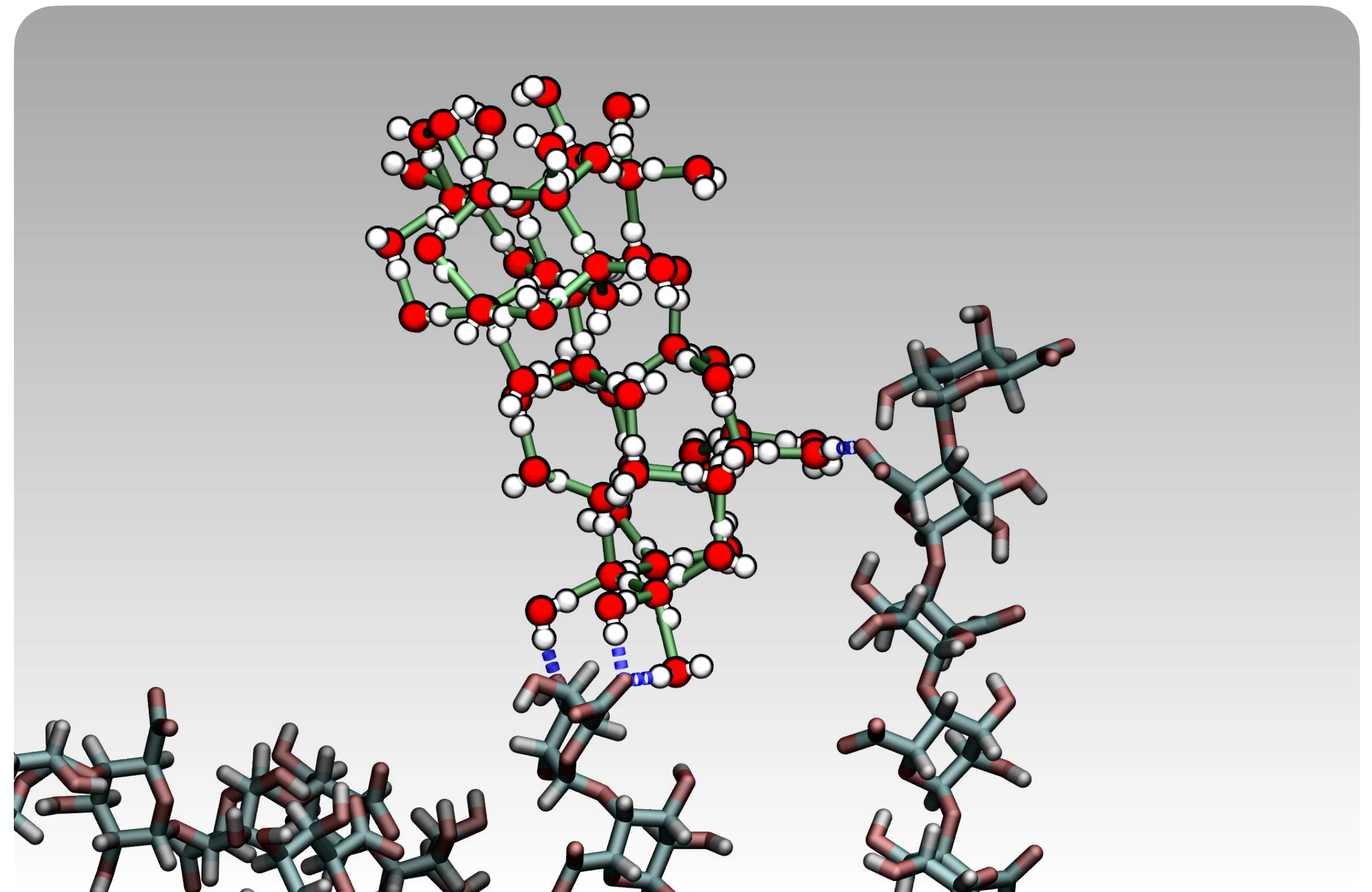



First steps toward Understanding Ice Formation in Plants



Prof. Gabriele C. Sosso
email: g.sosso@warwick.ac.uk

The good ol' days (2011): beta testing HECToR...

 HPC-Europa Transnational Access visit
To: gabriele.sosso@mater.unimib.it, Cc: Davide Donadio, Alexey Cheptsov, [Details](#)
Reply-To: Catherine Inglis

Dear Mr. SOSSO

HPC-Europa2 Transnational Access programme

With respect to your application to the HPC-Europa2 Transnational Access programme, I am pleased to inform you that the Scientific Users Selection Panel has approved your application to visit HLRS, subject to suitable dates being agreed.

Your visit has been approved for 8 weeks, as requested in your application.

You are advised to advance the code as much as possible before the start of your visit, in order to take full advantage of your host's expertise and the available computing facilities. Similarly, although the reviewers commented that you already had good knowledge of the use of parallel codes, you are nevertheless encouraged to further consolidate your parallel programming skills before the visit, if possible.

HLRS will contact you directly with further information.

In the meantime, if you have any questions please do not hesitate to contact the Transnational Access team at access@hpc-europa.org

Yours sincerely

Catherine Inglis
HPC-Europa2 Transnational Access Co-ordinator

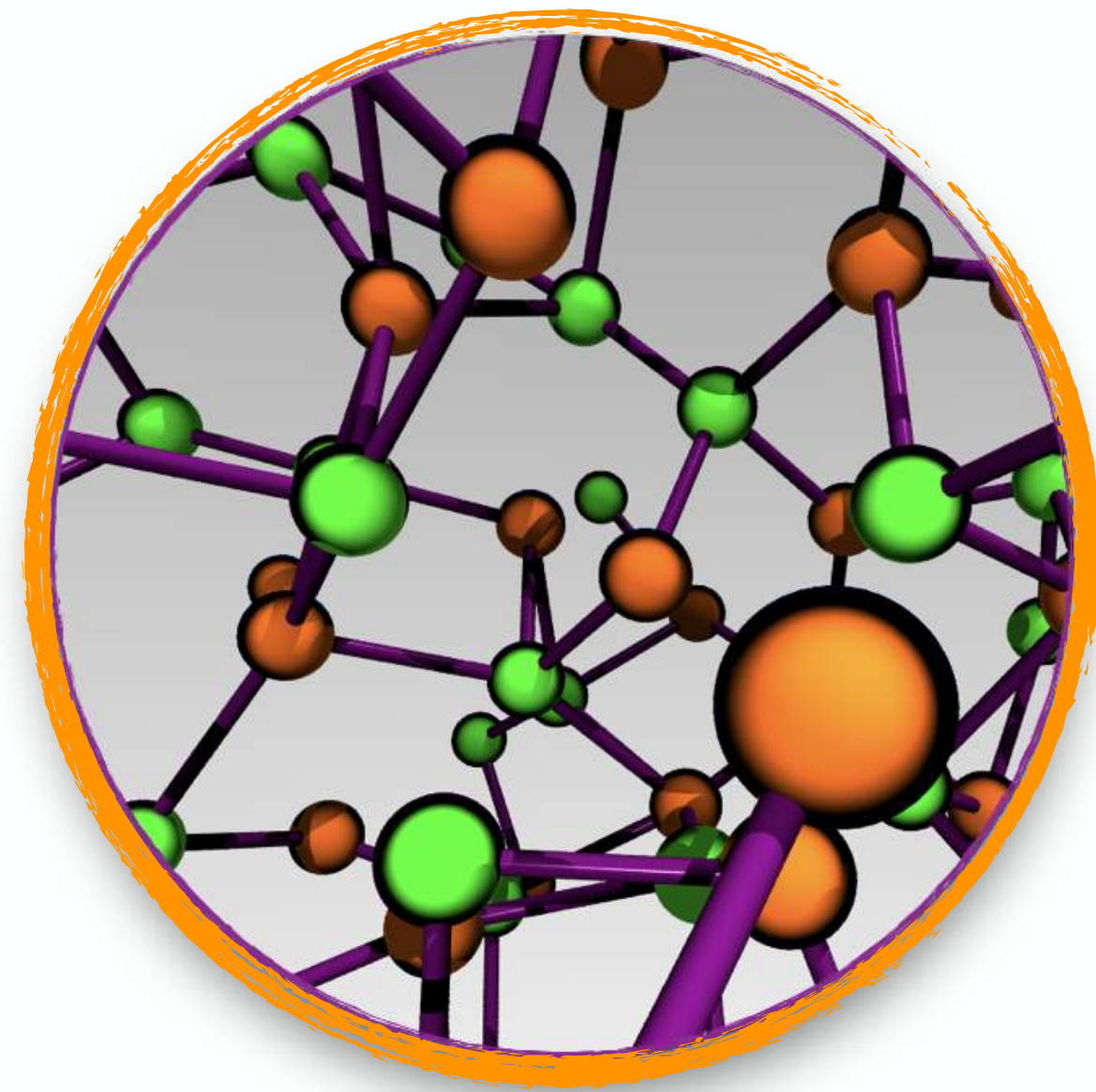
Top 500 – UK

Rank	Site	System	Cores	Rmax (TFlop/s)	Rpeak (TFlop/s)	Power (kW)
19	University of Edinburgh United Kingdom	HECToR - Cray XE6, Opteron 6276 16C 2.30 GHz, Cray Gemini interconnect Cray Inc.	90,112	660.2	829.0	

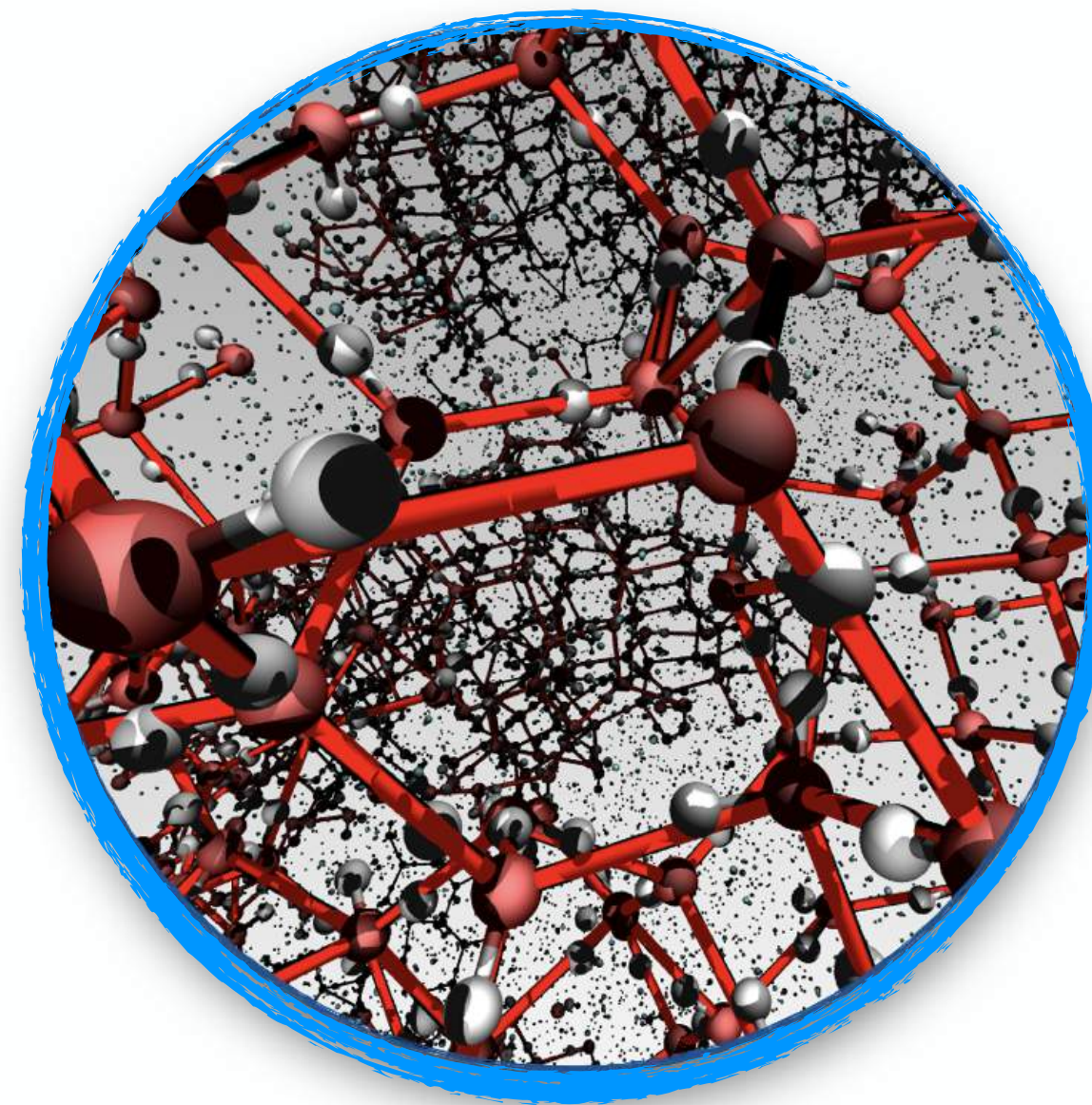
The SossoGroup - what do we do?



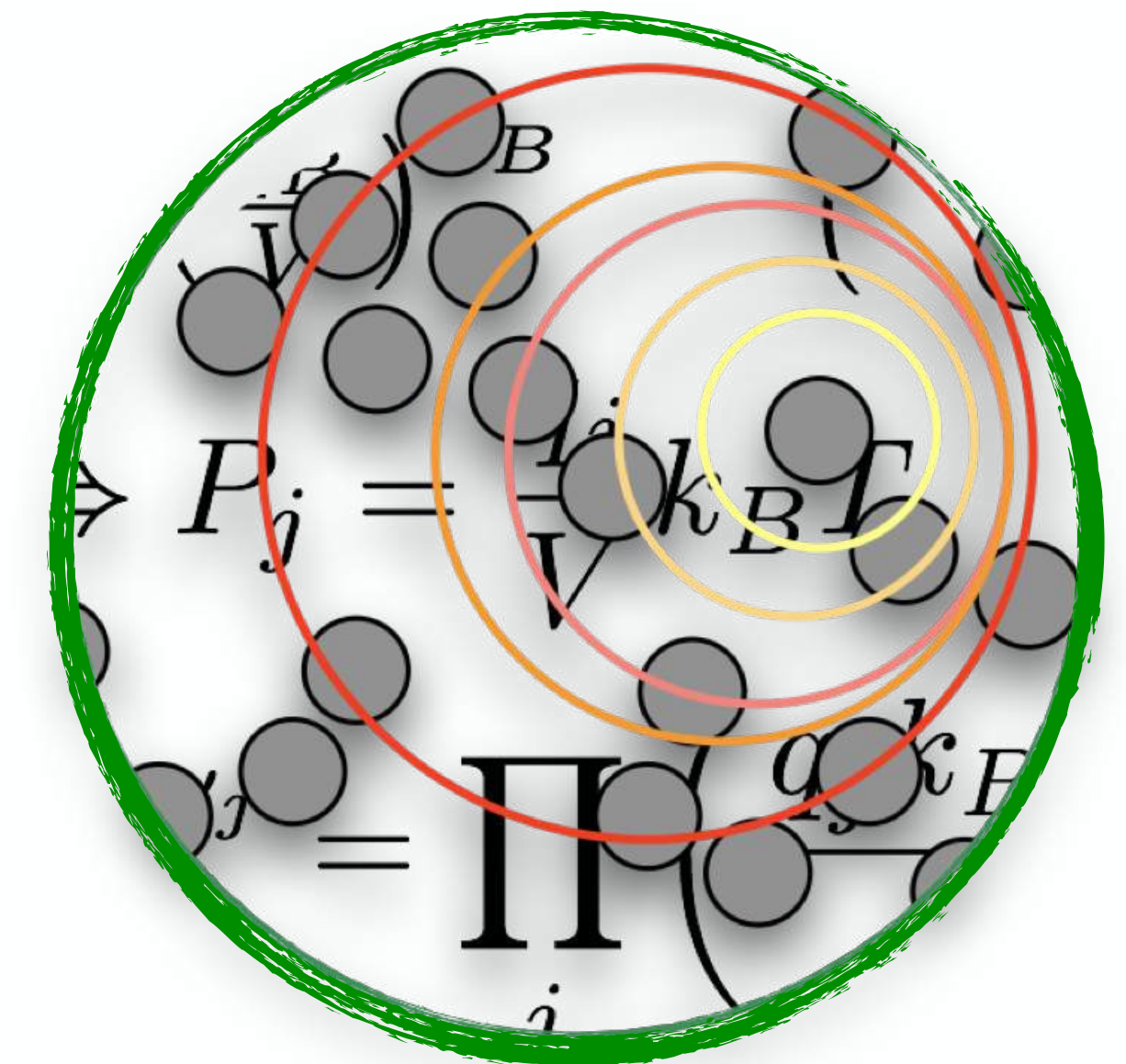
DimEnslON at Warwick
Molecular Simulations of
Disordered Systems and
Phase Transitions



Disordered
Systems



Phase
Transitions



Computational
Methods

- Methodological aspects
- Cryopreservation
- Heterogeneous ice nucleation

RESEARCH ARTICLE | MAY 08 2025

You reap what you sow: On the impact of nuclei morphology on seeded molecular dynamics simulations

Special Collection: Michele Parrinello Festschrift

Katarina E. Blow ; Gabriele C. Sosso ; David Quigley 



+ Author & Article Information

J. Chem. Phys. 162, 184503 (2025)

<https://doi.org/10.1063/5.0261353> Article history 

Article [Open access](#) | Published: 15 September 2024

Data-driven discovery of potent small molecule ice recrystallisation inhibitors

[Matthew T. Warren](#), [Caroline I. Biggs](#), [Akalabya Bissoyi](#), [Matthew I. Gibson](#)  & [Gabriele C. Sosso](#) 

Nature Communications 15, Article number: 8082 (2024) | [Cite this article](#)

7532 Accesses | 6 Citations | 86 Altmetric | [Metrics](#)

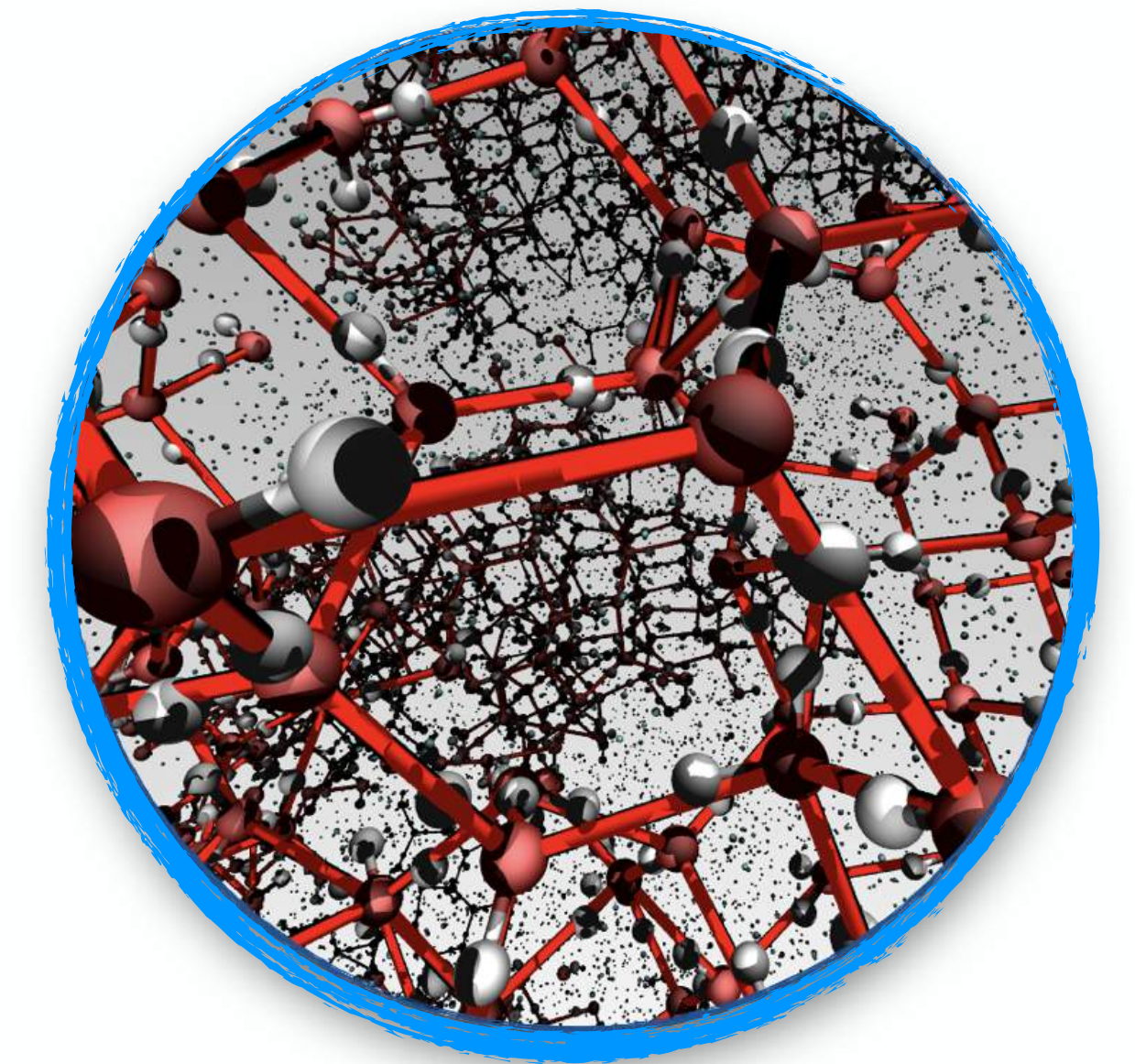
DOI: [10.1039/D3FD00097D](https://doi.org/10.1039/D3FD00097D) (Paper) *Faraday Discuss.*, 2024, 249, 114-132

Understanding the impact of ammonium ion substitutions on heterogeneous ice nucleation[†]

Katarina E. Blow ^{*a}, Thomas F. Whale ^b, David Quigley ^a and Gabriele C. Sosso ^b

^a Department of Physics, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK. E-mail: k.blow@warwick.ac.uk

^b Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK. E-mail: g.sosso@warwick.ac.uk



