SUPPORTING MATERIAL

Determining Valine Side-Chain Rotamer Conformations in Proteins from Methyl ^{13}C Chemical Shifts: Application to the 360 kDa Half-Proteasome

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Determination of force constants k_J and k_δ.

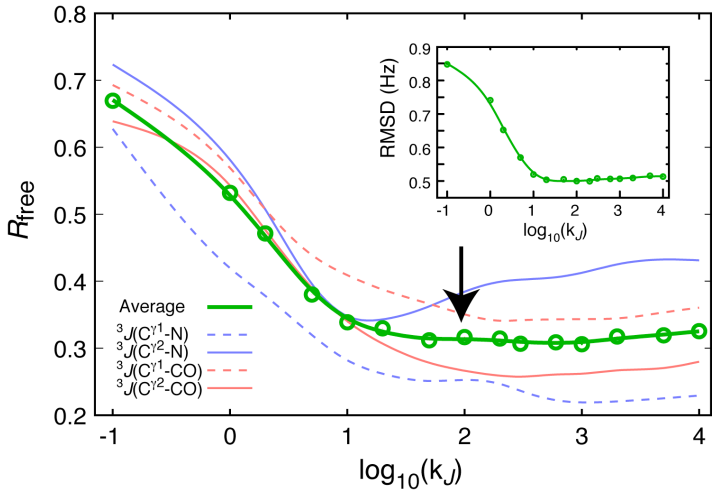

Figure S1: Determination of the force constant, k_J, used in Eq. 8 of Materials and Methods. Details are given in the text. Shown in green is the average of the four R_{free} profiles (Eq S1) obtained by removal of one of the four scalar couplings in the analysis. Blue dashed line, R_{free} of $^3J(C^{\gamma1},N)$; blue continuous line, R_{free} of $^3J(C^{\gamma2},N)$; red dashed line, R_{free} of $^3J(C^{\gamma1},CO)$; red continued line, R_{free} of $^3J(^{13}C^{\gamma2}, CO)$. The insert shows the average RMSD calculated by following the same procedure as for R_{free}. The black arrow indicates the value of $\log_{10}(k_J)$ that was chosen for all calculations.

The R_{free} values were calculated as[1,2]:

$$R_{free}(^3J_i) = \frac{1}{\sqrt{2}} \frac{\mathrm{RMSD}(^3J_{bc,i} - ^3J_{exp,i})}{\mathrm{RMSD}(^3J_{exp,i} - \langle ^3J_{exp,i} \rangle)} \qquad \text{(S1)}$$

where $J_{bc,i}$, i={($C^{\gamma1}$,CO), ($C^{\gamma2}$,CO), ($C^{\gamma1}$,N), ($C^{\gamma2}$,N)}, is the scalar coupling back-calculated from the ensemble derived from the three other couplings $\{J_{exp,j}\}_j$, $j \neq i$; $J_{exp,i}$ is an experimental observed scalar coupling, and RMSD the root-mean-square-deviation over all of the values from Val residues in the six proteins.

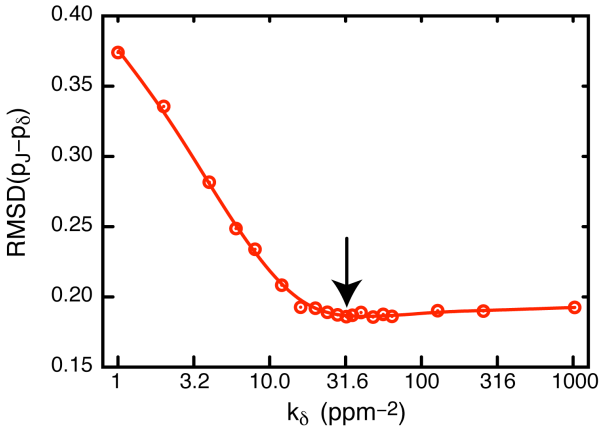

Figure S2. Root-mean-square-deviation (RMSD) between rotamer populations calculated from scalar couplings using the *J*-reference ensemble, p_J, and populations calculated from chemical shifts p_δ for different values of k_δ (see text for details). The RMSD($p_J - p_\delta$) is calculated as $\sqrt{1/3N \sum_{\text{Val}} \sum_{r \in \{g+,t,g-\}} (p_J(r) - p_\delta(r))^2}$, where N=22 is the number of Val residues included in the analysis. The black arrow shows the minimum (k_δ = 32 ppm^{-2}).

