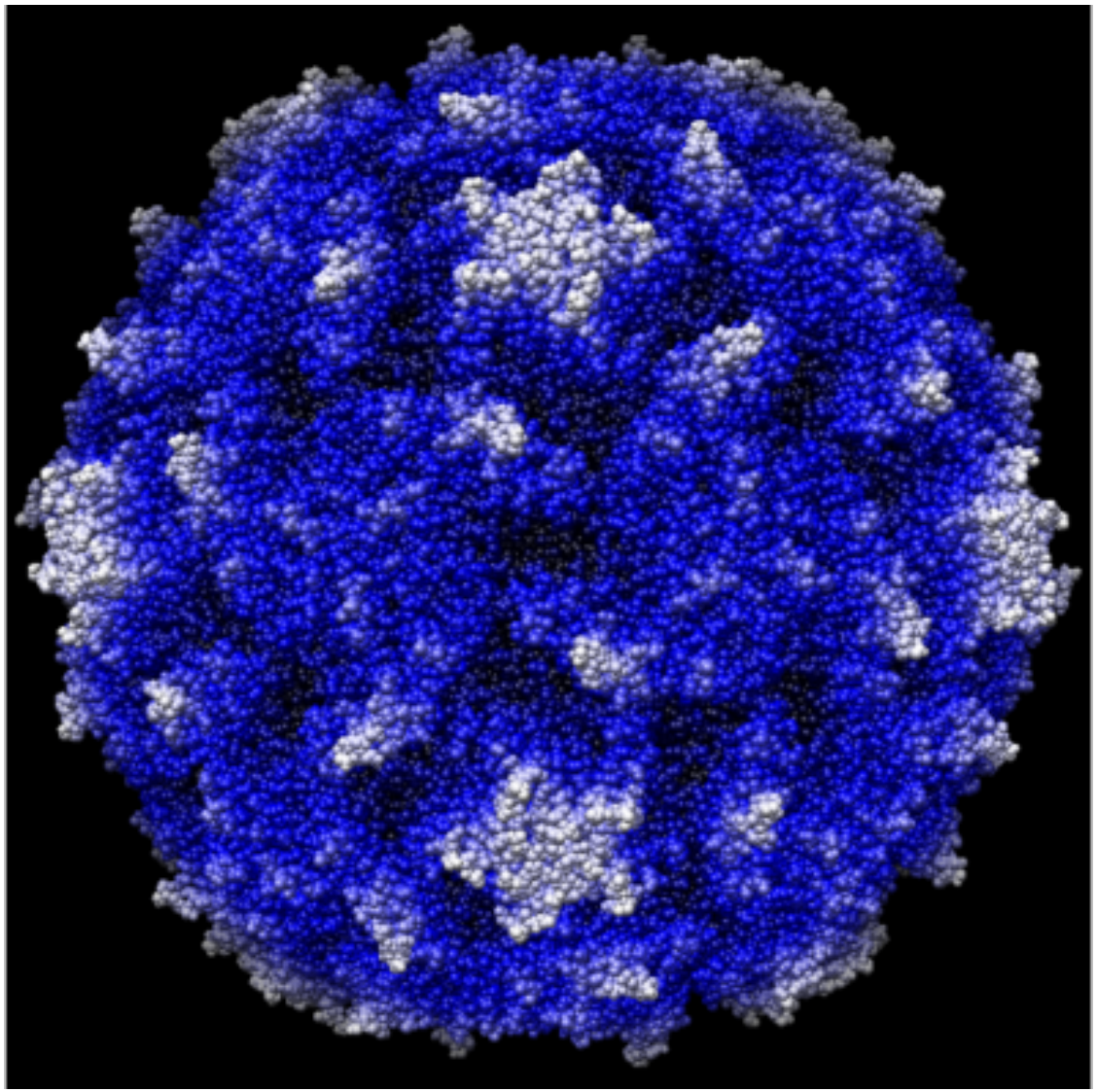
Poliovirus Structure and Function



How does poliovirus introduce its genome into a cell?

- Why this is important
  - We still do not understand how nonenveloped viruses facilitate crossing membranes
- Why this is hard
  - Infection is inefficient (high particle:pfu) -difficult to tell whether any particle is on productive pathway
  - Cell entry pathways far more complex than we knew

Visualizing poliovirus entry in HeLa cells

- **The idea:**

  Because of high particle to pfu ratio any visualization assay must incorporate an assay to identify productive pathways.

- **The precedent:**

  Zhuang et al. with influenza - assay for membrane fusion

- **The implementation with polio:** (Brandenburg et al. 2007)

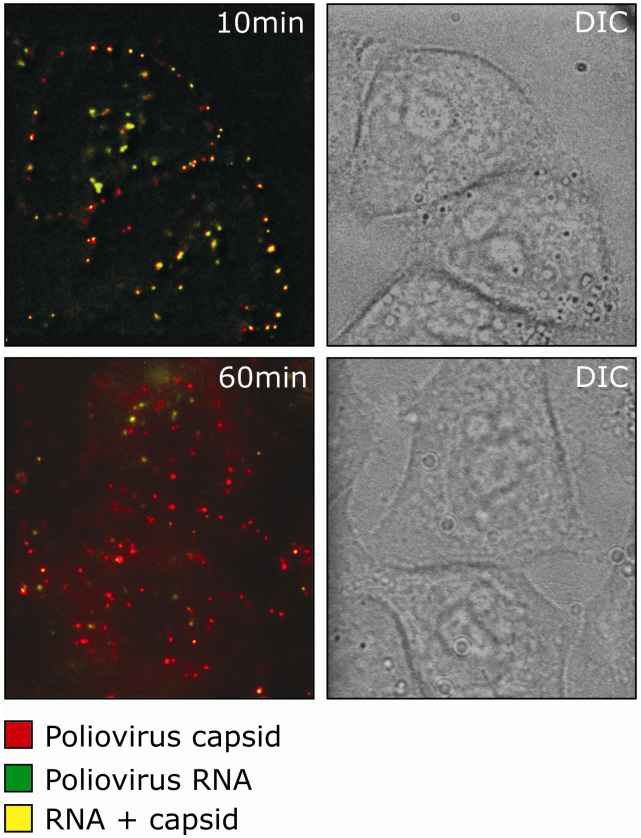
  Separately label virus capsid and RNA - allows to identify trajectories leading to RNA release.

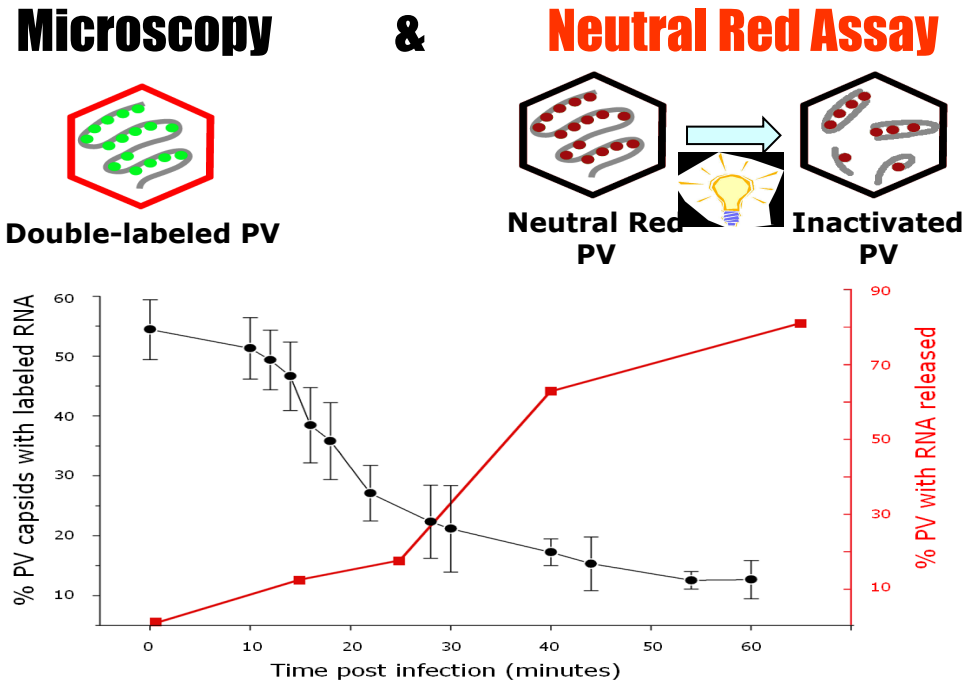
  - At single particle level
  - In live cells in real time
  - In low MOI infections (~1 pfu/cell)

  Develop parallel infectivity-based assay for RNA release

  Use both assays to measure kinetics of RNA release and effects of inhibitors of trafficking pathways

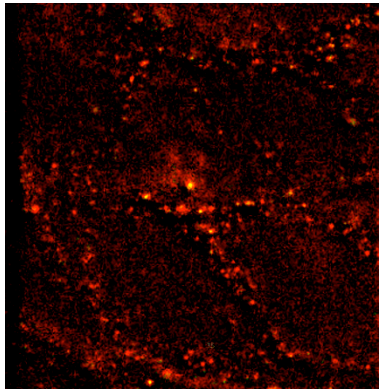
Agreement between visualization and biological assay



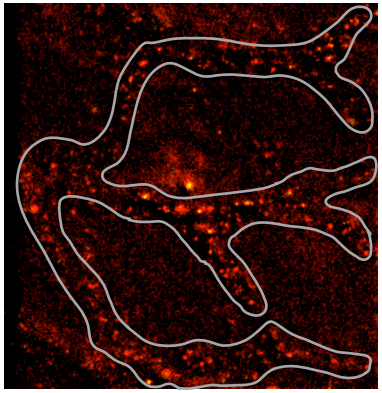


- Dual label detects RNA release and release is efficient.
- Kinetics parallel kinetics of biological assay for RNA release.
- In all cases tested effects of drugs on RNA release parallel their effect on infection.

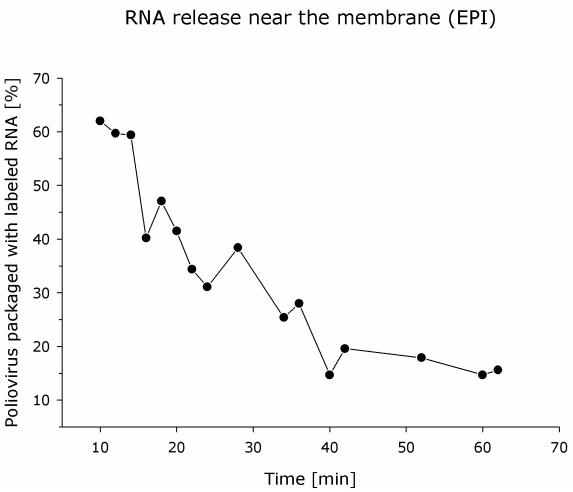
Poliovirus RNA Release Near the Cellular Membrane

