Combining CNCs and silk proteins: Revealing interactions and alignment in shear

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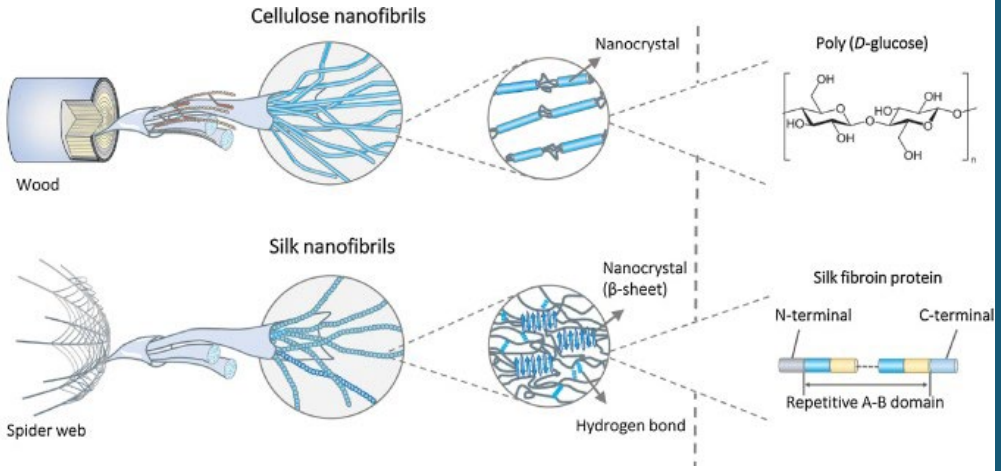
Combining properties of ordered CNC and mobile silk proteins

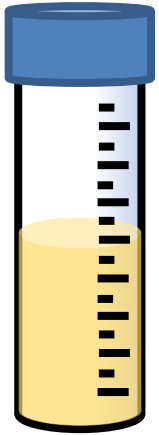


Objectives

- To explore their suitability for filament formation
  - Tuning and characterizing the attractive interactions between CNCs and silk proteins
  - Studying the shear-induced alignment of CNC and silk fibroins to promote the alignment in shear
- Preliminary spinning demonstration
  - Dry-spinning in aqueous conditions, no coagulant baths, ambient temperature and minimal energy
  - Exploit inherent features of CNCs and regenerated silk fibroin protein
    - Rigid ordered domains from cellulose
    - Soft aligned domains from silk

Materials

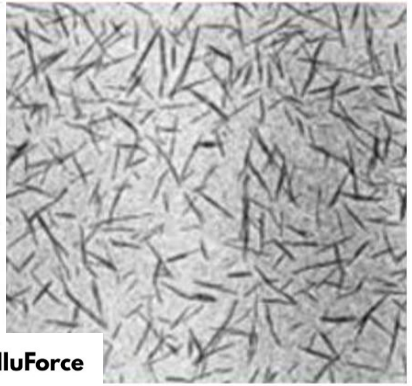
Regenerated silk fibroin (RSF)



Oxford Biomaterials Limited

- Produced from *Bombyx mori* silk skeins
- Degummed
- Dissolved in dissolving agent
- Softness and strain
- Regenerated silk fibroin (RSF)

Cellulose nanocrystals (CNC)



- CNCs from Celluforce
- Prepared by sulfuric acid hydrolysis
- 254 mmol/kg surface $-OSO_3^-$ groups
- Diameter ~4 nm
- Dispersed in wanted medium
- Order and rigidity

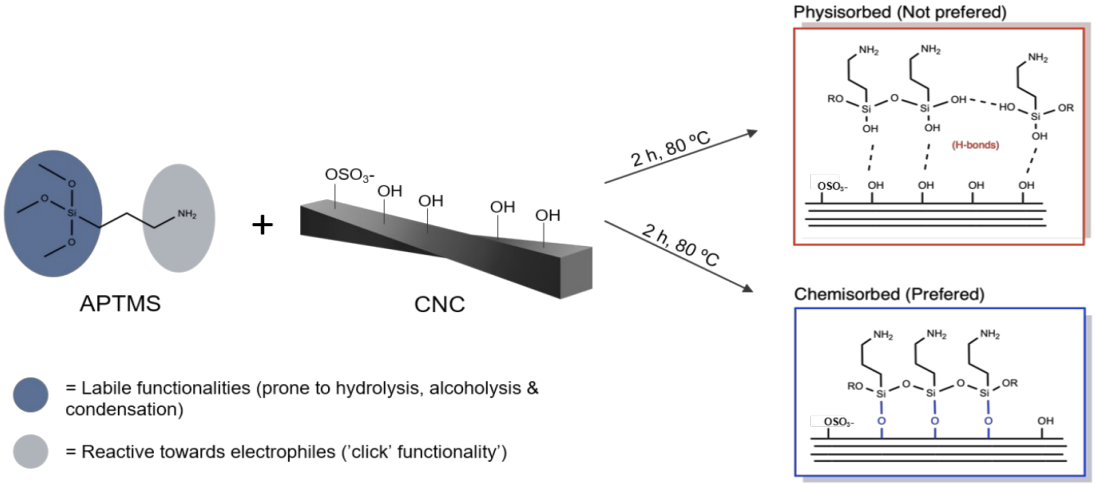
INTRODUCTION OF AMINOSILANES ON CNCs

Surface amination of cellulose nanocrystals (CNCs)

