**Introduction**

- This work focuses on 15N assignment in synthetic collagen model peptides and 15N-labelled mouse bone.
- 15N 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 15N relaxation to be characterized.
- The first part of the poster presents 15N T_1S of model peptides and mouse bone; the second focuses on as yet unassigned 15N resonances of bone.

**Model Samples**

- Graph represents relaxation of the following model peptides with labelling highlighted (A-Ala, G-Glycine, P-Proline, D-Hydroxyproline, R-Arginine):
  - DB256 (GPO)_3(GPO)NH_2
  - DB258 (GPO)_3(GPO)(GPO)NH_2
  - DB283 (GPO)P(GPO)(GPO)NH_2
  - DB284 (GPO)P(GPO)(GPO)NH_2
  - DB265 (GPO)P(GPO)(GPO)NH_2
  - DB289 (GPO)PFO(GPO)P(GPO)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]_3, and [GPO]P(GPO)NH_2 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 15N NMR of Labelled Bone*:

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

**Possible Glycosylation, Enzymatic and Non-Enzymatic Cross-Linking Species** with 13C predictions:

**Enzymatic Cross-Linking Products**

- Immature cross-links: Formed between two lysine and/or hydroxylysine side chains:
  - Hydroxylysine-ketonaemic (HLY-KNM)
  - Ketonaemic-hydroxylysine (KMN-HLY)
- Mature cross-links: Formed between three lysine and/or hydroxylysine side chains:
  - Glucosylpyranose (GPO)
  - Ketonaemic-glucosylpyranose (KGM)

**The Glycosylation Products**

- These species are formed from hydroxylysine residues reacting with galactose

**Non-Enzymatic Glycation Products**

- The initial glycation reaction with glucose:
  - Glucosylpyranose (GPO)
  - Glucosylpyranose (GPO)
- Some advanced glycation endproducts (AGEs). Most involve lysines:
  - N-halogenated L-lysine
  - N-substituted L-lysine

**Future Work**

- MD simulations to help understand collagen dynamics
- Other nucleus relaxation: 1H and 13C
- DNP NMR and model compound synthesis to help identify unknowns

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* Spectra were obtained during 15N NMR visit in Berlin
  - Ala arginine, G-Glycine, P-Proline, D-Hydroxyproline, R-Arginine

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