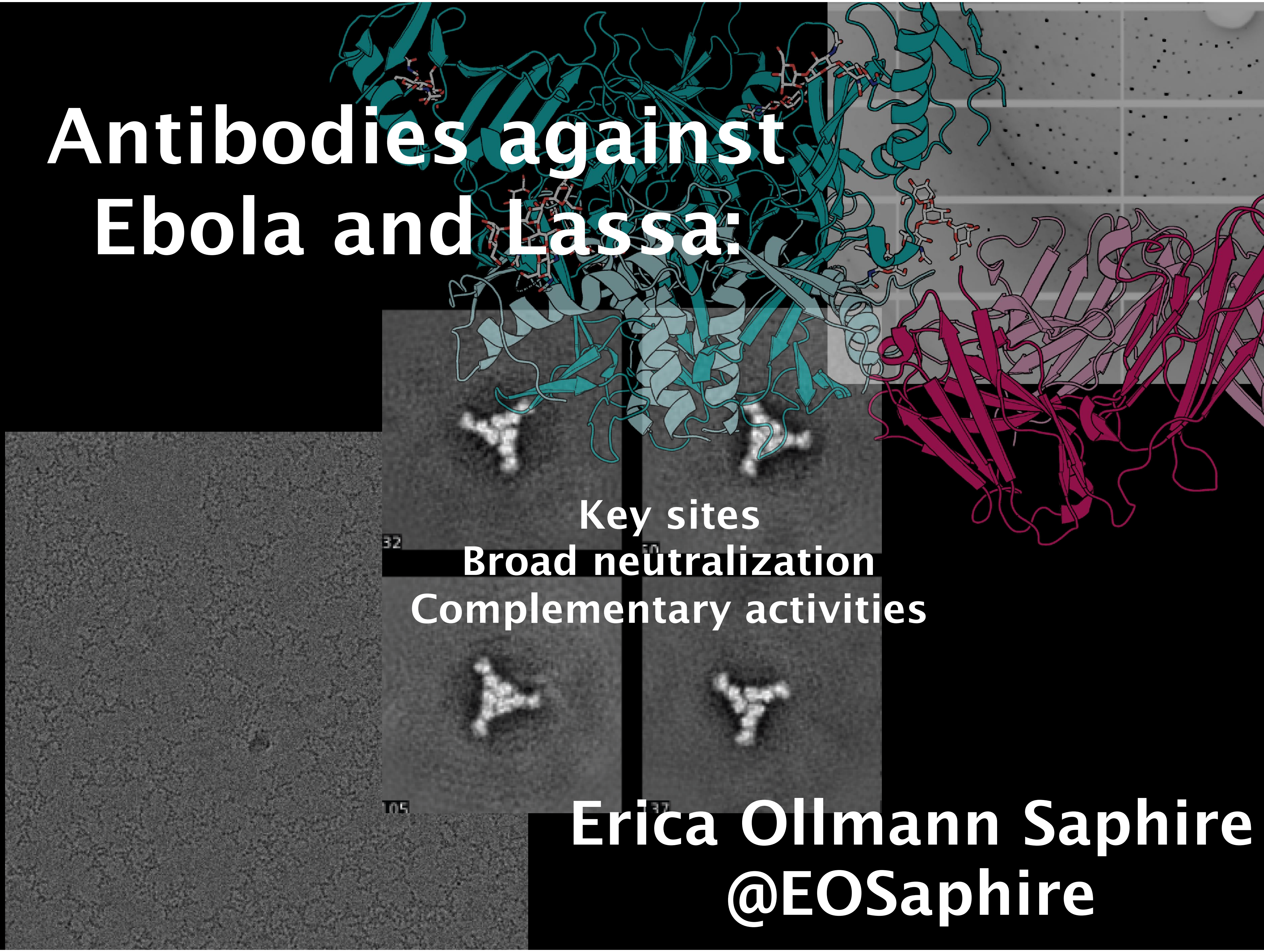
# Antibodies against Ebola and Lassa:

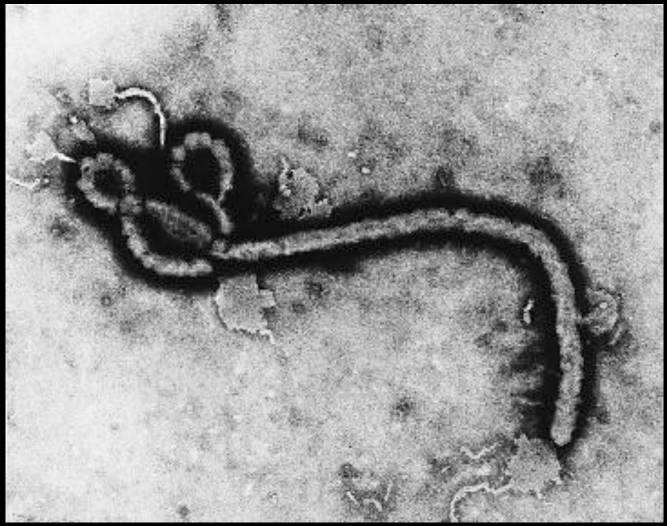


Key sites
Broad neutralization
Complementary activities


## Erica Ollmann Saphire
## @EOSaphire

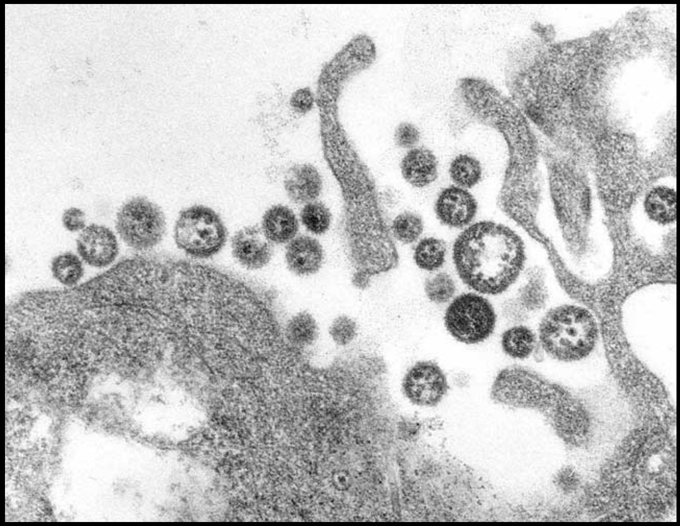
## Filoviruses

Ebola, Sudan, Bundibugyo, Reston
Marburg…



## Arenaviruses

Lassa, LCMV, Lujo
Junin, Machupo, Sabía…



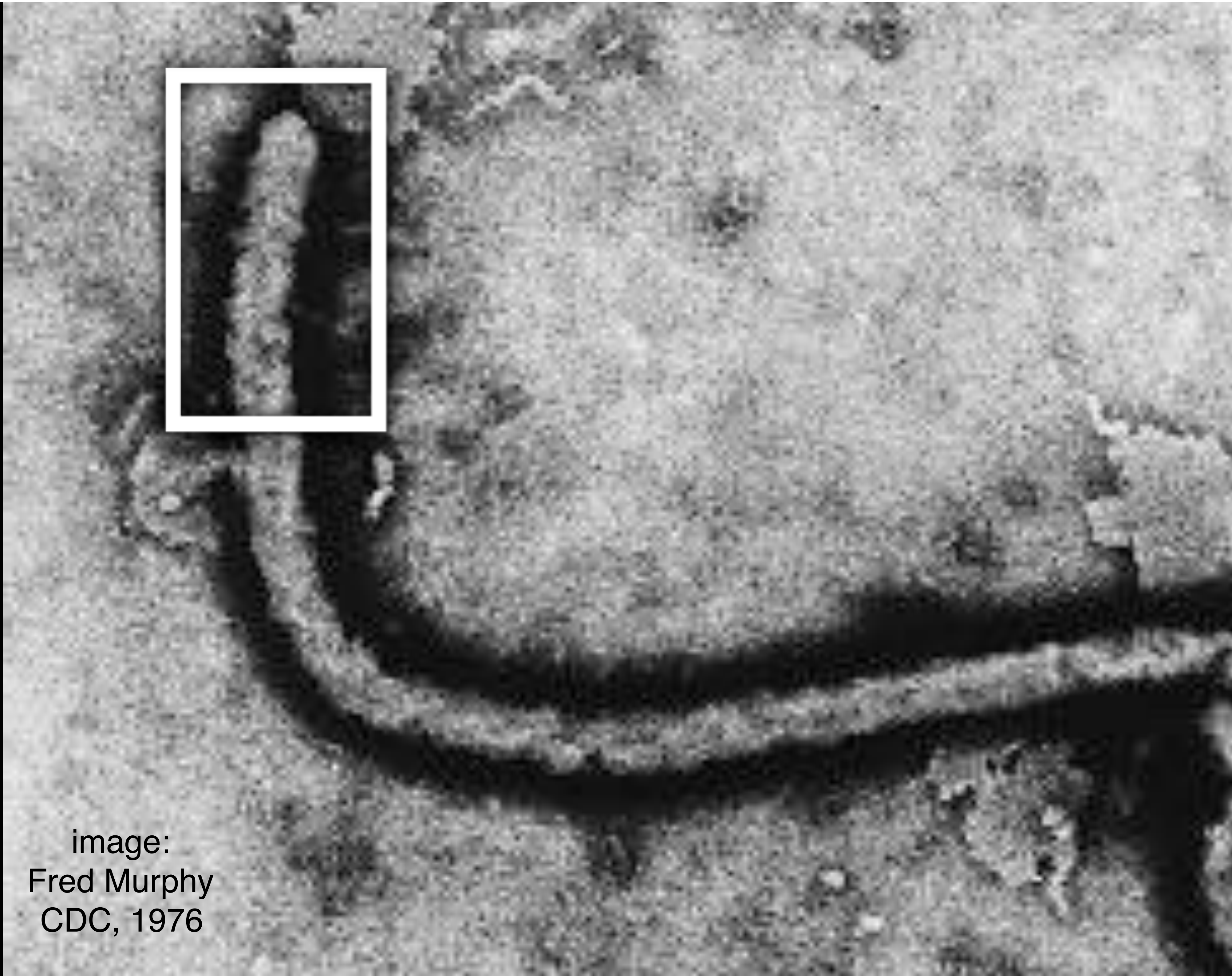
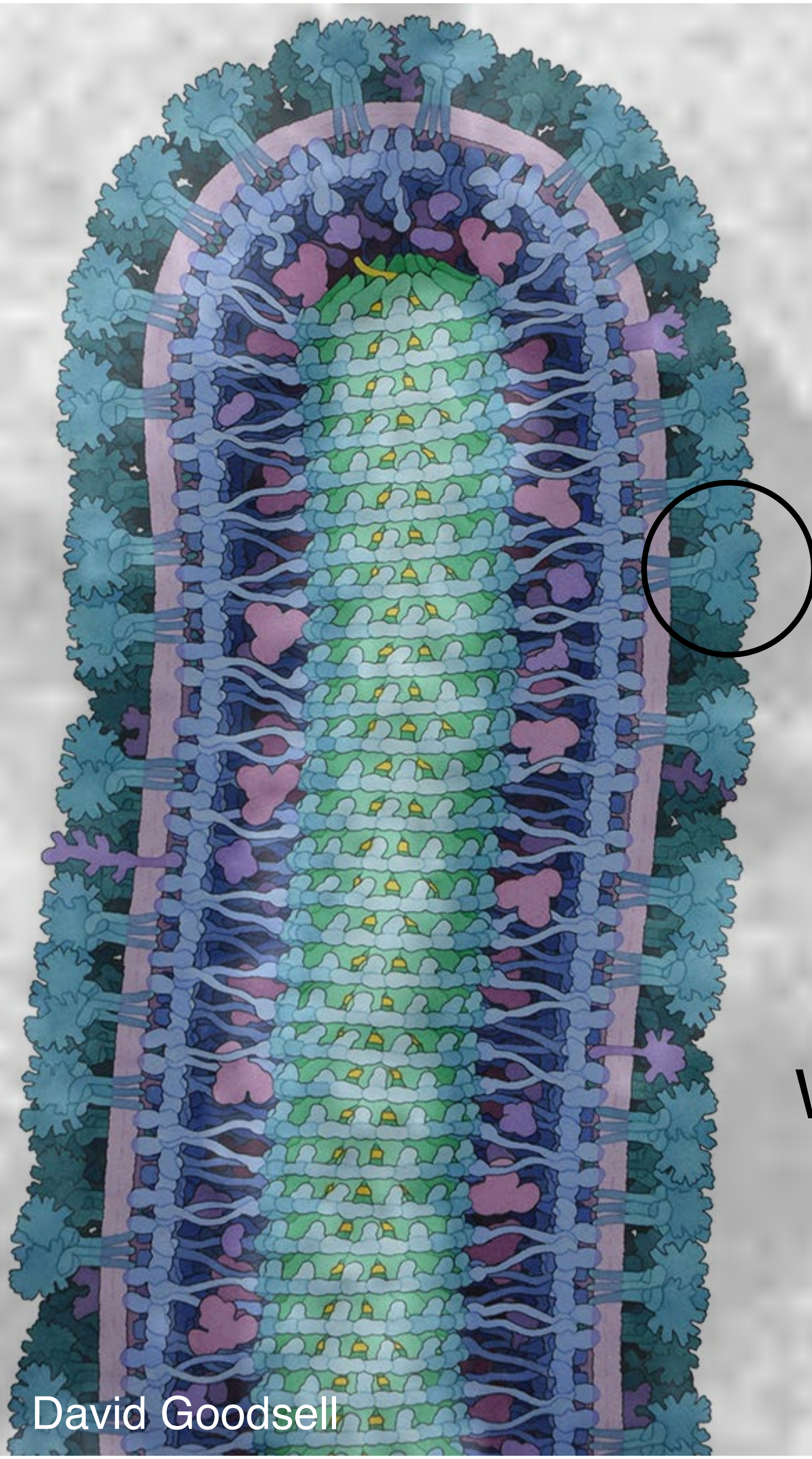


image:
Fred Murphy
CDC, 1976




# Ebola virus

**One surface protein, the glycoprotein GP**

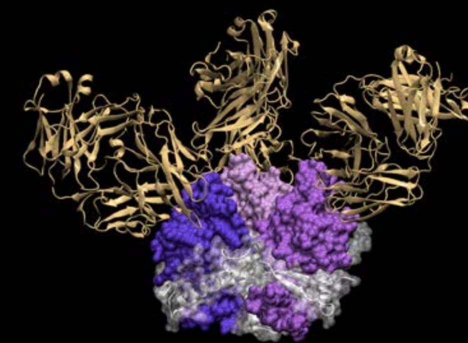
Attachment to and fusion with target cells

Component of vaccines
Target of antibodies

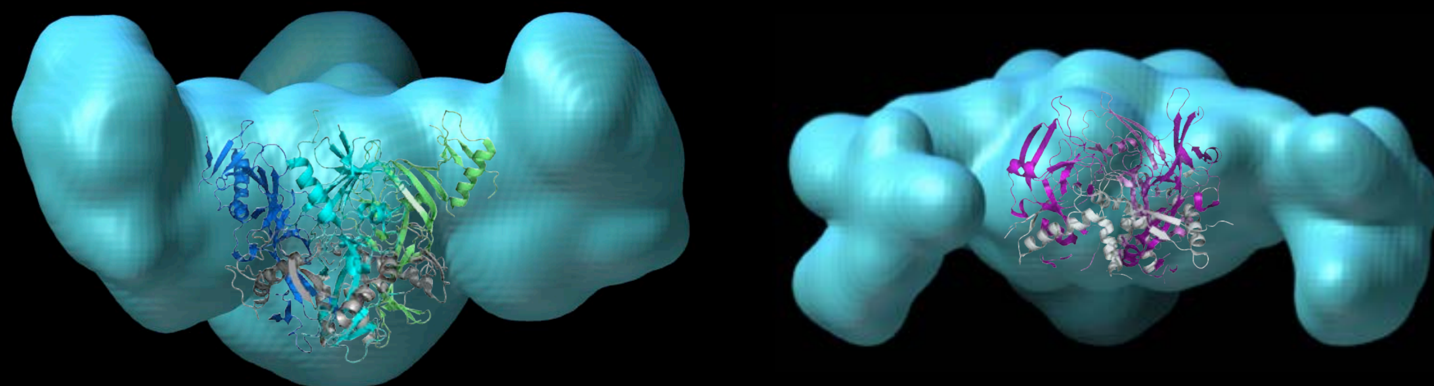
What are the best antibodies?
What makes them “good”?
How do we find them?

Ebola virus

Sudan virus



Marburg virus



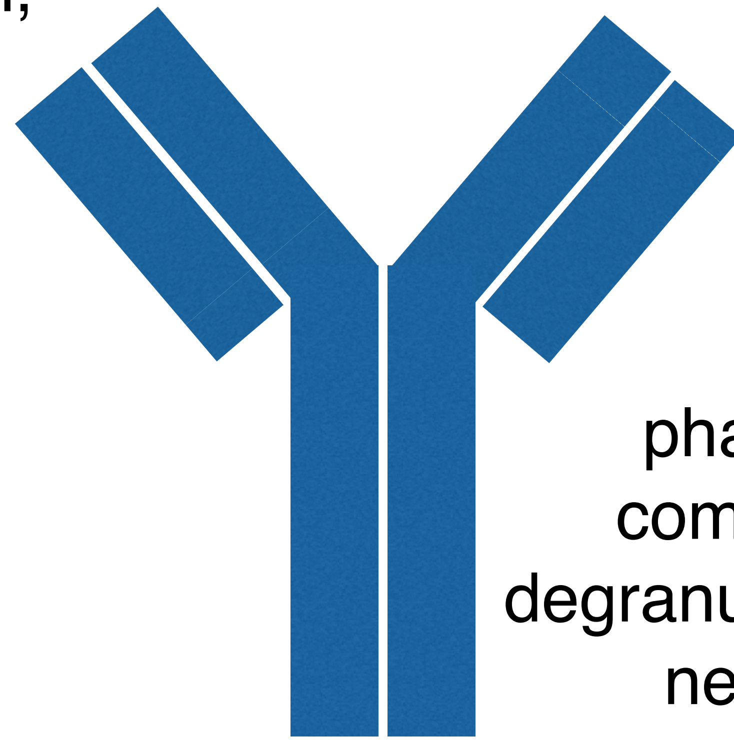
Fully glycosylated, mucin-containing
Ebola and Marburg GP

Use these structures to interpret antibody responses
Galvanize a global effort by previously competing groups

# Antibody

## Fab

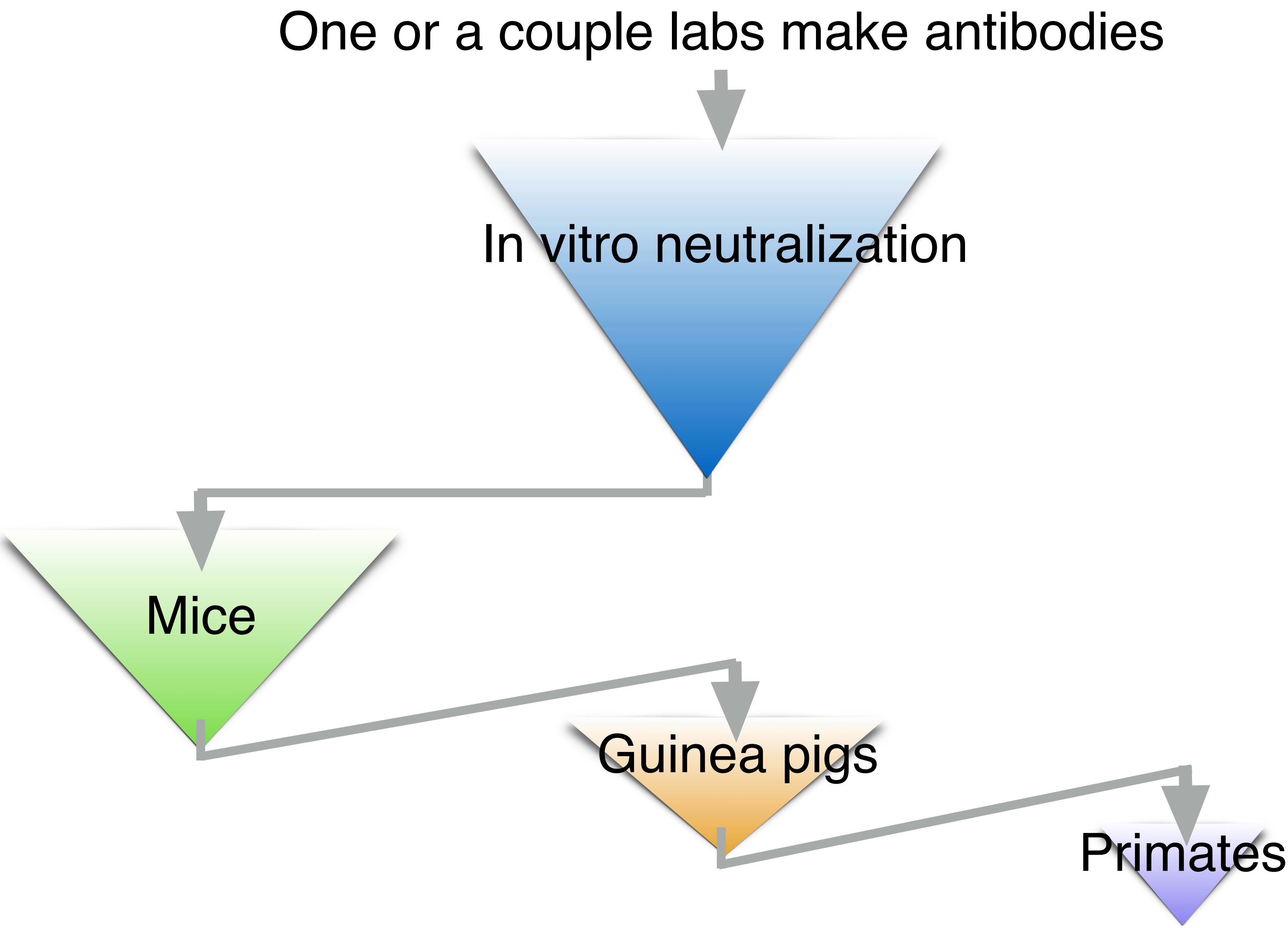
antigen recognition,
mechanical
neutralization



