BIOLOGICAL
CRYSTALLOGRAPHY

Volume 71 (2015)

Supporting information for article:

Bond distances in polypeptide backbone depend on the local conformation

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Table S1 PDB codes for the set of very high-resolution protein structures used in the statistical survey of peptide bond geometrical parameters.

PDB code#	**Resolution (Å)**
1EJG, 1UCS, 2VB1, 1US0, 2DSX, 1YK4, 3A38, 1R6J, 1HJE, 2B97, 2OV0, 2WFI, 3AL1, 4I8H, 1GCI, 1X6Z, 3W5H, 2PVE, 1PQ7, 1W0N, 2IXT, 3UI4	0.5-0.8
1NWZ, 2H5C, 2VXN, 2JFR, 2O9S, 2PWA, 1P9G, 2HS1, 2O7A, 2YKZ, 4EIC, 1M40, 1MC2, 1X8Q, 2F01, 2FMA, 2OL9, 3O4P, 3QPA, 4AYO, 1G6X, 1MUW, 2DDX, 3NJW, 3ZSJ, 1DY5, 1V6P, 3Q8J, 4HS1, 1GWE, 2P74, 3KLR, 3ZOJ, 4F1V, 1ETL, 1I1W, 3IP0, 1BYZ, 1ET1, 1G66, 1IX9, 1N9B, 1OEW, 1VYR, 2BW4, 2WUR, 2XU3, 3G21, 3KS3, 3VOR, 3ZR8, 4EA9	0.8-0.9

When multiple chains are present, chain A is selected.

Table S2 Experimental distributions of peptide bond lengths for residues grouped by ψ values. T-test results for pairwise comparisons among means from adjacent bins are also reported (see main text).

C-N statistics					
C-N distance	40° bin centred around **ψ= -60°**	40° bin centred around **ψ=0°**	40° bin centred around **ψ=60°**	40° bin centred around **ψ=120°**	40° bin centred around **ψ=180°**
Mean	1.3360	1.3319	1.3347	1.3315	1.3335
Variance (σ^2)	0.000100	0.000138	0.000126	0.000139	0.000147
Stand. Dev. (σ)	0.0100	0.0118	0.0112	0.0118	0.0121
Stand. Err. ($\sigma/\sqrt{N}$)	0.00026	0.00044	0.00087	0.00028	0.00051
Sample size (N)	1468	703	168	1732	565
T-test results					
C-N distance	**ψ= -60° / ψ=0°**	**ψ=60° / ψ=0°**	**ψ=60° / ψ=120°**	**ψ=180° / ψ=120°**	
P-value (α=0.05)	$5.4*10^{-16}$	0.0026	$5.2*10^{-4}$	$4.3*10^{-4}$	
Diff. of means #	yes	yes	yes	yes	
C-O statistics					
C-O distance	40° bin centred around **ψ= -60°**	40° bin centred around **ψ=0°**	40° bin centred around **ψ=60°**	40° bin centred around **ψ=120°**	40° bin centred around **ψ=180°**
Mean	1.2353	1.2347	1.2339	1.2351	1.2324
Variance (σ^2)	0.0000901	0.000128	0.000098	0.000126	0.000119
Stand. Dev. (σ)	0.00949	0.0113	0.0099	0.0113	0.0109
Stand. Err. ($\sigma/\sqrt{N}$)	0.00025	0.00043	0.00076	0.00027	0.00046
Sample size (N)	1468	703	168	1732	565
T-test results					
C-O distance	**ψ=0° / ψ= -60°**	**ψ=0° / ψ=60°**	**ψ=120° / ψ=60°**	**ψ=120° / ψ=180°**	
P-value (α=0.05)	0.105	0.195	0.0753	$4.0*10^{-7}$	
Diff. of means #	no	no	no	yes	

In the last row of T-test results "yes" and "no" indicate if the difference between means from two adjacent bins is statistically significant with the first mean (on the left) greater than the second (on the right) or not, respectively.

Table S3 Experimental distributions of bond lengths at C^{α} atom for residues grouped by ψ values. T-test results for pairwise comparisons among means from adjacent bins are also reported (see main text).

