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Abstract

The functions of biological macromolecules are highly correlated with their structures, which can often be determined at atomic resolution using X-ray crystallography or nuclear magnetic resonance (NMR) methods. However, these are not problem-free operations. For instance, suitable crystals might be difficult or impossible to obtain, and packing constraints might induce conformational changes, while current NMR methods still have size limitations. Lower-resolution solution parameters such as translational and rotational diffusion coefficients, and the sedimentation coefficient, are instead relatively easily measured. Computing these parameters from atomic-level resolution structures can then offer additional insights, like allowing the investigator to verify the correspondence between crystal and solution structures, or, in multi-resolution modeling, to discriminate between alternative models. The UltraScan SOlution MOdeler (US-SOMO) suite presented in this work provides a comprehensive, flexible interface for accurately computing solution parameters from atomic-level structures of biomacromolecules through beadmodeling and cubic grid approaches.

Introduction

Computing hydrodynamic parameters, such as the translational diffusion coefficient D_t , the sedimentation coefficient s, the rotational relaxation time τ_h , and the intrinsic viscosity [η] from atomic-level structures is not a straightforward task. Bead modeling, where a macromolecule is represented by an array of non-overlapping spheres of variable diameter, is the most widely used technique to compute the hydrodynamics, and various methods have been implemented in public-domain computer programs. To overcome the limitations of existing software, we have developed the freely available UltraScan Solution Modeler (US-SOMO) suite. US-SOMO provides a flexible interface with a user-friendly GUI (see Figure 1) for accurately computing a multitude of solution parameters from 3D structures using two bead-modeling approaches.

X SOMO Solution Modeler											
Lookup Tables SOMO Options PDB Optio	ns <u>C</u> onfigurations	<u>F</u> ile									
	PDB Functions:	All options set to default values PDB HEADER: HYDROLASE (NUCLEIC ACID.RNA) 13-AUG-91 8RAT									
Select Lookup Table	/usr/local/ultrascan/etc/somo.residue	PDB TITLE : EFFECTS OF TEMPERATURE ON PROTEIN STRUCTURE AND DYNAMICS: X- PDB TITLE : RAY CRYSTALLOGRAPHIC STUDIES OF THE PROTEIN RIBONUCLEASE-A PDB TITLE : AT NINE DIFFERENT TEMPERATURES FROM 08 TO 320 K									
Batch Mode Operation		Residue sequence from 8RAT.pdb:									
Load Single PDB File	/home/ultrascan/ultrascan/somo/structures/8RAT.pdb	ALA SER SER SER ASN TYR CYS ASN GLN MET MET LYS SER ARG ASN LEU THR LYS ASP ARG CYS LYS PRO VAL ASN THR PHE VAL HIS GLU SER LEU ALA ASP VAL GLN ALA VAL									
Please select a PDB Structure:	Model: 1	CYS SER GLN LYS ASN VAL ALA CYS LYS ASN GLY GLN THR ASN CYS TYR GLN SER TYR SER THR MET SER ILE THR ASP CYS ARG GLU THR GLY SER SER LYS TYR PRO ASN CYS ALA TYR LYS THR THR GLN ALA ASN LYS HIS ILE ILE VAL ALA CYS GLU GLY ASN PRO TYR VAL PRO VAL HIS PHE ASP ALA SER VAL Checking the pdb structure for model 1 Loaded pdb file : ok 8RAT models selected: 1									
View/Edit PDB File											
SAXS/SANS Functions											
Bea	d Model Functions:	Building the bead model for 8RAT model 1 Checking the pdb structure									
Bead Model Suffix:	A20R50hiOT-so	PDB structure ok There are 951 atoms in 2 chain(s) in this model Creating heads from atomic model									
Overwrite existing filenames	Add auto-generated suffix	Computing ASA via ASAB1 Return from Computing ASA									
Build SoMo Bead Model	Build AtoB (Grid) Bead Model	There are 246 beads in this model before popping Begin popping stage 1									
Grid Existing Bead Model	Automatically Calculate Hydrodynamics	Beads popped 0. Begin radial reduction stage 1 Begin popping stage 2									
View ASA Results	Visualize Bead Model	Beads popped 0. Begin radial reduction stage 2									
Batch Mode Operation	View Bead Model File	Begin popping stage 3 Beads popped 0. Beain radial reduction stage 3									
Load Single Bead Model File	not selected	Finished with popping and radial reduction Rechecking beads									
SAXS/SANS Functions		0 previously buried beads are exposed by rechecking Finished rechecking beads Build bead medal completed									
Hydrod	lynamic Calculations:	All options set to default values									
Calculate Hydrodynamics	Show Hydrodynamic Calculations	Begin hydrodynamic calculations Model 1 will be included									
	Open Hydrodynamic Calculations File	Processing model 1 bead count 246 vbar 0.71 Using 98 beads for the matrix									
Select Parameters to be Saved	Save parameters to file	Supermatrix inversion Cycle 1 of 3 Supermatrix inversion Cycle 2 of 3 Supermatrix inversion Cycle 3 of 3									
Stop	Close	Calculate hydrodynamics completed									
Help	100%										

