

Some research from my past

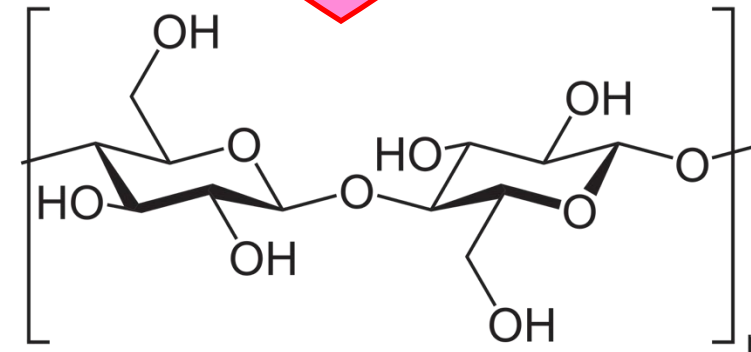
Prof. Tiffany Abitbol

2025

Honors undergrad project (how I started in research)

Chiral internal stresses in paper sheets

When you see kids posts "happy 3 week anniversary can't wait to spend the rest of my life with you baby"



Honors undergrad project – Chiral internal stresses in paper sheets



PROF. DEREK GRAY
2013 MARCUS WALLENBERG PRIZE WINNER

Gray group, ca. 2008



Prof Derek Gray
CNC guru
Physical Chemist

Elisabeth Kloser
Post doc 2008-2010
Set the bar on CNC
chemical
modification and
work up

Stephanie Beck
PhD 2007
FP Innovations

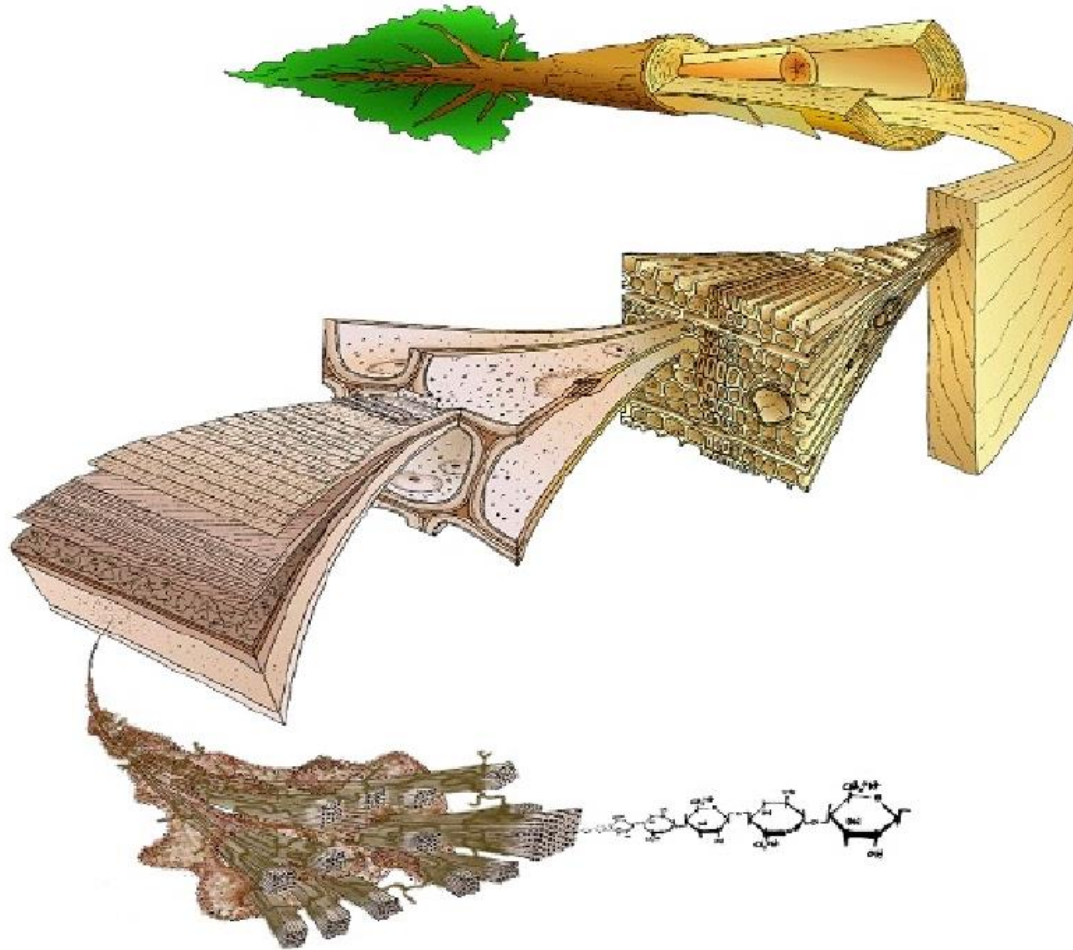
Maren Roman
Post Doc 2004
Prof at Virginia Tech

Emily Cranston
PhD 2008
Prof at UBC

Teri Dankovich
PhD 2012
CTO & Co-founder
at Folia Water

PhD 2011–
Cellulose-based
nanocomposite
materials

...nanomaterials
...quantum dots
...electrospinning
...hydrogels
...self-assembly



- Mostly from plants
- Glucose polymer
- Hierarchical
- Crystalline
- Hydrophilic
- Insoluble in most solvents
- Modifiable
- Building material, paper, plastics

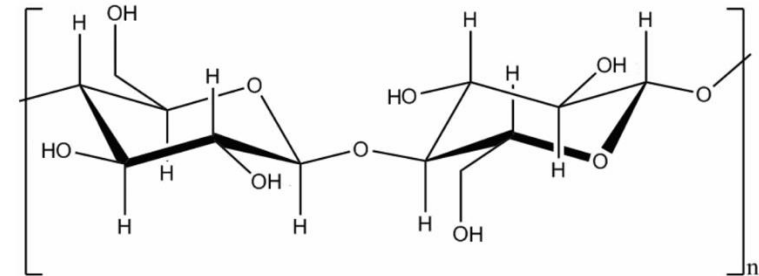
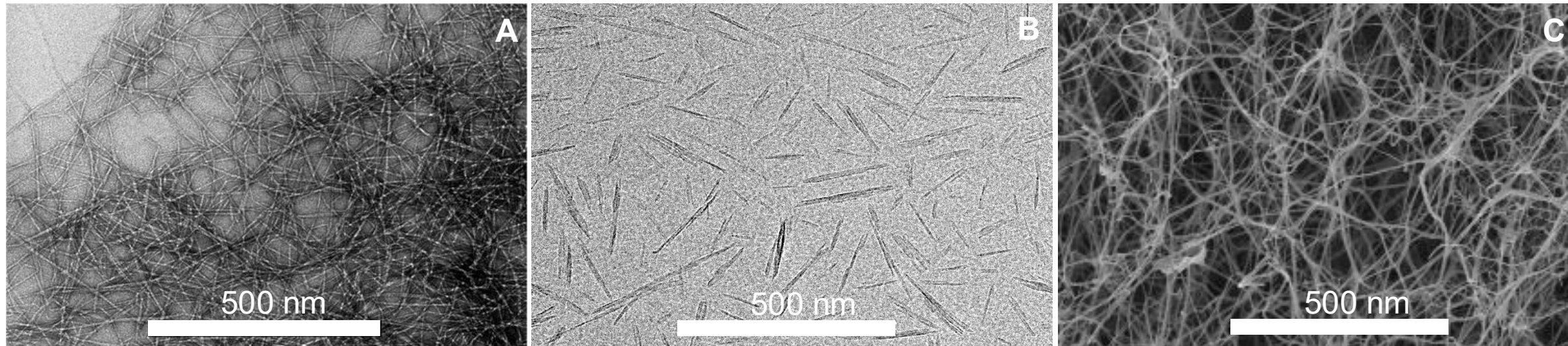


Figure 1.1: Chemical structure of cellulose.

Hierarchy of wood from JS Segmehl thesis, 2017
<https://doi.org/10.3929/ethz-b-000230544>

- Generic umbrella word
- Encompasses all forms of nanoscale cellulose particles
- Most common types:
 - Cellulose nanofibrils (CNF)
 - Cellulose nanocrystals (CNC)
 - Bacterial nanocellulose (BNC) – secreted as the ECM

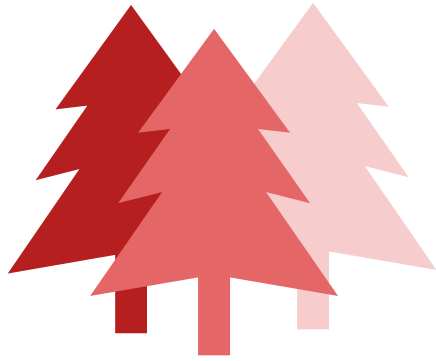


CNF

CNC

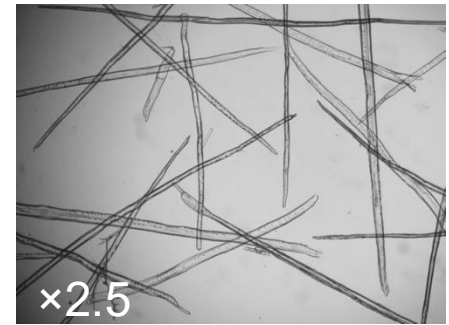
BNC

Nanocellulose



CELLULOSE CONTAINING BIOMASS

pulping

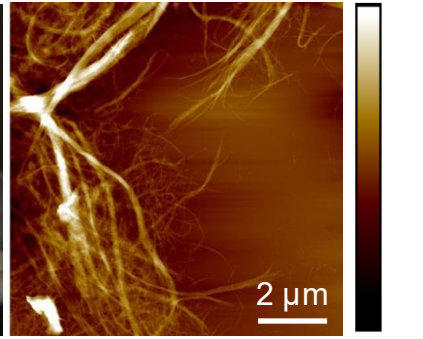


CELLULOSE-ENRICHED PULP

Enzymes & mechanical



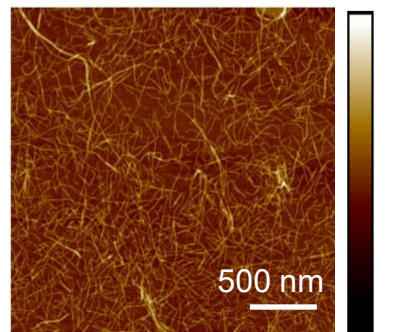
ENZYMATIC CNF



Chemicals & mechanical



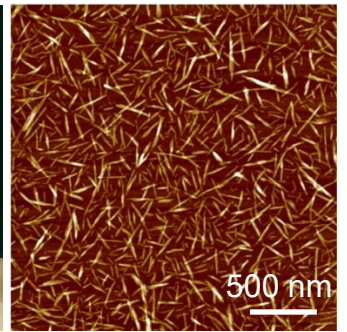
OXIDIZED CNF



Degradation



CNC



CNF = cellulose nanofibril
CNC = cellulose nanocrystal

1947

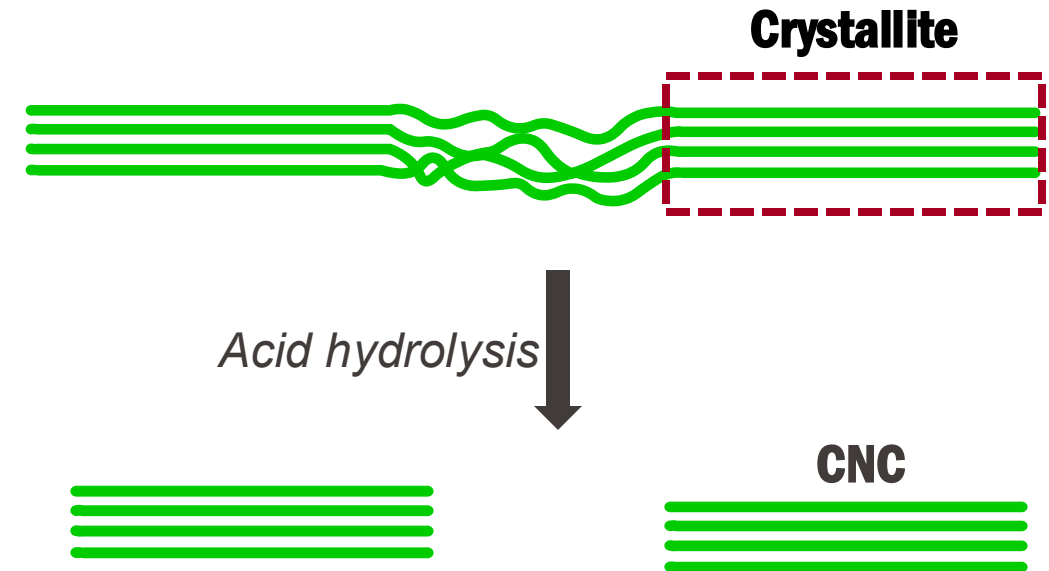
Cellulose Intercrystalline Structure

STUDY BY HYDROLYTIC METHODS

R. F. NICKERSON¹ AND J. A. HABRLE²

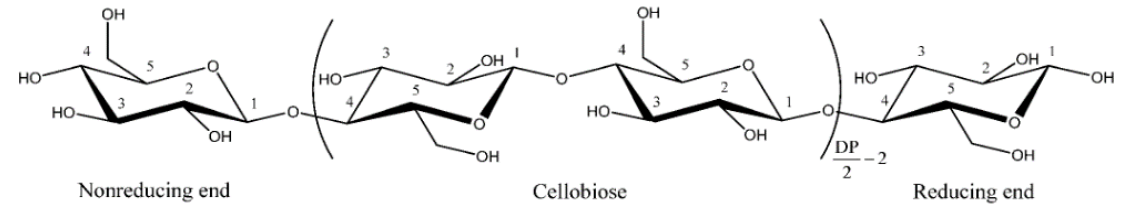
Mellon Institute, Pittsburgh 13, Pa.

