Multiscale simulation of actin filaments and actin-associated proteins

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Abstract

Actin is an important cytoskeletal protein that serves as a building block to form filament networks that span across the cell. These networks are orchestrated by a myriad of other cytoskeletal entities including the unbranched filament-forming protein formin and branched network-forming protein complex Arp2/3. Computational models have been able to provide insights into many important structural transitions that are involved in forming these networks, and into the nature of interactions essential for actin filament formation and for regulating the behavior of actin-associated proteins. In this review, we summarize a subset of such models that focus on the atomistic features and those that can integrate atomistic features into a larger picture in a multiscale fashion.

Keywords Coarse-graining · Cytoskeleton · Protein dynamics · Protein-protein interactions · Molecular dynamics · Enhanced sampling

Introduction

Actin is a filament-forming protein that contributes to various parts of the cellular cytoskeleton via complicated architectures. It co-exists as a monomer as well as in parallel, anti-parallel, and branched filament networks that assemble and disassemble to enable cell motility. The dynamic nature of the actin cytoskeleton results from the ability of actin to undergo transition between monomeric or globular (G-actin) and filamentous (F-actin) states, and it is critical to many important processes such as cell division (Pollard and Cooper 2009). The single-domain G-actin monomer consists of 375 residues, a nucleotide in its binding pocket, and divalent cations (Fig. 1a). The nucleotide is predominantly adenosine triphosphate (ATP) in G-actin. Polymerization of G-actin leads to the formation of actin filaments consisting of F-actin subunits that have a well-characterized structure well represented using a 13 subunit periodic repeat. The actin subunit structure is commonly divided into four major subdomains (labeled as 1, 2,

3, and 4 in Fig. 2d) and is asymmetric. G-actin has a more twisted structure that is typically characterized by a large dihedral angle between the subdomains 2-1-3-4 and a wide cleft represented by the distance between subdomains 2 and 4 (see Fig. 2d for labels). In F-actin, the structure is more flattened, characterized by a smaller dihedral angle and a narrower cleft. The filament made of the asymmetric F-actin subunits is polar in nature and consists of a “faster-growing” barbed end and a “slower-growing” pointed end (Fig. 1a). Moreover, the bound nucleotide ATP hydrolyzes in F-actin to form an intermediate structure consisting of adenosine diphosphate (ADP) with a bound inorganic phosphate (P_i) that is released at a much slower timescale to leave behind purely ADP-bound subunits (Fig. 1a). Due to the difference in growth rate of actin filaments at the two ends and the ATP hydrolysis, measuring growth kinetics of the filament is challenging. Seminal work by Pollard and co-workers established the macroscopic rate constants for the polymerization of ATP-, ADP- P_i -, and ADP-bound actin at the barbed and pointed ends of actin filaments and the rate constant for ATP hydrolysis and phosphate release (Fujiwara et al. 2007; Pollard and Weeds 1984; Pollard 1986).

The cell utilizes different active mechanisms to regulate actin filament formation within the cytoskeletal network. Such mechanisms usually involve regulation of the complex interactions between actin filaments and other cytoskeletal proteins (Campellone and Welch 2010). Thus, any imbalance in these delicate regulatory mechanisms such as a mutation disrupting a

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Fig. 1 Actin filaments and associated proteins. **a** A single actin filament and a magnified view of a section of the filament. The nucleotide in the binding pocket undergoes ATP hydrolysis and P_i release. In addition to the nucleotide-binding pocket, divalent cation binds at other sites to assist

polymerization and provide stiffness to the filament structure. **b** Formin-mediated growth of a straight actin filament. **c** The Arp2/3 complex binds to the side of a mother filament and grows a daughter filament, leading to a branched filament network

functional component of the system can result in malfunction of cells and occurrence of serious diseases. A central part of this regulatory system is composed of formin proteins and the actin-related protein (Arp) 2/3 complex. The hydrolysis of ATP has implications on binding kinetics of actin-associated proteins, including the proteins formin and Arp2/3 that play an important role in nucleation and growth of unbranched and branched actin filament networks (Fig. 1b, c). Breakthrough work from the Pollard lab, including the discovery of the Arp2/3 complex

and the gating mechanism in formins, has steered our understanding of how these proteins polymerize actin in different architectures (Espinoza-Sanchez et al. 2018; Machesky et al. 1994; Paul and Pollard 2009b).

Computer simulations have widely been used to study various aspects of actin filaments ranging from macroscopic properties of the actin network (Bidone et al. 2015; Popov et al. 2016) through single-filament properties (Baker and Voth 2013; Chu and Voth 2005; Chu and Voth 2006; Fan

Fig. 2 Computational modeling of actin. **a** Water crystals near the Mg^{+2} in G-actin versus F-actin observed in AA simulations (reproduced from Saunders and Voth (2011)). **b** Water wires observed in the metadynamics simulations and water wire length distributions in metadynamics simulations (reproduced from McCullagh et al. (2014)). **c** DB-loop

conformations observed in AA simulations, without and with additional divalent ions (reproduced from Hocky et al. (2016)). **d** Intuitive 12-sites-per-subunit CG model of actin, shown in a subunit and a section of the filament. **e** Cooperativity in phosphate release rate (reproduced from Katkar et al. (2018))

