Finanziato dall'Unione europea NextGenerationEU | Ministero dell'Università e della Ricerca | Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA | SUS-MIRRI.IT

The two-faced plant viruses: from plant pathogen to smart nanoparticles

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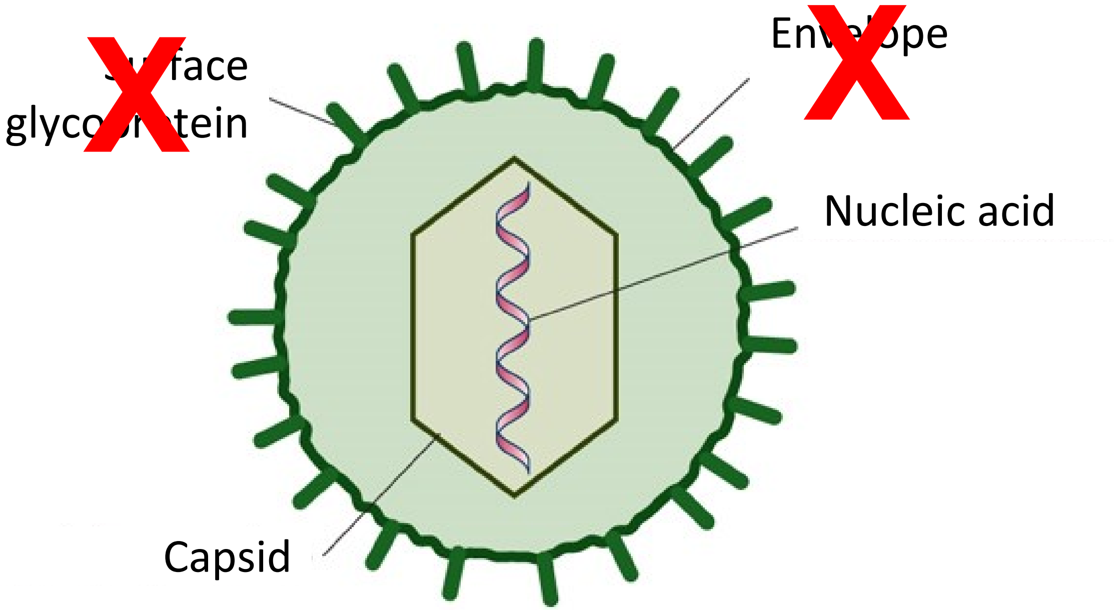
Biotechnologies Laboratory

November 28, 2023
Training course SUS-MIRRI.IT

Obligate intracellular parasite

Infectious agent capable of replicating exclusively within a cell (animal, vegetable, bacterial) in a highly host-specific manner



1- Cell entry, disassembly, viral genome release.

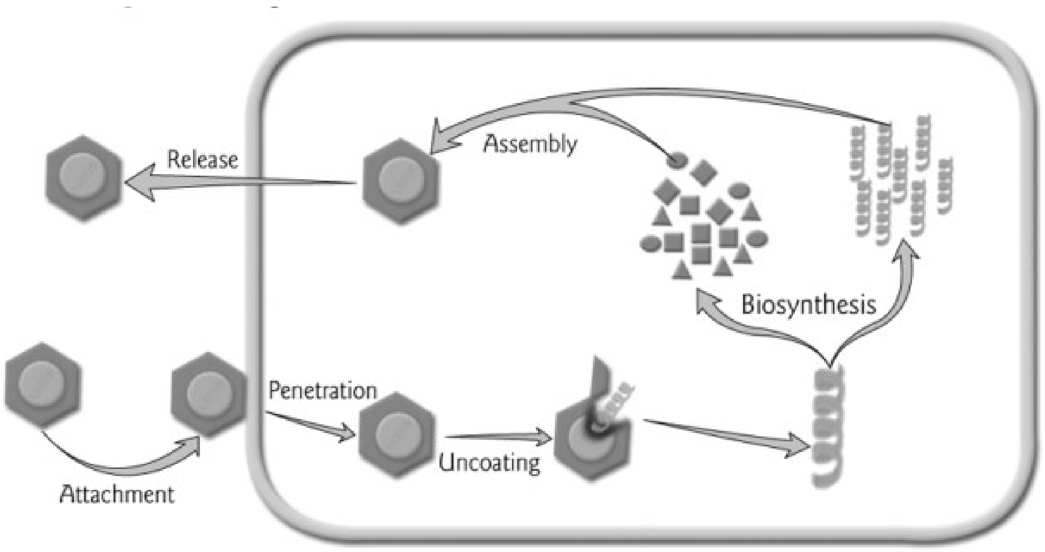
2- Viral genome translation to immediately produce viral proteins involved in replication.

3- Subgenomic RNAs production (if requested).

4- Production of proteins involved in movement and assembly.

5- Virion assembly.

6- Virion movement and systemic infection spreading.



- Viral genome organization

1- Few ORF coding for multifunctional proteins

2- Adoption of several strategies to produce different polyproteins, as leaky stop codons, frameshift….

3- Subgenomic RNAs

- Uses

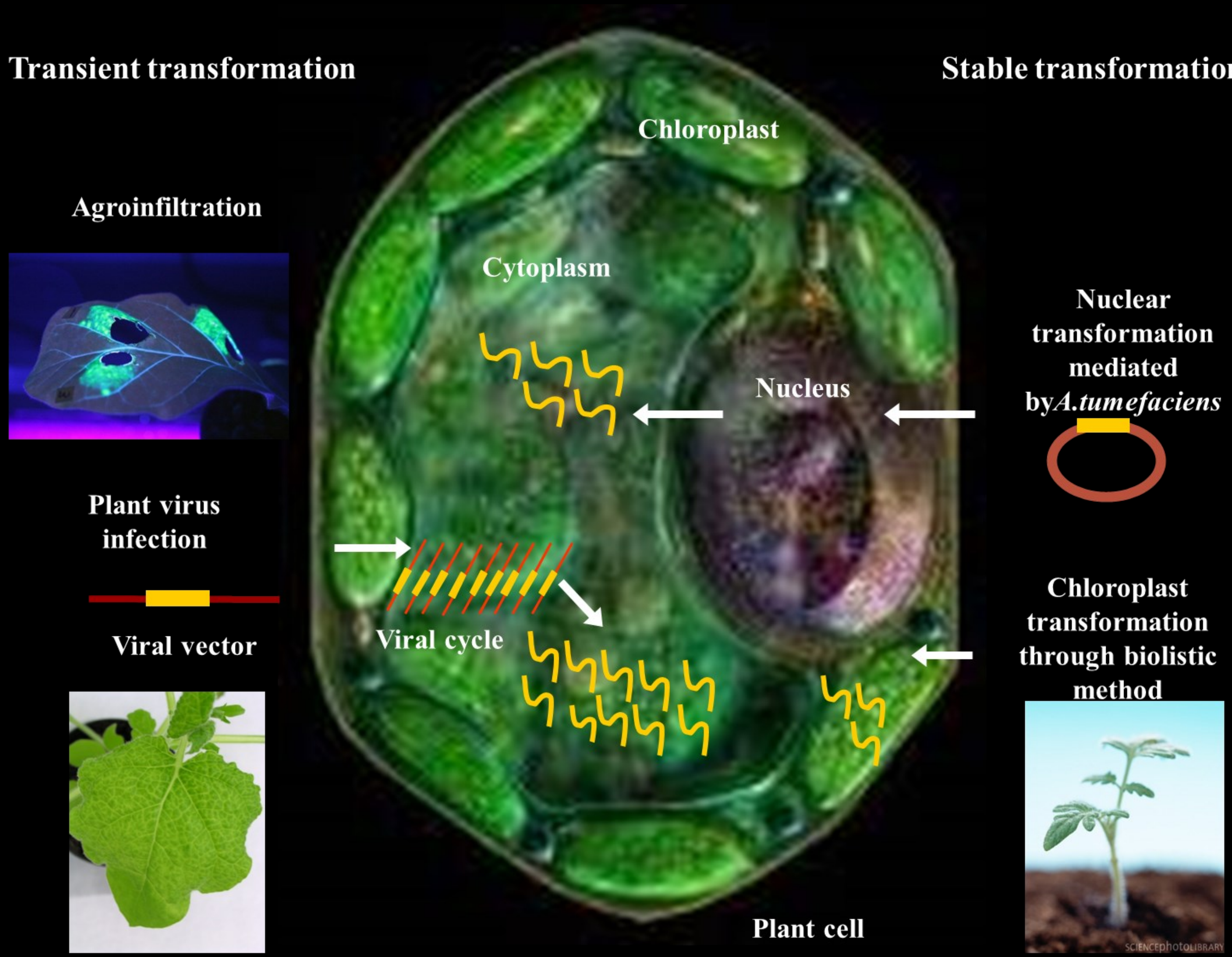
- "cross-protection" or "mild strain protection" systems

