

The background of the slide is a microscopic image showing a dense population of cells, likely fibroblasts, stained with a pinkish-red dye on a light blue background. The cells are elongated and spindle-shaped, typical of cells in culture.

Some research from my past

Prof. Tiffany Abitbol

2024

Honors undergrad project – Chiral internal stresses in paper sheets



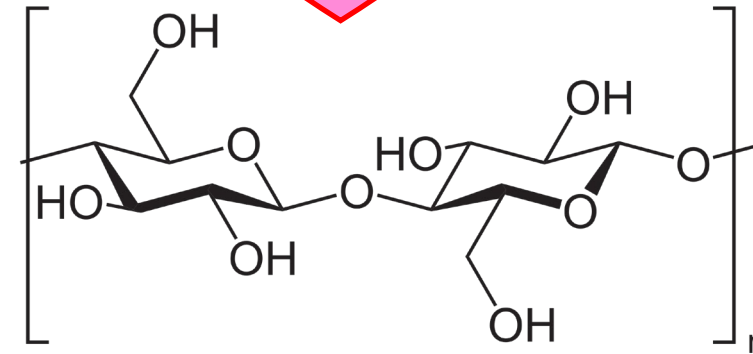
PROF. DEREK GRAY
2013 MARCUS WALLENBERG PRIZE WINNER

Honors undergrad project – Chiral internal stresses in paper sheets

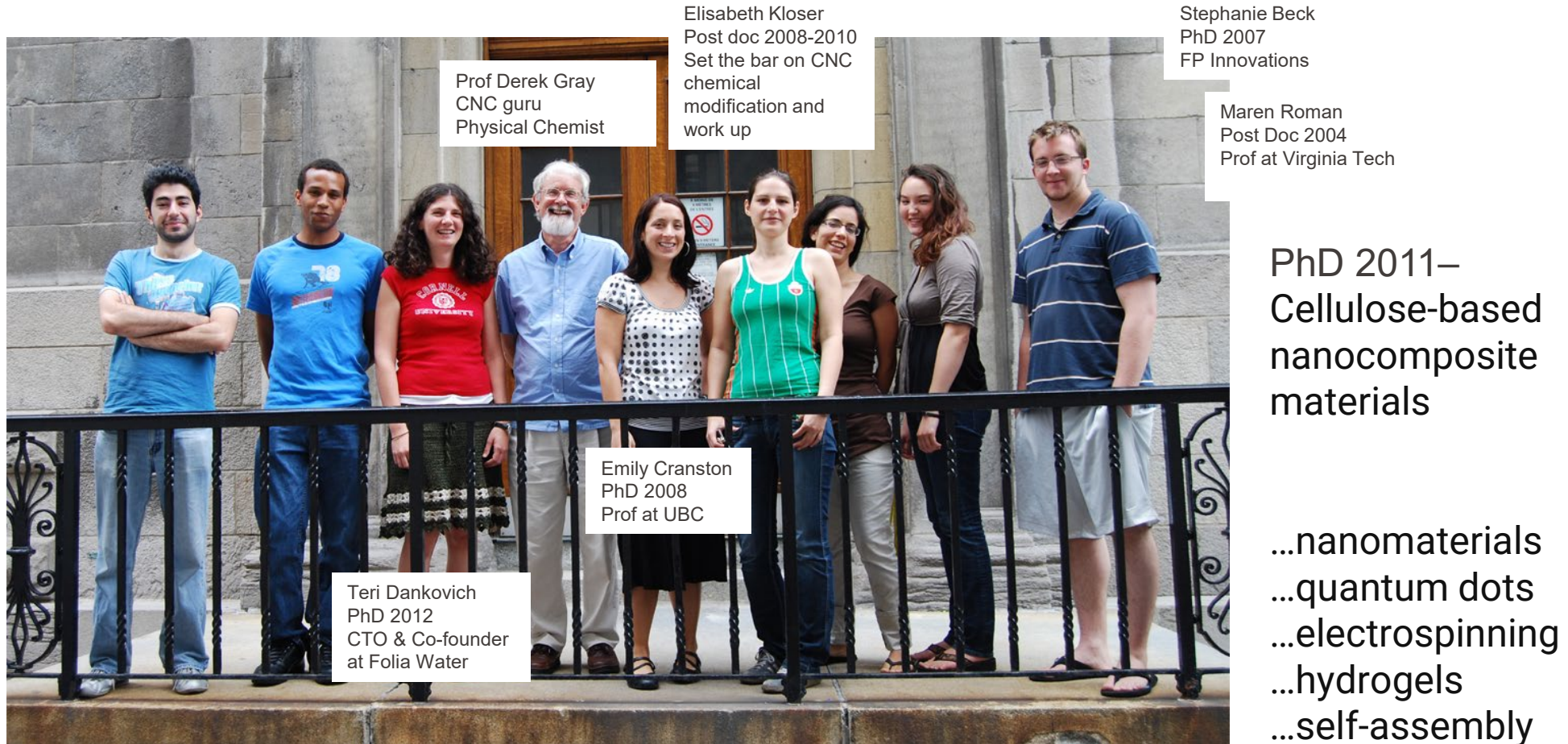
When you see kids posts "happy 3 week anniversary can't wait to spend the rest of my life with you baby"

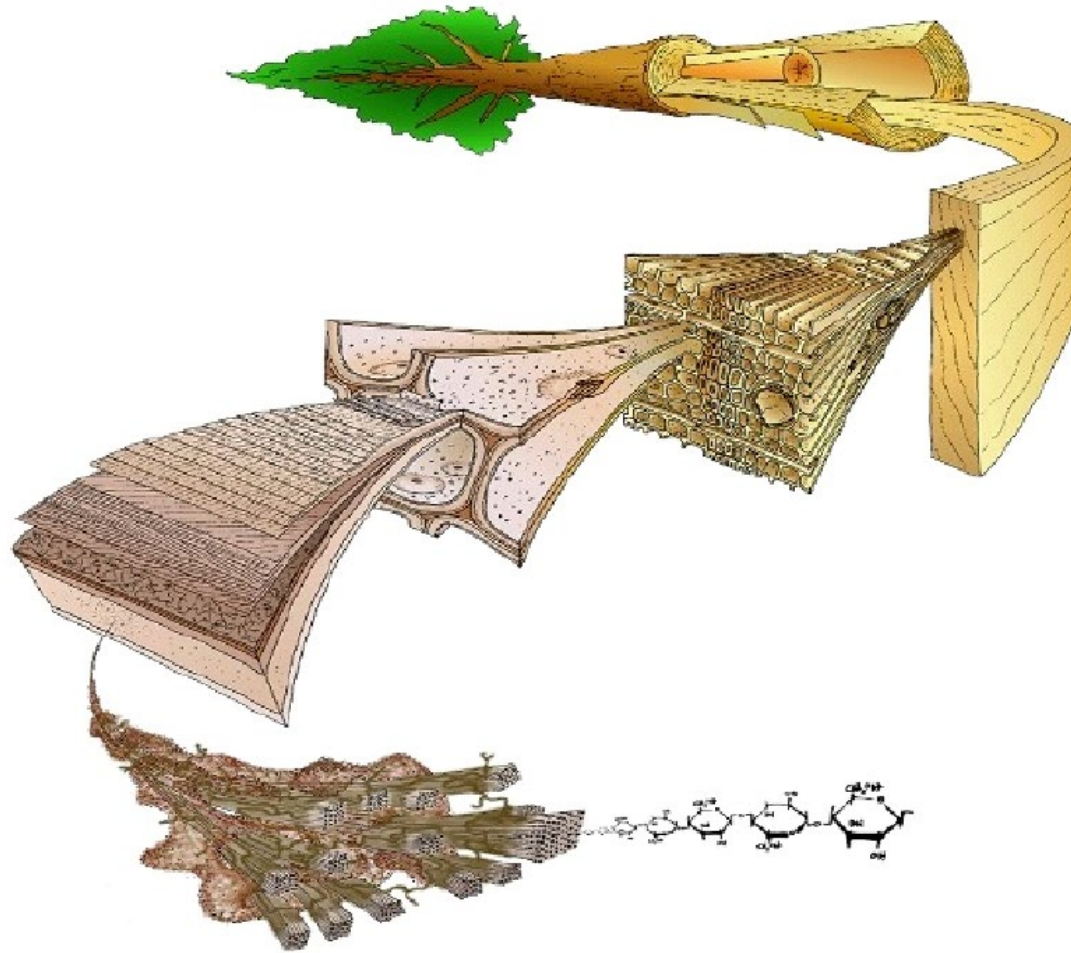


**1st time meeting
cellulose!**



Gray group, ca. 2008





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

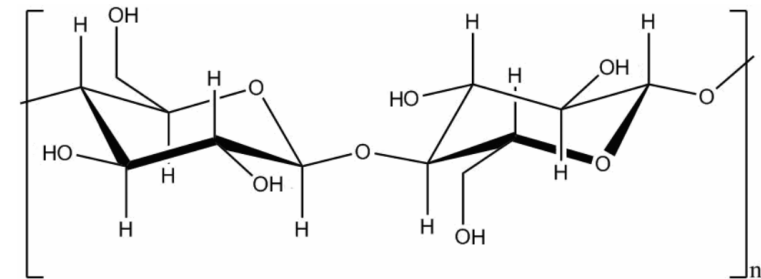
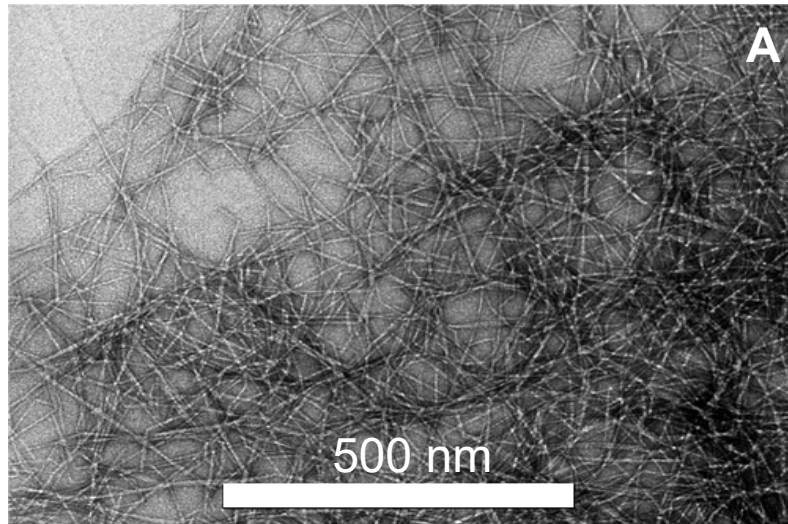


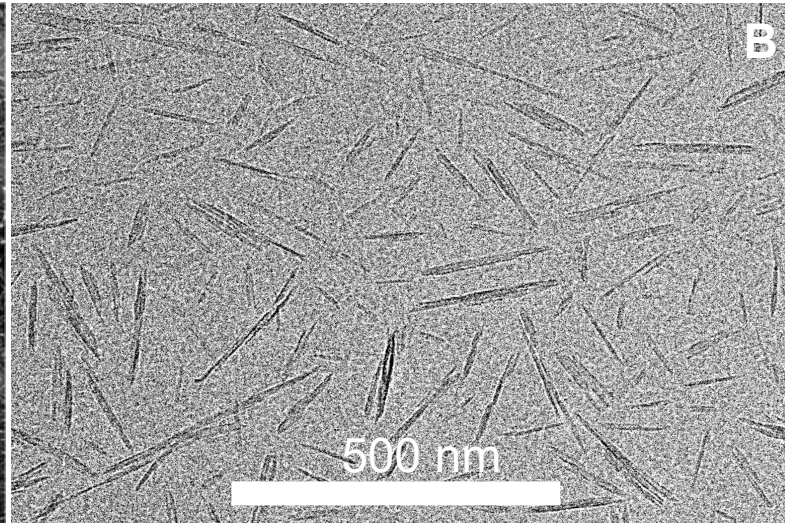
Figure 1.1: Chemical structure of cellulose.

