Insight into peptide self-assembly from anisotropic rotational diffusion derived from ^{13}C NMR relaxation

Davy Sinnaeve,*† Marc-André Delsuc,‡ José C. Martins† and Bruno Kieffer*‡

† NMR and Structure Analysis Unit, Ghent University, Krijgslaan 281 S4, B-9000 Gent, Belgium

‡ IGBMC, 1 rue Laurent Fries, BP 10142, 67404 Illkirch, France

Supporting information

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^{13}C relaxation rate constants

Table S1: Experimental relaxation rate constants at 0.5 mM concentration and 16.4 T, with 95% error

	R_1 [s^{-1}]	R_2 [s^{-1}]	R_2/R_1
Leu1 C^α	2.03 ± 0.14	12.3 ± 1.7	6.05 ± 0.77
Gln2 C^α	2.30 ± 0.19	10.9 ± 1.1	4.74 ± 0.60
Thr3 C^α	2.09 ± 0.23	13.9 ± 1.5	6.68 ± 0.95
Val4 C^α	2.00 ± 0.17	11.7 ± 0.9	5.87 ± 0.69
Leu5 C^α	2.07 ± 0.10	10.9 ± 1.0	5.28 ± 0.55
Ser6 C^α	2.16 ± 0.20	10.3 ± 0.8	4.78 ± 0.62
Leu7 C^α	2.16 ± 0.25	14.3 ± 1.0	6.63 ± 0.89
Ser8 C^α	1.88 ± 0.27	12.6 ± 1.2	6.68 ± 1.11
Ile9 C^α	2.10 ± 0.23	9.6 ± 1.1	4.59 ± 0.71
Thr3 C^β	2.19 ± 0.32	17.6 ± 2.1	8.05 ± 1.36
HDA C^β	1.85 ± 0.14	15.1 ± 1.9	8.14 ± 0.96

Table S2: Experimental relaxation rate constants at 6.6 mM concentration and 16.4 T, with 95% error

	R_1 [s^{-1}]	R_2 [s^{-1}]	R_2/R_1
Leu1 C^{α}	1.39 ± 0.08	25.2 ± 2.8	18.20 ± 2.28
Gln2 C^{α}	1.51 ± 0.07	24.9 ± 1.6	16.49 ± 1.33
Thr3 C^{α}	1.45 ± 0.09	27.9 ± 1.1	19.20 ± 1.44
Val4 C^{α}	1.36 ± 0.08	26.0 ± 1.3	19.10 ± 1.53
Leu5 C^{α}	1.56 ± 0.11	23.7 ± 1.1	15.24 ± 1.29
Ser6 C^{α}	1.52 ± 0.06	24.7 ± 2.0	16.23 ± 1.47
Leu7 C^{α}	1.35 ± 0.10	30.8 ± 1.4	22.88 ± 1.93
Ser8 C^{α}	1.27 ± 0.12	34.6 ± 2.7	27.26 ± 3.33
Ile9 C^{α}	1.47 ± 0.05	23.5 ± 1.8	15.95 ± 1.33
Thr3 C^{β}	1.45 ± 0.06	32.0 ± 2.2	22.01 ± 1.79
HDA C^{β}	1.46 ± 0.05	31.5 ± 3.4	21.60 ± 2.45

Table S3: Experimental relaxation rate constants at 16.4 mM concentration at 16.4 T and 11.7 T, with 95% error

