Supplementary Tables

Table S1. Methyl resonance assignments for hDHFR complexes.

Residue	hE:FOL:NADP$^+$		hE:NADPH		hE:THF	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
MET 0-ε	2.074	16.850	2.078	16.845	2.070	16.840
VAL 1-γ1	1.063	20.533	1.023	21.270	0.988	21.556
VAL 1-γ2	1.044	20.048	1.028	20.756	0.939	20.467
LEU 4-δ1	#N/A	#N/A	#N/A	#N/A	0.766	24.795
LEU 4-δ2	#N/A	#N/A	#N/A	#N/A	0.731	24.917
ILE 7-δ1	0.610	14.474	0.584	14.408	0.510	13.815
ILE 7-γ2	1.498	17.020	1.280	16.633	1.222	17.342
VAL 8-γ1	-0.093	18.337	-0.035	17.030	-0.062	17.922
VAL 8-γ2	0.508	21.308	0.589	21.237	0.536	21.114
ALA 9-β	1.434	21.544	1.595	21.365	1.194	21.600
VAL 10-γ1	0.616	21.513	0.761	21.606	0.642	22.030
VAL 10-γ2	0.867	21.291	0.797	21.145	0.833	20.789
MET 14-ε	2.233	18.511	2.242	18.452	2.209	18.555
ILE 16-δ1	0.271	13.554	1.195	14.214	1.187	13.466
ILE 16-γ2	1.020	18.906	1.386	19.937	1.321	19.939
LEU 22-δ1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
LEU 22-δ2	#N/A	#N/A	#N/A	#N/A	0.881	23.117
LEU 27-δ1	0.097	25.485	0.153	24.792	0.086	25.572
LEU 27-δ2	0.655	23.390	0.694	23.097	0.632	23.375
MET 37-ε	1.828	17.480	1.936	17.335	1.850	17.443
THR 38-γ2	-0.196	20.819	-0.139	20.507	-0.250	20.345
THR 39-γ2	1.189	21.751	1.211	21.554	1.197	21.801
THR 40-γ2	1.196	20.704	1.211	20.666	1.186	20.737
VAL 43-γ1	0.940	21.127	0.963	21.039	0.917	21.147
VAL 43-γ2	0.909	20.465	0.931	20.366	0.888	20.502
LEU 49-δ1	#N/A	#N/A	0.867	25.540	0.833	25.168
LEU 49-δ2	#N/A	#N/A	0.798	26.816	0.749	26.984
VAL 50-γ1	0.990	23.053	0.921	22.919	0.933	22.998
VAL 50-γ2	1.014	21.484	1.088	20.445	0.955	21.535
ILE 51-δ1	0.638	14.364	0.606	14.107	0.611	14.193
ILE 51-γ2	0.635	16.445	0.628	16.393	0.734	17.079
MET 52-ε	2.045	17.710	2.147	17.585	2.027	17.697
THR 56-γ2	1.356	21.469	1.372	21.065	1.309	20.916
ILE 60-δ1	0.238	13.554	0.714	13.744	0.312	13.129
ILE 60-γ2	0.156	15.832	0.741	16.850	0.493	16.640
LEU 67-δ1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
LEU 67-δ2	0.717	21.555	0.473	24.299	0.668	21.329
ILE 71-δ1	0.728	13.861	0.754	13.521	0.714	13.883

Table S1. Methyl resonance assignments for hDHFR complexes.

Residue	hE:FOL:NADP$^+$		hE:NADPH		hE:THF	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
ILE 71-γ2	1.059	18.932	1.072	18.812	1.035	18.877
LEU 73-δ1	#N/A	#N/A	#N/A	#N/A	0.731	23.798
LEU 73-δ2	#N/A	#N/A	0.702	24.917	0.700	25.387
VAL 74-γ1	0.527	20.423	0.561	20.234	0.516	20.273
VAL 74-γ2	0.128	21.810	0.177	21.738	0.152	21.765
LEU 75-δ1	0.083	26.482	0.089	26.283	0.864	25.452
LEU 75-δ2	0.164	23.884	0.157	23.803	0.447	24.876
LEU 79-δ1	0.930	25.374	0.968	25.403	0.885	25.517
LEU 79-δ2	1.086	22.338	1.126	22.319	1.027	22.380
ALA 86-β	0.227	17.101	0.275	17.040	0.236	17.222
LEU 89-δ1	0.850	24.952	0.869	25.035	0.812	24.705
LEU 89-δ2	0.454	25.562	0.480	25.533	0.421	25.708
LEU 93-δ1	#N/A	#N/A	0.844	23.487	#N/A	#N/A
LEU 93-δ2	#N/A	#N/A	0.801	25.240	#N/A	#N/A
ALA 96-β	1.451	19.593	1.458	19.355	1.427	19.444
LEU 97-δ1	0.636	25.017	0.687	23.960	0.654	25.139
LEU 97-δ2	0.700	22.193	0.733	22.108	0.700	21.791
LEU 99-δ1	0.671	24.029	0.719	23.936	0.719	23.904
LEU 99-δ2	0.451	24.182	0.506	24.256	0.517	24.266
THR 100-γ2	1.234	21.716	1.256	21.606	1.160	21.532
LEU 105-δ1	0.426	24.846	0.447	24.639	0.333	24.723
LEU 105-δ2	0.738	23.739	0.701	23.770	0.673	23.749
ALA 106-β	1.309	18.489	1.355	18.401	1.280	18.477
VAL 109-γ1	0.907	22.131	0.914	22.076	0.881	22.330
VAL 109-γ2	0.983	23.675	1.000	23.625	0.961	23.541
MET 111-ε	2.158	16.944	2.186	16.622	2.133	16.728
VAL 112-γ1	0.809	22.379	0.824	22.339	0.793	22.380
VAL 112-γ2	0.970	21.873	0.988	21.653	0.937	21.820
ILE 114-δ1	0.833	13.205	0.845	13.031	0.825	13.883
ILE 114-γ2	1.012	19.689	1.017	19.163	0.946	19.766
VAL 115-γ1	1.345	22.074	1.090	21.954	1.217	22.253
VAL 115-γ2	1.310	20.780	1.111	19.812	1.100	21.497
VAL 120-γ1	0.740	21.949	0.760	22.596	0.799	22.093
VAL 120-γ2	0.852	22.253	0.946	22.636	0.975	22.053
ALA 124-β	1.504	19.269	1.543	18.639	1.472	19.107
MET 125-ε	1.489	16.560	1.703	16.811	1.598	16.714
LEU 131-δ1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
LEU 131-δ2	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
LEU 133-δ1	0.152	24.872	0.382	24.744	0.192	25.089
LEU 133-δ2	0.649	24.429	0.613	24.118	0.675	24.387

Table S1. Methyl resonance assignments for hDHFR complexes.						
	hE:FOL:NADP$^+$		**hE:NADPH**		**hE:THF**	
Residue	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
VAL 135-γ1	0.527	20.687	0.666	21.052	0.520	20.775
VAL 135-γ2	0.604	20.670	0.672	21.290	0.584	20.738
THR 136-γ2	0.790	23.322	0.863	22.946	0.787	23.270
ILE 138-δ1	-0.884	10.230	-0.813	10.594	-0.921	10.399
ILE 138-γ2	0.530	17.067	0.677	17.447	0.544	17.424
MET 139-ε	2.005	16.680	2.035	16.407	1.978	16.622
THR 146-γ2	1.393	21.529	1.394	21.204	1.326	20.841
ILE 151-δ1	0.224	12.890	0.329	12.801	0.223	12.804
ILE 151-γ2	0.087	17.161	-0.022	16.723	0.016	17.198
LEU 153-δ1	1.099	25.613	1.122	25.473	1.062	25.694
LEU 153-δ2	0.957	22.215	1.019	22.213	0.980	22.500
LEU 158-δ1	0.295	22.228	0.339	22.081	0.273	22.149
LEU 158-δ2	0.778	26.030	0.815	25.936	0.761	26.059
LEU 159-δ1	1.007	25.834	1.000	25.812	0.967	25.822
LEU 159-δ2	1.012	22.463	1.003	22.636	0.965	22.791
VAL 165-γ1	1.049	21.732	1.057	21.560	1.021	21.764
VAL 165-γ2	1.324	22.449	1.344	22.353	1.310	22.452
LEU 166-δ1	1.071	25.536	1.092	25.131	1.036	25.819
LEU 166-δ2	0.991	22.734	1.025	23.210	1.005	22.782
VAL 169-γ1	0.887	21.590	0.920	21.516	0.859	21.640
VAL 169-γ2	1.007	22.754	1.037	22.622	0.994	22.787
ILE 175-δ1	1.044	14.261	1.078	13.852	1.008	13.913
ILE 175-γ2	0.825	18.430	0.895	18.332	0.837	18.435
VAL 181-γ1	-0.246	20.029	-0.274	19.927	-0.306	20.095
VAL 181-γ2	0.566	21.246	0.551	21.178	0.491	21.204

#N/A – resonances not assignable due to overlap ambiguity.

