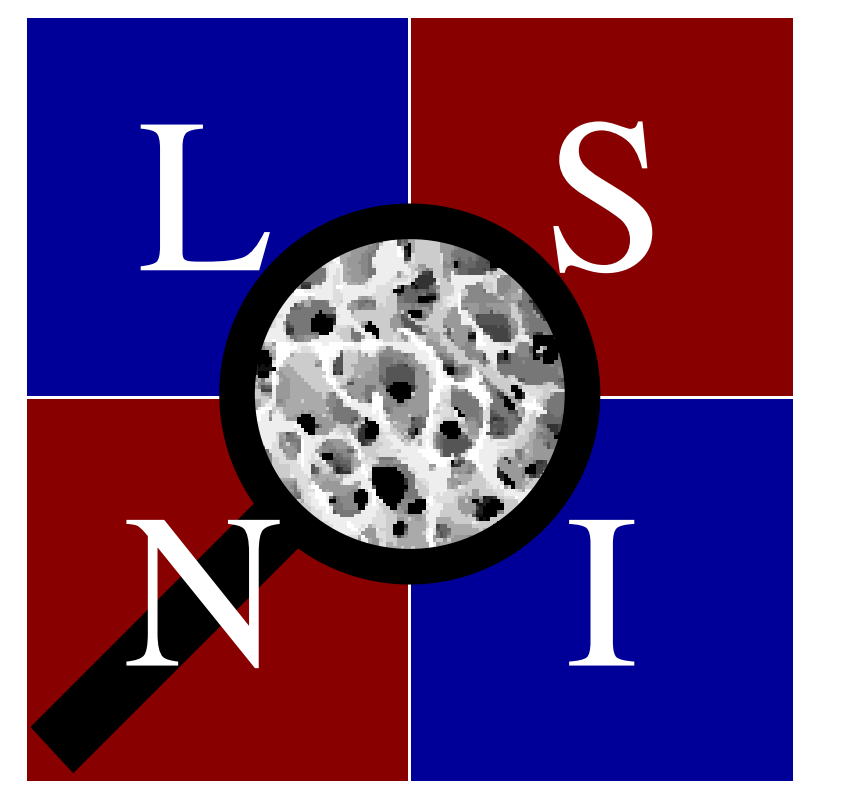


# NMR STUDY OF THE DYNAMIC PROPERTIES OF BONE WATER



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## INTRODUCTION

Water plays a pivotal role in the biology of bone. It serves as a solvent for transport of nutrients to and from osteocytes and largely determines the bone's viscoelastic properties. Despite its importance, the nature and binding properties of bone water are incompletely understood. Fernandez et al [2,3], were able to measure water diffusion in solid bone, by studying the H<sub>2</sub>O/D<sub>2</sub>O exchange kinetics, suggesting the presence of two water components of widely differing diffusion rate.

