Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Graphics and Modelling 26 (2008) 1333–1337
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

Model of the extracellular domain of the human α7 nAChR based on the crystal structure of the mouse α1 nAChR extracellular domain

Maria Konstantakaki [a], Socrates J. Tzartos [b,c], Konstantinos Poulas [b,*], Elias Eliopoulos [a,**]

[a] Department of Agricultural Biotechnology, Agricultural University of Athens, 75, Iera Odos, Votanikos, GR11855, Athens, Greece
[b] Department of Pharmacy, University of Patras, Rio-Patras, GR26504, Greece
[c] Department of Biochemistry, Hellenic Pasteur Institute, 127 Vas. Sofias Ave., GR11521, Athens, Greece

Received 15 September 2007; received in revised form 17 January 2008; accepted 23 January 2008
Available online 2 February 2008

Abstract

Neuronal nicotinic acetylcholine receptors (nAChRs) are important therapeutic targets for various diseases, including Alzheimer's disease, Parkinson's disease, and schizophrenia, as well as for cessation of smoking. Based on the recently determined crystal structure of the extracellular domain (ECD) of the mouse nAChR α1 subunit complexed with α-bungarotoxin at 1.94 Å resolution, we have constructed three-dimensional models of the ECD of the monomer, homodimer, and homopentamer of the human α7 nAChR and investigated in detail the interface between the two α7 subunits. The docking of the agonist in the ligand-binding pocket of the human α7 dimer was also performed and found consistent with results from labeling and mutagenesis experiments. Since the nAChR ligand-binding site is a useful target for mutagenesis studies and the rational design of drugs against diseases, these models provide useful information for future work.

Keywords: α7 nAChR; α1 nAChR; Homology model; Structure; Extracellular domain; Ligand-binding domain; Rational drug design

1. Introduction

Nicotinic acetylcholine receptors (nAChRs), integral membrane proteins that respond to the binding of acetylcholine (ACh), are composed of five subunits organized around a central pore perpendicular to the membrane, and consist of two groups: (a) the muscle type, found in fish electric organs and in vertebrate skeletal muscles, where they mediate neuromuscular transmission at the neuromuscular junction, and (b) the neuronal type, found mainly throughout the peripheral and central nervous system, but also in non-neuronal tissues. The muscle-type nAChR consists of five homologous subunits in the stoichiometry $(\alpha1)_2\beta1\gamma\delta$ (embryonic form) or $(\alpha1)_2\beta1\varepsilon\delta$ (adult form) with two ACh-binding sites per molecule. Neuronal nAChRs consist of a variety of subunits in different combinations. To date, nine α (α2–α10) and three β (β2–β4) neuronal subunit genes have been cloned. The α7 neuronal-type subunit can form homopentamer of five identical α7 subunits with five ACh-binding sites per molecule [1]. nAChRs exist in different states, closed, open or desensitized, while the binding of nicotinic ligands, agonists, or competitive antagonists, affects the equilibrium between the various states.

Extensive studies on nAChRs from various species have shown that each subunit consists of: (a) an N-terminal extracellular domain (ECD) of approximately 210–220 amino acids, of which a high proportion forms β strands, and bearing the domain that binds agonists and competitive antagonists [2,3], (b) four small (15–20 residues) hydrophobic transmembrane domains (M1–M4) and two small hydrophobic loops, linking segments M1–M2 and M2–M3, (c) a larger loop, which varies in size (100–150 residues) and sequence between subunits, and which lies between M3 and M4 and bears phosphorylation sites [4], and (d) the C-terminal region of each subunit, which consists of a small (4–28 residues) hydrophilic extracellular segment [1].

It is well documented that brain nAChRs, including α7, participate in complex functions, such as attention, memory,

Abbreviations: nAChR, nicotinic acetylcholine receptor; ECD, extracellular domain; ACh, acetylcholine.

* Corresponding author. Tel.: +30 2610 969953; fax: +30 2610 969954.
** Corresponding author. Tel.: +30 210 5294223; fax: +30 210 5294322.
E-mail addresses: kpoulas@upatras.gr (K. Poulas), eliop@aua.gr (E. Eliopoulos).

1093-3263/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmgm.2008.01.004

and cognition, while clinical data suggest their involvement in the pathogenesis of several disorders (Alzheimer's disease, Parkinson's disease, schizophrenia, and depression) [1]. Smoking is a major public health problem, and the α7 nAChR subtype is closely associated with nicotine addiction and nicotine-induced behavior [5]. α7 nAChRs are overexpressed in small cell lung carcinoma in smokers [6]. In order to treat these diseases, it would be useful to design drugs that can selectively activate α7 nAChRs and for this purpose it is important to have a detailed knowledge of the α7 ligand-binding site.

