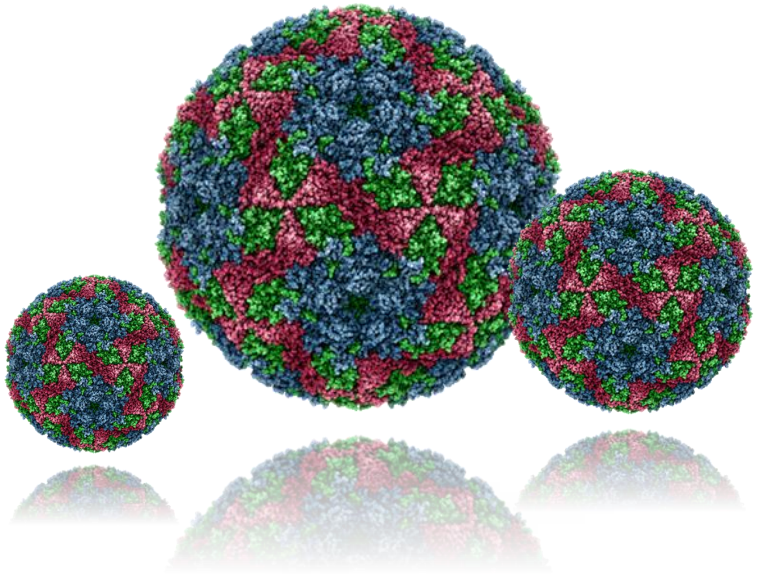
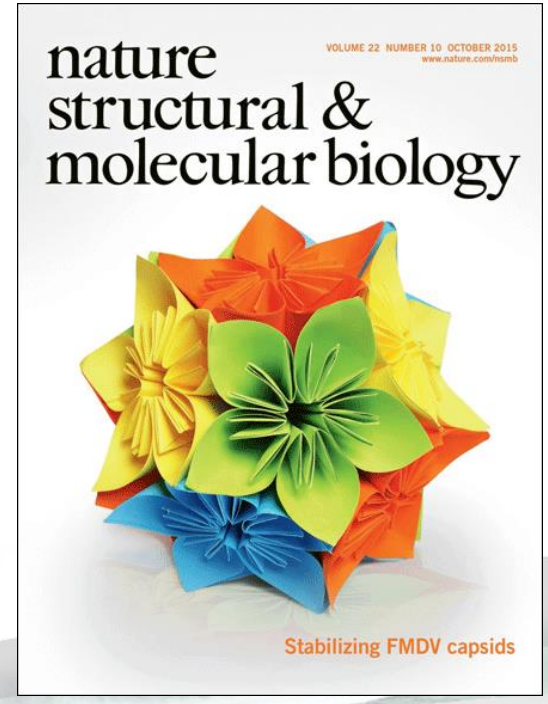
AN OVERVIEW OF REVERSE GENETIC APPROACHES TO ENHANCED FMD VACCINES IN AFRICA





Global Foot-and-Mouth Disease Research Alliance

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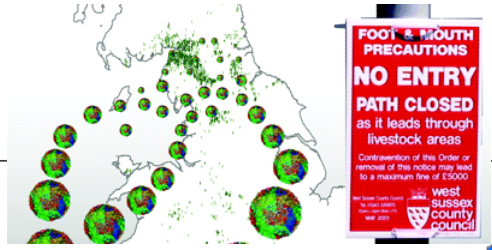
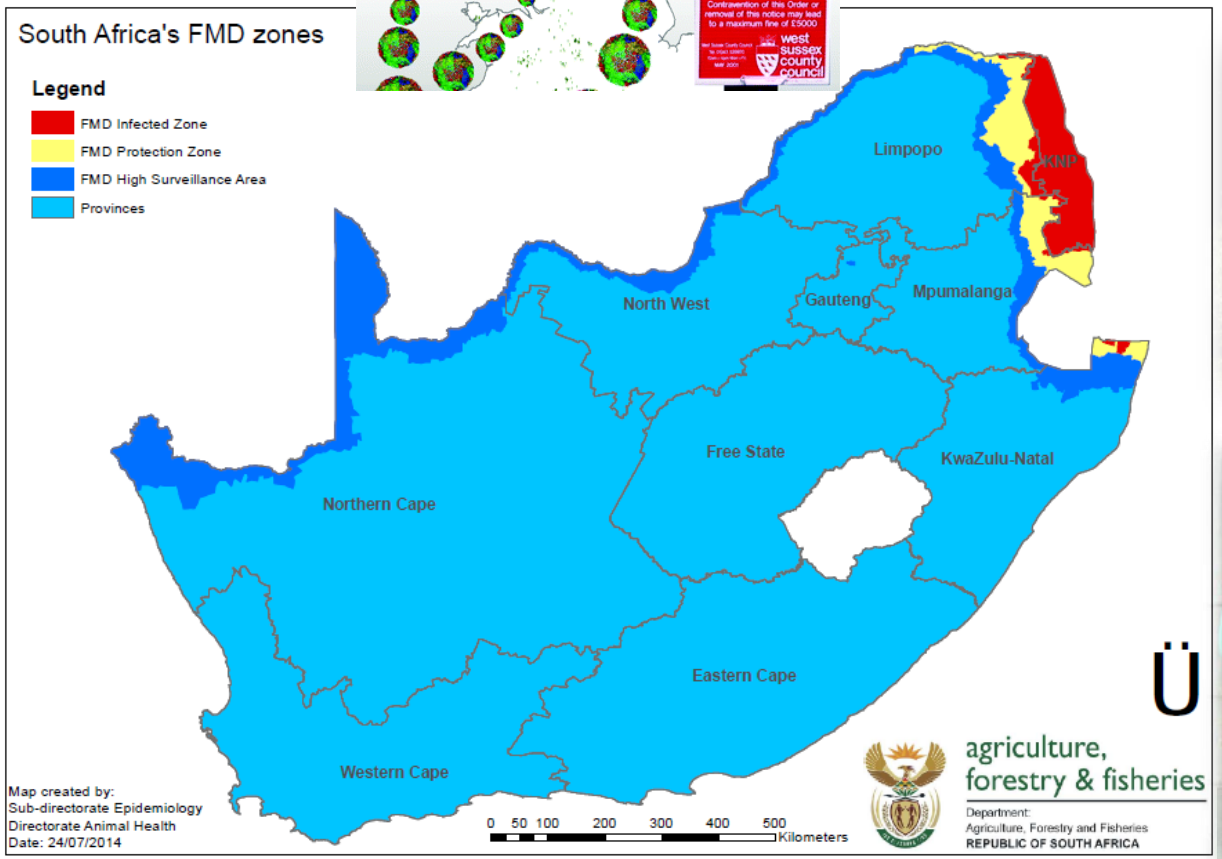
FMD in South Africa





