Supporting Information

Determination of Conformational Equilibria in Proteins Using Residual Dipolar Couplings

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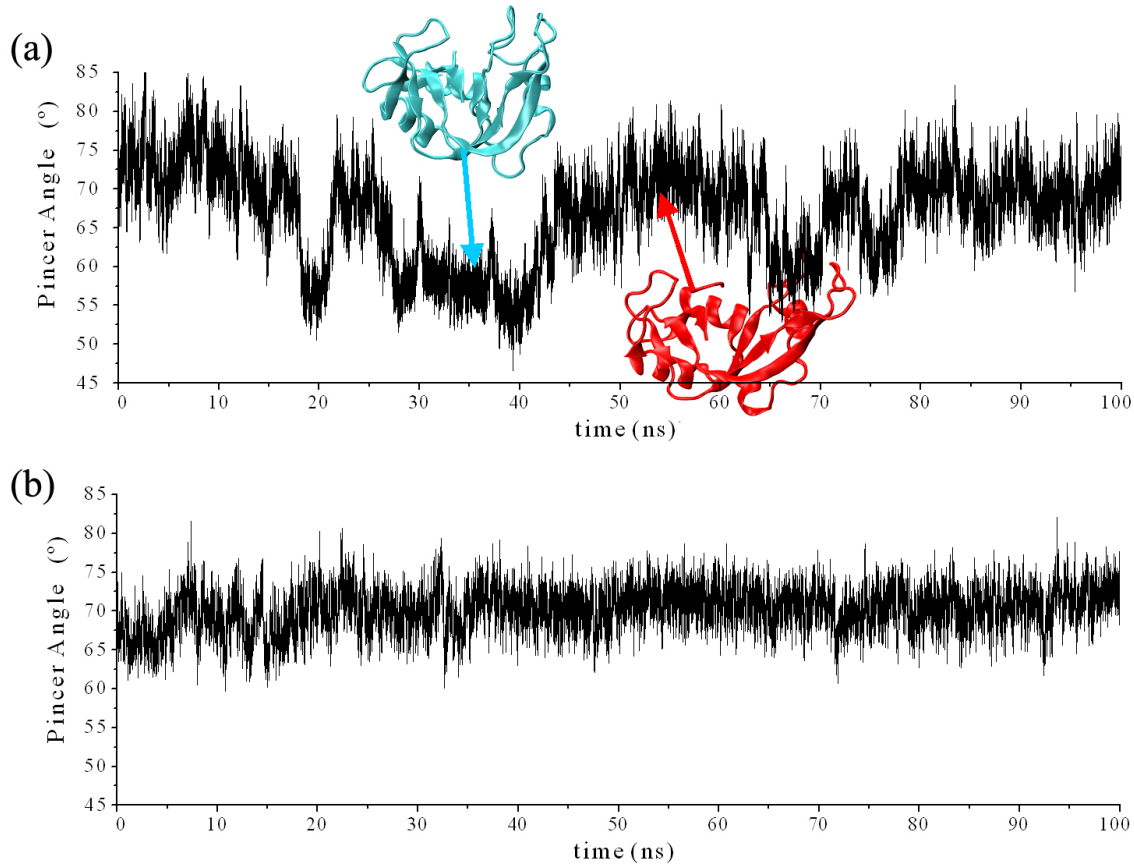

Figure S1

Time evolution of the pincer angle (see Figure 2a) in 100ns of unrestrained simulations. The pincer angle is defined as the angle formed by the three centres of mass of the Cα-atoms of three protein regions: region 1 (V2), which spans residues 42 and 43, region 2 (hinge), which spans residues 48, 49 and 80 and region 3 (V1), which spans residues 72 and 73. (Figure 2a), (A) Gromos96 force field; (B) Amber99SB force field. Representative structures of the closed (cyan) and open (red) conformations are also shown.

