Protein nanoparticles for molecular therapy

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Molecular Therapy

Therapy in general: Atenuate / Prevent / Cure } Disease

Specific Targeting

Efficient Delivery and Transport

↓

Development of new molecular vectors

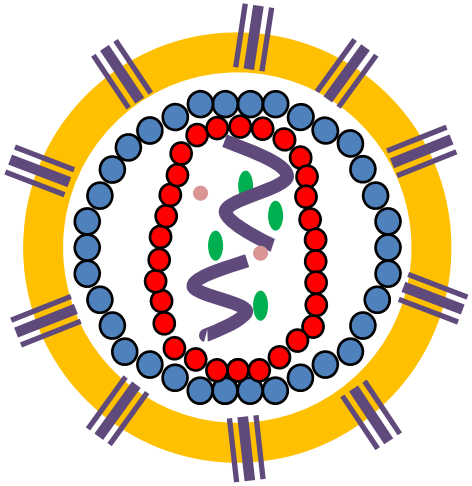
Molecular Therapy – VLP's

Aim: Transport and Delivery of Therapeutical Active Agents

VECTOR	EXAMPLES	ADVANTAGES	DISADVANTAGES
Non-viral	Liposomes; Polymer Nanoparticles	Safety; Easy to produce in large scale.	Low expression times; Low delivery efficiency; Low specificity.
Viral	Adenoviruses; Adeno-associated viruses; Retroviruses	High efficiency; High expression times.	Safety; Immune responses; Difficult to produce in large scale.
Hybrid methods	Fusogenic liposomes	More specific than non-viral vectors; Safer than viral vectors.	Difficult to produce in large scale.

Molecular Therapy – VLP's

E.g. Virus



REMOVE	MINIMIZE
Genome	Safety Issues
Envelope Proteins	Immune Responses
Accessory Proteins	Large-Scale Production Problems

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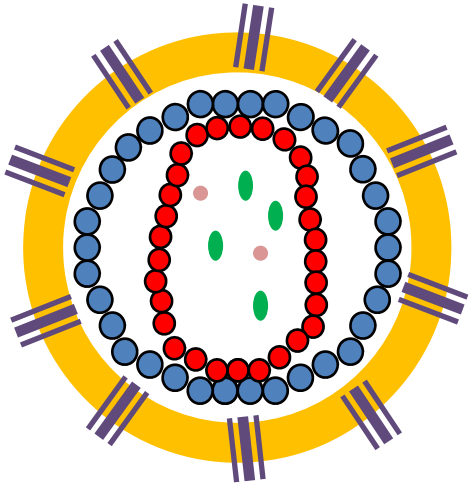

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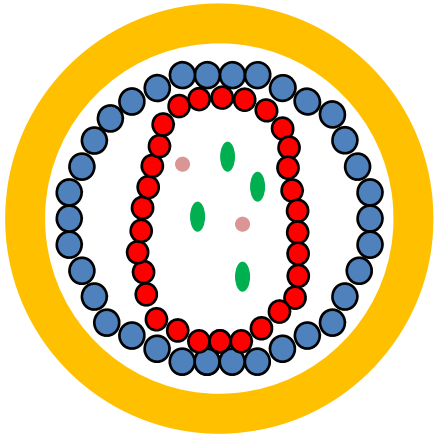

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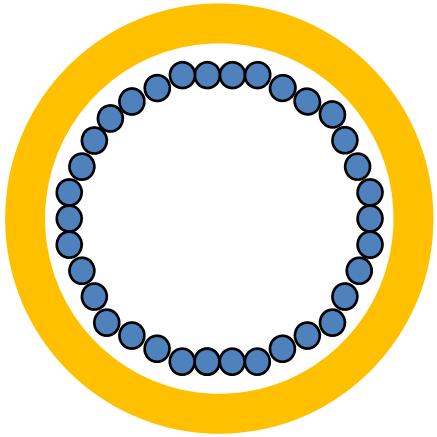

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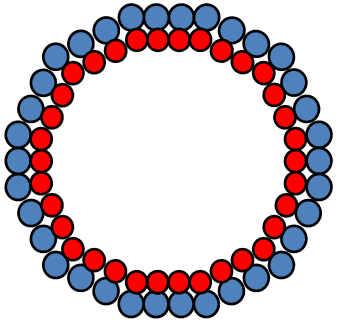
Virus-like particle with minimal viral protein content

Objectives

1. Construction of a viral nanoparticle
   a) Expression in animal cells
   b) Acess viral encapsulation with a lipidic membrane

