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Applications of the molecular dynamics flexible fitting method

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ABSTRACT

In recent years, cryo-electron microscopy (cryo-EM) has established itself as a key method in structural biology, permitting the structural characterization of large biomolecular complexes in various functional states. The data obtained through single-particle cryo-EM has recently seen a leap in resolution thanks to landmark advances in experimental and computational techniques, resulting in sub-nanometer resolution structures being obtained routinely. The remaining gap between these data and revealing the mechanisms of molecular function can be closed through hybrid modeling tools that incorporate known atomic structures into the cryo-EM data. One such tool, molecular dynamics flexible fitting (MDFF), uses molecular dynamics simulations to combine structures from X-ray crystallography with cryo-EM density maps to derive atomic models of large biomolecular complexes. The structures furnished by MDFF can be used subsequently in computational investigations aimed at revealing the dynamics of the complexes under study. In the present work, recent applications of MDFF are presented, including the interpretation of cryo-EM data of the ribosome at different stages of translation and the structure of a membrane-curvature-inducing photosynthetic complex.

1. Introduction

Advances in cryo-electron microscopy (cryo-EM) single-particle reconstruction have transformed the field of structural biology. Macromolecular assemblies are now routinely investigated from a structural perspective at ever increasing resolution. In many cases cryo-EM provides the first glimpse into the structure of macromolecular complexes, long before X-ray crystal structures become available. However, it is only when combined with such structures that the full power of cryo-EM can be realized. The bacterial ribosome is a prototypical example. Although many insights into the function of the ribosome were obtained from cryo-EM before crystal structures were available, the landmark achievements of ribosome crystallography dramatically increased the amount of information that could be derived from the cryo-EM reconstructions. However, cryo-EM remains a unique tool to study key structural conformers of biomolecular complexes not accessible to crystallography as it has the ability to capture such complexes in different functional states in a native environment, albeit at lower resolution. Thus, several independent methods have been developed recently for flexibly fitting atomic structures into electron microscopy density maps. Here we review the first applications of the molecular dynamics flexible fitting (MDFF) method (Trabuco et al., 2008), which illustrate the power of hybrid methods for investigating the biological function of macromolecular assemblies.

In the MDFF method (Trabuco et al., 2008), a molecular dynamics (MD) simulation is performed using an initial atomic model, often just a crystal structure. In addition to the standard MD force field, forces proportional to the gradient of the density map are applied to each atom, locally driving the structure to occupy high-density regions. Secondary structure restraints can be applied to proteins and nucleic acids in order to prevent overfitting, i.e., a close fit of the map at the cost of unphysical distortions of the molecular structure. Furthermore, any feature available in regular MD simulations can be employed in MDFF; for instance, a system can be simulated in environments containing water, ions, and even a membrane bilayer, mimicking in vitro or in vivo conditions. MDFF simulations are performed with NAMD, a highly scalable MD simulation package (Phillips et al., 2005), allowing for complexes of several megadaltons, i.e., systems comprising millions of atoms, to be studied with MDFF. The setup and analysis of MDFF simulations are performed with VMD, a molecular visualization package (Humphrey et al., 1996). A practical guide for setting up, performing, and analyzing MDFF simulations has been provided elsewhere (Trabuco et al., 2009).

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The challenge of morphing crystal structures of the ribosome into cryo-EM maps representing various functional states was the driving force for the development of MDFF (Trabuco et al., 2008). Thus, the majority of the initial applications of the method involved study of the ribosome. MDFF was first applied to investigate ribosome-induced conformational changes in elongation factor Tu, a critical step in the decoding of genetic information (Villa et al., 2009). MDFF also furnished the first atomic models of the ribosome bound to a protein-conducting channel, shedding light into how the ribosome modulates the channel's function (Gumbart et al., 2009; Becker et al., 2009). MDFF-derived atomic models of the ribosome stalled by the regulatory nascent chain TnaC revealed how it leads to translational arrest (Seidelt et al., 2009), as well as how it is recognized by the ribosomal exit tunnel (Trabuco et al., 2010a). In addition to studies of ribosome function, we review here an MDFF application addressing the process of protein-induced membrane curvature, which determines the morphology of bacterial photosynthetic membranes (Sener et al., 2009; Hsin et al., 2009; Hsin et al., 2010). Other MDFF applications published after the submission of this review, and thus not covered here, include investigations of the interactions between ribosomal L1 stalk and tRNAs (Trabuco et al., 2010b) and of the actin–myosin interface (Lorenz and Holmes, 2010), as well as recovery of atomic detail from coarse-grained structural models (Kim et al., in press).

2. Control of elongation factor Tu's GTPase activity

Elongation factor Tu (EF-Tu) delivers aminoacyl-tRNAs, i.e., transfer RNAs carrying an amino acid, to the elongating ribosome. EF-Tu is a GTPase, and it binds to aminoacyl-tRNAs with high affinity only in the GTP-bound state. When there is a cognate codon–anticodon interaction between the mRNA and the tRNA, GTP hydrolysis takes place and EF-Tu dissociates, allowing for the incoming aminoacyl-tRNA to be accommodated into the ribosome. The study of ribosome-induced conformational changes in EF-Tu was the first application of MDFF (Villa et al., 2009). A 6.7-Å cryo-EM reconstruction of an *E. coli* 70S ribosome in complex with an aminoacyl-tRNA:EF-Tu:GDP ternary complex (TC), stalled by the antibiotic kirromycin, was obtained. The cryo-EM data were interpreted by employing MDFF, which furnished the first atomic model of a 70S:TC complex (Fig. 1A).

