Research Article

Journal of Physical Organic Chemistry

Received: 20 February 2008, Revised: 29 April 2008, Accepted: 27 May 2008, Published online in Wiley InterScience: 10 July 2008

(www.interscience.wiley.com) DOI 10.1002/poc.1413

Binding of ciguatera toxins to the voltage-gated Kv1.5 potassium channel in the open state. Docking of gambierol and molecular dynamics simulations of a homology model

Francesco Pietra[a*]

Ciguatera poisoning is a toxinological syndrome from ingestion of seafood contaminated by dinoflagellate toxins which has serious social and economic consequences from the Indo-Pacific to the Caribbean. These polyannealed ethereal-ring toxins, which comprise ciguatoxins, maitotoxin, and gambierol, are known to affect ion channels. Reported here are the first indications at molecular level as to the mode of interaction of these toxins with ion channels. The study concerns gambierol, an eight-ring ladder polyether which is known to affect TRPV1-type of thermal and pain sensation channels, as well as to inhibit voltage-gated currents in K^+ channels of mouse taste cells. Automated docking of gambierol on a homology model of the voltage-gated Kv1.5 potassium ion channel in implicit solvent is followed by molecular dynamics (MD) simulation of the complex in a POPC membrane solvated with water. It is found that gambierol binds to the internal helices of the channel, unequally to the different subunits of the tetramer. Such unequal binding is a novel observation that should stimulate and aid developing a much demanded medical treatment of ciguatera poisoning. Copyright © 2008 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: gambierol; ciguatera poisoning; docking; molecular dynamics; transmembrane proteins

INTRODUCTION

Ciguatera poisoning is a toxinological syndrome resulting from ingestion of contaminated seafood from the tropics.[1–4] Typical symptoms comprise gastrointestinal, neurocutaneous and constitutional anomalies, including nervousness, cardiovascular upheaval, inverse temperature sensation, muscle cramps, headache and bewildering that may last from weeks to much longer. Worse, following the initial syndrome, subjects become hypersensitive to ciguateric seafood.[3]

No effective treatment of ciguatera poisoning is available, and, because of such enigmatic mixture of symptoms, misdiagnoses may occur.[3] With at least 50 000 cases of poisoning reported annually from the Indo-Pacific to the Caribbean, this syndrome has serious social and economic consequences, in particular job losses and banning of fish sale in certain areas and periods such as the barracuda in the Caribbean.[1]

Diagnosis of ciguatera is mostly based on etiology, knowing that the intoxication is caused by annealed (ladder) polyethers produced by the epiphytic dinoflagellate *Gambierdiscus toxicus* and spread along the marine food chain in otherwise edible fish and mollusk.[4] The toxin suite comprises ciguatoxins, gambierol, and maitotoxin.[4] These long molecules (if stretched, they are in the nanometer length range) show high affinity for transmembrane proteins, affecting the ion course in voltage-gated ion channels. It is well established that maitotoxin increases the Ca^{++} cytosolic concentration via activation of Ca^{++}-permeable, non-selective cation channels. Ciguatoxin CTX1B at low or moderate concentration partially blocks potassium currents, while even low doses suffice to block voltage-gated Na^+ channels by binding at receptor site 5, thus enhancing neuronal excitability.[2] The same receptor site 5 is chosen by toxic ladder polyethers (brevetoxins) released by the Caribbean dinoflagellate *Karenia brevis*.[5,6] This is counteracted by both brevenal (from the same dinoflagellate[5]) and gambierol.[7] Notably, gambierol is also known to affect TRPV1-type of thermal and pain sensation channels,[8] as well as to inhibit voltage-gated currents in K^+ channels of mouse taste cells.[9]

The binding sites of ladder polyethers to K^+ channels remain unknown. Therefore, to overcome present limits to experimentation, while providing a multifaceted approach to these complex phenomena, computational docking and molecular dynamics (MD) simulations are in order.

