



Multiscale Modeling of Silk and Silk-Based Biomaterials—A Review

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In celebration of David L. Kaplan, on the occasion of his 65th birthday

Silk embodies outstanding material properties and biologically relevant functions achieved through a delicate hierarchical structure. It can be used to create high-performance, multifunctional, and biocompatible materials through mild processes and careful rational material designs. To achieve this goal, computational modeling has proven to be a powerful platform to unravel the causes of the excellent mechanical properties of silk, to predict the properties of the biomaterials derived thereof, and to assist in devising new manufacturing strategies. Fine-scale modeling has been done mainly through all-atom and coarse-grained molecular dynamics simulations, which offer a bottom-up description of silk. In this work, a selection of relevant contributions of computational modeling is reviewed to understand the properties of natural silk, and to the design of silk-based materials, especially combined with experimental methods. Future research directions are also pointed out, including approaches such as 3D printing and machine learning, that may enable a high throughput design and manufacturing of silk-based biomaterials.

1. Introduction

Silk is one of the most impressive natural materials, due to its combination of outstanding physical, chemical, and biological properties endowed by its intricate hierarchical structure from the nanoscale up to the fiber scale.^[1,2] Especially remarkable are its mechanical properties, which outperform strong engineering materials, such as Kevlar or steel.^[3] This mechanical performance is even more impressive if we consider the

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thickness of silk fibers, its lightweight, and the fact that they are exclusively made out of proteins. This incredible strength arises from structural features along different length scales in a hierarchical fashion^[4] (Figure 1), in which silk fibroin protein presents elastic domains that are toughened by crystalline β -sheets. The structure of silk varies depending on the animal species that produces it. Some examples include *Bombyx mori* (*B. mori*) worms,^[5] or spiders from the orders of *Araneus* or *Nephila* (e.g., *N. edulis* or *N. clavipes*).^[6] These often use silk for specific objectives, such as spinning cocoons to protect the silkworm as it grows,^[7] or to spin webs that capture prey in the case of spiders.^[8]

Spider silk is a highly modular protein biopolymer,^[9] named silk fibroin, that is constituted of large repetitive sequences

of amino acids flanked by non-repetitive N- and C-terminus domains. The repetitive sequences are constituted of poly(glycine-alanine) and poly-alanine motifs that can form the already mentioned β -sheet crystalline domains along the structure of fibroin. These domains are intercalated between glycine-rich amorphous domains, such as β -turns or random coils. Differently, native silkworm silk is a block copolymer composed of hydrophobic crystalline and hydrophilic amorphous regions. It is formed by remarkably strong and tough fibers of silk fibroin coated with a glycoprotein called sericin (25–30 wt% of the total cocoon silk) that provides integrity to the proteinaceous complex.^[10] This silk fibroin is typically composed of heavy chains formed mainly by crystalline domains (350 kDa) linked by disulfide bonds to light chains (25 kDa).^[11,12] The crystalline regions consist of repeating glycine (G), alanine (A), and serine (S) sequences (GAGAGS) that form hydrophobic β -sheets.

Among the different animals that produce silk, the silkworm species *B. mori* received the most scientific attention as it is extensively domesticated for the mass production of silk since ancient times.^[3] However, although *B. mori* silk was used for centuries in the textile industry, several methods are currently being developed to convert silk into a wide array of new biomaterials.^[13] Being sustainable and highly biocompatible, silk is a promising material for tough and resistant hydrogels, membranes, or fibers,^[12,14,15] as well as for hybrid materials that incorporate other inorganic or organic compounds (e.g.,

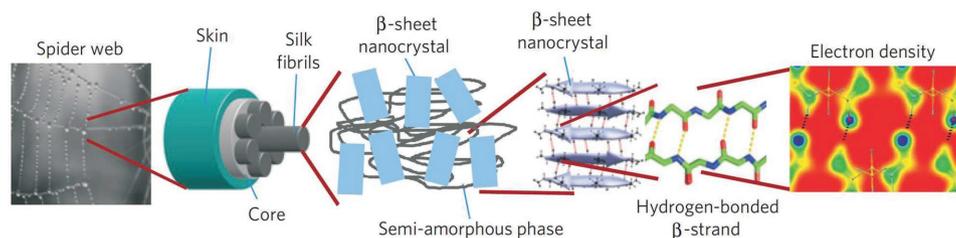


Figure 1. Schematic representation of the hierarchical structure of silk, from macro- to nanoscale (left to right): macroscopic silk fibers composed by assemblies of silk fibrils; stiff β -sheet nanocrystals in a softer semi-amorphous region; hydrogen bonds stabilizing these β -sheet nanocrystals; and electron density maps at the Angstrom scale (Reproduced with permission.^[2] Copyright 2010, Springer Nature).

