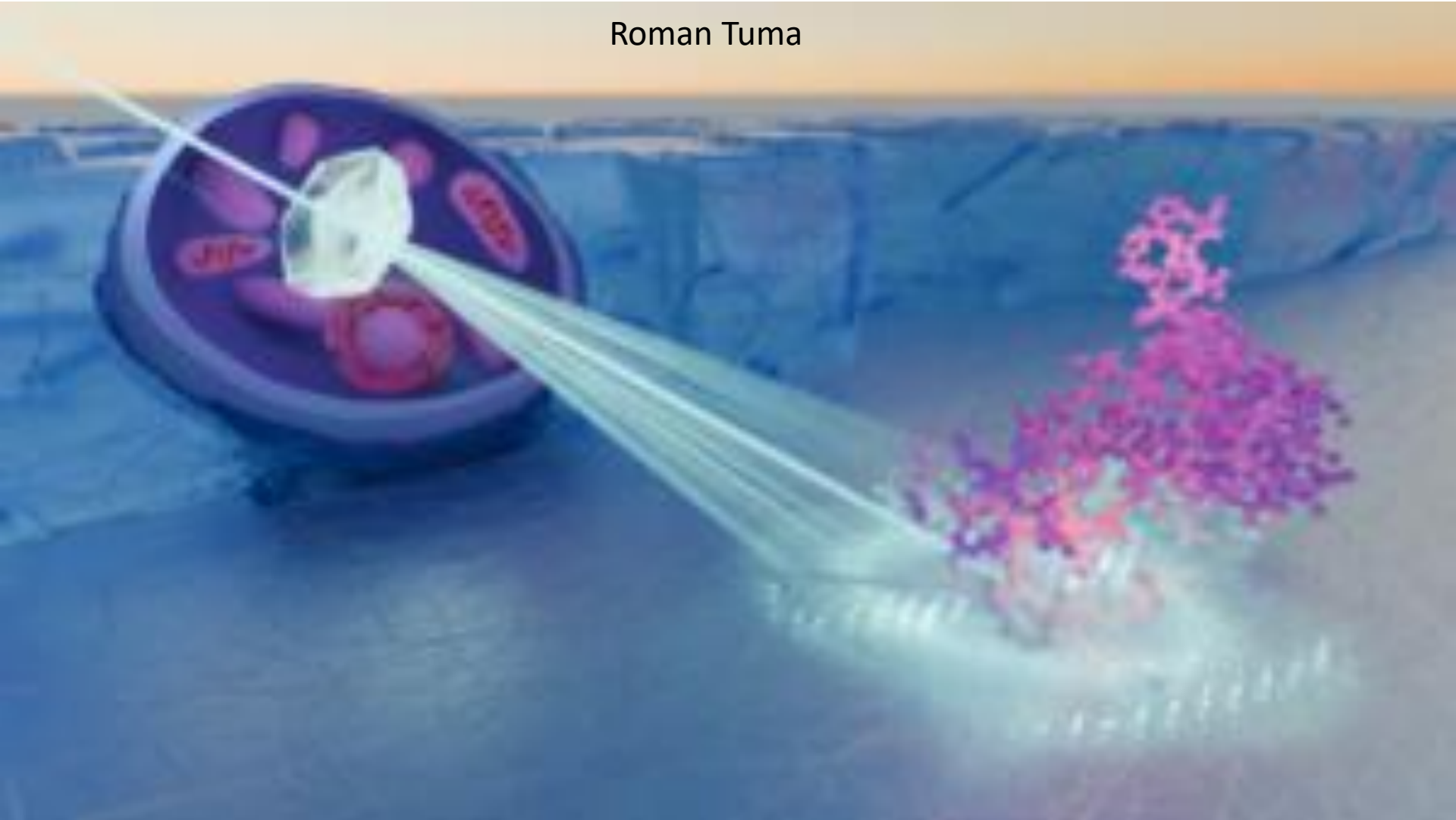


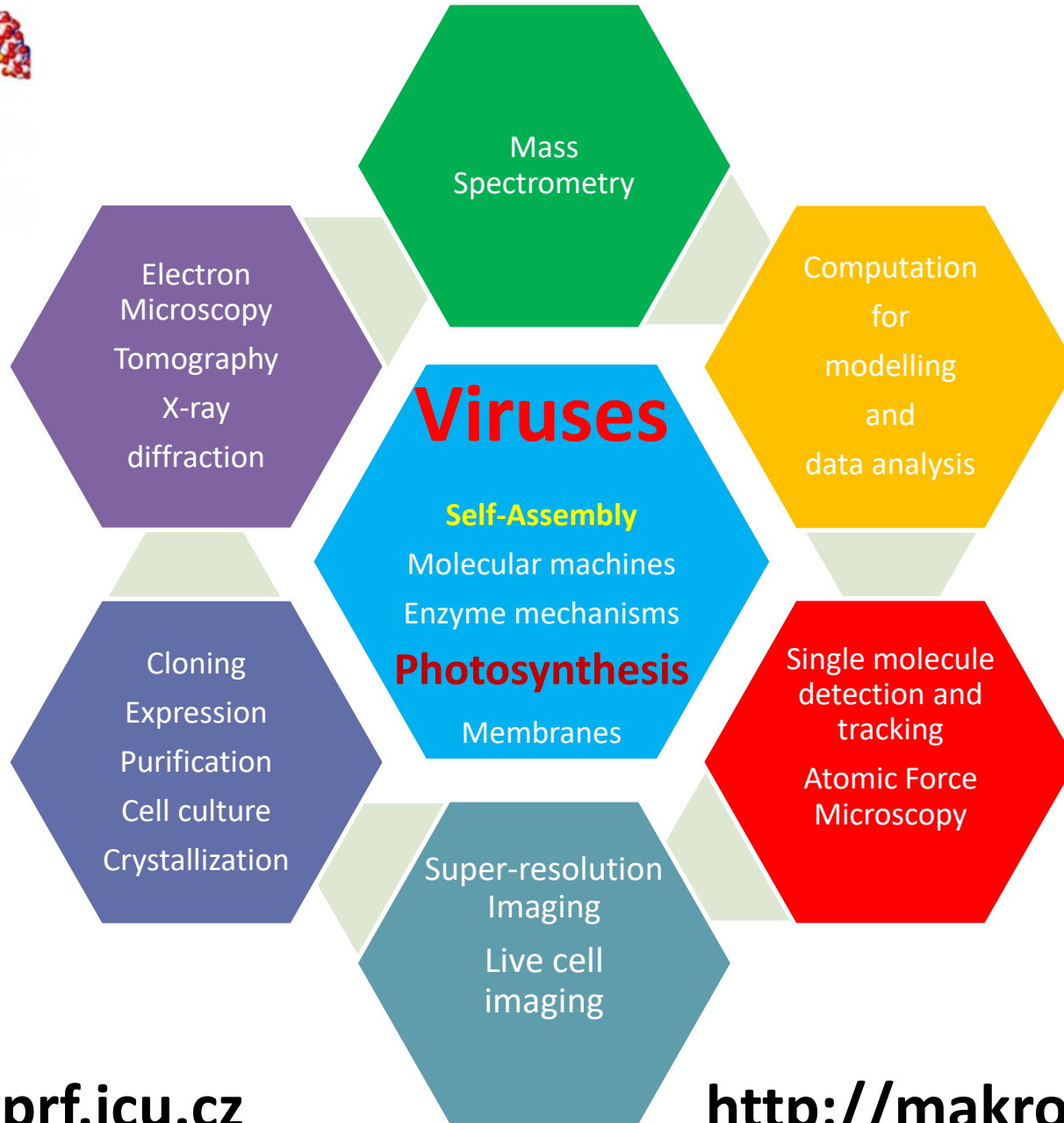
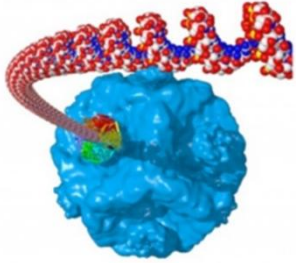
From Cells to Atoms: An interdisciplinary approach to biological mechanisms