2. Pseudotyping

3. Incorporation of a reporter protein



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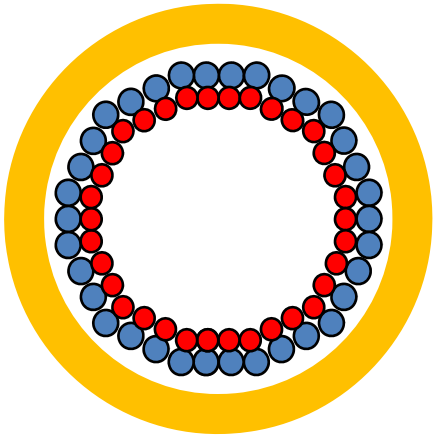

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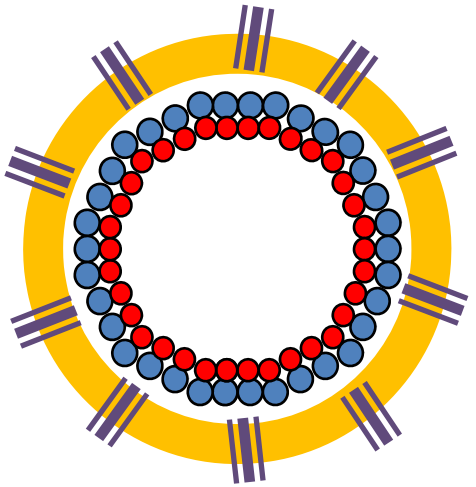

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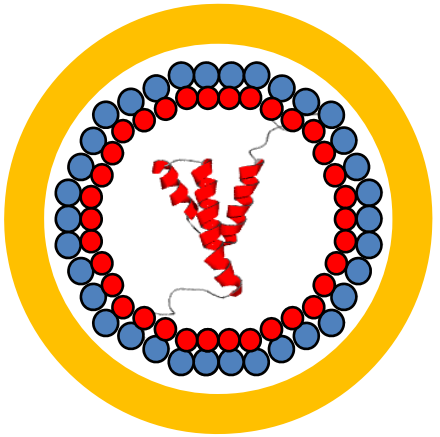
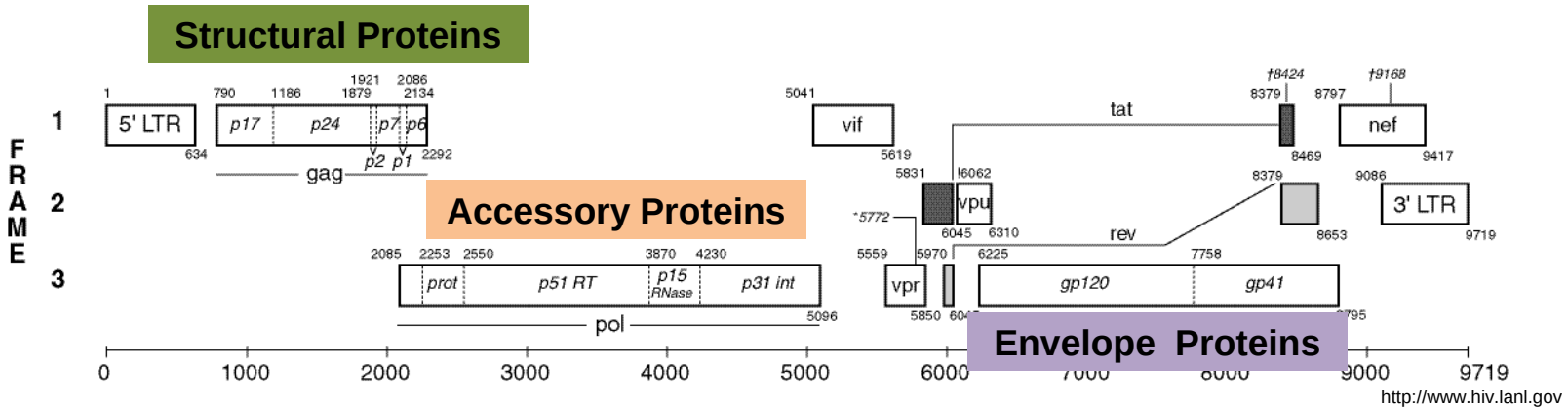


