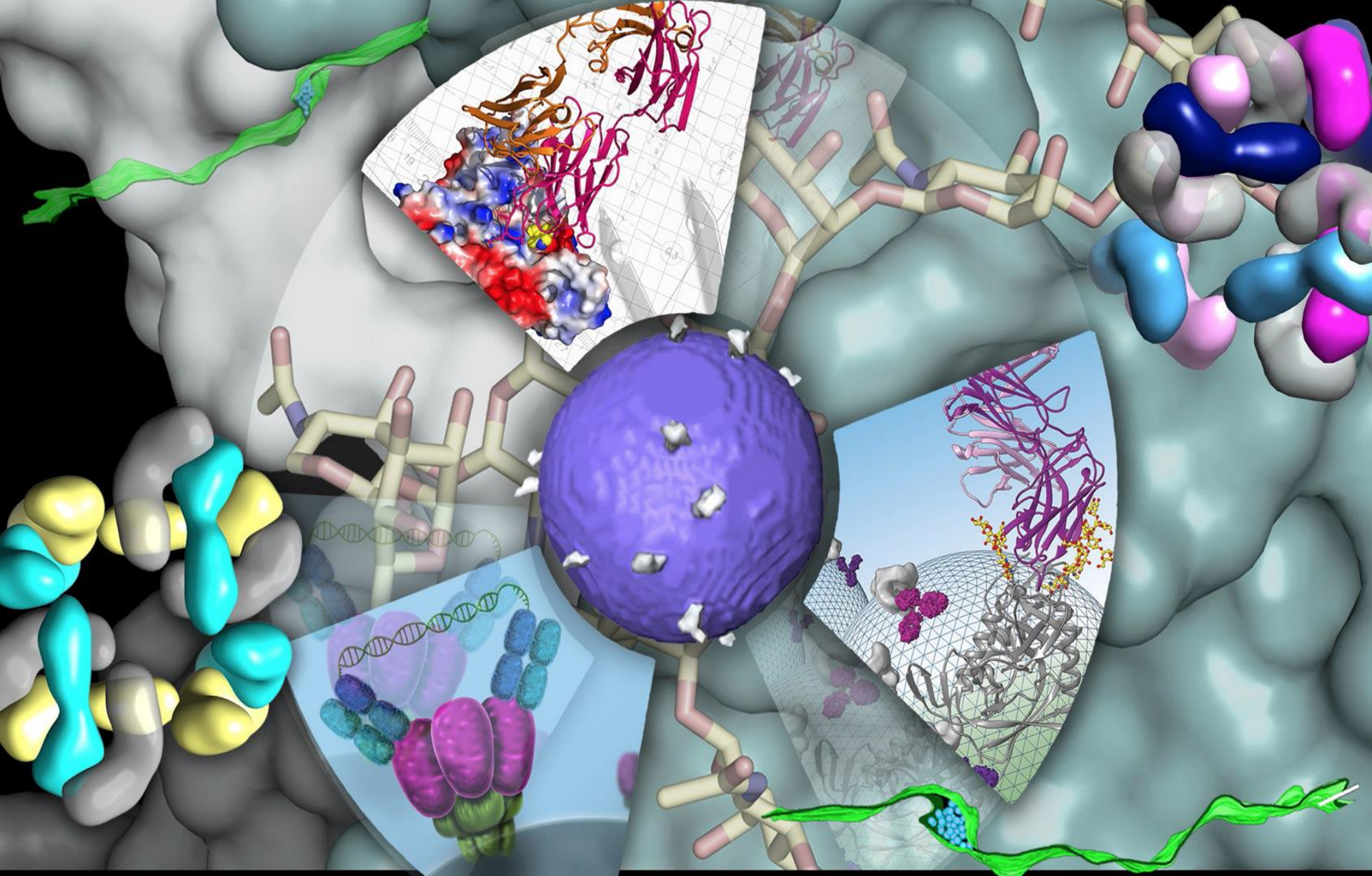
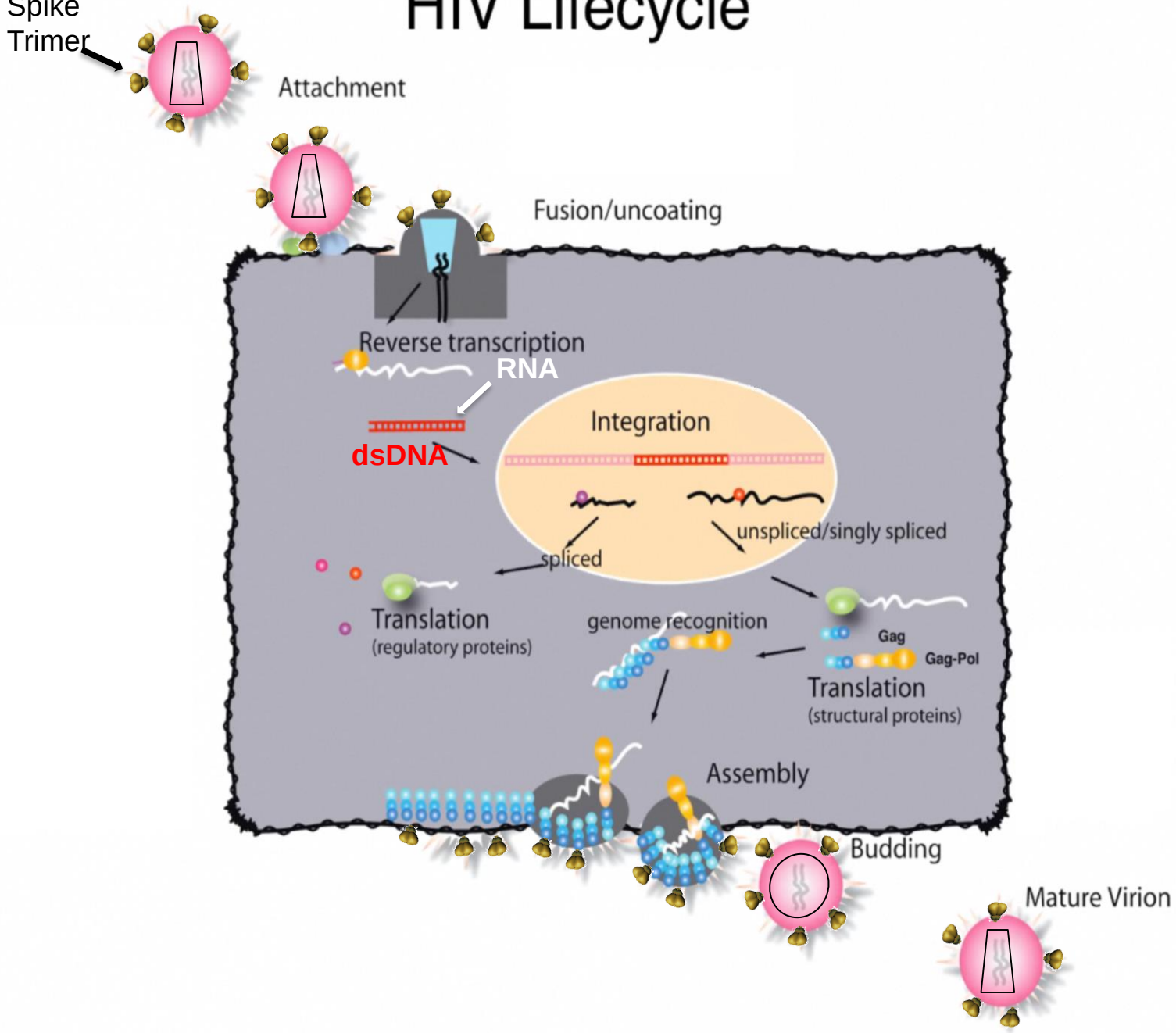


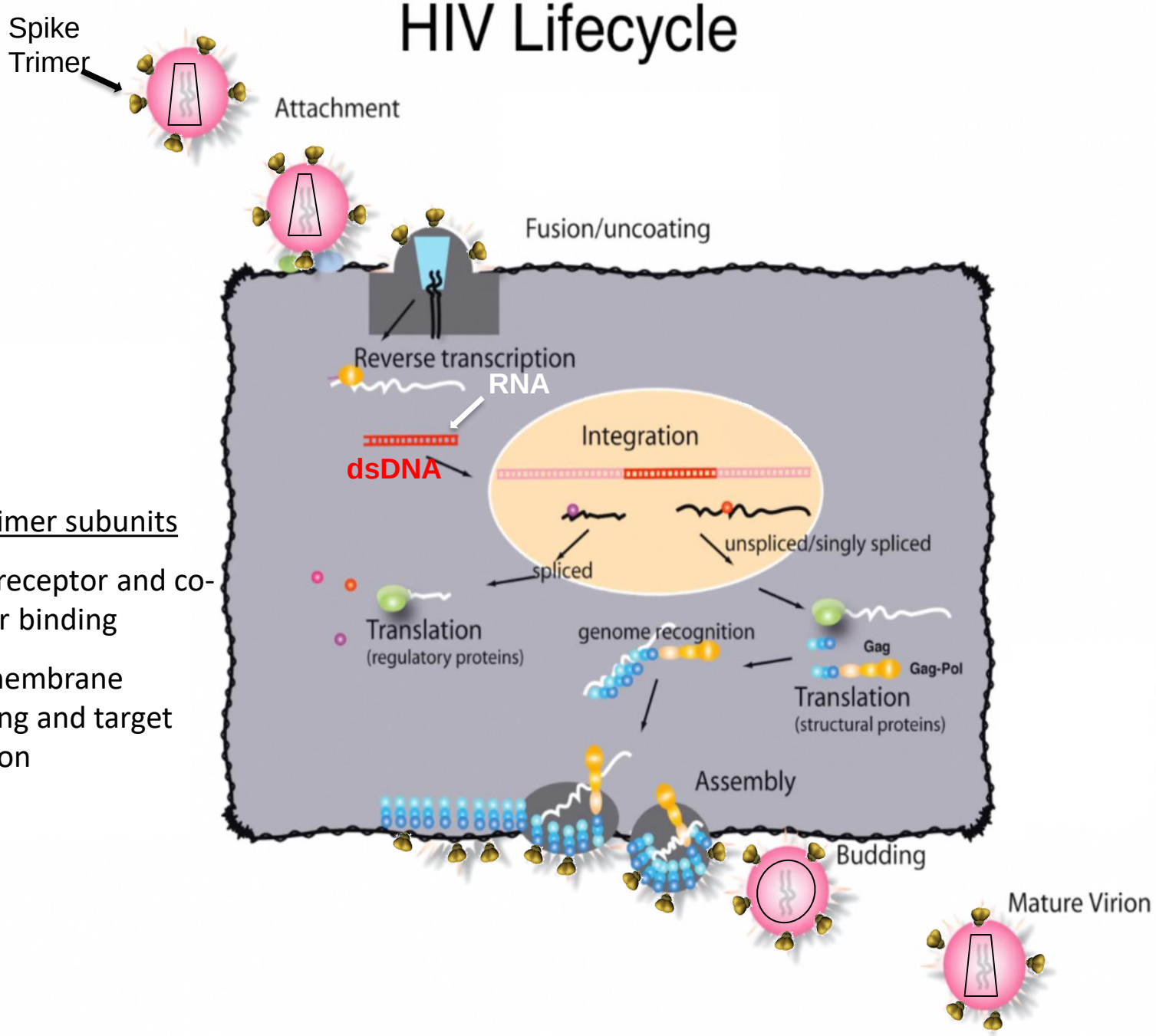
A Molecular Arms Race: The Immune System versus HIV

Pamela J. Bjorkman, Division of Biology and Biological Engineering, Caltech

HIV Lifecycle



HIV Lifecycle

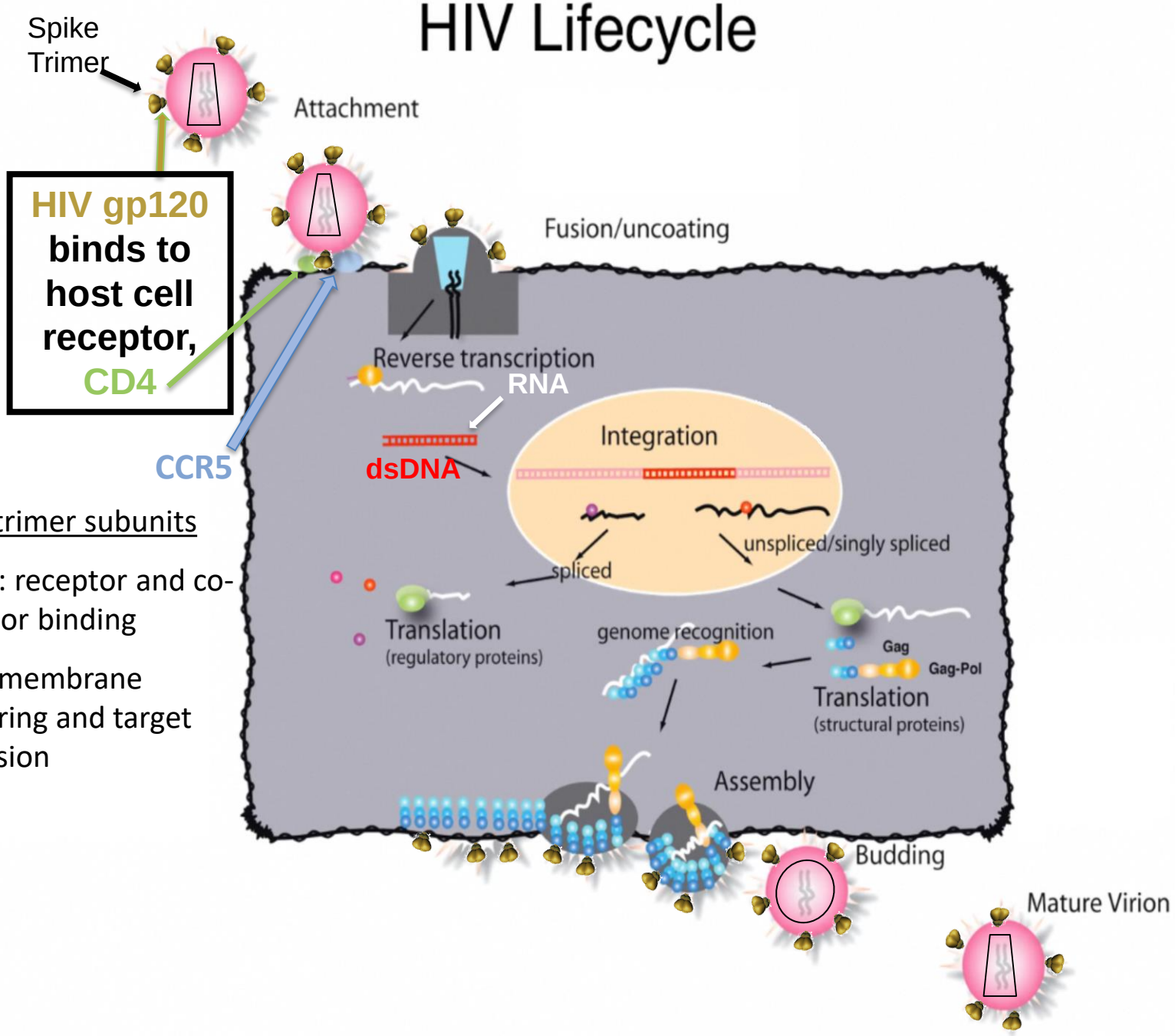


<u>Spike trimer subunits</u>

gp120: receptor and co-receptor binding

gp41: membrane anchoring and target cell fusion

HIV Lifecycle



Spike trimer subunits

gp120: receptor and co-receptor binding

gp41: membrane anchoring and target cell fusion

HIV Lifecycle

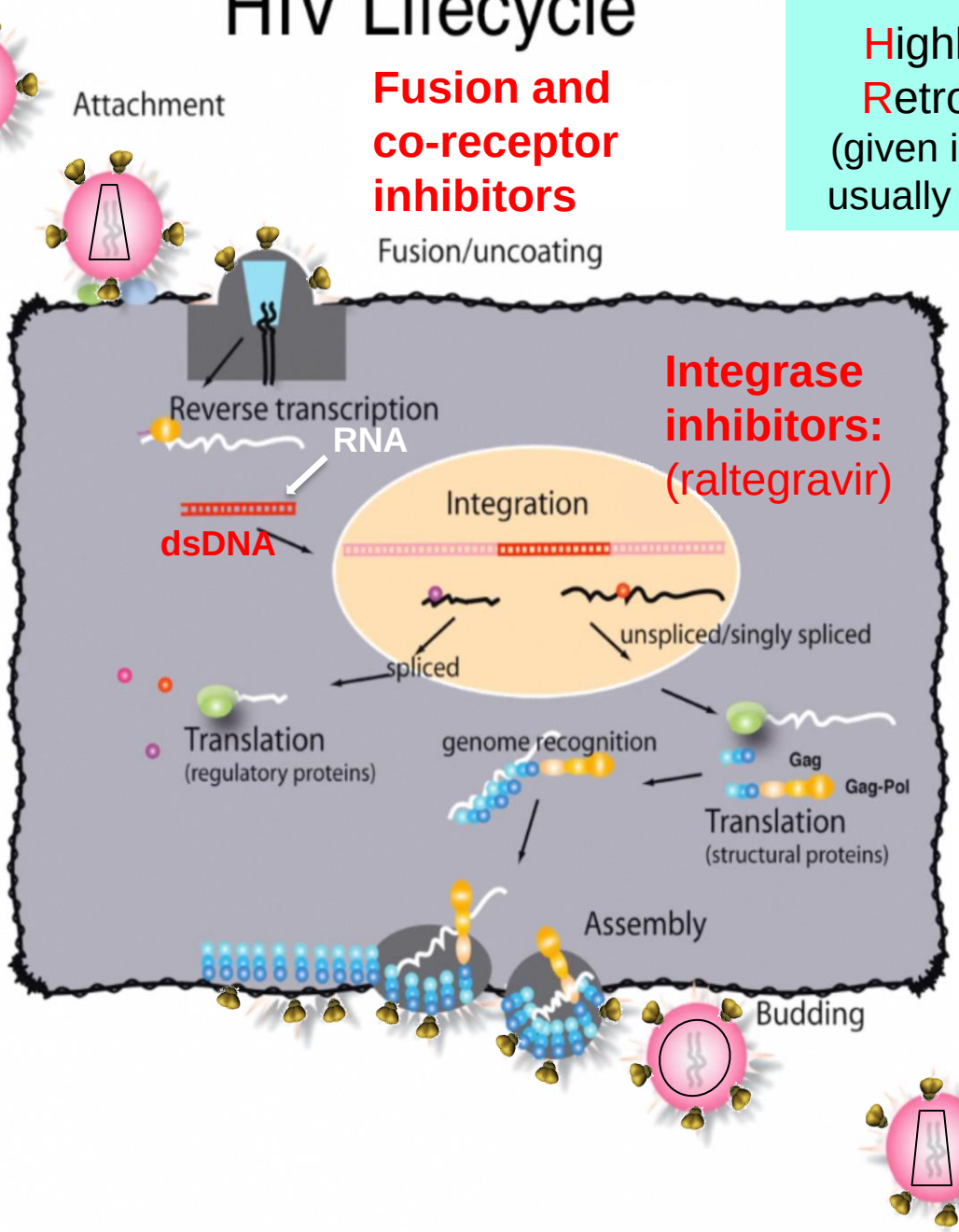
HAART
Highly-Active Anti-Retroviral Therapy
(given in combinations – usually 3 different drugs)

Fusion and co-receptor inhibitors

Reverse transcriptase inhibitors:

Nucleoside analogs (AZT)

Non-nucleoside analogs (nnRTIs) (Efavirenz)

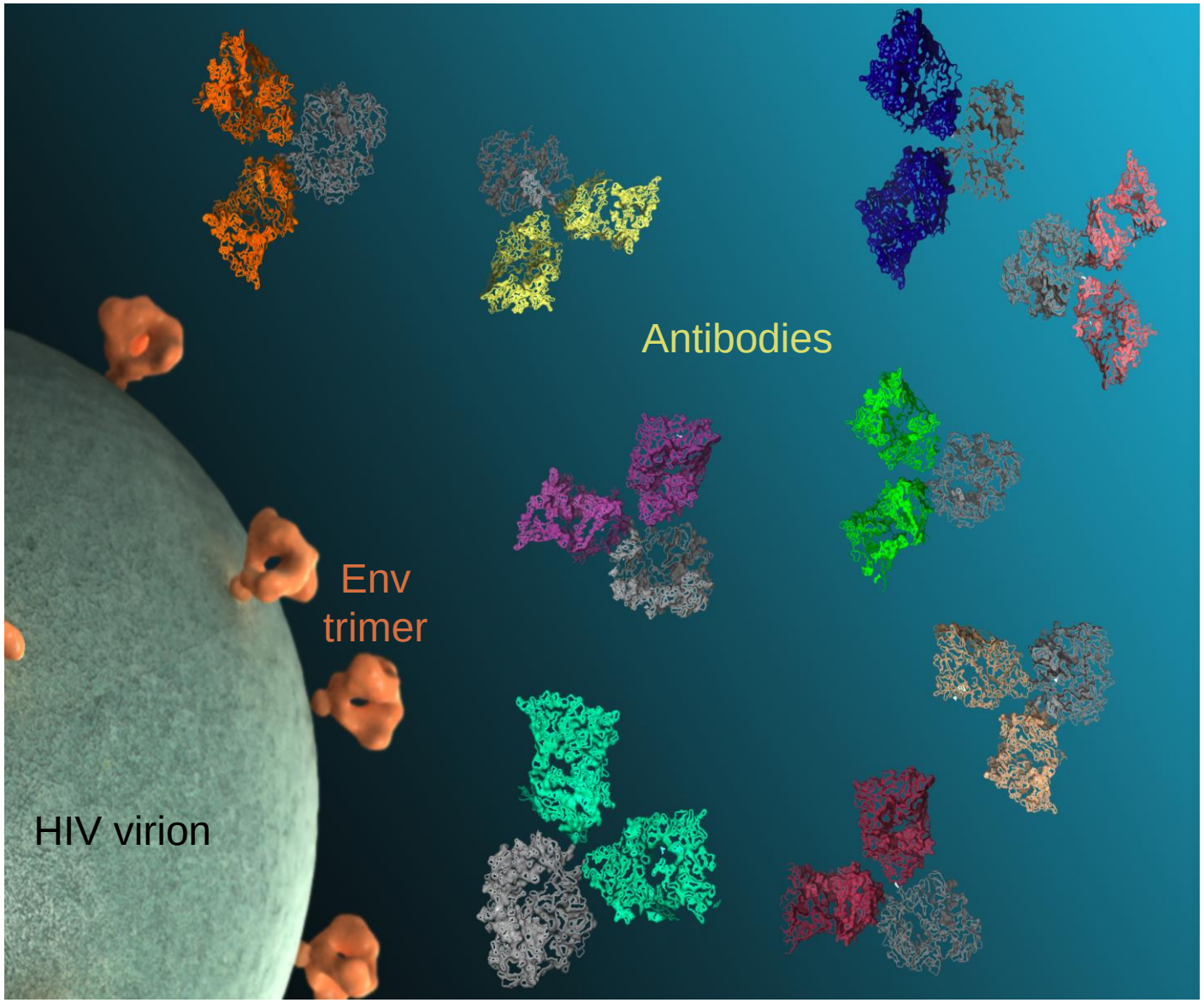


Integrase inhibitors:
(raltegravir)

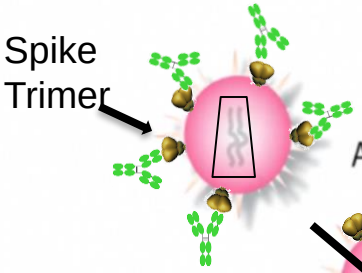
Only ~37% of infected people are being treated with HAART.

Protease inhibitors:
(crixivan, norvan)

Ideal way to protect against HIV/AIDS: A vaccine

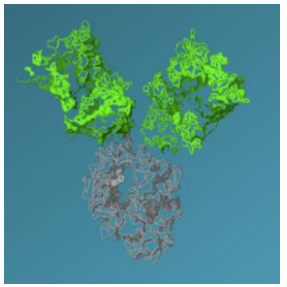


HIV Lifecycle

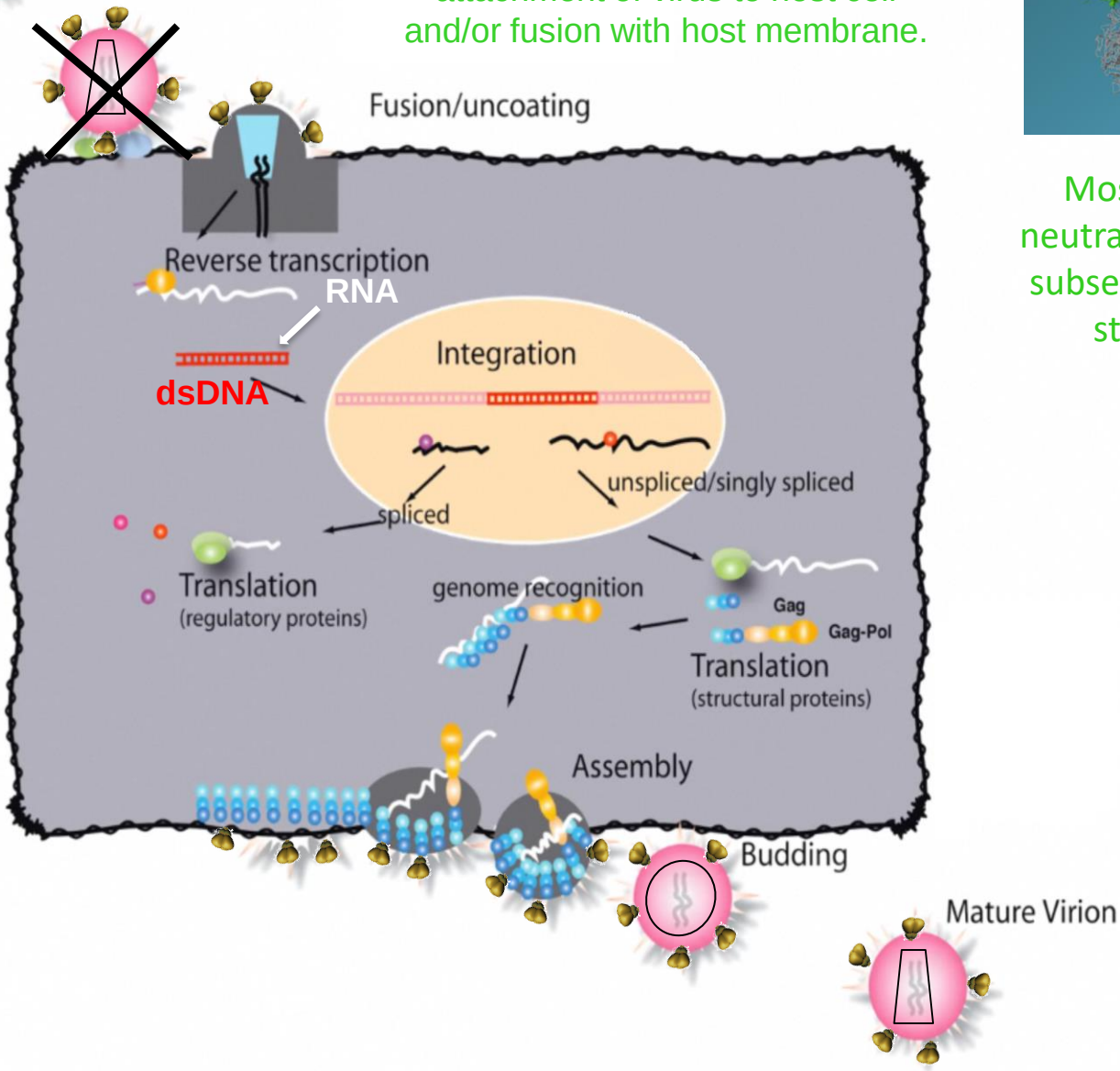


Attachment

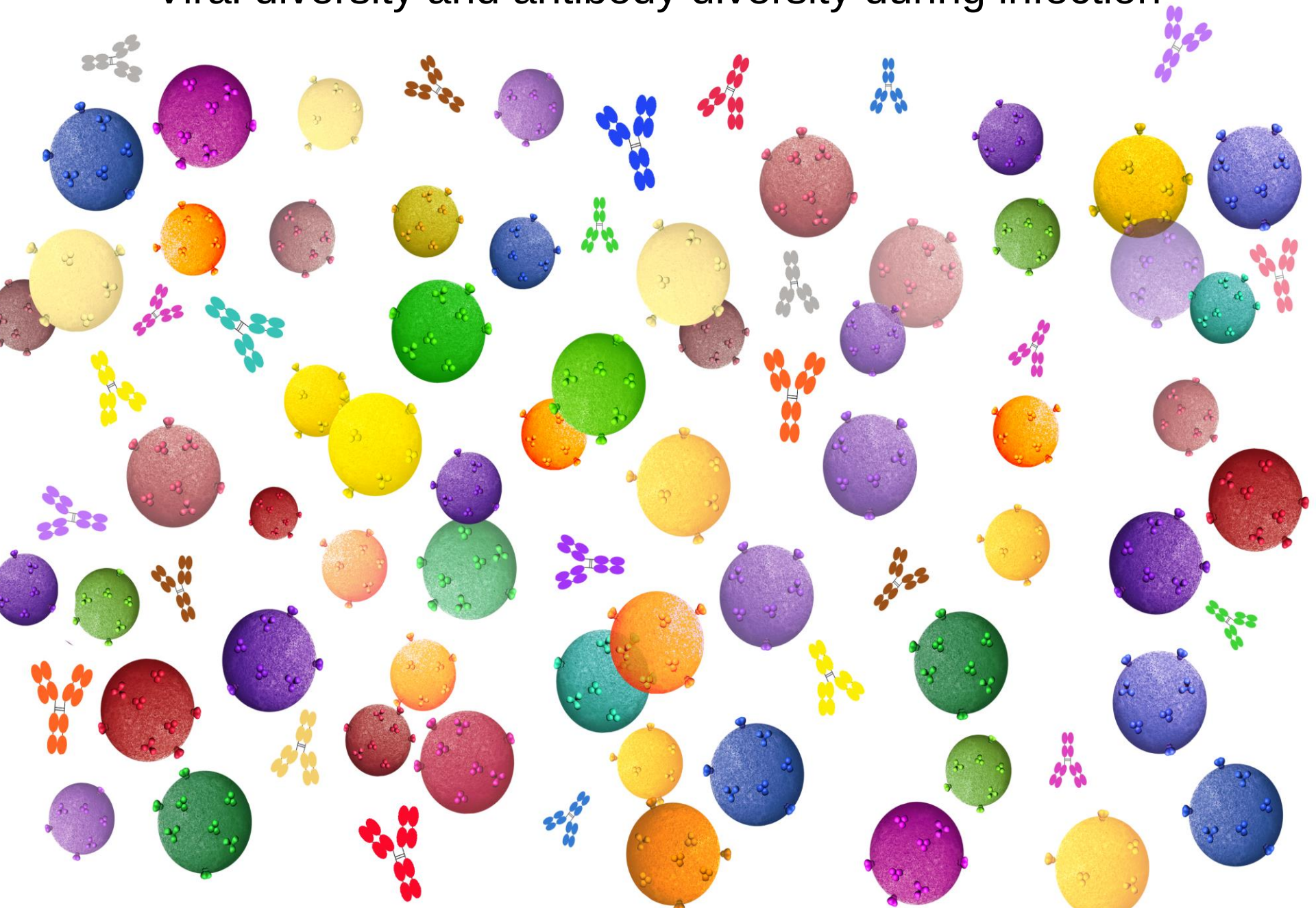
Neutralizing antibodies (NAbs) interfere with attachment of virus to host cell and/or fusion with host membrane.



Most NAbs neutralize only a subset of HIV-1 strains.

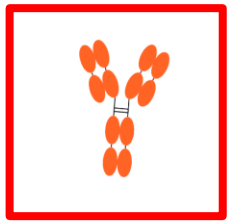


Viral diversity and antibody diversity during infection



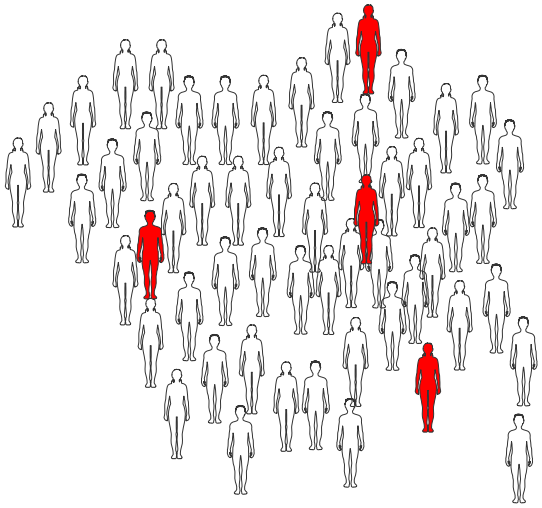
Characterize antibodies from HIV-infected patients

Most antibodies are strain-specific, but rare antibodies from rare patients are broadly neutralizing.



We call these **bNAbs** for **b**roadly **N**eutralizing **A**nti**b**odies.

Broadly neutralizing anti-HIV antibodies



5-10% of HIV+ individuals develop broadly neutralizing serum antibodies

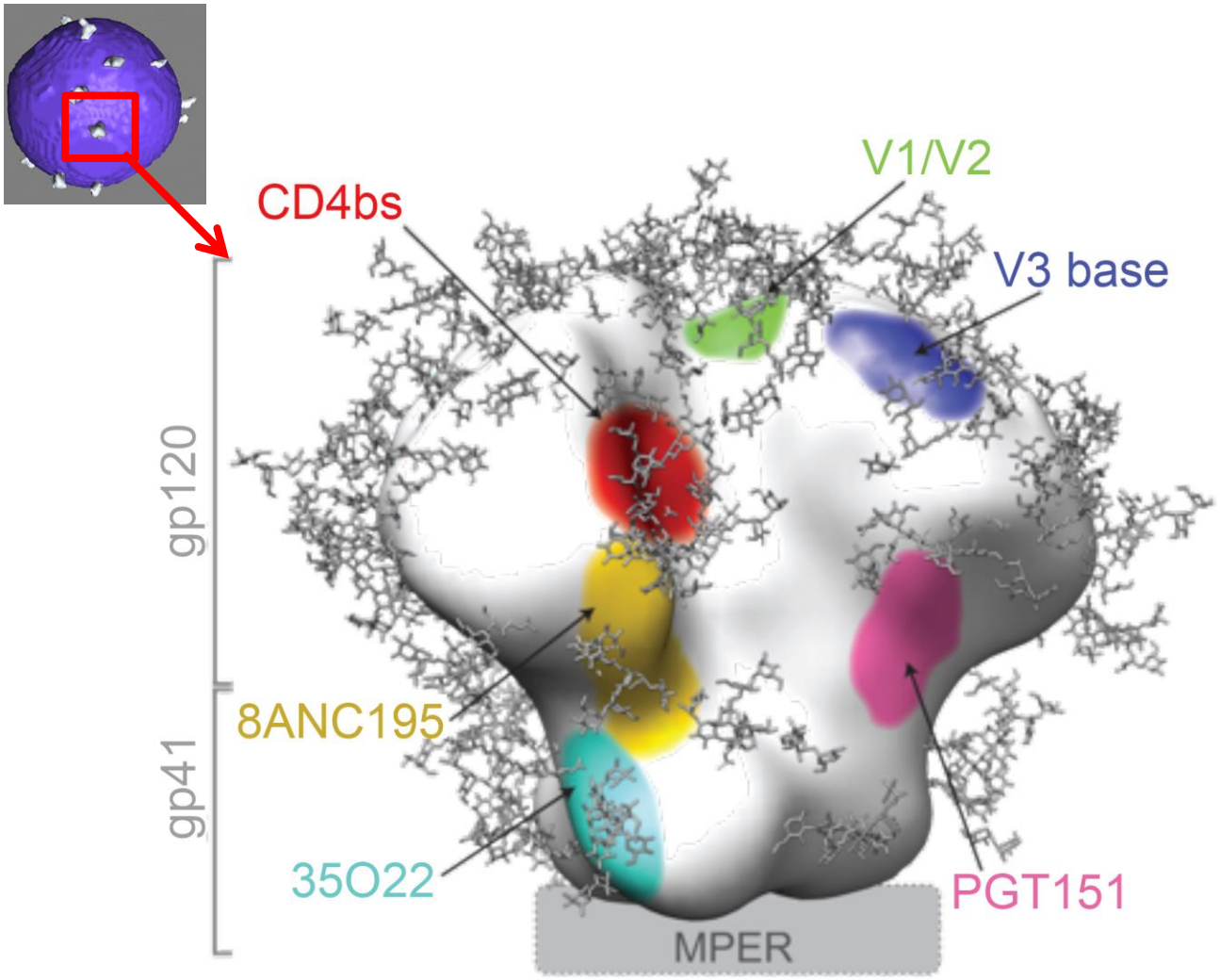
We can't (yet) make a vaccine to elicit bNAbs in animal models or humans.

bNAbs could be used for passive delivery-based prophylaxis or therapy in humans.

bNAbs protect primates in virus challenge experiments

Baba et al., Nat Med (2000); Mascola et al., Nat Med (2000)
Ferrantelli et al., JID (2004); Hessell et al., Nature (2007)
Hessell et al., PLoS Pathog (2009)

Structural studies of bNAb epitopes reveal mechanisms by which NAbs avoid or utilize the glycan shield on Env trimer



History of bNAbs against HIV-1

1990s – 2008:
b12, 4E10, 2F5, 2G12

2009-13:
dozens of new antibodies
Including
V1V2: PG9/PG16
V3: PGTs
CD4bs: VRC01 family
MPER: 10e8 (anti-gp41)

