

Molecular dynamics simulations

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Molecular dynamics simulations have become a standard tool for the investigation of biomolecules. Simulations are performed of ever bigger systems using more realistic boundary conditions and better sampling due to longer sampling times. Recently, realistic simulations of systems as complex as transmembrane channels have become feasible. Simulations aid our understanding of biochemical processes and give a dynamic dimension to structural data; for example, the transformation of harmless prion protein into the disease-causing agent has been modeled.

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Current Opinion in Structural Biology 2002, 12:190–196

0959-440X/02/\$ – see front matter

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Abbreviations

BPTI	bovine pancreatic trypsin inhibitor
EPR	electron paramagnetic resonance
FEP	free energy perturbation
LIE	linear interaction energy
MD	molecular dynamics
PBC	periodic boundary conditions
PME	particle mesh Ewald
rms	root mean square
TI	thermodynamic integration

Introduction

It is now 25 years since the report of the first molecular dynamics (MD) simulations of a protein, BPTI [1]. During this time, dynamics simulations have become an established tool in the study of biomolecules, complementary to experimental techniques.

Biomolecular dynamics simulations find three major areas of application today. Firstly, MD simulation is used to bring biomolecular structures alive, giving insights into the natural dynamics on different timescales of biomolecules in solution. Secondly, MD simulation affords thermal averages of molecular properties. According to the ergodic hypothesis, one can simulate a single molecule with its surroundings for a period of time and get time-averaged molecular properties that approach the experimentally measurable ensemble averages. This is used to calculate, for example, the bulk properties of fluids and the free energy differences for chemical processes such as ligand binding. Thirdly, MD can explore which conformations of a molecule or a complex are thermally accessible. This technique is used for exploring conformational space, for instance, in ligand-docking applications. Furthermore, if data from experiments are translated into restraining potentials that guide the dynamics calculations, MD can conveniently combine these experimental data with the

knowledge about the general properties of molecular structure that is embodied in the hundreds of parameters of a molecular mechanics force field. It is no news to readers of this journal that, today, virtually all structures of biomolecules obtained by X-ray crystallography or NMR spectroscopy are MD refined.

MD is not a mere historical phenomenon, but a rapidly developing field of science. In this article, we review the recent literature in the field of classical biomolecular dynamics simulations, discussing some of the new developments and methodological progress, as well as highlighting a few of the more exciting applications. For all kinds of simulation, a major question concerns the level of detail employed in modeling the system. We have chosen, due to space limitations, not to discuss the methods that take into account degrees of freedom of a quantum mechanical nature, but instead to focus on classical MD. We also exclude from this review the recent exciting developments in the application of MD to the protein folding problem, as a special contribution on folding by Gruebele is found elsewhere in this section (pp 161–168).

Bigger, better, faster

Like all computational branches of science, the MD field benefits from the seemingly never-ending improvements in computer hardware; simulations that were hard work for yesterday's supercomputers can be carried out today using standard office workstations.

This progression also allows improvements in the very basic parts of simulation techniques. The parametrization of present force fields was done using yesterday's methods in terms of system sizes, boundary conditions and the treatment of longer range electrostatic interactions. Today, we can afford much longer equilibration and sampling times — a microsecond protein simulation has been described [2]. We can thus optimize the force field to reproduce more intricate properties based on simulation averages, such as free energies of solvation, area per lipid head group or order parameters.

This is illustrated by three recent publications [3–5], in which the authors notice that the parameters describing alkanes are inadequate for reproducing the properties of lipid bilayer membranes in the long simulations of large systems that are possible today. This led to the derivation of better parameters for alkanes within the OPLS, CHARMM and GROMOS96 force fields, respectively.

The properties of established models may change when new boundary conditions are applied. This calls for the revisit of previously described problems. Mark and Nilsson [6] tested a series of models for liquid water

under their current set of boundary conditions. Similarly, Dixit and Chipot [7] return to the scene of the biotin–streptavidin system, as studied by Miyamoto and Kollman [8] in 1993, and get rather similar results with much longer simulations.

One problem that must still be said to be unsolved is the addition of polarization effects to the force fields. After adding polarization degrees of freedom to the problem, one needs to parametrize the model on much larger sets of experimental data. So far, polarizable models concentrate on neat liquids, for example, water [9,10].