Physical barriers, like fences

Immune barrier using vaccination

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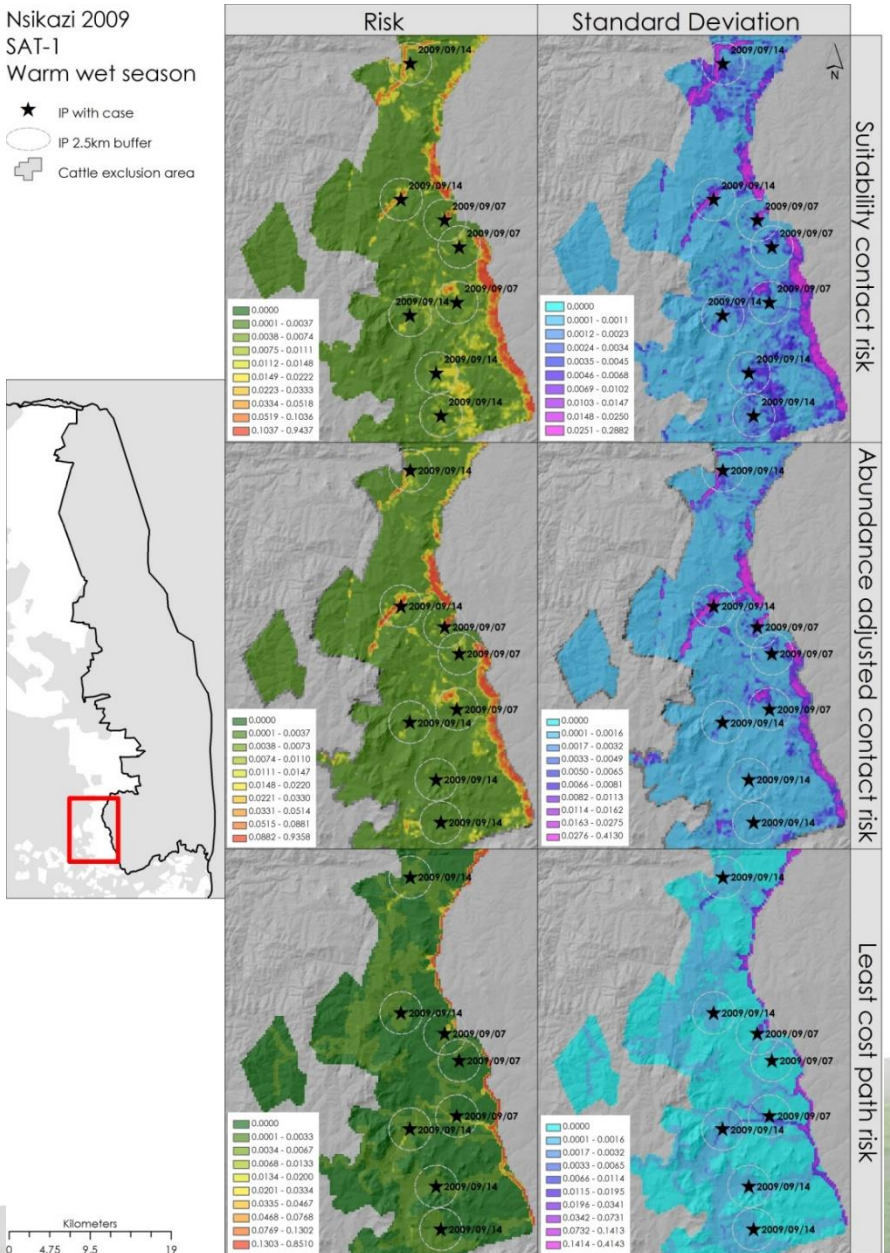
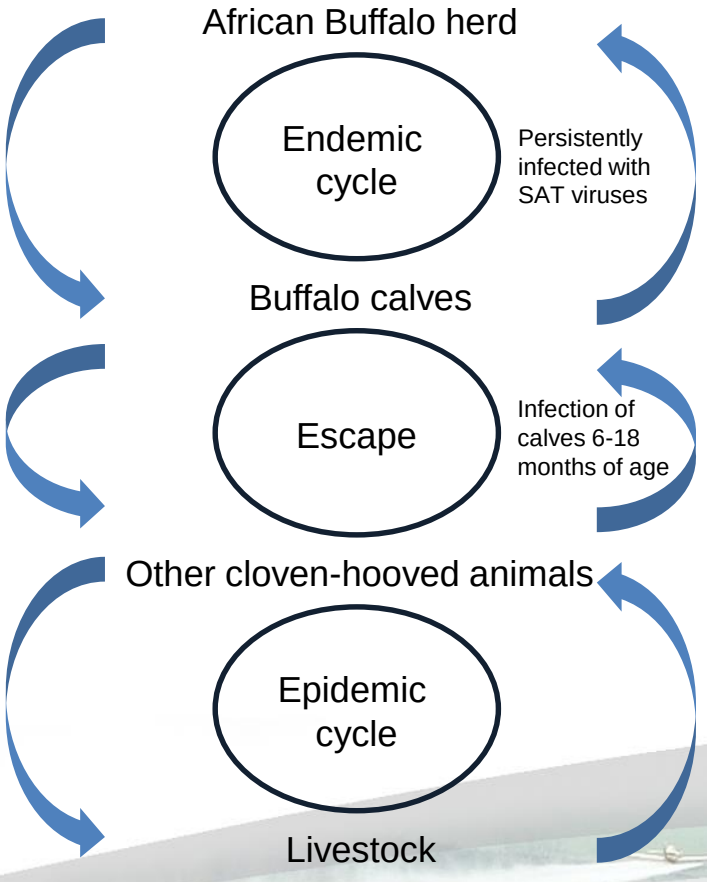
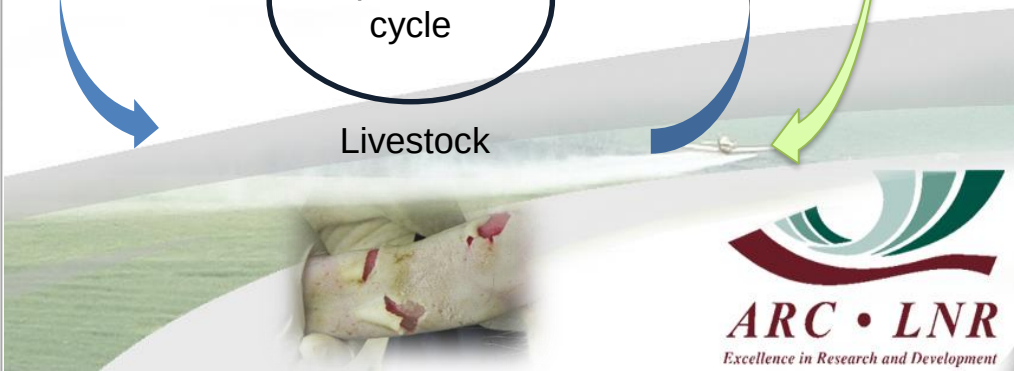
Interaction between buffalo, FMDV and cattle



The epidemiology of FMD in Africa is influenced by two different patterns





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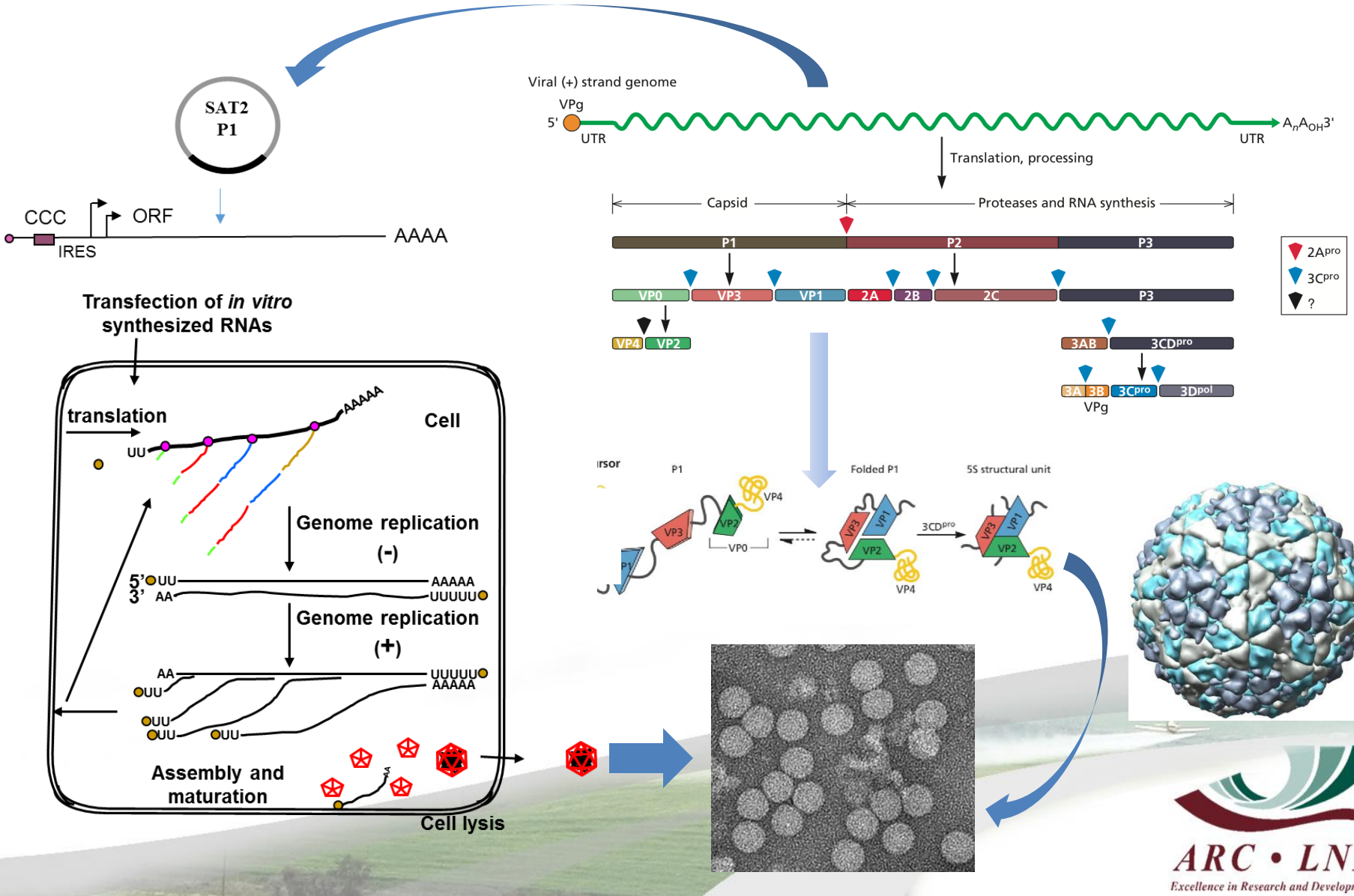
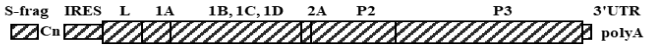
Reverse genetics for FMDV



