What's happened since 2014?

Prof. Tiffany Abitbol

2025

Another resilin-nanocellulose paper

BIOMACROMOLECULES
ACS AuthorChoice
Article
pubs.acs.org/Biomac

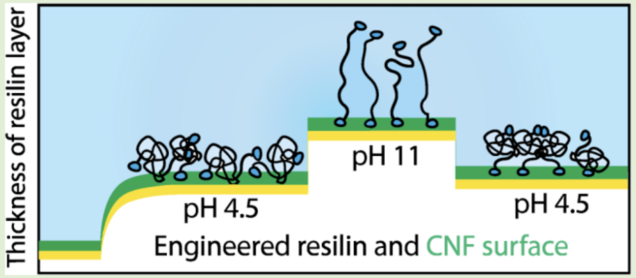
Elastic and pH-Responsive Hybrid Interfaces Created with Engineered Resilin and Nanocellulose

Wenwen Fang,[†] Arja Paananen,[‡] Marika Vitikainen,[‡] Salla Koskela,[‡] Ann Westerholm-Parvinen,[‡] Jussi J. Joensuu,[‡] Christopher P. Landowski,[‡] Merja Penttilä,[‡] Markus B. Linder,[†] and Päivi Laaksonen*,[†]

[†]Department of Bioproducts and Biosystems, Aalto University, Espoo, FI-00076 Aalto, Finland
[‡]VTT Technical Research Centre of Finland Ltd., Espoo, FI-02044 VTT, Finland

Supporting Information

ABSTRACT: We investigated how a genetically engineered resilin fusion protein modifies cellulose surfaces. We characterized the pH-responsive behavior of a resilin-like polypeptide (RLP) having terminal cellulose binding modules (CBM) and showed its binding to cellulose nanofibrils (CNF). Characterization of the resilin fusion protein at different pHs revealed substantial conformational changes of the protein, which were observed as swelling and contraction of the protein layer bound to the nanocellulose surface. In addition, we showed that employment of the modified resilin in cellulose hydrogel and nanopaper increased their modulus of stiffness through a cross-linking effect.



2017

Cited: 23 times; 3/y
IF: 5.5

More precise language:

RLP – resilin-like polypeptide with terminal CBM

What are they studying?

- Engineered resilin + CNF
- Effect of pH on conformation of protein construct

Main result:

CNF films and hydrogels become stiffer with resilin by **crosslinking**

Why this research?

Stiff building block: CNF

"Cellulose is the most abundant biopolymer in the biosphere … acting as **rigid building blocks**, which heavily contribute **to stiffness and strength**. Nanocelluloses have recently gained increasing attention because of their biodegradability, low cost, low density and good mechanical properties. Particularly, CNF can be employed as a **building block** for nanocomposites of water-soluble components because it is well dispersed in water without any chemical modifications."

Elastic building block: Resilin

"Resilin is an **elastomeric** protein found in specialized regions of the cuticle of most insects. It has outstanding elasticity and long fatigue life and is purported to be one of the most resilient elastic materials known. The high content of glycine and proline residues confers to resilin a high degree of flexibility and conformational disorder, which contributes to the mechanical properties of the protein. Resilin also shows **multistimuli responsiveness** to thermal and pH changes in aqueous solution…"

Why this research? Bio-inspired! (This is a new word since 2014…)

Stiff building block: CNF

+

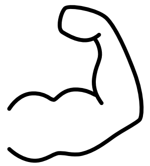
Elastic building block: Resilin

=

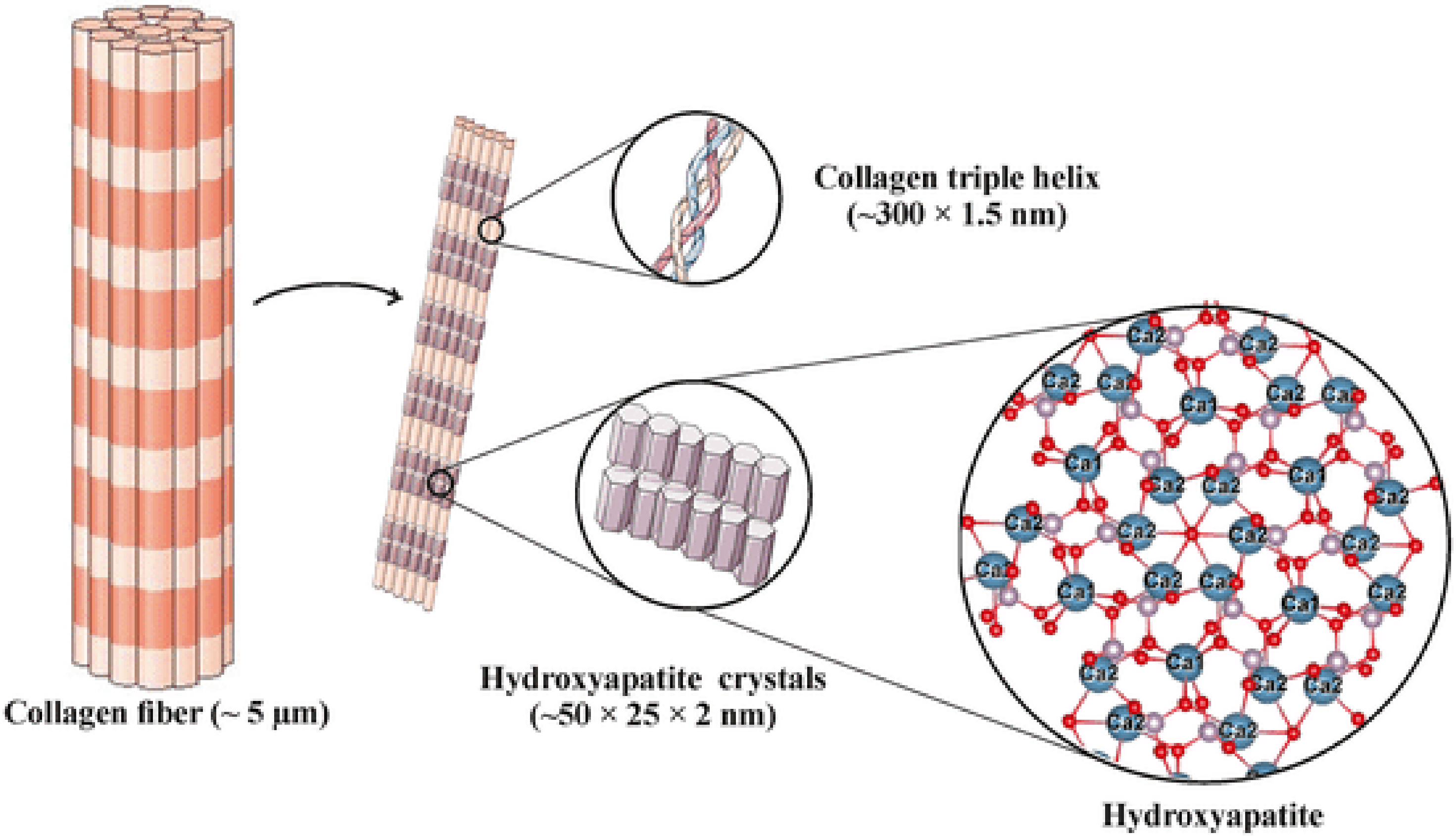
Something amazing, right???

STRONG
TOUGH
DAMAGE-RESISTANT

"In nature, biocomposites are often composed of **stiff building blocks** that are embedded in **a matrix of softer materials**. In such composite structures, the interfaces between the components carrying differing mechanical and chemical nature are in a key role for creating efficient transfer of mechanical stress, which has a major contribution in preventing damage through toughening of the material."



