Viruses in May
Katoomba, August, 2012

New Technology in Vaccine Engineering

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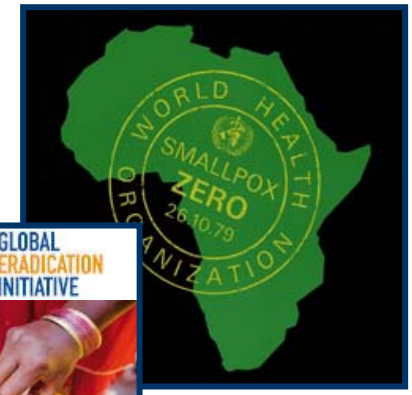
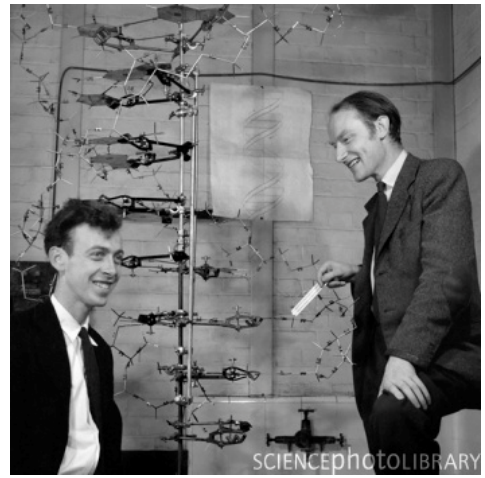
Introduction



- Vaccination enormously successful
  - Smallpox eradicated, polio close
- More to do
  - 15 M people still die annually, half children <5 yrs
  - Emergent and re-emergent disease
- Technological gap in approaches
  - Pasteur's "Isolate-Inactivate-Inject" dominates
  - Opportunity to engineer better systems



Modular Vaccine Design

http://www.ssa.ford.com/servlet/ContentServer?cid=1178863133817&pagename=FSSA%2FDFYPage%2FFord-Default&c=DFYPage&site=FSSA

Front guard

http://www.indiamart.com/ajantaautoacc/front-guard.html#ajanta-grill-guard-for-tavera

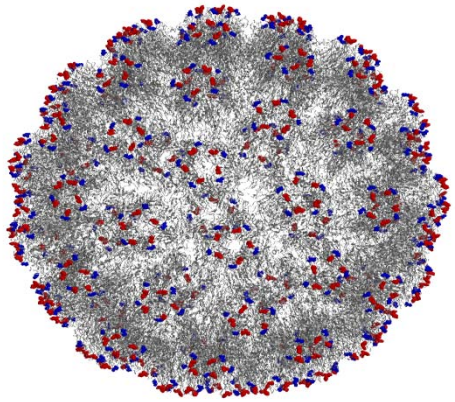
BASE
with sites for insertion of optional modules