- Purification of CNCs by Soxhlet extraction to remove carbonaceous contaminants from the CNC surface

- Amine modification of CNCs
  - Tune the attractive interactions between CNCs and silk
  - Further functionalization of the CNCs with RSF via bioconjugation

- Various dispersion systems and conditions tested
  - The highest DS_{surf} of ~0.2
    - 1 wt% CNC (Soxhlet purified) in DMAc with the addition of 1 w/w-% LiCl

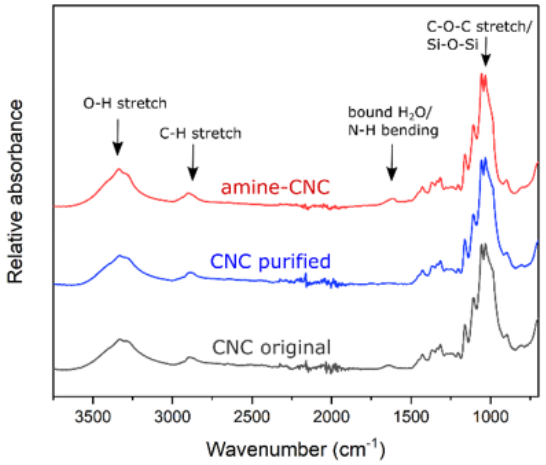


Peresin M. S. et al., *Carbohydr. Polym*. **2017**, 174: 309-317
Arola, S. et al. *Biomacromolecules* **2012,** *13* (3), 594-603.

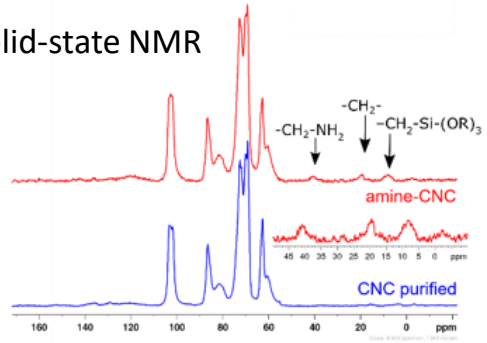
Chemical analysis of the amine-CNCs

- Fourier transform infrared spectroscopy (FT-IR)
  - Tentative information on the successful reaction
- Elemental analysis
  - Quantitative analysis of elemental composition (N, C, H, S) of samples, degree of substitution (DS)
- Solid-state NMR (^{13}C CP/MAS NMR)
  - Analysis of aliphatic signals form APTMS, cellulose I form retained
- X-Ray Photoelectron Spectroscopy (XPS)
  - Surface sensitive quantitative spectroscopic technique that measures the elemental composition on the topmost surface of CNC (C, N, O, S, Si), DSS degree of surface substitution
- Liquid-state NMR
  - Support for covalent attachment of the APTMS moiety

