## Supplementary Information

**Table S1.** Chemical shifts of stereospecifically assigned Leu methyl groups in Malate Synthase G (pH 7.1 37 ºC).[a]

| Res. | $H_{\delta1}$ | $C_{\delta1}$ | $H_{\delta2}$ | $C_{\delta2}$ |
|---|---|---|---|---|
| L25 | 0.30 | 24.63 | -0.28 | 20.08 |
| L30 | 0.76 | 25.86 | 0.82 | 22.15 |
| L46 | 0.81 | 26.31 | 0.83 | 22.90 |
| L53 | 0.31 | 25.99 | 0.74 | 22.31 |
| L54 | 1.10 | 24.95 | 0.68 | 19.98 |
| L64 | 0.90 | 25.43 | 0.76 | 24.58 |
| L85 | -0.08 | 24.82 | -0.20 | 20.09 |
| L88 | 0.83 | 23.55 | 0.67 | 25.35 |
| L91 | 0.90 | 25.36 | 0.91 | 24.62 |
| L128 | 0.57 | 24.97 | 0.61 | 24.33 |
| L138 | 0.77 | 23.22 | 0.75 | 26.31 |
| L142 | 0.46 | 27.96 | -0.44 | 21.85 |
| L178 | 0.62 | 26.79 | 0.73 | 26.76 |
| L180 | -0.08 | 24.40 | 0.36 | 22.73 |
| L198 | 0.67 | 22.63 | 0.82 | 26.10 |
| L202 | 0.77 | 24.82 | 0.51 | 22.69 |
| L210 | 1.09 | 28.08 | 0.91 | 22.54 |
| L230 | 0.68 | 21.76 | -0.49 | 24.06 |
| L231 | 0.88 | 25.43 | 1.07 | 24.84 |
| L236 | 0.81 | 25.17 | 0.70 | 22.50 |
| L240 | 0.63 | 24.91 | 0.72 | 24.41 |
| L269 | 0.88 | 26.90 | 0.65 | 22.43 |
| L285 | 0.95 | 24.64 | 0.90 | 23.63 |
| L286 | 0.88 | 25.70 | 0.77 | 26.17 |
| L291 | 0.60 | 25.30 | 0.77 | 26.28 |
| L293 | 0.93 | 25.49 | 0.78 | 24.03 |
| L298 | 1.06 | 23.58 | 0.83 | 25.24 |
| L329 | 0.21 | 25.28 | 0.63 | 22.27 |
| L334 | 0.83 | 21.44 | 0.78 | 27.66 |
| L335 | 0.82 | 25.21 | 0.64 | 22.08 |
| L343 | 0.99 | 23.01 | 0.74 | 25.79 |
| L375 | 0.88 | 25.65 | 0.73 | 22.07 |
| L420 | 0.47 | 24.80 | 0.38 | 22.51 |
| L433 | 0.98 | 27.20 | 0.89 | 25.45 |
| L454 | 0.39 | 24.89 | 0.46 | 21.61 |
| L471 | 0.92 | 26.00 | 1.08 | 22.21 |
| L491 | 0.57 | 25.10 | 0.29 | 20.69 |
| L494 | 0.63 | 25.95 | 0.67 | 22.34 |
| L498 | 0.76 | 25.09 | 0.62 | 23.33 |
| L514 | 1.07 | 25.99 | 0.94 | 22.39 |