et al. 2013; Fan et al. 2012; Hocky et al. 2016; Lyman et al. 2008; Pfaendtner et al. 2010a; Pfaendtner et al. 2010b; Pfaendtner et al. 2012; Saunders and Voth 2012a; Saunders and Voth 2012b; Schramm et al. 2017; Yogurtcu et al. 2012) and properties of the single subunit (Chu and Voth 2005; Mehrafrooz and Shamloo 2018; Pfaendtner et al. 2009; Saunders et al. 2014; Saunders and Voth 2011) to the hydrolysis of the bound nucleotide (Akola and Jones 2006; Freedman et al. 2012; McCullagh et al. 2014; Sun et al. 2017). At the highest resolution, *ab initio* molecular dynamics (AIMD), density functional theory, or quantum mechanics/molecular mechanics (QM/MM) simulations allow one to simulate the reactive nature of chemical bonds in ATP and surrounding species using electronic structure theory and the rest of the system using molecular mechanics to make the simulations computationally more efficient. The free energy landscape of the ATP hydrolysis reaction has been successfully studied using QM/MM simulations (McCullagh et al. 2014; Sun et al. 2017). At the next level of “resolution” are the classical all-atom (AA) molecular dynamics (MD) simulations, where atoms are represented by particles whose motions are governed by Newton’s second law and are typically simulated using a general empirical (and non-reactive) force-field such as CHARMM. These AA simulations have been used to simulate filaments of actin and have been able to provide valuable insights pertaining to the characteristics of single filaments, including their mechanical properties and the structural changes associated with polymerization (Baker and Voth 2013; Chu and Voth 2005; Chu and Voth 2006; Fan et al. 2013; Fan et al. 2012; Hocky et al. 2016; Lyman et al. 2008; Pfaendtner et al. 2010a; Pfaendtner et al. 2010b; Pfaendtner et al. 2012; Saunders and Voth 2012a; Saunders and Voth 2012b; Schramm et al. 2017). However, AA simulations are typically limited to tens of subunits long filaments. Coarse-grained (CG) models have been widely used to push beyond the limits of AA simulations in terms of the timescales and length scales that they can explore, by an extent that depends on the level of coarse-graining (Saunders and Voth 2013).

Top-down CG models, parameterized to reproduce macroscopic properties of the system, have been widely used to study actin (Caby et al. 2012; Freedman et al. 2017; Guo et al. 2011; Popov et al. 2016). Particle-based CG models, especially constructed using a bottom-up technique using underlying fine resolution simulations (e.g., AA), reproduce the collective behavior of the atomistic system with a reduced number of particles. Additionally, another class of models at a similar resolution is Markov state models, in which a state-based representation of the system is used. Such kinetic models that look at G-actin and F-actin with different states for each nucleotide have been widely used to study polymerization and ATP hydrolysis in actin filaments (Ranjith et al. 2010; Stukalin and Kolomeisky 2006; Vavylonis et al. 2005). In this review, we focus on QM/MM and AA simulations of

actin filaments, formins, and Arp2/3 complex and briefly talk about bottom-up CG models of these proteins. We specifically highlight computational studies from the Voth group that were highly motivated by the pioneering work of Tom Pollard, many of which were a direct result of either formal collaborations or informal interactions with him.

Actin

Many models of actin filament structure have been proposed in the literature (Galkin et al. 2011; Kabsch et al. 1990; Murakami et al. 2010; Narita et al. 2011; Oda et al. 2009). Although significant efforts were dedicated to obtain improved structures and to gain insights of the effect of states of the bound nucleotide (e.g., Galkin et al. (2011)), no high-resolution structure of either ATP- or ADP-P_i-bound actin filaments existed until recently. High-resolution structures of two ATP analogs were almost simultaneously solved using cryogenic electron microscopy (cryo-EM) in two recent independent studies, including one from the Pollard lab (Chou and Pollard 2018; Merino et al. 2018). In addition, the Pollard lab was successful in obtaining the only high-resolution structure of ADP-P_i-bound actin filament available today (Chou and Pollard 2018).

Prior to the availability of these structures from cryo-EM, Pollard helped to guide the efforts of us and others to use rigorous computer simulations to study the structural differences of actin filaments with different bound nucleotides. A typical method of constructing actin filament for a given nucleotide state in computational simulations was to superpose the ATP- (Graceffa and Dominguez 2003) or ADP (Otterbein et al. 2001)-bound G-actin structure in the filament structure model (e.g., Fujii et al. (2010), Kabsch et al. (1990), and Oda et al. (2009)), or for the nucleotide state ADP-P_i, by replacing P_γ from ATP (see Fig. 1a) with P_i with AA simulations to further refine the resulting structure (Chu and Voth 2005; Pfaendtner et al. 2009; Pfaendtner et al. 2010b). The effect of nucleotide state on structure and dynamics of actin subunits in G-actin and in F-actin was established using AA simulations accelerated with metadynamics in Pfaendtner et al. (2009). The DNase-binding loop (DB-loop, see Fig. 1a) in actin monomers as well as in F-actin has been shown to exhibit multiple conformations (Otterbein et al. 2001; Rould et al. 2006) and was studied using computer simulations in a series of collaborative work involving Thomas Pollard (Pfaendtner et al. 2009; Pfaendtner et al. 2010b). AA metadynamics simulations of G-actin revealed that DB-loop can coexist in unfolded and folded conformations in ADP-bound G-actin. Further, it was discovered that the lowest free energy corresponds to an unfolded DB-loop in ATP F-actin filaments, while in ADP F-actin, the folded DB-loop is stable and that folding of the DB-loop results into a softer filament with a lower persistence length (Hocky et al. 2016; Pfaendtner

et al. 2009; Pfandtner et al. 2010b). Key amino acid residues including Met44 involved in the folding of DB-loop were identified through these simulations. AA MD simulations of the Oda filament model (Oda et al. 2009) were found to be more stable compared to the Holmes filament model (Kabsch et al. 1990) based on the root-mean-square fluctuations in these simulations relative initial structures, and CG analysis of these simulations demonstrated the effect of DB-loop conformations and the bound nucleotide on the dihedral angle distributions. Motivated by these results, extensive self-learning adaptive umbrella-sampling simulations were performed on single-actin subunits starting from the G-actin and F-actin conformations, using the dihedral angle and cleft width as collective variables (Saunders et al. 2014). These simulations were not able to explore the full structural transformation between G-actin and F-actin structure of the subunit. Yet, several important aspects of the transformation were revealed. Based on the area of configurational space explored in these simulations, ADP-bound actin was found to be conformationally more mobile than ATP-bound actin. AA simulations of ADP-bound actin filaments were also used to refine two other binding sites—polymerization and stiffness sites—for divalent cations in addition to the nucleotide-binding pocket in the subunits and study their effects on the structure of subunits and properties of the filament (Hocky et al. 2016) (see Fig. 1a for locations). The DB-loop becomes relatively less flexible in the presence of a divalent ion at the stiffness site between the DB-loop of a given subunit and the binding cleft of the target interacting subunit (representative conformations are shown in Fig. 2c). Persistence length and torsional rigidity of actin filaments increase due to the presence of the additional cations (Hocky et al. 2016; Kang et al. 2013; Kang et al. 2012).