Early biochemical studies on muscle-type nAChR indicated that two separate parts of the nAChR ligand-binding domain are involved in the formation of the agonist/competitive antagonist-binding site [7–9]. The ACh-binding pocket of the nAChR is formed between loops A, B, and C of the α subunit and strands β5 and β6 of the β-sandwich core of the adjacent γ or δ subunit [10]. The residues in the loops known to be involved in the formation of the ACh-binding site are Tyr190, Cys192, and Tyr198 in the C loop, which is incorporated in the β9–β10 hairpin, and Trp149 in the B loop.

Detailed information on the atomic structure of an ACh-binding domain first became available following the elucidation of the crystal structure at 2.7 Å resolution of the acetylcholine binding protein (AChBP) from the glial cells of the mollusc, *Lymnaea stagnalis* [11]. This protein, a water-soluble homopentamer of a 210 amino acid subunit, is a functional homologue of the ECDs of nAChRs subunits and has been extensively used as a model for their ligand-binding domain.

Based on the crystal structure of the AChBP, the atomic model of the *Torpedo* muscle-type nAChR at 4 Å resolution allowed a detailed description of the whole receptor in its closed-channel form [10]. More recently, the co-crystallization of various mollusk AChBPs with several agonists and antagonists [12–16] has revealed details of the atomic interactions between ligands and specific residues in the ligand-binding site.

A further breakthrough in the investigation of the structure of the nAChRs was the recent crystal structure determination of the mouse muscle nAChR α1 subunit ECD [17]. Structural comparison of the ECDs of the α1 subunit of the mouse nAChR, the *Torpedo* nAChR, and the AChBP bound to either carbamylcholine [12] or α-cobratoxin [16] indicated a high overall similarity of main chain folding, with most side chains adopting a similar conformation [17]. Specifically, the predicted pentamer assembly interfaces of the mouse α1 subunit resemble the corresponding interfaces in the *Torpedo* nAChR and AChBP. On the minus surface, there is structural similarity both in main chain and side chain conformation. On the plus surface, only minor differences are observed. The side chains of Thr150, Tyr151, Asp152, and Ala155 (mutated from Val in the mouse α1 subunit) in loop B of mouse α1, are oriented as in the *Torpedo* nAChR α subunit with a slight offset, which is not seen in the comparison with the structures of AChBP bound to agonist or antagonist [17].

The structure of the ECD of the α1 subunit of the mouse nAChR is a good starting point for the modeling of the ECD of the human neuronal α7 receptor.

2. Methodology

2.1. Sequence alignment

The structure of the ECD of the mouse α1 subunit of the nAChR is the first X-ray structure obtained of a region of the nAChR and can be used to model the ECD of the human α7 subunit due to the high degree of homology between them, with 38% identity of residues (Fig. 1). This is about 50% higher than the degree of identity between the ECDs of the α7 subunit and AChBP, which is about 25%. The sequence alignment of the ECDs of the mouse α1 and human α7 subunits was performed using the ClustalW program [18].

2.2. Molecular modeling

Based on the sequence alignment with the ECD of the α1 mouse subunit, the atomic coordinates for the ECD of the human α7 monomer were derived using the MODELLER interface [19] from the QUANTA package [20].

The ECD of the homopentamer was modeled with five-fold symmetry operations using the X-ray structure of the AChBP pentamer [11] as the template. The models generated were subjected to overall energy minimization with respect to all the atoms and the final structures studied.

The docking of ACh with the homodimer was performed in two steps. The ligands were first manually positioned in the agonist-binding pocket of the receptor, according to Le Novère et al. [21], using QUANTA [20], then the plausible docking position was adjusted by local minimization with rigid body fit.