In the hydrolysis of cellulosic fibers with aqueous hydrochloric and sulfuric acids at boiling temperatures, the disordered intercrystalline chain network appears to be attacked first.

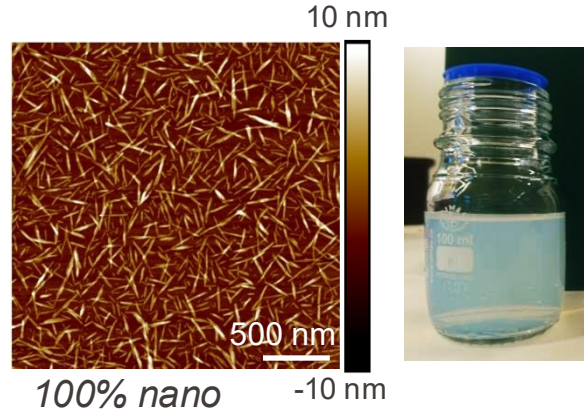


Ind. Eng. Chem., 39(11), 1507-1512 (1947).

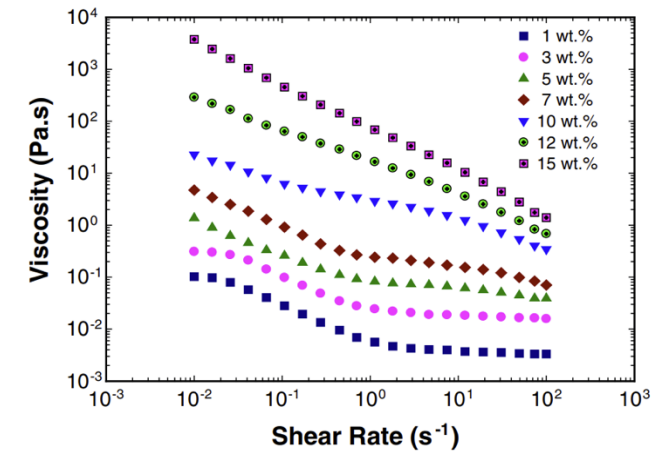
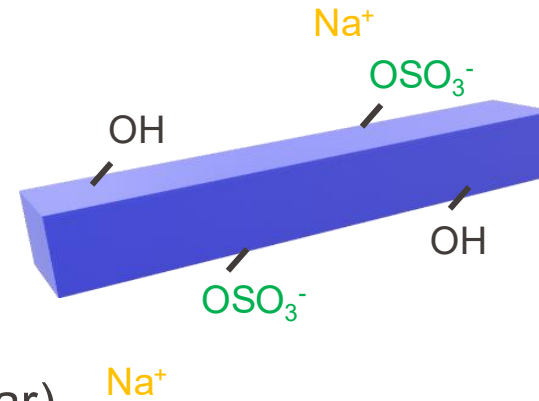
CNC properties



- Rod-shaped
- Chemically anisotropic
- High crystallinity
- High Young's modulus (1 GPa)
- 100% nano-yield
- Polydisperse
- Hydrophilic
- Surface charge groups
- Colloidally stable in water (EDL)
- Chemically modifiable
- Shear thinning viscosity
- Alignment in external fields (E, B, shear)
- Liquid crystalline self-assembly

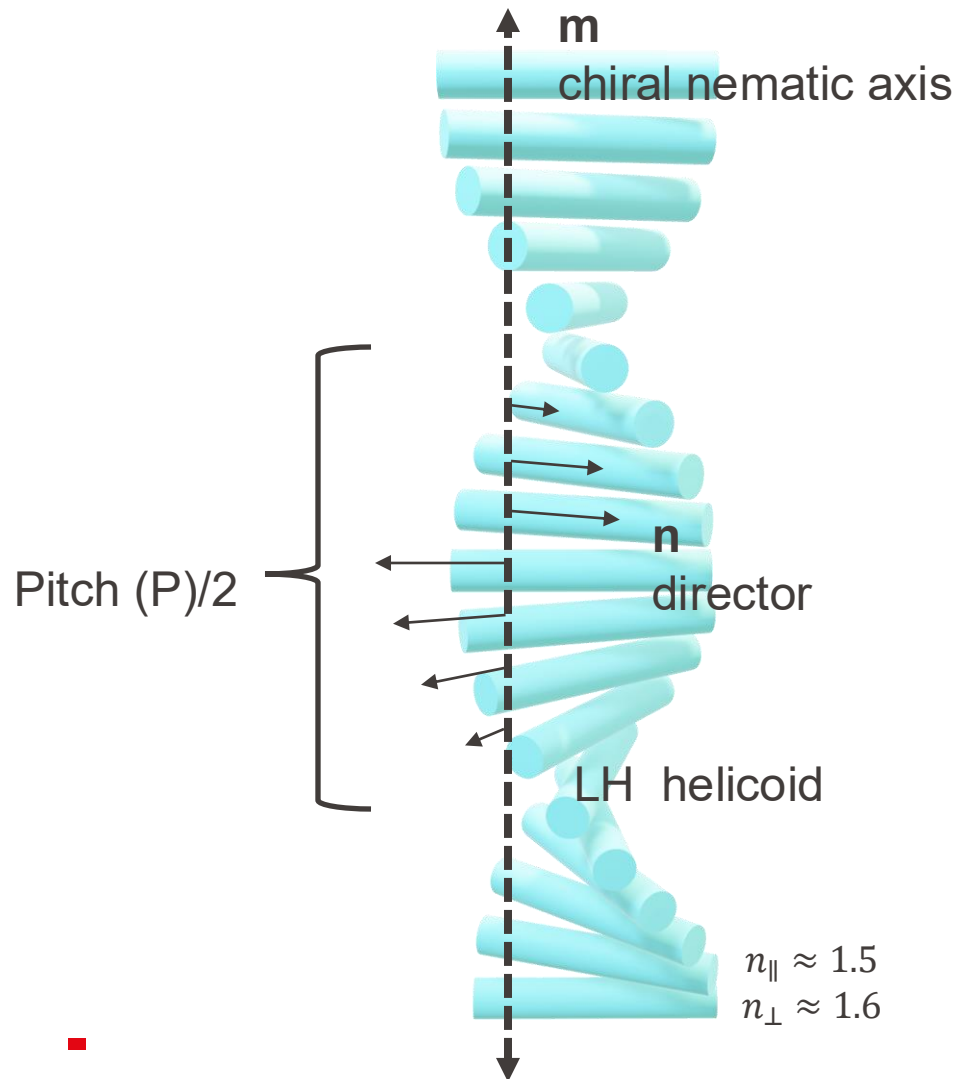


CNC = a bundle of cellulose chains with non-native surfaces

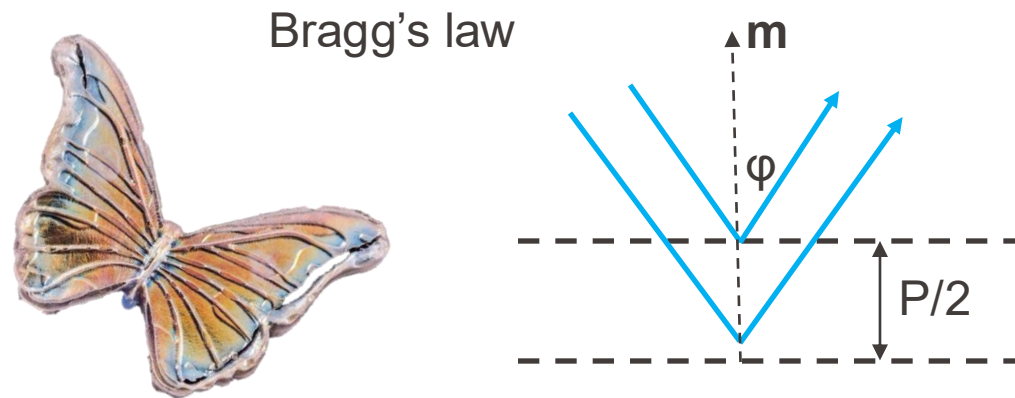


Shafiei-Sabet *et al.* Cellulose 2014, 21, 3347–3359.

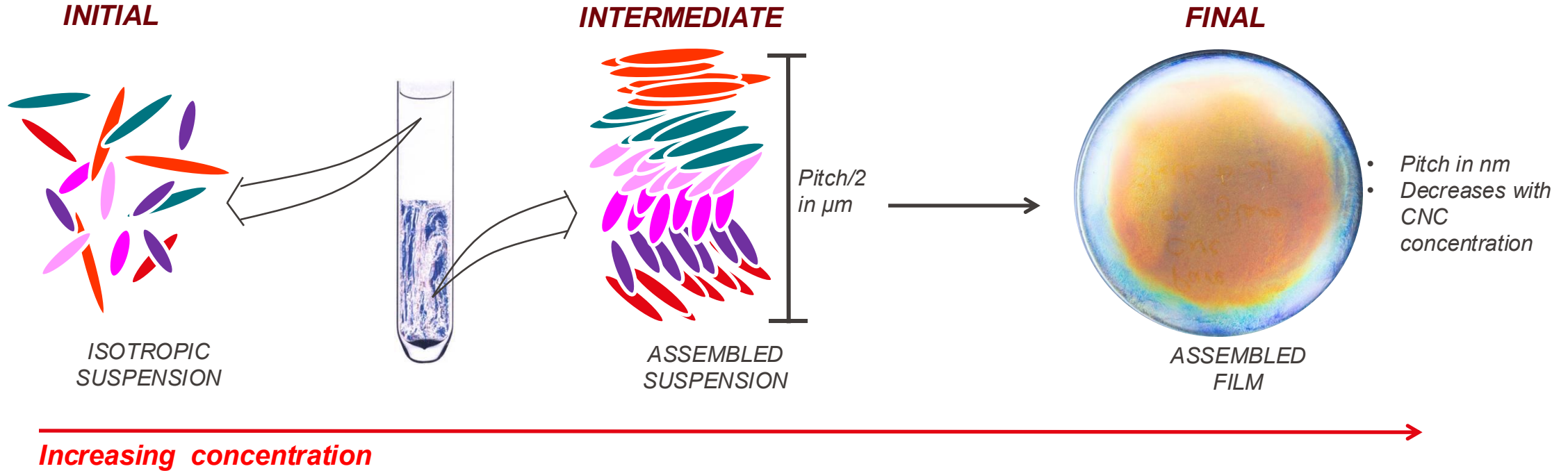
Chiral nematic liquid crystals



- CNCs form chiral nematic phases as they are concentrated
- The liquid crystal structure can be locked in films
- Structure colors occur when $P/2$ coincides with visible wavelengths

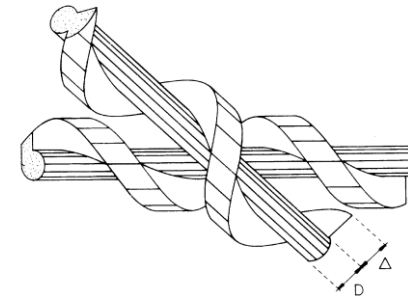


Basics of CNC Self-assembly



WHY DOES THIS HAPPEN?

- Stiff rods
- Translational entropy

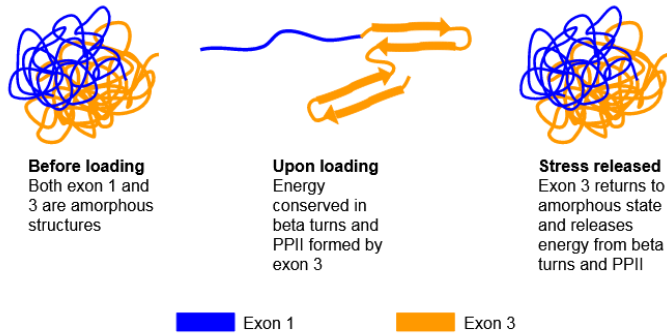


Dong et al., *Langmuir* **12**, 1996, p.2076
 Straley, *Phys. Rev. A* **14**, 1976, p.1835

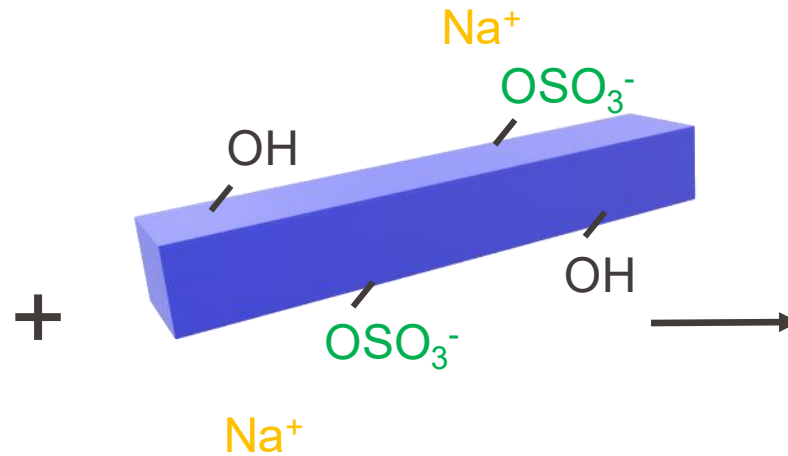
Protein-CNC hybrids: post doc with an ELM?



Drosophila melanogaster



<https://en.wikipedia.org/wiki/Resilin>



- Stiff nanoparticle
- *Young's Modulus of ca. 1 GPa*



Prof. Shoseyov

Springy and stiff?

