Electronic Supplementary Information

Sequential assignment of NMR spectra of peptides at natural isotopic abundance with Zero and Ultra Low-Field-TOCSY

Alexey S. Kiryutin,[*a] Ivan V. Zhukov,[a] Fabien Ferrage,[b] Geoffrey Bodenhausen,[b]

Alexandra V. Yurkovskaya,[a] Konstantin L. Ivanov[†a]

[a] *International Tomography Center, Siberian Branch of the Russian Academy of Sciences and Novosibirsk State University, Novosibirsk, 630090, Russia*

[b] *Laboratoire des Biomolécules, LBM, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France*

* **Corresponding authors**, emails: kalex@tomo.nsc.ru (A. S. Kiryutin)
† Konstantin L. Ivanov passed away on March 5th 2021 at the age of 44 years as a consequence of Covid-19, while the final revisions of this paper were being prepared.

This file contains:

1. Relaxation data for Boc-Met-enkephalin
2. 2D-NMR spectra of Boc-Met-enkephalin

1. Relaxation data for Boc-Met-enkephalin

Here we present relaxation data for the system under study, focusing on the carbon nuclei of interest (C' and Cα carbons) and Hα protons. These data are summarized in **Tables S1** and **S2**. The measurements at high field were done in a standard way (inversion-recovery experiments) at 9.4 and 16.4 T. Measurements at low fields were done using fast field-cycling. In such experiments, we first let the spins relax to equilibrium at $B_0 = 9.4$ T. Subsequently, the sample is shuttles to obtain a field switch $B_0 \rightarrow B_{rel}$ and spins start to relax to a new equilibrium at the B_{rel} field. The relaxation delay τ_{rel} is incremented to monitor the evolution of the magnetization at this field. Subsequently, the field is switched back $B_{rel} \rightarrow B_0$ and the NMR spectrum acquired. By integrating signals in the NMR spectra we obtain the relaxation behavior of nuclei as a function of τ_{rel}. Typical relaxation curves of this kind measured at $B_{rel} = 0.3$ T are shown in **Figure S1** for carbonyl carbons and Cα carbons of Boc-Met-enkephalin. To determine relaxation times these curves were fitted to mono-exponential functions, which provide good fits in most cases.

An unexpected initial increase of the polarization of the Cα carbon is caused by cross-relaxation in directly bound ^{1}H-^{13}C spin pairs. As has been shown before, cross-relaxation manifests itself in the ^{13}C relaxation trace, giving rise to a bi-exponential time dependence.[1, 2] Here, in most cases, we were able to follow only the slow component of such bi-exponential relaxation curves. The analysis of such bi-exponential curves is discussed at length in a previous publication[2].

Table S1. Apparent longitudinal relaxation times T_1^{app}(^{13}C) [s] of Boc-Met-enkephalin carbonyl- and α-carbons at 25 °C, $B_0 =$ 9.4 and 0.3 T.

Site	9.4 T	0.3 T
Met C’	5.9±0.4	3.5±1.9
Tyr C’	2.5±0.2	3±1.5
Phe C’	3.0±0.2	2.3±0.7
Gly-1 C’	3.0±0.3	4±2.5
Gly-2 C’	2.2±0.2	2.9±1.5
Tyr Cα	0.35±0.03	0.13±0.17*
Phe Cα	0.29±0.02	0.44±0.33*
Met Cα	0.45±0.02	0.7±0.76*
Gly-2,3 Cα	0.24±0.01	0.19±0.9*

Values marked with a star (*) were obtained by fitting relaxation curves with growing mono-exponential functions. These values correspond to the slow components of the bi-exponential cross-relaxation kinetics of Cα carbons.

Table S2. Apparent longitudinal relaxation times T_1^{app}(^{1}H) [s] of α-protons in Boc-Met-enkephalin at 25 °C, $B_0 =$ 9.4 and 16.4 T.

