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Morphology of native cellulose: the microfibril

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CHEM-E2140 Cellulose-based fibres

Recap from the previous lecture: assignment

What is hemicellulose?

- **Plant polysaccharides that are not cellulose or pectin**

Why does hemicellulose exist?

- **Not entirely sure**
- **Partially controls the water content**
- **Forms biomechanical hotspots with cellulose microfibrils**

What are the differences between different hemicelluloses?

- **Composition and arrangement of monosaccharides**

Assignment

Take a couple of minutes and write down a couple of bullet points to the query *Before the lecture*

Learning outcomes

After this lecture, the student will be able to:

- Describe how cellulose occurs in nature (in microfibrils)
- Possess command on basic facts about microfibrils
- Spot the gaps in knowledge of cellulose microfibrils
- Be aware of the disputes concerning microfibrils

Outline

(1) Facts about native cellulose microfibrils (CMFs)
- Quick view on biosynthesis
- Relatively undisputed facts about CMFs

(2) Disputed issues about CMFs
- Width of CMFs
- Number of chains in a CMF
- Twist along the CMF
- Longitudinal disorder: fringed fibrillar model and levelling off DP

(3) Bundling of CMFs

What do we know about the cellulose microfibril?

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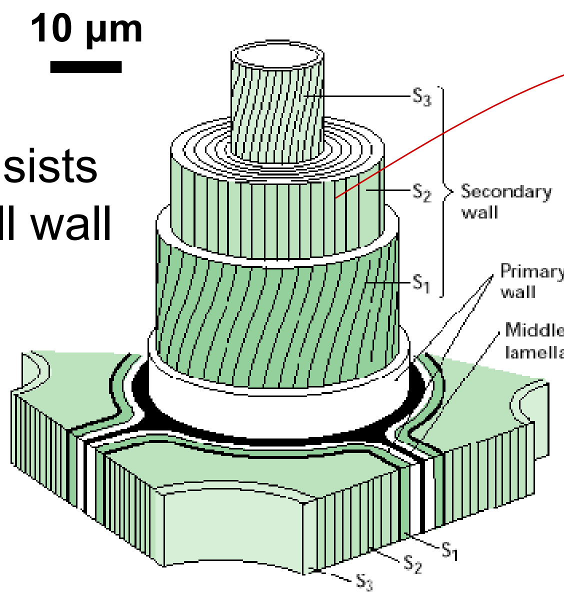
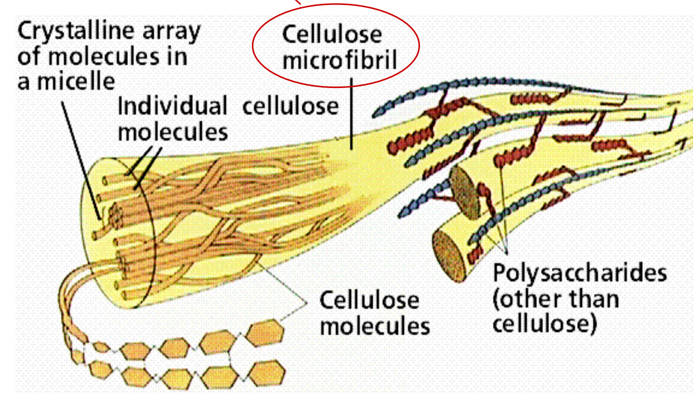
Cellulose microfibril in the cell wall

The cell walls of all plant fibres are reinforced by cellulose microfibrils

Wood fibre consists of a layered cell wall matrix

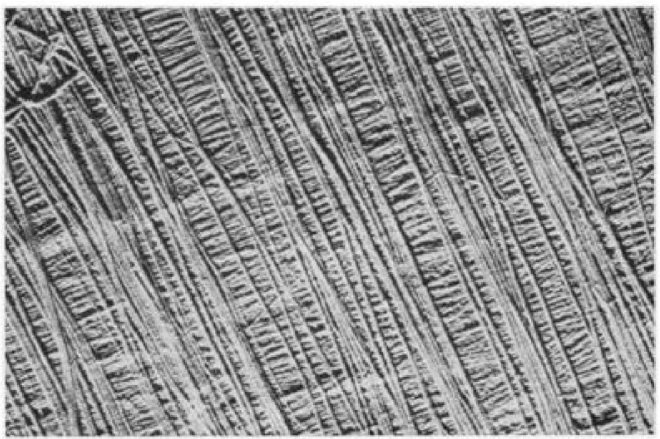


Cellulose microfibril
Diameter: **3-20 nm**
(In wood: 3-4 nm)

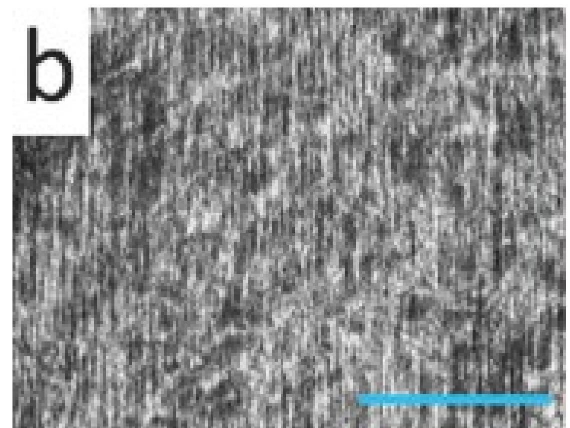


Appearance of microfibrils

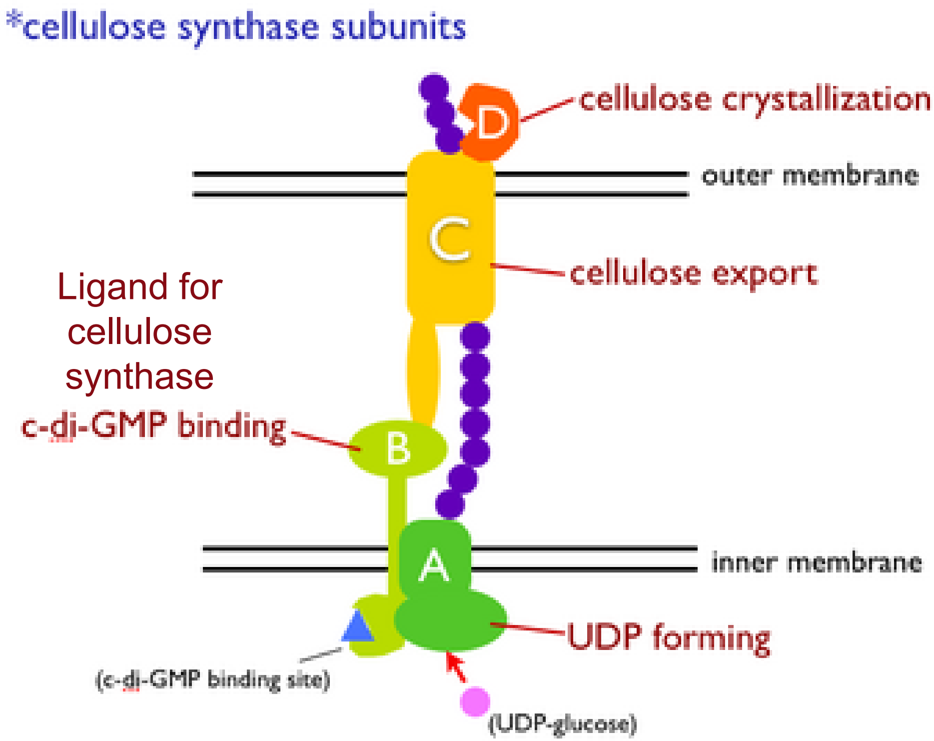
Algal microfibrils ~20 nm width

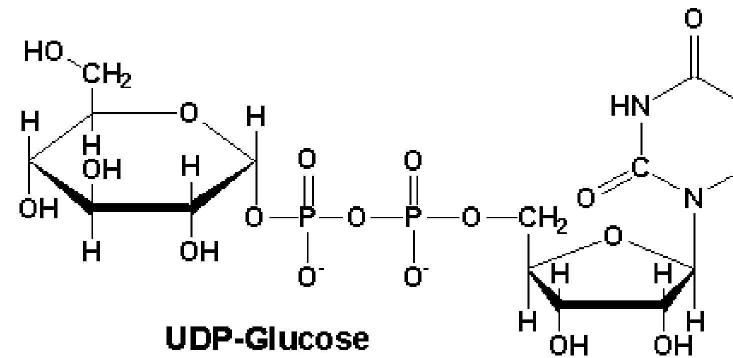


Ramie microfibrils ~6-7 nm width



Biosynthesis of cellulose microfibril

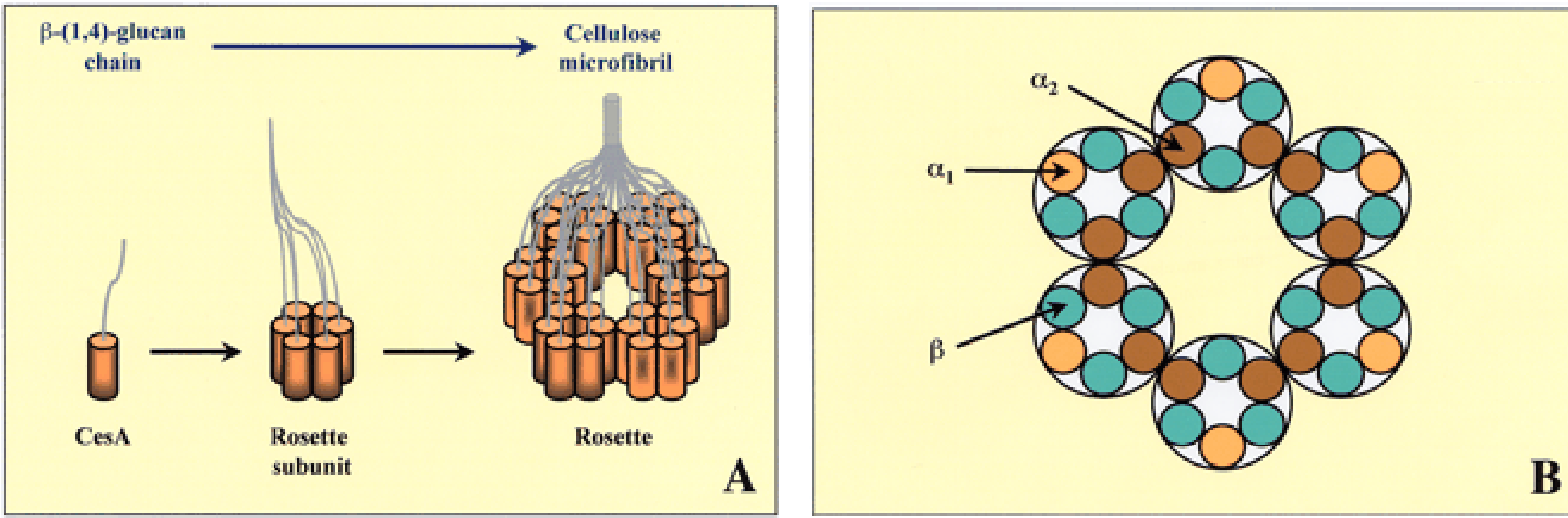




UDP-Glucose

Cellulose synthase: a rosette

- Cellulose synthase (CesA) complex is called a *rosette*
- 6 CesA units form a rosette subunit, 6 subunits form a complete rosette