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

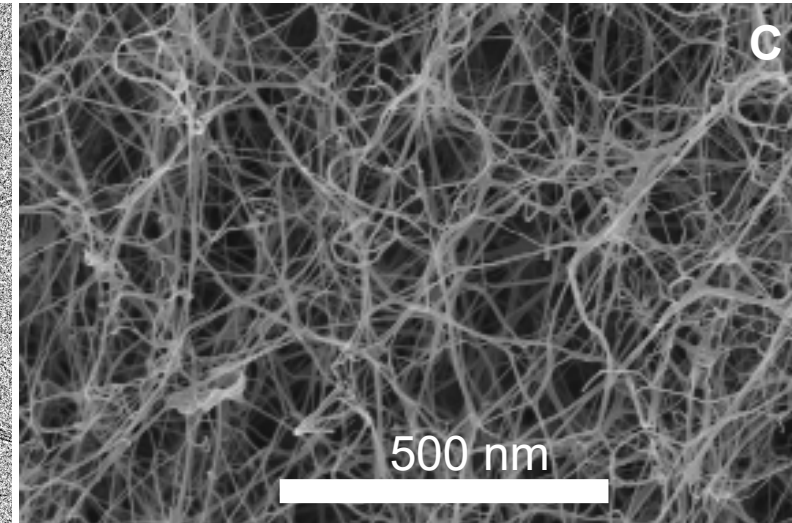
- Generic umbrella word
- Encompasses all forms of nanoscale cellulose particles
- Most common types:
 - Cellulose nanofibrils (CNF)
 - Cellulose nanocrystals (CNC)
 - Bacterial nanocellulose (BNC) – secreted as the ECM



CNF



CNC

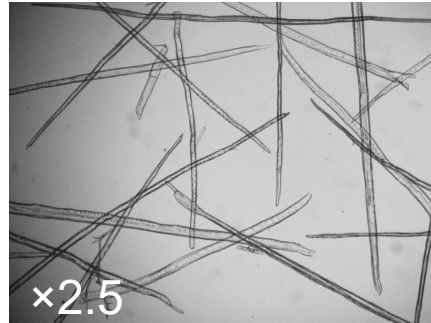


BNC



CELLULOSE
CONTAINING
BIOMASS

pulping →



CELLULOSE-ENRICHED
PULP

Enzymes &
mechanical



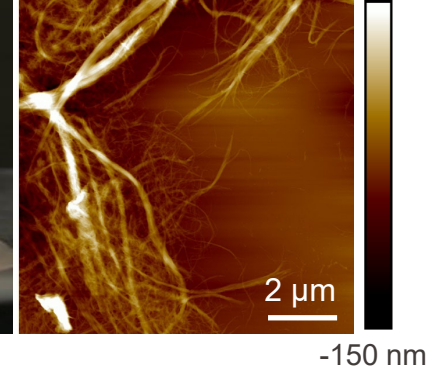
Chemicals &
mechanical



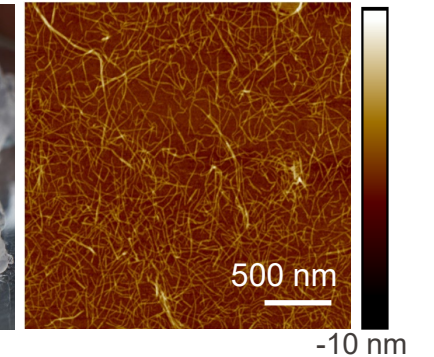
Degradation



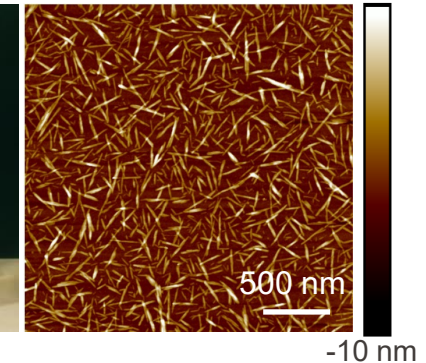
ENZYMATIC CNF



OXIDIZED CNF



CNC



CNF = cellulose nanofibril
CNC = cellulose nanocrystal

CNCs by acid hydrolysis

1947

Cellulose Intercrystalline Structure

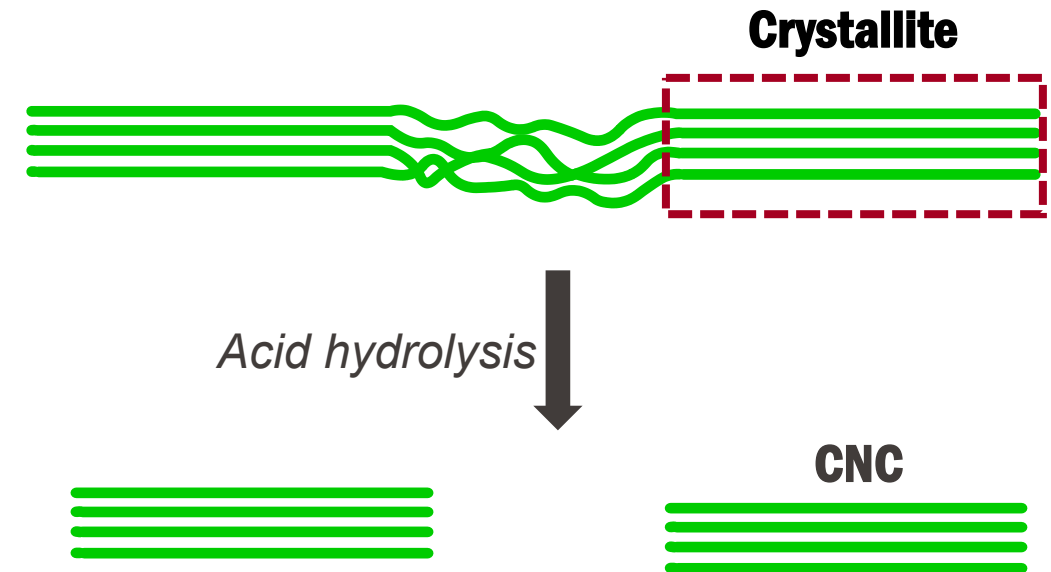
STUDY BY HYDROLYTIC METHODS

R. F. NICKERSON¹ AND J. A. HABRLE²

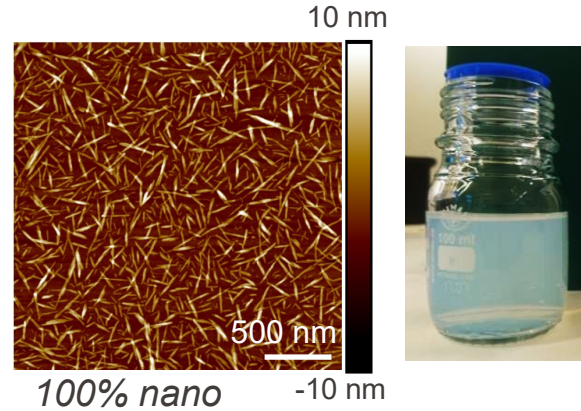
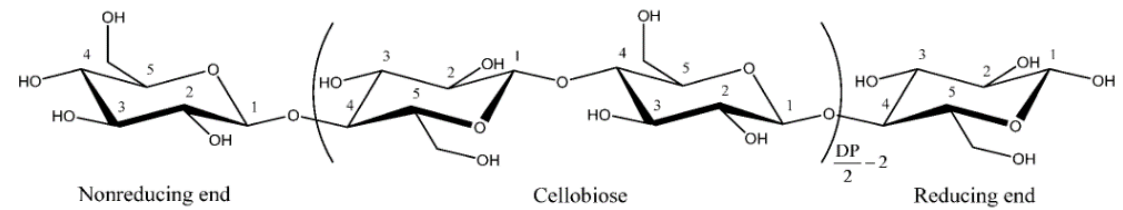
Mellon Institute, Pittsburgh 13, Pa.

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.

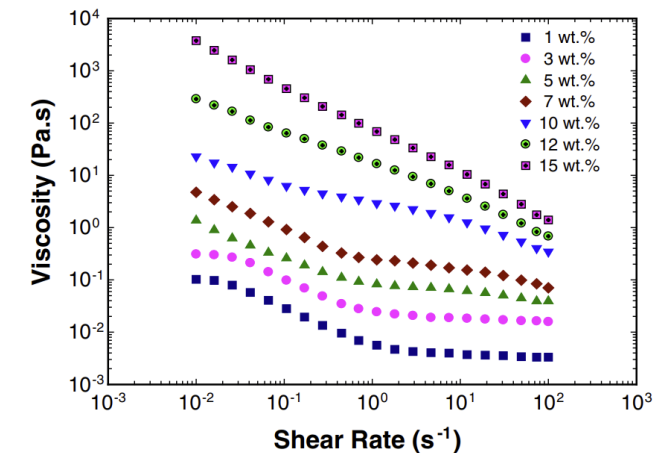
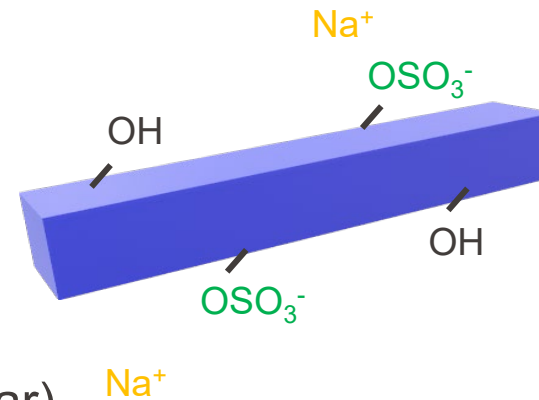
Ind. Eng. Chem., 39(11), 1507-1512 (1947).



