Cellulose degradation

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**Aalto University
School of Chemical
Engineering**

Learning outcomes

After this lecture, the student should be able to:

- Describe how the supramolecular features of native cellulose influence its hydrolytic degradation behaviour by acid and enzymes

Outline
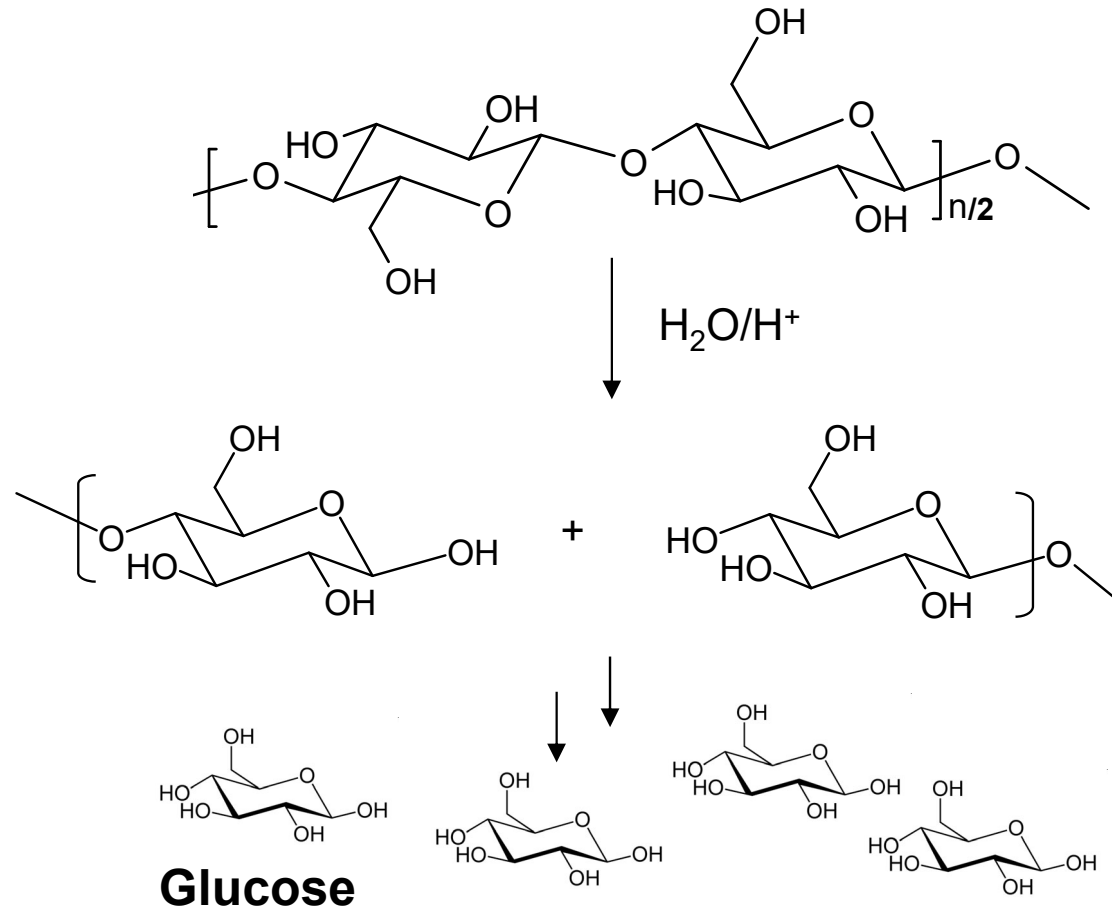
(1) Acid hydrolysis

- Basics on how supramolecular structure of cellulose influences acid hydrolysis
- Special case for research: hydrolysis with HCl gas

(2) Enzymatic hydrolysis

Acid hydrolysis

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)**
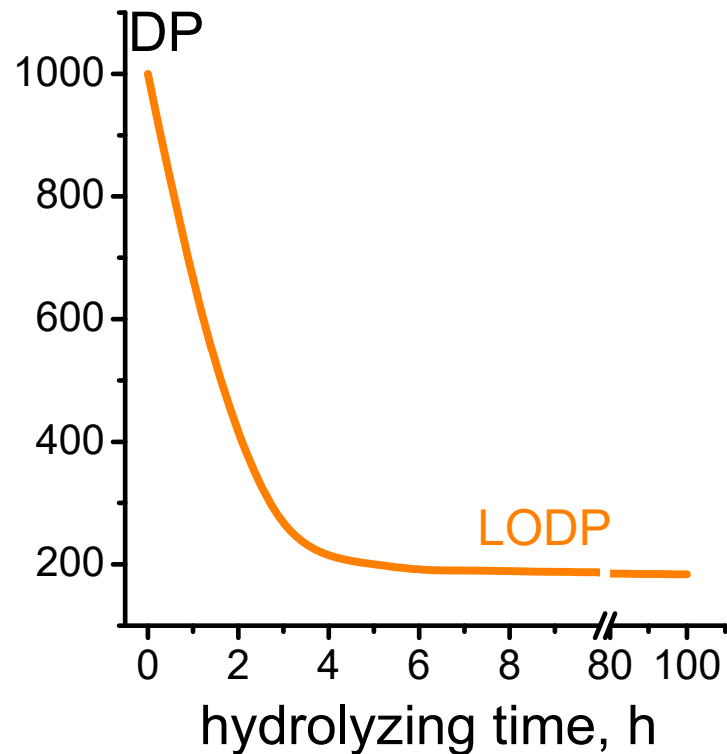

Glucose

Mechanism of acid hydrolysis

Cellulose hydrolysis is the reaction between water and cellulose

Acid is just a catalyst

+ H⁺ → $+ H^+$

+ H₂O / - ROH / - H⁺

-HOR

+ H₂O / - H⁺

+ H₂O / - ROH / - H⁺
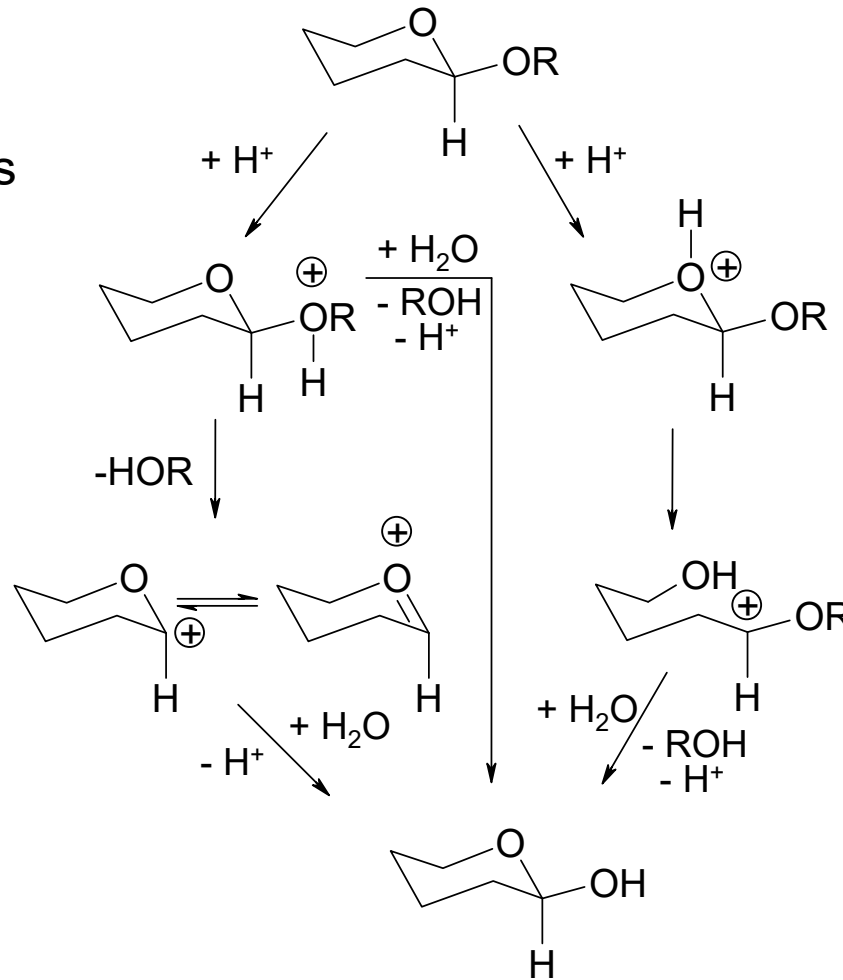
Adapted from:
Klemm et al. *Comprehensive Cellulose Chemistry*, Vol. 1; Wiley-VCH, 1998.

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: Levelling-off degree of polymerization**

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

Discrepancies within 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
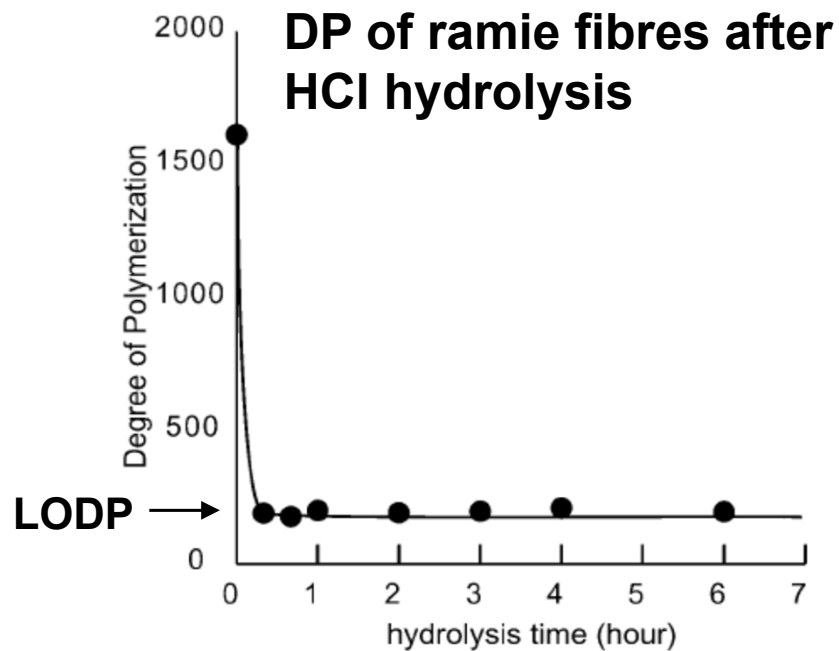- Amount of material lost during hydrolysis (yield loss) also varies a great deal

