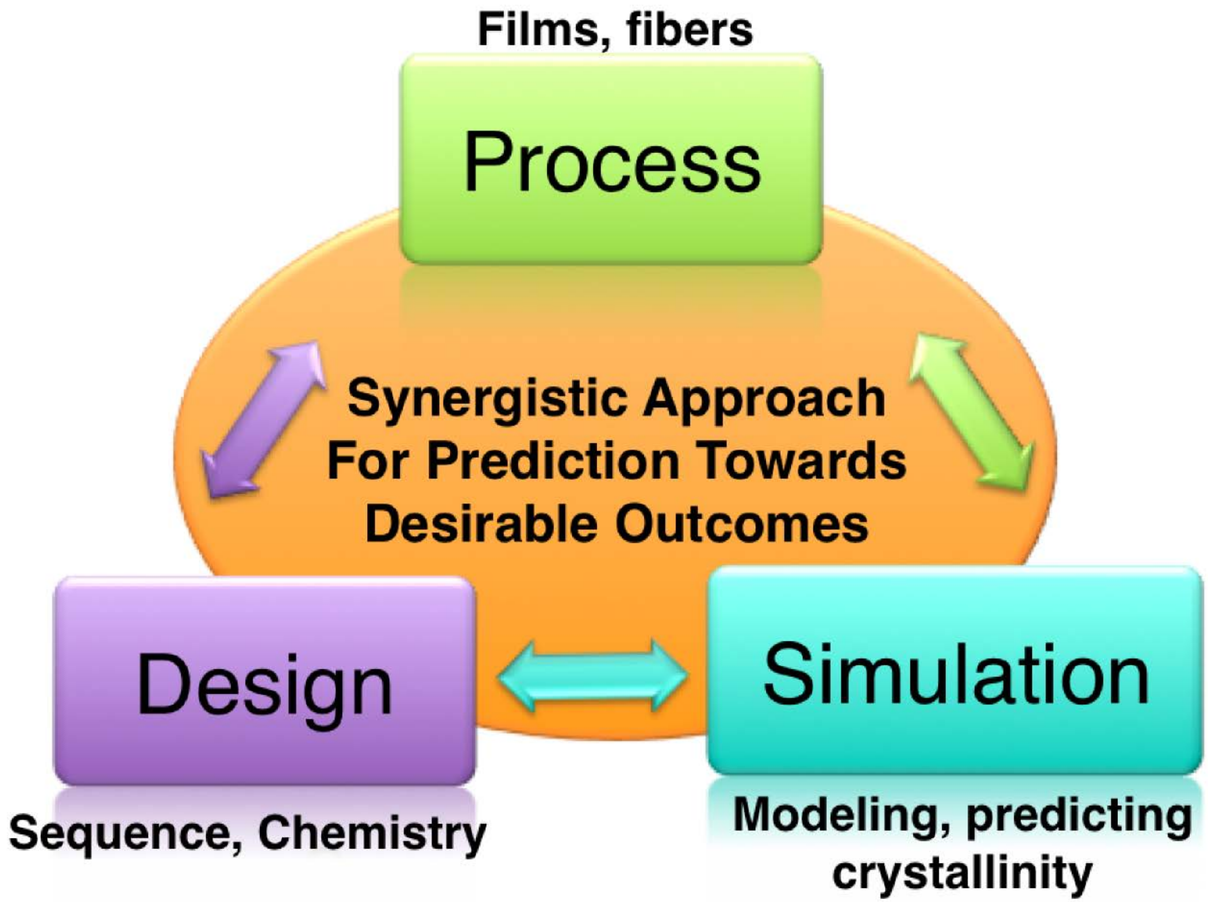
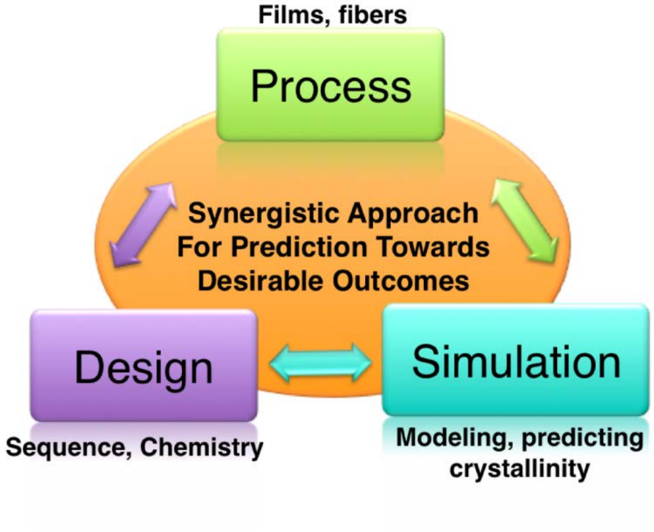
Models to Predict Protein Biomaterial Performance



Markus Buehler (MIT)
David L. Kaplan (Tufts University)
Joyce Wong (Boston University)

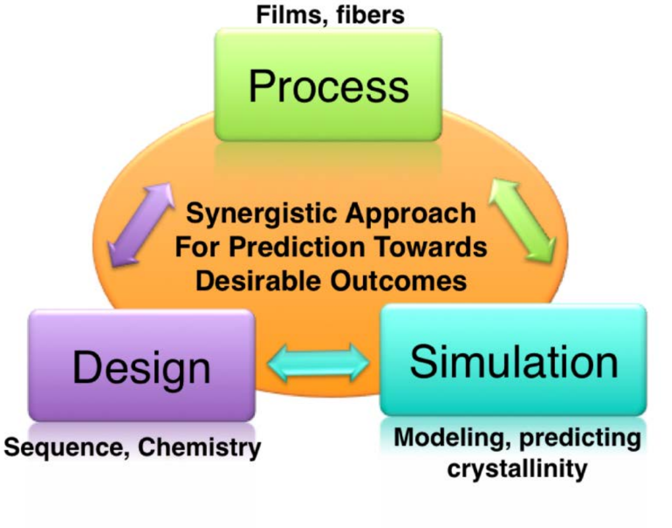
Objective



Develop **predictive assessments** of the in vivo performance of protein biomaterials

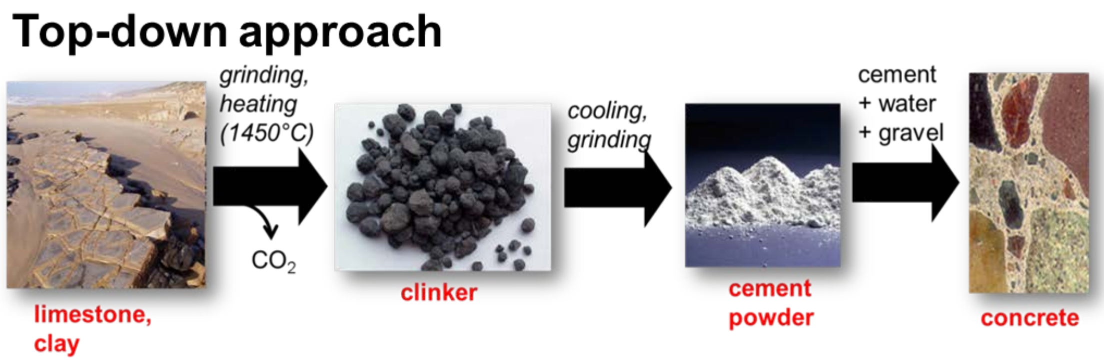
Reduce the current trial-and-error approach utilized in the field with a **more rational approach** based on **bottom-up multiscale modeling** to guide the preparation of the required materials

Hypothesis

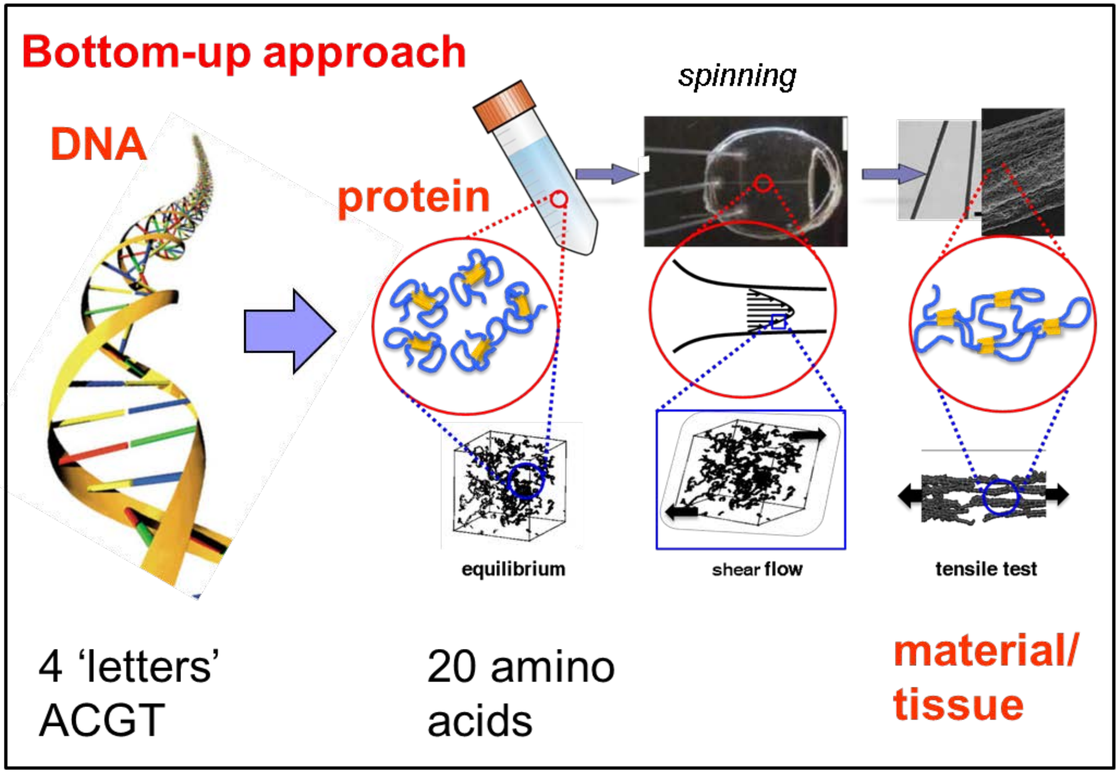


Predictions of biomaterials performance can be attained by the **combined use of suitable experimental models** to cover **polymer features** (chemistry, molecular weight), **processing** (fiber morphology, hierarchical structure) and **modeling** at different length scales of a **material's structural hierarchy** (from chemical to macroscopic scales)

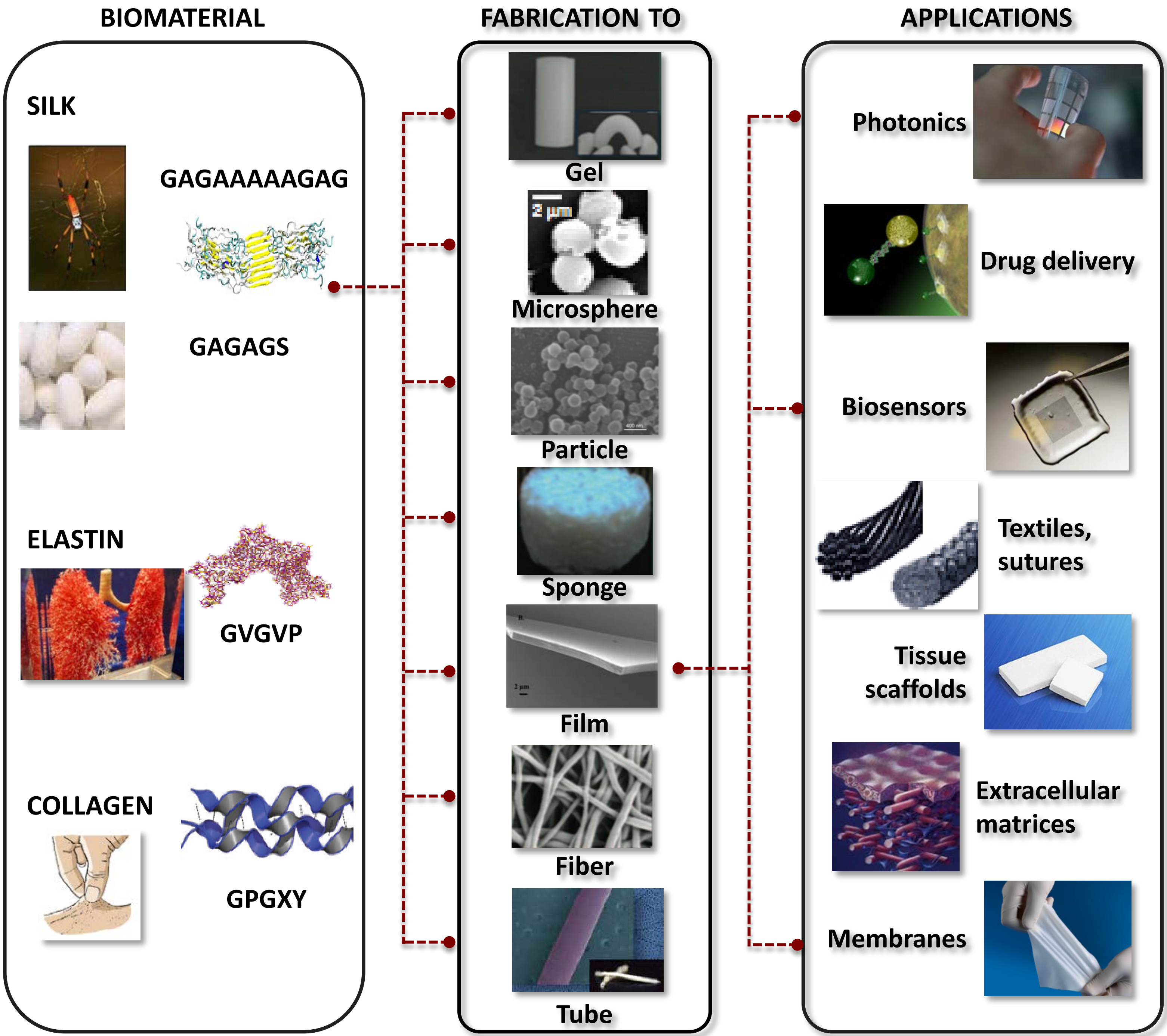
Conventional vs. new material design and manufacturing approaches



