Published in final edited form as:

J Mol Biol. 2008 October 17; 382(4): 1089–1106. doi:10.1016/j.jmb.2008.07.074.

Integration of small angle X-ray scattering data into structural modeling of proteins and their assemblies

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Summary

A major challenge in structural biology is to determine the configuration of domains and proteins in multi-domain proteins and assemblies, respectively. To maximize the accuracy and precision of these models, all available data should be considered. Small angle x-ray scattering (SAXS) efficiently provides low-resolution experimental data about the shapes of proteins and their assemblies. Thus, we integrated SAXS profiles into our software for modeling proteins and their assemblies by satisfaction of spatial restraints. Specifically, we model the quaternary structures of multidomain proteins with structurally defined rigid domains as well as quaternary structures of binary complexes of structurally defined rigid proteins. In addition to SAXS profiles and the component structures, we employ stereochemical restraints and an atomic distance-dependent statistical potential. The scoring function is optimized by a biased Monte Carlo protocol, including quasi-Newton and simulated annealing schemes. The final prediction corresponds to the best scoring solution in the largest cluster of many independently calculated solutions. To quantify how well the quaternary structures are determined based on their SAXS profiles, we used a benchmark of 12 simulated examples as well as an experimental SAXS profile of the homo-tetramer D-xylose isomerase. Optimization of the SAXS-dependent scoring function generally results in accurate models, if sufficiently precise approximations for the constituent rigid bodies are available; otherwise, the best scoring models can have significant errors. Thus, SAXS profiles can play a useful role in the structural characterization of proteins and assemblies, if they are combined with additional data and used judiciously. Our integration of a SAXS profile into modeling by satisfaction of spatial restraints will facilitate further integration of different kinds of data for structure determination of proteins and their assemblies.

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Keywords

small-angle X-ray scattering; quaternary structure; macromolecular assembly modeling; statistical potentials; protein structure prediction

Introduction

A comprehensive structural description of proteins, nucleic acids, and their assemblies will help us discover the principles that underlie cellular processes and bridge the gaps between genome sequencing, functional genomics, proteomics, and systems biology^{1; 2}. While X-ray crystallography and NMR spectroscopy can provide accurate high-resolution structures, these methods are limited by the difficulties in protein purification, stability of large complexes, crystallization (X-ray), and size (NMR). Single particle cryo-electron microscopy (cryo-EM) generally does not provide atomic-resolution structures and currently cannot be applied to systems smaller than approximately 250 kDa. While efficient, computational protein structure prediction methods are limited by their accuracy. These difficulties may be overcome by computational methods that effectively combine experimental, theoretical, and statistical information^{2; 3}.

Small angle X-ray scattering (SAXS) can rapidly provide low-resolution information about the shape of a macromolecule or a complex in solution^{4; 5; 6; 7}. A SAXS measurement determines the molecule's rotationally averaged scattering intensity as a function of spatial frequency, $I(q)$, typically at 1-3 nm resolution^{5; 7}. This profile can be readily transformed into an electron pair distance distribution function $P(r)$, which is essentially a histogram of all pairwise distances r of the electrons in the sample. Due to the rotational averaging, the information content of a SAXS profile is dramatically reduced compared to an X-ray crystallographic diffraction pattern or even a density map from cryo-EM. However, one of the advantages of SAXS is its applicability to a wide range of assemblies; its applications range from DNA fragments⁸ to whole virions⁹. Moreover, data collection and processing are very rapid (typically, from seconds to minutes), allowing high-throughput analyses of a large number of samples at many conditions. These advantages are in stark contrast to crystallography, cryo-EM, and NMR spectroscopy. The ease of altering solution conditions makes SAXS ideal for mapping structural differences between varied conformational states of a macromolecular system; if the structure of one conformational state is known, even the relatively sparse information content of a SAXS profile can be sufficient to determine structural rearrangements, such as hinge-motions in proteins^{10; 11; 12}.

Information from SAXS can in principle be incorporated into the modeling process in two ways. First, a SAXS profile can be used to assess different models that were produced based on other considerations. For example, experimental SAXS profiles have been used to choose one of the many different quaternary structure arrangements produced by molecular docking of the son-of-sevenless domains¹³. Similarly, simulations indicated that SAXS profiles can be used to choose a close-to-native solution from a large set of alternative homology models of a given protein¹⁴.

Second, a SAXS profile can be used during the model building stage itself. The first such calculation of a model based on a SAXS profile relied on representing a macromolecular surface using spherical harmonics¹⁵. However, this representation has a relatively low resolution, thus leading to the development of alternative methods. Due to the limited information content of SAXS profiles, virtually all subsequently developed methods have aimed to integrate additional information into structure determination to reduce the manifold of solutions consistent with a given SAXS profile to a usefully small number.

Depending on the nature and resolution of additional information, different representations have been proposed. Early approaches modeled the molecule's envelope enforcing compactness¹⁶. Early coarse-grained approaches represented the macromolecule as an assembly of unconnected beads on a grid^{17; 18; 19}. This representation enforces an overall mass by using a required number of beads; geometrical symmetry may also be incorporated through symmetric sampling. In addition, compactness of the models is ensured by restricting the sampling to the vicinity of a compact initial model^{17; 18} or by including appropriate terms into the scoring function^{19; 20}. Other modeling approaches represent a protein as a chain of beads rather than a set of disconnected beads on a grid²⁰. In a recent application, atomic models were fitted into 6-fold symmetric bead reconstructions to gain insights into domain rearrangements of the AAA-ATPase p97 during its functional cycle¹⁰. Higher resolution *a priori* structural information about some parts of the protein can also be integrated into the modeling process by focusing the conformational sampling only on the undefined segments, such as loops²¹, or on the configuration of structurally defined domains and their flexible linkers²². For example, rigid body modeling was applied to give qualitative insights into the conformation of the polypyrimidine binding protein²³. Recently, SAXS profiles have been incorporated into the modeling of protein structures based on NMR-derived restraints, which significantly increased the accuracy of models for multidomain proteins compared to the models based on NMR profiles alone²⁴. In addition, the inclusion of simulated SAXS profiles into folding simulations led to models of small helical proteins²⁵.

Here, we describe the newly developed SAXS module in *Integrative Modeling Platform* (IMP), our software platform for modeling macromolecular assemblies by satisfaction of spatial restraints (<http://salilab.org/imp>)^{3; 26}. This integration in turn allowed us to combine SAXS profiles with various other types of data already used by IMP. Specifically, we present a protocol for modeling multidomain proteins and complexes. In particular, the protocol calculates the quaternary structures of multidomain proteins with structurally defined rigid domains as well as quaternary structures for complexes of structurally defined rigid proteins. In addition to a SAXS profile and rigid-body constraints, we employ stereochemical restraints derived from a molecular mechanics force field²⁷, a simple non-bonded atom pair term²⁸, the atomistic distance-dependent statistical potential DOPE²⁹, and an optional symmetry-enforcing term²⁶. We quantify the performance of the protocol using a benchmark of simulated examples. Finally, to test the method in a realistic setting, we also model the quaternary structure of the homotetramer D-xylose isomerase (XI) based on an experimental SAXS profile. The method already revealed large domain rearrangements between the nucleotide-free and the nucleotide-bound forms of *Escherichia coli* Hsp90¹².

Theory and Methods

We developed a method to calculate atomic models of proteins and their assemblies that are consistent with experimental SAXS profiles as well as other spatial restraints. The solution is found by optimizing a scoring function that quantifies how consistent a model is with the SAXS profile and the other restraints. Next, the method is described by specifying its three components: (i) the representation of the modeled system, (ii) the terms that contribute to the scoring function, and (iii) the optimization protocol. We also describe how to evaluate the ensemble of models obtained from independent optimizations of the scoring function.

Representation of a protein or a complex

The system is represented by its N_{at} non-hydrogen atoms. A major problem that needs to be overcome is the large size of the search space compared to the amount of input data. To reduce the number of degrees of freedom, the model is partitioned into L rigid bodies, b_l , $l = 1, 2, \dots$,

$L \leq N_{at}$. Each rigid body $b_l \left(\begin{smallmatrix} \mathbf{r}_l \\ \theta_l \end{smallmatrix} \right)$ is characterized by its center of mass, $\mathbf{r}_l$ and its orientation

$\vec{r}_l$. The orientation is measured by three rotations ($\theta_x, \theta_y, \theta_z$) around the x -, y -, and z -axes of the coordinate system with its origin at the center of mass. A rigid body $b_l(\vec{r}_l, \theta_l)$ can be any set of atoms, including a single atom, a secondary-structure element, a domain, or the whole protein. Each atom of the whole macromolecule is a member of exactly one rigid body. The two extreme cases correspond to either the entire structure or each atom being a rigid body. Here, in our applications to proteins, we model user-defined domains as rigid bodies while the connecting linkers are flexible. In our application to assemblies of proteins, we model the whole proteins as rigid bodies.

