Supporting Information.

Supplemental Table 1. Exchange parameters obtained from fitting ^{13}C-^{1}H multiple-quantum CPMG relaxation dispersion data for Ile δ1, Val γ1/γ2 and Leu δ1/δ2 methyl groups of malate synthase G (MSG), 37°C, to a two-site exchange model[a].

Residue[b]	R_{ex} [s^{-1}] 14.1 / 18.8 T	p_B [%]	k_{ex} [s^{-1}]	$\Delta\varpi_C$ [ppm]	$\Delta\varpi_H$ [ppm][b]	χ^2 / #DF simple	χ^2 / #DF exch.	Secondary Structure / Domain
L 10[?]	3.46 / 5.64	0.67 ± 0.11 0.67 ± 0.12	982 ± 376 981 ± 399	1.83 ± 0.17 1.83 ± 0.19	- *0.000 ± 103.1*	163 / 28	6.4 / 25 6.4 / 24	βB strand / N-term. clasp
I 109	3.24 / 5.38	3.96 ± 2.87 3.96 ± 2.88	569 ± 98 569 ± 98	0.29 ± 0.12 0.29 ± 0.12	- *0.000 ± 0.389*	444 / 51	34.1 / 48 34.1 / 47	αE helix / N- clasp - barrel connection
L 117	9.31 / 11.70	2.39 ± 0.36 *13.1 ± 34.0*	982 ± 144 1027 ± 177	0.88 ± 0.10 *0.40 ± 0.50*	- 0.104 ± 0.092	431 / 28	11.6 / 25 9.2 / 24	β1 strand / barrel domain
V 119	3.38 / 3.73	0.74 ± 0.20 1.98 ± 1.53	546 ± 260 442 ± 256	1.23 ± 0.13 1.13 ± 0.76	- 0.102 ± 0.042	134 / 28	12.1 / 25 **7.9 / 24**	β1 strand / barrel domain
L 128	16.77 / 12.43	10.15 ± 3.84 11.08 ± 0.66	139 ± 57 350 ± 54	1.11 ± 0.06 0.75 ± 0.07	- 0.076 ± 0.005	680 / 28	16.0 / 25 **5.2 / 24**	α1 helix / barrel domain
L 142	11.58 / 15.07	12.00 ± 3.44 *20.29 ± 32.14*	137 ± 65 301 ± 193	0.53 ± 0.07 0.32 ± 0.29	- *0.062 ± 0.073*	307 / 28	10.8 / 25 10.5 / 24	αF helix / α/β domain
I 148	6.09 / 7.19	3.44 ± 0.25 *10.11 ± 14.14*	369 ± 38 414 ± 61	0.42 ± 0.03 0.25 ± 0.17	- 0.041 ± 0.021	946 / 51	17.1 / 48 **13.9 / 47**	αF - αG loop / α/β domain
V 155[*]	7.57 / 11.04	6.38 ± 1.17 16.61 ± 1.09	418 ± 47 474 ± 24	0.40 ± 0.05 0.26 ± 0.01	- 0.061 ± 0.002	999 / 28	11.7 / 25 **8.0 / 24**	αF - αG loop / α/β domain
V 166	4.91 / 7.35	4.83 ± 4.04 *15.67 ± 111.4*	369 ± 209 451 ± 391	0.37 ± 0.23 *0.22 ± 0.74*	- *0.063 ± 0.228*	177 /28	37.8 / 25 37.3 / 24	αG helix / α/β domain
I 167[*]	3.50 / 5.61	2.87 ± 0.40 3.36 ± 2.80	258 ± 82 373 ± 104	0.37 ± 0.06 0.34 ± 0.15	- *0.016 ± 0.040*	270 / 51	28.2 / 48 28.2 / 47	αG helix / α/β domain
