Supplemental Information (Figures 1S – 5S)

Investigating Bone Resorption in Atlantic Herring Fish Intermuscular Bones with Solid-State NMR

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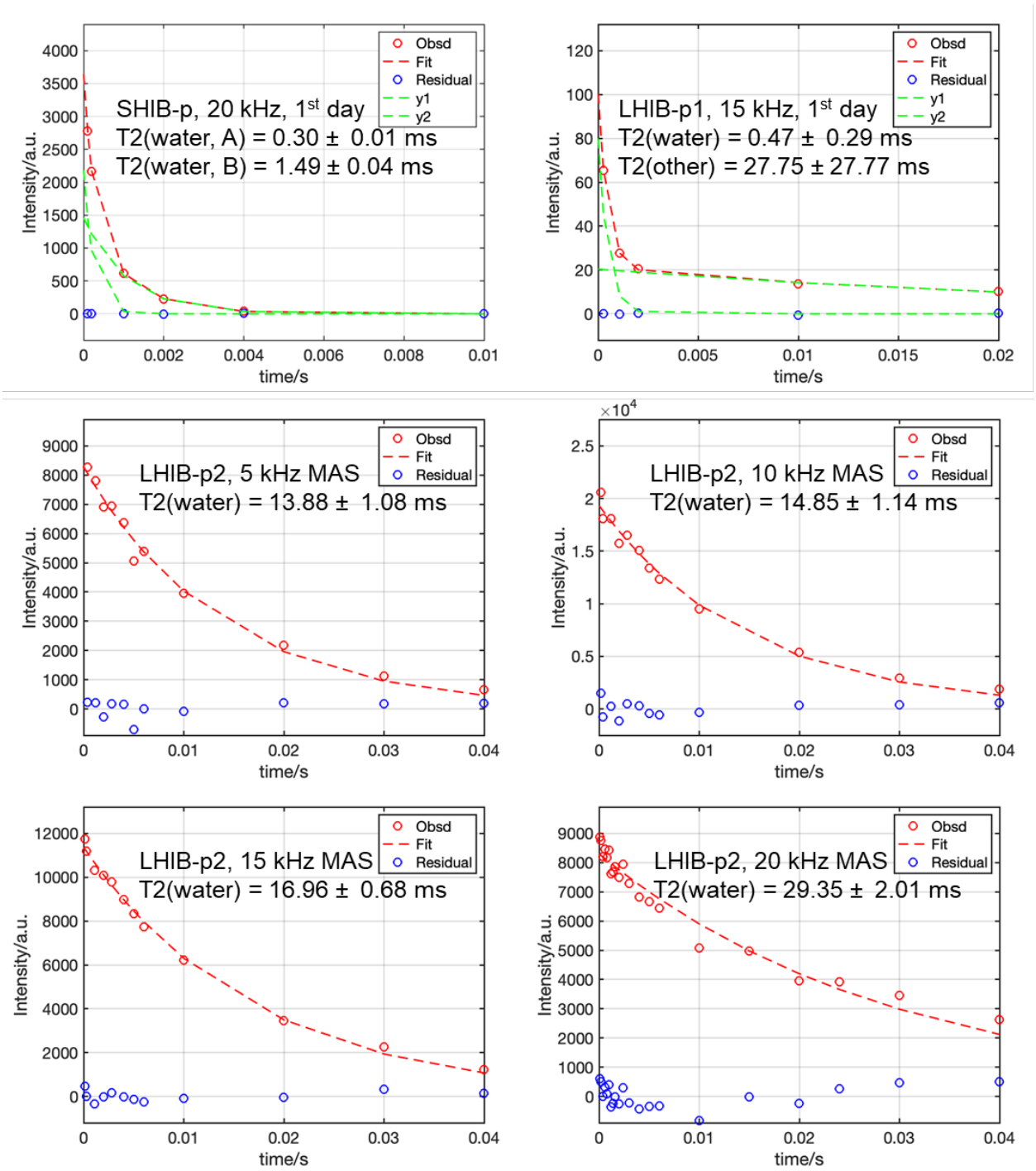

Figure 1S. T_2 of SHIB-p, LHIB-p1, and LHIB-p2a under various spinning conditions. The horizontal axis are spin echo times. The data were fitted with MATLAB. Top Panels required biexponential fits, whereas single exponential fits sufficed with the LHIB-p2a data. Data acquired with VT control at 25ºC. The actual temperature varies due to frictions as described in the main text.