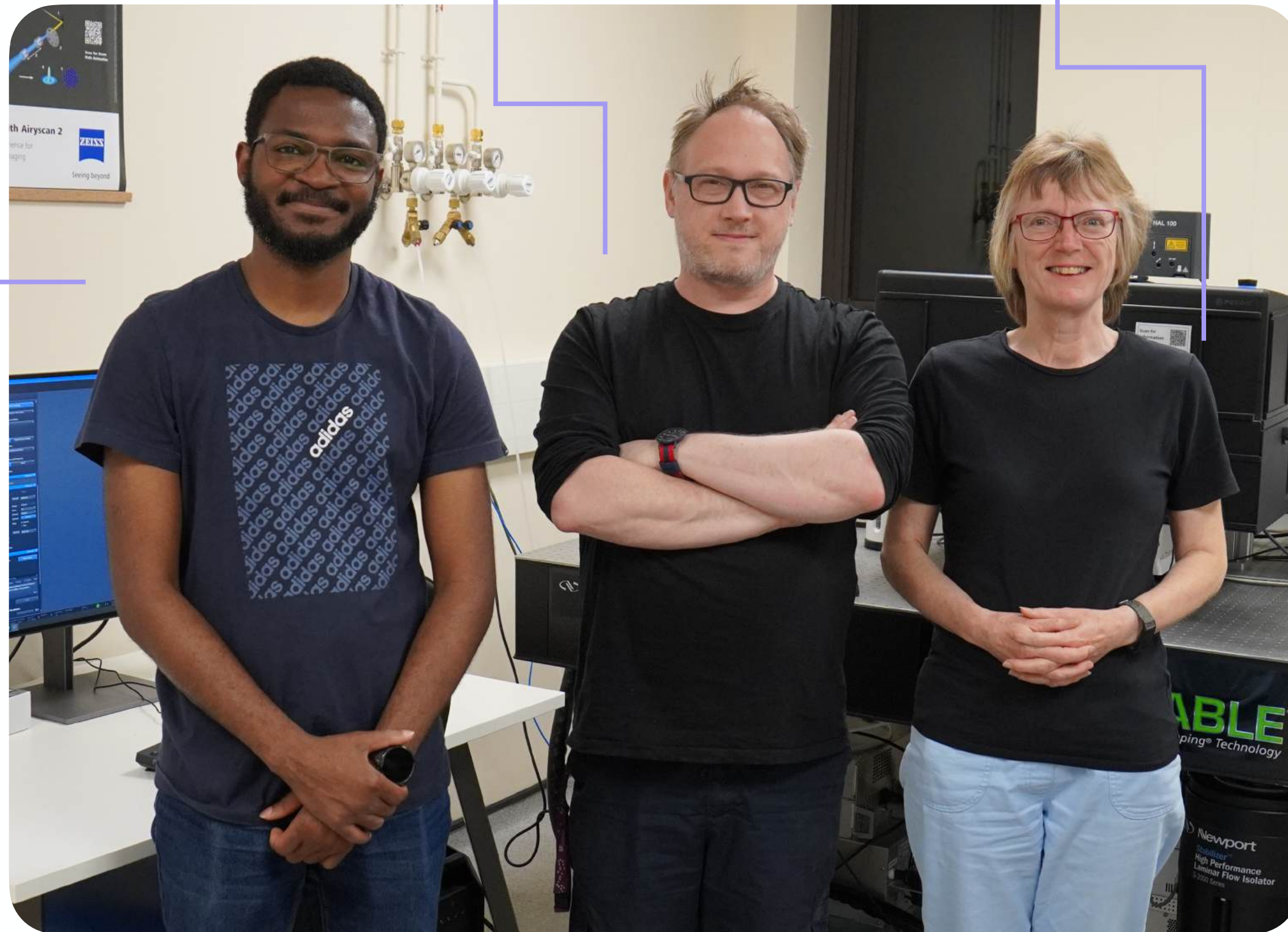
Ice

Osi Obomighie
(Durham)

Tim Hawkins
(Durham)

Heather Knight
(Durham)

Tom Whale
(Leeds)



[...]

- Ice formation in plants - why do we care?
- Ice formation through the plants cell wall
- What's next?



- **Protecting crops from frost damage**
- Enhance cold tolerance for specific plants
- Cryopreservation of seeds
- Transfer knowledge to ice formation in animal/human cells

French wine suffers worst hit in decades amid damage from frost and disease

Production forecast to plunge by 29% after unseasonal spring weather damages grapes on vine



📷 A man checks vine buds during the burning of anti-frost candles in the Luneau-Papin wine vineyard in Le Landreau, near Nantes, western France, in April. Photograph: Sebastien Salom-Gomis/AFP/Getty Images

The Guardian, 2021

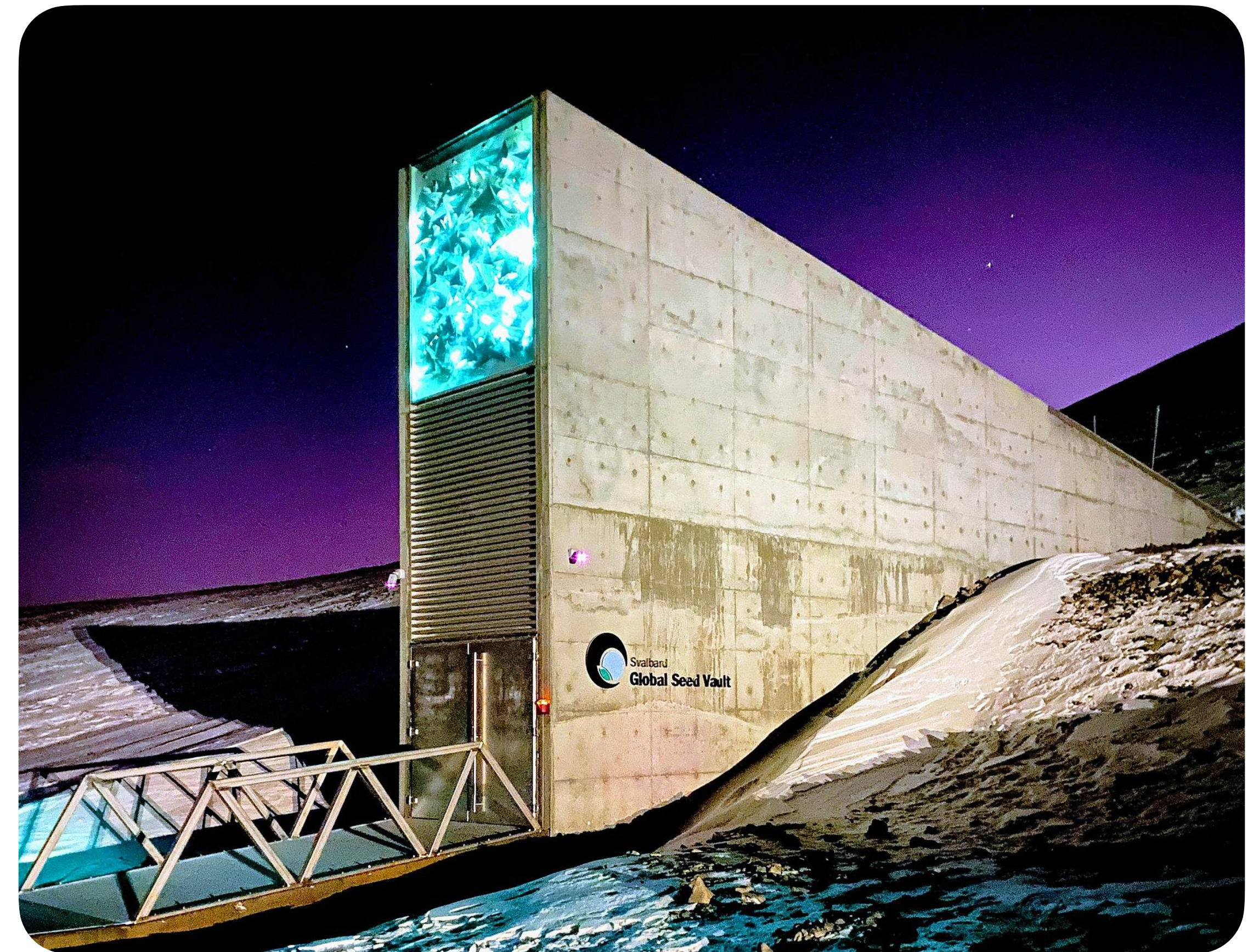
Ice formation in plants - why do we care?

- Protecting crops from frost damage
- **Enhance cold tolerance for specific plants**
- Cryopreservation of seeds
- Transfer knowledge to ice formation in animal/human cells



Ice formation in plants - why do we care?

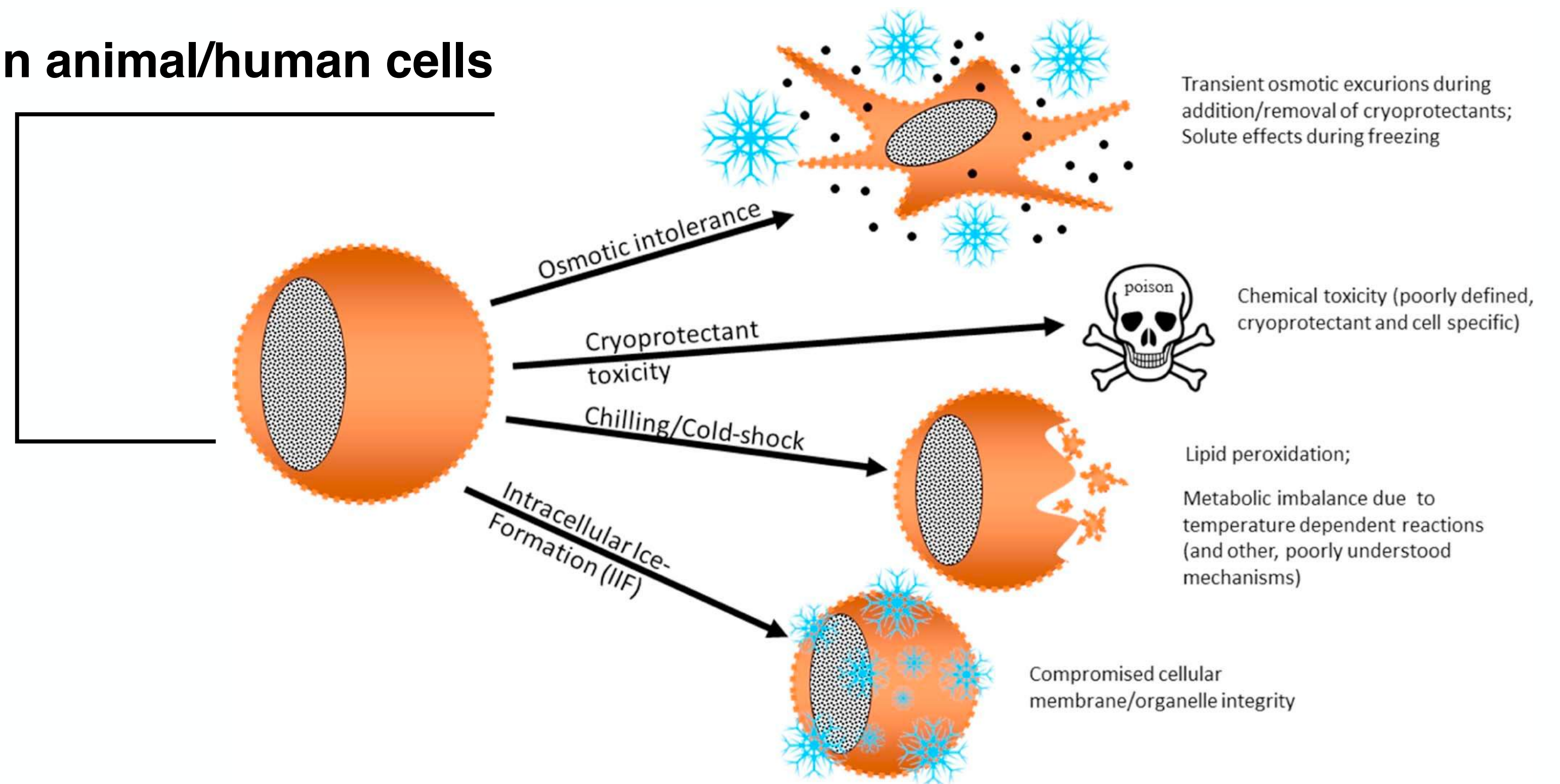
- Protecting crops from frost damage
- Enhance cold tolerance for specific plants
- **Cryopreservation of seeds** _____
- Transfer knowledge to ice formation in animal/human cells



Svalbard Global Seed Vault

Ice formation in plants - why do we care?

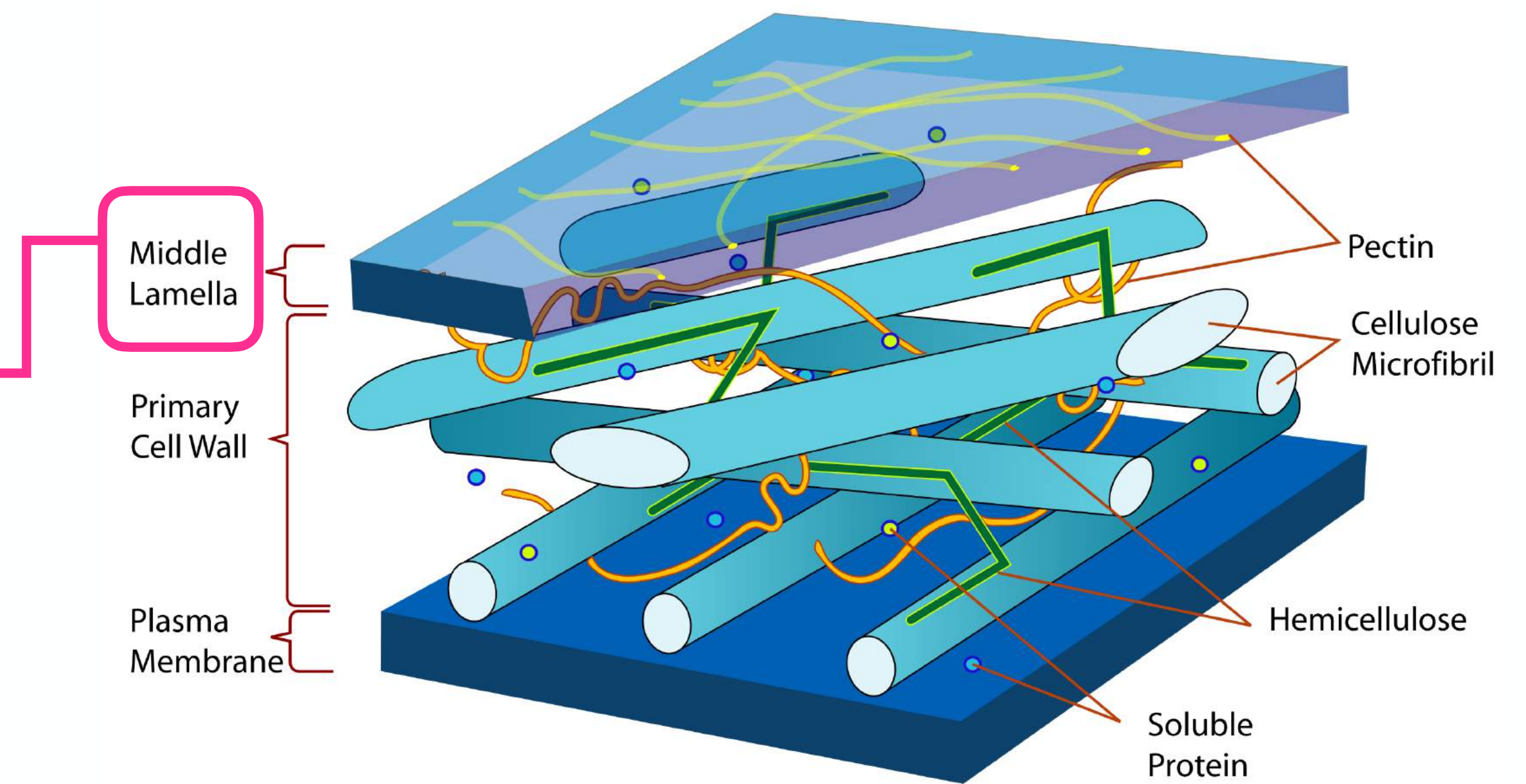
- Protecting crops from frost damage
- Enhance cold tolerance for specific plants
- Cryopreservation of seeds
- **Transfer knowledge to ice formation in animal/human cells**



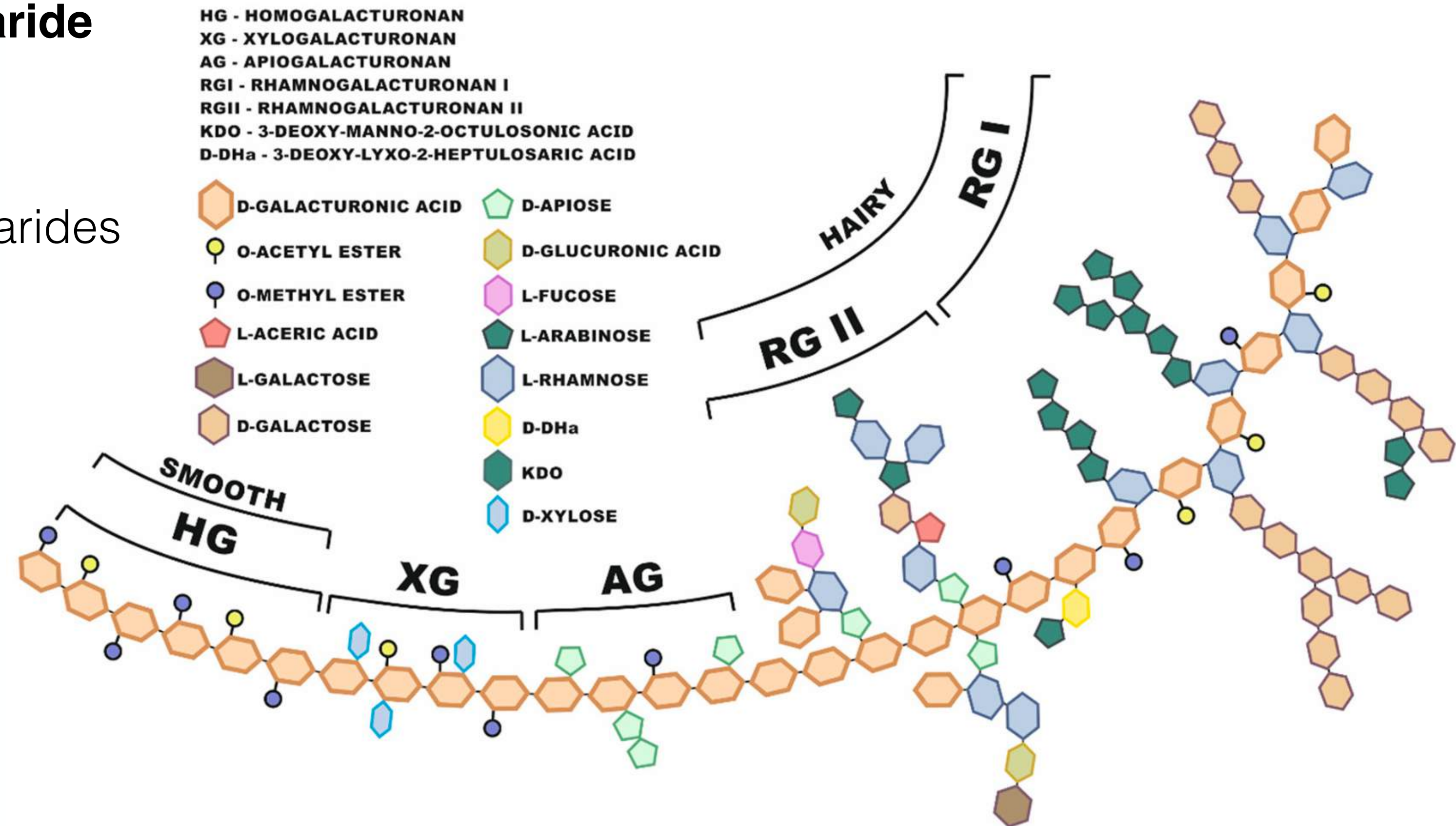
Cytherapy **2016**, 18 (6), 697–711

The Micro-scale perspective

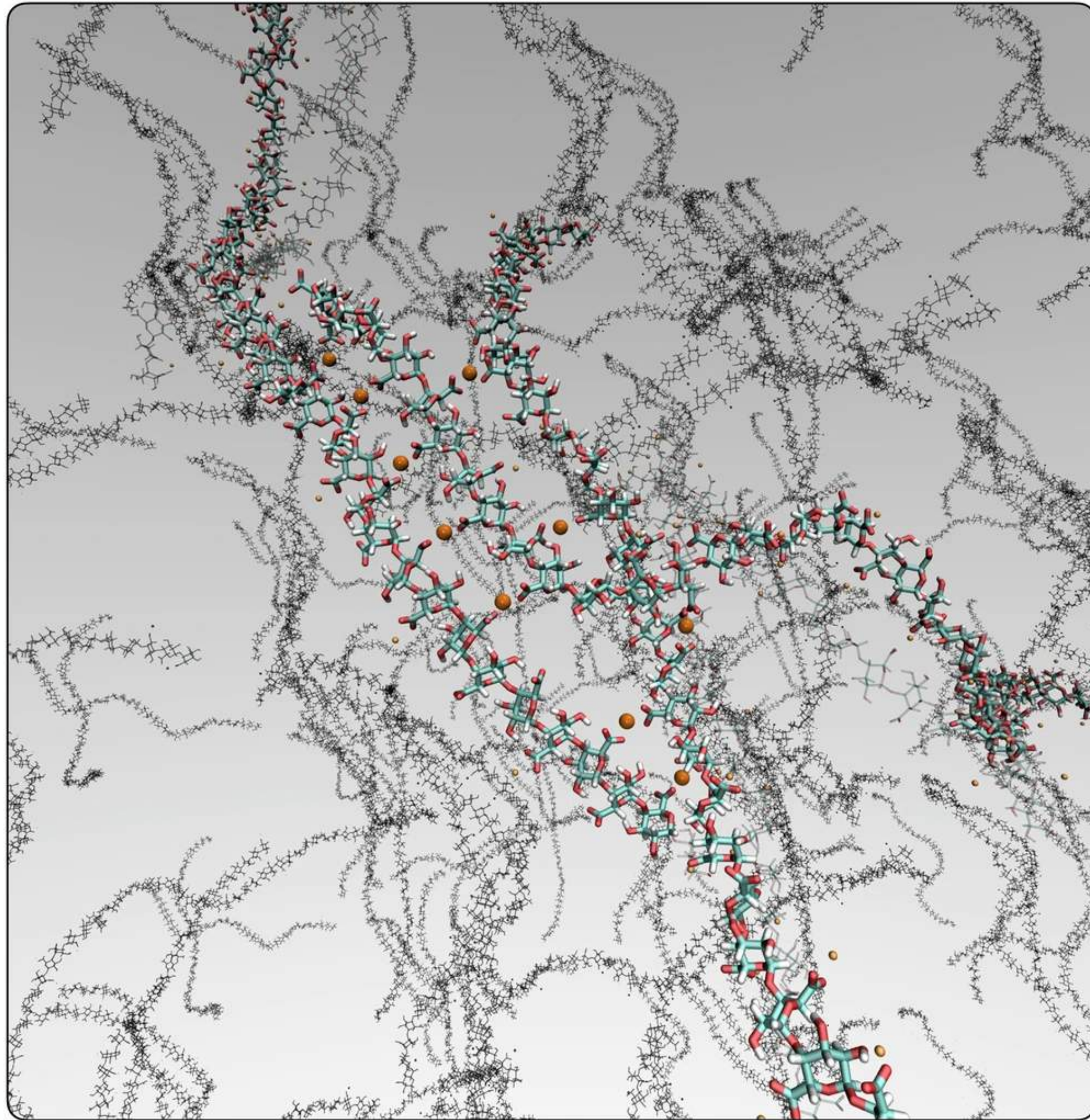
- The plant cell wall is a complex, heterogeneous system...
- We are interested in the middle lamella (the outer layer), as the ice forms in the extracellular domains
- The middle lamella is (mostly) made of pectin



