in fact somewhat nonintuitively.[148] This study is somewhat difficult to interpret, as mixed small-molecule force fields were used in addition to normal-mode entropy estimates for all the of data points. In a later publication it was noted that both of the above studies selected molecules with insufficiently wide potency ranges[149]; however, the ranges of potencies in the reports of Pearlman and Kuhn et al. are around 3 kcal/mol, consistent with our findings that MM-PBSA, in general, cannot be expected to reliably resolve compounds within 2–3 kcal/mol. For a sufficiently wide range of potencies careful application of MM-PBSA may provide help and bias discovery effort toward the more potent compounds.[150]

The literature reports other issues with MM-PBSA, for example, in systems including in the presence of a metal ion in the binding site.[151] Inadequacies of MM-PBSA to accurately represent first-solvation-shell effects were shown to introduce significant error into direct potency prediction.[170] However, despite the first-solvation-layer error, MM-PBSA was still able to successfully rank-order the ligands.

There appears to be an emerging consensus that MM-PBSA likely has some applicability and utility in drug discovery. However, results have ranged too widely, from promising to poor to difficult, to interpret unambiguously. There is as of yet insufficient data to conclusively demonstrate the scope, the limitations of MM-PBSA, and the fundamental reliability in industrial drug discovery research, though the studies described here demonstrate enough promise to focus more effort on these methods in the future.

## Alchemical calculations

Alchemical free energies are substantially more rigorous than MM-PBSA calculations but also significantly more computationally demanding. They were first applied to protein/ligand systems in the early to mid-1980s. Tembe and McCammon[152] laid out some of the basic theory for applying these calculations to protein/ligand interactions and used them to examine the "binding" of two Lennard–Jones spheres in a small bath of Lennard–Jones spheres in 1984. This was probably the first "alchemical" free-energy calculation, although the term itself originated somewhat later. The first applications to true protein/ligand complexes followed shortly, with Wong and McCammon computing relative binding free energies of three trypsin inhibitors[153] with some success, and Hermans and Subramaniam computing the binding free energy of xenon to myoglobin.[154] This and related work from others ushered in a wave of alchemical applications in the late 1980s and early 1990s.

However, in a recent review, David Pearlman noted that some of the early success with alchemical methods may have been simply luck. He argues, "[W]e are now at a point that is, in reality, where we thought we were 20 years ago!"[155] At the very least, performing accurate alchemical free-energy calculations has turned out to be a great deal more difficult and computationally demanding than originally thought,[8,156] so enthusiasm waned after the initial success before undergoing a resurgence since the early 2000s. This recent change has been described as a "coming of age."[165] Alchemical methods have yet to see widespread use in pharmaceutical applications, however.

Part of the recent increase in enthusiasm for free-energy calculations has been due to some of the methodological innovations addressed above, including the movement away from EXP, and another large part has been due to steadily increasing computer power bringing new problems into range. Together, these factors mean that much of the work on alchemical methods before the early 2000s is woefully out of date, so in this discussion we focus mostly on work since 2000. As noted above, alchemical free energies can be calculated by TI, EXP (exponential averaging), or BAR, among other methods, but the basic ideas remain the same. Here we highlight some key applications areas of alchemical methods without focusing on methodological issues highlighted above.

### Relative free energies

Relative binding free energies were one of the earliest applications of alchemical methods, and they have remained a traditional application of alchemical free-energy methods. Relative free-energy calculations involve alchemically transforming one ligand into another, allowing direct calculation of the relative binding free energies from an appropriate thermodynamic cycle. This may be substantially more efficient than computing two absolute binding free energies and subtracting in cases where the ligands are relatively similar, as it eliminates statistical noise due to transformation of the rest of the ligands. If the limiting factor in the precision of the calculations is a long time-scale conformational fluctuation of the protein, however, the relative efficiency of relative free-energy calculations may be lessened considerably.

There have been a number of practical success stories with these calculations. One particularly interesting and comprehensive set of studies has been work from the Jorgensen lab on binding of HIV-1 nonnucleoside reverse transcriptase inhibitors (NNRTIs).[157–163] One study used docking and molecular dynamics equilibration to generate a model structure of sustiva bound to HIV-1 reverse transcriptase and then alchemical free-energy methods with Monte Carlo conformational sampling to compute the change in binding affinity of sustiva due to several known drug resistance mutants. Because the computed drug resistance profile matched well with experiment (with relative binding free energies accurate to 1–2 kcal/mol), this suggested that the model binding mode was indeed correct, a fact that was subsequently confirmed crystallographically.[157] Two other studies examined effects of known drug resistance mutations on several inhibitors and derivatives[158,159]; both were accurate to within 1 kcal/mol in relative binding free energies. One issue with these calculations concerns the approximations made, including the