

**International Journal of Biomedicine | June 2019 - Volume 9, Issue Suppl\_1:  
Abstracts From the Second Russian International Conference "Cryo-electron  
microscopy 2019: achievements and prospects"**

**POSTER ABSTRACT PRESENTATIONS**

**SESSION TITLE: STRUCTURE OF MEMBRANE PROTEINS**

DOI: 10.21103/IJBM.9.Suppl\_1.P7

**Abstract P-7: Cryoelectron Microscopy Study of Water-Soluble Extracellular  
Domain of  $\alpha 7$  Nicotinic Acetylcholine Receptor**

Andrey V. Tsarev<sup>1,2</sup>, Roman A. Kamyshinsky<sup>3</sup>, Vasilii I. Mikirtumov<sup>4</sup>, Dmitriy S. Kulbatskii<sup>1</sup>, Eugene O. Yablokov<sup>5</sup>, Zakhar O. Shenkarev<sup>1,2</sup>, Olga S. Sokolova<sup>4</sup>, Ekaterina N. Lyukmanova<sup>1,2</sup>

<sup>1</sup>*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia;* <sup>2</sup>*Moscow Institute of Physics and Technology, Dolgoprudny, Moscow Region, Russian Federation;* <sup>3</sup>*National Research Center, Kurchatov Institute, Moscow, Russia;* <sup>4</sup>*Lomonosov Moscow State University, Moscow, Russia;* <sup>5</sup>*Institute of Biomedical Chemistry, Moscow, Russia*

**Background:** Nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel, which is widely distributed both in the central and peripheral nervous system, and in some of the non-neuronal tissues, including epithelium and immune cells. The pathophysiology of a number of diseases is associated with dysfunctions of this receptor, including neurodegenerative and mental disorders like Alzheimer disease (AD) and schizophrenia. The nicotinic receptor of  $\alpha 7$  type ( $\alpha 7$ -nAChR) plays important role in the memory and learning processes and is inhibited by soluble aggregates of  $\beta$ -amyloid peptide (A $\beta$ ). A $\beta_{1-42}$  is the most toxic form of the amyloid peptide.

**Methods:** The water-soluble analogue of the ligand-binding extracellular domain of  $\alpha 7$ -nAChR was produced in *Pichia pastoris* and purified from a culture medium by Ni-NTA and size-exclusion chromatography. The equilibrium dissociation constant (K<sub>d</sub>) of the complex of the recombinant domain with  $\alpha$ -bungarotoxin was measured on the optical SPR biosensor Biacore 3000. Structures of the  $\alpha 7$  domain alone and in the complex with oligomeric A $\beta_{1-42}$  peptide were studied by cryoelectron microscopy (cryo-EM).

**Results:** The  $\alpha 7$  ligand-binding domain has an increased stability in solution, and demonstrates ligand-binding characteristics similar to those of the native receptor (K<sub>d</sub> of the domain/ $\alpha$ -bungarotoxin complex 28 $\pm$ 2nM). Statistical analysis of the cryo-EM images of the individual domain particles revealed the presence of a pentameric structure, confirming intact subunit assembly. Unfortunately, the domain demonstrated the preferable orientation on grids with the top view. Nevertheless, the 3D structure of the domain with a height  $\sim$  7 nm, external diameter of  $\sim$  9 nm, and the pore diameter of  $\sim$  2 nm was reconstructed at 8.5 Å resolution. 2D classification of the cryo-EM images of the domain particles in the

complex with A $\beta$ <sub>1-42</sub> revealed the conformational changes appeared due to interaction with the amyloid peptide.

**Conclusion:** Obtained results open new perspectives for structural studies of the nAChR ligand-binding domains in complex with the ligands which escape crystallization.

**Key Words:** cryo-electron microscopy •  $\alpha$ 7-nAChR • beta-amyloid peptide

**Sources of Funding:** This work was supported by the Russian Science Foundation (project # 19-74-20163).

International Journal of Biomedicine. 2019;9 Suppl 1: S19. doi: 10.21103/IJBM.9.Suppl\_1.P7

©2019 International Medical Research and Development Corporation