Single-particle Reconstruction of a Prolate Mycobacteriophage Capsid via Negative Stain TEM

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What are Bacteriophage?

- Viruses that infect bacteria
- Most have dsDNA genomes
- Most abundant biological entities on the planet
- Vary widely in morphology
- 4 major structural components
  - Capsid (head)
  - Tail
  - Head-to-Tail connector
  - Baseplate
MooMoo

- Isolated from a water sample in Sandy Hook, KY in 2012
- Large, prolate capsid
- Host is *Mycobacterium smegmatis*
- Highly mosaic genome
- ~300nm from the end of the tail to the top of the capsid
MooMoo

- *In silico* protein folding was previously performed on the major capsid (A) and major tail (B) proteins using I-TASSER
- EM data needed to determine repeat structure and protein orientation within the phage particle
EM Methods

- Automated acquisition of negative stained particles (uranyl acetate)
- 200 kV, 74000x magnification
- Over fourteen thousand images!
Example Images
Data Analysis (ongoing)

- Single-particle reconstruction has been performed using negative stain data for Araucaria, another Mycobacteriophage
- We will follow the pipeline established by Sassi et al (2013) using either EMAN2 or RELION software packages
- End result will be an ~30 Å reconstruction
Issues Encountered

◦ Humidity and equipment issues/limitations meant no cryoEM data were obtained
◦ COVID-related delays
◦ Data analysis
References


Questions?