Site	9.4 T	16.4 T
Met Hα	1.3±0.1	2.0±0.1
Tyr Hα	1.3±0.1	2.1±0.1
Phe Hα	1.3±0.1	1.9±0.1
Gly-2,3 Hα	0.4±0.05	0.8±0.1

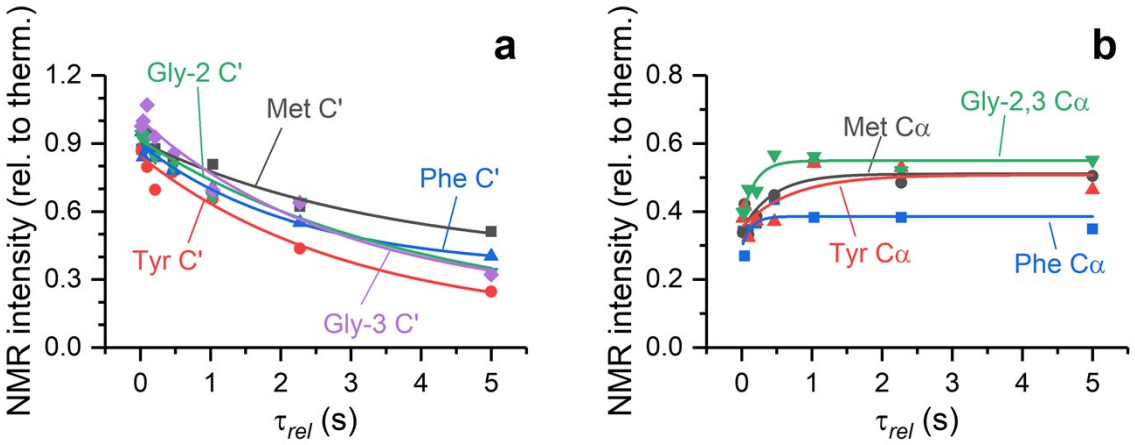

Figure S1. Relaxation curves of (a) carbonyl and (b) α-carbons of Boc-Met-enkephalin at 25° C, measured at $B_{rel} =$ 0.3 T. The field switching duration was 200 ms, the stabilization delay prior to detection at high field was 25 ms, the relaxation delay was 8 s; 864 scans were collected for each value of τ_{rel}. The overall duration of the experiments was ca. 24 hours.

2. 2D-NMR spectra of Boc-Met-enkephalin

In this section, we present complete 2D-NMR spectra of the compound under study, highlighting various ^{1}H-^{13}C correlations.

In **Figure S2** we present the complete ZULF-TOSCY spectrum, whereas in **Figure 3b** only part of this 2D spectrum is shown, highlighting the region corresponding to carbonyl carbons and Hα protons. In the full spectrum, one can see additional resonances, corresponding to correlations in aromatic rings of tryptophan and phenyl-alanine, some correlations in the methionine side chain, and all correlations within Boc moiety. Two distinct Boc conformations exist possibly the *cis* and *trans* conformation of the amide bond, each giving rise to a specific set of cross-peaks.