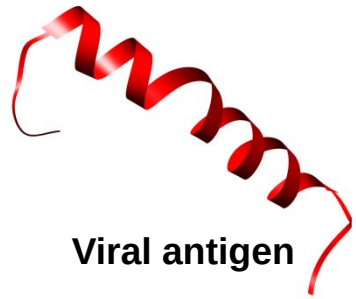
+

MODULE

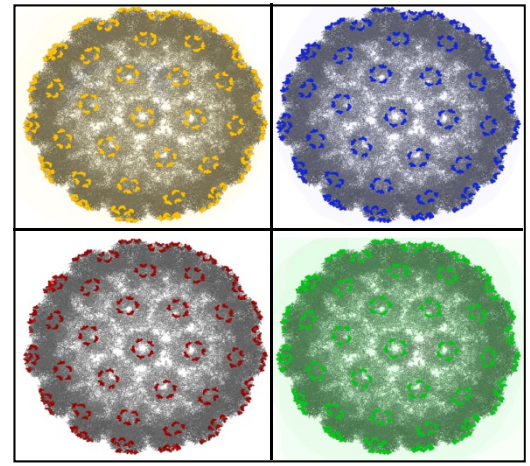
→

MODULAR DESIGN

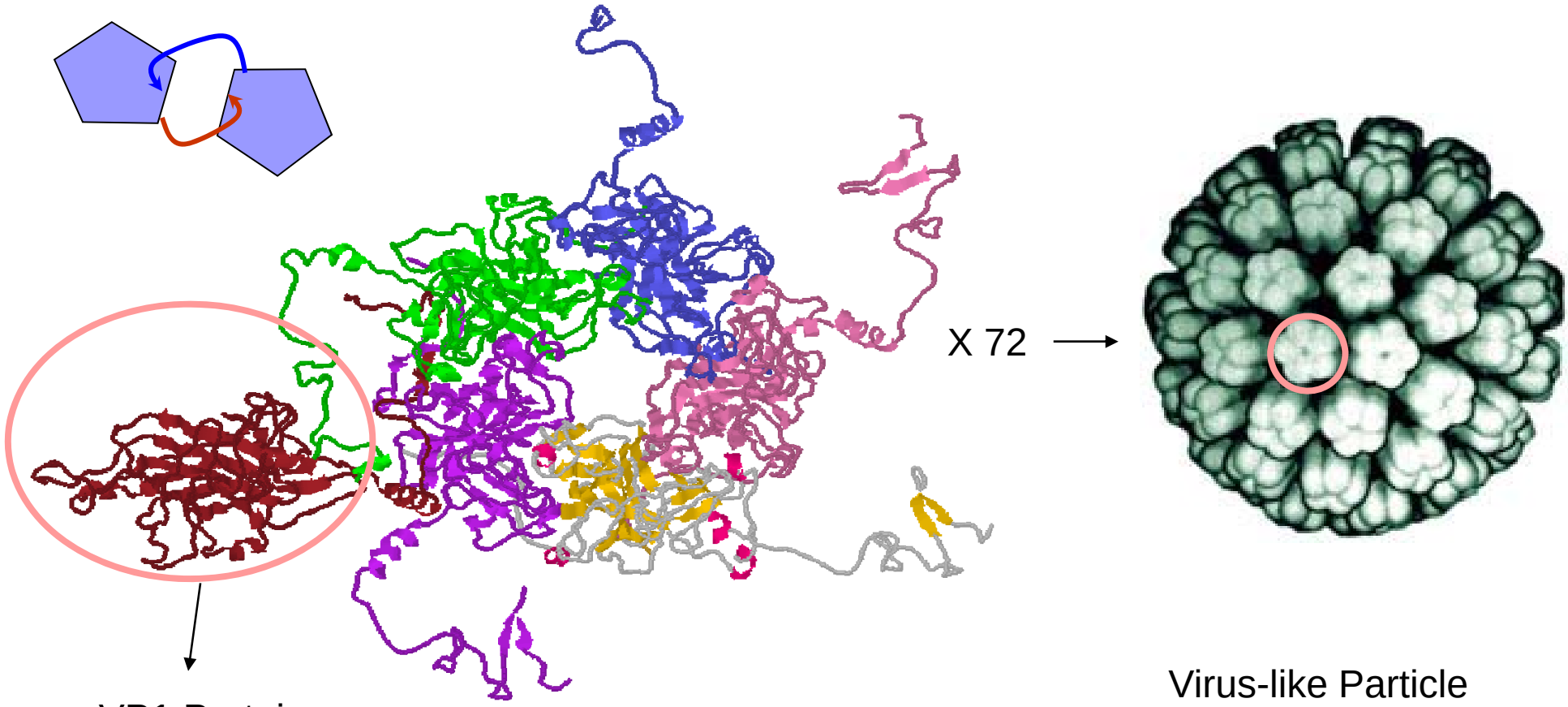




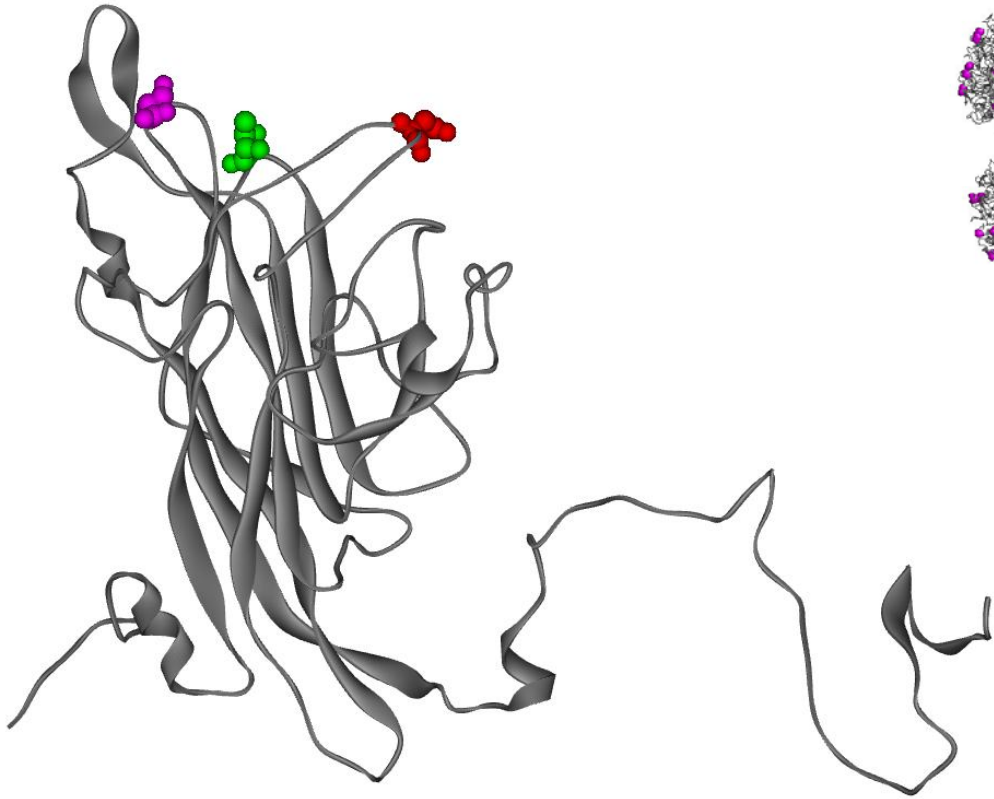
Viral antigen

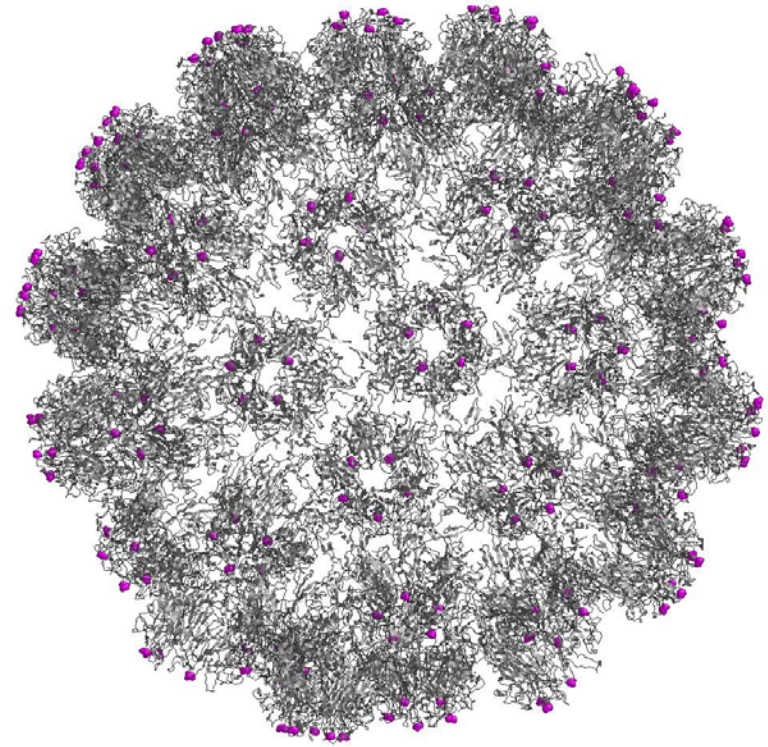


Murine Polyomavirus

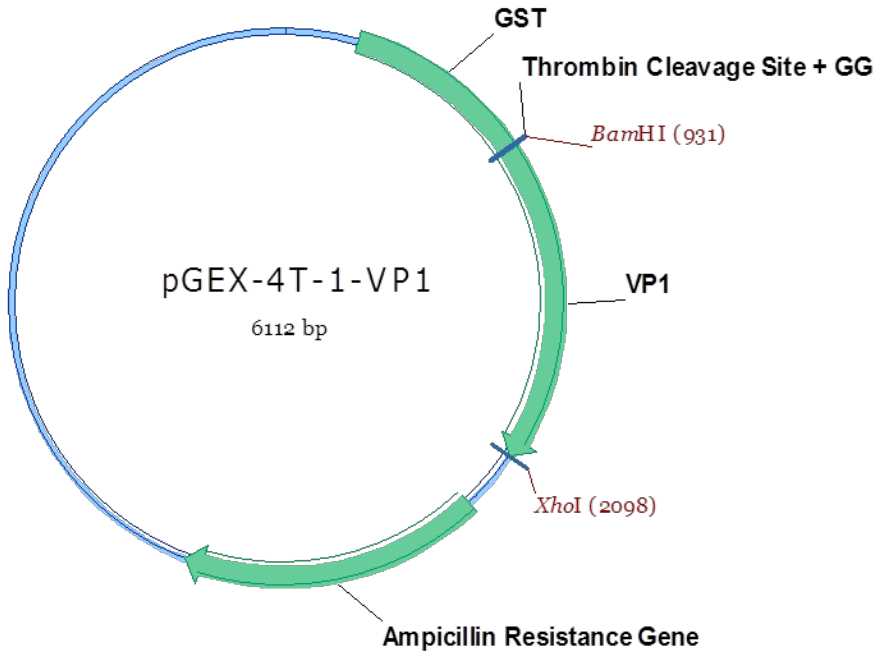


Antigen Insertion Sites on VLP Surface





Bioprocess Engineering



Best available expression in literature: 1 mg/L.OD

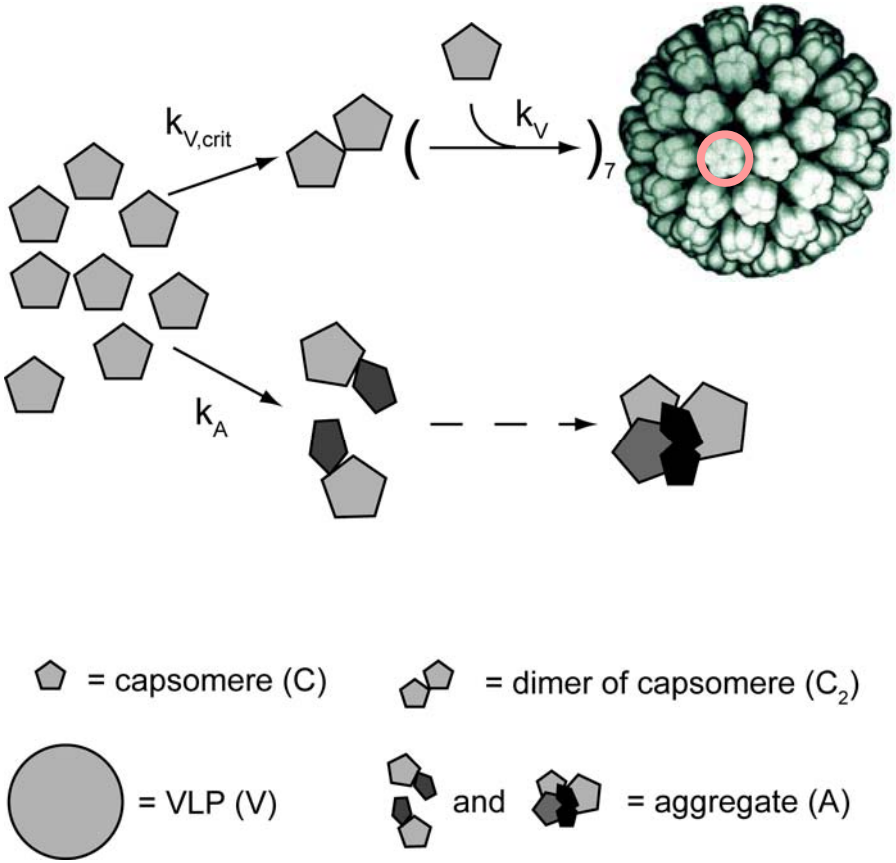
After factorial optimisation, Host selection and redesign: 15-20 mg/L.OD

J. Biotechnol. (2008), 134(1-2): 64-71

Confirmed 2-4 g/L in fed-batch *E. coli* fermentation.

J. Biotechnol. (2010), 150(2): 224-231

A practical model



$$C = C_{actual} - C_{critical} \quad (1)$$

$$\frac{dC}{dt} = -k_{V,crit}C^2 - k_A C^2 - k_V C\sum_{i=2}^{71} C_i \quad (2)$$

$$\frac{dC_2}{dt} = \frac{1}{2}k_{V,crit}C^2 - k_V C C_2 \quad (3)$$

$$\frac{dC_i}{dt} = k_V C\left(C_{i-1} - C_i\right), \mathrm{i} = 3 \sim 71 \quad (4)$$

$$\frac{dV}{dt} = k_V C C_{71} \quad (5)$$

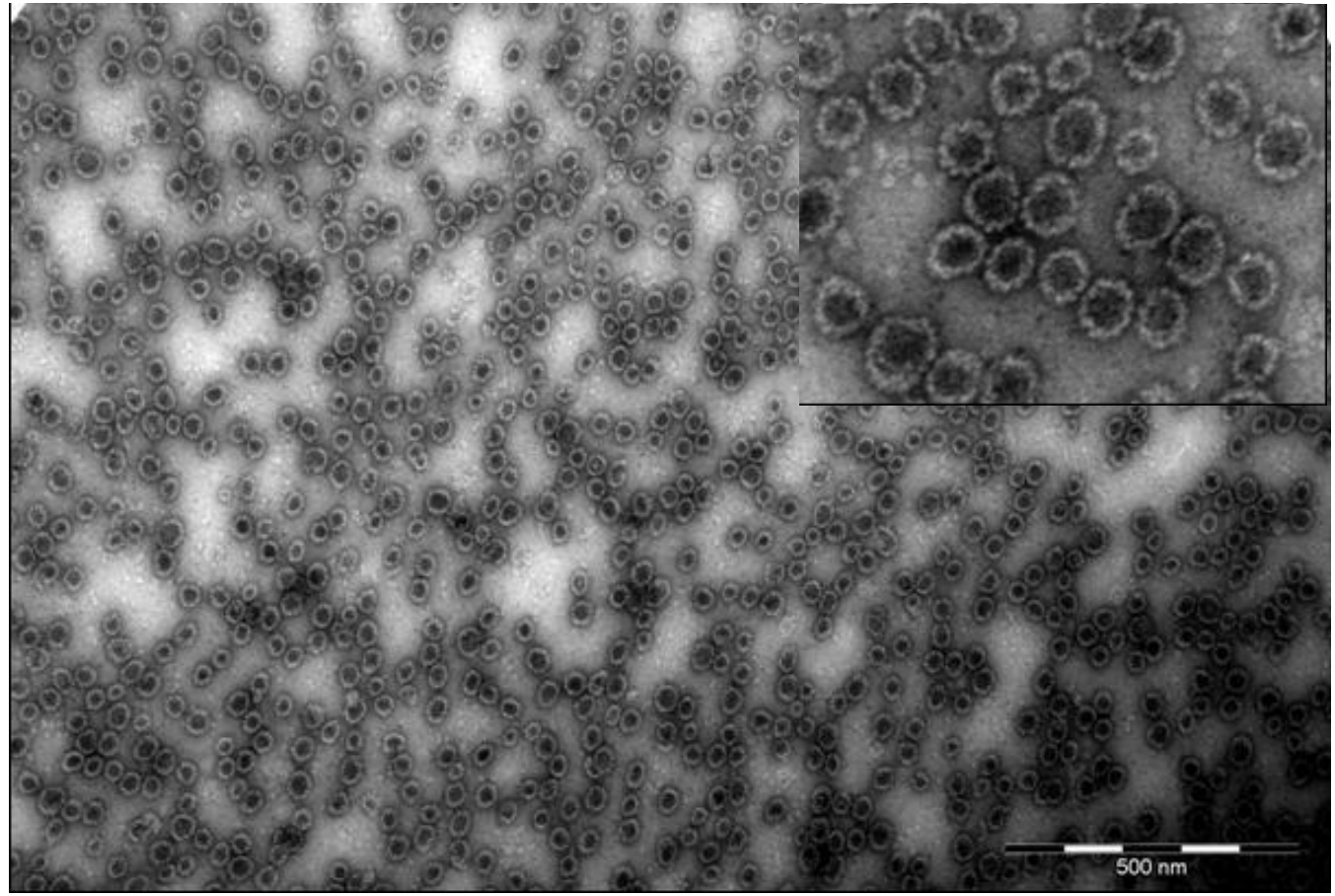
$$\frac{dA}{dt} = \frac{1}{2}k_A C^2 \quad (6)$$

