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Function and dynamics of macromolecular complexes explored by integrative structural and computational biology

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Three vignettes exemplify the potential of combining EM and X-ray crystallographic data with molecular dynamics (MD) simulation to explore the architecture, dynamics and functional properties of multicomponent, macromolecular complexes. The first two describe how EM and X-ray crystallography were used to solve structures of the ribosome and the Arp2/3–actin complex, which enabled MD simulations that elucidated functional dynamics. The third describes how EM, X-ray crystallography, and microsecond MD simulations of a GPCR:G protein complex were used to explore transmembrane signaling by the β-adrenergic receptor. Recent technical advancements in EM, X-ray crystallography and computational simulation create unprecedented synergies for integrative structural biology to reveal new insights into heretofore intractable biological systems.

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Introduction

The synergy among structural studies using X-ray crystallography, electron microscopy (EM) and image reconstruction is especially powerful for inferring the design and functional properties of multicomponent, macromolecular complexes. A common strategy is to fit high-resolution X-ray or NMR structures into lower-resolution EM-derived molecular boundaries of the entire complex. Another approach is to use low to moderate resolution EM maps or EM-derived models to obtain initial phases for higher resolution X-ray crystallographic structure determination, exemplified by determination of the 9 Å structure of the 50S ribosome from an electron cryomicroscopy (cryoEM) map [1]. To the best of our knowledge, the first example of this approach was determination of the 28 Å X-ray structure of tomato bushy stunt virus by the Harrison laboratory [2] using a phasing model provided by an EM map of negatively stained particles determined in the Crowther laboratory [3].

In the last decade there have been significant advances in EM technology and image processing methods, including improvements in microscope and stage stability, coherence and intensity of the electron beam, and, most recently, the development of direct electron detectors with a sensitivity that competes with film [4–8]. There is an expanding number of subnanometer resolution cryoEM maps of macromolecular complexes derived by single particle analysis, some approaching atomic resolution, exemplified by large megadalton (MDa) structures such as icosahedral viruses [9] and ribosomes [10–12] and smaller complexes such as the TRP channel [13,14] (reviewed in this issue [15]), the HIV ENV complex [16], and γ-secretase [17]. *Bona fide* atomic resolution structures have been derived from analysis of 2D crystals of membrane proteins reconstituted into lipid bilayers [18] and protein structures derived by electron crystallography of 3D crystals [19]. Meanwhile, X-ray crystallography has been used to solve structures of membrane proteins and their complexes in membrane mimetic environments such as bicelles [20] and lipidic cubic phases [21]. Most recently, X-ray crystallography using a free electron laser (XFEL) has been used to solve structures from sub-micron crystals of large, biologically active complexes [22] and membrane proteins in a lipid environment [23] (reviewed in this issue by Feld and Frank [24]). In addition to these impressive advances in experimental structural biology, there are increasingly accurate and accessible computational tools for modeling structures with limited or sparse experimental data and

investigating the functional dynamics of complex biological systems [25–28].

There are excellent historical and methodological reviews on the marriage of EM and X-ray crystallography, and on the myriad integrative structural biology approaches that include spectroscopic methods (e.g. NMR, EPR, FRET [29,30]). Here we focus on structural studies using EM and X-ray crystallography, which were brought into the realm of integrative structural biology by applications of molecular dynamics (MD) simulations. Also powerful, but not covered here, are macromolecular structural modeling methods that are guided or restrained by experimental data. These computational methods include the Integrative Modeling Platform from the Sali laboratory [25] and Rosetta from the Baker laboratory [31].

We highlight three vignettes that exemplify integrative structural and computational biology. In the first, studies of the ribosome employed cryoEM and X-ray crystallography to independently solve the same structure, from which MD studies were then possible. The second example describes how X-ray crystallography and electron tomography were used in tandem to solve the structure of the Arp2/3–actin complex, which enabled further analysis by MD simulation. In the third, an X-ray structure of the β-adrenergic receptor in a complex with the heterotrimeric G protein Gs was used to interpret structural flexibility of the complex by single particle EM image analysis. Microsecond MD simulations of the β-adrenergic receptor revealed the possible pathway of ligands to the orthosteric binding site and a mechanism for signaling across the membrane.