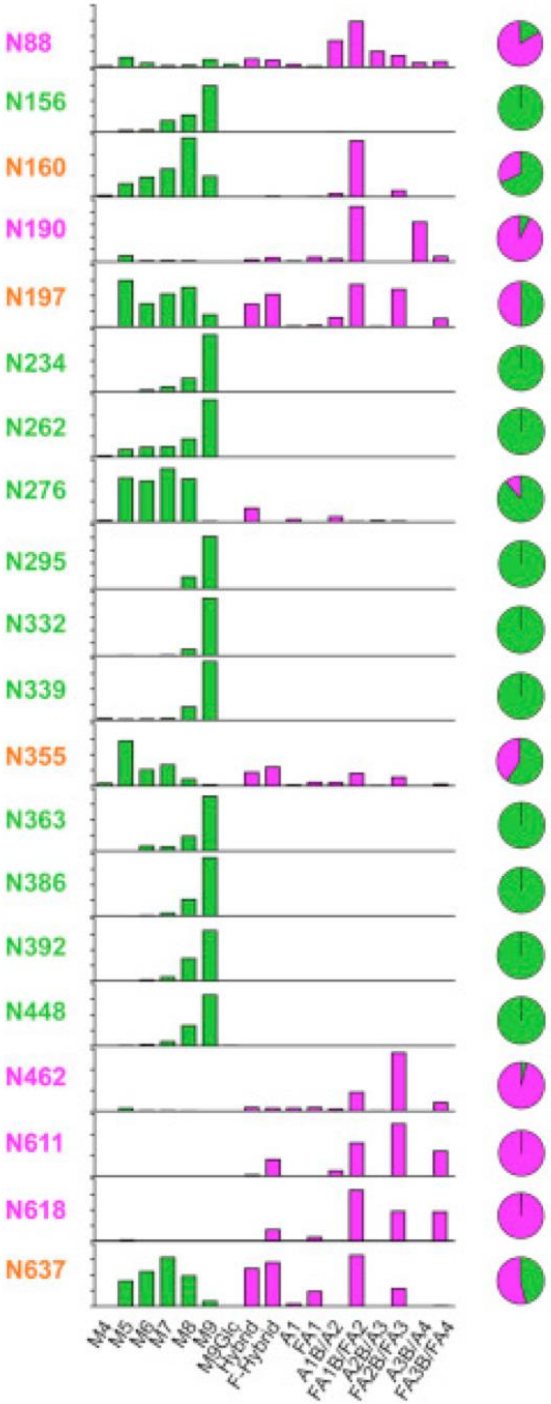
2014:
gp120/gp41 interface Abs
8ANC195
35O22
PGT151

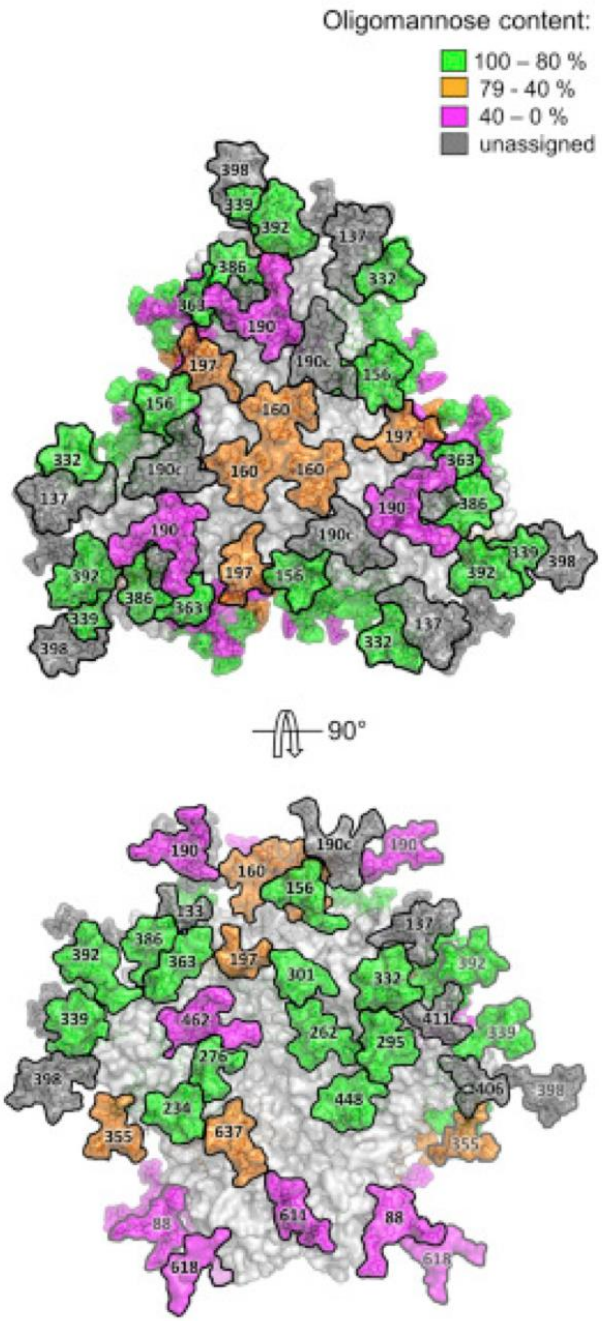
HIV-1 spikes are heavily glycosylated (~50% of mass; ~30 PNGSs per monomer).

Mass spectroscopy has identified *N*-glycans at individual PNGSs in soluble HIV-1 Env trimer (BG505 SOSIP.664)

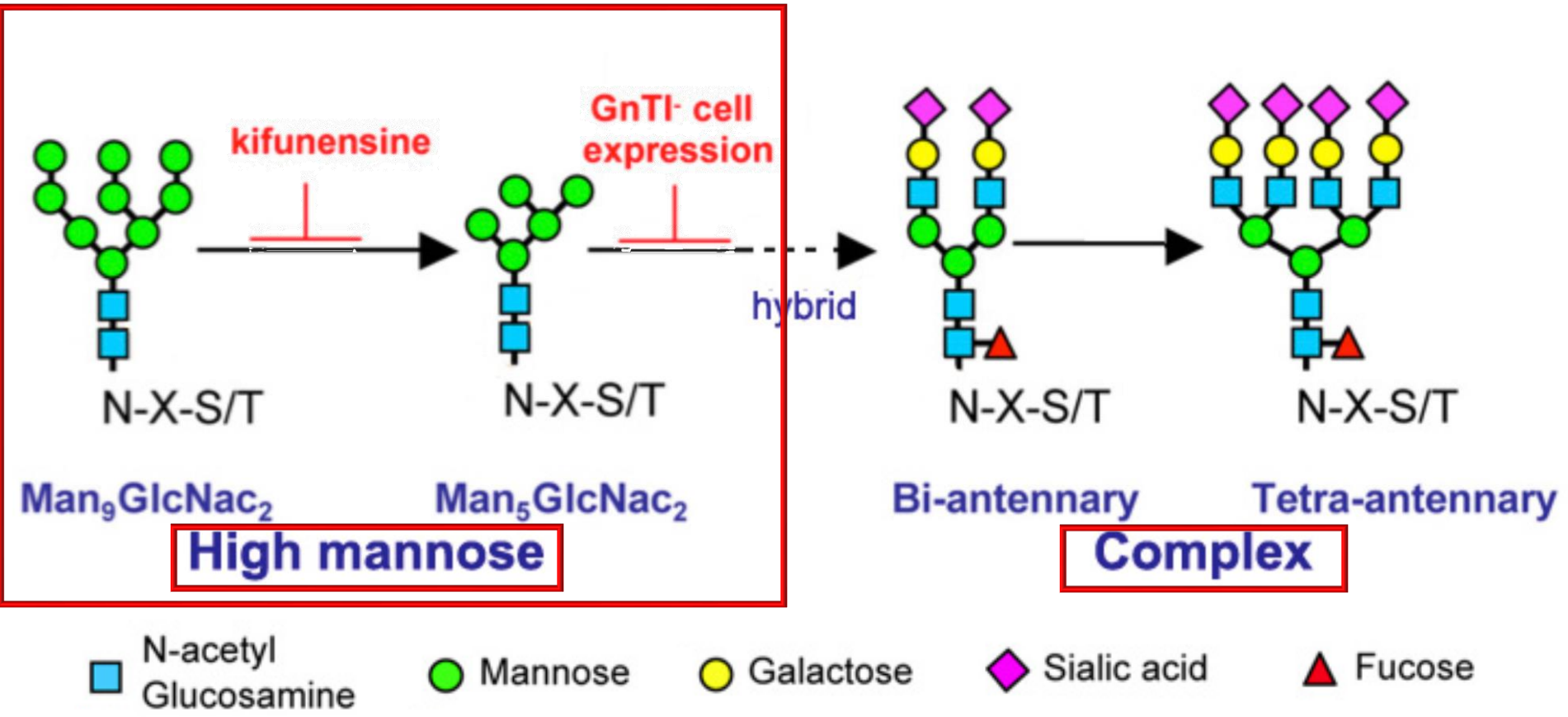
Behrens et al., 2016, *Cell Reports*

PNGS = Potential *N*-linked Glycosylation Site

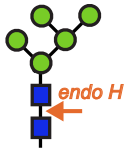
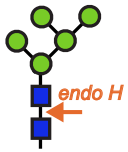
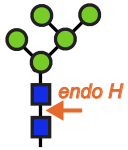
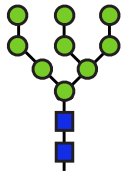
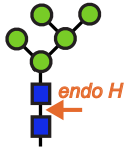
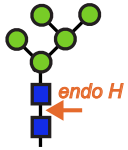
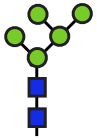


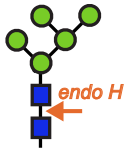
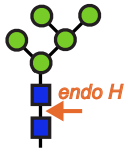
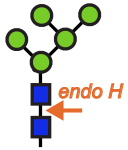
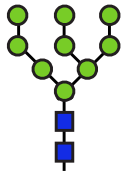
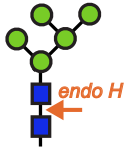
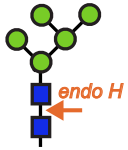
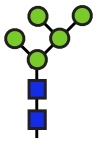
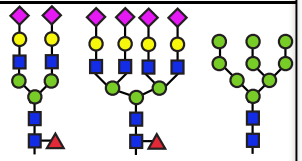


Glycoproteins in crystal structures are rarely natively glycosylated because heterogeneous glycosylation usually impedes crystallization

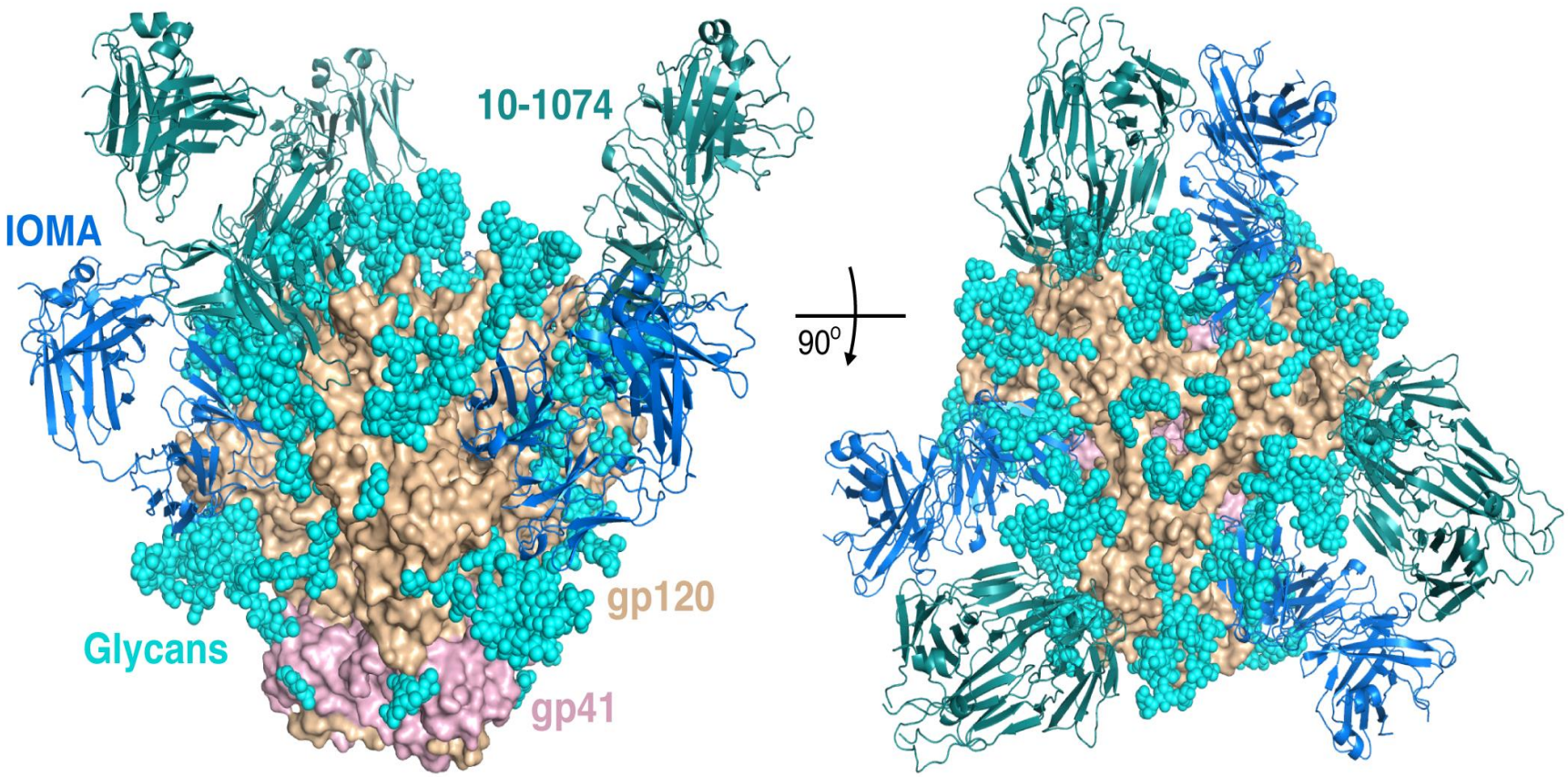


Adapted from Binley et al., 2010, *J Virol*

Paper	Env trimer	Fab(s)	Endo H?	Resolution	Form of glycan	Schematic glycan
Julien et al., 2013, *Science*	BG505 from GnTI-/- cells	PGT122	Yes	4.7 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Pancera et al., 2014, *Nature*	BG505 from GnTI-/- cells	PGT122, 35O22	Yes	3.5 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Do Kwon et al., 2015, *NSMB*	BG505 from GnTI-/- cells	None	Yes	3.72 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Scharf et al., 2015, *Cell*	BG505 from kif-treated cells	8ANC195	No	3.58 Å	High mannose; Man_9	
Kong et al., 2015, *Acta Cryst D*	BG505 from GnTI-/- cells	8ANC195, PGT128	Yes	4.6 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Garces et al., 2015, *Immunity*	BG505-N137A from GnTI-/- cells	3H/109L, 35022	Yes	3.0 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Stewart-Jones et al., 2016, *Cell*	SOSIPs from GnTI-/- cells	scFv VRC01, PGT122, 35O22	No	3.4 Å – 3.7 Å	High mannose; Man_{5-9} described as “fully glycosylated”	

Paper	Env trimer	Fab(s)	Endo H?	Resolution	Form of glycan	Schematic glycan
Julien et al., 2013, *Science*	BG505 from GnTI-/- cells	PGT122	Yes	4.7 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Pancera et al., 2014, *Nature*	BG505 from GnTI-/- cells	PGT122, 35O22	Yes	3.5 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Do Kwon et al., 2015, *NSMB*	BG505 from GnTI-/- cells	None	Yes	3.72 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Scharf et al., 2015, *Cell*	BG505 from kif-treated cells	8ANC195	No	3.58 Å	High mannose; Man_9	
Kong et al., 2015, *Acta Cryst D*	BG505 from GnTI-/- cells	8ANC195, PGT128	Yes	4.6 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Garces et al., 2015, *Immunity*	BG505-N137A from GnTI-/- cells	3H/109L, 35022	Yes	3.0 Å	High mannose; Man_{5-9} where protected from Endo H; otherwise core GlcNAc	
Stewart-Jones et al., 2016, *Cell*	SOSIPs from GnTI-/- cells	scFv VRC01, PGT122, 35O22	No	3.4 Å – 3.7 Å	High mannose; Man_{5-9} described as “fully glycosylated”	
Gristick, von Boehmer et al., 2016, *NSMB*	BG505 from wt HEK cells	10-1074, IOMA	No	3.5 Å and 3.9 Å	Fully AND **natively** glycosylated; Complex and high mannose glycans	

IOMA–10-1074–BG505 crystal structures (3.9 Å and 3.5 Å)

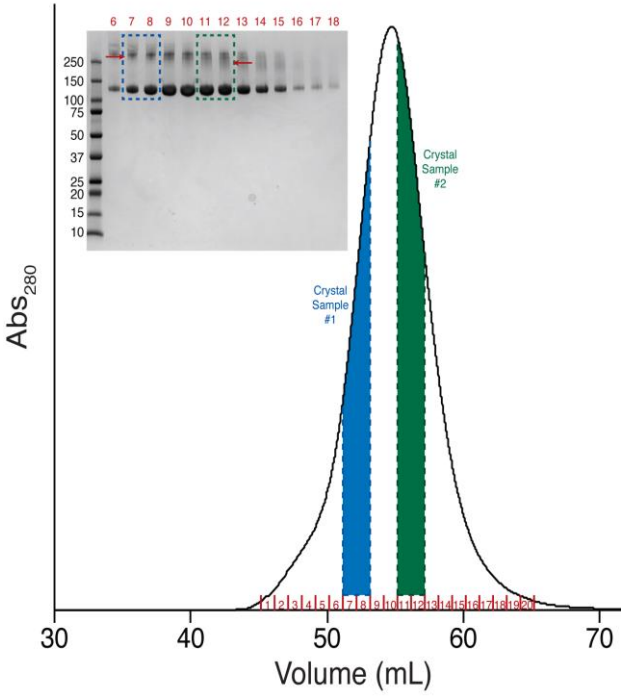


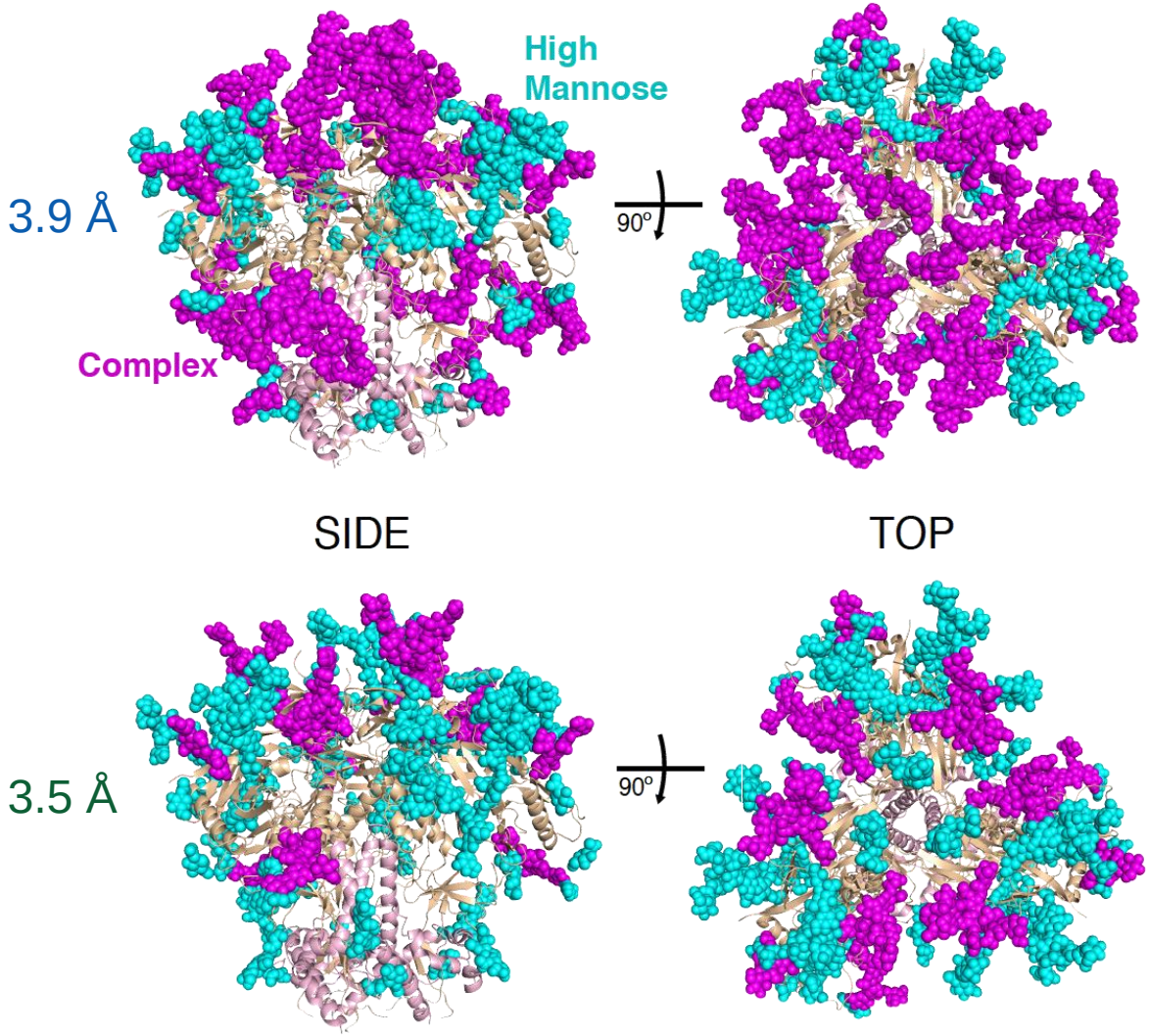
10-1074: Asn332$_{gp120}$ glycan-directed V3 loop bNAb in clinical trials
IOMA: New CD4bs bNAb with unusual CD4-mimetic properties
BG505: soluble native-like gp140 Env trimer (SOSIP)

