

A detailed 3D molecular model of a single-strand RNA virus assembly site. The central focus is a large, multi-colored protein shell (green, pink, and blue) with a yellow and orange RNA strand inside. To the right, a large, light-colored protein structure is shown in a semi-transparent view, revealing its internal structure. The background is a dark green, textured surface representing the host cell membrane. In the top left corner, there is a small yellow horizontal line.

Genome-regulated Assembly of a ssRNA Virus May Also Prepare It for Infection

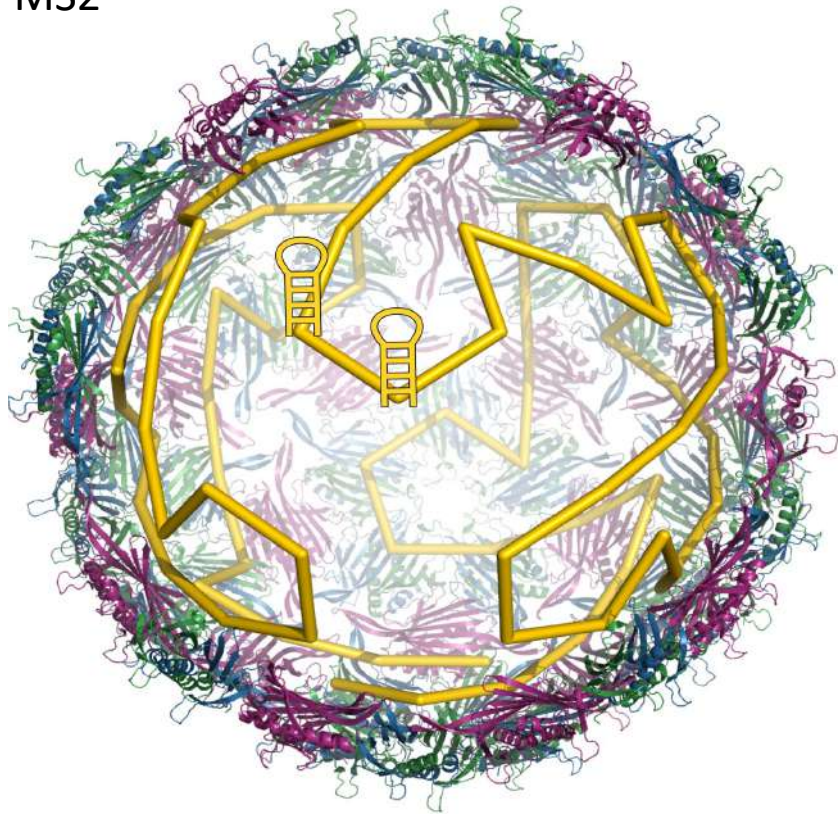
Rebecca Chandler-Bostock
University of Leeds

Packaging Signals



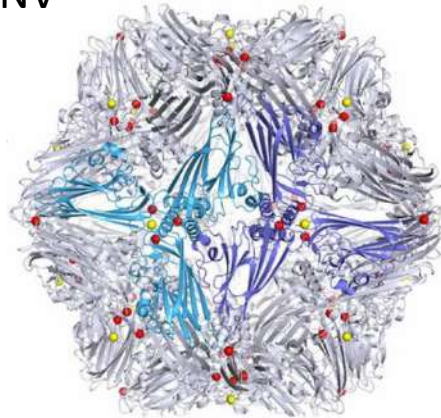
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MS2



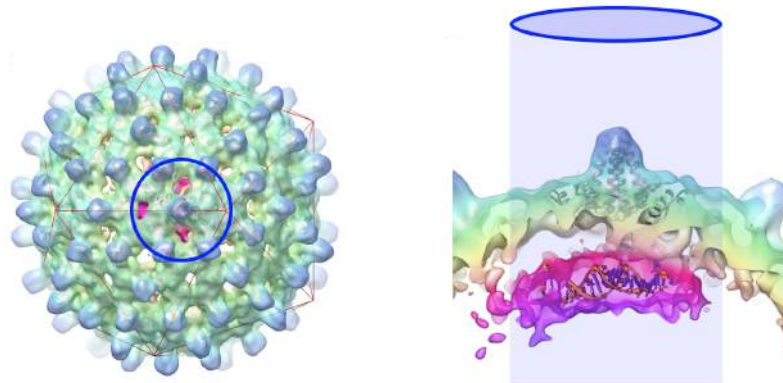
Dykeman *et al.* JMB 2013
Rolfsson *et al.* JMB, 2016
Twarock *et al.* Nat Comms 2018

STNV



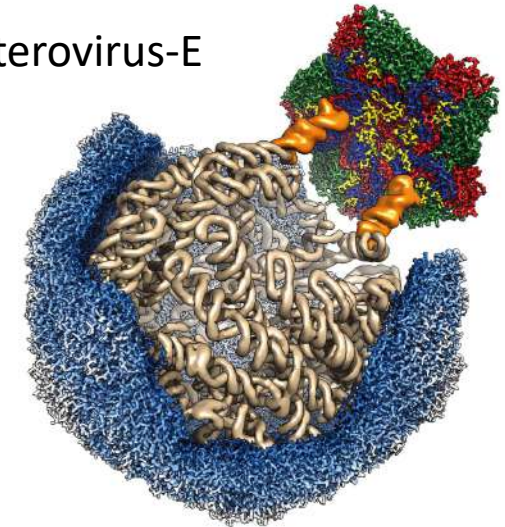
Bunka *et al.* JMB 2011;
Patel *et al.* PNAS 2015, 17

