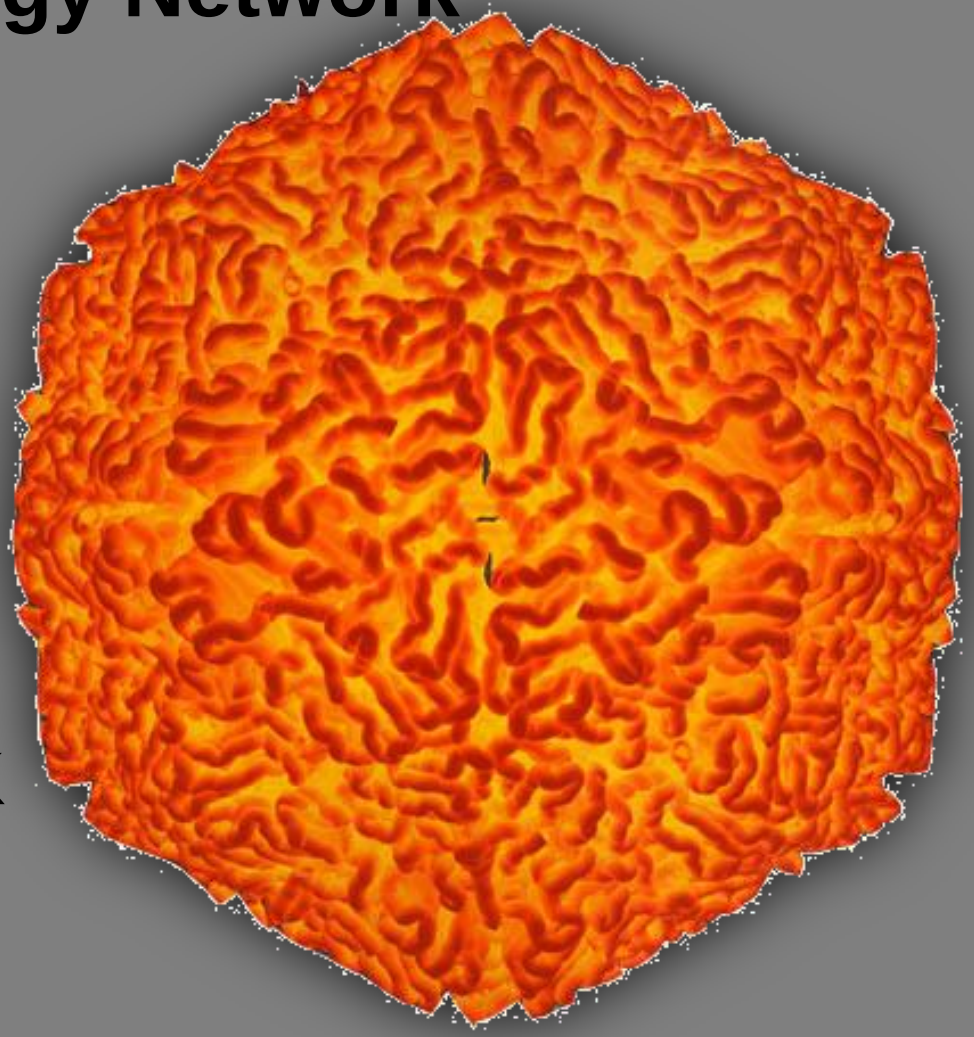
Structural Biology and Vaccinology

Veterinary Vaccinology Network

16th-17th February 2015



DAVID STUART
Diamond Light Source, UK
Oxford University, UK
Instruct, EU

Curr Top Med Chem. 2013;13(20):2629-37.

Structural vaccinology: a three-dimensional view for vaccine development.

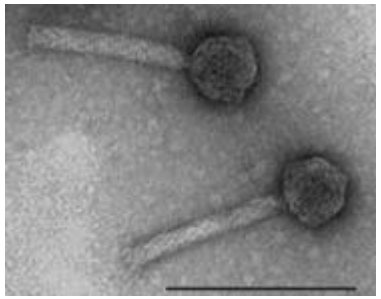
Cozzi R[1], Scarselli M, Ferlenghi I.

[1]Novartis Vaccines and Diagnostics, Siena, Italy.
ilaria.ferlenghi@novartis.com

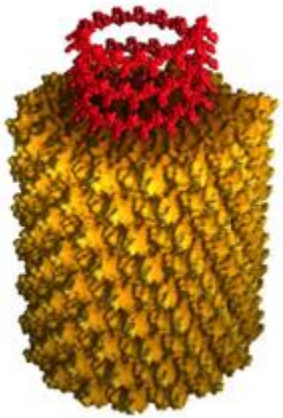
The Structural Vaccinology approach is the logical evolution of Reverse Vaccinology: a genome-based approach combined with structural biology, with the idea that protective determinants can be used to selectively engineer the antigens that can be re-designed and simplified for inclusion in vaccine combinations. The final objectives of the rational structure-based antigen optimization are the facilitation of industrial-scale production of the antigens combination, obtain a greater immunogenicity and a greater safety profile and finally expand the breadth of protection. Structural Vaccinology is particularly powerful in case of antigenic variation between closely related strains and species.

I will only consider viruses …

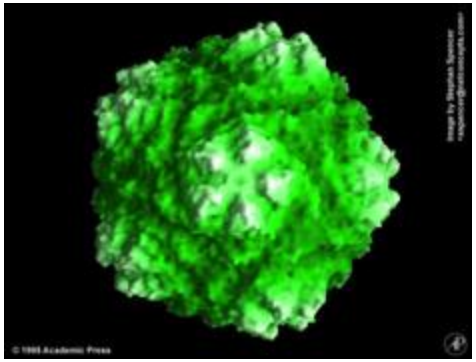
But viruses span a huge range of sizes and shapes ...



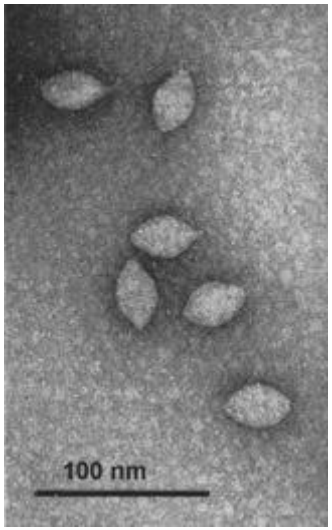
Enterobacteria virus P2



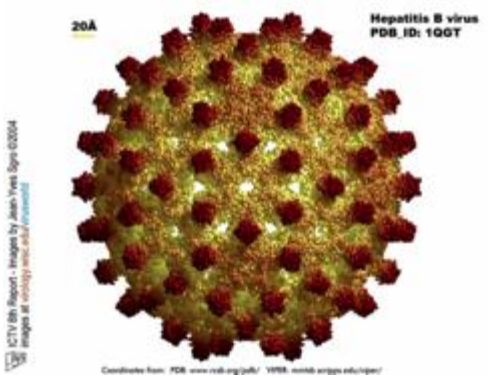
Tobacco mosaic virus



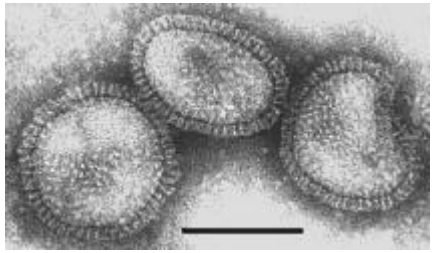
Satellite tobacco necrosis virus



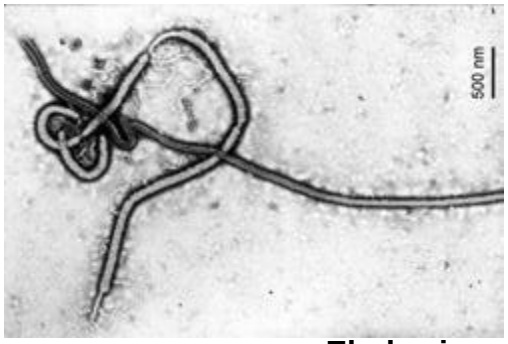
Sulfolobus spindle-shaped virus 1



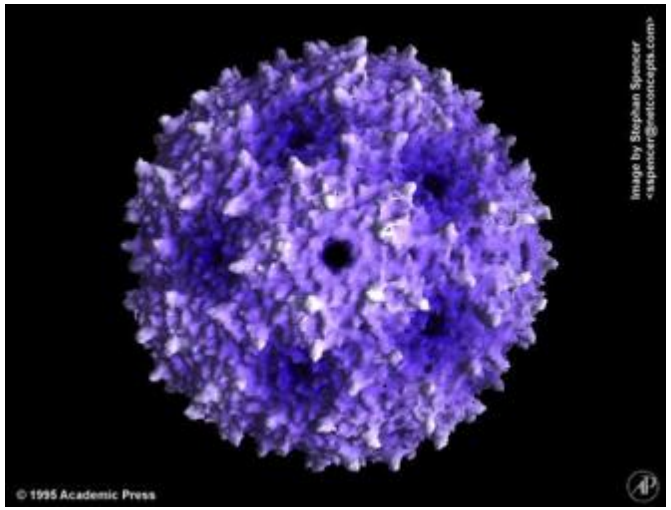
Hepatitis B virus



Influenza virus A



Ebola virus



Enterobacteria phage MS2

Images from: IMV Virus World and ICTVdB Picture Gallery

Some 10^{24} viral infections are thought to occur every second in the biosphere, a snapshot of a process likely to have been ongoing for several billion years… so the diversity is hardly surpising!

I will talk about work on only a minute fraction of these – picornaviruses – there is no silver bullet – each case will be a bit different

Pharma is used to structure based drug design, but vaccines?

We can now determine the atomic structure of COMPLETE viruses relatively easily, and the question is does this allow us to design new/better vaccines?

The technology advances to enable this are remarkable …

Dorothy Crowfoot Hodgkin

Fig. 1. TOBACCO NECROSIS VIRUS DERIVATIVE.

2½° oscillation photograph, crystal rotating about normal to (001), X-ray beam parallel to (100). 90 hours exposure.

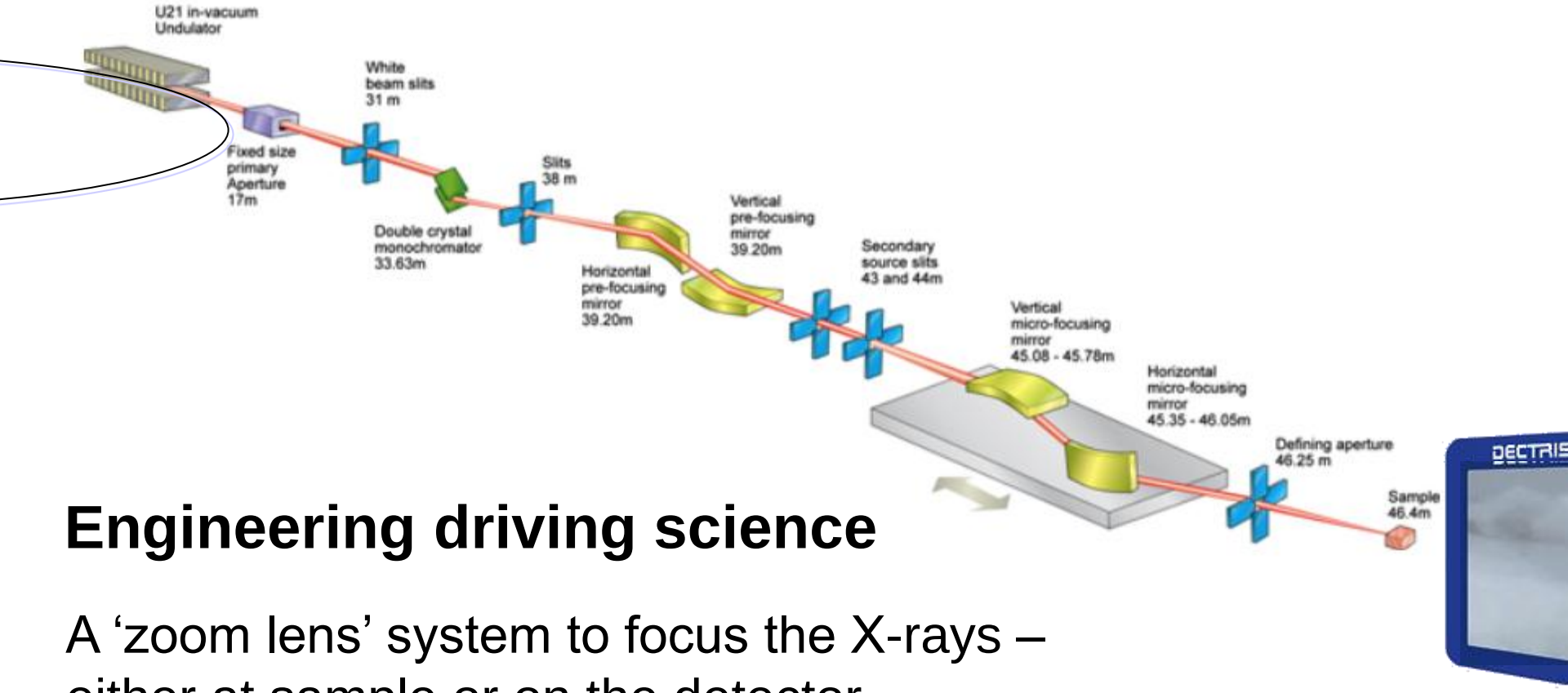
1945

2012

Diamond light source, UK

0.1 sec exposure – for a bigger virus!

A modern synchrotron beamline – 50 metres of optics



Engineering driving science

A ‘zoom lens’ system to focus the X-rays – either at sample or on the detector

10^{12} X-rays per second

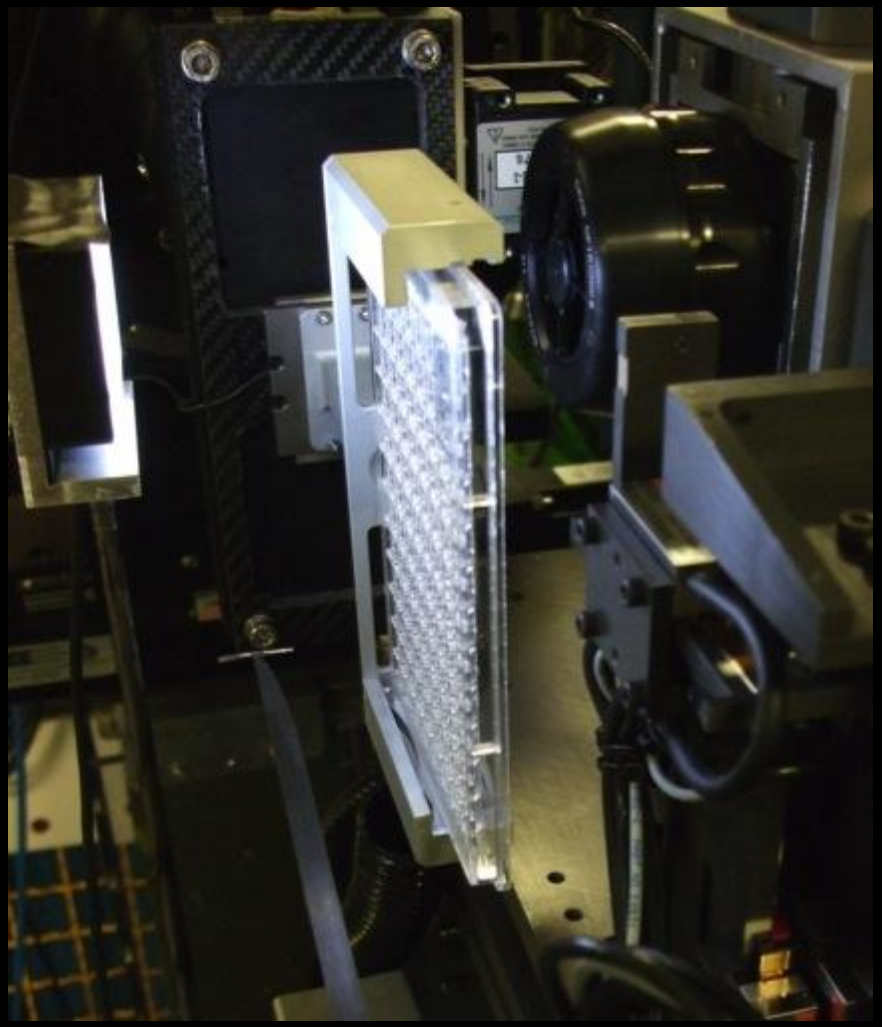
Beam size 5 – 50 µm

Gwyndaf Evans, Danny Axford, David Waterman, James Foadi, Jun Aishima, Robin Owen
Diamond Light Source

So we have gone from taking long exposure photographs with a pinhole camera to high speed movies on a sophisticated camera with a zoom lens … and there is more to come!

