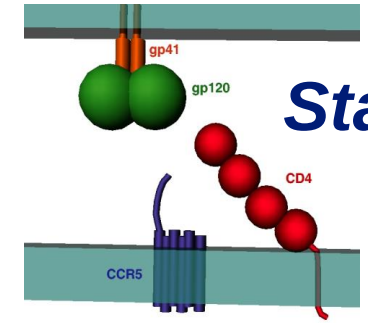
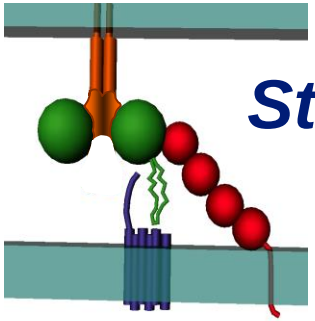
Long-acting BMS-378806 Analogues Stabilize the State-1 Conformation of the HIV-1 Envelope Glycoproteins

Joseph Sodroski

October 1, 2019



State 1



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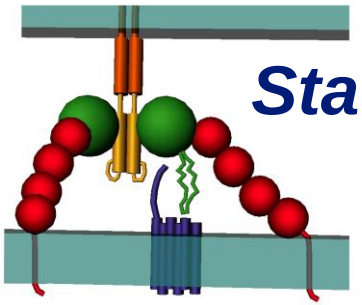
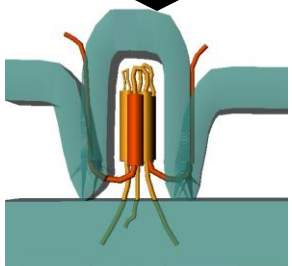
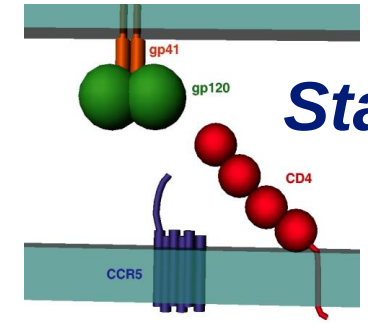