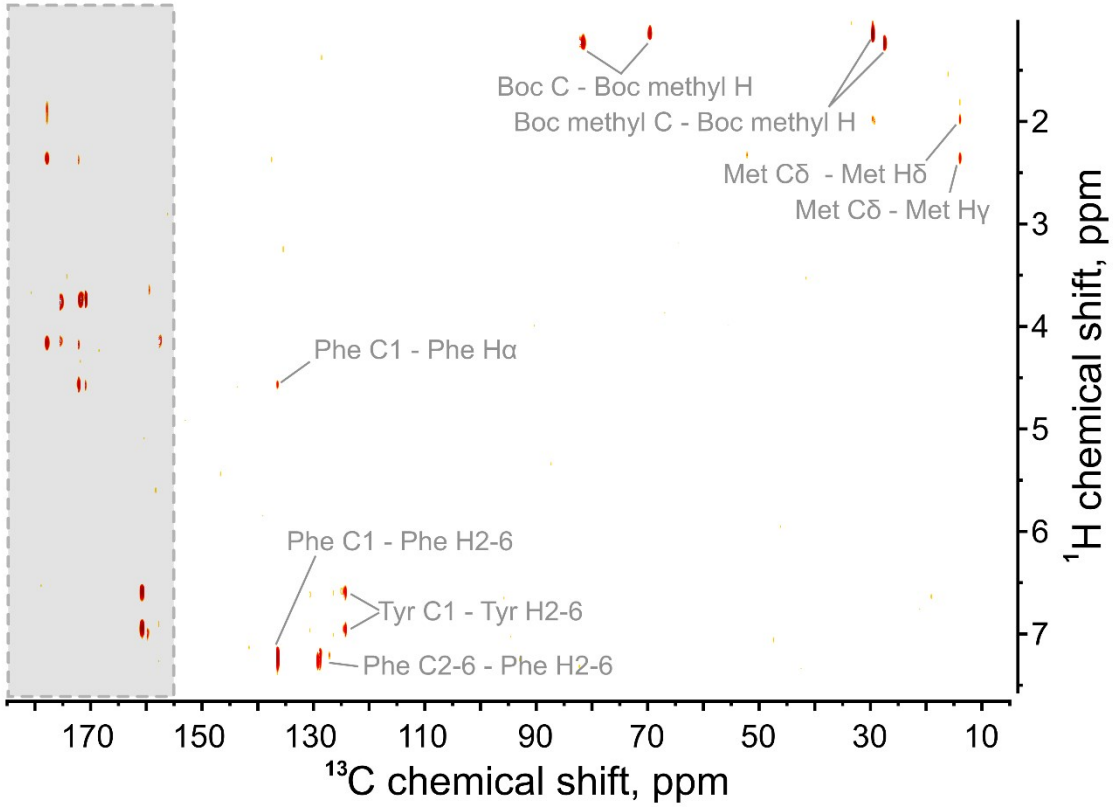

Figure S2. Complete ^{13}C-^{1}H ZULF-TOCSY spectrum of 83 mM Boc-Met-enkephalin, pH 10.9, 25°C, taken at $B_0 = 9.4$ T. The part of the spectrum shown in **Figure 3b** of the main text is highlighted by a grey rectangle. Experimental details: 80 increments in the indirect dimension, 256 scans per transient, relaxation delay 6 s, sample transfer time 403 ms in each direction, B_{UL}=50 nT, $\tau_{opt} = \tau_{mix}^{opt} = 100$ ms (corresponding to $J_{CH} = 2.5$ Hz), total acquisition time ca. 58 hours. The time-domain signal $S(t_1,t_2)$ was multiplied by a 90° shifted sine squared window function in both dimensions prior to 2D Fourier transformation. The resulting spectrum $S(f_1,f_2)$ is given by 512×1024 data points. Hα-Cα correlations are not visible due to fast relaxation of the α-carbons.

Likewise, in **Figure S3** we present the complete HMBC spectrum, highlighting some of the cross-peaks belonging to aromatic protons of tryptophan and phenylalanine, to correlations in the methionine side-chain, and to correlations within the Boc-moiety. Note that the two sets of cross-peaks correspond to two different Boc conformations.