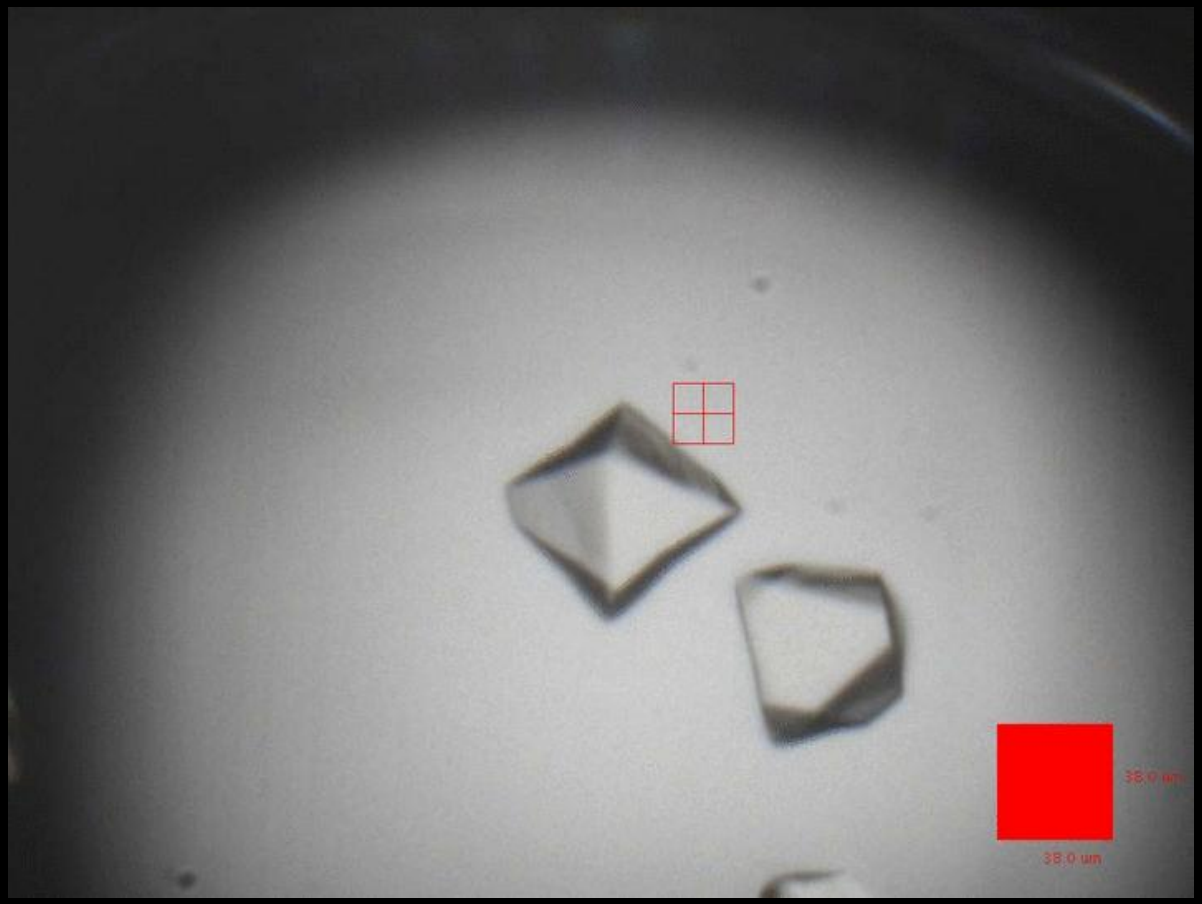
We can deal with fragile pathogenic crystals

- *In situ* in the tiny (nl) drops in which they are grown



We can deal with fragile pathogenic crystals

Looking along the X-ray beam – each crystal survives 0.4s – the X-ray beam is 1/50th of a millimetre across

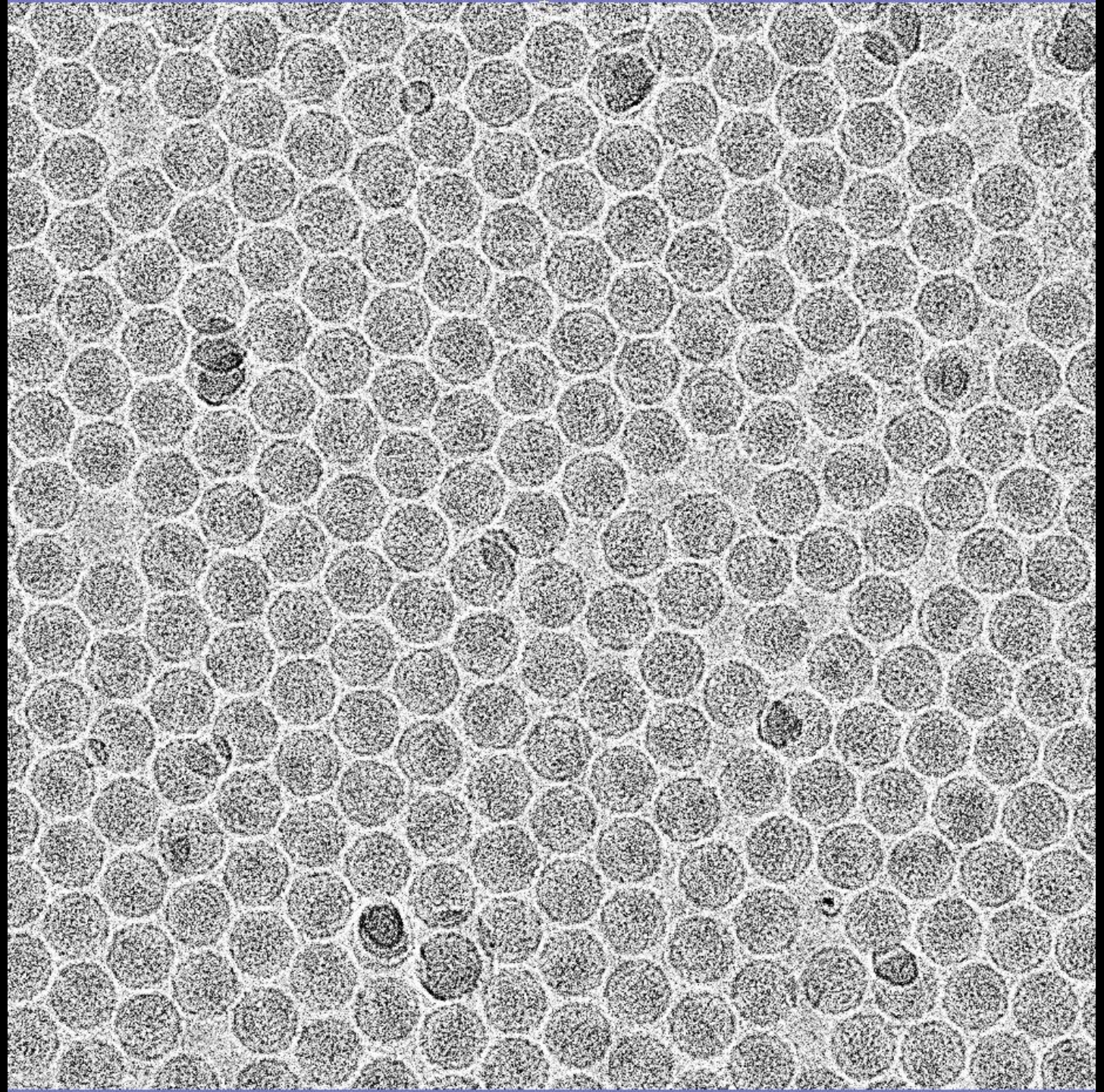


I24 staff, plus E Fry, JS Ren, A Kotcha DIS, Oxf (Axford et al 2012)

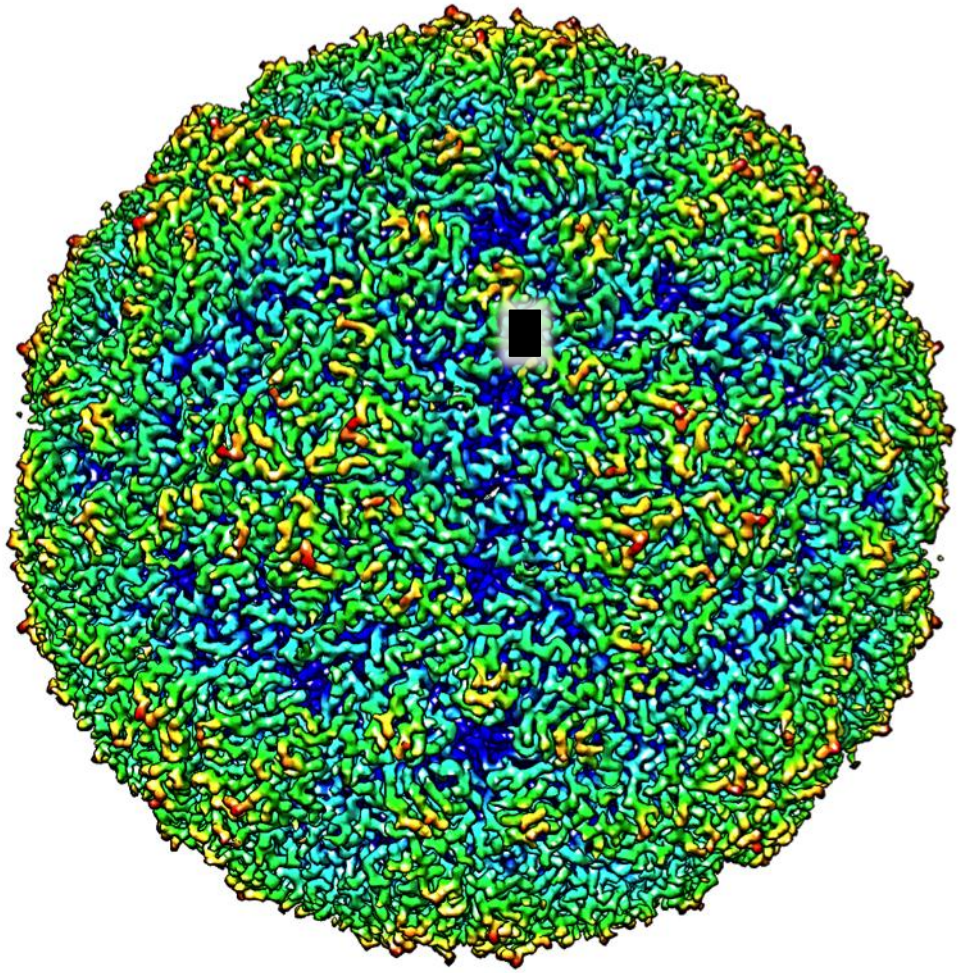
And there are revolutions in electron microscopy…

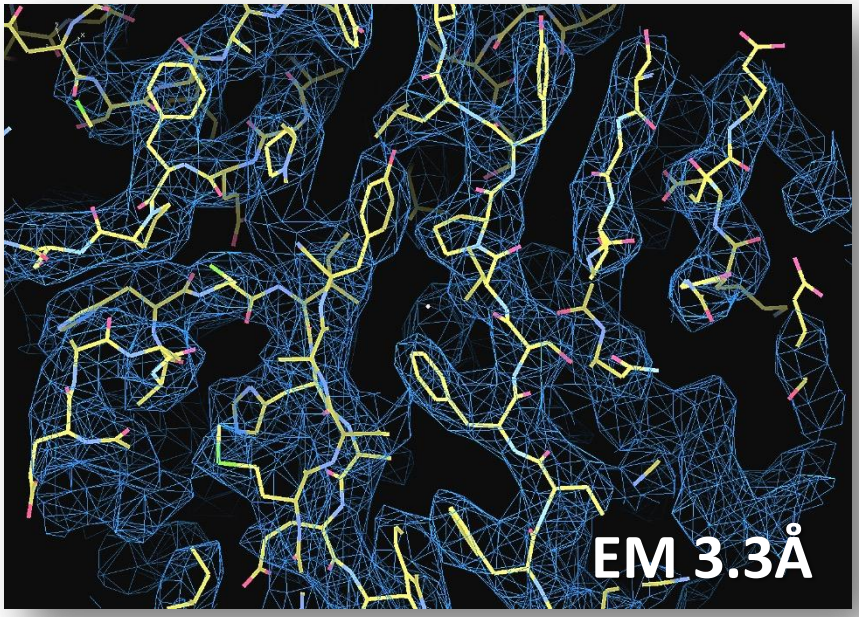
Wonderful new detectors, that collect 4k movies, and allow virus particle movements to be tracked in software … so we can see atomic detail
Live virus in cryo – in a water ‘glass’

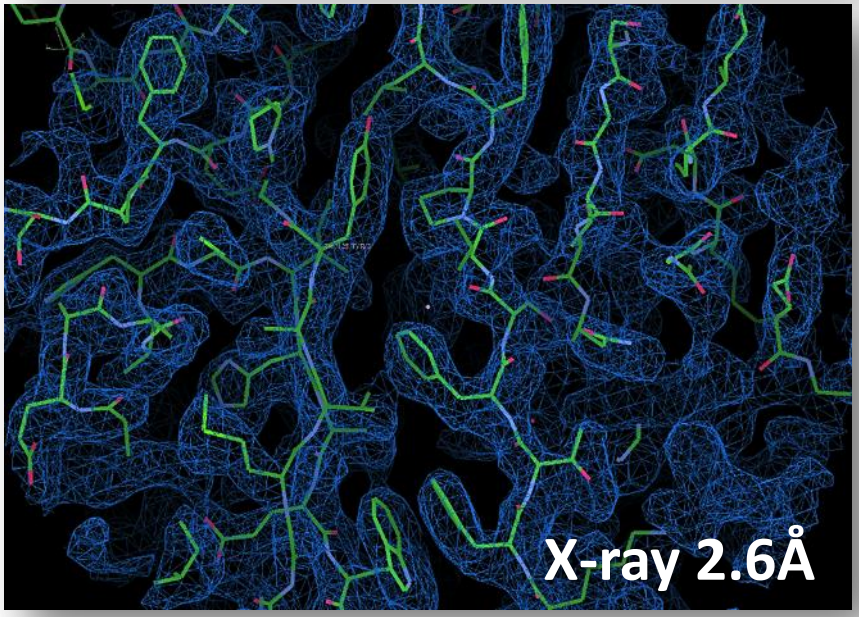
Expensive – hence a National Facility for the UK



Example from foot-and-mouth disease virus electron microscopy and X-ray structures







Both of these methods are now pretty quick (<1/2 day data collection), and require smaller amounts of sample than in the past

Our targets: Picornaviruses

- RNA viruses with an icosahedral protein capsid – no lipid!
- name is derived from *pico*, meaning small, and RNA – *ie* small RNA virus
- include many important pathogens of humans and animals ranging from acute "common-cold"-like illnesses, to poliomyelitis, to chronic infections in livestock

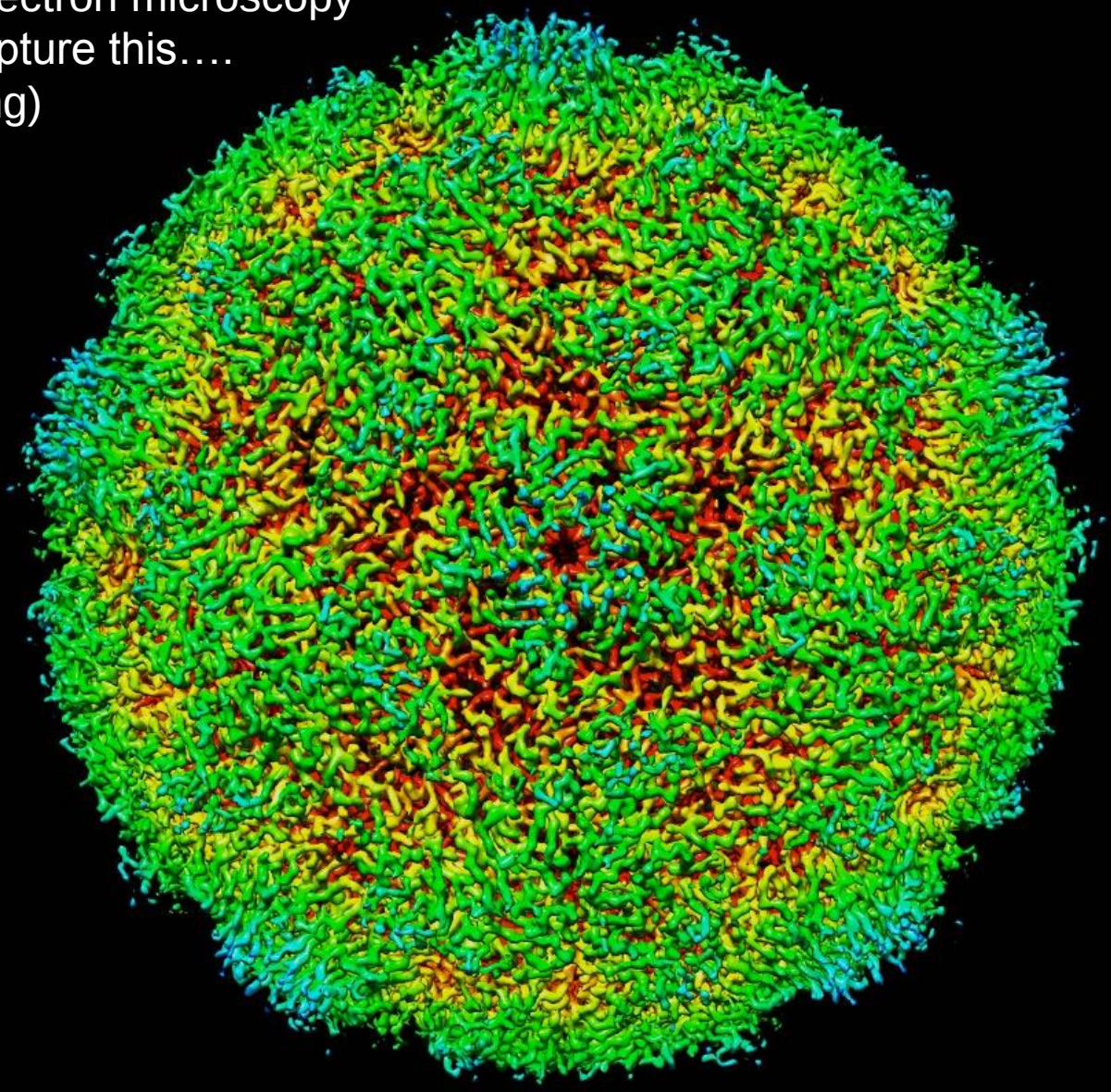
- **We have a smattering of vaccines, based on 50 year old technology and NO licensed drugs.**
- **B cell responses critical – complete virus particle required for proper antibody protection**

Our targets: Picornaviruses

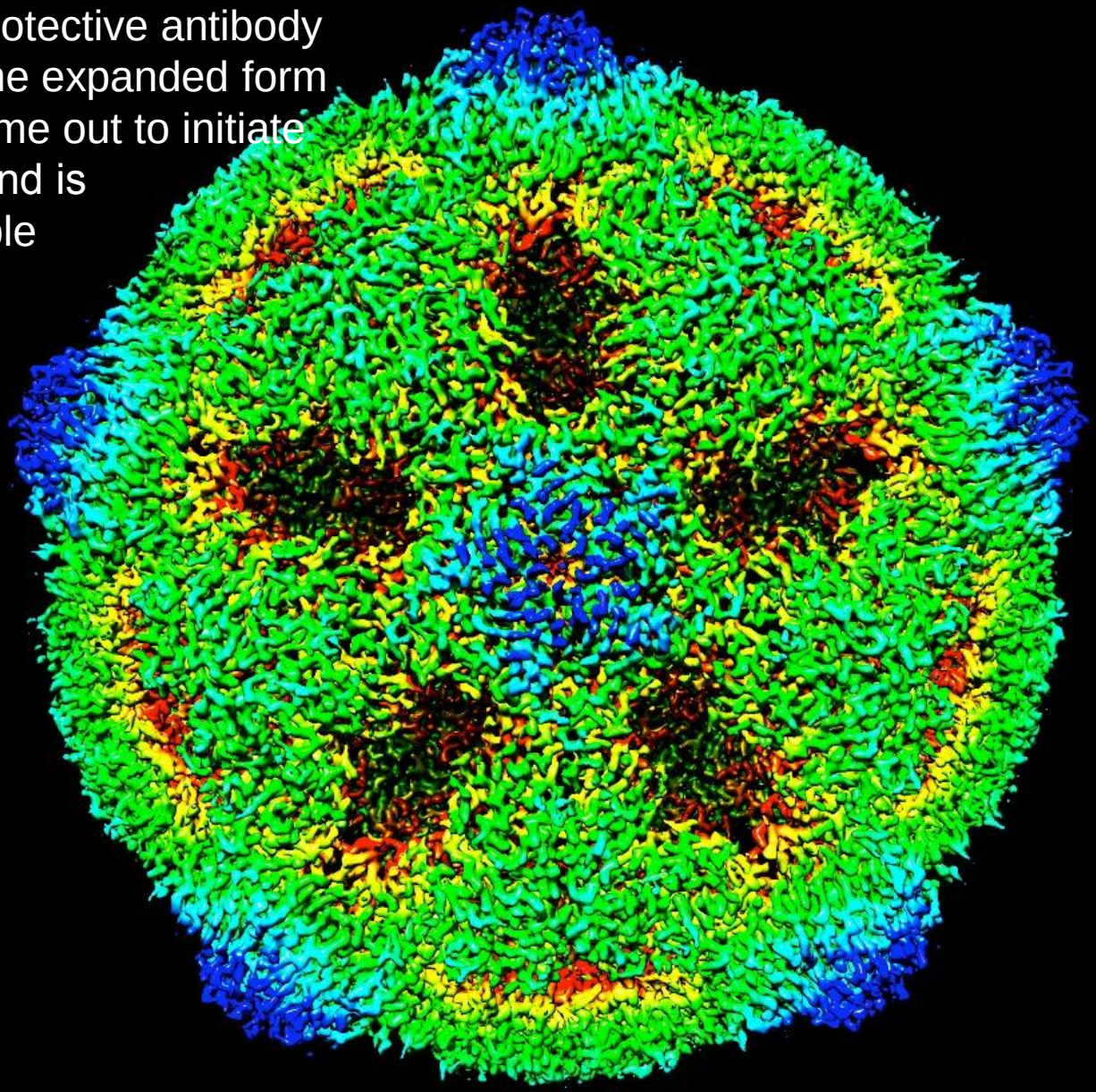
- **Aim to do two ‘simple’ things**
  - **make a synthetic version**
  - **make it stable enough to do the job**