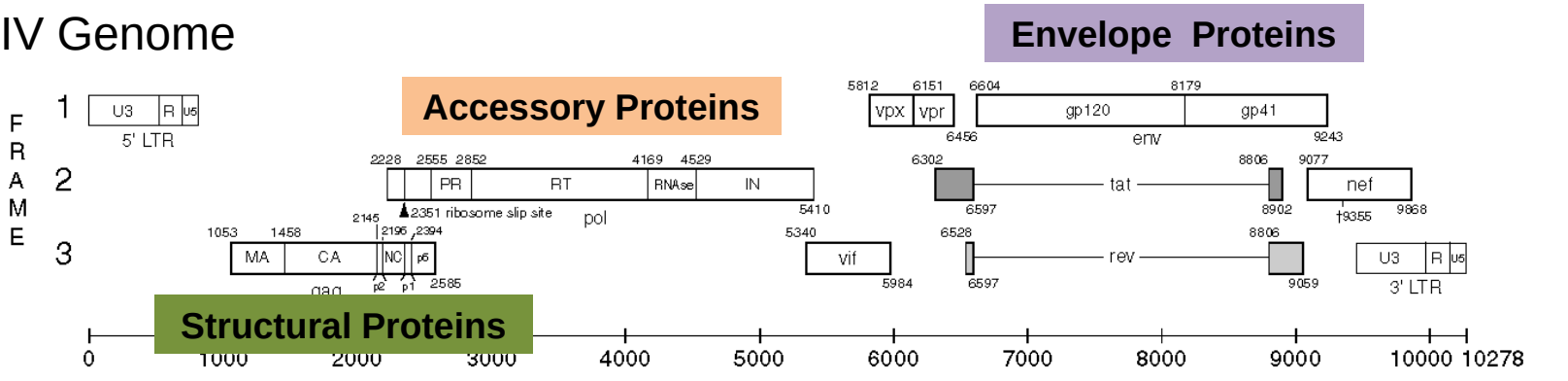
1. Nanoparticle Construction

HIV-1 Genome



http://www.hiv.lanl.gov

SIV Genome



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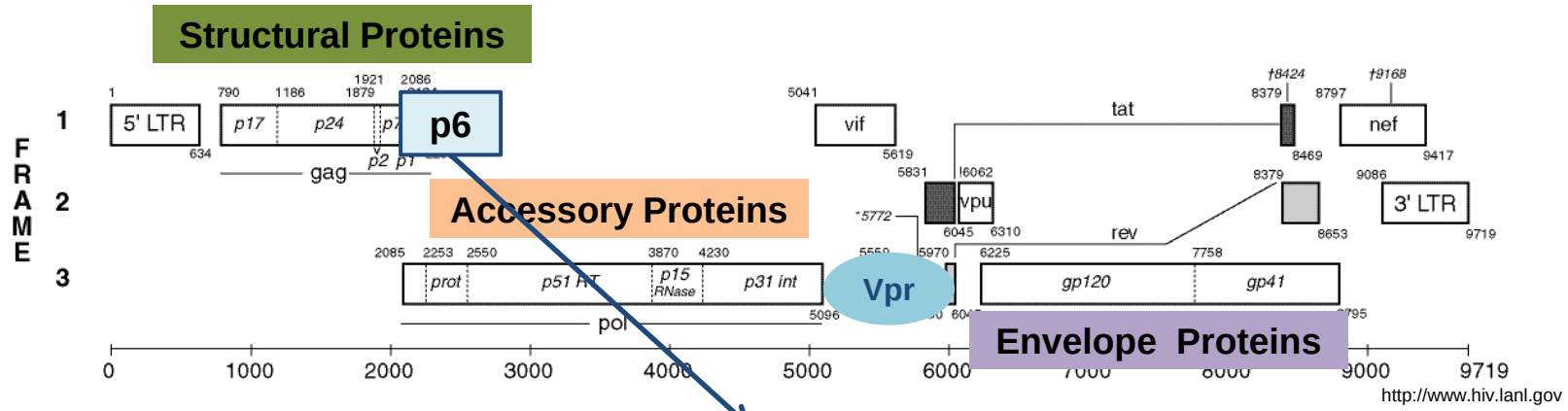