Coarse-grained modeling of actin

Simplified mechanistic models, typically parameterized using a top-down approach in which model parameters are tuned to obtain certain target filament properties, have been successfully used to study actin filaments and their mechanical properties (Bidone et al. 2015; Yogurtcu et al. 2012). Such models reduce large-scale complex process into simpler phenomenology, but typically have somewhat limited predictive power and can potentially lack a rigorous justification for the choice of parameters involved. On the other hand, “bottom-up” CG models of actin filaments with a CG force-field parameterized using reference AA simulations, such as the hetero-elastic network model (hENM), have been found to be adequate in studying actin filaments in terms of their structure and mechanical properties (Chu and Voth 2005; Chu and Voth 2006; Deriu et al. 2011; Katkar et al. 2018; Lyman et al. 2008; Saunders and Voth 2012b; Schramm et al. 2017; Voth

2017; Zhang et al. 2008). We limit this review below to such bottom-up CG models.

Bottom-up coarse-graining of actin involves two steps. The first step is to define a CG map that translates the AA structure into CG sites. The map can either be defined using biological intuition or knowledge of the structure of the protein, giving rise to the *intuitive* map, or can be obtained using information from the dynamics observed in the AA trajectory. An example of the latter approach is the essential dynamics coarse-graining (ED-CG) technique (Zhang et al. 2008). The idea behind ED-CG is to find the optimum mapping by variationally minimizing a mathematical residual, defined as the total difference between the displacement of pairs of CG beads averaged across AA data, with the idea that if the two CG sites move in a correlated fashion in the AA data, their contribution to the residual should be small. Numerical implementation of the minimization algorithm involves a combination of simulated annealing to obtain a global minimum of the residual, and a steepest descend algorithm to find the accurate local minimum. The mapping is constrained to the primary sequence of actin to limit the search space, with the belief that preserving the primary sequence can allow using the same mapping even with large conformational changes in the protein. (The latest iteration of this code is available for public use at <https://github.com/uchicago-voth/CGMGC.p>). Once the map is defined, the second step consists of parameterizing the force-field that governs the interactions between the CG sites. In the hENM (Lyman et al. 2008), the interactions between CG sites are assumed to be pair-wise and further approximated as harmonic potentials. The harmonic spring parameters—the spring constant and the equilibrium spring length—are optimized to reproduce the mean square fluctuations of CG sites between the mapped AA and the CG simulations.

A number of CG actin filament models with different levels of coarse-graining based on either ED-CG or intuitive mapping and hENM have been developed. The highest level of coarse-graining in actin filaments in this class of models is the four-sites-per-subunit model. The intuitive CG map at this resolution is readily obtained such that each subdomain in actin is represented by one CG site (Kabsch et al. 1990). A specific force-field consisting of three bonds between the CG sites representing the connectivity between the four subdomains along with additional angle and dihedral potentials defining the opening and closing of the nucleotide pocket in actin subunits can be parameterized using the AA simulations. CG modeling at this resolution can help gain insights into the subdomain level changes in the average structure of actin and more importantly, into how “stiff” or “loose” these subdomains are relative to each other, through the dynamics governing their collective motions. Such a CG model, along with the underlying AA simulations, was used to discover that the DB-loop conformation undergoes a loop-to-helix transformation as a result of ATP hydrolysis in actin filament. The

resulting CG model parameters also provided insights into key structural differences such as the softened dihedral angle in ADP-bound G-actin versus an ATP-bound G-actin (Chu and Voth 2005). Long CG actin filaments can be constructed by replicating the mapping from short actin filaments in the AA simulations. Certain choices can be made to keep the CG force-field local. However, it is essential to include interactions between CG sites that extend beyond the nearest neighboring actin subunits in order to reproduce the correct mechanical properties of the filaments (Chu and Voth 2006).

A more detailed CG model of actin consisting of 12-sites-per-subunit has also been developed (Saunders and Voth 2012b) (Fig. 2d). The novel CG map used in this model consists of four CG sites dedicated to the four major subdomains of actin, but also used seven other minor sites that represent various smaller structured domains in the subunit and a dedicated CG site for the bound nucleotide. Including these minor sites enables studying the relative orientation of the major subdomains in the subunit and across subunits in the actin filament. A systematic comparison of various models for the structure of actin filaments (Oda (Oda et al. 2009), Namba (Fujii et al. 2010), and Holmes (Kabsch et al. 1990) models) was performed using a CG model at this resolution to study the dynamics implied by each of these filament models in molecular simulations. This analysis identified the most accurate structure based on its stability in molecular simulations (Oda model). The key structural transformation between G-actin and F-actin subunit in the Oda model was identified to be the rotation of subdomain 2, and the positioning of the DB-loop within the subunit and across subunits in the filament was also identified to be important. The CG model further showed that largest change is observed in the relative position of subdomains 2 and 4 in actin, but other minor sites also rearrange (Saunders et al. 2014). A distinction between the flattening of ATP- and ADP-bound actin monomers was later discovered using this model. In presence of the nucleotide ATP, CG sites 1 and 2 shift and rotate relative to each other, and CG sites 3 and 1 rotate in order to flatten the structure. In presence of the nucleotide ADP, the flattening is characterized by a shift and rotation in CG sites 3 and 4 instead. Thus, in ATP bound subunits, flattening is coupled with twisting of the nucleotide-binding cleft, while in ADP reorientation of subdomain 2 results into flattening. A recent improvement of this CG model parameterized for ATP-, ADP-P_i-, and ADP-bound actin filaments was used as an input for the ultra-coarse-graining technique (Dama et al. 2017; Dama et al. 2013; Davtyan et al. 2014) to study the mechanism of ATP hydrolysis in actin filaments (Katkar et al. 2018).

