free energy can be computed directly from these end states. At least two methods for computing the configuration integrals in the neighborhood of minima have been developed that have been applied to ligand binding systems: mode integration (MINTA),[61] and the Mining Minima approaches of Gilson and coworkers. Both start out with a method for enumerating minima. In MINTA importance sampling Monte Carlo integration is used to calculate the configuration integrals. It has been used to screen for the free-energy difference between ligand enantiomers, where its accuracy was comparable to alchemical methods, but was more efficient,[62,63] though there are important caveats in the original implementation.[64] Gilson and coworkers have emphasized calculating the integrals in bond/angle/torsion coordinates to minimize errors.[68] They have applied such methods successfully to a number of simplified binding systems.[65–68]

The methods' two shared main problems are the need to find all minima contributing to the partition function and the correct computation of entropies of neighborhoods near minima. Minima searches of proteins the size of typical drug targets are notoriously difficult, and hence the studies noted above focus mostly on problems where many of the errors may cancel out or on smaller model systems. The problems of estimating entropy in these methods has much in common with the same problem in MM-PBSA calculations, though because these methods are perhaps more sensitive to the correct entropy calculation, the problems have been investigated to a significant degree.[45,61,69,70] However, significantly fewer people are investigating these alternative end-point methods than MM-PBSA, and they are generally more computationally expensive, so the short-term prospects are not necessarily particularly encouraging despite the strong theoretical underpinnings.

Alchemical methods

The methods described above are designed primarily for implicit solvent systems and represent relatively computationally cheap approximations to the binding free energy. However, implicit water models are unsuitable for a fully molecular description of phenomena such as the formation of correlated hydrogen bonding networks in binding active sites and there are many protein/ligand systems where the atomic detail of the water in the binding site plays an important role in the binding process.[71] For free-energy calculations to include these phenomena, more expensive explicit water simulations must be used. Using explicit water, the free-energy terms in MM-PBSA become dominated by statistical noise from the water. The standard approach for solvation free energies in explicit solvent simulations is instead to compute the free energy of a particular change of state directly, while holding the rest of the system fixed, which does not depend directly on the energies of the rest of the system. We note that although these methods are the techniques of choice for explicit water simulations, they can be performed equally easily for continuum water simulations.

"Free-energy perturbation" is a very common term for these methods that directly compute the free-energy difference as a function of changing molecular structure. "Perturbation" usually refers to an approximate theory that can be written as a series of more easily calculated terms. Free-energy perturbation (frequently abbreviated FEP), however, is exact. The term *perturbation* here instead refers to the changes in the chemical identity, as simulations frequently involve changes in chemical identity, such as an amine to an alcohol or a methyl group to a chlorine. Additionally, FEP is sometimes used to refer specifically to application of the Zwanzig relationship (discussed below). To avoid confusion, we will use the term *alchemical* to refer to this class of methods, as the chemical identity of the atomic models will change, appear, or disappear during the process, and use EXP to refer to the Zwanzig relationship.

Zwanzig relationship

The most well-known method historically for calculating free energies, and still a very common one, is the Zwanzig relationship.[72] The free energy between two Hamiltonians $H_0(\boldsymbol{x})$ and $H_1(x)$ over a coordinate and momentum space (x) can be calculated as

$$\Delta G = \beta^{-1} \ln \left\langle e^{-\beta[H_1(\boldsymbol{x}) - H_0(\boldsymbol{x})]} \right\rangle_0 = \beta^{-1} \ln \left\langle e^{-\beta \Delta H(\boldsymbol{x})} \right\rangle_0 , \qquad (5.10)$$

where $\beta = (kT)^{-1}$. We will denote this method EXP, for exponential averaging. Although the equation is exact, many studies have demonstrated that except in the case of rather small changes, EXP convergence as a function of the amount of data collected is far from ideal, and an average that appears to have converged may only indicate very poor overlap between the two states studied.[73,74]

Overlap in configuration space in the direction of decreasing entropy is usually greater, and thus EXP in this direction will generally be more efficient.[75,76] For example, inserting a molecule into a dense fluid is a more effective way to compute the chemical potential than deleting molecules from the same fluid, because the important conformations for both ensembles are actually easiest to sample in simulations without the molecule present.

Multiple intermediates

In some cases, such as computing the chemical potential of bulk fluids, the symmetry of the problem can be used to greatly improve computational efficiency of FEP.[77] However, in most instances where the states of interest are very far from having any phase-space overlap, the transformation can be broken into a series of free-energy calculations with nonphysical intermediates – for example, turning off the atomic charges or turning a carbon into an oxygen. The