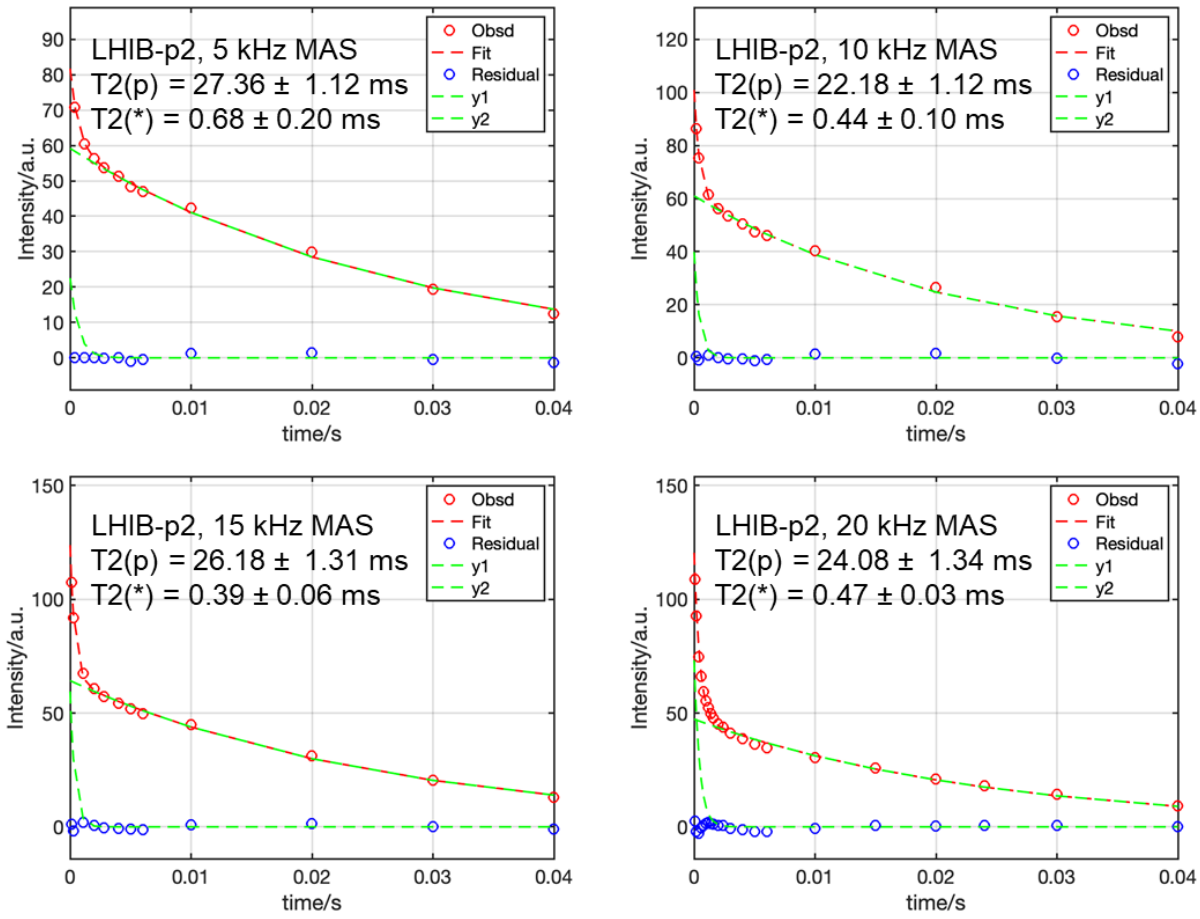

Figure 2S. T_2 of peptides (p, estimated from integrals from 0.4 to 2.4 ppm) in LHIB-p2a under various spinning conditions, * denotes baseline contributions from the water peak (see discussion below). The data were fitted with MATLAB with double exponentials. The horizontal axis are spin-echo times.

DISCUSSION - the need for biexponential fits. It should be recognized that water signal is so much larger than the peptide signals, its tails extend well over on either side of the spectra and contributed significantly to the integration of the peptide signals. We have selected a region away from water (0.4 to 2.4 ppm) to minimize this impact. However, the contribution of water tails is still considerable, especially at the initial time points. Hence it is necessary to use biexponential fits to delineate the two components. There is even an argument for triexponential fits. One can well imagine the broad 1H-signal from the solid collagen fibrils to be picked up as well at the initial time points, which is known from MRI studies to relax below sub-millisecond T2 times. We decide not to over-determine the fit and present the biexponential fits here. The weights of the two fits can be extracted by the individual fits (in green), but it is not clear whether the weight calculation is meaningful. We focus here on the long-lasting 1H-signals which we believe comes from the digested peptides.