* *Correspondence to: F. Pietra, Accademia Lucchese di Scienze, Lettere e Arti, founded in 1584, Palazzo Ducale, 55100 Lucca, Italy.*
E-mail: chiendarret@gmail.com

a *F. Pietra*
Accademia Lucchese di Scienze, Lettere e Arti, Palazzo Ducale, 55100 Lucca, Italy

For gambierol, the biophysical characteristics of the ionic currents suggest a delayed rectifier type of channel,[10] hence very likely of the Kv1.5 type.[11] Therefore, relying on the solid knowledge that all voltage-gated K^+ channels differ very little from one another in the channel portion, recourse is made here to a homology model (called 'teepee' in the following) of the Kv1.5 K^+ channel from available high-resolution X-ray diffraction data for mammalian Kv1.2.[12]

METHOD

All docking (serial mode) and MD simulations (parallel mode) were carried out on a computer based on AMD dual-core Opteron CPUs, with 4 GB RAM per cpu, driven by Linux Debian amd64 etch as operative system. Program DOCK, version 6.1,[13] was compiled with gcc version 4:4.1.1–15, and was used in combination with programs DMS,[14] SPHGEN-CPP, version 1.2,[15] SPHERE-SELECT, version 1.0,[16] and Chimera, version 1.2422.[17] Amber, version 9,[18] was compiled with Intel® ifort, version 9.1.036, and icc, version 9.1.042, which were also used to compile OpenMPI, version 1.2.3, as support for parallel execution of the simulation programs. The structure of gambierol[19,20] was minimized with the molecular mechanics program PCMODEL, version 9.1,[21] MMFF94 force field,[22] running on Linux Debian i386 etch with OpenGL graphic support. Simulated annealing MD *in vacuum* was carried out with Amber 9, driven by Xanneal Python script.[23]

DOCK 6.1[13] offers a wide variety of algorithms to predict binding poses, superimposing a potential ligand onto a negative image of the binding pocket (created with DMS[14]), starting from the crystal or NMR structure of the receptor. I choose grid scoring for the whole protein model, making recourse to SPHGEN-CPP[15] and SPHERE-SELECT[16] to generate and select spheres, as DOCK's own sphere generator and selector proved unable to perform with so many spheres (685) and grid points (10 536 750). As initial structures, I chose the crystal structure of the protein[12] and the least energy conformation of the ligand from simulated annealing MD *in vacuum*. The procedure was started with rigid body docking,[13] where both the protein and the ligand initial conformations were held fixed. Then, flex body docking[13] was carried out, where the ligand was allowed to move and which requires as much as 9GB memory. In either case, I relied on minimum ligand–protein binding energy for best scoring. Re-scoring (amber score[13]) was then carried out with the ligand best pose from flex body docking, using a sphere representation of the receptor for the distance-movable-region. This is an MD-conjugated-gradient minimization in implicit solvent, where the solvation energy is calculated using the Generalized Born solvation model. In this procedure, the ligand only is first allowed to move, then all the atoms in the ligand and receptor are allowed to move. The best scored complex ligand–protein from this procedure was embedded in a lipid membrane and MD simulation was carried out with program Amber.

In essential details, while closely following a guideline, [24] for compatibility of programs the Kv1.5 model had to be rebuilt from MacKinnons Kv1.2 X-ray diffraction data.[12] The model includes residues 417–527 (standard Kv1.5 numbering[25]) for segments S5 and S6, the selectivity filter, the pore protein, and a water molecule in the fourth position of the selectivity filter. This will be called 'teepee' from here on. For gambierol, Cartesian coordinates from the PCMODEL[21] minimized structure and related parameters from Antechamber,[26] force field GAFF,[27] as implemented in Amber 9,[18] were used to run a 10 000 cycles minimization *in vacuum* at constant volume, without any restraint on any atom, time step 0.001 ps. Although the structure of gambierol from PCMODEL minimization was strain free, this minimization/heating procedure was needed to get simulated annealing *in vacuum*[23] running correctly. This involved repetitive heating to 800 K and cooling down to 50 K, collecting 100 conformations, from which the least energy conformation was chosen and used for docking. To this end, the molecular surface of teepee, deprived of all hydrogen atoms, was calculated with DMS,[14] while spheres were generated[15] and selected[16] for a 25 Å radius centered on the oxygen atom of the water molecule at the fourth position of the selectivity filter. The spheres covered most teepee, only leaving out the short loops on the extracellular side and the tail of the truncated helices on the intracellular side. A grid for docking was set up for a box of $66 \times 67 \times 63$ Å, totaling 10 536 750 grid points. AM1-BCC charges for gambierol were MOPAC calculated with Antechamber,[26] as implemented in Chimera.[17] An 80×80 Å bilayer of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), with polar heads solvated by TIP3P water, was created with Membrane plugin.[28] Cartesian coordinates for POPC were extracted from the membrane pdb file, while parameters were obtained from divcon calculation with Antechamber,[26] force field GAFF,[27] and AM1-BCC charges. With Chimera,[17] pdb files were opened for both the membrane and the best-scored protein-ligand complex, the latter aligned along the main axis of the membrane and translated to the center. POPC and water molecules overlapping the complex were eliminated, with a 1.5 Å margin around the complex. The aligned complex and membrane were combined in Amber 9 LEaP, force fields ff99SB [18] and GAFF,[27] and solvated with TIP3P water, with 12 Å buffer, getting a $113 \times 110 \times 92$ Å box. This was minimized at constant volume with restraint by a harmonic potential with a force of 30 kcal/(mol Å^2) on the teepee-complex and lipid. Minimization was continued without any restraint on any other atom, initially for 1000 steps of steepest descent, time step 0.001 ps, followed by 1000 steps of conjugated gradient minimization. Further 1500 steps of steepest descent minimization were carried by removing all restraints. Langevin heating from 0.1 to 300 K was carried out in 50 ps with restraint by a force constant of 32 kcal/(mol Å^2) on the teepee-complex and the polar head of POPC, under SHAKE, with 0.002 ps time step. The system was simulated at constant temperature of 300 K and pressure of 1.0 atm for 550 ps, all restraints were removed, and simulation continued for 8 ns (0.0015 ps time step). Although no special attention was paid to lateral diffusion in the lipid bilayer,[29] no anomaly was observed. RMSD *versus* time was calculated with the RMSD Trajectory Tool,[30] from production MD, with respect to frame 0 and by first aligning the structures. Clustering was carried out with the Cluster plugin.[31] Mapping of protein residues around the ligand was accomplished with Chimera,[17] from which Figs 1 and 2 were also derived.