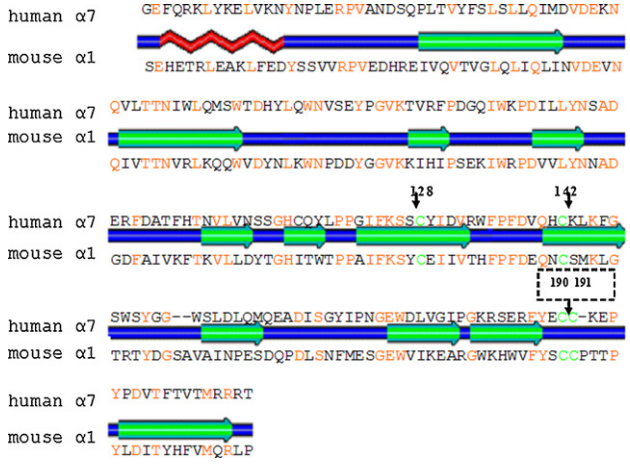

Fig. 1. Sequence alignment of the ECDs of the mouse α1 and human α7 nAChRs using ClustalW [24]. Identical residues are shown in red, while the key Cys residues in loop C (residues 190 and 191) or the Cys loop (residues 128 and 142) are shown in green. The line between the sequences shows the secondary structure, as determined by the SABLE server (http://sable.cchmc.org/). Green arrows represent β-strands, α-helix is shown in red and the blue line represents the loops.

3. Results

3.1. α7 nAChR: from monomer to pentamer

The model of the ECD of the α7 nAChR subunit monomer is shown in Fig. 2. It is composed of an N-terminal α-helix followed by seven strands that form a β-sandwich and comprise an immunoglobulin fold. A disulphide bond is formed between Cys128 and Cys142. Loops 29–62, 128–142 (Cys loop), and 156–189 form the interface with the following transmembrane segment.

As expected from the low number of insertions/deletions, the model of the ECD of the human α7 does not exhibit major differences with the template. Although several parts of the sequence of the α7 ECD show lower homology with the mouse α1 ECD, the 13-residue Cys loop, which is highly conserved in the superfamily, adopts a similar structure to that of the mouse α1 ECD. Phe135 seems to be a key residue for the whole Cys loop, being at the top of the loop structure (Fig. 2).

The model of the ligand-binding pocket of the dimer and its complex with the ligand ACh is shown in Fig. 3. The dimer interface is formed by an interlocking array of neighboring chain secondary structure elements with the result that a large surface area is buried upon interlocking of the neighboring chains of the homopentamer. The residues involved in the dimer interface are listed in Table 1. The interface consists of 29 residues, of which 17 are from chain A and 12 from chain B.

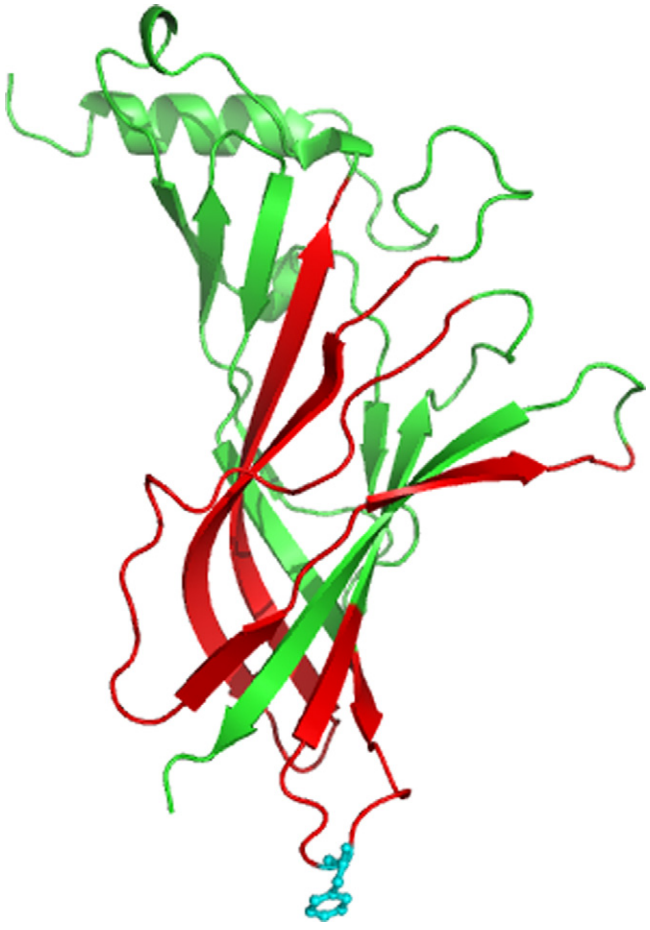

Fig. 2. Model of the ECD of the α7 nAChR monomer based on the sequence alignment shown in Fig. 1 and subsequent homology modeling. Loops 29–62, 128–142 (Cys loop), and 156–189, which form the interface with the transmembrane segment, are shown in red. Phe135 at the tip of the Cys loop is shown in ball-and-stick in cyan.

