Insights into Collagen Structure from ^{15}N-labelled Synthetic Model Peptides and Mouse Bone

Ieva Goldberga,[1] David G. Reid,[1] Wing Ying Chow,[2] H. Oschkinat,[2] Melinda J. Duer[1]

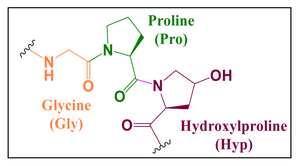
[1] *Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, United Kingdom (ig326@cam.ac.uk)*
[2] *Leibniz-Institut für Molekulare Pharmakologie, Campus Berlin-Buch, Robert-Roessle-Str.10, Berlin, Germany*

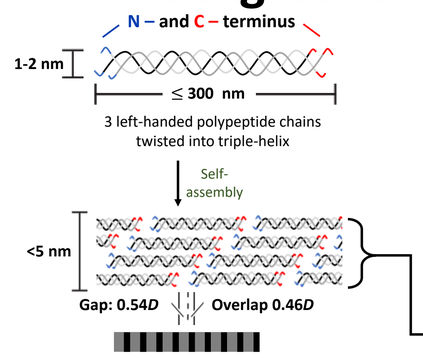
Introduction

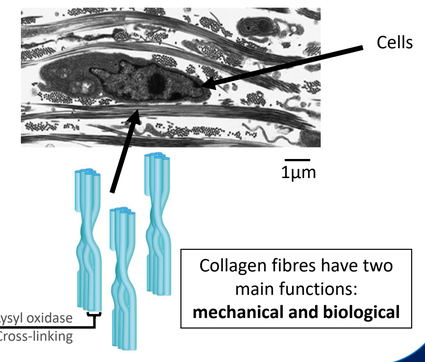
- This work focuses on ^{15}N assignment in synthetic collagen model peptides and ^{15}N-labelled mouse bone.
- ^{15}N relaxation is a sensitive probe of collagen backbone dynamics.
- Interpretation is assisted by selectively labelled amino acids in model collagen peptides which allow the sequence dependence and neighbour effects of ^{15}N relaxation to be characterized.
- The first part of the poster presents ^{15}N T_1's of model peptides and mouse bone; the second focuses on as yet unassigned ^{15}N resonances of bone.

Background

Collagen consists of Gly-X-Y repeats where X and Y are often Proline and Hydroxylproline (10.5%)[1]





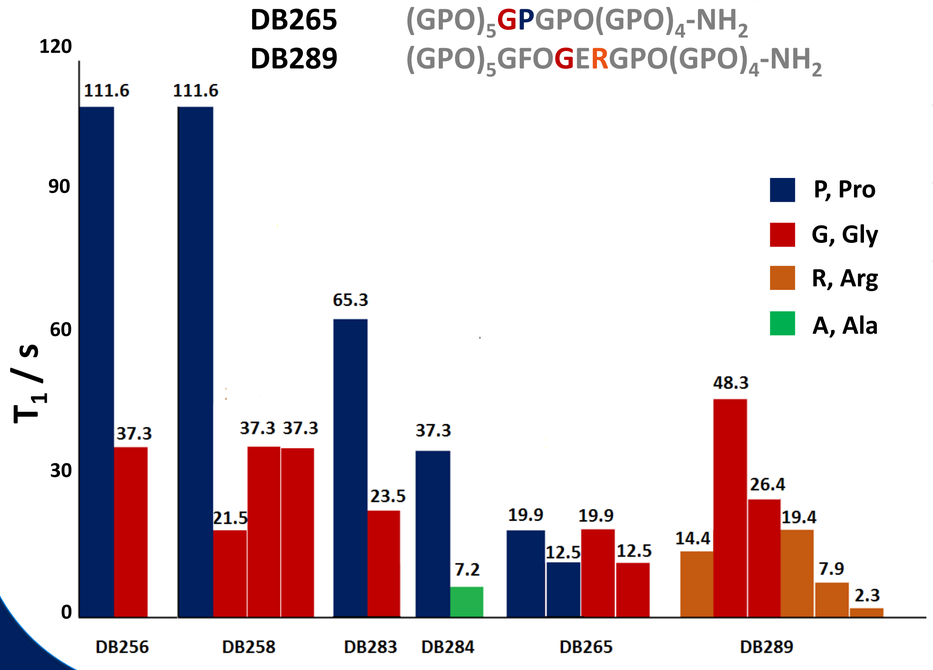


Collagen fibres have two main functions: **mechanical and biological**

Model Samples

- Graph represents relaxation of the following model peptides with labelling highlighted (A-Ala, G-Glycine, P-Proline, O-Hydroxyproline, R-Arginine):

