# Inducing HIV-1 neutralizing antibodies by stabilized native-like SOSIP envelope trimers

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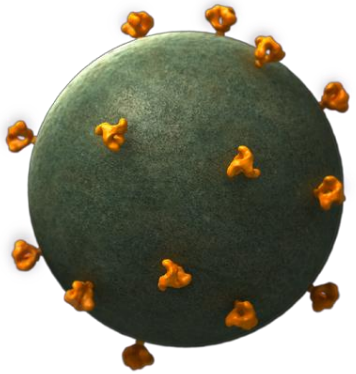
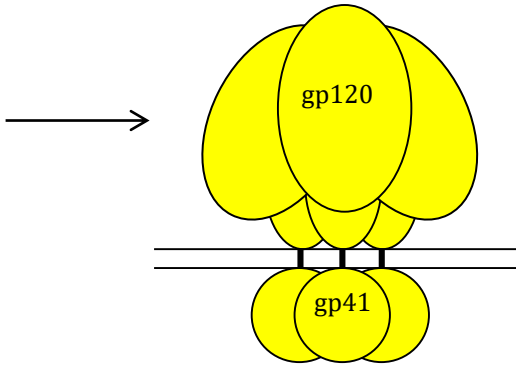
100 Gardes meeting, Veyrier-du-Lac, France, October 1st, 2019

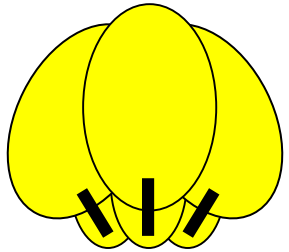
# Hypothesis:

## A stable structural and antigenic mimic of the native, cleaved envelope trimer should induce neutralizing antibodies



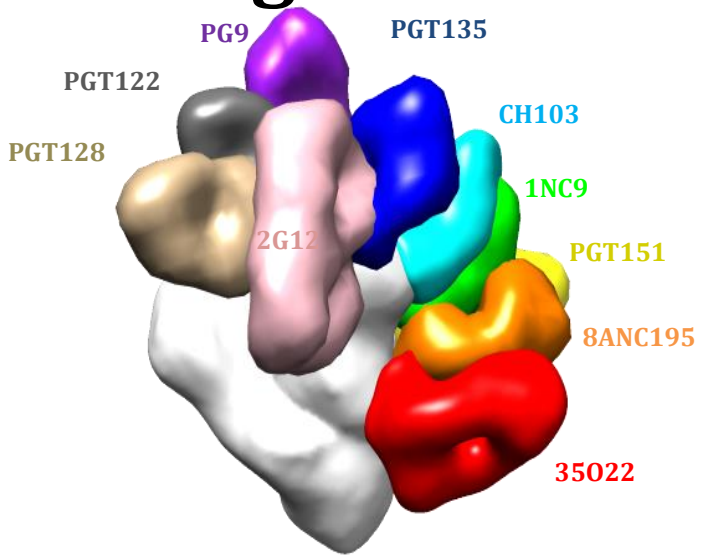



Native Env spike



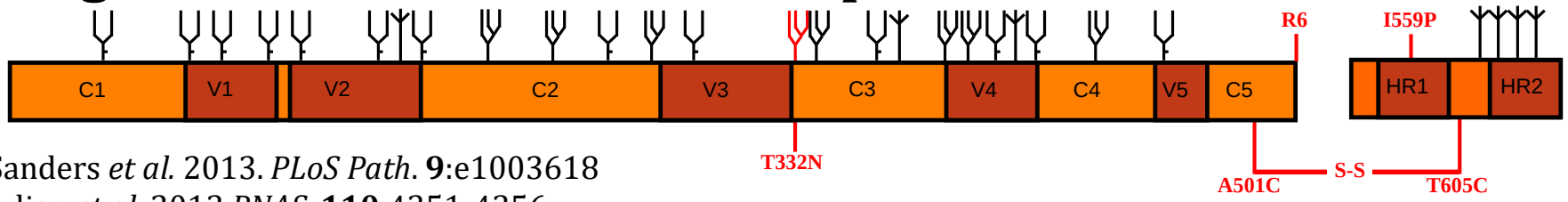
**SOSIP gp140**

Sanders *et al.* 2002
*J.Virol*. **76**:8875-8889



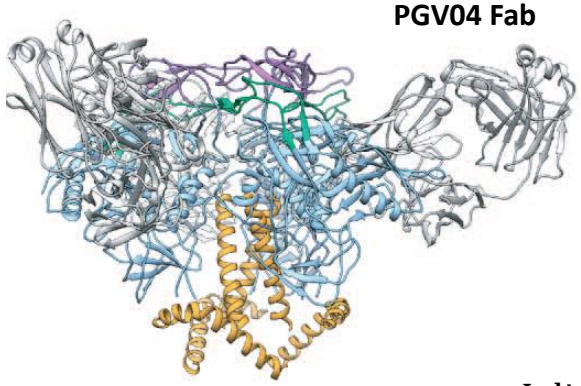

Derking *et al.* 2015. *PLoS Path*. **11**: e1004767

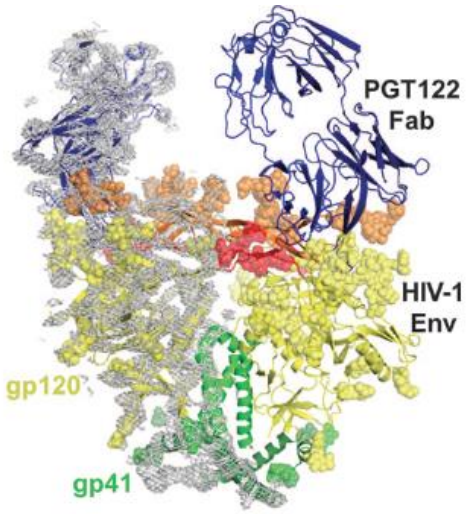
## 3rd generation native-like envelope trimer: BG505 SOSIP trimer



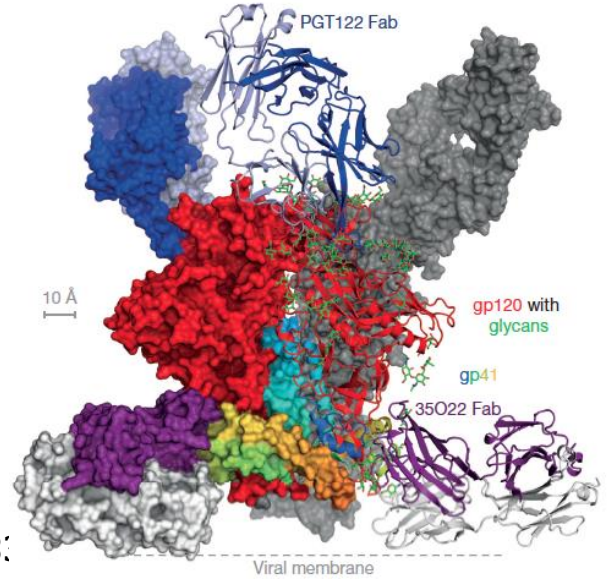

Sanders *et al.* 2013. *PLoS Path*. **9**:e1003618
Julien *et al.* 2013 *PNAS*. **110**:4351-4356

# The BG505 SOSIP trimer yielded the first high resolution structures of an HIV envelope trimer (2013-2014)



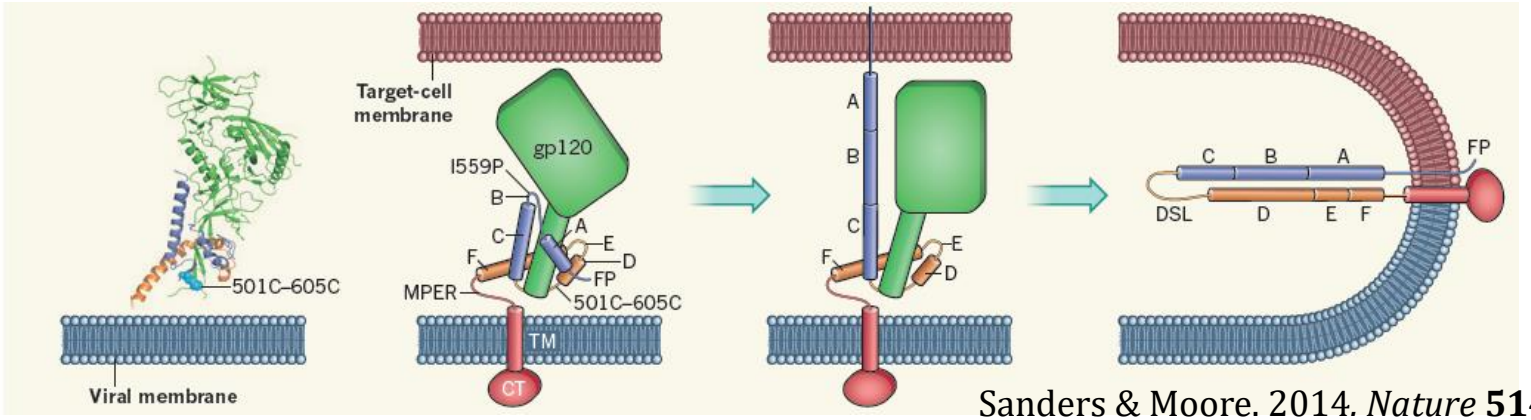




Julien *et al.* 2013. *Science.* **342:**1477-148



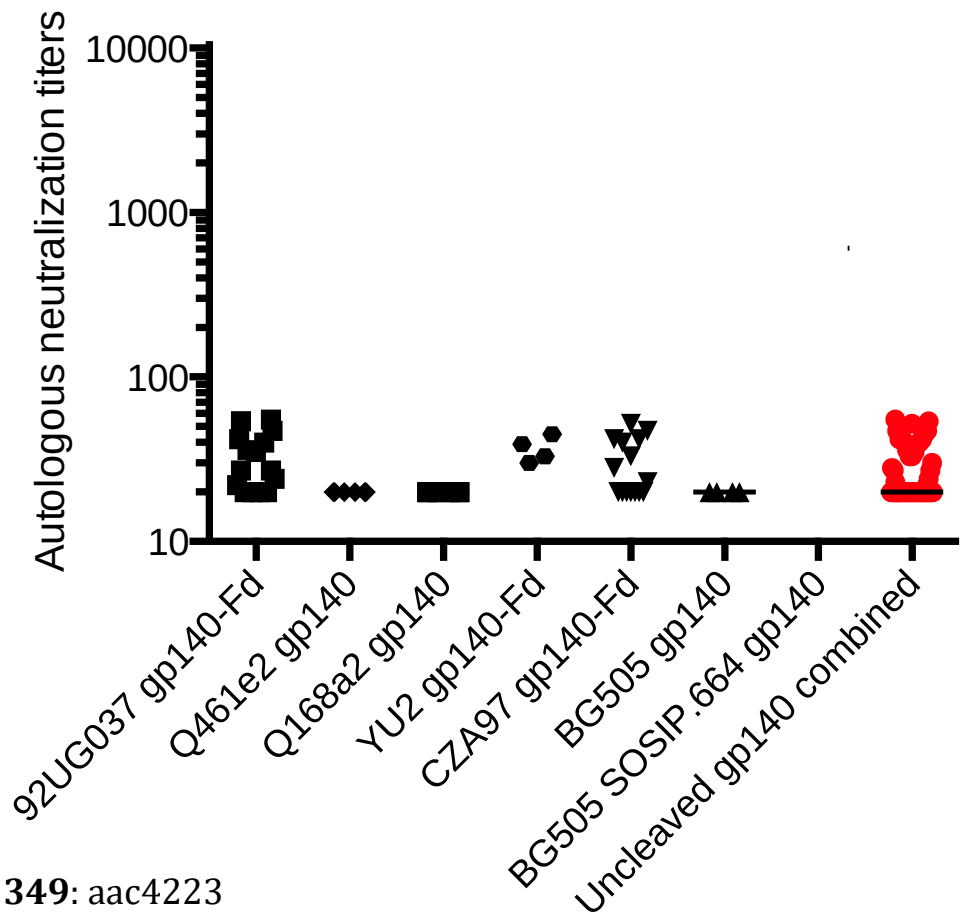

Pancera *et al*. 2014. *Nature* **524**:455-461

Lyumkis *et al*. 2013. *Science* **342**:1484-1490




Sanders & Moore. 2014. *Nature* **514**:437-438

# The BG505 trimer induces autologous Tier 2 NAbs in rabbits



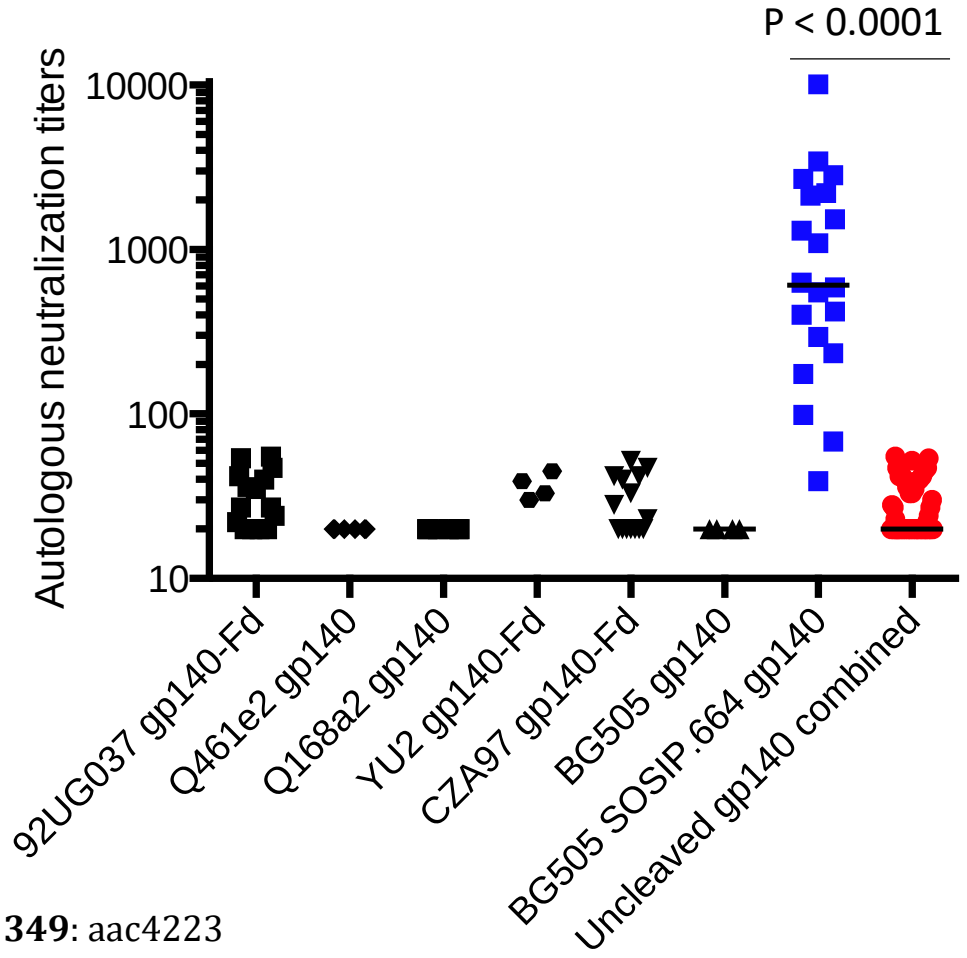

Sanders *et al.* 2015. *Science* **349**: aac4223

**Note 1:** Only data included for immunogens for which the autologous virus was tier 2
**Note 2:** Only rabbit or guinea pig data
**Note 3:** Only TZM-bl neutralization data
**Note 4:** Apples and oranges comparison: different isolates, species, neut assays, labs

**References:**


Nkolola *et al.* 2010. *J. Virol.* **84**:3270
-92UG037.8 gp140-Fd
-CZA97.012 gp140-Fd
Blish *et al.* 2010. *J. Virol.* **84**:2573
-Q461e2 gp140
-Q168a2 gp140
Sanders *et al.* 2015. *Science* **349**: aac4223
-YU2 gp140-Fd
-BG505 gp140 (=WT.SEKS)
-BG505 SOSIP.664 gp140

# The BG505 trimer induces autologous Tier 2 NAbs in rabbits

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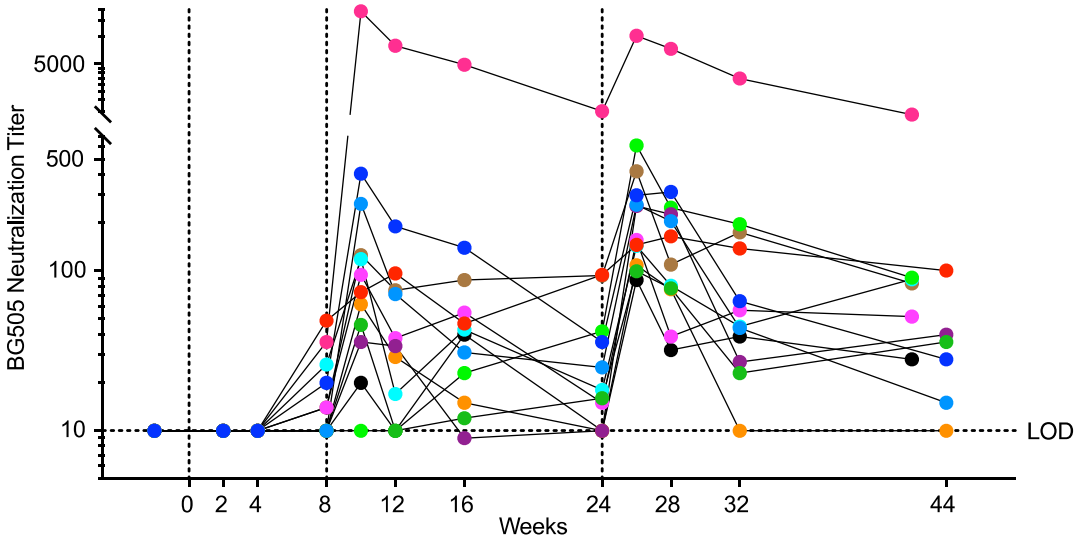
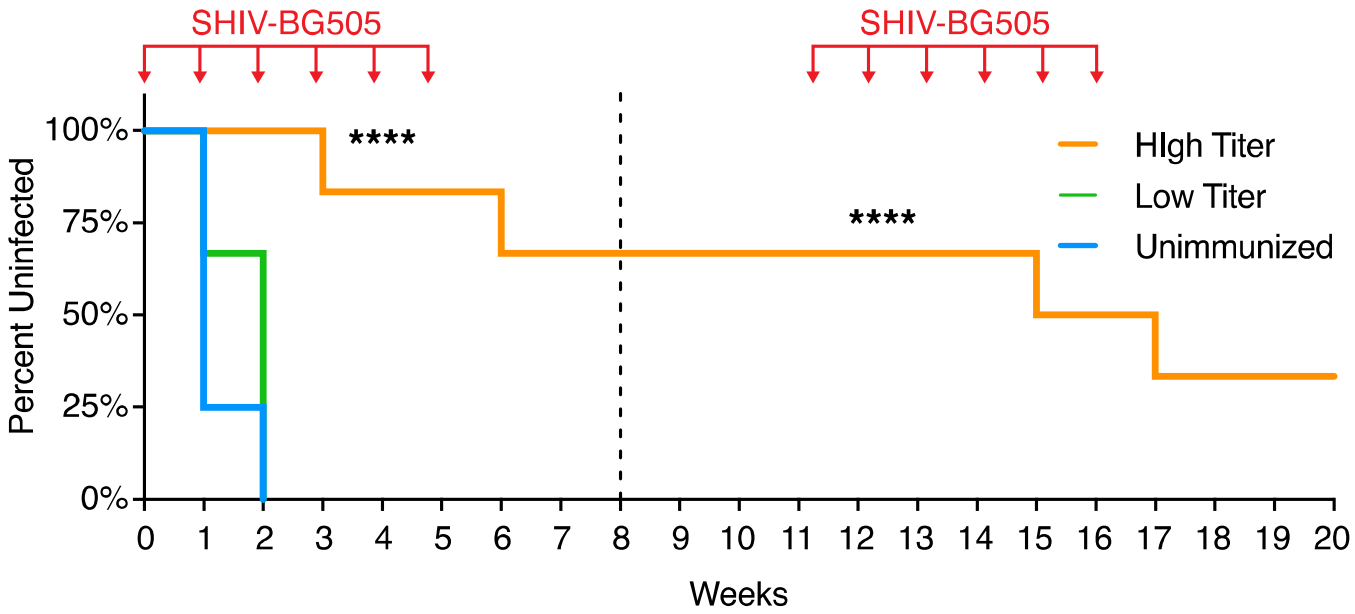
-BG505 SOSIP.664 gp140