- 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

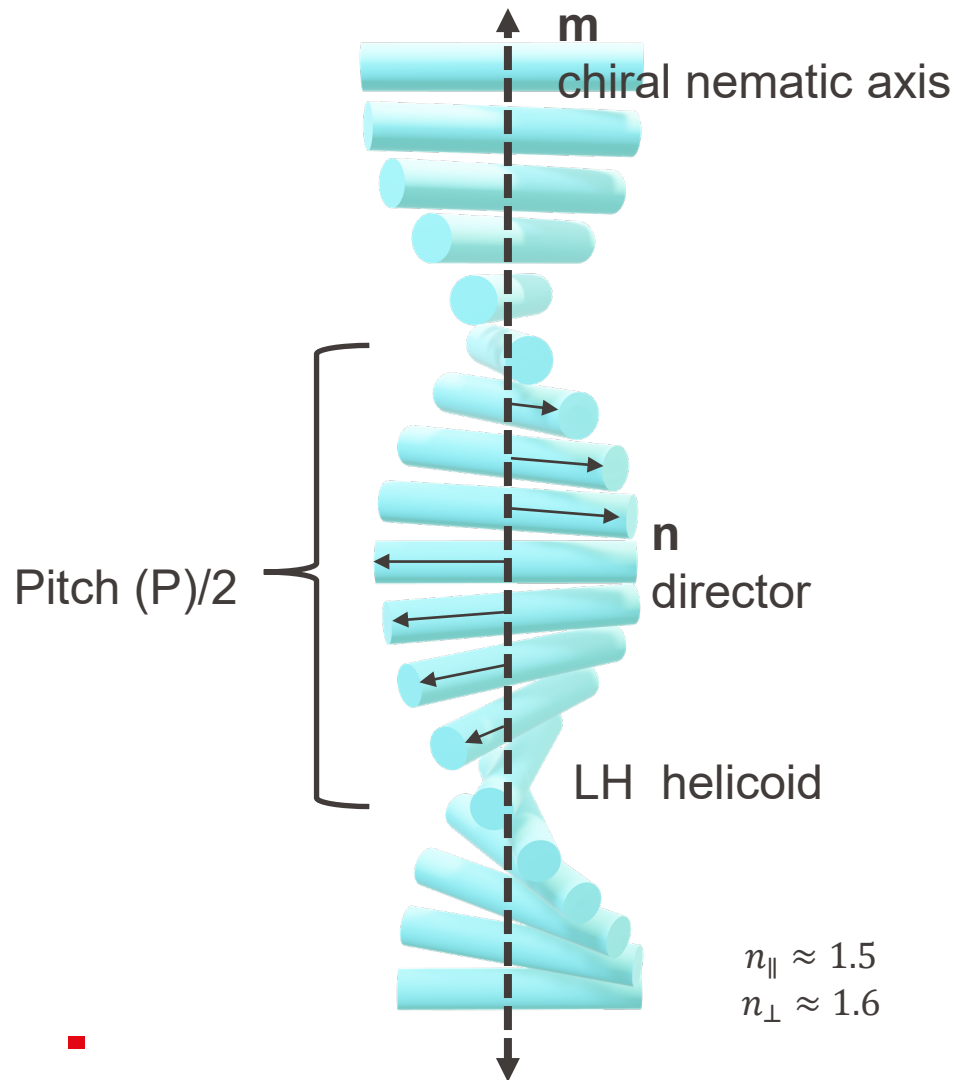


CNC = a bundle of cellulose chains with non-native surfaces

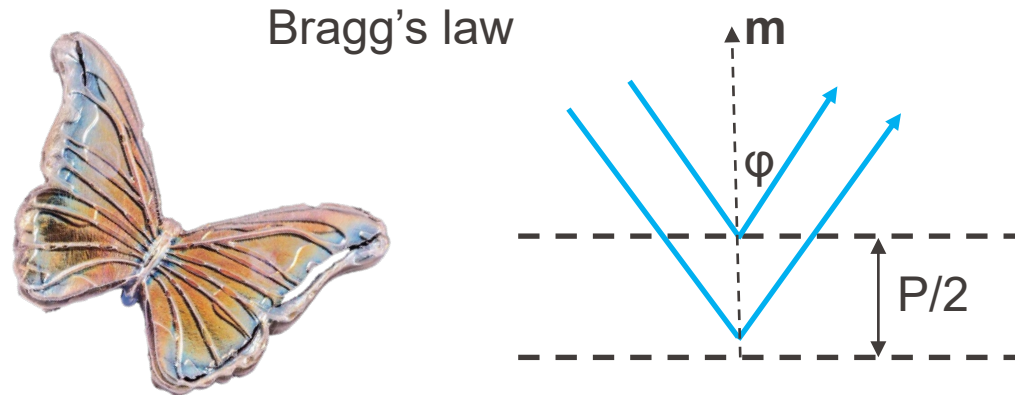


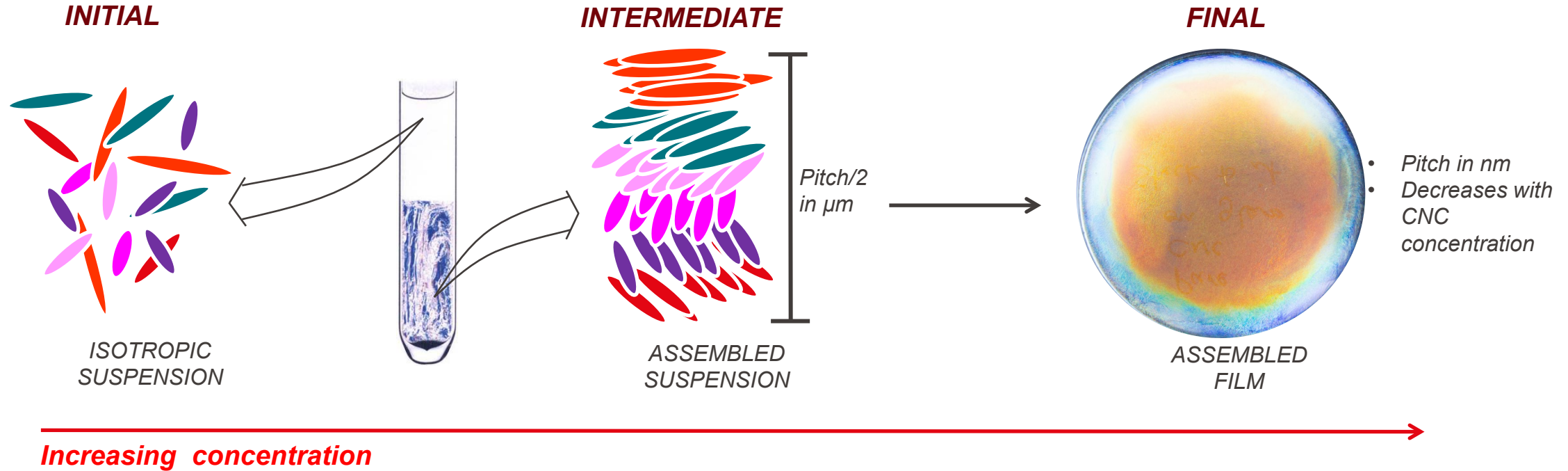
Shafiei-Sabet *et al.* Cellulose 2014, 21, 3347–3359.

Chiral nematic liquid crystals



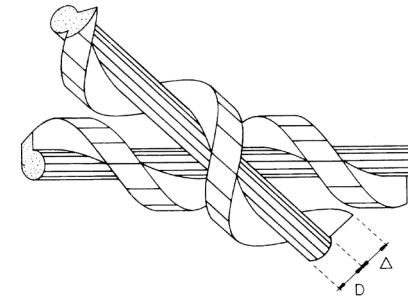
- CNCs form chiral nematic phases as they are concentrated
- The liquid crystal structure can be locked in films
- Structure colors occur when $P/2$ coincides with visible wavelengths





WHY DOES THIS HAPPEN?

- Stiff rods
- Translational entropy

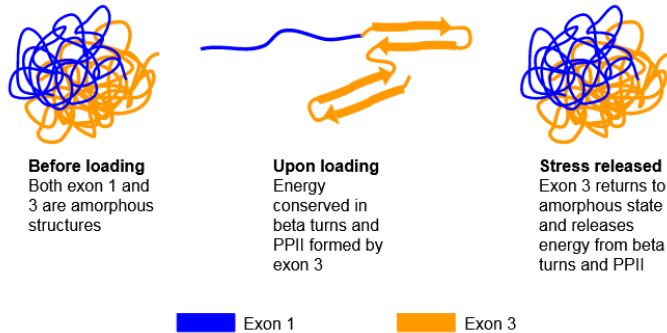


Dong et al., *Langmuir* **12**, 1996, p.2076
 Straley, *Phys. Rev. A* **14**, 1976, p.1835

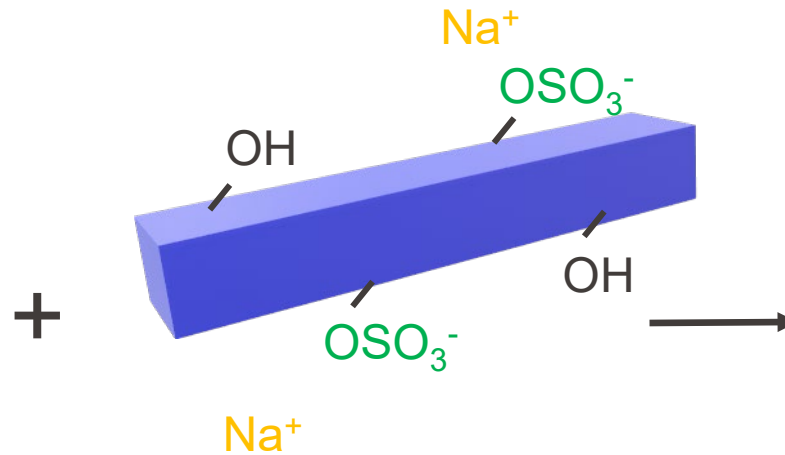
Protein-CNC hybrids: post doc with an ELM?



Drosophila melanogaster



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



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



Prof. Oded Shoseyov

Springy and stiff?

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

... a post doc within a post doc? (5 month exchange)

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?
- Oh! What a low IF! Mon dieu!
- Cited 35 times since 2015, not great
- One of the 1st reports of a nanocellulose-protein hybrid



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



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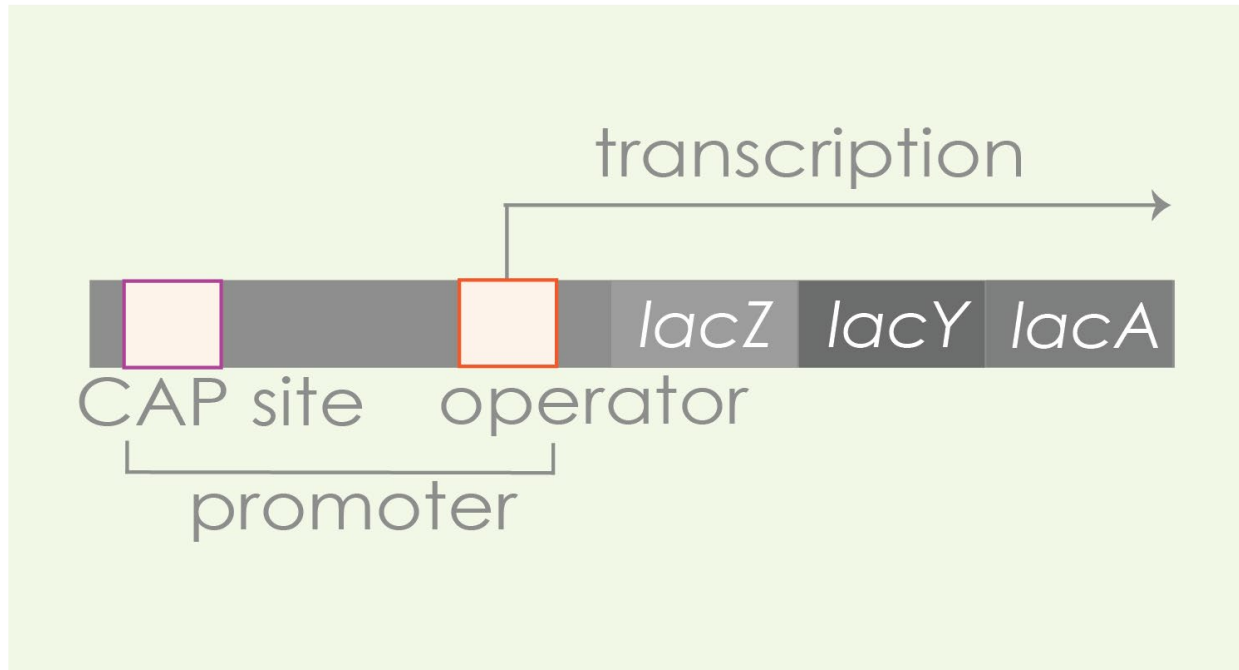
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- “...advances in bioengineering have provided scientists with the strategies needed to design recombinant proteins with specific functionality in mind, such as elastomeric recombinant proteins based on resilin, which can then be used to prepare well-defined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

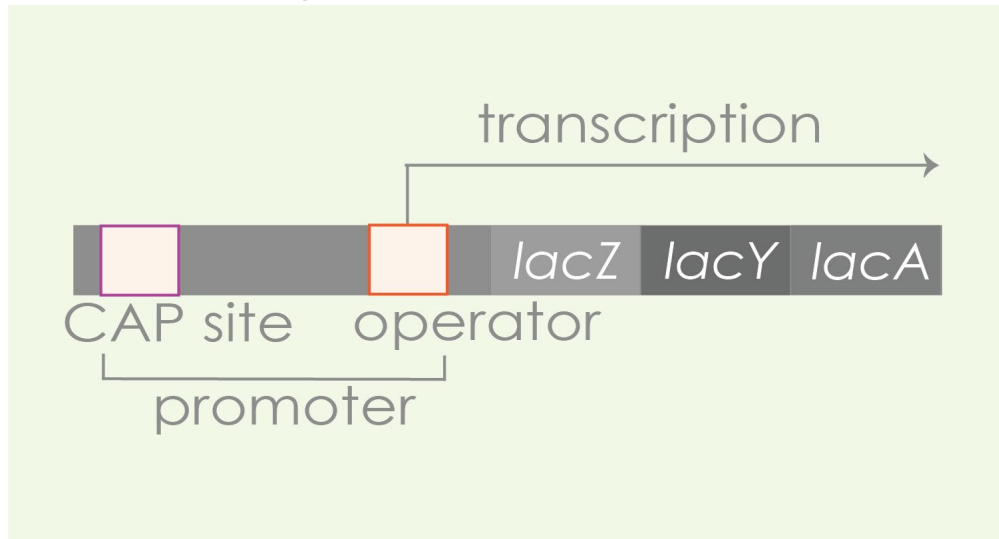
The Lac Operon



- 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)

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

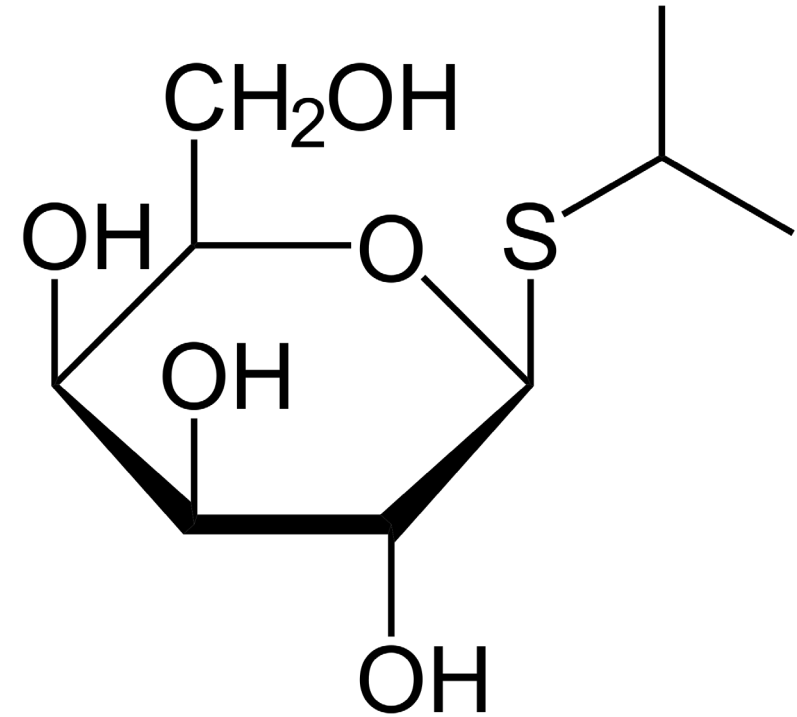
- Lactose (allolactose) binds to repressor, making it unbind from the operator
- Gene transcription can occur