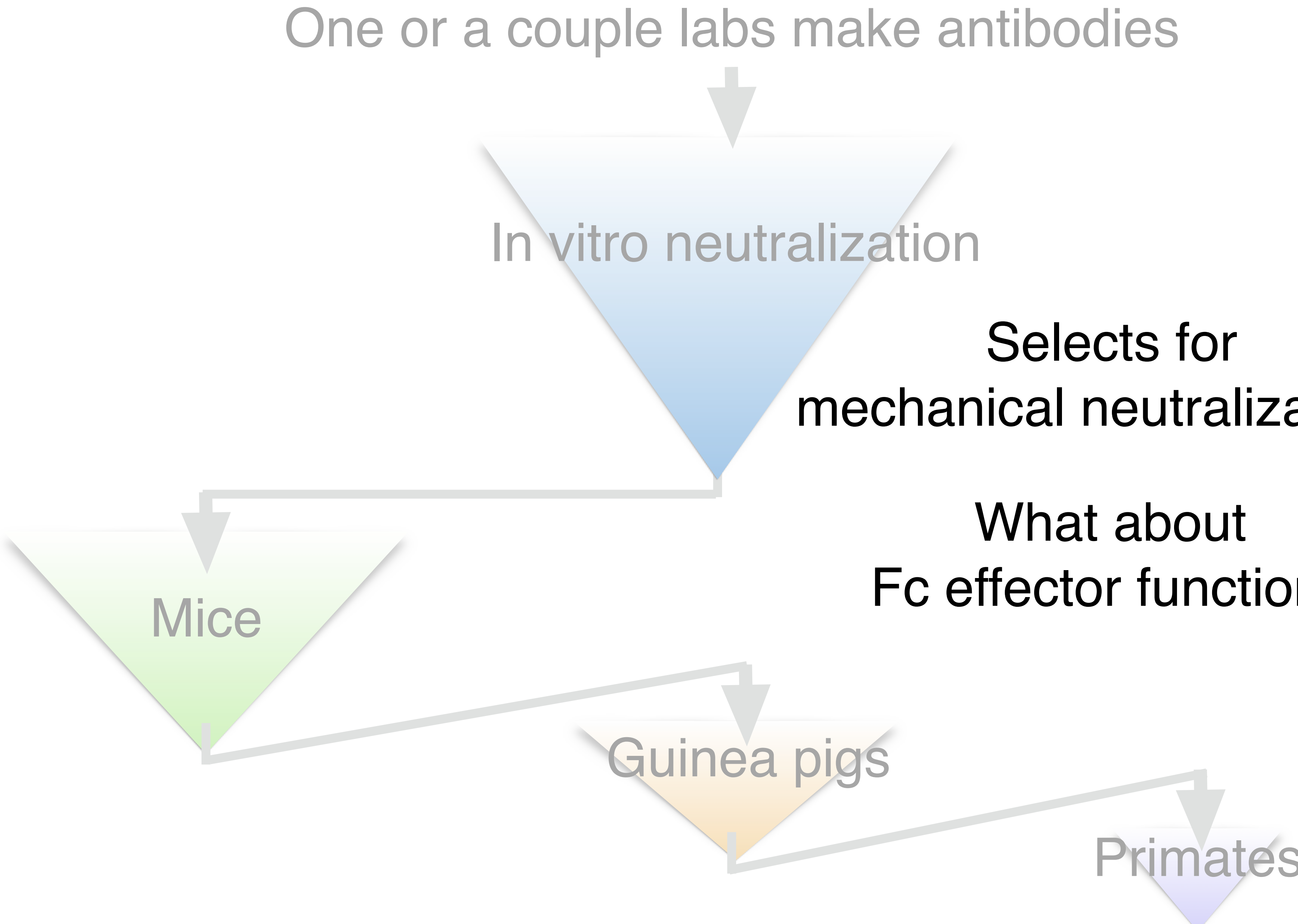
## Fc

phagocytosis,  ADCC
complement deposition
degranulation/cytokine release
neutrophil activation
mucus trapping
TRIM21 killing
supply Ag to APC
epithelial barrier
B cell survival, regulation
neutrophil activation

# Typically:


One or a couple labs make antibodies
In vitro neutralization
Mice
Guinea pigs
Primates

# Typically:

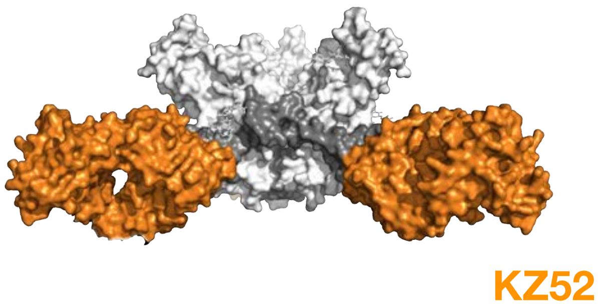


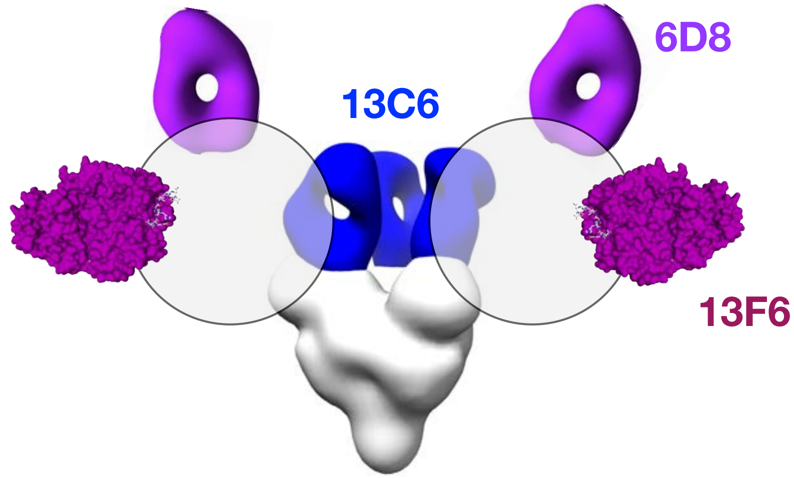

Selects for
mechanical neutralization?

What about
Fc effector function?

Neutralizes
Does not Protect

Protects
Does not Neutralize



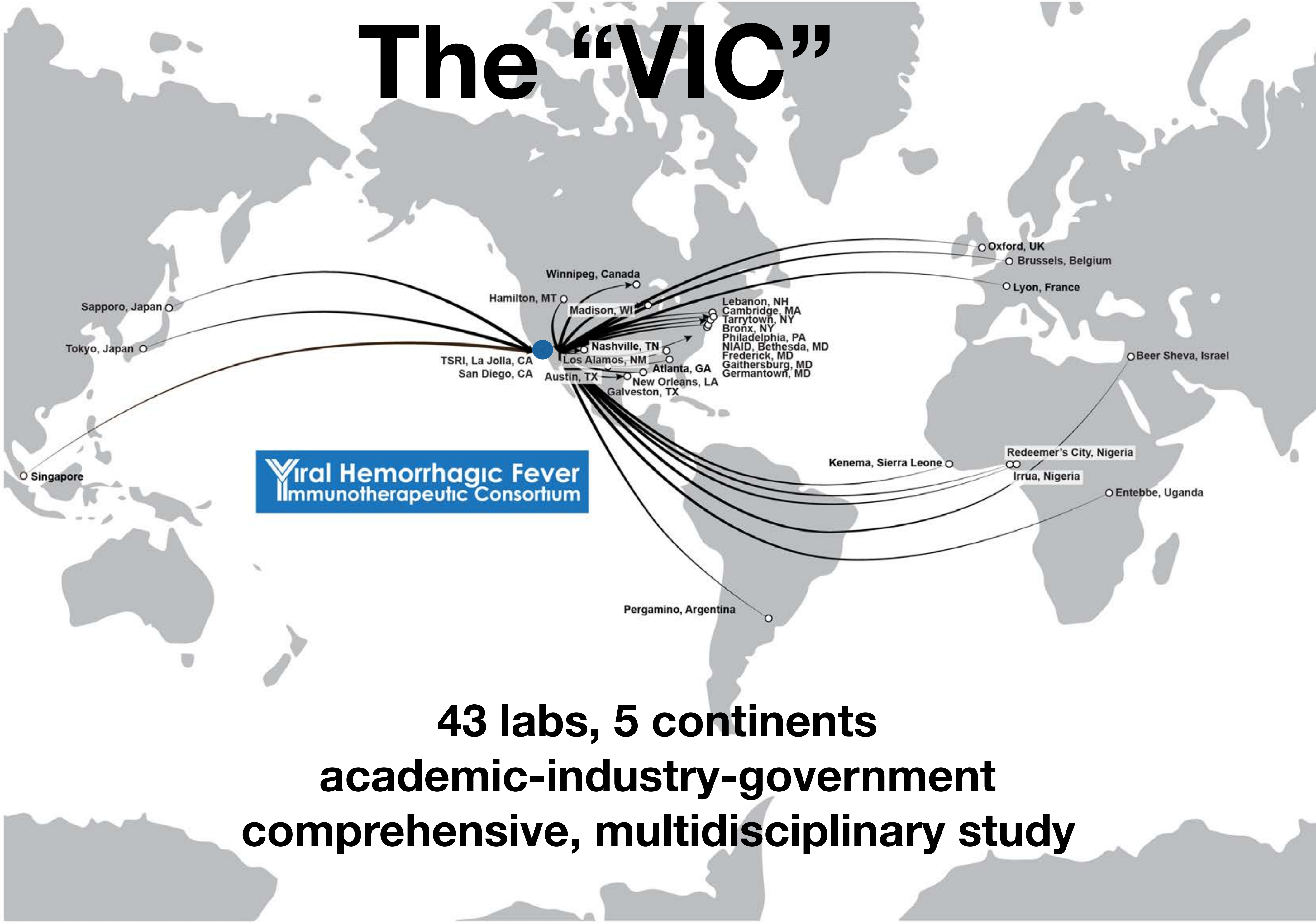




**KZ52 monotherapy**
CHO
50 mg/kg days -1, 4
**0% survival**

**MB-003 cocktail**
CHO or *Nicotiana*
50 mg/kg (CHO) or
16.7 mg/kg (*Nicotiana*) days 0,4,7
**50% (CHO) or 100% (*Nicotiana*) survival**

**Need to understand what leads to protection.**
**Need a statistically significant pool to do so.**

# The "VIC"

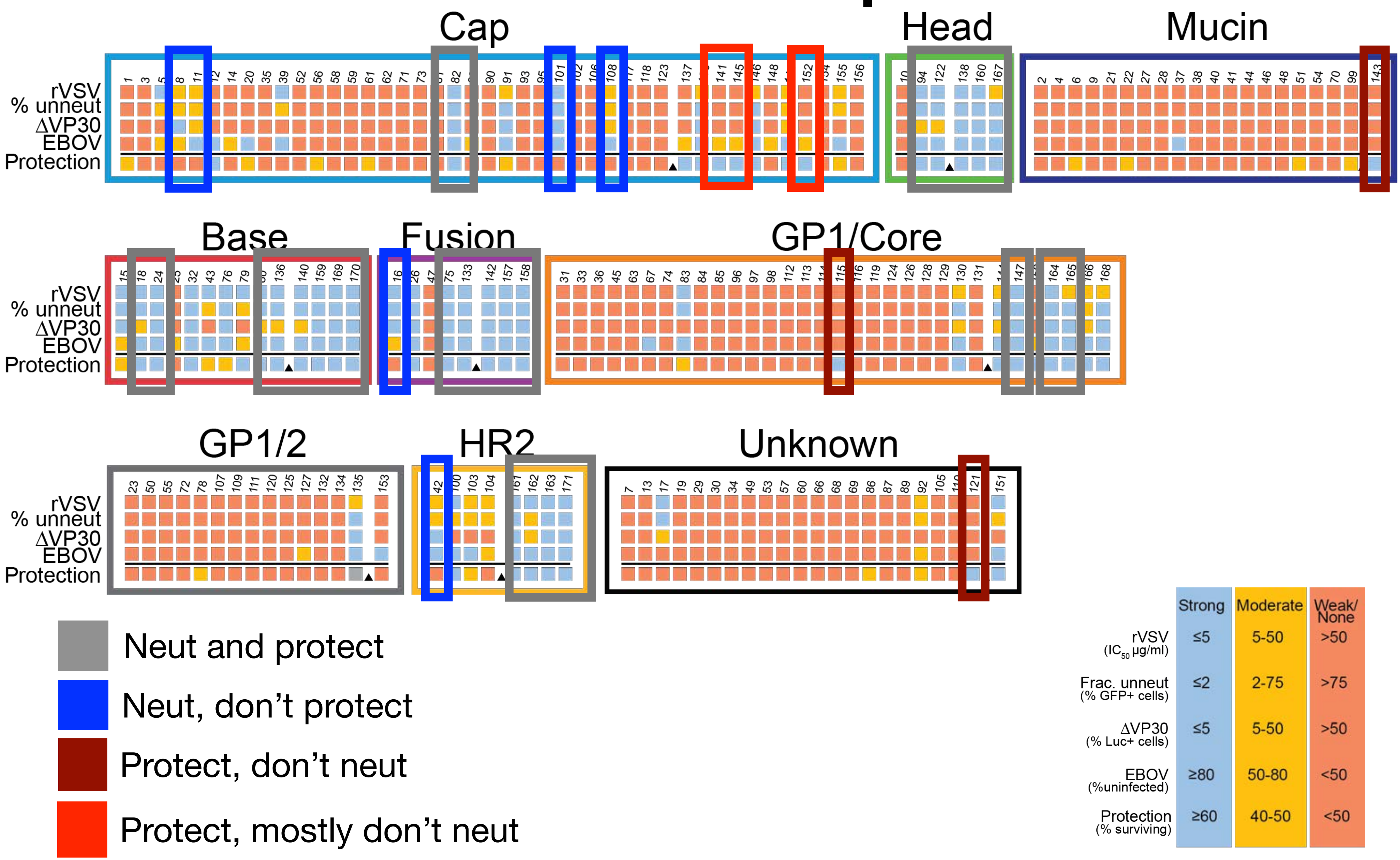



**43 labs, 5 continents**
**academic-industry-government**
**comprehensive, multidisciplinary study**

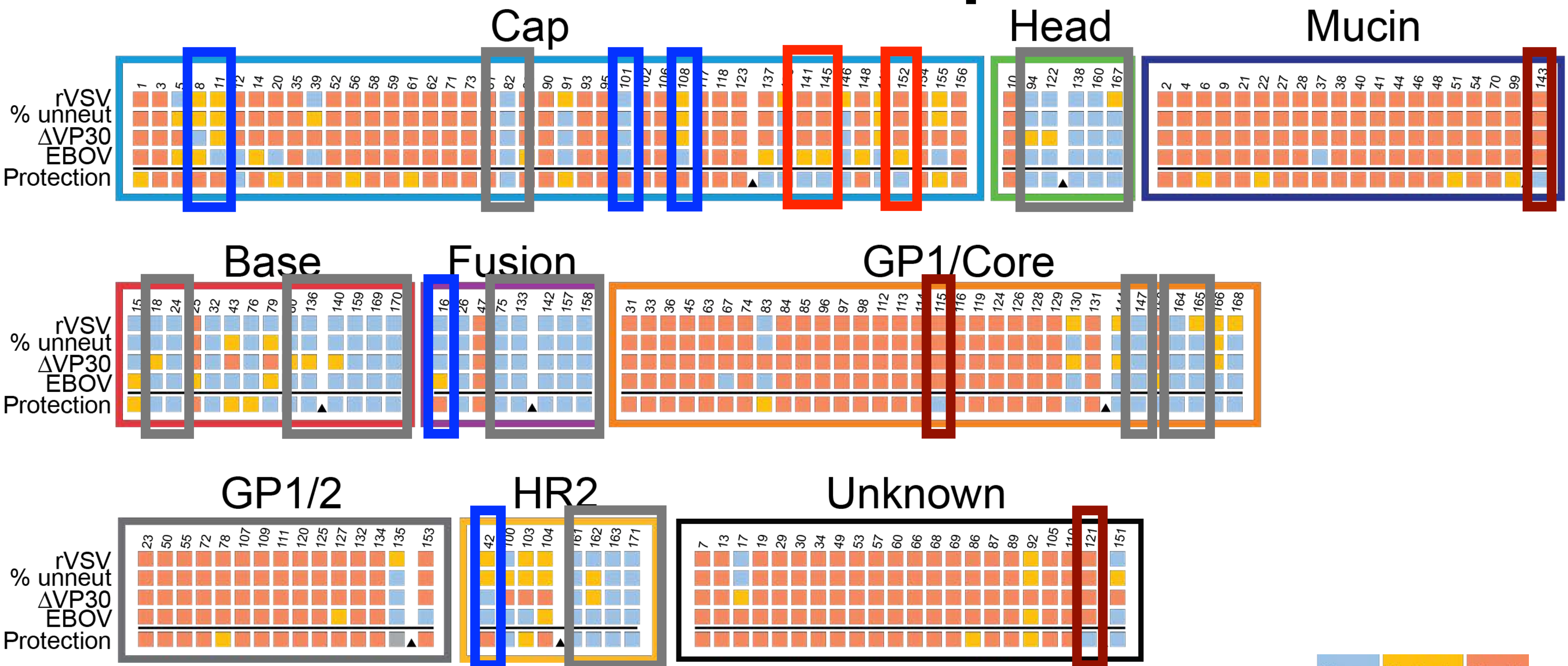
# Ebola virus project: 230 mAbs

- Species, Isotype
- Cross-reactivity
- Epitope
- Escape propensity and location
- GP and sGP binding affinity
- Neutralization (BSL-2, -3 and -4 systems, + un-neutralized fraction )
- Protection in mouse model
- Immune effector functions: Phagocytosis, CD107a, MIP-1β, IFNγ, human/murine, monocytes/macrophages
- Fc polyfunctionality
- Glycan modification (21 species)
- For a subset of antibodies:
    - Alanine scanning mutagenesis
    - Electron microscopy

# Relationship between neutralization and protection



# Relationship between neutralization and protection



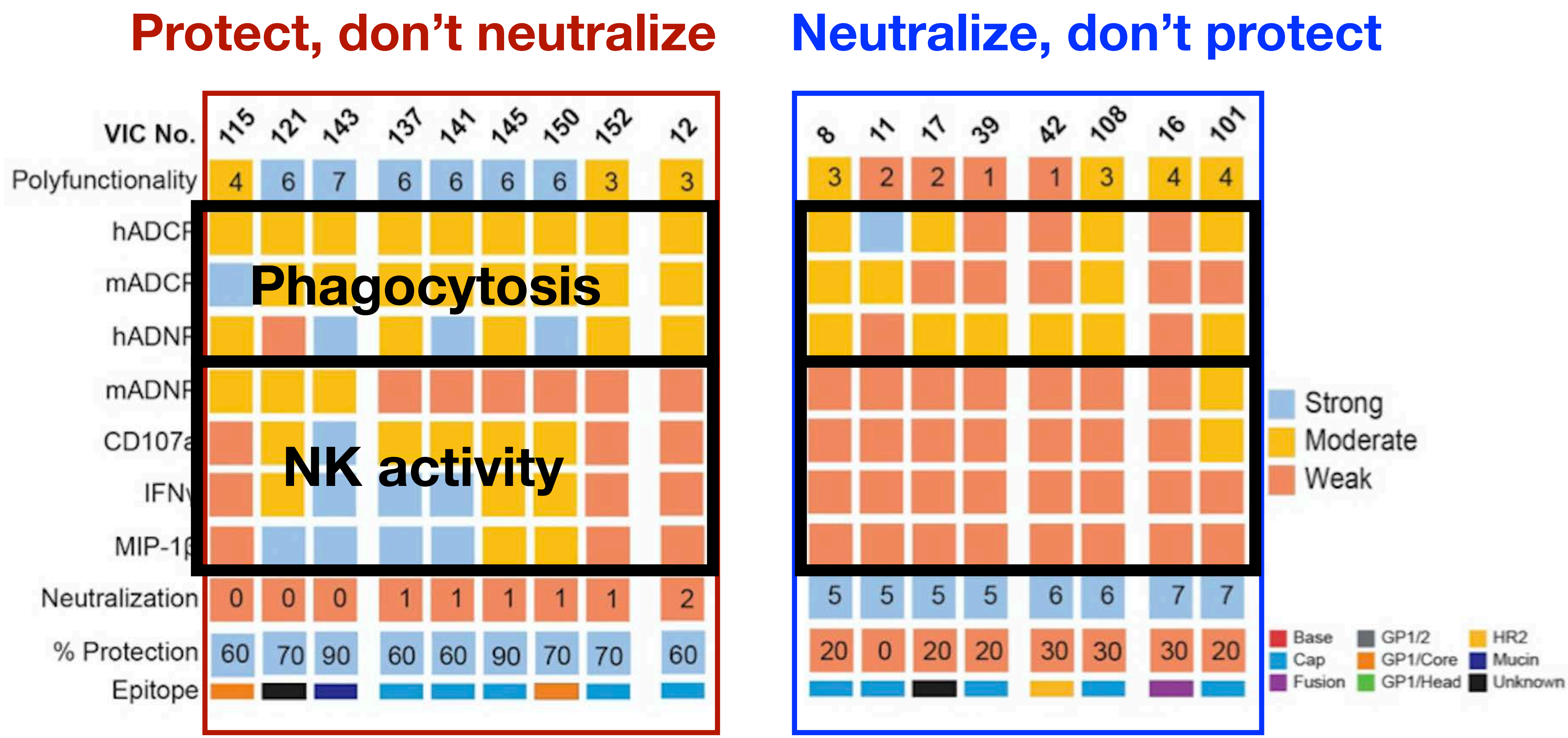

Neut and protect

Neut, don't protect

Protect, don't neut

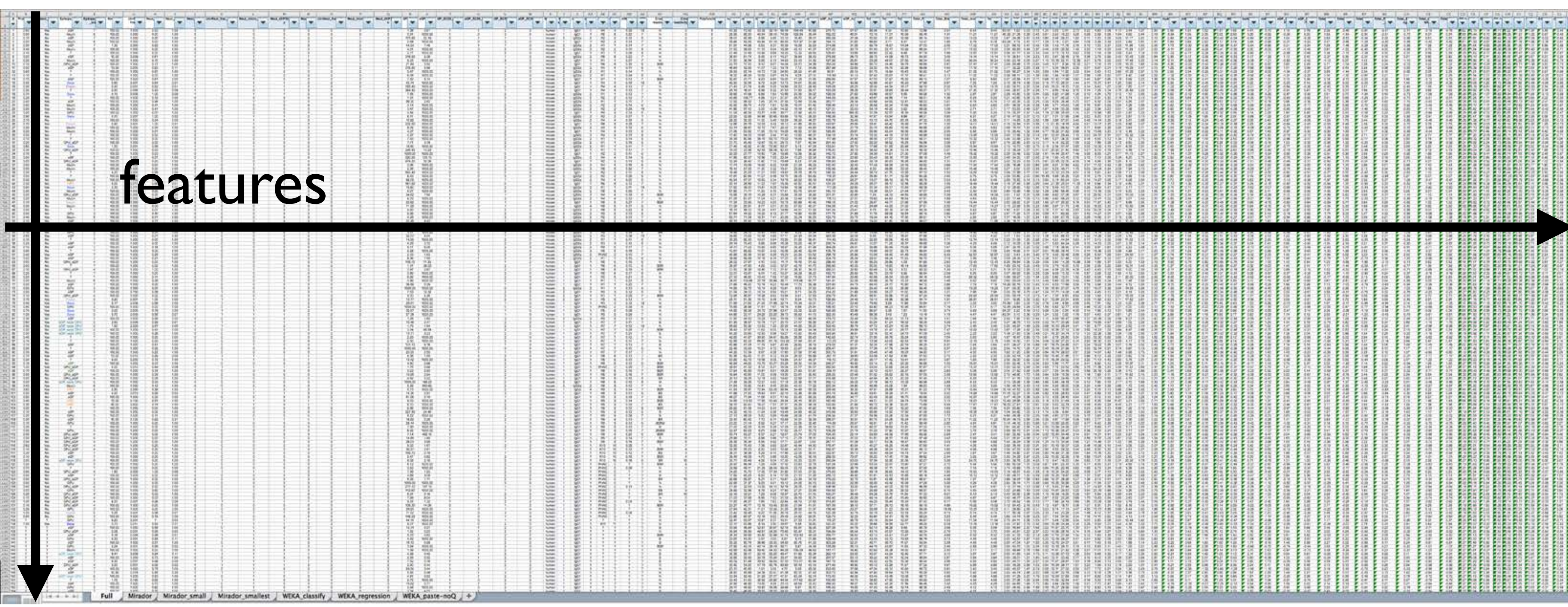
Protect, mostly don't neut

| | Strong | Moderate | Weak/ None |
|---|---|---|---|
| rVSV ($IC_{50}$ µg/ml) | ≤5 | 5-50 | >50 |
| Frac. unneut (% GFP+ cells) | ≤2 | 2-75 | >75 |
| ΔVP30 (% Luc+ cells) | ≤5 | 5-50 | >50 |
| EBOV (%uninfected) | ≥80 | 50-80 | <50 |
| Protection (% surviving) | ≥60 | 40-50 | <50 |