Protein-based biomaterials as the example

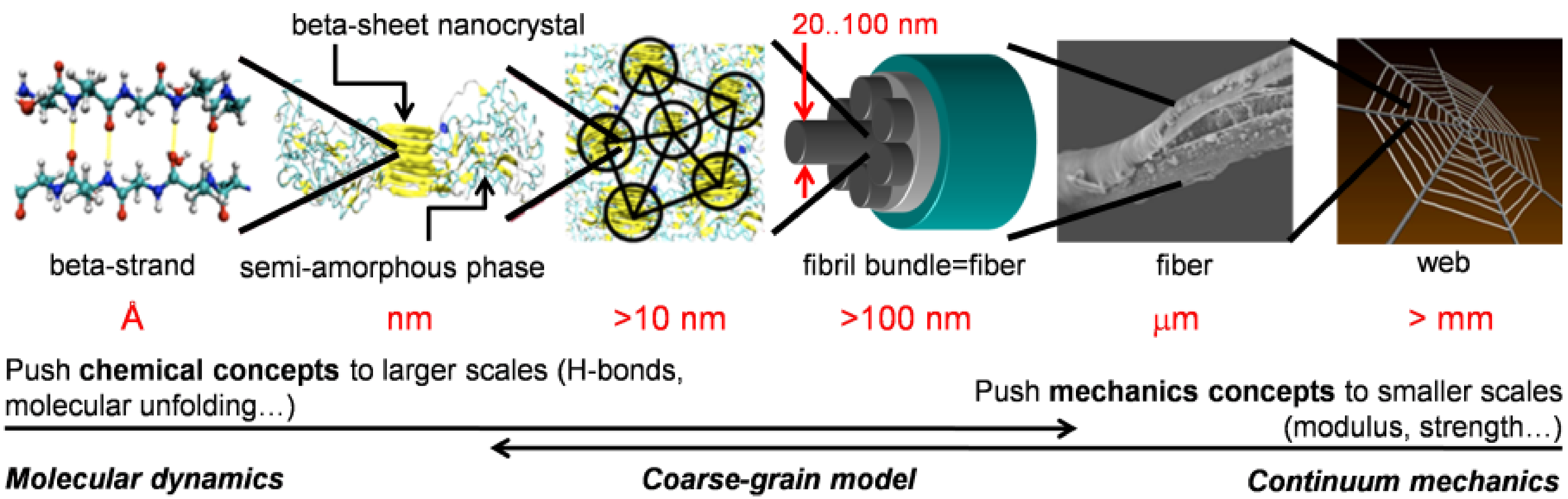


Wong *et al*., Nano Today, 2012



Kaplan, Wong, Buehler *et al.*, *Biomaterials*, 2012

Multiscale hierarchical structure illustrated for silk

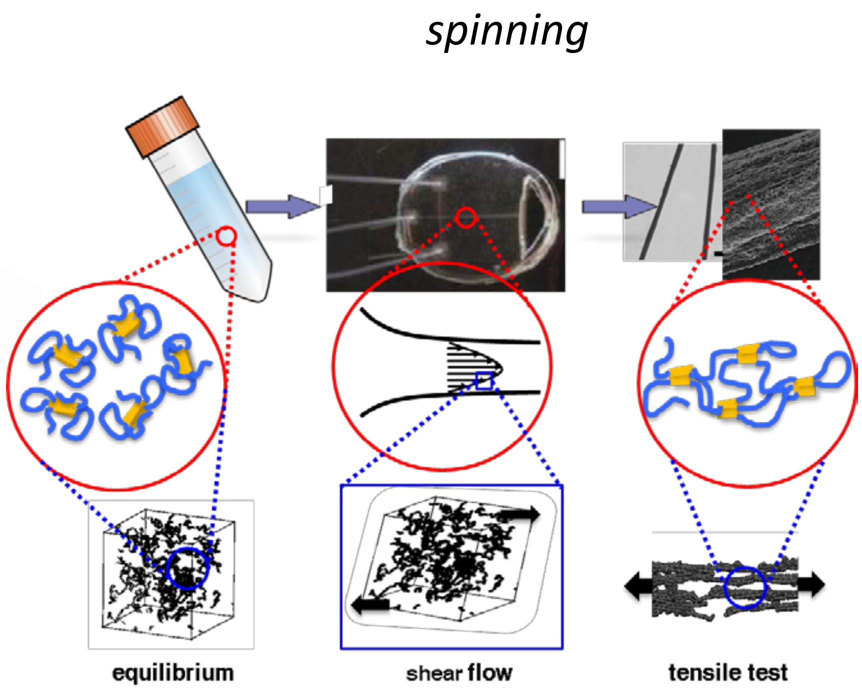


Giesa, Buehler *et al., Nano Letters*, 2011

Design, processing, and material properties are closely linked

DNA

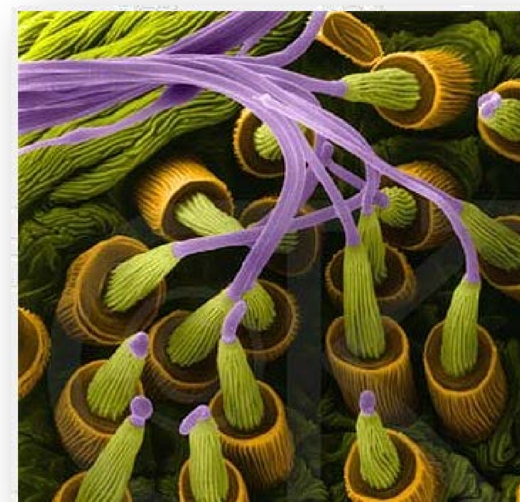
4 ‘letters’
ACGT



protein

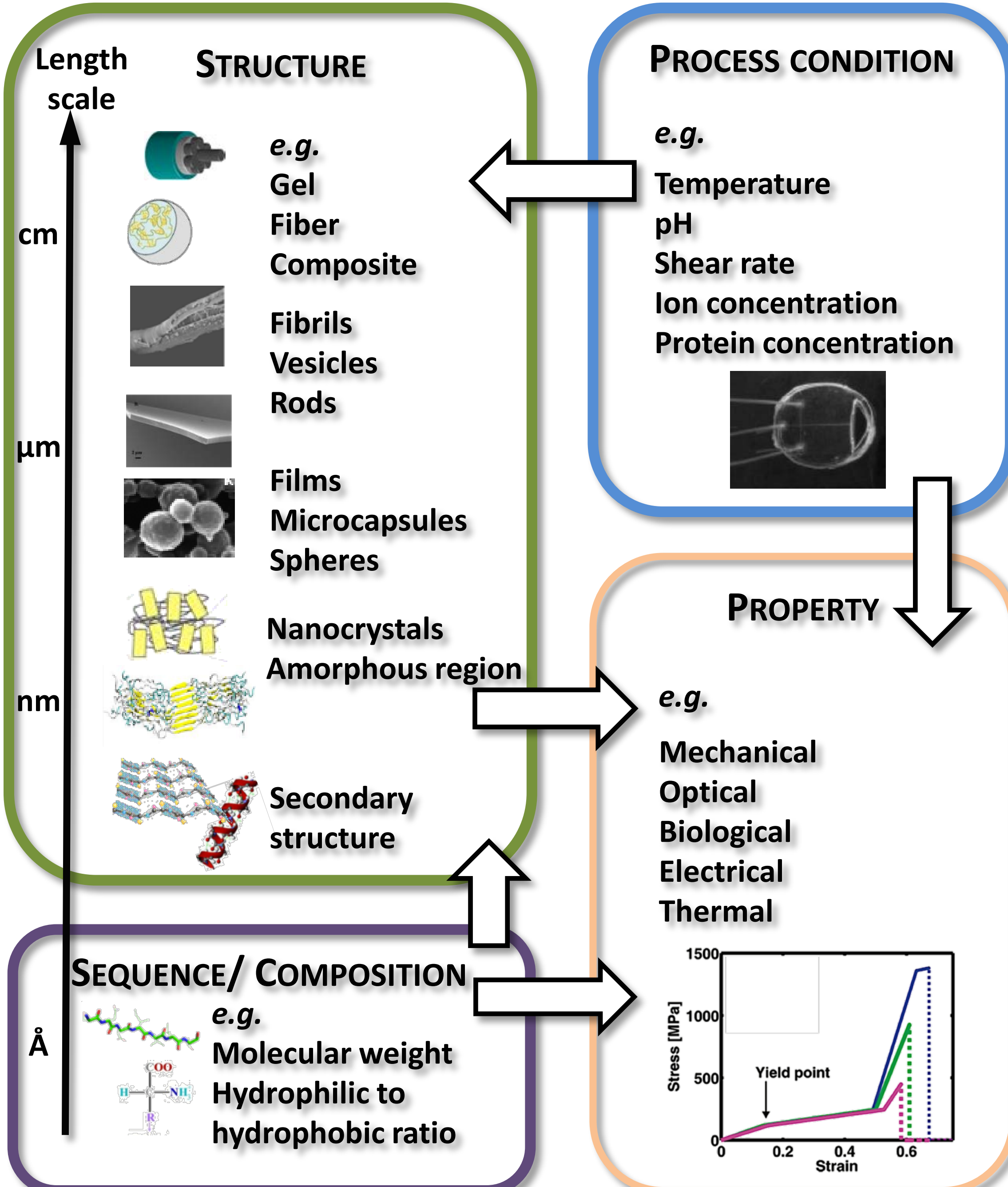
20 building blocks
(amino acids)

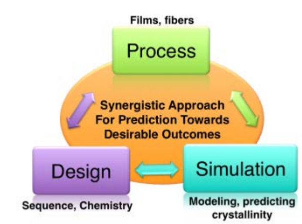
**material/
tissue**



(spider) silk spinning
liquid to solid

Biopolymer design: sequence, structure, process condition and properties





Kaplan, Wong, Buehler *et al.*, *Biomaterials*, 2012

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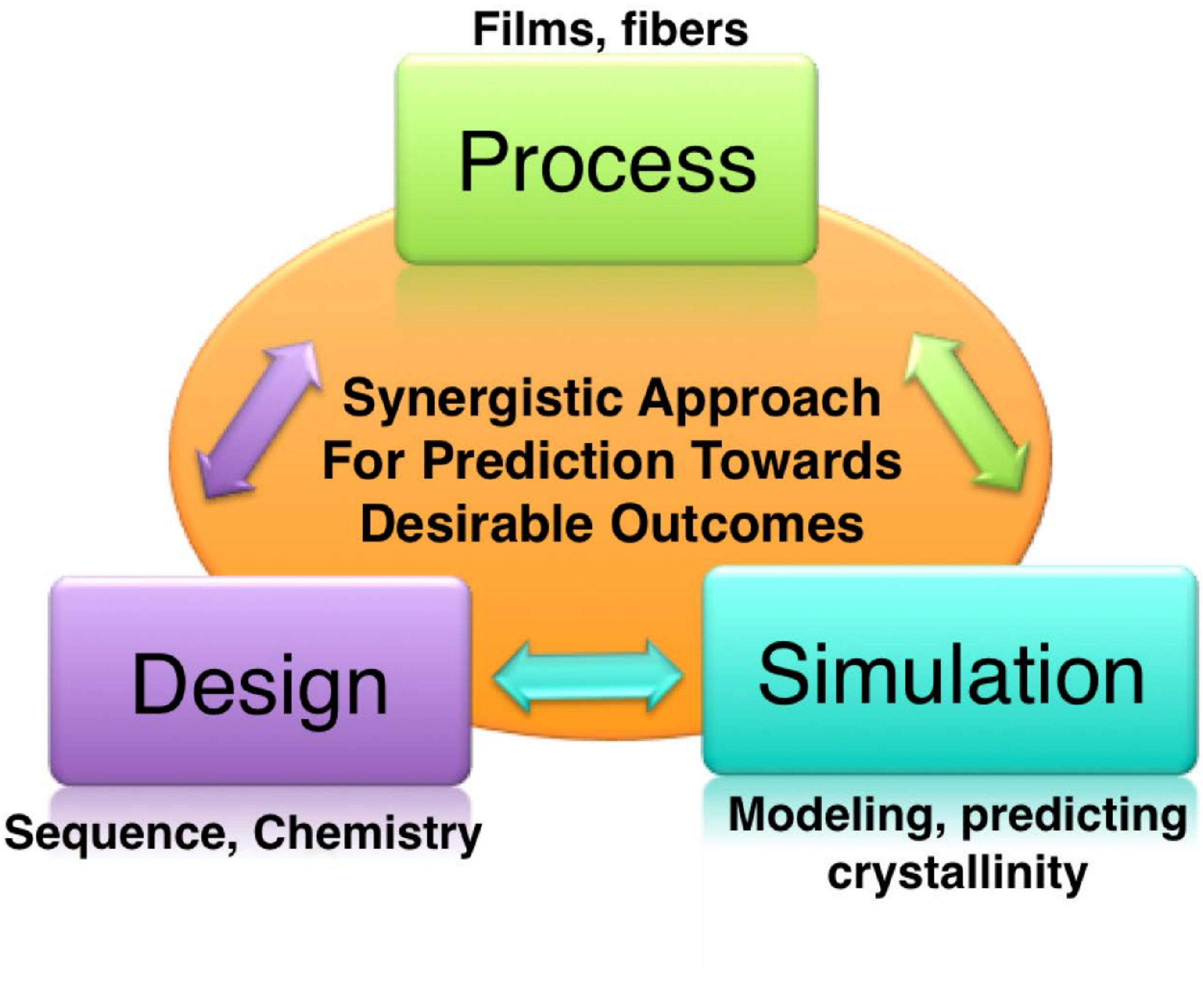

