ISSN 0006-3509, Biophysics, 2011, Vol. 56, No. 2, pp. 220–224. © Pleiades Publishing, Inc., 2011.
Original Russian Text © O.V. Levtsova, I.D. Davletov, O.S. Sokolova, K.V. Shaitan, 2011, published in Biofizika, 2011, Vol. 56, No. 2, pp. 242–247.

A Molecular Dynamics Study of the Interaction between Domain I-BAR of the IRSp53 Protein and Negatively Charged Membranes

O. V. Levtsova, I. D. Davletov, O. S. Sokolova, and K. V. Shaitan

Moscow State University, Moscow, 119991 Russia

Received December 28, 2010

Abstract—The methods of computer simulation in all-atom and coarse-grained approximations have been used to study specific interactions of the isolated domain I-BAR of the actin-binding protein IRSp53 with model membranes containing neutral phospholipids and those including negatively charged $PI(4,5)P_2$ phospholipids. It has been shown that the I-BAR domain does not interact with neutral lipids but induces bending of the synthetic membrane rich in negatively charged phospholipids. Clustering of charged lipids on the surface of the membrane at the sites of its interaction with the protein has been observed. This indicates that the interaction of I-BAR with negatively charged lipids is of electrostatic and hydrophobic nature.

Keywords: molecular dynamics, conformational changes, protein–lipid interactions

DOI: 10.1134/S0006350911020199

INTRODUCTION

The behavior of the actin cytoskeleton is regulated in cells by various actin-binding proteins. Every year there appear reports on the involvement of new proteins into the process of actin regulation [1, 2]. Actin-binding proteins associated with the plasma membrane may modify it, causing formation of filopodia [3]. This process is controlled by domains of actin-binding proteins called BAR (Bin, Amphiphysin, Rvs) and I-BAR (invert-BAR). Mutations in genes encoding a BAR domain may lead to various nervous and autoimmune diseases [4]. A classical BAR domain presents as a dimer in which each monomer consists of three bent antiparallel α-helices [5]. By its outer surface the BAR domain interacts with the membrane at the expense of electrostatic interactions and also anchoring of the N-terminal peptides in the lipid layer [6]. The results of a recent cryoelectron-microscopic study of the interaction of isolated F-BAR domains with a lipid membrane [7] have shown that the domains are capable of self-organization. Single domains interact with each other by their distal ends and sides to form a continuous helical «coating» on the surface of the lipid cylinder.

The protein IRSp53 (Insulin Receptor Substrate p53) takes part in regulating cell movement, modulating the formation of lamellopodia [8, 9]. At the N-end it contains an I-BAR domain and at the C-end, an actin-binding domain. Expression of an isolated I-BAR domain in cells causes a significant increase in the number of filopodia [10, 11], but the full-sized protein is inhibited in cells. A crystal structure of I-BAR has been published [12, 13], but the mechanisms of its binding with the membrane is unknown. As a rule, it is supposed that I-BAR interacts with the membrane from the inside by its convex side, by analogy to the BAR domain, which surrounds the tube from the outside by its concave side. This, however, does not correspond to the results obtained upon studying the interaction of isolated I-BAR domains with polar lipids in vitro [9, 14]. In these works it has been shown that a series of positively charged amino acids (K108, K130, K147, K171) of the I-BAR domain of IRSp53 draw up into a «lipid binding line» positioned at a significant distance from the convex surface [14, 15].

The tasks of the given work included a study of the mechanisms and dynamics of membrane deformation upon interaction with the I-BAR domain of protein IRSp53 by the methods of coarse-grained and all-atom molecular dynamics (MD).

DATA AND METHODS

In the case of all-atom approximation, the study was conducted in the Gromacs 3.3.1 package [16] with the OPS-AA force field [17]. Use was made of the following protocol:

- step, 2 fs;
- trajectory, 10 ns;
- Berendsen barostat;
- barostat pressure, 1 bar normal to and 30 bar along the membrane;

Editor's Note: I certify that this text exactly reproduces all factual statements and largely conveys the phrasing and style of the original Russian publication. *A.G.*

