



New opportunities in integrative structural modeling

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Abstract

Integrative structural modeling enables structure determination of macromolecules and their complexes by integrating data from multiple sources. It has been successfully used to characterize macromolecular structures when a single structural biology technique was insufficient. Recent developments in cellular structural biology, including in-cell cryo-electron tomography and artificial intelligence-based structure prediction, have created new opportunities for integrative structural modeling. Here, we will review these opportunities along with the latest developments in integrative modeling methods and their applications. We also highlight open challenges and directions for further development.

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Introduction

Integrative modeling allows macromolecular structure determination by combining data from multiple experimental and computational techniques [1]. In essence, empirical data guides the assembly of a protein complex from its component structures. For example, atomic structures may be fit into the 3D density obtained by cryo-electron microscopy (cryo-EM) or scattering profiles by small-angle X-ray scattering (SAXS) and

simultaneously orientated with distance restraints obtained by cross-linking mass spectrometry (XL-MS) (Figure 1). Depending on data quality, integrative models can inform the general architecture, find evolutionary relationships, localize active sites and near-atomic interactions, and generate hypotheses on the mechanism(s) of action.

Integrative modeling has resolved the structure of many complexes (Figure 2). A prime example is the nuclear pore complex, first published in 2007 [2]. Since then, nuclear pore complexes from various species have been modeled with increasingly precise data, culminating with near-atomic yeast and human models [3–5]. Other recent examples include the Fanconi anemia core complex [6], yeast Smc5/6 holo-complex [7], and mycobacterial type VII secretion system [8] built using cryo-EM and XL-MS data; a ribozyme using NMR and SAXS data [9]; RAGE complexed with S100B using XL-MS, hydrogen-deuterium exchange, and mass spectrometry data [10]; and histone H3-H4 and RNA polymerase sub-complexes using restraints from a novel genetic interaction mapping technique [11].

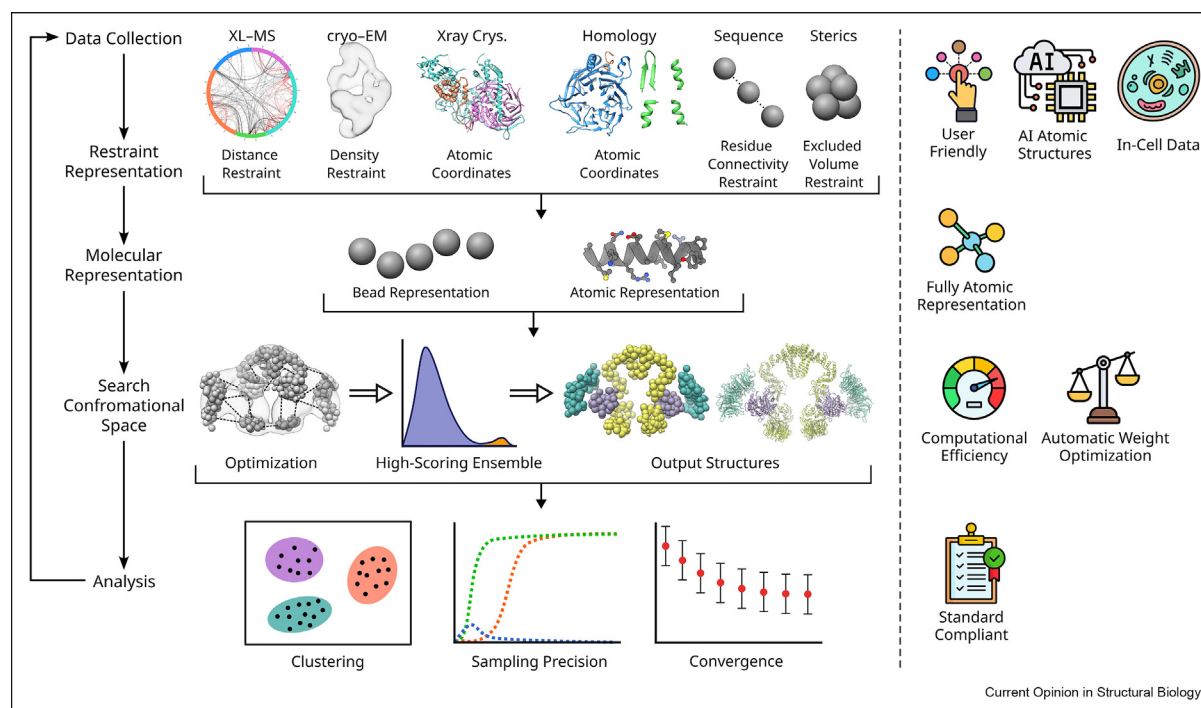
Many integrative modeling software tools are available, with Integrative Modeling Platform (IMP) [12] and HADDOCK [13] being the most popular. Derivative programs targeting different modeling challenges and methodological aspects include Python modeling interface [14] and our Assemblin protocol [15], both based on IMP; M3 [16] which is based on HADDOCK; or IMProv [17], a graphical interface to IMP.