- Roughly 70% of the pectin in the middle lamella is Homogalacturonan (HG)
- Roughly 5% of the pectin in the middle lamella is Rhamnogalacturonan-II (RG-II)
- **HG is a (mostly) linear polysaccharide**
- RG-II is a branched polysaccharide
- Pectin is a 3D network of polysaccharides

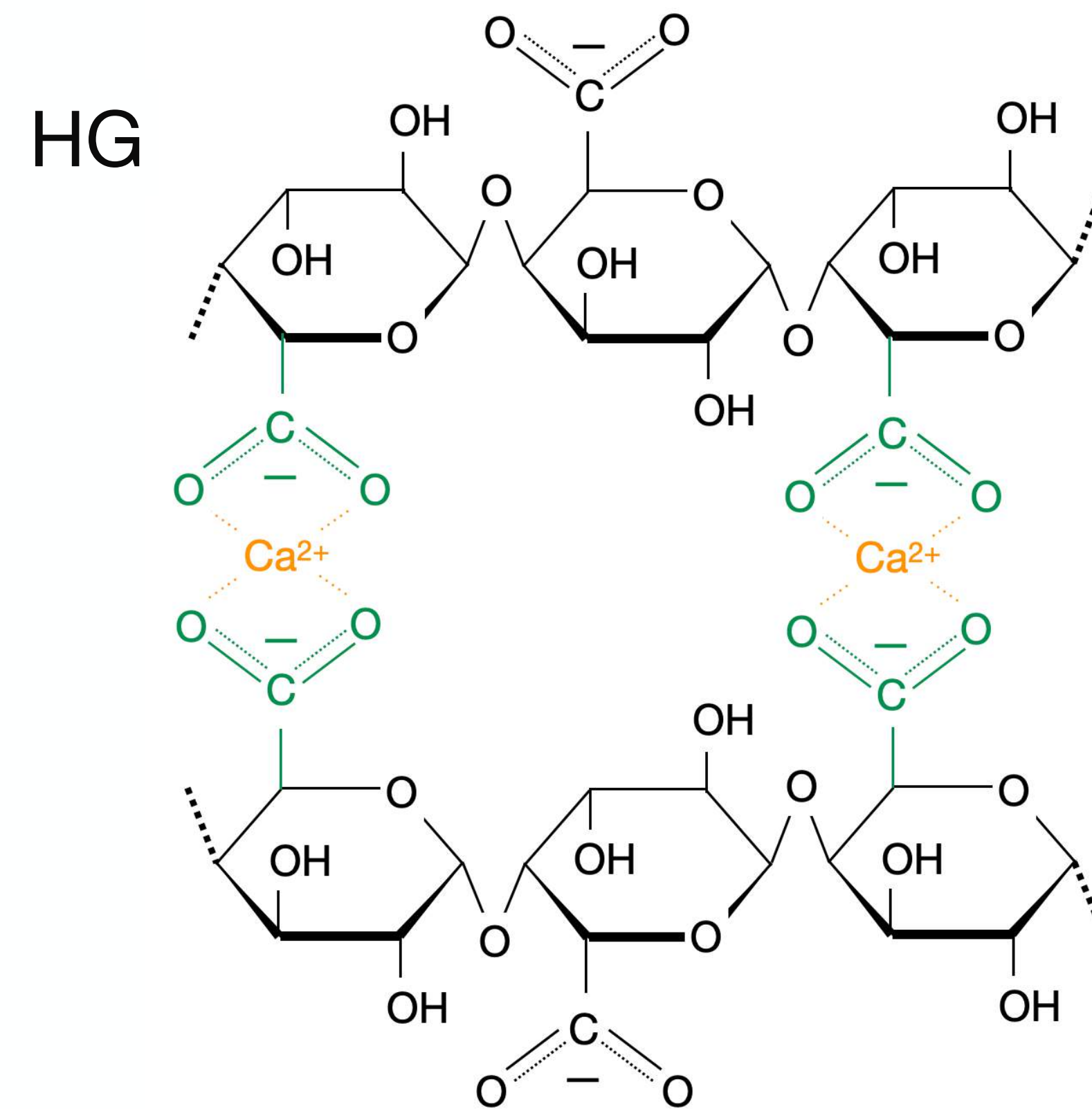


Cross-linking



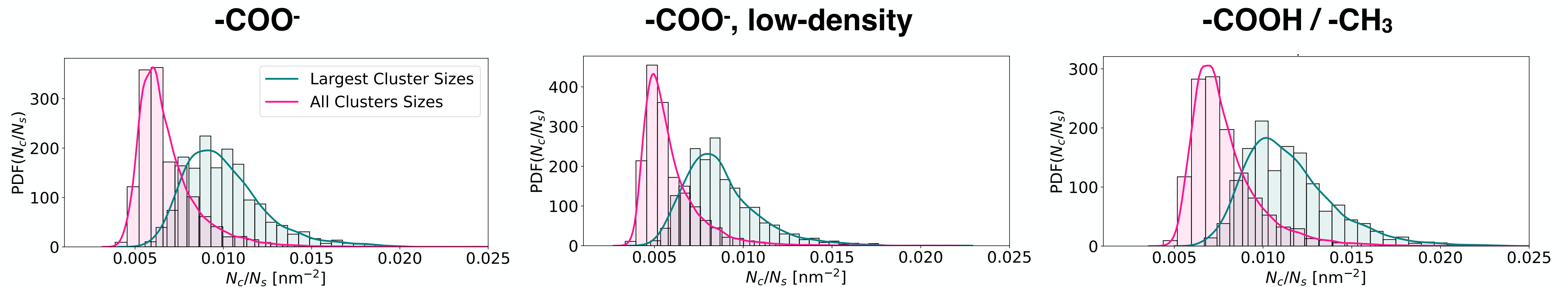


- HG chains can cross-link to each other (chiefly) thanks to Ca^{2+} ions





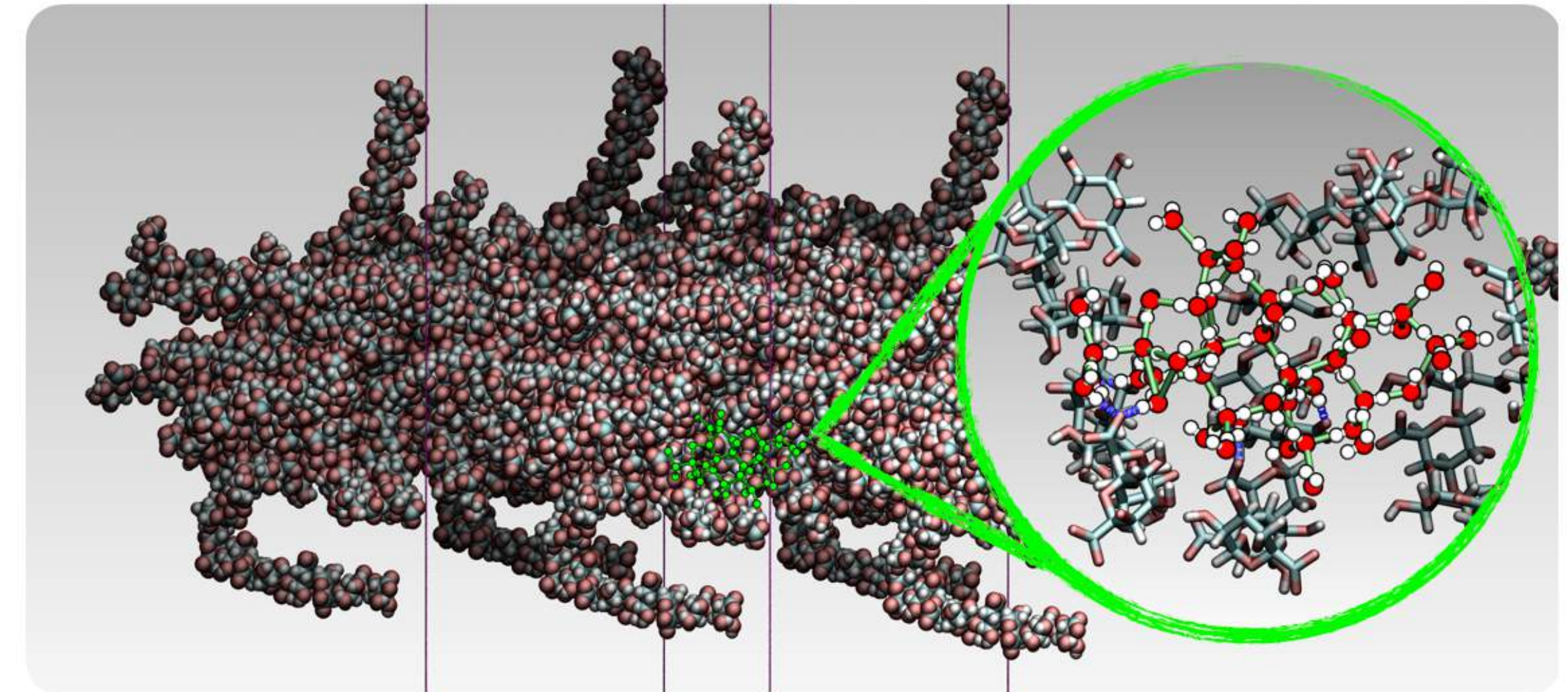
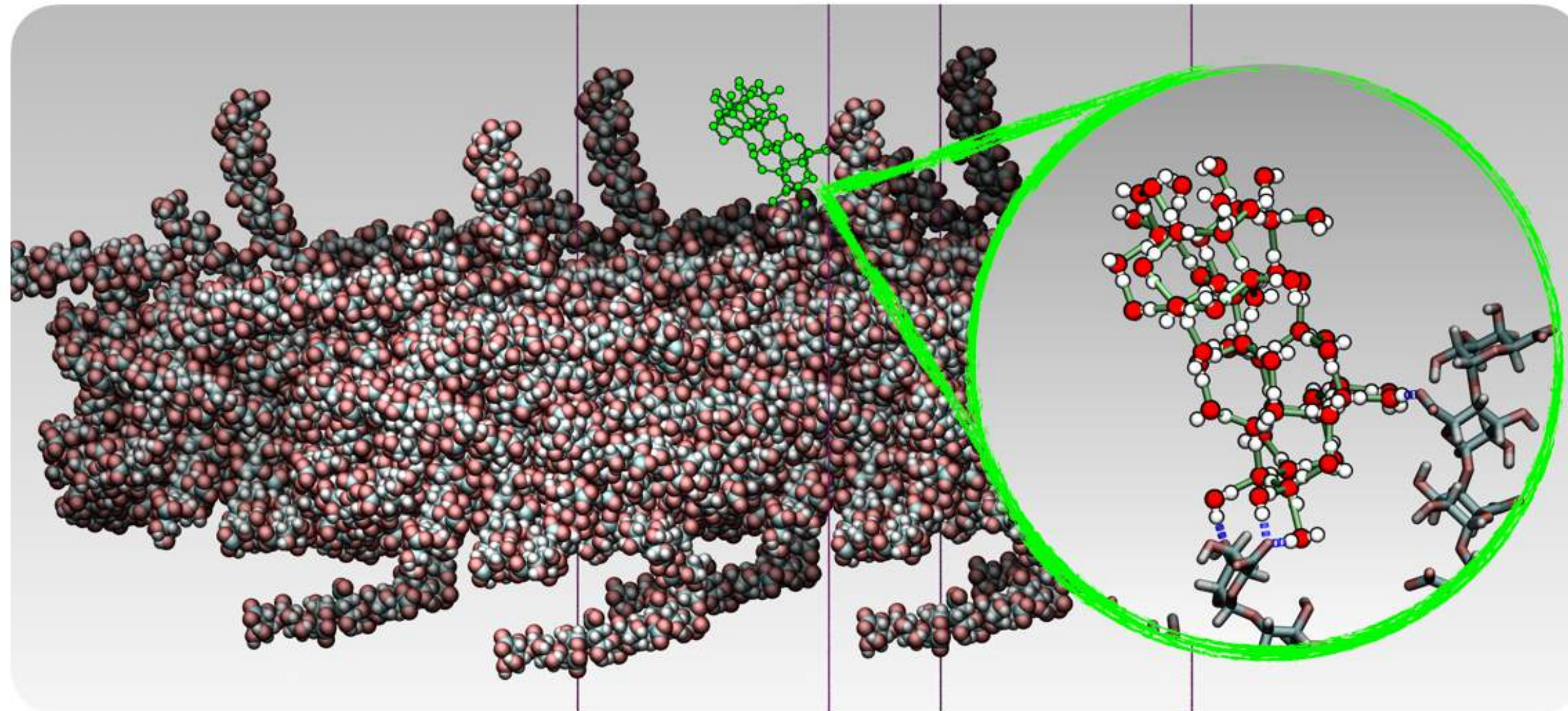
- As ice formation in plants triggers at rather mild supercooling (~ -5 °C), there must be something in the plant that acts as a ice nucleating agent (heterogeneous nucleation)
- It is not unthinkable to assume that HG itself can serve as an ice nucleating agent (INA) [*Lip. Bil. analogy]
- To assess whether that's the case, we have looked at the emergence of pre-critical ice nuclei in strongly supercooled water, by means of molecular dynamics simulations
- We have considered a number of different HG functionalizations and/or cross-linking topologies



No sizeable changes in the distribution of the size of the nuclei

HG as an ice-nucleating agent (INA)

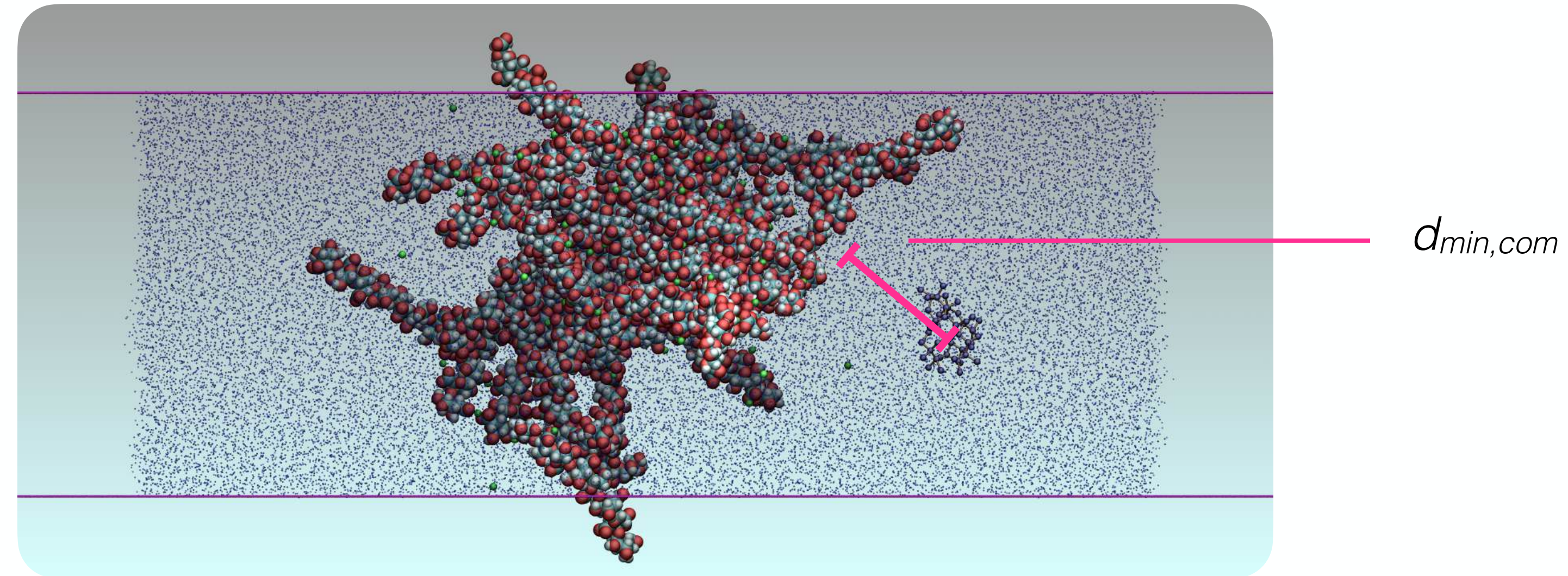
- HG is very hydrophilic
- HG is quite happy to accommodate ice nuclei at its interface with water



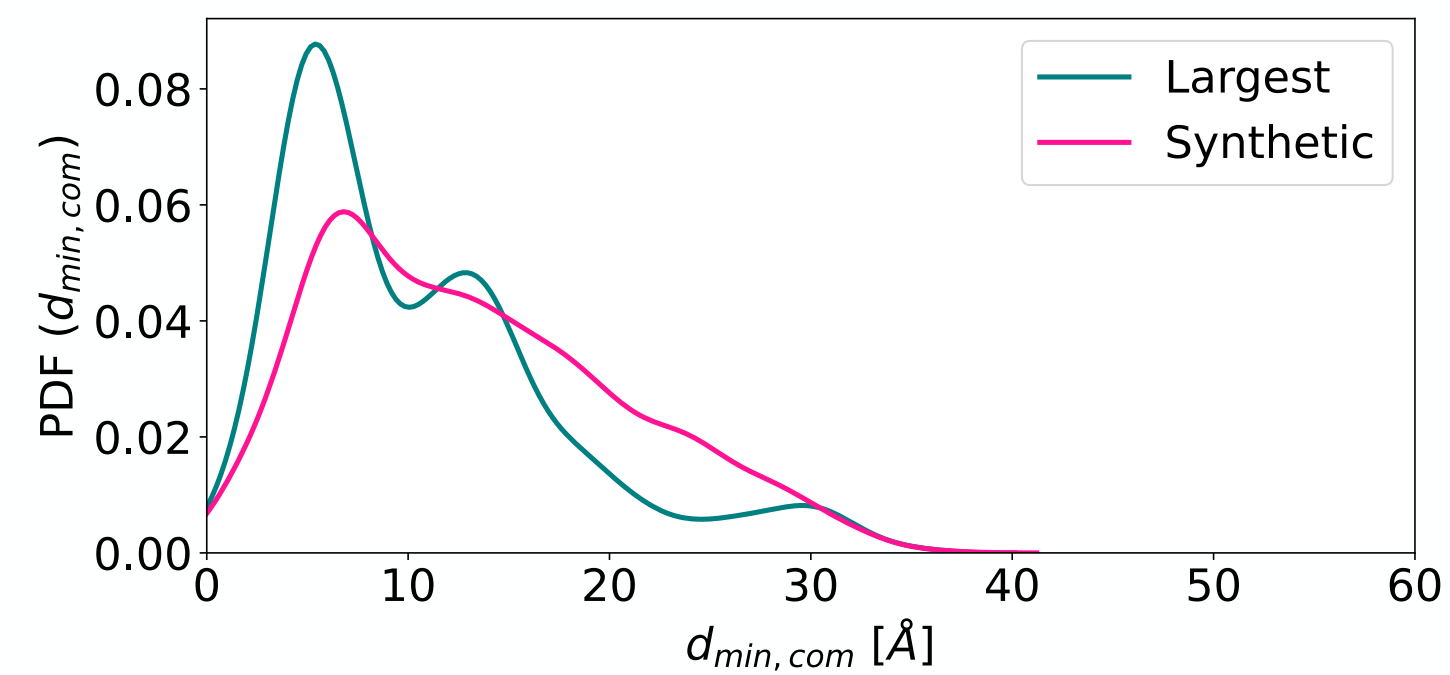
HG as an ice-nucleating agent (INA)



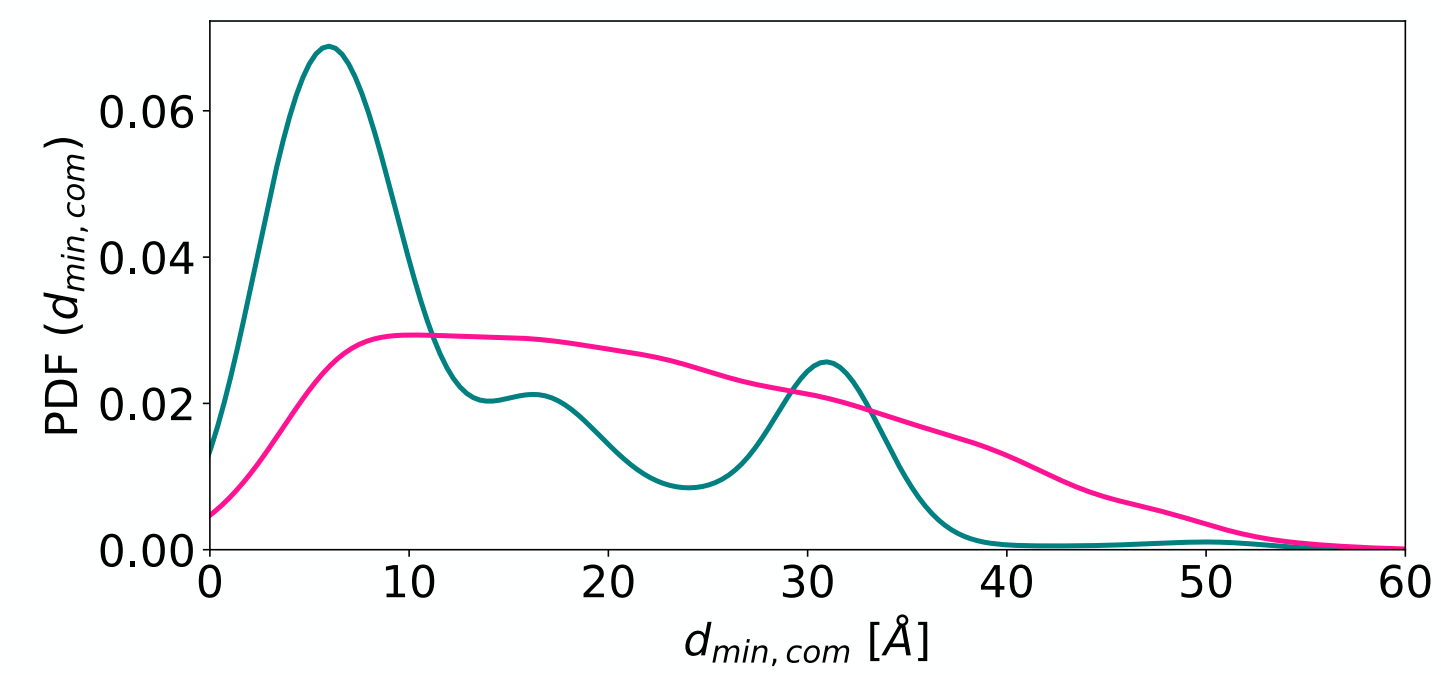
- Very active INAs facilitate the preferential formation on these nuclei in their proximity
- What about HG?