silica,^[16] hydroxyapatite,^[17] graphene oxide^[18]) or even additional peptide sequences to create fusion proteins.^[19] These materials have potential applications in biomedicine, energy harvesting, electronics, optoelectronics, and photonics, just to name a few.^[3]

Despite all these advances in the development of silk-based materials, it is still of the utmost importance to fully understand how targeted properties (e.g., toughness, strength, degradability, porosity) are achieved through specific amino acid sequences, protein secondary structures, and/or various processing conditions. In this regard, computational modeling at different length scales provides fundamental understanding of structure–property and sequence–property relationships that can greatly accelerate the development of novel silk structures and silk-based materials with tailored specific properties.^[9] Nevertheless, these computational models must also be validated and improved by iterating between simulations and experimental research on the synthesis and processing of silk biopolymers.^[20] The requirement of such iterative process highlights the need for interdisciplinary research consortia to accelerate the development of silk into a mainstream feedstock in the materials industry. Here, we review how multiscale computational modeling has aided the understanding of the outstanding mechanical properties of natural silk, as well as the study, design, and prediction of properties of synthetic silk-based materials.

2. Computational Methods for Multiscale Modeling of Silk

Multiscale computational modeling offers a bottom-up way to predict the structure–function relationships of silk and silk-based materials *in silico*, and from the level of chemical interactions. Thus, using different modeling and simulation techniques, we are able to design these materials along different length scales, from the amino acid sequence to the macroscopic structure, to predict their properties.^[4] These *in silico* approaches can enormously reduce the costs of experimental research.^[20] Many of these bottom-up models for silk and silk-based biomaterials have been extensively validated by experiments, showcasing their predictive power.^[8,16,17,21]

Although the focus has been so far on understanding the mechanical properties of natural silk materials, multiscale computational modeling also offers the possibility to create new, synthetic materials based on silk. For instance, computational modeling has been applied in the fabrication of hydroxyapatite-

silk membranes^[17] or in the creation of synthetic modular biopolymers that combine features from different protein materials, such as the stiffness of silk domains and the elasticity of elastin domains. In the latter case, computational models were used in combination with recombinant DNA technology to design and create silk-elastin-like proteins (SELPs) that form soft, stimuli-responsive biomaterials.^[9,19,21]

The main challenge associated with computational modeling and simulations is the trade-off between attainable time- and length-scales and the accuracy in reproducing actual phenomena. A detailed, atomistic description of the properties of a silk-based material cannot be applied to large time- and length-scales due to the large amount of computational resources required. Conversely, the description of phenomena that occur over larger time- and length-scales is only affordable computationally if the atomistic accuracy is reduced, and by using model systems that simplify and approximate the description of the material. These problems can potentially be addressed with multiscale modeling that considers different levels of detail depending on the length scale at which the system is described. Based on the fundamental laws of physics, it can describe different features of a material with tunable accuracy by using different simulation methods with varying levels of computational cost and accuracy, that is, quantum mechanical (QM) methods, full atomistic and coarse grained (CG) molecular dynamics (MD) methods, and finite elements (FE) methods. For instance, full atomistic MD simulations were used to study the performance of silk biomaterials (e.g., robust hydrogels, composite silk-silica materials, or silk-elastin protein polymers) at the nanoscale.^[16,19,22] The intermediate mesoscale is accessible through CG models that merge groups of atoms into single beads that are parameterized *ad hoc* to reproduce the behavior of these specific groups of atoms. Composite silk-hydroxyapatite membranes for water filtration^[17] and regenerated silk fibers (RSFs)^[23] were examined with CG models, as well as the effects of shear on spider silk spinning,^[24] just to name a few.

Predicting protein structures is typically a key aspect for these computational models (Figure 2). However, silk proteins are often very large molecules and studying the slow evolution of their secondary and higher-order structures with atomistic-level MD simulations may not be tractable, as most MD simulations are limited to timescales of a few hundred nanoseconds. Therefore, researchers are placing greater emphasis on developing new advanced sampling techniques to predict protein structures more rapidly. These techniques include replica exchange