Figure 1: The US-SOMO main GUI panel, with the commands module on the left side and the progress window on the right side (shown is the processing of the RNase A 8RAT.PDB structure).

The UltraScan SOlution MOdeler (US-SOMO) Software Suite and its application to the modeling of integrin $\alpha_{IIb}\beta_3$.

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US-SOMO layout and properties

As shown in Figure 1, the main US-SOMO GUI has three sections, dealing with PDB uploading/parsing and related operations, bead model(s) generation/editing/visualization, and hydrodynamic computations. US-SOMO operates by comparing atom and residue entries in the PDB file with those stored in lookup tables, and then properly assigning radii, masses and theoretical hydration values. The lookup tables are editable from a pull-down menu, and include a hybridizations table [TTCG99], an atom table (where atomic groups are defined), a SAXS coefficients table, and a residue table, where the global properties of each residue (e.g. the partial specific volume) and the rules governing their conversion to one or more beads are also defined. Structures and models are visualized with RasMol. Approximate methods to deal with structures with incomplete residues or with non-coded residues are provided, accessible from the PDB-options pull-down menu. Several finecontrol panels can be opened from the SOMO options pulldown menu.



The SoMo direct Figure correspondence method applied to the RNase A 8RAT.PDB structure.



Figure 3: The A2B grid (5 Å) method applied to the RNase A 8RAT.PDB structure.

US-SOMO bead generation routines

Two methods are available, a direct correspondence (SoMo) method (Figure 2) and a grid (A2B) approach (Figure 3). In the SoMo method (Figure 2), an ASA routine identifies exposed and buried side- and main-chain segments in the original structure (A); beads representing the exposed side-chains (cyan, red, yellow & green beads) are then generated (volume = sum of the atoms volumes + theoretical H2O volume) and placed according to specific rules (B); overlaps between beads are removed by fusion above a certain threshold (white beads) and then hierarchically or synchronously proportionally reducing the beads' radii, while moving their centers outwardly by the same amount (C; this preserves the original surface); the same procedure is then used, without the outward translation, for the main-chain exposed beads (D, blue beads); finally, buried residues beads (orange) are generated, placed and their overlaps removed (E).



US-SOMO A2B bead generation

The A2B grid method method (Figure 3) proceeds similarly to the direct correspondence method: all beads are placed according to the grid size (B); surface exposed beads (red) are identified (C); surface beads overlaps are removed (D); buried beads (orange) overlaps are removed and the ensemble is rescreened for exposed/buried beads (E).

Performance of US-SOMO

Table 1 lists the comparison between experimental and computed D_t , τ_h , and $[\eta]$ for the SoMo method using the hierarchical or the synchronous overlap removal procedures for a series of proteins ranging from ~14,000 to ~149,000 Daltons in molecular weight. Buried beads are not used in the computations, significantly reducing the computer load. Note the excellent agreement for D_t (usually < 2%) across the entire range, and the good performance for τ_h and [η] (usually within 5%). Similar results are obtained using the A2B approach (not shown). US-SOMO is fully described and validated in two publications [BDRR09],[BDR10].