In contrast to the intuitive CG maps used in the above models, a systematic ED-CG map for a given CG resolution can be obtained that best represents collective motions in AA simulations. The ED-CG map with four-sites-per-subunit was shown to preserve the ability of seeing the two most essential

modes in the structural transformation between G-actin and F-actin. However, a rigorous analysis of the resulting CG maps based on AA simulations of G-actin showed similarities with the intuitive map only at the level of seven-sites-per-subunit. A key strength of ED-CG over biological intuition is, for example, the identification of DB-loop as a separate CG site in the seven-site model, which is not separated out in any of the seven contiguous domains in the intuitive CG map. Although biological intuition can help in describing either four- or seven-site CG maps, ED-CG can be used to define arbitrary number of CG sites depending on the required resolution for the problem being studied. For example, ED-CG maps with four to twenty CG sites per actin subunit were generated from the same underlying AA simulations of actin filaments with either ATP or ADP as the bound nucleotide. This analysis showed that although the same ED-CG map can be used to define each subunit in the filament, heterogeneity is required in the hENM parameters for corresponding pairs of CG beads to reproduce mechanical properties of filament that are consistent with AA simulations (Fan et al. 2012).

ATP hydrolysis

The nucleotide ATP bound to actin subunits predominantly hydrolyzes in filaments rather than in G-actin. The rate of ATP hydrolysis in G-actin is about $7 \times 10^{-6} \text{ s}^{-1}$, which is about four orders of magnitude slower than in F-actin ($\sim 0.3 \text{ s}^{-1}$). Clearly, the difference between G-actin and F-actin structures was believed to be responsible for the dramatic increase in the rate of hydrolysis in F-actin. In spite of the availability of high-resolution structures of G-actin and filament models, no direct structural evidence at the residue-level resolution can be obtained to understand these differences. Molecular simulations are a perfect tool to build upon experimentally observed structures in order to probe into the important structural changes and the key residues involved in ATP hydrolysis.

Ordering of water around the nucleotide

Structured water has been identified in the nucleotide-binding pocket of actin (see Fig. 1 for location of the pocket). This water was shown to be stable in long AA simulations of G-actin (McCullagh et al. 2014). Moreover, ordering of the water around the divalent cation Mg^{+2} provides “lubrication” to assist the twisting of a single actin subunit, isolated from the filament, from F-actin structure to relax to a G-actin structure, highlighting the important role of water in preventing direct salt bridges between the key acidic residues of actin in the nucleotide-binding pocket. Instead, these residues are found to interact with the cation through water molecules in order to facilitate smoother transition between a twisted G-actin and a flat F-actin conformation.

The hydrolysis reaction involves the cleavage of the P_{γ} – O_{β} bond (see Fig. 1a) with a lytic water added to P_{γ} . In addition to organization of the first solvation shell around P_{γ} for the lytic water, the sub-sequent water solvation shells are important for facilitating proton transport via Grotthuss shuttling from the lytic water to P_{γ} and this process is catalyzed by key coordinating residues highlighted in the following paragraph. The structural transformation from G-actin to F-actin results into an extended network of stable waters in the nucleotide-binding pocket (Saunders and Voth 2011). This change in stability of waters between G-actin and F-actin in filaments was observed in AA MD simulations. Figure 2a shows waters in the nucleotide-binding pocket, colored in red and labeled as W1, W2, etc., and the Oda filament model is used to represent F-actin. These simulations highlighted the importance of including waters observed in the original crystal structure in the initial setup of MD simulations. In absence of these waters in the initial simulation setup, typical timescales of MD simulations were found to be insufficient to observe the formation of structured water. Although the translational diffusion of water is not affected to a significant degree in the structural transformation from G-actin to F-actin, the waters in the nucleotide-binding pocket are freer to rearrange rotationally in F-actin (McCullagh et al. 2014). These waters form shorter proton shuttling water wires in F-actin that facilitate the proton transport between the lytic water, the key residues involved, and the P_{γ} (Fig. 2b shows a representative snapshot of the AA simulation and the statistical distribution of water wire length).

Several important residues have been identified to be involved in structural transformation from G-actin to F-actin, including the nucleotide-binding Gln137 and His161, and the nearby bases Asp11, Asp154, Asp157 (Graceffa and Dominguez 2003; Iwasa et al. 2008; Oda et al. 2009; Otterbein et al. 2001; Schuler 2001; Schuler et al. 1999; Vorobiev et al. 2003; Wertman et al. 1992) (labeled in Fig. 2a). Of these residues, Gln137 is believed to stabilize the lytic water, while His161 is believed to act as a catalytic base (Graceffa and Dominguez 2003; Vorobiev et al. 2003). AA MD simulations have been successfully used to gain a better understanding of the connection between structural transformation and the ATP hydrolysis reaction. These simulations revealed that Asp11 has a closer coordination with the cation Mg^{+2} , while Gln137 moves relative to the P_{γ} . The detailed mechanism of ATP hydrolysis in both G-actin and F-actin involves the cleavage of the P_{γ} – O_{β} bond and the nucleophilic attack on P_{γ} by the lytic water. This involves transfer of the proton from the lytic water to the Asp154 carboxylate oxygen, followed by protonation of O_{γ} to form the species H_2PO_4 (Akola and Jones 2006; Freedman et al. 2012;

McCullagh et al. 2014). The flattened structure of F-actin and the resulting rearrangement of key residues help lower the free energy barrier, including altering the water wires in the nucleotide-binding pocket to be more favorable for the transport of this proton, as highlighted in the data shown in Fig. 2b (McCullagh et al. 2014).