	16.4 T			11.4 T
	R_1 [s^{-1}]	R_2 [s^{-1}]	R_2/R_1	R_2 [s^{-1}]
Leu1 C^α	1.23 ± 0.03	33.6 ± 2.1	27.29 ± 1.88	45.6 ± 5.0
Gln2 C^α	1.40 ± 0.03	32.6 ± 1.7	23.31 ± 1.29	34.7 ± 2.4
Thr3 C^α	1.25 ± 0.03	37.2 ± 1.1	29.82 ± 1.09	40.3 ± 3.1
Val4 C^α	1.20 ± 0.04	39.4 ± 2.7	32.91 ± 2.45	38.3 ± 3.4
Leu5 C^α	1.40 ± 0.04	31.6 ± 1.1	22.58 ± 1.03	34.0 ± 2.0
Ser6 C^α	1.39 ± 0.05	33.0 ± 1.5	23.82 ± 1.34	33.7 ± 3.1
Leu7 C^α	1.20 ± 0.05	43.3 ± 2.3	35.94 ± 2.39	39.4 ± 2.1
Ser8 C^α	1.09 ± 0.06	50.2 ± 2.8	46.01 ± 3.48	55.0 ± 8.5
Ile9 C^α	1.34 ± 0.03	31.2 ± 2.2	23.31 ± 1.73	31.3 ± 2.2
Thr3 C^β	1.38 ± 0.05	39.6 ± 2.8	28.64 ± 2.27	40.3 ± 3.3
HDA C^β	1.35 ± 0.05	38.7 ± 2.7	28.74 ± 2.28	42.8 ± 4.3

Table S4: Experimental relaxation rate constants at 44.8 mM concentration and 16.4 T, with 95% error

	R_1 [s^{-1}]	R_2 [s^{-1}]	R_2/R_1
Leu1 C^α	1.19 ± 0.11	43.4 ± 2.9	36.55 ± 4.10
Gln2 C^α	1.33 ± 0.10	45.8 ± 2.4	34.55 ± 3.06
Thr3 C^α	1.04 ± 0.08	49.1 ± 1.8	47.02 ± 3.92
Val4 C^α	1.05 ± 0.09	55.1 ± 3.5	52.65 ± 5.59
Leu5 C^α	1.38 ± 0.10	44.9 ± 3.3	32.58 ± 3.40
Ser6 C^α	1.23 ± 0.11	48.6 ± 4.3	39.67 ± 4.86
Leu7 C^α	0.98 ± 0.10	50.2 ± 7.2	51.33 ± 8.89
Ser8 C^α	0.92 ± 0.10	76.5 ± 7.2	83.51 ± 11.95
Ile9 C^α	1.25 ± 0.08	38.2 ± 3.5	30.44 ± 3.37
Thr3 C^β	1.19 ± 0.10	51.2 ± 2.6	42.97 ± 4.24
HDA C^β	1.21 ± 0.11	65.6 ± 5.1	54.41 ± 6.42

Experimental section: grid search

The grid search was performed using a script written in MatLab 7.5 (2007, The MathWorks Inc.). The atomic coordinates of the 11 C and H pairs were obtained from the pseudodesmin A solution structure[1]. The laboratory frame was chosen so that the Ser8 C^{α}–H^{α} vector was oriented along the z-axis. The initial orientation of the main axis of the rotational diffusion axis frame was also along the z-axis. It is only the angles θ between the C–H vectors and this main axis – from here on referred to as the reference vector – that determine the R_1 and R_2 relaxation rate constants.

The reference vector was rotated from its initial position by first performing a rotation by an angle α along the x-axis of the laboratory frame and subsequently a rotation by an angle β.along the z-axis

$$\mathbf{V}_{ref} = \mathbf{R}_z(\beta)\mathbf{R}_x(\alpha)\mathbf{V}_{ref}^{init} = \begin{bmatrix} \cos\beta & \sin\beta & 0 \\ -\sin\beta & \cos\beta & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & \sin\alpha \\ 0 & -\sin\alpha & \cos\alpha \end{bmatrix} \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} \tag{S.1}$$

The reference vector sampled all positions by varying α between 0° and 179.5° in steps of 0.25° and varying β between –180° and 179.5° in steps of 0.5°. For each orientation, the angles θ were calculated for each C–H pair.