Intra-serotype chimeric vaccine: clinical signs & viraemia

A) Parental FMDV:

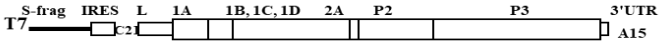
SAT2/ZIM/14/90



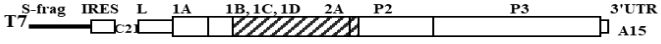
→ Vac

B) Chimeric FMDV constructs:

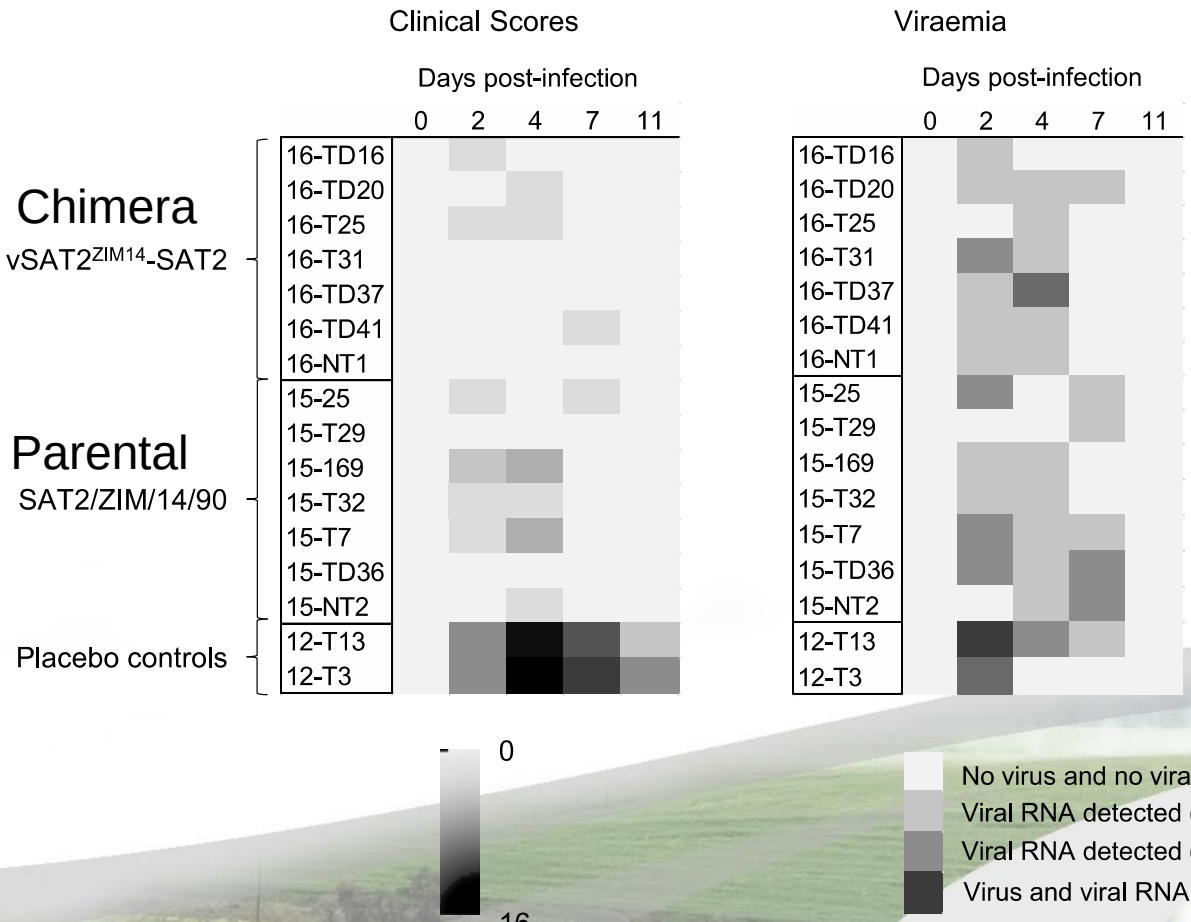
vSAT2



vSAT2^{ZIM14}-SAT2



→ Vac



Protected

Protected

- Severe tongue lesions 2 animals
- High temperatures 2 animals

Systemic spread

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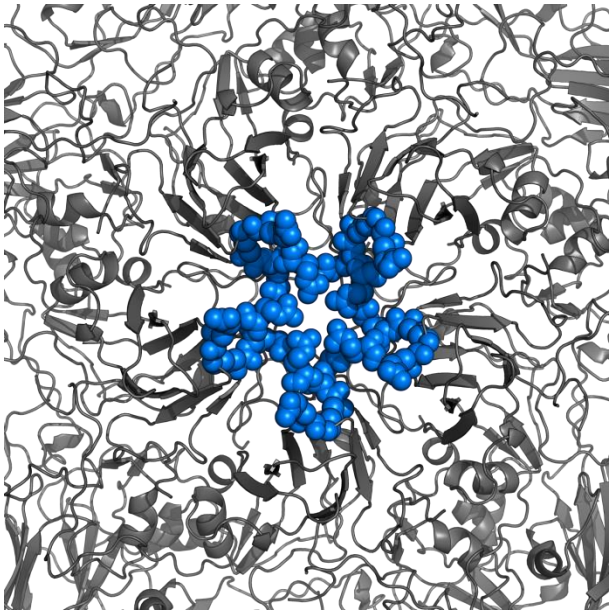
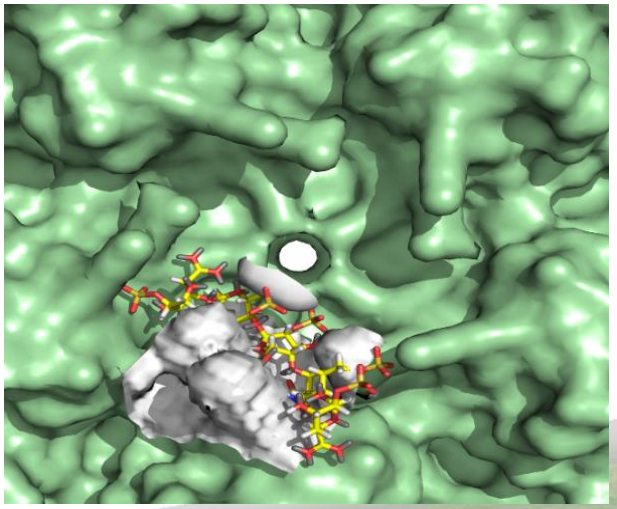
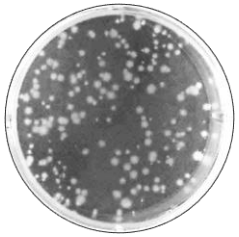
Energetically favorable binding of Heparin moiety