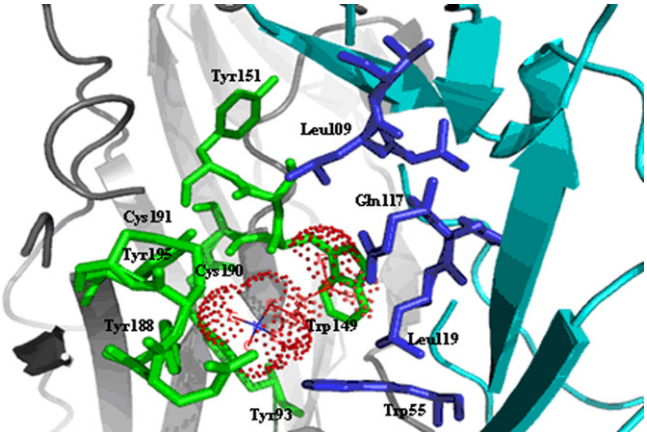

Fig. 3. Ligand-binding site of the human α7 homodimer model with ACh bound (shown in red). The amino acids of the principal component are shown in green and those of the complementary component in blue. All key residues discussed in the text are indicated.

The ACh-binding pocket of the chicken α7 nAChR, as modeled previously [21], is formed by loops A, B, and C of the principal component (chain A) and loops D and E of the complementary component (chain B) of the adjacent subunit. The key residues of the loops involved in the formation of the ACh-binding site are Tyr93 from loop A, Trp149 and Tyr151 from loop B, and Tyr188, Cys190, Cys191, and Tyr195 from loop C, which are incorporated in the β9–β10 hairpin (all these residues belong to chain A), and Trp55 from loop D and Leu109, Gln117, and Leu119 from loop E (residues from chain B) [21].

Table 1
Residues involved in the human α7 homodimer interface

Principal component (chain A)	Complementary component (chain B)
Arg20	Tyr8
Lys46	Gln39
Asn47	Met41
Gln48	Arg79
Tyr64	Ala102
Lys87	Phe104
Asp89 (loop A)	Leu119 (loop E)
Leu91 (loop A)	Ile123
Ala96 (loop A)	Tyr168 (loop F)
Glu98 (loop A)	Ile169
Ser127	Asn171
Cys128	Glu173
Tyr129	
Ile130	
Trp149 (loop B)	
Ser150 (loop B)	
Cys190 (loop C)	

Twenty-nine residues are involved in the dimer interface. Several amino acids that belong to ligand-binding site loops also participate in the dimer interface. Specifically, four of these amino acids belong to loop A, two to loop B, one to loop C, one to loop E, and one to loop F.

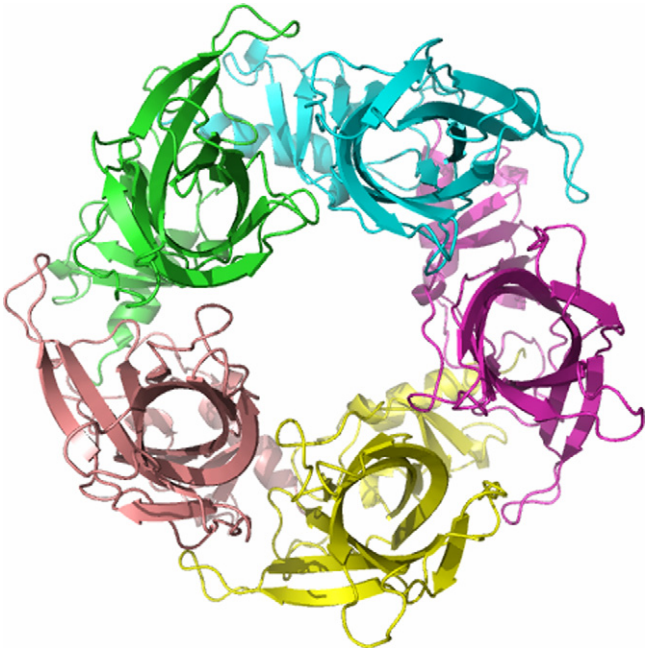

Fig. 4. Model of the ECD of the human α7 nAChR homopentamer, with chain A colored green, chain B cyan, chain C magenta, chain D yellow, and chain E pink.