Hydrolysis mechanism at the filament level

Thanks to the computational studies described in the previous paragraph, the detailed mechanism of hydrolysis of the nucleotide ATP is now believed to be well understood at the level of an individual actin subunit. However, two contradicting views have existed about the mechanism of collective ATP hydrolysis at the level of the actin filament. In a classic study that established the kinetic rate constants of ATP hydrolysis, Pollard and Weeds first proposed the random hydrolysis mechanism in actin filaments (Pollard and Weeds 1984). In this mechanism, ATP hydrolysis is assumed to be equally likely in all actin subunits in a filament and hence a single rate governs hydrolysis of ATP bound to any subunit throughout the filament. This implies that the conformation of the neighboring subunits does not significantly affect the rate constant for hydrolysis, even if the neighboring nucleotide state modulates the local conformations of each subunit.

In contrast to this view, the nucleotide state of neighboring subunits may instead be assumed to significantly modulate the rate constant through modulation of the local conformations that each subunit explores, giving rise to a spectrum of cooperative hydrolysis models. Among these cooperative models, the vectorial model is the most distinct from the random hydrolysis model, since it assumes that ATP hydrolysis can occur predominantly in those subunits in the filament that have an adjoining hydrolyzed subunit, and hence are at an interface between un-hydrolyzed and hydrolyzed sections of the filament (Pantaloni et al. 1985). A similar contradiction has existed for the P_i release reaction, which could be assumed to be either random or cooperative at the filament level. Several attempts have been made to perform a systematic comparison between these views (Burnett and Carlsson 2012; Burnett and Carlsson 2013; Carlier et al. 1987; Jégou et al. 2013; Korn et al. 1987; Ohm and Wegner 1994; Pieper and Wegner 1996; Ranjith et al. 2010; Stukalin and Kolomeisky 2006; Vavylonis et al. 2005). However, common experimentally measured quantities such as rate of filament elongation, fluctuations in filament length, and size of the un-hydrolyzed ATP cap near the filament end are found to be insensitive to the mechanism of hydrolysis, except for small quantitative difference near critical G-actin concentration. Using a mixture of ATP- and ADP-bound G-actin to introduce large number of interfaces in the filament suggested that the rate of hydrolysis at the interface must be less than 100 times faster than the rate of hydrolysis away from the

interface, although the predictions of a random hydrolysis mechanism were also shown to be able to explain the observed experimental data (Ohm and Wegner 1994; Pieper and Wegner 1996). By using the nearest-neighbor cooperativity in such a model as a parameter to fit experimentally measured P_i release profiles, it was shown that the possibility of a high degree of cooperativity in P_i release could not be completely excluded, however with some ambiguity in the data analysis involved (Burnett and Carlsson 2012; Burnett and Carlsson 2013; Jégou et al. 2013; Jégou et al. 2011).

Extracting a complex cooperative model in order to probe the true mechanism of ATP hydrolysis and P_i release is limited by the lack of any direct experimental evidence to empirically justify and parameterize a model constructed for any assumed cooperative mechanism. Although detailed computational simulations at the resolution of AIMD, QM/MM, and AA have proven to be useful in investigating the mechanism of ATP hydrolysis at the level of an actin subunit, these simulations cannot be used to directly investigate the mechanism at the filament level because these are typically limited to systems of the size of a few actin subunits. A bottom-up CG modeling approach is a promising candidate in this respect. In this approach, coarser resolution models are parameterized in a systematic way to reproduce essential behavior of underlying finer resolution models. A multiscale bottom-up CG model of the actin filament was recently successfully developed and employed to predict the nature and extent of such complex cooperativity present in both ATP hydrolysis and P_i release reactions (Katkar et al. 2018). AA simulations of actin filaments were used to predict the nature and extent to which neighboring nucleotide states modulate the local conformations of each subunit in the filament. Figure 1a shows a schematic highlighting the fluctuations of neighboring subunits relative to a given subunit. Using the state-of-the-art ultra-coarse-graining (UCG) technique (Dama et al. 2013; Davtyan et al. 2014; Voth 2017), these predictions from AA simulations were used to study how the rate constant for hydrolysis is modulated by the nucleotide state of the neighboring subunits. Both ATP hydrolysis and P_i release reactions were predicted to exhibit cooperativity, to the extent of up to $\pm 20\%$ change in local rate constants of these reactions in the filament relative to the average rate constant across the entire filament, depending on the local nucleotide state of neighboring subunits (Fig. 2e shows the variation in rate of phosphate release as a function of neighboring environment of a given subunit). The random and vectorial hydrolysis models are attempts to simplify a much more complex cooperativity into a simpler picture that looks at the difference between rate constants of hydrolysis and P_i release in subunits at interfaces and in the bulk of the actin filament. At the level of this simplification, the UCG model predictions showed no evidence in support of the vectorial mechanism and seemed to converge toward the random hydrolysis mechanism proposed by Pollard and Weeds.

Filament-forming proteins

The following sections will discuss the computational studies from Voth group in collaboration with Tom Pollard that have shed light on the structural and dynamical properties of formins, their interactions with the actin filaments, and important mechanisms related to nucleation and polymerization of actin.

Unbranched filaments

Formins are large proteins (120–220 kDa) with multiple domains that regulate the elongation of unbranched actin filaments. The dimeric formin homology 2 (FH2) domain forms the core structure of formins (Xu et al. 2004). They nucleate actin filament by stabilizing actin monomers and processively move with the growing filament (Paul and Pollard 2009b). FH2 domains, in the absence of FH1 domain and profilin-bound actin monomers, have been shown to slow down elongation of actin filament (Paul and Pollard 2009a). These domains are capable of polymerizing actin filament when they are in “open” state as opposed to “closed” state. The Pollard lab first suggested that two states of FH2 domain at the barbed end can have different effects on the conformation of terminal subunits, which would determine the addition of a new subunit (Paul et al. 2008; Paul and Pollard 2009b). Their single-molecule experiments demonstrated that filament ends with associated formins grow steadily; thus, they proposed that there is rapid fluctuation between an open and a closed state during actin filament growth at the barbed end. This phenomenon of the equilibrium between the open and closed states of the FH2 domain at the barbed end of the filament is called “gating” (Vavylonis et al. 2006). The fraction of time spent by the FH2 domain in an open state is expressed by a gating factor. FH2 domains that belong to different types of organisms have variability in gating factor although they have similar three-dimensional structure and mechanistic behavior (Kovar et al. 2006). For example, mDia1 (mammalian formin) has a gating factor of ~ 0.95 which means that it is mostly found in an open state and competent of polymerizing actin filament. On the other hand, Bni1p (budding yeast formin) and Cdc12 (fission yeast formin) have “gating” factors of ~ 0.6 and ~ 0.05 , respectively.

