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Supplemental Information

Active Site Breathing of Human Alkbh5 Revealed by Solution NMR and Accelerated Molecular Dynamics

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Figure S1. (**a**) MALDI-TOF spectra acquired for the 5'-GG(m^6A)CT-3' oligonucleotide before (top) and after (bottom) incubation with Alkbh5 for 20 minutes at room temperature (see *Materials and methods*). The peak at 1,504 Da observed after incubation with the enzyme is consistent with demethylation of the oligonucleotide.

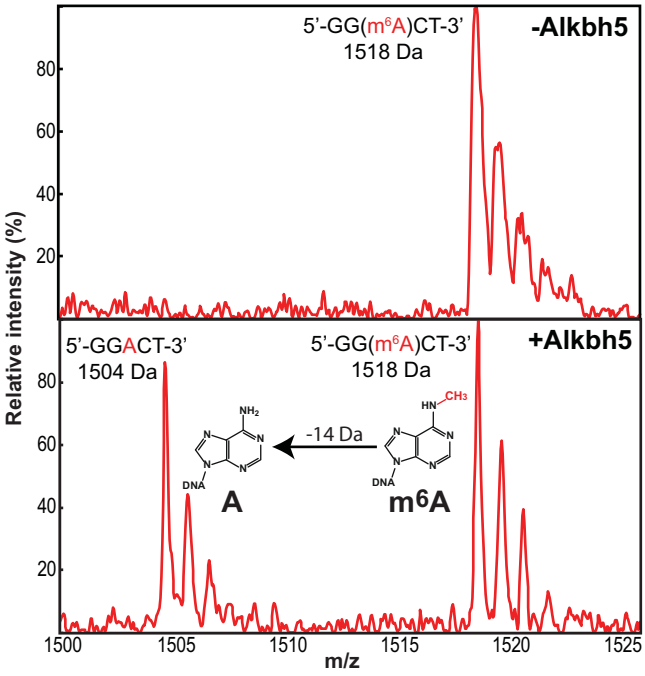


Figure S2. Methyl-TROSY spectrum of human Alkbh5 acquired on a U-[^{2}H,^{15}N]/Ile(δ1)-$^{13}CH_3$/Val,Leu-($^{13}CH_3$/$^{12}C^{2}H_3$)-labelled sample. Resonance Assignment was performed using out-and-back experiments (1). Assigned ^{1}H-$^{13}C_{methyl}$ resonances are labeled in the figure.