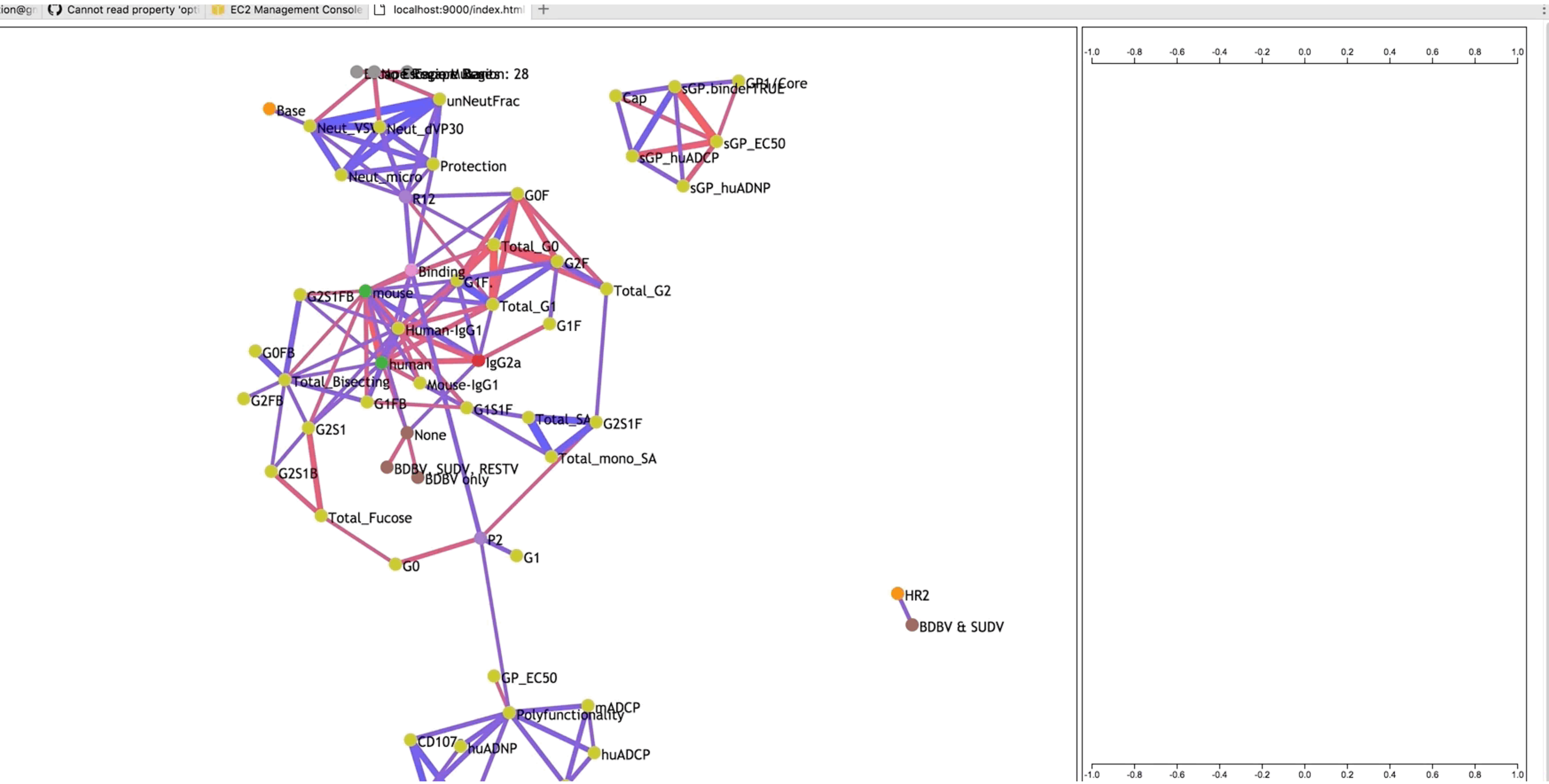

Protect, don't neutralize
Neutralize, don't protect
VIC No.
115
121
143
137
141
145
150
152
12
8
11
17
39
42
108
16
101
Polyfunctionality
hADCP
mADCP
hADNP
mADNP
CD107a
IFNγ
MIP-1β
Neutralization
% Protection
Epitope
Phagocytosis
NK activity
Strong
Moderate
Weak
Base
Cap
Fusion
GP1/2
GP1/Core
GP1/Head
HR2
Mucin
Unknown

# VIC dataset

antibodies

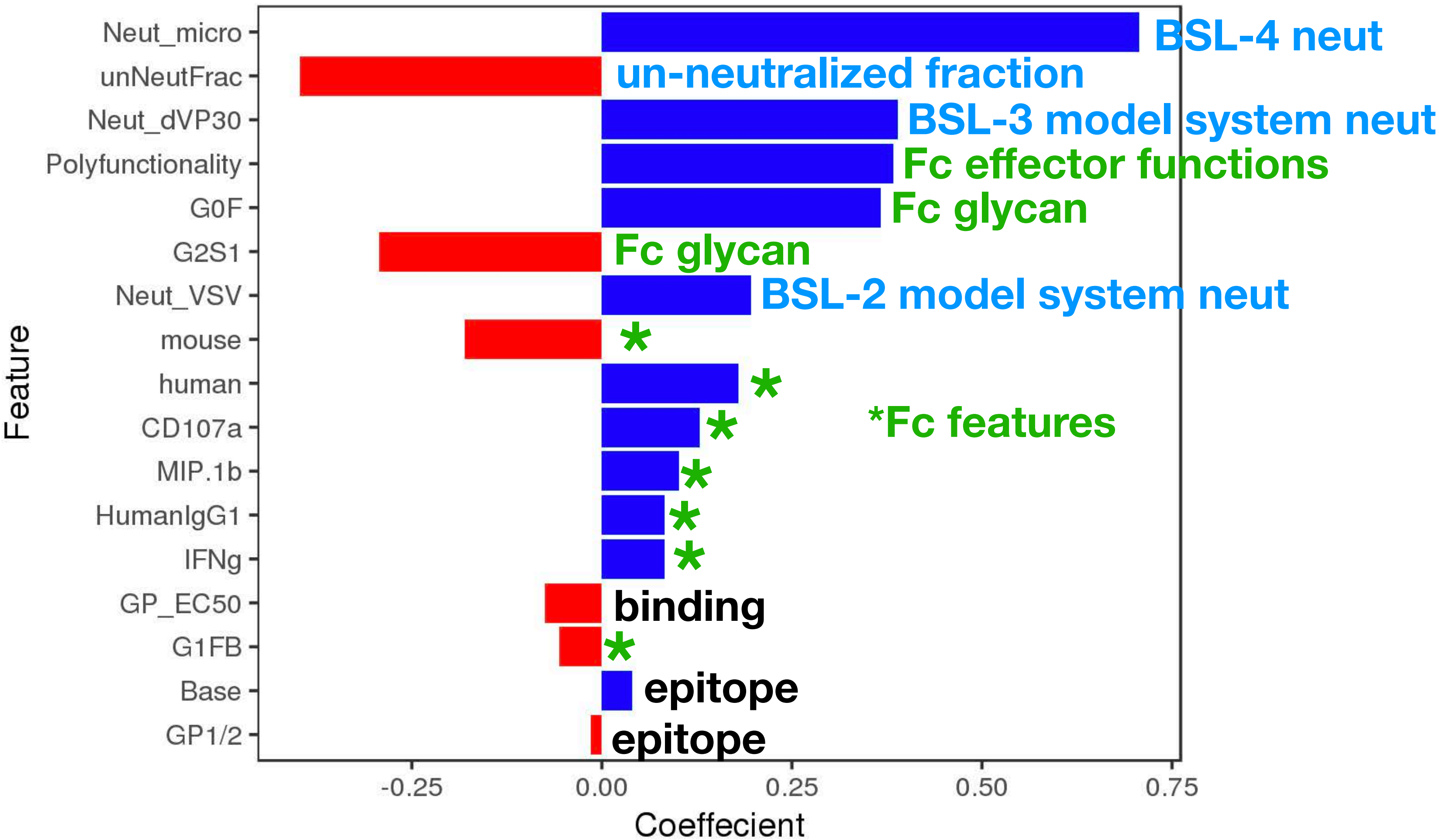



analysis:
Saphire, Schendel, Andersen, Gangavarapu at TSRI
Alter, Gunn, Yu at Ragon Korber, Wagh at LANL




**VIC Correlation Network: Karthik Gangavarapu, Kristian Andersen**

**Logistic regression: 17 features together predict protection (AUC 0.958)**

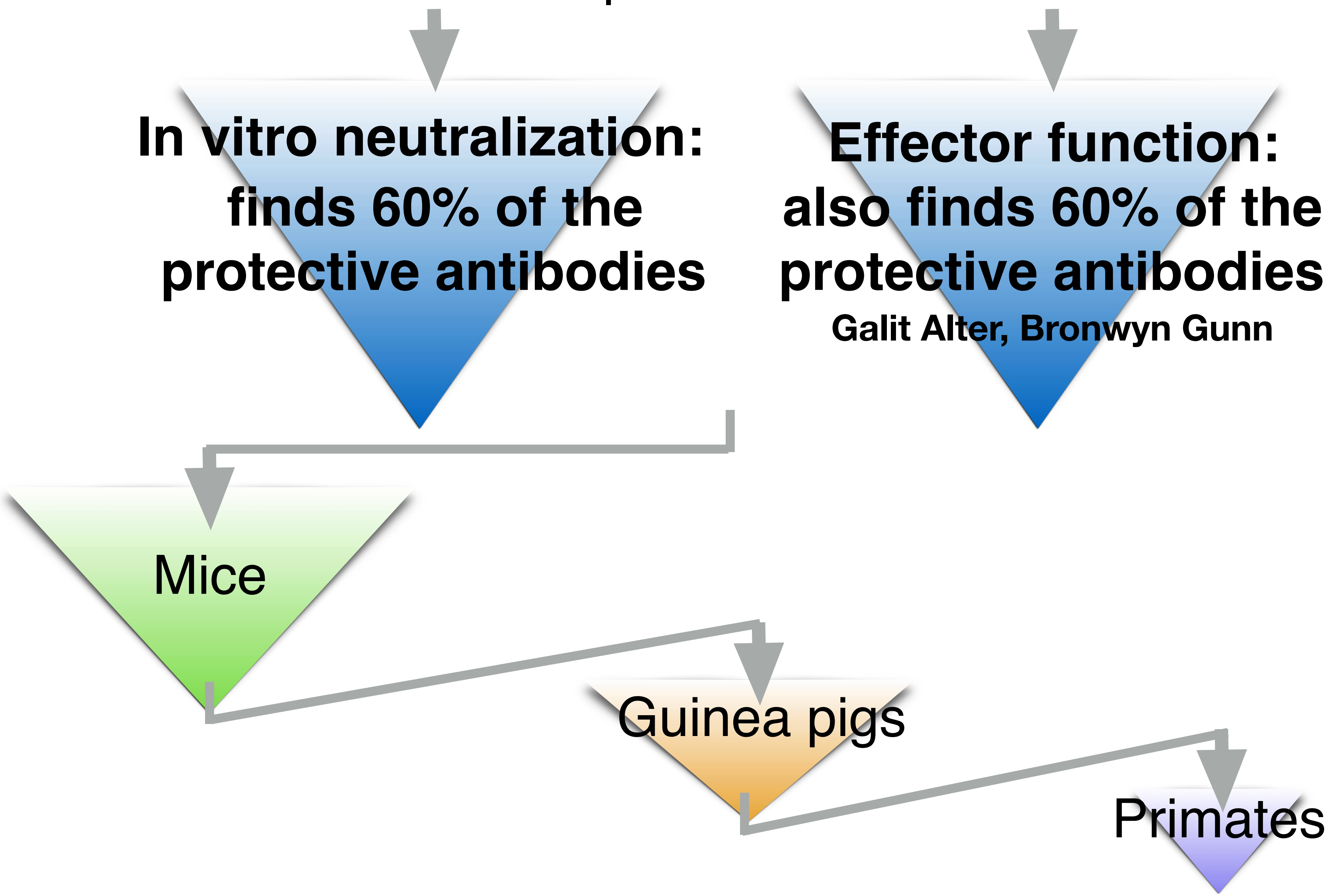



**Coefficients that weight individual features in the equation that predicts protection**

$$P = 1/1 + e^{-(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \ldots)}$$

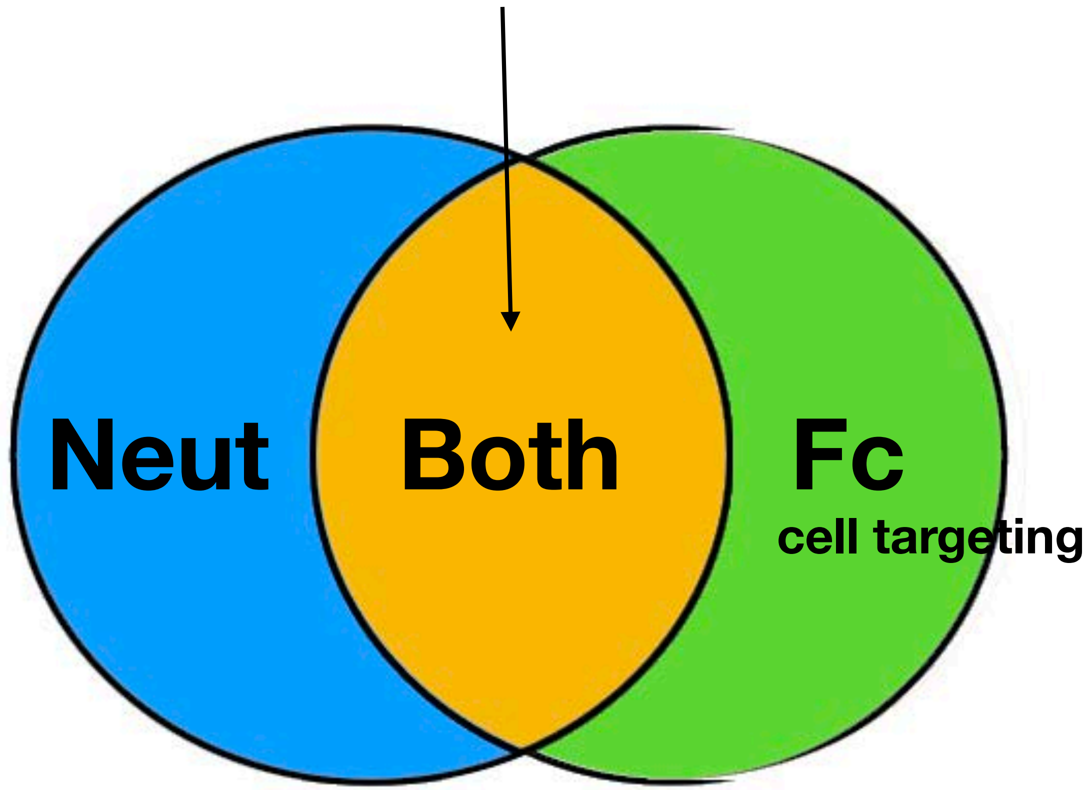
# Typically:

One or a couple labs make antibodies




***Nat. Immunol*, in press**

Sweet spot?




Better understand cell-targeting:
Improve immunotherapeutics
Steer/interpret vaccines
Natural infection

