Liquid-Liquid Phase Separation (LLPS) in Biological Materials and Bioinspired Engineering

École Thématique CNRS – Surface and Bio-Interfaces au Service d'une Santé

21-26 September 2025, Cargèse

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http://www3.ntu.edu.sg/home/ali.miserez/index.html

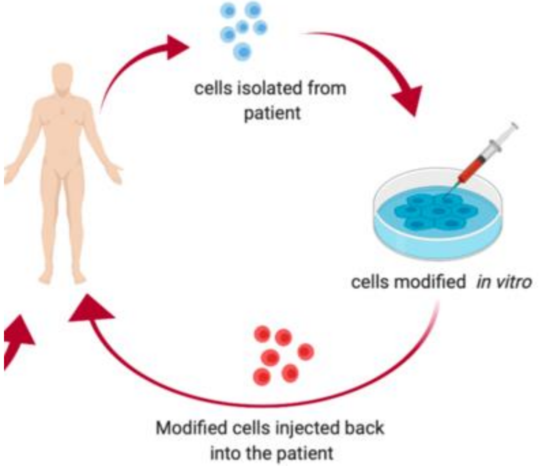
https://web.mse.ntu.edu.sg/susmat/

Jumbo squid

???

Sustainable plastic

???



- Intracellular delivery or macromolecular therapeutics
- Gene and cell therapy

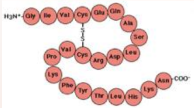
???

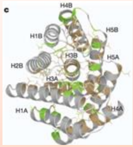
Eco-friendly coatings

Molecular Biomimicry: Grand Challenge

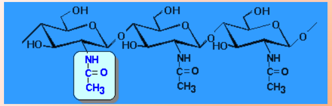
Biological model systems

- **Composition:**
  - Proteins (sequencing)

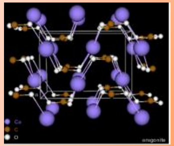




  - Polysaccharides



  - Biominerals

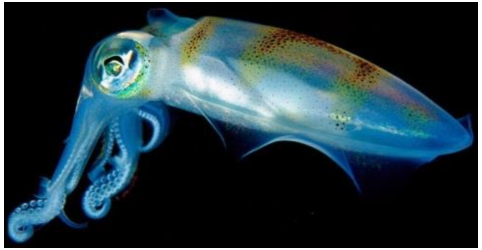


- **Mechanical performance**

How does Nature go from its basic building blocks to the final structure ?

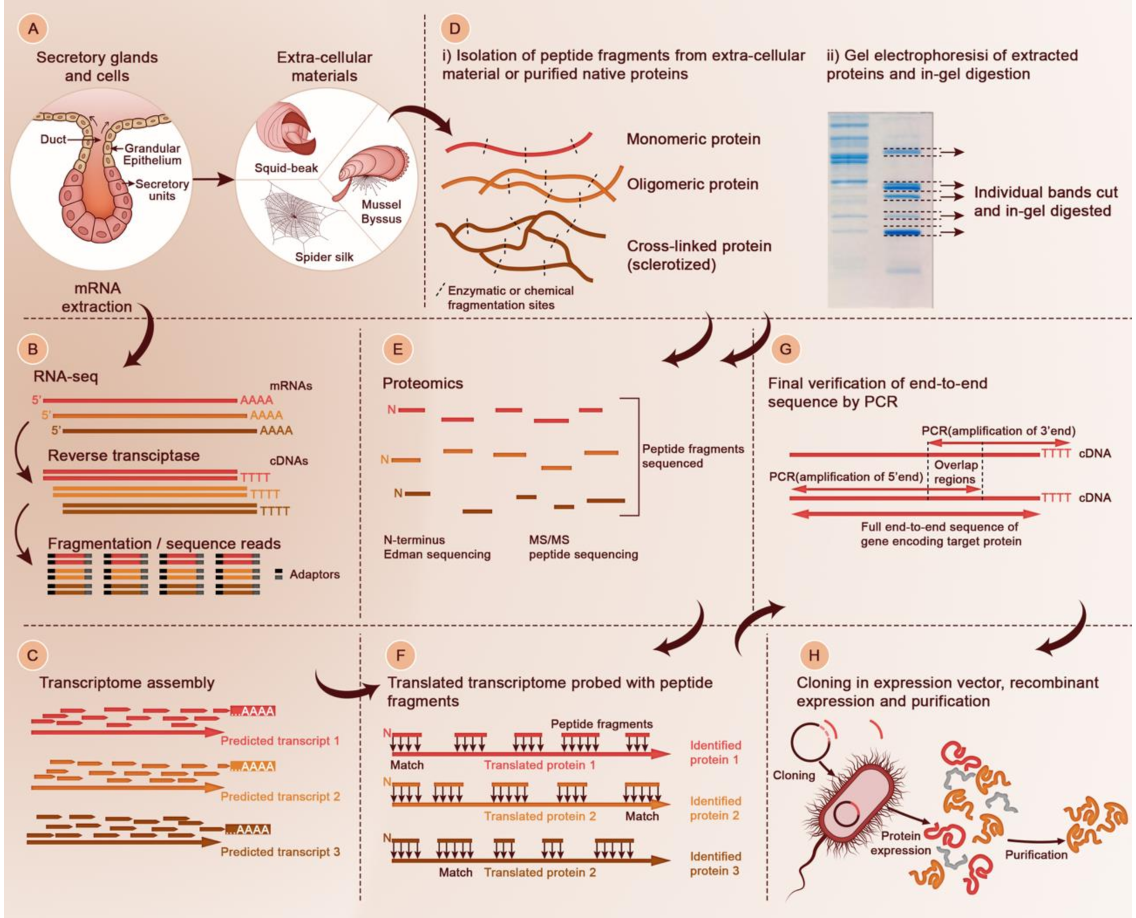
???

- **Optical properties (camouflage)**



➢ Biofabrication process ?
- From molecules to final structure
- Spatio-temporal growth

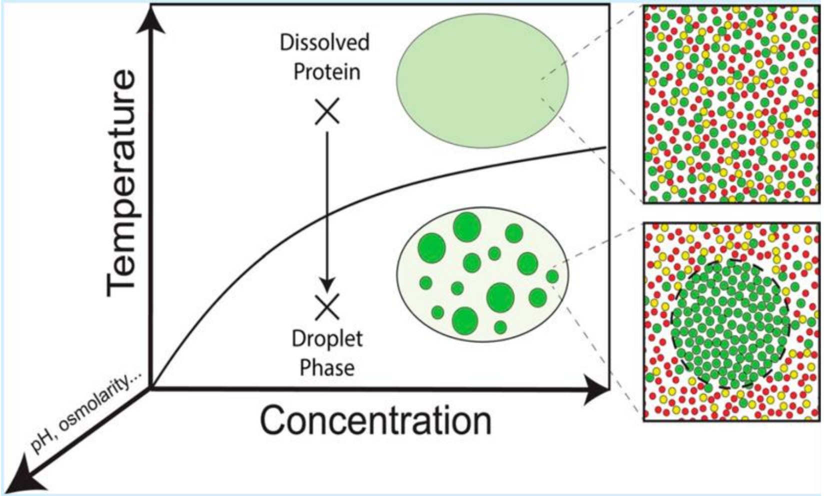
Molecular Biomimetic: Discovery Pathway



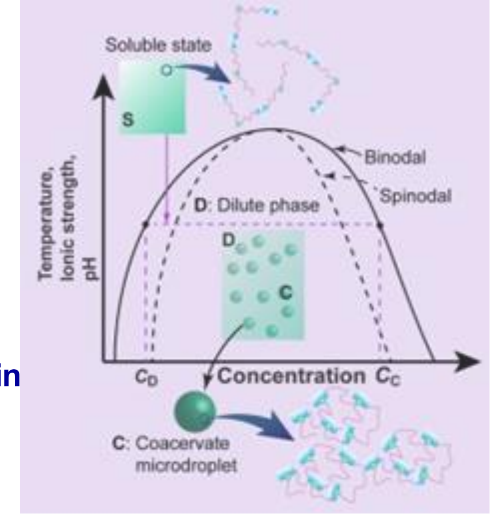
Guerette *et al*, **Nature Biotechnology** *2013, 31, 908-15.*

Miserez, Yu, Mohammadi, **Chemical Reviews** 2023, 123, 2049-111

Liquid-Liquid Phase Separation / Coacervation



Brangwynne, **JCB** 2013, 203, 875-81



- Sun *et al.*, **MRS Bulletin** 2020, 45, 1039-47

- Flory-Huggins free energy of mixing, F_{FH}

$$F_{FH} = (K_B T)\left[\frac{\phi}{N_p}\ln\phi + (1-\phi)\ln(1-\phi) + \chi\phi(1-\phi)\right]$$

$\boldsymbol{\phi}$: polymer volume fraction
$\boldsymbol{N_p}$: polymerization degree
$\boldsymbol{\chi}$: Flory-Huggins parameter
$\boldsymbol{z}$: coordination number

$$\chi = \frac{z}{K_B T}\left[u_{ps} - \frac{1}{2}(u_{pp} - u_{ss})\right]$$

χ: strength of enthalpic interactions

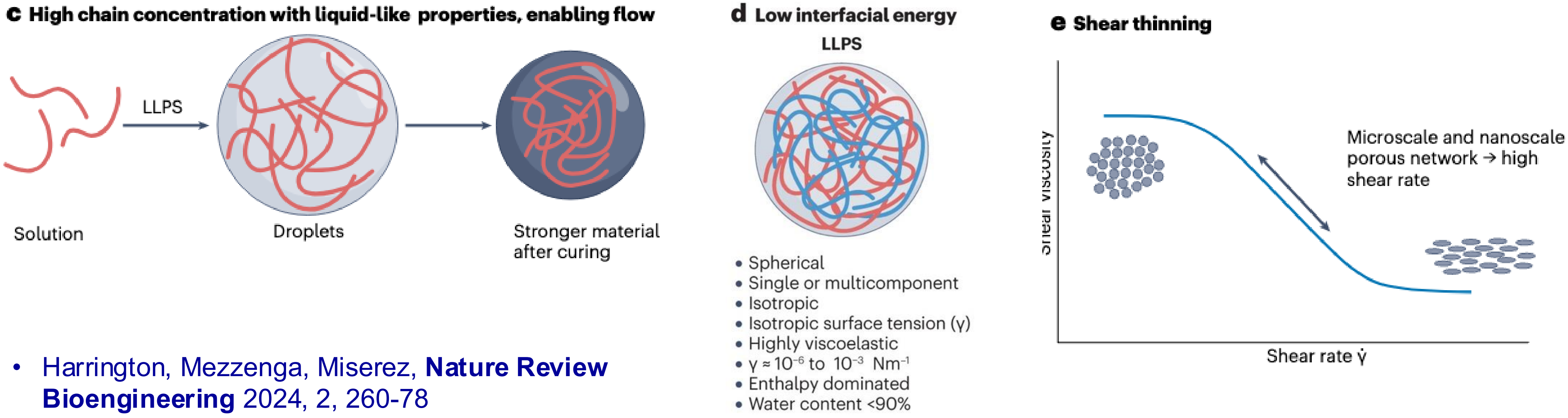
- Electrostatic interactions: Overbeek-Voorn

$$F_{FH} = (K_B T)\left[\frac{\phi}{N_p}\ln\frac{\phi}{2} + (1-\phi)\ln(1-\phi) - \alpha(\sigma\phi)^{3/2}\right]$$

$\boldsymbol{\alpha}$: charge per site
$\boldsymbol{\sigma}$: linear charge density of polyion

Unique Properties of Coacervates

- High biopolymer concentration with liquid-like properties → Viscous flow
- Low surface tension (10^{-6} to 10^{-3} N/m) → Good wetting properties
- Shear-thinning → Facilitates flow in micro-porous network



- Harrington, Mezzenga, Miserez, **Nature Review Bioengineering** 2024, 2, 260-78

Coacervation of Tropoelastin

Historical perspective

No. 4250 April 14, 1951 NATURE

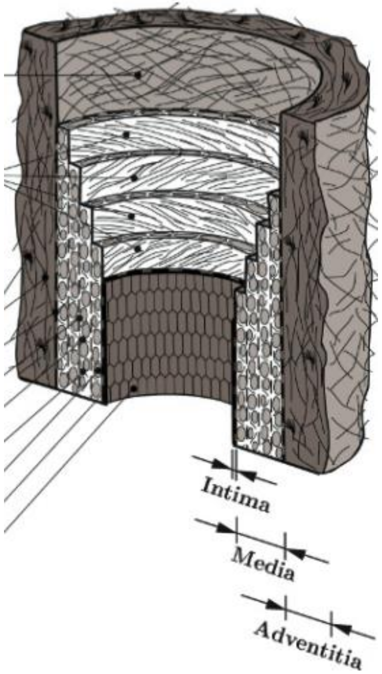
A Soluble Protein derived from Elastin

at any te
buffer of l

Adair, Davis, Partridge

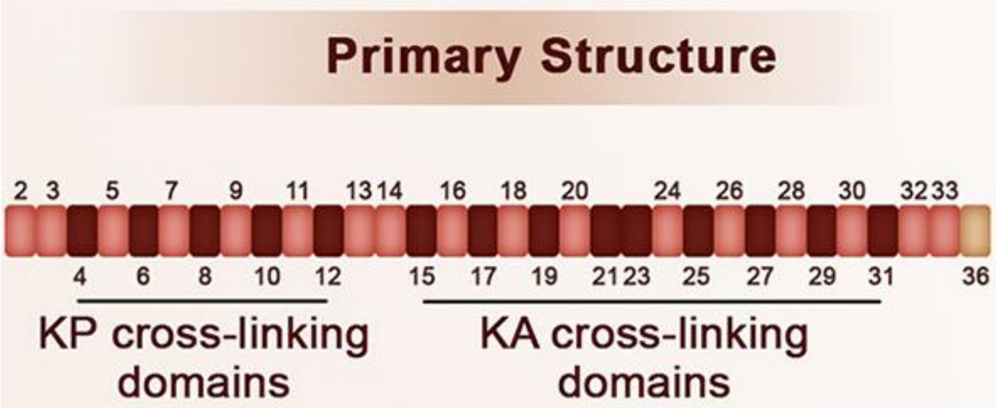
The non-diffusible protein was soluble in water at temperatures below 25° C., giving a pale yellow mobile solution. On raising the temperature to 25–30° C. in the presence of dilute buffer (*p*H 4–6), a precipitate consisting of liquid droplets separated. The droplets showed no birefringence under crossed nicols and immediately redissolved on reducing the temperature. On centrifuging at 37° C. the droplets coalesced to form a lower layer of viscous liquid; refractive index measurements showed the two liquid phases to consist of aqueous protein solutions of different concentration.

