Refinement when Amplitudes aren't enough: Real-Space, H-Bonding & Electrostatics

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Methodological improvements have reduced, but not eliminated over-fitting in macromolecular structure refinement. It is well known that over-fitting depends on freedom in the atomic model compared to the quality and quantity of the experimental data. The latter depends on resolution – 3 Å usually being considered the minimum.

Our goal is to improve the robustness of refinements at resolutions that are at best marginal, by using fully the available data, or by adding stereochemical restraints to the model freedom. Real-space refinement is most advantageous when model-independent phases can be used as additional data. The local nature of the refinement eliminates the over-fitting due to compensating errors that occurs in reciprocal-space where all atoms depend on all data points. Atomic density functions that rigorously incorporate resolution limits allow the method to be applied in both crystallography and electron microscopy at resolutions as low as 50 Å.

Additional stereochemical restraints have a modest positive impact in medium resolution crystallographic structures. A hydrogen-bonding restraint that is directionally targeted towards either dipolar or lone-pair interactions is beneficial, in contrast to prior attempts that optimized only dipolar effects. Minimization of the electrostatic potential energy is also beneficial, and more so when calculated by continuum methods rather than by the Coulombic methods previously used. This has been accomplished by combining refinement with numerical solution of the Poisson-Boltzmann equation.

Keywords: refinement, restraint, electrostatic potential