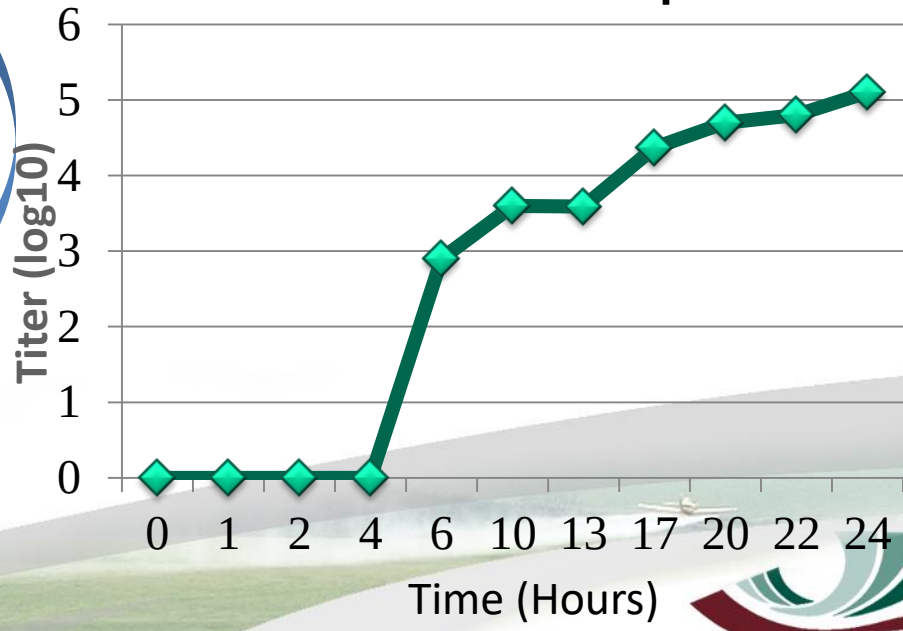
Chitray 2018 PhD

SAT2-SAT2 chimera with added positive charges at 5-fold axis



CHO-K1

Growth kinetics in BHK-21 **suspension** cells



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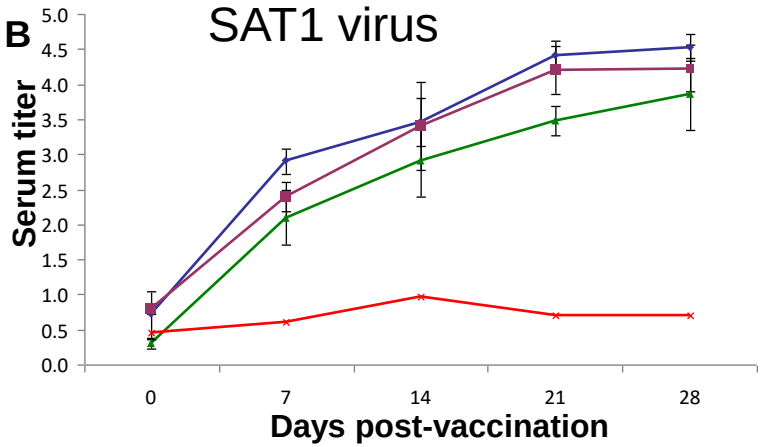
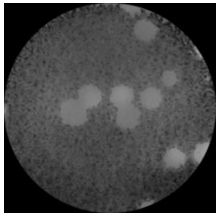
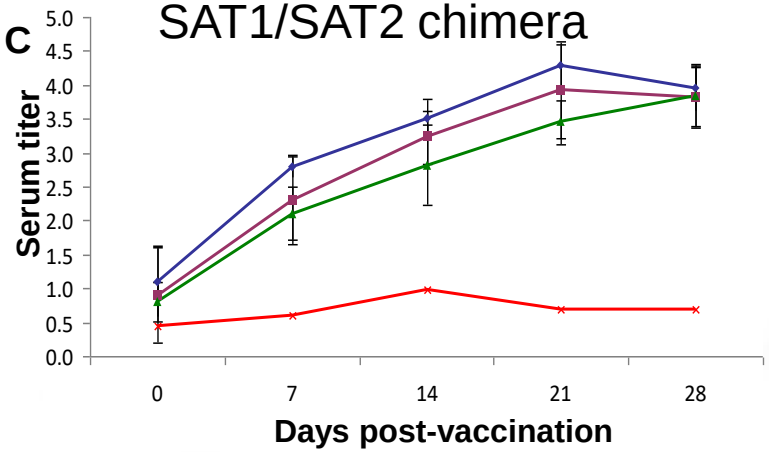
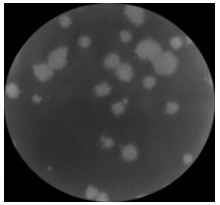
Inter-serotype chimeric vaccine: Ab & clinical scores





1.2 ug
0.6 ug
0.3 ug
controls





Blignaut et al., 2011. **J Virol.**

		0	2	4	7	11
SAT1/SAT2 chimera						
Full dose	16-1	0	3	1	0	0
	16-2	0	1	0	0	0
	16-3	0	0	0	0	0
	16-4	0	1	0	0	0
	16-5	0	1	1	0	0
Controls	11-1	0	10	9	2	2
	11-2	0	13	9	5	2
1/4 Dose	6-1	0	5	2	0	0
	6-2	0	3	1	0	0
	6-3	0	0	0	0	0
	6-4	0	0	0	0	0
	6-5	0	0	0	0	0
1/16 Dose	13-1	0	0	0	0	0
	13-2	0	5	3	0	0
	13-3	0	0	0	0	0
	13-4	0	0	0	0	0
	13-5	0	0	0	0	0
SAT1 virus						
Full dose	14-1	0	0	0	0	0
	14-2	0	0	0	0	0
	14-3	0	0	0	0	0
	14-4	0	0	0	0	0
	14-5	0	0	0	0	0
Controls	11-3	0	10	9	2	2
	11-4	0	13	9	5	2
1/4 Dose	8-1	0	0	0	0	0
	8-2	0	0	0	0	0
	8-3	0	0	0	0	0
	8-4	0	0	0	0	0
	8-5	0	0	0	0	0
1/16 Dose	5-1	0	0	1	1	0
	5-2	0	0	0	0	0
	5-3	0	0	1	1	0
	5-4	0	0	0	0	0
	5-5	0	0	0	0	0

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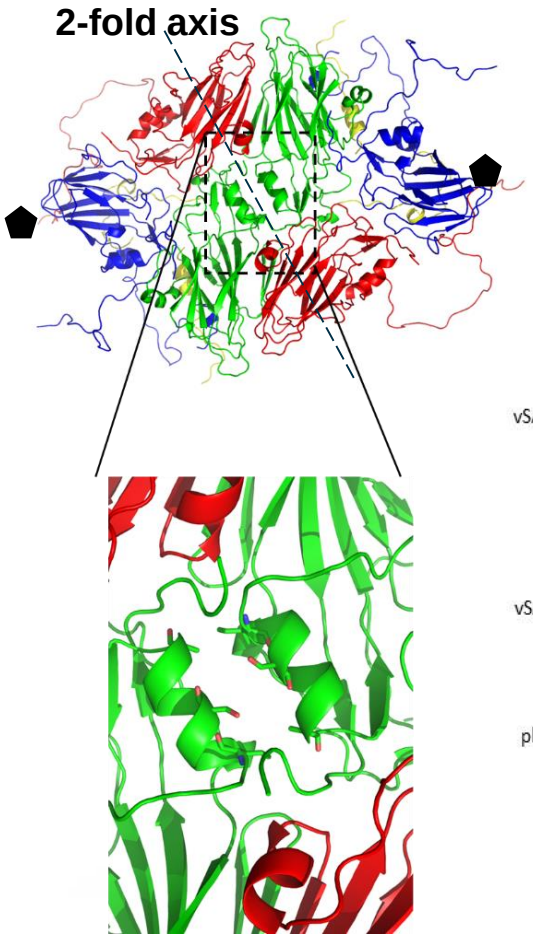
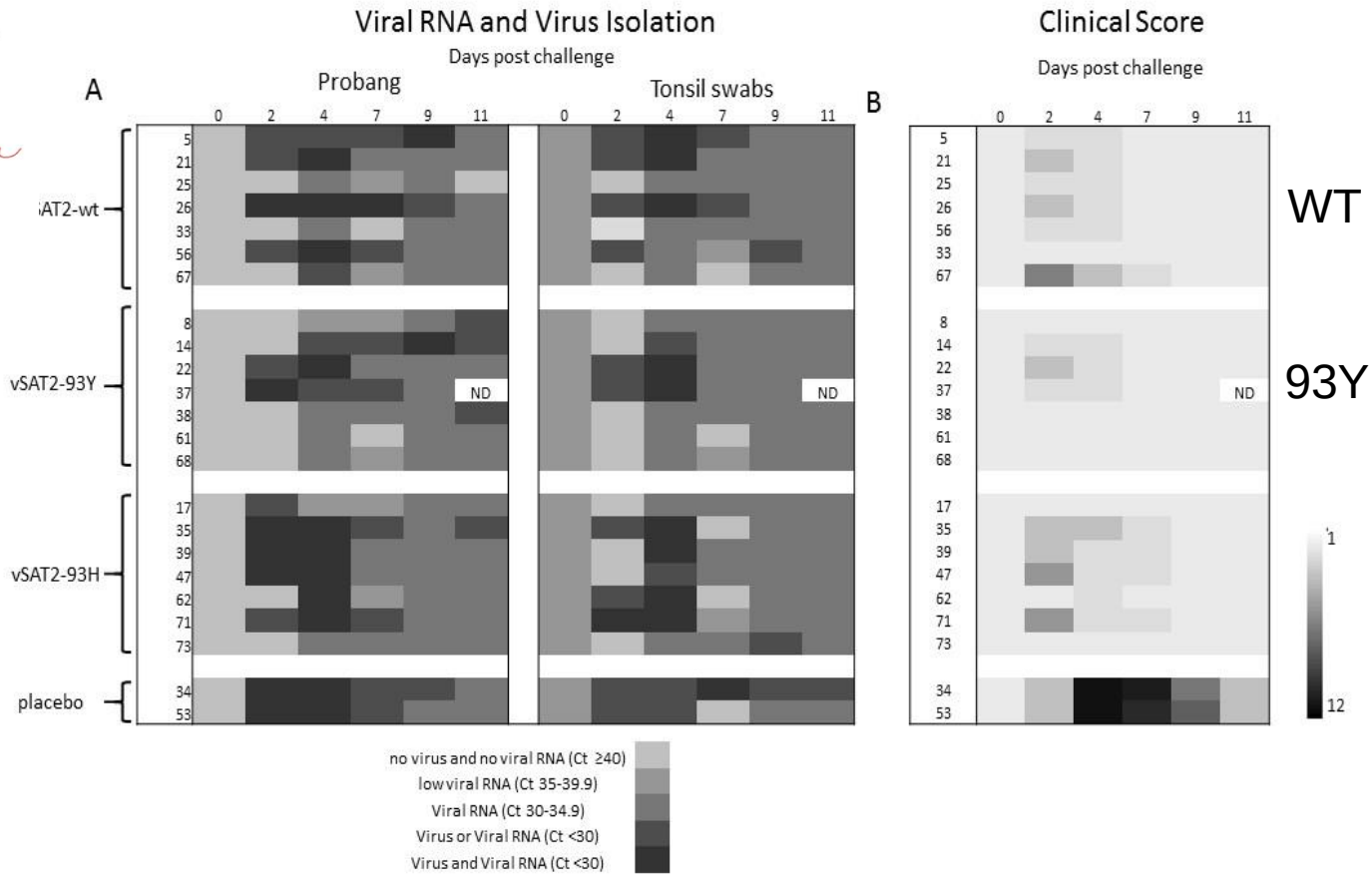
Interactions at the capsid 2-fold interface and stability





