Catalytic mechanism of cyclophilin as observed in molecular dynamics simulations: Pathway prediction and reconciliation of X-ray crystallographic and NMR solution data

DANIEL TRZESNIAK AND WILFRED F. VAN GUNSTEREN

Laboratory of Physical Chemistry, Swiss Federal Institute of Technology Zürich, ETH, CH-8093 Zürich, Switzerland

(RECEIVED May 18, 2006; FINAL REVISION July 6, 2006; ACCEPTED August 10, 2006)

Abstract

Cyclophilins are proteins that catalyze X-proline *cis-trans* interconversion, where X represents any amino acid. Its mechanism of action has been investigated over the past years but still generates discussion, especially because until recently structures of the ligand in the *cis* and *trans* conformations for the same system were lacking. X-ray crystallographic structures for the complex cyclophilin A and HIV-1 capsid mutants with ligands in the *cis* and *trans* conformations suggest a mechanism where the N-terminal portion of the ligand rotates during the *cis-trans* isomerization. However, a few years before, a C-terminal rotating ligand was proposed to explain NMR solution data. In the present study we use molecular dynamics (MD) simulations to generate a *trans* structure starting from the *cis* structure. From simulations starting from the *cis* and *trans* structures obtained through the rotational pathways, the seeming contradiction between the two sets of experimental data could be resolved. The simulated N-terminal rotated *trans* structure shows good agreement with the equivalent crystal structure and, moreover, is consistent with the NMR data. These results illustrate the use of MD simulation at atomic resolution to model structural transitions and to interpret experimental data.

Keywords: computer simulation; NMR; X-ray; molecular dynamics; GROMOS force field

Supplemental material: see www.proteinscience.org

Cyclophilins show diverse functions. One activity is the specific binding of Gly-Pro containing polypeptides. They also act as isomerases and catalyze the *cis-trans* interconversion of peptides that have a proline residue in their sequence, changing the dihedral angle between the proline and the preceding amino acid (Fischer et al. 1989; Takahashi et al. 1989; Ke and Huai 2004). This is a

reaction involved in numerous vital processes in nature (Gothel et al. 1996; Gothel and Marahiel 1999; Rovira et al. 2000; Stevens et al. 2001) and different structural mechanisms have been proposed (Harrison and Stein 1990; Kofron et al. 1991; Zhao and Ke 1996a,b). In principle, both *cis* and *trans* forms of the ligand should comparably bind the active site, but until some years ago only crystal structures of cyclophilin with the ligand in the *cis* conformation were available (Kallen et al. 1991; Ke et al. 1993; Zhao and Ke 1996a,b). The only exception was the cyclophilin/HIV-1 capsid complex, which had the ligand in the *trans* conformation (Gamble et al. 1996; Vajdos et al. 1997; Zhao et al. 1997). Three years ago,

Reprint requests to: Wilfred F. Van Gunsteren, Laboratory of Physical Chemistry, Swiss Federal Institute of Technology Zürich, ETH, CH-8093 Zürich, Switzerland; e-mail: wfvgn@igc.phys.chem.ethz.ch; fax: 41-1-6321-039.

Article and publication are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.062356406>.

