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Global Foot-and-Mouth Disease Research Alliance

DETERMINING THE EPITOPE DOMINANCE ON THE CAPSID OF A SAT2 FOOT-AND-MOUTH DISEASE VIRUS BY MUTATIONAL ANALYSIS

Opperman, P.A., Theron, J. and F.F. Maree

Introduction

- Strong link between the protection of cattle against FMDV and the levels of virus-neutralizing antibodies produced following vaccination
  - Most important factor imparting vaccine-induced protection against FMDV
  - Humoral immune response

- Monoclonal antibodies (MAbs) have been used extensively to identify several antigenic sites on the structural proteins of virions
  - Serotypes A, O, C and Asia-1
  - Located on structural protrusions on the virus surface
  - Loops connecting β-barrel structures of the three outer capsid proteins
  - βG-βH loop of 1D has been identified as immunodominant

- **SAT2 serotype is most prominent in southern Africa:**
  - **Three antigenic sites have been identified**
    - **βG-βH loop of 1D, downstream of the RGD motif, is analogous to site 1 of serotype O1BFS**
    - **Residue 210 at the C terminus of the 1D**
    - **Residue 154 of 1D in combination with residue 79 of 1B**
  - **Importance of each of these sites in SAT2 viruses is still undefined**

- **Knowledge of the residues that comprise the antigenic determinants**
  - **Structural design of vaccine seed strains**
  - **Improved protection against specific outbreak isolates**
  - **Lack of information concerning the neutralizing antigenic determinants of the SAT viruses**

Objective

Determine the role of known and predicted epitopes of SAT2 viruses and to present evidence of epitope dominance within the SAT2 serotype of FMDV

- Epitope-swapping approach in an infectious cDNA clone of a SAT2 virus
- Selected and mutated residues located in ten of the structurally exposed loops of 1B, 1C and 1D
- Measured the effect of these mutations on antigenicity with virus neutralization (VN) assays
  - Polyclonal antisera raised against SAT2/ZIM/7/83, used as the genetic background, and SAT2/KNP/19/89, the epitope-donor

Predicting of Antigenic Sites

- **Capsid-coding region of FMDV strains found in sub-Saharan Africa analyzed using one-way antigenic relationships (r1-values)**

- **Variable regions on the capsid proteins combined with structural data and serological relatedness to identify possible epitope**

- **Modelled SAT2 capsid structure using O1BFS as template**
  - **Based on the optimal alignment of the SAT2 virus, ZIM/7/83, P1 sequence corresponding to O1BFS**

- **Variable residues on surface-exposed loops were regarded as immune relevant and mapped to the SAT2 pentamer structure**
  - **Outside the 1D βG-βH loop were concentrated around the 5-fold and 3-fold axis of the virion and the C-terminal of 1D** (Maree *et al*., 2011)
  - **Previously identified neutralizing epitopes of type A and O**