Kotecha et al., 2015. **Nature Struct Mol Biol.**
Scott et al., 2017. **J Virol.**
Scott et al., 2017. **Vaccine.**

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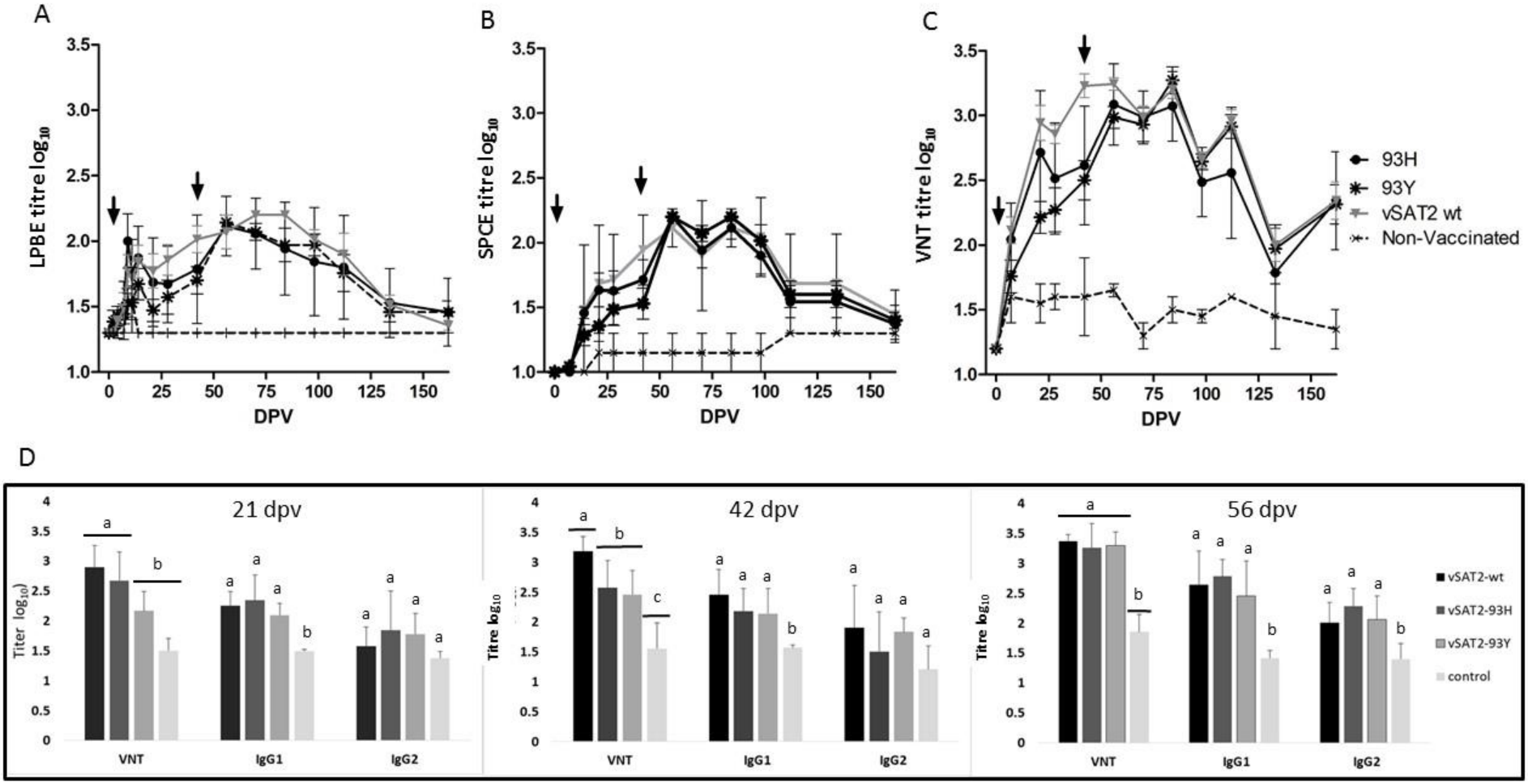
Antibody kinetics: wildtype and stabilised antigens



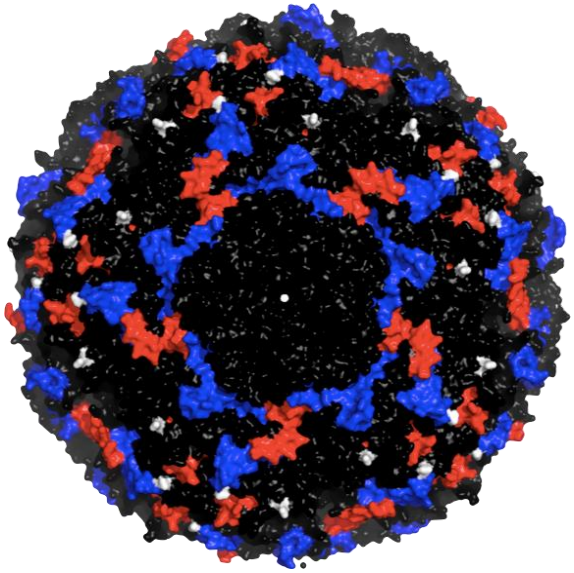
Product	Number	Culture adaptation	Engineered Stability	Antigenicity	Up-Scaled production	Vaccine trial
Infectious genome length clones	vSAT2 2x vSAT1	vSAT2 vSAT1^{vac}	vSAT2 -	VNTs VNTs	vSAT2 -	GP's, cattle -
Chimeric viruses						
Intra-type	7	v^{SAU}SAT2	-	VNTs, Mabs	v^{ZIM14}SAT2	Cattle
Inter-type	8	v^{KNP}SAT2; v^{ZAM}SAT2	-	VNT, challenge	v^{KNP}SAT2	Pigs
Capsid stabilized mutants	18 mono	vSAT2	vSAT2^{93Tyr} vSAT2^{93His}	Mabs Mabs	vSAT2^{93Tyr} vSAT2^{93His}	GP's, cattle Cattle
	2 triple		vSAT2^{93Trp}	Mabs	-	-
	2 quadruple		vSAT2$^{62Y87M\ 143V}$	Mabs	-	-
Cell culture adaptation	5 Inter-type	v$^{Nam(KRR)}$SAT	-	VNTs	-	-
		v^{KNP196}SAT	-	Challenge	v^{KNP}SAT2	Pigs
	8 Intra-type	v$^{Sau(KRR)}$SAT	-	VNTs, Mabs	-	-
		v$^{Sau(83RR)}$SAT	-	VNTs, Mabs	-	-
Antigenic refocus	18 Epitope replaced	-	-	vKNP^{S5}SAT vKNP^{S2A}SAT vKNP^{S2B}SAT	-	-
	8 charge dampened	-	-	vSAT2^{2A} vSAT2^{2b} vSAT2^{S4}	-	-
Total:	**79**	**26**	**4**	**6**	**5**	**5**

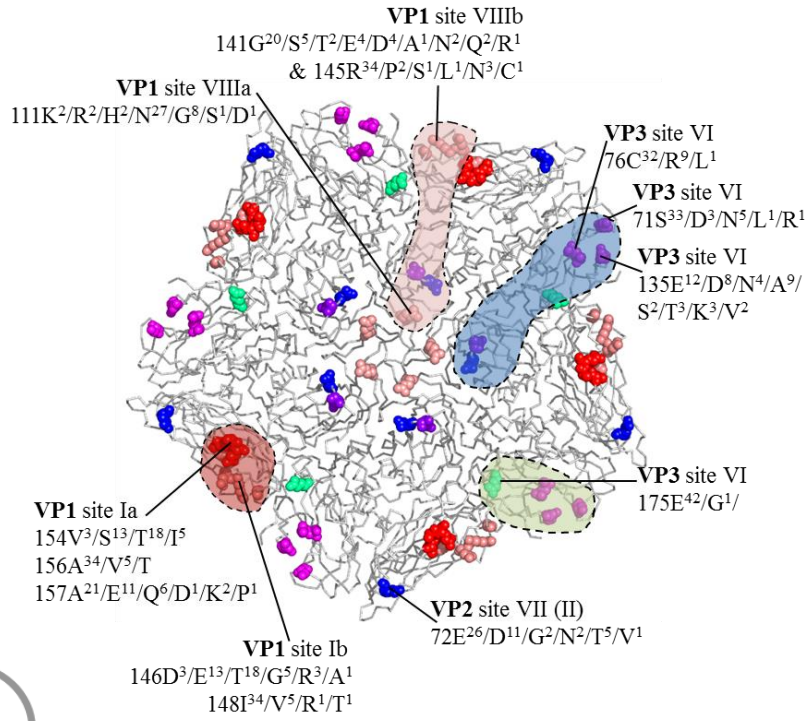
Is it possible to design a universal vaccine that will elicit protection for all serotypes or even within a serotype?