Here, we will review recent innovations and new opportunities arising from advances in experimental and computational techniques.

State-of-the-art in integrative structural modeling

The first step in integrative modeling, besides collecting experimental data, is choosing how to represent macromolecules during modeling (Figure 1). While small complexes can be represented at the atomic level, larger complexes must be represented more coarsely—such as spherical beads approximating individual or groups of residues—for computational efficiency. Multiple levels can be used simultaneously in a so-called multi-scale representation, with fine-grain details retained only for calculations that require accuracy [1,18]. IMP, PMI, and

Figure 1



Schematic of a typical integrative modeling workflow (left) with opportunities and key areas for development (right).

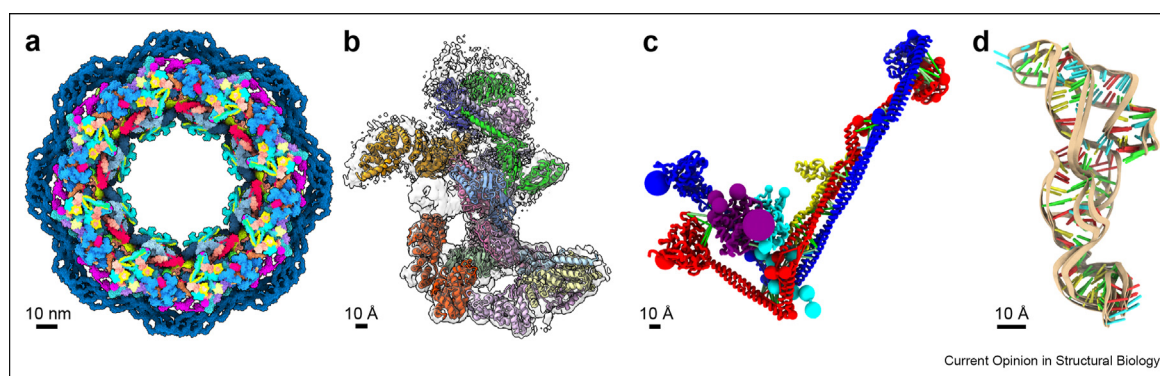
Assemblines use multi-scale representation to efficiently generate large models with atomic features. Though the choice of representation is usually dictated by the software and the modeler's intuition, an automatic optimization protocol based on local information density has been proposed [19].

The second step is representing experimental data as restraints—mathematical formulations that evaluate the fit of sampled models to the input data. Importantly, the

restraints must account for uncertainty in the data, such as EM map resolution or the expected cross-link distance distribution, and be suitable for the scale of representation. Many experimental data types, such as EM, SAXS, or XL-MS, have already been implemented as restraints, with more sophisticated forms being developed [20,21].

Third, an optimization procedure searches the space of structural configurations for models that best fit the data, based on a scalar scoring function constructed from

Figure 2



Examples of recent integrative models. **(a)** Human nuclear pore complex model (PDB ID: 7R5K). **(b)** Fanconi anemia core complex (PDB DEV ID: 00000055). **(c)** Smc5/6 complex showing multi-scale atomic and bead representation (PDB DEV ID: 00000081). Green bars indicate crosslinks used for modeling. **(d)** Neurospora Varkud satellite ribozyme (PDB DEV ID: 00000067).

the restraints. While optimization procedures have matured [1,13], computational efficiency is still lacking for large and fine-grained models. The scoring function is usually a linear combination of individual restraints [13,15] with manually selected weights. Methods based on Bayesian inference have been proposed [12–15,21] with weights optimized automatically based on the estimated quality of the data. However, only a few restraint types and software have Bayesian formulations, and the resulting function is often still a linear combination of Bayesian and conventional terms. Thus, an integrative model is not only a hypothesis over coordinates but also the relative weighing of data types. In our opinion, automated weighting is one of the biggest challenges in integrative modeling that requires further advancement.

