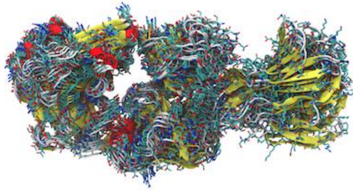

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Septin heterocomplex structure reveals the molecular determinants at G-interface

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Abstract:
Introduction and Objective. Septins are cytoskeletal proteins, which play a vital role in several cellular functions such as membrane remodeling, compartmentalization, and cell division. They bind GTP and able or not to hydrolyze it into GDP, interacting with each other forming filaments necessary to accomplish their functions. Thus, the septins studies have focused on the understanding of filament assembly and how GTP binding and hydrolysis drives it. Currently, the only heterocomplex structurally characterized available, formed by human septins 2-6-7 (1), displays very low resolution (4 Å), which has hampered conclusions on its structure and filament assembling mechanism. Therein, this study sought to obtain structural insights on the molecular determinants of the interaction between the GTP binding domains (G domains) of the septin 2-6 complex (SEPT6G-SEPT2G), which is essential to understand the filament assembly.
Materials and Methods. We co-expressed the septin heterocomplex in E. coli and co-purified it by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). We grew protein crystals using the vapor diffusion technique. Using the I24 line at Diamond Light Source (UK), we collected the diffraction patterns and processed data via XIA2DIALS pipeline. With integrated data, we determined the phases via molecular replacement, refined the model and validated it to make structural inferences. The importance of the G-interface formation on the thermal stability of the heterodimer was also analyzed and compared to SEPT6G alone in solution, using differential scanning calorimetry (DSC).
Results. The co-expressed and co-purified SEPT6G-SEPT2G complex consisted of a heterodimer in the solution, also observed in the asymmetric unit of the structure obtained at 2.6 Å. Analyzing the structure and aligning it with the structure previously characterized (1), it was possible to visualize many important regions (not only at the interface of interaction) which have not been observed even in structures of greater resolution of individual septins, due to their high flexibility. Scrutinizing the new structure interactions, we identified for the first time, a fundamental structural motif directly connecting the lack of catalytic activity of the septin 6 to specificity and sustainment of G-interface. The absence of SEPT2G as a partner of SEPT6G (to form the heterodimer) led to a significant loss in thermal stability (20 ºC) of the latter (denaturation starts bellow physiological temperatures), showing the importance of heterodimer formation as a starting point for filament assembly.
Conclusions. The structure of the SEPT6G-SEPT2G complex brought significant details about the molecular interactions at the G interface, linking them to the lack of activity in SEPT6. Additionally, it explains the enhanced thermal stability of heterodimer when compared to individual septin. These findings shed light on the molecular determinants of G-interface between septin 2 and septin 6 and its physiological significance as a core for the filament assembly.

References. (1) Sirajuddin et al. Structural insight into filament formation by mammalian septins. Nature 2007: 449: 311-315.e