Table S2. Average over all complexes for Ile, Thr, Val ^{3}J and rotamer populations [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Ile7	1.18 (0.51)	1.20 (0.43)	0.32 (0.00 - 0.75)	0.17 (0.00 - 0.32)	0.50 (0.00 - 1.00)	189 (6)
Ile16	0.59 (0.60)	3.66 (0.28)	0.05 (0.00 - 0.14)	**0.95** (0.85 - 1.00)	0.00 (0.00 - 0.15)	60 (7)
Ile51	1.75 (0.21)	0.78 (0.44)	**0.81** (0.56 - 0.95)	0.09 (0.00 - 0.22)	0.11 (0.00 - 0.44)	-67 (5)
Ile60[d]						-60 (4)
Ile71	2.08 (0.36)	0.65 (0.57)	**1.00** (0.73 - 1.00)	0.00 (0.00 - 0.20)	0.00 (0.00 - 0.27)	-56 (7)
Ile114[d]						-61 (12)
Ile138	1.77 (0.29)	0.57 (0.60)	**0.78** (0.50 - 1.00)	0.02 (0.00 - 0.19)	0.20 (0.00 - 0.50)	-63 (10)
Ile151	2.07 (0.25)	1.09 (0.82)	**1.00** (0.80 - 1.00)	0.00 (0.00 - 0.20)	0.00 (0.00 - 0.18)	-60 (2)
Ile175	1.97 (0.30)	1.03 (0.60)	**0.95** (0.68 - 1.00)	0.05 (0.00 - 0.27)	0.00 (0.00 - 0.32)	-62 (2)
Thr38	0.26 (0.37)	3.25 (0.24)	0.00 (0.00 - 0.00)	**0.95** (0.84 - 1.00)	0.05 (0.00 - 0.16)	62 (6)
Thr39	0.92 (0.28)	1.52 (0.14)	0.36 (0.17 - 0.56)	0.32 (0.19 - 0.46)	0.31 (0.02 - 0.61)	-46 (14)
Thr40	1.53 (0.16)	0.41 (0.35)	**0.89** (0.74 - 1.00)	0.06 (0.00 - 0.15)	0.05 (0.00 - 0.21)	-57 (2)
Thr56	1.51 (0.11)	0.61 (0.43)	**0.85** (0.73 - 0.98)	0.13 (0.00 - 0.27)	0.02 (0.00 - 0.18)	-63 (5)
Thr100	1.04 (0.18)	2.22 (0.17)	0.34 (0.21 - 0.41)	**0.64** (0.52 - 0.69)	0.02 (0.00 - 0.23)	65 (5)
Thr136	1.63 (0.07)	0.69 (0.46)	**0.97** (0.87 - 1.00)	0.03 (0.00 - 0.13)	0.00 (0.00 - 0.07)	-58 (3)
Thr146	1.29 (0.25)	0.96 (0.56)	**0.67** (0.44 - 0.90)	0.21 (0.00 - 0.36)	0.12 (0.00 - 0.44)	*
Val1-Cγ1	0.97 (0.51)	1.96 (0.07)				
Cγ2	0.65 (0.27)	1.93 (0.43)	0.43 (0.39 - 0.47)	0.17 (0.02 - 0.32)	0.40 (0.29 - 0.52)	179 (17)
Val8-Cγ1	0.67 (0.57)	1.41 (0.34)				
Cγ2	1.88 (0.51)	1.20 (0.42)	0.21 (0.03 - 0.35)	**0.67** (0.36 - 0.97)	0.12 (0.00 - 0.29)	68 (2)
Val10-Cγ1	0.89 (0.31)	2.60 (0.21)				
Cγ2	1.02 (0.19)	0.85 (0.66)	**0.62** (0.54 - 0.69)	0.21 (0.07 - 0.32)	0.17 (0.14 - 0.33)	*
Val43-Cγ1	1.51 (0.12)	1.27 (0.11)				
Cγ2	0.76 (0.19)	2.77 (0.04)	0.23 (0.19 - 0.27)	0.11 (0.06 - 0.15)	**0.66** (0.65 - 0.67)	178 (3)
Val50-Cγ1						
Cγ2[d]						*
Val74-Cγ1	1.82 (0.11)	0.46 (0.31)				
Cγ2	0.20 (0.23)	3.15 (0.15)	0.09 (0.08 - 0.27)	0.11 (0.00 - 0.11)	**0.80** (0.73 - 0.84)	180 (5)
Val109-Cγ1	1.47 (0.32)	0.94 (0.35)				
Cγ2	0.38 (0.16)	3.05 (0.15)	0.15 (0.04 - 0.21)	0.10 (0.00 - 0.24)	**0.75** (0.72 - 0.79)	177 (9)
Val112-Cγ1	1.80 (0.17)	0.55 (0.29)				
Cγ2	0.42 (0.11)	3.45 (0.26)	0.03 (0.01 - 0.06)	0.09 (0.00 - 0.19)	**0.88** (0.80 - 0.96)	177 (2)
Val115-Cγ1						
Cγ2[d]						-53 (7)
Val120-Cγ1	1.89 (0.20)	0.92 (0.54)				
Cγ2	0.63 (0.31)	3.09 (0.19)	0.16 (0.09 - 0.22)	0.07 (0.00 - 0.19)	**0.77** (0.72 - 0.83)	*
Val135-Cγ1	1.84 (0.23)	0.71 (0.61)				
Cγ2	0.31 (0.35)	2.90 (0.34)	0.15 (0.09 - 0.40)	0.12 (0.00 - 0.12)	**0.73** (0.60 - 0.91)	182 (7)
Val165-Cγ1	1.60 (0.13)	0.77 (0.73)				
Cγ2	0.89 (0.30)	3.60 (0.08)	0.00 (0.00 - 0.06)	0.08 (0.00 - 0.17)	**0.92** (0.83 - 0.94)	178 (4)
Val169-Cγ1	1.80 (0.15)	1.00 (0.26)				
Cγ2[c]			0.16 (0.05 - 0.24)	0.00 (0.00 - 0.17)	**0.84** (0.73 - 0.95)	*
Val181-Cγ1	1.56 (0.23)	0.16 (0.18)				
Cγ2	0.78 (0.36)	3.44 (0.18)	0.00 (0.00 - 0.00)	0.15 (0.08 - 0.25)	**0.85** (0.75 - 0.92)	189 (2)

[a] Coupling values (in Hz) represent an average over all complexes with standard deviations in parentheses; complexes with errors larger than 1 Hz were excluded from the average. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. The populations for Val residues represents a simultaneous fit of Cγ1 and Cγ2 couplings when both sets are available. [b] Average χ_1 angles from the X-ray structures with standard deviations in parentheses; * indicates multiple rotamer conformations present in the X-ray structures. [c] ^{3}J measurement not available due to overly broad resonance or overlap. [d] The coupling constants indicate that the residue undergoes different rotamer averaging in one or more complexes of hDHFR.