Harry Gristick

Structures of IOMA–10-1074-BG505 prepared from higher and lower MW SEC fractions show differences in glycosylation

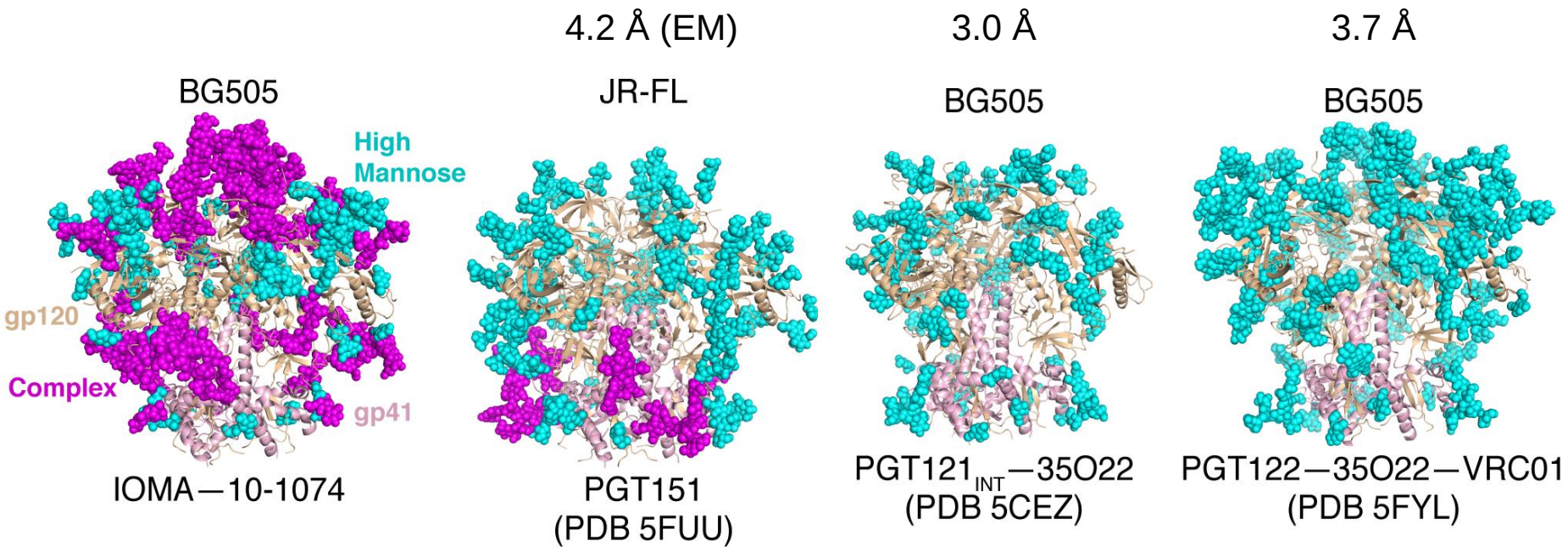
SEC of BG505 gp140 expressed in HEK-6E cells





Harry Gristick

Natively-/fully-glycosylated Env trimer in IOMA–10-1074–BG505 crystal structure reveals most complete view of ordered glycans yet obtained



Natively-/fully-glycosylated structures

HM-only structures

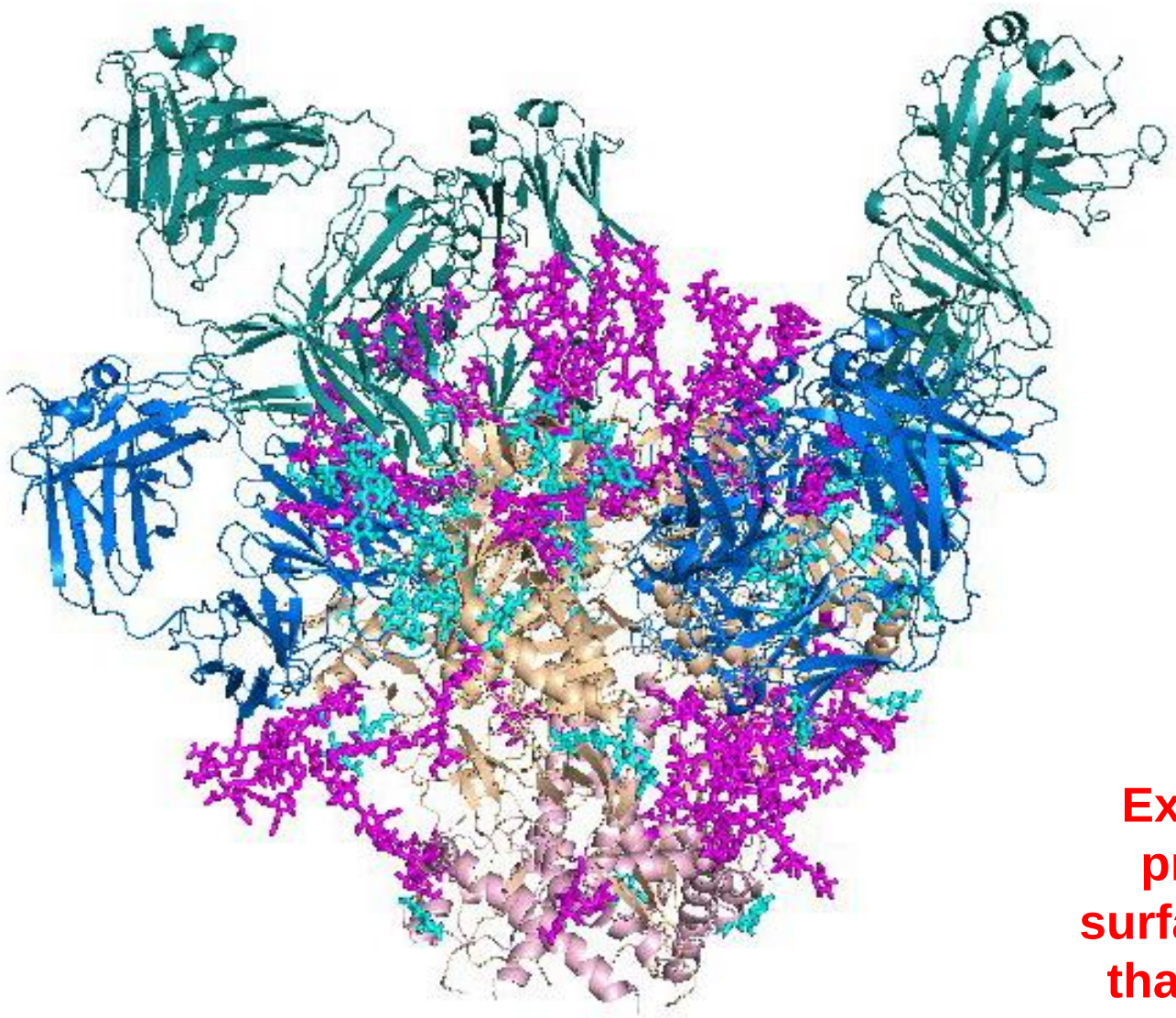
Harry Gristick

10-1074
Fab

IOMA
Fab

BG505
gp120

BG505
gp41

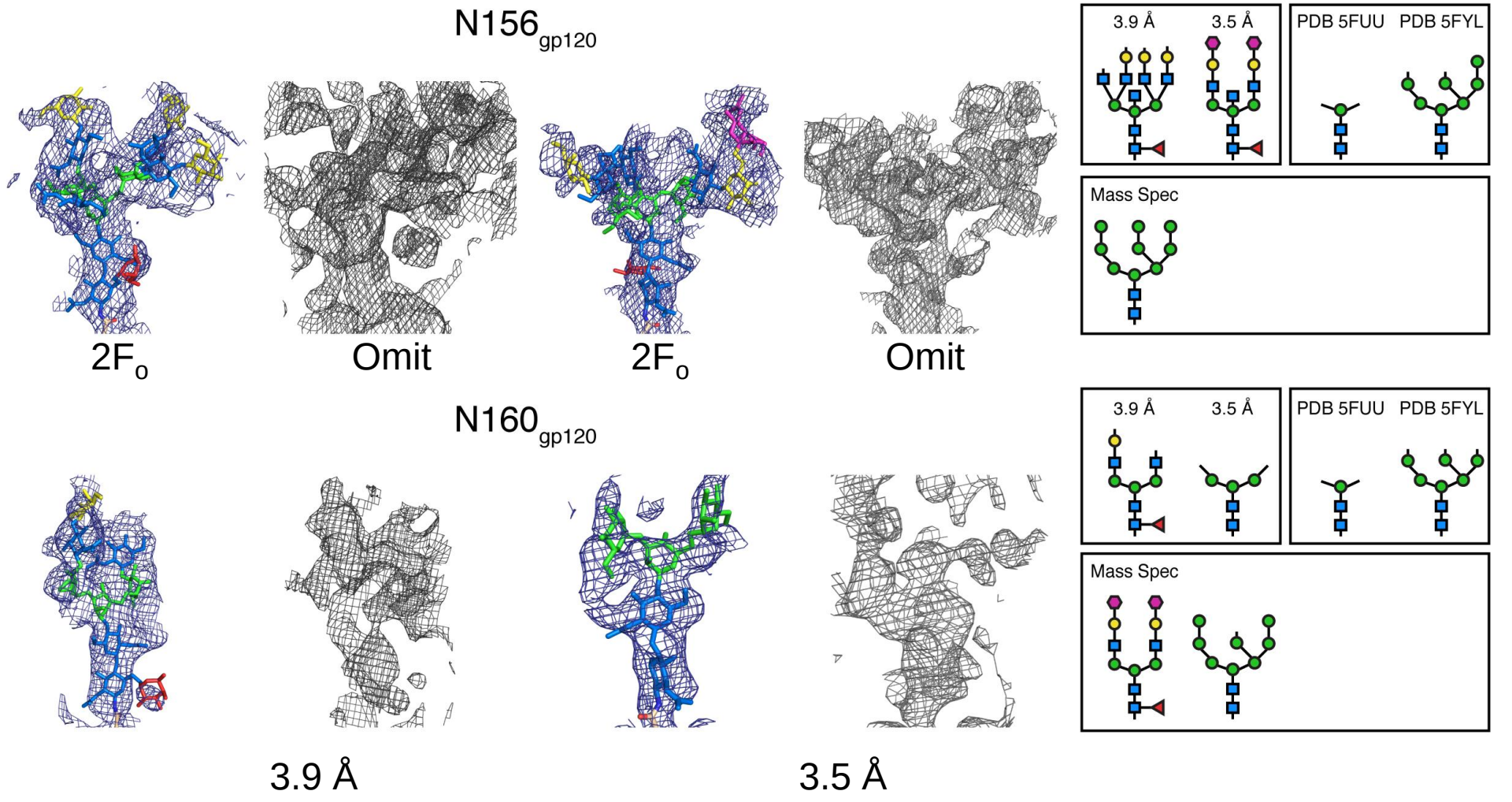


Complex
glycans

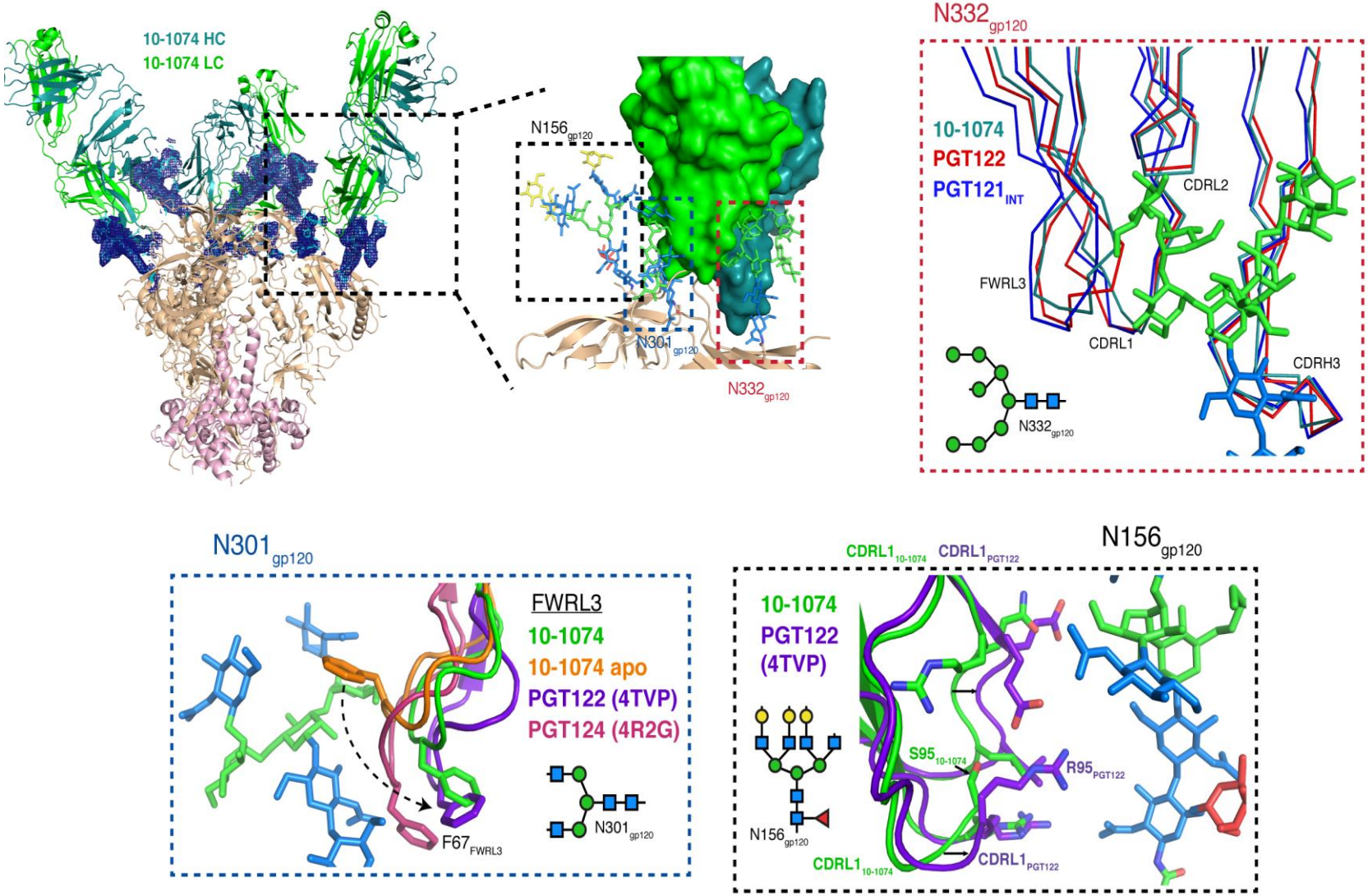
High
mannose
glycans

Exposed
protein
surface area
that is not
conserved

We compared ordered *N*-glycans at each PNGS in our two structures, the cryo-EM structure (5FUU), a BG505 HM crystal structure (5FYL), and mass spectroscopy assignments

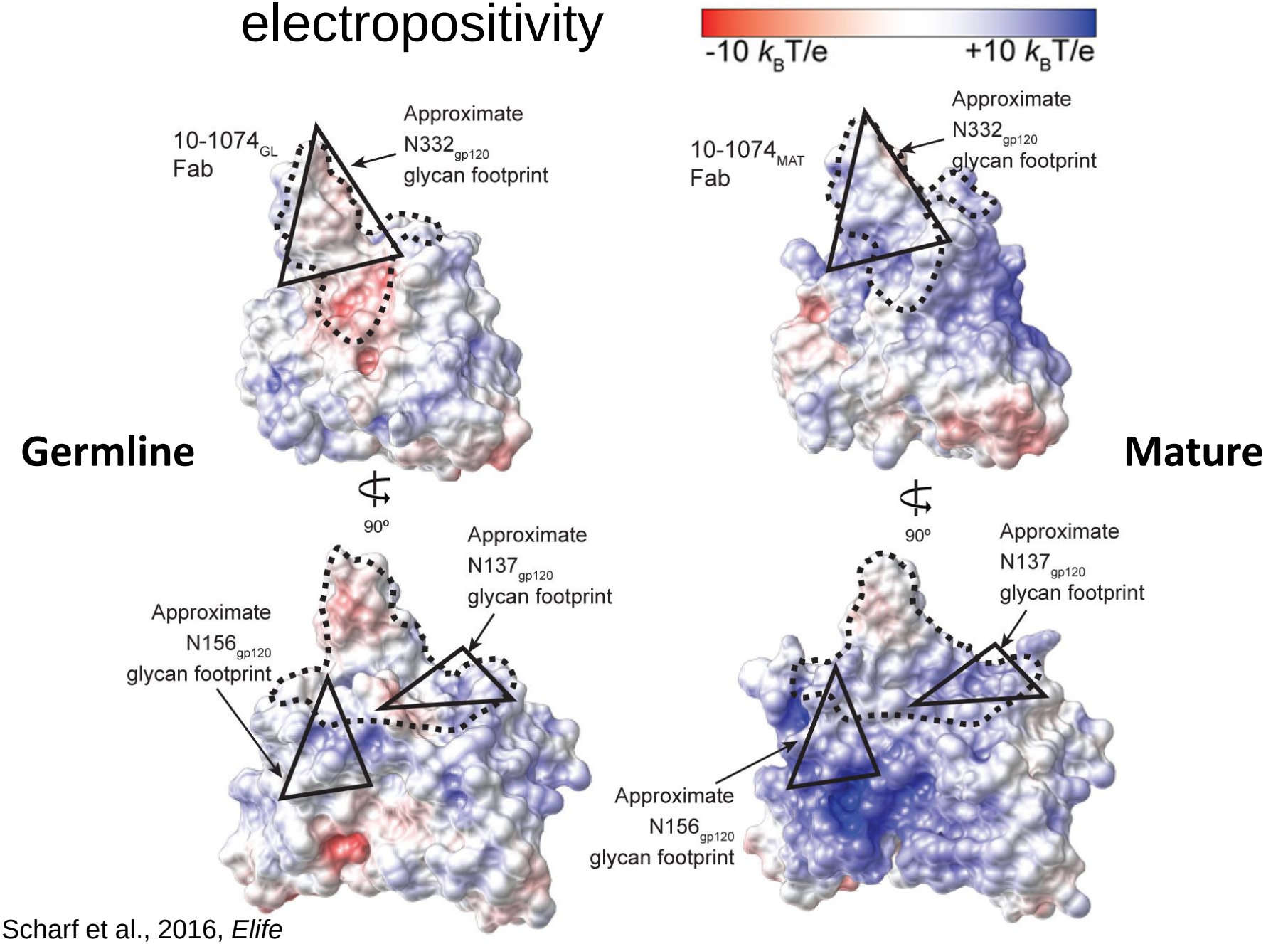


We resolved the complete epitope of 10-1074 in the context of native glycosylation