| | | | | |
|---|---|---|---|---|
| L526 | 0.11 | 23.50 | 0.11 | 22.39 |
| L543 | 0.57 | 25.18 | 0.25 | 21.50 |
| L546 | 0.79 | 25.26 | 0.26 | 20.50 |
| L572 | 0.73 | 26.50 | 1.06 | 23.40 |
| L573 | 0.99 | 24.35 | 0.96 | 25.65 |
| L576 | 0.83 | 24.89 | 1.22 | 24.67 |
| L604 | 0.92 | 27.78 | 0.76 | 23.07 |
| L628 | 0.90 | 25.55 | 0.68 | 23.48 |
| L651 | 1.20 | 28.48 | 1.06 | 25.34 |
| L660 | 0.72 | 25.92 | 0.48 | 23.37 |
| L696 | 0.74 | 26.71 | 0.91 | 24.15 |
| L699 | 0.91 | 25.05 | 0.89 | 22.17 |
| L711 | 0.81 | 25.83 | 1.02 | 22.84 |
| L712 | 0.18 | 24.74 | 0.06 | 22.96 |
| L717 | 0.98 | 25.20 | 0.96 | 23.71 |
| L724[b] | 0.94 | 24.80 | 0.87 | 22.98 |

[a]Stereospecific assignments were obtained with the method of fractional $^{13}$C-labeling of Wüthrich and coworkers.[1,2] Methyls of L10, L117, L174, L313, L362, L372, L413, L435, L577, L596, L635, and L646 could not be sterospecifically assigned due to either resonance overlap of both diastereotopic methyls and/or very low sensitivity. $^{1}$H and $^{13}$C chemical shifts are referenced against external TSP. [b]L724 belongs to the two-residue linker (-LE-) connecting the C-terminus of MSG with a hexa-histidine tag sequence.[3]

**Supplemental Figure Legends**

**Figure S1.** An alternative MQ-SED pulse scheme for the measurement of $^3J_{C\gamma C'}$ coupling constants in high molecular weight, deuterated, selectively methyl-protonated proteins. All pulse shapes, durations, delays and carrier positions are as described in the legend to Figure 3b. The durations and strengths of the pulsed field gradients are: G1 = (1.0 ms, 15 G/cm), G2 = (0.8 ms, 7.5 G/cm), G3 = (0.1 ms, 12 G/cm), G4 = (0.5 ms, -25 G/cm). The phase cycle employed is: $\phi 1 = 4(x),4(-x)$; $\phi 2 = x,-x$; $\phi 3 = 2(x),2(-x)$; rec = $4(x),4(-x)$. Quadrature detection in $F_1$ is achieved by States-TPPI[4] of phase $\phi 1$. A pair of subspectra are recorded in an interleaved manner with $^{13}C'$ pulses at positions *a* or *b* (see text). The value of *T* is set to a multiple of $1/^1J_{CC}$ (57 ms). $^3J_{C\gamma C'}$ values are obtained from the relation $I_b/I_a = \cos(\pi^3 J_{C\gamma C'}T)/\cos(\pi^4 J_{H\gamma C'}T)$ and we have assumed that $^4J_{H\gamma C'} = 0$ in the analysis.

**Figure S2**. Correlation plot between $^3J_{C\gamma C'}$ couplings of Val side chains in MSG measured using the pulse schemes of Figure 3b (*x*-axis) and of Figure S1 (*y*-axis). Only well separated correlations were included in the comparison (41 $^3J_{C\gamma C'}$ values). The bars drawn along the *x* and *y* axes near the origin of the plot correspond to average standard errors in the corresponding measurement estimated as described in the legend to Figure 5.

**Figure S3.** $^1$H-$^{13}$C MQ relaxation dispersion profiles for selected Val residues that do *not* show evidence of rotamer averaging from measured values of $^3J_{C\gamma N}$ and $^3J_{C\gamma C'}$ couplings, Table 1. Dispersion profiles were recorded at 800 (red) and 600 (black) MHz as described in Korzhnev *et al.*[5] (see legend to Figure 6 for additional details).

**References**

1)Neri, D.; Szyperski, T.; Otting, G.; Senn, H.; Wüthrich, K. *Biochemistry* **1989**, *28*, 7510-7516.

2)Szyperski, T.; Neri, D.; Leiting, B.; Otting, G.; Wüthrich, K. *J. Biomol. NMR* **1992**, *2*, 323-334.