# The BG505 SOSIP trimer induces NAb-mediated protection in rhesus macaques against the homologous Tier 2 SHIV virus




Pauthner *et al.* 2017. *Immunity* **46**:1073-1088

Pauthner *et al.* 2019. *Immunity* **50**:1-12

# Evaluating NAb induction by BG505 SOSIP trimers in humans

**Clinical trial 1 (IAVI W001): dose-ranging in AS01b (PI: J.McElrath)**

**Clinical trial 2 (HVTN137): adjuvant screening (PI: J. McElrath)**

**Immunogen: BG505 SOSIP.664**

**Vaccinations (months)**


**Goals:**

- Establish that the trimer is safe and well tolerated.
- Determine whether the trimer induces autologous NAbs (also heterologous NAbs, and undesirable Abs)
- Compare human Ab responses to the SOSIP trimer with responses in other animal models (e.g., NHP, rabbits, guinea pigs, rats)

| | |
|---|---|
| Lead scientists | Moore/Sanders |
| Funders | NIH (HIVRAD/HVTN) & BMGF (IAVI VxPDC) |
| Manufacturer | KBI Biopharma |
| GMP finished | Q2 2017 |
| Clinical trial start | Q1 2019 and Q4 2019 |
| Clinical sites | Ragon Institute, FHCRC, KAVI Kenia |
| | (J. McElrath, J Maenza, B. Walker, B. Juelg, O. Anzala) |

iavi
International AIDS Vaccine Initiative

BILL & MELINDA GATES foundation

NIH

# "The SOSIP design is flawed because SOSIP proteins are in State 2"

**From Lu M, ...., Sodroski JG, Mothes W. 2019. *Nature* 568:415-419**
**"BG505 sgp140 SOSIP.664 proteins are in a conformation that is distinct from the native Env"**, specifically conformational "State 2" and not the more appropriate "State 1".

**From Castillo-Menendez LR, Nguyen HT, Sodroski J. 2019. *J. Virol.* 93: pii: e01709-18**
**"[There are] differences in conformation between structurally well-characterized HIV-1 Env trimers (sgp140 SOSIP.664 and EnvΔCT complexed with the PGT151 antibody) and native, mature Envs on primary HIV-1."**

**From Nguyen HT, Alsahafi N, Finzi A, Sodroski JG. 2019. *J. Virol.* 93:pii: e00304-19**
**"The I559P and SOS changes have a profound impact on the conformation of Env, moving Env away from the native pretriggered Env conformation".**

# "The SOSIP design is flawed because SOSIP proteins are in State 2"

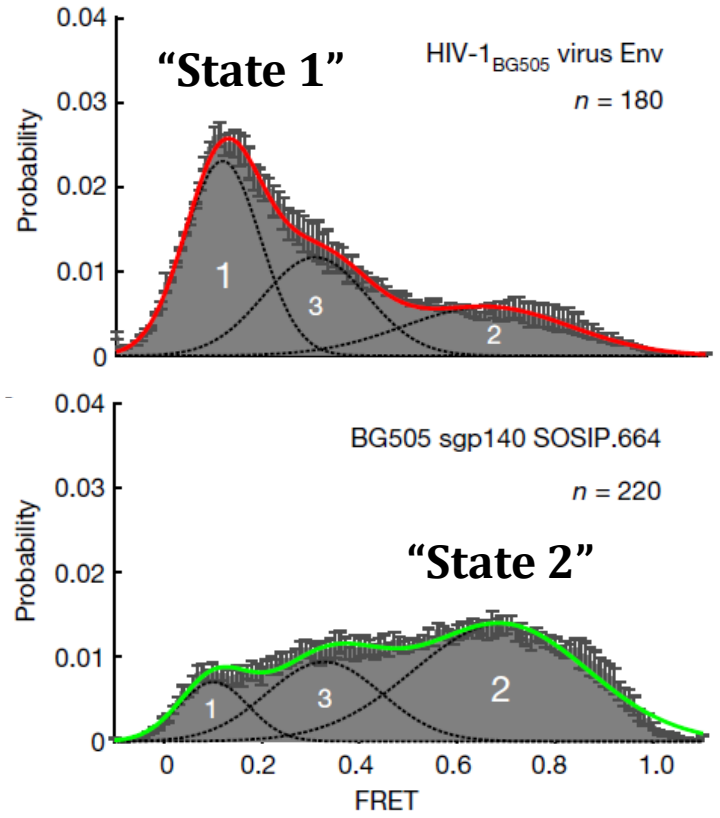
**From a grant review:**

"**A major concern is the proposed use of SOSIP trimers as immunogens.** The Sanders' lab (co-applicant) and others describe these soluble gp140s as being "native-like" trimers. …. How well SOSIPs really capture the "native-like" structure of Envs incorporated into infectious viral particles is unclear. ….. data presented at Cold Spring Harbor Retroviruses and the Institute of Human Virology meetings showing that **SOSIPs are stabilized in a conformation that differs from the native-like State 1 conformation**. Therefore, how good is an immunogen that does not recapitulate the structural properties of a real Env (functional incorporated Env) can be?"

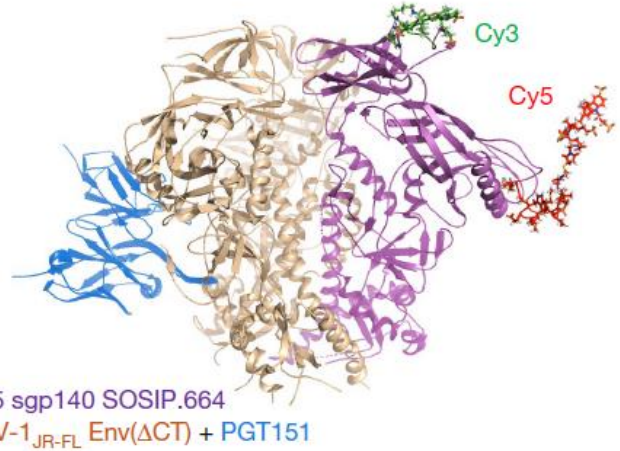
**From a manuscript review:**

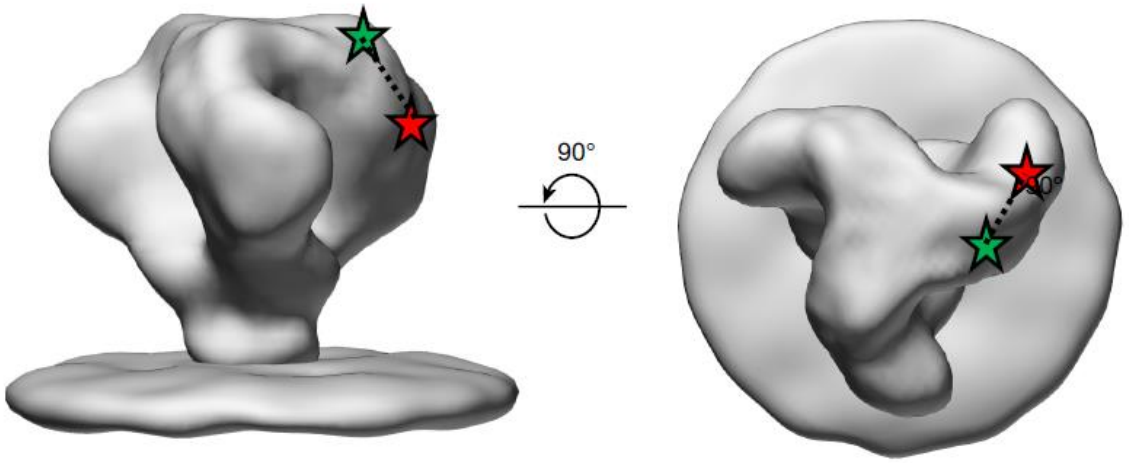
"This is another very clear example of how the Moore group continues to demonstrate repeatedly how to NOT elicit cross-reactive neutralizing antibodies to HIV-1…. This group of investigators … most likely fail to elicit efficiently cross-neutralizing HIV-1 antibodies because of subtle, but **critical, structural flaws inherent in the SOSIP design [as] recently and convincingly shown to result in a default State 2 conformation, rather than the State 1 that is the bonafide native state on the surface of the virus** by the Mothes group (Nature 2019)."

# “The SOSIP design is flawed because SOSIP proteins are in State 2”




smFRET measures movement of fluorescent labels attached to V1 and V4




Munro *et al.* 2014. *Science* **346**: 759-763
Lu *et al.* 2019. *Nature* **568**:415-419

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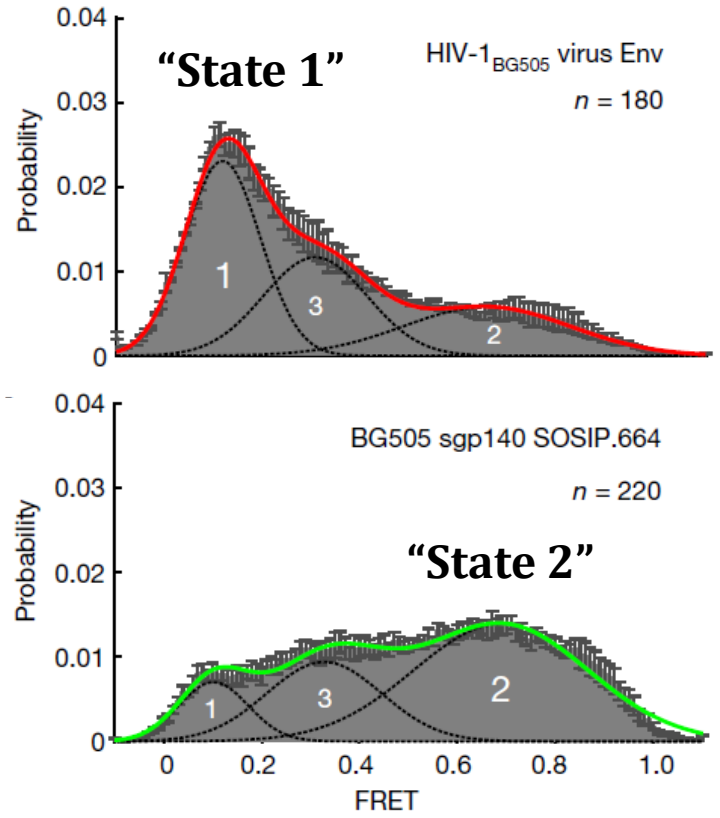



“**BG505 sgp140 SOSIP.664 proteins are in a conformation that is distinct from the native Env**”

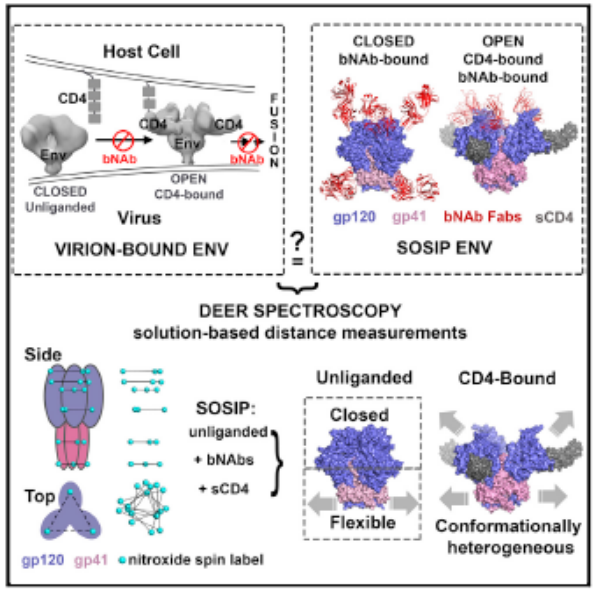
Lu *et al.* 2019. *Nature* **568**:415-419

Article

**Immunity**

## DEER Spectroscopy Measurements Reveal Multiple Conformations of HIV-1 SOSIP Envelopes that Show Similarities with Envelopes on Native Virions

### Graphical Abstract





### Authors

Beth M. Stadtmueller, Michael D. Bridges, Kim-Marie Dam, Michael T. Lerch, Kathryn E. Huey-Tubman, Wayne L. Hubbell, Pamela J. Bjorkman

### Correspondence

bjorkman@caltech.edu



### In Brief

HIV-1 Env, the only target of neutralizing antibodies, is highly dynamic, and only snapshots of static conformations are available. Stadtmueller et al. used DEER spectroscopy to map conformations of soluble Env and its complexes with antibodies or the CD4 receptor. Results reveal similarities to virion-bound Env and buried non-neutralizing antibody epitopes, advancing knowledge of Env function and vaccine design.


### Highlights

- SOSIP Env apex is 3-fold symmetric and consistent with closed prefusion structures
- Unliganded Env base and CD4-bound Env apex and base exhibit flexibility
- SOSIPs retain desired properties of immunogens; e.g., burying non-neutralizing epitopes
- Results allow interpretation of smFRET studies and SOSIP and virion Env structures

# “The SOSIP design is flawed because SOSIP proteins are in State 2”

“Results suggest similarities between SOSIPs and virion-bound Envs”

“Our experiments showed no evidence of multiple states with respect to V1V2–V4 separation distances”

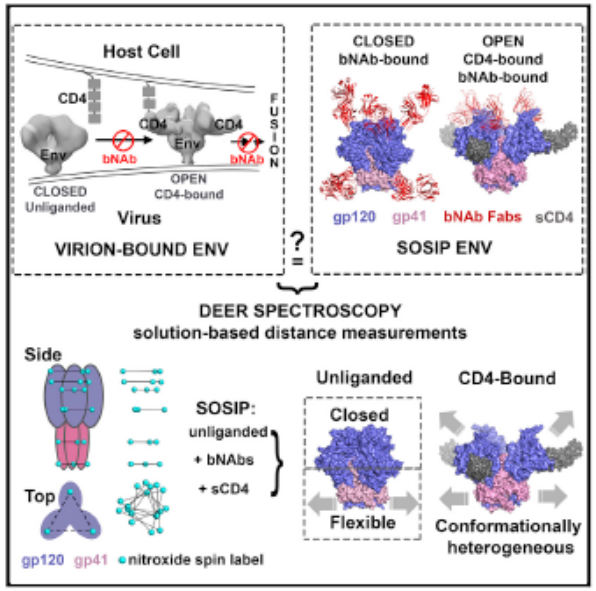
“[Our data] suggest that BG505 SOSIP exists in a single, symmetric conformation with respect to distances between the V1V2 and V4 regions”

Article

Immunity

## DEER Spectroscopy Measurements Reveal Multiple Conformations of HIV-1 SOSIP Envelopes that Show Similarities with Envelopes on Native Virions

### Graphical Abstract

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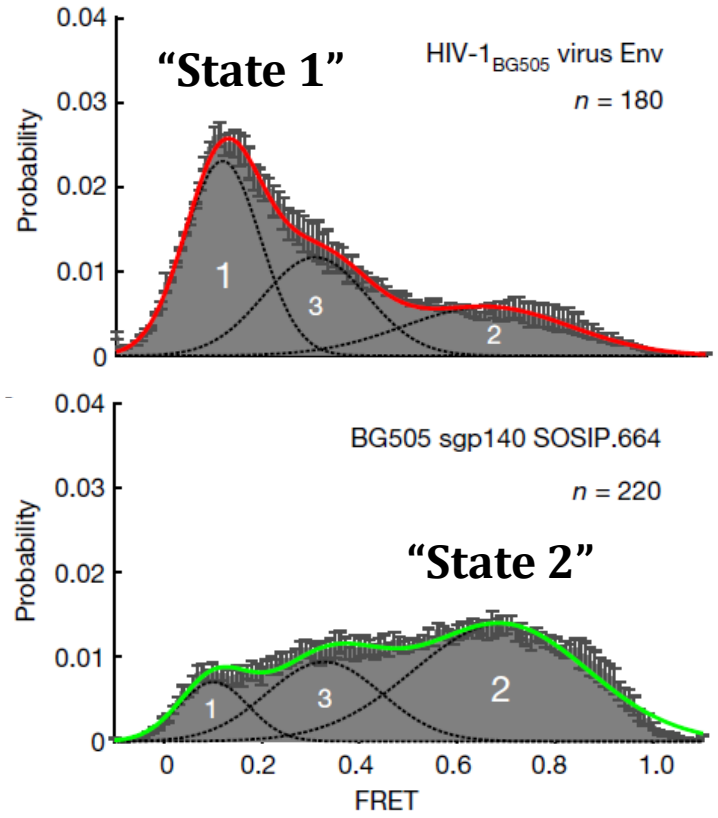
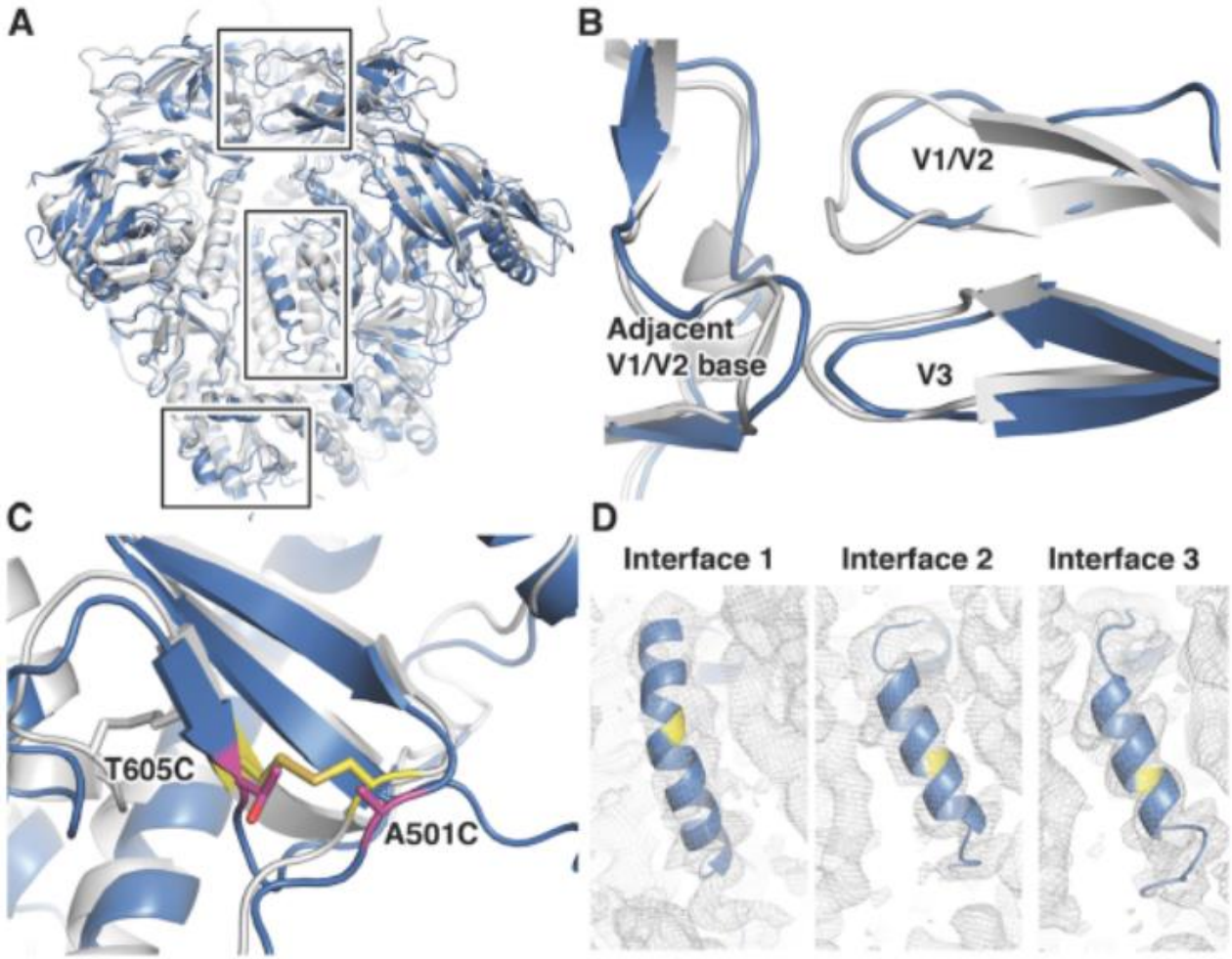
# SOSIP adopts a similar structure as native Env purified by PGT151



