in order to assess a compound's ability to interact with the target of interest. Virtual chemical libraries are often freely available from commercial vendors (the largest of which is the ZINC database; http://zinc.docking.org/) and, as with physical samples, pharmaceutical companies generally maintain virtual libraries of their proprietary compounds for internal use. Structural information on biological targets may be available through X-ray crystallography, as a large number of protein crystal structures are available through the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). If a structure is not readily available, it may be possible to create a homology model of the biological target using crystal structure data of a closely related macromolecules.[81] In either case, the individual compounds of the chemical libraries can then be "docked" in a hypothetical binding site in the target of interest to determine a relative rank order for the entire set of compounds. Automated data analysis tools are then employed that organize the predictions provided by the "docking" of the chemical libraries to the hypothetical binding sites of the biological targets. The predictions can then be used to select a smaller subset of a large library for physical biological screening as potential starting points.

Much like physical HTS, there are some important limitations that must be considered in evaluating virtual screening data. First and foremost, virtual screening results are predictions based on model system and not actual data on physical compounds. As such, the quality of the results will depend on the quality of the model. *In silico* models based on X-ray crystal structures tend to be stronger models than homology models built on related biological structures, but it is important to realize that there are limitations associated with X-ray crystal-based models as well. Crystal structures can provide exceptionally detailed structural information, with resolution as low as 1.5 Å, but by definition, X-ray crystal structures are solid state version of the desired target. It is possible that the structure provided by X-ray crystallography matches the biologically active form of the biological target of interest, but it may not. In "real-life" situations, biological targets are either dissolved in water or membrane bound, and it is possible that they may have a different configuration in these situations as compared to the close-packed structure of a crystal form. Also, in many cases, sections or a macromolecule must be altered or removed in order to generate a crystallizable form of the biological target (with or without a ligand). Given these limitations, virtual "hits" should also be physically validated in biological screening efforts in order to confirm that the predictions provided by molecular modeling are representative of the real system.

Irrespective of the initial screening method employed (physical or virtual), a successful screening effort will produce a subset of potential "hit" compounds that will need to be examined in order to determine whether or not follow-up efforts are warranted. This determination, also referred to as "lead discovery," (Figure 1.7) can be quite complex in itself depending on