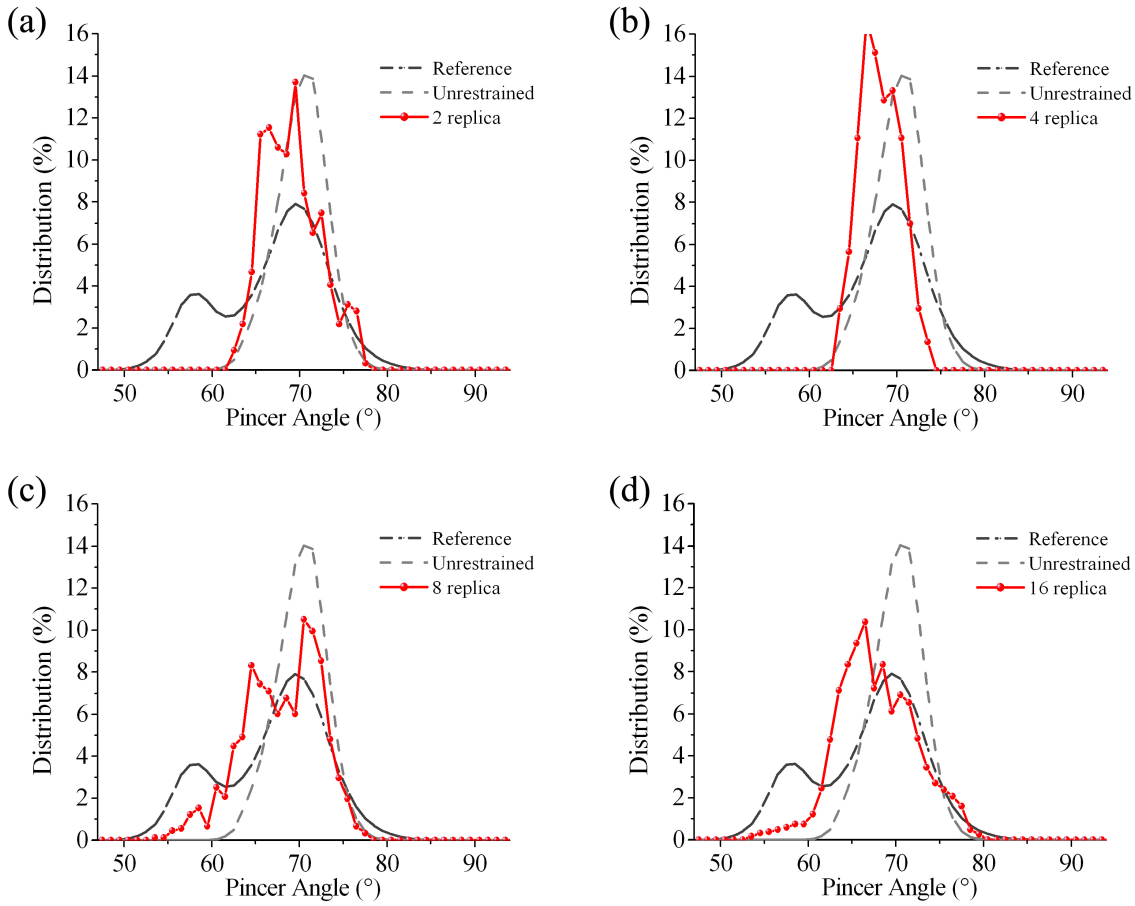

Figure S2

Comparison of the distribution of the values of the pincer angle (see Figure 2a) in the restrained ensemble using only the steric alignment medium (red) with the distributions in the reference (black dot-dashed) and unrestrained (grey dashed) ensembles: (A) 2-replica; (B) 4-replica; (C) 8-replica; (D) 16-replica simulations. These data indicate that the use of only one alignment medium does not enable the simultaneous sampling of the closed and open states through the restrained molecular dynamics protocol that we describe in this work.