ATR-FTIR

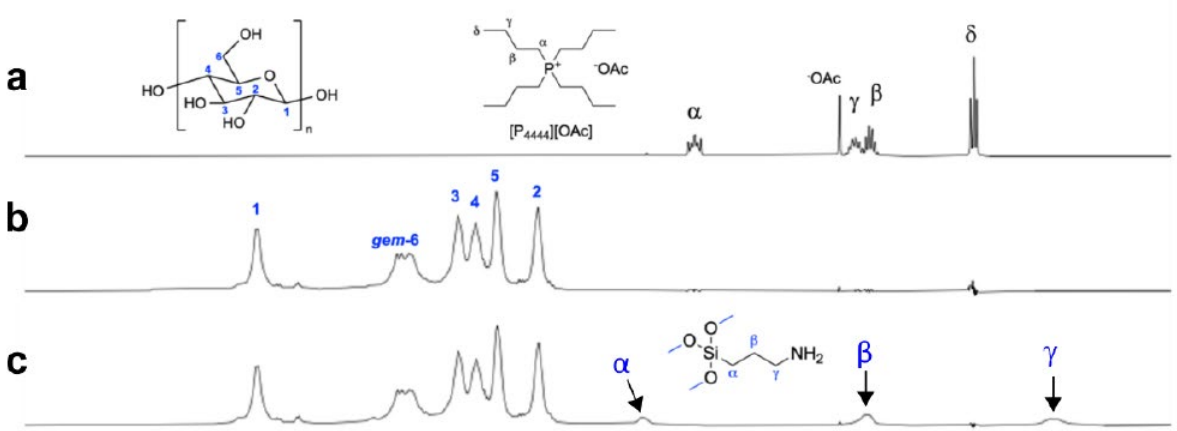


Solid-state NMR



Support for covalent binding of APTMS

- **Diffusion-edited ^{1}H NMR**

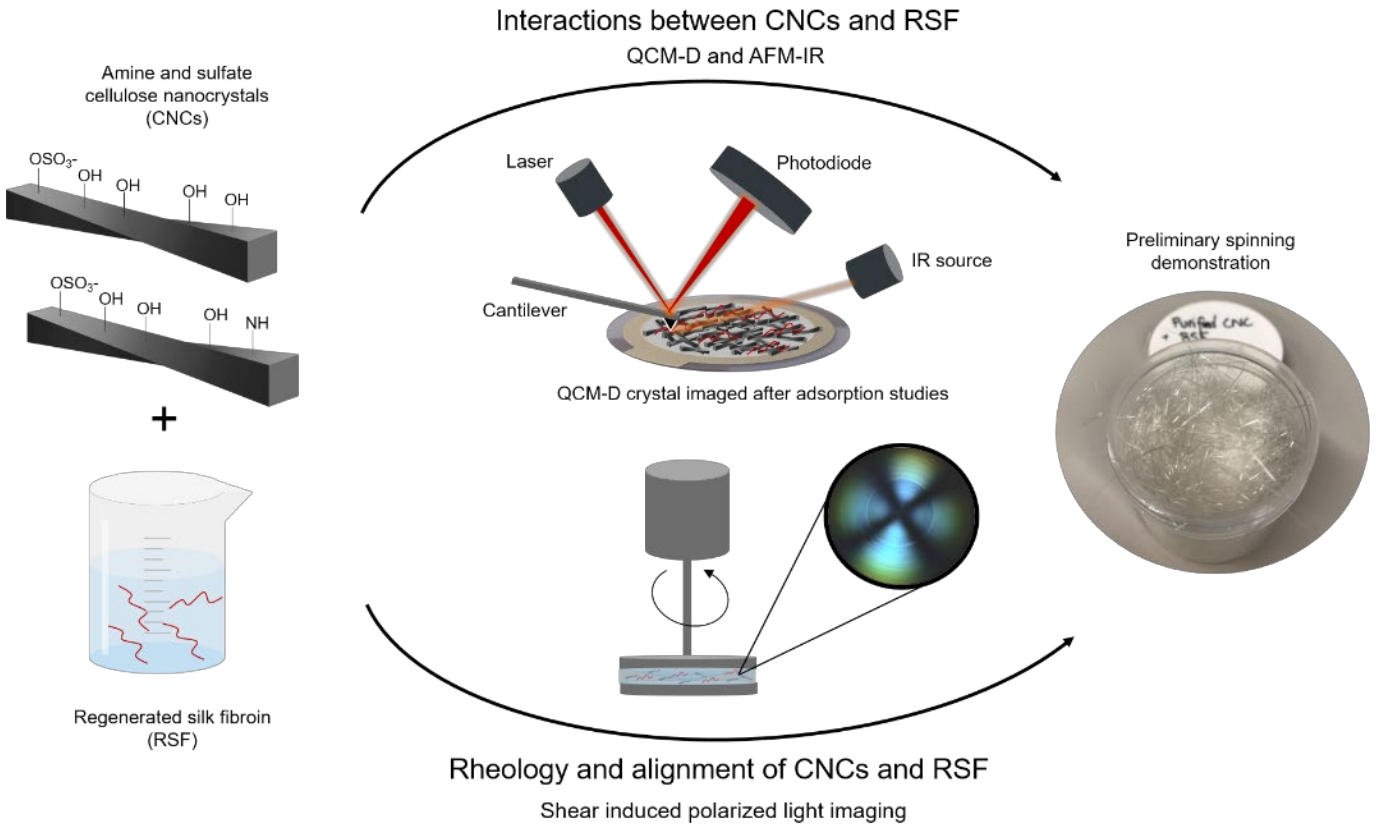


- Technique edits out the slow-diffusing species originating from solvent: only chemisorbed signals retained in the spectrum
- Clear alkyl resonances retained evidencing covalent binding

King, A. et al. *Biomacromolecules*, **2018**, *19*, 2708.