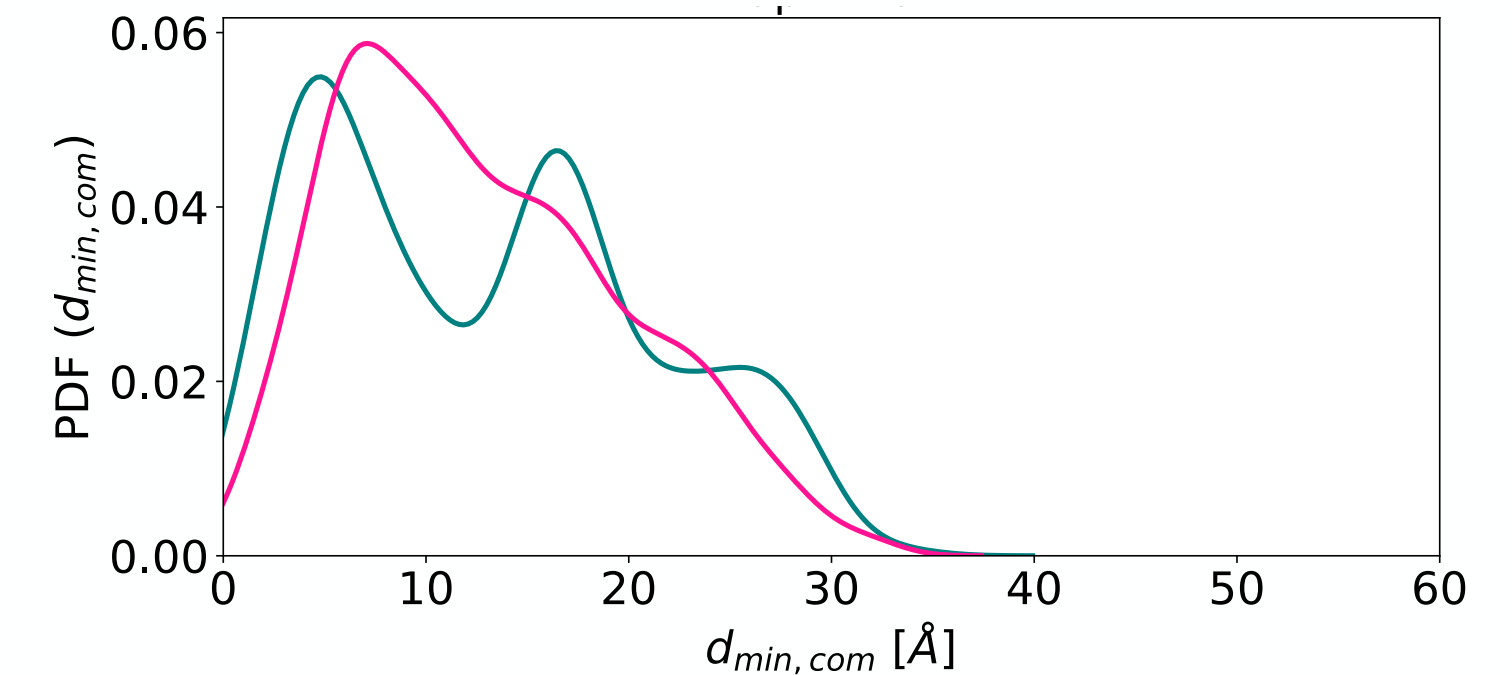
-COO-



-COO-, low-density

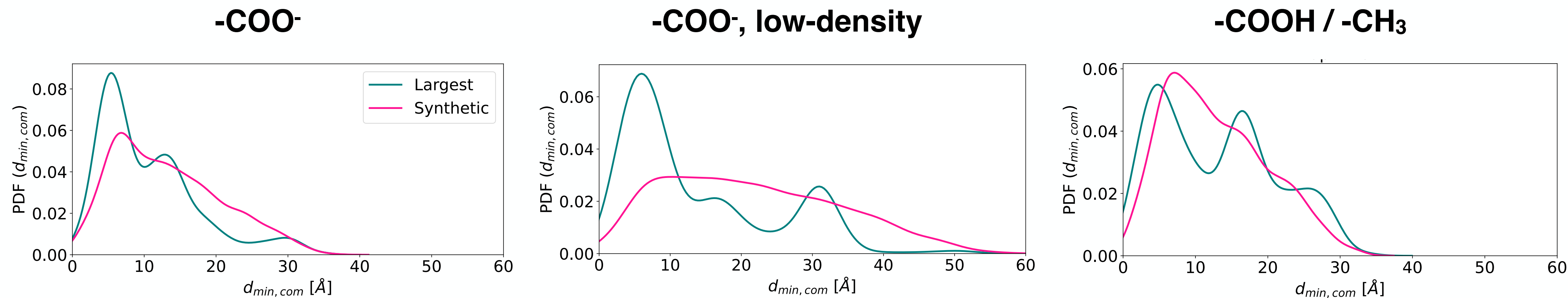


-COOH / -CH₃





- Very active INAs facilitate the preferential formation on these nuclei in their proximity
- What about HG?



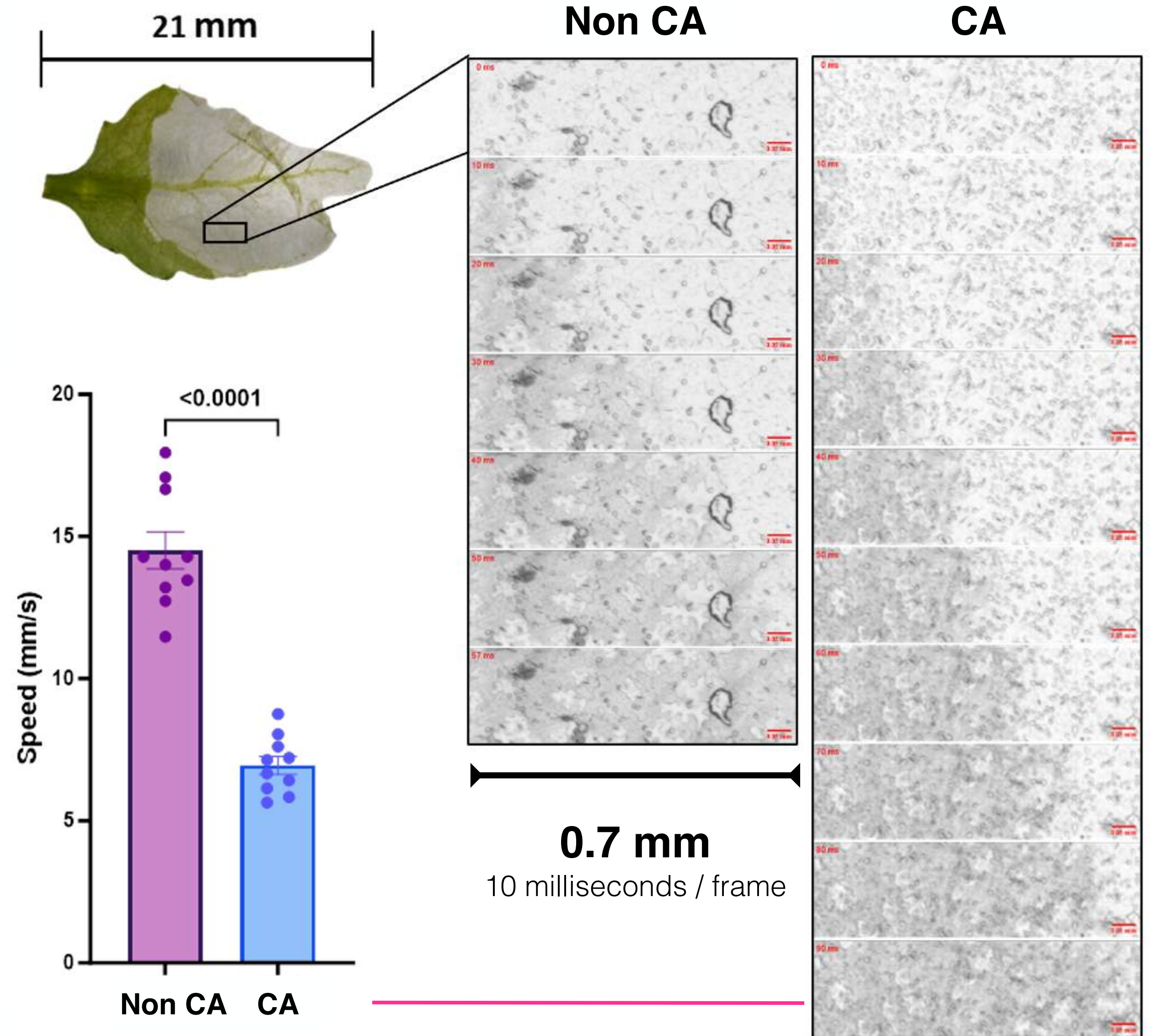
On these length scales, HG is not a “strong” INA, notwithstanding functionalizations and/or cross-linking topologies.

HOWEVER

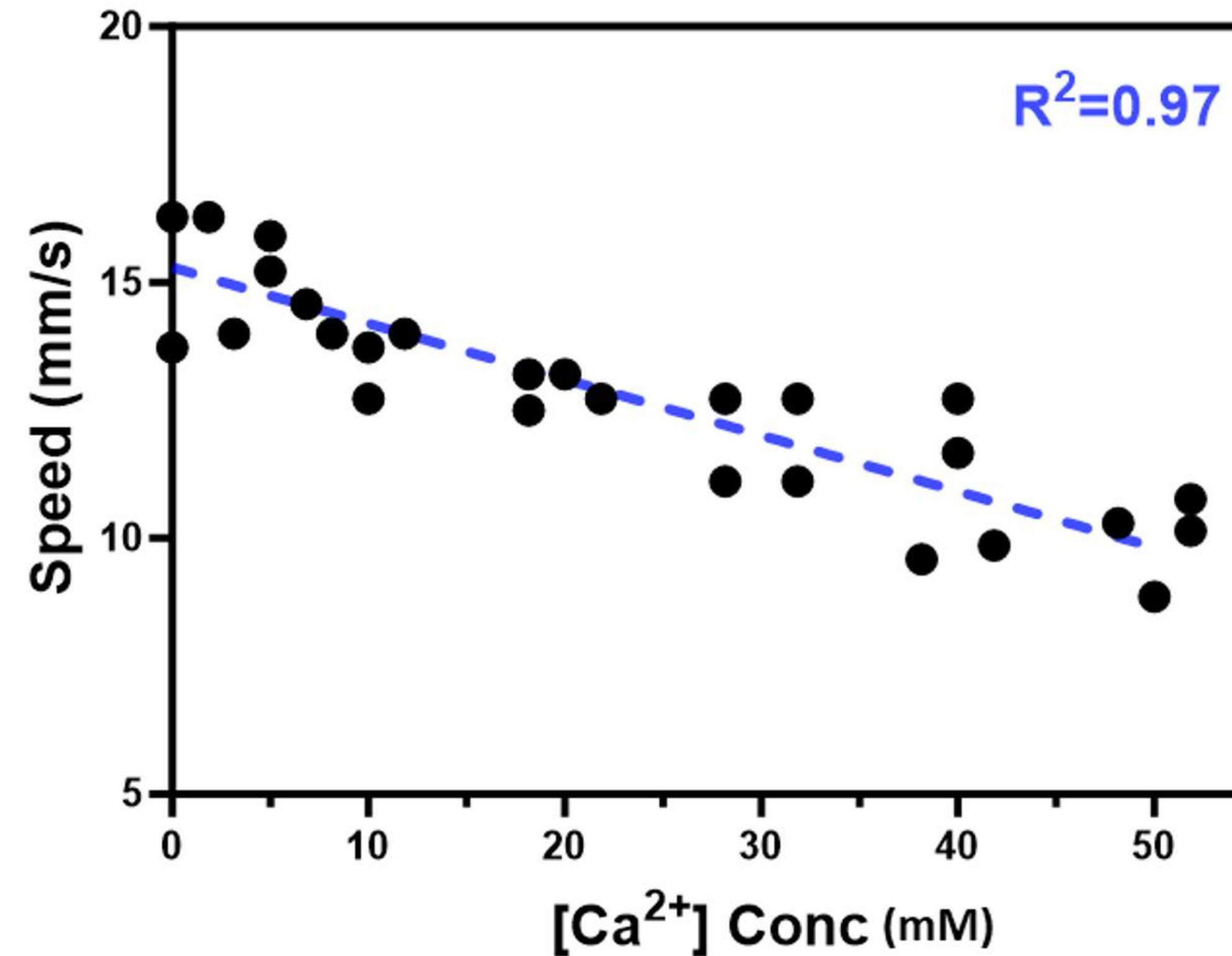
- Large(r) supramolecular motifs might be more active.
- The morphology of HG might be relevant in the context of ice *growth* as well

Ice growth rate - Acclimation

- Cold acclimation (**CA**):
 - Improves freezing tolerance
 - Reduces the ice growth rate
 - We suspect that acclimation reduces the porosity of the cell wall
- Custom microscope (Tim & Tom)



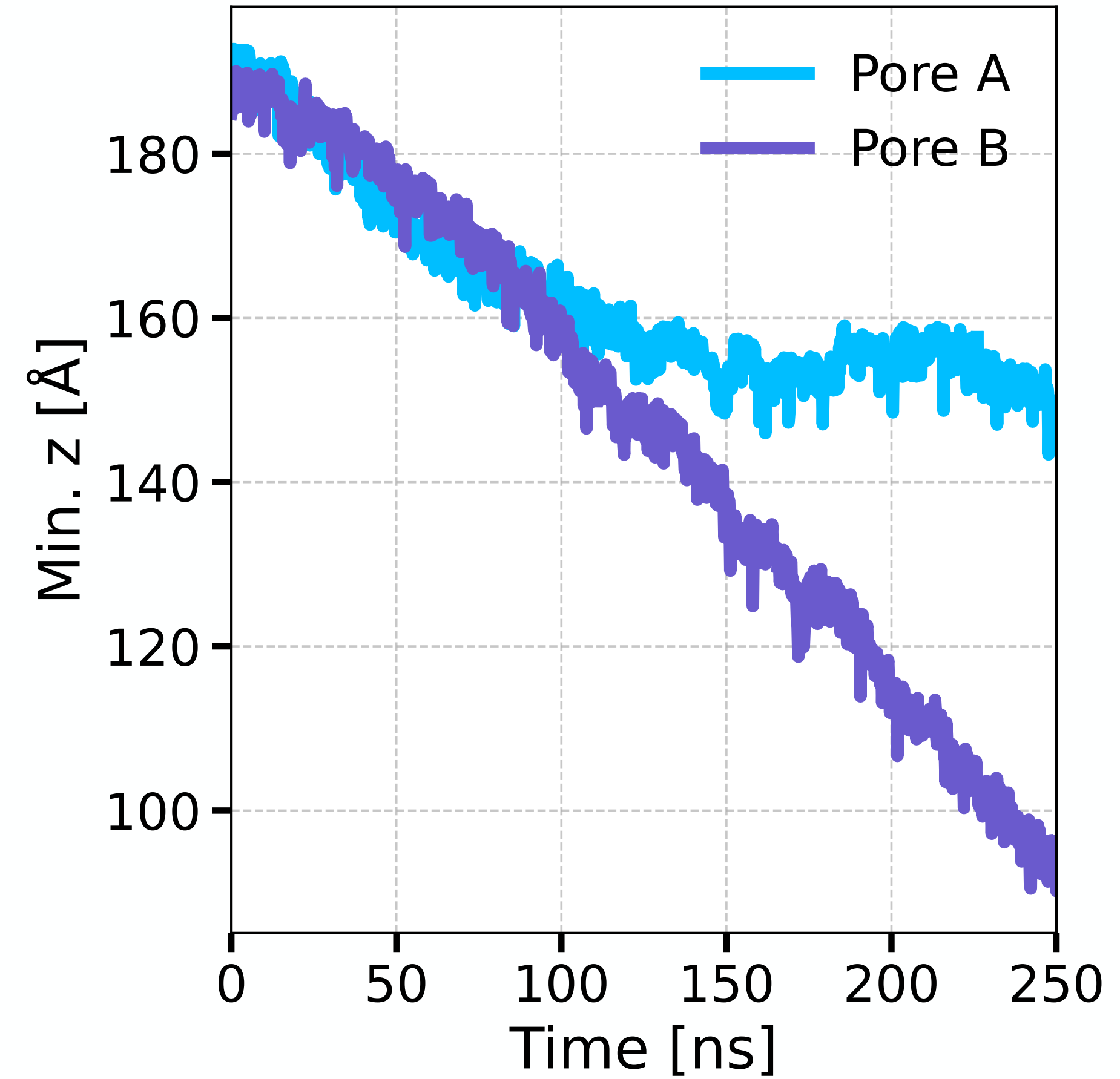
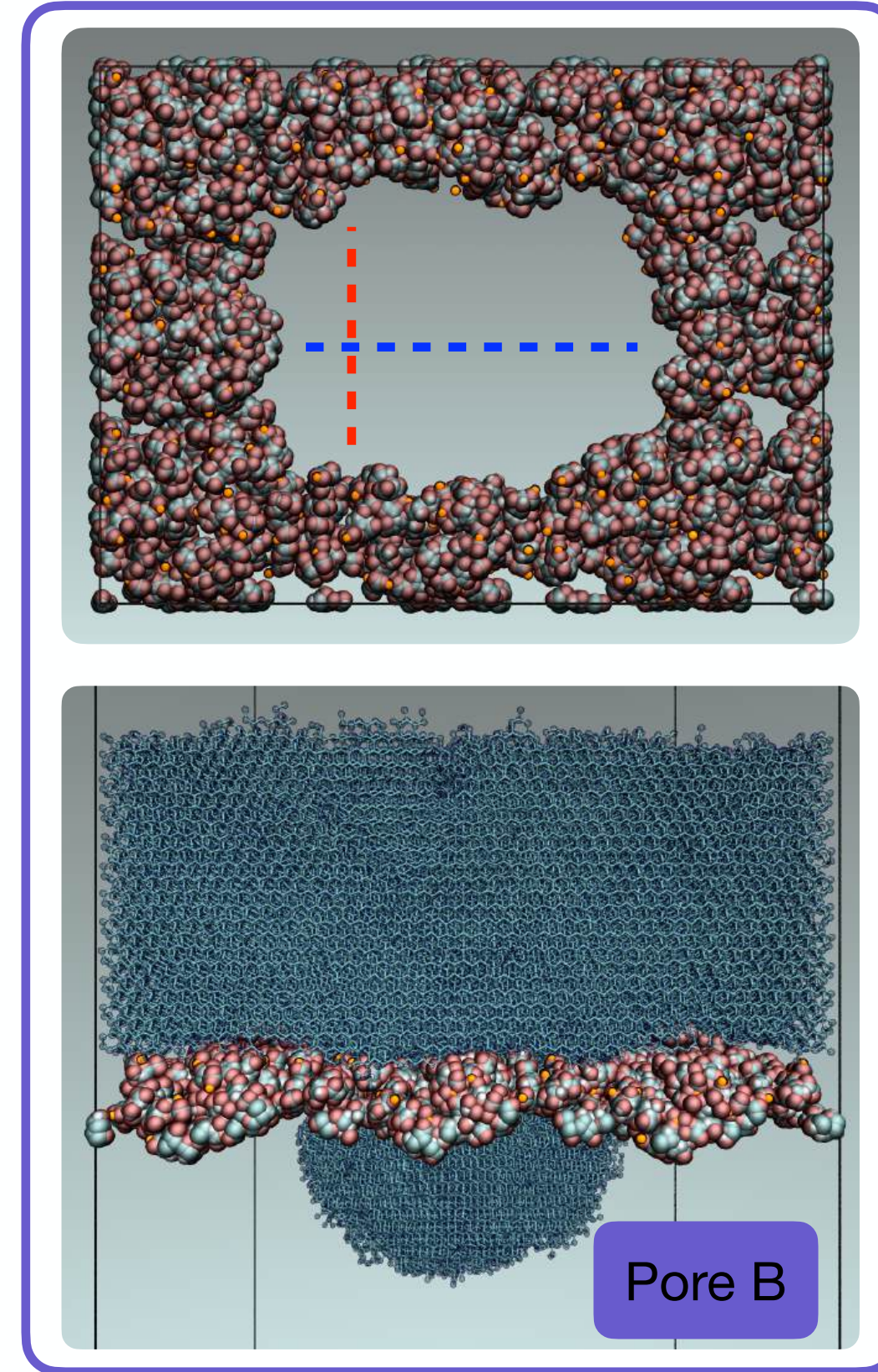
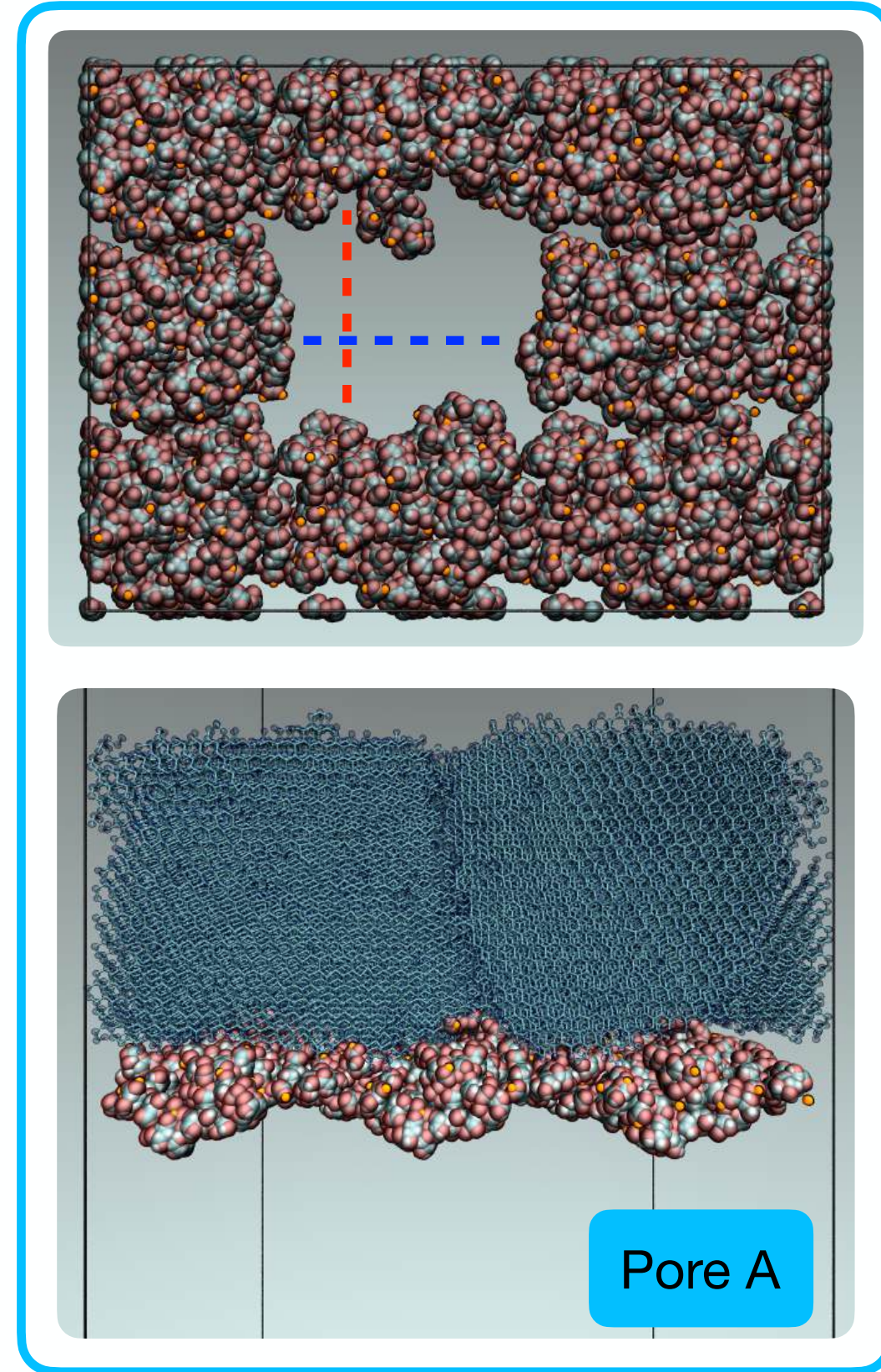
- As an alternative strategy to reduce frost damage, we supplemented the plants with extra Ca^{2+}
- We found that the content of Ca^{2+} is inversely correlated with the ice growth rate
- The more Ca^{2+} we add, the more HG cross links, thus reducing porosity



