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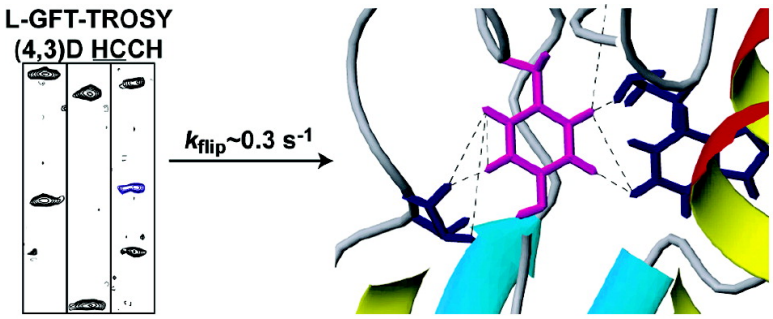

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Probing Structure and Functional Dynamics of (Large) Proteins with Aromatic Rings: L-GFT-TROSY (4,3)D HCCH NMR Spectroscopy

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The rings of aromatic amino acids are valuable probes to study protein structure, dynamics, and folding by using nuclear magnetic resonance (NMR) spectroscopy. They are often part of a protein's molecular core and give rise to a large number of $^1H-^1H$ nuclear Overhauser effects (NOEs),[1] as is required for high-quality structure determination. Ring flipping rates provide unique information on larger-amplitude motional modes which allow the rings to rotate about χ^2.[1-3] Hence, NMR assignment of rings is of central importance for structural biology of proteins. HCCH spectroscopy[4] employed in conjunction with NOE spectroscopy (NOESY) is most efficient,[5] and four-dimensional (4D) HCCH is attractive due to limited shift dispersion of Phe rings.[1] Despite the tremendous importance, however, an implementation that provides 4D information while being suited for both *sensitivity* and *sampling* limited data collection[6] is not available.

Here we present aromatic L-GFT and L-GFT-TROSY (4,3)D HCCH. *Sensitivity* is maximized by using (i) newly introduced longitudinal relaxation (L-)optimization[7] for aromatic protons ($^1H^{aromatic}$), (ii) pulsed field gradient (PFG) selection of coherences with preservation of equivalent pathways (PEP),[5,8] (iii) (semi-) constant time (*ct*) frequency labeling,[5] and (iv) employment of transverse relaxation optimized spectroscopy (TROSY).[9-11] Rapid *sampling* is accomplished by use of G-matrix FT (GFT) NMR[12] combined[13,14] with L-optimization, which thus serves to enhance sensitivity[7] and/or shorten relaxation delays.[13]

$^1H^{aromatic}$ L-optimization is feasible since (i) $^1H^{aromatic}/^1H_2O$ and $^1H^{aliphatic}$ chemical shift ranges do not overlap, which enables selective "flipping" of $^1H^{aliphatic}/^1H_2O$ magnetization while $^1H^{aromatic}$ magnetization is along *z* and (ii) a large number of dipolar $^1H^{aromatic}-^1H^{aliphatic}$ and Tyr $^1H^{aromatic}-^1H^{hydroxyl}$ interactions can increase R_1 of $^1H^{aromatic}$. For 14 kDa protein PfR13, a target of the Northeast Structural Genomics Consortium (NESG), we recorded a series of L- and *non*-L-*ct* 2D [^{13}C,^1H]-HSQC/TROSY spectra (Figure S1) at 20 and 4 °C with varying relaxation delay between scans. PFG-PEP coherence selection[8] was used.[15] HSQC spectra were recorded at 20 °C, where the approximate isotropic correlation time for overall tumbling of PfR13, τ_{iso}, is 8.5 ns. TROSY spectra with suppression of signal contributions from $^{13}C^{aromatic}$ polarization were acquired at 4 °C, where $\tau_{iso} = 12.5$ ns. Signal-to-noise (S/N) ratios were measured as a function of the relaxation delay between scans, t_{rel}, and divided by the square-root of the measurement time, t_{tot}, thus yielding SN_t, a measure for intrinsic sensitivity. A least-squares fit of

$$SN_t = A\frac{1 - \exp(-R_1(t_{rel} + t_{acq}))}{\sqrt{t_{rel} + t_{acq} + t_{seq}}} \qquad (1)$$