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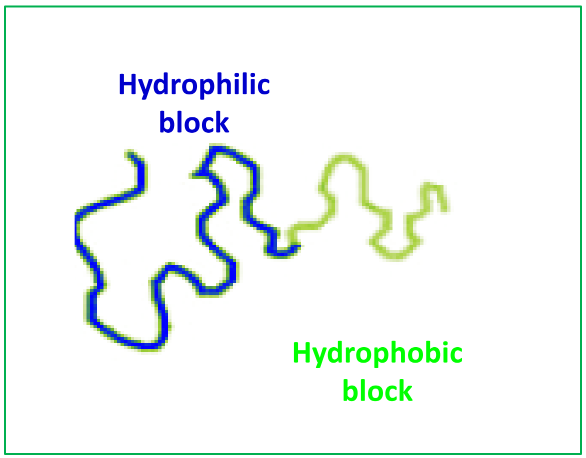
Silk Block Copolymers – Design & Self-Assembly

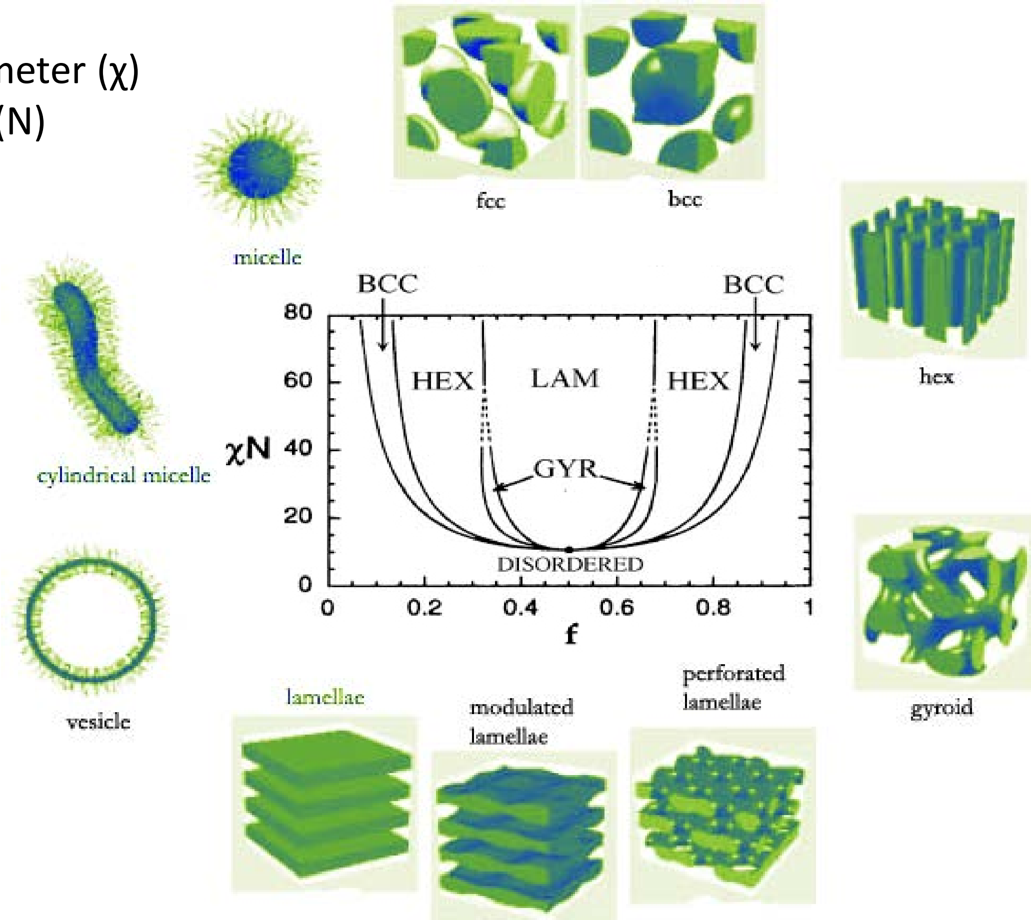
<u>Phase separation controlled by</u>:

-Block length ratio (f_A)

-Segregation (Flory-Huggins) parameter (χ)

-Overall degree of polymerization (N)