Scoring function

The scoring function is defined as:

$$S = S_{\text{stereo}} + S_{\text{overlap}} + S_{\text{DOPE}} + S_{\text{sym}} + S_{\text{SAXS}}. \quad (1)$$

S_{stereo} accounts for the inter-rigid body stereochemical features of the atoms in the protein (*ie*, chemical bond lengths, bond angles, *etc*) according to the CHARMM molecular mechanics force field ²⁷. The term S_{overlap} penalizes steric clashes in the model ²⁸; a harmonic potential penalizes distances between two atoms if they are closer than the sum of their van der Waals radii. The term S_{DOPE} is an atomic distance-dependent statistical potential called Discrete Optimized Protein Energy (DOPE) ²⁹. Additionally, a symmetry restraint S_{sym} can be imposed on the arrangement of the rigid bodies ²⁶; S_{sym} is the sum of the root-mean-squares of the differences between equivalent distances in two symmetry units. The term S_{SAXS} accounts for the SAXS profile and is described in detail next.

SAXS experiments measure the rotationally averaged X-ray scattering of the macromolecule under scrutiny. The measured quantity is a one-dimensional curve that gives the scattered intensity I_{exp} as a function of the momentum transfer $q = (4\pi/\lambda)\sin(\theta)$, where λ is the wavelength of the incident X-ray beam and 2θ is the scattering angle.

Similar to previous approaches to modeling a macromolecule based on its SAXS profile ^{17; 18; 20; 24}, we score a model based on the deviation between the calculated (I_m) and experimental SAXS profiles (I_{exp}):

$$\chi^2 = \frac{1}{Q} \sum_{k=1}^Q \frac{1}{\sigma_{\text{exp}}^2(q_k)} \cdot (I_{\text{exp}}(q_k) - c \cdot I_m(q_k))^2, \quad (2)$$

where k denotes the index of the measured frequency q , Q is the total number of frequencies, and σ_{exp} is the experimental error. The relative scaling of I_m with respect to I_{exp} cannot be determined precisely because the protein concentrations generally cannot be measured with sufficient accuracy. Thus, the profile I_m is scaled by a constant c , which is chosen by minimizing χ^2 (Supplementary Theory and Methods).

Moreover, we require S_{SAXS} to be comparable in size to the other four types of terms in S . We used the following rationale: S_{overlap} , S_{stereo} , S_{DOPE} , and S_{sym} are extensive functions in that they increase proportionally to the number of atoms N_{at} in the model. For the native structure, the value of χ^2 from Eq. (2) is on the order of 1. We choose the SAXS penalty of the native structure to be of comparable magnitude as the internal potential energy, defining the scaling as follows:

$$S_{\text{SAXS}} = N_{\text{at}} k_B \cdot T \cdot \chi^2 \quad (3)$$

where k_B is the Boltzmann constant and $T=100$ K.

The experimental SAXS profile of a macromolecule in solution is the difference between the scattering of the solution with and without the protein. Thus, the profile is approximately equal to the difference between the scattering intensities of the macromolecule and the solvent in the same volume. This model neglects the approximately 3 Å thin hydration layer around the macromolecule, which has a slightly higher density than bulk water. We neglected the hydration layer because the density difference between the hydration layer and bulk water is relatively small ($<0.060 \text{ e} \cdot \text{\AA}^{-3}$) compared to the densities of water ($0.334 \text{ e} \cdot \text{\AA}^{-3}$) and protein (approximately $0.440 \text{ e} \cdot \text{\AA}^{-3}$) and because the volume of the hydration layer is small compared to that of the macromolecule. We assess the error due to neglecting the hydration layer in Results by comparing our profiles to those using the program CRY SOL, which does account for the hydration layer.

We utilize the Debye Formula³⁰ to calculate the SAXS profile I_m of a given atomic model of a protein or assembly:

$$I_m(q) = \sum_{j=1}^{N_A} \sum_{i=1}^{N_A} f_i(q) f_j(q) \frac{\sin(qd_{ij})}{qd_{ij}}, \quad (4)$$

where $f_i(q)$ and $f_j(q)$ are the isotropic atomic form factors of the atoms i and j , respectively, and d_{ij} is the Euclidean distance between these atoms. In solution, the scattering of the protein is reduced by the contribution of the solvent in the protein excluded volume; we followed a previous formulation³¹ to account for this effect (Supplementary Theory and Methods). To accelerate computation, we did not include hydrogen atoms explicitly; their vacuum scattering and their excluded volumes were added to those of their bound atoms.

For optimization of S , we need the gradient of χ^2 . After some algebra, we obtain the partial derivative of χ^2 (Eq. (3)) with respect to the x -coordinate of atom i using Eq. (4):

$$\frac{\partial}{\partial x_i} \chi^2 = 4c \cdot \sum_{j \neq i}^{N_A} \frac{x_i - x_j}{d_{ij}^2} \sum_{k=1}^Q \frac{I_{\text{exp}}(q_k) - c \cdot I_m(q_k)}{\sigma_{\text{exp}}^2(q_k)} \cdot f_i(q_k) \cdot f_j(q_k) \cdot \left(\frac{\sin(q_k d_{ij})}{q_k d_{ij}} - \cos(q_k d_{ij}) \right). \quad (5)$$

The derivatives with respect to y and z are equivalent to those for x .

The calculation of both χ^2 and its derivatives is computationally demanding. However, we can substantially shorten the computation time for both quantities by approximating the modeled system with atoms of different scattering masses but equal shape (Supplementary Theory and Methods). Using this approximation, the computation time is reduced by a factor equal to the number of frequencies at which $I(q)$ is sampled, typically 100. Even at the resolution of $q_{\text{max}}=1 \text{\AA}^{-1}$, the loss in accuracy due to this approximation is marginal: The deviation between a profile calculated with and without the approximation is of the order of $\chi^2=10^{-3}$, which is at least two orders of magnitude below typical optimal χ^2 scores for our models (Supplementary Theory and Methods).

The rigidity constraints imply that atoms belonging to the same rigid body b_l are not allowed to move with respect to each other. Thus, all forces within b_l must be zero, which is achieved for S_{SAXS} by excluding all atoms that are part of the same rigid body as atom i from the summation in Eq. (5).

Modeling with multiple SAXS profiles

In IMP, we provide the option to add more than one SAXS profile term to S (Supplementary Theory and Methods). In addition to the profile of the macromolecule under scrutiny, SAXS profiles of the gold-labeled macromolecule or profiles of subsets of the original system can be acquired. When the corresponding structures are conserved in these constructs, these profiles provide additional information about the macromolecule.

Optimization

We optimize the model with respect to S to obtain an ensemble of good-scoring solutions (Fig. 1). To sample different minima of S , many independent optimizations are carried out starting from different random initial configurations. We differentiate between the global search mode, in which we sample 1000 different initial configurations, and the local search mode, in which we sample 100 initial configurations in the vicinity of a specific configuration. In both cases, the resulting models are clustered to make the final prediction.

Starting from an initial model of a monomeric protein or a complex (*eg*, a crystal structure or a homology model), the domains and the connecting linker residues (monomeric protein) or the proteins (complex) are individually rotated and translated by random values $\Delta\Phi$ and ΔT , respectively. Depending on the magnitude of these parameters, the protocol explores the intermediate neighborhood of the initial model (local search mode) or the whole space of solutions (global search mode). We chose $\Delta T=40\text{\AA}$ and $\Delta\Phi=180^\circ$ for global sampling and $\Delta T=1\text{\AA}$ and $\Delta\Phi=2^\circ$ for local sampling. In the case of monomers, the domains are initially brought into relative vicinity by optimization with respect to S_{stereo} using the method of conjugate gradients³², which reduces the sampling problem at a negligible computational cost.