- **I will give as a major example Foot-and-mouth disease virus (FMDV) – but even in this family there are differences, for instance swine vesicular disease virus is more like poliovirus**

These enteroviruses ‘breathe’
A series of electron microscopy structures capture this….
(Xiangxi Wang)

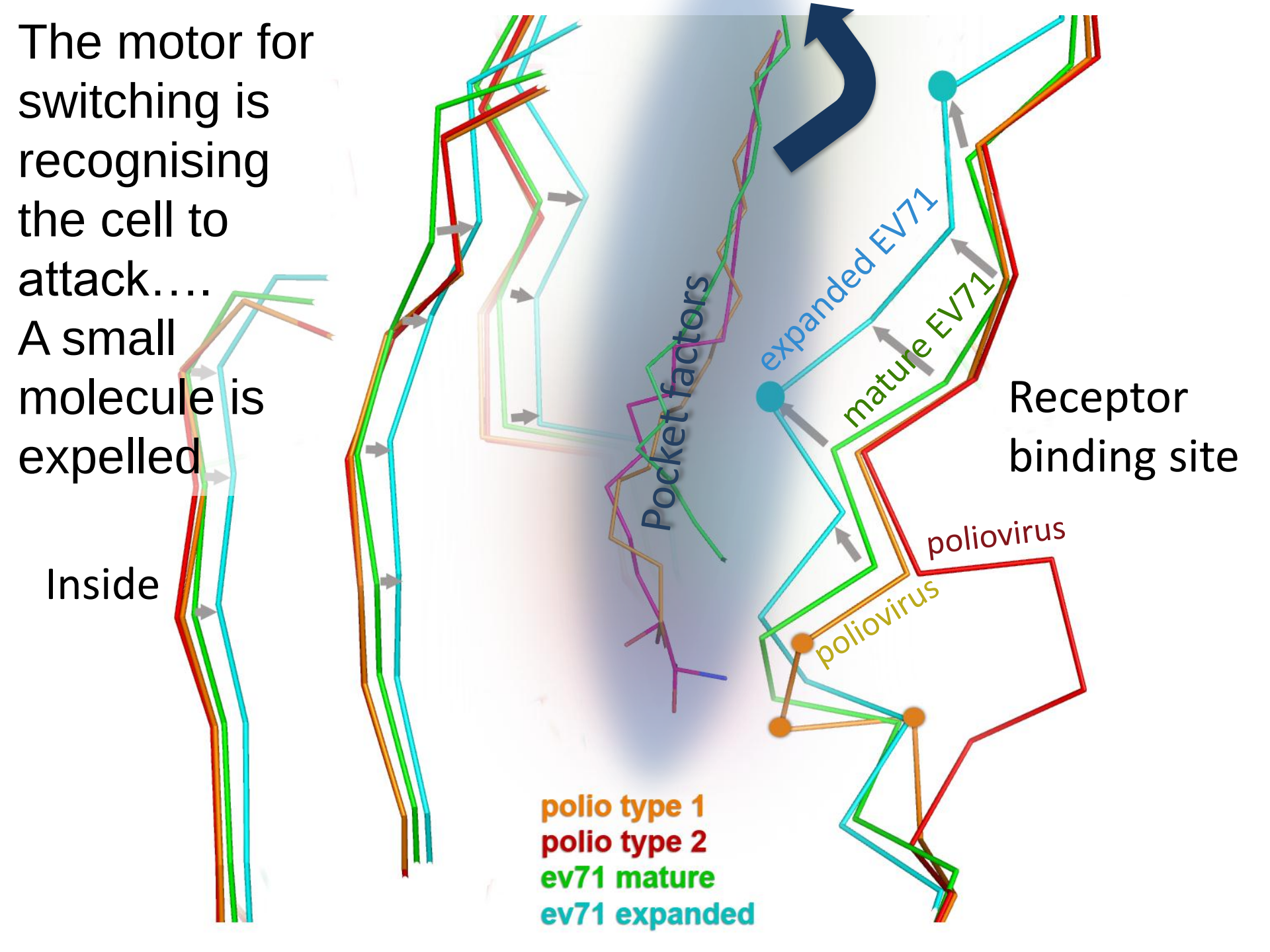


Only the unexpanded form can generate a protective antibody response – the expanded form lets the genome out to initiate infection … and is the most stable PROBLEM since minus RNA stability is reduced

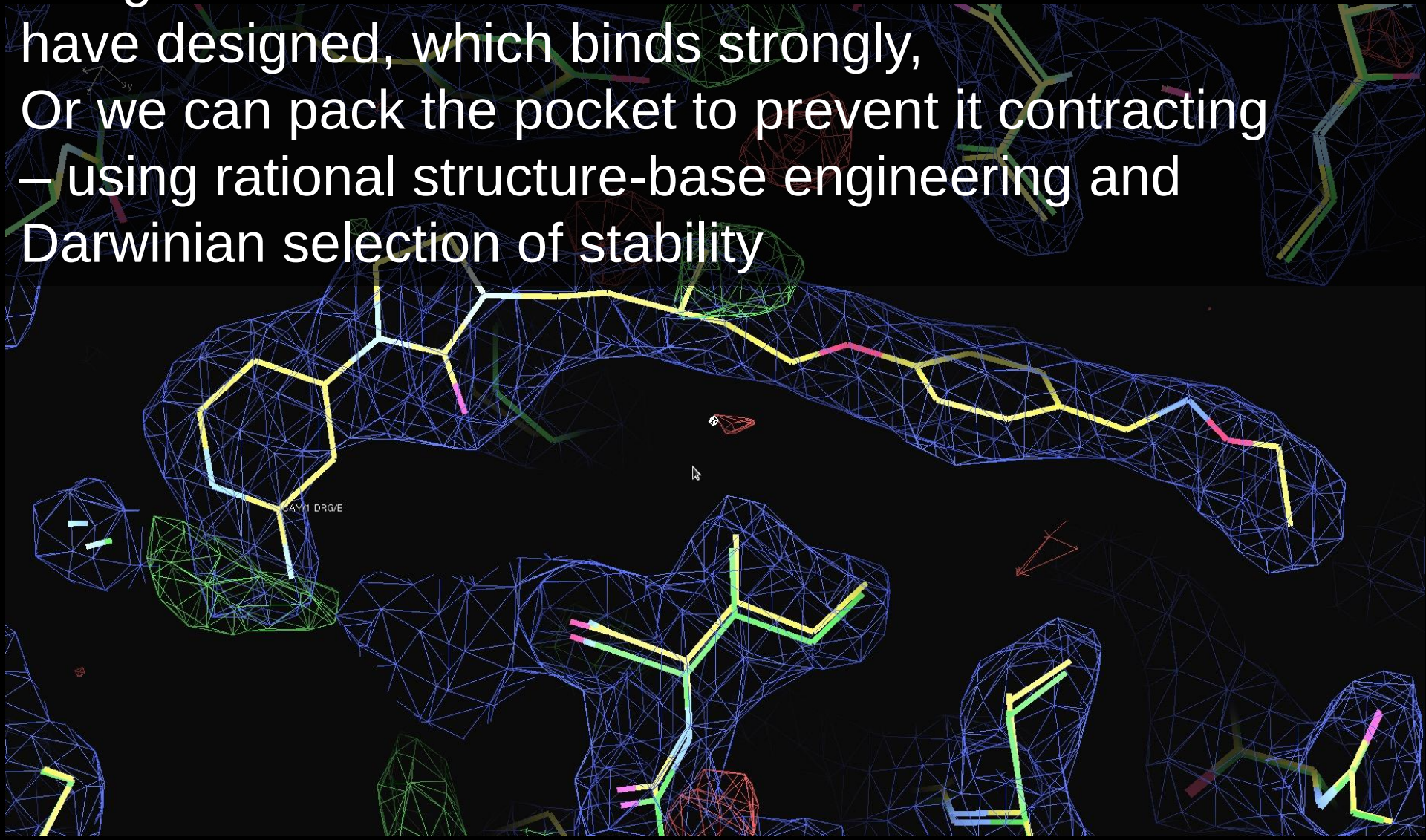


The motor for switching is recognising the cell to attack….
A small molecule is expelled

Inside

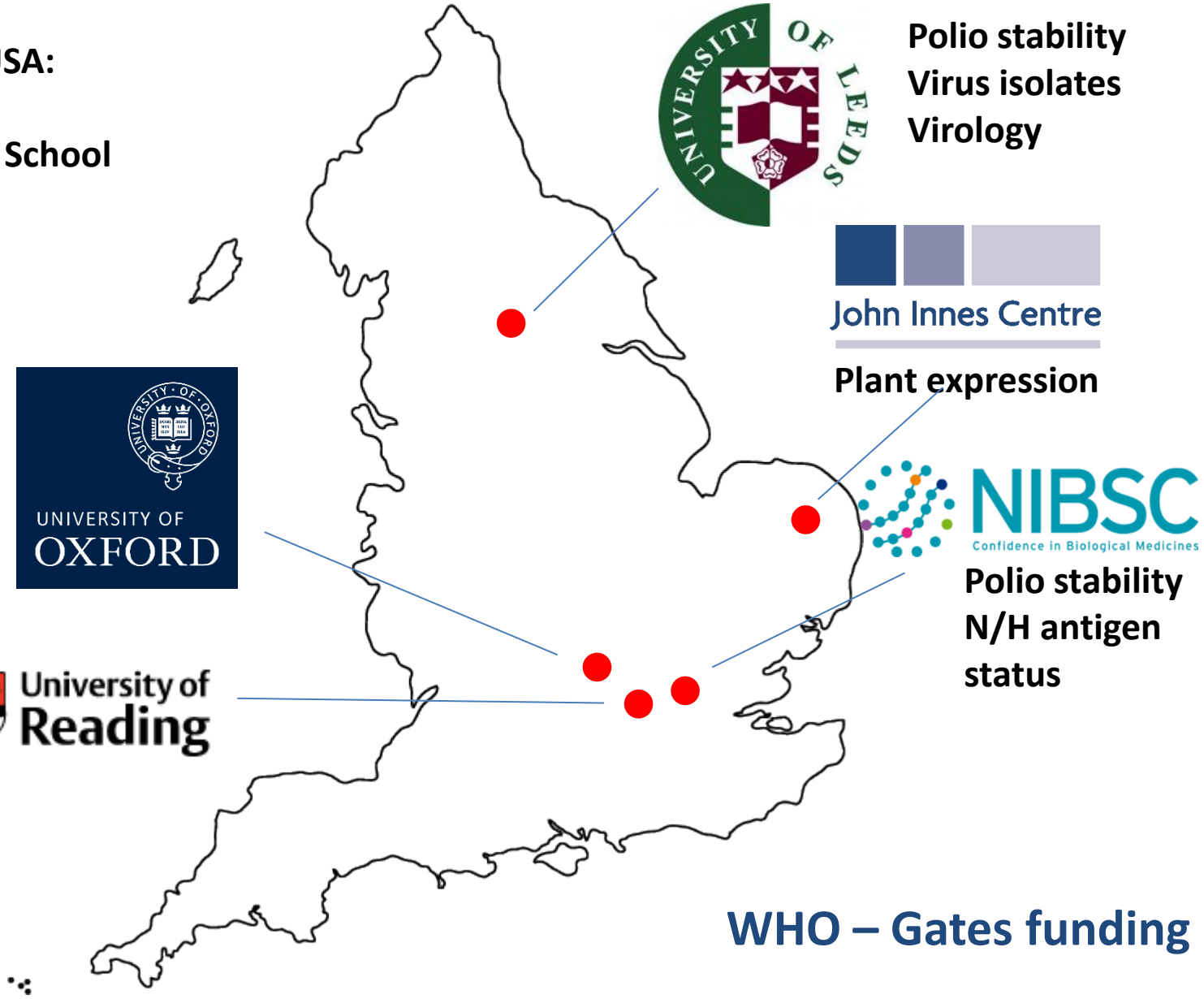


So we might lock the virus in the correct antigenic state using a small molecule – for instance this molecule we have designed, which binds strongly,
Or we can pack the pocket to prevent it contracting – using rational structure-base engineering and Darwinian selection of stability



Polio - collaborators

Collaborator in USA:
James Hogle,
Harvard Medical School



Polio stability
Virus isolates
Virology

Plant expression

Structure based prediction
Pocket binding drugs

Polio stability
N/H antigen status

Baculovirus expression
VLP synthesis

WHO – Gates funding

- **For FMDV we can use a different approach … as we will see …**

Foot-and-mouth disease – structure-based vaccine development

Supported by
wellcome trust

STRUBI

Liz Fy, Abhay Kotecha, Ren Jingshan, Claudine Porta (Pirbright), Tom Walter, Karl Harlos

WTCHG

Robert Esnouf

THE Pirbright INSTITUTE

Bryan Charleston, Julian Seago, Terry Jackson, Alison Burman, Clare Grant, John Hammond

Ian Jones, Reading University

ARC • LNR
Excellence in Research and Development

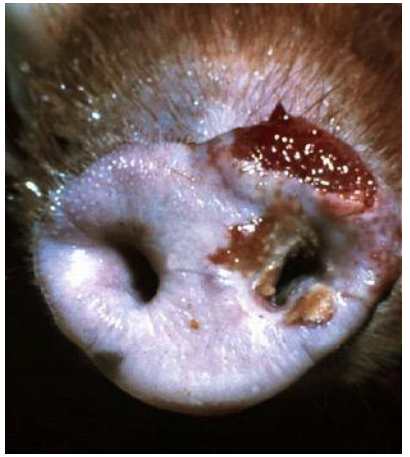
Francois Maree, Katherine Scott, SA

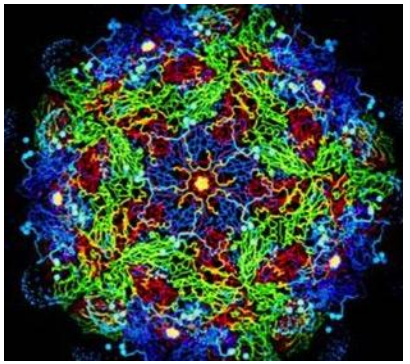
Dr Venkat, Bangalore

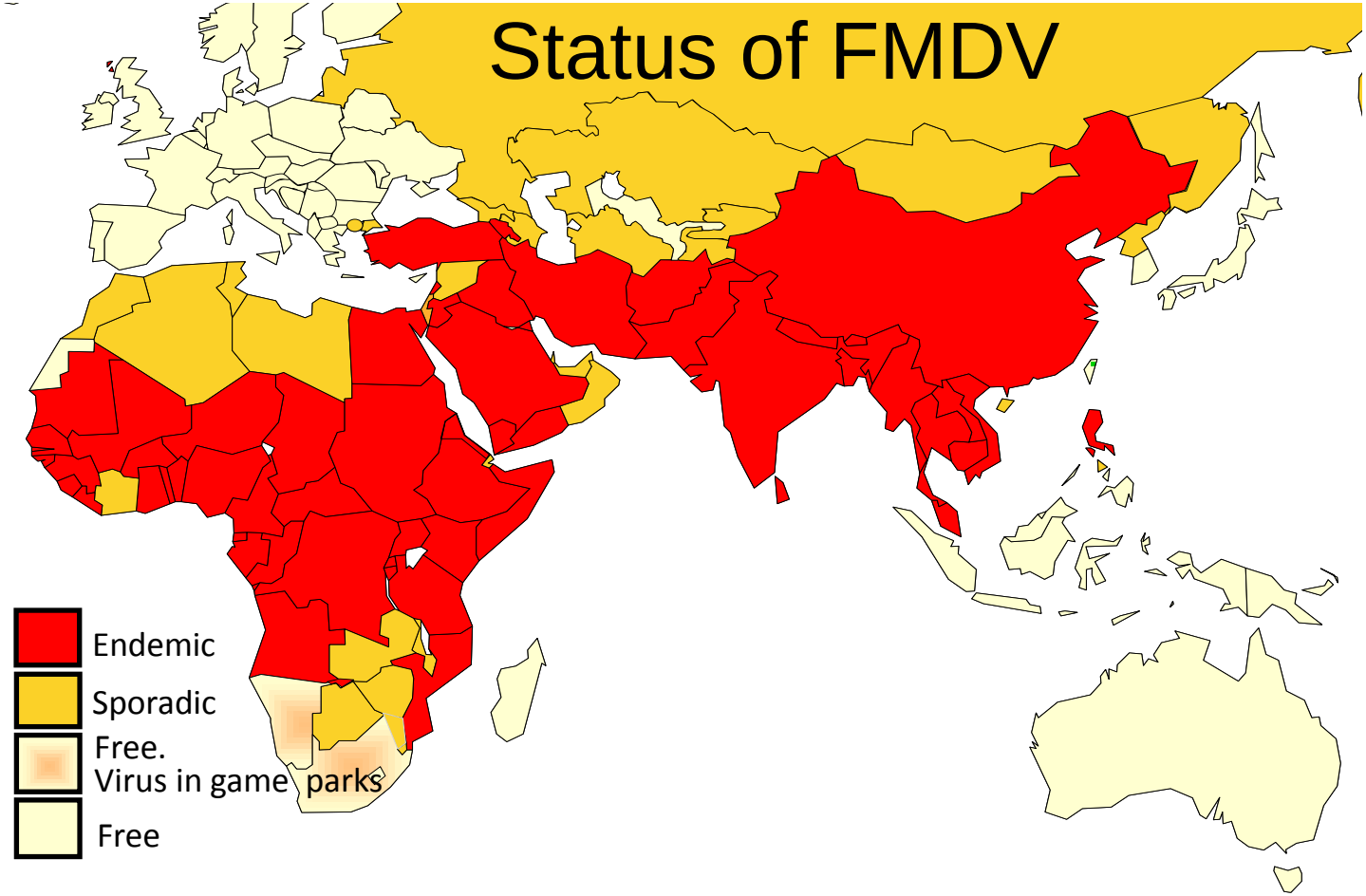
dp Intervet Schering-Plough Animal Health

MSD Intervet

Foot-and-mouth disease.
A global food security issue and impediment to wellbeing







- Much of the global FMD burden of production losses falls on the world's poorest communities, and those which are most dependent upon the health of their livestock.