One way of making even longer and bigger simulations possible is the use of specialized hardware. The Japanese MD engine project group [11] has built a machine that is specially designed for computing nonbonded interactions — the notorious bottleneck in MD simulations. Simulations have already been reported that use the capabilities of this hardware (e.g. [12]). On an unprecedented scale is the IBM Blue Gene Project, not yet finished, for which the aim is to build a petaflop computer for MD simulation applications [13].

On the border

The first protein simulation [1] 25 years ago involved a protein molecule in a vacuum. Here, clear progress has been made in the form of inclusion of a realistic description of the surrounding solvent. Four features of this development are the inclusion of explicit solvent molecules around the protein, the addition of counterions, a more realistic treatment of the system boundaries and a more accurate treatment of long-range electrostatic forces. Each of these points offers several alternative implementation options and it is still somewhat a matter of taste which options are chosen, but some common choices are emerging.

Many people, including the authors of this review, agree that periodic boundary conditions (PBC) elegantly ensure that the system does not have an abrupt border with a vacuum. The competing method is to enclose the system of interest in a sphere, with restraints and often stochastic forces acting at the boundary to mimic an extended system. Obviously, with a small sphere cutting into the protein, large-scale dynamics cannot be observed — with the domains partly constrained, there are no domain motions. Tarek *et al.* [14] report that neutron scattering data cannot be reproduced using stochastic boundary conditions, whereas PBC afford results in agreement with scattering experiments. On the other hand, PBC introduce an artificial periodicity into the system; especially in combination with Ewald summation methods, which include this periodicity in the long-range electrostatic interactions, artifacts are possible. For example, Weber *et al.* [15•] find that a peptide in a smaller periodic box seems to be a stable α helix, whereas the same peptide unfolds when the box size is larger and the periodicity artifacts are consequently smaller.

In the light of these findings, it is interesting to note that Zuegg and Gready [16] found a stabilization of the human prion protein in a short (<1 ns) particle mesh Ewald (PME) simulation, as compared to an unstable 0.8 nm cutoff simulation. In contrast, Alonso *et al.* [17••] use a longer cutoff of 1.0 nm and observe a stable structure for the Syrian hamster prion protein over 10 ns at neutral pH. In a 10 ns simulation at low pH, the expected loss of α helix and gain of β structure is observed, which is thought to constitute early steps in the biologically relevant transformation of the prion protein from the harmless PrP^C form to the disease-associated PrP^{Sc} form.

The current trend is to take long-range electrostatic interactions explicitly into account, which can be done in an exact but periodical manner using particle-particle particle-mesh (P3M)/PME/Ewald summation, as mentioned above. An alternative, and in our experience surprisingly accurate, method is the reaction field approach, in which the pair-wise electrostatic interactions are corrected for the effect of the polarizable surroundings outside a cutoff radius [18,19]. Different groups have studied the influence of the reaction field method on protein dynamics [20,21]. Recently, Rozanska and Chipot [22] investigated the effect of long-range electrostatics methods on an umbrella sampling/potential of mean force calculation. They found that a reaction field approach is sufficiently accurate in this context, but argue that, in many cases, an Ewald summation may be more computationally efficient compared to a larger cutoff radius. In this context, Norberg and Nilsson [23] surprisingly find that a plain cutoff scheme can be sufficient to preserve a DNA structure as well as the PME approach, provided that the cutoff is atom-based rather than group-based.

The simulation temperature can be regulated in several ways, for instance, with a simple Berendsen scaling of velocities [24]. The use of the Nosé–Hoover approach [25,26] is sometimes recommended because it is said to yield a perfect canonical distribution. Tuckerman *et al.* [27••], in a paper describing a rigorous treatment of non-Hamiltonian dynamics, have explained how the inner derivative arising from applying the chain rule for differentiation — the phase space compressibility — has been neglected in the original Nosé–Hoover argument.

One boundary condition that is sometimes taken for granted is the simulation pressure. Marchi and Akasaka [28] find that hydrogen-bonding patterns change drastically with pressure in simulations of BPTI. Interestingly, the choice between the CHARMM or Amber force fields makes a very large difference with respect to pressure response. The importance of pressure is highlighted by two recent papers, in which methane model atoms are simulated in water solution [29•,30•]. It is shown that, at normal pressure, the methanes aggregate, but when the pressure is increased, the hydrophobic effect seems to become weaker and the aggregates dissolve. This could model

pressure denaturation of proteins and also serves as a warning against NVT ensemble (constant number of particles, volume and temperature) calculations, in which the pressure in an even slightly too densely packed simulation box can reach thousands of atmospheres.

