

# Breaking the shackles of symmetry in structural studies of icosahedral viruses.

Structural insights into calicivirus attachment and entry

David Bhella

Tokyo Medical and Dental University

July 1<sup>st</sup> 2019

MRC-University of Glasgow Centre for Virus Research

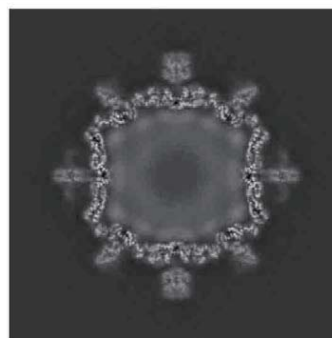
## Summary



- Brief introduction to Cryo-EM
- Asymmetry in icosahedral viruses
- Structural analysis of asymmetric features critical to calicivirus entry
- The Scottish Centre for Macromolecular imaging

MRC University of Glasgow Centre for Virus Research

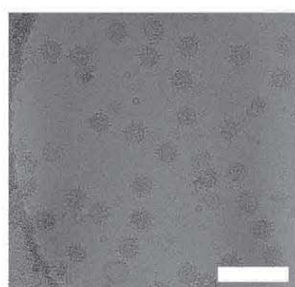
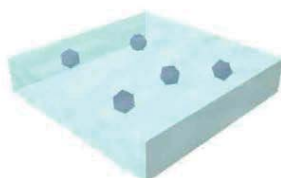
## Cryo-EM structure of *Macrobrachium Rosenbergii* nodavirus



MRC University of Glasgow Centre for Virus Research

Ho *et al.*, Structure of the *Macrobrachium rosenbergii* nodavirus: A new genus within the Nodaviridae? *PLoS Biol.* **16**, e3000038 (2018).

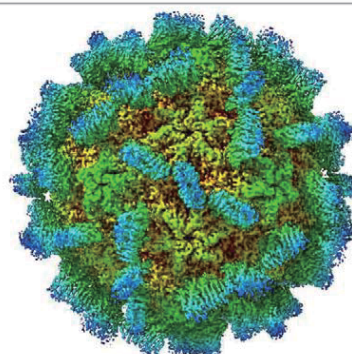
## Cryomicroscopy of purified macromolecular assemblies



*Macrobrachium Rosenbergii* nodavirus virus-like particles

MRC University of Glasgow Centre for Virus Research

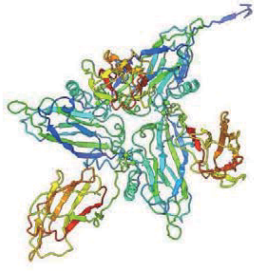
## Cryo-EM structure of *Macrobrachium Rosenbergii* nodavirus



MRC University of Glasgow Centre for Virus Research

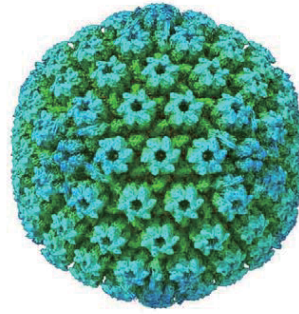
Ho *et al.*, Structure of the *Macrobrachium rosenbergii* nodavirus: A new genus within the Nodaviridae? *PLoS Biol.* **16**, e3000038 (2018).

### Structure of *MnV* capsid protein



MRC-University of Glasgow Centre for Virus Research  
Ho et al., Structure of the *Macrobrachium rosenbergii* nodavirus: A new genus within the *Nodaviridae*? *PLoS Biol.* 16, e3000038 (2018).

### Asymmetry in icosahedral viruses



MRC-University of Glasgow Centre for Virus Research  
McElwee et al. Structure of the herpes simplex virus portal-vertex. *PLoS Biol.* 16, e2006191 (2018).

### Structure of *MnV* capsid protein



MRC-University of Glasgow Centre for Virus Research  
Ho et al., Structure of the *Macrobrachium rosenbergii* nodavirus: A new genus within the *Nodaviridae*? *PLoS Biol.* 16, e3000038 (2018).

