Molecular dynamics simulations of a K^+ channel blocker: Tc1 toxin from *Tityus cambridgei*

Alessandro Grottesi, Mark S.P. Sansom*

Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

Received 18 November 2002; revised 10 December 2002; accepted 10 December 2002

First published online 19 December 2002

Edited by Maurice Montal

Abstract Toxins that block voltage-gated potassium (Kv) channels provide a possible template for improved homology models of the Kv pore. In assessing the interactions of Kv channels and their toxins it is important to determine the dynamic flexibility of the toxins. Multiple 10 ns duration molecular dynamics simulations combined with essential dynamics analysis have been used to explore the flexibility of four different Kv channel-blocking toxins. Three toxins (Tc1, AgTx and ChTx) share a common fold. They also share a common pattern of conformational dynamics, as revealed by essential dynamics analysis of the simulation results. This suggests that some aspects of dynamic behaviour are conserved across a single protein fold class. In each of these three toxins, the residue exhibiting minimum flexibility corresponds to a conserved lysine residue that is suggested to interact with the filter domain of the channel. Thus, comparative simulations reveal functionally important conservation of molecular dynamics as well as protein fold across a family of related toxins.

Key words: Voltage-gated channel; Channel blocker; Essential dynamics; Molecular simulation

1. Introduction

Potassium channels consist of a large family of integral membrane proteins that regulate the electrical properties of cells. K channels play a major role in many physiological processes such as cell excitability, release of neurotransmitters, secretion of hormones, regulation of fluid secretion and lymphocyte activation [1,2]. Recent advances in membrane protein crystallography have revealed structures of two bacterial K channels [3–5]. The structural conservation between bacterial and animal K channels [6] means that the bacterial channel structures may be used as templates in modelling studies of animal K channels [7,8]. However, such models are by their very nature approximations, and in need of refinement. In particular, we need to improve the quality of K channel homology models in the region of their extracellular loops (which contribute to the structure of the extracellular mouth/vestibule of the channels).

A large number of peptide toxins isolated from venomous animals interact with voltage-gated ion channels and either physically block ion conduction or modify voltage-dependent gating [9,10]. Some of these toxins have been used as molecular probes to map the outer vestibule molecular surface of voltage-gated K (Kv) channels [11–13]. Indeed, it is widely assumed that the three-dimensional structure of these peptide toxins provides useful insights to understand the role that specific residues of the channel outer vestibule play in the mechanism of toxin binding and blocking. Thus, it may be possible to use such toxins as an inverse template for models of the vestibule. However, before this can be done with any degree of certainty we need a better understanding of the balance between flexibility and rigidity in these relatively small proteins.

The aim of this work was to investigate the conformational behaviour of the α-KTx peptide toxin Tc1 from the scorpion venom of *Tityus cambridgei* [14] by means of molecular dynamics (MD) simulations. This K channel blocker interacts strongly with the *Shaker* Kv channel, blocking its physiological activity. Tc1 contains 23 amino acids with three disulphide bridges. Its three-dimensional structure has been elucidated recently [15], consisting of an α/β-sandwich, with one short N-terminal helix and a double-stranded β-sheet at the C-terminus. It is the smallest K channel toxin from scorpion venoms. Its small and compact structure makes it particularly suitable for a detailed analysis of structural dynamics.

In order to characterise more fully the dynamical behaviour of this channel blocker, 10 ns duration MD simulations of Tc1 have been compared with comparable simulations of three other K channel toxins. These include two other peptide toxins that share the same fold: agitoxin-2 and charybdotoxin (both from *Leiurus quinquestriatus* var. *hebraeus* [16,17]); and a toxin with a somewhat different fold, namely κ-conotoxin PVIIA from *Conus purpurascens* [18]. The simulations are analysed in terms of the possible biological relevance of the balance between rigidity and flexibility in these peptide toxins.

