

Title: Structure and mechanism of monkeypox virus (MPXV) helicase-primase complexes

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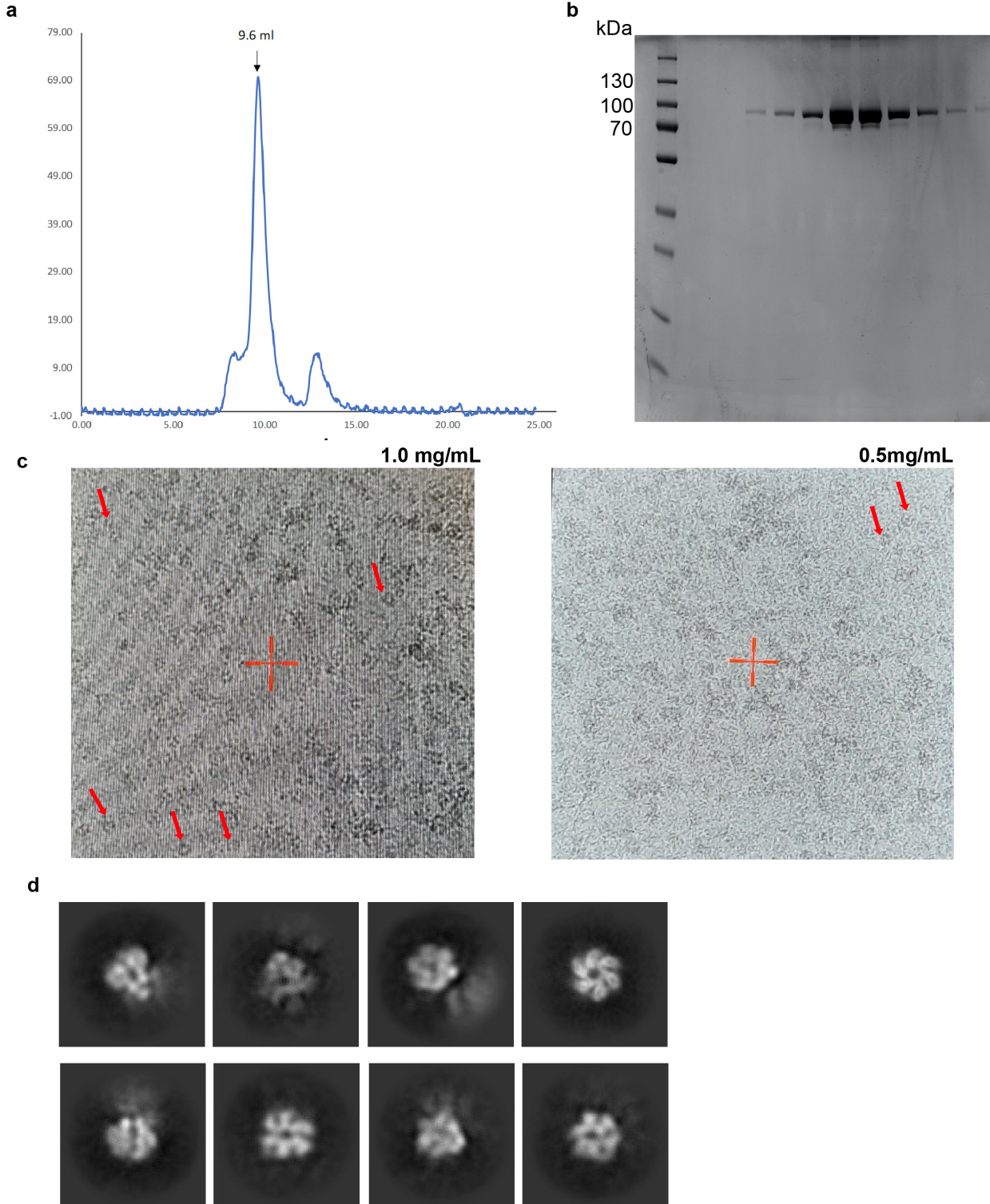


Figure 1. a. Size-exclusion chromatography (SEC) profile of MPXV helicase primase (apo form) showing single homogeneous peak (elution volume or V_e = 9.6 mL). The SEC peak fractions were checked for purity over a coomassie-stained SDS-PAGE (**b**). The purest fractions were used for grid preparation and cryoEM studies. **c.** Micrographs obtained from two grids (1 mg/mL and 0.5 mg/mL) clearly show single particles (red arrows). **d.** 2D class averages obtained from 400 micrographs collected at our in-house Glacios microscope (200 keV) suggest a ring-like architecture of this protein complex.

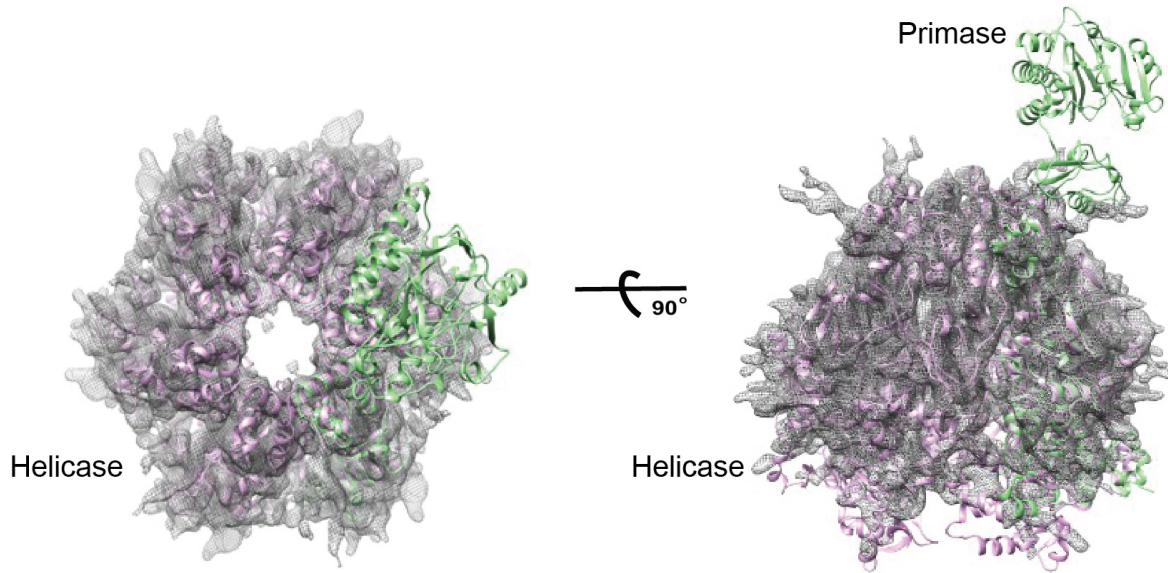


Figure 2. Single particle reconstruction derived from Glacios data reveals a hexameric assembly of full length apo MPXV D5 to $\sim 4.5\text{\AA}$ resolution. The KRIOS data may help us achieve higher resolution for central helicase core and may resolve the density for the primase and zinc-finger domains.