Title:

Deciphering poxvirus assembly: characterization of Viral Membrane Assembly Proteins and their interactions with model membranes

Keywords: poxviruses, enveloped virus assembly, biochemistry, structural biology, protein-membrane interactions

Description:

Our team is interested in poxviruses, a part of the expanding viral phylum *Nucleocytoviricota*, that include Variola virus, the causative agent of smallpox. Smallpox was eradicated after global vaccination using Vaccinia virus (VACV) as the first live vaccine. Vaccination was stopped and few antivirals are now available against emerging poxviruses that represent a threat to humans and wildlife like Mpox virus. VACV remains hence a crucial model for studying poxvirus biology and pathogenicity. This proposed project for a M2 internship will focus on VACV assembly, and particularly on a group of five Viral membrane Assembly Proteins (VMAPs). VACV membrane assembly is indeed unique, with no similarity to other known membrane biogenesis processes. During VACV assembly, endoplasmic reticulum (ER) membranes are extensively remodeled. In brief, VMAPs are supposed to recruit ER-derived vesicles to the viroplasm, where these membrane precursors are ruptured and form half-moon crescent structures with stabilized open ends. However, the underlying molecular mechanism is poorly understood and in particular, how VMAPs might stabilize these membrane open ends in the cell cytoplasm remain unclear.

The goal of this internship is to produce recombinant VMAPs in either cellular (E. coli) or cell-free expression (wheat germ extracts) systems. The expressed VMAPs will be purified using liquid chromatography methods after a solubilization step using detergent if necessary. These proteins will then be characterized using biophysical techniques to assess their purity, stability, oligomeric states and other biochemical properties. The main objective will then be to investigate VMAP-membrane interactions through reconstitution in model membranes (*e. g.* liposomes or ER-derived microsomes) and use flotation assays to assess membrane binding. The intern will have to optimize the preparation of protein-membrane samples as described above for subsequent structural studies by cryo-electron tomography. This internship offers an exciting opportunity to study the unique mechanisms of poxvirus assembly combining virology, membrane biology, biochemistry and structural biology. Ultimately, the expected findings are likely to provide new insights into virus-host interactions that could inspire antiviral strategies and enhance our understanding of viral pathogenicity.

Techniques used during the internship:

- Recombinant protein expression in *E. coli* or in cell-free expression system (wheat germ extracts)
- Protein purification (affinity chromatography and gel filtration)
- Protein quality control (SDS-PAGE, western-blot, negative-staining electron microscopy)
- Test of protein solubilization with detergents

- Liposome preparation
- Reconstitution in proteoliposomes
- Membrane binding assays using floatation experiments
- Sample preparation and optimization for cryo-electron tomography

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