## Neutralization

**18% of sample pool**

**Expected**

## Immune effector

**5-10% of sample pool**

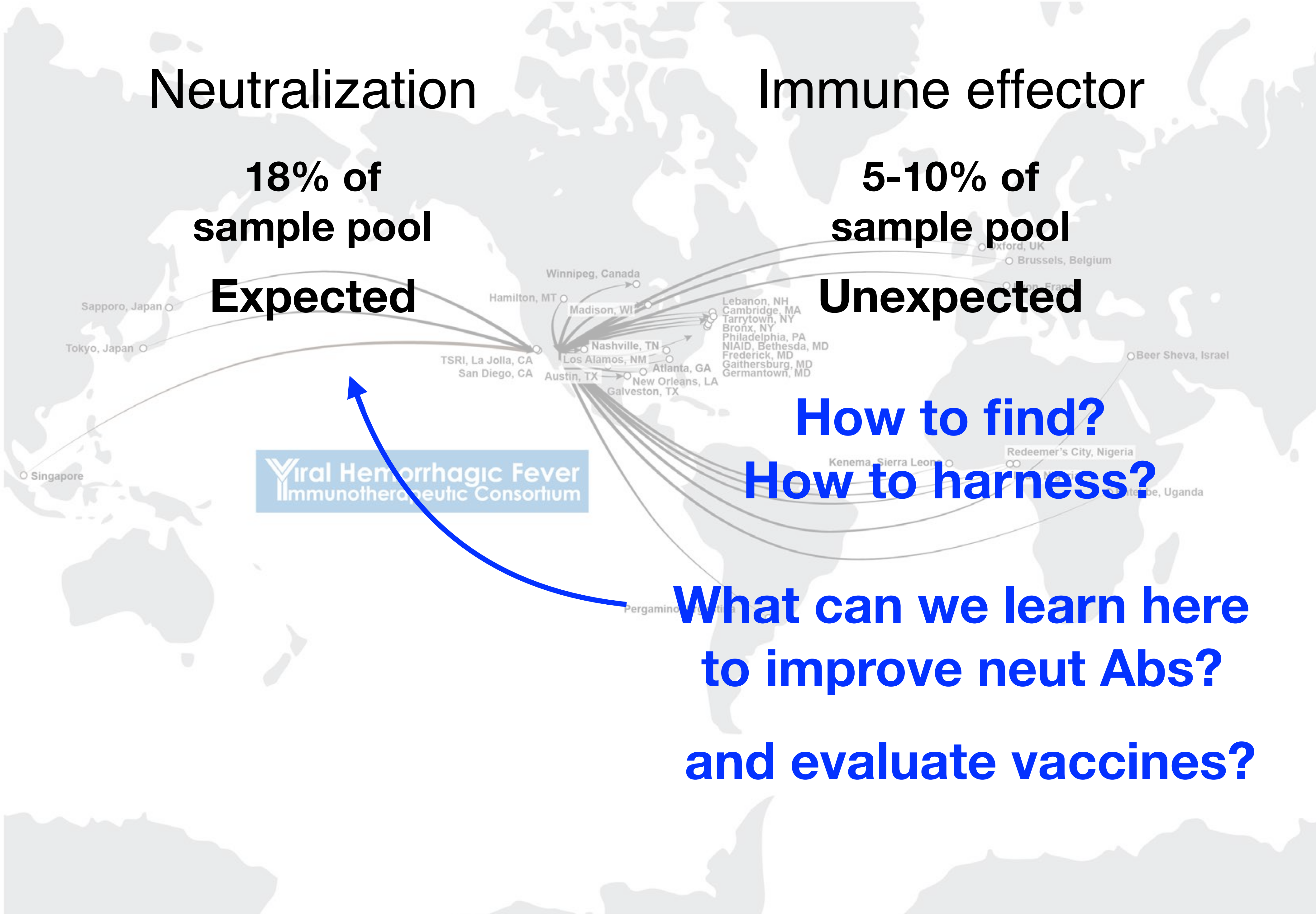
**Unexpected**

**How to find?**

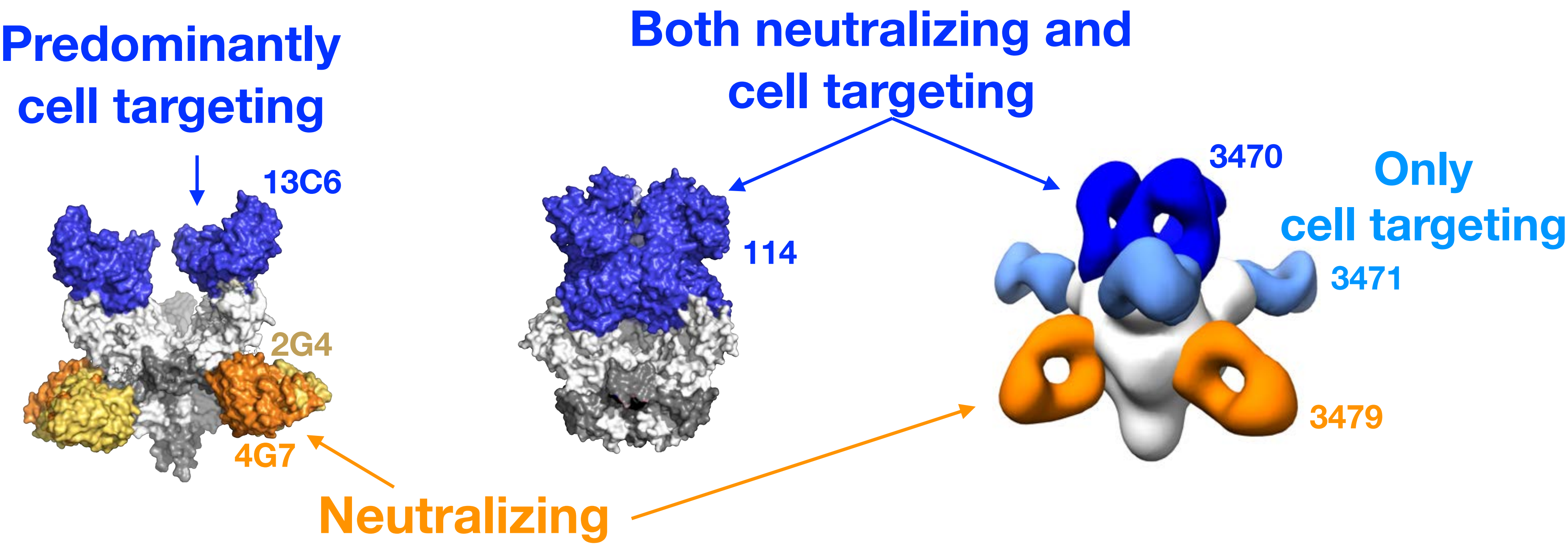
**How to harness?**

**What can we learn here to improve neut Abs?**

**and evaluate vaccines?**



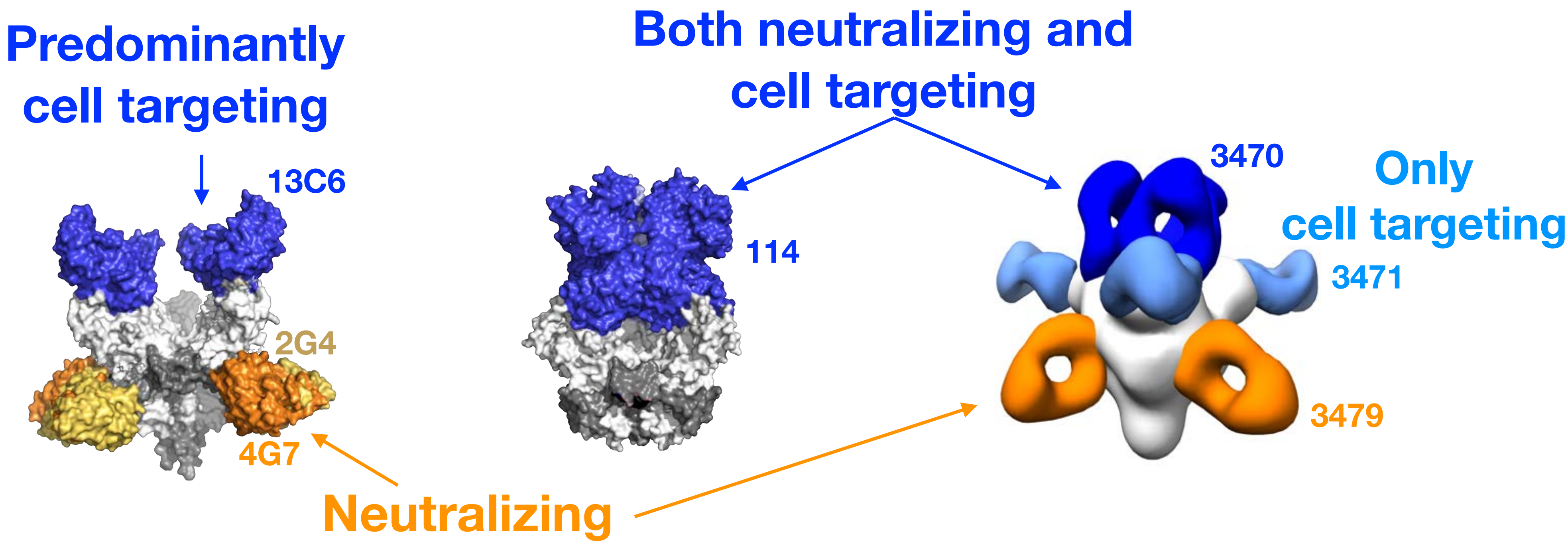
# 2018: WHO list for outbreaks in DRC




**ZMapp** **mAb 114** **REGN-EB3**

Saphire, *Nat. Immunol.*, 2018

# 2018: WHO list for outbreaks in DRC




**All of these active only against Ebola virus (Zaire)**

Saphire, *Nat. Immunol.*, 2018

# In the world, there are also…..

**Ebola virus**

**Bundibugyo virus**

**Sudan virus**

**Bombali virus**

**Taï Forest virus**

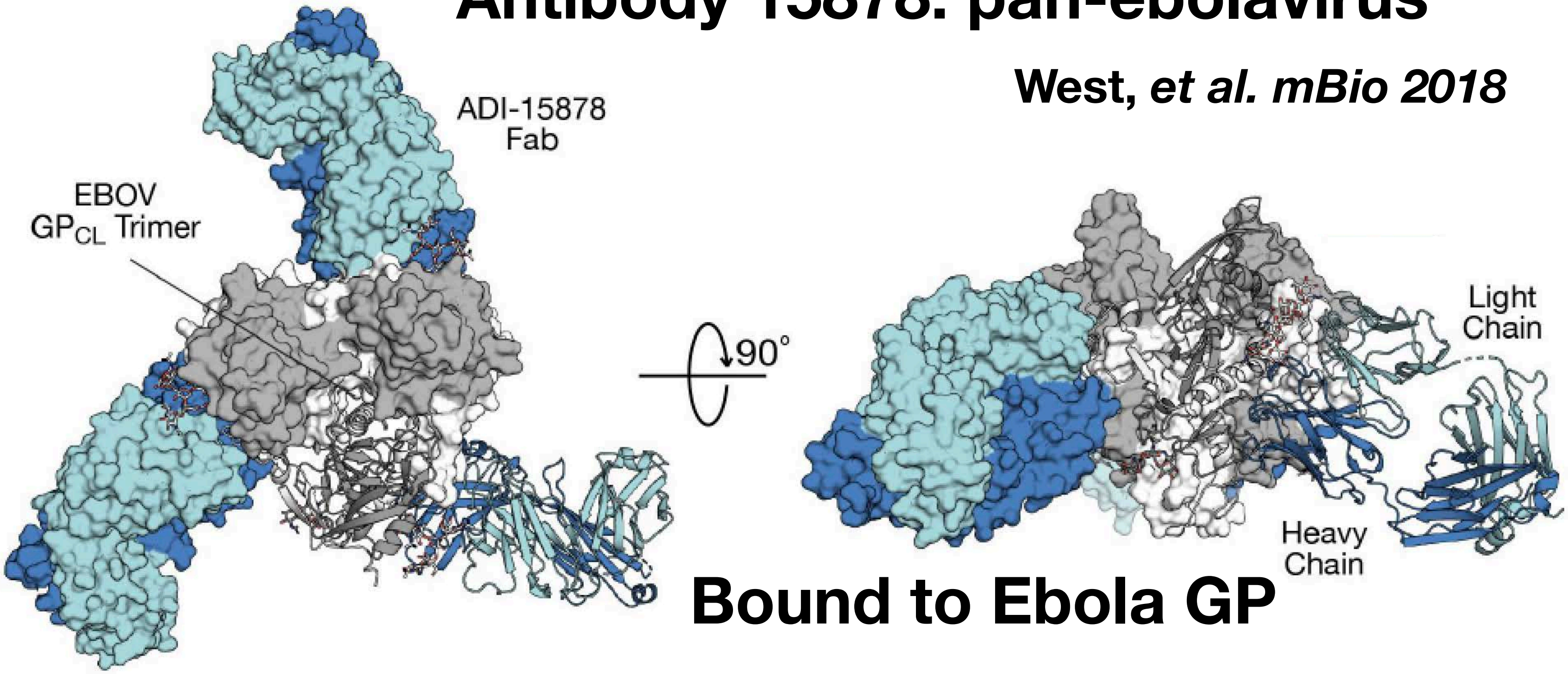
**Marburg virus**

**Cross reactivity would be ideal**

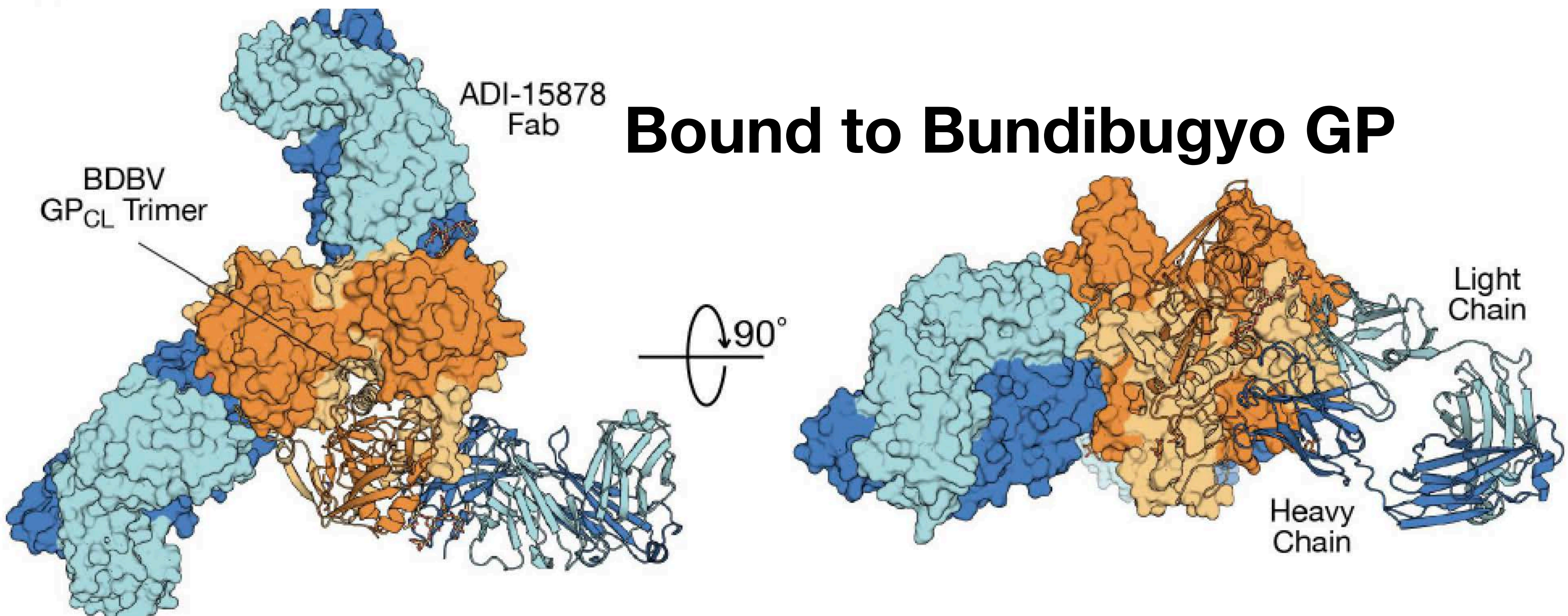
**Are there any antibodies that are broadly neutralizing?**

# Antibody 15878: pan-ebolavirus

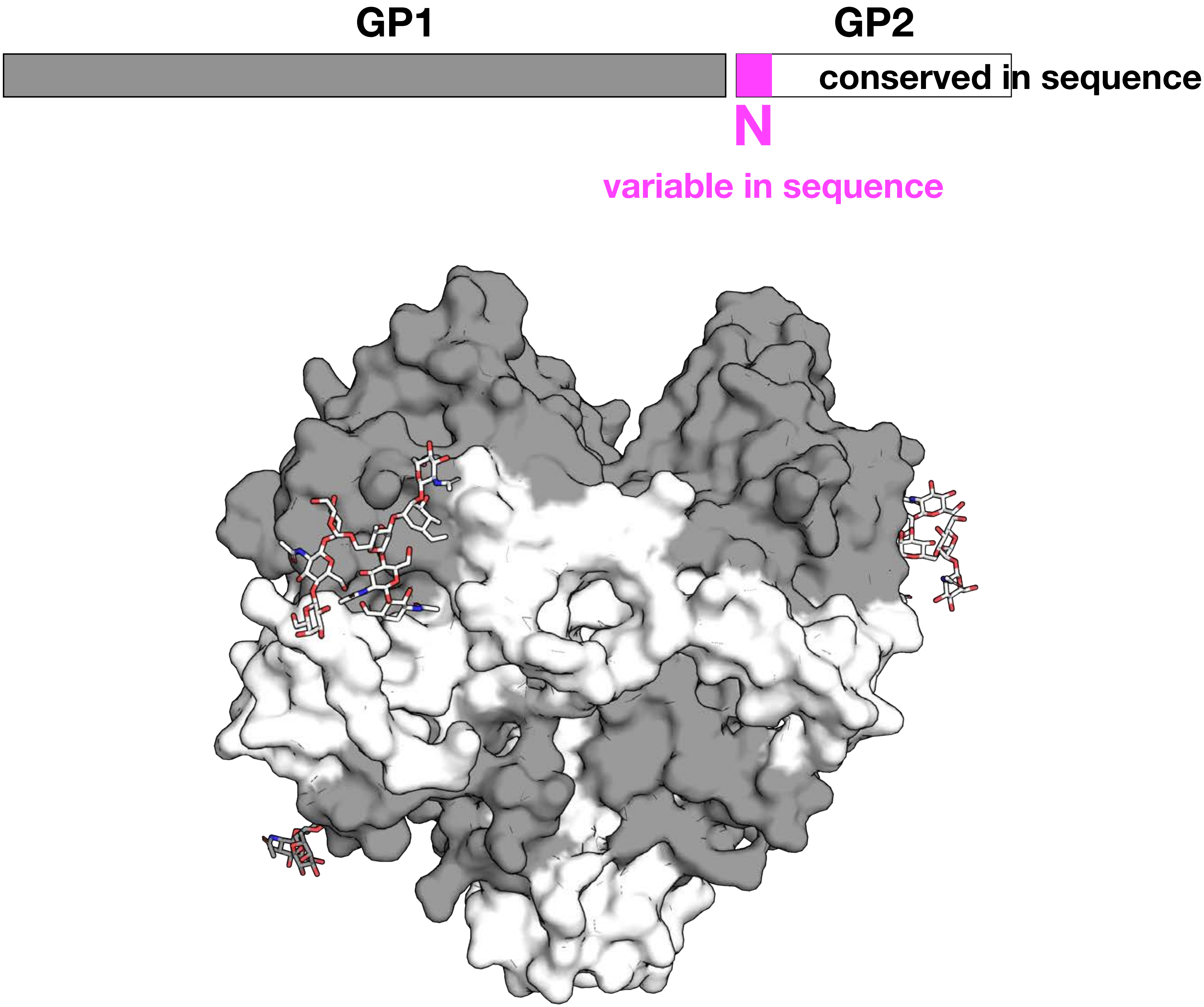
**West, *et al. mBio 2018***



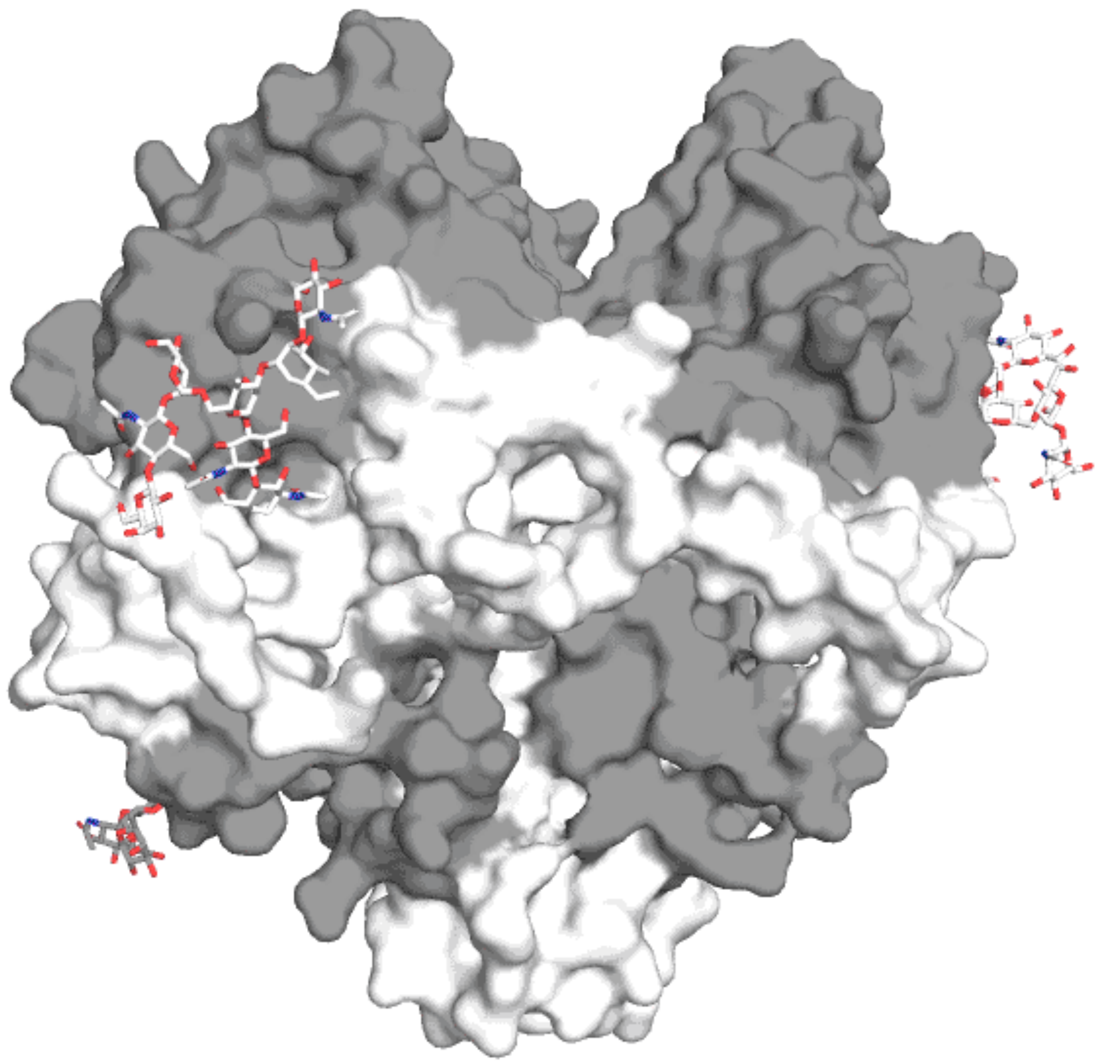

## Bound to Ebola GP




## Bound to Bundibugyo GP



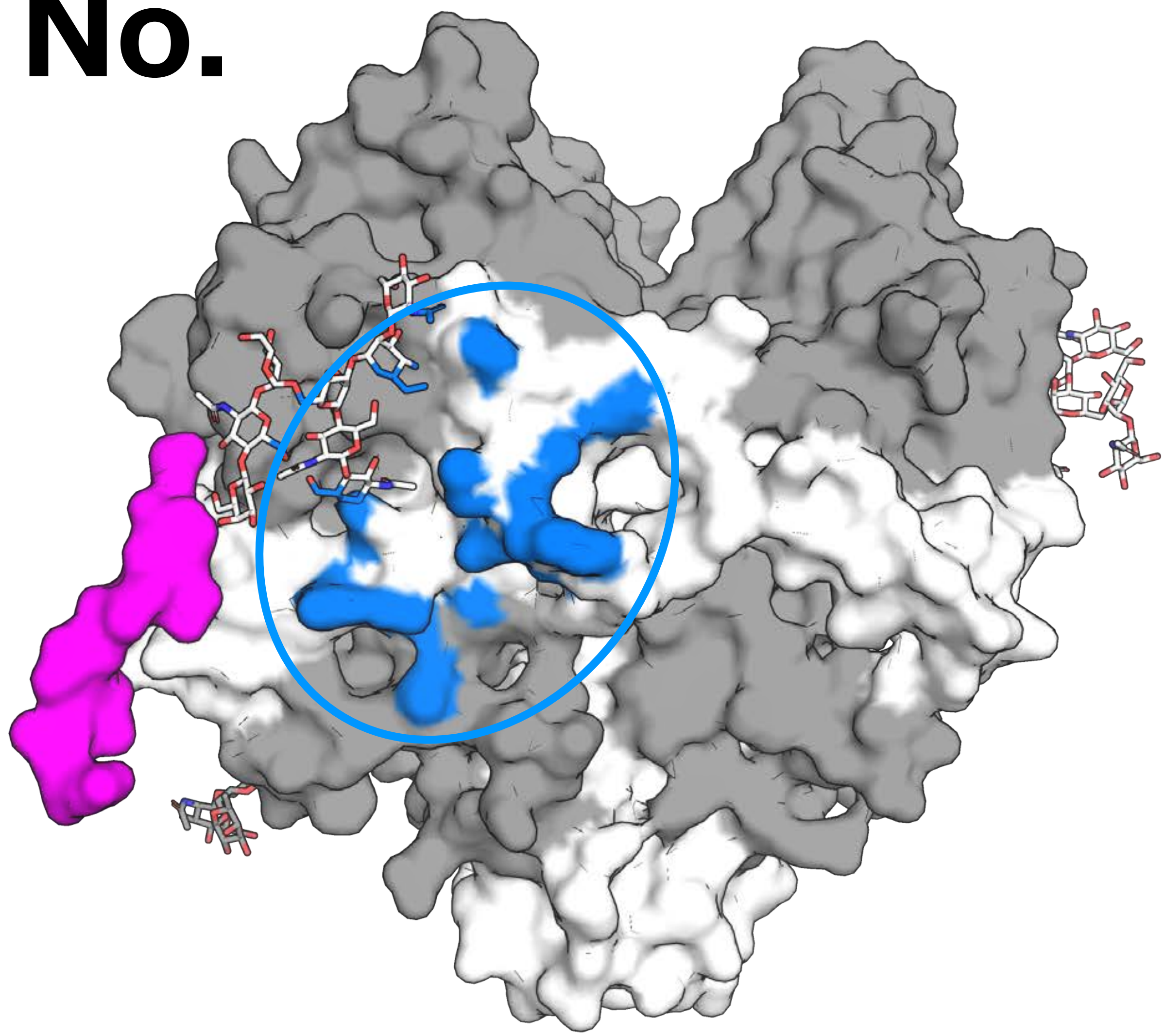

West, *et al. mBio 2018*



West, *et al. mBio 2018*

## Is 15878 unique?

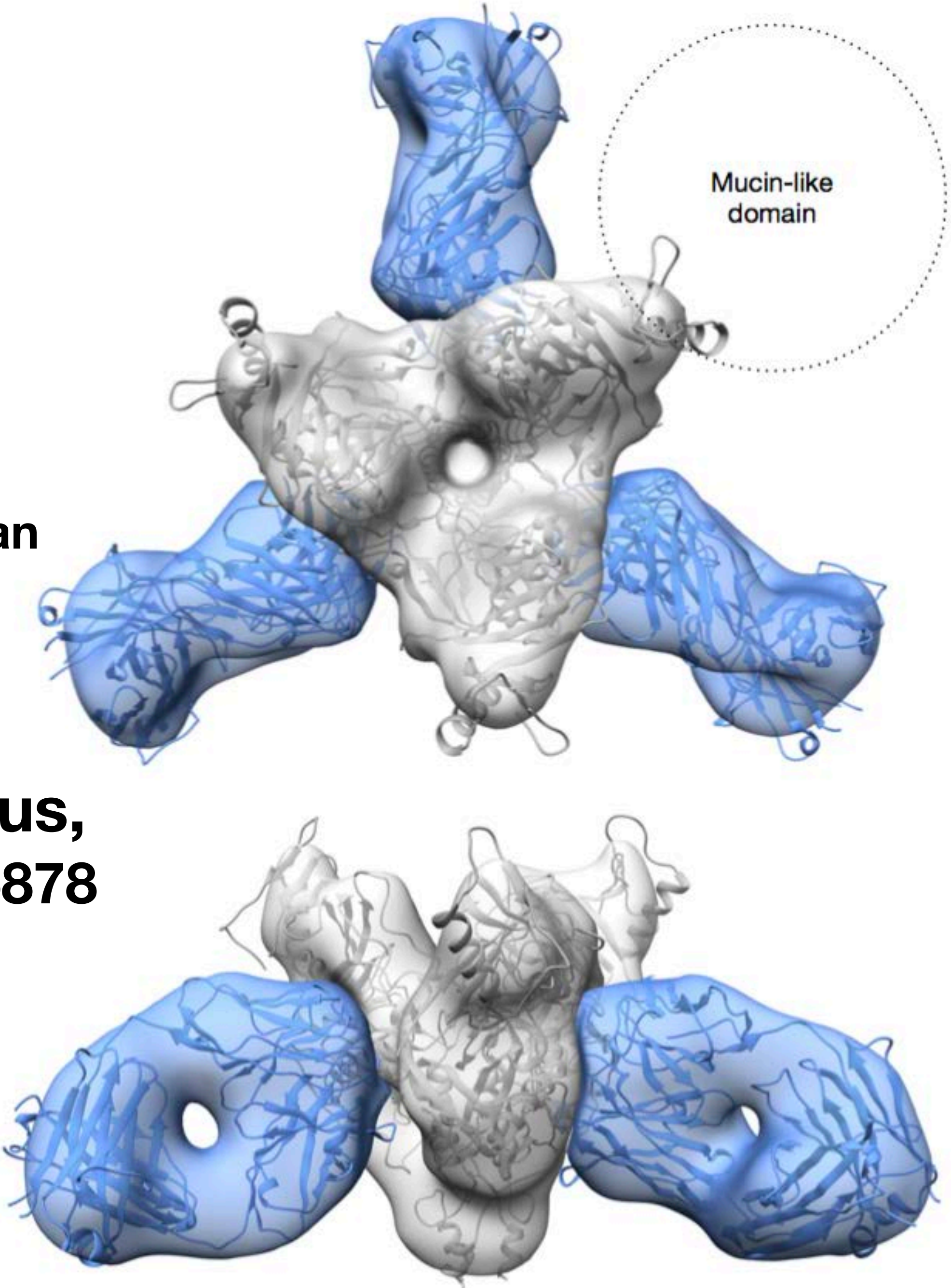
# No.