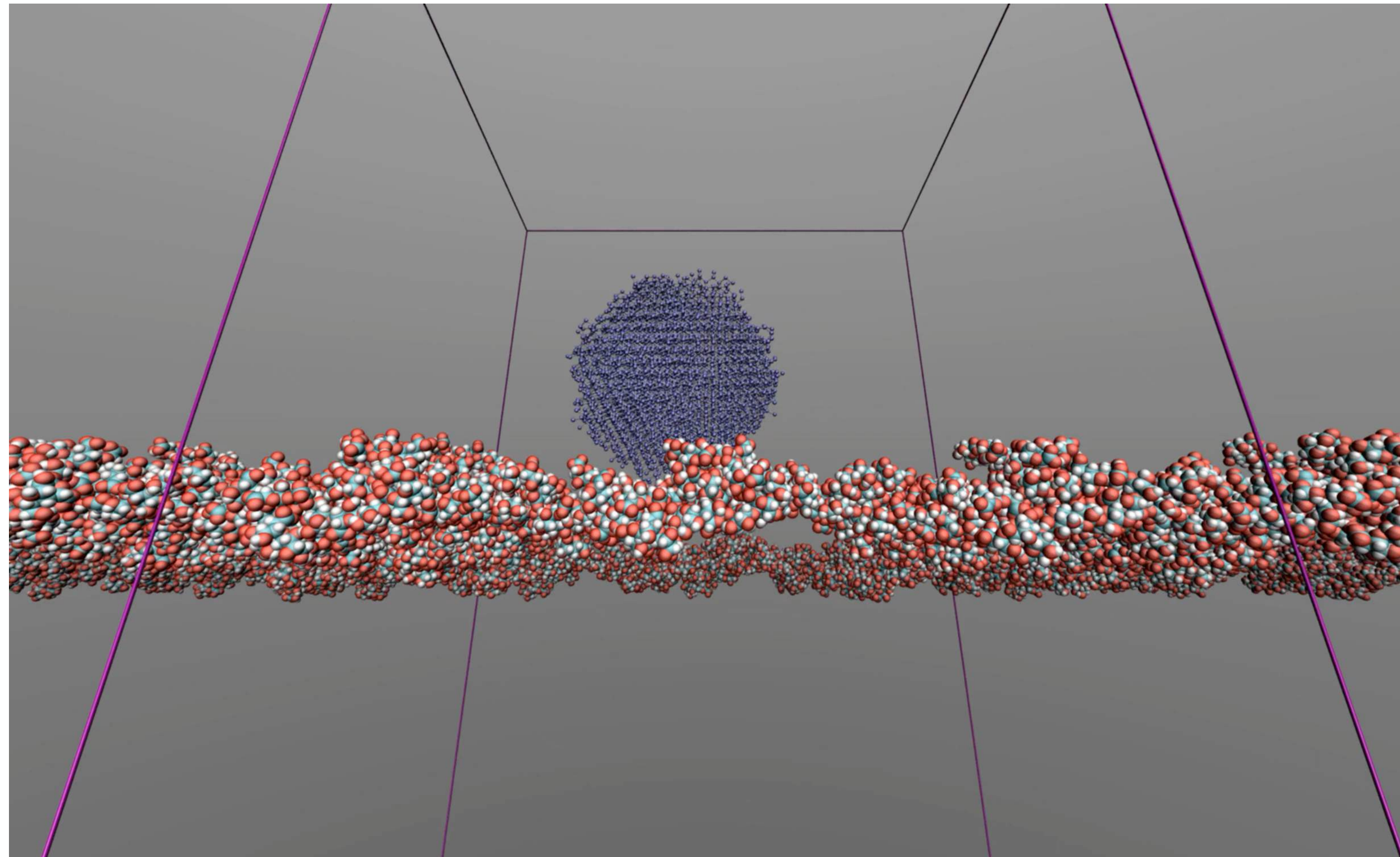
HG porosity & Ice Growth

$R_1 = \sim 2 \text{ nm}$
 $R_2 = \sim 3.5 \text{ nm}$

$R_1 = \sim 3.5 \text{ nm}$
 $R_2 = \sim 5.5 \text{ nm}$



- Seeded MD simulations of ice growth through HG pores



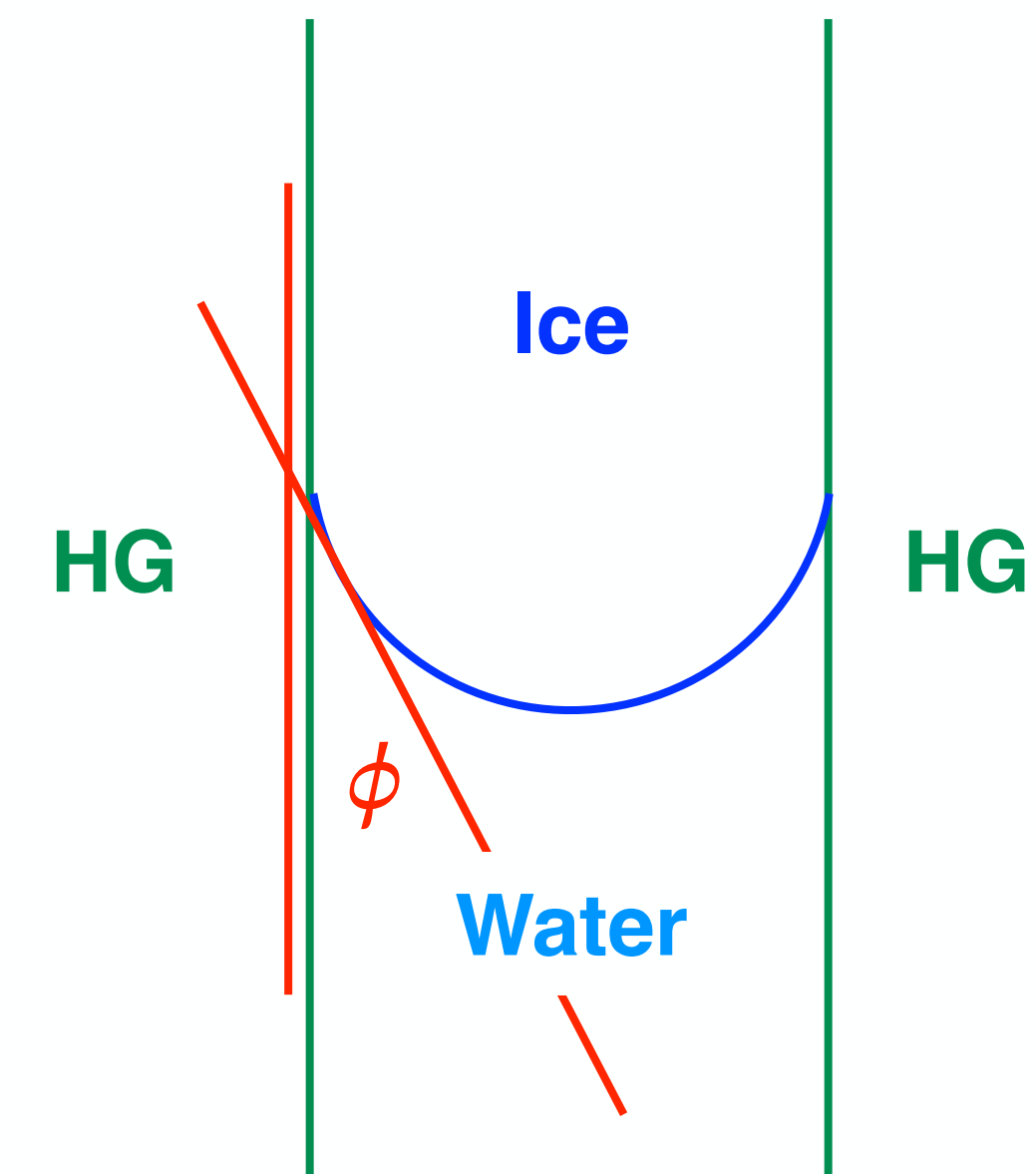
- “*The Ice Re-crystallization Inhibition Activity of Pectic Polysaccharides*”. Pump-priming application, 2019, 4 nodes, $24 \times 4 = 96$ CPUs
- “*Understanding the Freezing Tolerance of Plants: The Role of Pectin*”. Access to HPC 1 application, 2020, 2 nodes, $128 \times 2 = 256$ CPUs
- ~1M atoms, fully atomistic classical MD simulations
- CHARMM36 (HG) + TIP4P/Ice water
- Even the post-processing is not trivial (custom version of PLUMED [Gareth Tribello, QUB], high-memory nodes required)
- GPU acceleration (ONE GPU /node does it! ARCHER3? 🙄)



From the Gibbs-Thomson equation:

$$r_{\text{eff}}^* = -\frac{T_{\text{bulk}} \gamma_{\text{ice,water}} \cos \phi}{(T_s - T_{\text{bulk}}) L_f \rho_{\text{ice}}} \quad \text{with:} \quad r_{\text{eff}} = \left(\frac{1}{R_1} + \frac{1}{R_2} \right)^{-1}$$

- r_{eff}^* = critical “effective” radius of the pore, above which the ice can grow through the pore
- T_{bulk} = melting temperature in the bulk
- T_s = actual temperature of the system
- γ_{ice} = interfacial free energy between water and ice
- ϕ = angle formed by the ice-water interface and the walls of the HG pore
- L_f = latent specific heat of fusion
- ρ_{ice} = density of the ice phase
- $R_{1,2}$ = two principal radii of (mean) curvature characterising the pore



$$r_{\text{eff}}^* = -\frac{T_{\text{bulk}} \gamma_{\text{ice,water}} \cos \phi}{(T_s - T_{\text{bulk}}) L_f \rho_{\text{ice}}}$$



- Computed for the TIP4P/Ice model
- Qualitatively, $\phi \sim 20^\circ$
- Consistent with HG being very hydrophilic

- Let's assume:

- $\phi \sim 20^\circ$

- The HG *network* (as opposed to a single pore) is characterised by a tortuosity τ — $\tau = \frac{1}{\sqrt{\eta}}$ — Porosity (Bruggeman expression)

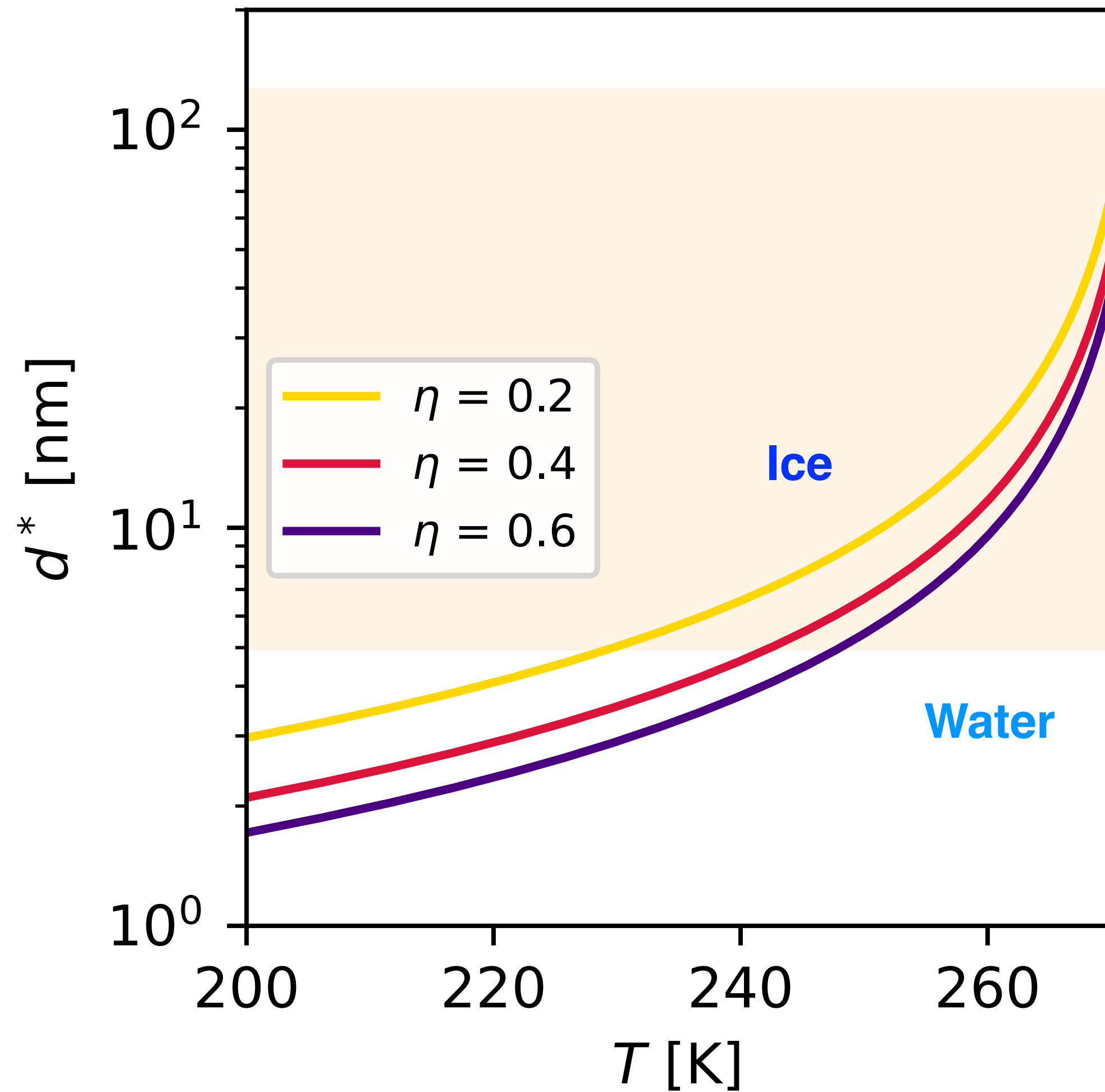
- A cylindrical pore of diameter d — $d = 2R = 4r_{eff}$



$$r_{eff}^{network}(T_s) = \frac{r_{eff}(T_s)}{\sqrt{\eta}} = - \frac{T_{bulk} \gamma_{ice,water} \cos \phi}{(T_s - T_{bulk}) L_f \rho_{ice} \sqrt{\eta}}$$



“Effective” critical pore diameter d^* as a function of temperature



- Consistent with experimental pore size distribution (orange/shaded region)
- Strong dependence on the porosity η of the HG network

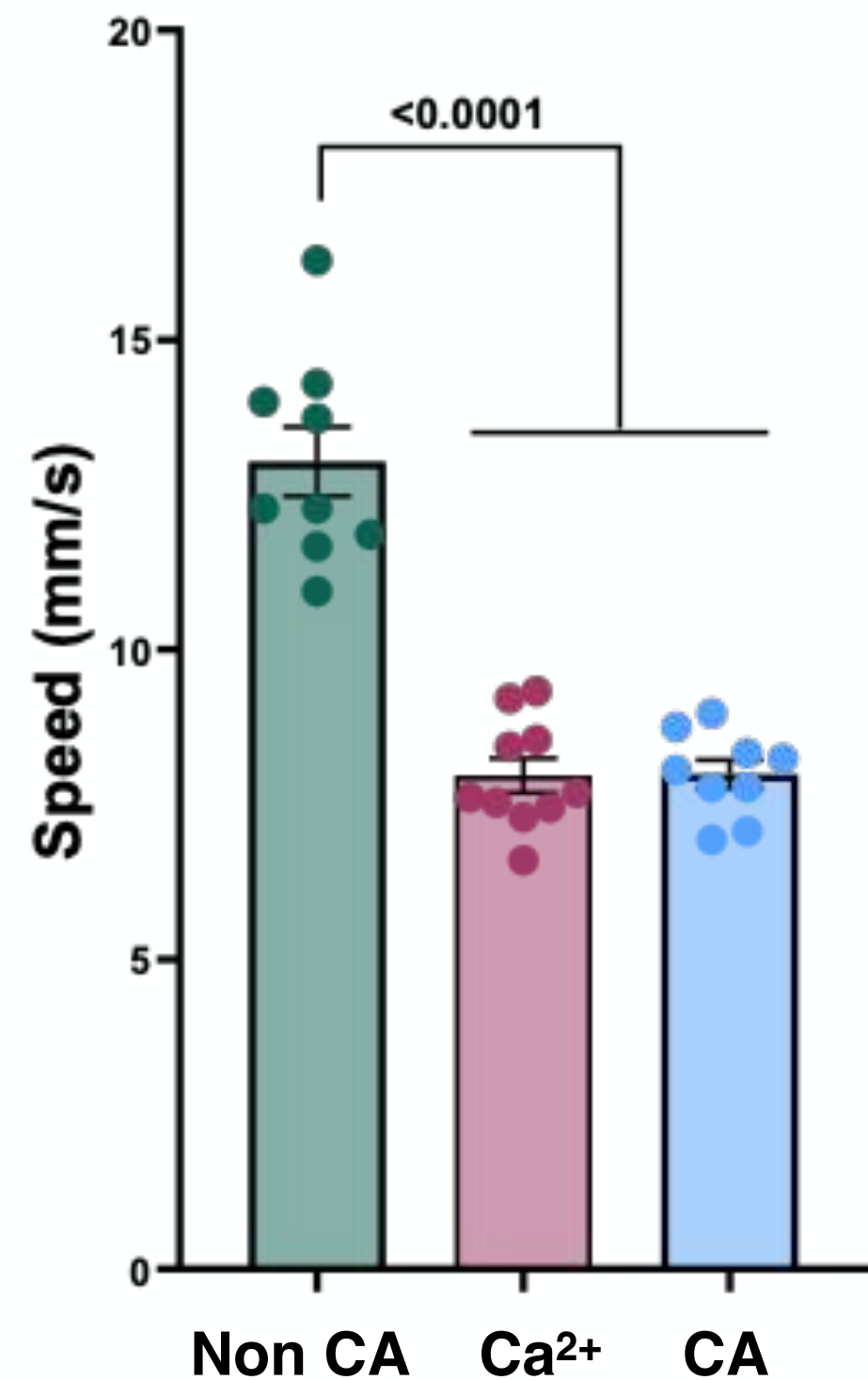


Even small changes in terms of HG cross-linking can make a difference in terms of ice growth through the cell wall*