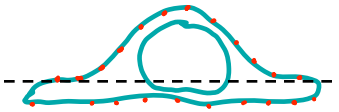


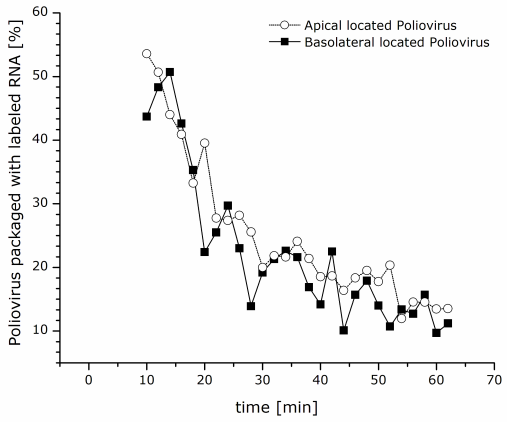
e.g. frame 35 of a z-stack



indication of membrane bound polioviruses

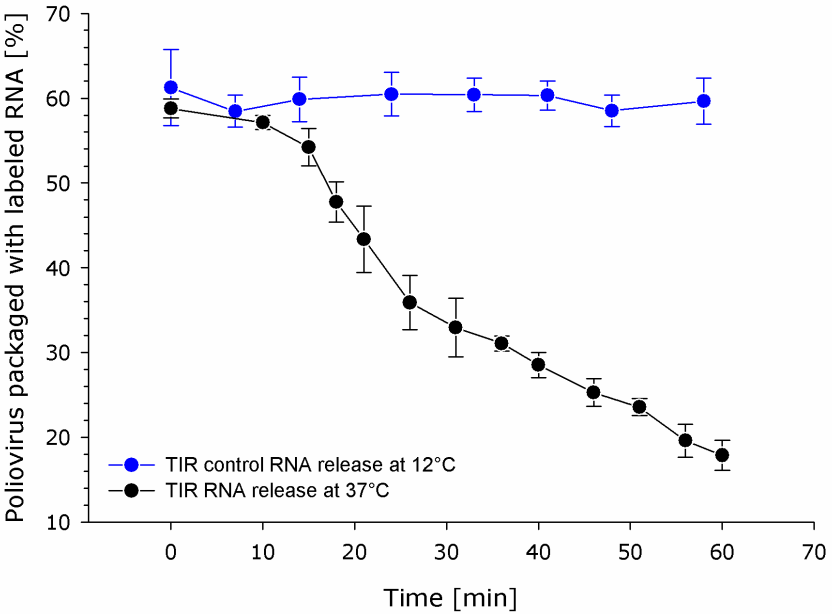


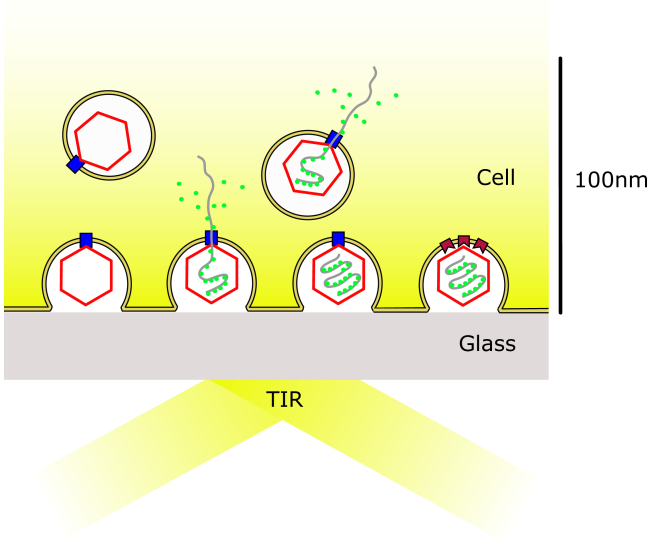




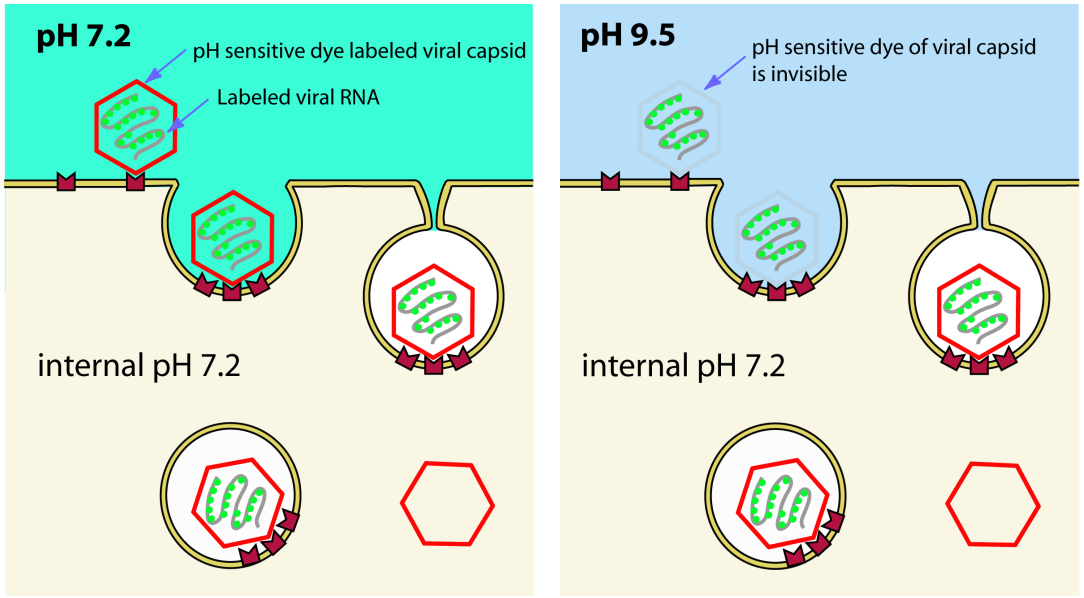
Counting the "membrane-bound" polioviruses and determine the RNA : capsid ratio

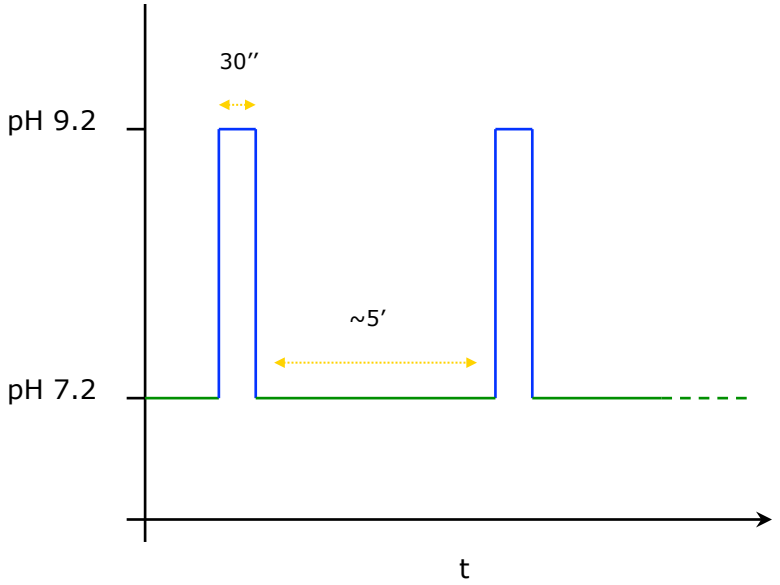
Total Internal Reflection - Fluorescence Microscopy of Poliovirus RNA release

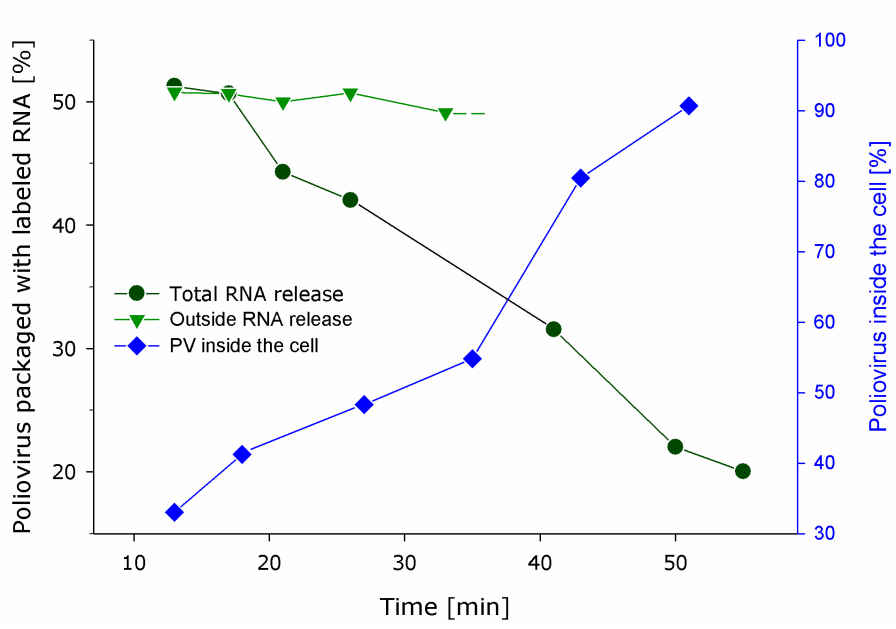




Live Epifluorescence Microscopy of pH-sensitive Poliovirus







- RNA release occurs in a site not accessible to changes in extracellular pH.
- Capsid binding drugs prevent both RNA release and movement to pH insensitive compartment (data not shown).

Conclusions to date - polio in Hela cell

Polio entry uses noncanonical pathway or multiple pathways that is (are):

- •Independent of clathrin, dynamin, caveoli, and microtubules
- •Dependent on energy, actin, intermediate filaments and an unknown tyrosine kinase(s)

After conversion to 135S particle is internalized into a compartment that is inaccessible to changes in extracellular pH.

After internalization, virus in vesicles undergoes very unusual rapid actin-dependent movement (Vaughan et al., 2009).

Soon after internalization, RNA is released.

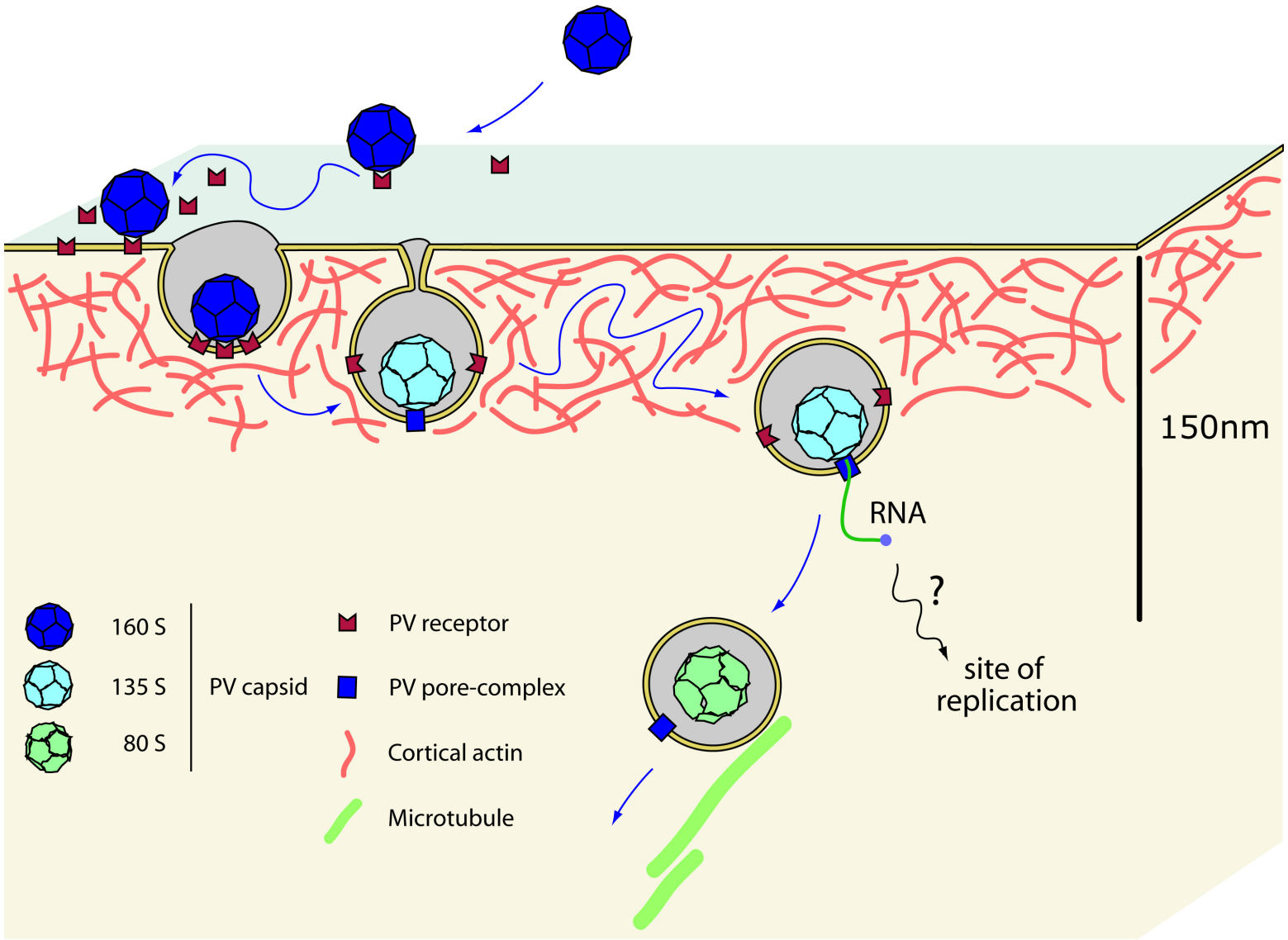
After RNA release vesicle-associated empty capsid moves rapidly to cell interior in microtubule dependent pathways.

Productive RNA release is efficient !!!!!!!!!