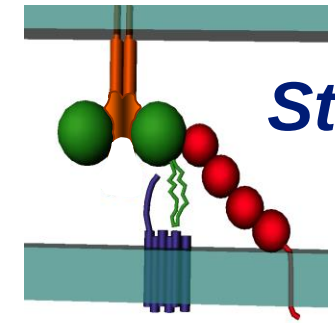
State 3





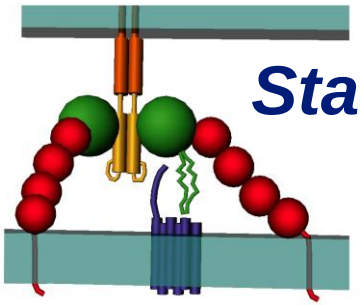
State 1

Destabilization

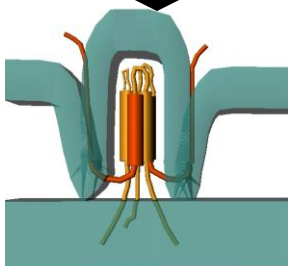


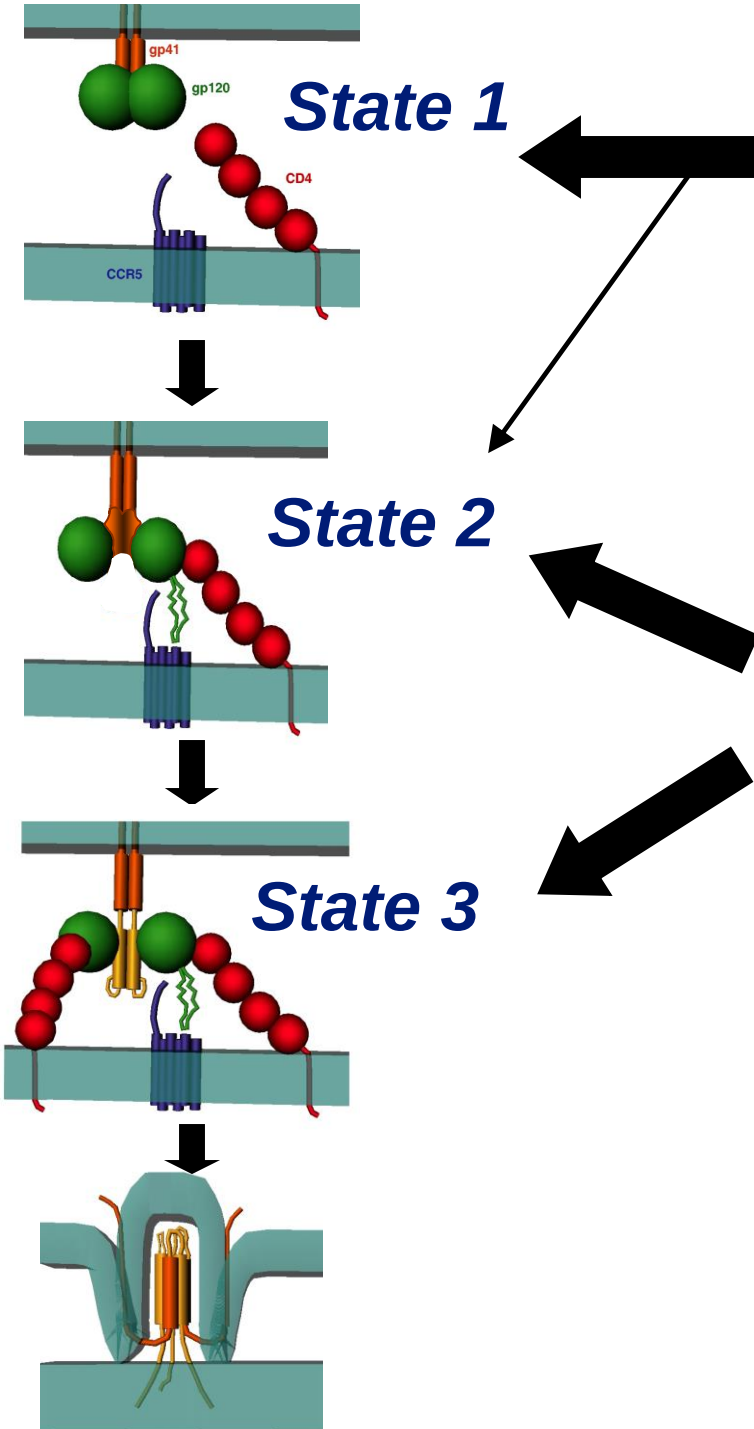
State 2

A "default" conformation



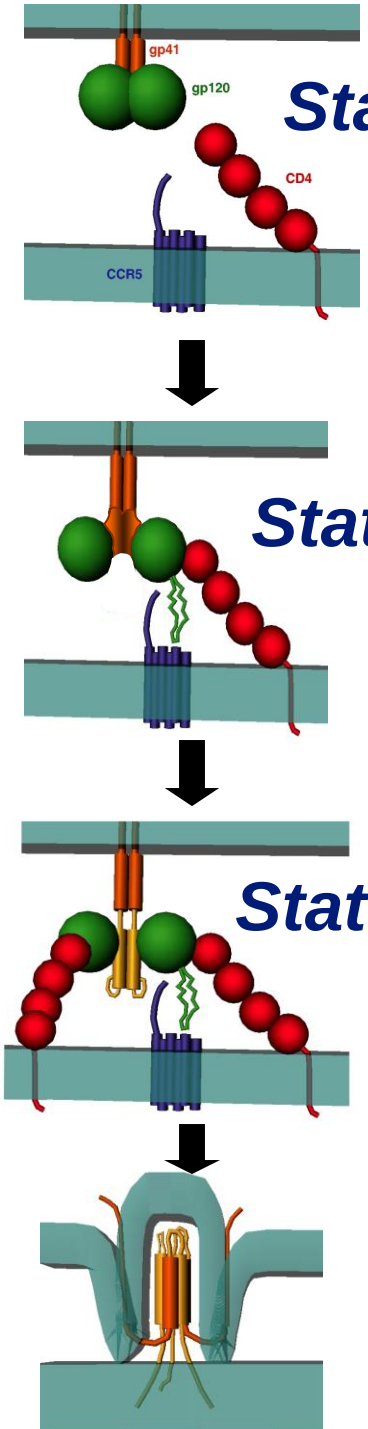
State 3





Potent and broad neutralizing antibodies

Poorly neutralizing antibodies



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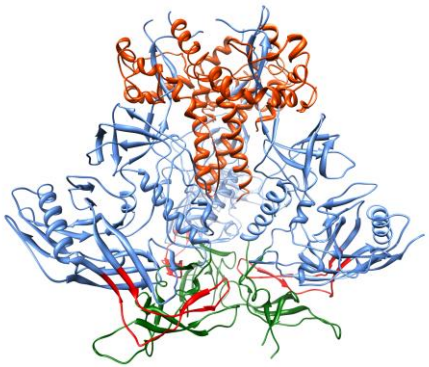
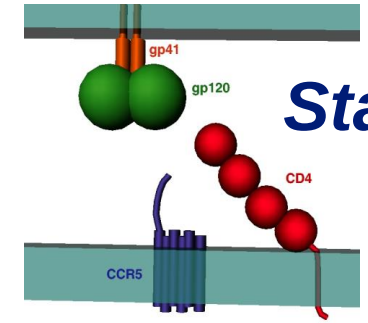
crosslinking/mass spectrometry

HIV-1 Env structures

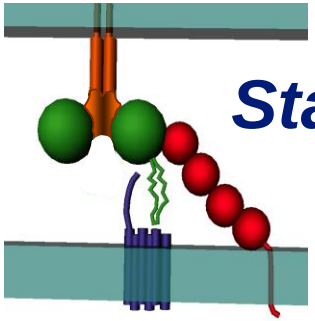


sgp140 SOSIP.664

PGT151-Env∆CT



State 1



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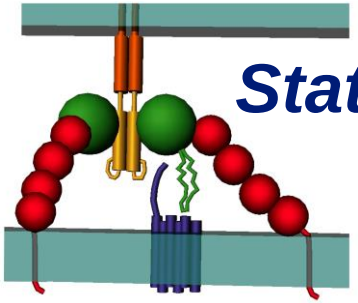

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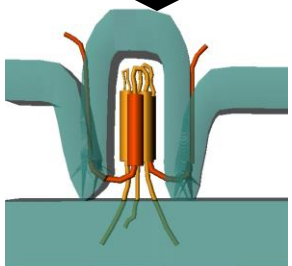
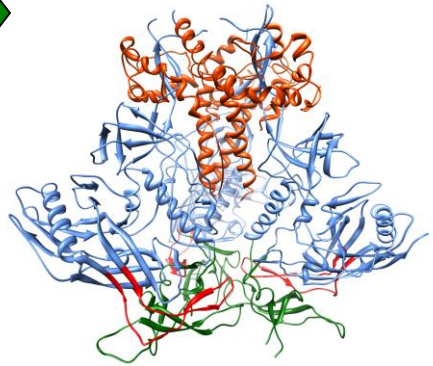


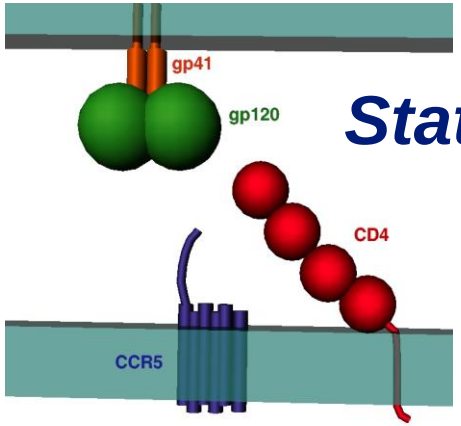
smFRET

HIV-1 Env structures



sgp140 SOSIP.664

PGT151-Env∆CT



State 1

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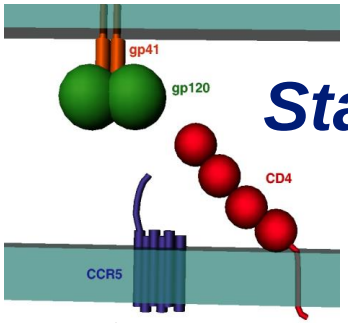
Potent and broad neutralizing antibodies

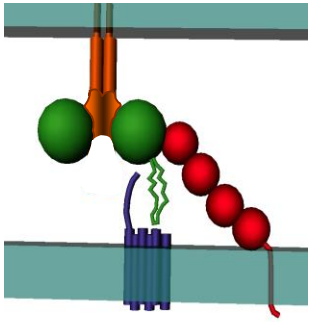
How can we stabilize the metastable State-1 conformation of the HIV-1 envelope glycoproteins?

What is a source of the State-1 HIV-1 Env? Cells? Virus-like particles (VLPs)?

