Molecular dynamics simulations of biomolecules

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Molecular dynamics simulations are important tools for understanding the physical basis of the structure and function of biological macromolecules. The early view of proteins as relatively rigid structures has been replaced by a dynamic model in which the internal motions and resulting conformational changes play an essential role in their function. This review presents a brief description of the origin and early uses of biomolecular simulations. It then outlines some recent studies that illustrate the utility of such simulations and closes with a discussion of their ever-increasing potential for contributing to biology.

It has been 25 years since the first molecular dynamics simulation of a macromolecule of biological interest was published[1]. The simulation concerned the bovine pancreatic trypsin inhibitor (BPTI), which has served as the 'hydrogen molecule' of protein dynamics because of its small size, high stability and relatively accurate X-ray structure available in 1975; interestingly, its physiological function remains unknown. Although this simulation was performed in vacuum with a crude molecular mechanics potential and lasted for only 9.2 ps, the results were instrumental in replacing our view of proteins as relatively rigid structures (in 1981, Sir D. L. Phillips commented, "Brass models of DNA and a variety of proteins dominated the scene and much of the thinking…"[2]) with the realization that they were dynamic systems, whose internal motions play a functional role. Of course, experimental data, such as those from the hydrogen exchange experiments of Linderstrom-Lang and his co-workers[3] already existed pointing in this direction, and the *Feynman Lectures on Physics*, published 14 years earlier contain the prescient sentence[4]:

> Certainly no subject or field is making more progress on so many fronts at the present moment than biology, and if we were to name the most powerful assumption of all, which leads one on and on in an attempt to understand life, it is that *all things are made of atoms* [italics in original], and that *everything that living things do can be understood in terms of the jigglings and wigglings of atoms.* [italics added]

Two years after the BPTI simulation, it was recognized[5,6] that thermal (B) factors calculated during X-ray crystallographic refinement could be used to infer the internal motions of proteins. Subsequently, plots of estimated mean-square fluctuations *versus* residue number (introduced in ref. 1) became a standard part of the analysis of high-resolution structures, even though the contribution to the B-factors from overall translation and rotation as well as crystal disorder continues to be a concern in their interpretation. During the following 10 years, a wide range of motional phenomena were investigated by molecular dynamics simulations of proteins and nucleic acids. Most of these studies focused on the physical aspects of the internal motions and the interpretation of experiments. They include the analysis of fluorescence depolarization of tryptophan residues[7], the role of dynamics in measured NMR parameters[8] and inelastic neutron scattering[9], the effect of solvent and temperature on protein structure and dynamics[10,11], and the now widely used simulated annealing methods for X-ray structure refinement[12] and NMR structure determination[13]. Simultaneously, several applications demonstrated the importance of internal motions in biomolecular function, including the hinge bending modes for opening and closing active sites[14], the flexibility of tRNA[15], the fluctuations required for ligand entrance and exit in heme proteins[16] and the role of configurational entropy in proteins and nucleic acids[17,18].

Why molecular dynamics simulations are important

Simulations can provide the ultimate detail concerning individual particle motions as a function of time. Thus, they can be used to address specific questions about the properties of a model system, often more easily than experiments on the actual system. For many aspects of biomolecular function, it is these details that are of interest (for example, by what pathways does oxygen enter into and exit from the heme pocket in myoglobin?). Of course, experiments play an essential role in validating the simulation methodology: comparisons of simulation and experimental data serve to test the accuracy of the calculated results and to provide criteria for improving the methodology. This is particularly important because theoretical estimates of systematic errors inherent in simulations have not been possible — that is, the errors introduced by the use of empirical potentials are difficult to quantify.

Another significant aspect of simulations is that, although the potentials used in simulations are approximate, they are completely under the control of the user, so that by removing or altering specific contributions their role in determining a given property can be examined. This is most graphically demonstrated by the use of 'computer alchemy' — transmuting the potential from that representing one system to another during a simulation — in the calculation of free energy differences[19].

There are three types of applications of simulation methods in the macromolecular area, as well as in other areas involving mesoscopic systems. The first uses simulation simply as a means of sampling configuration space. This is involved in the utilization of molecular dynamics, often with simulated annealing protocols, to determine or refine structures with data obtained from

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experiments, as mentioned above. The second uses simulations to obtain a description of the system at equilibrium, including structural and motional properties (for example, atomic mean-square fluctuation amplitudes) and the values of thermodynamic parameters. For such applications, it is necessary that the simulations adequately sample configuration space, as in the first application, with the additional condition that each point be weighted by the appropriate Boltzmann factor. The third area uses simulations to examine the actual dynamics. Here not only is adequate sampling of configuration space with appropriate Boltzmann weighting required, but it must be done so as to correctly represent the development of the system over time. For the first two areas, Monte Carlo simulations can be used, as well as molecular dynamics. By contrast, in the third area where the motions and their development with time are of primary interest, only molecular dynamics can provide the necessary information. The three sets of applications make increasing demands on simulation methods as to their required accuracy and precision.

Molecular dynamics today

The increase in the number of studies using molecular dynamics to simulate the properties of biological macromolecules has been fueled by the general availability of programs and of the computing power required for meaningful studies. The original simulation was less than 10 ps in length. By comparison, current simulations are often 1,000 times as long (10 ns) but take a factor of ~50 less time for a system of the same size. Much of the gain in simulation time is re-invested into studying much larger systems (10^4–10^6 atoms instead of 500) to include, for example, an explicit solvent and/or membrane environment. In addition to being able to perform simulations for a much longer period of time, another important consequence of the access to faster computers is that multiple simulation runs can be performed to obtain estimates of statistical errors. Although the widely used programs (such as CHARMM[20] and its direct descendants AMBER[21] and GROMOS[22]) have a great range of capabilities ("They can do everything", as implied by the descriptions of the programs), users often find that they cannot do exactly what is needed to solve their particular problem. Thus, developments in simulation methodology continue apace with applications[23–25].