Directed evolution
or rational engineering of antigenic sites may change the reactivity of antibodies to a heterologous virus within a serotype.

Antigenic structure of SAT viruses

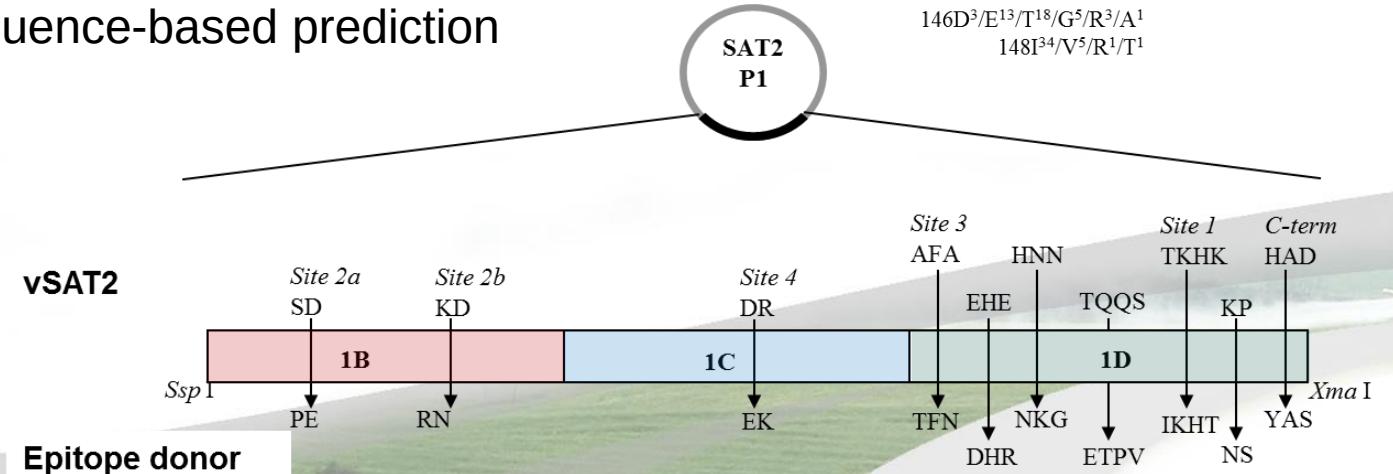
Discontinuous or multiple epitopes





Repetitive display of epitope

Sequence-based prediction



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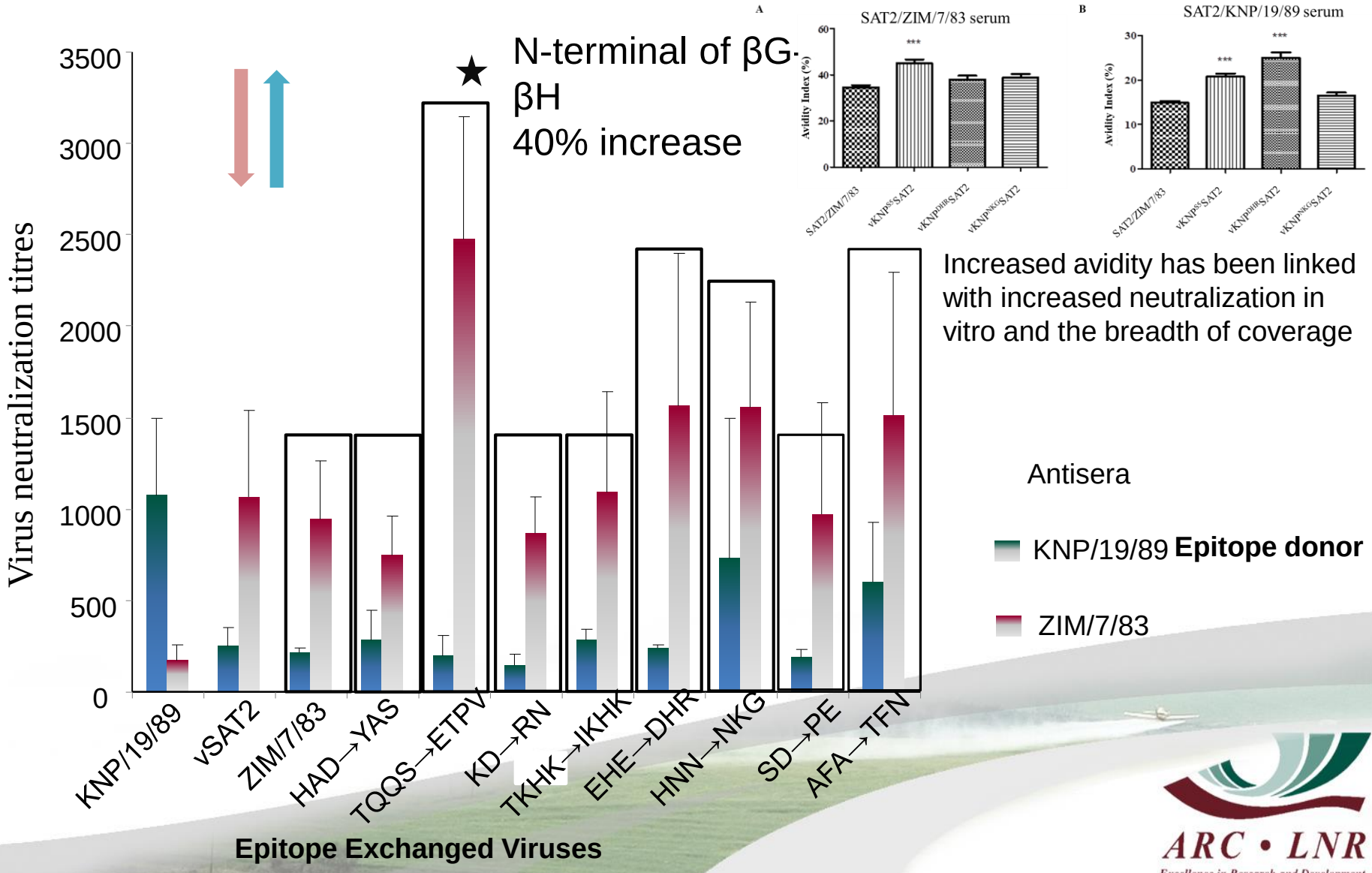
Reactivity of sera to epitope-replaced mutant viruses