Building computational models of formins bound to the barbed ends of actin filaments

With the combined efforts of the Pollard and Voth Labs, a detailed model of Bni1 FH2 domain bound to actin filament barbed end was created (Baker et al. 2015) by using a filament composed of seven actin subunits with a twist angle of 167° based on the bare actin filament structure solved by X-ray fiber diffraction (Oda et al. 2009) and intermolecular contacts

observed in the co-crystal structure of Bni1 FH2 domain—actin with a twist angle of 180° (Otomo et al. 2005) (Fig. 3a). Here, twist angle is defined as the angle by which consecutive subunits rotate along the filament axis. Baker et al. carried out 160 ns of AA MD simulation to refine the model, which resulted in conformational changes in the proteins and flattening of the helical twist at the barbed end of the filament from a

twist angle of 167° (bare actin filament) toward larger twist angles (Bni1-actin filament). Alternative modeling approaches can be used to generate the initial structures of formins that have no available crystal structures (such as Cdc12 or mDia1). For instance, Aydin et al. used template-based homology modeling to generate the initial structures of mDia1 and Cdc12 FH2 domains interacting with the barbed

Fig. 3 Formin FH2 domains associated with actin filament seven-mer. **a** Initial all-atom structure of the formin Bni1p FH2 domain interacting with actin filament composed of seven actin subunits. The structure corresponds to a state just after addition of a new actin subunit (shown in gray color) and before stepping of trailing FH2 domain (shown in red color) (reproduced from Baker et al. (2015)). **b** (Top) All-atom and CG hENM models of an actin subunit and Bni1p FH2 domain. (Bottom) Two different CG models of the FH2 domains associated with the actin filament seven-mer: one residue per CG site (left) and multiple residues per CG site (right) (reproduced from Baker et al. (2015) and Aydin et al. (2018)). **c** Structural alignment of the FH2 domains of Bni1, Cdc12, and

mDia1 at the barbed end of the actin filament seven-mer (top), and separate structural alignment of the FHL and FHT domains (bottom) (reproduced from Aydin et al. (2018)). **d** Individual alignment of important regions in FHL and FHT domains (reproduced from Aydin et al. (2018)). **e** (Top) Twist angles between actin subunits A2 and A3 as a function of time during all-atom MD simulations of FH2 domains associated with actin filament five-mer. (Middle and bottom) The distributions of helical twist angles (A2–A3) during the last time intervals of MD simulations from two replicas domains (reproduced from Aydin et al. (2018))

end of actin filament by using the existing structure of Bni1 FH2 domain bound at the barbed end of the filament as a template (Aydin et al. 2018). These atomistic models of Cdc12, Bni1, and mDia1 correspond to the conformations just after the addition of a new actin subunit (labeled A1 in Fig. 1b). To build structures corresponding to the conformation prior to the addition of a new subunit, actin subunit A1 was removed from the barbed ends of the seven-mer filaments. Overall, these works led to generation of the structures of different FH2 domains bound to the barbed ends of filaments with 167° twist angle, which was not known previously.

AA simulations are not suitable to study phenomena occurring at large length and time scales, as mentioned in the preceding sections. This limitation can be overcome by generating CG models of formins at the barbed end of the filament. Baker et al. used a CG model based on the hENM approach with a resolution of one-CG bead-per-residue to investigate the effect of the point mutations on the interactions between Bni1 and actin (Fig. 3b). The intramolecular interactions within proteins were described by hENM, and intermolecular interactions between formin and actin filament were modeled by the combination of van der Waals and electrostatic interactions, via the use of Lennard Jones (LJ) and screened Coulomb (Yukawa) potentials, respectively. This high-resolution CG model (each residue is represented by a single CG site) enabled mutating a residue and studying its effect on the interactions between formin and actin on longer time scales. Aydin et al. built a lower-resolution CG model by using the ED-CG method (Fig. 3b). CG sites of formin and actin were determined based on the collective protein motions via ED-CG, and each CG site contained about eight to nine residues. The intramolecular and intermolecular interactions were described similar those to the model in Baker et al. by using hENM, and LJ and Yukawa, respectively. The lower-resolution CG model smoothens free energy surface further, and this enables observing conformational differences between three formins.

Structural and dynamical properties of formin—actin complexes: identification of interfacial binding regions that affect nucleation and polymerization of actin

Baker et al. simulations enabled the initial structure of Bni1-actin complex to undergo conformational changes, which brought the leading (FHL) and trailing (FHT) FH2 domains into closer to the actin subunits at the barbed end. They investigated the contacts between formin and actin residues in the refined structure by measuring distances between alpha-carbon atoms. The most extensive interactions were found to be located between the KnA and KnB helices in the FH2 knob region and the actin target binding cleft (TBC, see Fig. 1a) and

DB-loop, and these contacts are stronger in the refined structure of Bni1-Oda actin seven-mer than the starting Otomo structure. These regions were investigated in more detail by measuring salt bridges and hydrogen bonds between actin and the charged residues in the FH2 knob helices. Two residues (R1423 in KnA and K1467 in KnB) were selected to test the importance of these electrostatic interactions between Bni1 and actin by making point substitutions at these residues both via experiments and simulation. The mutations reduced the total non-bonded interaction energy between FH2 knob helices and actin TBC by making the interactions in this region less favorable. The CG simulations of both wild type (WT) and mutant Bni1 FH2 domain interacting with actin seven-mer and actin dimer show that there is a larger difference in number of contacts between the WT and mutant Bni1 FH2 for actin dimer than actin seven-mer. These results suggest that these two residues can be considered more important for the nucleation than elongation. Experiments done in the Pollard lab confirmed this prediction by showing that mutant Bni1 has no effect on the elongation of actin but drastically reduces the nucleation activity. Thus, the combined experimental and computational findings indicate that the electrostatic interactions between the knob helices of formin FH2 domains and TBC of actin filament are critical for the nucleation activity of formins (Baker et al. 2015).

