Supporting Information

Structural Assembly of Multidomain Proteins and Protein Complexes Guided by the Overall Rotational Diffusion Tensor

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A. Parameters of the overall rotational diffusion tensor for Maltose Binding Protein (MBP)

Experimental data (^{15}N T_1, $T_{1\rho}$ and ^{1}H-^{15}N NOE) collected at 37° C on 600 MHz Varian Inova spectrometer were kindly provided by the authors of ref.[1] (Prof. N. Skrynnikov). The data were analyzed with in-house computer program RotDif [2] using the first structure of the NMR ensemble (PDB code 1EZP) as a representative MBP structure. With few exceptions, only those amino acid residues identified by MolMol program [3] as belonging to secondary structure elements of MBP were included in the analysis. Here is the list of the residue numbers corresponding to these amino acids:

7, 8, 10, 19, 20, 23, 24, 25, 26, 27, 28, 36, 37, 38, 44, 46, 47, 49, 50, 59, 60, 61, 62, 63, 69, 70, 71, 85, 93, 94, 95, 96, 98, 99, 103, 106, 108, 114, 116, 117, 118, 135, 136, 138, 140, 145, 146, 147, 155, 156, 157, 158, 160, 161, 163, 167, 168, 169, 170, 171, 176, 177, 181, 182, 187, 188, 190, 191, 192, 193, 194, 196, 198, 200, 211, 212, 213, 217, 222, 223, 224, 225, 226, 230, 245, 249, 250, 253, 255, 258, 264, 265, 266, 275, 276, 279, 281, 288, 289, 291, 292, 293, 294, 296, 301, 302, 305, 306, 307, 308, 309, 317, 318, 320, 324, 326, 328, 329, 339, 341, 344, 346, 347, 348, 349, 351, 357, 358, 360, 362, 363, 365, 367

The RotDif analysis of ^{15}N relaxation data using computer program resulted in the following parameters of the overall rotational diffusion tensor:

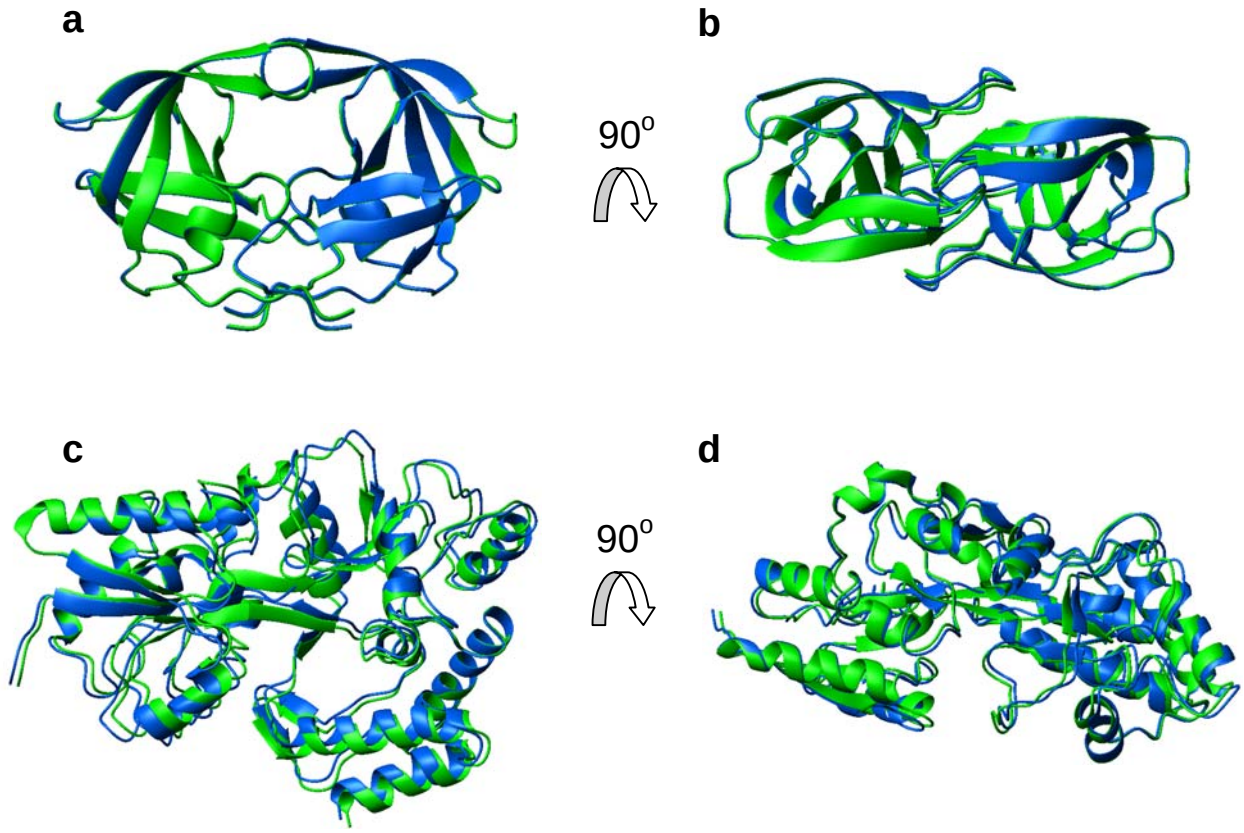
$D_x = 0.81 \pm 0.04\times10^7\ s^{-1}$; $D_y = 0.83 \pm 0.05\times10^7\ s^{-1}$; $D_z = 1.08 \pm 0.08\times10^7\ s^{-1}$.