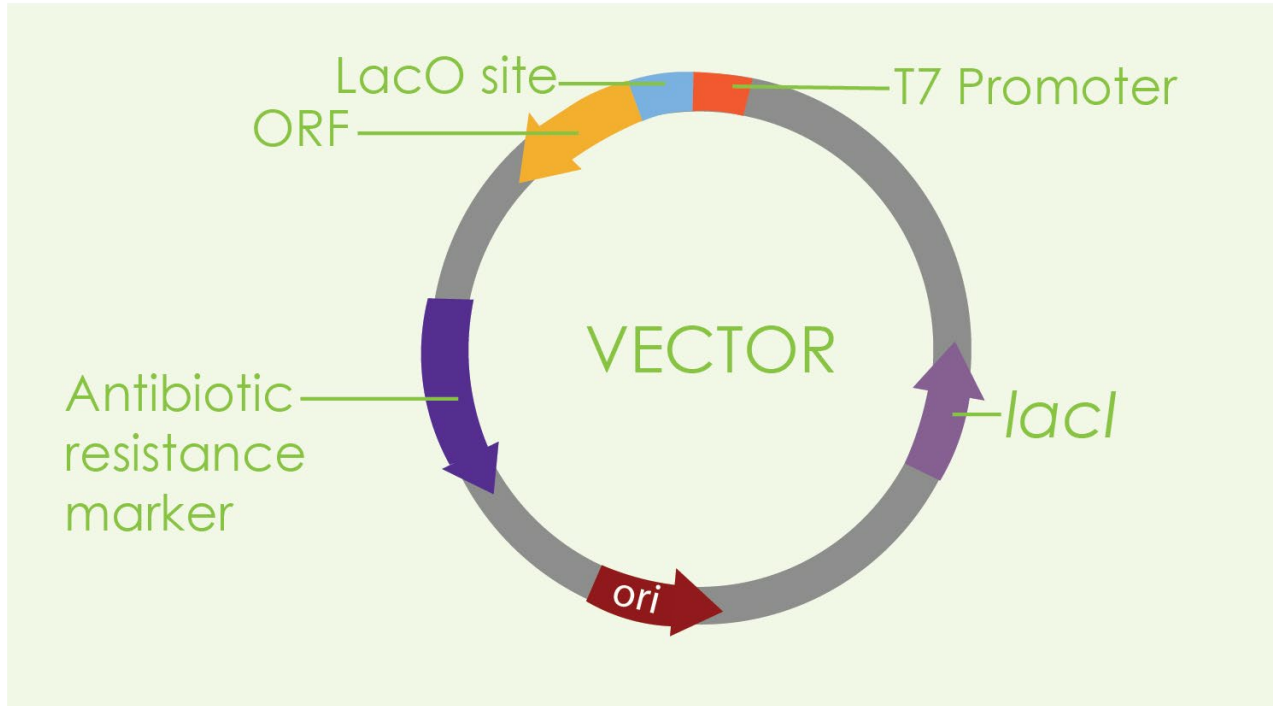
Glucose control (catabolite repression)

- 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)

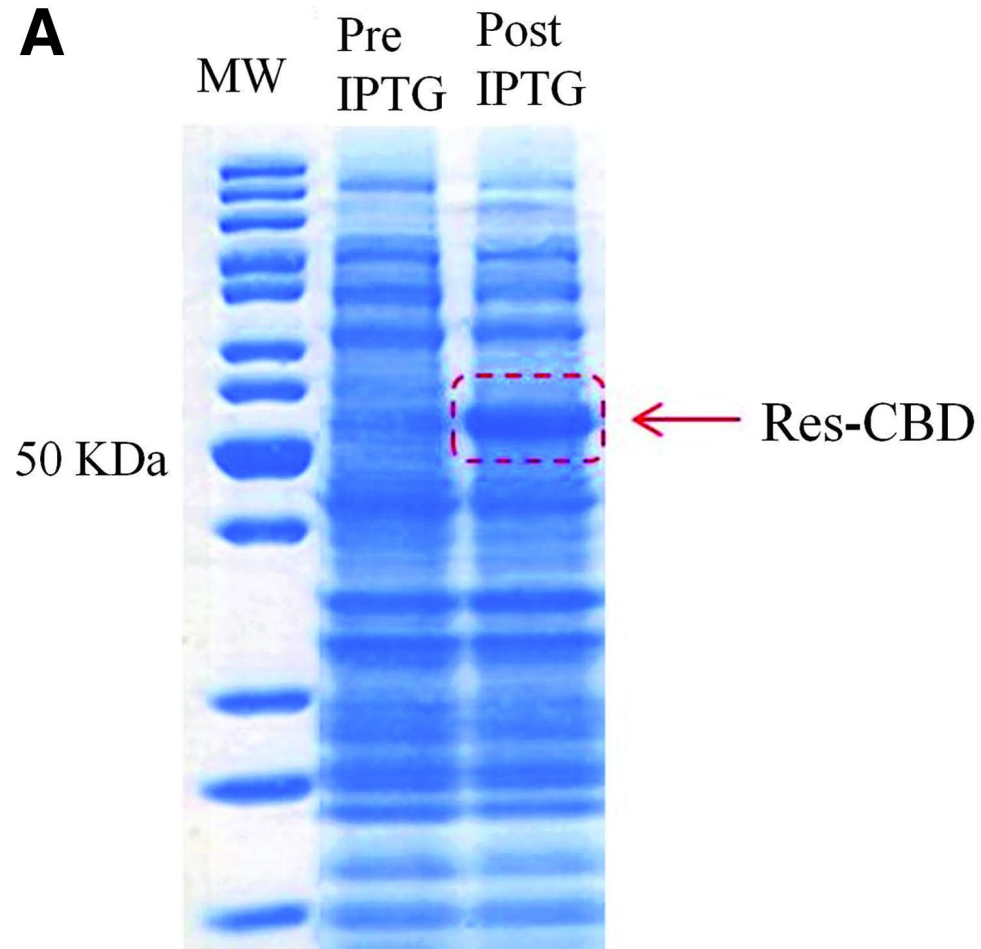
- IPTG is a molecular analog of allolactose (lactose metabolite) – binds to repressor
- Triggers transcription of the *lac operon*
- Used to induce protein expression where the gene is under control of the *lac operon* (often used in recombinant protein)





- 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

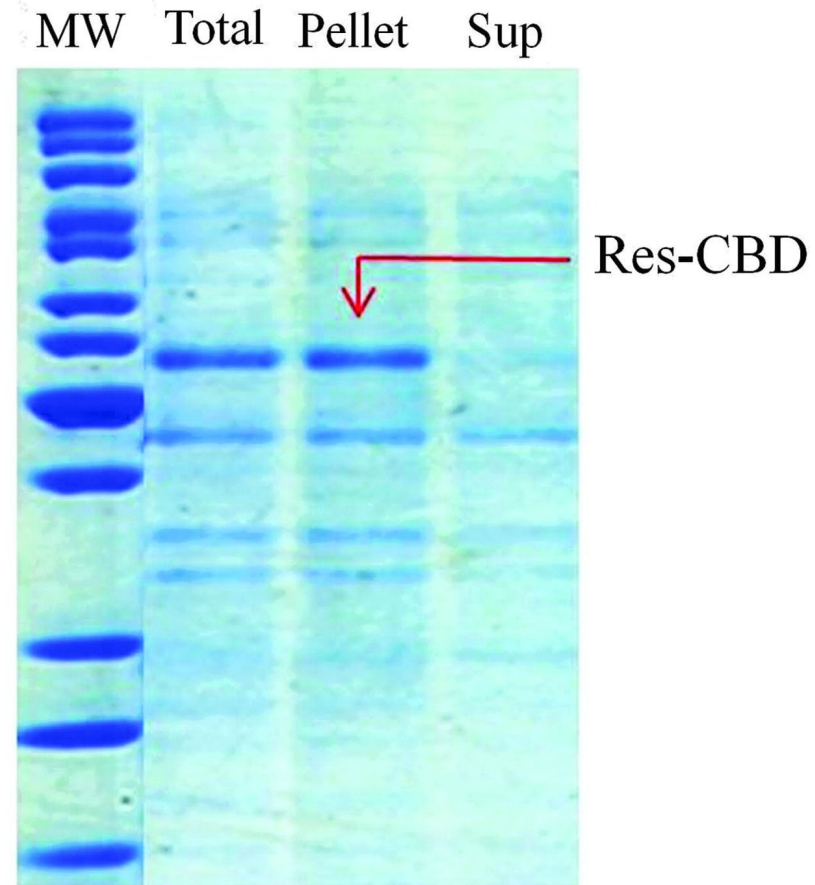
Step 1. Isolation and extraction of res-CBD



“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”

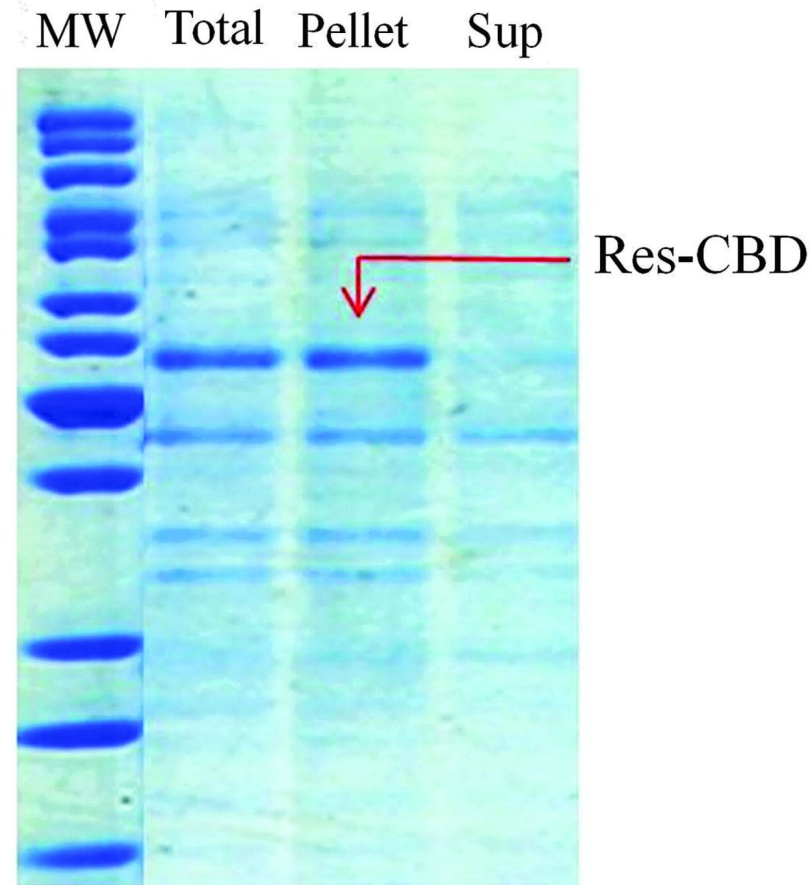
- Figure shows SDS-PAGE of total bacteria - with IPTG induction, a more distinct res-CBD band is present