- **Viral vectors for heterologous proteins expression or epitope presentation**

-Vectors for host gene silencing and functional genomics analysis

- **Nanotechnology**

Plant viruses as expression vectors



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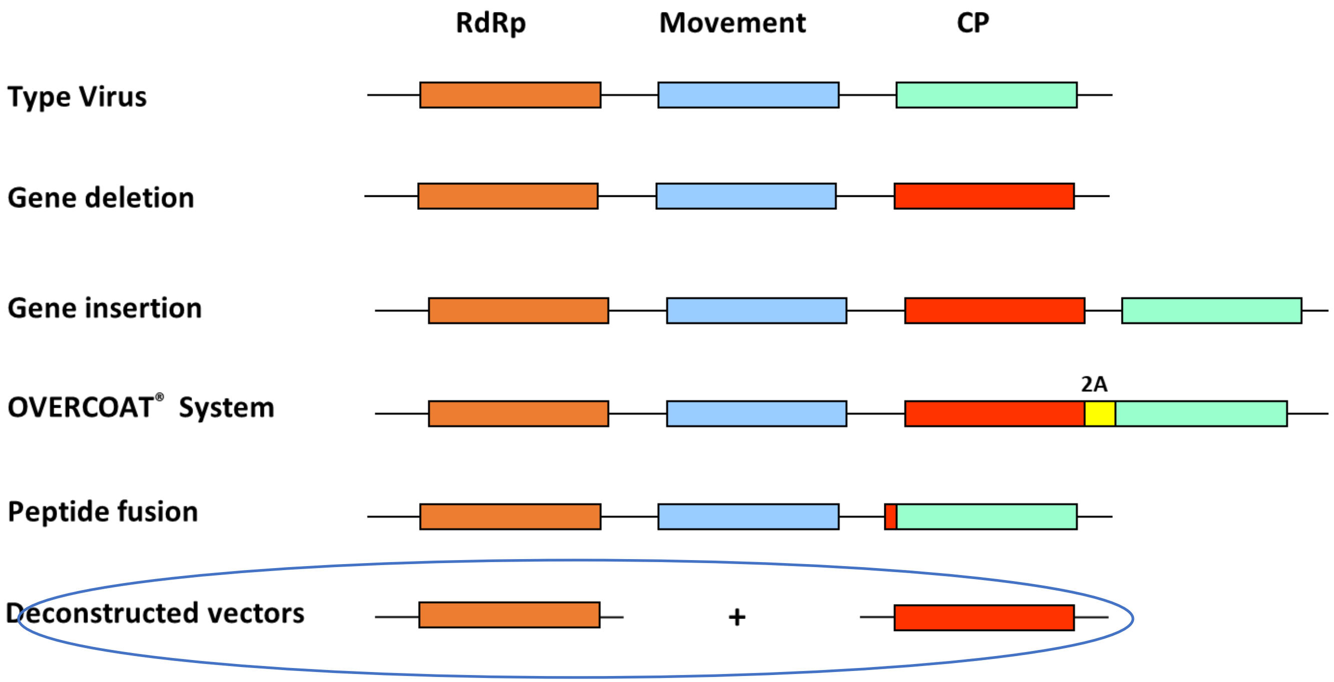
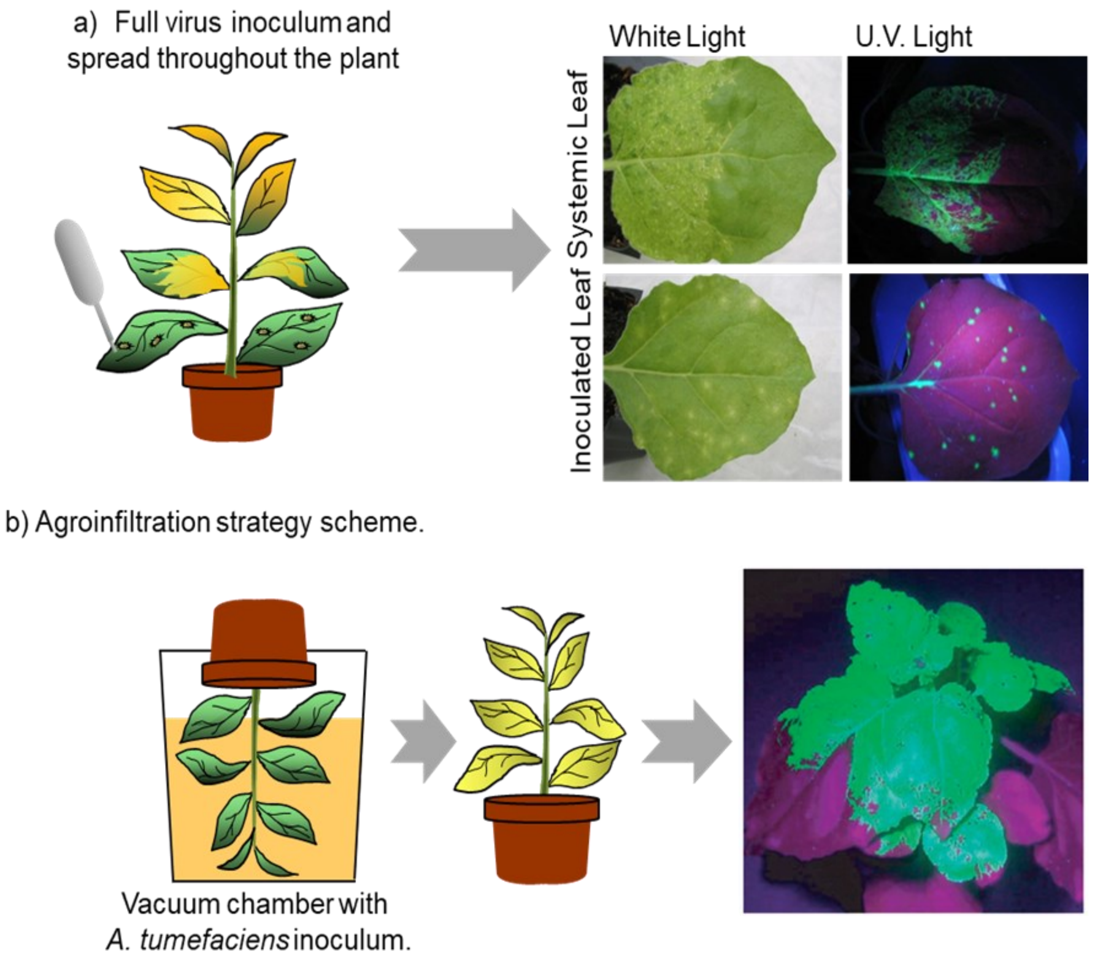
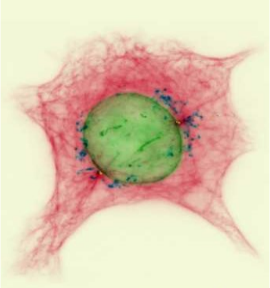




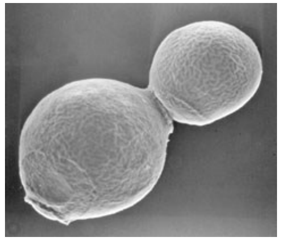
Plant viruses for biopharmaceuticals production