- Elastin: main component of arterial walls (with collagen)



G.A. Holzapfel *Nonlinear Solid Mechanics. A Continuum Approach for Engineering, 2000.*

Coacervation of tropoelastin



Miserez, Yu, Mohammadi, **Chemical Reviews** 2023, 123, 2049-111

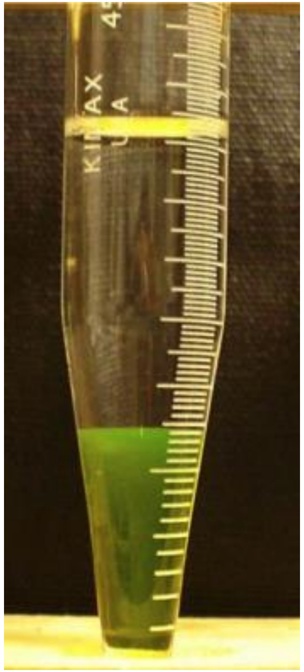
➢ Biochemical characteristics:

- Hydrophobic domains (G,V,P, A) in repeats of 3-6: GVGVP, GVGVAP, etc
- Hydrophilic domains rich in Lys and Ala. K typically separated by 2-3 A residues, AAAKAAKAA
- C-terminal highly conserved, basic (2 Cys)
- Overall 72 kDa in humans

Coacervation of tropoelastin

Elastin processing: coacervation

- Tropoelastin: soluble < 20ºC
- Higher (physiological) *T* : cloudy solution forms → Lower Critical Solution Temperature (LCST)
- Solution phase-separates: coacervation
- Not a precipitate: both liquid phases co-exist
  - Bottom layer forms sticky viscoelastic phase containing tropoelastin and only 60% water
  - Top layer is an aqueous equilibrium solution

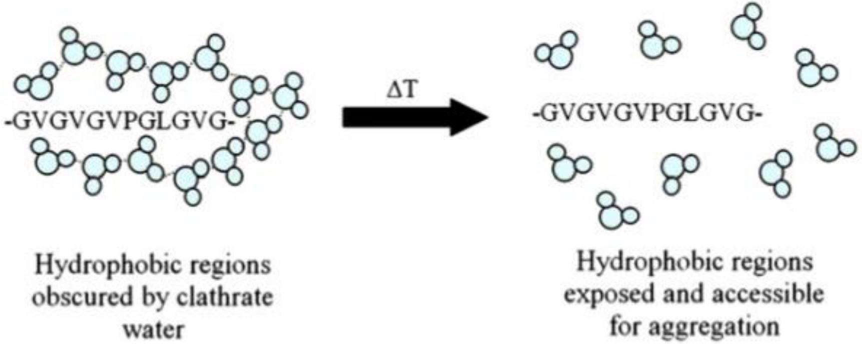


Coacervation of tropoelastin

Coacervation of tropoelastin: physico-chemical mechanism

- LCST behavior: inverse observed for many proteins
- Due to hydrophobic interactions of tropoelastin
- Low *T*: water molecules closest to the protein cannot form interactions with the hydrophobic domains of tropoelastin

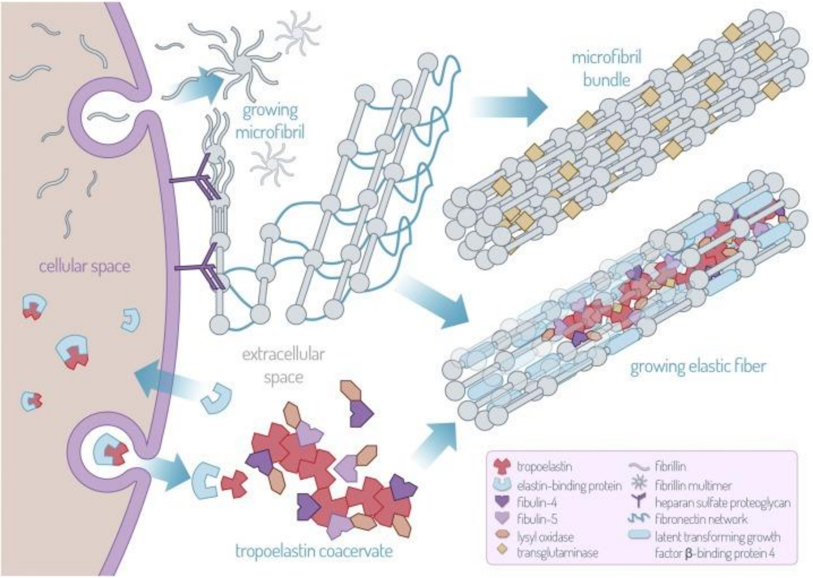
H-bond water forms a clathrate configuration around the domains



- T increases: H-bonding network disturbed and tropoelastin free to interact → coacervation
- This behavior is important during fibrillogenesis :
  - Concentrates and aligns tropoelastin molecules prior to cross-linking

Coacervation of tropoelastin

- Transcribed from a single gene and alternatively spliced in the nucleus



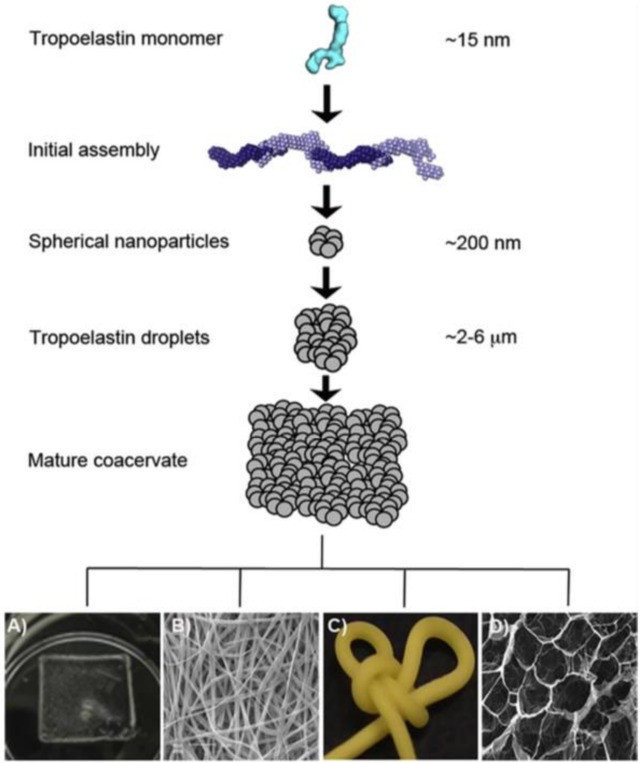
A. Tropoelastin associates with EBP and FKBP65 in the rough endoplasmic reticulum. The tropoelastin-EBP complex moves through the Golgi and is secreted to the cell surface

B. Secreted tropoelastin coacervates, is oxidized by LOX, and associates with microfibrils to generate the nascent elastic fiber

C. Continued secretion, oxidation, and deposition of tropoelastin occupy the bulk of elastin synthesis

Biomimetic Applications of Elastin

I. Recombinant elastin: Production and Self-Assembly



Wise *et al*, **Acta Biomat. 2014**

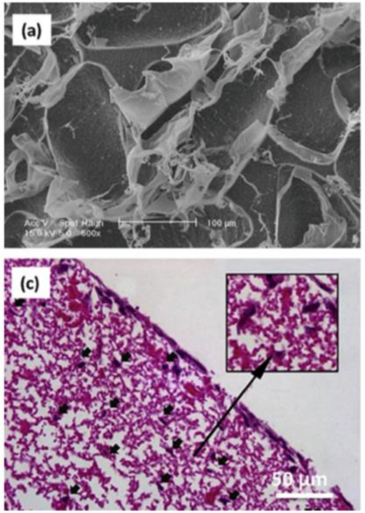
- Gene encoding full-length human tropoelastin cloned in *E. coli*: now routine
- Purification is straightforward by exploiting coacervation (heat/cooling cycles)
- Multi-scale assembly close to native tropoelastin
- No immune response, no cell cytotoxicity: highly biocompatible materials

- Example of applications:
  - Hydrogels
  - Electro-spun fibers
  - Sutures

Biomimetic Applications of Elastin

I. Recombinant elastin: Tissue Engineering Applications

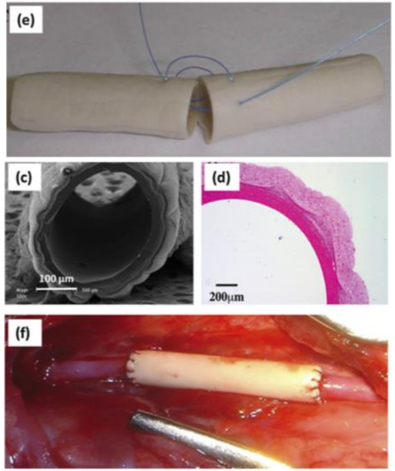
- Hydrogels



Annabi *et al.*, **Biochem. Eng J**. 2013

Skin fibroblast penetration and growth within Porous 3D hydrogels

- Electrospun fibers / hollow tubular grafts



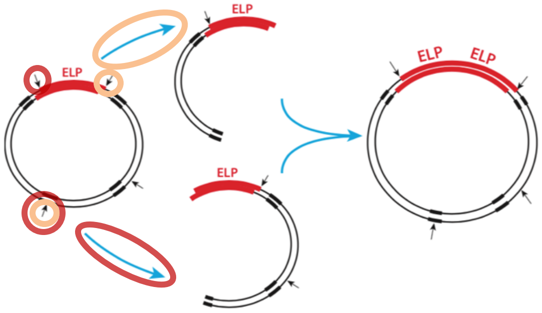
✓ Attachment and proliferation of various cell types (endothelial cells, dermal fibroblast, etc)

- Cross-linking curing and mechanical stabilization:
  - Chemical cross-linking: succinimidyl, glutaraldehyde
  - Enzymatic cross-linking: lysyl oxidase
  - Physical cross-linking (pH change induced)

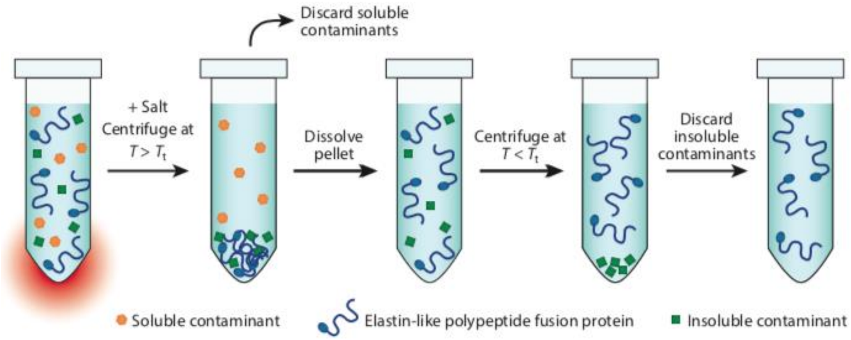
Elastin-Like Polypeptides (ELPs)

- Basic sequence design based on pentapeptide: **(VPGXG)$_n$**
- Production by genetic engineering:

X: guest residue (anything by Pro)



- Recursive direction ligation with enzymes cleaving at specific locations
- Re-ligation → doubling the length of gene encoding ELPs

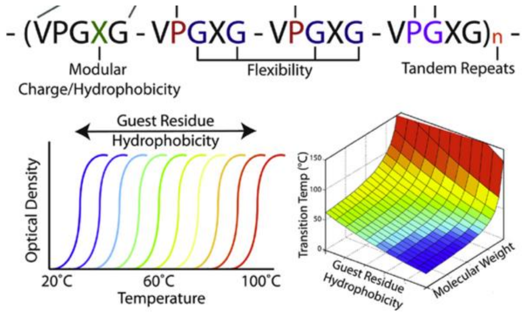


- Simple purification by temperature cycling across T_t (ELP used as a purification tag for many other recombinant proteins)

Varamko *et al.* **Ann. Rev. Biom. Eng** 2020

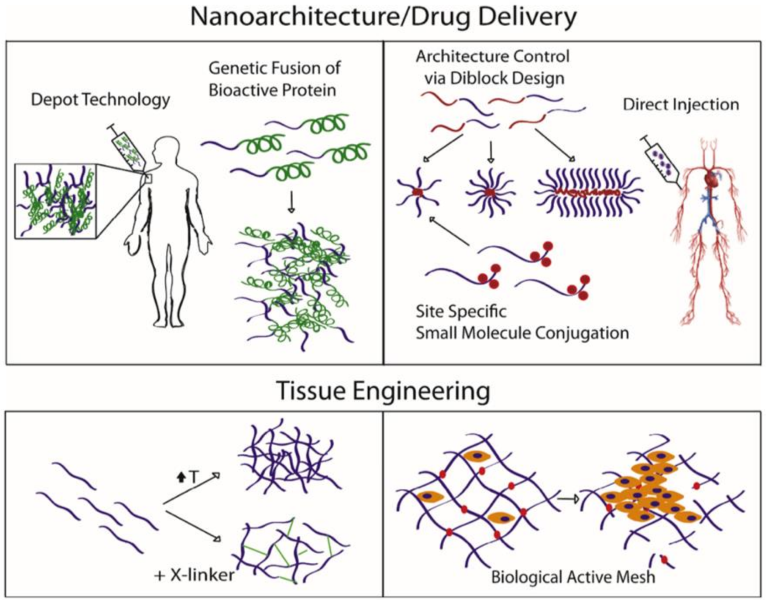
Biomolecular Engineering of ELPs

Tuning Phase-Transition of ELPs



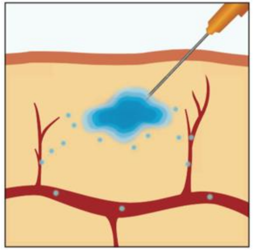
Roberts *et al*, FEBS Letters, 2015

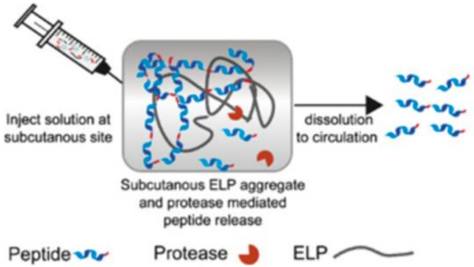
- Phase-transition temperature is tunable:
  - Guest residue
  - Peptide length
  - "Smart" stimuli-responsiveness in biomedical applications

