NOVEL HIS-TAG MARKER FOOT-AND-MOUTH DISEASE VIRUS VACCINE BOUND TO NANOLIPOPROTEIN ADJUVANT VIA METAL IONS: UTILITY ON VACCINE AND DIAGNOSTIC DEVELOPMENTS

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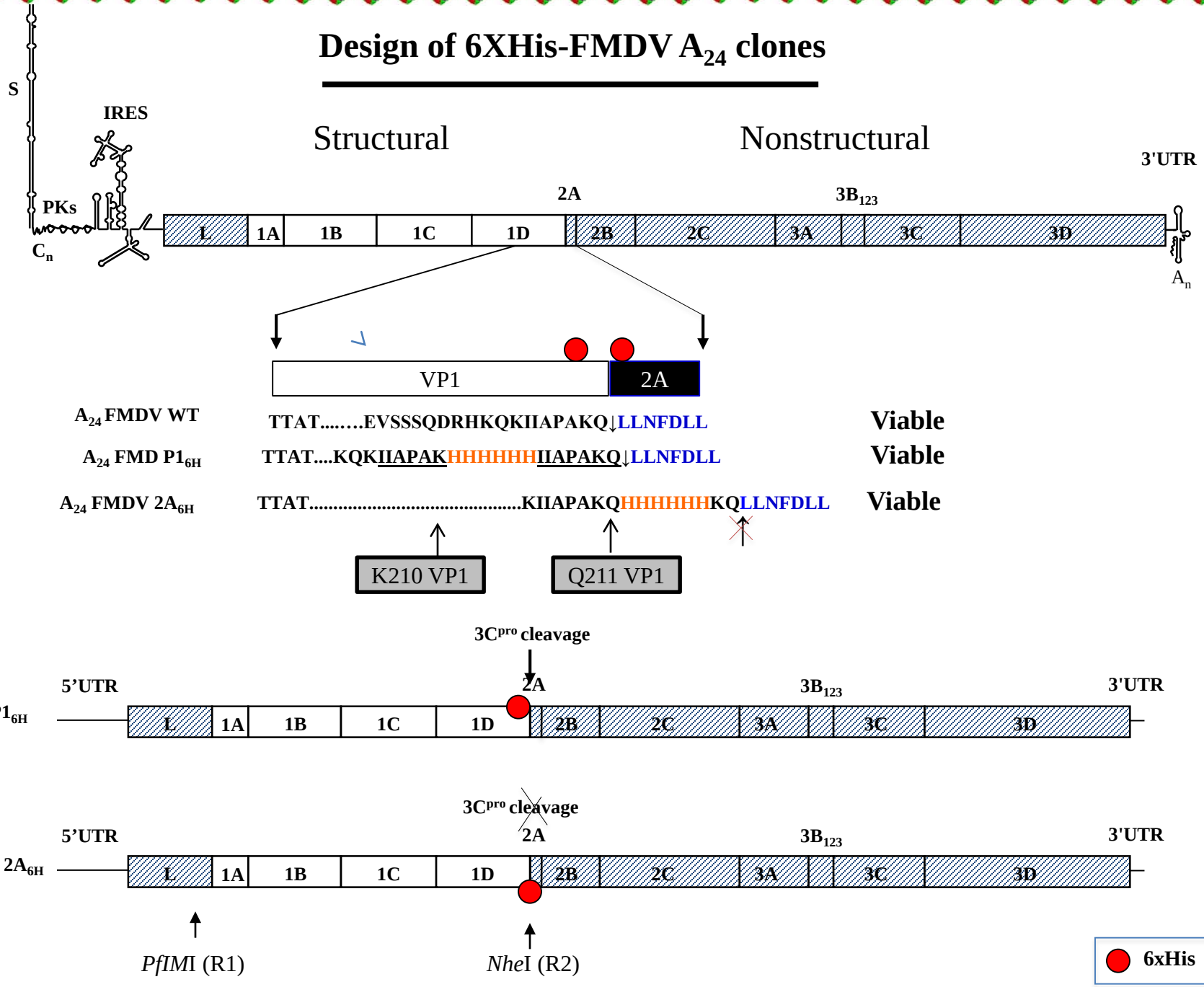
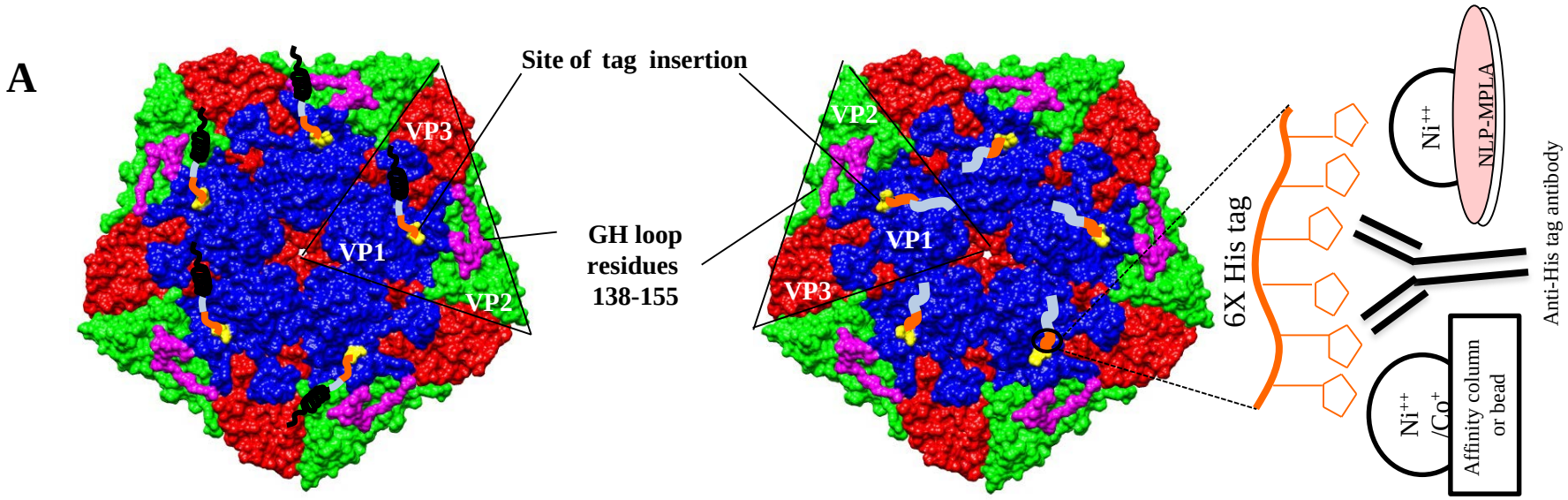
Global Foot-and-Mouth Disease Research Alliance

