# SUPPLEMENTARY MATERIAL

## The NMR signature of gluconoylation - a frequent N-terminal modification of isotope-labeled proteins

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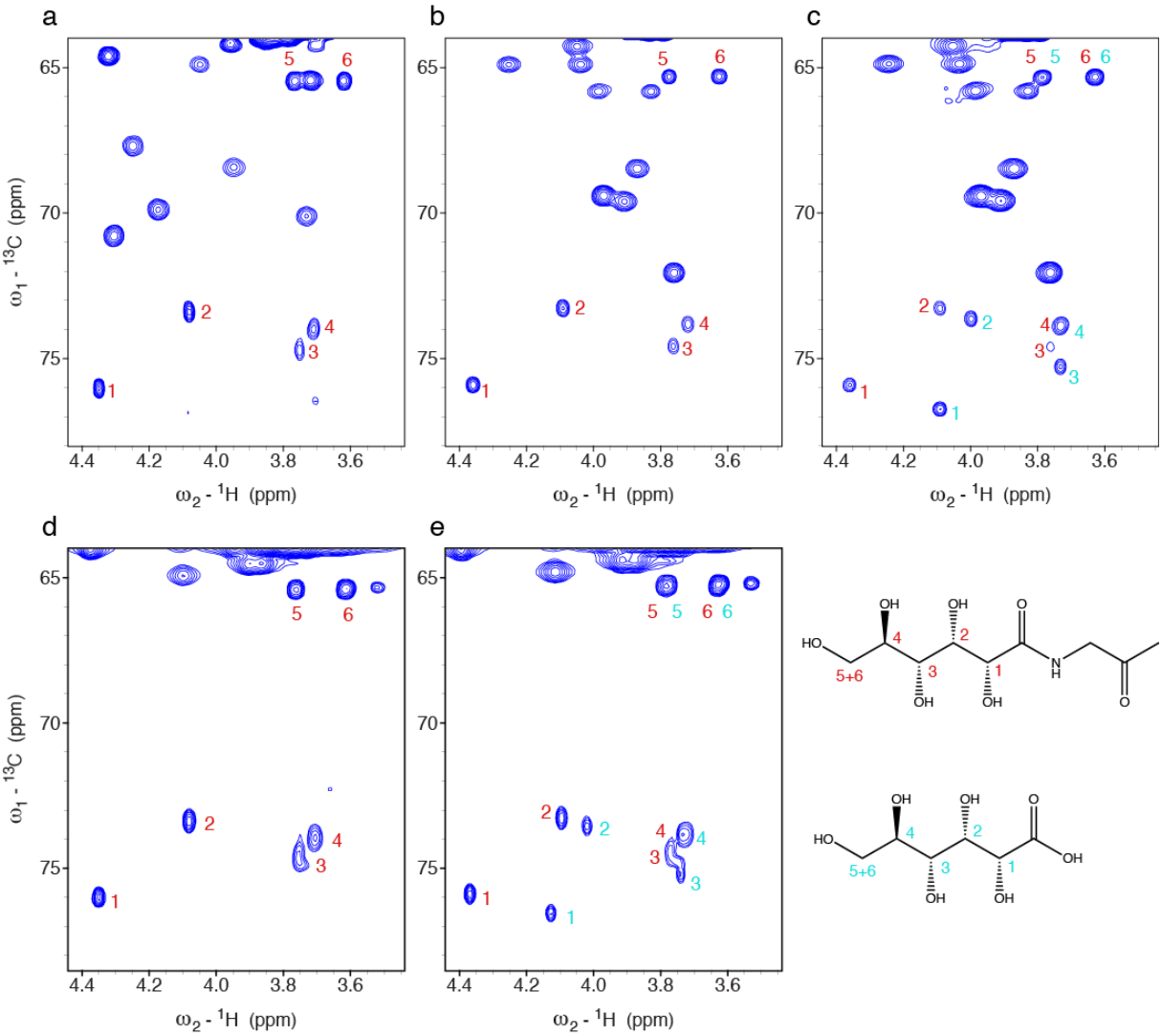
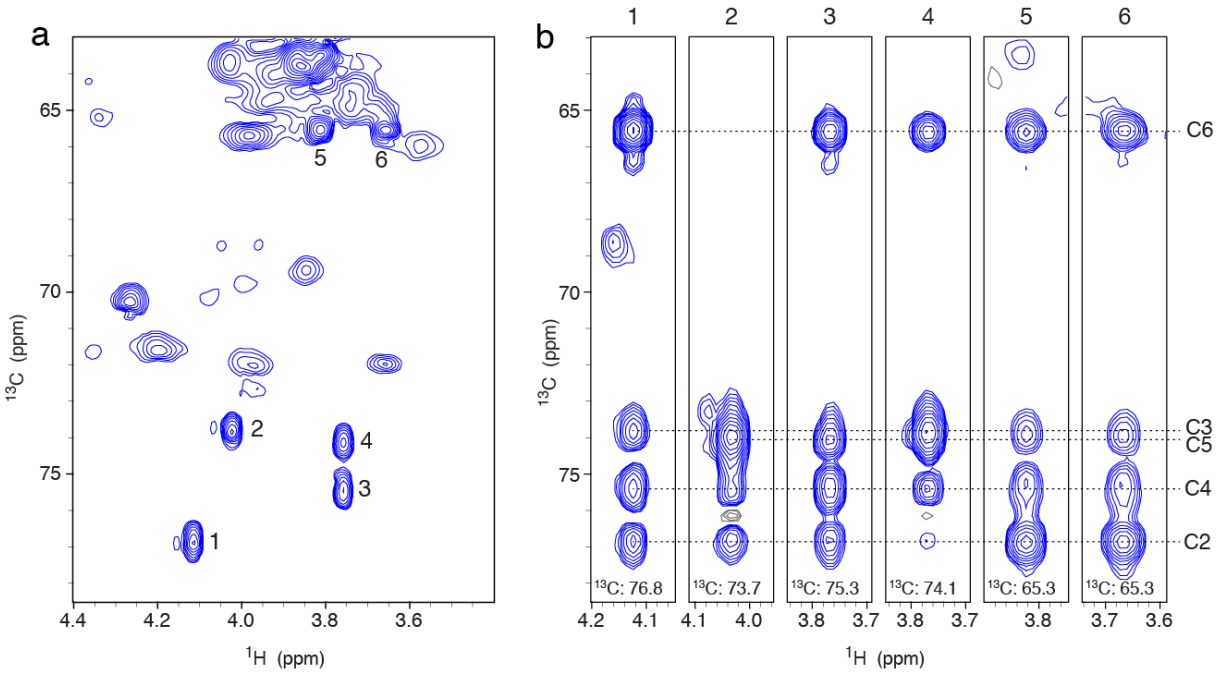
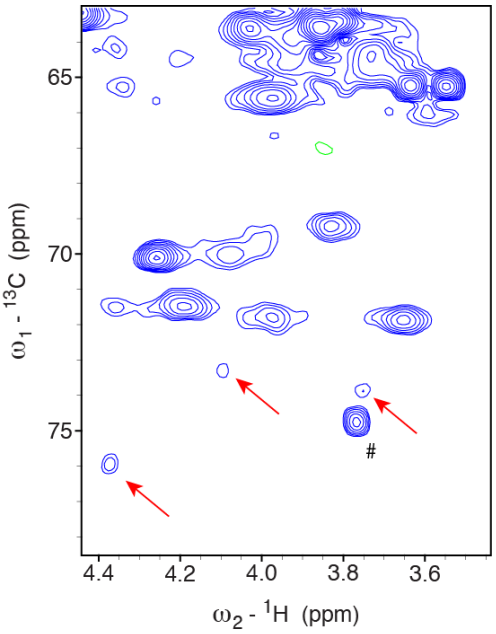
**Content:**