### The *caliciviridae*

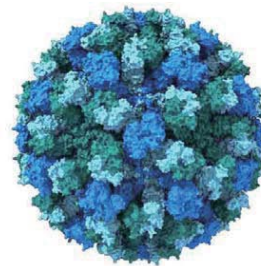


### Feline Calicivirus



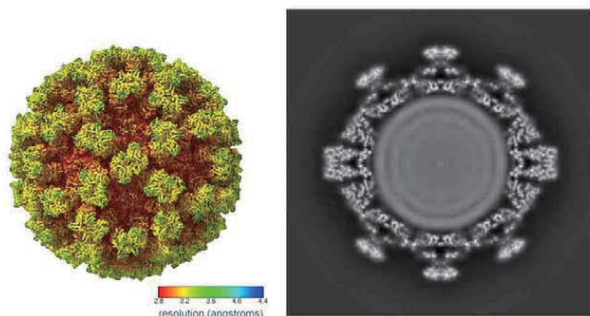
MRC-University of Glasgow Centre for Virus Research

### Calicivirus architecture



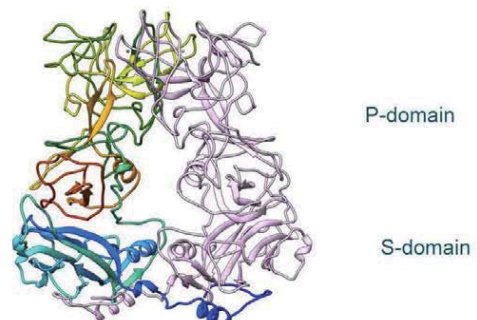
MRC-University of Glasgow Centre for Virus Research

### CryoEM structure of Feline Calicivirus

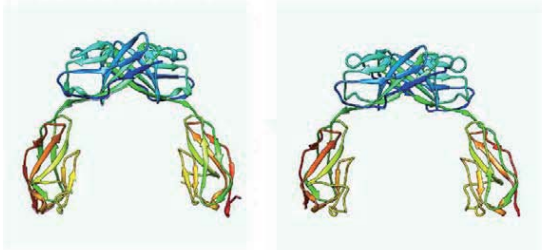


MRC-University of Glasgow Centre for Virus Research

### Structure of Feline Calicivirus



### Junctional Adhesion Molecule A

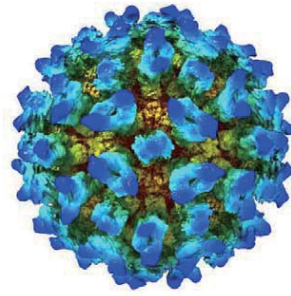


Murine JAM-A  
Kostrewa *et al.* (2001) EMBO J.

Human JAM-A  
Prota *et al.* (2003) PNAS

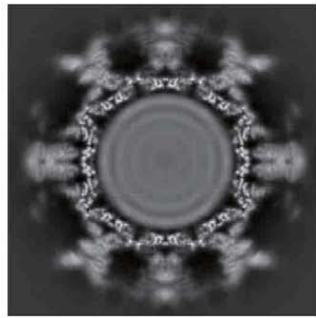
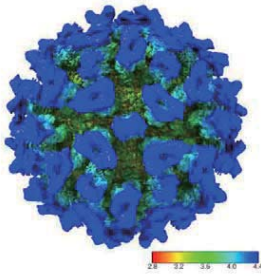
MRC University of Glasgow Centre for Virus Research

### FCV-fJAM-A binding



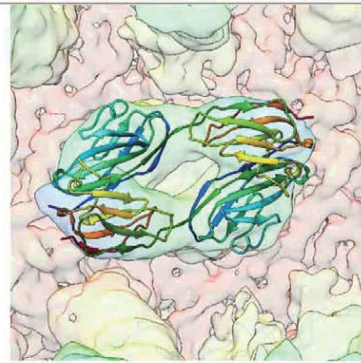
MRC University of Glasgow Centre for Virus Research

### 3.5 angstrom structure of FCV-fJAM-A



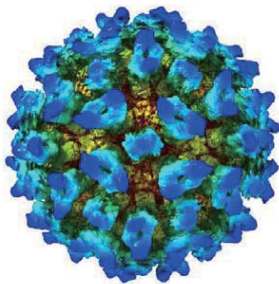
MRC University of Glasgow Centre for Virus Research