- Each CesA synthesizes one cellulose chain
- 6×6 rosette is held as circumstantial evidence for 6×6 chain model for the cellulose microfibril

Major implication of cellulose biosynthesis

- Cellulose crystallizes as it synthesizes
- Native cellulose is *always* in the form of microfibrils
- There are no individual chains of cellulose in nature
- There is no amorphous cellulose in nature

Relatively undisputed facts on native microfibrils

- Smallest supramolecular unit of cellulose in the plant cell wall
- Monodisperse width (nm range)
- The width depends on the botanical source
- Very long (µm range, owing to high DP of native cellulose)

Presemo

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https://presemo.aalto.fi/e2140microfibril

Disputed issues about cellulose microfibril (CMF)

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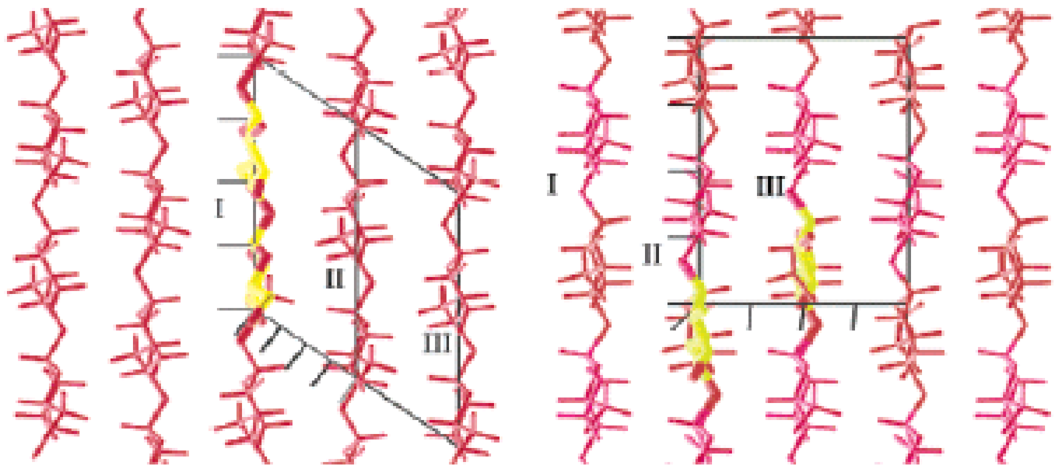
Cellulose microfibril (CMF) – major controversies

- Width of the CMF
- How many chains make up the CMF
- Twist (chirality) along the CMF
- Longitudinal disorder: the fringed-fibrillar model

Unit cell vs. crystallite width



I_α: one chain triclinic I_β: two chain monoclinic

Crystallographic details in 1Å resolution (cellulose I_α ja I_β):

Nishiyama et al.
J. Am. Chem. Soc. **2002**, *124*, 9074.
J. Am. Chem. Soc. **2003**, *125*, 14300.

In general, the unit cell of crystalline cellulose is recognized and agreed upon but the width of the crystallite and general morphology of the microfibril is still elusive

Note on terms

- 36 and 24 chain models refer to the smallest CMFs, such as those present in wood cells
- Often these smallest CMFs are referred to as elementary fibrils
- In many species, the CMFs are larger but they are multiplicates of the elementary fibrils
- Often the CMFs (or elementary fibrils) aggregate, forming larger CMF bundles

Measured CMF widths

Source	Degree of crystallinity	Microfibril width*	Microfibril width**
Algal cellulose	>80 %	10 nm	10-35 nm
Bacterial cellulose	65-79%	5 nm	4-7 nm
Cotton linters	56-65%	5 nm	7-9 nm
Ramie	44-47%	5 nm	3-12 nm
Hemp	60%	3-5 nm	3-18 nm
Flax	56%	4-5 nm	3-18 nm
Dissolving pulp	43-56%	4-5 nm	10-30 nm

*) Deduced from X-ray diffraction (reflection from 110 lattice plane)
**) Deduced from transmission electron microscopy images

Zugenmaier, In: *Crystalline cellulose and cellulose derivatives;* Springer: 2008; p. 208.

Models for CMF

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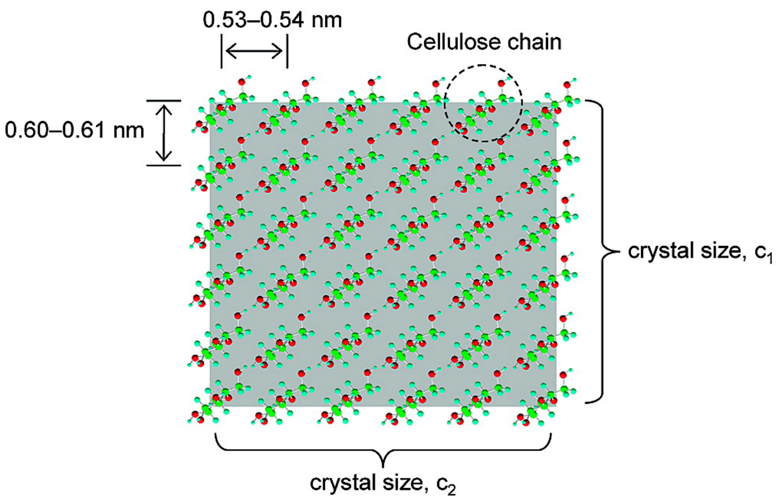
Note on the models

- **These models deal with the size, shape and number of cellulose chains in the smallest cellulose microfibrils, i.e., those residing in wood**

- **CMFs residing in other plant fibres are considered multiplicates of these smallest microfibrils**

Models for cellulose crystallite / microfibril: the 6×6 model

Figure taken from:
Okita et al. *Biomacromoleuels* **2010**, *11*, 1696.

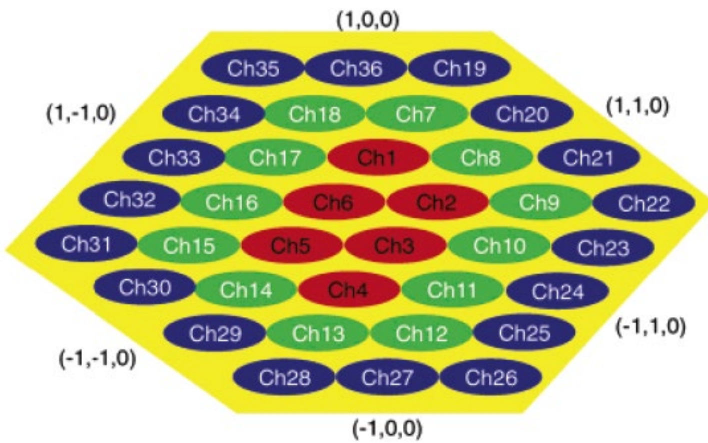


Endler and Persson *Mol. Plant.* **2011**, *4*, 199 (Review)

The 6×6 model (rectangular)

- Most common model for CMF
- Based on circumstantial evidence on the 6×6 organisation of the rosette in biosynthesis
- Roughly fits the evidence (XRD and microscopy data)
- Much of the molecular modelling of CMFs is performed with this model

Alternative 6×6 model



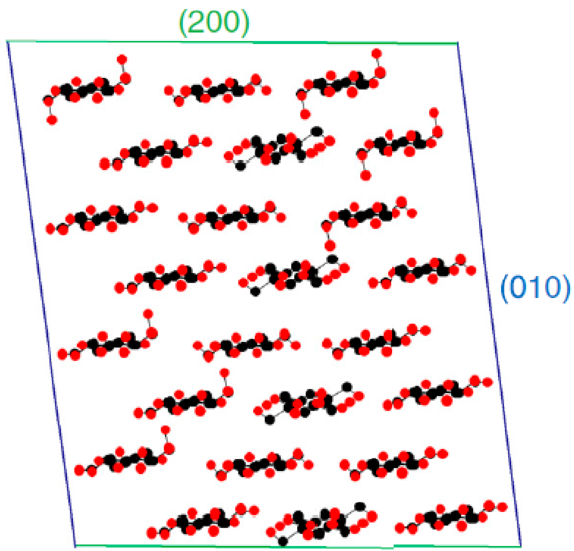
The 6×6 model (irregular hexagon)

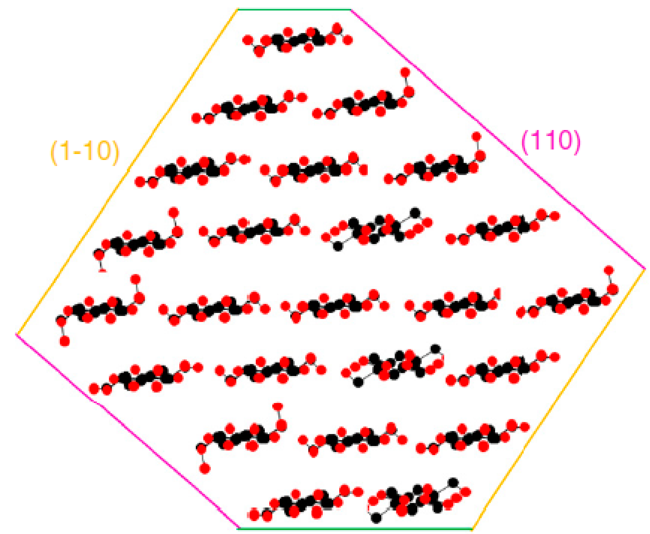
- Based entirely on atomic force microscopy (AFM) data
- Widely used despite the fairly weak experimental evidence
- Used in some molecular modellings of CMFs