USDA

Design of 6XHis-FMDV A_{24} clones

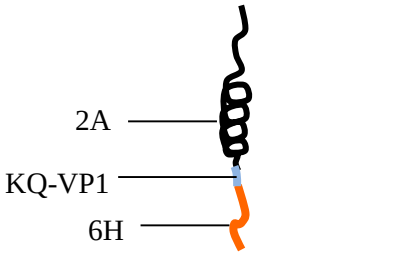


Modeling of FMDV A24 capsid for selecting the site of insertion and schematic representation of different components of virus-adjuvant complex



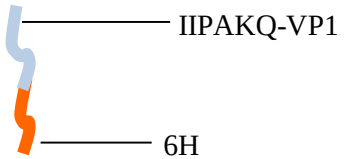
A_{24} FMDV $2A_{6H}$

A_{24} FMDV $P1_{6H}$



$2A_{6H}$ tag

HHHHHHKQLLNFD
LLKLAGDVESNPG

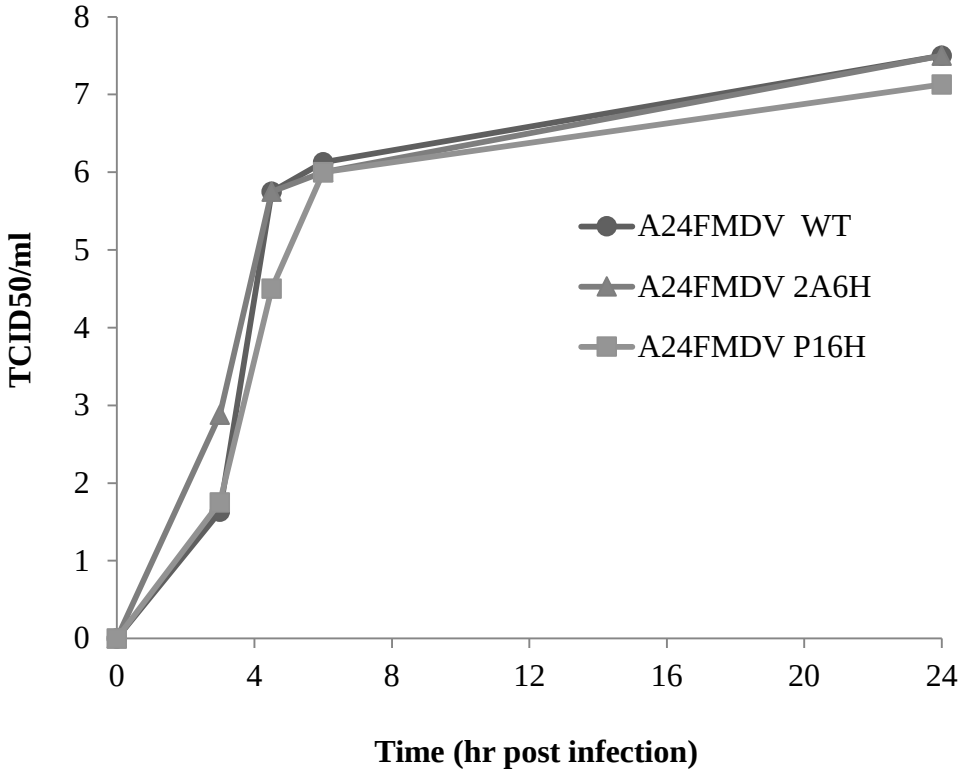


$P1_{6H}$ tag

HHHHHHIIAPAKQ

Growth curve and phenotype of 6xHis-FMDVs

A



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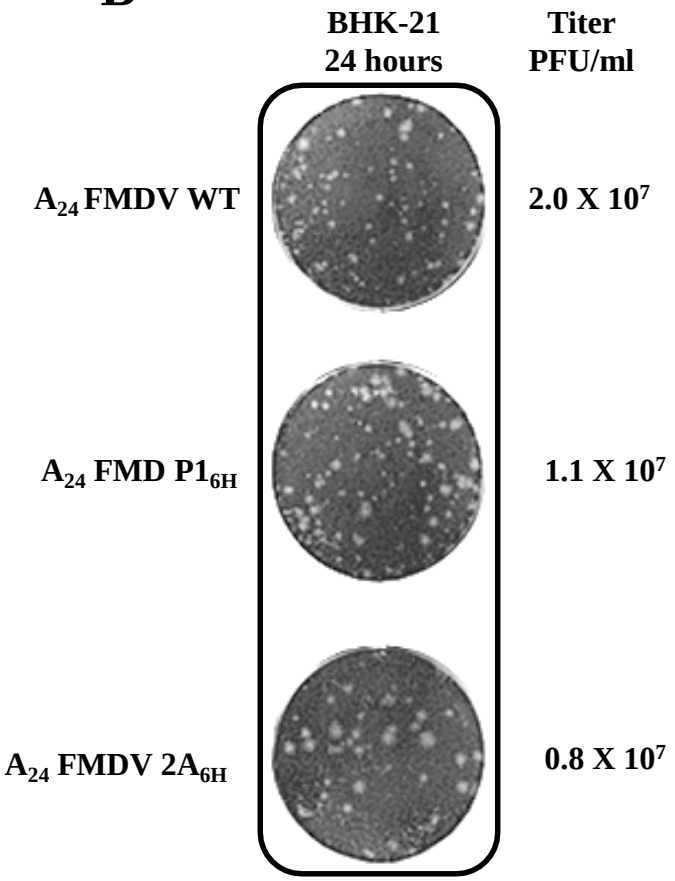


Both A24 FMDV$_{2A}$6H and A24 FMDV$_{P1}$6H follow WT FMDV 24 like growth kinetics and produce Plaque phenotypes similar to the later.

Determination of specific neutralizing antibody response of parental and mutant viruses to cattle FMDV A_{24}Cru antisera

Virus	Bovine anti A_{24}WT sera Neutralization Titers[a]	r_1-values[b]
A_{24}WT	3.15	1.00
A_{24}FMDV $2A_{6H}$	3.45	1.99
A_{24}FMDV $P1_{6H}$	3.00	0.7

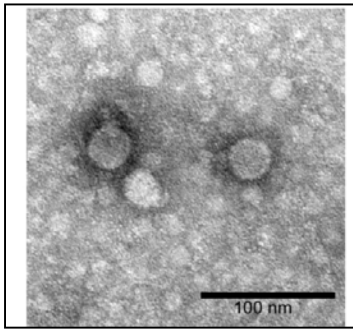
Virus neutralizing titers (log_{10} of reciprocal of the last serum dilution to neutralize 100 $TCID_{50}$ of virus in 50% of the wells).
Sensitivity > 0.9. Sera was produced in a bovine infected by intradermolingual inoculation of 4 log_{10} bovine tongue infectious doses of FMDV A_{24}WT and collecting blood 21 days post-infection.
[b]The mean r1-values as measured against the reference strain A_{24}WT.

R1 vallue from virus neutralization titer experiiment proves that the 6xHis FMDVs retain antigenicity of parental FMDV A24 virus.

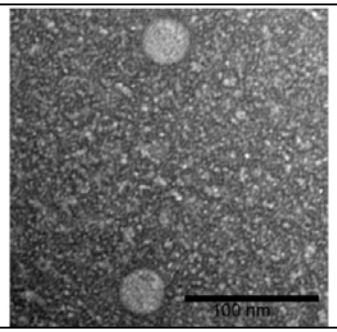
EM shows that 6xHis tagged FMDVs form complex with MPLA-NiNLP