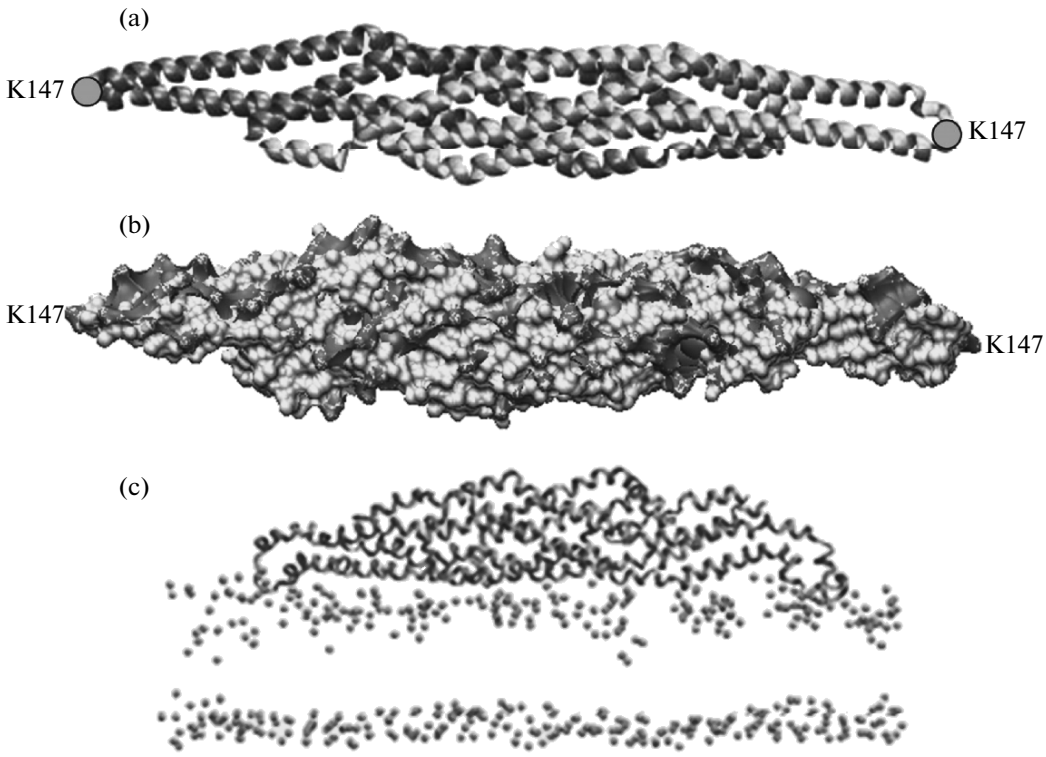

Fig. 1. (a) Crystal structure of the I-BAR domain; (b) protein surface with charged amino acids; (c) pattern of protein interaction with negatively charged membrane in 10 ns (all-atom MD).

- cutoff radius for Coulomb and van der Waals interactions, 2 nm;

- thermostat, stochastic dynamics;

- thermostat temperature, 300 K.

In the case of coarse-grained MD, use was made of the MARTINI force field [18] and the following protocol:

- step, 40 fs;

- trajectory, 500 ns;

- Berendsen barostat;

- barostat pressure, 1 bar;

- cutoff radius for Coulomb and van der Waals interactions, 1.2 nm;

- permittivity, 15;

- Berendsen thermostat;

- thermostat temperature, 325 K.

Model lipid bilayers consisted of 448 molecules of phosphatidylcholine lipids. For a neutral membrane, only palmitoyloleyl phosphatidylcholine (POPC) was used; for a negatively charged membrane, one layer was supplemented with phosphoinositide(4,5)phosphatidylcholine (PIP(4,5)PC) at a ratio 1 PIP(4,5)PC: 4 POPC.

The crystal structure of the I-BAR domain of IRSp53 was retrieved from the Protein Data Bank (code 1Y2O [9]). I-BAR was placed 0.4 nm above the membrane surface in three positions: conventional, with C-ends directed away from the membrane (system 1); I-BAR facing the membrane by the «lipid binding line» (system 2); and C-ends towards the membrane (system 3). Results were visualized using *scilab* (http://www.scilab.org) and VMD (http://www.ks.uiuc.edu/Research/vmd).

RESULTS

Interaction of I-BAR with nonpolar membrane. No interaction was observed within 25 ns of simulation; the domain did not approach the membrane surface and did not penetrate the lipid head region.

Interaction of I-BAR with polar membrane. *All-atom MD.* Interaction was observed as soon as in 4 ns of simulation. In 10 ns the distal ends of the dimer submerged into the lipid head region (Fig. 1c).