Ding and Himmel *J. Agric. Food Chem.* **2006**, *54*, 597

24 chain model

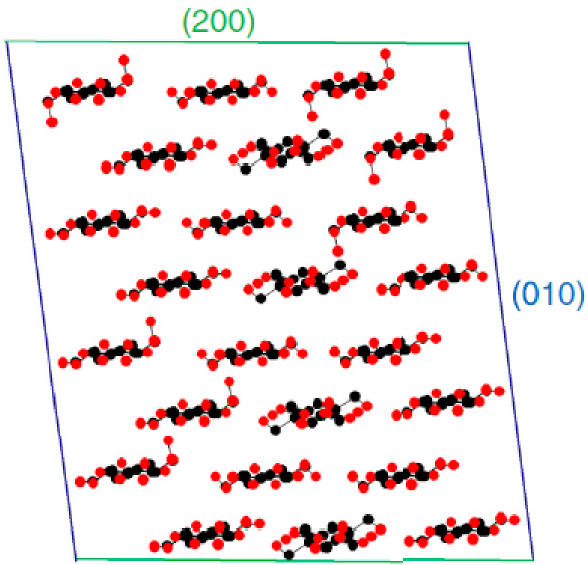
Two possibilities: rectangle or “diamond” model





Fernandes et al. *PNAS* **2011**, *108*, E1195

24 chain model

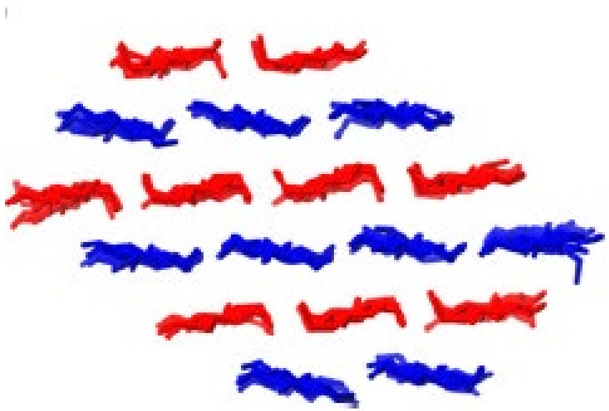


Fernandes et al. *PNAS* **2011**, *108*, E1195

24 chain model

- Based on several techniques: FTIR, NMR, and diffraction
- A credible alternative
- A disputed model but has the most substantial experimental data of all CMF models
- Suggests that only 4 of the 6 rosettes are simultaneously active during biosynthesis

18-24 chain model



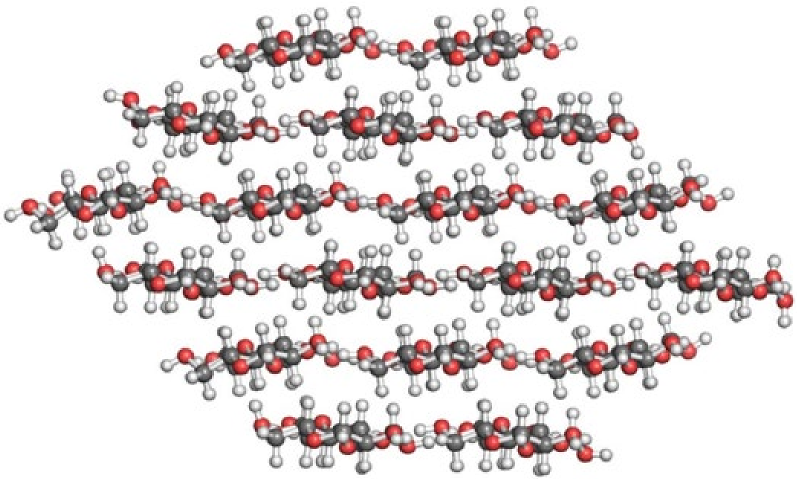
Oehme et al. *Plant Physiol.* **2015**, *168*, 3

18-24 chain model

- Based on molecular dynamics simulations in aqueous environment
- Comparison with previously published experimental data suggests that a 36 chain model is highly unlikely
- Proposes that CMF is made of either 18 or 24 chains
- Endorsed by the researchers who originally came up with the 24 chain model

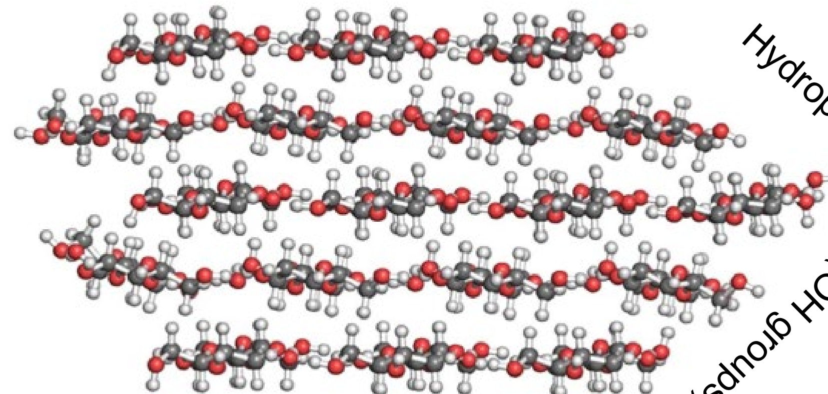
18-chain models

234432 model



34443 model

Hydrophobic plane (C-H groups)



Hydrophilic plane (OH groups)

Deemed as the most probably option according computer simulations

Kubicki et al. *Sci. Rep.* **2018**, *8*, 13983.

Presemo

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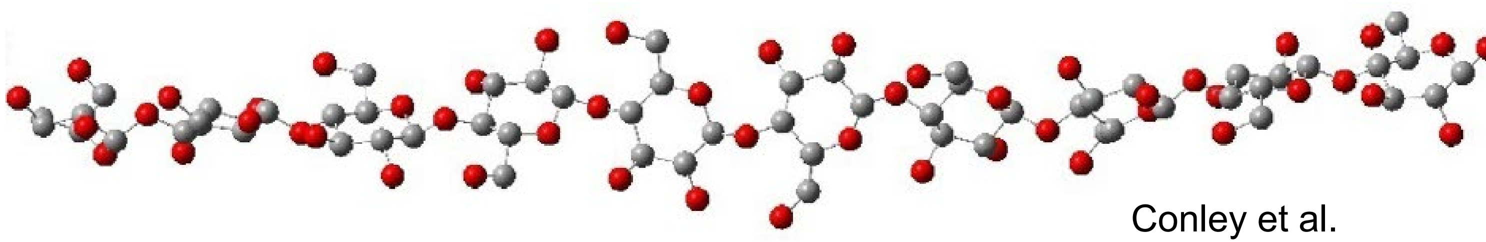
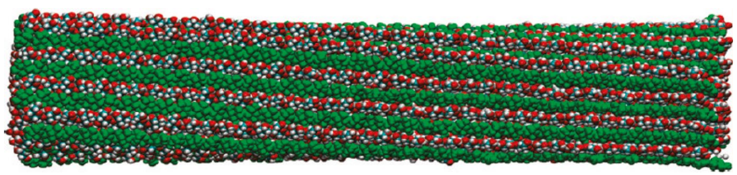
Twist along CMF

Simulations suggest twist



Isolated cellulose chains twist in simulations

Conley et al.
Carbohydr. Polym. **2016**, *135*, 285.

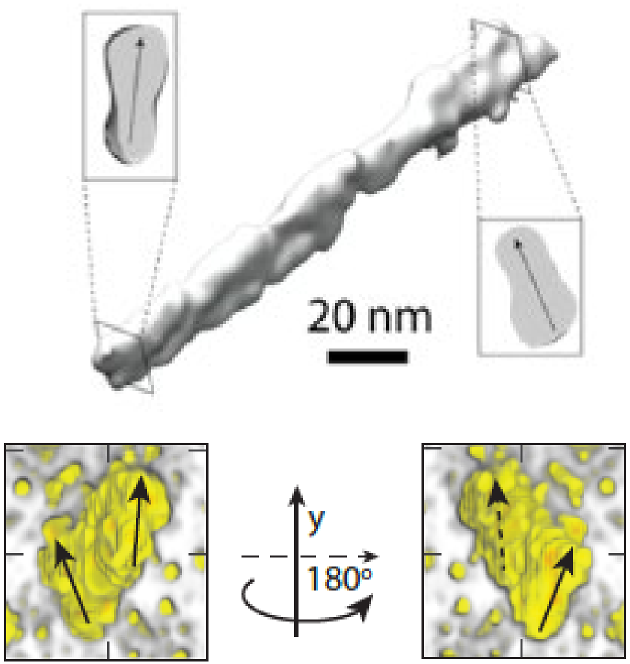


Paavilainen et al.
J. Phys. Chem. B **2011**, *115*, 3747.

- Computational models of very small cellulose crystals are twisting
- The periodicity of the twist is longer than with individual chains
- The twist is right-handed

CMF twist: experimental evidence

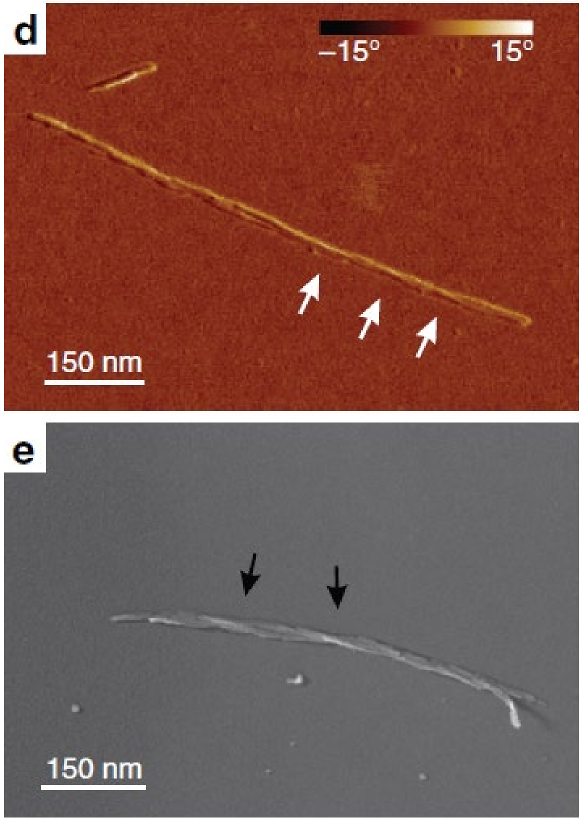
Electron tomography on a cellulose nanocrystal



- Experimental evidence on CMF twist is not unambiguous
- Many images abound in literature but quantitative data is largely missing

Majoinen et al. *J. Am. Chem. Soc.* **2014**, *136*, 866.

