NACHRDB: A Web Resource of Structure-Function Annotations to Unravel the Allostery of Nicotinic Acetylcholine Receptors

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Abstract
Summary: Due to their paramount importance, near-ubiquitous presence, and complex nature, nicotinic acetylcholine receptors (nAChRs) have remained the focus of intensive research for over 50 years. The vast amount of knowledge accumulated on the topic has become extremely difficult to navigate. NACHRDB addresses this challenge by providing web-based, real-time access to curated residue-level functional annotations of neuromuscular nAChRs with interactive 3D visualization and sequence alignment. NACHRDB provides systematic access to experimental observations and predictions from computational studies reported in the literature or performed specifically to complement current knowledge, which allows new findings to be interpreted in a more holistic context, both from a structural and a functional perspective. NACHRDB aims to serve as an invaluable resource for identifying gaps in knowledge and for guiding discovery through structural and molecular biology experiments, especially when exploring the allosteric mechanisms underlying neuromuscular nAChR function and pathology.

Availability and Implementation: NACHRDB is freely available online, with a self-explanatory interface and useful tool tips (https://crocodile.ncbr.muni.cz/Apps/NACHRDB/). No installation or user registration is required. NACHRDB content is stored in .json format, queried using Python, and rendered in browser using Javascript and WebGL (LiteMol). NACHRDB is highly responsive and accessible through any modern Internet browser on desktop and mobile devices.

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Supplementary information: Supplementary data are available online.

1 Introduction
The nicotinic acetylcholine receptor (nACHR) is an evolutionarily ancient allosteric membrane protein mediating synaptic transmission (Changeux, 2018). This prototypic member of the pentameric ligand-gated ion-channel family is involved in many physiological and pathological processes including neurological diseases and addictions. NACHRs convert the chemical signal of acetylcholine into ion current by allowing sodium and calcium ions to enter the cell and potassium ions to exit the cell. This fast conversion of chemical signal to ion current relies heavily on allosteric regulation, which links the agonist-binding pocket in the extracellular domain to a gating mechanism lying approximately 60 Å away in a central channel spanning the entire length of the molecule. Hundreds of nACHR-binding compounds have been found to modulate nerve impulse transmission by regulating this coupling of agonist binding and channel gating (Gündisch and Eibl, 2011; Reyes-Parada and Iturriaga-Vasquez, 2016). Not surprisingly, nAChRs have remained the focus of intensive research for more than 50 years (Brown, 2019). The effort to understand structure-function relationships in nAChRs has resulted in huge amounts of structural and functional information. However, the cumulative knowledge on nAChRs is not systematically accessible, with thousands upon thousands of experimental and/or computational studies reporting data on different receptor types, using different residue numbering schemes, and different terminology. Furthermore, because nAChRs are very large and difficult to crystalize, structural studies have focused on individual parts of the molecule, which makes it challenging to compile the current knowledge in an efficient manner and to promote further discoveries (Changeux, 2012; Halliwell, 2007). Finally, in the absence of comprehensive and unified structural annotation, it is almost impossible to identify gaps in knowledge or areas of controversy. We developed NACHRDB to address such limitations by providing web-based, real-time access to curated
residue-level functional annotations of neuromuscular nAChRs, with interactive 3D visualization and sequence alignment.

2 Data Sources and Database Coverage

Functional annotations were collected from a semi-systematic literature review of Medline through PubMed (see Supplementary Materials). Upon manually scanning over 3000 papers on neuromuscular nAChRs, we selected 117 studies published between 1982 and 2019 and providing functional annotations of specific residues or parts of the protein. Currently, NACHRDB contains approximately 2000 unique annotation records describing the role of specific residues, as inferred based on experimental observations or computational predictions reported for nAChRs of organisms from 6 genera. These studies, which cover 41 experimental or computational methods, were conducted at 92 institutions from 25 countries. Additionally, we conducted two computational studies to complement current knowledge, which resulted in the addition of 741 annotation records. NACHRDB thus provides a comprehensive view on structure-function relationships in the neuromuscular nAChR (Fig. 1).