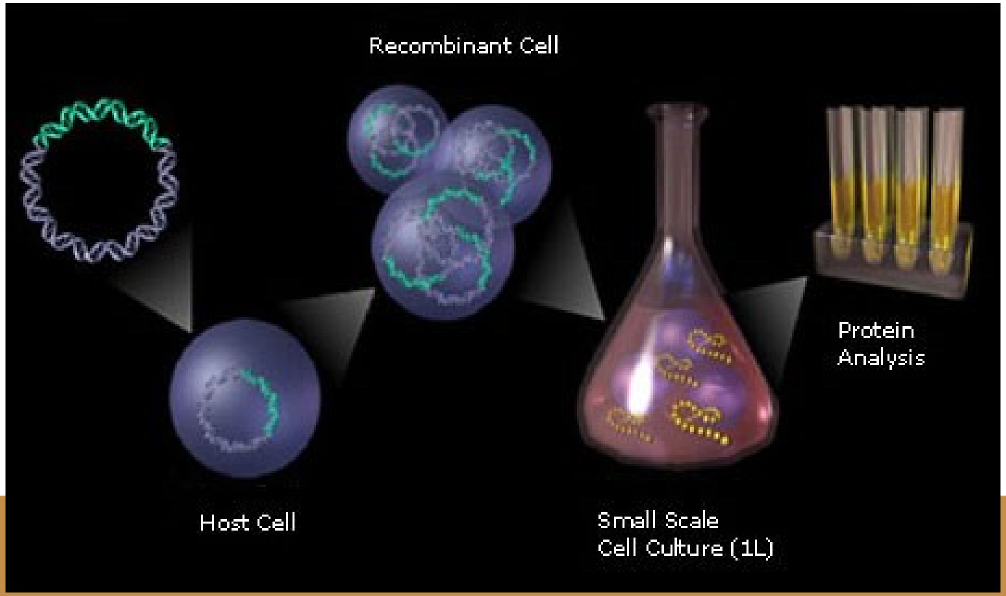
Bioreactors


Mammals cells lines

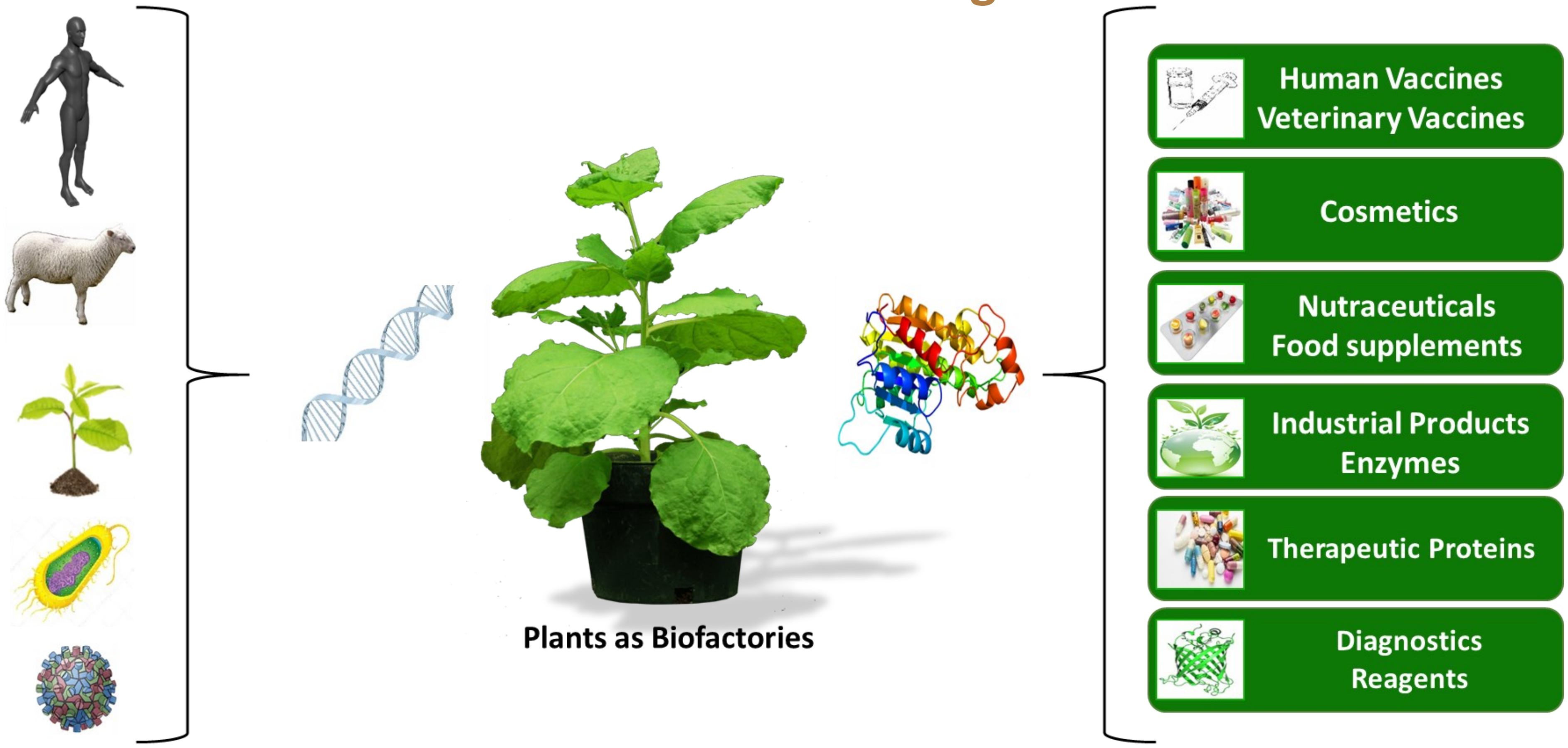

E. coli


Insect cells lines


Yeast



Plant Molecular Farming



- Eukaryotic expression system
- Easy and rapid scale-up
- Biosafety
- Low costs

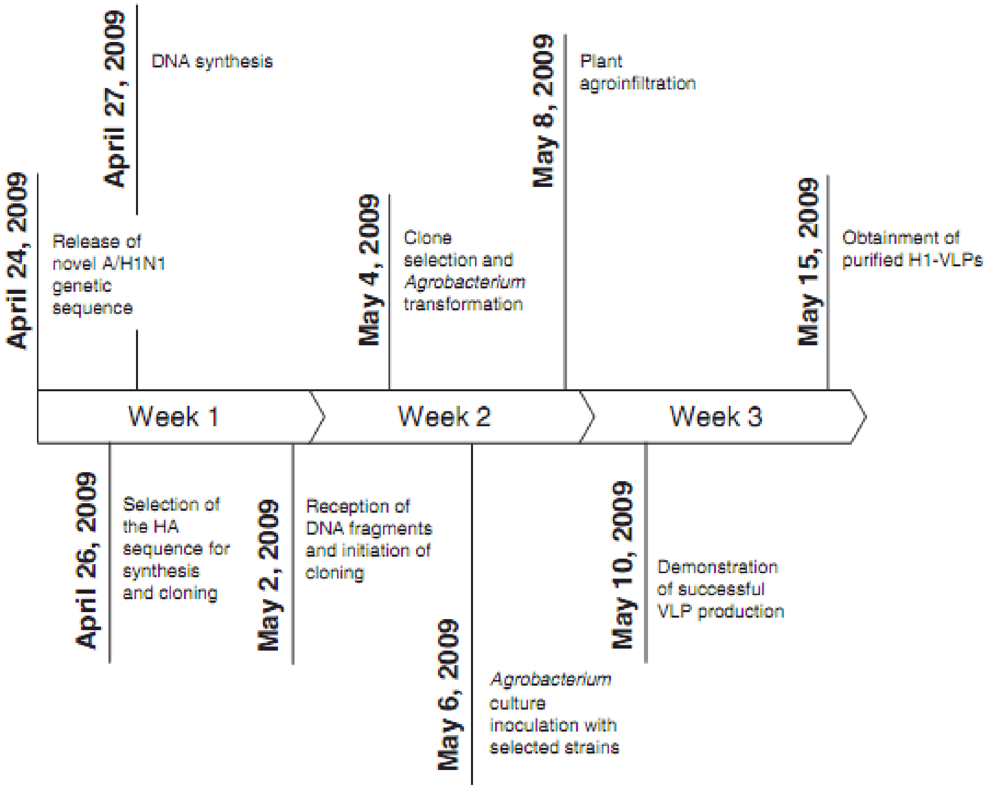
Table 1. Comparison of production and effective cost for three countries and two presentations

	Korea or India	United States		Korea		India	
	Yeast derived 10-dose vials	Plant-derived single dose packet	Plant-derived 10-dose packet	Plant-derived single dose packet	Plant-derived 10-dose packet	Plant-derived single dose packet	Plant-derived 10-dose packet
Cost	$0.27	$0.15	$0.06	$0.09	$0.04	$0.075	$0.03
Effective Cost	$0.42	$0.16	$0.08	$0.10	$0.05	$0.08	$0.04
% savings for plant derived vaccine against yeast derived for effective cost		62%	81%	76%	88%	81%	90%

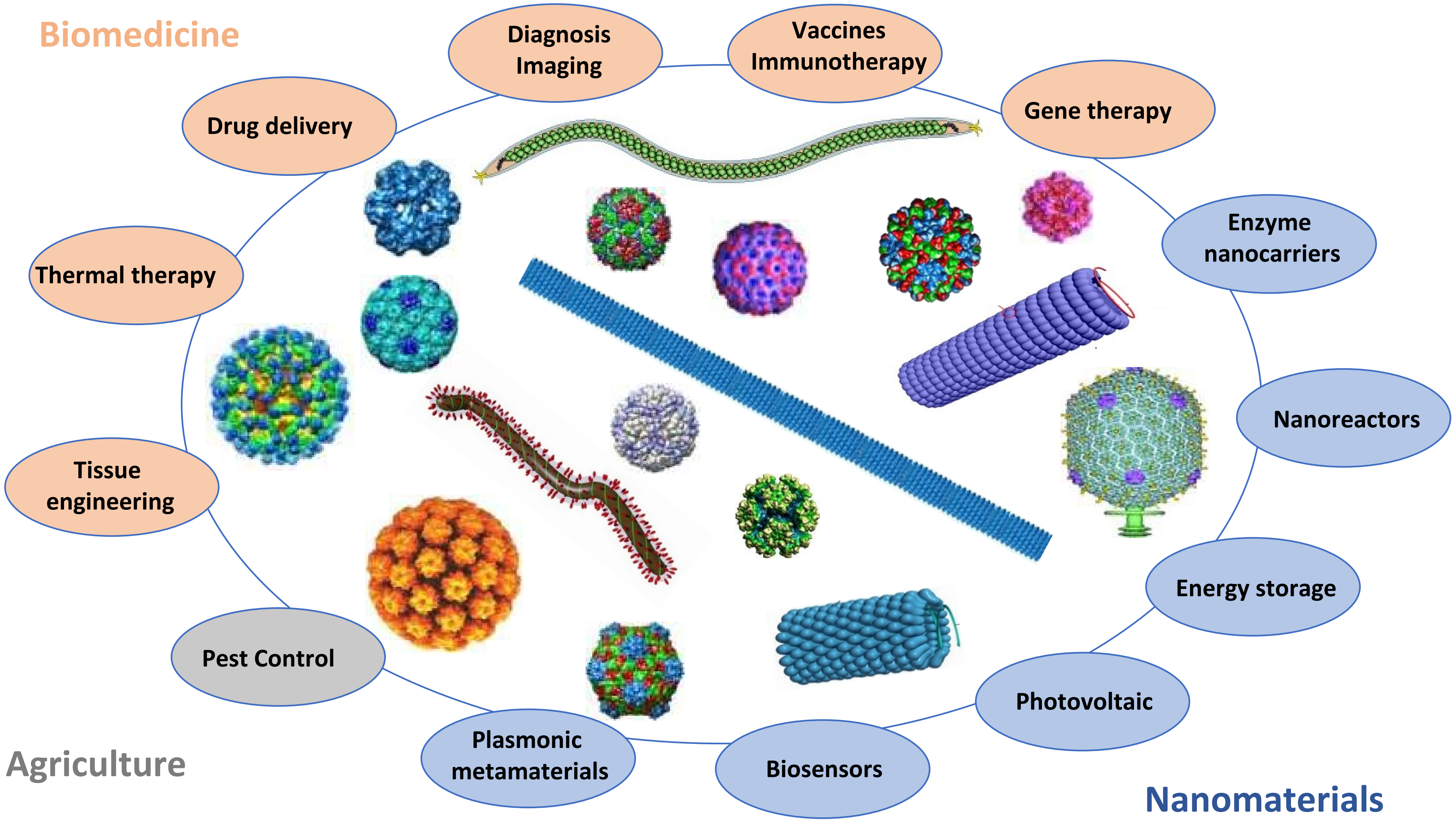
Arntzen C, R Mahoney, A Elliott, B Holtz, A Krattiger, CK Lee, S Slater. 2006. Plant-derived Vaccines: Cost of Production. The Biodesign Institute, Arizona State University: Tempe. www.biodesign.asu.edu/centers/idv/projects/provacs

