Empty virus-like particles (eVLPs) for plant synthetic biology

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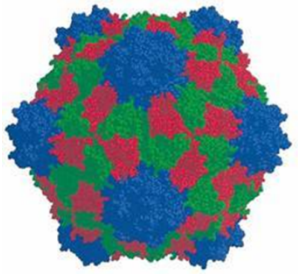
Introduction to Opportunities in Plant Synthetic Biology
21-22 May 2013
University of Nottingham



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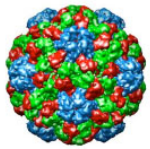
Why use virus particles in synthetic biology?

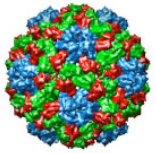
- They have dimensions on the nanoscale
- They are generally highly regular
- They can self-assemble
- They are bio-compatible
- They can be genetically and chemically manipulated
- They can be used as nanoscale containers

Virus particles that have been used

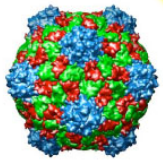
Icosahedral plant viruses



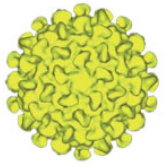
BMV



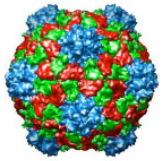
CCMV



CPMV

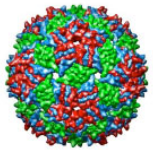


HCRSV

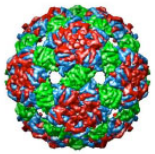


RCNMV

Icosahedral bacteriophages



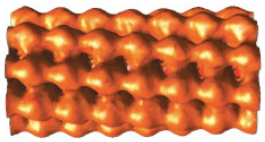
MS2



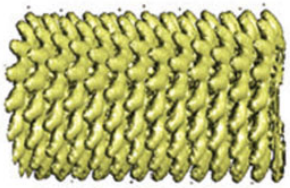
Qβ

..and odd shaped ones

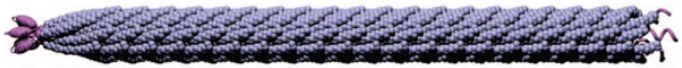
Rod-shaped plant viruses and a filamentous phage



PVX



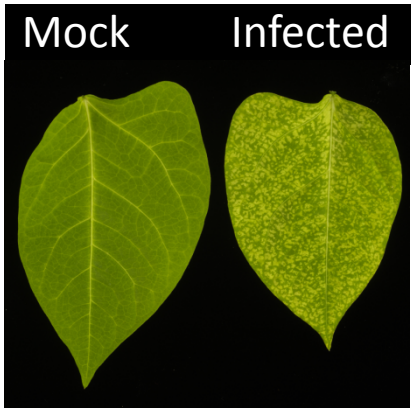
TMV



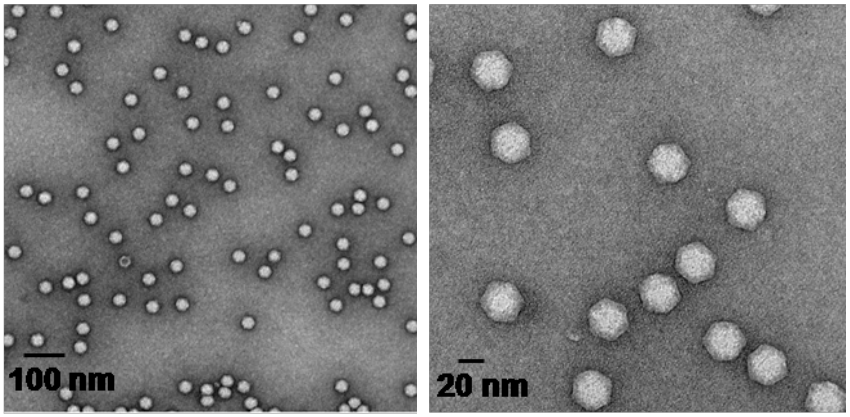
M13

Cowpea Mosaic Virus (CPMV)

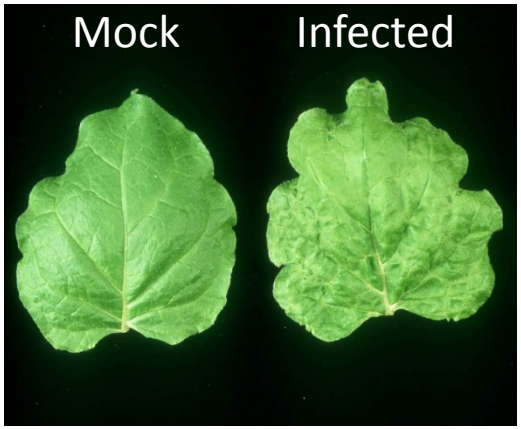
- Type member of family *Comoviridae*
- Natural host: Cowpea
- Causes chlorotic spots upon infection
- Grows to high titres (1g/kg) in cowpea



Symptoms of CPMV infection in cowpea



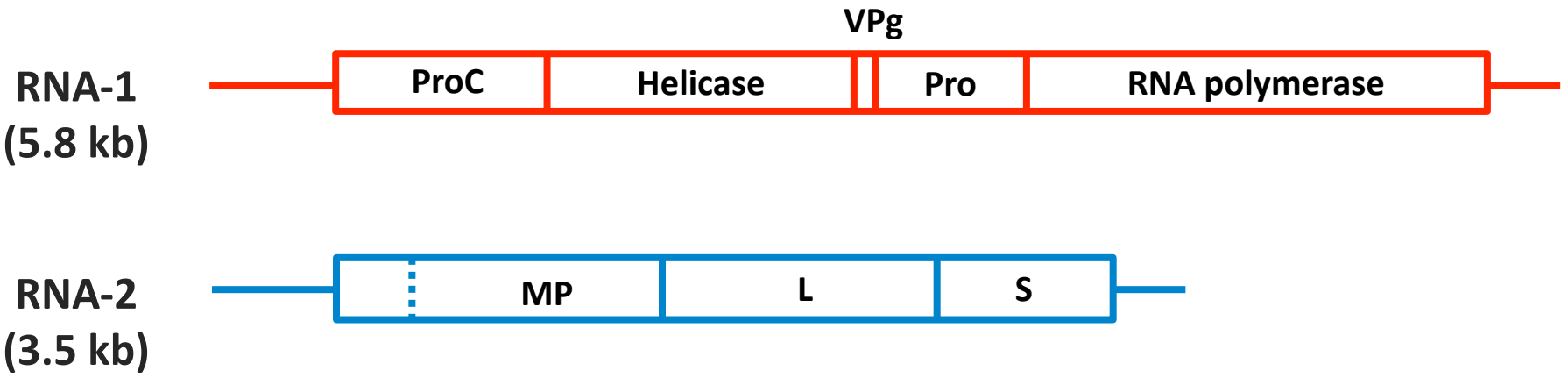
CPMV visualised using electron microscopy;
Stained with 2% uranyl acetate



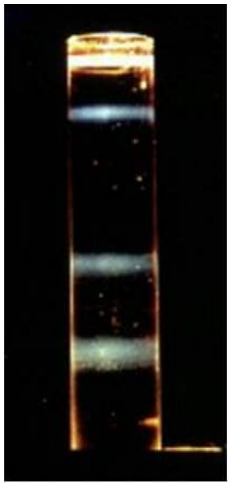
Symptoms of CPMV infection in *Nicotiana benthamiana*

Cowpea Mosaic Virus Genome

The CPMV genome consists of two positive-sense RNA.



