Nanocarriers: From Theory to Practice

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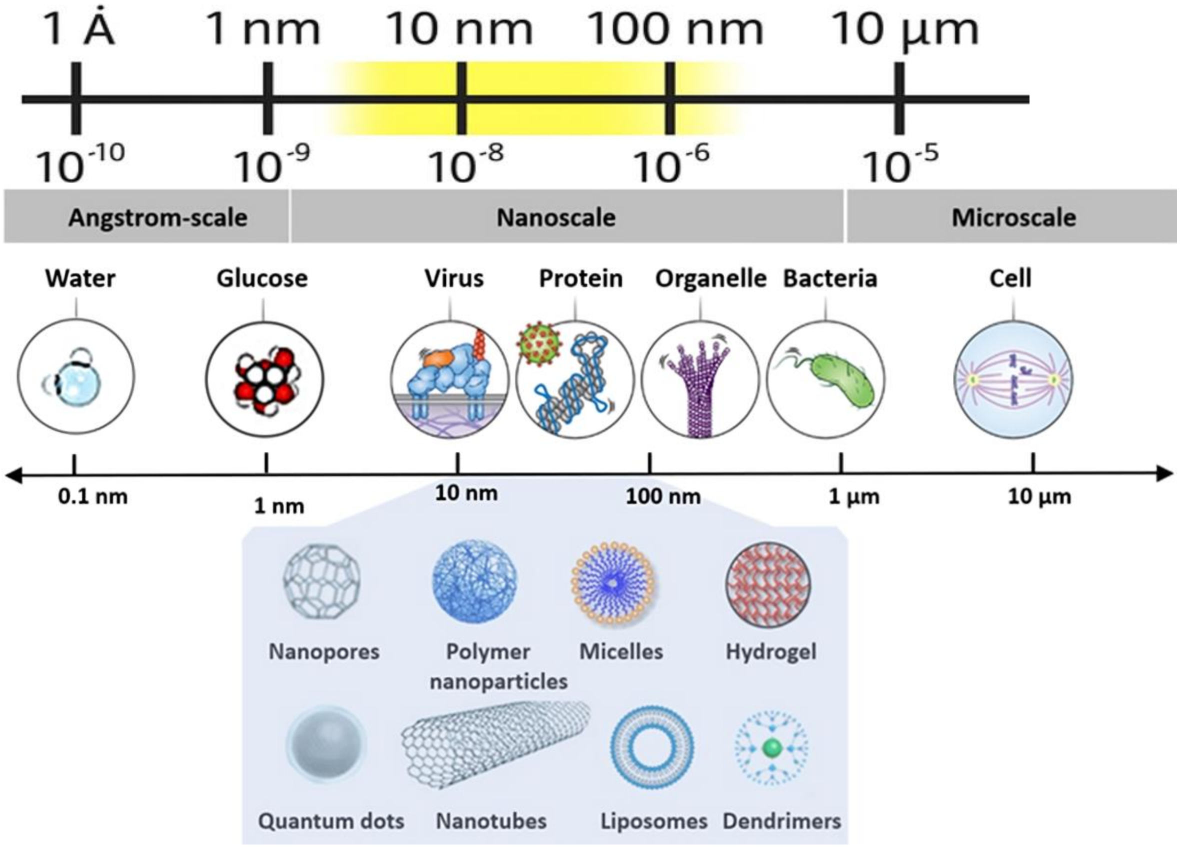
What Are Nanocarriers?

- Tiny delivery systems (1–1000 nm)
- Transport active ingredients (e.g., drugs, fertilisers, pesticides)
- Improve stability, uptake, and targeting
- Allow controlled or sustained release

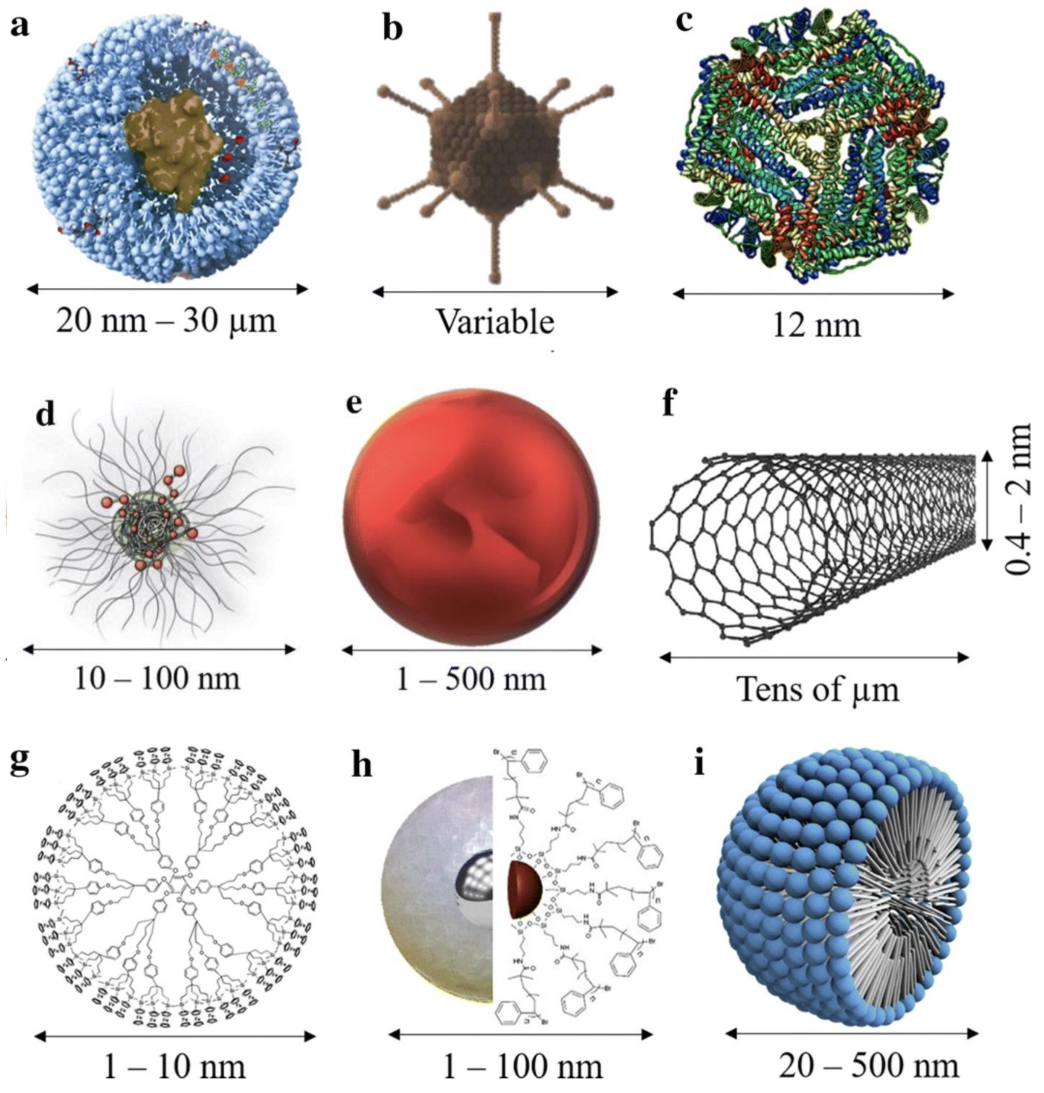
How small is nanoscale small?



Adapted from Yicong Wu and Hari Shroff, *Histochemistry and Cell Biology*, 2022.

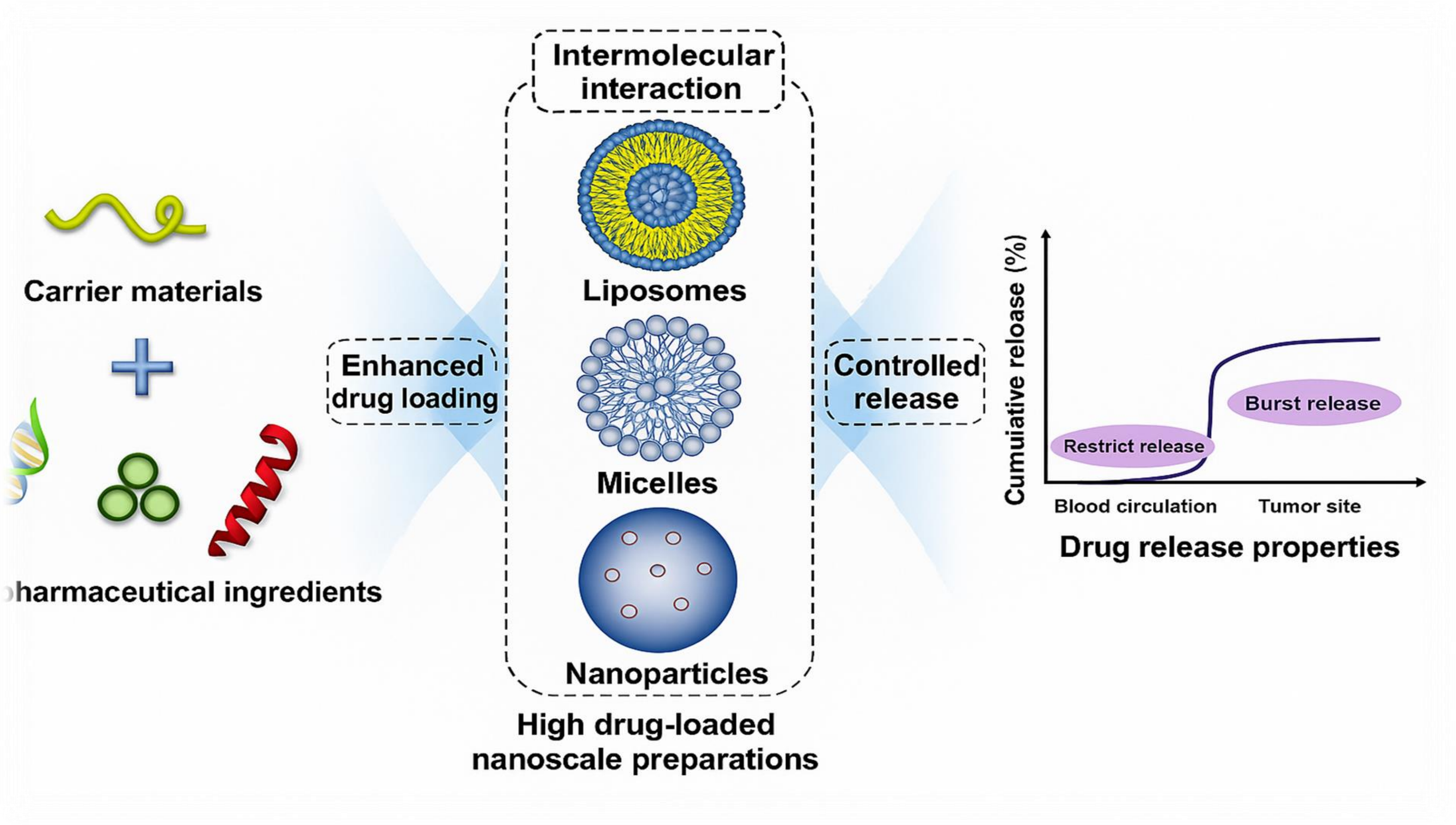
Nanoparticles commonly used for drug delivery in mammalian systems

- Liposomes
- Viral vectors/Virus-Like Particles
- Self-assembled proteins: ribbon diagram representing the structure of the ferritin protein.
- Polymeric nanoparticle
- Metallic nanoparticle
- Single-walled carbon nanotube
- Astruc's 54-ferrocene dendrimer
- Polystyrene-coated magnetic NPs with core/shell structure
- Micelle formulation as a drug delivery system



Cancer Nano **10**, 11 (2019). https://doi.org/10.1186/s12645-019-0055-y

How Nanocarriers Work: Structure, Cargo, and Release Profile



Virus-Like Particles as Nanocarriers

Reducing Pesticide Usage with VLP Protein Cages,

VLPs as enzyme systems and many more applications…………..