For each reference vector orientation, the rotational diffusion coefficients were fitted to the experimental R_1/R_2 values at each pseudodesmin A concentration separately by minimizing the sum of square difference SS

$$SS = \sum_i \left(\frac{\left(\frac{R_1^{calc}}{R_2^{calc}}\right)_i - \left(\frac{R_1^{\exp}}{R_2^{\exp}}\right)_i}{s_{R1/R2,i}} \right)^2 \tag{S.2}$$

with *i* varying over all ^{13}C nuclei considered at the particular concentration, and $s_{R1/R2,i}$ the 95% error of the respective experimental R_1/R_2 value divided by 1.96. The expressions for the calculated R_1 and R_2 values are given by[2, 3]

$$R_1^{calc} = \frac{K_{DD}^2}{4}\left[\mathrm{J}\left(\omega_H - \omega_C\right) + 3\,\mathrm{J}\left(\omega_C\right) + 6\,\mathrm{J}\left(\omega_H + \omega_C\right)\right] + K_{CSA,C}^2\,\mathrm{J}\left(\omega_C\right) \tag{S.3}$$

$$\begin{aligned} R_2^{calc} = {} & \frac{K_{DD}^2}{8}\left[4\,\mathrm{J}\left(0\right) + \mathrm{J}\left(\omega_H - \omega_C\right) + 3\,\mathrm{J}\left(\omega_C\right) + 6\,\mathrm{J}\left(\omega_H\right) + 6\,\mathrm{J}\left(\omega_H + \omega_C\right)\right] \\ & + \frac{K_{CSA}^2}{6}\left[4\,\mathrm{J}\left(0\right) + 3\,\mathrm{J}\left(\omega_C\right)\right] \end{aligned} \tag{S.4}$$

The Larmor frequencies ω_H and ω_C are given by

$$\begin{aligned} \omega_H &= 2\pi\gamma_H B_0 \\ \omega_C &= 2\pi\gamma_C B_0 \end{aligned} \tag{S.5}$$

with γ_H and γ_C the gyromagnetic ratios of the ^{1}H (2.6752×10^8 $s^{-1}\cdot T^{-1}$) and ^{13}C (6.7283×10^7 $s^{-1}\cdot T^{-1}$) nuclei respectively and B_0 the magnetic field strength (16.4 T). The constant K^2_{DD} for dipolar relaxation is given by

$$K_{DD}^2 = \left(\frac{\mu_0}{8\pi^2}\right)^2 \frac{\gamma_H^2\gamma_C^2 h^2}{r_{CH}^{\ 6}} \tag{S.6}$$

with μ_0 the permeability of the vacuum ($4\pi\times10^7$ $T\cdot m\cdot A^{-1}$), h Planck's constant (6.626×10^{-34} $m^2\cdot kg\cdot s^{-1}$) and r_{CH} the distance between the ^{13}C and ^{1}H nucleus (here assumed to be 1.09 Å) [4]. The constant K^2_{CSA} for chemical shift anisotropy relaxation is given by

$$K_{CSA}^2 = \frac{\gamma_C^2 B_0^2 \Delta\sigma^2}{3} \tag{S.7}$$

with $\Delta\sigma$ the chemical shift anisotropy, taken to be 10 ppm, which is a compromise value for the various $^{13}C^{\alpha}$ chemical shift anisotropy values found in proteins and more particularly in α-helices[4]. Note that it is assumed here that the main axis of the (axially symmetric) CSA tensor is parallel to the C–H bond vector. Note that, compared to the dipolar relaxation mechanism, the CSA mechanism for $^{13}C^{\alpha}$ nuclei has only a very small impact on the R_1 and R_2 values and especially on their ratio.

The spectral density function for axially symmetric anisotropic rotational diffusion is given by[2, 3]

$$\mathrm{J}(\omega)=\frac{2}{5}\left[\frac{1}{4}\left(3\cos^2\theta-1\right)^2\frac{\tau_A}{1+\omega^2\tau_A^2}+3\cos^2\theta\sin^2\theta\frac{\tau_B}{1+\omega^2\tau_B^2}+\frac{3}{4}\sin^4\theta\frac{\tau_C}{1+\omega^2\tau_C^2}\right]\quad\text{(S.8)}$$

with the correlation time constants τ_A, τ_B and τ_C determined by the rotational diffusion coefficients

$$\begin{aligned}\tau_A&=\frac{1}{6D_r^{\perp}}\\ \tau_B&=\frac{1}{5D_r^{\perp}+D_r^{\parallel}}\\ \tau_C&=\frac{1}{2D_r^{\perp}+4D_r^{\parallel}}\end{aligned}\quad\text{(S.9)}$$