Crystallite length vs. LODP

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

* X-ray diffraction

- 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

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Modern comparison between LODP and crystallite length

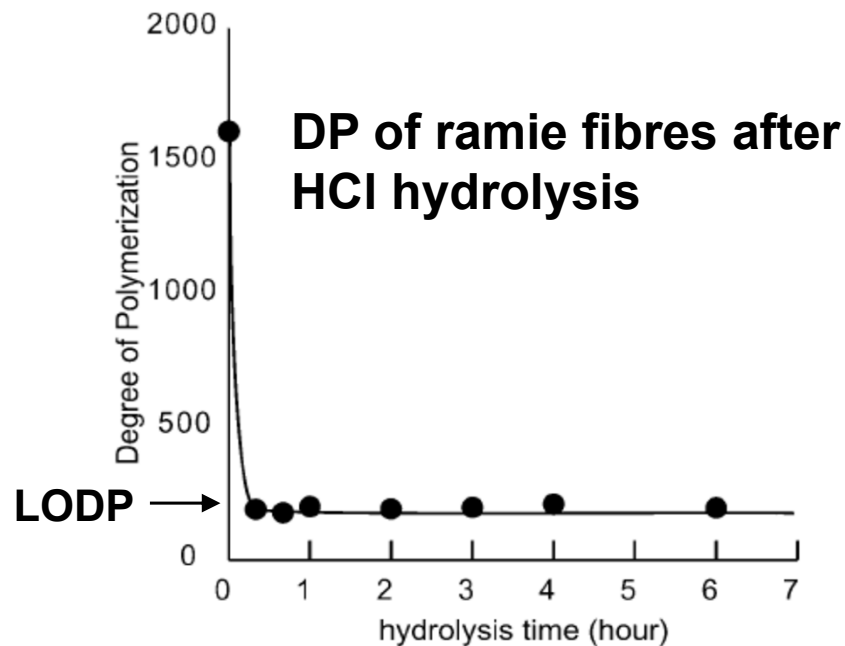**DP of ramie fibres after HCl hydrolysis**

Small angle neutron scattering (SANS) pattern of untreated ramie
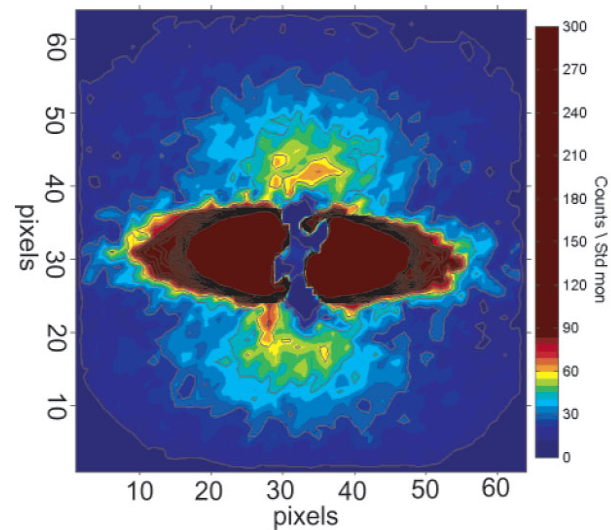
Crystallite length (i.e. length of crystalline domains) by SANS agrees with the level-off degree of polymerization (LODP).

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

Modern comparison between LODP and crystallite length

DP of ramie fibres after HCl hydrolysis

- 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.
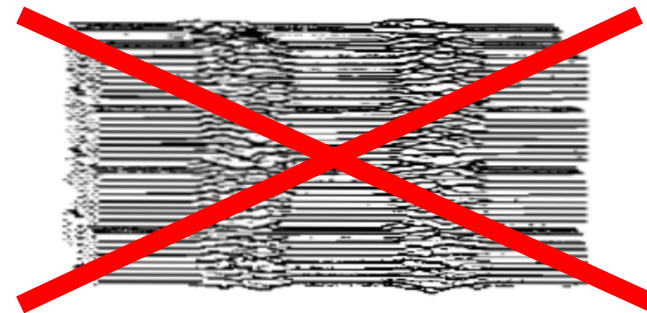
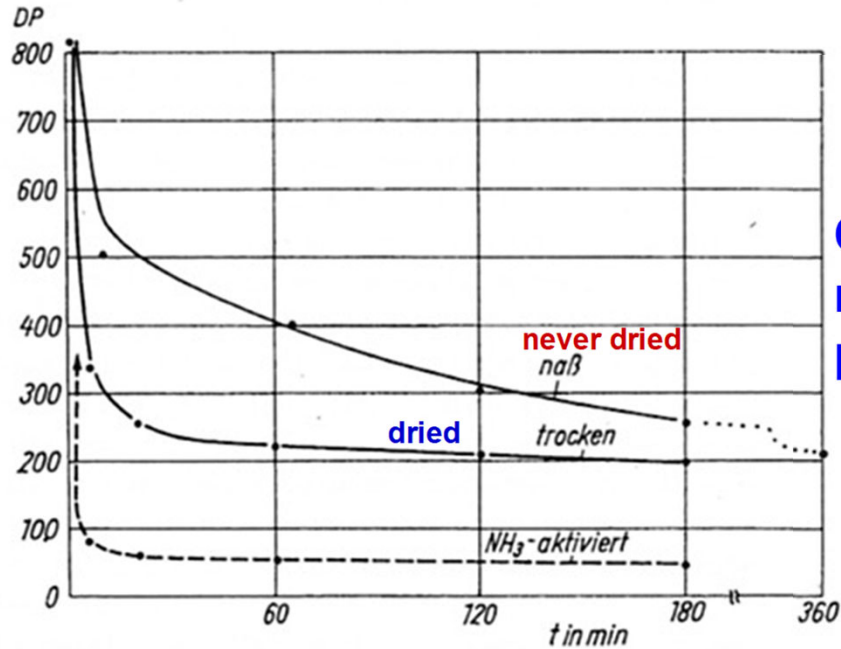# Parameters affecting kinetics of acid hydrolysis

Obvious parameters: *acid concentration and temperature*

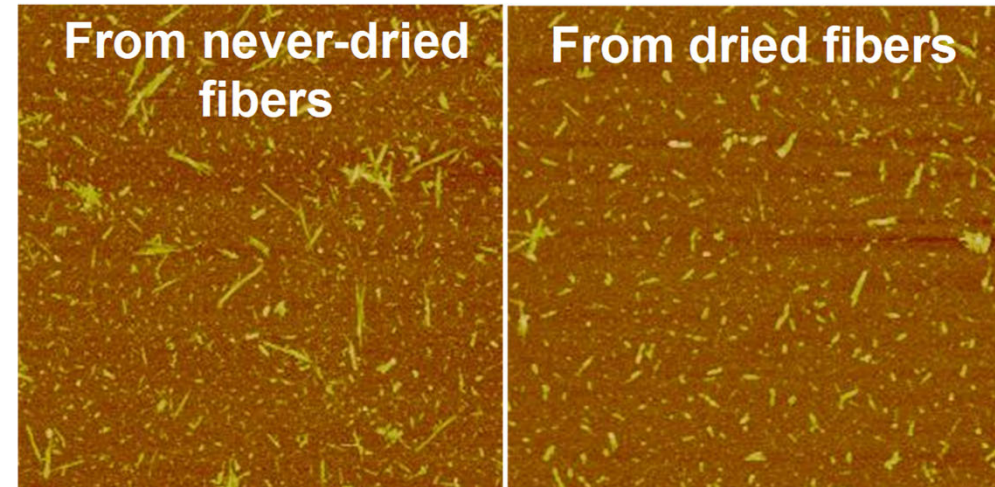NOTE: the acid concentrations are huge, far beyond the catalytic amounts

NOTE: very large concentrations are required to fully degrade cellulose (e.g., 72 wt% sulphuric acid)

Battista *Ind. Eng. Chem.* **1950**, 42, 502.
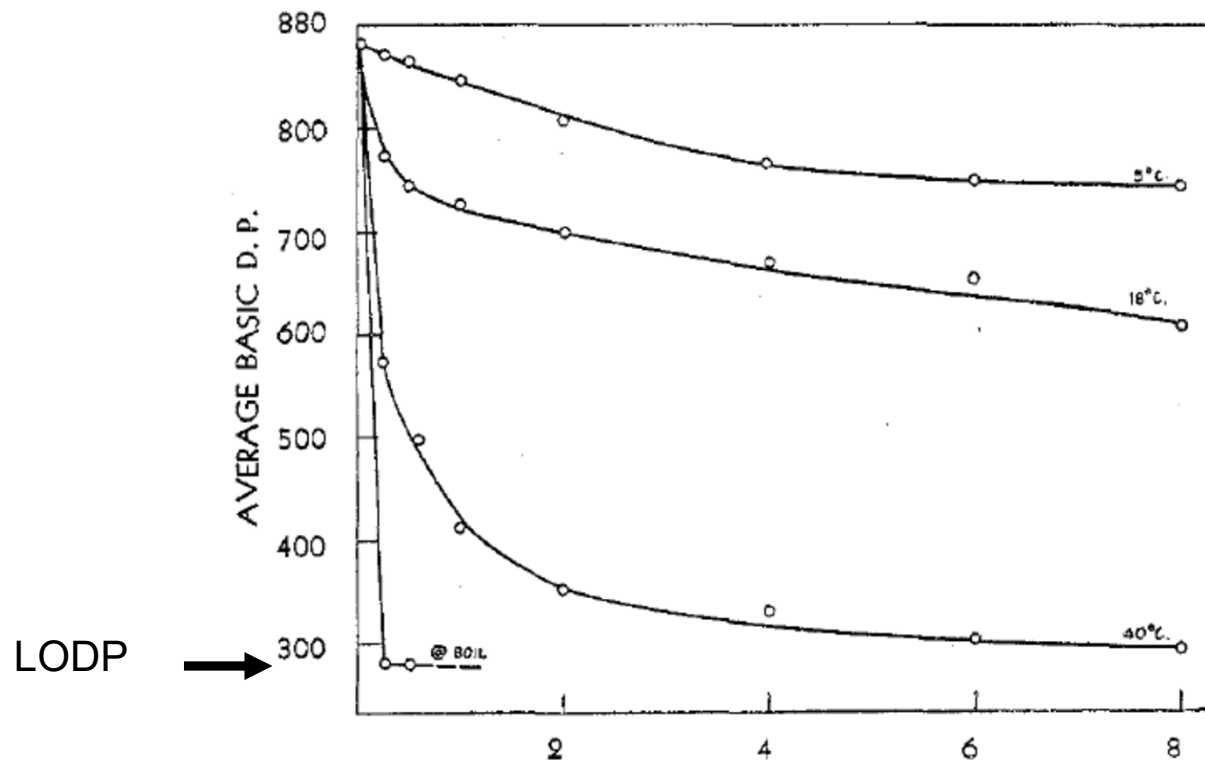
Accessibility effect

Steege and Philipp *Zellst. Pap.* **1974**, *23*, 68.