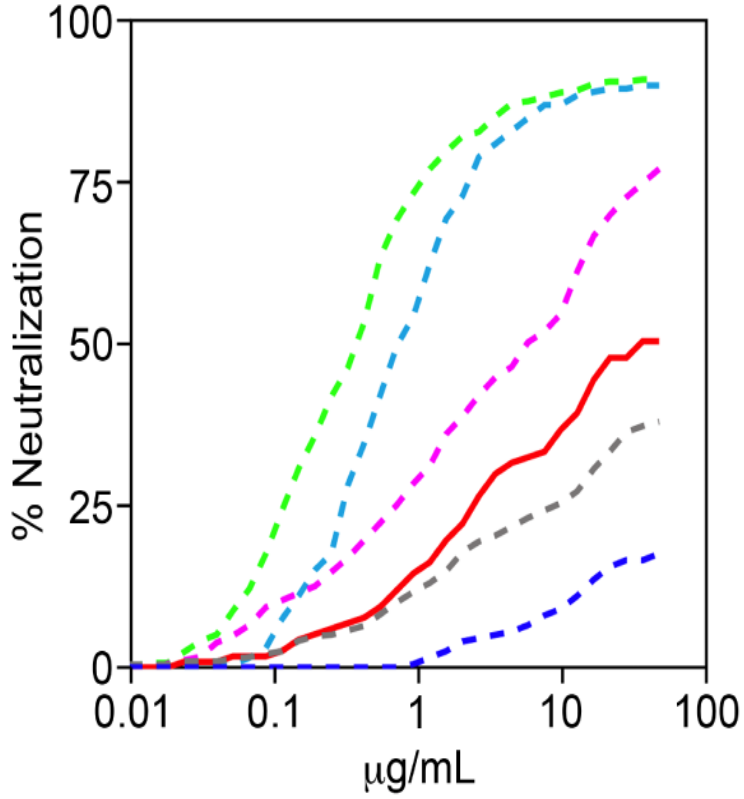
Harry Gristick

10-1074 shows maturation towards increased electropositivity



Scharf et al., 2016, *Elife*

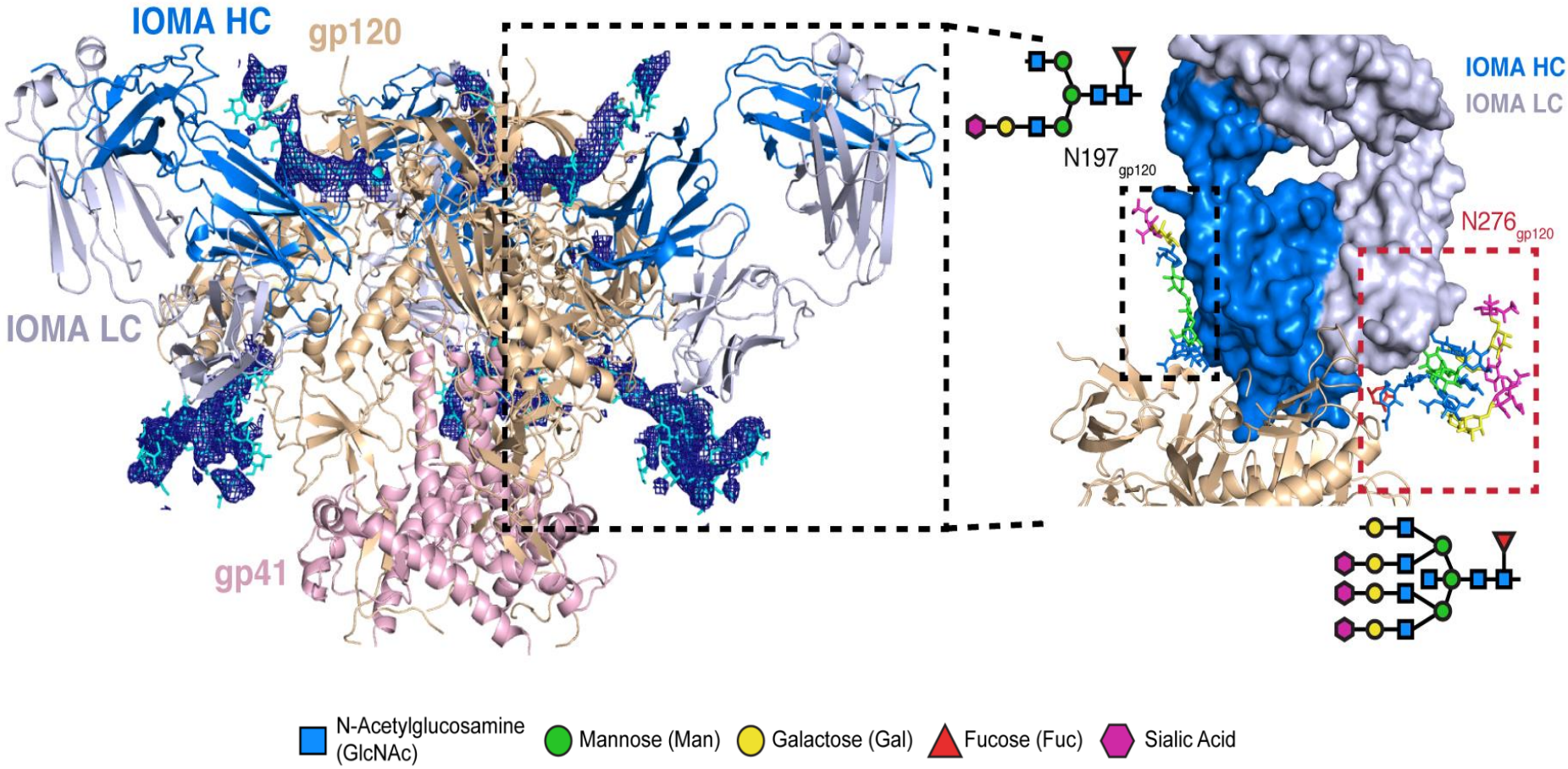
IOMA is not as potent as more heavily mutated VRC01-class bNAbs, but is more potent than CD4bs bNAbs with similar levels of SHM



	VH SHM aa/nucl	VL SHM aa/nucl
VRC01	41/93	25/56
CH235.12	42/74	21/40
8ANC131	39/79	28/60
IOMA	22/35	15/23
b12	20/35	20/35
CH235	15/23	10/14

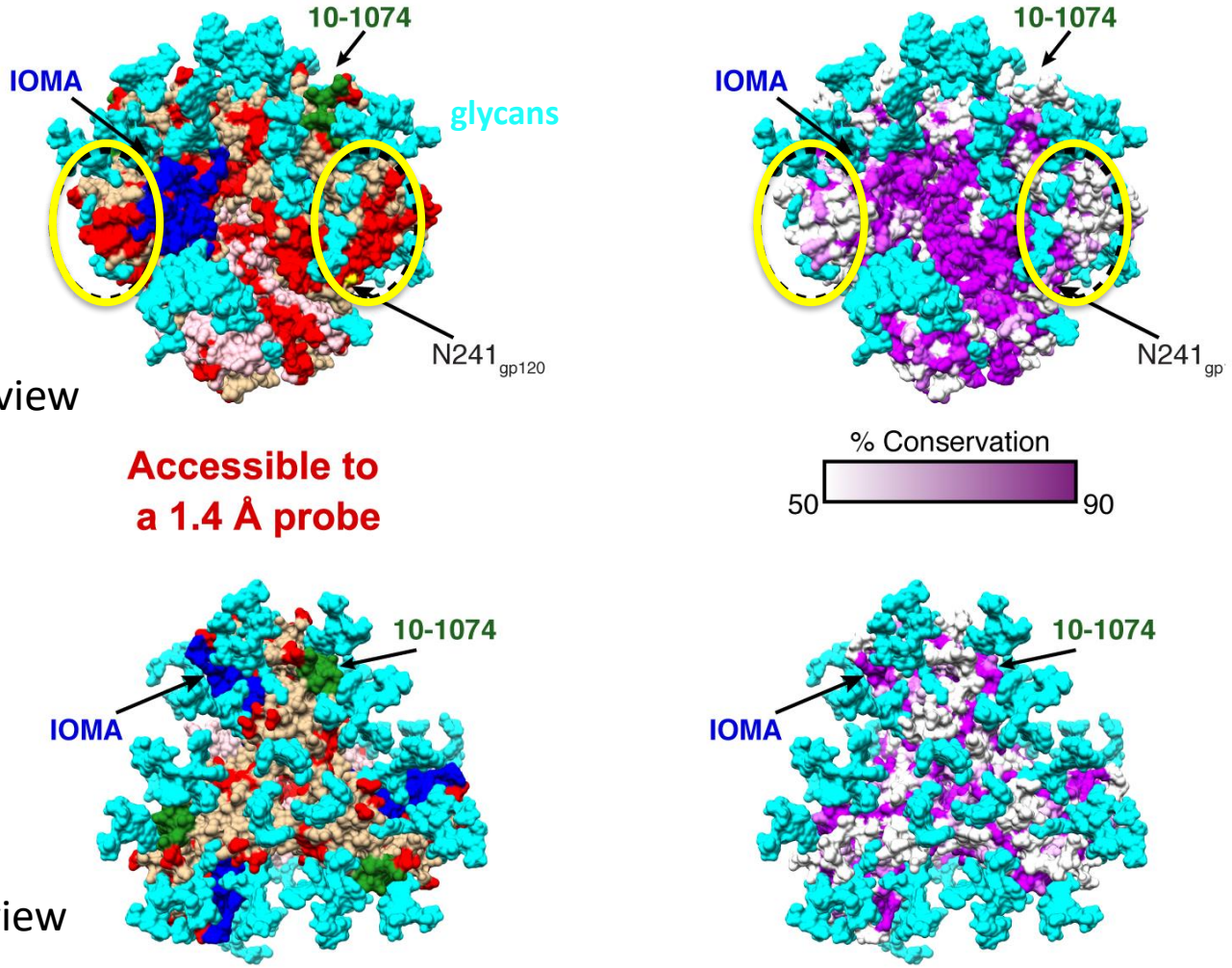
Lotta von Boehmer
Michel Nussenzweig
Rockefeller Univ

IOMA is framed by complex-type *N*-glycans attached to $N197_{gp120}$ and $N276_{gp120}$



Harry Gristick

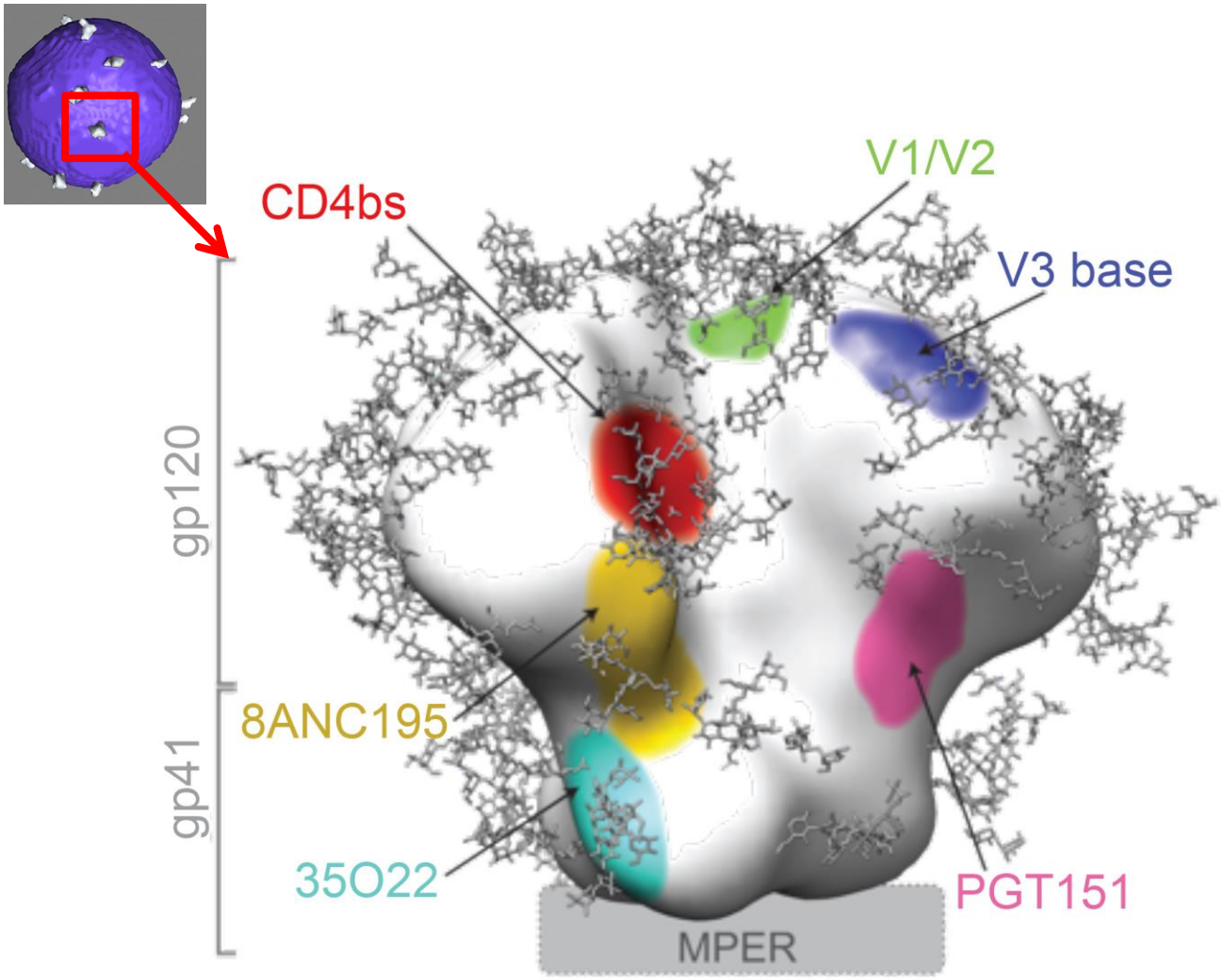
Mapping accessible areas onto natively-glycosylated BG505 reveals antibody-vulnerable glycan holes that can be targeted by strain-specific antibodies



Areas of contiguous red (left) that are white/light purple (right) are sites of low sequence conservation potentially accessible to antibodies. Can find one such site on BG505 adjacent to $N241_{gp120}$.

See McCoy et al., 2016, "Holes in the Glycan Shield of the Native HIV Envelope Are a Target of Trimer-Elicited Neutralizing Antibodies, *Cell Reports*.

The HIV-1 Env utilizes multiple strategies to avoid antibodies

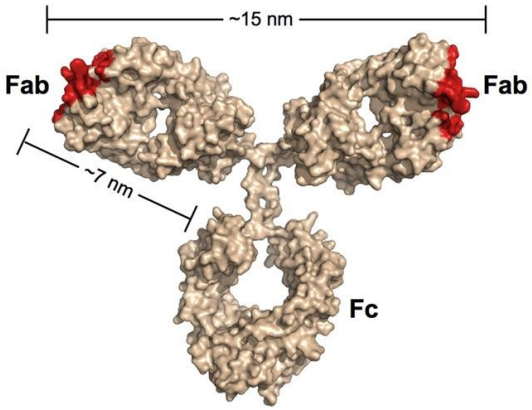


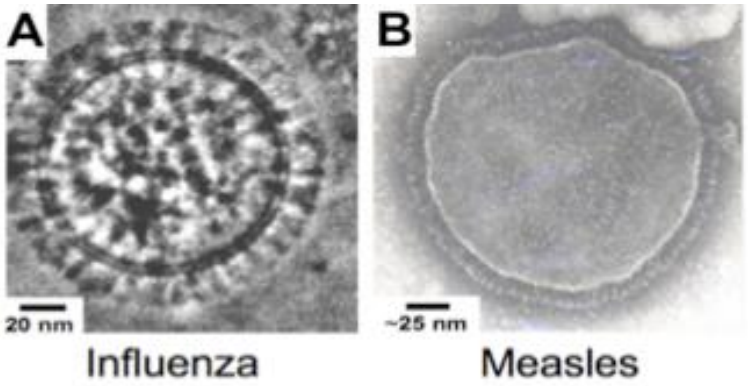
- Glycan shield
- Rapid mutation
- Hide conserved regions in interfaces
- **Low density of Env spikes**

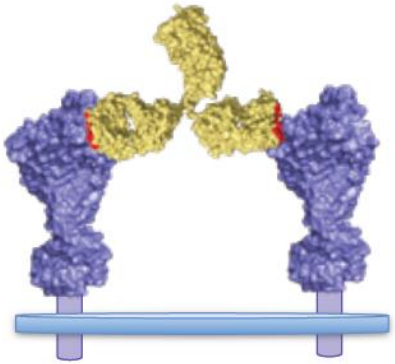
Few and far between: how HIV may be evading antibody avidity

Klein and Bjorkman, 2010, *PLoS Pathogens*

For most viruses, two identical Fabs in IgGs permit bivalent binding through inter-spike crosslinking.



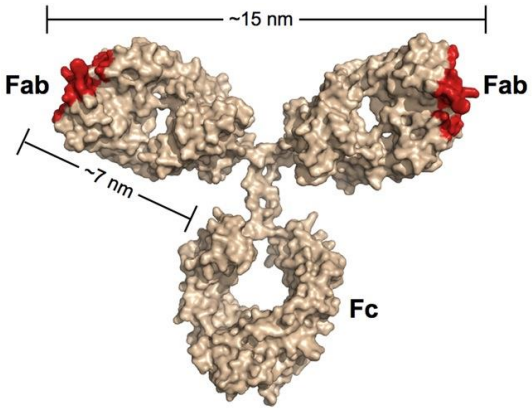


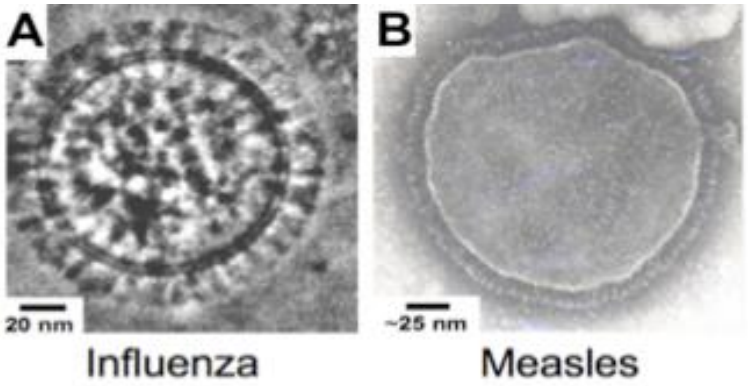


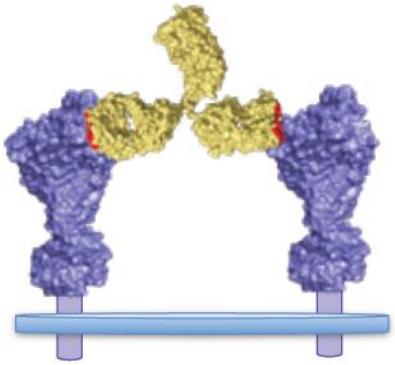
Few and far between: how HIV may be evading antibody avidity

Klein and Bjorkman, 2010, *PLoS Pathogens*

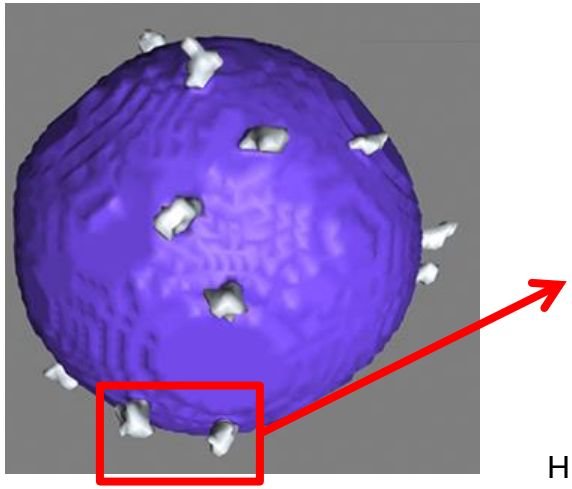
For most viruses, two identical Fabs in IgGs permit bivalent binding through inter-spike crosslinking.