The number of publications using molecular dynamics is now in the thousands (using 'molecular dynamics' and 'proteins' as key words to search the ISI 'Web of Science' (*http://www.isinet.com/isi/products/citation/wos/*) yielded >260 papers published in the first six months of 2002), so a comprehensive listing in a short review is not possible, nor would it be useful for readers of this journal. Instead, we have chosen to describe a small number of specific examples, some from our own work, that illustrate particularly clearly the uses of molecular dynamics simulations to obtain functionally relevant information that complements experimental data. However, before doing so, we mention some of the scientists, whose work is not illustrated in this review, but who have made significant contributions to the development of molecular dynamics. Among them are H.J.C. Berendsen, one of the pioneers in molecular dynamics, and N. Gō, who have contributed particularly to extended[26] and normal mode[27] dynamical methodology, respectively; R. Elber, who has introduced many original developments, including methods for studying long-time scale processes[28]; W. Jorgenson and the late P. Kollman, who are both major contributors to the development and application of free energy simulations[29,30]; W. van Gunsteren, who has been crucial in stressing the need for standards of accuracy in molecular dynamics simulations[31]; J. Gao, who has been concerned particularly with the study of enzymatic reactions by mixed quantum mechanical/molecular mechanics (QM/MM) methods[32]; and M. Levitt, who did an early simulation of B-DNA[33].

Nuclear magnetic resonance. One area where there has been and continues to be a symbiotic relationship between simulations and experiment is nuclear magnetic resonance (NMR), which in addition to its use for structure determinations[13] is a powerful technique for the study of the dynamics and thermodynamics of biological macromolecules. Interpretations of relaxation rates (T_1 and T_2) and NOE measurements by molecular dynamics, initiated in the early 1980s[8], are now widespread[34]. It is nevertheless useful to emphasize this because, in a recent paper[35] on the use of NMR to study motional properties, Lee and Wand state, "Despite its importance, the precise nature of the internal motions of protein molecules remains a mystery." Lee and Wand[35] focus on internal protein motions (particularly librations of side chains) and the correlation of the measured flexibility based on order parameters with configuration entropy. In fact, molecular dynamics simulations have taught us much about these motions[36,37] and have been used for many years to calculate and interpret order parameters measured by NMR[8,34]. It is interesting to note also that already in 1983 normal mode calculations demonstrated that the motional contribution to the internal entropy of BPTI is equal to about 600 kcal mol^{-1} (including quantum corrections, which so far have been neglected in NMR analyses)[17] and that the value per residue of a protein is very similar to that for an isolated peptide[38]. More recent simulations have shown the importance of the residual motions to the entropy of binding of internal waters, using BPTI as the model system[39], and of antiviral drugs to the capsid of rhinovirus[40]. A recent molecular dynamics analysis[34] of the relationship between NMR order parameters and protein entropy suggests that although there is a correlation, the NMR results correspond to only a fraction (25%) of the total entropy.

Role of solvent in protein dynamics. A particularly striking example of the utility of molecular dynamics simulations is provided by their use to resolve the contentious question concerning the role of solvent in determining the internal motions of proteins[41], particularly at temperatures below the so-called glass transition[42] when many proteins cease to be active. It has not been possible to determine experimentally whether or not solvent fluctuations 'drive' the internal motion of a protein, but this was accomplished by using an attribute of the simulation methodology to create a physical system not accessible in Nature. Simulations can be performed with one part of the system (for example, the protein) at one temperature and another part of the system (for example, the surrounding solvent) at a different temperature. The amplitudes of the atomic fluctuations (corresponding to the B-factors) in carbonmonoxymyoglobin were calculated from simulations with the temperature of the protein and the solvent at either 300 K or 180 K — that is, above and

Table 1 Average mean square fluctuations

Temperature[1]	Backbone (Å²)	All heavy atoms (Å²)
P300 / S300	0.23	0.36
P180 / S300	0.18	0.28
P300 / S180	0.09	0.13
P180 / S180	0.09	0.13

[1]Temperature is in Kelvin. 'P' refers to the protein and 'S' to solvent.

