Effect of the membrane proximal region on the antigenic structure of herpes simplex virus (HSV) glycoproteins gD, gC, and gE

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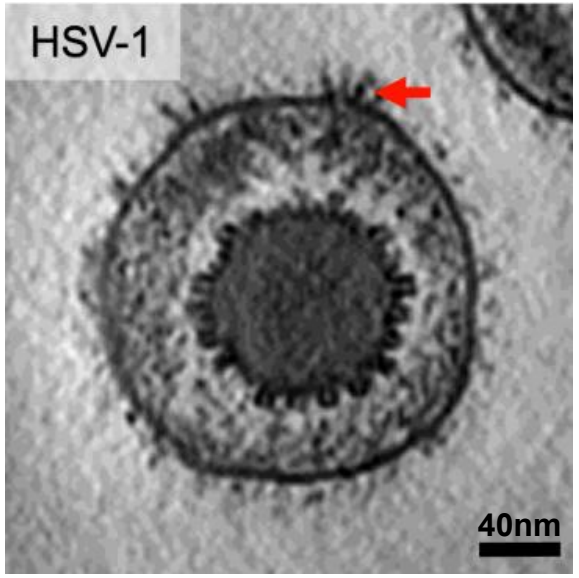
May 6, 2025

Herpesviruses

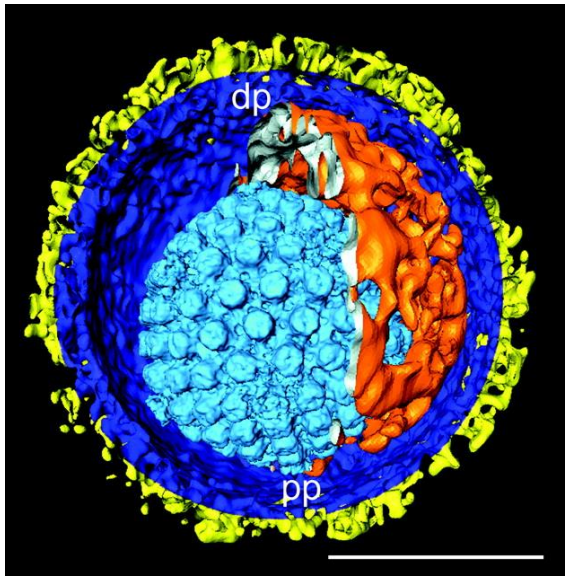
- DNA genome, icosahedral capsid, tegument, lipid envelope with glycoproteins
- Large, approximately 100 genes
- Co-evolve with their hosts, well adjusted pathogens
- Ubiquitous; infect all vertebrate species and have been found in invertebrates
  - 9 different human herpesviruses
  - TODAY: herpes simplex virus (HSV)

    two types: HSV-1 & HSV-2



HSV-1 cryogenic electron tomography

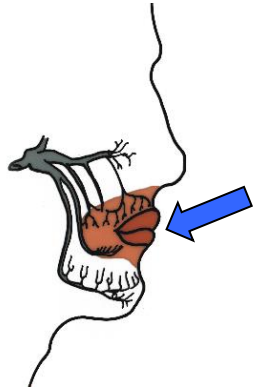
(Zhen et al. 2024)



HSV-1 tomogram cutaway

(Grunewald et al. 2003)

Herpes Simplex Virus Infections

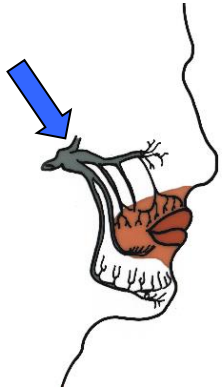


1) Primary infection

type-1: mouth, skin, eye

type-2: genital

*HSV-1 is capable of infecting genital region and vice versa

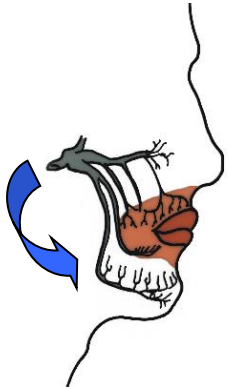


2) Latent infection

neurons

type-1: trigeminal

type-2: sacral

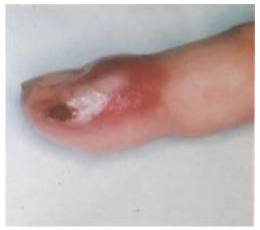


3) Recurrent infection

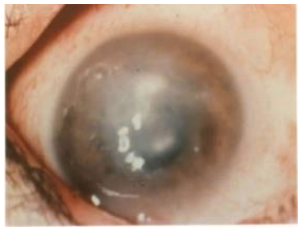
leads to disease or shedding

Virus survives in immune host

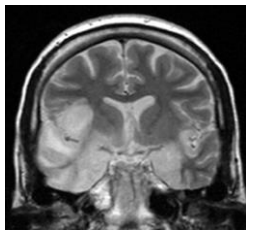
- Infects many types of cells
- Multiple entry glycoproteins
- Multiple receptors on cells



Whitlow's Finger



Keratitis



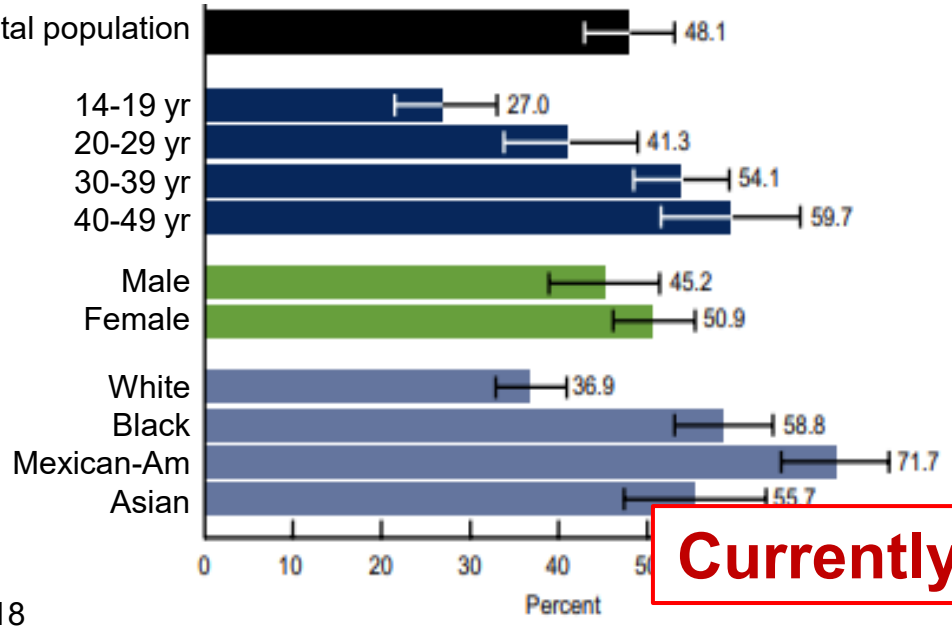
Encephalitis

Neonatal Herpes

Sero-epidemiology of HSV in USA

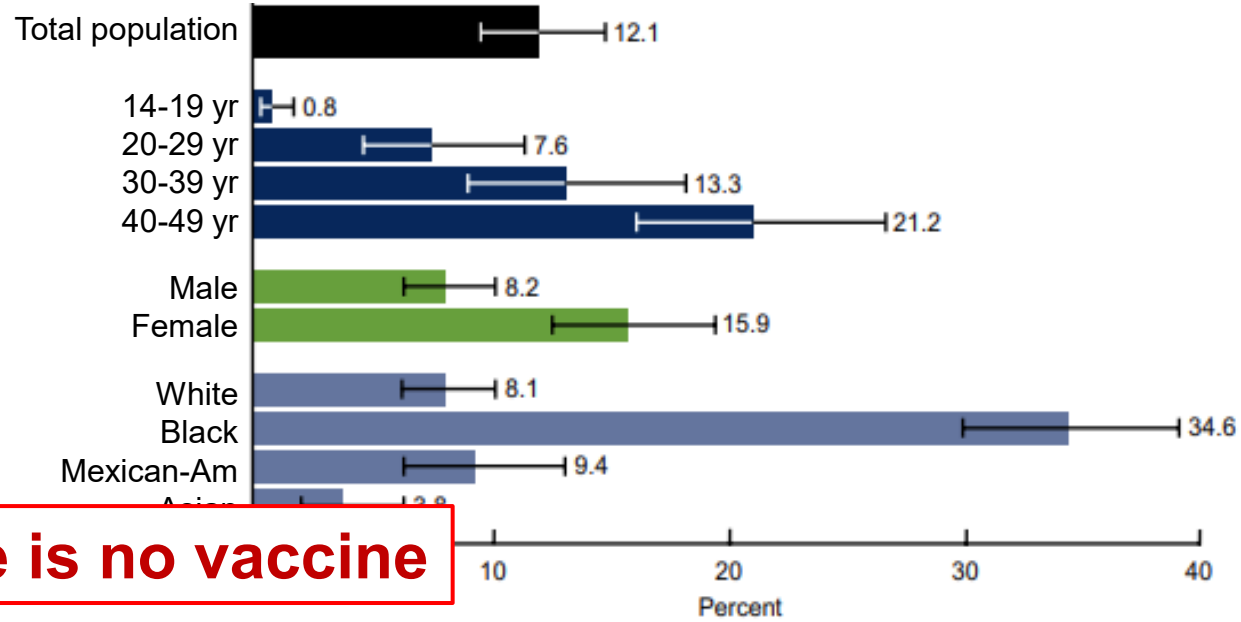
HSV-1

- 48% in people 14-49 years old
- Sero-presence increases with age
- Highest sero-presence in women and Mexican-Americans
- HSV-1 increasingly seen as cause of genital herpes



HSV-2

- 12% in people 14-49 years old
- Sero-presence increases with age
- Highest sero-presence in women and non-Hispanic black populations
- Association with acquisition of HIV



Currently, there is no vaccine

CDC, 2018

Currently there are at least 10 HSV vaccine candidates

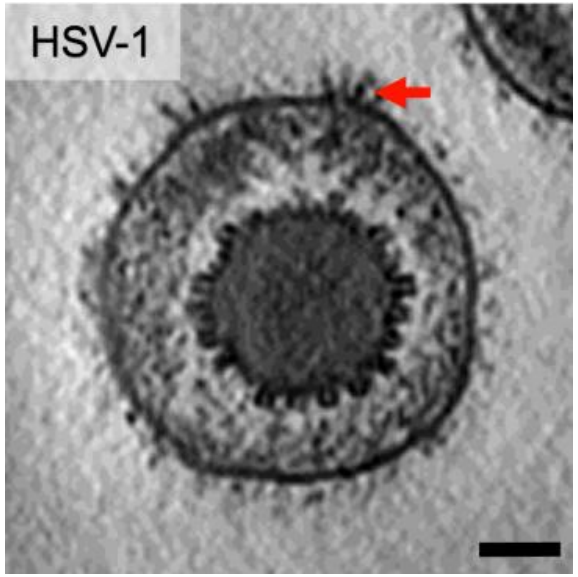
based on DNA, mRNA, protein subunit, killed virus, & live-attenuated virus vaccine technologies

Sci Immunol. 2019 Sep 20;4(39):eaaw7083. doi: 10.1126/sciimmunol.aaw7083.