A

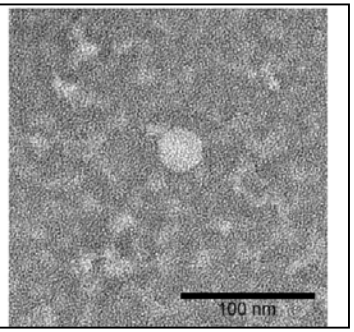
A_{24} FMDV WT



A_{24} FMDV $2A_{6H}$

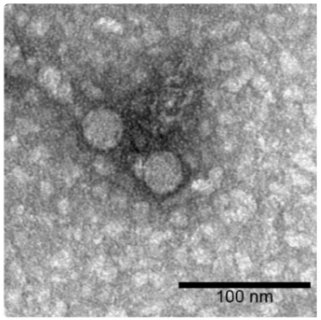


A_{24} FMDV $P1_{6H}$

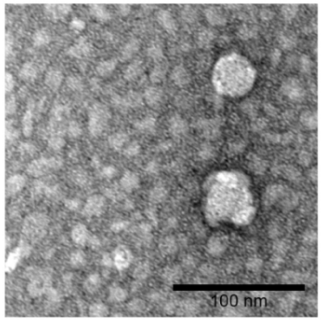


B

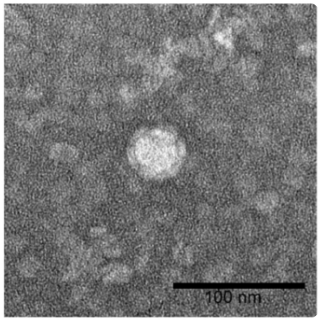
A_{24} FMDV WT+ NiNLP-MPLA



A_{24} FMDV $2A_{6H}$ + NiNLP-MPLA

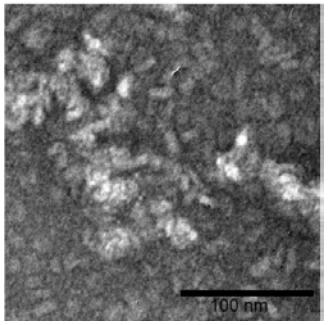


A_{24} FMDV $P1_{6H}$ + NiNLP-MPLA



C

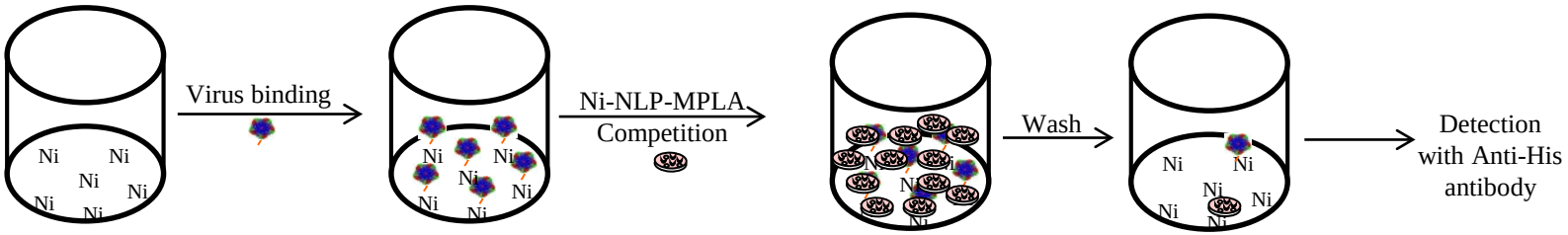
NiNLP-MPLA

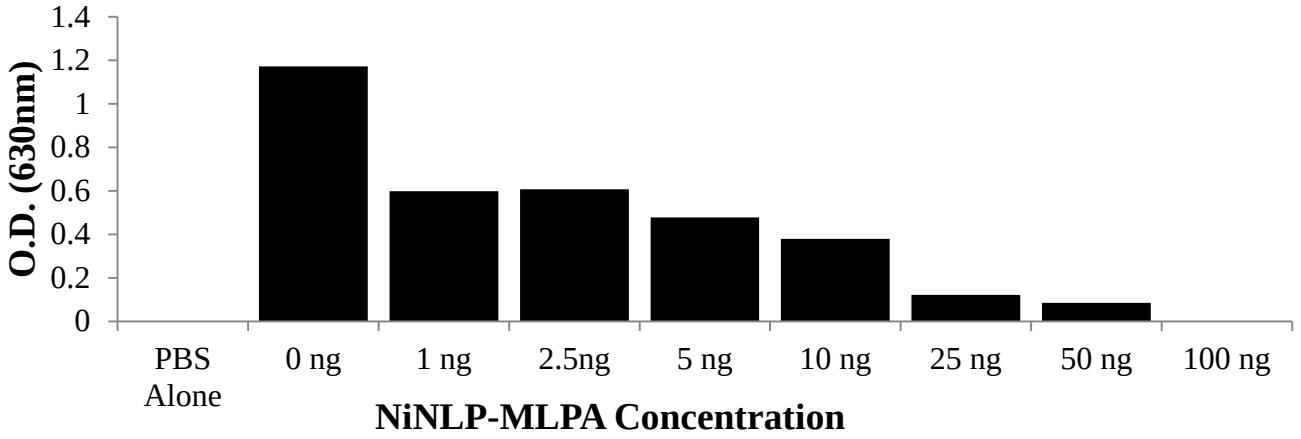


D

No. of NiNLP-MPLA bound	A24 FMDV WT	A24 FMDV P16H
0	124	15
1	9	23
2	0	16
3	0	22
4	0	14
5	0	9

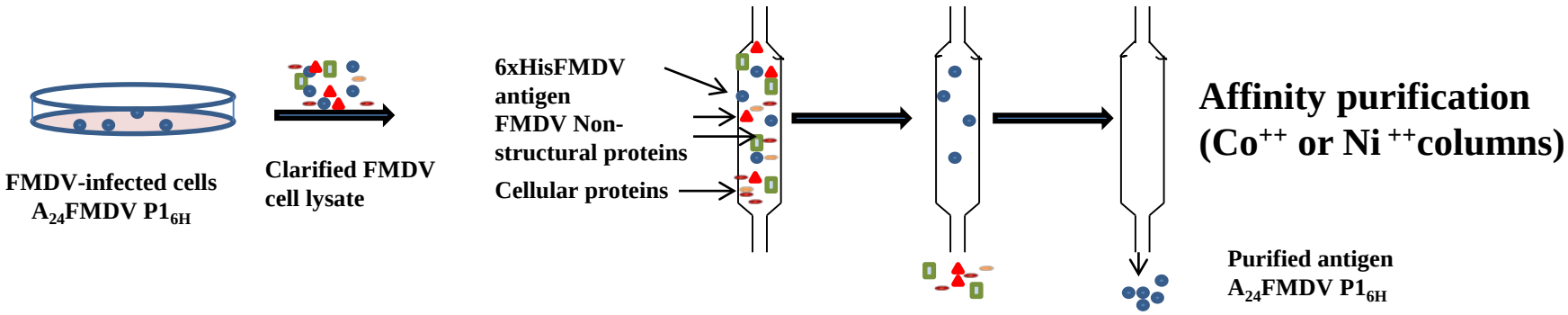
Determination of 6xHistag mediated binding of FMDV capsid to MPLA-NiNLP using Ni-NTA coated plate ELISA plate: implication in diagnostic assay development