- Exon 1 with C-terminal cellulose binding module (CBD) expressed in *E. Coli*
- CNC + exon 1 linked via CBD

(started as a 5-month exchange while I was a post doc at McMaster)

Original Research

Bionanocomposite Films from Resilin-CBD Bound to Cellulose Nanocrystals

Amit Rivkin^{1,*}, Tiffany Abitbol^{1,2,*}, Yuval Nevo¹, Ronen Verker³, Shaul Lapidot¹, Anton Komarov⁴, Stephen C. Veldhuis³, Galit Zilberman⁵, Meital Rechtes⁶, Emily D. Cranston², and Oded Shoseyov¹

Abstract

*This research explores the properties of bionanocomposite films prepared by binding recombinant resilin-like protein (res) consisting of the exon 1 resilin sequence from *Drosophila melanogaster*, engineered to include a cellulose binding domain (CBD), to cellulose nanocrystals (CNCs). The optimal binding of res-CBD to CNCs was 1:5 by mass, and the resulting res-CBD-CNCs remained colloidally stable in water. Res-CBD-CNCs were solvent cast into transparent, free-standing films, which were more hydrophobic than neat CNC films, with water contact angles of 70–80° compared to 35–40° for the latter. In contrast to the multi-domain orientation typical of chiral nematic CNC films, res-CBD-CNC and CBD-CNC films exhibited long-range, uniaxial orientation that was apparently driven by the CBD moiety. Glycerol was studied as an additive in the films to determine whether the addition of a wet component to solvate the recombinant protein improved the mechanical properties of the res-CBD-CNC films. In comparison to the other films, res-CBD-CNC films were more elastic with added glycerol, in the range of 0.5–5 wt% (i.e., the films responded more elastically to a given strain and/or were less plastically deformed by a given mechanical load), but became less elastic at 25 wt% glycerol. Overall, films made of res-CBD-CNCs plus 0.5 wt% glycerol displayed improved mechanical properties compared to neat CNC films, with an increase in toughness of 150% and in elasticity of 100%.*

- Had not heard of the word ELM: is it an ELM?
- IF = 1 ish
- Oh! What a low IF! Mon dieu!
- Cited 36 times since 2015, not great (ca. 3.6/y)
- One of the 1st reports of a nanocellulose-protein hybrid



Industrial Biotechnology

Editor-in-Chief: Richard A. Gross, PhD

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- Recombinant resilin protein, genetically engineered to include a cellulose binding domain (part that sticks to cellulose)
- Studied the properties of this resilin construct bound to CNCs in films



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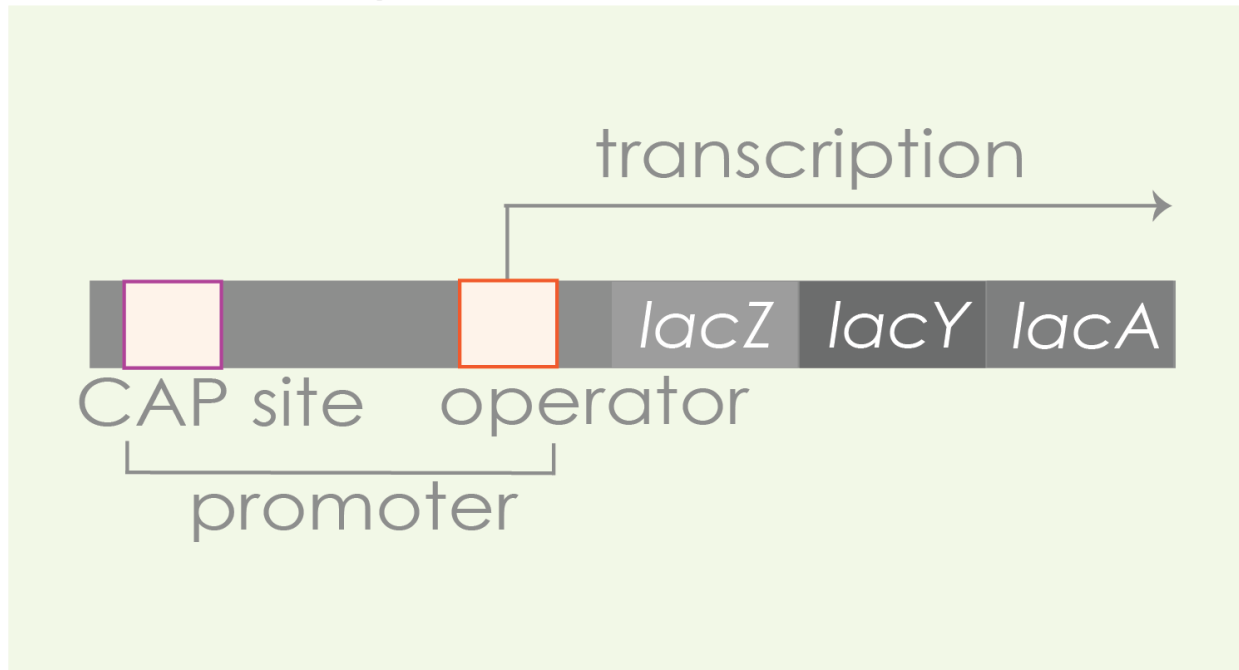
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- “...advances in bioengineering have provided scientists with the strategies needed to **design recombinant proteins with specific functionality** in mind, such as elastomeric recombinant proteins based on resilin, which can then be used to prepare well-defined biomaterials.”

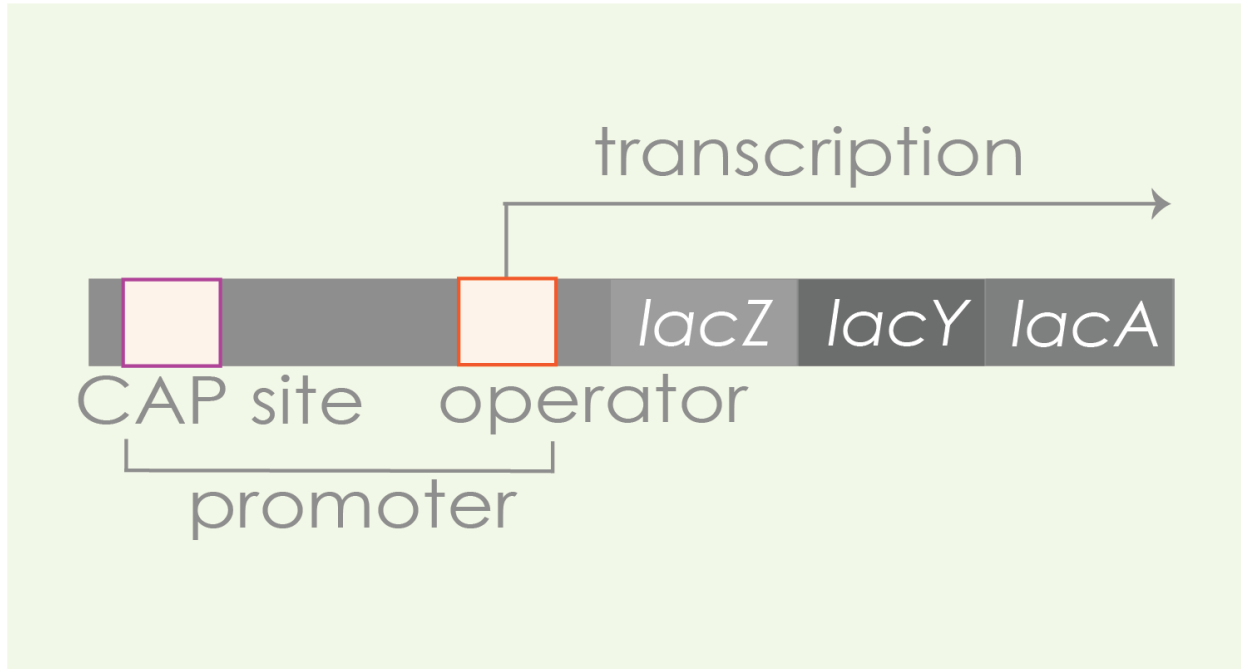
Lac operon – remember me?

The Lac Operon



- A group of genes in *E. Coli* that control the metabolism of lactose
- Three genes involved *lacZ*, *lacY* and *lacA*
- Genes transcribed when glucose is low to enable lactose metabolism (preference is for glucose)

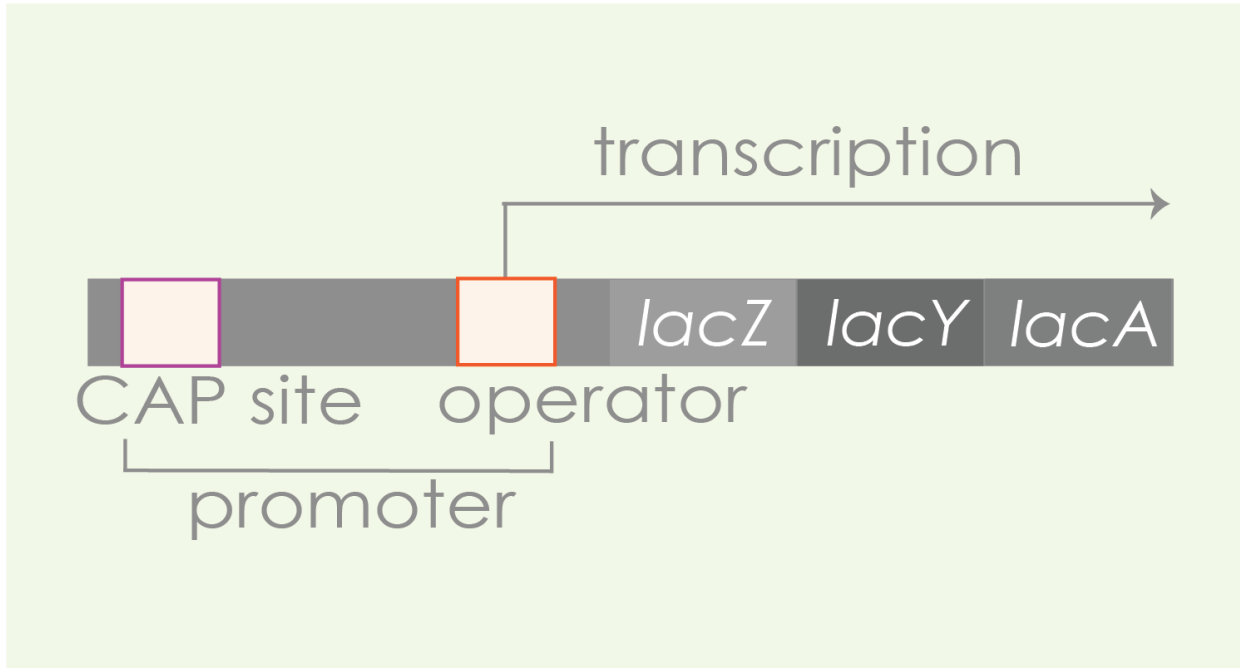
The Lac Operon



- Promoter – where RNA polymerase binds to start gene transcription
- Operator – where the repressor protein binds to block transcription: *with low glucose and with lactose present, lactose binds to the repressor protein, changing its shape so it can't bind to the operator, allowing transcription of the proteins responsible for lactose metabolism*
- CAP (catabolite activator protein) site – where the CAP protein binds when glucose is low

Lac operon – more on CAP and CAP site

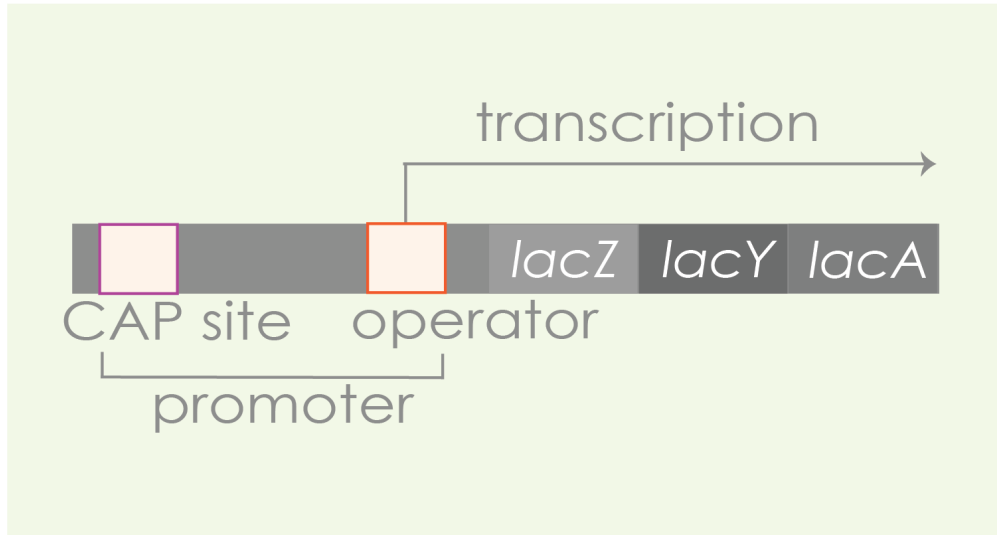
The Lac Operon



- When glucose is low, CAP (cyclic adenosine monophosphate) levels rise
- CAP binds to CAP site, activating the protein
- This enables cells to digest lactose
- Thus, cell only invests in lactose metabolism when glucose is low
- “**AND gate**” system only works when **lactose is present** to remove the repressor protein **AND glucose is not present** (CAP is activated)

Lac operon – gene regulation

The Lac Operon



No lactose (default)

- Repressor is active and bound to operator
- No gene transcription for lactose metabolizing enzymes