Howard et al. (2003) successfully obtained crystal structures of both ligand conformations for cyclophilin/HIV-1 capsid complex mutants. These structures suggest a reaction mechanism in which the ligand makes a counterclockwise N-terminal rotation (viewed from the N-terminal) as had previously been suggested based on the *trans* cyclophilin/HIV-1 capsid structures. Simulation studies on the mechanism were performed by Hur and Bruice (2002). They simulated cyclophilin A starting from the *cis* and from the *trans* conformers and proposed a mechanism similar to the one proposed by Howard et al. (2003). However, a twist of $\sim 20^\circ$ from planarity was observed for both the *cis* and *trans* isomers, which is not present in the crystal structures. Agarwal and colleagues (Agarwal 2004, 2005; Agarwal et al. 2004) also investigated cyclophilin bound to diverse ligands using computer simulations and computed free energy profiles for the *cis*–*trans* rotation. Their structural analyses agree with the proposed N-terminal reaction mechanism. Additionally, they examined the protein response to the *cis*–*trans* isomerization reaction by performing a quasi-harmonic analysis of atomic vibrations. A complex of cyclophilin and the small ligand Ala-Ala-Pro-Phe (AAPF) in aqueous solution was studied by Eisenmesser et al. (2002) who measured NMR chemical shifts and relaxation rates. Based on their findings, they proposed a completely different reaction mechanism. They observed conformational change for protein residues L98 and S99 during catalysis and concluded that these residues would be involved in the process. Their observation could be explained assuming a C-terminal rotation of the ligand in the active site that would bring some ligand atoms closer to these two residues, altering their local chemical environment and thus producing the observed signal. The authors suggested that a study that provides a detailed picture of what is happening at the atomic level would be required to clarify the seeming inconsistencies between the crystal and solution data. This motivated us to initiate molecular dynamics (MD) simulations of cyclophilin A bound to the AAPF ligand in solution in an effort to resolve the seeming inconsistency between the X-ray crystal (Howard et al. 2003) and the NMR solution data (Eisenmesser et al. 2002). First we use MD simulation to obtain a *trans* ligand conformer (which was not available when the present study was started) through turning off the standard dihedral-angle potential energy term in the force field and applying a dihedral-angle restraint. We show the importance of thermal relaxation of the environment when modeling conformational transitions. Second, our simulations corroborate the mechanism proposed by Howard et al. (2003) and offer an alternative interpretation of the NMR data, showing that even an N-terminal rotation would explain the observations by Eisenmesser et al. (2002).

Results

Obtaining the ligand in the trans conformation

When this project started, almost all model structures available had the ligand bound to cyclophilin in the *cis* conformation, except the model for the cyclophilin/HIV-1 capsid complex (Gamble et al. 1996; Vajdos et al. 1997; Zhao et al. 1997), which shows an N-terminal rotated *trans* ligand conformation. We decided to start from a structure with the ligand in a *cis* conformation, because of its abundance over *trans* structures. The molecular coordinates were taken from the Protein Data Bank, PDB entry 1RMH (Zhao and Ke 1996a), which comprises cyclophilin A bound to the *cis* isomer of the Ala-Ala-Pro-Phe (AAPF) peptide. In order to obtain coordinates of a *trans* conformer of the ligand, we needed to devise a procedure to obtain these without a bias toward any mechanistic assumption (C-terminal or N-terminal rotation) regarding the *cis*–*trans* isomerization. Several approaches were available. One possibility would be to use a method similar to the one used by Hur and Bruice (2002), who modeled the N-terminal rotated ligand into the protein active site. In a first approach, we rotated the peptide ω dihedral angle to 180° and reconstructed or optimized conformations of side chains involved in steric hindrances. This was done for a C-terminal rotation and for an N-terminal rotation. However, such a procedure would easily result in a conformation that would never occur in reality and would not allow the protein environment to relax properly. Another possibility would be to use the approach by Agarwal (2004) who applied harmonic dihedral angle restraints centered at reference angles that were after given time periods increased by a 5° increment, covering the whole ω dihedral angle transition in 36 steps (Agarwal 2004). Using such a procedure, one obtains many discontinuous simulation trajectories and the relaxation of the environment is restricted by the limited motion of the ω dihedral angle. To avoid such problems, we opted for performing a MD simulation while forcing the dihedral angle ω between peptide residues A2 and P3 from 0° to 180° using only one nonphysical harmonic dihedral-angle restraining term centered at 180° with a very weak force constant. First, the physical dihedral-angle potential-energy term for this dihedral angle (transition barrier of 67 kJ mol^{-1} in the GROMOS force field (van Gunsteren et al. 1996; Schuler et al. 2001) was switched off and, already by just doing so, the ω angle quickly adopts an equilibrium value around 70° , exhibiting a counterclockwise N-terminal rotation when viewed from the N-terminal. This means that the protein active site naturally forces the ligand to this intermediate state. Then, a harmonic potential-energy term with its minimum at 180° was applied to the ω