A natural virus preparation contains three kinds of particles which can be separated on a density gradient.



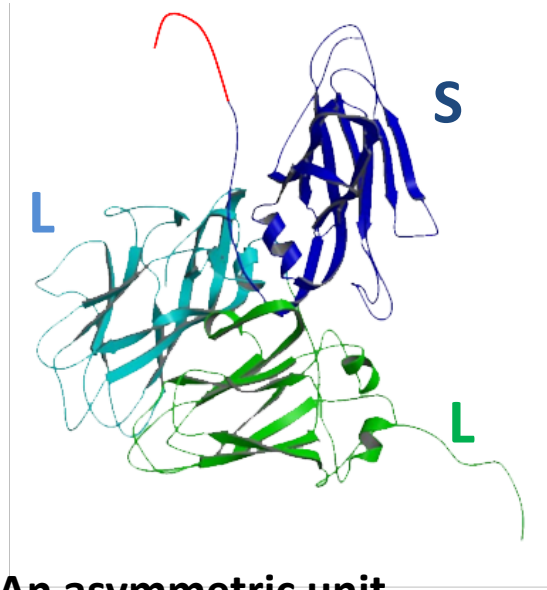
Top (RNA-free)

Middle
(RNA-2 containing particles)

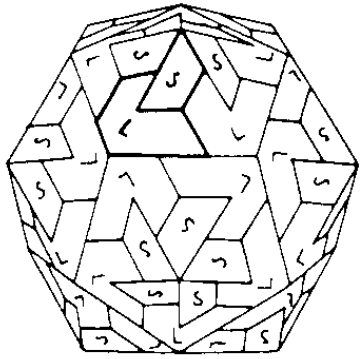
Bottom
(RNA-1 containing particles)

Cowpea Mosaic Virus Capsid

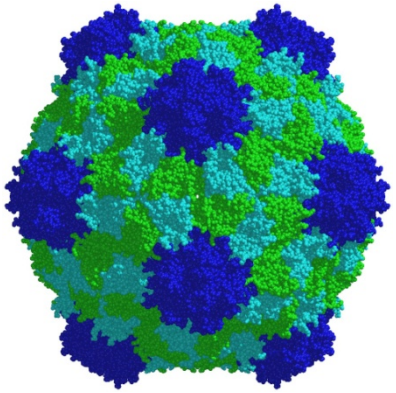
- The capsid consists of 60 copies of two subunits - **L**arge subunit (two domains) and **S**mall subunit (one domain).
- 60 asymmetric units are arranged in icosahedral symmetry.
- Diameter of the capsid is 28-30 nm.



An asymmetric unit
(before cleavage = VP60)



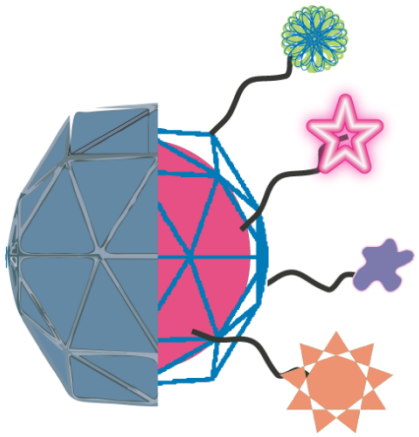
Icosahedral symmetry



CPMV full capsid

Infectious CPMV is a versatile particle

- the virus can be genetically and chemically manipulated
- organic, inorganic & biological molecules can be bound
- electro-active nanoparticles can be prepared
- multi-layer arrays can be constructed
- it can serve as a template for mineralization and metallization



Nanomaterials
Nanoelectronics
Nanoreactors

Imaging
Biosensors
Catalysis

Biomedicine

Problems using CPMV particles produced via infection

- Limits to the genetic changes you can make to the particle surface without losing viability
- Particles contain viral nucleic acid and are therefore infectious (regulatory issues).
- The presence of the viral RNA inside particles means there is no room to put anything else
- Thus particles cannot be loaded

SOLUTION – create empty virus-like particles (eVLPs)

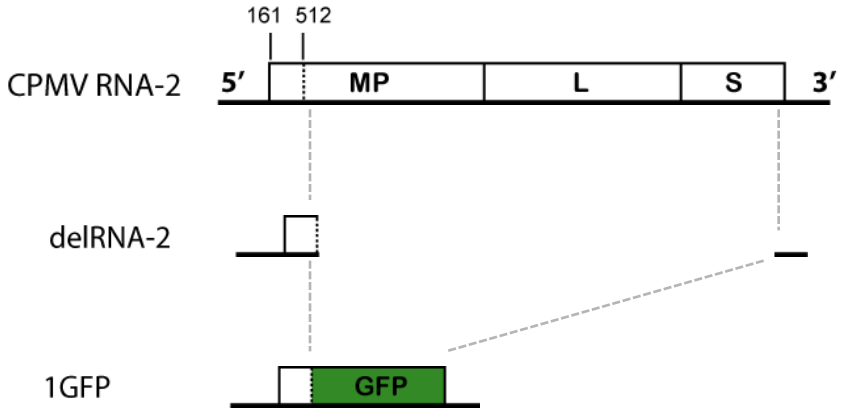
Potential advantages of empty virus-like particles (eVLPS)

- They are non-infectious therefore no danger of spreading a plant disease in the environment
- The fact that they are empty (no RNA inside) means that they can potentially be loaded with other material
- They do not have to be functional in a virological sense meaning more radical changes can be made.
- Therefore open up new possibilities for the particle technology

But how to produce these?

Use CPMV –*HT*!