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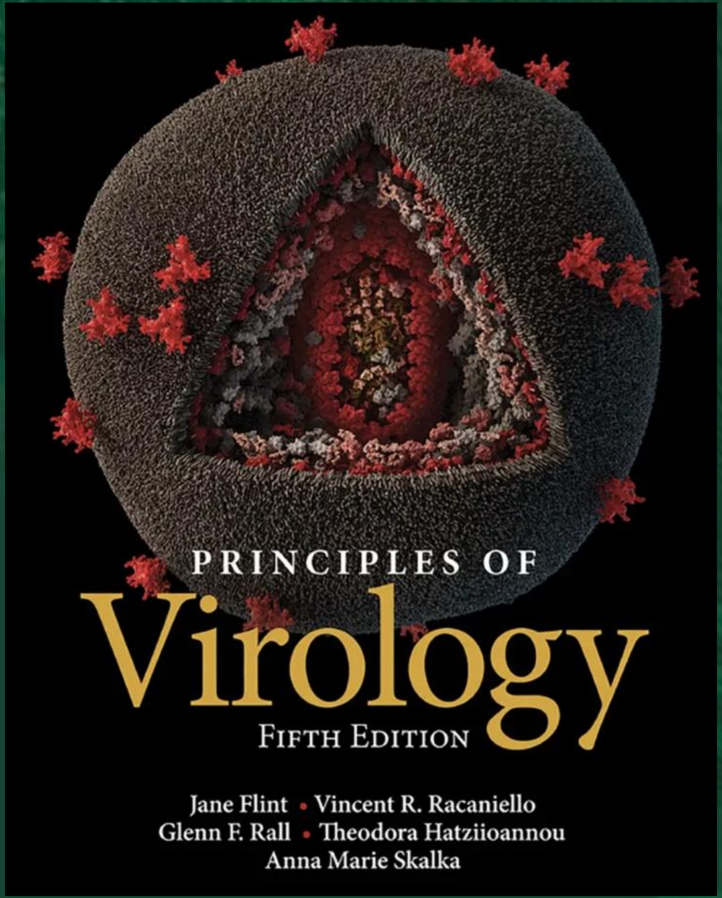
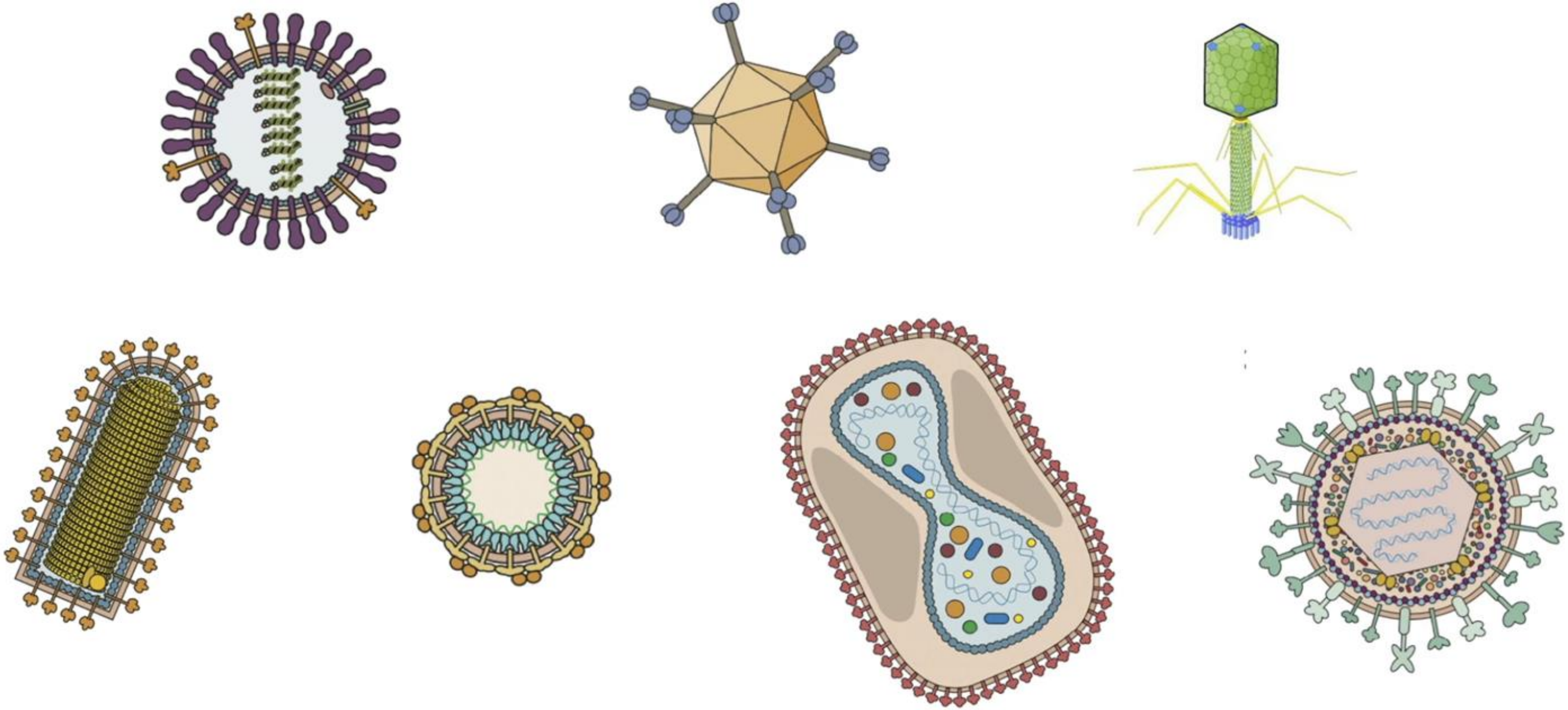
Structure of Viruses



To create something that functions properly- A container, a chair, or a house – its essence has to be explored, for it should serve its purpose to perfection i.e., it should be durable, inexpensive and beautiful – Walter Gropius

What are the functions of structural proteins of virus particles?



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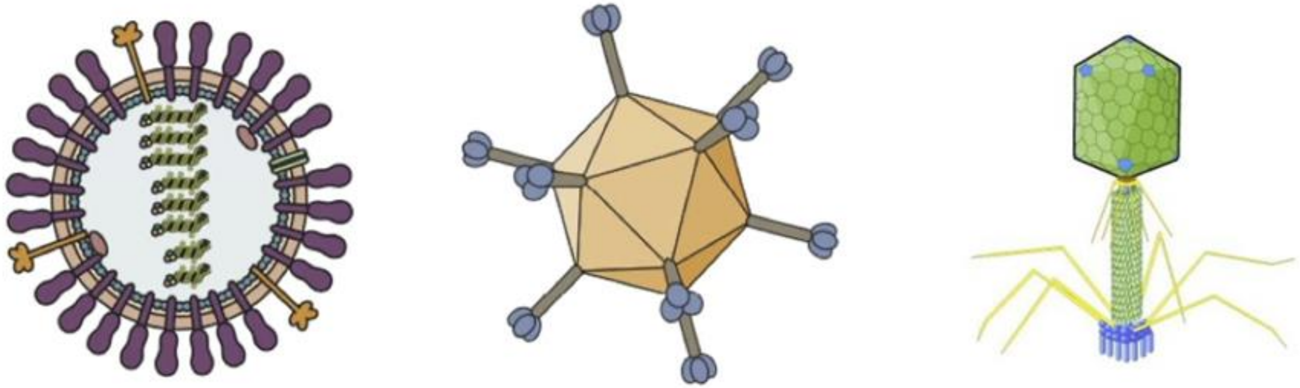
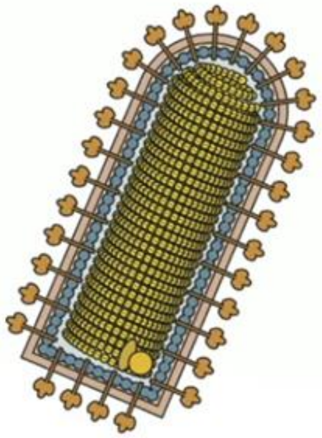
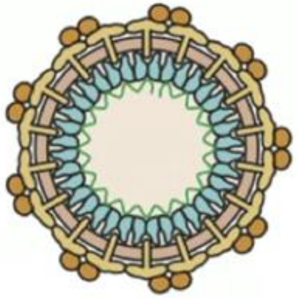


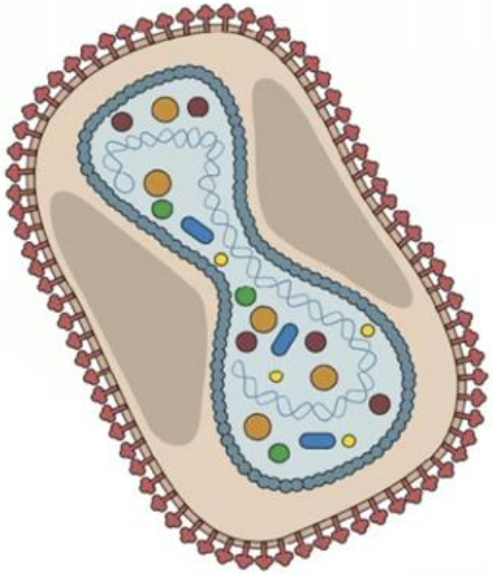
Protection of the genome

- Assembly of a stable protective protein shell
- Specific recognition and packaging of the nucleic acid genome
- Interaction with host cell membranes to form the envelope

What are the functions of structural proteins of virus particles?





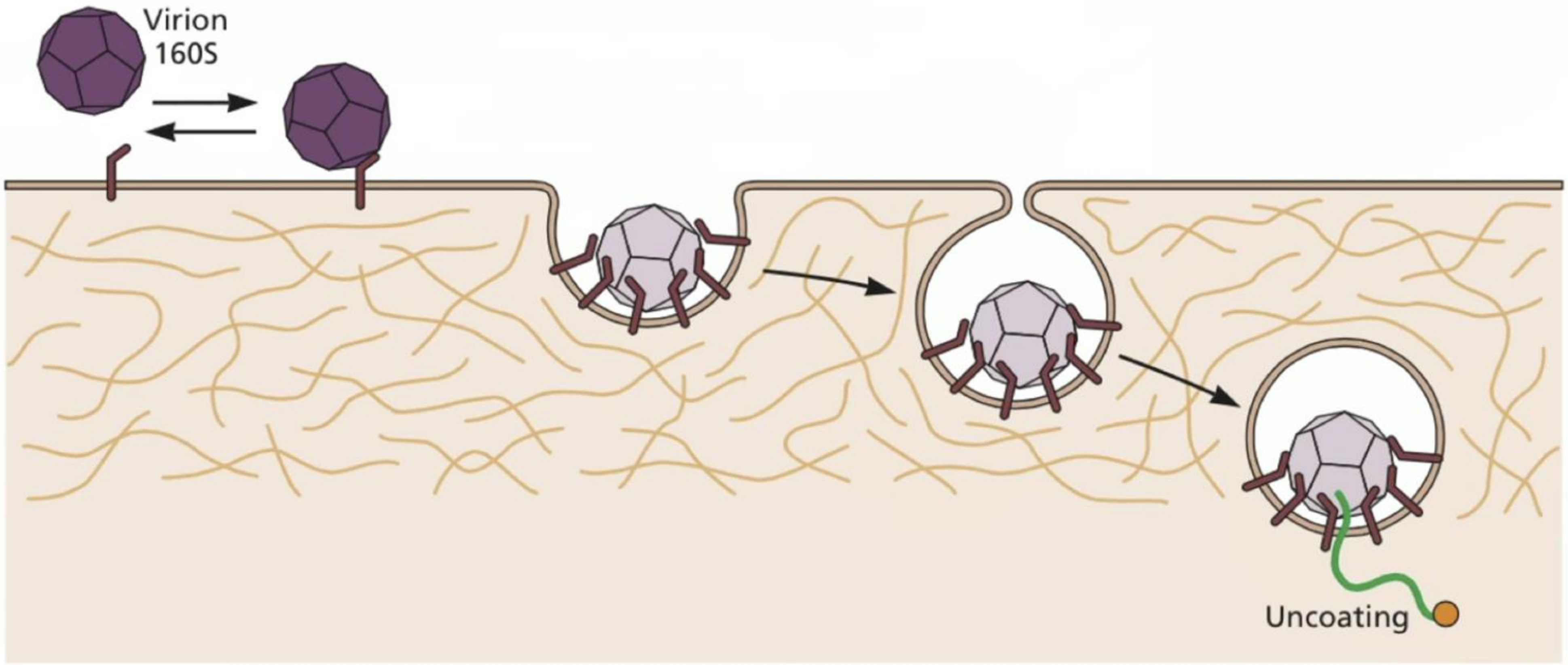


Delivery of the genome

- Bind host cell receptors
- Uncoating of the genome
- Fusion with cell membranes
- Transport of genome to the appropriate site

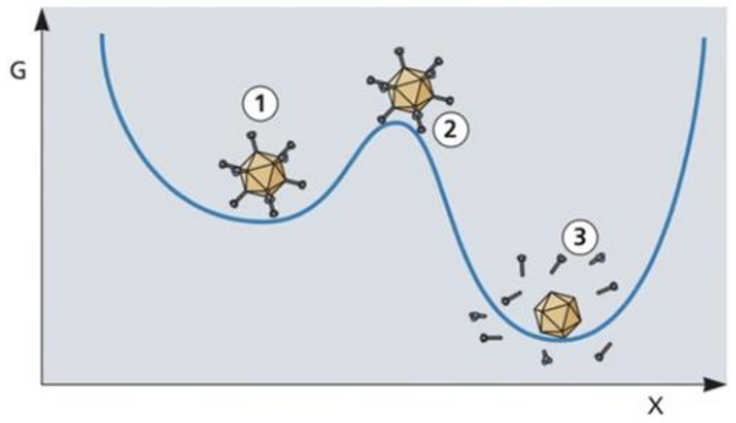
Virus particles are metastable

- Must protect the genome (stable)
- Must come apart on infection (unstable)



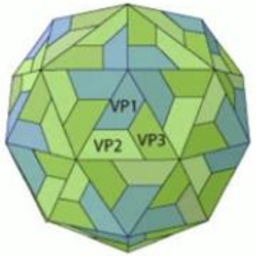
Virus particles are metastable

- Virus particles have not attained minimum free energy conformation
- Unfavorable energy barrier must be surmounted



- Energy put into virus particle during assembly (*spring loaded*)
- Potential energy used for disassembly if cell provides proper signal

How is metastability achieved?