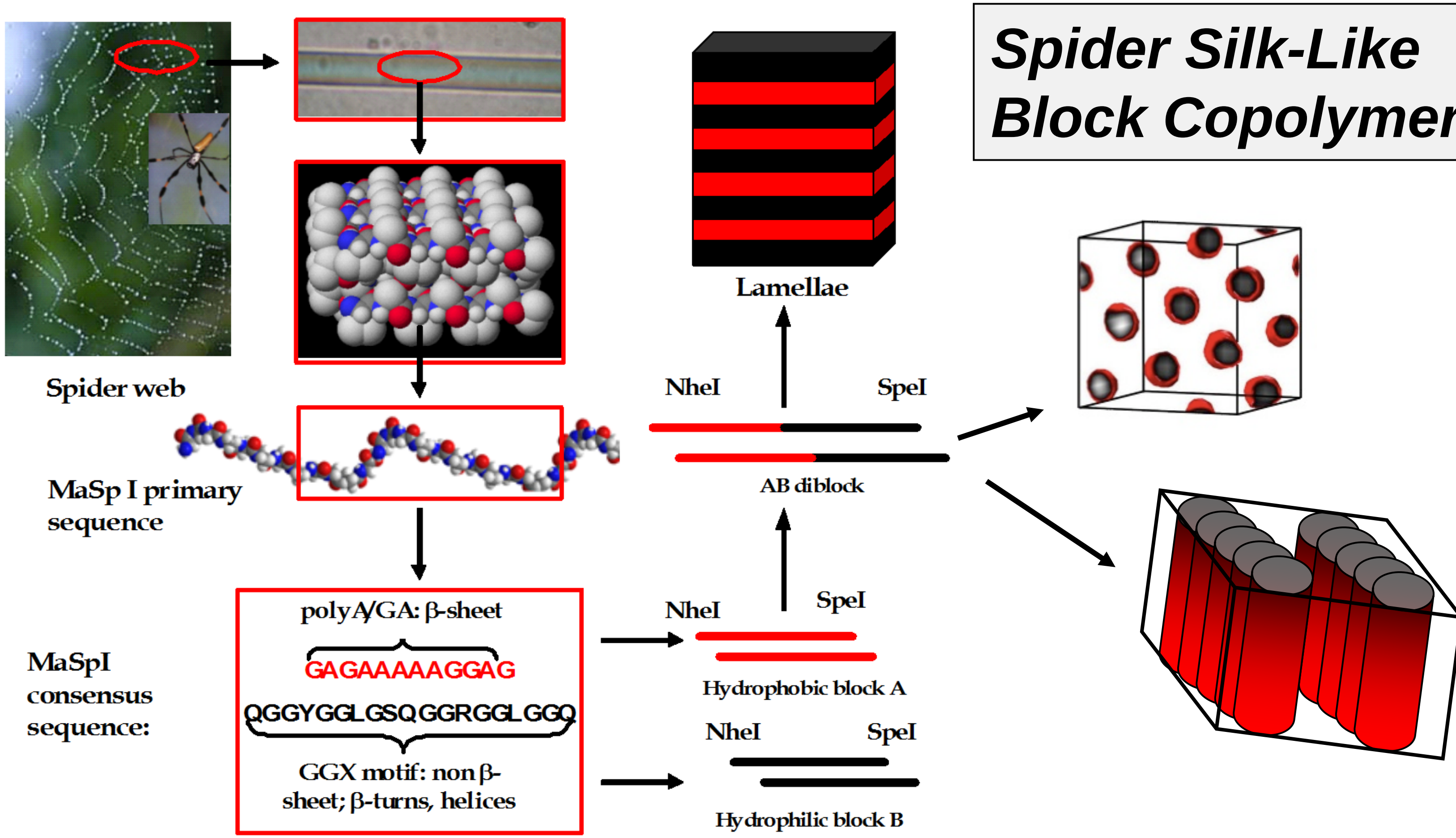


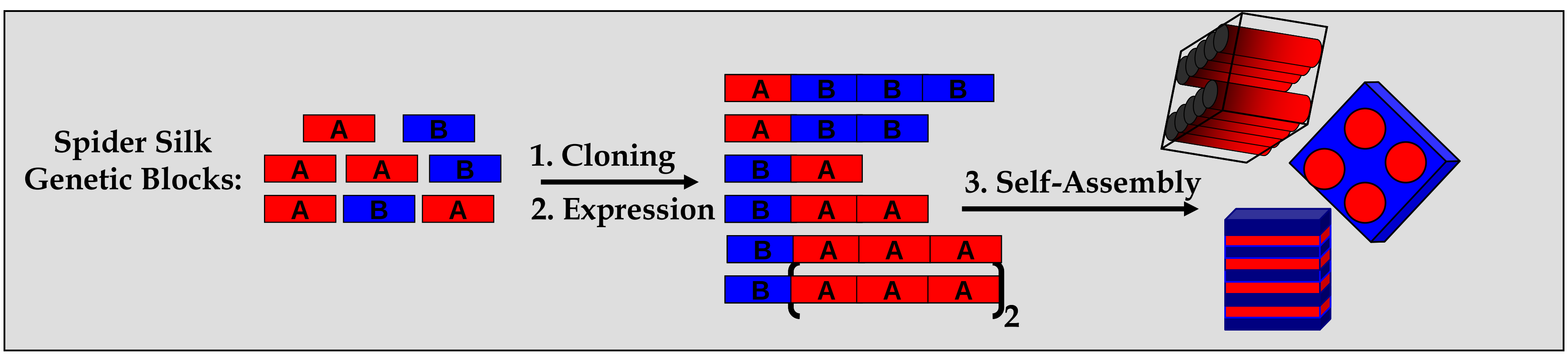


Self-assembled structures of polymeric materials

Ikkala *et al.*, *Chem. Comm*. **2004** *19*, 2131

Spider Silk-Like Block Copolymers



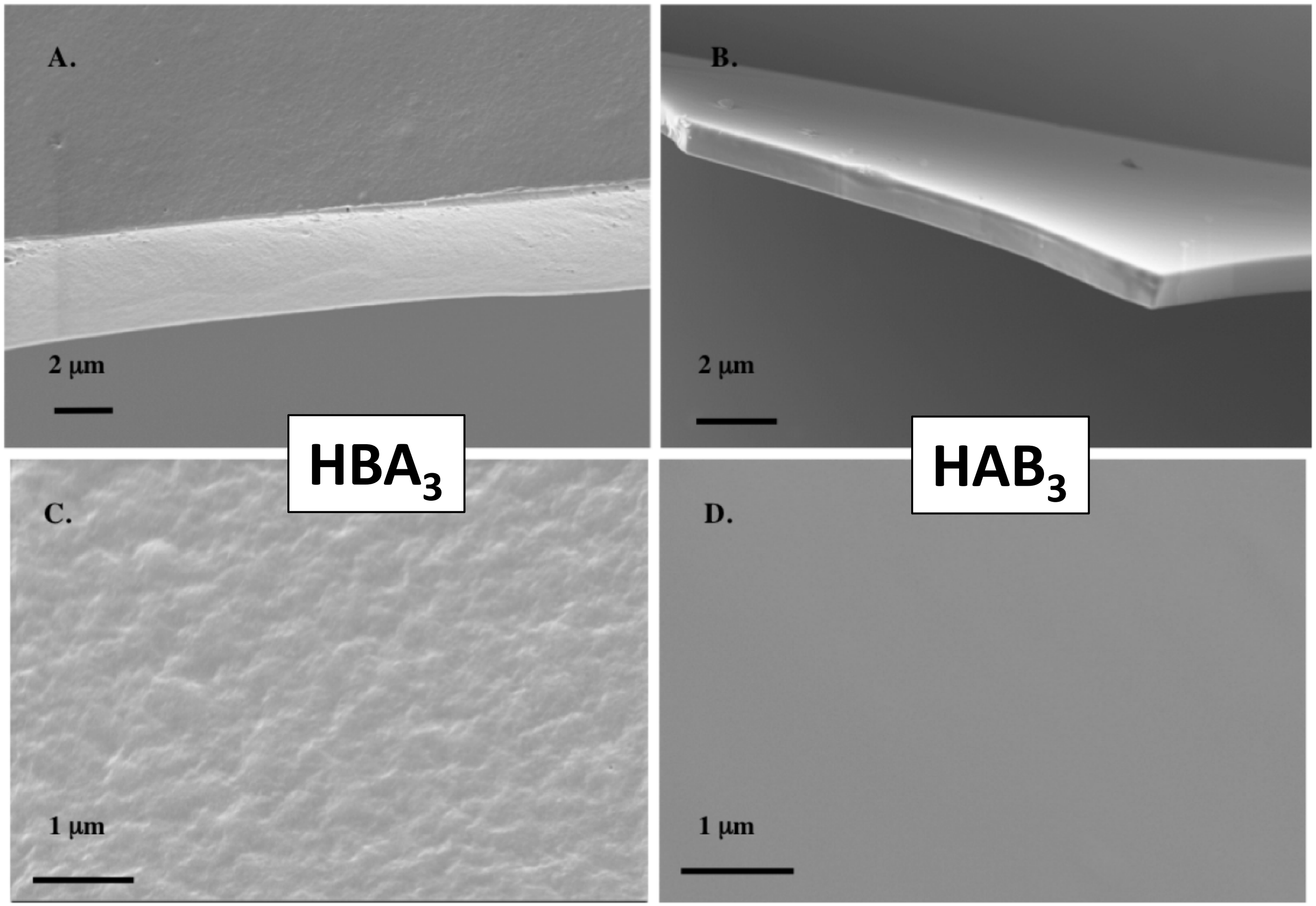


Example – Biosynthesis of protein variants based on spider silk

Code	Spider silk-like block copolymers	Mw [Da]
HBA$_3$	*M H H H H H H S S G L V P R G S G M K E T A A A K F E R Q H M D S P D L G T D D D D K A M A A S* <u>*Q G G Y G G L G S Q G S G R G G L G G Q T S*</u>***G A G A A A A A G G A G T S G A G A A A A A G G A G T S G A G A A A A A G G A G T S***	10,068
HAB$_3$	*M H H H H H H S S G L V P R G S G M K E T A A A K F E R Q H M D S P D L G T D D D D K A M A A S*___G A G A A A A A G G A G T___<u>*S Q G G Y G G L G S Q G S G R G G L G G Q T S Q G G Y G G L G S Q G S G R G G L G G Q T S Q G G Y G G L G S Q G S G R G G L G G Q T S*</u>	11,967

hydrophilic block B is underlined
hydrophobic Block A bold
hexahistidine tag italicized.

Films – materials formation from spider silk block copolymers



Scanning Electron Microscopy

Characterization of Spider Silk Block Copolymers

Percentage of structures after FTIR and Deconvolution

Code	Beta sheet	Turn	Alpha helix	Random coil
HBA_3	21.1±5	24.8±3	13.2±5	12.6±5
HAB_3	10.8±3	27.4±5	8.7±4	11.8±5

Mechanical properties - water annealed films

Code	Linear Elastic Modulus @ 2-3% strain [MPa]	Ultimate Tensile Strength [MPa]	Failure strength [%]
HBA_3	161.9±27.5	13±0.7	43.8±16.9
HAB_3	553±31.3	20.9±7	6.1±3.4

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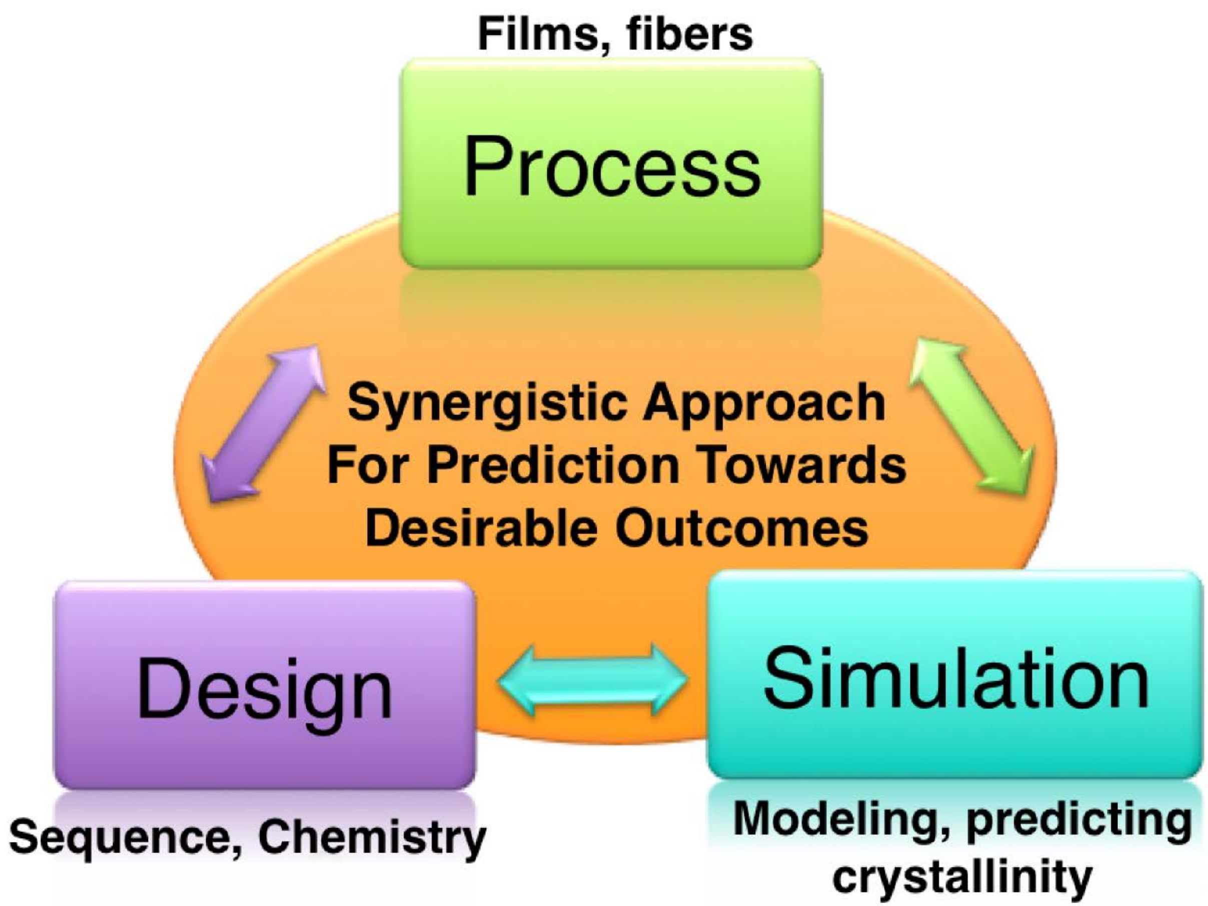

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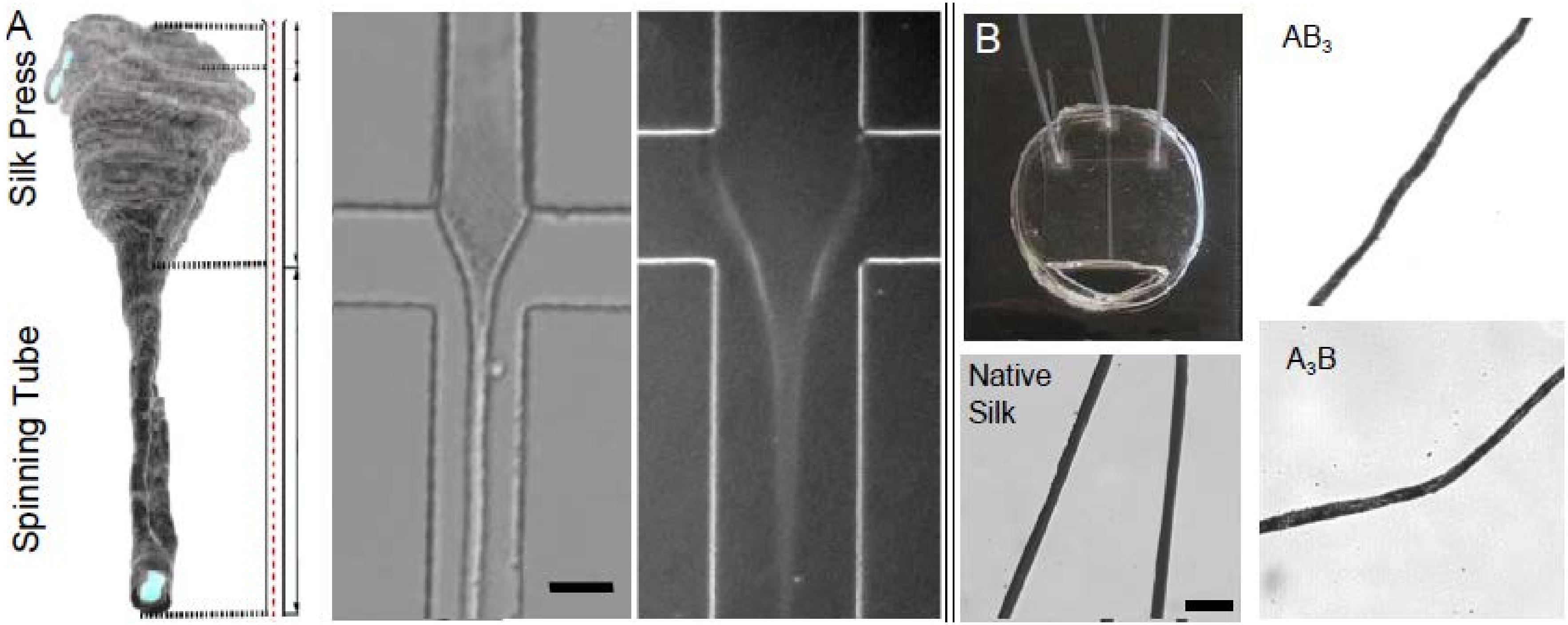
Regenerated silk fibers + block copolymers



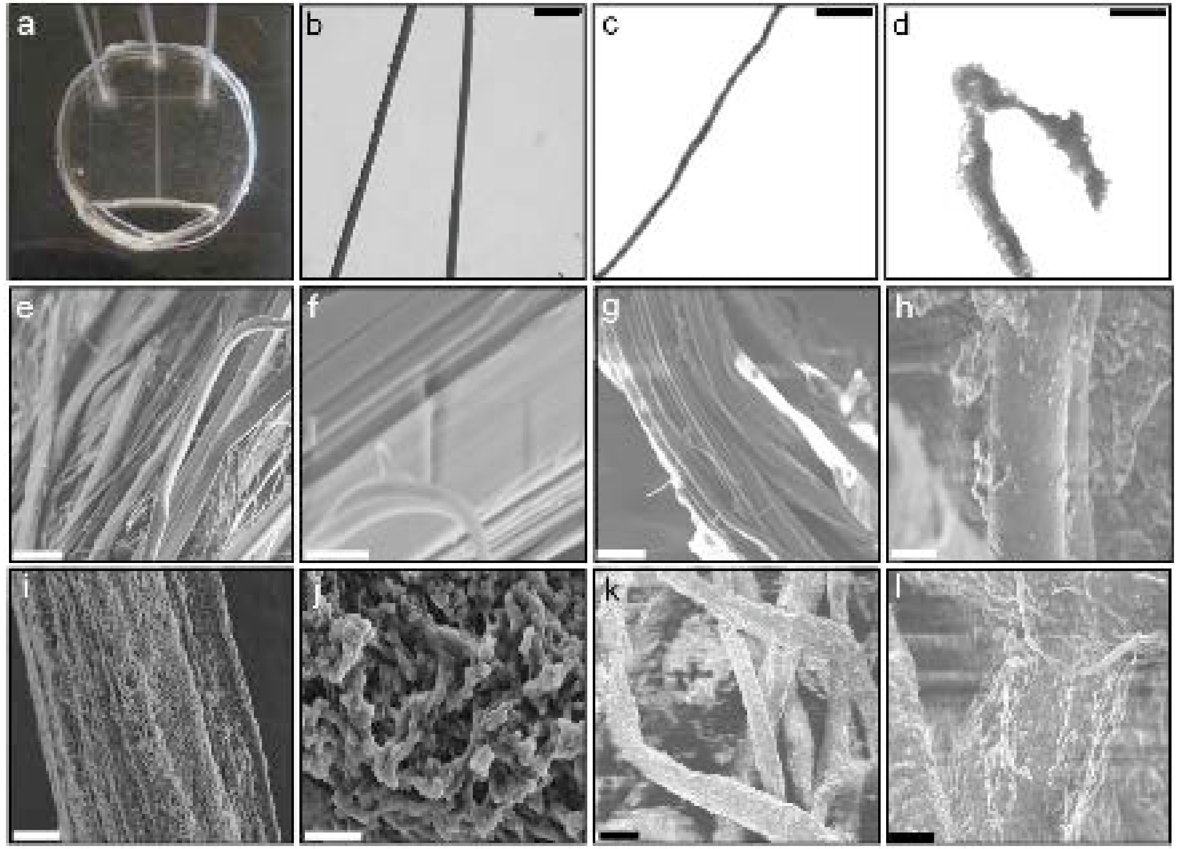
gland

microfluidic device
(fluid focusing)

spun fibers
(AB$_3$ & BA$_3$)