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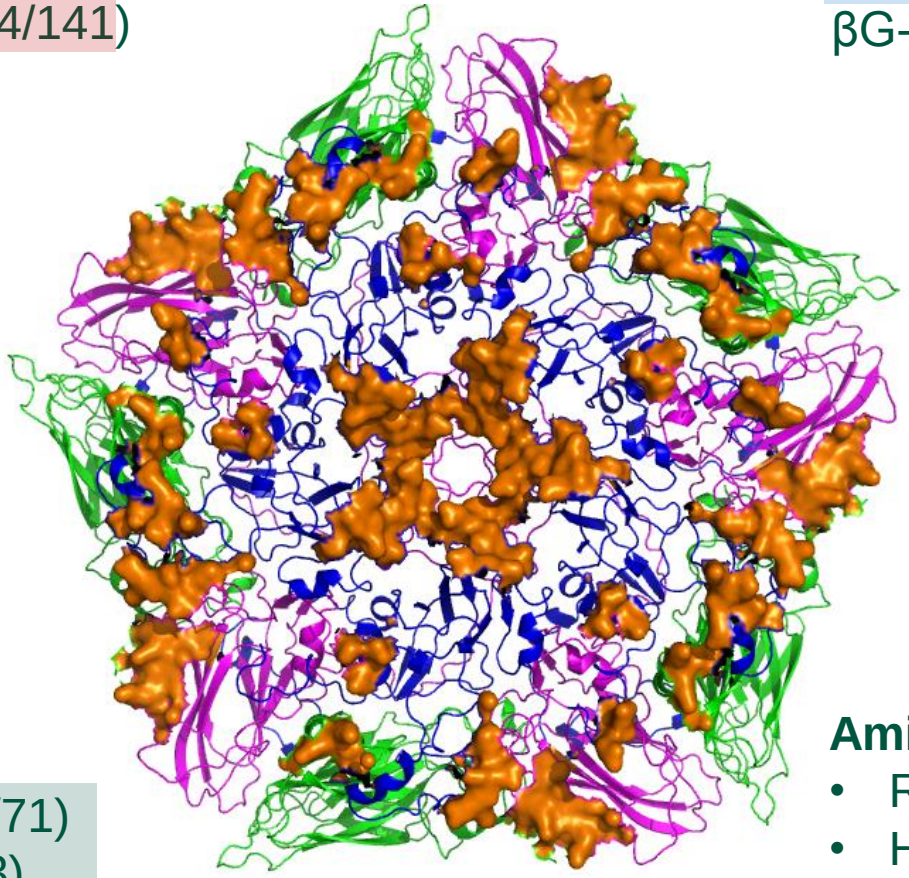
1B (4)
βA-βB loop (31-45)
βB-βC loop (64-82)
βC-βD loop (93-101)
βE-βF loop (130-134/141)

1C (4)
N-terminus (30-45)
βB-βC loop (63-77)
βE-βF loop (125-142)
βG-βH loop (165-183/172)