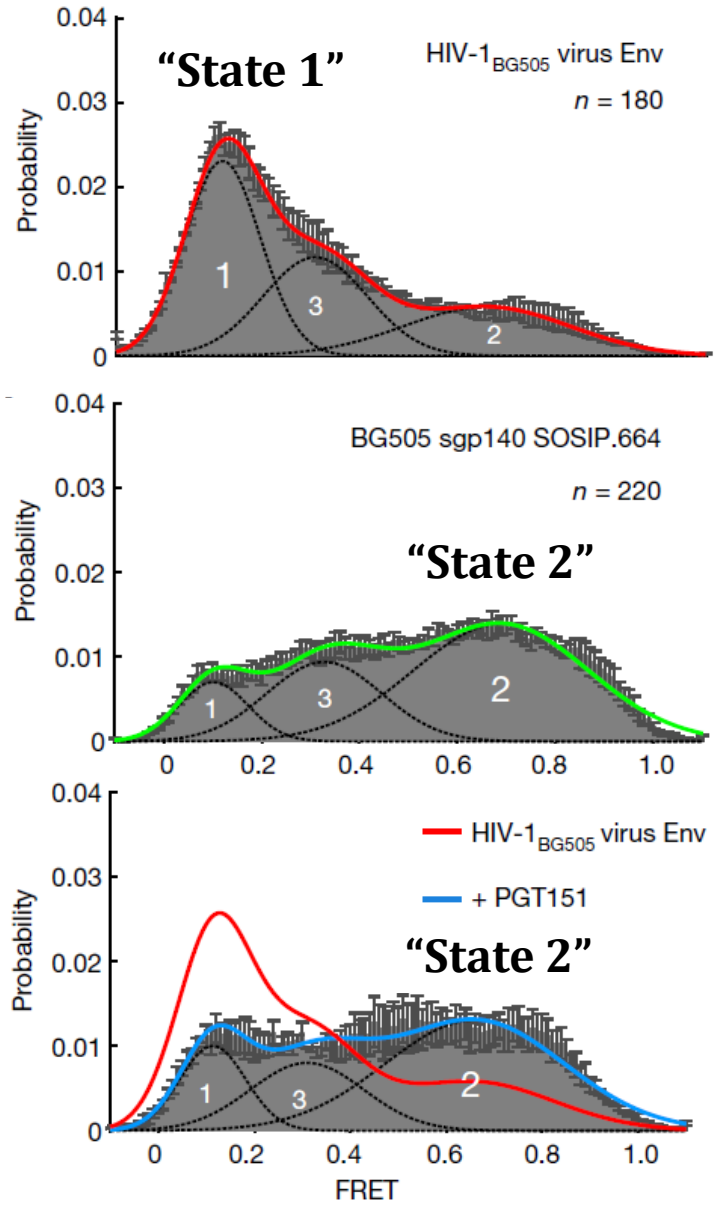

**Blue: JR-FL gp160**

White: BG505 SOSIP




Lee *et al.* 2016. *Science* **351**:1043

# "PGT151-purified native Env is also in State 2, not State 1"

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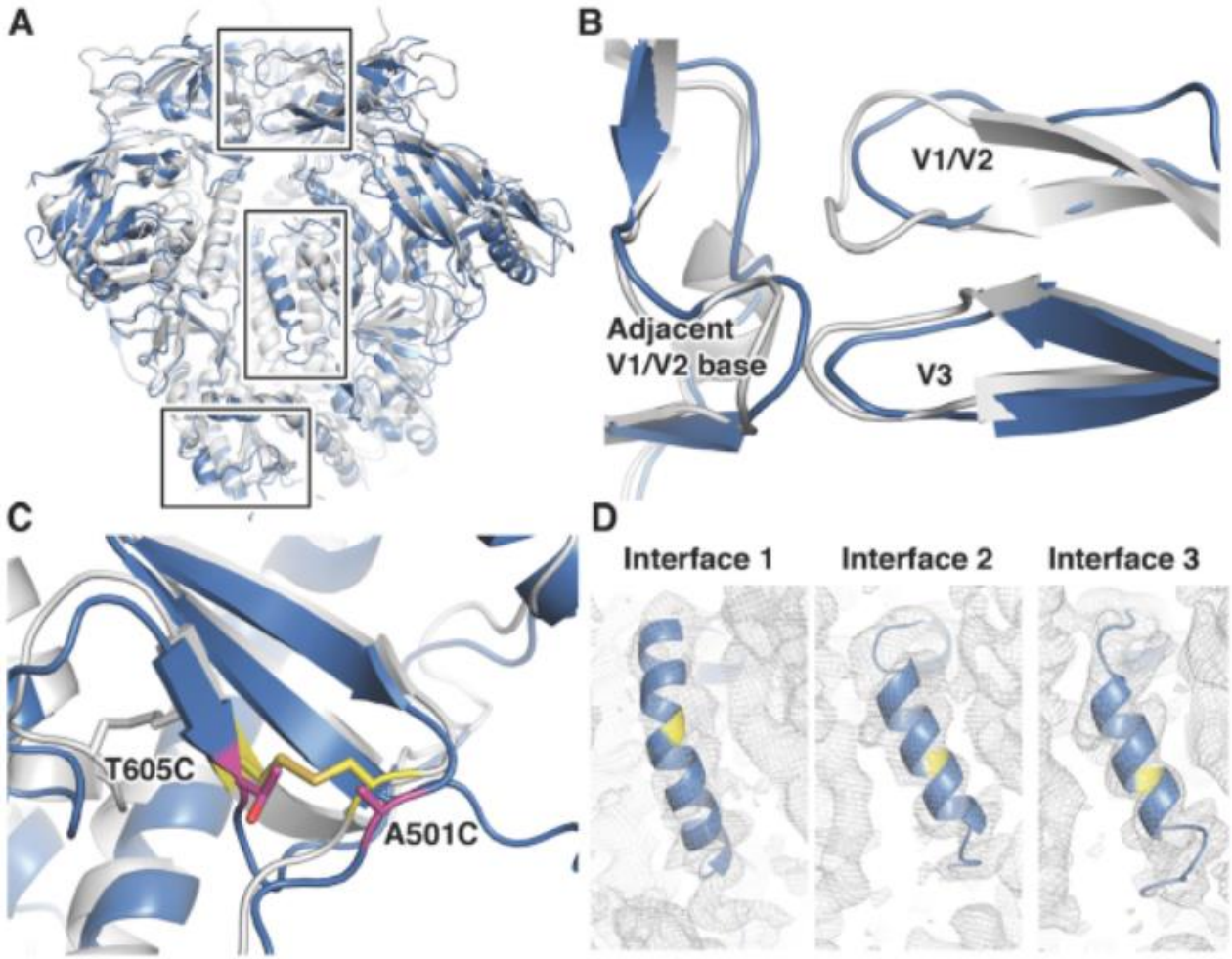
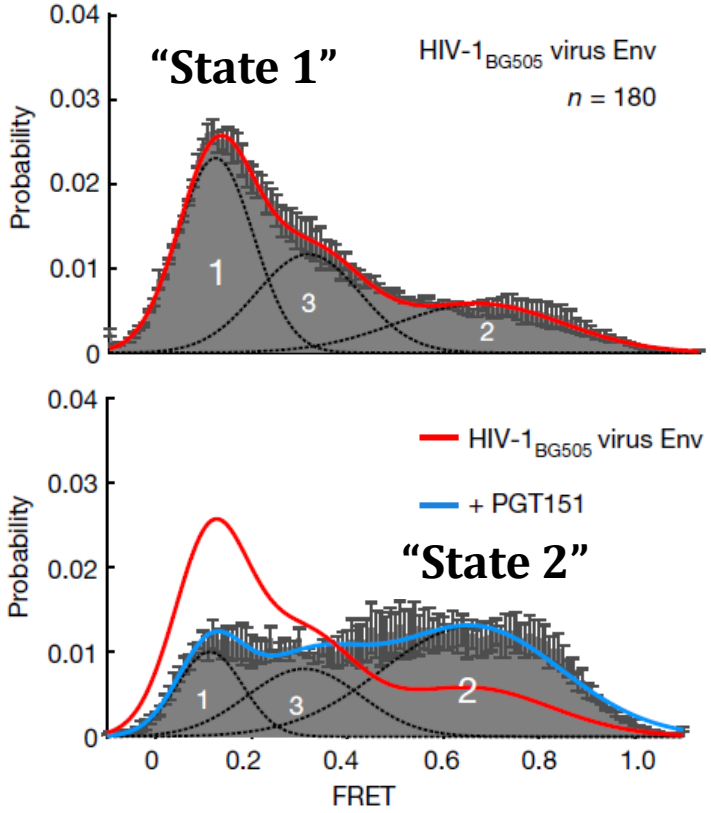
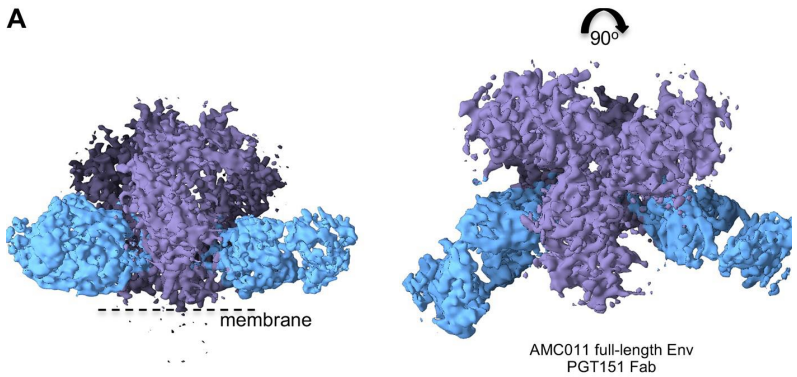
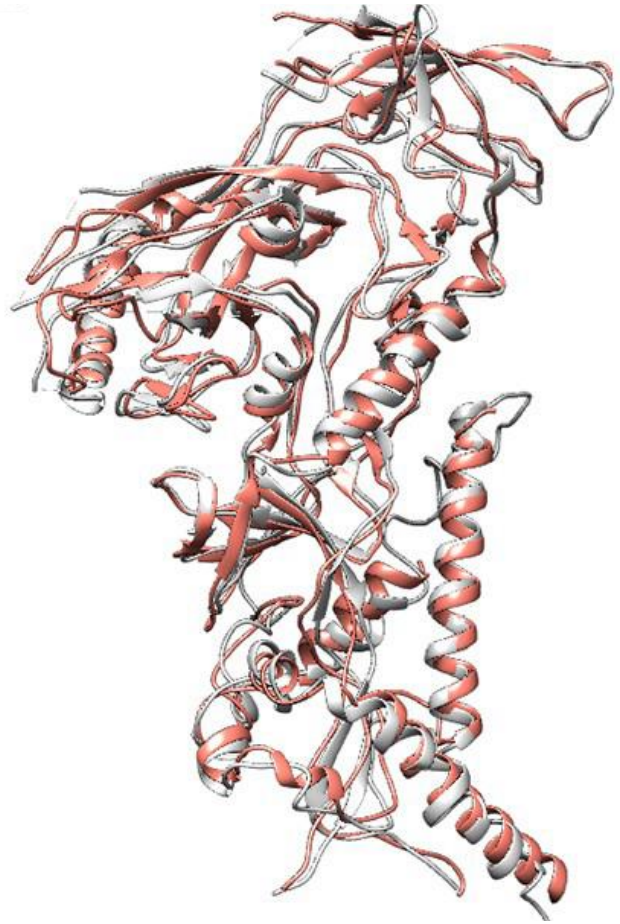



Lee *et al.* 2016. *Science* **351**:1043

# Full length native Env purified by “State 2” preferring PGT151 is structurally similar to SOSIP gp140



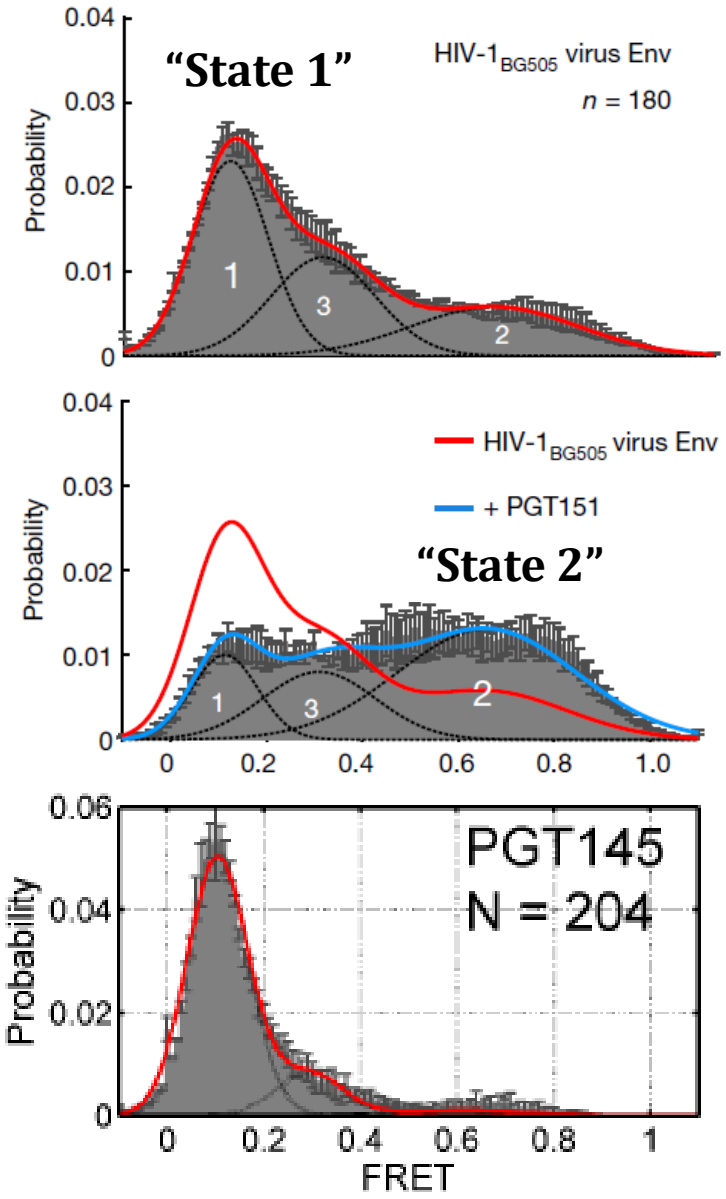



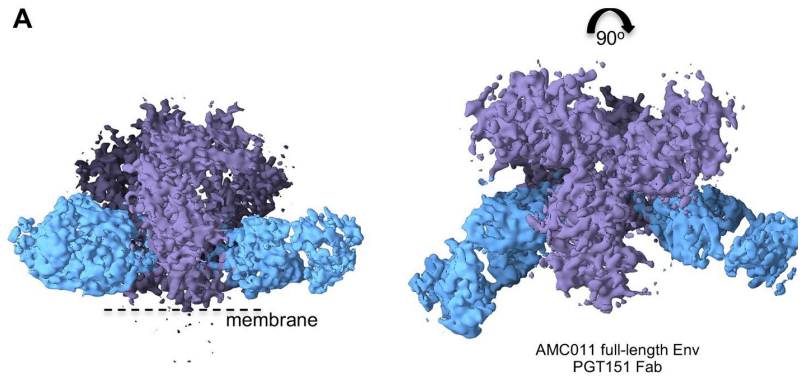



Full-length AMC011 PGT151 Fab
AMC011 SOSIP.v4.1 ACS202 Fab

Torrents de la Peña *et al.* 2019. *PLoS Pathogens* **15**:e1007920.

# Full length native Env purified by “State 2” preferring PGT151 is structurally similar to SOSIP gp140




Torrents de la Peña *et al.* 2019. *PLoS Pathogens* **15**:e1007920.