Coarse-grained MD. In system 1 (C-ends away form membrane) in 25 ns of simulation one distal end of the domain entered the lipid head region (Fig. 2a). The symmetrical distal end began interacting with membrane in 50 ns but did not submerge into the lipid bilayer throughout the trajectory. The first deformation of the membrane was noted in 85 ns, however the membrane bend was unstable, and 50% of the time the membrane remained flat. Further the protein turned relative to the membrane about its long axis and lay on its side. As a result, in 500 ns one of the domains was lying on the membrane surface (Fig. 3a), and the fol-

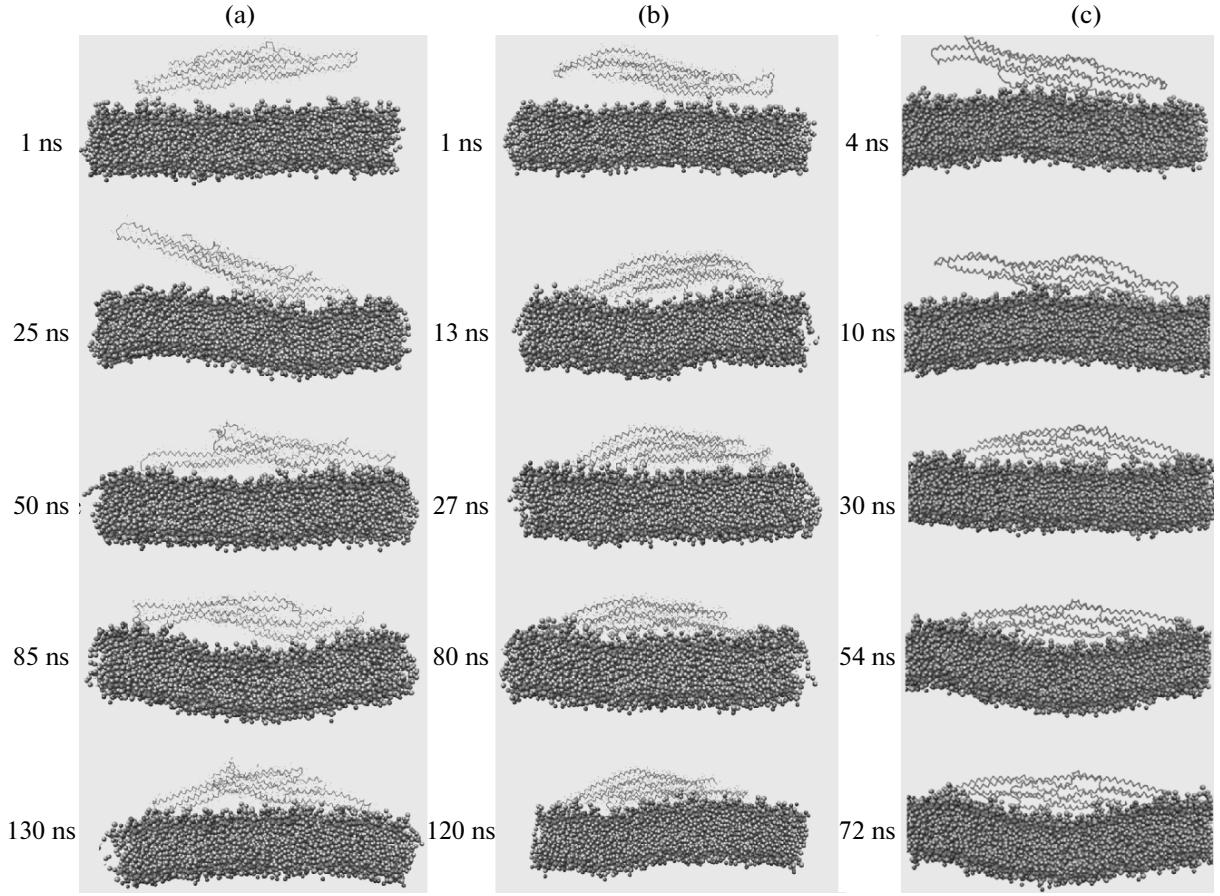

Fig. 2. Interactions of I-BAR domain with a polar membrane as dependent on the initial position of the domain relative to membrane (coarse-grained MD); (a) C-ends directed away from the membrane (system 1); (b) I-BAR oriented along the «lipid binding line» (system 2); (c) C-ends directed toward the membrane (system 3).

lowing positively charged amino acids interacted with $PI(4,5)P_2$ lipids: R29, K121, R128, K136, K143, R145, K146, K147. The latter four residues were submerged in the lipid bilayer. The second monomer contacted the membrane by the following residues: K142, K143, K146, K147, which did not penetrate the membrane in the course of 500 ns.