C^{α}-C^{β} distance	40° bin centred around **ψ= -30°**	40° bin centred around **ψ=60°**
Mean	1.5312	1.5259
Variance	0.000172	0.000146
Stand. Dev. (σ)	0.0131	0.0121
Stand. Err. (σ/√N)	0.00026	0.00093
Sample size (N)	2609	168

T-test results

C^{α}-C^{β} distance	**ψ= -30° / ψ=60°**	**ψ=150° / ψ=60°**
P-value (α=0.05)	$1.3*10^{-7}$	$5.4*10^{-14}$
Diff. of means #	yes	yes

N-C^{α} statistics

N-C^{α} distance	40° bin centred around **ψ=0°**	40° bin centred around **ψ=90°**
Mean	1.4559	1.4612
Variance	0.000117	0.000131
Stand. Dev. (σ)	0.0108	0.0114
Stand. Err. (σ/√N)	0.00041	0.00068
Sample size (N)	703	281

T-test results

N-C^{α} distance	**ψ=90 / ψ=0°**	**ψ=90° / ψ=180°**
P-value (α=0.05)	$6.8*10^{-12}$	$9.4*10^{-24}$
Diff. of means #	yes	yes

C^{α}-C statistics

C^{α}-C distance	40° bin centred around **ψ= -30°**	40° bin centred around **ψ=60°**
Mean	1.5254	1.5306
Variance	0.000130	0.000170
Stand. Dev. (σ)	0.0114	0.0131
Stand. Err. (σ/√N)	0.00022	0.0010
Sample size (N)	2609	168

T-test results

C^{α}-C distance	**ψ=60° / ψ= -30°**	**ψ=60° / ψ=150°**
P-value (α=0.05)	$7.5*10^{-7}$	$4.4*10^{-10}$
Diff. of means #	yes	yes

In the last row of T-test results "yes" and "no" indicate if the difference between means from two adjacent bins is statistically significant with the first mean (on the left) greater than the second (on the right) or not, respectively.

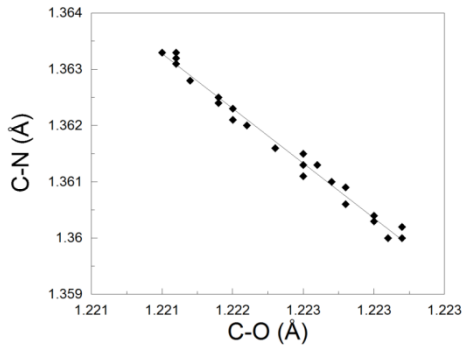


Figure S1 Pep model in vacuo: Correlation of C-O/C-N peptide bond distances. The regression line is also shown (correlation coefficient R=-0.99; $y_{C\text{-}N}$= 3.37-1.94 $x_{C\text{-}O}$).

