Mini review in Current Opinion in Structural Biology: Macromolecular Assemblies

Title: Structural Highlights of Macromolecular Complexes and Assemblies

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Abstract

The structures of macromolecular assemblies have given us deep insights into cellular processes and have profoundly impacted biological research and drug discovery. We highlight the structures of macromolecular assemblies that have been modeled using integrative and computational methods and describe how open access to these structures from structural archives has empowered the research community. The arsenal of experimental and computational methods for structure determination ensures a future where whole organelles and cells can be modeled.

Keywords

Integrative modeling; Computational modeling; Protein structure prediction; PDB; PDB-Dev; ModelArchive; AlphaFold; RoseTTAFold; PDBx/mmCIF; ModelCIF; IHMCIF; Computed structure models

Introduction

A vast majority of biological macromolecules function as part of assemblies in the cell. The first structures of large assemblies such as viruses and ribosomes were determined using X-ray crystallography [1,2]. These structures provide insights into the function and interactions of macromolecular machines involved in cellular processes and have profoundly impacted

biomedical research and drug discovery. Structures of viral protein complexes have enabled vaccine and drug development to combat existing and emerging viral pathogens, including the SARS-COV-2 virus [3]. Furthermore, structures of many integral membrane protein complexes (e.g., G-protein coupled receptors and ion channels) have aided in successful structure-guided drug design investigations [4]. With the advancement of structural biology methods, three-dimensional electron microscopy (3DEM) methodology has emerged as a powerful tool for structure determination of large macromolecular machines. For example, recent 3DEM investigations determined the structure of bacterial expressome (complexes of RNA polymerase, ribosome, and associated molecules) in atomic detail (Figure 1A) [5,6] and elucidated the role of transcription elongation factors in stabilizing the complex and enabling coupling between transcription and translation. Additionally, 3DEM has enabled exploration of macromolecular assemblies in their natural cellular environment as seen in recent investigations of ribosomes that revealed multiple translational states [7,8], provided insights into the dynamics of the ribosome-translocon complex at the endoplasmic reticulum membrane [9] and visualized how anticancer drugs inhibit protein biosynthesis [10].

Experimentally-determined structures of macromolecules and their complexes obtained using single methods such as macromolecular crystallography (MX), nuclear magnetic resonance (NMR) spectroscopy, and 3DEM are archived in the Protein Data Bank (PDB) [11,12]. Recently, structures of several important macromolecular complexes have been determined using integrative approaches, where experimental data from multiple complementary methods are combined [13]. In addition to MX, NMR, and 3DEM, methods contributing to integrative structural biology include small angle scattering (SAS), chemical crosslinking mass spectrometry (CX-MS), Forster resonance energy transfer (FRET), hydrogen-deuterium exchange mass spectrometry (HDX-MS), electron paramagnetic resonance spectroscopy (EPR), and atomic force microscopy (AFM). An advantage of integrative structure determination is that it is capable of modeling structures of large, complex, heterogenous, and dynamic macromolecular systems spanning multiple spatiotemporal scales and conformational states (Figure 2).

In addition to experimental and integrative approaches, modeling protein complexes using purely computational methods has been an active area of research in the structure prediction community [14]. After the success of AlphaFold2 [15] in the Critical Assessment of methods for protein Structure Prediction (CASP) 14 challenge held in 2020, researchers have been exploring mechanisms to expand the application of deep learning methods to model higher order protein assemblies. AlphaFold-Multimer [16] has been developed by training the AlphaFold2 system with multimeric proteins of known stoichiometry. Application of AlphaFold-Multimer or a variation of the tool in the CASP15 challenge held in 2022 demonstrated significant progress in the community regarding prediction of multimeric protein complexes [17].

In the following sections, we highlight structures of complexes from recent studies, modeled using integrative and computational approaches and summarize the mechanisms developed to archive these structures in structural model repositories and make them Findable, Accessible, Interoperable, and Reusable (FAIR) [18].

Structures of macromolecular complexes obtained from integrative and computational methods

The nuclear pore complex (NPC) plays a key role in regulating transport across the nuclear membrane. The structure and assembly mechanism of NPC have been elucidated by integrative modeling (Figure 1B) [19,20] and have led to the creation of a spatiotemporal model of the complex including the postmitotic intermediates involved in the assembly pathway. The modeling was carried out using data from 3DEM, CX-MS, SAS, and fluorescence correlation spectroscopy. The studies reveal that the assembly pathway of NPC follows two distinct molecular mechanisms, where the order of incorporation of structural components is reversed and shed light on the role of NPCs in the evolution of endomembrane systems in eukaryotes.

The in-cell structure of an actively transcribing-translating expressome was determined using integrative modeling, where experimental data obtained entirely from in-cell experiments (CX-MS and 3DEM) were combined to obtain a multi-scale structure of the assembly (Figure 1C). The study adds insights into the mechanism of transcription-translation coupling and highlights the role of auxiliary factors in mediating the coupling [21].