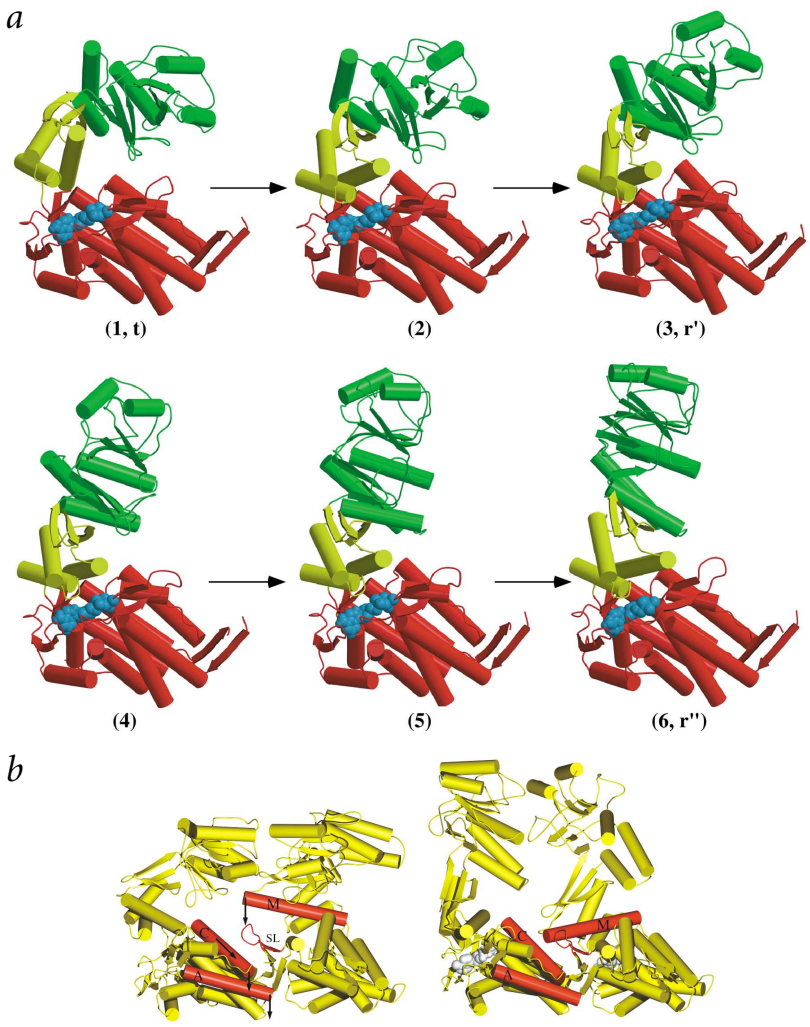

Fig. 1 Conformational change in the functional cycle of GroEL obtained by simulation of a single subunit (see ref. 45). ***a***, A set of structures on the reaction path showing the behavior of a single subunit (apical domain, green; intermediate domain, yellow; equatorial domain, red; and ATP, blue). Structures 1–3 correspond to the first stages associated with ATP binding; 4–6 correspond to the second stage involving GroES binding. The early downward motion of the intermediate domain (compare [1,t] with [2] and [6,r′′]) is the trigger for the overall transition. ***b***, The mechanism of the intermediate domain trigger. Two adjacent subunits from the crystal structures are shown (viewed from inside the central cavity). The key structural elements are in red; they are helices A, C and M, and the stem-loop (SL). The downward motion of helix M of the intermediate domain frees the apical domain for its upward movement and twist, and stabilizes the inclination of the equatorial domain with respect to the axis defined by the helix direction. The arrows indicate the direction of the motion of the helices; the arrow along the axial direction of the C helix corresponds to an axial translation. For details, see ref. 45.

below the protein glass transition at ~220 K. The results of the four possible temperature combinations (Table 1) revealed that the magnitudes of the fluctuations in the protein are only weakly dependent on protein temperature. Instead, the fluctuations are large when the solvent is at 300 K and small when the solvent is at 180 K, independent of the protein temperature. This result demonstrates that the temperature of the solvent and thus its mobility is the dominant factor in determining the functionally important protein fluctuations in this temperature range. Additional simulations have shown that at still lower temperatures (~80 K), the protein 'freezes' — that is, has only small fluctuations — even if the solvent is at 300 K. At this temperature, the internal motional barriers become important and dominate the dynamics of the protein atoms.

Conformational change in the functional mechanism of GroEL. Chaperones are essential for the correct folding of many proteins in the highly concentrated milieu of the cell[43]. One of the best-characterized chaperones is the bacterial chaperonin GroEL, which is estimated to assist the folding of 10% of cytosolic proteins in *Escherichia coli* and is required for growth of the bacteria. The chaperonin consists of two rings, each with seven identical subunits stacked back to back[44]. During its functional cycle, GroEL undergoes large conformational changes[44] that are regulated by the binding and hydrolysis of ATP and involve the co-chaperonin GroES. The seven subunits in each ring are in a 'closed' structure in the absence of ATP and GroES but adopt an 'open' conformation when bound to these cofactors (Fig. 1). Experiments have shown that ATP binding and hydrolysis is cooperative (as discussed below).

Molecular dynamics simulations[45] have been used to find a pathway between the open and closed conformations, which has been impossible to determine by experimental techniques. Because the actual transition between the open and closed structures is believed to be on the millisecond timescale (the full GroEL cycle takes ~15 s), a direct simulation of the opening and closing motion in the presence and absence of ATP by molecular dynamics is not possible as yet. Nevertheless, several methods have been developed to follow the transition between two known states on the nanosecond time scale accessible to simulations. One of these, targeted molecular dynamics, was used to determine the transition pathway of GroEL (Fig. 1). The results indicate that the subunits adopt an intermediate conformation with ATP bound but in the absence of GroES. Notably, the early downward motion of the small intermediate domain induced by nucleotide binding triggers the larger movement of the apical and equatorial domains (Fig. 1*b*). Moreover, the simulations demonstrated that steric interactions and salt bridges between different subunits are the source of the observed positive cooperativity of ATP binding and hydrolysis within one ring and the negative cooperativity between the two rings. Recent cryo-electron microscopy results[46] support the molecular dynamics prediction that the intermediate domain plays a key role in the conformational transition of GroEL.