2. Materials and methods

2.1. Systems

Initial coordinates were taken from the Protein Data Bank (http://www.rcsb.org) entries 1JLZ (for Tc1 [15]), 1AGT (for agitoxin-2, AgTx [19]), 2CRD (for charybdotoxin, ChTx [20,21]) and 1KCP (for κ-conotoxin, PVIIA [22]). For Tc1 two different starting structures were used, corresponding to the two closest models to the average nuclear magnetic resonance (NMR) structure.

2.2. Simulations

MD simulations were performed with GROMACS 3.0 [23] (http://www.gromacs.org) with a modified version of the GROMOS-87 forcefield [24]. Simulations were carried out in the NVT ensemble. A fixed-

*Corresponding author. Fax: (44)-1865-275182.
E-mail address: mark@biop.ox.ac.uk (M.S.P. Sansom).

0014-5793/02/$22.00
doi:10.1016/S0014-5793(02)03849-8

volume rectangular box with periodic boundary conditions was used. All toxins were simulated in the presence of explicit SPC-water molecules [25]. Each system contained approximately 1200–1800 water molecules and totalled about 3000–5000 atoms. Counterions were added where needed to keep all systems electrically neutral. The initial velocities were taken randomly from a Maxwellian distribution at 300 K. Non-bonding electrostatic interactions were calculated using the Particle Mesh Ewalds summation methods [26]. Lennard-Jones interactions were calculated using a cut-off of 0.9 nm. The pair lists were updated every 10 steps. The LINCS algorithm [27] was used to constrain bond lengths. The temperature was held constant by coupling to an external bath [28] using a coupling constant ($\tau = 1$ fs) equal to the integration time step. Configurations in all trajectories were saved every 0.1 ps.

A temperature coupling constant equal to the integration time step (1 fs) was used since we wished to minimise the effect of the temperature fluctuations on the convergence of the essential degrees of freedom. This value of coupling constant will result in a narrower temperature fluctuation distribution, resulting in a more accurate phase-space sampling in the NVT ensemble [29,30]. This approach has been used successfully in previous protein MD simulations [31,32]. However, we are aware that this approach could introduce some non-realistic effects. In order to test this we performed an additional 10 ns simulation on Tc1 using the Berendsen temperature-coupling scheme with a coupling constant $\tau = 100$ fs (i.e. weak coupling). We found no significant qualitative differences in the dynamic behaviour (as measured by essential dynamics analysis) between this simulation and the simulation performed using the strong coupling ($\tau = 1$ fs) scheme.

For all systems the solvent was relaxed, following energy minimisation, by 50 ps of MD at 300 K, while restraining protein atomic positions with a harmonic potential. The systems were then minimised without restraints and their temperature brought to 300 K in a stepwise manner: 10 ps long MD runs were carried out at 50, 100, 200 and 250 K, before starting the *production* runs at 300 K. All production runs were 10 ns long. Average properties were computed after discarding the first 1 ns of simulation for each system. Secondary structure content was calculated using DSSP [33]. Other analyses were performed using GROMACS and/or local code. Molecular graphics images were prepared using Molscript [34] and Raster3D [35].

2.3. Essential dynamics analysis

Quantitative characterisation of the dynamical properties of each system was performed using principal component analysis of the covariance matrix of the positional fluctuations of the Cα atoms, as described previously [36–38]. This matrix was built from the equilibrated portion of the trajectories (i.e. beyond the first nanosecond), and its diagonalisation afforded the principal directions of the large-amplitude concerted motions (principal eigenvectors) that characterise the essential subspace of each protein's internal dynamics.

3. Results and discussion

3.1. Molecular dynamics of Tc1

The structures of the four toxins are shown in Fig. 1. Tc1, AgTx and ChTx share a common fold, with the proposed channel-blocking lysine residue attached to the first strand of the β-hairpin. The helix is somewhat shorter in Tc1 than the other two related toxins. The PVIIA toxin has a different fold, albeit still containing a short α-helix plus two antiparallel β-strands.

