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Predicting the Rotational Tumbling of Dynamic Multidomain Proteins and Supramolecular Complexes**

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Protein motion plays a key regulatory role in fundamental cellular processes, such as signaling, molecular recognition, cell-cycle control, replication, and transcription.[1] In higher organisms, many proteins contain long flexible tails and disordered linkers connecting folded domains.[2] Interdomain mobility is important for the interaction with different partners as it allows the adjustment of the relative distance and orientation that is required for binding.[3] In addition, post-translational modifications, ligand binding, protein–protein, and protein–nucleic acid interactions induce disorder-to-order transitions of flexible regions and switch proteins between different functional modes.[3,4] Interdomain contacts and ordering of disordered linkers introduce a correlation between the rotational motion of the protein domains and alter their rotational diffusion properties, in particular the rotational correlation time (τ_c). Thus, measurement of rotational correlation times by NMR spin relaxation, electric birefringence, fluorescence depolarization, and dielectric relaxation can provide insight into the function of biomolecules.[5] In addition, several NMR methods have been developed to describe conformational heterogeneity of flexible multidomain proteins.[6]

Interpretation of experimentally determined rotational correlation times in terms of protein interactions and disorder-to-order transitions relies on the ability to predict τ_c values from known three-dimensional structures. For rigid molecules, τ_c can be accurately determined through standard bead and boundary element hydrodynamic methods.[7] In addition, the presence of short disordered segments can be taken into account using ensemble methods.[7] In contrast, in flexible multidomain proteins and supramolecular complexes the overall tumbling of the protein, interdomain mobility, and local protein dynamics are coupled:[8] interdomain dynamics can lead to drastic changes in the shape of the biomolecule and thus its rotational tumbling. If in addition global and interdomain motions take place on comparable timescales, hydrodynamic coupling will occur and the protein diffusion model will be time-dependent.[8b] Thus, for many systems the extended model-free approach, which relies on the assumption that fast and slow motions are decoupled from each other and from the overall tumbling,[9] is not applicable. Moreover, a treatment of interdomain dynamics as jumps between two distinct conformational states[8a] is successful only if the overall diffusion tensor of the two states is highly similar and coupling between overall and interdomain motion is small. In a general case, rotational motion of rigid domains connected through flexible linkers is very complex, and parameters describing their orientational correlation function as a sum of exponentials do not have the same physical interpretation as in the model-free approach.[8b]

Herein we describe a method, named HYCUD (HYdrodynamic CoUpling of Domains), to calculate the rotational correlation time of flexible modular biomolecules. We apply the approach to homodimeric heterochromatin protein 1 (HP1), two and three copper-binding-domain constructs of Wilson disease protein (ATP7B), and different two-domain GB1 variants. The calculations demonstrate that HYCUD can accurately predict rotational correlation times in a wide variety of complex multidomain proteins. Comparison of rotational correlation times predicted by HYCUD for the 300 kDa HP1-nucleosome complex with experimental values revealed that HYCUD is applicable to large and dynamic supramolecular complexes.

The HYCUD method starts by construction of an ensemble of conformers for the multidomain protein or supramolecular complex (Supporting Information, Figure S1). To this end, a model of the protein/-protein complex is built using known high-resolution structures of the individual domains and arbitrary backbone conformations for flexible segments. Next, a large ensemble of conformations (up to 5000 conformers) without steric clashes is built using the flexible-meccano approach of the ensemble optimization method.[10] Folded domains are treated as rigid bodies, while random configurations of the Cα backbone are created for flexible segments using an empirical database of backbone torsion angles.[11] Borders between folded and disordered parts are defined on the basis of experimental data, such as NMR relaxation data. Alternatively disorder predictions may be used to define domain boundaries.[12] Subsequently, backbone and side-chain conformations are reconstituted on the basis of Cα coordinates using REMO.[13] Next, the rotational correlation time τ_{c0} and intrinsic viscosity $[\eta]$ for each globular domain in its isolated state is calculated using the atomic-level shell-model implemented in the program HYDROPRO10.[14]

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To this end, non-hydrogen atoms of the protein are replaced by spheres with the same atomic effective radius (AER). In the HYCUD calculations reported herein, the adjustable parameter AER of the globular domains was set to a value that gave the best agreement with the known experimental τ_c of the "isolated" domains. For disordered linkers and tails, an AER of 3.0 Å was used. Disordered segments exceeding 14 residues are divided into non-overlapping fragments of approximately 14 residues, that is, twice the persistence length of coil-like polypeptide chains.[15] At the end of this procedure, rotational correlation times and intrinsic viscosities for each domain or disordered segment in the conformer ensemble of the multidomain protein are available.