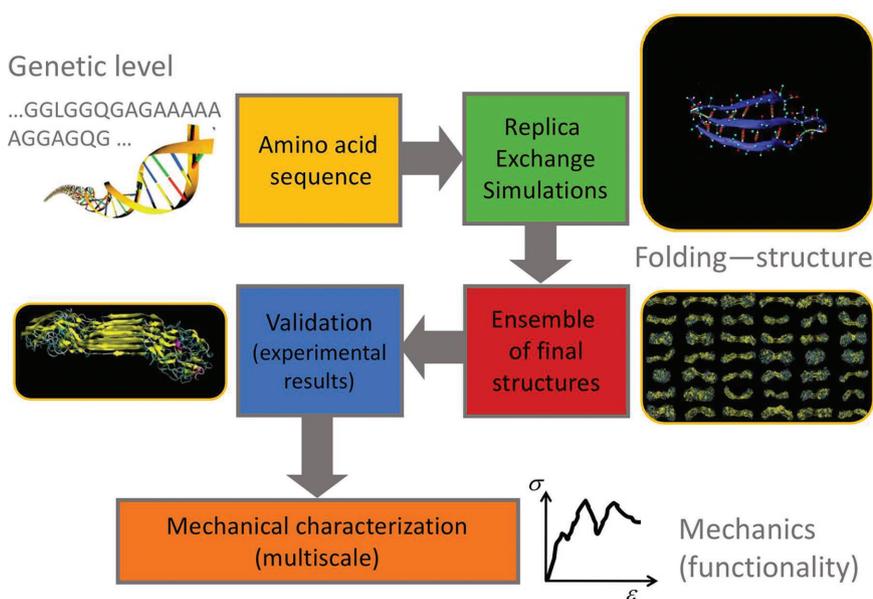


Figure 2. Predicting the mechanical properties of silk: the genetic code determines the sequence of amino acids in silk proteins. A key issue of silk materials is the understanding of the process by which it is formed. REMD simulations can be used to predict the most energetically favorable conformation. The resulting structure can be validated with experiments and used for computational modeling to predict the properties of silk (Adapted with permission.^[4] Copyright 2012, Springer Nature).

molecular dynamics (REMD),^[25] autonomous basin climbing,^[26] or TIGER2A.^[27] REMD in implicit water is typically used with subsequent solvation in explicit water to refine the protein structure.^[9,16,28] CHARMM^[21,28–30] and AMBER^[31,32] have been typical force fields applied for atomistic simulations.

3. Computational Studies of Natural Silk

Silk fibroin is expressed by spiders or silkworms, emerging as a liquid material before it is concentrated upon entering the spinning duct. Ionization of the environment and shear stress guide the extrusion process, thereby promoting and stabilizing the formation of β -sheet crystals of fibroin.^[33,34] The sequence of amino acids constituting the fibroin protein gives rise to certain physical interactions, specifically hydrogen bonds and electrostatic interactions, which control the self-assembly into delicate hierarchical structures with extremely high toughness and strength. The exact process by which spiders or silkworms convert an aqueous solution of amino acids into a robust silk fiber remains poorly understood. Learning the key mechanisms that govern the production and performance of natural silk has an importance that goes beyond just satisfying scientific curiosity, as it can also enable the bottom-up design and manufacture of complex biomaterials. To this end, multiscale computational modeling is a great tool for advancing this understanding.

The structural resilience of silk does not arise from the materials that constitute it, but from how these materials are hierarchically arranged in the 3D space.^[4] The several levels of hierarchy are governed by the protein sequence, which in turn governs how the proteins self-assemble into fibrils that form

fibers to yield a tough and strong structure with properties that are different from those of its building blocks. The origin of the excellent mechanical properties of spider silk^[2,6,35–39] and silkworm silk^[40] has been examined with fully atomistic MD simulations. Using such detailed models, computational modeling has contributed to elucidate the mechanism by which a specific combination of elastic regions and strong crystalline β -sheet domains, facilitated by hydrogen bonding,^[2,35,36] provides such remarkable mechanical performance to silk. This is counterintuitive, as hydrogen bonds are among the weakest chemical interactions in nature. However, multiscale modeling approaches have provided explanations for the nanoscale effects that maximize macroscale performance.^[2,4,41] Full-atomistic MD simulations showed that small nanocrystals (2–5 nm) of β -sheets were the key for the strength of silk, failing in a concerted manner with hydrogen bonds breaking in a stick-slip motion that enhanced the energy dissipation upon loading. Larger crystals led to material failure through bending, forming a crack-like flaw that

caused stress concentration and reduced the mechanical strength of silk.^[2]