Lactose present

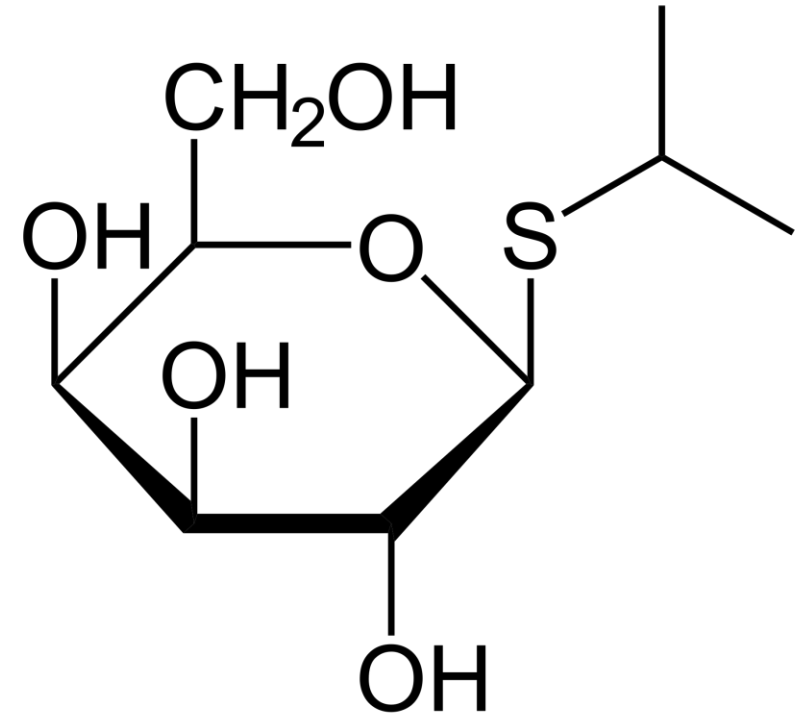
- Lactose (allolactose) binds to repressor, making it unbind from the operator
- Gene transcription can occur

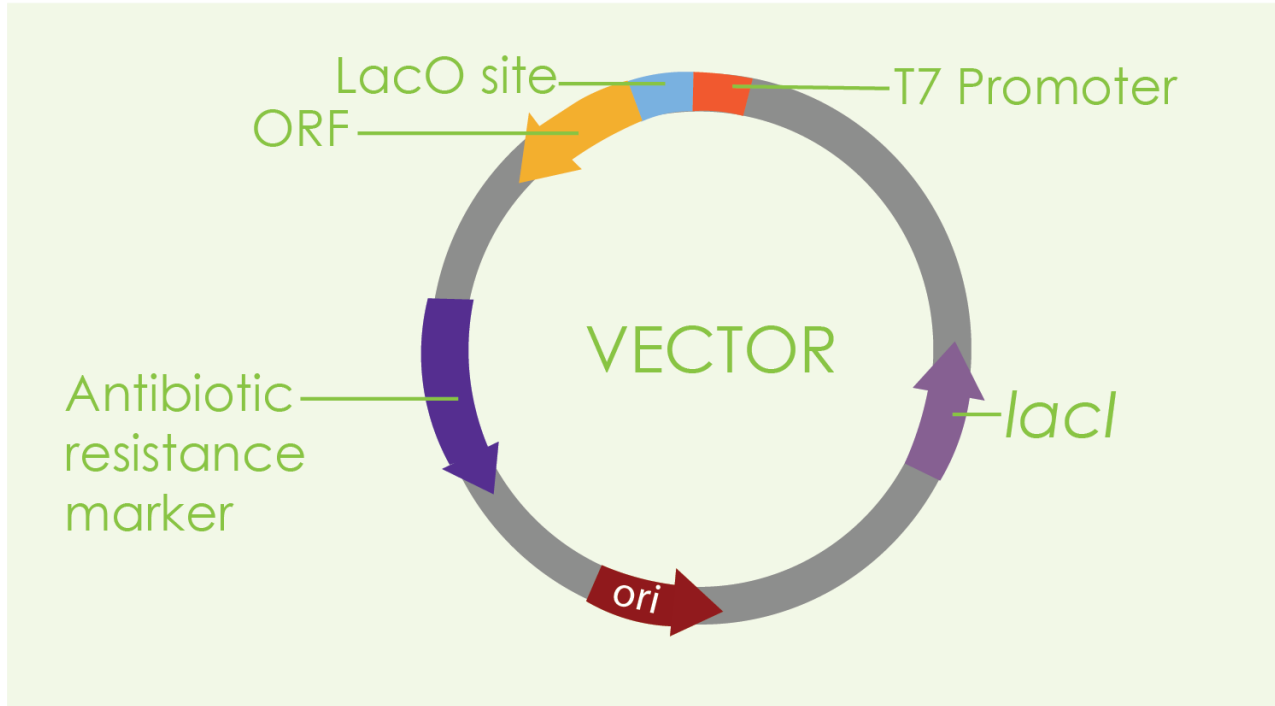
Glucose control (catabolite repression)

- CAP is inactive when glucose present, minimal transcription
- CAP becomes active when glucose absent/low, enhancing transcription
- Glucose is preferred carbon source; cells only use lactose when glucose is not present

“**AND gate**” system only works when **lactose is present** to remove the repressor protein **AND glucose is not present** (CAP is activated)

- IPTG is a molecular analog of allolactose (lactose metabolite) – binds to repressor
- Triggers transcription of the *lac operon* (sufficiently low glucose levels)
- Used to induce protein expression where the gene is under control of the *lac operon* (often used in recombinant protein)





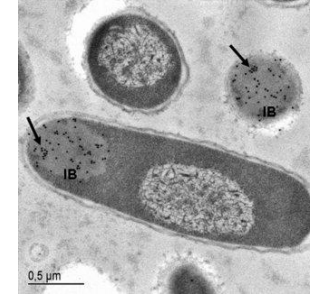
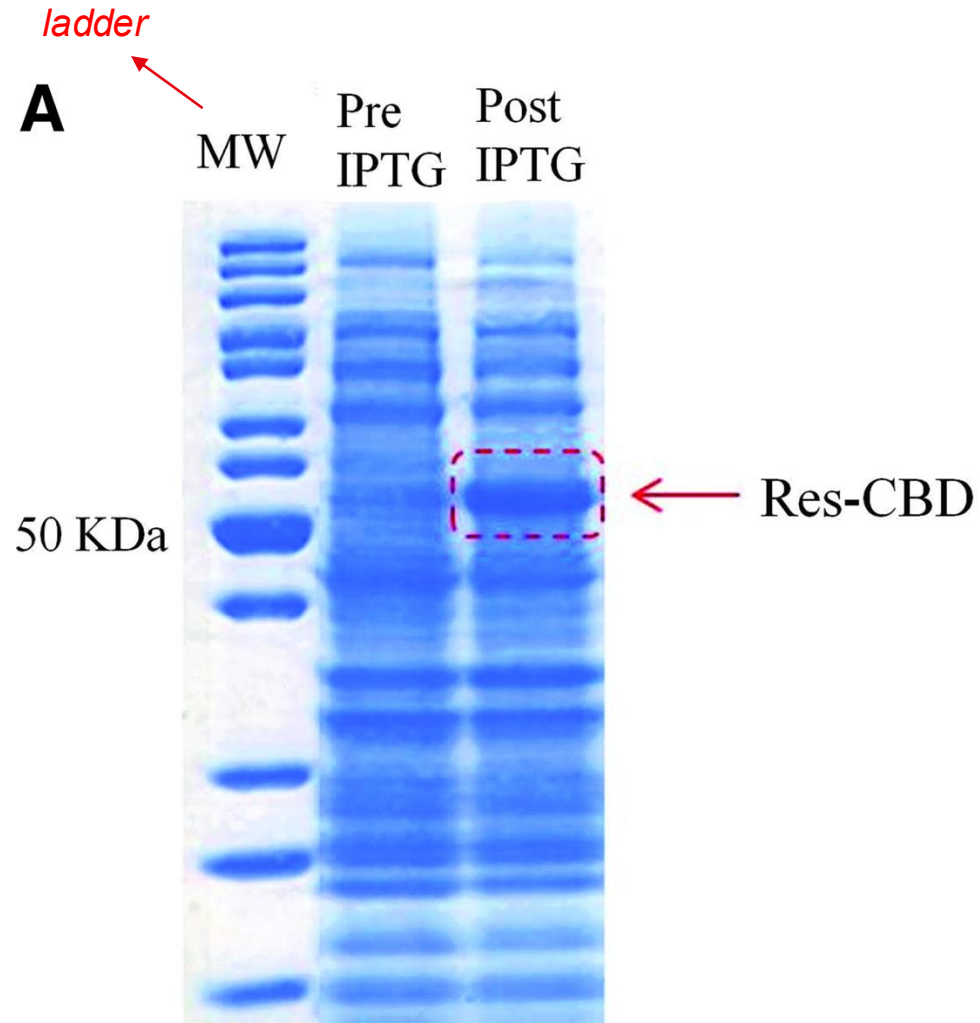
- Clone gene for exon 1 fused to CBD into plasmid vector
- Introduce in bacteria (*E. Coli*)
- Use *lac operon* to induce the transcription of gene of interest to get the desired protein
- Without IPTG, no transcription or protein expression

What is the gist of the overall experiment

- (1) Create the plasmid
- (2) Introduce in bacteria
- (3) Grow the bacteria
- (4) Add IPTG to induce expression of the desired protein
- (5) Purify the desired protein (eventually)
- (6) Bind the desired protein to CNC
- (7) Make CNC-resilin materials
- (8) Characterize their material properties and check our hypothesis of springy and stiff!



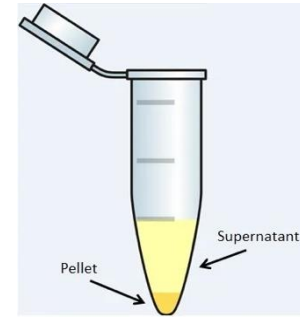
Step 1. Expression of **res-CBD**?



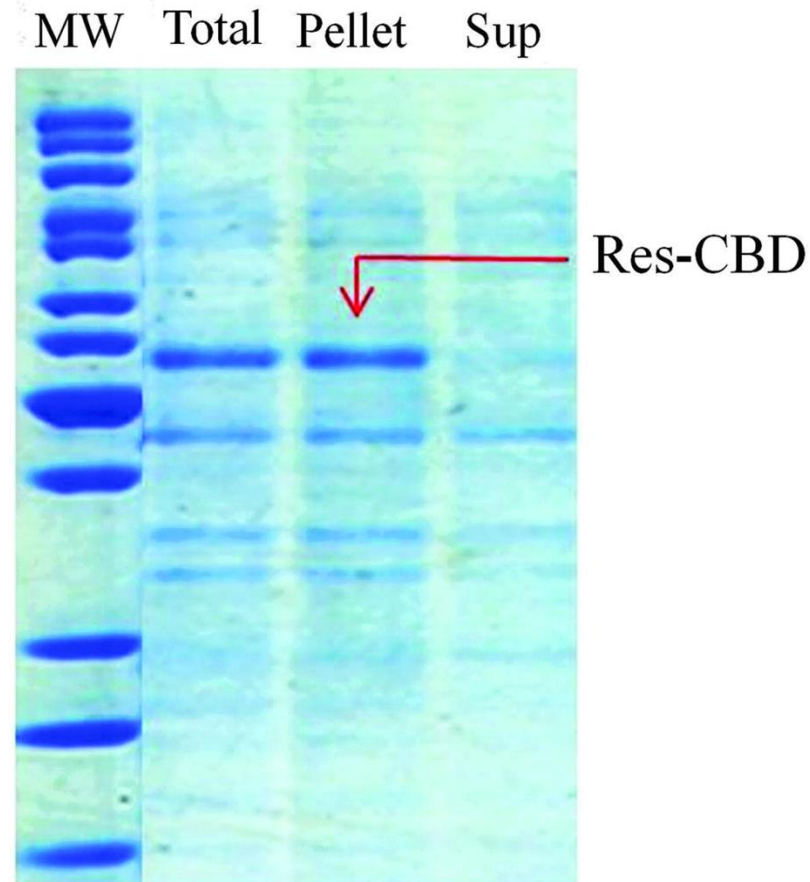
“The **chimeric** gene, consisting of 17 elastic repeats from the exon 1 of the *D. melanogaster* resilin gene, which was C-terminal fused to a *C. cellulovorans* CBD gene, was successfully cloned, expressed in *E. coli*, and purified from the **inclusion bodies of the bacteria**”

- Figure shows SDS-PAGE of **total bacteria** - with IPTG induction, a more distinct res-CBD band is present
- Total bacteria = total cell lysate