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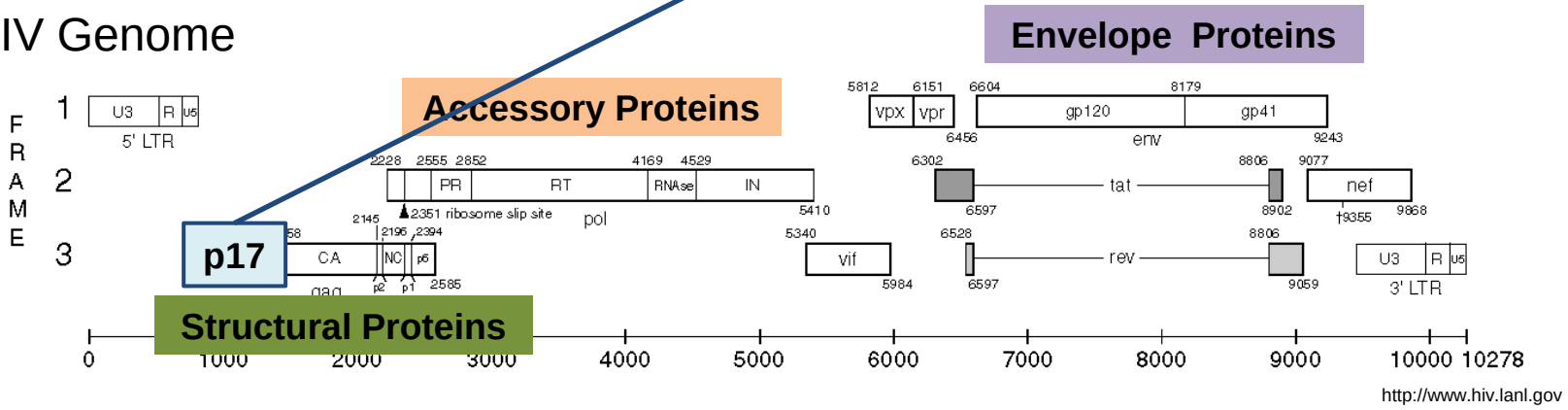
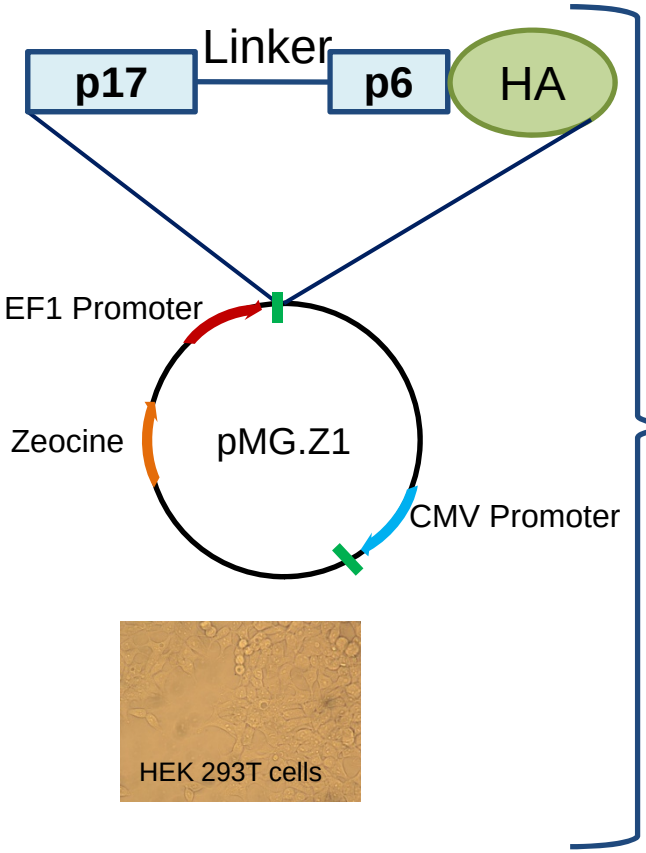
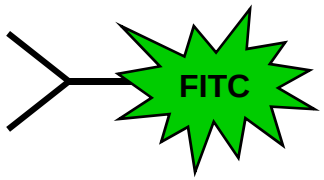
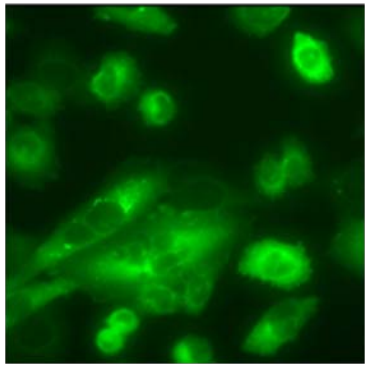