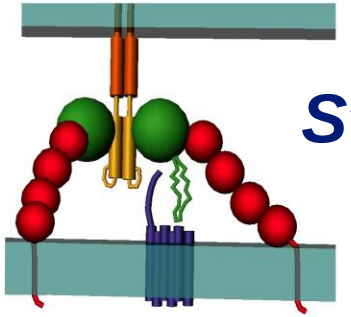


State 1

BMS-806 analogues, **484, 18A**

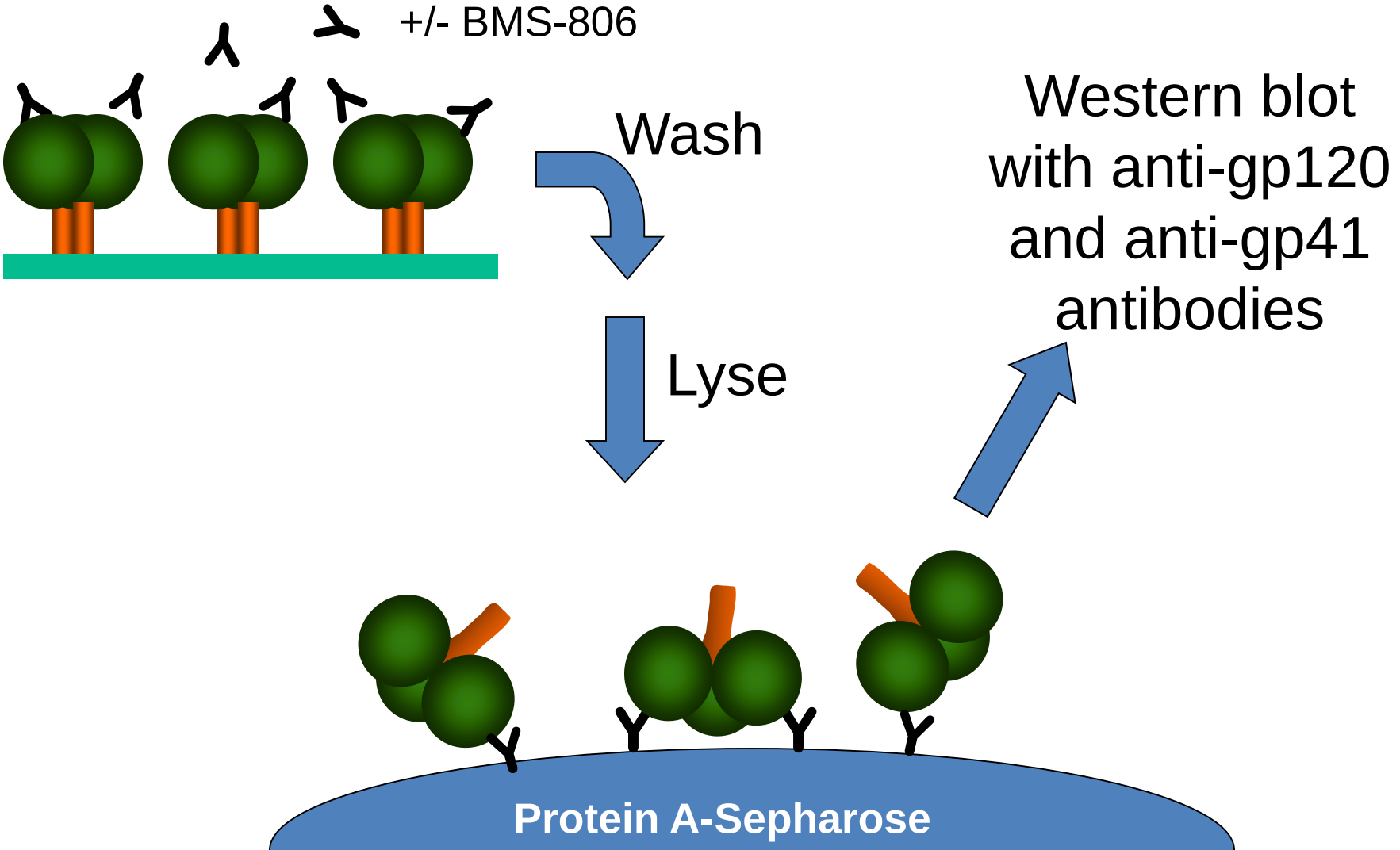


State 2

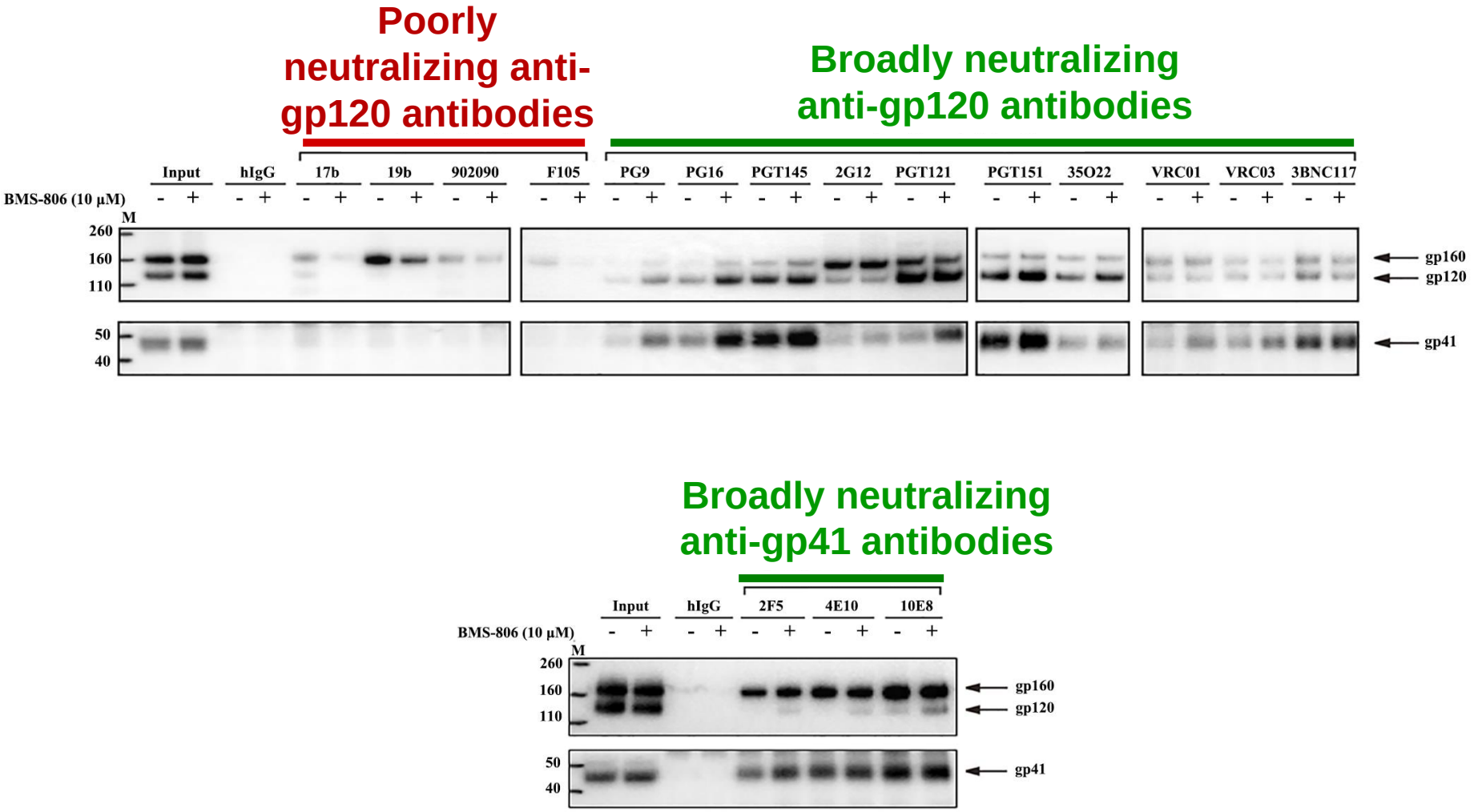


State 3

