Quantitative Computer Simulations of Biomolecules: A Snapshot

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Abstract: A recent workshop titled "Quantitative Computational Biophysics" at Florida State University provided an overview of the state of the art in quantitative modeling of biomolecular systems. The presentations covered a wide range of interrelated topics, including the development and validation of force fields, the modeling of protein–protein interactions, the sampling of conformational space, and the assessment of equilibration and statistical errors. Substantial progress in all these areas was reported.

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Introduction

A workshop on "Quantitative Computational Biophysics" (QCBP2007), held February 17–21 at Florida State University, brought together scientists working in the field of biomolecular simulations and modeling to discuss their most recent research. Talks and posters presented at the workshop detailed substantial progress along different directions, which can be broadly divided into four areas: 1. New force fields and their validation by experiment; 2. Protein–protein interactions and electrostatics; 3. Enhanced sampling techniques; and 4. Statistical convergence. Most speakers reported on their research in one or several of these areas, including work that has not yet been published, which was often followed by extensive discussions. Because of the format of this review we focus on work that has appeared in the literature or is in press.

New Force Fields and Their Validation by Experiment

In a new generation of AMBER and CHARMM molecular mechanics force fields, the polypeptide backbone φ,ψ Ramachandran map was refined to provide an improved representation of the conformational ensemble of folded protein systems.[1–3] In the case of the AMBER99SB force field these modifications were made based on quantum-chemical energy calculations of glycine- and alanine tetrapeptides.[2] To assess the consequences of these force field modifications, comparison with high quality experimental data is essential. For sub-ns time-scale dynamics, NMR spin relaxation parameters are well suited for this task. It is quite common to compare model-free S^2 order parameters[4] of protein backbone N—H bond vectors, reflecting the restriction in angular motion, with those extracted from MD trajectories. For short trajectories in the hundreds of ps using previous force fields, agreement with experiment was often found to be reasonable, but trajectories in the ns range and beyond, made possible by the ever increasing computer power, revealed overestimation of motion, especially for loop regions.[5] The recent modifications have largely overcome this problem, showing much better agreement.[1,2] Because NMR spin relaxation data are sensitive to the internal motional timescales, direct back-calculation of relaxation parameters provides a stringent test of the trajectory and its underlying force field as is demonstrated by a MD simulation of ubiquitin using AMBER99SB in explicit SPC water.[6] Although overall molecular tumbling is still too rapid in the simulation, it

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is found that the internal motional aspects of the correlation functions are reproduced with nearly quantitative agreement.

Recently introduced enhanced sampling methodology in the form of accelerated MD (AMD)[7] has been applied to the calculation of spin-relaxation S^2 N—H order parameters of the immunoglobulin binding domain GB3. The observed good convergence of the computed values toward experimental values[8] reflects the high quality of these new force fields and shows that substantial time savings are possible using enhanced sampling techniques once the acceleration parameters are properly adjusted.

In contrast to NMR spin relaxation data, which are only sensitive to sub-ns motions, NMR residual dipolar couplings (RDCs), which occur when proteins are weakly aligned in the external magnetic field, probe a much wider range of timescales, from ps to ms.[9] Because RDCs can be measured with high accuracy for many spin pairs and in different alignment media and because they simultaneously reflect structure and dynamics, these parameters represent benchmarks that are both rigorous and comprehensive. Agreement is achieved between RDCs calculated from the ensemble of ubiquitin conformations generated using extended MD trajectories with AMBER99SB, mentioned above, and RDCs measured in multiple alignment media that is comparable to or better than for the highest resolution X-ray or NMR structural model.[10] Taken together, the canonical ensemble of ubiquitin based on this latest force field shows a high level of consistency with experimental NMR data reflecting both rapid and slower motional processes.

Although similar comparisons will need to be carried out for other protein systems, the results for GB3 and ubiquitin suggest that the most recent generation of MD force fields has made a formidable stride toward the quantitative structural dynamic description of protein behavior at ambient conditions. As computer power continues to increase, MD ensembles will become available that will probe dynamics on an ever wider range of timescales and thereby will continue to allow stringent assessments of the quality of force fields by comparison with experimental RDCs, which, in turn, may guide future force-field improvements.