Step 1. Isolation and extraction of res-CBD

B

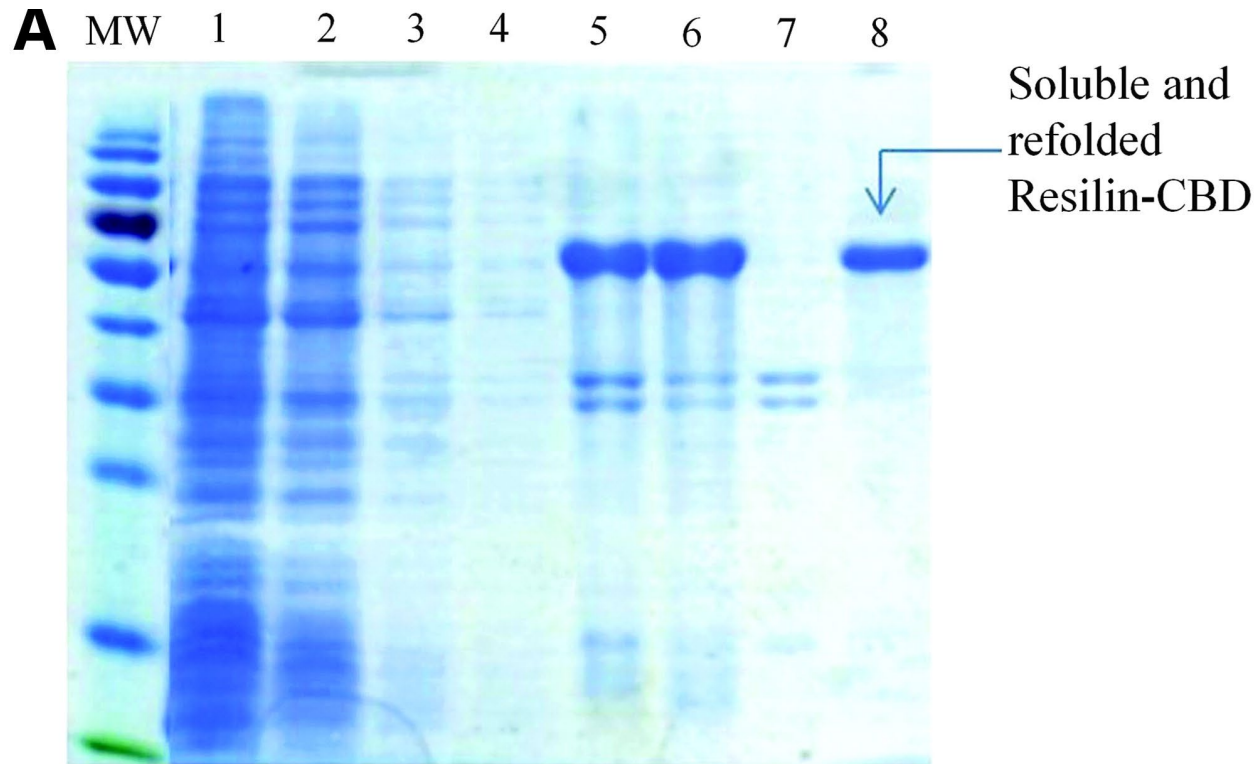
- Bacteria sonicated (lysed) and total, supernatant and pellet were analyzed by SDS-PAGE
- Res-CBD found in pellet, confirming its expression in inclusion bodies
- IB's = particles of aggregated protein (common for recombinant protein)
- So purification is usually 2 steps –1. extraction of IB's and 2.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

Step 1. Isolation and extraction of res-CBD

B

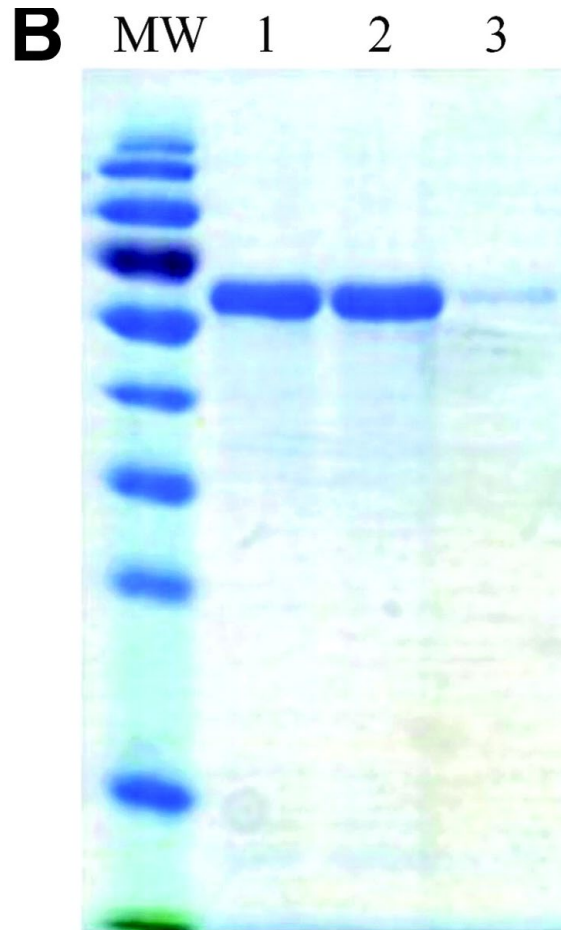
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After solubilization and refolding



- Lanes 1-4: supernatants after IB washing steps (soluble protein is removed leaving only IBs) – (supernatant is depleted in protein with washing)
- Lanes 5-7: Urea dissolved IB's – total, supernatant and pellet (res-CBD is now in the supernatant)
- Lane 8: Supernatant after refolding (looks clean, pretty much only res-CBD)

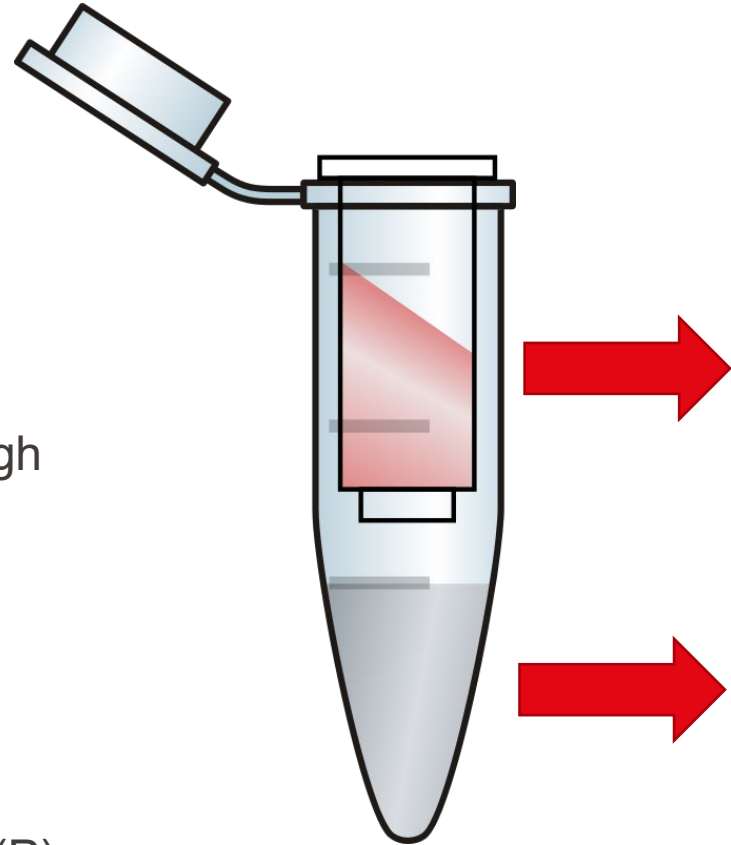
Checking proper refolding



- 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
- 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 the retentate (ultrafiltration)
- Study crude (C) before filtration, and retentate (R) and permeate (P) after filtration