In system 2 (along the «lipid binding line») the protein lay on the membrane surface in 13 ns and one distal end entered the lipid bilayer (Fig. 2b). I-BAR started bending the membrane in 80 ns, but the bend was local and did not spread over the whole membrane. After 500 ns the observed orientation was similar to the above-described system 1 (Fig. 3b). The amino acid residues interacting with the membrane were R128, K142, K143, K143, K146, K147 of one monomer and K142, K145, K146 of the other.

In system 3 (C-ends toward membrane) the interaction of the protein with the membrane began from its central part where both C-ends were (Fig. 2c). Within 10 ns both distal ends turned to the membrane (outer loops between R145 and E165). In 30 ns the distal ends of both monomers entered the lipid head region. The bend of distal ends caused a conformational stress that spread over the whole protein and led to conformational alterations that caused membrane bending at 60 ns. This bend persisted for the subsequent 440 ns. In the course of the remaining time both distal ends were deeply submerged into the membrane polar region (Fig. 3c). Such protein position was stabilized by interaction of the following amino acid residues with the membrane: K145, K147, K148, K152, K156, K160.

Oscillations of membrane bend. To estimate the stability of membrane bends upon interaction with the I-BAR domain we studied the time dependence of the bend (mean curvature radius) for all three systems (data not shown). Figure 3d presents the mean membrane curvature induced by I-BAR. The maximal bend was observed in system 3 (C-ends toward membrane), with the mean radius about 90 nm. In system 1 (C-ends away from membrane) the mean radius was about 120 nm. In system 2 the bend radius was the least stable: it varied over 100–200 nm. The membrane bend oscillations of different amplitude in the three systems may tentatively be explained by that one domain is incapable of forming a stable membrane bend, this requires a complex of several domains.

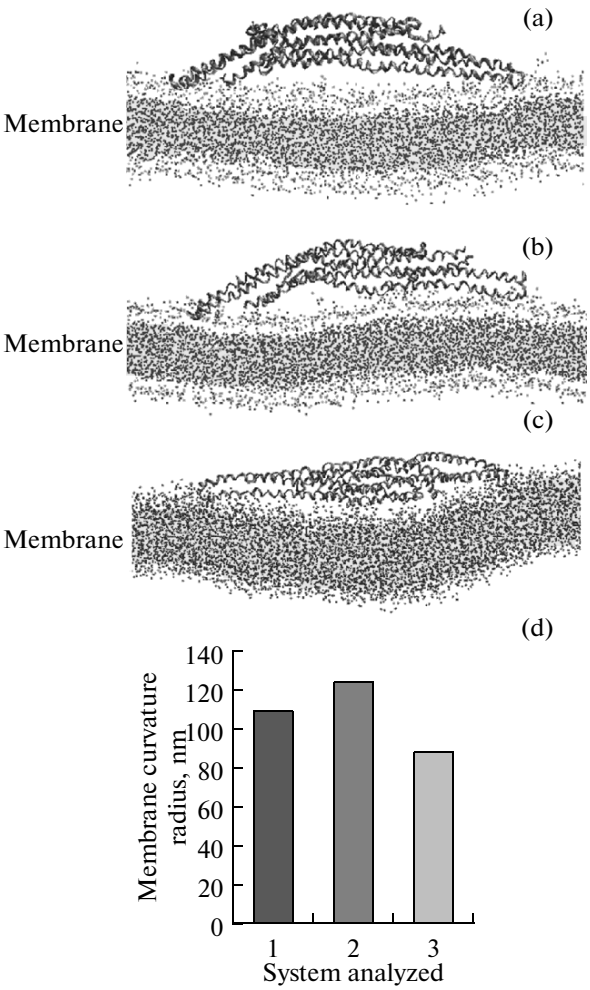

Fig. 3. Interactions of I-BAR domain with a polar membrane in 500 ns in different systems (coarse-grained MD); (a) C-ends directed away from the membrane (system 1); (b) I-BAR oriented along the «lipid binding line» (system 2); (c) C-ends directed toward the membrane (system 3); (d) mean membrane curvature induced by interaction with I-BAR.

