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Docking to flexible nicotinic acetylcholine receptors: A validation study using the acetylcholine binding protein

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ABSTRACT

Computational docking to nicotinic acetylcholine receptors (nAChRs) and other members of the Cys-loop receptor family is complicated by the flexibility of the so-called C-loop. As observed in the large number of published crystal structures of the acetylcholine binding protein (AChBP), a structural surrogate and homology modeling template for the nAChRs, the conformation of this loop is controlled by the ligand present in the binding pocket. As part of the development of a protocol for unbiased docking to the nAChRs, we here present the results of docking of ligands with known binding modes to an AChBP ensemble with systematic variations in C-loop closure generated via a series of targeted geometry optimizations. We demonstrate the ability to correctly predict binding modes for 12 out of 15 ligands and induced degrees of C-loop closure for 14 out of 15 ligands. Our approach holds a promising potential for structure based drug discovery within nAChRs and related receptors.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) constitute a pharmaceutically important class of pentameric ligand-gated ion channels called Cys-loop receptors, to which also the $GABA_A$, glycine and $5\text{-}HT_3$ receptors belong [1]. The rational design of new ligands and application of virtual screening towards these receptors is hampered by the lack of a high-resolution X-ray structure of the ligand-binding site of the nAChRs. To date, the only nAChR structures available are the 4 Å electron microscopy of the muscle type nAChR from *Torpedo marmorata* [2] and the X-ray structure of the N-terminal part of the mouse α_1 monomer [3]. Augmented with structural information from the acetylcholine binding protein (AChBP) isolated primarily from the freshwater snails *Lymnaea stagnalis* (*Ls*) and *Aplysia californica* (*Ac*) [4–16], these structures form the basis for homology models of nAChRs and other Cys-loop receptors [17]. The AChBP is homologous to the ligand binding extra-cellular domain of the nAChR and, equally importantly, binds key nicotinic ligands such as ACh, nicotine, epibatidine, and methyllycaconitine (MLA) (structures shown in Table 1). Hence, they are regarded as functional and structural surrogates of the nAChR [9,18], and apart from being used extensively as templates for the construction of homology models they have also been used directly in virtual screening with the purpose of discovering novel nAChR ligands [15]. Until now 26 structures of the AChBP in the apo form, in complex with buffer molecules, toxins and small molecule nicotinic agonists, partial agonists, antagonists and allosteric modulators have been deposited in the Protein Data Bank (PDB) [19]. Despite low overall sequence identity to nAChRs [20] the ligand-binding site is much better conserved and the protein thus provides detailed insights into the protein–ligand interactions of nicotinic ligands. An interesting observation from the many AChBP structures is the large flexibility of the binding site, in particular originating from the 10 to 12 residue hairpin shaped C-loop being able to move away from, or close in on, the site to accommodate differently sized ligands (Fig. 1). Comparing AChBP structures co-crystallized with agonists to those with antagonists it is clear that agonists stabilize full C-loop closure while antagonists hold the loop in an open conformation. Several studies have indicated C-loop closure to be involved in the receptor activation process which eventually leads to opening of the ion channel in nAChRs [21–24]. Obviously, the C-loop flexibility has a large impact on the size and shape of the binding pocket, and a prerequisite for successful application of structure based design, automated docking and virtual screening to nAChRs and other Cys-loop receptors is the ability to account for this flexibility.

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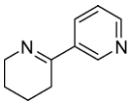
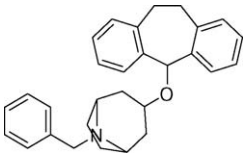
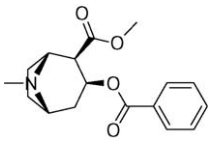
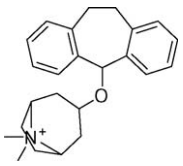
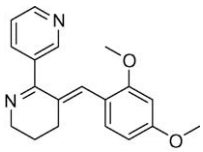
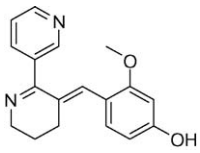
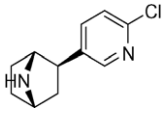
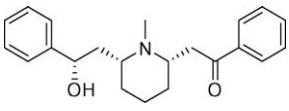
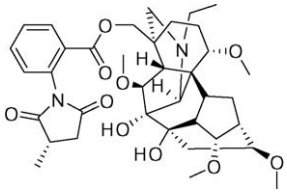
Table 1
Ligand names, structures, AChBP complex details, binding affinities, and cross-docking results.

Ligand			Structure	Sp.	Affinity[a]		Self-docking[b]		Cross-docking[c]		
PDB code	Closure	Ref.			pK_i/pK_D	Ref.	Gscore	RMSD	Gscore	RMSD	PDB
Ac-AChBP											
	Anabaseine			*Ac*	<6	[51]	−10.43	1.2 Å	−11.13	1.1 Å	2byq
2wnl	12.1 Å	[16]		*Ls*	6.6	[51]	–	–	−11.61	0.8 Å	2zjv
	BDSAO[d]			*Ac*	6.0	[15]	−7.79	2.2 Å	−7.79	2.2 Å	2w8g-DE
2w8g	18.3–19.8 Å	[15]		*Ls*	7.0	[15]	–	–	None	–	–
	Cocaine			*Ac*	5.7	[11]	−10.24	0.8 Å	−11.08	1.7 Å	2wnc
2pgz	14.0 Å	[11]		*Ls*	–		–	–	None	–	–
	DDSAO			*Ac*	6.3	[15]	−6.60	1.5 Å	−7.06	1.8 Å	2w8g-DE
2w8f	18.3 Å	[15]		*Ls*	7.2	[15]	–	–	None	–	–
	DMXBA			*Ac*	6.5	[51]	−11.83	0.5 Å	−12.31	0.9 Å	2wn9
2wnj	13.8 Å	[16]		*Ls*	7.7	[51]	–	–	−5.79	7.0 Å	2zju
	4-OH-DMXBA			*Ac*	8.5	[51]	−12.90	0.5 Å	−12.90	0.5 Å	2wn9
2wn9	13.9 Å	[16]		*Ls*	9.4	[51]	–	–	−6.18	8.1 Å	2zju
	Epibatidine			*Ac*	7.9	[52]	−10.46	0.4 Å	−10.46	0.4 Å	2byq
2byq	12.0 Å	[9]		*Ls*	9.8	[18]	–	–	−11.02	0.6 Å	1uw6
	Lobeline			*Ac*	9.5	[9]	−11.64	1.2 Å	−11.64	1.2 Å	2bys
2bys	12.3 Å	[9]		*Ls*	7.5	[9]	–	–	−5.01	6.6 Å	2zju
	MLA			*Ac*	8.6	[52]	−10.48	1.2 Å	−10.56	1.3 Å	2w8g-DE
2byr	18.2 Å	[9]		*Ls*	9.4	[52]	–	–	None	–	–