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Elucidating membrane protein function through long-timescale molecular dynamics simulation

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***Abstract*—Recent advances in algorithms, software, and hardware for molecular dynamics (MD) simulations have brought previously inaccessible simulation timescales within reach, allowing the use of MD simulation to address a substantially broader set of questions regarding protein function. MD has proved particularly useful in elucidating the functional mechanisms of membrane proteins, whose dynamics are especially difficult to characterize experimentally. Here, we illustrate the utility of state-of-the-art high-performance MD simulations in the study of membrane proteins, using as examples a G-protein–coupled receptor, an aquaporin, and an antiporter. In each case, we used MD either to deduce an atomic-level mechanism for protein function or to reconcile apparent discrepancies among recent experimental observations.**

I. Introduction

Although proteins are frequently visualized as static structures, their function is in fact closely tied to their dynamical properties. Molecular dynamics (MD) simulations provide an established technique for the characterization of protein motions, offering insight into the behavior of biomolecular systems at spatial and temporal scales that are difficult to access experimentally. The major conformational changes that underlie some of the most important aspects of protein function, however, typically take place on timescales ranging from microseconds to seconds. Because all-atom MD simulations must be discretized at the level of femtoseconds, even microsecond timescales have until recently been beyond the reach of the longest such simulations.

The timescales accessible to MD simulation, however, have grown steadily over the years, due in large part to improvements in the performance of commodity hardware. Over the last few years, such hardware improvements have been accompanied by the development of significantly improved software and algorithms for parallel MD [1–4], resulting in particularly rapid growth in the maximum speed attainable in biomolecular simulations. All-atom simulations of more than a microsecond have become practical, and simulations encompassing hundreds of nanoseconds of biological time can now be completed in a few days. These advances have substantially increased the utility of MD simulation as a tool for elucidating the mechanisms underlying protein function.

MD simulations can be particularly illuminating in the case of membrane proteins, since experimental characterization of the structural dynamics of such proteins is especially difficult. Moreover, many membrane proteins are of great scientific and pharmaceutical interest. A number of research groups, including our own, have recently applied high-performance MD simulations to the study of membrane proteins [e.g. 5–12]; for a review, see [13].

Here, we illustrate the utility of MD for describing atomic-level functional mechanisms of membrane proteins through three studies completed within our group [6, 9, 12]. The simulations in each of these studies were performed on a commodity Linux cluster using Desmond, a software package we developed for efficient, parallel MD [1].

II. Identification of two distinct inactive conformations of a G-protein–coupled receptor

The G-protein–coupled receptors (GPCRs) constitute the largest class of drug targets, but until 2007, the only GPCR whose structure had been determined crystallographically was that of rhodopsin, which is an unusual GPCR in that it is activated by absorption of light rather than by the binding of a ligand. The recent determination of crystal structures for the β_2-adrenergic receptor (β_2AR) in a presumably inactive state constituted a major step toward understanding the atomic-level mechanisms of ligand-activated GPCRs, but also raised new questions [14, 15]. Although earlier biochemical observations had suggested that this receptor possessed a network of salt bridges known as the ionic lock, which was believed to form a molecular switch for receptor activation, this network was disrupted in the crystal structures. The unexpectedly broken ionic lock raised questions about the true conformation(s) of the inactive state and the role of the ionic lock in receptor activation and signaling.

To address these questions, and to better characterize the inactive state, we performed microsecond-timescale MD simulations of β_2AR in multiple wild-type and mutant forms [12]. In wild-type simulations, the ionic lock formed reproducibly, with the salt bridge network matching that suggested by earlier biochemical studies [16, 17]. During microseconds of simulation, the lock remained formed most of the time but occasionally broke for tens or even hundreds

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of nanoseconds. Mutations associated with increased receptor activity increased the fraction of time the lock was broken in our simulations. In order to obtain the high-resolution crystal structure of β_2AR, the receptor was stabilized through substitution of a small, rigid protein in place of a flexible intracellular loop; in our simulations, this substitution caused the conformation with the lock broken to predominate. Our results suggest that inactive β_2AR exists in equilibrium between conformations with the lock formed and the lock broken, and that the conformation with the lock formed may predominate in the inactive wild-type receptor in its native environment, despite the broken lock in the crystal structures.

III. Control of Water Transport by Aquaporin 0 in Junctional and Non-Junctional Forms

Aquaporin 0 (AQP0), which serves as the primary water channel in the lens fiber cells of the mammalian eye, is unusual among the aquaporin water transporters in two regards: it transports water about 10-fold more slowly than other aquaporins, and it mediates adhesive contacts between cells. Recent crystal structures [18, 19] have shown that AQP0 homotetramers in adjacent membranes can come together to form octameric junctions, but the permeability of this junctional form is difficult to measure experimentally and has been the subject of some debate [20].