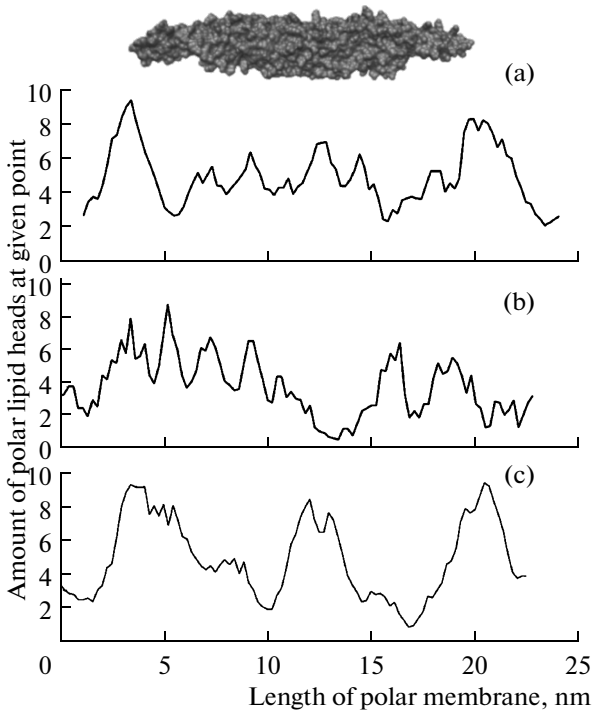

Fig. 4. Clustering of polar lipids in the membrane. The profile of density of PI(4,5)PC polar heads along the membrane upon interaction with the I-BAR domain; (a) C-ends directed away from the membrane (system 1); (b) I-BAR oriented along the «lipid binding line» (system 2); (c) C-ends directed toward the membrane (system 3).

Clustering of charged PIP(4,5) lipids. We have also studied the lateral diffusion of $PI(4,5)P_2$ lipids upon membrane interaction with the I-BAR domain. Figure 4 displays the curves for lipid density along the abscissa (parallel to the long axis of the I-BAR domain) for the three system. We observed no detectable lipid clustering in system 1 with the C-ends directed away from the membrane surface (Fig. 4a). With the C-ends oriented toward the membrane and along the «lipid binding line» we observed three peaks of lipid density: two in the region of distal ends and one in the central part of the dimer (Fig. 4b,c).

DISCUSSION

For studying the interaction of two lipid membranes (consisting of POPC and POPC/$PIP(4,5)P_2$) with the I-BAR domain of the IRSp53 protein we used different MD modifications (all-atom and coarse-grained approximation). Coarse-grained MD is used to build simplified molecular models, which significantly speed ups computer calculations at the expense of that the quantity of atoms representing one amino acid is decreased and the integration step is increased [18]. This permits simulating significantly longer processes (100–1000 ns). In our simulation the initial steps of protein interaction with the membrane proceeded identically in both systems (all-atom (Fig. 1c) and coarse-grained approximation (Fig. 2a–c)), which permitted using the coarse-grained model for calculating the protein interaction with the membrane in the course of 500 ns.

The I-BAR domain is formed by two monomers (Fig. 1a). Most of the positively charged amino acid (mainly lysine and arginine) reside on the distal ends of the dimer (Fig. 1b) and along the side opposite to the C-end. It is believed that the positively charged amino acids prevalently interact with negatively charged lipids [14]. However, as shown above, the orientation of I-BAR domain (C-ends toward the membrane, C-ends away from the membrane, along the «lipid binding line») does not tell on its ability to interact with PIP-rich membranes. This may be evidence in favor of hydrophobic interactions between membrane and protein. At the same time we observed no

I-BAR interaction with a neutrally charged membrane (data not shown). This is evidence for the presence of electrostatic interaction between the protein and negatively charged lipids. In all systems examined the protein lay down on the membrane in 10 ns and its distal ends submerged into the lipid head region (Figs. 1c, 2). This may be explained by that on the distal ends of both monomers there is a positively charged amino acid lysine in position 147 (K147), which is equally accessible at any position of the dimer relative to the membrane (Fig. 1a,b).

The observed interaction of I-BAR with the membrane differed significantly from the interaction of related BAR domains [19]. For the BAR domains the interaction with membrane starts from distal ends and spreads onto the central part [20]. For I-BAR, apart of that, we observed interaction between lipids and the central part of the dimer (Fig. 2). This interaction leads to formation of a positive membrane curvature at the early steps of protein–lipid interaction (Fig. 2a,c). The nature of this interaction is electrostatic, and it is not stabilized by interaction of N-terminal helices with lipids as it has been predicted for other proteins [21].