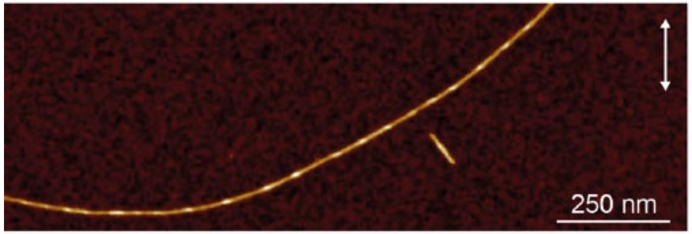
CMF twist: experimental evidence

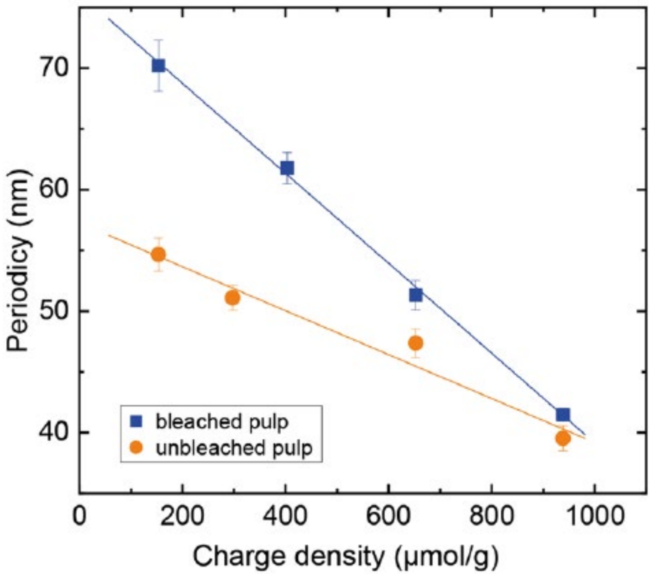


- Visualization on CMF twist is not unambiguous
- Many images abound in literature but quantitative data is largely missing

Usov et al. *Nature Commun.* **2015**, *6*, 7564.

CMF twist: experimental evidence



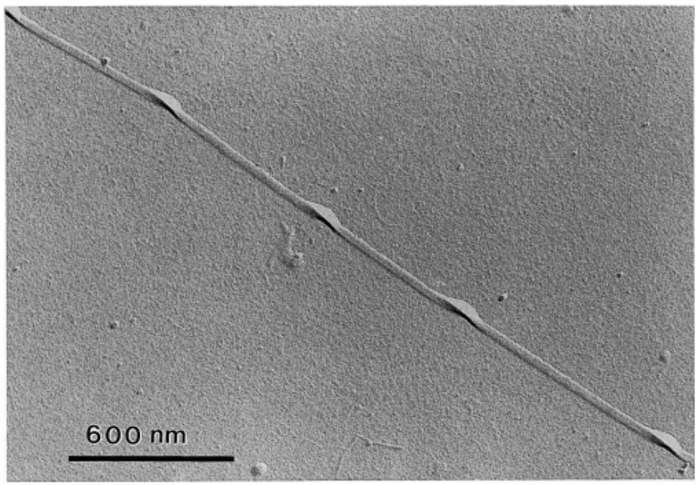


- Quantitative data found for isolated microfibrils (cellulose nanofibres)
- Periodicity of the twist (pitch) varies from 40-70 nm
- Criticism: twist is altered when the microfibril is isolated from cell wall

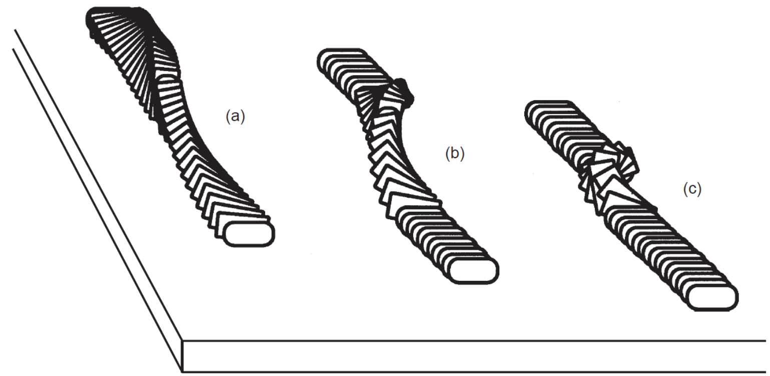
Arcari et al.
Biomacromolecules **2019**, *20*, 1288.

Proposed alteration of CMF twist

- Some accounts suggest that the twist is altered and “localized” upon drying



CMFs of *M. denticulata* alga after drying



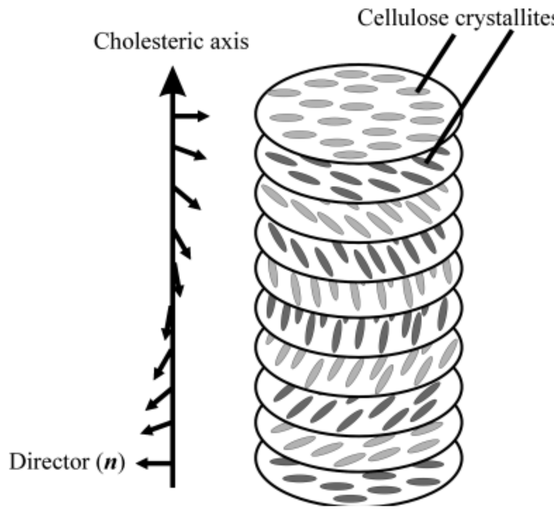
Suggestion on what happens to a pristine CMF (a) upon drying (b) and in a totally dried state (c)

Hanley et al. *Cellulose* **1997**, *4*, 209.

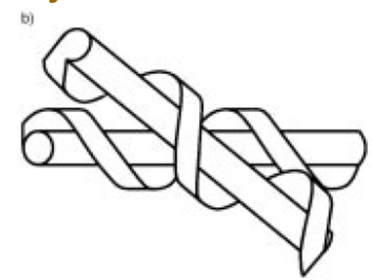
Implication of the CMF twist

Cellulose nanocrystals spontaneously forms a liquid crystal phase in solution

Photograph of liquid crystal suspension of cellulose nanocrystals



Chiral nematic phase formed by cellulose crystallites



Tight packing by the chiral interaction of screwlike rods

Revol et al. *Int. J. Biol. Macromol.* **1992**, *14*, 170.
Fleming et al. *Chem. Eur. J.* **2001**, *7*, 1831.
Habibi et al. *Chem. Rev.* **2010**, *110*, 3479.

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Fringed fibrillar model

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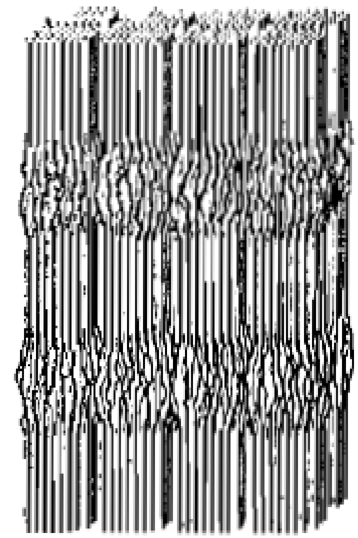
Longitudinal disorder: semi-crystallinity

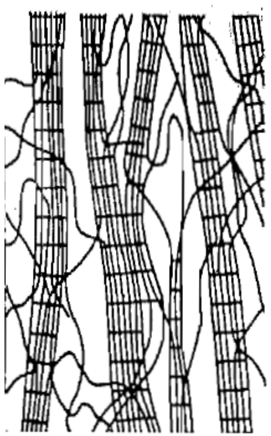
Crystallographic data presents evidence that cellulose within microfibrils is not totally crystalline.



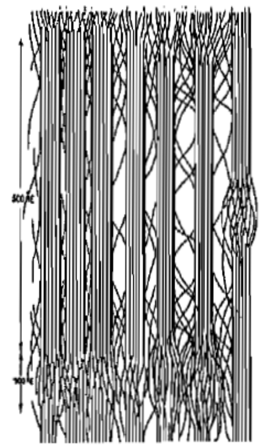
Proposition:
cellulose runs through alternating crystalline and “ amorphous” regions.

Fringed-fibrillar model of CMFs

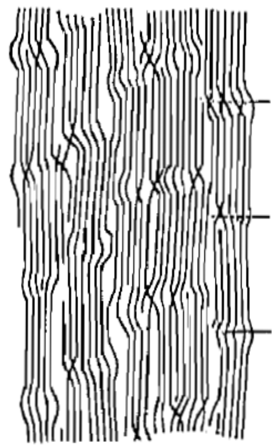
- According to various models, **disordered** cellulose segments coexists with crystalline cellulose in native cellulose microfibrils.