to the experimental SN_t values yields t_{rel}^{opt} at which, for a given proton, intrinsic sensitivity is maximal (Tables S1-S3). t_{acq} and t_{seq} represent the acquisition time and length of the pulsing period, respectively, while scaling factor *A* and effective longitudinal relaxation rate R_1 are fitted (Figure 1). Analysis of resolved peaks (Tables S1, S2) reveals that at 20 °C (4 °C) (i) the average $t_{rel}^{opt} = 1.9$ s (1.8 s) *without* is longer than the average $t_{rel}^{opt} = 1.1$ s (1.0 s) *with* L-optimization, and (ii) the average gain in intrinsic sensitivity arising at t_{rel}^{opt} due to L-optimization (Figures 1, S2) is ~20% (~15%). For the L-optimized experiment acquired at t_{rel}^{match} ~0.4-0.6 s, intrinsic sensitivity matches the one of the *non*-L congener at its t_{rel}^{opt}, that is L-optimization allows one to reach the maximum sensitivity achieved *without* L-optimization at about 4-fold increased sampling speed.

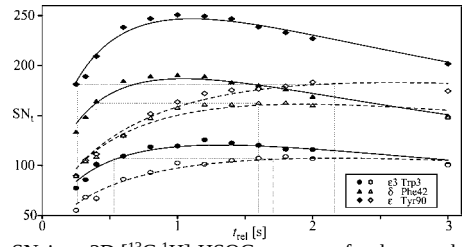

Figure 1. SN_t in *ct* 2D [^{13}C,^1H]-HSQC versus t_{rel} for three peaks of protein PfR13 (20 °C; 750 MHz). Filled (open) symbols and solid (dashed) lines correspond to spectra acquired *with* (*without*) L-optimization. Vertical lines at ~1.6-2.2 s indicate t_{rel}^{opt} *without* L-optimization, and those at ~0.2-0.5 s indicate t_{rel}^{match} (Table S1) where L-optimized congeners reach the same intrinsic sensitivity.

Exploration of L-optimization with 2D NMR (Figures 1, S2; Tables S1-S3) enabled implementation of L-GFT-TROSY (4,3)D HCCH (Figure 2), which is based on highly efficient $^1H^{(1)}(t_1) \rightarrow {}^{13}C^{(1)}(t_1) \rightarrow {}^{13}C^{(2)}(t_2) \rightarrow {}^1H^{(2)}(t_3)$ transfer via large $^1J\{^{13}C^{aromatic}-{}^{13}C^{aromatic}\}$ (~50 Hz) and $^1J\{^1H^{aromatic}-{}^{13}C^{aromatic}\}$ (~160 Hz) couplings. The shifts of $^1H^{(1)}$ and $^{13}C^{(1)}$ are jointly sampled in a GFT dimension. Frequency labeling with the shifts of $^1H^{(1)}$, $^{13}C^{(1)}$, and $^{13}C^{(2)}$ is accomplished within only ~11.6-13.5 ms during polarization transfer. Short maximal evolution times of ~4.5 ms suffice in the GFT dimension since peaks are dispersed over the sum of $^1H^{aromatic}$ and $^{13}C^{aromatic}$ spectral widths.[13] At 4.5 ms *ct* delay, TROSY yields higher sensitivity only for large proteins ($\tau_{iso} > 23$ ns; Figure S4) but allows one to use $^{13}C^{(1)}$ polarization[10] for acquiring central peaks[12,17] without compromising on INEPT delays.[18] These peaks provide (H)CCH information defining centers of peak pairs at $\Omega(^{13}C^{(1)}) \pm \Omega(^1H^{(1)})$ in basic spectra.