Next, in Stage I of optimization, a coarse relaxation of the model is carried out with 10 cycles of a quasi-Newton method³³, relying on the scoring function consisting only of S_{stereo} , $S_{overlap}$, S_{sym} , and S_{SAXS} ; the weight of $S_{overlap}$ is gradually increased from 0.005 to 1. The low initial weight of $S_{overlap}$, allows large variations of the model during the initial iterations.

In Stage II, we further optimize the model with respect to the sum of S_{stereo} , $S_{overlap}$, S_{sym} , and S_{SAXS} using a biased Monte Carlo algorithm: all atoms of the model are randomly displaced with a standard deviation of 5 Å, maintaining the rigid-body constraints. This perturbed model is then relaxed using one cycle of quasi-Newton optimization. Twenty-five Monte-Carlo steps are carried out, decreasing the temperature exponentially. At each step, the resulting model is accepted or rejected according to the Metropolis criterion.

In Stage III, additional 25 Monte-Carlo steps are finally carried out to optimize the models with respect to the complete S , including S_{DOPE} .

On average, more than 200 different models are generated for each of the 1,000 initial independent optimizations, resulting in the total of ~200,000 evaluated models. On a Linux-based cluster of 100 processors, the computations take from a few hours to two days, depending on the size of the modeled system.

Analysis of optimized models

Because each optimization of a random starting configuration samples only a fraction of the configuration space, it generally ends in a local minimum. Thus, we rank all solutions by S_{SAXS} and retain only the top 10% for further analysis. These solutions are hierarchically clustered³⁴ with MATLAB (The Mathworks Inc.), relying on Ca root-mean-square deviations (Ca RMSDs) for all pairs of structures. Each Ca RMSD is obtained by superposing the largest domains and then computing the deviation between the whole structures. As a threshold level t for clustering, we use the resolution of the experimental SAXS profile, $t=2\pi/q_{\text{max}}$.

Benchmark of multidomain proteins and binary complexes

To assess the method with statistical rigor, we applied it to a benchmark set of 12 known structures with calculated SAXS profiles. The benchmark includes 9 multidomain proteins (Tables 1, S1); their domains are treated as rigid bodies in our calculations. The domain definitions for the native structures were taken from the CATH database. Domains for five of the 9 proteins were represented by experimentally determined structures with identical sequences in a different state. Domains for the other 4 proteins were modeled by comparative modeling based on related structures. The alignments for comparative modeling were obtained from our comprehensive database of structural alignments, DBAli³⁵. The models were built with the ‘automodel’ class in MODELLER-9.0. Except for 1o0vA, the models cover the whole sequence. The benchmark also includes 3 protein complexes, each consisting of 2 proteins (Table 2), which were obtained from ‘Docking Benchmark 2.0’³⁶. Here, the rigid bodies for each protein corresponded to crystallographic structures of the same sequence in a different state³⁶.

We aimed to map the accuracy of the predicted configurations as a function of rigid body accuracy. We modeled the configurations of rigid bodies as described above, employing the global and local search modes. For comparison, we also created an initial model with all rigid bodies superposed onto their counterparts in the native structure (RBNC, rigid bodies in the native configuration) using Chimera³⁷ and optimized the linker segments with respect to S_{stereo} and S_{overlap} . Starting from this initial model, we performed 10 independent local optimizations (“RBNC refined” in Supplementary Table S1). Similarly, we also performed 10 local optimizations starting with the native state (“native refined” in Supplementary Table S1).

Assessment of similarity to the native state

The models were compared to the native state using two measures: (i) Ca RMSD with respect to the native structure and (ii) rigid-body translation Δr and rotation $\Delta\alpha$ of the rigid bodies relative to their positions in the native state. The Ca RMSD was calculated using the ‘superpose’ command of MODELLER-9.0. For calculation of Δr and $\Delta\alpha$, the reference frame was defined by first superposing the largest rigid body from the model on its equivalent fragment in the native structure. Next, each of the remaining rigid bodies was rotated around its center of mass and subsequently translated such that it superposed with the equivalent part of the native structure. The corresponding rotation and translation define $\Delta\alpha$ and Δr , respectively. If the model consisted of more than two rigid bodies, we computed the mean values of $\Delta\alpha$ and Δr to characterize the similarity between two configurations of rigid bodies, always using the largest rigid body to define the reference frame.

Acquisition of experimental XI profiles

We prepared XI solutions with concentrations of 0.55, 1.1, 2.7, and approximately 20 mg/ml from XI crystals (Hampton Research, Aliso Viejo, CA) and a buffer solution containing 10mM Hepes (pH 7.4) and 150 mM NaCl. SAXS profiles were recorded at Beam Line 4-2 at the Stanford Synchrotron Radiation Laboratory³⁸. Each solution was placed in a cuvette, which

was maintained at 20 °C and located 2.5 m from a MarCCD165 detector (MarUSA, Evanston, IL). Twenty 15 second exposures (X-ray wavelength $\lambda=1.381$ Å) were acquired in series for each concentration. For a range of concentrations, we obtained approximately constant radii of gyration R_G in the $R_G \cdot q_{\min}$ - $R_G \cdot q_{\max}$ range of 0.37-1.27, indicating no protein aggregation or changes in the homotetramer quaternary structure: R_G of 32.7 ± 0.4 Å⁻¹ at 0.55 mg/ml, 32.9 ± 0.2 Å⁻¹ at 1.1 mg/ml, and 32.8 ± 0.1 Å⁻¹ at 2.7 mg/ml; these R_G values are similar to those reported previously³⁹. SAXS profiles were computed from the scattering images using MarParse³⁸ and profiles recorded at 2.7 mg/ml (0.01 Å⁻¹ $< q < 0.10$ Å⁻¹) and 20 mg/ml (0.055 Å⁻¹ $< q < 0.27$ Å⁻¹) concentrations were scaled and merged using Primus⁴⁰. The complete profiles ranged from $q=0.01$ Å⁻¹ to $q=0.27$ Å⁻¹. We finally resampled the profile on a uniform grid of 100 mesh points using linear interpolation.

Results

Accuracy of calculated SAXS profiles

We determined that it is not necessary to include the hydration shell of a protein in the Debye model for calculating SAXS profiles because the inclusion of the hydration shell has a much smaller impact on χ^2 than the errors in an experimentally measured SAXS profile. Specifically, we compared the experimentally measured lysozyme SAXS profile with the profiles computed by our method and CRY SOL⁴¹ (PDB 6lyz) (Fig. 2). The profiles calculated by our program and by CRY SOL agree with the experimental profile within its error, though the CRY SOL profile is a slightly better fit ($\chi^2=0.20$ versus $\chi^2=0.26$). We also determined that using a single consensus atomic shape has no notable effect on the accuracy of calculated SAXS profiles; that is, the χ^2 difference between profiles calculated with the consensus and specific atomic shapes is approximately 0.001 (Supplementary Theory and Methods).

Modeling Diphteria toxin using a simulated SAXS profile

We first illustrate the method by its application to monomeric Diphteria toxin (Fig. 3A, PDB code 1mdt). We calculated the SAXS profile of the crystallographic structure: The q values ranged from $q_{\min}=0.02$ Å⁻¹ to $q_{\max}=0.5$ Å⁻¹ and the $I(q)$ values were sampled on 100 equidistant grid points. Experimental error was simulated by adding white Gaussian noise with a standard deviation of 0.3 times $I(q_{\max})$. The two Diphteria toxin domains were treated as rigid bodies; they were taken from the dimeric form of the protein, which possesses a dramatically different domain arrangement (Fig. 3B, PDB code 1ddtA).

In our approach, the predicted quaternary structure depends primarily on the experimental S_{SAXS} term and the modeling terms S_{overlap} and S_{DOPE} . The main role of the additional term S_{stereo} is to keep domains in relative proximity and thus restrict the number of possible conformations. To assess the effect of combining experimental information (S_{SAXS}) and modeling information (S_{overlap} and S_{DOPE}) on the predicted quaternary structures, we calculated models with different combined scoring functions consisting of (i) only S_{stereo} , S_{overlap} , and S_{DOPE} ; (ii) only S_{stereo} and S_{SAXS} ; and (iii) the complete S . One thousand independent optimizations were carried out for each scoring function variant in the global sampling mode. For each scoring function, we evaluated the score distribution and the C α RMSD values of the models with respect to the native state (Fig. 3C-E). The first scoring function, which lacks S_{SAXS} , has minima for configurations that differ substantially from the native state (Fig. 3C); none of the models has a C α RMSD below 10 Å. For the scoring function including only S_{SAXS} and S_{stereo} , a set of different configurations is consistent with the SAXS profile ($\chi^2 < 1$) (Fig. 3D); the lowest scoring configurations are not near-native (their C α RMSD is between 10 and 15 Å). In contrast, optimization of the complete S successfully results in near-native models (Fig. 3E). Thus, the combination of the SAXS and modeling terms results

in a global minimum close to the native state, while either of the individual terms do not. This synergy justifies the integration of SAXS and protein structure modeling.