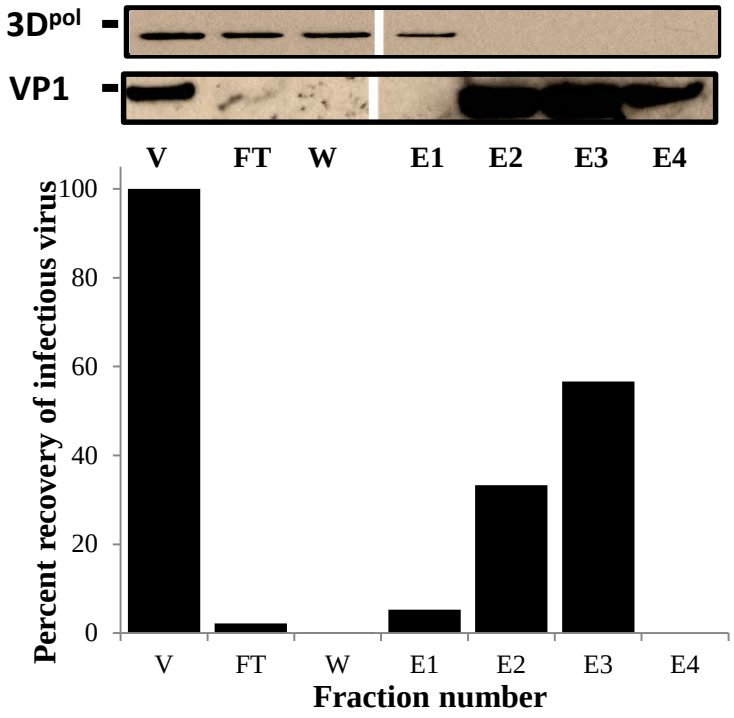


ELISA assay further confirmed a specific interaction between 6xHis FMDV capsid and MPLA-NiNLP.

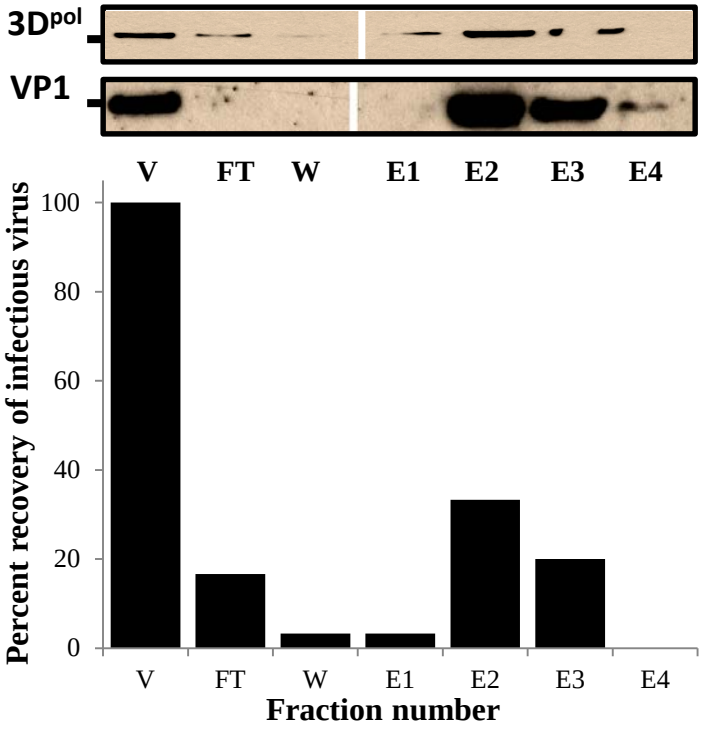
Co-NTA column purification of A24 FMDV P16H