Fiber Morphology

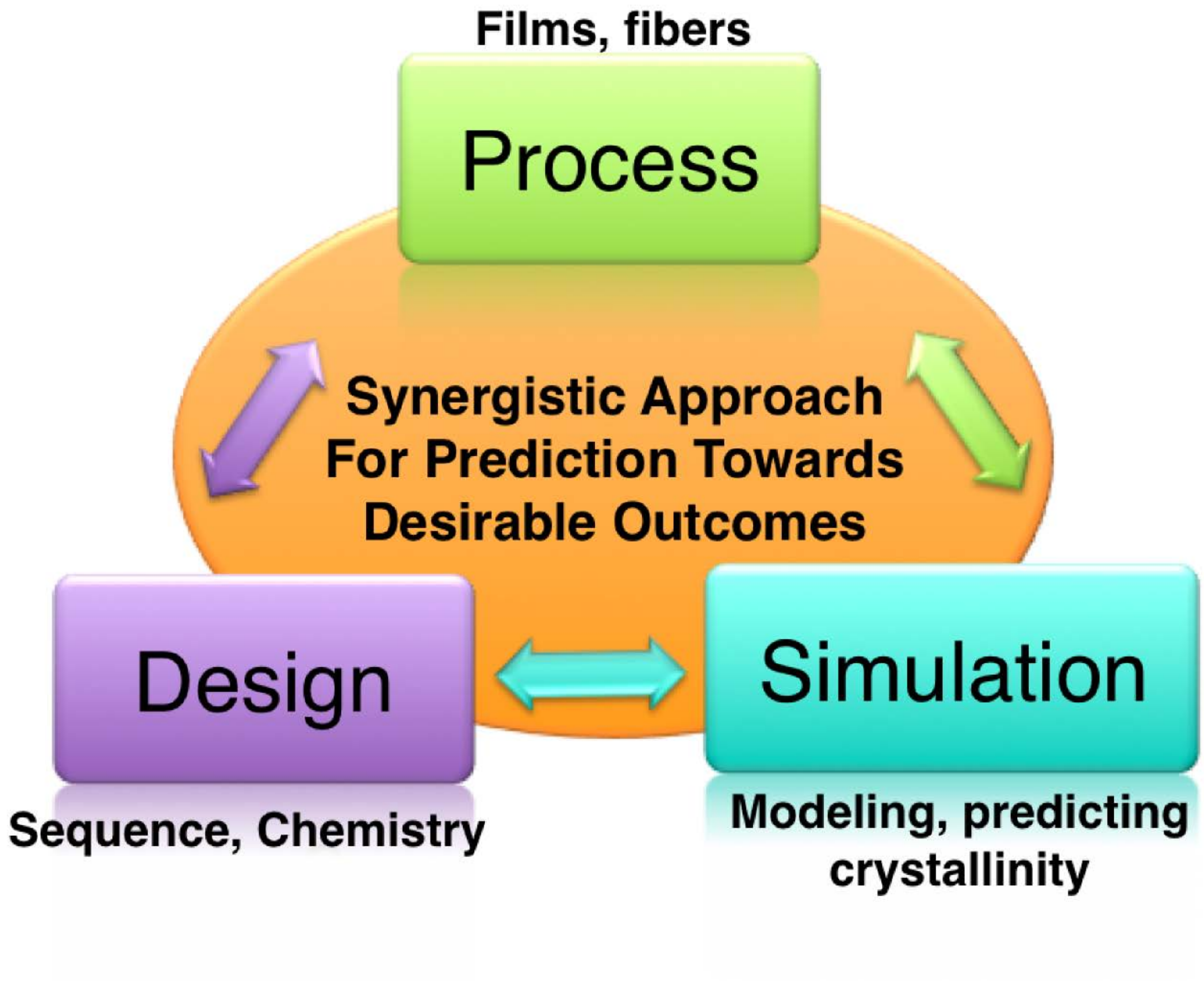
spun from regenerated silk fibroin (RSF) and mixtures with HAB_3 and HBA_3 (30% RSF/70% copolymer)



Brightfield images [RSF (B, scale bar 150 mm) RSF/HAB_3 blend (C, scale bar 150 mm) and RSF/HBA_3 blend (D, scale bar 150 mm) fibers]

E-L - SEM images of fibers [(E) Native silk, scale bar 30 mm, (F) Native silk, scale bar 2 mm,(g) RSF fibers, scale bar 8 mm, (H) RSF fibers, scale bar 4 mm, (I) RSF/HAB_3 blend, scale bar 30 mm, (J) RSF/HAB_3 blend, scale bar 2 mm,(K) RSF/HBA_3 blend, scale bar 20 mm, (L) RSF/HBA_3 blend, scale bar 5 mm]

Models to Predict Protein Biomaterial Performance



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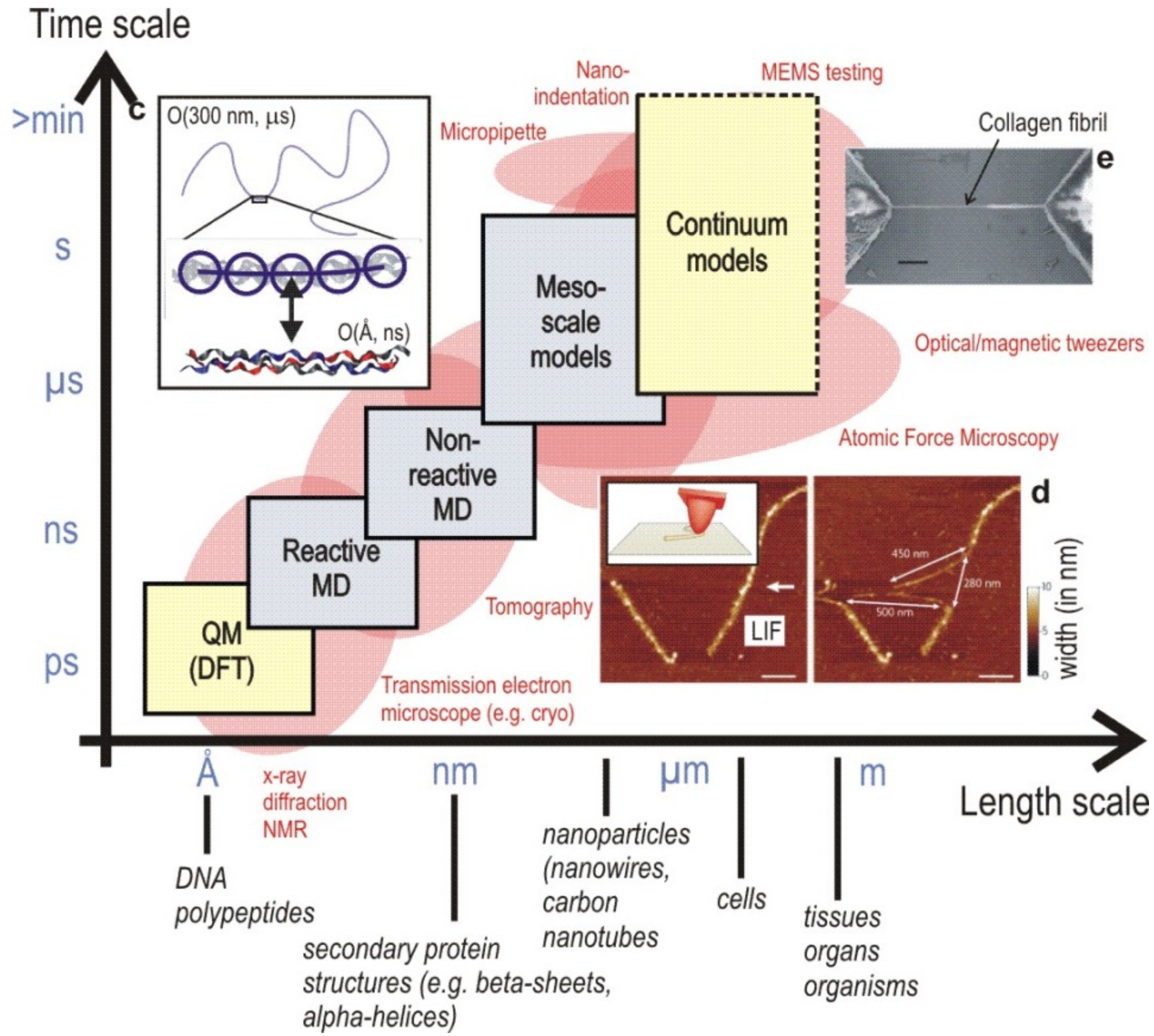
Multi-scale integration of computational and experimental tools

→ structure-property-process relationships in protein materials



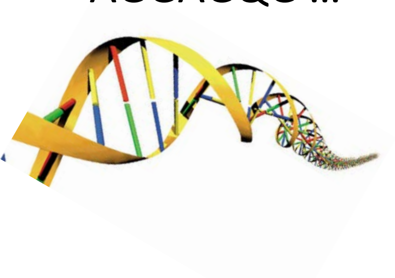
Yung & Buehler, *Nature Materials*, 2009

Reactive, nonreactive molecular dynamics (MD), mesoscale (coarse-grained) and continuum models to span Angstrom to micrometers and meters and time-scales from femtoseconds to seconds

Structure prediction and functional properties

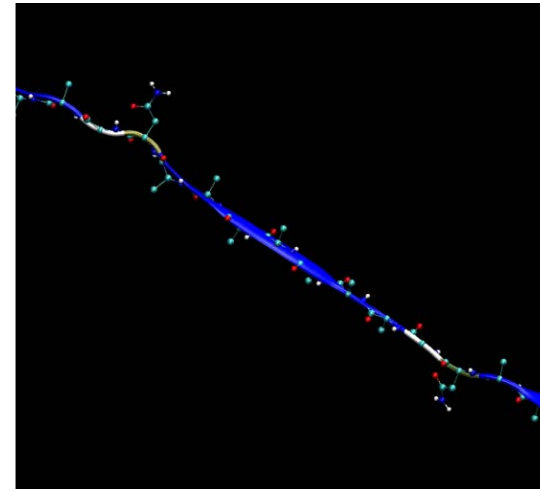
Sequence information

...GGLGGQGAGAAAAA
AGGAGQG ...