HBV



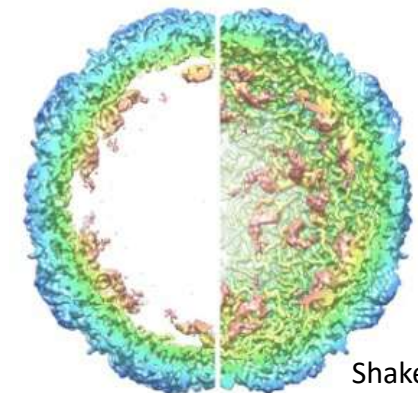
Patel *et al.* 2017 Nat Micro

Enterovirus-E



Chandler-Bostock *et al.* PLoS Pathog. 2020

Human Parechovirus 1

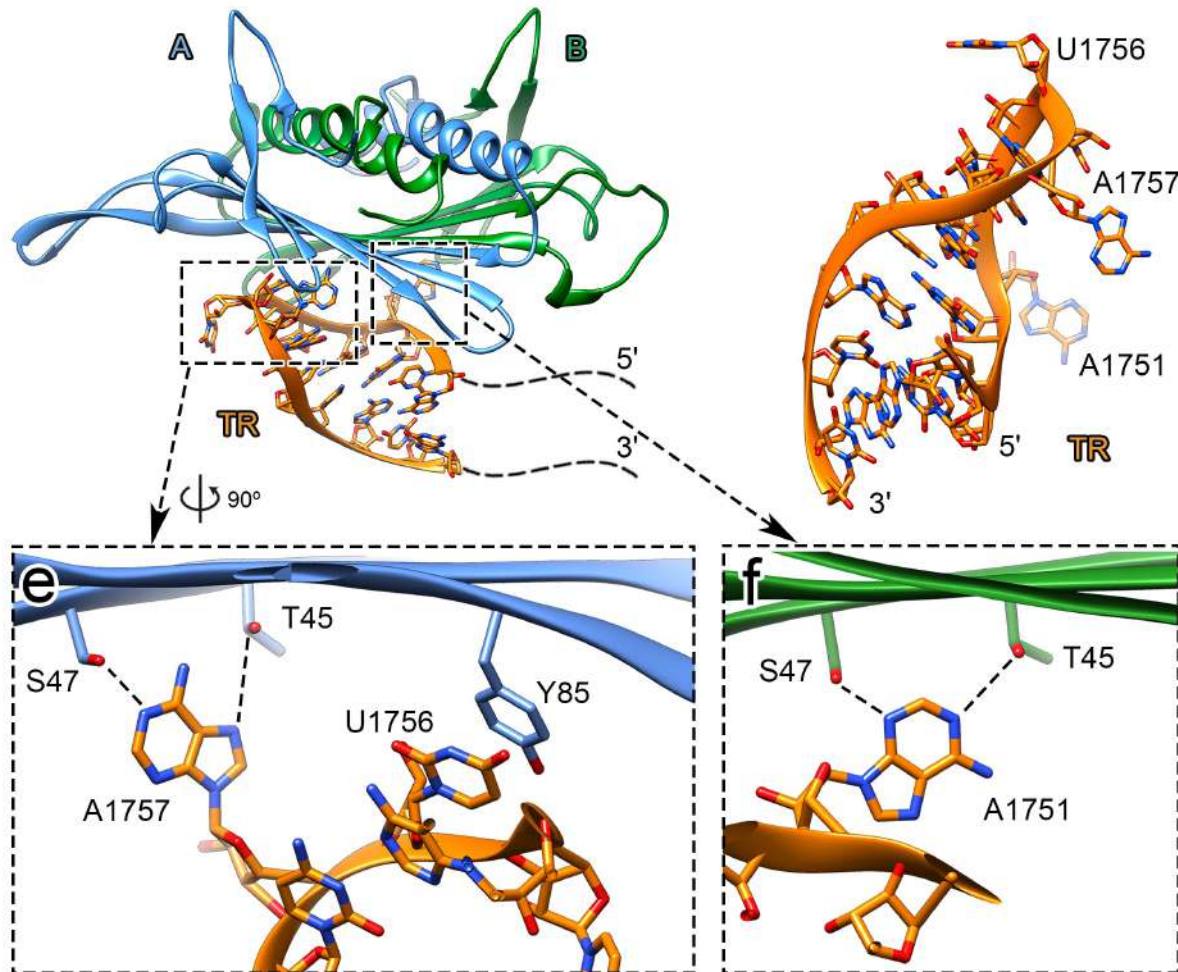


Shakeel *et al.*
Nat Comms 2017

PS-Mediated Assembly of MS2

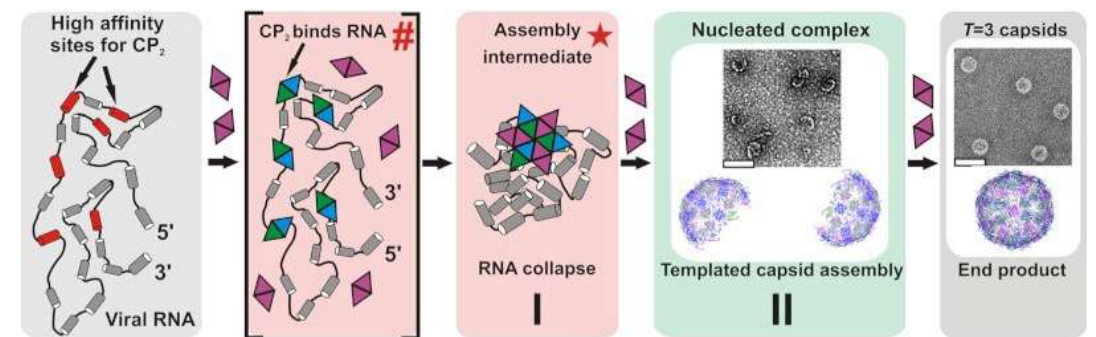


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Chandler-Bostock *et al.* 2022 JMB

- TR is the most well characterised PS in MS2
- Viral assembly takes seconds in presence of RNA containing packaging signals
- Coat protein-alone assembly takes days

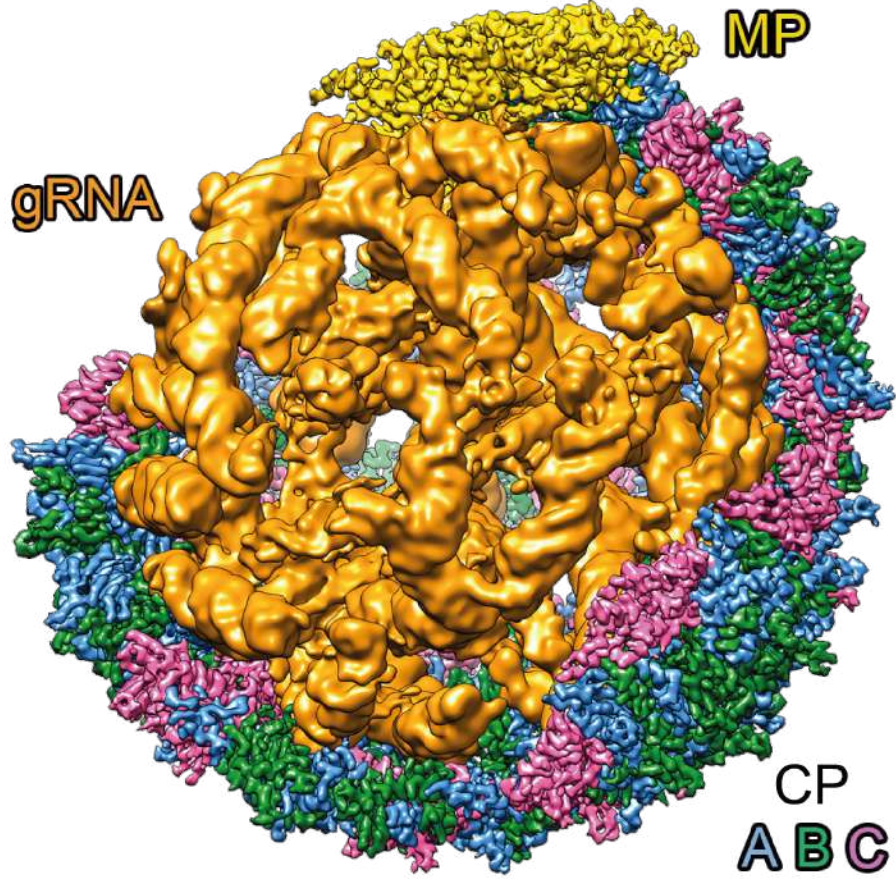
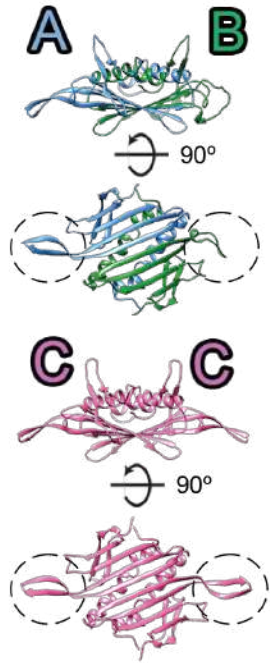
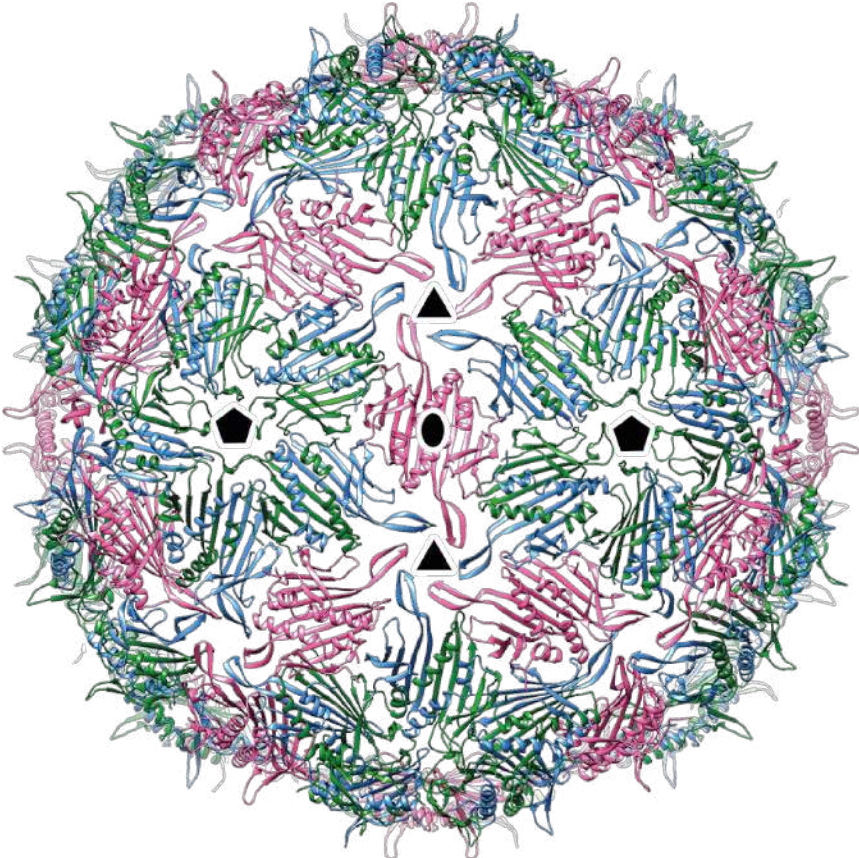