The resulting SS values were added together to form the total sum of square difference E, which is divided by the number of data points N considered (35) minus the number of estimated parameters n (10, which is two angles and 2 diffusion coefficients at each concentration). E/(N – n) is plotted in Figure S1. Note that the color profile of this surface is determined by the distribution of the C–H bond vector orientation in the monomer conformation and should not be interpreted in terms of deviation of axial symmetry. The minimum represents the optimal orientation of the monomer conformation in the axial symmetric rotational diffusion tensor frame. This corresponds to a value of 1.52, which can be interpreted in terms of a χ^2 goodness of fit test. The p-value of the χ^2 test (25 degrees of freedom) is 0.05. This shows that the current mathematical model adequately fits the data (χ^2 value is not significantly above 1.0) and thus demonstrating that there is potentially little room for a full anisotropic model to significantly improve the goodness of fit. In addition, there is no overfitting (χ^2 value is not significantly below 1.0).

A Monte Carlo error analysis was performed to estimate the precision of results of the grid search. For this, Gaussian noise was added to the fitted R_1 and R_2 values with a standard deviation of the 95% error bars divided by 1.96 of the particular relaxation rate constant. Like this, 200 new datasets were generated. For each of these, the grid search was repeated, with a narrower interval for α (0° to 4.75° in steps of 0.25°) to save computation time. The resulting positions of the minimum total sum of squares value for each Monte Carlo iteration are shown in Figure S2. The obtained maximum value for α for the optimized position was 2.25°, justifying the narrower interval considered during the grid search. The 95% error bars of the rotational diffusion coefficients, the correlation time constants, the θ angles and cylinder dimensions were calculated as the standard deviation of 200 values obtained multiplied with 1.96.

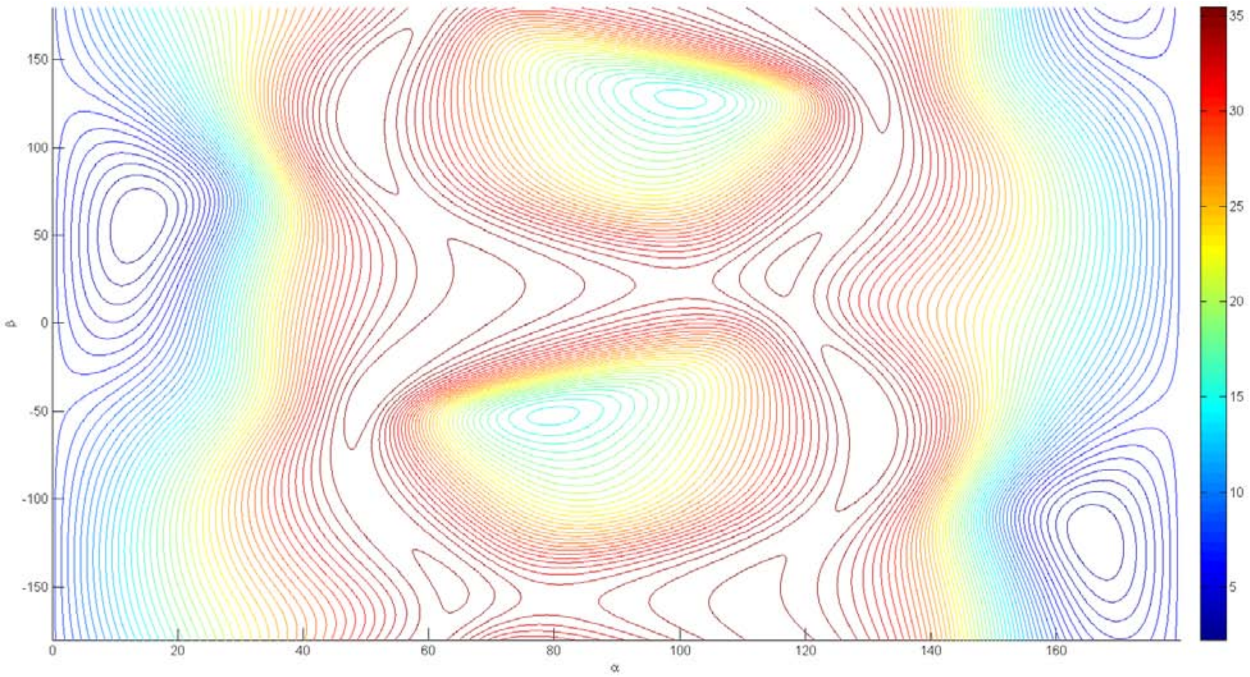

Figure S1. contour plot representing the E/(N – n) value as a function of α and β.