3)Tugarinov, V.; Muhandiram, R.; Ayed, A.; Kay, L. E. *J. Am. Chem. Soc.* **2002**, *124*, 10025-10035.

4)Marion, D.; Ikura, M.; Tschudin, R.; Bax, A. *J. Magn. Reson.* **1989**, *85*, 393-399.

5)Korzhnev, D. M.; Klober, K.; Kanelis, V.; Tugarinov, V.; Kay, L. E. *J. Am. Chem. Soc.* **2004**, 126, 3964-3973.

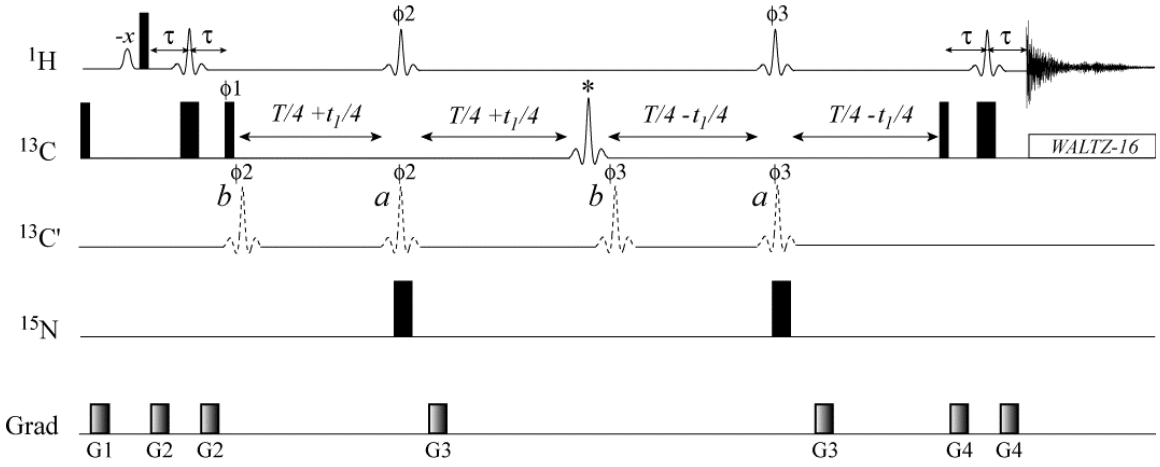
1H
-x
τ
τ
ϕ2
ϕ3
τ
τ
13C
ϕ1
T/4 +t1/4
T/4 +t1/4
*
T/4 -t1/4
T/4 -t1/4
WALTZ-16
13C'
ϕ2
b
ϕ2
a
ϕ3
b
ϕ3
a
15N
Grad
G1
G2
G2
G3
G3
G4
G4