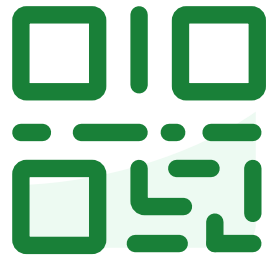
Roman Tuma



<https://www.nature.com/articles/s41467-026-69205-6>

Mechanisms and dynamics of macromolecular complexes





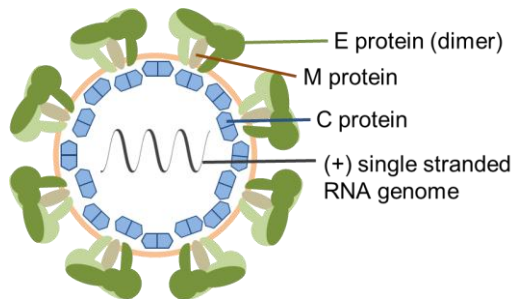
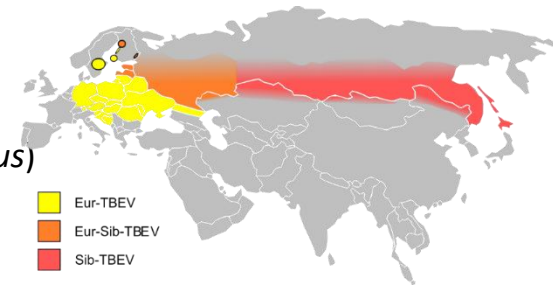
**Join at slido.com
#3870698**

ⁱ The Slido app must be installed on every computer you're presenting from

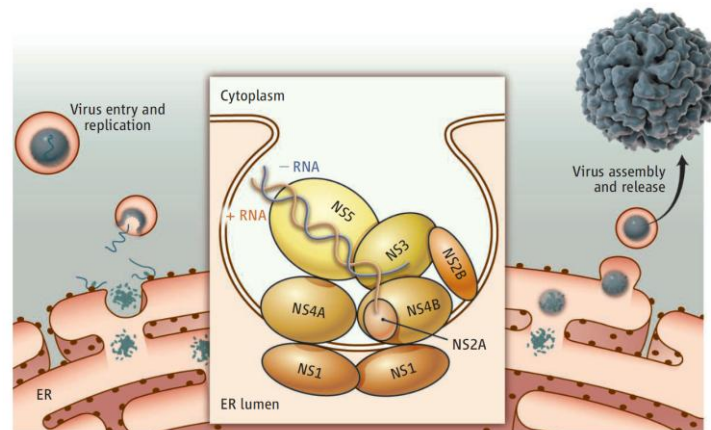
We want to know how viruses replicate

Tick-borne encephalitis virus (TBEV)

- Arthropod-borne virus (arbovirus) belongs to the family *Flaviviridae* (genus *Flavivirus*)
- **Tick-borne encephalitis (TBE)** affects human CNS in Europe and northeastern Asia
- A safe and effective vaccine against the TBEV is available but **NO specific treatment**
- a small enveloped virus containing positive ssRNA genome (~11kb) encoded a single large polyprotein (3414 AA)



3 structural proteins



(Shi, *Science* 343: 849, 2014)

Tick-transmitted *Flavivirus*



family *Ixodidae*

7 nonstructural proteins (NS) – Replication complex, enzymes
Where chemistry meets biology

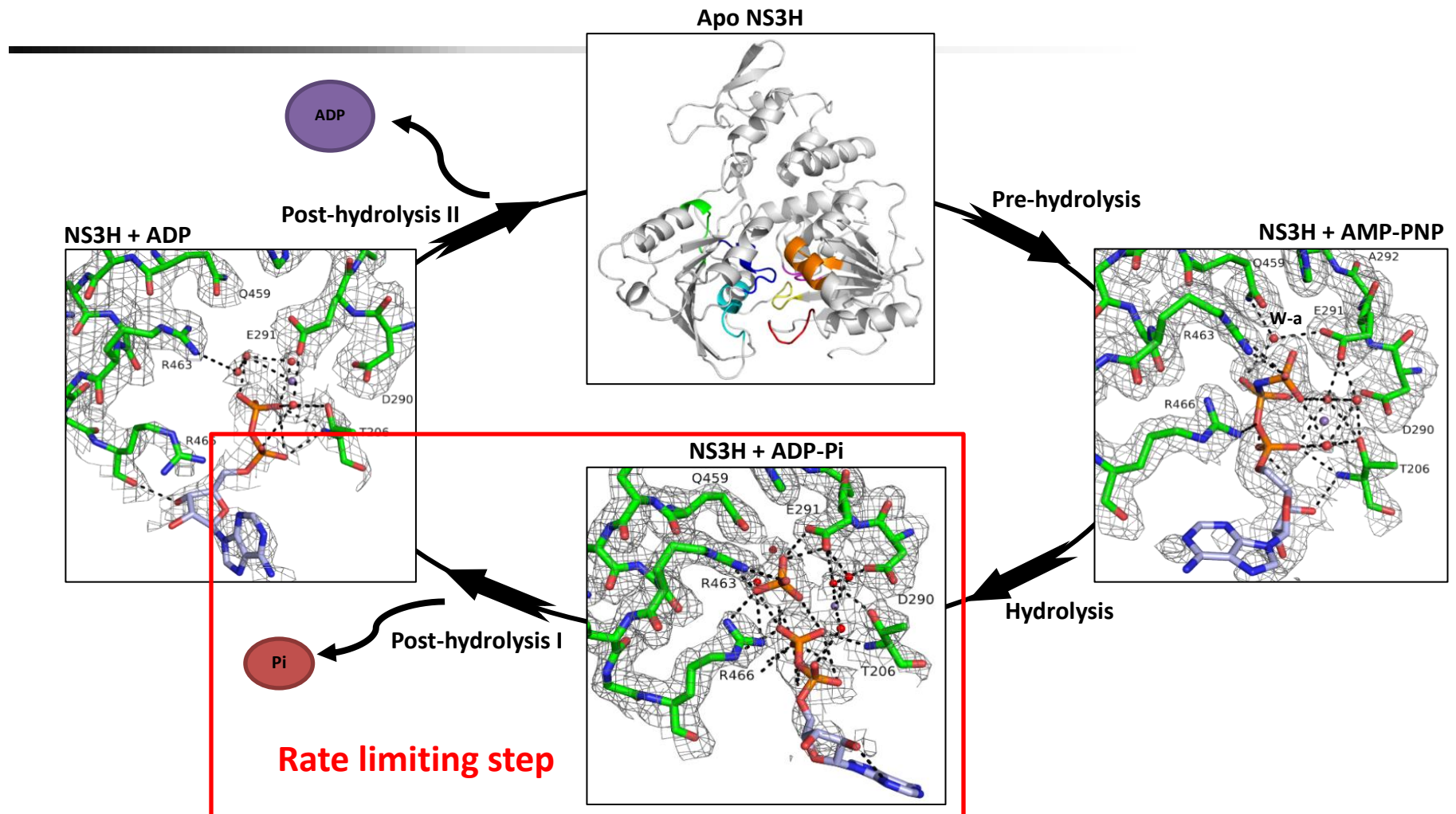
Viral NS3 helicase uses ATP hydrolysis (ATPase) to remodel RNA genome

- is an essential enzyme during replication – target for inhibition

Research is starting with asking questions (formulating working hypotheses):

How is RNA remodeling coupled to its ATPase activity?

Which step in the enzymatic mechanism is coupled to RNA remodeling?



Molecular dynamics provides mechanistic insights into steps which are difficult or impossible to capture by structural techniques – e.g. post hydrolysis inorganic phosphate release



**Outstanding related questions
(current and future projects):**

- 1) How is the phosphate release coupled to RNA movement?
- 2) How is the helicase activity regulated by other viral proteins within the replication complex
- 3) How does the replication complex assemble onto the membrane surface?



Which catalytic step is likely coupled to RNA remodeling?