- Overall the direct losses **limit livestock productivity** creating a **food security** issue and contributing to **malnutrition**

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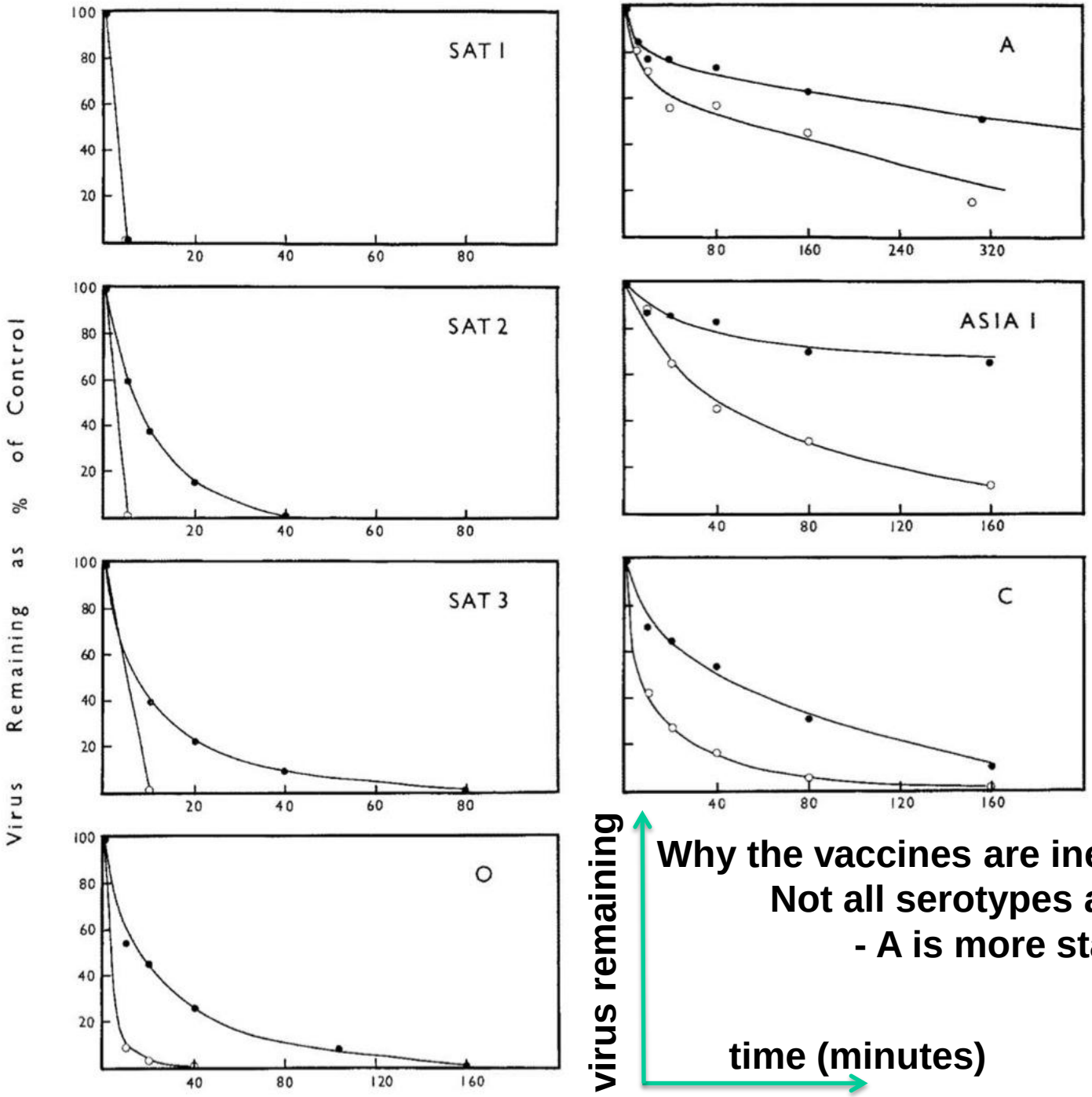
- Overall the direct losses **limit livestock productivity** creating a **food security** issue and contributing to **malnutrition**

- **Current vaccines** are inactivated live virus formulated with adjuvant
- Globally there is a **shortage of vaccine** – billions of doses needed.
- **This is 50 year-old technology**
- We are trying to bring this up-to-date: safe, cheap, effective (for some types of FMDV there is NO effective vaccine)
- (i) **How to make a synthetic virus-free vaccine?**
- (ii) **How to make it stable enough to be effective?**



FMDV Serotype stability

Thermal stability at 49 degrees,

Untreated - filled circles)

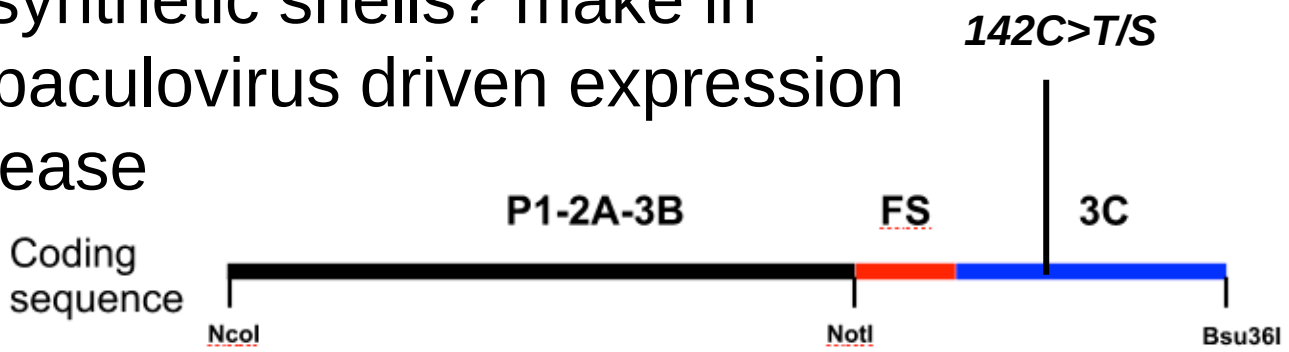
Chemically inactivated (BEI) - open circles

Why the vaccines are ineffective - instability
Not all serotypes are equally unstable
- A is more stable than O or SAT2

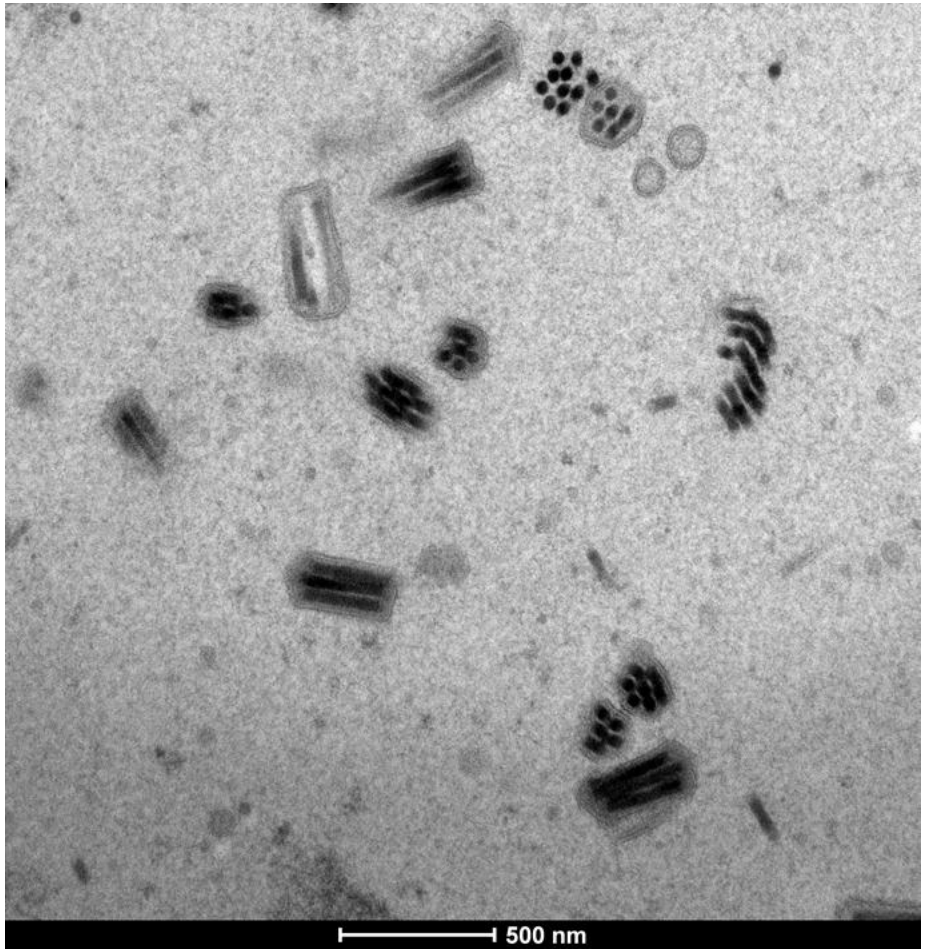
Data: Tim Doel

Q1: can we make synthetic shells? make in insect cells, using baculovirus driven expression – detune toxic protease

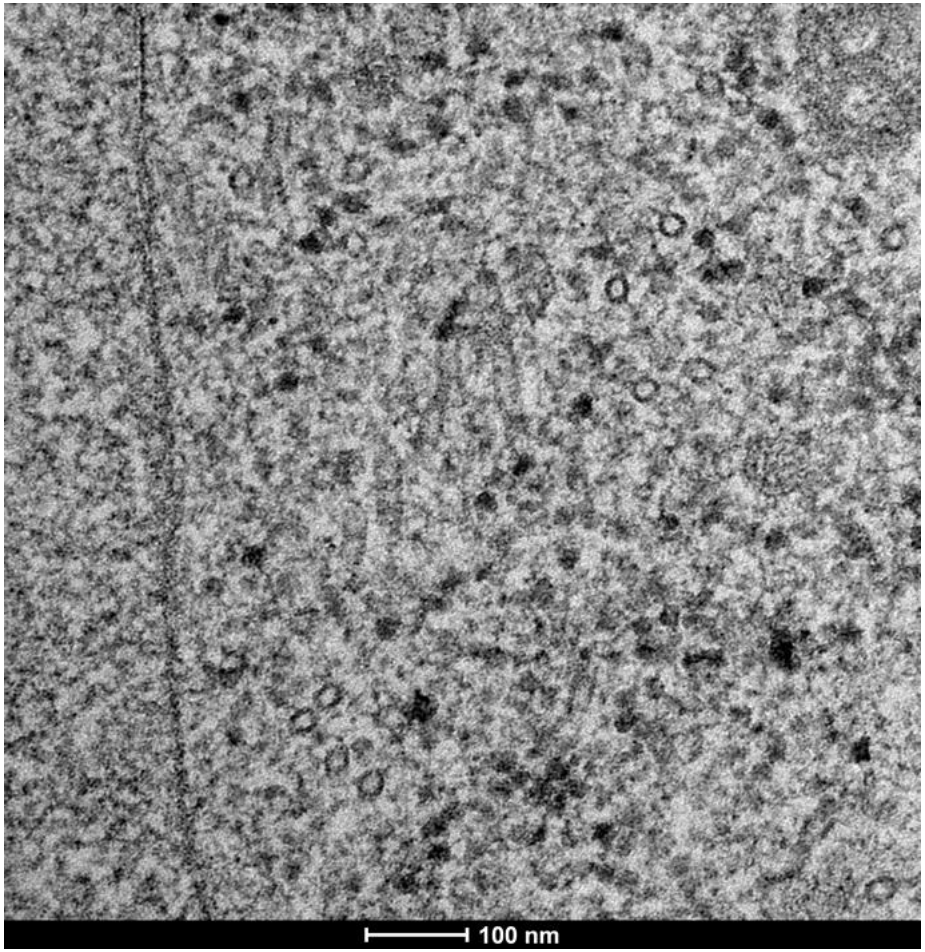
(Ian Jones, Reading)



Nucleus

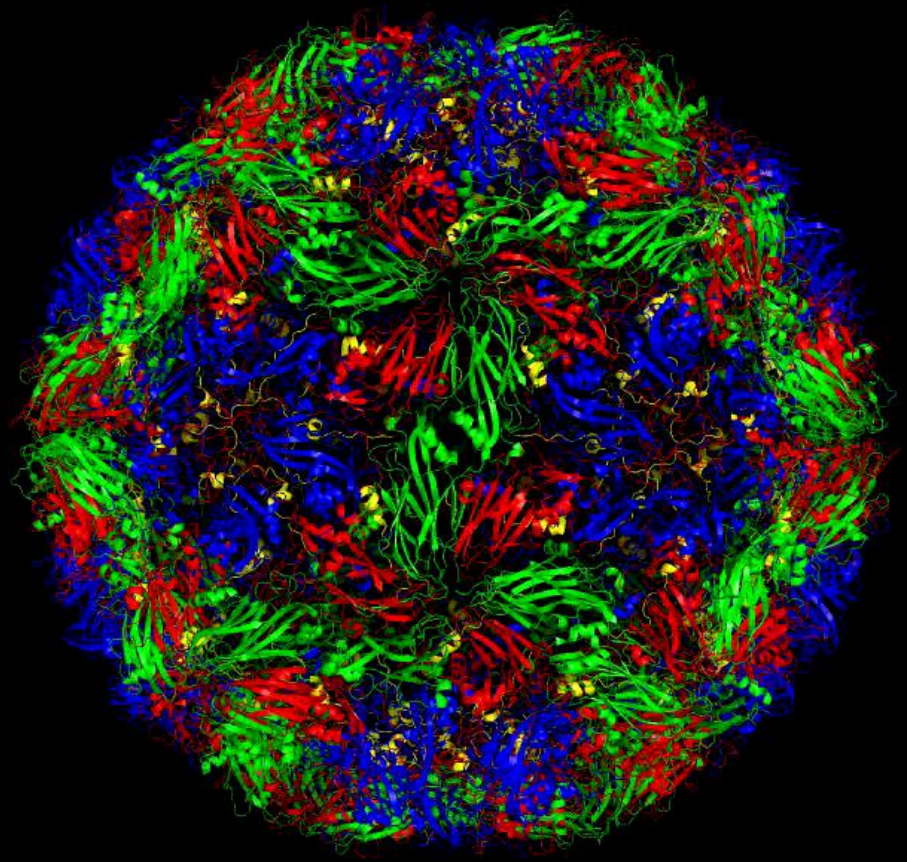


Cytoplasm



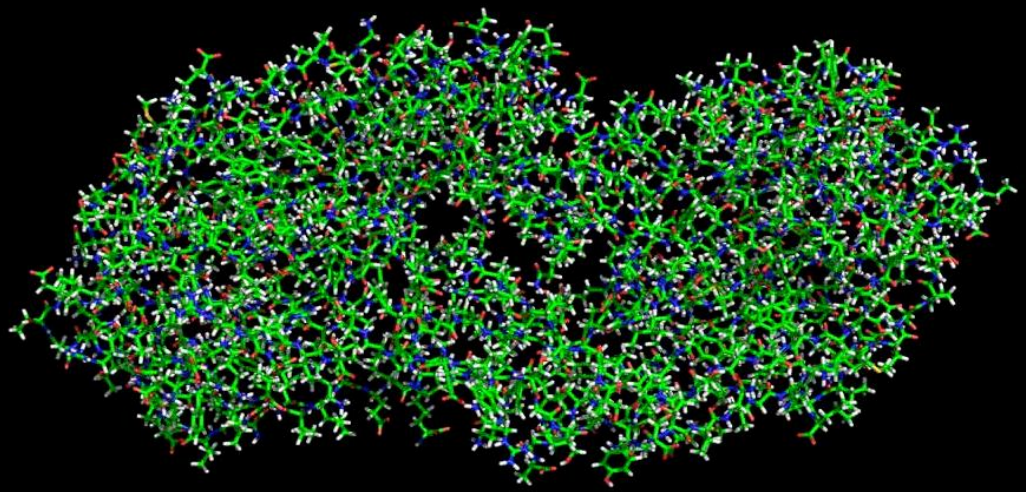
- Q2: Can structure help us re-engineer the particles to make them more stable?

FMD vaccines are fragile - capsids are unstable

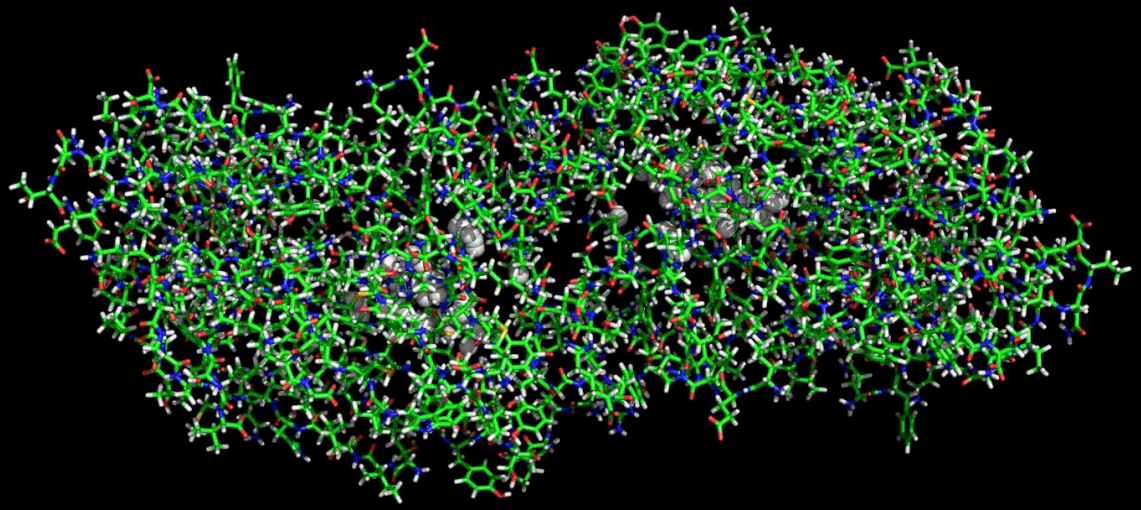


- Use *in silico* modelling to help solve the problem of what to re-engineer

- We first cut out the part that is the weak link …



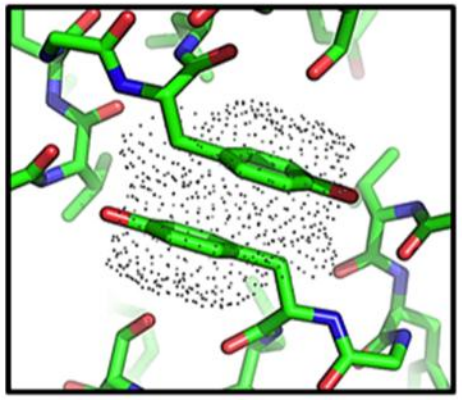
"Textbook" simulation – noise dominates



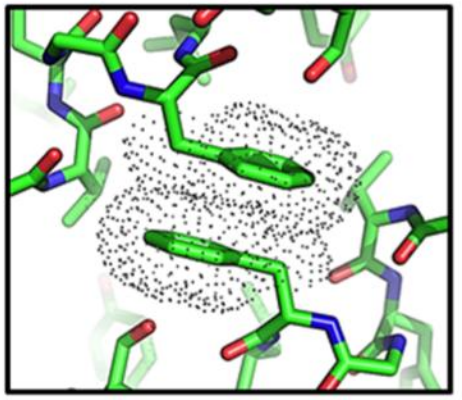
Modified simulation - target the weakspot

- We designed a series of single point mutations to stabilise the symmetry point at the centre of the interface between pentamers

and the predicted structures….