Kontturi and Vuorinen, *Cellulose* **2009**, *16*, 65.

$\rightarrow$ Acid hydrolysis is known to proceed faster with dried fibres
$\rightarrow$ More tension dissipated by the disordered regions

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Presemo
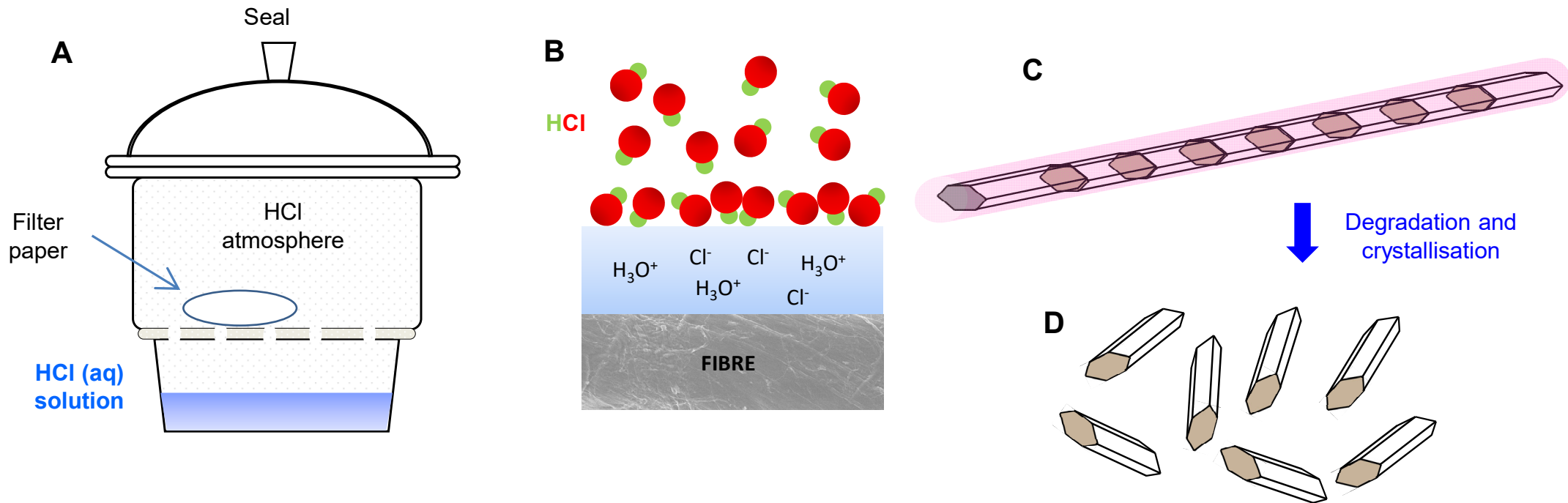
Take out your smartphones or laptops:

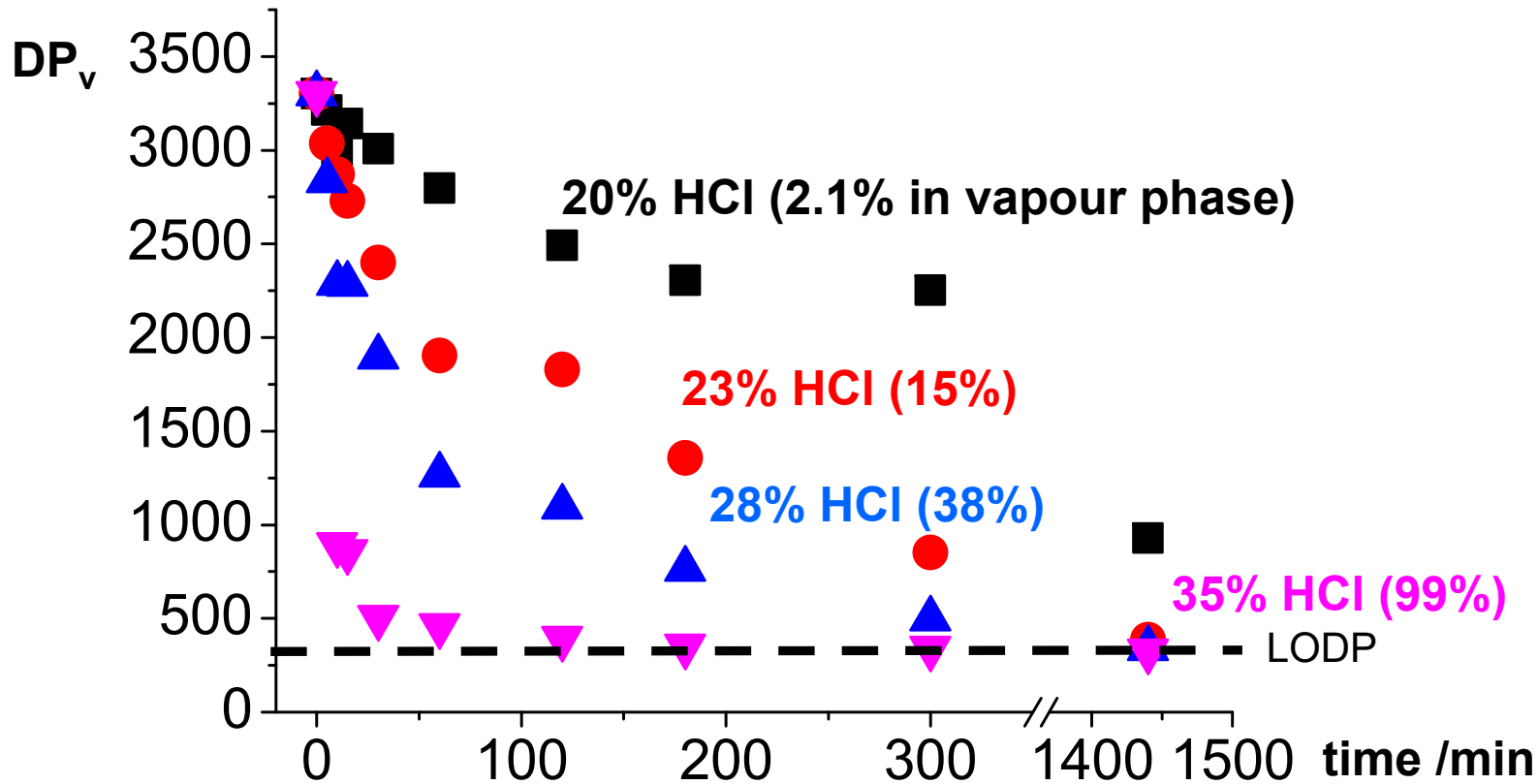**https://presemo.aalto.fi/l2010degradation**

Acid hydrolysis of dry fibres: use of HCl gas

Basic concept

- Gaseous HCl molecules adsorb on water-covered fibers

- Adsorbed HCl dissociates and catalyzes cellulose hydrolysis to LODP
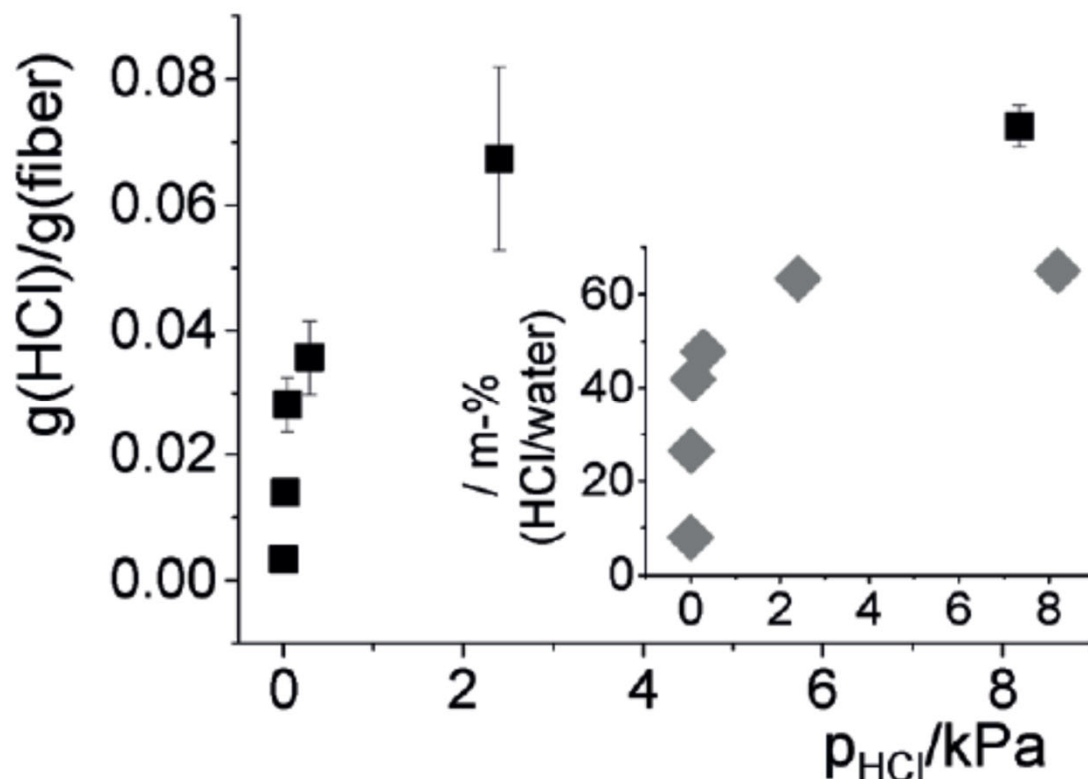
- CNCs can be dispersed from hydrolyzed fibres

DP reduction with HCl (g)

→ LODP value is exactly the same with HCl (g) and HCl (aq)

Kontturi et al. *Angew. Chem. Int. Ed.* **2016**, *54*, 14455.