Density Functional Theory Calculations of $^{13}C^{\gamma 1}$ and $^{13}C^{\gamma 2}$ Chemical Shifts:

All density functional theory calculations described below were carried out with Gaussian 03 Rev. B.05 (ref[3]), following a procedure similar to that explained previously[4].

Generation of model complexes

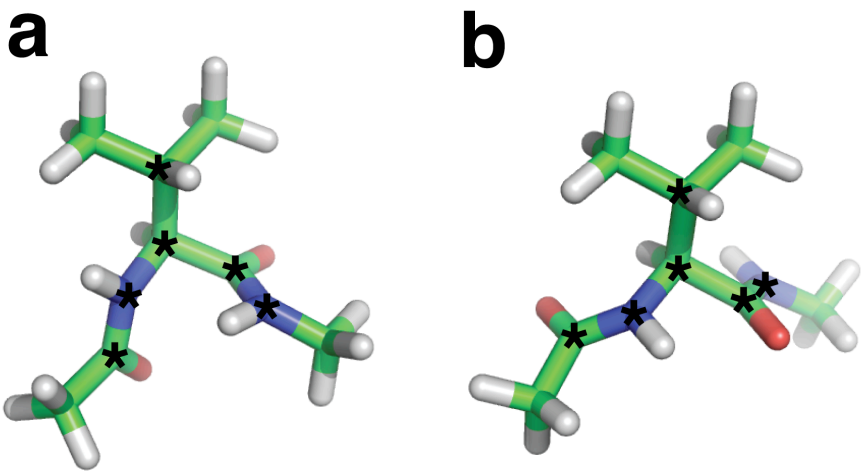

Figure S3. Structures of the model complexes used for calculating chemical shifts of the $^{13}C^{\gamma 1}$ and $^{13}C^{\gamma 2}$ nuclei of Val residues as a function of χ_1. The model complexes were generated with Molden 4.8 (http://www.cmbi.ru.nl/molden/), details are below.

Initial geometry optimizations

An initial geometry optimization of the model complex structure was performed using the three-parameter Becke-Lee-Yang-Parr (B3LYP) exchange-correlation-functional in the 6-31G*[5,6] basis set (resulting in 212 basis functions). Initially, χ_1=170° and the backbone was placed in an α-helix (model a: φ= −60°, ψ= −40°) or β-sheet extended conformation (model b: φ= −120°, ψ= +140°). The χ_1 dihedral angle and the position of all atoms marked with * in Figure S3 were frozen during the geometry optimization. The structures shown in Figure S3 were obtained after the initial optimizations, where the χ_1 angle is 170°.

Model complexes with different χ_1 dihedral angles, χ_1={10, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 110, 130, 150, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 230, 250, 270, 280, 285, 290, 295, 300, 305, 310, 315, 320, 330, 350} degrees were generated from the initially optimized model using an in-house written program and structures were subsequently optimized using a B3LYP functional in the 6-31G* basis set, fixing the χ_1 dihedral angle and the atoms marked with * in Figure S3.

Calculation of chemical shifts

Chemical shifts were calculated using the geometry optimized structures of each of the 39 model complexes using the EPR-III[7] basis on all atoms (resulting in 656 basis functions). The EPR-III basis is particularly advantageous for calculating chemical shifts since it has been optimized to enhance the description of electron density in the nuclear region. Chemical shifts were calculated using the gauge-independent atomic orbital (GIAO)[6] approach implemented in Gaussian 03 (see Figure 1b).

The chemical shieldings provided by the GIAO approach have to be referenced in order to obtain chemical shifts. Thus, the chemical shifts $\delta(^{13}C^{\gamma 1})$ and $\delta(^{13}C^{\gamma 1})$ were calculated as $\delta(\chi_1) = -(\sigma(\chi_1)-\sigma_{ref})$, where σ is 1/3 the trace of the calculated chemical shielding tensor and σ_{ref} was determined so that the chemical shift corresponding to random coil, $\delta_{RC}= 0.06\delta_{63} + 0.74\delta_{174} + 0.20\delta_{296}$, agrees with the experimentally observed random coil chemical shift of 21.30 ppm and 20.80 ppm for $^{13}C^{\gamma 1}$ and $^{13}C^{\gamma 2}$ respectively, where δ_x is the chemical shift calculated for χ_1=x. The experimental random coil values were calculated as the average of the values obtained from the BMRB database(http://www.bmrb.wisc.edu) and those observed from small peptides[8]. Of note is the very good agreement between the theoretically derived difference for random coil chemical shifts ($\delta(^{13}C^{\gamma 1})-\delta(^{13}C^{\gamma 1})$), 0.57 ppm, and that obtained experimentally, 0.50 ppm, which further supports the theoretical calculations.