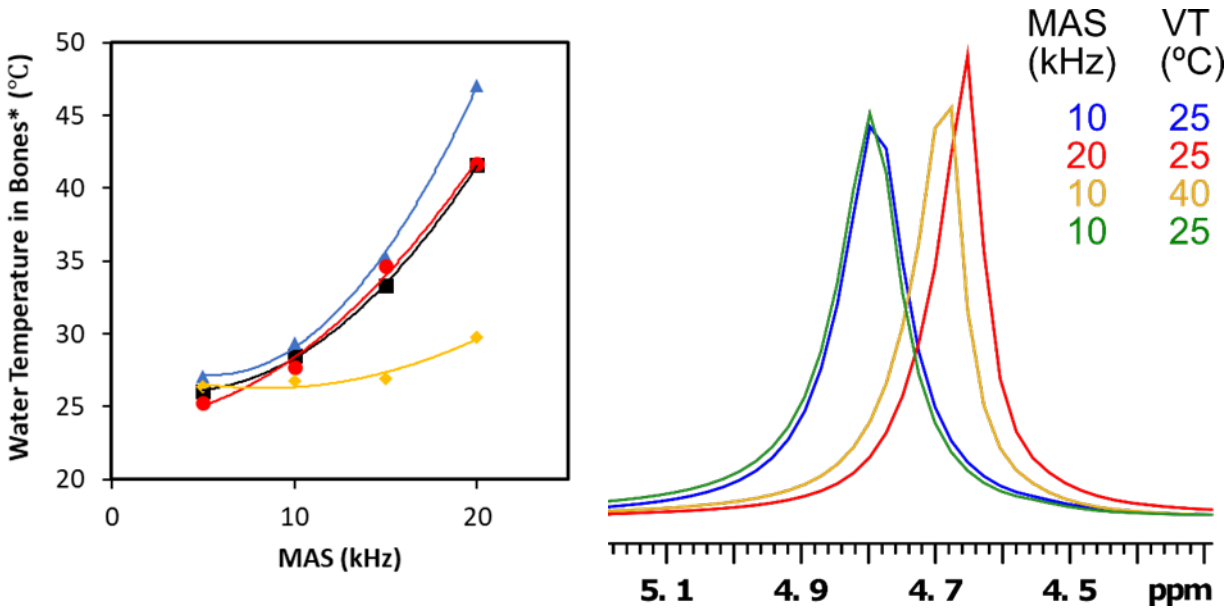

Figure 3S. Impact of MAS spinning on water chemical shift and temperature in Atlantic herring intermuscular bones. Left Panel: Temperature calculated from water chemical shifts, LHIB-p2a (▲), LHIB-p2b (●), and SHIB-p (♦, studied previously[17]), all with variable temperature control set to 25ºC and compared with values predicted by literature[25] (■). A quadrature trendline is plotted with each curve to guide to eye. Right Panel, zoomed-in water chemical shift region of LHIB-p2b at various MAS and variable temperature controls.