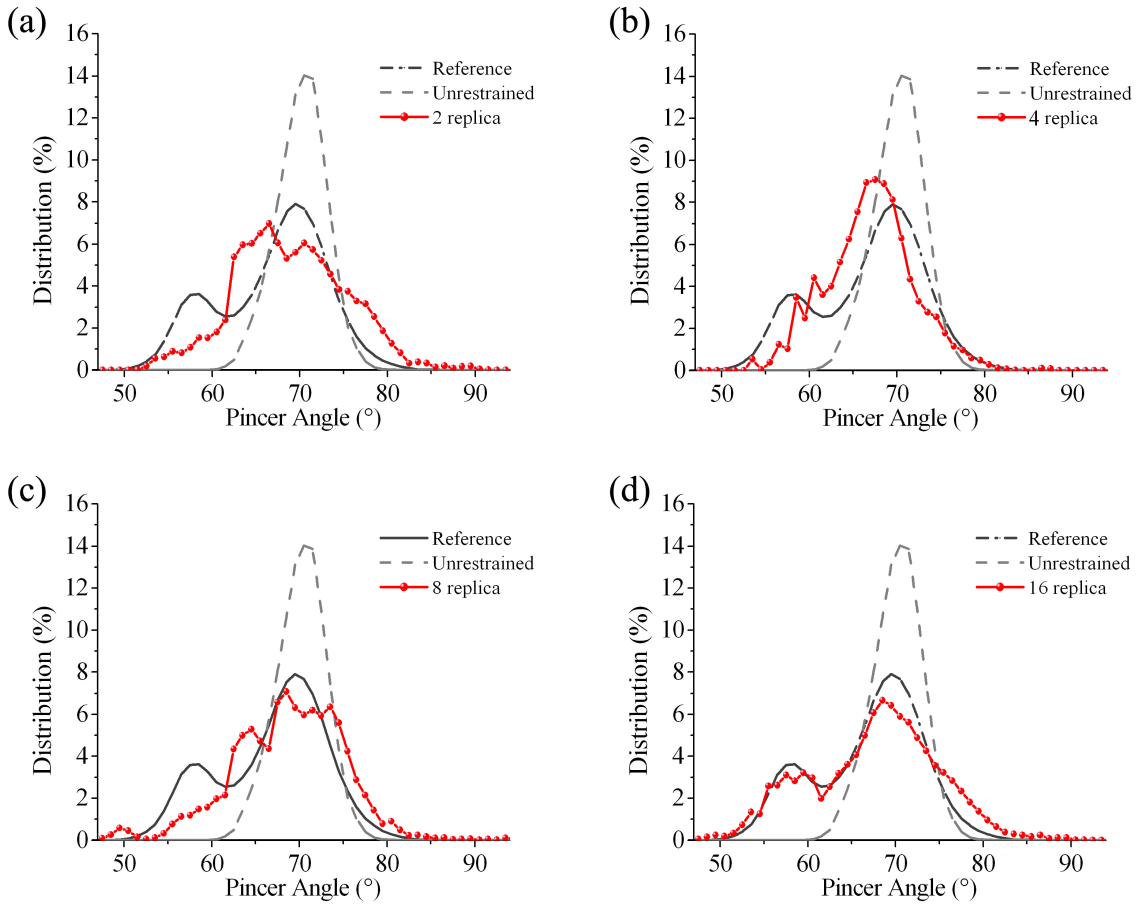

Figure S3

Comparison of the distribution of the pincer angle (see Figure 2a) in the restrained simulations (red) using three alignment media (two steric and one electrostatic) with the distributions from the reference (black dot-dashed) and unrestrained (grey dashed). (A) 2-replica; (B) 4-replica; (C) 8-replica; (D) 16-replica simulations. These data show that the simultaneous sampling of the open and closed states is achieved through the molecular dynamics protocol that we describe in this work when 16 replicas are used. The use of two alignment media (one steric and one electrostatic) in the case that we studied here also enables one to obtain a restrained ensemble closely resembling the reference ensemble.