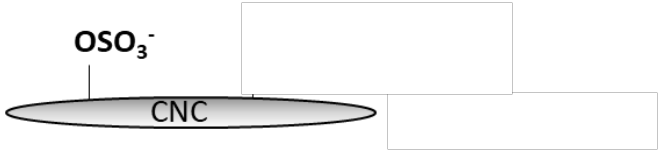
INTERACTIONS BETWEEN
CNCs AND SILK

Approach and the methodology

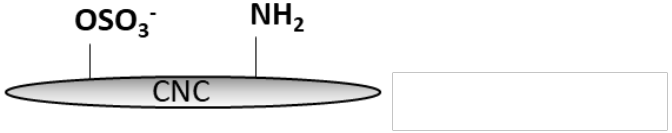


Interactions between CNCs and silk (RSF) – QCM-D

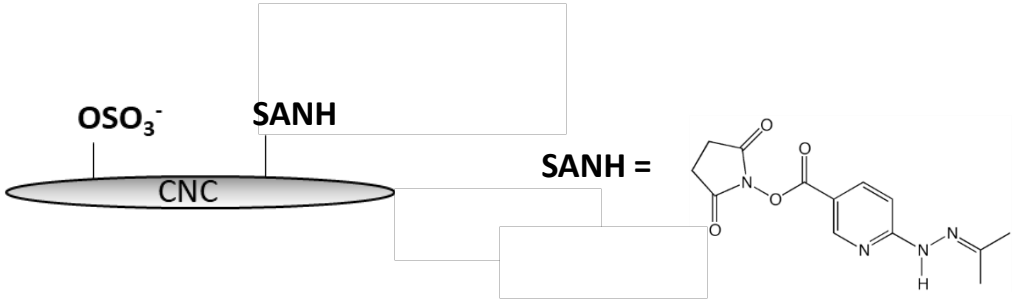
- Sulfated CNCs → ionic interactions



- Amine-CNCs (DS:0.2) → ionic interactions

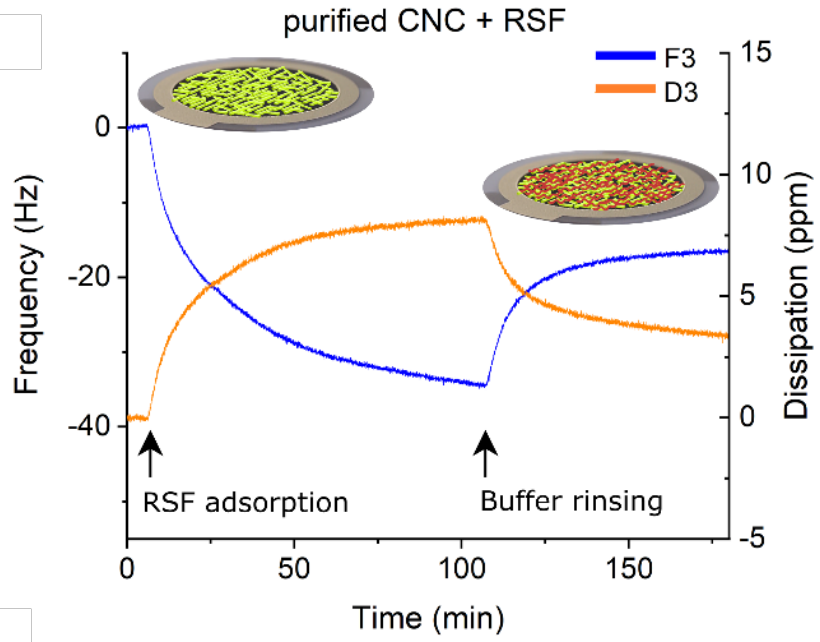


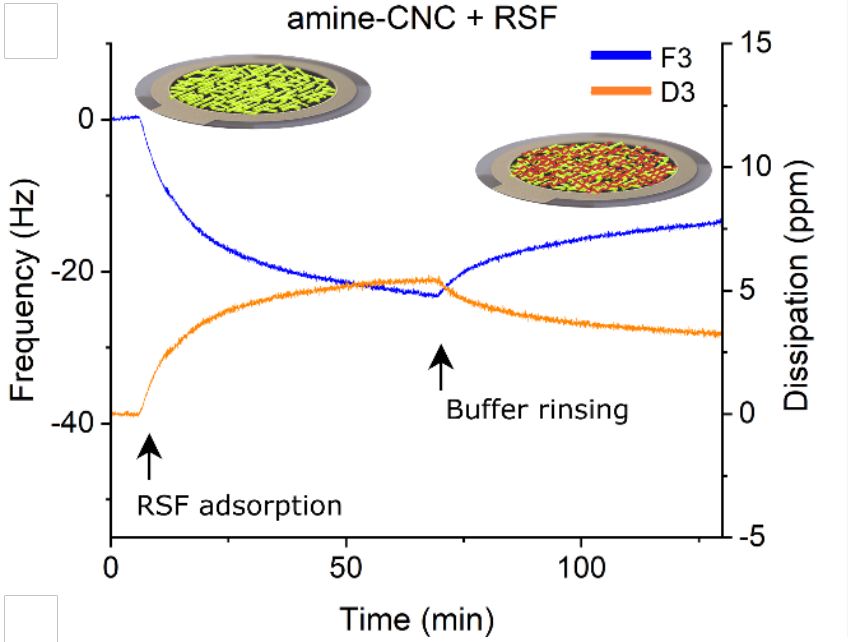
- SANH-modified amine-CNC → covalent crosslinking via amine-amine coupling



- **QCM-D** – Quartz crystal microbalance with dissipation monitoring
  - To reveal silk **adsorption** on CNC surfaces
  - How does the introduction of an amine group affect adsorption?
  - Is there any benefit of covalent coupling?
  - Are attractive physico-chemical interactions enough?