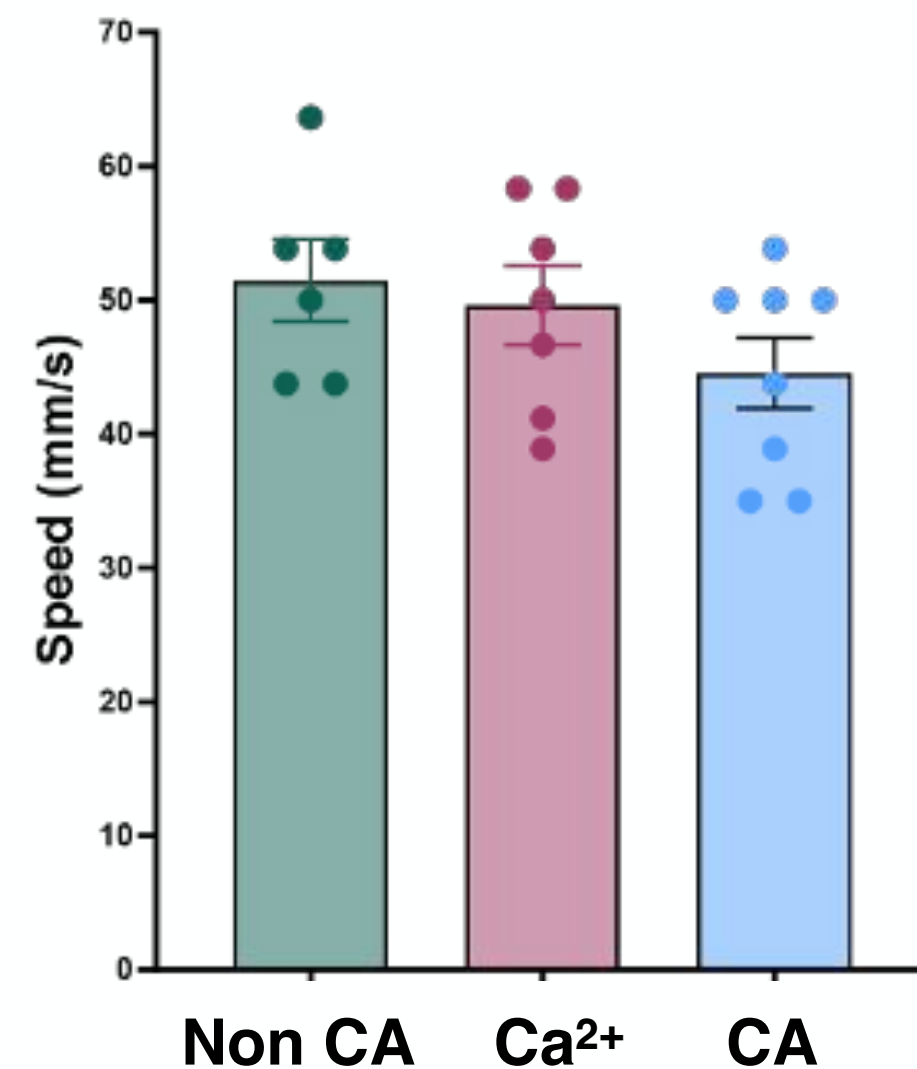
- Mutation
- Cold acclimation
- Ca^{2+} supplement
- And...

* From *qualitative* results!

Original samples



“Flooded” samples

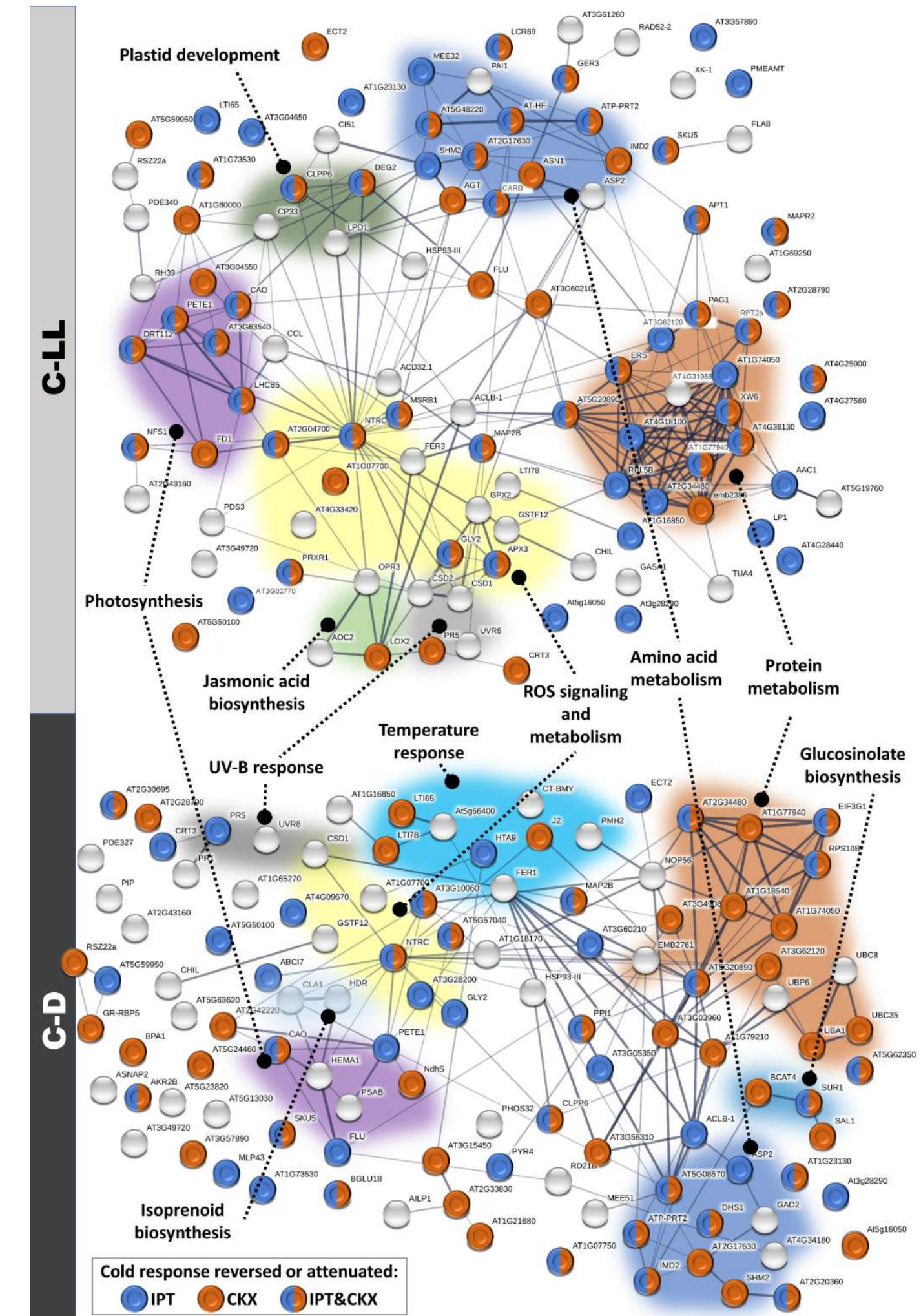


CA = Cold acclimated

- HG (and pectin in general) swells (a lot) in water
- “Flooding” the cell walls with water swells the HG
- Swelling increases the porosity of the HG network, thus increasing the ice growth rate
- At the mesoscale, “cryosuction” (e.g., Robert Style, ETH)



- Biology is complicated (what else is new...)
- Freezing tolerance in plants seems to be largely determined by the extent of ice growth
- The structural features of the pectin within the cell wall have an impact on ice growth
- There is scope to modify pectin so as to improve freezing tolerance
- Maybe we can leverage some of these findings in the context of animal cells/tissues as well
- Multi-disciplinary efforts are needed!
- **The complexity of the system poses a though computational challenge (multi-scale simulations, etc.)**



communications biology

Article



<https://doi.org/10.1038/s42003-025-07495-0>

Understanding pectin cross-linking in plant cell walls

 Check for updates

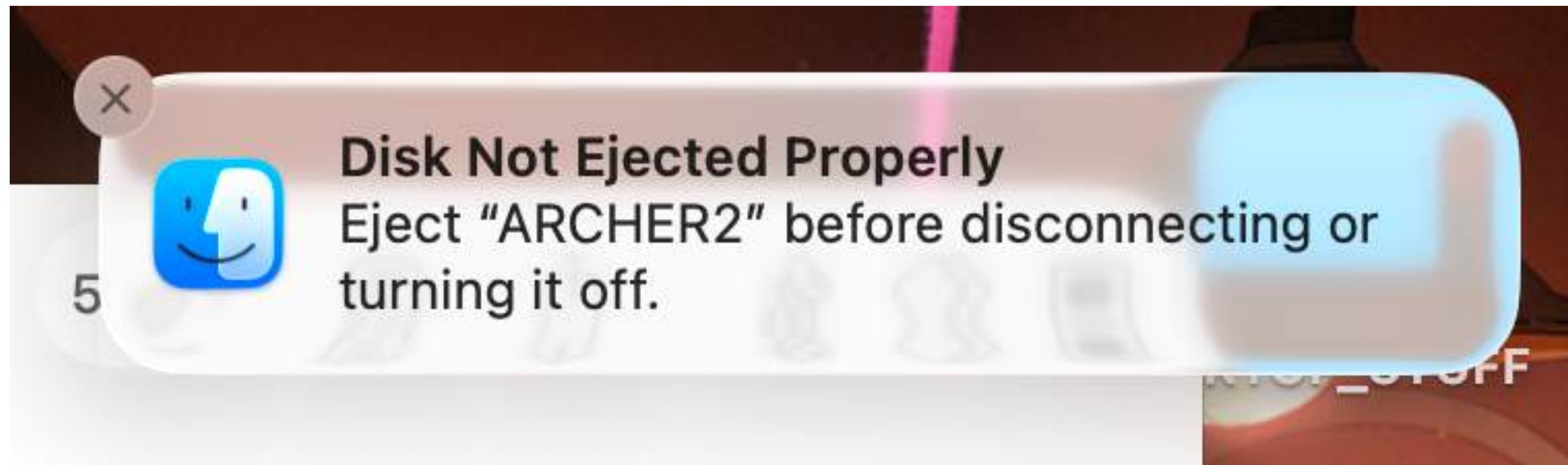
Irabonosi Obomighie ¹, Iain J. Prentice², Peter Lewin-Jones ³, Fabienne Bachtiger², Nathan Ramsay¹,
Chieko Kishi-Itakura¹, Martin W. Goldberg¹, Tim J. Hawkins¹, James E. Sprittles ³,
Heather Knight ¹  & Gabriele C. Sosso ² 

Thanks!

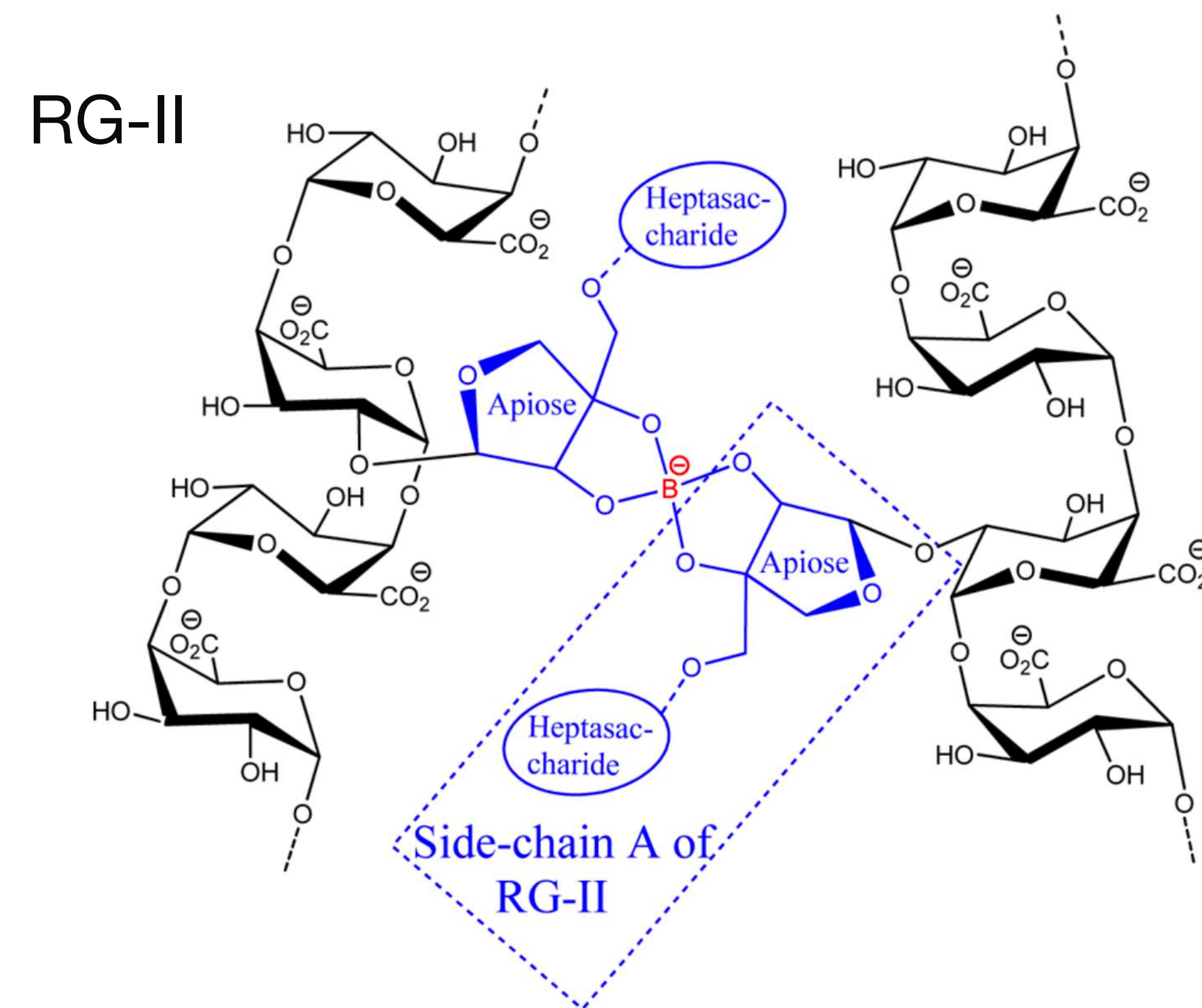
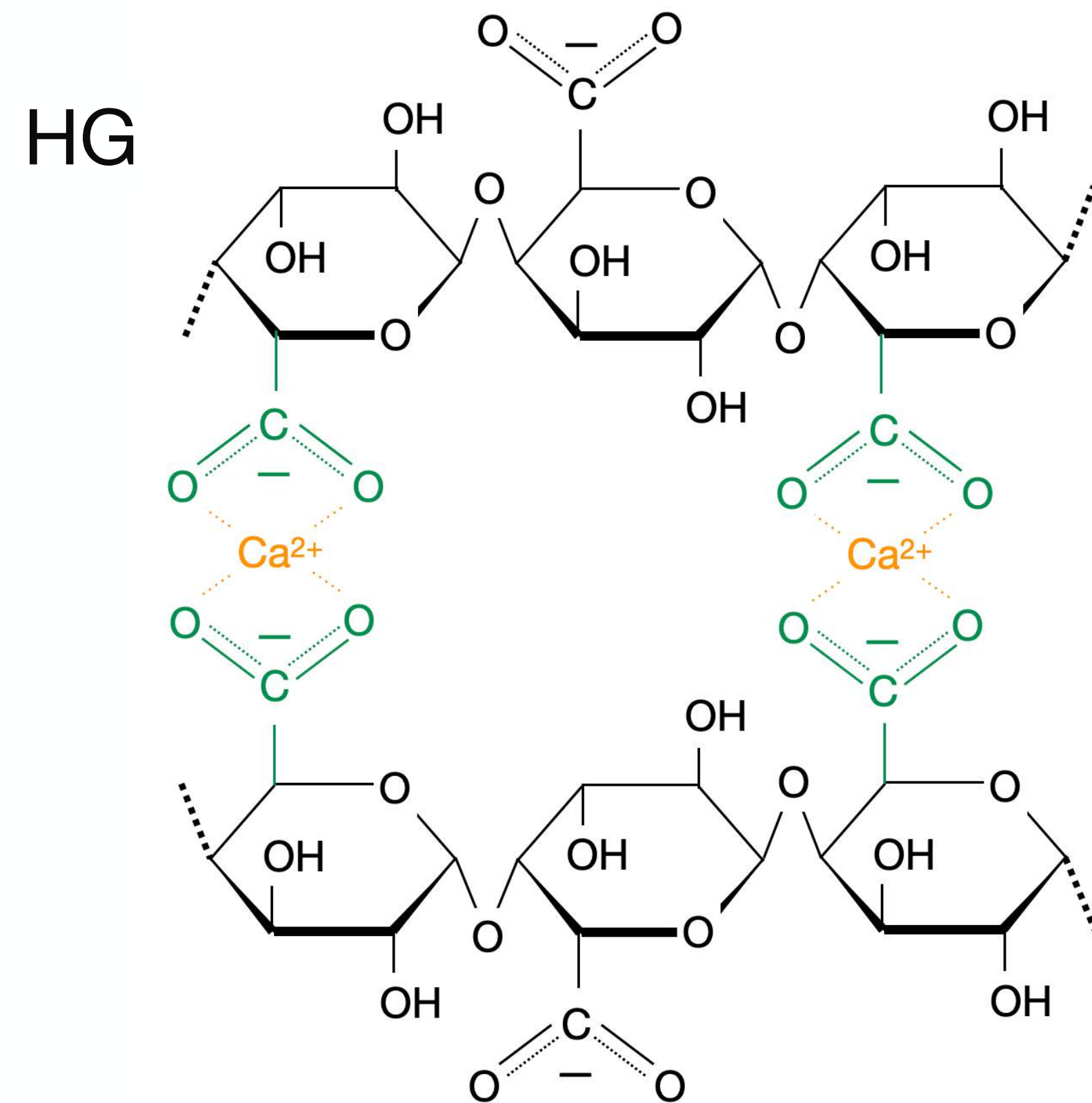


**Biotechnology and
Biological Sciences
Research Council**



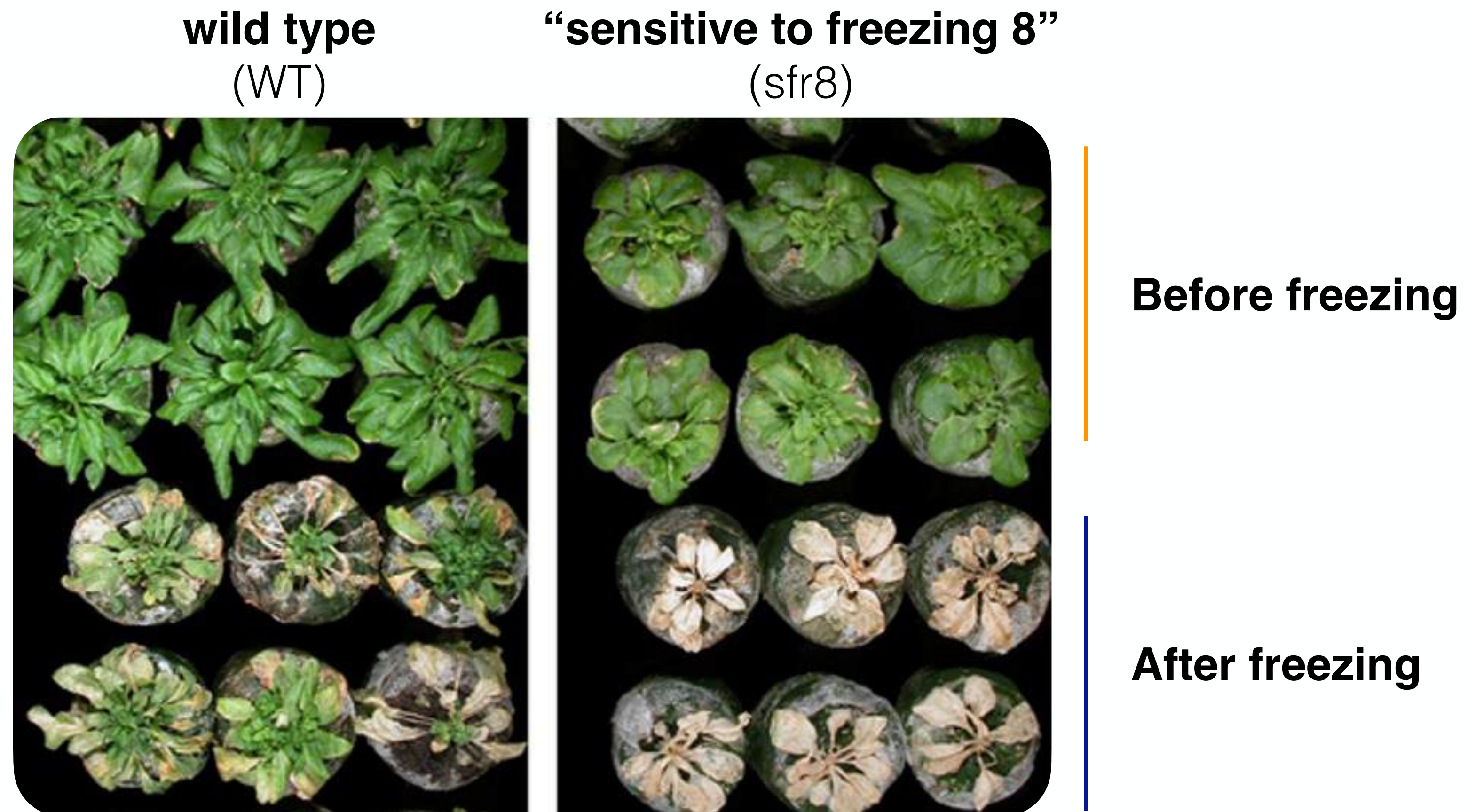


- HG chains can cross-link to each other (chiefly) thanks to Ca^{2+} ions
- RG-II chains can cross-link to each other (chiefly) thanks to boron bridges