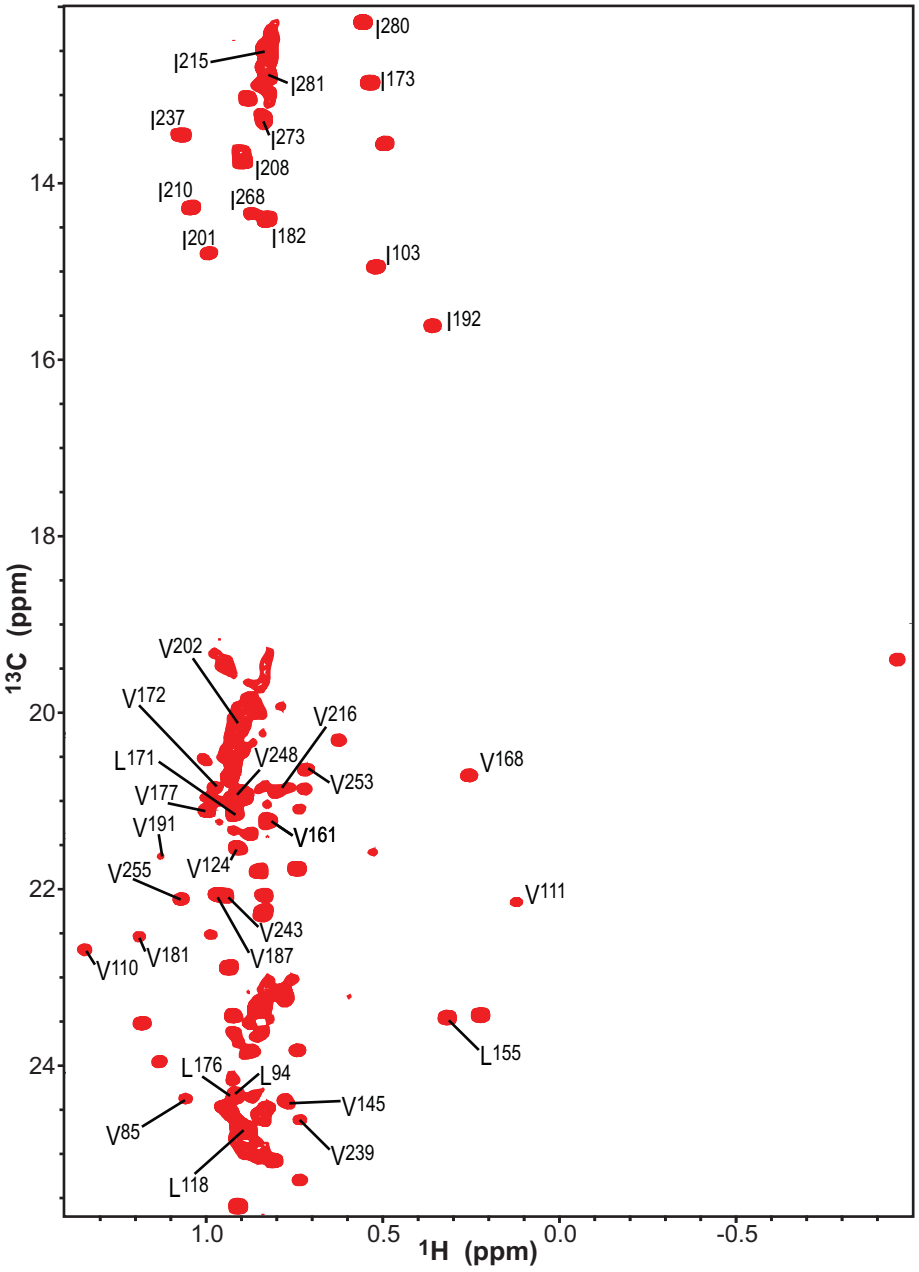


Figure S3. (**a**) Evolution of the backbone r.m.s.d. during the 200 ns GaMD run. Clustering analysis was performed from 20 ns (dashed line) onward. (**b**) Overlay of the 20 Alkbh5 structure in the final aMD/RDC conformational ensemble. Each ensemble member is highlighted with a different color.

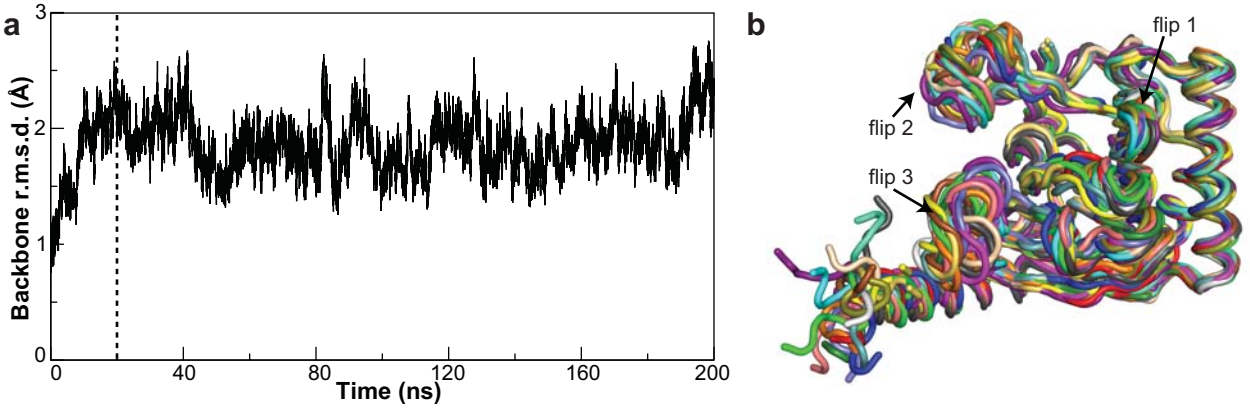


Figure S4. Global fitting of the twenty-four ^{15}N and $^{13}C_{methyl}$ relaxation dispersion curves at 15 (blue), 20 (green) and 25 (red) °C that describe µs-ms dynamics in the catalytic domain of Alkbh5. Experimental data are reported as diamonds. Results of the global fit are shown as solid lines. Relaxation dispersion curves measured at 800 MHz are shown.