V 170	6.23 / 8.16	6.23 ± 1.13 6.23 ± 1.14	203 ± 103 203 ± 104	0.43 ± 0.10 0.43 ± 0.10	- *0.000 ± 35.7*	105 / 28	6.6 / 25 6.6 / 24	αG helix / α/β domain
V 188[*]	3.86 / 5.86	3.14 ± 0.54 18.90 ± 1.37	332 ± 87 464 ± 44	0.45 ± 0.08 0.19 ± 0.02	- 0.078 ± 0.03	153 / 28	6.6 / 25 5.5 / 24	βD strand / α/β domain
V 189	4.89 / 6.73	4.84 ± 0.60 7.05 ± 5.74	205 ± 59 278 ± 106	0.44 ± 0.06 0.34 ± 0.16	- 0.038 ± 0.032	314 / 28	10.0 / 25 9.9 / 24	βD strand α/β domain
V 189[*]	4.30 / 5.94	4.92 ± 1.44 16.90 ± 2.32	222 ± 89 331 ± 48	0.35 ± 0.10 0.19 ± 0.02	- 0.063 ± 0.005	277 / 28	18.6 / 25 16.6 / 24	βD strand / α/β domain
I 229	3.15 / 3.92	1.94 ± 0.38 *3.85 ± 4.79*	220 ± 75 326 ± 119	0.51 ± 0.04 0.34 ± 0.25	- 0.040 ± 0.016	351 / 51	25.0 / 48 23.6 / 47	βH strand / α/β domain
L 240[*]	6.62 / 9.46	5.34 ± 1.31 21.28 ± 1.41	372 ± 83 468 ± 41	0.41 ± 0.08 0.22 ± 0.02	- 0.077 ± 0.003	268 / 28	9.7 / 25 7.8 / 24	βI strand / α/β domain
I 242	21.11 / 21.50	7.22 ± 0.40 9.59 ± 0.58	343 ± 31 402 ± 18	0.82 ± 0.02 0.72 ± 0.03	- 0.047 ± 0.004	6961 / 51	29.8 / 48 **13.4 / 47**	βI strand / α/β domain
I 248	10.62 / 12.31	3.69 ± 0.14 4.10 ± 0.49	435 ± 39 450 ± 39	0.71 ± 0.02 0.67 ± 0.04	- 0.026 ± 0.014	1780 / 51	16.4 / 48 16.1 / 47	βI - ηD loop / α/β domain
I 260	25.46 / 23.65	5.13 ± 0.68 6.09 ± 0.17	552 ± 55 669 ± 19	1.64 ± 0.02 1.50 ± 0.02	- 0.076 ± 0.004	12465 / 51	44.3 / 48 **11.4 / 47**	βJ strand / α/β domain
I 265	22.31 / 26.33	9.27 ± 0.21 15.06 ± 4.47	385 ± 22 420 ± 39	0.56 ± 0.01 0.45 ± 0.08	- 0.041 ± 0.009	4318 / 51	18.1 / 48 **15.3 / 47**	βJ - β2 loop / α/β-barrel connection
L 291	5.15 / 7.44	7.00 ± 1.38 *21.95 ± 80.08*	212 ± 52 305 ± 230	0.33 ± 0.06 *0.19 ± 0.33*	- *0.059 ± 0.155*	203 / 28	5.1 / 25 4.9 / 24	α2 helix / barrel domain
L 293[*]	4.86 / 5.66	1.58 ± 0.17 15.43 ± 0.52	516 ± 126 633 ± 43	0.73 ± 0.09 0.25 ± 0.11	- 0.097 ± 0.002	162 / 28	6.7 / 25 **3.5 / 24**	α2 helix / barrel domain
L 298[?]	9.89 / 6.30	3.71 ± 2.39 7.13 ± 1.85	232 ± 170 196 ± 69	1.36 ± 0.12 1.29 ± 0.09	- 0.059 ± 0.013	423 / 28	15.4 / 25 **7.7 / 24**	α2 - βK loop / α/β - barrel connection