Table S3. Ile, Thr, Val ^{3}J and rotamer populations for hDHFR:FOL:NADP^{+} [a]

Residue	$^{3}J_{C\gamma N}$	$^{3}J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Ile7	0.61 (0.33)	0.63 (1.10)	0.00 (0.00 - 0.19)	0.13 (0.02 - 0.97)	**0.87** (0.03 - 0.98)	187
Ile16	0.58 (0.65)	3.32 (0.83)	0.15 (0.00 - 0.41)	**0.85** (0.56 - 1.00)	0.00 (0.00 - 0.44)	66
Ile51	1.58 (0.12)	0.70 (0.64)	**0.62** (0.48 - 0.81)	0.04 (0.00 - 0.25)	0.34 (0.00 - 0.52)	-63
Ile60	1.71 (0.42)	1.12 (0.43)	**0.81** (0.32 - 1.00)	0.19 (0.00 - 0.31)	0.00 (0.00 - 0.67)	-61
Ile71	1.81 (0.26)	0.84 (0.84)	**0.88** (0.56 - 1.00)	0.11 (0.00 - 0.30)	0.00 (0.00 - 0.44)	-62
Ile114	2.03 (0.36)	0.30 (0.51)	**1.00** (0.69 - 1.00)	0.00 (0.00 - 0.09)	0.00 (0.00 - 0.31)	-52
Ile138	1.55 (0.43)	0.53 (0.46)	**0.57** (0.16 - 0.98)	0.00 (0.00 - 0.09)	0.43 (0.00 - 0.84)	-57
Ile151	1.85 (0.35)	0.98 (0.16)	**0.85** (0.56 - 0.95)	0.15 (0.05 - 0.18)	0.00 (0.00 - 0.37)	-59
Ile175	1.72 (0.23)	0.53 (0.46)	**0.73** (0.51 - 0.94)	0.00 (0.00 - 0.13)	0.27 (0.00 - 0.49)	-61
Thr38	0.78 (1.07)	3.45 (0.15)	0.00 (0.00 - 0.02)	**1.00** (0.97 - 1.00)	0.00 (0.00 - 0.03)	60
Thr39	0.90 (0.17)	1.48 (0.13)	0.35 (0.23 - 0.48)	0.30 (0.20 - 0.40)	0.34 (0.16 - 0.53)	-55
Thr40	1.42 (0.25)	0.66 (0.58)	**0.79** (0.55 - 1.00)	0.12 (0.00 - 0.28)	0.09 (0.00 - 0.35)	-59
Thr56	1.44 (0.16)	0.91 (0.95)	**0.77** (0.54 - 0.94)	0.23 (0.00 - 0.46)	0.00 (0.00 - 0.27)	-67
Thr100	0.92 (0.23)	2.18 (0.08)	0.27 (0.12 - 0.40)	**0.59** (0.49 - 0.66)	0.14 (0.00 - 0.37)	64
Thr136	1.68 (0.23)	0.36 (0.63)	**1.00** (0.77 - 1.00)	0.00 (0.00 - 0.23)	0.00 (0.00 - 0.14)	-60
Thr146	1.08 (0.12)	0.66 (0.23)	**0.58** (0.47 - 0.67)	0.02 (0.00 - 0.15)	0.40 (0.23 - 0.50)	-52
Val1-Cγ1	0.44 (0.57)	1.87 (0.24)				
Cγ2	0.63 (0.75)	1.58 (0.41)	0.39 (0.30 - 0.48)	0.32 (0.11 - 0.52)	0.29 (0.18 - 0.41)	167
Val8-Cγ1	0.66 (0.59)	1.65 (0.13)				
Cγ2	1.30 (0.16)	1.08 (0.46)	0.31 (0.26 - 0.36)	**0.49** (0.36 - 0.58)	0.21 (0.16 - 0.30)	68
Val10-Cγ1	0.84 (0.17)	2.42 (0.09)				64,
Cγ2	1.11 (0.29)	0.80 (0.22)	**0.55** (0.51 - 0.58)	0.36 (0.30 - 0.43)	0.09 (0.06 - 0.13)	182
Val43-Cγ1	1.37 (0.05)	1.31 (0.04)				
Cγ2	0.63 (0.18)	2.74 (0.10)	0.24 (0.22 - 0.26)	0.12 (0.08 - 0.16)	**0.64** (0.61 - 0.66)	180
Val50-Cγ1	1.06 (0.10)	2.42 (0.01)				
Cγ2	1.13 (0.04)	1.93 (0.02)	**0.57** (0.56 - 0.57)	0.07 (0.06 - 0.08)	0.36 (0.35 - 0.37)	-68
Val74-Cγ1	1.71 (0.06)	0.65 (0.49)				
Cγ2	0.40 (0.40)	3.30 (0.12)	0.00 (0.00 - 0.19)	0.16 (0.00 - 0.20)	**0.84** (0.80 - 0.87)	178
Val109-Cγ1	1.32 (0.21)	1.06 (0.18)				
Cγ2	0.48 (0.43)	2.94 (0.09)	0.17 (0.11 - 0.23)	0.11 (0.03 - 0.20)	**0.72** (0.70 - 0.74)	170
Val112-Cγ1	1.82 (0.22)	1.05 (1.08)				
Cγ2	0.30 (0.28)	3.41 (0.03)	0.14 (0.13 - 0.15)	0.00 (0.00 - 0.00)	**0.86** (0.85 - 0.87)	177
Val115-Cγ1	0.83 (0.72)	3.30 (0.08)				
Cγ2	0.23 (0.40)	0.67 (0.82)	**0.83** (0.81 - 0.87)	0.12 (0.00 - 0.12)	0.05 (0.05 - 0.19)	-54
Val120-Cγ1	1.92 (0.25)	1.24 (0.21)				
Cγ2	0.85 (0.65)	3.22 (0.30)	0.20 (0.12 - 0.20)	0.00 (0.00 - 0.12)	**0.80** (0.72 - 0.88)	72
Val135-Cγ1	1.68 (0.22)	0.91 (0.24)				
Cγ2	0.68 (0.43)	3.27 (0.11)	0.13 (0.04 - 0.15)	0.05 (0.00 - 0.16)	**0.82** (0.80 - 0.85)	178
Val165-Cγ1	1.74 (0.20)	1.26 (0.25)				
Cγ2	0.55 (0.48)	3.56 (0.10)	0.09 (0.06 - 0.13)	0.00 (0.00 - 0.00)	**0.91** (0.87 - 0.94)	180
Val169-Cγ1	1.75 (0.16)	1.11 (0.17)				
Cγ2 [c]			0.19 (0.11 - 0.24)	0.00 (0.00 - 0.16)	**0.81** (0.70 - 0.89)	177
Val181-Cγ1	1.71 (0.09)	0.31 (0.27)				
Cγ2	0.59 (0.65)	3.33 (0.16)	0.00 (0.00 - 0.16)	0.17 (0.00 - 0.21)	**0.83** (0.79 - 0.89)	187

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^{3}J_{C\gamma N}$ and $^{3}J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^{3}J \pm \sigma$. The populations for Val residues represents a simultaneous fit of Cγ1 and Cγ2 couplings when both sets are available. [b] χ_1 angles from the X-ray structure (4M6K).[1] [c] ^{3}J measurement not available due to overly broad resonance or overlap.