dihedral angle starting with a weak force constant (broad potential energy well) that was gradually increased with time. This was done slowly to allow the environment to relax and to avoid sudden atomic displacements that could eventually produce local strain. The force constants are listed in Table 1. The dihedral angle change during the whole process is shown in Figure 1 and exhibits three distinct stages. The first one is an immediate jump from 0° to 70° . After ~ 100 psec, there is another transition to an equilibrium value of 135° , where the ω angle stays for about 400 psec when it makes another transition to the target value of 180° . Figure 2 pictures snapshots of the active site at five time points, the values of the dihedral angle ω between ligand residues A2 and P3 and the distances from the NH1 atom of residue R55 of cyclophilin to the oxygen (O) and nitrogen (N) atoms of the proline residue P3 of the AAPF peptide ligand. Panels A to E show a counterclockwise rotation of the dihedral angle ω and how the side chain of R55 of cyclophilin responds to this forced rotation. R55 is thought to be fundamental to this reaction because it stabilizes the transition state geometry of the ligand by interacting with the lone pair that is formed at the N atom of P3 and with the carbonyl oxygen atom of P3, restricting conformational changes to the N-terminal (Zhao and Ke 1996a; Hur and Bruice 2002; Li and Cui 2003; Agarwal et al. 2004). Thus, the general belief is that these interatomic protein–ligand distances should get shorter when the ligand is in the transition state conformation. A slight shortening of the mentioned distances is observed. We also see rotation and displacement of the whole ligand in the active site. In principle, after 600 psec, we have obtained a *trans* rotated ligand in the protein active site

Table 1. Force constant applied to force the *cis*→*trans* isomerization of the dihedral angle ω (A2-P3) of the AAPF ligand

Time period (psec)	Force constant ($\text{J mol}^{-1} \text{ degree}^{-2}$)
0–100	1
100–200	5
200–600	10
600–1400	50

(the two panels D and E show a very similar situation). However, at this early stage of the *cis* → *trans* transition, relaxation of the environment might not be complete yet. Therefore, the transition simulation including the forcing potential energy term was extended until 1400 psec. To investigate the degree of relaxation, we performed two simulations (without the forcing potential energy term and with the standard dihedral-angle potential energy term restored) starting from different *trans* conformations, one called *trans_early* at 600 psec and another called *trans* at 1400 psec. Besides these, a simulation starting from the *cis* isomer was also performed. Table 2 shows a summary of the simulations done. Details about the simulation setups can be found in the Materials and Methods section. Figure 3 shows Ramachandran plots (Ramachandran and Sasisekaran 1968) for the ligand residues A2 and P3. We observe that the relaxation of the environment plays a significant role within a few nanoseconds. In the top left panel we see that the *cis* simulation compares well with the experimental values obtained from the crystal structure. The top right panel shows the *cis* → *trans* transition, in which the ψ angle in

Figure 1. Time series of the peptide dihedral angle ω between the A2 and P3 residues of the AAPF ligand during the *cis* → *trans* simulation. The vertical dashed lines (from A to E) indicate the time points singled out for structural analysis (shown in Fig. 2).

Figure 2. Configurations at five time points (see Fig. 1) taken from the *cis* $\rightarrow$ *trans* trajectory. The protein is shown in ribbon representation colored according to its secondary structure, while the AAPF ligand is shown in stick representation (backbone thick sticks, side chains thin sticks) colored according to atom type. The four atoms that define the dihedral angle ω between A2 and P3 residues of the ligand that is forced to rotate from *cis* to *trans* are shown as yellow balls. The residue R55 of the protein is colored green. On the *left* the ligand is viewed from the side (N-terminal to the *left*) and on the *right* the ligand is viewed from the front (N-terminal to the front). Tables on the *left*: label, time point, dihedral angle ω between residues A2 and P3 and interatomic distances of the NH1 atom of residue R55 of the protein and the N and O atoms of residue P3 of the ligand. C and N stand for C-terminal and N-terminal, respectively, and indicate the orientation of the AAPF peptide.