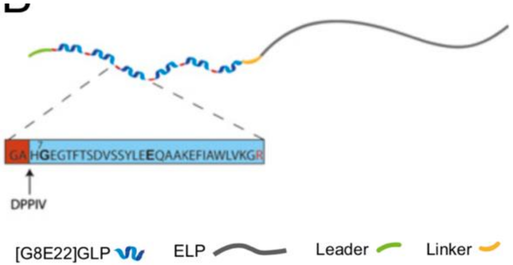


- Two main areas of applications:
  - Drug delivery
  - Tissue Engineering

Biomolecular Engineering of ELPs: Injectable Depots



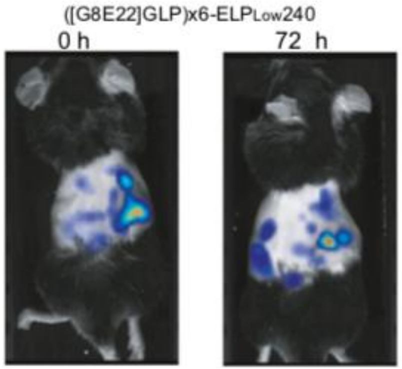




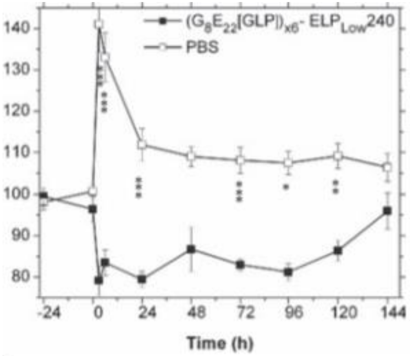
Amiram, **PNAS** 2013

Varanko, **Ann. Rev. Biom. Eng.** 2020

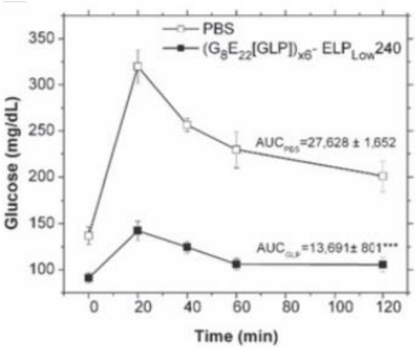
- Injectable glucagon-ELP depots for diabetes II treatment
- GLP: glucagon-like peptides (for glucose management)



In vivo injection in diabetic mouse model

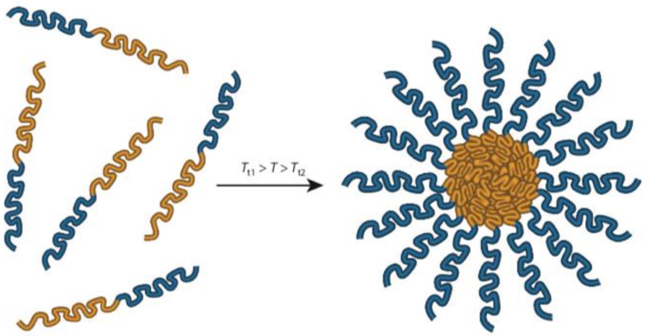


Glucose reduction after single injection



Blood glucose level 52 h after injection

Biomolecular Engineering of ELPs: Drug Nanocarriers



Varanko, **Ann. Rev. Biom. Eng**. 2020

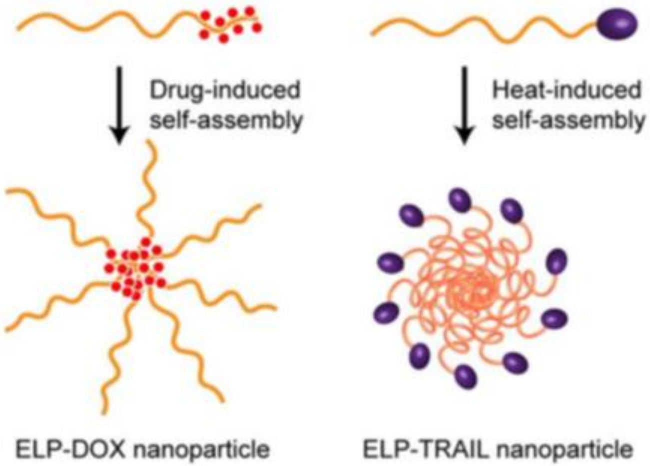
Orange: more hydrophobic ELP block (T_{t2})
Blue: more hydrophilic ELP block (T_{t1})

$T_{t1} > T > T_{t2}$: orange block aggregates but blue block is soluble: micelle-like

Ligands (RGD) within the ELP → cell uptake with targeting ability

- **Self-assembled ELPs/drug nanoparticles**

  - DOX conjugated with Cys
  - Enhanced plasma circulation
  - Tumor targeting

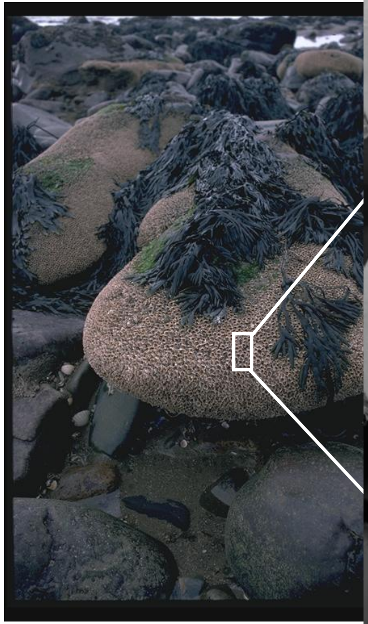


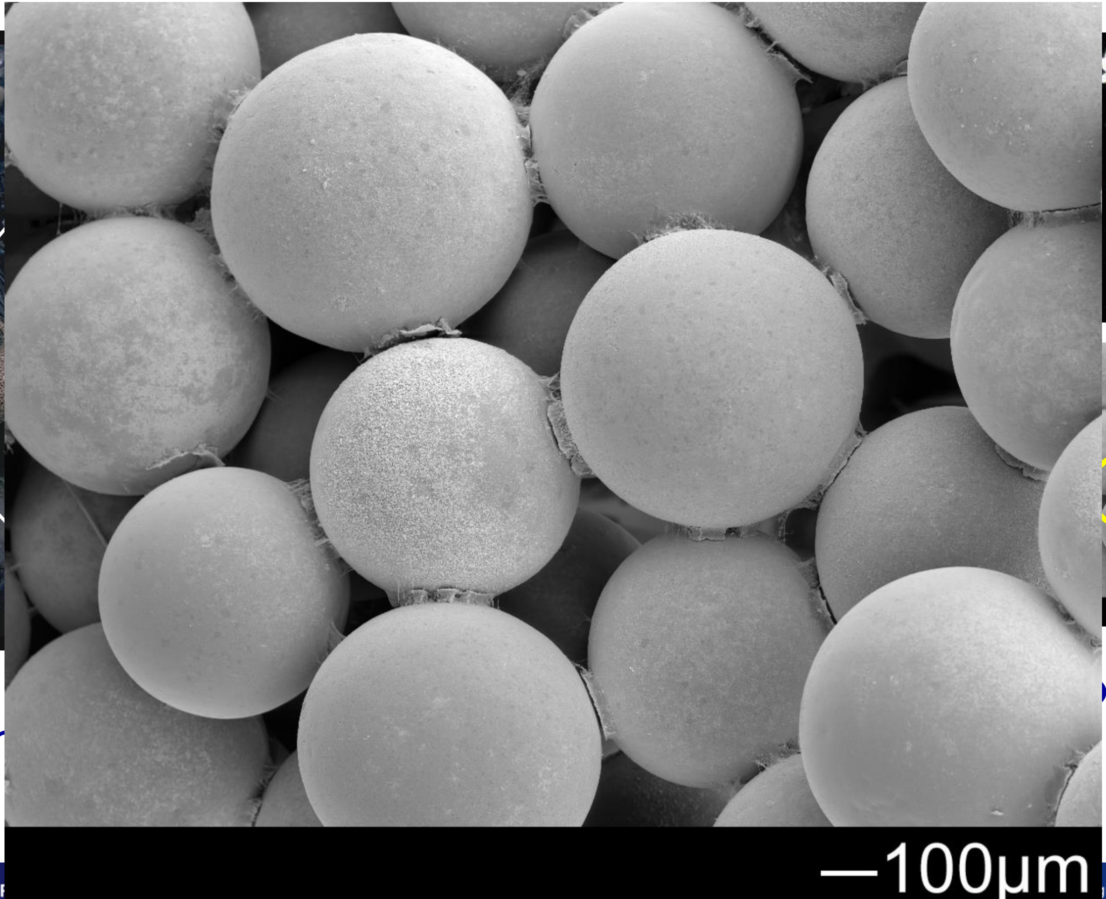
- **ELP** fused to TRAIL
  TRAIL: Ligand inducing tumor necrosis

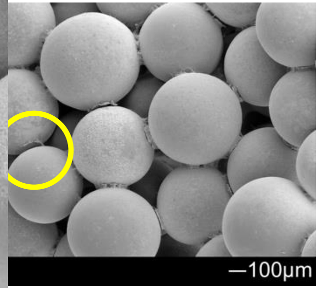
Le *et al.*, **Mol. Syst. Des. Eng**, 2019

Coacervation/LLPS in Biological Adhesives

Protective Tubes of the sandcastle worm







~ 30 sec set ur
cold seawater

unded with
the cement

Coacervation/LLPS in Biological Adhesives

Worms can build protective tubes with many materials

Stewart, Weaver, Morse & Waite, **J. Exp. Biol.** 20024

Tube Cement: Proteins with Dopa and P-serine

Pc-1; MW = 18,240 kDa; pI = 9.74 +

YGCGVGIGC- AGGRCGGACG GKGYGYGG-K LGYGAYGKGG IGGYGYGKGC VGGYGYGGLG
AGK------- LGGYGYGGSK CGGYGYGGQK LGGYGYGGKK LGGYGYAAKK VGGYGYGAKK
VGGYGYGAKK VGGYGYGAKK VGGYGYGAKK VGGYGYGAKK VGGYGYGAKK VGGYGYGVKK
VGGYGYG---

Pc-2; M = 21,116 kDa; pI= 9.91 +

CGGAGG WRSGSCGG
RWGHPAV----HKALGGYG-G YGAHPAVHAAVHKALGGYGAGAYGAG
AWGHPAV----HKALGGYGAGA WGHPAV----HKALGGYG-G
YGAHPAVHVAVHKALGGYGAGACGHKTGGYGG
YGAHP-VAV---KA--AY-NHGFNYGANNAIKSTKRFGG
YGAHP-V-VK--KAFSRGLSHGAY-AGSKAATGYGYGSGKAAGGYGY

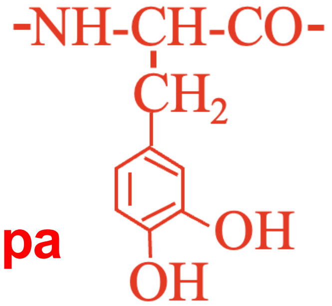
Pc-4; MW = 24,330 kDa; pI = 9.5 +

GYPTYSPSGGTHSGYNGPHGNVVKK TYRGPYGAGAAK AWNGYHGAGYT SVHHGPASTSWHTS
WSNKKGGYGYGLKNKG YGYGLKKVGYGVGLHAAGW HGVGPYGAGYHGAGW NGLGYHGAGYGV
HGVGLHGAGYGL HGVGLHGVGYGL HGVGLHGAGYGL HGVGLHGAGYGI HGVGLHGAGYGI
HGVGLHGVGYGL HGVGLHGAGYGL HGVGLHGAGYGI HGVGLHGAGCGI HKTACYGVGL-- HGHY

Pc-3; M = 30,000 kDa; pI = 2.5 (80% serine, with phospho-serine groups) –

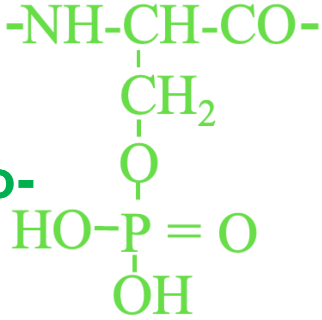
CCKRYSSSSYSSSSSSSSSYSSSSSSSYSSSSSSSSYSSSSSSSSSYSSSSSYSSSSSSSY
SSSSSSSSSYSSSSSYSSSSSSSSSYSSSSSSSSSYSSSSSSSSSYSSSSSYSSSSSSYSS
SSSSSSSSYSSSSSYSSSSSSSSSYSSSSSSSSSYSSSSSYSSSSSYSSSSSSSSSSSSYSSS
SSSSSSYSSSSSSYSSSSSSSSSYSSSSSSSSSYSSSSSSSSSYSSSSSSSSSSSYSSSSSSYS
SSSSSYSSSSSSSSSYSSSSSSSSYSSSSSSSSSSSYSSSSSSSSSSSSSSYSSS

GGYGYG



Dopa

SSSSY



Phospho-
serine

Complex Coacervation of Cement Proteins

Processing is as important as chemistry: Cross-linkable coacervates

Herb Waite
UCSB

- **Pc1, Pc2; pos. charged proteins**
(pI = 9.5 - 10, with dopa)

