DOI 10.18699/SBB-2022-179

Membrane environment regulates interaction of three-finger toxins with nicotinic acetylcholine receptors

Shenkarev Z.O.[1, 2], Chesnokov Y.[3], Zaigraev M.M.[1, 2], Chugunov A.O.[1], Kulbatskii D.S.[1], Kocharovskaya M.V.[1, 2], Paramonov A.S.[1], Bychkov M.L.[1], Shulepko M.A.[1], Kirpichnikov M.P.[1, 4], Lyukmanova E.N.[1, 2, 4]*

[1] *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia*

[2] *Phystech School of Biological and Medical Physics, Moscow Institute of Physics and Technology (National Research University), Dolgoprudny, Moscow Region, Russia*

[3] *National Research Center "Kurchatov Institute", Moscow, Russia*

[4] *Interdisciplinary Scientific and Educational School of Moscow University "Molecular Technologies of the Living Systems and Synthetic Biology", Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia*

* *ekaterina-lyukmanova@yandex.ru*

Key words: snake neurotoxin, nicotinic acetylcholine receptor, three-finger protein, Ly6/uPAR, cryo-EM, membrane catalysis, membrane binding site

Nicotinic acetylcholine receptor of α7 type (α7-nAChR) presented in the nervous and immune systems and epithelium is a promising therapeutic target for cognitive disfunctions and cancer treatment. WTX is a non-conventional three-finger neurotoxin from Naja kaouthia venom, targeting α7-nAChR with weak affinity. There is no data on interaction mode of non-conventional neurotoxins with α7-nAChR. Using α-bungarotoxin (classical three-finger neurotoxin with high affinity to α7-nAChR), we showed applicability of cryo-EM to study interactions of α7-nAChR extracellular ligand-binding domain (α7-ECD) with its ligands. Cryo-EM structure of the α7-ECD/WTX complex together with NMR data on membrane active site in the WTX molecule and mutagenesis data allowed to reconstruct the α7-nAChR/WTX structure in the membrane environment. WTX interacts at the entrance to the orthosteric site located at the receptor intersubunit interface and simultaneously forms the contacts with the membrane surface. WTX interaction mode with α7-nAChR significantly differs from α-bungarotoxin's one, which do not contact the membrane. Our study provides evidence of the 'membrane catalysis' mechanism for non-conventional neurotoxins.

Acknowledgements: This study made under financial support of Russian Science Foundation (project No. 19-74-20163).

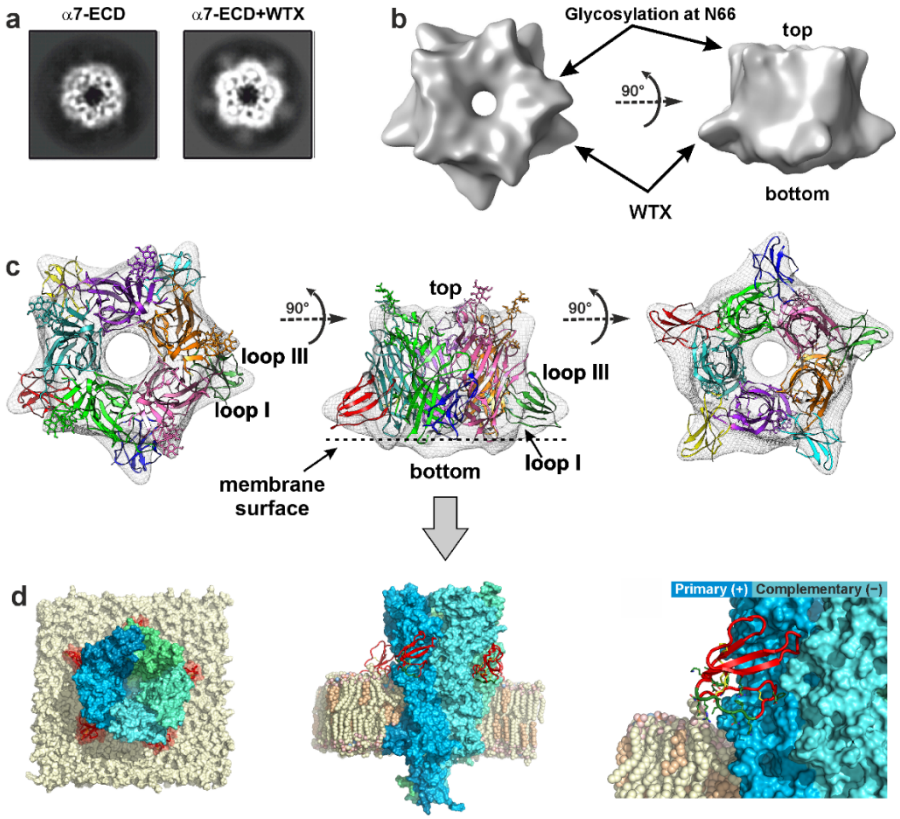

Fig. 1. 3D reconstruction of α7-nAChR/WTX complex determined by cryo-EM and computer modeling. **a** Comparison of the representative top-view 2D class averages for α7-ECD and α7-ECD/WTX complex. The fine features of pentameric domain complex and density corresponding to five WTX molecules are visible. **b** 3D density map of α7-ECD/WTX complex. The features corresponding to the linked glycan fragments and bound WTX molecules are indicated. **c** Model of α7-ECD/WTX complex fitted into experimental 3D density map. The position of the toxin's loops I and III and expected position of membrane interface in the full-length channel are shown. **d** Views of the α7-nAChR/WTX complex. nAChR subunits are individually colored in a *blue-green spectrum*; five WTX molecules at the interfaces of two adjacent receptor's subunits are shown by *red*. Carbon atoms of the membrane lipids are colored as follows: POPC and POPE, *pale yellow*; cholesterol, *pale orange*. Other lipid atoms: phosphorus, *orange*; oxygen, *red*; nitrogen, *blue*. Lipids of the proximal part of the membrane are removed for clarity. Membrane-interacting residues of WTX are colored by *forest green*. WTX interacts by the loops II and III with α7-nAChR and by loop I, head-1, and *N*- and *C*-termini with the membrane