Natural biocomposites: bone



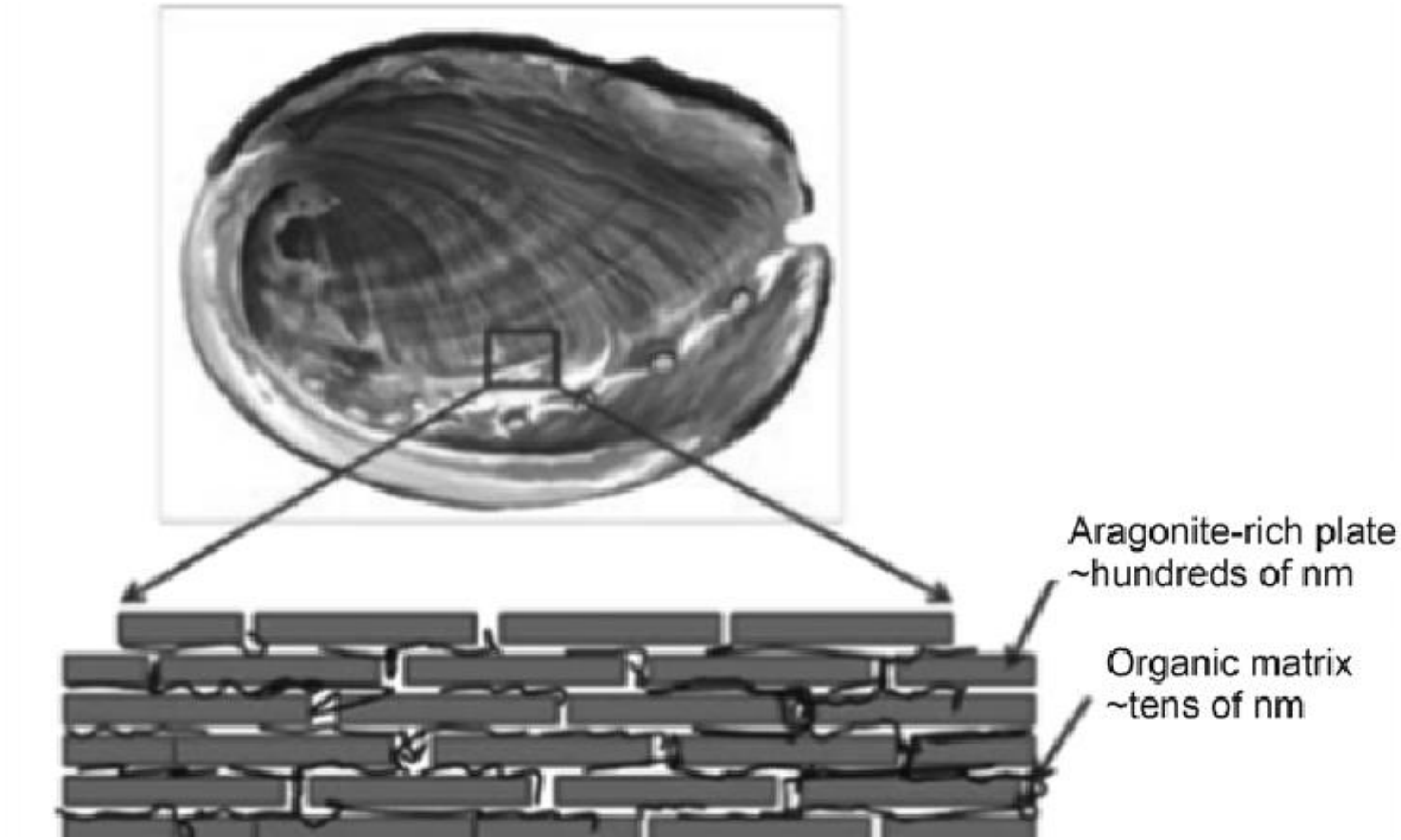
Strong: Hydroxyapatite

Soft: Collagen

$Ca_5(PO_4)_3(OH)$

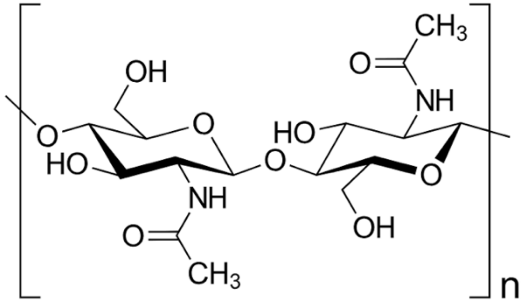
DOI:10.1016/j.oceram.2021.100122

Natural biocomposites: nacre



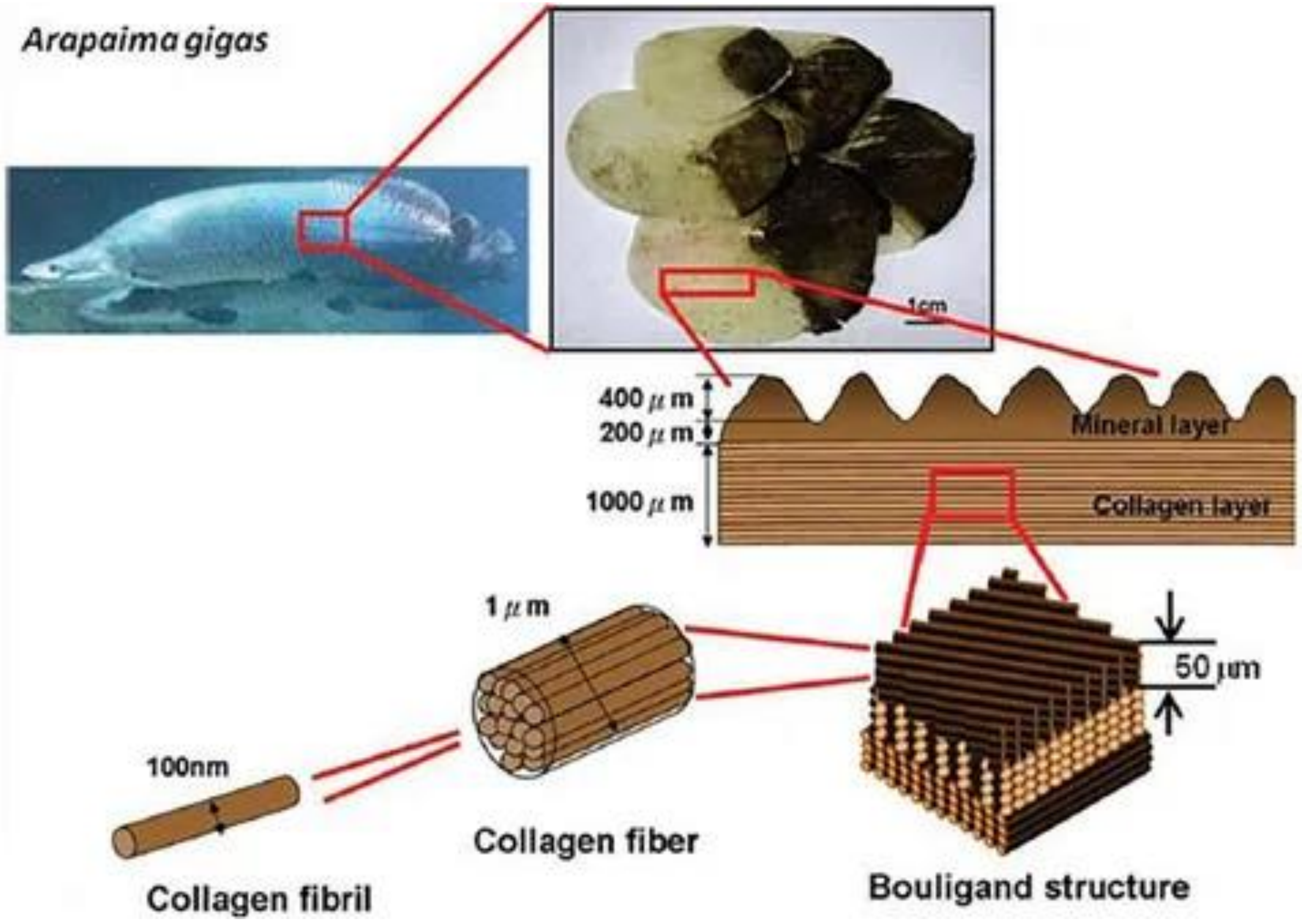
Strong: Aragonite

Soft: chitin and protein



DOI:10.5937/savteh2001069R

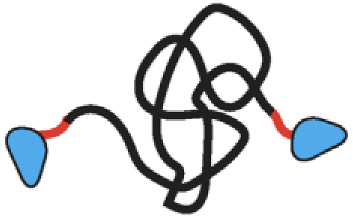
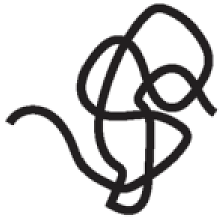
Natural biocomposites: fish scales



Strong: Mineralized collagen

Soft: hydrated collagen

CBMs on both ends!