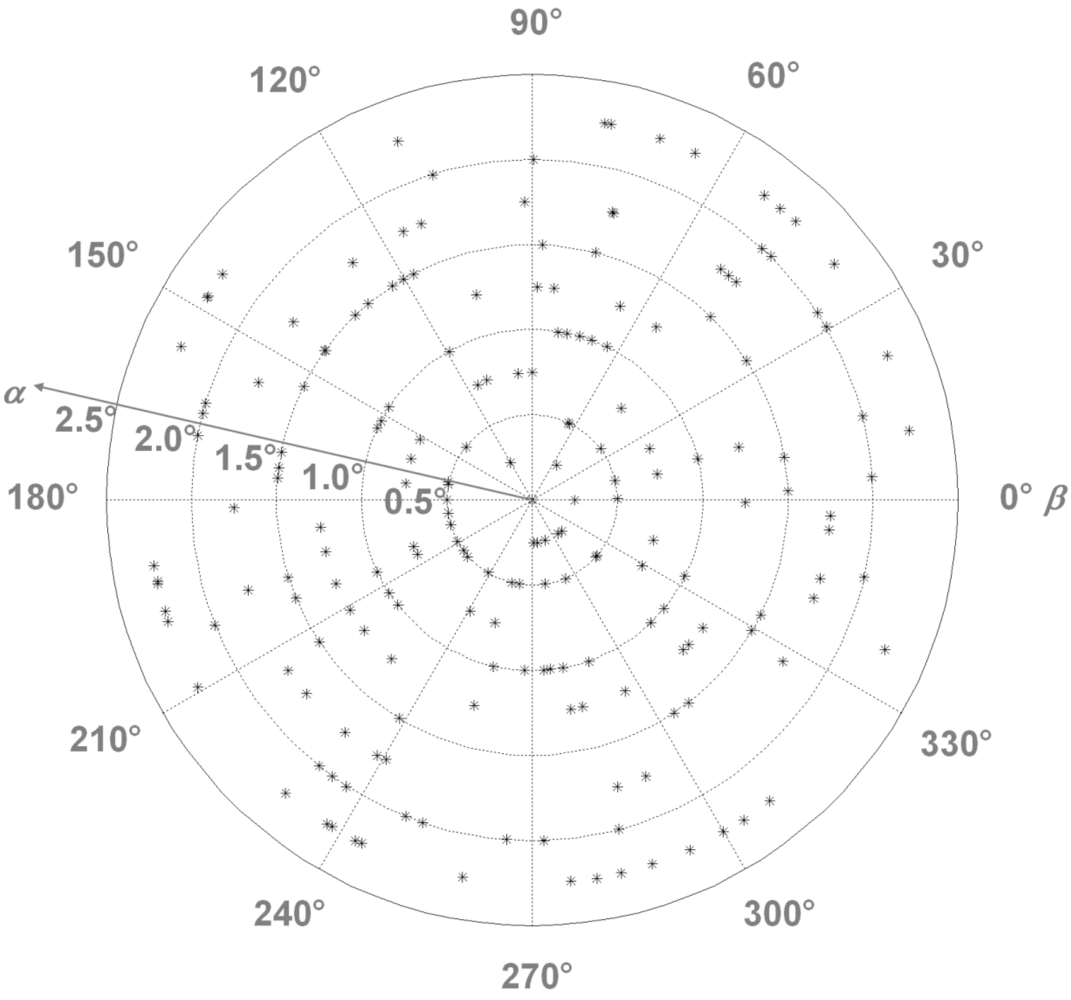

Figure S2. A polar plot representing the 100 α and β angle combinations obtained during the Monte Carlo error analysis. Each point represents the deviation of the newly optimized main axis direction during the Monte Carlo analysis from the optimal direction after the grid search using all datasets. The distance from the middle point represents the α coordinate while the polar position along the circle represents the β coordinate.