Scalar couplings and chemical shifts

Below are listed, in tabular form, the data used in this study. Residues with significant errors in the scalar couplings were excluded in order to ensure that the derived algorithm is not affected by these errors. Thus we only included residues for which,

$$\frac{\sigma_{J(\mathrm{N,C}\gamma 1)} + \sigma_{J(\mathrm{N,C}\gamma 2)}}{\mathrm{RMSD}(J(\mathrm{N,C}^{\gamma}))} + \frac{\sigma_{J(\mathrm{CO,C}\gamma 1)} + \sigma_{J(\mathrm{CO,C}\gamma 2)}}{\mathrm{RMSD}(J(\mathrm{CO,C}^{\gamma}))} < 1 \qquad \text{(S2)}$$

where RMSD(J(N,C$^{\gamma}$)) is the RMSD of all the experimental J(N,C$^{\gamma 1}$) and J(N,C$^{\gamma 2}$) couplings, and RMSD(J(CO,C$^{\gamma}$)) is the RMSD of all the experimental J(CO,C$^{\gamma 1}$) and J(CO,C$^{\gamma 2}$) values; σ_{Ji} is the experimental error of the ^3J_i scalar couplings.

Protein L[9]

Residue	^3J(CO,C$^{\gamma 1}$) (Hz)	^3J(CO,C$^{\gamma 2}$) (Hz)	^3J(N,C$^{\gamma 1}$) (Hz)	^3J(N,C$^{\gamma 2}$) (Hz)	$\delta(^{13}C^{\gamma 1})$ (ppm)	$\delta(^{13}C^{\gamma 2})$ (ppm)
V49	1.0±0.1	0.8±0.1	0.3±0.1	1.6±0.2	18.6	23.1
V51	0.5±0.1	3.2±0.1	1.8±0.1	0.1±0.1	21.5	21.1

Human ubiquitin[10]

Residue	^3J(CO,C$^{\gamma 1}$) (Hz)	^3J(CO,C$^{\gamma 2}$) (Hz)	^3J(N,C$^{\gamma 1}$) (Hz)	^3J(N,C$^{\gamma 2}$) (Hz)	$\delta(^{13}C^{\gamma 1})$ (ppm)	$\delta(^{13}C^{\gamma 2})$ (ppm)
V5	0.05±0.02	3.66±0.02	1.83±0.02	0.46±0.06	20.8	23.2
V17	3.93±0.03	0.96±0.03	0.25±0.13	0.68±0.05	22.3	19.1
V26	0.82±0.04	4.18±0.01	2.16±0.03	0.62±0.04	23.7	21.5

Maltose Binding Protein (measured in-house)

Residue	^3J(CO,C$^{\gamma 1}$) (Hz)	^3J(CO,C$^{\gamma 2}$) (Hz)	^3J(N,C$^{\gamma 1}$) (Hz)	^3J(N,C$^{\gamma 2}$) (Hz)	$\delta(^{13}C^{\gamma 1})$ (ppm)	$\delta(^{13}C^{\gamma 2})$ (ppm)
V8	0.02±0.02	3.36±0.03	1.74±0.05	0.77±0.12	20.9	21.2
V50	3.41±0.02	0.94±0.09	0.59±0.10	0.69±0.13	21.1	19.7
V261	0.48±0.36	2.67±0.03	1.68±0.09	0.34±0.26	20.3	21.1
V343	1.05±0.11	3.61±0.03	1.99±0.06	0.24±0.36	21.3	23.3

C-terminal SH2 domain of phospholipase C-γ1 (peptide free)[11]

Residue	$^{3}J(CO,C^{\gamma1})$ (Hz)	$^{3}J(CO,C^{\gamma2})$ (Hz)	$^{3}J(N,C^{\gamma1})$ (Hz)	$^{3}J(N,C^{\gamma2})$ (Hz)	$\delta(^{13}C^{\gamma1})$ (ppm)	$\delta(^{13}C^{\gamma2})$ (ppm)
V28	0.83±0.11	3.11±0.03	1.75±0.04	0.57±0.10	21.0	20.0
V36	0.50±0.50	3.63±0.06	0.84±0.08	0.25±0.25	21.7	23.7
V60	1.10±0.12	3.36±0.04	1.73±0.06	0.56±0.18	21.8	21.4
V67	2.71±0.03	1.73±0.05	0.57±0.09	0.69±0.09	22.0	20.9
V78	1.48±0.05	3.13±0.03	1.59±0.03	0.25±0.25	21.1	22.7