A2 changes from 120° to -30° . In the bottom left panel (*early_trans*), we see that for the P3 the ϕ and ψ angles correspond to the experimental values, but for A2 the simulation results are off. However, after relaxation, the *trans* simulation in the bottom right panel corrects the results and brings them to match the crystallographic values.

Comparison with experimental X-ray crystal and NMR solution data

The structural comparisons with X-ray crystal data were done using the coordinates from PDB entry 1M9Y (Howard et al. 2003) because of the availability of a *cis* and a *trans* conformer of the ligand, even though in the

Table 2. Summary of simulations performed

Simulation label	Starting structure	A2-P3 ω dihedral angle (degree)	Simulation length (psec)
<i>cis</i> $\rightarrow$ <i>trans</i>	1RMH <i>cis</i> crystal (Zhao and Ke 1996a)	10 $\rightarrow$ 177	1400
<i>cis</i>	1RMH <i>cis</i> crystal	3 $\pm$ 9	2500
<i>early_trans</i>	<i>cis</i> $\rightarrow$ <i>trans</i> (after 600 psec)	180 $\pm$ 10	2500
<i>trans</i>	<i>cis</i> $\rightarrow$ <i>trans</i> (after 1400 psec)	173 $\pm$ 9	2500
1RMH <i>cis</i> (Zhao and Ke 1996a)	—	17 $\pm$ 4	—
1M9Y <i>cis</i> (Howard et al. 2003)	—	11 $\pm$ 5	—
1M9Y <i>trans</i> (Howard et al. 2003)	—	177 $\pm$ 5	—

The last column shows the average value and fluctuations during the simulation for the A2-P3 dihedral angle ω of the ligand, except for simulation *cis* $\rightarrow$ *trans*, where only the initial and final values are shown. The ω values of the crystal structures 1RMH (Zhao and Ke 1996a) and 1M9Y (Howard et al. 2003) are shown in the bottom three rows for comparison (for these, the fluctuations were estimated from the different copies of the protein in the crystal).

latter structure the ligand consists of a whole HIV-1 protein mutant with a corresponding amino acid binding sequence AAPI (the remainder of the HIV-1 protein was cut away). The first property to compare is the A2-P3 ligand ω dihedral angle. The average values in the different simulations are listed in Table 2 and show that neither the *cis* nor any of the *trans* structures show a large twist as proposed before by Hur and Bruice (2002). Our results rather agree with the crystal structures (Howard et al. 2003) that show almost no deviation from planarity for the *trans* conformer (177°) and a small twist toward the transition state (11°) for the *cis* conformer. Figure 4 shows the overlap of the crystal structures with the last structures from the simulations. The experimental and simulated protein structures exhibit a very good overlap, even in the flexible loops. The backbone atom-positional root-mean-square differences (RMSDs) from the X-ray structures are 0.14 nm for the *cis* ligand conformer and 0.12 nm for *trans* one at the end of the simulations. The ligand conformations also show a reasonably large overlap between the experimental and simulated structures (a final backbone RMSD of 0.06 nm in *cis* and 0.10 nm in *trans*). However, especially when in the *trans* conformation, the simulated ligand shifts its position inside the active site despite the good conformational agreement. One should bear in mind that in the case of the crystal structures, a whole protein was the molecule binding to cyclophilin. The replacement of the terminal groups of the tetra-peptide by the amino acid sequence of the remaining parts of the HIV-protein may of course lead to different results. However, here we aimed at simulating the complex as measured by NMR in order to interpret those solution data on the complex. To characterize the cyclophilin–AAPF association, we calculated protein–ligand interatomic distances. This would allow us to verify whether key interactions were still present and whether the NMR data that was supposed to be contradicting a N-terminal rotation (Eisenmesser et al. 2002) could be explained by our simulations. We calculated many interatomic distances involving key protein–ligand

