

25.2. PROGRAMS IN WIDE USE

new ideas in refinement. Since its source code is designed to allow easy reordering of tasks and simple modifications, a number of innovations in refinement made their first appearance in *TNT*. These features include the following.

25.2.4.5.1. *Identifying and restraining symmetry-related contacts (1982)*

Without a search for symmetry-related bad contacts, it was quite common to build atoms into the same density from two different sides of the molecule. A number of models in the PDB contain these types of errors because neither the refinement nor the graphics programs available at that time would indicate this type of error.

25.2.4.5.2. *The ability of a single package to perform both individual atom and rigid-body refinement (1982)*

Prior to *TNT*, one often started a refinement with rigid-body refinement using *CORELS* and then switched to another program. *TNT* was the first refinement package to allow both styles of refinement. One was not required to learn about two different packages when running *TNT*.

25.2.4.5.3. *Space-group optimized FFTs for all space groups (1989)*

This innovation allowed *TNT* to run efficiently in all space groups available to macromolecular crystals.

25.2.4.5.4. *Modelling bulk solvent scattering via local scaling (~1989)*

With a simple and quick model of the scattering of the bulk solvent in the crystal (Tronrud, 1997), the low-resolution data could be used in refinement for the first time. The inclusion of these data in the calculation of maps greatly improved their appearance.

25.2.4.5.5. *Preconditioned conjugate-gradient minimization (1990)*

This method of minimization (Axelsson & Barker, 1984; Tronrud, 1992) allows the direct inclusion of the diagonal elements of the second-derivative matrix and the indirect inclusion of its off-diagonal elements. An additional benefit is that it allows both positional parameters and *B* factors to be optimized in each cycle. Previously, one was required to hold one class of parameter fixed while the other was optimized. It is much more efficient and simpler for the user to optimize all parameters at once. This method, because it incorporates the diagonal elements directly, produces sets of *B* factors that agree with the diffraction data better than those from the simple conjugate-gradient method.

25.2.4.5.6. *Restraining stereochemistry of chemical links to symmetry-related molecules (~1992)*

It is not uncommon for crystallization enhancers to be found on a special position in the crystal. In addition, cross-linking the molecules in a crystal is often done for various reasons. In both cases, the model contains chemical bonds to a molecule or atoms in another asymmetric unit of the crystal. In order for the stereochemistry of these links to be properly restrained, it must be possible to describe such a link to the refinement program.

25.2.4.5.7. *Knowledge-based B-factor restraints (~1994)*

When the resolution of the diffraction data set is less than about 2 Å, the individual *B* factors of a refined model are observed to vary wildly from atom to atom, even when the atoms are bonded to one another. This pattern is not reasonable if one interprets the *B* factor

as a measure of the vibrational motion of the atom. Traditionally, one applies an additional restraint on the *B* factors of the model, where the ideal value for the difference in *B* factor for two bonded atoms is zero.

Since it is clear from examinations of higher-resolution models that the *B* factors generally increase from one side of a bond to the other (e.g. moving from the main chain to the end of a side chain), the traditional restraint is flawed. A restraint library was generated (Tronrud, 1996) where each bond in a residue is assigned a preferred increment in *B* factor and a confidence (standard deviation) in that increment.

25.2.4.5.8. *Block-diagonal preconditioned conjugate-gradient minimization with pseudoinverses (1998)*

With this enhancement, *TNT*'s minimizer treats the second-derivative matrix as a collection of 5×5 element blocks along its diagonal, one block for each atom. While this method improves the rate of convergence for noncrystallographic symmetry restraints, its most significant feature is that it allows the refinement of atoms located on special positions without special handling by the user.

25.2.4.5.9. *Generalization of noncrystallographic symmetry operators to include shifts in the average B factor (1998)*

It is rather common in crystals containing multiple copies of a molecule in the asymmetric unit for one or more molecules to have a higher *B* factor than the others. If the transformation that generates each copy of the molecule consists only of a rotation and translation of the positions of the atoms, the difference in *B* factors cannot be modelled. The transformations used in *TNT* now consist of a rotation, translation, a *B*-factor shift and an occupancy shift.

25.2.4.6. *TNT as a research tool*

TNT was intended not only as a tool for performing refinement, but as a tool for developing new ideas in refinement. While most of the latter has been done by *TNT*'s authors, several others have made good use of *TNT* in this fashion. If one has an idea to test, the overhead of writing an entire refinement package to perform that test is overwhelming. *TNT* allows modification at a number of levels, so one can choose to work at the level that allows the easiest implementation of the idea. Several examples follow.

25.2.4.6.1. *Michael Chapman's real-space refinement package*

At Florida State University, Chapman has implemented a real-space refinement package, principally intended for the refinement of virus models, using *TNT*. He was able to use *TNT*'s minimizer and stereochemical restraints unchanged along with programs he developed to implement his method. More information about this package can be found at <http://www.sb.fsu.edu/~rsref>.

25.2.4.6.2. *Gerard Bricogne's Buster refinement package*

Bricogne & Irwin (1996) have developed a maximum-likelihood refinement package using *TNT*. Not only are *TNT*'s minimizer and stereochemical restraints used, but many of the calculations of the maximum-likelihood residual's derivatives are performed using *TNT* programs. While Bricogne and co-workers have not needed to modify *TNT* programs to implement their ideas, there is ongoing collaboration between them and *TNT*'s authors on the development of commands that allow access to some previously internal calculations. More information about *Buster* can be found at <http://lagrange.mrc-lmb.cam.ac.uk/>.