Step 1. Isolation and extraction of res-CBD

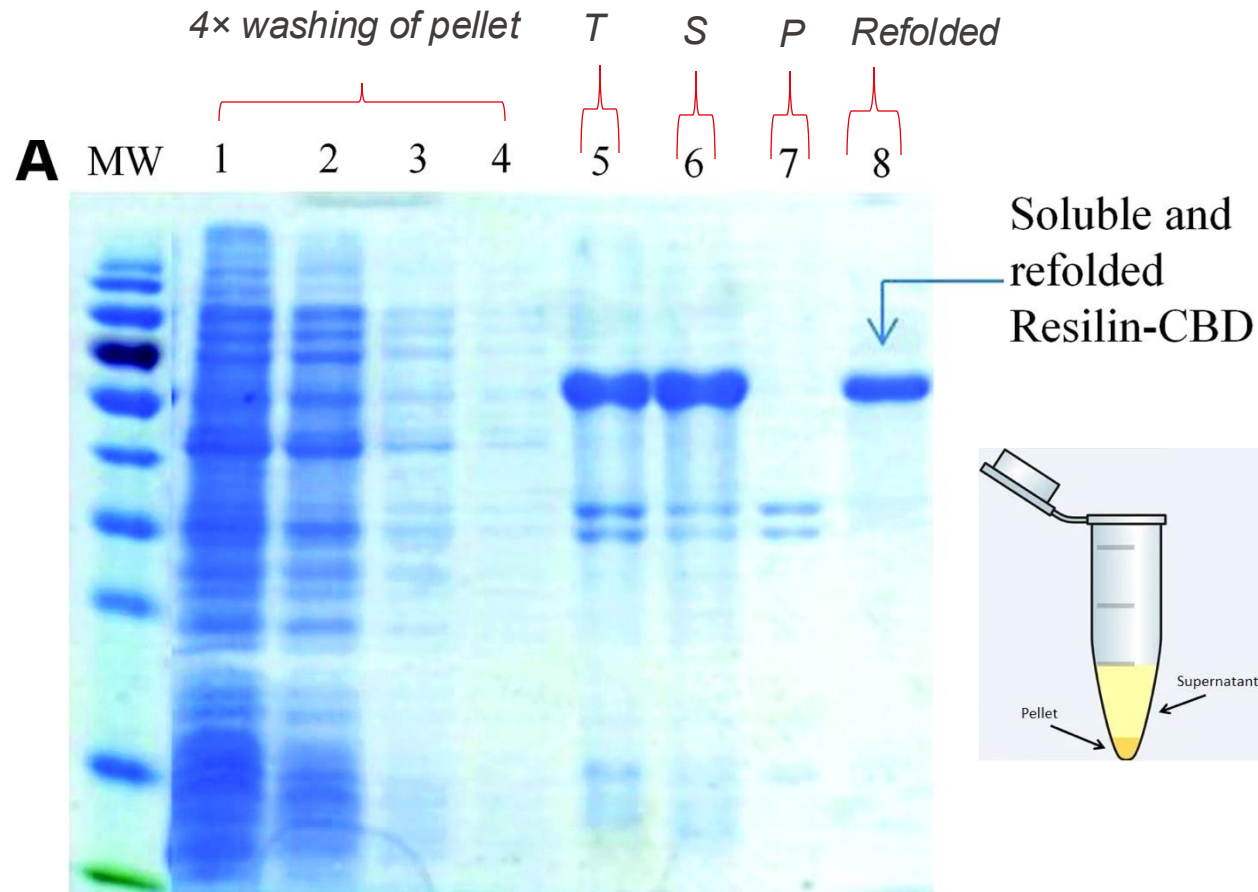


- 1) Centrifuge lysed bacteria
- 2) Analyze protein fraction in pellet and supernatant

B

- Bacteria sonicated (lysed) and total, supernatant and pellet were analyzed by SDS-PAGE
- Res-CBD found in pellet, confirming its expression in inclusion bodies
- IB's = particles of aggregated protein (common for recombinant protein)
- So, purification is usually 2 steps –1. extraction of IB's and 2. solubilization of expressed protein and refolding

After solubilization and refolding



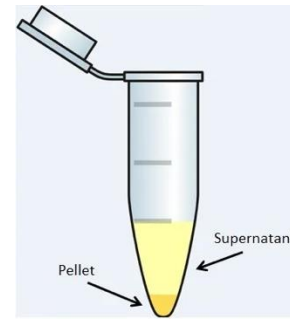
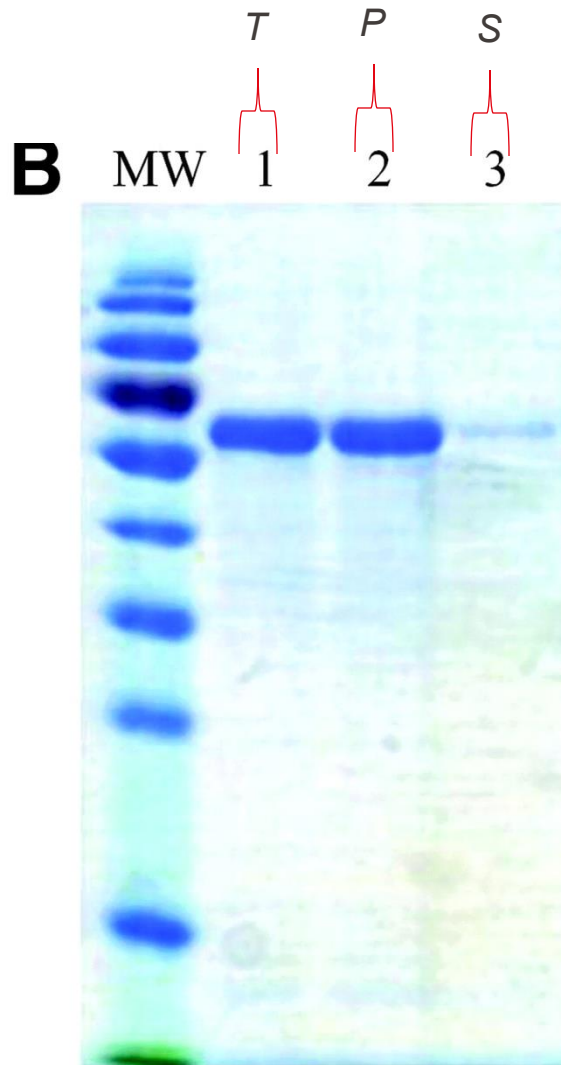
(1) 4× washing of IB (lanes 1-4)

(2) Dissolve IB's in urea (Total - lane 5)

(3) Centrifuge Total (Supernatant - lane 6 and Pellet - lane 7)

(4) Refold supernatant to target pure and folded res-CBD (lane 8)

Checking proper refolding and binding

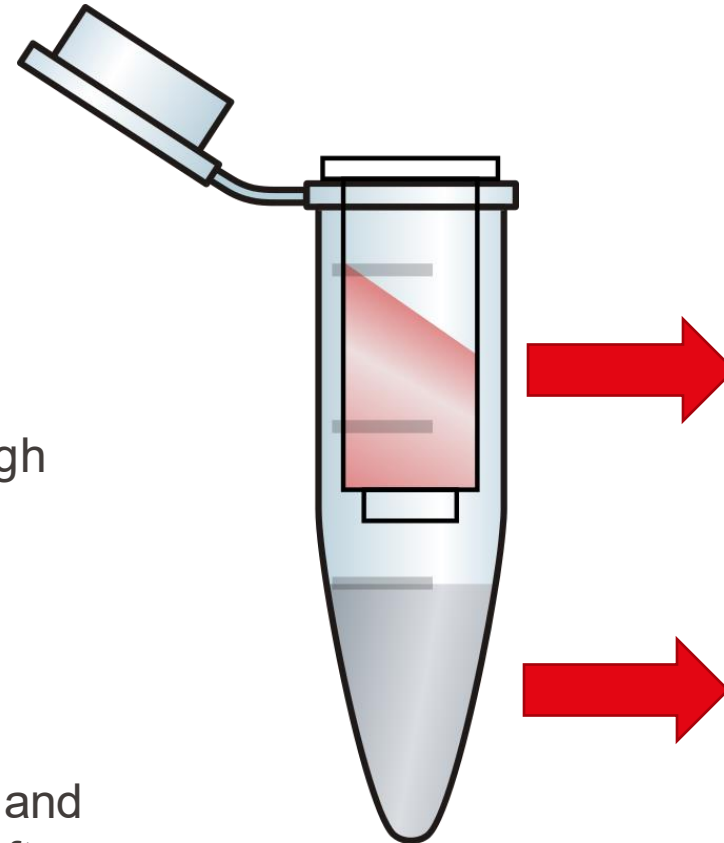


- 1) *Incubate/mix folded protein with cellulose powder*
- 2) *Centrifuge to see if it works = protein should be in pellet*

- Tested binding with a commercial pure cellulose microcrystalline powder (Avicel) at 1:300 w/w protein to cellulose
- Binding is only possible if refolded correctly
- Lane 1: total (before centrifugation)
- Lane 2: pellet – shows res-CBD with the cellulose in the pellet
- Lane 3: shows no/little res-CBD in the supernatant because it is bound to the cellulose powder

What about binding to cellulose nanocrystals?

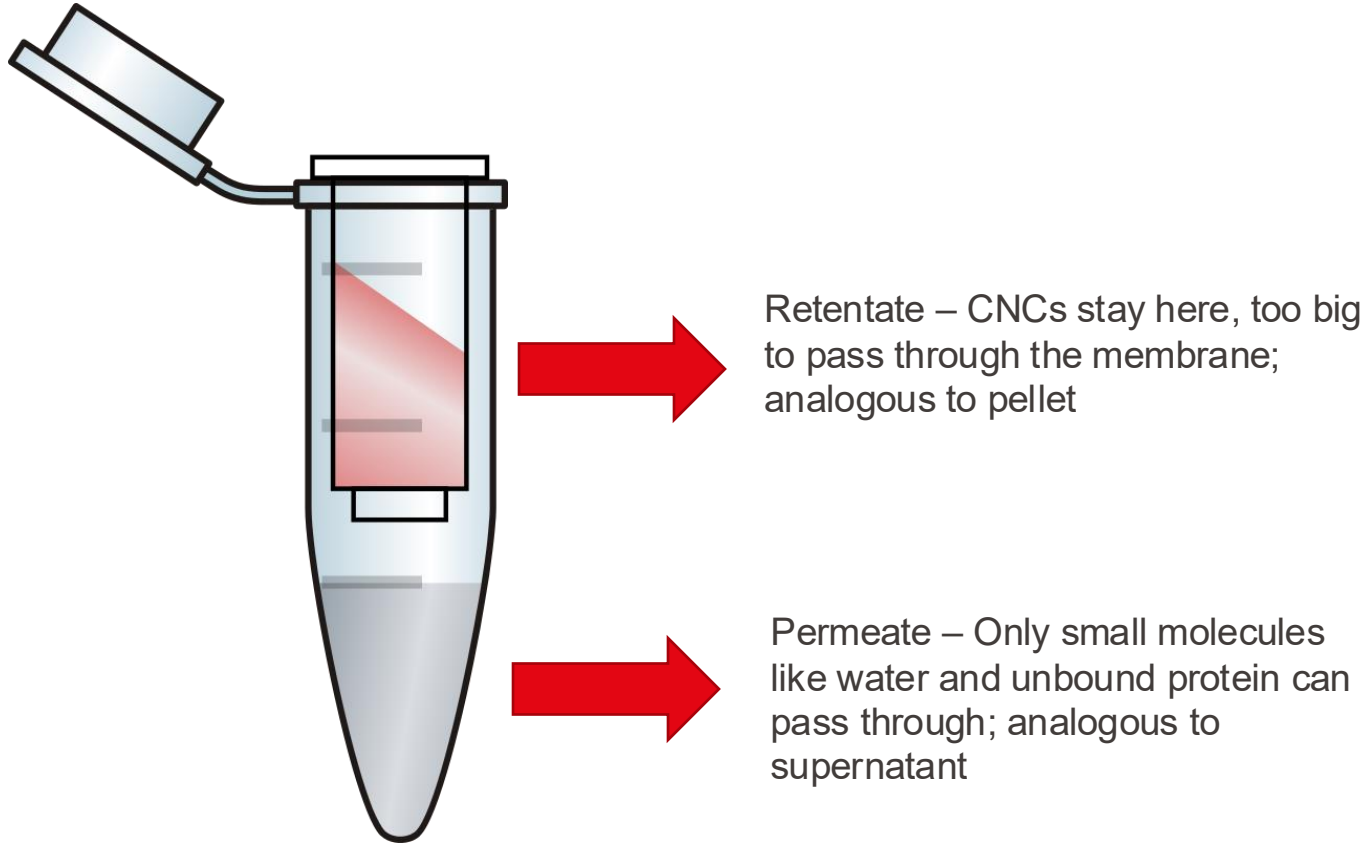
- Different ratios of protein to cellulose nanocrystals
- 1:1, 1:5, 1:10, 1:50
- Incubated together
- Filtered with centrifugation through a 0.2 μm filter such that any unbound protein will be in the permeate; CNC stays in in the retentate (ultrafiltration)
- Study crude (C) before filtration, and retentate (R) and permeate (P) after filtration



Retentate – CNCs stay here, too big to pass through the membrane; analogous to pellet

Permeate – Only small molecules like water and unbound protein can pass through; analogous to supernatant

