Experimental and computational study of the protein-membrane interaction of the proposed N-terminal domain MetY of Hepatitis E Virus pORF1

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Positive-sense single-stranded RNA ((+)RNA) viruses form membranous replication vesicles in infected cells where synthesis of new viral RNAs takes place. In the Alphavirus-like superfamily of (+)RNA viruses, vesicle formation is mediated by the N-terminal domain of the replication polyprotein (nsP1 in CHIKV), that also harbours the RNA capping enzyme. Cryo-electron microscopy (CryoEM) and tomography (CryoET) studies of three viruses, including Chikungunya virus (CHIKV), have shown that this N-terminal domain self-assembles as a membrane-associated dodecameric ring, recruits other viral proteins to the replication complex, and subsequently forms a gate between the replication vesicle and the cytosol[1].

While not very well studied, there are convincing indications that the methyltransferase N-terminal domain (MetY) of the Hepatitis E Virus (HEV – an Alphavirus-like virus) replication polyprotein (pORF1) performs a similar dual function of membrane-bound gate and capping enzyme. This hypothesis is supported by AlphaFold modelling and structural homology with CHIKV nsP1[2], and by in vitro studies.

During my PhD, I investigate this through *in vitro* (biochemistry) and *in silico* (molecular dynamics) analysis of the MetY membrane interactions and oligomerisation. I have identified membrane interactions using both approaches, with simulations revealing two short α-helices involved in binding and possibly membrane deformation. I am currently working on extending these results to include structural (CryoEM) and cellular biology approaches.

Bibliography :

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