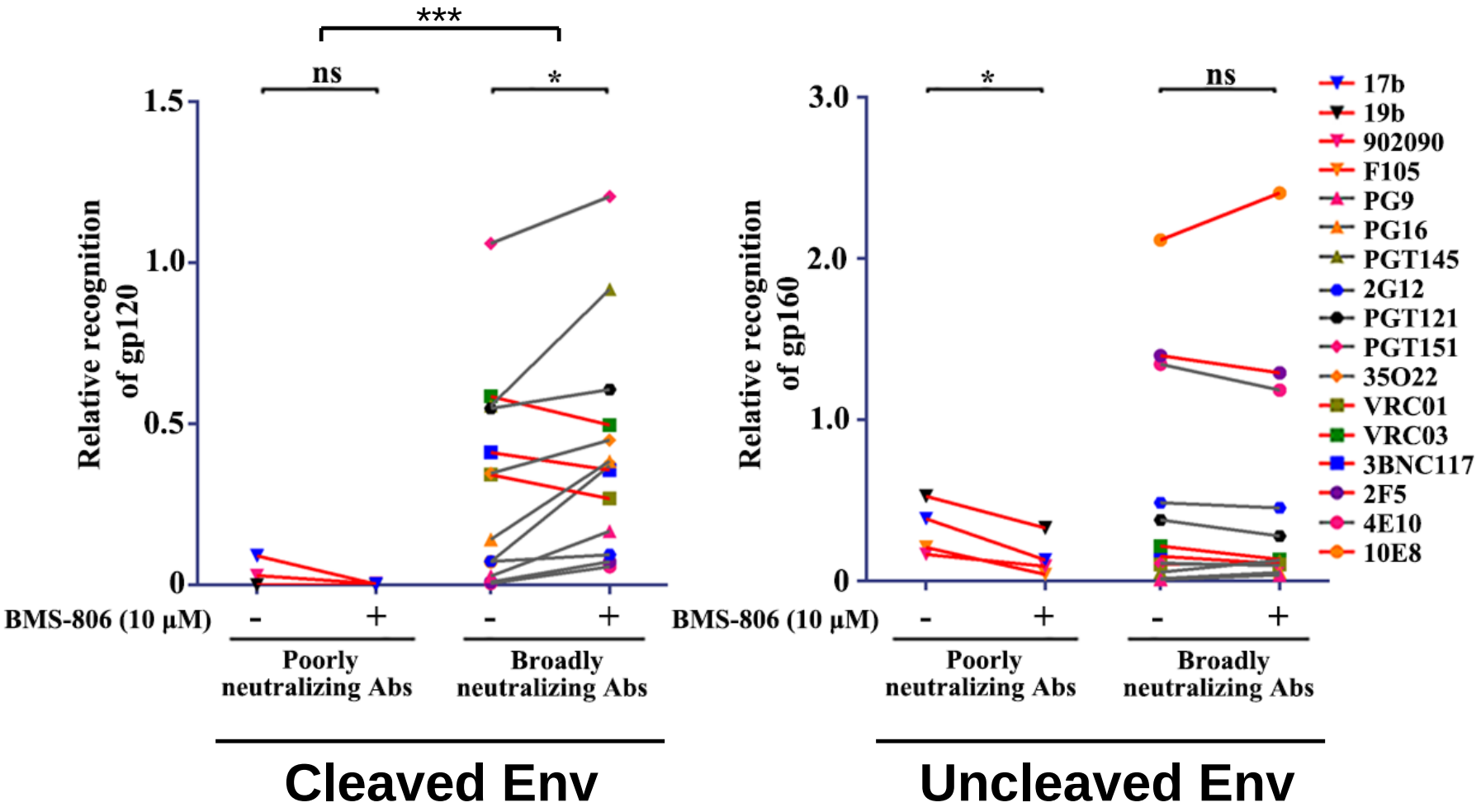
Immunoprecipitation of Env from the surface of expressing cells



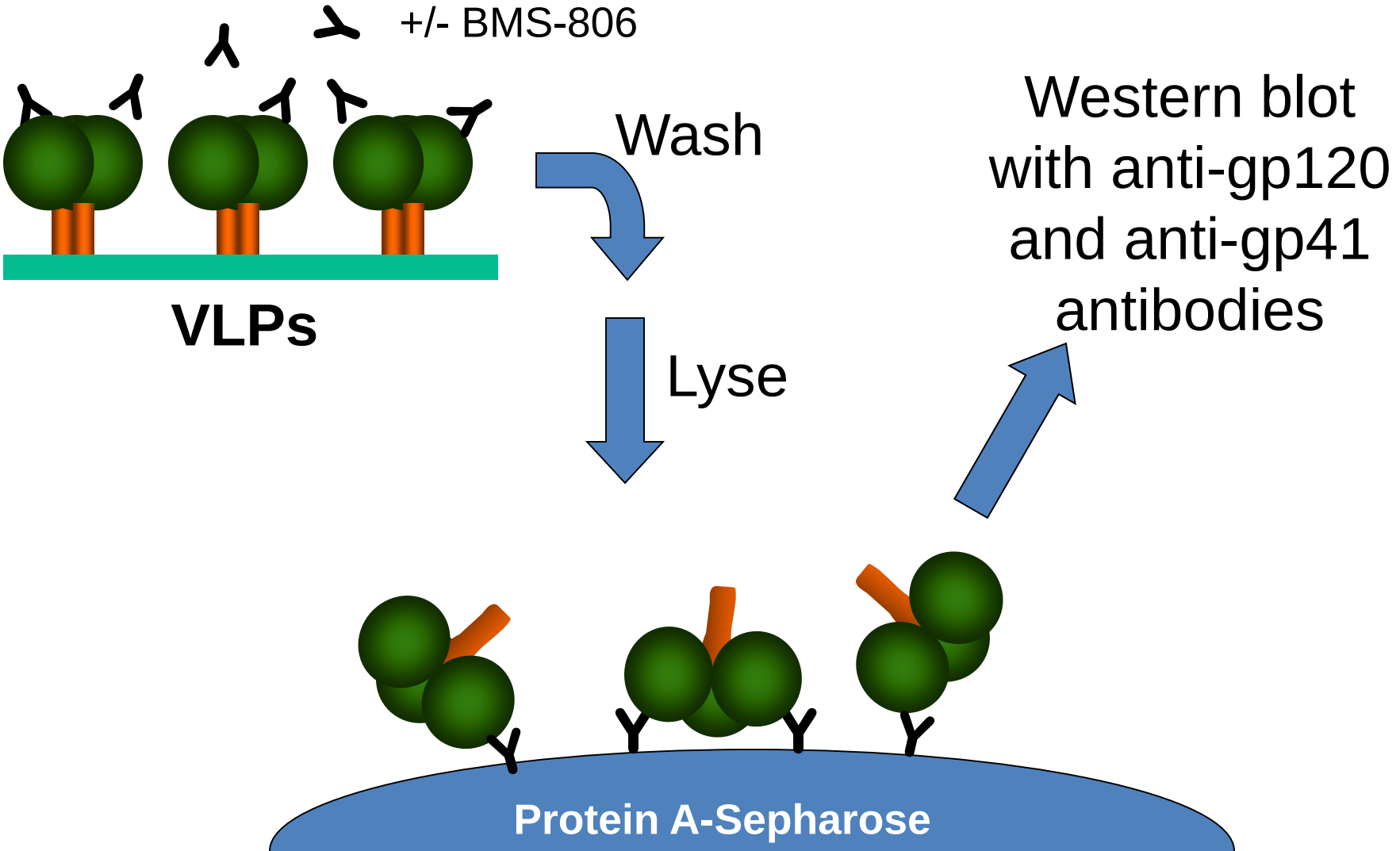
Effect of BMS-806 on immunoprecipitation of Env from the cell surface