Structure and conformational dynamics of ribosomes

Ribosomes are massive MDa-sized ribonucleoprotein complexes that serve as the universal translator of genetic information, responsible for the conversion of messenger RNA (mRNA) transcripts to the polypeptides they encode. Prokaryotic ribosomes are typically formed by two subunits, constituting the 2.3 MDa 70S assembly: the larger 50S subunit is composed of 34 proteins and 3000 ribosomal RNA (rRNA) nucleotides, and the smaller 30S subunit is formed from 21 proteins and 1500 rRNA nucleotides. The mRNA transcript travels through a channel in the small subunit, which mediates the interactions between the anticodon-tRNA and the codons of the transcript. Catalytic activity for peptide bond formation resides in the large subunit. Together, the 50S and 30S subunits form the three sites for binding unique transfer RNA (tRNA) molecules [32].

In 1955, ribosomes were first visualized in cells by Palade using EM of fixed and stained thin sections of tissues [33]. The overall architecture of the prokaryotic ribosome and the quaternary structure of many of its component proteins were gleaned from EM and immunolabeling of negatively stained preparations [34,35]. These studies provided the first, albeit low resolution, images of isolated ribosomes. Significant advances were made possible by cryoEM and three-dimensional image reconstruction of two-dimensional crystalline sheets of the eukaryotic 80S ribosome, which revealed the polypeptide exit pathway [36,37]. In 1995, cryoEM of 70S ribosome particles embedded in vitreous ice [38,39] yielded maps at 23–25 Å resolution, revealing potential pathways in both subunits for the movement of the mRNA and the growing polypeptide, thereby enabling the first simple models of translation (Figure 1a). Five years later, cryoEM and single particle analysis of 70S ribosomes showed conformational changes that accompany binding of elongation factor G and subsequent GTP hydrolysis that permit mRNA translocation in the active site: rotation of the 30S subunit with respect to the 50S subunit and a subsequent widening of the mRNA channel [40••]. This study demonstrated the significant capability of EM to directly detect mechanistically relevant conformational intermediates that may not be amenable to X-ray crystallography.

Due to the heterogeneity, asymmetrical assembly, flexibility, and immense size of ribosomes, well-ordered, isotropic crystals were difficult to obtain. In the 1980s and 1990s the Yonath laboratory was able to obtain 3D crystals that exhibited diffraction to ∼3 Å resolution [41,42]. However, pathological defects such as twinning effectively truncated the data sets to medium resolution. A major breakthrough came in the late 1990s when Steitz and colleagues determined a 9-Å resolution X-ray structure of the *H. marismortui* 50S ribosomal subunit (Figure 1b). Initial low-resolution phasing to 20-Å was performed by molecular replacement, with phases provided by a 20-Å cryoEM map [1]. The EM-derived phases allowed determination of the substructure of bound heavy atom clusters in the crystals and subsequent phase extension to 9-A resolution. The ability to phase the X-ray crystallographic data beginning only with the EM maps validated the accuracy of the EM reconstructions. Heretofore unseen features of the ribosome were revealed by the 9-Å electron density maps, yet the precise demarcation between the protein and RNA components was elusive.

In 2000, fundamental insight into the molecular basis of translation was first revealed in atomic detail by determination of the 2.4-Å X-ray structure of the 50S ribosomal subunit [43] (Figure 1c). This leap in resolution was made possible by modifications to crystal growth and harvesting conditions that significantly improved diffraction and prevented twinning. Additional prokaryotic ribosome subunit crystal structures followed quickly, permitting further understanding of the translation machinery [44,45]. These initial structures demonstrated that the active site was devoid of protein, and the enzymatic function of the ribosome was entirely due to RNA, a

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