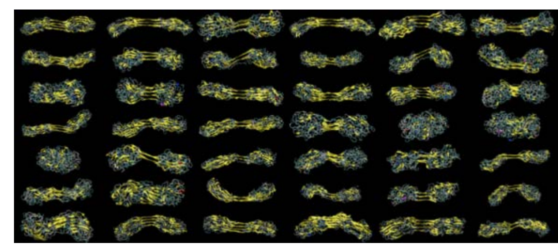
Amino acid sequence

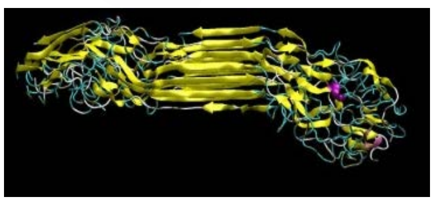
Replica Exchange Simulations



Folding —structure

Ensemble of final structures

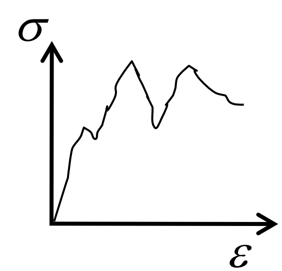




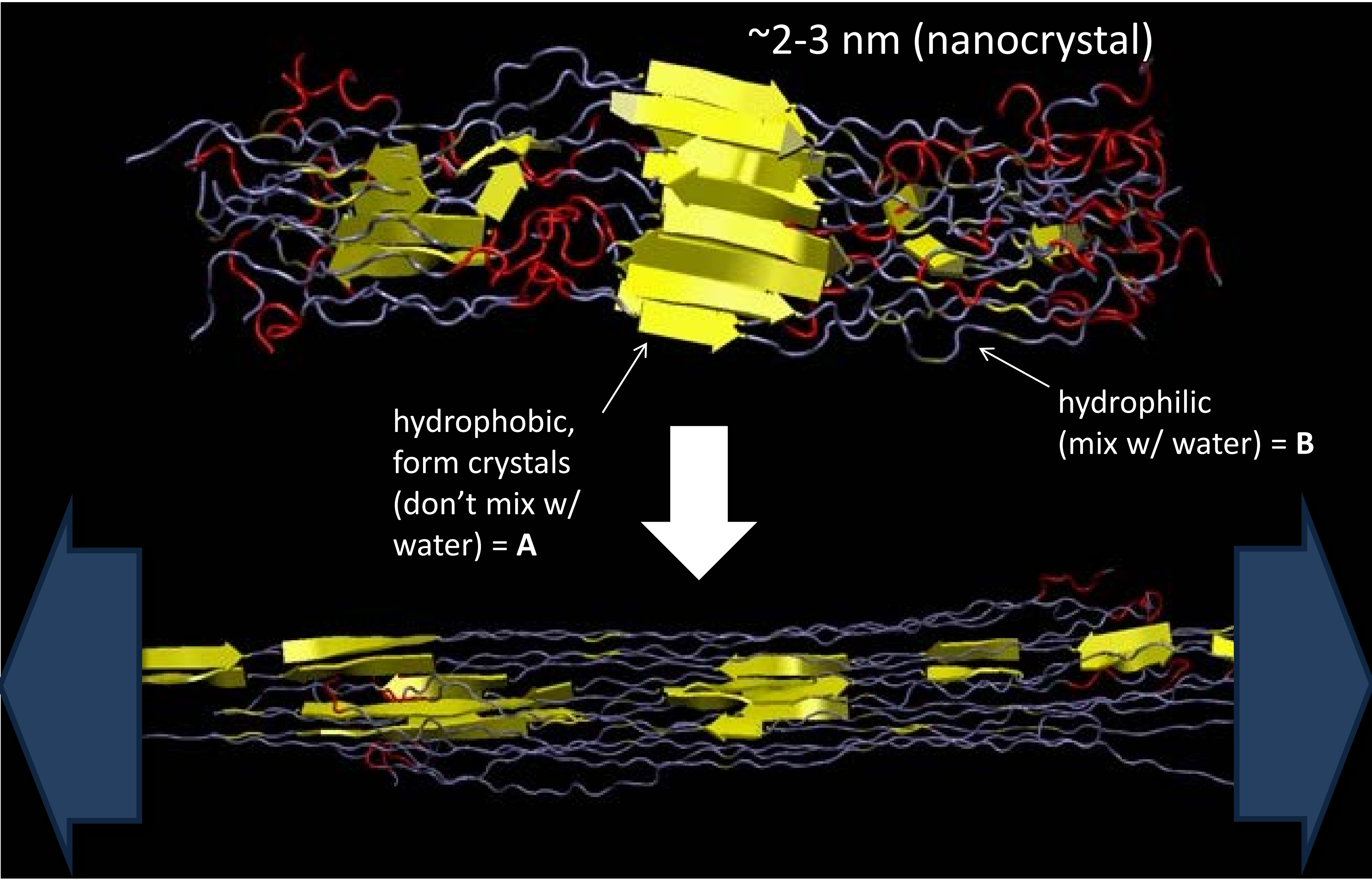
Validation (experimental results)

Mechanical characterization (multiscale)

Mechanics (functionality)



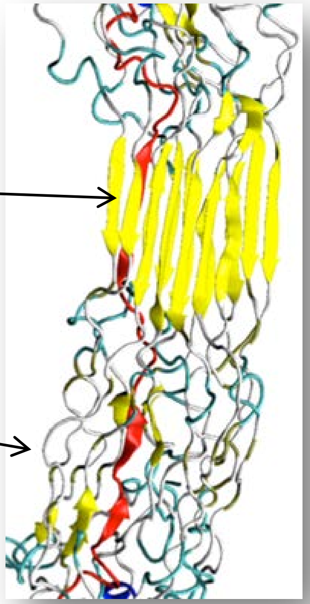
Example: molecular mechanics of silk

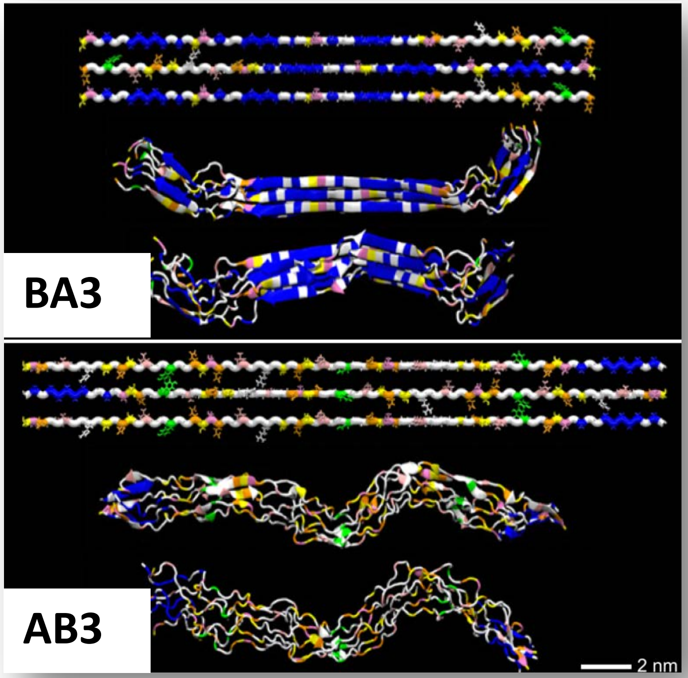


De novo sequences: in silico model

AB3 vs. BA3

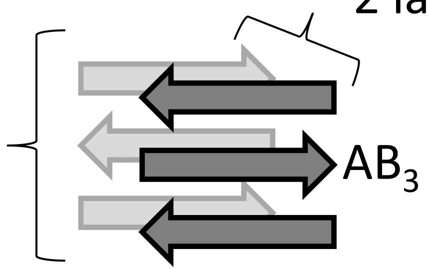
- A = hydrophobic domain
- B = hydrophilic domain

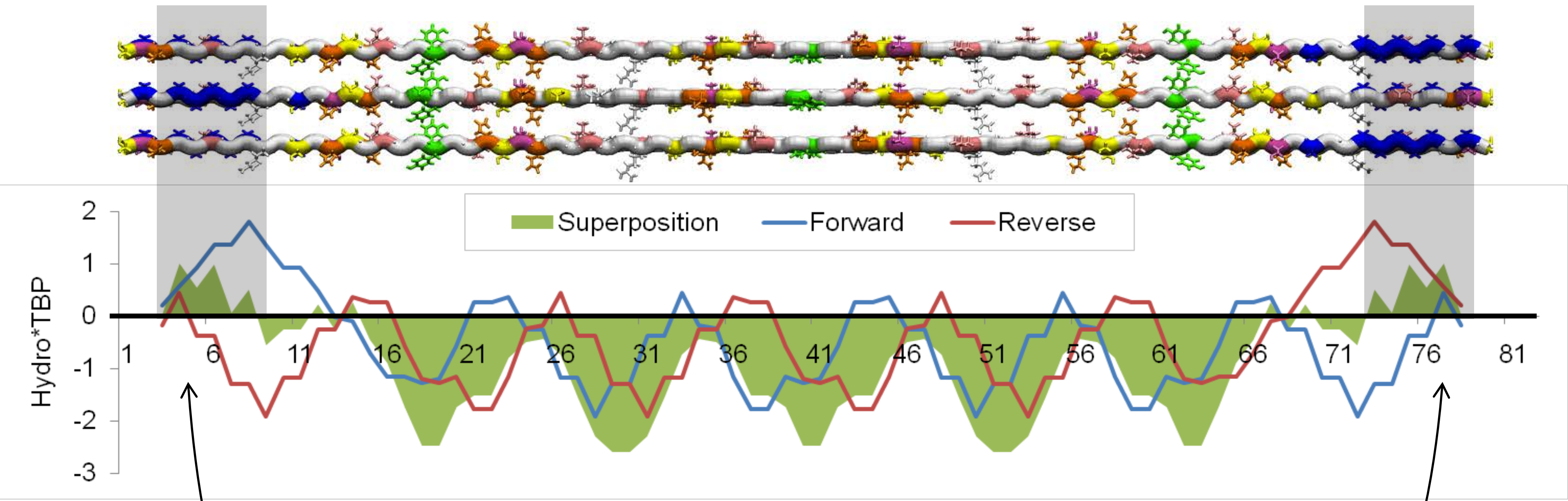




AB_3 Lattice 3x2

AB_3
Length: 80 res, 27 nm



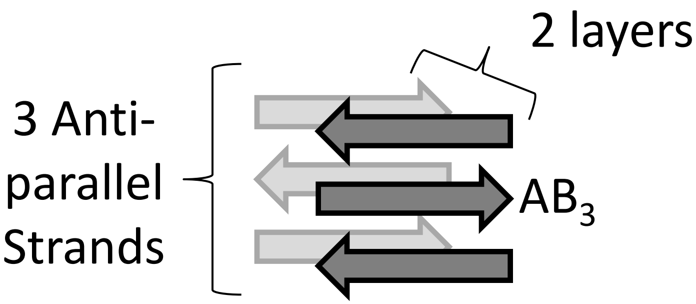


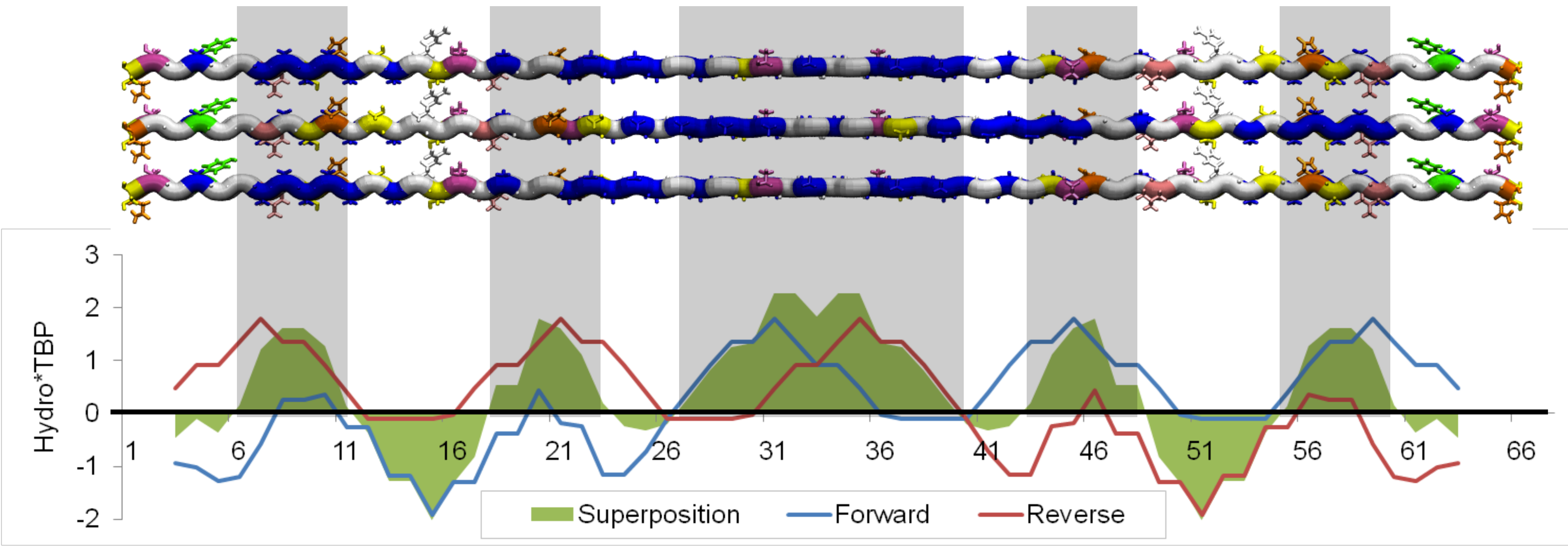
Slight chance of β-sheet at A (poly-Ala)