Nucleoside-modified mRNA encoding HSV-2 glycoproteins C, D, and E prevents clinical and subclinical genital herpes

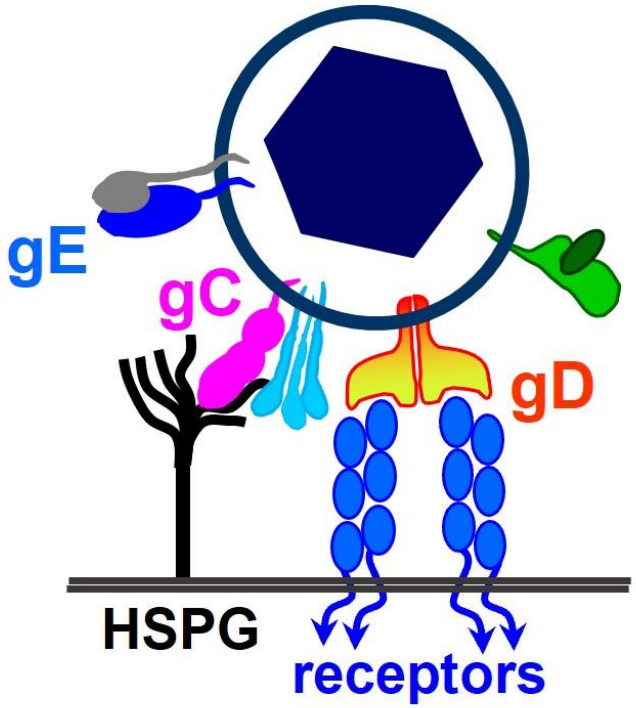
Sita Awasthi [1], Lauren M Hook [1], Norbert Pardi [1], Fushan Wang [1], Arpita Myles [2], Michael P Cancro [2], Gary H Cohen [3], Drew Weissman [1], Harvey M Friedman [4]

Our vaccine candidate uses mRNA that encodes 3 viral glycoproteins as antigens



HSV-1 cryogenic electron tomography

(Zhen et al. 2024)



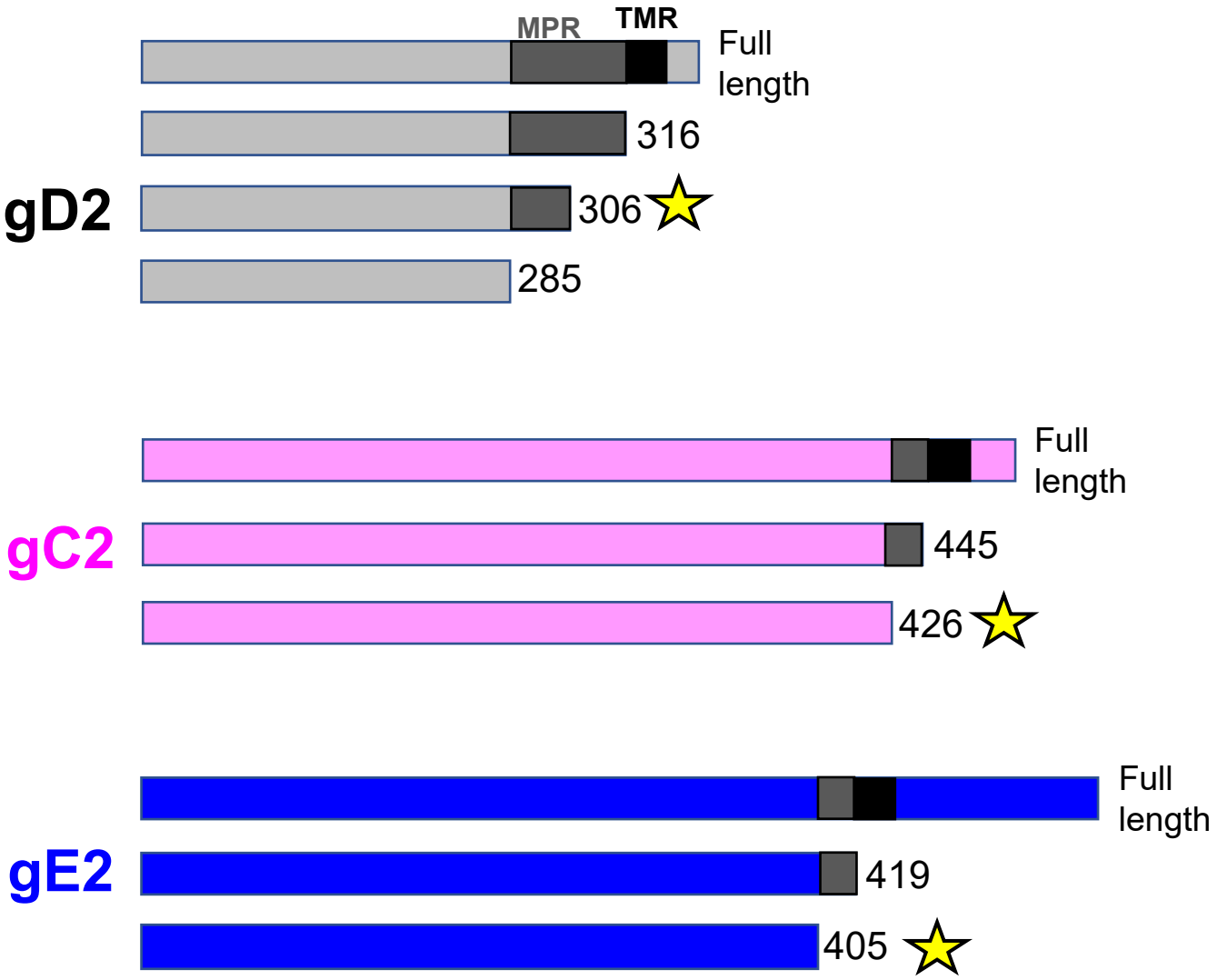
gD is the receptor binding protein, one of 4 glycoproteins absolutely required for virus entry

gC facilitates the initial virus-cell attachment (binds heparan sulfate proteoglycan) and contributes to immune evasion

gE has a role in cell-cell spread, neuronal transport, and immune evasion

Our current vaccine formulation expresses soluble gD2, gC2, and gE2

Does size matter?

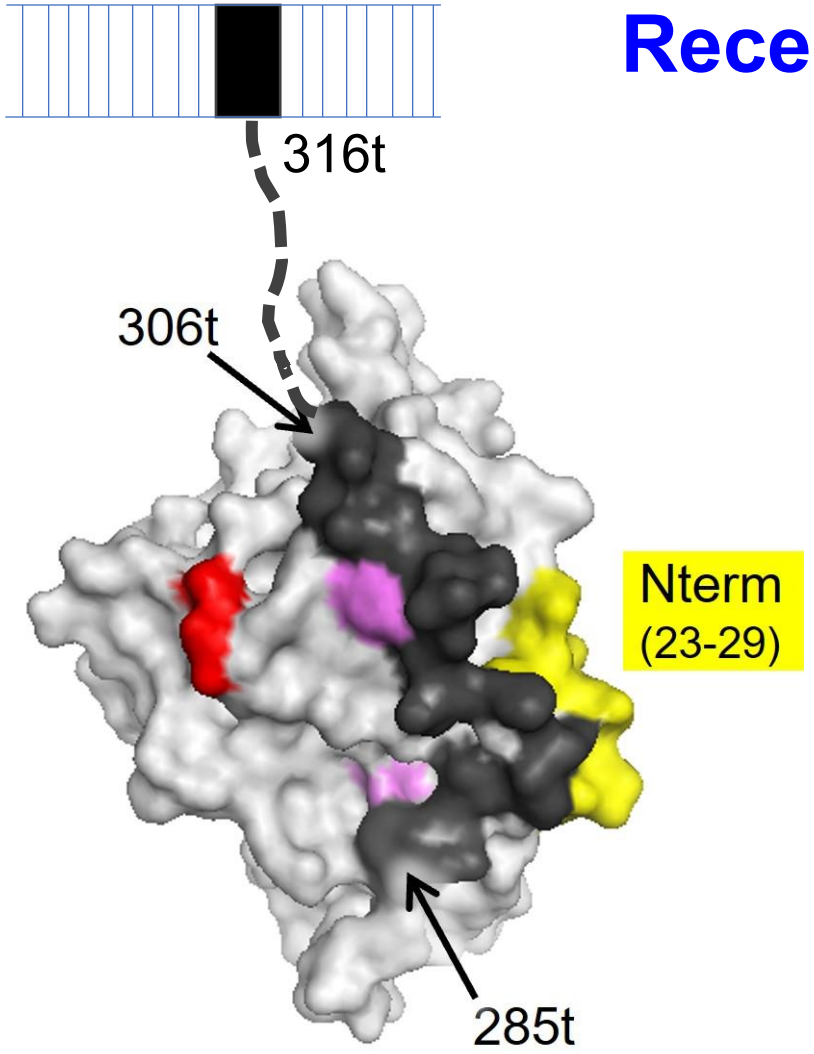


★ Glycoprotein forms that lack all or most of the membrane proximal region (**MPR**) are in our current vaccine formulation

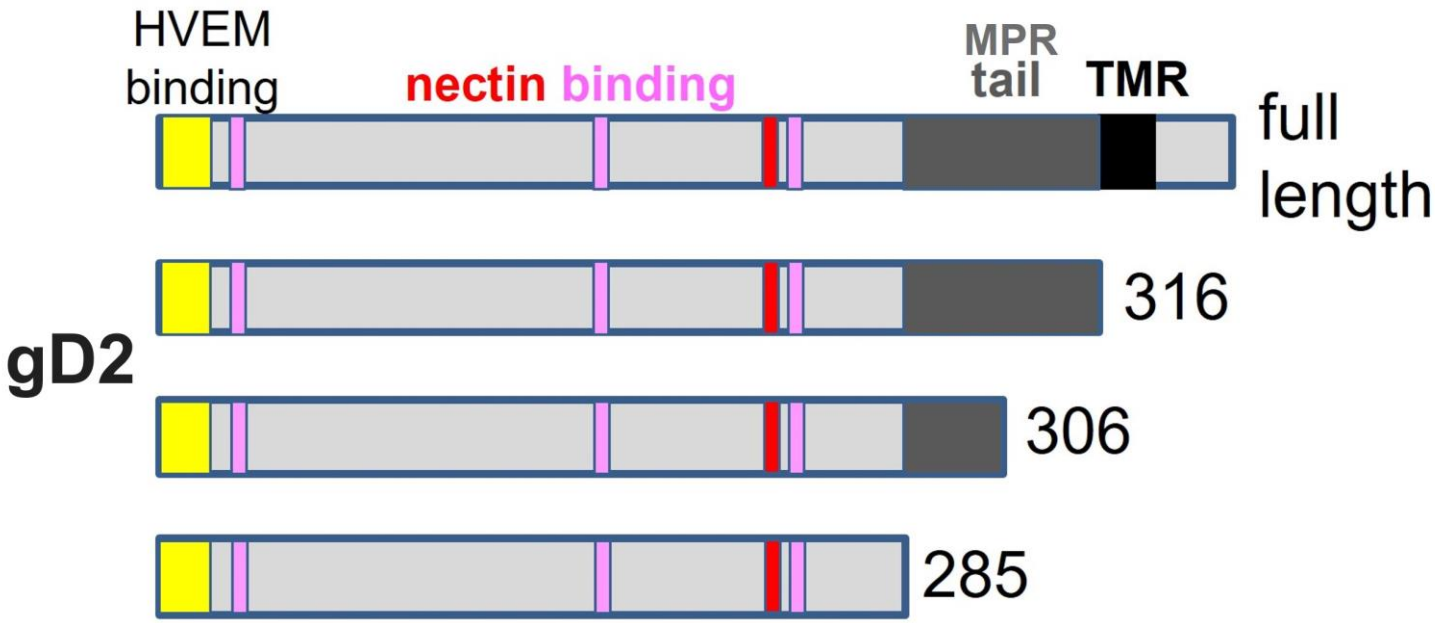
Would inclusion of the MPR affect the antigenic properties of these proteins?

Would size affect what kinds of antibodies are produced when used as a vaccine antigen?