Last, integrative models must be assessed for precision, consistency with the data, and exhaustiveness of conformational sampling. A formalized solution is included in the *imp-sampcon* toolkit [22], recently improved to determine local precision [23].

New opportunities from artificial intelligence-based structure prediction

Traditionally, atomic structures for integrative modeling came mainly from X-ray crystallography, NMR, and cryo-EM. If no experimental structures existed, homology modeling could be used to predict structures based on homologous proteins as templates. Yet, this was a relatively time-consuming process limited by the data available. If no atomic structures could be obtained, spherical beads would be used to approximate residues or entire subunits. As a result, integrative models were limited in precision, with some subunits retaining “bead” representation, or having uncertain localization and unresolved steric clashes. Although fully atomic integrative modeling programs exist, such as HADDOCK, they are limited by the quality of input structures and the accuracy of the optimization algorithms.

The advent of artificial intelligence (AI) protein structure prediction programs, such as AlphaFold2 [24] and RoseTTAFold [25], has revitalized the potential of atomic integrative structural modeling. These programs can model the atomic structure of proteins and small complexes at a precision comparable to experimental structures. In some cases, AlphaFold structures have fit experimental data better than crystal structures [4] and may exhibit conformations resembling the complexed structure. Almost all proteins cataloged in UniProt are available for download from the EBI’s AlphaFold database. Together with user-friendly implementations of these programs [26], we can predict atomic structures with relative ease.

Thus, the current state-of-the-art of integrative modeling is to build sub-complexes via AI-based

structure prediction prior to assembling the higher-order structure. In our work on the human nuclear pore complex [4], we used AlphaFold to generate models of single proteins and sub-complexes that were assembled using our Assembline integrative modeling pipeline. The study exemplified several advantages of AlphaFold models for integrative modeling, such as an excellent fit to cryo-EM densities even if subunits are modeled outside the complex context, the surface complementarity of independently modeled proteins, and minimal steric clashes. Many studies have used AlphaFold models as starting structures for cryo-EM model building (for example: [27–30]), and some have begun using AlphaFold in cross-linking [31] and SAXS [32] studies. The structure of SMG1 kinase with its protein cofactors was determined by integrating cryo-EM and cross-linking data with AlphaFold model fitting and refinement [33]; cross-links were also used to evaluate the quality of AlphaFold models [34]. A new Phenix procedure integrated AlphaFold modeling and iterative refinement with high-resolution cryo-EM maps [35]. Although computation costs are still an issue, we can, nevertheless, confidently discontinue approximating structure with mere “beads” in integrative modeling.

Can AI modeling programs reduce the number of orthogonal techniques needed for integrative modeling? On the one hand, it might be sufficient to obtain accurate models of sub-complexes with AlphaFold, put them together using a single experimental data set, such as cross-links, and only then validate against EM or SAXS data. This would simplify integrative modeling by eliminating the need for tedious restraint weight adjustments. On the other hand, the diversity of perspective is a key advantage of integrative modeling. A limitation of current AI-modeling tools is the nontrivial assessment of model accuracy [36]. Though they provide quality scores to estimate model accuracy, these are, nevertheless, just predictions with an inherent level of error. Modeling complexes is still challenged by unknown stoichiometry, alternative homo-oligomeric states (e.g., a tetramer vs. pentamer), and asymmetry. When AI-based models have wrong conformations or contain errors, additional experimental information will be required for refinement. For example, AlphaFold predicts an interaction between NUP160 and ELYS proteins in the human nuclear pore complex. However, the complex contains the NUP160 subunit on both sides of the nuclear membrane, but ELYS is present and interacts with NUP160 only on the nuclear side. In another example, XL-MS was used to guide the modeling of multi-domain SARS-CoV-2 proteins in conformations that could not be predicted using AlphaFold2 alone [37]. Thus, the biological context provided by techniques such as EM, cross-linking, SAXS, and biochemical experiments on interactions may still be necessary to clarify nuances and validate the