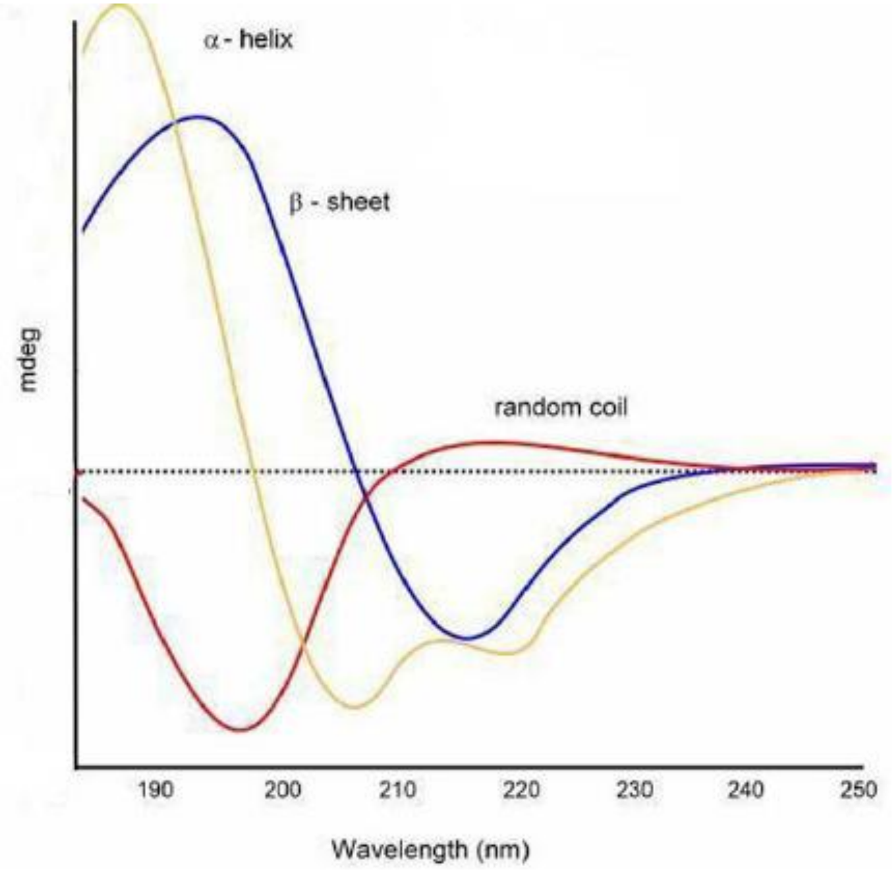
QACSSVWGQCGGQNWSGPTCCASGSTCVYSNDYYSQCLPGASTSTG MGPGGPEPPVNSYLPPSDSYGAPGQSGPGGRPSDSYGAPGGGNGG RPSDSYGAPGQGQGQGQGQGGYAGKPSDSYGAPGGGNGNGGRPS SSYGAPGGGNGGRPSDTYGAPGGGNGGRPSDTYGAPGGGGNGNG GRPSSSYGAPGQGQGNGNGGRPSSSYGAPGSGNGGRPSDTYGAPG GGNGGRPSDTYGAPGGGNNGGRPSSSYGAPGGGNGGRPSDTYGAP GGGNGNGSGGRPSSSYGAPGQGQGGFGGRPSDSYGAPGQNQKPSD SYGAPGSGNGNGGRPSSSYGAPGSGPGGRPSDSYGPPASGGAGGGS GGGQSHYGQCGGIGYSGPTVCASGTTCQVLNPYYSQCL	CBM-RLP-CBM 38.9 kD 
QACSSVWGQCGGQNWSGPTCCASGSTCVYSNDYYSQCLPGANPPG TTTTSRPATTTGSSPGPPGANPPGTTTTSRPATTTGSSPGPTQSHYGQC GGIGYSGPTVCASGTTCQVLNPYYSQCL	dCBM 12.1 kD 
PEPPVNSYLPPSDSYGAPGQSGPGGRPSDSYGAPGGGNGGRPSDSYG APGQGQGQGQGQGGYAGKPSDSYGAPGGGNGNGGRPSSSYGAPG GGNGGRPSDTYGAPGGGNGGRPSDTYGAPGGGGNGNGGRPSSSYG APGQGQGNGNGGRPSSSYGAPGSGNGGRPSDTYGAPGGGNGGRPS DTYGAPGGGNNGGRPSSSYGAPGGGNGGRPSDTYGAPGGGNGNGS GGRPSSSYGAPGQGQGGFGGRPSDSYGAPGQNQKPSDSYGAPGSG NGNGGRPSSSYGAPGSGPGGRPSDSYGPPAS	RLP 29.6 kD 

- CBM to bind to CNF
- Linker
- Exon 1 of resilin

- CBM Dimer

- Resilin-like polypeptide (RLP)