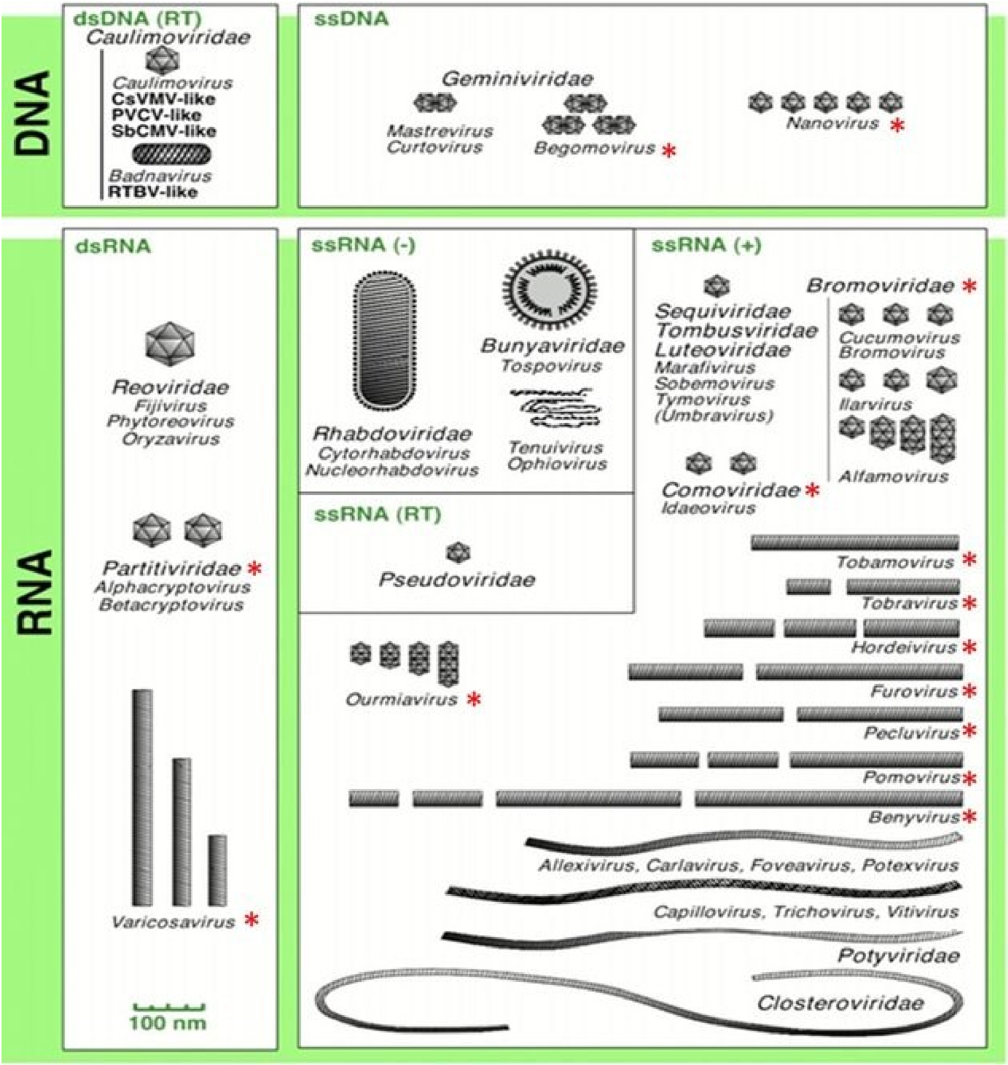
Vertical farming

medicago



Plant viruses in nanotechnology

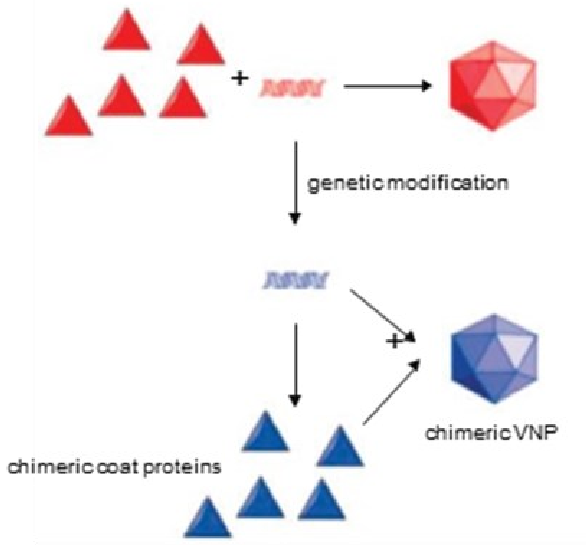




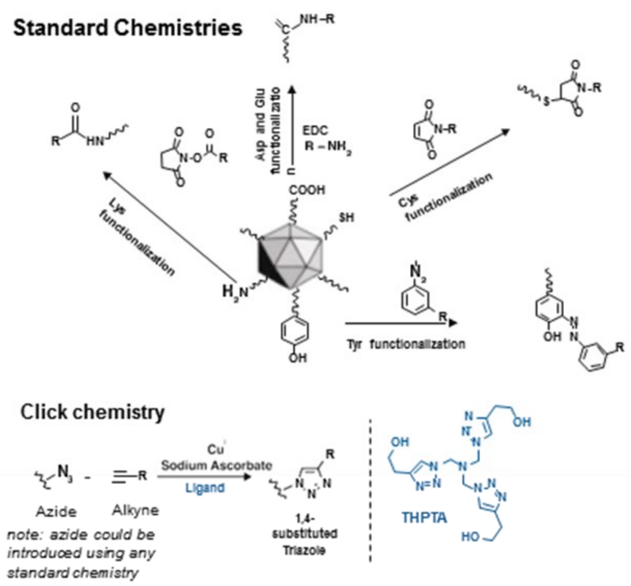
- **Very different in shape and dimension**
- **Easy, safe, rapid production in plants**
- **Self-assembly**
- **High surface/volume ratio**
- **Modification of the surface**
- **Modification of the inner**
- **Genetic engineering**
- **Bioconjugation**
- **Standard chemistries**

How can we modify plant viruses ?

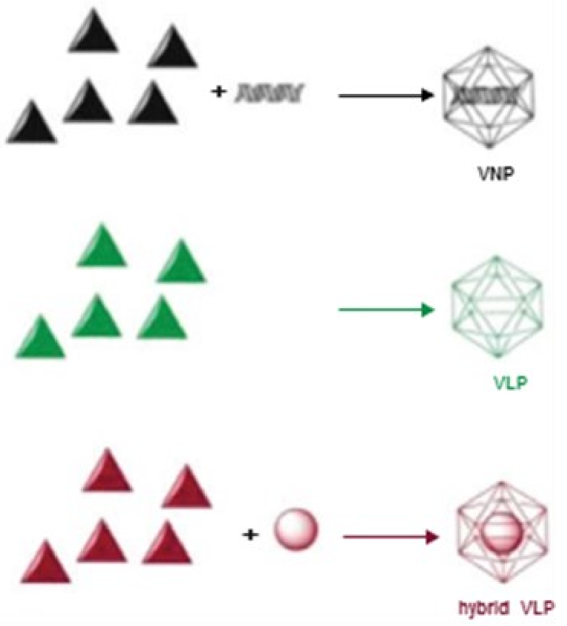
Genetic modification



Bioconjugation



Encapsulation



Plant Viruses @ ENEA

POTATO VIRUS X - PVX

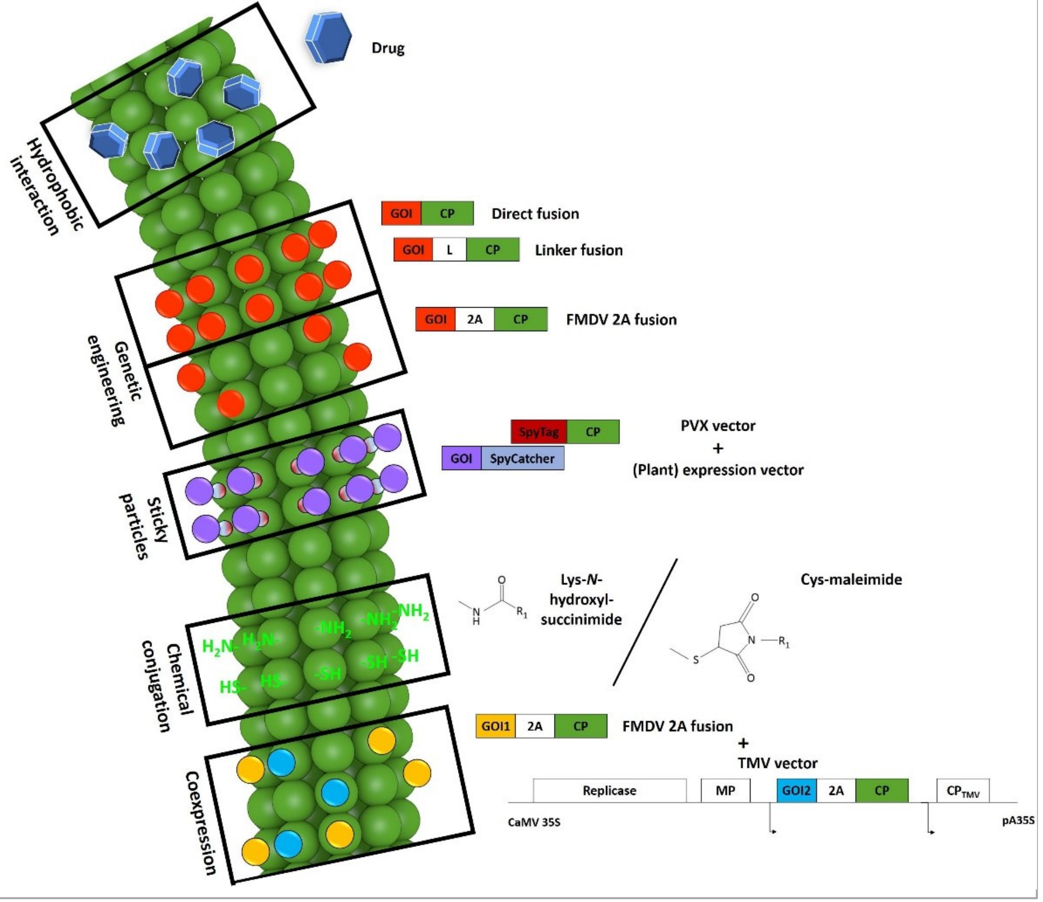
- Alphaflexiviridae
- Potexvirus

- Filamentous, flexible

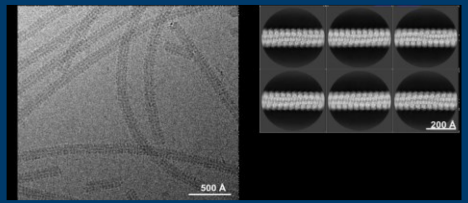
- Lenght: 500 nm
- Diameter: 13 nm

- ss(+) RNA 6400 nt

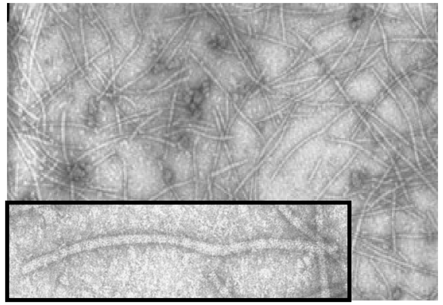
- 1300 Coat Protein (CP)