With regard to the starting structure, Lee and Kollman [31•] give fuel to an old rivalry by stating that they consistently find that crystallographically determined structures are more accurate than those determined by NMR.

Never enough time

Because of the simulation timescales available with current technology, many biomolecular properties can be computed that were not within reach a few years ago. For instance, data from NMR experiments are, in reality, averages over all of the molecules in the measurement vessel and over a given measurement time window. In order to reproduce such averages, simulations have to be long enough to sample the relevant conformations of the system in an adequate fashion. In our laboratory, Daura *et al.* [32] found striking similarities between computed J-coupling constants and nuclear Overhauser effect (NOE) distances when evaluated as MD averages of β -heptapeptide conformations over a 50 ns dynamics simulation. It is notable that many unfolded conformations contribute to this simulation average and that these properties deviate markedly from experimental values if computed from single snapshots of the simulation. By calculating the exact spectral densities for the interproton vectors and the full relaxation matrix, we have been able to compute entire ROESY (rotating Overhauser effect spectroscopy) spectra of peptides in solution from dynamics simulations [33].

Sometimes properties converge very rapidly. For example, Kolmodin and Åqvist [34] describe a rapid backbone flip away from the X-ray structure of apo-Cdc25 when a sulfate ion is added to model a phosphorylated serine or threonine substrate. Here, the property in question — structural deviation from the typical tyrosine phosphatase binding loop — converges to its final small value within the first 2 ps of the simulation.

One can also use enhanced sampling methods, such as umbrella sampling [35] or the technique known as either local elevation [36] or conformational flooding [37]. Another example is self-guided MD, which adds a guiding force (calculated from the trajectory so far) to each atom to enhance the systematic motions in the system. This method was applied to the dynamics of a pentapeptide [38] and within 2 ns reproduced the reversible folding of the peptide in accordance with experimental results. Conventional 2 ns simulations from both the extended and the folded conformations failed to achieve folding or unfolding.

Steinhoff *et al.* [39] present the first combination of MD simulations and electron paramagnetic resonance (EPR) spectroscopy. Using a combination of unusual restraints

and boundary conditions, simulations of 2 ns enabled them to calculate EPR spectra for nitroxide sidechains attached to cysteine substitution mutants of bacteriorhodopsin and find excellent correlation with experimental spectra.

However, there are still properties that turn out to require even longer timescales. One example is the calculation of the dielectric constant of a protein solution [40]. It was shown that a 5 ns simulation of ubiquitin is not sufficient to sample all of the protein orientations, with respect to the box, that are needed to calculate the protein–water cross-correlation contribution.

Another example involves pK calculations of residues in bacterial xylanases [41]. It shows that, even though the backbone atom positional rms difference between the monomers is only 0.029 nm, the calculated pK values of corresponding residues differ by up to six pK units.

In simulations carried out by Takaoka *et al.* [42], very long simulation times were required to equilibrate a crystal-structure-based membrane simulation of an L- α -dimyristoyl-phosphatidylcholine bilayer. After a first high temperature pulse, 4.7 ns were required to equilibrate the lateral exchange of nearest lipids in the membrane.

As a final example, it was recently demonstrated that simulations of relatively large proteins that are being analyzed using principal component methods are sometimes too short. Very often, the first principal components resemble half, full or one and a half cosines. Hess [43••] showed that the principal components of random diffusion display exactly this behavior, concluding that the systems do not repeatedly cross barriers on the free energy landscape, but rather exhibit random diffusion.

Computation of free energies

Reinhardt *et al.* [44] suggest a new way of calculating free energies for alchemical transformations based on non-equilibrium thermodynamics theory. They start from the Clausius inequality [45], which states that the integral of the heat flow during an irreversible process places a lower bound on the entropy change for that process. By simulating both the forward and the reverse process, upper and lower bounds to the free energy can be obtained.

Using ordinary free energy perturbation (FEP) calculations, Lu and Kofke [46] claim that, rather than taking the average value of forward and backward simulations as an estimate of the free energy change, one should select the direction of the process that increases the entropy of the system. In an additional paper [47], they introduce a special measure to determine if a FEP calculation is to be trusted or if it should rather be carried out in the opposite direction.

Free energies for different compounds can be calculated from a simulation of a common unphysical reference state by taking a single perturbation step. The free energies of

nine compounds binding to the estrogen receptor were calculated using this approach, with good agreement to experimental data [48].