At a larger length scale, mesoscale CG models identified a combination of crystalline phase and semi-amorphous protein matrix as a key factor to endow silk with such superior mechanical performance.^[24,42,43] The semi-amorphous regions unraveled first under load, while the crystalline regions provided strength.^[42] CG simulations also demonstrated that the threads in silk webs had a nonlinear response to stress: the fibers softened at the yielding point, while they increased their stiffness dramatically at large deformations, until failure was achieved.^[43] However, silk's outstanding performance was attributed not only to the merit of the constitutive fibers, but also to their geometric arrangements that confined stress in the vicinity of the loading point, thereby resisting impacts.^[44] The relationship between mechanical behavior of web structures and their key architectural features such as type (radial or spiral), number, and diameter of the threads, were also studied with CG models.^[4,8] It was reported that a homogeneous distribution of threads within webs was better suited for resisting point loads (such as the impact of an insect), whereas stronger radial threads combined with weaker spiral threads performed better for distributed loadings (such as wind loads). These studies showed that the variation in spider web architectures found in nature was related to their adaptation to the different loading conditions in their respective environments. The variable strength of different individual silk threads was also researched computationally, explaining how different amino acid sequences could vary the mechanical properties of silk by altering its secondary structure. Silk proteins rich in alanine were prone to generate stiff and strong crystals that provide



mechanical strength. Conversely, silk with high amounts of glycine in the protein sequence tended to become more disorganized and softer.^[2,42]

One particularly interesting property of spider silk is supercontraction: some silk fibers have the capacity to contract up to 50% in humid environments.^[30] This is a rare effect in biological materials, which tend to swell in humid environments. The biological role of this effect is still under debate, but multiscale modeling and simulations have provided possible explanations for its mechanism. It was shown that the process was highly influenced by the presence of tyrosine.^[30] The –OH group in tyrosine's sidechain acted as a donor or an acceptor of hydrogen bonds under dry conditions, whereas the –OH group became an acceptor in wet states. This switching happened at a lesser extent for arginine and serine as well. Under wet conditions, the presence of water affected the interactions between protein chains, especially where tyrosine was present, making them fold onto themselves. This effect was enhanced by the presence of the hydrophobic amino acid, glycine, in the amorphous areas of the protein. This induced the formation of coiled or helical structures that resulted in supercontraction.

Another effect of supercontraction has been proposed at the structural, macroscopic scale, where webs have been shown to stiffen and possibly yield better signal transduction through vibrations across the web fibers that help the spiders to orient themselves in the web.^[45–47] It was suggested that the deformation of a spider web under gravity loading as a result of water droplets in morning dew is strongly interconnected to the supercontraction phenomenon, offering one possible biological reasoning.

The spinning of dragline silk was examined with MD simulations to study how silk's secondary structures changed into fibrous structures under shear flow.^[33] Quantitative relationships were established between shear stress and secondary structure, which is relevant for the development of microfluidic devices that aim at mimicking the natural process of silk spinning. It was found that structures of at least six polyalanine repeats were needed to generate “silk-like” structures. Shear stresses of 20–50% of the failure stress of silk induced α - β transitions. By applying atomistic, CG, and well-tempered metadynamics simulations, researchers also investigated the biophysical mechanisms by which spiders store a solution of highly concentrated small repeats of silk proteins in their silk-extruding glands while preventing their self-assembly into fibers, so that silk can be produced on demand.^[29] The self-assembly of fibers can be achieved through the control of the salt concentration and pH in these glands, which affects the interactions within the protein's N-terminal domain that ultimately govern their polymerization into fibers. The simulations indicated that high salt concentrations weakened the salt bridges between protein monomers and prevented premature fiber formation during storage. Once the salt concentration was reduced, dimerization of N-terminal became more energetically favorable, thus silk fibers could be formed.

4. Computational Modeling of Silk-Based Biomaterials

Developments in materials science devote a great deal of attention to the design and production of novel functional

and tunable polymeric materials. To this end, silk appears as excellent feedstock due to its light weight, easy processing, biocompatibility, and excellent mechanical properties. Therefore, several research groups are actively developing methods to convert natural silk into synthetic biomaterials. Two typical approaches are 1) to develop novel processing techniques for native silk forms and 2) to genetically control the protein sequence of silk and to express it using microorganisms. In the first case, native silk must be disassembled into silk nanofibrils (SNF) in a solvent, and then self-assembled into supramolecular structures in the shape of, for example, membranes, fibers, or hydrogels, by casting or spinning the suspension and removing the solvent. In the second approach, recombinant DNA can add domains to silk proteins that enhance the interaction with different materials for the self-assembly of the fusion proteins into hybrid materials in combination with other components. This section provides an overview of how multiscale computational modeling has been applied to the design and production of these silk-based materials by following the above-mentioned approaches.