Interactions between CNCs and silk (RSF) – QCM-D





Interactions between CNCs and silk (RSF) – AFM and photothermal AFM-IR

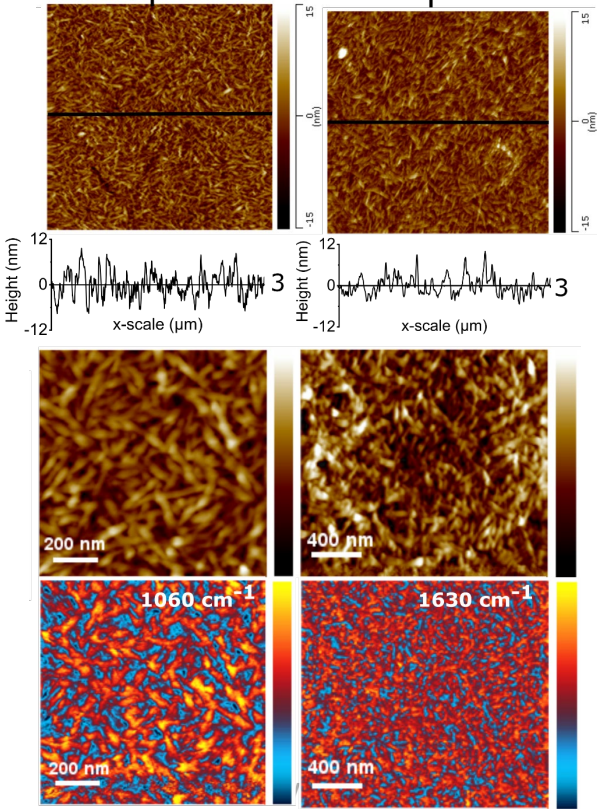
VTT

BRUKER

CNC

Before RSF adsorption

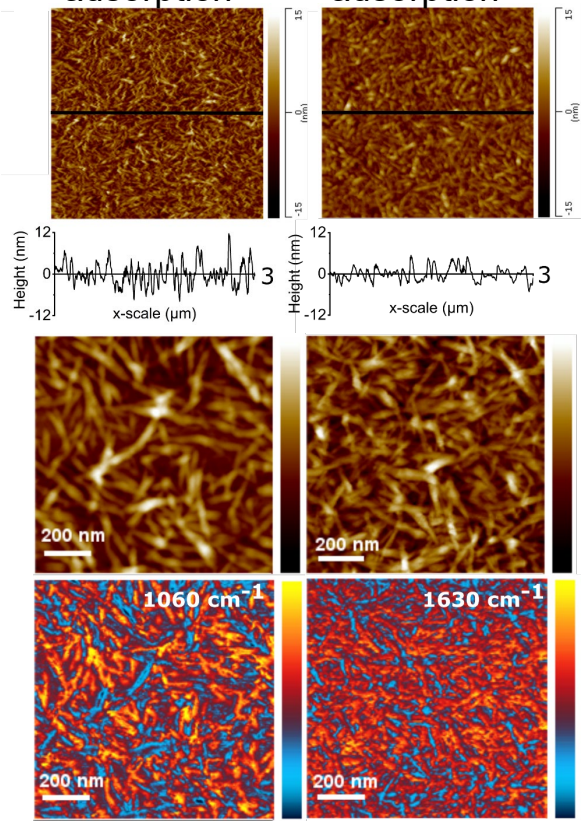
After RSF adsorption



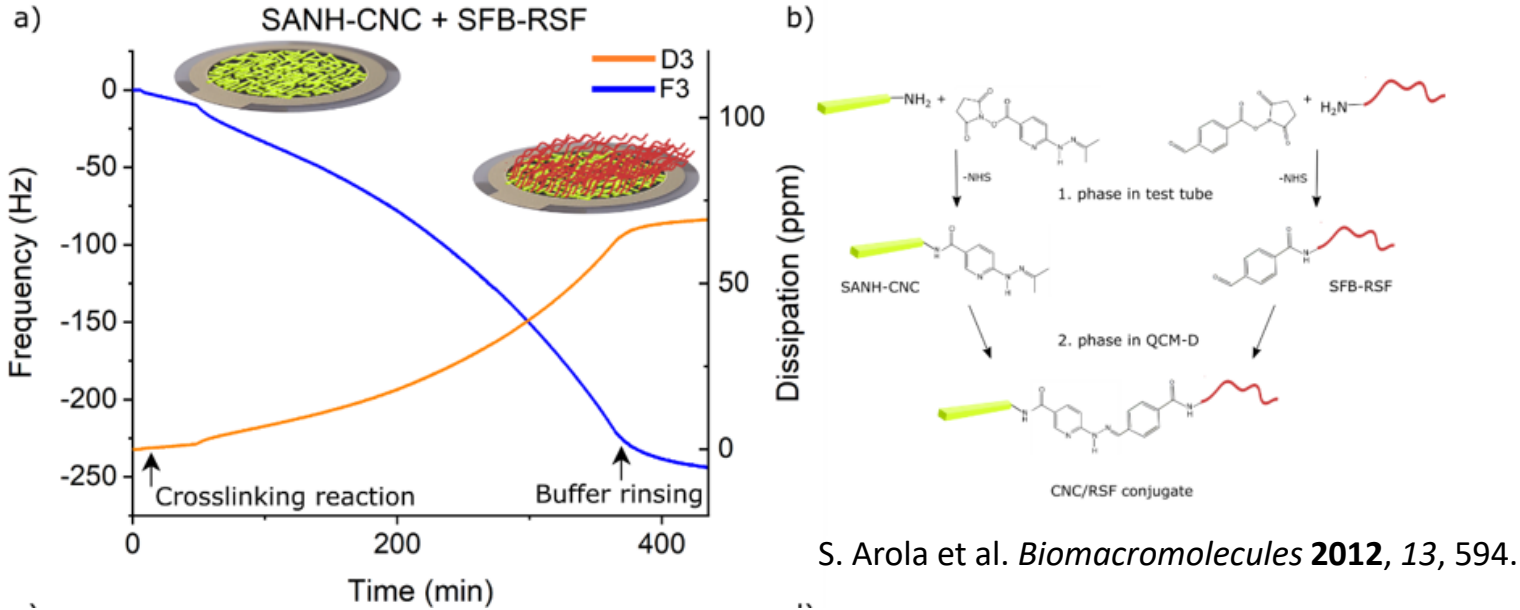
amine-CNC

Before RSF adsorption

After RSF adsorption



Interactions between CNCs and silk – Bioconjugation

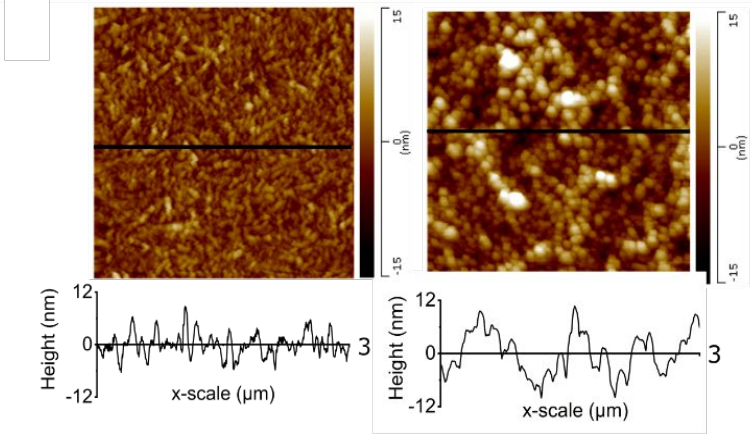


S. Arola et al. *Biomacromolecules* **2012**, *13*, 594.

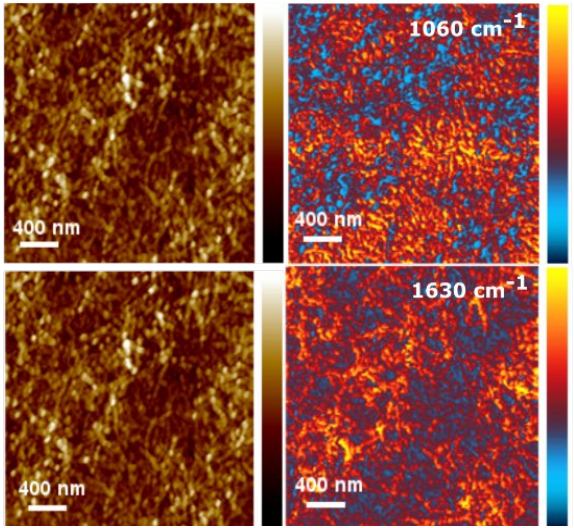