The key step in HYCUD is the calculation of the scaling factor that approximately describes the "relative viscosity" experienced by each domain/disordered segment i in the full-length protein. To this end, the effective concentration of domain/segment j in the vicinity of domain i is calculated on the basis of the distance, r_{ij}, between the center of mass of the two domains. The effective concentration of domain j, c_j, is defined as the concentration for which the expected value to find the domain j at the distance r_{ij} from domain i is 1 (see Supporting Information for further details). The effective "relative viscosity" (η/η_0) is then obtained as a first-order approximation through Equation (1):

$$\frac{\eta_i}{\eta_0} = 1 + \sum_{j \neq i} [\eta]_j c_j \quad (1)$$

The rotational correlation time τ_c of domain i is calculated according to Equation (2):

$$\frac{\tau_{ci}}{\tau_{coi}} = \frac{\eta_i}{\eta_0} \quad (2)$$

τ_{ci} in the flexible multidomain protein/complex is finally obtained by taking the average over the ensemble of protein models.

HYCUD was first applied to heterochromatin protein HP1. HP1 is a key regulator of chromatin structure and function and contains two globular domains, chromodomain (CD) and chromoshadow domain (CSD). The two domains are connected by a 37-residue linker. In addition, two flexible tails are attached to the N- and C-terminal sides of the protein (Figure 1a and Figure S2).[16] The CSD provides an interface through which HP1 dimerizes.[17] Using NMR spectroscopy, we recently demonstrated that there are no stable domain–domain or domain–linker contacts, and the interdomain linker and the tails are disordered.[18] To investigate the influence of different modular arrangements of HP1 on the τ_c of its CD domain, we compared τ_c in full-length HP1,[18] with those of the isolated CD with a short tail (residues 19–79) and the HP1 variant hHP1βI161A, which is monomeric (Figure 1a).[17b,19] At 25 °C, the average τ_c of CD(19–79) was (5.2 ± 0.3) ns. In monomeric HP1, the τ_c of CD rose to (9.8 ± 1.3) ns (Figure S3), a value similar to that observed for the CD in wild-type, that is, dimeric, HP1 (τ_c = (10.2 ± 1.2) ns).[18] Corresponding values for the CSD were (10.5 ± 1.1) and (24.6 ± 5.0) ns in monomeric (Figure S3) and dimeric HP1,[18] respectively. The similarity of CD domains' τ_c values in monomeric and dimeric HP1 indicates that the rise of τ_c with respect to the isolated CD is mainly caused by the N-terminal tail and the hinge region of the same HP1 molecule.