ⁱ The Slido app must be installed on every computer you're presenting from

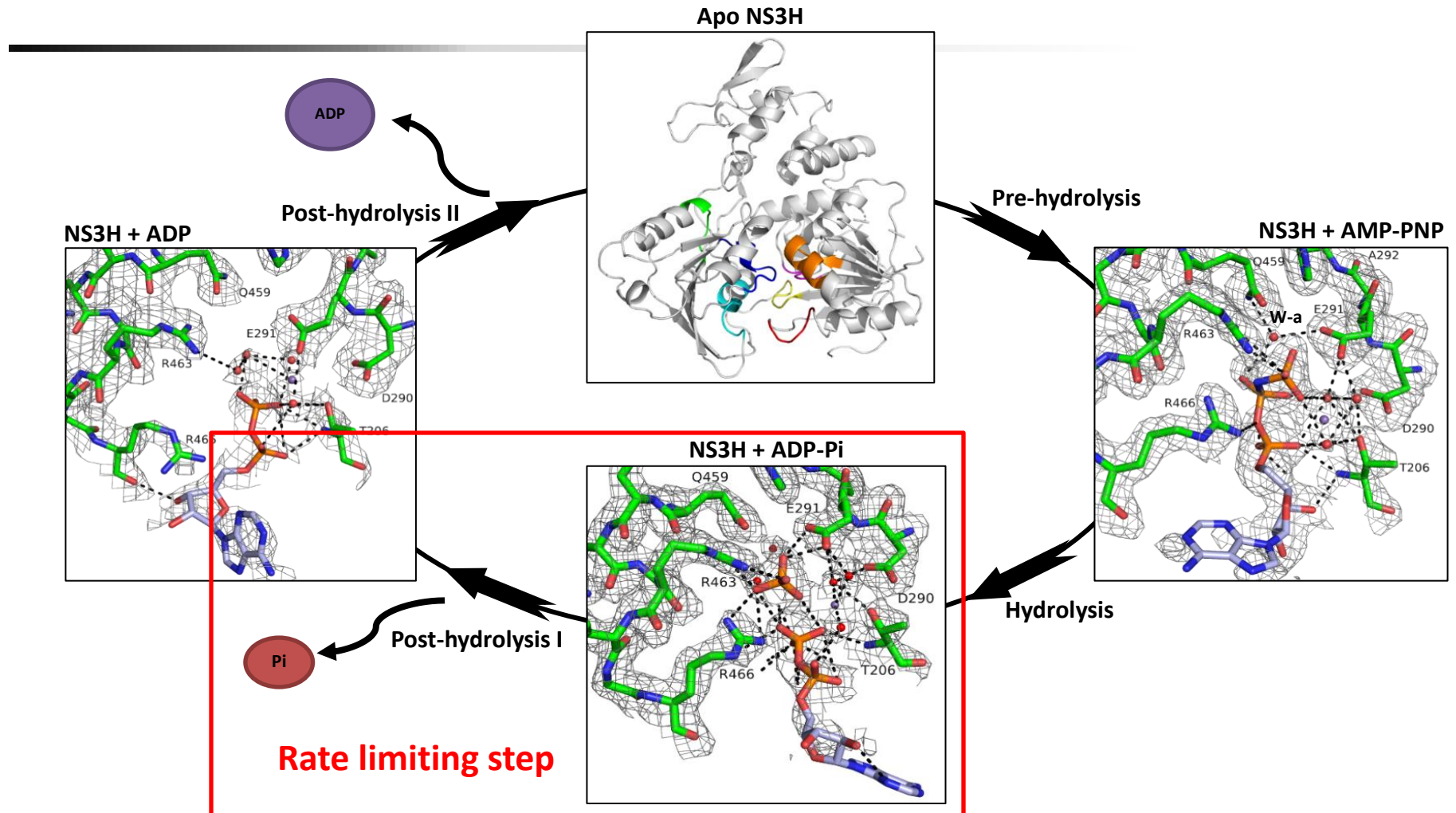
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Research is about asking questions:

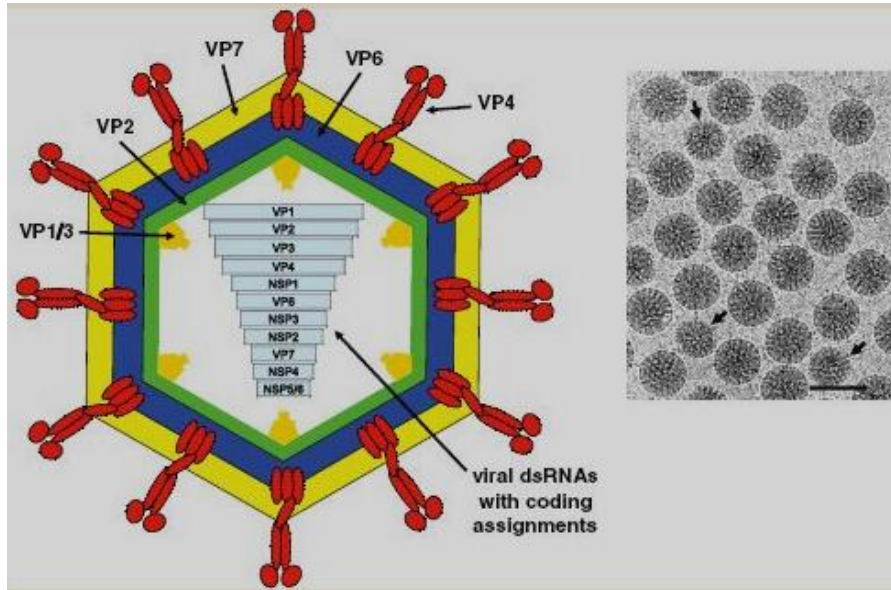
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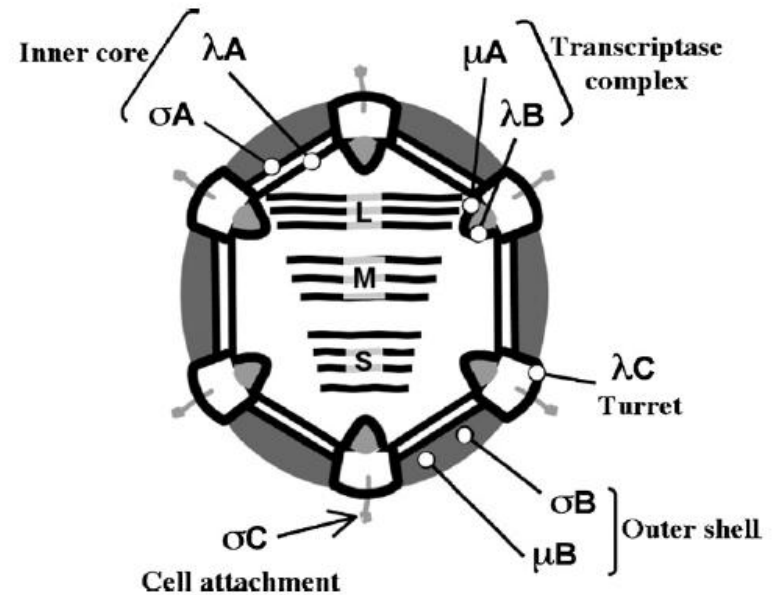


Reoviridae – viruses with segmented dsRNA genome

Rotavirus (RV)



Avian reovirus (ARV)