Borodavka *et al.* PNAS 2012

Structure of MS2



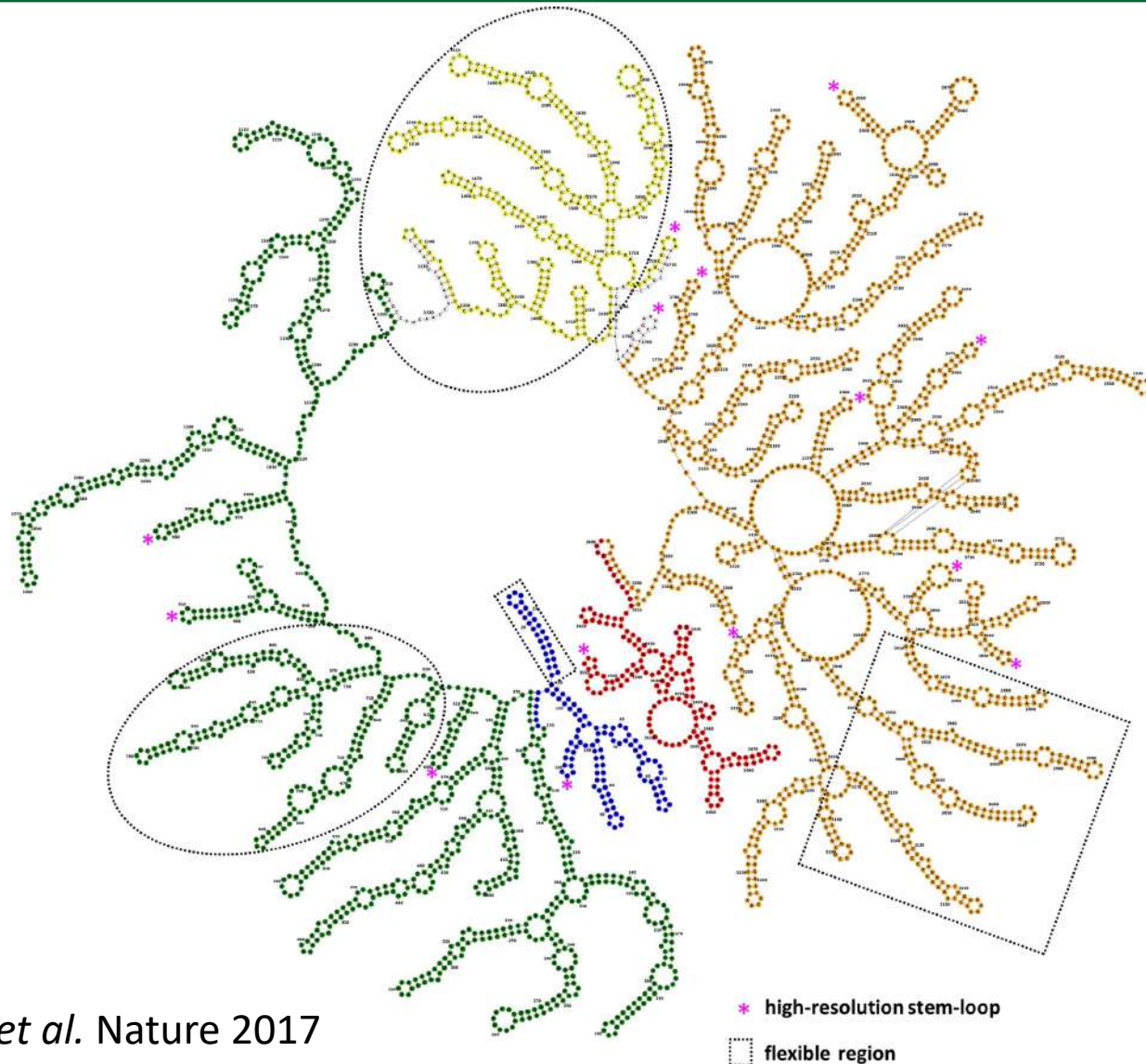
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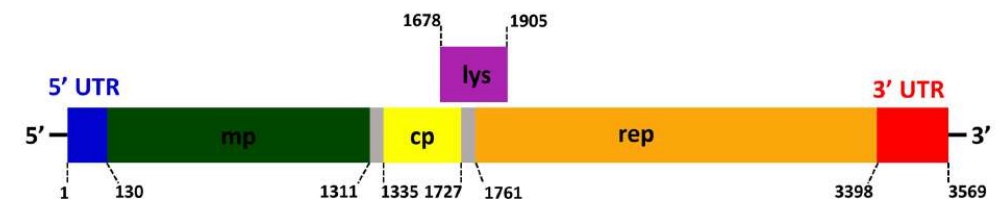
RNA Structure by Cryo-EM



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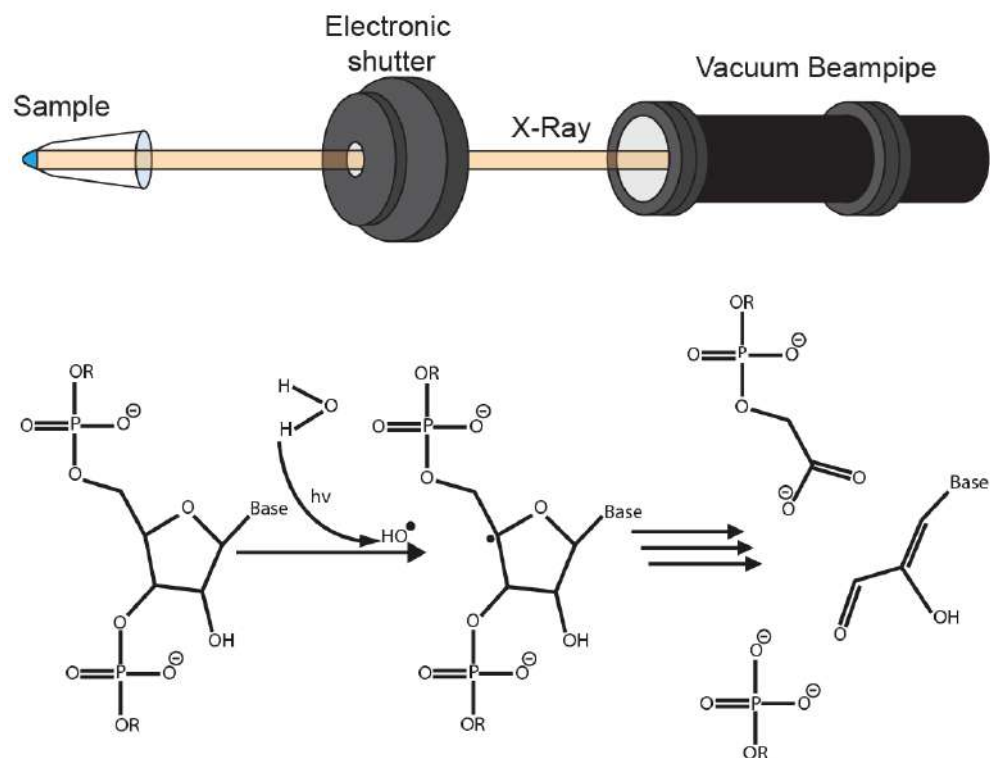
- RNA structure mapped inside MS2 virion
- Flexible regions within dashed lines were not resolved