interactions as a function of time and selected those that are more meaningful for display in Figure 5. In general, there is a fairly good agreement between the values calculated from the simulation and from the corresponding crystal structure. The black lines refer to the *cis* simulation and the results agree very well with the crystallographic values (magenta lines) for almost all protein–ligand contact pairs. For some pairs, e.g., involving side chain atoms of R55, the values deviate slightly in the beginning but quickly come back to the reference value. The comparisons of the *trans* simulations to the crystal data and to one another show more variation in Figure 5. We can see that interatomic AAPF–cyclophilin distances that reside in the N-terminal part of the ligand, e.g., A1(O)–R55(NH₂), A1(O)–N101(N), A1(O)–N102(N,O), A2(N,O)–N101(N,O), A2(N,O)–N102(N,O), A2(O)–Q63(NE₂), A2(CB)–R55(N,NH₁,NH₂), do not

Figure 3. Ramachandran plots for the ligand residues alanine 2 (A2) (black dots: simulation, green squares: crystal structures) and proline 3 (P3) (red dots, simulation; blue squares, crystal structures). The *top left* panel corresponds to the *cis* simulation, the *top right* to the *cis* $\rightarrow$ *trans* simulation, the *bottom left* to the *early_trans* simulation, and the *bottom right* to the *trans* simulation.

Figure 4. Overlay of the three-dimensional fold of the protein–ligand structures at the end of the MD simulations (green for the protein; black for the ligand) and the crystallographic X-ray structures (Howard et al. 2003) (blue for the protein; yellow for the ligand) of cyclophilin A with the ligand in the *cis* (top) and *trans* (bottom) conformations. The superposition was done using the atoms in the protein secondary structure elements. The protein is shown in ribbon representation, while the ligand is shown in stick representation (side chains are omitted for clarity). C and N stand for C-terminal and N-terminal, respectively, and indicate the orientation of the ligand (black) and the protein (green).

match the crystal values (cyan) in the *trans_early* simulation (red). However, in the *trans* simulation (green), many of these distances change to agree with the crystallographic values. This is the part of the system that suffers the most significant alterations during the ω dihedral-angle rotation process. This reflects that the environment had not completely relaxed yet when we did start the *trans_early* simulation. Some distances, e.g., A2(N,O)–N102(N,O), do not make it to the crystallographic values, most likely due to relaxation slower than a few nanoseconds. As a matter of fact, all distances concerning protein amino acids located in the loop between residues 100–110 do not satisfy the crystallographic distance bounds. This is due to the displacement of the ligand away from this loop during the ω dihedral-angle turning process (see Fig. 2). Next, we consider residues that show signal changes in the NMR experiment, the protein amino acids L98 and S99. It can be seen that in the *trans* conformation, residue A2 of the ligand is significantly further away from those amino acids than in the *cis* conformation, inducing a changing in their local chemical environment during catalysis.

This could be the explanation for the NMR observations made by Eisenmesser et al. (2002). Instead of the proposed C-terminal rotation of the ligand (which would bring the X4 ligand amino acid close to L98 and S99) we see that the simulated N-terminal rotation also fulfills the NMR data. The MD simulations seem to agree well with both crystallographic and NMR data and, moreover, reconcile the seeming inconsistency between the two.

Conclusions

In this study, we illustrate the predictive and the interpretative power of MD simulations based on a thermodynamically calibrated force field through a structural study of the catalytic mechanism of the *cis*–*trans* isomerase cyclophilin A with an AAPF peptide ligand. A simulation starting from the *cis* conformer showed that the simulation parameters and the force field used describe the complex with the ligand in the *cis* conformation satisfactorily. Second, using MD simulation in conjunction with a gently forcing potential energy term, we obtained a structure of the system with the ligand in the *trans* conformation that overlaps well with the corresponding crystal structure. An essential feature of the modeling procedure is to let the system sufficient time to relax and adapt to the changing dihedral angle. Third, the *cis* and *trans* simulations were used to resolve seeming contradictions between different experimental data. Our *trans* simulation indicates that a N-terminal ligand rotation would also be in agreement with the NMR experimental data presented by Eisenmesser et al. (2002) who suggested a C-terminal rotation. The N-terminal rotation moves the ligand A2 away from protein residues L98 and S99, modifying their local chemical environment, which can explain the NMR signal. Our simulations also support the hypothesis that both the *cis* and the *trans* conformations bind to cyclophilin with hardly any distortion from planarity. They corroborate the proposed N-terminal rotation mechanism proposed by Howard et al. (2003). This once more illustrates that MD simulations may provide the atomic detail and time resolution needed to interpret experimental data.