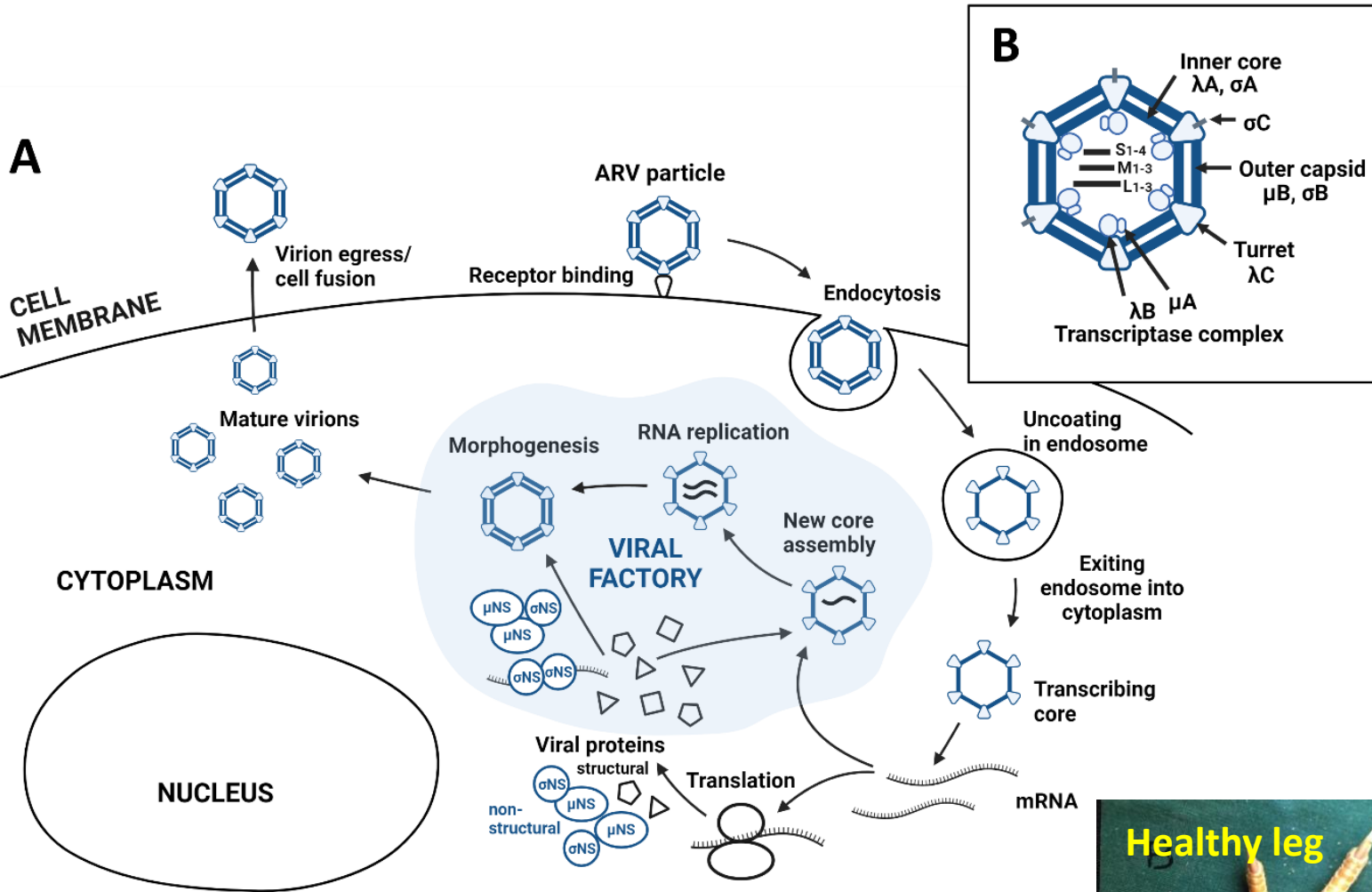


RV causes gastroenteritis

Knipe, David M.; Howley, Peter. Fields Virology (Knipe, Fields Virology)

ARV causing gastroenteritis, hepatitis, malabsorption, myocarditis, and pneumonia in poultry birds

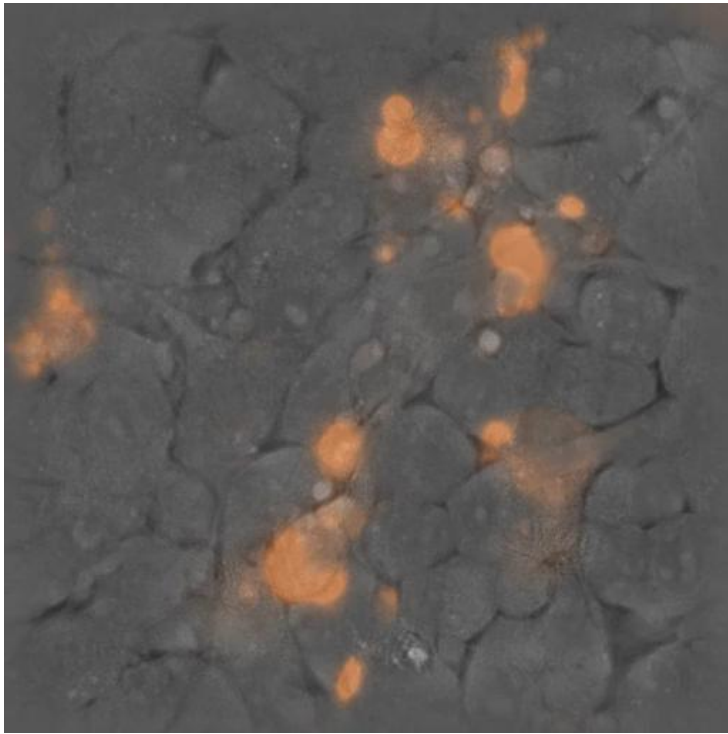
Avian reovirus (ARV) is a common farm fowl pathogen



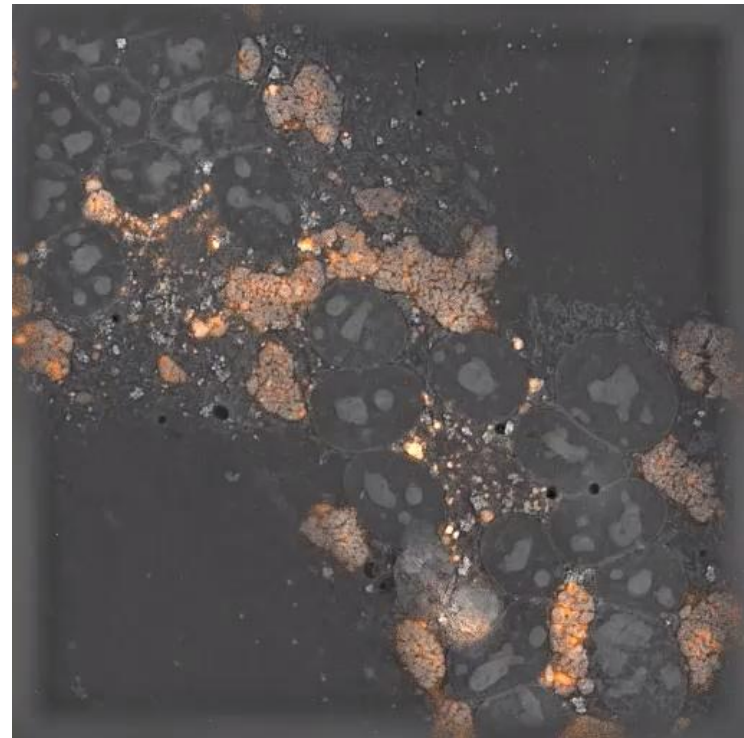
What are viral factories/viroplasm/viral inclusions?

- Virally induced **membrane-less** “organelles” in cytoplasm
- formed by liquid-liquid phase separation (akin water/oil)
 - biological condensates (both phases are hydrated)

Cells 9 hours after infection (early)



20 hours after infection (late)