Receptor binding protein: gD

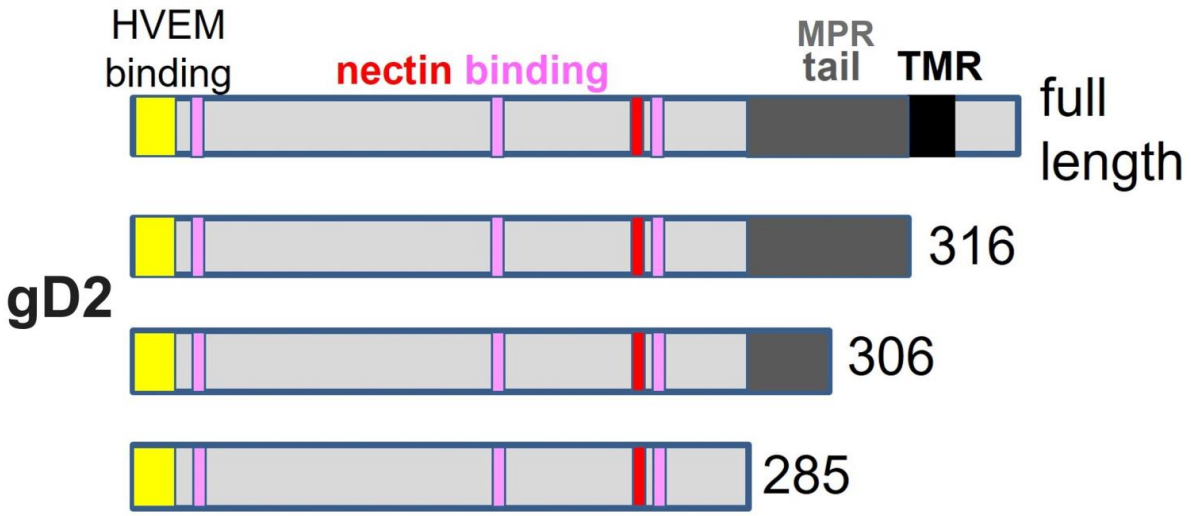


gD2 crystal structure

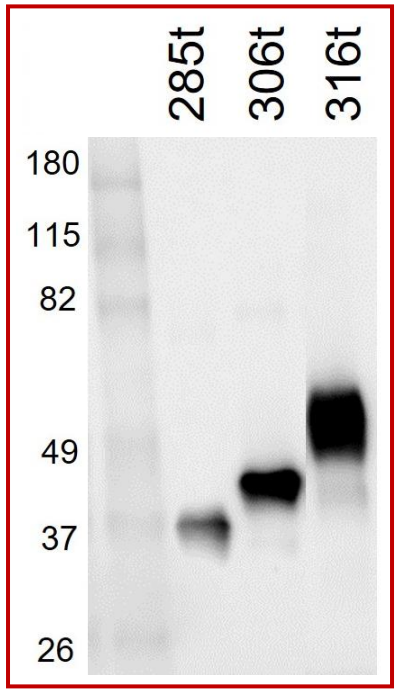


- **gD** binds the receptor HVEM along a linear stretch at its N-term (yellow) and receptor nectin-1 on an adjacent face of the folded protein (**red**, pink). Similarly color-coded MAbs have epitopes in these regions.
- All 3 soluble forms of gD bind nectin-1 but 306t & 316t have lower affinity due to the MPR tail partially obscuring the binding site; pink MAb binding is also affected.

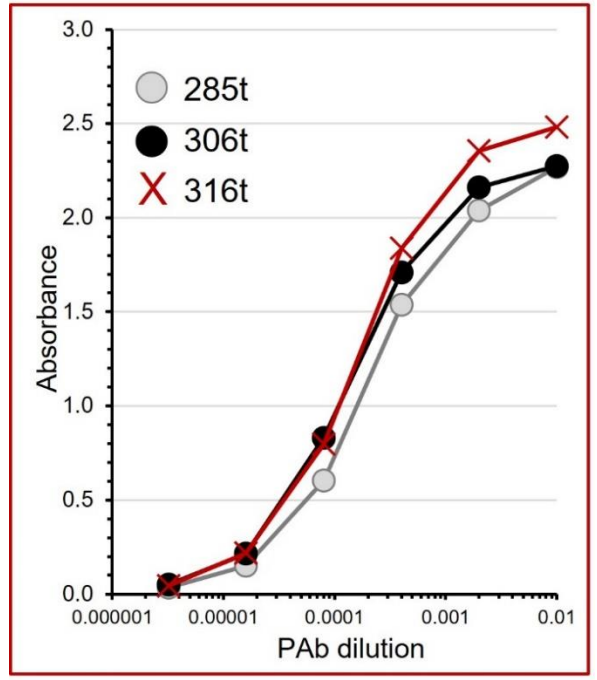
Multiple soluble forms of gD



gD2 Western blot



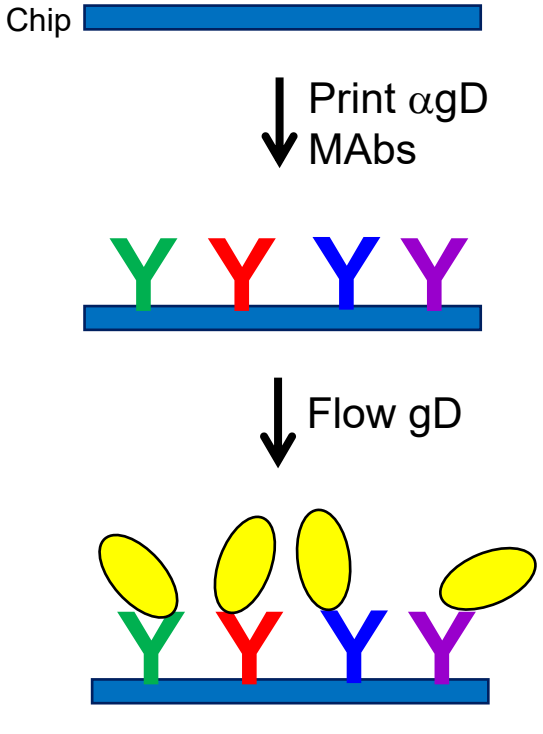
gD2 ELISA



- Western shows size and glycosylation differences between the proteins
- ELISA using polyclonal antibody shows no difference in signals

Next: Binding to monoclonal antibodies (MAbs) using the LSA

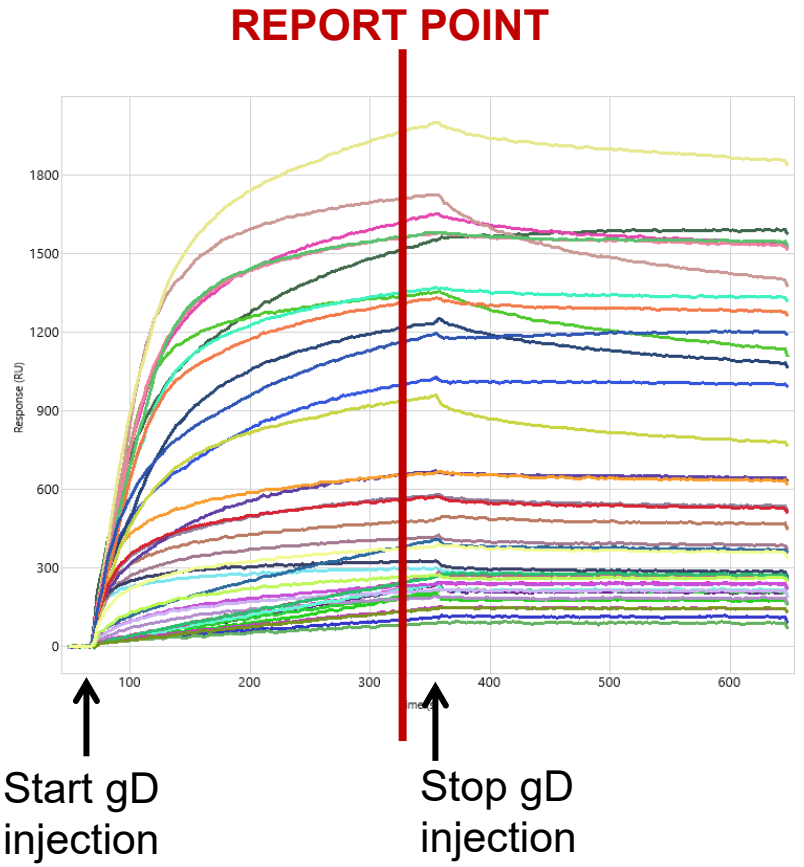
Evaluating gD-MAb binding with the Carterra LSA



Evaluate gD binding by:

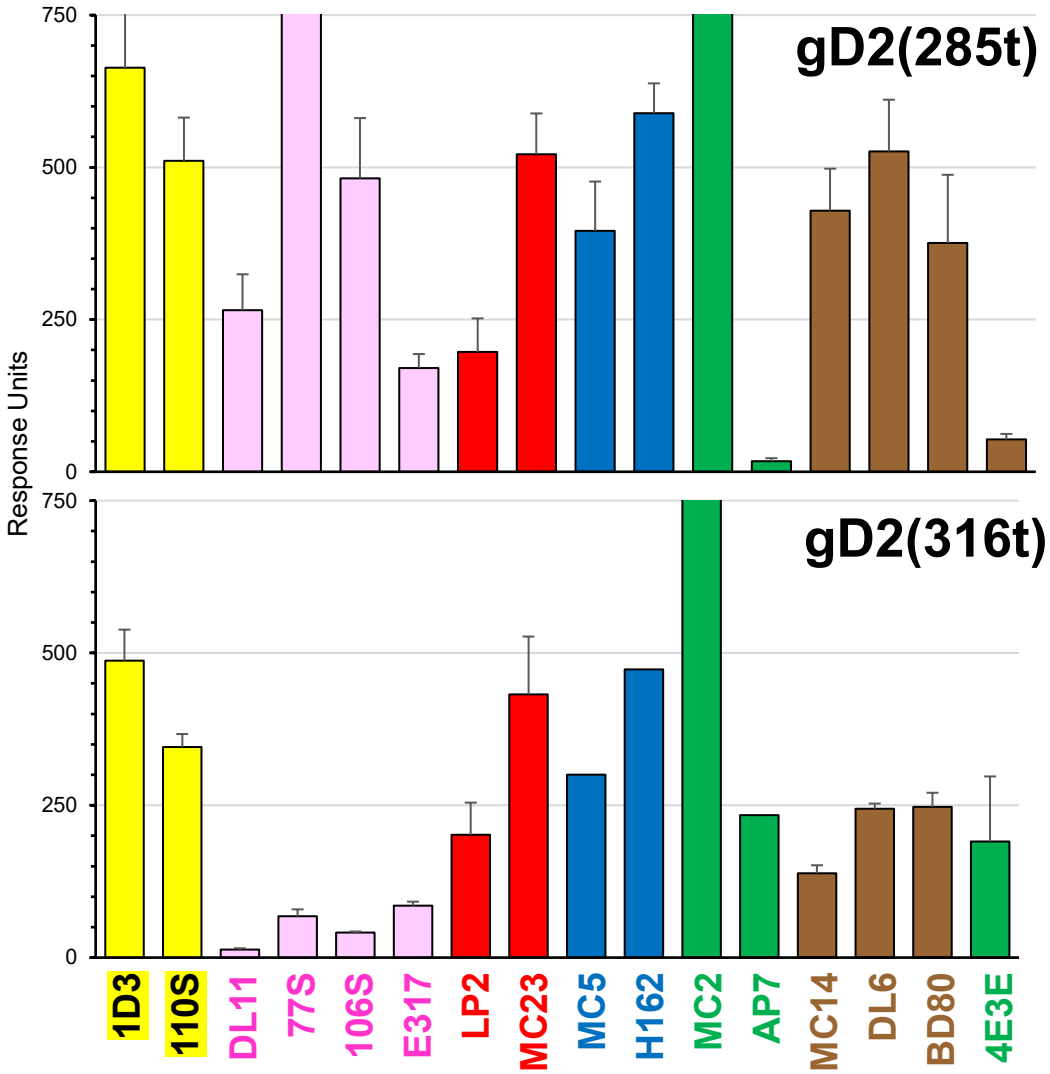
1) Max binding
2) Kinetics

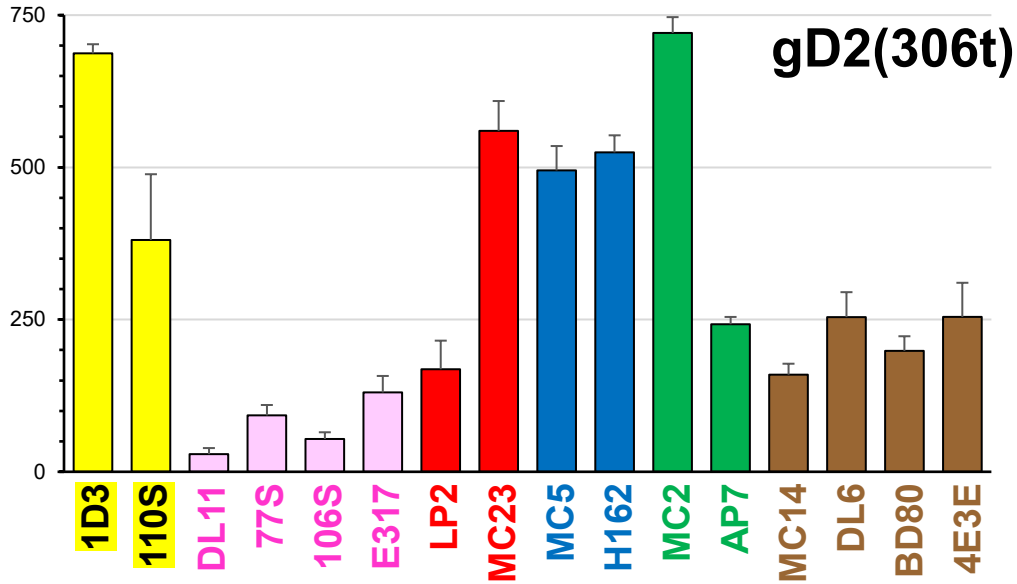
gD binding curve



Can generate bar graph from REPORT POINTS of each MAb (MAX binding)

MAb binding to different forms of gD

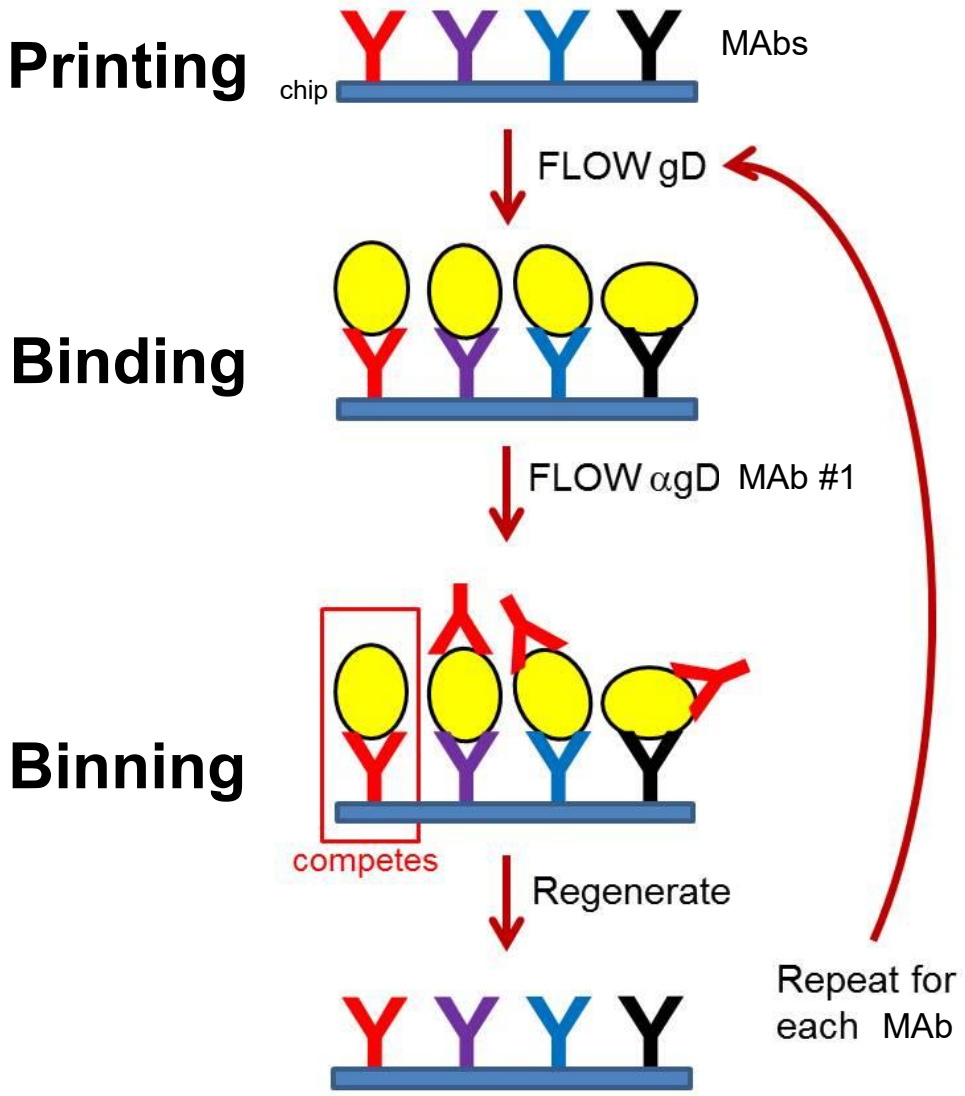


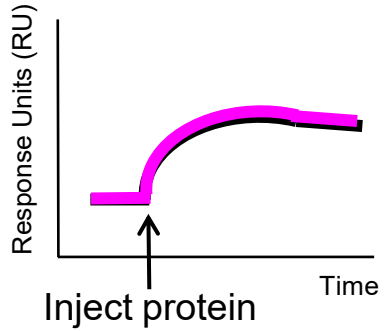


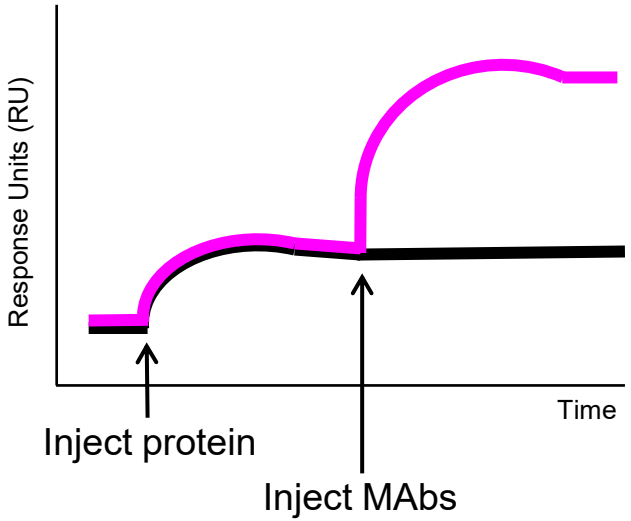
- MAb binding pattern similar between 306t & 316t
- **Pink** MAbs bind poorer to 306t/316t due to MPR tail interference
- MAbs **AP7** & **4E3E** behave the opposite: bind poorer to 285t due to epitopes within/near MPR tail

Next: Generation of MAb community maps

Binning of MAbs (Community Mapping Through Competition)