The complicated nature and relatively slow convergence of the typical alchemic transformations associated with FEP and thermodynamic integration (TI) calculations make them unsuitable for the calculation of differences in the free energy of interaction between dissimilar molecules. This has prompted the development of several MD-based schemes whereby the trajectories are analyzed on the basis of assumptions about the response of the environment to the molecules. The MM/PBSA method analyzes frames from the trajectory with Poisson–Boltzmann electrostatic calculations and surface area analysis, and seems to be successful in many applications. A recent example is the analysis of the binding free energies of HIV protease dimers [49], for which experimental ranking was reproduced and alternative mutations suggested to enhance dimer binding. In the linear interaction energy (LIE) method, MD simulation averages of the electrostatic and Lennard–Jones interaction energies in the bound and free states are calculated and multiplied by empirically determined factors to give a binding energy estimate. Recently, the interaction free energy between trypsin and 13 BPTI mutants was well reproduced [50] using this method.

Empirical scoring functions of weaker theoretical foundation can also be used for this purpose. Marelius *et al.* [51] investigated the sensitivity of such a scoring function to conformational variations generated by MD simulation. They show that good scoring functions are relatively insensitive to the conformation, but argue that such a function is correspondingly less useful for docking purposes, because the energy minimum basin is more shallow and less well defined.

Drug design and docking applications

It has been known for years, and was restated recently [52], that free energy calculations from MD simulations can be a powerful tool in the process of computer-aided drug design. Lee and Kollman [53] propose a new compound as a promising inhibitor of thymidylate synthase from TI calculations and a pictorial representation of the free energy changes. Using the LIE approach, Graffner-Nordberg *et al.* [54] examined the selectivity of three compounds that bind to human and *Pneumocystis carinii* dihydrofolate reductase. MM/PBSA calculations were performed on a series of derivatives of TIBO (a substituted tetrahydroimidazole benzodiazepine thione) as inhibitors of HIV-1 reverse transcriptase [55]. In the same study, the binding mode of a known drug was predicted with excellent agreement to the X-ray structure, which was determined later. Other examples of studies into the dynamics of active sites include the work by Okimoto *et al.* [12], who examined the role of water molecules in HIV-1 protease–substrate complexes, and the work done by Pak *et al.* [56], who smoothed the energy landscape using a method called

q-jumping in order to investigate the binding modes of ligands to protein kinase C.

It is notable that many of the most interesting applications of MD and related techniques, especially in the context of drug design, are still being performed without a more advanced treatment of long-range interactions and often with rather small spherical stochastic boundary conditions [50,52,55]. Apparently, the longer sampling times available with simpler methods are regarded as worth the lower accuracy of the model. To alleviate this type of error, Simonson [57] presents a consistent way of correcting an alchemic free energy calculation for the electrostatics outside a sphere with a continuum electrostatic model.

Membranes and channels

With increasing computational power, larger and more complex systems have become accessible to MD simulations. Marrink *et al.* [58] studied the spontaneous formation of dodecylphosphocholine surfactant micelles during simulations up to 50 ns. Their impressive systems contain up to 54 surfactant molecules solvated in more than 20 000 water molecules. The next step towards the simulation of membrane-bound proteins is described by Tieleman and Sansom [59], who discuss different aspects of simulating a peptide in a lipid bilayer. Gao and Wong [60] show that an adrenocorticotropin peptide in a solvated micelle moves to a position parallel to the micellar surface, regardless of whether it starts inside the micelle or at the surface. Membrane simulations can model yet another aspect of natural membranes by the inclusion of sterol molecules, as was done by Smondyrev and Berkowitz [61] using a dimyristoylphosphatidylcholine bilayer containing cholesterol, ergosterol or lanosterol.

The availability of X-ray structures of membrane-bound proteins opens the way to MD simulations of such systems as well. For instance, three monomeric bacteriorhodopsin molecules, 28 lipid molecules and about 2800 water molecules under PBC were simulated for 1 ns to predict the movement of water molecules during the photocycle of the protein [62]. Structural and dynamic properties of the potassium channel were reported by Allen *et al.* [63], who mimicked the lipid bilayer using constraints in the outer region of the protein. The same channel was studied by Åqvist and Luzhkov [64], in a sphere of 3.3 nm containing water molecules and hydrocarbon-like atoms representing the membrane. They calculated free energy differences between different possible ion configurations within the channel to elucidate the ion transport mechanism. A complete explicit treatment of the same channel inserted in a phospholipid bilayer in aqueous solution, using PME for long-range electrostatic interactions, was reported by Bernèche and Roux ([65]; see also Update).