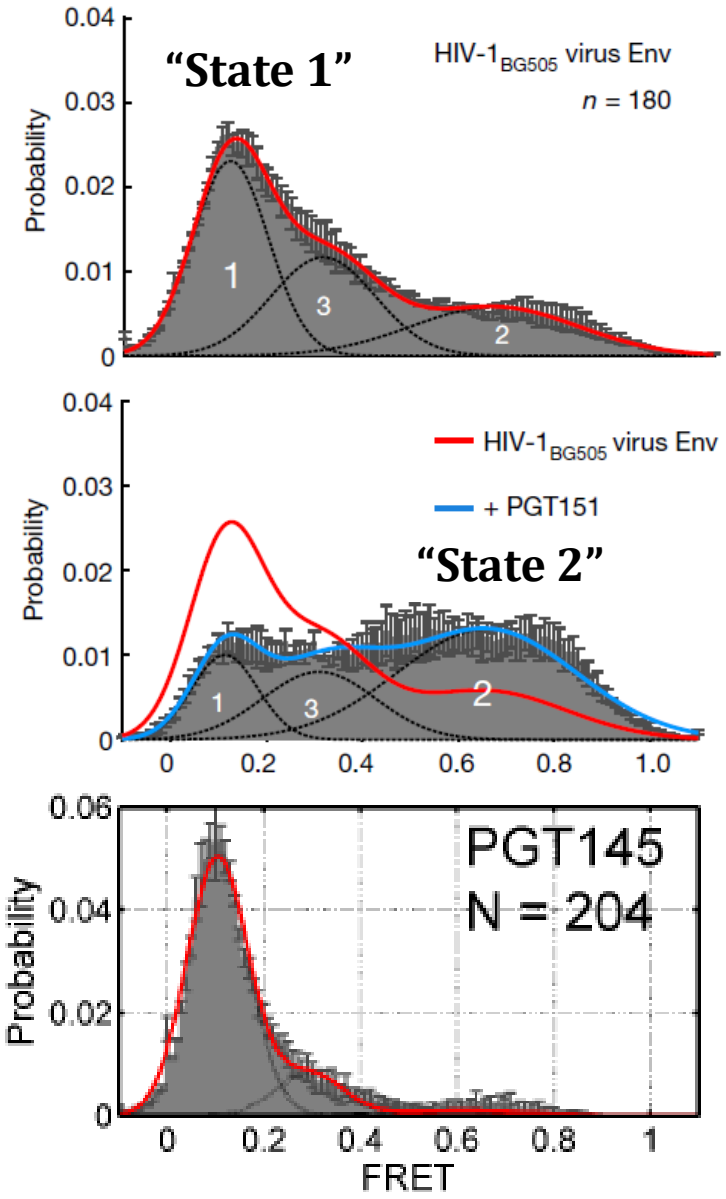
**Full lenth native Env has a similar structure as SOSIP when purified by “State 2” preferring bNAb PGT151**

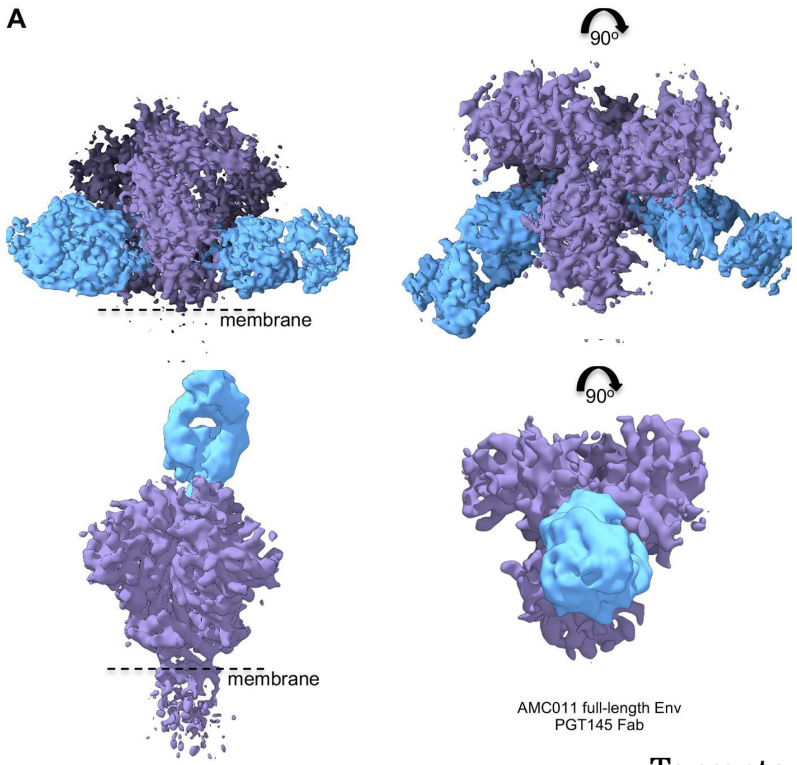
**Question to the audience:**

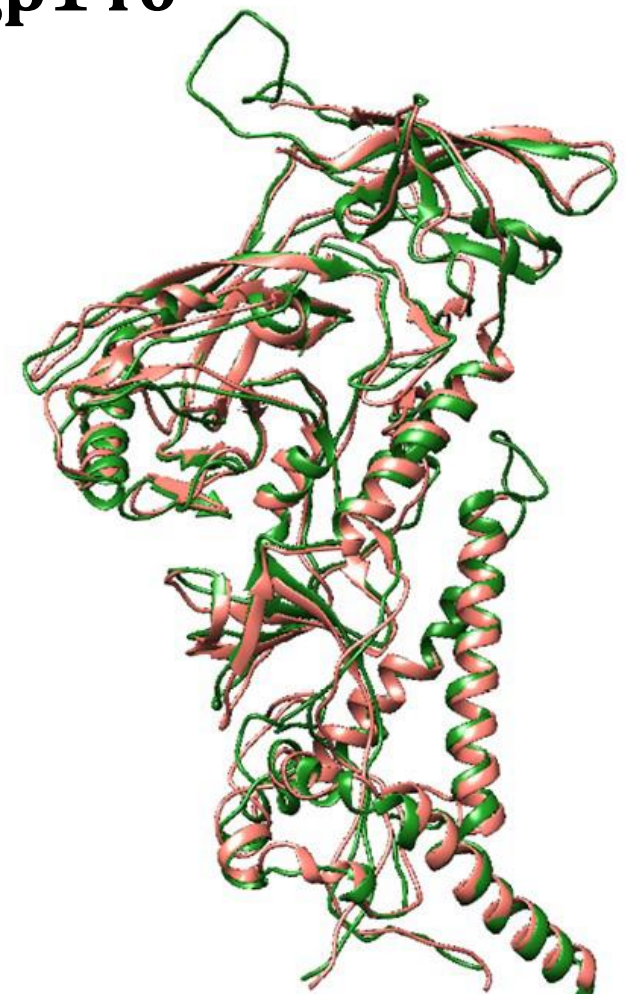
**Does full length native Env have a similar or a different structure as SOSIP when purified by “State 1” preferring bNAb PGT145?**

**A. Similar (hands up)**
**B. Different (hands down)**

# Full length native Env purified by “State-1” preferring PGT145 is structurally similar to SOSIP gp140

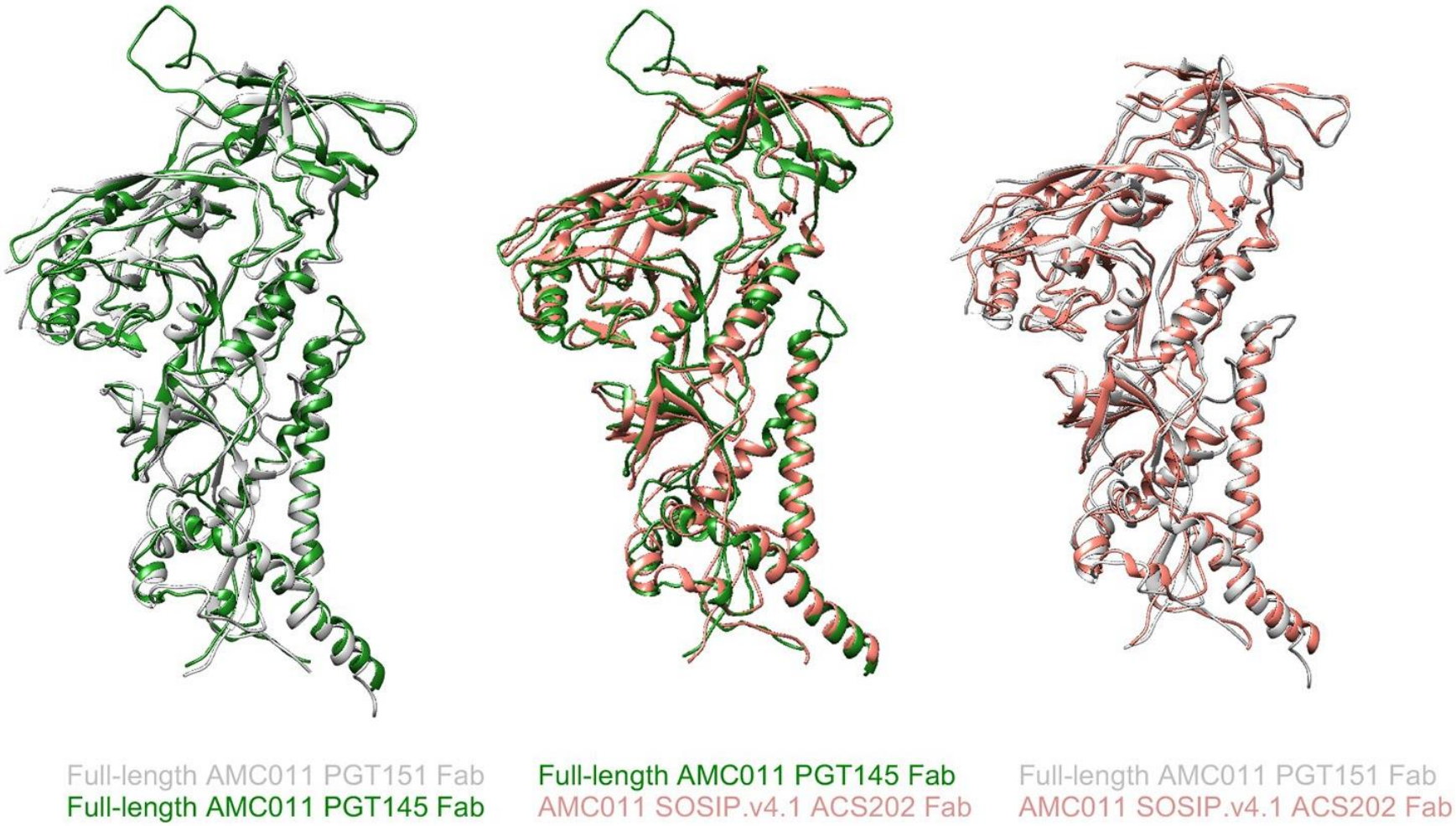



Torrents de la Peña *et al.* 2019. *PLoS Pathogens* **15**:e1007920.

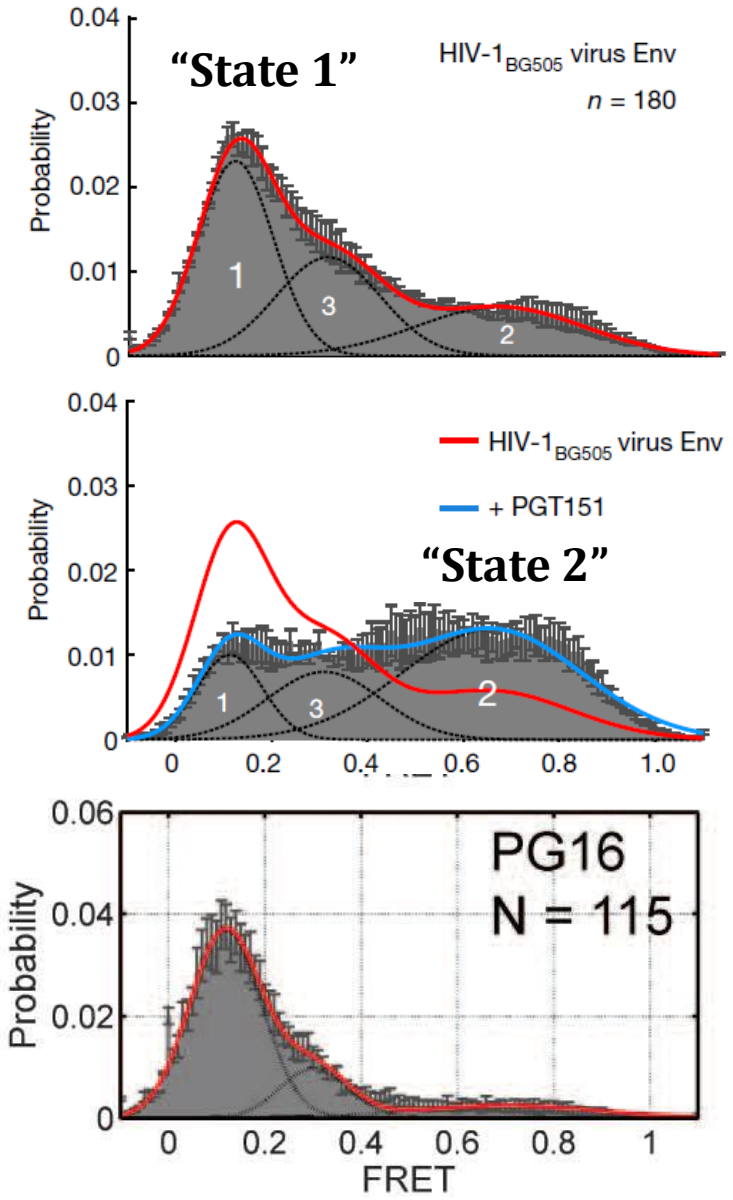
# Full length native Env purified by “State-1” preferring PGT145 is structurally similar to SOSIP gp140




**Data were corroborated by bNAb binding studies using BLI**

Torrents de la Peña *et al.* 2019. *PLoS Pathogens* **15**:e1007920.

# Structure of native Env in complex with “State-1” preferring bNAb PG16




Structure of 92UG037.8 gp160 in complex with PG16
(courtesy of Steve Harrison)

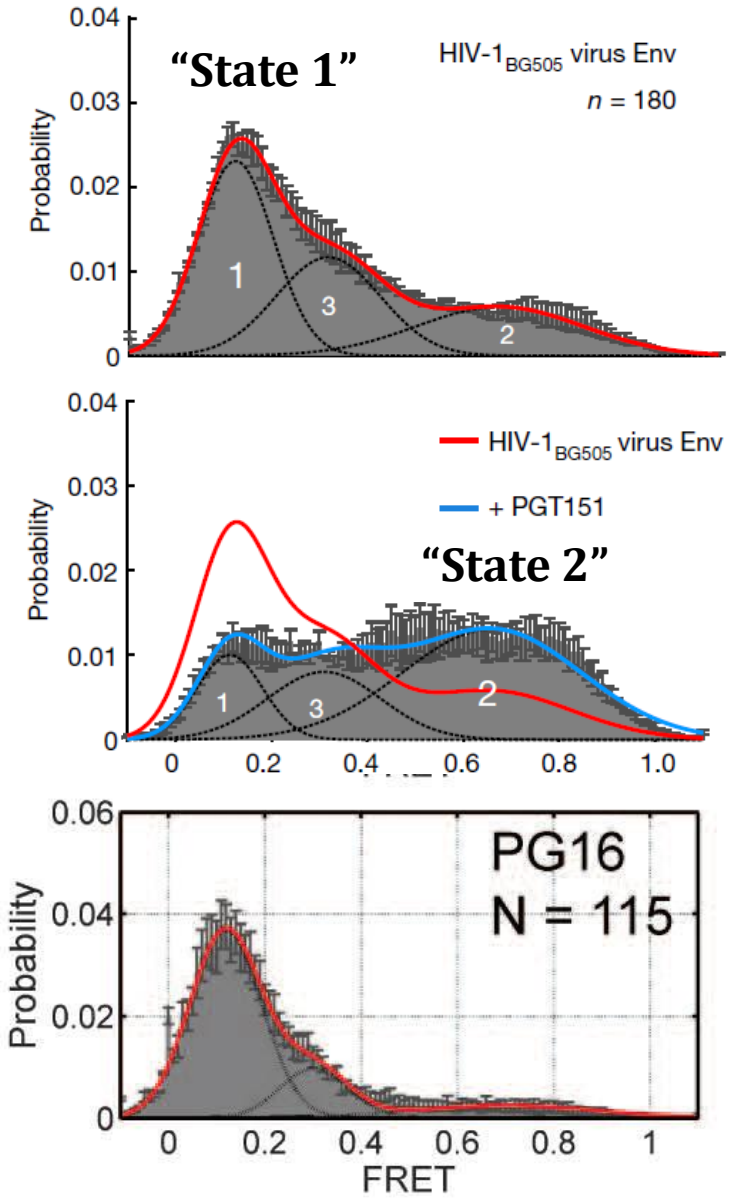
Pan, Peng, Chen, Harrison. 2019. *JMB* in revision
bioRxiv, http://dx.doi.org/10.1101/730333

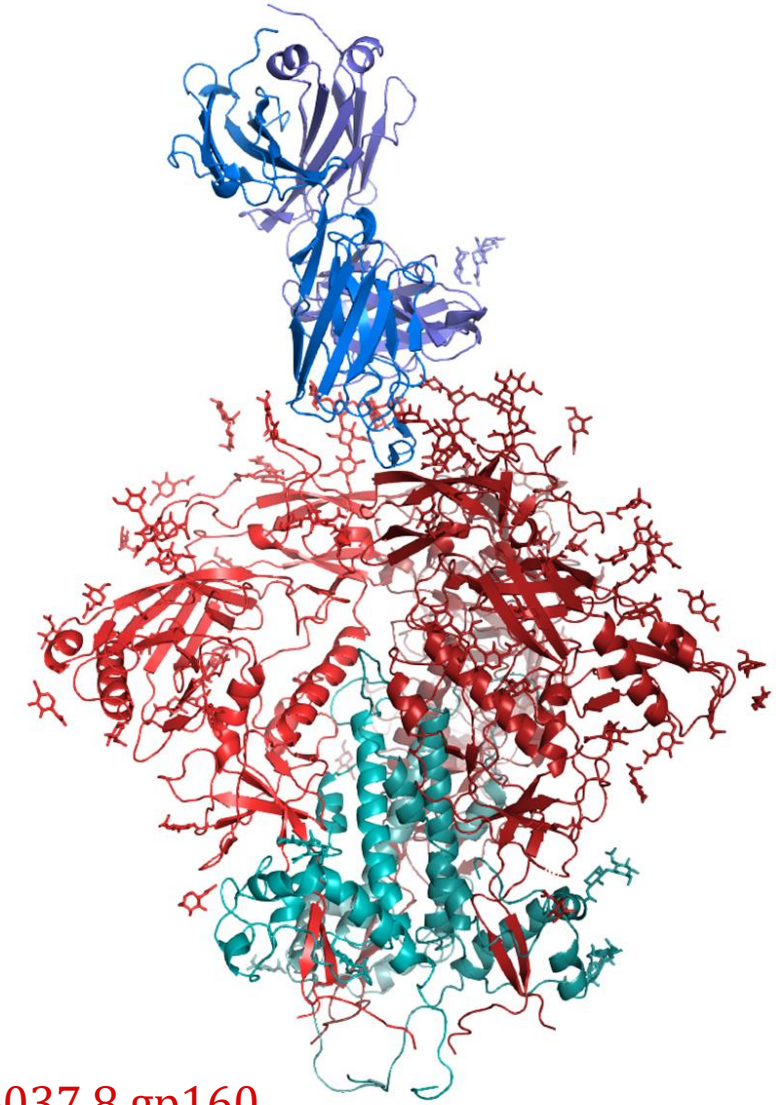
**Question to the audience:**

**Does full length native Env have a similar or a different structure as SOSIP when purified by “State 1” preferring bNAb PGT16?**

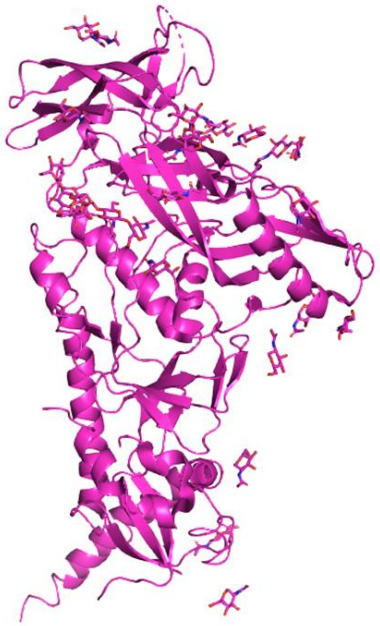
**A. Similar (hands up)**
**B. Different (hands down)**