Imidazole Elution

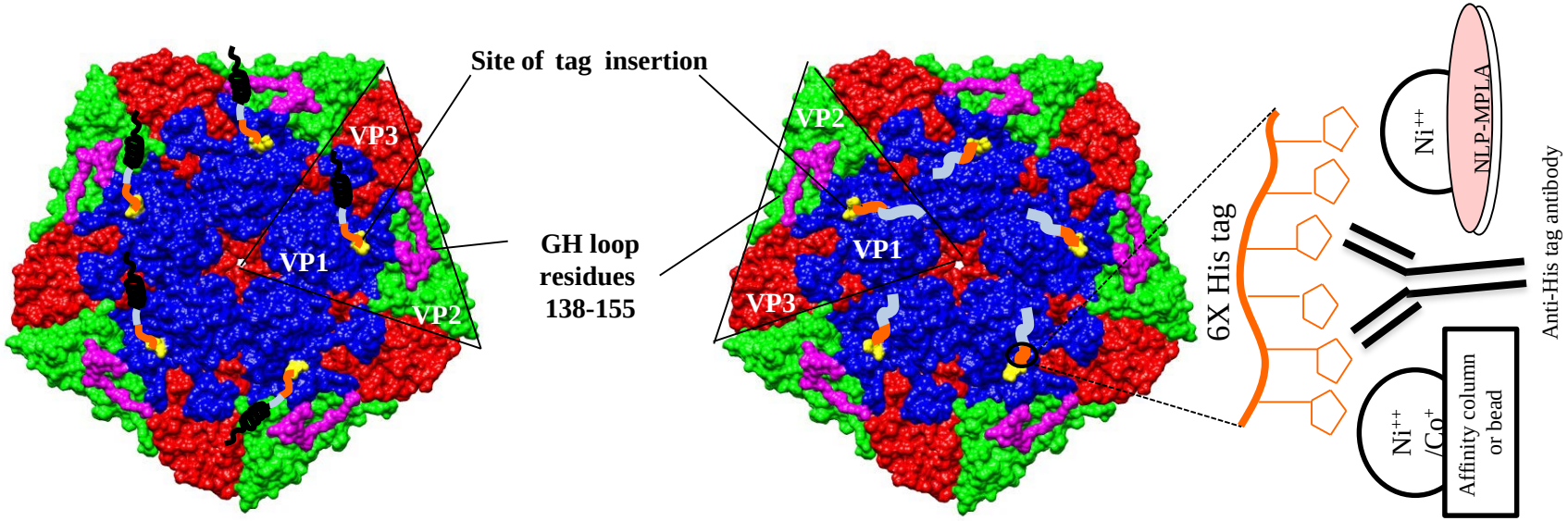


EDTA Elution



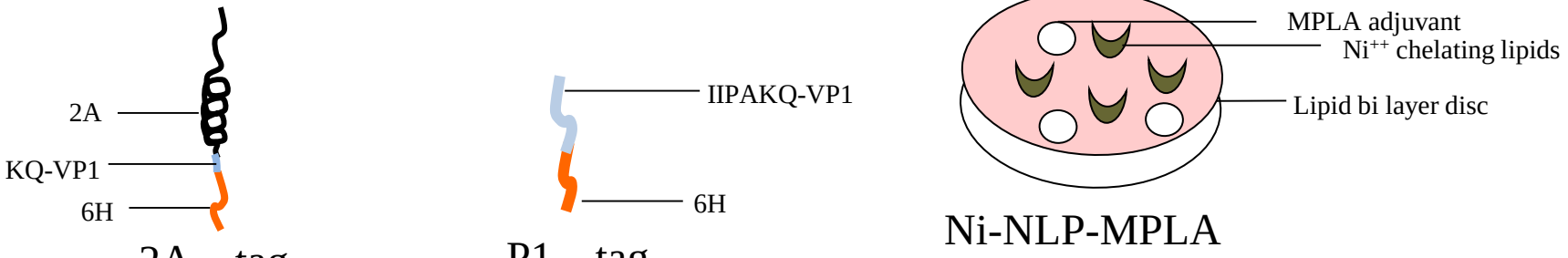
Modeling of FMDV A24 capsid for selecting the site of insertion and schematic representation of different components of virus-adjuvant complex