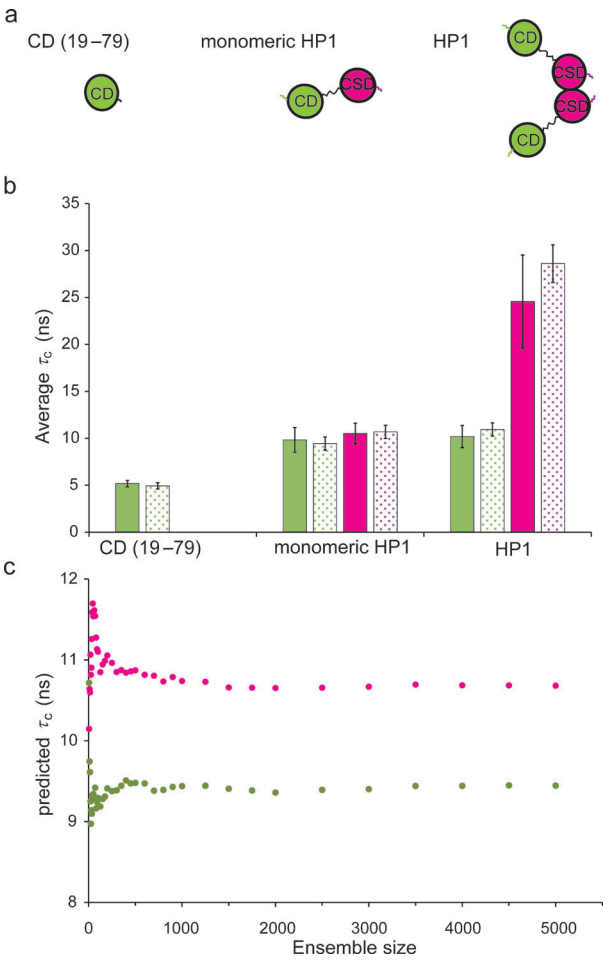

Figure 1. Calculation of rotational correlation times in flexible multidomain proteins using HYCUD. a) Schematic representation of heterochromatin protein 1 (HP1) variants, containing chromo (CD) and chromoshadow domains (CSD). b) Effective rotational correlation time (τ_c) of CD (green) and CSD (magenta) in three states: CD(19–79), monomeric HP1, and full-length dimeric HP1. Experimental τ_c values (filled columns) and values calculated by HYCUD (dotted columns). c) Influence of ensemble size on τ_c values calculated by HYCUD for CD (green) and CSD (magenta) in monomeric HP1.

Next, the τ_c values of CD and CSD in the different multidomain arrangements were predicted using HYCUD. The predicted τ_c of CD (19–79), that is, the globular domain (residues 19–72) with a short C-terminal tail was in agreement with the experimental value when using an AER of (3.3 ± 0.2) Å. Using the same AER but without the C-terminal extension, the predicted τ_c was (4.1 ± 0.3) ns, indicating that inclusion of the short C-terminal tail increases τ_c by about 20%. For CD and CSD in monomeric HP1, HYCUD predicted τ_c values of (9.4 ± 0.7) ns and (10.7 ± 0.7) ns, respectively, in close agreement with the experimental results (Figure 1b). In dimeric HP1, τ_c values of (10.9 ± 0.7) ns for CD and (28.6 ± 2.0) ns for CSD were obtained. Thus, the HYCUD approach was able to reproduce the non-trivial similarity of τ_c of CD in monomeric and dimeric HP1.

We then investigated how HYCUD results depend on adjustable parameters, such as ensemble size, AER, and the size of fragments in disordered segments. Figure 1c demonstrates that HYCUD calculations converged with 500–1000 conformers. In addition, HYCUD predictions changed only slightly when the AER for disordered tails and linkers was varied between 2.75 and 3.25 Å (Figure S4a). This is an important finding, because there is little experimental basis for the selection of the AER for disordered polypeptides—in contrast to globular protein domains, where the AER can be chosen on the basis of an experimentally known τ_c value. Finally, the specific fragment size in disordered regions in the range from 6–18 residues showed little impact on HYCUD predictions (Figure S4b). Altogether, the data indicate that HYCUD predictions are robust with respect to the choice of the available flexible parameters.

Wilson disease protein (ATP7B) is a membrane protein playing an important role in copper homeostasis.[20] The two- and three-domain constructs of this protein, WLN56 and WLN4–6, respectively, have been characterized by NMR spectroscopy.[21] In WLN4–6, domain 4 (72 residues) is connected to domain 5 (72 residues) through a 58-residue flexible linker and a short linker of 8 residues joins domain 5 to domain 6 (69 residues; Figure S5).[21c] An NMR study has shown that at 25 °C, the isolated domain 6 tumbles with a τ_c of (4.5 ± 0.3) ns, while the two-domain construct (WLN56: domains 5 and 6) reorients in solution as a single dumbbell-shaped molecule with a τ_c of (9.1 ± 0.6) ns.[21b] In contrast, in the three-domain protein WLN4–6, domains 4, 5, and 6 follow distinct rotational correlation times of (6.3 ± 0.1), (9.7 ± 0.1), and (8.2 ± 0.1) ns at 35 °C.[21c] The difference between the observed τ_c values of domains 5 and 6 suggests that the connecting 8-residue linker is at least partially flexible.[21c]