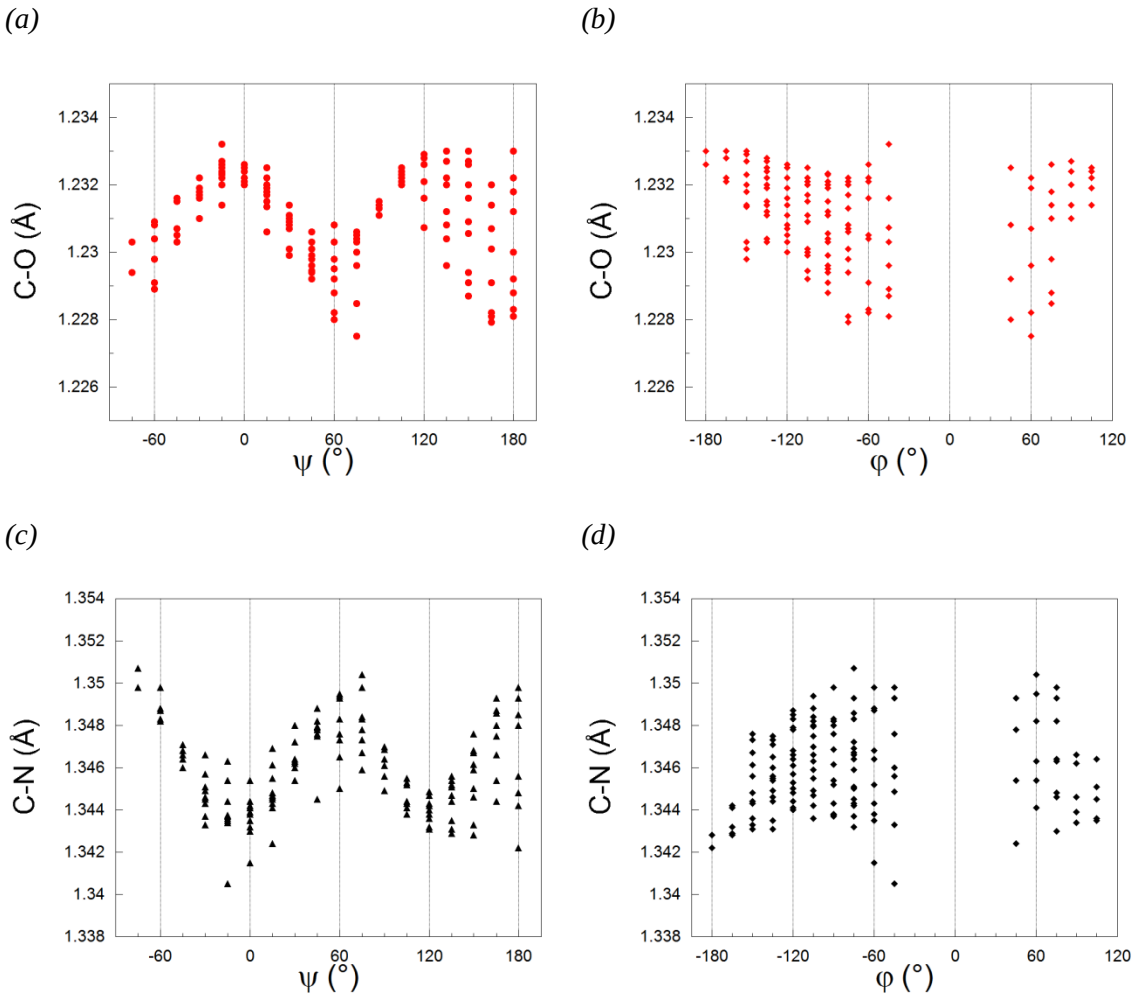


Figure S2 Ala1 model: Variability of CO/CN bond distances with the ψ and the φ angle. *(a)* C-O vs. ψ. *(b)* C-O vs. φ. *(c)* C-N vs. ψ. *(d)* C-N vs. φ.

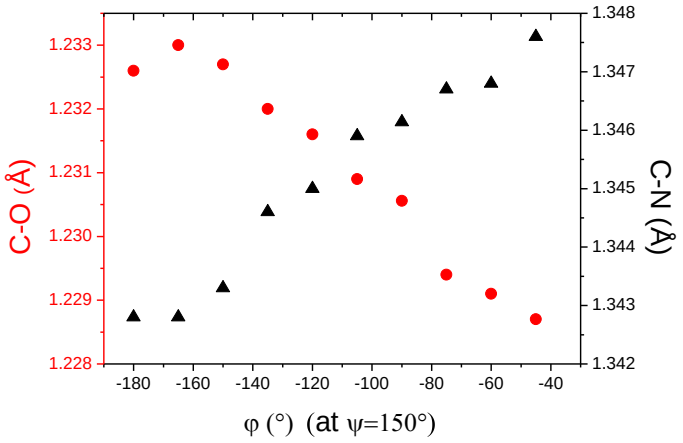


Figure S3 Ala1 model: Distance dependence on φ in the extended conformation region (fixed ψ=150°).

