criteria used for validity of protein parameters is usually proper structural conformational preferences, which may not be sufficiently accurate for their use in protein/ligand binding calculations. A recent version of the GROMOS force field, 53A6 has parameters that were fit to free-energy calculations of the free energies of transfer between water and cyclohexane, a first for an atomistic force field.[232] It seems likely that parameterizing to phase transfer properties should result in higher accuracy for binding affinity calculations, which are essentially transfers to a heterogeneous liquid phase, though direct comparisons between force fields optimized to pure liquid properties and transfer free energies have yet to be made.

Even more problematic are sufficiently accurate ligand parameters, as the number of functional groups is significantly higher than for protein systems and the amount of time that has been invested for parameterization significantly lower. Typical ligand parameters might be taken from the generalized Amber force field (GAFF),[225] usually using the ANTECHAMBER program in the AMBER distribution for atomtyping, although it is also sometimes done by hand, and the AM1-BCC method[226,227] (implemented in a variety of software codes) to determine the charges. Schrödinger also has automatic tools to assign atom types of novel compounds within the OPLS-AA parameterization system. Relatively few force fields have associated tools or even algorithms for determining compatible (let alone validated) ligand parameters.[228]

A number of research groups are actively developing polarizable potential functions,[228] which have the potential to greatly improve macromolecular force fields' abilities to predict binding affinities by adding an extra level of physical detail. However, at this point, it is not clear that any of them yet are quantitatively better than fixed charge force fields, as few of them have been validated to the extent that fixed charge models have. Polarizable molecular models are significantly slower than fixed charge models, and therefore both the iterative improvement of such force fields and the development of tools useful for production runs for free-energy calculations will be substantially behind that of fixed change force fields.

CONCLUSIONS

As we have seen in this chapter, free-energy calculations are not at the present time generally reliable methods to predict binding affinity and are not currently truly a part of standard structure-based drug design methods. And they certainly are not, and will not anytime in the near future, "black-box" methods that will "automagically" allow determination of free energies without significant investment in the physical chemistry and biology of the system. Although many papers are being published computing binding free energies in retrospective case studies, there is still a lack of comparative studies presenting results over large numbers of systems, with even fewer purely predictive studies. Partly for these reasons, quantitative free-energy calculations are not a vital part of the discovery work flow of most major pharmaceutical company as far as we aware.

However, such calculations are certainly much closer to usability than they have been in the past. In particular, several recent studies mentioned in this chapter have highlighted the advantages of alchemical relative free-energy calculations compared to approximate methods like MM-GBSA and docking.[130,178,229] Alchemical methods have also been successfully applied in a lead-optimization context[161,230] (and see references therein). It appears that in this area free-energy calculations are already becoming useful but with large remaining hurdles to their more widespread adoption. Such hurdles include not only the computer time needed but also the human time and biochemical knowledge required to set up these simulations.[156,176,218,229]

As we have presented in this chapter, the methods used for free-energy calculations are changing rapidly. Major molecular simulation codes, such as AMBER, CHARMM, NAMD, GROMACS, and GROMOS, are undergoing major improvements and changes in the features used to compute binding free energies. Although these changes will likely greatly improve the ability to perform free-energy calculations, ongoing changes make it difficult to put together stable work flows for preparing ligands and simulation structures and determining ideal free-energy protocols without significant human effort, and it is difficult to recommend particular codes for the easiest use at the present time. MCPRO, developed in the Jorgensen lab, which has recently begun to be distributed by Schrödinger as MCPRO+, although not including much of the most recent methodology (such as Bennett Acceptance Ratio methods or soft-core alchemical pathways), is likely the easiest to use and set up and was used successfully for the HIV-1 NNRTI work in the Jorgensen lab described in this chapter.

One ongoing problem has been the lack of extensive experimental high-accuracy ligand binding affinities. As we have discussed, a desirable goal for free-energy calculations is to reach an accuracy threshold of 1 kcal/mol. However, the majority of experimental measurements, unless using highly accurate methods like ITC or SPR or extremely well-tuned competitive binding affinity assays, many not be more accurate than this, making large-scale validation of computational methodologies difficult. A number of academic databases of protein/ligand structures and interactions have been created, such as http://www.bindingmoad.org/ and http://lpdb.chem.lsa.umich.edu/ at the University of Michigan, http://www.bindingdb.org at Johns Hopkins, http://www.agklebe.de/affinity at Phillips-Universität Marburg,[230] and http://www.pdbcal.org at Indiana, but the degree of validation and utility of these databases are not well established.

In the United States, a recently announced NIH NIGMS RFA to establish a national Drug Docking and Screening Data Resource represents an attempt to increase the public availability of high-quality experimental data sets required