Table S4. Ile, Thr, Val ^{3}J and rotamer populations for hDHFR:THF:NADP$^+$ [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Ile7	1.59 (0.72)	1.52 (0.01)	**0.68** (0.07 - 0.69)	0.32 (0.25 - 0.32)	0.00 (0.00 - 0.68)	184
Ile16	0.82 (1.42)	3.62 (0.49)	0.06 (0.00 - 0.22)	**0.94** (0.77 - 1.00)	0.00 (0.00 - 0.23)	62
Ile51	1.65 (0.36)	0.38 (0.54)	**0.67** (0.32 - 1.00)	0.00 (0.00 - 0.08)	0.33 (0.00 - 0.68)	-64
Ile60	1.61 (0.83)	0.97 (1.37)	**0.71** (0.00 - 1.00)	0.14 (0.00 - 0.53)	0.15 (0.00 - 0.75)	-58
Ile71[d]	1.94 (0.26)	0	**0.94** (0.70 - 1.00)	0.00 (0.00 - 0.00)	0.06 (0.00 - 0.30)	-56
Ile114[d]	2.02 (0.42)	0	**1.00** (0.62 - 1.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.38)	-53
Ile138	2.10 (0.12)	1.19 (0.48)	**1.00** (0.92 - 1.00)	0.00 (0.00 - 0.08)	0.00 (0.00 - 0.00)	-56
Ile151	1.86 (0.33)	1.44 (0.02)	**0.71** (0.70 - 0.72)	0.29 (0.28 - 0.30)	0.00 (0.00 - 0.00)	-59
Ile175	1.79 (0.24)	0.50 (0.18)	**0.80** (0.58 - 1.00)	0.00 (0.00 - 0.03)	0.20 (0.00 - 0.42)	-62
Thr38	0.52 (0.68)	2.99 (0.23)	0.00 (0.00 - 0.19)	**0.83** (0.73 - 0.95)	0.17 (0.00 - 0.27)	56
Thr39	1.12 (0.27)	1.54 (0.02)	0.49 (0.32 - 0.58)	0.39 (0.31 - 0.44)	0.12 (0.00 - 0.37)	-47
Thr40	1.65 (0.16)	0.16 (0.23)	**1.00** (0.87 - 1.00)	0.00 (0.00 - 0.04)	0.00 (0.00 - 0.12)	-58
Thr56	1.59 (0.63)	0.31 (0.43)	**0.94** (0.49 - 1.00)	0.03 (0.00 - 0.03)	0.03 (0.00 - 0.50)	-65
Thr100	0.95 (0.22)	2.07 (0.37)	0.31 (0.12 - 0.50)	**0.55** (0.34 - 0.70)	0.14 (0.00 - 0.44)	66
Thr136	1.58 (0.39)	1.01 (0.04)	**0.74** (0.59 - 0.75)	0.26 (0.18 - 0.27)	0.00 (0.00 - 0.21)	-58
Thr146	1.19 (0.28)	0.61 (0.08)	**0.65** (0.48 - 0.84)	0.04 (0.00 - 0.15)	0.31 (0.03 - 0.52)	55, -59
Val1-Cγ1	0.68 (0.57)	1.98 (0.03)				
Cγ2	1.04 (0.66)	1.55 (0.18)	0.42 (0.41 - 0.44)	0.29 (0.23 - 0.37)	0.28 (0.23 - 0.33)	191
Val8-Cγ1[d]	0	1.17 (0.02)				
Cγ2	2.17 (0.48)	1.14 (0.36)	0.13 (0.12 - 0.15)	**0.87** (0.66 - 0.88)	0.00 (0.00 - 0.19)	69
Val10-Cγ1	1.22 (0.43)	2.44 (0.05)				-60,
Cγ2	1.24 (0.20)	1.00 (0.36)	**0.55** (0.53 - 0.58)	0.33 (0.21 - 0.44)	0.12 (0.04 - 0.21)	62
Val43-Cγ1	1.45 (0.06)	1.41 (0.02)				
Cγ2	0.89 (0.22)	2.72 (0.03)	0.28 (0.27 - 0.28)	0.08 (0.07 - 0.10)	**0.64** (0.63 - 0.65)	180
Val50-Cγ1	1.03 (0.16)	2.36 (0.14)				-72,
Cγ2	1.01 (0.16)	1.86 (0.02)	**0.55** (0.50 - 0.60)	0.09 (0.04 - 0.14)	0.36 (0.36 - 0.36)	177
Val74-Cγ1	1.82 (0.17)	0.51 (0.72)				
Cγ2	0.40 (0.38)	3.24 (0.19)	0.11 (0.00 - 0.13)	0.07 (0.00 - 0.23)	**0.82** (0.77 - 0.91)	177
Val109-Cγ1	1.40 (0.30)	1.04 (0.18)				
Cγ2	0.41 (0.37)	3.00 (0.04)	0.17 (0.11 - 0.22)	0.10 (0.03 - 0.16)	**0.74** (0.73 - 0.74)	176
Val112-Cγ1	1.63 (0.16)	0.89 (0.32)				
Cγ2	0.88 (1.53)	3.70 (0.07)	0.05 (0.00 - 0.07)	0.00 (0.00 - 0.07)	**0.95** (0.93 - 0.98)	179
Val115-Cγ1	1.24 (0.47)	3.40 (0.03)				
Cγ2	0.48 (0.83)	0.86 (1.22)	**0.87** (0.85 - 0.88)	0.00 (0.00 - 0.15)	0.13 (0.00 - 0.13)	-59
Val120-Cγ1	1.84 (0.28)	0.85 (0.38)				
Cγ2[c]			0.12 (0.00 - 0.22)	0.00 (0.00 - 0.28)	**0.88** (0.69 - 1.00)	186
Val135-Cγ1	1.82 (0.29)	0.10 (0.15)				
Cγ2	0.24 (0.41)	3.10 (0.35)	0.00 (0.00 - 0.03)	0.19 (0.00 - 0.32)	**0.81** (0.68 - 0.97)	179
Val165-Cγ1	1.58 (0.29)	1.51 (0.10)				
Cγ2	1.11 (0.53)	3.50 (0.06)	0.13 (0.11 - 0.15)	0.00 (0.00 - 0.00)	**0.87** (0.85 - 0.89)	181
Val169-Cγ1	1.62 (0.14)	0.69 (0.17)				
Cγ2[c]			0.05 (0.00 - 0.12)	0.21 (0.06 - 0.34)	**0.74** (0.64 - 0.84)	179
Val181-Cγ1	1.68 (0.28)	0.33 (0.47)				
Cγ2	0.83 (0.54)	3.26 (0.25)	0.00 (0.00 - 0.10)	0.18 (0.00 - 0.29)	**0.82** (0.71 - 0.90)	189

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. The populations for Val residues represents a simultaneous fit of Cγ1 and Cγ2 couplings when both sets are available. [b] χ_1 angles from the X-ray structure (4M6L).[1] [c] ^{3}J measurement not available due to overly broad resonance or overlap. [d] 0 valued coupling indicates measurement at limit of detection due to low signal-to-noise.

Table S5. Ile, Thr, Val ^{3}J and rotamer populations for hDHFR:NADPH [a]

Residue	$^{3}J_{C\gamma N}$		$^{3}J_{C\gamma CO}$		p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Ile7	1.63	(1.52)	1.57	(1.36)	0.69 (0.00 - 0.94)	0.31 (0.00 - 0.70)	0.00 (0.00 - 1.00)	187
Ile16	1.20	(0.12)	4.00	(0.20)	0.00 (0.00 - 0.06)	**1.00** (0.94 - 1.00)	0.00 (0.00 - 0.00)	61
Ile51	2.05	(0.57)	1.25	(0.77)	**0.91** (0.50 - 1.00)	0.09 (0.00 - 0.44)	0.00 (0.00 - 0.50)	-64
Ile60	2.22	(1.32)	1.72	(0.64)	**0.67** (0.01 - 1.00)	0.33 (0.00 - 0.55)	0.00 (0.00 - 0.90)	-56
Ile71	1.98	(1.12)	0.44	(0.44)	**0.98** (0.00 - 1.00)	0.00 (0.00 - 0.03)	0.02 (0.00 - 0.99)	-61, -56
Ile114	1.43	(0.33)	1.28	(0.23)	**0.59** (0.21 - 0.83)	0.23 (0.11 - 0.29)	0.18 (0.00 - 0.67)	-59
Ile138	1.65	(0.66)	1.35	(1.24)	**0.76** (0.03 - 1.00)	0.24 (0.00 - 0.63)	0.00 (0.00 - 0.97)	-64, -48
Ile151	2.34	(0.24)	1.93	(0.09)	**0.60** (0.56 - 0.64)	0.40 (0.36 - 0.44)	0.00 (0.00 - 0.00)	-62
Ile175	1.98	(0.11)	1.42	(0.51)	**0.96** (0.87 - 1.00)	0.04 (0.00 - 0.13)	0.00 (0.00 - 0.00)	-61
Thr38[c]								60
Thr39	1.14	(0.23)	1.36	(0.56)	**0.52** (0.30 - 0.74)	0.32 (0.04 - 0.50)	0.15 (0.00 - 0.51)	-55
Thr40	1.11	(1.15)	0.61	(1.06)	**0.60** (0.00 - 1.00)	0.02 (0.00 - 0.34)	0.38 (0.00 - 0.77)	-55
Thr56[c]								-66
Thr100	1.25	(0.40)	2.40	(0.48)	0.31 (0.14 - 0.50)	**0.69** (0.46 - 0.85)	0.00 (0.00 - 0.27)	69
Thr136[c]								-60
Thr146	1.65	(0.33)	1.38	(1.25)	**0.86** (0.33 - 1.00)	0.14 (0.00 - 0.67)	0.00 (0.00 - 0.24)	-55, 192
Val1-Cγ1	1.57	(0.26)	1.95	(0.08)				
Cγ2	0.43	(0.44)	2.26	(0.20)	0.44 (0.40 - 0.46)	0.07 (0.00 - 0.16)	0.50 (0.44 - 0.55)	#N/A
Val8-Cγ1	0.61	(0.59)	1.37	(1.19)				
Cγ2	2.18	(0.31)	1.80	(0.61)	0.00 (0.00 - 0.59)	**0.90** (0.20 - 0.95)	0.10 (0.05 - 0.21)	66
Val10-Cγ1	0.61	(0.45)	2.72	(0.30)				190,
Cγ2	0.80	(0.74)	1.59	(0.06)	**0.66** (0.56 - 0.72)	0.07 (0.00 - 0.17)	0.27 (0.26 - 0.28)	-64
Val43-Cγ1	1.62	(0.08)	1.20	(0.10)				
Cγ2	0.96	(0.24)	2.81	(0.07)	0.21 (0.18 - 0.24)	0.11 (0.06 - 0.16)	**0.68** (0.66 - 0.70)	179
Val50-Cγ1	0.99	(1.16)	3.17	(0.05)				
Cγ2	0.85	(1.47)	1.66	(0.73)	**0.80** (0.76 - 0.82)	0.00 (0.00 - 0.24)	0.20 (0.00 - 0.20)	-65
Val74-Cγ1	1.94	(0.04)	0.67	(0.61)				
Cγ2[d]	0		2.97	(0.30)	0.23 (0.10 - 0.23)	0.00 (0.00 - 0.00)	**0.77** (0.77 - 0.90)	180
Val109-Cγ1	1.23	(0.53)	0.42	(0.38)				
Cγ2	0.49	(0.84)	2.99	(0.68)	0.00 (0.00 - 0.09)	0.28 (0.00 - 0.49)	**0.72** (0.51 - 0.94)	171
Val112-Cγ1	1.28	(1.14)	0.36	(0.63)				
Cγ2	0.51	(0.89)	3.11	(0.19)	0.00 (0.00 - 0.23)	0.21 (0.00 - 0.27)	**0.79** (0.73 - 0.83)	176
Val115-Cγ1	1.21	(0.48)	3.67	(0.26)				
Cγ2	0.45	(0.78)	0.55	(0.96)	**0.95** (0.85 - 1.00)	0.00 (0.00 - 0.15)	0.05 (0.00 - 0.05)	-55
Val120-Cγ1	2.15	(0.41)	0.18	(0.20)				
Cγ2	0.41	(0.71)	2.95	(0.15)	0.00 (0.00 - 0.18)	0.25 (0.00 - 0.25)	**0.75** (0.68 - 0.92)	183
Val135-Cγ1	1.69	(0.59)	0.34	(0.49)				
Cγ2	1.09	(1.10)	2.60	(0.57)	0.00 (0.00 - 0.10)	0.32 (0.00 - 0.54)	**0.68** (0.46 - 1.00)	180
Val165-Cγ1	1.49	(0.29)	0.29	(0.51)				
Cγ2	0.73	(0.73)	3.67	(0.34)	0.00 (0.00 - 0.00)	0.11 (0.00 - 0.18)	**0.89** (0.82 - 1.00)	181
Val169-Cγ1	1.85	(0.23)	0.91	(0.53)				
Cγ2[c]					0.12 (0.00 - 0.29)	0.00 (0.00 - 0.25)	**0.88** (0.71 - 1.00)	176
Val181-Cγ1[d]	1.30	(0.72)	0					
Cγ2	0.43	(0.74)	3.49	(0.47)	0.00 (0.00 - 0.00)	0.17 (0.00 - 0.25)	**0.83** (0.75 - 1.00)	189