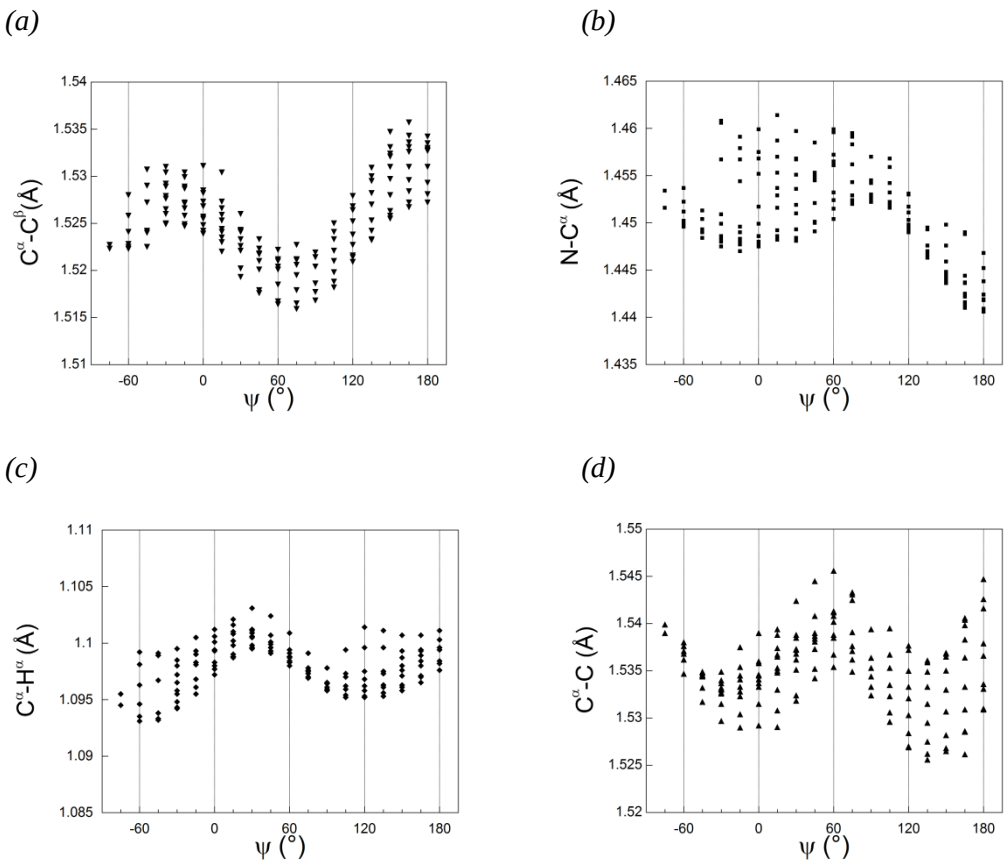


Figure S4 Ala1 model: Dependence of bond distances at C^{α} atom on the ψ angle. *(a)* C^{α}-C^{β}. *(b)* N-C^{α}. *(c)* C^{α}-H^{α}. *(d)* C^{α}-C.

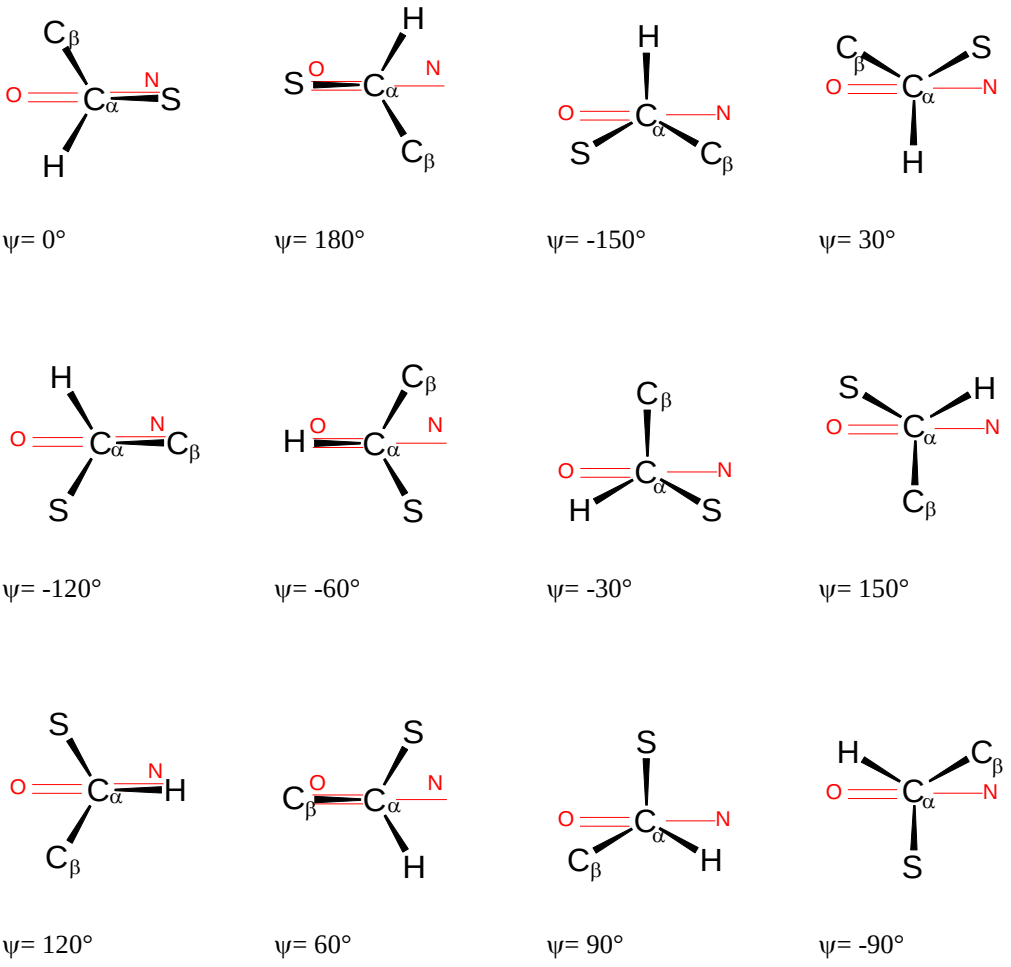


Figure S5 Schematic drawings of conformers characterized by different ψ values. The projections are drawn by looking along the C^{α}-C bond. The S substituent stands for the CH_3-CO-NH- group in Ala1 model. Going from left to right: conformers with one C^{α} bond (i) eclipsed with the C-N bond, (ii) eclipsed with C-O bond, (iii, iv) perpendicular to the CO-NH plane. The figure is adapted from Fig S2 of Improta et al. 2011.