Another explicit treatment was performed by Elmore and Dougherty [66], who did three simulations (>1 ns) of the *Mycobacterium tuberculosis* homolog of the bacterial

mechanosensitive channel of large conductance. Their very large systems consisted of a 495-residue protein immersed in 290 palmitoyl-oleoyl-phosphatidylethanolamine molecules and 17 851 water molecules, leading to a total of 73 313 atoms.

Conclusions

Driven by improvement in simulation methodology, increasing accuracy of biomolecular force fields and the ever-increasing power of computers, MD simulation is rapidly progressing. Since the first simulation of a protein 25 years ago, which followed one small protein in a vacuum for 8.8 ps, we have progressed towards an ever more realistic representation of the solvent and surroundings of biomolecules, and towards larger systems and longer equilibration times. We can now parametrize our models using hundreds of nanoseconds of sampling time, allowing accurate tuning to reproduce average equilibrium properties, such as free energies. We can study complicated systems, such as fully solvated membrane protein complexes. And we can apply MD to attacking the protein folding problem, to explaining spectroscopic data and to the design of novel ligands.

Update

Simulations of a very large system, consisting of the tetrameric human water channel aquaporin-1 and 271 phospholipid molecules fully solvated in water, with a total system size of 87 644 atoms, as well as simulations of a similar 101 448 atom system involving the related bacterial glycerol facilitator, have been reported [67]. Multiple events of diffusional permeation of water molecules through the pores were observed on a simulation timescale (10 ns).