We further analyzed the models obtained from the optimization of S . Using C α RMSD, we hierarchically clustered the 10% models with the best χ^2 (Fig. 4A). The models clearly cluster into 3 distinct groups, separated by more than 12.5 Å C α RMSD from each other. Each cluster is represented by the model with the best S (Fig. 4B-D). Albeit all models correspond to distinct configurations, their shapes and calculated profiles are similar, as expected from the nature of the SAXS restraint. The model from cluster III (Fig. 4D) has the lowest S and the lowest SAXS penalty ($\chi^2=1.3$ compared to 1.5, and 1.4 for the models from clusters I and II, respectively; Fig. 4E) and is closest to the native state in terms of its C α RMSD (1.4 Å compared to 10.4 and 14.0 Å, respectively). Interestingly, cluster III does not constitute the largest cluster nor does it include the models with the lowest S_{DOPE} (Supplementary table S1). Thus, in this case S and χ^2 are indicative of the cluster with near-native models whereas S_{DOPE} and the cluster size are not.

Benchmark on multidomain proteins

To assess the modeling protocol with statistical rigor, we applied it to our benchmark set of 9 multidomain proteins (Theory and Methods). The benchmark comprised proteins with 2 domains (5 cases), 3 domains (3 cases), and 4 domains (1 case) (Tables 1, S1). For five proteins, the domains were represented by experimentally determined structures, which are identical in sequence but part of a different assembly. The rigid bodies of the remaining four proteins were modeled by comparative modeling based on related template structures. Thus, the benchmark covered different scenarios in terms of protein size and available structural information.

After optimization and clustering, the cluster with the most accurate model (in terms of C α RMSD) was termed the most accurate cluster. In the global search mode, the best-scoring models in the most accurate cluster were close to the native state (C α RMSD < 4 Å) for 6 proteins, of medium accuracy for one protein (C α RMSD = 12.8 Å), and of low accuracy in only two cases (C α RMSD > 18 Å) (Tables 1, S1). When crystallographic domain structures were used, the resulting configurations were always highly accurate. In contrast, when comparative models were used, high accuracy configurations could only be obtained for 1cb6 and medium accuracy configurations for 1lknA. In our benchmark, these proteins also possessed the highest sequence identity to their template structures (63% and 46%, respectively). It is well established that the accuracy of a comparative model is correlated with the sequence identity on which it is based⁴². Therefore, not surprisingly, the accuracy of whole protein models produced by our method is correlated with the sequence identity of the structural template for the modeling of the individual domains.

In real applications, we do not have the modeled native structure. Thus, we don't know which one of the generated models is most accurate, but need to select it based on some criterion. Possible criteria include the S and S_{SAXS} (χ^2) scores, as well as the cluster size. In all but one high- and medium-accuracy case (global sampling for 1mdtA; Figs. 3 and 4), the most accurate model was found in the largest cluster. Similarly, in all but one case (global sampling for 1cb6), the most accurate model was also the one with the best S and S_{SAXS} scores. Thus, the selection of the final prediction by either one of these three criteria is likely to identify the most accurate of the generated models.

In almost all cases, the local search produced more accurate models than the global search. Only for 1mdtA, the local search resulted in a less accurate model than the global search, consistent with the largest distortion in the initial structure relative to the native state (C α RMSD > 20 Å) among all benchmark cases. Remarkably, for the four-domain protein 1cb6 (C α RMSD of the initial model is 8.2 Å), local sampling yielded more accurate results than

global sampling, albeit global sampling results in models with lower scores. Thus, in this case, the near-native configurations are within the radius of convergence for the local sampling, whereas the global minimum, which is not a near-native configuration, is not. Based on the examples of 1mdtA and 1cb6, we conclude that the radius of convergence for local sampling is on the order of 10 Å.

Benchmark on binary protein complexes

We also applied our protocol to 3 binary protein complexes (Theory and Methods). The accuracy of binary complex models is generally lower than that for the individual two-domain proteins (Table 2). The highest accuracy was achieved for 1avxAB, whose best-scoring models had C α RMSD of approximately 10 Å. This target was classified as ‘easy’ in Docking Benchmark 2.0³⁶. The high C α RMSD is due largely to inaccurate relative orientation of the two proteins ($\Delta\alpha = 81.6^\circ$); in comparison, the relative position is quite accurate ($\Delta r = 5.1$ Å). Interestingly, local sampling in the vicinity of the RBNC state resulted in a model similar to the configuration using global sampling, which also scored better than the RBNC state according to S and χ^2 , but not S_{DOPE} (Table S2).

The results for target 1ibr are similar to those for 1avx; the best scoring models were different from the native state and scored better than the RBNC state. The target 1ibr is classified as ‘difficult’ in Docking Benchmark 2.0³⁶.

For the third example, 1fq1 (‘difficult’ in Docking Benchmark 2.0³⁶), the largest three clusters produced by the global sampling were of similar size and different from the native state (C α RMSD > 30 Å). Moreover, the individual S , S_{SAXS} , and S_{DOPE} scores for the RBNC configuration were worse than those for optimized models.

If we assemble the native protein structures instead of their models, the local sampling around the native state always finds a configuration close to the native state (C α RMSD < 1.2 Å); moreover, the optimized S , S_{SAXS} , and S_{DOPE} are significantly better than those for the assembly of non-native rigid bodies (Table S2).

Quaternary structure of D-xylose Isomerase from an experimental SAXS profile

To test our methodology on experimental rather than simulated profiles, we modeled the quaternary structure of D-xylose isomerase (XI) from *Streptomyces rubiginosus*. The model was calculated using three different approximations for the monomers: (i) the native subunit structure in the complex, (ii) a comparative model based on 4xim as a template (67% sequence identity), and (iii) a comparative model derived from 1a0d (28% sequence identity). The best possible superpositions of four copies of the monomer comparative models onto the native structure of the XI complex, *ie*, the C α RMSD values of the RBNCs, were 2.7 Å and 5.1 Å for 4xim and 1a0d, respectively.

To reproduce the 222 symmetry of the XI tetramer consisting of identical subunits A, B, C, and D, we added a symmetry term S_{sym} to S . Specifically, we restrained equivalent intra-subunit C α atom distances between equivalent chain pairs to be similar to each other^{26; 43}.

We acquired an experimental XI SAXS profile ranging from $q_{min}=0.01$ Å⁻¹ to $q_{max}=0.27$ Å⁻¹ (Theory and Methods). For each of the three assembly calculations, we built 500 configurations using the global sampling mode and clustered the 10% best-scoring (χ^2) configurations (Table 3). Because XI is a homotetramer, the labels on the four subunits are irrelevant when comparing two configurations; *ie*, swapping monomers has absolutely no impact on the structure. Therefore, we calculated C α RMSDs between a model and the native state for all subunit permutations in the model and chose the minimum value as the final C α RMSD.

For the native monomer, the largest cluster contains the models with the lowest score S as well as the most native-like models ($C\alpha$ RMSD = 3.5 Å), indicating an accurate prediction. However, if we only consider S_{SAXS} , the best scoring models are not closest to the native state (Fig. 5A-D), illustrating the positive role played by the other terms in the combined scoring function S (Fig. 5E, F). The agreement between the model SAXS profiles and the experimental profile is generally lower than that in the benchmark simulations ($\chi^2_{\min} \approx 8$ versus $\chi^2_{\min} \approx 1$). We also checked our χ^2 against that implemented in CRY SOL ⁴¹, confirming that the CRY SOL χ^2 likewise favors non-native solutions and correlates well with our χ^2 (Supplementary Fig. S1). Comparison of the SAXS profiles of the best scoring models in the largest cluster with the experimental and simulated SAXS profiles of the native state reveals that deviations from the experimental profile are most significant in the range of $0.11 < q < 0.12 \text{ \AA}^{-1}$ (Fig. 5A-C). Similar observations were reported previously for XI ³⁹. In contrast to S_{SAXS} , S_{DOPE} is substantially lower for models in the cluster close to the native state than for the models in the other clusters.