L-GFT-TROSY (4,3)D HCCH (data set "I") was recorded in 24 h with $t_{rel} = 1$ s (t_{rel}^{opt} for PfR13; Table S4) for 21 kDa protein HR41 (Figure 3; 25 °C; $\tau_{iso} \approx 11$ ns; 95% peak detection yield), an NESGC target for which data collection is *sensitivity* limited. For sensitivity comparison, L-GFT ("II"), GFT ("III"); $t_{rel} = 1.5$ s with presaturation of water line), and L-GFT-TROSY with 13.5 ms *ct* delay[10,11] ("IV") were recorded. The relative sensitivity for *basic spectra* is ~2.5:3.8:2.1:1 (Table S5). Thus, at $\tau_{iso} \approx 11$ ns one has that (i) TROSY is ~20% less sensitive[19] when also taking into account that ^{13}C polarization yields central peaks in TROSY (see Supporting Information), (ii) L-optimization increases sensitivity by ~20-60% (the variation is due to nonuniform ^1H density),

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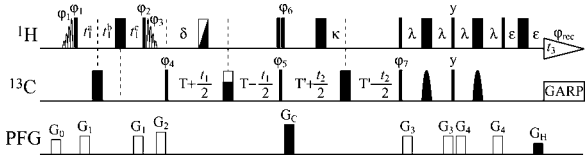

Figure 2. Radio frequency (rf) pulse scheme of L-GFT-TROSY (4,3)D HCCH. 90° and 180° pulses are thin and thick bars. Selective 90° ^{1}H pulses with rising and falling shapes are 1.1 ms E-BURP2 and time-reversed E-BURP2 pulses[16] applied at 2 ppm. Those flip ^{1}H^{aliphatic}/^{1}H$_2$O magnetization while ^{1}H^{aromatic} magnetization is along *z*. During reverse INEPT, flip-back pulses are not required since (i) hard ^{1}H pulses yield a 720° rotation of ^{1}H magnetization and (ii) selective 180° ^{13}C^{aromatic} REBURP pulses[16] of 610 μs duration (at 750 MHz) decouple ^{1}H^{aliphatic} from ^{13}C^{aliphatic}. Phases of ^{1}H rf pulses are adjusted such that ^{1}H^{aliphatic}/^{1}H$_2$O magnetization is along $+z$ at the beginning of t_3. Decoupling of ^{13}C^{aromatic} during t_3 is accomplished using GARP.[5] Delays: $\lambda = 1.3$ ms, $\kappa = 1.5$ ms, $T = T' = 2.25$ ms, $\delta = T - \kappa + t_1/2$, $\epsilon = 0.3$ ms. Semi-*ct* ^{1}H$^{(1)}$ shift evolution[5] is implemented with $t_{1,max} = 2T$, $t_1{}^a(0) = \lambda$, $t_1{}^b(0) = 1$ μs, $t_1{}^c(0) = \lambda + 1$ μs, and $\Delta t_1{}^a = t_1/2$, $\Delta t_1{}^b = \Delta t_1{}^a + \Delta t_1{}^c$, $\Delta t_1{}^c = -\lambda$ $t_1/2T$. An S^3-filter[11] implements TROSY: the black-and-white 180° pulse on ^{1}H is applied only every other step of the phase cycle. To decouple $^1J(^{13}C^\gamma - ^{13}C^\beta)$ for enhancing signals at $\Omega(^{13}C^\gamma)$ in central peak subspectra (Figure 3), the 180° pulse during $t_1(^{13}C^{(1)})$ is applied with[5] $\gamma B_1 = \Delta\omega/\sqrt{3}$ ($\Delta\omega$ is difference between ^{13}C^{aromatic} carrier and average $^{13}C^\beta$ shift). GFT NMR phase-cycle: $\varphi_1 = x,y,-x,-y$ to obtain basic subspectra from ^{1}H and central peak subspectra from ^{13}C polarization.[12] For L-GFT (4,3)D HCCH: $\delta = \kappa$, the black-and-white 180° pulse on ^{13}C during $t_1(^{13}C^{(1)})$ is at high power and a ^{13}C 90° pulse before PFG G_0 is added (not shown). GFT NMR phase cycle: $\varphi_1 = x,y$ for basic subspectra; the central peak subspectrum is recorded by omitting ^{1}H$^{(1)}$ shift evolution. For definitions of PFGs and phase cycles see Figure S5.