I 309	18.25 / 19.93	7.64 ± 0.25 9.94 ± 1.01	308 ± 19 345 ± 20	0.64 ± 0.01 0.56 ± 0.03	- 0.036 ± 0.006	6921 / 51	22.1 / 48 **<u>16.9 / 47</u>**	α2 - βK loop / α/β - barrel connection
L 313*	4.29 / 6.06	4.94 ± 5.61 21.45 ± 2.49	578 ± 146 580 ± 72	0.32 ± 0.20 0.18 ± 0.02	- 0.084 ± 0.005	130 / 28	9.1 / 25 8.0 / 24	α2 - βK loop / α/β - barrel connection
L 329*	4.81 / 7.08	4.59 ± 1.44 15.79 ± 1.40	358 ± 77 429 ± 36	0.37 ± 0.09 0.22 ± 0.02	- 0.067 ± 0.003	380 / 28	13.1 / 25 **9.6 / 24**	βL strand / α/β domain
L 343	3.14 / 4.54	2.06 ± 0.46 *15.72 ±315.2*	317 ± 154 *484 ±1067*	0.51 ± 0.13 *0.19 ±1.91*	- *0.081 ±0.552*	86 / 28	10.8 / 25 9.7 / 24	β3 - βM loop / barrel domain
V 377*	12.06 / 15.68	9.18 ± 0.74 17.55 ± 0.64	209 ± 38 339 ± 21	0.52 ± 0.04 0.34 ± 0.01	- 0.059 ± 0.001	1575 / 28	18.4 / 25 15.6 / 24	α3 helix / barrel domain
I 424	11.40 / 17.08	3.85 ± 0.39 3.85 ± 0.39	1067 ± 68 1067 ± 75	0.78 ± 0.05 0.78 ± 0.05	- *0.000 ±67.5*	3564 / 51	38.4 / 48 38.4 / 47	β5 strand / barrel domain
L 491	7.82 / 9.92	6.60 ± 1.02 *12.96 ±18.18*	245 ± 67 331 ± 119	0.41 ± 0.07 0.28 ± 0.20	- 0.047 ± 0.047	369 / 28	14.0 / 25 13.8 / 24	α6 helix / barrel domain
L 498	4.20 / 4.08	2.65 ± 1.77 *12.8 ±63.57*	138 ± 107 557 ± 380	0.90 ± 0.11 *0.26 ±0.68*	- *0.096 ±0.112*	129 / 28	7.3 / 25 **<u>4.4 / 24</u>**	α6 - β7 loop / barrel domain
V 553	3.31 / 4.45	4.64 ± 1.22 4.63 ± 1.23	165 ± 84 165 ± 84	0.35 ± 0.10 0.35 ± 0.10	- *0.000 ±2.50*	145 / 28	9.4 / 25 9.4 / 24	αI helix / C-term. - barrel connection
L 696?	3.31 / 3.79	0.78 ± 0.27 *2.05 ±2.33*	523 ± 312 434 ± 386	1.18 ± 0.16 *1.10 ±1.11*	- 0.100 ± 0.062	93 / 28	12.6 / 25 9.9 / 24	αN helix / C-term. domain
L 712	5.47 / 6.66	0.93 ± 0.17 2.32 ± 1.91	937 ± 348 1149 ± 348	1.87 ± 0.16 1.23 ± 0.66	- 0.147 ± 0.020	164 / 28	11.8 / 25 10.9 / 24	αO helix / C-term. domain