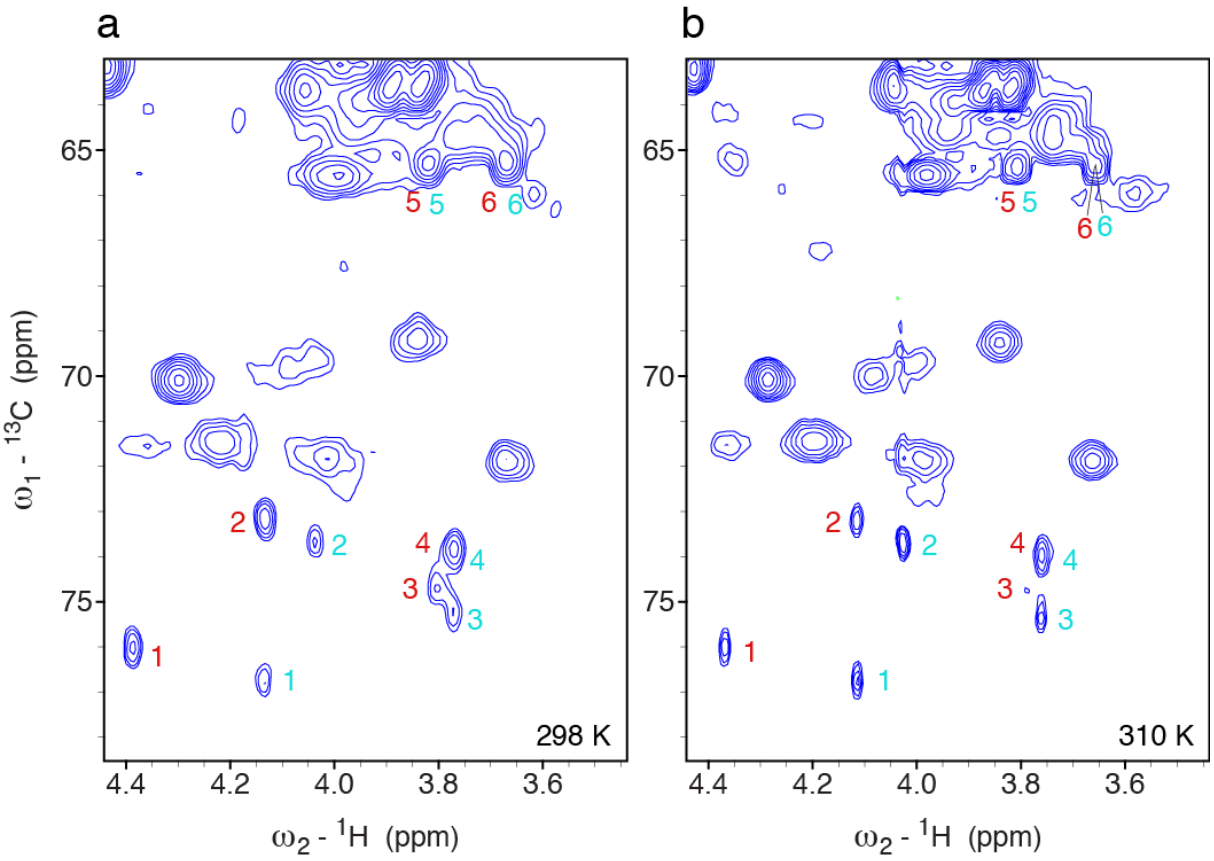

**Supplementary Figure S1.** $^{1}$H-$^{13}$C HSQC spectra of a variety of additional $^{13}$C/$^{15}$N labeled proteins. (a) Spectrum of a fresh sample of the RRM1 domain of hnRNP A1 in complex with the RNA UUAGGUC [1] recorded at 303 K, pH 6.5 and 750 MHz. (b) Spectrum of a fresh sample of the RRM2 domain of hnRNP A1 in complex with the RNA UCAGUU [1] recorded at 303 K, pH 6.5 and 900 MHz. (c) Spectrum of the same sample of panel b recorded after 5 days of triple resonance measurements at 303 K, pH 6.5 and 900 MHz. (d) Spectrum of a fresh sample of the two zinc knuckles of Lin28 in complex with the RNA AGGAGAU recorded at 303 K, pH 5.6 at 700 MHz in [2]. (e) Spectrum of the same sample of panel d after 4 days at 303 K.