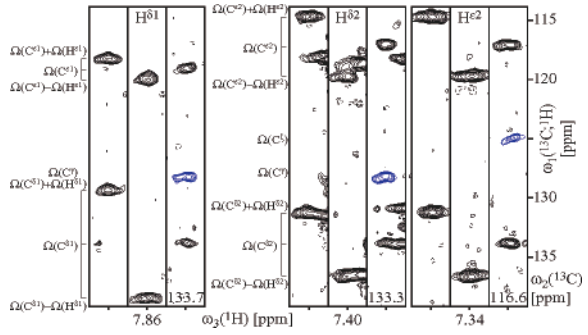

Figure 3. [$\omega_1(^{13}C^{(1)};^1H^{(1)}),\omega_3(^1H^{(2)})$]-strips taken along the GFT dimension of L-GFT-TROSY (4,3)D HCCH recorded at 750 MHz for 21 kDa HR41 with $t_{rel} = 1$ s. Peaks belong to the slowly flipping ring of Tyr 90. Central peaks arising from $^{13}C^{\gamma/\zeta}$ polarization are depicted in blue.

and (iii) $t_{1,max}(^{13}C^{(1)}) \sim 4.5$ ms vs 13.5 ms (often required without GFT) increases TROSY sensitivity ~2.5-fold.

HR41 contains 6 Phe, 6 Tyr, and 6 Trp, and nearly complete aromatic resonance assignment[20] enabled high-quality NMR structure determination.[21] Correlation of $^{13}C^\gamma$ and Tyr $^{13}C^\zeta$ shifts with, respectively, $^{13}C^\delta/^1H^\delta$ and Tyr $^{13}C^\epsilon/^1H^\epsilon$ shifts supports assignment of slowly flipping rings: the same C^γ/C^ζ shifts are detected on $CH^{\delta/\epsilon}$-moieties belonging to an immobilized ring (Figure 3). Nearly complete analysis of ^{1}H line widths was afforded with (4,3)D HCCH (Tables S6, S7), which is important to explore flipping of all rings in the protein. From ^{13}C-resolved [^{1}H,^{1}H]-NOESY,[5] k_{flip} (Tyr 90) ≈ 0.3 s^{-1} reflects a slow motional mode on the seconds time scale, which proves the absence of faster large-amplitude motions enabling ring flipping. This indicates remarkable rigidity of the substructure in which the ring is embedded. Tyr 90 is conserved among ubiquitin-conjugating enzymes E2 (to which HR41 belongs as inferred from structure[21b]) and is located in spatial proximity to the interface between E2 and the ubiquitin protein ligase E3.[22] Hence, the rigidity and/or the slow motional mode might be important for E2−E3 dimerization and thus for cellular protein degradation.

L-optimization for rapid data acquisition (Figure 1) is exemplified for 13 kDa protein MaR11[21b] (1 mM; Table S4; Figure S6), an NESG target for which data collection is *sampling* limited:[23] L-GFT (4,3)D HCCH was acquired with $t_{rel} = 0.3$ s in 25 min (Table S4; 94% peak detection yield).

Overall, for proteins up to ~25 kDa, PFG-PEP *ct* L-2D [^{13}C,^{1}H]-TROSY and L-GFT (4,3)D HCCH are most sensitive, while the TROSY congener is attractive for large proteins and slowly flipping (nearly stalled) rings, which are unique reporters of slow protein dynamics.[1−3] Aromatic L-optimization includes "flip-back" of ^{1}H$_2$O polarization,[24] which is important for systems >100 kDa.[25] We thus expect that L-GFT(-TROSY) (4,3)D HCCH NMR will play a key role for high-quality structure determination of large (membrane[26]) proteins and for studying the quite unexplored (functional) dynamics of their molecular cores.

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Supporting Information Available: 1. On NMR of aromatic rings. Details of 2. L-optimization, 3. PFG-PEP and TROSY sensitivity enhancement, 4. (4,3)D HCCH, 5. ^{1}H^{aromatic} line width analysis for HR41. This material is available free of charge via the Internet http://pubs.acs.org.