Productive RNA release takes place with 200nm of surface

(Note that details of entry pathway vary in different cells Coyne&Bergelson, EMBO J. 2007)

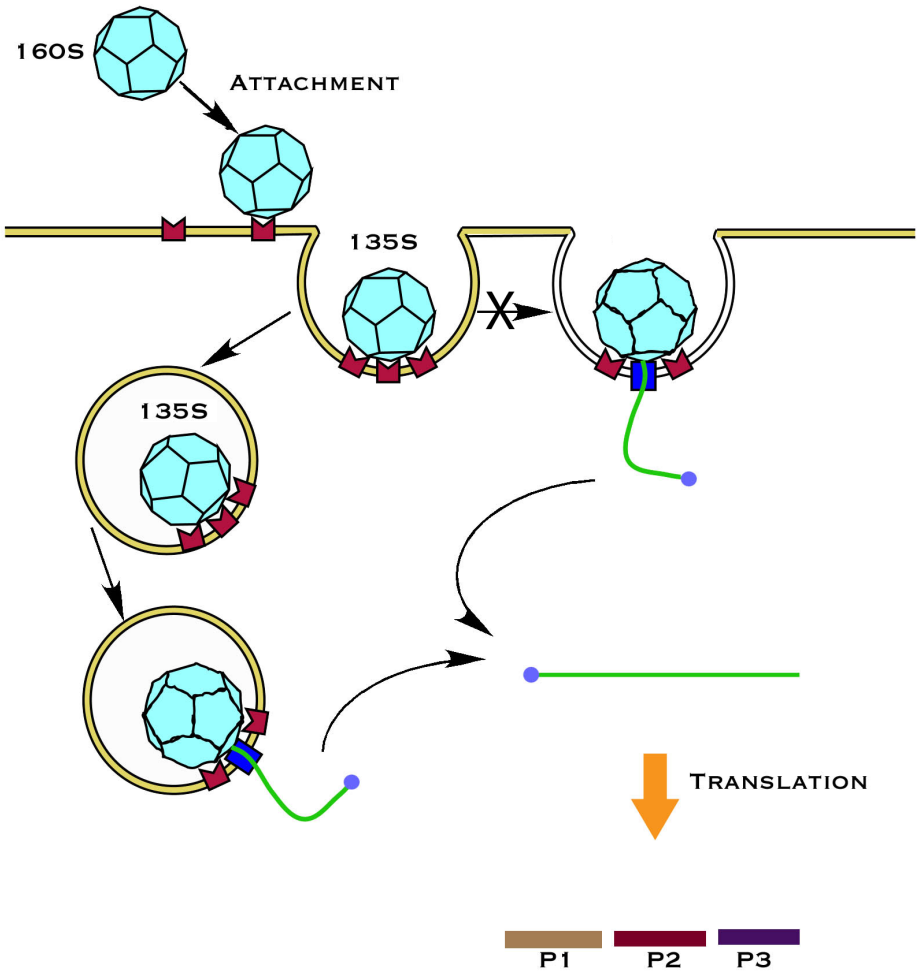
Poliovirus entry: cartoon summary



Is entry machinery more conserved than entry pathway?

- Picornaviruses utilize a number of endocytic mechanisms: dependent on cell type, passage history, receptor utilization etc.
- Promiscuity of entry pathways used by picornaviruses raises questions about what generalities can be learned
- Hypothesis: limited conformational repertoire of picornaviruses will lead to commonality in mechanisms used to translocate RNA once inside the cell within each picornavirus genus and perhaps family-wide.
- Is this true? Ask me at end!!!!

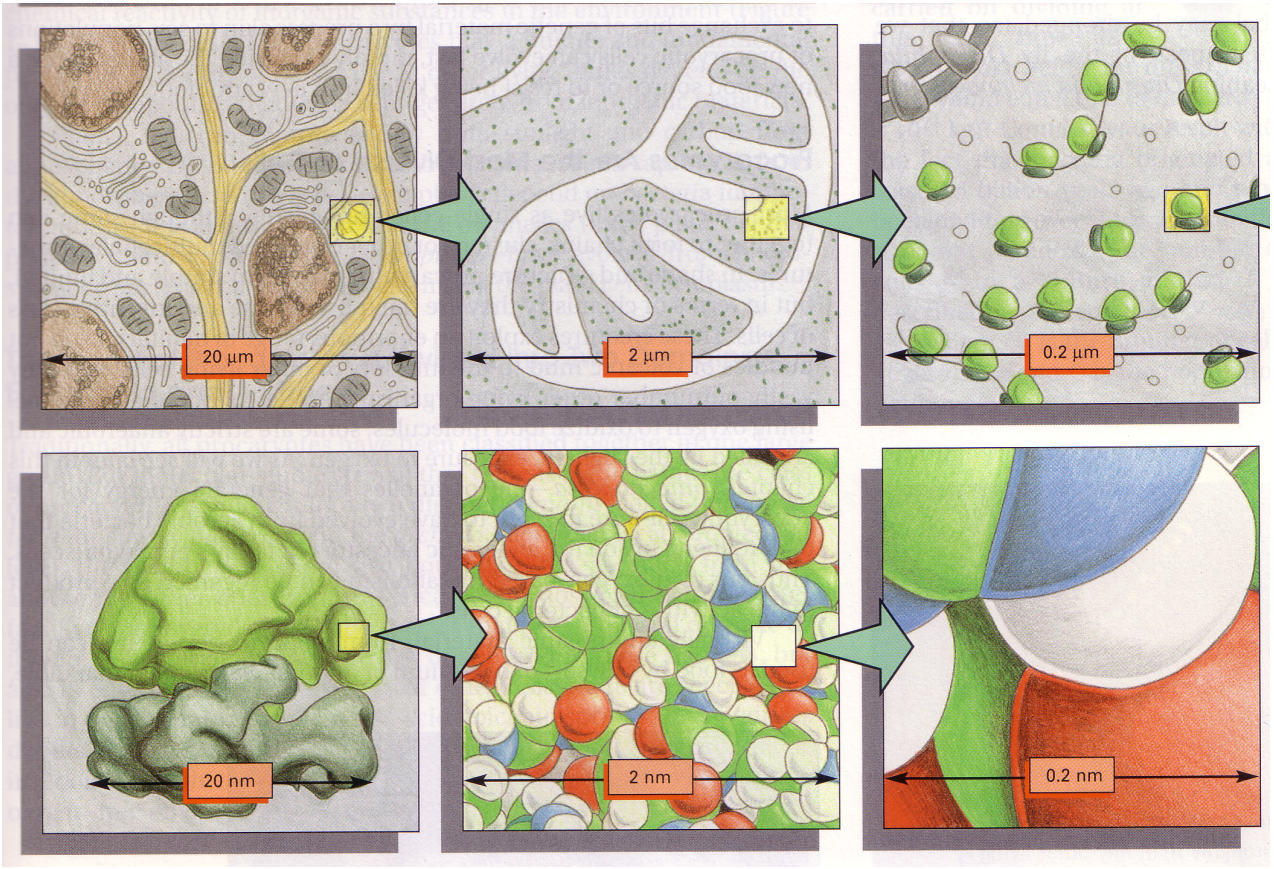
Early events in poliovirus entry



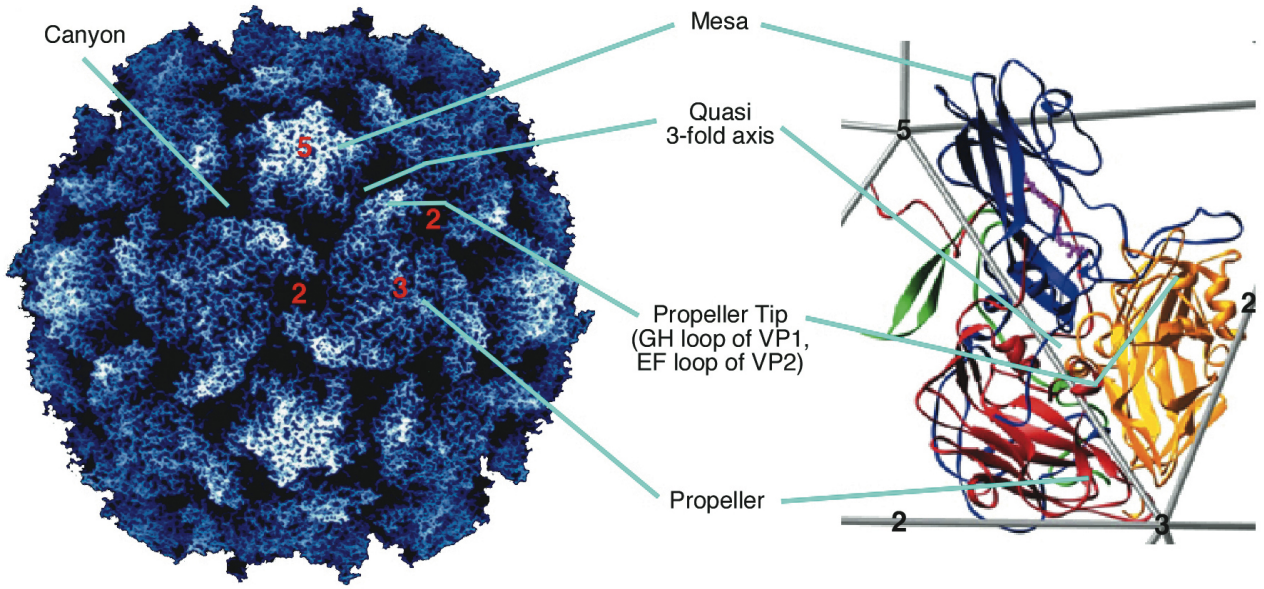
Adapted from Flint et al.

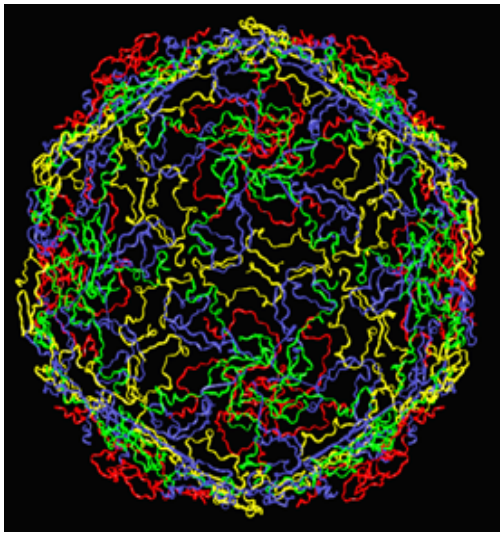
- Virus binds to specific receptors (CD155 aka Pvr)
- At physiological temperature receptors induce conformational changes to 135S particle, resulting in externalization of VP4 (which is myristoylated) and N-term of VP1, and the insertion of these peptides into the cell membrane.
- Electrophysiology experiments demonstrate that peptide insertion results in formation of pores and channels (Tosteson and Chow)
- Genetic experiments indicate that ability to form channels correlates with ability to release RNA into cytoplasm to initiate infection (Danthi and Chow)
- Working model: interaction of peptides with membrane form pores that facilitate translocation of RNA across endosomal membrane into cytoplasm.

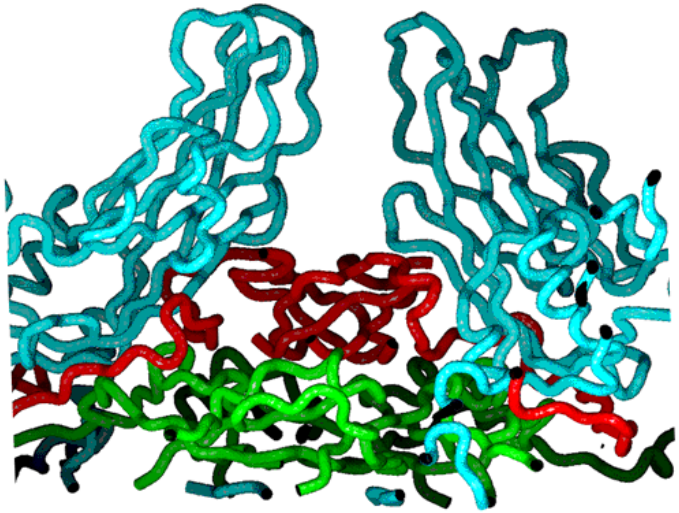
Structural understanding the machinery used in cell-entry is a problem that spans 6 orders of magnitude in scale



This range is beyond the scope of any one structural method and requires the development of hybrid approaches combining multiple methods (figure from MBOC).