Hearle 1958



Dolmetsch 1968

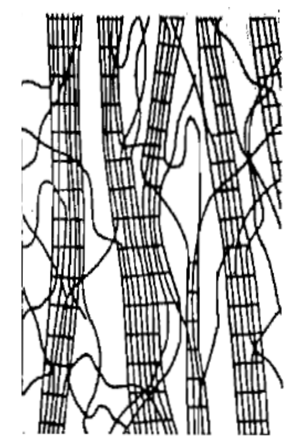


Hess and Kiessig 1953

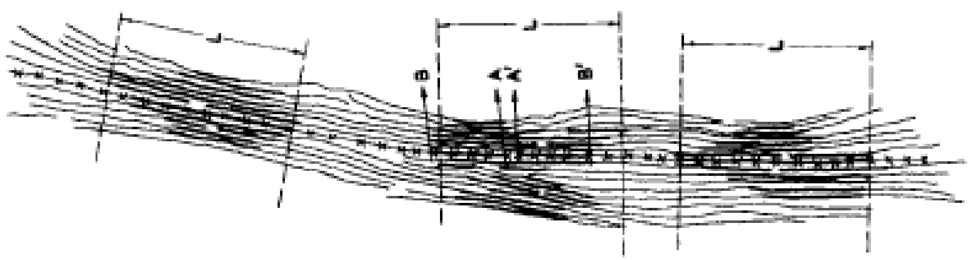
Semicrystallinity of microfibrils

- Original models were designed for all polymeric fibrils: synthetic, regenerated and native alike

Fringed fibrillar model



Fringed micellar model



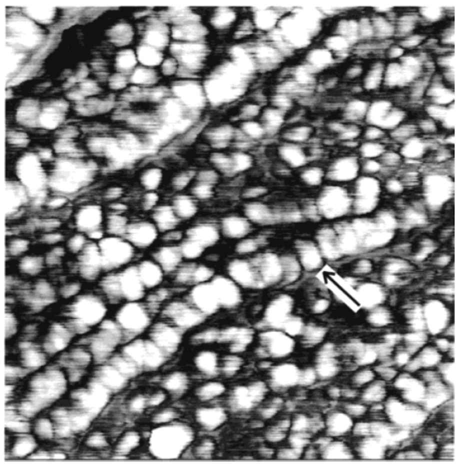
Mark *J. Phys. Chem.* **1940**, *44*, 764.

Hearle *J. Polym. Sci.* **1958**, *28*, 432.

Synthetic polymers vs. cellulose

- High resolution morphology by AFM

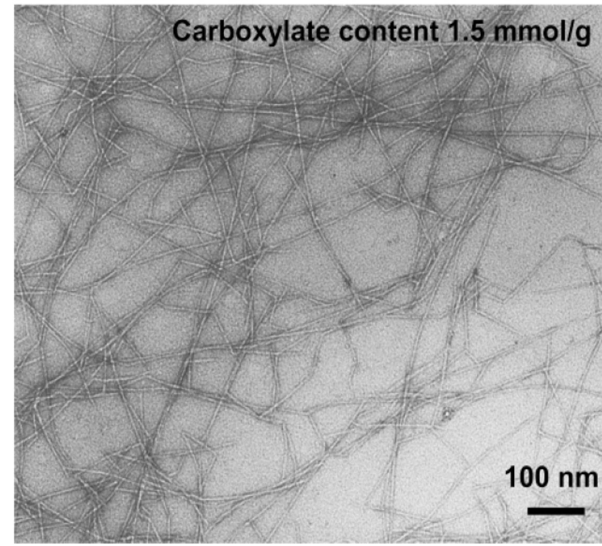
Polyethylene



Amorphous segments visible

Loos et al. *Macromolecules* **1999**, *32*, 8910.

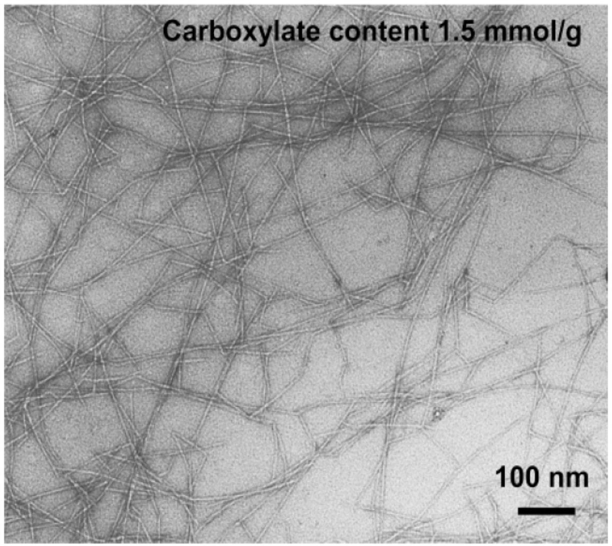
Cellulose microfibrils isolated from wood pulp



Amorphous segments ***NOT*** visible

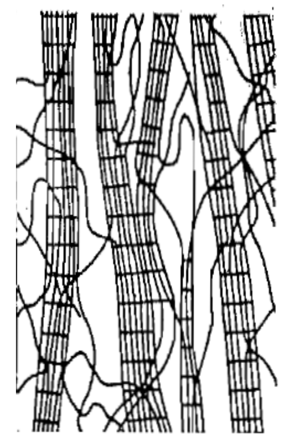
The original fringed fibrillar model

- Modern TEM images of microfibrils isolated by TEMPO-mediated oxidation do not support the Hearle model where fibrils are branched and polydisperse in width



Saito et al. *Biomacromolecules* **2006**, *7*, 1687.
Saito et al. *Biomacromolecules* **2007**, *8*, 2485.

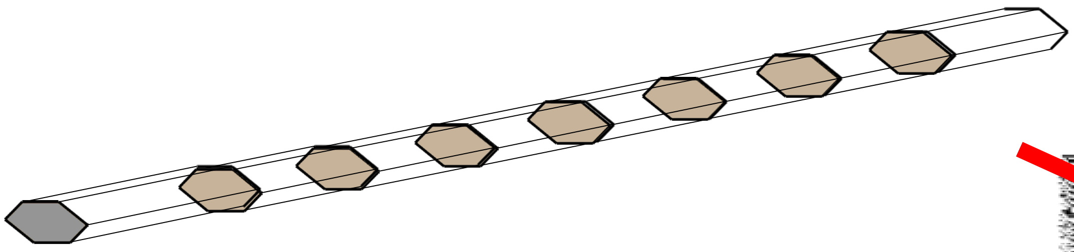
Fringed fibrillar model



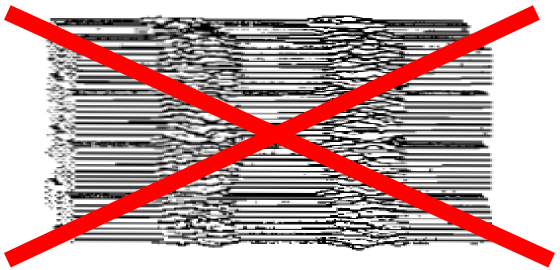
Hearle *J. Polym. Sci.* **1958**, *28*, 432.

More realistic picture of a microfibril

- “Amorphous” regions are more like defects between the crystallites
- Their length is probably very small (maybe 1-2 nm)



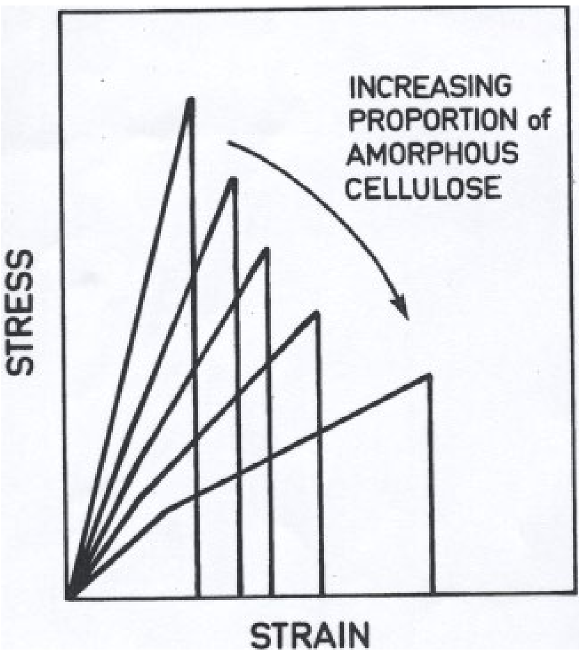
Defects: more realistic



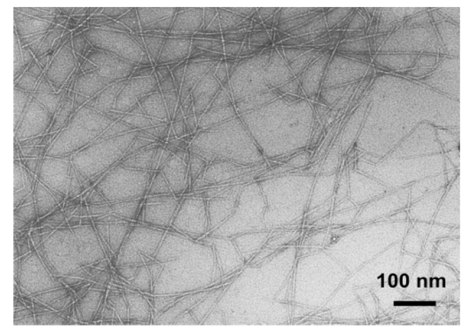
Bulky amorphous regions: less realistic

Implications of fringed-fibrillar model

Alternating crystalline-amorphous regions explain well
the macroscopic mechanical properties of cellulosic materials.



The length and width of the crystalline domains depend on the native source of the material.



Elastic properties of isolated cellulose nanofibrils depend on their native source.

Page *J. Pulp Paper Sci.* **1983**, *3*, TR15.

Reservations with fringed fibrillar model

- When you see data of the *degree of crystallinity* or *crystallinity index* of cellulose, its physical meaning is unclear
- If the degree of crystallinity is, e.g., 64%, it does not mean that 64% of the cellulose is crystalline and 36% is ”amorphous”
- Probably much of the material responsible for the ”amorphous” response resides on the microfibril surface
- Cellulose I and cellulose II degrees of crystallinity should *not* be compared with each other
- Systematic sets of data can be compared with each other if the crystalline forms, the analytical method, and the raw materials are similar