Calculation of cylinder dimensions

The cylinder dimensions were calculated from the rotational diffusion coefficients according to the formulas described by Ortega *et al*[5] that relate them to the NMR correlation time constants

$$\tau_A = \frac{\pi\eta L^3}{4k_B T p^2} \times \begin{cases} \Big[1.18 + 0.1744\left(\ln p + 0.2877\right)^2 - 0.2417\left(\ln p + 0.2877\right)^3 \\ -0.03882\left(\ln p + 0.2877\right)^4\Big] \ldots\ldots\ldots\ldots\ldots\ldots \text{if } p < 0.75 \\ \\ \Big[1.18 + 1.116\left(\ln p + 0.2877\right)^2 - 0.9729\left(\ln p + 0.2877\right)^3 \\ -0.4954\left(\ln p + 0.2877\right)^4\Big] \ldots\ldots\ldots\ldots\ldots\ldots \text{if } p > 0.75 \end{cases} \tag{S.10}$$

$$\tau_B = \frac{\pi\eta L^3}{4k_B T p^2} \times \Big[1.183 + 0.2902\ln p + 0.4406\left(\ln p\right)^2 - 0.05850\left(\ln p\right)^3 \\ -0.009544\left(\ln p\right)^4\Big] \tag{S.11}$$

$$\tau_C = \frac{\pi\eta L^3}{4k_B T p^2} \times \left[0.9833 + \frac{0.06532}{p} + \frac{0.05168}{p^2} - \frac{0.003234}{p^3}\right] \tag{S.12}$$

with $p = L/d$, k_B the Boltzmann constant (1.381×10^{-23}), T the temperature (283.0 K) and η the solvent viscosity (0.631 mPa·s) for chloroform at 10°C). The rotational diffusion coefficients are related to the correlation time constants as

$$\begin{aligned} D^{\parallel} &= \frac{5}{18\tau_C} - \frac{1}{9\tau_B} \\ D^{\perp} &= \frac{1}{6\tau_A} \end{aligned} \tag{S.13}$$

The dimensions can then be numerically fitted from these expressions.

Results of the anisotropic analysis of the heteronuclear relaxation data and cylinder dimension calculations

Table S5: Rotational diffusion coefficients, correlation time constants and cylinder dimensions

	0.5 mM	6.6 mM	16.4 mM	44.8 mM
$D^{\parallel}$ [μs^{-1}]	92.69 ± 10.40	49.29 ± 2.87	43.53 ± 1.63	38.16 ± 2.57
$D^{\perp}$ [μs^{-1}]	56.20 ± 4.14	27.31 ± 1.14	20.75 ± 0.63	15.44 ± 0.97
$D^{\parallel}/D^{\perp}$ [μs^{-1}]	1.65 ± 0.29	1.81 ± 0.17	2.10 ± 0.14	2.47 ± 0.31
cov($D^{\parallel}$–$D^{\perp}$) [μs^{-2}]	-8.90	-0.68	-0.21	-0.54
τ_A [ns]	2.97 ± 0.22	6.10 ± 0.25	8.03 ± 0.24	10.79 ± 0.68
τ_B [ns]	2.68 ± 0.10	5.38 ± 0.11	6.79 ± 0.10	8.67 ± 0.23
τ_C [ns]	2.07 ± 0.15	3.97 ± 0.15	4.64 ± 0.12	5.45 ± 0.26
L cylinder [Å]	32.56 ± 4.05	43.57 ± 2.81	51.51 ± 2.05	60.93 ± 4.12
d cylinder [Å]	21.88 ± 1.86	26.36 ± 1.18	26.40 ± 0.77	26.46 ± 1.40