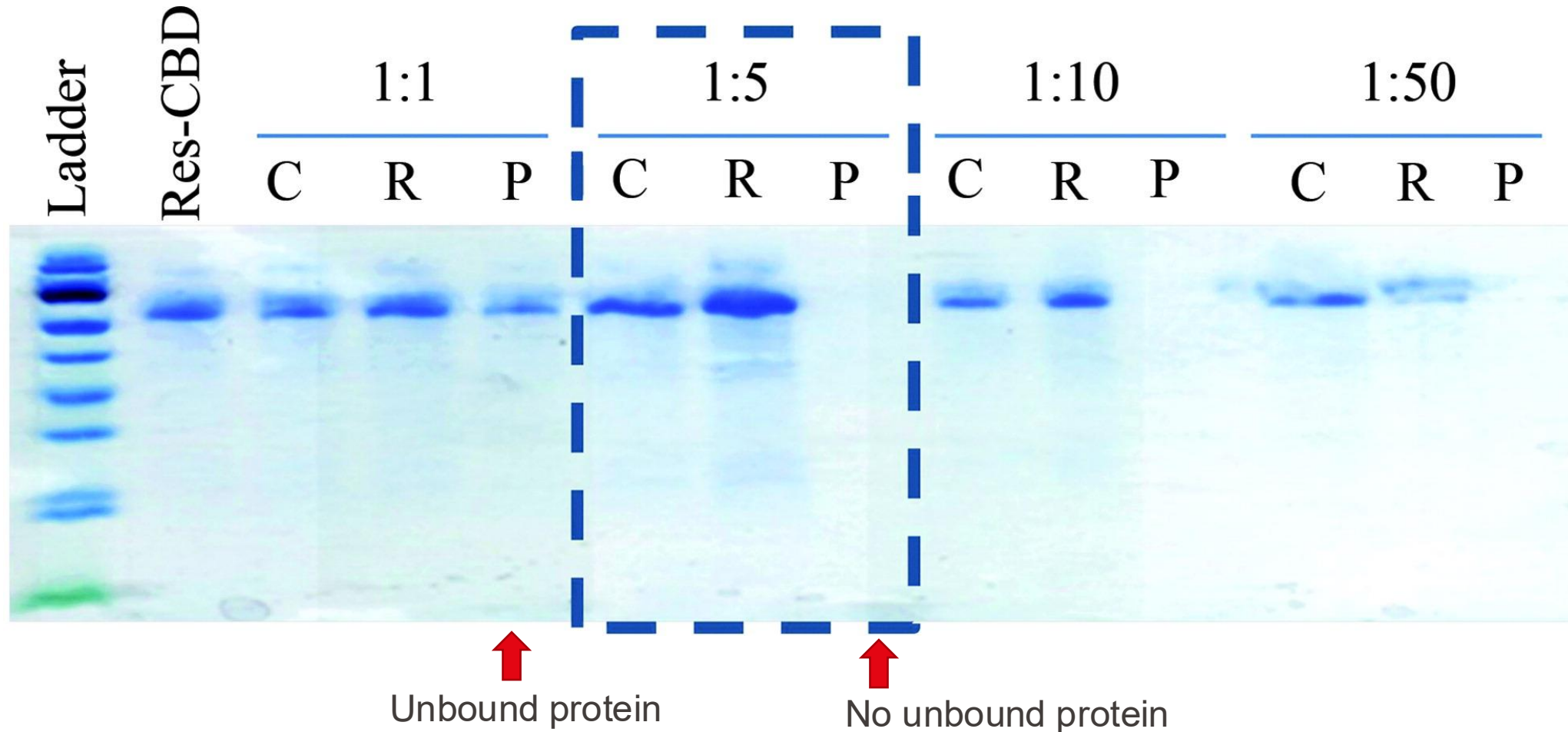
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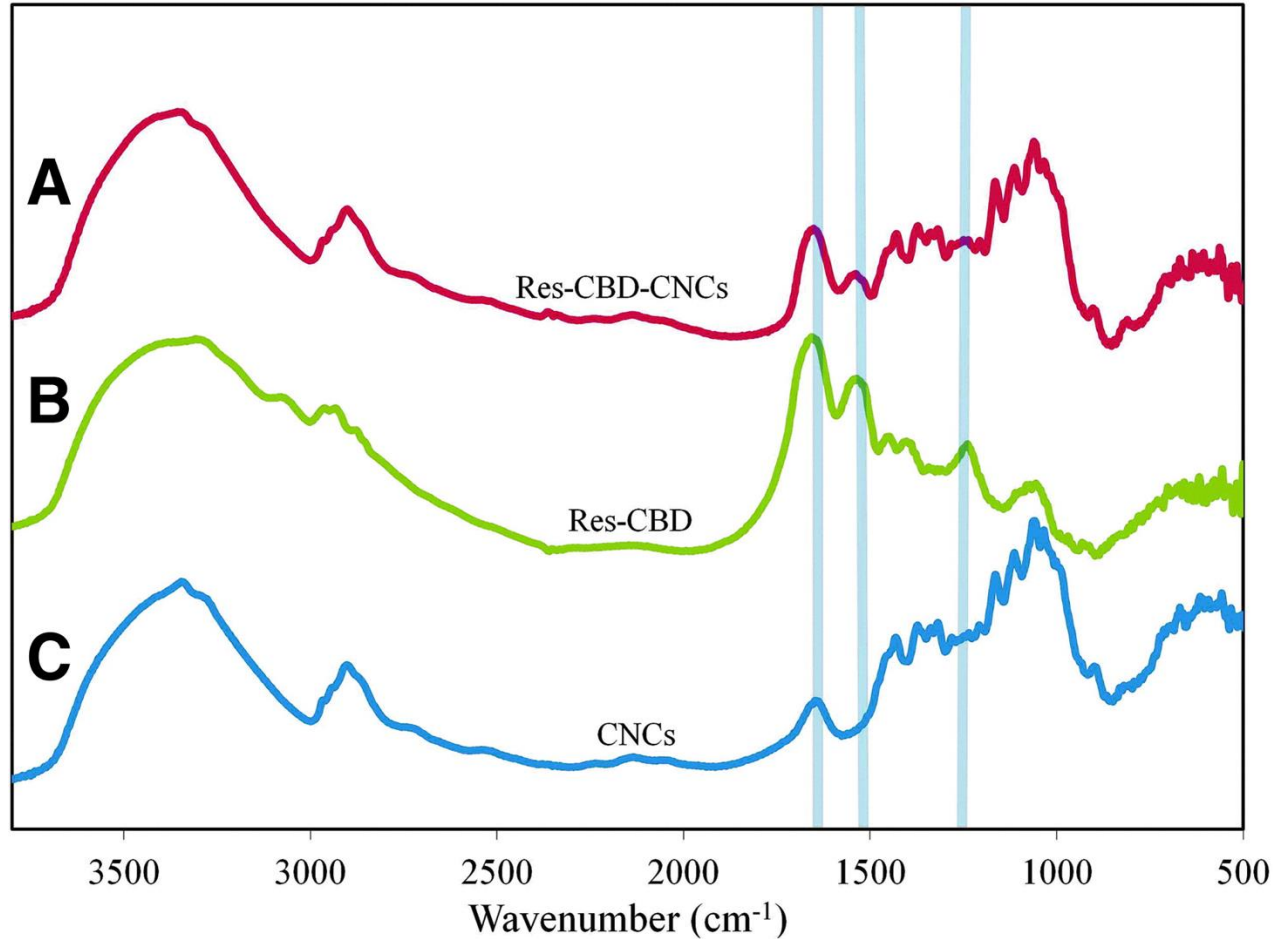


- If we see Res-CBD in the permeate, that means that we overloaded the binding capacity of the CNCs
- We try different ratios to home in on the actual binding capacity

Binding capacity; protein:cellulose ratios

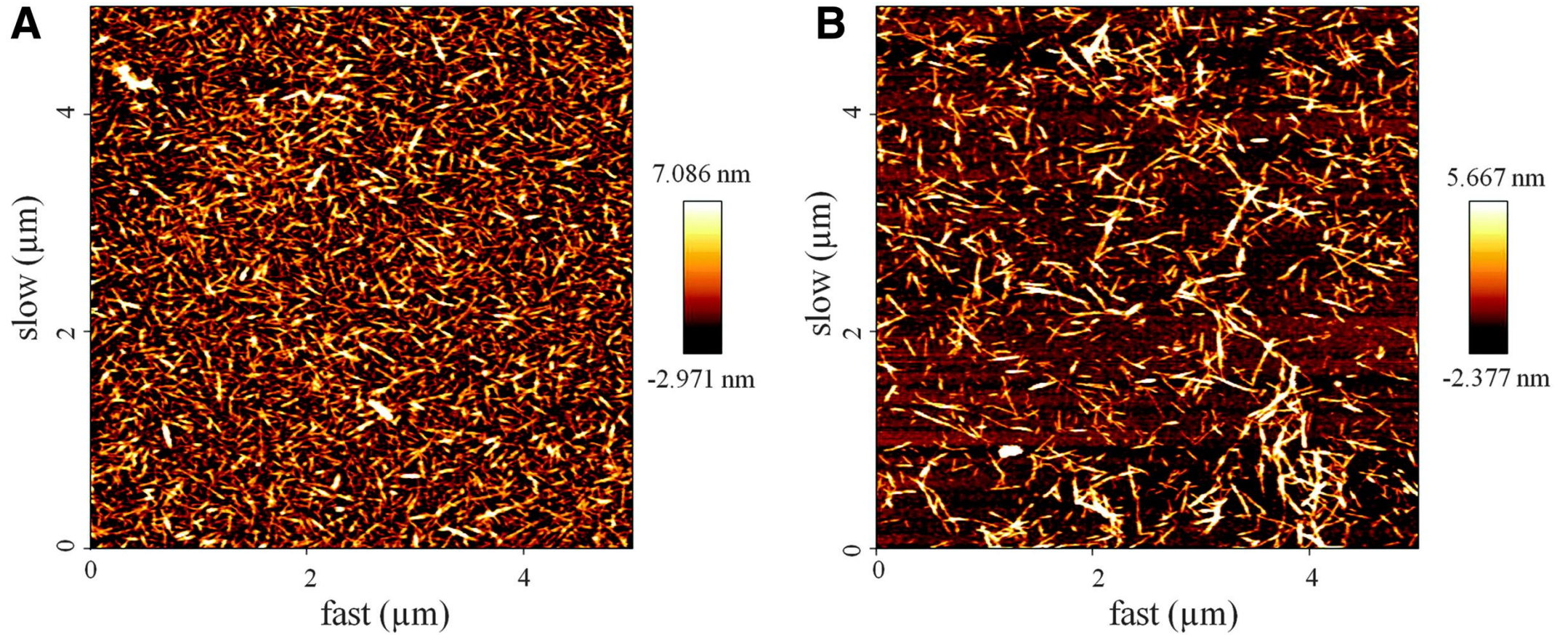
- 1:1 – too much protein, since we have unbound protein in permeate
- 1:5 – optimal, no protein in permeate
- 1:10 – the condition that was used for the rest of the study



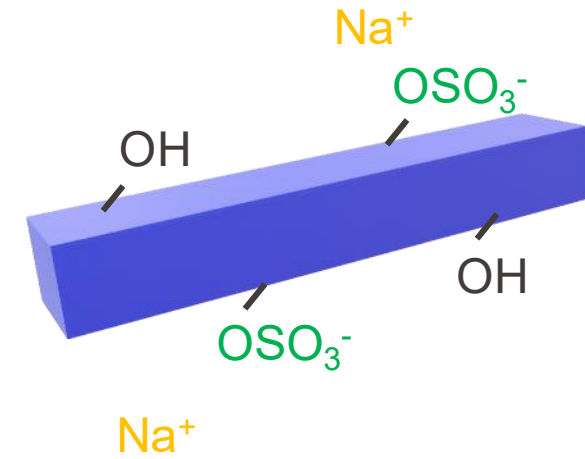


- Bonds related to protein amino acids show up in bound construct... makes sense
- Really looks like a blended spectra

What does the bound construct look like?

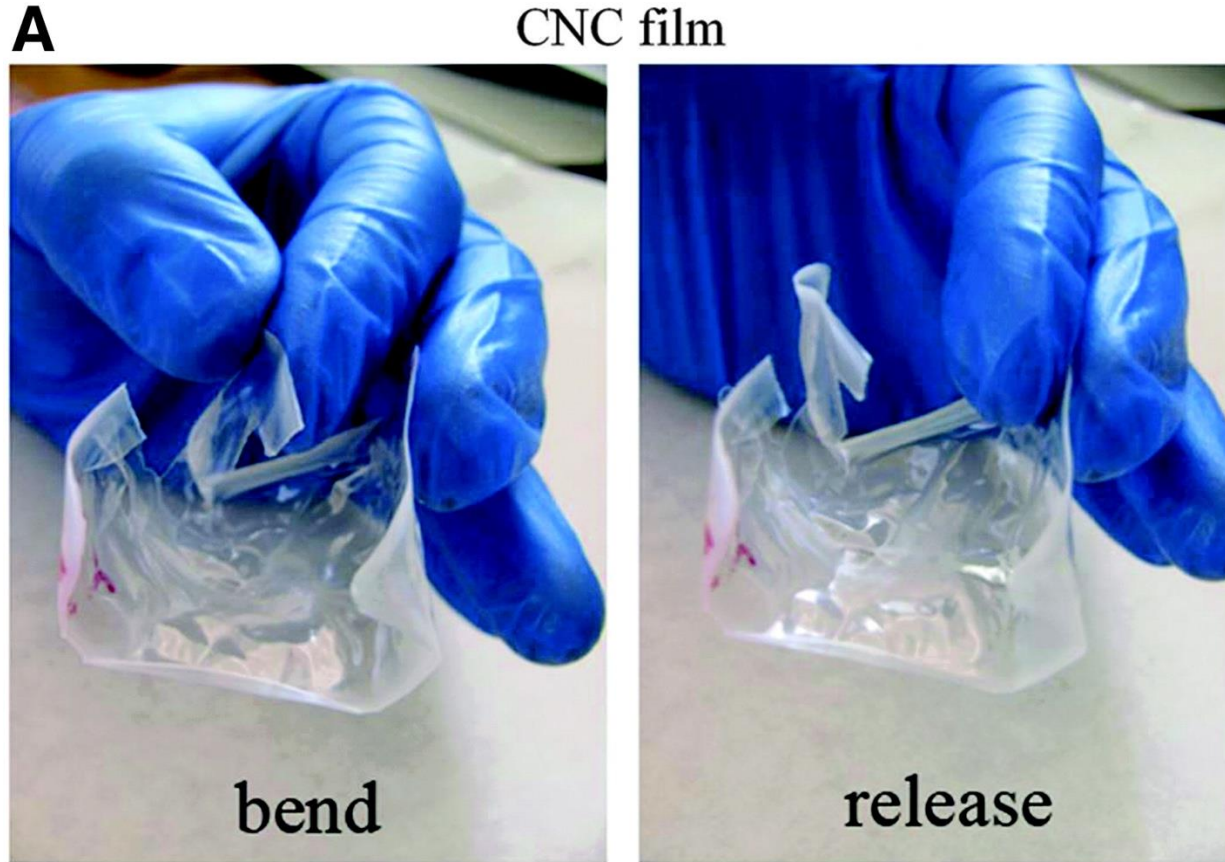


What about colloidal properties?