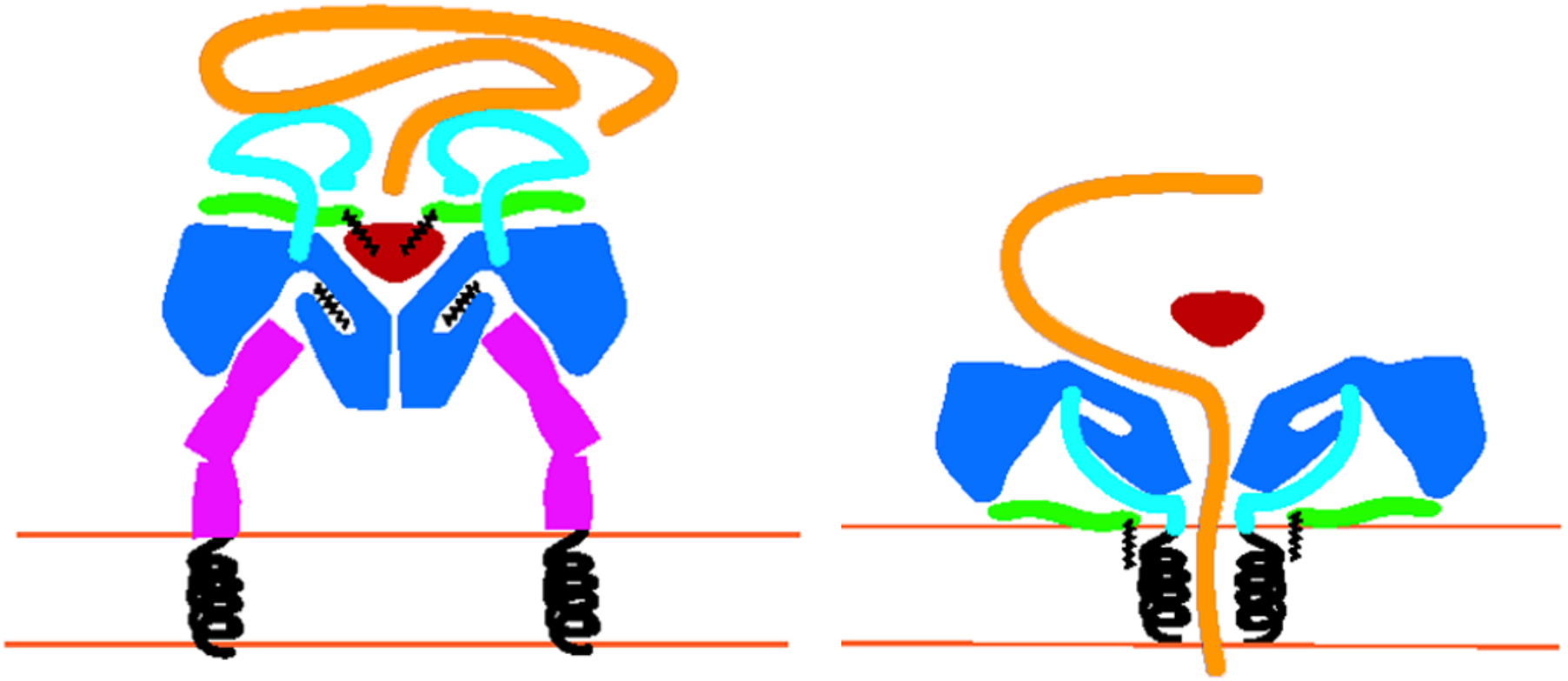




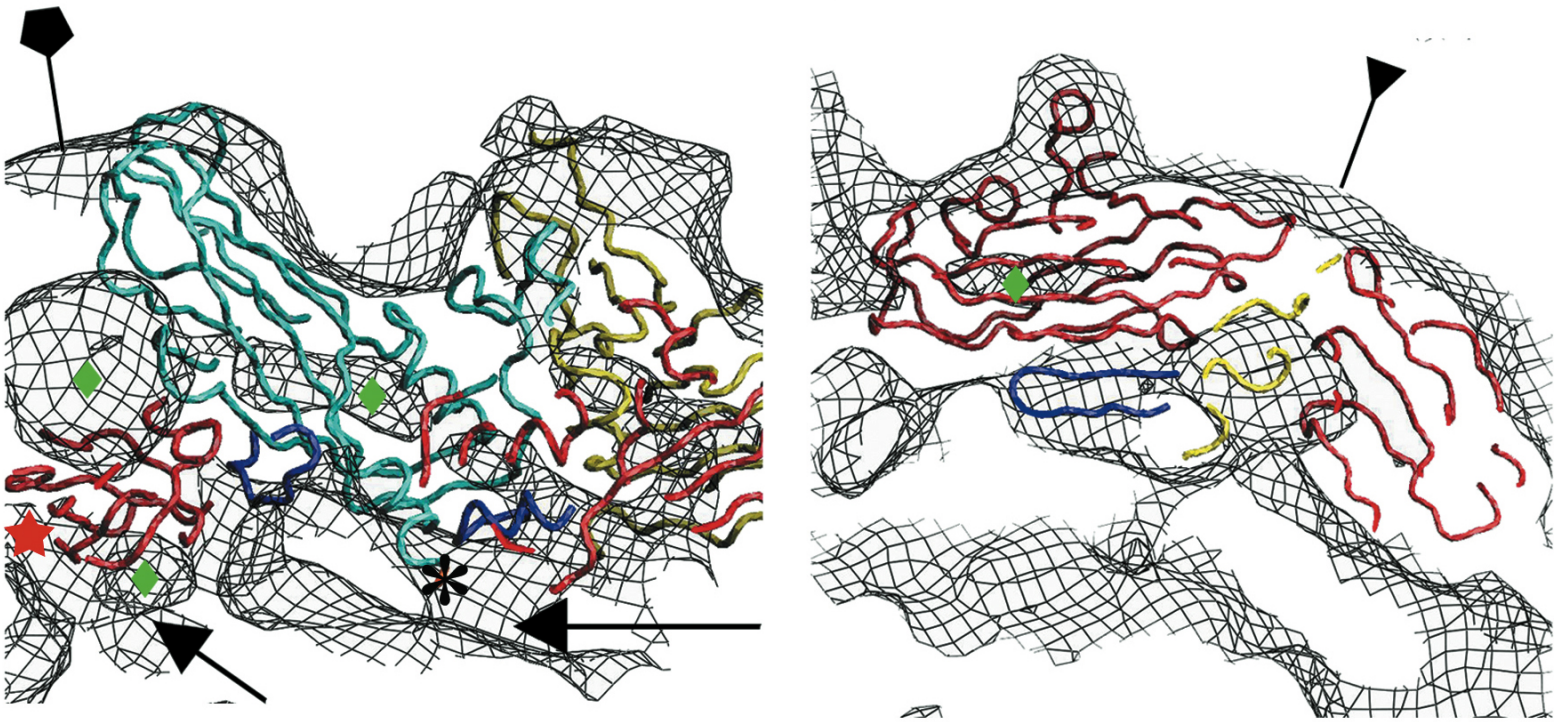


Virus structure currently at 2.2Å resolution

Cartoon model for RNA release



Cryo-EM structures



Current state-of--art 3.5Å, sufficient to build independent models.
At lower resolution: Build pseudoatomic models based on high-resolution structure (not quite a free lunch).

Virus-receptor complex

First structures (type 1) reported at ~23Å

Belnap et al. 2000, He et al. 2000, Casasnovas et al. 2000

Fit homology models (but three models differed)