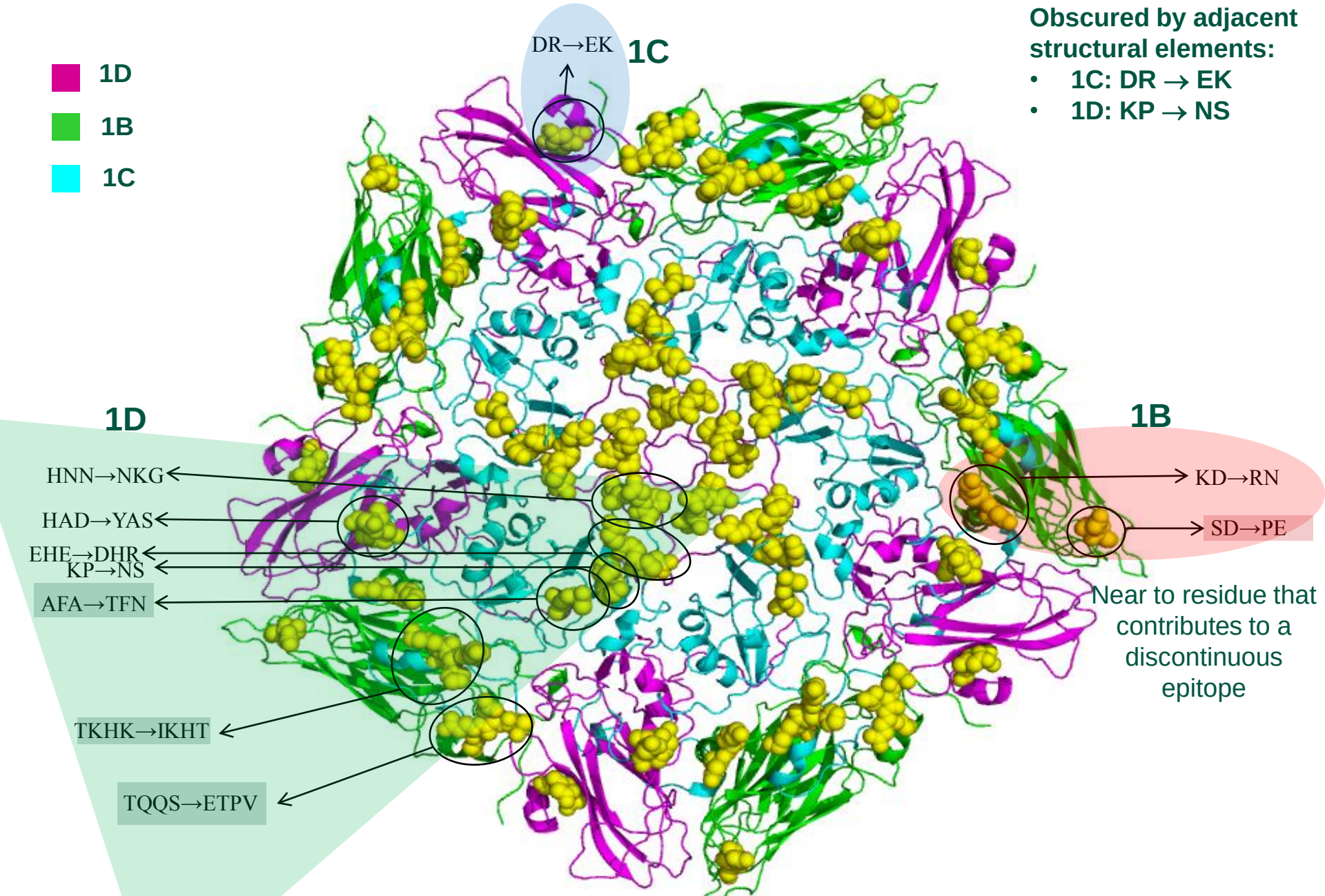


1D
1B
1C
Predicted epitopes

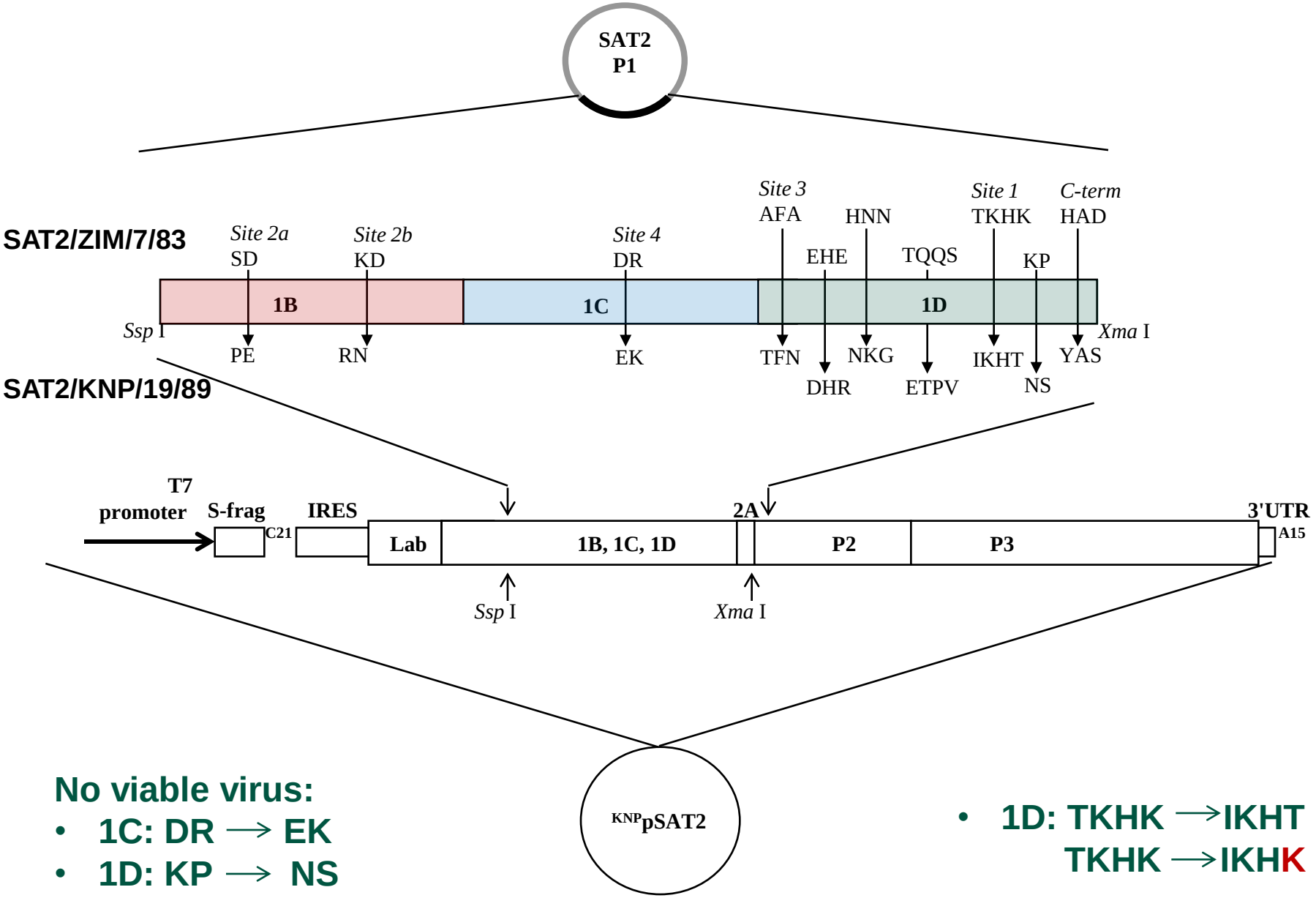
1D (7)
N-terminus (9-40)
βB-βC loop (43-62/71)
βE-βF loop (80-103)
βF-βG loop (110-122)
βG-βH loop (136-167)
βH-βI loop (176-187)
C-terminus (192-212)