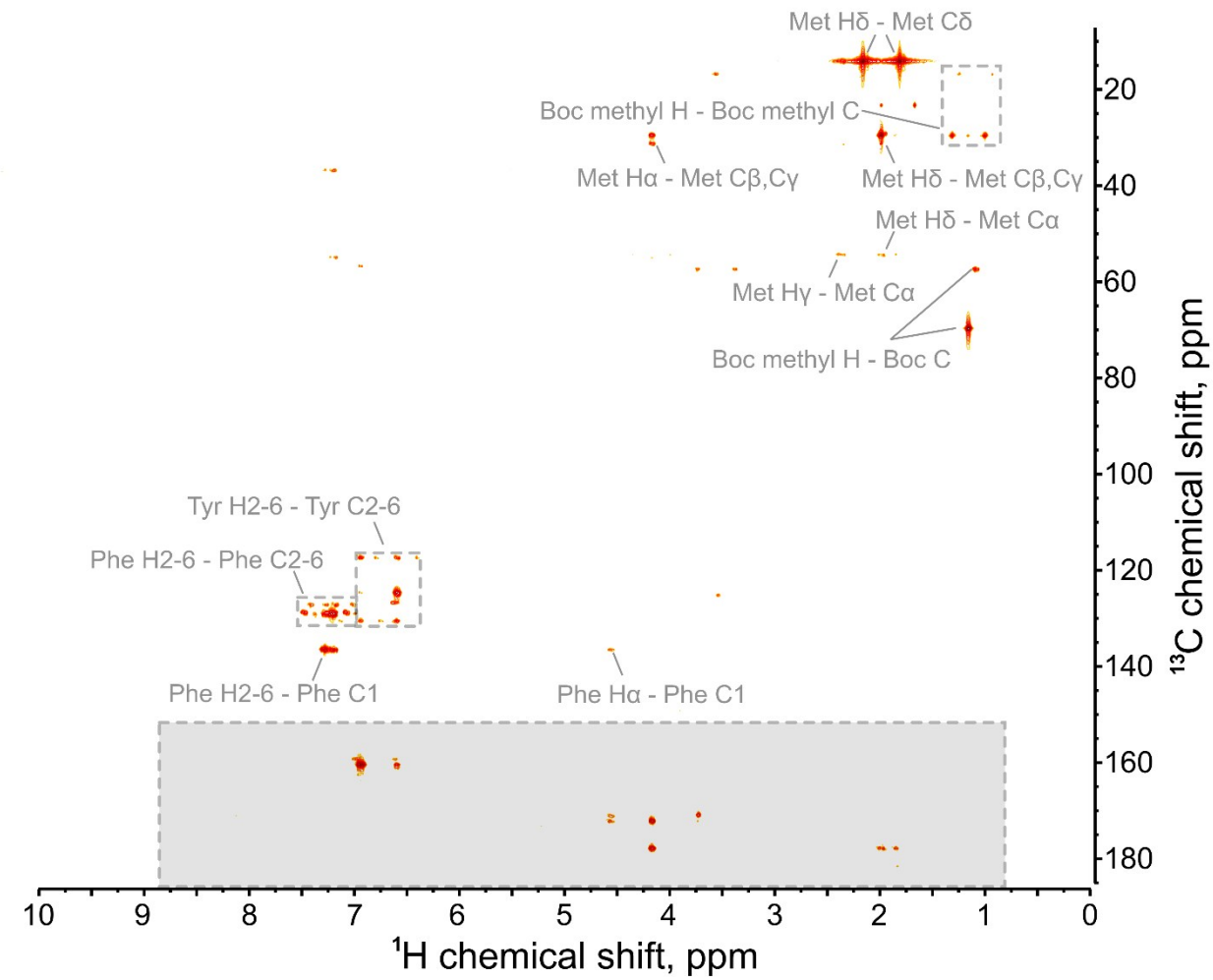

Figure S3. Complete ^{1}H-^{13}C HMBC spectrum of 83 mM Boc-Met-enkephalin, pH 10.9, 25 °C, $B_0 = 9.4$ T. The indirect dimension was sampled with 1024 values of t_1 were over 180 ppm (resolution 17.7 Hz/point), 56 scans per transient, the long range high-pass $J^L(^{13}C - ^{1}H)$-filter delay was optimized to 1 Hz, the low-pass $J^1(^{13}C - ^{1}H)$-filter delay was optimized to 100 Hz to suppress direct correlations, the relaxation delay was 3 s, the total acquisition time ca. 70 hours. The time-domain signal $S(t_1,t_2)$ was multiplied by a 90° shifted sine squared window function in both dimensions, the spectrum $S(f_1,f_2)$ is represented by 2048×4096 data points.

