for improving sampling in atomistic simulations is umbrella sampling,[111] where bias terms are added to constrain the simulation in some way and their effect is then removed. This procedure can be used to either lower potential energy barriers or to restrain simulations to slow-interconverting configurations that are relevant to the binding affinity (for example, different torsional states), allowing each of the component energies to be properly computed and then combined.[97,112,113] Another application is computing the free energy of constraining the free ligand into the bound conformation directly before computing the free energy of binding and then releasing these restraints, usually decreasing the correlation times for sampling of the intermediate states and thus increasing the efficiency of the simulation.[97,108]

Expanded ensemble, Hamiltonian exchange, and λ dynamics

Alchemical simulations usually include a number of intermediate states. It is possible to bring these intermediates together in a single simulation system, either as series of coupled simulations of the intermediate states, usually called Hamilton exchange, or as a single simulation that visits all of the intermediate states, called expanded ensemble simulation. A number of studies have shown that Hamiltonian exchange and expanded ensembles can speed up simulations by allowing the system to go around barriers by going through alchemical states where those barriers are not as pronounced, significantly speeding up free-energy simulations.[114–120] Alternatively, the alchemical variable λ can be treated as a dynamic variable, which adds complications by introducing a fictitious mass corresponding to the λ degree of freedom but is essentially equivalent to Monte Carlo techniques.[118,121–123] There are a number of variations of sampling in λ that may show promise in the future, but such methods are still in the preliminary stages of development.[124–128]

Multiple ligands simulations

If binding calculations of multiple ligands with a single protein target can be performed in the same simulation, this can significantly speed up the efficiency of calculations. This has been most successfully done by running a single simulation of a nonphysical reference state and then computing the free energy via EXP to a large number of potential ligands.[97,129,130] However, this frequently fails to work when the ligands are too dissimilar.[131] More sophisticated multiligand approaches will most likely be necessary.

RECENT HISTORY IN LIGAND BINDING CALCULATIONS FOR PHARMACEUTICALLY RELEVANT SYSTEMS

MM-PBSA calculations

MM-PBSA has been used in a range of applications for exploring the free energetics of biologically relevant molecules, and reports in the literature appear for a variety of nontrivial, structure-related problems. Although early applications focused on rationalizing relative conformational stabilities in DNA[132] and RNA,[133] it was not long before attempts to analyze protein/ligand interactions began to appear. For instance, MM-PBSA was used to verify a hypothesis that electrostatic interactions were the primary driver for hapten association with Fab fragments of antibody 48G7.[134] It was also used to elucidate situations in which hydrogen bonding was postulated to be an important contributor to protein/ligand association,[135] to gain insights into the role of hydrophobic interactions in cAMP-dependent protein kinase,[47] and to investigate carbohydrate recognition in concanavalin.[37] Other uses of MM-PBSA include rationalizing the role of pK_a shifts in protein/ligand binding,[137] and demonstrating the importance of the choice of proper protonation states in the active site.[138] Structure-based ligand design methods have also been built on top of MM-PBSA. One technique, called computational alanine scanning,[139] probes potential interaction sites in receptor binding pockets, and an analogous method was developed for small molecules called fluorine scanning.[140]

The wide range of applications of MM-PBSA reported in the literature reflect its increasing penetration into the scientific community. Based on results from a search of a life sciences citation database maintained by *Entrez*, the number of publications reporting some use of MM-PBSA to perform binding analysis has steadily increased from fourteen total in the two years from 2001 to 2002 to fifty total in the years 2006–2007. The consistent increase in the use of MM-PBSA is likely due to several factors. MM-PBSA has relatively low computational cost compared to other (more rigorous) binding free-energy methods, which broadens the number of systems to which it can be reasonably applied. Several initial reports on MM-PBSA appearing in the literature showed significant potential for the method. In particular, one of the earliest results demonstrated impressive affinity predictions for the protein target avidin binding a set of biotin analogs.[141] Subsequent reports showed equally promising results for affinity predictions for other systems.[142–146]

Because of the early reports a large number of groups have applied MM-PBSA to a wide variety of systems. We can investigate one aspect of the evolution of the use of MM-PBSA by inspecting the literature reports that have appeared over the years from 2001 to 2007. Shown in Figure 5.2 are values (estimated where possible from the publications found in the aforementioned citation search) for the mean-square error (MSE) in reported affinity predictions, as a function of the publication year.

From 2000 to 2003, MM-PBSA reports contained significantly smaller average MSE values in the literature, compared to averages from the span of 2004 to 2007. There are a number of possible explanations for this trend. Early applications may have been restricted to more well-behaved systems appropriate for initial verification and later studies were more representative of the average over many systems.