RESULTS AND DISCUSSION

The validity of the homotetramer homology models of Kv1.5 channel, derived from the crystal structure of mammalian potassium channel Kv1.2 in the open state[12] was already discussed exhaustively.[24] It is only worth reminding here that

Journal of Physical Organic Chemistry

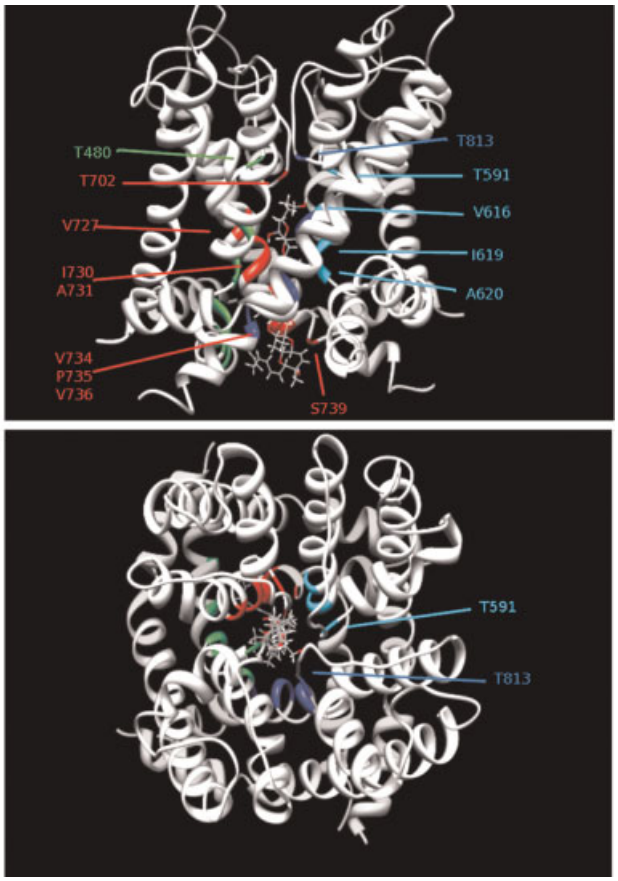

Figure 1. **A**: side view of minimum-energy Kv1.5 K^+ channel homology model–gambierol complex, hiding the POPC/H_2O membrane used for MD simulation. Extracellular zone on top and cytoplasmic side at the bottom. Protein residues at $\leq$3.0 Å from gambierol (oxygen atoms in red and hydrogen atoms in white) are represented in different colors for the different subunits of the tetramer and the related filter: green, cyan, red, and blue for subunits 1, 2, 3, and 4, respectively. Numbering of the amino acid sequence is fully indicated (by the residue name, according to standard Kv1.5 numbering,[25] and pointing line of same color) for subunits 2 and 3, while for subunits 1 and 4 the filter only is indicated to avoid overcrowding of labels. However, the position of all other residues of subunits 1 and 4 can be easily appreciated by recalling (as in the text) that descending along Fig. 1A corresponds to descending along Table 1, for the teepee from the short loop to the truncated helix for each subunit, and for gambierol from the carbinol head to the polyene tail.
B: top view corresponding to side view A. The hydroxyl oxygen of gambierol is seen in red, along with the adjacent methylene group in white, at the southeastern part of the channel. Filter residues to which this hydroxyl hydrogen atom is most close (T591 and T813) are indicated by the residue names and pointing lines of the same color