	UNBOUND CNCs	RES-CBD-CNCs
Tiffany Abitbol (tiffany.abitbol@epfl.ch) is signed in		
%S by conductometric titration	1.04 (0.03) ^a	^b
Zeta-potential (mV)	-56.29 (1.41)	-42.2 (1.6)
Electrophoretic mobility (m ² /Vs)	-4.40 (0.11)	-3.3 (0.1)
Mean particle size by NanoSight (nm)	105 (11)	143 (10)

- No huge difference in surface charge
- Small increase in “apparent” size



- OK, I confess, this is the ugliest CNC film that ever was and we can do better...
- What you can trust – CNC films are really brittle and they crack easily

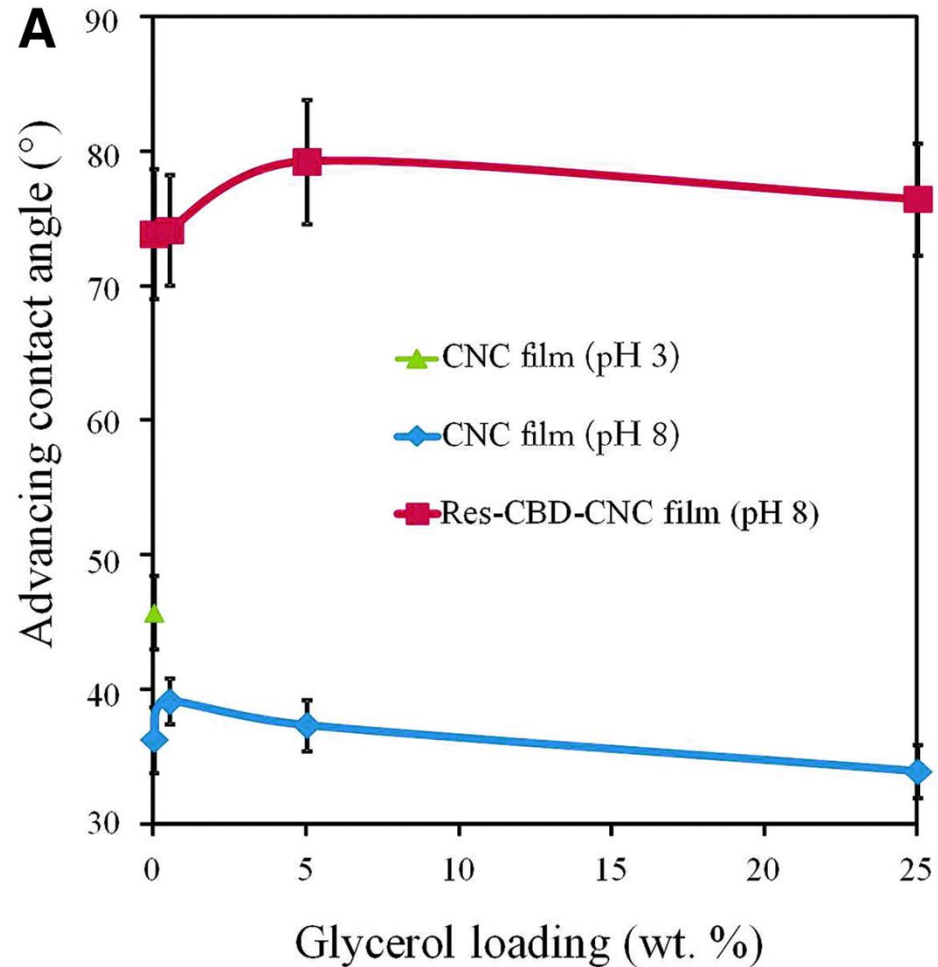
B

Res-CBD-CNC film

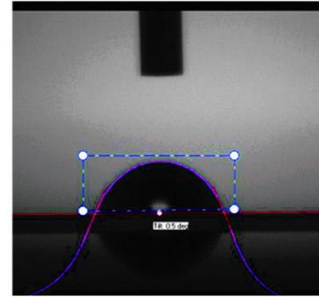


- Point is – the construct film (cast under the same conditions as the film on the previous slide) is more bendy
- Remember – we are just trying to find evidence of resilin elasticity! (We sometimes add glycerol as a plasticizer to make it even more bendy, but not yet)
- Remember, glycerol is in the ELM toolkit!

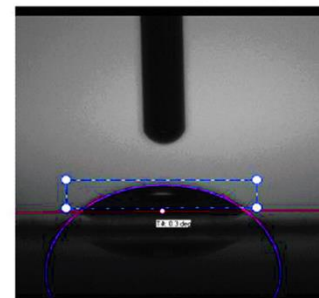
What about surface wetting?



Res-CBD-CNC film

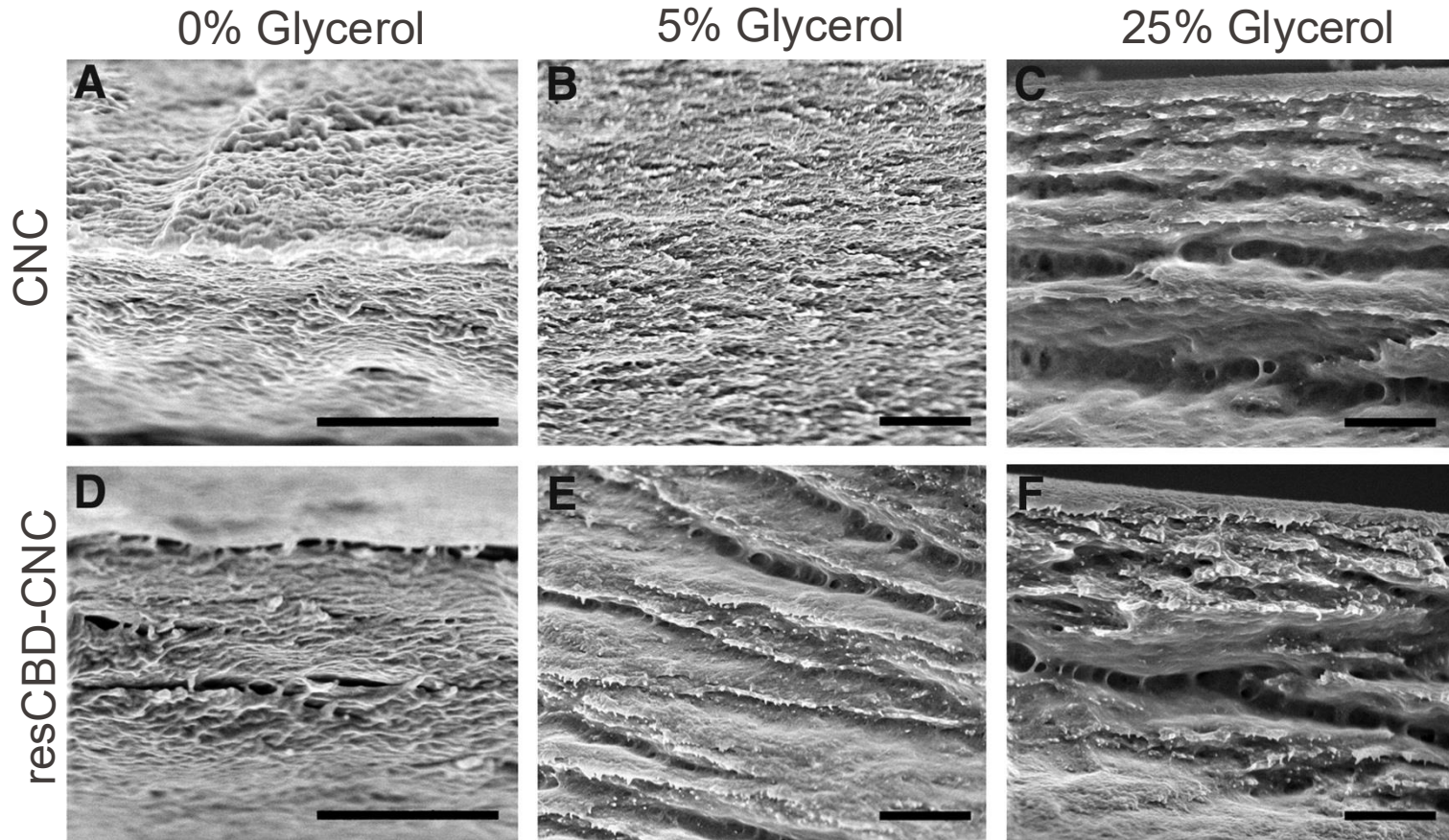


CNC film (pH 8)



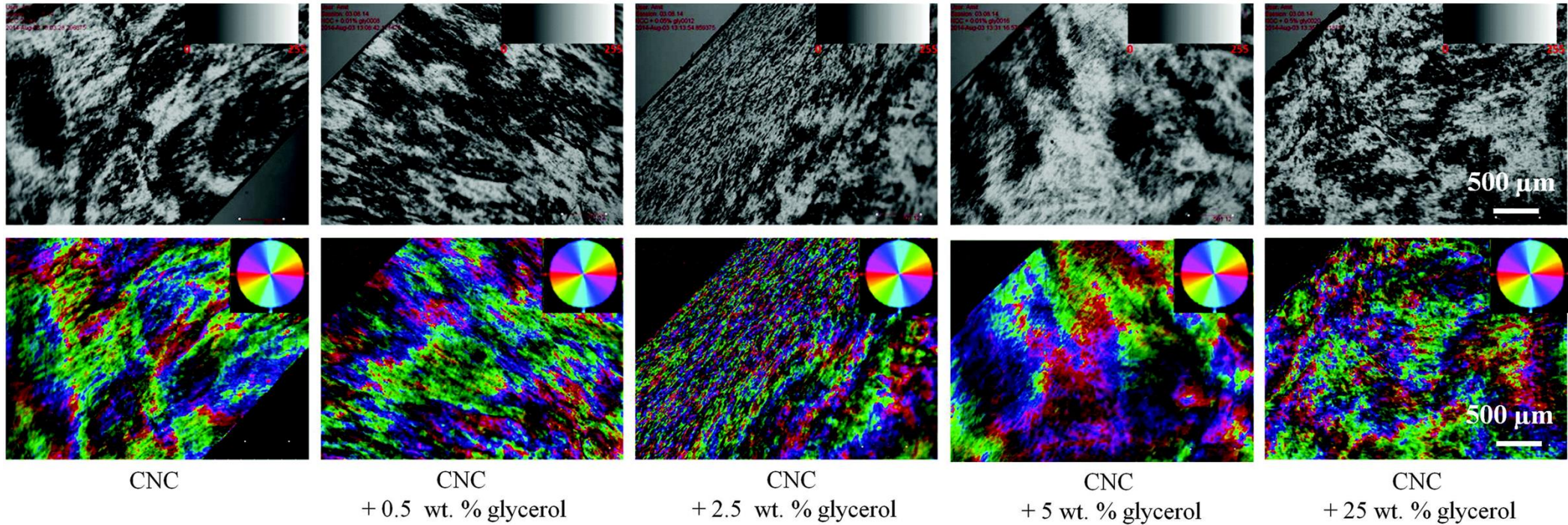
- Res-CBD films are much more hydrophobic than pure CNC films!
- Something is different!

What's it look like inside the films?



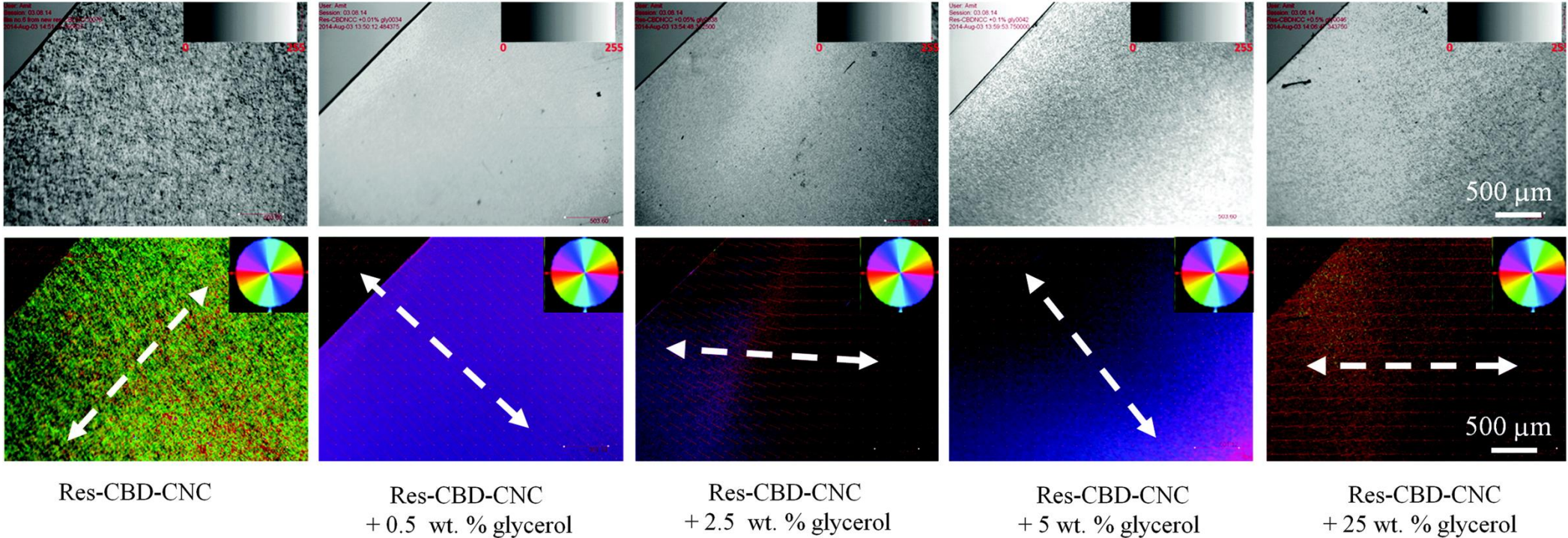
- Cross-sectional SEM
- 500 nm scale bars
- Glycerol films look sticky
- Typical mille-feuille look. Yum!
- Hard to see a big diff with and without protein