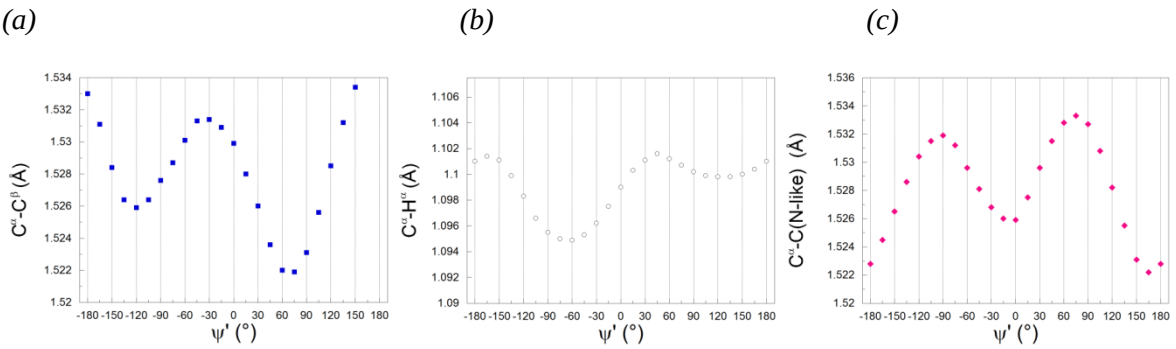


Figure S6 Pep model: Dependence of bond distances at C^{α} atom on the ψ' angle. *(a)* C^{α}-C^{β}. *(b)* C^{α}-H^{α}. *(c)* C^{α}-CH_3(N-like).

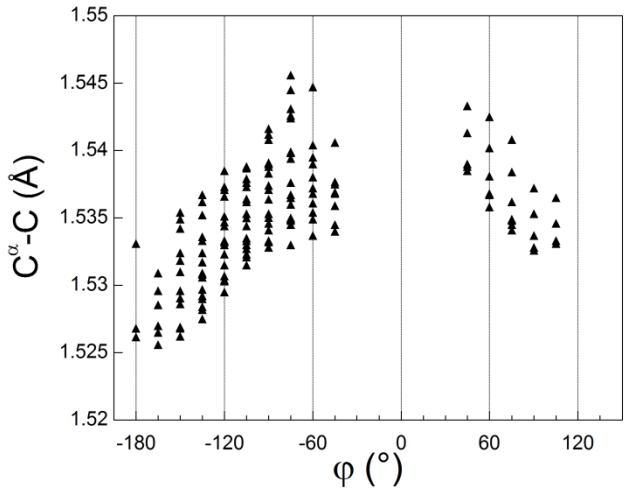


Figure S7 Ala1 model: Dependence of C^{α}-C bond distance on the φ angle.