with initial condition, at $t = 0$

$$C_{actual} = C_O,\ C_1 = C_2 = \ldots = C_{71} = V = A = 0$$

Biotechnol. Bioeng. (2010), 107:550

VP1 self-assembly *in vitro*



The UQ Microbial Vaccine Platform (MVP)

- Speed
  - same process for different viruses
  - time from DNA to purified antigen < 1 week
  - processing can be automated
- Scale
  - Makes protein using industrial biotechnology tools
  - 100M doses per kL of bacterial culture in 24 h
- Safety
  - we make protein, not virus
  - we can sterile filter before virus assembly

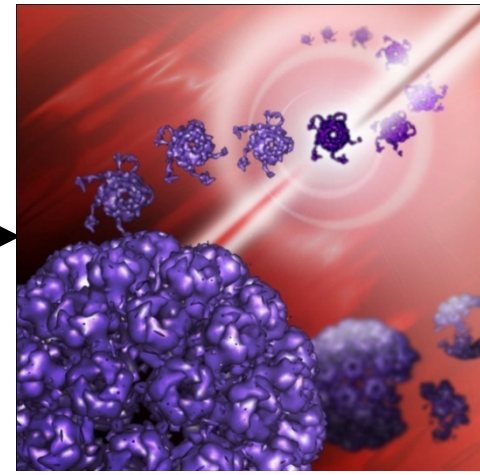
The UQ Microbial Vaccine Platform (MVP)

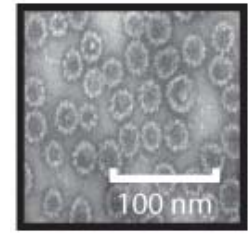
Water
Glucose
Salts
Bacteria

2 g/l Soluble Antigen

Within 24 h

Purification and Assembly





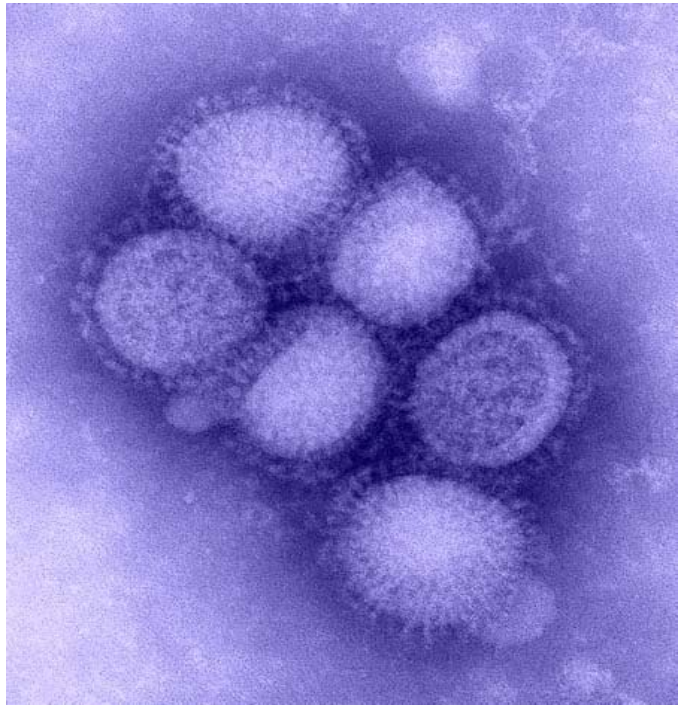
>100,000 doses per litre

1000L pilot
= 100M doses
In 24 h

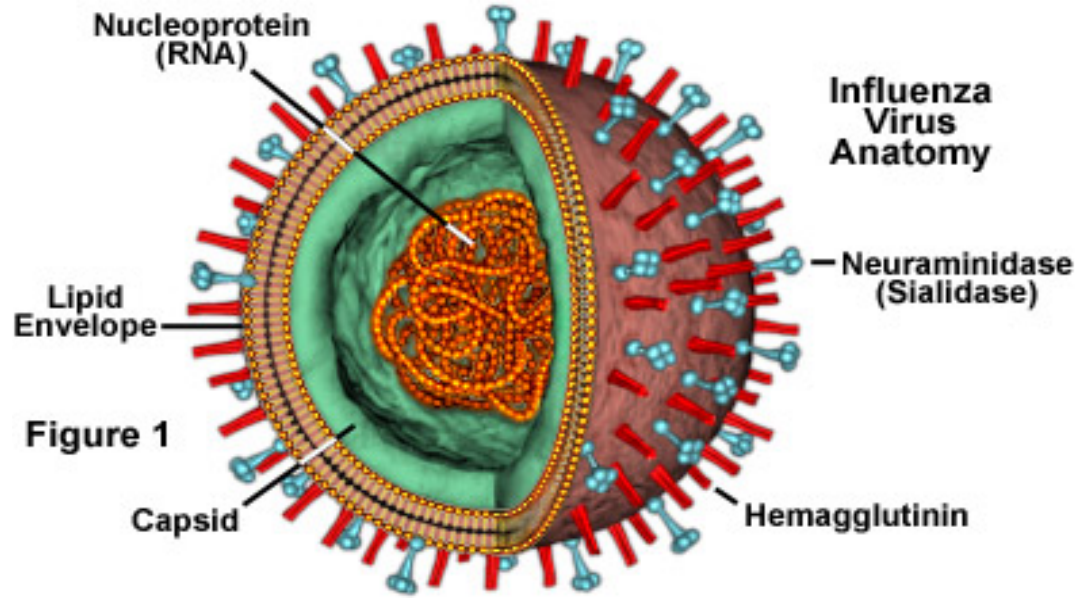
"Dose Excess" regime.
Everyone can cultivate bacteria.

Application of the Platform: Influenza

Influenza



www.cdc.gov

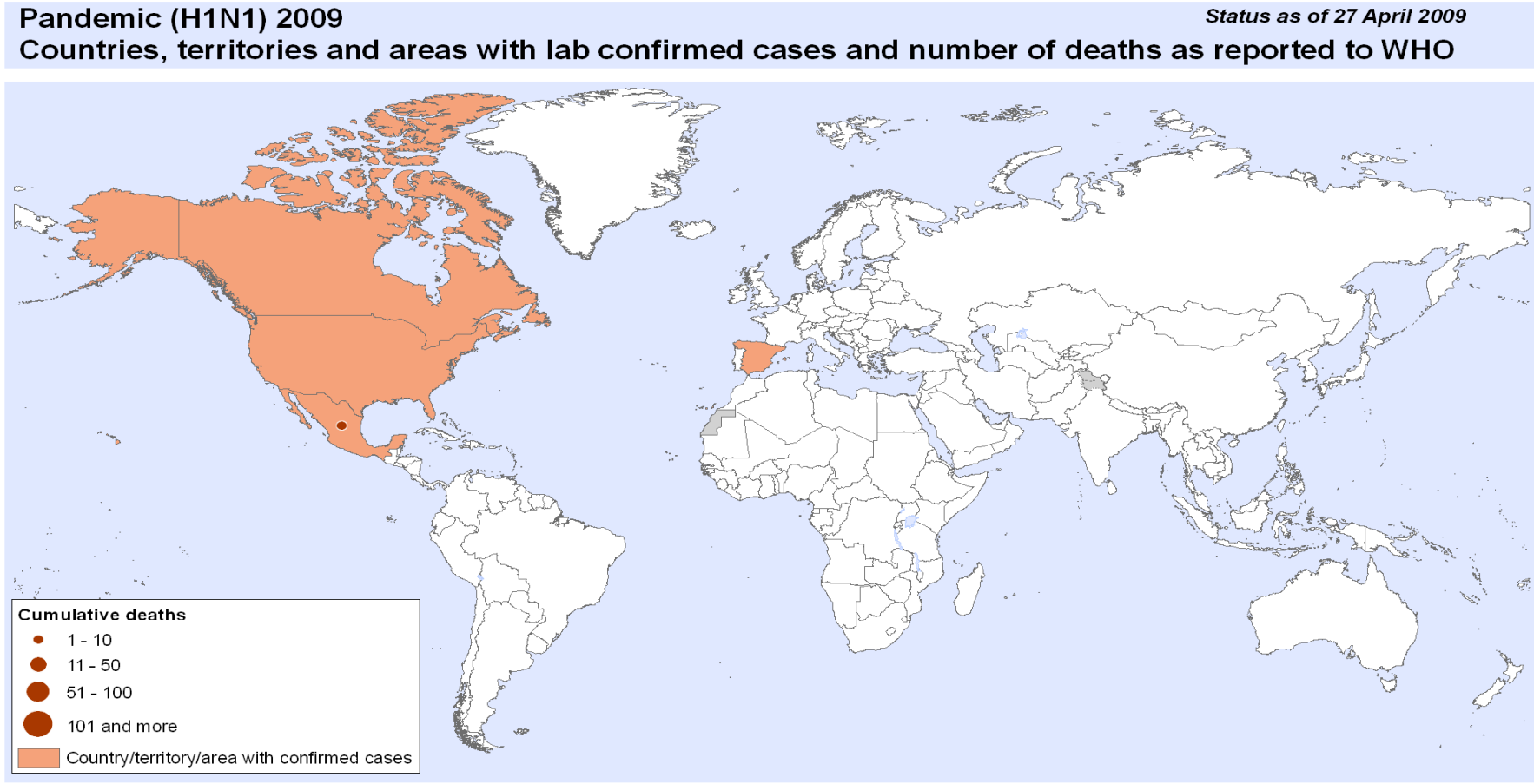


http://microbiology2009.wikispaces.com/Influenza

Global Challenge

When the virus changes, existing vaccine does not work.