A



A_{24} FMDV $2A_{6H}$ A_{24} FMDV $P1_{6H}$

B



$2A_{6H}$ tag

HHHHHHKQLLNFD LLKLAGDVESNPG

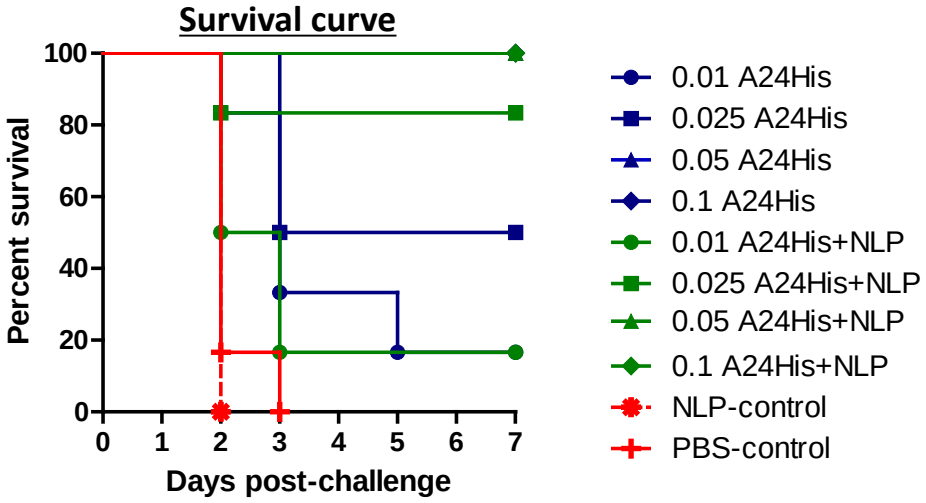
$P1_{6H}$ tag

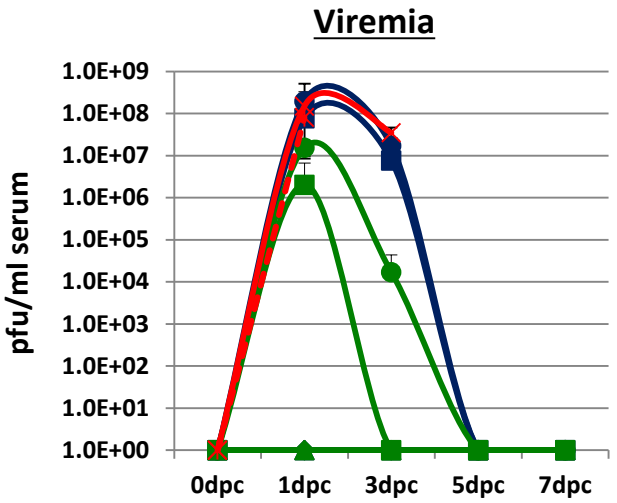
HHHHHHIIAPAKQ

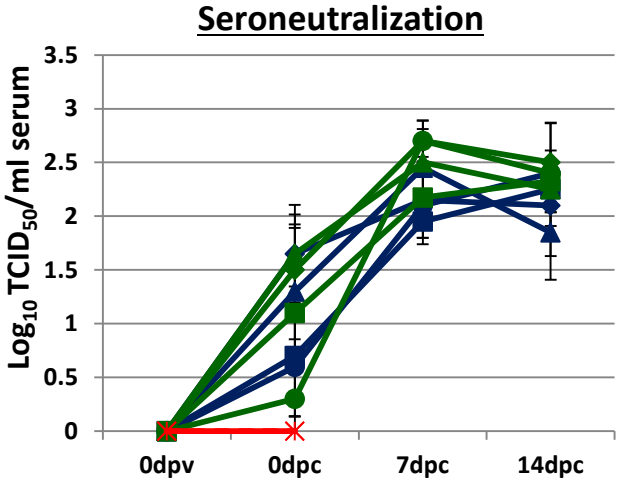
Ni-NLP-MPLA

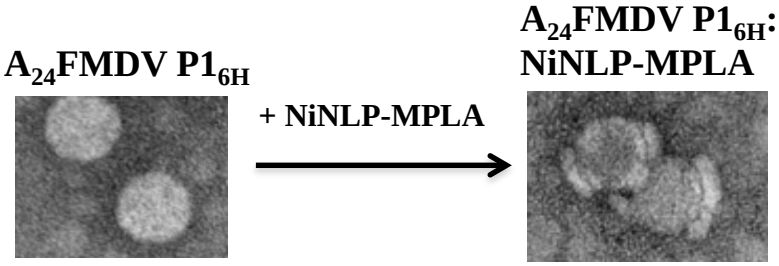
Lawrence P, Pacheco JM, Uddowla S, Hollister J, Kotecha A, Fry E, Rieder E. Foot-and-mouth disease