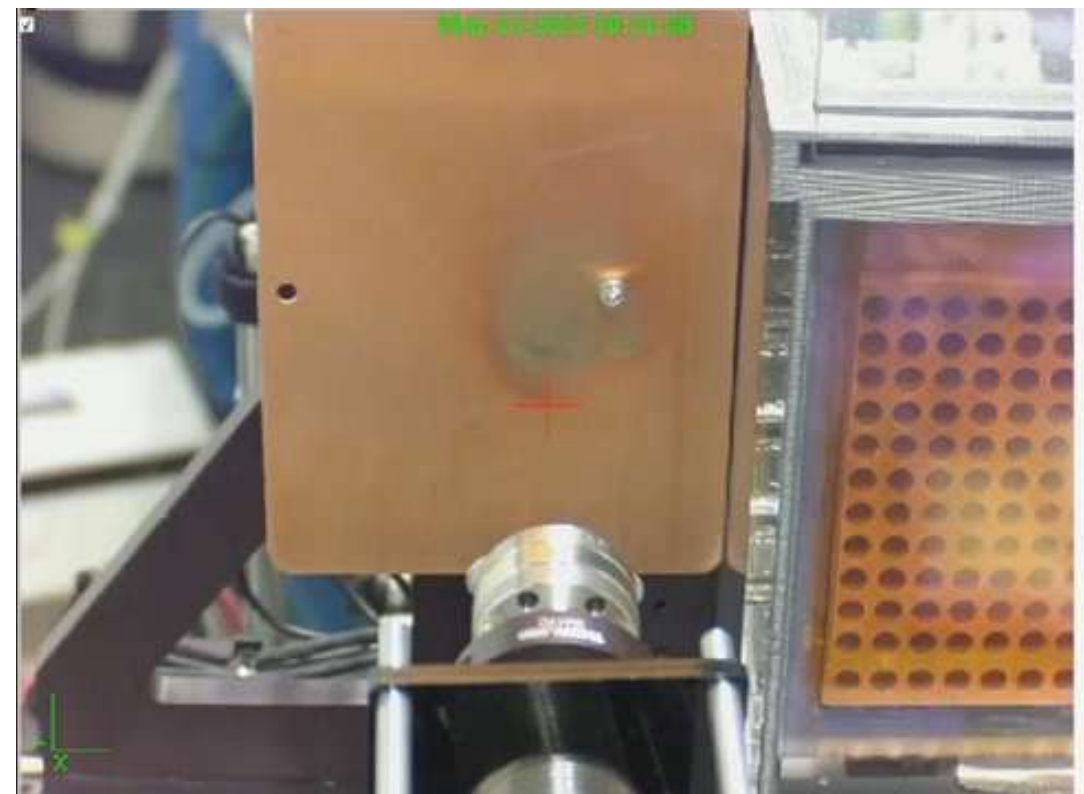
X-Ray Footprinting



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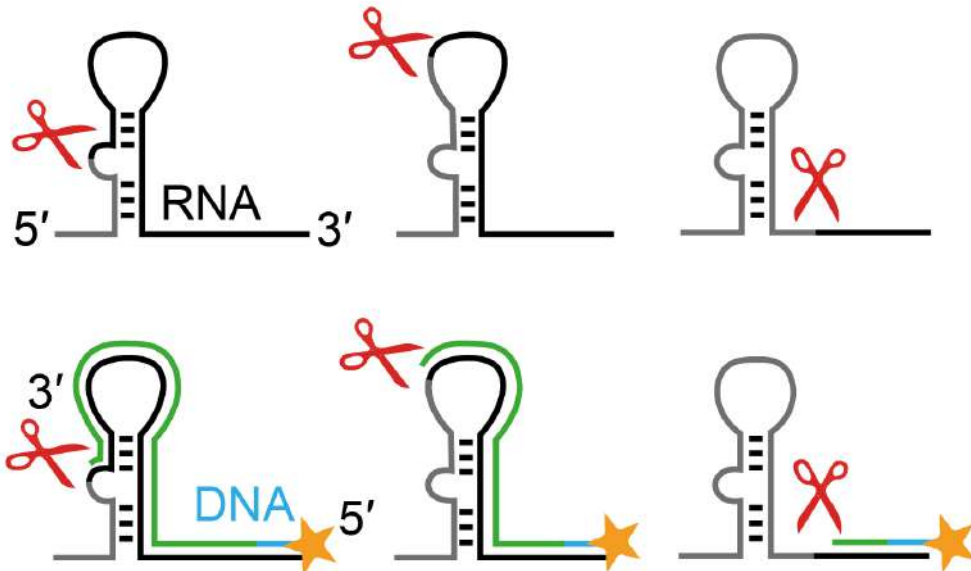
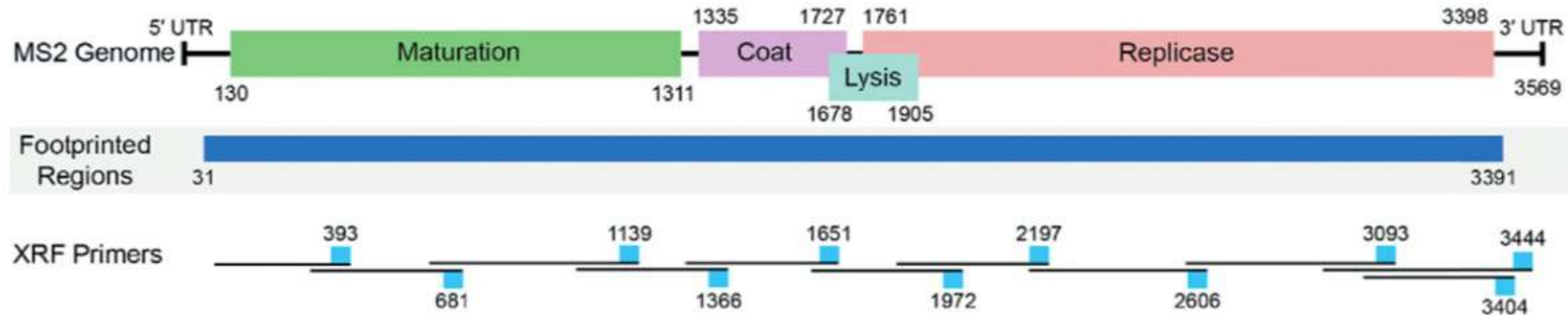
- Photolysis of solvent water molecules creates hydroxyl radicals.
- RNA backbone is cleaved in flexibility-dependent manner.



Capillary Electrophoresis



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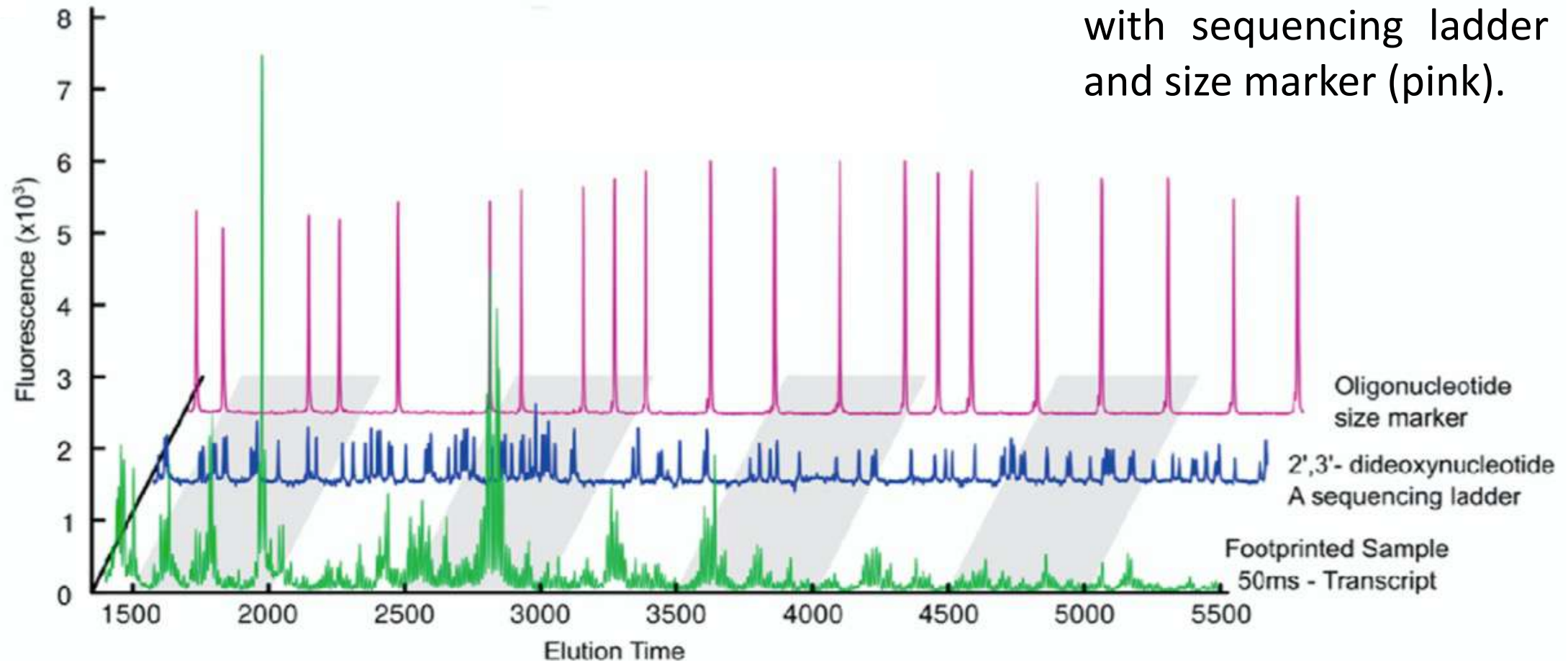
- Eleven sequence-specific primers dispersed through MS2 genome
- Primers extend in RT reaction as far as break point in RNA

Capillary Electrophoresis



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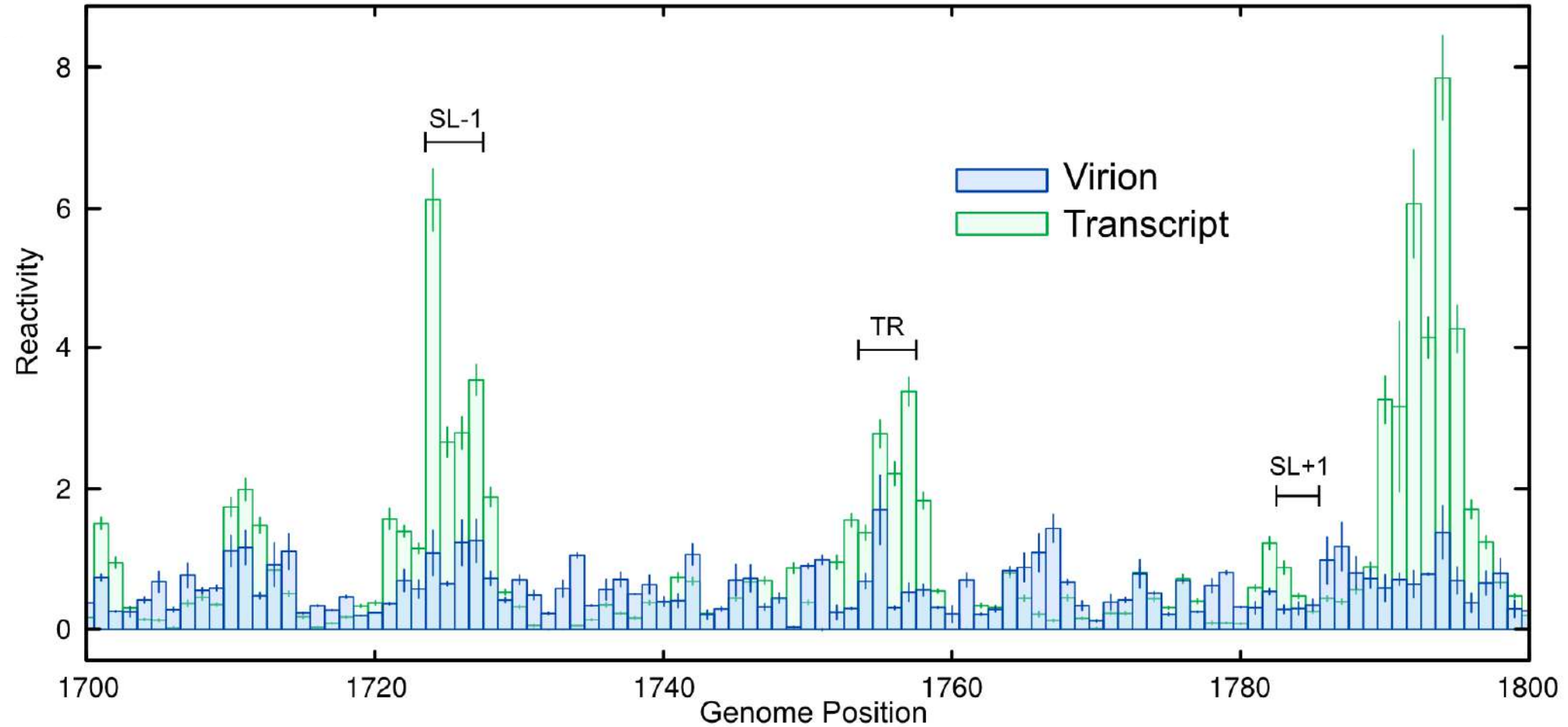
X-Ray Footprint (green) aligns with sequencing ladder (blue) and size marker (pink).