In order to assess the stability of the MD trajectories, the root mean square deviations (RMSD) of Cα atoms with respect to their initial coordinates (from the NMR structures) were monitored for all the systems as a function of time (Fig. 2). It seems that the timescale of the simulations was sufficient to allow relaxation of all the systems towards an equilibrated state. For Tc1 the RMSDs for the two simulations are very similar, and so in the remainder of this paper the results from the two Tc1 simulations were merged. In all of the simulations, especially that of AgTx (Fig. 2C), there is evidence from the RMSD that a toxin may switch away from and back towards the NMR model. This gives us some confidence in the extent of our conformational sampling during these multiple 10 ns simulations.

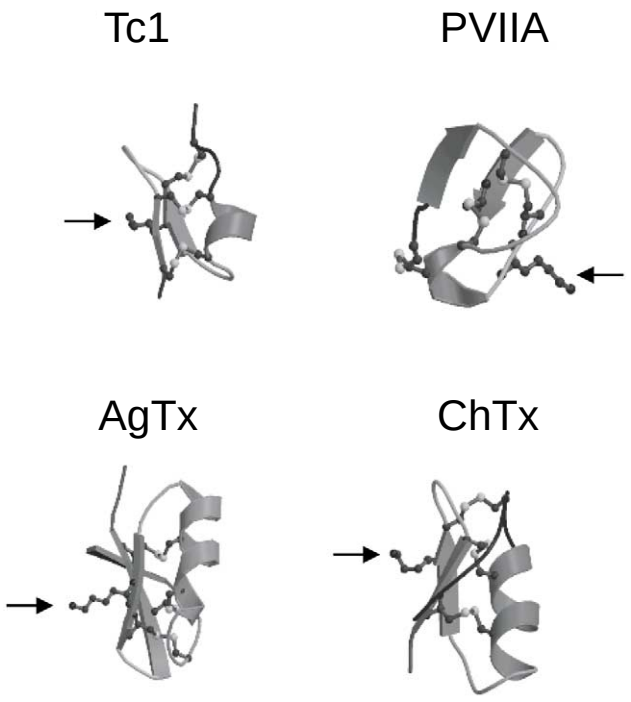

Fig. 1. Fold diagrams for the four toxins showing the disulphide bridges *and* the key Lys residues implicated in block (for AgTx, ChTx, and PVIIA) or suggested to be involved in block (for Tc1) of the K channel filter (the latter highlighted via arrows).

In order to further assess the stability of the trajectories, the time-averaged secondary structure content of the Tc1 system was checked (data not shown). The probability of each residue to be in the α, β or random coil conformation was analysed: the N-terminal α-helix was located at residues 5–8; a double-stranded β-sheet was formed from residues 13 to 16 and from 19 to 22. The position and length of each element of secondary structure was comparable with that in both of the starting structures.

Since Tc1 has three disulphide bridges the overall flexibility of the molecule is expected to be low. The root mean square fluctuations (RMSFs) of the backbone atoms of the Tc1 toxin are shown in Fig. 3A. It is evident that, with the exception of the N-terminal region, the two turns form the most flexible regions of the protein. All the residues within the α-helix and β-sheet have comparable low fluctuations. As might be expected, the flexibility of cysteines at positions 5, 9, 15, 20 and 22 was low, reflecting the constraints on the backbone due to the disulphide bridges. Interestingly, Lys-14 is the residue that displays the lowest RMSF in the Tc1 simulation(s). Lys-14 is homologous to Lys-27 in ChTx and AgTx. For the latter two toxins, the homologous lysine is quite rigid (Fig. 3C,D). Indeed, the overall flexibility profiles for Tc1, AgTx and ChTx show strong similarities. Since Lys-27 in ChTx and AgTx has been shown to be functionally relevant for Kv channel block by these toxins [13], Lys-14 in Tc1 would seem to also play a major role in the interaction with the outer vestibule of *Shaker* channels.

