended to folded conformations. RMSD values for the X = K sequence suggest a suppressed transition in the elastin domain. The analysis suggests that the inverse temperature transition is governed by the "X" residue in elastin domain. (C) Differential scanning calorimetry and turbidity profiles of SELP solution demonstrated that SELPs can be designed to respond to a variety of stimuli (e.g., temperature, pH, and phosphorylation). (D) Optical and SEM images of SELP, below and above T_t, exemplified by SE8Y with sequence of $[(GVGVP)_4(GYGVP)(GVGVP)_3(GAGAGS)]_{14}$. Result demonstrated that SELPs can be fabricated into hydrogels for stimuli-responsive actuators. (E) Snapshots of the elastin domain, (GVGVP)(GXGVP)(GVGVP), from REMD simulation, below and above T_t. An extended conformation is observed below T_t, and a folded configuration is present above T_t. Adapted with permission from ref 24. Copyright 2016 WILEY-VCH.

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4. EXAMPLE 2: OPTIMIZATION OF SILK DESIGNS FOR BIOMATERIAL INTERFACES: SILK MINERALIZATION−PEPTIDE FUSION PROTEINS

Organic−inorganic interfaces are integral to biomaterial functions in many areas of tissue repair and regeneration.[39,40] Fundamental insight into interfacial mineralization of proteins related to calcification of medical materials would permit insights into the fine-tuning of biomaterials to either promote the formation of inorganic−organic hybrid materials as a route to stiffer and stronger materials for bone regeneration or to prevent the mineralization to sustain flexible and dynamic material functions, such as for heart valves and blood vessels.[11,24,26]

To address the challenge of biomineralization interfaces and the needs derived from hardened/stiffer biomaterial systems, silks were modified via genetic engineering with different functional domains to control biomineralization (Figure 4).[41,42] A number of key features were elucidated from these studies, including domain types and distributions related to mineralization, combined with the mechanical properties of the silks where the presence of charged termini on silk protein assembly improved mechanical/functional properties. For instance, the silica-binding peptide R5 (SSKKSGSYSGSKGSKRRIL) derived from *Cerithiopsis fusiformis* silaffin gene was fused to the N- or C-terminus of the silk polymer, $(SGRGGLGGQGAGAAA-AAGGAGQGGYGGLGSQGT)_{15}$, derived from the consensus repeat of *N. clavipes* dragline silk protein.[43] Genetically engineered constructs (nh-silk, nh-silk-R5, silk-ch, R5-silk-ch, and nh-silk-sillafin, where nh and ch stand for N- or C-terminally fused histidine tag) were produced and processing parameters (solvating agent, β-sheet induction method, temperature) that affected protein secondary structure were defined.[26] The presence of positively charged amino acids was essential for supporting biomineralization of the silk fusion proteins; however the process was not amino acid specific. Moreover, the location of the charged domain involved in the biomineralization in the fusion protein affected protein folding and consequently surface exposure of the charged amino acids.[26] In another example, artificial silk block copolymers were decorated with a VTKHLNQISQSY (VTK) domain, identified via phage display with preferential adsorption on bone-like mineral and hydroxyapatite, where the silk domain was critical to the material properties and the VTK domain for biomineralization.[44,45] Understanding the mechanism by which these key factors impact biomineralization allows for the rational design of silk-based materials to generate new inorganic/organic hybrid systems for biomedical applications.

The integrated modeling−experimental approach allowed for the efficient identification of key parameters in material design to control function, here related to mineralization. Through the integration of modeling, insight was gained into key factors such as protein folding and exposure and alignment of charged units, fostering the fine-tuning of protein sequence and processing parameters to support biomineralization. The computational procedure started with initial predictions of the protein structure from homology modeling. Replica exchange simulations in implicit water were performed to identify protein folding at ambient (300 K) temperature.[26] For each case, the most probable structure was selected using a single linkage clustering algorithm. The representative structures were then refined in explicit water, and average properties were reported from the last part of the simulation. The results showed that the extent of silicification in the experiments was correlated with the amount of solvent accessible surface area of positively charged amino acids in the folded, assembled structures.