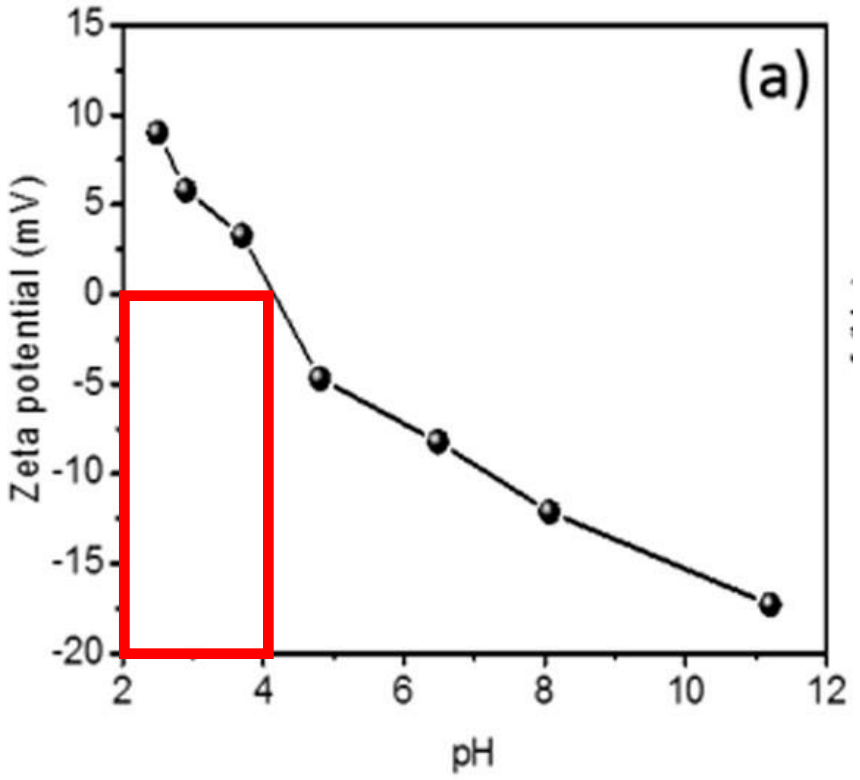
Protein structure: Circular dichroism



DOI:10.2741/3676

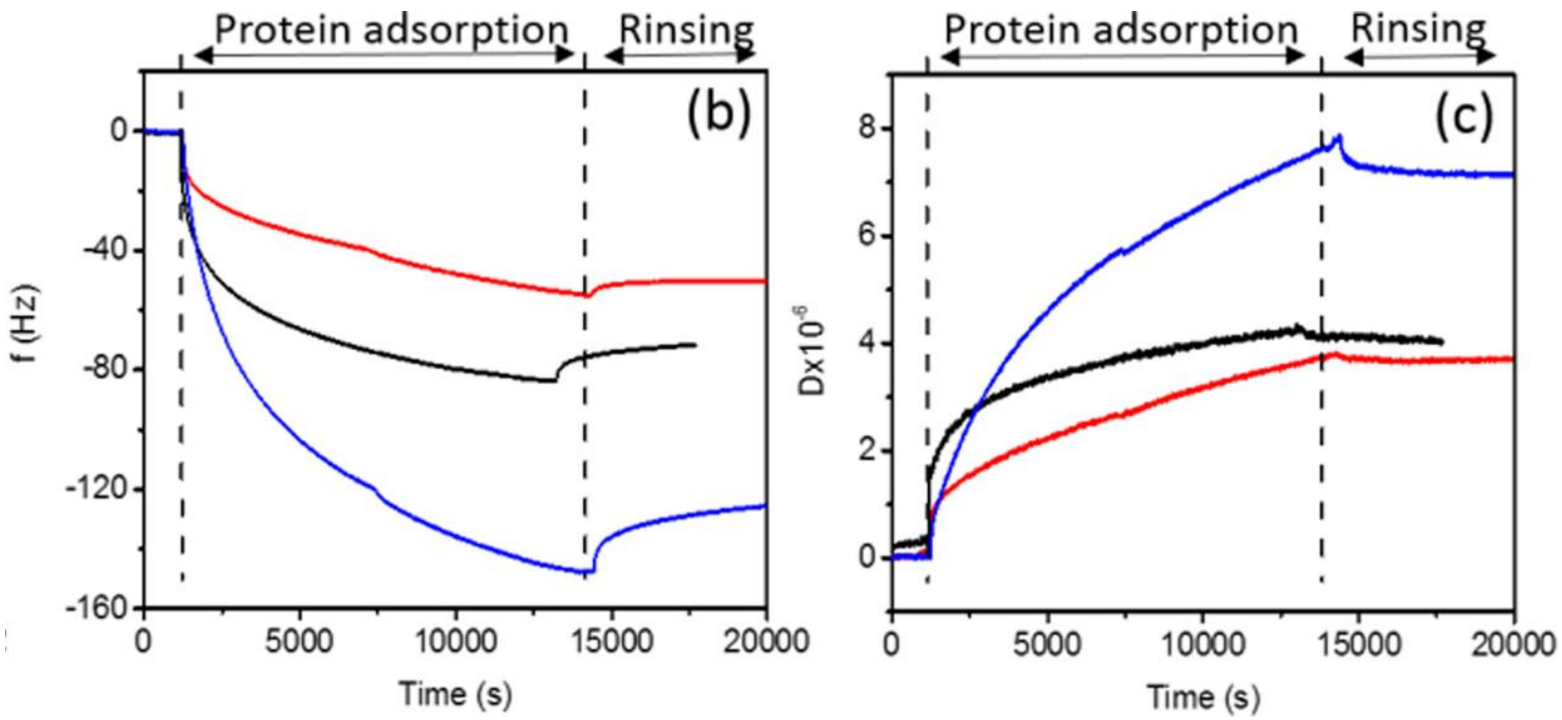
- CD is used to distinguish protein secondary structures

CBM-RPL-CBM: zeta potential



- ZP was measure to determine the isoelectric point (around pH 4.5)
- Theoretical IEP based on sequence is 7.9
- Why the discrepancy? Positively charged residues are in the core of the folded structure and no on the surface
- **What about the CNF?**

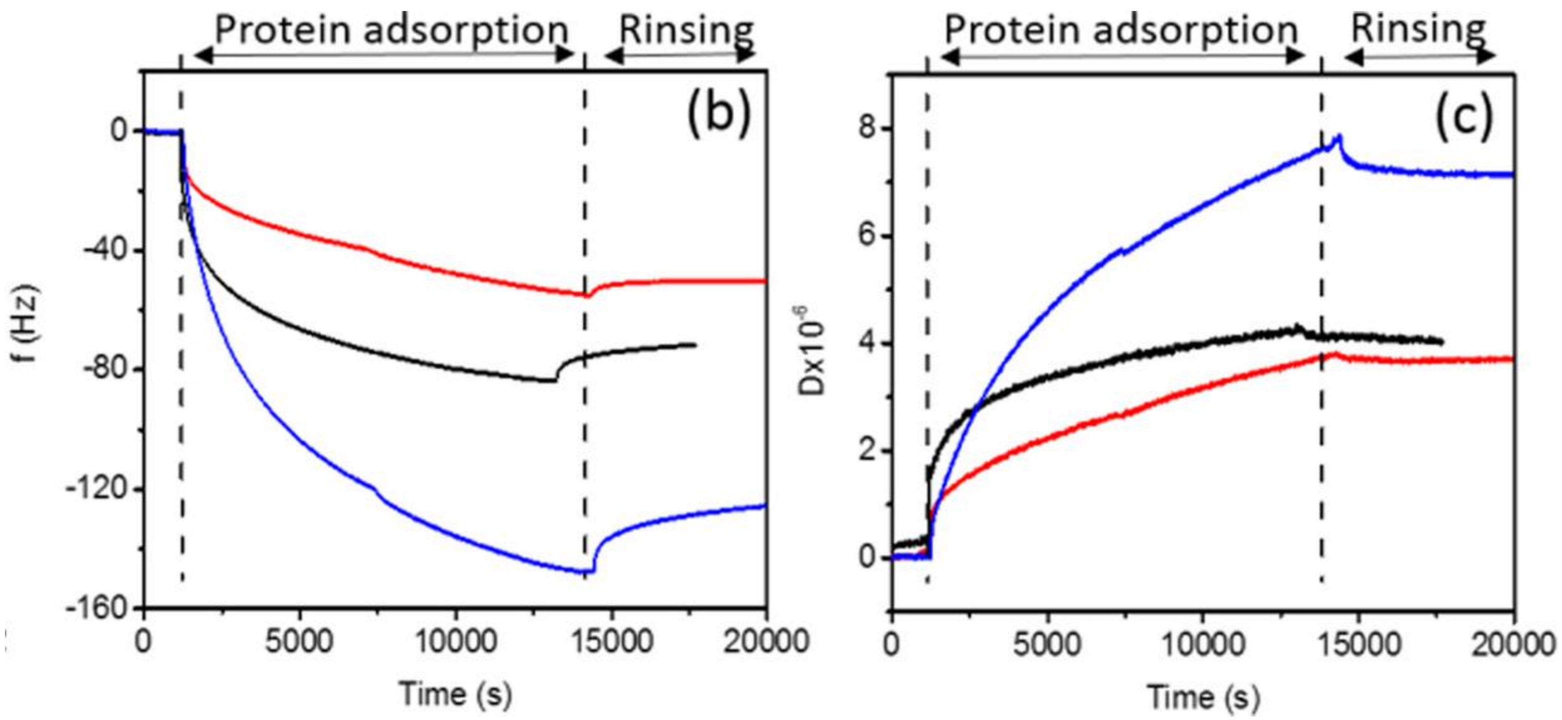
Interaction of protein with CNF surface: QCM



pH 3 – protein is net cationic
pH 4.5 – protein is net neutral
pH 8 – protein is net anionic

- 3 pH values = different charges on protein
- CNF is slightly anionic **(they assume)**
- Binding happens in all cases because CBM binding is pH insensitive
- Electrostatics can enhance (pH 3) or deter the binding (pH 8)