Presemo

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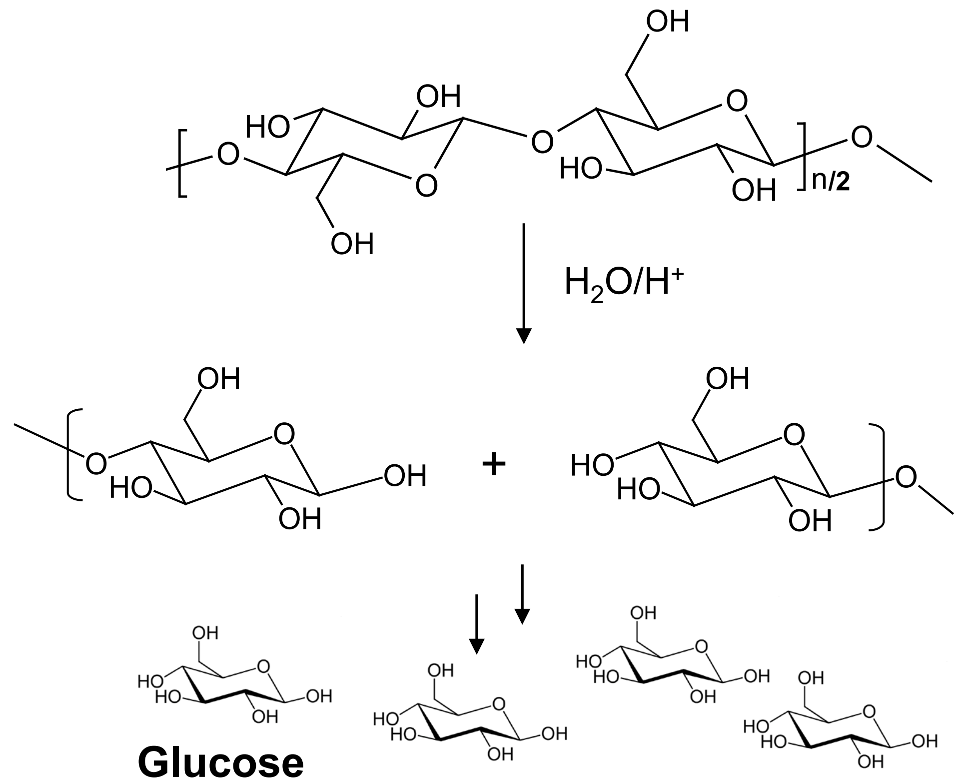
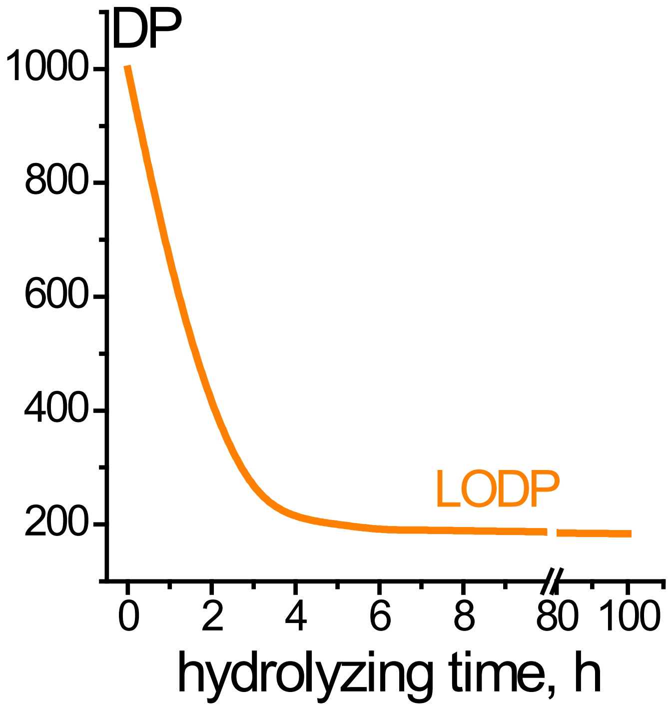
Levelling-off degree of polymerization (LODP)

Acid hydrolysis of cellulose

- **Acid hydrolysis involves the breakage of glycosidic bond by addition of water, catalyzed by acid**
- **High concentrations are required for complete degradation (e.g., 72% (w/w) H_2SO_4)**



Kinetics of acid hydrolysis of cellulose



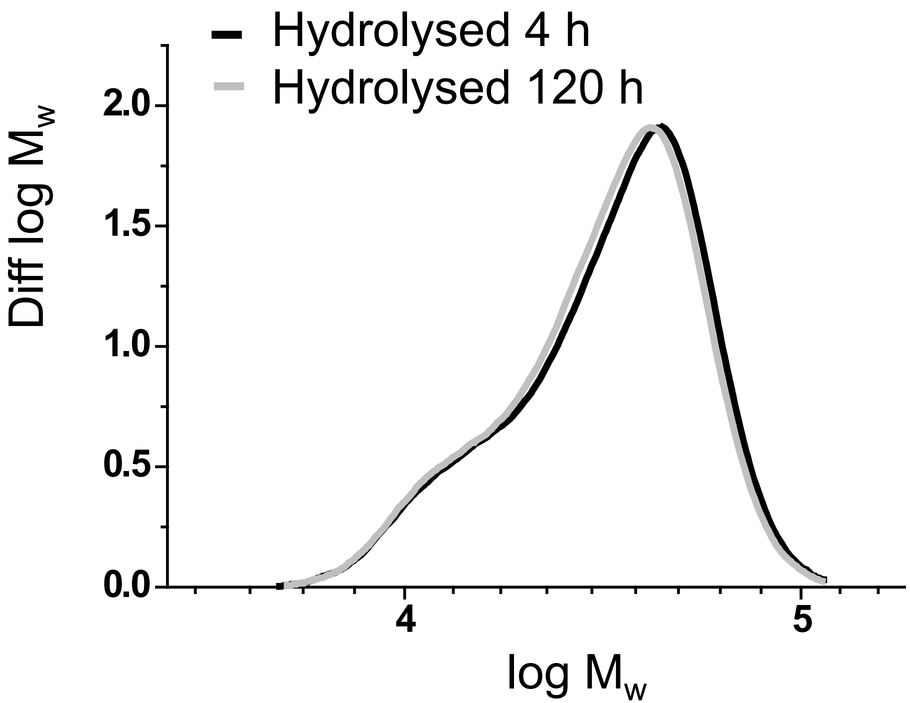
- When milder acid concentrations are used, DP first drops fast, after which it almost halts, hitting the LODP
- Traditionally LODP is determined with 2-3 M HCl at around 100ºC
- Common explanation for LODP: “amorphous” regions are hydrolysed and crystallites are left intact

LODP of different cellulose sources

Material	LODP
Wood pulp	100-250
Cotton linters	100-250
Ramie	200-350
Valonia	7000

Notice the large variation in numbers for the same source

Molecular weight distribution at LODP



Cotton linters LODP ~150

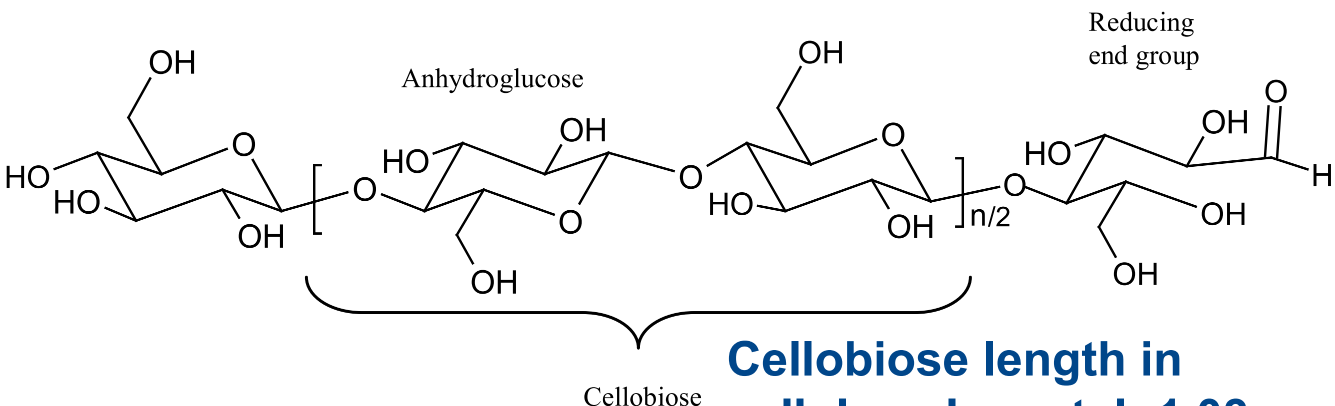
Notice: bimodal distribution

Discrepancies with LODP

Cellulose substrate and reference	LODP	Yield loss (%)	Conditions for determining LODP
Cotton linters	200-250	n.a.	2.5 N HCl, 105ºC, 15 min
Cotton linters	187	7	2.5 N H_2SO_4, 96ºC, 6 h
Cotton linters	253	2	2.5 N H_2SO_4, 100ºC, 30 min
Cotton linters	190	4.4	2.4 N HCl, 100ºC, 1 h
Cotton linters	100	6	6.5 N HCl, 108ºC
Cotton linters	200	3.5	2.5 N HCl, 100ºC, 30 min
Cotton linters	162	5	5% HCl, 95ºC, 1 h

- No standard method to measure LODP exists
- Many different values for similar cellulose grades have been reported
- Amount of material lost during hydrolysis (yield loss) also varies a great deal

Does LODP represent the length of the crystalline region?



Cellobiose length in cellulose I crystal: 1.03 nm

- Length calculated from LODP should correspond to crystallite length measured by XRD or NMR

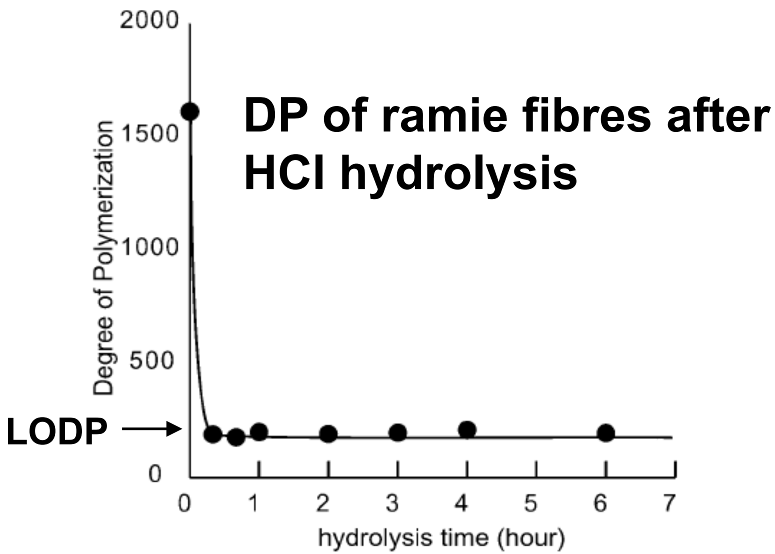
Crystallite length vs. LODP

Material	LODP	Crystal length by XRD*
Wood pulp	100-250	23 nm
Cotton linters	100-250	35 nm

