Supplementary information

Teilum, K.; Brath, U.; Lundström, P.; Akke, M.

“Biosynthetic $^{13}$C labeling of aromatic side-chains in proteins for NMR relaxation measurements”.
Supplementary Figure 1. The pulse sequence used to measure aromatic $^{13}$C $R_{1p}$ relaxation rates. Narrow and wide bars indicate RF-pulses of 90° and 180° flip-angles, respectively, while very wide bars indicate spinlock or broad-band decoupling. All pulses are applied with phase $x$, unless otherwise indicated. Pulses on $^1$H are applied with the carrier frequency centered on the water resonance. Pulses on $^{13}$C were centered at 132.5 ppm, except during the spin-lock period when the carrier was shifted to the desired offset. Adiabatic alignment of the magnetizations with their respective effective fields during the spin-lock relaxation period, and subsequent return to the $z$-axis, were achieved using tan/tanh RF frequency/amplitude ramps. The adiabatic frequency sweep was initiated 25 kHz downfield of the final spin-lock carrier frequency. Decoupling during acquisition was achieved with the GARP-1 sequence using an RF field strength of $\gamma B_1/2\pi = 2150$ Hz. A continuous-wave RF field was applied far off resonance on the $^{13}$C channel to avoid differential heating for different relaxation delays and field strengths (not shown). The delay $\tau_{xh}$ was adjusted to $1/J_{CH}$ and $\Delta$ was set to 0.6 ms. The phase cycle was $\phi_1 = x, -x$; $\phi_2 = x$; $\phi_3 = x, x, y, -x, -x, -y, -y$; $\phi_4 = x$; $\phi = x, -x, -x, x$. Gradient strengths in units of G/cm (lengths in units of ms) were: $g_0 = 5000$ (1.0); $g_1 = 4000$ (0.8); $g_2 = 13000$ (1.0); $g_3 = 5000$ (0.8); $g_4 = -17000$ (1.2); $g_5 = 13000$ (0.8); $g_6 = 500$ ($t_1$); $g_7 = 15000$ (1.0); $g_8 = 4000$ (1.0); $g_9 = 500$ (1.0); $g_{10} = 15000$ (1.0).
4000 (1.0); g10 = 14920 (0.25). Sensitivity-enhanced quadrature detection in $t_1$ was achieved by inverting $\phi_1$ and reversing the sign of $g_{10}$. The phases $\phi_2$ and $\phi$ were inverted in every other increment to achieve States-TPPI phase cycling. $R_{1\rho}$ rates were measured at eleven effective fields and the decays were sampled at eight points including one duplicate. The maximum relaxation delay at each field was adjusted to approximately $t_{SL} = 1/R_{1\rho}$. On-resonance data points were recorded with spin-lock field strengths of 559, 732, 1142, 1796, 2729, 3760 and 4648 Hz, and off-resonance points with a spin-lock field of 4648 Hz and offsets of 1000, 2000, 4000, 8000 and 20000 Hz.
Supplementary Figure 2. Relaxation dispersion curves for all aromatic $^{13}$C nuclei in E140Q-Tr2C expressed on [1-$^{13}$C]-glucose. $^{13}$C$^{\delta}$ in Phe (F89, F92 and F141) and Tyr (Y99 and Y138), and $^{13}$C$^{\varepsilon 1}$ in His (H107). All data were processed using the NMRPipe/NMRDraw software package. Mono-exponential functions were fitted to the $R_{1p}$ decay curves at each tilt angle, with errors in peak intensities estimated from duplicate measurements. $R_{1p}$ dispersion curves were fitted using simplex routines in Matlab (The Mathworks, Inc.). Two models were fitted to each dispersion curve: a two-parameter model including $R_1$ and $R_{2,0}$, and a four-parameter model including $R_1$, $R_{2,0}$, $\tau_{ex}$ and $\phi_{ex} = p_A p_B (\Delta \omega)^2$. The statistically significant improvement of the four-parameter model over the two-parameter model was assessed from the $F$-statistic, with the more complex model being accepted if $p < 0.05$. Errors in the fitted parameters were estimated by the jackknife procedure.
References


(4) Shaka, A. J.; Barker, P. B.; Freeman, R. J. Magn. Reson. 1985, 64, 547-552.