- SANH-CNC surfaces spincoated from 0.1 w/w-% solution on QCM-D crystal
- Surface stabilized in phosphate buffer (10 mM pH 7) for 2 min
- 0.1 mg/ml SFB-RSF in phosphate buffer circulated through QCM-D cell

Bioconjugation of CNCs and silk (RSF) – AFM and photothermal AFM-IR

Before and after bioconjugation



AFM-IR maps

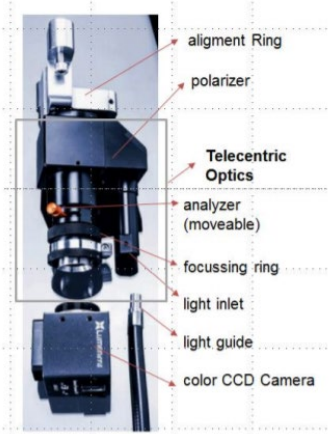


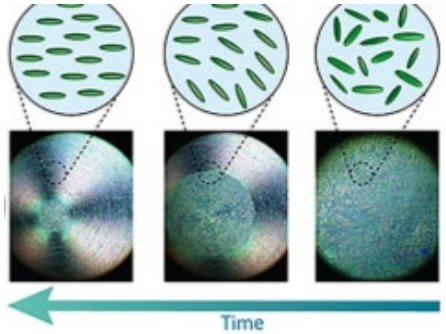
Cellulose assignment (1060 cm^{-1}): blue area -> proteins
Silk fibroin assignment (1630 cm^{-1}): blue area -> cellulose