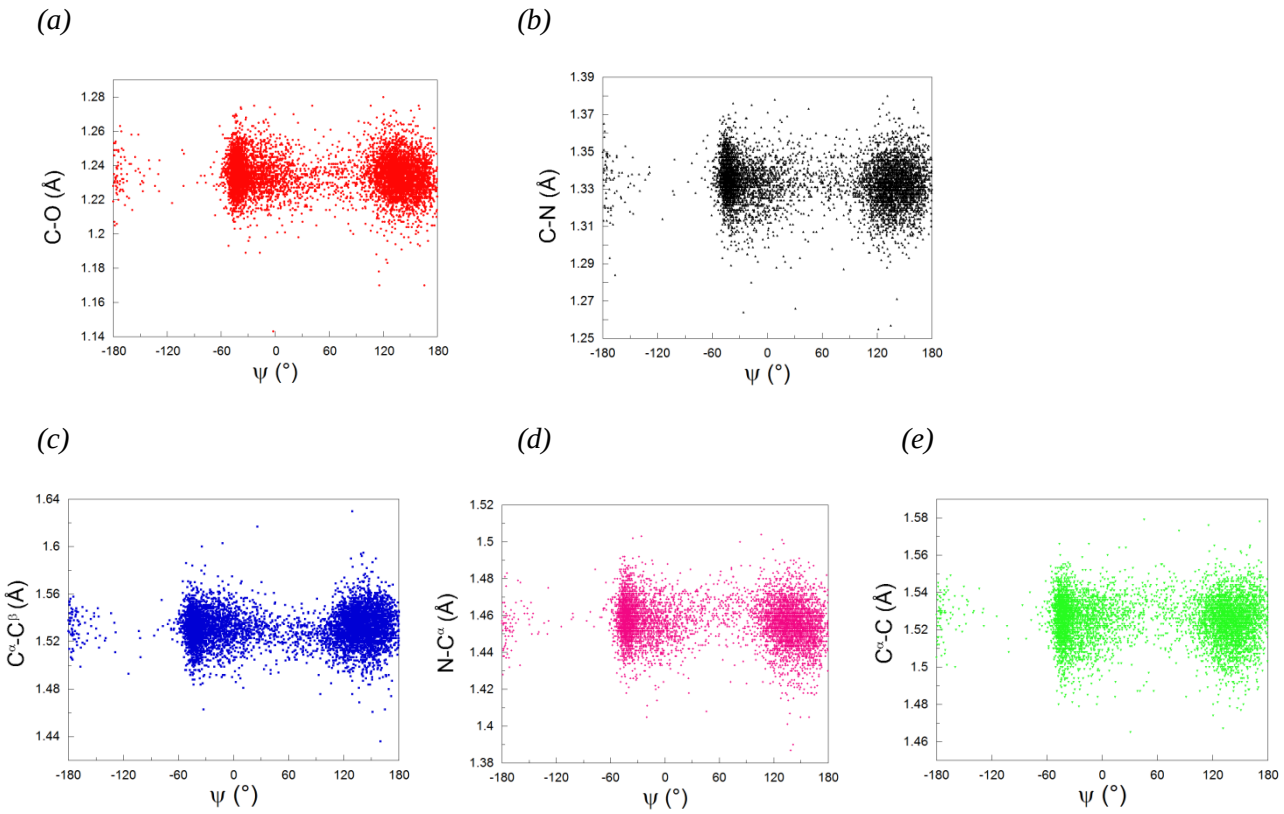
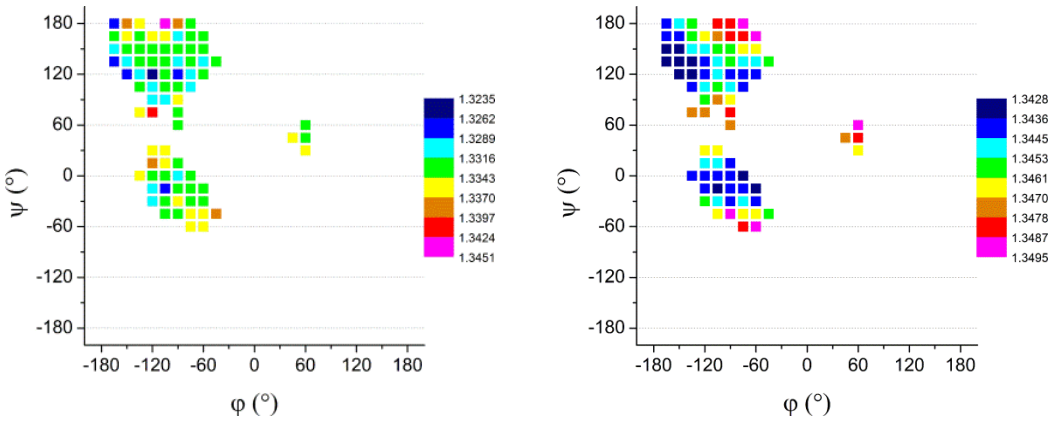
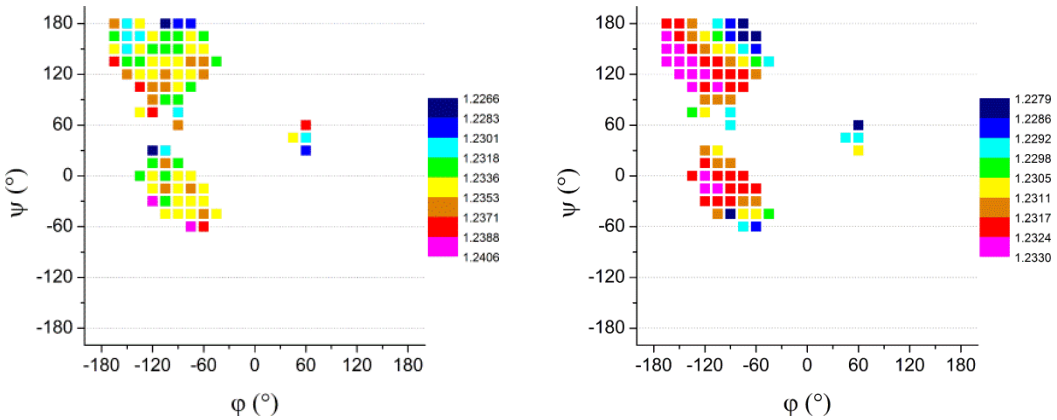


