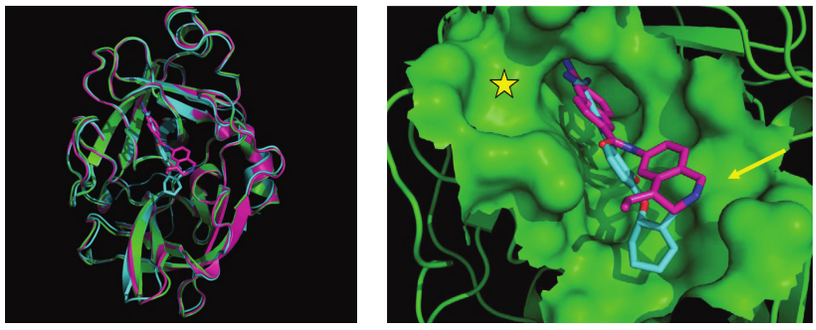

a) b)

Figure 7.3. (a) Overlay of urokinase crystal structures provided for virtual screening (cyan and magenta ribbons) and cross-docking challenges (green ribbons and surface); **(b)** ligands in crystal structures 1o5c (cyan carbons) and 1owd (magenta carbons) in cross-docking structure.

where rmsd is the root-mean-squared deviation between docked pose and crystallographically identified conformation and DPI is the coordinate precision error of the crystal structure.[149–151] For urokinase twenty-seven of the thirty-four compounds were active and had been crystallized in the protein; for JNK3 fifty-two of the sixty-two compounds were active and had been crystallized in the protein. Results are presented here for only the seventy-nine compounds with protein/ligand crystal structures.

Manual docking process. Although other practitioners might use other specific computational tools at each stage of the process, in broad outline the manual docking protocol used here matches standard practice for supporting a drug discovery team. Before beginning any actual docking, all available structural data were examined closely. For the SAMPL-1 challenge, available data included the four ligand-bound structures from the virtual screening challenge along with the three ligand-free structures provided for the cross-docking exercise; no structural information from the public domain or from in-house drug discovery efforts was sought or used. Structures for each individual protein target were aligned and compared to assess the degree of protein flexibility, to identify protein features likely to be important for interactions with ligands, and to contemplate protonation states and side-chain conformations within the binding site. Before any docking began, 2D representations of the ligands to be docked were also closely examined. The goal here was to divide the ligands into related classes that would be expected to bind similarly, to identify ligand features likely to be important for interactions with the protein, and to identify ligand features – for example, unusual functional groups, possible tautomers, protonation states – that might cause difficulties or require special treatment during docking.

In preparation for semiautomated docking, 3D conformations for all ligands were generated with OMEGA 2.1.0[152] using default parameters, the standard omega fragment library, and provided SD files as input. To generate a good starting point for docking, Rocs 2.2[153] was used to overlay the omega ligand conformers to each ligand in the ligand-bound protein structures from the virtual screening challenge; overlays were optimized using the Implicit Mills–Dean color force field provided with Rocs, and overlays were ranked using the combo shape-and-color score. All overlays were visually inspected to select one or more starting points for docking and to decide which molecules would need to be built by hand. For JNK3, overlays were visually inspected in the context of the provided protein structures to decide into which structure each ligand should be docked.

Starting from the selected Rocs overlays, the February 2003 version of Flo/qxp[154] was used to explore pose space for each of the ninety-six ligands in the cross-docking challenge. For some of the ligands, the initial overlay was considered sufficiently likely, and the ligand was therefore merely minimized in the binding-site environment. For most of the other ligands, the central scaffold was allowed to minimize and the mcdock algorithm in Flo was used to search conformation space for substituents on that scaffold. For a very few molecules for which no good starting alignment was identified, a full docking run was carried out using mcdock with 1,000 or 2,000 search cycles. And a final few molecules were hand built; the core scaffold was manually placed at a desired location in the protein and conformations explored as substituents were added to that core. For JNK3, constraints were included during docking and minimization to enforce expected interactions between ligands and backbone atoms in the hinge region of the kinase.

The final computational step was to clean the small-molecule conformations for the selected docked pose for each ligand by minimization in MOE. All hydrogen atoms were added to the protein and to each ligand in turn. For JNK3, constraints were imposed on the hinge-binding interactions; for both JNK3 and urokinase, the protein was kept rigid during minimization using the MMFF. In those few cases where it appeared necessary to do so, ligand atoms were held rigid and selected portions of the protein were allowed to minimize or side-chain conformations were optimized using the CHARMM force field.

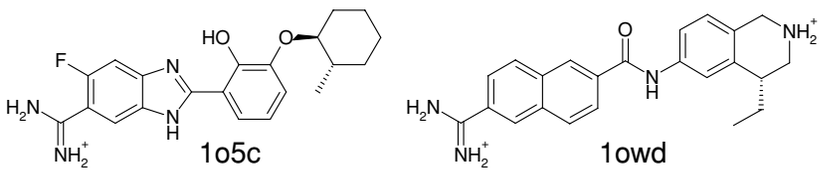

Figure 7.4. Small molecules from urokinase crystal structures provided for the virtual screening challenge.

At the end of this semiautomated, semimanual docking process, a single docked