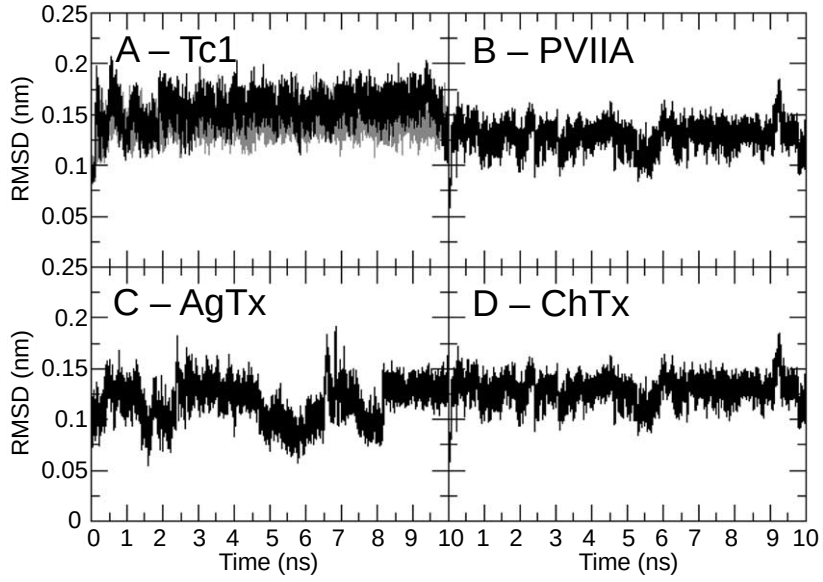

Fig. 2. Cα RMSDs vs. time for simulations of (A) Tc1, (B) PVIIA, (C) AgTx and (D) ChTx. For Tc1 the two lines (black and grey) correspond to the two simulations performed. Note that in the case of Tc1, the first residue has been discarded in this and further analyses because of its exceptional flexibility as a result of its N-terminal location.

3.2. Essential dynamics analysis of Tc1

In order to investigate in greater depth the motions that take place in Tc1 and the other toxins a comparative principal component analysis of the Cα atoms was performed. In Fig. 4 we show the eigenvalue spectra of the diagonalised covariance matrices. Only the first 10 eigenvalues (corresponding to the essential eigenvectors) are plotted. It can be seen that for Tc1 the protein's main degrees of freedom are confined within the first 10 eigenvectors. In particular, the first and second eigenvectors of the fluctuations covariance matrix accounted for 50% of the overall fluctuations of the protein (data not shown).

To probe further the details of the dynamic behaviour of Tc1, a per residue basis displacement along the first two eigenvectors has been calculated (Fig. 4B). A concerted motion between the N-terminal region and the β-turn is clearly visible in the first eigenvector. This is in agreement with inspection of the three-dimensional structure, which reveals these two regions to be close in space, and thus their motion is likely to

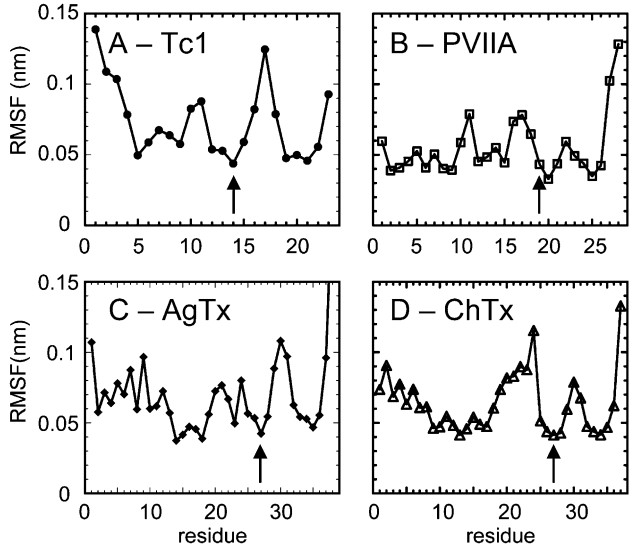

Fig. 3. RMSFs vs. residue number for (A) Tc1, (B) PVIIA, (C) AgTx and (D) ChTx. In each case the arrow indicates the lysine residue implicated in channel block.