The calculation with the 4xim-based monomer models follows the same trends as those with the native subunit (Table 3 and Fig. 5E). Therefore, in a realistic setting, our modeling protocol would have determined correctly the quaternary arrangement of four subunits of the XI monomer, using its experimental SAXS profile and a high accuracy subunit model based on 67% sequence identity to the template structure.

In contrast, the results for the comparative model based on the remotely related 1a0d are qualitatively different. In this case, the largest cluster does not contain the most native-like models ($C\alpha$ RMSD = 49.1 Å). If we select the cluster according to S or S_{DOPE} , the model is closer to the native state, albeit far from it in terms of $C\alpha$ RMSD (15.3 Å). Nevertheless, this model still predicts correctly many of the residues at the subunit interfaces: 14% of the native contacts (the number of correctly predicted residue–residue contacts in the model divided by the number of contacts in the native structure) are identified correctly, which is considered ‘acceptable’ in blind assessments of protein docking methods at CAPRI meetings ⁴⁴.

Scoring versus sampling

We now analyze the extent to which model accuracy is limited by sampling and scoring; sampling is limiting when configurations close to the native state are not generated during optimization and scoring is limiting when the most native-like configurations do not correspond to the global minimum of S .

For three of our benchmark cases (1ha0, 1ibr, and 1ko9), we plotted the best score ($S_{\min}$), the corresponding $C\alpha$ RMSD ($\text{RMSD}(S_{\min})$), and the minimum $C\alpha$ RMSD ($\text{RMSD}_{\min}$) for a set of structures resulting from global sampling against the number of independent optimizations (Fig. 6). For the two-domain protein 1ha0, $S_{\min}$, $\text{RMSD}(S_{\min})$, and $\text{RMSD}_{\min}$ do not improve substantially beyond 100 independent optimizations. Moreover, global sampling performs as well as local sampling starting from the RBNC (Fig. 6A; Supplementary Table S1). For the protein complex 1ibr, $S_{\min}$ and $\text{RMSD}(S_{\min})$ reach a plateau at approximately 100 optimizations. $\text{RMSD}(S_{\min})$ asymptotically reaches the value for local sampling around the RBNC, which is well above 10 Å, showing that a non-native configuration scores better than the RBNC (Fig. 6B; Table S2). The values for $\text{RMSD}_{\min}$ asymptotically approach a value of approximately 3 Å ($C\alpha$ RMSD of RBNC is 2.3 Å), indicating that near-native configurations are sampled, but do not score well. In summary, we observed for all two-component systems (two-domain proteins and binary complexes) that the best-scoring models scored approximately the same as the refined RBNC configurations; therefore, sampling does not appear to be limiting the accuracy of our predictions in these cases.

For the three-domain protein 1ko9, $S_{\min}$, $\text{RMSD}(S_{\min})$ and $\text{RMSD}_{\min}$ improve slowly with an increase in the number of independent optimizations, beginning to reach a plateau at 400

independent optimizations. $S_{\min}$ from global optimization exceeds $S_{\min}$ of the models obtained by refining the RBNC, indicating that in this case the accuracy of the predicted quaternary structures is limited by sampling. Similar results are obtained for the other three- and four domain proteins; the global minimum of S could only be reached using global sampling if we performed at least 1,000 independent optimizations. However, when a sufficiently accurate starting configuration is available (eg, from a similar template protein), highly accurate configuration models can be obtained using only 100 independent optimizations (C α RMSD < 2 Å) (Supplementary Table S1).

Discussion

We incorporated information from a SAXS profile into protein structure modeling by satisfaction of spatial restraints, implemented in our *Integrative Modeling Platform* (IMP)^{3; 26}. We then benchmarked IMP by modeling quaternary structures of multidomain proteins and protein assemblies using rigid domains and proteins, respectively. We discuss here (i) the relationship between our method and those of others, (ii) the benefits of integrating protein structure modeling and SAXS fitting, (iii) the limitations arising from inaccurate scoring, imperfect sampling, and errors in rigid bodies, as well as (iv) the scope for integration of additional information into the modeling process.

Comparison with other methods

Recently, experimental SAXS profiles have also been used to calculate atomic models of proteins by BUNCH²² and CNS, which relies on NMR-derived restraints as well as a SAXS profile²⁴. We now outline similarities and differences between these two approaches and that of ours.

Our SAXS penalty is similar to the score implemented previously in CNS²⁴. Identically, both scores employ the Debye formula to treat the excluded solvent and rely on χ^2 as the SAXS penalty. Moreover, they both calculate the partial derivatives allowing them to use gradient-based optimization techniques. However, the computation of the SAXS penalty and its derivative in our approach is significantly faster compared to the original implementation because we employ the electron pair distribution function $P(r)$ for the calculation of χ^2 and its derivative (Supplementary Theory and Methods). The gain in efficiency depends on the granularity of $I(q)$ sampling. For example, we reduce the computation time by two orders of magnitude for a dataset with 100 data points and more for finer-sampled profiles. This gain in computational speed does not reduce the precision of the calculated SAXS profiles by more than the typical precision of experimental SAXS profiles. Moreover, we can gain additional efficiency relative to CNS through the use of rigid bodies, which required changes in the calculation of the partial derivatives of S_{SAXS} (Supplementary Material). The gains in computational efficiency are important because they allowed us to sample the space of possible solutions more exhaustively.

Our SAXS penalty is different from the penalty in BUNCH²², which is calculated by CRY SOL⁴¹ and up-weights high frequency components in χ^2 . While we also tested such a scoring function, it did not result in a significant improvement for our benchmark. For example, we modeled the Diphtheria toxin using a χ^2 that weights frequencies according to q^2 . The corresponding S_{SAXS} term did not result in a more accurate model if used only in conjunction with S_{stereo} (Supplementary Fig. S1). It also did not produce a higher yield of near-native configurations when using the full score S , compared to the uniform weighting (Supplementary Fig. S1). Also in our experimental test case XI, the IMP and BUNCH scores are highly correlated (Supplementary Fig. S2). Therefore, for parsimony, we used the scoring function with uniform weighing of the SAXS profile at different frequencies. Moreover, unlike our score, the BUNCH SAXS penalty includes two additional fitting parameters that modify I_m

41, corresponding to the average displaced volume per atomic group and the density of the hydration layer. The slightly better fit between the CRY SOL and experimental profiles of lysozyme (Fig. 2) is likely due to these additional parameters, but their effect is negligibly small ($\Delta\chi^2=0.05$). Thus, the additional fitting parameters in CRY SOL cannot explain the errors in the calculated profile of XI for $0.11\text{\AA}^{-1} < q < 0.12\text{\AA}^{-1}$ (Fig. 5A). Likewise, the program solX⁴⁵ calculates SAXS profiles of known atomic structures that fit experimental data as well as CRY SOL's profiles⁶ without employing fitting parameters other than the protein concentration similarly to our approach. It is unlikely that errors in our experimental data collection are responsible for this discrepancy because independent SAXS measurements of XI yielded comparable differences between experimental profiles and profiles computed from the crystallographic XI structure³⁹. Moreover, qualitatively similar discrepancies could be observed for *E. coli* aspartate transcarbamylase (ATCase), a 303 kDa homo-dodecamer, in the original CRY SOL publication⁴¹. The calculated ATCase profile has a pronounced local minimum at $q \approx 0.08\text{\AA}^{-1}$, while the corresponding minimum in the experimental SAXS profile is significantly less prominent. In addition to the imperfect calculation of the SAXS profile for a given structure, these discrepancies might also reflect structural differences between the crystallographic and solution structures.

Our optimization protocol consists of independent minimizations of the scoring function from many random starting configurations, with the aim to sample the entire configuration space (for the global optimization mode) (Fig. 1). For each minimization, we use a simulated annealing biased-Monte Carlo algorithm; each Monte Carlo step is followed by a local quasi-Newton relaxation, for which the first derivatives of the scoring function are needed. Thus, the biased-Monte Carlo process samples only local minima. In contrast, BUNCH²² employs a conventional simulated annealing Monte Carlo protocol, in which the sampling is not restricted to local minima. However, in many applications, such as X-ray crystallography⁴⁶, NMR spectroscopy⁴⁷, comparative protein structure modeling²⁸, and *ab initio* structure prediction of proteins⁴⁸ and assemblies⁴⁹, optimization methods that employ the first derivatives are known to be significantly more efficient than Monte Carlo schemes. Thus, we efficiently implemented and used the first derivatives in our optimization.