[a]Only those residues with a positive exchange contribution R_{ex} to $R_{2,eff}$ are listed. k_{ex} - exchange rate constant for a two-site exchange process, $k_{ex}=k_{AB}+k_{BA}$; p_B - population of the minor state, $\Delta\varpi_C$ / $\Delta\varpi_H$ - ^{13}C / ^{1}H chemical shift difference between the exchanging states, χ^2 / #DF - χ^2 value obtained in fits of experimental CPMG dispersion profiles to a model describing $R_{2,eff}(\nu_{cpmg})$ / number of degrees of freedom associated with the fit. The models considered are: a 'simple' model, which assumes no chemical exchange and a two-site exchange model. Each column of the table is divided into two rows containing the results of a minimization with $\Delta\varpi_H$ fixed to 0 (first row) and with an adjustable $\Delta\varpi_H$ value (second row).

[b]A very conservative approach has been taken in which the methyls listed in the Table satisfy the following criteria: (i) the methyl resonance is well resolved in ^{13}C-^{1}H spectra, (ii) the exchange contribution R_{ex} to $R_{2,eff}$, estimated as $R_{2,eff}(\nu_{cpmg}^{min})$ - $R_{2,eff}(\nu_{cpmg}^{max})$ exceeds 3 s^{-1} for data recorded at 600 and 800 MHz, (iii) relaxation dispersion profiles cannot be fit to a model that assumes no conformational exchange (χ^2 confidence is less than 0.1%), (iv) profiles can be fit to a two-site exchange model with a χ^2 confidence level of at least 0.1% (v) A statically significant reduction in (χ^2) is associated with the fit involving a two-site exchange model relative to a fit with the model which assumes no exchange based on the F-test at a 99.9% confidence level, (vi) uncertainties of k_{ex}, p_B and $\Delta\varpi_C$ with $\Delta\varpi_H$ fixed to 0 as estimated using the covariance matrix method[1] are less than 100% of the extracted value (as a rule, uncertainties of k_{ex}, p_B and $\Delta\varpi_C$ are higher when $\Delta\varpi_H$ is adjustable; values in italics have greater than 100% uncertainty). Criteria (i) - (vi) lead to the selection of 34 methyl groups (15 Leu, 9 Val, 10 Ile) and one unassigned Val/Leu resonance (data not shown). For 12 methyl groups (5 Leu, 4 Val, 1 Ile) satisfying criteria (i) - (v), the uncertainties in one or more exchange parameters exceeds 100% of the values (not listed in the Table). With the exception of V166, for which 2.5%<p(χ^2)<5%, and the 5 Ile δ1 groups listed in Table 2 with negative R_{ex}, it is not possible to eliminate model 1 on the basis of χ^2 statistics. Indeed, p(χ^2)>90% for 28 of the 34 methyls in Table 1, while for the remaining 5 (excluding V166) p(χ^2)>50%. However, the model with adjustable $\Delta\varpi_H$ often results in a significant (according to F-statistics criteria) improvement in fit relative to the corresponding model with $\Delta\varpi_H$=0. Those residues for which this is the case at the 99% (99.9%) confidence level are shown in bold (bold underlined).

*Minimization with adjustable $\Delta\varpi_H$ was stopped at $R_{2,eff}(\infty)$ close to 0 (using an additional energy term introduced to prevent negative values). In this case the uncertainties in the extracted parameters are likely to be underestimated.

?Ambiguous assignment.

Supplemental Table 2. Exchange parameters obtained from fitting ^{13}C-^{1}H multiple-quantum CPMG relaxation dispersion profiles of Ile δ1 methyls of malate synthase G (MSG) with negative exchange contributions, R_{ex}, to $R_{2,eff}$[a].

Residue	R_{ex} [s^{-1}] 14.1 / 18.8 T	p_B [%]	k_{ex} [s^{-1}]	$\Delta\varpi_C$ [ppm]	$\Delta\varpi_H$ [ppm]	χ^2 / #DF simple	χ^2 / #DF exch.	Secondary Structure / Domain
I 42	-1.91 / -1.51	1.11 ± 0.21	595 ± 117	2.04 ± 0.07	0.399 ± 0.013	294 / 51	53.8 / 47	αC helix / N-term. clasp
I 284	-0.78 / -0.38	7.70 ± 1.64	133 ± 17	0.90 ± 0.03	0.141 ± 0.005	124 / 51	46.4 / 47	α2 helix / barrel domain
I 592	- 1.14 / -0.86	4.35 ± 0.88	298 ± 74	0.52 ± 0.03	0.245 ± 0.009	225 / 51	38.8 / 47	αK helix / C-term. domain
I 623	-0.76 / -0.31	2.73 ± 0.38	406 ± 65	0.73 ± 0.04	0.406 ± 0.011	102 / 51	20.3 / 47	βO - βP loop / C-term. domain
I 697	-1.36 / -2.16	0.89 ± 0.20	935 ± 315	2.68 ± 0.15	0.476 ± 0.030	99 /51	42.7 / 47	αN helix / C-term. domain

[a]See captions to Supplemental Table 1 for details. The methyl groups listed in the Table satisfy the same criteria as in Table 1, with criterion (ii) reformulated as: $R_{ex} < 0$ at 600 and 800 MHz.

References

1)Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Recipes in C*; Cambridge University Press: Cambridge, 1988.