Biomaterials: Predictive Design, Synthesis and Material Properties

Markus J. Buehler[1], Joyce Y. Wong[2], David L. Kaplan[3]

mbuehler@mit.edu jywong@bu.edu david.kaplan@tufts.edu

[1]Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[2]Department of Biomedical Engineering and Materials Science and Engineering, Boston University, Boston, MA 02215, USA

[3]Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA

PROJECT SUMMARY

MOTIVATION: New integrated modelling and experimental approaches are needed to improve biomaterial design and functional outcomes. Scalable computational modelling tools are required to integrate with and guide the experimental design of polymers in order to generate new biomaterials with predictable functions.

APPROACH: We utilize mesoscopic simulations with a coarse-grained description of silk proteins as multiblock copolymers, synergistically integrated with genetic protein sequence bioengineering and bio-inspired shear-flow focusing processing, to systematically demonstrate how biological silk spinning and the resulting features can be understood, predicted, and optimized *in silico* (**Fig.1**).

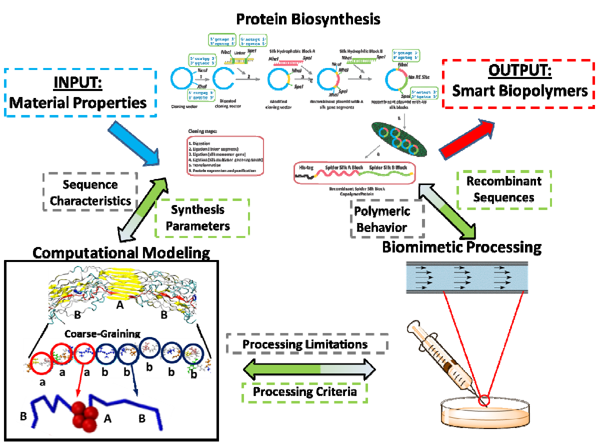

Figure 1. Synergetic integrations of multi-scale modeling, silk polymer synthesis, and microfluidic fiber spinning to study the hierarchical structure of silk polymers and their self-assembly process.

AIM 1: ***in vitro preparation and characterization of fiber-based biomaterials.***
AIM 2: ***in silico multiscale modeling and design of fiber-based biomaterials.***
AIM 3: ***in vivo characterization of fiber-based biomaterials.***

- ❑ The proposed study allows us to design biomaterials *denovo* by establishing a predictive modeling toolkit.
- ❑ Inputs such as the amino acid sequence, molecular weight, and processing conditions are used to predict new outcomes: hierarchical structure from the molecular level upwards and mechanical properties at all relevant scales.
- ❑ The same "universal" library of elements (amino acids) can be extended to many biomaterials as diverse as spider silk, tendon, cornea, blood vessels, or cells, each of which displays greatly variegated functional properties.
- ❑ We anticipate that our insights from the planned study would have broad impact and utility in a range of biomaterial and regenerative medicine needs.

Methods: In order to fully harness the advantage in natural silk spinning processes to generate defined material features, our integrated research group combines state-of-the-art multi-scale computational modeling and experimental synthesis techniques to produce artificial silk-based fibers using regenerated and recombinant silks from spiders and silkworms (**Fig. 2**). We also addresses the synthetic imitations by unifying the knowledge about the interplay between structure, processing, and properties.

PROGRESS AND MILESTONE

- ❑ Dissipative Particle Dynamics (DPD) simulation snapshots of (b) HAB_3, (c) $H(AB)_2$, and (d) HA_3B confirm that higher "A"-"B" domain ratio leads to larger aggregates both before and after shear flow (**Fig. 2**).
- ❑ Only the H(AB)2 sequence exhibit weak network connectivity, while the other two sequences do not form any network (**Fig. 3** (a)).
- ❑ The median size of each aggregate or cluster **Fig. 3** (b) is increased during the shear flow, while the total number of bridges **Fig. 3** (c) is reduced for H(AB)2 during the shear flow and remains zero for both HAB3 and HA3B.
- ❑ Using the optimal "A"-"B" domain ratio predicted above (1:1), we pursue the effect of polymer chain length by simulating the H(AB)n (n = 2, 4, 8, and 12) systems after equilibration ((a)-(b)) and shear flow ((c)-(d)) (**Fig. 4**).
- ❑ We find stronger polymer networks for longer polymer chains before shear flow. Longer polymer chains also exhibit better network conductance along the fiber direction after shear flow.

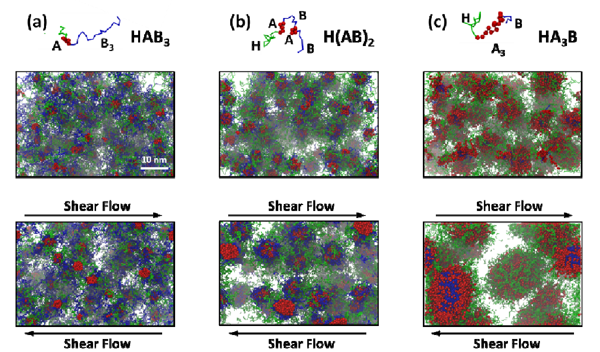

Figure 2. The Effect of "A"-"B" domain ratios. Color code: amorphous phase in blue and green and ordered phase (β-sheets) in red.