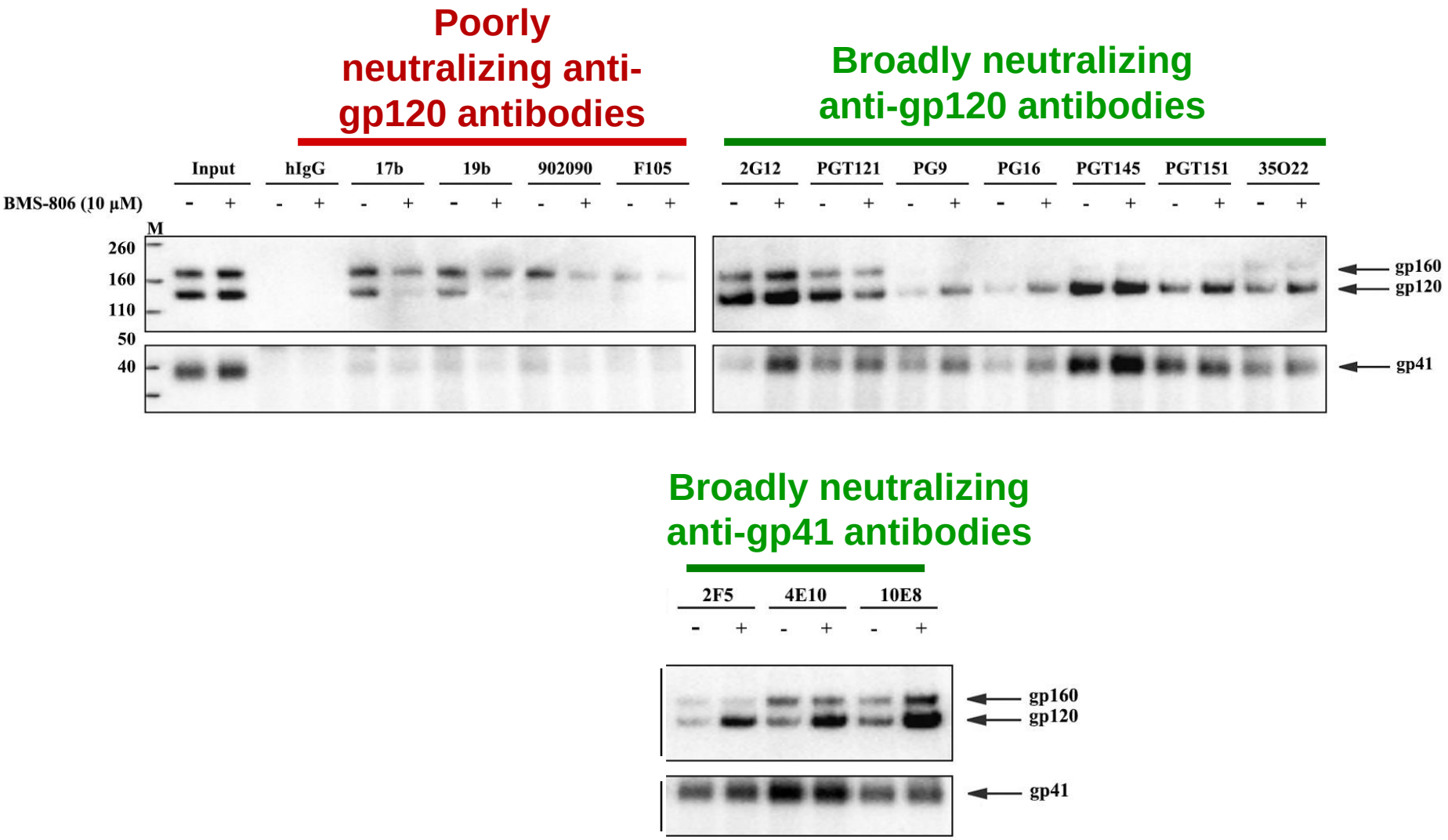
Effect of BMS-806 on immunoprecipitation of Env from the cell surface