HCl (g) adsorption

- Monolayer (Langmuir) adsorption

- When scaled to the amount of water, the amount of HCl adsorbed exceeds the solubility limit (~ 40 w-%)

→ Concentration of HCl (aq) on the surface is ~40 w-%

Crystallinity development

Degree of crystallinity as determined by x-ray diffraction

[%]

HCl partial pressure / kPa
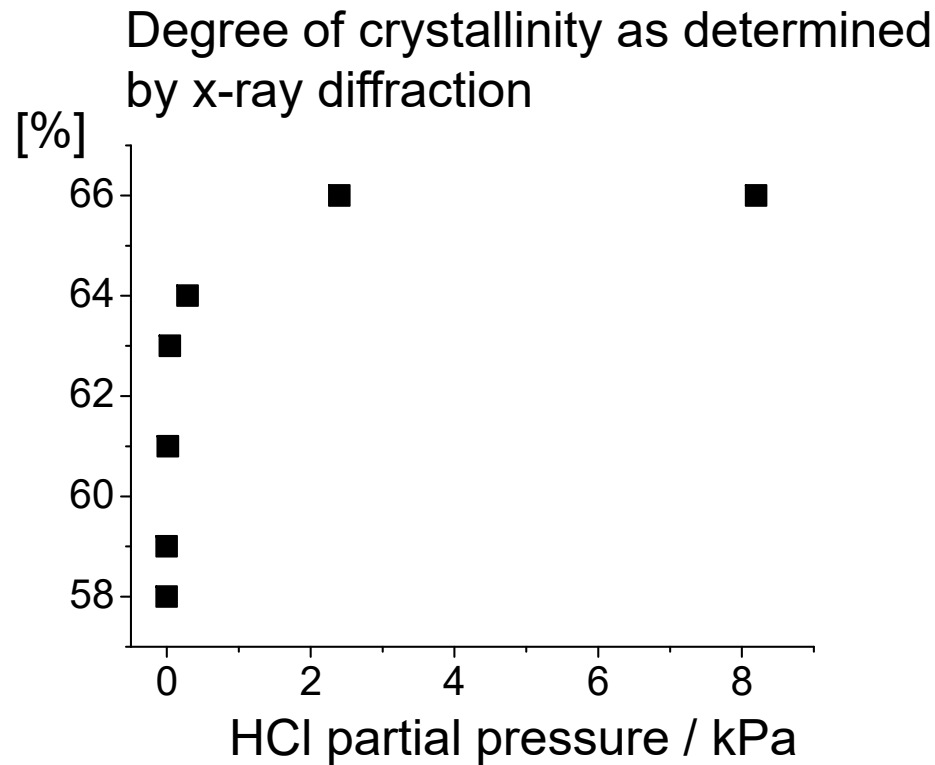
- Acid hydrolysis of cellulose usually results in formation of extractable sugars
- Hot water extraction of the hydrolyzed filter paper failed to extract *anything*

REASON: vapour phase acid causes crystallization of cellulose simultaneously with its degradation.

NOTE: No change in morphology of the fibres
NOTE: No mass transfer out of the fibres

Kontturi et al. *Angew. Chem. Int. Ed.* **2016**, *54*, 14455.

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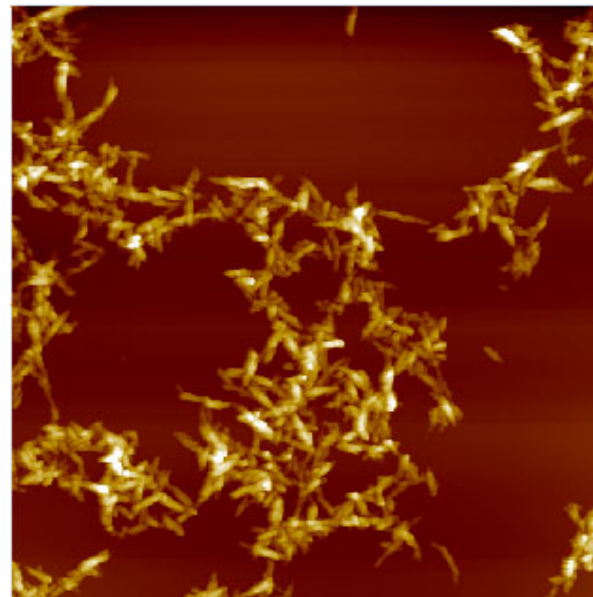# Preparation of cellulose nanocrystals – proof of concept

(1) Hydrolysis down to LODP with HCl vapor
(2) Dispersion in formic acid (72 h sonication)
→ **Over 97% yield of CNCs**
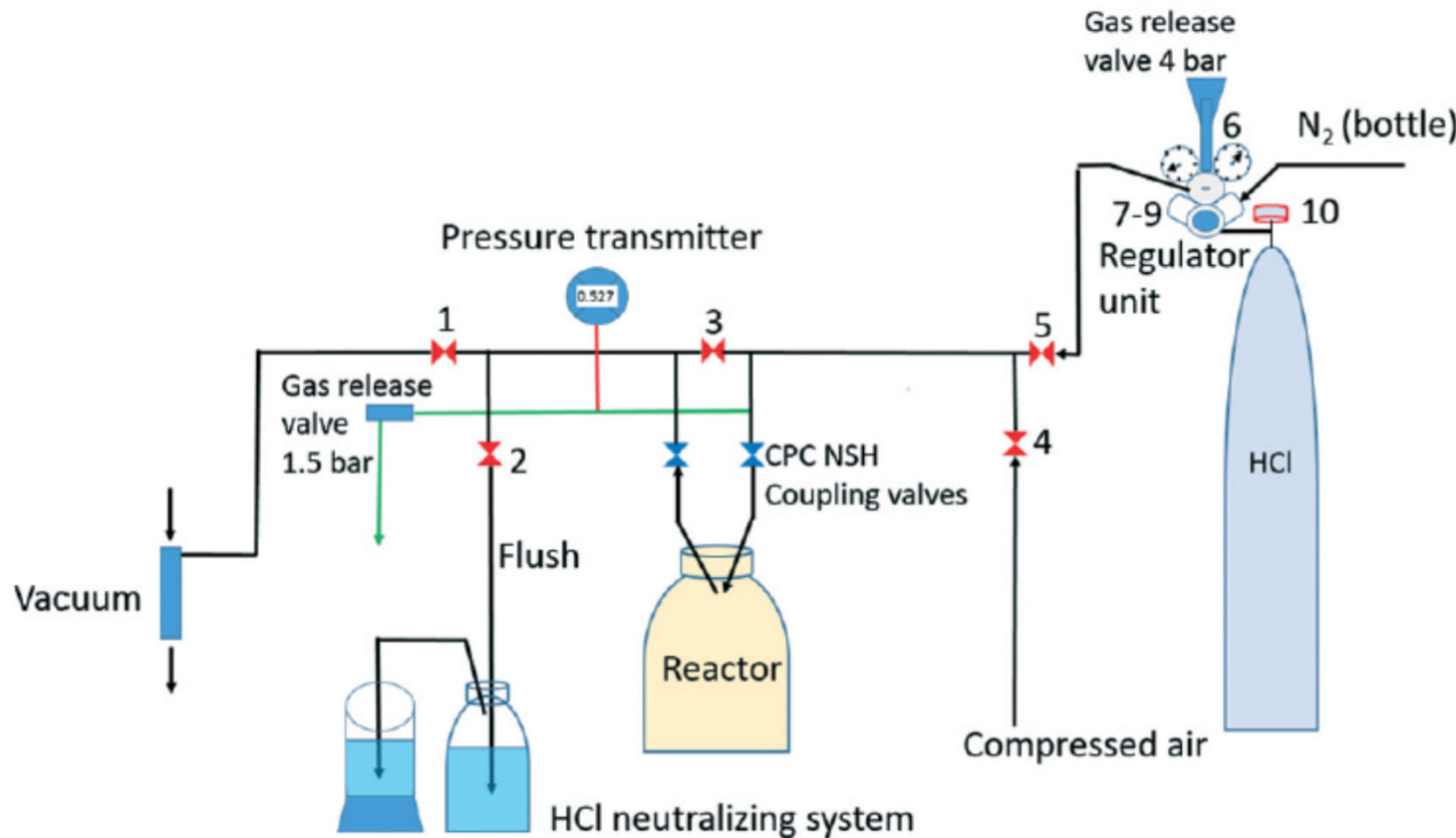
The challenge is in the dispersion.

5×5 µm^2 AFM

cryo TEM

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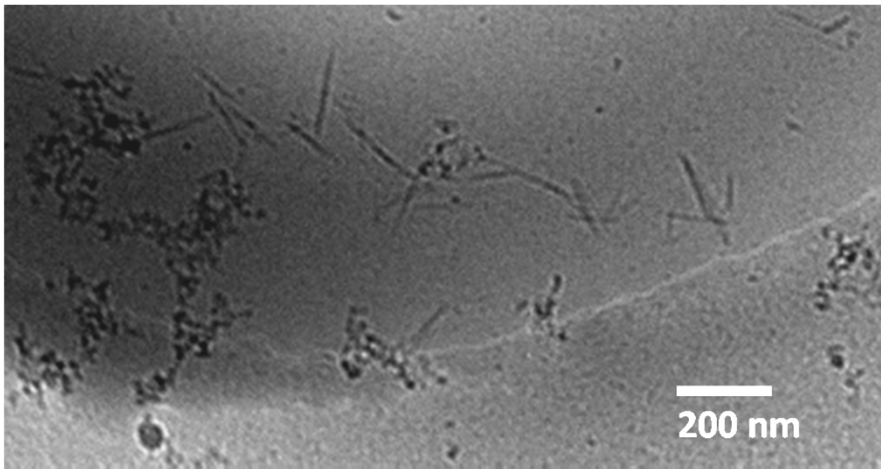
Upscale of HCl (g) hydrolysis

- Custom built reactor enables upscaling from gram scale to hundreds of grams

- HCl (g) pressure can be rised to several bars instead of vapor pressure (<0.1 bar at most)

Pääkkönen et al. *React. Chem. Eng.* **2018**, *3*, 312.