Seawaterr

secs

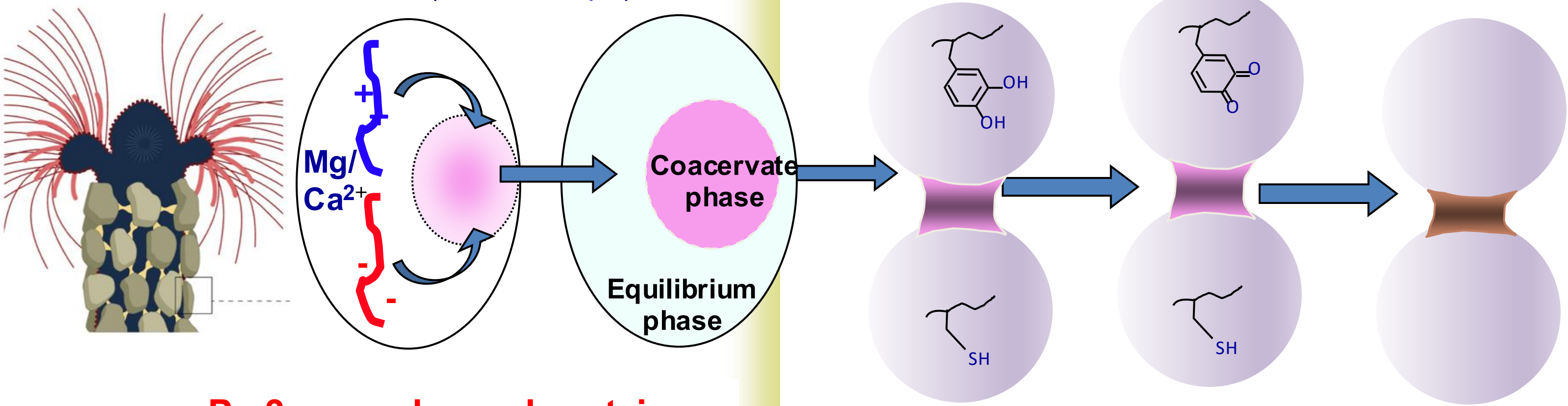
Mg-Phosphate Gelation

Oxidation

min-hours

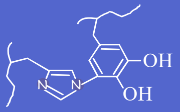
Cross-linking Hardening

Pc-1 + Pc-2 (both w/ Dopa)

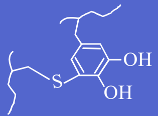


- **Pc-3: neg. charged proteins**
(pI = 2.5, with 80 mol.pct serine!)

Zhao, Sun et al, ***J. Biol. Chem.*** 2005



Histidyl dopa
(331.1 Da)
at 5 mole%
in cement



Cysteinyl dopa
(316.1 Da)
at 0.5 mole%
in cement

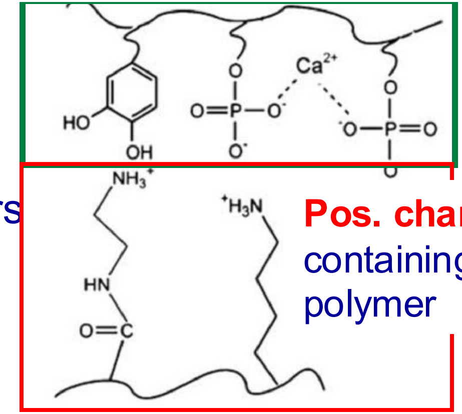
Coacervation/LLPS in Biological Adhesives

➢ The tricks of complex coacervation worth mimicking

- Oppositely charged polyelectrolytes
- Viscous dispersion: spreads on wet surface, fills gaps, but no dispersion in seawater
- Trigger for curing: pH change (~ 5 to 8.2 for seawater)

Neg. charged: phosphate group and dopamine methacrylate

Acrylic copolymers

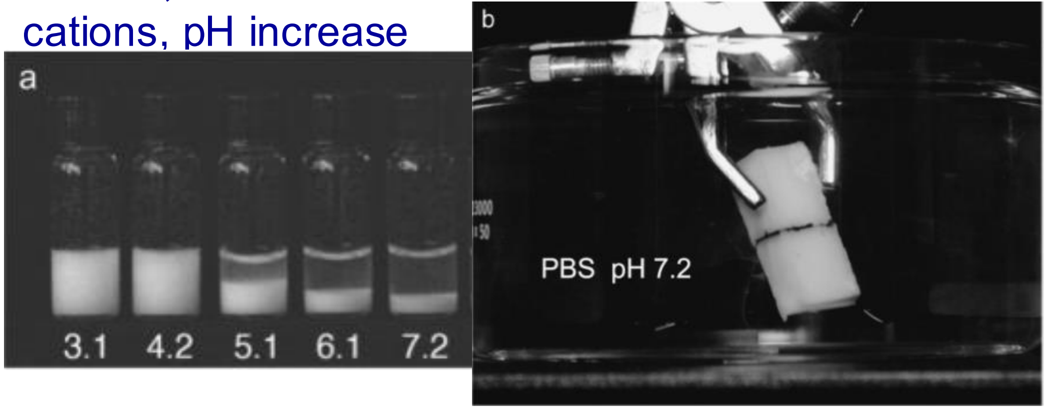


Pos. charged: amine containing acrylic polymer

Shao and Stewart, **Adv. Materials**, 2010

➢ Coacervation

Mixture, addition of cations, pH increase



Shao, Bachus, Stewart, **Macromol. Biosci**, 2009

Coacervation/LLPS in Biological Adhesives

Complex coacervation now a "hot" topic of synthetic bioadhesives

Progress in Polymer Science 139 (2023) 101649

Contents lists available at ScienceDirect

Progress in Polymer Science

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

ELSEVIER

Soft underwater adhesives based on weak molecular interactions

Mehdi Vahdati[a,*], Dominique Hourdet[b], Costantino Creton[b]

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[b] Soft Matter Sciences and Engineering (SIMM), ESPCI Paris, PSL University, Sorbonne University, CNRS, 75005 Paris, France

Vadhati *et al*, **Prog. Pol. Science**, 139, 2023

REVIEW

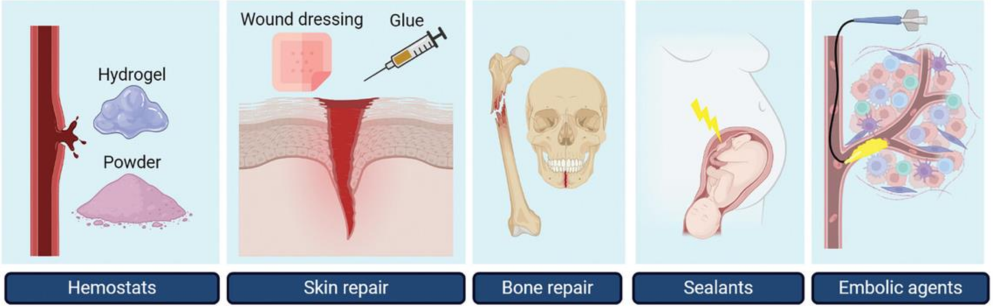
ADVANCED HEALTHCARE MATERIALS
www.advhealthmat.de

Sticky Science: Using Complex Coacervate Adhesives for Biomedical Applications

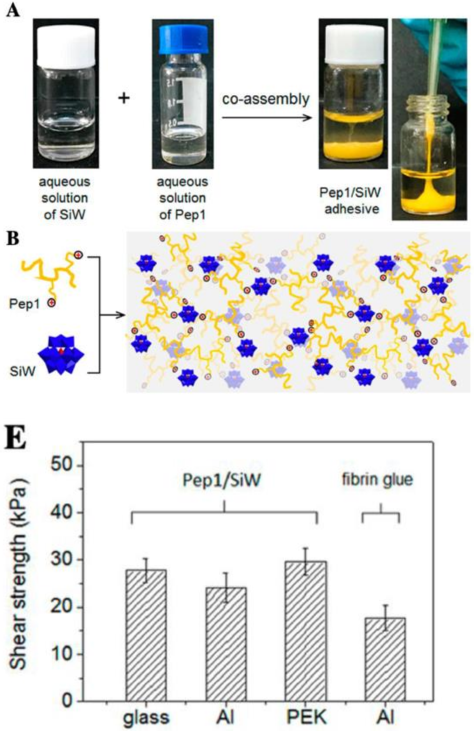
*Ayla N. Kwant, Julien S. Es Sayed, Marleen Kamperman, Janette K. Burgess, Dirk-Jan Slebos, and Simon D. Pouwels**

Kwant *et al*, **Adv. Health Care Mat**, 14, 2025

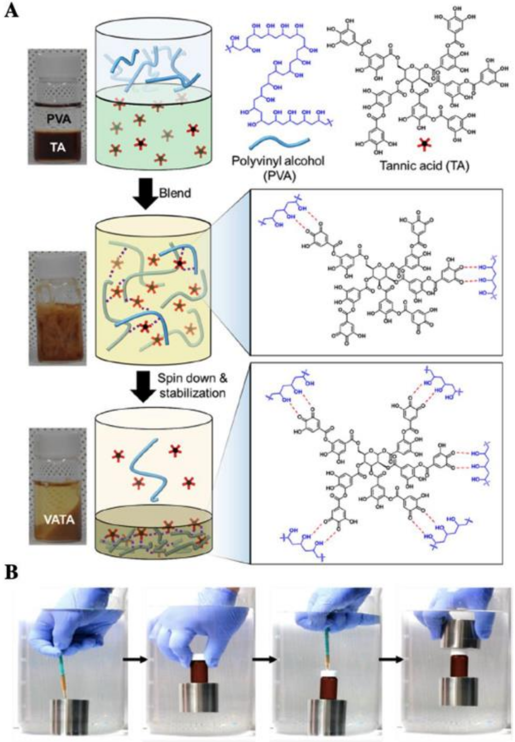
"In this review, we critically discuss the state-of-the-art in the design and characterization of soft viscoelastic coacervates and gels based on specific weak molecular interactions for underwater adhesion"



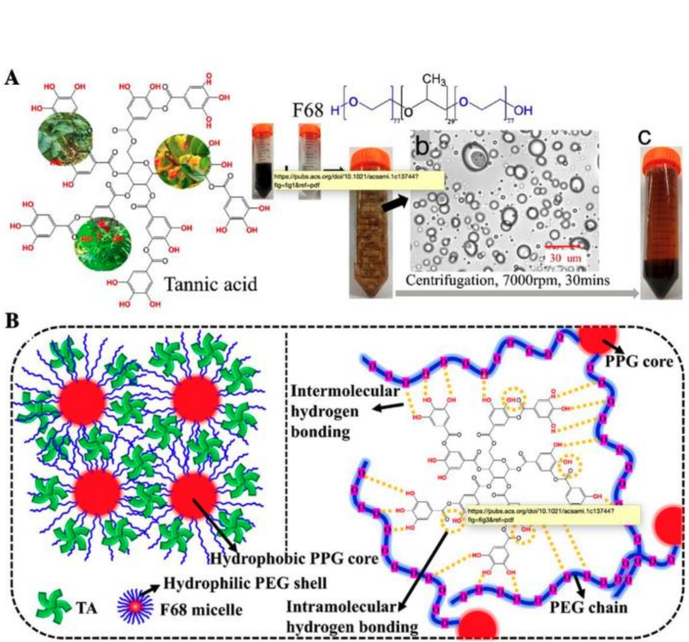
Synthetic Bioadhesives of Complex Coacervates



Polyoxymetalate / Gly-His-Lys tripeptide
(Li *et al*, **Langmuir** 2019, 4995)

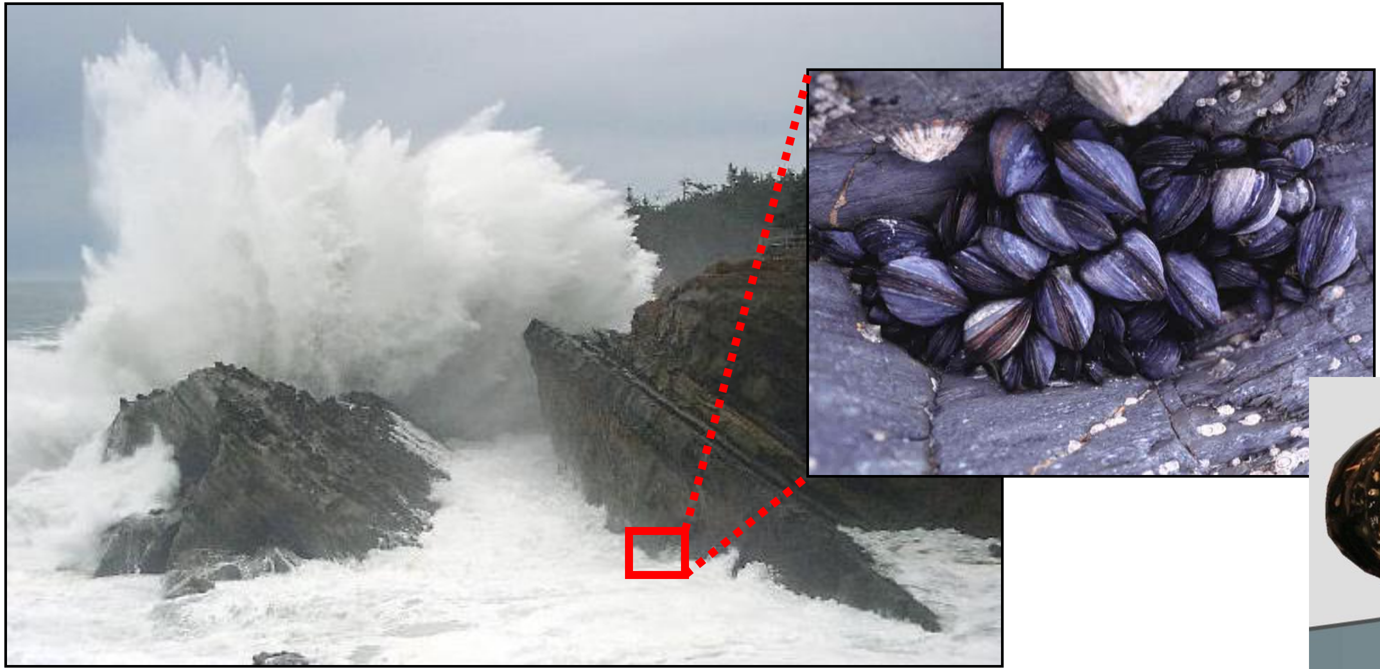


Tannic acid/poyvinly acid (H-bonded coacervates)
(Lee *et al*. **ACS AMI**, 2020, 20933)