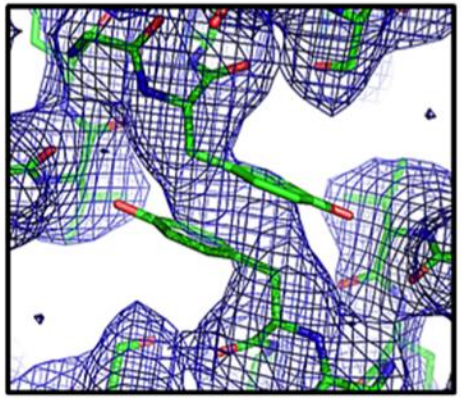


O1M VP2 S93Y

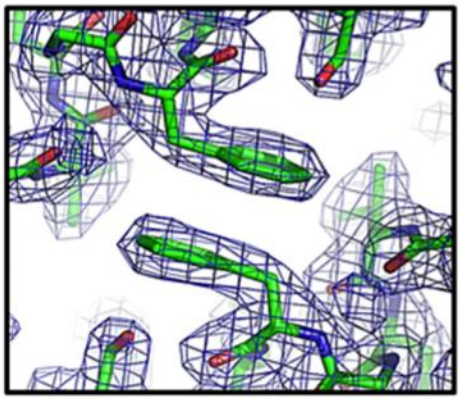


O1M VP2 S93F

look like the X-ray…



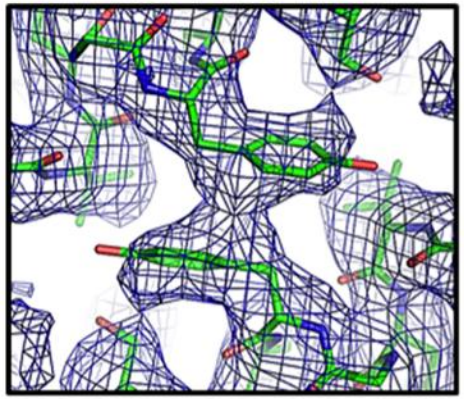
O1M VP2 S93Y



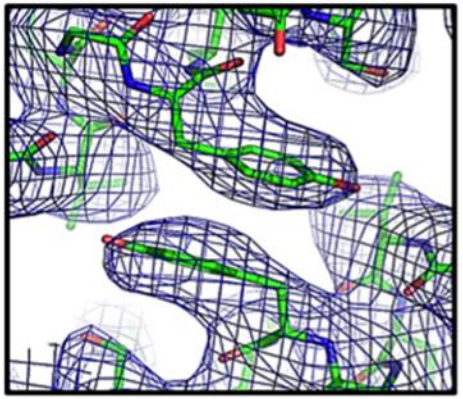
A22 VP2 S93F

and EM structures….

for two of the most unstable serotypes, O and SAT2



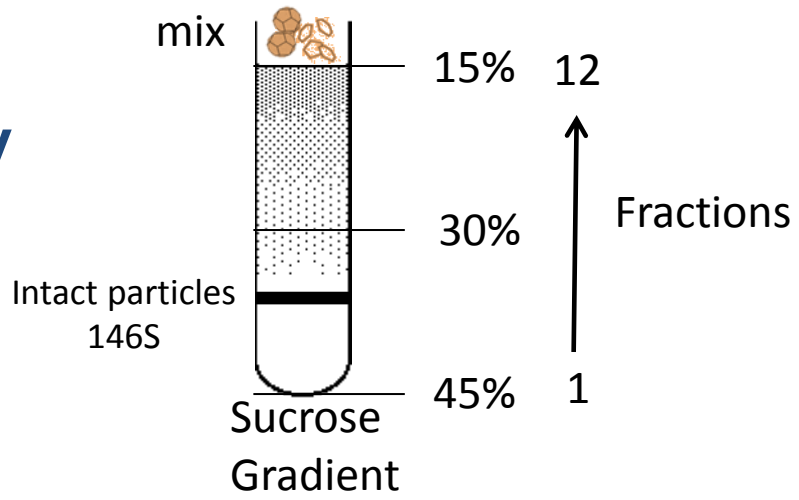
O1M VP2 S93Y

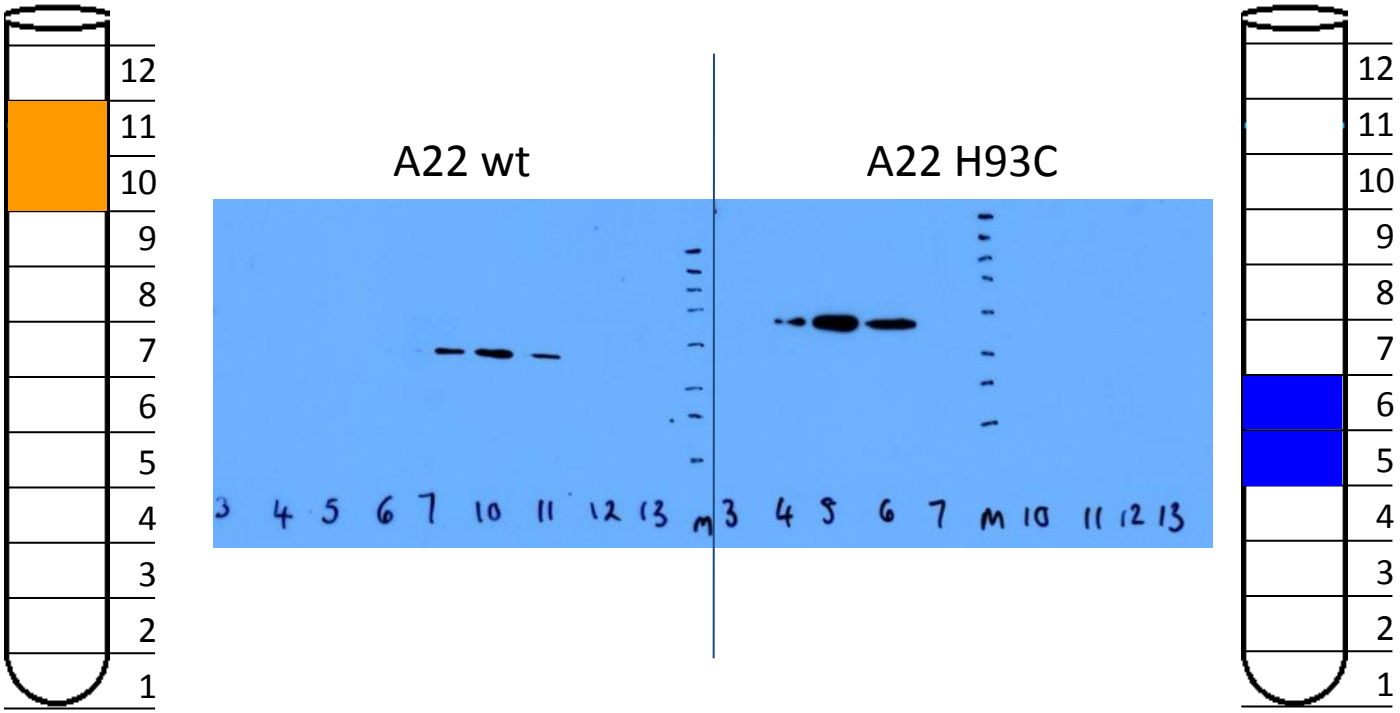


SAT2 VP2 S93Y

Validation: Temperature stability

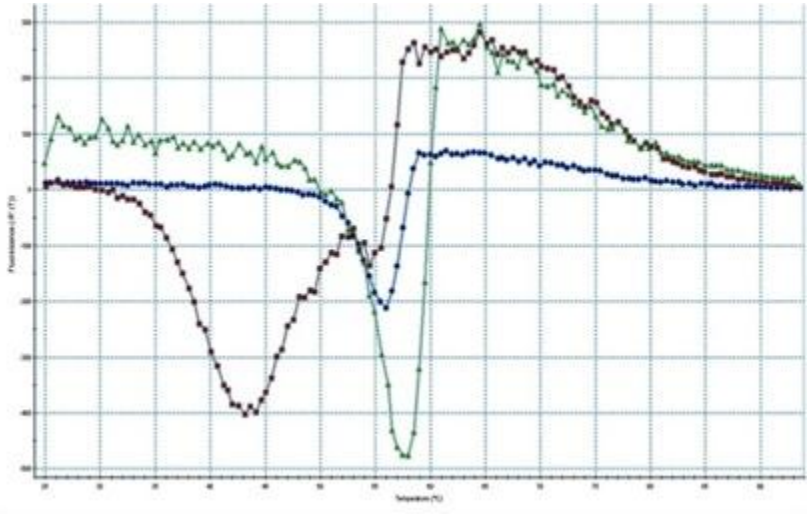
Heating at 56^0C for 2 hrs



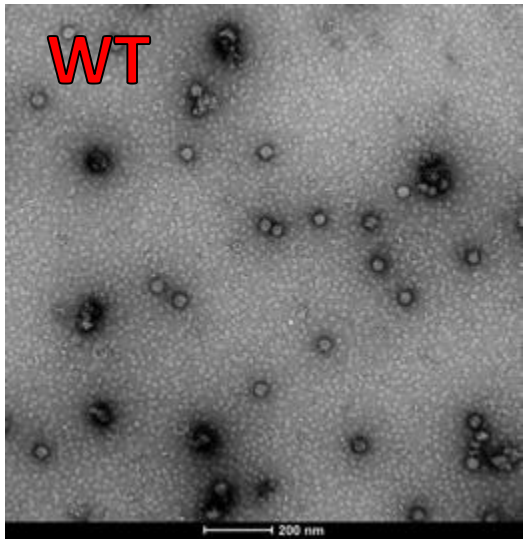


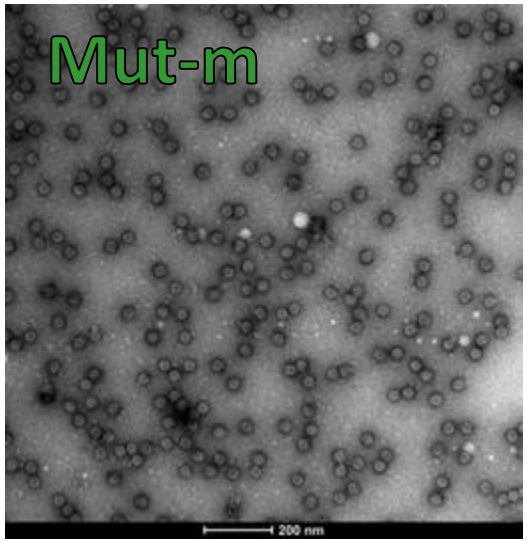
and they show *in vitro* stability (O serotype example)

Mutant	$\Delta\Delta G_{PB}$	Results
i	-5kcal/mol	Capsids stable
l	-3kcal/mol	Capsids stable
m	-5kcal/mol	Capsids stable
o	-3 kcal/mol	Few capsids, stable
p1	-2 kcal/mol	Not stable
p2	-3 kcal/mol	Poor yield & stability
p3	-.3 kcal/mol	Poor yield & stability



Thermoflour assay

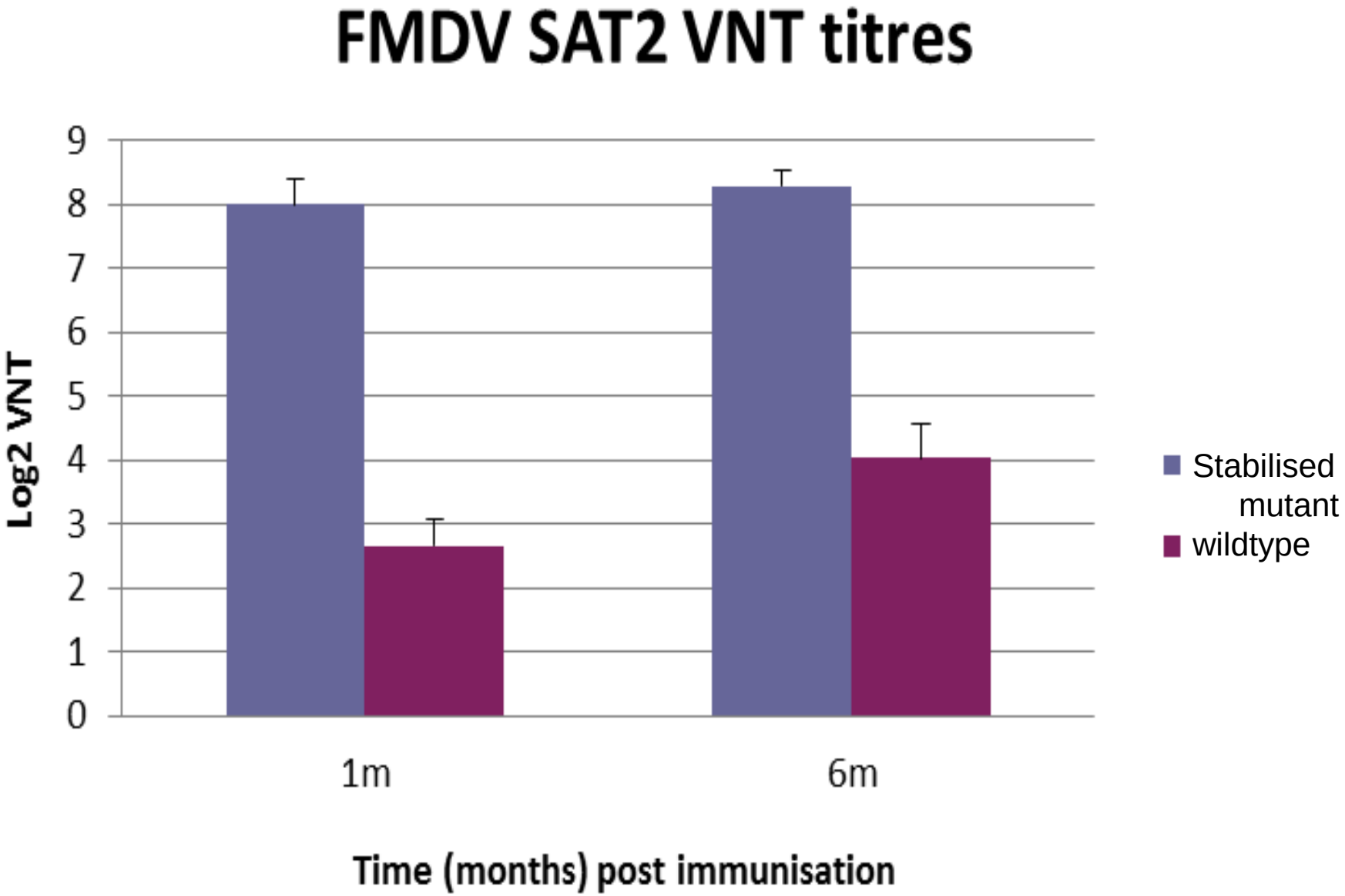




Purified capsids after 10 days in PBS at 4°C

Inactivated WT largely dissociates into pentamers

… and protect animals (SAT2 serotype example)



FMDV Stabilised empty capsids, summary

- Improved storage characteristics

- Safe production - no live virus required

- Smaller production plants could be built locally altering the economics of vaccine production

- Vaccine can be quickly produced to new virus variants

- Simple diagnostic to discriminate vaccinated and infected animal

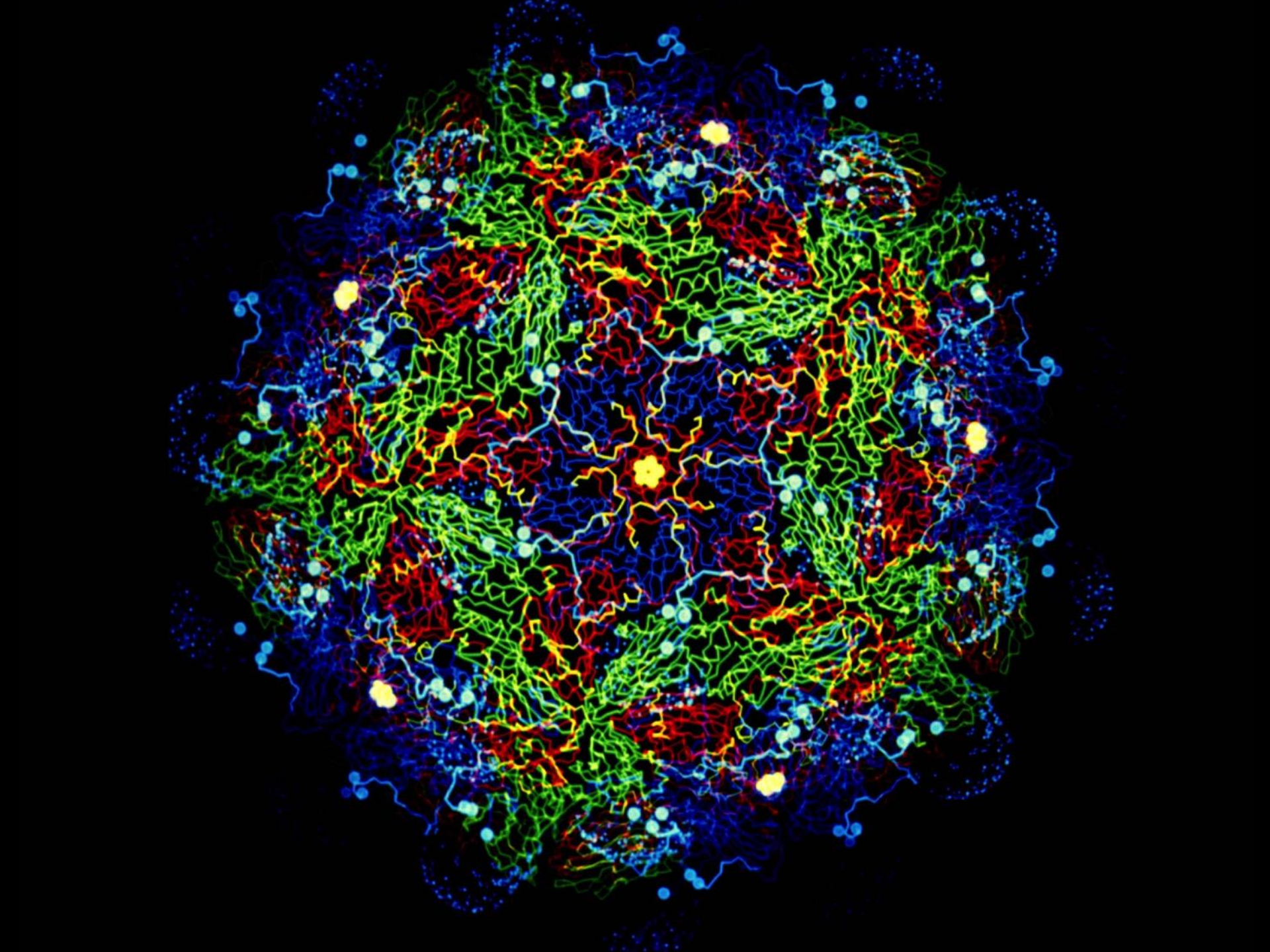
- Further animal tests and commercial viability tests underway