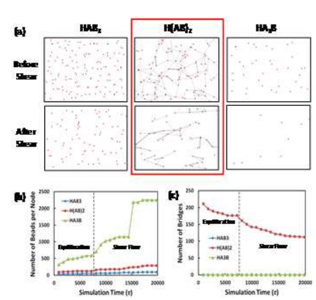

Figure 3. polymer Network analysis.

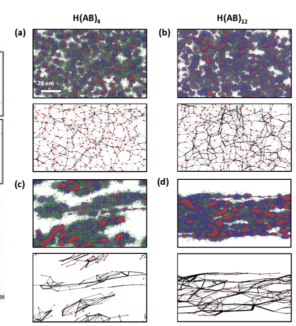

Figure 4. Polymer network diagrams for H(AB)n sequences.

❑ **EIGHT new spider silk block copolymers were engineered for this study:**

- **HAB_2, HAB_3, and HBA_{2-6}**
- **$H(AB_3)_2$, $H(AB_3)_4$, and $H(AB_3)_8$**
- **$H(A_2B_2)$ and $H(A_2B_2)_2$**
- **$H(AB)_2$, $H(AB)_3$, and $H(AB)_{12}$**

$H(AB)_n$ family is the most promising system based on simulation data

- ❑ H(AB)2 self-assembled into small micelles with an average diameter of 2 nm ± 0.5 nm; whereas, H(AB)12 formed larger micelles with an average diameter of 32 nm ± 5 nm as detected by AFM and DLS (**Fig. 5**).
- ❑ Successful spinning of macroscopic fibers (~ 1 cm long) using the $H(AB)_{12}$ sequence is confirmed by both bright field optical microscope and SEM images (**Fig. 6**).
- ❑ The spun $H(AB)_{12}$ fibers exhibit enhanced stiffness modulus of 1 ~ 8 Gpa (**Fig. 6**).
- ❑ The experimental findings agree well with the model predicted enhancement in the polymer network stiffness modulus after shear flow treatment, confirming that flow-focusing and shearing in the spinning process can facilitate the assembly of silk fibers by aligning individual polymer chains.

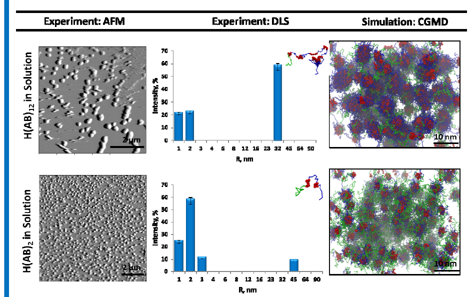

Figure 5. AFM, DLS, and coarse-grained molecular dynamic (CGMD) simulation results on aggregate morphologies of H(AB)12 and H(AB)2 recombinant spider silk block copolymers in aqueous solution.

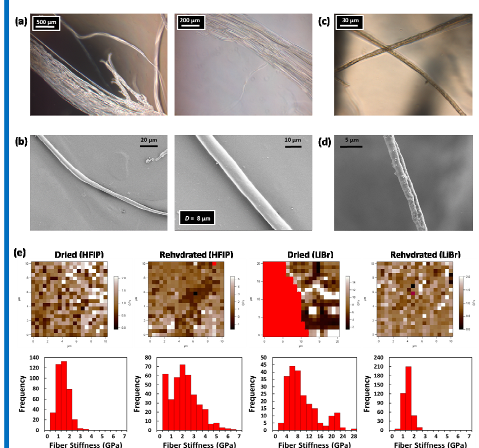

Figure 6. Recombinant silk fibers with the $H(AB)_{12}$ polymer: (a) bright field microscopic images of spun fibers in bundle in the coagulation bath and (b) SEM images of the separated fiber after rehydration. (e) The stiffness maps (top) and the corresponding stiffness distributions (bottom) of the four different types of fibers as probed by nanoindentation.

PUBLICATIONS BY THE GROUP

[1] Lin, S.; Ryu, S.; Tokareva, O. S.; Gronau, G.; Jacobsen, M.; Huang, W.; Rizzo, D.; Li, D.; Staii C.; Pugno, N.; Wong, J.Y.; Kaplan, D.L.; Buehler M.J., Predictive design, synthesis and spinning of biological silk fibers, *Nature Communication,* 2015, 6.

[2] Tokareva, O.S.; Lin, S.; Huang, W.; Jacobsen, M.; Wong, J.; Cebe, P.; Buehler, M.; Kaplan, D.L., Effect of sequence features on fiber formation with spider silk block copolymers, *Journal of Structural Biology,* 2014, 186 (3), 412-419.

[3] Tokareva, O.S.; Jacobsen, M.; Buehler, M.; Wong, J.; Kaplan, D.L., Structure-function-property-design interplay in biopolymers: spider silk. *Acta Biomaterialia* 2014, 10 (4), 1612-1626.