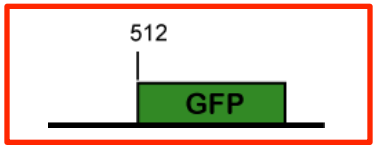
CPMV-*HT*

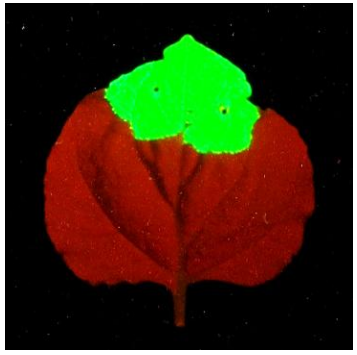


WT 5′

~0.1 g/kg GFP

CPMV-*HT* 5′





~1.5 g/kg GFP

CPMV-*HT* expression system

- Expression cassette: gene of interest flanked by
  - CaMV 35 S promoter
  - modified 5' UTR of Cowpea Mosaic Virus RNA-2
  - 3' UTR of CPMV RNA-2
  - *nos* Terminator

- *Agrobacterium*-mediated transient expression

Hyper-trans (HT)
expression cassette

Sainsbury *et al*. (2009) *Plant Biotech. J.* **7**, 682–693

Scalability

Bench scale (syringe), **µg (to mg)**

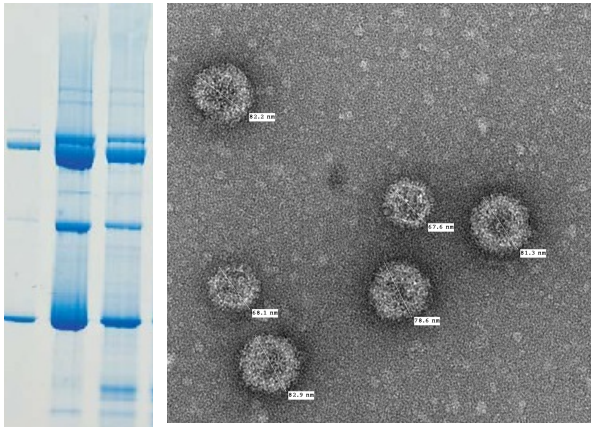
Pilot scale (vacuum), **mg**

→Industrial (vacuum), **g – kg!!**

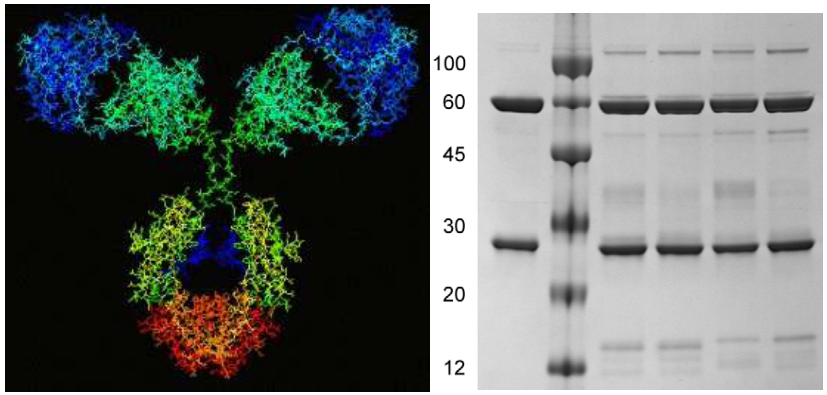
Successes using CPMV-*HT* system

Single protein (DsRed)

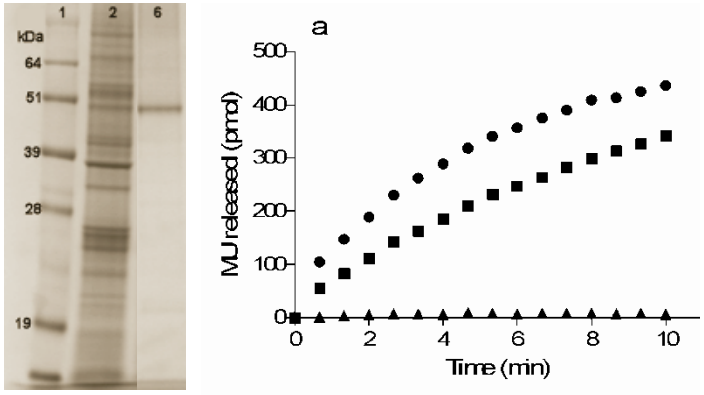
Four proteins (BTV VLPs)



Two proteins (anti-HIV antibody 2G12)



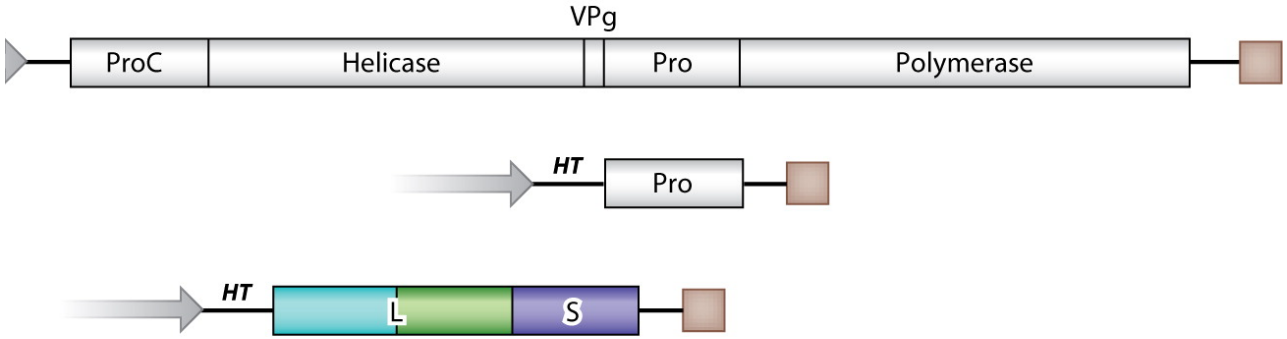
Active enzyme (hGL)



To produce CPMV eVLPs in plants

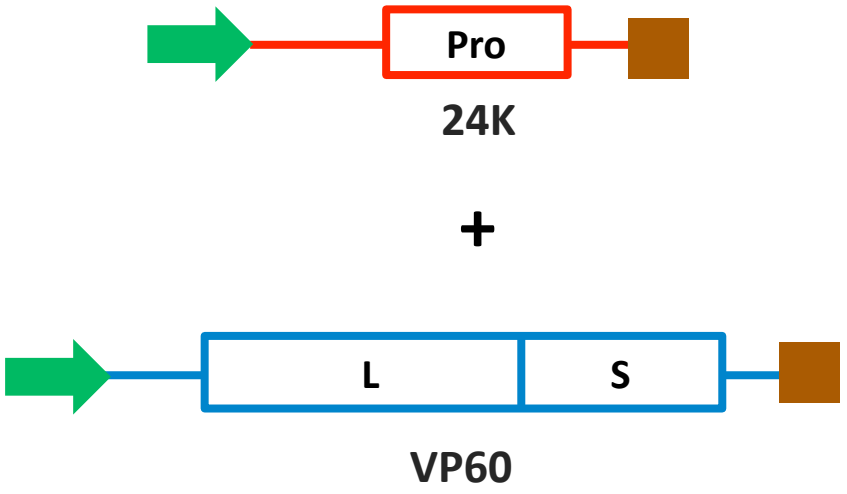
Need:

- source of coat protein precursor (VP60)
- 24K proteinase to achieve processing
- Co-expression of the two



Production of CPMV VLPs

Co-expression of coat protein precursor VP60 and the viral proteinase 24K produces empty virus-like particles or **eVLPs**.