4.1. Silk Depolymerization and Self-Assembly

In order to fabricate silk-based materials, silk must be first depolymerized into micro- or nanofibrils that can be subsequently rearranged into different material formulations (e.g., membranes, hydrogels, or fibers). Several methods for creating biomaterials from silk include chemical methods with solvents (e.g., formic acid, hexafluoroisopropanol (HFIP)),^[23,48] physical methods (e.g., ultrasonication),^[49] or a combination of both.^[50]

From the modeling perspective, different levels of molecular simulations have been used to study the depolymerization of silk. A CG dissipative particle dynamics method was developed to understand the dynamics of silk polypeptide chains under ultrasonication and to track their evolution from a condensed assembled form into independent chains.^[50] MD results indicated that ultrasonication was effective in disrupting the amorphous (hydrophilic) regions in silk SNFs, but not the crystalline (hydrophobic) regions. Given the previously reported weaker connectivity of spun fiber bundles in the radial direction,^[24] the authors concluded that ultrasonication could effectively exfoliate fiber bundles along the fiber axis. MD simulations also helped to understand how to confer specific properties to the regenerated silk materials, in order to facilitate its manufacture into silk-based wearable electronics. For such an application, it is critical to obtain a silk material that is flexible enough to adapt to body motion and skin deformation. MD simulations proved that the addition of CaCl_2 made silk films more amenable to deformation, due to the coordinating capacity of CaCl_2 with water in the environment, acting as a plasticizing agent that kept the membrane humid and thus flexible.^[48,51]

The length of hydrophilic and hydrophobic peptide blocks proved to be critical in determining their self-assembly, which is vital for predicting block copolymer behavior. The self-assembly process was modeled with CG dissipative particle dynamics^[52] and validated with atomic force microscopy (AFM) data. Longer chains assembled into larger micelles of

a diameter of 32 ± 5 nm, whereas small chains led to small micelles of 2 ± 0.5 nm. Furthermore, not only the mechanical properties of silk-based materials, but also its biodegradability, were studied through MD^[53]: shorter lengths resulted in less ordered molecular structures in the silk, facilitating the access of solvents, which facilitates its degradation. Longer chains had more ordered domains with a lower solvent accessible area. These results were validated experimentally in *in vivo* assays.^[53]

4.2. Fibers

The experimental production of RSFs that mimic the mechanical performance of natural silk is a major research area in silk materials.^[15,23,50,54–56] The main focus of this research direction is on wet spinning techniques where the spinning dope (a silk suspension) is ejected through the tip of a syringe into a coagulation bath (e.g., water or methanol) that consolidates the fiber. Recently, a dry spinning technique using hexafluoroisopropanol (HFIP), a highly volatile solvent, was reported, yielding silk fibers with improved mechanical properties.^[23] Regardless of the technique (wet or dry), they all aimed at mimicking the spinning process of silk in nature, such that the isotropic and homogeneous nature of the silk suspension before spinning became anisotropic and heterogeneous after being subjected to shear flow.

The mechanical properties of RSFs were studied computationally (**Figure 3**) using an atomistic model^[57,58] to study how their mechanical properties were affected by different ratios of a polyalanine domain (susceptible to forming β -sheets) and segments of tripeptides with the repeats of the sequence GGX,

(being X typically alanine (A), tyrosine (Y), leucine (L), or glutamine (Q)), that formed helices and provided elasticity to the material. Pulling tests were performed computationally, and three stages were identified during deformation^[58]: 1) extension of the protein, 2) straightening of the two pulled strands, and 3) final shearing and failure. Having more hydrophobic domains increased the mechanical strength of the material due to the stacking of β -sheet crystallites and the shear strength increased due to the presence of more hydrogen bonds. Moreover, the straightening and alignment of fibers during the pulling test caused a marked increase in stiffness due to the formation of more β -sheets. When the fibers were rich in hydrophobic domains, they failed at lower strains, but at a much higher failure strength (3.45 nN). Conversely, fibers rich in amorphous domains could be stretched almost 1.45 times more but with a lower failure strength (2.40 nN).^[58] These simulations proved useful in identifying trends in the secondary structure and hydrophobic packing among domains under stress.

The properties of dry-spun RSFs were studied with CG models representing silk microfibrils^[23] to analyze the sliding of microfibrils within a silk bundle. Half of the silk microfibrils in the bundle were fixed on their left end, while the other half were pulled on their right end. Defects were included by randomly deleting some of the beads constituting the microfibrils. The results showed that the toughness of silk bundles increased with increasing diameters but at the expense of reducing the Young's modulus. RSF bundles with smaller diameters experienced brittle fracture, while thick RSFs led to ductile fracture. As the RSF diameter is directly related to the spinning speed, these studies provide guidelines for tuning the design of silk fibers to achieve targeted mechanical properties.