The heterodimeric CLOCK-BMAL1 complex belonging to the basic helix-loop-helix (bHLH) family of transcription factors, is an essential component of the molecular clock and plays a crucial role in managing the circadian rhythm. The integrative structure of the CLOCK-BMAL1 complex bound to native nucleosome consisting of DNA wrapped around histones, has been determined using 3DEM and CX-MS data (Figure 1D) [22]. This multi-structure investigation addresses how different classes of bHLH transcription factors structurally and functionally interact with nucleosomes and identify specific DNA motifs within chromatin.

The pentraxin protein PTX3 is a member of a family of soluble pattern recognition molecules that play an important role in innate immune defense by facilitating responses to infections and triggering processes such as inflammation. The complete structure of the PTX3 complex was built by integrative modeling using 3DEM and mass spectrometry data, and AlphaFold-based starting models (Figure 1E) [23]. The study elicits the details of functional interaction sites of PTX3 involved in immune defense and exemplifies the use of AlphaFold models as starting models in integrative structure determination.

Protein-protein interactions play an important role in cellular processes, but the structures of many eukaryotic protein complexes are still unknown. A combination of RoseTTAFold and AlphaFold2 [24] has been developed and applied to systematically identify and build structures of core protein complexes in eukaryotes that carry out key functions. The prediction algorithm involves the use of deep learning methods to build a coevolution guided interaction identification pipeline, which is applied on a proteome-wide scale. This study on the yeast interactome evaluates ~8.3 million pairs of yeast proteins to obtain computed structure models (CSMs) of ~800 complexes that have been previously identified but were structurally uncharacterized and ~100 complexes that are novel interactions not previously identified. These complexes (examples shown in Figure 3) provide insights into a wide variety of biological processes

including transcription, translation, mitosis, meiosis, DNA damage and repair, protein and ion transport, protein translocation and modification, and enzyme function.

Like the investigation on the yeast interactome, AlphaFold2 has been applied to model structures of complexes involving cancer driver proteins [25] and create the structural landscape of the cancer protein-protein interactome. The authors investigated over 100,000 putative human protein-protein interactions identified from various databases and supported by multiple high throughput experiments. Using AlphaFold2, they developed a methodology to generate CSMs for about ~1700 complexes. About 1000 modeled complexes are novel interactions and provide insights into many cancer-related biological processes such as the MAP kinase cascade and the Fanconi anemia pathway, revealing mechanisms for cancer development and new targets for cancer treatment.

Archiving structures of macromolecular complexes

PDB is the archive for experimental structures [11,12], ModelArchive was created to host CSMs referenced in publications (https://www.modelarchive.org), and PDB-Dev has been developed for archiving integrative structures [26,27]. All three repositories follow a common language for describing data standards with shared definitions [26,28,29] that enable interoperation among experimental and integrative structures, and CSMs. Integrative structures and CSMs highlighted in the previous section are archived in PDB-Dev and ModelArchive respectively.

PDB and PDB-Dev have been created using methods for curation and validation that are based on community recommendations. These methods ensure data standardization and completeness and include model quality assessments that facilitate appropriate utilization of these structures in downstream applications. PDB-Dev was implemented separately from the PDB to facilitate an agile development platform, with the goal of eventually unifying the two resources and work is currently in progress in this direction.

To facilitate interoperability, the integrative structural biology community made recommendations [30,31] for creating a federation consisting of a network of experimental data and structural model resources that support integrative modeling (illustration in Figure 4). Such a network would foster the creation of independent data repositories within different scientific domains that can exchange data with each other. Although the creation of such a federation requires concerted and collaborative effort within and across different scientific disciplines, it has the potential to transform how research is conducted by providing seamless access to diverse scientific data.

Broader insights and future perspectives

The structures highlighted in this review provide rich insights into mechanisms of biological processes such as transcription, translation, and nuclear transport. Although static structures of macromolecules enhance our understanding of macromolecular function, these molecules rarely exist in isolation in the cell. It is important to examine how they interact with other molecules and cellular components to gain a deeper understanding into their function and reveal the dynamics of “molecules in action”.

An arsenal of many experimental, integrative, and computational methods is now available for structure determination of single molecules and large macromolecular assemblies. MX is still the predominant method for experimental structure determination and will continue to be important for structural investigations, especially for those involved in understanding drug and small molecule binding to macromolecules. Increasing the availability of well-curated and validated co-crystal structures of macromolecules bound to small molecules will facilitate the application of machine learning algorithms to predict the effects of drug binding and can potentially impact molecular medicine. 3DEM is the method of choice for obtaining the structures of large macromolecular assemblies, both as a single method and as part of integrative modeling investigations. NMR spectroscopy provides information about conformational ensembles observed in solution, thus enhancing our understanding of macromolecular dynamics.