References

(1) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(2) Wagner, G. *Q. Rev. Biophys.* **1983**, *16*, 1−57.
(3) Skalicky, J. J.; Mills, J. L.; Sharma, S.; Szyperski, T. *J. Am. Chem. Soc.* **2001**, *123*, 388−397.
(4) Kay, L. E.; Ikura, M.; Bax, A. *J. Am. Chem. Soc.* **1990**, *112*, 888−889.
(5) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G.; Skelton, N. J. *Protein NMR Spectroscopy: Principles and Practice*; Academic Press: New York, 1996.
(6) Szyperski, T.; Yeh, D. C.; Sukumaran, D. K.; Moseley, H. N. B.; Montelione, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8009−8014.
(7) Pervushin, K.; Vogeli, B.; Eletsky, A. *J. Am. Chem. Soc.* **2002**, *124*, 12898−12902.
(8) Kay, L. E.; Keifer, P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663−10665.
(9) Pervushin, K.; Riek, R.; Wider, G.; Wüthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12366−12371.
(10) Pervushin, K.; Riek, R.; Wider, G.; Wüthrich, K. *J. Am. Chem. Soc.* **1998**, *120*, 6394−6400.
(11) Meissner, A.; Sørensen, O. W. *J. Magn. Reson.* **1999**, *139*, 447−450.
(12) Kim, S.; Szyperski, T. *J. Am. Chem. Soc.* **2003**, *125*, 1385−1393.
(13) Atreya, H. S.; Szyperski, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9642−9647.
(14) Atreya, H. S.; Szyperski, T. *Method Enzymol.* **2005**, *394*, 78−108.
(15) PFG-PEP is recommended for all but very large proteins; sensitivity is enhanced up to $\tau_{iso} \approx 35$ ns (Figure S3) with excellent water suppression. TROSY is generally preferred in 2D *ct* [^{13}C,^{1}H]-spectroscopy since sensitivity can be enhanced by using ^{13}C^{aromatic} polarization.[10]
(16) Geen, H.; Freeman, R. *J. Magn. Reson.* **1991**, *93*, 93−141.
(17) Szyperski, T.; Braun, D.; Banecki, B.; Wüthrich, K. *J. Am. Chem. Soc.* **1996**, *118*, 8146−8147.
(18) In aliphatic (4,3)D HCCH, central peaks can be derived from ^{13}C polarization since INEPT is tuned for CH, CH_2, and CH_3 groups.[5] Aromatic spin systems contain only CH, and delays are tuned to (1/2*J*). Hence, ^{13}C polarization yields central peaks in aromatic *non*-TROSY (4,3)D HCCH only if sensitivity of basic spectra is compromized.
(19) Theory predicts that at *ct* delays of 4.5 and 13.5 ms, TROSY becomes more sensitive at $\tau_{iso} \gtrsim 23$ ns and $\tau_{iso} \gtrsim 8$ ns, respectively (Figure S4).
(20) Liu, G.; Aramini, J.; Atreya, H. S.; Eletsky, A.; Xiao, R.; Acton, T. A.; Ma, L. C.; Montelione, G. T.; Szyperski, T. *J. Biomol. NMR* **2005**, *32*, 261−261.
(21) (a) Shen, Y.; Atreya, H. S.; Liu, G.; Szyperski, T. *J. Am. Chem. Soc.* **2005**, *127*, 9085−9099. (b) Liu, G.; Shen, Y.; Atreya, H. S.; Parish, D.; Shao, Y.; Sukumaran, D. K.; Xiao, R.; Yee, A.; Lemak, A.; Bhattacharya, A.; Acton, T. A.; Arrowsmith, C. H.; Montelione, G. T.; Szyperski, T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10487−10492.
(22) (a) Huang, L.; Kinnucan, E.; Wang, G.; Beaudenon, S.; Howley, P. M.; Huibregtse, J. M.; Pavletich, N. P. *Science* **1999**, *286*, 1321−1326. (b) VanDemark, A. P.; Hill, C. P. *Curr. Opin. Struct. Biol.* **2002**, *12*, 822−830.
(23) With $t_{rel} = 0.3$ s, the minimal measurement times of L-4D HCCH and L-GFT (4,2)D HCCH[13] are 4.9 h and 3 min, respectively.
(24) Grzesiek, S.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 12593−12594.
(25) Wider, G. *Methods Enzymol.* **2005**, *394*, 382−398.
(26) Atreya, H. S.; Eletsky, A.; Szyperski, T. *J. Am. Chem. Soc.* **2005**, *127*, 4554−4555.

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