

# Human diseases and fungal adaptation: using solid-state NMR to probe extracellular matrices



Ananya Singh<sup>1</sup>, Sabeeha Malek<sup>2</sup>, Abi Hession<sup>1</sup>, Kacper Babiarz<sup>1</sup>, Josiah Idogun<sup>2</sup>, Teresa Massam-Wu<sup>2</sup>, Dariusz Köster<sup>2</sup>, Mohan Balasubramanian<sup>2</sup>, Wing Ying Chow<sup>1</sup>

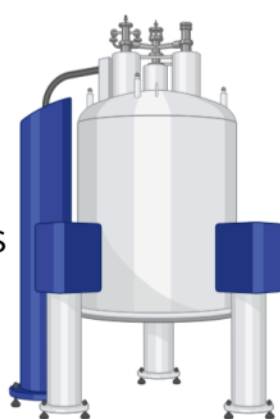
1 Department of Physics, University of Warwick

2 Centre for Mechanochemical Cell Biology and Warwick Medical School, University of Warwick

## Solid-state NMR

Why solid-state NMR?

- Solid/Gel-like samples without extraction or purification
- Can investigate proteins, sugars, including in tissues and intact cells.
- Obtain quantifiable information at the atomic lengthscale.



Biological solid-state NMR is often used to solve protein structures and probe protein dynamics, giving detailed insight on one or a few purified biomolecule(s).

Instead, we investigate the extracellular matrix (ECM), a complex and heterogeneous biological environment, and ask:

- what is the composition?
- What is the distribution of molecular features with different mobility regimes?

Solid-state NMR can pinpoint ECM structure change in biological processes in bone biomineralisation (Chow 2014) and rare genetic diseases, e.g. alkaptonuria (Chow 2020). Recent developments expanded applications to fungal and plant cell walls (Ghassemi 2022)



## Ehlers-Danlos syndromes

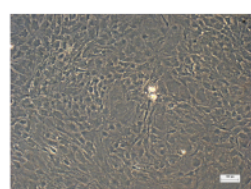
Ehlers-Danlos syndrome (EDS) is a genetic disease that leads to tissue fragility. The genetic cause of the most common form (1 in ~5000), hypermobile EDS (hEDS), is still unclear, resulting in slow diagnosis (average 10 years) and limited treatment options for patients.

Culture of human fibroblasts with three genetic backgrounds:

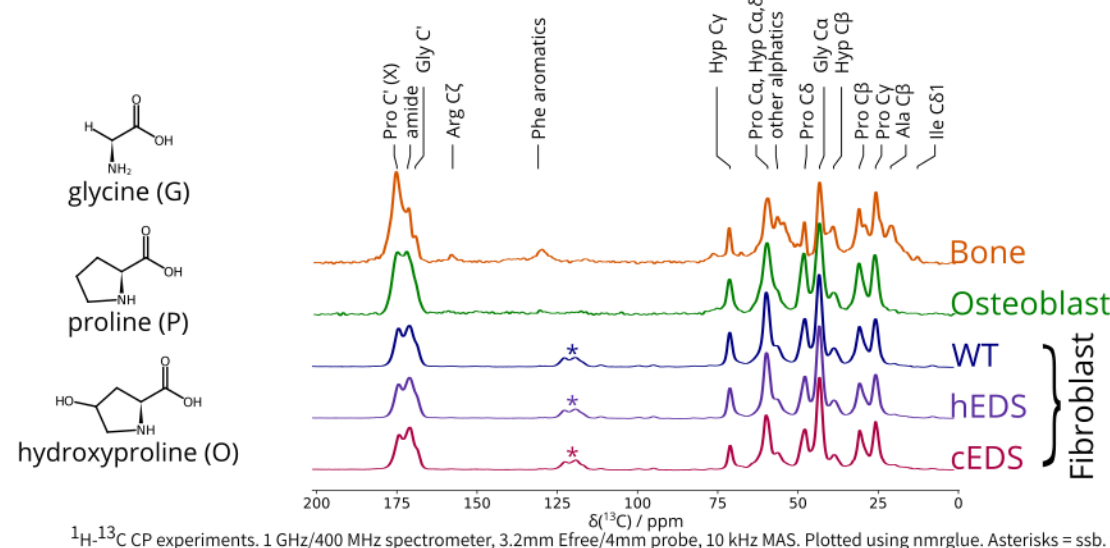
1. BJ fibroblast cell line (WT)
2. hypermobile Ehlers-Danlos syndrome (hEDS)
3. classical Ehlers-Danlos syndrome (cEDS)