## OBJECTIVES

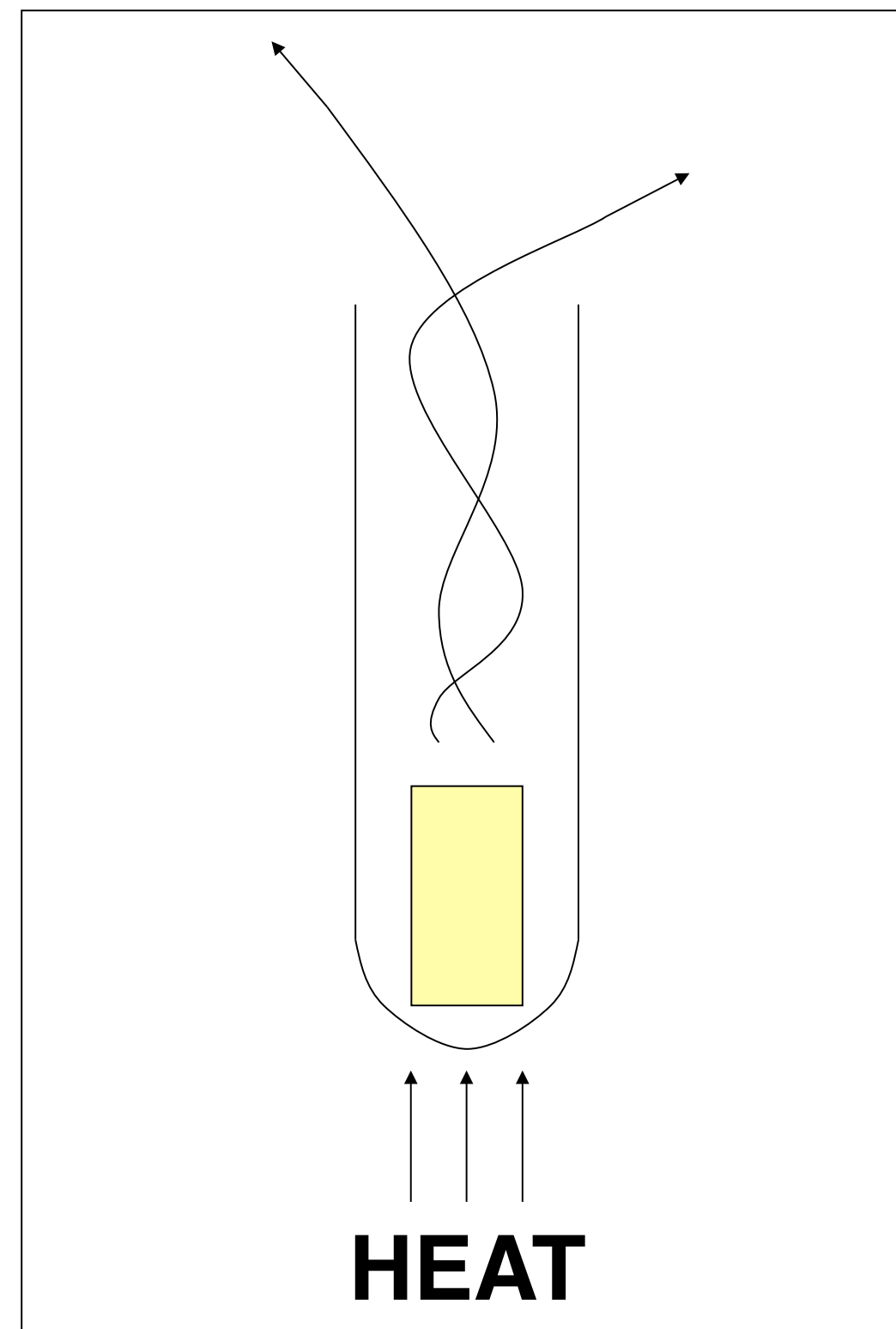
- To investigate the properties of the two bone water fractions by monitoring the NMR signal while the water is gradually expelled by dehydrating the bone at elevated temperature.
- To further validate these results by thermogravimetric analysis (TGA) - following the mass of the bone as a function of heating time.

## METHODS AND MATERIALS

**Sample:** rectangular section (10×4×1mm<sup>3</sup>) of cortical bone, with the marrow removed, harvested from the mid-shaft of the tibia of a 14 week old New Zealand white rabbit.

### NMR Experiment:

- the sample was placed in a 5mm NMR tube with no lid in the spectrometer and maintained at 100°C for 48 hours.
- vertical-bore spectrometer operating at 9.4T (DMX-400, Bruker)
- after 20 minutes, to allow equilibration, the FID (30 scans, 20° flip angle = 1.72 [ms], T<sub>R</sub> = 1.5s, dwell time = 5 [ms]) was acquired over 48 hours (every 5, 15 and 60 minutes for time periods 0-1, 1-6 and 6-48 hours).
- T<sub>1</sub> was measured with inversion recovery at various times throughout the 48 hours (Table 2, τ<sub>90</sub> = 7.75 [ms], τ<sub>180</sub> = 15.5 [ms], T<sub>1</sub> = 0.1 – 3s).



**Figure 1: Schematic of experimental set up for the NMR experiment.** The sample is heated to 100°C inside the spectrometer in air for 48 hours while the FID and T<sub>1</sub> of the bone water are measured periodically.