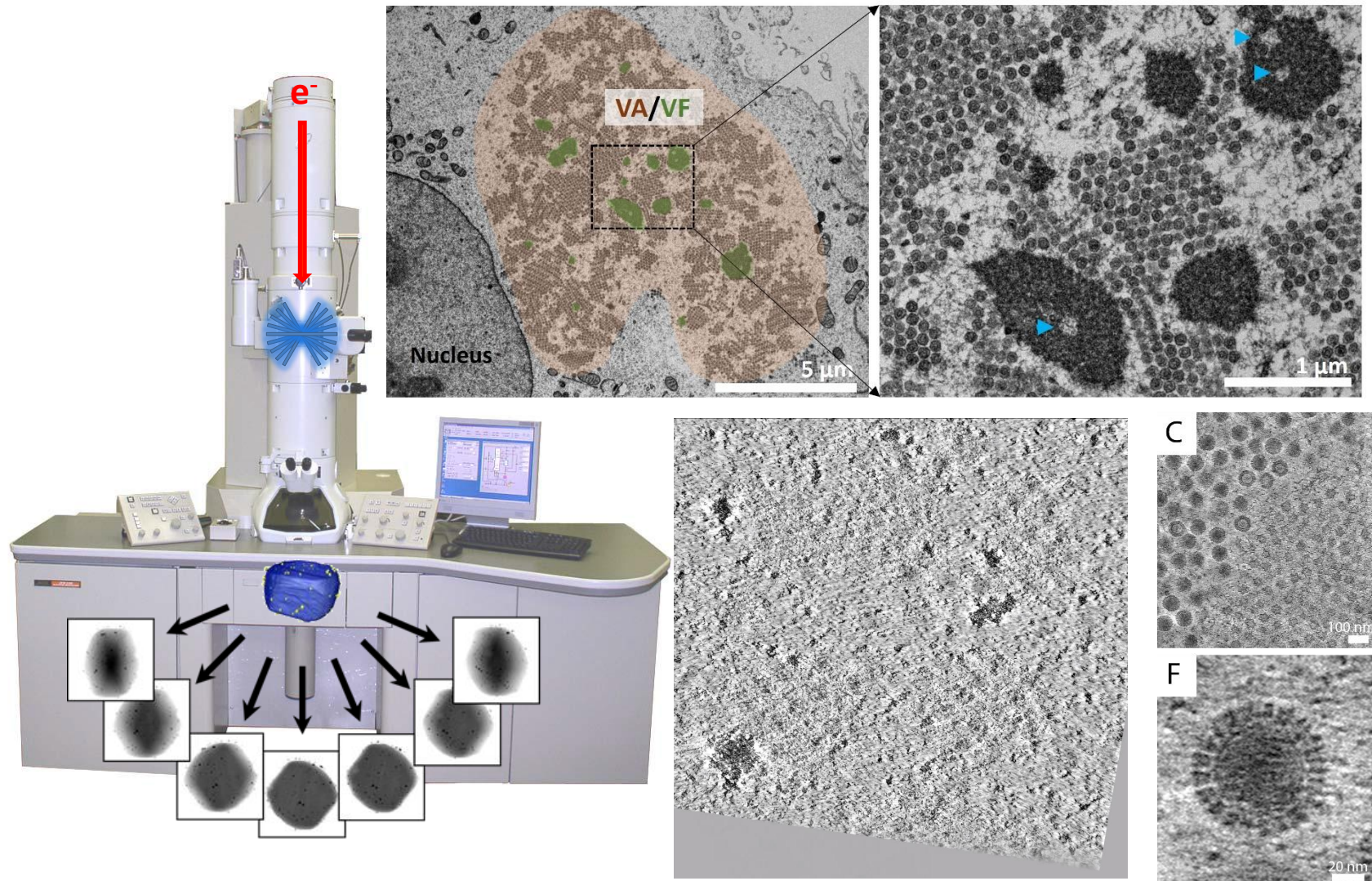


Lice cell imaging – fluorescence and refractive index microscopy

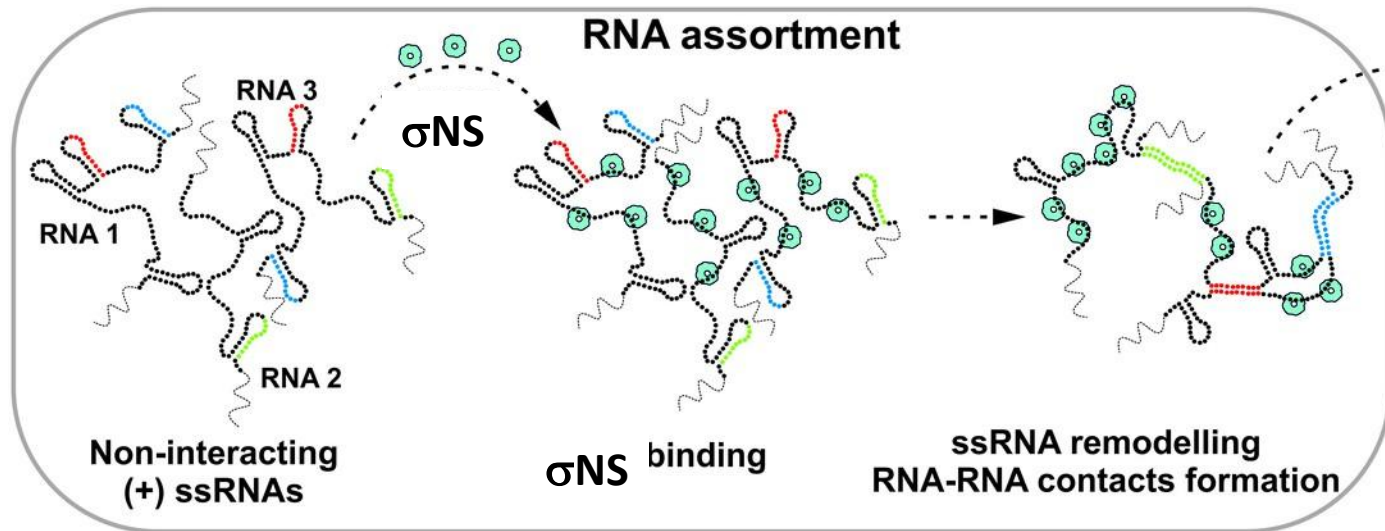
- round droplets with fluid-like behavior form ~9 h post transfection
- After 20 hours cells are fused into multinucleated syncytia with inclusions immobile

Making of a virus at nanoscale— electron microscopy view

Viroplasm (VF) are embedded within para-crystalline arrays (VA) of virions



Non-structural RNA-binding proteins are abundant in viroplasm and play essential role in genomic RNA segment assortment and packaging



Viral RNA binding protein σ NS is abundant in viroplasm

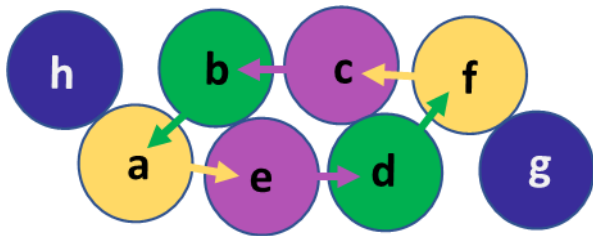
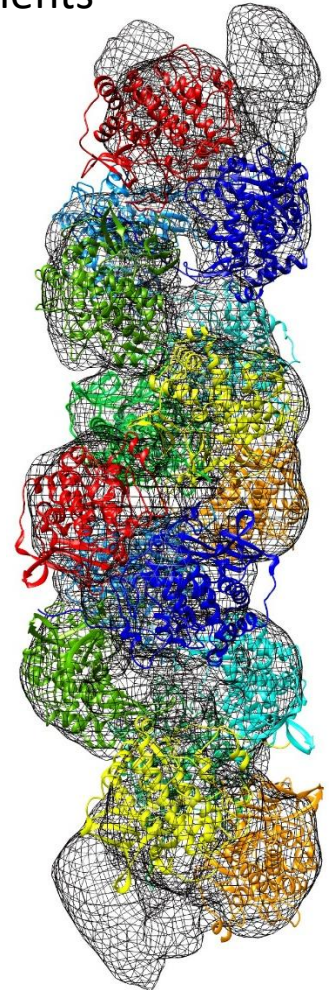
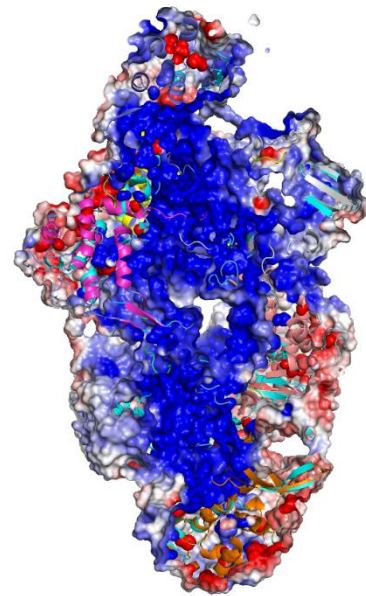
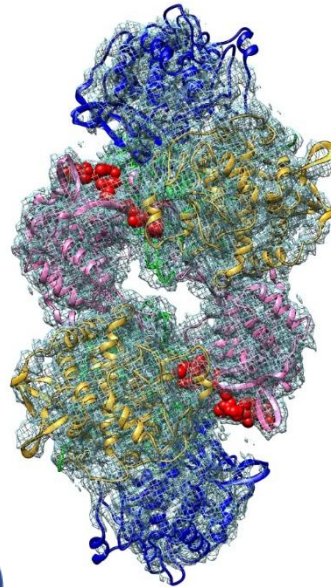
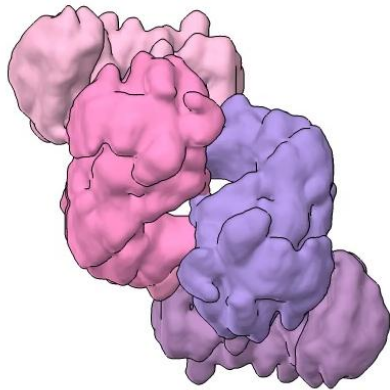
What is the molecular mechanism of RNA remodeling?