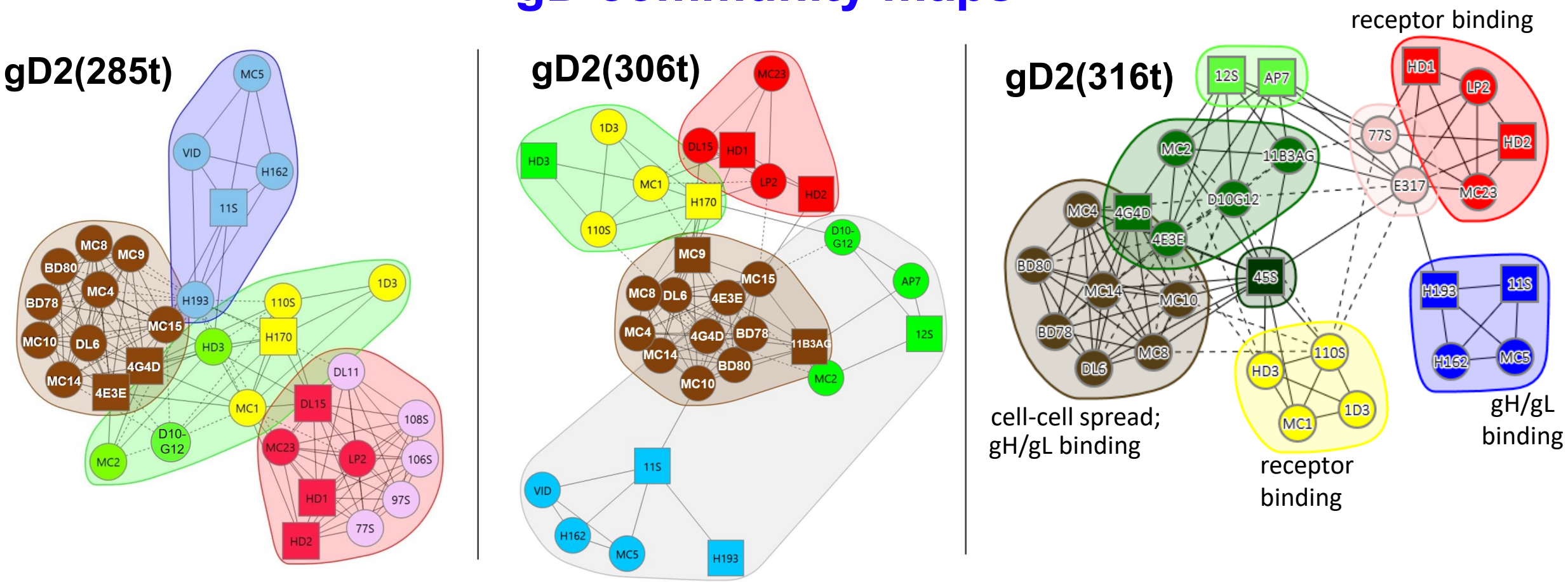
Binding = no competition

MAbs bind to different epitopes

No binding = competition

MAbs bind to same/overlapping epitopes

gD community maps

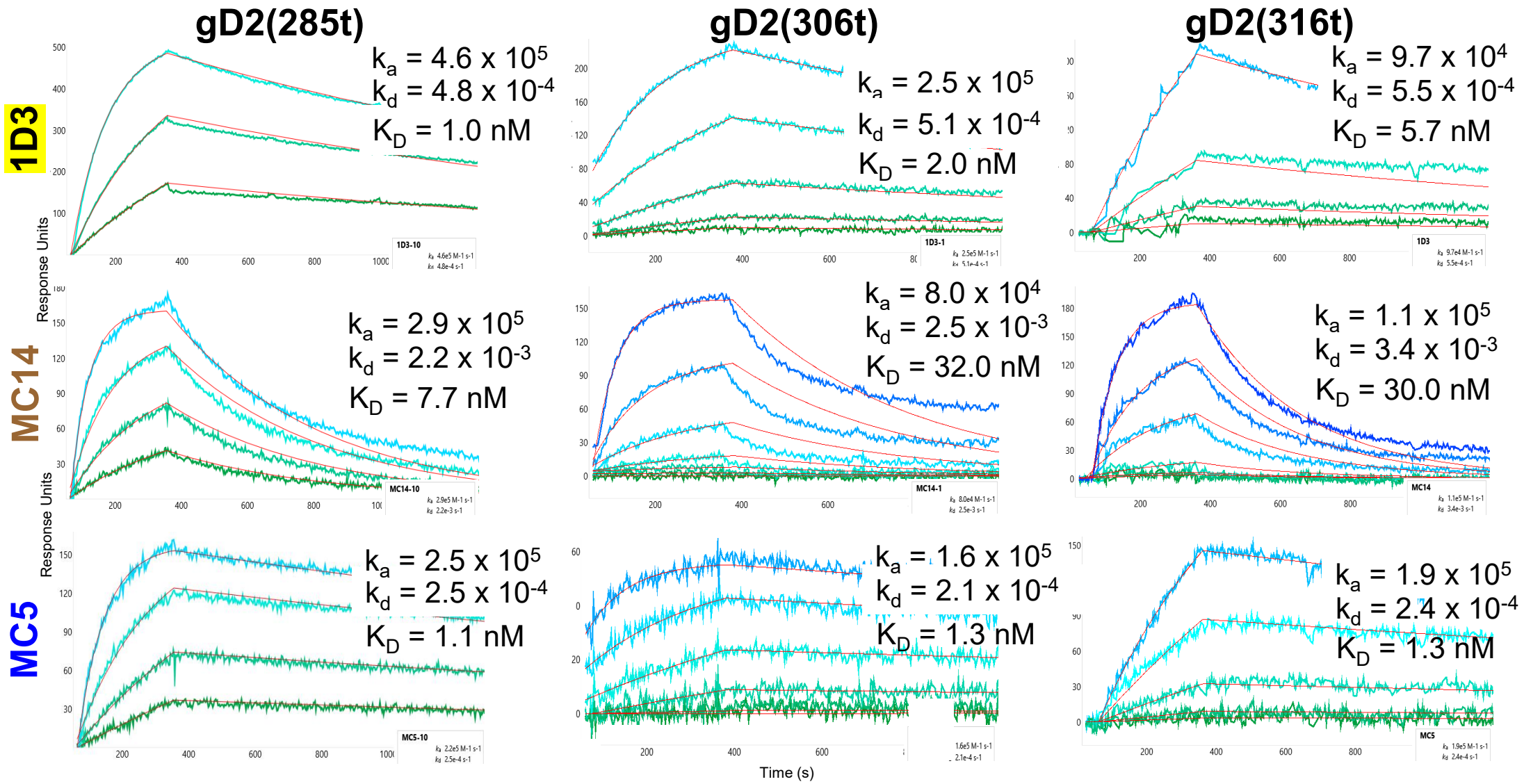


- Distribution of the communities on the map depends on the size of gD
- **Pink** MAbs lost on 306t/316t due to MPR tail occluding epitopes, while **AP7/12S** lost on 285t bc epitope partially deleted
- MAb functions can be overlaid onto communities

285t, 316t maps were generated with SPRi competition using the Wasatch CFM/Ibis biosensor and published previously (Cairns et al. 2015)

Next: Kinetics of binding

Kinetics of binding for each gD protein are similar



- **Brown** community MAbs (**MC14**) have slight kinetic difference between forms
- These epitopes are located near the gD C-term so this makes sense

gD Summary

Comparing the 285t, 306t, 316t forms:

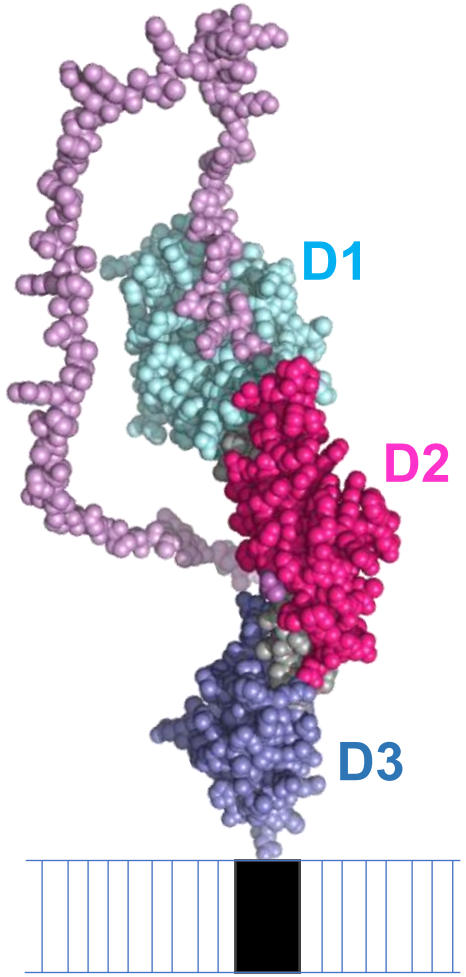
- No difference in ELISA signals when using PAbs
- Major difference in binding between 285t vs longer gD forms for **pink** community MAbs
  - * C-terminal gD tail occludes binding in longer gD forms
- Some **green** & **brown** community MAbs (e.g. **AP7**, **4E3E**) do not bind 285t (epitopes overlap the MPR tail)
- Kinetic analysis shows minor difference in K_D for gD forms when binding to **brown** community MAbs
  - * **brown** community located near the gD C-terminus

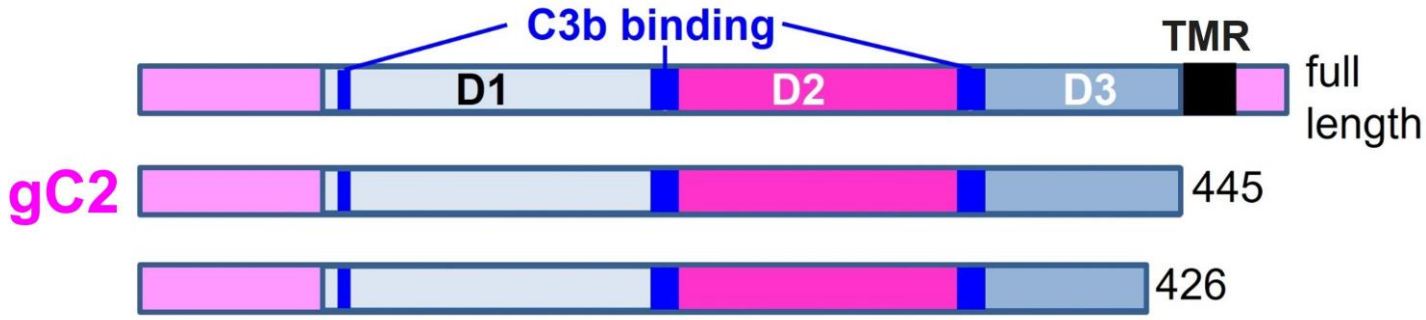
gD proteins clearly behave differently based on size

Next: gC2

Attachment & immune evasion protein: gC

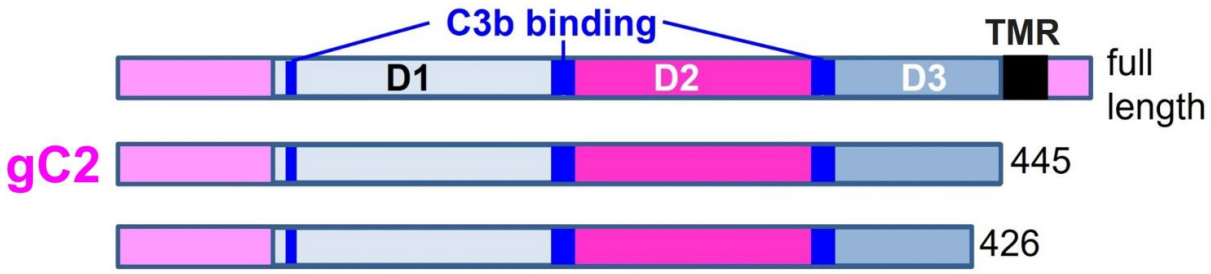
gC2 predicted structure
(AlphaFold)



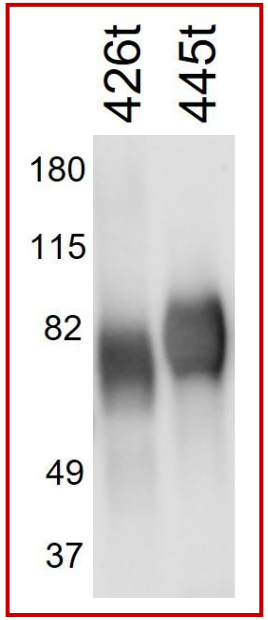


- **gC** has role in virus-cell attachment, binding to cell surface heparan sulfate proteoglycan
- **gC** binds C3b, inhibiting complement activation and promoting immune evasion