# Structure of native Env in complex with “State-1” preferring bNAb PG16



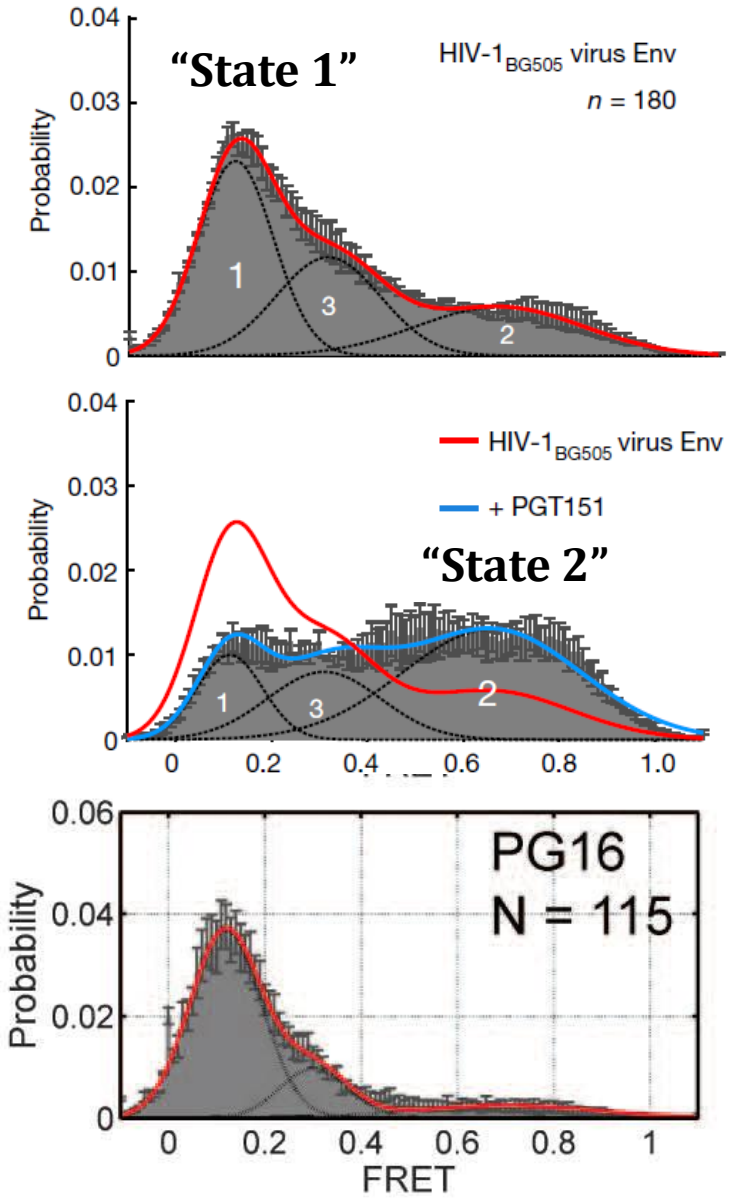
PG16
92UG037.8 gp160
(gp120/gp41)

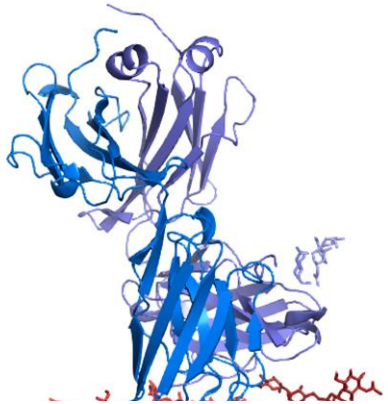


BG505 SOSIP (4zmj)

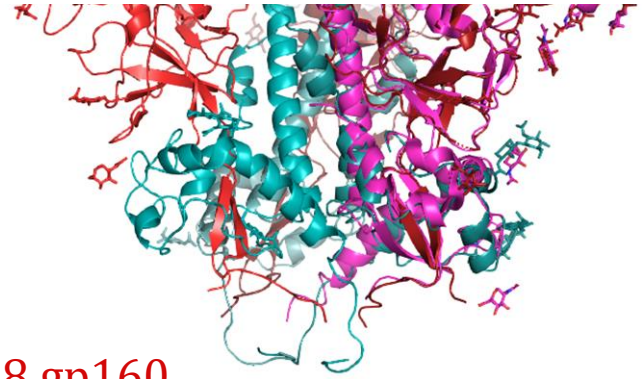
Pan, Peng, Chen, Harrison. 2019. *JMB,* in revision
bioRxiv, http://dx.doi.org/10.1101/730333

# Structure of native Env in complex with "State-1" preferring bNAb PG16



"The principal conclusion from our analysis is that a clade A gp160 has an overall conformation (with a few local exceptions) indistinguishable from that of BG505 SOSIP.664"



PG16
92UG037.8 gp160
(gp120/gp41)

BG505 SOSIP (4zmj)

Pan, Peng, Chen, Harrison. 2019. *JMB,* in revision
bioRxiv, http://dx.doi.org/10.1101/730333

# Why is the interpretation of smFRET data in disagreement with DEER spectroscopy and cryo-EM structures?

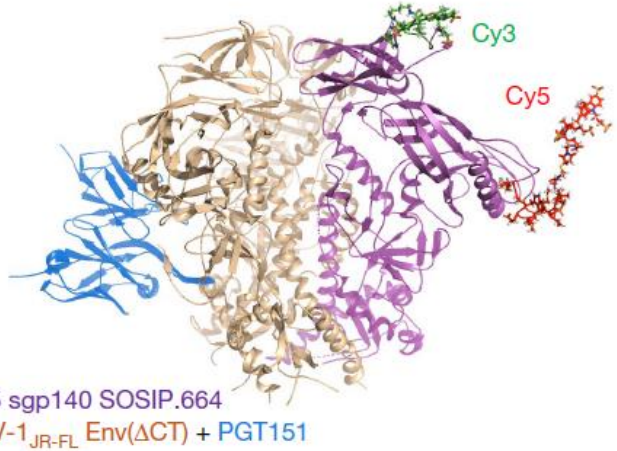
**smFRET signal derives from functional Env AND non-functional Env**

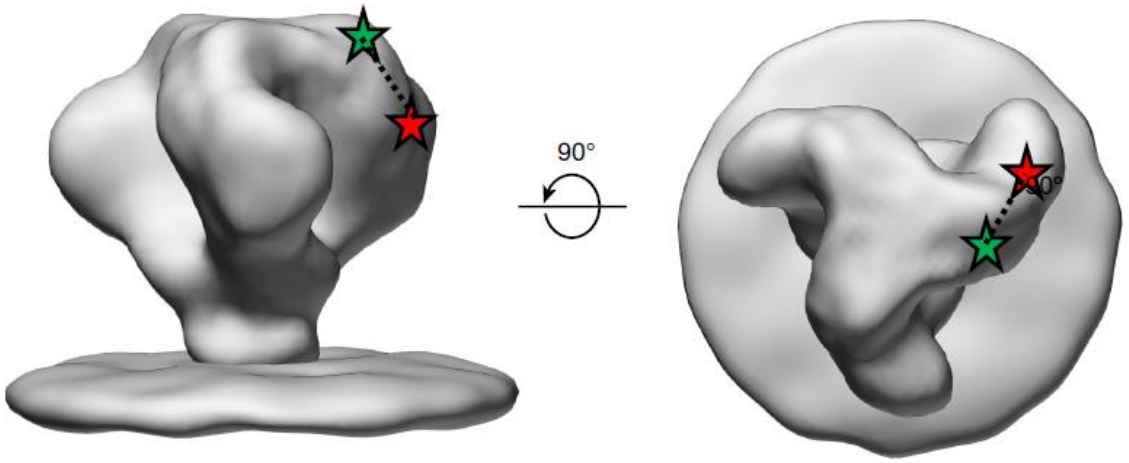
**smFRET uses large flexible labels**

Pamela Bjorkman:
"discrepancy could result from the size, hydrophobicity, and/or flexibility differences in DEER and smFRET labels"
(Stadtmueller *et al.* 2018. *Immunity* **43**:235-246)

Steve Harrison:
"depending on the orientation of the tether through which the acceptor fluorophore is attached, its distance can vary over 30-40 A, enough to span the difference between high and low FRET configurations"
(Pan *et al.* 2019. *JMB,* in revision
bioRxiv, http://dx.doi.org/10.1101/730333)

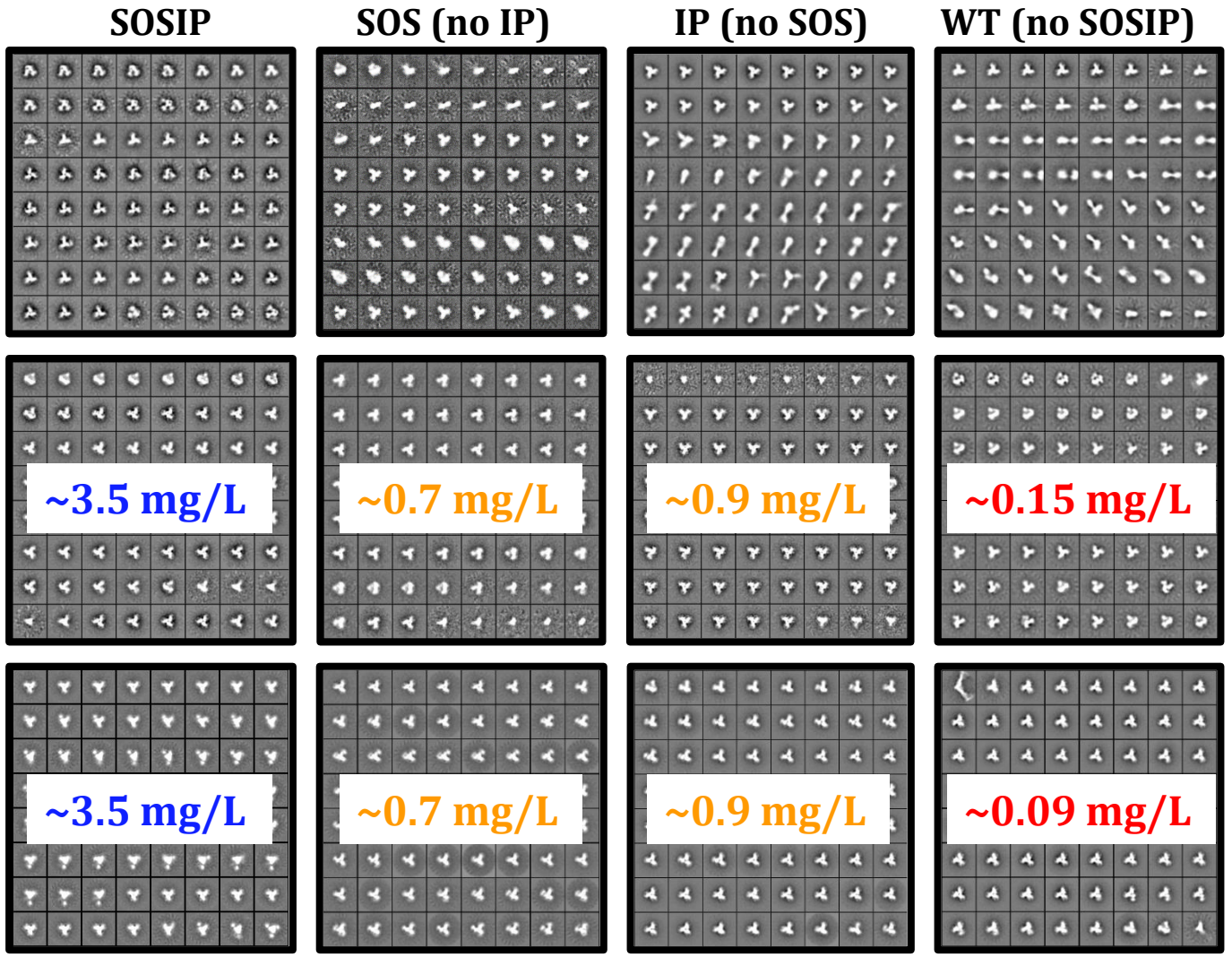
smFRET measures movement of fluorescent labels attached to V1 and V4




Munro *et al.* 2014. *Science* **346**: 759-763
Lu *et al.* 2019. *Nature* **568**:415-419

# The SOSIP mutations do not alter the overall conformation of native-like gp140 but improve the proportion and yield of native-like trimers




BG505 SOSIP.664 variants were made that lacked the SOS and/or I559P changes and expressed in ExpiCHO cells. Trimers were then purified via 2G12/SEC columns, or a PGT151 column, or a PGT145 column.

For each construct, the PGT151- and PGT145-purified trimers had comparable NS-EM appearance, melting temperatures (DSC) and antigenicity for bNAbs and non-NAbs (SPR; not shown).

However, trimer yields were substantially lower when the stabilizing changes were omitted.

Ringe, Moore *et al.* 2019. *submitted*

## Conclusions (part I)

## The structures of full length native Env and SOSIP gp140 are very similar

Native Env trimers purified by “State-1” preferring bNAbs or “State-2” preferring bNAbs are very similar structurally

The conformations of SOSIP trimers purified by “State-1” preferring bNAbs or “State-2” preferring bNAbs are very similar

**One should be cautious with using Env smFRET data to make inferences about Env structure**

The SOSIP mutations do not affect the overall conformation of native-like Env gp140 trimers, only their yields

The SOSIP trimer represent an appropriate mimic of the native Env trimer and therefore a suitable platform for immunogen design, including germline-targeting

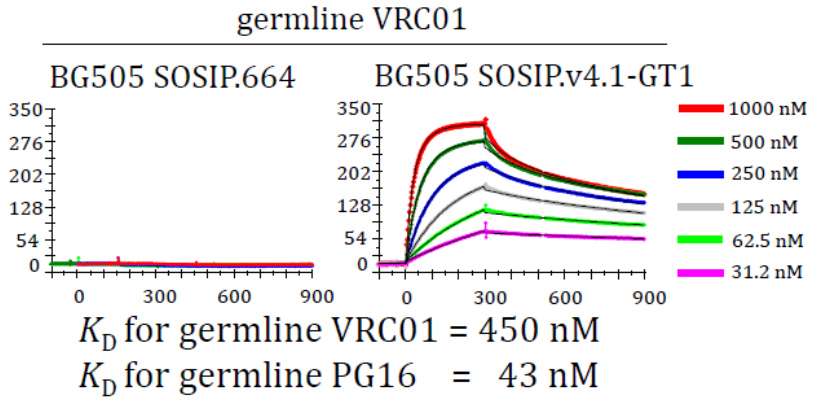
# BG505 SOSIP based germline-targeting immunogens

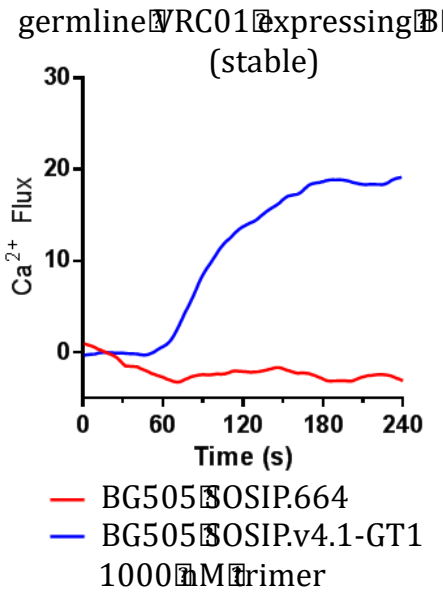
**Preclinical observations *in vitro***

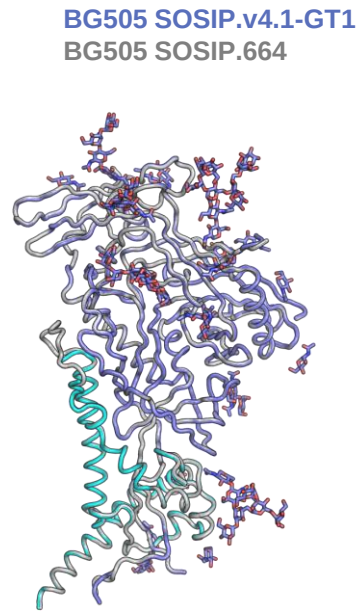
- BG505 SOSIP.v4.1-GT1 (GT1) engages multiple bNAb germline precursors in vitro
- GT1 activates B cells expressing bNAb germline precursors as their BCR
- GT1 crystal structure allowed refinement of the trimer: GT1.1 and GT1.2
- GT1.1 engages 7 in a million naïve human B cells, mostly CD4bs-specific

**Preclinical observations *in vivo***

- GT1, GT1.1 and GT1.2 activate multiple bNAb germline precursors in multiple knock-in mouse models
- GT1.1 primes CD4bs-specific responses in macaques



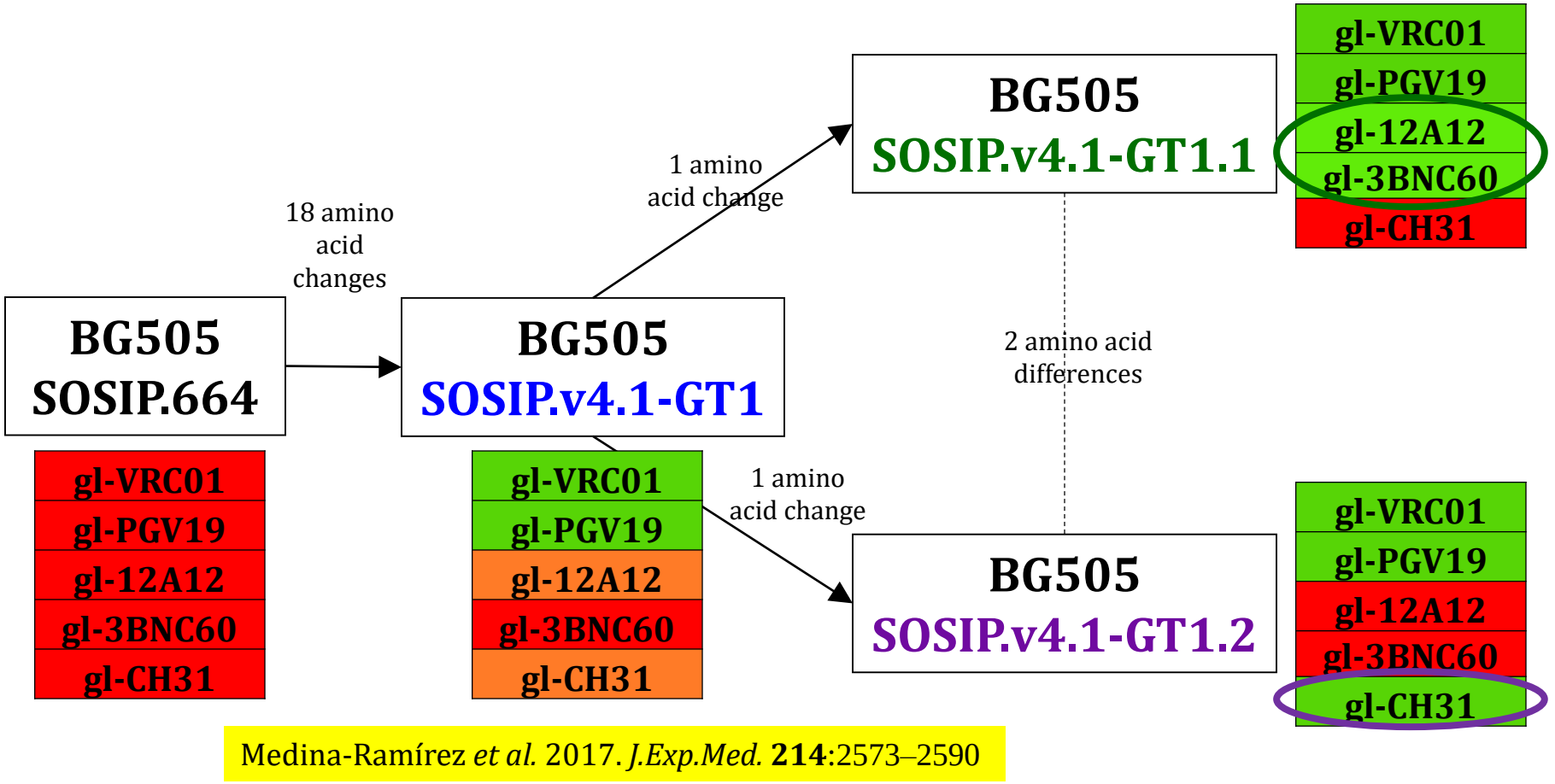




Medina-Ramírez *et al.* 2017. *J.Exp.Med.* **214**:2573-80
and unpublished obervations