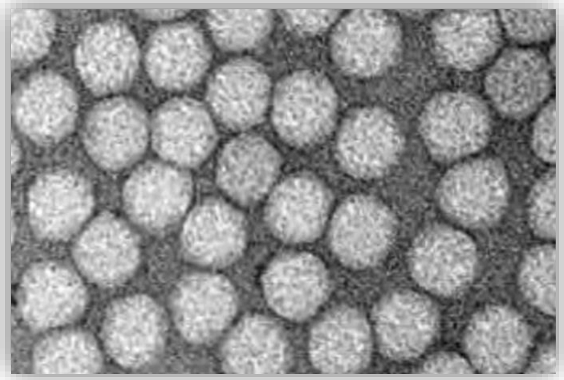


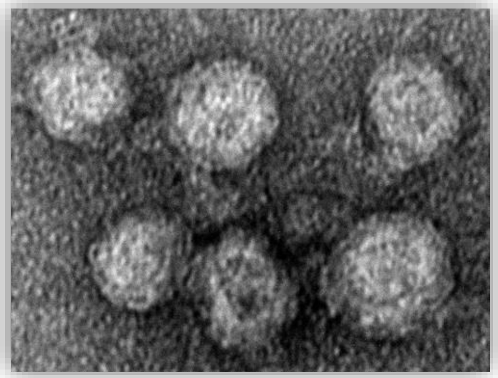
- *Stable structure*
  - Created by symmetrical arrangement of many identical proteins to provide maximal contact
- *Unstable structure*
  - Structure is not usually permanently bonded together
  - Can be taken apart or loosened on infection to release or expose genome

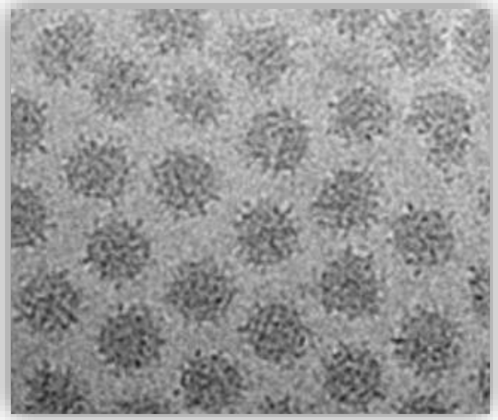
Electron microscopy

- Biological materials have little inherent contrast: need to be stained
- Negative staining with electron-dense material (uranyl acetate, phosphotungstate), scatter electrons (1959)
- Resolution 50-75 Å (alpha helix 10 Å dia; 1 Å = 0.1 nm)

Detailed structural interpretation impossible



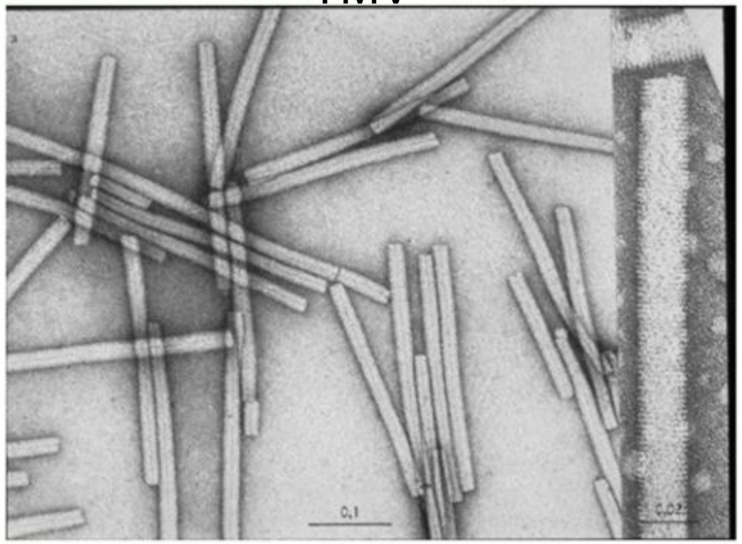


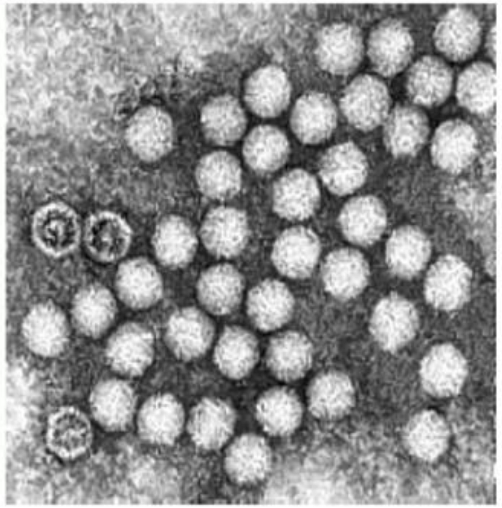


Building virus particles: Symmetry is key

- Watson and Crick did more than discover DNA structure
- Their seminal contribution to structural virology:
  - Noted that most virus particles were spherical or rod-shaped
  - Idea: as virus genomes are small (!) particles would be built with many copies of a few proteins (genetic economy)
  - Identical protein subunits are distributed with *helical symmetry* for rod-shaped viruses
  - *Icosahedral symmetry* for round viruses

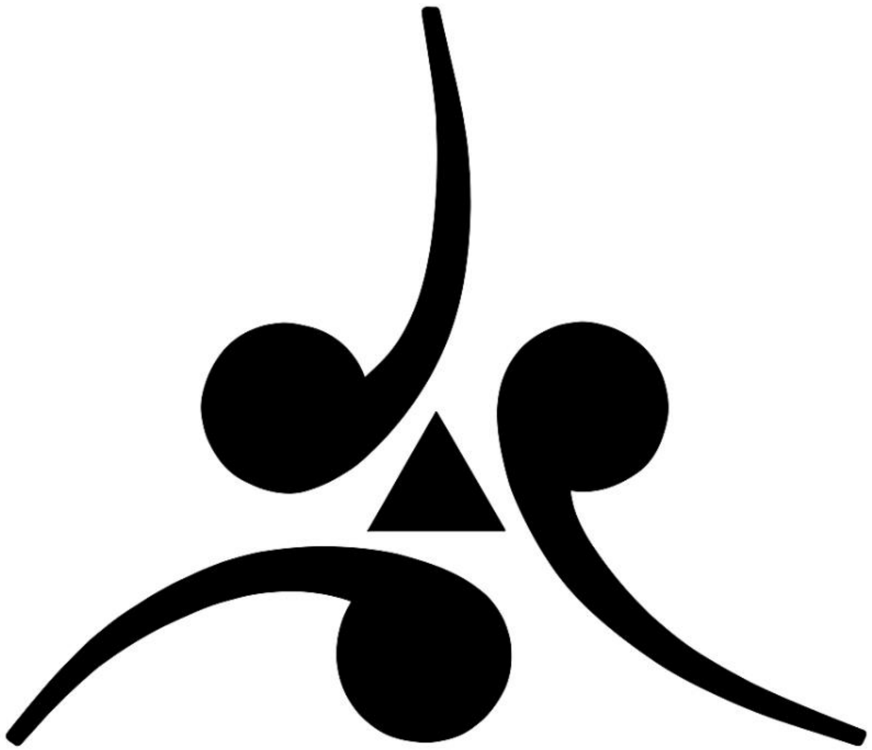
TMV





Polioviru

What is Symmetry



Basic 3-fold Axis

Repetition of Identical Units

- During capsid formation, each protein subunit forms the same interactions with its neighbours.
- This uniform pattern ensures that when many copies come together, they naturally fit into a symmetric, closed shell, like an icosahedron.

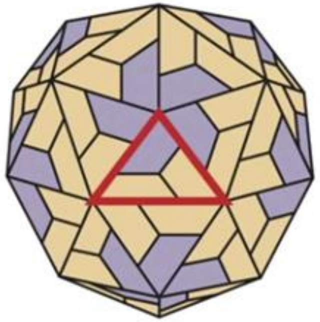
Energetic Favourability Through Symmetry

- Symmetrical assemblies allow multiple pattern-matching contacts with minimal genetic complexity.
- Instead of having different proteins for every corner or edge, **viruses** use one type of subunit that repeats itself to create a stable, highly ordered structure.

There are rules for this SYMMETRY….

The symmetry rules are elegant in their simplicity

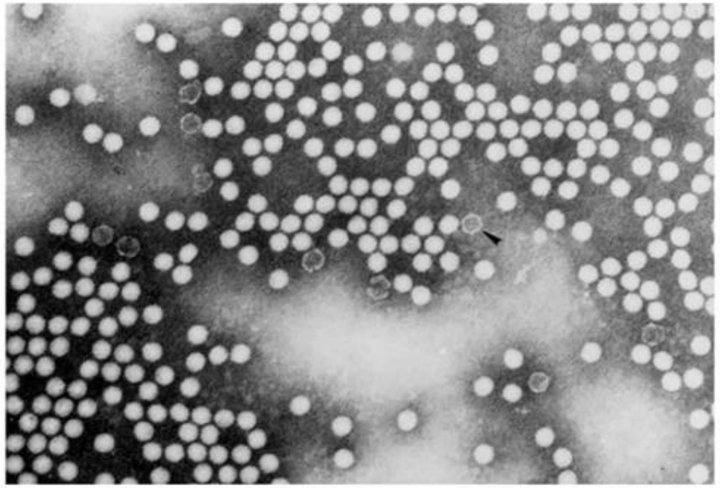
They provide rules for "self-assembly"

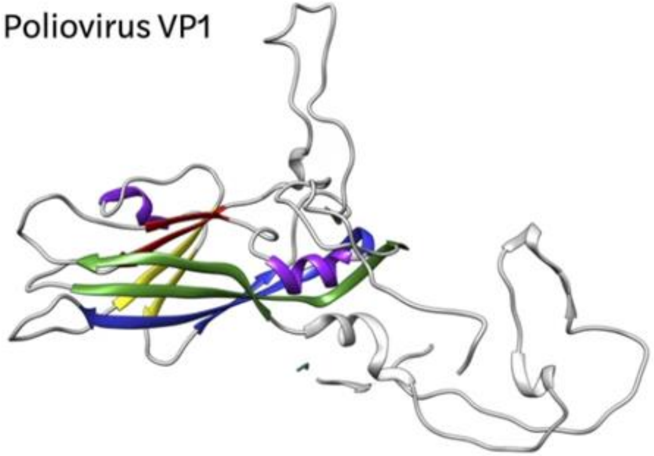


- **Rule 1:** Each subunit has 'identical' bonding contacts with its neighbors
  - Repeated interaction of chemically complementary surfaces at the subunit interfaces naturally leads to a symmetric arrangement
- **Rule 2:** These bonding contacts are usually non-covalent
  - Reversible; error-free assembly

How can you make a round capsid from proteins with irregular shapes?