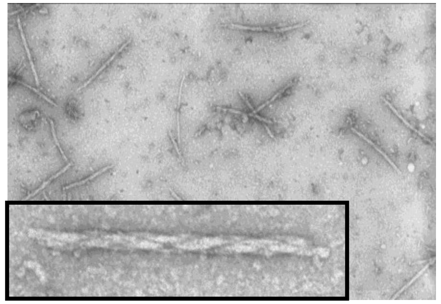
PVX in Biotechnology @ ENEA



PVX wt

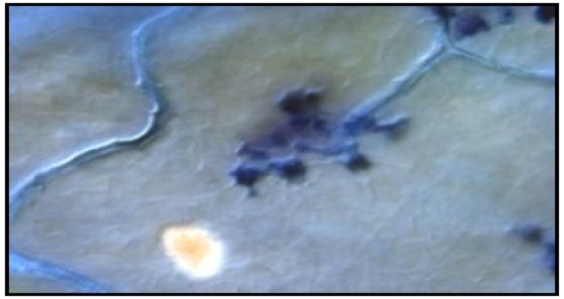


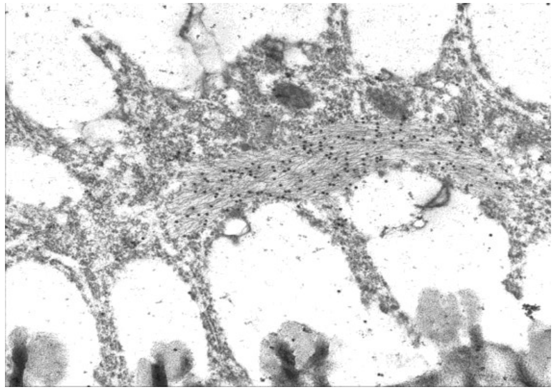
PVX mutant



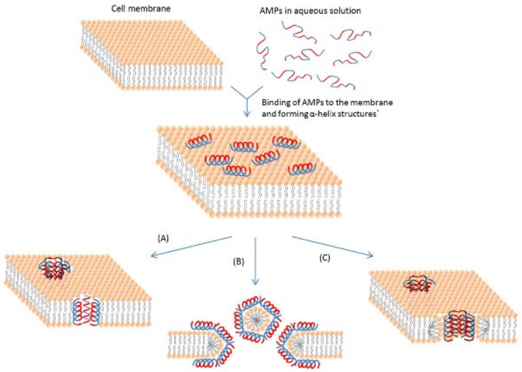
Plant virology

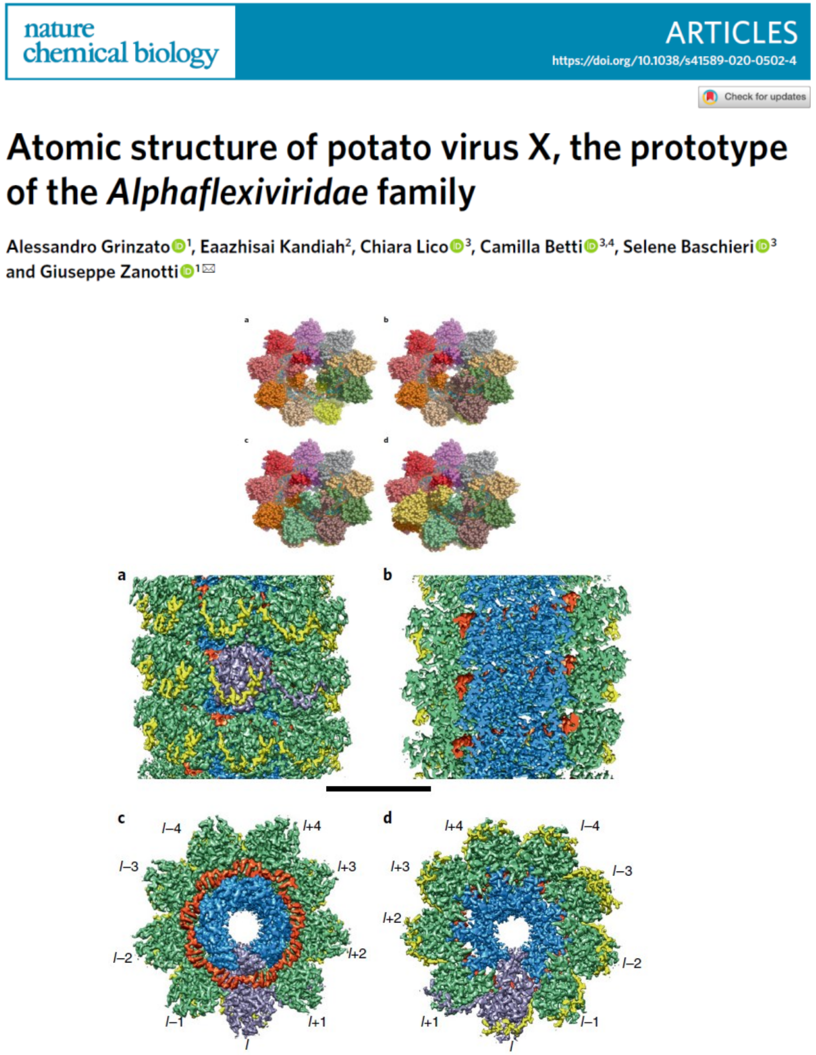
CVPs stability
Virus movement
CP-Virion structure