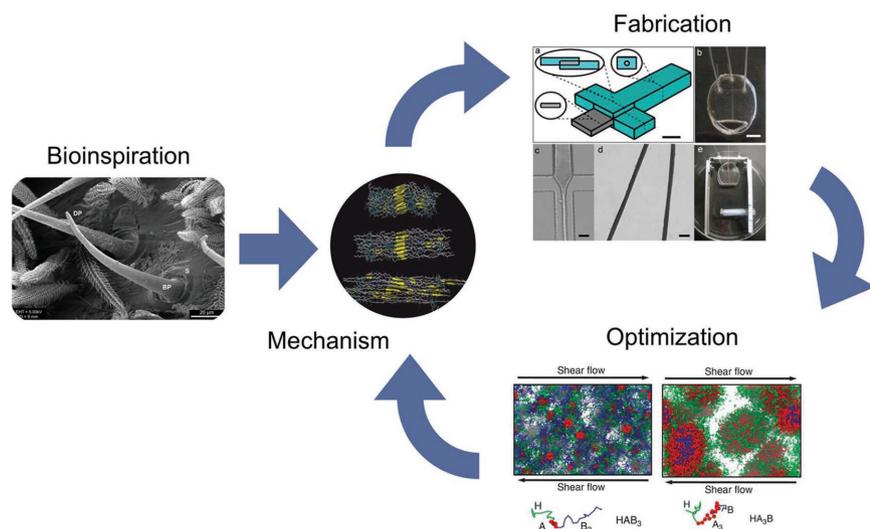


Figure 3. Production of silk fibers bioinspired from the spinning process in the spinnerets of spiders (Reproduced with permission.^[59] Copyright 2017, Elsevier). Molecular dynamics simulations can unravel the mechanisms that endow silk with its outstanding mechanical properties due to a combination of stiff β sheets imbedded an amorphous soft phase (Reproduced with permission.^[41] Copyright 2011, Wiley). This provides guidelines for the fabrication of strong and tough RSFs through processes that mimic nature, like microfluidics (Reproduced with permission.^[60] Copyright 2011, American Chemical Society). Coarse-grain simulations can be used to optimize the fabrication process by, for example, optimizing mixing ratios between soft and stiff domains (Reproduced with permission.^[24] Copyright 2015, Springer Nature).

4.3. Silk Composites

Silk can be used in combination with other materials to create hybrid materials (e.g., hydroxyapatite, silica, graphene oxide)^[16–18,31,54,61] and mechanically robust systems. These hybrid materials have applications in different areas like water filtration, strain measurements, and bone regeneration. In water filtration, silk-based materials have been shown to achieve excellent performance in retaining pollutants at the molecular level. In a different field, strain sensors made from these hybrids, in combination with 3D printing of a silk-fluorescent dye mixture, were capable of quantifying the deformation of biological tissues under mechanical loading. Several studies also reported combining hybrid materials with silica to enhance silicification for bone regeneration, or with graphene for producing conductive silk. In all these cases multiscale computational modeling was very helpful in understanding the interplay between

silk and the materials used to create the composites, and how these hybridizations affected protein folding and other related properties, such as the solvent accessible surface area. Thus, the formation of multilayered structures in silk-based membranes with an inorganic material was examined with CG models. Silk-inorganic interactions with a mild surface energy were critical parameters that dictated whether the resulting membrane would be a multilayered material or a homogeneous mixture of two materials. These interactions had to be weaker than silk–silk fibril and inorganic–inorganic interactions. This information was critical for the selection of suitable materials to form multilayered structures, as several protein–inorganic combinations have strong interfacial interactions. According to computational results, the interaction of silk with hydroxyapatite fulfilled the requirements to create multilayered structures, and filtration membranes were thus synthesized experimentally. The resulting membranes were confirmed to have a multilayered structure with good mechanical flexibility and toughness, as well as an outstanding retention capability for several organic pollutants. The formation of a multilayered structure was also suggested for the interaction of silk-graphene oxide membranes,^[18] although no computational proof was provided. However, some studies have used atomistic MD to study the properties of hybrid materials made of silk and carbon allotropes such as graphene^[31,62] or carbon nanoscrolls (CNS).^[32] It was reported that the interaction of silk with graphene was different for the various protein domains in silk fibroin. On the one hand, graphene tended to compete with the intramolecular interactions in crystalline β -sheet domains, weakening the interchain interactions and thus reducing the β -sheet content. On the other hand, random or less oriented secondary structures in silk fibroin became more stable in the presence of graphene, which increased the concentration of helical structures.^[31] It was also found that the strength of the silk crystalline domains was greatly enhanced by the addition of CNS, which shield the crystallites from water, reducing their hydration level. The CNS-silk interaction appeared to be highly affected by external stimuli, such as electric fields, which can be useful for the design of drug-delivery systems that use silk-CNS composites to protect the drug from degradation in water, while enabling the release of the drug through controlled external stimuli.^[32]