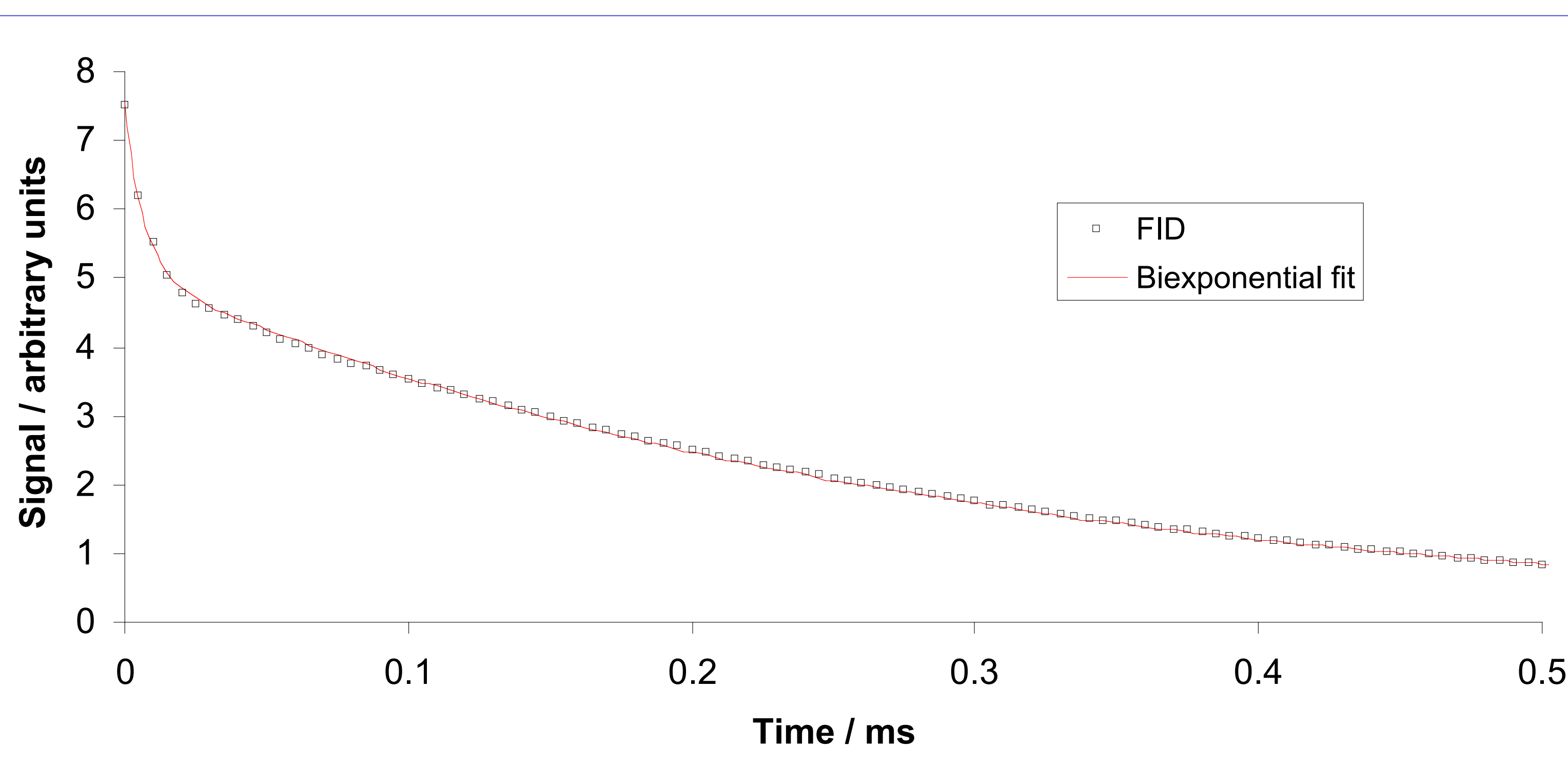
### TGA Experiment:

- the sample was placed in the TGA machine and heated to 100°C for 15 hours.
- the total mass and heat flow into/out of the sample were measured as a function of heating time.