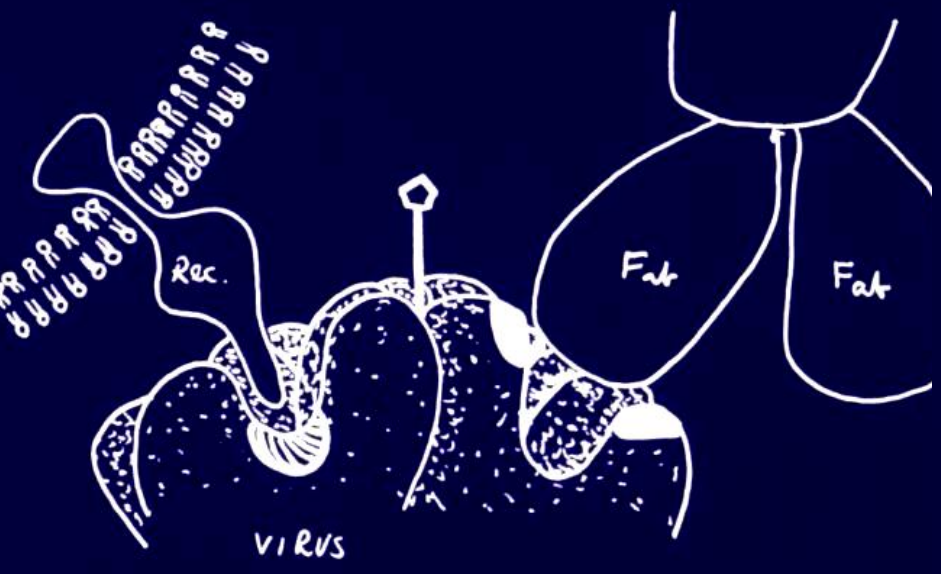
But can we think about making more cross-reactive responses?

Do we understand mechanisms for neutralisation?

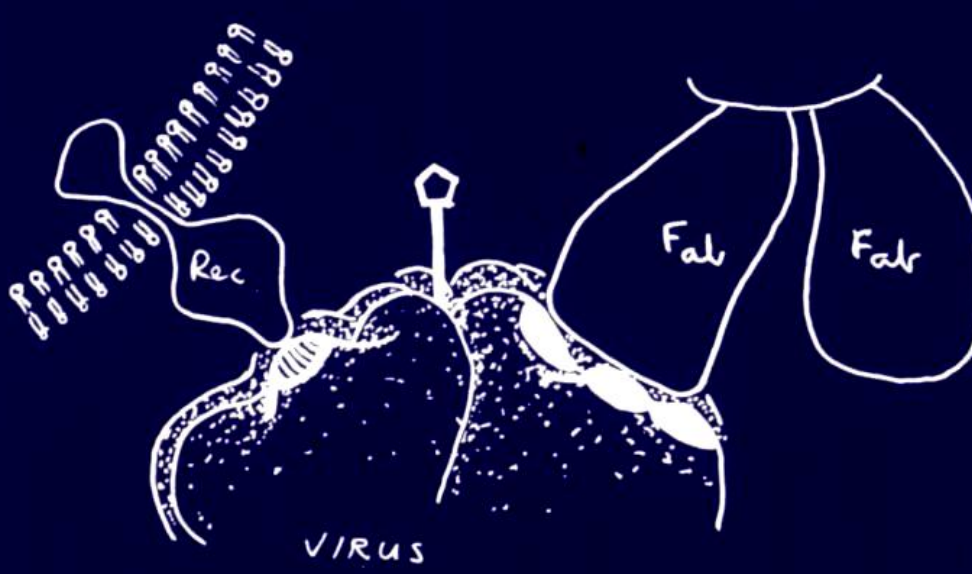
It appears that, although all of the virus surface should be visible there is one specific loop that seems to dominate.

The famous VP1 or ‘FMDV loop’
The mechanism of neutralisation here is probably very straightforward – the loop binds the internalisation receptor – the αvβ6 integrin.

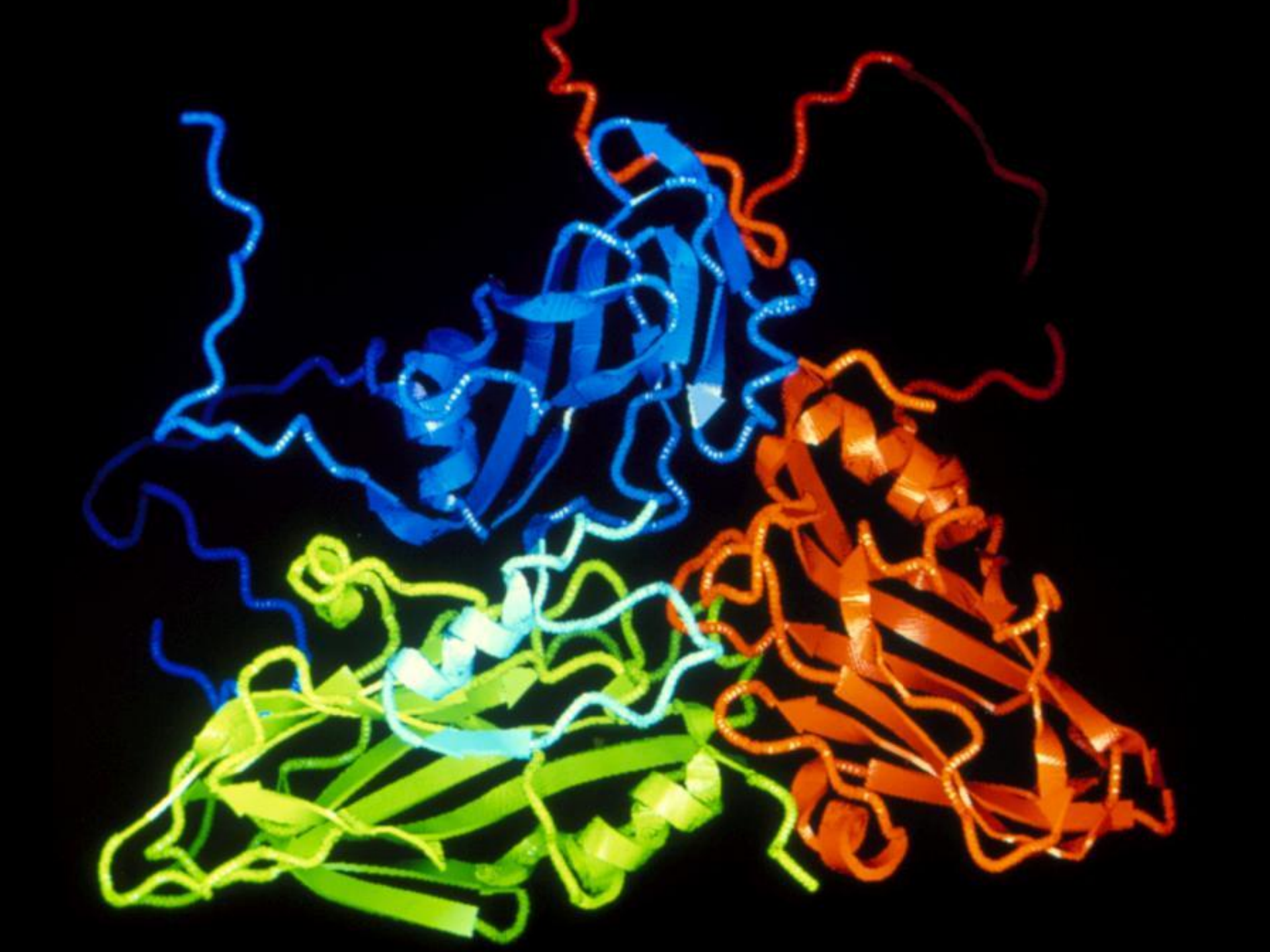


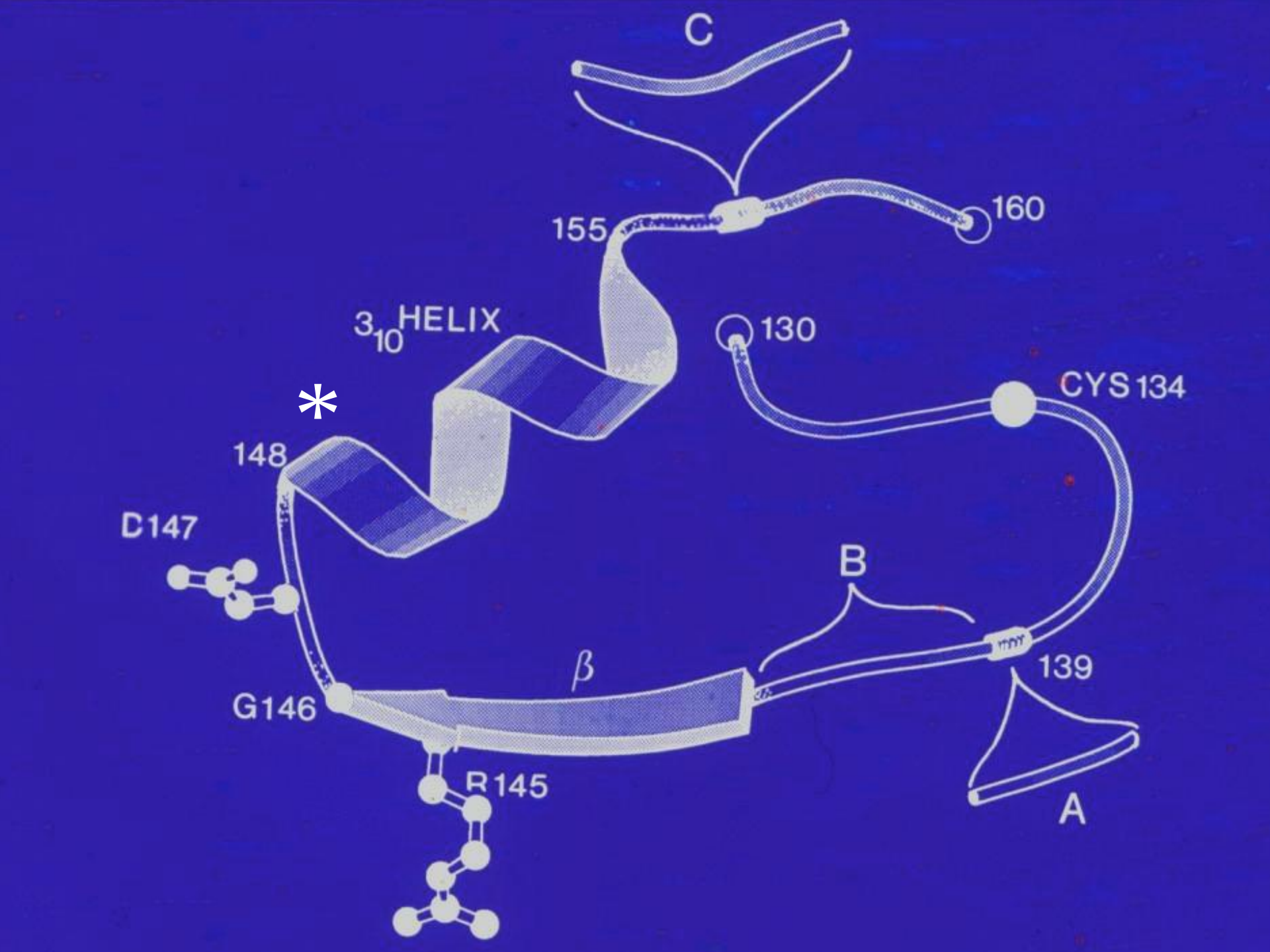


The Canyon Hypothesis

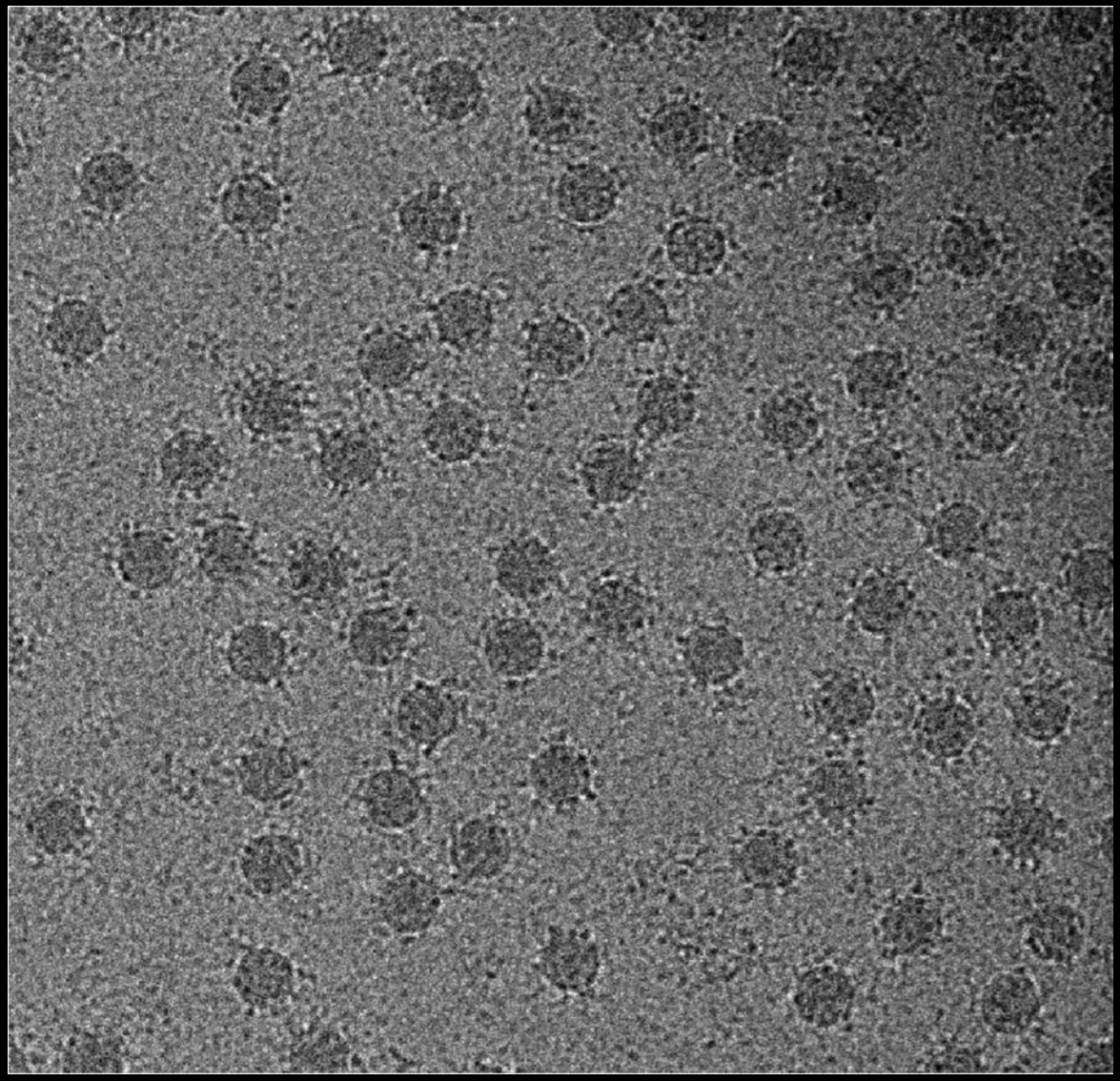


The FMDV Method




C
155
160
3_{10} HELIX
130
CYS 134
*
148
D147
B
β
139
G146
R145
A

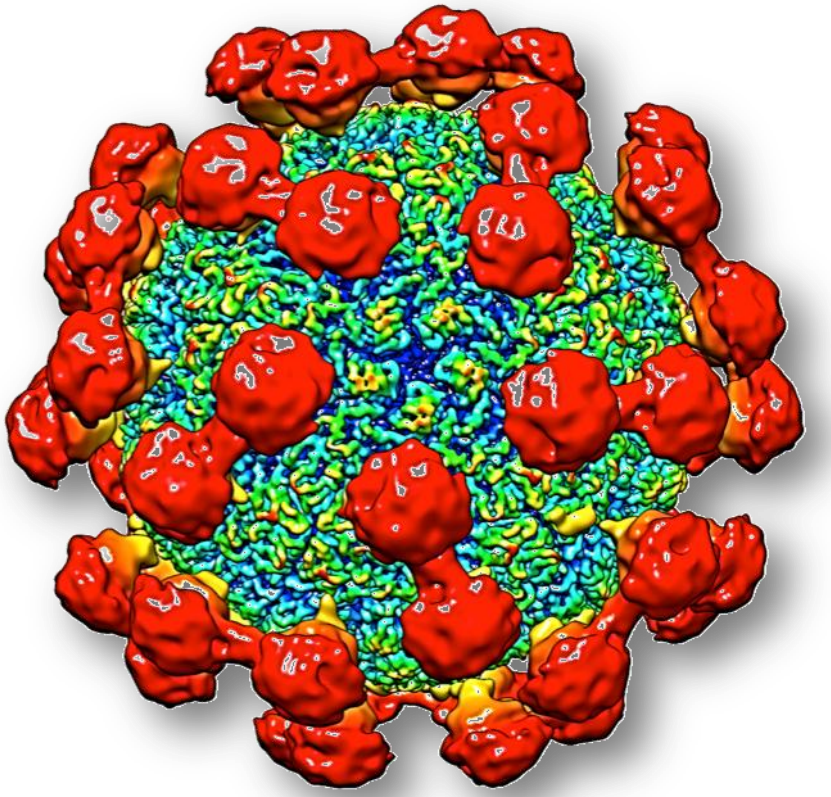
FMDV – αvβ6 complex + 2mM Mn



small headpiece
No Hybrid domain

O1M – αvβ6 complex

(collaboration with Prof. Tim Springer, Harvard)