Remember it's CNC – we care about self-assembly



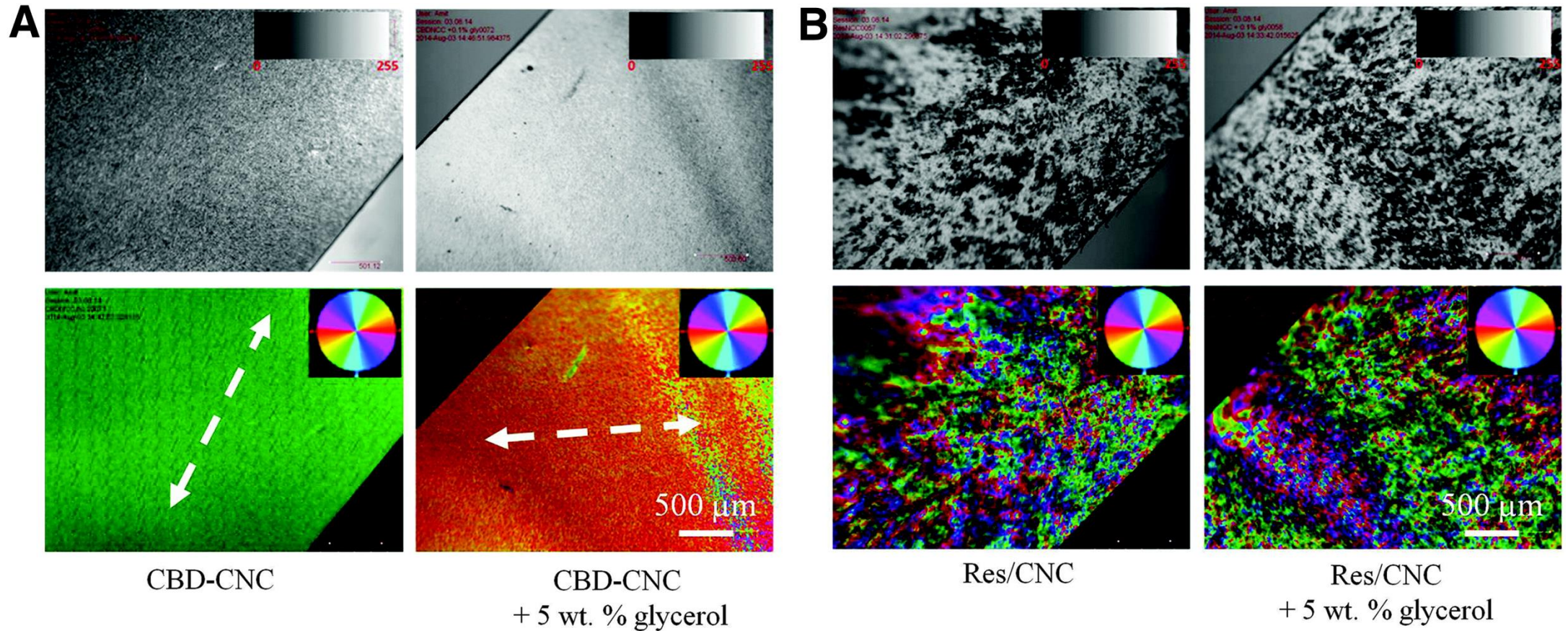
- Fancy polarized optical microscope
- CNC films show lots of different orientations (typical)

Remember it's CNC – we care about self-assembly



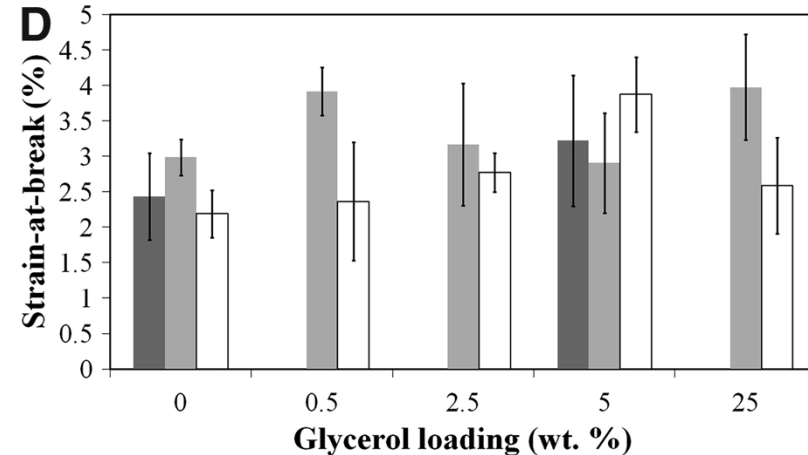
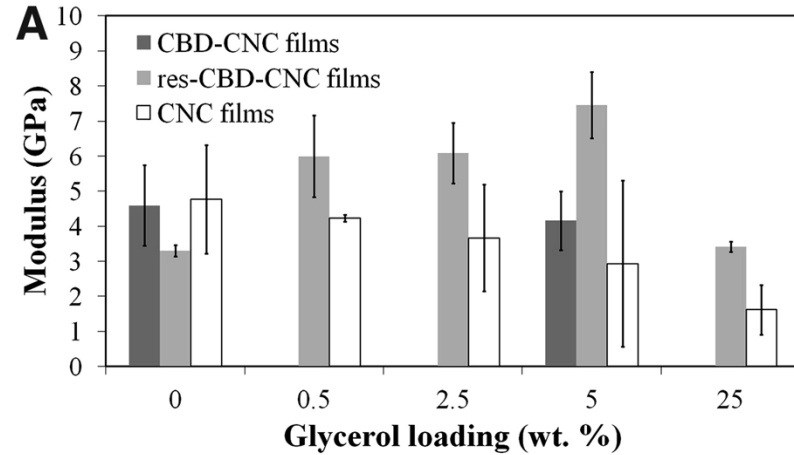
- Fancy polarized optical microscope
- Hmm... one color means all the rods are pointing in the same direction?

What's going on with the alignment?



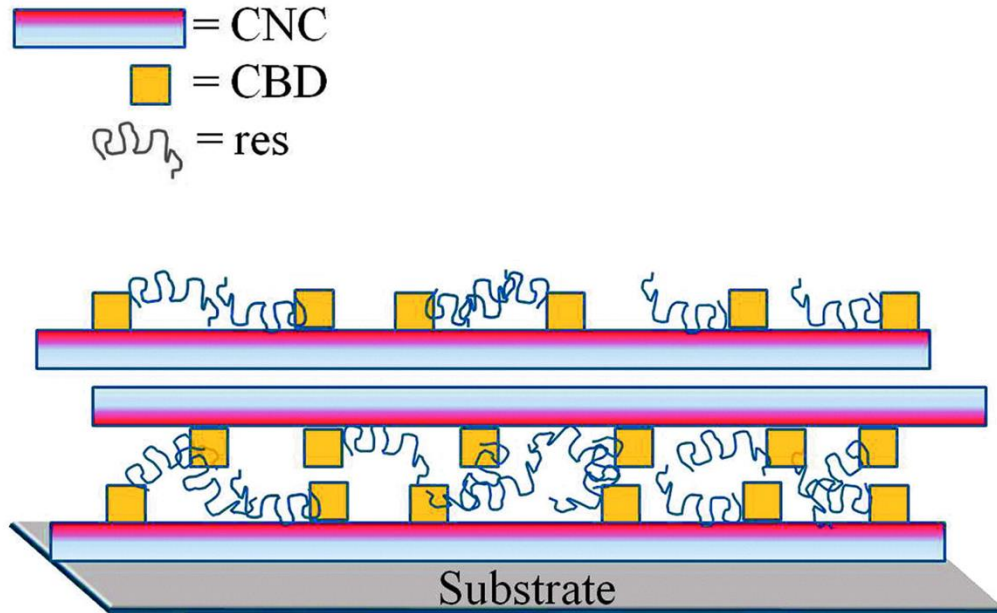
- Seems related to the CBD, not so much the resilin...this is so interesting!

So are these films springy and stiff?



- CNC (white): lower modulus and higher strain at break with added glycerol (intuitive)
- resCBD-CNC (light grey): higher modulus and less clear trend with strain at break with added glycerol (counterintuitive)
- Res seems important for modulus – resilin makes CNC stiffer? (What now?)
- @0.5% glycerol – films are indeed stiffer (er) and (more) springy
- For the mechanicians – we did a whole bunch more tests...read the paper if interested

What's the big picture? (We think...I used PP in 2014)



Compared to CNC films -

- Tougher (+150%)
- More elastic (+100%)
- Less hydrophilic
- Long-range (cm) unidirectional alignment (this is actually a hard thing to achieve)

- Hydrophobic crystal face of CNC probably matters and is where the protein binds

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Modular assembly of proteins on nanoparticles

Wenwei Ma, Angela Saccardo, Danilo Roccatano, Dorothy Aboagye-Mensah, Mohammad Alkaseem, Matthew Jewkes, Francesca Di Nezza, Mark Baron, Mikhail Soloviev & Enrico Ferrari

Nature Communications 9, Article number: 1489 (2018) | Cite this article

17k Accesses | 78 Citations | 181 Altmetric | Metrics

Abstract

Generally, the high diversity of protein properties necessitates the development of unique nanoparticle bio-conjugation methods, optimized for each different protein. Here we describe a universal bio-conjugation approach which makes use of a new recombinant fusion protein combining two distinct domains. The N-terminal part is Glutathione S-Transferase (GST) from *Schistosoma japonicum*, for which we identify and characterize the remarkable ability to bind gold nanoparticles (GNPs) by forming gold-sulfur bonds (Au-S). The C-terminal part of this multi-domain construct is the SpyCatcher from *Streptococcus pyogenes*, which provides the ability to capture recombinant proteins encoding a SpyTag. Here we show that SpyCatcher can be immobilized covalently on GNPs through GST without the loss of its full functionality. We then show that GST-SpyCatcher activated particles are able to covalently bind a SpyTag modified protein by simple mixing, through the spontaneous formation of an unusual isopeptide bond.

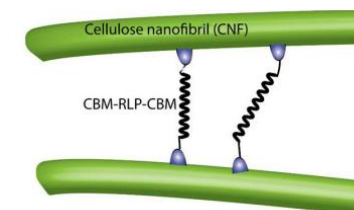
2018 thesis from Aalto (and papers – better IF)

Nanocellulose for bio-inspired nanocomposites - surface modification with recombinant proteins

Wenwen Fang

Department of Bioproducts and Biosystems

Research output: Thesis > Doctoral Thesis > Collection of Articles



Overview Fingerprint Infrastructure (2)

Reading options

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Article

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

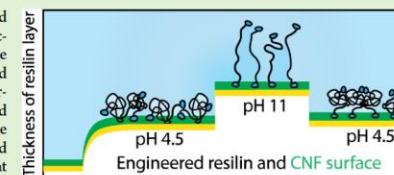
Wenwen Fang,[†] Arja Paananen,[‡] Marika Vitikainen,[‡] Salla Koskela,[‡] Ann Westerholm-Parvinen,[‡] Jussi J. Joensuu,[‡] Christopher P. Landowski,[‡] Merja Penttilä,[‡] Markus B. Linder,[†] and Päivi Laaksonen^{*,†}

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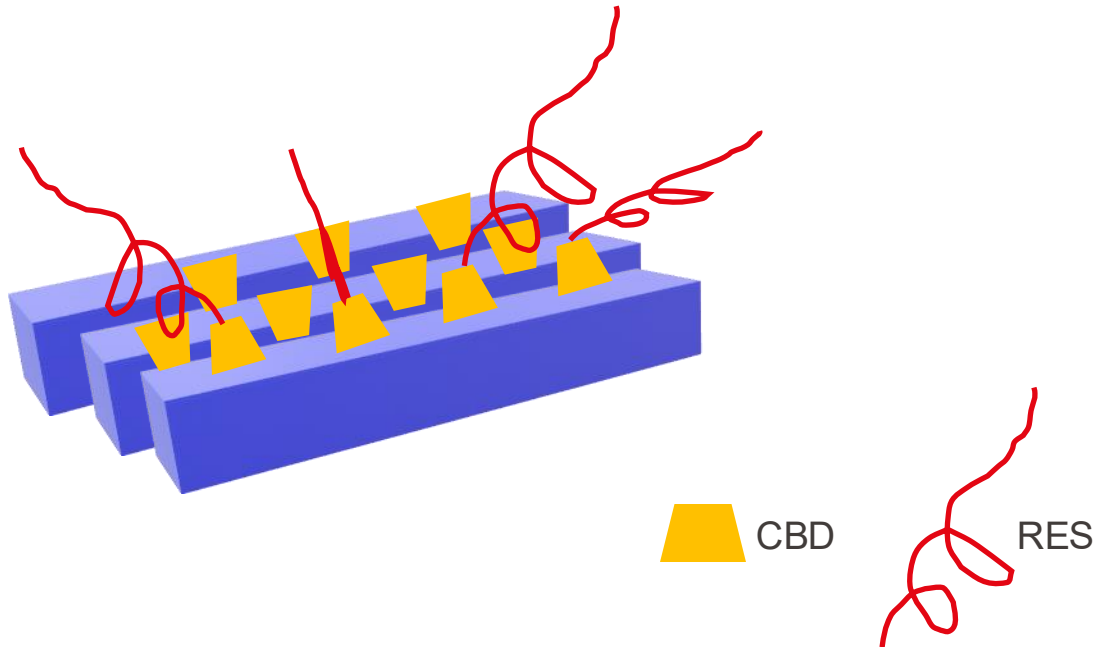
[‡]VTT Technical Research Centre of Finland Ltd., Espoo, FI-02044 VTT, Finland

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.



What's exciting for me?



CBD zips up CNCs!

- Directed alignment of a nanomaterial (usually we need strong forces to get a unidirectional alignment)
- That we can use a CBD moiety to bind stuff to cellulose nanocrystals (much nicer than conventional surface modifications) – remember: I am a chemist
- It could be better (always) – different modifications, crosslinking the resilin to get an elastic network, anything you can think of?
- Not a high impact publication, or in an easy to find journal, but an early instance of biotech to modify a nanomaterial surface – more examples?

Do you like this paper? Why or why not? (Just kidding, don't answer) Is this an ELM?



YES



NO