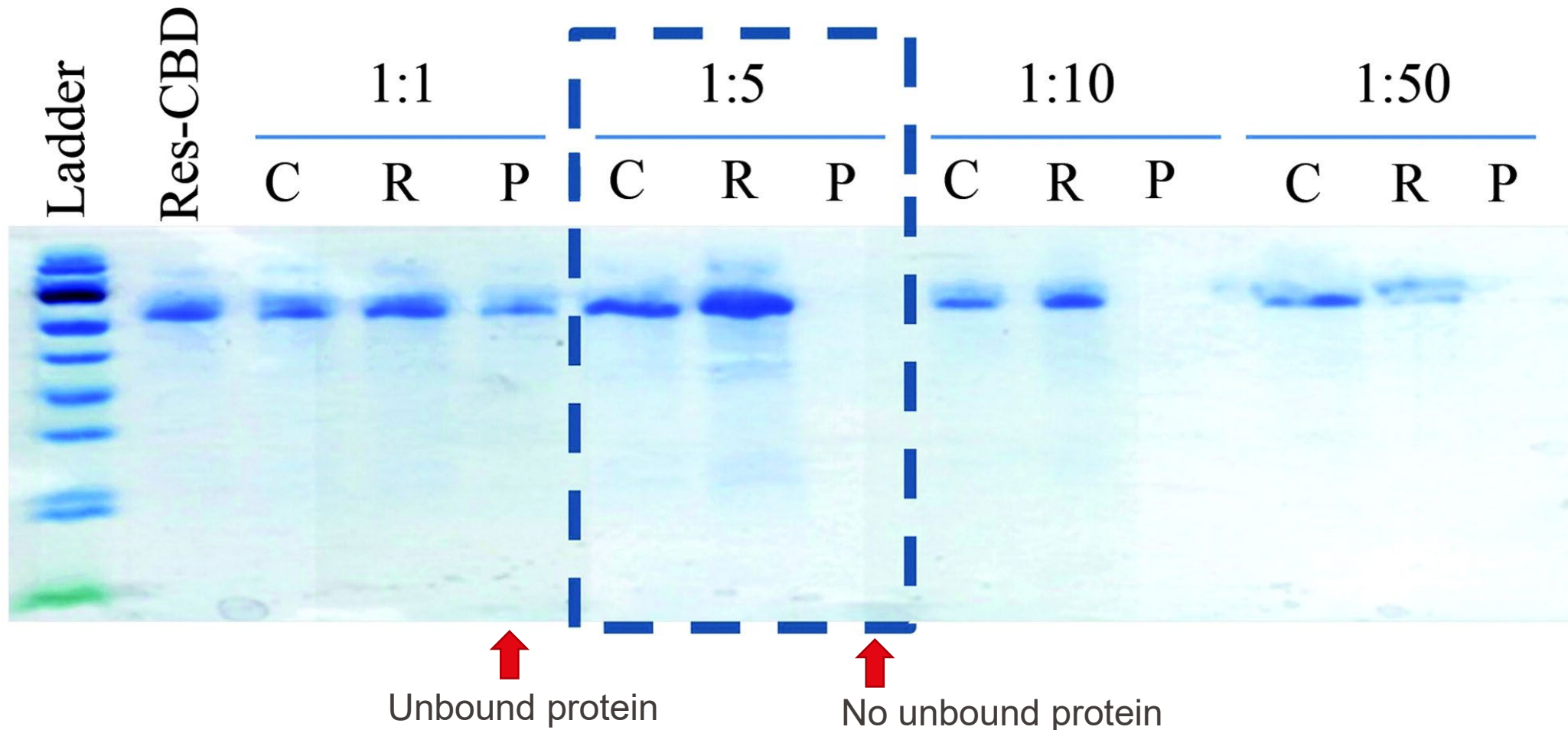


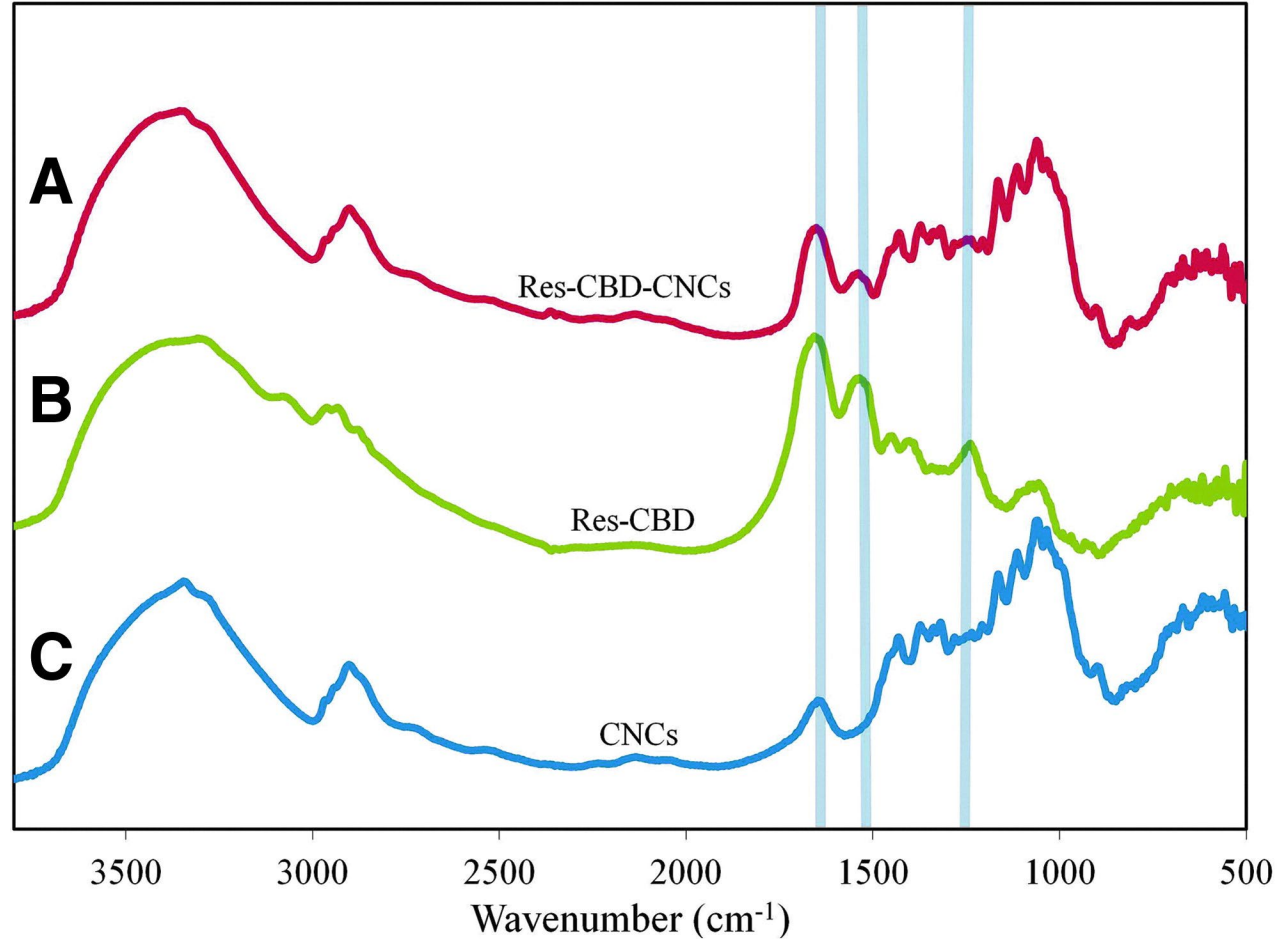
Retentate – CNCs stay here, too big to pass through the membrane

Permeate – Only small molecules like water and unbound protein can pass through

What about binding to cellulose nanocrystals

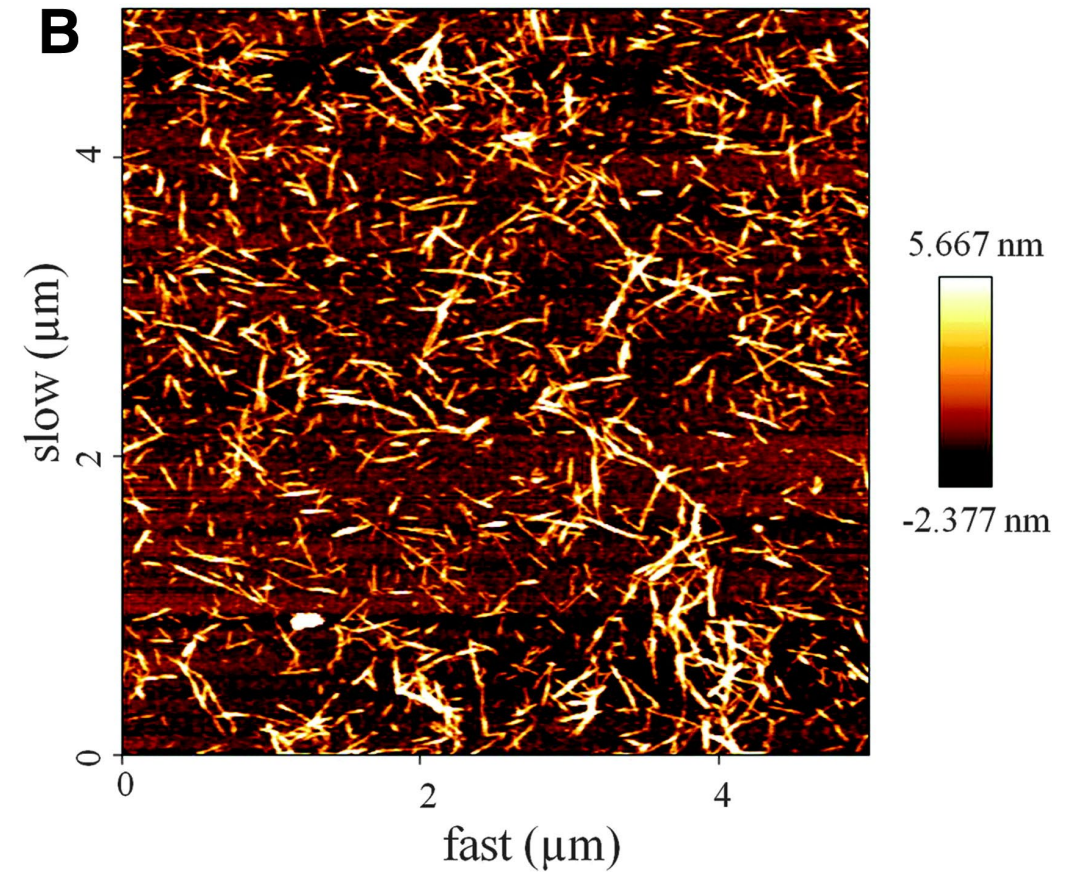
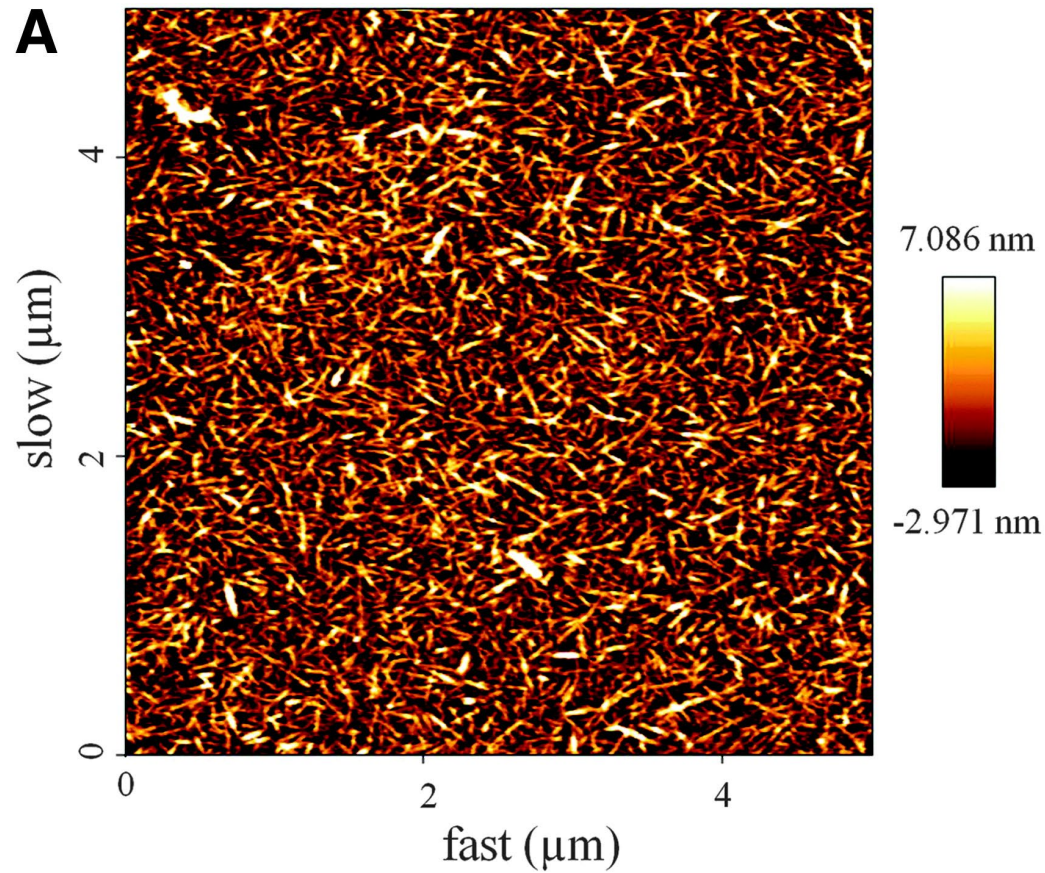
- 1:1 – too much protein, since we have unbound protein in permeate 28
- 1:5 – optimal, no protein in permeate
- 1:10 – the condition that was used for the rest of the study



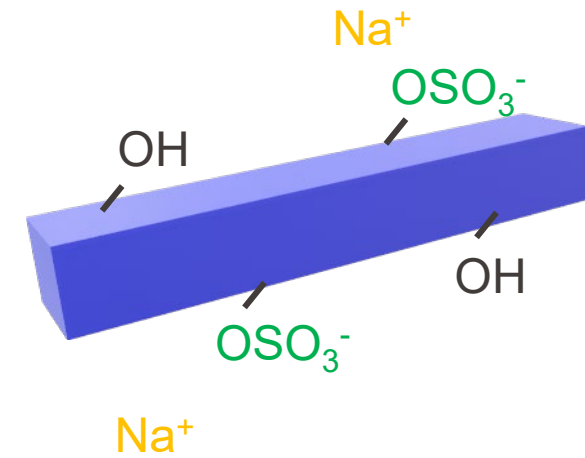


- Bonds related to protein amino acids show up in bound construct... makes sense
- Really looks like a blended spectra

What does the bound construct look like?



What about colloidal properties?



Tiffany Abitbol (tiffany.abitbol@epfl.ch) is signed in	UNBOUND CNCs	RES-CBD-CNCs
%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

