Expression, Purification, and Biophysical Characterization of Monkeypox Virus Protein I1

Even after the eradication of smallpox, poxviruses remain a global health threat. The 2022 monkeypox outbreak, with over 94,000 cases worldwide and no approved treatments, alongside fears of weaponized poxvirus use due to reduced immunity post-vaccination cessation, prompts new studies for drug target discovery. I1, a 35-kDa protein from poxvirus, has been shown to bind DNA in a nonspecific manner and may facilitate DNA-packaging required for late-stage virion morphogenesis. Despite being required for DNA encapsidation and virion maturation, little is known about I1, including its structural, oligomeric, and biochemical properties, as well as its activity towards DNA.

Using a battery of orthogonal biophysical methods including dynamic light scattering (DLS), analytical ultracentrifugation (AUC), size-exclusion chromatography in line with multi-angle light scattering (SEC-MALS), and synchrotron size-exclusion chromatography small-angle X-ray scattering in line with MALS (SEC-SAXS-MALS), we established the solution properties of recombinant I1. Bioinformatic analysis and limited proteolysis experiments established the presence of an N-terminal oligomerization domain that mediates the tetramerization of the protein at concentrations below 30 μM. At higher concentrations, SAXS analyses provide evidence for the formation of octamers. SAXS data combined with AlphaFold predictions yield a low-resolution quaternary model for this protein. This data provides a point of departure for future studies focusing on its interactions with nucleic acids and high-resolution structural studies.