In order to understand the gating mechanism and, therefore, factors affecting polymerization of actin, the homology models of Cdc12 and mDia1 were generated as discussed in the previous section. AA MD simulations were run to investigate differences in the structures of three FH2 domains (Cdc12, Bni1, and mDia1) and their interactions with the barbed end of actin filament (Aydin et al. 2018). All FH2 domains have similar three-dimensional structures except small variations in some regions (Fig. 3c, d). In addition, the differences in the number of contacts between formin and actin residues do not correlate with their gating factors. The most notable difference was found in the number of salt bridges formed between the knob helices and actin TBC. The number of salt bridges inversely correlates with the gating factors: Cdc12 and mDia1 have the highest and lowest number of salt bridges, respectively, and the number of salt bridges for Bni1 varies between them. These findings also predicted that Cdc12 might be the best actin nucleating formin, consistent with the idea proposed by Baker et al. that the electrostatic interactions between knob helices and actin TBC play a critical role in the nucleation of actin filament.

Effect of formins on the barbed end configurations of the actin filaments in relation with the gating mechanism

FH2 domains cause flattening of actin subunits at the barbed end of the actin filaments, as hypothesized by the Pollard lab

to explain the gating mechanism (Aydin et al. 2018; Baker et al. 2015). Extensive AA simulations were used to test this hypothesis. Twist angles between actin subunits A1 and A2 (see Fig. 1b for definition) were stable at 167° during a control AA MD simulation of an Oda actin filament 30-mer without FH2 domain (Baker et al. 2015). On the other hand, twist angles between actin subunits bound to Bni1 FH2 were found to shift toward larger values (from ~ 167 to $\sim 172^\circ$) at the end of long simulations by Baker et al. These AA simulations captured the effect of formin at the barbed end of the filament and sampled transition between twisted “open” state and flatter “closed” state.

Although formin was shown to influence the flattening of the barbed end in simulations by Baker et al., these simulations did not rigorously test Paul and Pollard’s hypothesis on the gating mechanism. This is because Bni1 has a gating factor of ~ 0.6 and the barbed end bound to Bni1 is in equilibrium between twisted “open” and flattened “closed” states. A more reliable test would be comparing the twist angle distributions of actin subunits bound to formins with different gating factors. Toward this aim, Aydin et al. investigated flattening of the barbed end by Cdc12, Bni1, and mDia1 FH2 domains with gating factors of ~ 0.05 , ~ 0.6 , and ~ 0.95 respectively, by comparing their distributions of twist angles (Aydin et al. 2018). The findings from this investigation support Pollard’s hypothesis: The filaments interacting with Cdc12 and mDia1 have the most flattened and twisted barbed ends, respectively, while the flattening of the barbed end bound to Bni1 varies between them (Fig. 3e). In addition, the AA and CG simulations revealed an additional contribution to gating, steric blocking, by showing that some overlaps exist between alpha-carbon atoms of FH2 domains and incoming actin subunits during the simulations, and the extent of overlap is consistent with the gating factors of formins (steric blocking is the largest for Cdc12 and the smallest for mDia1) (Aydin et al. 2018). Moreover, metabasin metadynamics (a recently developed variant of metadynamics) simulations showed that Cdc12 FH2 domains encounter higher energy barriers than Bni1 and mDia1 FH2 domains to undergo conformational changes, which would be necessary to escape from a state with steric blocking.

Overall, these investigations demonstrate the important contributions of both helix distortion and steric blocking to the gating with implications for two main models of processive elongation: “stair-stepping” and “stepping second.” Aydin et al. revealed that the formins can switch between “open” and “closed” states without dissociation of an FH2 domain (Aydin et al. 2018), as proposed by the stepping second model of the Pollard Lab. On the other hand, these simulations have no large-scale conformational changes so they do not completely rule out the “stair-stepping” model, which requires dissociation of one FH2 domain from the end of the filament for a transition to “open” state. Indeed,

different energy barriers obtained from metadynamics simulations might be relevant for the “stair-stepping” model, as the magnitude of energy barrier for a conformational change can affect ability of FH2 domains to dissociate from the barbed end of the filament.

Branched filament networks

One of the key components of the branched cytoskeletal network is the actin-related protein complex Arp2/3, which consists of seven proteins. Arp2 and Arp3 are the two actin-like subunits that form the core of the complex, and the remaining five subunits, ArpC1-5, are considered to play a role in helping with activation of Arp2/3 and binding of the complex to a mother filament. Arp2/3 binds to a preexisting actin filament and nucleates a daughter filament by forming the first two monomers. The Arp2/3 complex is an inactive state in its native state so that it cannot initiate filament nucleation without getting activated by other cellular components. There are only small number of computational studies that studied the Arp2/3 complex, given the size and complexity of this system, despite its importance in the cytoskeletal system and role in the cell motility.

Effect of bound nucleotide on the structure and dynamics of the Arp2/3 complex

Pfaendtner and Voth (2008) performed the first AA MD simulations of the Arp2/3 complex and investigated the role of the Arp3-bound nucleotide on the dynamics of the entire complex by using ATP-bound, ADP-bound, and nucleotide free systems. Moreover, they generated the missing subdomains (subdomains 1 and 2) of Arp2 by using a homology model and studied the effect of bound nucleotide in Arp2 as an additional analysis and further verification of the effect of nucleotide. The stability of Arp3 with different nucleotide states was investigated by analyzing the width of nucleotide-binding cleft. It was found to remain in closed state for ADP- and ATP-bound Arp3 within Arp2/3 complex, whereas adopted an open conformation in nucleotide free Arp2/3 and ATP-bound Arp3-only simulations. A CG analysis further demonstrated that the shape of ATP-bound Arp3 within the complex is much more compact than ATP-bound Arp3-only. The investigations on the nucleotide-binding cleft showed that ADP-bound Arp3 is much more stable. This study also found that the contacts between adjacent subunits are not affected by hydrolysis of the nucleotide ATP. Therefore, the bound nucleotide has no role in initiating the movements between the subunits for the activation process of Arp2/3. Overall, this work supported the hypothesis suggesting that conformational differences in the ATP- and ADP-bound structures point to the role of ATP hydrolysis in regulating Arp2/3 function and structure (Espinoza-Sanchez et al. 2018; Goley et al. 2004).