### FCV-fJAM-A binding



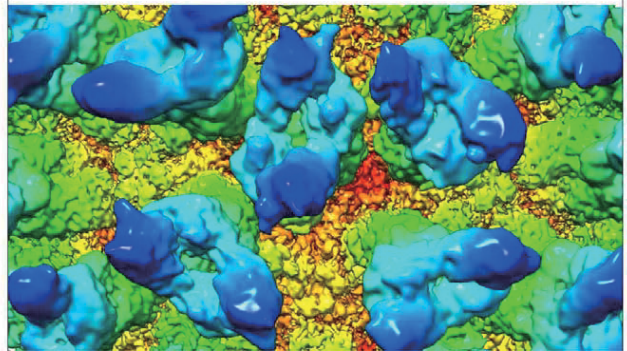
MRC University of Glasgow Centre for Virus Research

### fJAM-A binding induces major conformational changes



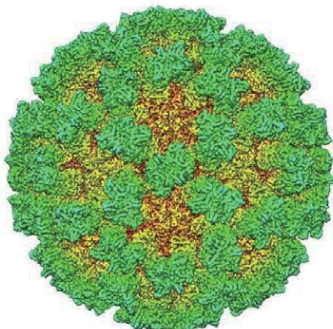
MRC University of Glasgow Centre for Virus Research

### Focused classification - CC dimer



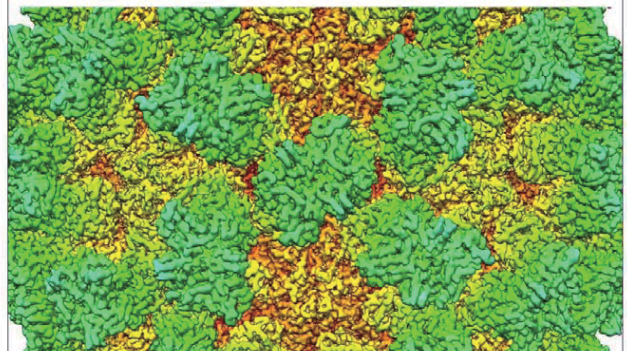
MRC University of Glasgow Centre for Virus Research

### Focused classification - CC dimer



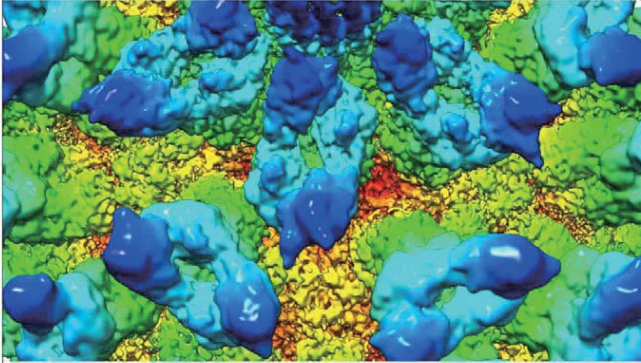
MRC University of Glasgow Centre for Virus Research

### Focused classification - AB dimer



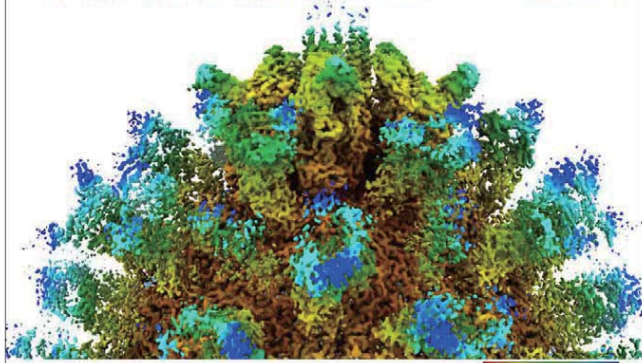
MRC University of Glasgow Centre for Virus Research

A novel feature at the icosahedral three-fold axis (CC-dimer analysis)



MRC-University of Glasgow Centre for Virus Research

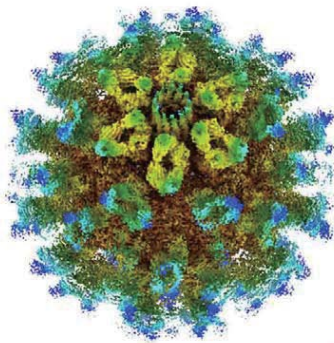
Focused classification at the 3-fold



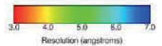
MRC-University of Glasgow Centre for Virus Research