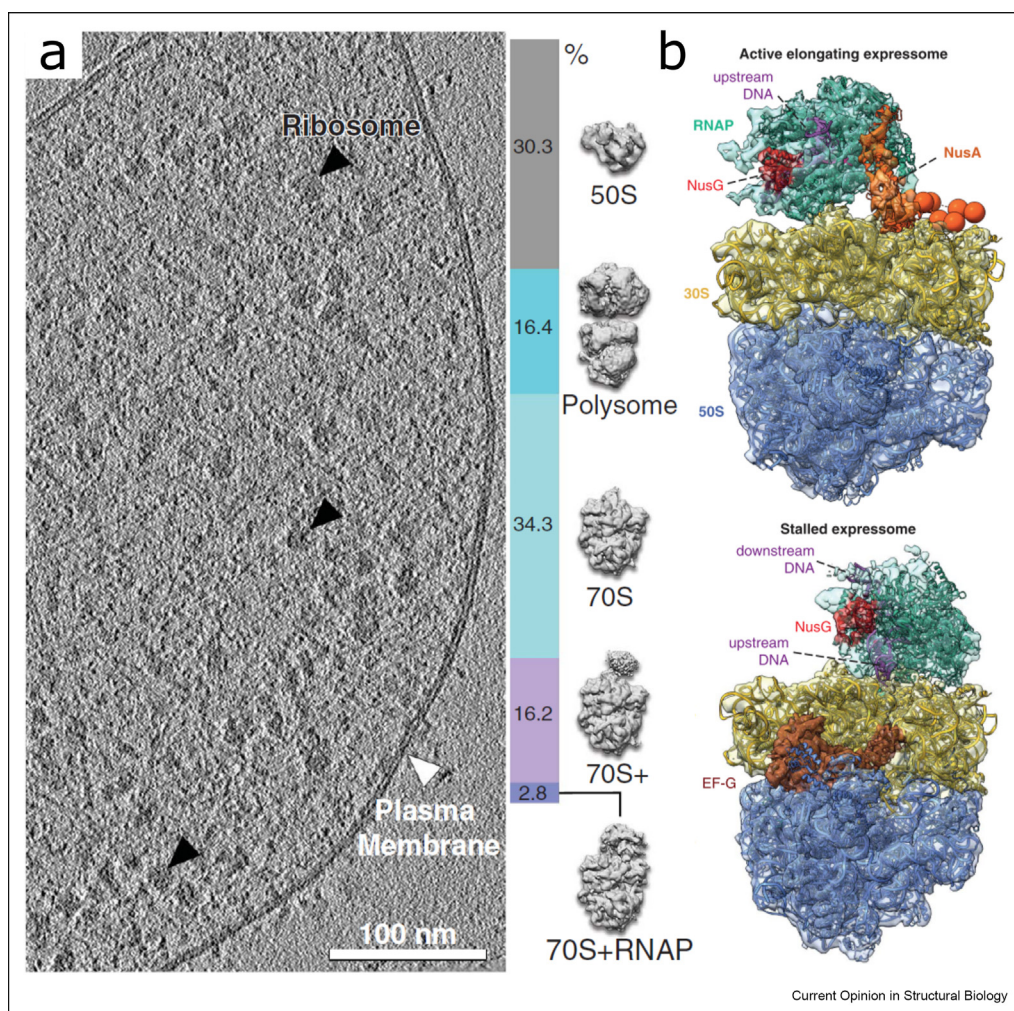
predicted models. Finally, the incorporation of additional data, such as EM densities, cross-link distance restraints, or SAXS profiles, into the AI-based structure prediction itself could create a new generation of integrative modeling programs, made possible by the availability of open-source codebases.

New opportunities in cellular structural biology

Cellular structural biology, whereby macromolecular structures are solved in the cellular environment, has gained traction thanks to recent advances in cryo-ET [38]. In this technique, cells are placed on a grid, vitrified, and, optionally, thinned into lamellae using focused ion beam (FIB) milling. Lamellae are imaged under a transmission electron microscope across a range

of tilt angles, and the images are reconstructed into a 3D tomogram. Individual macromolecular particles can be computationally extracted, aligned, and averaged in a process called sub-tomogram averaging, resulting in 3D density maps with a resolution of 10–50 Å. FIB-milling is now an automated process and becoming faster and more reproducible [39], electron detectors are becoming more sensitive still, image acquisition is becoming more efficient [40], and processing software for finding and averaging particles in tomograms is becoming rapidly more streamlined and accurate [41,42]. With these advances in cellular cryo-ET, we can expect an explosive increase in cryo-ET output, akin to the ‘resolution revolution’ in single-particle cryo-EM [43]. Despite these advances and the first examples of high-resolution in-cell cryo-ET maps [41,42], cryo-ET