Amino Acids
- Regions of hypervariability
- High entropy
- Structurally exposed loops
- Antibody recognition sites

10 structurally exposed loops of SAT2/KNP/19/89 were introduced into the pSAT2 plasmid, a SAT2/ZIM/7/83 infectious clone



Cloning Strategy



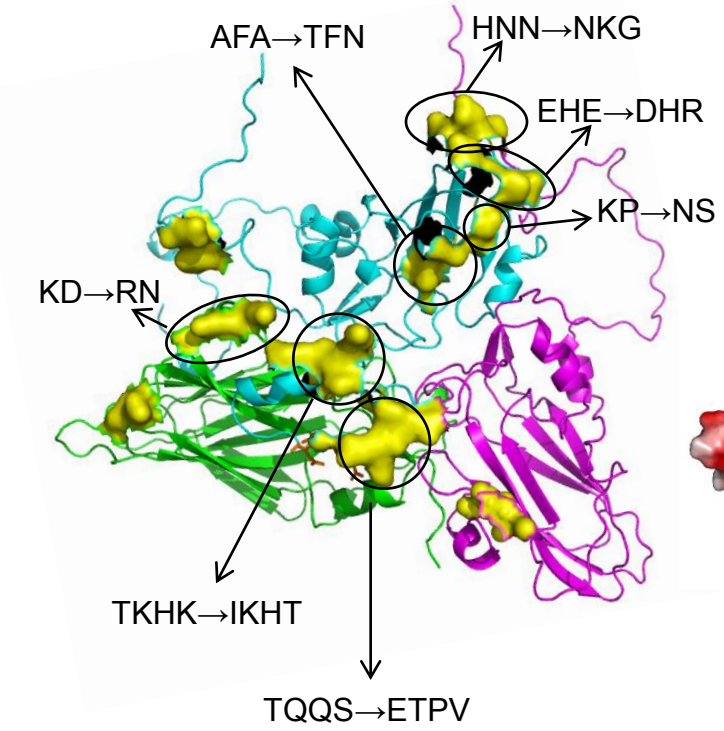
No viable virus:

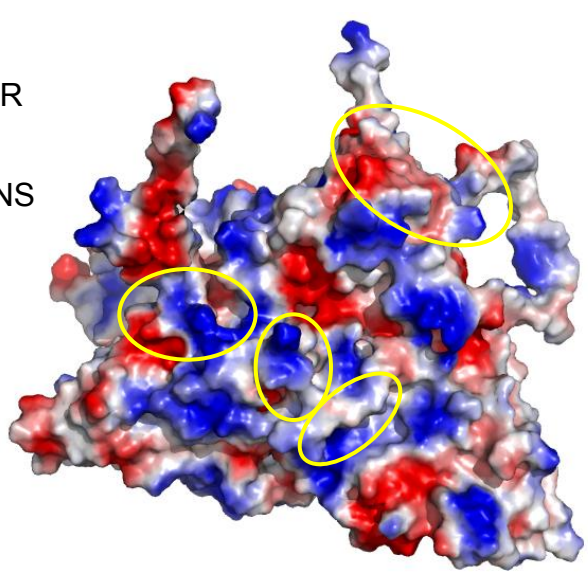
- **1C: DR ⟶ EK**
- **1D: KP ⟶ NS**

- **1D: TKHK ⟶ IKHT**
  TKHK ⟶ IKHK

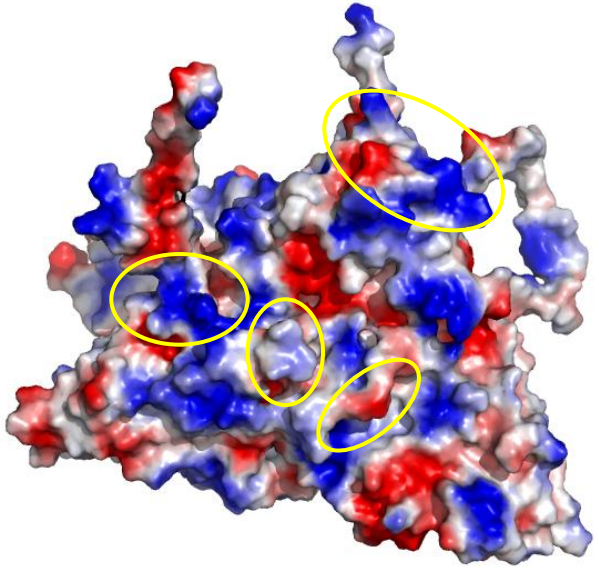
Electrostatic Potential Of Mutations

- Increase in the net positive charge in the 1D protein of the derived recombinant virus
- EHE→DHR (84-86) and HNN→NKG (109-111) mutations of 1D
- Strong effect on the local surface potential of the capsid
- Distinct patch of surface area that was predominantly positively charged