MS2 Virion and RNA reactivities



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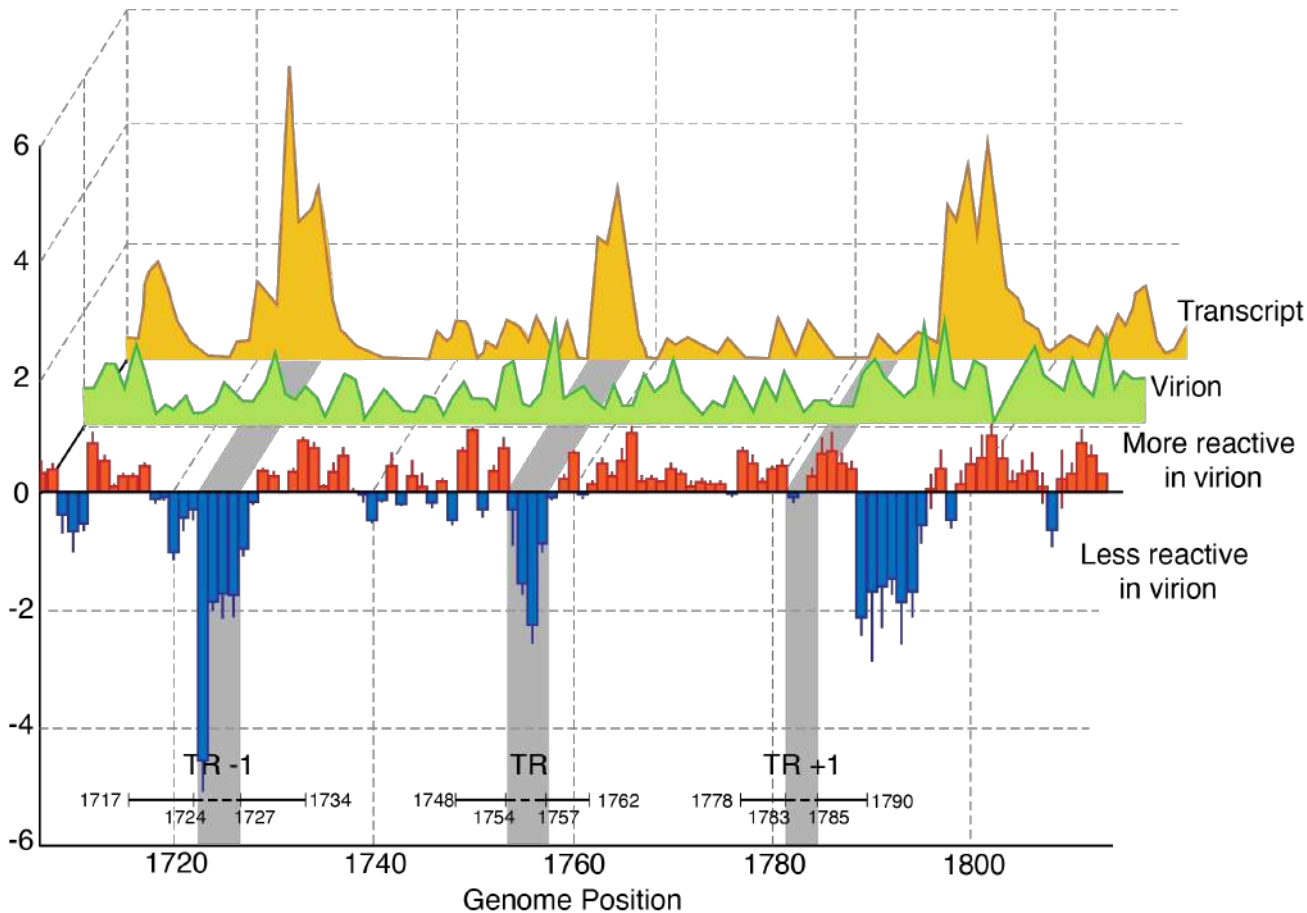


Chandler-Bostock *et al* 2022 JMB

MS2 Virion and RNA reactivities

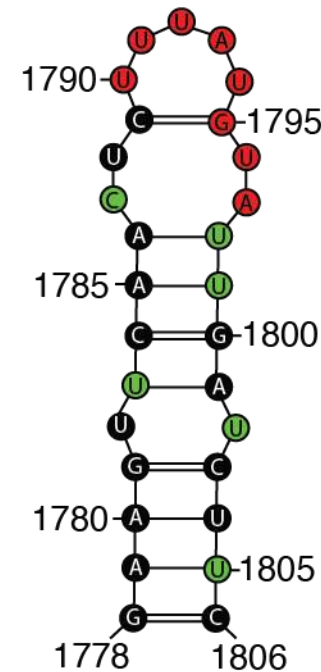


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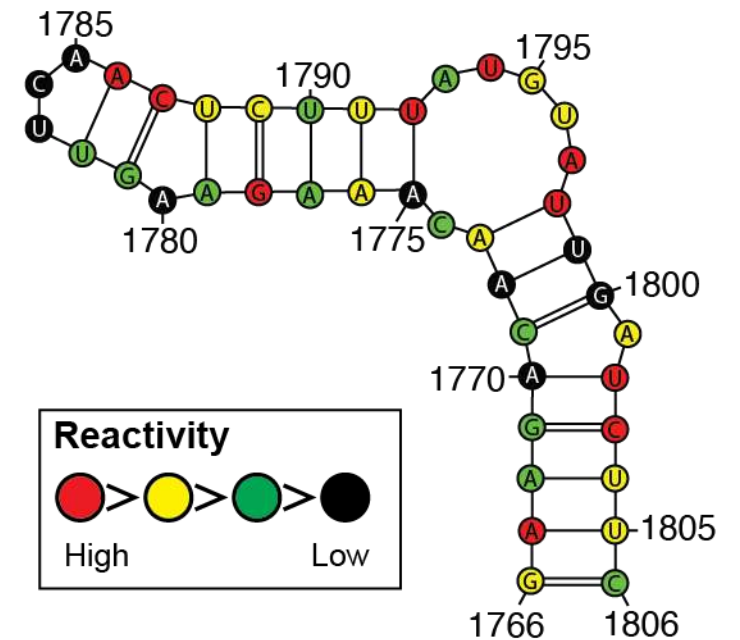