Finally, **Figure S4** shows the HSQC spectrum of Boc-Met-enkephalin. One can see that the signals of interest, corresponding to correlations between the carbonyl carbons and the Hα-protons, are missing because the inter-pulse delays in the polarization transfer block are not optimal for detecting these cross-peaks.

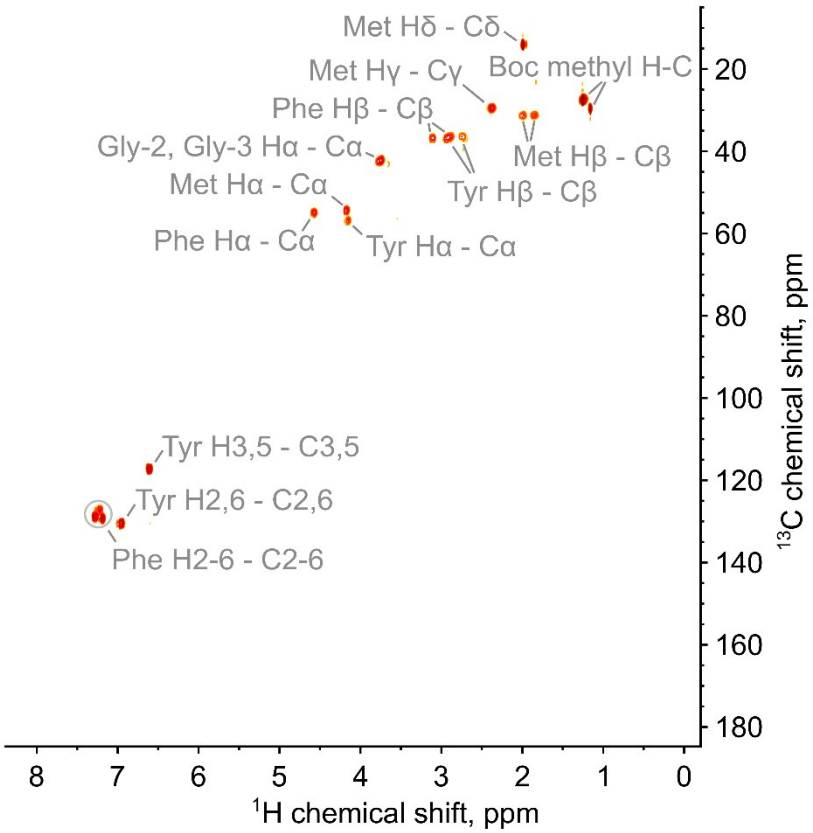

Figure S4. ^{1}H-^{13}C HSQC spectrum of 83 mM Boc-Met-enkephalin, pH 10.9, 25 °C, 9.4 T. Parameters: 256 increments of t_1 were used to sample the indirect dimension over 180 ppm (resolution 247.6 Hz/point), 32 scans per transient, delays in INEPT transfer optimized for $J^1(^{13}C - ^1H)$=130 Hz, relaxation delay 3 s, total acquisition time ca. 8 hours. The time-domain signal $S(t_1,t_2)$ was multiplied by a 90° shifted sine squared window function in both dimensions, the spectrum $S(f_1,f_2)$ is represented by 2048×2048 data points.

1. I. V. Zhukov, A. S. Kiryutin, A. V. Yurkovskaya, Y. A. Grishin, H.-M. Vieth and K. L. Ivanov, *Phys. Chem. Chem. Phys.*, 2018, 20, 12396-12405.
2. N. Bolik-Coulon, P. Kaderavek, P. Pelupessy, J. N. Dumez, F. Ferrage and S. F. Cousin, *J. Magn. Reson.*, 2020, 313.