Dynamic coupling of protein modules in protein kinases. The activation of tyrosine kinases of the Src family has been shown by molecular dynamics simulations to be governed by the flexibility of the short peptide linker between the SH2 and SH3 domains of these molecules[47]. Crystallographic studies show that the inactive state is one in which these domains are 'assembled', with the SH2 domain bound to a phosphorylated tyrosine at the C-terminus of the kinase domain, and the SH3 domain

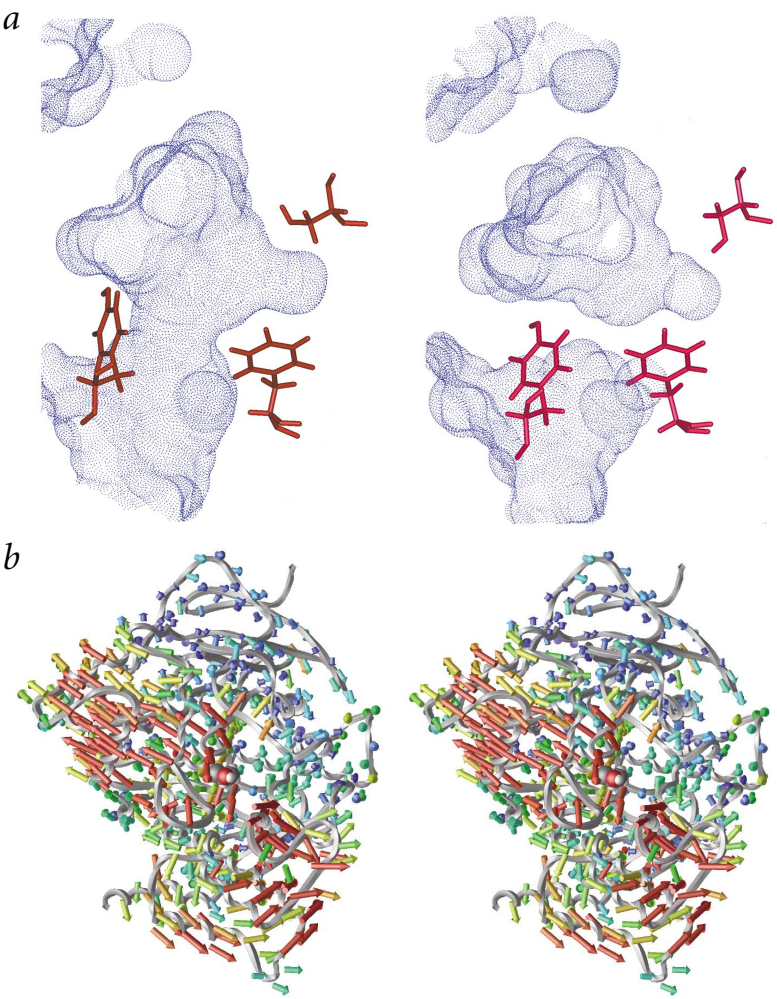

Fig. 2 Fluctuation of the width of the bottleneck in the main channel or 'gorge' of mouse acetylcholinesterase. This substrate access channel to the active site chamber is closed most of the time, but opening fluctuations occur frequently enough to allow the very fast kinetics observed for the endogenous substrate acetylcholine. The opening fluctuations result from local displacements of aromatic side chains and collective displacements that include shifts of helices. ***a***, Close-up views of the gorge in closed (left) and relatively open conformations (right). The outer face of the gorge is indicated by the surface at the left. At the local level, rapid (ps) modulation of the opening results from reorientations of side chains such as those of the two residues shown, Phe 338 and Tyr 124. The catalytic Ser 203 is shown adjacent to the active site chamber. Less frequent openings *via* a 'back door' connect the active site chamber to the outer surface at the top of the figure. ***b***, Larger fluctuations of the bottleneck on longer timescales result from collective motions of the enzyme. The arrows in the stereo image indicate the correlation of the Cα displacements with the fluctuations of the bottleneck width. The view is down into the 'gorge'; the catalytic Ser 203 is shown in a space-filling representation. (For more details, see *http://mccammon.ucsd.edu/* and ref. 54.)

bound to the peptide that connects the SH2 domain to the kinase domain. In the active state, these inhibitory interactions are absent, and the SH2 and SH3 domains are free to move about and interact with other ligands. It has remained a mystery how the activation signals originate from, for example, dephosphorylation of the tyrosine at the C-terminus of the kinase domain, which is 40 Å from the active site.

Molecular dynamics simulations of several nanoseconds duration showed that the motion of the SH2 and SH3 domains is highly correlated in the inactive state. Subsequent simulations in which the tyrosine at the C-terminus of the kinase domain was dephosphorylated showed increased motion in both the SH2 and SH3 domains, and a loss of inter-domain correlation. The results suggested that, over longer time scales, the protein would evolve into the disassembled, activated state. Targeted molecular dynamics simulations, corresponding to those used for GroEL (see above), were used to drive the protein toward the active form and pointed to the key role of the SH2–SH3 linker peptide in transmitting the effects of ligation from one domain to the other. The suggested importance of the SH2–SH3 linker was supported by subsequent experiments.

Dynamic gating in acetylcholinesterase. In acetylcholinesterase, analysis of simulations several nanoseconds in length have revealed dynamic features relevant to function that were not anticipated from experiment[48] (Fig. 2). Particularly interesting is the fact that fluctuations in the structure of the enzyme lead to intermittent opening and closing of the long channel from the surface of the enzyme to the buried active site. In the crystal structures, and in the vast majority of the structures seen in the molecular dynamics simulations, there is a bottleneck in the channel that prevents entry of the endogenous substrate, acetylcholine (Fig. 2). Opening of this 'gate' occurs on the picosecond timescale, which is often enough to allow the enzyme to maintain the high speed of action ($k_{cat} / K_M > 10^7\ M^{-1}\ s^{-1}$) needed for the destruction of acetylcholine in the function of cholinergic synapses. These opening events are due in part to reorientation of aromatic side chains that form the bottleneck. It is interesting to note that early molecular dynamics simulations had focused on aromatic side chains (such as in BPTI) and revealed such motions in a similar time range[36,37]. The recent results suggest that such fast motions are important in the activity of acetylcholinesterase.

