recent review of absolute free-energy calculations is provided by Deng and Roux.[181]

Despite the fact that these issues were raised in the 1986 work, a variety of "absolute" binding free-energy calculations since then have neglected one or both of these issues. Nevertheless, beginning in the mid-1990s, there have been a number of successful applications of absolute binding free-energy calculations. These cluster in three general categories that each merit individual discussion: (1) binding of water within buried sites or binding sites of proteins, (2) binding of nonpolar ligands in a designed binding cavity within T4 lysozyme, and (3) binding of ligands to FKBP.

Water binding. The area of water binding is particularly interesting (and challenging) because it is extremely difficult to access the thermodynamics of water binding experimentally, meaning that computation uniquely provides access to important and interesting information. It represents an important application of free-energy calculations that does not simply serve to predict a binding affinity but also adds physical insights about the binding process that can also be used in the further design. One important computational challenge is to ensure that the water molecule being removed is not replaced by any other water molecules while being removed, or else the net result of the calculation will simply be to remove a water molecule from bulk. This methodological issue is not always addressed in work on water binding and in some cases may be a concern.

Work in the area of water binding was begun with the absolute binding free-energy calculations of Helms and Wade in 1995. They found that a crystallographic water bound in a cavity in cytochrome P450cam with a particular inhibitor had a binding free energy around −2.8±1.6 kcal/mol while transferring a water into the cavity with the natural substrate (camphor) would cost 3.8±1.2 kcal/mol. A follow-up study found the preferred number of water molecules in the cavity in the absence of ligand,[182] finding that six waters is thermodynamically preferable over five and seven or eight by 1–2 kcal/mol. A third study then computed the absolute binding free energy of camphor by replacing it with six waters in the binding site.[183] More recently, Deng and Roux applied alchemical free-energy calculations in combination with a grand canonical Monte Carlo scheme to replace camphor with water molecules while removing camphor from the cavity.[184] Their computed binding free energy for camphor agreed fairly well with that of Helms and Wade, but they differed slightly on the number of water molecules in the cavity.

Another water binding free-energy study examined the binding of crystallographic waters in the subtilisin Carlsberg complex with eglin-C and found that only some of the waters appeared to bind favorably.[185] Another study (on bovine pancreatic trypsin inhibitor and a barnase mutant) reached similar conclusions about crystallographic waters,[186] and a more recent study has also observed unfavorable binding energies for crystallographic waters.[187] The reasons for this apparent discrepancy are so far unclear, but one suggestion has been limitations in the force field,[186,187] partly because the crystallographic waters are often conserved across several different structures of the same complex or binding site, suggesting that crystal structure uncertainties may not be the problem.

Several studies have examined binding of a specific water (water 301) in the complex of HIV-1 protease with inhibitor KNI-272. Two studies agreed on the binding free energy of this water (around −3.3 kcal/mol for one protonation state of the protease),[188,189] while a third study disagreed by about 7 kcal/mol,[187] possibly due to methodological differences relating to the treatment of protein flexibility. The active site protonation state appears to substantially modulate the binding free energy of this water molecule.[189]

There have been a variety of other examinations of water energetics as well. Roux et al. looked at binding of several waters within bacteriorhodopsin and found that transfer of waters from bulk to the proton channel was thermodynamically favorable (in some cases by up to 6 kcal/mol), suggesting implications for proton transfer.[190] De Simone et al. looked at water binding within the prion protein.[191] An extensive study looked at binding free energies of fifty-four water molecules in binding sites of six proteins, with and without ligands present. As validation, some results were compared with previously published work before moving to new binding sites. Overall, water binding free energies varied substantially, with a mean binding free energy of −6.7 kcal/mol, substantially more favorable than the mean binding free energy of water molecules that are displaced by ligands (−3.7 kcal/mol). The range of binding free energies runs from slightly positive to around −10 kcal/mol.[187] One major conclusion from this and the other studies in this area is that water molecules make a highly variable contribution to the thermodynamics of ligand binding, and factoring water molecules into ligand design is likely not to be an intuitive process, thus increasing the need for computational methods that can account for variable contributions of bound waters.

A final study worthy of note for its novelty and potential practicality for drug discovery is the work of Pan et al., which used a grand canonical Monte Carlo technique to qualitatively predict locations around binding sites where waters can easily be displaced, suggesting routes for lead optimization.[192] Although this is not an application of absolute alchemical free-energy methods, it is nevertheless an extremely interesting application of free-energy methods to water binding.

T4 lysozyme ligand binding. Another important set of systems for studying binding free-energy calculations are the two model binding sites in T4 lysozyme created by point mutations. The first of these, the L99A mutant, introduces a simple nonpolar cavity, while the second (L99A/M102Q) adds a polar group at the margin of the cavity and introduces the possibility of more hydrogen bonding. Both have been extensively characterized