HIV Protease[10]

Residue	$^{3}J(CO,C^{\gamma1})$ (Hz)	$^{3}J(CO,C^{\gamma2})$ (Hz)	$^{3}J(N,C^{\gamma1})$ (Hz)	$^{3}J(N,C^{\gamma2})$ (Hz)	$\delta(^{13}C^{\gamma1})$ (ppm)	$\delta(^{13}C^{\gamma2})$ (ppm)
V75	1.75±0.10	2.64±0.10	1.44±0.10	0.22±0.22	21.7	21.0
V77	1.11±0.30	3.54±0.10	1.73±0.12	0.4±0.30	22.0	22.1
V82	1.73±0.04	1.48±0.05	0.89±0.07	1.09±0.05	19.8	20.2

Protein GB3[10]

Residue	$^{3}J(CO,C^{\gamma1})$ (Hz)	$^{3}J(CO,C^{\gamma2})$ (Hz)	$^{3}J(N,C^{\gamma1})$ (Hz)	$^{3}J(N,C^{\gamma2})$ (Hz)	$\delta(^{13}C^{\gamma1})$ (ppm)	$\delta(^{13}C^{\gamma2})$ (ppm)
V6	0.77±0.03	3.69±0.01	1.99±0.02	0.53±0.06	20.9	21.2
V21	2.52±0.01	1.09±0.02	0.69±0.03	1.08±0.02	20.9	19.9
V39	0.86±0.02	3.27±0.01	1.86±0.02	0.58±0.04	21.5	21.4
V42	1.27±0.01	3.04±0.01	1.73±0.01	0.74±0.03	21.3	21.3
V54	0.70±0.05	1.01±0.04	0.83±0.05	1.85±0.03	20.2	20.9

Rotamer populations derived from scalar couplings and chemical shifts

Protein L:

	Scalar couplings			Chemical Shift			
	p_{g+}	p_t	p_{g-}	p_{g+}	p_t	p_{g-}	O
V49	0.83	0.15	0.02	0.82	0.18	0.00	0.99
V51	0.16	0.84	0.00	0.00	0.89	0.11	0.97

Human Ubiquitin:

	Scalar couplings			Chemical Shift			
	p_{g+}	p_t	p_{g-}	p_{g+}	p_t	p_{g-}	O
V5	0.06	0.94	0.00	0.24	0.76	0.00	0.97
V17	0.07	0.04	0.90	0.00	0.41	0.59	0.84
V26	0.00	1.00	0.00	0.00	0.77	0.23	0.96

Maltose Binding Protein:

	Scalar couplings			Chemical Shift			
	p_{g+}	p_t	p_{g-}	p_{g+}	p_t	p_{g-}	O
V8	0.15	0.85	0.00	0.15	0.75	0.10	0.99
V50	0.10	0.10	0.80	0.13	0.40	0.47	0.84
V261	0.30	0.70	0.00	0.30	0.56	0.15	0.97
V343	0.00	0.90	0.10	0.11	0.90	0.00	0.99

C-terminal SH2 domain of phospholipase C-γ1:

	Scalar couplings			Chemical Shift			
	p_{g+}	p_t	p_{g-}	p_{g+}	p_t	p_{g-}	O
V28	0.20	0.75	0.05	0.15	0.45	0.41	0.80
V36	0.20	0.80	0.00	0.06	0.94	0.00	0.98
V60	0.06	0.80	0.15	0.00	0.93	0.08	0.99
V67	0.16	0.27	0.58	0.00	0.81	0.19	0.60
V78	0.01	0.75	0.24	0.10	0.90	0.00	0.95

HIV Protease:

	Scalar couplings			Chemical Shift			
	p_{g+}	p_t	p_{g-}	p_{g+}	p_t	p_{g-}	O
V75	0.07	0.60	0.34	0.00	0.86	0.14	0.94
V77	0.02	0.85	0.14	0.00	1.00	0.00	0.99
V82	0.42	0.30	0.28	0.49	0.14	0.38	0.95

Protein GB3:

	Scalar couplings			Chemical Shift			
	p_{g+}	p_t	p_{g-}	p_{g+}	p_t	p_{g-}	O
V6	0.05	0.90	0.05	0.15	0.76	0.10	0.99
V21	0.35	0.16	0.50	0.20	0.39	0.41	0.88
V39	0.15	0.80	0.06	0.00	0.95	0.05	0.98
V42	0.14	0.71	0.16	0.01	0.94	0.06	0.97
V54	0.82	0.19	0.00	0.35	0.47	0.19	0.73

Full author list for reference number 11 of text

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