*Figure S1*

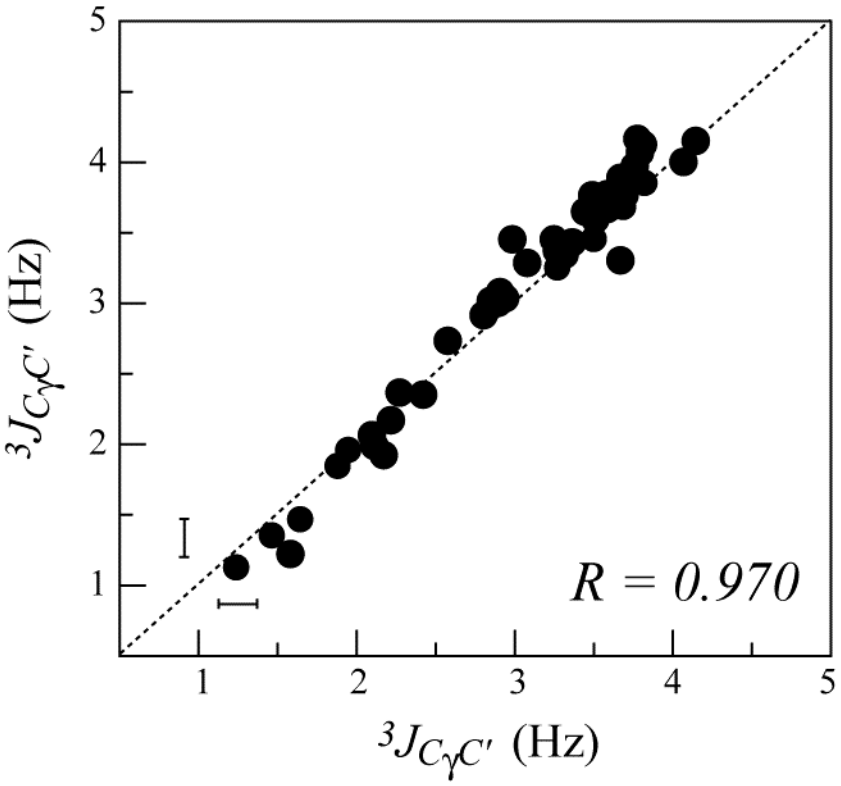


*Figure S2*

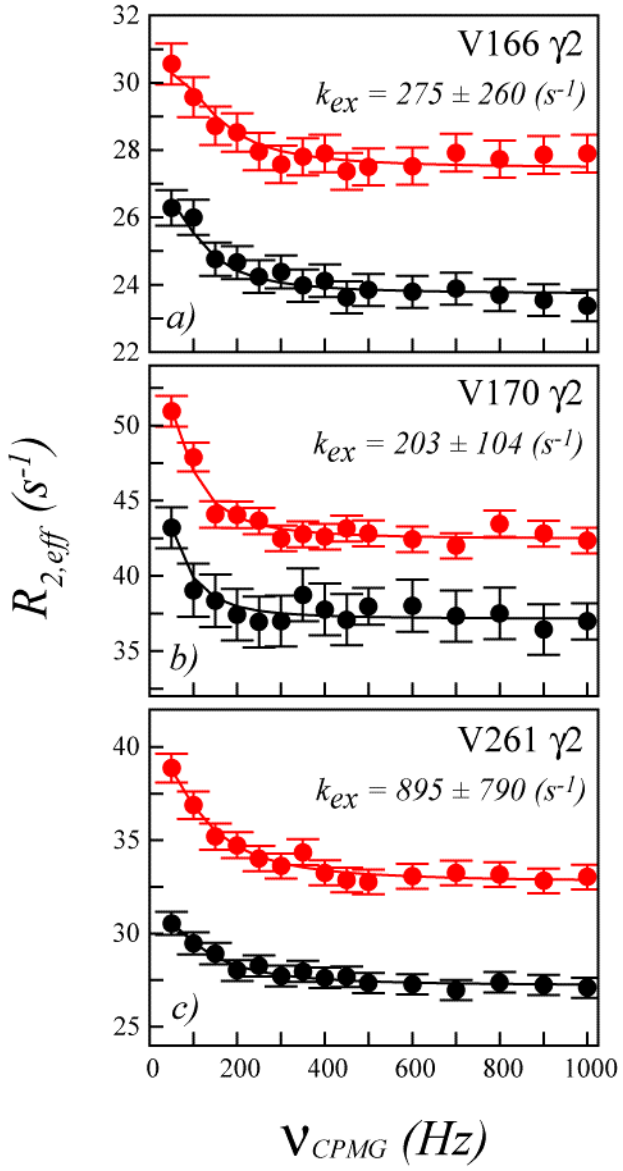
V166 γ2
$k_{ex}$ = 275 ± 260 ($s^{-1}$)
a)
V170 γ2
$k_{ex}$ = 203 ± 104 ($s^{-1}$)
b)
V261 γ2
$k_{ex}$ = 895 ± 790 ($s^{-1}$)
c)
$R_{2,eff}$ ($s^{-1}$)
$\nu_{CPMG}$ (Hz)

*Figure S3*