As the field of structural biology evolves, the outlook for applying Integrative modeling to address future challenges looks promising. There is a growing trend that shows increasing use of 3DEM maps in combination with restraints from CX-MS or similar experiments and starting structural models of subunit components to obtain structures of large macromolecular assemblies. The scope of such studies has expanded further with the availability of >200,000 experimental structures in the PDB and >200 million CSMs in AlphaFoldDB [32], which together provide a massive pool of starting models for integrative modeling investigations.

The enormous wealth of well-curated and validated structural data provided freely by the PDB to all users worldwide, has played a crucial role in the successful development and application of machine learning algorithms for protein structure prediction [33] and showcases the importance of making scientific data FAIR. With the success of AlphaFold2 [15] and RoseTTAFold [24], the field of protein structure prediction is looking towards developing computational methods to address newer challenges such as modeling multimeric complexes [17], conformational ensembles [34], and RNA structures [35,36]. Recent advances reported by Google DeepMind and Isomorphic Labs indicate remarkable progress in computational modeling of macromolecular complexes including proteins, nucleic acids, small molecules, and post-translational modifications [https://deepmind.google/discover/blog/a-glimpse-of-the-next-generation-of-alphafold/].

Expansion of deep learning-based modeling algorithms to include experimental restraints is an active area of research. AlphaLink [37] is a new method that integrates experimentally determined CX-MS restraints into the network architecture of AlphaFold2 to improve performance and predict distinct conformations of proteins. New integrative modeling methods are also being developed to understand structural dynamics of macromolecules. For example, integrative modeling of large GTPases using restraints from FRET, SAS, and EPR, illustrates different conformers involved in oligomerization and reveals important mechanistic and kinetic information regarding conformational transition between multiple states [38]. Furthermore, molecular dynamics simulation has become an important tool in integrative structural biology, where its application in combination with other experimental data is leading to deeper

understanding of the mechanisms and dynamics of macromolecular function as elucidated in the study of antibiotic-ribosome interactions [39].

Since the first structures of small single domain proteins were determined more than 60 years ago, the scope of structural biology has expanded allowing for larger and more complex structures to be determined using a wide variety of methods. Today, integrative modeling has led to determination of multi-scale and dynamic structures of macromolecular assemblies such as the nuclear pore complex. The current trend points to a future that will involve tackling newer and more sophisticated structure determination challenges, such as elucidation of three-dimensional structures of complete genomes [40,41] and creation of spatiotemporal models of the whole cell [42,43].

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Figure Captions

Figure 1: Structures of macromolecular complexes. (A) Structure of the expressome determined using 3DEM archived in the PDB (6X9Q; image taken from the “Molecule of the Month” [44] article by David S. Goodsell made available by RCSB PDB [11]); (B) Multi-scale structure of the nuclear pore complex obtained by integrative modeling (PDBDEV_00000012); (C) Integrative structure of expressome from in-cell modeling (PDBDEV_00000049); (D) Atomic structure of the CLOCK-BMAL1 transcription factor bound to the nucleosome obtained by integrative modeling (PDBDEV_00000210); and (E) Atomic structure of the octameric pentraxin core along with tetrameric coiled coil region obtained by integrative modeling (PDBDEV_00000141). Images B, C, D, and E are obtained using Mol* [45] based on structures archived in PDB-Dev.

Figure 2: Spanning the length scale from cells to molecules. (A) *Saccharomyces cerevisiae* cell highlighting nucleus dimension (2 μm); (B) Nuclear Pore Complex (98 nm) [19]; (C) Integrative structure of the Nup-84 sub-complex (40 nm) [46], with the black arrow indicating the Seh-1 subunit; (D) Cartoon representation of the atomic structure of the Seh-1 subunit (5 nm). The double-headed red arrows show the dimensions of the components shown.

Figure 3: Structures of core eukaryotic protein complexes involved in transcription, translation, and DNA repair modeled using a combination of RoseTTAFold and AlphaFold2 [24] and archived in the ModelArchive (https://www.modelarchive.org, accession code: ma-bak-cepc). Figure taken from Humphreys IR, et al. Computed structures of core eukaryotic protein complexes. Science. 2021; 374(6573):eabm4805. doi: 10.1126/science.abm4805. Reprinted with permission from AAAS.

Figure 4: Schematic representation of federating structural models and experimental data. At the center are the three structural biology model repositories: PDB for experimentally determined structures [11,12]; the ModelArchive for computed structure models (https://www.modelarchive.org); and PDB-Dev for integrative structures [26,27]. The outer circle shows different kinds of experimental data contributing to integrative structural biology. Existing data exchange mechanisms for MX, NMR, 3DEM, and SAS data and among the structural model repositories are represented by black arrows. Data exchange with other types of experimental data that are yet to be developed are shown by gray arrows. Collaborative activities are in progress in these communities to create benchmarks, data standards, and other mechanisms to promote FAIR data practices [47-52].

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