The dynamics of the gate have been shown to reflect global fluctuations in the enzyme: such coupling may serve as a mechanism for allostery in acetylcholinesterase and other macromolecules[48]. Moreover, the detailed dynamics of the gating fluctuations have been shown to make possible 'dynamic selectivity' for substrates that differ in size. In this scenario, potential substrates that are larger than acetylcholine have to wait a long time for a correspondingly larger opening of the gate; these molecules would likely diffuse away from the entrance back into the surrounding solution rather than reacting. This suggests that because the dynamics of proteins, as well as their structures, have functional roles, there may be evolutionary selection for dynamics. More generally, because both the structure and dynamics are determined by the potential energy surface[49], or 'energy landscape' in more pictorial language, it is the latter that is governed by evolution.

Excited states in biology. Excited states of molecules play an essential role in biological systems from vision to photosynthesis. Molecular dynamics simulations have been particularly useful in increasing our understanding of the coupling between the excited state behavior and the electron and/or energy transfer that results. One important example concerns the photosynthetic complexes of bacteria, which consist of two protein–pigment

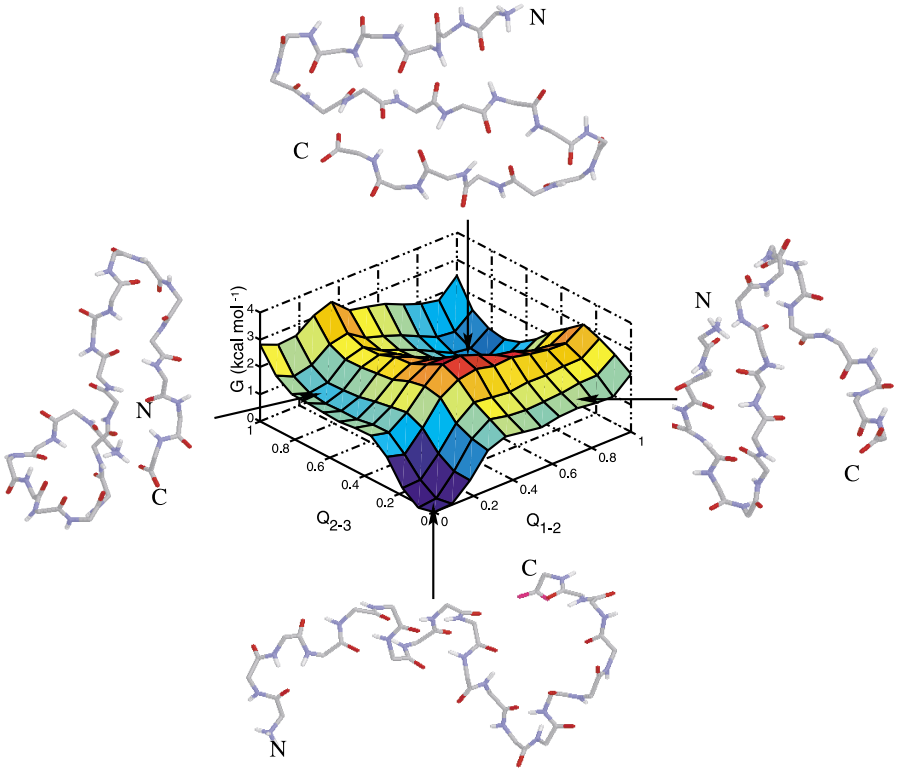

Fig. 3 Folding simulations of a three-stranded β-sheet peptide. Free energy surface at 330 K as a function of the fraction of native contacts between residues in strands 1 and 2 (Q_{1-2}) and between residues in strands 2 and 3 (Q_{2-3}). A total of 2.0×10^5 conformations sampled during the 20 runs at 330 K (totaling >2 μs) were used. The backbone of a random structure ($Q_{1-2} = Q_{2-3} = 0$) used as the starting conformation for one of the runs is shown at the bottom; two conformations along the trajectories are on the side; and the folded state is at the top. (For details, see ref. 56.)

assemblies: the photosynthetic reaction center and the light-harvesting complex. Both of these have been studied extensively by experiments and simulations. It has been shown that the protein motions play an essential role in the electron transfer that takes place in the photosynthetic reaction center[50–52] and a model for the light-harvesting complex has been developed using molecular dynamics simulations. Other cases of interest, which we do not have space to describe here, concern the light-triggered events in bacteriorhodopsin[53] and green fluorescent protein[54].

Protein folding. Although the question of how proteins fold has been of fundamental interest to researchers for many years, it is only rather recently that the spotlight of scientific research has focused on this problem, due in part to the interest generated by the numerous protein sequences available from the genome projects and the realization that the misfolding of proteins can lead to disease. The close interaction between sophisticated experiments and specialized simulations has led to a general understanding of the mechanism of protein folding[49]. However, much remains to be learned about the folding of individual proteins, including the prediction of the structure of a protein from its sequence (the 'other' protein folding problem, which is far from being solved). Because of the difficulty of folding a protein by brute force techniques (the fastest protein folding reactions require ~10 μs to complete, which is at the limit of accessible simulation times), simplified models — for example, lattice models[49] and Cα off-lattice models[55] — have been used to obtain insights concerning the mechanism of protein folding. Recently, it has become possible to follow the folding and unfolding of model peptides, such as a three-stranded β-sheet with an all-atom representation for the peptide and a simplified treatment for the solvent[56] (Fig. 3). Such simulations are likely to be extended to study the folding of actual proteins before long.