- RNA binding - stoichiometry
- Structure
- RNA secondary structure remodeling without the need of ATP hydrolysis

σ NS octamer

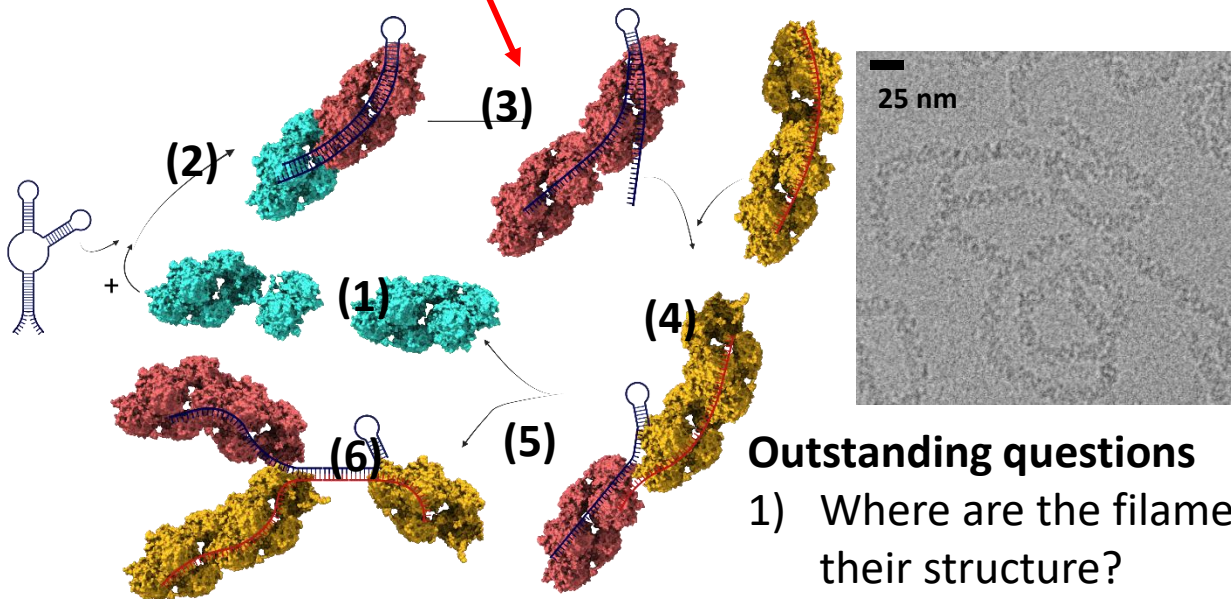
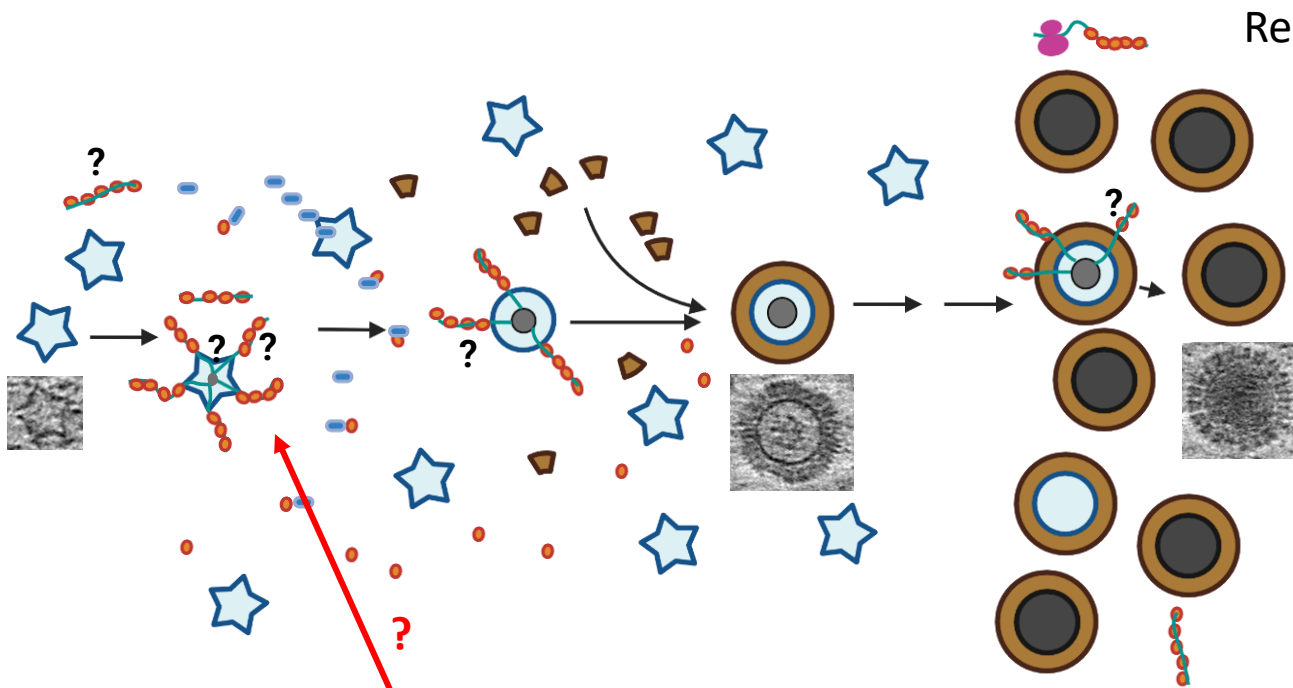
Reovirus σ NS
N-terminal domain swapped
octamer built from dimers

Octamer model reveals positively charged tunnels that bind ssRNA but are too small to accommodate dsRNA
Longer RNA promotes assembly into filaments



Single particle cryo-EM reconstruction at 4 Å
Bravo et al 2023 submitted

Reovirus assembly pathway



Outstanding questions

- 1) Where are the filaments within viroplasm and what is their structure?
- 2) Structure of the assortment complex?
- 3) Does the assortment complex trigger assembly?

Light-driven chemistry inside a phage head

Adding colours to virus assembly

David Kaftan¹, Zdeno Gardian^{1,2}, Juraj Dian³, Jakub Psencik³, Roman Tuma¹

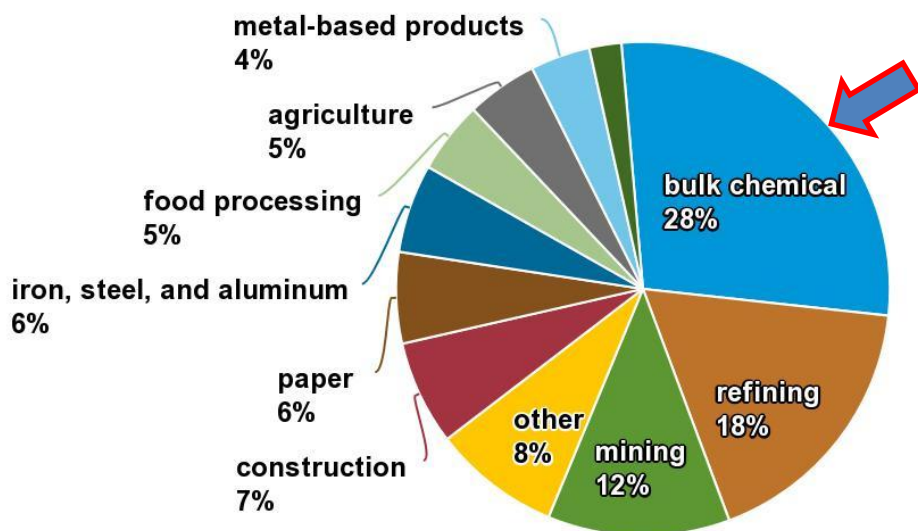
1. Faculty of Science, University of South Bohemia, Ceske Budejovice, Czechia.