The ligand-binding site of the human α7 nAChR is formed essentially by the same amino acids as those in the chicken α7 homodimer. However, some additional residues are located close to the ACh molecule and seem also to be important. These are Gly147, Ser148, and Ser150 from loop B (chain A). Furthermore, Val108 and Tyr118 (loop E) from the complementary component (chain B) may also be involved in forming the ligand-binding site. Like the AChBP pentamer, the homopentamer, or $(\alpha 7)_5$, model shows five-fold symmetry when viewed along the axis (Fig. 4).

4. Discussion

The model of the ECD of the homodimer of the human α7 nAChR based on the recently determined structure of the ECD of the α1 subunit of the mouse nAChR [17] is in good agreement with the chicken α7 model produced by Le Novère et al. [21]. In addition, the human α7 homodimer bound to ACh provides a refined reference model for ligand design studies. Notably, the position of the ligand in the current model is in agreement with the results of biochemical experiments performed on *Torpedo* nAChR. Labeling experiments by Cohen et al. [22] have shown that the methyl groups forming bonds with the ammonium of ACh are close to Tyr93. Moreover, photolabeling experiments by Grutter et al. [23] have shown that the ester bond of ACh is close to cystine and to Tyr195. In addition, according to mutagenesis experiments [24], the charged ammonium is able to establish a π-cation interaction with Trp149. Finally, the estimated binding free energy of the ligands in the selected position is in agreement with experimentally determined binding efficiencies [25].

For several decades, the nAChR has served as the prototypic molecule for neurotransmitter receptors. The solution of the three-dimensional structure of the α1 nAChR is a breakthrough, as it serves as an improved template for the modeling of other types of nAChR and provides detailed knowledge of the structure of the ligand-binding site and helpful information on the gating mechanism. However, in order to design drugs against diseases mediated by neuronal-type nAChR, such as schizophrenia or Alzheimer's disease, it is necessary to determine the 3D structures of the neuronal nAChRs in various states of oligomerization. The knowledge obtained will permit an in-depth understanding of the structure–function relationships of the nAChR and the design of selective ligands for therapeutic purposes. Furthermore, knowledge of the 3D structure of the nAChR makes it much simpler to perform mutagenesis experiments aimed at designing novel therapeutic agents. Although the model of the homopentameric α7 nAChR was not experimentally derived, it can serve as a tool to further advance the design of novel drugs against diseases involving neuronal-type nAChRs.

5. Conclusions

Models of the ECD of the monomer, homodimer and pentamer of the human α7 nAChR, based on the recently determined structure of the ECD of the α1 subunit of the mouse nAChR are presented in detail. Ligand-binding is also modeled and discussed. Since the α7 homopentamer is a very important drug target, because it is implicated in a number of human diseases, the models presented can serve as tools for further experiments.

Acknowledgements

Part of the work was supported by grants of the Pythagoras EPEAEK programme (EU and Greek Ministry of Education), PEP W. Greece (EU and GSRT) and Karatheodoris grant from University of Patras. We would like to thank Dr. N. Papandreou for useful information on energy minimization.

References

[1] D. Kalamida, K. Poulas, V. Avramopoulou, E. Fostieri, G. Lagoumintzis, K. Lazaridis, A. Sideri, M. Zouridakis, S.J. Tzartos, Muscle and neuronal nicotinic acetylcholine receptors, Structure, function and pathogenicity, FEBS J. 274 (2007) 3799–3845.
[2] M. Dennis, J. Giraudat, F. Kotzyba-Hibert, M. Goeldner, C. Hirth, J.Y. Chang, C. Lazure, M. Chretien, J.P. Changeux, Amino acids of the *Torpedo* marmorata acetylcholine receptor subunit labeled by a photoaffinity ligand for the acetylcholine binding site, Biochemistry 27 (1988) 2346–2357.
[3] J.L. Galzi, J.P. Changeux, Neuronal nicotinic receptors: molecular organization and regulations, Neuropharmacology 34 (1995) 563–582.
[4] R.L. Huganir, P. Greengard, Regulation of neurotransmitter receptor desensitization by protein phosphorylation, Neuron 5 (1990) 555–567.
[5] B. Buisson, D. Bertrand, Nicotine addiction: the possible role of functional upregulation, Trends Pharmacol. Sci. 23 (2002) 130–136.
[6] M.A. Sciamanna, G.E. Griesmann, C.L. Williams, V.A. Lennon, Nicotinic acetylcholine receptors of muscle and neuronal (alpha7) types coex-

