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ORAL ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF MEMBRANE PROTEINS

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Abstract OR-3: Integrative Structural Study of the Complex of Snake Toxin WTX with α7-type Nicotinic Acetylcholine Receptor

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Background: Nicotinic acetylcholine receptors are ligand-gated ion channels present in the nervous system, epithelium, and the immune system. The α7-type nicotinic receptor (α7-nAChR) is a homopentameric membrane protein containing five ligand binding sites located at the interface between subunits in the extracellular domain of the receptor. α7-nAChR is considered a promising target for the treatment of cancer and cognitive dysfunction in Alzheimer's disease, schizophrenia, and depression. WTX is a non-conventional three-finger neurotoxin from the *Naja kaouthia* venom inhibiting α7-nAChR. WTX structure consists of three loops protruding from the "head" (core) stabilized by a system of disulfide bonds.

Methods: The complex of the α7-nAChR extracellular domain with a recombinant analogue of WTX was studied by cryo-electron microscopy. The structure of the complex of full-length α7-nAChR with the toxin in the membrane environment was reconstructed by *in silico* molecular modeling. Interaction of WTX with the lipid membrane was confirmed by NMR-spectroscopy.

Results: Analysis of electronic images confirmed the homopentameric organization of the extracellular domain with a diameter of ~ 9 nm and a height of ~ 7 nm. On the electron density map, additional regions corresponding to five WTX molecules located at the intersubunit interfaces of the domain were observed. Fitting the known spatial structures of the extracellular domain and

the WTX toxin into the obtained electron density made it possible to reconstruct the structure of the complex (although with a low resolution of ~ 8 Å due to the predominant orientation of particles in the ice) and to determine the topology of the toxin-receptor interaction. It was revealed that WTX interacts with the extracellular domain of α7-nAChR by the loop II, while the loop I and the toxin's head seem to interact with the surface of the lipid membrane surrounding the receptor. Model of the complex of the full-length α7-nAChR receptor with WTX in the membrane environment corresponding to the neuronal membrane was constructed using computer simulation methods. Molecular dynamics for >1500 ns confirmed the stability of the complex. The predicted membrane-active site of the WTX molecule includes residues Lys13 and Arg18. The study of WTX and its mutants Lys13Ala and Arg18Ala by NMR-spectroscopy confirmed the importance of these residues for interaction with lipid membrane.

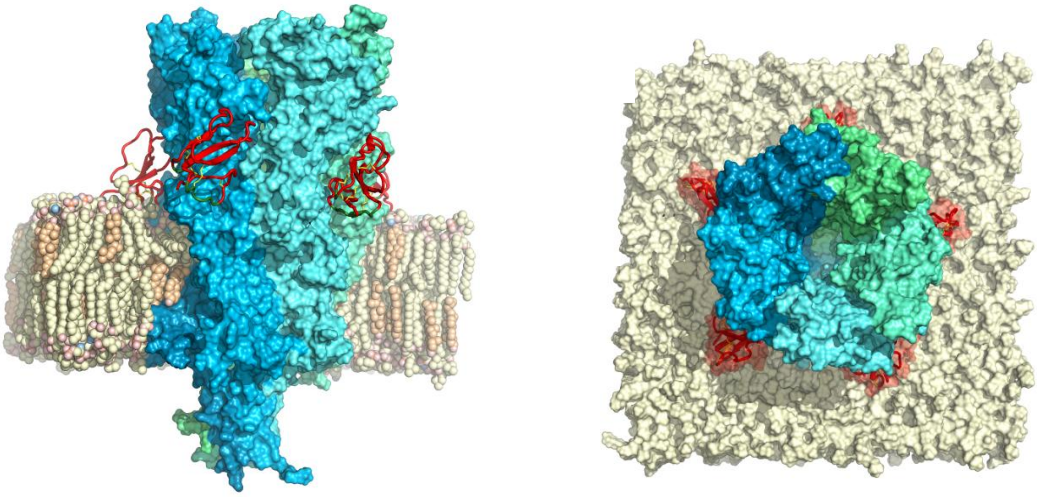

Figure 1. *The model of the complex of α7-nAChR/WTX in the membrane environment. Side and top views.*

Conclusion: Interaction mode of non-conventional neurotoxins with nAChR has been determined for the first time.

Key Words: Three-finger toxin • α7-nAChR • cryo-EM • neuronal membrane

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