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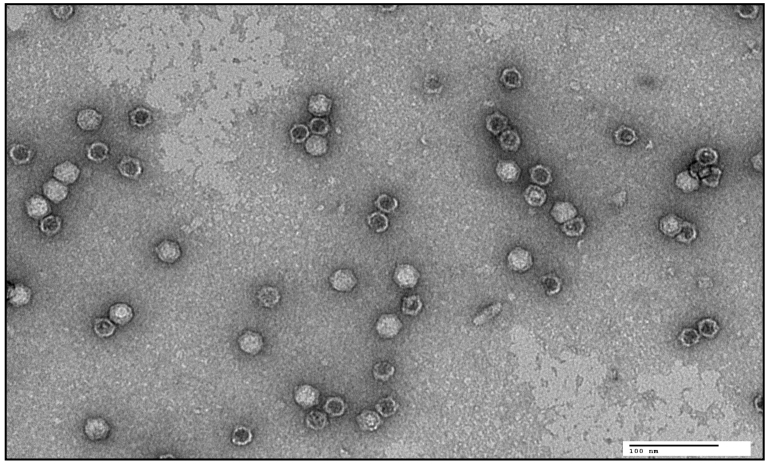
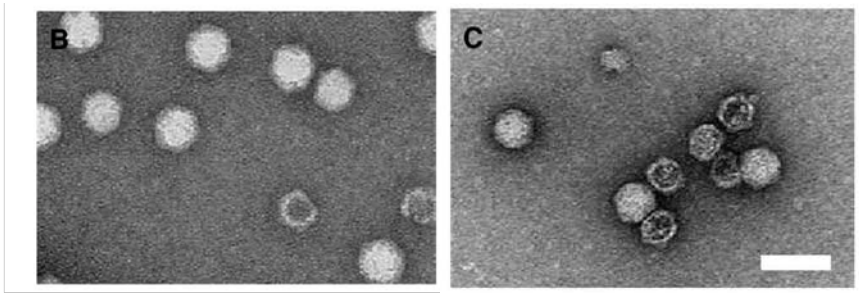


RNA-free particles of CPMV visualised under TEM

Can express VP60 from separate plasmids or from same plasmid

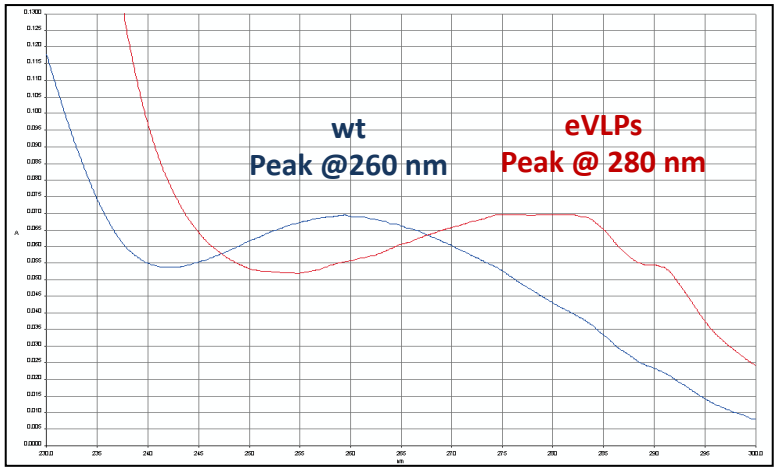
Saunders, Sainsbury and Lomonossoff (2009). *Virology* **393**, 329–337.