West, *et al. mBio 2018*

# 6D6

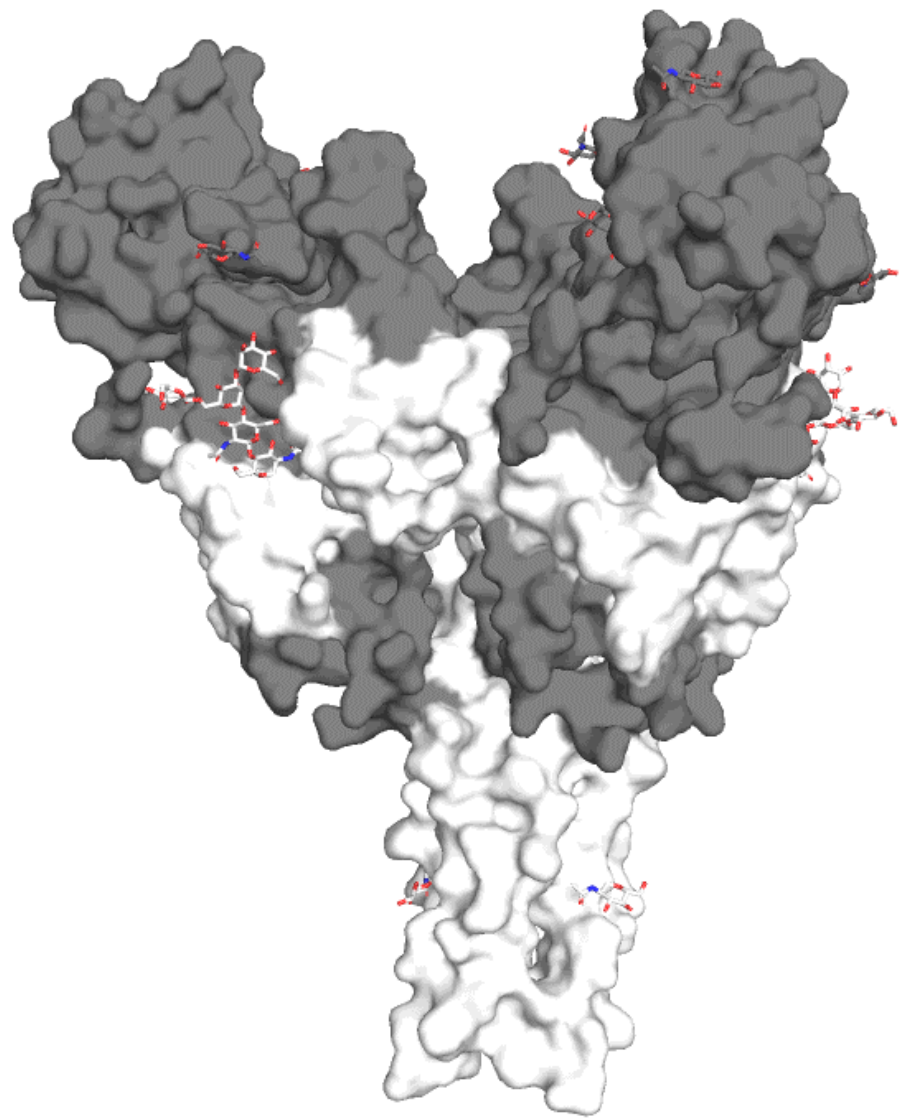
**(Takada, Hokkaido)**
**mouse immunized**
**with Ebola, then Sudan**



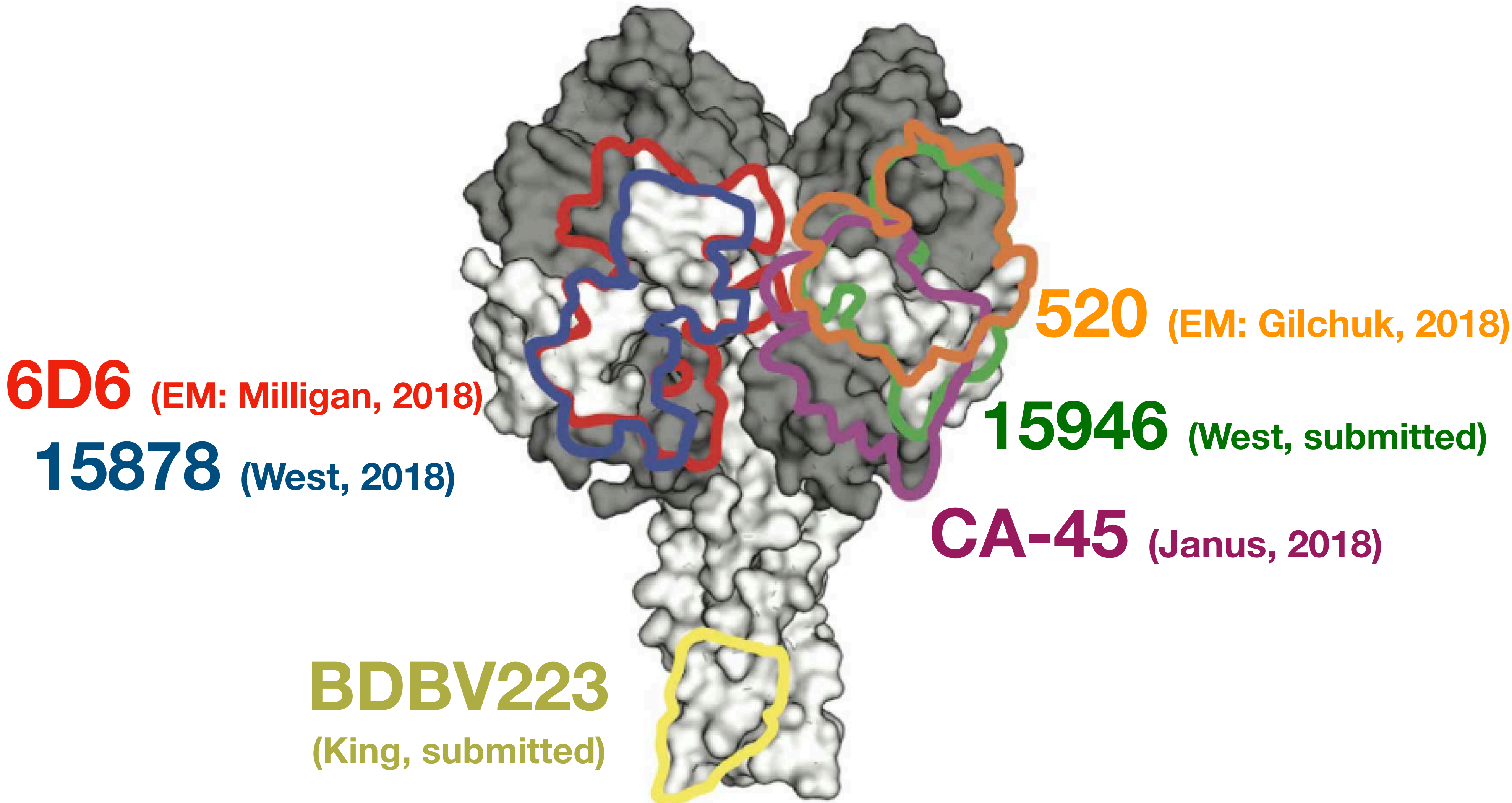

**also pan-ebolavirus,**
**Same epitope as 15878**

**Milligan, *et al. JID 2018***

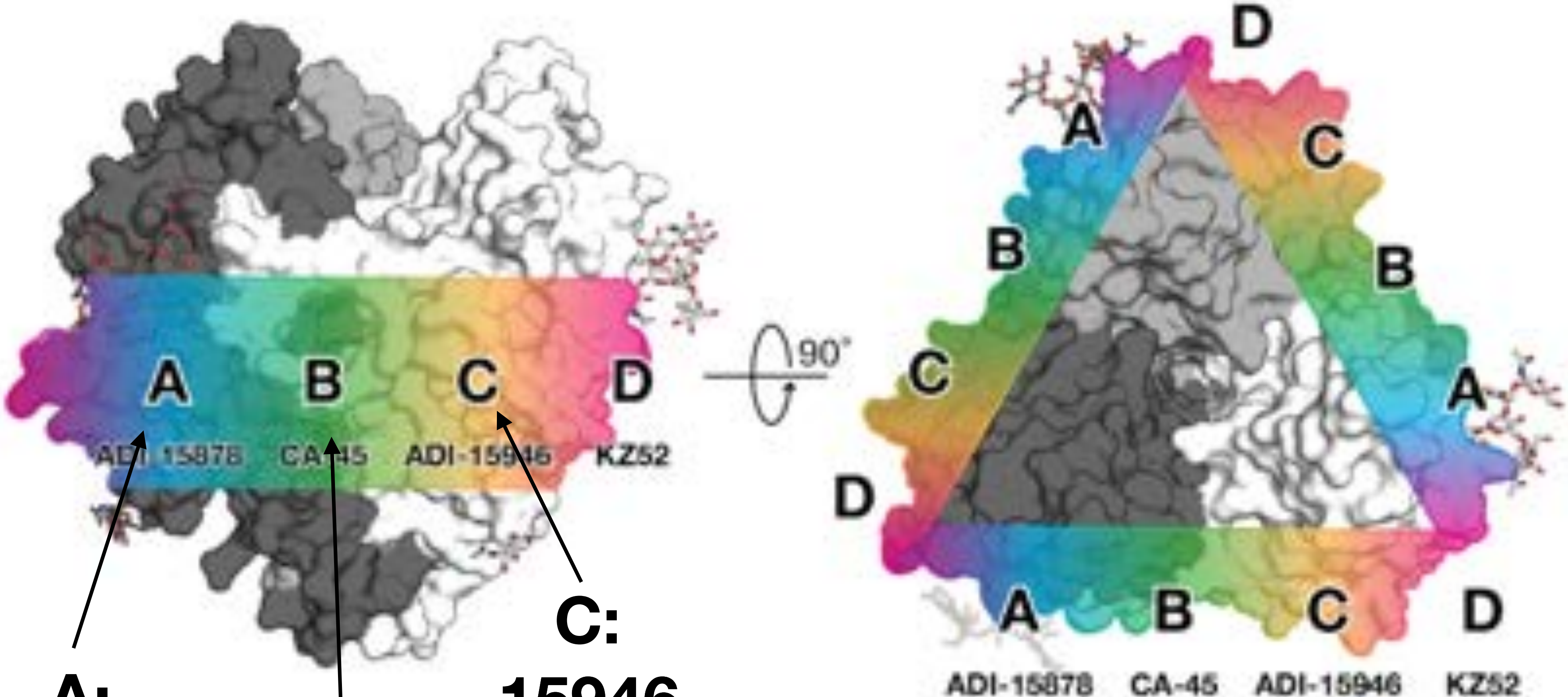
# Antibody 15946
# similar concept, different site



Brandyn West



6D6 (EM: Milligan, 2018)
15878 (West, 2018)
520 (EM: Gilchuk, 2018)
15946 (West, submitted)
CA-45 (Janus, 2018)
BDBV223
(King, submitted)



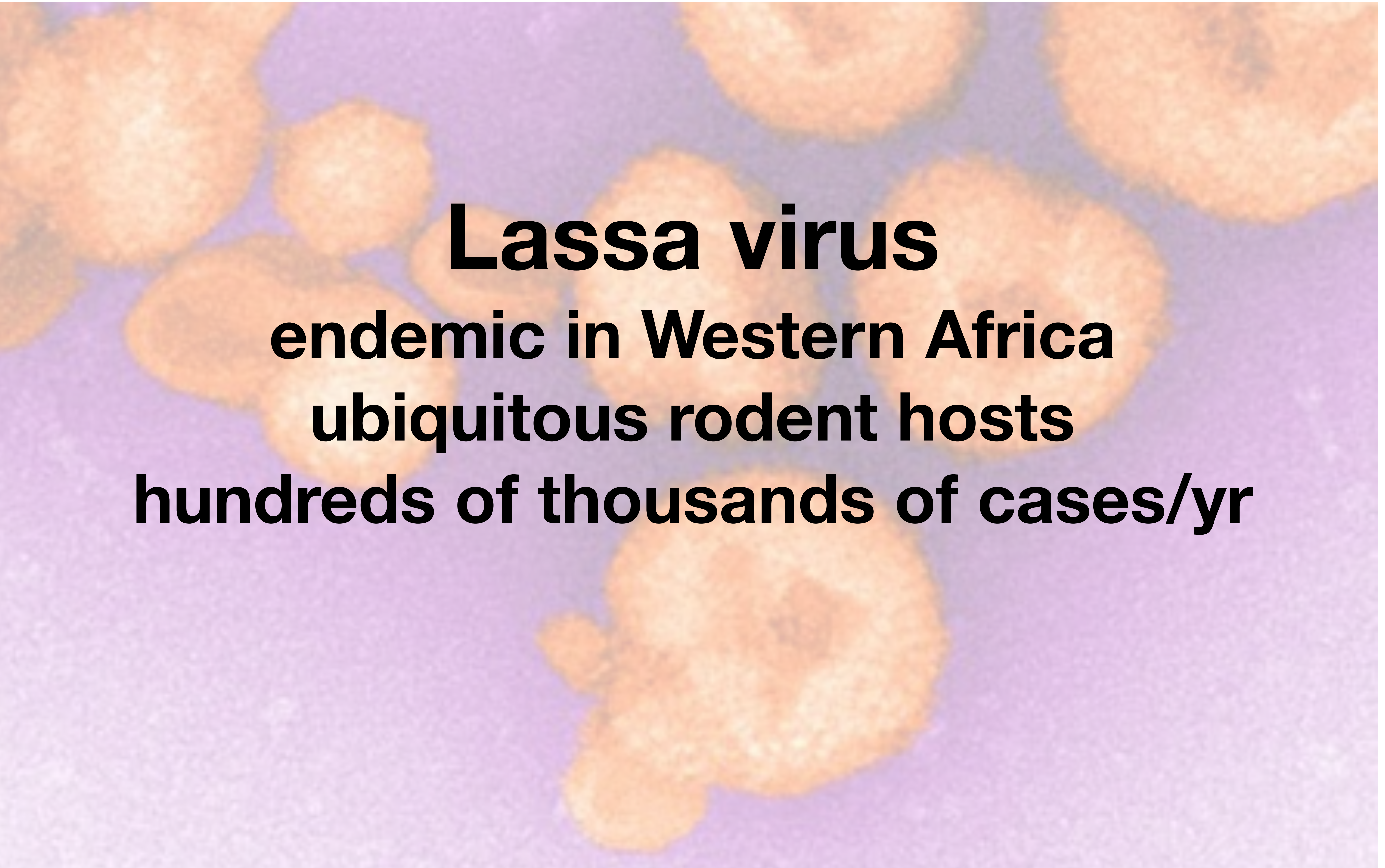

**A:**
**15878**
**6D6**

**B:**
**CA-45**

**C:**
**15946**
**520**

**“Waist” of GP -**
**broadly reactive antibodies**

# In the world, there is also…..



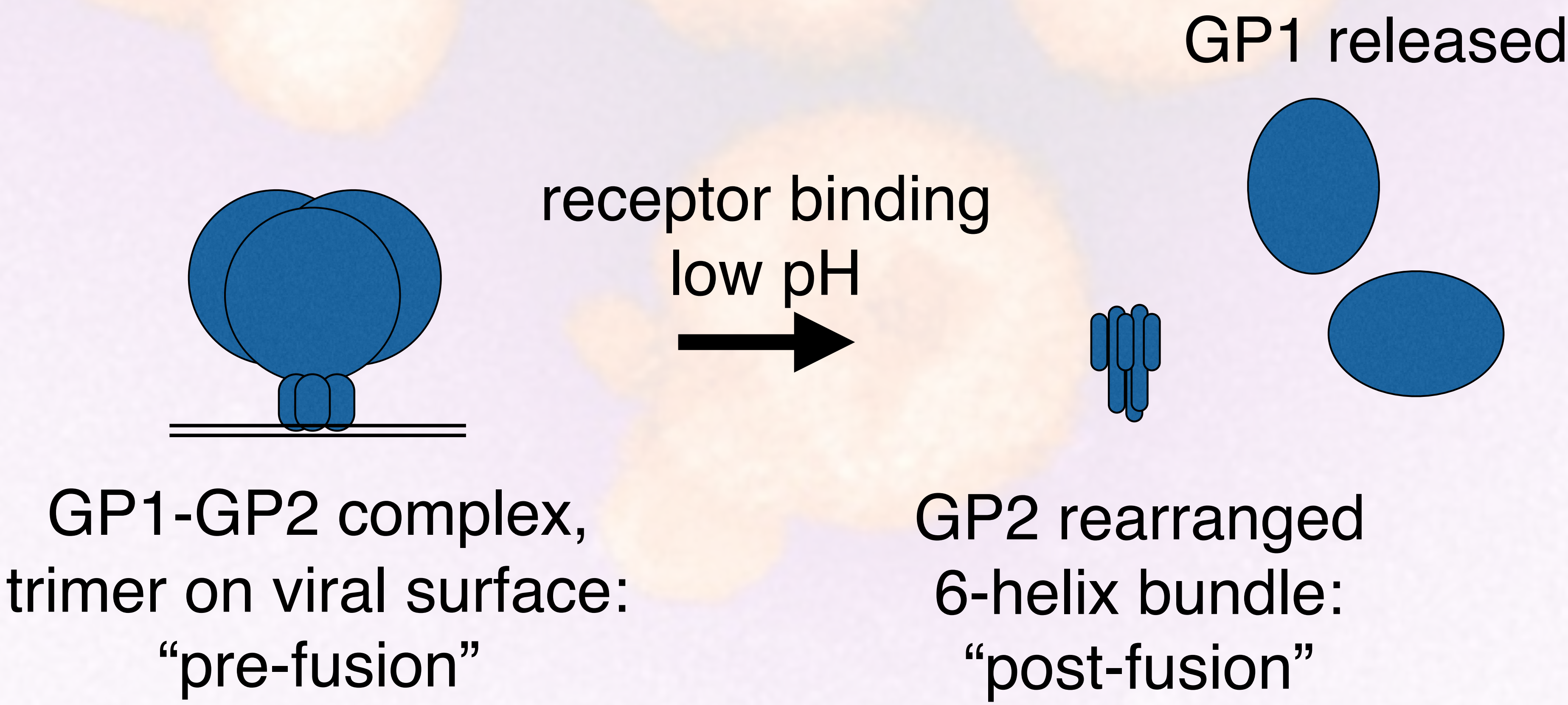
## Lassa virus

**endemic in Western Africa**

**ubiquitous rodent hosts**

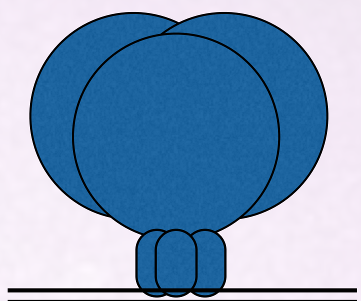
**hundreds of thousands of cases/yr**

# Lassa virus Glycoprotein GPC

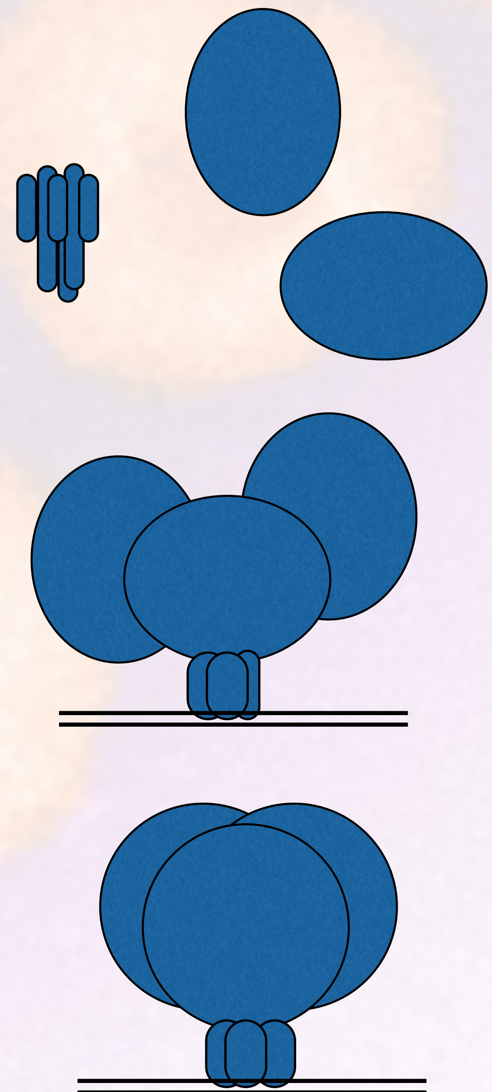


Range of conformers
from wild-type GPC

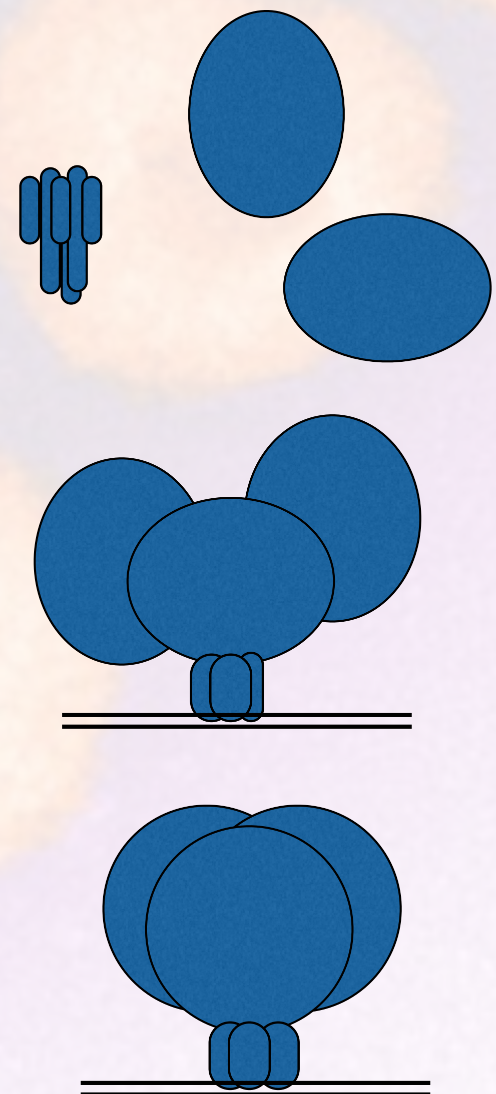
Unstable
May be presented rarely
Heavily glycosylated



GP1-GP2 complex,
trimer on viral surface:
“pre-fusion”