Tannic acid/PEG/PPG
(Peng *et al*, **ACS AMI** 2021, 48239

Coacervation/LLPS in Biological Adhesives



Coacervation/LLPS in Biological Adhesives

WOOD PILINGS

ROCKS, CONCRETE

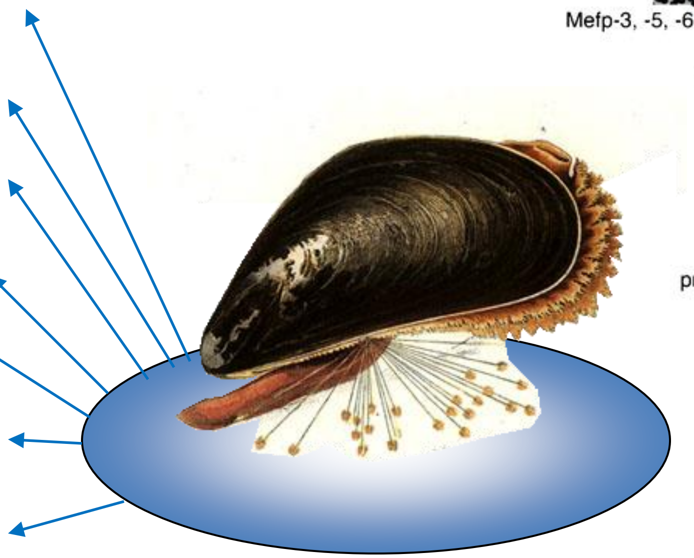
STEEL HULLS

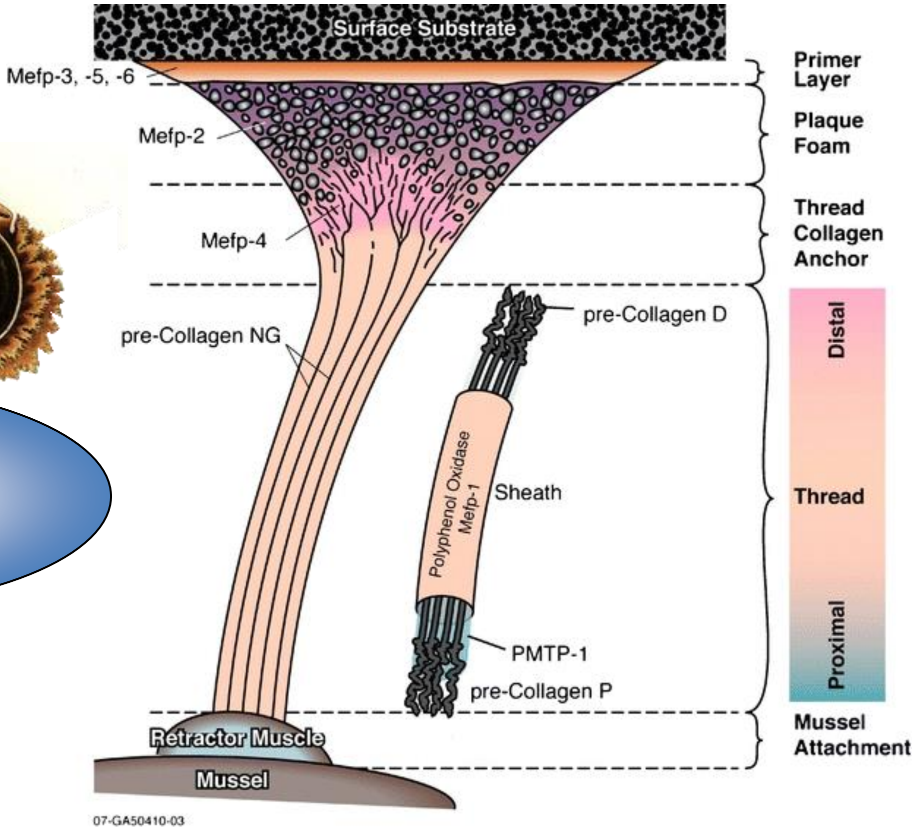
GLASS

OTHER MUSSELS

VARIOUS POLYMERS

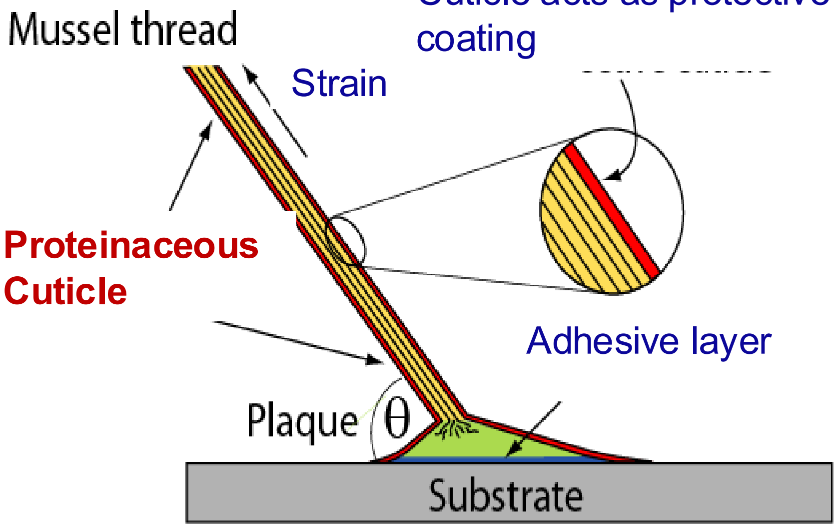
ALUMINIUM

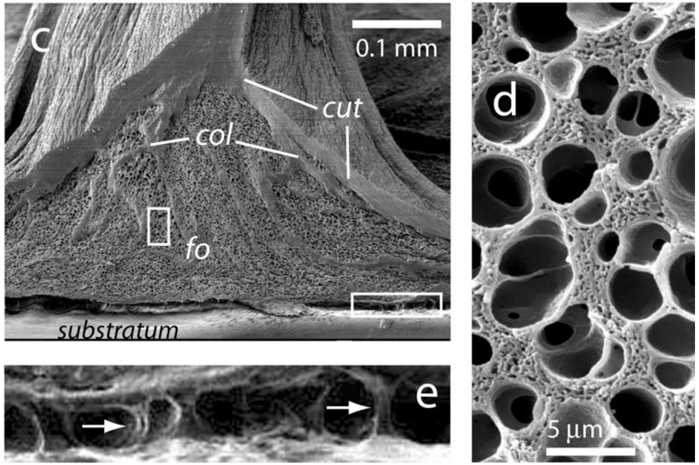




Coacervation/LLPS in Biological Adhesives

- Mussel byssus: a multi-protein fiber with various levels of Dopa
  - Core of the fiber: collagen-like and silk-like proteins
  - Adhesive plaque: disordered proteins with high Dopa content
  - Protective cuticle: Deca-peptide repeat with 20% Dopa

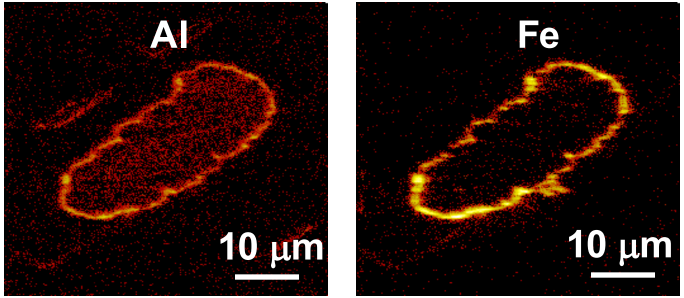


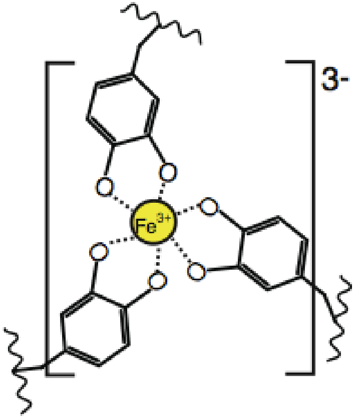


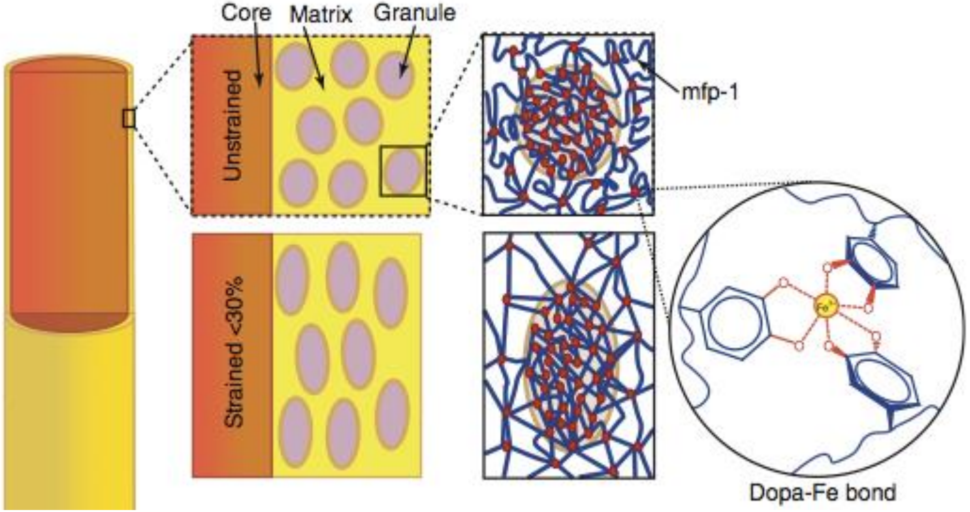
Waite *et al.* ***Ann Rev Mat Res***, 2011

Coacervation/LLPS in Biological Adhesives

Protective cuticle: Metal-Protein Complex



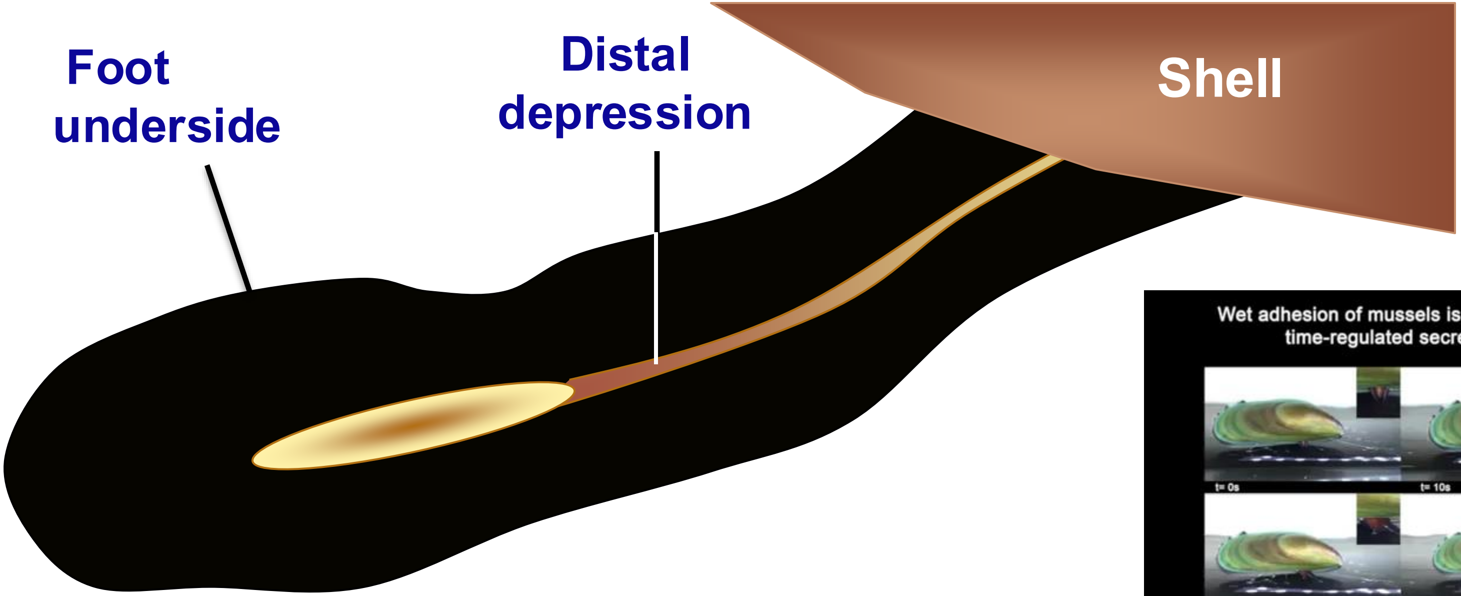


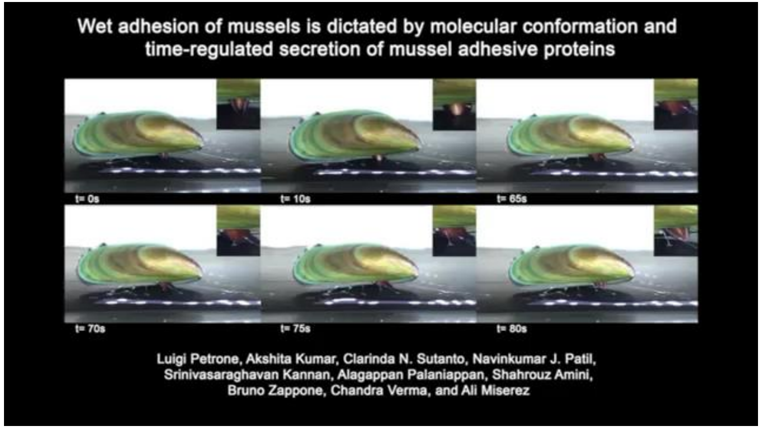


Harrington *et al.*, **Science 328,** 206–211 (2010)