A perennial problem in structure modeling is whether or not an optimization scheme finds all the good scoring solutions. To at least partly address this problem, we run many minimizations in parallel and independently on a large computer cluster (*ie*, hundreds of nodes). The resulting large sample of solutions is then clustered, to present them more parsimoniously for subsequent analysis. "By construction, the structures in one cluster are generally distinct from the structures in another cluster; they involve dissimilar interfaces and have C α -RMSD values worse than 12 Å (Figs. 4, 5). An analysis of the sampling shows that our protocol usually finds the global minimum for up to four rigid bodies (below); thus, most good-scoring local minima are also expected to be sampled in these cases. A large computer cluster with at least 100 processors is currently needed for an efficient use of our method, perhaps limiting its practical utility. Nevertheless, such computing clusters are becoming increasingly available to many users. In addition, our software is being adapted to run on graphics processing units, such as Nvidia's Tesla with 240 processors (http://www.nvidia.com/object/tesla_c1060.html), which might enable efficient application on a single desktop computer.

Integration of SAXS and protein structure modeling

Integrative computational methods can exploit various kinds of spatial information to determine the assembly structures at higher accuracy and precision than is possible based on each individual type of data^{3; 26}; in conjunction, pieces of data that are relatively uninformative by themselves can still result in accurate and precise models of proteins and assemblies.

Here, we combined a SAXS profile with information about protein structures that can be calculated only from their sequence. Specifically, we supplemented the SAXS term (S_{SAXS}) by the penalties for steric clashes ($S_{overlap}$), an atomic distance-dependent statistical potential (S_{DOPE}), stereochemistry restraints from a molecular mechanics force field (S_{stereo}), and symmetry restraints (S_{sym}). Importantly, an integration of these different types of information can significantly improve modeling accuracy relative to relying on a SAXS profile or protein structure modeling alone (eg, Fig. 3; 1cb6 in Tab. 1; XI in Fig. 5). Moreover, the sum of the different terms was not observed to be less accurate than any of the individual terms; in other words, when the final configurations were inaccurate, no optimization of the individual terms, including S_{SAXS} , produced an accurate configuration.

Our method does not necessarily predict unique best-scoring solutions; due to the low information content of the input restraints, models from different clusters can have comparable scores. For example, S_{SAXS} is completely invariant to rotations of a spherical rigid body. For the benchmark case 1cb6, models from the near-native cluster and a non-native cluster have similar scores (Supplementary Table S1). Nevertheless, due to the combination of different types of information, the number of distinct configurations compatible with the input data is generally much smaller compared to using only a single type of information (eg, Fig. 3).

Influence of scoring, sampling, and rigid bodies on model accuracy

Our benchmark allows us to assess the limitations of the protocol and highlight opportunities for future research. Modeling by optimization depends on two conditions: (i) The scoring function needs to have a global minimum at the native or near-native state and (ii) the sampling needs to be sufficiently thorough to find the global or near-global minimum. Therefore, we tested the degree to which our method is limited by the accuracy of the scoring function and the thoroughness of sampling. We also asked how accurate do the rigid bodies need to be so that the scoring function still has the global minimum at the native state. The assessment of sampling allowed us to judge when a global search without reliance on a suitable initial structure can be successful; or, conversely, when we need a sufficiently accurate initial model so that at least a near-native state can be found by local sampling alone. We also analyzed the accuracy of the method as a function of the number of rigid bodies, which allows us to further qualify sampling and scoring limitations.

For systems of two rigid bodies (ie, two-domain proteins and binary protein complexes), even the global sampling produced numerous configurations close to the global minimum of the scoring function S (Fig. 6A, B; Supplementary Tables S1 and S2). Thus, the accuracy of these models is largely determined by the accuracy of S . The global minimum of S corresponded to the native or near-native state only if the rigid bodies were not too distorted (ie, C α RMSD of less than 3 Å). Therefore, as expected, the accuracy of the rigid bodies crucially influences the landscape of S .

Among the individual terms of S , it is not surprising that S_{DOPE} sometimes favors non-native configurations if sufficiently distorted rigid bodies are used. Errors in the positions of exposed atoms interfere with their packing, which is the aspect scored by S_{DOPE} . Moreover, distorted rigid bodies can cause steric clashes and thus increase $S_{overlap}$. In contrast to S_{DOPE} , a strong dependence of S_{SAXS} on the rigid body accuracy is somewhat surprising; in all cases, the rigid domains and proteins possessed the correct fold, but S_{SAXS} did not always favor near-native configurations. If the C α RMSD errors of the rigid bodies were above approximately 3 Å, configurations with minimum S_{SAXS} were significantly different from the native state (ie, higher than 18 Å for C α RMSD). This result can be explained by the ambiguity of the SAXS restraint; many different quaternary structures have a similar envelope and therefore similar SAXS profiles. Subtle structural differences can then make S_{SAXS} of near-native configurations worse than that for some non-native configurations. The deteriorating effect of errors in the

rigid bodies on the accuracy of the predicted configurations turns out to be most pronounced for complexes (Table 2, Supplementary Table S2). For multidomain proteins, the connecting linker limits the number of possible configurations. Thus, the number of possible false minima of S is generally larger for protein complexes than for monomeric proteins. We anticipate that the precise degree of tolerable structural differences will also depend on the shape of the rigid body: If a rigid body does not have a distinctive shape (*eg*, it is a sphere or a cylinder), small distortions can be sufficient to alter the position of the global minimum; for more distinct shapes, the sensitivity of the prediction accuracy to the rigid body errors is expected to be smaller.

For three or more rigid bodies, the typical number of independent optimizations we used (1000) was insufficient to reliably find the global minimum of S in a global search (Fig. 6C). For four domains (benchmark case 1cb6), we increased the number of initial configurations to 5,000, requiring 3 days on 200 CPUs. Such an exhaustive sampling is impossible without employing a large computer cluster. However, configurations close to the global minimum of S could be found at dramatically reduced computational cost employing local sampling, if the template (initial) configuration was sufficiently close to the native state ($C\alpha$ RMSD < 10 Å). In such a case, the local search is more efficient than the global search because no computing time is wasted on searching far from the native configuration. In the future, the development of computationally more expensive and sophisticated sampling strategies may allow sampling the configurations of five or more domains with a larger radius of convergence than that of our present optimization.

To overcome the limitations on prediction accuracy arising from rigid body errors, we probably need to abandon the rigid body approximation. In the field of protein-protein docking, simultaneous sampling of different component conformations and their configuration has been described⁴⁹. Similar approaches could be used for fitting a configuration to a SAXS profile, requiring both more sophisticated scoring functions and sampling algorithms than those described here. The use of high-angle scattering data might allow us to compute atomic structures more accurately, but this goal would require flexible modeling of the atomic structures. Structural changes at the domain level result in signals at frequencies beyond $q = 0.5$ Å⁻¹, which we did not consider in our calculations because we represented domains and proteins as rigid bodies.

Our protocol currently relies on the sample protein or complex existing in a single state. However, proteins and complexes can exist in equilibrium among different states, corresponding to varied packing between secondary structure segments, domains, and proteins as well as variations in unstructured regions such as long domain linkers. An approach to score a given ensemble of models has been proposed recently⁵¹. Our protocol could also be extended to optimize an ensemble of models using a similar score. However, the increased computational effort as well as the limited information in SAXS profiles will limit such approaches.