TEM Polio virus

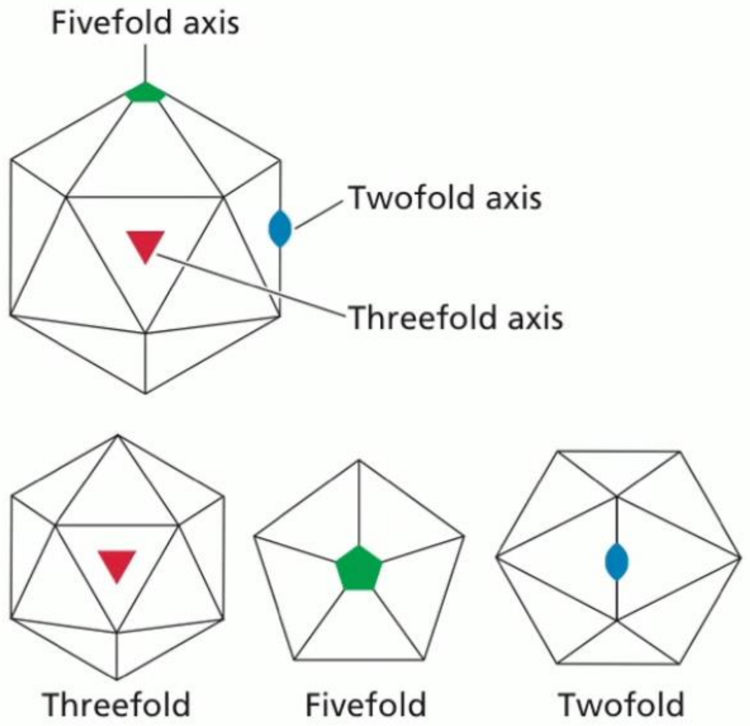




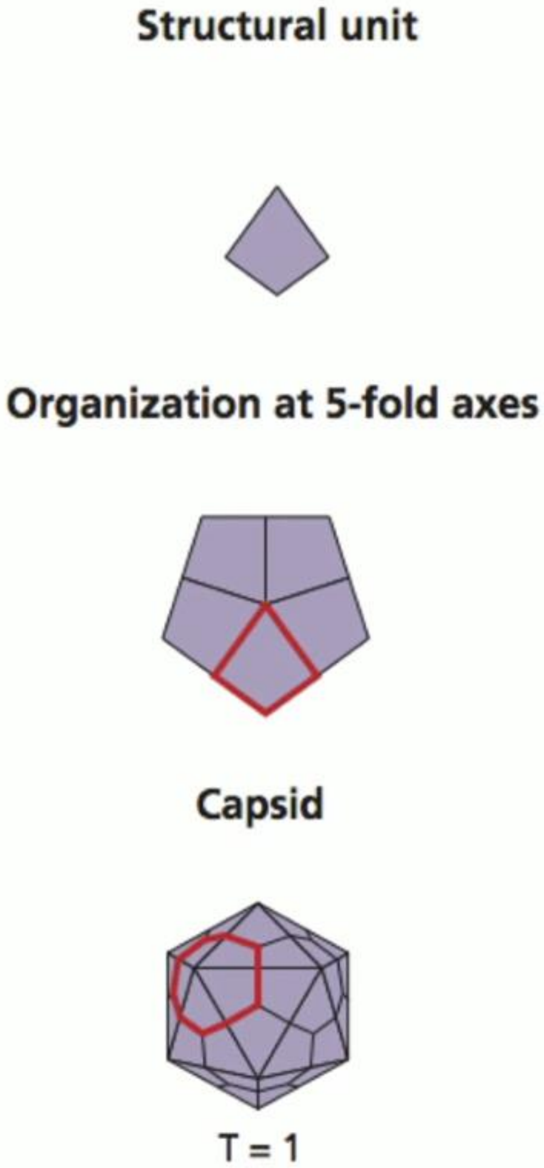
- **Clue 1:** All round capsids have precise numbers of proteins; multiples of 60 are common (60, 180, 240, 960)
- **Clue 2:** Spherical viruses come in many sizes, but capsid proteins are 20-60 kDa average
- Watson & Crick concluded that these are built with *icosahedral symmetry*

Icosahedral symmetry

- Icosahedron: solid with 20 faces, each an equilateral triangle
- 5x, 3x, 2x axes of symmetry (12 each)
- Allows formation of a closed shell with smallest number (60) of identical subunits



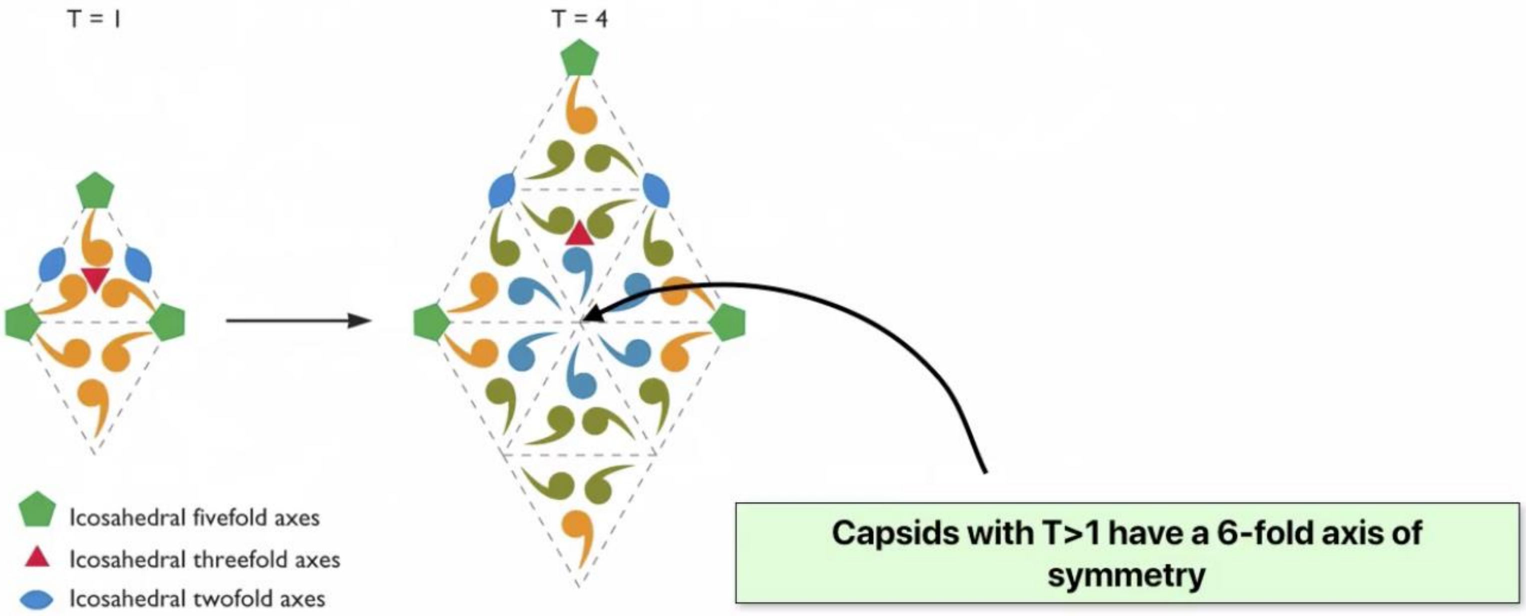
Simple icosahedral capsids



- 60 structural units made of one protein
- Total of 60 identical protein subunits
- Interactions of all molecules with their neighbors are identical
- The particles are spherical, not icosahedra!

Triangulation number, T

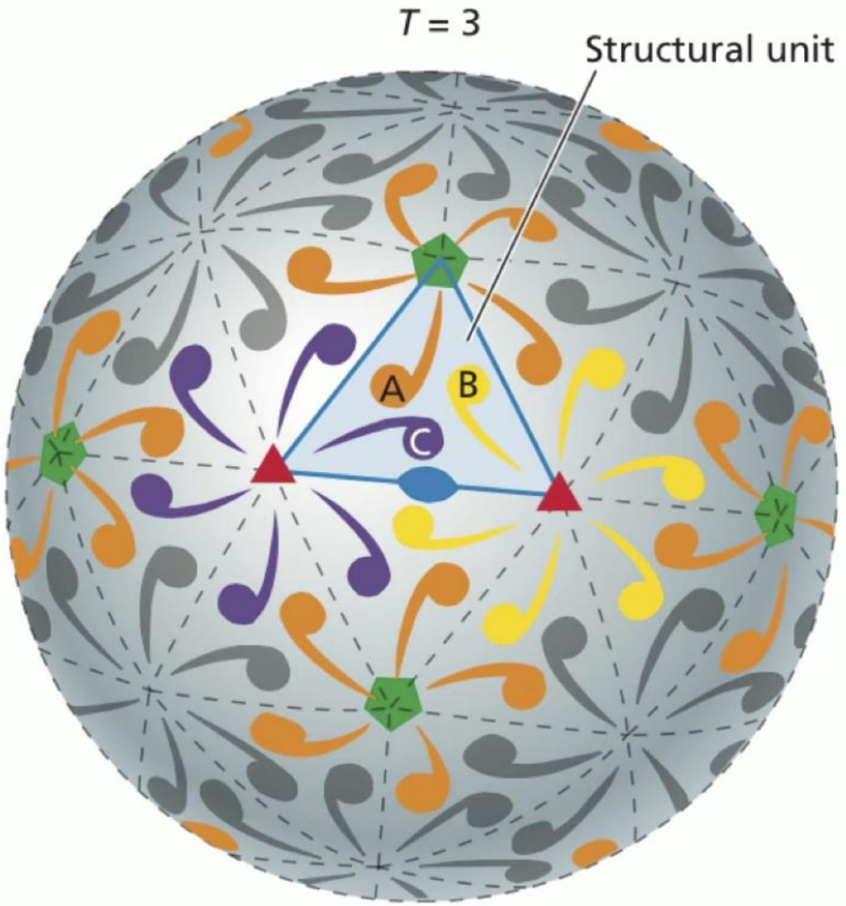
- The number of facets per triangular face of an icosahedron
- Combining several triangular facets allows assembly of larger face from same structural unit



How do you make bigger Viruses?

Quasiequivalence

- When a capsid contains more than 60 subunits, each occupies a *quasiequivalent* position
- The noncovalent binding properties of subunits in different structural environments are similar, but not identical

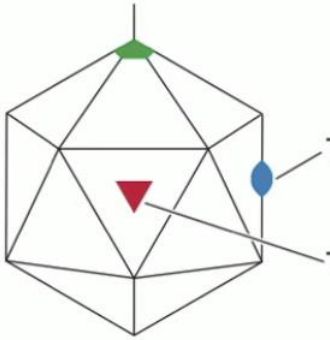


Viral capsid proteins are arranged in nearly identical chemical environments, which is known as ***quasi-equivalence.***

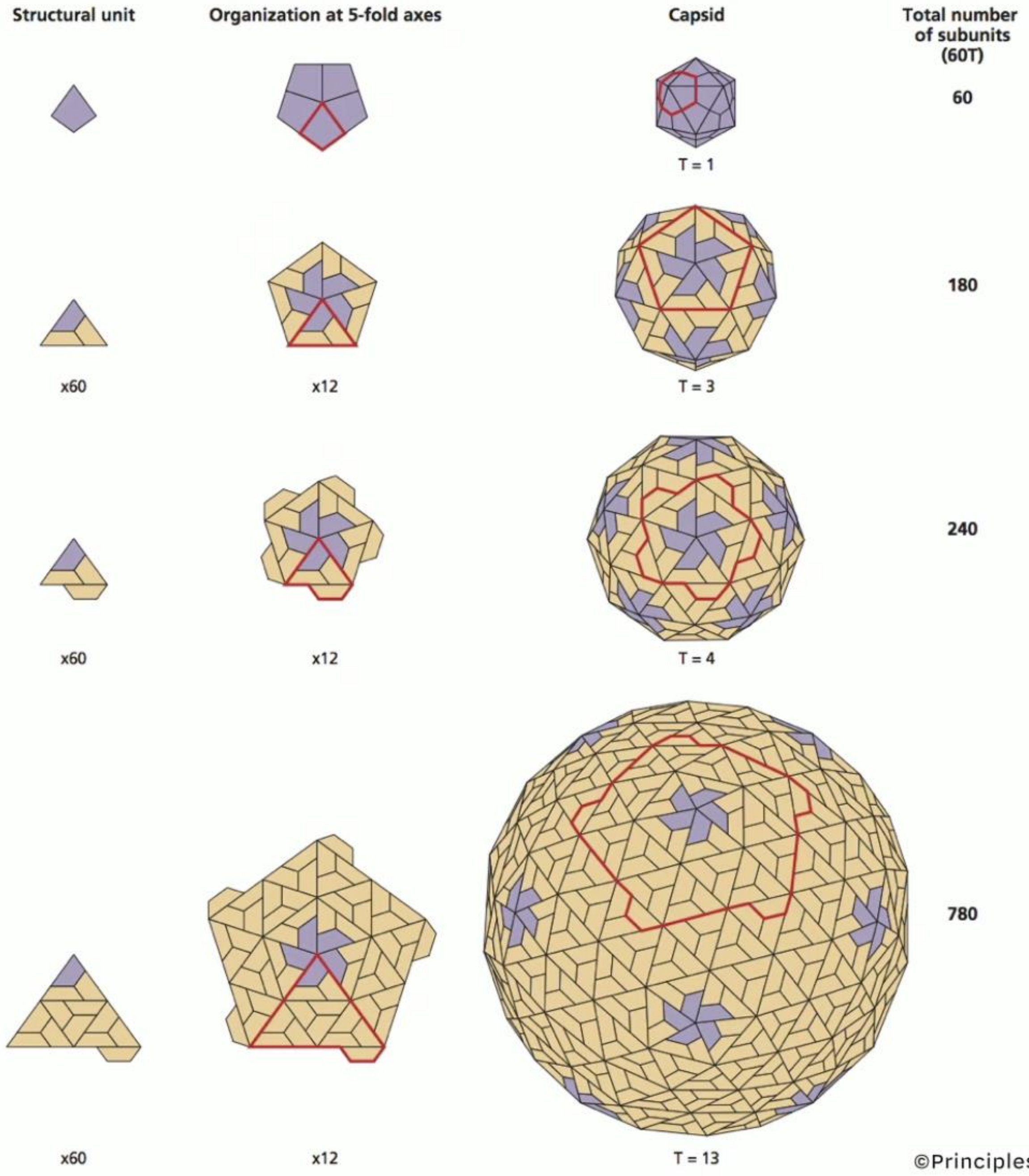
Viruses would not make bigger proteins but make more subunits per particle

T, triangulation number

The number of subunits comprising the structural unit



Capsids with T>1 have a 6-fold axis of symmetry

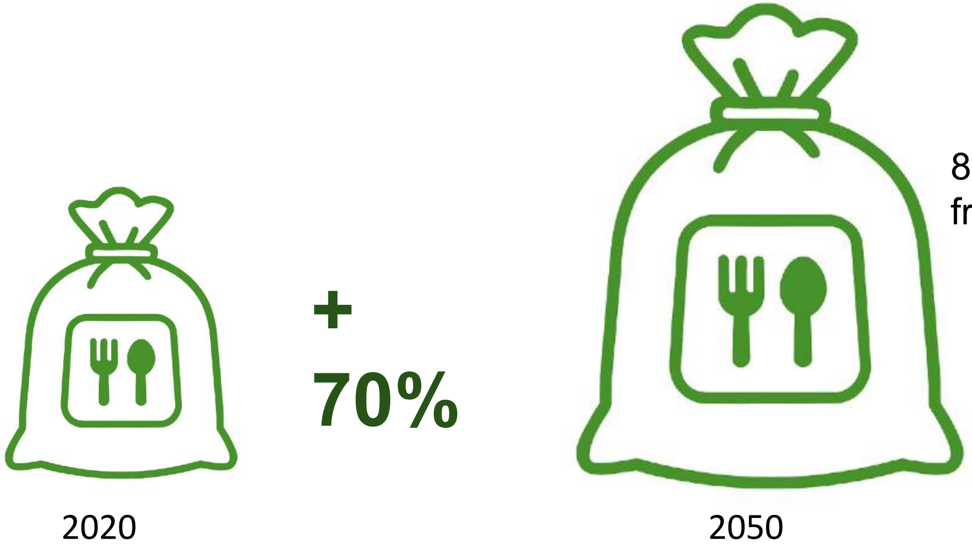


How are we applying this knowledge of structural virology in Agriculture?