# BG505 SOSIP based germline-targeting immunogens

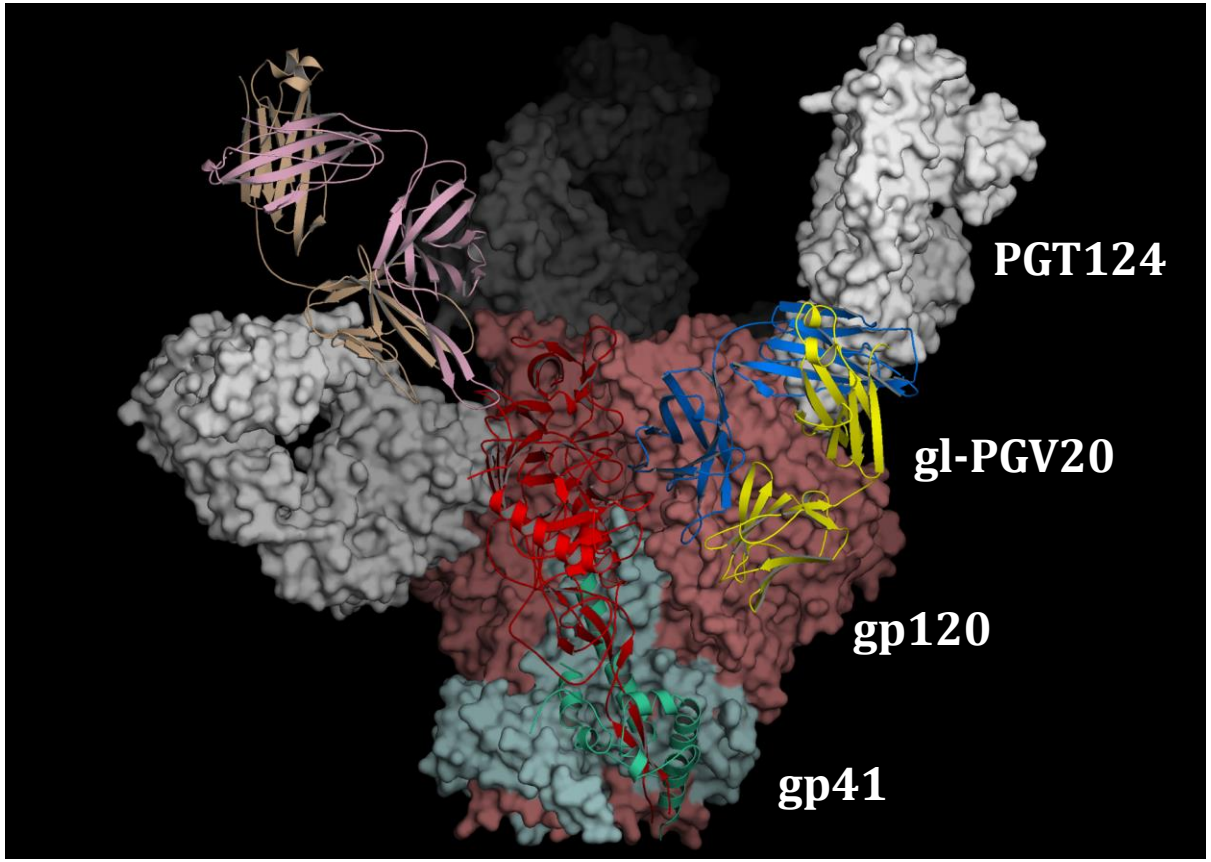
## Engagement of VRC01-class precursors




Medina-Ramírez *et al.* 2017. *J.Exp.Med.* **214**:2573–2590

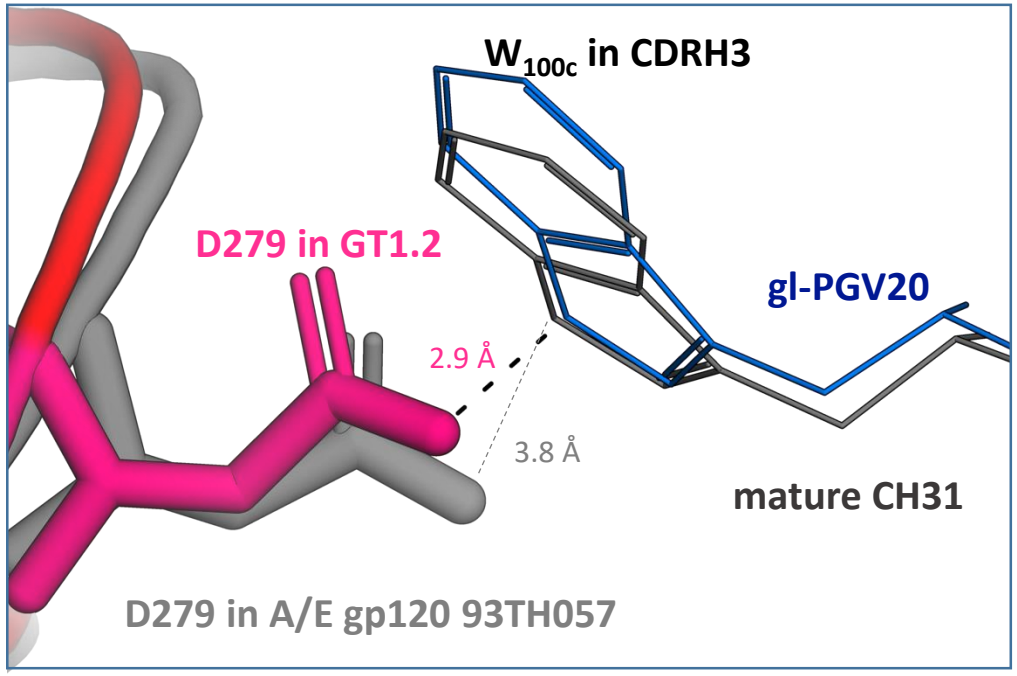
# Crystal structure of germline-targeting trimer bound to a VRC01-class germline bNAb

**BG505 SOSIP.v4.1-GT1.2 in complex with gl-PGV20**




**$P2_1$: 3.8 Å**

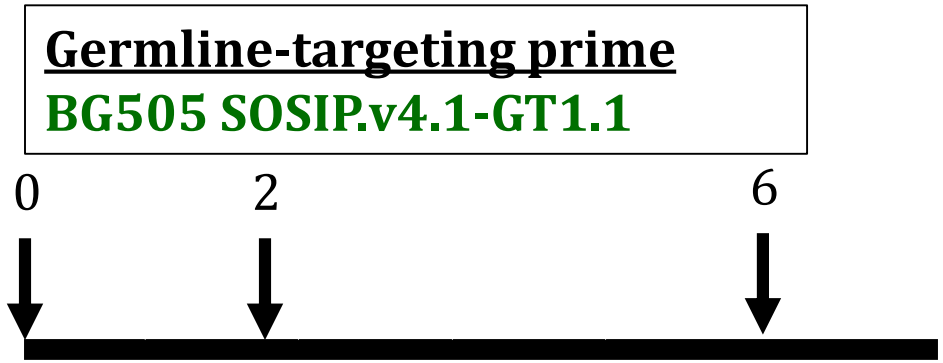
**Structure validates design features: N279D**




Anita Sarkar, Ian Wilson

# Evaluating BG505 SOSIP germline-targeting in humans

## Clinical trial (IAVI C101): dose-ranging in AS01b (PI: M.Caskey)




**Vaccinations (months)**

**Goals:**

Evaluate the safety and immunogenicity of two doses of GT1.1 / AS01b in healthy HIV uninfected adults

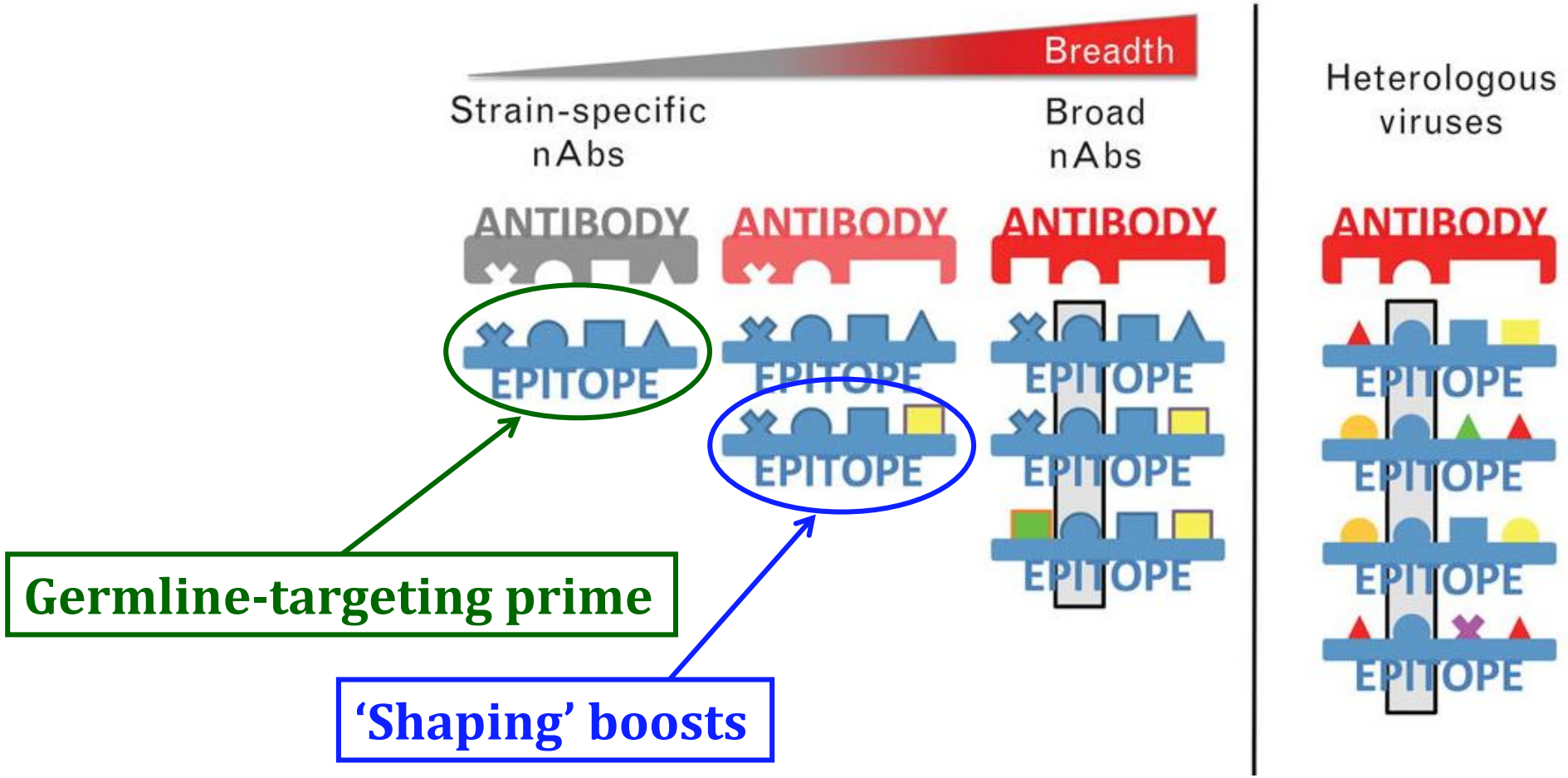
Evaluate whether the GT1.1 trimer can activate CD4bs-class and V2-apex class precursor B cells in humans

| | |
|---|---|
| Lead scientists | Sanders/Moore |
| Funders | NIH (HIVRAD) & BMGF (IAVI VxPDC) |
| Manufacturer | KBI Biopharma |
| GMP finished | Q4 2019 |
| Clinical trial start | Q1 2020 |
| Clinical sites | RU, GWU, AMC |

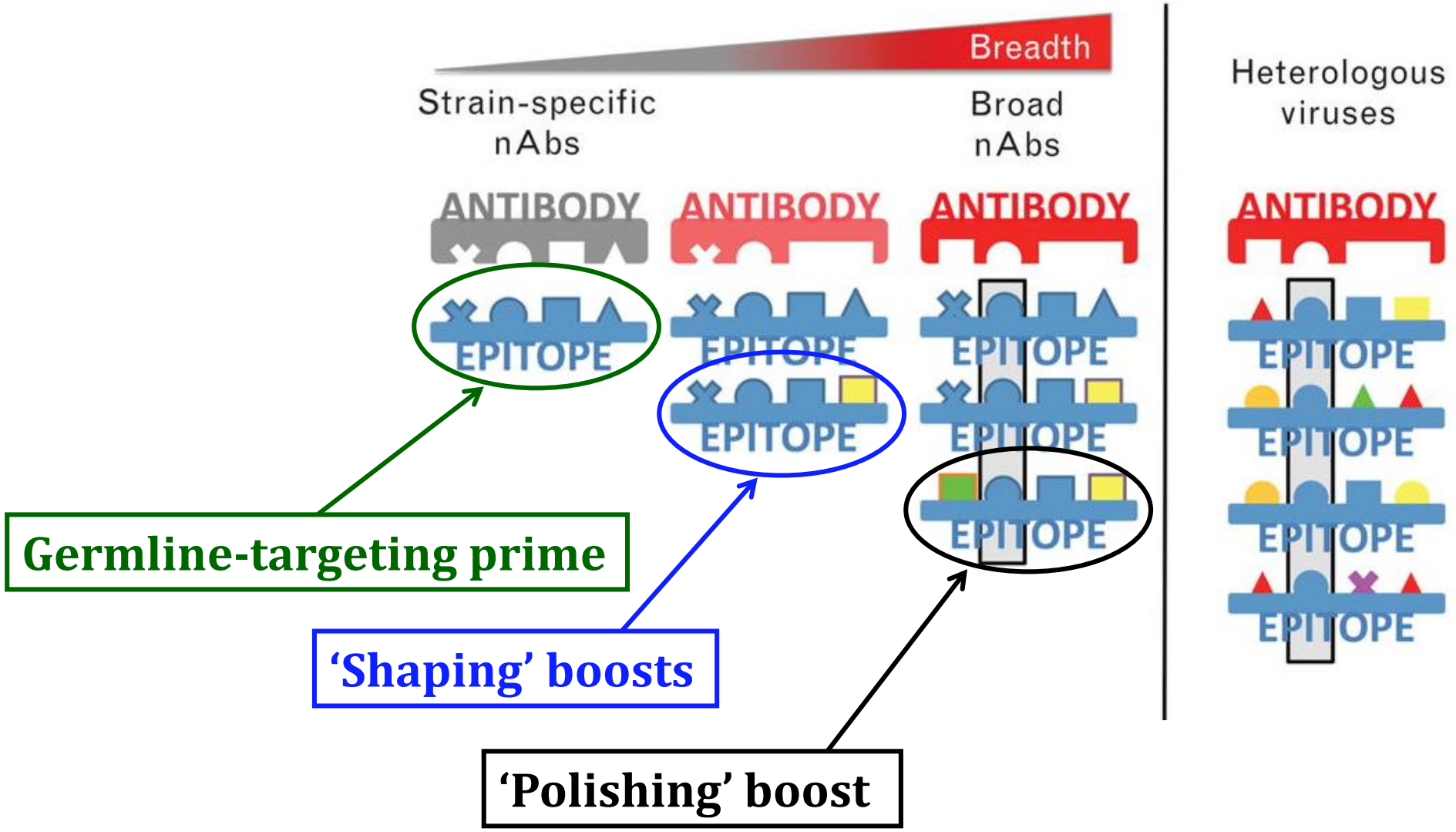
iavi
International AIDS Vaccine Initiative

BILL & MELINDA GATES foundation

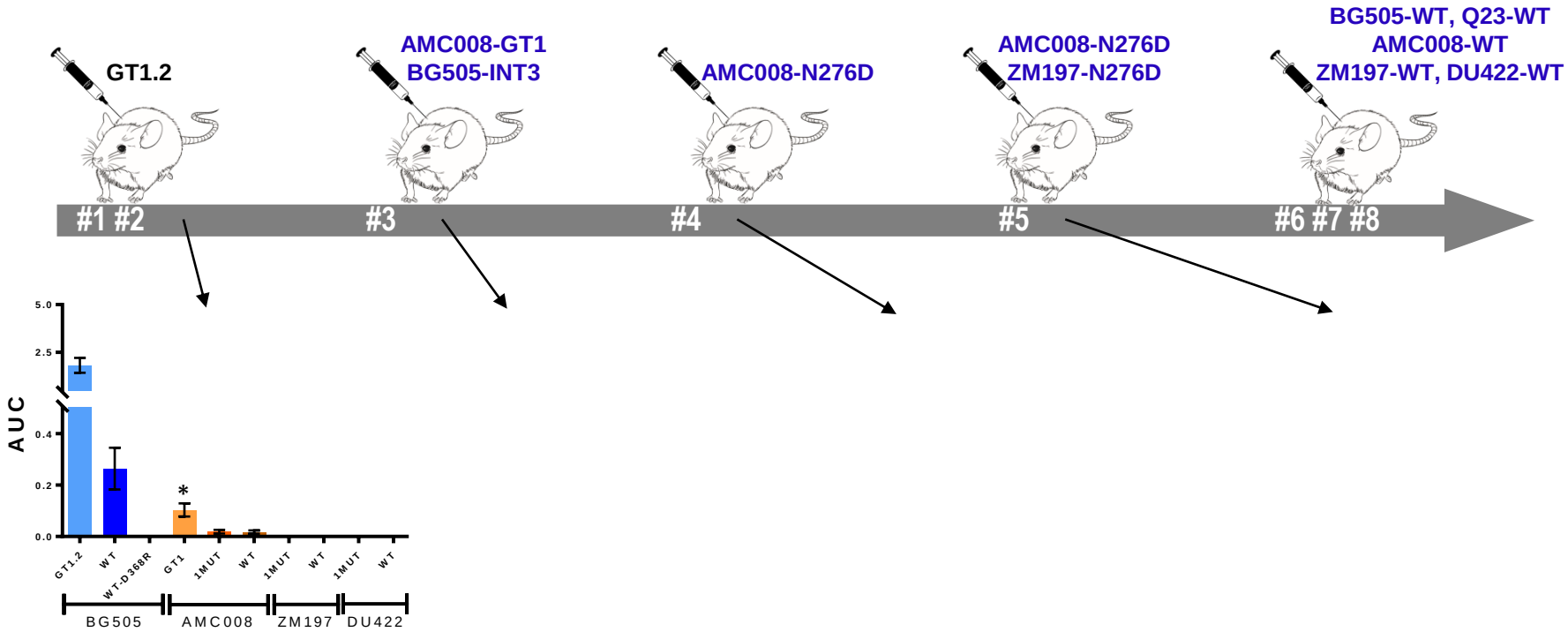
# ‘Shaping’ appropriately primed B cell responses