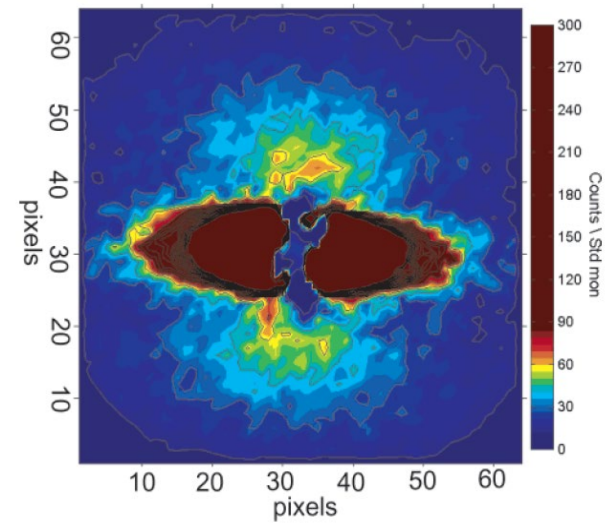
- Crystal length determined from CMFs does not correlate with LODP
- Possible reason: diffraction and/or spectroscopy cannot detect the CMF twist and interprets it for a shorter crystallite

* X-ray diffraction

Careful comparison of LODP and small angle neutron scattering



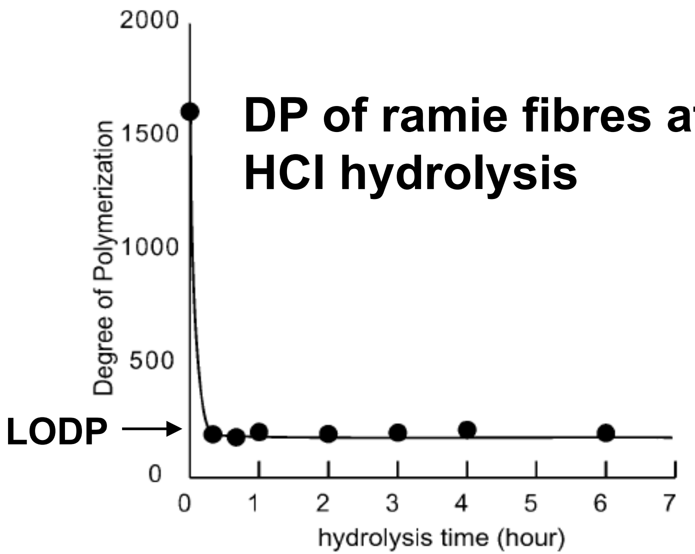
Small angle neutron scattering (SANS) pattern of untreated ramie



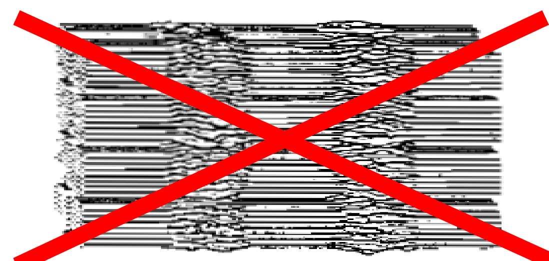
➡ Crystallite length (i.e. length of crystalline domains) by SANS agrees with the level-off degree of polymerization (LODP).

Nishiyama et al. *Biomacromolecules* **2003**, *4*, 1013.

Careful comparison of LODP and small angle neutron scattering

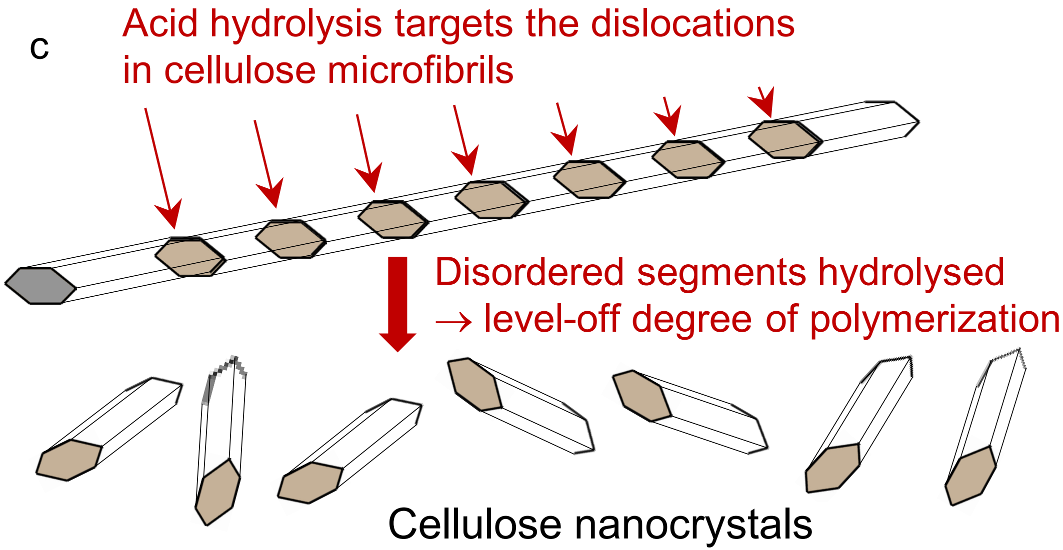


- The yield loss upon controlled acid hydrolysis is very small (~1%)
- This implies a very short disordered region (4-5 anhydroglucose units)
- Disordered – *not* amorphous

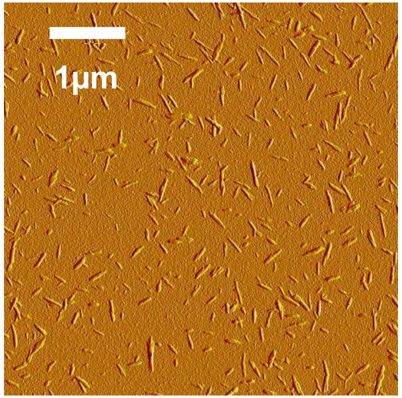


Nishiyama et al. *Biomacromolecules* **2003**, *4*, 1013.

Implications of LODP: Cellulose nanocrystals



Cellulose nanocrystals



AFM image

Acid hydrolysis targets the disordered regions in a cellulose microfibril. → **Result: cellulose nanocrystals**

Presemo

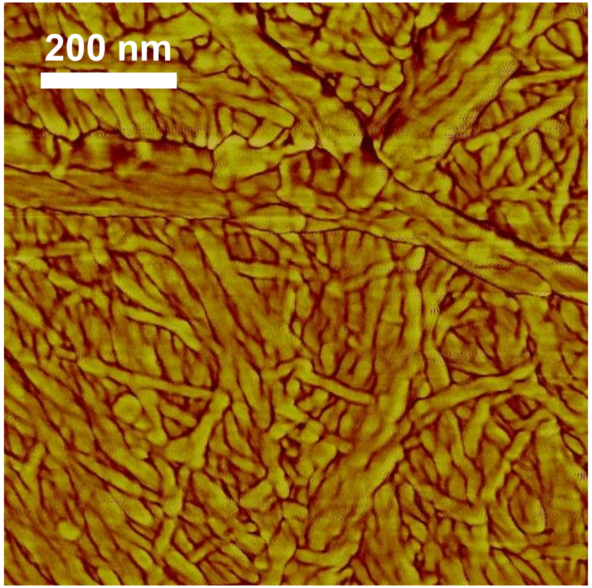
Take out your smartphones or laptops and open the webpage:

https://presemo.aalto.fi/e2140microfibril

Bundling of CMFs

Appearance of CMFs

Aggregates: 12-20 nm



AFM image of a surface of bleached birch kraft pulp; sample untreated.

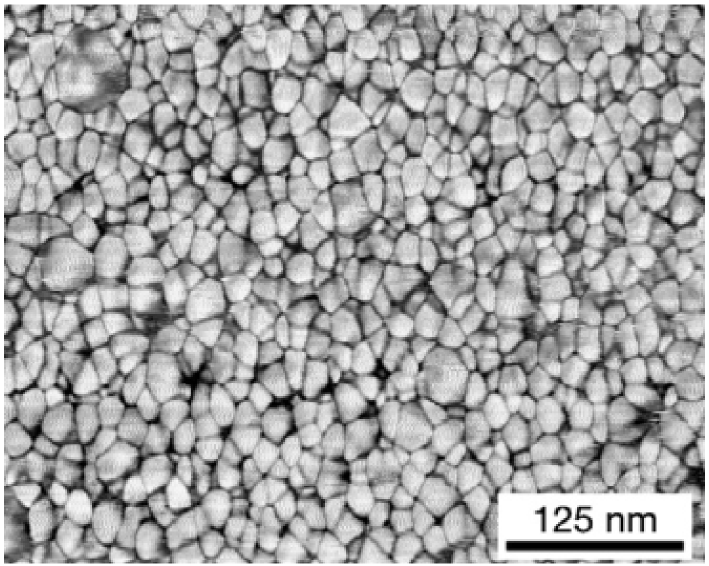
Individual microfibrils: ~3.5 nm

TEM image of longitudinal cross-section of chlorite delignified pine cell wall; freeze-dried and stained

Heyn *J. Ultrastructure Res.* **1969**, *26*, 52.

Appearance of CMFs

Aggregates: 12-20 nm



TEM image of radial cross-section of wood cell wall.