Table 1. Comparison between experimental (Exp.) and computed (Comp.) values of $D_{t(20,w)}^{o}$, $\tau_{h(20,w)}^{0}$ and $[\eta]$ for several test proteins using the two different overlap removal procedures (HI, hierarchical; SY, synchronous) within the direct correspondence SoMo bead modeling method (ASA cutoff 20 Å: peptide bond rule on; fusion threshold 70%; outward translation on; ASA re-check threshold 50%).

Protein	PDB	MW	$D_{t(20,\mathbf{w})}^{0}$, % diff. from exp.		$ au_{\mathbf{h}(20,\mathbf{w})}^{0}$, % diff. from exp.			$[\eta]$, % diff. from exp.			
			F			ns			$cm3 \cdot g^{-1}$		
			Exp.	Comp., HI	Comp., SY	Exp.	Comp., HI	Comp., SY	Exp.	Comp., HI	Comp., SY
RNase A	8RAT	13 682	11.6±0.3	11.8 (+1.7)	11.8 (+1.7)	8.05 ± 0.51	7.79 (-3.2)	7.74 (-3.9)	3.30±0.04	3.24 (-1.82)	3.22 (-2.42)
α -Lactalbumin	1A4V ^{a)}	15 793	10.9	10.9 (0)	10.9 (0)	10.3 ± 2.7	10.25 (-0.5)	10.14 (-1.6)	na ^{b)}	3.63 (nd ^{c)})	3.59 (nd)
Myoglobin (CO)	1DWR	17 521	10.7	10.9 (+1.9)	10.9 (+1.9)	10 ± 1	10.0 (0)	9.88 (-1.2)	na	3.24 (nd)	3.20 (nd)
Chymotrypsinogen A	2CGA	25 666	9.5	9.64 (+1.5)	9.64 (+1.5)	na	13.8 (nd)	13.7 (nd)	3.21	3.13 (-2.5)	3.10 (-3.4)
β-Lactoglobulin	1BEB	36 608	7.85 ± 0.08	7.92 (+0.9)	7.92 (+0.9)	23.2	25.1 (+8.2)	24.8 (+7.1)	na	3.92 (nd)	3.88 (nd)
Ovalbumin	10VA	43 157	$\textbf{7.73} \pm \textbf{0.04}$	7.78 (+0.7)	7.78 (+0.7)	20.9	26.2 (+25.4)	25.9 (+24.1)	4.0 ± 0.5	3.48 (-13.0)	3.44 (-14.0)
Hemoglobin CO	1HCO	64 557	6.9	6.98 (+1.2)	6.98 (+1.2)	na	35.6 (nd)	35.4 (nd)	na	3.28 (nd)	3.25 (nd)
Hemoglobin oxi	1GZX	64 573	7.21	6.99 (-3.1)	6.99 (-3.1)	35.4	34.9 (-1.1)	34.7 (-1.8)	3.16 ± 0.2	3.18 (+0.6)	3.16 (0.0)
Citrate synthase	1CTS	97 838	5.8	5.86 (+1.0)	5.86 (+1.0)	na	59.5 (nd)	59.1 (nd)	3.95	3.51 (-11.1)	3.49 (-11.7)
G3PD apo	2GD1	143540	5.0	5.08 (+1.6)	5.08 (+1.6)	na	90.2 (nd)	89.6 (nd)	na	3.59 (nd)	3.56 (nd)
G3PD holo	1GD1	146431	5.3	5.10 (-3.8)	5.10 (-3.8)	na	88.9 (nd)	88.6 (nd)	3.45	3.48 (+0.9)	3.46 (+0.3)
Lactate	5LDH	148636	$\textbf{5.06} \pm \textbf{0.15}$	5.14 (+1.6)	5.14 (+1.6)	na	89.1 (nd)	88.3 (nd)	na	3.46 (nd)	3.43 (nd)
dehydrogenase											

^{a)}Modified with the addition of a Nag₄Man₃Gal₂ biantennary carbohydrate chain attached to Asn45.^{[11] b)}na: not available; ^{c)}nd: not done.

Future

As we continue to improve US-SOMO, we are developing the ability to simulate SAXS and SANS data from atomic structures. We will also implement Brownian Dynamics and Discrete Molecular Dynamic methods to compute hydrodynamic parameters for structures presenting local or larger scale flexibility issues.

References

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