Interaction of protein with CNF surface: effect of pH by QCM



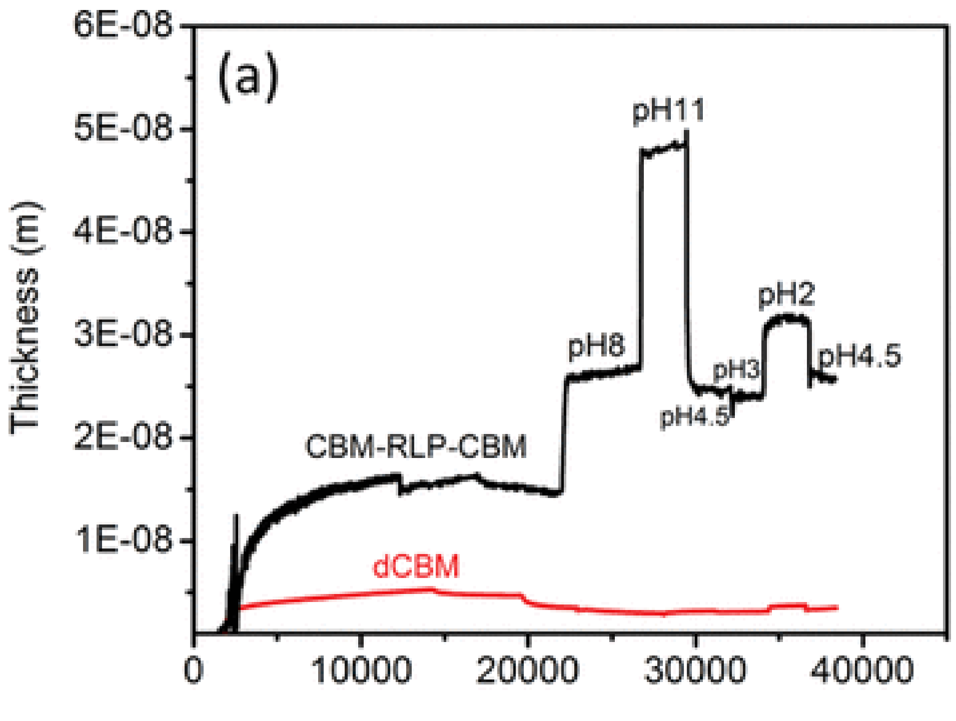
pH 3 – protein is net negative
pH 4.5 – protein is net neutral
pH 8 – protein is net cationic

Used the Sauerbrey equation to estimate the packing density of protein on the surface:

pH 3 = 2334 ng/cm^2
pH 2 = 1317 ng/cm^2
pH 8 = 999 ng/cm^2

- Monolayer is suggested
- What about conformation?

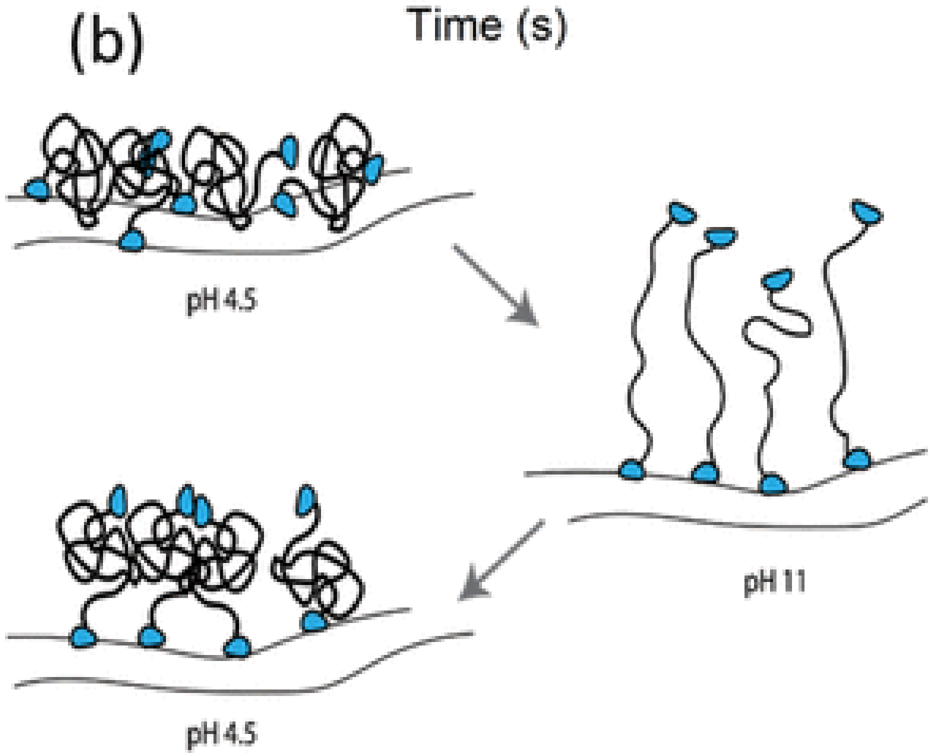
Interaction of protein with CNF surface: QCM



dCBM as control: nothing happens

- To understand conformation, changed pH after initial absorption and stabilization at pH 4.5 – **Key: the amount of protein does not change, only it's conformation**
- Start = pH 4. 5
- pH 8 = swells
- pH 11 = swells more
- pH 4.5 = deswells
- pH 3 – same
- pH 2 = swells
- pH 4.5 = deswells, thicker than start

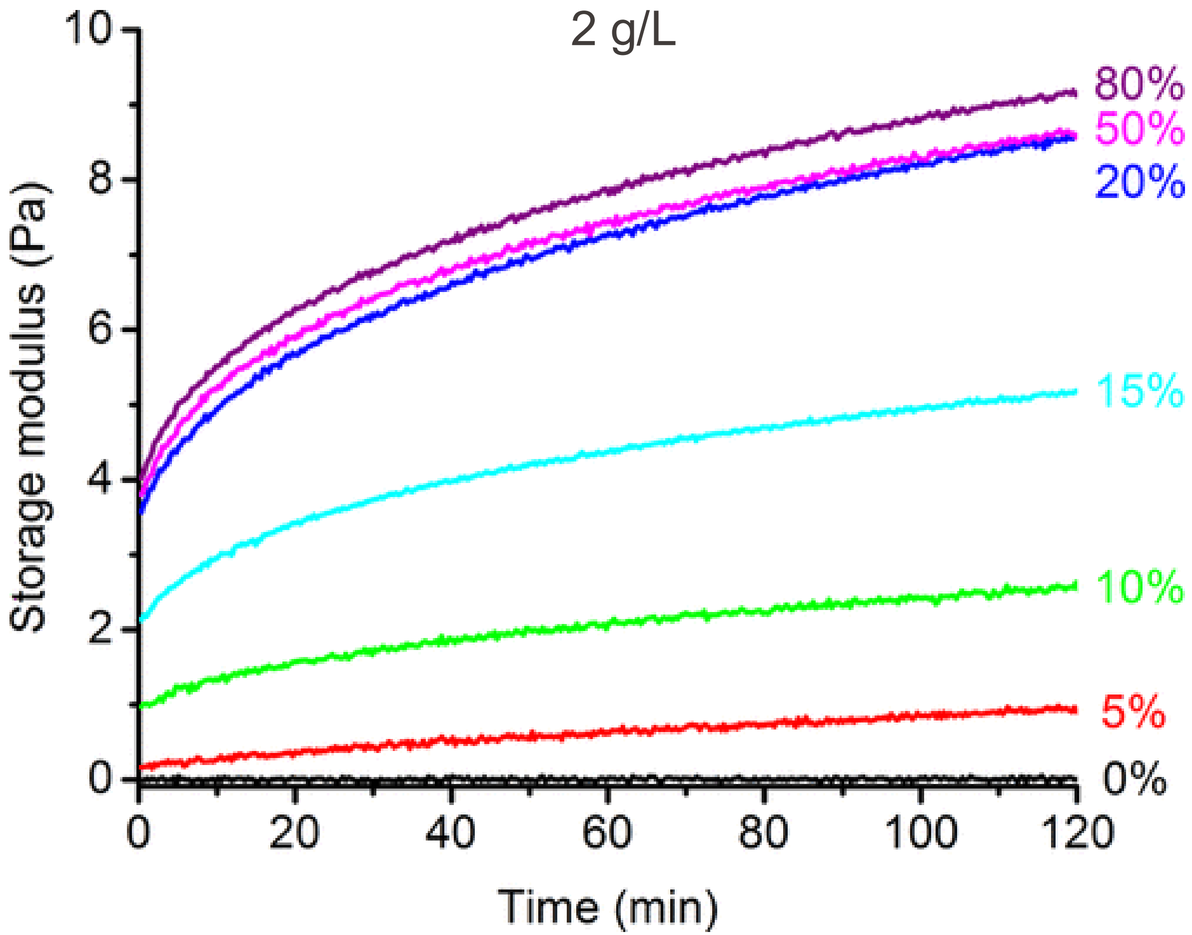
How can we understand this? Schematic.