VLPs as Nano Carriers in Spray-Induced Gene Silencing (SIGS)

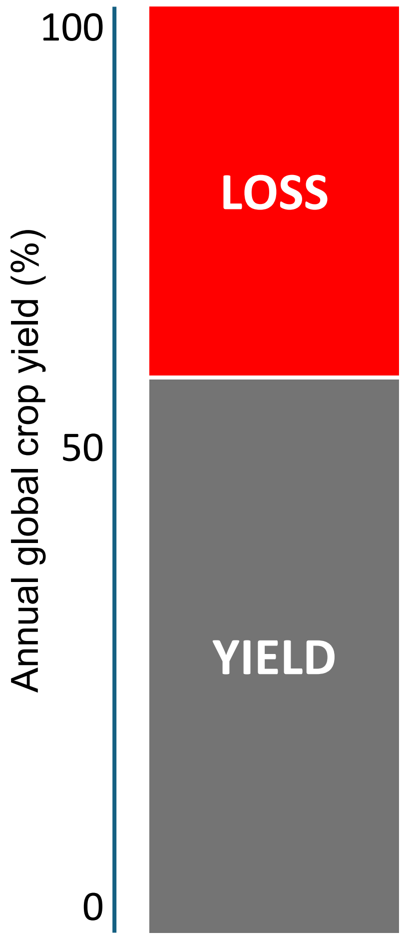
1 **Rising global food demand**

2 **Crop diseases & pests threaten yields**



80% of this increase must come from plant products

50-70% more food required by 2050
-To feed nearly 10 billion people



Up to 40% of global crop yields are lost annually to pests and diseases, costing over $220 billion

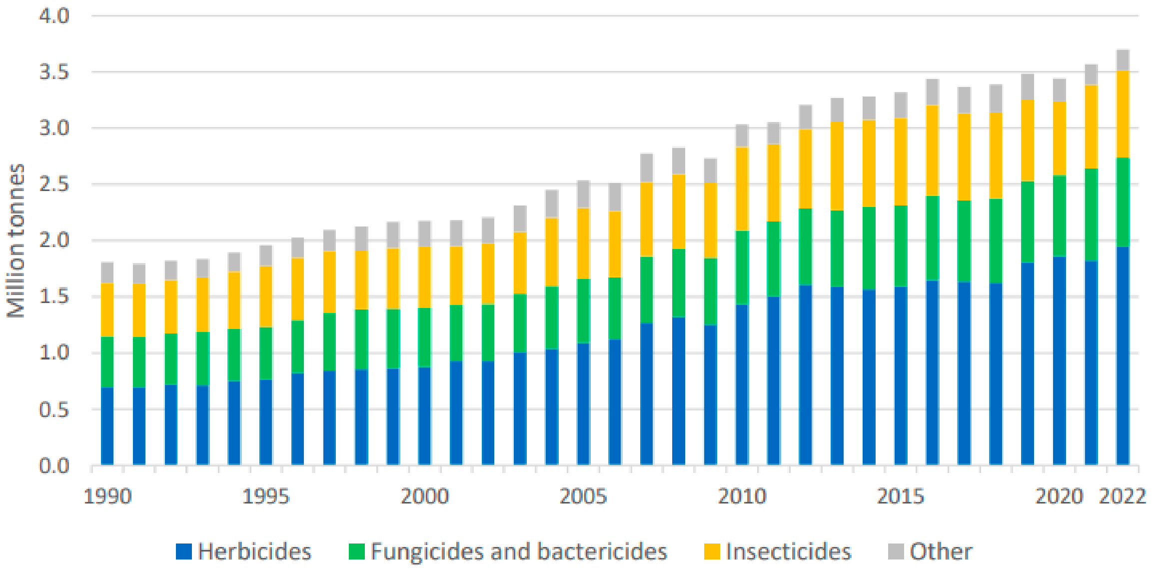
1 **Rising global food demand**

2 **Crop diseases & pests threaten yields**

3 **Heavy reliance on chemical pesticides**

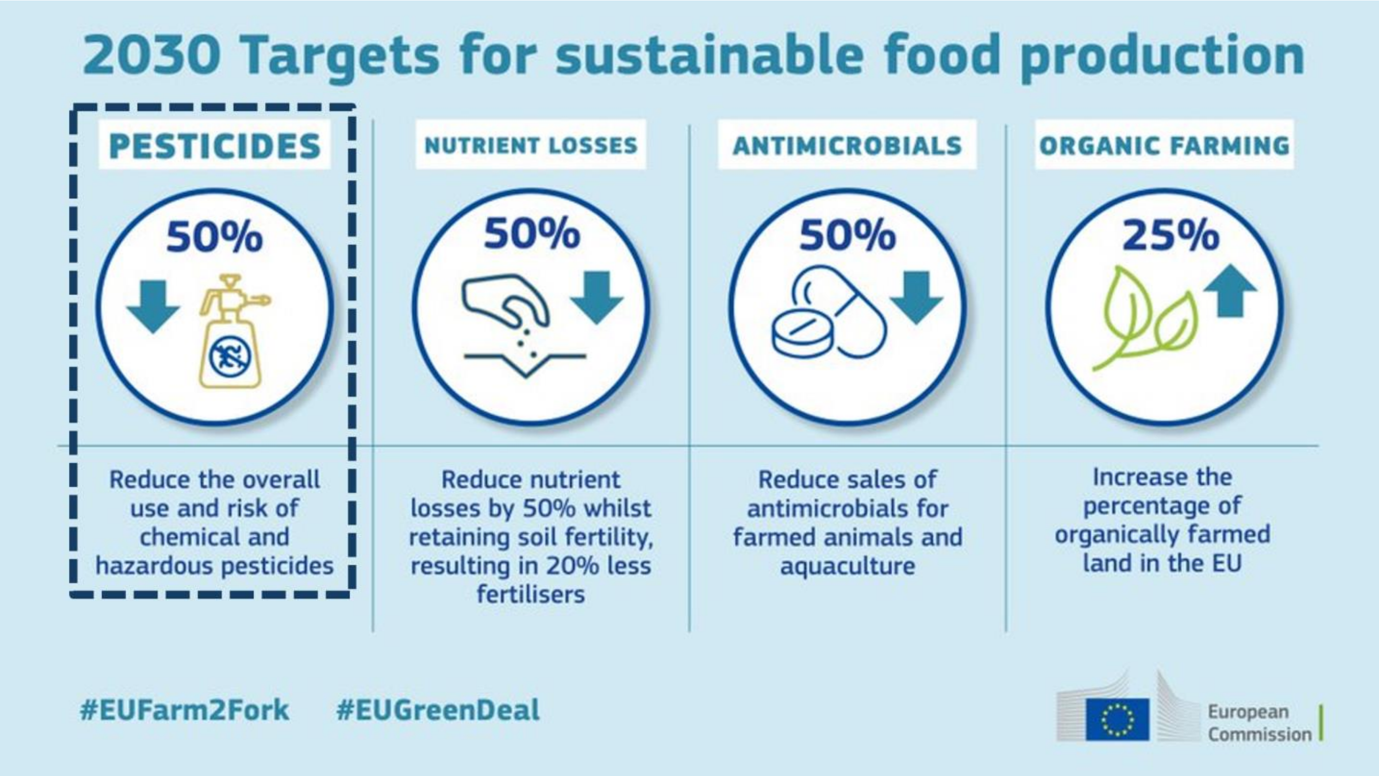
4 **Breeding is time-consuming and limited**

5 **Pathogen evolution challenges control**



Source:FAOSTAT

Global pesticide use has risen significantly since 1990, now reaching nearly 4 million tonnes annually



6 **Sustainable solutions needed**

- ❑ EU Directive 2009/128/EC : Sustainable use of pesticides

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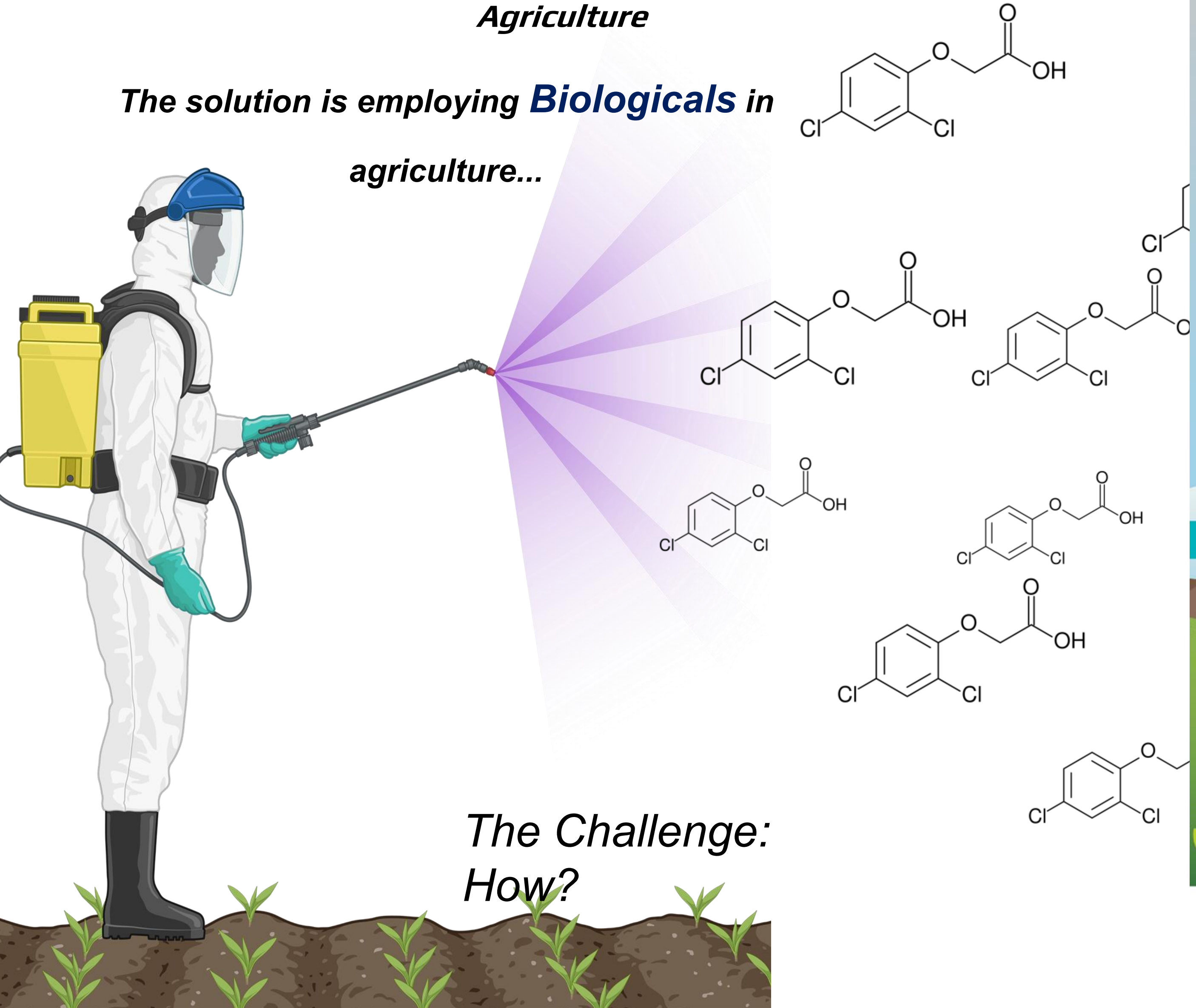
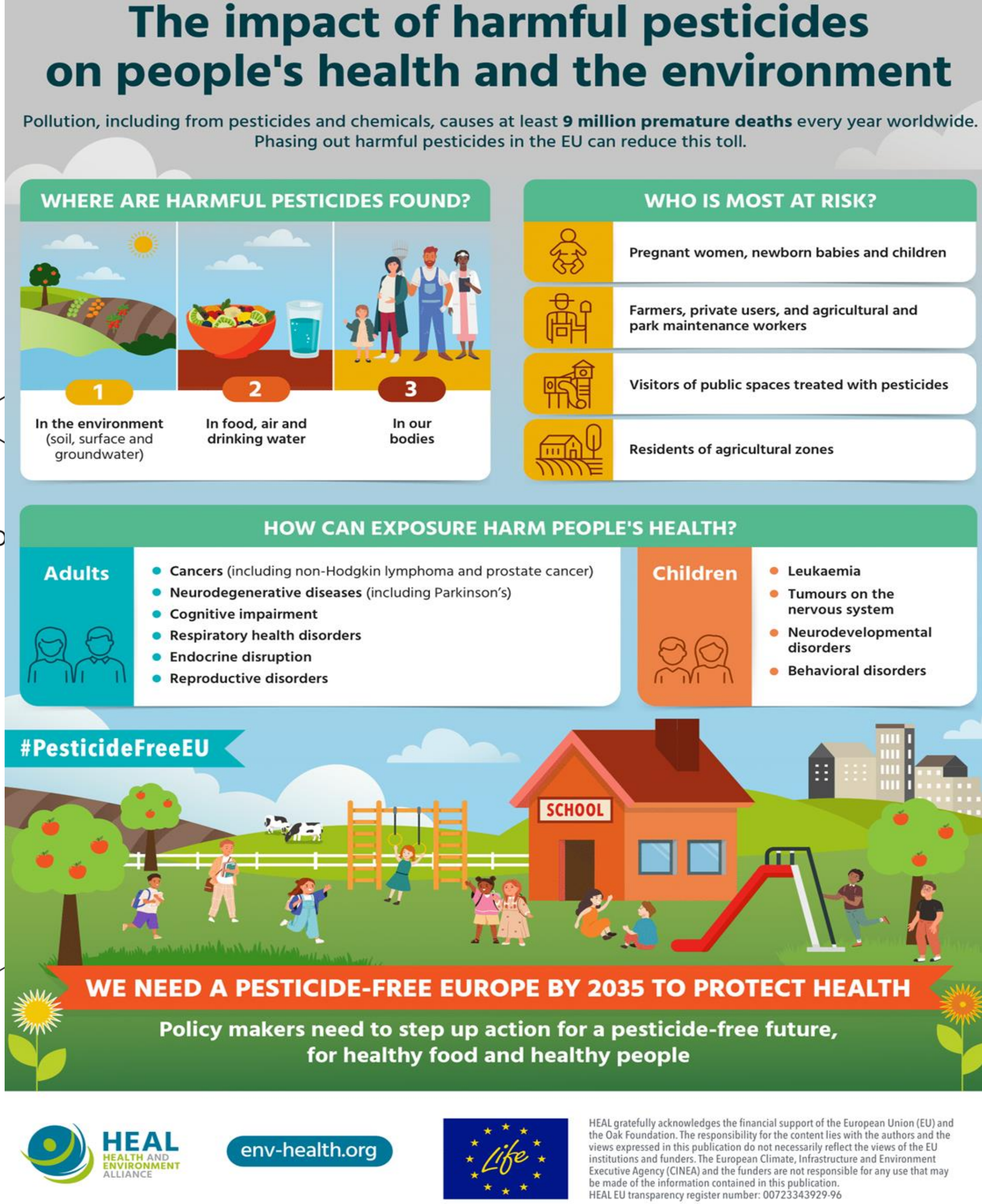
The problem is Synthetic Pesticide application in Agriculture