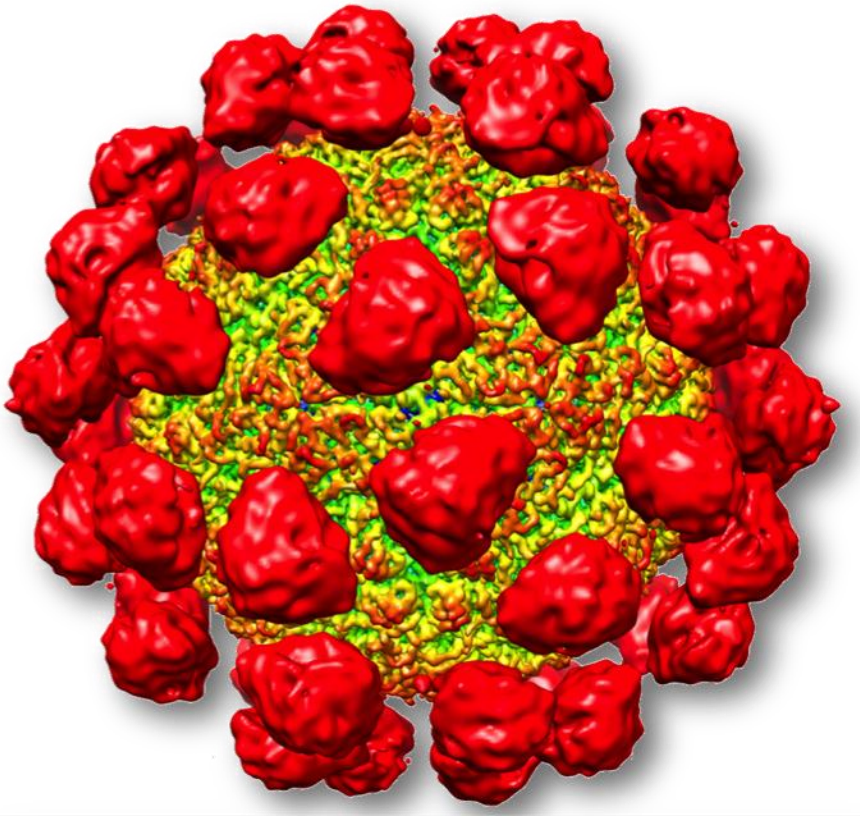


10900 Particles

Capsid resolution 3.4Å

Integrins are flexible - filtered at 10Å

2mM Mn

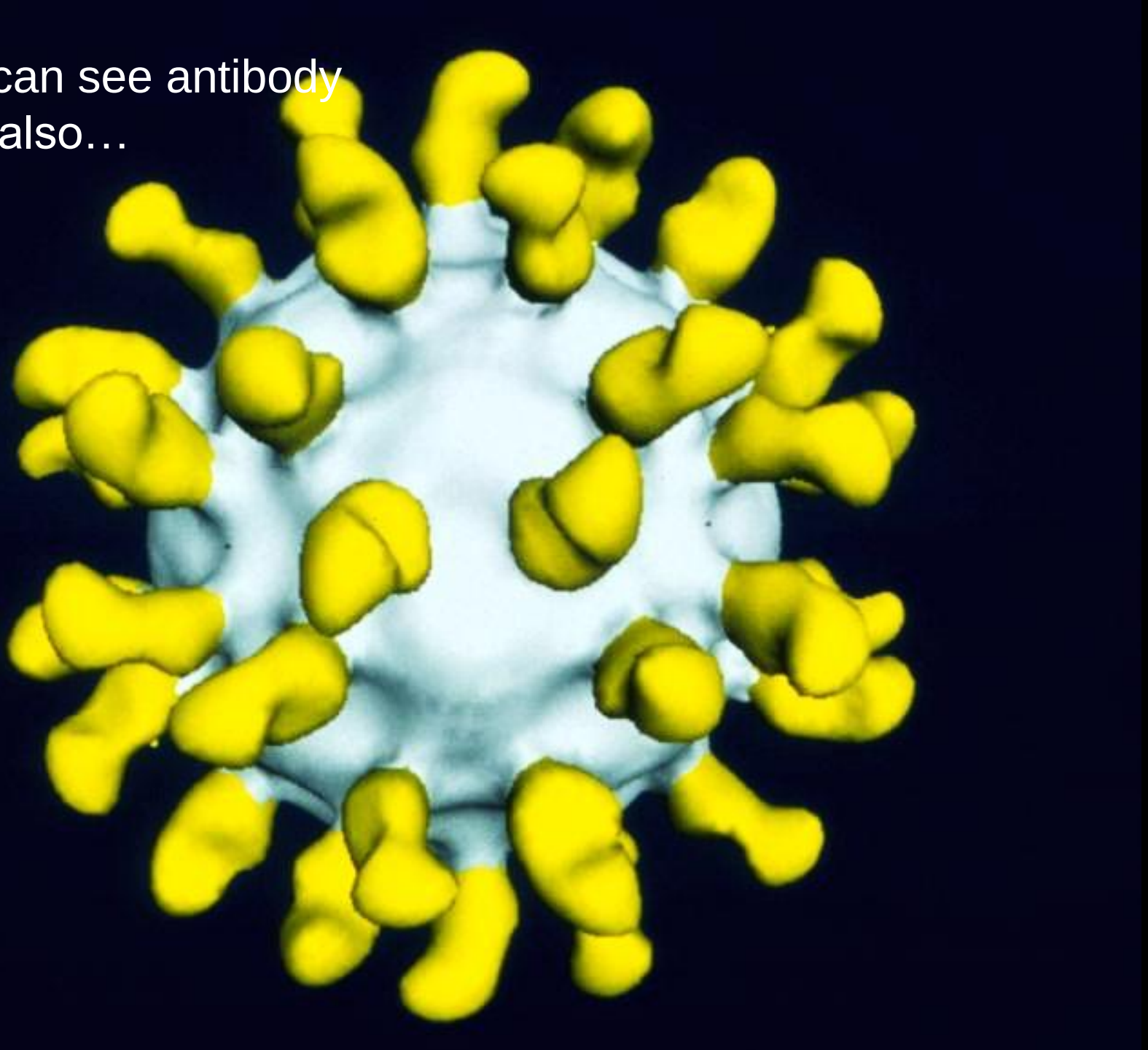


1649 Particles

Capsid resolution 3.4Å

Integrins are flexible - filtered at 10Å

But we can see antibody binding also…



We (Bryan Charleston leading) have set up a pipeline to investigate the immune response to

Deep IgG sequencing, to follow changes with vaccination (delta T, delta serotype)

Bioinformatics

Selection of Abs

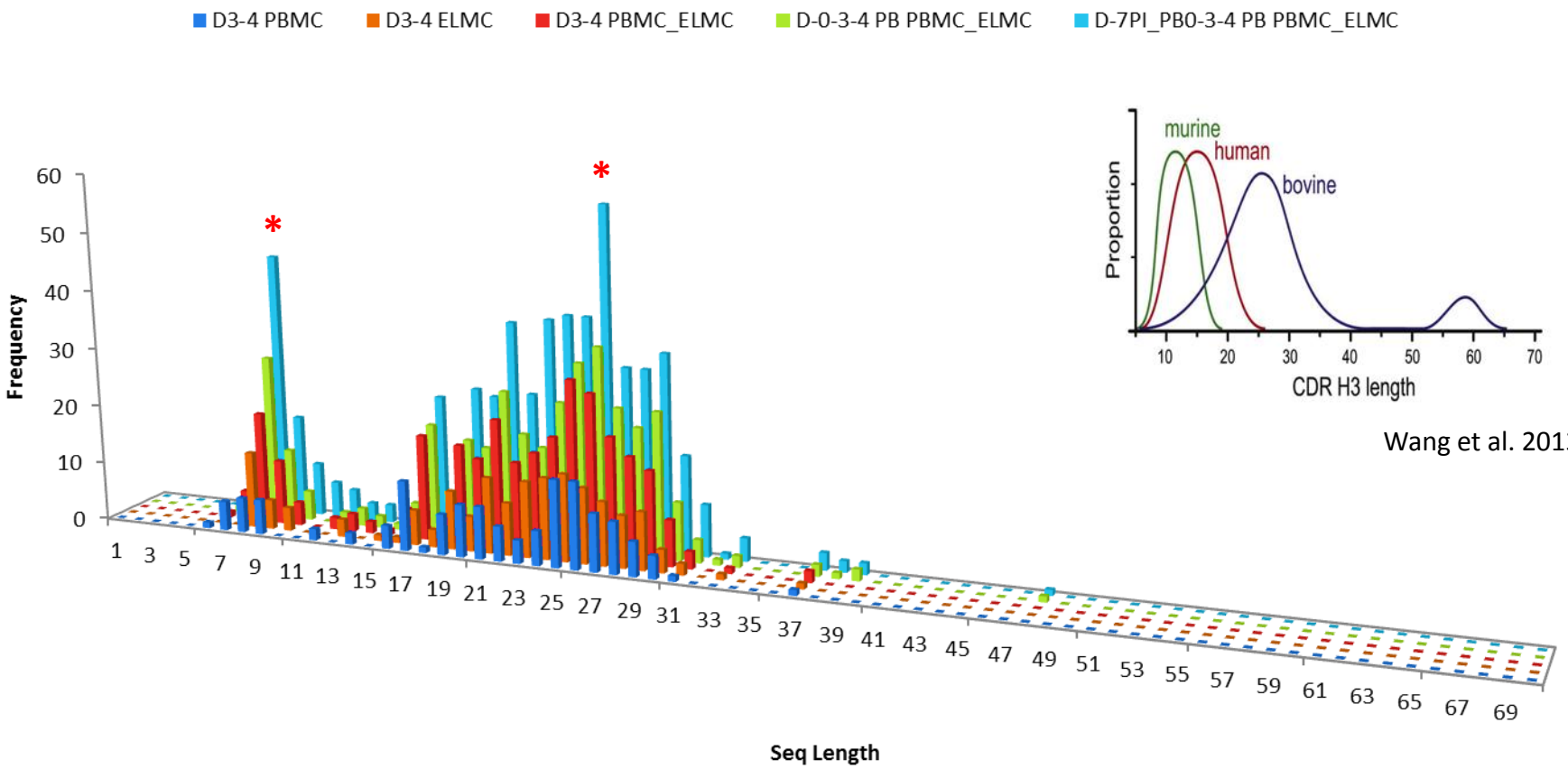
Synthetic gene blocks

Express / Purify

Ag Binding?

Structure Ab (X-ray) and Ab/Ag (EM)

Distribution of CHD3 length of Vh Post Boost

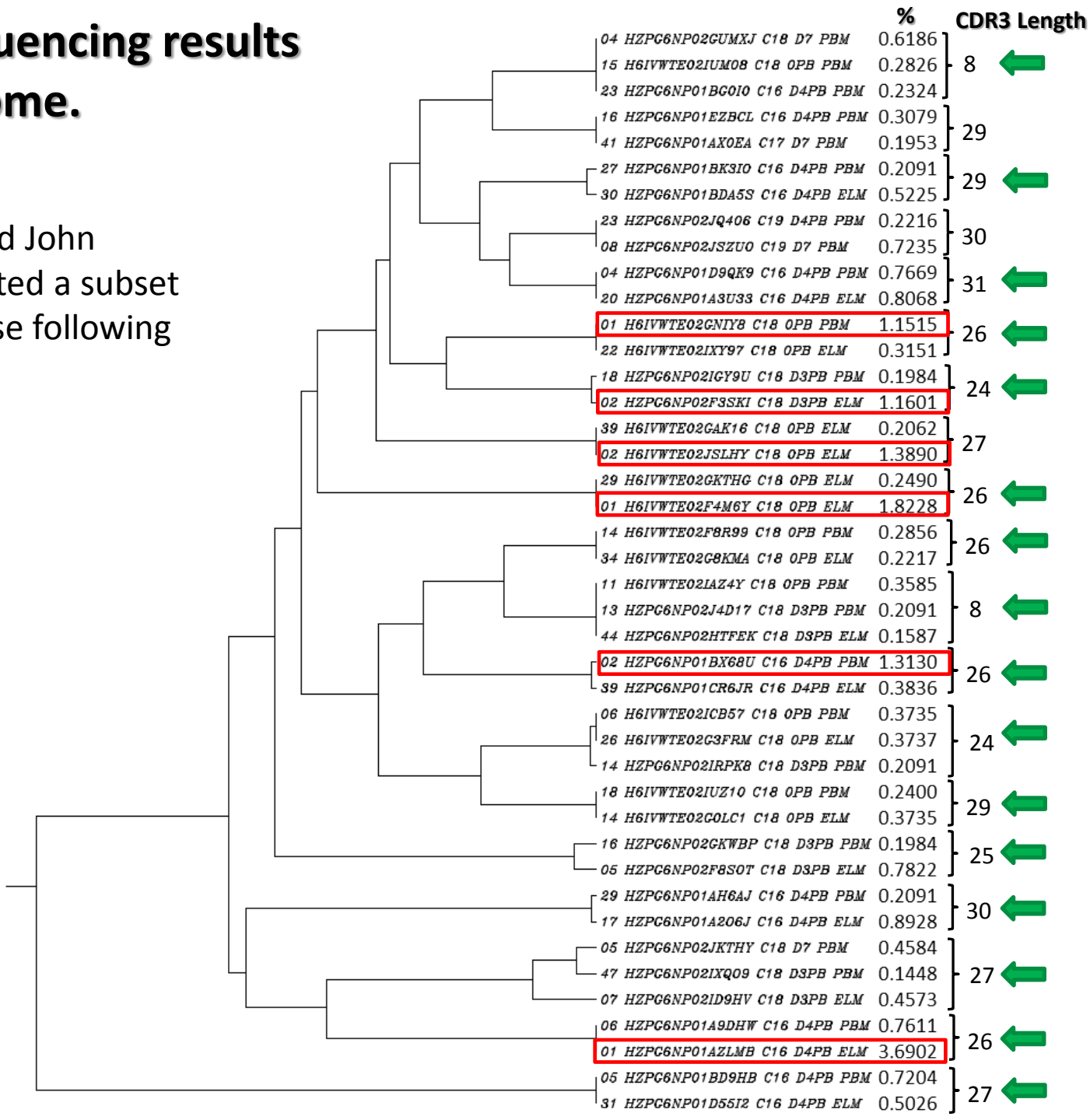


Wang et al. 2013

The small fraction of sequences with ultralong CHD3 completely disappears post immunisation

Preliminary sequencing results –lots more to come.

With Clare Grant and John Hammond we selected a subset that seem to increase following vaccination



Selected Heavy Chain sequences for scale up

Sequences	CHR3 Length	% Abundance
(01) >04_HZPG6NP02GUMXJ_FMDC18_D7_PBMC	8	0.6186
(02) >44_HZPG6NP02HTFEK_FMDC18_D3PB_ELMC	8	0.6620
(03) >02_HZPG6NP02F3SKI_FMDC18_D3PB_ELMC	24	1.1601
(04) >26_H6IVWTE02G3FRM_FMDC18_0PB_ELMC	24	0.3737
(05) >05_HZPG6NP02F8SOT_FMDC18_D3PB_ELMC	25	0.7822
(06) >01_HZPG6NP01AZLMB_FMDC16_D4PB_ELMC	26	3.6902
(07) >01_H6IVWTE02GNIY8_FMDC18_0PB_PBMC	26	1.1515
(08) >34_H6IVWTE02G8KMA_FMDC18_0PB_ELMC	26	0.2218
(09) >01_H6IVWTE02F4M6Y_FMDC18_0PB_ELMC	26	1.8228
(10) >31_HZPG6NP01D55I2_FMDC16_D4PB_ELMC	27	0.5026
(11) >05_HZPG6NP02JKTHY_FMDC18_D7_PBMC	27	0.4584
(12) >07_HZPG6NP02ID9HV_FMDC18_D3PB_ELMC	27	0.4573
(13) >30_HZPG6NP01BDA5S_FMDC16_D4PB_ELMC	29	0.5225
(14) >14_H6IVWTE02G0LC1_FMDC18_0PB_ELMC	29	0.3735
(15) >17_HZPG6NP01A2O6J_FMDC16_D4PB_ELMC	30	0.8928
(16) >20_HZPG6NP01A3U33_FMDC16_D4PB_ELMC	31	0.8068

