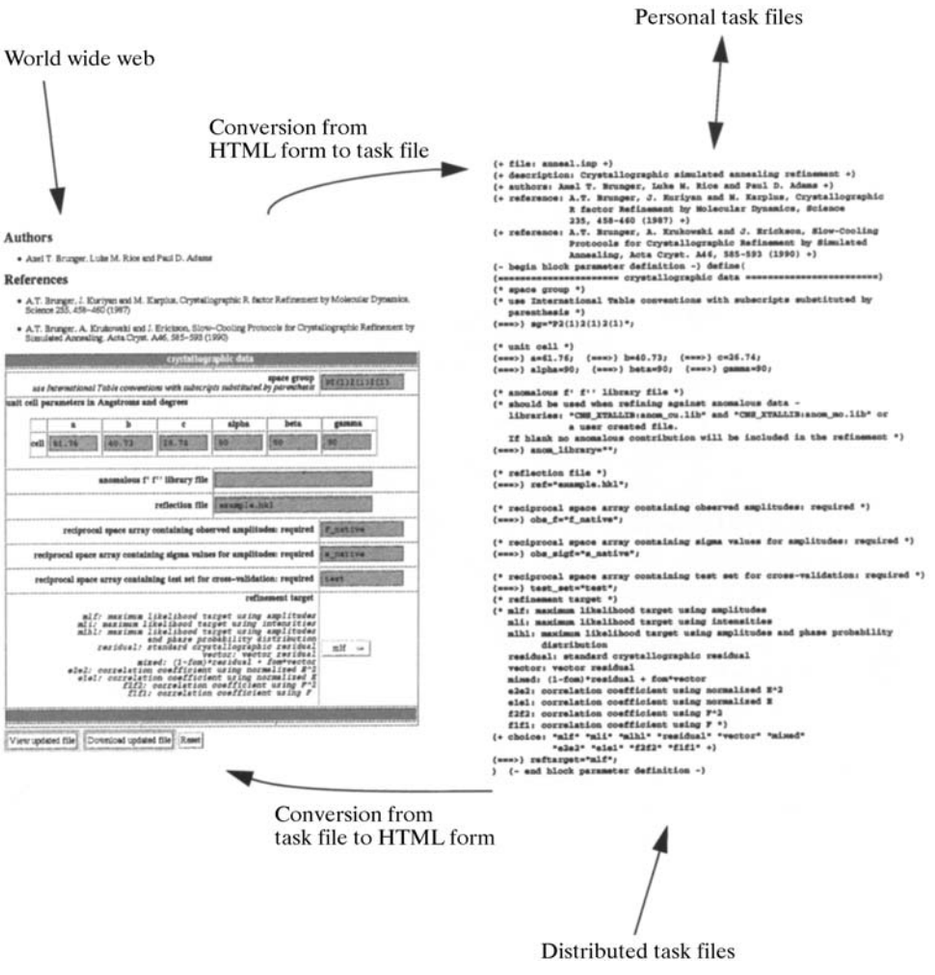

Fig. 25.2.3.10. Use of the CNS HTML form page interface, emphasizing the correspondence between input fields in the form page and parameters in the task file.

validated σ_A estimates, determines the weighting scheme between the X-ray refinement target function and the geometric energy function (Brünger *et al.*, 1989), refines a flat bulk solvent model (Jiang & Brünger, 1994) and an overall anisotropic B value for the model by least-squares minimization, and subsequently refines the atomic positions by simulated annealing. Options are available for specification of alternate conformations, multiple conformers (Burling & Brünger, 1994), noncrystallographic symmetry constraints and restraints (Weis *et al.*, 1990), and 'flat' solvent models (Jiang & Brünger, 1994). Available target functions include the maximum-likelihood functions MLF, MLI and MLHL (Pannu & Read, 1996*a*; Adams *et al.*, 1997; Pannu *et al.*, 1998). The user can choose between slow cooling (Brünger *et al.*, 1990) and constant-temperature simulated annealing, and the respective rate of cooling and length of the annealing scheme. For a review of simulated annealing in X-ray crystallography, see Brünger *et al.* (1997).

During simulated-annealing refinement, the model can be significantly improved. Therefore, it becomes important to recalculate the cross-validated σ_A error estimates (Kleywegt & Brunger, 1996; Read, 1997) and the weight between the X-ray diffraction target function and the geometric energy function in the course of the refinement (Adams *et al.*, 1997). This is important for the maximum-likelihood target functions that depend on the cross-validated σ_A error estimates. In the simulated-annealing task file, the recalculation of σ_A values and subsequently the weight for the crystallographic energy term are carried out after initial energy minimization, and also after molecular-dynamics simulated annealing.

25.2.3.10. *Conclusions*

CNS is a general system for structure determination by X-ray crystallography and solution NMR. It covers the whole spectrum of methods used to solve X-ray or solution NMR structures. The multi-layer architecture allows use of the system with different levels of expertise. The HTML interface allows the novice to perform standard tasks. The interface provides a convenient means of editing complicated task files, even for the expert (Fig. 25.2.3.10). This graphical interface makes it less likely that an important parameter will be overlooked when editing the file. In addition, the graphical interface can be used with any task file, not just the standard distributed ones. HTML-based documentation and graphical output is planned in the future.

Most operations within a crystallographic algorithm are defined through modules and task files. This allows for the development of new algorithms and for existing algorithms to be precisely defined and easily modified without the need for source-code modifications.

The hierarchical structure of CNS allows extensive testing at each level. For example, once the source code and CNS basic commands have been tested, testing of the modules and task files is performed. A test suite consisting of more than a hundred test cases is frequently evaluated during CNS development in order to detect and correct programming errors. Furthermore, this suite is run on several hardware platforms in order to detect any machine-specific errors. This testing scheme makes CNS highly reliable.

Algorithms can be readily understood by inspecting the modules or task files. This self-documenting feature of the modules provides a powerful teaching tool. Users can easily interpret an algorithm and compare it with published methods in the literature. To our knowledge, CNS is the only system that enables one to define symbolically any target function for a broad range of applications, from heavy-atom phasing or molecular-replacement searches to atomic resolution refinement.

25.2.4. The *TNT* refinement package

(D. E. Tronrud and L. F. Ten Eyck)

25.2.4.1. *Scope and function of the package*

TNT (Tronrud *et al.*, 1987) is a computer program package that optimizes the parameters of a molecular model given a set of observations and indicates the location of errors that it cannot correct. Its authors presume the principal set of observations to be the structure factors observed in a single-crystal diffraction experiment. To complement such a data set, which for most macromolecules has limitations, stereochemical restraints such as standard bond lengths and angles are also used as observations.

A molecule is parameterized as a set of atoms, each with a position in space, an isotropic B factor and an occupancy. The

references