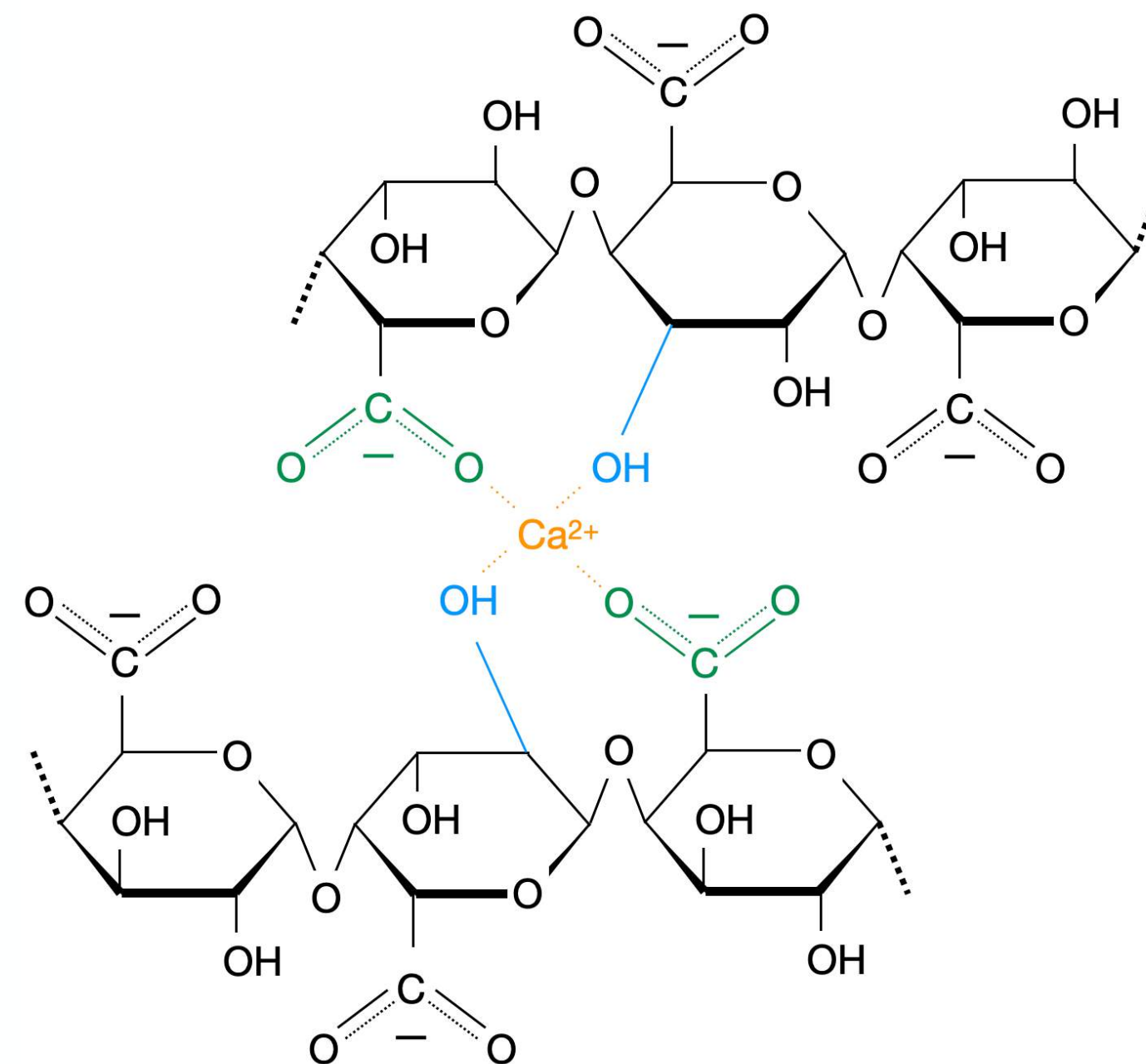


The “sensitive to freezing 8” mutant

- Heather found a mutant of *Arabidopsis thaliana*, *sfr8*, with reduced cross-linking
- The facts: *sfr8* suffers upon freezing a lot more (compared to the wild type)
- The hypothesis: *sfr8* can link fewer RG-II, which in turn brings the HG chains further apart

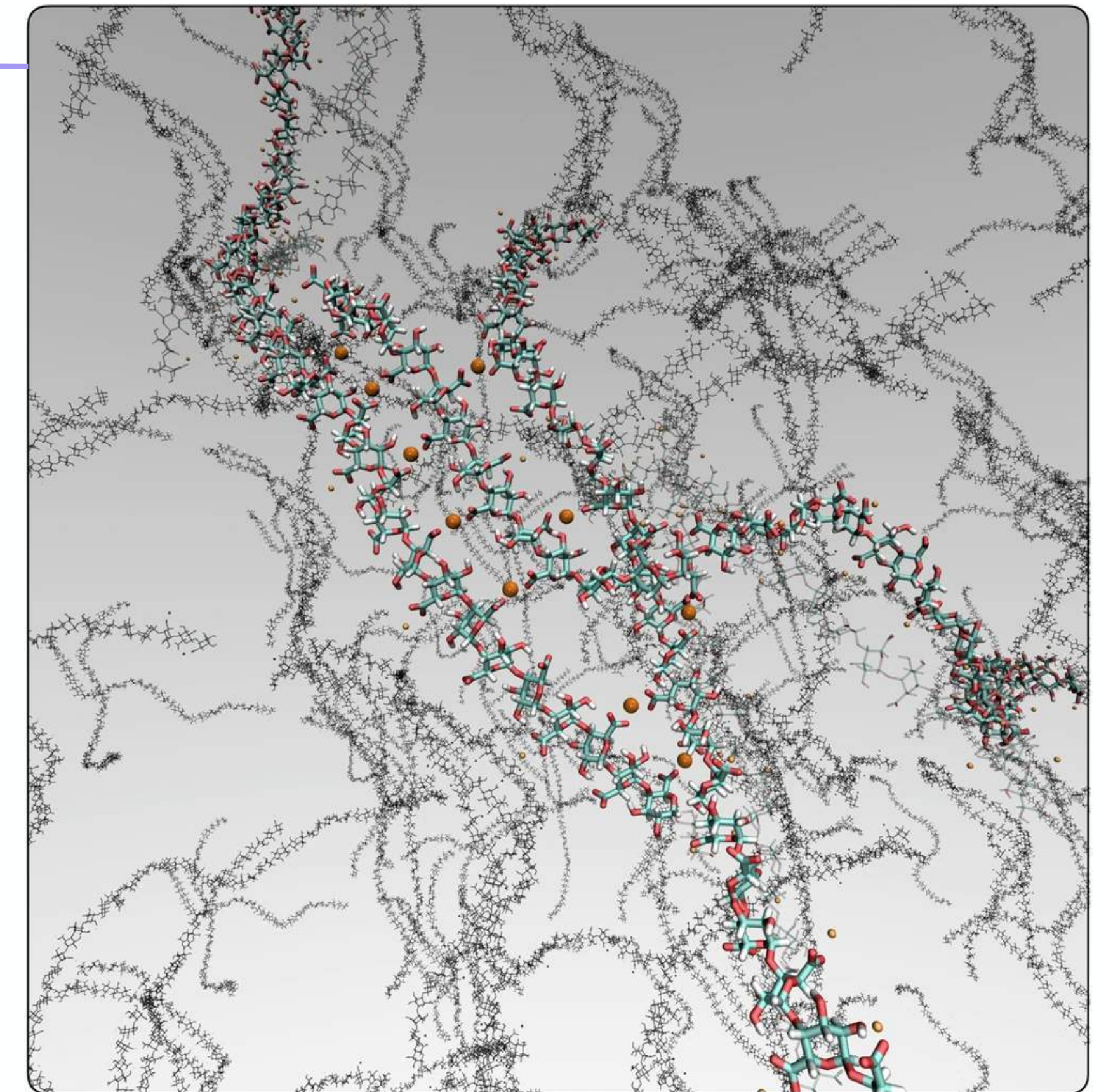
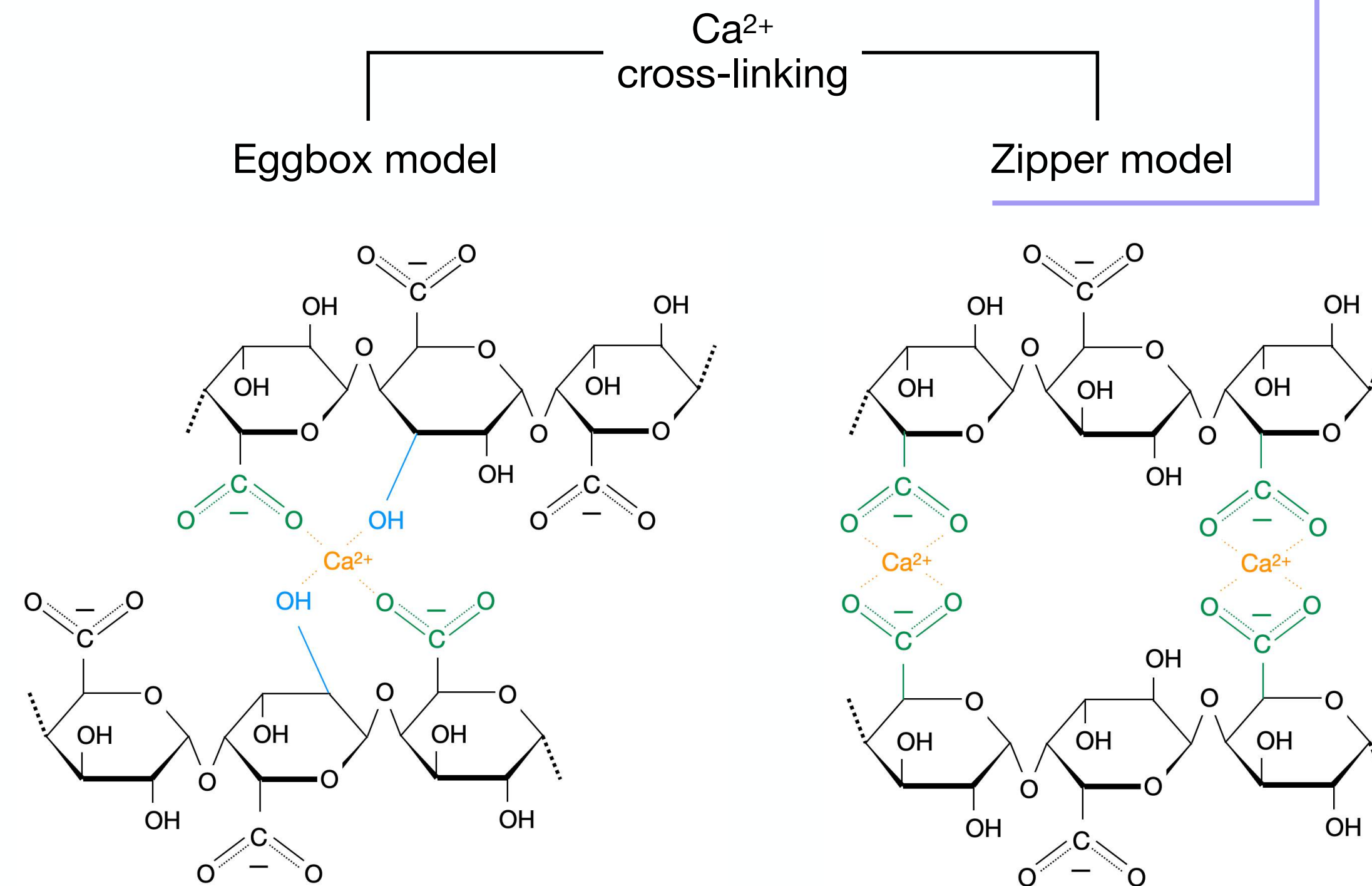


- Reduced cross linking → more porous HG network → ice can grow through the cell wall
- First step: understand HG cross-linking
- 80% of the literature on HG cross-linking adopts the famous “eggbox” model for pectin
- In the eggbox model, each link involves a Ca^{2+} ion, two $-\text{COO}^-$ groups *and* two $-\text{OH}$ groups

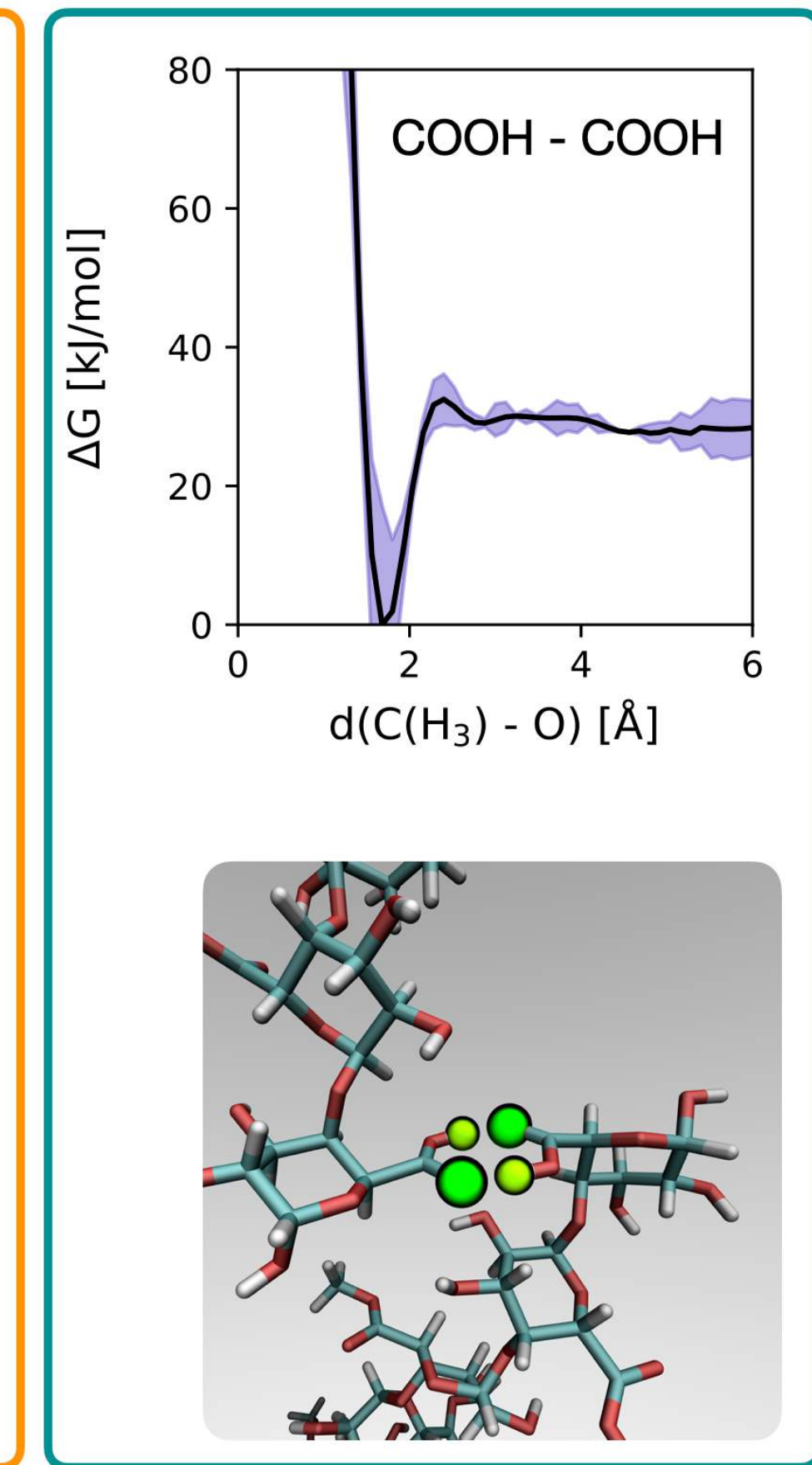
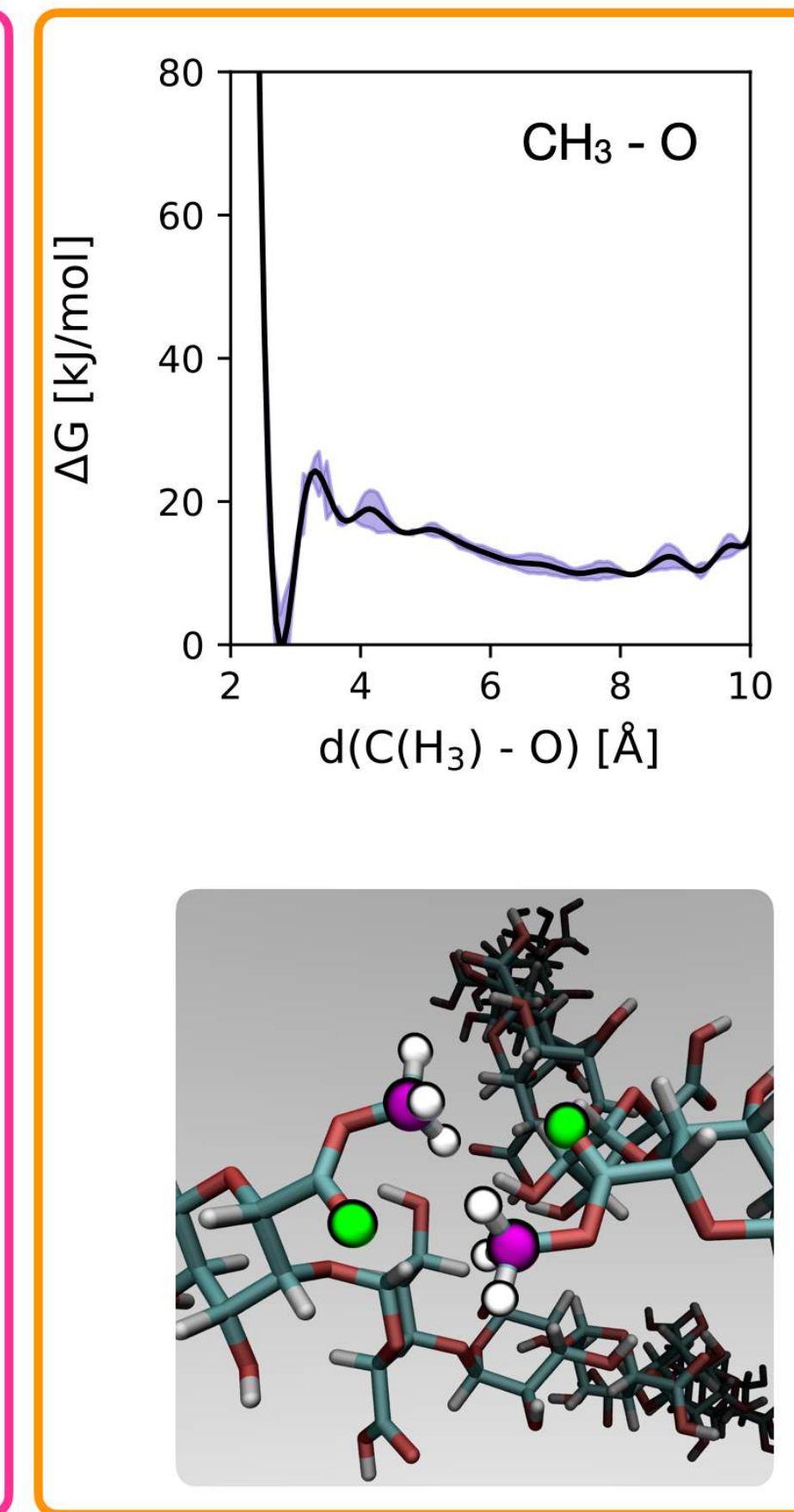
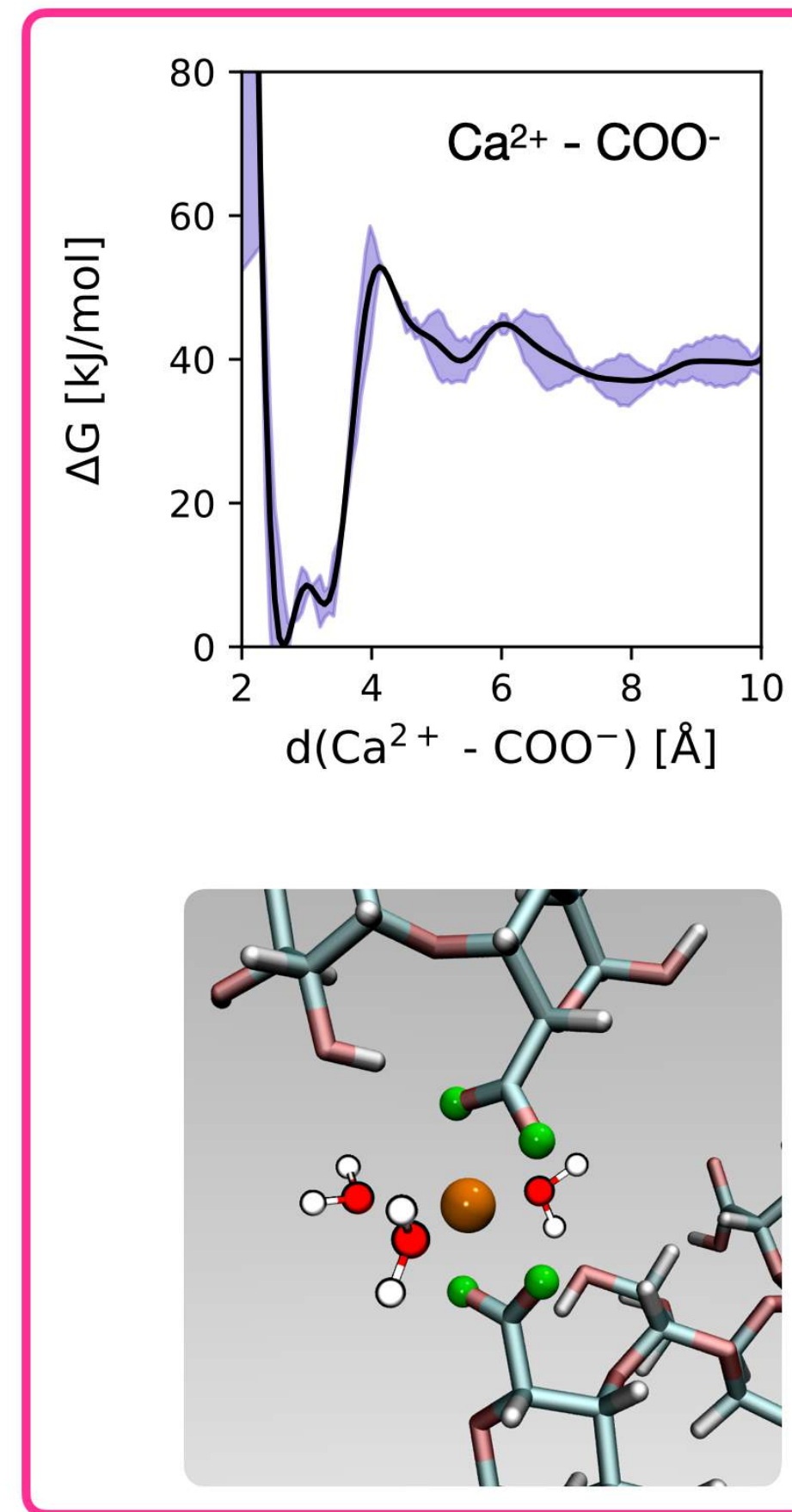
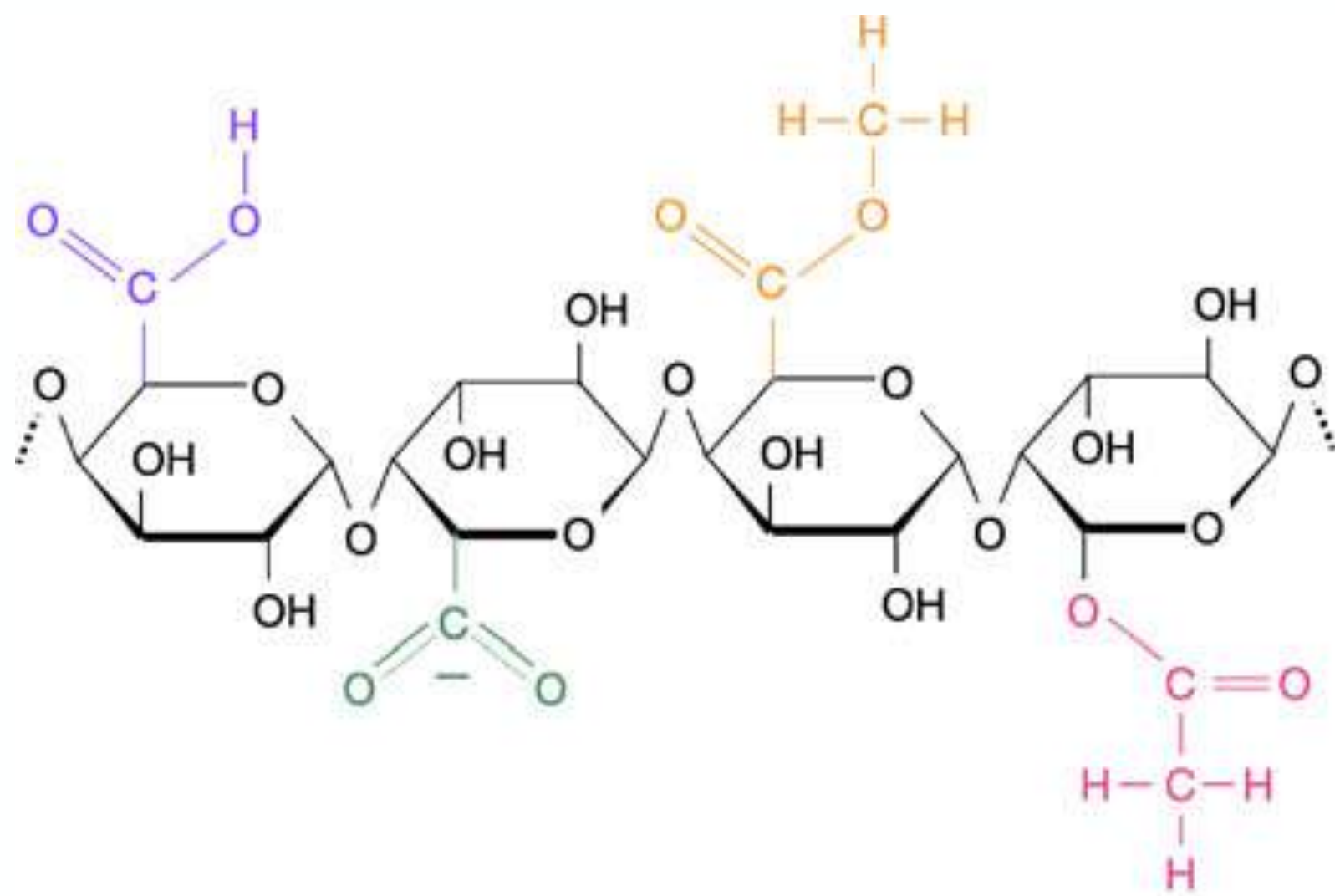