- The differences in pH that we see might be related to a partial unbinding of 1 CBM, that never quite reorder as they were at the start

- Most differences are from the resilin part of the construct

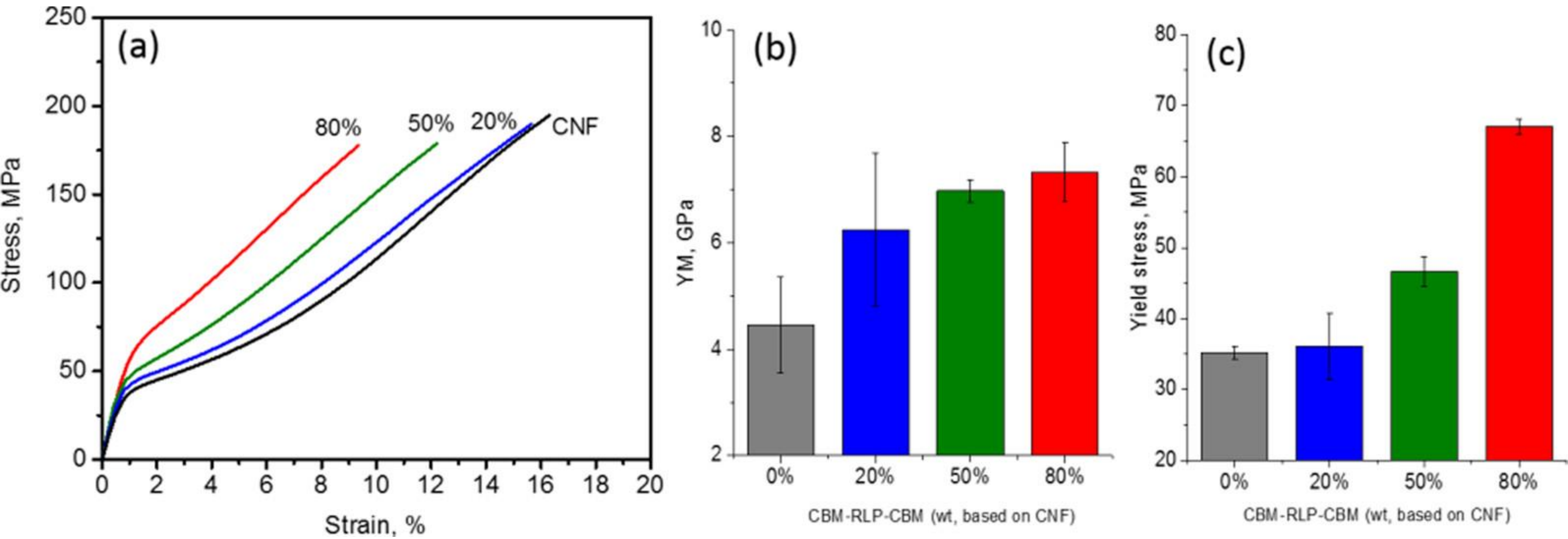
Rheology (more bulk stuff!)



- Different ratios of CNF to protein, up to 80% protein
- Storage modulus increase up to 20% protein, at which point it saturates (only slight increase from 20 to 80%)
- Attributed to binding of protein to CNF/ crosslinking of the CNF network

Mechanical properties of films (no photos?)

Data at 80% RH!



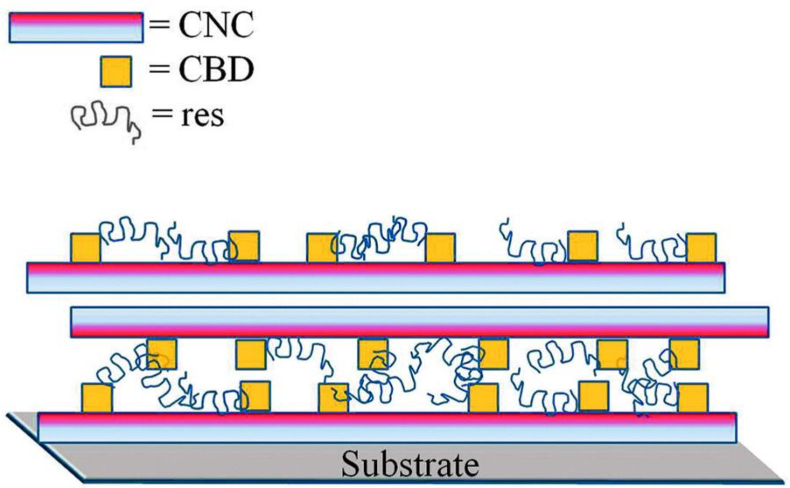
Increase in properties taken as indicative of CNF crosslinking by protein

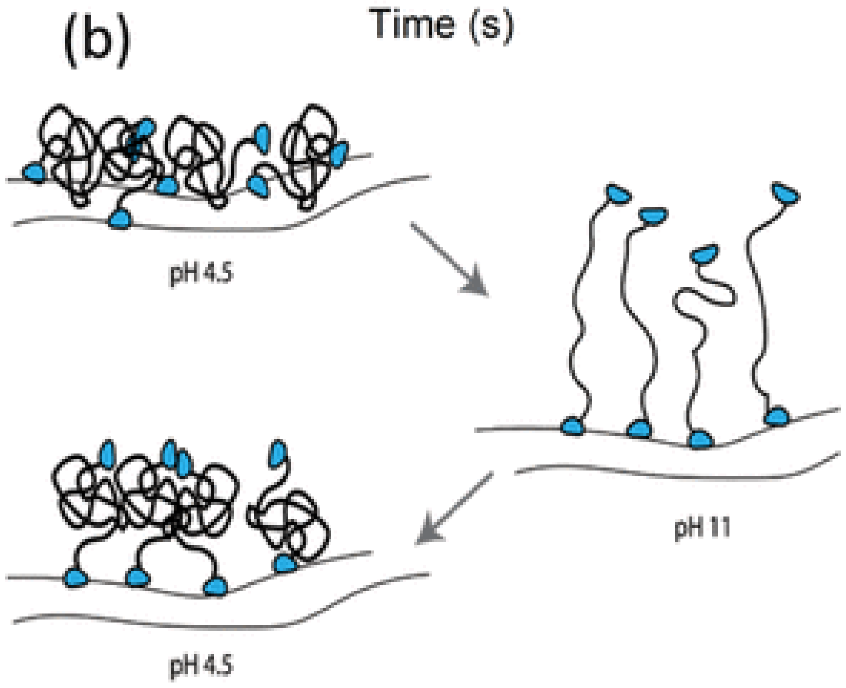
Conclusions

A **very elastic** cellulose binding fusion protein and its interaction with nanocellulose was investigated. Two terminal cellulose binding domains were fused to a Rec1 resilin exon to create a cellulose binding resilin. **Attachment of the protein via CBMs to the surface of nanocellulose films was confirmed**. **It was also shown that the elasticity and pH responsive behavior of the resilin was maintained at the surface-bound state (?)**. When embedded as a cross-linker in the CNF matrix, **the resilin fusion protein increased the rigidity of the CNF network both in a hydrogel form and in the dried cellulose nanopapers**. **Surprisingly enough, the resilin did not soften the nanocellulose materials, but its main contribution was in increasing the stiffness and yield strength**. In comparison, Rec1-resilin without the CBMs was embedded into the nanopaper but it did not change the properties when compared with pure CNF. The main conclusion of the work was that the resilin-like behavior was transferred into a fusion protein. **The fusion protein was able to bind to cellulose, cross-link cellulose nanofibrils, and modify the interactions between the cellulose nanofibrils.**