While traditional molecular mechanics potentials use fixed atomic charges, new force fields are emerging that include polarizability effects. Of the two major models presently employed in biophysical applications, one is based on the fluctuating charge method[11] and the other on the classical Drude oscillator treatment.[12] The former has been developed to treat not only solvent molecules[13] but also protein systems,[14] while the latter has mostly been used to treat various solvent molecules.[15–17] Significant efforts have been made in the parameterization of polarizable force fields[11,18] to provide more accurate physical descriptions, such as small-molecule liquid-vapor interfaces[19–21] and atomic level anisotropy,[22] so as to better reproduce various molecular properties in the condensed phase.

Advances in computer power and methodology have also led to new trends in quantum mechanical (QM) calculations. First, semi-empirical QM methods have been further improved, such as the PDDG/PM3 method,[23] which improves upon the traditional PM3 method via the employment of a pairwise distance directed Gaussian modification. Recently, this method has been extended to the treatment of various elements[24,25] such as halogen atoms, sulfur, phosphor, and silicon. Because of the fact that the PDDG/PM3 method shows competitive capability against B3LYP/6-31G(d) and overall better performance than SCC-DFTB and AM1 methods in reproducing the experimental enthalpy changes,[26] efficient and reliable studies on various chemical reactions can be achieved.[27–29] The classical specific reaction parameter (SRP) method still shows its usefulness, in particular for chemical reactions, which are challenging to study. For instance, based on the newly developed AM1/d Hamiltonian, the parameterization of hydrogen, oxygen, and phosphor atoms allows the modeling of the phosphorylation reactions to be in agreement with the model chemistry of B3LPY/6-311++G(3df,2p).[30] Besides the improvement in QM methods, a more accurate treatment of the interactions between the QM portion and the classical portion in the hybrid quantum mechanical and molecular mechanical (QM/MM) approach is provided in the QM/MM based generalized solvent boundary potential (GSBP) method,[31] which can more rigorously treat the long-range electrostatic interactions. In terms of property calculations, efficient semi-empirical NMR chemical shift prediction was recently made possible using a divide-and-conquer scheme[32] with application to structural refinement and drug discovery.[33–35]

Protein–Protein Interactions and Electrostatics

Many proteins operate in the context of multi-component complexes whereby both binding affinity and binding rate are likely to play important roles in their proper functioning.[36] Progress in the quantitative understanding of both binding affinity and binding rate were reported at the Workshop.

In principle, the binding constant of two proteins can be calculated from configurational integrals of the proteins in the unbound state and in the bound state.[36,37] While a rigorous implementation of such a formulation poses a significant challenge, considerable insights into binding affinity can be gained from studying effects of pH, ionic strength, and point mutations.[38–40] These effects can be calculated from the electrostatic free energies (solute Coulombic interaction energies plus solvation energies) of the unbound proteins and their complex, based on the Poisson–Boltzmann equation. These calculations often are able to quantitatively rationalize experimental data on binding affinity and can suggest mutations for achieving desired binding properties. An important technical detail is the specification of the boundary between the low protein dielectric and the high solvent dielectric. The solvation energy is apparently very sensitive to the precise specification of the dielectric boundary. In particular, changing the dielectric boundary from the van der Waals surface to the molecular surface typically changes the electrostatic interaction free energy between two oppositely charged proteins from negative to positive.[38,39,41] Comparison against experimental data and against results from MD simulations in explicit solvent will hopefully bring a resolution to this rather fundamental issue in the near future.

An essential step in the formation of a stereospecific complex between two proteins is the diffusional process which brings them together in appropriate relative orientations. Reaching such

a transient complex becomes rate-limiting, typically for proteins with binding rates higher than $\sim 10^5$ M^{-1} s^{-1}. Long-range electrostatic attraction can enhance the binding rate; this rate enhancement, as shown analytically, can be predicted from the electrostatic interaction free energy in the transient complex.[42] Predictions based on this theory are found to agree closely with experimental data in a comprehensive study of protein–protein binding rates.[36]

It should be emphasized that dynamics is a critical element in the complex formation process, with potentially significant effects on both the binding affinity and binding rate. Motions up to tens of ns can now be readily modeled by molecular dynamics simulations to study their specific role in the binding process.[40,43] After reaching the transient complex, conformational rearrangements toward the stereospecific complex appear to occur on these timescales.[43] Such conformational rearrangements are found to be highly cooperative, a behavior that is closely analogous to protein folding.