The GTPase activity of EF-Tu is very low when the factor is not bound to the ribosome (Fasano et al., 1982). Crystal structures of EF-Tu (Kjeldgaard et al., 1993; Berchtold et al., 1993) suggested that a hydrophobic gate, formed by switch I and the P-loop, blocks access of the catalytic residue His84 (Daviter et al., 2003) to the GTP molecule (Fig. 1B; *E. coli* numbering is used throughout this paper), raising the question of how binding to the ribosome and recognition of the codon induces gate opening. This question was partially resolved by the MDFF-derived model of a 70S:TC complex, which revealed that EF-Tu's switch I moves away from the gate and interacts with the 16S rRNA (Fig. 1C). The other side of the gate, the P-loop, stays in place due to its interaction with the sarcin–ricin loop, a conserved region of the 23S rRNA. The model also displays a reorientation of the catalytic residue His84 toward the nucleotide, yielding a state consistent with GTP hydrolysis by EF-Tu. Thus, application of MDFF shed light onto ribosome-induced conformational changes in EF-Tu relevant for the control of its GTPase activity; however, the model of the 70S:TC complex by itself did not reveal yet how the codon–anticodon match signals to the hydrophobic gate.

More recently, a *T. thermophilus* 70S:TC structure was determined by X-ray crystallography at 3.6 Å resolution (Schmeing et al., 2009), confirming the mechanism of hydrophobic gate opening resolved via MDFF (Villa et al., 2009). This X-ray structure can now be used to evaluate the accuracy of MDFF-derived models. The original *E. coli* 70S:TC model was obtained through MDFF performed in vacuo (Villa et al., 2009). More accurate results, albeit at a much higher computational cost, can be expected from MDFF in explicit solvent (Trabuco et al., 2009). A compromise can be achieved by fitting the entire complex in vacuo and refining in explicit solvent the region of interest, in this case the ternary complex and its surroundings. For comparison purposes, the region encompassing the ternary complex was refined with explicit-solvent MDFF with no secondary structure restraints using the same cryo-EM data as in Villa et al. (2009). Thus, the *T. thermophilus* 3.6-Å crystal structure can be compared with MDFF-derived models, one in vacuo and one in explicit solvent, obtained using the *E. coli* 6.7-Å cryo-EM reconstruction.

As shown in Table 1, using the ribosomal protein S12 as an example, many interactions between the ternary complex and the ribosome seen in the crystal structure are also captured by MDFF, both in vacuo and in explicit solvent. However, some of the contacts are only reproduced by MDFF when explicit solvent is used. This observation underlies the importance of solvent in the interpretation of cryo-EM data by flexible fitting methods. In MDFF, the force field contains not only intramolecular potentials, but also describes intermolecular interactions between water and solute as long as water is included in the simulation.

When compared to an isolated TC, the tRNA from the 70S:TC crystal structure shows a distinct conformational difference, namely a swing of the tRNA's D loop (Fig. 2A). Such a swing is not present in MDFF-derived models. We note, however, that the tRNA present in the 70S:TC crystal structure is $tRNA^{Thr}$, whereas the isolated TC and 70S:TC cryo-EM reconstruction both contain

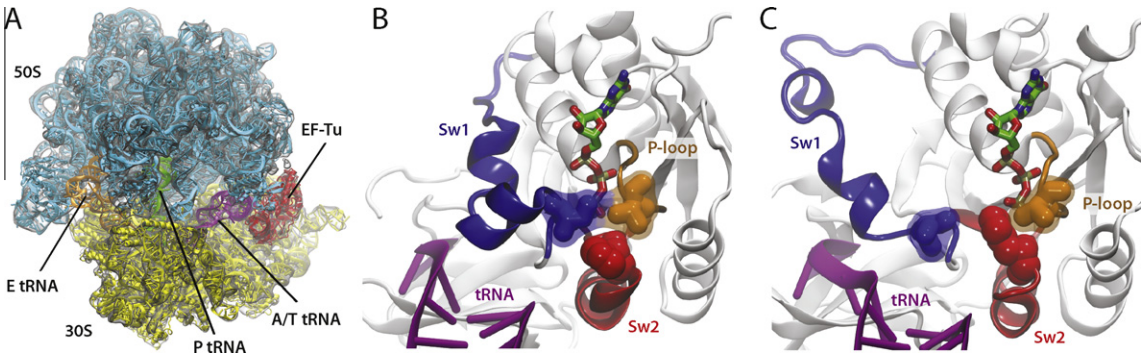

Fig. 1. Ribosome-induced GTPase activation of EF-Tu. (A) 6.7-Å cryo-EM reconstruction of a 70S:EF-Tu:aminoacyl-tRNA:GDP complex stalled with kirromycin, together with an MDFF-derived model. (B) EF-Tu contains a hydrophobic gate formed by Val20 (P-loop, orange) and Ile60 (switch 1, blue), which prevents the catalytic residue His84 (switch 2, red) from accessing the GTP molecule. (C) When bound to the ribosome, switch 1 moves away from the P-loop, opening the hydrophobic gate; His84 can then reorient toward the nucleotide in a conformation conducive to GTP hydrolysis.