We performed a set of MD simulations to determine the permeability of junctional AQP0, as well as the mechanism of the low permeability of AQP0 in its non-junctional form [9]. Each simulation included either one AQP0 tetramer in a single membrane or two AQP0 tetramers in adjacent membranes. Simulations of at least 100 ns were required to obtain converged estimates of the water permeability, but once converged, the calculated permeability values of the junctional and non-junctional forms were within error of each other as well as within error of the experimental value for AQP0 in a single membrane. This suggests that the junctional form of AQP0 not only contributes to the organizational structure of the lens but also transports water. Our simulations also indicated that two conserved tyrosine residues in the channel lumen are primarily responsible for its low permeability, one through a static gating mechanism and one through a dynamic gating mechanism. These tyrosine residues are absent in faster-conducting aquaporins, but direct experimental investigation of their roles in mammalian AQP0 through mutagenesis has been precluded by the lack of an appropriate expression system.

IV. Mechanism of Na^+/H^+ Antiporting

Sodium-proton antiporters, membrane proteins found in all kingdoms of life, expel sodium ions from the cell by harnessing the energy from the cotransport of protons down their electrochemical gradient into the cell. A crystal structure of the most heavily studied sodium-proton antiporter, NhaA, was determined in 2005 [21]. We undertook a set of MD simulations that led to the development of a model for NhaA's transport mechanism [6], which was substantiated using experimental studies of NhaA mutants and which provides a molecular realization of the "alternate accessibility" mechanism for membrane transport suggested by Jardetzky more than 40 years ago [22]. Additional simulations led to models for the mechanism of NhaA's experimentally observed pH regulation and cation selectivity.

Experimentally, transport of each sodium ion by NhaA takes place on a timescale of roughly one millisecond. Because MD simulations of this length were infeasible when we performed this study, we instead relied on shorter simulations of different steps of the antiporting cycle. Although the individual simulations were only 12 to 100 nanoseconds in length, multiple sequential rounds of simulation were required to develop and test mechanistic hypotheses, and our approach would have been impractical without the ability to complete each of these simulations within hours or days.

V. Concluding Remarks

The three studies presented here illustrate how MD simulation can provide insights into membrane protein function that would be difficult to obtain through experiment. These studies, which were performed on commodity clusters using the Desmond MD code, would have been difficult if not impossible prior to the introduction just a few years ago of Desmond and other fast, highly scalable software packages, which have significantly extended the achievable length of such simulations. Even now, however, MD simulations of membrane proteins on high-performance commodity clusters and other general-purpose hardware have been limited to a length of about ten microseconds. The recent completion of Anton [23], a specialized machine we constructed for the execution of ultra-high-speed MD simulations, extends the range of such simulations to the millisecond timescale—roughly two orders of magnitude beyond the reach of the fastest conventional machines running the fastest current MD codes. The introduction of Anton is thus likely to further expand the power of MD to illuminate the functional mechanisms of membrane proteins at an atomic level of detail.

References

[1] K. J. Bowers, E. Chow, H. Xu, R. O. Dror, M. P. Eastwood, B. A. Gregersen, J. L. Klepeis, I. Kolossváry, M. A. Moraes, F. D. Sacerdoti, D. E. Shaw, "Scalable algorithms for molecular dynamics simulations on commodity clusters," in *Proc. 2006 ACM/IEEE Conf. on Supercomputing (SC06)*, 2006.

[2] A. Bhatelé, S. Kumar, C. Mei, J. C. Phillips, G. Zheng, L. V. Kalé, "Overcoming scaling challenges in biomolecular simulations across multiple platforms," in *Proc. IEEE Int. Parallel and Distributed Processing Symp.*, 2008.

[3] B. G. Fitch, A. Rayshubsky, M. Eleftheriou, T. J. C. Ward, M. E. Giampapa, M. C. Pitman, J. W. Pitera, W. C. Swope, and R. S.

Germain, "Blue matter: scaling of N-body simulations to one atom per node," *IBM J. Res. Dev.*, vol. 52, pp. 145–158, 2008.

[4] B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, "GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation," *J. Chem. Theory Comput.,* vol. 4, pp. 435–447, 2008.

[5] K. Martinez-Mayorga, M. C. Pitman, A. Grossfield, S. E. Feller, and M. F. Brown, "Retinal counterion switch mechanism in vision evaluated by molecular simulations," *J. Am. Chem. Soc.,* vol. 128, pp. 16502–16503, 2006.

[6] I. T. Arkin, H. Xu, M. Ø. Jensen, E. Arbely, E. R. Bennett, K. J. Bowers, E. Chow, R. O. Dror, M. P. Eastwood, R. Flitman-Tene, B. A. Gregersen, J. L. Klepeis, I. Kolossváry, Y. Shan, and D. E. Shaw, "Mechanism of Na^+/H^+ antiporting," *Science*, vol. 317, pp. 799–803, 2007.

[7] V. Jogini and B. Roux, "Dynamics of the Kv1.2 voltage-gated K^+ channel in a membrane environment," *Biophys. J*., vol. 93, pp. 3070–3082, 2007.

[8] A. Grossfield, M. C. Pitman, S. E. Feller, O. Soubias, and K. Gawrisch, "Internal hydration increases during activation of the G-protein-coupled receptor rhodopsin," *J. Mol. Biol.*, vol. 381, pp. 478–486, 2008.