Coacervation/LLPS in Biological Adhesives

Mussel Adhesives Biofabrication

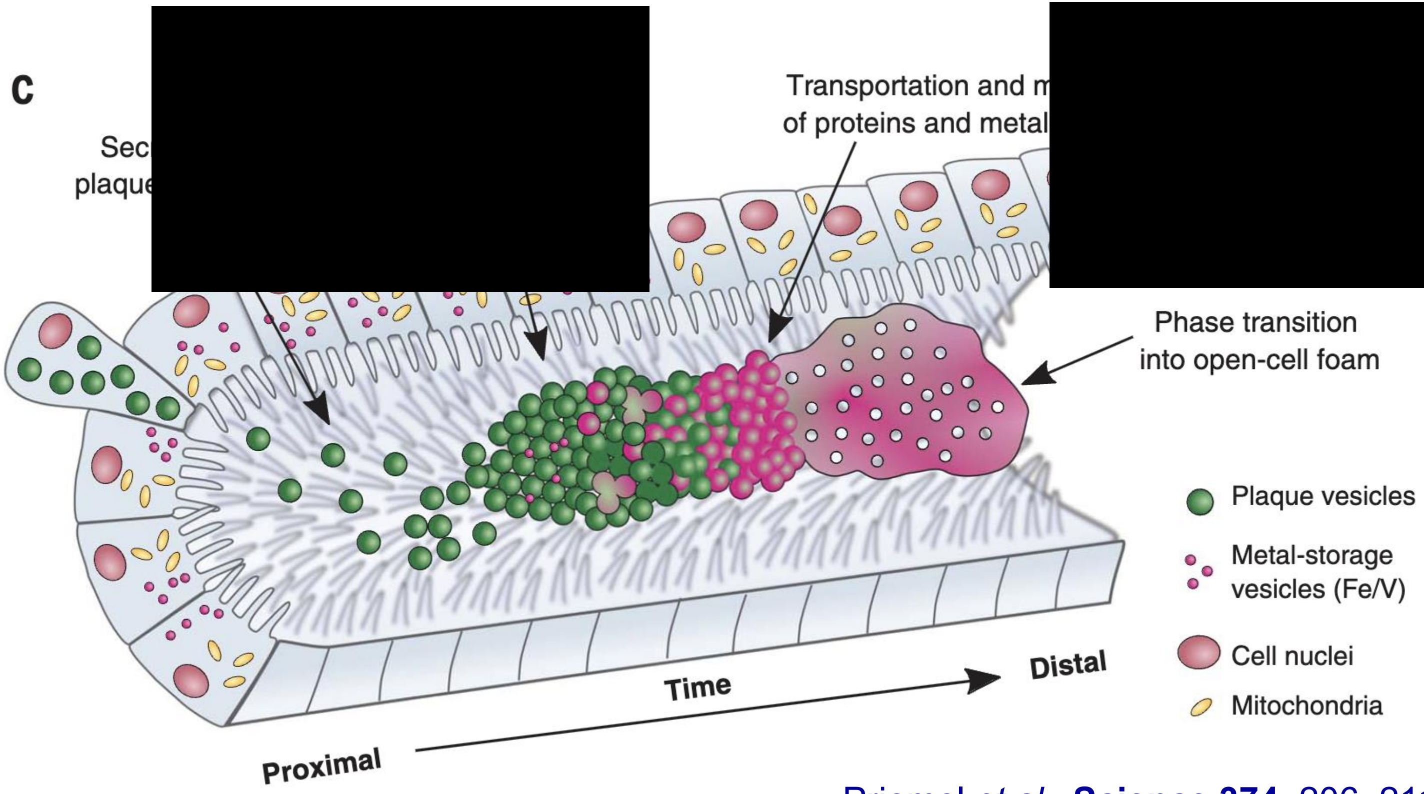




Byssal plaque formation is often compared to reaction injection molding

Coacervation/LLPS in Biological Adhesives

Mussel Adhesives Biofabrication (Harrington Lab, McGill)

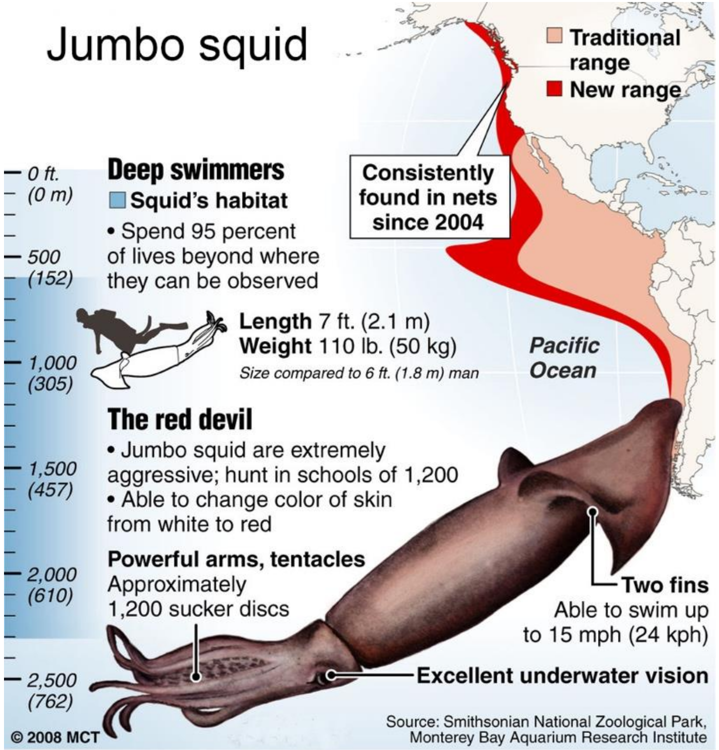


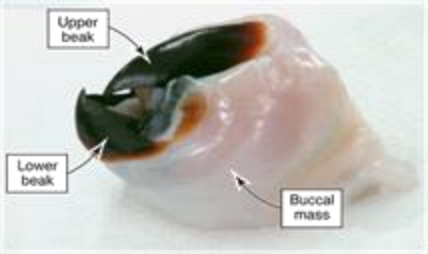
Secreting glands, vesicles, and microfluidic network in the mussel foot

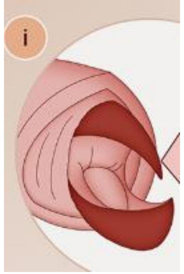
Priemel *et al.*, **Science 374,** 206–211 (2021)

Coacervation in Biological Composites: The Squid Beak

Humboldt squid, *Dosidicus gigas*







Miserez *et al.*, **Acta. Biomat.** 2007, 3, 139-49
Miserez *et al.*, **Science** 2008**,** 319,. 1816-19
Sun *et al.*, **Acc. Chem. Res** 2024, 57, 164-75

➢ Function

- Hard "biotool"
- Mechanical function (chewing)
- Prey handling

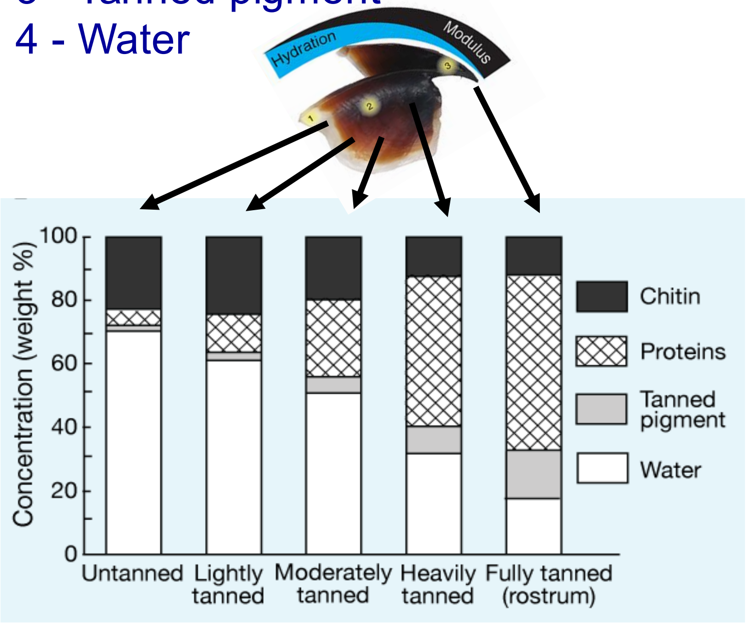
➢ Unique Characteristics

- Not calcified
- No metal ions
- The composition is FULLY organic
- Graded structure of proteins/chitin/water

Squid beak: A Biomolecular Graded Composite

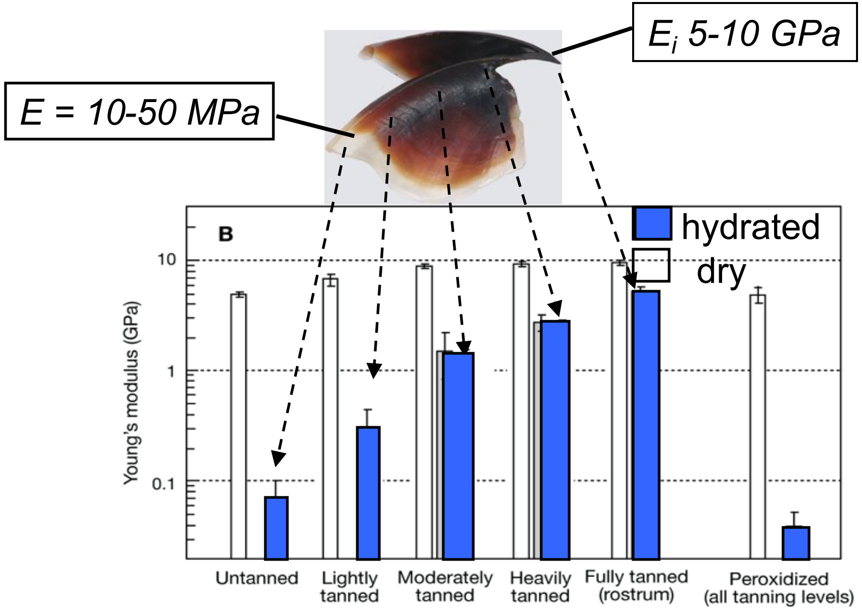
- The beak is a graded composite of

1 - Chitin
2 - Gly, Ala, His-rich proteins
3 - Tanned pigment
4 - Water



Miserez *et al*., **Acta. Biomat**. vol 3, pp. 139, (2007)
Miserez *et al*., **Science**, vol. 319, pp. 1816, (2008)

- Elastic modulus



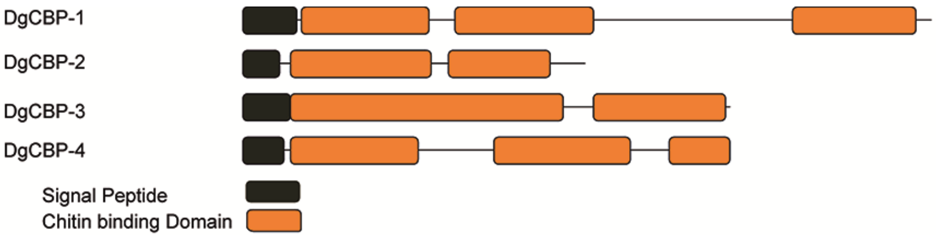
Spans 2 orders of magnitude (hydrated conditions)

- Interfacial stresses are minimized by the mechanical gradient
- One of the strongest fully organic material known (stronger than most tough polymers)

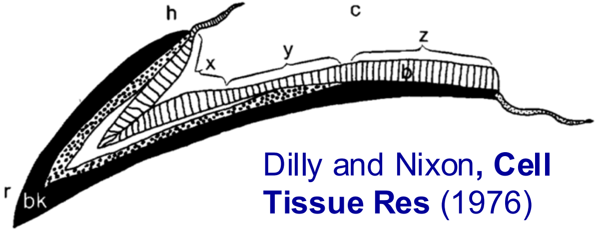
Squid Beak Synthesis and Biofrabrication

Beak transcriptome (beccublast cells) + proteomics

- Beaks are made of 2 protein families
  - Chitin-binding proteins (CBPs)

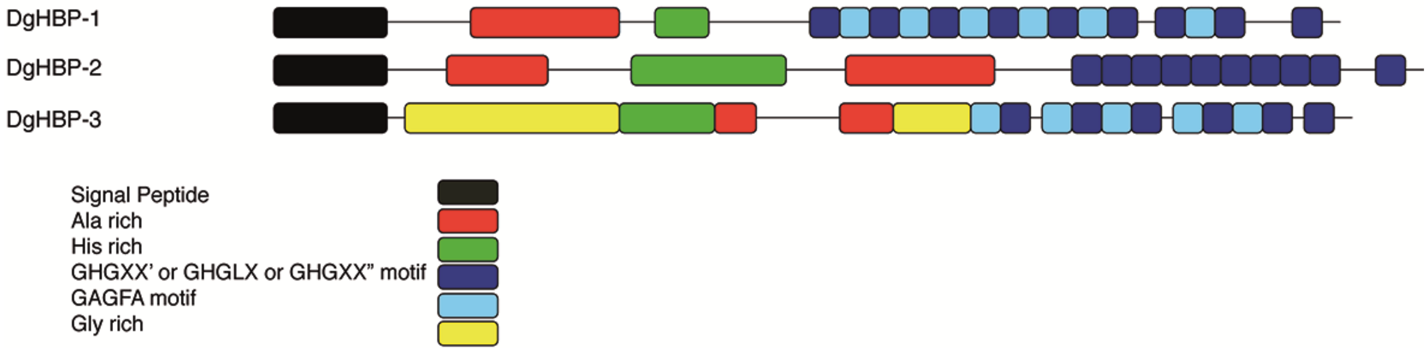


Beccublast cells



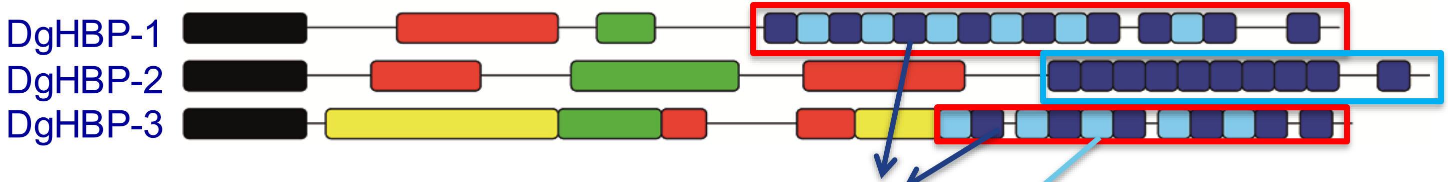
Dilly and Nixon, **Cell Tissue Res** (1976)