virus (FMDV) with a stable FLAG epitope in the VP1 G-H loop as a new tool for studying FMDV pathogenesis. Virology. 2013 Feb 5;436(1):150-61.

Mice inoculation with inactivated A24 FMDV $P1_{6H}$:MPLA-NiNLP adjuvanted vaccine and subsequent challenge with A24 FMDV WT



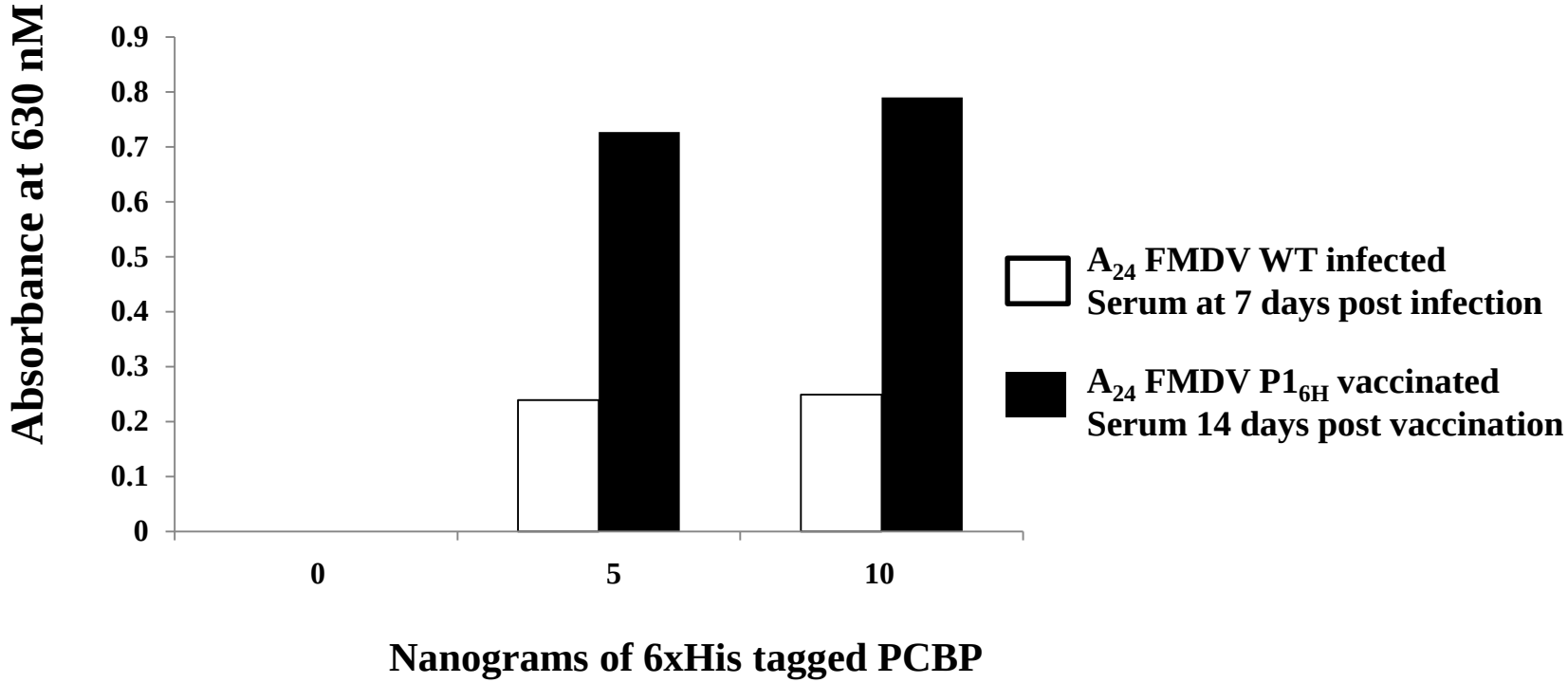






Vaccine:adjuvant complex prevented the disease in mice in a dose dependent manner.