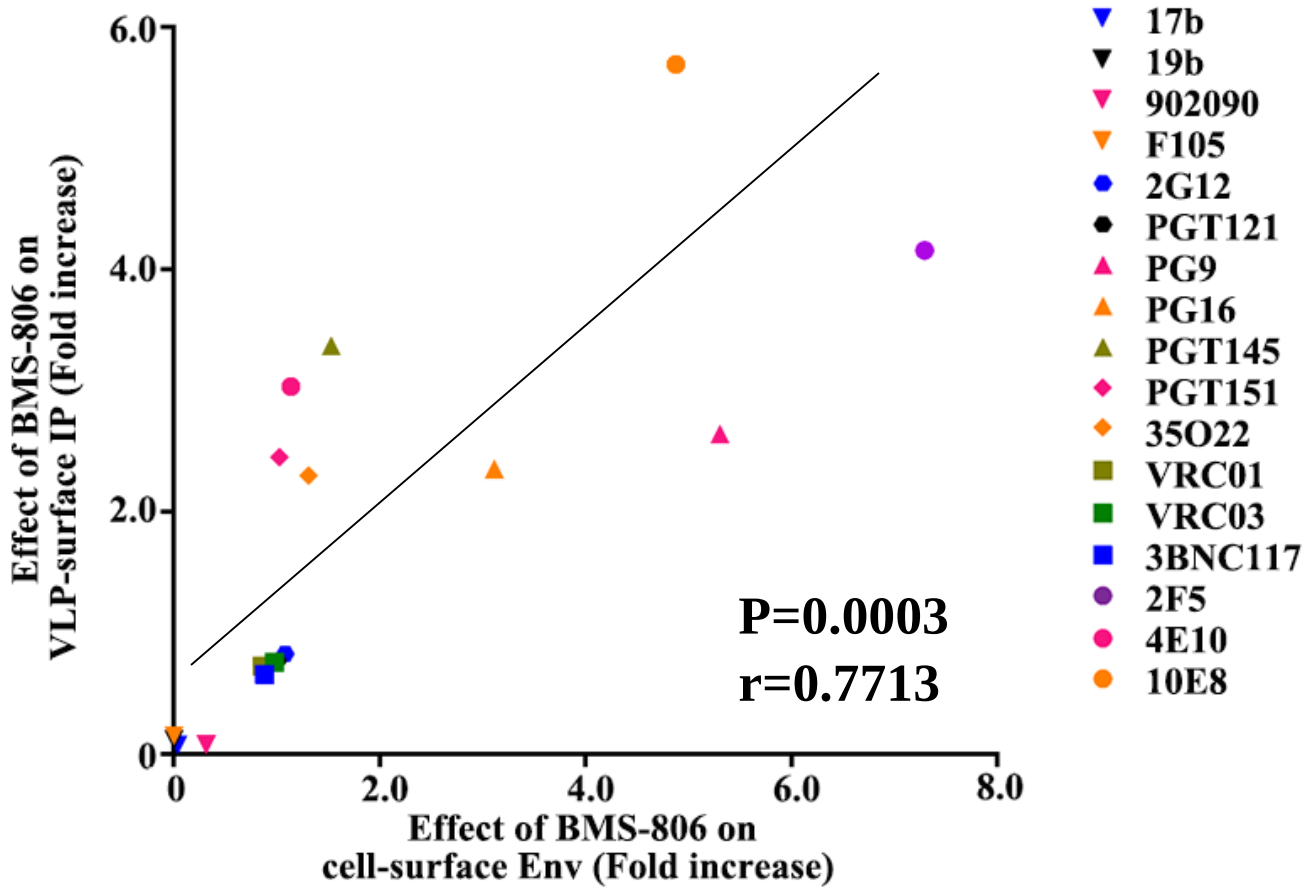
Immunoprecipitation of Env from the surface of virus-like particles



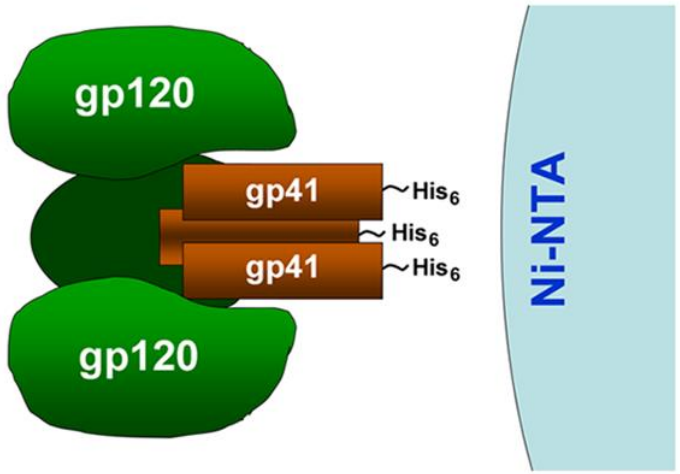
Effect of BMS-806 on immunoprecipitation of Env from the surface of virus-like particles



Effect of BMS-806 on immunoprecipitation of gp120 Env from the cell surface correlates with the effect of BMS-806 on recognition of gp120 on virus-like particles



Effect of BMS-806 on the association of gp120 with Env complexes in detergent-solubilized cell lysates