2. Nanoparticle Expression

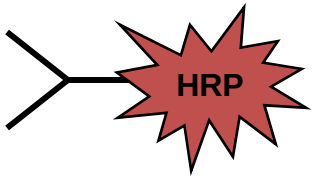


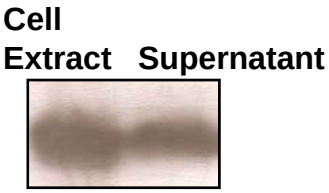
Fluorescence Microscopy



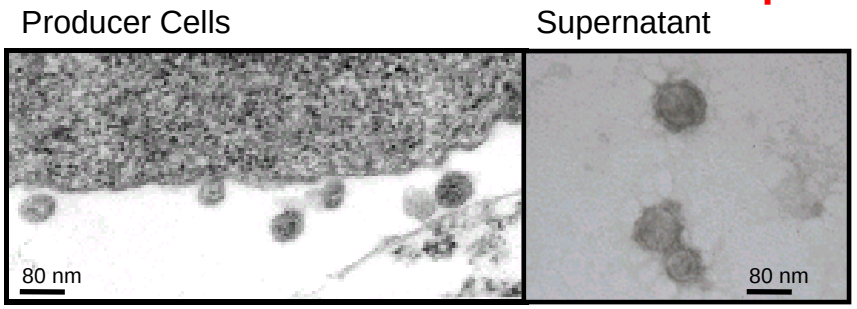


Western Blot





Electron Microscopy



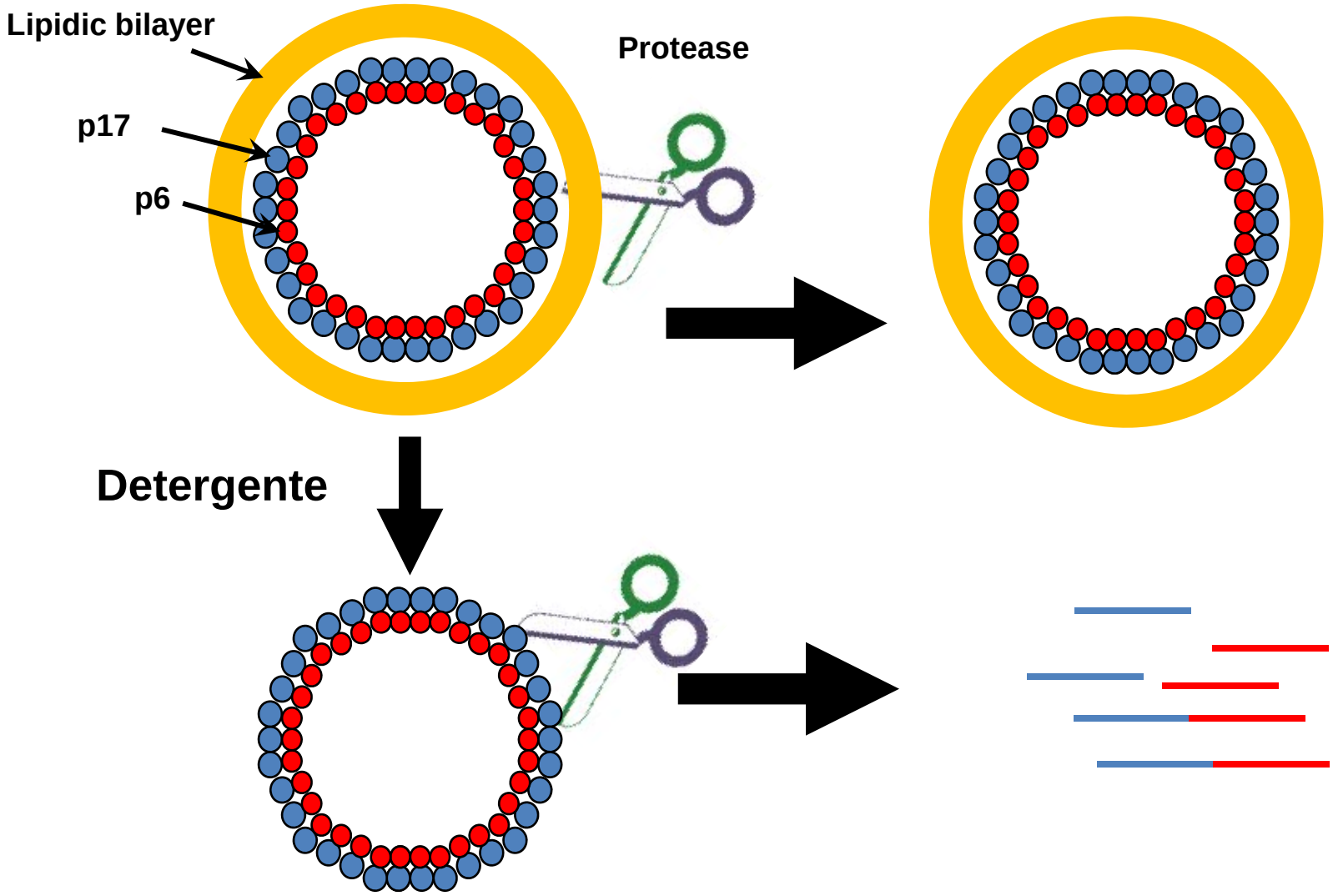
Summarizing:

Proteins expressed to the culture medium as spherical particles of 80nm.
Surrounded by a lipidic membrane??

Costa et al. (2007) Biotechnol. Appl. Biochem., **48**: 35–43.

2. Nanoparticle Characterization

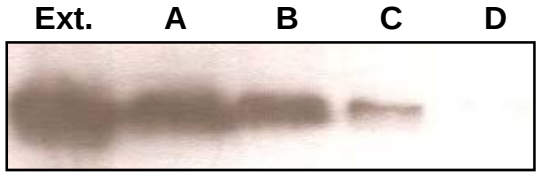
Protease Protection Assay



2. Particle Characterization

Protease Protection Assay

(Protease Degradation)



Ext. – Cell Extract

A - Untreated

B- 1 % Triton X-100

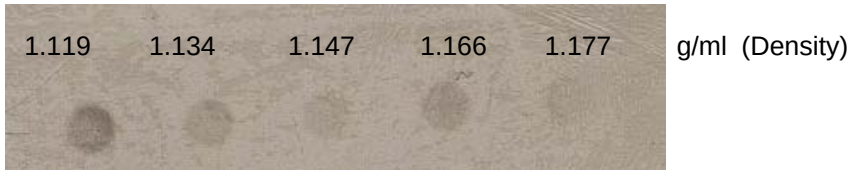
C - Proteinase K 1 μg/ml

D - 1 % Triton X-100 and Proteinase K 1 μg/ml

(with Anti-HA HRP)

Density Analysis

(20-80%(w/v) Sucrose Gradient Ultracentrifugation)



Top Bottom

Average density: 1.147 g/ml

Summarizing:

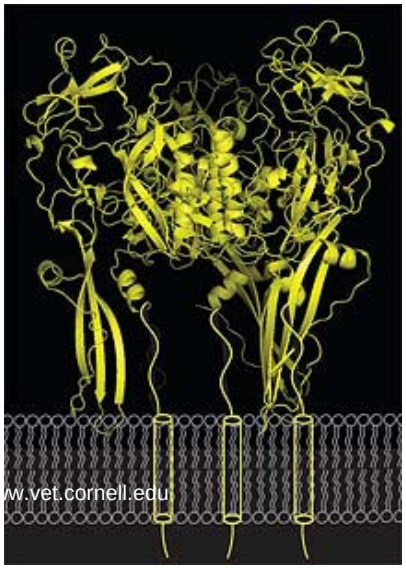
Dense spherical particle with 80nm;
Density of 1.147 g/ml;
Surrounded by a lipidic membrane.

Association between the particle and envelope protein?

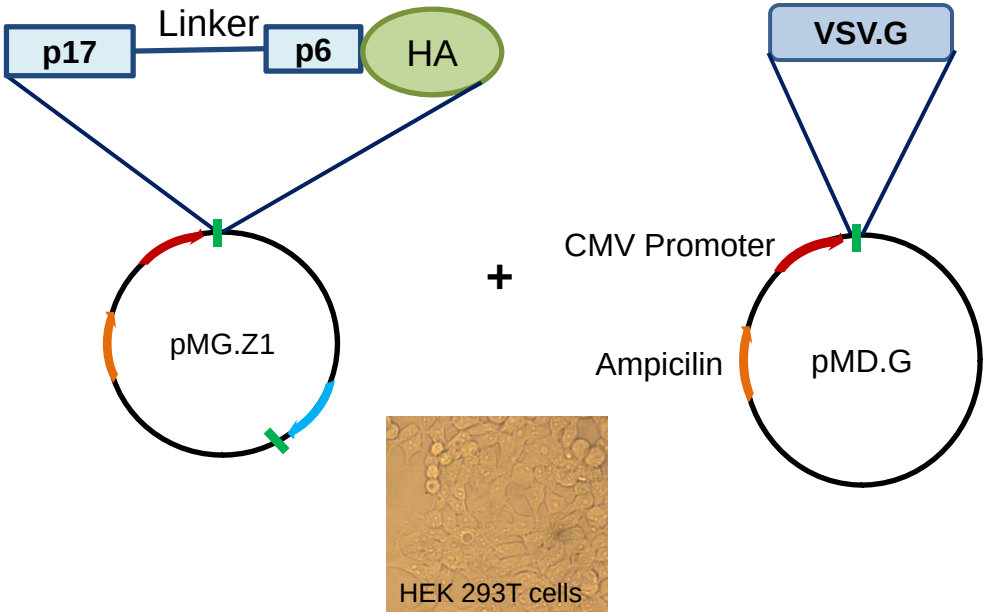
Costa et al. (2007) Biotechnol. Appl. Biochem., **48**: 35–43.

