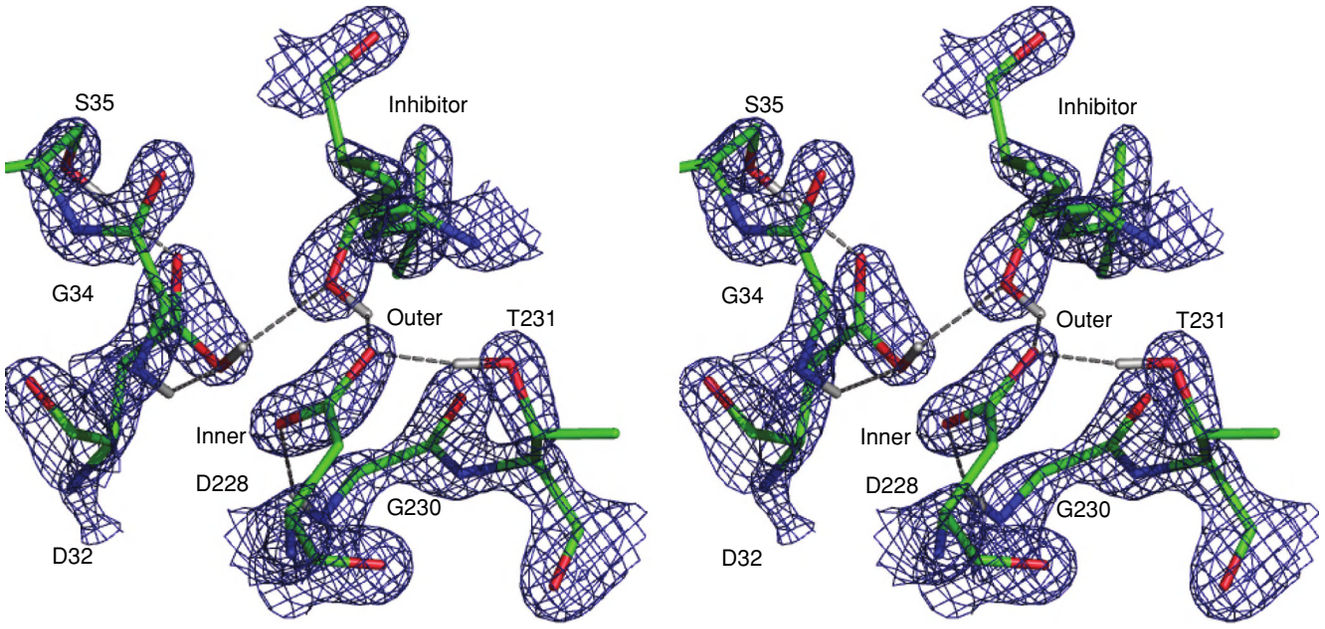

Figure 8.2. Cross-eye stereo view of the key residues of inhibitor-bound β-secretase in a state with Asp32 protonated and Asp228 deprotonated suggested as being most probable by QM/MM x-ray refinement, together with the σ_A-weighted 2Fo-Fc electron density maps contoured at 2.7σ level.

E_{chem} and $E_{x\text{-}ray}$. Although the electron density map computed from the x-ray data can be used to determine the structure on a larger scale, the energy function in Equation (8.1) is necessary to control the stereochemical details of the structure. However, though not yet well recognized, if E_{chem} is accurate enough the EREF formalism allows the use of energetic information to filter out unlikely tautomeric and protonation states, which may not otherwise be clear based solely on the coordinates of nonhydrogen atoms. Conversely, if E_{chem} is represented by approximate or even inaccurate energy functions, the refined structure can be significantly biased.[9,10] Unfortunately even though highly accurate parameters for bond lengths, angles, and torsions are available for amino and nucleic acids,[11–13] those for the small molecules are partially lacking, especially when extremely rare or novel chemical moieties are encountered.[14] QM constitutes an ideal choice for E_{chem} and a major improvement over MM because it does not require a priori knowledge of the potential energy surface of the ligand, which may be actual or virtual, and it is generally more accurate and reliable. Refinement studies on proteins[15,16] and complexes[17–21] have shown that QM-based energy restraints performed comparably with or, in some cases, showed some improvements over the MM-based ones.

Applications involving QM refinements of cocrystal structures have mostly being carried out in the QM/MM manner, which have been focused on two major areas. First, accurate energies calculated with QM and QM/MM have been used to suggest the probable protonation states of the key protein residues[22] and of the metal-bound ligands[17,23] in the context of the crystalline environment. For example, QM/MM x-ray structure refinement was employed to construct realistic all-atom models of a complex of human β-secretase bound to a peptidic inhibitor and the relative stability of the resulting structures for different protonation states was evaluated by QM/SCRF calculations, which suggested one of the key aspartates, Asp32, was preferentially protonated in the cocrystal structure.[22] Although the nonhydrogen atom coordinates of the refined structure are not substantially different from those in the crystal structure, QM/MM refinement provided an all-atom model as a reasonable starting point for structure-based virtual screening and de novo design of β-secretase inhibitors. Second, energy restraints derived from high-level QM calculations have been used to refine ligand geometries to enhance the quality of low-resolution structures. Ryde et al. applied this approach to refine a 1.70Å structure of cytochrome c_{553} from *Bacillus pasteurii*. The refined structure was in better agreement with the same structure solved at 0.97Å and also reduced the *R* value of the lower-resolution structure by 0.018 (Figure 8.2).

A combined molecular dynamics (MD) potential of mean force (PMF) and QM/MM x-ray refinement study[24] has helped further our understanding of the binding preference for 1,6-dihydroxynaphthalene (DHN) to Orf2.[25] From the MD/PMF simulations three minima were located for the binding of DHN to Orf2 [C1 (the x-ray structure) C2 and C3]. C1 leads to the preferential product for the prenylation of DHN, whereas C3 leads to the minor product.[25] Each of these structures were then subjected to QM/MM x-ray refinement using the semiempirical PM3 Hamiltonian. The outcome of the QM/MM refinement versus a standard