*International Tables for Crystallography* (2006). Vol. F, Figure 20.1.3.1, p. 482.



the experimental density of 1.35 g cm$^{-3}$, leading to a system size of 3044 protein atoms and 5120 atoms total.

The crystal structure of ubiquitin [Protein Data Bank (Bernstein *et al.*, 1977) code 1UBQ] solved at 1.8 Å resolution (Vijay-Kumar *et al.*, 1987) was used as a starting point. To achieve the appropriate total density, noncrystallographic water molecules were added, using a minimum distance of 0.220605 nm between non-hydrogen protein atoms or crystallographic water oxygen atoms and the oxygen atoms of the added water molecules, which were taken from an equilibrated water configuration (van Gunsteren *et al.*, 1996). Initial velocities were assigned from a Maxwell–Boltzmann distribution at 300 K. The protein and solvent were coupled separately to temperature baths of 300 K with a coupling time of 0.1 ps (Berendsen *et al.*, 1984). No pressure coupling was applied. Another simulation (results not shown) including pressure coupling showed no significant change in the box volume. Bonds were kept rigid using the *SHAKE* method (Ryckaert *et al.*, 1977), with a relative geometric tolerance of $10^{-4}$. Long-range forces were treated using twin range cutoff radii of 0.8 and 1.4 nm (van Gunsteren & Berendsen, 1990). The pair list for non-bonded interactions was updated every 10 fs. No reaction field correction was applied. All simulations were performed using the *GROMOS*96 package and force field (van Gunsteren *et al.*, 1996).

The system was initially minimized for 20 cycles using the steepest-descent method. The protein atoms were harmonically restrained (van Gunsteren *et al.*, 1996) to their initial positions with a force constant of 25000 kJ mol$^{-1}$ nm$^{-2}$. This minimized structure was then pre-equilibrated in several short MD runs of 500 steps of 0.002 ps each, gradually lowering the restraining force constant from 25000 kJ mol$^{-1}$ nm$^{-2}$ to zero. The time origin was then set to zero, and the entire unit cell was simulated for 2 ns. The time step was 0.002 ps, and every 500th configuration was stored for evaluation. The first 400 ps of the run were treated as equilibration time, the remaining 1.6 ns were used for analysis.

## 20.1.3. Results

### 20.1.3.1. *Energetic properties*

In Fig. 20.1.3.1, the non-bonded contributions to the total potential energy are shown. The non-bonded interactions comprise Lennard–Jones and electrostatic interactions. Solvent–solvent, solute–solute and solute–solvent interaction energies are shown separately. All of these appear converged after approximately 100 ps. The solvent–solvent energy remains close to its initial value during the whole simulation, the water molecules having relaxed during the pre-equilibration period, while the protein was restrained. The protein internal energy increases during the first few hundred picoseconds, but this is compensated by a decrease in the protein–solvent energy as the protein adapts to the force field and the pre-relaxed solvent environment. This effect becomes negligible after about 200 ps, from which time point the system can be viewed as equilibrated with respect to the energies. The distribution of kinetic *versus* potential energy and the total (bonded and non-bonded) energy of the system relaxes even faster (results not shown).

### 20.1.3.2. *Structural properties*

Not all properties converge as fast as the energies. Fig. 20.1.3.2 shows the root-mean-square atom-position deviation (RMSD) from the X-ray structure for each of the four individual chains for both C$\alpha$ atoms and all atoms. Here, several relaxation periods can be distinguished. After the initial increase, which occurs during the first 50 ps of the simulation, a plateau is reached, and the system is apparently stable until 300 ps. The values reached are 0.12 nm for the C$\alpha$ atoms and 0.20 nm if all atoms are considered. These numbers are comparable with results obtained in crystal simulations of other proteins of equivalent length reported in the literature (van Gunsteren *et al.*, 1983; Berendsen *et al.*, 1986; Shi *et al.*, 1988; Heiner *et al.*, 1992; Levitt *et al.*, 1995). After 300 ps, however, the values increase slowly again. For the C$\alpha$ atoms, there is apparently a second plateau from 300 to 850 ps, but during this period the RMSD for all atoms continues to increase monotonically. After 850 ps, a final plateau is reached. During the second nanosecond of the simulation (1000–2000 ps), the RMSDs are 0.21 nm for the C$\alpha$ atoms and 0.29 nm for all atoms. The RMSD of chain 1 is an exception. There is a strong increase after 1200 ps due to a movement of a particular part of the chain which will be addressed later. To ensure that the RMSD values have converged, longer runs would be required.

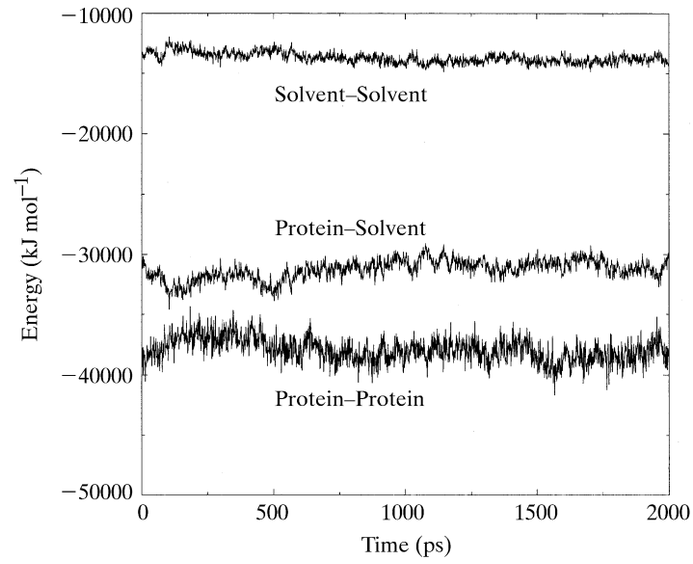


Fig. 20.1.3.1. Non-bonded energies (in kJ mol$^{-1}$) of the simulated system as a function of time.

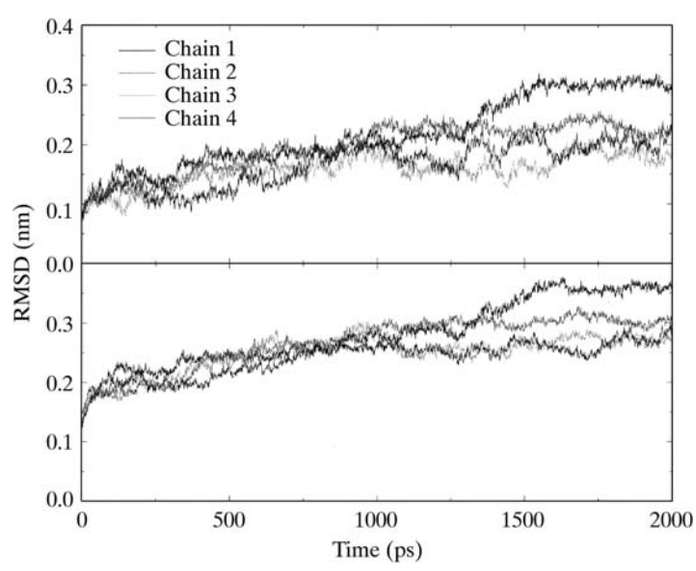


Fig. 20.1.3.2. Root-mean-square atom-positional deviations (RMSD) in nm from the X-ray structure of the four different protein molecules in the unit cell as a function of time. Rotational and translational fitting was applied using the C$\alpha$ atoms of residues 1–72. The upper and lower graphs show the deviations for the C$\alpha$ atoms and for all atoms, respectively.





references