Materials and Methods

The MD simulations were performed using the GROMOS96 simulation program (van Gunsteren et al. 1996). The GROMOS 45A3 force field (van Gunsteren et al. 1996; Schuler et al. 2001) was used for modeling cyclophilin A and the ligand Suc-AAPF-NA. The nonstandard building blocks for the ligand ending groups Suc (succinyl) and NA (*p*-nitroanilide) were made based on the same set of parameters (for details, see Supplemental Materials). The number of (united) atoms was 1636 for the protein and 58 for the ligand. Residues with dissociative protons had the charge state chosen according to the most probable state at pH 7 (histidine residues protonated), yielding an overall

Figure 5. Time series of the distances from selected atoms of the protein (labels on the *right*) to selected atoms of the ligand (labels on the *top*) during the 2500 psec of simulation. The labels show the residue name and number, with the atom name within parentheses. In last column, X denotes phenylalanine (F) for the simulations and isoleucine (I) for the crystal structures. The black lines represent the *cis* simulation, whereas the red and green lines correspond to the *early_trans* and *trans* simulations, respectively. The lines in magenta and cyan stand for the values in the two X-ray crystallographic structures with the ligand in *cis* and *trans* conformation, respectively.

charge of $+5 e$ for the protein and $-1 e$ for the ligand. The initial coordinates of cyclophilin complexed with the ligand in the *cis* conformation were taken from the Protein Data Bank entry 1RMH (Zhao and Ke 1996a). The complex was placed at the center of a periodic truncated octahedral box with 11,033 water molecules equilibrated at 298 K and 1 atm. The model chosen for the solvent was SPC water (Berendsen et al. 1981). Bond lengths were kept rigid using the SHAKE (Ryckaert et al. 1977) algorithm with a relative geometric tolerance of 10^{-4} . A time step of 2 fsec was used to integrate the equations of motion and configurations were stored for analysis every 2 psec. Nonbonded interactions were treated by a triple-range cutoff scheme with a short-range cutoff radius of 0.8 nm and a long range cutoff of 1.4 nm. Inside the short-range cutoff, interactions were updated at every time step whereas between the short- and the long-range cutoff radii interactions were updated only every fifth step to save computational time. Beyond 1.4 nm a reaction-field force was applied with dielectric permittivity of 61 (Heinz et al. 2001). Simulations were carried out under constant temperature and pressure using the weak coupling technique (Berendsen et al. 1984). Solute and solvent were coupled to separate temper-

ature baths at 298 K with a coupling constant of 0.1 psec. The coupling constant to the pressure bath was 0.5 psec and an isothermal compressibility of $4.575 \times 10^{-4} (\text{kJ mol}^{-1} \text{nm}^{-3})^{-1}$ was used. Before starting the simulations the system was submitted to an equilibration process in which the first step consisted of two sequential steepest descent energy minimizations to adapt the system coordinates to the force field and to relax eventual geometric strain. The protein atoms were positionally restrained in the first energy minimization by a harmonic force (force constant of $250 \text{ kJ mol}^{-1} \text{nm}^{-2}$), which was removed in the second energy minimization. The second step was a thermalization, which was divided into five steps where the protein atoms were again harmonically positionally restrained with the force constant being decreased at each step ($5.0, 3.75, 2.5, 1.25 \times 10^4 \text{ kJ mol}^{-1} \text{nm}^{-2}$ and no restraints in the last one). Initial velocities were assigned in the first thermalization step based on a Maxwellian distribution at 300 K. The whole thermalization process was completed after a time period of 170 psec. The final structure obtained was the starting point for the *cis* $\rightarrow$ *trans* and the *cis* simulations. The *trans_early* and *trans* simulations had distinct starting points as specified in Table 2 and Figures 1 and 2 by the letters D and E, respectively.