The overall rotational correlation time τ_c=18.43 ± 0.69 ns.

The Euler angles (in the Y-convention) for the orientation of the diffusion tensor eigenvectors with respect to the molecular reference frame of 1EZP.pdb are:

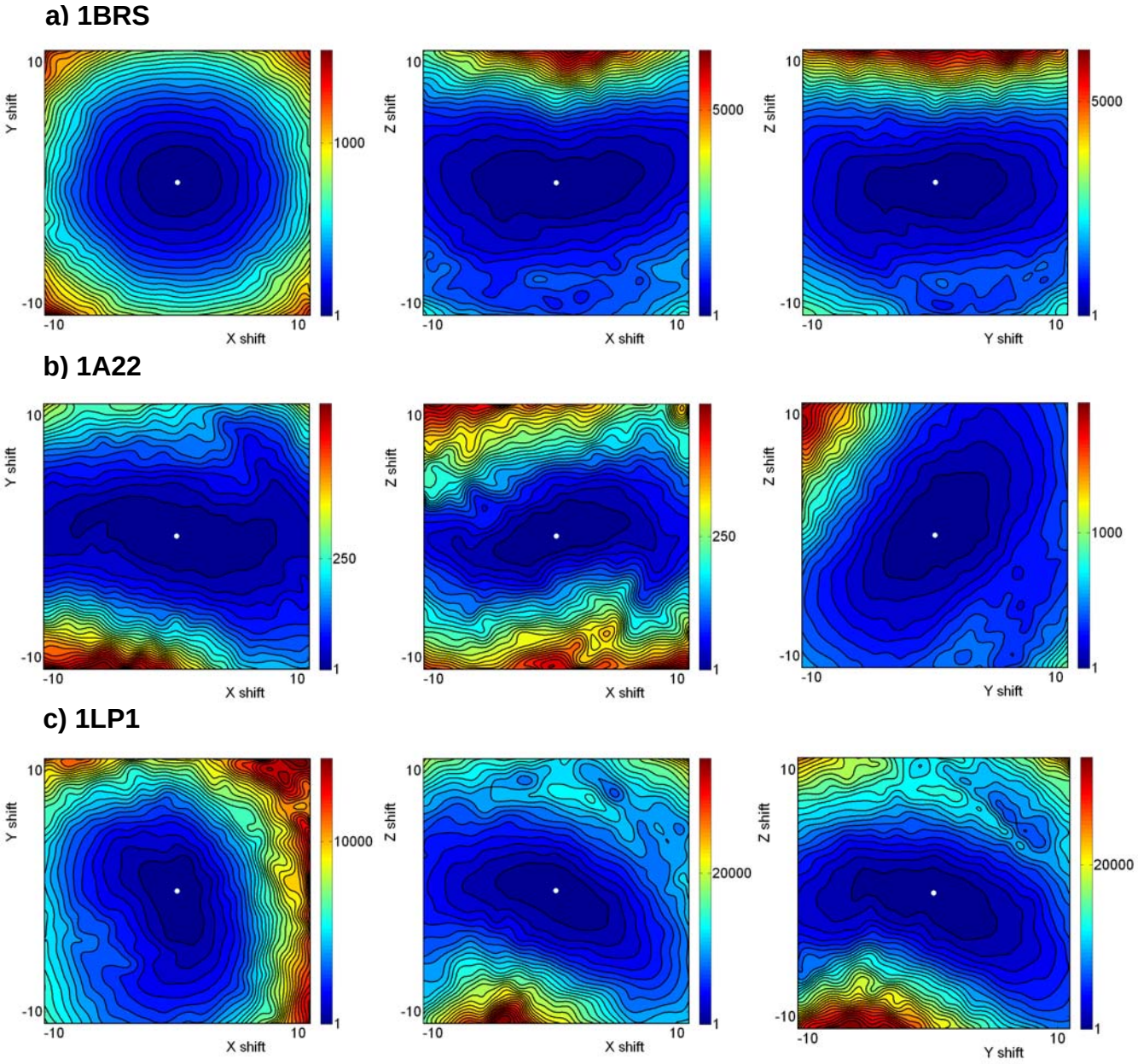
$\alpha = 178^o \pm 6^o$; $\beta = 81^o \pm 6^o$; $\gamma = 172^o \pm 54^o$.