### Data Analysis:

- Each FID was fitted to a biexponential function of the form  $M_1 \exp\{-t/T_2^*(1)\} + M_2 \exp\{-t/T_2^*(2)\}$
- Diffusion constants were calculated by modelling the bone as a one-dimensional object of length  $d$  and diffusion constant,  $D$ . In this case the signal,  $I(t)$ , at long times is given by  $I(t) \approx 8I(0)/\pi^2 \exp\{-D\pi^2 t / d^2\}$  [4].
- Finite element simulations were run based on the same one dimensional model using the calculated values of  $D$  and  $I(0)$  (Figure 3).

## RESULTS



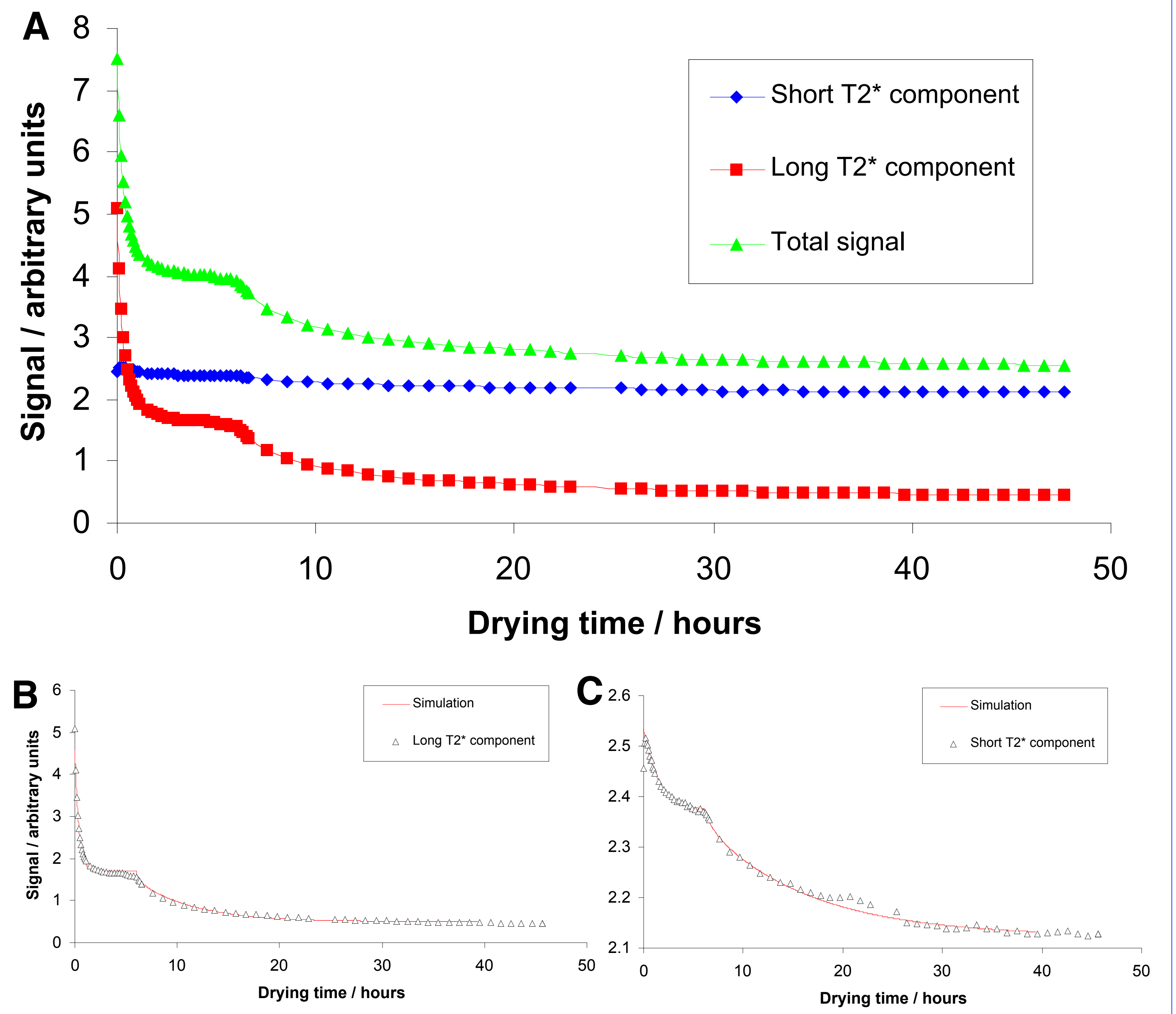
**Figure 2: The FID of bone water at 100°C** shows distinct biexponential decay which we associate with two water components. The short T<sub>2</sub><sup>\*</sup> component (T<sub>2</sub><sup>\*</sup> = 6.5 [ms]) corresponds to protein bound water, while the long T<sub>2</sub><sup>\*</sup> component (T<sub>2</sub><sup>\*</sup> = 250 [ms]) is associated with 'free' water in the pore space.