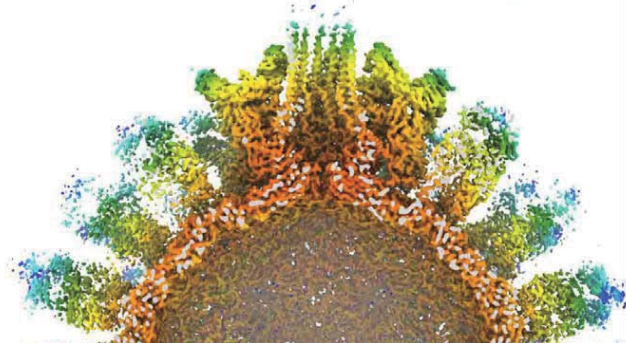
Focused classification at the 3-fold



MRC-University of Glasgow Centre for Virus Research



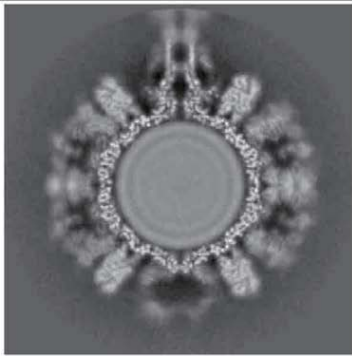
Focused classification at the 3-fold



MRC-University of Glasgow Centre for Virus Research

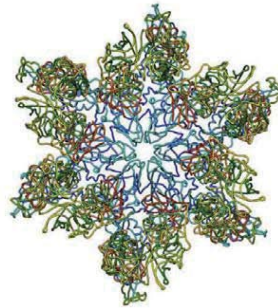


z-slices through the asymmetric reconstruction



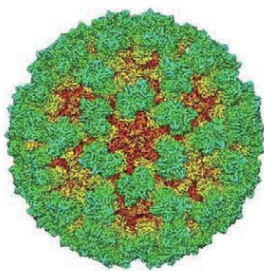
MRC-University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1

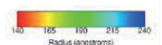


MRC-University of Glasgow Centre for Virus Research

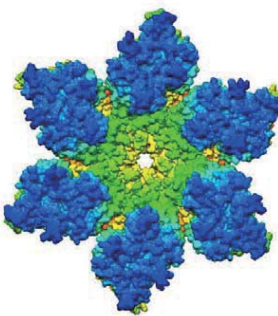
fJAM-A binding induces conformational changes in VP1



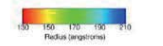
MRC-University of Glasgow Centre for Virus Research



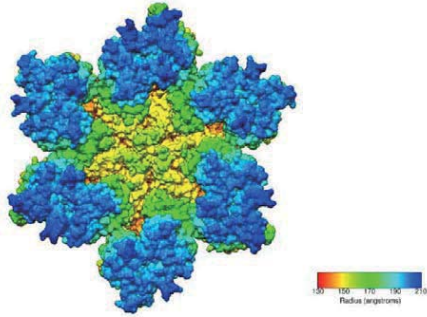
fJAM-A binding induces conformational changes in VP1



MRC-University of Glasgow Centre for Virus Research

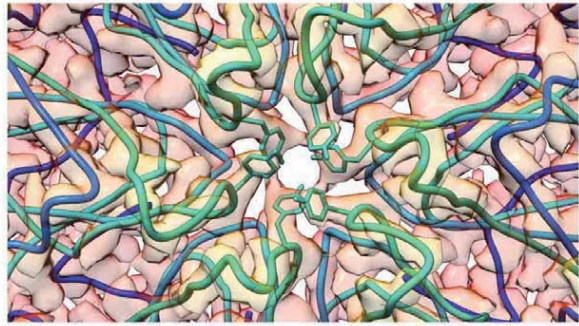


fJAM-A binding induces conformational changes in VP1



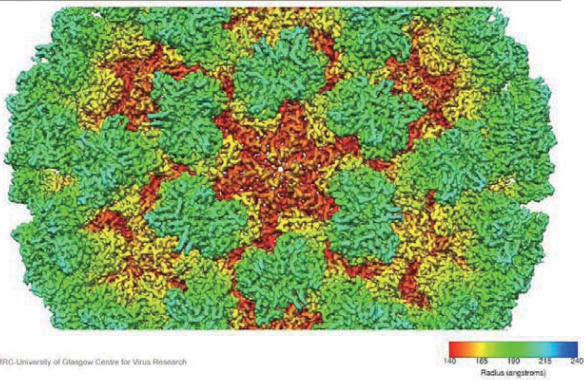
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1