Structures for all three serotypes at 16-20Å

He et al. 2003

homology model for receptor

Structure for all three serotypes at 8.5Å

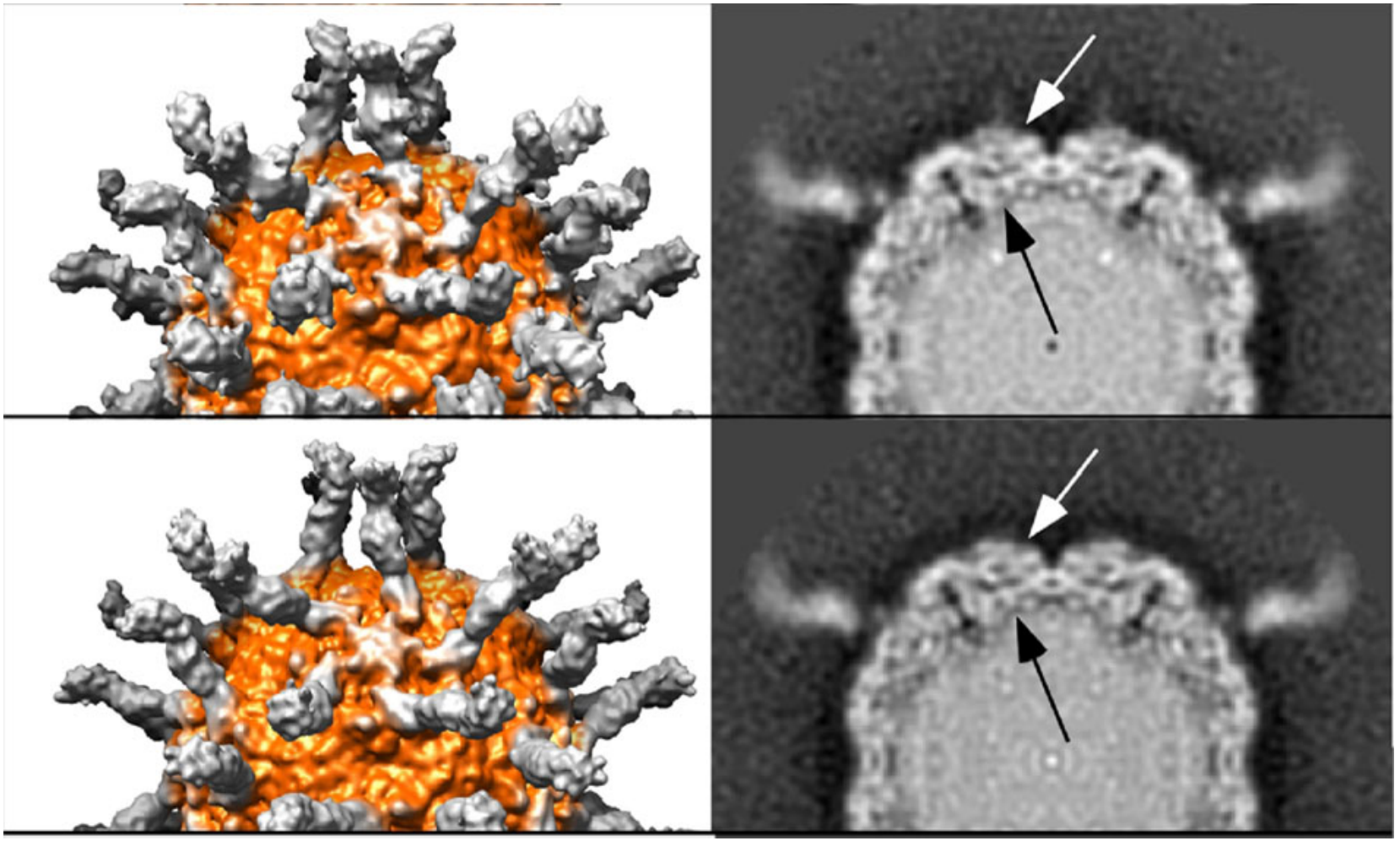
Zhang et al. 2008

crystal structure of receptor

Structure for type 1 at ~7Å resolution

Belnap et al. in prep

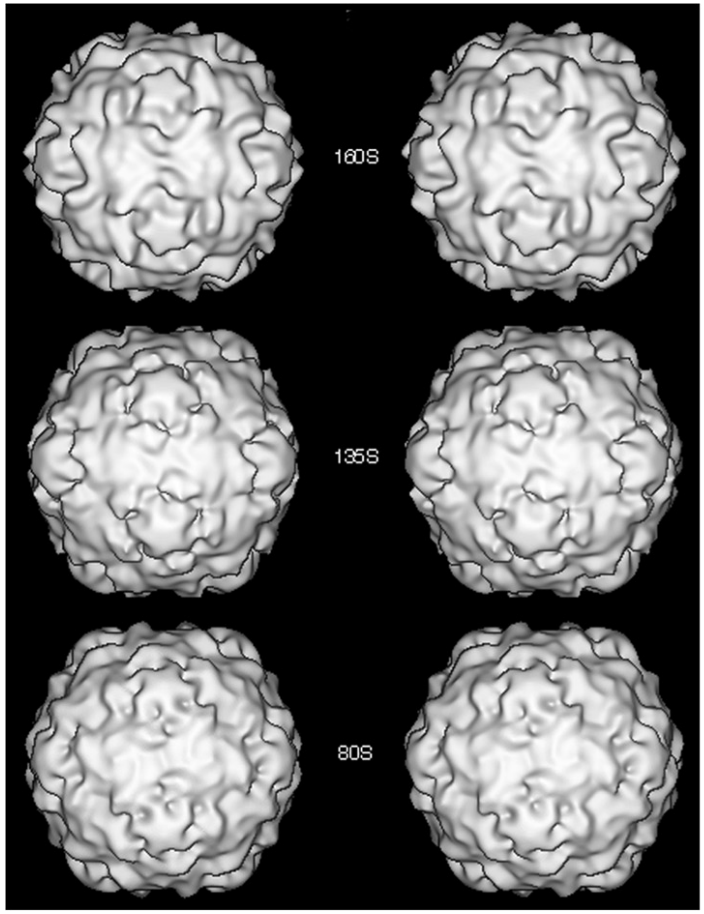
crystal structure for receptor

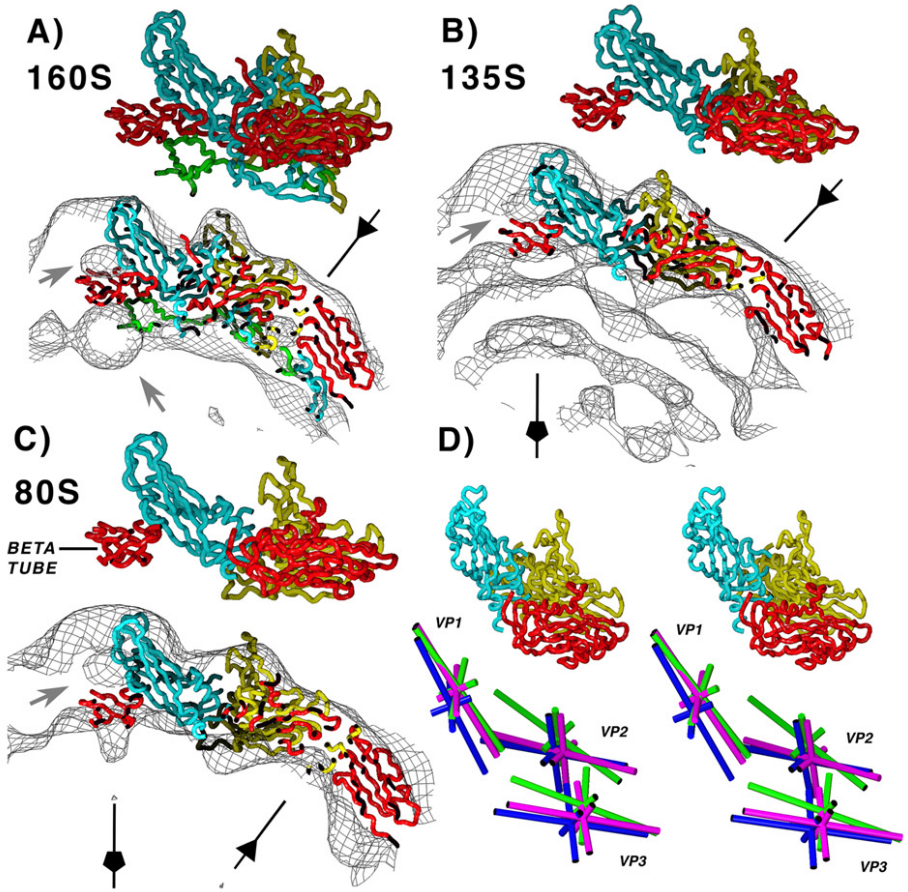


Fitting crystal structures of receptor and virus produces model with excellent shape and chemical complimentarity

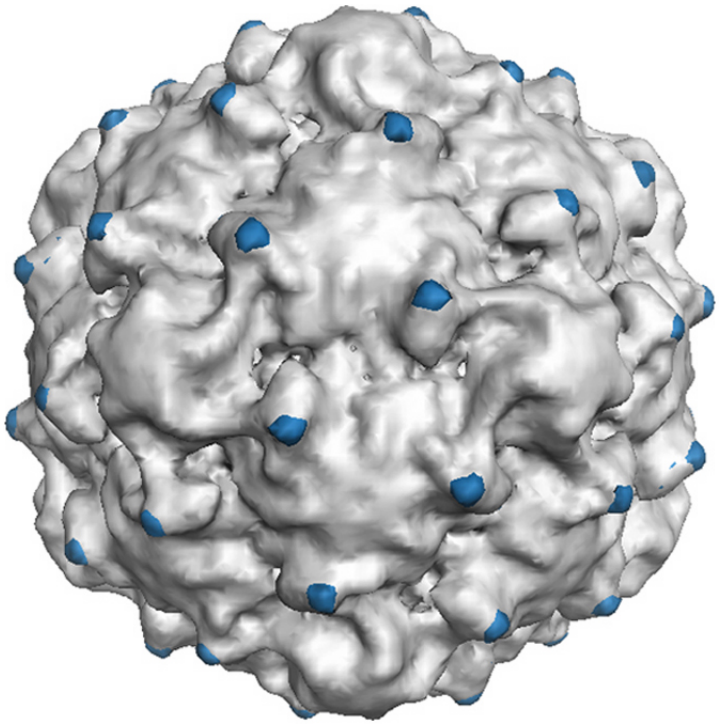
Current resolution 6-7 Å (Belnap, et al. in prep.)

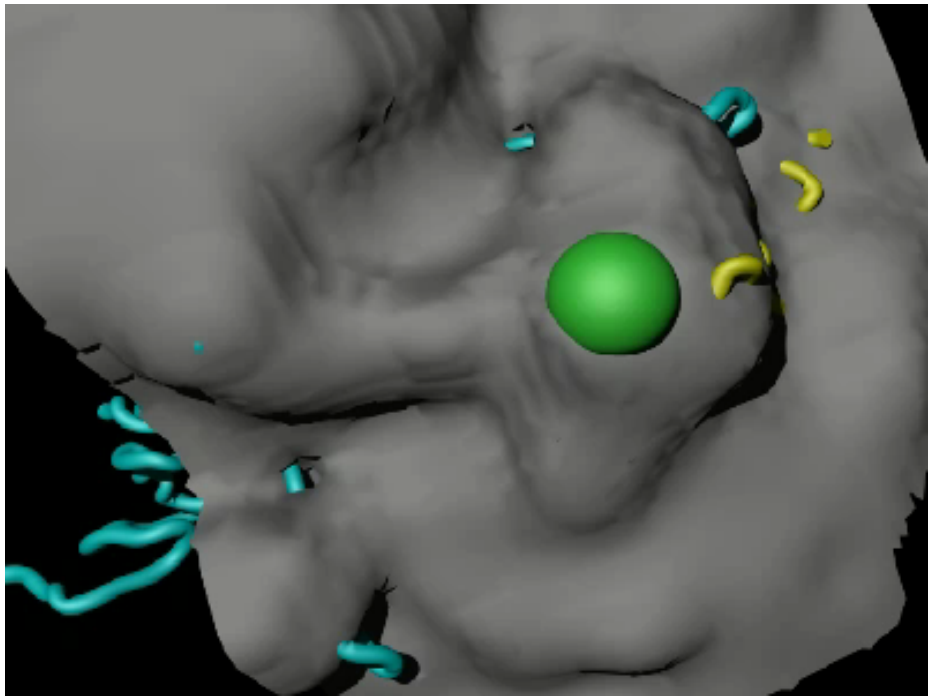
135S particle and 80S particle at 23Å resolution

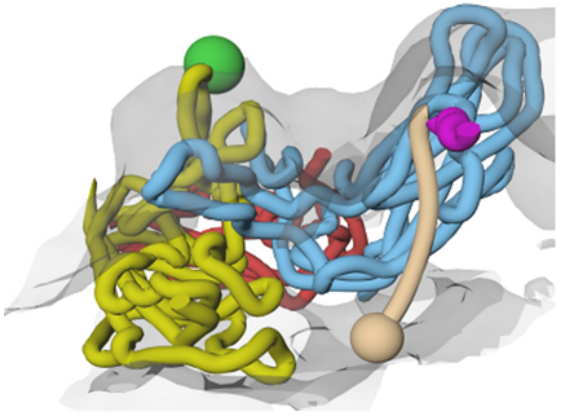


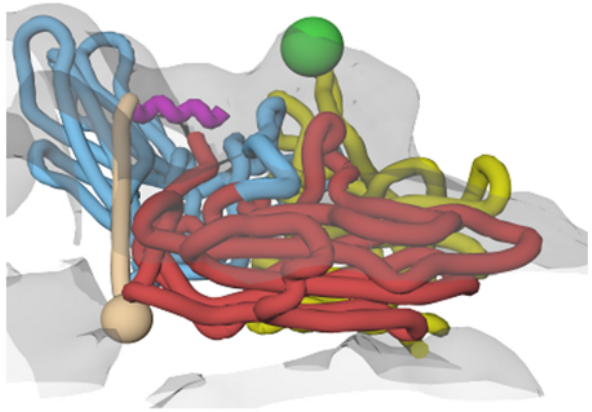


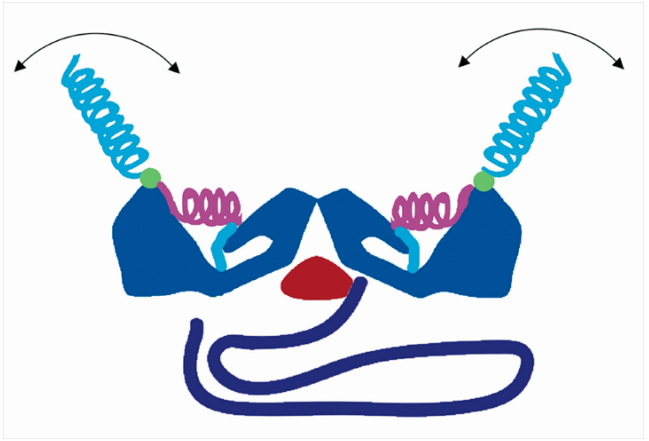
- Plug in place - no room for N-term of VP1 to exit at fivefold
- No definitive answer where VP4 and N-term VP1 come out
- No holes in either structure - must be additional intermediates