Krishnaji, Bratzel, *et al., Advanced Functional Materials*, 2012

BA_3 Lattice 3x2

BA_3
Length: 64 res, 22 nm

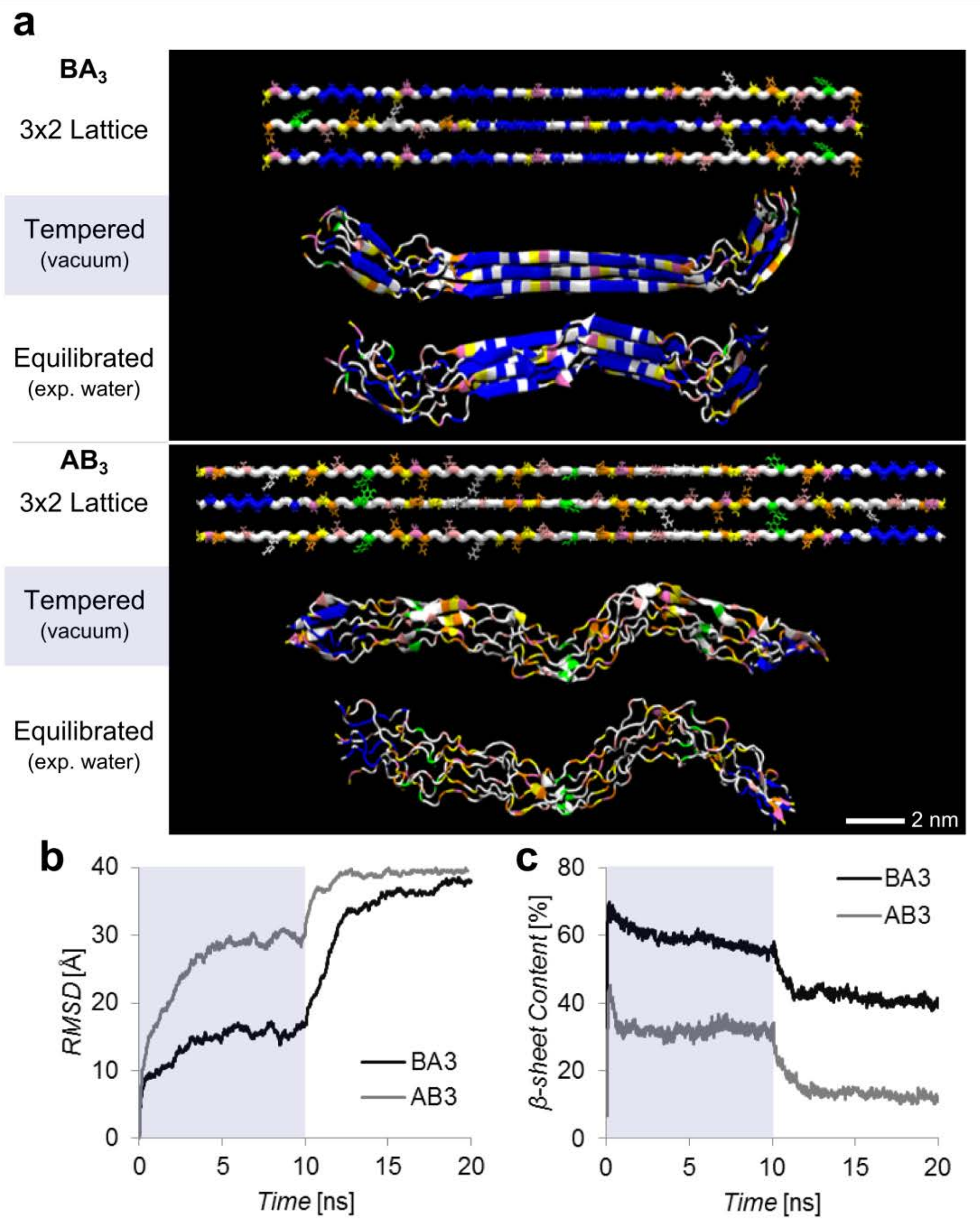




Strong chance of many β-sheets because of overlapping A

Krishnaji, Bratzel, *et al., Advanced Functional Materials*, 2012

Equilibration & structure convergence - computation



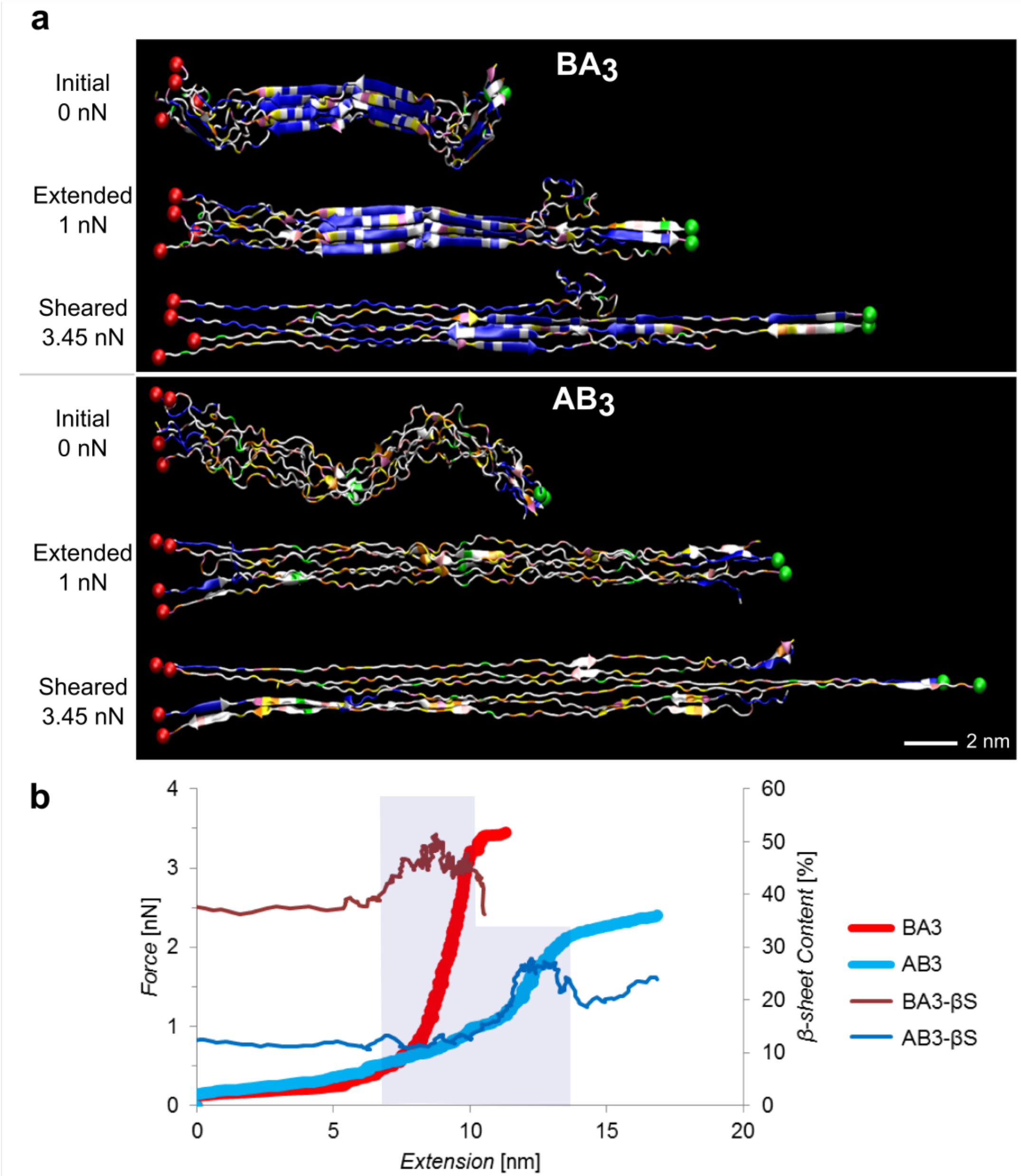
Length and secondary structure change visible during tempering in implicit water and equlibration in explicit water (water molecules hidden for clarity)

Residues are colored by secondary structure: **β-sheet is in blue**.

Convergence for each stage is determined by:
(b) RMSD and
(c) changes in total β-sheet content.

Krishnaji, Bratzel, *et al., Advanced Functional Materials*, 2012

Pull out testing - computation



Force-control pullout testing of the equilibrated structures in explicit water

Length and secondary structure change visible during deformation

Residues are colored by secondary structure: **β-sheet is in blue**.

Total instantaneous β-sheet content (thin line) correlated with the force-extension curves (thick line) for each structure: **AB3 stiffer than AB3**

Comparison: Experimental modulus

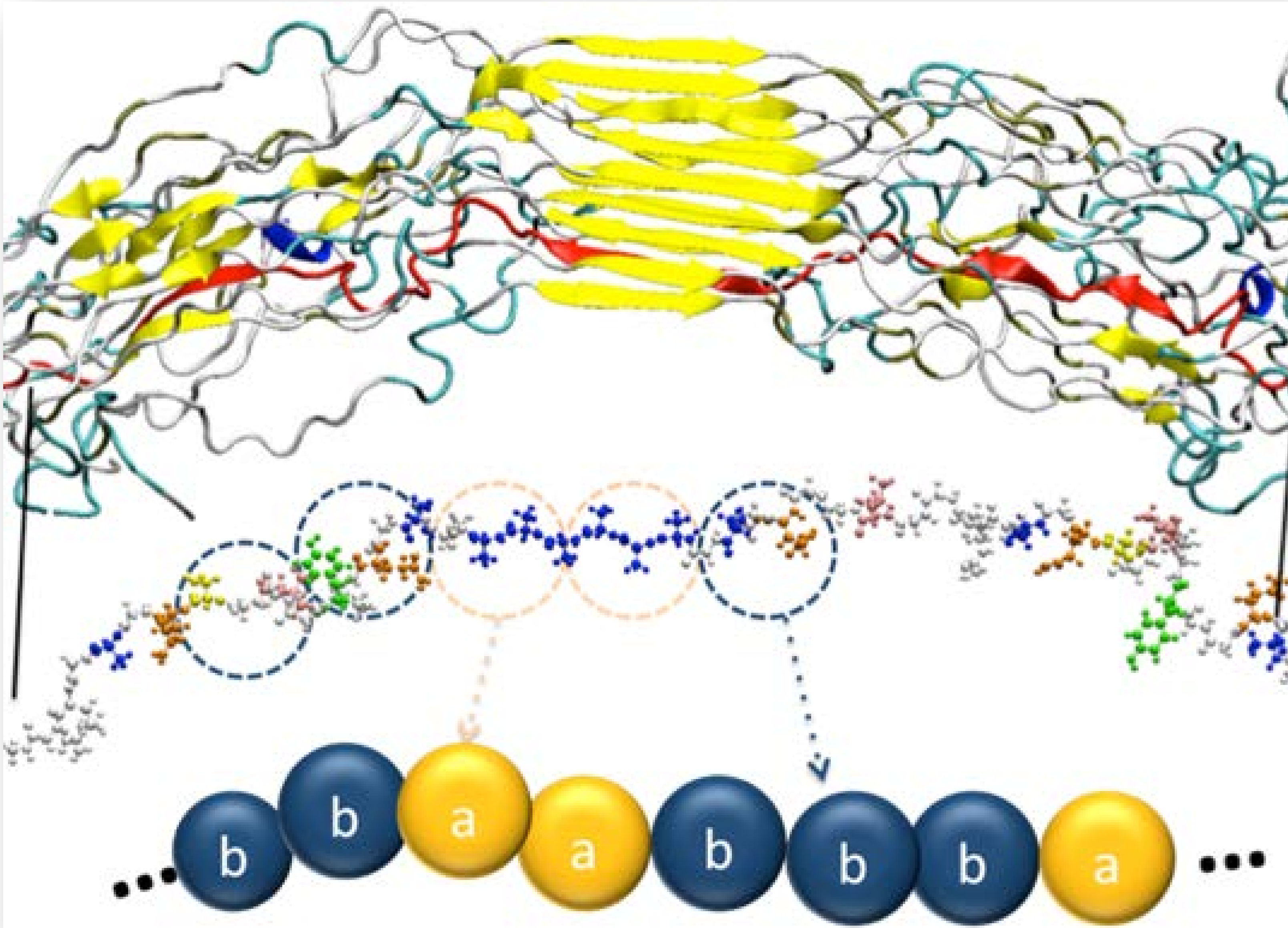
E_{AB3} = 0.16±0.03 GPa

E_{BA3} = 0.44±0.1 GPa

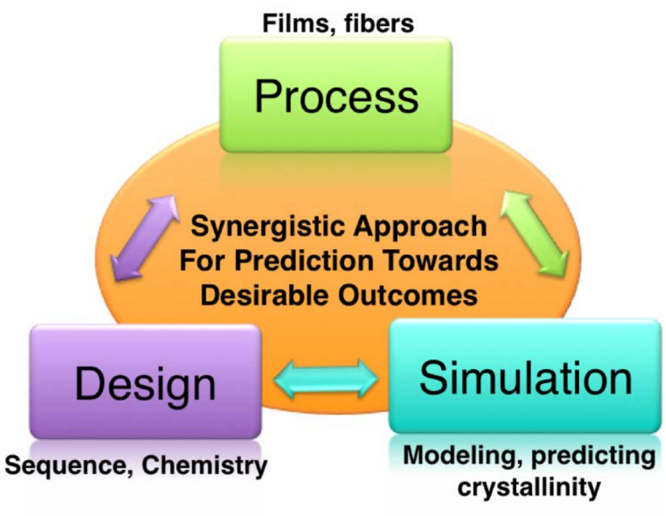
Krishnaji, Bratzel, *et al., Advanced Functional Materials*, 2012

Scaling up: Dissipative Particle Dynamics (DPD) model

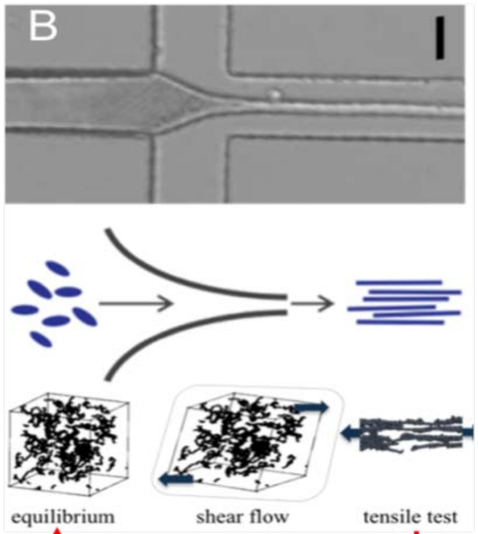
Gronau, Kaplan, Wong, Buehler *et al.*, *Biomaterials*, 2012