H1N1 (2009): April 27th



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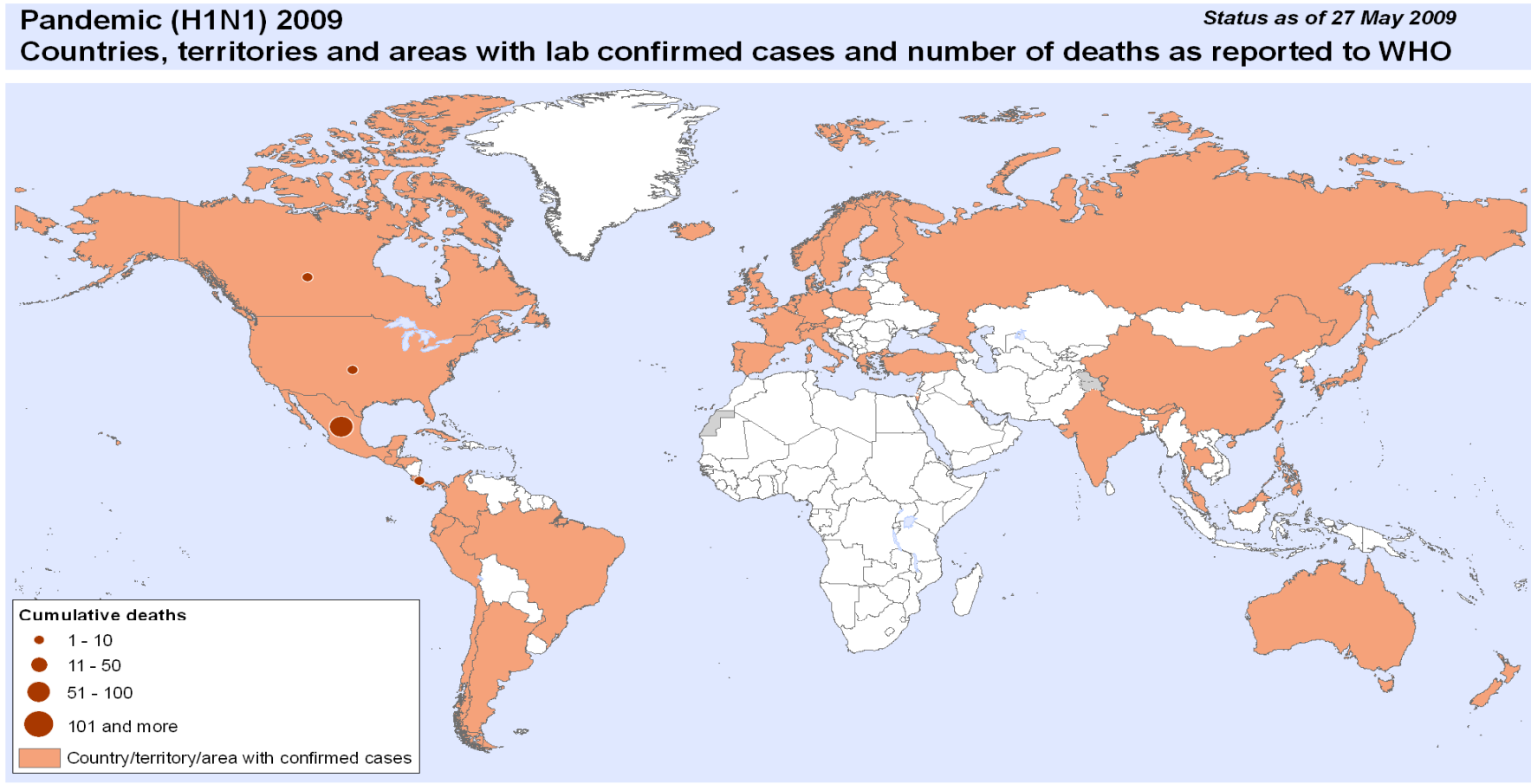
Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Map produced: 08 October 2009 09:56 GMT

H1N1 (2009): May 27th



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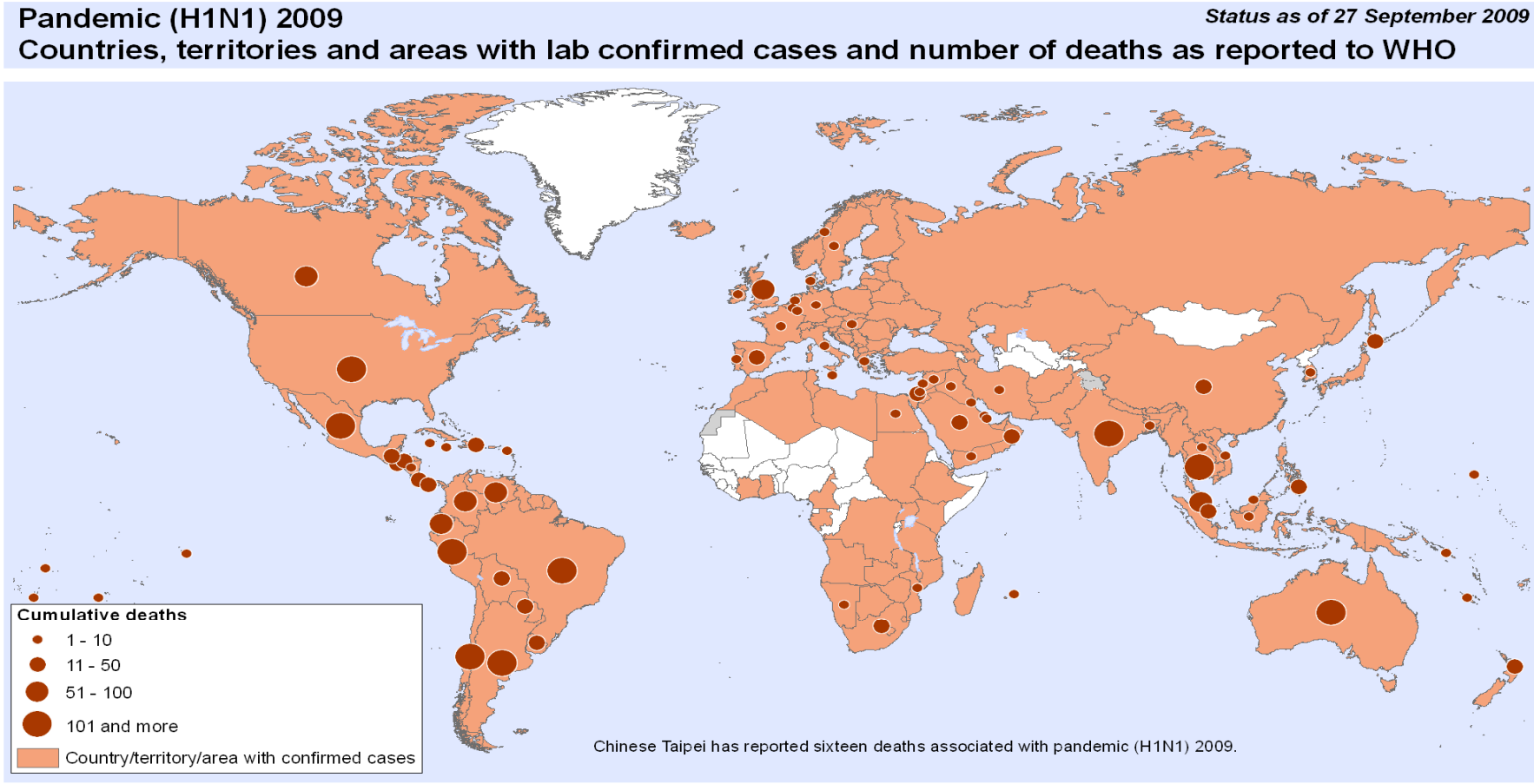

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Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Map produced: 30 September 2009 07:54 GMT