# ‘Polishing’ appropriately ‘shaped’ B cell responses



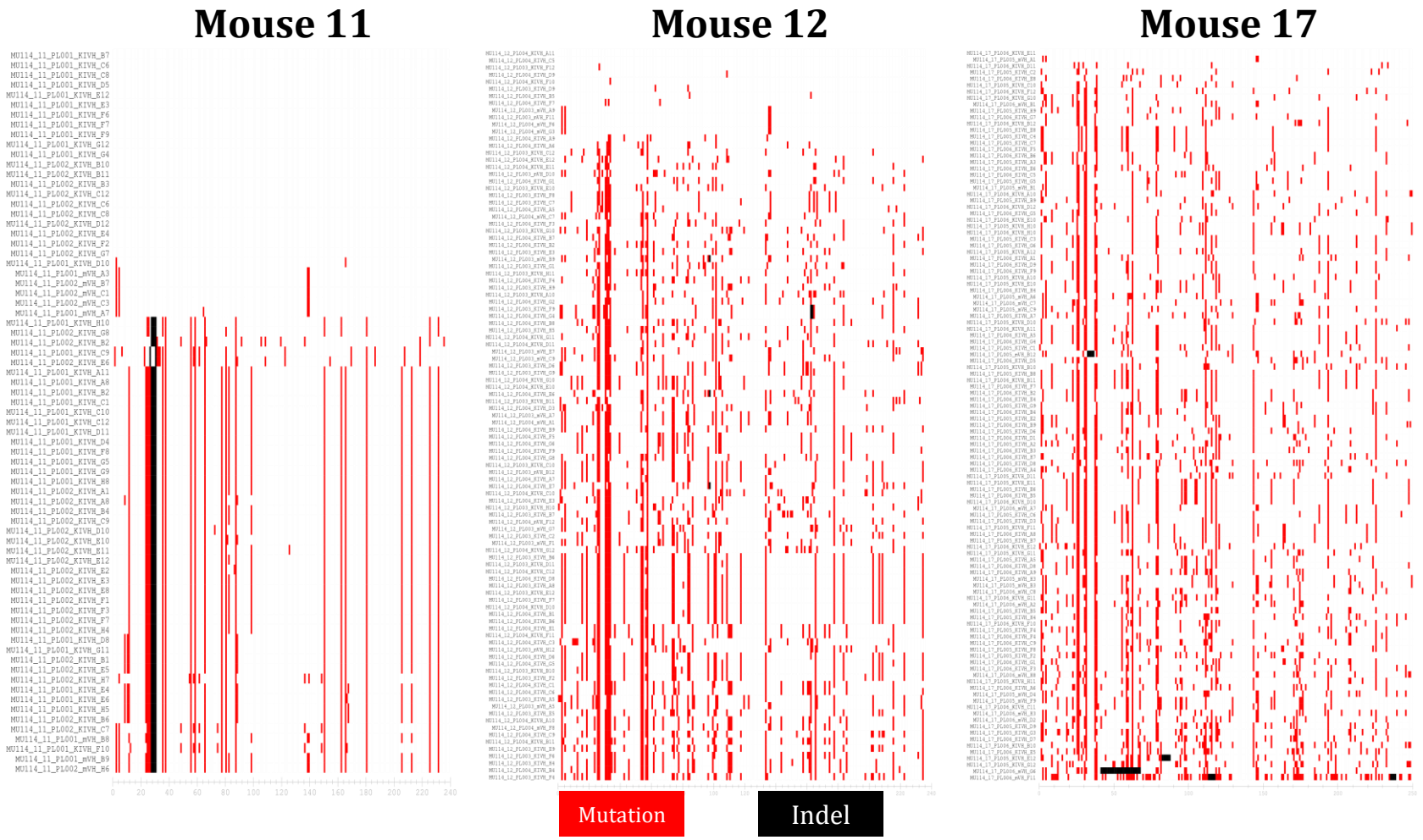
# ‘Shaping’ and ‘polishing’ appropriately primed B cell responses




- 1MUT = N276D

- Experiments performed in germline CH31 KI mice (Laurent Verkoczy)
- VRC01-like Ab induction confirmed in VRC01-class signature neutralization (David Montefiori)
- Similar results obtained with GT1.1 in $V_H1$-2/$J_H2$/LC chimeric mice (‘Alt mice’)

# Germline-targeting ‘shaping’ and ‘polishing’ selects for VRC01-class somatic mutations and indels

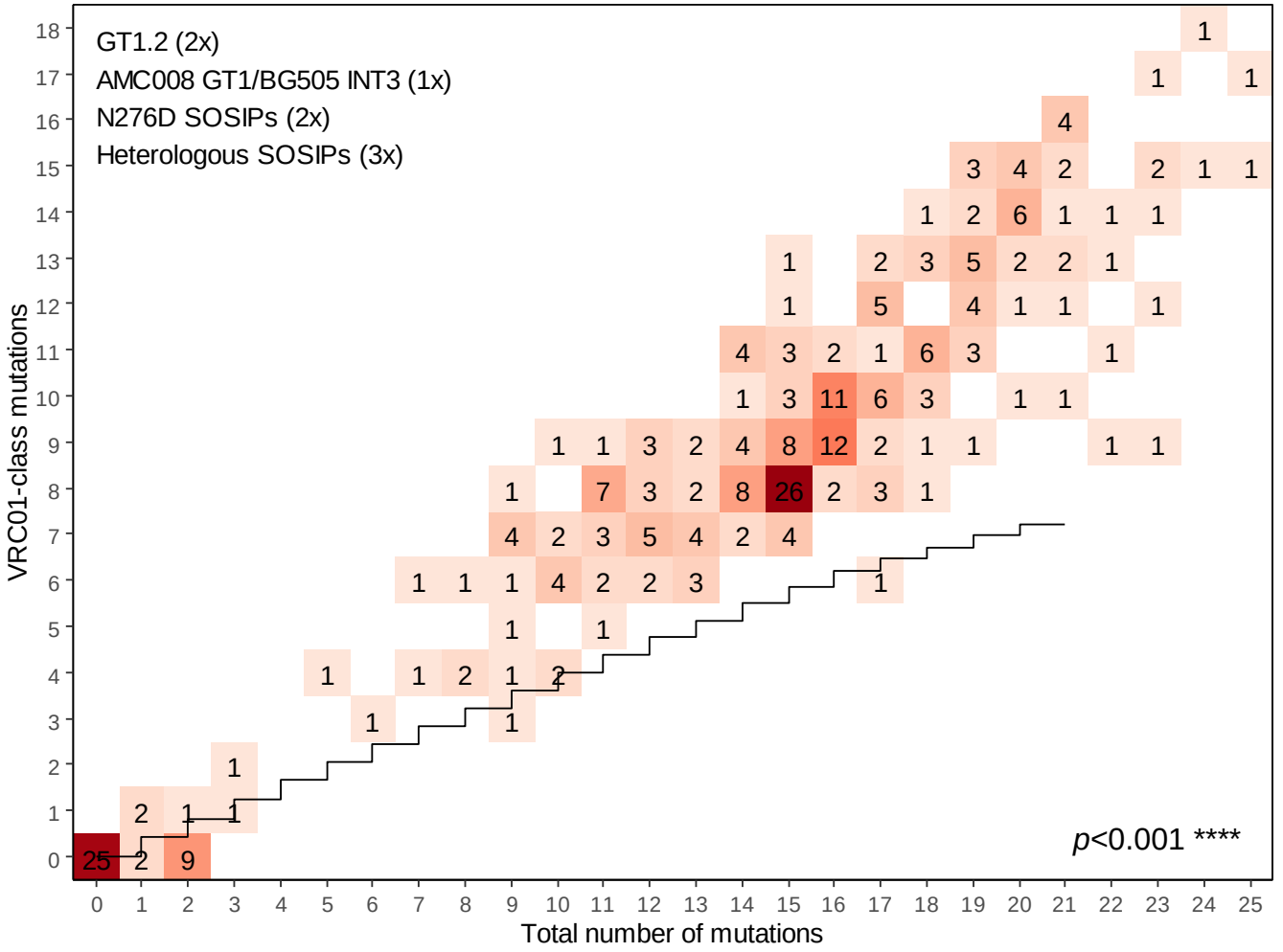



**Sequence analysis of single sorted memory B cells.**
Heavy and Light Chain (combined) pixel plots for individual mice

- SHM levels of up to 15% of HC and 10% of LC
- Many shared mutations with mature CH31 and/or VRC01
- Including mutations in contact sites (V58, R74), and at AID coldspots (e.g. V58)
- Indels shared with mature VRC01-class bNAbs

Laurent Verkoczy, Kevin Wiehe, Chuancang Jiang, Bart Haynes *et al.*

# Germline-targeting ‘shaping’ and ‘polishing’ selects for VRC01-class somatic mutations and indels




Number of VRC01-class mutations in VH1-2 induced by random SHM (from Briney *et al.* 2016; courtesy of Brian Briney and Bill Schief)

Tom Caniels
Maarten Pater

# Germline-targeting, ‘shaping’ and ‘polishing’ selects for rare insertions found in mature VRC01-class bNAbs

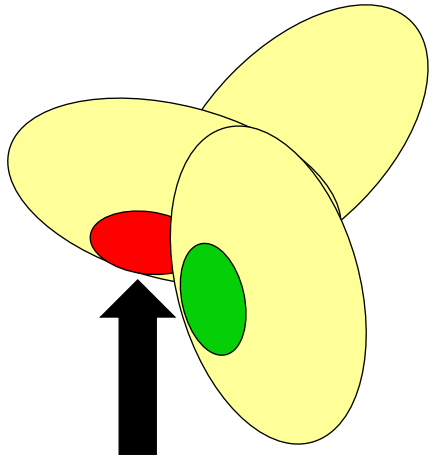
CDRH1

```
                 glCH31 CKASGY---------TFT-GYYMHW
                 CH31   ..FAEDDDYSPYWVNPAP.EHFI.F
MU114 11 PL001 KIVH C9 .E..-........DILI..K.I..
MU114 11 PL001 KIVH E4 ..VADF.....SPED.....K.F..
MU114 11 PL001 KIVH F10...TEF.....MPGD.....K.I..
MU114 11 PL001 mVH B9  ..IAEF.....SPED.....K.F..
MU114 11 PL002 KIVH B2 .....T....FTGY-......I..
MU114 11 PL002 KIVH G8 ...TE......TPEY.....K.I..
MU114 11 PL002 KIVH H7 ..VTEF.....IPEN.....K.I..
```

FWR3 insertion

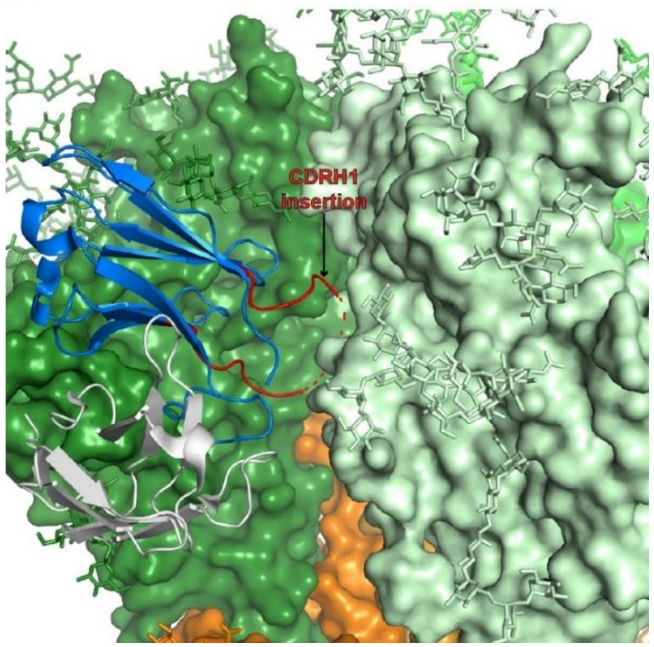
```
glCH31  RVTMTRDTSIS------TAYMELSRLRSDDTAVYYC-AR
CH31    ...A...R.MT.....FL.VKS.............
PGV20   .......VYRE.....V..LD.RS.TFA.....F....
PGV04   ..SL...RDLF.....H.DIRG.TQG...T.F....
12A12   .INFD..IYRE.....I.F.D..G.......L.F....
VRC01   .......VYSD.....FL..RS.TV......F..T.
3BNC60  ..SL..QA.WDFDTY..SF..D.KAV......I.F....

MU114 17 PL005 KIVH E12.IA......MIPDYMDI......N..TF..........
```

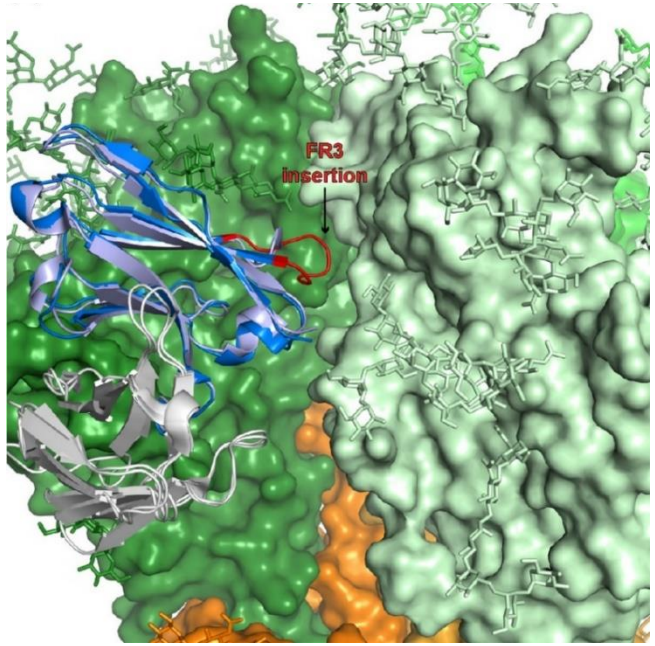


Red: primary contact
(CD4 binding site)
Green: secondary contact
(neigboring protomer)

**CDRH1 insertion in CH31**




**FWR3 insertion in 3BNC60**




Kepler & Wiehe, 2017. *Immunol. Rev.* **275**:129

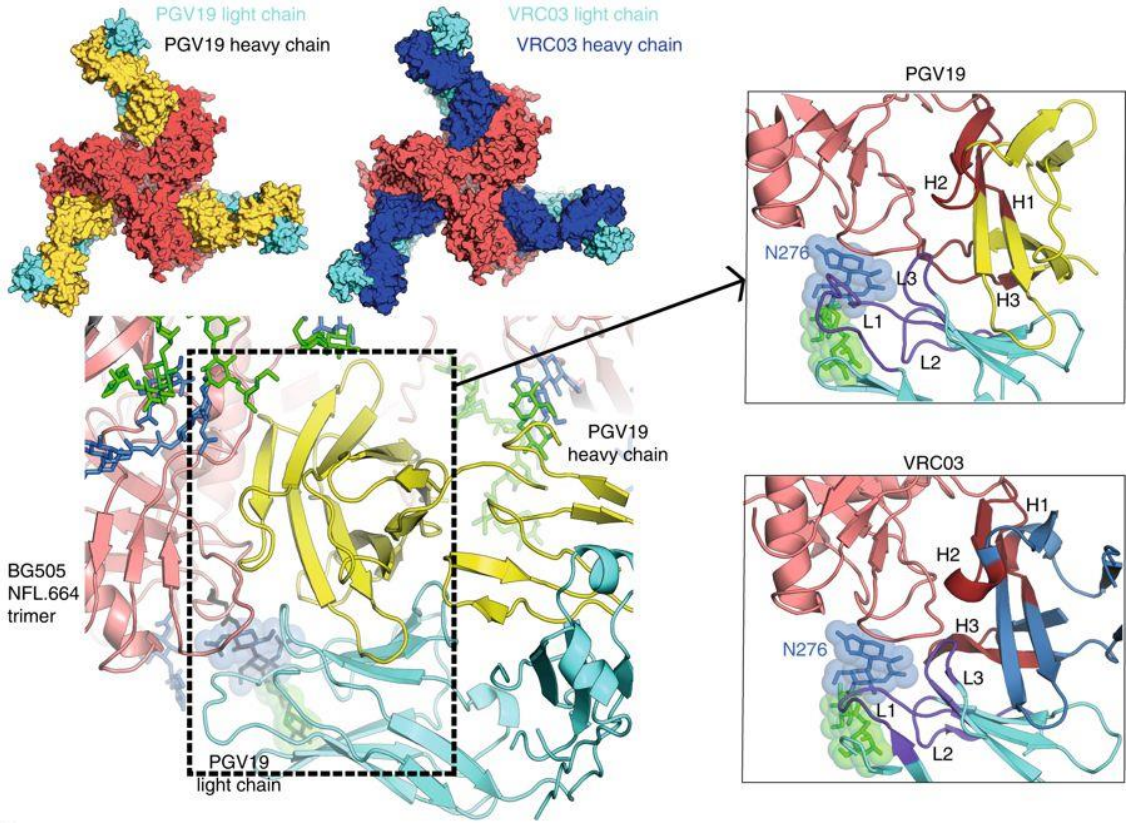
# Germline-targeting, ‘shaping’ and ‘polishing’ selects for glycines and rare deletions found in mature VRC01-class bNAbs

```
                    CDRL1
CH31.UCA TITCQASQDISNYLNWYQQKPGKAPK
CH31     .......RG.GKD.......A.....
VRC01    I.S.RT..YG--S......R..Q..R
Seq 1    .....T.HG.N.F............E
Seq 2    ....R....FG..............N
Seq 3    ..A.....G.IK...........Q...
Seq 4    .......A.G..............
Seq 5    ..........--............
Seq 6    ..........--............
```

| HC V gene | Antibody | LC V gene | Deleted residues | Extra glycines |
|---|---|---|---|---|
| VH1-2 | VRC01 | KV3-20*01 | 3 | 0 |
| | NIH45-46 | KV3-20*01 | 3 | 0 |
| | VRC-PG04 | KV3-20*01 | 3 | 0 |
| | VRC-CH31 | KV1-33*01 | 0 | 2 |
| | 12A12 | KV1-33*01 | 0 | 3 |
| | 3BNC117 | KV1-33*01 | 4 | 0 |
| | VRC-PG20 | LV2-14*01 | 6 | 0 |
| | VRC18 | KV3-20*01 | 0 | 1 |
| | VRC23 | KV3-15*01 | 0 | 2 |
| | VRC27 | KV1-5*01 | 0 | 2 |
| | IOMA | LV2-23*02 | 0 | 1 |
| | DRVIA7* | KV1-5*03 | 0 | 0 |