The simulations were all 2500 psec long except the *cis* → *trans* simulation that was 1400 psec long. The trajectories and the crystal structures were analyzed using the GROMOS05 analysis software (Christen et al. 2005).

Acknowledgments

We thank Dorothee Kern for suggesting that we simulate this system. We also thank the National Center of Competence in Research (NCCR) in Structural Biology and the Swiss National Science Foundation for financial support.

References

- Agarwal, P.K. 2004. *Cis/trans* isomerization in HIV-1 capsid protein catalyzed by cyclophilin A: Insights from computational and theoretical studies. *Proteins* **56**: 449–463.
- Agarwal, P.K. 2005. Role of protein dynamics in reaction rate enhancement by enzymes. *J. Am. Chem. Soc.* **127**: 15248–15256.
- Agarwal, P.K., Geist, A., and Gorin, A. 2004. Protein dynamics and enzymatic catalysis: Investigating the peptidyl-prolyl *cis-trans* isomerization activity of cyclophilin A. *Biochemistry* **43**: 10605–10618.
- Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., DiNola, A., and Haak, J.R. 1984. Molecular-dynamics with coupling to an external bath. *J. Chem. Phys.* **81**: 3684–3690.
- Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., and Hermans, J. 1981. Interaction models for water in relation to protein hydration. In *Intermolecular forces* (ed. B. Pullman) pp. 331–342. Reidel, Dordrecht.
- Christen, M., Hünenberger, P.H., Bakowies, D., Baron, R., Bürgi, R., Geerke, D.P., Heinz, T.N., Kastenholz, M., Kräutler, V., Oostenbrink, C., et al. 2005. The GROMOS software for biomolecular simulation: GROMOS05. *J. Comput. Chem.* **26**: 1719–1751.
- Eisenmesser, E.Z., Bosco, D.A., Akke, M., and Kern, D. 2002. Enzyme dynamics during catalysis. *Science* **295**: 1520–1523.
- Fischer, G., Wittmann-Liebold, B., Lang, K., Kieffhaber, T., and Schmid, F.X. 1989. Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. *Nature* **337**: 476–478.
- Gamble, T.R., Vajdos, F.F., Yoo, S.H., Worthylake, D.K., Houseweart, M., Sundquist, W.I., and Hill, C.P. 1996. Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* **87**: 1285–1294.
- Gothel, S.F., Herrler, M., and Marahiel, M.A. 1996. Peptidyl-prolyl *cis-trans* isomerase of *Bacillus subtilis*: Identification of residues involved in cyclosporin A affinity and catalytic efficiency. *Biochemistry* **35**: 3636–3640.
- Gothel, S.F. and Marahiel, M.A. 1999. Peptidyl-prolyl *cis-trans* isomerases, a superfamily of ubiquitous folding catalysts. *Cell. Mol. Life Sci.* **55**: 423–436.
- Harrison, R.K. and Stein, R.L. 1990. Mechanistic studies of peptidyl prolyl *cis-trans* isomerase—Evidence for catalysis by distortion. *Biochemistry* **29**: 1684–1689.
- Heinz, T.N., van Gunsteren, W.F., and Hünenberger, P.H. 2001. Comparison of four methods to compute the dielectric permittivity of liquids from molecular dynamics simulations. *J. Chem. Phys.* **115**: 1125–1136.
- Howard, B.R., Vajdos, F.F., Li, S., Sundquist, W.I., and Hill, C.P. 2003. Structural insights into the catalytic mechanism of cyclophilin A. *Nat. Struct. Biol.* **10**: 475–481.