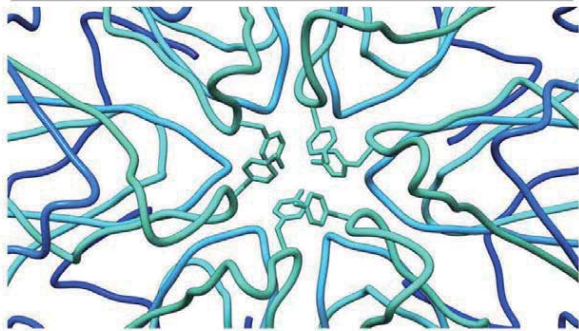
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1



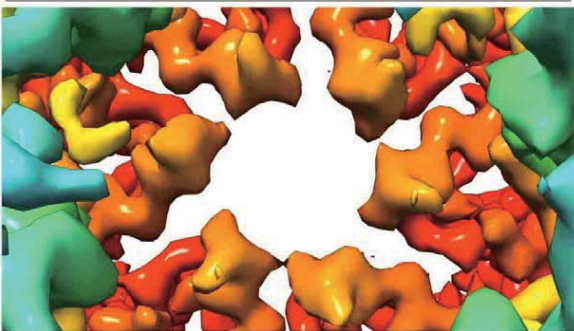
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1



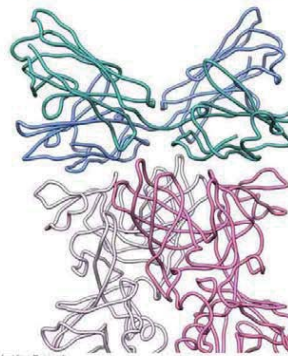
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1



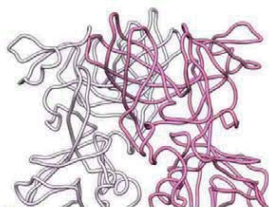
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1 P-domain - AB dimer



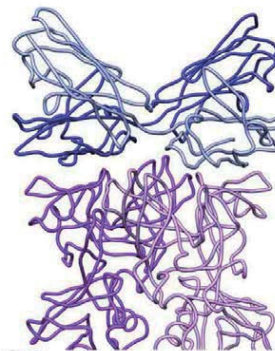
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1 P-domain - AB dimer



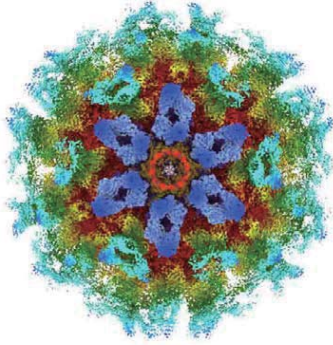
MRC University of Glasgow Centre for Virus Research

fJAM-A binding induces conformational changes in VP1 P-domain - CC dimer



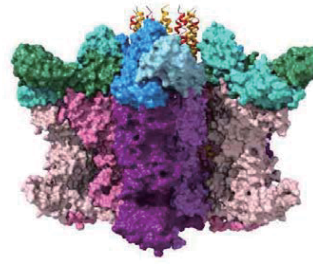
MRC University of Glasgow Centre for Virus Research

An atomic model of the portal vertex



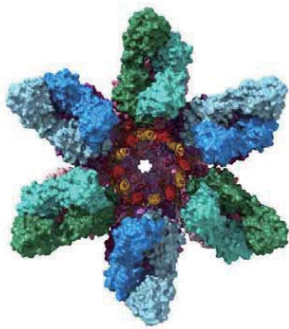
MRC University of Glasgow Centre for Virus Research

An atomic model of the portal vertex



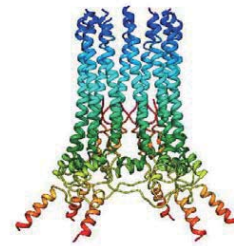
MRC University of Glasgow Centre for Virus Research

An atomic model of the portal vertex



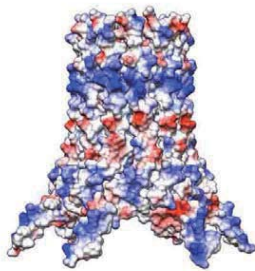
MRC University of Glasgow Centre for Virus Research