Zoom out comparison

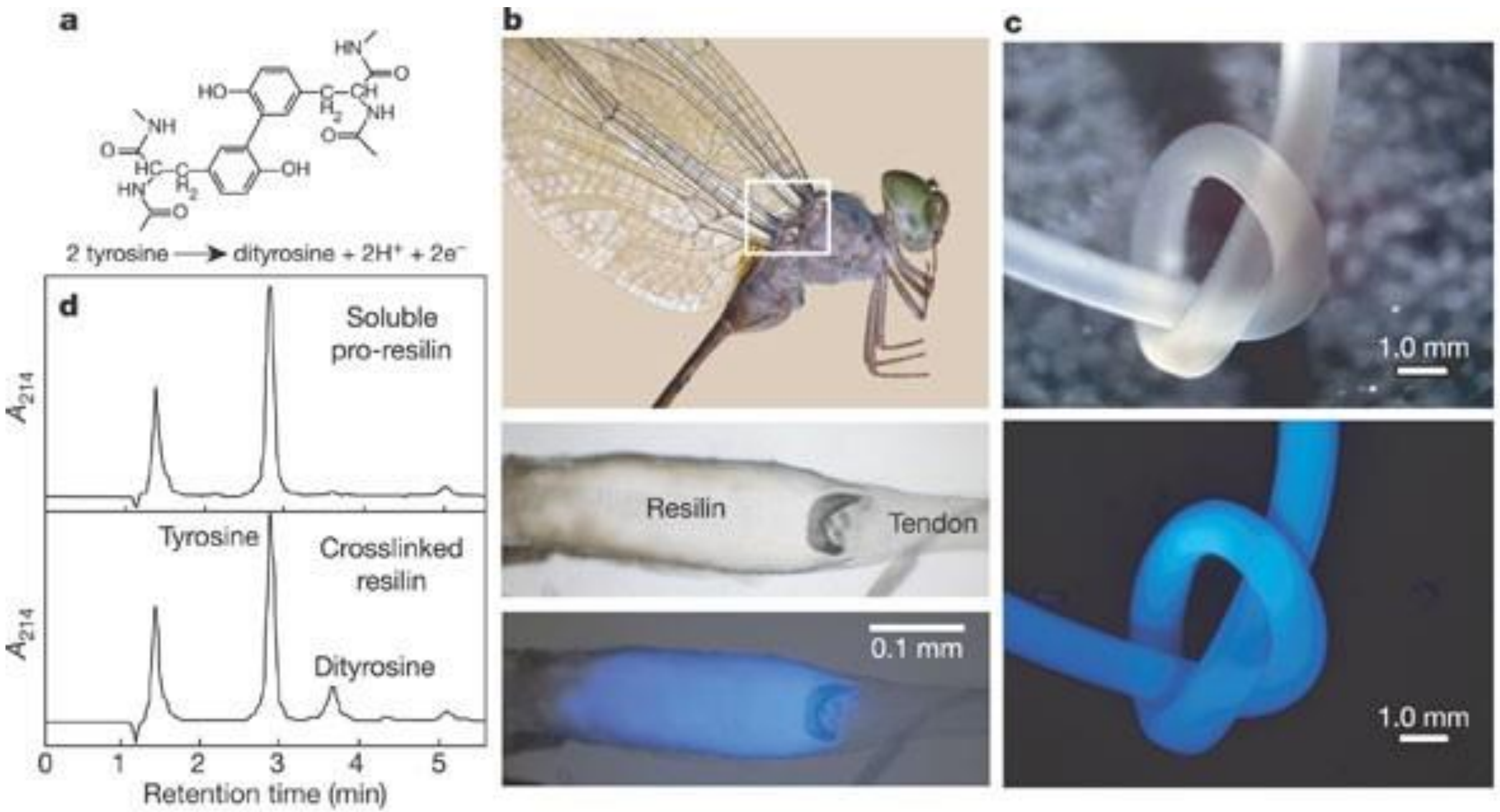
- Both papers show a stiffening with resilin-CBM constructs
- One paper attributes most changes to resilin, the other to CBD
- Natural resilin is crosslinked – this is key to its elastomeric recovery; neither study has crosslinked the resilin





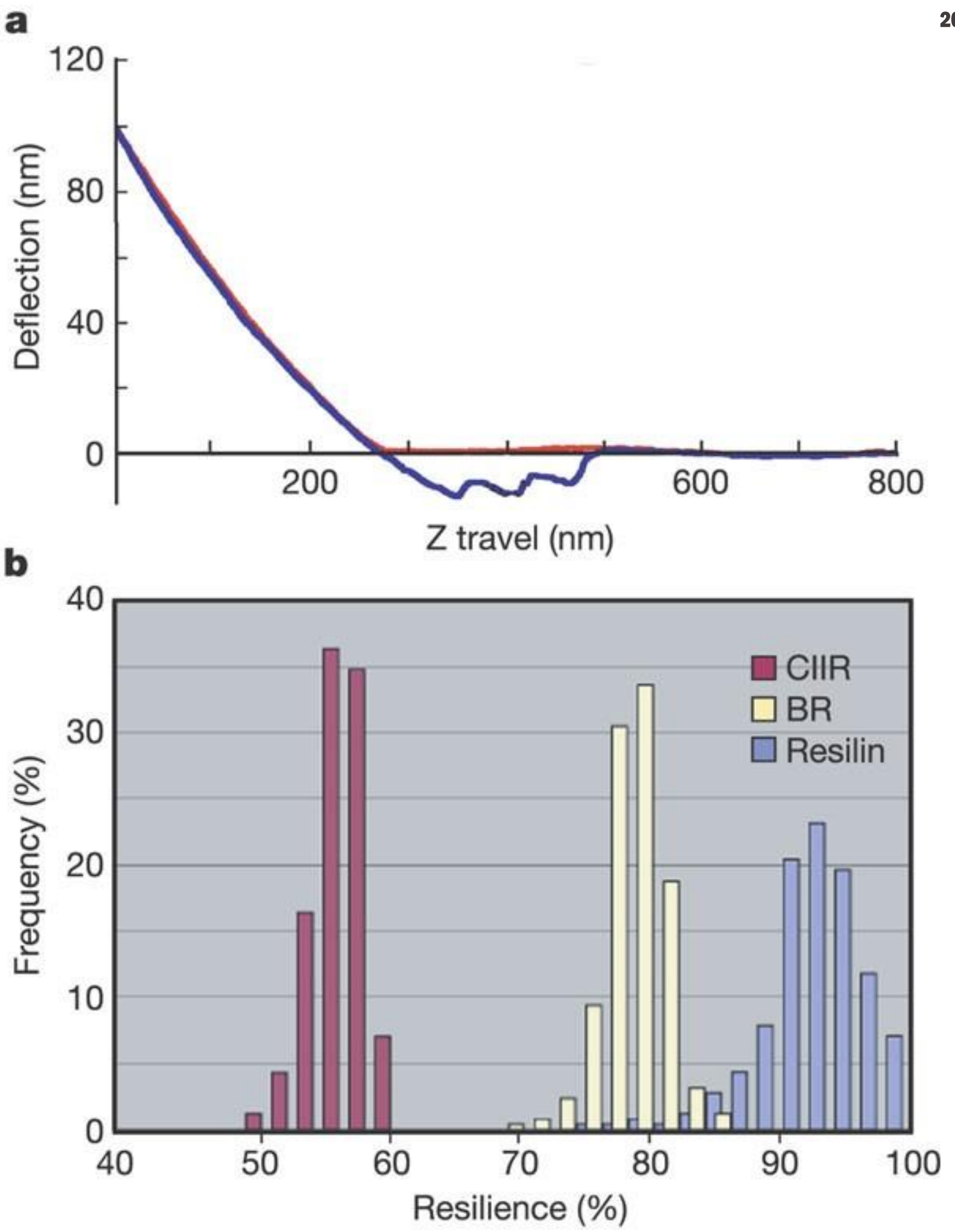
Resilin needs to be crosslinked - 2005

Elvin, C., Carr, A., Huson, M. *et al.* Synthesis and properties of crosslinked recombinant pro-resilin. *Nature* **437**, 999–1002 (2005). https://doi.org/10.1038/nature04085