Recent development with HCl (g) hydrolysis

- Coupled with TEMPO-oxidation or other suitable charge addition method leads to good dispersion of eventual CNCs

- Higher moisture contents lead to DP reductions below LODP

- Other polymorphs (cellulose II and cellulose III) result in high glucose yields

- Building of a reactor for kg scale is underway (Business Finland R2B project H-Cel)

Note: visualization of LODP

HCl (g) | Hydrolysis
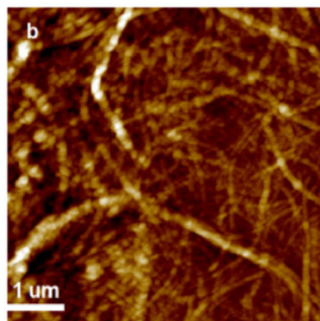
100 nm

Separated crystallites
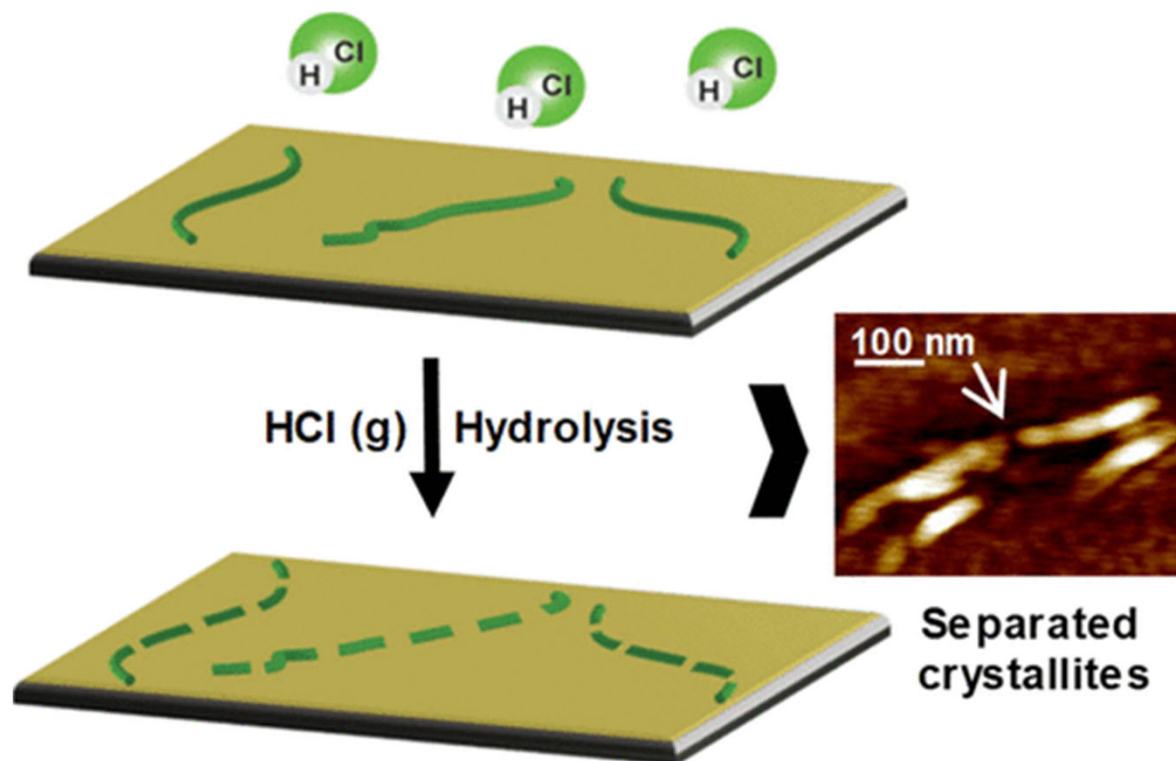
Unlike liquid, HCl gas does not induce any morphological changes on a cellulose substrate

→ Hydrolysis by HCl gas enables visualization of the LODP phenomenon

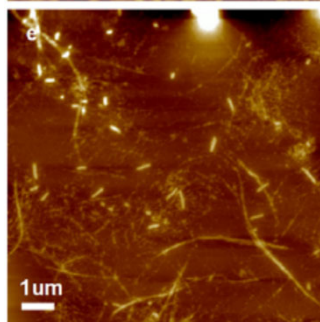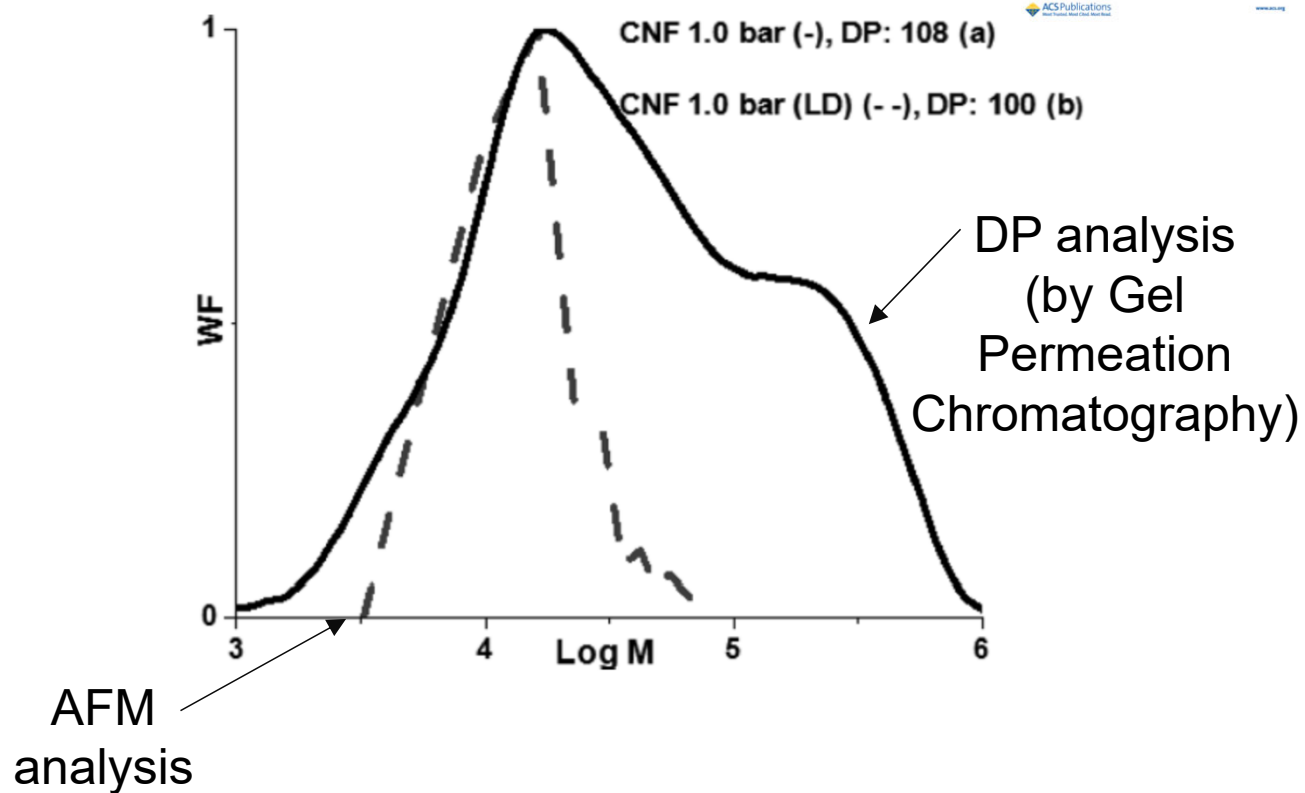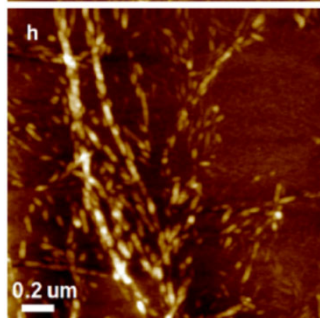Spiliopoulos et al. *Biomacromolecules* **2021**, 22, 1399.

Visualization of LODP

Control reference

0.6 bar HCl 30 min

1.0 bar HCl 30 min

CNF 1.0 bar (-), DP: 108 (a)

CNF 1.0 bar (LD) (- -), DP: 100 (b)

DP analysis (by Gel Permeation Chromatography)

AFM analysis

Spiliopoulos et al. *Biomacromolecules* **2021**, *22*, 1399.

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Presemo

Take out your smartphones or laptops:

https://presemo.aalto.fi/l2010degradation
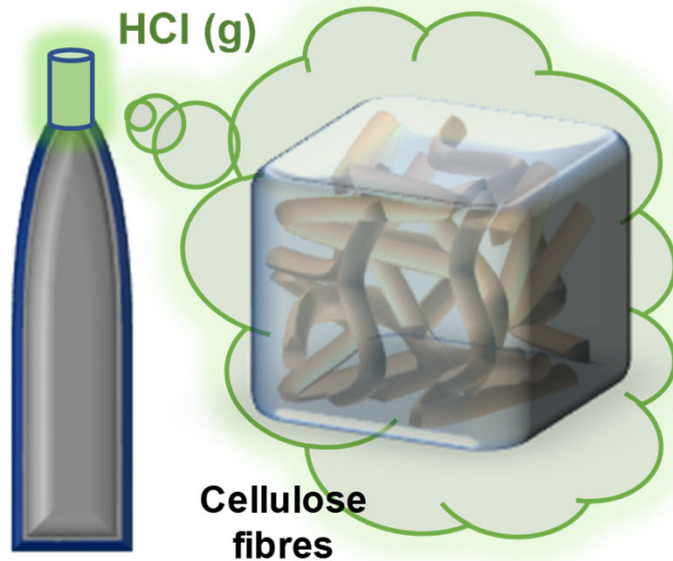
Case study: how HCl (g) hydrolysis influences accessibility / hydrolysis