3. Pseudotyping

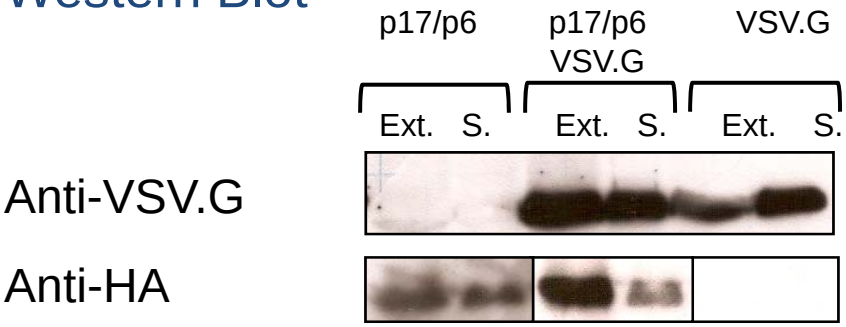
Vesicular Stomatities Virus Glycoprotein G (VSV.G)



Co-transfection



Western Blot



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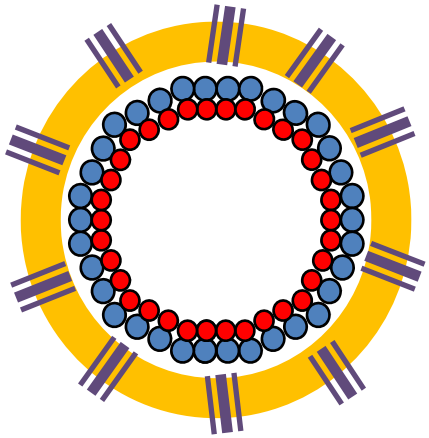
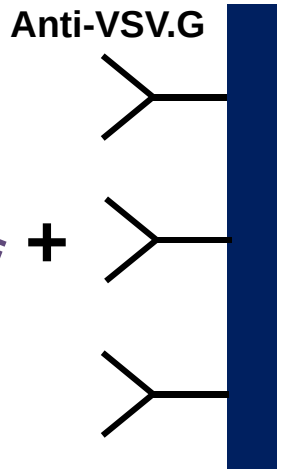
3. Pseudotyping

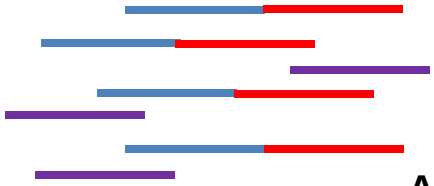
Co-Immunoprecipitation Assay



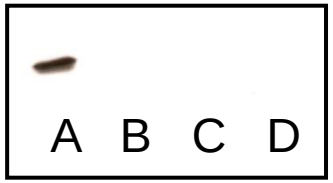
Anti-VSV.G



Wash and boile



Anti-HA



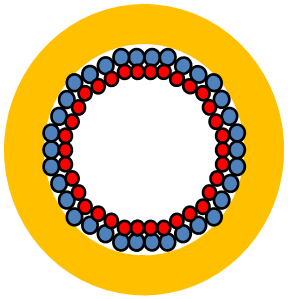
Transfections:

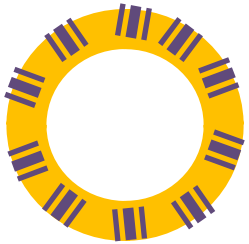
A – p17/p6 + VSV.G

B – p17/p6

C – VSV.G

D – no transfection





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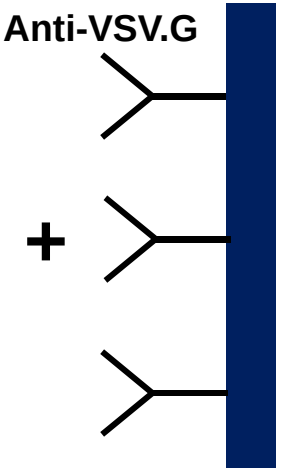


Wash and boile

Anti-HA

Summarizing:

Co-transfected cells produce particles with both p17/p6 and the envelope protein to the culture medium.