the sequence identity of Kv1.2 with Kv1.5, for which there is no crystal structure available, is about 90% in the pore region. The latter is of our concern. This Kv1.5 homology model was already used successfully for docking small ligands, such as *ortho,ortho*-disubstituted bisaryls.[24] These small ligands fulfill the rule of thumb of drug hunting that docking within 2 Å RMSD of the crystallographic pose can only be achieved with less than eight rotatable bonds.

Nature has no such restraints, being able to dock selectively large, non-polymeric ligands to proteins, which is the basis of many processes. Present work aimed at nature's capability in docking a large, flexible natural ligand. To achieve that, recourse was made to a docking computational program, DOCK 6.1,[13] with contributed software to treat large ligands,[15,16] as explained above. Docking was just a starting point for MD simulation with the complex. Thus, the best scored teepee–gambierol complex from automated docking in implicit solvent[13] was embedded in a phospholipid bilayer of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), solvated with water, and equilibrated, followed by standard MD simulation. This constitutes a more realistic environment than the water-solvated octane box used for MD simulations with anti-fibrillation drugs.[24] The minimum energy structure (quite similar to the averaged structure) after 8 ns of MD simulation, freed of the lipid bilayer for clarity, is shown in Fig. 1A, where the teepee residues in closest contact with gambierol, characterized by the same color for the same unit of the tetramer, are indicated with pointers for subunits 2 and 3 and in part also for subunits 1 and 4. At any event, the amino acid sequence can also be easily distinguished with the aid of Table 1. In doing so, one has to bear in mind that descending along Table 1 corresponds to descending along Fig. 1A, for the teepee from the short loop to the truncated helix for each subunit, and for gambierol from the carbinol head to the polyene tail.

It should be remarked that the single most critical residue, T480, and other important residues, V505, V512, V516, identified by alanine scanning for anti-fibrillation drugs that block K^+ channels,[32] are the most relevant ones with gambierol too. What's more, Table 1 and Fig. 1A reveal unequal interaction of gambierol with the different subunits of the tetramer. This has no precedent in either experimentation with Kv1.5 channels[32] or simulations with homology models,[24] where no distinction was ever made as to docking to the tetramer subunits.

Unequal docking to the teepee is most dramatically corroborated by mapping around the gambierol hydroxyl hydrogen atom. Within a distance of 2.5 Å, this hydrogen atom turns out to point to T591 (filter/2) and T813 (filter 4). The shortest distance (2.18 Å) is found between the gambierol hydroxyl hydrogen atom and the T591 hydroxyl oxygen atom, with an O—H—O angle of *ca.* 153°. This situation is compatible with strong H-bonding,[33] which can be viewed to contribute to the orientation of gambierol with its polar head on the filter side. Figure 1B best illustrates the point, with the hydroxyl group of gambierol pointing to the right toward cyan-colored T591.

It is also worth noticing that the polyene chain of gambierol is selectively oriented toward the S3 helix (the *cis* C38 proton of gambierol is closest ($\leq$3.0 Å) to V736 (S3/3)). All adaptations of the ligand to the protein make gambierol quite strained, while the RMSD between teepee after MD simulation and the crystal structure is less than 1 Å. The strained, cyan-colored structure (as from Fig. 1A, B) is aligned in Fig. 2 with the *in vacuum* minimum strain energy, shown in red. It should be noticed from the former that the polyene chain has taken a disfavored orientation, while rotation around the C18—C19 bond at the central oxepane ring has induced repulsive contacts between the C21 methyl group and the C16 proton, forcing a twist in the whole ladder moiety, up to the polar end. This amounts to a strain energy increase of *ca.* 90 kcal/mol, from single-point energy molecular mechanics calculation *in vacuum*[21] with MMFF94 force field.[22]

The tentative mode of interaction of a representative ciguatera toxin with K^+ channels described here should stimulate experimenting and aid devising a much needed specific remedy for such a socially and economically relevant disorder as ciguatera.