Absence of RNA within particles

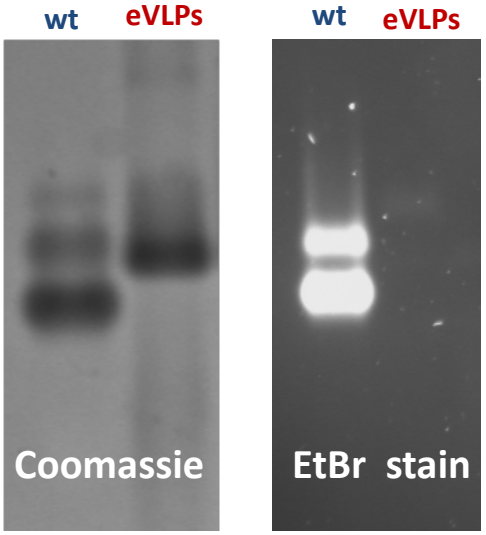


wt eVLPs

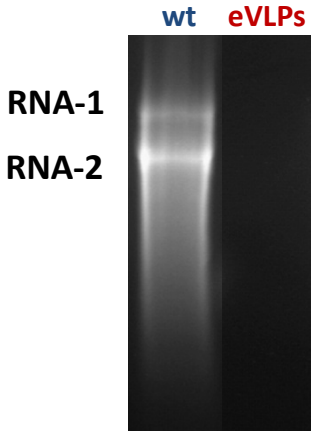
Transmission electron microscopy



UV-Vis spectra of particles

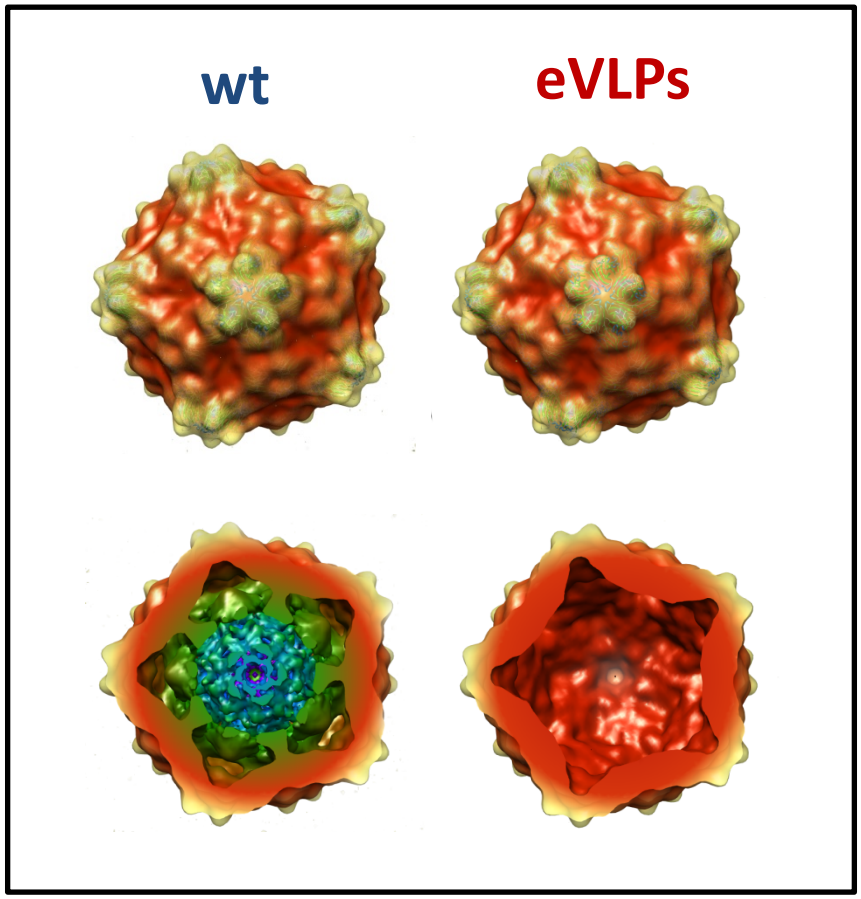


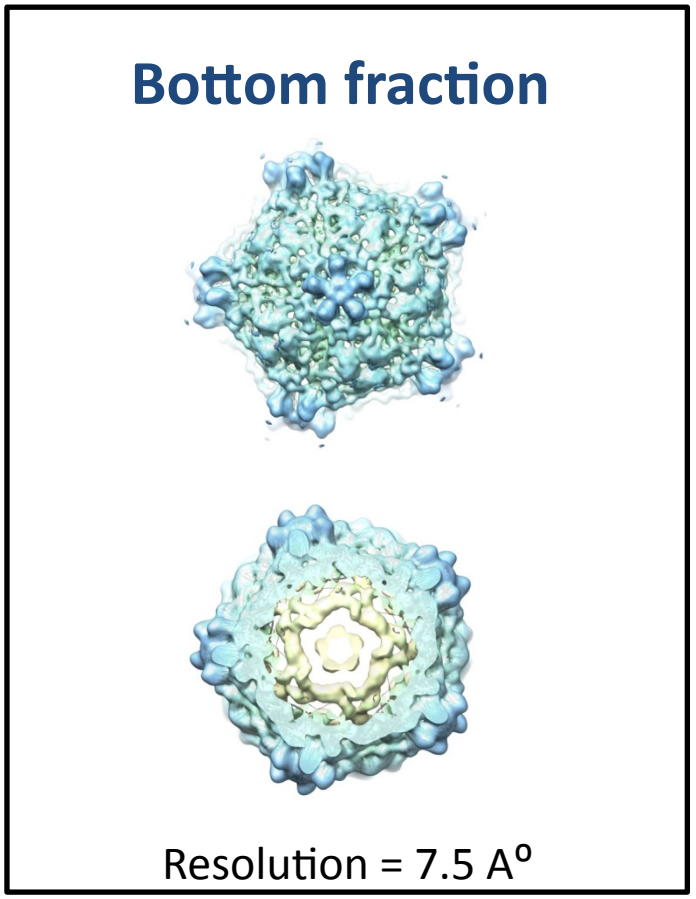
Particle migration in agarose gels



Electrophoresis of extracted RNA

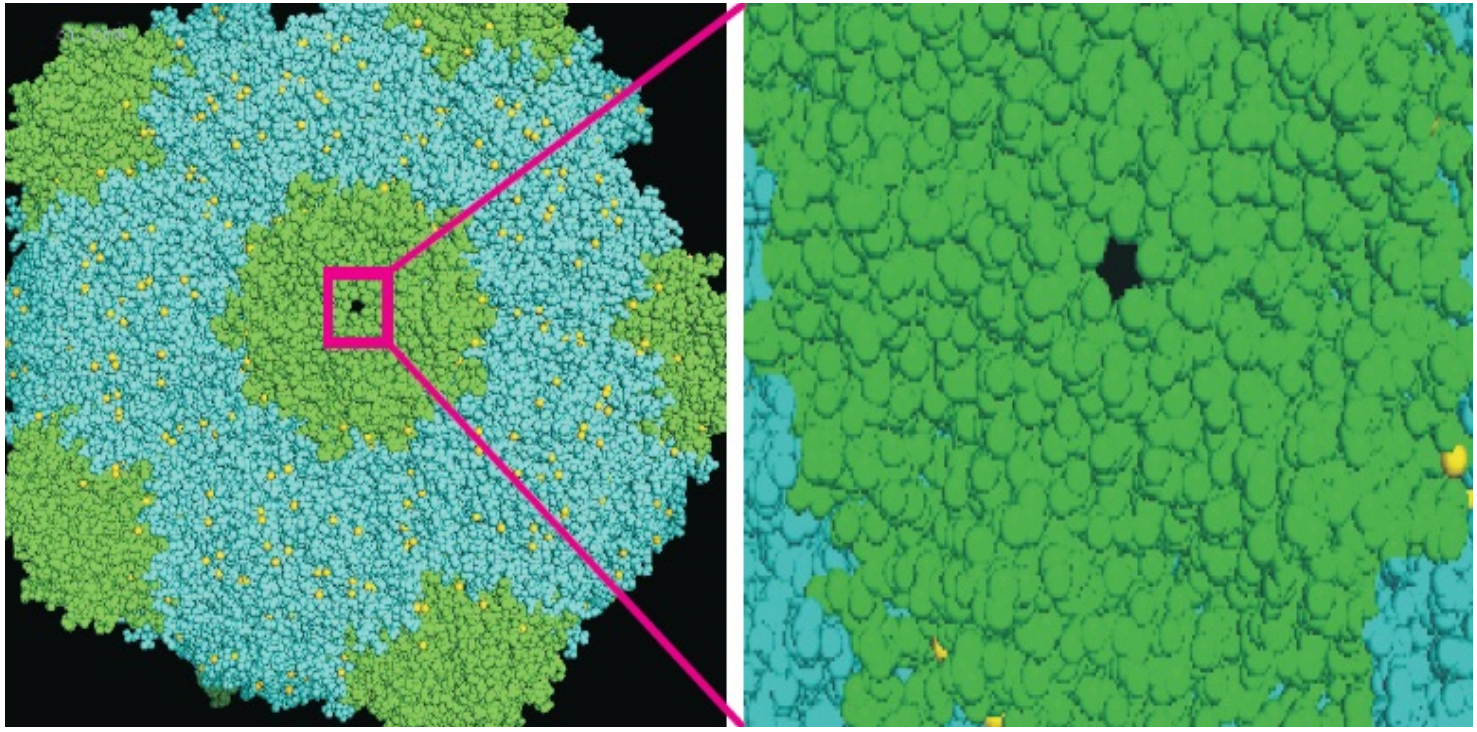
Cryo-EM reconstructions of wt CPMV and eVLPs