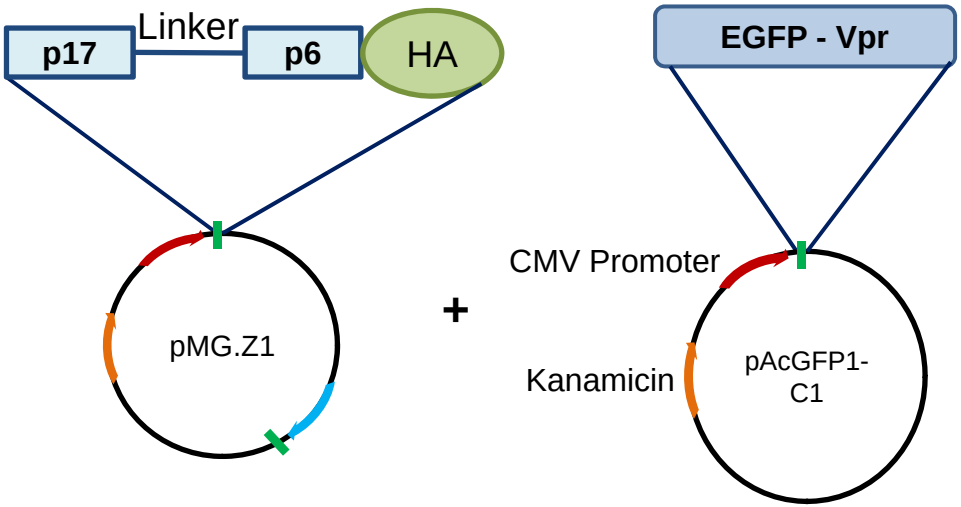
Is protein incorporation possible?

4. Reporter Protein – Viral Protein R

http://bioafrica.mrc.ac.za/

- Incorporation in the viral particles;
- Present in the citoplasm and nucleous of the infected cells.

Co-transfection



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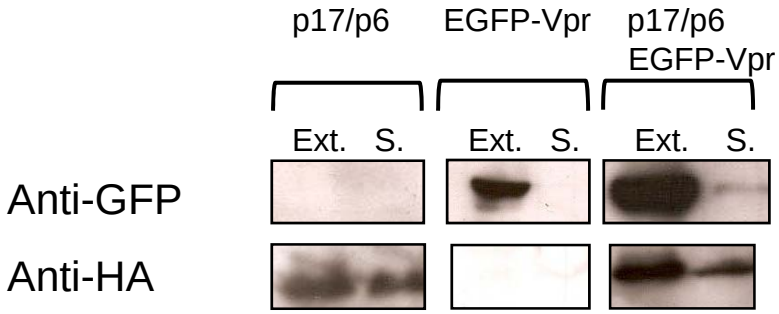
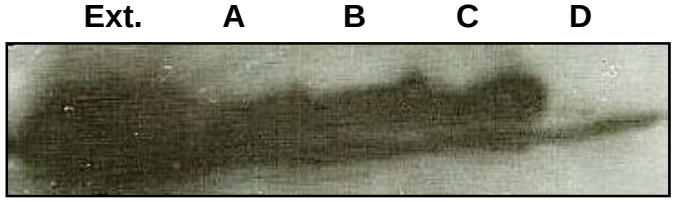


Ext. – Cell Extracts; S. – Supernatants

Protease Protection Assay

(Protease Degradation)



Summarizing:

Co-transfected cells produce particles with both p17/p6 and the EGFP-Vpr protein to the culture medium.

Conclusions

- p17/p6 surrounded by a lipidic membrane is produced to the culture medium;
- Forms a dense particle with 80nm and a density of 1.147 g/ml;
- p17/p6 particles can be pseudotyped with VSV.G;
- p17/p6 particles can incorporate cargoe proteins such as Vpr.

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Colaborators

Prof. João Gonçalves

Faculdade de Farmácia, Universidade de Lisboa

Co-workers

Luis Rosa

Raul Baltazar

Rogério Rodrigues