Determination of anti-6xHis TAG antibody in A24 FMDV P16xH treated mice serum



Successful generation of anti-6H response in vaccinated animals can lead us to further design assays to differentiate vaccinated from infect animals.

SUMMARY

- Engineered FMD viruses with histidine residues inserted into or fused to the FMDV capsid. 6xHis FMDVs exhibited growth kinetics, plaque morphologies and antigenic characteristics similar to wild-type virus.

- Electron microscopy and biochemical assays revealed that the 6xHis FMDVs readily assembled into antigen: adjuvant complexes with Ni^{2+} chelated nanolipoprotein and monophosphoryl lipid A adjuvant (MPLA:NiNLP).

- The 6xHis tag allowed one-step purification of the mutant virions by Co2+ affinity columns.

- Animals Immunized with the inactivated 6xHisFMDV:MPLA:NiNLP vaccine acquired enhanced protective immunity against FMDV challenge compared to virions alone.

- Induction of anti-6xHis in the immunized animals could be exploited in the differentiation of vaccinated from infected animals needed for the improvement of FMD control measures.

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Dr.Tom Burrage, Ben Clark – DHS

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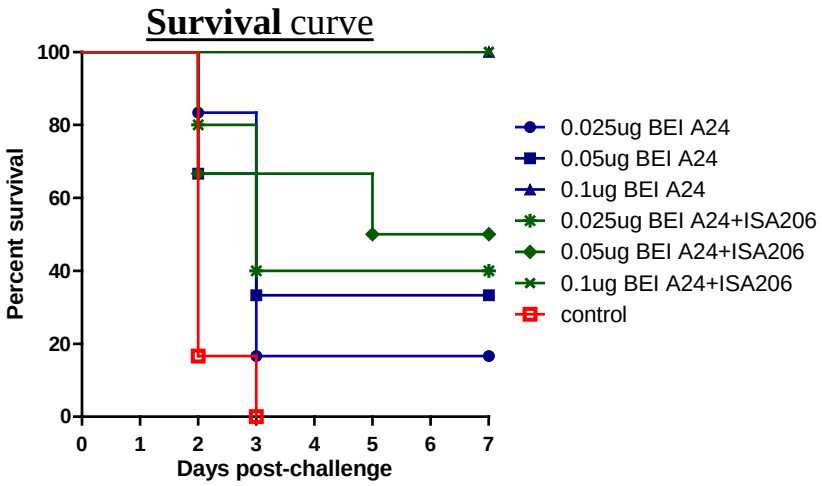
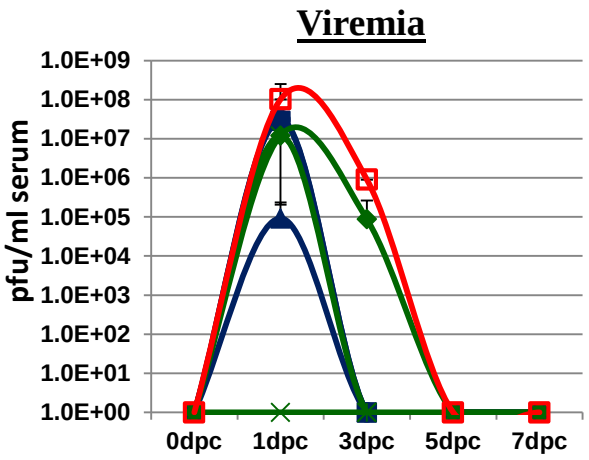
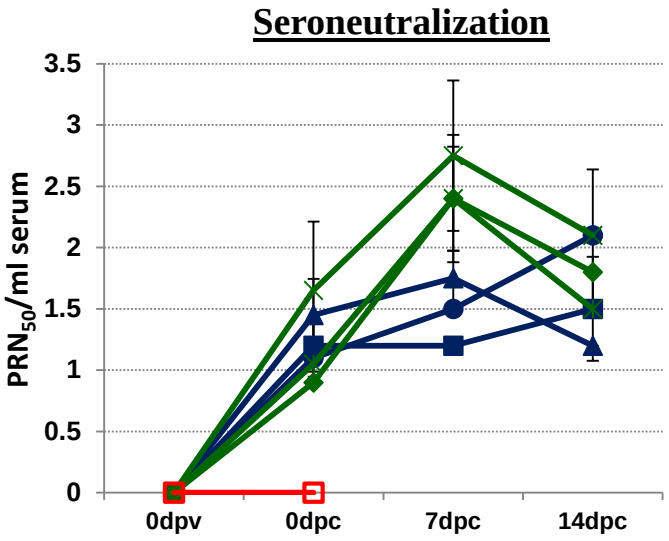
Lawrence Livermore National Laboratory, Livermore, CA

Mice inoculation with inactivated A24 FMDV WT and ISA206 adjuvant and challenge with A24 FMDV WT







Virus caused lethal disease was prevented by vaccine adjuvant preps in mice in a dose dependent manner.