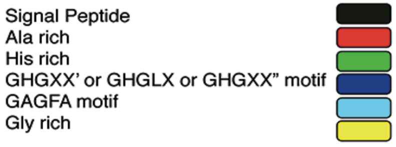
  - His-rich beak proteins (HBPs) with repetitive motifs on the C-terminal



Tan et al., **Nature Chem. Biol.** 2015

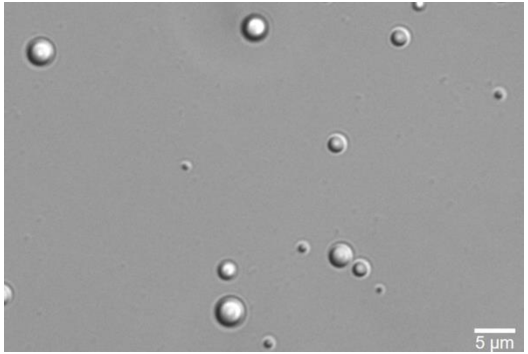
Squid Beak Synthesis and Biofabrication



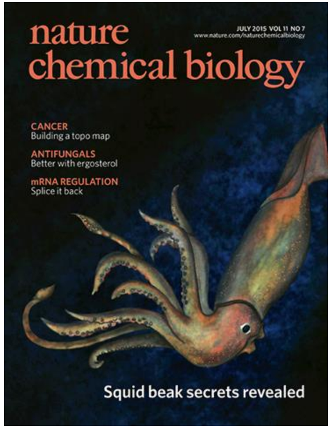


- **GHGXY** (X: P, L, V)
- **GAGFA**
- Elastin polypeptides: **$(VGVXP)_n$**

Histidine-rich beak proteins (HBPs)

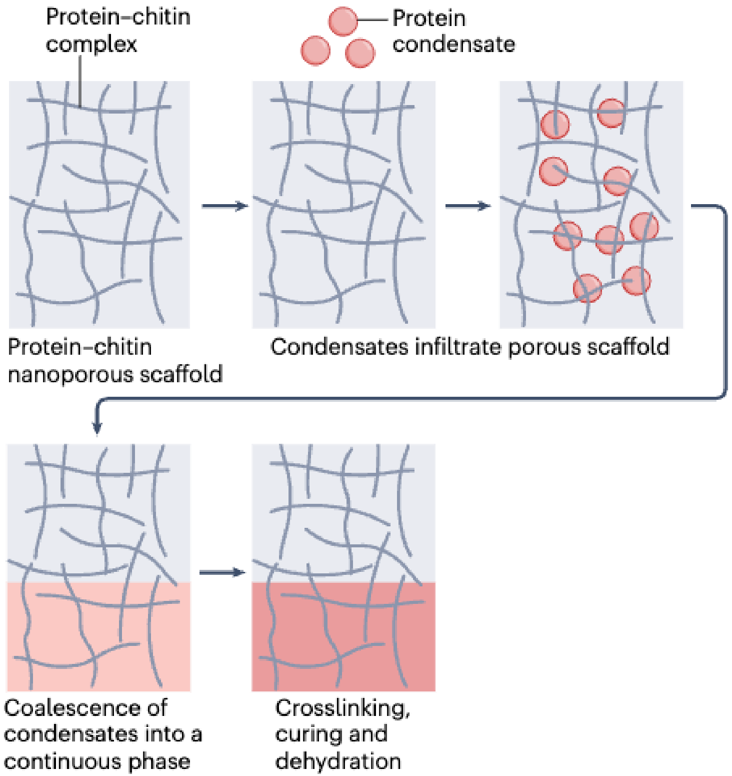


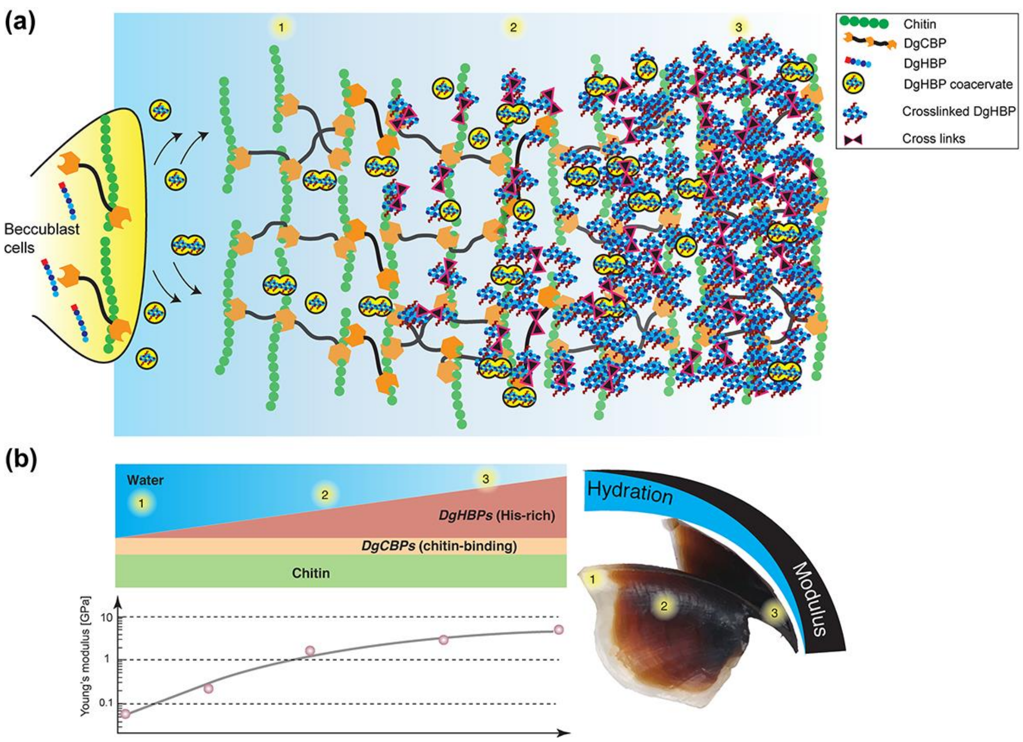
Coacervate microdroplets



Tan *et al.*, **Nature Chem. Biol**. vol. 11, pp. 488, 2015

Squid Beak Synthesis and Biofabrication





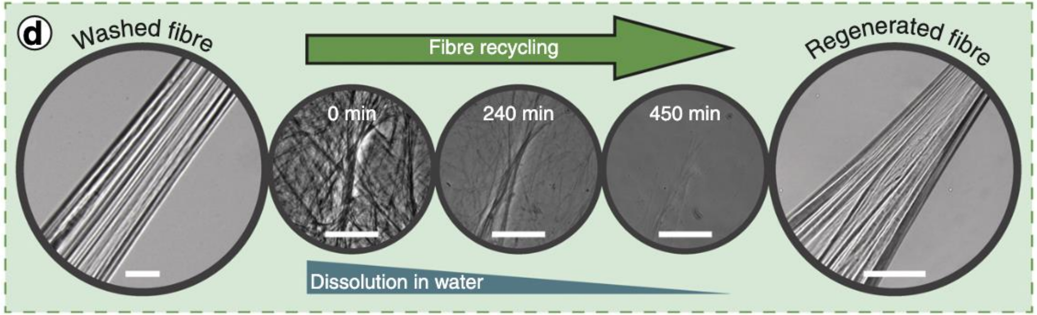
Harrington, Mezzenga, Miserez, **Nature Review Bioengineering** 2024, 2, 260-78

Tan *et al.*, **Nature Chem. Biol**. vol. 11, pp. 488, 2015

LLPS in the slime of the velvet worm

Velvet Worm Slime: Fully Recyclable Biopolymer

- **Unique characteristics:**
  - Dried slime is as stiff and strong as synthetic polymer...
  - ... but can be re-solubilized in water
  - Resolubilized slime: a solution of protein-based nanoglobules surrounded by lipids
  - Fibers can be redrawn from the colloidal solution

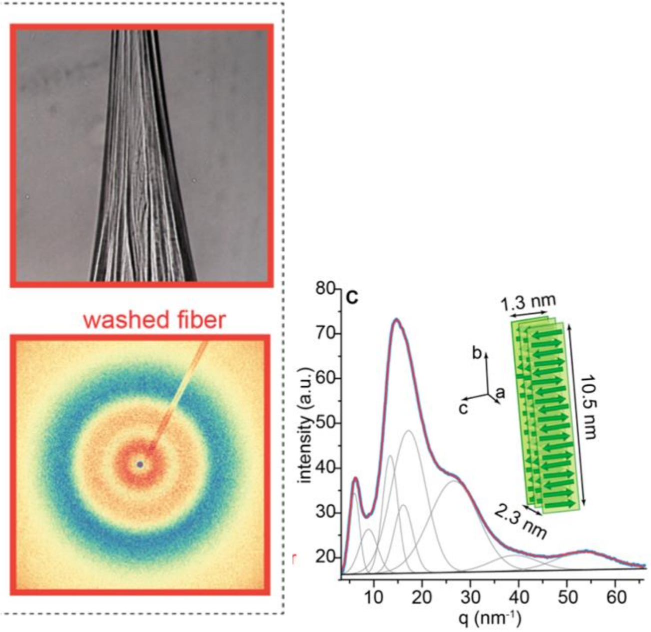


Baer *et al.*, **Nature Commun 8,** 974, 2017

Yang, Sharma *et al*, **Advanced Science 9 (18),** 2022

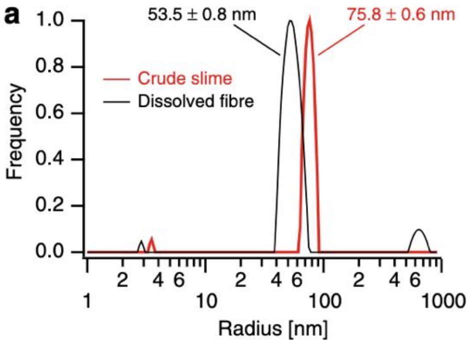
Velvet Worm Slime: Fully Recyclable Biopolymer

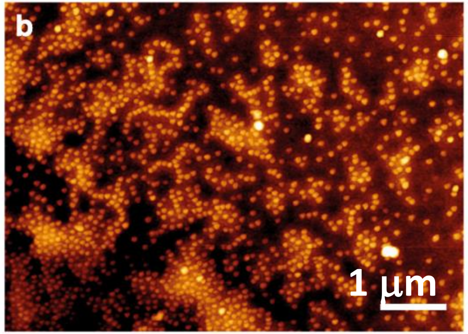
- WAXS of hardened fibers



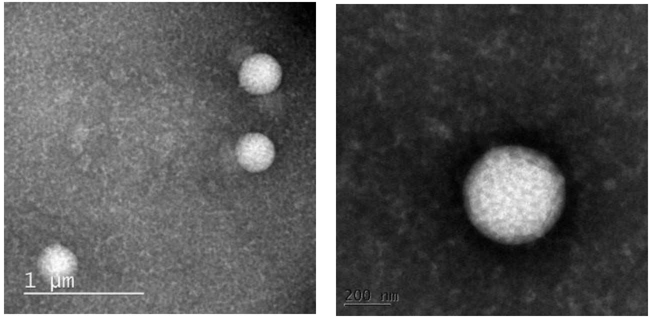
Baer *et al*, **ACS Nano 13**. 4992, 2019

- DLS and AFM of re-dissolved slime





Baer *et al.*, **Nature Commun 8,** 974, 2017

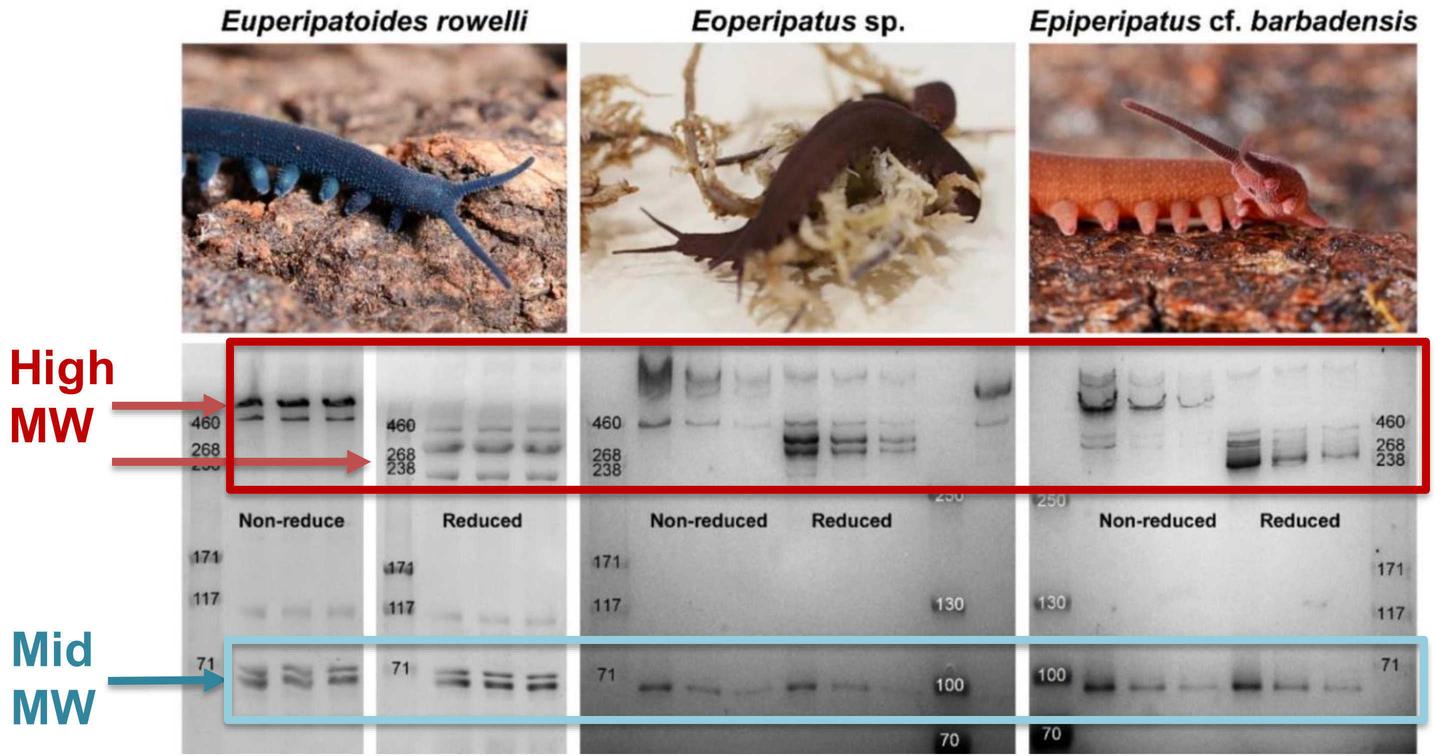


TEM micrographs of re-dissolved slime (negative stain)