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^{3}J_{C\gamma N}$ and $^{3}J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^{3}J \pm \sigma$. The populations for Val residues represents a simultaneous fit of Cγ1 and Cγ2 couplings when both sets are available. [b] χ_1 angles from the X-ray structure (4M6J). [c] ^{3}J measurement not available due to overly broad resonance or overlap. [d] 0 valued coupling indicates measurement at limit of detection due to low signal-to-noise.

Table S6. Ile, Thr, Val ^{3}J and rotamer populations for hDHFR:THF [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Ile7	0.90 (1.38)	1.10 (1.09)	0.01 (0.00 - 1.00)	0.10 (0.00 - 0.46)	**0.88** (0.00 - 1.00)	198
Ile16	0	3.71 (0.71)	0.00 (0.00 - 0.21)	**0.96** (0.73 - 1.00)	0.04 (0.00 - 0.27)	51
Ile51	1.71 (0.70)	1.76 (1.24)	**0.70** (0.06 - 1.00)	0.30 (0.00 - 0.74)	0.00 (0.00 - 0.94)	-75
Ile60	1.44 (1.63)	2.33 (1.41)	0.46 (0.00 - 1.00)	**0.54** (0.00 - 0.97)	0.00 (0.00 - 0.95)	-64
Ile71	2.49 (0.54)	1.35 (0.94)	**1.00** (0.64 - 1.00)	0.00 (0.00 - 0.36)	0.00 (0.00 - 0.06)	-45
Ile114	1.85 (0.63)	2.48 (1.11)	**0.55** (0.11 - 1.00)	0.45 (0.00 - 0.89)	0.00 (0.00 - 0.37)	-78
Ile138	1.27 (1.12)	0	0.30 (0.00 - 1.00)	0.00 (0.00 - 0.06)	**0.70** (0.00 - 0.96)	-77
Ile151	2.22 (0.26)	0	**1.00** (0.95 - 1.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.05)	-62
Ile175	2.39 (0.36)	1.67 (0.57)	**0.97** (0.66 - 1.00)	0.03 (0.00 - 0.34)	0.00 (0.00 - 0.00)	-65
Thr38[d]	0	3.32 (0.47)	0.00 (0.00 - 0.00)	**0.98** (0.77 - 1.00)	0.02 (0.00 - 0.23)	70
Thr39	0.53 (0.52)	1.69 (0.28)	0.10 (0.00 - 0.46)	0.28 (0.11 - 0.54)	**0.63** (0.07 - 0.89)	-26
Thr40	1.27 (1.25)	1.21 (1.47)	**0.62** (0.00 - 1.00)	0.30 (0.00 - 0.69)	0.08 (0.00 - 0.81)	-56
Thr56[c]						-56
Thr100	1.37 (1.19)	3.09 (1.18)	0.09 (0.00 - 0.65)	**0.91** (0.34 - 1.00)	0.00 (0.00 - 0.66)	58
Thr136	1.63 (1.42)	1.34 (1.53)	**0.68** (0.00 - 1.00)	0.32 (0.00 - 0.78)	0.00 (0.00 - 1.00)	-53
Thr146	1.25 (0.22)	1.61 (0.90)	**0.56** (0.28 - 0.82)	0.44 (0.03 - 0.72)	0.00 (0.00 - 0.42)	-70
Val1-Cγ1	1.18 (0.22)	2.04 (0.11)				
Cγ2	0.50 (0.67)	2.35 (0.02)	0.47 (0.43 - 0.48)	0.02 (0.00 - 0.06)	**0.51** (0.51 - 0.52)	#N/A
Val8-Cγ1	1.40 (0.80)	1.48 (1.28)				
Cγ2	1.22 (1.08)	0.80 (0.80)	0.28 (0.00 - 0.54)	**0.51** (0.00 - 0.67)	0.21 (0.12 - 0.50)	69
Val10-Cγ1	0	2.85 (0.27)				
Cγ2	0.91 (0.81)	0	**0.70** (0.54 - 0.76)	0.10 (0.10 - 0.46)	0.20 (0.00 - 0.20)	-60
Val43-Cγ1	1.58 (0.38)	1.18 (0.49)				
Cγ2	0.57 (0.53)	2.79 (0.16)	0.21 (0.03 - 0.29)	0.12 (0.00 - 0.33)	**0.67** (0.64 - 0.71)	174
Val50-Cγ1	1.12 (0.93)	2.35 (0.23)				
Cγ2	0.88 (0.24)	2.12 (0.27)	**0.56** (0.47 - 0.61)	0.01 (0.00 - 0.17)	0.43 (0.36 - 0.45)	-65
Val74-Cγ1[d]	1.49 (1.36)	0				
Cγ2	0	3.09 (0.43)	0.00 (0.00 - 0.00)	0.22 (0.00 - 0.34)	**0.78** (0.66 - 1.00)	188
Val109-Cγ1	1.93 (0.20)	1.22 (0.67)				
Cγ2	0.15 (0.73)	3.28 (0.16)	0.18 (0.04 - 0.20)	0.00 (0.00 - 0.17)	**0.82** (0.79 - 0.87)	190
Val112-Cγ1	1.97 (0.99)	0.41 (0.41)				
Cγ2	0.44 (0.76)	3.59 (0.16)	0.00 (0.00 - 0.05)	0.07 (0.00 - 0.12)	**0.93** (0.88 - 0.97)	174
Val115-Cγ1	0.87 (1.51)	2.71 (0.37)				
Cγ2	0	0.81 (1.14)	**0.64** (0.52 - 0.77)	0.32 (0.00 - 0.38)	0.04 (0.04 - 0.30)	-43
Val120-Cγ1	1.66 (0.63)	1.39 (0.35)				
Cγ2[c]			0.28 (0.11 - 0.35)	0.00 (0.00 - 0.53)	**0.72** (0.31 - 0.89)	188
Val135-Cγ1	2.17 (0.67)	1.47 (0.70)				
Cγ2	0	2.62 (0.68)	0.30 (0.06 - 0.30)	0.08 (0.00 - 0.50)	**0.61** (0.44 - 0.82)	193
Val165-Cγ1	1.59 (1.38)	0				
Cγ2	1.15 (0.90)	3.66 (0.41)	0.00 (0.00 - 0.00)	0.09 (0.00 - 0.18)	**0.91** (0.82 - 1.00)	172
Val169-Cγ1	1.97 (0.19)	1.29 (0.32)				
Cγ2[c]			0.14 (0.00 - 0.28)	0.00 (0.00 - 0.01)	**0.86** (0.72 - 1.00)	5
Val181-Cγ1	1.43 (1.33)	0				
Cγ2	1.25 (0.60)	3.66 (0.37)	0.00 (0.00 - 0.00)	0.10 (0.00 - 0.17)	**0.90** (0.83 - 1.00)	193