TR+1 Stem Loop Reactivities

Transcript



Virion



RNA Structures from XRF Data

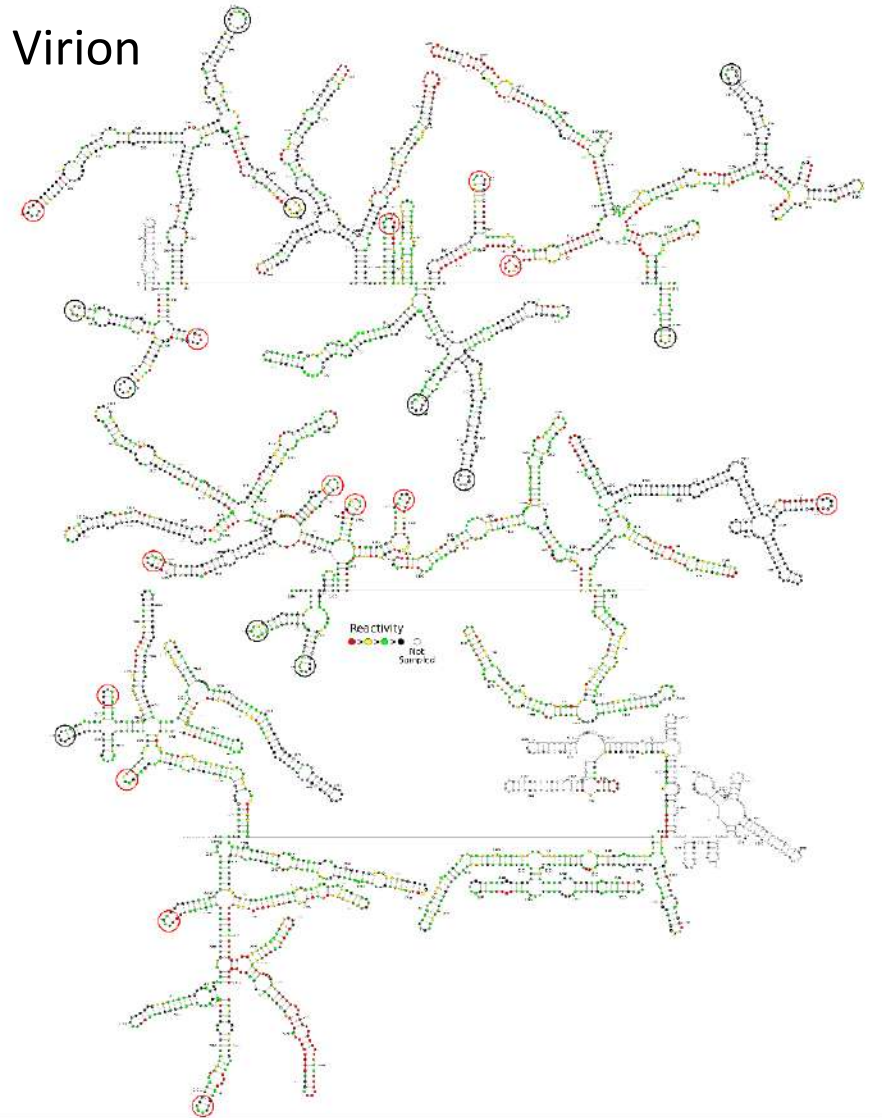


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Transcript



Virion



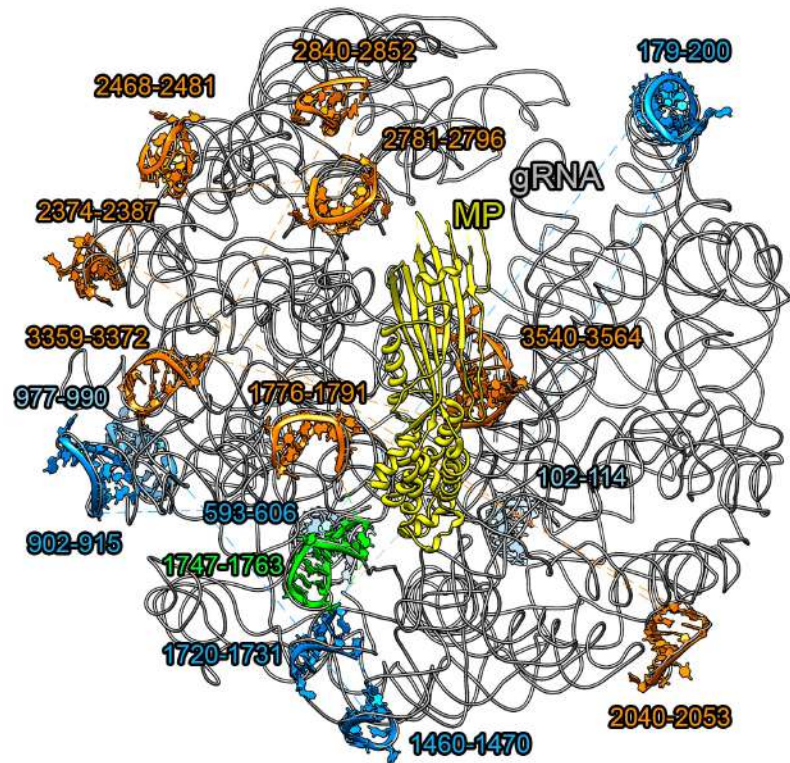
- XRF constrained S-fold structures
- MS2 genome as transcript RNA and *in virio*
- Sites thought to act as PSs are circled