Biom mineralization is a key process for many biological functions in which silk can also play a relevant role.^[16,63] Mechanisms that induced silicification with silk materials were studied with atomistic simulations, and the knowledge gleaned was useful for preparing cell-instructive surfaces experimentally for tissue repair and regeneration. Simulations helped in elucidating how functional domains present in the fusion proteins that combined silk with other protein domains enhanced its interaction with silica. It was found that the key parameter for triggering silicification was the solvent accessible surface area of positively charged amino acids in the folded, assembled structures of the fusion proteins. Subsequent research used atomistic computational modeling to understand the binding between silk/silica-integrin,^[16] showing that the integrin was activated in contact with silk/silica complexes, and that the silica surface activated the integrin, rather than the silk-silica chimera. Ultimately, this was useful for understanding the mechanisms that stimulate

osteogenesis, by understanding how human mesenchymal stem cells initially adhere to the silk scaffold.

4.4. Hydrogels

Hydrogels are lightweight polymeric networks that are insoluble in water while accumulating a significant part of the aqueous medium within their porous network. They are usually mechanically weak materials, due to their low density and the low friction of the constitutive polymer chains, but offer important functions as biomaterials for replacement, tissue engineering, or soft robotics. Their mechanical weakness, however, hinders their implementation in applications that demand robust hydrogels. Recently, the preparation of strong, yet flexible silk hydrogels was reported using a binary-solvent-induced conformation transition (BSICT). This method avoided the complex procedures of inducing cross-links in the hydrogel network and based its strength solely on hydrogen bonds. Atomistic modeling elucidated the mechanism by which BSICT induced the gelation of silk fibers: the dissolution of silk in HFIP seeded the formation of β -sheets. These seeds formed larger β -sheets when water was added. The simulations also indicated the importance of adding HFIP before adding water to maximize the content of β -sheets in the hydrogels.^[22]

A particular type of stimuli-responsive hydrogels was manufactured using recombinant DNA techniques.^[19] These materials combined the stiffness of the crystalline domains of silk (GAGAGS) with the elasticity of elastin motifs (GXGVP) to create SELPs. SELPs could be assembled into a biopolymer that contracted when heated above a transition temperature. This phenomenon is known as the inverse temperature transition and it is typical for elastin proteins.^[21] The design of the genetic sequences encoding different types of SELPs was guided by computational modeling to predict the responses of SELPs to different environmental stimuli, such as temperature, pH, ionic strength or biological triggers. The authors reported the X amino acid in the elastin block to be key to the stimuli-responsiveness of the material: hydrophobic residues (e.g., C or F) conferred responsiveness to temperature changes; negatively charged residues (e.g., E) conferred a temperature response only at high pH, but not in water; using a biological recognition site (with the amino acid sequence RGYSLG) conferred an elevated inverse temperature transition temperature only after phosphorylation. Overall, the result proved that MD simulations could be used to design fusion protein-based biopolymers rationally.

The mechanical functions of SELPs and their unfolding process were studied at the molecular scale in atomistic simulation with steered MD at different pulling speeds and two different temperatures (7 and 57 °C). The simulations confirmed experimental observations of the material collapsing at higher temperatures, leading to a more compact conformation. It was found that the amount of hydrogen bonds in SELPs was larger at high temperatures. Enhanced energy dissipation of the biopolymer at experimentally relevant pulling speeds was predicted. Confirmed experimentally, this effect is counterintuitive as one would expect that the thermal effects at high temperatures will make the structure more easily perturbed.

Recent research provided further explanation of the molecular mechanisms that govern the stimuli-responsiveness of SELPS through a CG model.^[28] The development of CG models for SELPs was a significant advance, as it provided a tool to study the structural and mechanical properties of SELPs at a much lower computational cost than advanced sampling methods for fully atomistic systems, such as REMD. The CG model clearly showed the transition temperatures previously reported for SELPs and was extensively validated with experimental data. This work showed evidence that cross-links in SELPs could forestall deswelling at high temperatures. In this work, all-atoms simulations were used to obtain initial SELP structures, which were then CG with the PLUM potential that fully represented the protein backbone, while coarse-graining the side chains into a single bead.

5. Conclusions and Outlook

This paper explores different research efforts to use multiscale modeling and simulations to understand the outstanding mechanical properties of silk, as well as to develop predictive computational frameworks for the design and development of silk-based biomaterials. Multiscale computational modeling provides an excellent platform to support such developments by understanding the mechanisms and time-evolution of the building blocks of silk-based biomaterials, which is needed to optimize their production conditions. Both all-atoms and CG MD simulations have proven useful in elucidating molecular-scale mechanisms that affect the mechanical and biological properties of silk fibers. Especially useful is the development of more accurate CG methods to study the properties of silk materials at the mesoscale with reduced computational cost. The focus of computational modeling and simulations of silk was initially placed on understanding the mechanical properties of spider silk and spider webs. Their mechanical performance was identified to be closely related to the presence of β -sheet nanocrystals and a well-balanced ratio of hydrophilic and hydrophobic domains in the silk fibroin protein. These developments enabled the study of how to process silk into biomaterials, such as silk membranes, composites, or hydrogels. Subsequent computational studies enabled also the prediction of the properties of non-natural silk-protein biomaterials that combine domains from different proteins through recombinant DNA techniques, for example, to improve the interaction with inorganic surfaces for tissue engineering, or the creation of thermoresponsive hydrogels.