**Supplementary Figure S2.** Assignment of the hydrolysis product gluconate. (a) 2D $^{1}$H-$^{13}$C HSQC of $^{13}$C/$^{15}$N-labeled CCL2 (pH 5.8) expressed in M9 minimal medium after 6 weeks of NMR measurements at 310 K, recorded at 700 MHz and 310 K. (b) 3D (H)CCH-TOCSY spectrum of the same sample as in panel a.




**Supplementary Figure S3.** $^{1}$H-$^{13}$C HSQC of unlabeled CCL2 expressed in LB medium (pH 5.8), measured at $^{13}$C natural abundance, at 600 MHz and 310 K. The arrows indicate characteristic and well isolated signals of gluconoyl that are present with low intensity.




**Supplementary Figure S4.** $^1$H-$^{13}$C HSQC of $^{13}$C/$^{15}$N-labeled CCL2 (pH 7.5, 0.25 mM) measured at 600 MHz and two different temperatures. (a) Spectrum measured at 298 K with 4 transients and 1024×350 points. (b) Spectrum recorded at 310 K with 16 transients and 2024×512 points.

**Supplementary Table S1.** $^{1}H$ and $^{13}C$ chemical shifts of gluconoyl and gluconate at different pH values and different temperatures.

| | Gluconoyl | | | | Gluconate | | | |
|---|---|---|---|---|---|---|---|---|
| Nucleus | pH 5.6<br>303 K | pH 6.5<br>303 K | pH 7.5<br>310 K | pH 7.5<br>298 K | pH 5.6<br>303 K | pH 6.5<br>303 K | pH 7.5<br>310 K | pH 7.5<br>298 K |
| C2 | 75.9 | 75.9 | 76.0 | 76.0 | 76.7 | 76.7 | 76.8 | 76.8 |
| C3 | 73.3 | 73.3 | 73.2 | 73.2 | 73.6 | 73.6 | 73.7 | 73.7 |
| C4 | 74.5 | 74.6 | 74.7 | 74.7 | 75.2 | 75.3 | 75.4 | 75.2 |
| C5 | 73.9 | 73.8 | 74.0 | 73.8 | 73.9 | 73.9 | 74.0 | 73.8 |
| C6 | 65.3 | 65.3 | 65.4 | 65.3 | 65.3 | 65.3 | 65.4 | 65.3 |
| H2 | 4.36 | 4.36 | 4.37 | 4.39 | 4.13 | 4.09 | 4.11 | 4.14 |
| H3 | 4.09 | 4.09 | 4.12 | 4.13 | 4.02 | 4.00 | 4.03 | 4.04 |
| H4 | 3.76 | 3.76 | 3.79 | 3.80 | 3.74 | 3.73 | 3.76 | 3.77 |
| H5 | 3.72 | 3.72 | 3.76 | 3.77 | 3.73 | 3.73 | 3.76 | 3.77 |
| H6 | 3.62 | 3.63 | 3.66 | 3.67 | 3.62 | 3.63 | 3.66 | 3.67 |
| H6 | 3.77 | 3.78 | 3.81 | 3.82 | 3.77 | 3.79 | 3.81 | 3.82 |

## SUPPLEMENTARY REFERENCES


1. Beusch, I., Barraud, P., Moursy, A., Clery, A. & Allain, F.H.T. Tandem hnRNP A1 RNA recognition motifs act in concert to repress the splicing of survival motor neuron exon 7. *Elife* **6**(2017).
2. Loughlin, F.E. et al. Structural basis of pre-let-7 miRNA recognition by the zinc knuckles of pluripotency factor Lin28. *Nature Structural & Molecular Biology* **19**, 84-U105 (2012).