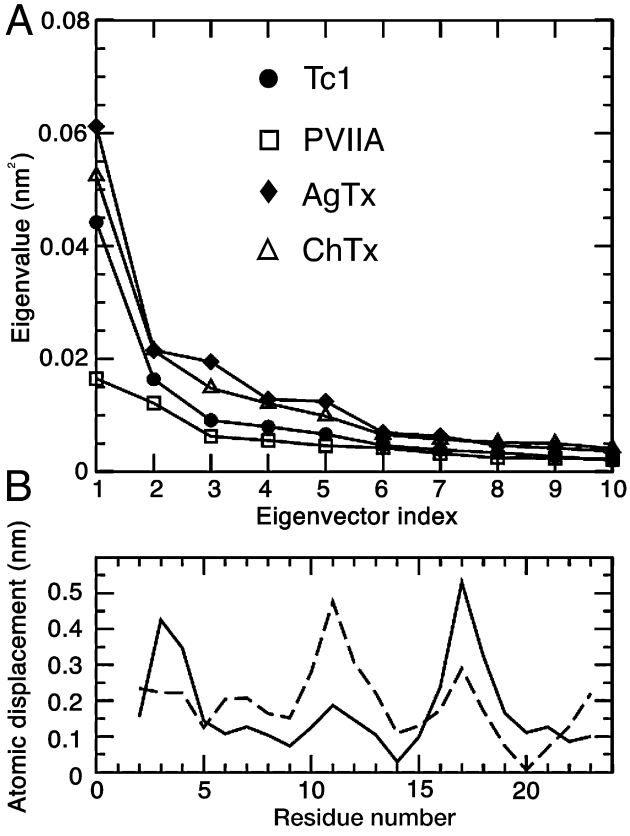

Fig. 4. A: Eigenvector spectra for the four simulations. B: Atomic displacements along the first (solid line) and second (dashed line) eigenvectors for the Tc1 simulation.

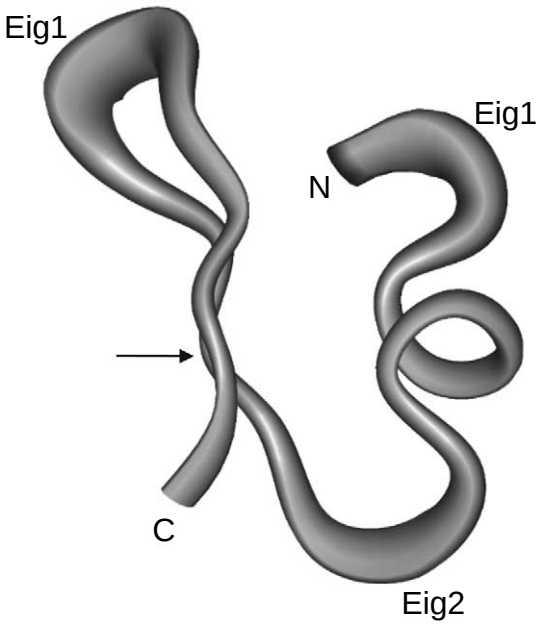

Fig. 5. Tc1 fold, displayed as a Cα trace with the trace radius proportional to the atomic displacements due to the first two eigenvectors. The approximate position of Lys-14 is shown by the arrow.

be mechanically correlated (see also Fig. 5). The second eigenvector is mainly dominated by the motion of the loop between the α-helix and the first β-strand. It is worth noting that in both eigenvectors, Lys-14 is one of the two least flexible residues in the toxin.

3.3. Comparison with other toxins

It is of interest that the eigenvalue spectra are very similar for the three toxins that share the same α/β-fold (i.e. Tc1, AgTx and ChTx). In particular for all three of these toxins the first and second eigenvectors accounted for about 50% of the motion. This suggests that at least some aspects of dynamic behaviour are conserved across a protein fold class.