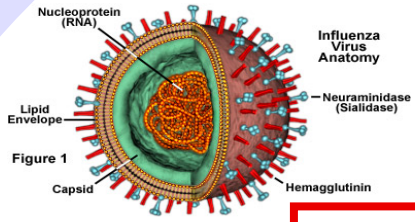
Influenza in a Connected World

World Health Organization

Control

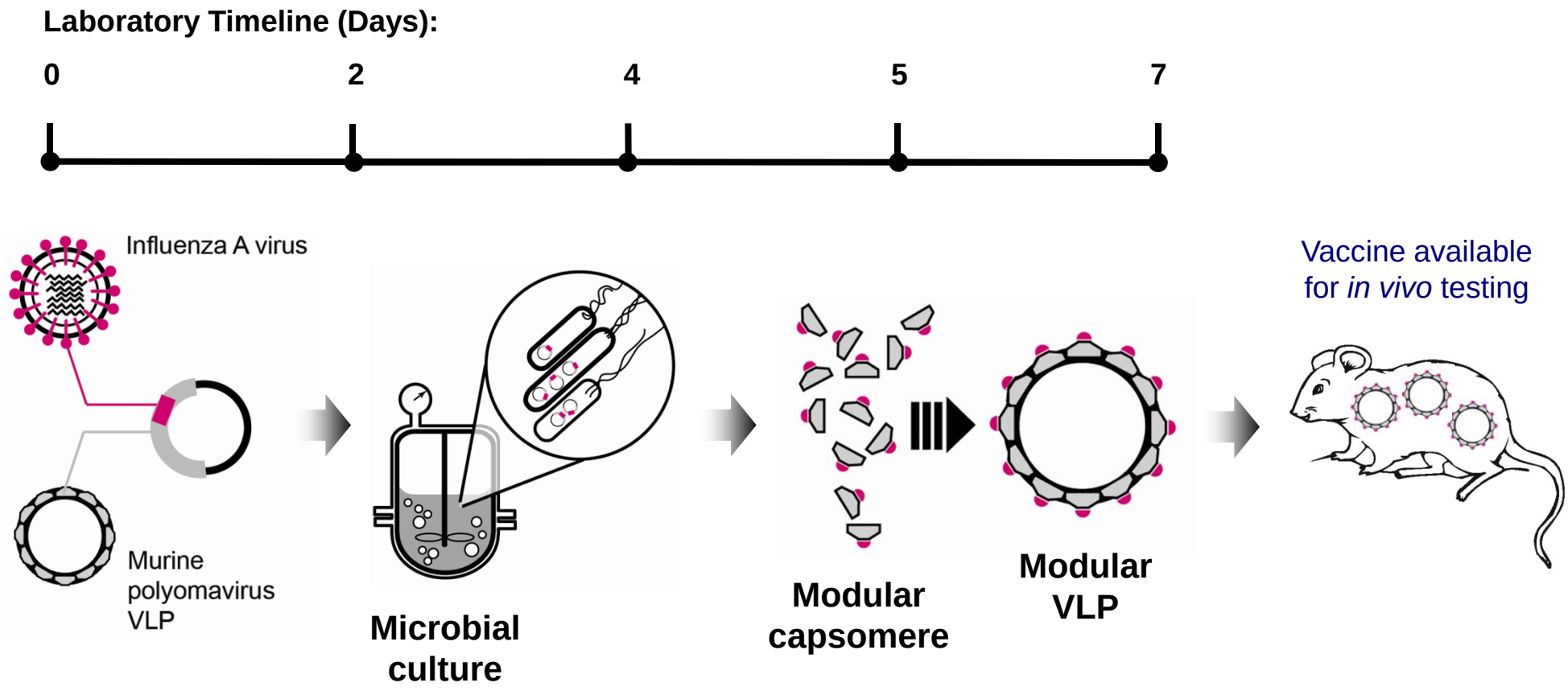
Share

Identify



People die while they wait for the new vaccine

Rapid response for emergent virus



Target Epitopes

- Strain-specific
  - **HA1 – receptor binding regions**
  - Other HA1 epitopes

Assume Viruses Change

- Broadly cross-protecting
  - **M2e of matrix protein 2**
  - HA stalk regions

Assume Viruses are Static

Target Epitopes

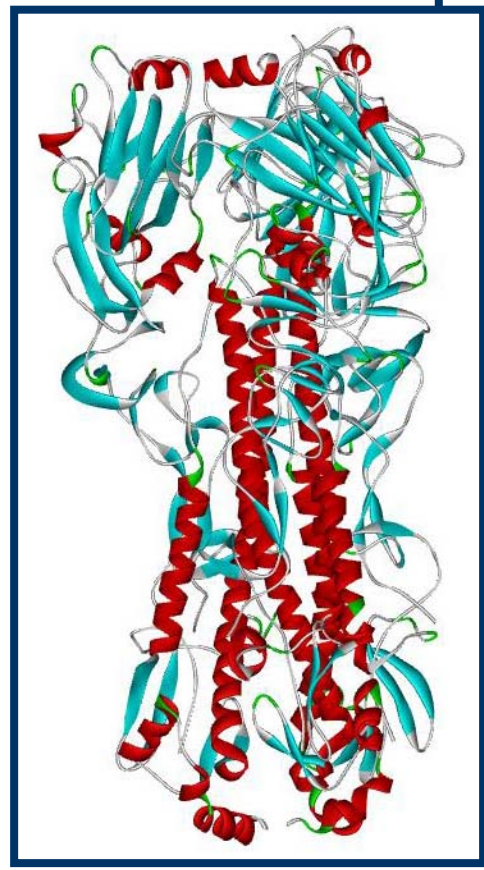
- Strain-specific
  - **HA1 – receptor binding regions**
  - Other HA1 epitopes

Assume Viruses Change

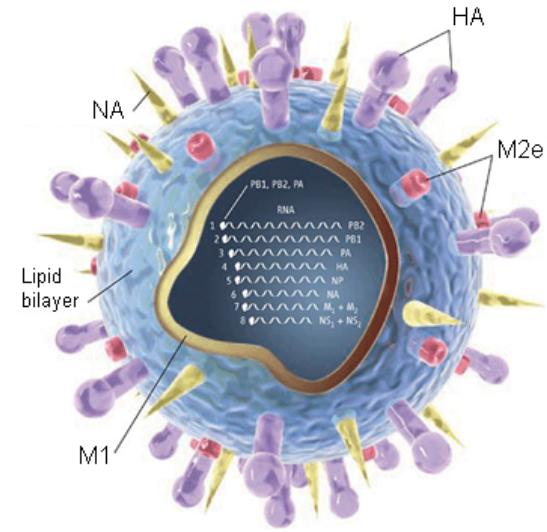
- Broadly cross-protecting
  - **M2e of matrix protein 2**
  - HA stalk regions

Haemagglutinin Helix 190

- Receptor binding site.
- Biology 101 – blocking the receptor binding site will block viral entry.
- Glycosylation?
- Structure?