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. The populations for Val residues represents a simultaneous fit of Cγ1 and Cγ2 couplings when both sets are available. [b] χ_1 angles from the X-ray structure (1DHF).[2] [c] ^{3}J measurement not available due to overly broad resonance or overlap. [d] 0 valued coupling indicates measurement at limit of detection due to low signal-to-noise.

Table S7. Average aromatic ^3J and rotamer populations for hDHFR complexes [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Trp24[c]						47 (5)
Phe31[e]						*
Tyr33	2.68 (0.04)	0.58 (0.82)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	**1.00** (1.00 - 1.00)	172 (8)
Phe34	2.76 (0.51)	0.33 (0.48)	0.00 (0.00 - 0.07)	0.00 (0.00 - 0.16)	**1.00** (0.84 - 1.00)	168 (3)
Trp57[e]						170 (2)
Phe58[e]						-74 (2)
His87	0.45 (0.10)	5.46 (0.47)	**1.00** (1.00 - 1.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	-63 (5)
Phe88	0.65 (0.39)	3.92 (0.30)	**0.87** (0.79 - 0.95)	0.01 (0.00 - 0.21)	0.12 (0.00 - 0.21)	-67 (6)
Trp113	0.29 (0.08)	4.08 (0.30)	**0.91** (0.83 - 0.99)	0.09 (0.01 - 0.17)	0.00 (0.00 - 0.00)	-72 (1)
Tyr121	0.43 (0.36)	3.51 (1.14)	**0.76** (0.47 - 1.00)	0.21 (0.00 - 0.53)	0.02 (0.00 - 0.19)	-76 (3)
His127[d,e]						192 (13)
His130	2.63 (0.21)	0.78 (1.10)	0.00 (0.00 - 0.14)	0.05 (0.00 - 0.14)	**0.95** (0.86 - 1.00)	187 (5)
Phe134	0.58 (0.37)	4.14 (0.21)	**0.92** (0.87 - 0.98)	0.00 (0.00 - 0.13)	0.08 (0.00 - 0.13)	-70 (1)
Phe142	0.42 (0.32)	4.22 (0.06)	**0.95** (0.93 - 0.96)	0.03 (0.00 - 0.07)	0.02 (0.00 - 0.07)	-76 (4)
Phe147	0.54 (0.21)	4.80 (0.61)	**1.00** (0.93 - 1.00)	0.00 (0.00 - 0.06)	0.00 (0.00 - 0.07)	-58 (9)
Phe148[d]	2.70 (0.23)	n/a Pro			**1.00** (0.93 - 1.00)	191 (5)
Tyr156[e]						-76 (5)
Tyr162[d]	2.12 (0.09)	n/a Pro			**0.78** (0.74 - 0.82)	194 (7)
Tyr177	0.64 (0.14)	0.86 (0.88)	0.08 (0.00 - 0.31)	**0.81** (0.51 - 0.95)	0.12 (0.05 - 0.18)	62 (1)
Phe179	0.78 (0.34)	4.18 (0.41)	**0.93** (0.82 - 1.00)	0.00 (0.00 - 0.14)	0.07 (0.00 - 0.18)	-64 (7)
Tyr182	0.60 (0.28)	3.81 (0.26)	**0.84** (0.77 - 0.91)	0.06 (0.00 - 0.23)	0.10 (0.00 - 0.22)	-64 (2)

[a] Coupling values (in Hz) represent an average over all complexes with standard deviations in parentheses; complexes are excluded from average if error ranges are non overlapping or errors are larger than 1 Hz. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. Rotamer averaging for aromatics is interpreted as reporting on in-well rotamer averaging and/or a skewed χ_1 conformation. [b] Average χ_1 angles from the X-ray structures with standard deviations in parentheses; * indicates multiple rotamer conformations present in the X-ray structures. [c] ^3J measurement not available due to overly broad resonance or overlap. [d] $^3J_{C\gamma CO}$ coupling is not available since the *i*+1 residue is a proline. [e] Unique average χ_1 conformation in one or more complexes of hDHFR.

Table S8. Aromatic ^3J and rotamer populations for hDHFR:FOL:NADP$^+$ [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Trp24[c]						41
Phe31	0.54 (0.39)	4.09 (0.46)	**0.91** (0.79 - 1.00)	0.01 (0.00 - 0.21)	0.07 (0.00 - 0.21)	-89
Tyr33	2.66 (0.12)	1.16 (0.60)	0.00 (0.00 - 0.05)	0.00 (0.00 - 0.03)	**1.00** (0.95 - 1.00)	173
Phe34	2.46 (0.12)	1.01 (0.31)	0.07 (0.02 - 0.14)	0.00 (0.00 - 0.09)	**0.93** (0.86 - 0.98)	168
Trp57	2.79 (0.06)	0.97 (0.84)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	**1.00** (1.00 - 1.00)	170
Phe58	0.21 (0.33)	4.44 (0.44)	**1.00** (0.89 - 1.00)	0.00 (0.00 - 0.11)	0.00 (0.00 - 0.07)	-73
His87	0.42 (0.30)	4.99 (0.16)	**1.00** (0.96 - 1.00)	0.00 (0.00 - 0.04)	0.00 (0.00 - 0.04)	-59
Phe88	0.23 (0.27)	4.14 (0.20)	**0.92** (0.87 - 0.98)	0.08 (0.00 - 0.13)	0.00 (0.00 - 0.05)	-67
Trp113	0.21 (0.29)	4.08 (0.30)	**0.91** (0.83 - 0.99)	0.09 (0.00 - 0.17)	0.00 (0.00 - 0.06)	-71
Tyr121	0.09 (0.06)	3.12 (0.24)	**0.66** (0.60 - 0.72)	0.34 (0.28 - 0.40)	0.00 (0.00 - 0.00)	-76
His127[d]	2.80 (0.10)	n/a Pro			**1.00** (0.98 - 1.00)	189
His130	2.81 (0.08)	1.56 (0.85)	0.00 (0.00 - 0.01)	0.00 (0.00 - 0.00)	**1.00** (0.99 - 1.00)	184
Phe134	1.16 (1.22)	4.28 (0.12)	**0.96** (0.93 - 0.99)	0.00 (0.00 - 0.07)	0.04 (0.00 - 0.07)	-69
Phe142	0.09 (0.17)	4.18 (0.16)	**0.94** (0.90 - 0.98)	0.06 (0.02 - 0.10)	0.00 (0.00 - 0.00)	-79
Phe147	0.50 (0.34)	3.98 (1.49)	**0.88** (0.50 - 1.00)	0.06 (0.00 - 0.50)	0.06 (0.00 - 0.21)	-66
Phe148[d]	2.51 (0.15)	n/a Pro			**0.95** (0.89 - 1.00)	194
Tyr156	0.08 (0.15)	3.11 (0.46)	**0.66** (0.54 - 0.78)	0.34 (0.22 - 0.46)	0.00 (0.00 - 0.00)	-78
Tyr162[d]	2.08 (0.04)	n/a Pro			**0.76** (0.74 - 0.78)	184, 197
Tyr177	0.67 (0.26)	1.76 (0.52)	0.31 (0.18 - 0.45)	**0.56** (0.31 - 0.81)	0.13 (0.02 - 0.25)	61
Phe179	0.42 (0.41)	4.26 (0.27)	**0.96** (0.89 - 1.00)	0.02 (0.00 - 0.11)	0.02 (0.00 - 0.11)	-60
Tyr182	0.83 (0.30)	3.50 (0.56)	**0.76** (0.61 - 0.91)	0.04 (0.00 - 0.32)	0.20 (0.07 - 0.33)	-66

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. Rotamer averaging for aromatics is interpreted as reporting on in-well rotamer averaging and/or a skewed χ_1 conformation. [b] χ_1 angles from the X-ray structure (4M6K).[1] [c] ^3J measurement not available due to overly broad resonance or overlap. [d] $^3J_{C\gamma CO}$ coupling is not available since the *i*+1 residue is a proline.