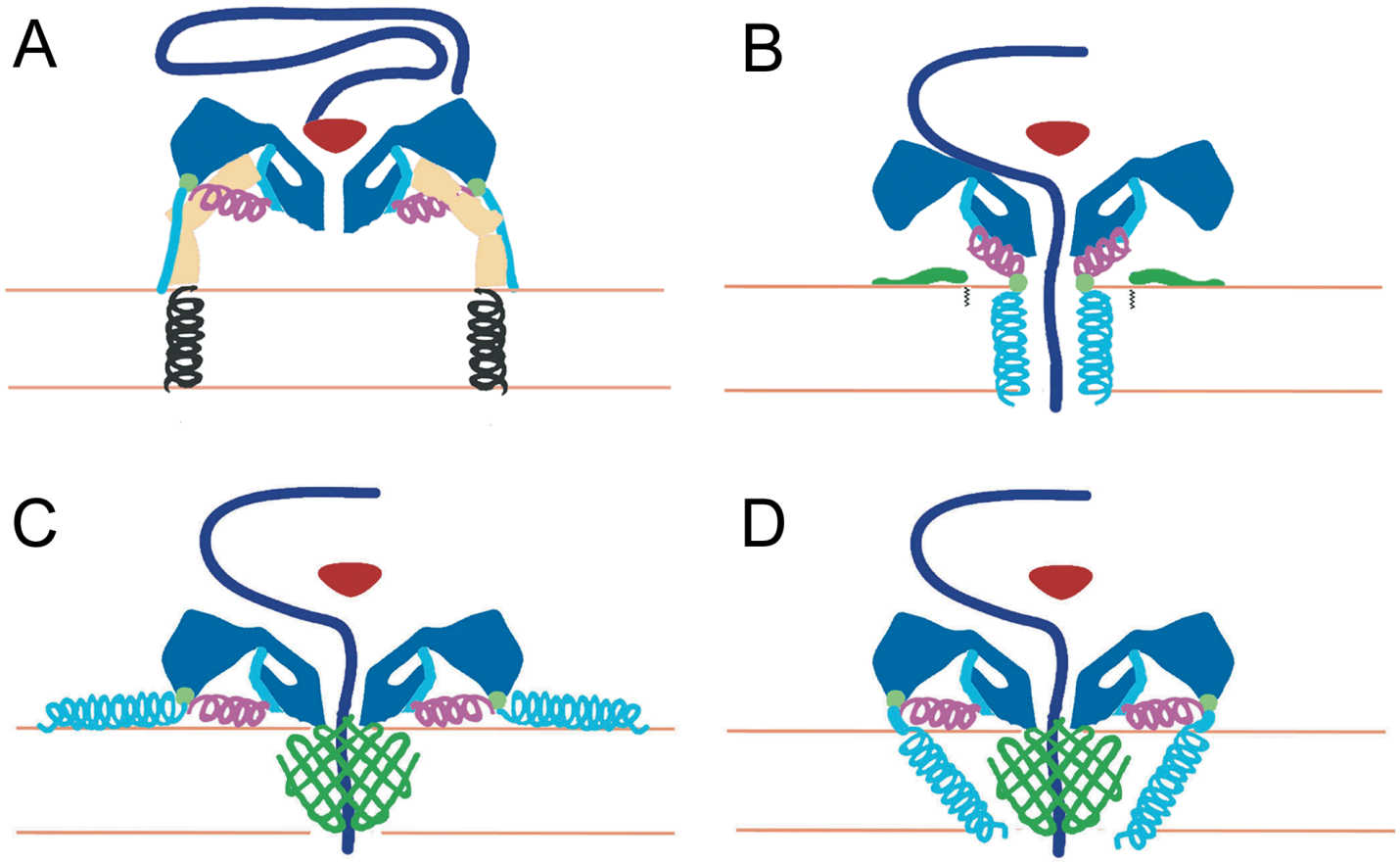




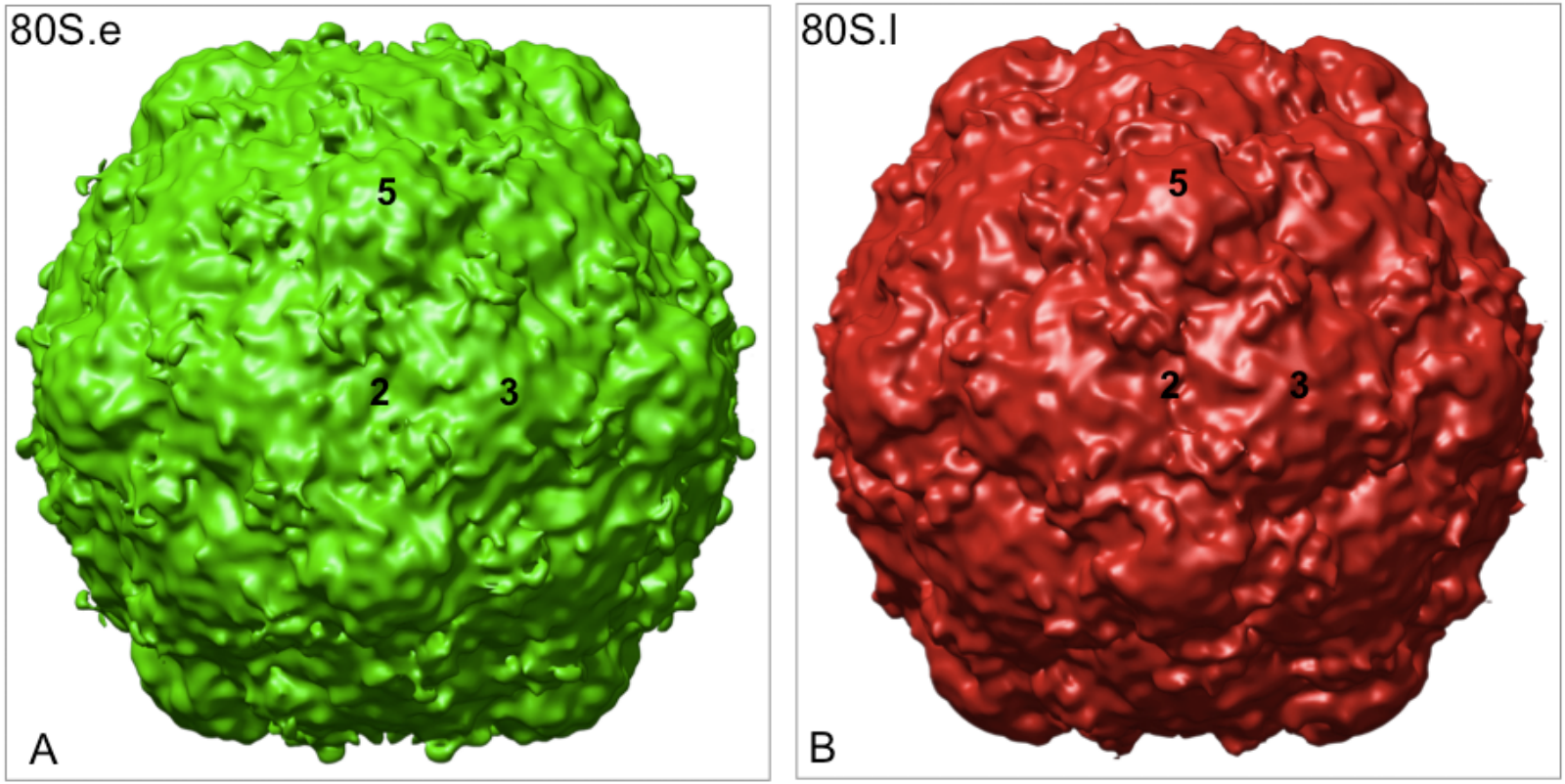


135S structure at 10Å (Bubeck, Filman, Hogle, and Belnap (2005)

Revised models for RNA release

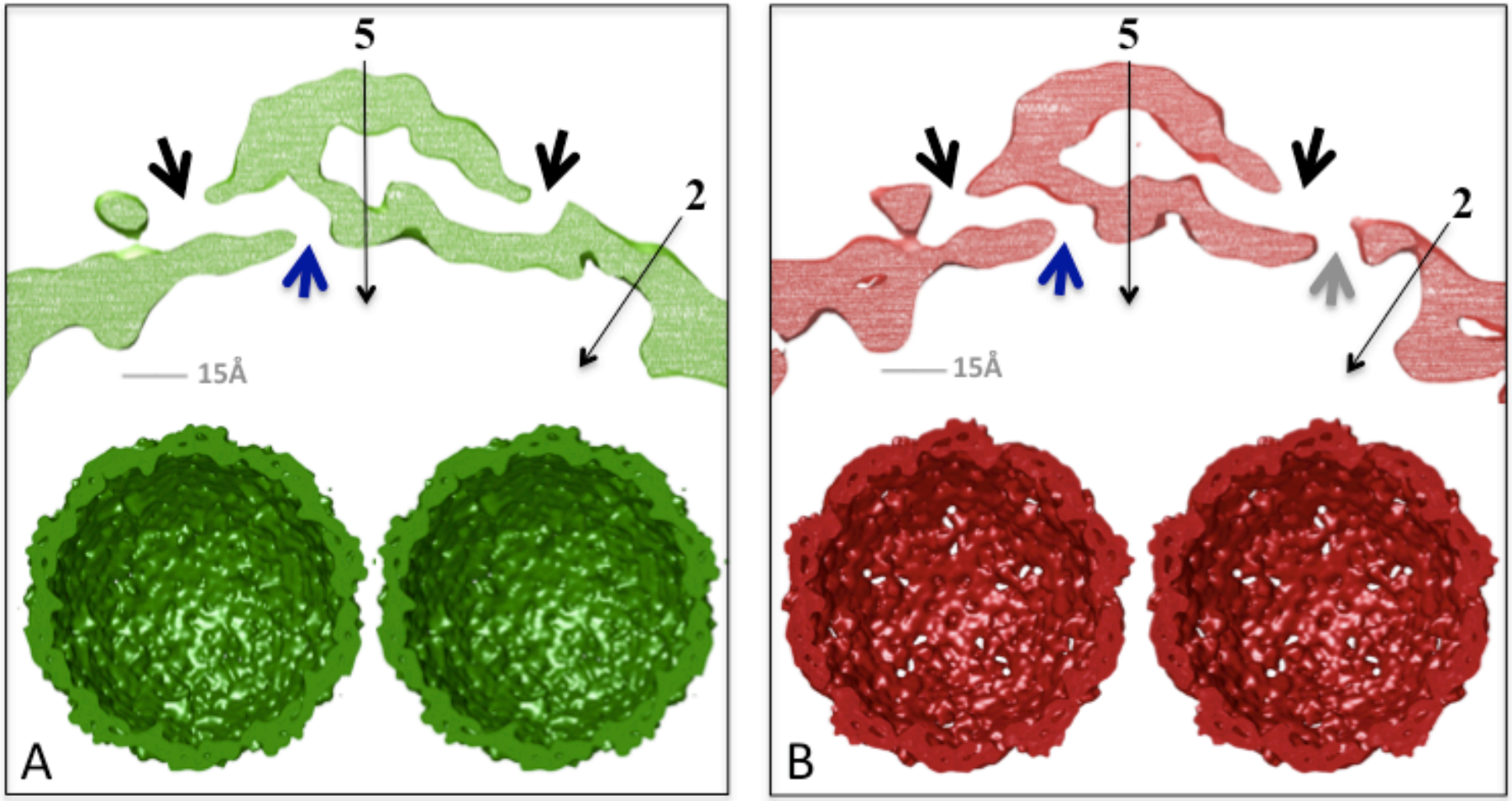


Empty capsids at 9.5Å

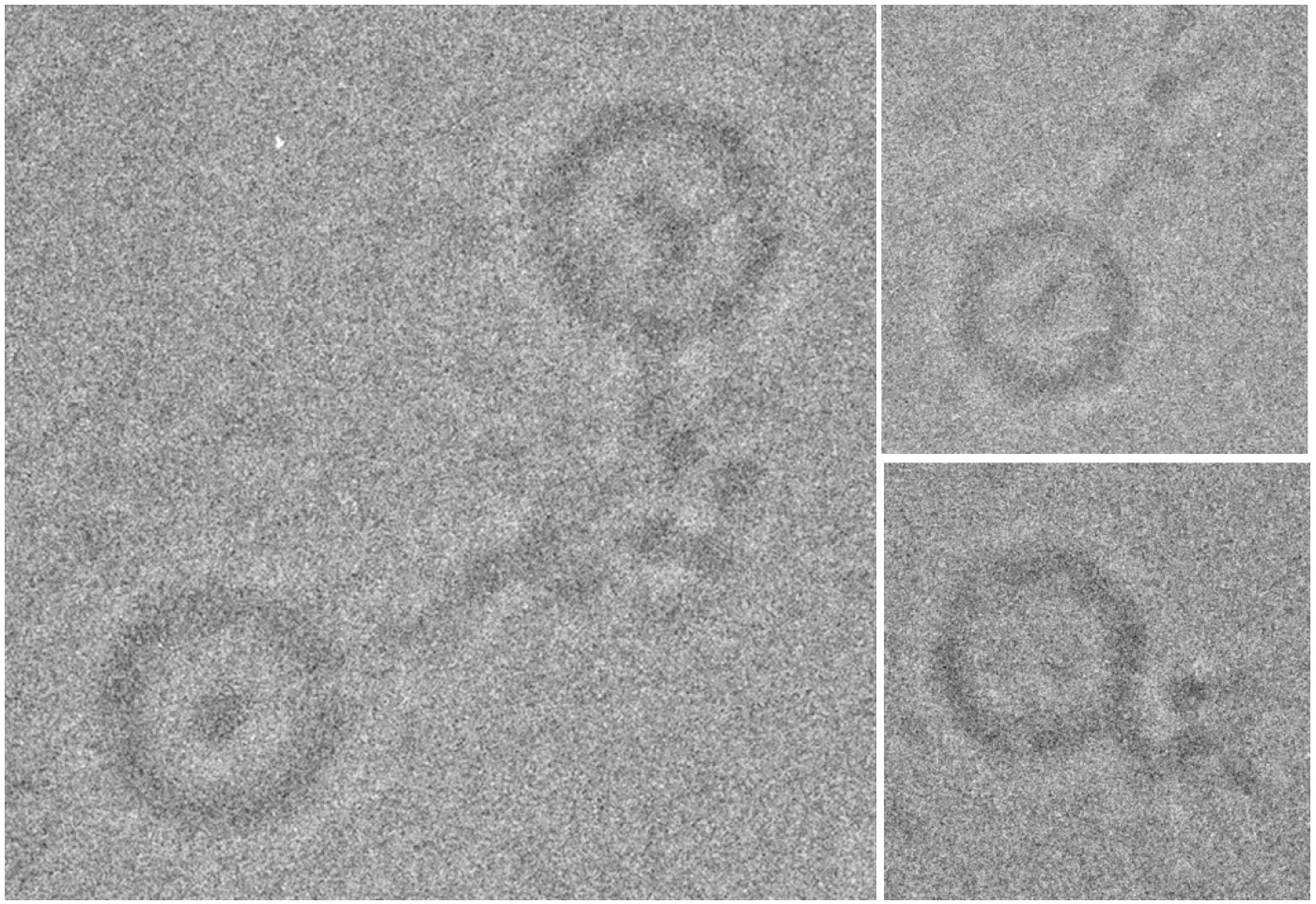


- Attempts to extend resolution converged at ~17Å
- Visual inspection showed variable RNA content
- Classification analysis sorted particles into two distinct classes (previously observed by Hewat et al. with rhinoviruses)