Component	D / 10 <sup>-7</sup> cm <sup>2</sup> s <sup>-1</sup>	
	1 <sup>st</sup> stage	2 <sup>nd</sup> stage
Total signal	6.50	0.45
Short T <sub>2</sub> <sup>*</sup>	1.83	0.27
Long T <sub>2</sub> <sup>*</sup>	7.17	0.50

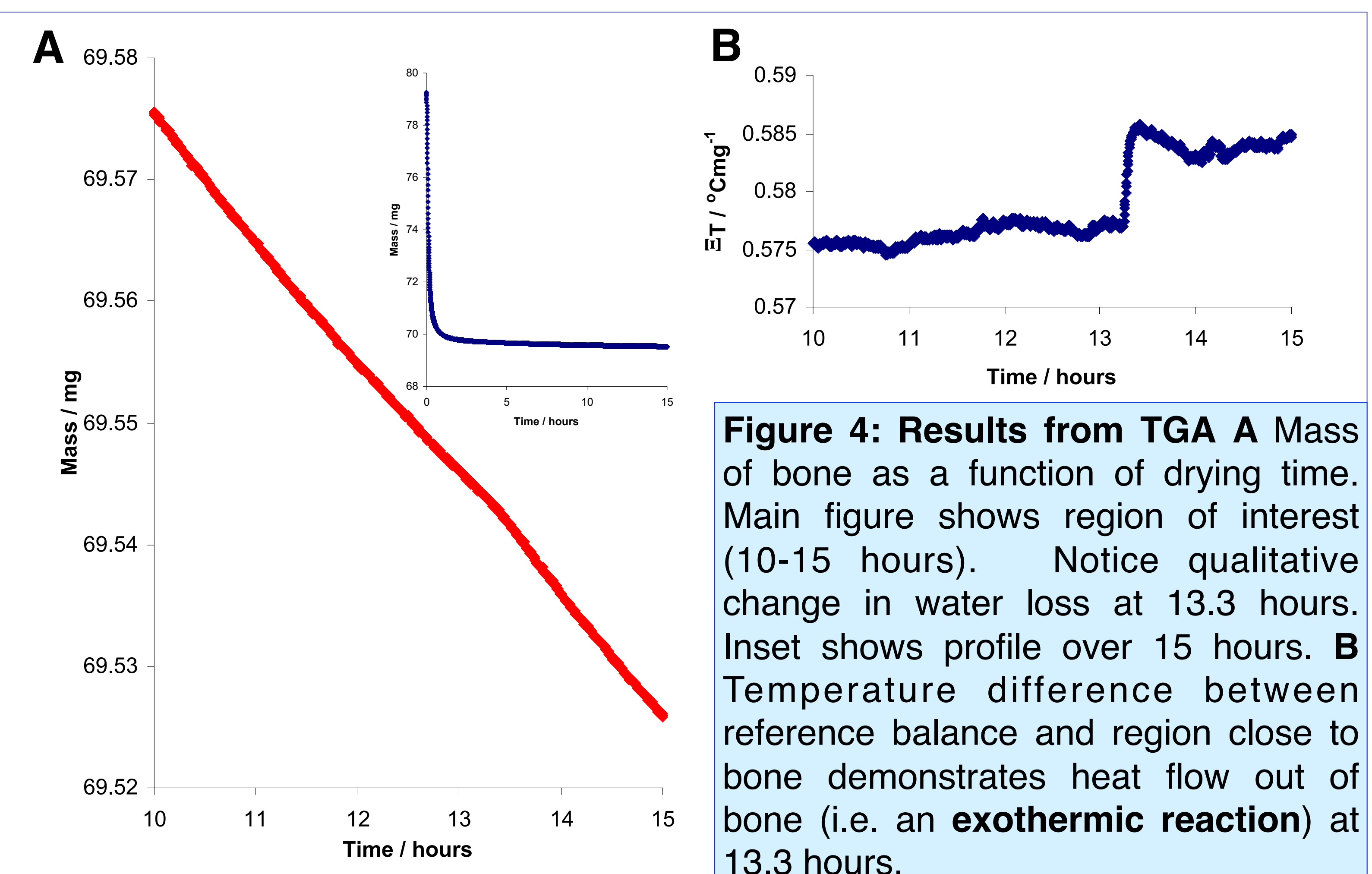
**Table 1: Diffusion constants of each water component at each stage of the drying process.** Compared with bulk water which has D ~8.0 × 10<sup>-5</sup> cm<sup>2</sup>s<sup>-1</sup> just below 100°C.