Table S5: θ angles of the C–H bonds within the rotational diffusion tensor frame

Leu1 C^{α}	138.1	±	2.0
Gln2 C^{α}	72.9	±	2.4
Thr3 C^{α}	47.0	±	1.7
Val4 C^{α}	42.4	±	2.1
Leu5 C^{α}	74.2	±	2.1
Ser6 C^{α}	69.2	±	2.0
Leu7 C^{α}	34.3	±	2.3
Ser8 C^{α}	14.0	±	2.1
Ile9 C^{α}	79.5	±	2.4
Thr3 C^{β}	132.8	±	1.7
HDA C^{β}	35.7	±	1.8

Pseudodesmin A monomer solution conformation with N–H bonds exemplified

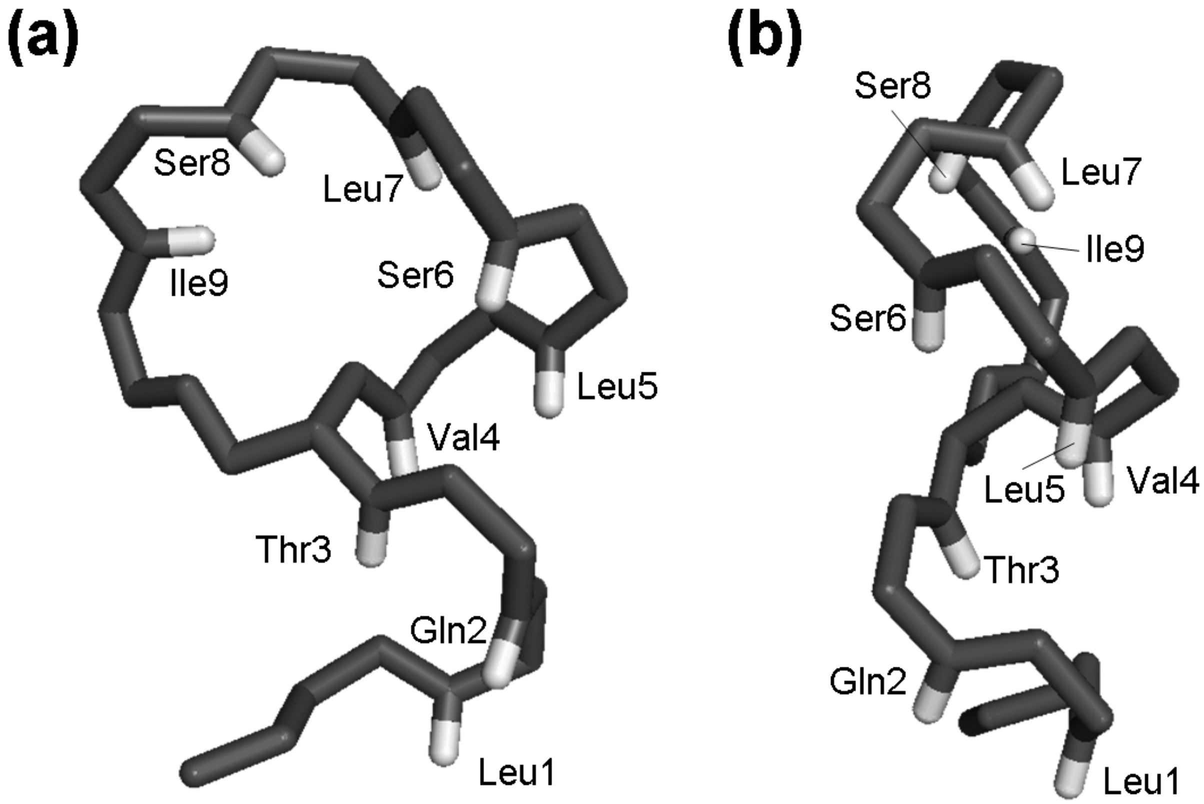

Figure S3. The solution structure of pseudodesmin A with only the main chain in black and the H^N nuclei in white, thereby emphasising the N–H bond vectors being mostly aligned along a single direction. (a) Side view from the helix, N→C bottom to top, with the loop structure positioned to the left of the helix; (b) different side view from the helix, N→C bottom to top, with the loop structure positioned behind the helix.

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