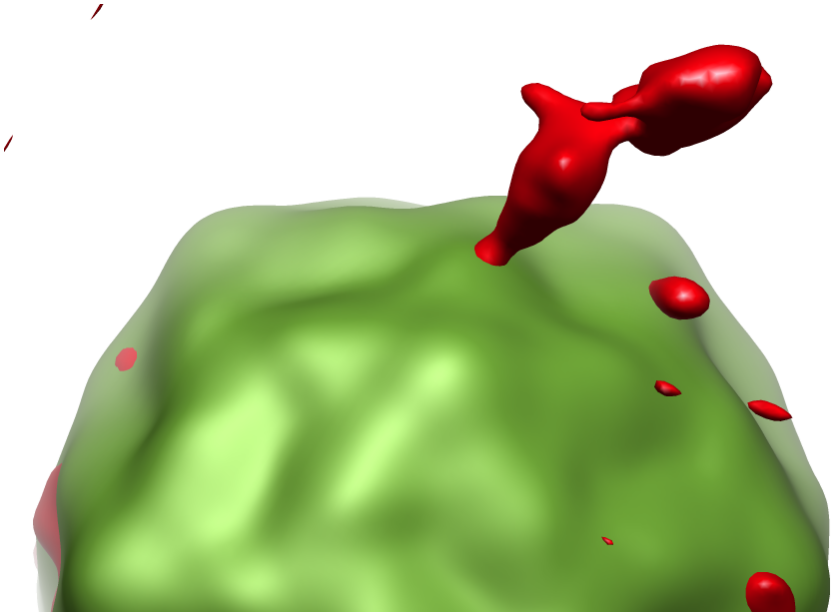
Holes in the empty capsid shells

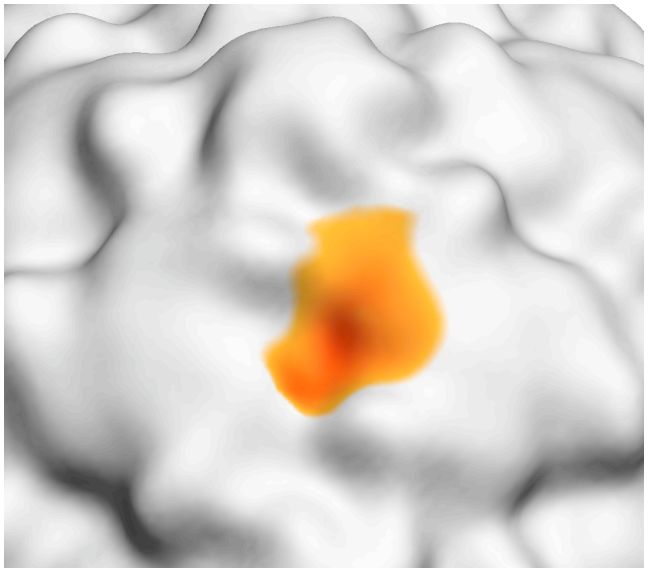


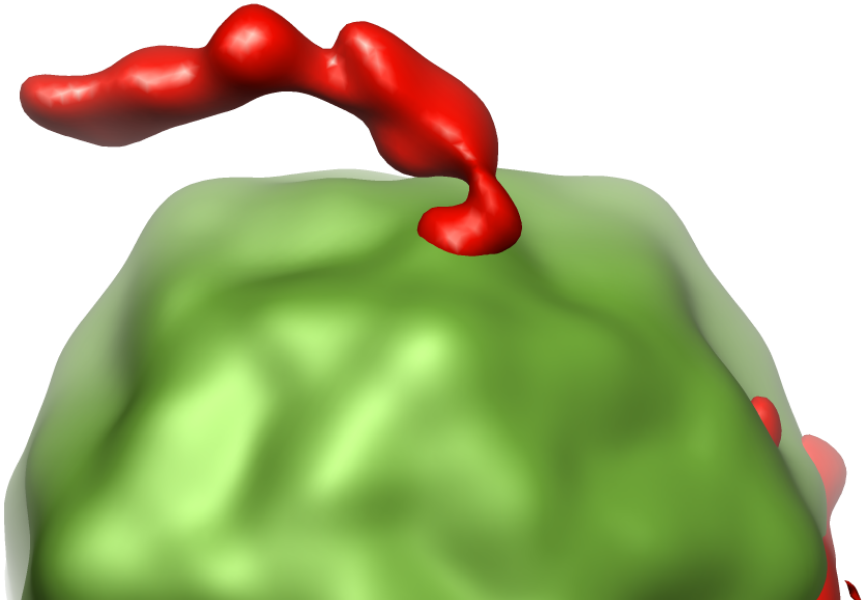
Big surprise: some particles have been caught in the act of releasing RNA

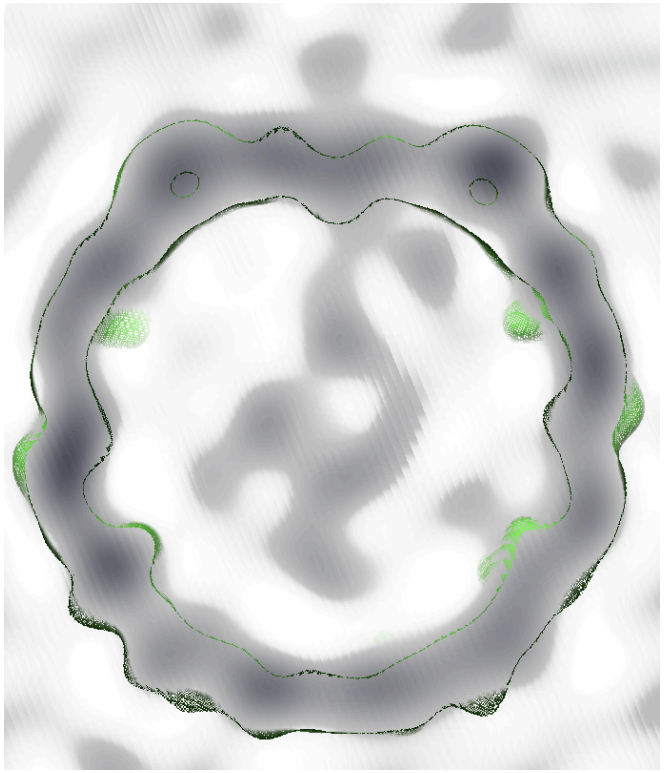


The particles are all classified with the 80Se particles





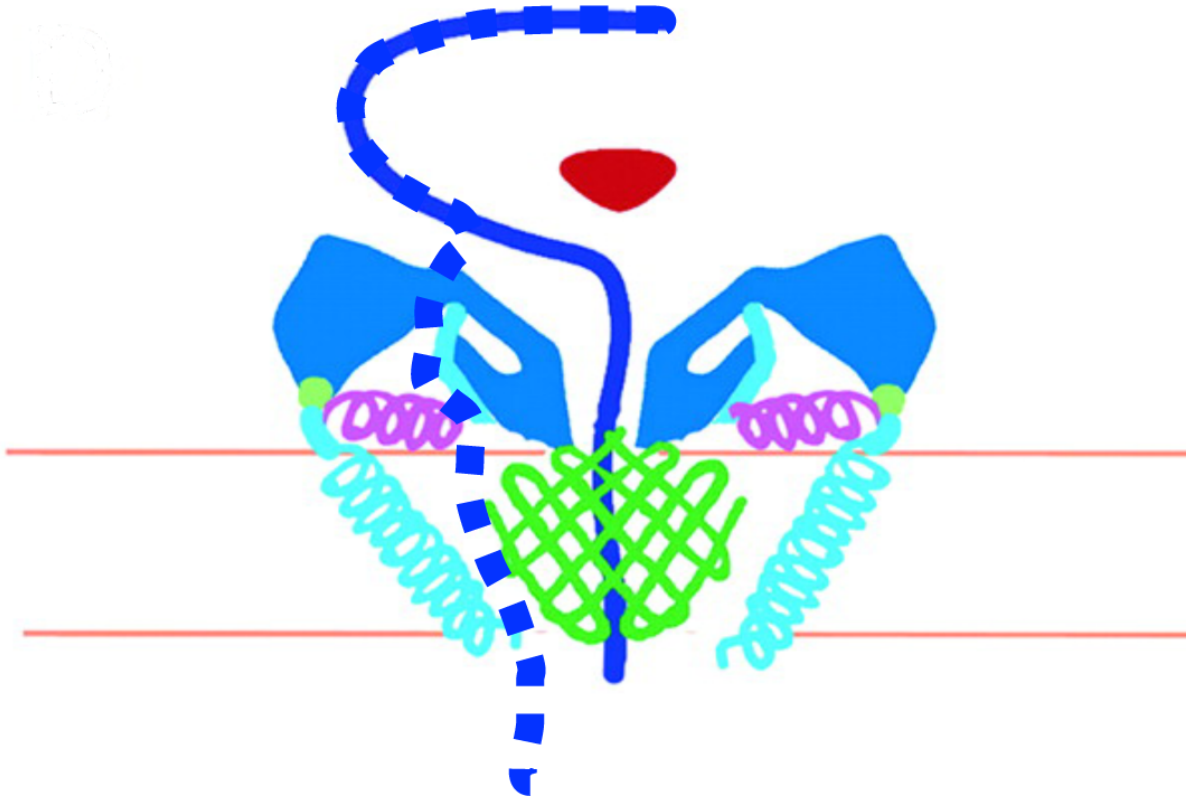




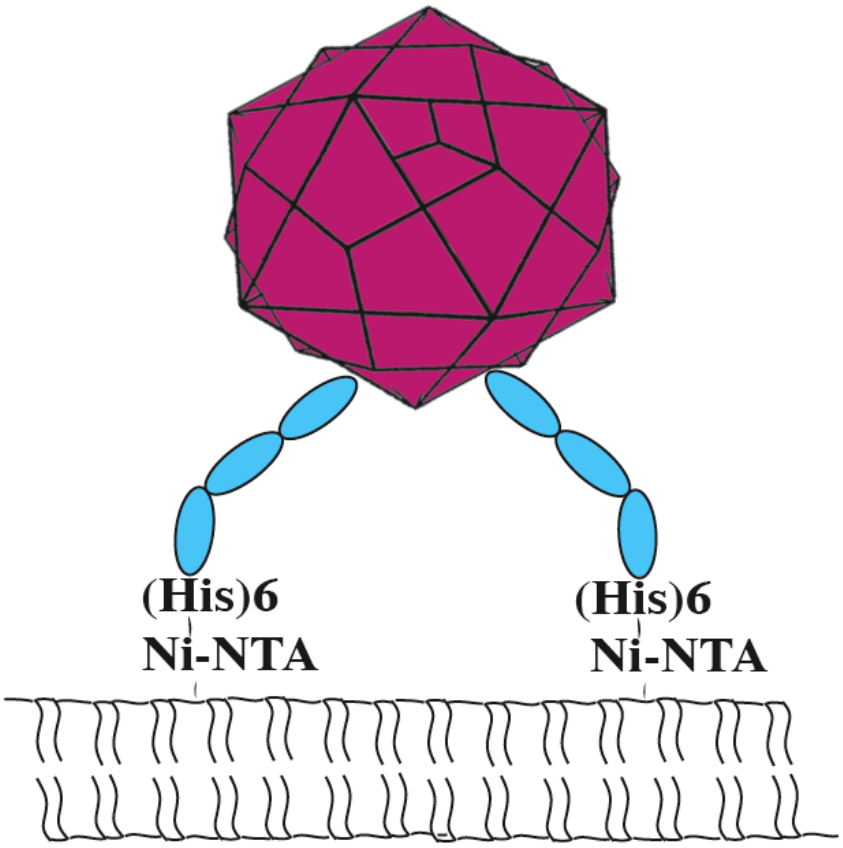
Current thinking about 80S

- Variable RNA content inside with no visible RNA outside in most particles may indicate hydrolysis of externalized RNA.
- 80Se particles represent particles that are currently releasing their RNA and RNA remains engaged with externalization machinery even after hydrolysis
- 80Sl particles represent particles that have completed RNA release or particles where the RNA has disengaged from externalization machinery after hydrolysis
- Efforts to extend resolution of all structures including those with RNA inside and outside underway with a very large data set (500,000 particles)

Re-revised model for RNA release

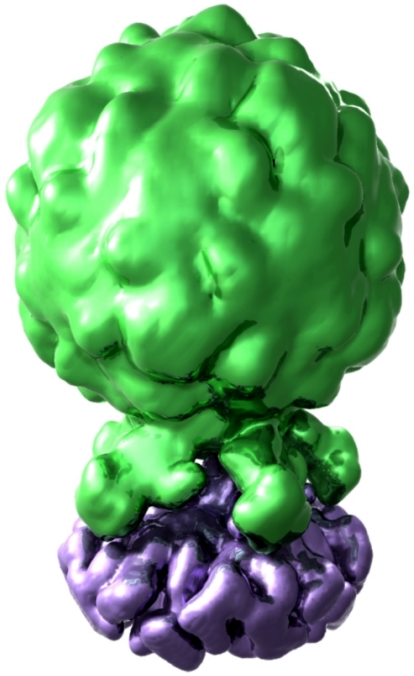


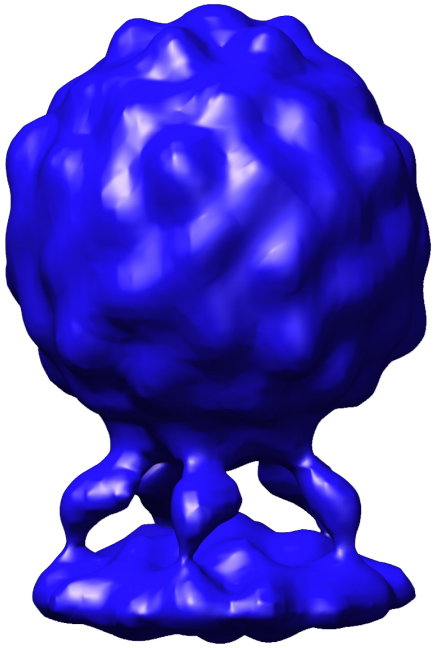
A simple liposome-based model for structural and biochemical studies.