The solution is employing ***Biologicals*** *in agriculture...*



The Challenge: How?



Target pathosystem: Potato and late blight

- Potato (*Solanum tuberosum*)
  - Global staple crop
  - Key role in global food security

- Late blight
  - Oomycete, *Phytophthora infestans*
  - Plant wilting, stem lesions & tuber rot
  - Irish famine in the 19th century

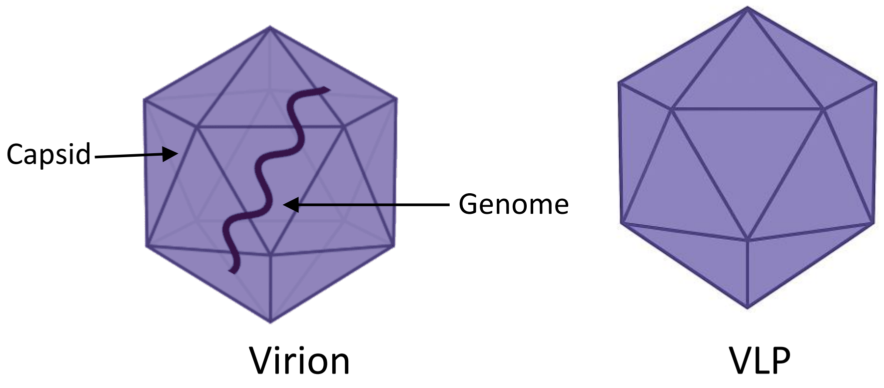
Source: NDSU

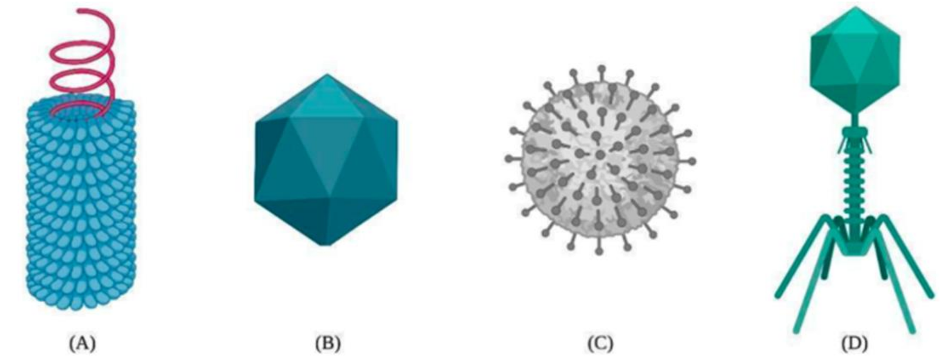
Source: Bayer crop science

Virus-like particles (VLPs) as delivery vehicles

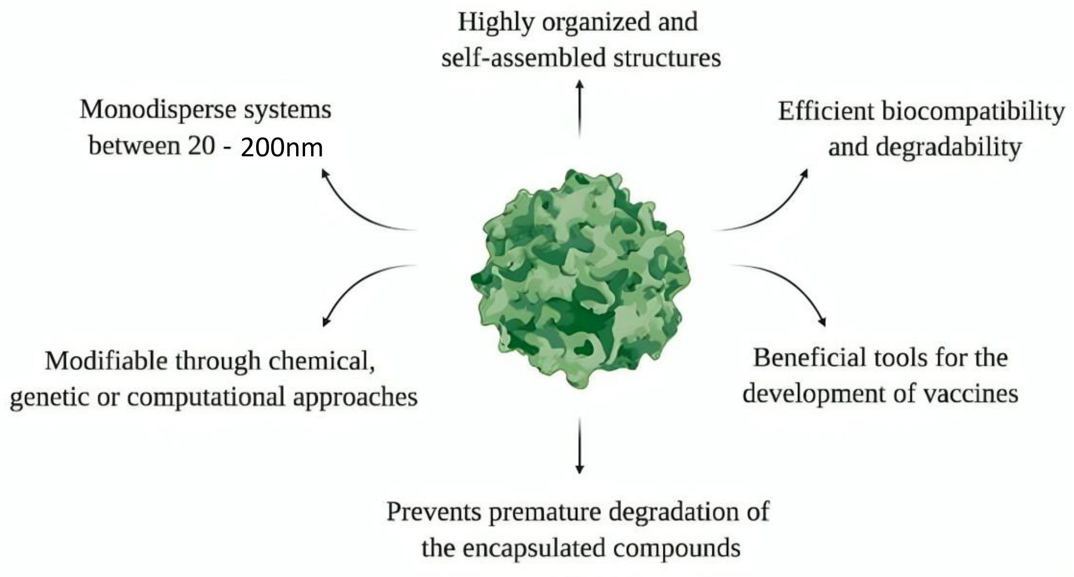
What are VLPs?

- Empty protein shells derived from viral capsid proteins
- Lack genetic material and are non-infectious
- High surface to volume ratio, so concentrated delivery → Potential cost reduction
- High shelf life, stable over a wide range of pH & temperatures



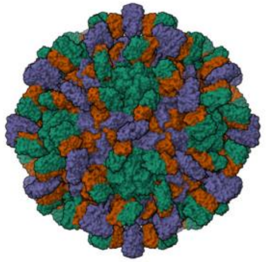


Different types of viral capsids: (A) helical, (B) icosahedral, (C) spherical, and (D) complex.



Mejía-Méndez et al. 2022

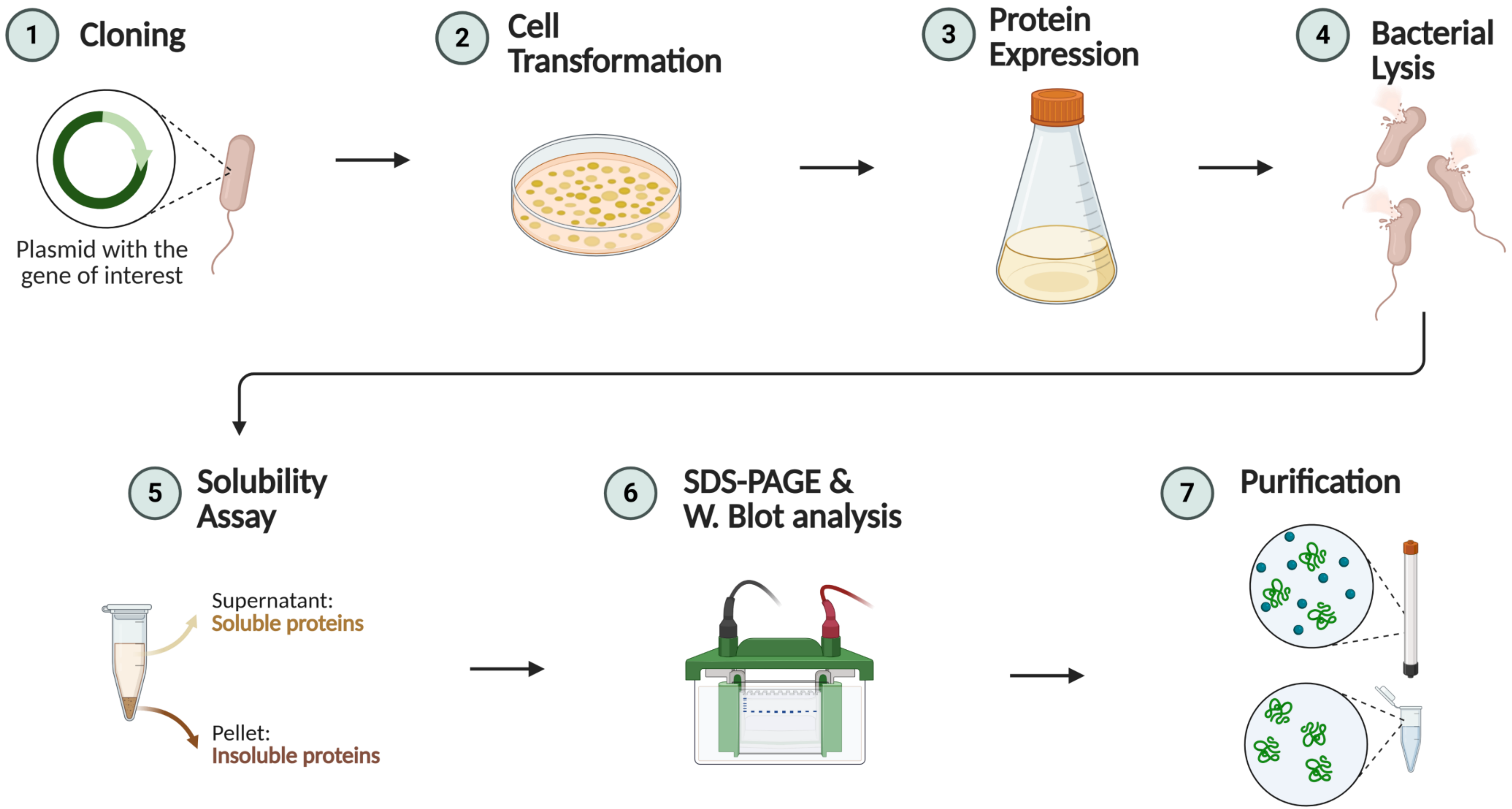
VLPs used in this study

Structure	Non-enveloped, icosahedral 
Capsid composition	180 coat protein subunits, T=3 symmetry
Size	~30 ± 3 nm diameter

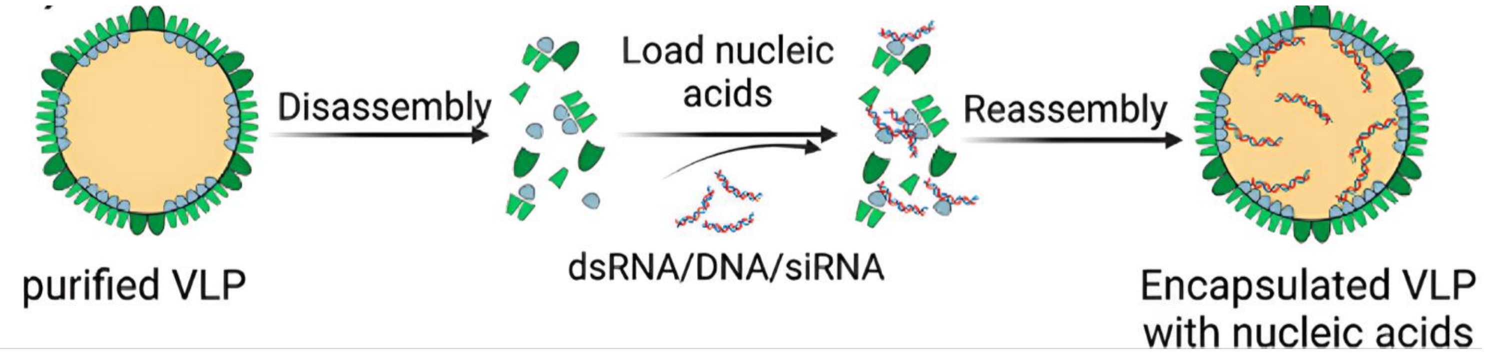
Why these VLPs?