Figure S8 PDB ultrahigh resolution protein structure survey. Dependence of bond lengths on ψ dihedral angle**.** *(a)* C-O *vs.* ψ. *(b)* C-N *vs.* ψ. *(c)* C^{α}-C^{β} *vs.* ψ. *(d)* N-C^{α} *vs.* ψ. *(e)* C^{α}-C

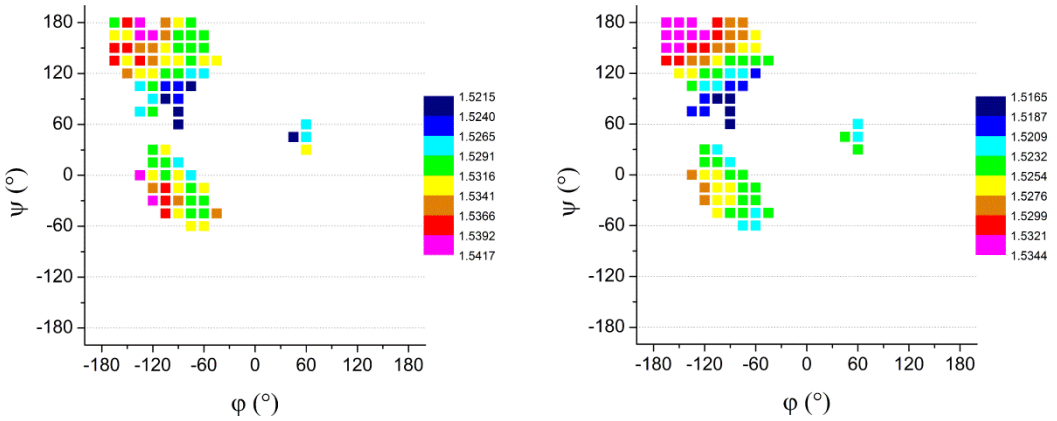
(a)


ψ (°)
φ (°)
180
120
60
0
-60
-120
-180
-180 -120 -60 0 60 120 180
1.3235
1.3262
1.3289
1.3316
1.3343
1.3370
1.3397
1.3424
1.3451
1.3428
1.3436
1.3445
1.3453
1.3461
1.3470
1.3478
1.3487
1.3495

(b)


ψ (°)
φ (°)
180
120
60
0
-60
-120
-180
-180 -120 -60 0 60 120 180
1.2266
1.2283
1.2301
1.2318
1.2336
1.2353
1.2371
1.2388
1.2406
1.2279
1.2286
1.2292
1.2298
1.2305
1.2311
1.2317
1.2324
1.2330

(c)


ψ (°)
φ (°)
180
120
60
0
-60
-120
-180
-180 -120 -60 0 60 120 180
1.5215
1.5240
1.5265
1.5291
1.5316
1.5341
1.5366
1.5392
1.5417
1.5165
1.5187
1.5209
1.5232
1.5254
1.5276
1.5299
1.5321
1.5344