PSs Identified by XRF

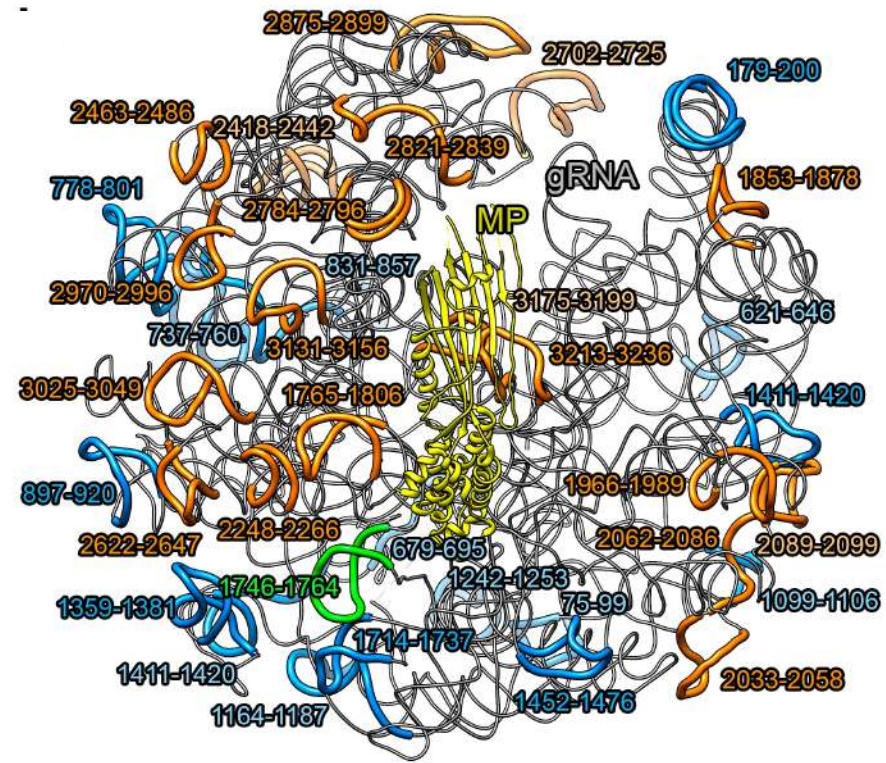


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Cryo-EM



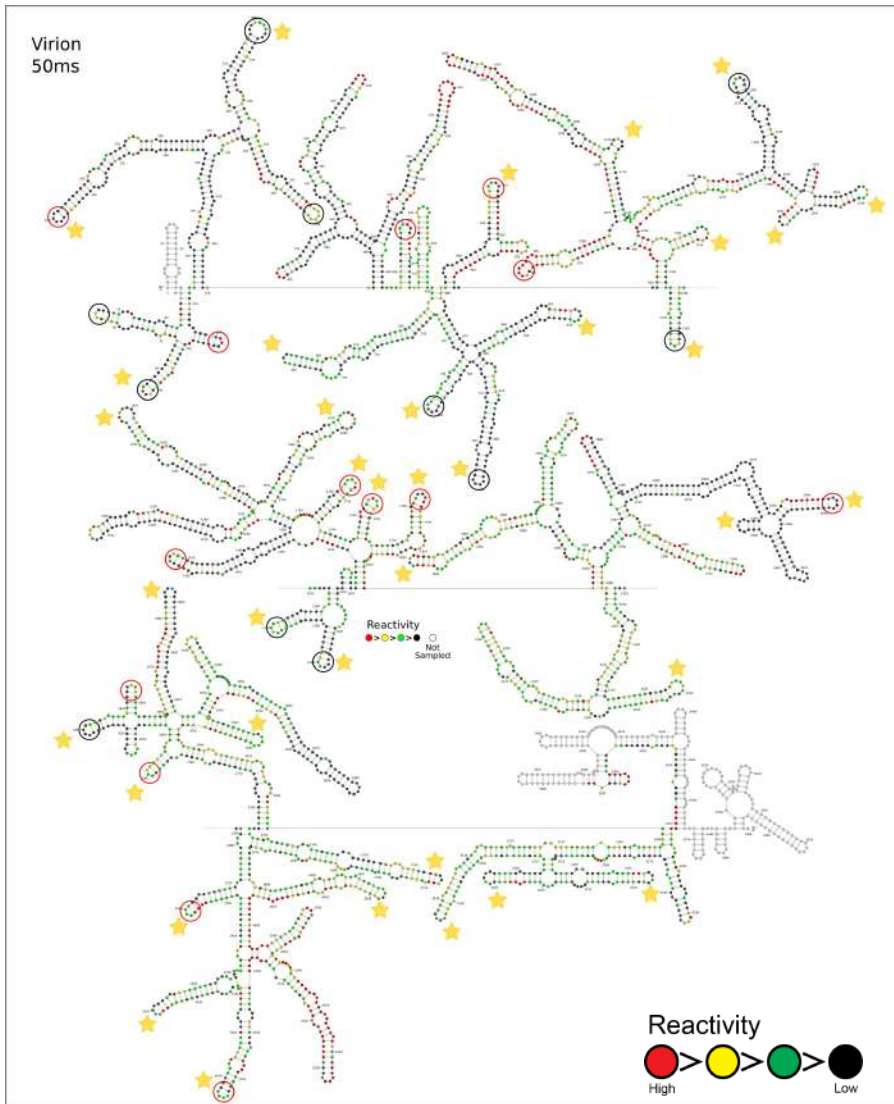
XRF



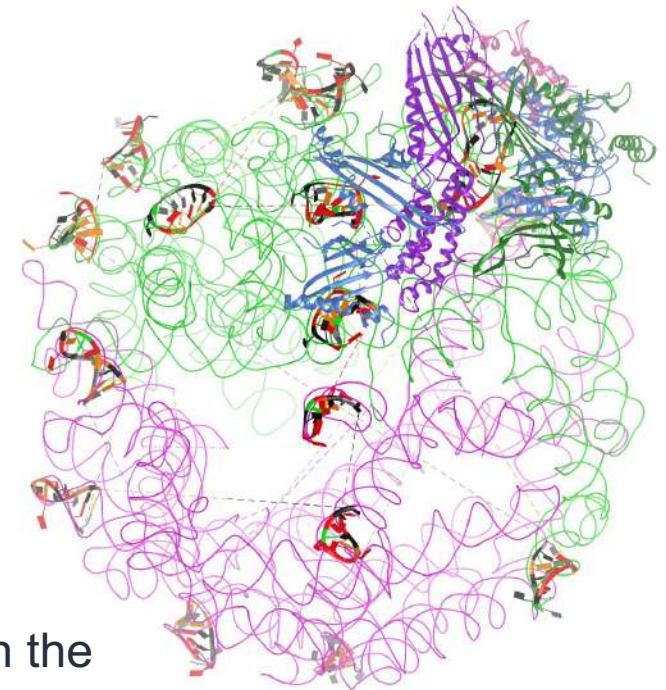
Transient Packaging Signals



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XRF identifies **31 PSs** in contact with capsid, comprising the 15 PSs determined via cryo-EM.



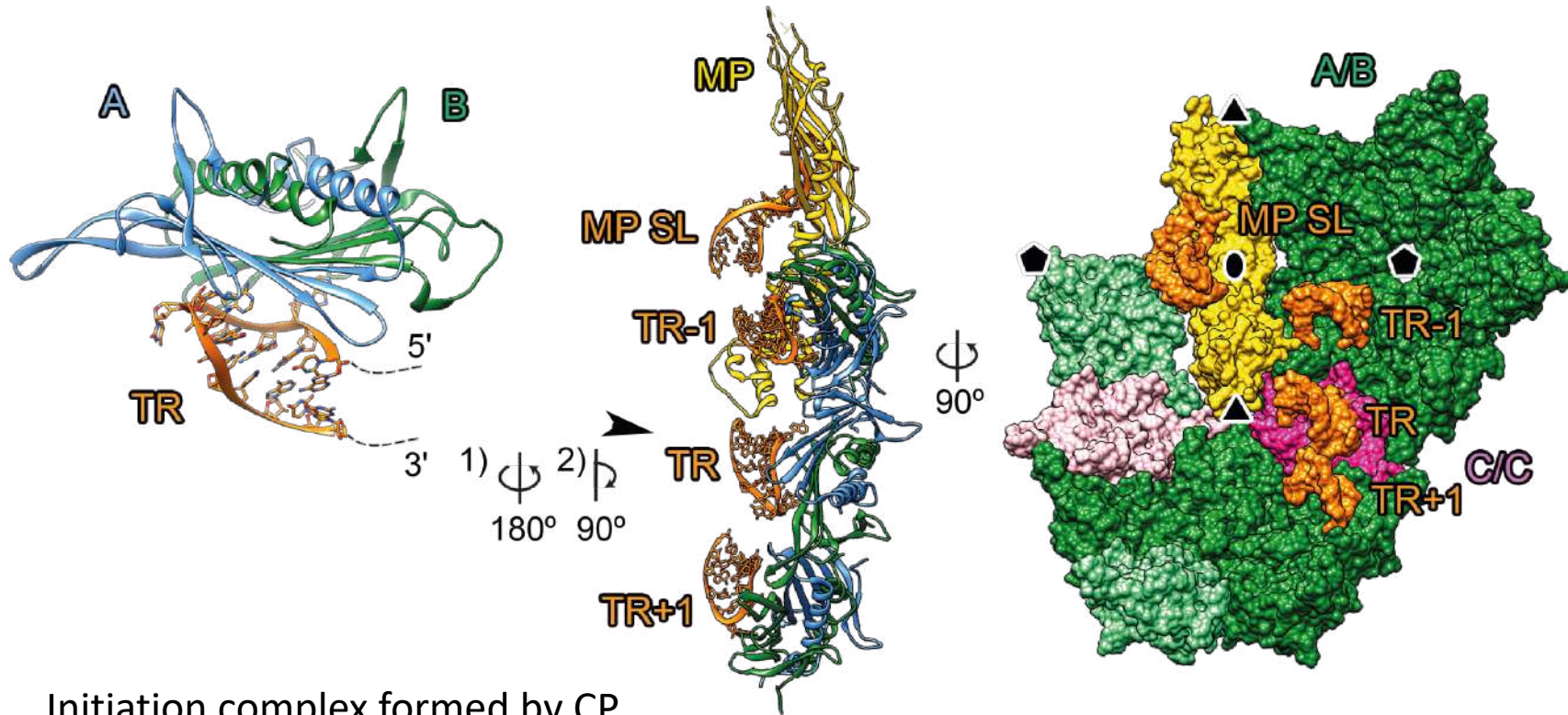
An additional **34 unbound SLs** with the molecular characteristics of the PSs are seen.

Could these correspond to PSs that have acted transiently during assembly?

Implications of CP-RNA contacts for Assembly



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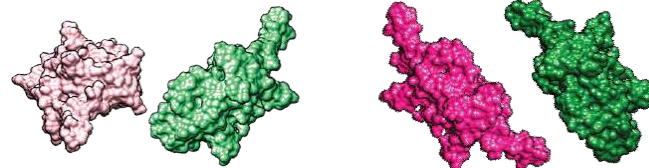
Initiation complex formed by CP dimer binding at TR and maturation protein binding the 3' stem loop

This determines the size and symmetry of the capsid that will assemble.

It blocks replicase access to its 3' binding site and commits the gRNA molecule to assembly.

Dissociated from RNA

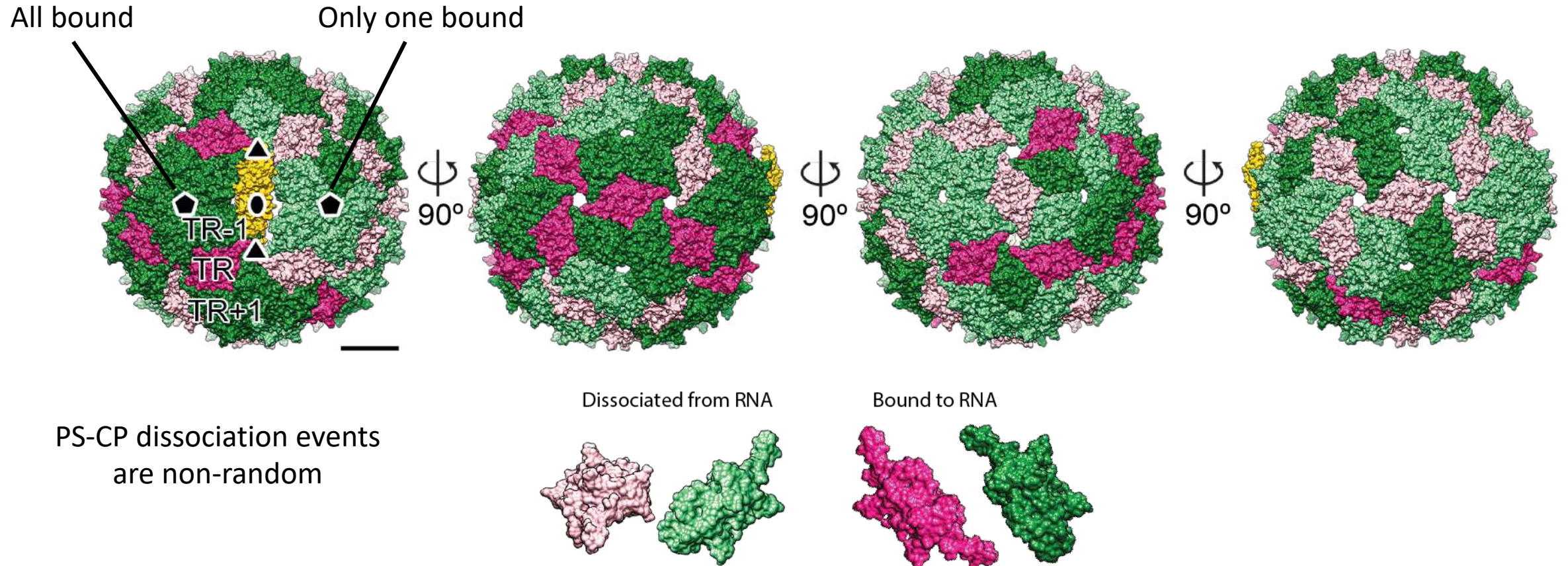
Bound to RNA



Roles of PS Dissociation for Phage Infection



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More information on the mechanism explaining interplay of capsid geometry and packaging signals:

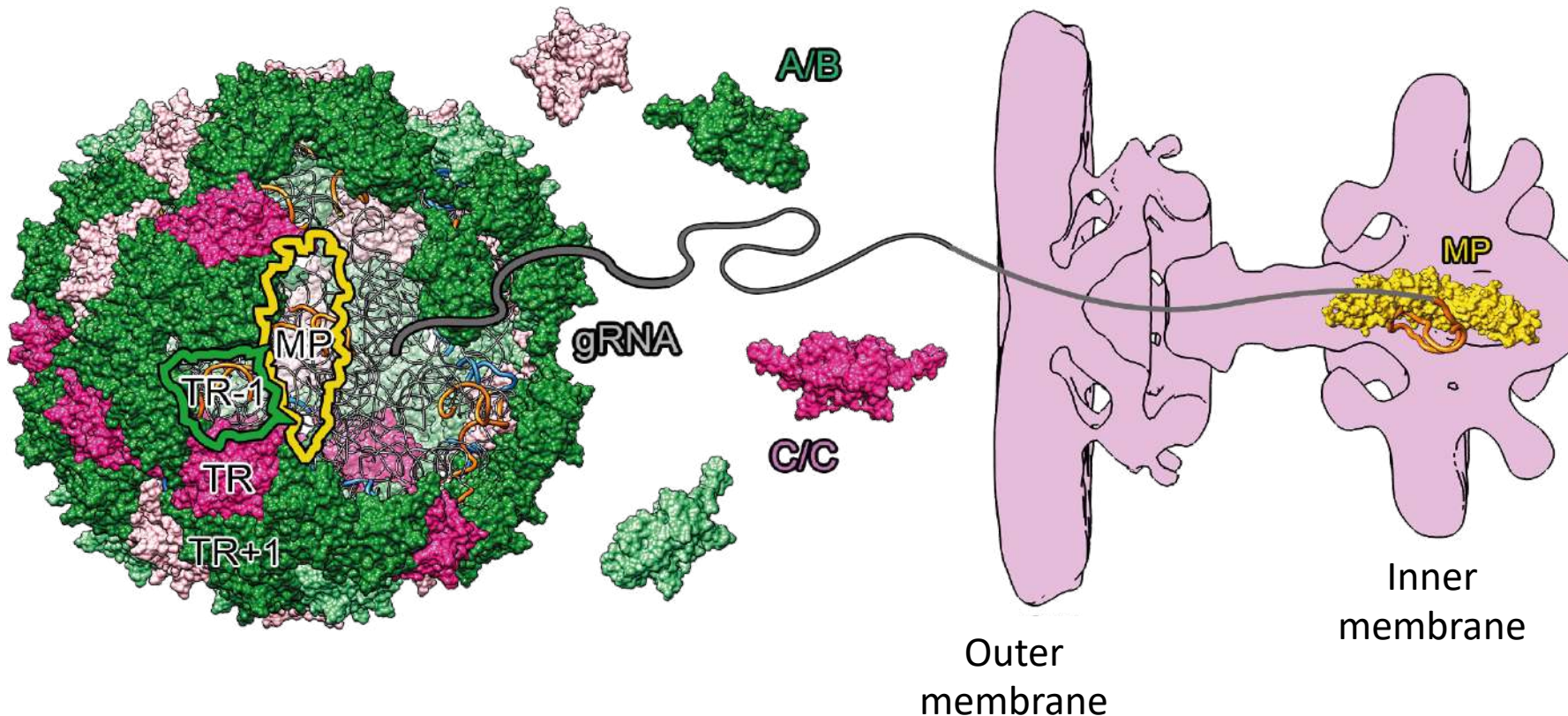
R. Twarock, G.J. Towers, and P.G. Stockley, Molecular frustration: a hypothesis for regulation of viral infections, *Trends in Microbiology*, 2023

Roles of PS Dissociation for Phage Infection



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Dissociation of PS contacts makes RNA extrusion easier



Capsomer lacking PS contacts is under structural strain and so more likely to rupture

Maturation protein occupies $\sim 2/3$ channel volume and can “tow” RNA through

- Genome condensation and molecular crowding could provide the driving force for an internal conformational rearrangement resulting in the loss of PS contacts.
- These transient PSs could play a significant role in phage infectivity.
- The molecular details of virion assembly and disassembly are important for understanding natural infections.
- Transient PSs provide unique perspectives on previously poorly understood aspects of viral life-cycles.
- They also have implications for VLP design for gene therapy applications.

Acknowledgements



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 - ▣ Drew Scott
 - ▣ Simon White



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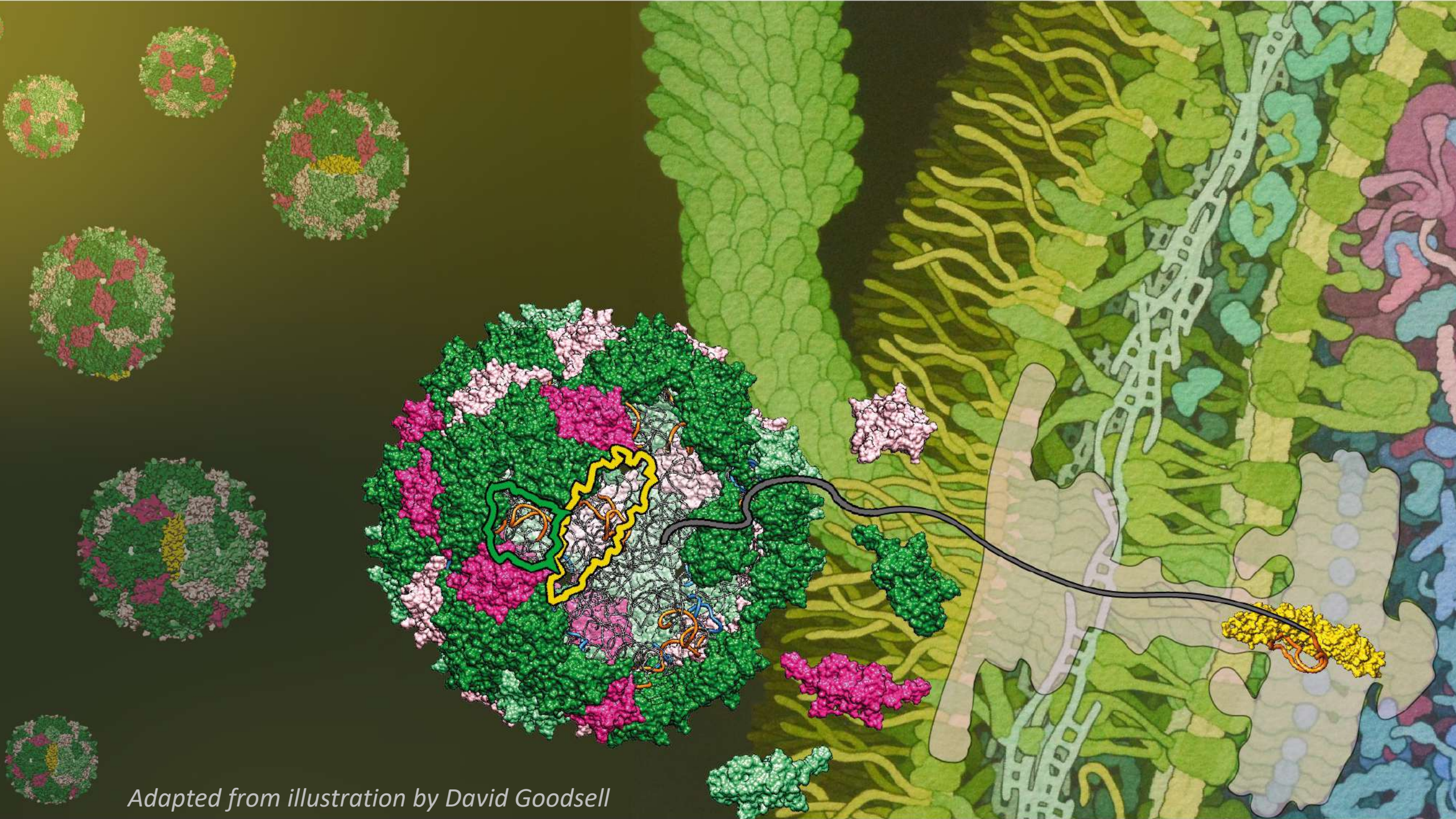
- Reidun Twarock
 - ▣ Rich Bingham
 - ▣ Sam Clark
 - ▣ Eric Dykeman



- Erik Farquhar
- Jen Bohon

wellcome trust





Adapted from illustration by David Goodsell