2. Biology Center, Czech Academy of Sciences, Ceske Budejovice, Czechia.

3. Faculty of Mathematics and Physics, Charles University, Prague, Czechia.

Motivation – chemical industry is extremely energy demanding

U.S. industrial sector energy consumption by type of industry, 2018



Even modest improvement will have sizeable impact on the environment

Note: Includes electricity purchases and energy sources used as feedstocks for making products. *Other* includes wood products (2%), plastics products (1%), and all others (6%).

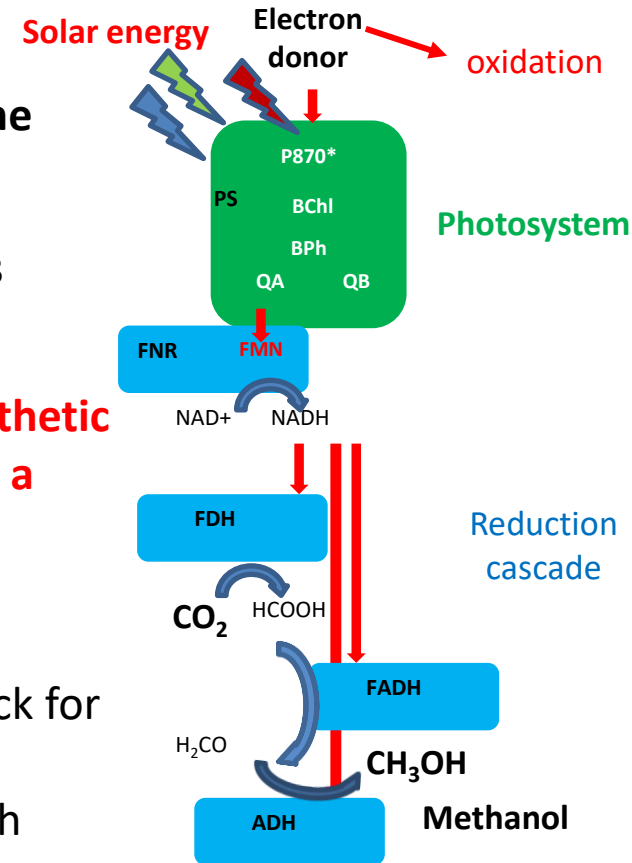
Source: U.S. Energy Information Administration, *Annual Energy Outlook 2019*, Tables 25-35, January 2019

- Most industrial chemistry employs high pressure and temperature as external driving forces to steer reactions towards desired products, resulting in **indirect coupling to the chemical reaction coordinate** and high energy consumption
- Transformation to more efficient, **directly coupled processes** would bring considerable energy savings

We propose to tap the electrochemical potential of photosynthetic reaction centre (RC) for driving redox reactions by light inside a phage head

Potential applications:

- **reduction of carbon dioxide** to methanol - fuel and feedstock for synthetic reactions.
- **reduction of N_2 to NH_3** (nitrogenase complex) – Haber Bosch process replacement (used since 1910)



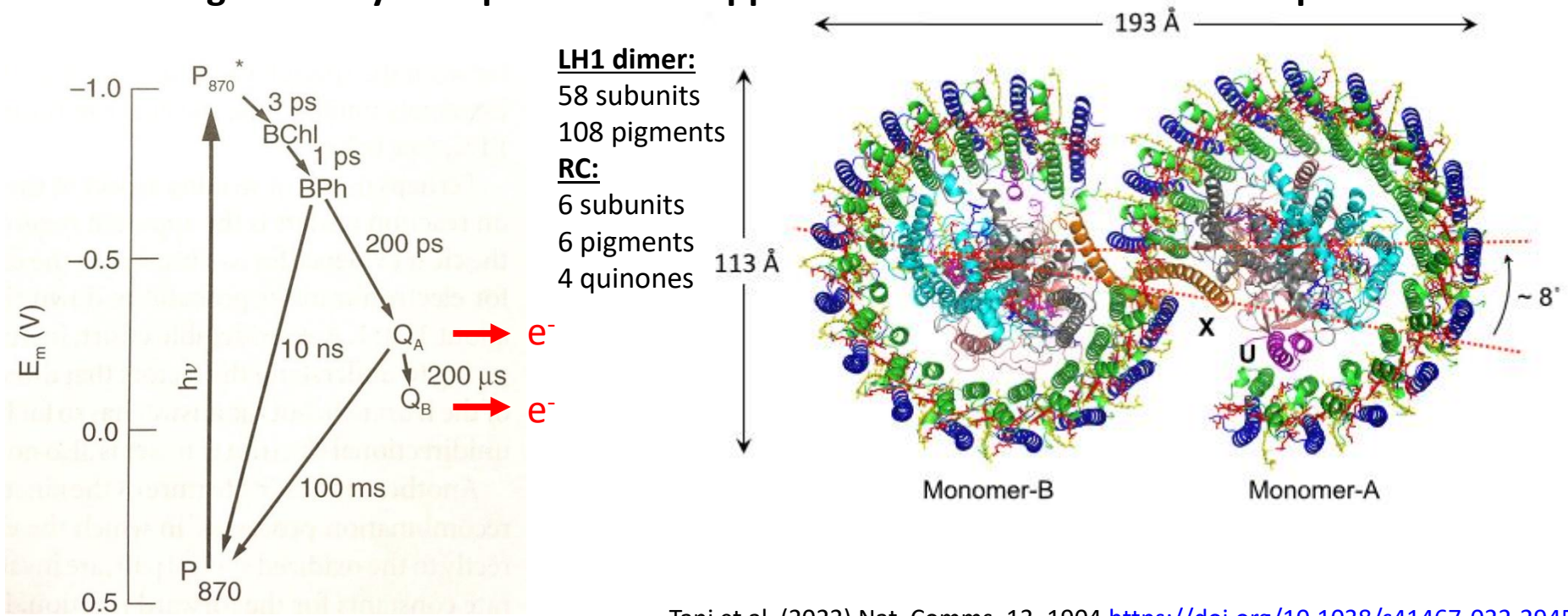


If a molecule or atom is oxidized what happens?

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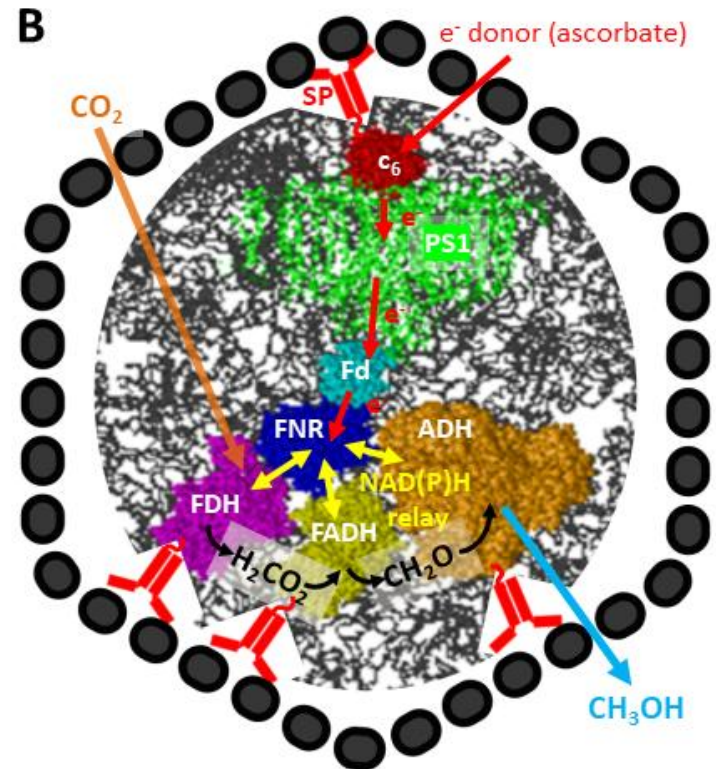
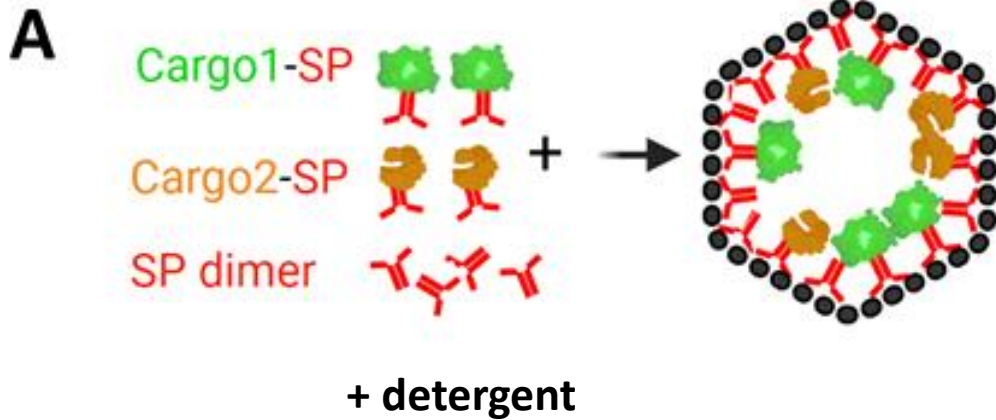
Photosystem from *Rhodobacter sphaeroides* contains reaction centre (RC) enclosed within a belt of light harvesting subunits (LH1) – two-for-one package