Rheology – to reveal possible flow induced alignment

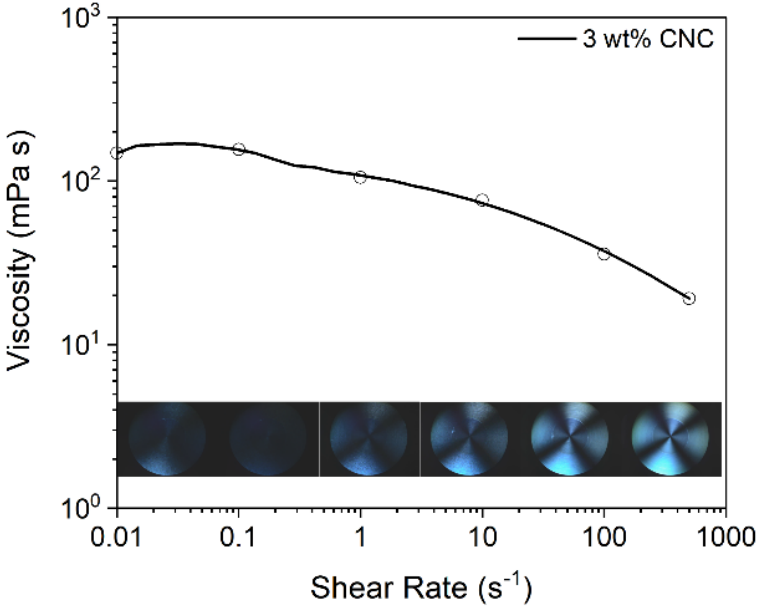
- Why rheology measurements?
  - From surface interactions to bulk behaviour
  - Possible to qualify & visualize the alignment

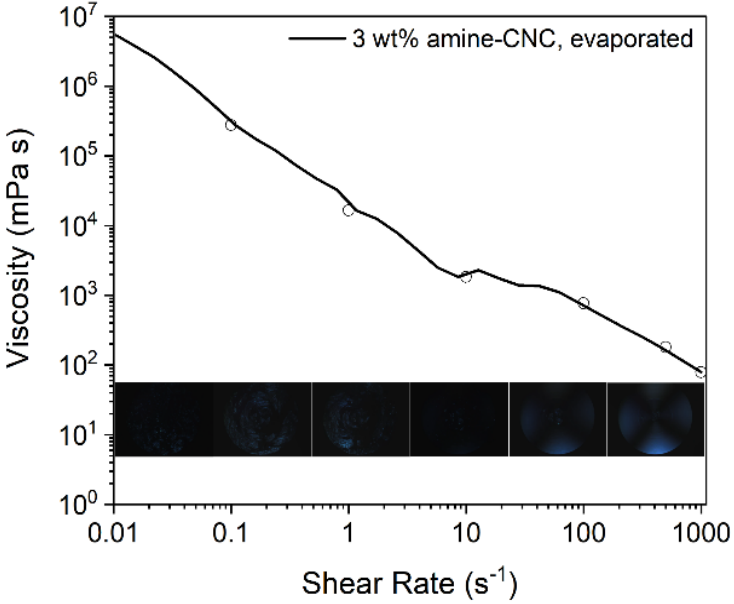
- Anton Paar MCR302
  - Cone & plate geometry (50 mm)
  - Steady-state shear viscosity measurements
  - **Shear induced polarized light imaging (SIPLI)**
    - Cross polarization
    - Detection of birefringence
      - appearance of a Maltese cross





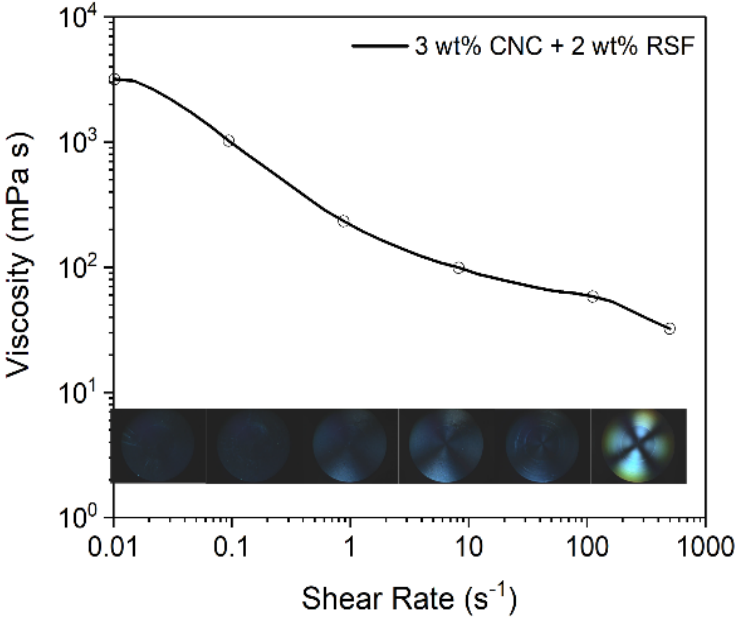
Rheology – Viscosity and SIPLI imaging of CNCs