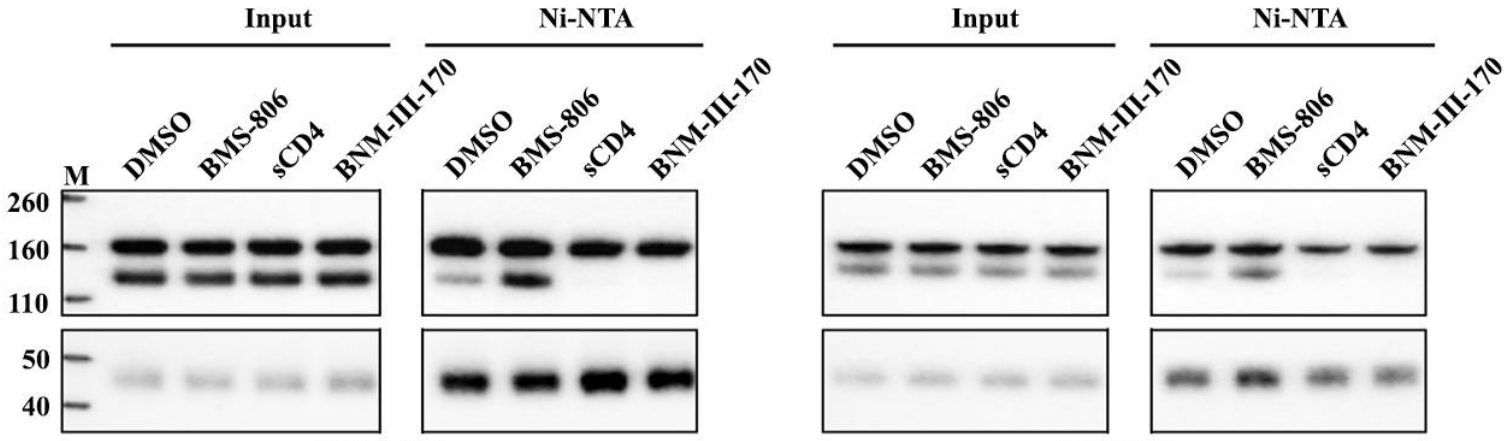


+DMSO

+BMS-806

+sCD4

+BNM-III-170



HIV-1_{AD8} HIV-1_{CH848}

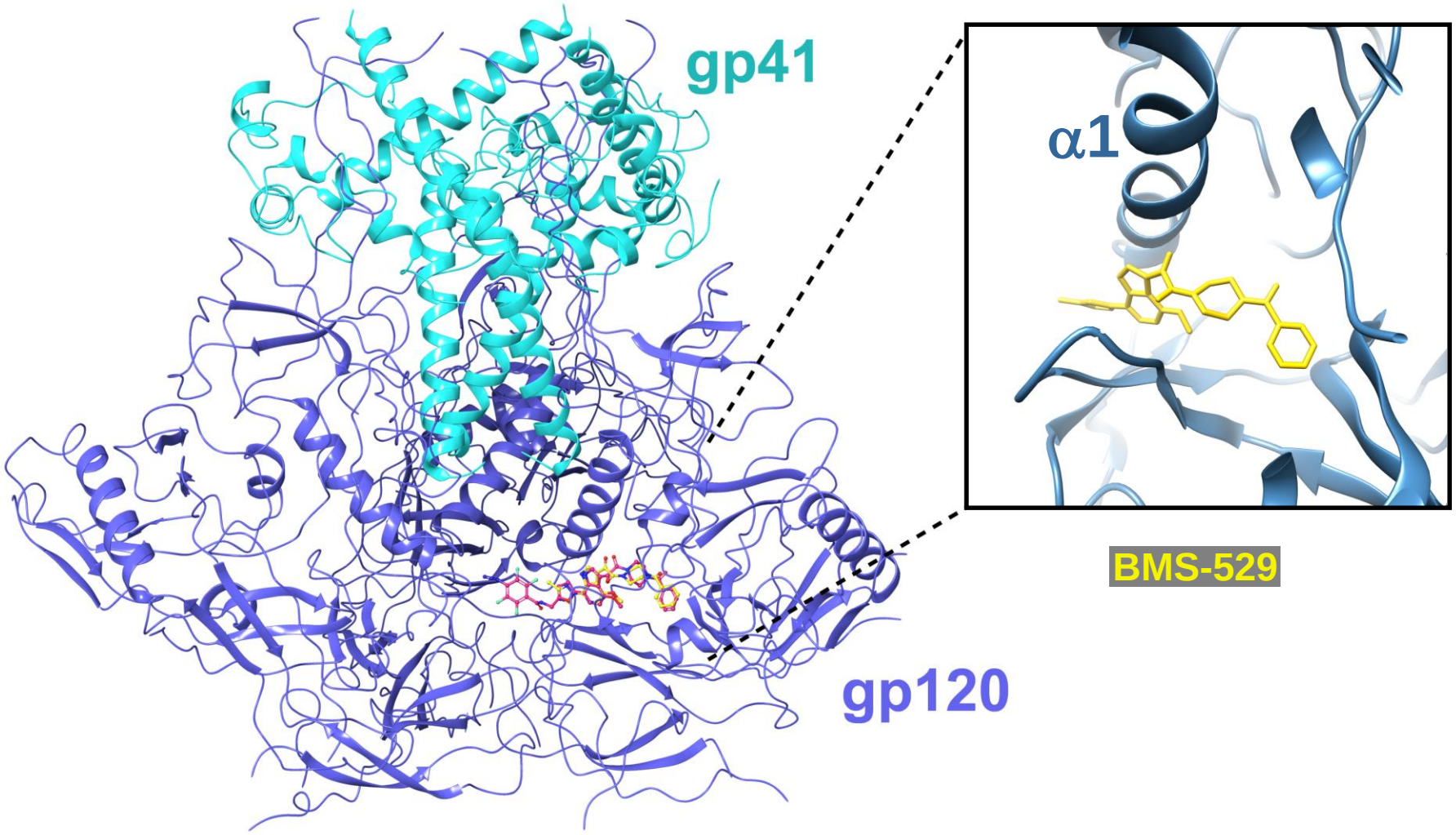
Conclusions

- **The mature (cleaved) HIV-1 Env trimer on the cell surface mimics the functional State-1 Env on the virus**
- **BMS-806 decreases the exposure of epitopes for poorly neutralizing antibodies and maintains or increases the exposure of the epitopes for most bNabs → consistent with stabilization of a State-1-like Env conformation**
- **BMS-806 stabilizes the non-covalent association of gp120 with the Env complex**