Integration of SAXS profiles with additional information

Given the relatively low information content of a SAXS profile and the limitations in our protein structure modeling terms, incorporating additional information into the scoring function S is desirable. Using supplementary information is further justified by the sensitivity of S to rigid body errors and possible systematic experimental errors in a SAXS profile (*eg*, due to aggregation of macromolecules in solution). Further integration is facilitated by our implementation of SAXS fitting in IMP, which can already produce models by simultaneously satisfying a large variety of other spatial restraints. In addition to the SAXS and modeling terms used here, IMP can incorporate (i) restraints implied by an alignment between the modeled sequence and related structures²⁸, (ii) restraints implied by an alignment of the modeled sequence and many short segments of known structure, (iii) bioinformatics analysis of protein

interaction modes ⁵², (iv) protein-protein docking that is restrained by the composition of interacting surfaces determined by NMR spectroscopy ⁵³, (v) symmetry and density from a cryo-EM map of the assembly ⁵⁴, and (vi) proximity of subunits inferred from immuno-affinity purifications, yeast two-hybrid system, footprinting, and chemical cross-linking ²⁶. The global shape information from SAXS is especially complementary to local restraints, such as the atomic distance restraints derived from chemical cross-linking detected by mass spectrometry ⁵⁵. Another attractive application is the integration of SAXS for structural characterization of component structures of a large assembly whose overall density is determined by cryo-EM.

Conclusions

An accurate quaternary structure model of a protein or a protein assembly can be obtained using only a SAXS profile, stereochemistry restraints from a molecular mechanics force field, and an atomic distance-dependent statistical potential, provided sufficiently accurate approximations for the constituent domain and protein structures are available. Otherwise, the predictions are generally ambiguous and have large errors. Our integration of a SAXS profile into modeling by satisfaction of spatial restraints will facilitate further integration of different kinds of data for structure determination of proteins and their assemblies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Maya Topf, Narayanan Eswar, Frank Alber, Fred Davis, Min-Yi Shen, and Marc Marti-Renom for fruitful discussions. FF is grateful to a long-term fellowship from the Human Frontier Science Project Organization (HFSPO). KAK was supported by a NSDEG Graduate Fellowship. SSRL is funded by DOE BES, and the SSRL Structural Molecular Biology Program is supported by DOE, OBER and NIH NCRR BTP Grant (P41 RR001209). DAA has been supported by the Howard Hughes Medical Institute, DAA and AS have been supported by a UC Discovery Grant (bio03-10401/Agard). AS has also been supported by The Sandler Family Supporting Foundation, NIH (R01 GM54762, R01 GM083960, U54 RR022220, and PN2 EY016525), NSF (EIA-032645 and IIS-0705196), Hewlett-Packard, NetApps, IBM, and Intel.

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Fig. 1.

Flowchart for the modeling protocol. As input, we need a SAXS profile, an initial model, the definition of rigid bodies, and optionally the symmetry. Initialization yields N random configurations, which are subsequently optimized in three stages. For the final analysis, only the 10% of the N models with the best S_{SAXS} score are retained and clustered.

Fig. 2. Accuracy of the Debye model for calculating SAXS profiles. We compare an experimental profile of lysozyme (black, obtained from <http://www.embl-hamburg.de/ExternalInfo/Research/Sax/crysol.html>) to the profiles calculated using our methodology (green) and the program CRY SOL ⁴¹ (red).

Fig. 3.

Modeling Diptheria toxin using a simulated SAXS profile. A: Native monomeric Diptheria toxin (PDB code 1mdtA) has two domains (blue and red). B: We approximated these domains by their structures in the dimeric form (PDB code 2ddtA). C: The sum of S_{DOPE} and $S_{overlap}$ of all models optimized with respect to $S_{DOPE} + S_{overlap} + S_{stereo}$, plotted against their C α RMSD relative to the crystallographic structure. The yellow and orange circles depict the native structure and the RBNC, respectively. D: The χ^2 of all models optimized with respect to $S_{SAXS} + S_{stereo}$, plotted against C α RMSD. The χ^2 for the native structure and the RBNC are outside the plotted range ($\chi^2=1.21$ and $\chi^2=1.57$, respectively). E: Overall score S of all models optimized with respect to the complete S , plotted against C α RMSD. The best-scoring models from the three clusters in Fig. 4 are highlighted in red, blue, and green.

Fig. 4. Clustering of Diptheria toxin models. A: Hierarchical tree of the 10% best-scoring models according to their χ^2 values. To cluster the models, the tree is cut at a pairwise C α RMSD of 12.5 Å. B, C, D: Models with the best S within clusters I, II, and III, respectively. E: The $I(q)$ profiles of the best scoring models from cluster I, II, and III compared to the calculated SAXS profiles. The χ^2 values are 1.4, 1.5, and 1.3, respectively.

Fig. 5.

Models of XI and their calculated SAXS profiles compared to the experimental profile. To facilitate visual comparison of the different models, chain A (red) of all models is oriented identically. A: Native XI ($\chi^2=17.7$; PDB code 1xib). B: The XI model, based on the native subunit, with the best S_{DOPE} ($\chi^2=9.6$). C: The XI model, based on the native subunit, with the best S_{SAXS} ($\chi^2=7.5$). D: The χ^2 of the top 10% models (*ie*, clustered models) plotted against their C α RMSD with respect to the crystallographic structure. The models with the best S_{DOPE} and S_{SAXS} are indicated in red and green, respectively. E: S_{DOPE} of the top 10% models plotted against their C α RMSD with respect to the crystallographic structure. F: Combined score S of the top 10% models plotted against their C α RMSD. G: The XI configuration of subunit comparative models based on 4xim (67% sequence identity); left, best S_{DOPE} ; right, best S_{SAXS} . H: The XI configuration of subunit comparative models based on 1a0dA (27% sequence identity); left, best S_{DOPE} ; right, best S_{SAXS} .

Fig. 6. Scoring *versus* sampling. A: The minimum score S_{min} (left), the corresponding C α RMSD (middle), and the minimum C α RMSD for all models ($RMSD_{min}$; right) are plotted as a function of the number of independent optimizations for the benchmark case 1ha0. For comparison, the values for the best scores achieved for local sampling in the vicinity of the RBNC state (RBNC refined) and for local optimization of the native bodies in the vicinity of the native configuration are also shown. B: Dimeric complex 1ibr. C: Three-domain protein 1ko9.

Benchmark of multidomain proteins. ^a We modeled 9 different proteins (targets) using rigid bodies for the specified amino-acid residue ranges, connected by flexible linkers. ^b The rigid bodies corresponded to comparative models built using the specified template structures with different sequence identities, except for 100% sequence identity cases for which the specified template structure was used. ^c We predicted domain configurations using protocols that differed by the initial configuration (Near-template or RBNC) and the optimization (local or global). ^d The models from each protocol were then clustered. The most accurate cluster was identified as the cluster with the model that was closest to the native configuration in terms of Ca RMSD. The size of the cluster is the percentage of all models in it. Min RMSD is the Ca RMSD of the most accurate model in the most accurate cluster. We also list Ca RMSD as well as Δr and $\Delta\alpha$ (the translation and rotation of the rigid bodies for the optimal superposition on their native counterparts) for the models with the best S , S_{SAXS} , and S_{DOPE} in the most accurate cluster.

Table 1

Target ^(a)	Template ^(b)	Optimization protocol ^(c)				Most accurate cluster ^(d)											
		PDB	Rigid bodies (residues)	PDB	Seq Id%	Initial model	Sampling	Best model by S		Best model by S_{saxs}		Best model by S_{DOPE}					
								Size (%)	Min RMSD (Å)	RMSD (Å)	Δr (Å)	$\Delta\alpha$ (°)	RMSD (Å)	Δr (Å)	$\Delta\alpha$ (°)		
1ha0	1-316	2viuA	100	Near-template	Global	52	2.2	2.9	0.5	0.8	2.7	0.7	1.2	2.9	0.5	0.8	
326-494		2viuB	100	Near-template	Local		100	2.4	2.4	0.6	0.9	2.4	0.6	0.9	2.4	0.6	0.9
				Near-template	None		n.a.	2.8	2.8	0.1	0.1	2.8	0.1	0.1	2.8	0.1	0.1
				RBNC	None		n.a.	2.4	2.4	0.2	0.2	2.4	0.2	0.2	2.4	0.2	0.2
1gv2	89-136	1h88	100	Near-template	Global	37	1.2	2.8	5.0	24.6	2.6	4.3	25.0	5.0	3.8	45.2	
149-90		1h88	100	Near-template	Local		100	3.0	3.0	2.2	36.9	10.7	10.9	112.4	3.0	2.2	36.9
				Near-template	None		n.a.	9.2	9.2	7.8	101.9	9.2	7.8	101.9	9.2	7.8	101.9
				RBNC	None		n.a.	0.7	0.7	0.2	1.2	0.7	0.2	1.2	0.7	0.2	1.2
1mdtA	1-375	1ddtA	100	Near-template	Global	17	1.4	1.5	1.5	6.7	1.9	2.0	6.8	1.5	1.5	6.7	