Molecular dynamics: what the future holds

One of the very exciting recent developments in molecular dynamics simulations is that, with modern computers, the simulation time is extended to a range from 100 ns to μs, making it possible to study biological phenomena as they happen. This is analogous — but in an inverse sense — to the observation that, although experiments on the ps time scale were an important development, it is only when the time resolution was extended to femtoseconds that the actual events involved in chemical reactions could be observed[57,58]. A striking recent result is that, by running multiple simulations of 10 ns duration, the 'real time' visualization of water molecule migrating through a model of the aquaporin channel has been achieved[59] (Fig. 4).

It is becoming increasingly evident that essential functions in the cell are executed not by individual proteins, but by protein complexes. The structures of such large multisubunit complexes are now being determined. In most complexes (they are almost all 'molecular machines') conformational change is directly involved in function. Two such systems that have already been simulated by molecular dynamics are the nicotinic acetylcholine receptor (R. Henchman and J.A. McCammon, work in progress) and ATP synthase[60,61]. Another complex that is waiting to be studied by molecular and normal mode dynamics is the ribosome, whose structure was determined recently. With the pace of advance in both computer hardware and algorithms, simulation of such large systems for the time required to obtain meaningful results is expected to be possible in the very near future.

The next stage for molecular dynamics simulations is the evolution from molecular and supramolecular systems to the cellular level. Studies of the formation of such assemblages will certainly be more demanding. An interesting example of structure formation studied in real time by molecular dynamics concerns the formation of phospholipid bilayers[62,63]. The simulation of more complex cellular activities, such as synaptic transmission[64] and the dismantling of the nuclear membrane on cell division by the cytoplasmic motor protein dynein[65], are two examples of interest. Much of this work will build on the detailed knowledge of the structure and dynamics of channels, enzymes and other cellular components. However, more global

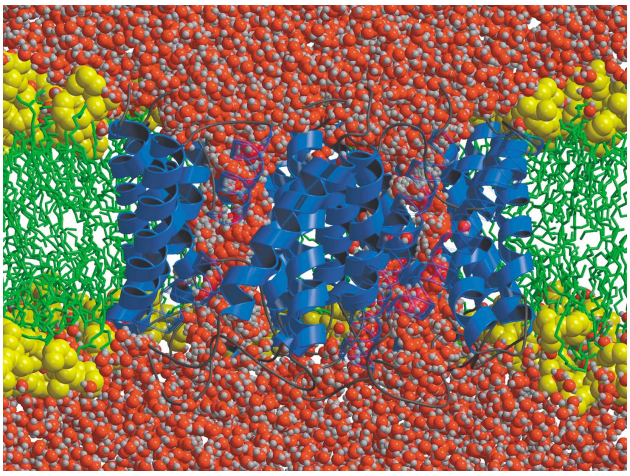

Fig. 4 The permeation of water through a model of aquaporin (blue) in a lipid bilayer membrane (head groups in yellow and hydrocarbon tails in green). The permeation of the water molecules (red and gray) through the aquaporin tetramer during the 10 ns simulation is indicated by an overlay of 100 snapshots (see ref. 59 for details).

simulations are likely to be initiated with less detailed models. A recent example of this type of approach is the use of simplified normal mode calculations for the cowpea chlorotic mottle virus as a way of interpreting low-resolution (28 Å) cryo-electron microscope data, which indicates swelling of this virus at low pH[66]. However, the ultimate descriptions, which will necessarily include such details as the possible effects of mechanical stress in a contracting neuromuscular synapse upon its channels and other components (as one example) will require atomistic simulations.

Given the continuing improvements in molecular dynamics simulations, another development will be their routine use by experimentalists as a tool, like any other, for improving the interpretation and understanding of data. This has, of course, been true for many years as part of high-resolution structure determinations[12,13] and it is now beginning to occur, as illustrated above[47], in the interpretation of the structural results by the scientists who obtained them[47]. We expect that before long such an interplay between experiments and simulations will be an integral part of molecular biology, as it is now in chemistry.

Conclusions

Molecular dynamics simulations of biological macromolecules have provided many insights concerning the internal motions of these systems since the first protein was studied over 25 years ago. With continuing advances in the methodology and the speed of computers, which is still doubling every eighteen months or less (according to Moore's law), molecular dynamics studies are being extended to larger systems, greater conformational changes and longer time scales. This makes possible the investigation of motions that have particular functional implications and to obtain information that is not accessible from experiment. The results available today make clear that the applications of molecular dynamics will play an even more important role for our understanding of biology in the future.

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Competing interests statement

The authors declare that they have no competing financial interests.