Velvet Worm Slime: Biochemical Features

- Sub-groups of Onychophora: *Peripatidae* and *Peripatopsidae*
- Diverted ~ 380 Million years ago

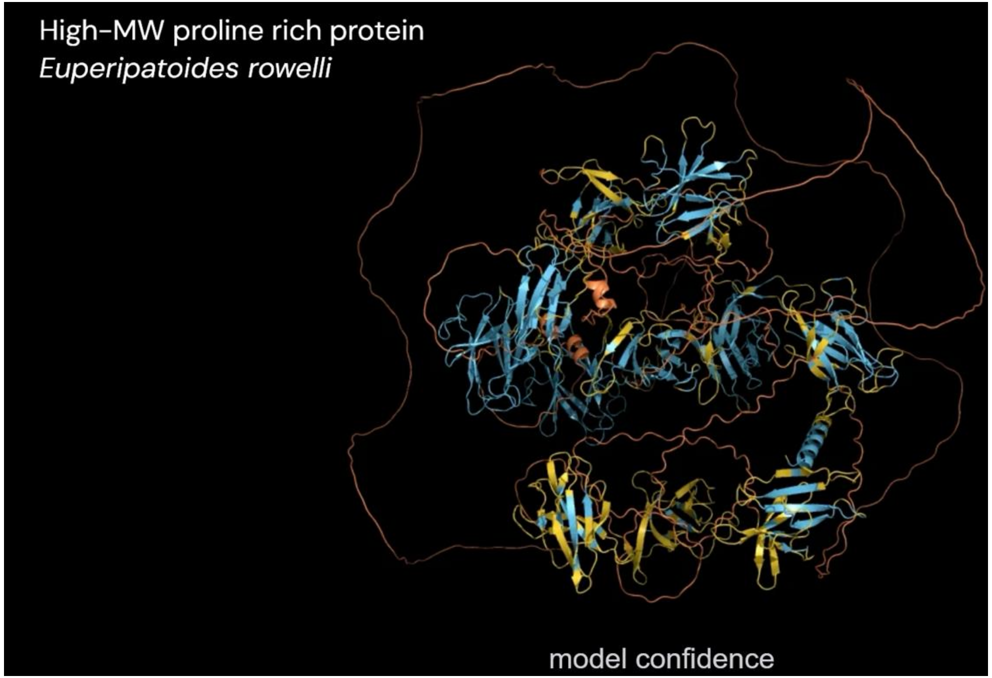
With Harrington Lab
McGill University



- High MW bands: Multi-protein complex held by disulfide bonds
- Phosphorylation
- Glycosylation

Hu, Baer *et al*, PNAS **122,** e2416282122, 2025

High Molecular Weight Proteins of the slime



➢ Key primary features

- High Pro (17%, many hydroxylated) and Lys (10%)
- Disordered regions dominate
- Repeat domains: Di-basic peptides (RR/RK/KK) **|** PP/IIP/PI motifs **|** single acidic residues
- FUS-like (GS-rich) domain at N-termini
- A few Cys residues, near the termini

Yang, Sharma *et al*, **Advanced Science** 2022
Hu, Baer *et al*, PNAS **122,** e2416282122, 2025

LLPS HMW Slime Constructs

- N-terminus of ES_P1 has a <u>predicted</u> IDP structure

```
10         20         30         40         50         60
MKILLSVLVL LIVVECGNSR KIRHRGGSRR GSGGGSGGSS GGSSGGSDGS YGGSDGGSGG

        70         80         90        100        110        120
SYGDSGGGSG DSTGSNGGPG DSYSESGGSS GDGGSGGSYG GSDGGPGGSY GGSGGSGGGG

       130        140
GGGGGGGGGG SGSDNNPPEG
```

- Homology with FUS → LLPS ?

```
FUS-LC   SQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQS- 119
ES_P1-NT --------GSGGGSGGSSGGS--SGG----------------------SDGSYGGSDGGS 58
ES_P2-NT --------GSSGGSYGSTGGS--YDG----------------------SGGLYGGSSGGS 63
                  *   . **:**     .                      :.* **.*. .

FUS-LC   -SSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGGNYG 178
ES_P1-NT GGSYGDSGGGSGDSTGSNGG-------------------PGDSYSESGGSSGDGGSGGSYG 100
ES_P2-NT ---YGELGSGG-------------------------------LFGGS--GGGGFGPGGSYG 88
            **:  .*.                               :. *  ..*. * **.**

FUS-LC   QDQSSMS-----------------------SGGGSGGGYGNQDQSGGGGSGGYGQQDRG- 214
ES_P1-NT GSDGGPGGSYGG--------------------SGGSGGGGGGGGGGGGGSGSDNNPPEGY 141
ES_P2-NT GFDGGLGGSSGGSQGLPGNGWILQPDGSYLKYEGSG--GGGGGGGGGGSGSDGPPGNGW 146
           :.. .                          ***   *. . .******. .   .*
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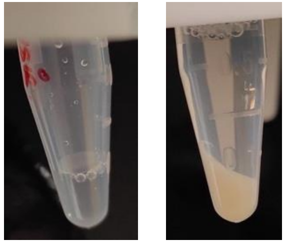
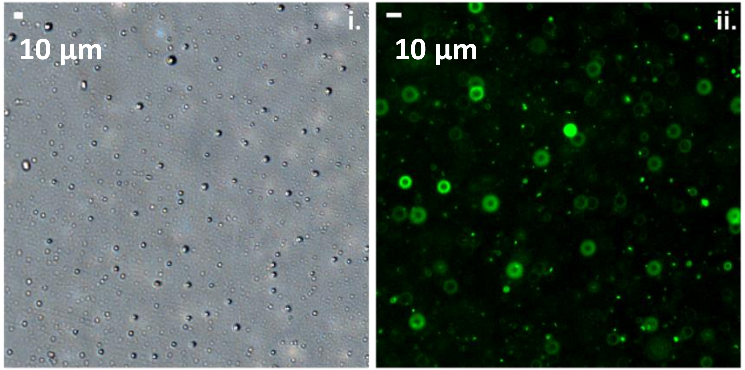
Yang, Sharma *et al*, **Advanced Science 9 (18),** 2022

- Recombinant construct of N-terminus region

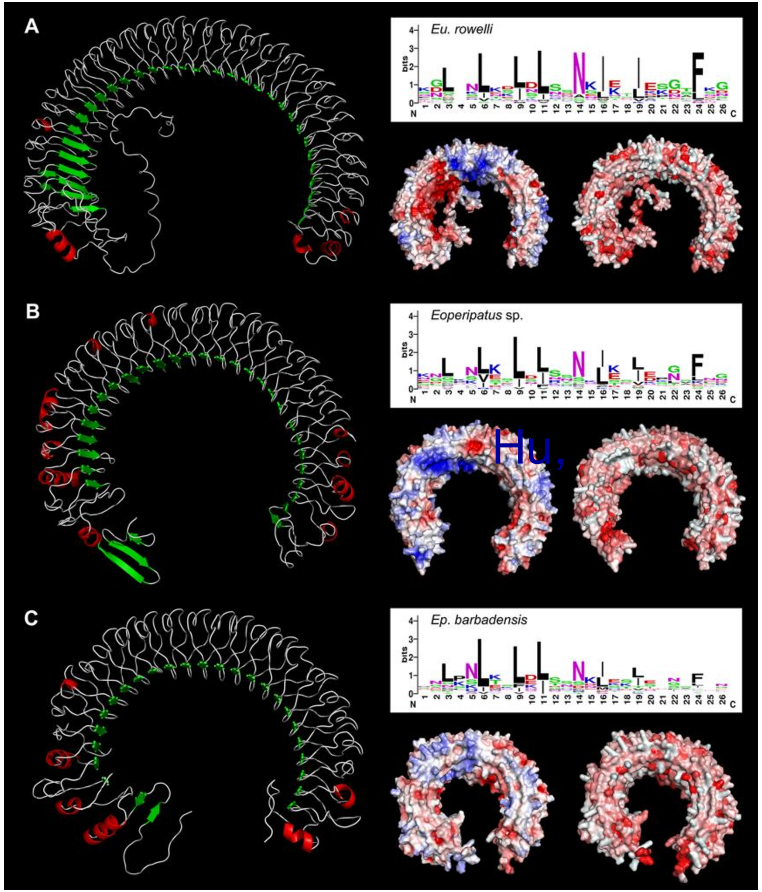


4ºC

> 20ºC (Centrifuged)

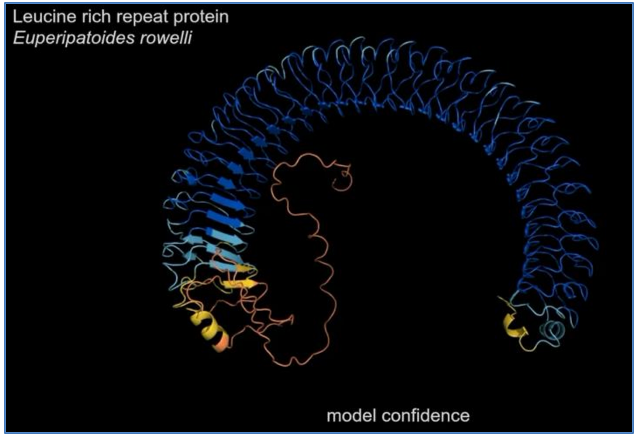


Mid Molecular Weight Proteins of the slime



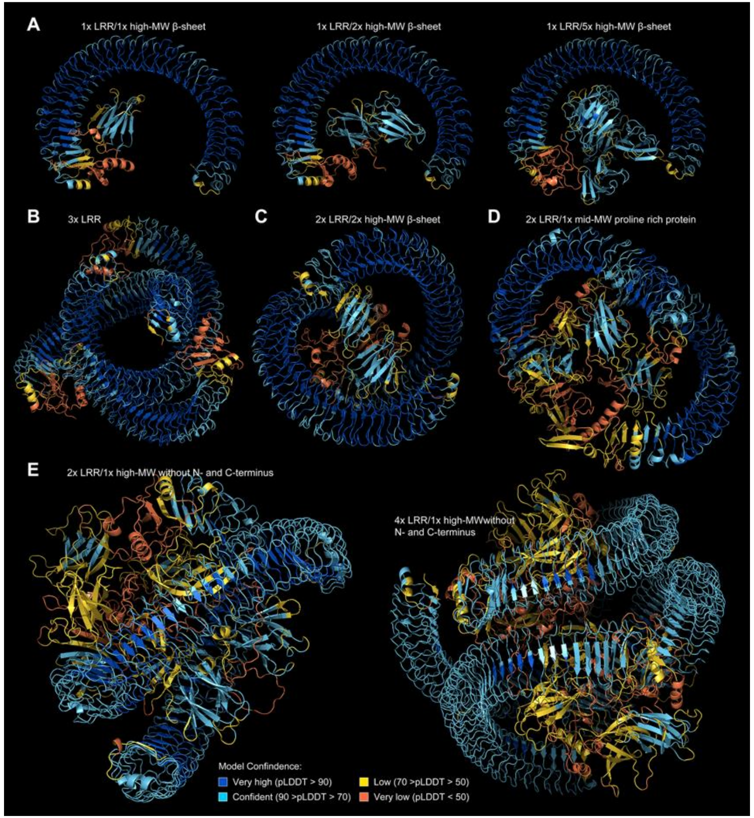
➢ Leucine-rich repeat (LRR)

- Strong homology to Toll-like receptor: “horseshoe-like” structure
- LRR involved in innate immunity, development, cell adhesion and signaling
- First example of LRR in an extra-corporeal biological materials



Hu, Baer *et al*, PNAS **122,** e2416282122, 2025

High-MW / Mid-MW Complex Predictions



Hu, Baer *et al*, PNAS **122,** e2416282122, 2025

- Protein-protein interactions: AlphaFold 3
  - High confidence of high-MW / Mid-MW interactions (β-sheet rich regions)
  - Receptor/ligand interactions → supramolecular biopolymer
- Possible implications for slime biofabrication
  - High MW and Mid MW segregated in the slime
  - Shear during secretion: elongate high MW and expose ligands → Network formation based on receptor/ligand interactions
  - In water: concentration of receptor/ligand decreases

  → Returns to the lowest energy state: the slime

Take Home Message

- Living World / Biology
  - Sustainable, “green” chemistry principles: protein-based materials provide countless peptide design principles
- LLPS / Coacervation involved in biogenesis of
  - Extra-cellular materials (elastin)
  - Bioadhesives (sandcastle worm cement, mussel adhesives)
  - Biological composites (squid beak, insect cuticles)
  - Extra-corporeal fibers (velvet worm slime, spider silk)
- Sustainability
  - Making sustainable materials using synthetic biology, and aqueous-based chemistry
- Biomedical field applications
  - Bio-based materials are (usually) safe and can be used in numerous healthcare applications → **Lecture 2**

Acknowledgements

Students and researchers that have contributed to coacervates research

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- Mr. Congxi Huan**
- Mr. Filipe Fereira **
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** Current PhD students

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- Prof. Yu Jing (NTU)
- Prof. Bertrand Czarny (NTU)
- Prof. Atul Parikh (UC Davis/NTU)
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