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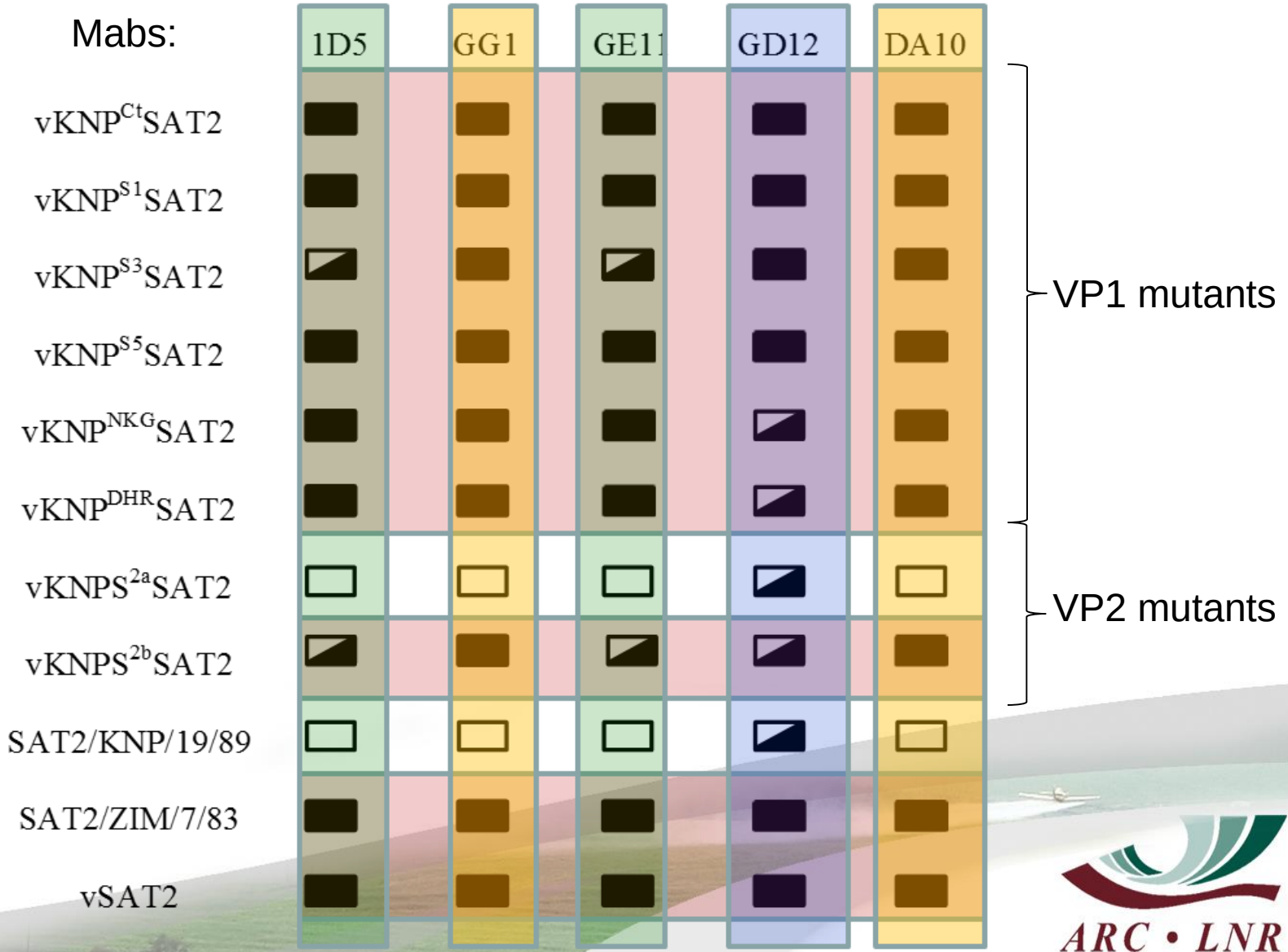
Antigenic profiles of epitope-replaced mutant viruses



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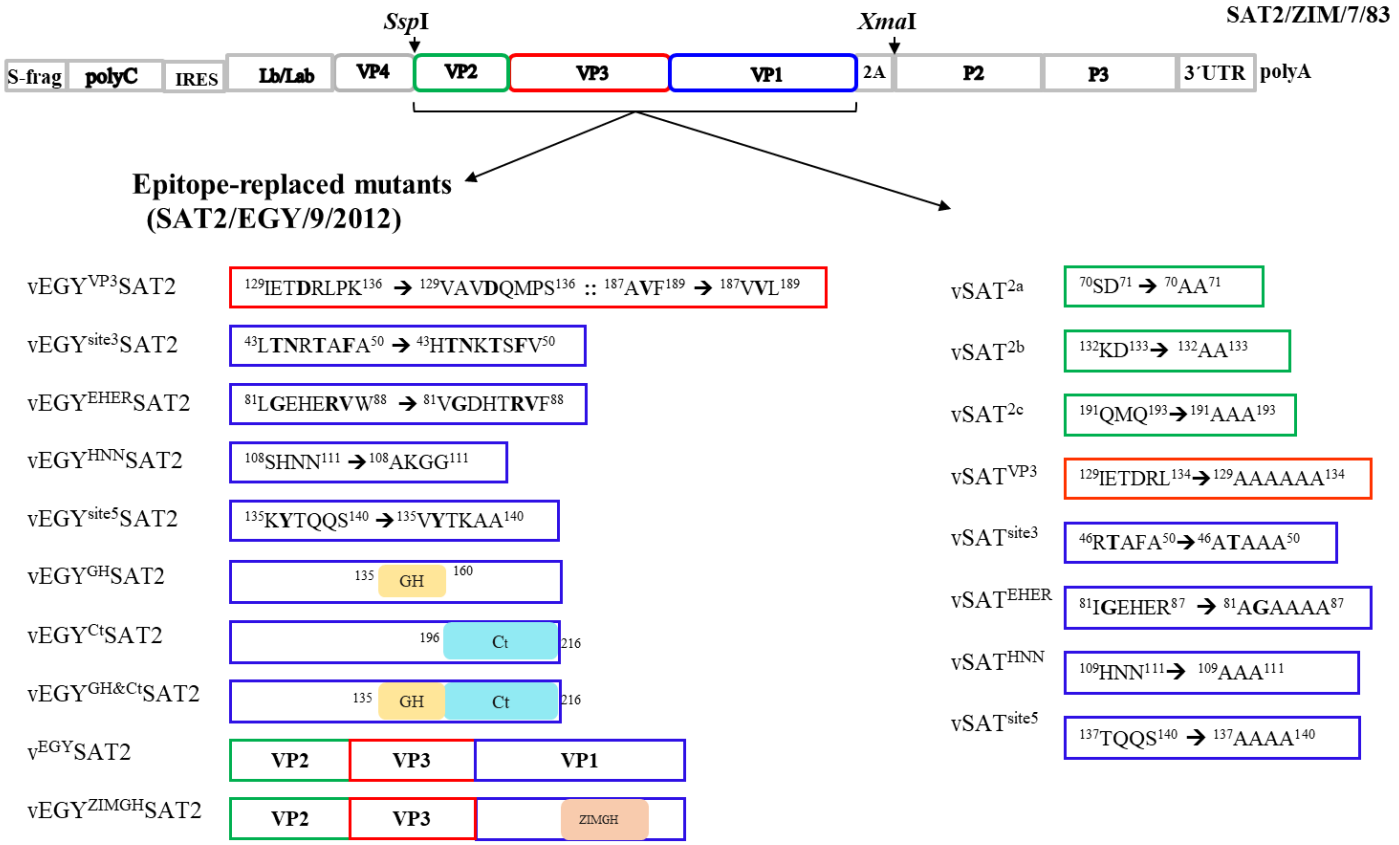
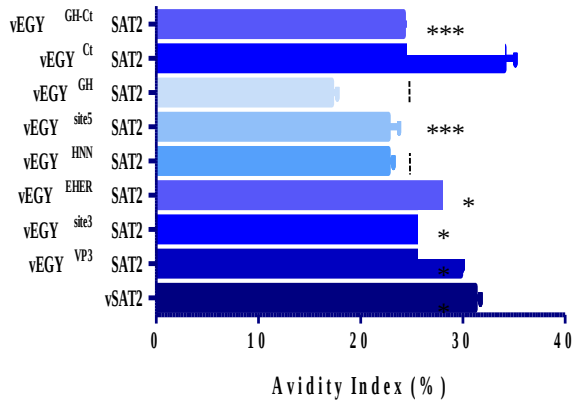
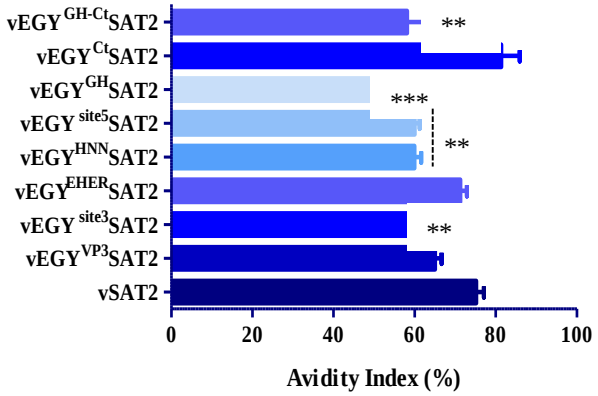
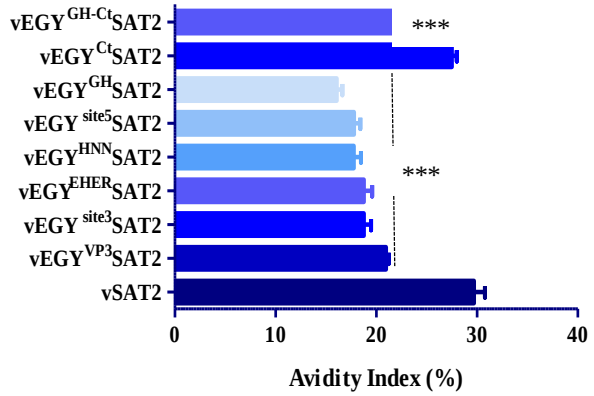