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1. McCammon, J.A., Gelin, B.R. & Karplus, M. Dynamics of folded proteins. *Nature* **267**, 585–590 (1977).
2. Phillips, D.C. in *Biomolecular Stereodynamics II* (ed. Sarma, R.H.) 497 (Adenine Press, Guilderland, New York; 1981).
3. Linderstrom-Lang, K. Deuterium exchange between peptides and water. *Chem. Soc. (London)* **2**, 1–20 (1955).
4. Feynman, R.P. Leighton, R.B. & Sands, M. in *The Feynman Lectures in Physics* Vol. I, 3–6 (Addison-Wesley, Reading; 1963).
5. Frauenfelder, H., Petsko, G.A. & Tsernoglou, D. Temperature-dependent X-ray diffraction as a probe of protein structural dynamics. *Nature* **280**, 558–563 (1979).
6. Artymiuk, P.J. *et al.* Crystallographic studies of the dynamic properties of lysozyme. *Nature* **280**, 563–568 (1979).
7. Ichiye, T. & Karplus, M. Fluorescence depolarization of tryptophan residues in proteins: a molecular dynamics study. *Biochemistry* **22**, 2884–2893 (1983).
8. Dobson, C.M. & Karplus, M. Internal motion of proteins: Nuclear magnetic resonance measurements and dynamic simulations. *Methods Enzymol.* **131**, 362–389 (1986).
9. Smith, J., Cusack, S., Pezzeca, U., Brooks, B.R. & Karplus, M. Inelastic neutron scattering analysis of low frequency motion in proteins: A normal mode study of the bovine pancreatic trypsin inhibitor. *J. Chem. Phys.* **85**, 3636–3654 (1986).
10. Brünger, A.T., Brooks, C.L. III, & Karplus, M. Active site dynamics of ribonuclease. *Proc. Natl. Acad. Sci. USA* **82**, 8458–8462 (1985).
11. Frauenfelder, H. *et al.* Thermal expansion of a protein. *Biochemistry* **26**, 254–261 (1987).
12. Brünger, A.T., Kuriyan, J. & Karplus, M. Crystallographic R factor refinement by molecular dynamics. *Science* **235**, 458–460 (1987).
13. Nilsson, L., Clore, G.M., Gronenborn, A.M., Brünger, A.T. & Karplus, M. Structure refinement of oligonucleotides by molecular dynamics with nuclear Overhauser effect interproton distance restraints: application to 5′ d(C-G-T-A-C-G)$_2$. *J. Mol. Biol.* **188**, 455–475 (1986).
14. Colonna-Cesari, F. *et al.* Interdomain motion in liver alcohol dehydrogenase: structural and energetic analysis of the hinge bending mode. *J. Biol. Chem.* **261**, 15273–15280 (1986).
15. Harvey, S.C., Prabhakaran, M., Mao, B. & McCammon, J.A. Phenylalanine transfer RNA: molecular dynamics simulation. *Science* **223**, 1189–1191 (1984).
16. Case, D.A. & Karplus, M. Dynamics of ligand binding to heme proteins. *J. Mol. Biol.* **132**, 343–368 (1979).
17. Brooks, B.R. & Karplus, M. Harmonic dynamics of proteins: normal modes and fluctuations in bovine pancreatic trypsin inhibitor. *Proc. Natl. Acad. Sci. USA* **80**, 6571–6575 (1983).
18. Irikura, K.K., Tidor, B., Brooks, B.R. & Karplus, M. Transition from B to Z DNA: contribution of internal fluctuations to the configurational entropy difference. *Science* **229**, 571–572 (1985).
19. Simonson, T., Archontis, G. & Karplus, M. Free energy simulations come of age: protein-ligand recognition. *Accts. Chem. Res.* **35**, 430–437 (2002).
20. Brooks, B.R. *et al.* CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **4**, 187–217 (1983).
21. Weiner, P.W. & Kollman, P.A. AMBER: assisted model building with energy refinement. A general program for modelling molecules and their interactions. *J. Comput. Chem.* **2**, 287–303 (1981).
22. Scott, W.R.P. *et al.* The GROMOS biomolecular simulation program package. *J. Phys. Chem. A* **103**, 3596–3607 (1999).
23. Tuckerman, M.E. & Martyna, G.J. Understanding modern molecular dynamics: techniques and applications. *J. Phys. Chem.* **104**, 159–178 (2000).
24. van Gunsteren, W.F., Weiner, P.K. & Wilkinson, A.J. Computational Simulation of Biomolecular Systems: Theoretical and Experimental Applications Vol. 2 (ESCOM, Leiden; 1993).
25. Becker, O.M., MacKerell, A.D. Jr, Roux, B. & Watanabe, M. *Computational Biochemistry and Biophysics* (Marcel Dekker, New York; 2001).
26. Berendensen, N.J.C. & Hayword, S. Collective protein dynamics in relation to function. *Curr. Opin. Struct. Biol.* **10**, 165–169 (2000).
27. Hayward, S., Kitao, A. & Gō, N. Harmonicity and anharmonicity in protein dynamics. *Proteins Struct. Funct. Genet.* **23**, 177–186 (1995).
28. Zaloj, V. & Elber, R. Parallel computations of molecular dynamics trajectories using the stochastic path approach. *Comput. Phys. Commun.* **128**, 118–127 (2000).
29. Lamb, M.L. & Jorgenson, W. Computational approaches to molecular recognition. *Curr. Opin. Struct. Biol.* **1**, 449–457 (1997).
30. Kollman, P. Free energy calculations: Application to chemical and biological phenomena. *Chem. Rev.* **93**, 2395–2421 (1993).
31. van Gunsteren, W.F. & Mark, A.E. Validation of molecular dynamics simulations. *J. Chem. Phys.* **108**, 6109–6116 (1998).
32. Gao, J. & Truhlar, D.G. Quantum mechanical methods for enzyme kinetics. *Annu. Rev. Phys. Chem.* **53**, 467–505 (2002).
33. Levitt, M. Computer simulations of the DNA double helix dynamics. *Cold Spring Harb. Symp. Quant. Biol.* **47**, 231–252 (1983).