Despite current advances in biotechnology, synthetic biology, and nanotechnology, the still-limited capacity to control some material properties at the nanoscale restricts our ability to optimize the properties of silk materials at larger scales. Nevertheless, the opportunities for research toward bottom-up design and manufacture of silk-based materials expand far beyond aiming at just trying to imitate nature, but rather using self-assembly of protein-based biopolymers to manufacture novel silk-based materials with properties that outperform current engineering counterparts, and even natural materials. Recent research has shown how novel processing techniques (e.g., microfluidics, solvent-biopolymer interactions or dry spinning) could help to tune the hierarchical structure and

mechanical properties of, for example, silk fibers,^[23] silk hydrogels,^[22] or nanocellulose.^[64] These techniques open opportunities in supramolecular chemistry to create silk-based biomaterials with novel electrical, optical, or mechanical properties, alone or in combination with, for example, hydroxyapatite,^[17] nanocellulose,^[65] or graphene and graphene oxide.^[18,61,66] Some of these applications include, just to name a few, biophotonic devices with structural color changes for biological^[67] or mechanical^[48] sensing; flexible electronic devices for monitoring in soft and curved biological systems^[68]; stimuli responsive biomaterials based on protein folding–unfolding for drug delivery^[21]; or silk-reinforced green elastomeric composites for soft robotics and flexible tubes.^[69] Using recombinant DNA techniques to create non-natural proteins (like the aforementioned work with SELPs)^[19] is another promising approach to develop new silk-based materials. This last option can greatly benefit from the developments in gene editing techniques (e.g., CRISPR-Cas9) that provide a much easier way to modify the DNA sequence.

Future challenges also include finding innovative methods to design complex hierarchical materials, in which algorithms based on machine learning can offer new pathways to accelerate the design process. Recent work has demonstrated the design of composites against fracture, and we envision that similar methods can be applied to the design of protein sequences, processing, and other manufacturing parameters.^[70,71] Another exciting area of investigation is the study of 3D spider webs, and the design of innovative additive manufacturing processes. For instance, whereas folding, assembly, and similar methods focus on smaller scale aspects, the deposition of material in space is achieved by additive manufacturing (3D printing), which enables the creation of larger-scale hierarchical features. The spider itself can be viewed as an autonomous 3D printer, which could serve as a template for future technology.^[8,72–74] **Figure 4** shows an image of a

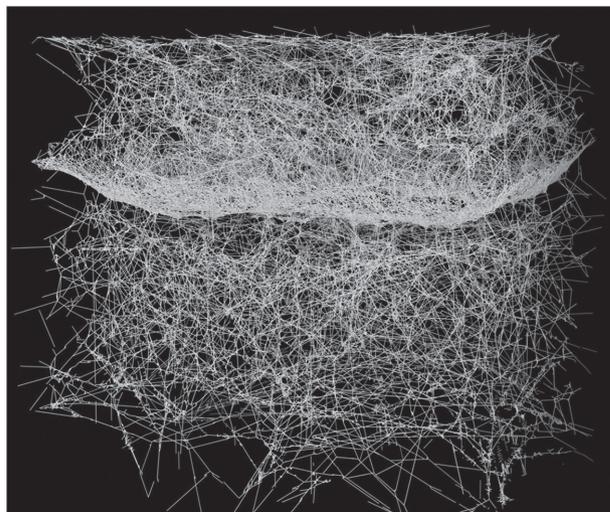


Figure 4. Computational model for the reconstruction of 3D spider webs from scanning a real 3D web, as reported in ref. [74]. These models provide insight into how spiders optimize their webs for impact and loading resistance. This can inspire engineers to design new, more reliable, and resistant synthetic structures and composites.



complex 3D spider web that offers exciting new challenges in modeling, simulation, and translation of design from the web to engineered, synthetic systems. The future challenges, together with the already proven benefits of using multi-scale modeling for understanding of structure–processing–property relationships of silk and silk-based biomaterials, make this an ever-growing field that holds great promise for the development of sustainable, biocompatible, and functional biomaterials.

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Conflict of Interest

The authors declare no conflict of interest.

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