Table S9. Aromatic ^3J and rotamer populations for hDHFR:THF:NADP$^+$ [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Trp24[c]						43
Phe31[e]	0	4.06 (0.35)	**0.90** (0.82 - 0.99)	0.10 (0.01 - 0.18)	0.00 (0.00 - 0.00)	-75
Tyr33	2.71 (0.06)	1.11 (1.28)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	**1.00** (1.00 - 1.00)	176
Phe34[e]	2.48 (0.10)	0	0.00 (0.00 - 0.00)	0.06 (0.02 - 0.10)	**0.94** (0.90 - 0.98)	170
Trp57	2.95 (0.13)	0.98 (1.69)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	**1.00** (1.00 - 1.00)	168
Phe58	0.17 (0.29)	4.28 (0.19)	**0.96** (0.91 - 1.00)	0.04 (0.00 - 0.09)	0.00 (0.00 - 0.03)	-71
His87	0.35 (0.31)	5.19 (0.32)	**1.00** (0.97 - 1.00)	0.00 (0.00 - 0.03)	0.00 (0.00 - 0.03)	-64
Phe88	0.43 (0.10)	3.71 (0.39)	**0.82** (0.71 - 0.92)	0.16 (0.02 - 0.29)	0.02 (0.00 - 0.07)	-63
Trp113	0.37 (0.45)	4.84 (1.33)	**1.00** (0.76 - 1.00)	0.00 (0.00 - 0.24)	0.00 (0.00 - 0.20)	-72
Tyr121	0.19 (0.32)	2.63 (0.14)	**0.54** (0.50 - 0.57)	0.46 (0.37 - 0.50)	0.00 (0.00 - 0.06)	-75
His127[d]	2.81 (0.03)	n/a Pro			**1.00** (1.00 - 1.00)	192
His130[e]	2.69 (0.09)	0	0.00 (0.00 - 0.00)	0.02 (0.00 - 0.06)	**0.98** (0.94 - 1.00)	186
Phe134	0.31 (0.27)	3.99 (0.21)	**0.89** (0.83 - 0.94)	0.11 (0.00 - 0.17)	0.00 (0.00 - 0.09)	-71
Phe142	0.24 (0.35)	4.27 (0.08)	**0.96** (0.94 - 0.98)	0.04 (0.00 - 0.06)	0.00 (0.00 - 0.06)	-79
Phe147	0.36 (0.61)	4.80 (0.61)	**1.00** (0.94 - 1.00)	0.00 (0.00 - 0.06)	0.00 (0.00 - 0.06)	-62
Phe148[d]	2.51 (0.02)	n/a Pro			**0.95** (0.94 - 0.96)	191
Tyr156	0.24 (0.42)	2.60 (0.38)	**0.53** (0.43 - 0.62)	0.47 (0.25 - 0.57)	0.00 (0.00 - 0.13)	-75
Tyr162[d]	2.08 (0.05)	n/a Pro			**0.76** (0.74 - 0.78)	195
Tyr177[e]	0.43 (0.19)	0	0.00 (0.00 - 0.00)	**0.98** (0.89 - 1.00)	0.02 (0.00 - 0.11)	63
Phe179	0.60 (0.34)	4.08 (0.23)	**0.91** (0.85 - 0.97)	0.00 (0.00 - 0.15)	0.09 (0.00 - 0.15)	-59
Tyr182	0.68 (0.17)	3.75 (0.59)	**0.83** (0.67 - 0.98)	0.04 (0.00 - 0.27)	0.14 (0.02 - 0.21)	-64

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. Rotamer averaging for aromatics is interpreted as reporting on in-well rotamer averaging and/or a skewed χ_1 conformation. [b] χ_1 angles from the X-ray structure.[1] [c] ^3J measurement not available due to overly broad resonance or overlap. [d] $^3J_{C\gamma CO}$ coupling is not available since the *i*+1 residue is a proline. [e] 0 valued coupling indicates measurement at limit of detection due to low signal-to-noise.

Table S10. Aromatic ^3J and rotamer populations for hDHFR:NADPH [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Trp24[c]						41
Phe31	2.64 (0.73)	1.20 (1.06)	0.03 (0.00 - 0.40)	0.00 (0.00 - 0.32)	**0.97** (0.60 - 1.00)	170
Tyr33	2.63 (0.47)	1.36 (2.35)	0.00 (0.00 - 0.36)	0.00 (0.00 - 0.20)	**1.00** (0.64 - 1.00)	178
Phe34[e]	3.35 (0.05)	0	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	**1.00** (1.00 - 1.00)	172
Trp57	2.07 (0.12)	1.51 (2.13)	0.24 (0.00 - 0.32)	0.00 (0.00 - 0.30)	**0.76** (0.68 - 0.81)	172
Phe58	0.76 (0.69)	3.33 (1.68)	**0.72** (0.28 - 1.00)	0.11 (0.00 - 0.72)	0.17 (0.00 - 0.48)	-75
His87	0.47 (0.81)	6.05 (0.55)	**1.00** (1.00 - 1.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	-60
Phe88	1.07 (0.35)	4.47 (1.09)	**0.96** (0.66 - 1.00)	0.00 (0.00 - 0.12)	0.04 (0.00 - 0.34)	-63
Trp113	0.36 (0.62)	5.20 (1.89)	**1.00** (0.71 - 1.00)	0.00 (0.00 - 0.29)	0.00 (0.00 - 0.27)	-71
Tyr121	0.86 (0.74)	5.40 (2.19)	**1.00** (0.59 - 1.00)	0.00 (0.00 - 0.32)	0.00 (0.00 - 0.41)	-72
His127[d]	3.00 (0.27)	n/a Pro			**1.00** (1.00 - 1.00)	177
His130[c]						194
Phe134	1.01 (0.88)	4.27 (1.10)	**0.95** (0.62 - 1.00)	0.00 (0.00 - 0.32)	0.05 (0.00 - 0.38)	-69
Phe142[c]	0.79 (0.70)				0.18 (0.00 - 0.50)	-72
Phe147	1.17 (1.35)	4.87 (1.45)	**1.00** (0.62 - 1.00)	0.00 (0.00 - 0.26)	0.00 (0.00 - 0.38)	-59
Phe148[d]	3.00 (0.21)	n/a Pro			**1.00** (1.00 - 1.00)	194
Tyr156	0.25 (0.44)	3.84 (0.07)	**0.85** (0.83 - 0.87)	0.15 (0.00 - 0.17)	0.00 (0.00 - 0.13)	-69
Tyr162[d]	2.25 (0.29)	n/a Pro			**0.84** (0.71 - 0.96)	196
Tyr177	0.70 (0.63)	1.05 (1.23)	0.13 (0.00 - 0.45)	**0.73** (0.13 - 1.00)	0.14 (0.00 - 0.42)	61, 63
Phe179	0.94 (0.35)	3.68 (0.80)	**0.80** (0.60 - 1.00)	0.00 (0.00 - 0.31)	0.20 (0.00 - 0.40)	-61
Tyr182	0.69 (0.60)	3.87 (0.75)	**0.86** (0.66 - 1.00)	0.01 (0.00 - 0.34)	0.14 (0.00 - 0.34)	-66

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. Rotamer averaging for aromatics is interpreted as reporting on in-well rotamer averaging and/or a skewed χ_1 conformation. [b] χ_1 angles from the X-ray structure.[1] [c] ^3J measurement not available due to overly broad resonance or overlap. [d] $^3J_{C\gamma CO}$ coupling is not available since the *i*+1 residue is a proline. [e] 0 valued coupling indicates measurement at limit of detection due to low signal-to-noise.