Water retention after HCl (g)
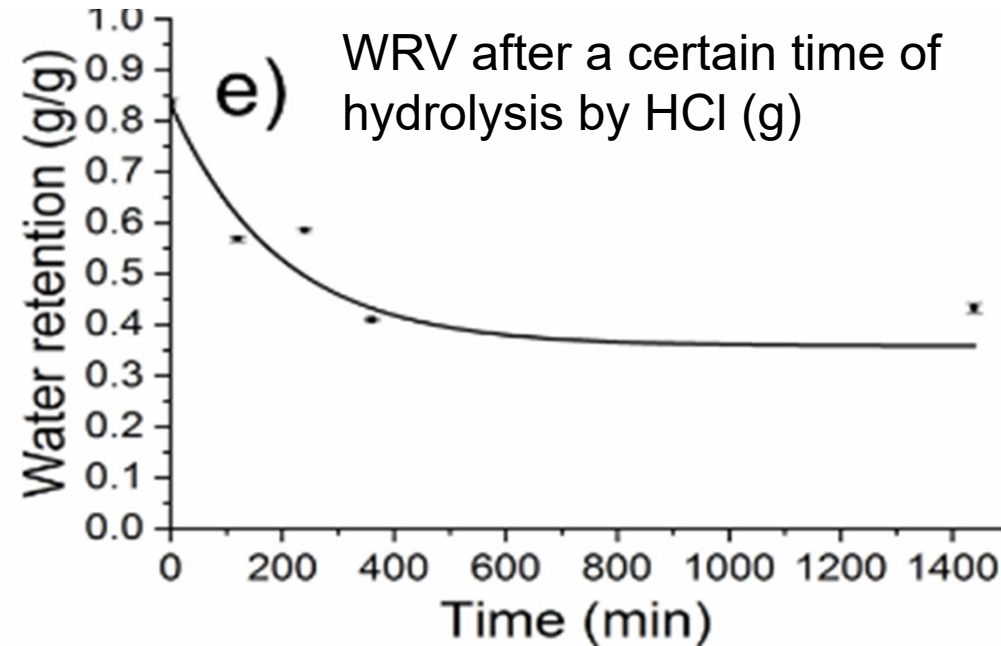
WRV after a certain time of hydrolysis by HCl (g)
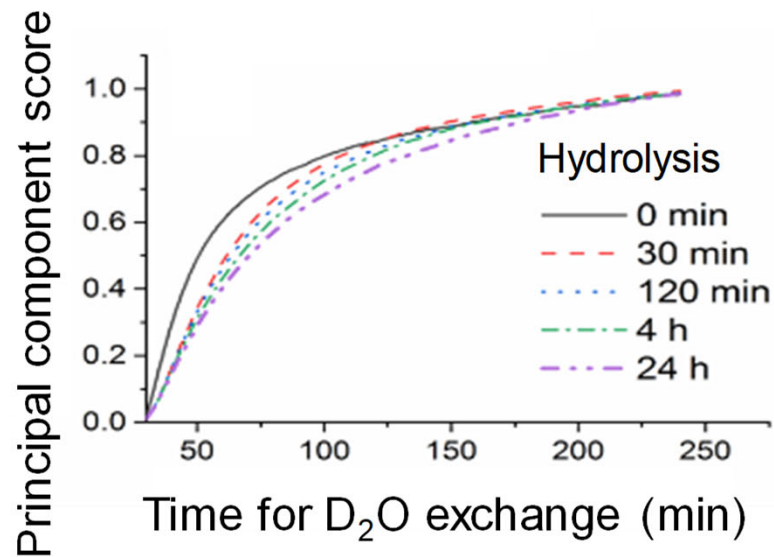
- Hydrolysis of dry (~95%) cellulose fibres to levelling off degree of polymerization by HCl (g)
→ Hydrolysis leads to reduced water uptake

Substrate: cotton linters

Further results on water uptake

Exposure to D_2O vapour in FTIR

Principal component score vs. Time for D_2O exchange (min)

Hydrolysis
— 0 min
- - - 30 min
····· 120 min
-·-·- 4 h
-··-··- 24 h

Dynamic vapour sorption

$m/m0$ vs. RH (%)
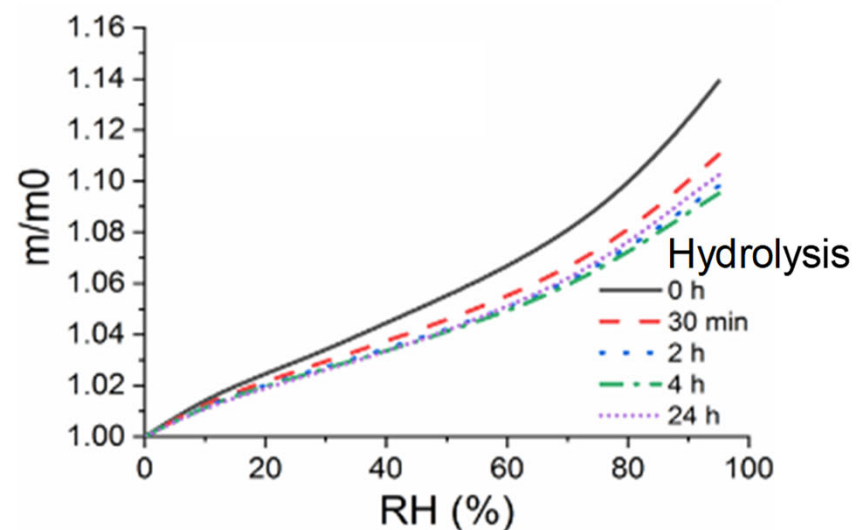
Hydrolysis
— 0 h
- - - 30 min
····· 2 h
-·-·- 4 h
····· 24 h

- Hydrolysis of dry (~95%) cellulose fibres to levelling off degree of polymerization by HCl (g)
→ Hydrolysis leads to reduced water uptake
- Reduced water uptake monitored by: WRV, D_2O exchange, dynamic vapor sorption, and thermoporosimetry

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Solala et al. *Macromol. Rapid Commun.* **2021**, *42*, 2100092
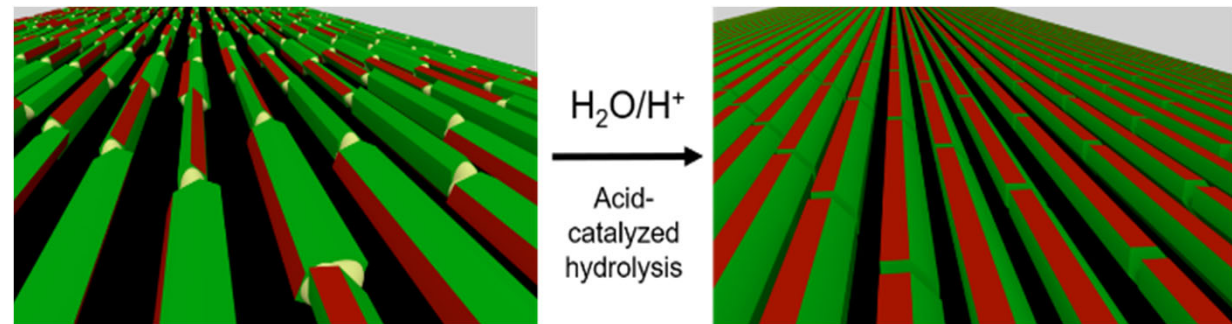
Reasoning reduced water uptake

Small angle X-ray scattering (SAXS) of centre-to-centre distance between cellulose microfibrils

Hydrolysis time, min	d, nm
0	11.6
30	9.9
120	9.4
240	9.1
360	8.9
1440	8.6

→ Clear rearrangement on the mesoscale

Hypothesis on rearrangement

H_2O/H^+

Acid-catalyzed hydrolysis

Hydrophobic planes on cellulose crystals coalesce and the microfibrils move closer to each other.

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# Effect of ultrastructure and accessibility on enzymatic hydrolysis

Basics of enzymatic hydrolysis

Cellulose degrading enzymes consist of: - cellulose binding module
- catalytic core
- linker between the two

linker

binding
module
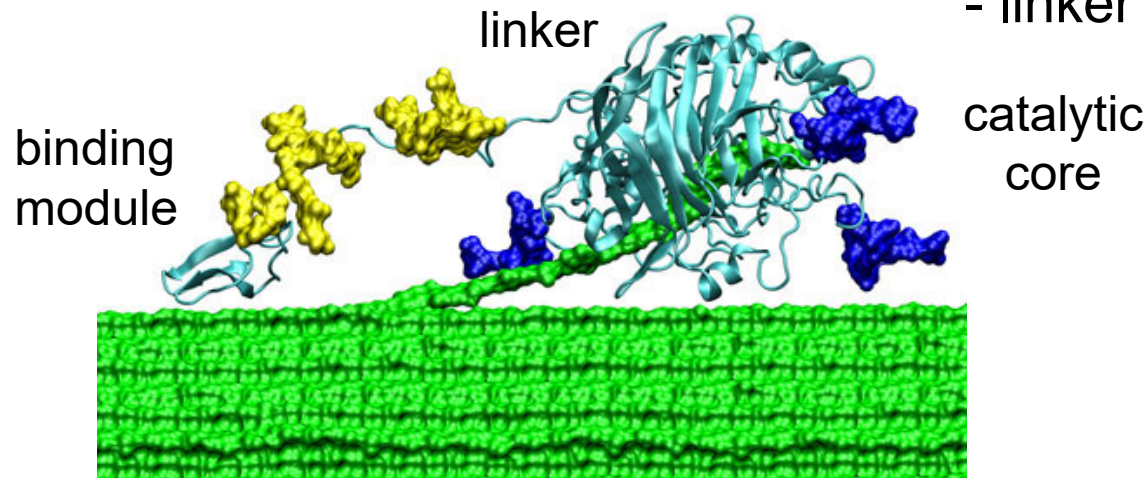
catalytic
core

(1) The enzyme undergoes *specific adsorption* on cellulose via binding module
(2) Cellulose is degraded by the catalytic core

