experimentally.[113,193–199] Because these binding sites are relatively simple and rigid, and structural data are so easy to obtain, they have been attractive sites for the development and testing of absolute free-energy methods. Most of the work has been on the apolar version of this cavity.

One early absolute free-energy study, in 1997, examined the binding of benzene in the apolar cavity.[200] Depending on the computational details, calculated values range from −4.0 to −5.1 kcal/mol, and the experimental absolute binding free energy is −5.2 kcal/mol. A follow-up study examined binding of noble gases in the cavity, especially xenon, which binds under pressure, in agreement with computed binding free energies.[201]

The lysozyme cavity was also used as a test system for the methodological work of Boresch et al. in 2003, which fell short of actually computing a binding free energy, but laid out a clear and straightforward thermodynamic cycle for computing binding free energies; the cycle involves the use of both orientational restraints and distance restraints.[202] Work on the polar cavity extended some of the arguments in favor of using orientational restraints and found that large kinetic barriers can separate ligand orientations, so considering multiple candidate bound orientations can help when computing absolute binding free energies.[179]

More recent work done by Deng and Roux[203] looked at a series of known binders to the lysozyme cavity, with somewhat mixed success; some binding free energies were too negative by a few kcal/mol. Another study used grand canonical Monte Carlo techniques on the same system, but while neglecting protein flexibility,[204] again with mixed success. A third looked at binding of a single ligand in the lysozyme cavity and quantified the contributions of a slow side-chain motion to ligand binding. It found that a single side-chain rearrangement could affect binding free energies by a few kcal/mol and that including the free energies associated with this conformational change was key for obtaining accurate binding free energies.[205]

The most extensive study to date has been the joint theory-experiment work of Mobley, Graves, and others,[113] which studied a variety of previously measured ligands and reached a root-mean-square error of roughly 1.9 kcal/mol after dealing with problems relating slow sampling of ligand orientational changes, protein conformational changes, and ligand electrostatics parameters. A unique feature of this study was that bound crystal structures were not used as starting points for the calculations, except for comparison purposes. Absolute free-energy methods were then applied successfully to predict binding affinities, with errors less than 0.7 kcal/mol, and orientations of five previously untested small molecules. The contribution of protein flexibility was also assessed and turned out to be key for the accuracy of the results.

Overall, a message from the lysozyme work has been that even simple binding sites can present significant sampling problems for molecular simulations, especially concerning ligand orientations and side-chain degrees of freedom and that a proper accounting of the thermodynamics here is key for obtaining predictive results.

FKBP binding calculations. The FK506 binding protein (FKBP) has been another popular test system for absolute free-energy methods, in part because of the relative rigidity of its backbone. FKBP-12 was important in the development of the immunosuppresive drug cyclosporin and has remained popular because of its role in the development of the field of chemical biology. A series of ligands studied experimentally by Holt et al. have been studied particularly closely by a number of researchers.[206]

Absolute alchemical free-energy methods were first applied to the system by Shirts,[207] who obtained root-mean-square error of 2.0 kcal/mol and a correlation coefficient (R^2) of 0.75. A follow-up study by Fujitani and collaborators[208] achieved a root-mean-square difference from a linear fit of only 0.4 kcal/mol, but with a large offset of −3.2 kcal/mol relative to experiment. As previously noted,[10] care must be taken when comparing this study directly with other absolute free-energy studies, because it neglects any treatment of the standard state, which could be part of the reason for the offset.[179]

A further study by Wang, Deng, and Roux using the same parameters as Shirts obtained a root-mean-square error around 2.0–2.5 kcal/mol,[97] but despite the fact that the results used the same parameters, they were significantly different from those of Shirts,[207] suggesting methodological or convergence differences. Another study by Jayachandran and coworkers obtained a root-mean-square error of 1.6 kcal/mol using a novel parallelized free-energy scheme that allows for contributions of multiple kinetically distinct ligand orientations.[209]

Two other smaller studies also applied absolute free-energy methods to the same system, although not alchemical free-energy methods. The work of Lee and Olson used PMF techniques to compute binding free energies of two inhibitors[106] with accuracies of 1–2 kcal/mol depending on the solvent model, and Ytreberg used nonequilibrium pulling techniques for two inhibitors with accuracies around 1 kcal/mol.[109]

In many cases, computed values have varied substantially, even when using the same parameters. Likely, full convergence has not yet been truly reached. This provides a warning for computations on the many systems that have significantly more conformational flexibility and an indication that higher accuracy with systems that have not been studied as systematically might result from some degree of coincidence.

Other interesting binding calculations. Absolute free-energy methods have also been applied in several other interesting cases where there is less of a body of work. Recently, Jiao et al. calculated the absolute binding free energy of benzamidine binding to trypsin using AMOEBA, a polarizable force field, and then computed the relative binding free energy of a benzamidine derivative[172] with accuracies to within than 0.5 kcal/mol. Due to the small size