Antimicrobial peptides

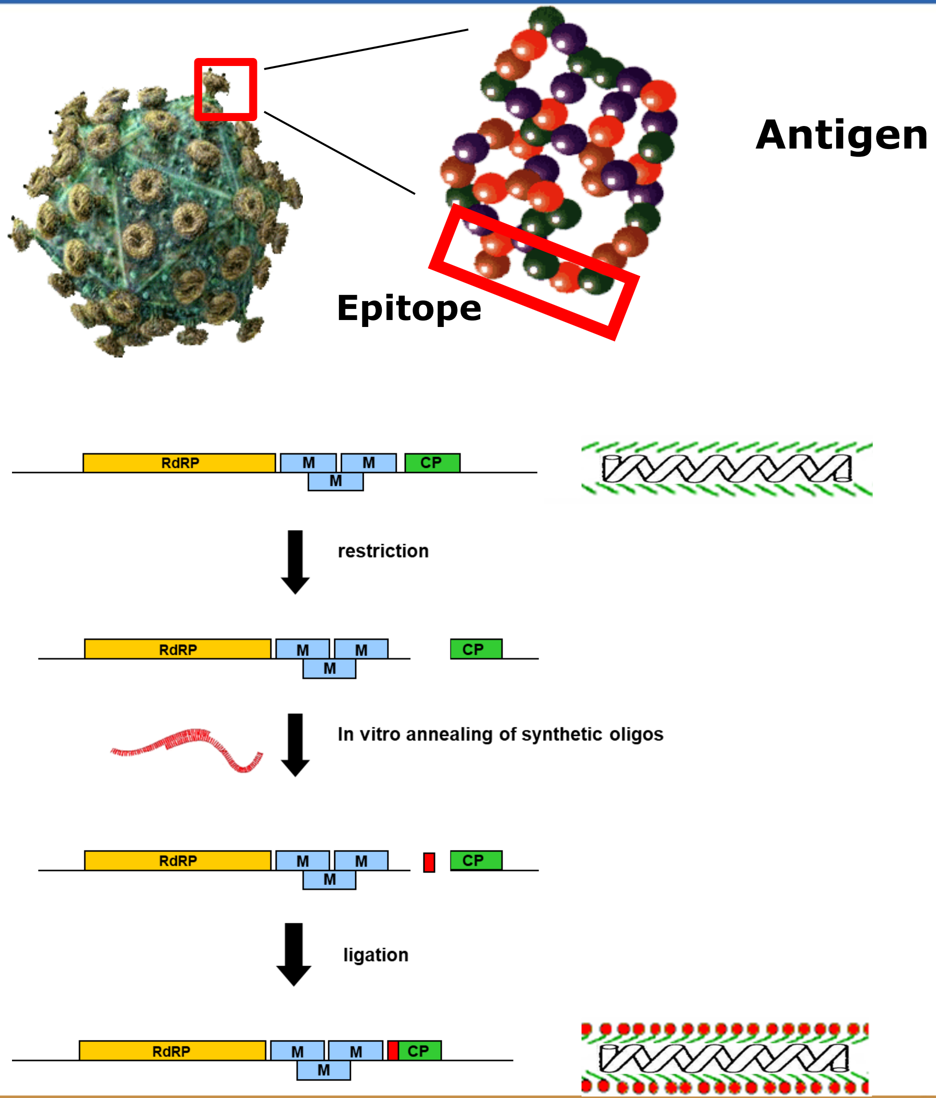


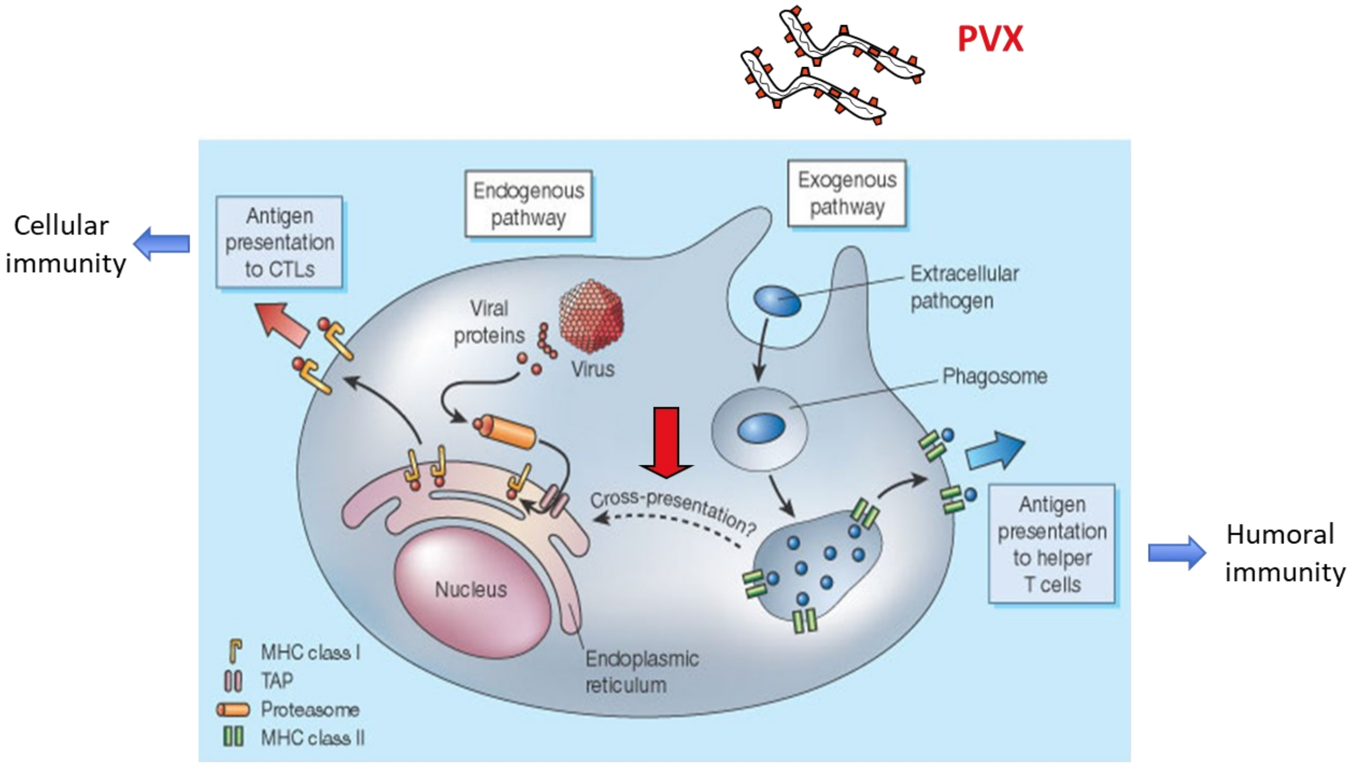

nature chemical biology ARTICLES
https://doi.org/10.1038/s41589-020-0502-4
Check for updates

Atomic structure of potato virus X, the prototype of the *Alphaflexiviridae* family

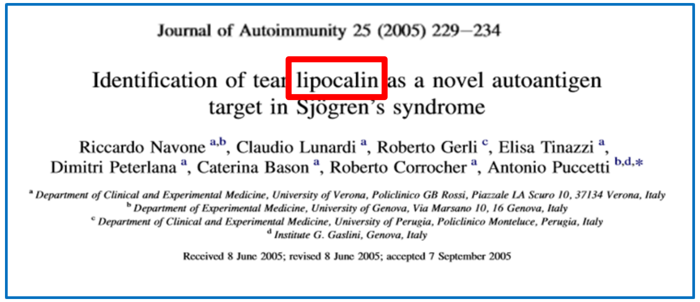
Alessandro Grinzato[1], Eaazhisai Kandiah[2], Chiara Lico[3], Camilla Betti[3,4], Selene Baschieri[3] and Giuseppe Zanotti[1]

New Vaccine Formulations





Diagnostic kit for Sjogren's Syndrome



Journal of Autoimmunity 25 (2005) 229–234

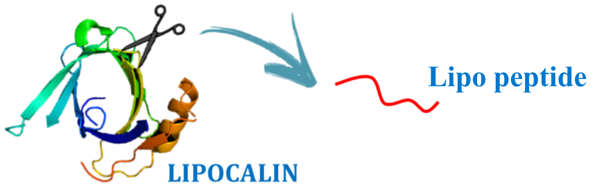
Identification of tear lipocalin as a novel autoantigen target in Sjögren's syndrome

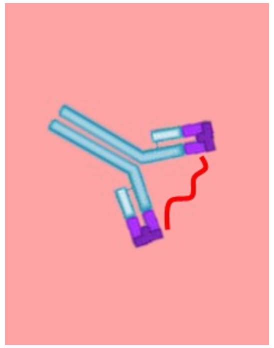
Riccardo Navone [a,b], Claudio Lunardi [a], Roberto Gerli [c], Elisa Tinazzi [a], Dimitri Peterlana [a], Caterina Bason [a], Roberto Corrocher [a], Antonio Puccetti [b,d,*]

[a] Department of Clinical and Experimental Medicine, University of Verona, Policlinico GB Rossi, Piazzale LA Scuro 10, 37134 Verona, Italy
[b] Department of Experimental Medicine, University of Genova, Via Marsano 10, 16 Genova, Italy
[c] Department of Clinical and Experimental Medicine, University of Perugia, Policlinico Monteluce, Perugia, Italy
[d] Institute G. Gaslini, Genova, Italy

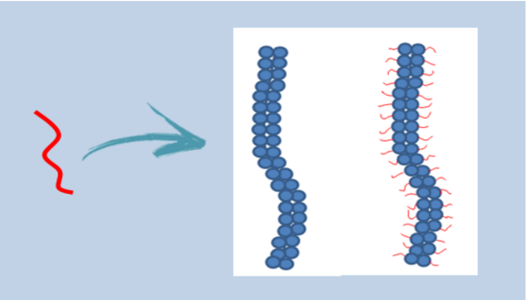
Received 8 June 2005; revised 8 June 2005; accepted 7 September 2005

Lipocalin is the major lipid binding protein in human tear fluid



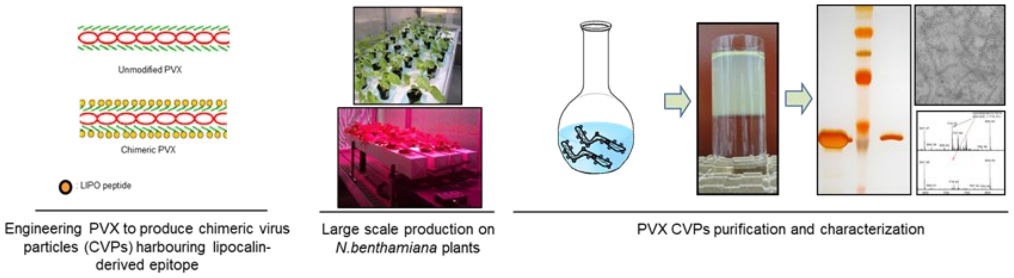


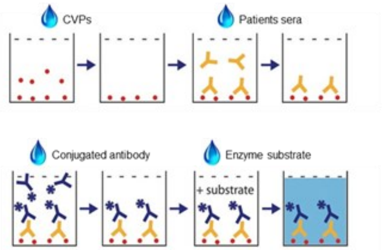
Lipo peptide is recognized by autoantibodies present in patients sera



Fusion of Lipo peptide to PVX CP

How does it work ?





Sjögren Patient

Healthy Patient

Does it work ?

SPECIFICITY

Ability to identify healthy people

False positives

Specificity

90%

SENSITIVITY

Ability to identify sick people

False negatives

Sensitivity

98,8%

Easy, rapid, not invasive → ELISA test

Reliable, sensible, specific, stable for at least 60 days → lipocalin peptide, PVX

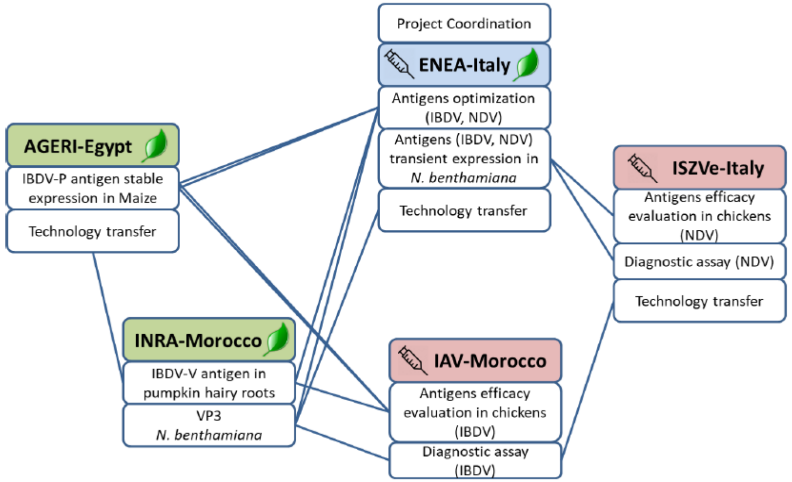
Cheap → *in planta* production

Pi-greekit

DIAMANTE
Biotechnology Company
nanotecnologie per la diagnosi di malattie autoimmuni

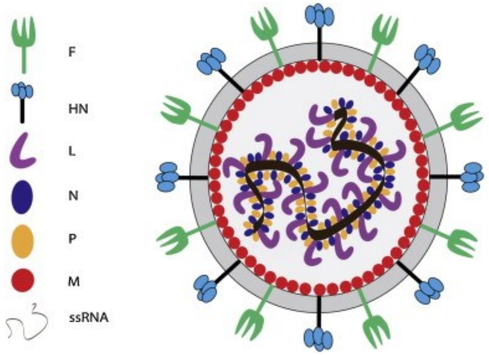
AVIan viral disease prevention and control with plant vaccines for the MEDiterranean area (AVIAMED)