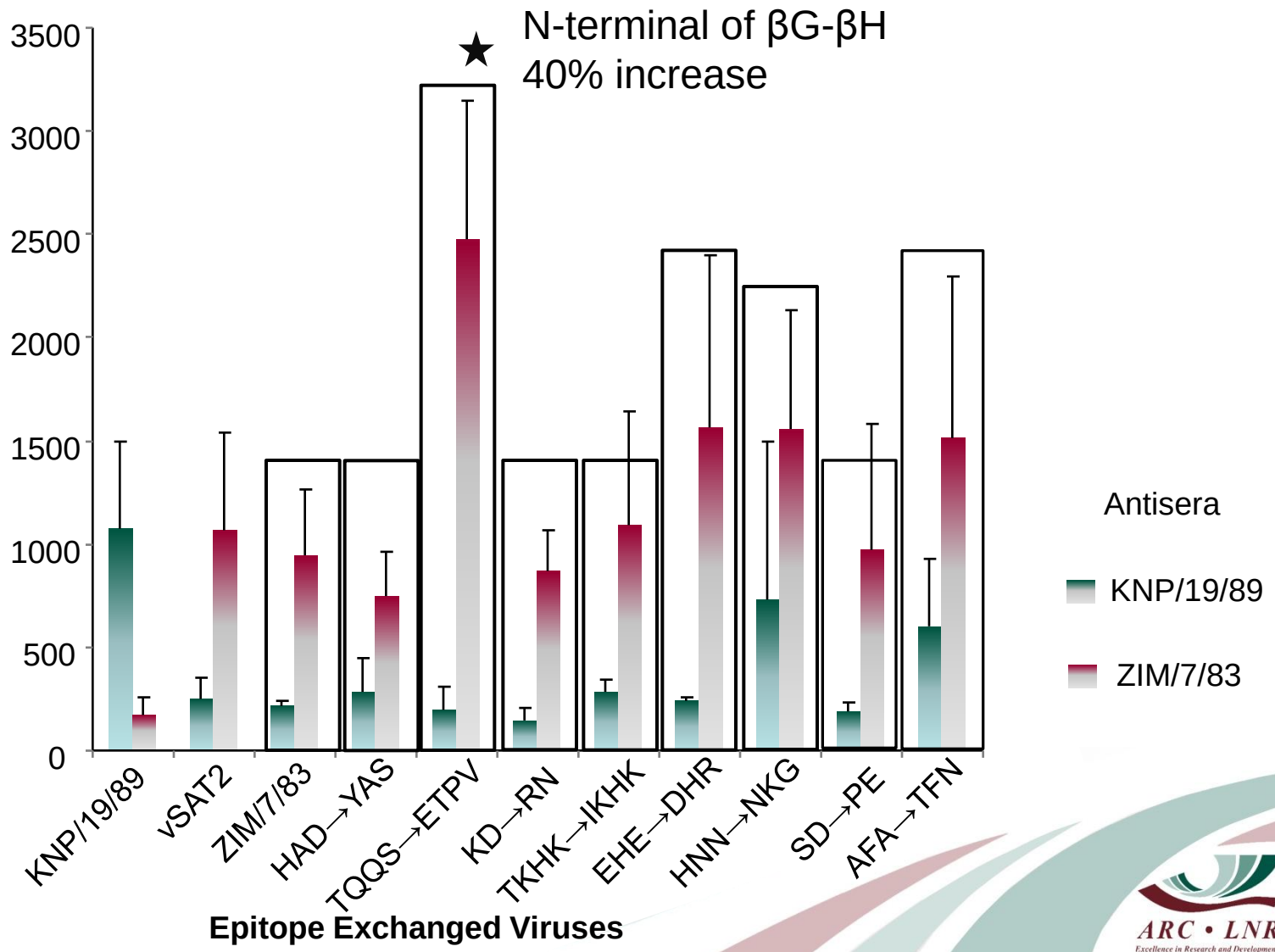


SAT2/ZIM/7/83



v^{KNP}SAT2

Antigenic Profiles Of The Epitope-Replaced Viruses



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Conclusions

- Similar neutralization profiles obtained as for vSAT2 and SAT2/ZIM/7/83
  - SD→PE and the KD→RN mutations in 1B
  - TKHK→IKHK mutation in the βG-βH loop of 1D
  - HAD→YAS mutation at the C-terminus of 1D

- Slight increase in neutralizing titre against the SAT2/KNP/19/89 antiserum and not significantly reduced against SAT2/ZIM/7/83
  - HNN→NKG and the AFA→TFN mutations in the 1D protein
  - Larger relative amount of neutralizing antibody against that particular epitope in the polyclonal serum.

- Higher neutralization titres with SAT2/ZIM/7/83 antisera
  - EHE→DHR; HNN→NKG, AFA→TFN, TQQS→ETPV
  - Mutated epitope alters the binding of high affinity antibodies to the capsid in such a way that the binding sites of lower affinity antibodies become available, resulting in a different neutralization kinetics/profiles.

- **HNN→NKG change resulted in a predominantly positively-charged local surface potential**
  - **Linked to the binding of SAT viruses to alternative receptors for cell entry**
  - **Viruses propagated in cell culture**
    - **Adversely affect vaccine seed stocks**
    - **Selection of viruses that are altered at multiple sites on the capsid**

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MSD Animal Health

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Department:
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