We used HYCUD to obtain insight into the rotational motional behavior of the ATP7B domains. Assuming that the 8-residue linker between domains 5 and 6 is fully flexible, HYCUD estimates the τ_c values of domain 5 and 6 in WLN56 as (8.1 ± 0.5) ns and (7.6 ± 0.5) ns, respectively (Figure S6). On the other hand, if the linker was fully rigid and domains 5 and 6 moved as a single rigid body (protein databank (PDB) code: 2EW9), a τ_c of (10.9 ± 0.7) ns would be expected. The experimentally observed τ_c value (9.1 ± 0.6) ns is within these two limits, indicating that the movement of the two domains is partially coupled. Consistent with this conclusion, ^{1}H,^{15}N-heteronuclear NOEs (nuclear Overhauser effects) in the linker are above zero.[21b] For the three-domain construct WLN4–6, HYCUD predicts at 35 °C τ_c values of (6.6 ± 0.4), (8.9 ± 0.6), and (7.0 ± 0.5) ns, for domains 4–6, respectively, when both interdomain linkers are considered as being fully flexible (Figure S5). The predicted τ_c value of domain 4 is in excellent agreement with the experimental value, but those of domains 5 and 6 are slightly smaller. We repeated our calculation assuming this time that the linker between domains 5 and 6 was fully rigid. The newly calculated values were (6.7 ± 0.4) ns for domain 4, with no significant change with respect to the previous calculation, and (13.8 ± 0.9) ns for the combined domains 5 and 6. The predicted combined τ_c values of domains 5 and 6 goes far beyond the experimental values, indicating that the linker between them must be highly flexible. Partial correlation of reorientational motion of globular domains could also be caused by short interdomain linkers, as it is shown in the case of GB1 domains connected through 3- or 6-residue linkers (see Supporting Information and Figure S7).

The interaction of HP1 with nucleosomes that are methylated at Lys9 in histone 3 (H3K9me3) is important for heterochromatin assembly.[22] We recently demonstrated that the CD of human HP1β bound to the H3 tail in the context of the mono-nucleosome particle constitutes the only anchoring point for the hHP1β-H3K9me3 nucleosome complex.[19] However, despite the absence of a direct contact between dimeric CSD of HP1 and the mono-nucleosome (Figure S8), rotational tumbling of the CSD was significantly slowed down in the hHP1β-H3K9me3 nucleosome complex.[19] The measured rates of cross-correlation between ^{15}N chemical shift anisotropy and ^{15}N, ^{1}H dipole–dipole coupling relaxation mechanisms suggested that the τ_c of CSD in the complex is approximately 2.2 times larger than in free HP1.[19] The average τ_c of CSD in free hHP1β has been reported as (24.6 ± 5.0) ns,[18] therefore τ_c of CSD in the complex is estimated to be (54.1 ± 10.9) ns.

To test if the increased τ_c value of CSD in the hHP1β-H3K9me3 nucleosome complex can be explained based on hydrodynamic coupling, we employed the HYCUD approach. We treated the nucleosome core (histone octamer + DNA as in 3LZ1.pdb),[23] two CDs in complex with the two modified H3 tails, which are present in a mono-nucleosome (PDB code: 1GUW),[24] and the CSD dimer (chains A,B of 2FMM.pdb)[25] as rigid bodies. The two hinge regions between CD and CSD as well as the two H3 tails (residues 18–38) were modeled as random conformations (Figure S8, see Supporting Information for further details). Fluorescence anisotropy decay data have shown that nucleosome core particles tumble with a τ_c of approximately 140 ns (in H_2O, 25 °C).[26] This value was used to calibrate the AER of mono-nucleosome, and the translational diffusion coefficient of nucleosome obtained with this AER was well matched to the experiment.[27] HYCUD then predicted a τ_c value of (46.3 ± 3.0) ns for the CSD within the HP1-nucleosome complex (Figure 2). Notably, in an alternative scenario, called "bridging model", in which one CD of HP1 binds to the H3K9me3 H3-tail of one mono-nucleosome and the second CD binds to another mono-nucleosome,[19,28] a similar τ_c value of (50.9 ± 3.3) ns was predicted for CSD. The predicted values agree well with the experimental τ_c, considering the large experimental error of τ_c inherent in the NMR analysis of this large system. We conclude that HYCUD can predict the rotational behavior of protein domains within supramolecular assemblies.