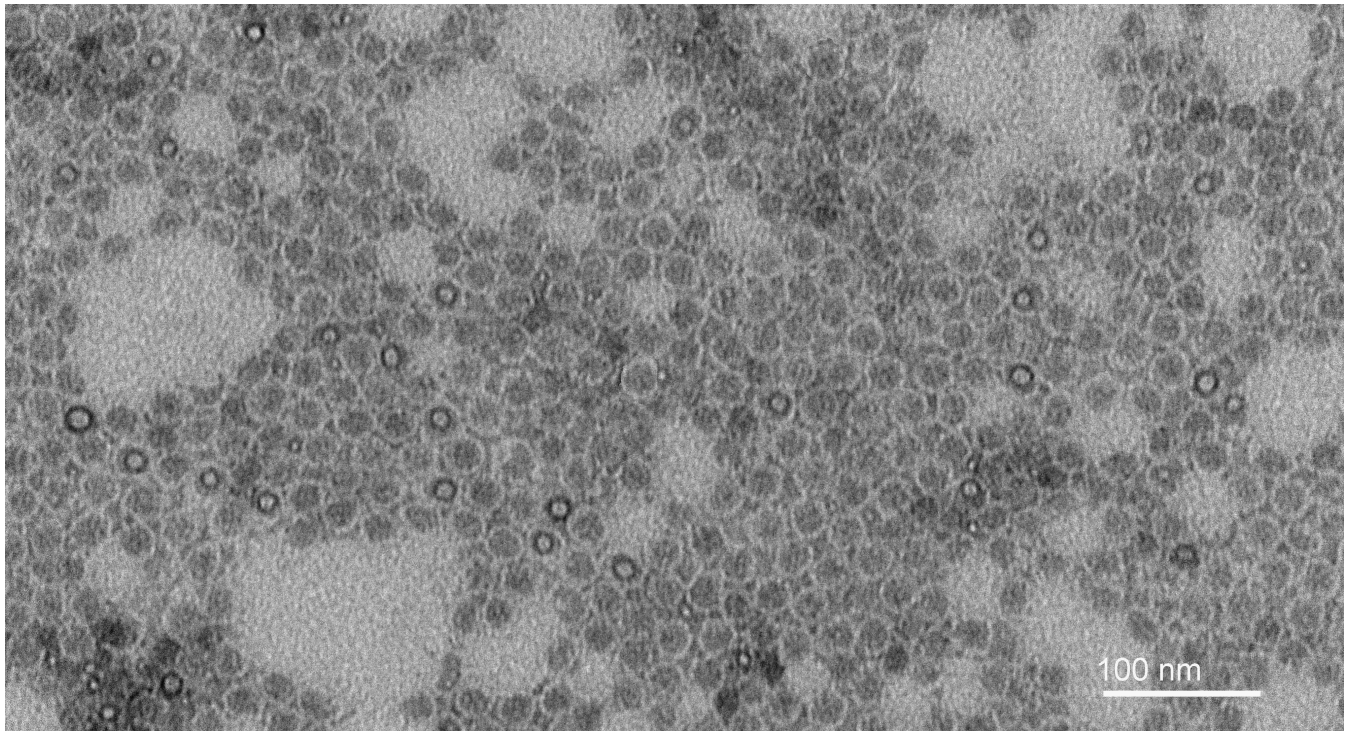
Surface view (top row) and cutaway view (bottom row) of particles.
Work done at the University of Leeds.

Possibility of loading eVLPs via pores



Filling eVLPs with Cobalt

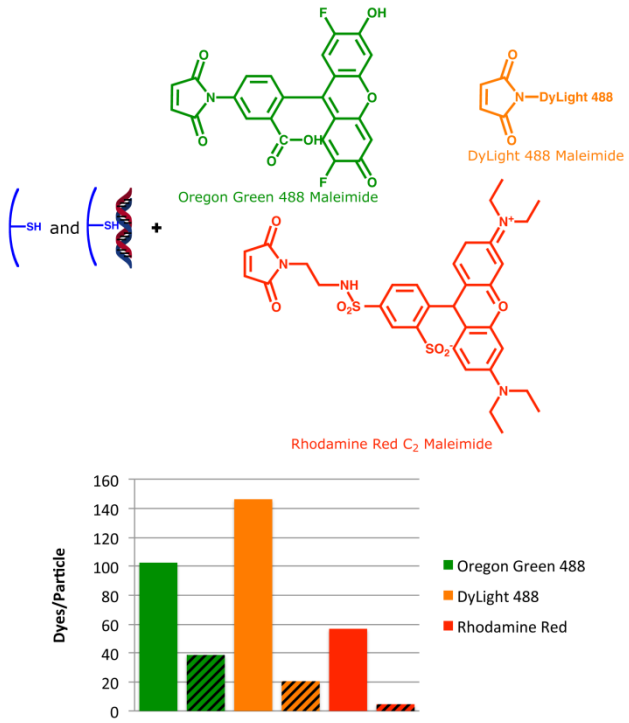
- eVLPs soaked in Cobalt chloride, washed and treated with sodium borohydride



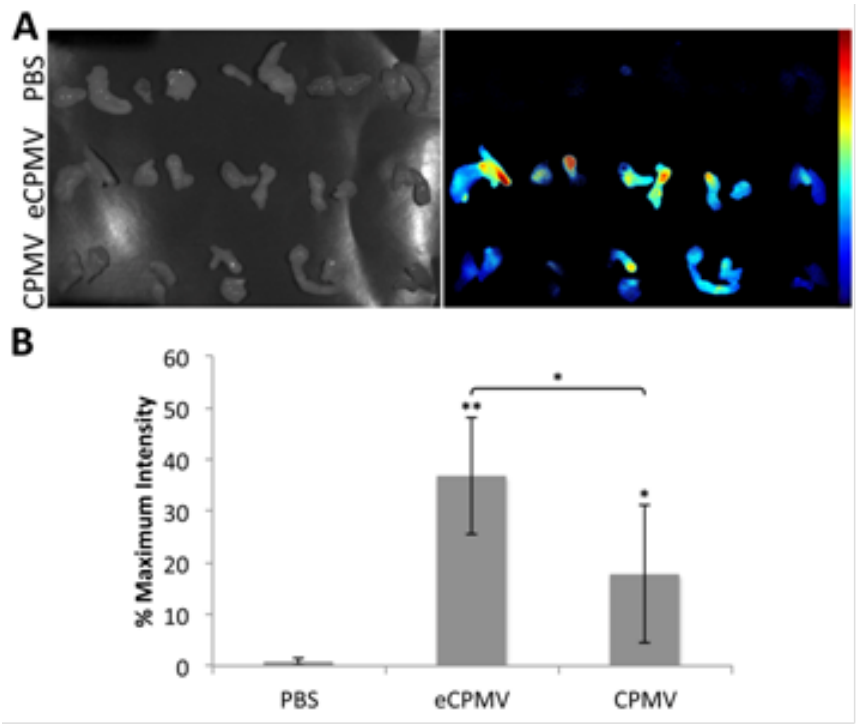
Unstained TEM image

Aljabali *et al*. (2010). *Small* **6**, 818-821.