A

CNC film



bend

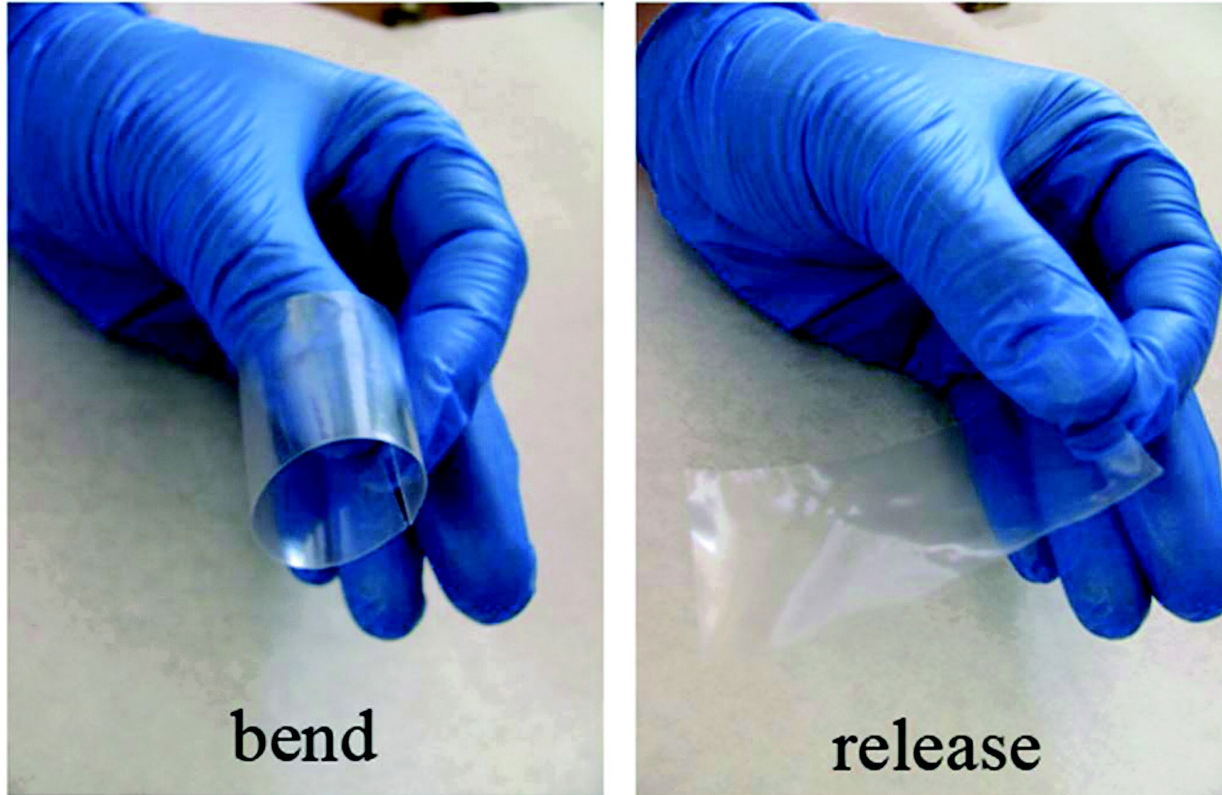


release

- 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

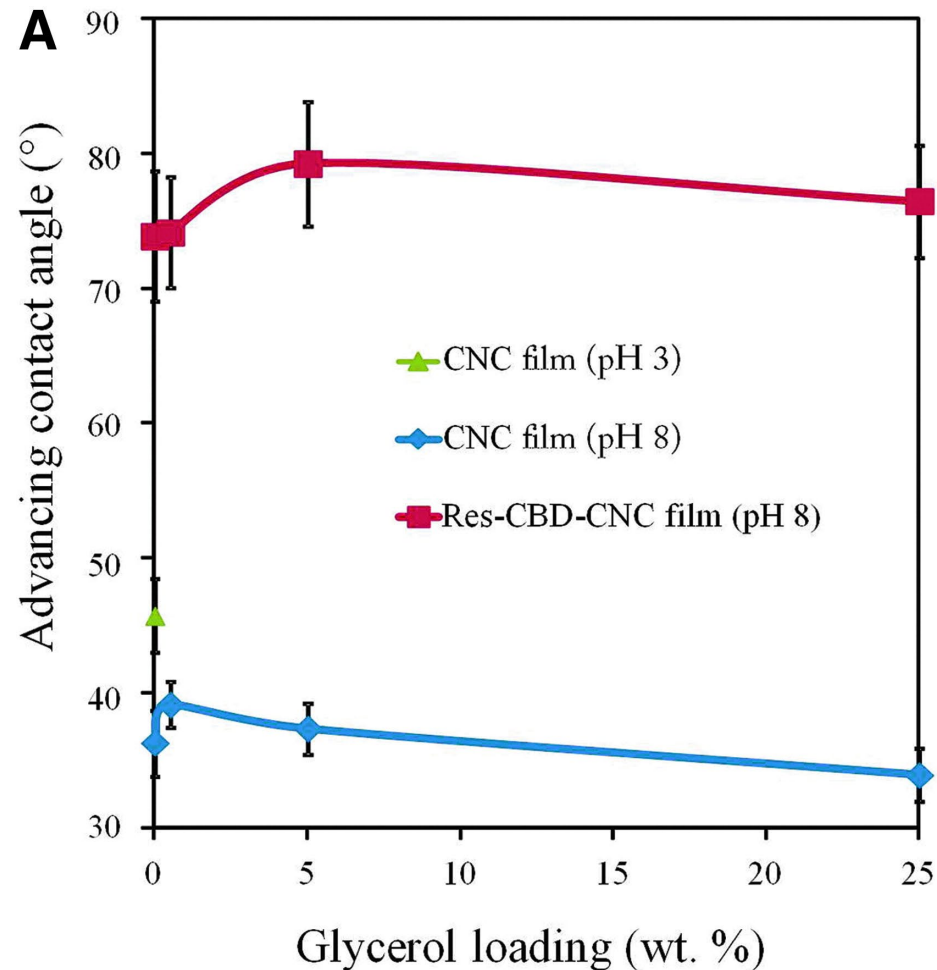
B

Res-CBD-CNC film

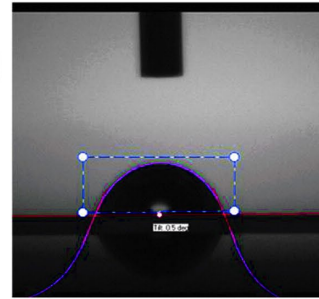


- Point is – the construct film (cast under the same conditions as the film on the previous slide) is more bendy
- Remember – we are just dying to find evidence of resilin elasticity! (We sometimes add glycerol as a plasticizer to make it even more bendy, but not yet)

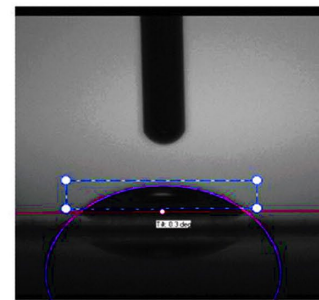
What about surface wetting?



Res-CBD-CNC film

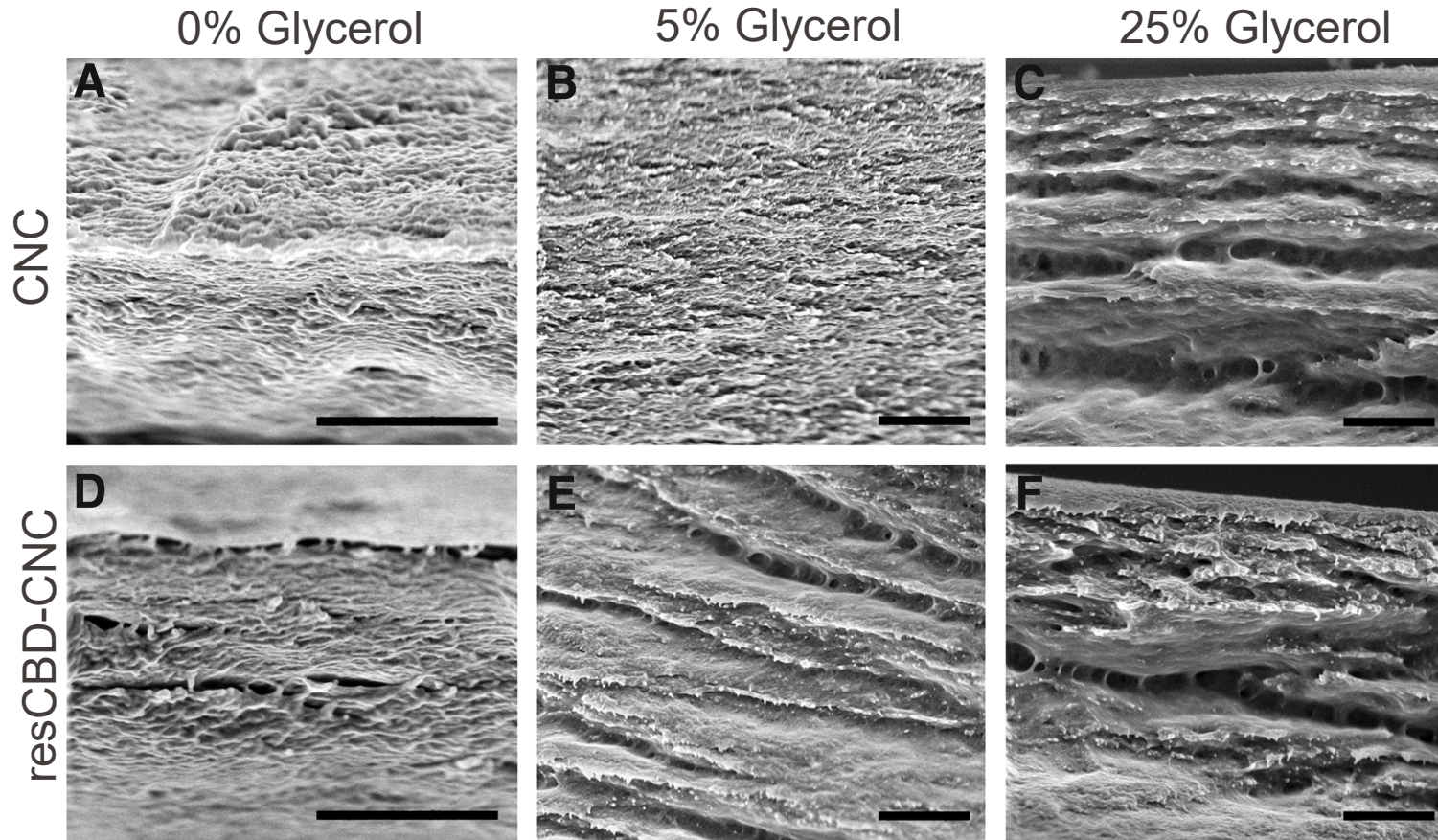


CNC film (pH 8)



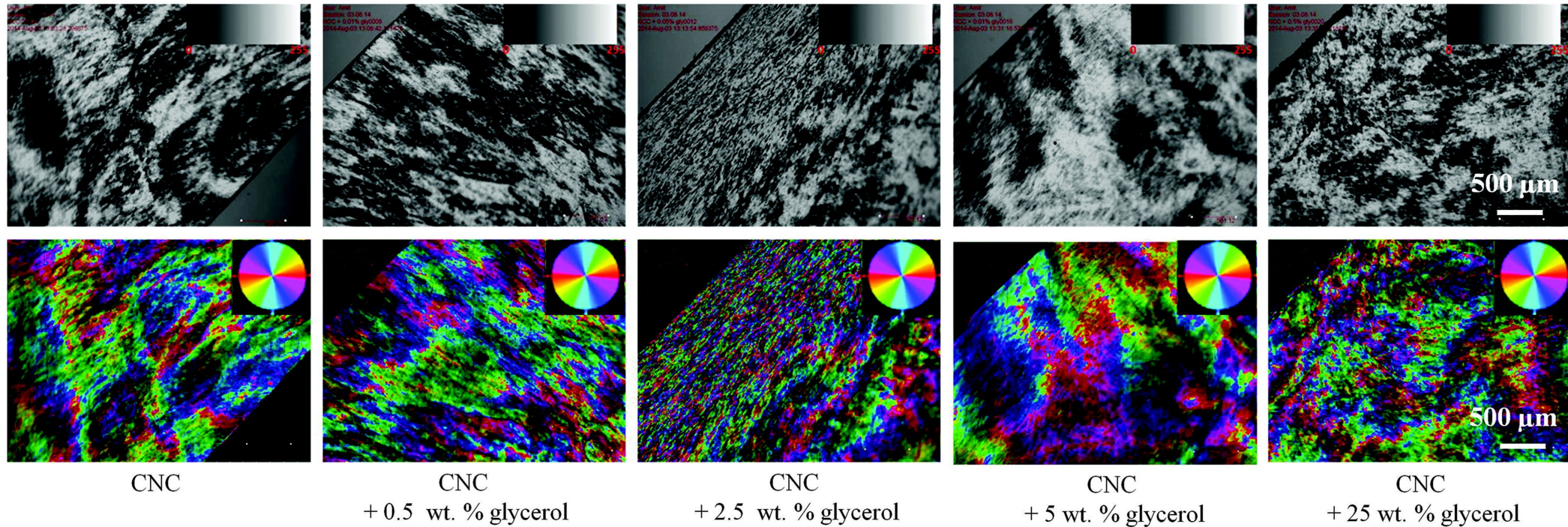
- Hello glycerol = wanted to keep the dried protein hydrated! Helps elasticity!
- Res-CBD films are much more hydrophobic than pure CNC films!
- Something is different!

What's it looks like inside the films?



