Poster

Open and closed conformations of a sub-80 kDa Chagas vaccine candidate defined by a cryo-EM led integrative approach

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Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, remains a significant health concern in Latin America. A potential breakthrough in Chagas disease vaccine development lies in targeting Trypanosoma cruzi 80kDa prolyl oligopeptidase (TcPOP), a validated antigen. In this study, TcPOP was expressed in Escherichia coli, purified, and utilized as an immunogen in mice to investigate the resulting immune response.

Recombinant TcPOP was successfully purified to homogeneity and analysed using SEC-MALS and SEC-SAXS. Secondly, the purified TcPOP was used to immunise mice in conjugation with an adjuvant, eliciting a robust immune response. Monoclonal and polyclonal antibodies were purified from immunized mice using hybridoma technology and assessed for their reactivity against TcPOP and exhibited a strong and specific response confirmed using Enzyme-Linked Immunosorbent Assay (ELISA), BLI and Mass photometry. Several crystallization trials were performed at Diamond light source but crystallization remains unsuccessful due to high solubility of TcPOP in solution.

Despite sub-80 kDa size of TcPOP, the antigen was employed on cryoEM grids to be screened under the CryoTEM. I collected data on Krios 300 KeV and after processing the dataset I was able to resolve the structure of apo-Tc80 at 2.5 Å resolution. Interesting, I found that the structure exhibits multiple conformation specifically closed, and open states, therefore, I was able to deduce both the conformation at 2.5Å and 3Å resolution, respectively. This outcome demonstrated the structural variability and dynamics of TcPOP prevented the crystal formation, moreover the result represented the dynamics of not just TcPOP but family of prolyl oligopeptidases.

Future research endeavours will focus on utilizing the vaccine potential of TcPOP-mAb complex, to later pin down the epitope that could be crucial for antigen recognition and parasite invasion. These structural insights will provide valuable information for rational vaccine design and therapeutic development against Chagas disease.