34. Wrabl, J.O., Shortle, D. & Woolf, T.B. Correlation between changes in nuclear magnetic resonance order parameters and conformational entropy: molecular dynamics simulations of native and denatured *Staphylococcal* nuclease. *Proteins Struct. Funct. Genet.* **38**, 123–133 (2000).
35. Lee, A.L. & Wand, A.J. Microscopic origins of entropy, heat capacity and the glass transition in proteins. *Nature* **411**, 501–504 (2001).
36. McCammon, J.A. & Harvey, S. *Dynamics of Proteins and Nucleic Acids* (Cambridge University Press, Cambridge, UK; 1987).
37. Brooks, C.L. III, Karplus, M. & Pettitt, B.M. *Proteins: A Theoretical Perspective of Dynamics, Structure, & Thermodynamics* (John Wiley & Sons, New York; 1988).
38. Karplus, M., Ichiye, I. & Pettitt, B.M. Configurational entropy of native proteins. *Biophys. J.* **52**, 1083–1085 (1987).
39. Fischer, S. & Verma, S. Binding of buried structural waters increases the flexibility of proteins. *Proc. Natl. Acad. Sci.* USA **96**, 9613–9615 (1999).
40. Phelps, D.K. & Post, C.B. A novel basis for capsid stabilization by antiviral compounds. *J. Mol. Biol.* **254**, 544–551 (1995).
41. Vitkup, D., Ringe, D., Petsko, G.A. & Karplus, M. Solvent mobility and the protein 'glass' transition. *Nature Struct.* Biol. **7**, 34–38 (2000).
42. Hagen, S.J., Hofrichter, J. & Eaton, W.A. Protein reaction-kinetics in a room-temperature glass. *Science* **269**, 959–962 (1995).
43. Ellis, R.J. & Hartl, F.U. Principles of protein folding in the cellular environment. *Curr. Opin. Struct. Biol.* **9**, 102–110 (1999).
44. Xu, A., Horwich, A.L. & Sigler, P.B. The crystal structure of the asymmetric GroEL-GroES-(ADP)$_7$ chaperonin complex. *Nature* **388**, 741–750 (1997).
45. Ma, J.P., Sigler, P.B., Xu, Z.H. & Karplus, M. A dynamic model for the allosteric mechanism of GroEL. *J. Mol. Biol.* **302**, 303–313 (2000).
46. Ranson, N.A. *et al.* ATP-bound states of GroEL captured by cryo-electron microscopy. *Cell* **107**, 869–879 (2001).
47. Young, M.A., Gonfloni, S., Superti-Furga, G., Roux, B. & Kuriyan, J. Dynamic coupling between the SH2 and SH3 domains of c-Src and hck underlies their inactivation by C-terminal tyrosine phosphorylation. *Cell* **105**, 115–126 (2001).
48. Tai, K., Shen, T., Borjesson, U., Philippopoulos, M. & McCammon, J.A. Analysis of a ten-nanosecond molecular dynamics simulation of mouse acetylcholinesterase. *Biophys. J.* **81**, 715–724 (2001).
49. Dobson, C.M., Šali, A. & Karplus, M. Protein folding: a perspective from theory and experiment. *Angew. Chem. Int. Ed. Engl.* **37**, 868–893 (1998).
50. Warshel, A. & Parson, W.W. Dynamics of biochemical and biophysical reactions: insight from computer simulations. *Q. Rev. Biophys.* **34**, 563–679 (2001).
51. Gehlen, J.N., Marchi, M. & Chandler, D. Dynamics affecting the primary charge-transfer in photosynthesis. *Science* **263**, 499–502 (1994).
52. Balabin, I.A. & Onuchi, J.N. Dynamically controlled protein tunneling paths in photosynthetic reaction centers. *Science* **290**, 114–117 (2000).
53. Ben-Nun, M. *et al.* Quantum dynamics of the femtosecond photoisomerization of retinal in bacteriorhodopsin. *Faraday Discuss.* **110**, 447–462 (1998).
54. Weber, W., Helms, V., McCammon, J.A. & Langhoff, P.W. Shedding light on the dark and weakly fluorescent states of green fluorescent proteins. *Proc. Natl. Acad. Sci. USA* **96**, 6177–6182 (1999).
55. Zhou, Y. & Karplus, M. Interpreting the folding kinetics of helical proteins. *Nature* **401**, 400–403 (1999).
56. Cavalli, A., Ferrara, P. & Caflisch, A. Weak temperature dependence of the free energy surface and folding pathways of structured peptides. *Proteins Struct. Funct. Genet.* **47**, 305–314 (2002).
57. Polanyi, J. & Zewail, A.H. Direct observation of the transition-state. *Accts. Chem. Res.* **28**, 119–132 (1995).
58. Lim, M., Jackson, T.A. & Anfinrud, P.A. Binding of CO to myoglobin from a haem pocket docking site to form nearly linear Fe-C-O. *Science* **269**, 962–966 (1995).
59. de Groot, B.L. & Grubmüller, H. Water permeation across biological membranes: Mechanism and dynamics of aquaporin-1 and GlpF. *Science* **294**, 2353–2357 (2001).
60. Ma, J. *et al.* A dynamic analysis of the rotation mechanism for conformational change in F1-ATPase. *Structure* **10**, 921–931 (2002).
61. Bückmann, R.A. & Grubmüller, H. Nanosecond molecular dynamics simulation primary mechanical energy transfer steps in F1-ATP synthase. *Nature Struct. Biol.* **9**, 196–202 (2002).
62. Marrink, S.J., Tieleman, D.P. & Mark, A.E. Molecular dynamics simulation of the kinetics of spontaneous micelle formation. *J. Phys. Chem. B* **104**, 12165–12173 (2000).
63. Bogusz, S., Venable, R.M. & Pastor, R.W. Molecular dynamics simulations of octylglucoside micelles: dynamic properties. *J. Phys. Chem. B* **10**5, 8312–8321 (2001).
64. Cowan, W.M., Sudhof, T.C. & Stevens, C.F. Synapses (Johns Hopkins University Press, Baltimore; 2000).
65. Lippincott-Schwartz, J. Ripping up the nuclear envelope. *Nature* **416**, 31–32 (2002).
66. Tama, F. & Brooks, C.L. III The mechanism and pathway of pH induced swelling in cowpea chlorotic mottle virus. *J. Mol. Biol.* **318**, 730–747 (2002).