Summary

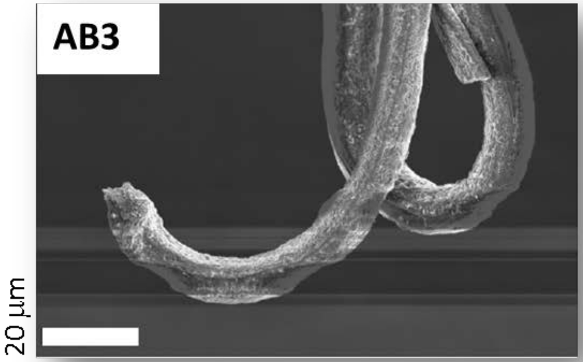


Microfluidic model of the spider's spinning duct

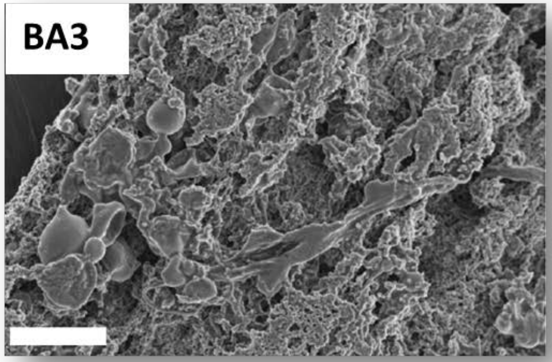


Design sequence AB3 vs. BA3

Novel biomaterial



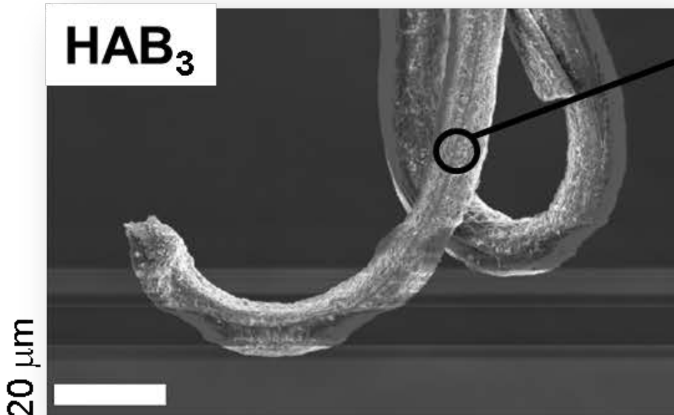
Forms fibers from microfluidic spinning device



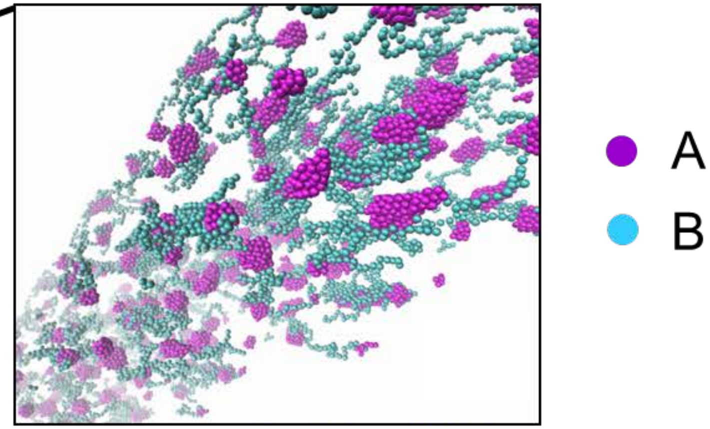
Clogging and no fiber formation

Wong, Kaplan, Buehler, *et al., Nano Today, 2012*

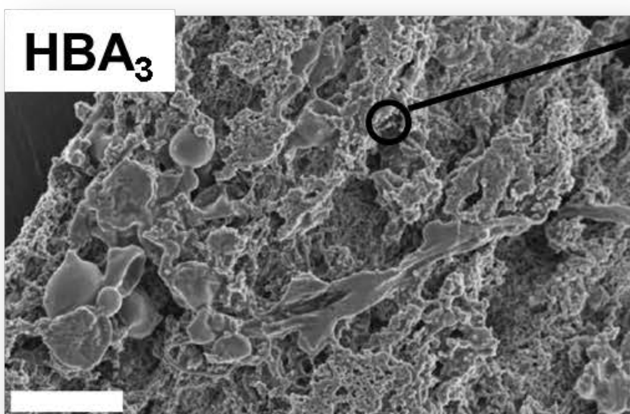
Microscopic insight from molecular simulation



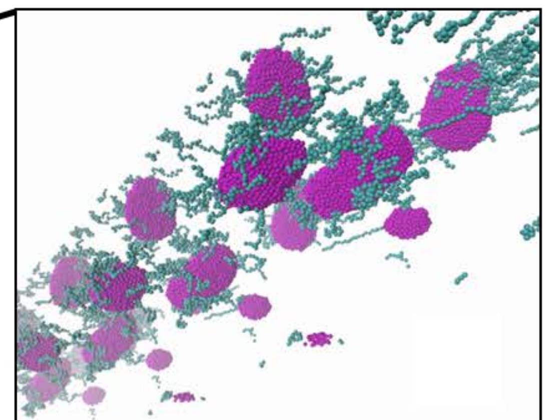
Forms fibers



Increased hydrophilic residues has positive effect on assembly process



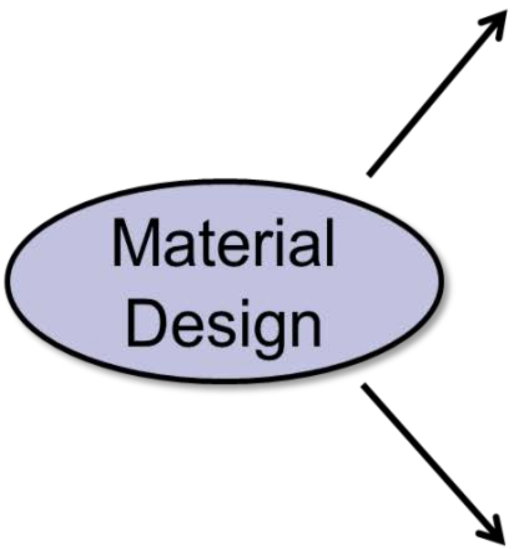
Clogging, no fiber formation

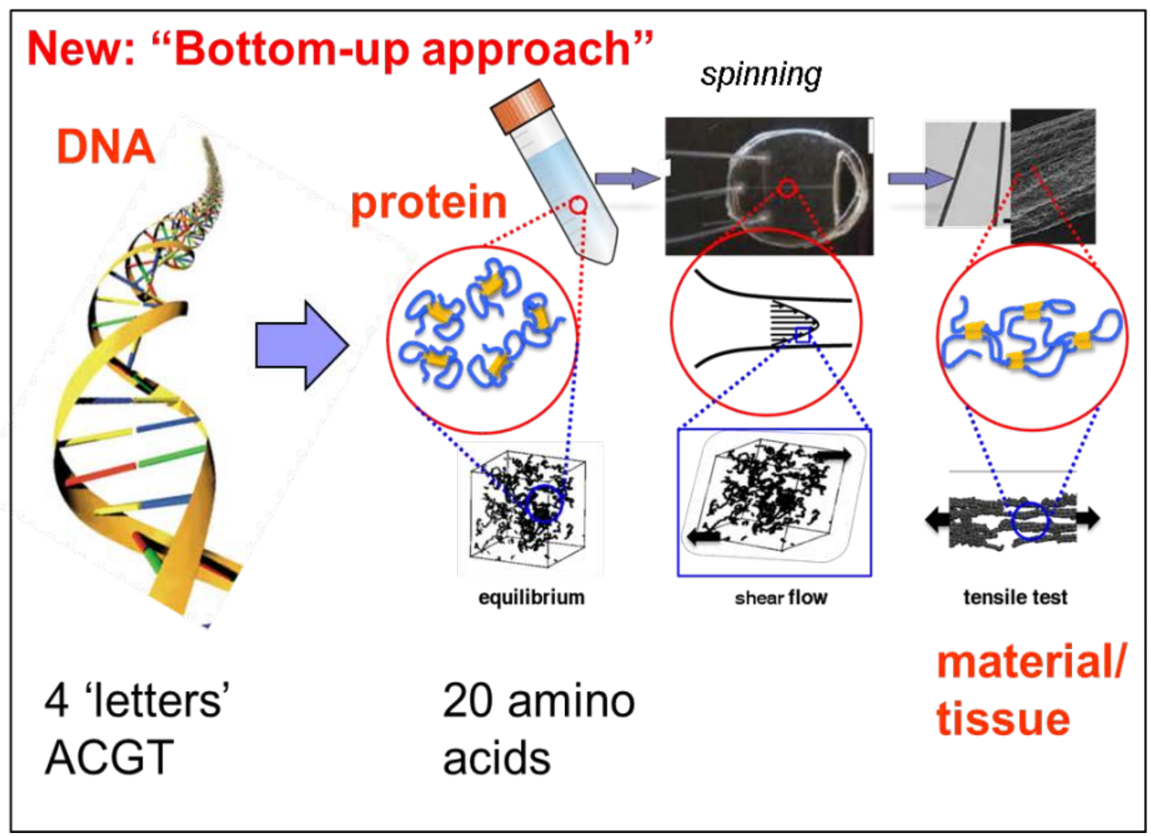


Proteins form 'globular structures', hence few/no cross-links = no fiber

Wong, Kaplan, Buehler, *et al., Nano Today, 2012*

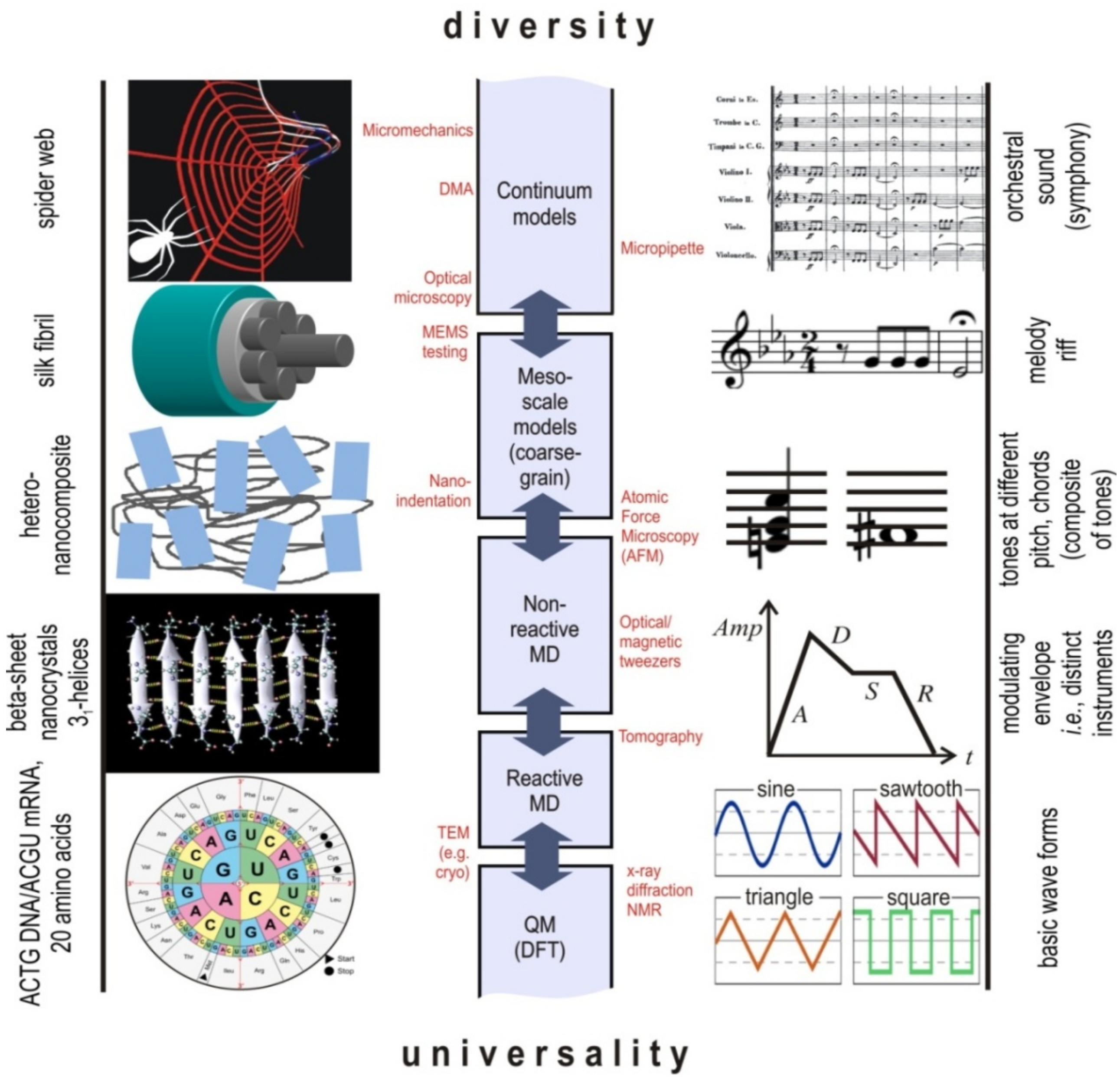
Conclusion – A New Approach to Materials Design





Wong, Kaplan, Buehler, *et al.*, *Nano Today, 2012*

Conclusions & Broader implications



M. Buehler, *Nano Today*, 2010

Multifunctionality (**diversity**) created by changing structural arrangements of few (**universal**) constituents

No need to invent new building blocks

Powerful biocompatible paradigm for materials innovation

- Fewer resources
- More flexibility
- Wider design space