Structural and functional properties of the Arp2/3 branch junction complex

As an extension of this work, Pfaendtner et al. (2012) performed large-scale MD simulations of the Arp2/3 complex branch junction based on the electron microscopy (EM) structure proposed by Rouiller et al. (2008) to gain insight into the molecular level details of the interfacial binding region between Arp2/3 complex and mother/daughter filaments and understand the structural and dynamical properties of the branch junction. This simulation system consisted of a periodic actin mother filament with 13 ADP-actin subunits, a daughter filament with 11 ADP-actin subunits, and the Arp2/3 complex in a solvated simulation box (Fig. 4a). Two different branch junction models (each system has ~3 million atoms) were investigated by using MD simulations totaling 175 ns. One of the models (Branch08) was based on the EM structure obtained by a fitting procedure (Rouiller et al. 2008), and the other model (Branch10) was constructed by using the same Arp2/3 branch junction complex but different subunits in the mother filament and daughter filament based on the Namba filament model (Fujii et al. 2010), except mother filament subunits M6 and M8 that bind Arp2/3 complex. No significant differences between two models were detected at the end of the MD simulations of Pfaendtner et al. (2012).

The MD simulations of the full branch junction resulted in a relatively low root-mean-squared deviation (RMSD), which verifies the quality of the initial EM structure. In addition, the angles between the mother filament and daughter filament from the simulations were found to be in good agreement with the angle derived from experimental imaging techniques. The binding interface between the Arp2/3 complex and neighboring actin subunits was further seen to be dynamic despite a comparably low RMSD of the Arp2/3 complex. The difference contact map was used to track the change in the contacts during both the Branch08 and Branch10 simulations. The contacts between the Arp2/3 complex and the mother filament were overall lost during the MD simulations due to structural relaxation. A large number of salt bridges between charged residues and hydrophobic contacts between hydrophobic residues were found in the interface between the Arp2/3 complex and the mother filament, and these strong interactions were considered to stabilize the binding interface so that the branch junction can reliably transmit forces during cell motility (Fig. 4c).

The Pfaendtner et al. simulations revealed that the DB-loop in actin and Arp2/3 complex play an important role in stabilizing interface between Arp2/3 and the mother filament. In addition, strong contacts between the D-loops in the first two subunits of daughter filament (D1 and D2 in Fig. 4a, b) and

Arp2/3 result in a binding interface at the region where the daughter filament and mother filament link together. Finally, this work established a new method for a more reliable comparison between simulation and experiments by filtering the MD simulations with experimentally measured structural data (Fig. 4d, e). Combining MD simulations of large biomolecules with low-resolution experimental structure can be helpful to overcome the timescale problems of the simulations, and also to test the structures of large biomolecular complexes obtained by using fitting methods.

Summary and future prospects

This review mostly highlights the work done in the Voth group to understand the actin cytoskeleton from multiscale computational approaches, as well as the important contributions of Tom Pollard, both in collaboration with his group and also inspired by his other contributions. We have summarized the key structural and chemical aspects of actin subunits at various resolutions, ranging from quantum mechanical modeling to study ATP hydrolysis to detailed AA simulations to characterize the structural transformations that actin subunits undergo as a result of (a) polymerization and (b) hydrolysis of the bound nucleotide ATP and phosphate release. In addition, a summary of CG models of actin at various resolutions of coarse-graining and the insights gained through these models were also discussed, including the methods used, such as ED-CG, hENM, and the UCG modeling approaches. Moreover, we discussed the modeling of actin-associating proteins such as formins and the Arp2/3 complex to understand the important mechanisms at the molecular level affecting the architecture of actin network at larger length scales by using these approaches. These collective works also highlight the fruitful collaborations between the Voth and Pollard labs, e.g., by providing direct evidence at the molecular level that supports the random hydrolysis mechanism for ATP hydrolysis in filaments as well as the gating mechanism of formins.

Many mysteries still remain to be unraveled related to the cytoskeleton. For example, there are numerous studies showing seemingly contradicting effects of applying external force on formin-mediated polymerization of actin filaments. One of the important challenges in modeling this system is connecting the residue-level properties with responses at the filament level while overcoming the size limitations in simulating this system at atomistic detail. In spite of recent advances in multiscale modeling approaches that have been able to push the limits of the system sizes that one can simulate, a real challenge is in cleverly combining these approaches, while carefully following the fundamental physical and

Fig. 4 Structure and interactions of the Arp2/3 branch junction domains (reproduced from Pfaendtner et al. (2012)). **a** The Arp2/3 complex subunits (colored) associated with mother (M1 to M13) and daughter filaments (D1 to D11). **b** Close up views of the Arp2/3 complex associated with mother and daughter filaments. **c** (Top) Salt bridge formation between ARPC2 (colored in cyan) and mother filament subunits M6 and M8 during all-atom MD simulations. (Bottom) Close up views of residues forming salt bridges. **d** Comparison between the density maps of the Arp2/3 complex from electron microscopy (EM) tomography (colored in gray) and the starting model based on EM data (blue) and the best-fitting molecular dynamics (MD) model (red). **e** Local changes in number of alpha-carbons inside the density map shown in (d)

chemical laws, to be able to address more challenging problems that would require, for example, simulating the full actin network dynamics at the mesoscopic level of a cell, including polymerization and depolymerization, ATP hydrolysis, and P_i release at the level of individual subunits, binding/unbinding

of actin binding proteins (cross-linkers, severing proteins, etc.) with precise dependence on the nucleotide state, all while accurately capturing the competition between these actin binding proteins. All of these challenges await combined simulation and experimental work in the future.

Compliance with ethical standards

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