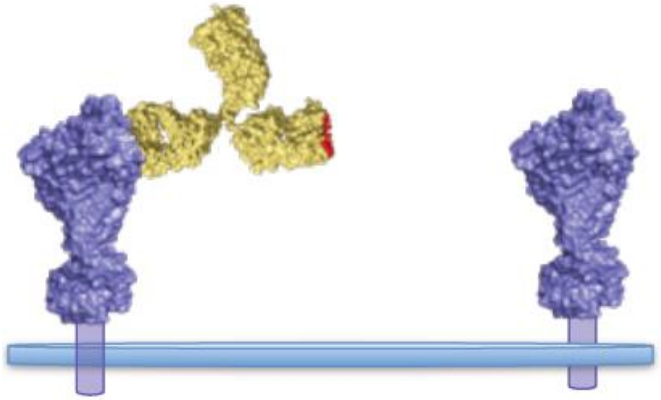




Unlike other viruses, HIV has very few spikes, and the spikes are far apart – most antibodies can't bind with both Fabs.

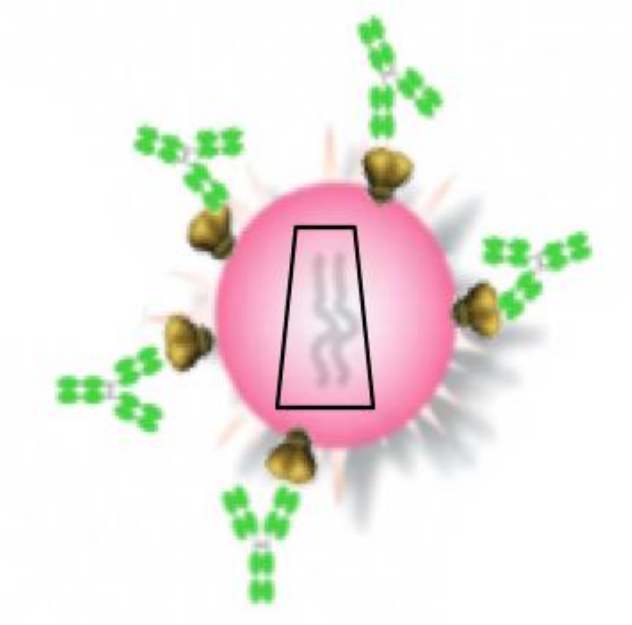


Zhu et al., 2006, *Nature*

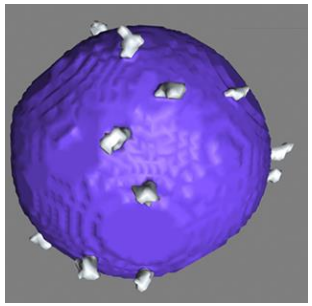


HIV spikes are relatively immobile in virus membrane

HIV's low spike density plus its rapid mutation – a deadly combination

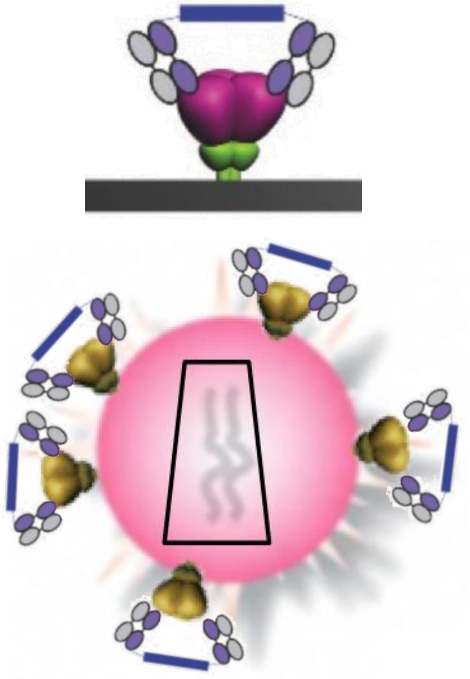


Engineer Abs that can crosslink between spikes?



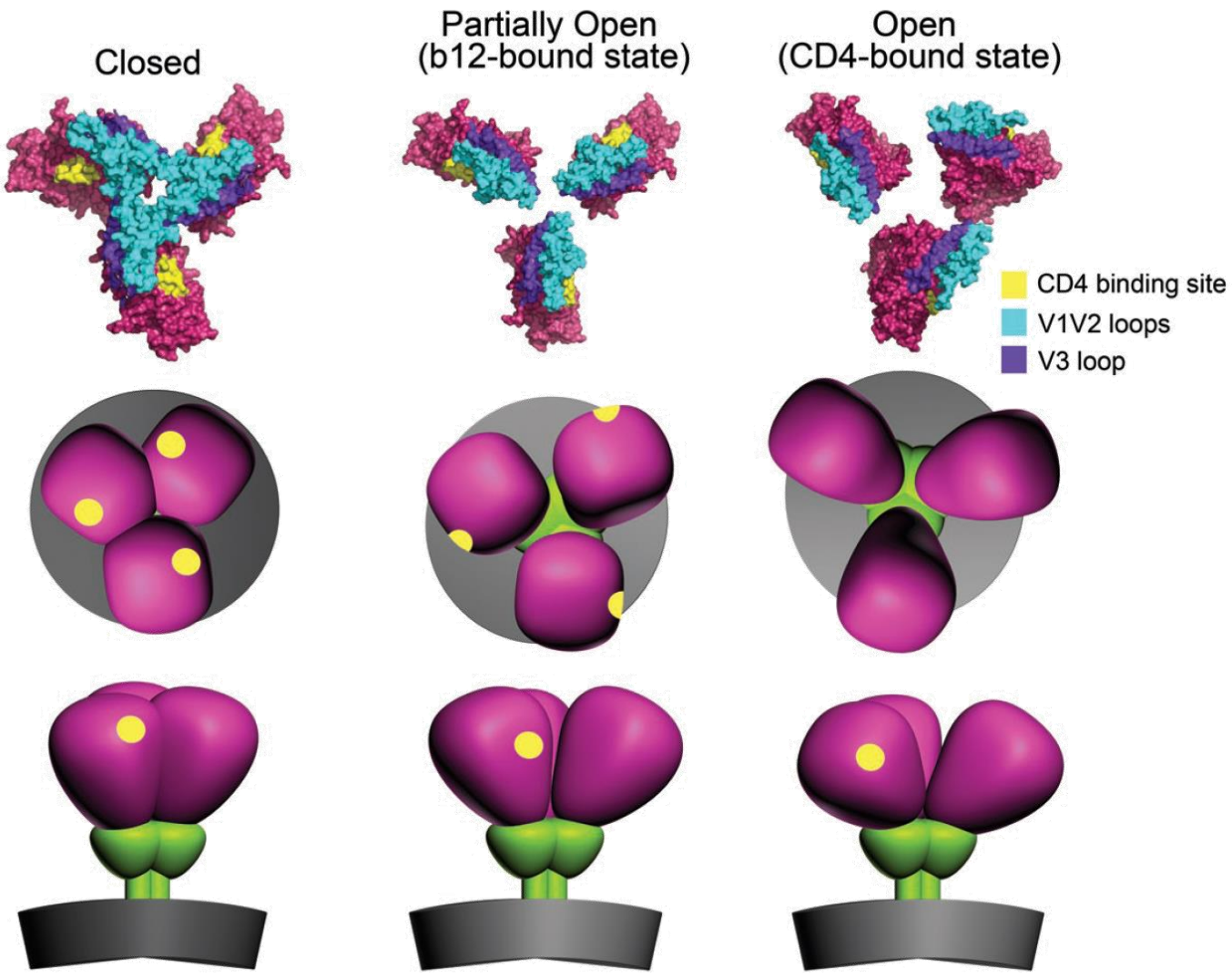
Can't make inter-spike crosslinking reagents that consistently crosslink because inter-spike distances vary even on a single virion.

Intra-spike cross-linking would allow bivalent binding (avidity) despite low spike density.



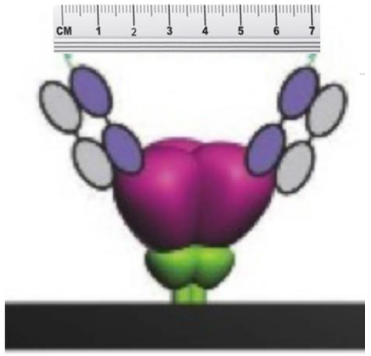
How to make bivalent reagents that bind to ≥2 epitopes within a single spike trimer? Modeling based on Env trimer structures?

HIV-1 Env exists in multiple conformations on virions



We use dsDNA as a ruler to measure distances on Env spike trimers on virions.

Persistance length ~500 Å;
3.4 Å/bp increments

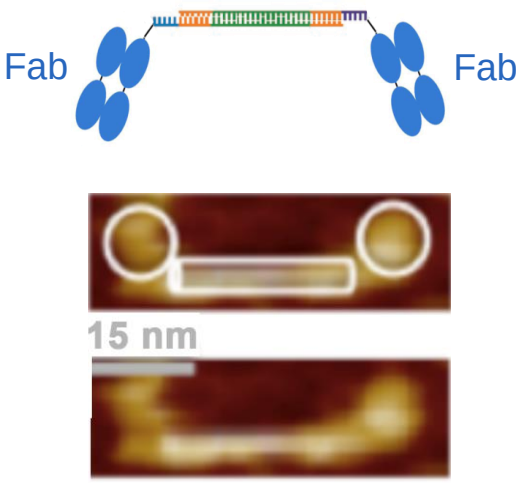


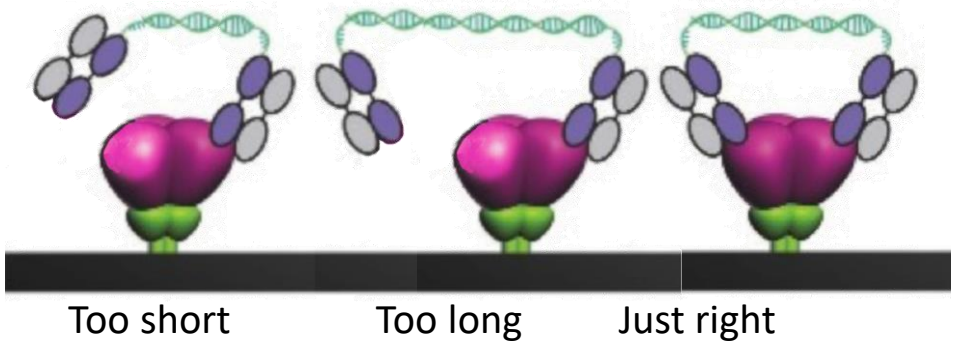
Liu et al., 2008, *Nature*; Merk & Subramaniam, 2013, *Curr Opin in Struct Biol*

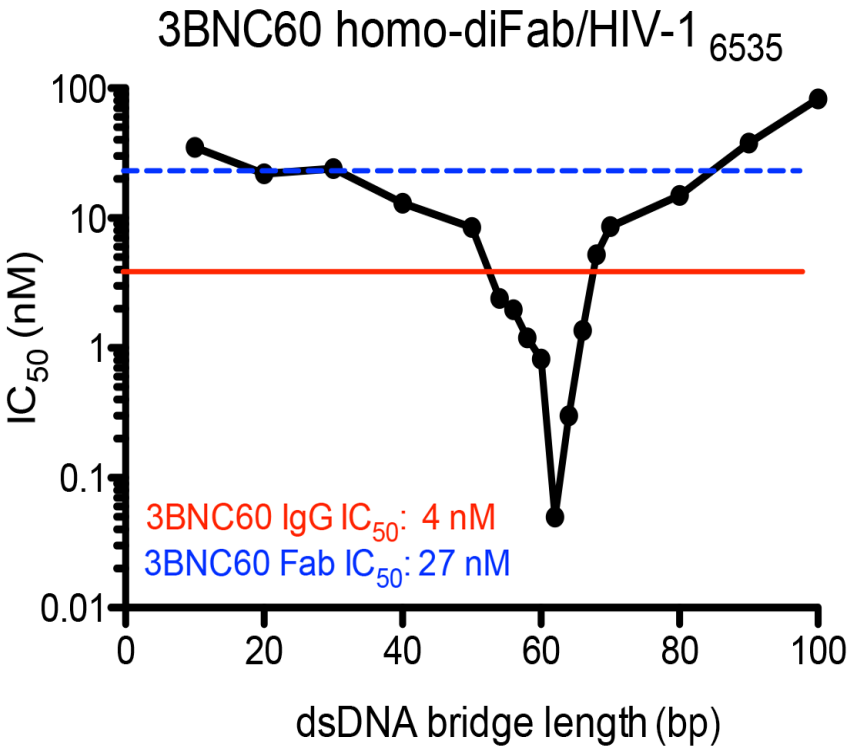
Galimidi et al., 2015, *Cell*

Compare neutralization potencies of CD4bs homo-diFab as a function of dsDNA linker length

Homo-diFabs with different lengths of dsDNA



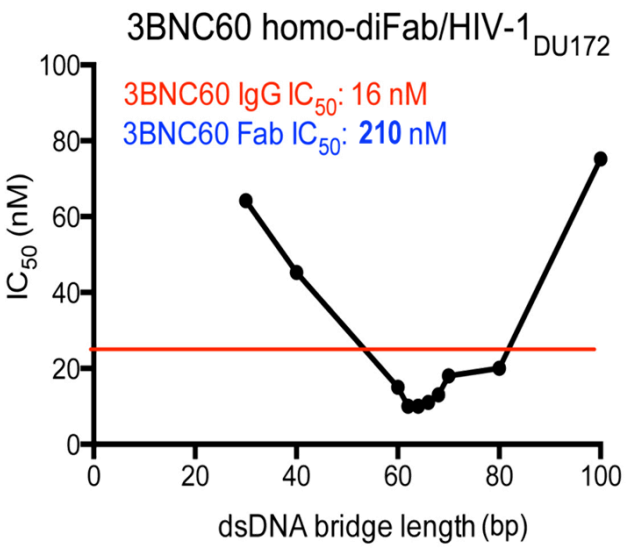


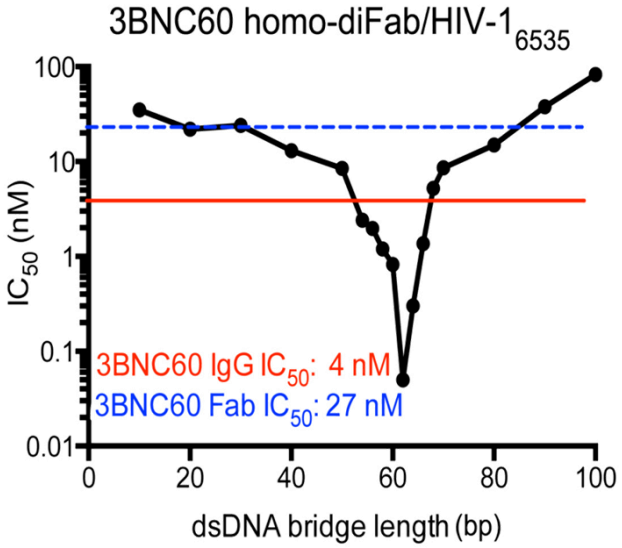


Most potent neutralization seen with a 62 bp DNA linker.
62 bp x 3.4 Å/bp = 211 Å

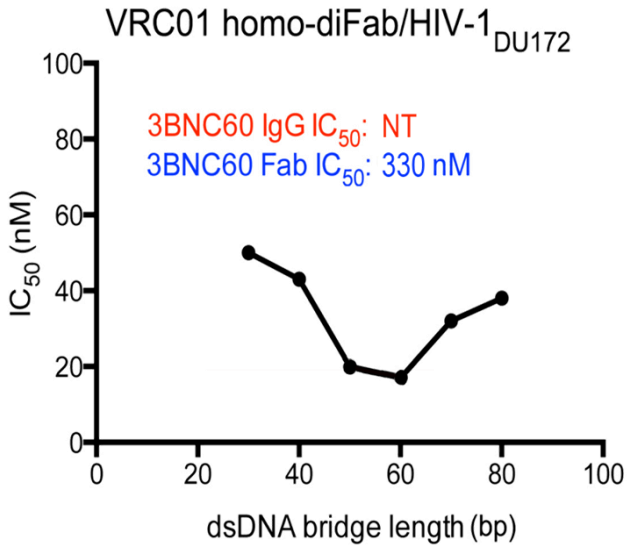
Galimidi et al., 2015, *Cell*

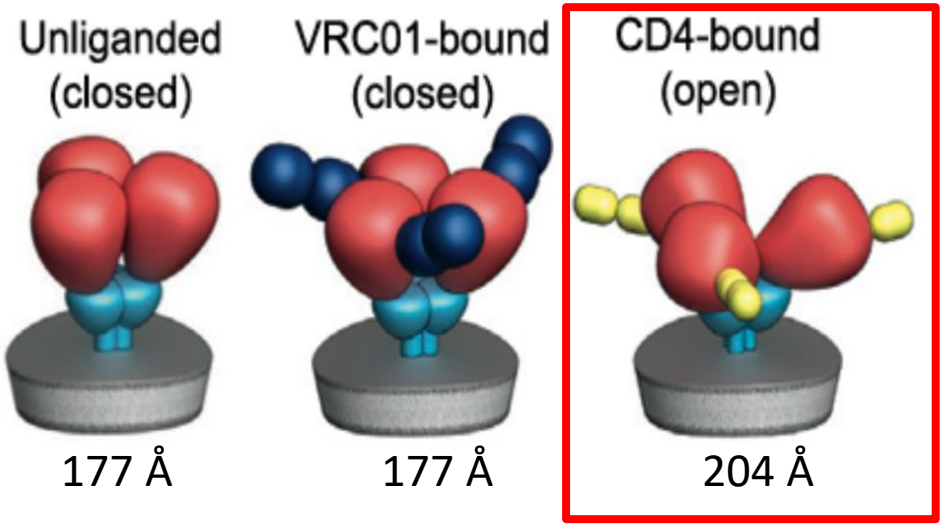
Best separation distance for CD4bs homo-diFabs: ~210 Å





