The refinement of a macromolecular structure from crystal diffraction data can be formulated as follows: find the most likely mini-ensemble of structures given molecular energetics and observed data. The (posterior) conditional probability to be maximized can be expressed via Bayes' law as the multiplicative product of two distributions, prior and data likelihood. The prior assigns probability to each mini-ensemble, viewed as a single state, via Boltzmann's distribution of states for a canonical ensemble at ambient temperature; the mini-ensemble energy is the mean of the energy values computed for individual structures under an energetic model. For a given mini-ensemble, a virtual asymmetric unit is constructed by averaging the structures and a virtual crystal by symmetric replication. The data likelihood is the probability that the measurement of x-rays delivered to the model probability. As energy depends upon the log of the energetic model, the factors in the distribution agrees precisely with the Fo-Fc difference maps; in the BPTI case, interleukin-1 beta, the computed energies of mobile water molecules has emerged since the initial temperature (typically 400°K) through assigning different initial velocities to each water molecule of the different copies. Then the system is very slowly cooled (5° steps) until it reaches the desired temperature (300°K). This dynamics simulation, implemented in XPLOR, includes the usual modeling forces and an X-ray term. The free R-factor is used to monitor the validity of the process. The method was applied to X-ray diffraction data from BPTI and RNA crystals. The results show that while some water molecules are highly localized (the different copies remain clustered in specific hydration sites), the rest are more widely distributed, sometimes forming water channels. The shape of the multiplicity distribution agrees precisely with the Fo-Fc difference maps; in the BPTI case, simulations and difference maps using neutron data were used to cross-check the results. The obtained models agree with the crystallographic data and are more compatible with other experimental observations than the ones with single fixed sites. This work is supported by the CNRS through the UPR 9004, by the CONICET through the IFILYSIB and by the EU through the collaborative project CII-CT 93 0014 (DG 12 HSMU); JRG is an invited fellow financed by the MENESRIP (France).

Water molecules inside cavities in proteins constitute integral parts of the structure. We have sought a quantitative measure of the hydrophilicity of the cavities by calculating energies and free energies of introducing a water molecule into these cavities. The computations required to survey the atomic coordinates of a protein molecule in terms of low-energy water positions are rapid. A proper assessment of hydration should be based on free energy, not energy; however, much experimentation indicates that the computed energies support the presence of 8 water molecules, where the x-ray structure reports 12 sites, some of them rather weak. In the other case, interleukin-1 beta, the computed energies and free energies of transferring one or two water molecules into the cavity are insufficiently low, and this suggests that the cavity is not hydrated, as reported in crystallographic studies, and at odds with a report based on nmr experiments that the cavity is hydrated.

The program and instructions for rapidly locating possible water interior water positions and discriminating between these on the basis of the energy of transfer are available from the authors (request DOWSER program from xia@femto.med.unc.edu).

Hydration models of biomacromolecular crystals, as obtained by crystallographic diffraction studies, usually position water molecules on precisely defined sites. Other experimental results, such as NMR, indicate that a large part of the water content has high mobility and is delocalized. The objective of this work is to find a hydration model that describes these mobile water molecules, while keeping the agreement with the observed diffraction amplitudes. A multiplicity model is proposed to describe the mobility. A set of water molecules, positioned by conventional methods, is used to generate several non-interacting copies. The system is set to an initial temperature (typically 400°K) through assigning different initial velocities to each water molecule of the different copies. Then the system is very slowly cooled (5° steps) until it reaches the desired temperature (300°K). This dynamics simulation, implemented in XPLOR, includes the usual modeling forces and an X-ray term. The free R-factor is used to monitor the validity of the process. The method was applied to X-ray diffraction data from BPTI and RNA crystals. The results show that while some water molecules are highly localized (the different copies remain clustered in specific hydration sites), the rest are more widely distributed, sometimes forming water channels. The shape of the multiplicity distribution agrees precisely with the Fo-Fc difference maps; in the BPTI case, simulations and difference maps using neutron data were used to cross-check the results. The obtained models agree with the crystallographic data and are more compatible with other experimental observations than the ones with single fixed sites. This work is supported by the CNRS through the UPR 9004, by the CONICET through the IFILYSIB and by the EU through the collaborative project CII-CT 93 0014 (DG 12 HSMU); JRG is an invited fellow financed by the MENESRIP (France).