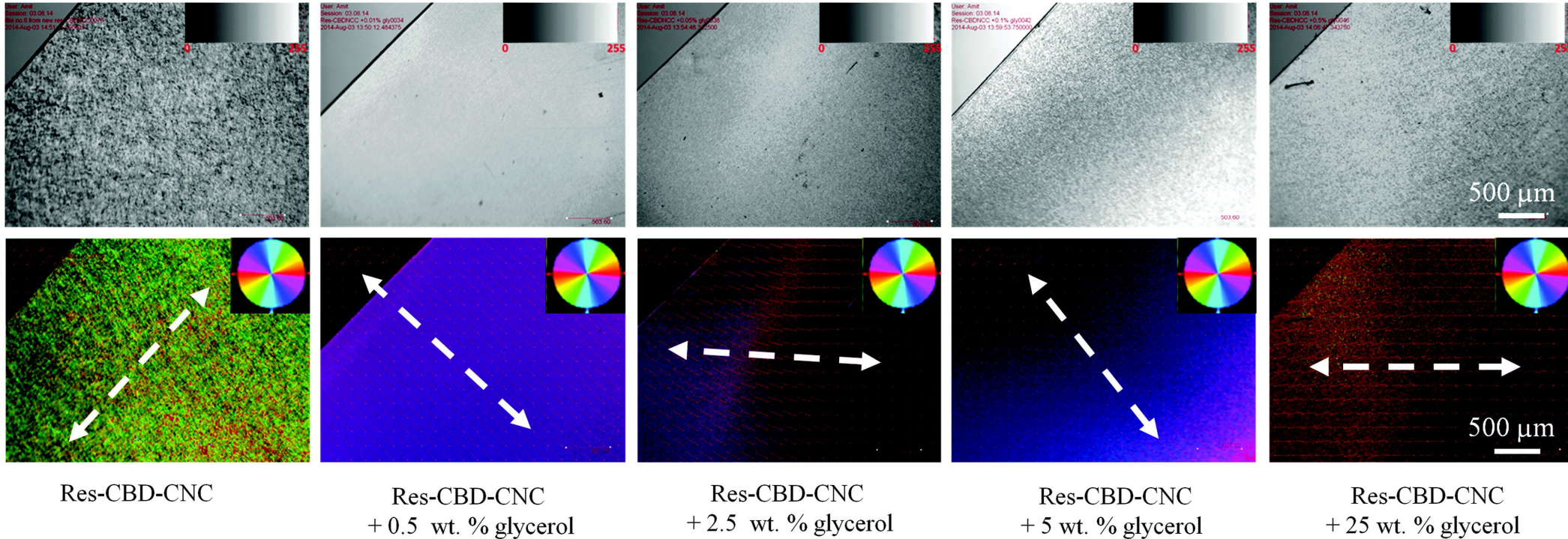
- Cross-sectional SEM
- 500 nm scale bars
- Glycerol films look sticky
- Typical mille-feuille look. Yum!
- Hard to see a big diff with and without protein

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



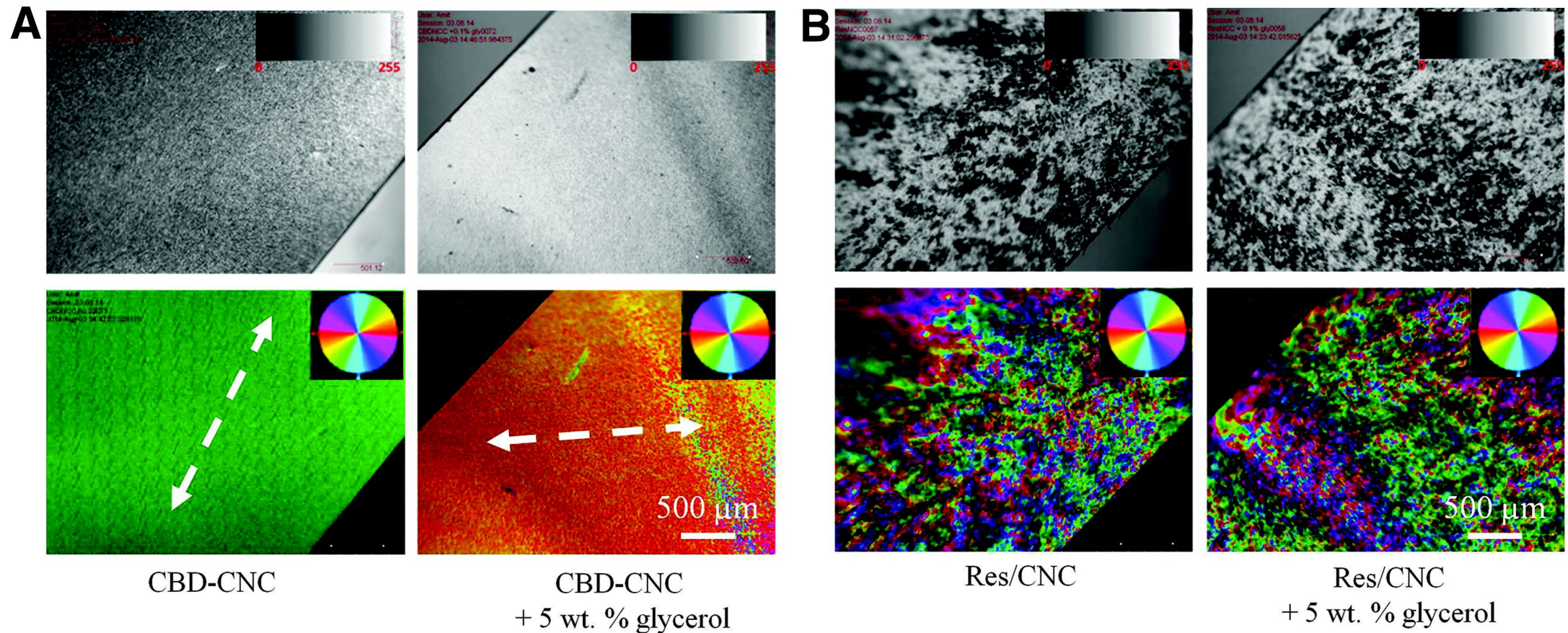
- Fancy polarized optical microscope
- CNC films show lots of different orientations (typical)

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



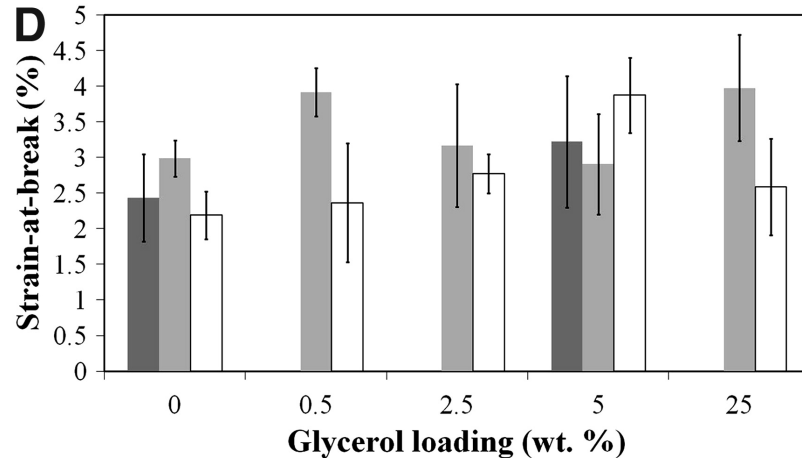
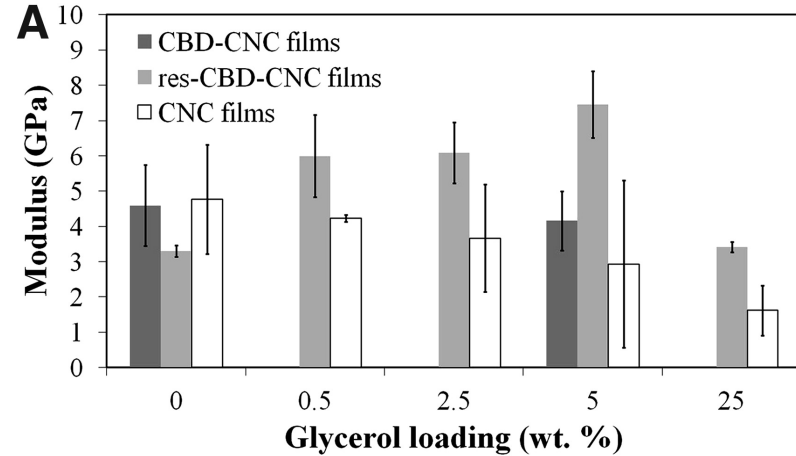
- Fancy polarized optical microscope
- Hmmm... one color means all the rods are pointing in the same direction?

What's going on with the alignment?



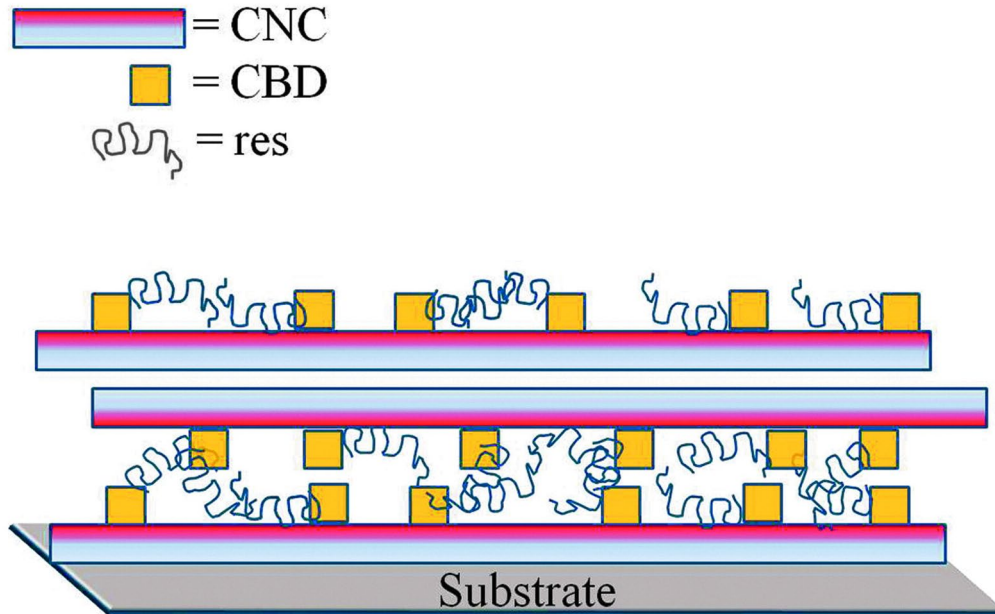
- Seems related to the CBD, not so much the resilin

So are these films strong and elastic?



- Tensile tests
- CNC: lower modulus and higher strain at break with added glycerol (intuitive)
- resCBD-CNC: higher modulus and less clear trend with strain at break with added glycerol (counterintuitive)
- Res seems important for modulus
- @0.5% glycerol – films are indeed strong and elastic
- 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

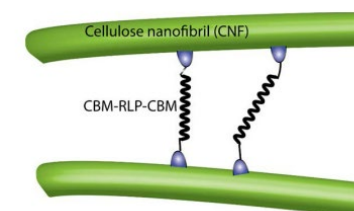
Department of Bioproducts and Biosystems

Research output: Thesis > Doctoral Thesis > Collection of Articles

Overview

Fingerprint

Infrastructure (2)



Reading options

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Article

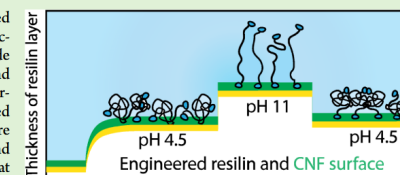
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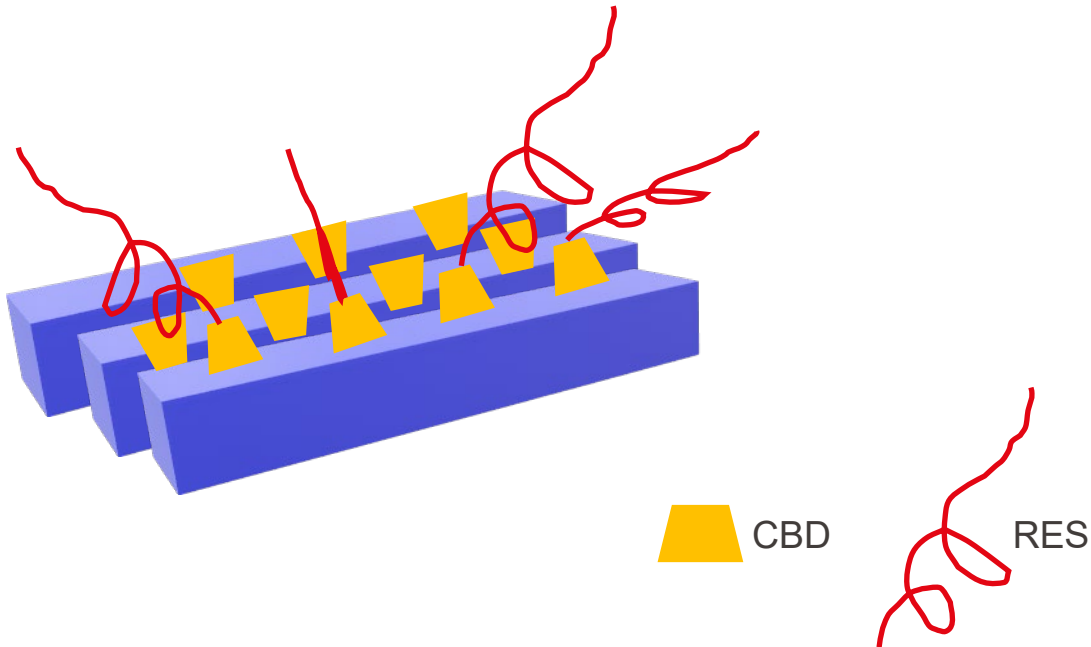
Elastic and pH-Responsive Hybrid Interfaces Created with Engineered Resilin and Nanocellulose

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^{*,†} [†]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.



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)
- 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?