- High dsRNA binding via arginine- and lysine-rich domains
- Scalable, cost-effective production in *E. coli*
- Stable under varied environmental conditions
- Tailorable surface for ligand or peptide functionalization
- Proven nucleic acid delivery in other systems
- Biodegradable and non-infectious for safe field use

Expression of VLPs in *E.coli*



Loading of VLPs



- Incorporating plant cell wall penetrating components into VLP assembly enhances **cell wall penetration**
- Making the resulting VLPs effective nanocarriers
- **Osmotic Shock for Loading:** We can also load the dsRNA into already-formed VLPs.
- One common method is using osmotic shock. By placing pre-assembled VLPs in a low-ionic-strength solution, the spaces between the VLP surface subunits increase.
- This change in conditions allows the dsRNA to enter the VLPs. The positively charged areas inside the VLPs core attract the dsRNA , effectively pulling them inside.

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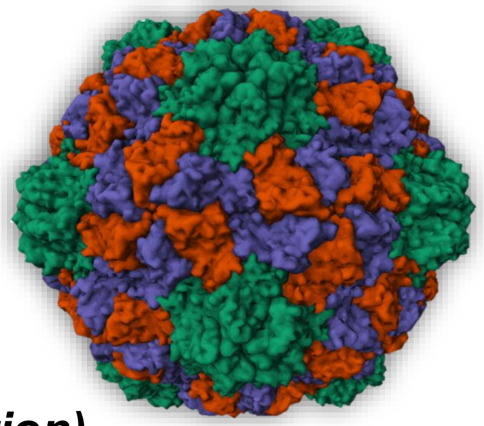
Our Innovation is using tiny yet powerful Virus-Like-Particles

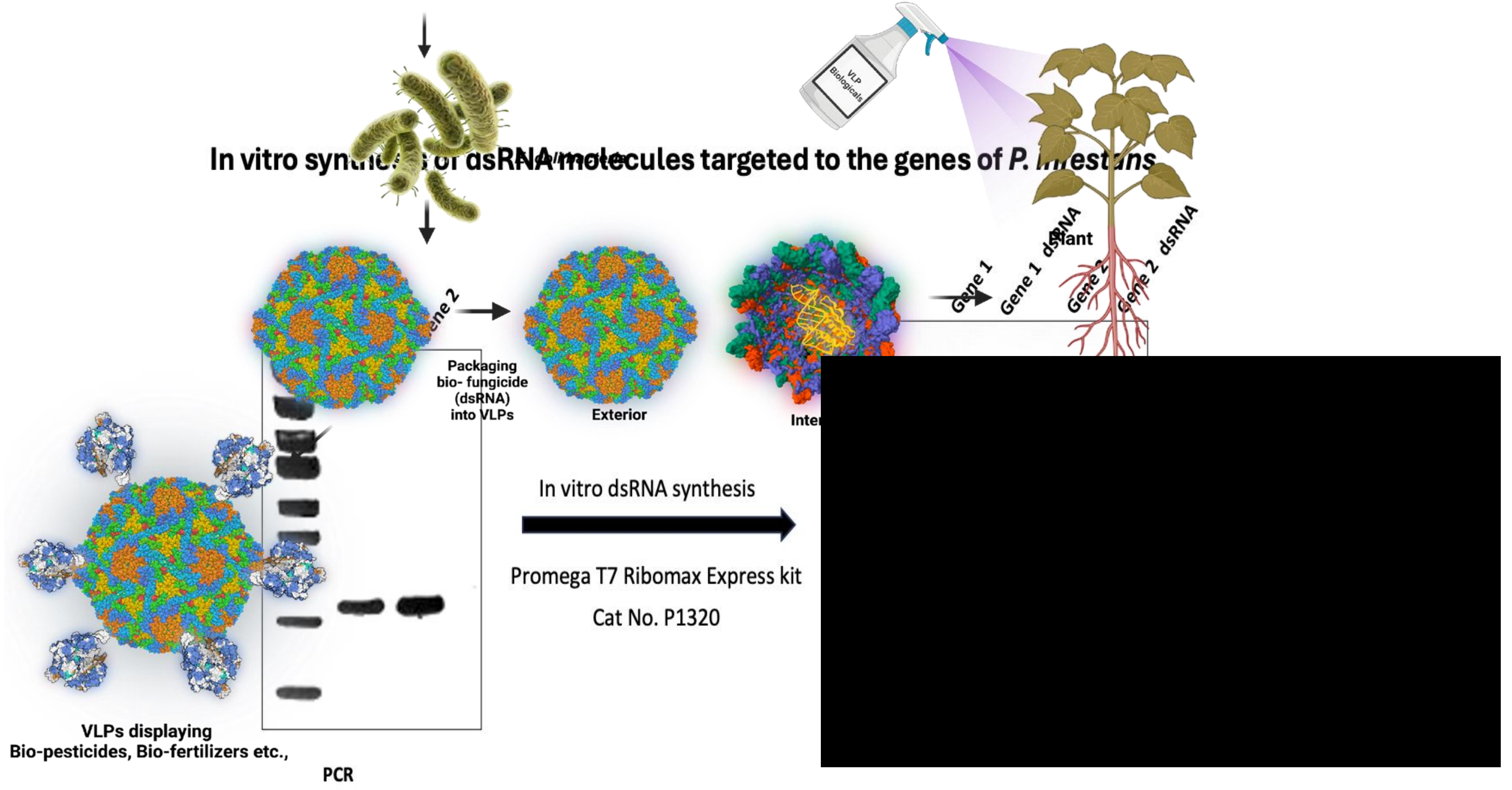
- ***Viruses** have thermodynamically very stable protein structures known as capsids*
- *These cages can be **Engineered** to be produced in bacteria*
- ***VLPs resemble viruses in their structure but are NOT VIRUSES***

We make VLPs as Precise Delivery Vehicles by loading them with pesticides or fungicides



- ***Non-infectious, biocompatible, and biodegradable in the crop fields***
- ***High shelf life of up to 5 years***
- ***70% reduction in pesticide usage***
- ***We made it at a very low cost (approx. 1/100th of making a pesticide formulation)***

Our Innovation



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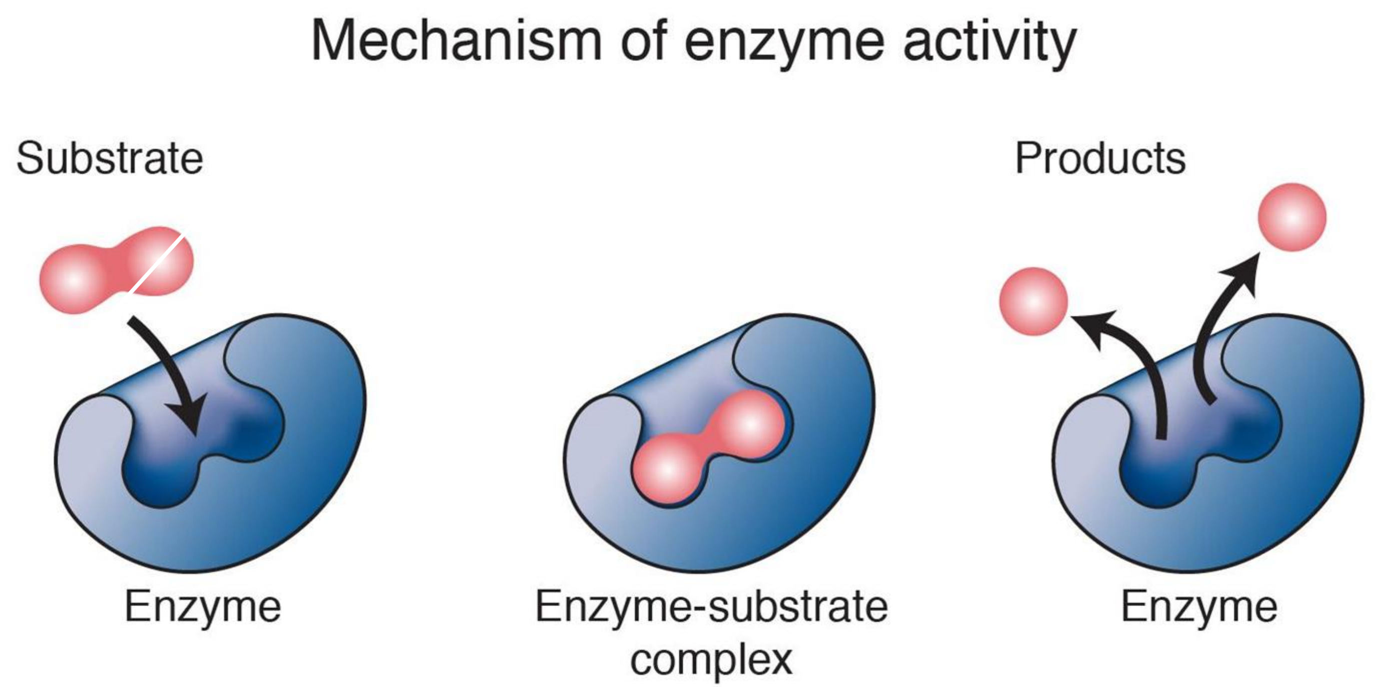
Ongoing/Future projects

VLPs as Enzyme Carriers

What are Enzymes? How do they work?

- Enzymes are proteins that help speed up metabolism, or the chemical reactions in living organisms
- They build some substances and break others down
- All living things have enzymes. Enzymes are employed in various industries from food to pharmaceuticals



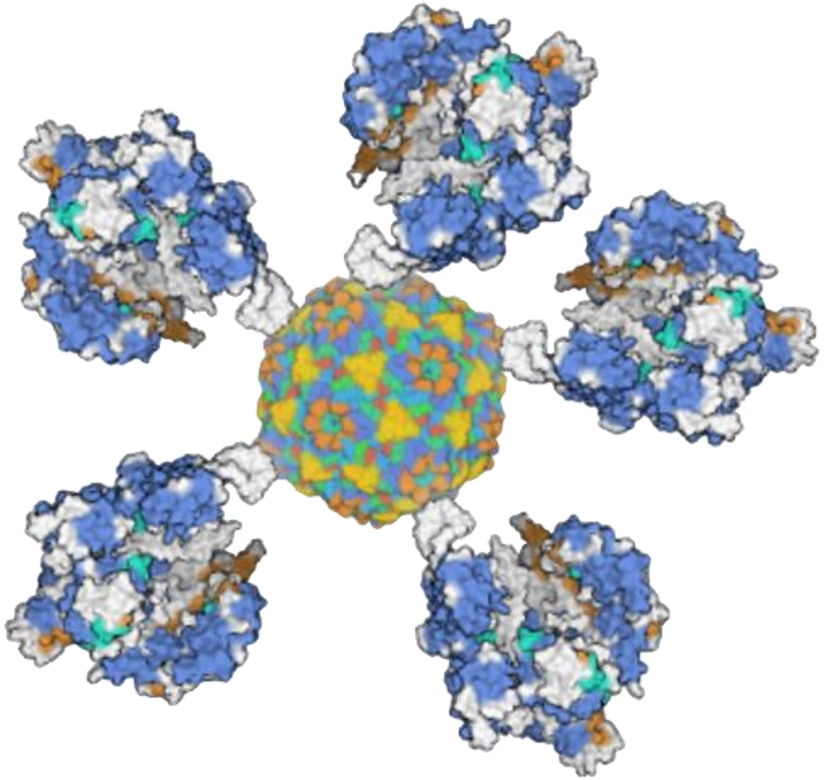
Virus Like Particles as Enzymes

- ***Viruses*** *have thermodynamically very stable protein structures known as capsids*
- ***These proteins can be engineered*** *as* ***enzymatic agents***
- *We developed* ***Protein cages*** *that can be* ***working as enzyme carriers***
- *We made it at a* ***very low cost (1% cost compared to making an enzyme formulation)***