SAT2/ZIM/7/83
SspI
XmaI
S-frag
polyC
IRES
Lb/Lab
VP4
VP2
VP3
VP1
2A
P2
P3
3´UTR
polyA
Epitope-replaced mutants
(SAT2/EGY/9/2012)
vEGY^VP3SAT2
129IETDRLPK136 → 129VAVDQMPS136 :: 187AVF189 → 187VVL189
vEGY^site3SAT2
43LTNRTAFA50 → 43HTNKTSFV50
vEGY^EHERSAT2
81LGEHERVW88 → 81VGDHTRVF88
vEGY^HNNSAT2
108SHNN111 → 108AKGG111
vEGY^site5SAT2
135KYTQQS140 → 135VYTKAA140
vEGY^GHSAT2
135 GH 160
vEGY^CtSAT2
196 Ct 216
vEGY^GH&CtSAT2
135 GH Ct 216
v^EGYSAT2
VP2 VP3 VP1
vEGY^ZIMGHSAT2
VP2 VP3 ZIMGH
vSAT^2a
70SD71 → 70AA71
vSAT^2b
132KD133 → 132AA133
vSAT^2c
191QMQ193 → 191AAA193
vSAT^VP3
129IETDRL134 → 129AAAAAA134
vSAT^site3
46RTAFA50 → 46ATAAA50
vSAT^EHER
81IGEHER87 → 81AGAAAA87
vSAT^HNN
109HNN111 → 109AAA111
vSAT^site5
137TQQS140 → 137AAAA140


SAT2/ZIM/07/83 sera
vEGY GH-Ct SAT2
vEGY Ct SAT2
vEGY GH SAT2
vEGY site5 SAT2
vEGY HNN SAT2
vEGY EHER SAT2
vEGY site3 SAT2
vEGY VP3 SAT2
vSAT2
***
***
*
*
*
0 10 20 30 40
Avidity Index (%)


SAT2/SAR/03/04 sera
vEGY GH-CtSAT2
vEGY CtSAT2
vEGY GHSAT2
vEGY site5SAT2
vEGY HNNSAT2
vEGY EHERSAT2
vEGY site3SAT2
vEGY VP3SAT2
vSAT2
**
***
**
**
0 20 40 60 80 100
Avidity Index (%)


SAT2/ZIM/14/90 sera
vEGY GH-CtSAT2
vEGY CtSAT2
vEGY GHSAT2
vEGY site5SAT2
vEGY HNNSAT2
vEGY EHERSAT2
vEGY site3SAT2
vEGY VP3SAT2
vSAT2
***
***
0 10 20 30 40
Avidity Index (%)

Antigenic profiles of epitope-replaced mutant viruses

	Mutants	ID5	GG1	GE11	GD12	DA10	Capsid position
Alanine replacements	vSAT2^{site3}						VP1; 46-50
	vSAT2^{EHER}	57	60	58	72	67	**VP1; 81-87**
	vSAT2^{HNN}				80	80	VP1; 109-111
	vSAT2^{site5}						VP1; 137-140
	vSAT2^{2A}	22	9	12		8	**VP2; 70-71**
	vSAT2^{2B}	64	55	70		64	**VP2; 132-133**
	vSAT2^{2C}						VP2;191-193
	vSAT2^{VP3}	65	66	59	60	59	**VP3; 129-134**
Epitope replacements	vEGY^{VP3}SAT2						VP3; 129-136 :: 187-189
	vEGY^{site3}SAT2					30	**VP1; 43-50**
	vEGY^{EHER}SAT2						VP1; 81-88
	vEGY^{HNN}SAT2						VP1; 108-111
	vEGY^{TQQS}SAT2						VP1; 135-140
	vEGY^{GH}SAT2						VP1; 135-160
	vEGY^{Ct}SAT2						VP1; 135-216
	vEGY^{GH-Ct}SAT2				71		VP1;196-216
	vEGY/SAT2		41	57	33		**VP1, VP2 and VP3**
	vSAT2						-

0-25 25-65 65-80 80-100

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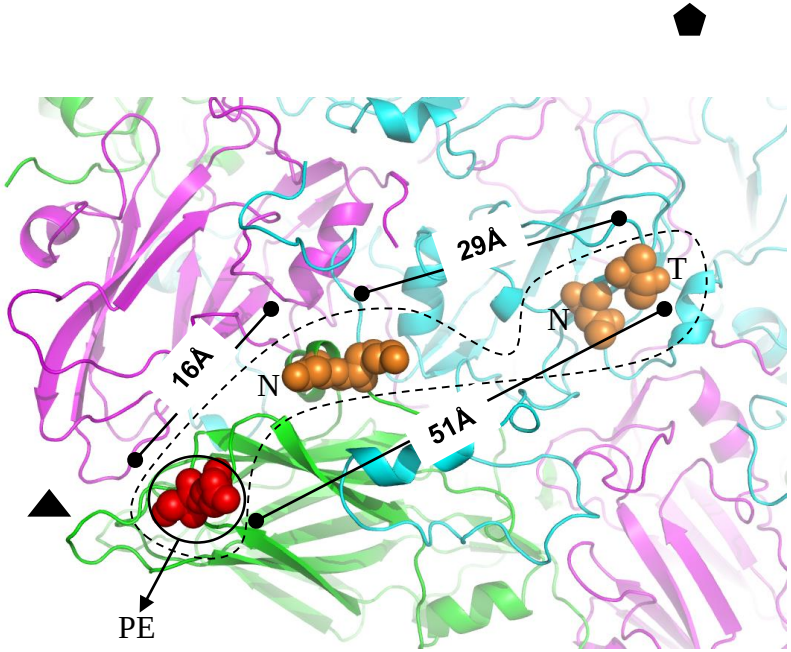
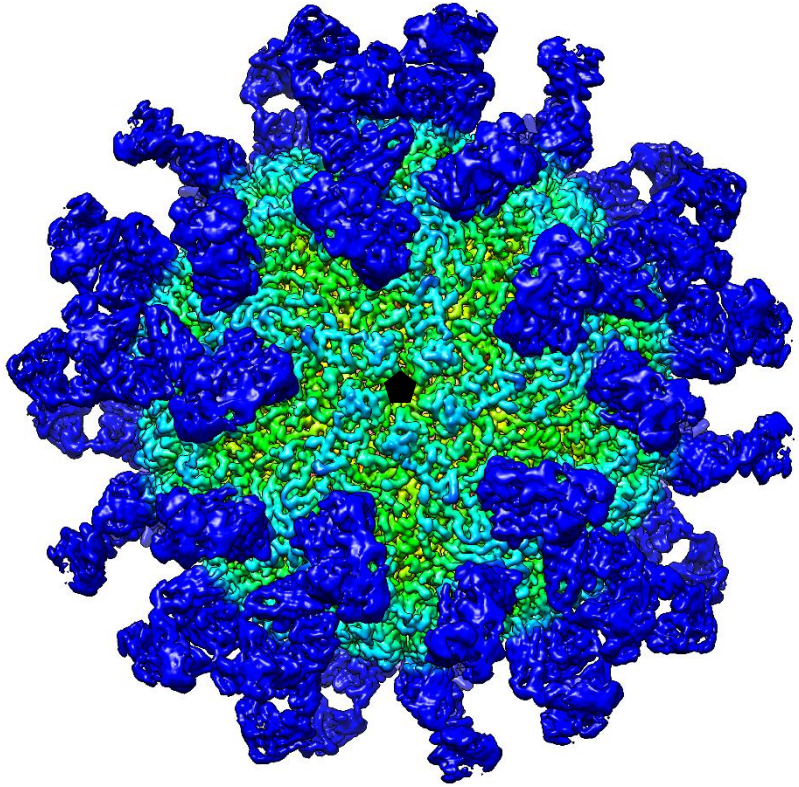
Monoclonal Ab footprint to SAT capsid



Opperman et al., 2014. J Virol. 88, 8307-18.



DA10 and GE11

Courtesy of Dr Kotecha, Oxford.

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Summary

- Reverse genetics technology combined with conventional inactivation to produce custom-made vaccines.
  - Developing countries: lack of vaccines tailored to their conditions
- Inter-serotype chimeric vaccine elicited immune responses similar to that of the parental vaccine and protect host animals against challenge.
- To circumvent a tedious adaptation process on BHK21 cells, the cell culture adaptation phenotype can be rapidly transferred – increased antigen yield.
- Functional residues involved in the protein-protein interactions at the pentameric interfaces in natural occurring viral capsids can be mutated to improve stability of the virion – improve vaccine shelf life.
- The antigenic properties of a vaccine seed virus can be modified to reflect those properties of an antigenically disparate virus.
  - Control of multiple genetic lineages (and antigenic variants) in different geographical regions.

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Katherine Scott
Melanie Chitray
Peninah Nsamba
Raksha Bhoora
Sonja Maree
Lia Rotherham
Kedibone Mawela
Tovho Ramulongo
Liz Botha
Maclaughlin Rathogwa
Lorens Maake

Jan Esterhuysen

P. Mutowemba and stables team

TAD Diagnostic team