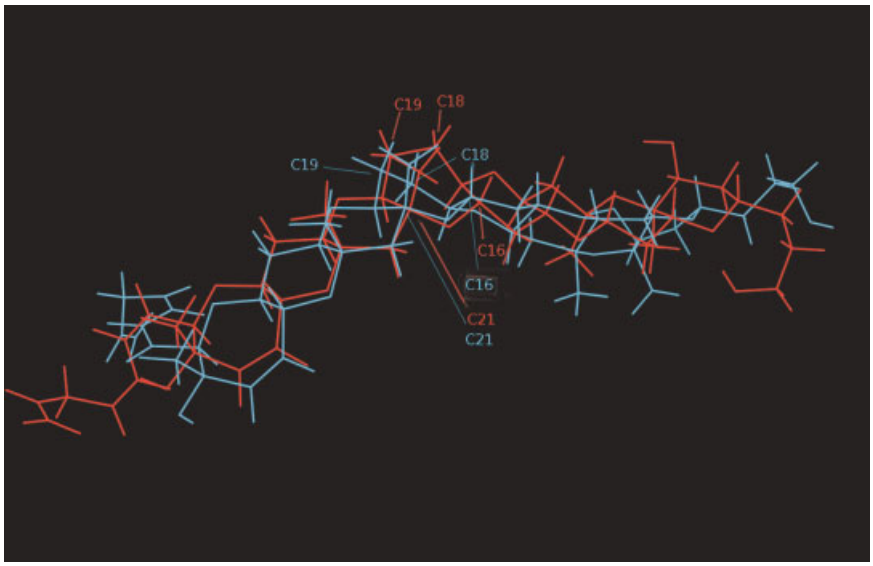

Figure 2. Gambierol structure (cyan) from minimum energy ensemble MD (as in Fig. 1), aligned with minimum strain-energy structure from simulated annealing in vacuum (red). RMSD 2.5 Å

Table 1. Mapping, from least energy ensemble, of teepee protein residues around ligand gambierol

Residue[a] within distance z (Å) from ligand				
$z \leq 2.0$	$z \leq 2.5$	$z \leq 3.0$	Substruct./unit	Color in Fig. 1A, B
	T480	T480	Filter/1	Green
		V505	S6/1	Green
		A509	S6/1	Green
	V512	V512	S6/1	Green
	P513	P513	S6/1	Green
	V516	V516	S6/1	Green
		N520	S6/1	Green
	T591	T591	Filter/2	Cyan
	V616	V616	S6/2	Cyan
	I619	I619	S6/2	Cyan
		A620	S6/2	Cyan
	T702	T702	Filter/3	Red
	V727	V727	S6/3	Red
		I730	S6/3	Red
	A731	A731	S6/3	Red
V734	V734	V734	S6/3	Red
	P735	P735	S6/3	Red
		V736	S6/3	Red
S739	S739	S739	S6/3	Red
	T813	T813	Filter/4	Blue
	I841	I841	S6/4	Blue
	V845	V845	S6/4	Blue
	P846	P846	S6/4	Blue
		V849	S6/4	Blue
		S850	S6/4	Blue

[a] Standard Kv1.5 numbering.[25]

SUPPORTING INFORMATION

Supporting information: (i) Cartesian coordinates for gambierol final pose from docking and MD, (ii) Parameters for the lipid of the membrane, in Amber prep format.

Acknowledgements

I thank Professor J. Åqvist and Dr M. Andér for a preprint of Reference [24] and for advice in tailoring the model as well as Dr S. Brozell for guidance in the use of DOCK6. All expenses for this work were borne by the author.