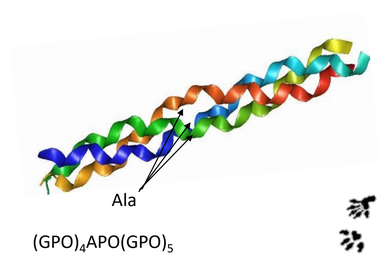
DB256	$(GPO)_5GPO(GPO)_5$-NH_2
DB258	$(GPO)_5GPP(GPO)_5$-NH_2
DB283	$(GPO)_5APPGPO(GPO)_4$-NH_2
DB284	$(GPO)_5GPPAPO(GPO)_4$-NH_2
DB265	$(GPO)_5GPGPO(GPO)_4$-NH_2
DB289	$(GPO)_5GFOGERGPO(GPO)_4$-NH_2



Relaxation Results

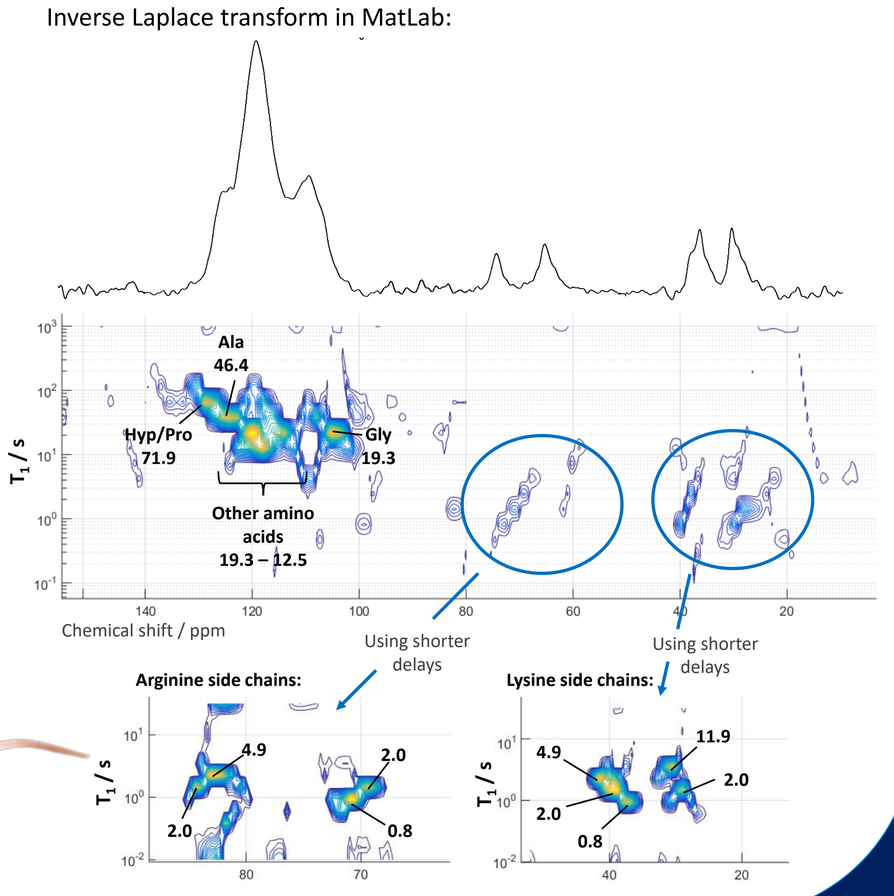
- Relaxation is affected not only by neighbouring amino acids within the chain but also by adjacent chains in the triple-helix structure:

- Schematic representation of chain stagger in $(GPO)_5GPO(GPO)_5$ and $(GPO)_5GPP(GPO)_5$ peptides sequence above (cross sections highlighted)
- Abundant GPO triplets provide stability to collagen triple-helix
- Ala and other substitutions 'loosen' the collagen triple helix
- Relaxation values of bone material are in good agreement with model samples



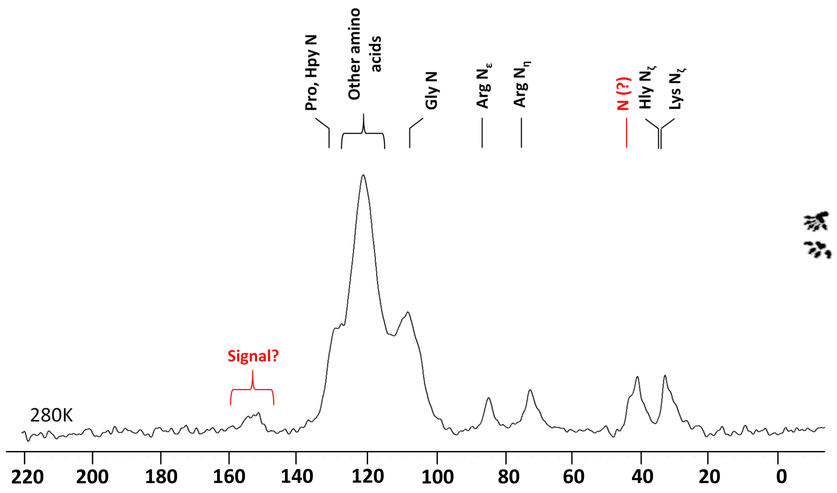
Bone Material

- Graphic representation of the relaxation results of bone material using Inverse Laplace transform in MatLab:

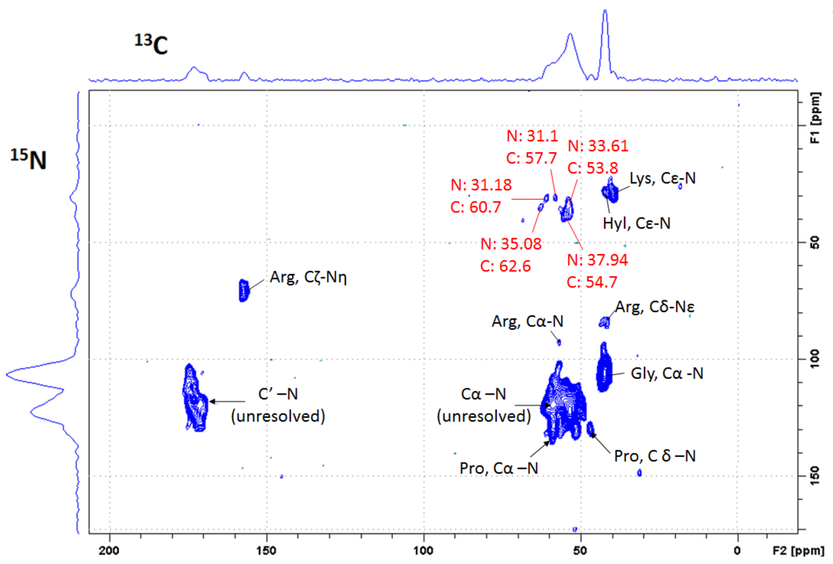


Less Abundant Nitrogen Species in the Bone Material: What are the Unidentified Signals?

1D ^{15}N NMR of Labelled Bone*:



2D Double CP of Labelled Bone*; Unassigned Resonances in Red:

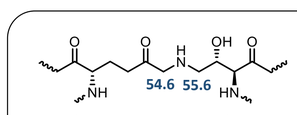


* Spectra were obtained during *iNEXT* visit in Berlin
* Arg-Arginine; Gly-Glycne; Hyl-Hydroxylysine; Lys-Lysine; Pro-Proline

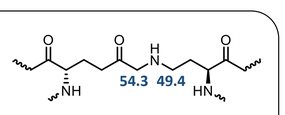
Possible Glycosylation, Enzymatic and Non-Enzymatic Cross-Linking Species[2] with ^{13}C predictions:

Enzymatic Cross-Linking Products

- Immature cross-links: Formed between two lysine and/or hydroxylysine side chains:

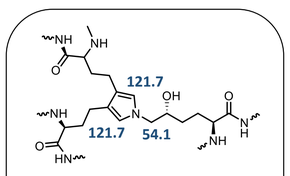


Hydroxylysine-ketonorleucine (HLKNL)

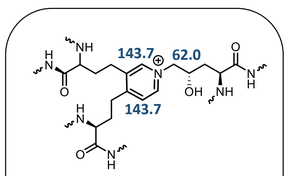


Lysino-ketonorleucine (LKNL)

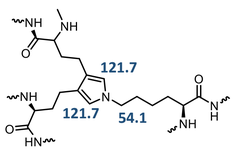
- Mature cross-links: Formed between three lysine and/or hydroxylysine side chains:



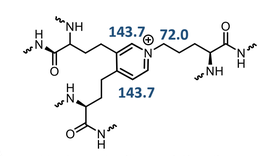
Lysil-pyrrole



Lysil-pyrridinoline

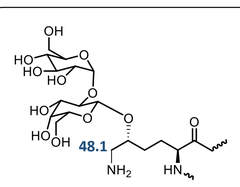


Hydroxylysil-pyrrole

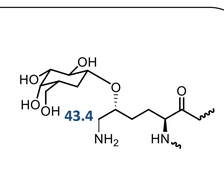


Hydroxylysil-pyrridinoline

The Glycosylation Products



α-D-glucosyl-β-D-galactosyl-5R-O-hydroxylysine

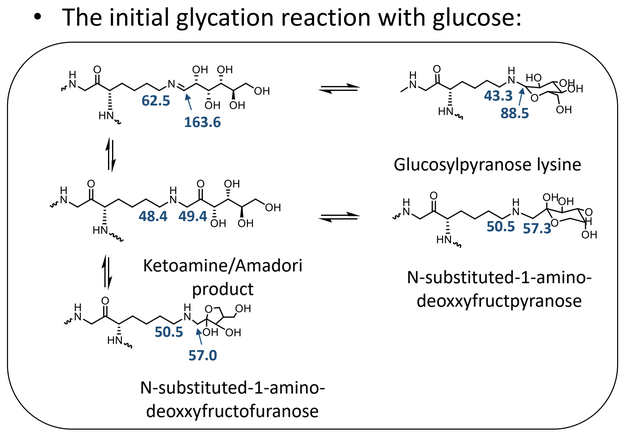


α-D-glucosyl-5R-O-hydroxylysine

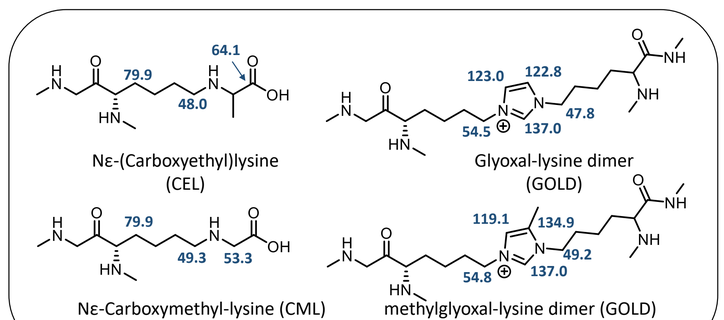
- These species are formed from hydroxylysine residues reacting with galactose

Non-Enzymatic Glycation Products

- The initial glycation reaction with glucose:



- Some advanced glycation endproducts (AGEs). Most involve lysines:



Future Work

- MD simulations to help understand collagen dynamics
- Other nuclei relaxation: ^{2}H and ^{13}C
- DNP NMR and model compound synthesis to help identify unknowns

[1] Shoulders, R. Raines, Collagen structure and stability. *Annu. Rev. Biochem*. **78**, 929-958 (2009)
[2] Bailey, A. J. *et al.* Mechanisms of maturation and ageing of collagen. *Mech. Ageing Dev.* **106,** 1–56 (1998)

UNIVERSITY OF CAMBRIDGE
EPSRC
iNEXT