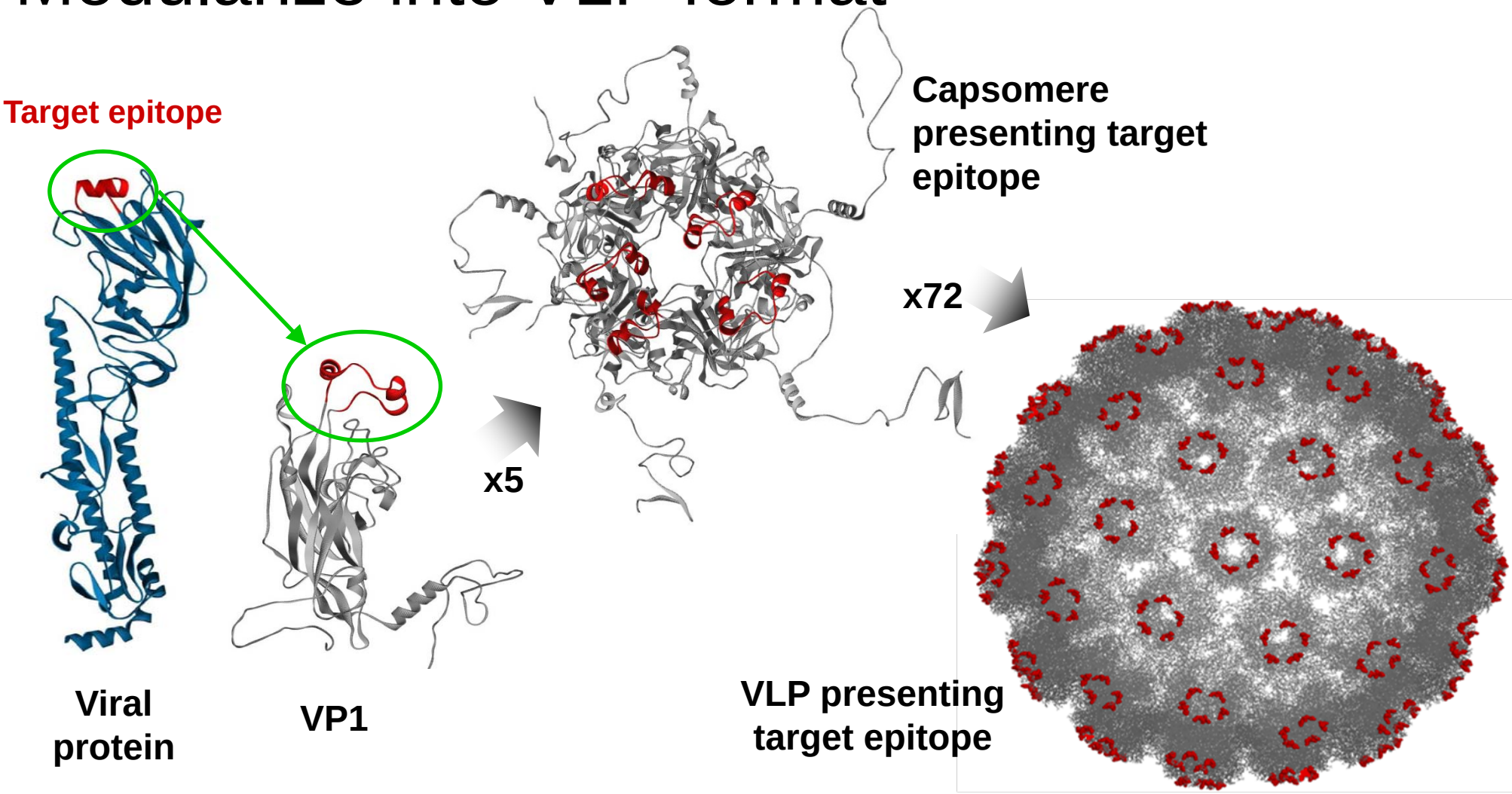


Influenza A virus

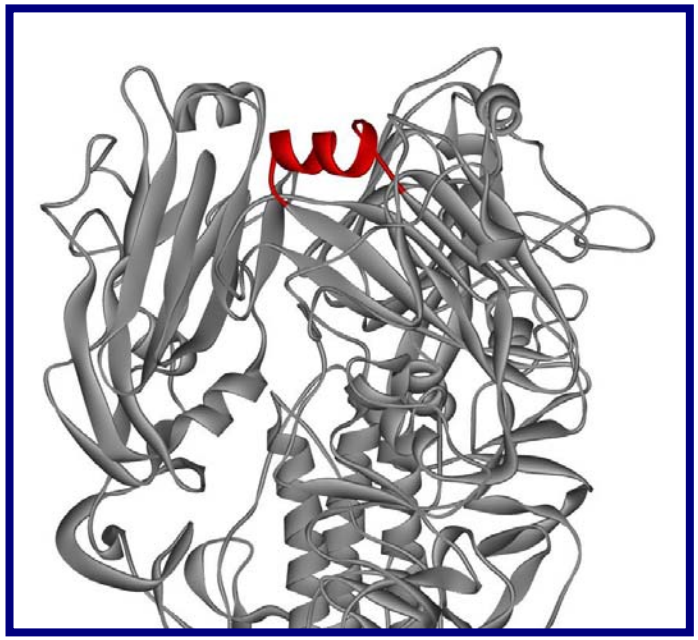


http://viromag.files.wordpress.com/2009/02/influenza-virus-diagram.jpg

Modularize into VLP format



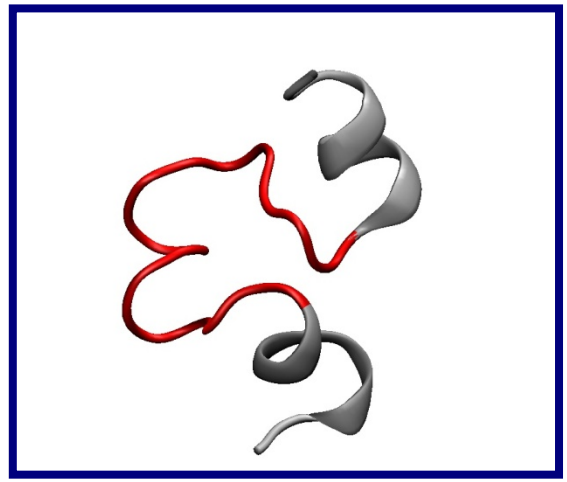
Structural analysis of helix 190 peptide



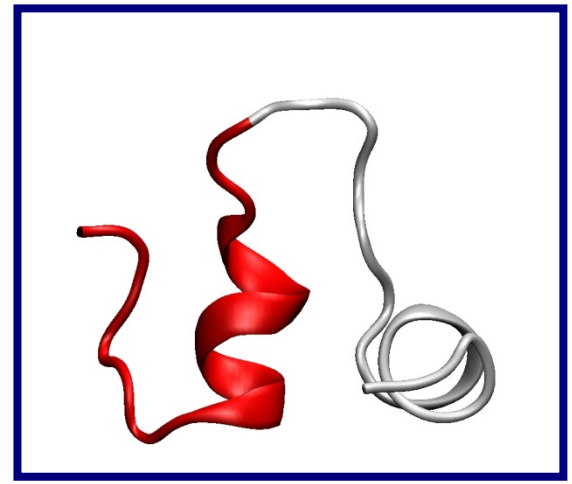
Helix 190
in native HA

MD simulation

- Gromacs
- In PBS solution
- 20 ns



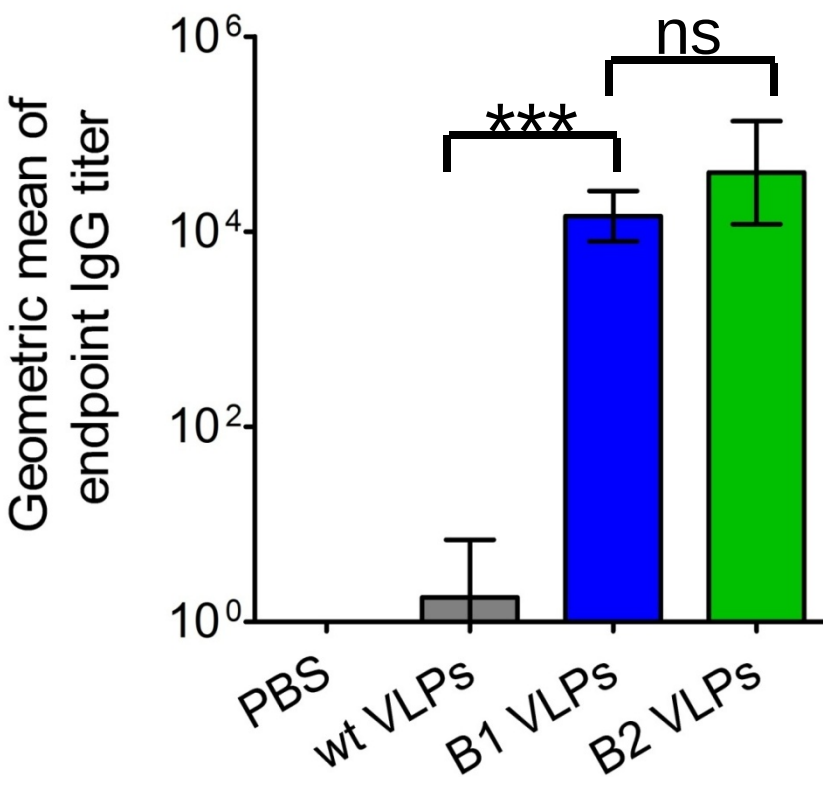
Peptide B1



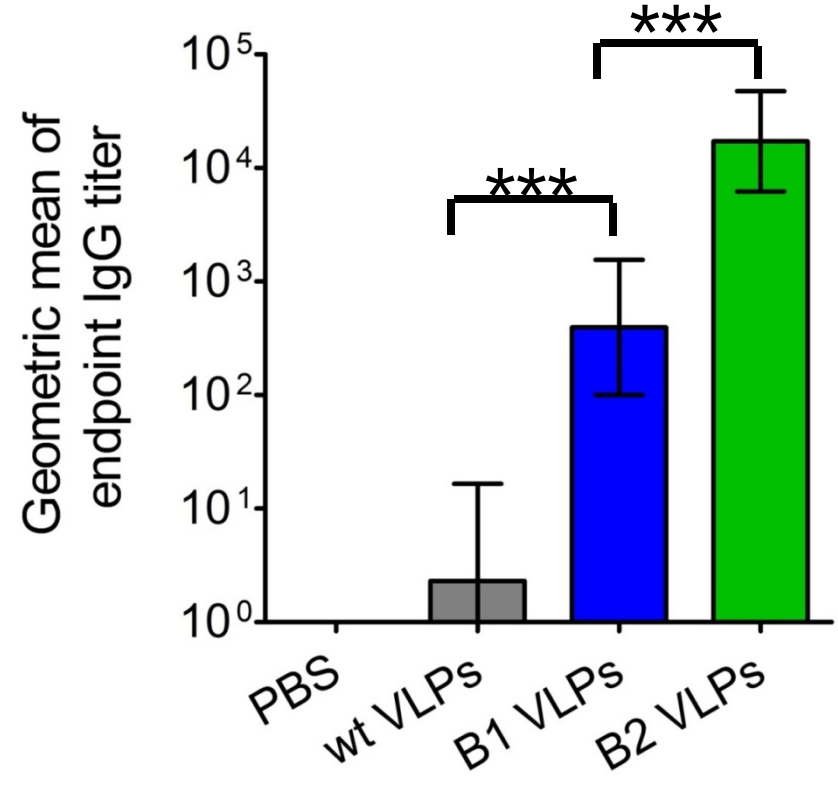
Peptide B2

Module Structure Matters

Peptide



Recombinant HA



ns = not significant

Initial Target Epitopes

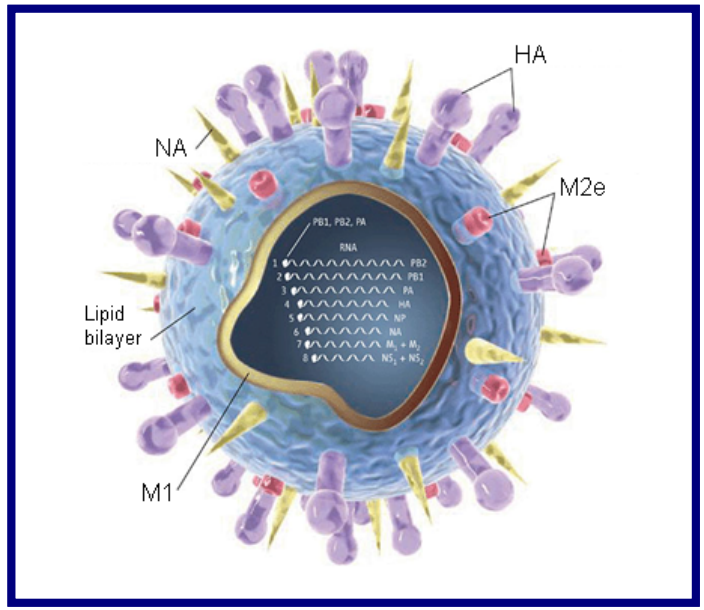
- Strain-specific
  - HA1 – receptor binding regions
  - Other HA1 epitopes
  - Biology 101
- Broadly cross-protecting
  - **M2e of matrix protein 2**
  - HA stalk regions

