**Study of mechanism of proteolysis and a selective ligand binding to gamma-secretase complex**

## The most important completed tasks

The main results of the project are summarized in Fig. 1. The γ-secretase complex (consisting of four membrane proteins: presenilin PS-1, PEN-2, APH-1 and NCT) was tested in all-atom water-membrane environment. A series of molecular dynamics (MD) simulations were performed with two different concentrations of cholesterol, 10% and 50%. In these simulations, the structurally similar amyloidogenic substrates Aβ43 and Aβ45 were tested - they lead in subsequent γ-secretase cleavage event to the less toxic Aβ40 and the more toxic peptide Aβ42, respectively. Simulations revealed similarities and differences in their binding to γ-secretase and mobility, and how cholesterol affected their properties. By running a large number of long MD simulations, a detailed map of the cholesterol binding sites of individual γ-secretase subunits was constructed (Fig. 1a). It was found that at high cholesterol levels, the substrate is repositioned by its direct contact with two cholesterol molecules, preventing bending of TM3 PS-1 helix (Fig. 1b). Comparing the mean fluctuations of individual residues, statistically significant differences were found in the mobility of Aβ43 and Aβ45 at the PS-1 binding site, especially at high cholesterol levels (Fig. 1c). Differences between Aβ43 and Aβ45 in the active site of PS-1 were also found in terms of the degree of residue packing in the active site and in the distance between the cleaved bond and the catalytic residues (Fig. 1d).

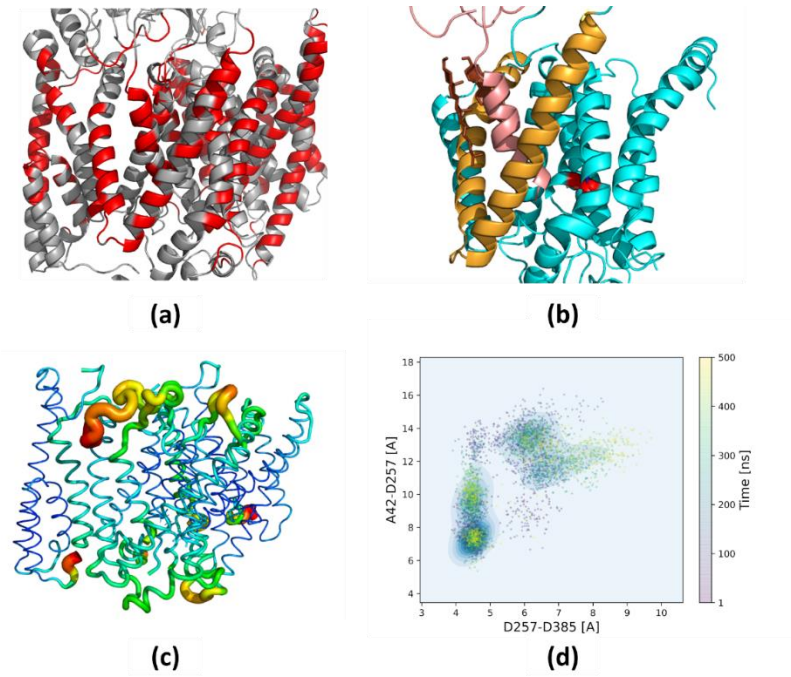


**Fig. 1.** Illustration of the most important results of the project. (**a**) Detailed map of cholesterol binding sites; (**b**) change of location of the substrate at the binding site by its direct contact with cholesterol, preventing bending of PS-1 helix; (**c**) assignment of differences in substrate mobility in the binding site, especially at high cholesterol concentrations; (**d**) study of differences in packing of residues in the active site of PS-1.

## Significance of the project

The recently determined cryo-EM structures of the γ-secretase complex allowed to obtain a dynamic picture of this membrane protease by using MD simulations and studying the binding of amyloidogenic substrates. The constructed maps of γ-secretase-cholesterol contacts may be useful for other researchers to design new experiments to understand the effect of cholesterol on substrate cleavage. Alzheimer's disease (AD) is the only disease among the top 10 killer diseases that does not have effective treatments, so basic research to understand the mechanisms of action of the proteins involved in AD could advance this field and ultimately lead to the discovery of new drugs.