Basics of enzymatic hydrolysis
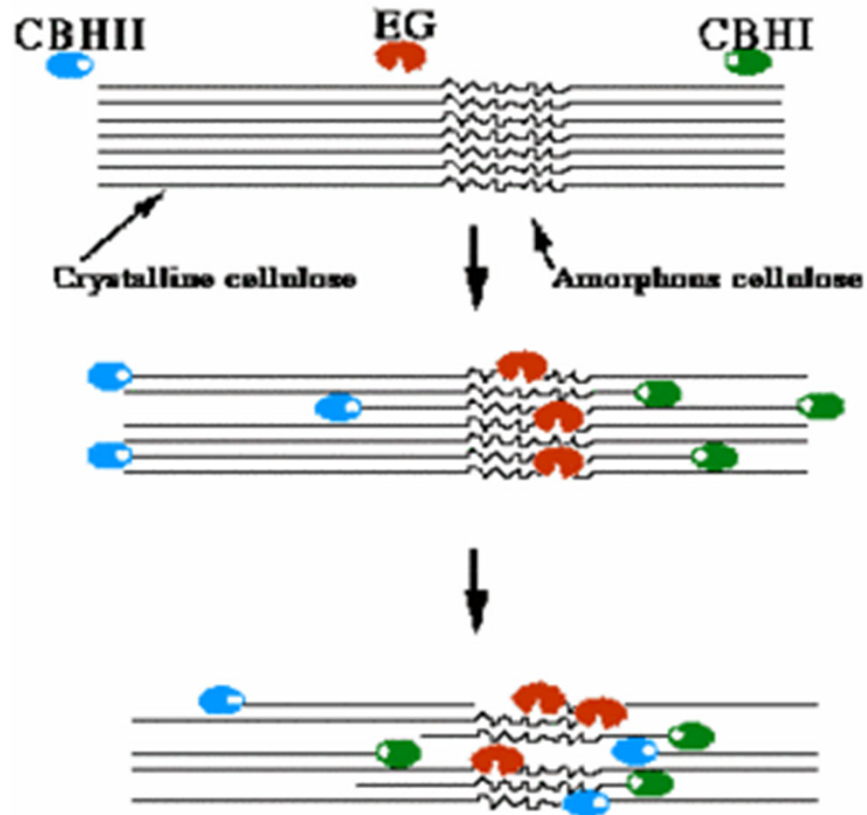
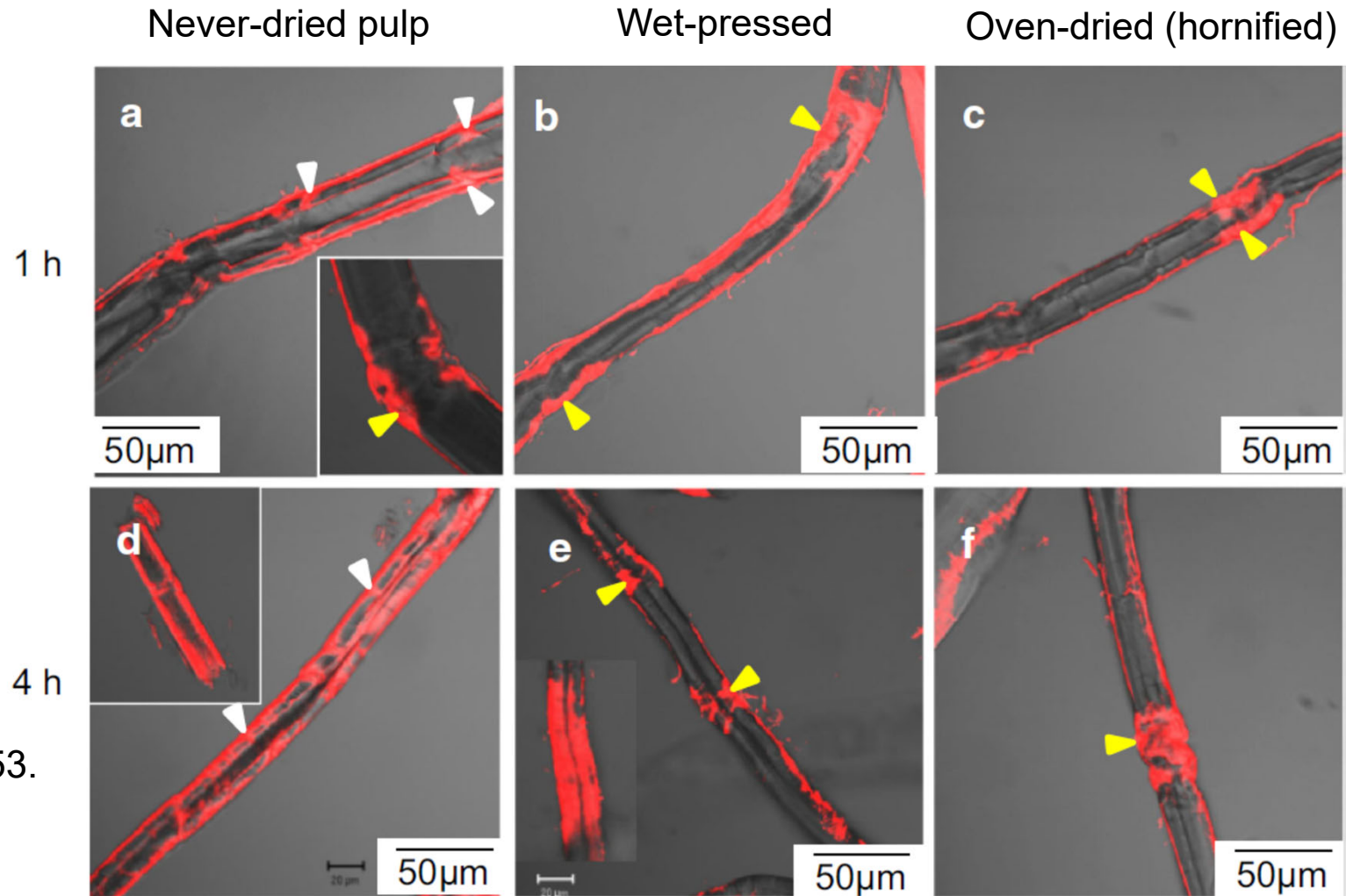CBH I works from the reducing end of the cellulose chain.

CBH II works from the non-reducing end of the cellulose chain.

EG works on the amorphous segments of the microfibril.

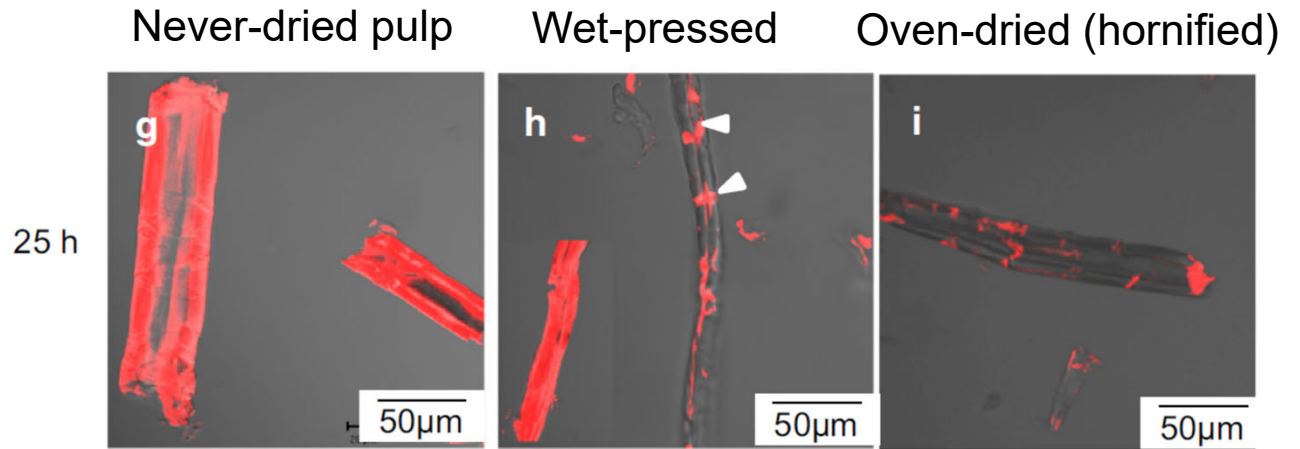**NOTE: A mixture or CBH, EG and β-glucanase is always required to completely convert cellulose to glucose.**
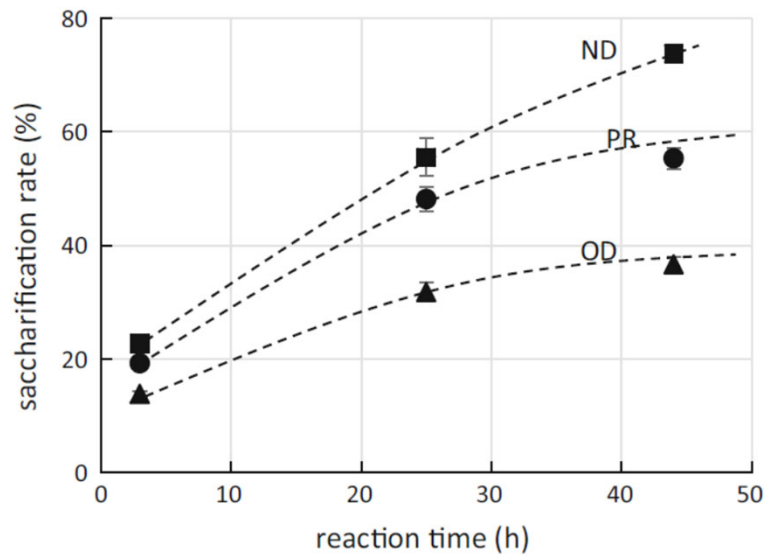
Hornification and hydrolysis

Never-dried pulp

Wet-pressed

Oven-dried (hornified)

Fluorescent labelling of cellulase enzymes

1 h

4 h

Imai et al.
Cellulose **2019**, *26*, 7653.

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Hornification and hydrolysis

Never-dried pulp   Wet-pressed   Oven-dried (hornified)

25 h

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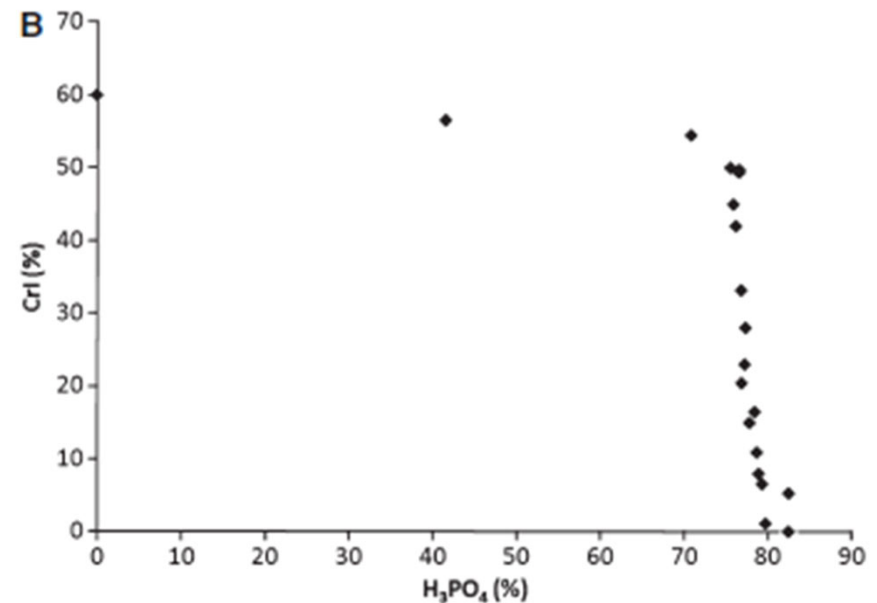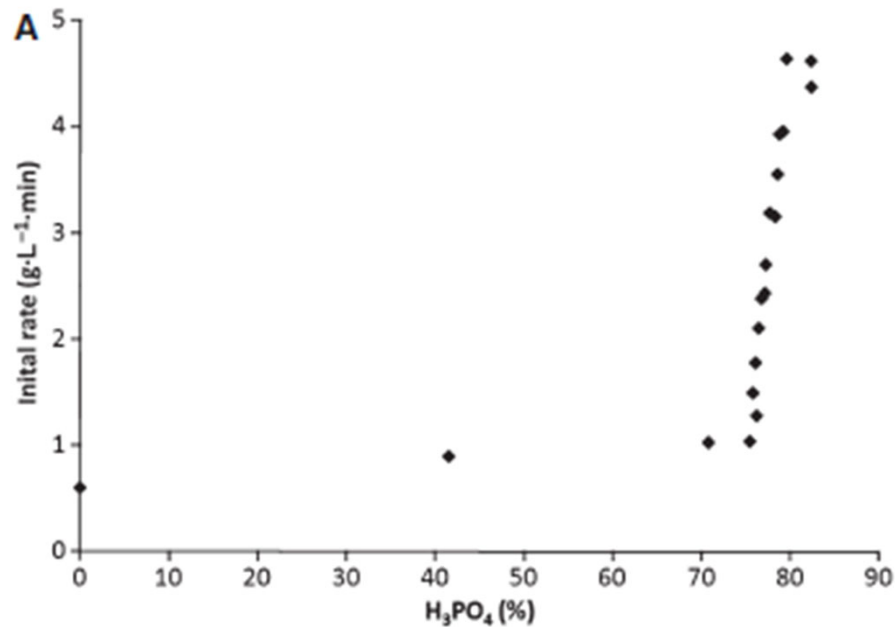Engineering