[9] M. Ø. Jensen, R. O. Dror, H. Xu, D. W. Borhani, I. T. Arkin, M. P. Eastwood, and D. E. Shaw, "Dynamic control of slow water transport by aquaporin 0: implications for hydration and junction stability in the eye lens," *Proc. Natl. Acad. Sci. USA*, vol. 105, pp. 14430–14435, 2008.

[10] V. Vásquez, M. Sotomayor, J. Cordero-Morales, K. Schulten, and E. Perozo, "A structural mechanism for MscS gating in lipid bilayers," *Science*, vol. 321, pp. 1210–1214, 2008.

[11] P. Bjelkmar, P. S. Niemalä, I. Vattulainen, E. Lindahl, "Conformational changes and slow dynamics through microsecond polarized atomistic molecular simulation of an integral Kv1.2 ion channel," *PLoS Comp. Biol*., vol. 5, pp. 1–14, 2009.

[12] R. O. Dror, D. H. Arlow, D. W. Borhani, M. Ø. Jensen, S. Piana, and D. E. Shaw, "Identification of two distinct inactive conformations of the β_2-adrenergic receptor reconciles structural and biochemical observations," *Proc. Natl. Acad. Sci. USA*, vol. 106, pp. 4689–4694, 2009.

[13] F. Khalili-Araghi, J. Gumbart, P.-C. Wen, M. Sotomayor, E. Tajkhorshid, and K. Schulten, "Molecular dynamics simulations of membrane channels and transporters," *Curr. Opin. Struct. Biol*., vol. 19, pp. 128–137, 2009.

[14] S. G. F. Rasmussen, H. J. Choi, D. M. Rosenbaum, T. S. Kobilka, F. S. Thian, P. C. Edwards, M. Burghammer, V. R. P. Rratnala, R. Sanishvili, R. F. Fischetti, G. F. X. Schertler, W. I. Weis, and B. K. Kobilka, "Crystal structure of the human β_2 adrenergic G-protein-coupled receptor," *Nature*, vol. 450, pp. 383–387, 2007.

[15] V. Cherezov, D. M. Rosenbaum, M. A. Hanson, S. G. F. Rasmussen, F. S. Thian, T. S. Kobilka, H. J. Choi, P. Kuhn, W. I. Weis, B. K. Kobilka, and R. C. Stevens, "High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor," *Science*, vol. 318, pp. 1258–1265, 2007.

[16] J. A. Ballesteros, A. D. Jensen, G. Liapakis, S. G. F. Rasmussen, L. Shi, U. Gether, and J. A. Javitch, "Activation of the β_2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6," *J. Biol. Chem*., vol. 276, pp. 29171–29177, 2001.

[17] X. Yao, C. Parnot, X. Deupi, V. R. P. Ratnala, G. Swaminath, D. Farrens, and B. Kobilka, "Coupling ligand structure to specific conformational switches in the β_2-adrenoceptor," *Nat. Chem. Biol*., vol. 2, pp. 417–422, 2006.

[18] T. Gonen, P. Sliz, J. Kistler, Y. F. Cheng, and T. Walz, "Aquaporin-0 membrane junctions reveal the structure of a closed water pore," *Nature*, vol. 429, pp. 193–197, 2004.

[19] T. Gonen, Y. Cheng, P. Sliz, Y. Hiroaki, Y. Fujiyoshi, S. C. Harrison, and T. Walz, "Lipid-protein interactions in double-layered two-dimensional AQP0 crystals," *Nature*, vol. 438, pp. 633–638, 2005.

[20] W. E. C. Harries, D. Akhavan, L. J. W. Miercke, S. Khademi, and R. M. Stroud, "The channel architecture of aquaporin 0 at a 2.2-Å resolution," *Proc. Natl. Acad. Sci. USA*, vol. 101, pp. 14045–14050, 2004.

[21] C. Hunte, E. Screpanti, M. Venturi, A. Rimon, E. Padan, and H. Michel, "Structure of a Na^+/H^+ antiporter and insights into mechanism of action and regulation by pH," *Nature*, vol. 435, pp. 1197–1202, 2005.

[22] O. Jardetzky, "Simple allosteric model for membrane pumps," *Nature*, vol. 211, pp. 969–970, 1966.

[23] D. E. Shaw, M. M. Deneroff, R. O. Dror, J. S. Kuskin, R. H. Larson, J. K. Salmon, C. Young, B. Batson, K. J. Bowers, J. C. Chao, M. P. Eastwood, J. Gagliardo, J.P. Grossman, C. R. Ho, D. J. Ierardi, I. Kolossváry, J. L. Klepeis, T. Layman, C. McLeavey, M. A. Moraes, R. Mueller, E. C. Priest, Y. Shan, J. Spengler, M. Theobald, B. Towles, and S. C. Wang, "Anton, a special-purpose machine for molecular dynamics simulation," in *Proc. 34th Ann. Int. Symp. on Computer Architecture (ISCA07)*, 2007.