In fact, binding and folding meet when one or both of the binding partners, originally unfolded, become folded upon binding. Consider the simpler case of one partner staying folded. That partner can be viewed as providing a template which modifies the energy function of the unfolded partner through their interactions.[44] This approach allows for the identification of a dominant pathway, along which folding and binding are coupled that leads from the unfolded, unbound state to the folded, bound state. However, it neglects the fact that overall translational and rotational motions become increasingly restricted as binding progresses[36]; the overall binding equilibrium also requires consideration of the unbound partners that are free to translate and rotate. It has been proposed that coupled folding and binding offer advantages in specificity.[45]

Enhanced Sampling Techniques

Conformational sampling plays an essential role in computational biophysics. Because of quasi-ergodicity problems in the simulations of complex systems, sampling enhancement is often required to acquire information on longer timescale events, including ones that are of biological importance. New developments and applications of generalized ensemble based enhanced sampling techniques are flourishing, as was clearly observed at this Workshop.

The basic idea of the generalized ensemble originated in the umbrella sampling method[46] in the Markov chain Monte Carlo (MCMC) framework. This concept became more practically useful with the introduction of the multicanonical approach,[47] where many orders of magnitudes of efficiency improvements were demonstrated. Interestingly, the efficiency of another early variant of generalized ensemble methods, replica exchange,[48] was realized even later. A unified view of these approaches was recently presented.[49]

In terms of molecular applications, generalized ensemble methods are catalyzing novel solutions to both old and new biophysical problems. Revisiting a classical problem, a MCMC calculation based on multi-canonical ensemble algorithm has accurately resulted in the residual entropy of ordinary ice and can shed light on possible understanding of the ice-like clusters in the protein interiors by the same approach.[50] Replica exchange sampling methods have been implemented in continuous constant pH molecular dynamics (CPHMD) simulations, allowing for reliable reproduction of pKa values of titratable groups in proteins.[51] As noted earlier, application of AMD[7] improves the accuracy of NMR order parameter calculations.[8] As can be anticipated, the novel applications represented by these examples will motivate wider application of generalized ensemble algorithms to deal with other biophysical problems that have been mainly treated by regular canonical simulation methods.

With deeper understanding of the existing generalized ensemble methods, various strategies have been proposed to further optimize sampling efficiency. In particular, with the realization of the fact that diffusion of the highest temperature (or "effective temperature") replica limits the sampling efficiency in replica exchange simulations, various "reservoir" based methods have been proposed to pre-generate the highest temperature samples for the structural exchanges.[52–55] Another way to improve the diffusion speed is to coarse-grain the potential model; motivated by this idea, resolution replica exchange was proposed to facilitate the entropy barrier crossing by performing replica exchanges between the potential models with different resolutions.[52] How to set up the temperature distribution in temperature replica exchange method is a crucial issue. Recently, a method was introduced to optimize temperature distributions for the efficient exploration of complex landscapes by systematically shifting computational resources towards the bottlenecks of a simulation, which are typically in the vicinity of free energy barriers.[56] With the advancement of QM potential based simulation methods, there is an urgent need for the corresponding sampling enhancement methods. The conflict between the required activations (such as increased temperature) for the sampling enhancements and the electronic structural self-consistent-field calculation instability caused by the resulting twisted structures must be reconciled. A hybrid replica exchange method is designed to guarantee robust sampling propagations by combining the regular replica exchange method, and the resolution replica exchange strategy, in which the model resolutions are varied between the MM and QM-based treatments.[57]