Bernèche and Roux [68*] expand their studies of the KcsA potassium channel [65] with a set of rigorous potential of mean force calculations, arriving at conclusions similar to those of Åqvist and Lushkov [64*].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. McCammon JA, Gelin BR, Karplus M: Dynamics of folded proteins. *Nature* 1977, **267**:585-590.
2. Duan Y, Kollman PA: Pathways to a protein folding intermediate observed in a 1-microsecond simulation in aqueous solution. *Science* 1998, **282**:740-744.
3. Shinoda W, Okazaki S: Molecular dynamics study of the dipalmitoyl phosphatidylcholine bilayer in the liquid crystal phase: an effect of the potential force fields on the membrane structure. *J Mol Liq* 2001, **90**:95-103.
4. Feller SE, MacKerell AD Jr: An improved empirical potential energy function for molecular simulations of phospholipids. *J Phys Chem B* 2000, **104**:7510-7515.
5. Schuler LD, Daura X, van Gunsteren WF: An improved GROMOS96 force field for aliphatic hydrocarbons in the condensed phase. *J Comput Chem* 2001, **22**:1205-1218.
6. Mark P, Nilsson L: Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K. *J Phys Chem A* 2001, **105**:9954-9960.
7. Dixit SB, Chipot C: Can absolute free energies of association be estimated from molecular mechanical simulations? The biotin-streptavidin system revisited. *J Phys Chem A* 2001, **105**:9795-9799.
8. Miyamoto S, Kollman PA: Absolute and relative binding free energy calculations of the interaction of biotin and its analogs with streptavidin using molecular dynamics/free energy perturbation approaches. *Proteins* 1993, **16**:226-245.
9. van Maaren PJ, van der Spoel D: Molecular dynamics simulations of water with novel shell-model potentials. *J Phys Chem B* 2001, **105**:2618-2626.
10. Guillot B, Guissani Y: How to build a better pair potential for water. *J Chem Phys* 2001, **114**:6720-6733.
11. Toyoda S, Miyagawa H, Kitamura K, Amisaki T, Hashimoto E, Ikeda H, Kusumi A, Miyakawa N: Development of MD engine: high-speed accelerator with parallel processor design for molecular dynamics simulations. *J Comput Chem* 1999, **20**:185-199.
12. Okimoto N, Tsukui T, Kitayama K, Hata M, Hoshino T, Tsuda M: Molecular dynamics study of HIV-1 protease-substrate complex: roles of the water molecules at the loop structures of the active site. *J Am Chem Soc* 2000, **122**:5613-5622.
13. Butler D: IBM promises scientists 500-fold leap in supercomputing power and a chance to tackle protein structure. *Nature* 1999, **402**:705-706.
14. Tarek M, Martyna GJ, Tobias DJ: Amplitudes and frequencies of protein dynamics: analysis of discrepancies between neutron scattering and molecular dynamics simulations. *J Am Chem Soc* 2000, **122**:10450-10451.
15. Weber W, Hünenberger PH, McCammon JA: Molecular dynamics simulations of a polyalanine octapeptide under Ewald boundary conditions: influence of artificial periodicity on peptide conformation. *J Phys Chem B* 2000, **104**:3668-3675.
- Using Ewald summation methods, a peptide is shown to be a stable α helix in a small periodic box but not in larger boxes, thereby demonstrating periodicity artifacts.
16. Zuegg J, Gready JE: Molecular dynamics simulations of human prion protein: importance of correct treatment of electrostatic interactions. *Biochemistry* 1999, **38**:13862-13876.
17. Alonso DOV, DeArmond SJ, Cohen FE, Daggett V: Mapping the early steps in the pH-induced conformational conversion of the prion protein. *Proc Natl Acad Sci USA* 2001, **98**:2985-2989.
- The conformation of the prion protein is seen to transform from the harmless Pr^{PC} form to the disease-associated Pr^{Sc} form at low pH. This sheds light on the pathogenic mechanism of amyloid-deposit-related diseases.
18. Barker JA: Reaction field, screening, and long-range interactions in simulations of ionic and dipolar systems. *Mol Phys* 1994, **83**:1057-1064.
19. Tironi IG, Sperb R, Smith PE, van Gunsteren WF: A generalized reaction field method for molecular dynamics simulations. *J Chem Phys* 1995, **102**:5451-5459.
20. Walser R, Hünenberger PH, van Gunsteren WF: Comparison of different schemes to treat long-range electrostatic interactions in molecular dynamics simulations of a protein crystal. *Proteins* 2001, **44**:509-519.
21. Gargallo R, Oliva B, Querol E, Avilés FX: Effect of the reaction field electrostatic term on the molecular dynamics simulation of the activation domain of procarboxypeptidase B. *Protein Eng* 2000, **13**:21-26.
22. Rozanska X, Chipot C: Modeling ion-ion interactions in proteins: a molecular dynamics free energy calculation of the guanidinium-acetate association. *J Chem Phys* 2000, **112**:9691-9694.
23. Norberg J, Nilsson L: On the truncation of long-range electrostatic interactions in DNA. *Biophys J* 2000, **79**:1537-1553.
24. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR: Molecular dynamics with coupling to an external bath. *J Chem Phys* 1984, **81**:3684-3690.
25. Nosé S: A molecular dynamics method for simulations in the canonical ensemble. *Mol Phys* 1984, **52**:255-268.
26. Hoover WG: Canonical dynamics—equilibrium phase-space distributions. *Phys Rev A* 1985, **31**:1695-1697.