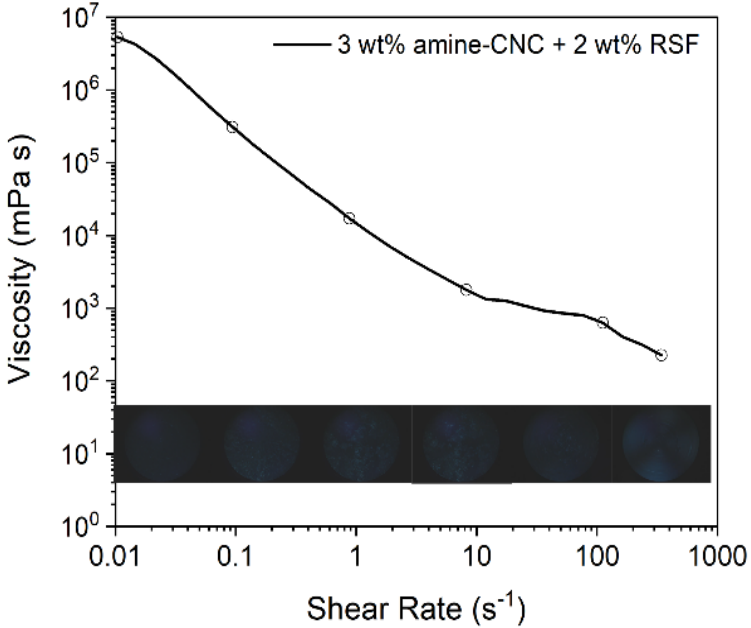




- CNC: Clear alignment of liquid crystals
- Amine-CNC: viscosity significantly increased (1000x) and a weak/medium birefringence detected depending on the concentration and sample preparation history

Rheology – Viscosity and SIPLI imaging of CNCs and silk (RSF)



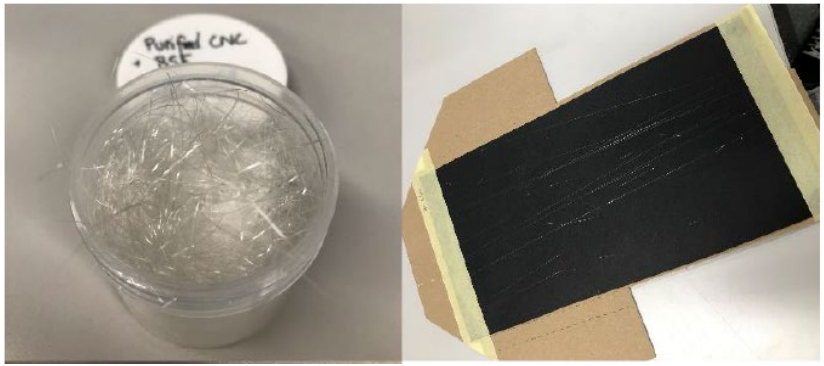


- Birefringence and shear-induced alignment retained even in the presence of RSF
- Pure RSF does not show birefringence and bioconjugation induced significant clustering
- Silk can orientate with CNCs when NOT covalently bound

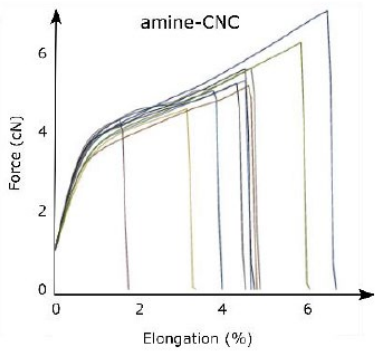
DRY-SPINNING OF FILAMENTS

Preliminary test for filament dry-spinning

CNC + RSF filaments



100% amine-CNC filaments



- Indirect evidence on crystal crosslinking between amine-CNCs
- How to directly prove the chemistry involved?

Spinnova
The Sustainable Fibre Company.

Conclusions – Parameters for bioinspired filament formation

- Filament formation takes place when the interactions between highly ordered CNCs and soft silk proteins are attractive (induced by electrostatic interactions) and clear alignment under shear is existing

Sample	Interaction	Alignment under shear	Filament dry-spinning
100% amine-CNC	attractive (rheology & XPS)	Medium	successful
CNC + RSF	attractive (QCM-D)	strong	successful
amine-CNC + RSF	attractive (QCM-D, rheology)	weak	challenging
SANH-CNC + SFB-RSF	Highly attractive (QCM-D)	clustering, no alignment	not possible

Thank you!

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