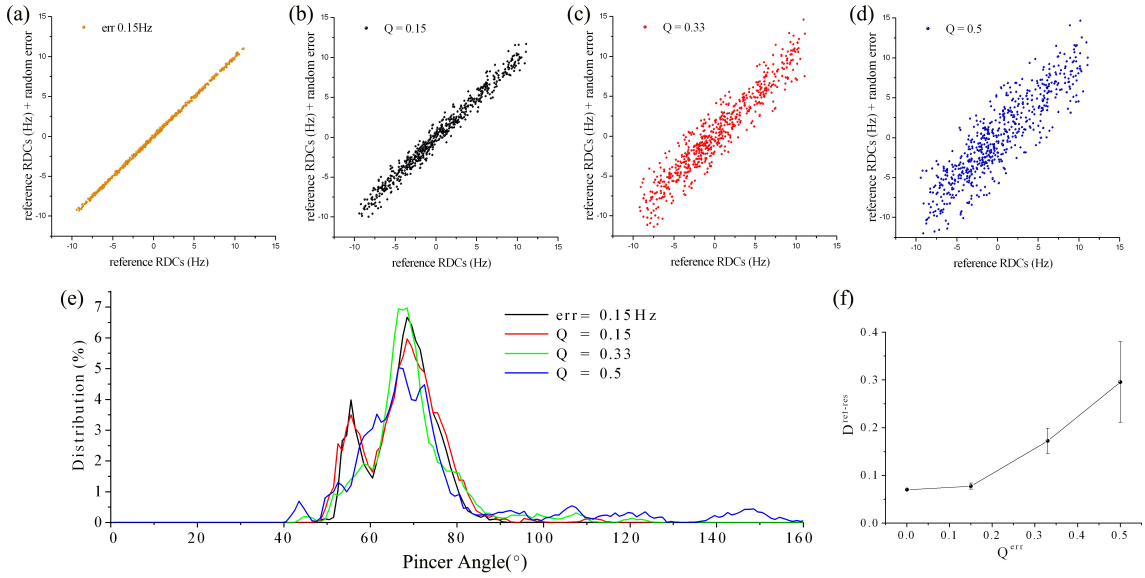

Figure S4

In order to assess the errors in the performance of the structure-based alignment prediction, four restrained simulations have been performed by perturbing the RDCs back-calculated from the reference ensemble. (a) A random error with a uniform distribution in [-0.15,0.15] Hz was added to the reference RDCs. (b-d) Random errors were generated with a distribution leading to Q factors between reference RDCs with and without errors of 0.15 (b), 0.33 (c) and 0.5 (d). (e) Pincer angle distributions of the four samplings. (f) Similarity of the pincer angle distributions of the restrained and reference ensembles. The similarity index ranges from 0 (for completely equal distributions) to 1 for completely different distributions). The error bars indicate the amount of population of distorted structures calculated as the structures presenting pincer angles lower than 50 or larger than 90.