- Hur, S. and Bruice, T.C. 2002. The mechanism of *cis-trans* isomerization of prolyl peptides by cyclophilin. *J. Am. Chem. Soc.* **124**: 7303–7313.
- Kallen, J., Spitzfaden, C., Zurini, M.G.M., Wider, G., Widmer, H., Wüthrich, K., and Walkinshaw, M.D. 1991. Structure of human cyclophilin and its binding site for cyclosporin A determined by X-ray crystallography and NMR spectroscopy. *Nature* **353**: 276–279.
- Ke, H.M. and Huai, Q. 2004. Crystal structures of cyclophilin and its partners. *Front. Biosci.* **9**: 2285–2296.
- Ke, H.M., Mayrose, D., and Cao, W. 1993. Crystal-structure of cyclophilin-A complexed with substrate Ala-Pro suggests a solvent-assisted mechanism of *cis-trans* isomerization. *Proc. Natl. Acad. Sci.* **90**: 3324–3328.
- Kofron, J.L., Kuzmic, P., Kishore, V., Colonbonilla, E., and Rich, D.H. 1991. Determination of kinetic constants for peptidyl prolyl *cis-trans* isomerases by an improved spectrophotometric assay. *Biochemistry* **30**: 6127–6134.
- Li, G.H. and Cui, Q. 2003. What is so special about Arg 55 in the catalysis of cyclophilin A? Insights from hybrid QM/MM simulations. *J. Am. Chem. Soc.* **125**: 15028–15038.
- Ramachandran, G.N. and Sasisekharan, V. 1968. Conformation of polypeptides and proteins. *Adv. Protein Chem.* **23**: 283–438.
- Rovira, P., Mascarell, L., and Truffa-Bachi, P. 2000. The impact of immunosuppressive drugs on the analysis of T-cell activation. *Curr. Med. Chem.* **7**: 673–692.
- Ryckaert, J.-P., Ciccotti, G., and Berendsen, H.J.C. 1977. Numerical integration of the Cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. *J. Comput. Phys.* **23**: 327–341.
- Schuler, L.D., Daura, X., and van Gunsteren, W.F. 2001. An improved GROMOS96 force field for aliphatic hydrocarbons in the condensed phase. *J. Comput. Chem.* **22**: 1205–1218.
- Stevens, S.Y., Sanker, S., Kent, C., and Zuiderweg, E.R.P. 2001. Delineation of the allosteric mechanism of a cytidyl-transferase exhibiting negative cooperativity. *Nat. Struct. Biol.* **8**: 947–952.
- Takahashi, N., Hayano, T., and Suzuki, M. 1989. Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin-A-binding protein cyclophilin. *Nature* **337**: 473–475.
- Vajdos, F.E., Yoo, S.H., Houseweart, M., Sundquist, W.I., and Hill, C.P. 1997. Crystal structure of cyclophilin A complexed with a binding site peptide from the HIV-1 capsid protein. *Protein Sci.* **6**: 2297–2307.
- van Gunsteren, W.F., Billeter, S.R., Eising, A.A., Hünenberger, P.H., Krüger, P., Mark, A.E., Scott, W.R.P., and Tironi, I.G. 1996. *Biomolecular simulation: The GROMOS96 manual and user guide*. vdf Hochschulverlag AG, Zürich.
- Zhao, Y.D. and Ke, H.M. 1996a. Crystal structure implies that cyclophilin predominantly catalyzes the *trans* to *cis* isomerization. *Biochemistry* **35**: 7356–7361.
- Zhao, Y.D. and Ke, H.M. 1996b. Mechanistic implication of crystal structures of the cyclophilin-dipeptide complexes. *Biochemistry* **35**: 7362–7368.
- Zhao, Y.D., Chen, Y.Q., Schutkowski, M., Fischer, G., and Ke, H.M. 1997. Cyclophilin A complexed with a fragment of HIV-1 gag protein: Insights into HIV-1 infectious activity. *Structure* **5**: 139–146.