62 bp x 3.4 Å/bp = 211 Å

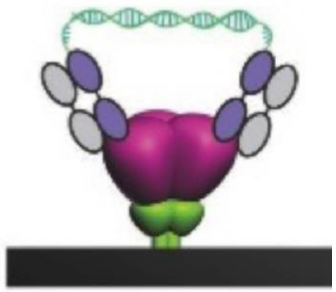


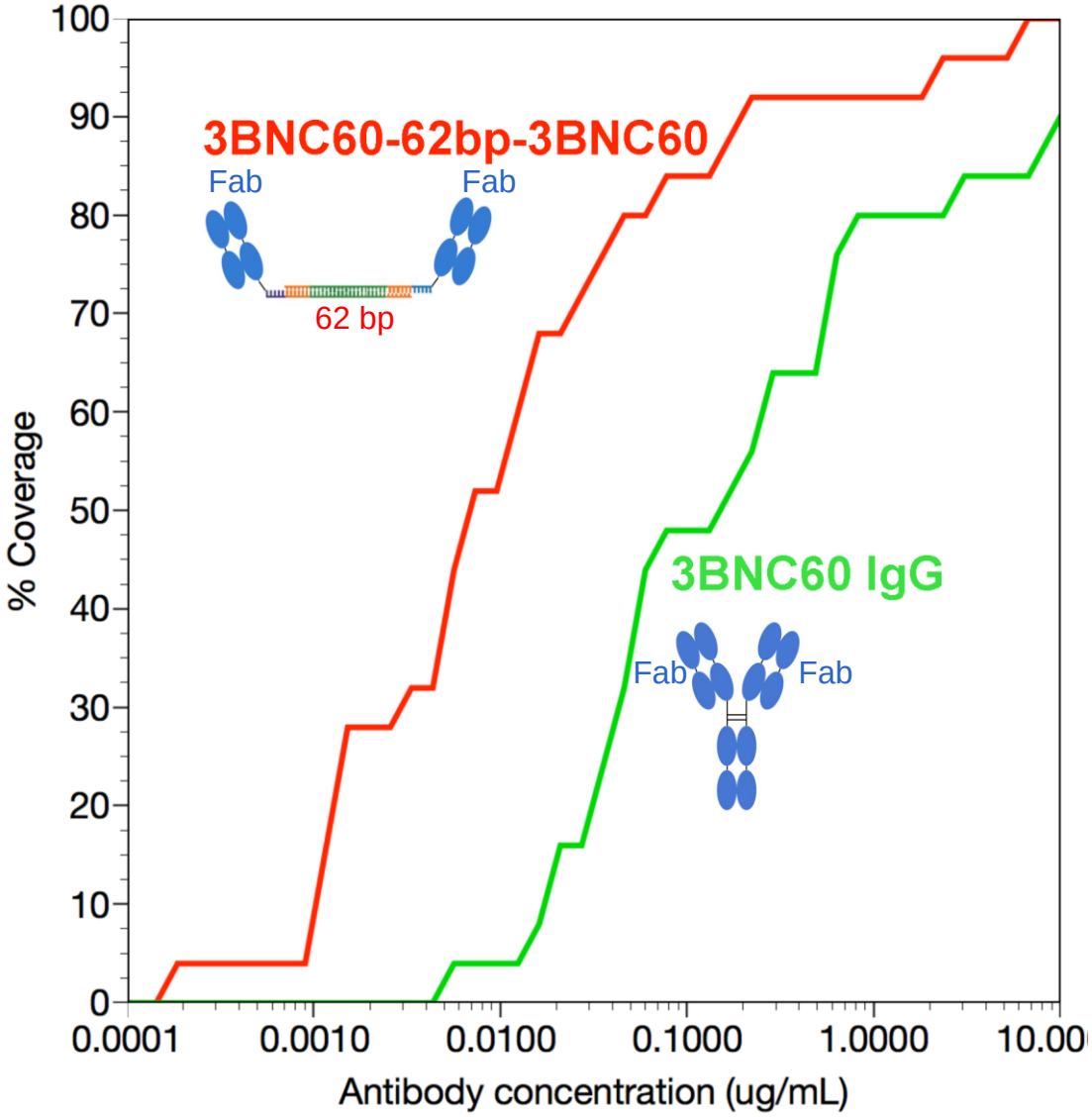


Images from Merk & Subramaniam, 2013, *Curr Opin in Struct Biol* 23:268-76

Rachel Galimidi

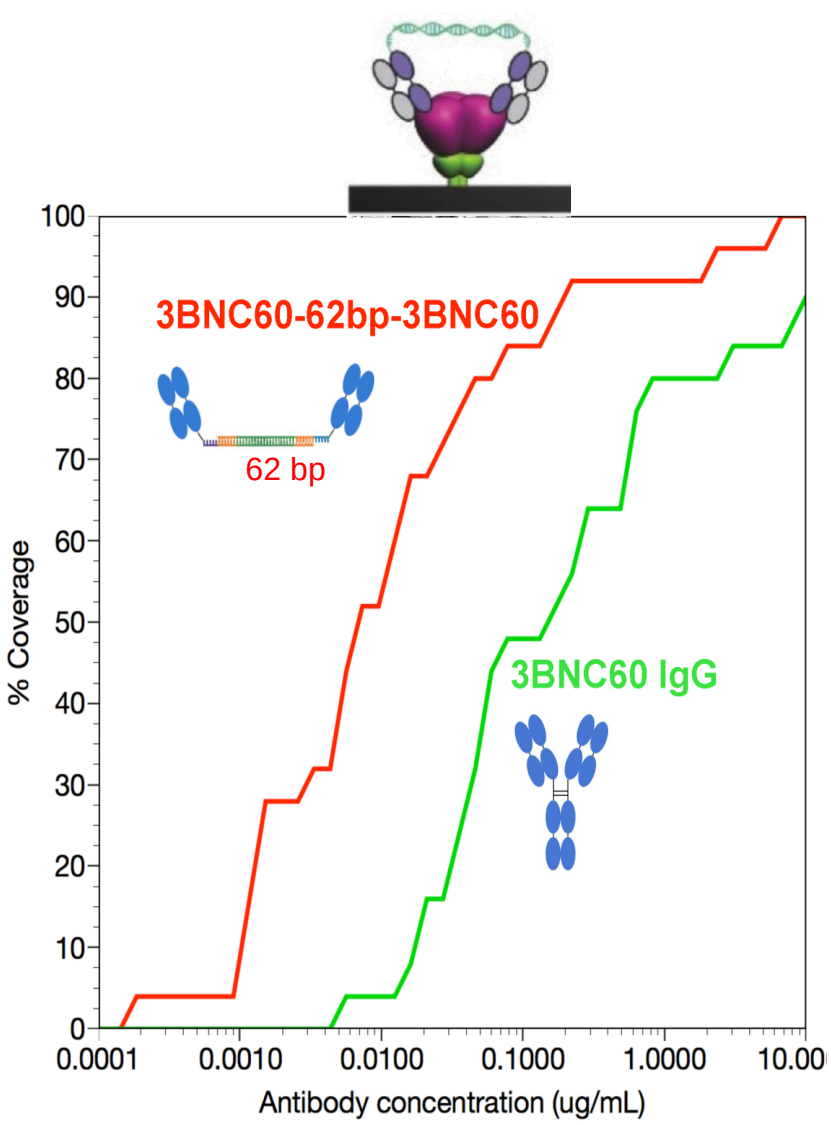
Optimal 3BNC60 homo-diFab is much more potent than 3BNC60 IgG when compared against many HIV strains

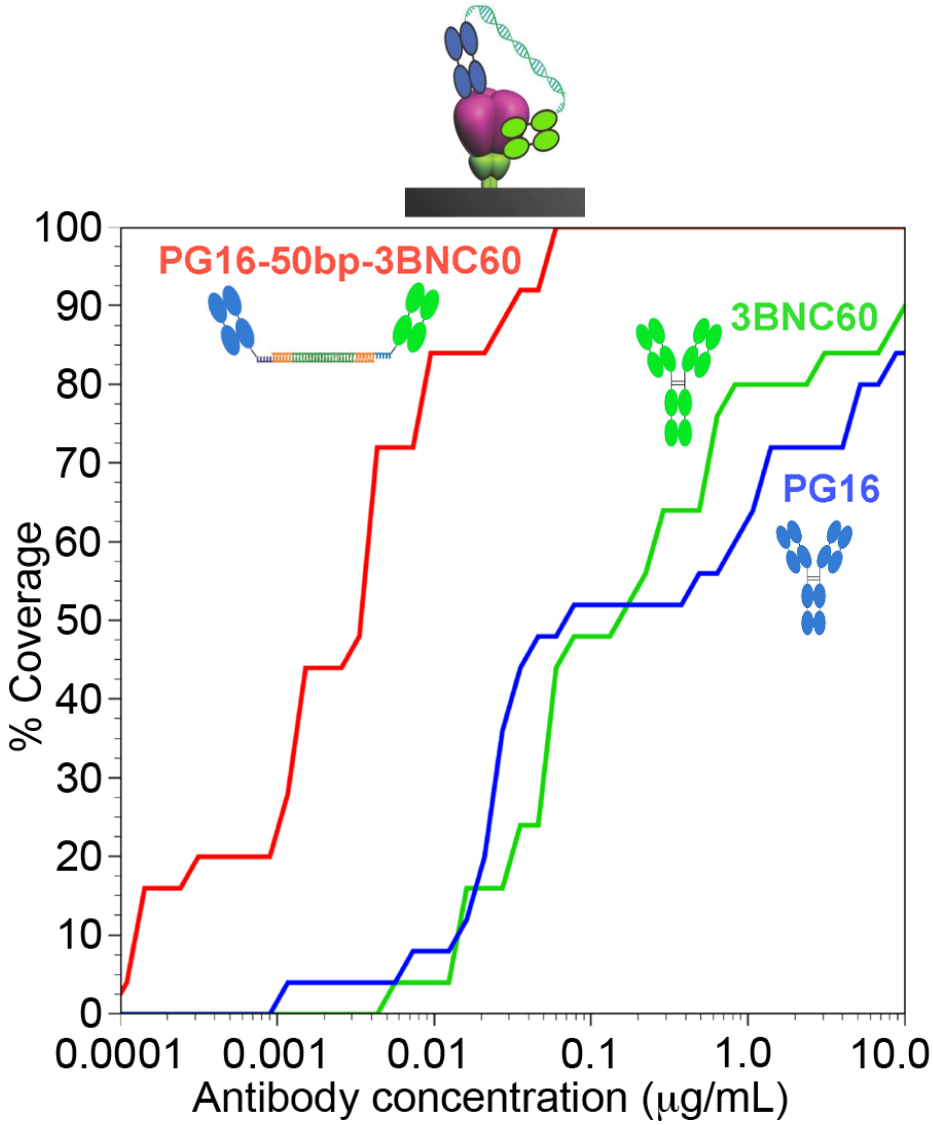




Galimidi et al., 2015, *Cell*

Optimal homo-diFab and hetero-diFab reagents show there is enormous potential for improving bNAbs by using intra-spike cross-linking to achieve synergistic neutralization

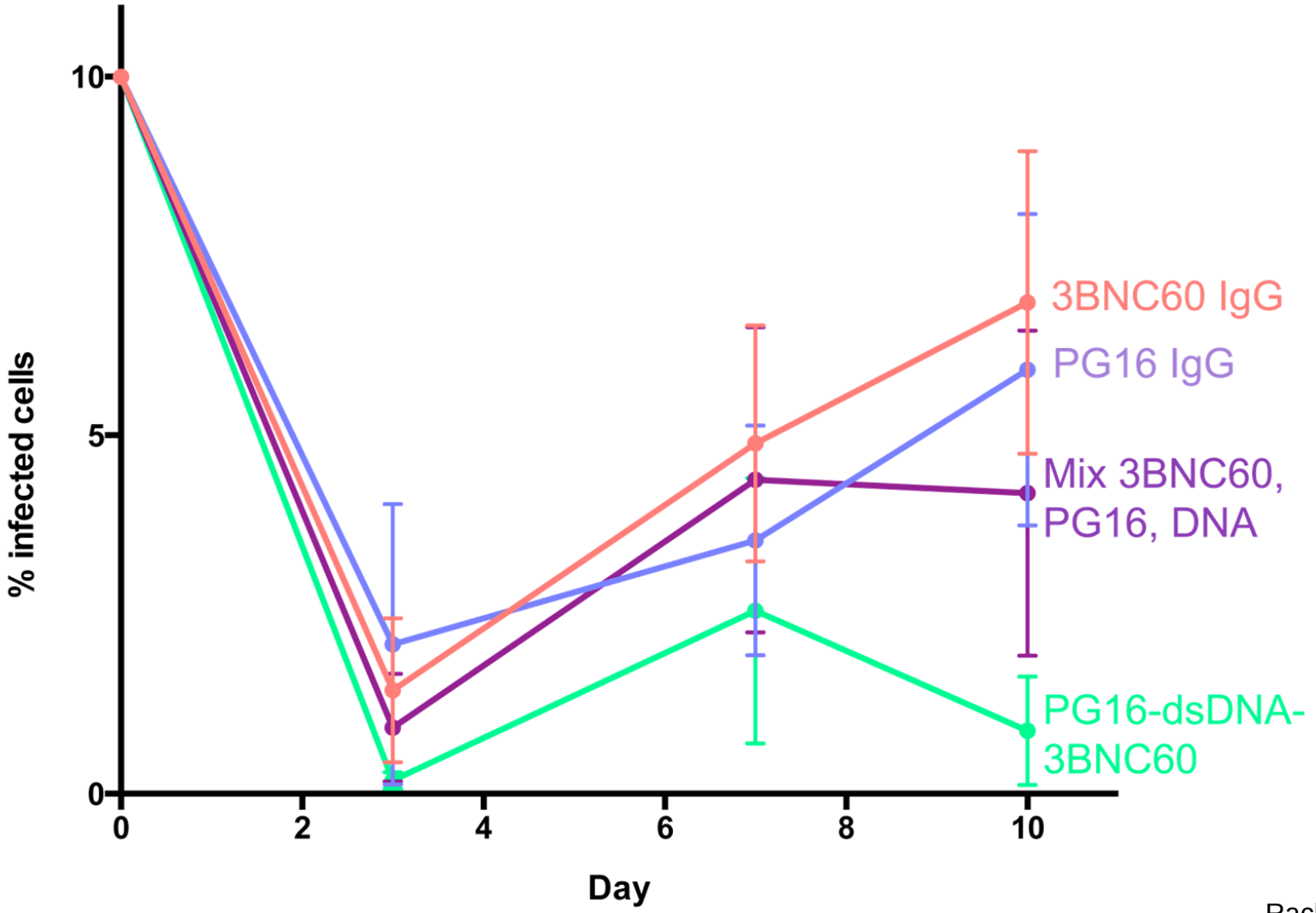




Galimidi et al., 2015, *Cell*

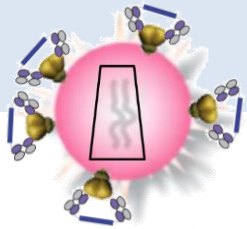
What about using avidity to suppress viral escapes?

Preliminary *in vitro* evolution data suggest suppression of HIV-1 escape mutations by PG16-50bp-3BNC60 hetero-diFab