27. Tuckerman ME, Liu Y, Ciccotti G, Martyna GJ: **Non-Hamiltonian molecular dynamics: generalizing Hamiltonian phase space principles to non-Hamiltonian systems.** *J Chem Phys* 2001, **115**:1678-1702.
- In this seminal paper, the Nosé-Hoover approach is shown to be incorrect and a new thermostat is suggested.
28. Marchi M, Akasaka K: **Simulation of hydrated BPTI at high pressure: changes in hydrogen bonding and its relation with NMR experiments.** *J Phys Chem B* 2001, **105**:711-714.
29. Ghosh T, Garcia AE, Garde S: **Molecular dynamics simulations of pressure effects on hydrophobic interactions.** *J Am Chem Soc* 2001, **123**:10997-11003.
- When increasing the pressure on a methane aggregation in water, the hydrophobic effect becomes less pronounced and the aggregates dissolve. This underlines the importance of regulating simulation pressure.
30. Rick SW: **Free energy, entropy and heat capacity of the hydrophobic interaction as a function of pressure.** *J Phys Chem B* 2000, **104**:6884-6888.
- When increasing the pressure on a pair of methane model atoms in water, the potential of mean force for the solvent-separated configuration is stabilized with respect to the contact configuration. This should serve as a warning against the use of NVT simulations.
31. Lee MR, Kollman PA: **Free-energy calculations highlight differences in accuracy between X-ray and NMR structures and add value to protein structure prediction.** *Structure* 2001, **9**:905-916.
- X-ray structures are consistently found to be more accurate than NMR-derived structures.
32. Daura X, Antes I, van Gunsteren WF, Thiel W, Mark AE: **The effect of motional averaging on the calculation of NMR-derived structural properties.** *Proteins* 1999, **36**:542-555.
33. Peter C, Daura X, van Gunsteren WF: **Calculation of NMR-relaxation parameters for flexible molecules from molecular dynamics simulations.** *J Biomol NMR* 2001, **20**:297-310.
34. Kolmodin K, Åqvist J: **Prediction of a ligand-induced conformational change in the catalytic core of Cdc25A.** *FEBS Lett* 2000, **465**:8-11.
35. Torrie GM, Valleau JP: **Nonphysical sampling distributions in Monte Carlo free-energy estimation: umbrella sampling.** *J Comput Phys* 1977, **23**:187-199.
36. Huber T, Torda AE, van Gunsteren WF: **Local elevation: a method for improving the searching properties of molecular dynamics simulation.** *J Comput Aided Mol Design* 1994, **8**:695-708.
37. Schulze BG, Grubmüller H, Evanseck JD: **Functional significance of hierarchical tiers in carbonmonoxy myoglobin: conformational substates and transitions studied by conformational flooding simulations.** *J Am Chem Soc* 2000, **122**:8700-8711.
38. Wu X, Wang S: **Folding studies of a linear pentamer peptide adopting a reverse turn conformation in aqueous solution through molecular dynamics simulation.** *J Phys Chem B* 2000, **104**:8023-8034.
39. Steinhoff HJ, Müller M, Beier C, Pfeiffer M: **Molecular dynamics simulation and EPR spectroscopy of nitroxide side chains in bacteriorhodopsin.** *J Mol Liquids* 2000, **84**:17-27.
40. Boresch S, Höchtel P, Steinhäuser O: **Studying the dielectric properties of a protein solution by computer simulation.** *J Phys Chem B* 2000, **104**:8743-8752.
41. Koumanov A, Karshikoff A, Friis EP, Borchert TV: **Conformational averaging in pK calculations: improvement and limitations in prediction of ionization properties of proteins.** *J Phys Chem B* 2001, **105**:9339-9344.
42. Takaoka Y, Pasenkiewicz-Gierula M, Miyagawa H, Kitamura K, Tamura Y, Kusumi A: **Molecular dynamics generation of nonarbitrary membrane models reveals lipid orientational correlations.** *Biophys J* 2000, **79**:3118-3138.
43. Hess B: **Similarities between principal components of protein dynamics and random diffusion.** *Phys Rev E* 2000, **62**:8438-8448.
- A principal components analysis of a random diffusion process resembles the results of such an analysis of protein dynamics, leading to the conclusion that the observed principal components may be meaningless.
44. Reinhardt WP, Miller MA, Amon LM: **Why is it so difficult to simulate entropies, free energies and their differences?** *Accounts Chem Res* 2001, **34**:607-614.
45. Clausius R: **Über eine veränderte Form des zweiten Hauptsatzes der mechanischen Wärmertheorie.** *Poggendorffs Annalen der Physik und Chemie* 1854, **93**:481-506. [Title translation: On a modified form of the second law of the mechanical theory of heat.]
46. Lu N, Kofke DA: **Accuracy of free-energy perturbation calculations in molecular simulation. I. Modeling.** *J Chem Phys* 2001, **114**:7303-7311.
47. Lu N, Kofke DA: **Accuracy of free-energy perturbation calculations in molecular simulation. II. Heuristics.** *J Chem Phys* 2001, **115**:6866-6875.