B. Superimposition of the original and fitted structures of HIV-1 protease and MBP



Supporting Figure 1. Superimposition of the structures of (a,b) HIV-1 protease and (c,d) maltose binding protein determined using the proposed method with their NOE-based NMR structures. Shown is the backbone of the original (green) and fitted (blue) structures. The RMSDs are 0.36 Å (HIV-1) and 1.34 Å (MBP).

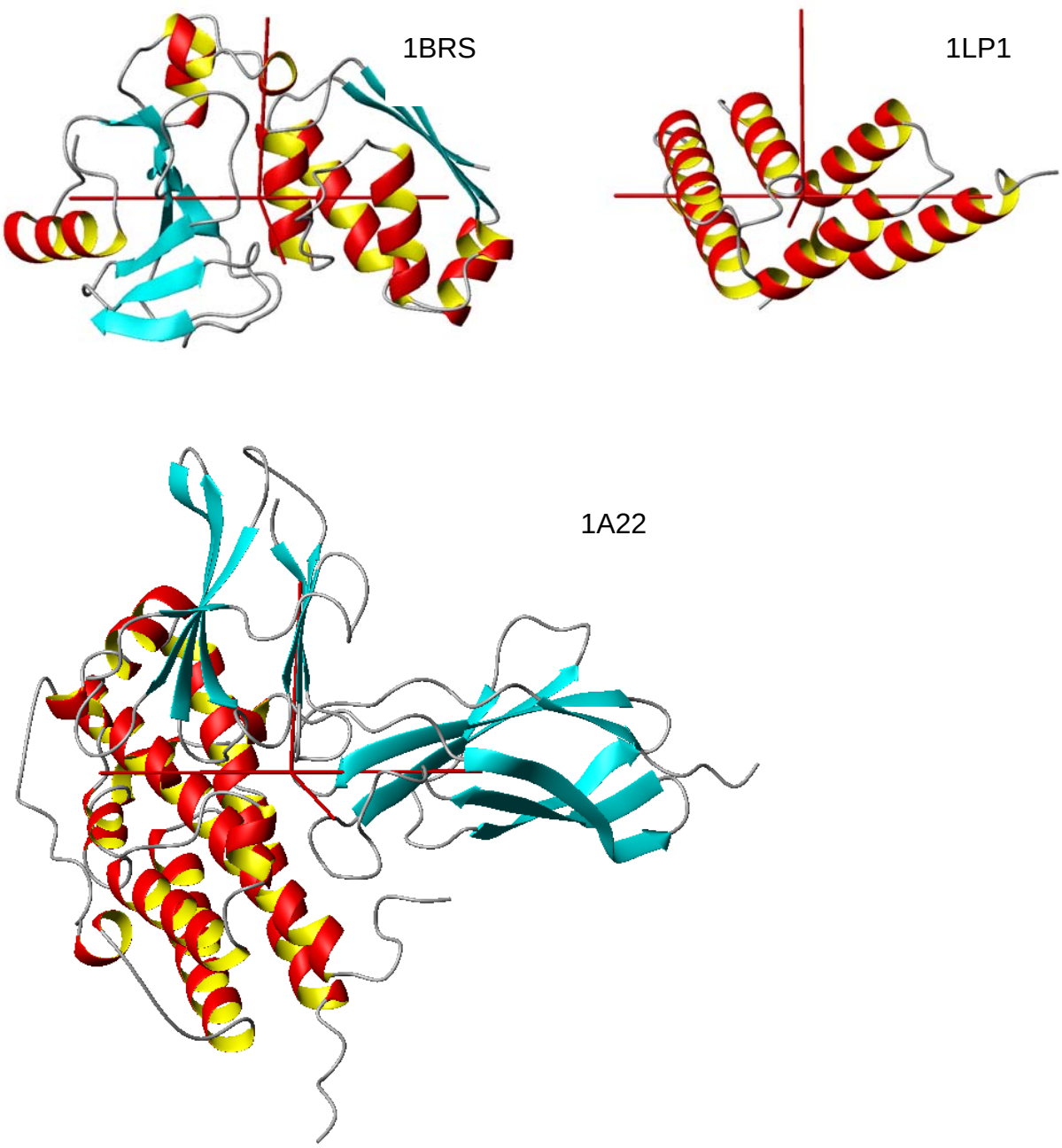
C. Analysis of proteins with unknown overall diffusion tensor parameters