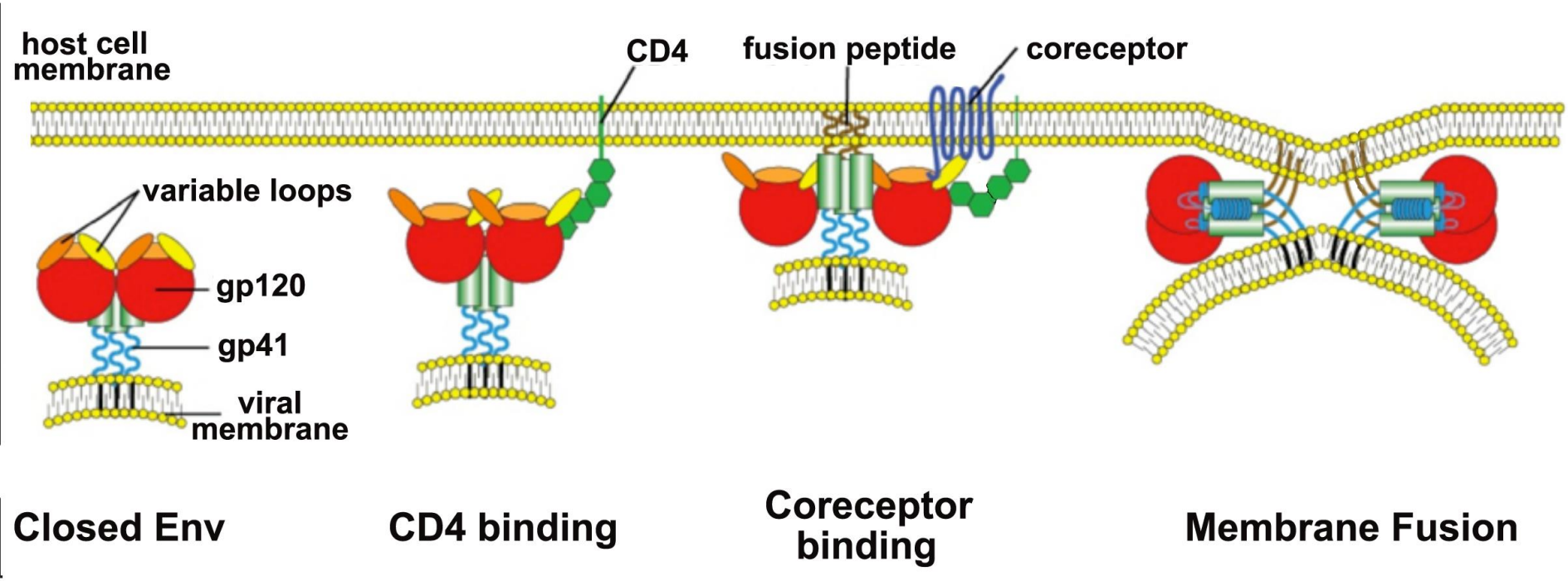
Rachel Galimidi

Intra-spike cross-linking can overcome HIV evasion of avidity



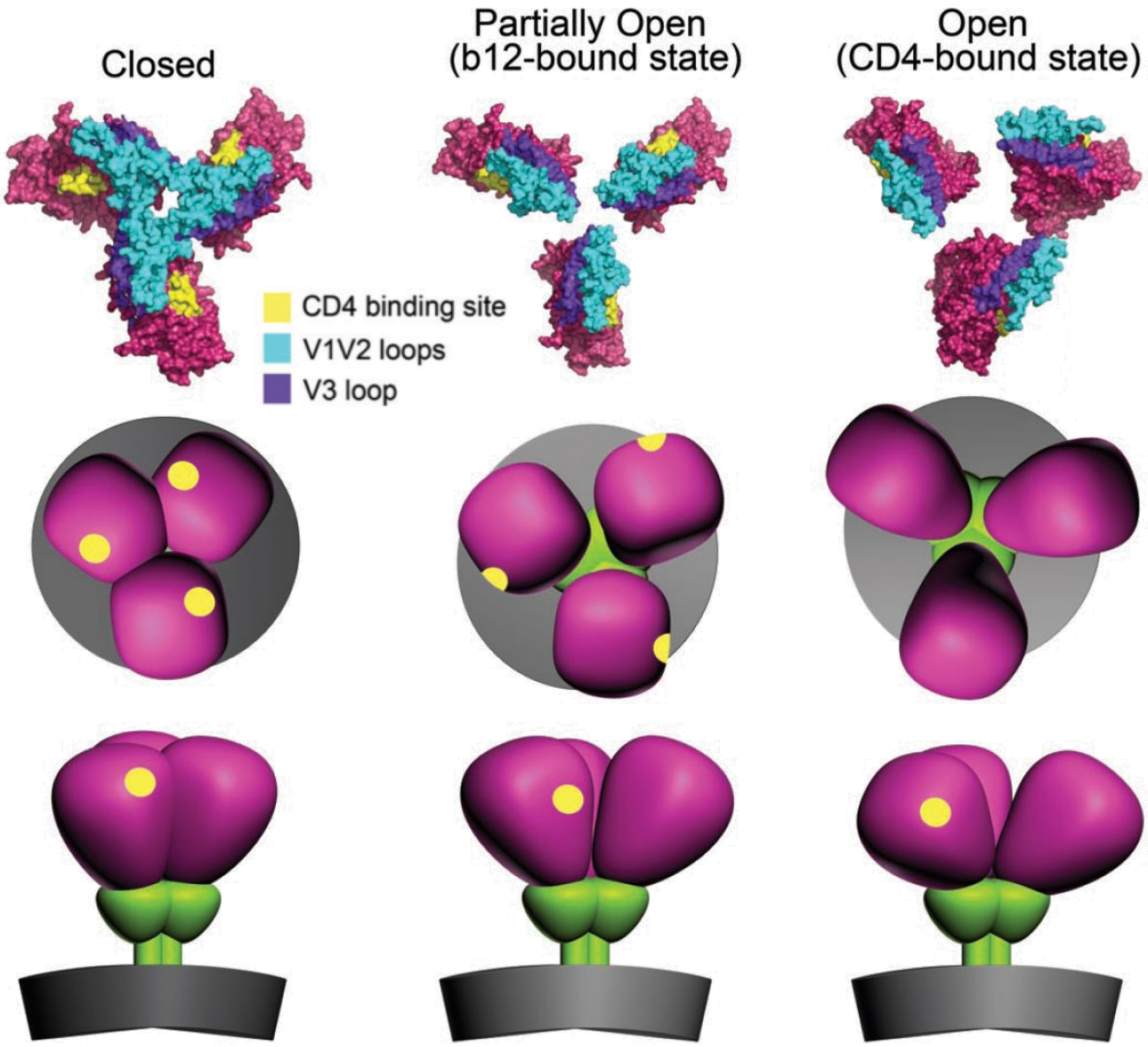
- Measuring method reveals dynamic information about Env conformations during neutralization
  - Compare optimal distances for Tier 1 versus Tier 2; CD4-dependent versus CD4-independent strains
- Up to 100-fold increases in geometric mean potency achieved with first generation intra-spike crosslinking reagents (homo- and hetero-diFabs)
  - Supports hypothesis that low spike density contributes to vulnerability of anti-HIV antibodies to spike mutations
  - Ideal anti-HIV therapeutic for passive delivery would utilize avidity to achieve intra-spike crosslinking (using protein-based linkers, not DNA)
    - Reduce the concentration required for sterilizing immunity
    - Render HIV's low spike density irrelevant
    - Hopefully would be resistant to Env mutations (in vitro evolution results)
  - Analogous to using several drugs or antibodies during ART, simultaneous binding to different epitopes would also reduce/abrogate sensitivity to Env mutations

Understanding structural changes in Env induced by CD4 and coreceptor binding could facilitate design of new therapeutic targets



Adapted from Didigu and Doms, *Viruses* **2012**, *4*, 309-324

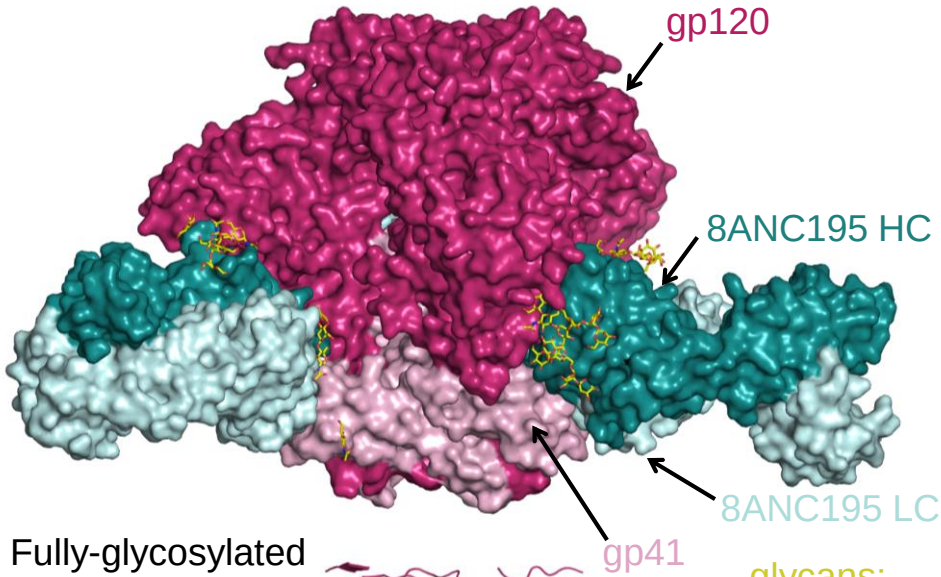
HIV-1 Env exists in different conformations on virions



Liu et al., 2008, *Nature*; Merk & Subramaniam, 2013, *Curr Opin in Struct Biol*

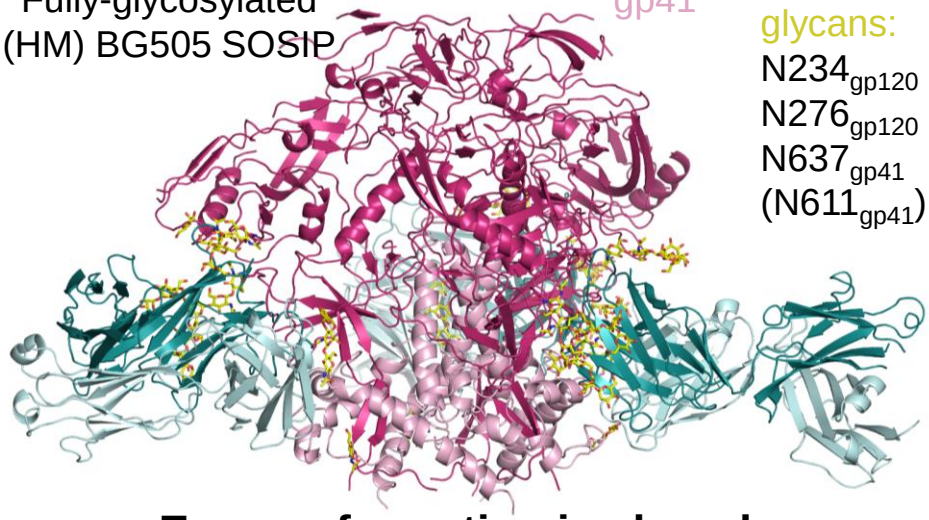
Another conformational state of HIV-1 Env

3.58 Å crystal structure of
8ANC195-Env trimer complex



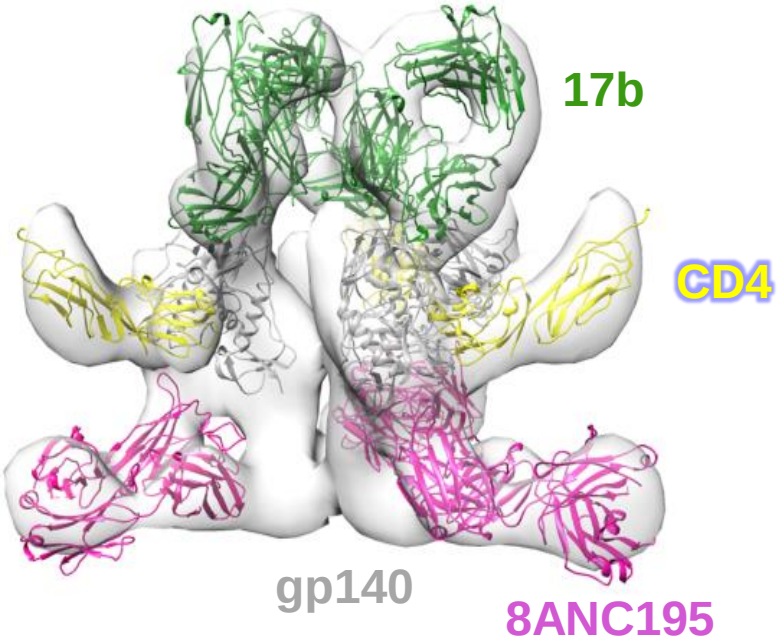
Fully-glycosylated
(HM) BG505 SOSIP

glycans:
$N234_{gp120}$
$N276_{gp120}$
$N637_{gp41}$
$(N611_{gp41})$



Env conformation is closed

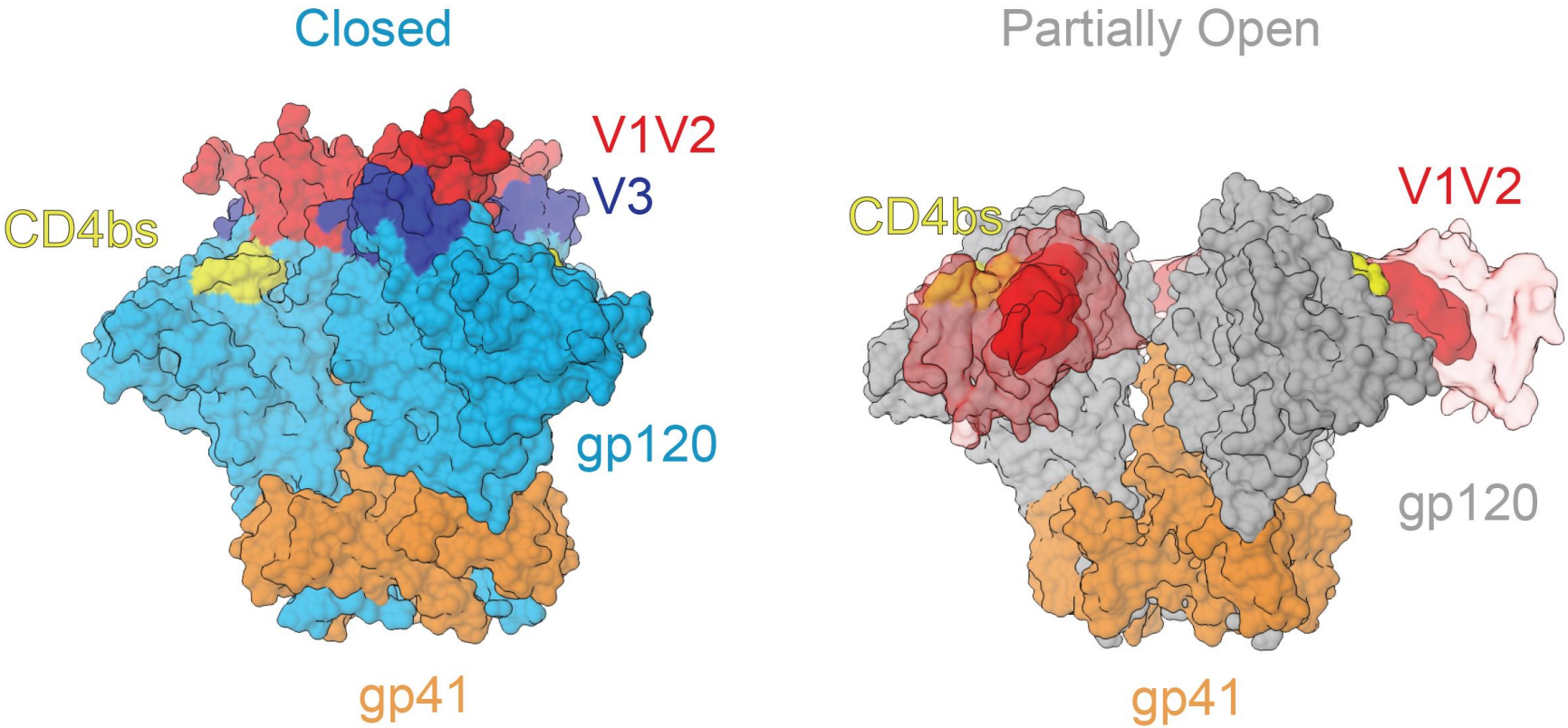
~17Å single particle EM structure of
17b-CD4-8ANC195-Env complex



Env conformation is mid-way between partially open and open

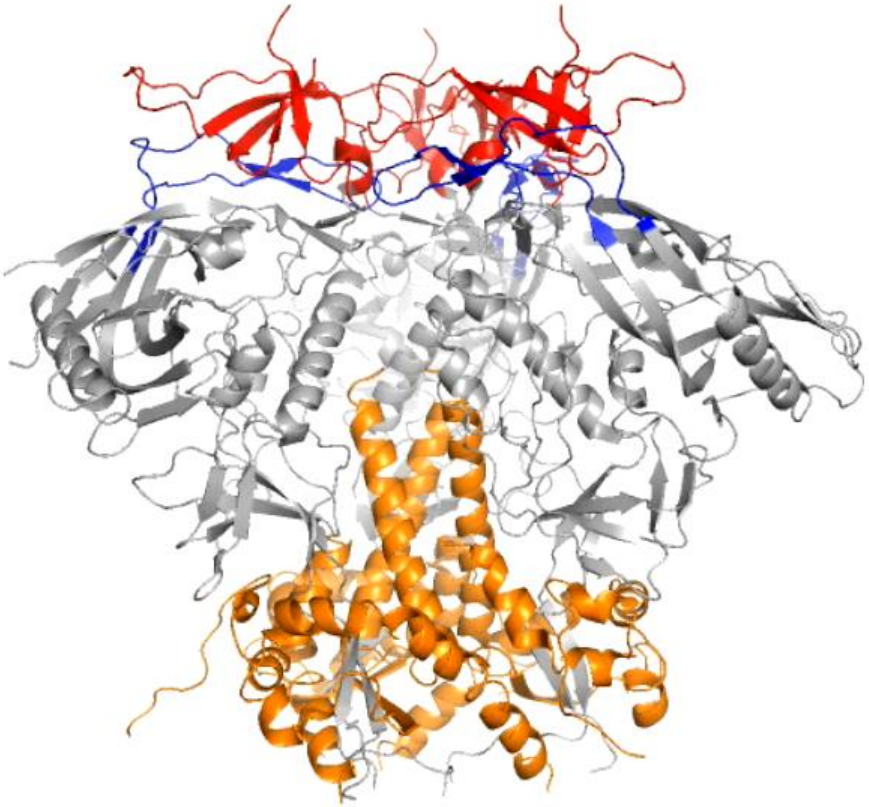
Scharf et al., 2015, *Cell*

Env trimer in CD4-bound partially-open 8.9 Å cryo-EM structure: V1V2 undergoes ~40 Å conformational change and interacts with CD4



Wang et al., 2016, *PNAS*

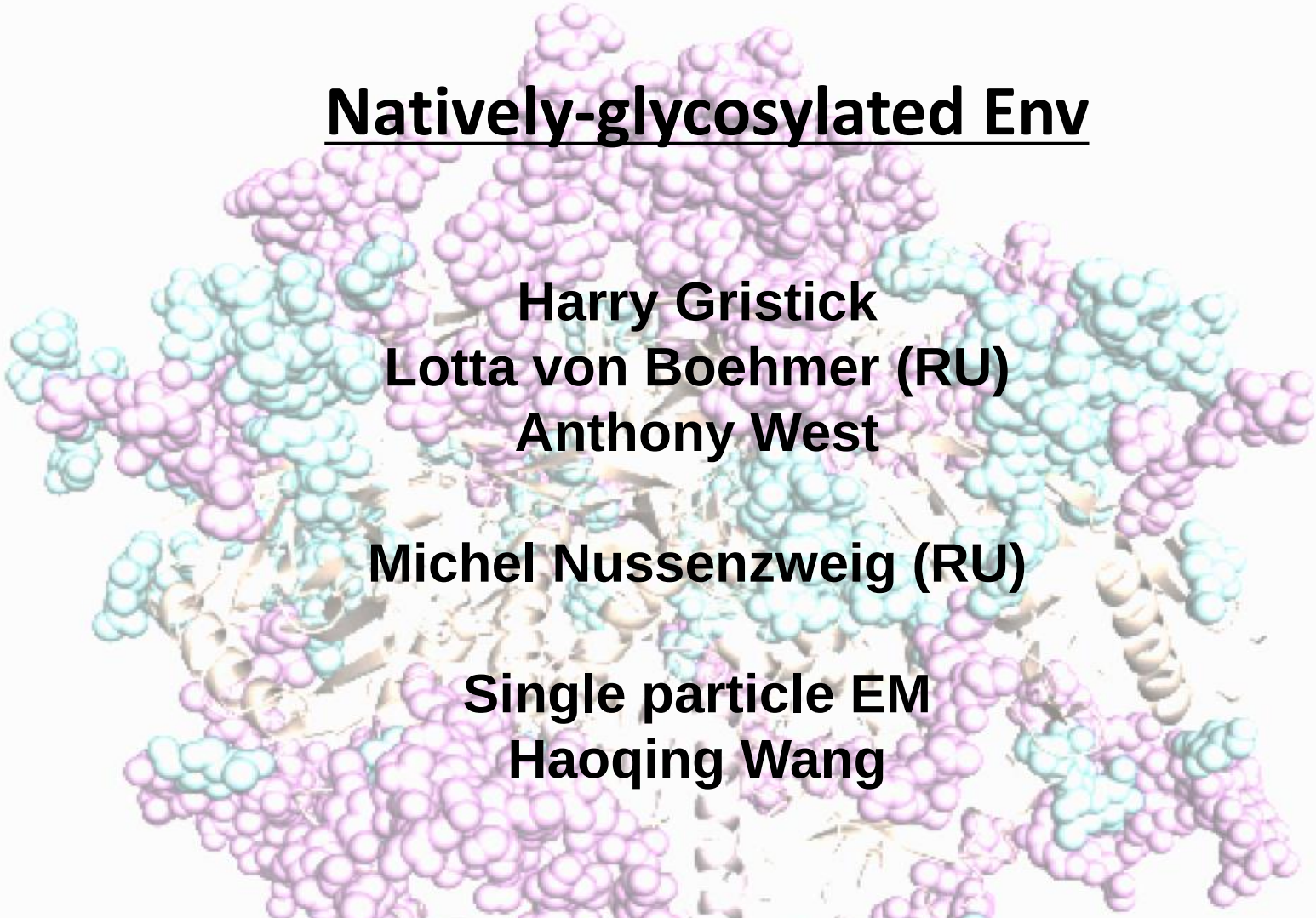
Env trimer in CD4-bound partially-open 8.9 Å cryo-EM structure: V1V2 undergoes ~40 Å conformational change and interacts with CD4



V1V2
V3
gp120
gp41
sCD4

Wang et al., 2016, *PNAS*

Natively-glycosylated Env



Harry Gristick
Lotta von Boehmer (RU)
Anthony West

Michel Nussenzweig (RU)

Single particle EM
Haoqing Wang

Michael Schamber
Michael Seaman, CAVD Neutralization Facility
Donor samples: Florian Klein, Gerd Fätkenheuer (U of Cologne)

Funding: HIVRAD P01, BMGF
Funding for EM: NIH P50 Cheetah center

Intra-spike crosslinking reagents

Rachel Galimidi
Anthony West
(Joshua Klein → Google)

X

Devashish Joshi, Maria Politzer,
Shiyu Bai, Pri Gnanapragasam
Caltech Protein Expression Center
Michael Seaman, CAVD Neutralization Facility

Funding: BMGF, NIH Director's Pioneer Award