eVLPs can be efficiently labelled at internal Cysteines



Comparison of internal labelling of eVLPs with wt CPMV

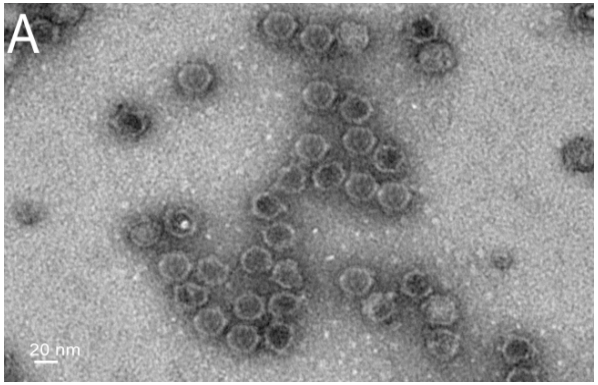


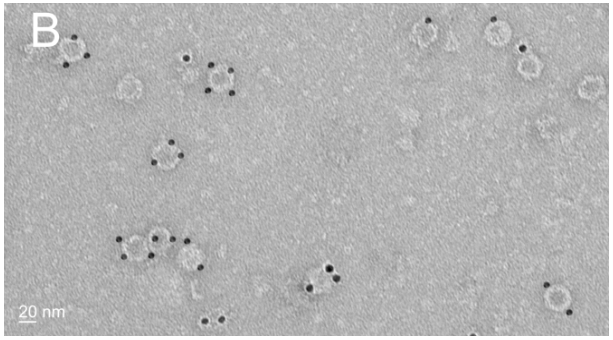
Tumour homing of internally labelled eVLPs and wt CPMV

Wen *et al*. (2012). *Biomacromolecules* **13**, 3990-4001

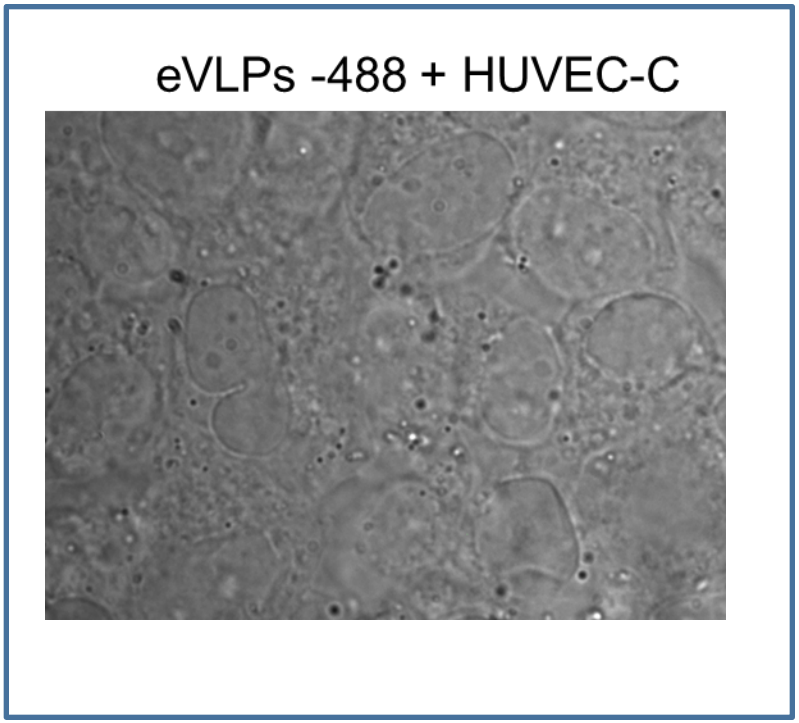
Simultaneously modifying inside and outside

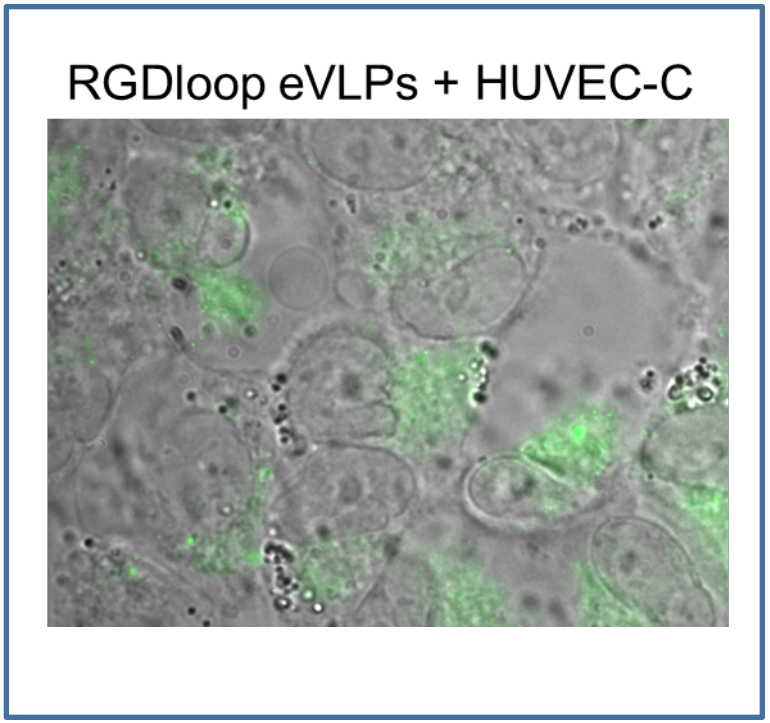
- eVLPs loaded with iron oxide (A)
- Outer surface coupled to biotinylated targeting oligopeptide
- Presence of peptide detected by streptavidin functionalised gold nanoparticles (B)