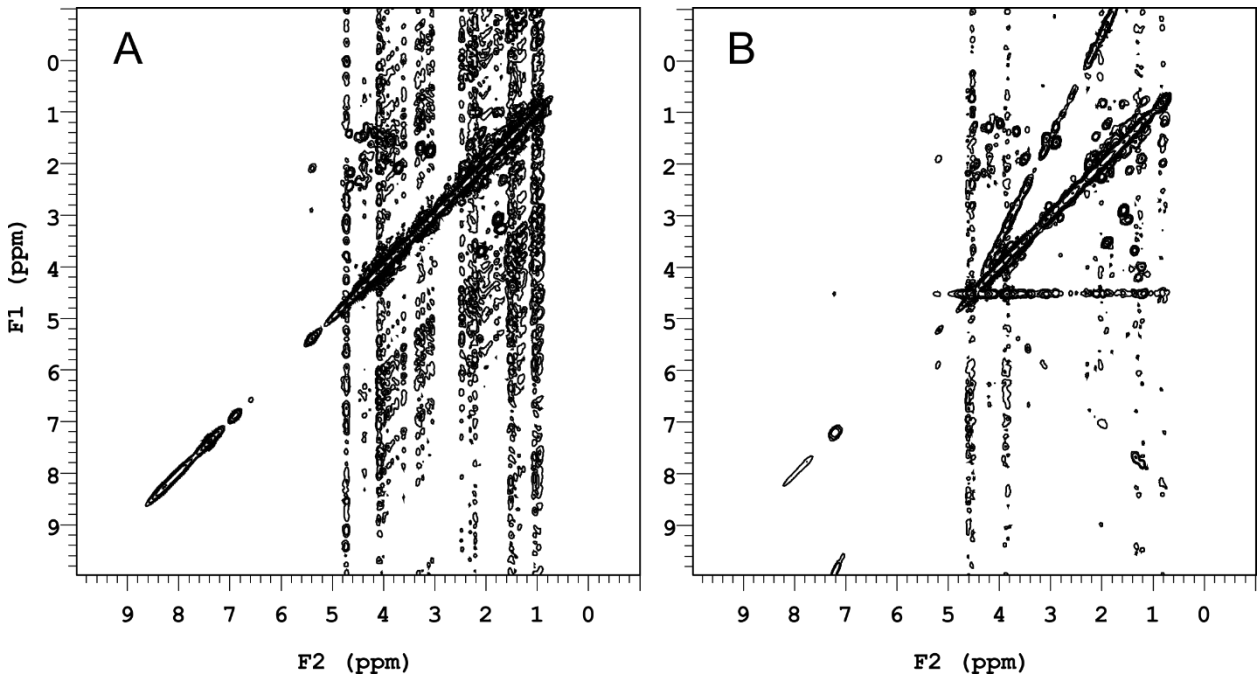

Figure 4S. $^{1}H/^{1}H$-COSY spectra of LHIB-p2a at 20 kHz MAS: (A) COSY-45, which suffers from pervasive artifacts likely due to the residual anisotropic interactions that were not sufficiently removed by magic angle spinning (MAS); and (B) DQF-COSY, which cleared up substantial amount of these interactions but added an undesirable 26.6°-diagonal (with a 2:1 offset ratio in F1 and F2 frequencies) as well as the 45°-antidiagonal artifacts, due to the lack of pulsed-field gradient capabilities in our solids probes.

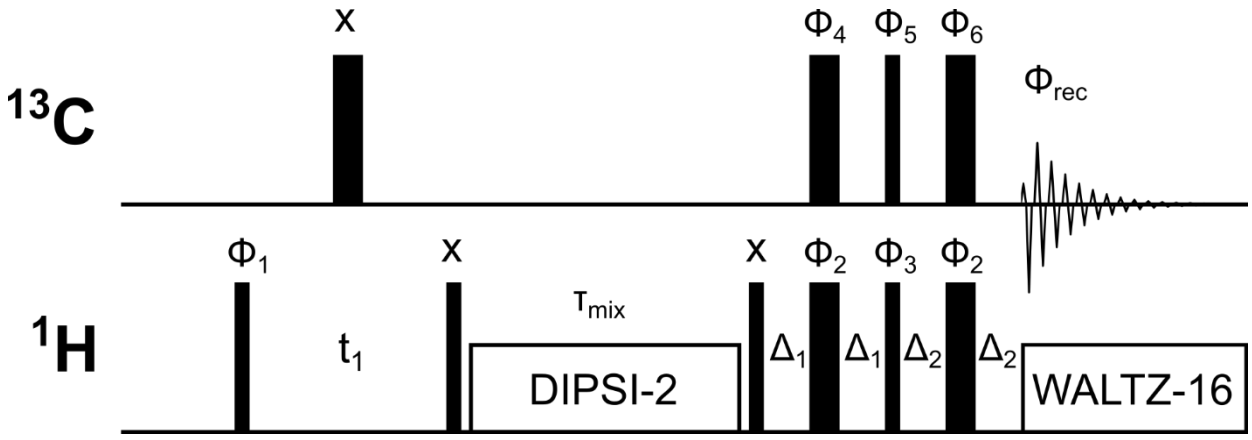

Figure 5S. Two dimensional refocused ^{1}H-DIPSI2-^{1}H/^{13}C-INEPT2D heteronuclear correlation pulse sequence. The delays τ_{mix}, Δ_1, and Δ_2 are rotor-synchronized with MAS. The phase cycles used are: ϕ_1 = 8(x), 8(-x); ϕ_2 = 8(x, -x); ϕ_3 = 4(y, y, -y, -y); ϕ_4 = 8(x, -x); ϕ_5 = 4(x), 4(y), 4(-x), 4(-y); ϕ_6 = 2(x, -x, x, -x, y, -y, y, -y); ϕ_{rec} = 2(x, x, -x, -x, y, y, -y,-y).