- GPI anchored ectodomain is functional receptor (Tosteson et al., 2004).
- Pvr from Racaniello has C-terminal (membrane proximal) His-tag.
- Avanti sells NTA lipids.
- Biochemical studies show receptor decorated liposomes bind virus and induce transition from virion to liposome bound 135S (Tuthill et al. J.Viol. 2006).

Poliovirus-receptor-liposome complex



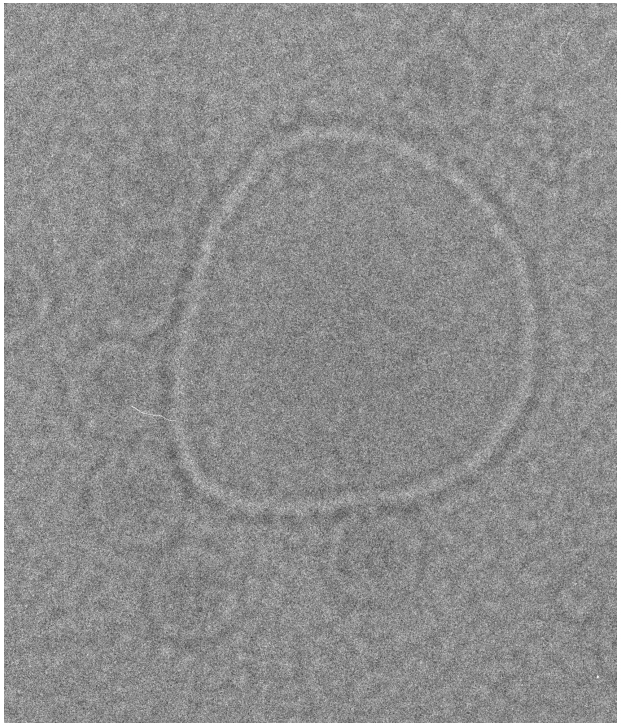


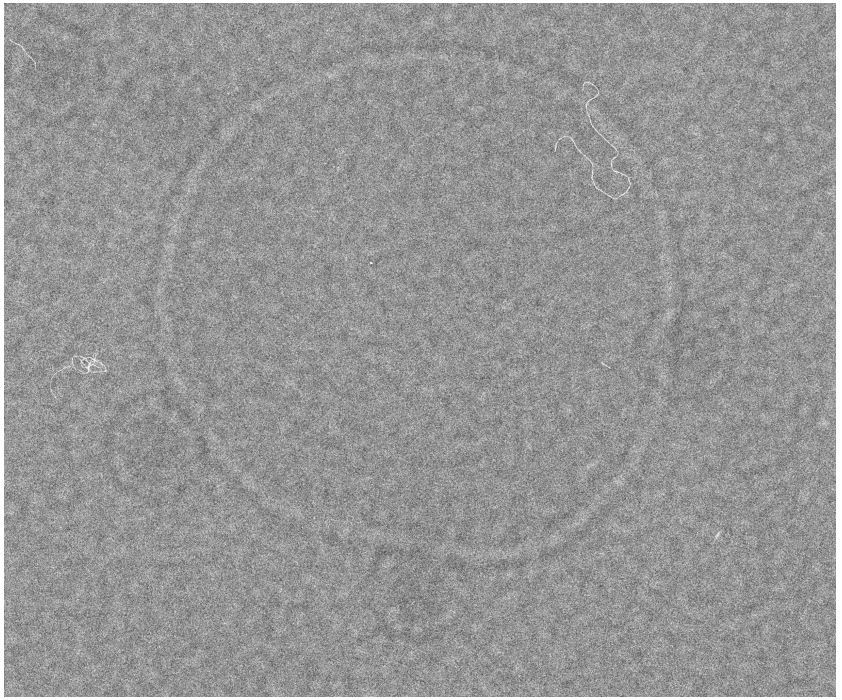
Single Particle:
Bubeck, et al. (2005)

Tomography:
Bostina et al. (2007)

Both at 30Å resolution

Images of membrane-associated 135S and 80S

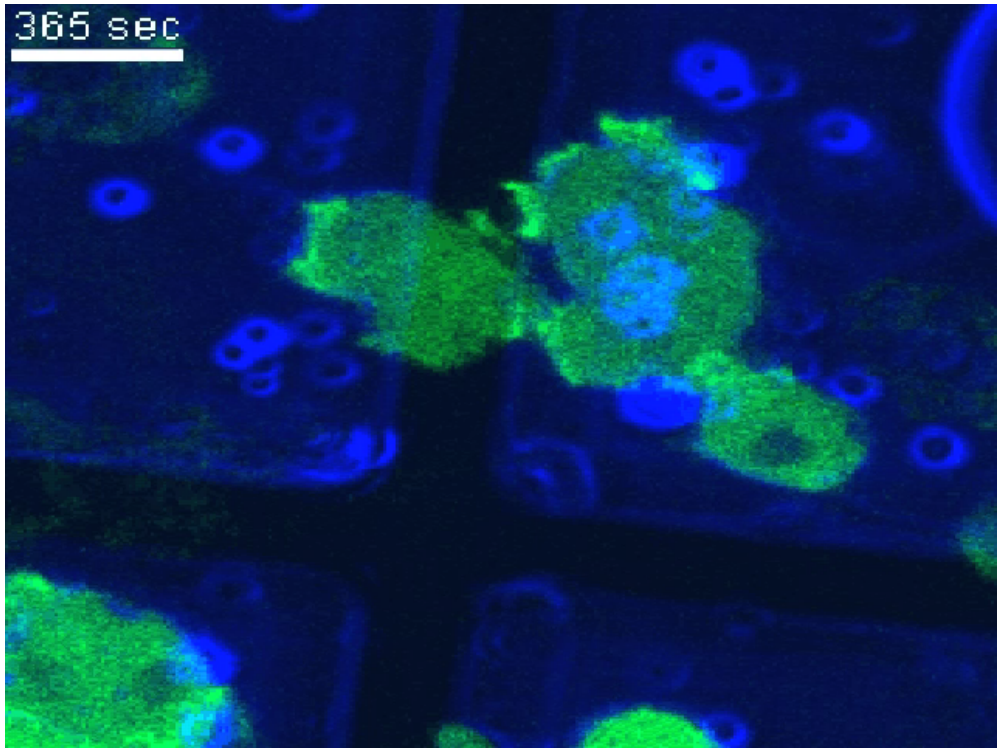


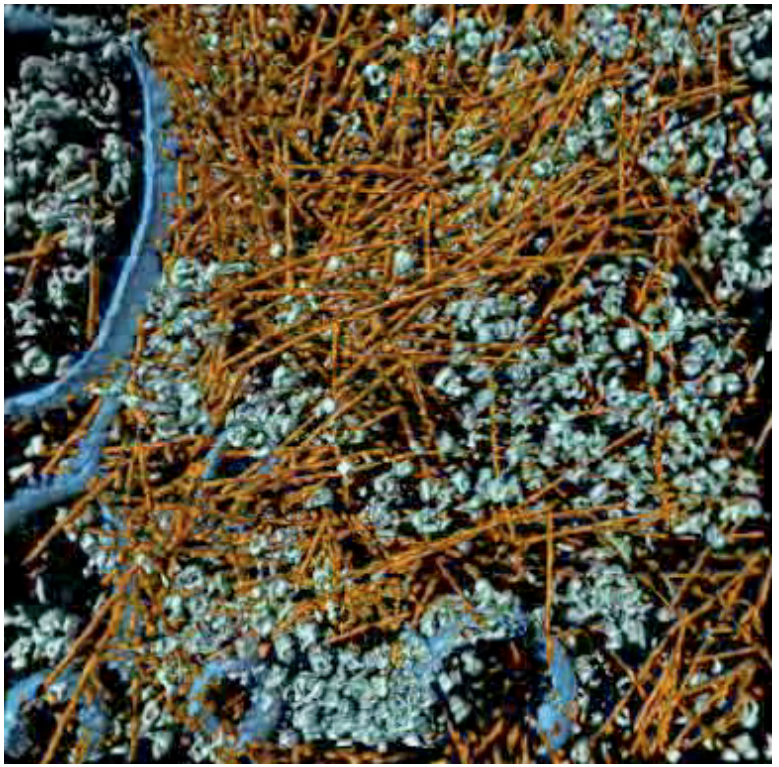


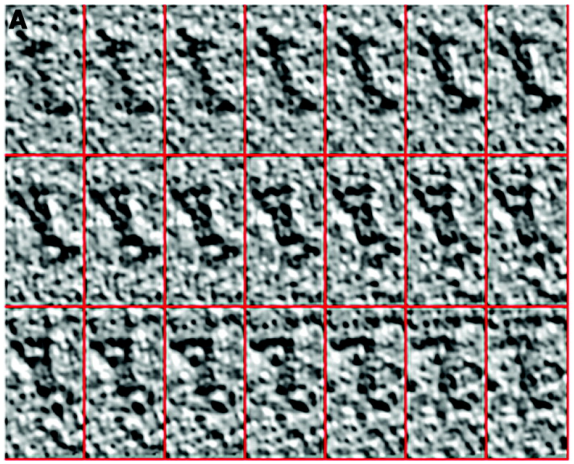
Sample optimization is complete, currently acquiring data.

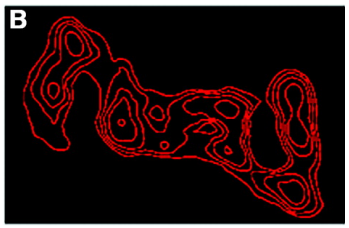
Also attempting to enrich for poliovirus containing vesicles from infected cells.

Bridging the gap: Cryotomography in cells









Cryoelectron tomography:

From Medalia, Baumeister et al. Science 298:1209-13 (2002).

Also 47Å structure of ribosome in *Spriroplasma Ortiz et al. JSB (2006)*

Can we do this for poliovirus?

Tomographic reconstructions require thin (<500 nm) samples

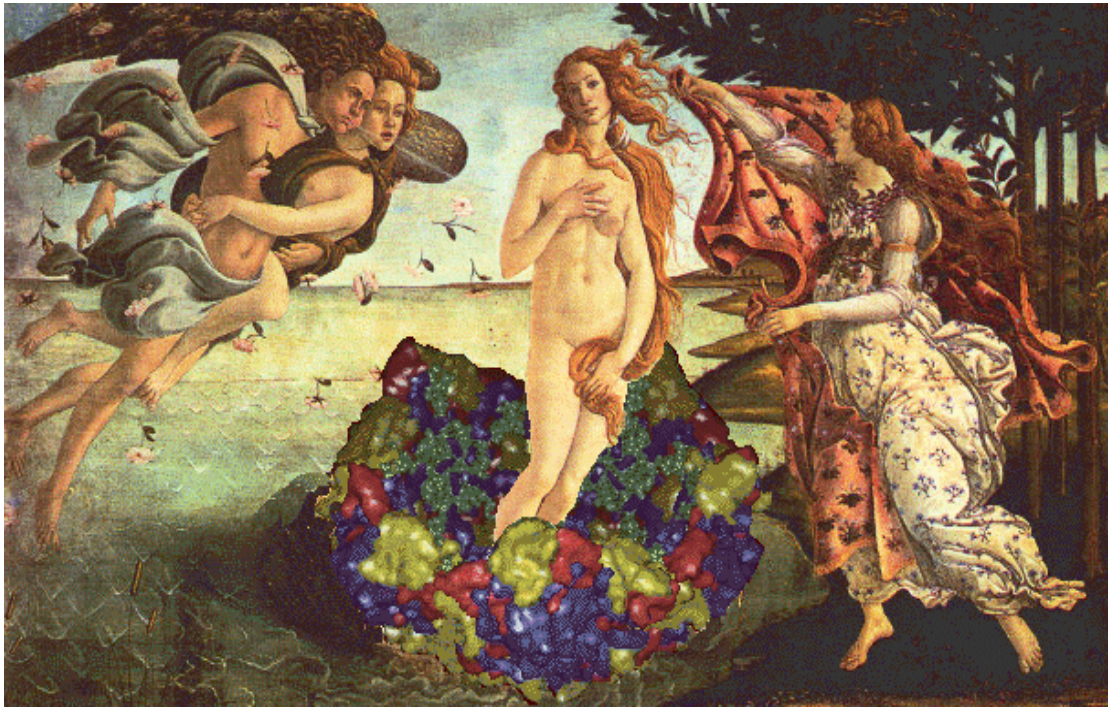
Productive steps in poliovirus entry take place efficiently in regions of cells that can be made to be very thin. May be possible to make thinner.

Efficiency of RNA release allows us to look without having to worry about high particle-to-pfu ratio

A potential problem

At low multiplicity the limited number of particles will be hard to locate in the large and crowded volume of the cell.

Solution: correlative cryomicroscopy: combine fluorescence microscopy and cryotomography of infected cells, embedded in vitreous ice



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