Enzymatic hydrolysis proceeding

Contrary to what is commonly thought, crystallinity of cellulose stays constant throughout enzymatic hydrolysis with a cellulase mixture.
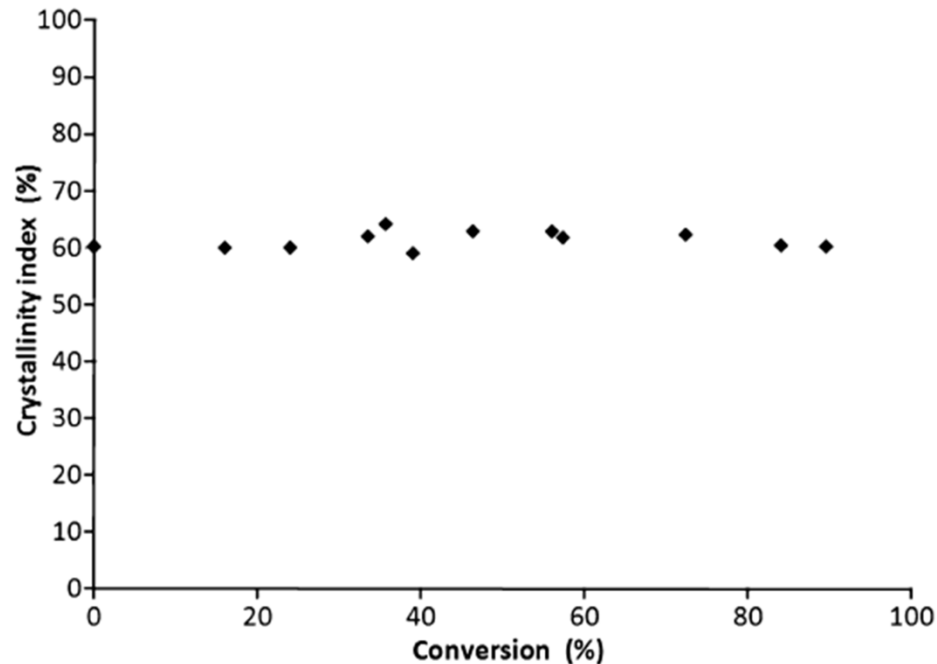
Amorphous segments
are NOT hydrolysed first.

Hall et al. *FEBS J.* **2010**, *277*, 1571.

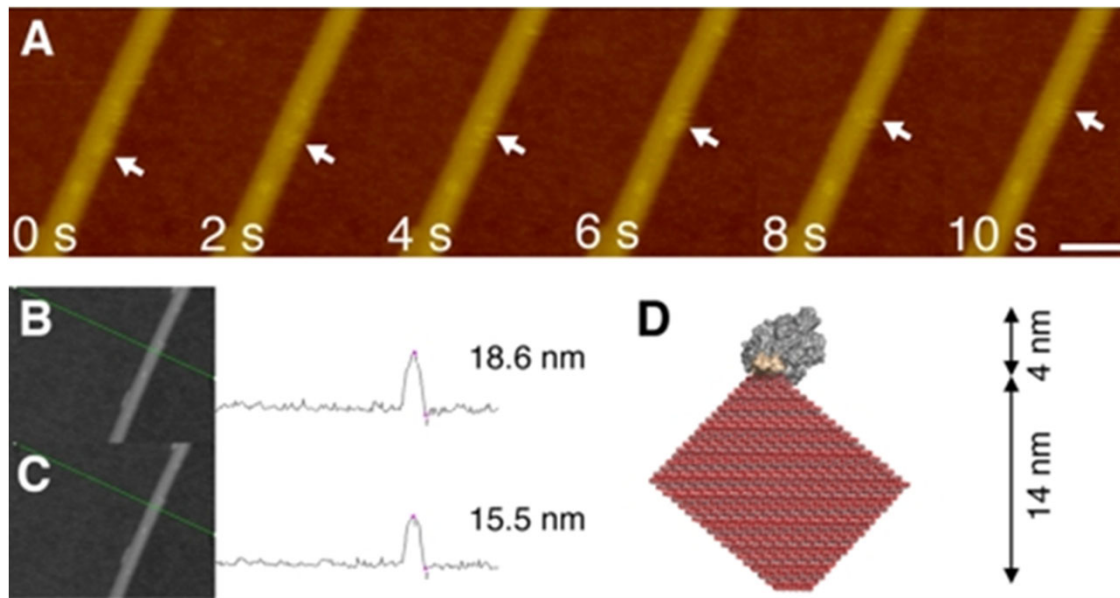# Enzymatic hydrolysis proceeding

Hydrolysis rate depends
on the crystallinity.

→ **Decrease in crystallinity of cellulose → increase in hydrolysis rate.**

Enzymatic hydrolysis: visualization

- Cellulose binding domains are not the selective components that, for example, recognize reducing or non-reducing chain ends
- Rather, the enzyme can adsorb at any point on the microfibril and the catalytic core causes the enzyme to move according to its selectivity.

Example: movement of CBH I sliding on crystalline cellulose towards the reducing end. (Visualized by high-speed AFM.)

Igarashi et al. *J. Biol. Chem.* **2009**, *284*, 36186.

Enzymatic hydrolysis: visualization
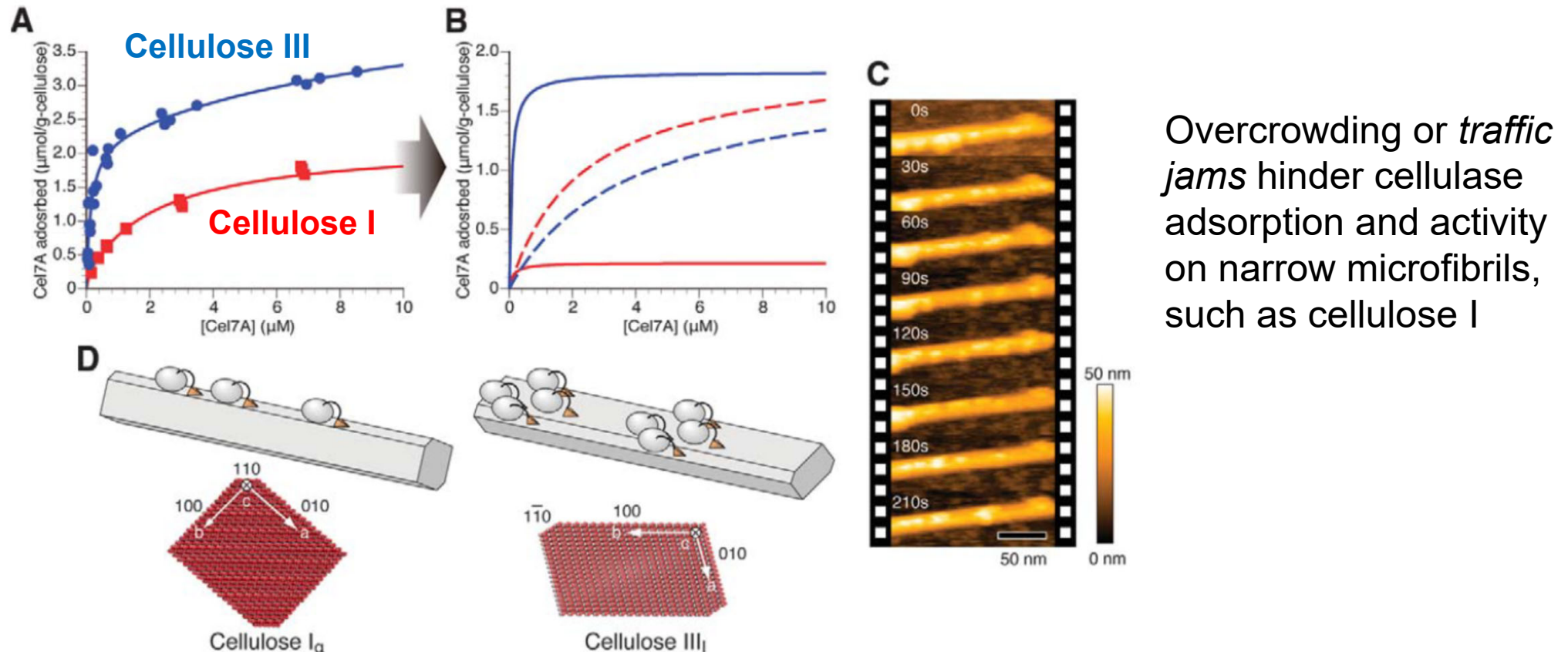
Overcrowding or *traffic jams* hinder cellulase adsorption and activity on narrow microfibrils, such as cellulose I

Igarashi et al. *Science* **2011**, *333*, 1279

Presemo

Take out your smartphones or laptops:

https://presemo.aalto.fi/l2010degradation

Summary

- **Acid hydrolysis is an anomaly within cellulose reactions: reduced accessibility results in an increased hydrolysis rate**

- **Hydrolysis in solid/gas system (HCl (g)) is not governed by accessibility**

- **Enzymatic hydrolysis is governed by a complex interplay between crystallinity, crystallite size, and basic accessibility**