Assume Viruses are Static

Matrix Protein M2e

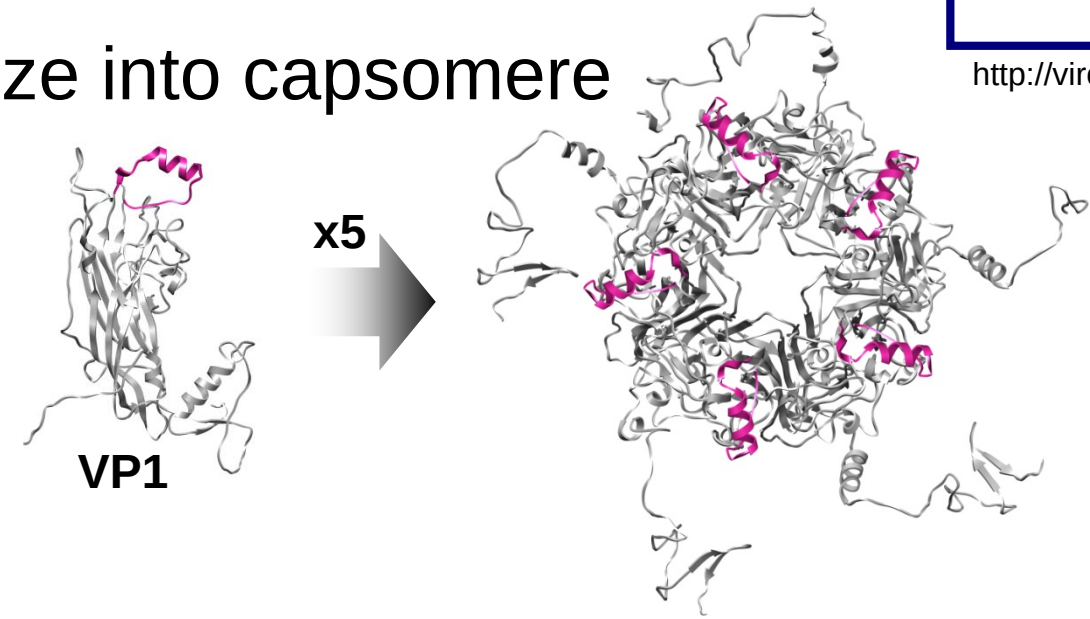
- Immunogenic
- Broad cross protection
- Complementary mechanism