Table S11. Aromatic ^3J and rotamer populations for hDHFR:THF [a]

Residue	$^3J_{C\gamma N}$	$^3J_{C\gamma CO}$	p_{-60}	p_{+60}	p_{180}	χ_1 [b]
Trp24[c]						51
Phe31	0.30 (0.47)	4.13 (1.59)	**0.92** (0.51 - 1.00)	0.08 (0.00 - 0.49)	0.00 (0.00 - 0.17)	-70
Tyr33[c]	2.72 (0.28)				**1.00** (0.92 - 1.00)	160
Phe34	2.47 (1.69)	0.31 (0.70)	0.00 (0.00 - 0.12)	0.07 (0.00 - 0.82)	**0.93** (0.18 - 1.00)	164
Trp57	2.89 (0.51)	1.82 (2.55)	0.00 (0.00 - 0.36)	0.00 (0.00 - 0.11)	**1.00** (0.64 - 1.00)	170
Phe58	0.62 (0.84)	4.68 (0.54)	**1.00** (0.92 - 1.00)	0.00 (0.00 - 0.07)	0.00 (0.00 - 0.08)	-76
His87	0.58 (0.90)	5.61 (0.07)	**1.00** (0.99 - 1.00)	0.00 (0.00 - 0.01)	0.00 (0.00 - 0.00)	-70
Phe88	0.87 (0.73)	3.72 (1.18)	**0.81** (0.50 - 1.00)	0.00 (0.00 - 0.49)	0.19 (0.00 - 0.50)	-75
Trp113	0.24 (0.35)	4.24 (1.77)	**0.95** (0.49 - 1.00)	0.05 (0.00 - 0.51)	0.00 (0.00 - 0.09)	-72
Tyr121	0.60 (0.85)	4.80 (0.64)	**1.00** (0.92 - 1.00)	0.00 (0.00 - 0.07)	0.00 (0.00 - 0.08)	-80
His127[d]	2.29 (0.16)	n/a Pro			**0.80** (0.73 - 0.87)	209
His130	2.40 (0.42)	1.57 (1.56)	0.12 (0.00 - 0.40)	0.02 (0.00 - 0.34)	**0.85** (0.60 - 1.00)	184
Phe134	0.42 (0.58)	5.33 (1.42)	**1.00** (0.85 - 1.00)	0.00 (0.00 - 0.13)	0.00 (0.00 - 0.15)	-70
Phe142[e]	0.57 (0.52)	>2.6 (1.83)	**>0.52** (0.05 - 0.99)	0.40 (0.00 - 0.95)	0.08 (0.00 - 0.32)	-72
Phe147	0.77 (0.75)	4.28 (2.67)	**0.95** (0.28 - 1.00)	0.00 (0.00 - 0.72)	0.05 (0.00 - 0.51)	-46
Phe148[d]	2.76 (0.37)	n/a Pro			**1.00** (0.90 - 1.00)	183
Tyr156	0.40 (0.59)	3.75 (1.11)	**0.82** (0.54 - 1.00)	0.17 (0.00 - 0.46)	0.01 (0.00 - 0.27)	-80
Tyr162[d]	2.07 (0.15)	n/a Pro			**0.76** (0.69 - 0.82)	202
Tyr177	0.75 (0.70)	0.82 (0.81)	0.07 (0.00 - 0.28)	**0.77** (0.24 - 1.00)	0.17 (0.00 - 0.48)	63
Phe179	1.18 (0.42)	4.68 (0.83)	**1.00** (0.78 - 1.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.22)	-75
Tyr182	0.18 (0.41)	4.13 (0.64)	**0.92** (0.76 - 1.00)	0.08 (0.00 - 0.24)	0.00 (0.00 - 0.10)	-63

[a] Coupling values (in Hz) represent an average over three or more measurements with standard deviations in parentheses. Rotamer populations were fit by minimizing the squared difference between measured and calculated $^3J_{C\gamma N}$ and $^3J_{C\gamma CO}$ couplings for each residue: $\Sigma(1/\sigma_{Jmeas})^2 (J_{calc} - J_{meas})^2$, where σ is the standard deviation; p_{major} is shown in bold. Ranges for populations given in parentheses were calculated using $^3J \pm \sigma$. Rotamer averaging for aromatics is interpreted as reporting on in-well rotamer averaging and/or a skewed χ_1 conformation. [b] χ_1 angles from the X-ray structure.[1] [c] ^3J measurement not available due to overly broad resonance or overlap. [d] $^3J_{C\gamma CO}$ coupling is not available since the *i*+1 residue is a proline. [e] $^3J_{C\gamma CO}$ coupling is a lower bound due to resonance overlap.

Table S12. Leucine chemical shifts (ppm) and calculated χ_2 rotamer populations [a]

	hE:FOL:NADP$^+$					hE:NADPH					hE:THF				
Res	$^{13}C\delta1$	$^{13}C\delta2$	$\delta1^{aro}$	$\delta2^{aro}$	p_{180}	$^{13}C\ \delta1$	$^{13}C\ \delta2$	$\delta1^{aro}$	$\delta2^{aro}$	p_{180}	$^{13}C\ \delta1$	$^{13}C\ \delta2$	$\delta1^{aro}$	$\delta2^{aro}$	p_{180}
4			-0.10	-0.14				-0.16	-0.52		24.79	24.92	0.01	-0.04	0.49
22			-0.02	0.07				-0.10	-0.12				-0.06	-0.84	
27	25.48	23.39	-0.52	-0.04	0.71	24.79	23.10	-0.53	-0.06	0.67	25.57	23.37	-0.37	-0.08	0.72
49			0.01	-0.04		25.54	26.82	0.05	0.04	0.37	25.17	26.98	-0.01	-0.03	0.32
67			0.06	-0.11				0.15	0.04				-0.03	-0.18	
73			-0.07	-0.08				-0.08	-0.09		23.80	25.39	-0.07	-0.02	0.34
75	26.48	23.88	-0.52	-0.34	0.76	26.28	23.80	-0.63	-0.43	0.75	25.45	24.88	-0.02	-0.03	0.56
79	25.37	22.34	0.01	0.06	0.80	25.40	22.32	0.06	0.1	0.81	25.52	22.38	-0.02	0.05	0.81
89	24.95	25.56	-0.05	-0.03	0.44	25.03	25.53	-0.06	-0.02	0.45	24.70	25.71	-0.10	-0.05	0.40
93			-0.01	0.08		23.49	25.24	-0.03	0.06	0.33			-0.06	-0.11	
97	25.02	22.19	-0.27	-0.10	0.78	23.96	22.11	-0.07	-0.09	0.69	25.14	21.79	-0.33	-0.11	0.84
99	24.03	24.18	-0.16	-0.25	0.49	23.94	24.26	-0.23	-0.76	0.47	23.90	24.27	-0.31	-0.49	0.46
105	24.85	23.74	-0.52	-0.13	0.61	24.64	23.77	-0.02	-0.1	0.59	24.72	23.75	0.08	-0.13	0.60
131			0.05	0.21				0.10	0.26				0.07	0.25	
133	24.87	24.43	-0.66	-0.05	0.54	24.74	24.12	-0.58	0.08	0.56	25.09	24.39	-0.50	0.07	0.57
153	25.61	22.21	0.06	0.08	0.84	25.47	22.21	0.08	0.16	0.83	25.69	22.5	0.11	0.15	0.82
158	22.23	26.03	-0.63	-0.11	0.12	22.08	25.94	-0.58	-0.11	0.11	22.15	26.06	-0.41	-0.18	0.11
159	25.83	22.46	0.12	0.07	0.84	25.81	22.64	0.08	0.05	0.82	25.82	22.79	0.14	0.06	0.80
166	25.54	22.73	0.07	0.15	0.78	25.13	23.21	0.03	0.03	0.69	25.82	22.78	0.09	0.08	0.80

[a] Not all leucines could be assigned. This approach for chemical shift based rotamer populations assumes there is no significant population for the leucine χ_2 -60° rotamer. The prevalence of just two χ_1,χ_2 rotamer pairs in leucines implies a similar level of rotamer averaging for the corresponding χ_1 rotamer. The most populated χ_1,χ_2 pairs are (-60°, 180°) and (180°, +60°). $\delta1^{aro}$ and $\delta2^{aro}$ are the aromatic contributions to chemical shift based on the corresponding X-ray structure using Shifts-4.3.[3] Populations were determined using $^{13}C\delta_{corr} = {}^{13}C\delta - \delta^{aro}$ as the corrected chemical shift (ppm).

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