Range of conformers
from wild-type GPC

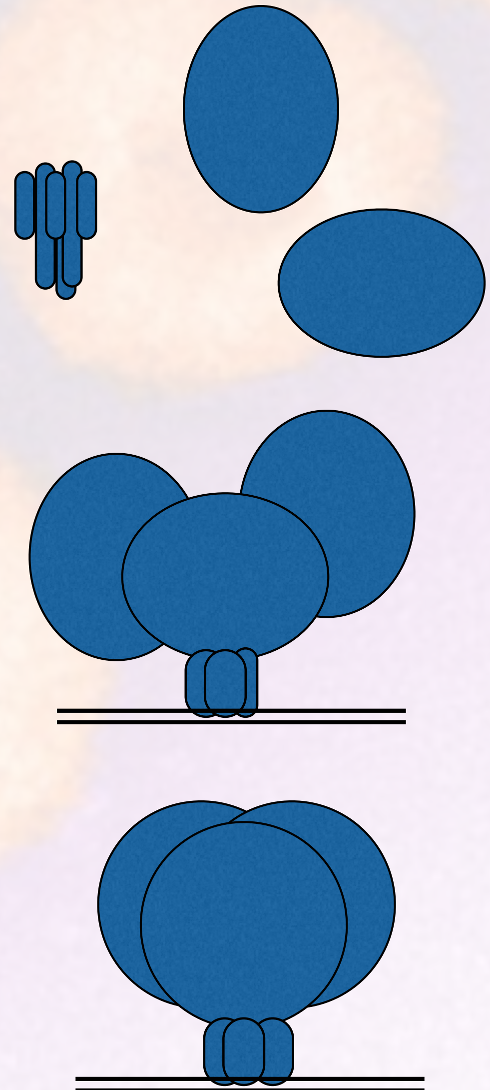


Antibodies from
with wild-type GPC
are elicited against all of these

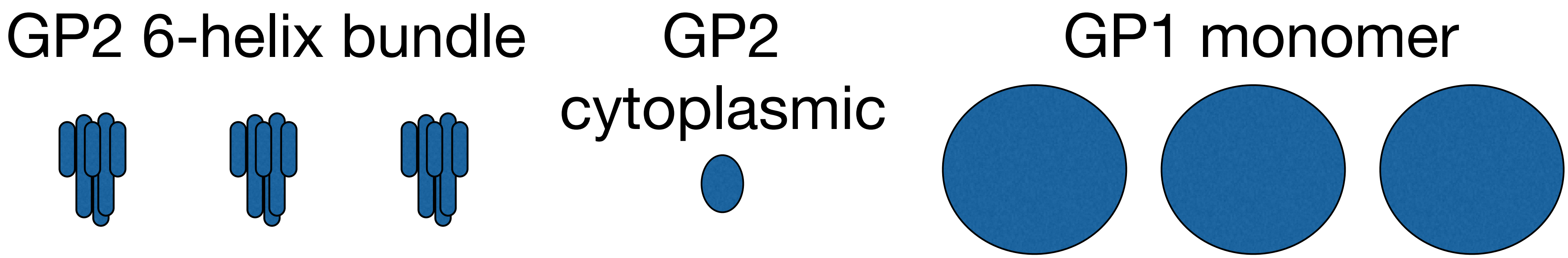
Neutralizing antibody rare
T cells current corr. of protection

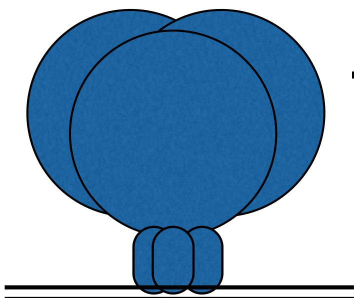
Range of conformers
from wild-type GPC

Instability, heavy glycosylation:
No structure of prefusion trimer



# < 2017: Structures Available

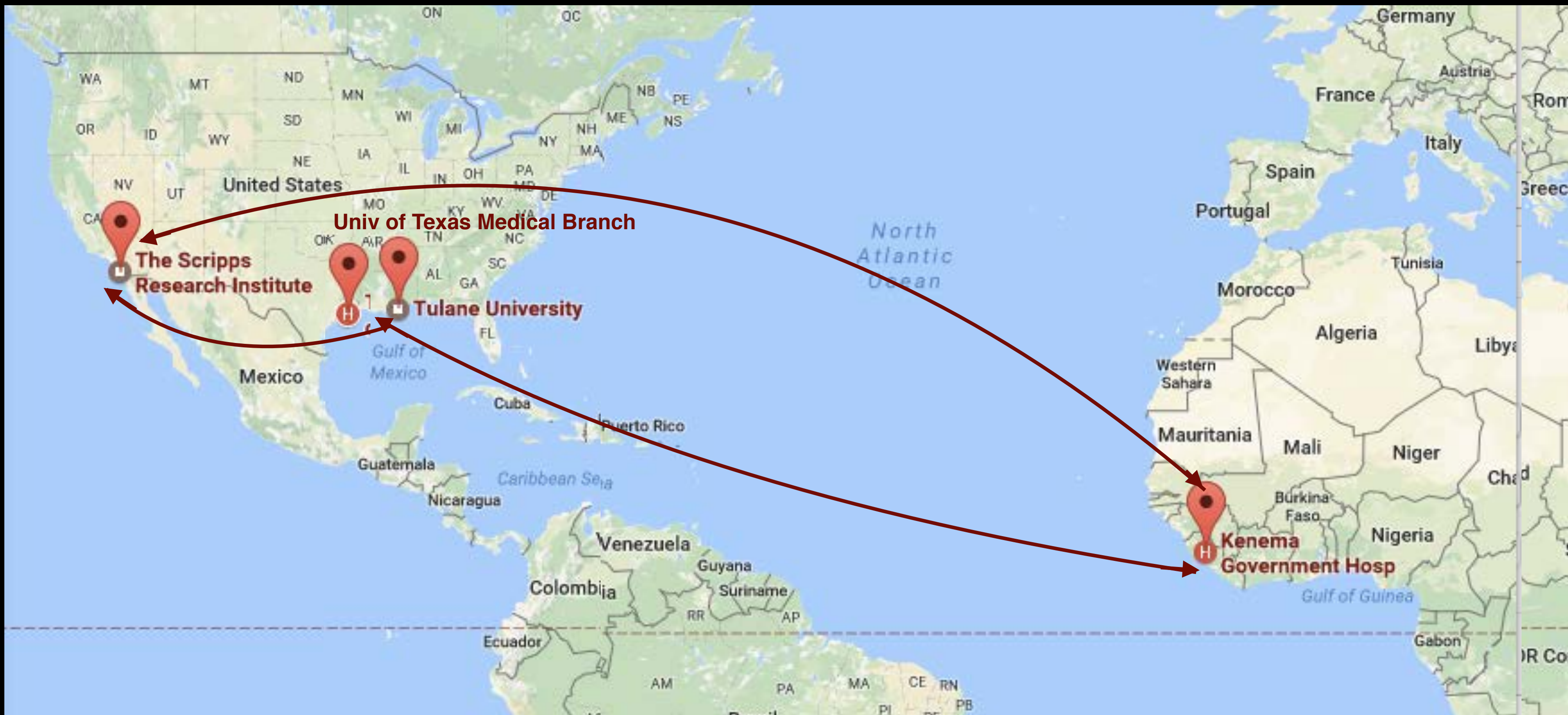


This one obtained after 10-year effort

Sought mAbs from survivors
to learn how to engineer the GP
to solve that structure…

# 2007-2017




My lab
Kate Hastie
Michelle Zandonatti

Tom Geisbert
Robert Cross

Robert Garry
James Robinson
Luis Branco
John Scheiffelin
Rachel Yenni

S. Humarr Khan
Donald Grant
Augustine Goba
Mambu Momoh
Mohammed Fullah
Michael Gbabkie
Lansana Kaneh
Veronica Koromeh
Richard Fonnie

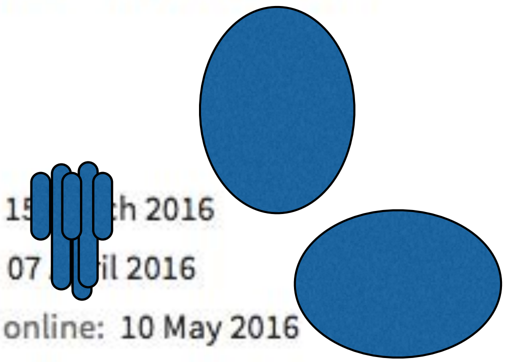
Simbirie Jalloh
Brima Kargbo
Mohamed Vandi
Momoh Gbetua
Odia Ikpanmwosa
Danny Asogun
Peter Okohere
Onikepe Folarin

MENU

nature COMMUNICATIONS

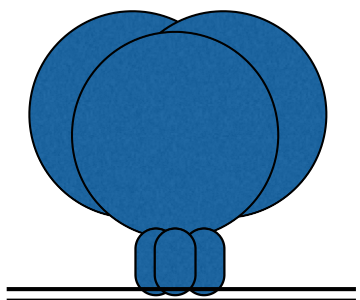
# Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits

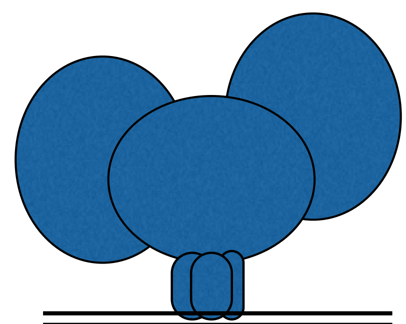
James E. Robinson, Kathryn M. Hastie […] Robert F. Garry



*Nature Communications* **7**,
Article number: 11544 (2016)
doi:10.1038/ncomms11544

Received: 15 [illegible] h 2016
Accepted: 07 [illegible] il 2016
Published online: 10 May 2016





Neutralizing antibodies bind prefusion GP

not these

nature medicine

Brief Communication | Published: 04 September 2017

# Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever

Chad E Mire, Robert W Cross, Joan B Geisbert, Viktoriya Borisevich, Krystle N Agans, Daniel J Deer, Megan L Heinrich, Megan M Rowland, Augustine Goba, Mambu Momoh, Mathew L Boisen, Donald S Grant, Mohamed Fullah, Sheik Humarr Khan, Karla A Fenton, James E Robinson, Luis M Branco, Robert F Garry & Thomas W Geisbert

*Nature Medicine* **23**, 1146–1149 (2017) | Download Citation

Cocktail of three neutralizing mAbs: protective in NHPs

**late stage** (day 8 of 9)

**low dose** 1.5 mg/kg

nature medicine

Brief Communication | Published: 04 September 2017

# Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever

Chad E Mire, Robert W Cross, Joan B Geisbert, Viktoriya Borisevich, Krystle N Agans, Daniel J Deer, Megan L Heinrich, Megan M Rowland, Augustine Goba, Mambu Momoh, Mathew L Boisen, Donald S Grant, Mohamed Fullah, Sheik Humarr Khan, Karla A Fenton, James E Robinson, Luis M Branco, Robert F Garry & Thomas W Geisbert

*Nature Medicine* **23**, 1146–1149 (2017) | Download Citation

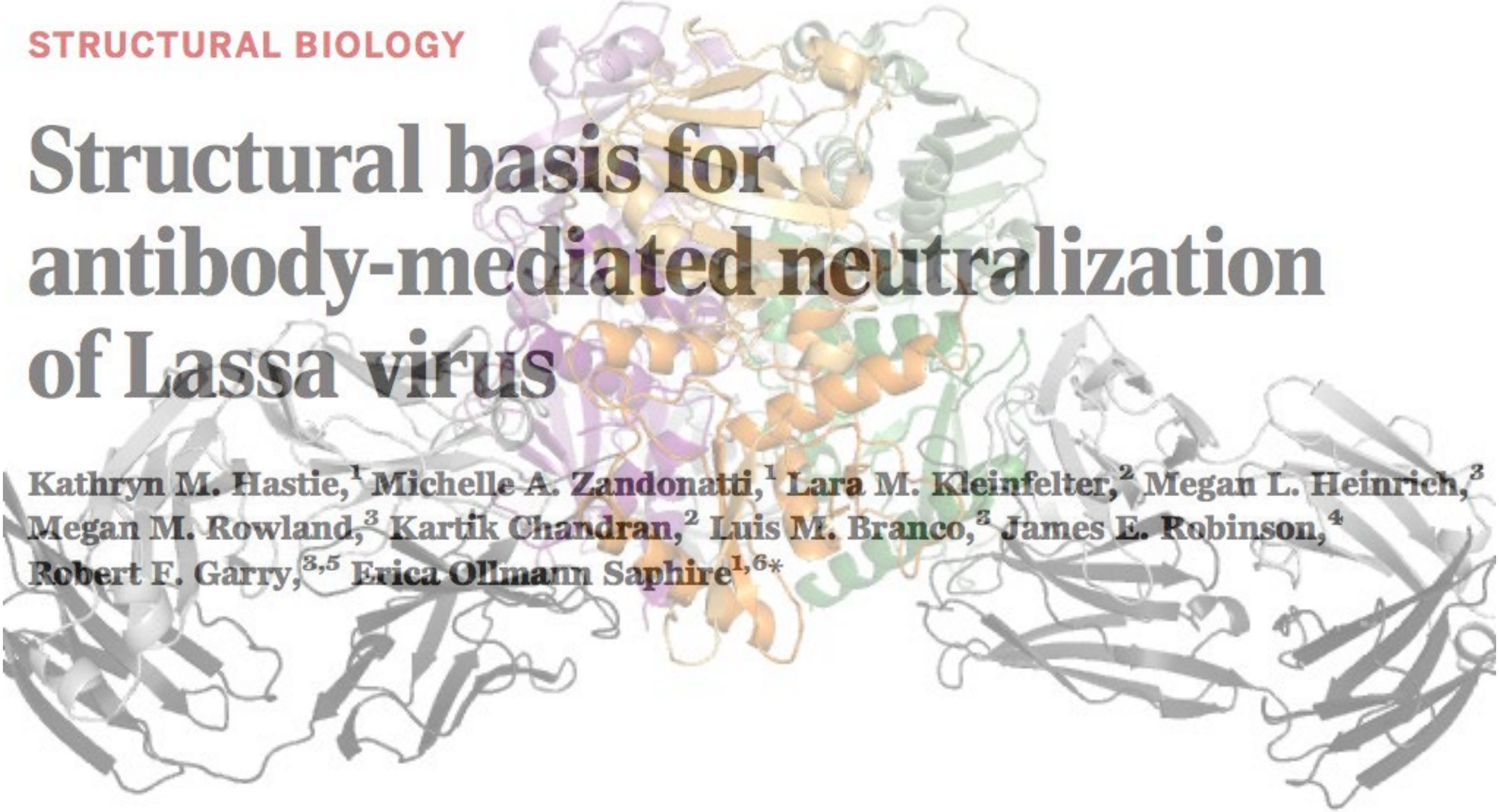
(a) Neut mAbs possible for Lassa virus
(b) Sufficient for protection
(c) Need certain GP structure to elicit/detect

RESEARCH ARTICLE

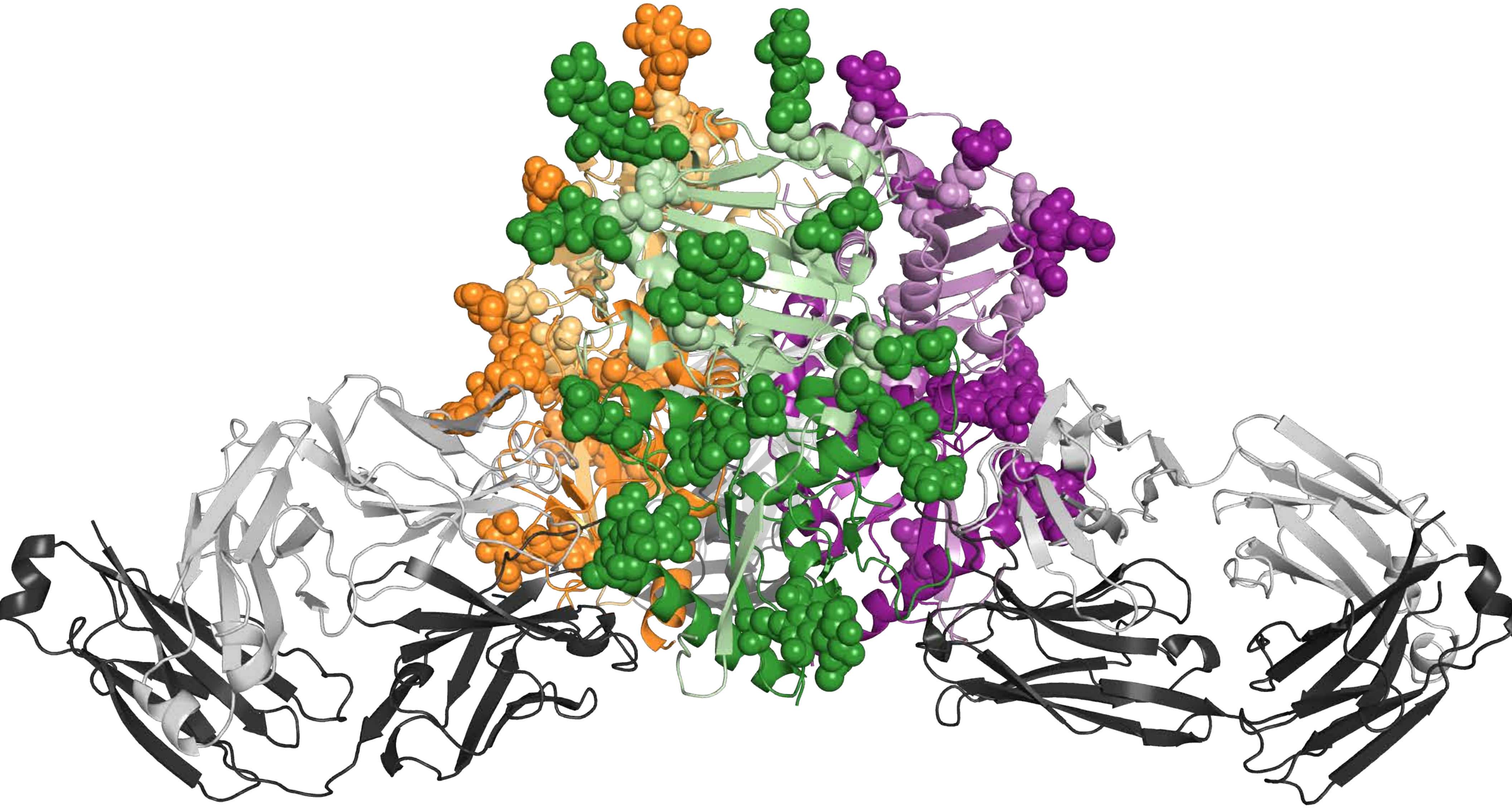
STRUCTURAL BIOLOGY

# Structural basis for antibody-mediated neutralization of Lassa virus

Kathryn M. Hastie,[1] Michelle A. Zandonatti,[1] Lara M. Kleinfelter,[2] Megan L. Heinrich,[3] Megan M. Rowland,[3] Kartik Chandran,[2] Luis M. Branco,[3] James E. Robinson,[4] Robert F. Garry,[3,5] Erica Ollmann Saphire[1,6]*