Influenza A virus

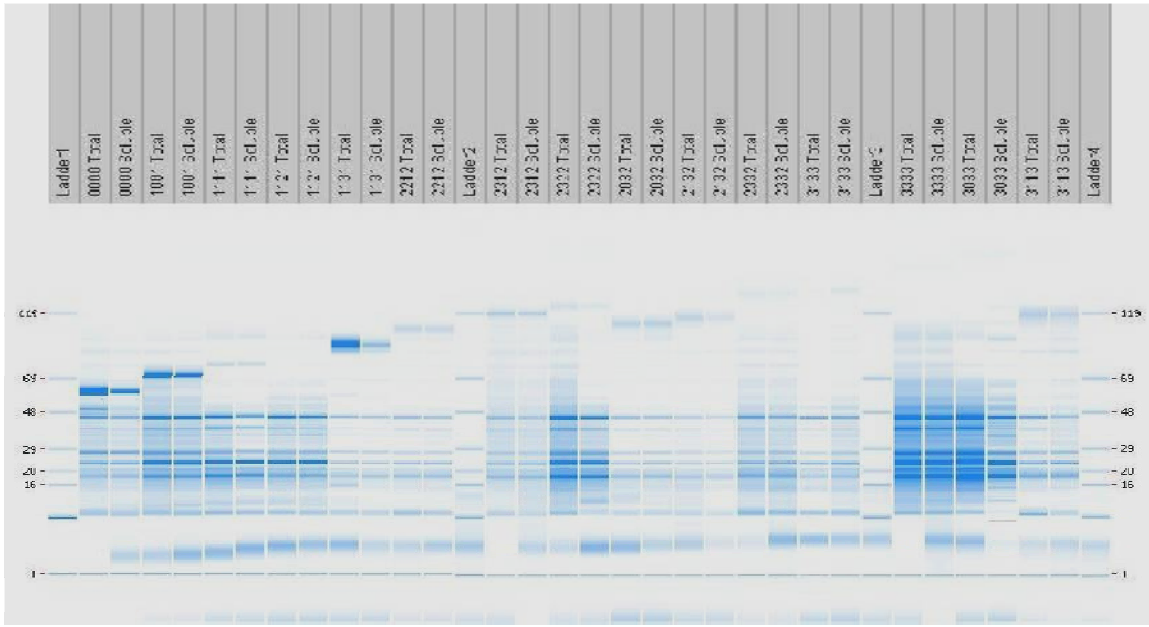


http://viromag.files.wordpress.com/2009/02/influenza-virus-diagram.jpg

Modularize into capsomere format



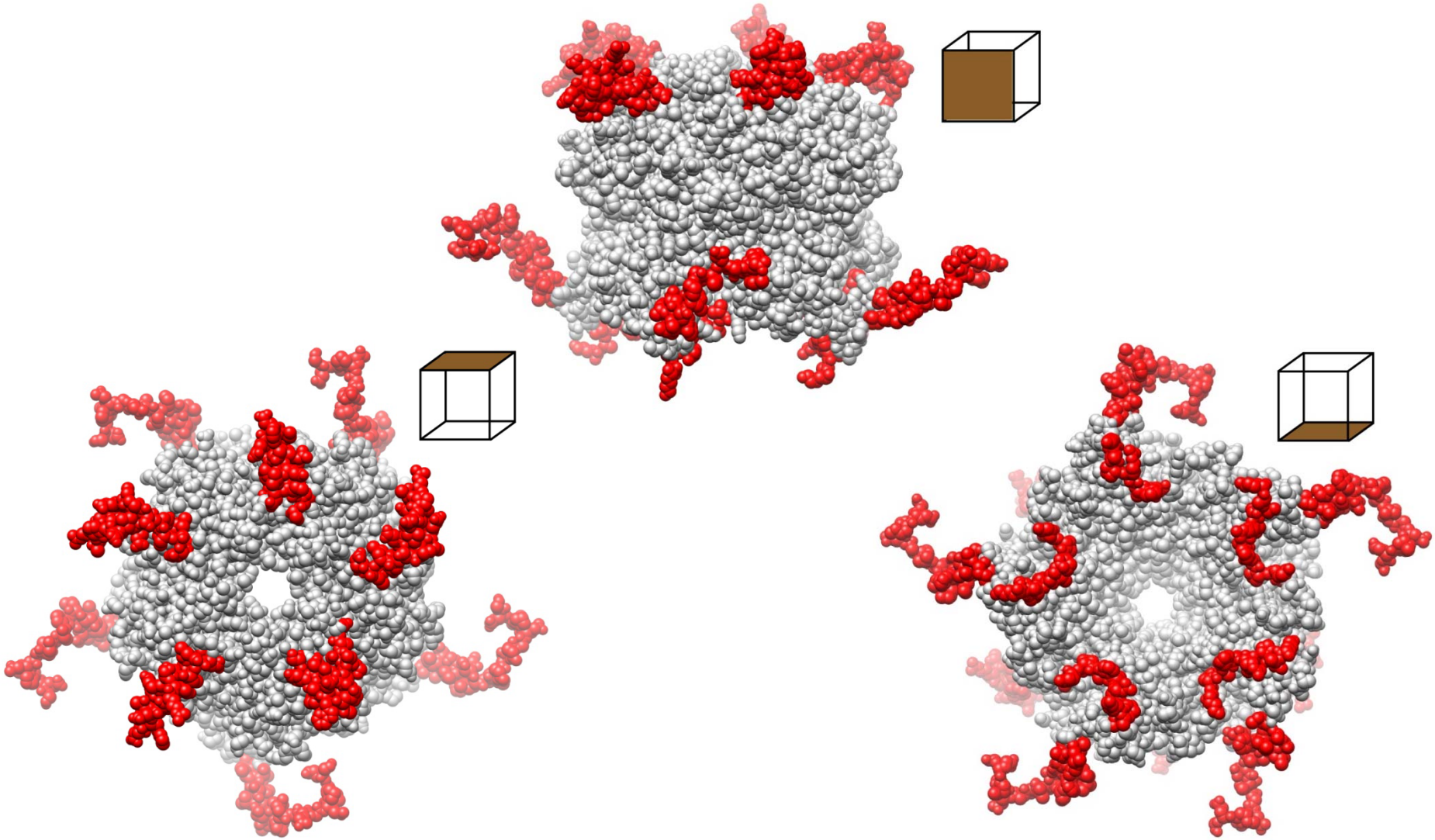
Screening of modularized capsomere



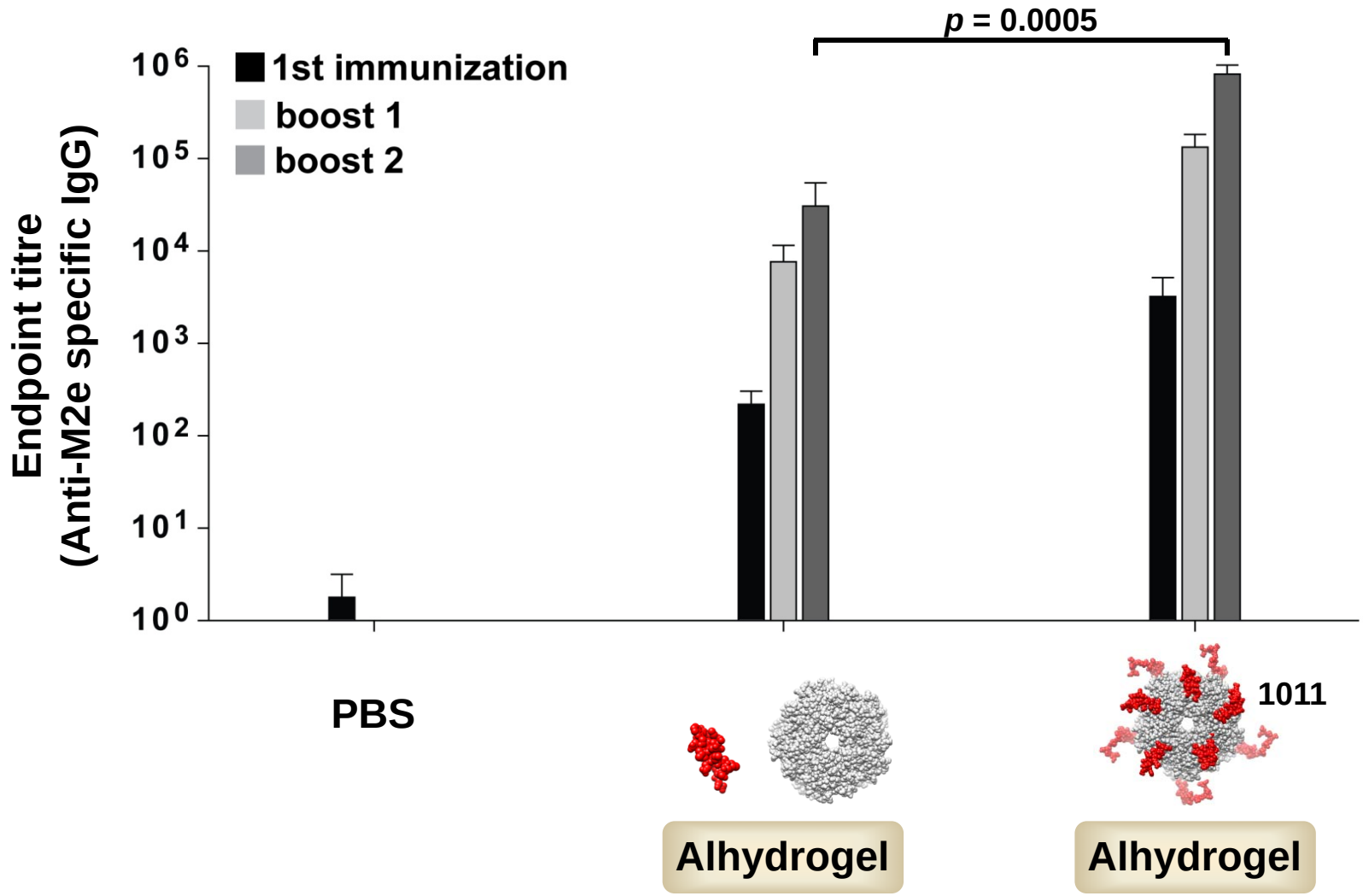
- Expression level
- Solubility level
- Downstream bioprocessing yield

1011 and 2022

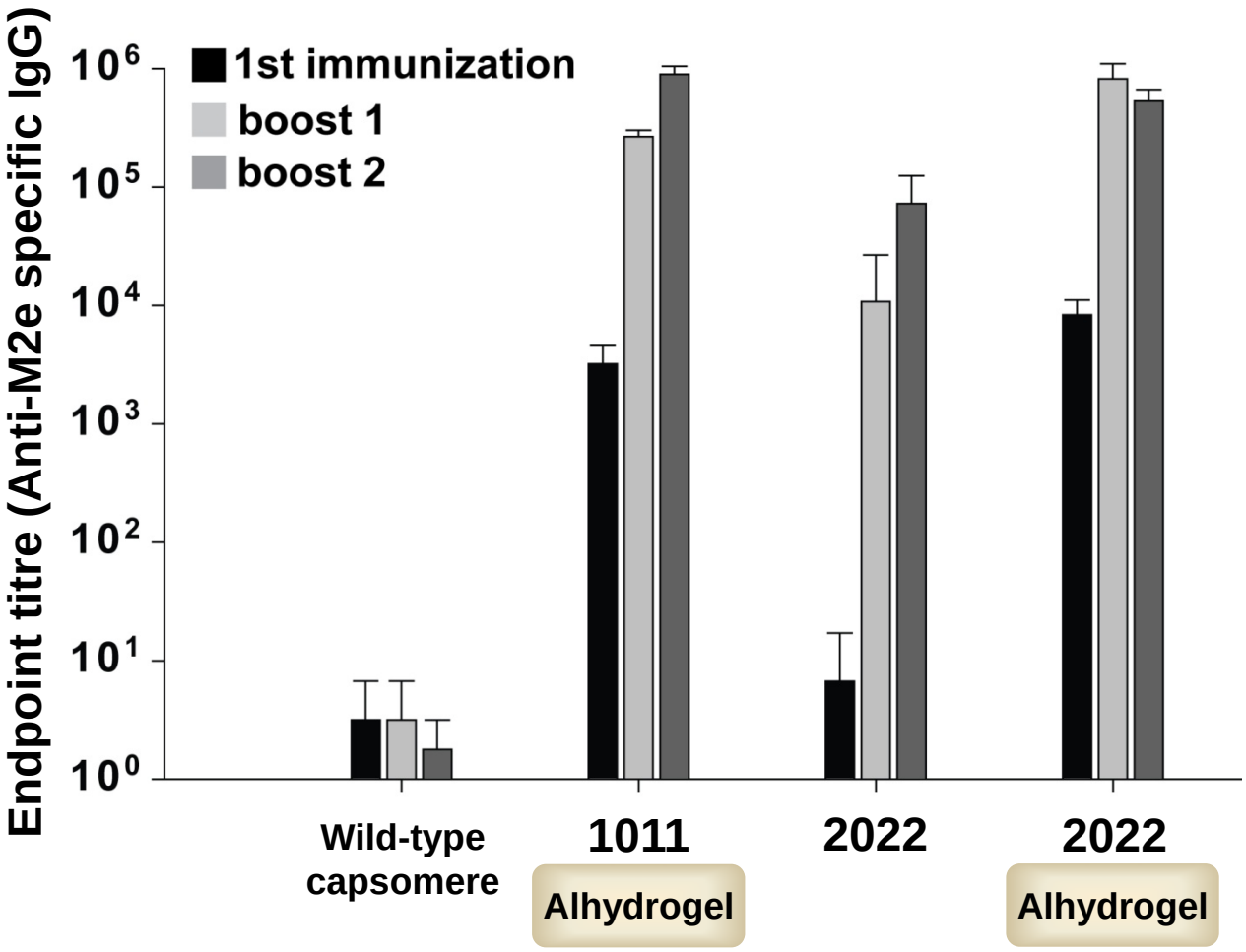
Modular capsomere



Capsomere format improves immunogenicity



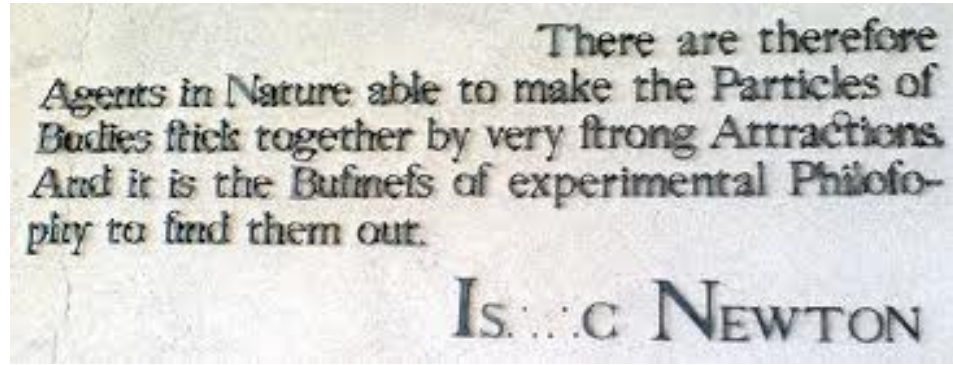
Modular capsomere 1011 vs 2022



- Excellent IgG titres
- Little sensitivity to more modules
- Adjuvant necessary for capsomere format
- Excellent epitope tolerance

Conclusions

- VLP and capsomere platform developed
  - Remarkable productivity, protein not virus based
- Excellent developability and manufacturability
- Excellent end point titres
  - Moving to protection studies
- Multitude of insertions successfully handled
  - Flexibility afforded by VLP and Capsomere formats



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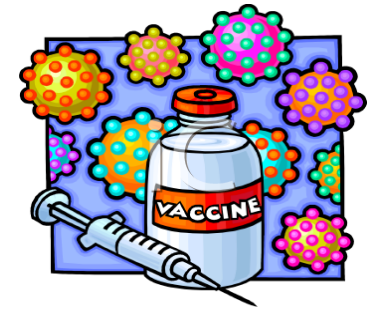

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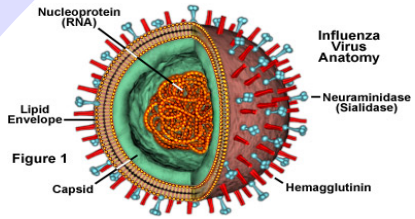

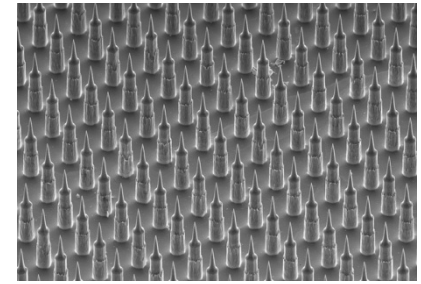

Acknowledgements

Dr Linda Lua & UQ Protein Expression Facility

Queensland Government

Australian Government
Australian Research Council

Australian Government

ansto
Nuclear-based science benefiting all Australians

EPSRC

BBSRC
bioscience for the future

NIST

FULBRIGHT

BILL & MELINDA GATES foundation