pressed in a small cell lung carcinoma, J. Neurochem. 69 (1997) 2302–2311.
[7] P.J. Corringer, N. Le Novère, J.P. Changeux, Nicotinic receptors at the amino acid level, Annu. Rev. Pharmacol. Toxicol. 40 (2000) 431–458.
[8] H.R. Arias, Localization of agonist and competitive antagonist binding sites on nicotinic acetylcholine receptors, Neurochem. Int. 36 (2000) 595–645.
[9] J.L. Galzi, D. Bertrand, A. Devillers-Thiery, F. Revah, S. Bertrand, J.P. Changeux, Functional significance of aromatic amino acids from three peptide loops of the α7 neuronal nicotinic receptors site investigated by site directed mutagenesis, FEBS Lett. 294 (1991) 198–202.
[10] N. Unwin, Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution, J. Mol. Biol. 346 (2005) 967–989.
[11] K. Brejc, W.J. van Dijk, R.V. Klaassen, M. Schuurmans, J. van De roost, A.B. Smit, T.K. Sixma, Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, Nature 411 (2001) 269–276.
[12] P. Celie, S.E. van Rossum-Fikkert, J.W. van Dijk, K. Brejc, A.B. Smit, Nicotine, and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures, Neuron 41 (2004) 907–914.
[13] P.H. Celie, I.E. Kasheverov, D.Y. Mordvintsev, R.C. Hogg, P. van Nierop, R. van Elk, S.E. van Rossum-Fikkert, M.N. Zhmak, D. Bertrand, V. Tsetlin, T.K. Sixma, A.B. Smit, Crystal structure of nicotinic acetylcholine receptor homolog AChBP in complex with an alpha-conotoxin PnIA variant, Nat. Struct. Mol. Biol. 12 (2005) 582–588.
[14] C. Ulens, R.C. Hogg, D. Bertrand, V. Tsetlin, A.B. Smit, T.K. Sixma, Structural determinants of selective alpha-conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP, Proc. Natl. Acad. Sci. USA 103 (2006) 3615–3620.
[15] S.B. Hansen, G. Sulzenbachre, T. Huxford, P. Marchot, P. Taylor, Y. Bourne, Structures of *Aplysia* AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations, EMBO J. 24 (2005) 3635–3646.
[16] Y. Bourne, T.T. Talley, S.B. Hansen, P. Taylor, P. Marchot, Crystal structure of Cbtx-AChBP complex reveals essential interactions between snake alpha-neurotoxins and nicotinic receptors, EMBO J. 24 (2005) 1512–1522.
[17] C.D. Dellisanti, Y. Yao, J.C. Stroud, Z.Z. Wang, L. Chen, Crystal structure of the extracellular domain of nAChRα1 bound to α-bungarotoxin at 1.94 Å resolution, Nat. Neurosci. 10 (2007) 953–962.
[18] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix-choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[19] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[20] Quanta, Accelrys, Inc., San Diego, California, USA, 2005.
[21] N. Le Novère, T. Grutter, J.P. Changeux, Models of the extracellular domain of the nicotinic receptors and of agonist- and Ca^{2+}-binding sites, Proc. Natl. Acad. Sci. USA 99 (2002) 3210–3215.
[22] J.B. Cohen, S.D. Sharp, W.S. Liu, Structure of the agonist-binding site of the nicotinic acetylcholine receptor. [3H] acetylcholine mustard identifies residues in the cation-binding subsite, J. Biol. Chem. 266 (1991) 23354–23364.
[23] T. Grutter, L. Ehret-Sabatier, F. Kotzyba-Hibert, M. Goeldner, Photoaffinity labeling of *Torpedo* nicotinic receptor with the agonist [^{3}H]DCTA: identification of amino acid residues which contribute to the binding of the ester moiety of acetylcholine, Biochemistry 39 (2000) 3034–3043.
[24] W. Zhong, J.P. Gallivan, Y. Zhang, L. Li, H.A. Lester, D.A. Dougherty, From *ab initio* quantum mechanics to molecular neurobiology: a cation–pi binding site in the nicotinic receptor, Proc. Natl. Acad. Sci. USA 95 (1998) 12088–12093.
[25] R. Anand, X. Peng, J. Lindstrom, Homomeric, and native alpha 7 acetylcholine receptors exhibit remarkably similar but non-identical pharmacological properties, suggesting that the native receptor is a heteromeric protein complex, FEBS Lett. 327 (1993) 241–246.