The next stage of deformation includes a conformational alteration of the protein molecule, as a result of which the distal ends of the dimer come close to the charged membrane and submerge into it. These changes proceed at the expense of Coulomb interactions between positively charged amino acids (lysine, arginine) and negatively charged lipids. A negative (resultant) bend of the membrane forms only after interaction of the distal ends (amino acid K147) with lipids. In this way, interaction with the membrane and formation of a bend therein do not present as combined processes and are induced by different conformations of the protein molecule.

The membrane curvature radius varied in our MD experiments from 100 to 200 nm. This apparently is connected with that a single domain is not capable of effectively bending a membrane. This is confirmed by recent investigations having shown that in an artificial system in vitro the domains form a continuous cylinder around a lipid tubule only upon interaction with each other [7, 22].

In this way, we have shown that a single I-BAR domain stably binds to the membrane not by its convex side but along the «lipid binding line» (Fig. 3b). In the case if several such domains are disposed close enough, they may interact with each other, turning to the membrane. Therewith clusters of negatively charged lipids migrate to the distal ends of the domains (Fig. 4), which further penetrate into the polar regions of the membrane and cause formation of filopodia. Actin-binding domains residing at the C-ends of monomers stabilize the arising filopodium upon interaction with actin.

ACKNOWLEDGMENTS

The authors express their gratitude to Professor P. Lappalainen (University of Helsinki) for fruitful discussions that preceded the writing of this paper, and to S.I. Trifonov for calculating the membrane bend radius.

The work was supported by the Russian Foundation for Basic Research (08-04-91759-AF_a, 10-04-01587-a) and Rosnauka (GK 16.740.11.0373).

REFERENCES

1. P. K. Mattila, M. Salminen, T. Yamashiro, and P. Lappalainen, J. Biol. Chem. **278** (10), 8452 (2003).
2. S. Suetsugu, S. Kurisu, T. Oikawa, et al., J. Cell Biol. **173**, 571 (2006).
3. J. Zimmerberg and M. M. Kozlov, Nat. Rev. Mol. Cell Biol. **7**, 9 (2006).
4. A. S. Nicot, A. Toussaint, V. Tosch, et al., Nat Genet. **39**, 1134 (2007).
5. B. J. Peter, H. M. Kent, I. G. Mills, et al., Science **303**, 495 (2004).
6. J. L. Gallop, C. C. Jao, H. M. Kent, et al., EMBO J. **25**, 2898 (2006).
7. A. Frost, R. Perera, A. Roux, et al., Cell **132**, 807 (2008).
8. H. Nakagawa, H. Miki, M. Nozumi, et al., J. Cell Sci. **116**, 2577 (2003).
9. S. Suetsugu, K. Murayama, A. Sakamoto, et al., J. Biol. Chem. **281**, 35347 (2006).
10. G. Bompard, S. J. Sharp, G. Freiss, and L. M. Machesky, J. Cell Sci. **118**, 5393 (2005).
11. A. Yamagishi, M. Masuda, T. Ohki, et al., J. Biol. Chem. **279**, 14929 (2004).
12. T. H. Millard, G. Bompard, M. Y. Heung, et al., EMBO J. **26**, 240 (2005).
13. S. H. Lee, F. Kerff, D. Chereau, et al., Structure **15** (2), 145 (2007).
14. P. K. Mattila, A. Pykalainen, J. Saarikangas, et al., J. Cell Biol. **176**, 953 (2007).
15. J. Saarikangas, H. Zhao, A. Pykalainen, et al., Curr. Biol. **19**, 95 (2009).
16. H. J. C. Berendsen, D. van der Spoel, and R. van Drunen, Comp. Phys. Comm. **91**, 43 (1995).
17. W. L. Jorgensen and J. Tirado-Rives, J. Am. Chem. Soc. **110**, 1657 (1988).
18. S. J. Marrink, H. J. Risselada, S. Yefimov, et al., J. Phys. Chem. **111**, 7812 (2007).
19. S. Ferguson, A. Raimondi, S. Paradise, et al., Dev. Cell **17**, 811 (2009).
20. A. Arkhipov, Y. Yin, and K. Schulten, Biophys. J. **95**, 2806 (2008).
21. J. Saarikangas, H. Zhao, A. Pykalainen, et al., Curr. Biol. **19**, 95 (2009).
22. A. Shimada, H. Niwa, K. Tsujita, et al., Cell **129**, 761 (2007).