Target ^(a)	Template ^(b)	Optimization protocol ^(c)		Most accurate cluster ^(d)									
				Size (%)		Best model by S		Best model by S_{saxs}		Best model by S_{DOPE}			
				Min	RMSD (Å)	RMSD (Å)	Δr (Å)	$\Delta\alpha$ (°)	RMSD (Å)	Δr (Å)	$\Delta\alpha$ (°)	RMSD (Å)	Δr (Å)
PDB	Rigid bodies (residues)	PDB	Seq Id%	Initial model	Sampling								
389-535	1ddtA			Near-template	Local	100	10.4	11.8	8.9	123.6	13.3	3.8	141.7
				Near-template	None	n.a.	20.3	20.3	31.0	177.4	20.3	31.0	177.4
				RBNC	None	n.a.	1.0	1.0	1.0	4.0	1.0	1.0	4.0
1a62	1-45			Near-template	Global	53	1.3	1.6	3.2	19.2	1.5	2.7	19.2
				Near-template	Local	100	10	1.1	1.1	0.7	6.6	1.1	0.7
				Near-template	None	n.a.	1.0	1.0	1.0	4.0	1.0	1.0	4.0
49-125	1pv0			Near-template	Local	100	10	1.1	1.1	0.7	6.6	1.1	0.7
				Near-template	None	n.a.	1.0	1.0	1.0	4.0	1.0	1.0	4.0
				RBNC	None	n.a.	1.0	1.0	1.0	4.0	1.0	1.0	4.0
1knA	19-184			Near-template	Global	46	8.3	12.6	14.1	166.3	12.7	15.0	160.1
				Near-template	Local	100	12.0	12.8	14.2	167.0	12.2	13.3	178.0
				Near-template	None	n.a.	13.3	13.3	16.0	133.4	13.3	16.0	133.4
1ko9A	12-100			Near-template	Global	27	1.8	2.1	1.7	10.8	2.4	2.6	15.3
				Near-template	Local	100	1.7	1.9	1.8	7.3	1.9	1.8	7.3
				Near-template	None	n.a.	1.5	1.5	0.9	5.1	1.5	0.9	5.1

Target ^(a)	Template ^(b)	Optimization protocol ^(c)		Most accurate cluster ^(d)																				
				Size (%)		Best model by <i>S</i>		Best model by <i>S_{saxs}</i>		Best model by <i>S_{Dope}</i>														
				Min	RMSD (Å)	RMSD (Å)	Δ <i>r</i> (Å)	Δ <i>α</i> (°)	RMSD (Å)	Δ <i>r</i> (Å)	Δ <i>α</i> (°)	RMSD (Å)	Δ <i>r</i> (Å)	Δ <i>α</i> (°)										
PDB	Rigid bodies (residues)	PDB	Seq Id%	Initial model	Sampling																			
1o0vA	1-102	1a6tB	33	RBNC	Global	17	27.2	50.6	58.2	172.5	46.9	55.2	164.2	50.6	58.2	172.5								
						n.a.	1.5	1.5	0.9	2.0	1.5	0.9	2.0	1.5	0.9	2.0								
115-204	116xA	116xA	31	RBNC	None	n.a.	5.6	5.6	4.6	21.4	5.6	4.6	21.4	5.6	4.6	21.4								
215-313	116xA	116xA	31	RBNC	None																			
1exmA	7-211	1f60A	35	Near-template	Global	24	25.4	29.8	37.8	157.6	29.6	37.6	149.6	25.4	31.8	117.8								
217-308	1f60A	1f60A	Near-template	Local	100	11.6	18.2	20.7	61.3	17.7	21.8	56.1	18.2	20.7	61.3									
312-405	1f60A	1f60A	Near-template	None	n.a.	9.1	9.1	7.1	33.5	9.1	7.1	33.5	9.1	7.1	33.5									
1cb6	1-87,252-330	1h76	63	Near-template	Global	n.a.	6.4	0.3	0.5	1.6	0.3	0.5	1.6	0.3	0.5	1.6								
89-249	1h76	1h76	Near-template	Local	100	3.4	3.5	4.6	11.2	3.9	4.2	12.6	3.5	4.6	11.2									
333-428,596-687	1h76	1h76	Near-template	None	n.a.	8.2	8.2	5.0	24.0	8.2	5.0	24.0	8.2	5.0	24.0									
433-592	1h76	1h76	RBNC	None	n.a.	2.1	2.1	0.2	1.3	2.1	0.2	1.3	2.1	0.2	1.3									

Table 2 Benchmark of binary complexes. ^a ‘Principal axes’ indicates that the initial model was obtained by centering the respective rigid protein structures on their centers of mass and aligning their moments of inertia. See Table 1 for definitions of the other terms.

Target		Template	Optimization protocol		Most accurate cluster										
					Size (%)	Min RMSD (Å)	Best model by S			Best model by S_{saxs}			Best model by S_{DOPE}		
PDB	Rigid bodies	PDB	Initial model	Sampling		RMSD (Å)	Δr (Å)	Δα (°)	RMSD (Å)	Δr (Å)	Δα (°)	RMSD (Å)	Δr (Å)	Δα (°)	
lavxAB	16-245:A	1qquA	Principal axes ^(a)	Global	45	9.3	10.5	5.1	81.6	10.5	5.1	81.6	14.6	8.8	116.1
				None	n.a.	2.6	2.6	0.2	1.0	2.6	0.2	1.0	2.6	0.2	1.0
libr	9-177:A	1qg4A	Principal axes ^(a)	Global	51	12.6	13.0	18.5	66.5	13.2	18.9	66.6	13.2	19.0	66.4
				None	n.a.	3.4	3.4	0.3	1.2	3.4	0.3	1.2	3.4	0.3	1.2
lfql	23-200:A	1fpzF	Principal axes ^(a)	Global	28	46.8	50.1	67.7	94.4	50.1	67.7	94.4	46.9	68.5	101.8
				None	n.a.	4.0	4.0	1.6	1.2	4.0	1.6	1.2	4.0	1.6	1.2

Table 3

Modeling of the XI tetramer, using monomer models based on a range of sequence identity with the templates. ^a: Clusters of all models are characterized by their relative sizes and the minimum Cα RMSD's. ^b: From each cluster, the best-scoring model according to S , χ^2 , S_{DOPE} , and $S_{overlap}$ is chosen and its respective score and Cα RMSD are shown.

Template		Cluster ^(a)			Best S in cluster ^(b)			Best χ^2 in cluster ^(b)			Best S_{DOPE} in cluster ^(b)			Best $S_{overlap}$ in cluster ^(b)		
PDB	seq. id. (%)	Size (%)	Min RMSD (Å)	S	RMSD (Å)	χ^2	RMSD (Å)	S_{DOPE}	RMSD (Å)	S_{DOPE}	RMSD (Å)	$S_{overlap}$	RMSD (Å)	S_{DOPE}	RMSD (Å)	
1xib	100	8	51.9	202190	53.5	8.4	51.9	-437	51.9	-437	51.9	174417	53.5			
		50	2.0	5828	2.3	9.6	2.3	-20171	2.1	1965	2.3					
		16	49.0	196400	50.1	7.5	50.1	5292	49.7	172413	50.1					
		20	27.3	380590	32.9	7.9	29.3	-513	27.3	351926	32.9					
4xim	67	48	3.4	53800	3.5	12.8	3.6	-9242	3.4	31709	3.5					
		14	50.6	191787	51.1	9.5	50.6	7396	50.8	155453	51.1					
		8	28.0	526269	29.4	10.8	28.0	2799	30.4	494592	29.4					
1a0d	28	20	43.2	118494	46.7	7.0	46.6	-1031	46.0	101594	46.7					
		22	47.8	92027	50.2	6.9	50.1	-1938	50.1	75363	50.2					
		16	10.9	86644	14.5	8.0	14.5	-2715	15.3	69575	14.5					
		10	47.2	394099	50.5	8.8	50.5	3591	47.2	368160	50.5					
		8	51.7	136284	51.8	8.3	51.7	3316	51.7	112201	51.8					
		18	26.6	116515	29.9	7.1	29.8	-1148	30.9	99543	29.9					