Structure of VP2



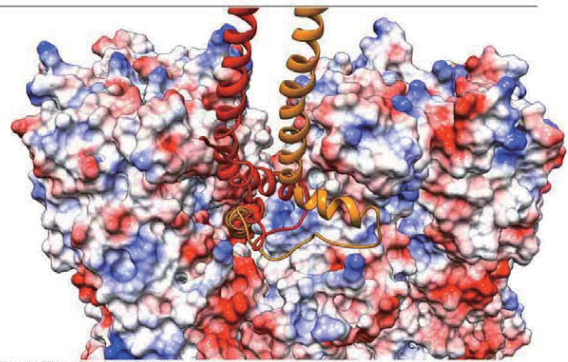
MRC University of Glasgow Centre for Virus Research

Structure of VP2



MRC University of Glasgow Centre for Virus Research

Anchoring VP2 to the capsid surface



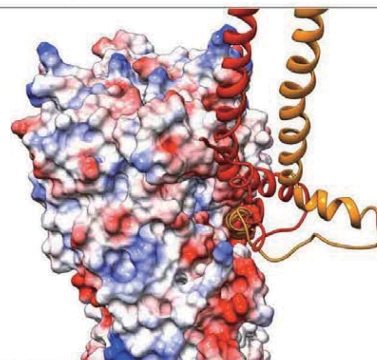
MRC University of Glasgow Centre for Virus Research

Anchoring VP2 to the capsid surface



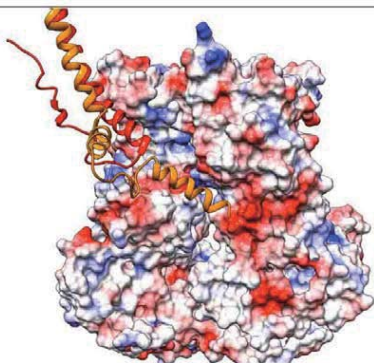
MRC University of Glasgow Centre for Virus Research

Anchoring VP2 to the capsid surface



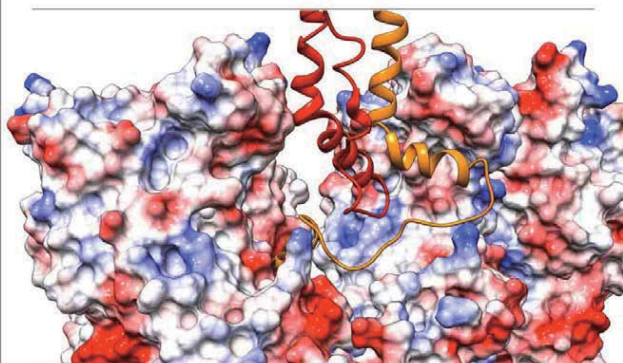
MRC University of Glasgow Centre for Virus Research

## Anchoring VP2 to the capsid surface



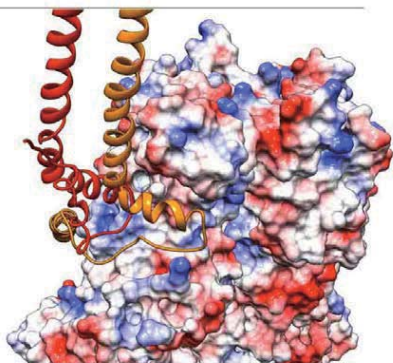
MRC-University of Glasgow Centre for Virus Research

## Anchoring VP2 to the capsid surface



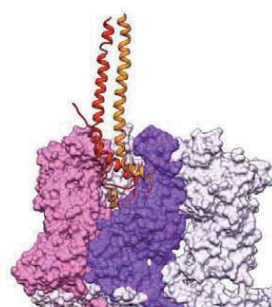
MRC-University of Glasgow Centre for Virus Research

## Anchoring VP2 to the capsid surface



MRC-University of Glasgow Centre for Virus Research

## An atomic model of the portal vertex



MRC-University of Glasgow Centre for Virus Research

The Scottish Centre for  
Macromolecular ImagingA national centre for structural biology research  
by Cryo-EM