Time / hours	T <sub>1</sub> / s	
	Short T <sub>2</sub> <sup>*</sup>	Long T <sub>2</sub> <sup>*</sup>
0	0.841	0.8397
5.6	0.9865	0.9855
21.5	1.2149	1.2019
27.4	1.2899	1.329
45.5	1.3656	1.4532

**Table 2: T<sub>1</sub> of remaining bone water as a function of drying time at 100°C.** T<sub>1</sub> of both components increases with heating time due to decreased H<sub>2</sub>O-H<sub>2</sub>O interaction. Note also that both components have similar T<sub>1</sub> suggesting that they are both water.



**Figure 3: Results from NMR** A Signal from bone water as a function of drying time for each component. All three curves show a two-stage behaviour, with the transition occurring around 6 hours after the start of heating. B, C time course of long (B) and short (C) T<sub>2</sub><sup>\*</sup> components compared with finite element simulations.



**Figure 4: Results from TGA** A Mass of bone as a function of drying time. Main figure shows region of interest (10-15 hours). Notice qualitative change in water loss at 13.3 hours. Inset shows profile over 15 hours. B Temperature difference between reference balance and region close to bone demonstrates heat flow out of bone (i.e. an exothermic reaction) at 13.3 hours.

## DISCUSSION

- All the FIDs decay in a biexponential fashion with T<sub>2</sub><sup>\*</sup> = 6.5 and 250 [ms] (Figure 2)
- T<sub>1</sub> was similar for both components and increased with drying time (Table 2). This change reflects reduced H<sub>2</sub>O-H<sub>2</sub>O interaction as the water content falls.
- All the decay curves (Figure 3) followed a two-stage behaviour with the transition point between the two stages being around 6 hours.
- Despite this unexpected result, the decay of each stage is approximately mono-exponential enabling diffusion coefficients to be calculated using [4].
- The results (Table 1) show that the long T<sub>2</sub><sup>\*</sup> component is more mobile than the short T<sub>2</sub><sup>\*</sup> component, consistent with their assignments as free and bound water.
- Simulations using these values of  $D$  are in good agreement with experiment for each stage and each component (Figure 3 B,C).
- Thermogravimetric analysis suggests that the two-stage behaviour is repeatable and that the change between stages is accompanied by an exothermic reaction.
- It is likely that the two-stage behaviour is dependent on complex binding properties of water and is suggested that it could involve collagen denaturing, a process that occurs around 70°C.

## CONCLUSIONS

Time-resolved NMR experiments during drying of bone can provide insight into the dynamics of water in different binding states.

## ACKNOWLEDGEMENTS

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