The HYCUD method is distinct from previous approaches, which modeled the rotational correlation time of proteins with disordered segments using the boundary element method.[29] In these previous approaches, the perturbation of the velocity field by the disordered ensemble was described by an exponential decay. The exponential decay is characterized by three parameters: δ, the distance to the rigid surface element, ε, a modulator for the decay rate for velocity

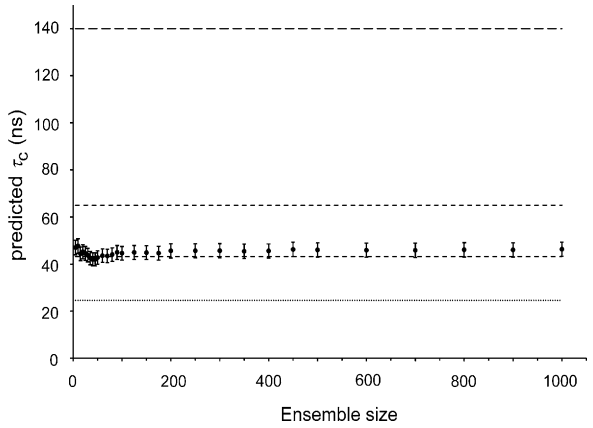

Figure 2. Calculation of the rotational correlation time of CSD in the HP1-nucleosome complex as a function of the number of conformers in the HP1-nucleosome ensemble. The experimental τ_c value of CSD in dimeric HP1[18] (dotted line), the average(±standard deviation) τ_c value of CSD in HP1 bound to nucleosome[19] (short dashed lines), and the τ_c value measured by fluorescence anisotropy for the nucleosome core particle at 25 °C in H_2O[26] (long dashed line) are shown.

correlation, and γ, a distance margin that expands the surface boundary and accounts for possible variations in the hydration between rigid and disordered parts. The two parameters ε and γ are variable, δ can be defined in three different ways. Although prediction of rotational correlation times in proteins with short disordered segments is robust with respect to the choice of the three parameters, proteins with longer disordered tails/linkers show a severe dependence on the choice of the parameters, strongly limiting the predictive capabilities of these previous approaches. In contrast, HYCUD has only one variable parameter, the atomic effective radius AER. The AER can be optimized based on the experimental τ_c values observed in the isolated domain. Alternatively, translational diffusion data of the full-length protein can be used to estimate the AER. In the latter case, the relevant thickness of the hydration layer should be reduced by about 1 Å for τ_c prediction when compared to the value that fits best to the translational diffusion data.[30] In addition, the range of physically acceptable AER values is constrained,[30,31] and theoretical considerations put a lower limit on the AER-dependent intrinsic viscosity.[31] Thus, HYCUD is a very robust method to predict rotational correlation times in flexible multidomain proteins and biomolecular complexes (Figures 1 and 2).

The function of supramolecular machines relies on their translational and rotational motions as a whole and the relative motions of their different parts with respect to each other. The complex of HP1 with nucleosome (Figure S8) is a very modular and dynamic assembly. It comprises several domains and flexible segments: a rigid nucleosome core, flexible histone tails, rigid chromo- and chromoshadow domains of HP1, a flexible linker connecting the CD and CSD, and flexible N- and C-terminal tails of HP1. In addition, the chromoshadow domain dimerizes resulting in two CD domains in each HP1 molecule. In the bridging mode of the HP1-nucleosome complex, the molecular weight of the complete complex exceeds 500 kDa. The success of HYCUD to reproduce the rotational motional behavior of CSD in the context of the HP1-nucleosome complex opens the way to analyze functional motions, disorder-to-order transitions, and interactions in protein–nucleosome complexes and other flexible supramolecular assemblies.

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