Beyond general methods designed solely for the target-state conformational sampling, a few new sampling methods have been proposed to specifically target certain property calculations. For "alchemical" free energy simulations, dual-topology strategies based on potential-scaling sampling methods (Hamiltonian replica exchange[58] and simulated scaling methods[59]) have been developed to synergistically improve the sampling enhancement and the free energy convergence[59,60]; here, the scaling parameter λ plays two roles: improving the phase space overlap and enhancing the conformational sampling. This technique was also extended to QM-based descriptions using the hybrid replica exchange treatment.[57] On the free energy surface (potential of mean force) mapping, the multi-overlap technique was generalized to enforce the transitions between the reference configurations.[61] In contrast to the replica exchange algorithm based on the ordinary Gaussian ensembles,[62] which was formulated in the framework of Monte Carlo simulation with a fixed Gaussian unit

function description, a Wang–Landau metadynamics method[63] was developed using MD to permit both optimal simulation efficiency and the quality of mapped free energy surface by recursively updating the unit Gaussian height. This Wang–Landau metadynamics method was further generalized to realize essential energy space random walk and so robustly permit AMD simulations.[64] Extending equilibrium reweighting schemes to the calculation of dynamical properties, such as time-correlation functions, continues to be a challenging problem. A path-integral based method was developed to recover the dynamic properties of the system from the simulation sped up through potential scaling strategies.[65]

Statistical Convergence

With the improvements in force fields and sampling methods, attention is increasingly turning to the issues of statistical error and convergence. Convergence estimates are critical for quantitative analysis, but convergence is difficult to assess because of the possible *nonobservance* of perfectly viable conformational states. Such states may be unobserved because they have relatively high free energies, or because they are separated by high free energy barriers from other regions so that a given simulation may be simply too short to see transitions into those states. This may introduce significant uncertainty into free energy estimates.

Despite these caveats, it is typically assumed that the simulated data is sufficiently complete to estimate meaningful errors. Starting from this assumption, a number of approaches exist for the quantitative estimation of errors. In particular, if the average value $\bar{f}$ of a quantity f is estimated from a simulation, then the variance of $\bar{f}$ scales with τ_{int}/T,[66,67] where τ_{int} is the auto-correlation time and T is the total simulation time (this is tantamount to the notion that the *effective* number of samples in the simulation is proportional to T/τ_{int}). Estimating τ_{int} reliably is difficult unless $T \geq 100\ \tau_{int}$, although efficient, semi-automated methods do exist for determining τ_{int}, when sufficient data is available. A popular method by Flyvbjerg and Petersen[67,68] uses block averages to estimate the amount of simulation time necessary for var($\bar{f}$) to become block-size independent.

The auto-correlation time for different quantities is not guaranteed to be the same, however, because the relaxation of most quantities is strongly dependent on specific conformational rearrangements. Recent work[52,67] has shown how to measure this type of structural parameter by dividing conformational space into equivalence classes according to their distance from prechosen reference structures and block averaging to determine when the populations of these equivalence classes become decorrelated.

Rigorous approaches to "measuring" equilibration have not been widely applied to computations of biophysical systems, because it has only recently become possible to routinely simulate longer than 10–100 ns or to systematically repeat simulations using different initial conditions. Although modern sampling methods have made these types of calculations possible, they can deceive one into believing that equilibration exists because rapid equilibration is achieved along a single coordinate. For replica exchange MD simulations, for example, if one starts a replica exchange simulation with half of the replicas in the native state and half of the replicas in the unfolded state, then the temperature coordinates will rapidly equilibrate giving *the appearance* of equilibration. Real equilibration, however, requires multiple folding and unfolding events. Recent replica calculations on large peptides and small proteins have demonstrated that even with replica exchange methods, equilibration often requires the simulation of several hundreds of nano-seconds per replica. For example, a simulation of the trpzip peptide in implicit solvent required at least 140 ns per replica,[55] a simulation of the trpcage protein in explicit solvent required at least 100 ns per replica,[69] and a simulation of Chignolin in explicit solvent required more than 500 ns per replica.[70]

Despite these successes, there is clearly much to be gained by applying more rigorous measures of equilibration to these and other kinds of problems, and cross comparisons of existing methods to eventually fulfill the promise of the title of the workshop. These advances in force fields and methods will make quantitative simulations possible for a rapidly increasing number of systems and further establish computational biophysics as an integral tool for the understanding of biomolecular function.

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