Resilin needs to be crosslinked - 2005

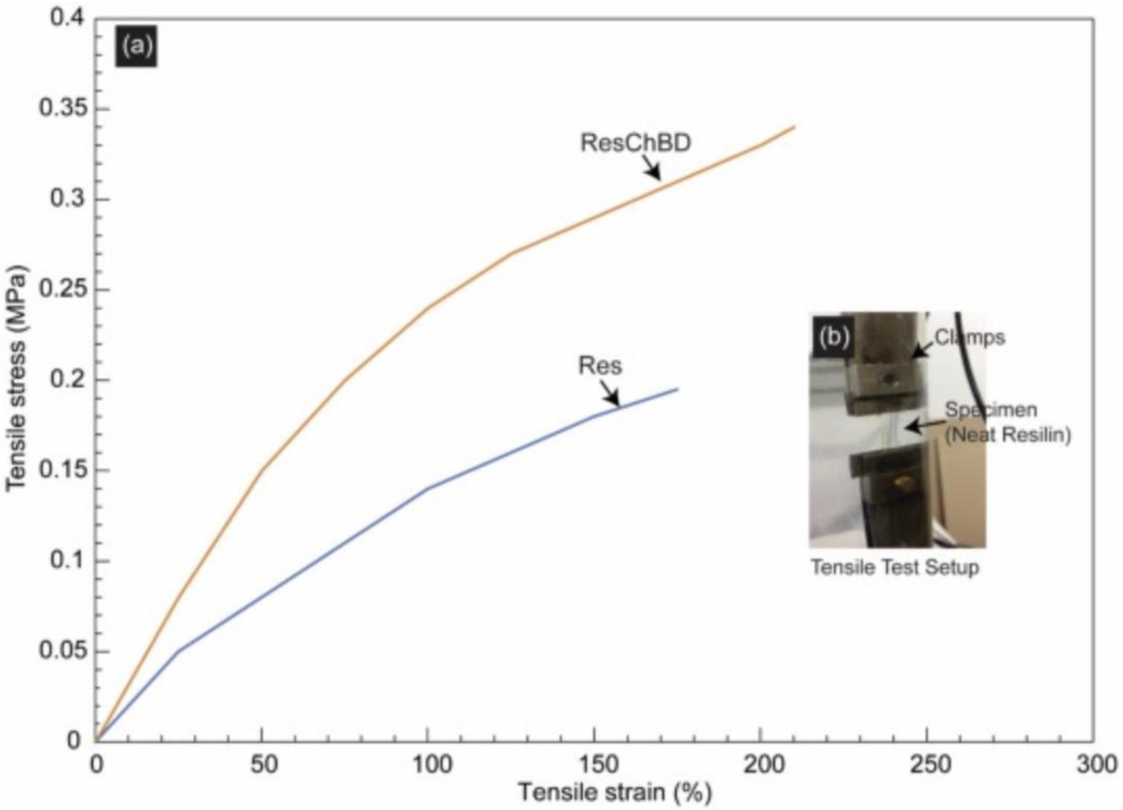
- Comparing crosslinked recombinant resilin to rubbers
- Resilin has better resilience



Elvin, C., Carr, A., Huson, M. *et al.* Synthesis and properties of crosslinked recombinant pro-resilin. *Nature* **437**, 999–1002 (2005). https://doi.org/10.1038/nature04085

What's happening in this space?

Binding to Chitin 2025 publication (Crosslinking!)



ELSEVIER
International Journal of Biological Macromolecules
Available online 28 October 2025, 148607
In Press, Journal Pre-proof What's this?

Soft, bio-inspired chitin/protein nanocomposites – mechanical behavior and interface interactions between recombinant resilin-like proteins and chitin nanofibrils

Ngesa E. Mushi [a 1], Ghasem Nurani [a], Simon Utsel [a], Shaul Lapidot [b], Oded Shoseyov [b], Harry Brumer [a 2], Qi Zhou [a c], Lars A. Berglund [a c]

Show more

EPFL

What's happening in this space?

ELSEVIER

Advances in Colloid and Interface Science

Volume 342, August 2025, 103530

Nano-structured antibiofilm coatings based on recombinant resilin

Nisal Wanasingha [a], Rajkamal Balu [a], Sheeana Gangadoo [a], Amanda N. Abraham [b], Agata Rekas [c], Jitendra P. Mata [d e], Anton P. Le Brun [d], Naba K. Dutta [a], Namita Roy Choudhury [a]

Show more

Add to Mendeley Share Cite

https://doi.org/10.1016/j.cis.2025.103530

Get rights and content

Full text access

- Antibiofilm!

ARTICLE | September 26, 2025

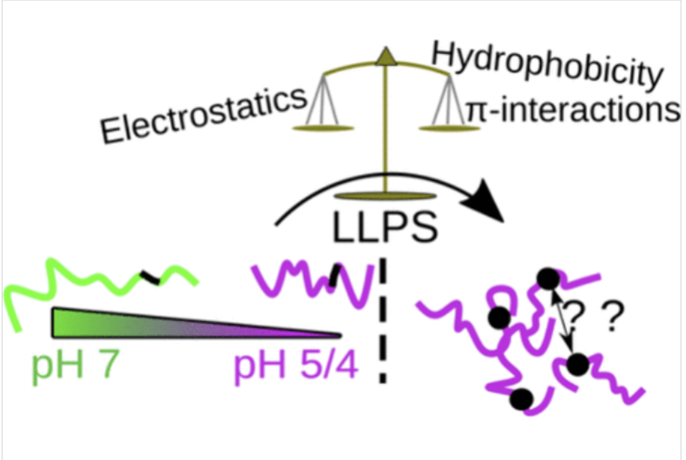
Conformation and Dynamics of Monomeric, Phase-Separated, and Cross-Linked Resilin Biomaterials

James B. Otis, Joerg Reichenwallner, Sean E. Reichheld, Aaron Au, Christopher M. Yip, Oliver P. Ernst, and Simon Sharpe*

Open PDF | Supporting Information (6) | open URL

Abstract

Resilin is an elastomeric protein found in insects that provides flexibility and locomotive function in numerous biological contexts. Recombinant resilin and resilin-derived proteins share resilin's capacity for liquid−liquid phase separation (LLPS) and formation of materials with high elasticity and biocompatibility, making it a promising candidate for regenerative medicine and tissue engineering applications. While prior research has focused on domain 1 of resilin, the role of domain 3 (D3) in resilin self-assembly and material properties is not well understood. Here, we used nuclear magnetic resonance, electron paramagnetic resonance, and small-angle X-ray scattering to study the conformation, dynamics, and intermolecular interactions of D3 as a monomer, in the phase-separated state, and as a cross-linked gel. We show that D3 remains unusually dynamic and is primarily disordered in all three states. In elucidating the mechanism of D3 LLPS, we find a complex set of electrostatic and π-based interactions complemented by the hydrophobic effect that finely tune the solution sensitivity of D3 and its capacity for LLPS. Overall, these results highlight the complex mechanisms governing resilin LLPS with implications for utilizing resilin-derived sequence features in the rational design of self-assembling biomaterials.



The list goes on…

TBC