AVIAMED

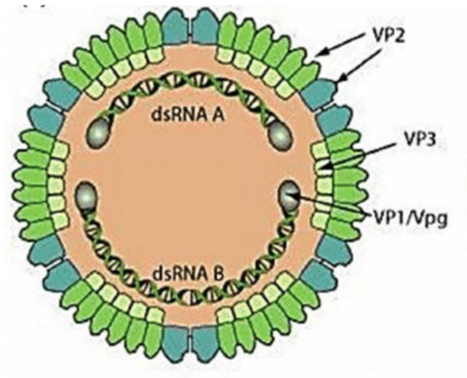


http://aviamedproject.net/

NDV



IBDV



Vaccine
DIVA test
(Differentiating Infected from Vaccinated Animals)

Plant Viruses @ ENEA

Tomato Bushy Stunt Virus
TBSV

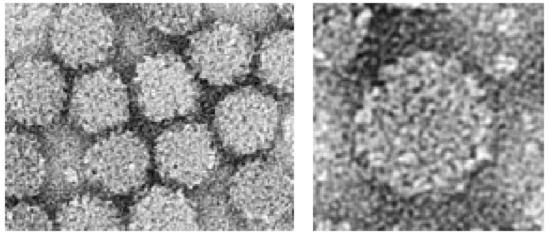
- Tombusviridae
- Tombusvirus

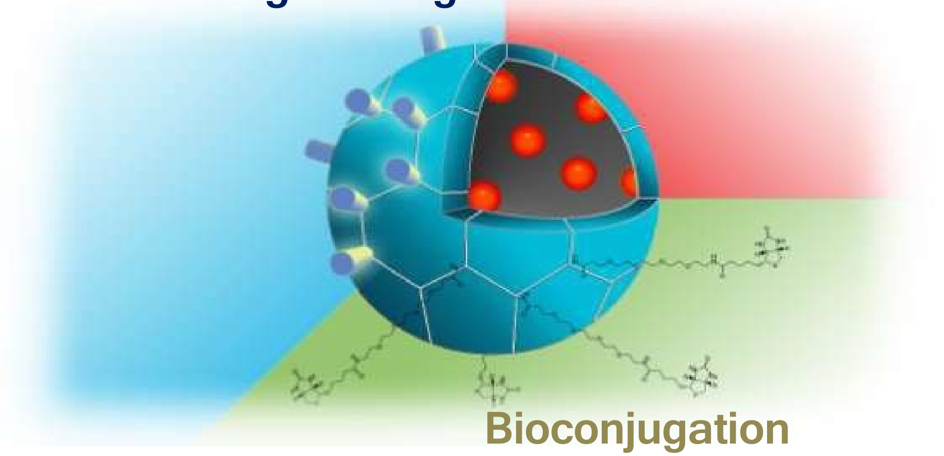
- Icosahedral
- Symmetry T=3

- Diameter: 30 nm

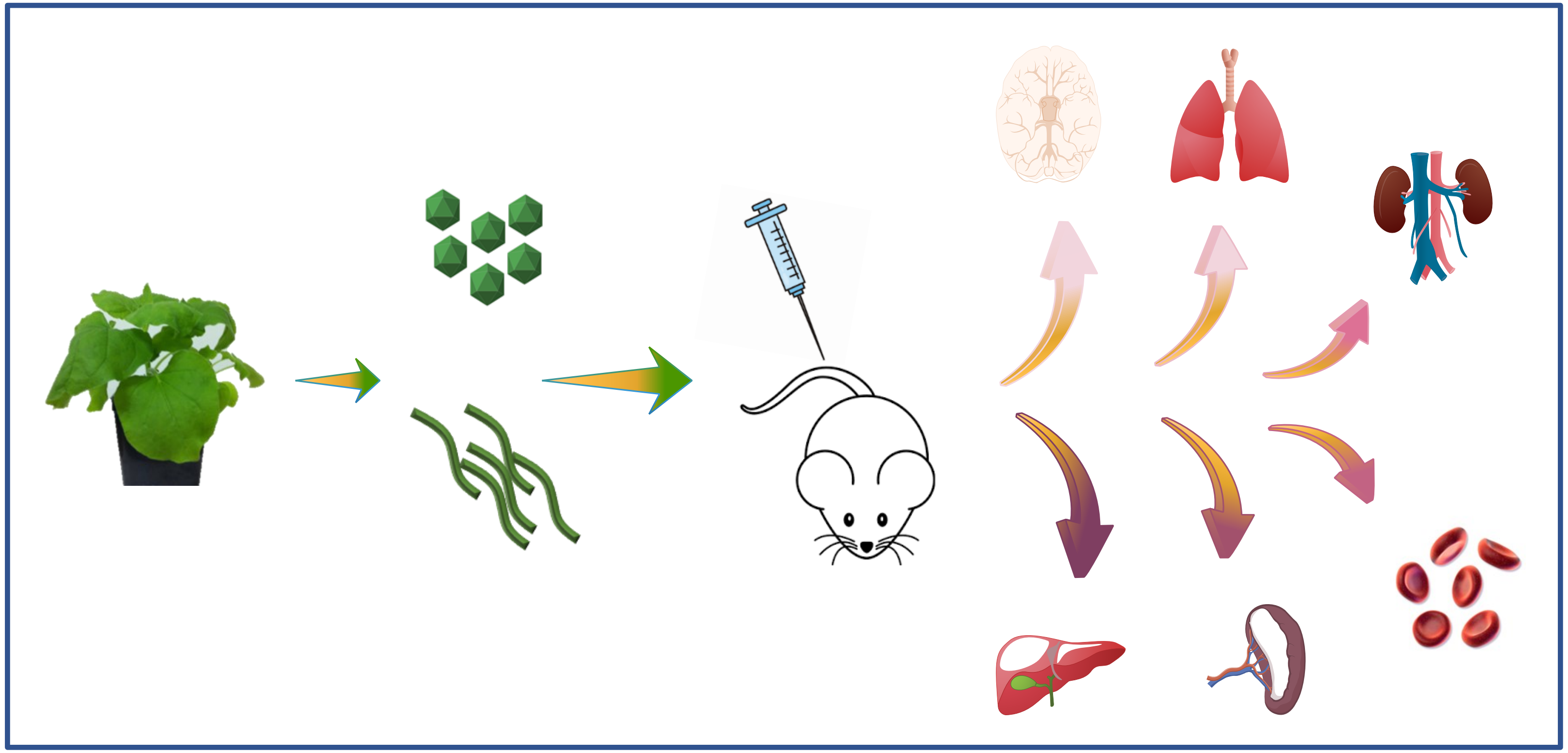
- ss(+) RNA 4788 nt

- 180 Coat Protein (CP)