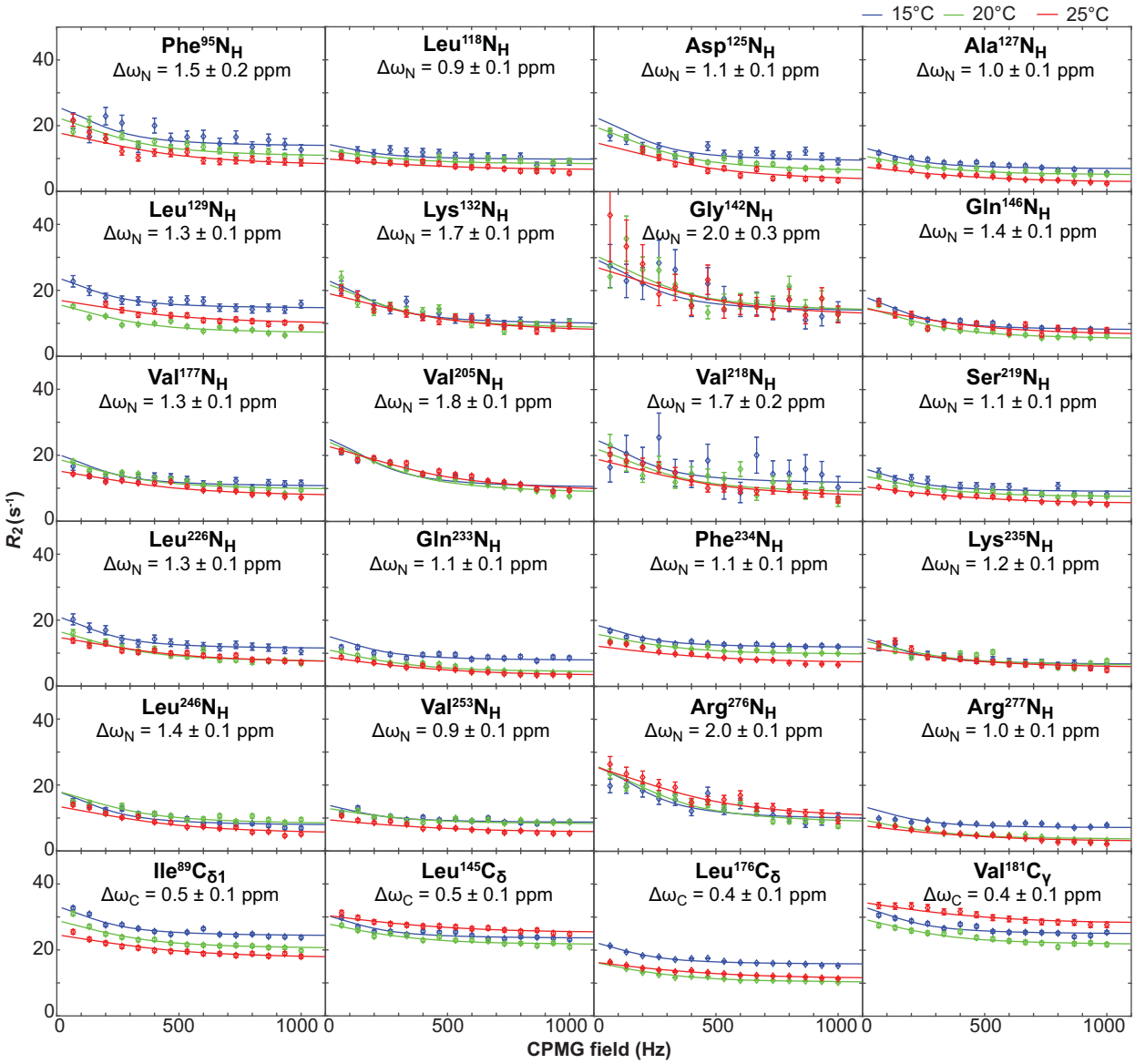
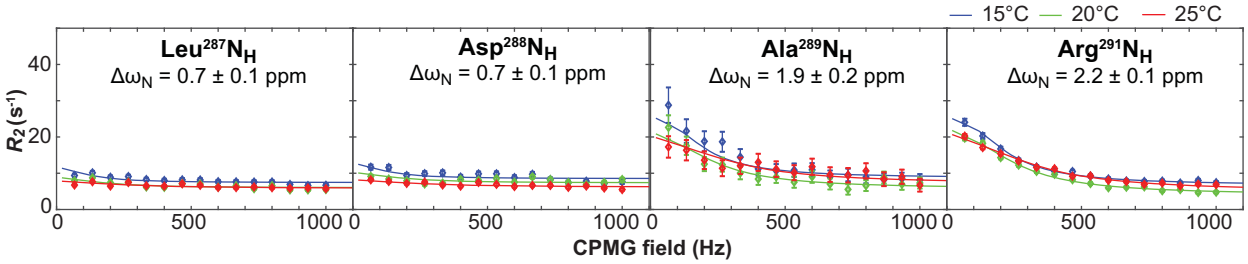


Figure S5. Global fitting of the four ^{15}N relaxation dispersion curves at 15 (blue), 20 (green) and 25 (red) °C that describe µs-ms dynamics in the C-terminal region of Alkbh5. Experimental data are reported as diamonds. Results of the global fit are shown as solid lines. Relaxation dispersion curves measured at 800 MHz are shown.



References

1. Tugarinov V., Venditti V., Marius Clore G. 2014. A NMR experiment for simultaneous correlations of valine and leucine/isoleucine methyls with carbonyl chemical shifts in proteins. J Biomol NMR. 58:1-8.