HG cross-linking - the “zipper” model

- We performed extensive, unbiased molecular dynamics simulations of HG cross-linking
- We found no evidence supporting the eggbox model [*FF]
- We found that the link involves a Ca^{2+} ion and two $-\text{COO}^-$ groups (but not the $-\text{OH}$ groups)
- We call this the “zipper” model



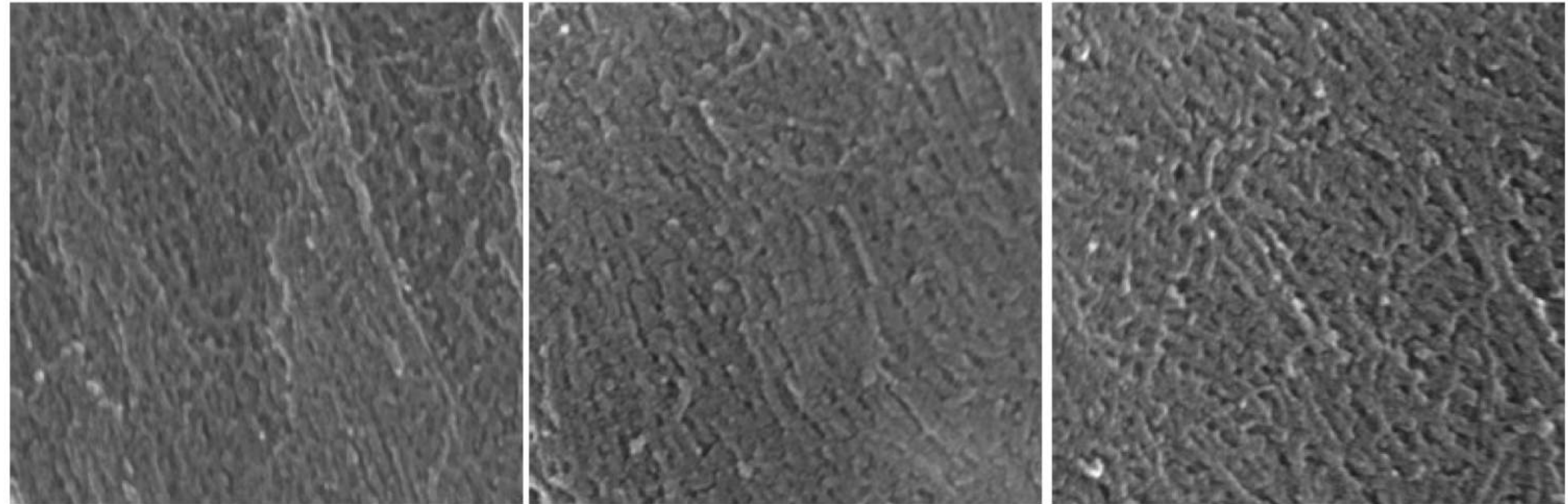
- There are other mechanisms by which HG can cross link, even without Ca^{2+}
- The Ca^{2+} -mediated linkages are the strongest ones, however
- Recent paper under review confirms the zipper model!



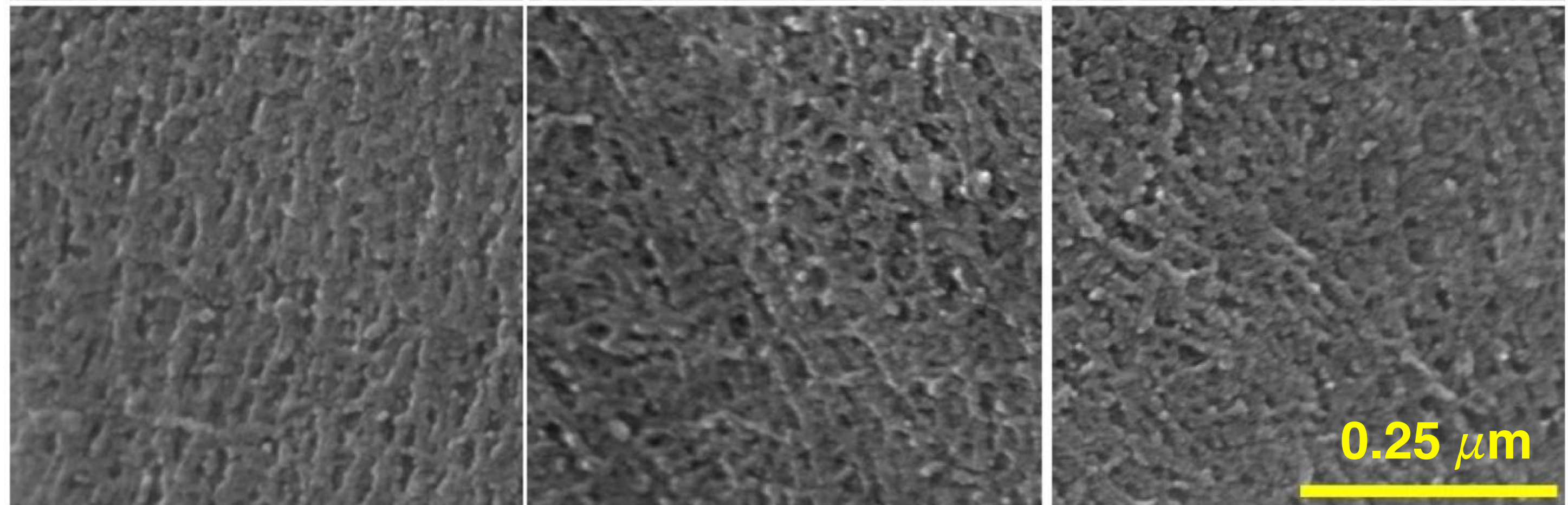
HG cross-linking & Cell wall porosity

- Heather found that, indeed, *sfr8* cell walls are more porous compared to the wild type
- **SEM images**, three different leaves

wild type Col0



“sensitive to freezing 8” *sfr8*



- Heather found that, indeed, *sfr8* cell walls are more porous compared to the wild type
- **Fluorescence measurements:** the fluorescence (**F**) of the dye decreases in time as the quencher molecules percolate through the cell wall. The more porous the cell wall, the more quencher gets into the membrane (hence the fluorescence decreases faster)

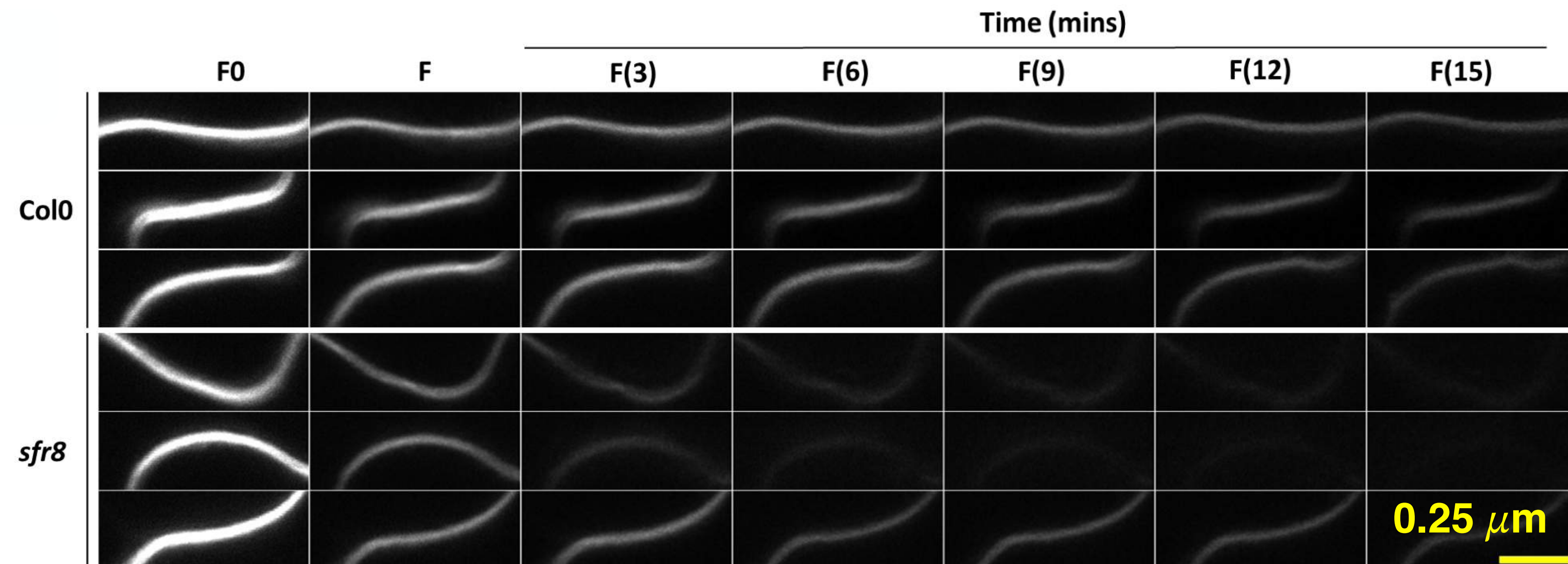
Quencher molecules
(trypan blue)



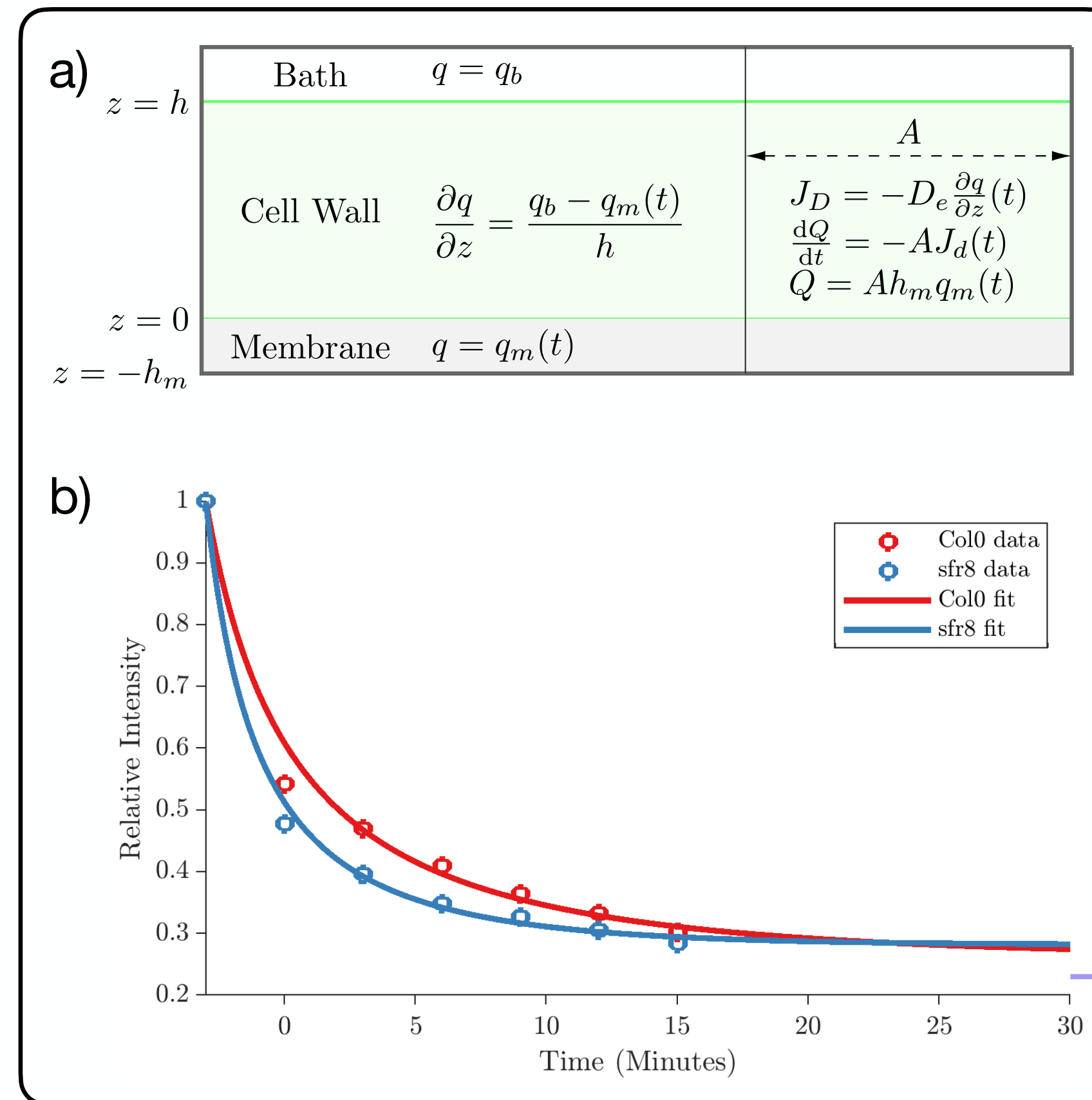
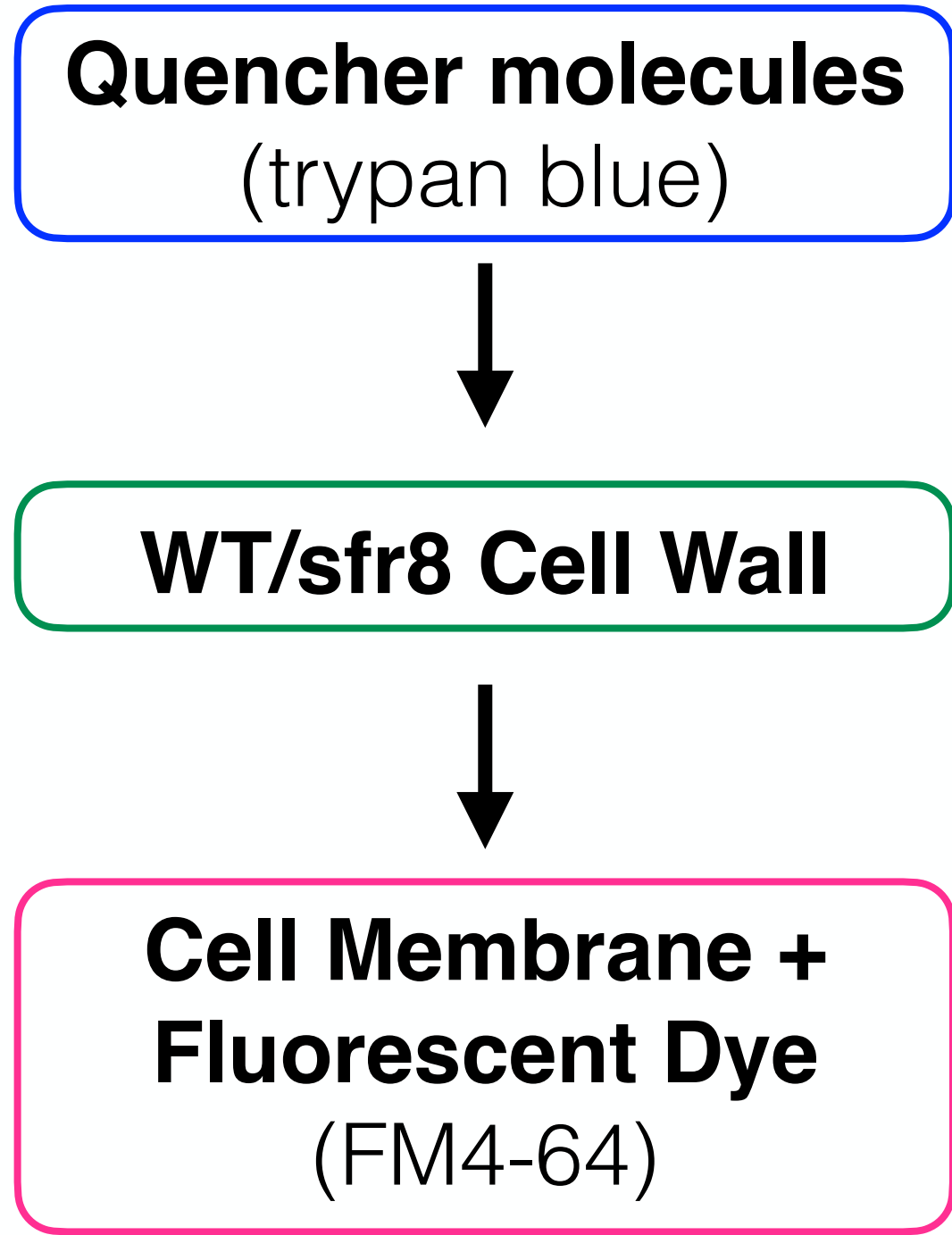
WT/*sfr8* Cell Wall



Cell Membrane +
Fluorescent Dye
(FM4-64)



- Heather found that, indeed, *sfr8* cell walls are more porous compared to the wild type
- **Mathematical modelling:** from basic fluid dynamics, we can model the fluorescence decay in time. By comparing WT and *sfr8*, we can estimate the (relative) porosity ϕ .



$$\frac{\phi_{sfr8}}{\phi_{Col0}} = 1.4717$$

- No need/evidence for “clogging” mechanisms