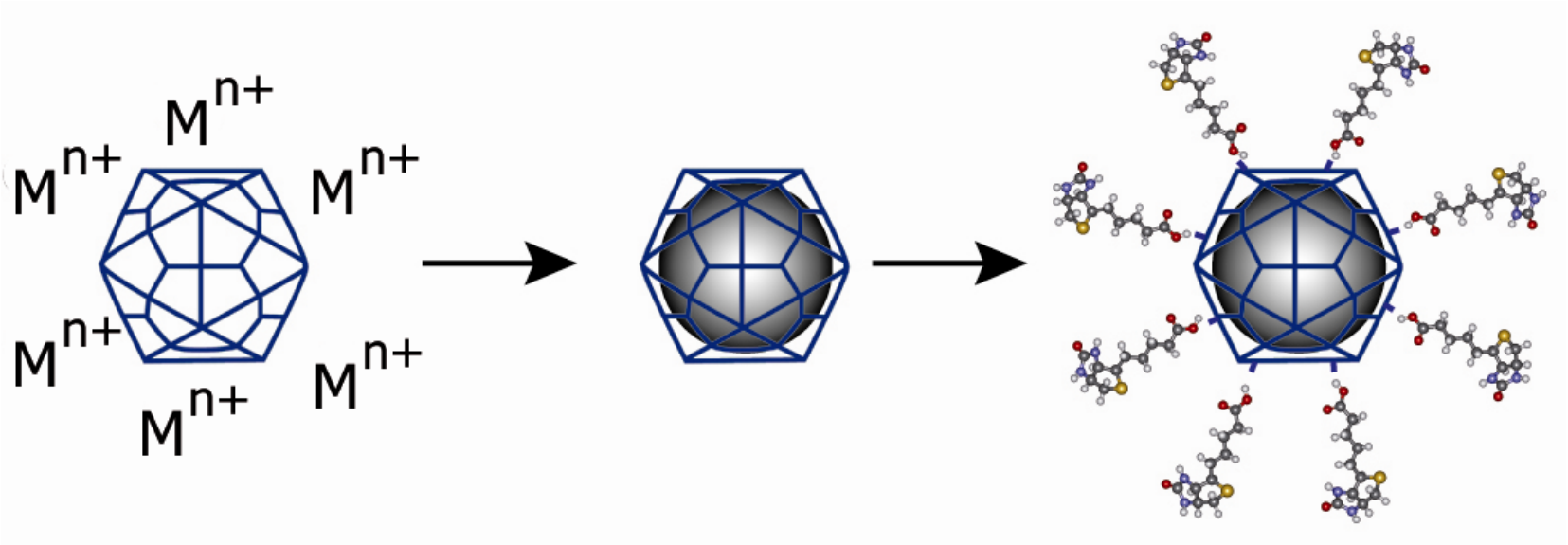
Targeting sequences can be genetically fused to outer surface of eVLPs



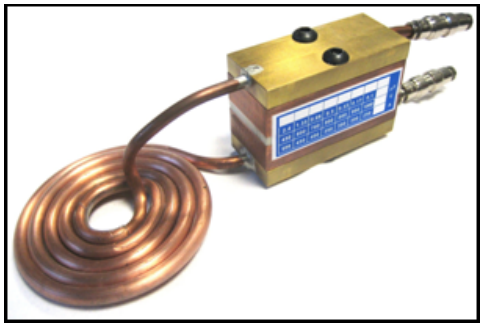


Comparison of binding of fluorescently labelled eVLPs without (left) and with (right) the RGD motif in the βB-βC loop of the S-protein.

Possible use of loaded, targeted eVLPs

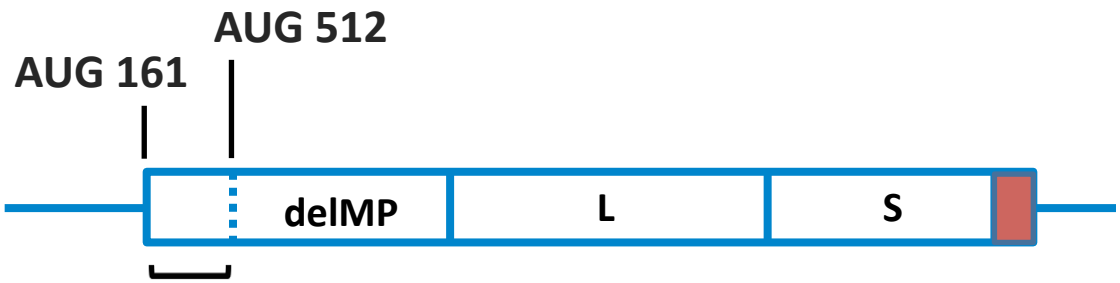


Applications for such targeted molecules in magnetic field hyperthermia therapeutics

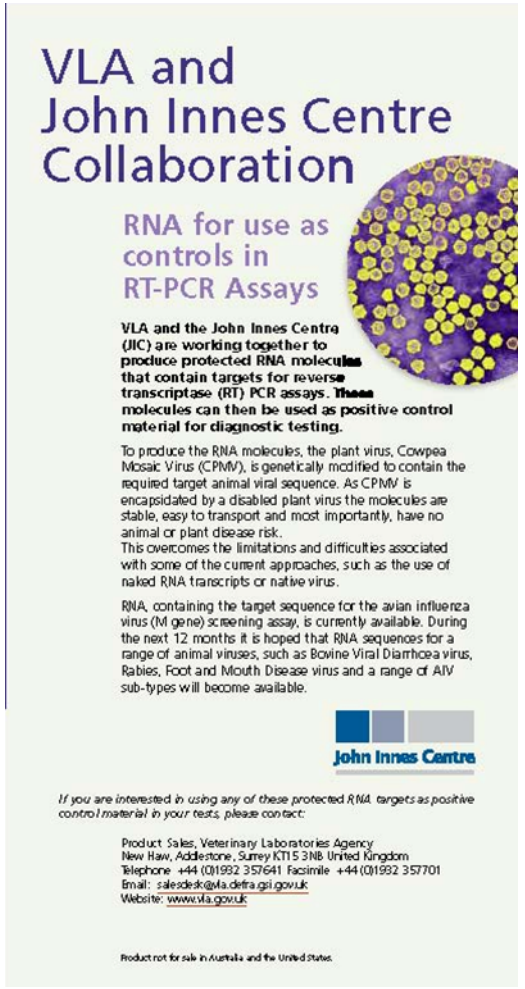


Potential applications of eVLPs: DNA/RNA delivery vehicle

RNA mimics



- Disarm movement protein
- Genetically modify coding sequence to include additional sequences for RNA of interest
- Express in presence of RNA-1
- Purify particles using a CsCl gradient



VLA and John Innes Centre Collaboration

RNA for use as controls in RT-PCR Assays

VLA and the John Innes Centre (JIC) are working together to produce protected RNA molecules that contain targets for reverse transcriptase (RT) PCR assays. These molecules can then be used as positive control material for diagnostic testing.

To produce the RNA molecules, the plant virus, Cowpea Mosaic Virus (CPMV), is genetically modified to contain the required target animal viral sequence. As CPMV is encapsidated by a disabled plant virus the molecules are stable, easy to transport and most importantly, have no animal or plant disease risk.
This overcomes the limitations and difficulties associated with some of the current approaches, such as the use of naked RNA transcripts or native virus.

RNA, containing the target sequence for the avian influenza virus (M gene) screening assay, is currently available. During the next 12 months it is hoped that RNA sequences for a range of animal viruses, such as Bovine Viral Diarrhoea virus, Rabies, Foot and Mouth Disease virus and a range of AIV sub-types will become available.

John Innes Centre

If you are interested in using any of these protected RNA targets as positive control material in your tests, please contact:

Product Sales, Veterinary Laboratories Agency
New Haw, Addlestone, Surrey KT15 3NB United Kingdom
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