5. EXAMPLE 3: EXTENDING SILK DESIGNS TOWARD DYNAMIC MATERIALS: SILK−ELASTIN-LIKE PROTEINS

Their impressive material properties render silks as ideal candidate materials for responsive composites. By this motivation, we examine silk−elastin composite materials for controlled mutability design. The representative sequence of *Bombyx mori* silkworm silk, GAGAGS, was fused with the elastin-like peptide, GXGVP (where X is an interchangeable amino acid), via genetic engineering to encode and then express silk−elastin-like proteins (SELPs) as templates for stimuli-responsive materials. The robust mechanical properties inherent to silk and the dynamic nature originating from the elastin domains formed the basis for these designs.[46,47]

A series of SELPs with different silk-to-elastin ratios,[24,47,48] molecular weights,[29] and protein chemistry[24,29,49] were constructed to address sequence−structure−function relationships and control of dynamic properties (Figure 5). The study of self-assembly mechanisms of SELP micelles for drug delivery revealed that the incorporation of a silk domain into SELP facilitated micelle formation.[47] Chemical modification of SELPs with retinal for photoresponsive biopolymers suggested that the protein chemistry in the elastin domain can be exploited to expand SELP constructs for new and specific functional responses.[50] Actuating properties of SELP hydrogels revealed that elastin chain folding−unfolding at the molecular level during inverse temperature transition (T_t) could be translated into macroscopic reversible materials where the physical properties changed based on cross-linking the elastin domain.[24] Studies of the responsive properties of SELP solutions and SELP hydrogels suggested that incorporation of different guest residues in the X position of the elastin domain modulated the protein chemistry and shifted the inverse transition temperature as a function of the silk-to-elastin ratio, chain length, protein concentration, and environmental factors, expanding the thermal sensitivity of these materials to a range of other stimuli-responsive properties, including pH, ionic strength, electric field, and enzymatic (phosphorylation) responses.[24,29] Though these studies provided further insight into sequence−function relationships of SELPs, fine-tuning of the inverse temperature transitions of SELPs per functional need remains a key issue to be resolved for dynamic biomaterial designs.

The incorporation of computational modeling provides a useful tool to reduce the trial-and-error outcomes and accelerate the rational design of stimuli-responsive materials to meet specific functional goals. Multiscale molecular modeling has been employed for the study of elastin-like peptides (ELPs),[51] tropoelastin,[52] silk protein,[53] and most recently SELPs. Drawing inspiration from ELP and silk models, SELP models were created to combine the unique mechanical and stimuli-sensitive characteristics of the two main component parts. These models describe molecular mechanisms of structural transitions and shifts in mechanical properties in response to temperature. An important future direction is the scale-up of the phase transition effects to macroscales to guide future design of novel stimuli-responsive SELPs.

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6. CONCLUDING REMARKS AND PROSPECTS

Tailored biomaterials with tunable functional properties are desirable for a variety of task-specific applications ranging from drug delivery, tissue engineering, dynamic biomaterial implants, material coatings, components for robotic devices, and implantable devices, among others. Silks, due to the genetic basis of encoded protein sequence control, mechanical properties, biocompatibility, and biodegradability, were selected as a prototype model to emulate in the pursuit of the *de novo* design of functional biomaterials to bridge experimental and modeling strategies in unison. In this Account, we demonstrated the power of the integrated simulation and experimental approaches on biopolymer design through three prominent examples: (i) spider silks to understand sequence−function relationships for mechanically robust biopolymer designs, (ii) silk−silica fusion proteins for functionalization of silk system for biomineralization interfaces, and (iii) silk−elastin-like proteins for stimuli responsive, shape-changing materials. The integrated modeling−experimental approach elucidated several key parameters to address the fundamental challenges in biopolymer designs, including specific insights into the role of molecular weight, hydrophobic/hydrophilic partitioning, and shear stress for silk fiber formation, as well as on protein folding, silk volume fraction, and controlling mutability for functional material designs. With the above examples of integrating genetic engineering, experiment, processing, and modeling, synergy was demonstrated for improved predictive outcomes for the materials.

While silk-mimetic peptides suggested useful insights for predicting function from the molecular building blocks, the mechanical properties of these silks remain inferior to the native materials, in part due to low molecular weight and the truncation of some critical domains such as the N- and C-termini. The integrated approach to generate predictive outcomes for full length silks, which has not yet been realized, combined with synergistic feedback loops of experiment and computation, should advance the understanding of basic mechanisms and trigger more optimized material designs to address current limitations.

Understanding the assembly of amino acid building blocks into multifunctional structures remains in its infancy. The integrated experimental and modeling approach provides new insights into structure−function relationships. Further understanding and expansion of this approach to add functionality to silk-based materials systems, including copolymers and composites as summarized earlier, will continue to propel utility for these methods. The approach also offers significant implications for other protein materials and eventually nonprotein polymer systems.

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The authors declare no competing financial interest.

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