lished study in which different functions have been applied to the same data sets. Second, experimental data are often not measured under the same conditions but collected from various literature references. This retrieval from various sources usually implies larger uncertainties within the experimental data

The task of ranking sets of 10–100 related ligands with respect to one target can also be handled by computationally more demanding methods. The most general approaches are probably force field scores complemented by electrostatic desolvation and surface area terms. An example is the MM-PBSA method that combines Poisson-Boltzmann electrostatics with AMBER molecular mechanics calculations and MD simulations (341,342). This method has recently been applied to an increasing number of examples, showing quite promising results (343–346). Poisson-Boltzmann calculations have been performed on a variety of targets with many related computational protocols (280–282, 347–350). Alternatively, extended linear response protocols (263) can be used. The OWFEG grid method by Pearlman has also shown very promising

VIRTUAL SCREENING

As outlined in section, virtual screening is a multistep process. Although, in principle, the whole process can be fully automated, it is highly advisable to allow for manual interventions, in that visual inspection and selection still play a major role.

The process usually starts with a detailed analysis of the available 3D protein structures. If possible, highly homologous structureswill also be analyzed, either to generate additional ideas about possible ligand structural motifs or to gain some insight on how to achieve selectivity against other proteins of the same class. A superposition of different protein–ligand complexes provides some ideas about key interactions repeatedly found in tight-binding protein–ligand complexes. Such an overlay will also highlight flexible parts of the protein or recurring water molecules in the binding site that could be included in the docking process. Tools such as Relibase (322, 323) may be used to perform these comparative analyses of protein–ligand complexes in an efficient way. Subsequently, programs like GRID (218), LUDI (108, 109), Superstar (351, 352), or DrugScore (318) are used to visualize potential binding sites ("hot spots") in the active site; in principle, any scoring function could be used for this purpose.

An important result of the 3D structure analysis is usually the identification of one or more key interactions that all ligands should satisfy. In aspartic proteases, for example, inhibitors should form at least one hydrogen bond to the catalytic Asp side-chains, whereas in metalloproteinases a coordination to the metal seems mandatory. Sometimes, a known ligand portion is used as initial scaffold based on which virtual screening techniques search for optimal side-chains. In principle, this step is not required, and instead one could fully rely on the docking and scoring step. However, following a pragmatic approach, it is important to use any well-founded information that is available about the system under consideration because more valuable results can usually be expected this way.

Once a reasonable hypothesis about the binding-site requirements has been generated, the next level of virtual screening is approached. Whether databases of commercially available compounds or "virtual" libraries of designed compounds are screened, it is advisable not to dock every possible compound, but only those that pass a series of hierarchical filters (cf. also Fig. 7.3). Simple preliminary filters remove

- compounds with reactive groups such as $-SO_2Cl$ or $-CHO$ because they are expected to cause problems in some biological assays as a result of unspecific covalent binding to the protein.
- compounds with molecular weights below 150 or above 500. Small molecules such as benzene are known to bind to proteins rather unspecifically at several sites. Large molecules such as polypeptides are difficult to optimize subsequently, given that good