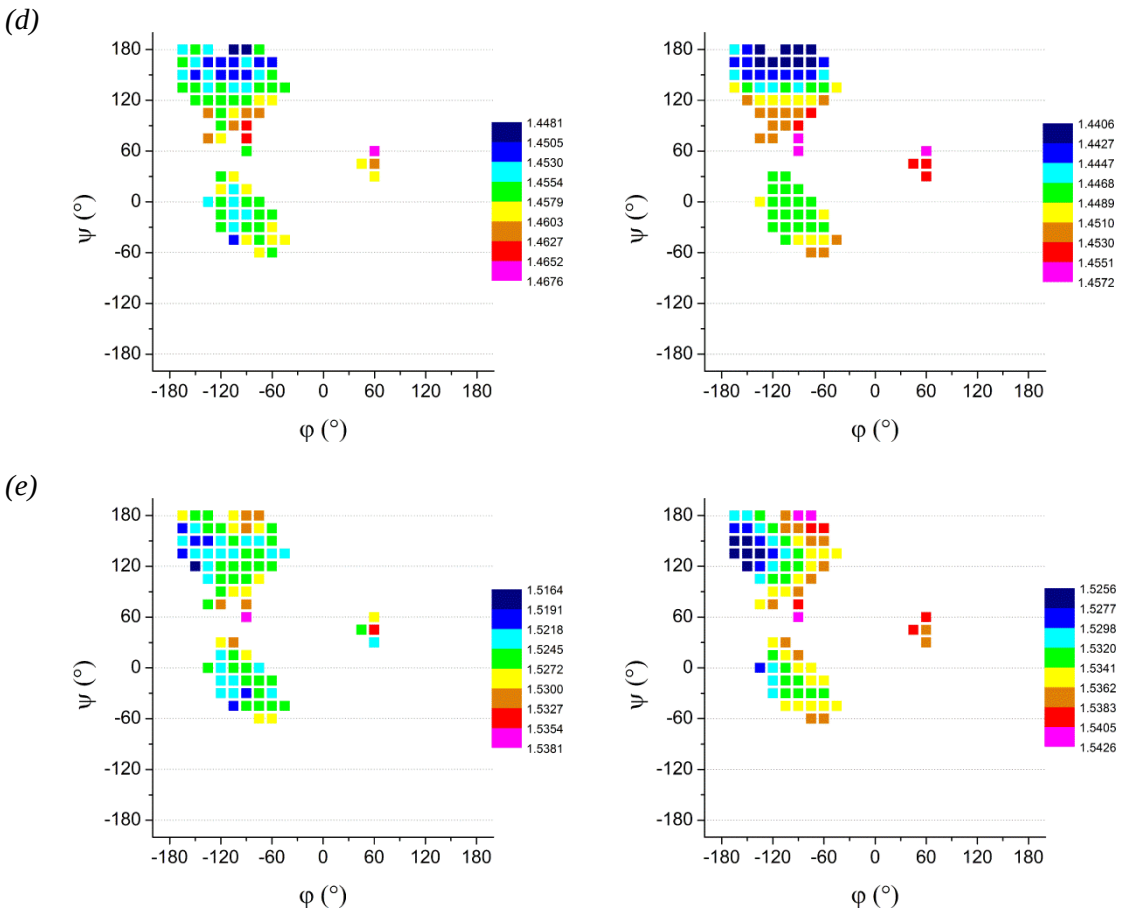


Figure S9 Comparison of conformational dependences of distance lengths. Results from experimental structures (left) and theoretical calculations (right). The experimental values are calculated by averaging the distances (all residue types except Gly) in (φ, ψ) bins of (15°x15°) size, centred on fixed values used for calculations. Only bins containing more than 10 measures are considered. *(a)* C-N, *(b)* C-O, *(c)* C^{α}-C^{β}, *(d)* N-C^{α}, *(e)* C^{α}-C.

a) *(b)*

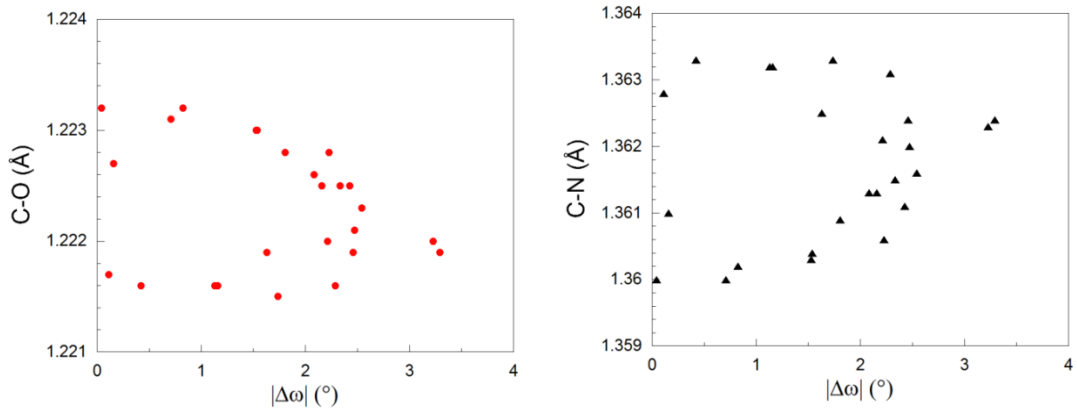


Figure S10 Pep model *in vacuo*: CO/CN distances *versus* |Δω|. *(a)* CO *versus* |Δω|. *(b)* CN *versus* |Δω|. It is worth noting that Δω is defined as follows: Δω= ω-180° (mod 360°).