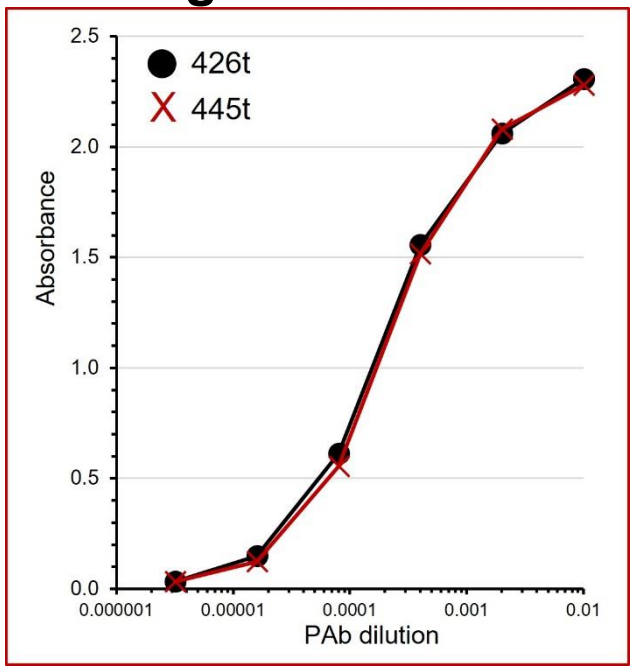
Soluble forms of gC



gC2 Western blot



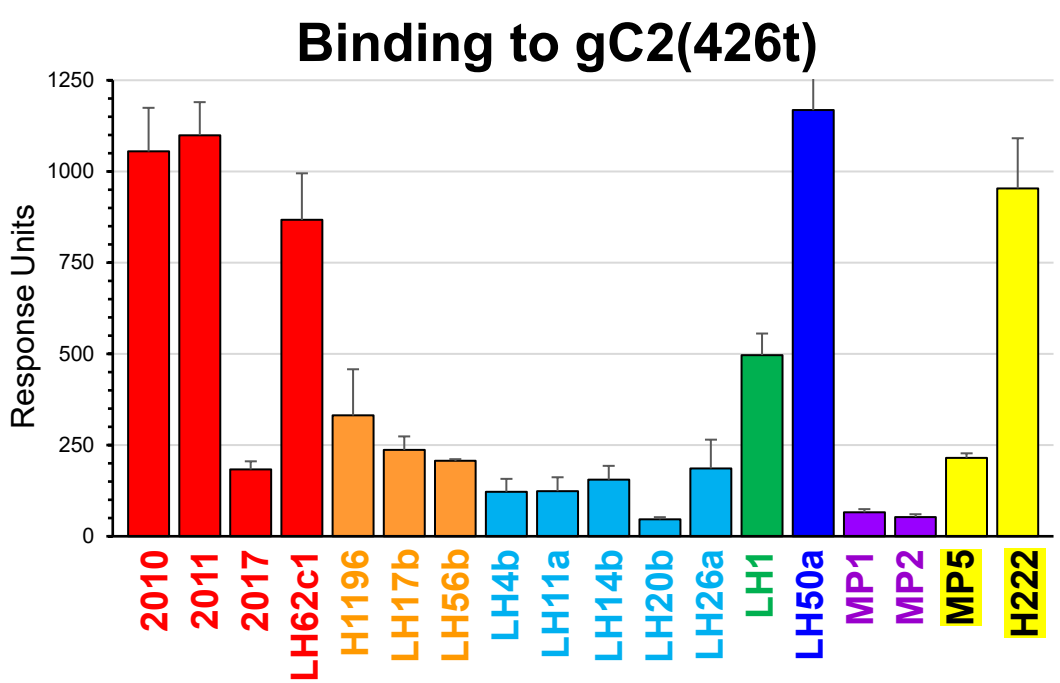
gC2 ELISA

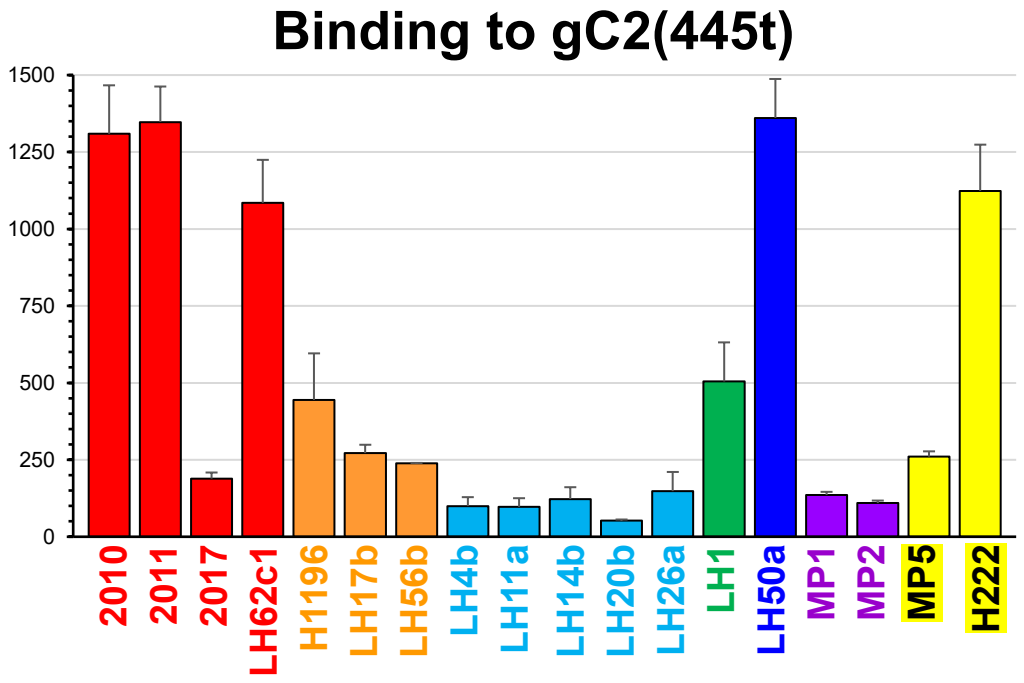


- Western shows size and glycosylation differences between the 3 proteins
- ELISA shows **no differences** in signals

Next: Binding to MAbs

MAb binding to different forms of gC

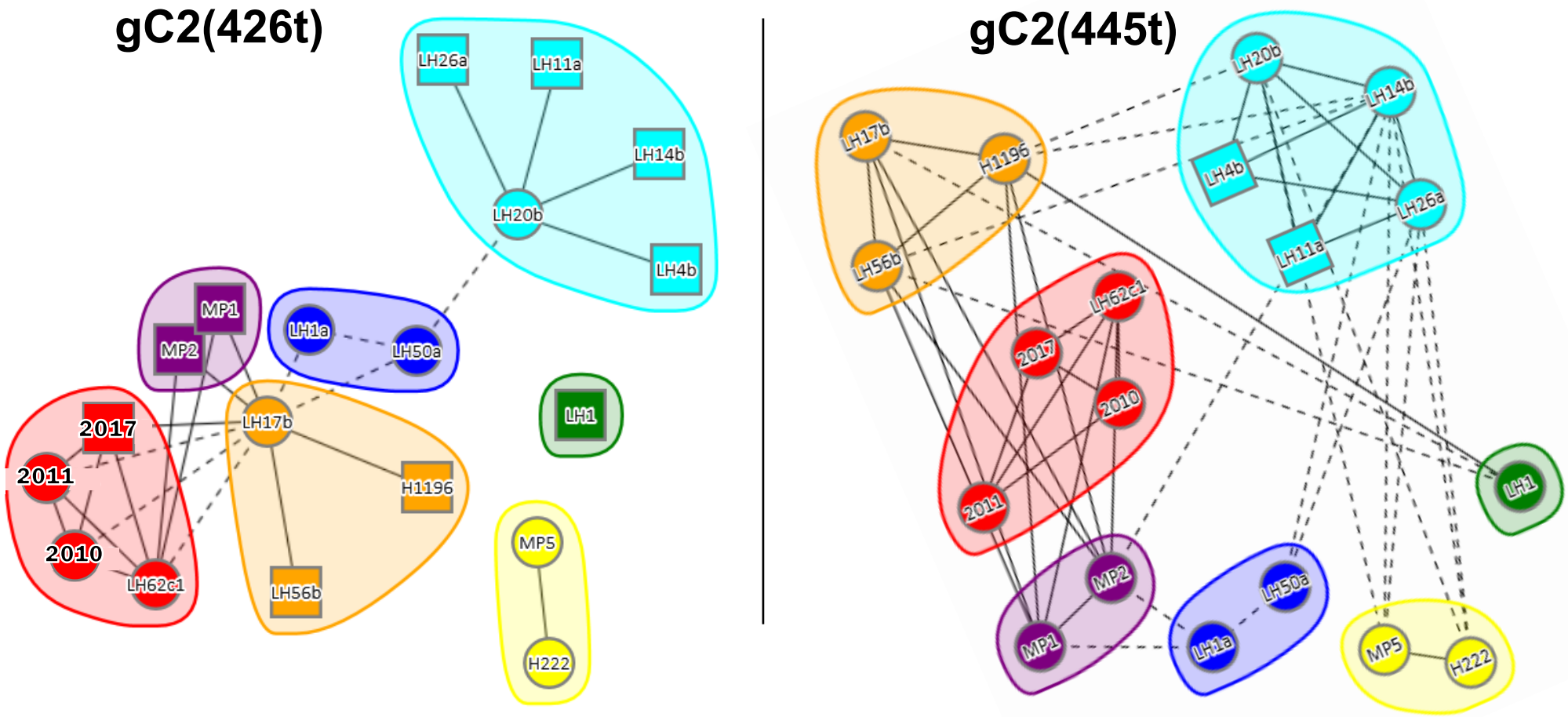




Pattern of binding is the same between gC proteins;
they see MAbs similarly no matter the form

Next: Generation of MAb community maps

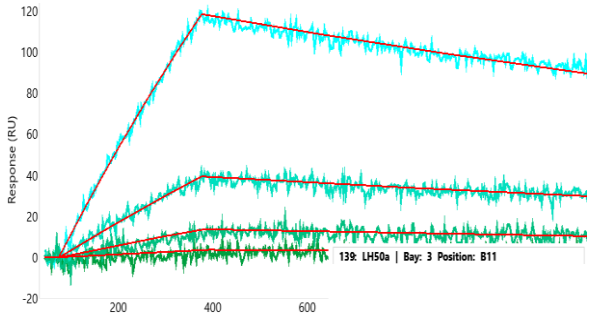
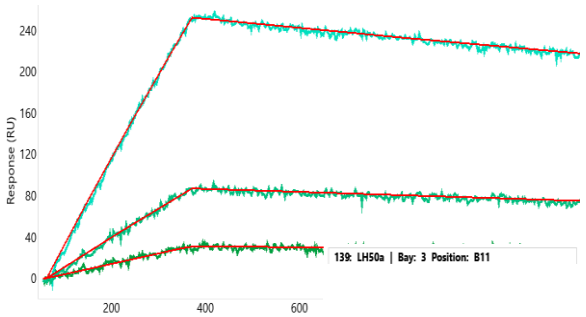
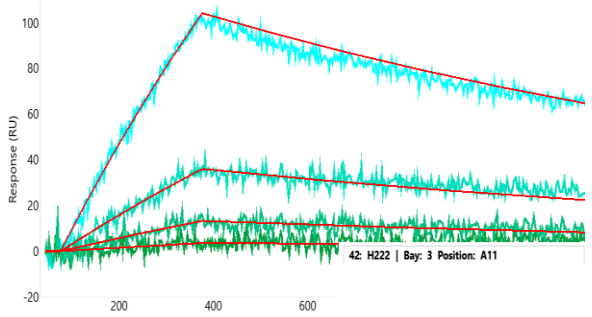
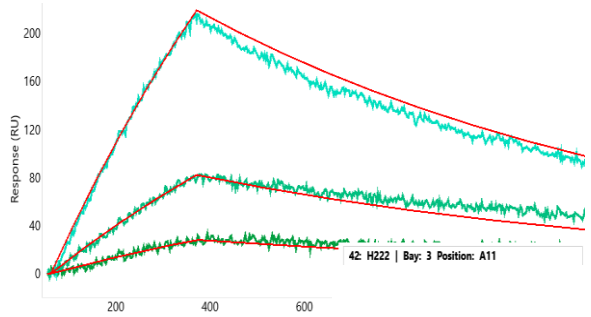
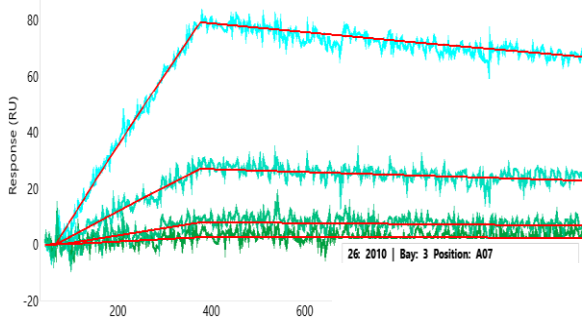
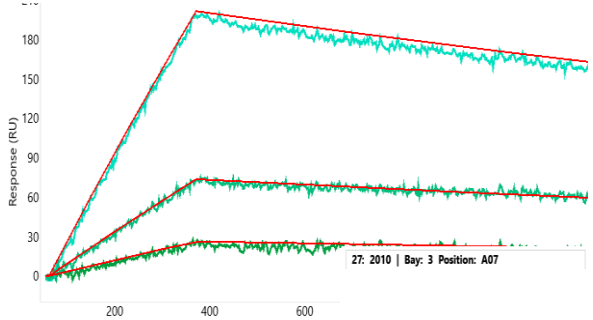
gC community maps



Community groupings the same between gC proteins but number of inter-community competitions is greater on 445t (likely due to less “square”/analyte only MAbs in the 445t mapping)

Next: Kinetics

Kinetics of binding for each gC protein are similar

	gC2(426t)		gC2(445t)	
LH50a		k_a = 1.6 x 10^5 k_d = 3.4 x 10^{-4} K_D = 2.2 nM		k_a = 2.3 x 10^5 k_d = 1.8 x 10^{-4} K_D = 0.8 nM
H222		k_a = 1.3 x 10^5 k_d = 5.9 x 10^{-4} K_D = 4.6 nM		k_a = 2.8 x 10^5 k_d = 9.8 x 10^{-4} K_D = 3.5 nM
2010		k_a = 5.9 x 10^4 k_d = 2.1 x 10^{-4} K_D = 3.5 nM		k_a = 1.8 x 10^5 k_d = 2.6 x 10^{-4} K_D = 1.4 nM

gC Summary

For gC2(426t) and gC2(445t):