- His-tagged, high yield of stable dimers, remains functional in detergent
- can be genetically manipulated and tapped at different electrochemical potentials



Tani et al. (2022) Nat. Comms. 13, 1904 <https://doi.org/10.1038/s41467-022-29453-8>.
Jun et al. (2019) Photosynth Res 137, 227-39. doi: 10.1007/s11120-018-0493-0.

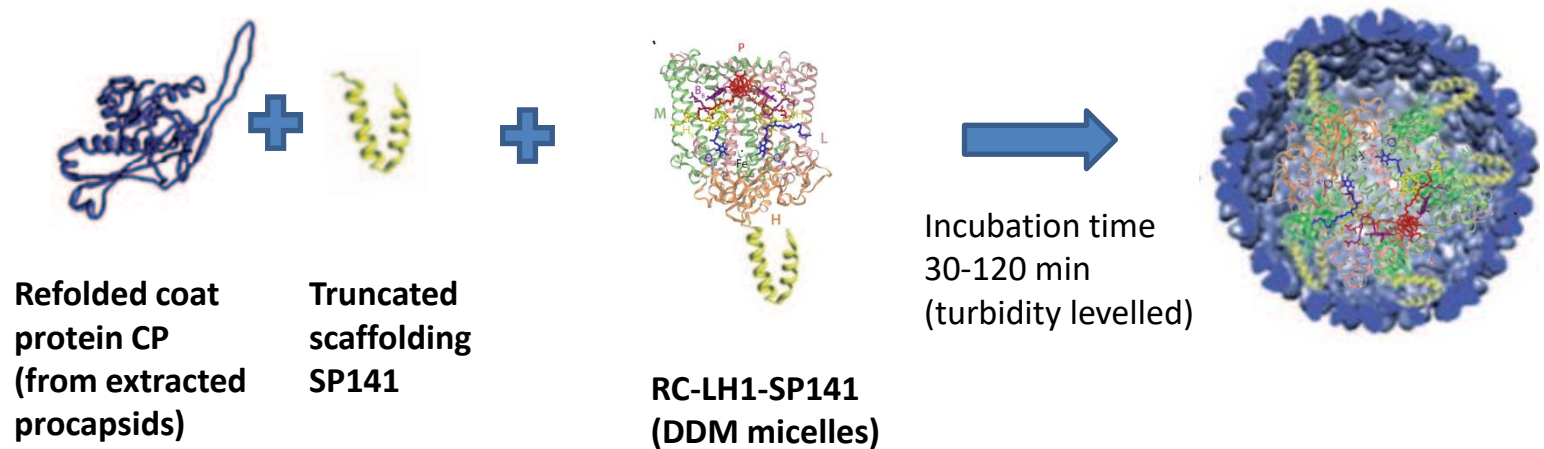
Nanocontainer for efficient photochemical reduction pathway



In vitro assembly optimization

Initial parameters for optimization

- 1) CP:SP141:RC-LH1-SP141 ratio
- 2) Order of addition
- 3) Absolute concentrations – limited by CP refolding efficiency (10 - 20 μM)



Assembly reactions contain 0.03% n-dodecyl- β -D-maltopyranoside (DDM) to keep RC-LH1 in solution

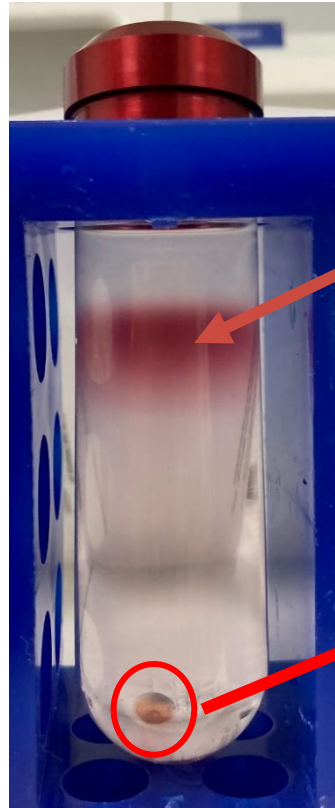
Potential applications are sample hungry – scaling up the preparation

Bucket biochemistry for purple phage (procapsids)

1. Assembly started by mixing the components in optimal ratios and volume scaled up to 6 ml
2. After 60 min the reaction was put on Amicon 10,000 MWCO filter and concentrated down to 0.5 ml
3. Layered on top of 10% sucrose cushion (with DDM) and centrifuged
4. Pellet resuspended in Tris buffer (no DDM)
5. Electron microscopy & SEC-MALLS

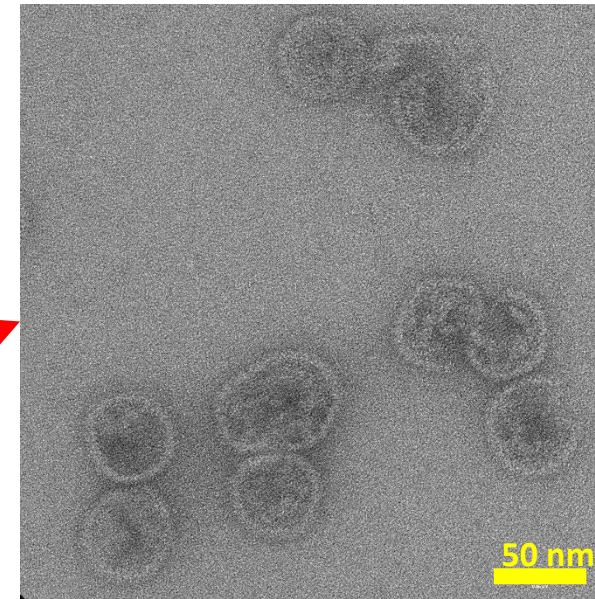
↓

Electrochemistry and fast spectroscopy
Structural biology, proteomics



Free RC-LH1-SP141
(recycled)

Negative stain EM



Opportunities for projects (applied biochemistry, bio-nanotechnology, chemical physics):

- 1) Preparation and assembly of redox enzymes and photosystems
- 2) Characterization of redox reactions catalyzed by virus based nanocontainers

Thank you for listening

Acknowledgments

University of South Bohemia:

David Kaftan – photosystems

Šárka Petáková – technical support

Guy Wolf – project student

Zdeno Gardian, Tomáš Bílý

– electron microscopy

Filip Dyčka, Peter Koník- MS facility

Štěpánka Tomková

Zdeněk Franta

Tom Fessl

Charles University:

Juraj Dian Jakub Pšenčík -

electrochemistry and

photophysics

Peter Mojzeš – Raman imaging

University of British Columbia:

Thomas Beatty

University of Oxford:

Alex Borodavka

University of Leeds:

Louie Aspinnall