48. Oostenbrink BC, Pitera JW, van Lipzig MMH, Meerman JHN, van Gunsteren WF: **Simulations of the estrogen receptor ligand-binding domain: affinity of natural ligands and xenoestrogens.** *J Med Chem* 2000, **43**:4594-4605.
49. Wang W, Kollman PA: **Free energy calculations on dimer stability of the HIV protease using molecular dynamics and a continuum solvent model.** *J Mol Biol* 2000, **303**:567-582.
50. Brandsdal BO, Åqvist J, Smalås AO: **Computational analysis of binding of P1 variants to trypsin.** *Protein Sci* 2001, **10**:1584-1595.
51. Marelus J, Ljungberg KB, Åqvist J: **Sensitivity of an empirical affinity scoring function to changes in receptor-ligand complex conformations.** *Eur J Pharm Sci* 2001, **14**:87-95.
- A simple scoring function for the free energy is applied to a MD trajectory to investigate scoring sensitivity to structural variation.
52. Reddy NR, Erion MD: **Calculation of relative binding free energy differences for fructose 1,6-bisphosphatase inhibitors using the thermodynamic cycle perturbation approach.** *J Am Chem Soc* 2001, **123**:6246-6252.
53. Lee TS, Kollman PA: **Theoretical studies suggest a new antifolate as a more potent inhibitor of thymidylate synthase.** *J Am Chem Soc* 2000, **122**:4385-4393.
54. Graffner-Nordberg M, Kolmodin K, Åqvist J, Queener SF, Hallberg A: **Design, synthesis, computational prediction, and biological evaluation of ester soft drugs as inhibitors of dihydrofolate reductase from *Pneumocystis carinii*.** *J Med Chem* 2001, **44**:2391-2402.
55. Wang J, Morin P, Wang W, Kollman PA: **Use of MM-PBSA in reproducing the binding free energies to HIV-1 RT of TIBO derivatives and predicting the binding mode to HIV-1 RT of Efavirenz by docking and MM-PBSA.** *J Am Chem Soc* 2001, **123**:5221-5230.
- The binding mode of Efavirenz to HIV-1 reverse transcriptase was predicted from free energy calculations and is seen to correspond well to a subsequently derived X-ray structure.
56. Pak Y, Enyedy IJ, Varady J, Kung JW, Lorenzo PS, Blumberg PM, Wang S: **Structural basis of binding of high-affinity ligands to protein kinase C: prediction of the binding modes through a new molecular dynamics method and evaluation by site-directed mutagenesis.** *J Med Chem* 2001, **44**:1690-1701.
57. Simonson T: **Electrostatic free energy calculations for macromolecules: a hybrid molecular dynamics/continuum electrostatics approach.** *J Phys Chem B* 2000, **140**:6509-6513.
58. Marrink SJ, Tieleman DP, Mark AE: **Molecular dynamics simulation of the kinetics of spontaneous micelle formation.** *J Phys Chem B* 2000, **104**:12165-12173.
- Micelles are formed spontaneously from random aggregates in simulations of large systems during long sampling times. This paper gives a first taste of the possibilities that modern methodology offers in understanding complex processes in large systems of biological importance.
59. Tieleman DP, Sansom MSP: **Molecular dynamics simulations of antimicrobial peptides: from membrane binding to trans-membrane channels.** *Int J Quant Chem* 2001, **83**:166-179.
- Different aspects of simulating a peptide in a lipid bilayer are discussed.
60. Gao X, Wong TC: **Molecular dynamics simulation of adrenocorticotropin (1-10) peptide in a solvated dodecylphosphocholine micelle.** *Biopolymers* 2001, **58**:643-659.
61. Smondyrev AM, Berkowitz ML: **Molecular dynamics simulation of the structure of dimyristoylphosphatidylcholine bilayers with cholesterol, ergosterol, and lanosterol.** *Biophys J* 2001, **80**:1649-1658.
62. Baudry J, Tajkhorshid E, Molnar F, Phillips J, Schulten K: **Molecular dynamics study of bacteriorhodopsin and the purple membrane.** *J Phys Chem B* 2001, **105**:905-918.

63. Allen TW, Bliznyk A, Rendell AP, Kuyucak S, Ching SH: **The potassium channel: structure, selectivity and diffusion.** *J Chem Phys* 2000, **112**:8191-8204.
64. Åqvist J, Luzhkov V: **Ion permeation mechanism of the potassium channel.** *Nature* 2000, **404**:881-884.
Free energy differences between different ion configurations in the potassium channel were calculated in order to elucidate the transport mechanism.
65. Bernèche S, Roux B: **Molecular dynamics of the KcsA K⁺ channel in a bilayer membrane.** *Biophys J* 2000, **78**:2900-2917.
66. Elmore DE, Dougherty DA: **Molecular dynamics simulations of wild-type and mutant forms of the *Mycobacterium tuberculosis* MscL channel.** *Biophys J* 2001, **81**:1345-1359.
67. De Groot BL, Grubmüller H: **Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF.** *Science* 2001, **294**:2353-2357.
68. Bernèche S, Roux B: **Energetics of ion conduction through the K⁺ channel.** *Nature* 2001, **414**:73-77.
A complete explicit potential of mean force calculation gives an insight into the ion transport mechanism of the KcsA channel.