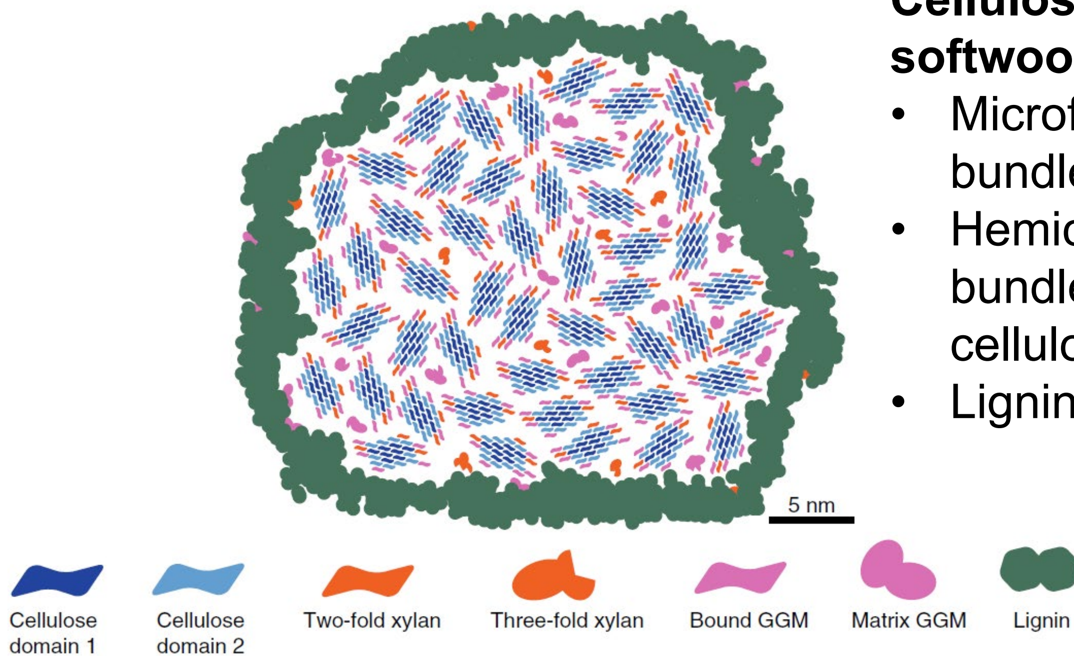
Individual microfibrils: ~3.5 nm

TEM image of longitudinal cross-section of chlorite delignified pine cell wall; freeze-dried and stained

Zimmermann et al. *J. Struct. Biol.* **2006**, *156*, 363.

Heyn *J. Ultrastructure Res.* **1969**, *26*, 52.

Native microfibril bundle: modern view

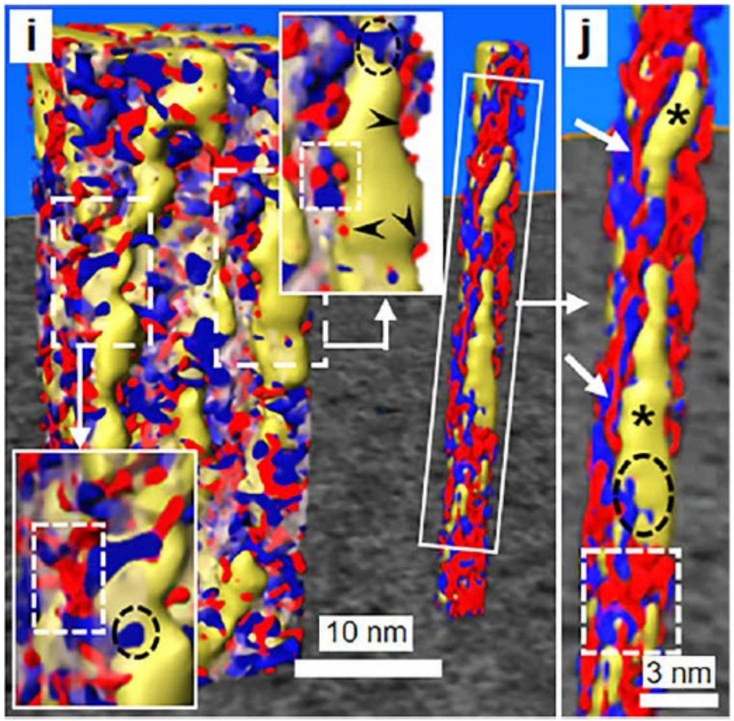


Cellulose microfibril bundle in a softwood cell wall:

- Microfibrils form larger entities called bundles or macrofibrils
- Hemicellulose resides inside the bundle, intimately connected to cellulose
- Lignin lies outside the bundle

Simmons et al. *Nature Commun.* **2016**, *7*, 13902
Terrett et al. *Nature Commun.* **2019**, *10*, 4978.

Native microfibril bundle: modern view



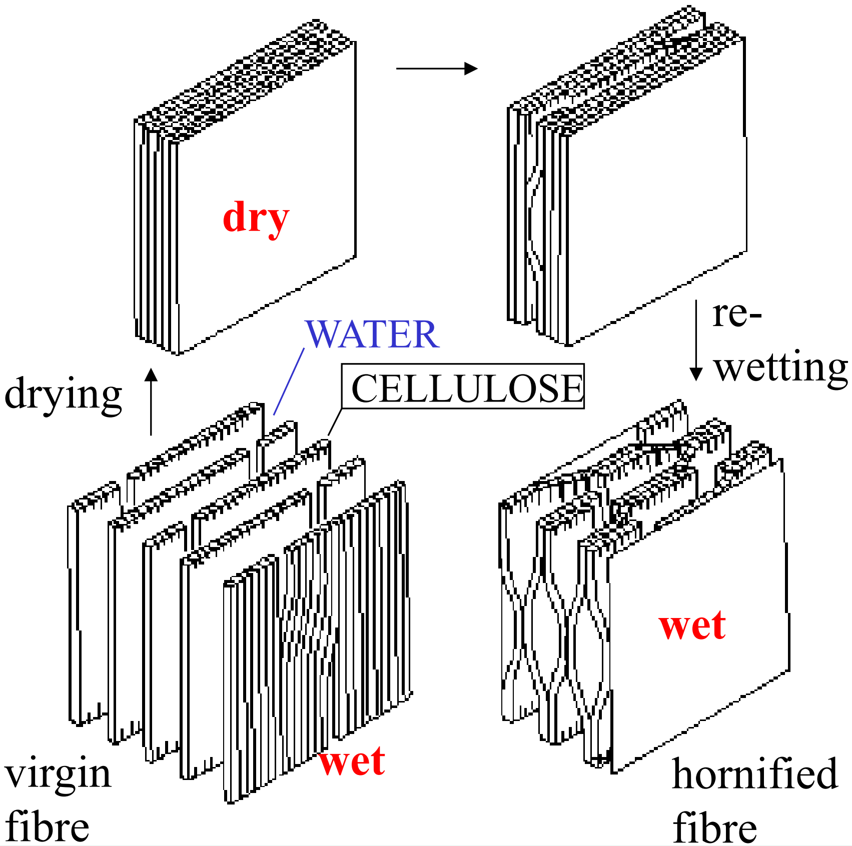
Electron tomography enables the imaging of the ultrastructure in unprecedented detail

- Bundle size: 6-25 nm
- Individual microfibrils also observed (outside bundles)

Cellulose
Hemicellulose
Lignin

Fernando et al. *Sci. Rep.* **2023**, *13*, 2350.

Additional CMF bundling: hornification



Hornification

- Well-known phenomenon with chemical pulp fibres
- Water swells the fibres by penetrating between CMFs

→ Fibres are porous in water

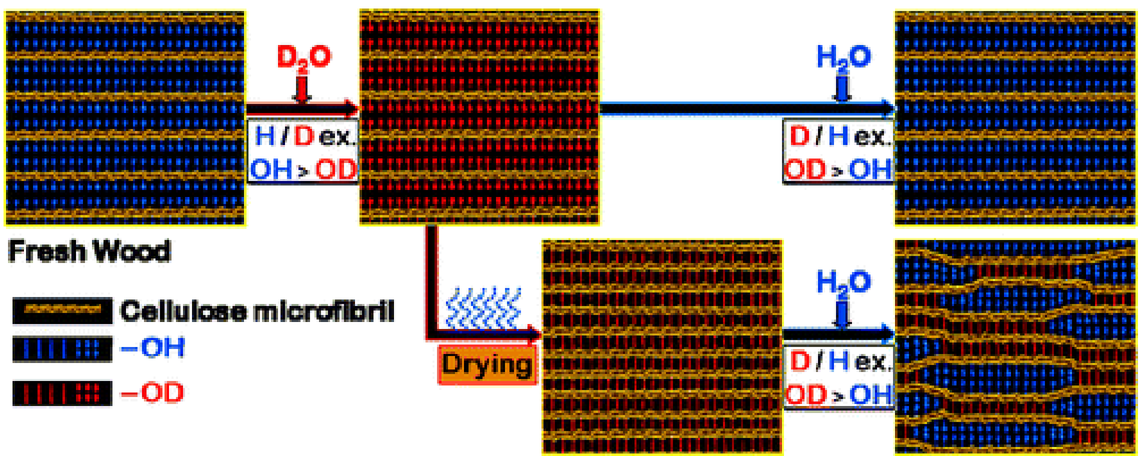
- When dried, the pores disappear
- Upon rewetting, the swelling is not restored to the same level

→ Porosity is irreversibly decreased upon drying

→ CMFs have bundled

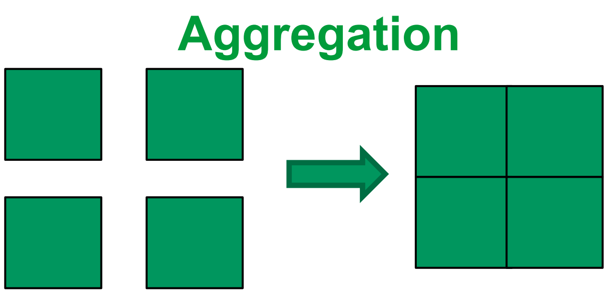
Additional CMF bundling: dehydration

- Just removing a plant from its native growth environment causes CMF bundling (aggregation)



Suchy et al.
Biomacromolecules **2010**, *11*, 515.

Implications of CMF bundling



Schematic cross sections of microfibrils

Why is this important?

↓

Reduced surface area

↓

Reduced accessibility

Fewer reaction sites

Difficulties to extract cellulose nanofibrils

Presemo

Take out your smartphones or laptops and open the webpage:

https://presemo.aalto.fi/e2140microfibril

Summary

- Native cellulose resides exclusively in cellulose microfibrils (CMFs)
- Width of CMFs is monodisperse but difficult to analyse unambiguously
- Number of cellulose chains in a CMF is not agreed upon
- Longitudinal disorder in CMF (fringed fibrillar model) does exist but the disordered regions are rather dislocations than bulky amorphous regions
- Levelling-off degree of polymerization, cellulose nanocrystal length, and the measured length of CMF crystalline regions do not match together perfectly
- CMFs have a tendency to bundle together upon drying

Assignment

Take a couple of minutes and write down a couple of bullet points to the query *After the lecture*

Leave the query paper on the desk next to the lecture hall exit