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Solving the structural puzzle of neurotrophin signaling

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Motivation and Aim: Neurotrophins and their receptors are involved in many important processes in neurons like proliferation, differentiation, neuroplasticity, apoptosis, etc. Incorrect functioning of any element in these cascades leads to the development of various neurodegenerative and mental diseases, including Alzheimer's and Huntington's diseases, depression and schizophrenia. A detailed understanding of the mechanisms of neurotrophin receptor activation, their interaction with ligands and adapter proteins can explain the diversity of biological responses and give an approach for the creation of novel drugs based on the rational design. Here we present our structural findings in the big puzzle of neurotrophin signaling.

Methods: We used bacterial and cell-free expression systems to produce the transmembrane domains of P75NTR and TrkA neurotrophin receptors, full intracellular domain and death domain (DD) of P75NTR, and receptor P75NTR without extracellular region. To investigate the transmembrane-containing fragments we used different membrane-mimetic media (micelles, bicelles, nanodiscs and liposomes). To characterize the behavior, dimerization propensity and structure of the proteins we used site-directed mutagenesis, light scattering, and solution NMR spectroscopy.

Results: First, we showed that there is no interaction between DDs of P75NTR in any context. We proposed a novel model of P75NTR functioning, suggesting that P75NTR dimerization occurs in the complex of two P75NTR molecules and “helper” protein, and ligand binding leads to the “helper” release, which restores the ability of P75NTR dimer to interact with adapter proteins. Second, we identified and characterized the direct interaction between p75NTR and TrkA through their transmembrane domains. Third, we investigated the dimerization of transmembrane domain of the TrkA and found two dimer interfaces that we believe correspond to active and inactive states of the receptor. Finally, we demonstrate that eJTM region of TrkA are intrinsically disordered and most likely that signal transduction occurs after direct interaction between ligand and this domain.

Conclusion: Membrane protein investigation is a big challenge for structural biology. To date, there are no experimental methods to investigate the structure of full-length receptor, like Trk or P75, therefore the researchers are trying to understand the activation mechanisms based on a lot of different indirect data. All our findings presented here open an additional part of the puzzle, but the other studies are required to see the full picture.

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