Selection of Light Chain

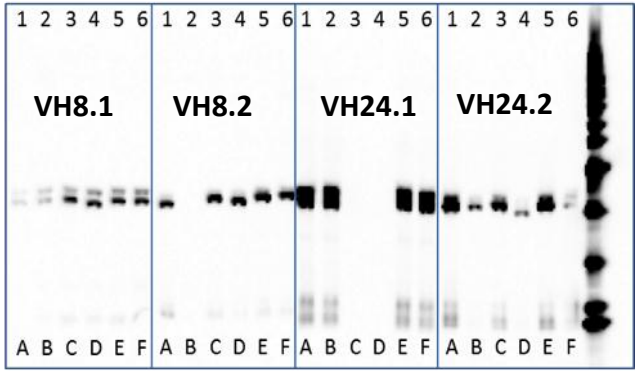
Classified into 14 groups

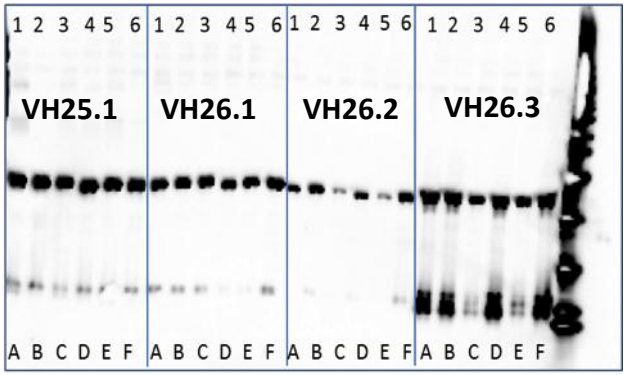
```
A    QAVLTQPSSVSGSLGQRVSITCSGSSSNVGTGNYVSWFQQIPGSAPRTLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASYQSG--NTAVFGSGTTLTVLGQPKSP-
B    QAVLTQPSSVSGSLGQRVSITCSGSSNNIG-SYGVGWYQQVPGSGLRTIIYGSSSRPSGVPDRFSGSKSGNTATLTISSLQAEDEADYFCATGDYSS-STAVFGSGTTLTVLGQPKSAP
C    QAVLTQPSSVSGSLGQRVSITCSGSSSNVGNG-YVSWYQLIPGSAPRTLIYGDTSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASAEDSS-SNAVFGSGTTLTVLGQPKSPP
D    QAVLTQPSSVSGSLGQRVSITCSGSSSNIG-SYNVGWYQQVPGSGLRTIIYGSSSRPSGVPDRFSGSKSGNTATLTISSLQAEDEADYFCVAYDSSS-STAVFGSGTTLTVLGQPKSP-
E    QAVLTQPSSVSGSLGQRVSITCSGSSSNVGYGNYVSWFQQIPGSAPRMLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASPDSSSS--GVFGSGTTLTVLGQPKSPP
F    QAVLTQPSSVSGSLGQRVSITCSGSSNNIG-RYGVGWYQQVPGSGLRTIIYGSSSRPSGVPDRFSGSKSGNTATLTISSLQAEDEADYFCAAGDSSS-STAVFGSGTTLTVLGQPKSPP
G    QAVLTQPSSVSGSLGQRVSITCSGSSNNIG-SYGVGWYQQVPGSGLRTIIYGSSSRPSGVPDRFSGSKSGNTATLTISSLQAEDEADYFCAAGDSSS-STAVFGSGTTLTVLGQPKSPP
H    QAVLTQPSSVSGSLGQRVSITCSGSSSNIG-SYDVGWYQQVPGSGLRTIIYGSSSRPSGVPDRFSGSKSGNTATLTISSLQAEDEADYFCAAGDSSS-STAVFGSGTTLTVLGQPKSPP
I    QAVLTQPSSVSGSLGQRVSITCSGSSSNVGTGNYVSWFQQIPGSAPRTLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASYQSD--NTAVFGSGTTLTVLGQPKSAP
J    QAVLTQPSSVSGSLGQRVSITCSGSSSNVGYGNYVSWFQQIPGSAPRMLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASPDSSSSGYAVFGSGTTLTVLGQPKSPP
K    QAVLTQPSSVSGSLGQRVSITCSGSSSNVGRGNYVNWFQQIPGSAPRTLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCAAYDSSS-NNAVFGSGTTLTVLGQPKSPP
L    SYELTQPTSVSVALGQTAKITCSG---DLLDEQYTQWYQQKPGQGPVRVIYKDSERPSGISDRFSGSSSGKTATLTISGAQTEDEADYYCQSADSSD--NAVFGSGTTLTVLGQPKSPP
M    SYELTQPTSVSVALGQTAKITCSG---DLLDEQYTQWYQQKPGQGPVRVIYKDSERPSGISDRFSGSSSGKTATLTISGAQTEDEADYYCQSADSSD--NPVFGSGTTLTVLGQPKSPP
N    QAVLTQPSSVSGSLGQSVSITCSGSSSNVGNG-YVSWYQMTPGSAPRTLIYGDTSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASAEDSS-SNAVFGSGTTLTVLGQPKSP-
     .  ****:*** :*** ..*****   ::     . *:*  **..   :**  :.*.**:.****** **:*******. *:******:* : : .     ****************.
```

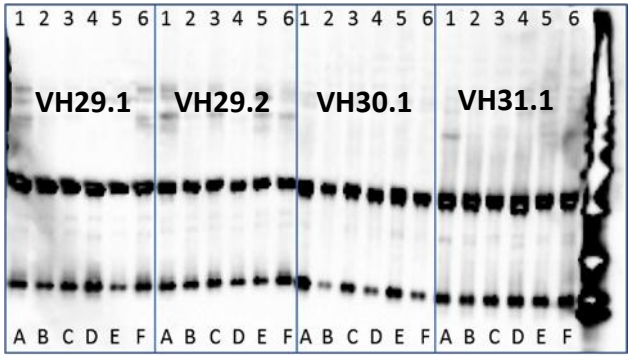
Wang *et al.* VL is closely related to group C

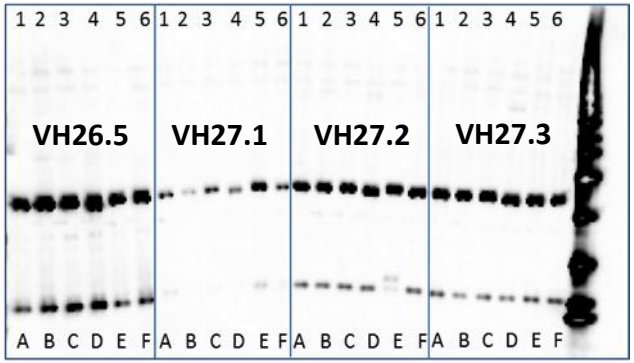
```
C              QAVLTQPSSVSGSLGQRVSITCSGSSSNVGNGYVSWYQLIPGSAPRTLIYGDTSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASAEDSSSNAVFGSGTTLTVLGQPKSPP
4K3E           EAVLNQPSSVSGSLGQRVSITCSGSSSNVGNGYVSWYQLIPGSAPRTLIYGDTSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCASAEDSSSNAVFGSGTTLTVLGQPKSPP
               :***.**********************************************************************************************************
```

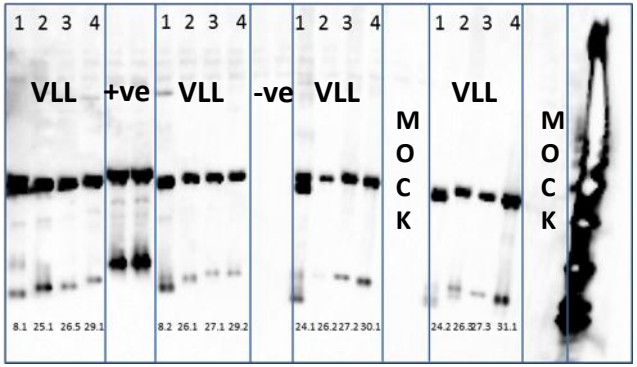
Expression test of recombinant Fabs (all vs all)





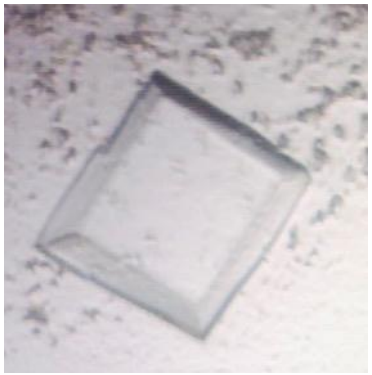






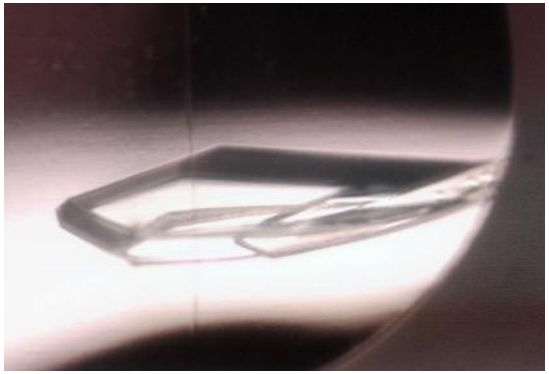
Bovine Fab Crystals of selected Vh paired with Vl group E

AK8.2_VLE



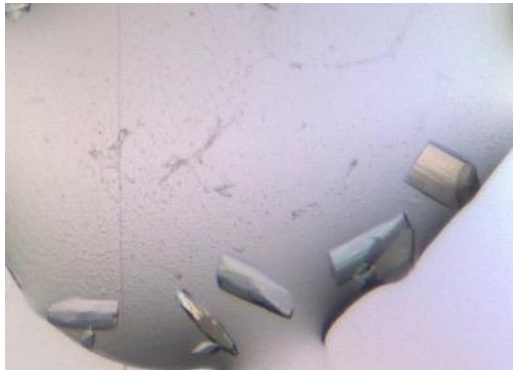
0.1M citric acid **pH 4.0**
30 %w/v PEG 6000

AK24.1_VLE



25 %w/v PEG 1500
0.100 M SPG System **pH 5.0**

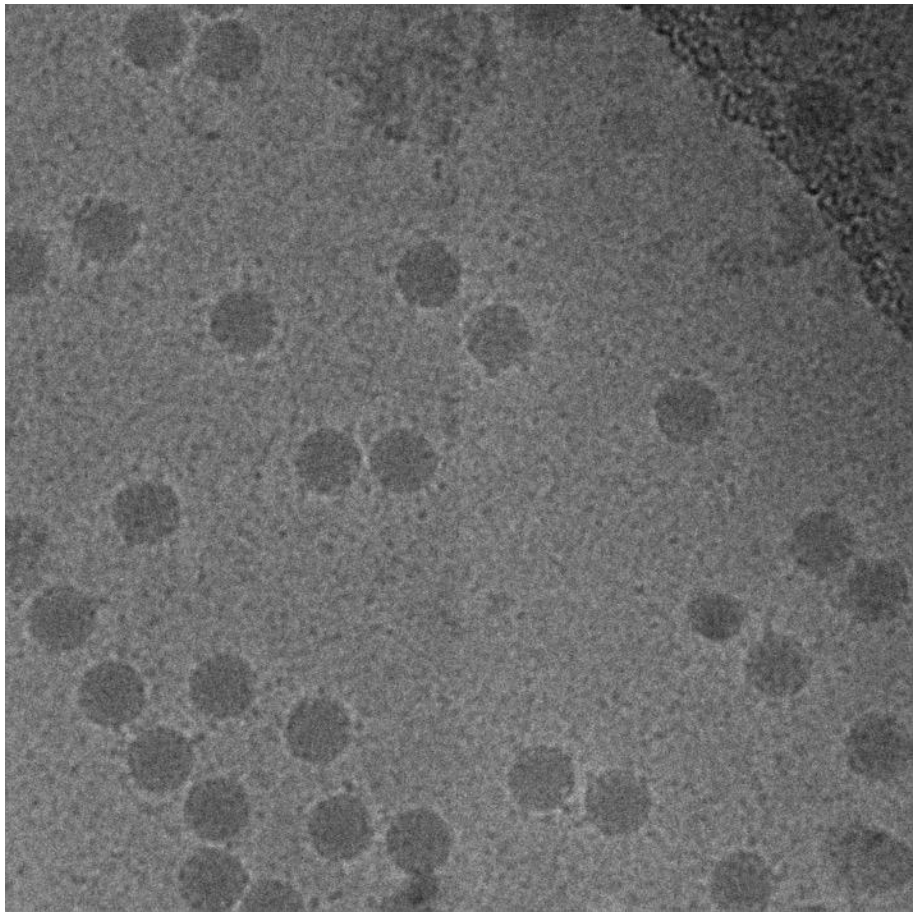
AK26.1_VLE



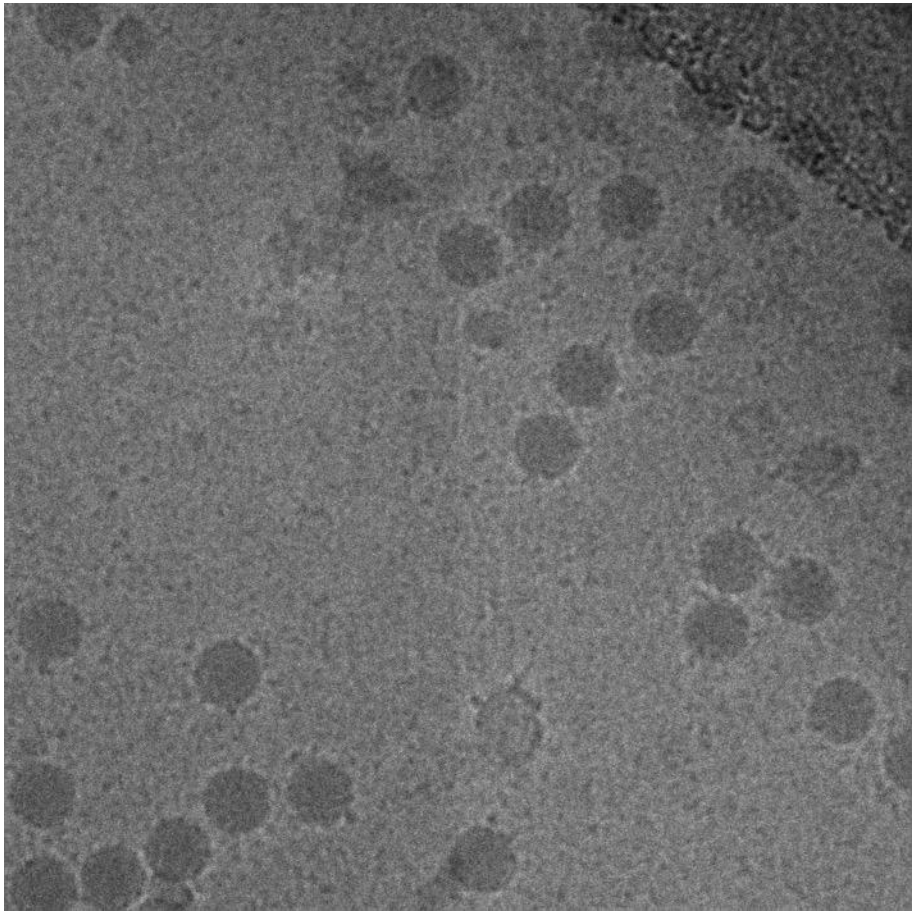
28.0% w/v PEG MME 2000
0.1 M bis-Tris **pH 6.5**

Diffraction data measured at I03, I04 and I24 beamlines at the Diamond Light Source

Fabs bind

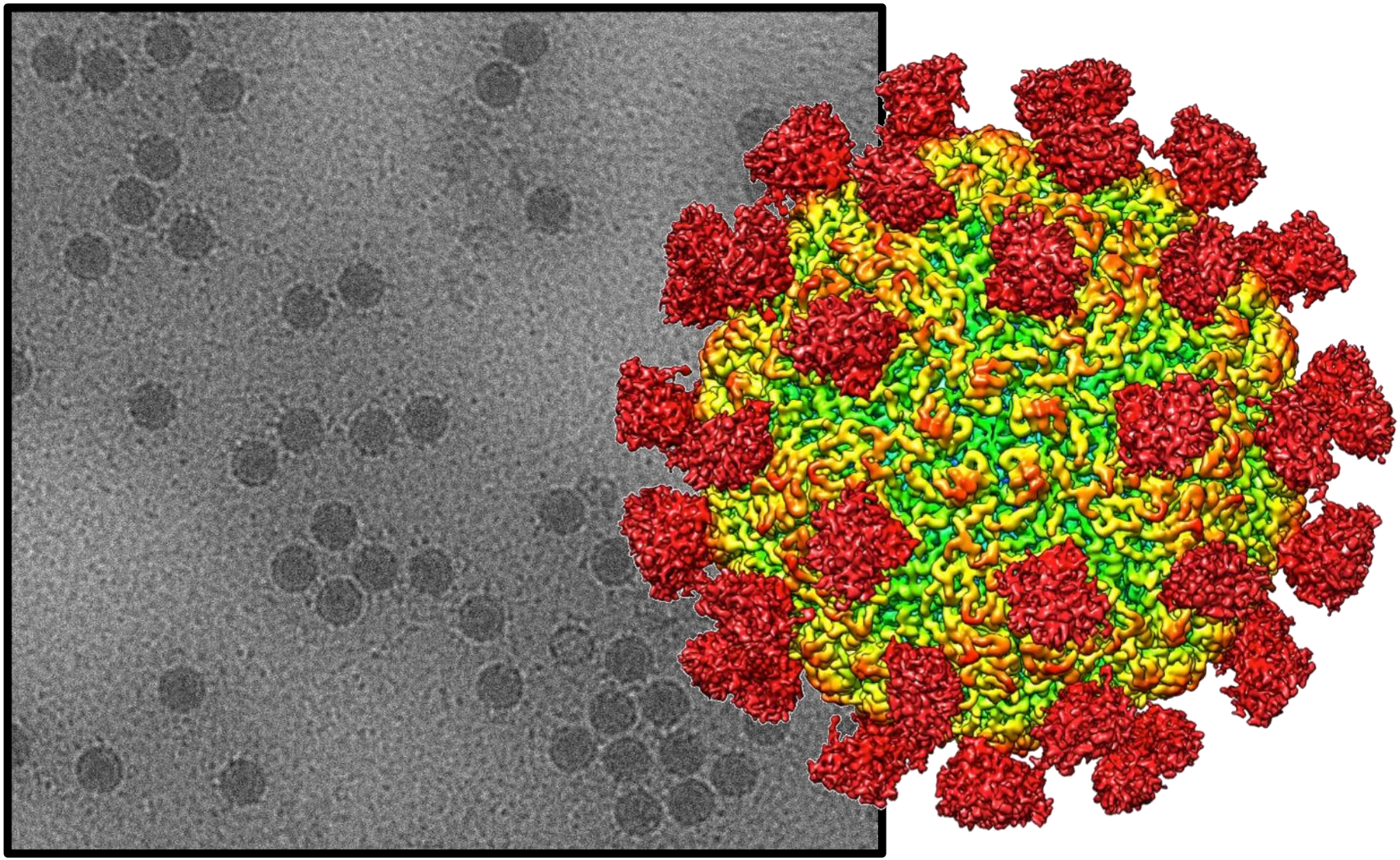


O1M – IB11 complex



O1M – rFab(3) complex

70hrs Collection on Polara



We now have EM data for 12 Fabs (CRD3 heavy chain loop form 8 to 31 residues) and apart from one, all are binding! - despite no careful light chain matching … (structure determinations heating our building as I speak…)

So our aim to to investigate the nature of the epitopes for different serotypes and see if there is scope for either

i) Ablating dominant serotype specific sites (eg GH loop)

ii) Assembling a chimeric surface taking distinct sites from different serotypes stitching them together (*cf* Novartis)

Of course we have no idea if any of this will work!

So perhaps we are beginning to understand complex pathogens well enough to apply computational tools and chemistry to fight them more effectively.

But there will be plenty of challenges – not least how do we produce more cross-protective vaccines!

Additional acknowledgements

Diamond:
I24 team: Gwyndaf Evans, Danny Axford, David Waterman, James Foadi, Jun Aishima, Robin Owen

I03 team: Katherin McAuley, James Nicolson, Mark Williams (& from I04: Dave Hall)

Martin Walsh

Strubi Oxford: Liz Fry, Karl Harlos, Jon Grimes, Jingshan Ren, Claudine Porta, Abhay Kotcha

Others: OPPF-UK (Ray Owens *et al*, RC@H), MPL (Isabel de Moraes *et al*, Diamond/RC@H), groups from Leeds IMM, IAH Pirbright, Rao Zihe group, Beijing, Kay Grunewald, Helen Saibil….

Funders: *Usual Diamond funders (STFC, Welcome Trust) Plus: MRC, Wellcome, DEFRA, BBSRC, EU.*

Thank you