In contrast, PVIIA displayed a somewhat different behaviour, with no single component dominating the peptide fluctuations, with the first two eigenvectors accounting for 40% of the motion. This is interesting, as both experimental [39–41] and simulation studies [42] on PVIIA suggest that no particular residue is a key to its blocking activity.

4. Conclusions

On the basis of both sequence alignment [10] and solution structure (NMR) data [15], it has been postulated the Lys-14 in Tc1 is likely to play a major role in the blocking mechanism of the voltage-gated *Shaker* Kv channel. Our simulation data support this hypothesis, as the flexibility of the polypeptide backbone reaches a minimum at residue 14, which is located within a secondary structure element. Thus the immediate vicinity of the blocking moiety, which is expected to enter the outer mouth of the Kv pore, is relatively fixed in its geometry. This may aid use of docking studies with Tc1 and related toxins [43] to refine homology models [8,44] of the Kv vestibule and pore. Essential dynamics analysis of Tc1 suggests the presence of concerted motions in the toxin structure, and that these are conserved across the family of related α/β toxins acting on Kv channels. Such conservation of the pattern of flexibility suggests it may have some functional importance. Further studies of toxin/Kv interactions will be required to reveal the exact nature of the relationship between peptide rigidity/flexibility and peptide toxin/channel interactions.

Acknowledgements: This work was supported by grants from the Wellcome Trust. Our thanks to colleagues for valuable discussions, especially Dr P.C. Biggin and Dr A. Amadei.

References

[1] Hille, B. (2001) Ionic Channels of Excitable Membranes, Sinauer Associates, Sunderland, MA.
[2] Ashcroft, F.M. (2000) Ion Channels and Disease, Academic Press, San Diego, CA.
[3] Doyle, D.A., Cabral, J.M., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Cahit, B.T. and MacKinnon, R. (1998) Science 280, 69–77.
[4] Zhou, Y., Morais-Cabral, J.H., Kaufman, A. and MacKinnon, R. (2001) Nature 414, 43–48.
[5] Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B.T. and MacKinnon, R. (2002) Nature 417, 515–522.
[6] MacKinnon, R., Cohen, S.L., Kuo, A., Lee, A. and Chait, B.T. (1998) Science 280, 106–109.
[7] Capener, C.E., Shrivastava, I.H., Ranatunga, K.M., Forrest, L.R., Smith, G.R. and Sansom, M.S.P. (2000) Biophys. J. 78, 2929–2942.
[8] Capener, C.E., Kim, H.J., Arinaminpathy, Y. and Sansom, M.S.P. (2002) Hum. Mol. Genet. 11, 2425–2433.
[9] Miller, C. (1995) Neuron 15, 5–10.
[10] Tytgat, J., Chandy, K.G., Garcia, M.L., Gutman, G.A., Martin-Eauclaire, M.-F., van der Walt, J.J. and Possani, L.D. (1999) Trends Pharmacol. Sci. 20, 444–447.
[11] MacKinnon, R., Heginbotham, L. and Abramson, T. (1990) Neuron 5, 767–771.
[12] Gross, A. and MacKinnon, R. (1996) Neuron 16, 399–406.
[13] Naranjo, D. and Miller, C. (1996) Neuron 16, 123–130.
[14] Batista, C.V.F., Gòmez-Lagunas, F., Lucas, S. and Possani, L.D. (2000) FEBS Lett. 486, 117–120.
[15] Wang, I., Wu, S.-H., Chang, H.-K., Shieh, R.-C., Yu, H.-M. and Chen, C. (2002) Protein Sci. 11, 390–400.
[16] Gimenez-Gallego, G., Navia, M.A., Reuben, J.P., Katz, G.M., Kaczorowski, G.J. and Garcia, M.L. (1988) Proc. Natl. Acad. Sci. USA 85, 3329–3333.
[17] Garcia, M.L., Garcia-Calvo, M., Hidalgo, P., Lee, A. and MacKinnon, R. (1994) Biochemistry 33, 6834–6839.
[18] Olivera, B.M. (1997) Mol. Biol. Cell 8, 2101–2109.
[19] Krezel, A.M., Kasibhatla, C., Hidalgo, P., Mackinnon, R. and Wagner, G. (1995) Protein Sci. 4, 1478–1489.
[20] Bontems, F., Gilquin, B., Roumestand, C., Menez, A. and Toma, F. (1992) Biochemistry 31, 7756–7764.
[21] Bontems, F., Roumestand, C., Gilquin, B., Menez, A. and Toma, F. (1991) Science 254, 1521–1523.
[22] Savarin, P., Guenneugues, M., Gilquin, B., Lamthanh, H., Gasparini, S., Zinn-Justin, S. and Menez, A. (1998) Biochemistry 37, 5407–5416.
[23] Lindahl, E., Hess, B. and van der Spoel, D. (2001) J. Mol. Model. 7, 306–317.
[24] van Gunsteren, W.F. and Berendsen, H.J.C. (1987) Gromos-87 manual, Biomos BV, Groningen.
[25] Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F. and Hermans, J. (1981) Intermolecular Forces, Reidel, Dordrecht.
[26] Darden, T., York, D. and Pedersen, L. (1993) J. Chem. Phys. 98, 10089–10092.
[27] Hess, B., Bekker, H., Berendsen, H.J.C. and Fraaije, J.G.E.M. (1997) J. Comp. Chem. 18, 1463–1472.
[28] Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., Di Nola, A. and Haak, J.R. (1984) J. Chem. Phys. 81, 3684–3690.
[29] D'Alessandro, M., Tenenbaum, A. and Amadei, A. (2002) J. Phys. Chem. B 106, 5050–5057.
[30] Evans, D.J. and Morriss, G.P. (1983) Phys. Lett. A 98, 433–436.