Supporting Figure 2. Dependence of the normalized target function, $\chi^2/\chi^2_{\min}$, on domain displacements (in Å) along the principal axes of the predicted diffusion tensors: (a) for the barnase-barstar complex (PDB code: 1BRS), (b) for G120R mutant of human growth hormone (hGH) in complex with receptor extracellular domain (hGHbp) (PDB code: 1A22), and (c) for protein Z with an in vitro selected affibody (PDB code: 1LP1). White dots in all panels mark position of minimum, which corresponds to the original domain positions in these complexes.

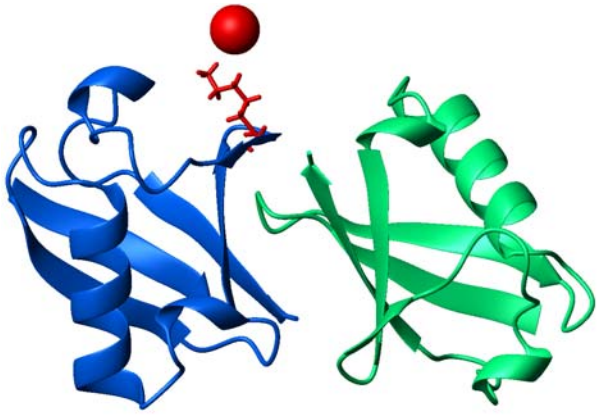
Supporting Table 1. Parameters of the overall rotational diffusion tensors and the quality of fit for 1BRS, 1A22, and 1LP1 structures, calculated using computer program ELM [4]. For all the structures we used Hydration Layer Thickness (HLT) 2.8 Å and evaluated diffusion tensor parameters at 293 K.

Structure	1BRS	1A22	1LP1
D_x [10^7 s^{-1}]	1.14	0.55	1.93
D_y [10^7 s^{-1}]	1.20	0.62	2.06
D_z [10^7 s^{-1}]	1.56	0.69	2.53
Euler angles specifying the orientation of the diffusion tensor eigenvectors with respect to the corresponding molecular frame			
α	149°	-28°	-46°
β	63°	60°	64°
γ	129°	77°	97°
Domain shift (0.5 $\|\Delta R\|$), [Å]	$8.6458 \cdot 10^{-4}$	0.0107	0.0011
Superimposed RMSD, [Å]	$6.3232 \cdot 10^{-4}$	0.0085	$8.0940 \cdot 10^{-4}$
χ^2_{min}	$1.7270 \cdot 10^{-9}$	$4.3780 \cdot 10^{-9}$	$6.1200 \cdot 10^{-9}$



Supporting Figure 3. Cartoon representation of the original 1BRS, 1A22, and 1LP1 structures, with the diffusion tensor axes shown as red rods. The structures are oriented such that the Z-axis of the diffusion tensor is horizontal and in the plane of the Figure. The original and fitted structures of these complexes are practically indistinguishable (see Supporting Table 1).

D. Validation of the Ub_2 structure using spin labeling data



Supporting Figure 4. Validation of the derived structure of the closed conformation of Ub_2 using site-specific spin labeling. Shown is the Ub_2 structure in the closed conformation, with the location of the unpaired electron of the spin label (red sphere) reconstructed from the measured signal attenuations in both Ub domains. Also shown (in red stick) is the side chain of Lys48 of the distal Ub – this residue was mutated to a cysteine in this study that served as a spin label attachment site. The distal domain is colored blue, the proximal is green. The procedures of attaching the spin label (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)methanesulfonate), measuring relaxation rate enhancement, and reconstructing its location from the observed signal attenuations are detailed elsewhere[5-7]. The open conformation is not shown – due to the low occupation probability (~10%) of this conformation and the significantly greater distance of the proximal domain from the spin label in this conformation, its effect on the position of the spin label is negligible, see ref [7].

References

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(3) Koradi, R.; Billeter, M.; Wuthrich, K. *J. Mol. Graph.* **1996**, *14*, 51-55.
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