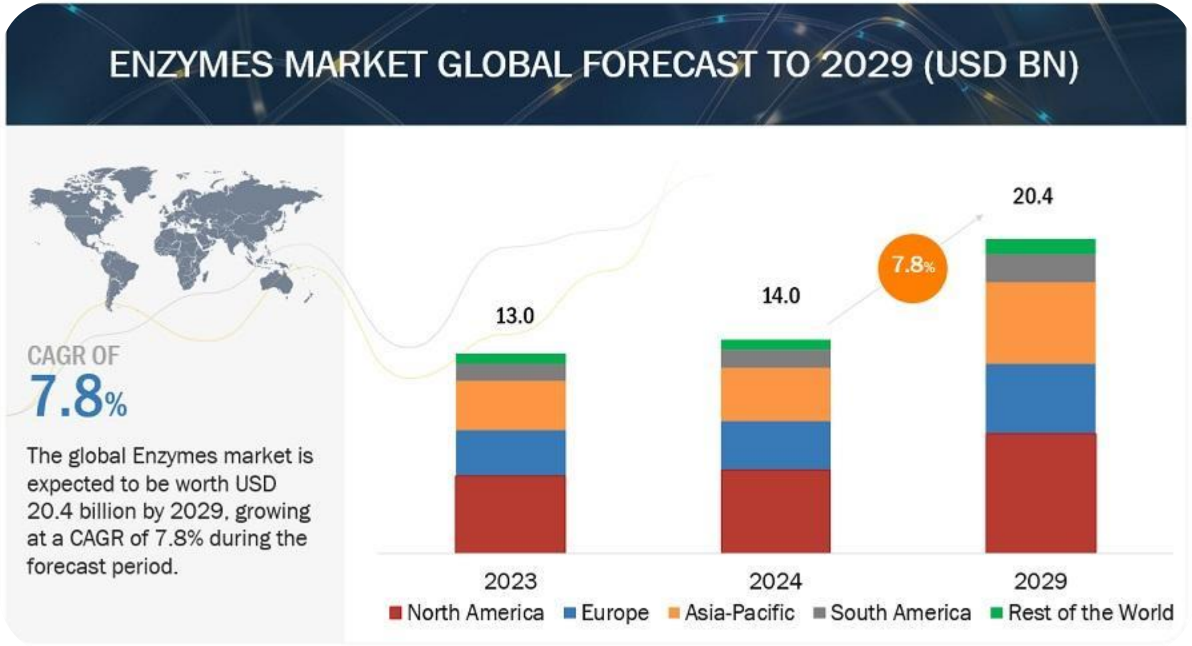
✓ Non-infectious, biocompatible, biodegradable and non-GMO

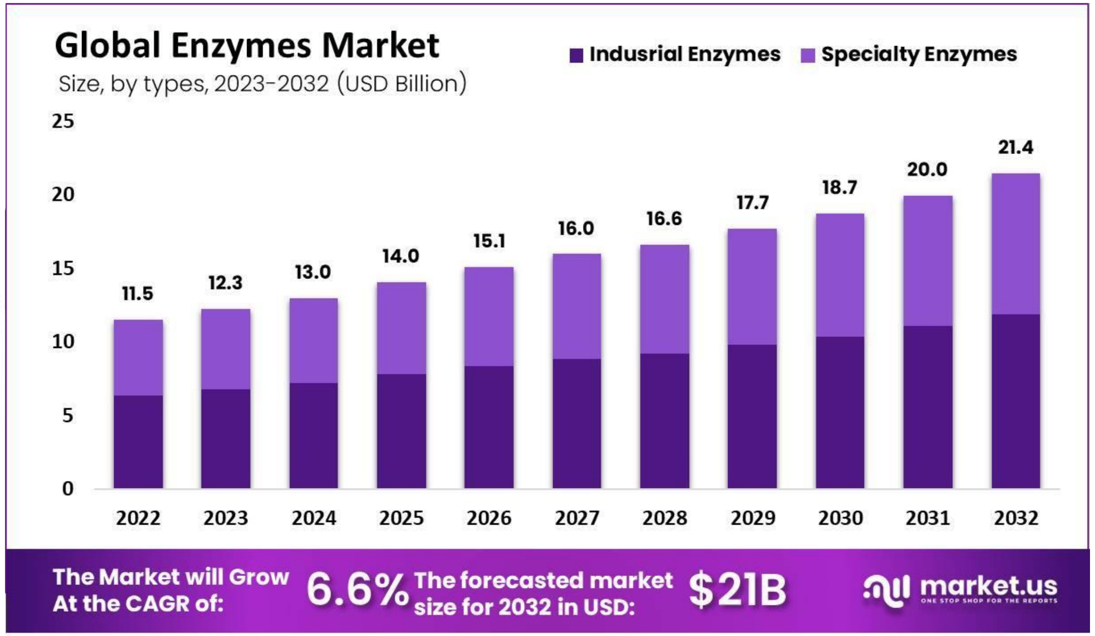
✓ High shelf life of up to 5 years

✓ Production of enzymes at very low costs and industrial scale is possible



VirEnzyme can revolutionize the Enzyme industry…..





Ongoing/Future projects

Revolutionary Spray-On Gene Editing: Harnessing Virus-Like Particles as Efficient CRISPR Nano-Carriers for Plants

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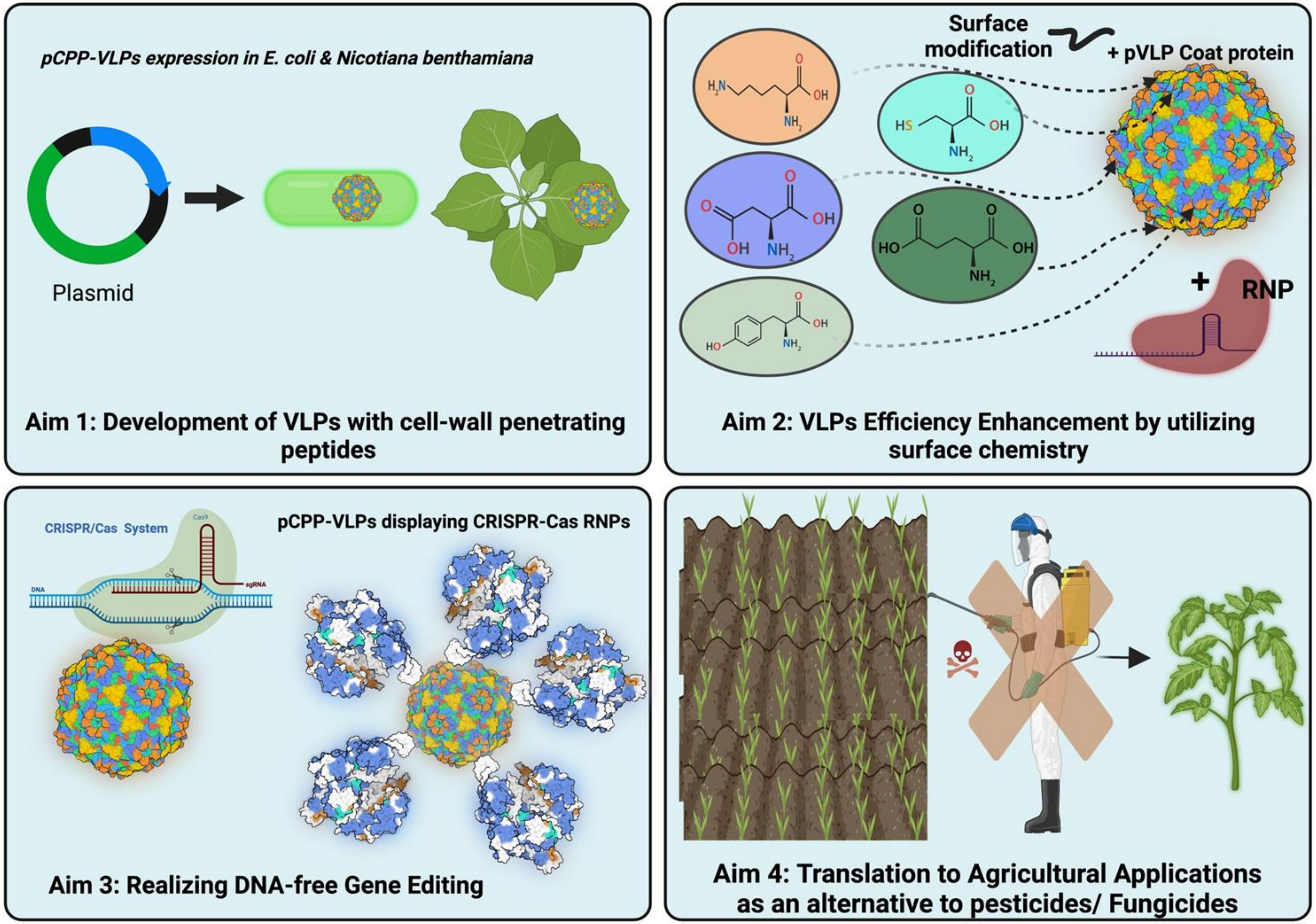

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VLPs for pest management ????

viruses

MDPI

Opinion

Can Virus-like Particles Be Used as Synergistic Agent in Pest Management?

Caroline Deshayes [1], Anne-Sophie Gosselin-Grenet [2], Mylène Ogliastro [2], Bruno Lapied [1,*] and Véronique Apaire-Marchais [1]

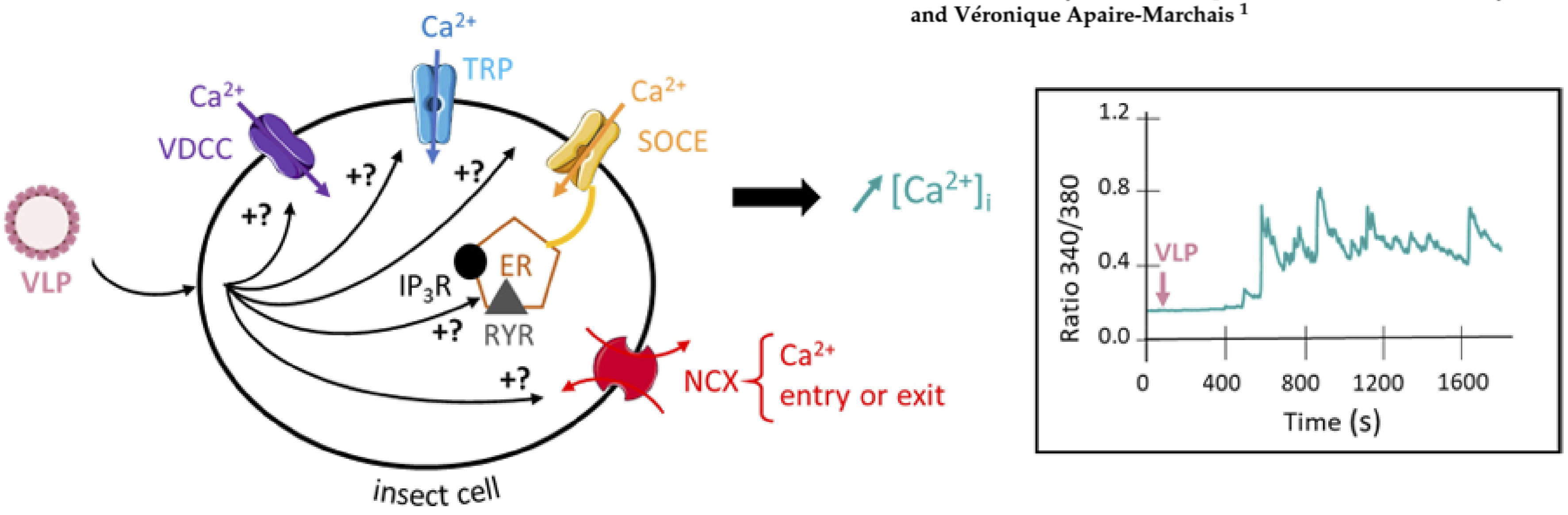

Figure 2. VLPs induce a multicomponent intracellular calcium rise. The scheme summarizes the hypothetic mechanisms by which VLPs increase intracellular calcium concentration. Inset: Representative multicomponent effect of VLPs on intracellular calcium concentration in Fura-2 loaded isolated insect neuron cell body using the calcium imaging ratiometric method (C. Deshayes, unpublished data). VDCC, voltage-dependent calcium channel; TRP, transient receptor potential channel; SOCE, store-operated calcium entry; NCX, sodium–calcium exchanger; RYR, ryanodine receptor; IP3R, inositol triphosphate receptor; ER, endoplasmic reticulum.

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Thank You

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SCIENCE AND EDUCATION FOR SUSTAINABLE LIFE


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