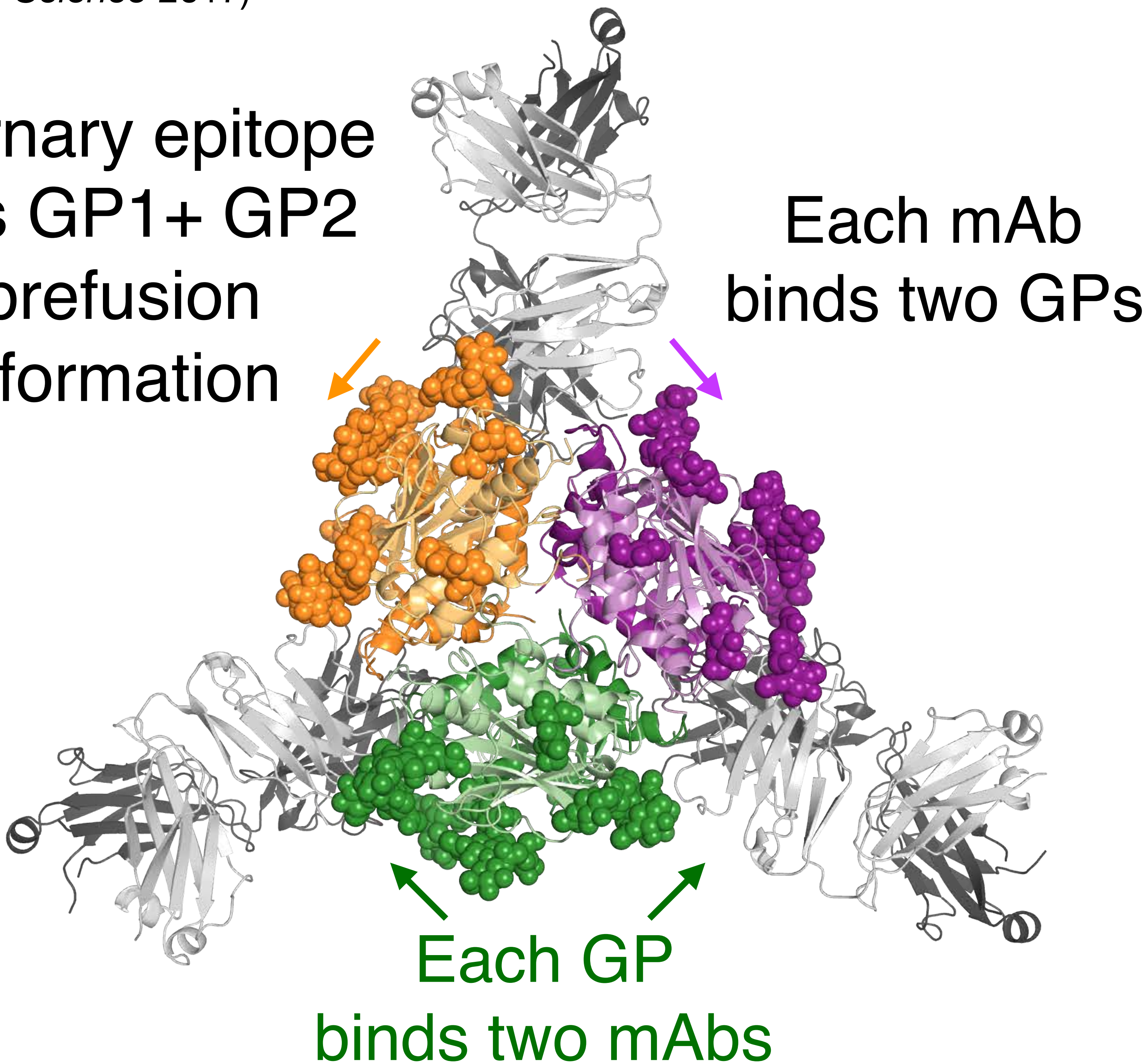


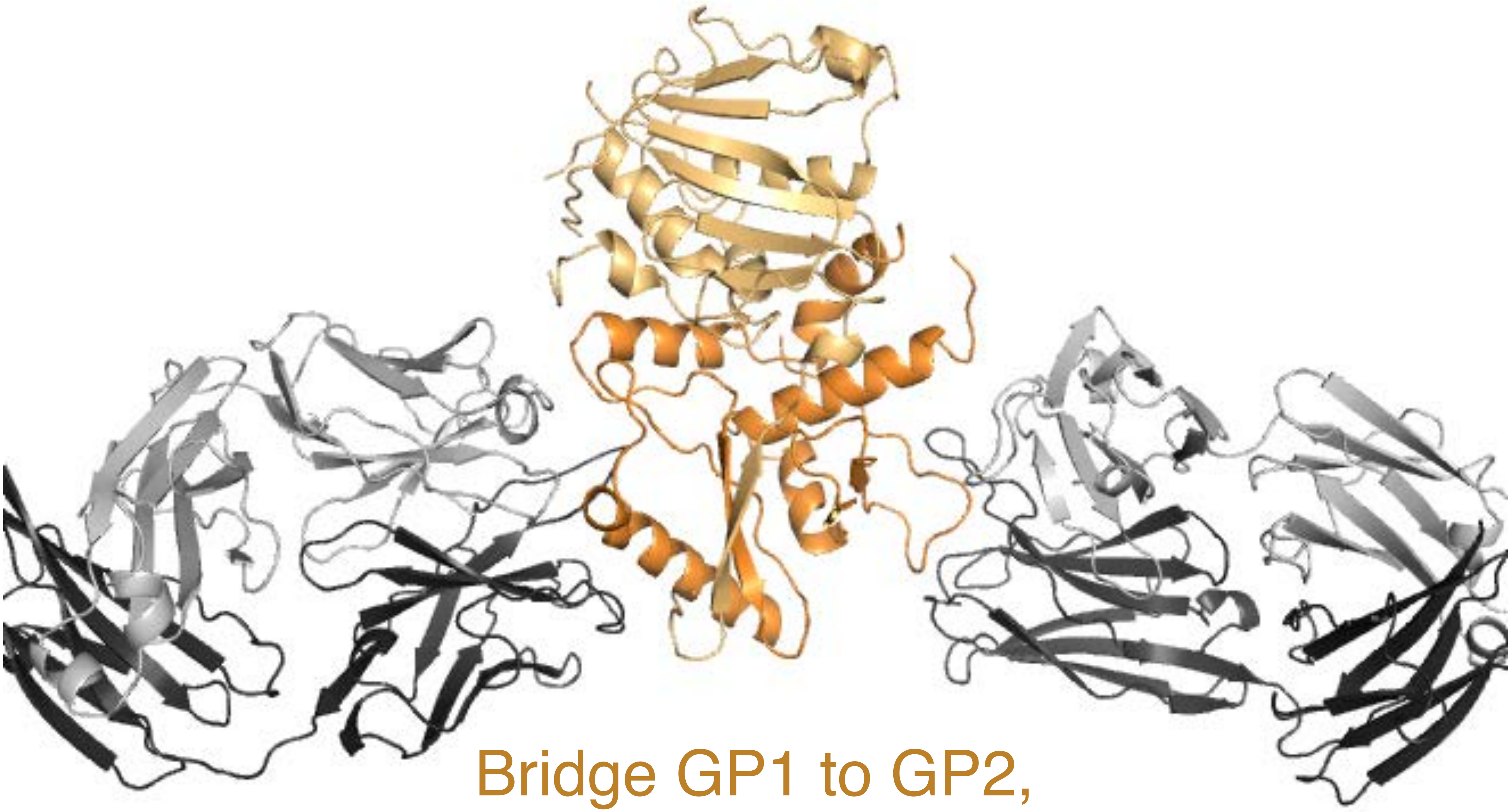
3.2 Å structure Lassa GP



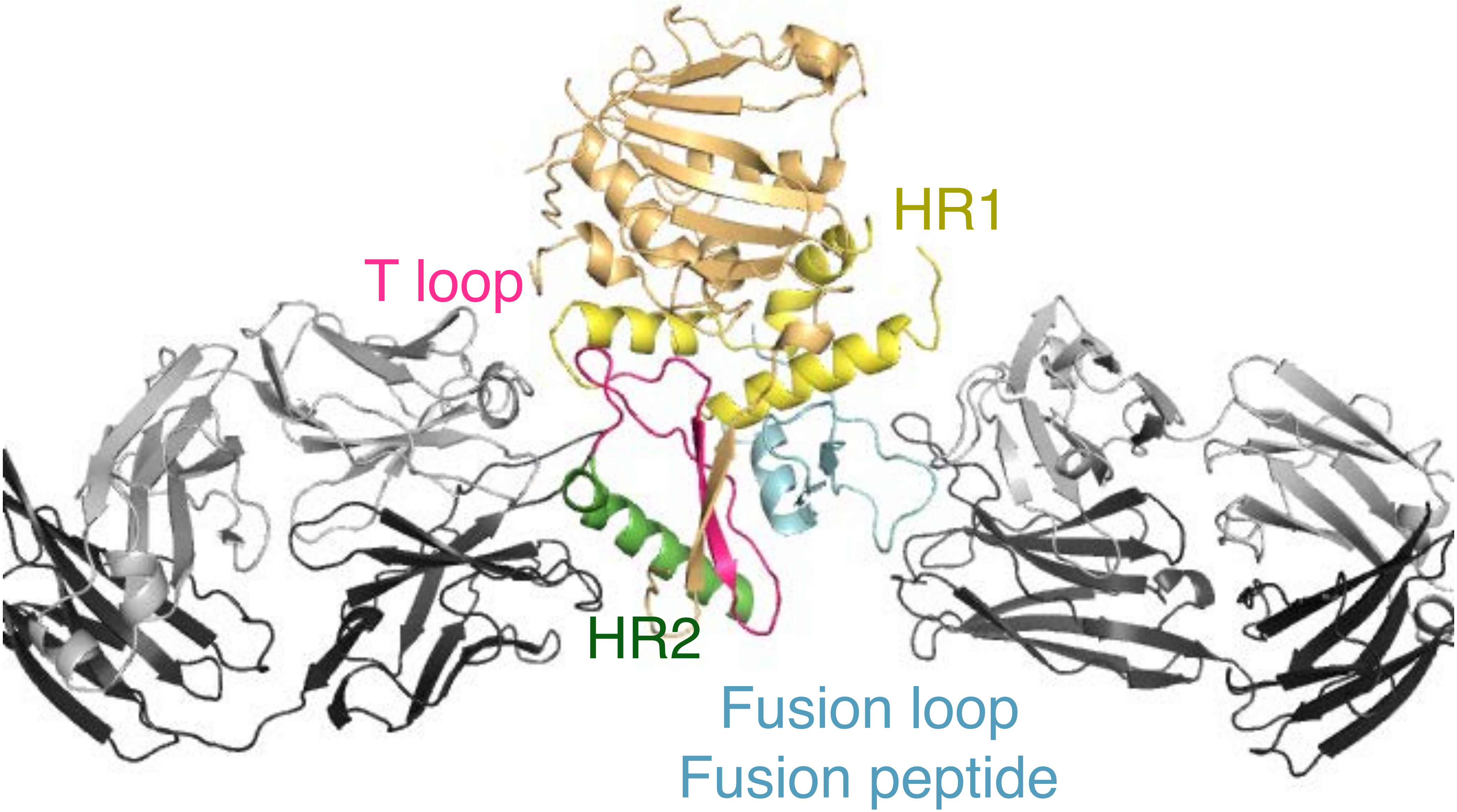
neutralizing human mAb 37.7H

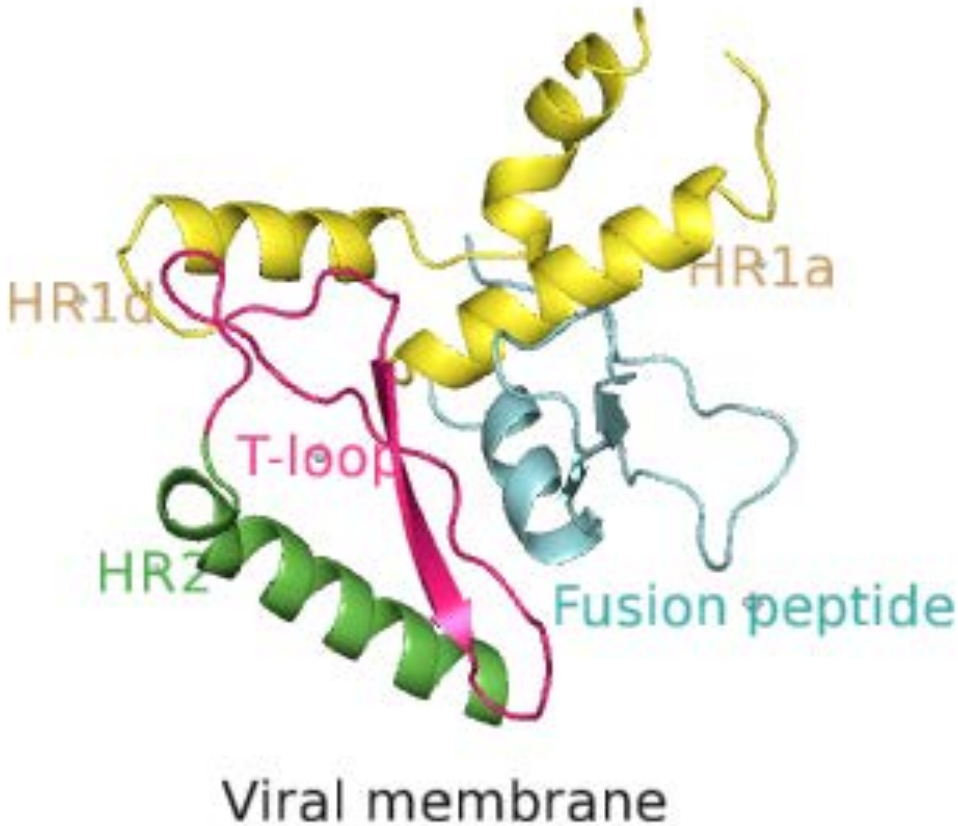
(Hastie, *et al. Science* 2017)



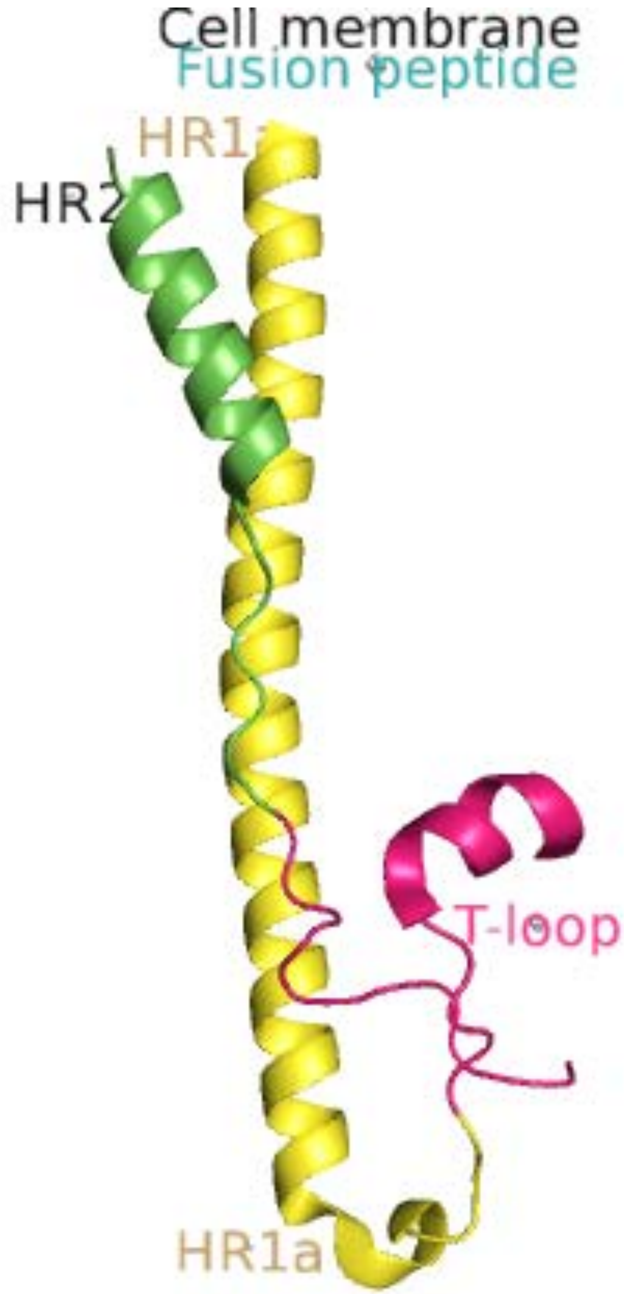


Bridge GP1 to GP2,
both in pre-fusion conformation


HR1
T loop
HR2
Fusion loop
Fusion peptide

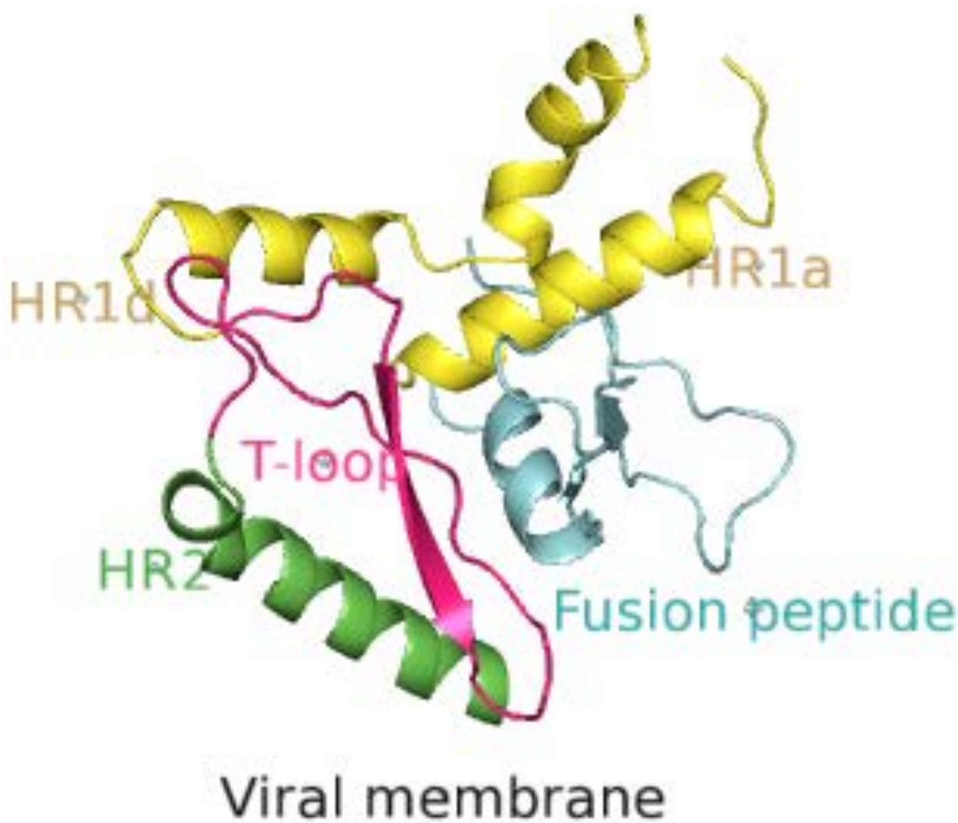


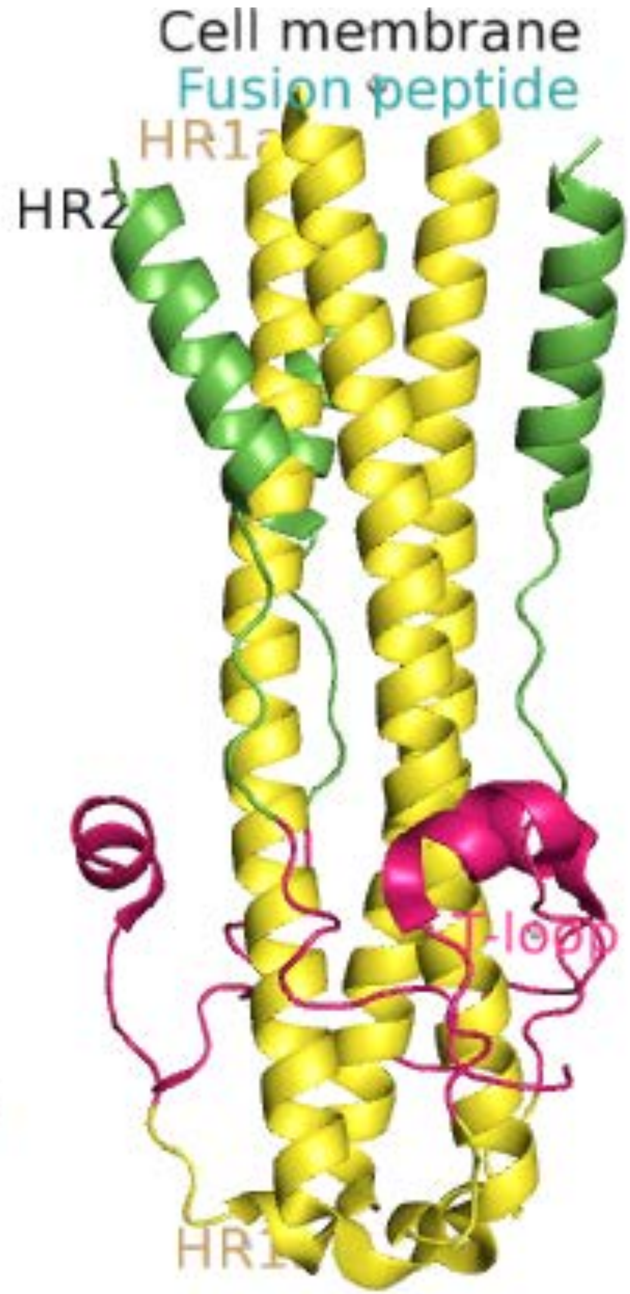

Pre-fusion




Post-fusion

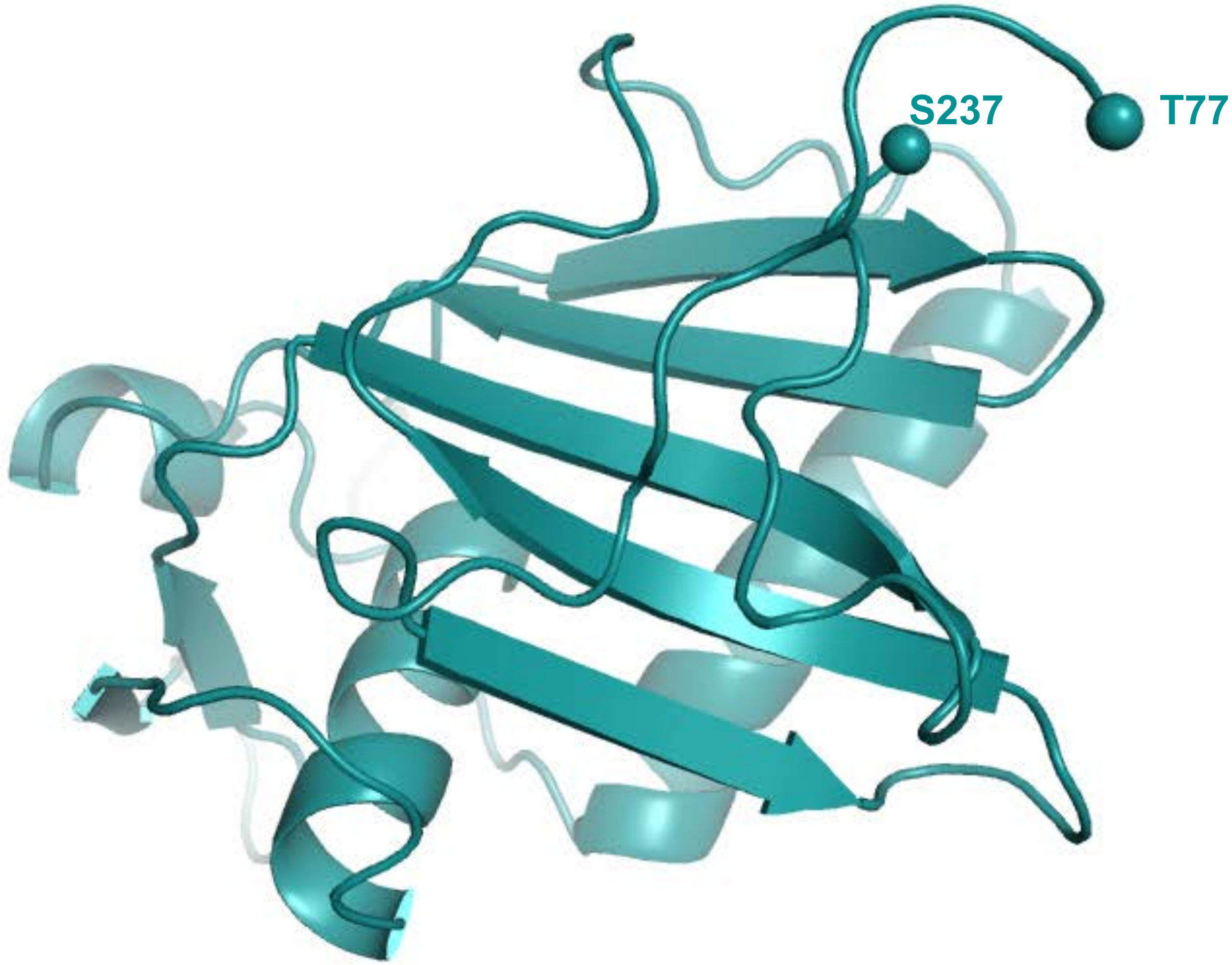
We expected conformational change in GP2…



…but there is also conformational change in GP1.