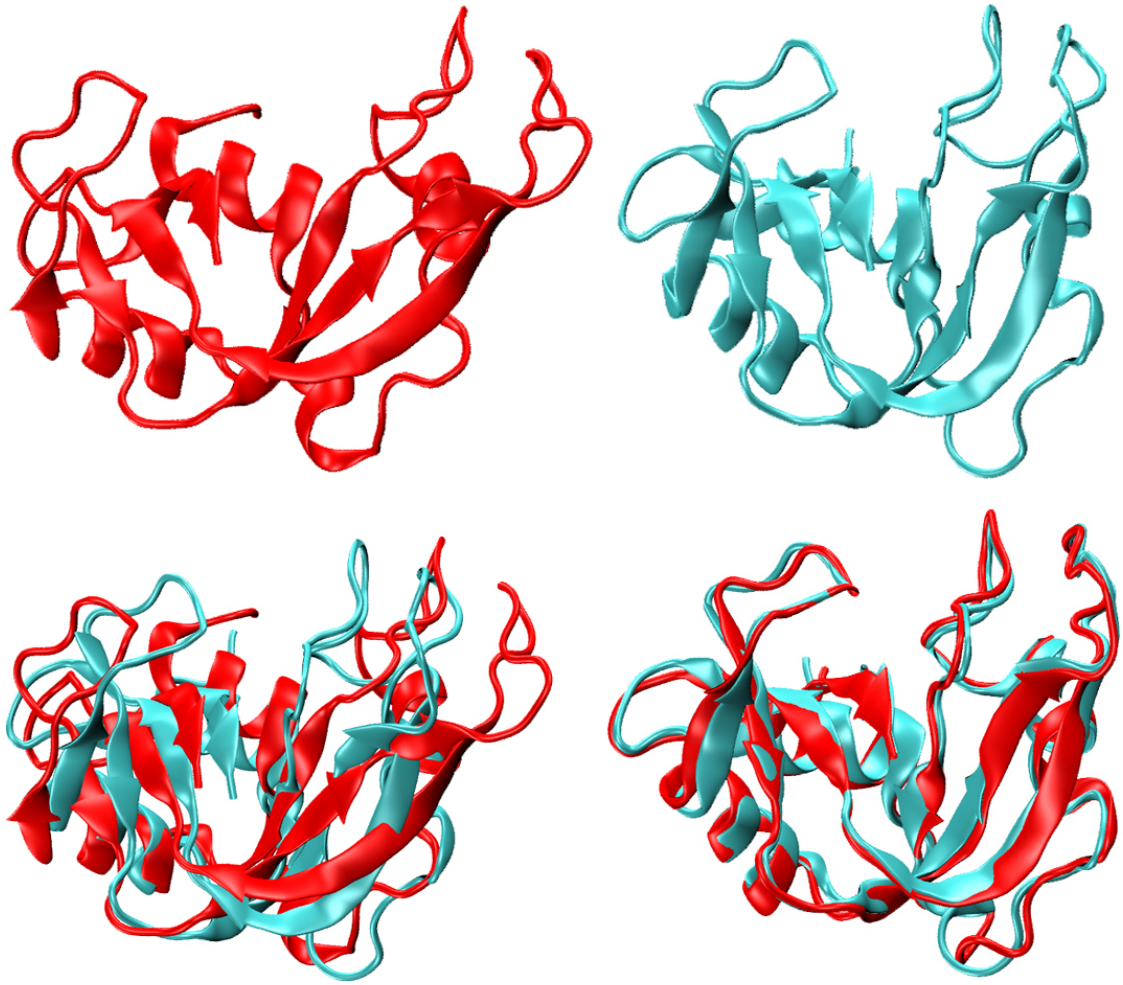

Figure S5

Structures obtained from a 2ns one-replica simulation with RDC restraints extracted from a closed conformation of the Gromos96 reference ensemble on the force field Amber99SB. The pincer angle of the target structure is 52.5^{o}. (A) Starting conformation (crystal structure 7RSA). (B) Target closed conformation from which RDCs data have been determined. (C) Overlay of the starting (red ribbons) and target (cyan ribbons) conformations; the RMSD between the two structures is 5.3Å. (D) Overlay of a representative conformation from the restrained simulations after imposing the restraints (red ribbons) and the target structure (cyan Ribbons); the RMSD between the two structures is 0.5Å.

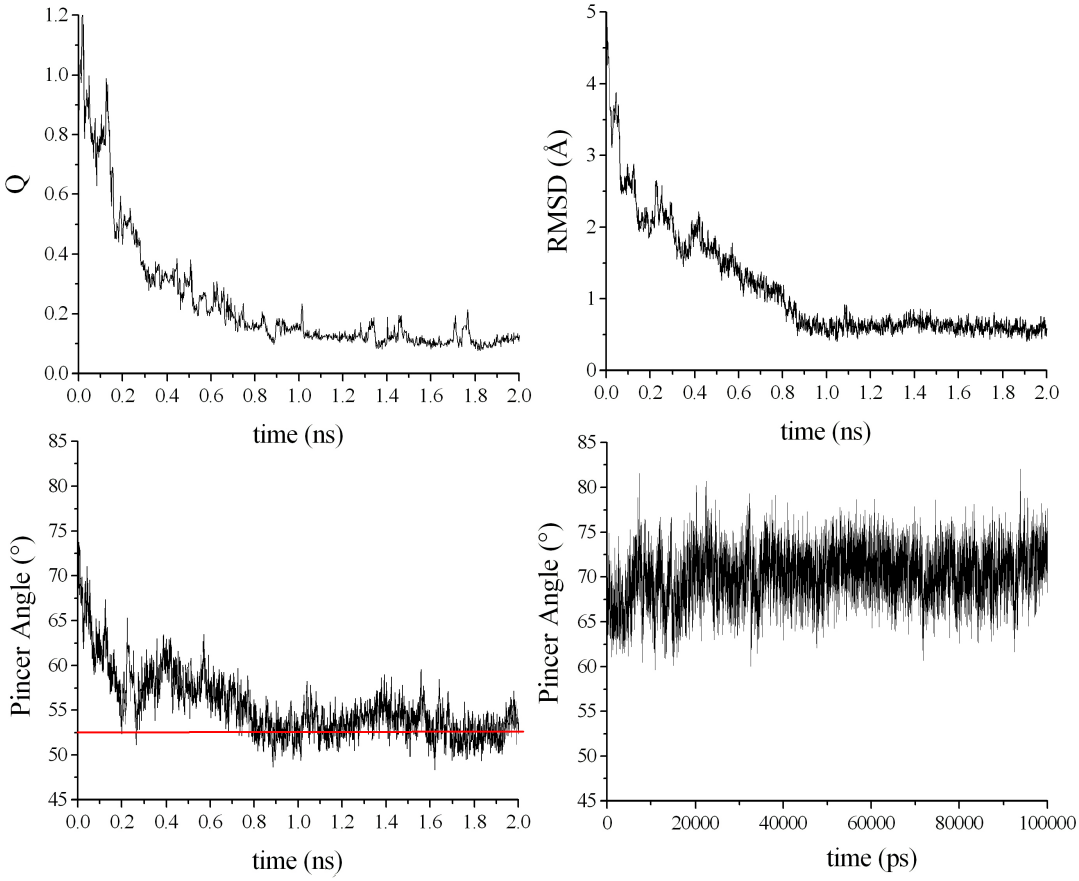

Figure S6

Time evolution of representative parameters in 2ns restrained molecular dynamics simulations starting from an open conformation and ending in a closed one (see the caption of Figure S2 for details). (a) Q factor. (b) RMSD from the target closed structure (i.e. the structure from which the RDCs imposed in the simulations were extracted). (c) Pincer angle in a restrained simulation; the pincer angle value for the target structure is indicated by a red line. (d) Pincer angle in an unrestrained simulation carried out with the same settings (including the Amber99SB force field, temperature, pressure, number of water molecules, titration of electrostatic interactions, see Methods) of the restrained simulations.