The structures and sequences of relevant nAChRs were obtained from the Protein Data Bank and from Uniprot, respectively. Sequence alignments were performed using Clustal W (Thompson et al., 1994). Residues potentially involved in charge transfer networks facilitating channel gating were predicted using a modified charge profile analysis (Ionescu et al., 2012). Briefly, the following steps were employed: (i) conformers were generated based on normal mode analysis using eNemo (Suhre and Sanejouand, 2004); atomic charges for each conformer were computed using AtomicChargeCalculator (Ionescu et al., 2015) with several different settings; residue charges were then compared across conformers; outliers were reported based on robust statistical analysis (see Supplementary Materials). Channel lining residues were predicted using ChannelsDB (Pravda et al., 2018) (see Supplementary Materials). All predictions were added to NACHRDB as annotation records, with full reference to the source of information. In-house scripts were employed for data processing, but all annotations were curated manually.
3 Implementation

NACHRDB content is stored in .json format, queried using Python, and rendered in browser using Javascript and LiteMol, a WebGL-based technology for real-time in-browser rendering of large-scale macromolecular structures (Sehnal et al., 2017) (Fig. 2). NACHRDB is freely available online (https://crocodile.ncbr.muni.cz/Apps/NACHRDB/), with no requirement for installation or user registration, with self-explanatory interface and useful tool tips. NACHRDB is highly responsive and accessible through any modern Internet browser on desktop and mobile devices.

4 Case studies

- A simple text search in NACHRDB helps to quickly gauge the potential role of specific residues, as well as to identify opportunities for further study. For example, certain residues found to link different domains, bind certain drug molecules, contribute to channel gating, form N-glycosylation sites, or regulate agonist binding were never studied in human neuromuscular nAChR (Fig. 3A).
- When conducting new computational studies, NACHRDB helps to put the computational predictions into context, as well as to identify gaps in current knowledge and thus opportunities for further study. For example, among the residues predicted to line the channel’s inner surface in neuromuscular Torpedo nAChR, 28.5% have been studied to date; among the residues predicted to contribute to protein-wide charge transfer networks in Torpedo nAChR that facilitate channel gating, 18.5% have been studied (Fig. 3B).
- Additionally, NACHRDB helps to identify potential areas of ambiguity or controversy in the current knowledge. For example, much remains to be clarified regarding the relationship between channel opening kinetics and voltage dependency, especially in nAChRs where key ASP residues are mutated to non-charged residues (Fig. 3C).

Armed with such information, it is easy to identify understudied areas and design further experiments that can cover the current gaps and provide a more meaningful contribution to the current knowledge. Many residues highlighted by state-of-the-art computational analyses have not been investigated to date, mainly because experimental studies are expensive and thus typically focus on areas thought to be of utmost importance. Furthermore, due to reporting bias, negative results are scarce. Thus, in addition to serving as an easy reference for structure-function information, we hope that NACHRDB will help promote the reporting of both positive and negative results, so that the scientific community may form a comprehensive picture of the functioning of nAChRs.

5 Limitations

At present, the annotation records in NACHRDB refer mainly to the neuromuscular receptor. Extending NACHRDB to cover neuronal receptors is our current focus. Moreover, users can submit suggestions for new annotations directly via NACHRDB; we will review these suggestions manually and update NACHRDB accordingly. We are also working on a text mining tool that will enable us to automatically expand the coverage of NACHRDB as soon as new information becomes available.
6 Conclusions
NACHRDB helps to quickly summarize the knowledge about specific parts of neuromuscular nAChRs, as well as to detect conflicting results reported for the same or homologous residues, and even to identify the parts of the protein not studied to date. NACHRDB provides systematic access to experimental observations and predictions from computational studies reported in the literature or performed specifically to complement current knowledge. New findings can thus be interpreted in a more holistic context, both from a structural and a functional perspective. Ultimately, NACHRDB aims to serve as an invaluable resource for guiding discovery through structural and molecular biology experiments, especially when exploring the allosteric mechanisms underlying nAChR function and pathology. In addition, NACHRDB can serve as a key starting point for the unification of the state-of-art knowledge in the broad field of pentameric ligand-gated ion channels.

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