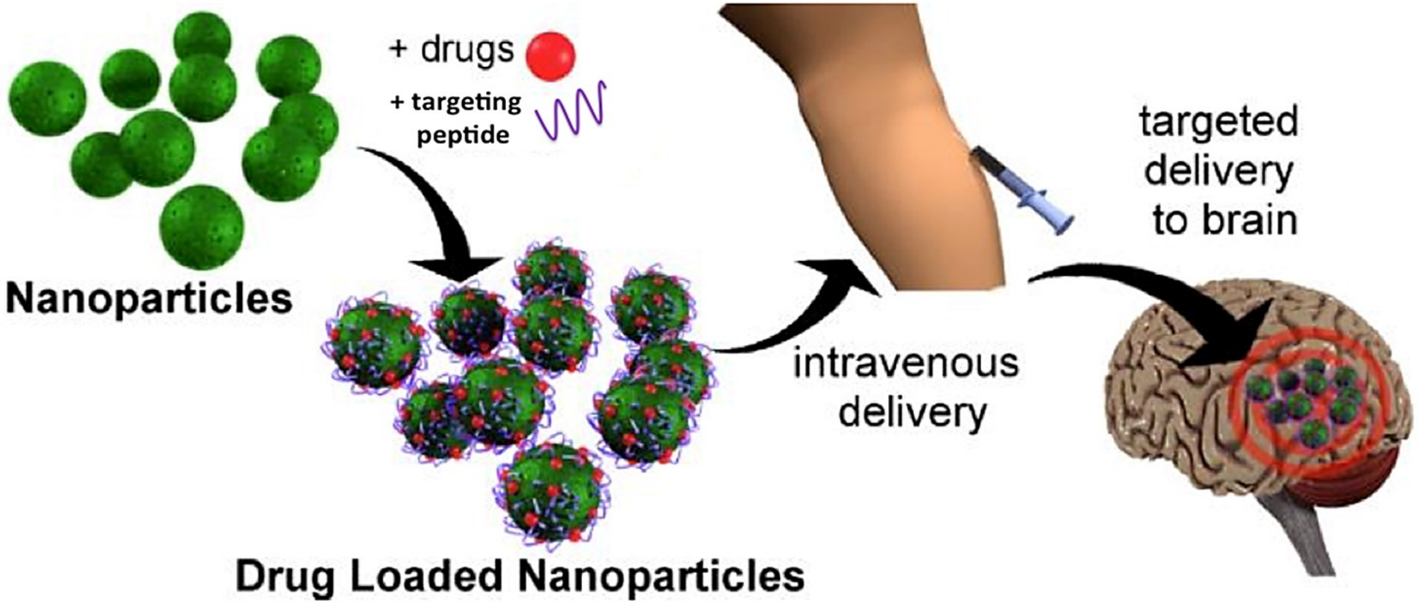


Biodistribution Evaluation



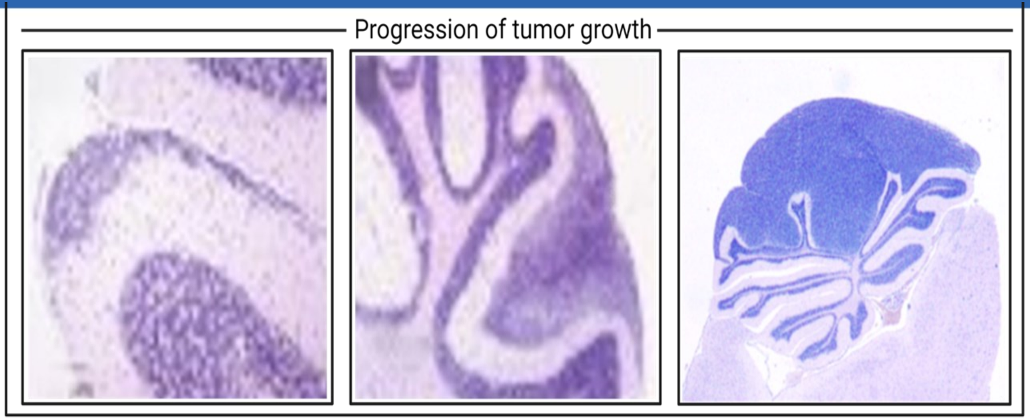
The NANOCROSS project

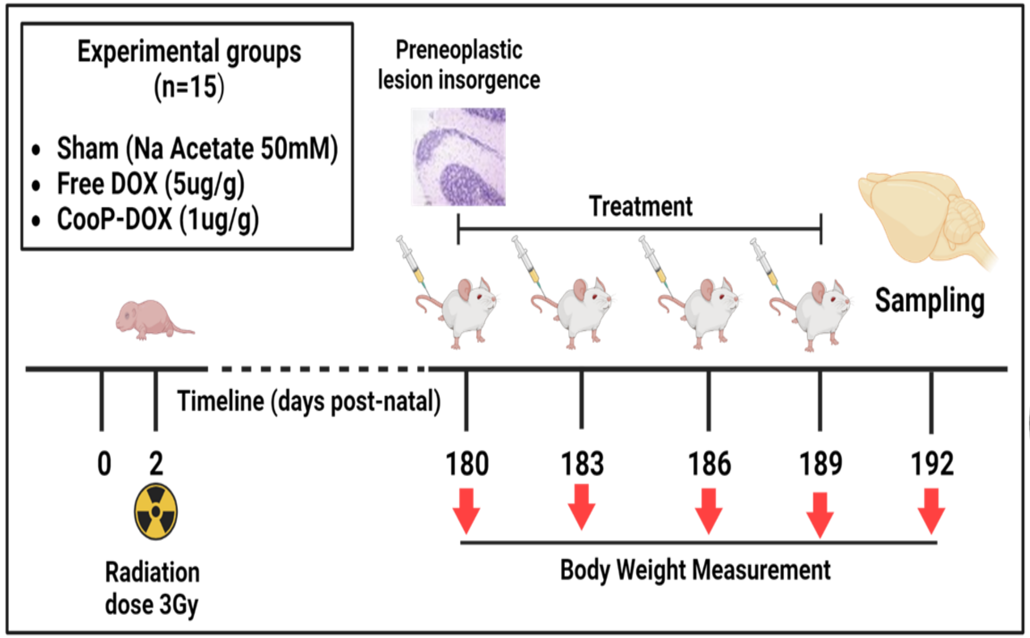
Plant virus nanoparticles for blood-brain barrier crossing and medulloblastoma targeting

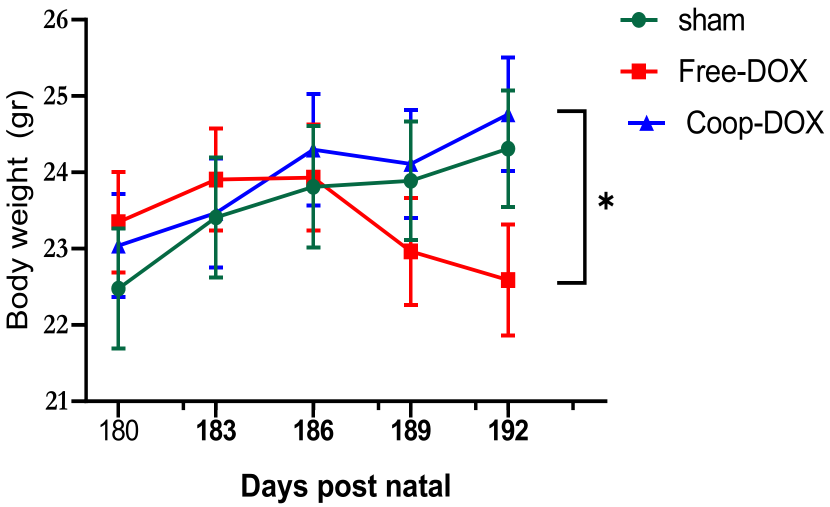


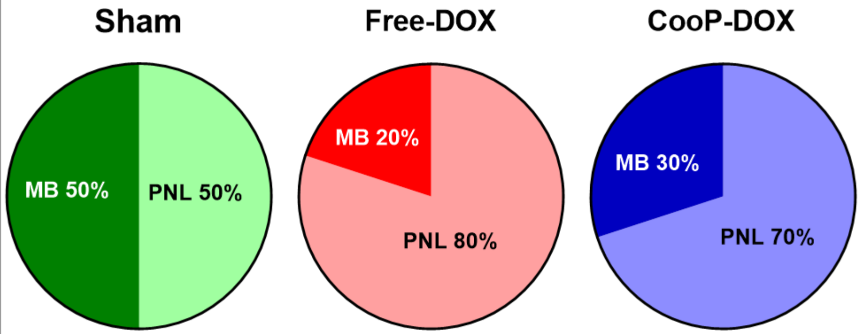
Medulloblastoma is a brain tumor affecting mainly young children, with the highest risk of unfavorable outcome and/or severe and life-long side effects for the aggressive nature of currently used therapy (surgery/radiotherapy/chemiotherapy)

In vivo therapeutic efficacy





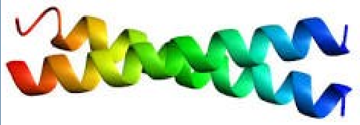


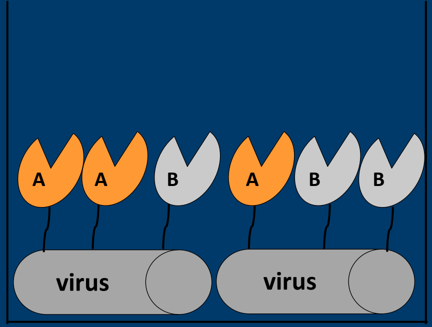


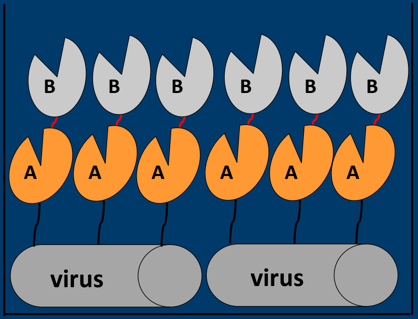
Enzyme nanocarriers (ENCs)

Coiled-coil interaction

Prof. Heribert Warzecha

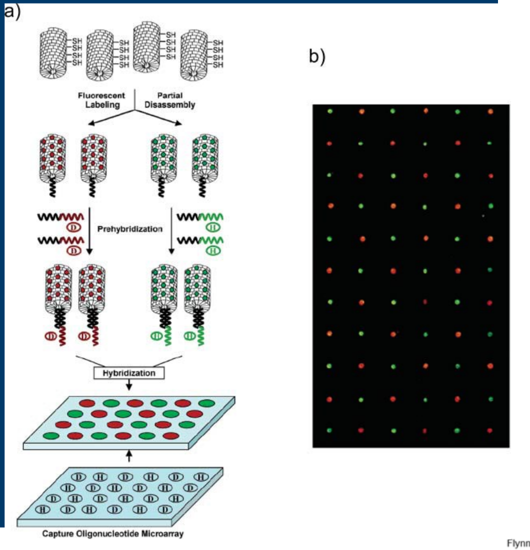


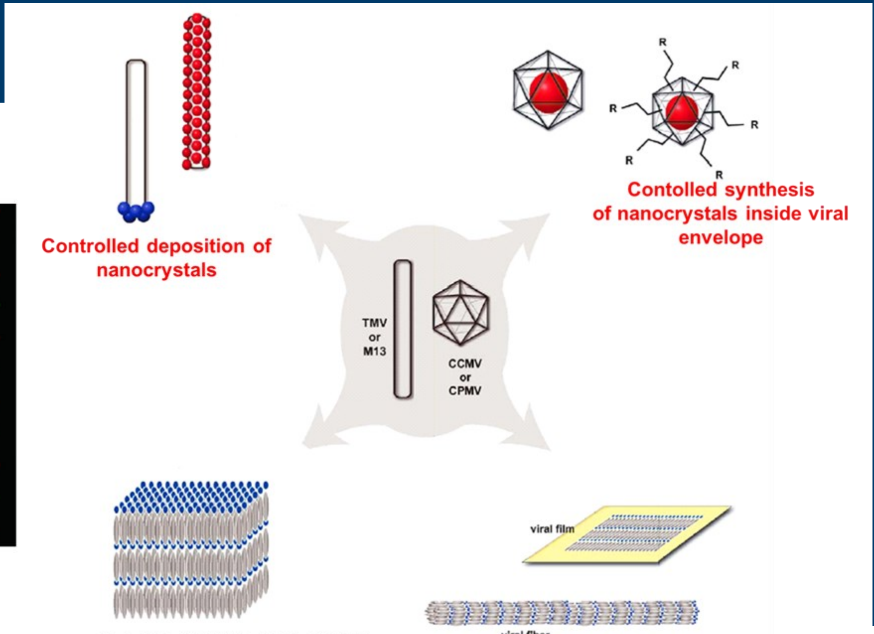




Viruses as nanomaterials

- *Tissue Egineering*
- *Nucleation cages, mineralization, crystals production*
- *Liquid crystals systems*
- *Biosensors*
- *Photovoltaic devices*





Flynn C.E., Lee S-W, Peelle, Belcher A.M. (2003) *Acta Materialia* **51**, 5867-5880.

Than you for your attention

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https://bioag.sspt.enea.it/