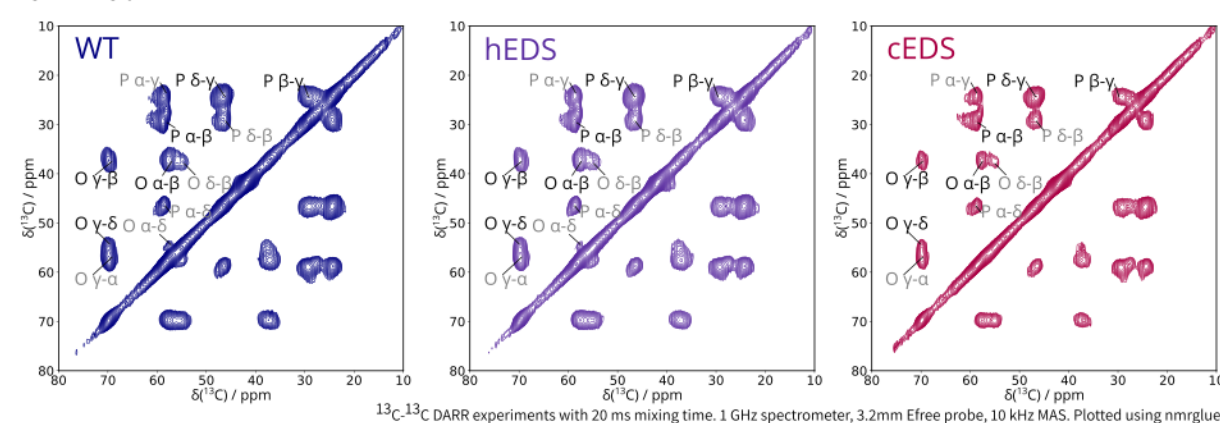
Fibroblasts are cultured to confluence (covering the surface) on standard media with added ascorbic acid, then switched to media containing <sup>13</sup>C-glycine and <sup>13</sup>C-proline. This protocol is based on previously published work on osteoblasts (Chow 2014).



After 2-3 weeks of cell culture, the fibroblasts produced a layer of ECM ("jelly" texture). The cells are removed by freeze-thawing and washing, the ECM layer is collected, dried, and used directly in ssNMR experiments.



Spectra obtained is consistent with previous work on osteoblast ECM and bone (Chow 2014). Signals arise from labelled glycine, proline, and hydroxyproline.



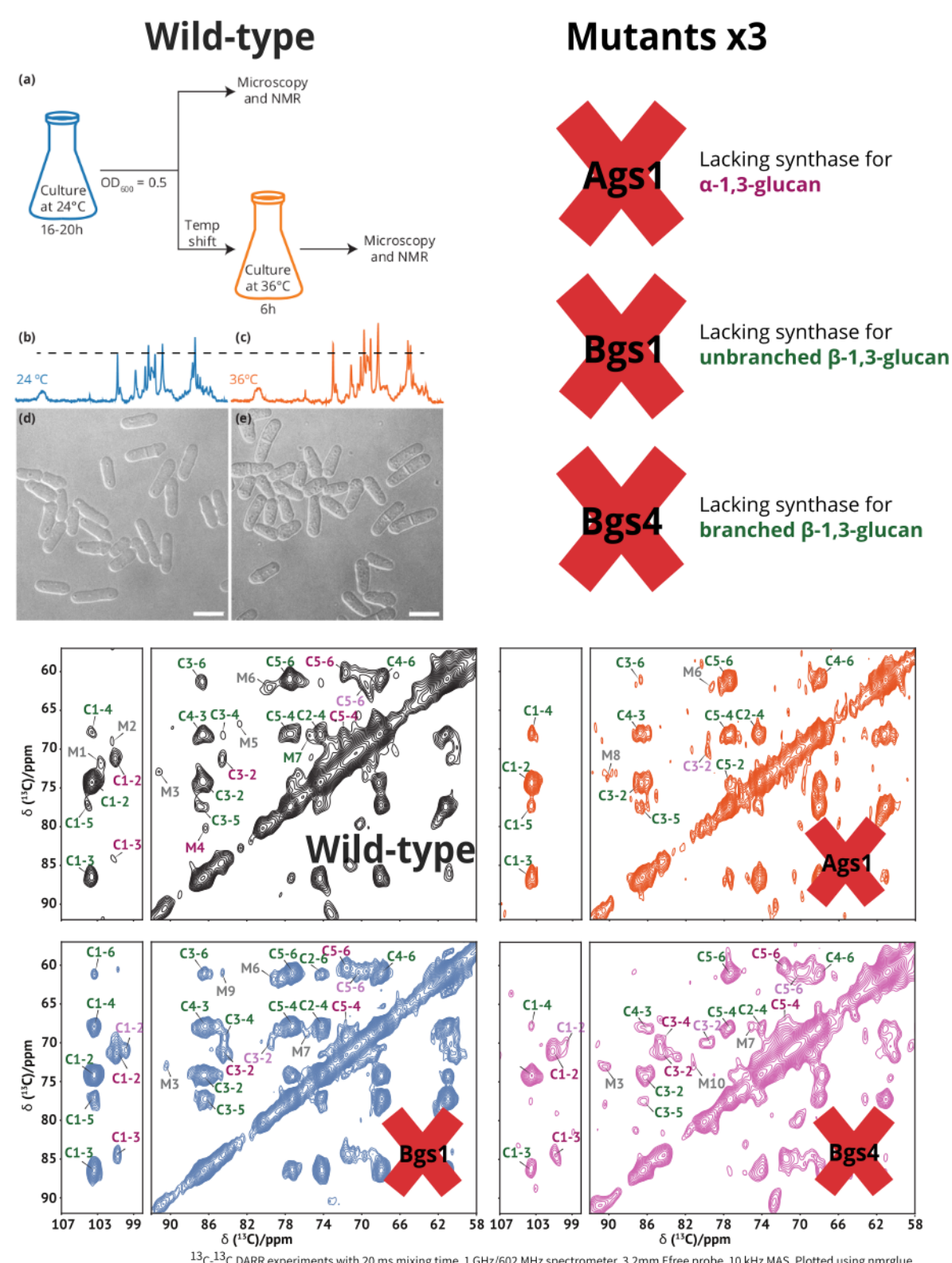
Summary: a feasible approach for modelling and investigating EDS ECM using cell culture and solid-state NMR

