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Type	Oral communication
Session	Structural Bioinformatics
Title	Using AlphaFold to delve into signal transduction mechanisms
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Motivation

Receiving and responding to external stimuli is one of the fundamental functions of a cell. This process involves a variety of proteins that interact with each other to transmit the signal across the cell membrane and ultimately elicit a cellular response. Understanding the structural details of these protein interactions is critical to understand the molecular mechanisms underlying signal transduction.
Experimental methods to determine the structure of protein complexes are expensive and time-consuming so in recent years computational tools have been developed to perform this task. The state-of-the-art method to predict the protein structure is the AlphaFold, a deep learning-based protein structure prediction tool developed by DeepMind. AlphaFold-Multimer is an extension of AlphaFold that predicts the structure of protein complexes, including multimeric assemblies, by combining deep learning with evolutionary information. AlphaFold-Multimer has demonstrated high accuracy in predicting the structure of protein complexes, making it a valuable tool for understanding the molecular basis of complex biological processes.

Methods

In our study we used AlphaFold-Multimer to predict the structure of interacting protein complexes, which were subsequently analyzed using a range of bioinformatics and data analysis approaches. Specifically, we calculated the Root Mean Square Deviation (RMSD) of the structures of the same protein bound to different interactions of the same class to compare the different predicted docking modes. Furthermore, we considered the predicted interface contact networks between a transducer and different partners, and determined the most central nodes in these networks to identify the positions that are more likely to contribute to interaction specificity. We also employed Rosetta InterfaceAnalyzer on the predicted structures to evaluate the stability of the complexes.

Results

In our study, we demonstrate the potential of AlphaFold-Multimer to provide novel insights into the mechanisms of signal transduction by predicting the structure of crucial complexes in two systems of biomedical interest: G-protein-coupled receptor (GPCR) and leucine-rich repeat kinase 2 (LRRK2). The structures generated by AlphaFold Multimer can be utilized to identify the structural determinants responsible for the interaction specificity between a transducer and its ligands.
GPCRs are a large family of cell-surface receptors that play a crucial role in intracellular signaling pathways and are targeted by many pharmacological drugs. β-arrestins are involved in controlling the downstream activity of GPCRs by desensitizing their activity, thereby adding another layer of signaling modulation.
We predicted the structure of all known interacting complexes between GPCR-G-protein interacting complexes. Our findings confirmed existing knowledge regarding G protein coupling specificity and shed light on potential new signaling mechanisms for individual G-protein family members, as well as for less studied G-proteins groups such as the G12/13 one. Additionally, we identified differences in the binding energy of different G-protein classes, with Gs and Gq/11 forming more stable complexes, while Gi/o and G12/13 formed less stable ones. This observation is consistent with the fact that these G proteins are more promiscuous and likely to form more transient complexes. We are also extending multimer predictions to predict the structures of the complexes of GPCRs with another fundamental class of receptor transducers, i.e. β-arrestins. Predictions are instrumental in shedding structural insights about different β-arrestins binding modes with the receptor (i.e. tail, core or full engagement) that can be useful for the interpretation of experimental data, as well as for the design of new precision, transducer-biased therapeutics.
We are also investigating the role of the Parkinson's disease-linked kinase LRRK2 in RAB-mediated signaling pathways. LRRK2 is a multi-domain protein that integrates multiple signals to spatiotemporally regulate the activity of the kinase domain. By using Alphafold Multimer to predict the interacting complex of LRRK2 with all human RABs, we were able to gain insights into the interaction sites of different RABs, discriminating those involved in the binding of modulators (e.g. RAB29) from the substrates (e.g. RAB10), and the different conformations that LRRK2 adopts while interacting with various partners. These predictions are coherent with crosslinking mass spectrometry experimental data.
In this study, we provide some examples of the usage of AlphaFold Multimer to better understand the structural basis of signaling pathways. The information provided by these models can be helpful to guide future experimental studies and facilitate the development of new therapeutic strategies for treating diseases.

Info

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