Figure 3



The structure of an actively transcribing-translating expressome determined by integrative modeling using in-cell cryo-ET and XL-MS data. (a) Slice through a tomogram of *M. pneumoniae*. Example ribosome particles are indicated by black arrows. Classification of particles on the right. (b) Models of the active elongating (top) and stalled (bottom) expressome. From O'Reilly FJ, Xue L, Graziadei A, Sinn L, Lenz S, Tegunov D, Blötz C, Singh N, Hagen WJH, Cramer P et al.: In-cell architecture of an actively transcribing-translating expressome. *Science* (80-) 2020, 369:554–557 [48]. Reprinted with permission from AAAS.

still suffers from comparably sparse data sets, resulting in low-resolution structures on average (10–50 Å). Cryo-ET is, therefore, in a prime position to benefit from integrative modeling. The most recent nuclear pore complex models are based on the in-cell cryo-ET maps [3,4,44–46]. Other examples include the Parkinson's disease-linked LRRK2 [47] and the complex of bacterial RNA polymerase in complex with a ribosome [48] (Figure 3).

A notable benefit of AI-based structure prediction for cellular cryo-ET structural biology is in providing structures for template matching—the identification of macromolecules in a tomogram based on a 3D search with a starting “template” structure [38]. Predicted models of complexes [49] could be used as better templates, enabling the identification of smaller and less abundant complexes, and a more complete interpretation of tomograms. Nonetheless, cryo-ET must still overcome many technical limitations. Even if 3D maps can be reconstructed, they may have an unknown identity and protein composition [50,51], unassigned densities [45], and unclear stoichiometry. Protein paralogs that exchange in the same complex in a context-dependent manner may not be distinguishable in 10–50 Å maps (e.g., different paralogs of tubulins in microtubules), leading to false positives in AlphaFold2 [36]. Thus, as with integrative structure determination of purified complexes, orthogonal in-cell techniques will be necessary for confident structure determination. These may include in-cell cross-linking [52], super-resolution microscopy [53], and cryo-super-resolution light and electron tomography [54]. Another opportunity lies in parallel single-particle cryo-EM analyses of cellular lysate fractions, containing dozens or hundreds of different complexes from the same cells that can be computationally identified and reconstructed, often at high resolution [55]. If high resolution cannot be obtained, XL-MS of the fractions can be used with integrative modeling, as was done for the 10-MDa eukaryotic pyruvate dehydrogenase complex [56]. Integrative modeling does not necessarily end when a structural ensemble is obtained. Rather, further data can be integrated into the model, such as biophysical simulations in the context of the native, cellular environment. For example, an integrative model of the nuclear pore complex was placed in the double membrane of the nuclear envelope to simulate pore dynamics [4].

Conclusions

Integrative structural modeling is an essential technique for structural biology. It combines the strengths of many techniques to resolve the structures of large and complex macromolecular systems which could not otherwise be achieved by a single experimental approach. Modern

AI structure prediction tools have yielded structural models of proteins and small complexes at unprecedented precision, enabling us to overcome limitations in the scale and detail with which we can model macromolecular structures. To support the growth of integrative structural biology, we must also advance several key areas. It is imperative to integrate AI-based structure prediction tools with experimental restraints; develop a consistent rationale for automatic weighting of different data types; improve the computational efficiency of optimization algorithms; conform to accepted standards of integrative models, such as the inclusion of data and restraints in structural representations; and develop computational representations of novel in-cell data types. These developments will pave the way for ever larger and more detailed structural modeling studies, perhaps one day integrating with atomic biophysical simulations. Integrative structural modeling is well poised to solve problems in cellular structural biology, where high-precision data is more scarce, and multidisciplinary approaches are a necessity. We expect that future studies will broaden our perspective of integrative models beyond a structure in isolation and toward the cellular horizon.

Conflict of interest statement

Nothing declared.

Data availability

No data was used for the research described in the article.

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