MRC-University of Glasgow Centre for Virus Research

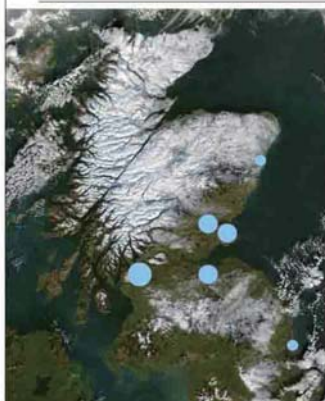
## JEOL JEM Z300FSC



MRC-University of Glasgow Centre for Virus Research

- "CryoARM 300"
- 12 slot specimen autoloader
- Automation
- 'Omega' - energy filter
- Phase plate
- Cold field emission gun
- STEM
- Direct Electron DE-64

## A network for structural biology research



- SCMI is envisaged as a hub and spoke network
  - 200 keV FEG feeder instruments at three partner institutions
    - Glasgow - JEOL F2 Cryo
    - Edinburgh - FEI Tecnai F20
    - Dundee - JEOL 2200

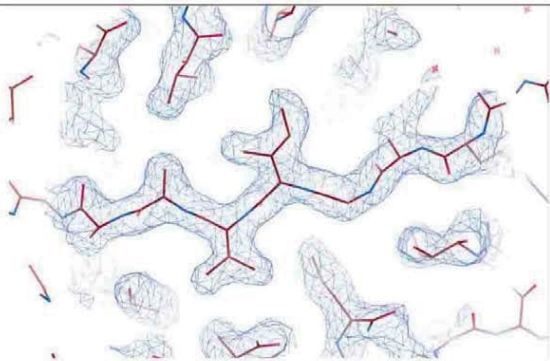
## JEOL JEM F200



MRC-University of Glasgow Centre for Virus Research

- "F2-Cryo"
- Automation
- Phase plate
- Cold field emission gun
- ARM lenses
- STEM
- Direct Electron DE-20

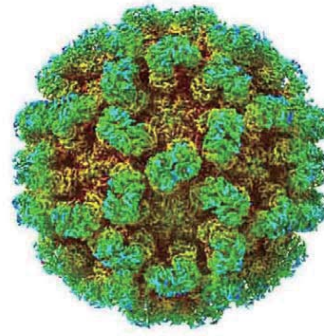
## Lumazine Synthase



MRC University of Glasgow Centre for Virus Research

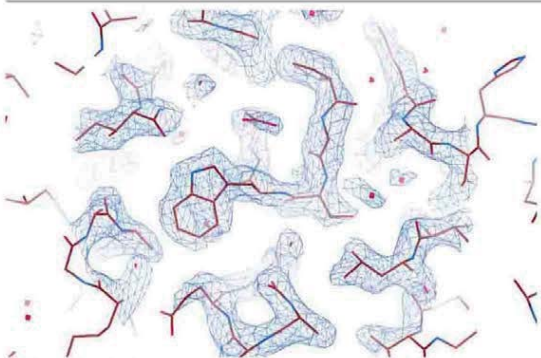
PDB 1HQK - Zhang et al. 2001 JMB 306 (5) pp1099-1114

## Vesivirus 2117



MRC University of Glasgow Centre for Virus Research

## Lumazine Synthase



MRC University of Glasgow Centre for Virus Research

PDB 1HQK - Zhang et al. 2001 JMB 306 (5) pp1099-1114

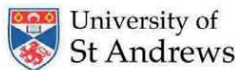
## Summary



- CryoARM 300 selected on grounds of novel technologies and innovative design
- CryoARM 300 installation started April 2018
- Software setup and hardware/software troubleshooting has taken time
- Workflow development ongoing
- SCMI is ready to move to service delivery

MRC University of Glasgow Centre for Virus Research

## SCMI funders



MRC University of Glasgow Centre for Virus Research

## Acknowledgements



- SCMI
  - James Streetley
  - Mairi Clarke
- FCV
  - Michaela Conley
  - Ian Goodfellow
- MrNV
  - Kok Lian Ho
  - Wen Siang Tan
  - Mads Gabrielsen
  - Poay Ling Beh
- Lumazine Synthase
  - Arvind Patel
  - Vanessa Cowton
  - Sarah Cole
- HSV portal
  - Marion McElwee
  - Frazer Rixon
- Vesivirus 2117
  - Ian Goodfellow
  - Ed Emmott

MRC University of Glasgow Centre for Virus Research