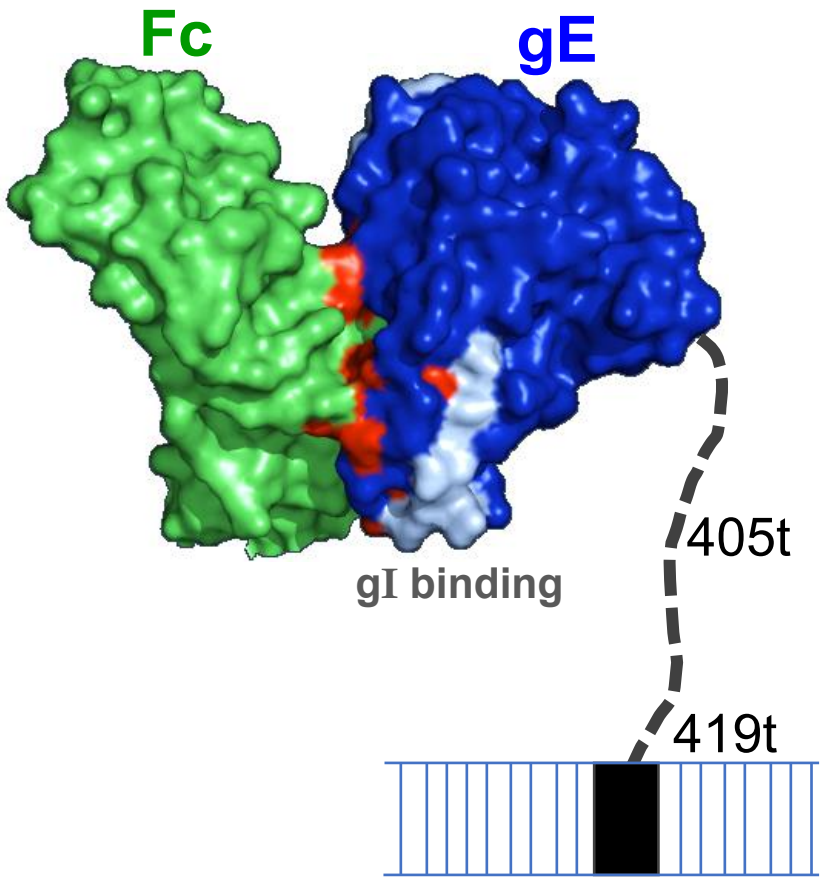
- No difference in ELISA signals when using PAbs
- No difference in MAb binding pattern
- Very similar community maps
- No difference in kinetics

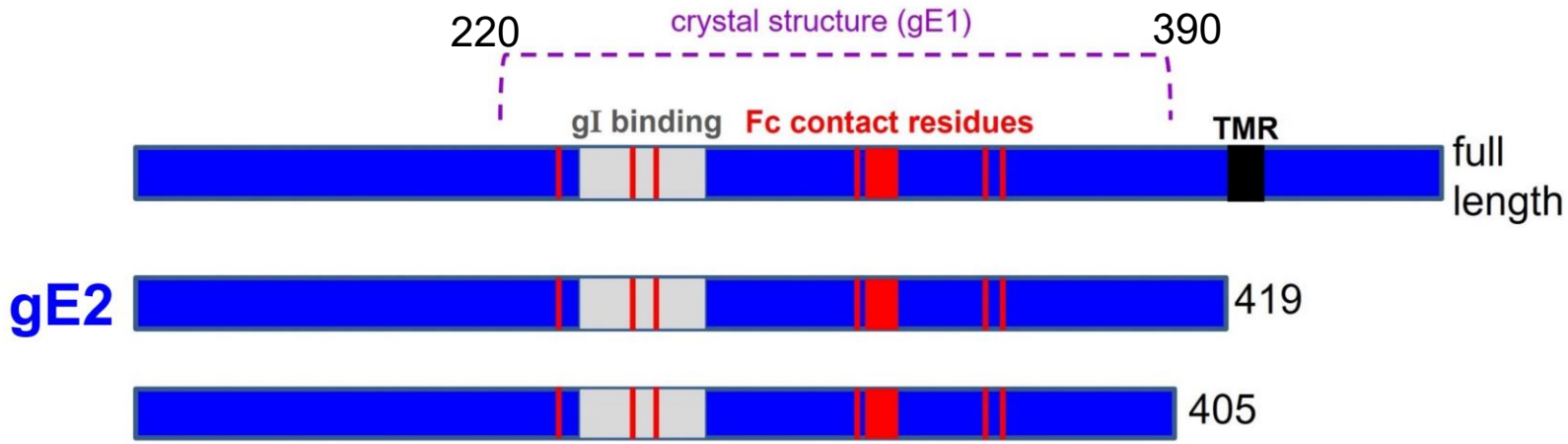
So far the two soluble gC forms are interchangeable

Next: gE2

Immune evasion protein: gE2

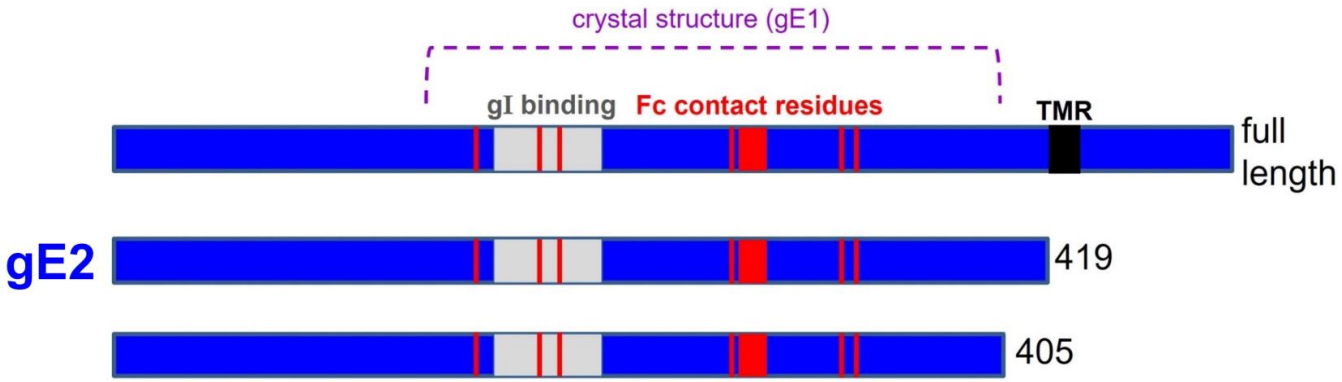
gE1 crystal structure



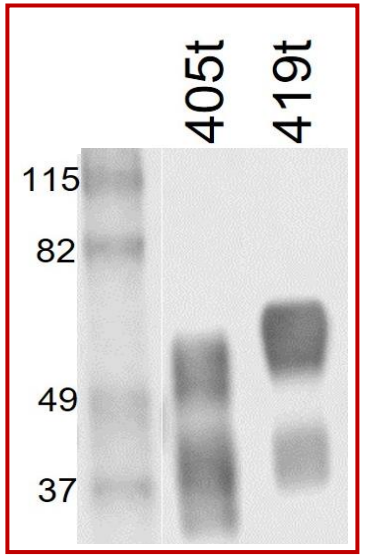


- **gE** forms a heterodimer with **gI** and has a role in cell-cell spread, neuronal transport, and **immune evasion**
- **gE** contains an Fc binding domain; by binding the Fc end of immune IgG, it protects the virus from antibody- and complement-dependent neutralization

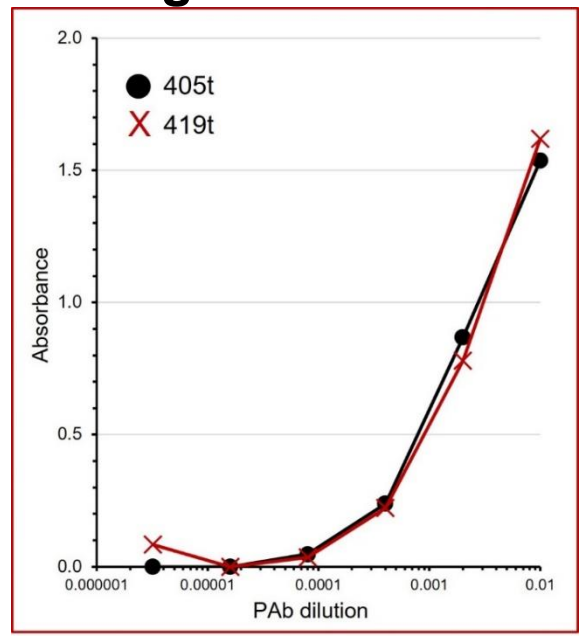
Soluble forms of gE



gE2 Western blot



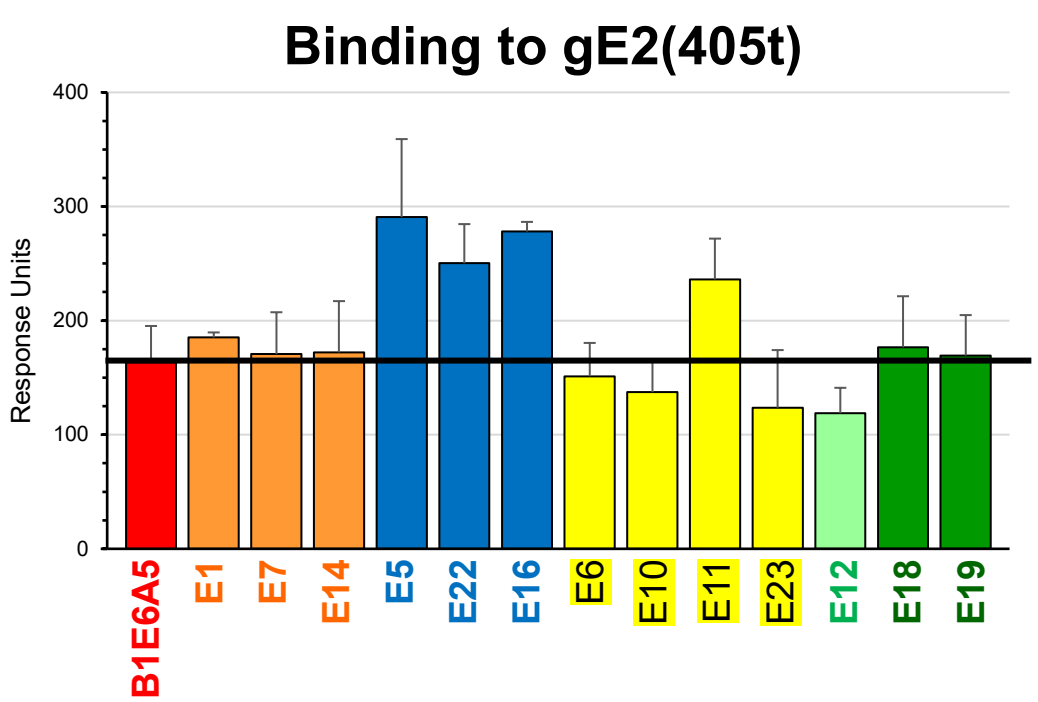
gE2 ELISA

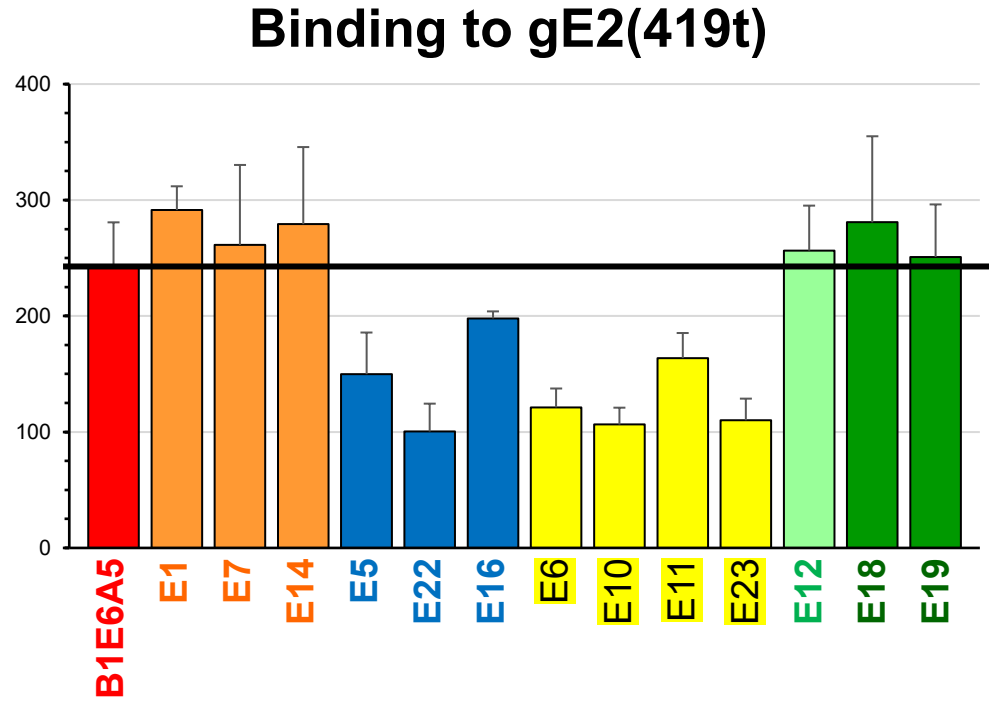


- Western shows size and glycosylation differences between gE proteins
- ELISA shows no difference in signals