Current work:

- combining solid-state NMR, Raman spectroscopy, and proteomics
- using statistical/machine learning approaches to compare spectra across samples and methods

## Fission yeast fungal cell wall

We studied the cell wall of *Schizosaccharomyces pombe* (fission yeast) by carrying out NMR experiments on intact whole cells. First, we established the culture methodology with <sup>13</sup>C glucose and demonstrated that <sup>13</sup>C signal does not significantly change the composition and overall glucan signal intensity. We then applied the same sample preparation procedure to 3 mutants, each lacking one enzyme for synthesising a specific cell wall glucan.



Summary: established a cost-effective workflow on *S. pombe*, and probed the structural and functional consequences of mutations in three important glucan synthases. Rigid (not shown) and mobile (not shown) cell wall glucans can be identified.

Current work:

- investigating synthase inhibitors
- linking to yeast cell cycle
- resulted in award of Wellcome funding in fungal adaptation

## Summary

- Two new areas of biological applications of solid-state NMR spectroscopy: Ehlers-Danlos syndrome and fission yeast (model organism with comprehensive genomic/genetic database).
- New insights on intact, hydrated ECM samples, including whole cells.
- Mobility-based spectral filtering is another unique advantage of solid-state NMR.

## References

- Singh A, Massam-Wu T, Balasubramanian M, and Chow WY. 2026. Cost-Effective Solid-State NMR Of Fungal Glucans: A Case Study On *Schizosaccharomyces pombe*. *Chemistry - a European Journal*, e71081.
- Malek S, Köster DV. 2021. The Role of Cell Adhesion and Cytoskeleton Dynamics in the Pathogenesis of the Ehlers-Danlos Syndromes and Hypermobility Spectrum Disorders. *Front Cell Dev Biol* 9:649082.
- Chow WY, Norman BP, Roberts NB, Ranganath LR, Teutloff C, Bittl R, Duer MJ, Gallagher JA, Oschkinat H. 2020. Pigmentation Chemistry and Radical-Based Collagen Degradation in Alkaptonuria and Osteoarthritic Cartilage. *Angew Chem Int Ed Engl* 59:11937-11942.
- Chow WY, Rajan R, Muller KH, Reid DG, Skepper JN, Wong WC, Brooks RA, Green M, Bihan D, Farndale RW, Slatter DA, Shanahan CM, Duer MJ. 2014. NMR spectroscopy of native and in vitro tissues implicates polyADP ribose in biomineralization. *Science* 344:742-746.
- Ghassemi, Nader, Alexandre Poulhazan, Fabien Deligey, Frederic Mentink-Vigier, Isabelle Marcotte, and Tuo Wang. 2022. "Solid-State NMR Investigations of Extracellular Matrixes and Cell Walls of Algae, Bacteria, Fungi, and Plants." *Chemical Reviews* 122 (10): 10036-10086.

UNIVERSITY OF WARWICK



W.Ying.Chow@warwick.ac.uk  
https://wychowlab.org/