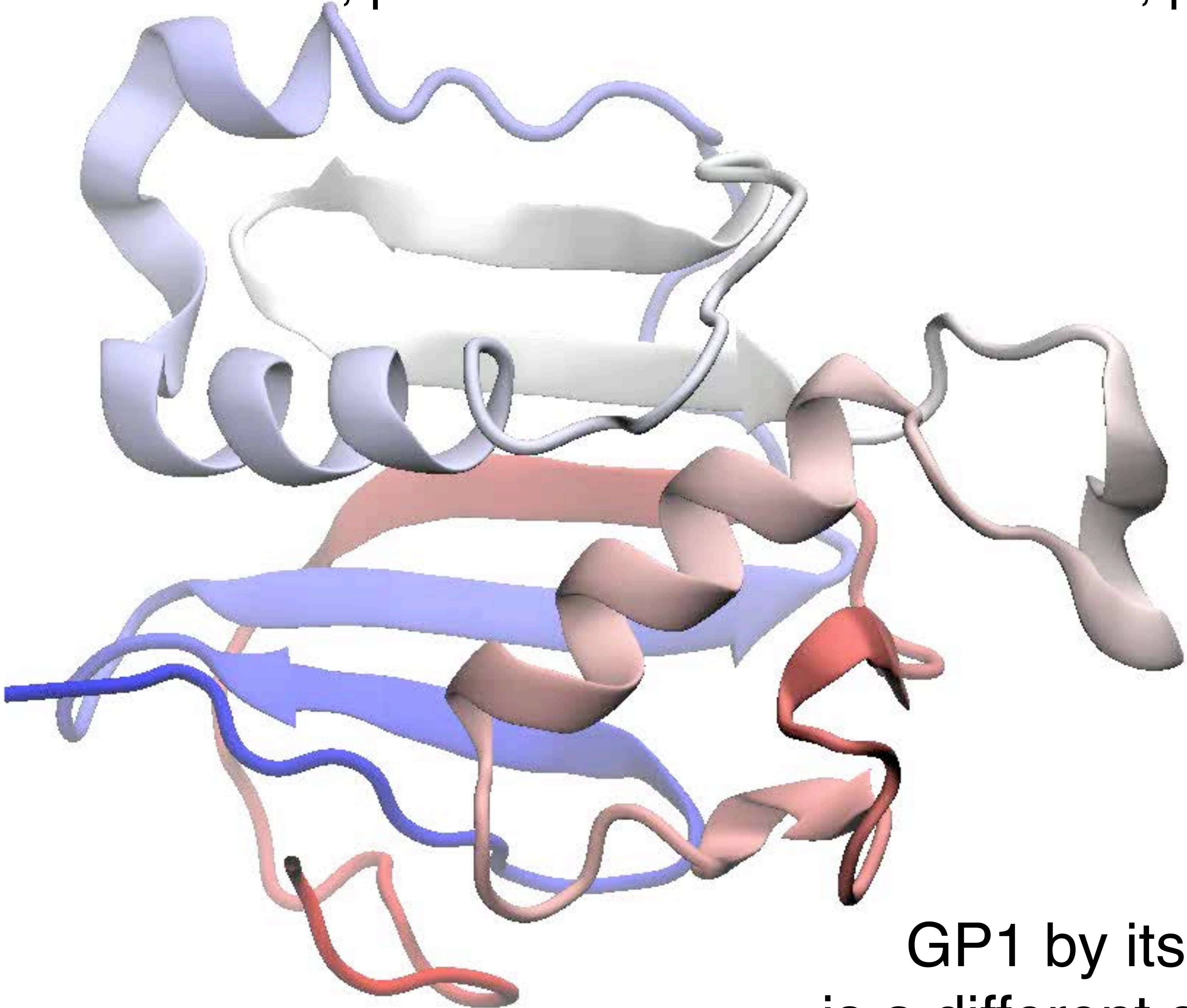
# Lassa GP1 solved in isolation at pH 5




Ron Diskin's lab: Cohen-Dvashi, H., *et al.* (2015) *J Virol.*

Start: GP1 in trimer, pH 8

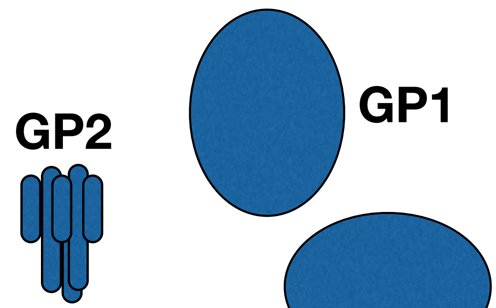
Finish: GP1 alone, pH 5



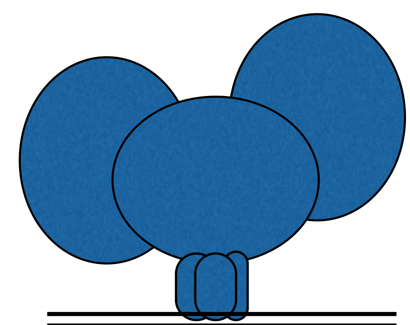
GP1 by itself
is a different shape

# For Lassa, more or less:

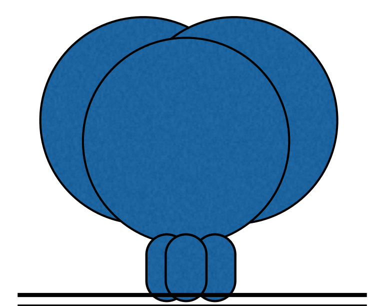
Non-Neutralizing




Non-Neutralizing

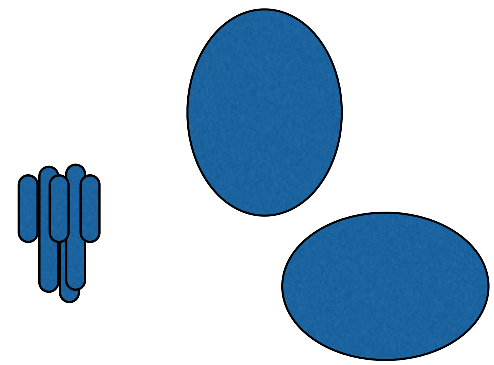


Neutralizing

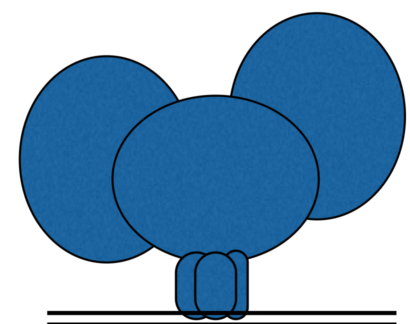


# From the wild-type glycoprotein:

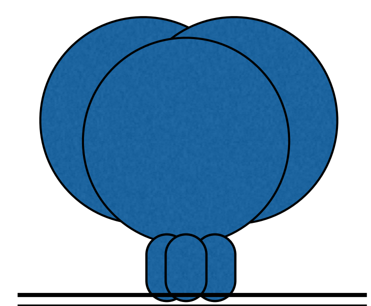
More common



More common

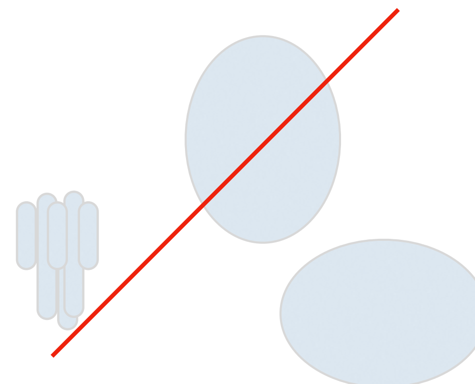


Rare

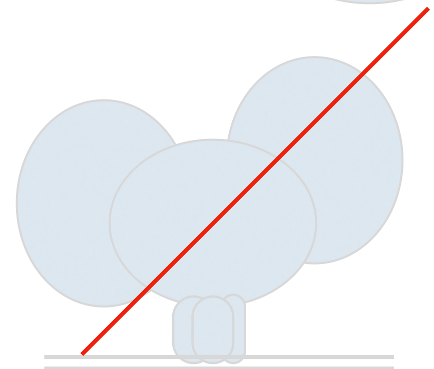


# Protein Engineering:
# Make Lassa GPC that is…

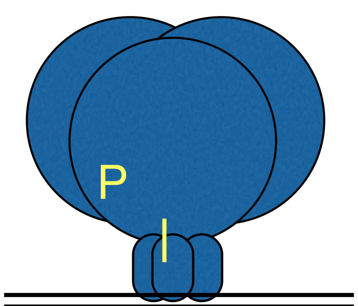
Never



Never

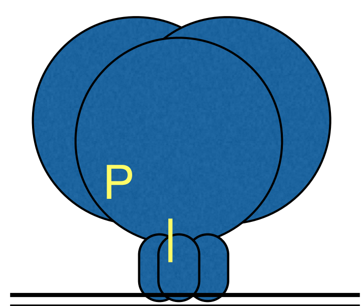


Always

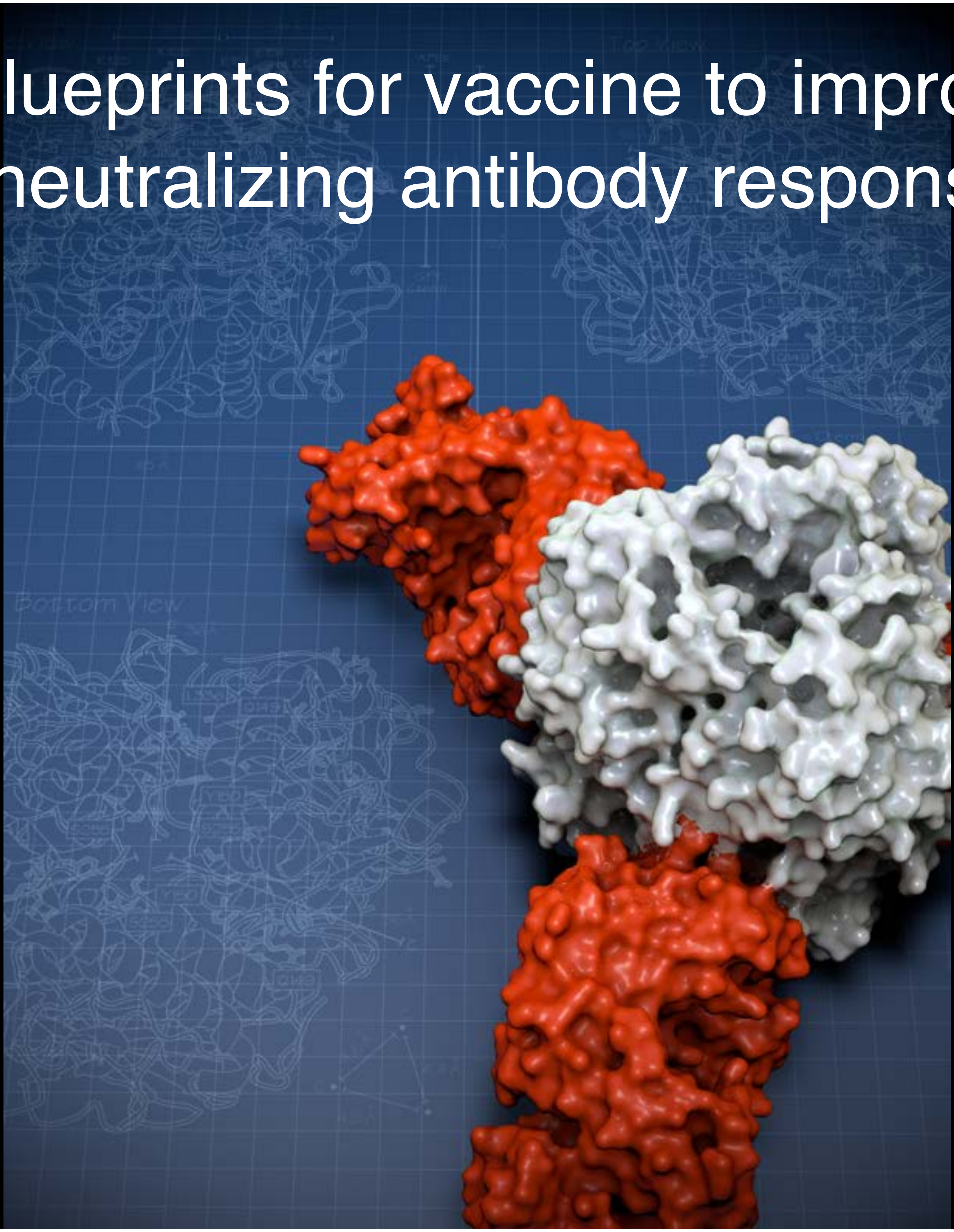


* Needs its wild-type S1P cleavage event
Not all cell types express enough S1P
Talk to us if you're trying to make this

**Immunize with this***
**to boost neut Abs**

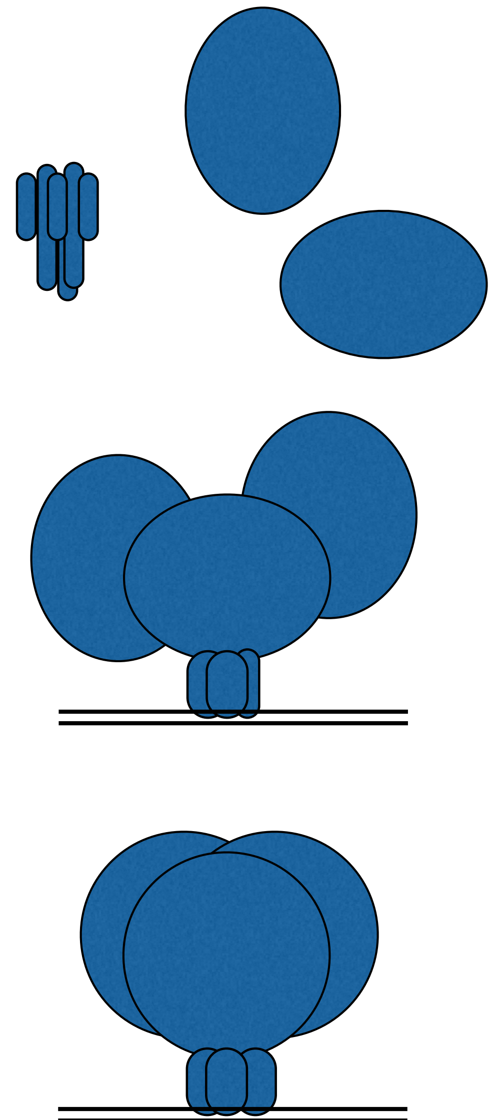


# Blueprints for vaccine to improve neutralizing antibody response



# Wild-Type GPC elicits non-neutralizing mAbs

These can be protective:



nature COMMUNICATIONS

Article | OPEN | Published: 11 October 2018

## Non-neutralizing antibodies elicited by recombinant Lassa–Rabies vaccine are critical for protection against Lassa fever

Tiago Abreu-Mota, Katie R. Hagen, Kurt Cooper, Peter B. Jahrling, Gene Tan, Christoph Wirblich, Reed F. Johnson & Matthias J. Schnell

## Do we want
## neutralizing or non-neutralizing antibodies?

# Yes.

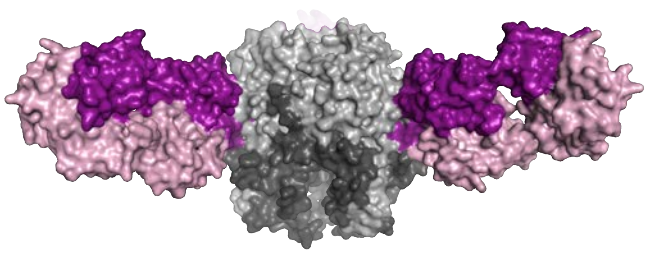
**Not an either/or.**
**Not black and white.**

**<u>Many kinds of neut Abs</u>**

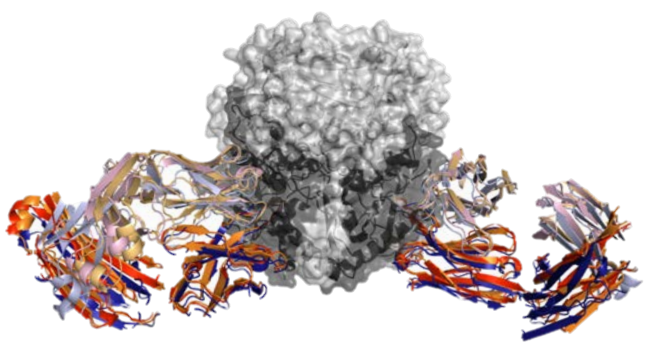
**Different epitopes**

**Different activities**

**Different potency**



**Neutralizes, does not protect**



**Neutralize, <u>do</u> protect**

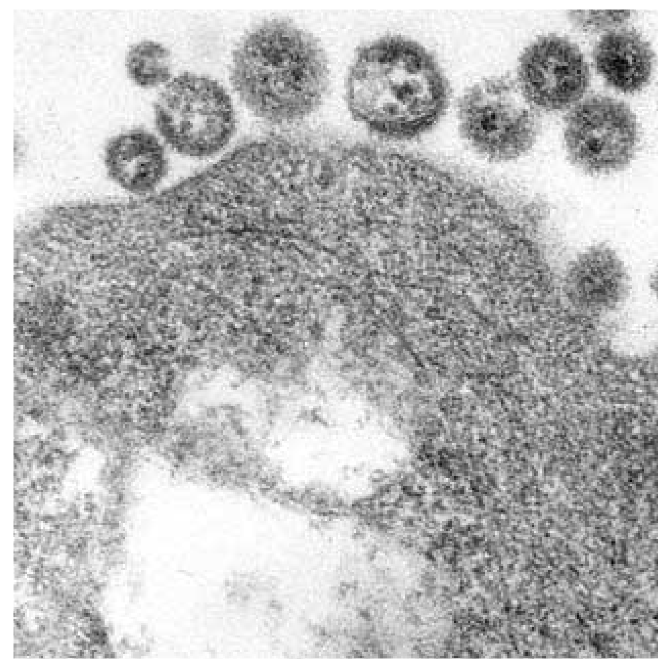
**<u>Many non-neut Abs</u>**

**Different epitopes**

**Different activities**

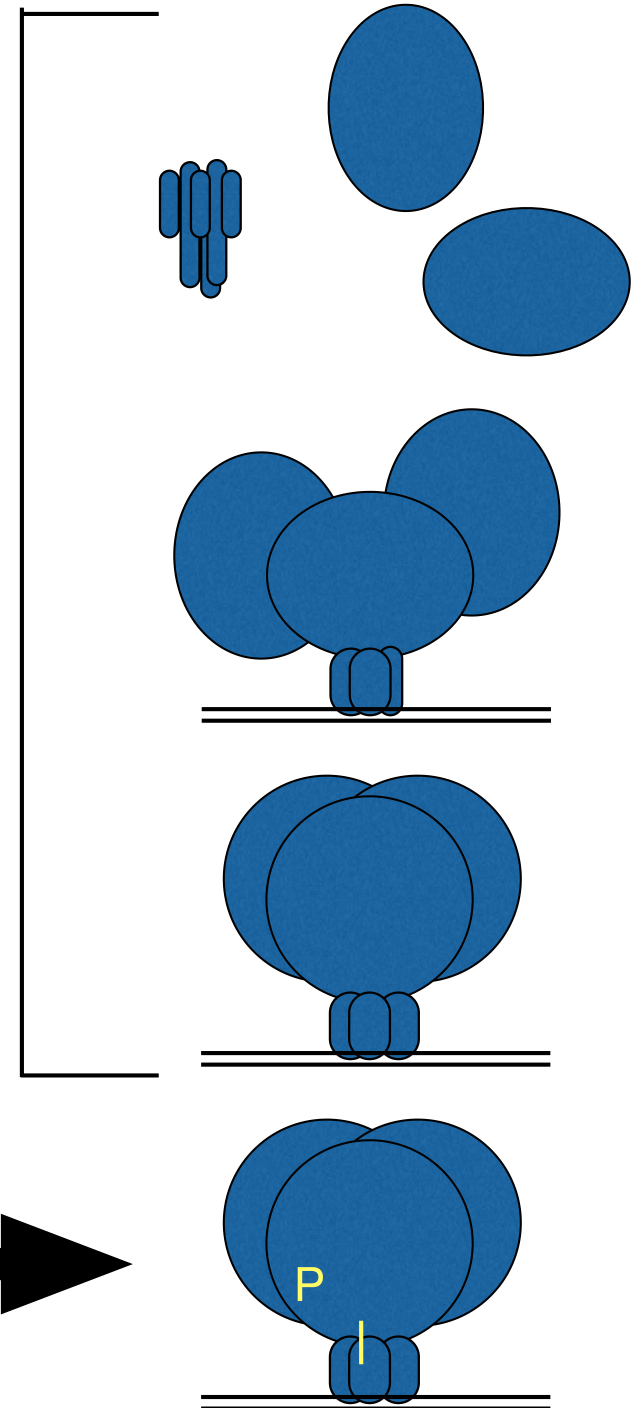
**Fc cell targeting?**

**Viral spread?**



**We will probably get both anyway**

# Adding
# a fusogenic GP
# gives you all these too:

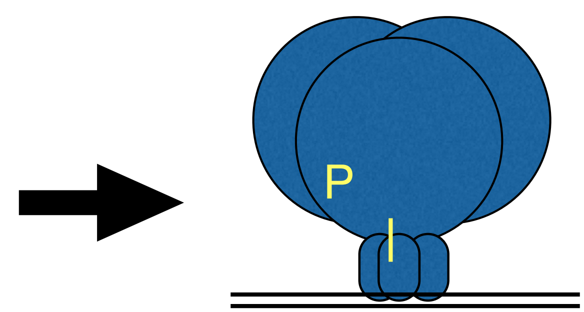



# Non-fusogenic

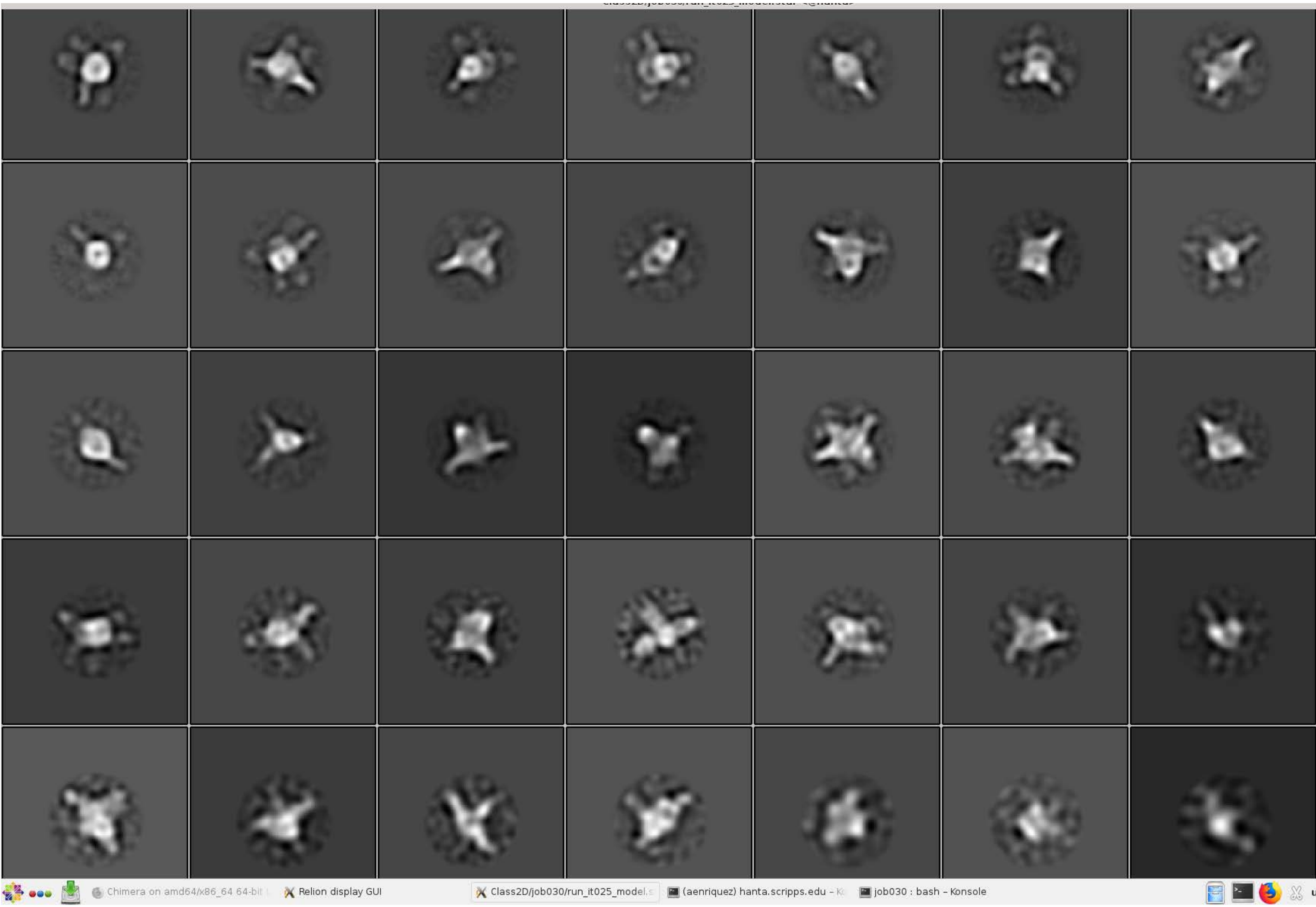
# Having potent neutralization is better than not having potent neutralization

Neutralization is hard to achieve with wild-type GP

**Immunize with this to boost neut Abs**



**We will get a mix of antibodies**
**We need to understand a mix of antibody responses**




**Class averages from polyclonal anti-Lassa antibody, cryoEM**

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Robert Garry
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Hannah Turner
Viktor Volchkov
Cheng-I Wang
Andrew Ward
Kelly Warfield
Kshitij Wagh
Anna Wec
Brandyn West
Gary Wong
Larry Zeitlin
and you?

NIAID for support

"If you want to go fast, go alone.
If you want to go far, go together."

*–African proverb*