[31] Grottesi, A., Ceruso, M.A., Colosimo, A. and Di Nola, A. (2002) Proteins Struct. Func. Genet. 46, 287–294.
[32] Ceruso, M.A., Amadei, A. and Di Nola, A. (1999) Protein Sci. 8, 147–160.
[33] Kabsch, W. and Sander, C. (1983) Biopolymers 22, 2577–2637.
[34] Kraulis, P.J. (1991) J. Appl. Crystallogr. 24, 946–950.
[35] Merritt, E.A. and Bacon, D.J. (1997) Methods Enzymol. 277, 505–524.
[36] Garcia, A.E. (1992) Phys. Rev. Lett. 68, 2696–2699.
[37] Wong, C.F., Zheng, C., Shen, J., McCammon, A. and Wolynes, P.G. (1993) J. Phys. Chem. 97, 3100–3110.
[38] Amadei, A., Linssen, A.B.M. and Berendsen, H.J.C. (1993) Proteins Struct. Funct. Genet. 17, 412–425.
[39] Jacobsen, R.B., Koch, E.D., Lange-Maleck, B., Stocker, M., Verhey, J., Van Wagoner, R.M., Vyazovkina, A. and Olivera, B.M. (2000) J. Biol. Chem. 275, 24639–24644.
[40] Naranjo, D. (2002) Biophys. J. 82, 3003–3011.
[41] Shon, K.J., Stocker, M., Terlau, H., Stuhmer, W., Jacobsen, R., Walker, C., Grilley, M., Watkins, M., Hillyard, D.R., Gray, W.R. and Olivera, B.M. (1998) J. Biol. Chem. 273, 33–38.
[42] Moran, O. (2001) Eur. Biophys. J. 30, 528–536.
[43] Eriksson, M.A.L. and Roux, B. (2002) Biophys. J. 83, 2595–2609.
[44] Ranatunga, K.M., Smith, G.R., Law, R.J. and Sansom, M.S.P. (2001) Eur. Biophys. J. 30, 295–303.