REFERENCES

[1] J. Y. S. Ting, A. F. T. Brown, *Eur. J. Emerg. Med.* **2001**, *8*, 295–300.
[2] M. Estacion, W. P. Schilling, *BMC Physiol.* **2001**, *1*, 2.
[3] J. Hidalgo, J. L. Liberona, J. Molgó, E. Jaimovich, *Brit. J. Pharmacol.* **2002**, *137*, 1055–1062.
[4] F. Pietra, *Biodiversity and Natural Product Diversity*, Elsevier, Oxford, **2002**.
[5] A. J. Bourdelais, S. Campbell, H. Jacocks, J. Naar, J. L. C. Wright, J. Carsi, D. G. Baden, *Fourth Symposium of Harmful Algae in the US.*, October 29-November 1, 2007, Woods Hole, Massachusetts.
[6] M. Inouea, M. Hirama, S. Masayuki, K. Sugiyama, T. Yasumoto, *Toxicon* **2003**, *41*, 469–474.
[7] K. T. LePage, J. D. Rainier, H. W. B. Johnson, D. G. Baden, T. F. Murray, *J. Pharmacol. Exp. Ther.* **2007**, *323*, 174.
[8] E. Cuypers, A. Yanagihara, J. D. Rainier, J. Tytgat, *Biochem. Biophys. Res. Commun.* **2007**, *361*, 214–217.
[9] V. Ghiaroni, M. Sasaki, H. Fuwa, G. P. Rossini, G. Scalera, T. Yasumoto, P. Pietra, A. Bigiani, *Toxicol. Sci.* **2005**, *85*, 657–665.
[10] Personal communication from Prof. A. Bigiani, University of Modena, Italy.
[11] L. Liu, D. R. Hansen, I. Kim, T. A. Gilbertson, *Am. J. Physiol. Cell Physiol.* **2005**, *289*, C868–C880.
[12] S. B. Long, E. B. Campbell, R. MacKinnon, *Science* **2005**, *309*, 897–903.

[13] P. T. Lang, D. Moustakas, S. Brozell, N. Carrascal, S. Mukherjee, S. Pegg, K. Raha, D. Shivakumar, R. Rizzo, D. Case, B. Shoichet, I. Kuntz, *DOCK*, Version 6.1, University of California, San Francisco, USA, **2006**.
[14] UCSF Computer Graphics Lab, University of California at San Francisco, CA.
[15] A. Magis, P. Sayeski, *Sphgen_cpp*, 28 July 2006, Version 1.2, University of Florida, Department of Physiology and Functional Genomics, USA.
[16] A. Magis, P. Sayeski, *Sphere_select*, 1 August 2006, Version 1.0, University of Florida, Department of Physiology and Functional Genomics, USA.
[17] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605–1612.
[18] D. A. Case, T. A. Darden, T. E. Cheatham, III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, K. M. Merz, D. A. Pearlman, M. Crowley, R. C. Walker, W. Zhang, B. Wang, S. Hayik, A. Roitberg, G. Seabra, K. F. Wong, F. Paesani, X. Wu, S. Brozell, V. Tsui, H. Gohlke, L. Yang, C. Tan, J. Mongan, V. Hornak, G. Cui, P. Beroza, D. H. Mathews, C. Schafmeister, W. S. Ross, P. A. Kollman, *AMBER*, Version 9, University of California, San Francisco, USA, **2006**.
[19] A. Morohashi, M. Satake, T. Yasumoto, *Tetrahedron Lett.* **1998**, *39*, 97–100.
[20] I. Kadota, H. Takamura, K. Sato, A. Ohno, K. Matsuda, Y. Yamamoto, *J. Am. Chem. Soc.* **2003**, *125*, 46–47.
[21] K. Gilbert, *PCMODEL, Molecular Modeling Software,* Version 9.1, Serena Software, Bloomington, Indiana 47402-3076, USA, **2006**.
[22] T. A. Halgren, *J. Comp. Chem.* **1996**, *17*, 616–641.
[23] J. E. Bushnell, *Xanneal*, Version 0.7.2, University of California, Santa Barbara, CA, USA, **2007**.
[24] M. Andér, V. B. Luzhkov, J. Åqvist, *Biophysical J.* **2008**, *94*, 820–831.
[25] http://www.expasy.org/uniprot/P22460
[26] J. Wang, W. Wang, P. A. Kollman, D. A. Case, *J. Mol. Graph. Model.* **2006**, *25*, 247–260.
[27] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, *J. Comput. Chem.* **2004**, *25*, 1157.
[28] I. Balabin, *Membrane Plugin*, Version 9.1, implemented in *Visual Molecular Dynamics*, Version 1.8.6, NIH, University of Illinois at Urbana-Champaign.
[29] E. Falck, T. T. Róg, M. Karttunen, I. Vattulainen, *J. Am. Chem. Soc.* **2008**, *130*, 44–45.
[30] L. Gracia, *RMSD Trajectory Tool*, Version 2.0, Department of Physiology and Biophysics, Cornell University, New York, NY.
[31] L. Gracia, *Clustering Tool*, Department of Physiology and Biophysics, Cornell University, New York, NY.
[32] N. Decker, P. Kumar, T. Gonzalez, B. Pirard, M. C. Sanguinetti, *Mol. Pharmacol.* **2006**, *70*, 1204–1211.
[33] http://journals.iucr.org/services/cif/hbonds.html