Next: Binding to MAbs

Differences in MAb binding between 405t & 419t

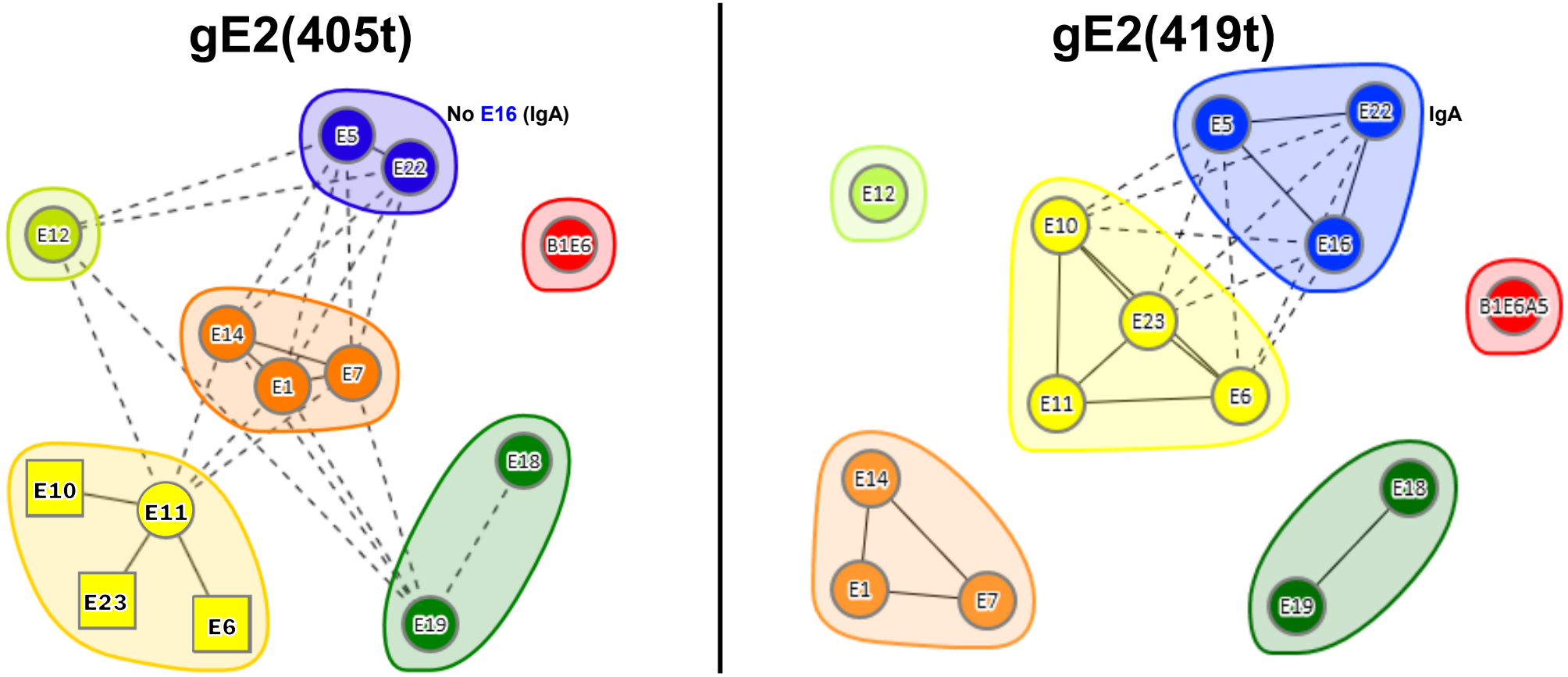




All MAbs bind to each gE protein, but the **blues** and yellows show a dip in relative signal on 419t

Next: Generation of MAb community maps

gE Community Maps



- MAbs communities remain the same but inter-community competitions change depending on gE size
- Positions of **orange** & yellow communities have changed

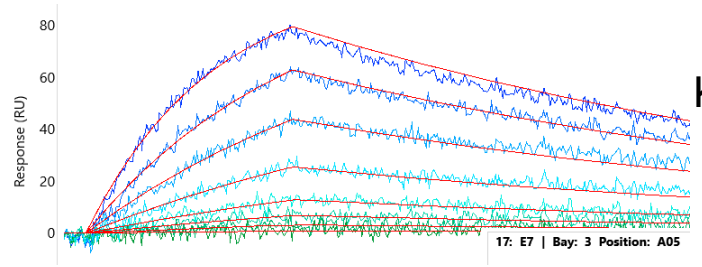
Next: Kinetics

Kinetics

gE2(405t)

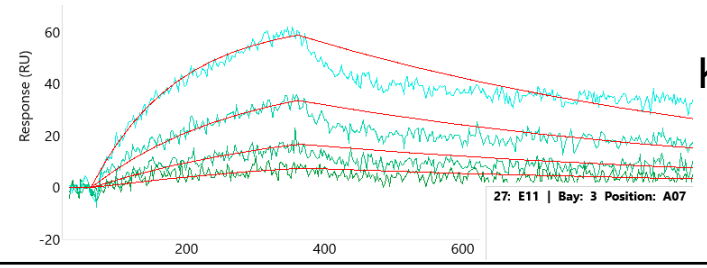
E7

$k_a = 4.8 \times 10^4$
$k_d = 7.9 \times 10^{-4}$
$K_D = 16.0$ nM



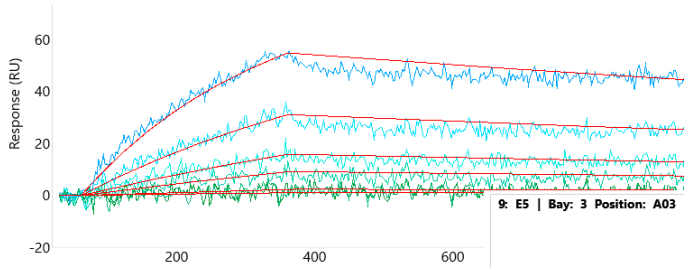
E11

$k_a = 1.0 \times 10^6$
$k_d = 1.3 \times 10^{-3}$
$K_D = 1.3$ nM



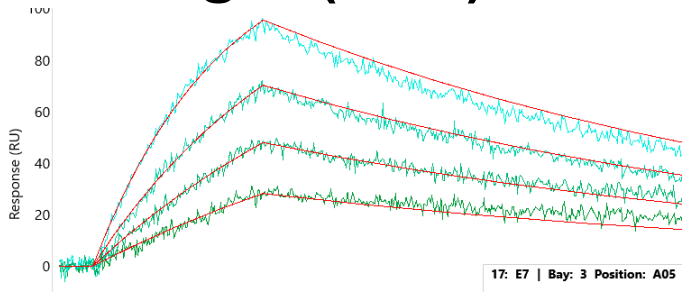
E5

$k_a = 1.3 \times 10^5$
$k_d = 3.6 \times 10^{-4}$
$K_D = 2.7$ nM

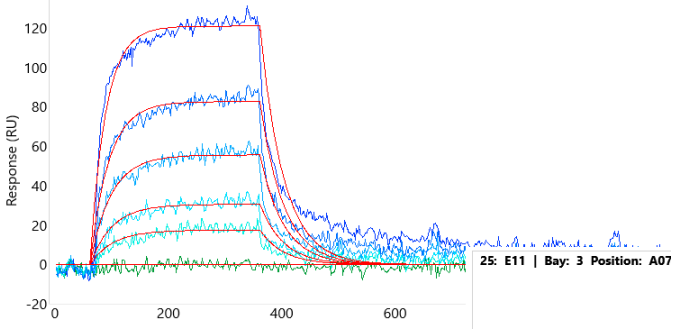


gE2(419t)

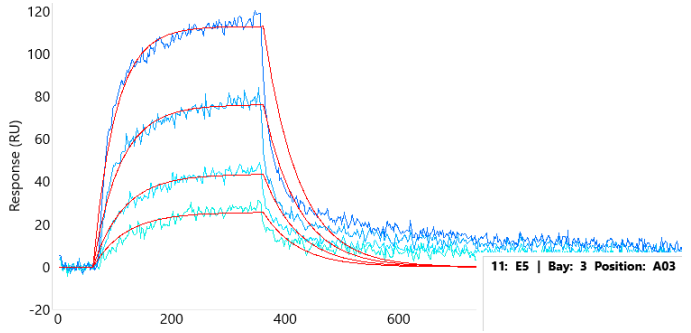
$k_a = 4.0 \times 10^5$
$k_d = 7.2 \times 10^{-4}$
$K_D = 1.8$ nM



$k_a = 1.7 \times 10^5$
$k_d = 1.5 \times 10^{-2}$
$K_D = 87.0$ nM



$k_a = 1.8 \times 10^5$
$k_d = 1.6 \times 10^{-2}$
$K_D = 90.0$ nM



- Striking kinetic difference between gE forms for all **blue** community MAbs and one yellow MAb (E11)
- This tracks with the differences seen in max MAb binding

gE Summary

For gE2(405t) and gE2(419t):

- No difference in ELISA signals when using PAbs
- Difference in level of MAb binding for all **blue** & yellow MAbs
- Community groups are the same but position of communities/inter-community relationships are different
- Striking kinetic difference on all **blue** MAbs & E11

The two gE proteins clearly behave differently based on size

OVERALL SUMMARY

- **gD**: SIZE MATTERS
  * **pink** MAbs bind poorly to longer forms due to MPR tail obscuring the epitopes
  * some **green** MAbs do not bind short (285t) form; epitopes partly located within the tail
  * some **brown** MAbs bind poorly to 285t due to epitopes being near this truncation
  * gD binding kinetics to MAbs remain fairly consistent between forms

- **gC**: no antigenic difference detected between long & short forms

- **gE**: SIZE MATTERS
  * blue & yellow MAbs have lower max binding RU to longer gE form
  * kinetics of **blue** MAbs & **E11** differ between long & short forms
  * position of gE epitopes currently unknown, but presence of the MPR affects the antigenic structure in the region of these MAb epitopes

Does size matter when used as vaccine antigens? When gC binds to C3b? When gE binds to Fc?

****to be determined****