Gristick *et al.* 2016. *Nat.Struct.Mol.Biol.* **23**:906

**Accommodation of the N276 glycan by glycine substitutions or deletions in CDRL1**




Sarkar *et al.* 2018. *Nat.Comm.* **9**:1956

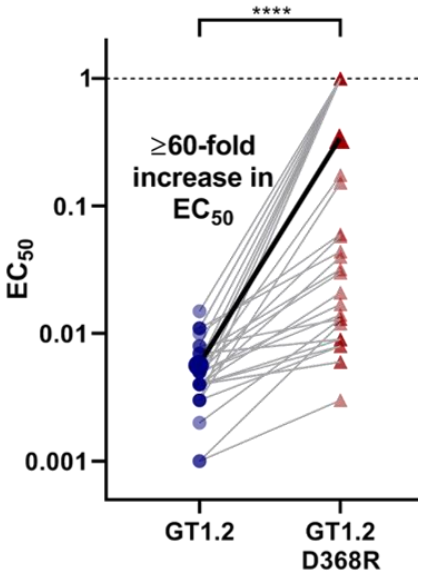
# Germline-targeting, ‘shaping’ and ‘polishing’ selects for antibodies with cross-binding activity

| | max. concentration tested (µg/mL) | | | | 1 | 1 | 50 | 50 | 10 | 10 | 50 | 10 | 50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ab ID | total # | VRC01-class # | ratio | indels? | GT1.2 | GT1.2 D368R | BG505 SOSIP | BG505 D368R | AMC008 GT1 | AMC008 N276D | AMC008 | ZM197M N276D | ZM197M |
| 28 | 23 | 15 | 0.65 | | 0.015 | >1 | >50 | >50 | >10 | >10 | >50 | >10 | >50 |
| 15 | 11 | 7 | 0.64 | | 0.011 | >1 | >50 | >50 | 0.037 | >10 | >50 | >10 | >50 |
| 20 | 15 | 9 | 0.60 | | 0.005 | >1 | >50 | >50 | 0.021 | >10 | >50 | >10 | >50 |
| 21 | 16 | 10 | 0.63 | | 0.011 | >1 | >50* | >50 | 0.042 | >10 | >50 | >10 | >50 |
| 31 | 17 | 12 | 0.71 | | 0.008 | >1 | >50 | >50 | 0.042 | >10 | >50 | >10 | >50 |
| 2 | 17 | 9 | 0.53 | in H1 (4) | 0.004 | 0.021 | >50 | >50 | <0.01 | <0.01 | >50 | >10 | >50 |
| 4 | 16 | 8 | 0.50 | in H1 (4) | <0.001 | 0.012 | >50 | >50 | <0.01 | <0.01 | >50 | >10 | >50 |
| 6 | 10 | 6 | 0.60 | in H1 (4) | 0.003 | 0.153 | >50 | >50 | <0.01 | <0.01 | >50 | >10 | >50 |
| 8 | 15 | 9 | 0.60 | | 0.003 | 0.99 | >50 | >50 | 0.041 | 0.13 | >50 | >10 | >50 |
| 10 | 15 | 9 | 0.60 | | 0.004 | 0.04 | >50 | >50 | <0.01 | 0.13 | >50 | >10 | >50 |
| 17 | 17 | 6 | 0.35 | | 0.005 | 0.014 | >50 | >50 | <0.01 | <0.01 | >50 | >10 | >50 |
| 24 | 15 | 10 | 0.67 | | 0.003 | 0.008 | >50 | >50 | 0.042 | 0.071 | >50 | >10 | >50 |
| 35 | 18 | 14 | 0.78 | | 0.011 | 0.044 | >50 | >50 | 0.041 | 0.052 | >50 | >10 | >50 |
| 11 | 20 | 9 | 0.45 | | 0.002 | 0.013 | >50 | >50 | 0.04 | 0.021 | >50 | 0.61 | >50 |
| 12 | 15 | 10 | 0.67 | | 0.010 | 0.060 | >50 | >50 | 0.04 | 0.037 | >50 | 0.17 | >50 |
| 13 | 20 | 11 | 0.55 | | 0.004 | 0.006 | >50 | >50 | 0.03 | 0.029 | >50 | 0.12 | >50 |
| 16 | 25 | 14 | 0.56 | | 0.006 | 0.03 | >50 | >50 | 0.052 | 0.05 | >50 | 0.19 | >50 |
| 38 | 15 | 10 | 0.67 | | 0.005 | 0.058 | >50 | >50 | 0.029 | 0.043 | >50 | 0.078 | >50 |
| 23 | 15 | 11 | 0.73 | del L1 (2) | 0.005 | >1 | 7.3 | >50 | 0.034 | >10 | >50 | >10 | >50 |
| 33 | 21 | 13 | 0.62 | | 0.003 | >1 | 0.25 | >50 | 0.058 | 0.41 | >50 | >10 | >50 |
| 18 | 17 | 12 | 0.71 | | 0.006 | >1 | 10.2 | >50 | <0.01 | <0.01 | >50 | >10 | >50 |
| 26 | 12 | 7 | 0.58 | | 0.004 | 0.008 | 4.6 | >50 | 0.053 | 0.067 | >50 | >10 | >50 |
| 3 | 19 | 8 | 0.42 | in H1 (4) | 0.004 | 0.006 | >50 | >50 | n.d. | n.d. | 0.67 | n.d. | >50 |
| 5 | 10 | 7 | 0.70 | in H1 (4) | 0.005 | 0.17 | >50 | >50 | <0.01 | <0.01 | 25 | >10 | >50 |
| 7 | 16 | 8 | 0.50 | in H1 (4) | 0.007 | 0.009 | >50 | >50 | 0.039 | 0.04 | 2.9 | >10 | >50 |
| 9 | 20 | 14 | 0.70 | | 0.007 | 0.009 | >50 | >50 | <0.01 | <0.01 | 0.18 | 0.037 | >50 |
| 22 | 18 | 11 | 0.61 | | 0.007 | 0.032 | >50 | >50 | 0.064 | 0.044 | >50 | 0.22 | 3.3 |
| 29 | 12 | 5 | 0.42 | in H1(5) | 0.005 | 0.017 | >50 | >50 | 0.059 | 0.048 | >50 | 0.11 | 0.15 |
| 30 | 19 | 13 | 0.68 | | 0.008 | 0.013 | >50 | >50 | 0.064 | 0.074 | >50 | 0.049 | 5.0 |
| 19 | 19 | 13 | 0.68 | | 0.004 | 0.009 | 5.5 | >50 | <0.01 | 0.026 | 7.2 | 0.17 | >50 |
| 27 | 24 | 15 | 0.63 | in FR3 (6) | <0.001 | 0.003 | 8.2 | >50 | <0.01 | <0.01 | 4.3 | 0.069 | 0.82 |
| 34 | 21 | 16 | 0.76 | | 0.004 | <0.001 | 0.56 | >50 | <0.01 | <0.01 | 0.74 | 0.038 | 0.038 |

*VRC01c is defined as those shared with VRC01, PGV04, PGV20, CH31, 3BNC60, 12A12

**EC50 values were only calculated from sigmoidal curves with >0.5 OD450 values

MAbs from FACS sorted memory B cells were tested for binding by ELISA

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# Germline-targeting, ‘shaping’ and ‘polishing’ selects for antibodies with cross-neutralizing activity

| | | Viruses from SOSIPs in immunization regimen | | | | | | heterologous tier 1B | | | | | heterologous tier 2 clade C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| amino acid pos. 276 | | D | D | D | N | N | N | D | D | N | N | N | N | N | N |
| Ab ID | indels? | BG505 GT1.2 | BG505 N276D N462D | BG505 N276D | BG505 WT | AMC008 | ZM197M | Pt45 dG5.2 | QG984 21M ENV.A3 | Pt45 pH1.1 | conS | Q23 env17 | Ce704810 053_2B7 | 3728 | B0055 |
| 3 | in H1 (4) | <0.001 | 0.01 | 0.2 | >200 | >200 | >200 | 0.03 | >2.5 | | | | | | |
| 5 | in H1 (4) | <0.001 | 0.4 | 0.5 | >200 | >200 | >200 | 0.03 | >2.5 | >200 | | | | | |
| 7 | in H1 (4) | <0.001 | >2.5 | >2.5 | >200 | >200 | >200 | 0.11 | >2.5 | >200 | | | | | |
| 9 | | <0.001 | <0.001 | 0.003 | >200 | >200 | >200 | 0.02 | >2.5 | | | | | | |
| 18 | | 0.001 | 0.007 | 0.025 | >200 | 160* | >200 | 0.02 | >2.5 | 204* | | | | | |
| 19 | | <0.001 | 0.009 | 0.04 | 35* | >200 | 120* | | 0.01 | | >200 | 59 | 190 | 37 | 64 |
| 22 | | <0.001 | 0.009 | 0.05 | 123* | >200 | 215* | | 0.04 | | | | | | |
| 23 | del L1 (2) | >0.1 | >2.5 | >2.5 | 68 | 187* | 68 | 16 | >2.5 | 35* | 153 | 53 | 55 | 79 | 38 |
| 26 | | 0.004 | 0.004 | 0.2 | 2 | >200 | >200 | | >2.5 | | | | | | |
| 27 | in FR3 (6) | <0.001 | 0.04 | 0.06 | >200 | >200 | >200 | 0.002 | >2.5 | >200 | | | | | |
| 29 | in H1(5) | 0.004 | 0.01 | 0.1 | >200 | >200 | >200 | | | | | | | | |
| 30 | | <0.001 | 0.003 | 0.01 | >200 | >200 | >200 | 0.01 | 0.02 | >200 | | | | | |
| 33 | | 0.4 | >2.5 | >2.5 | 7 | >200 | 78 | 1.6 | >2.5 | >200 | >200 | | >200 | >200 | >200 |
| 34 | | 0.005 | 0.02 | 0.06 | >200 | >200 | >200 | 0.09 | 0.01 | >200 | | | | | |

$IC_{50}$ (µg/mL)
- 0-25
- 25-100
- 100-250
- >200/>2.5
- n.d.

* estimated $IC_{50}$ by forcing curve through 0

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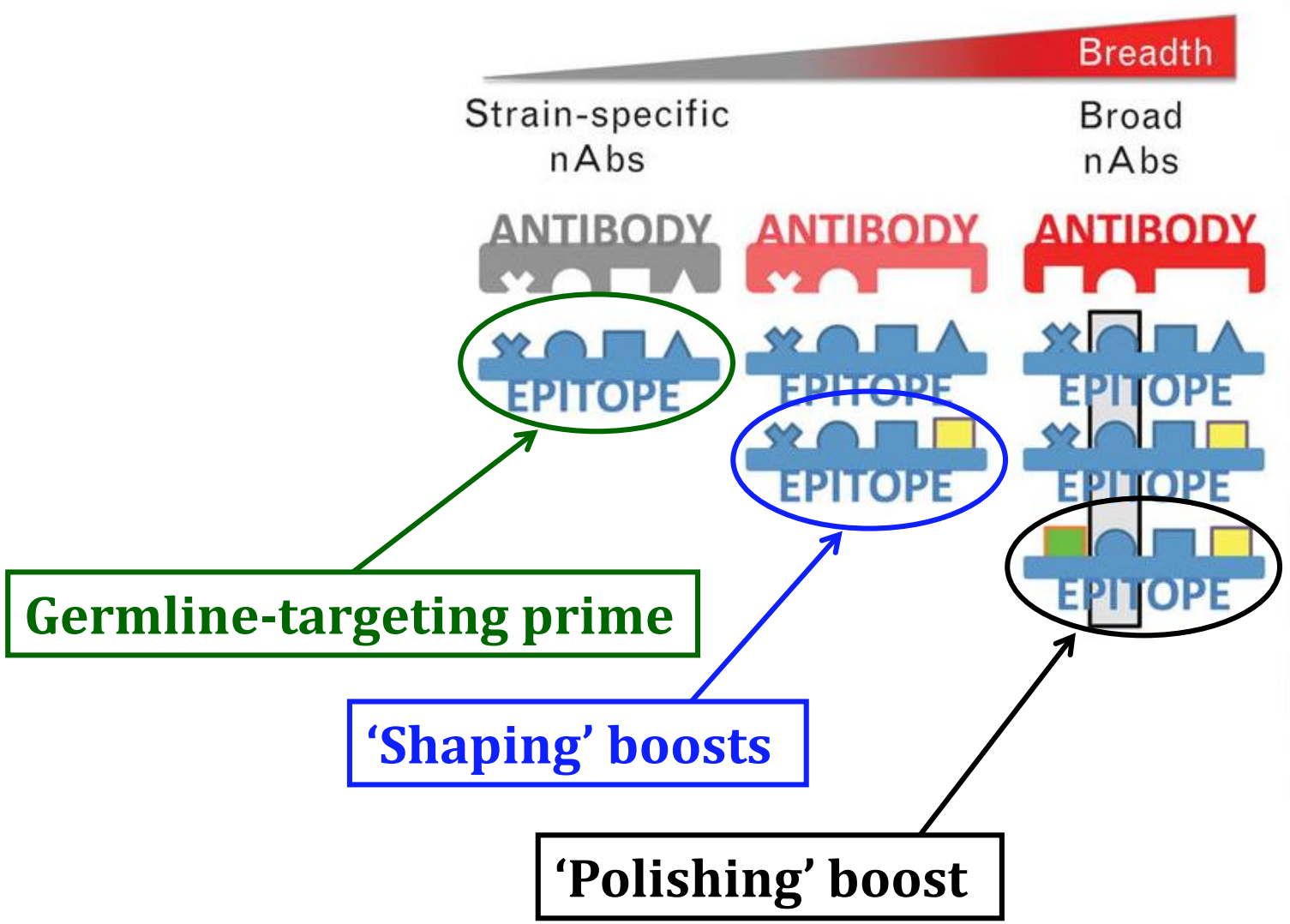
Proof-of-concept that priming, ‘shaping’ and ‘polishing’ of VRC01-class germline precursors by SOSIP-based immunogens can lead to accommodation of the N276 glycan and heterologous neutralization

# Evaluating priming, 'shaping' and 'polishing' regimens in humans




**Germline-targeting prime**
**BG505 GT1.1**

**'Shaping' boost 1**
**AMC008 GT1**
**BG505 INT3**

**'Shaping' boost 2**
**AMC008 N276D**
**ZM197M N276D**

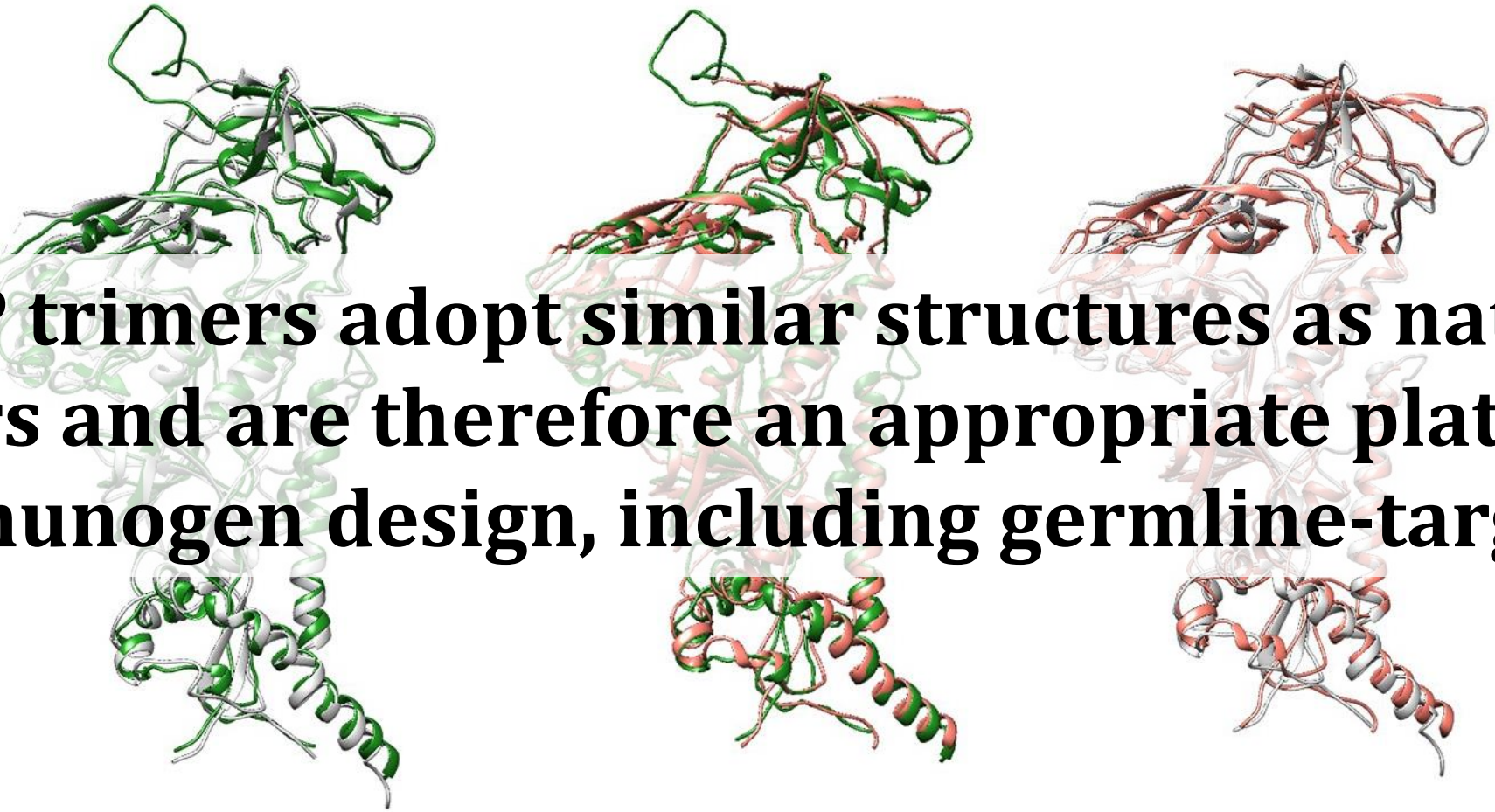
**'Polishing' boost**
**BG505 SOSIP.664**
**ConM SOSIP.v7**
**AMC011 SOSIP.v8**
**#763 SOSIP.v8**

## Conclusions (part II)

SOSIP gp140 can serve as a platform for germline targeting, ‘shaping’ and ‘polishing’

SOSIP-based germline targeting, ‘shaping’ and ‘polishing’ in VRC01-class knock-in mice leads to:

- The accumulation of VRC01-class mutations
- The selection of VRC01-class insertions and deletions
- The establishment of contacts with the neighboring protomer (?)
- The development of Abs that can accommodate the N276 glycan
- The development of Abs that can neutralize heterologous wild-type viruses



# SOSIP trimers adopt similar structures as native Env trimers and are therefore an appropriate platform for immunogen design, including germline-targeting

Full-length AMC011 PGT151 Fab
Full-length AMC011 PGT145 Fab

Full-length AMC011 PGT145 Fab
AMC011 SOSIP.v4.1 ACS202 Fab

Full-length AMC011 PGT151 Fab
AMC011 SOSIP.v4.1 ACS202 Fab

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