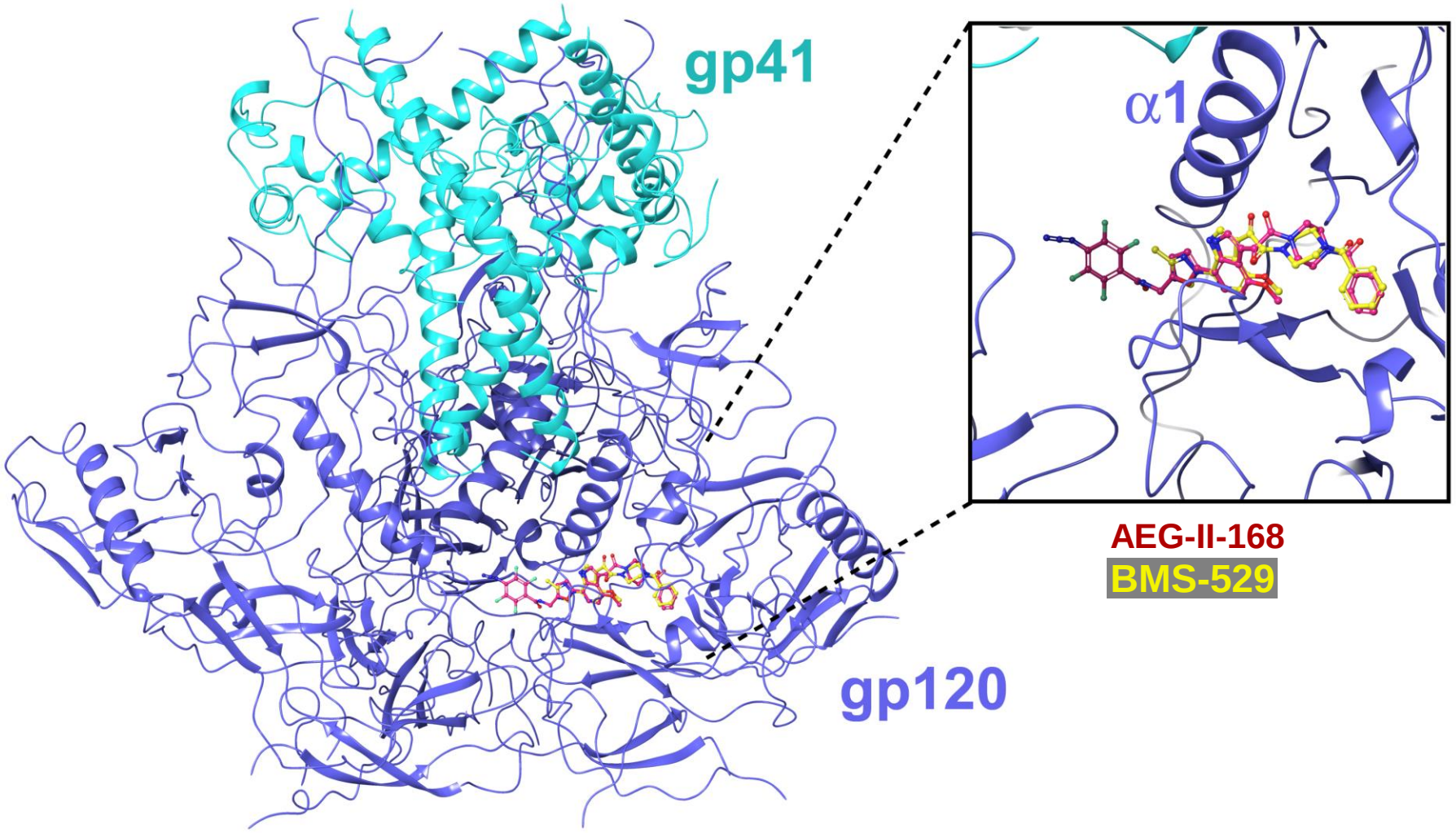
Objective

- **Develop small-molecule blockers of State 1-to-State 2 transitions in Env with long-acting effects**

BMS-806 and BMS-529 bind soluble gp140 SOSIP.664 trimers in a hydrophobic pocket (PDB 5U7O)



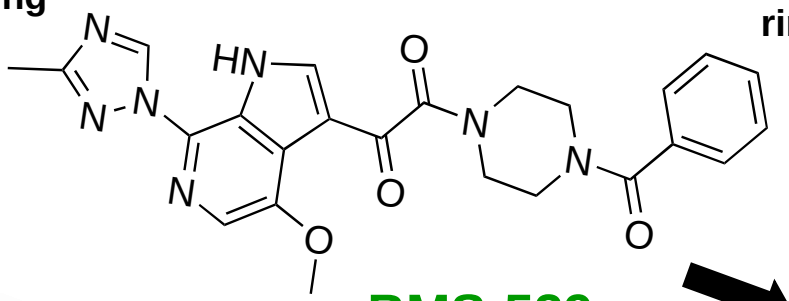
Computational docking of AEG-II-168 into a soluble gp140 SOSIP.664 trimer structure (PDB 5U7O)



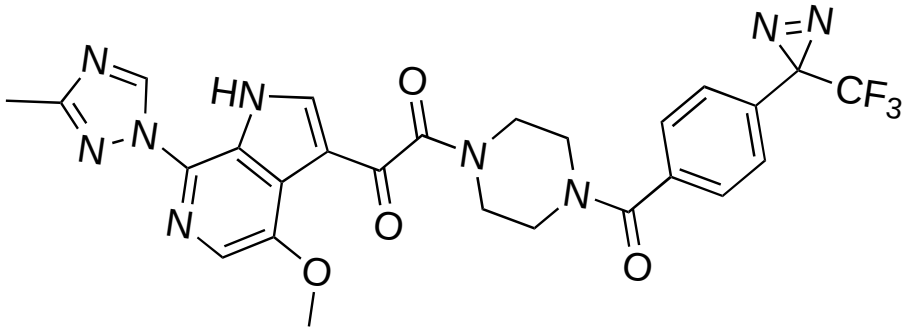
Modification of the methyltriazole but not the phenyl ring of BMS-529 allows retention of antiviral activity

Methyltriazole ring

Phenyl ring

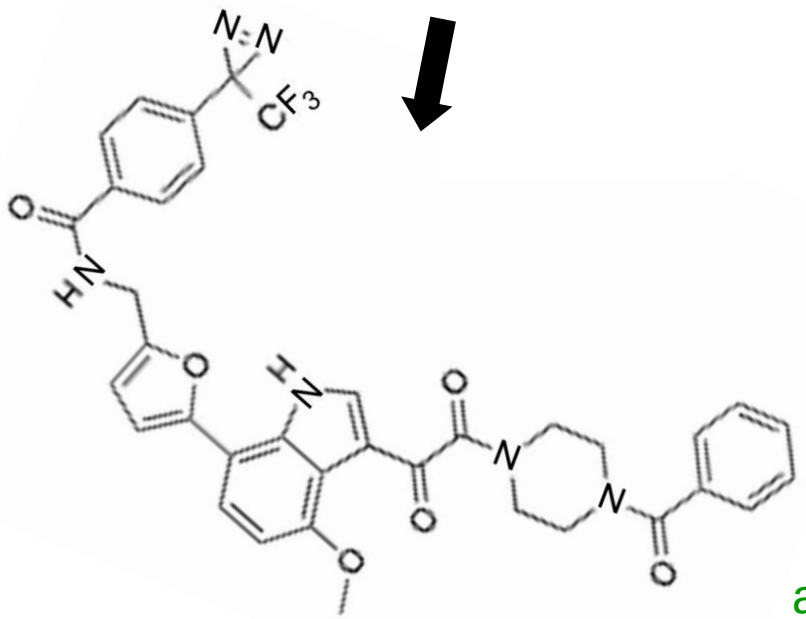


BMS-529



MF463

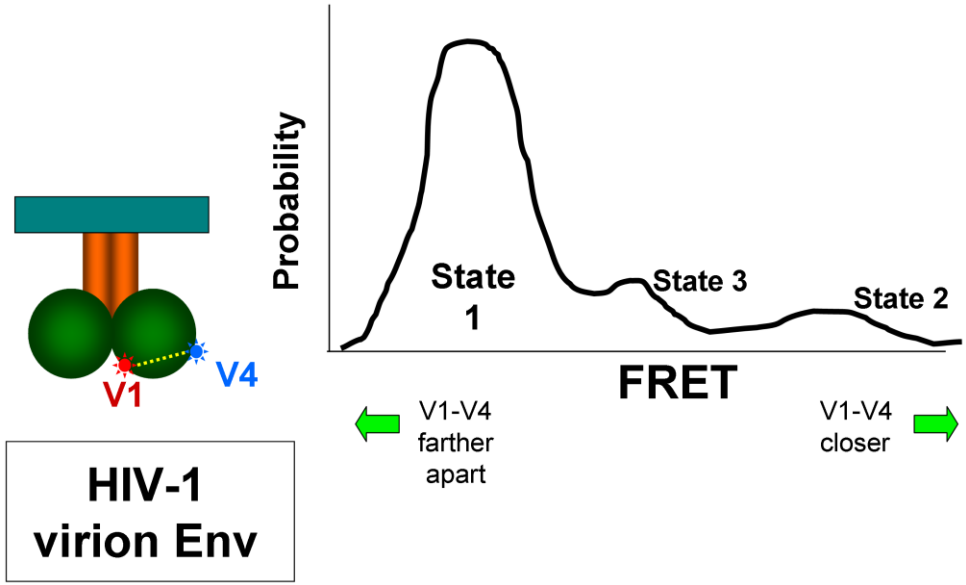
Complete loss of antiviral activity!

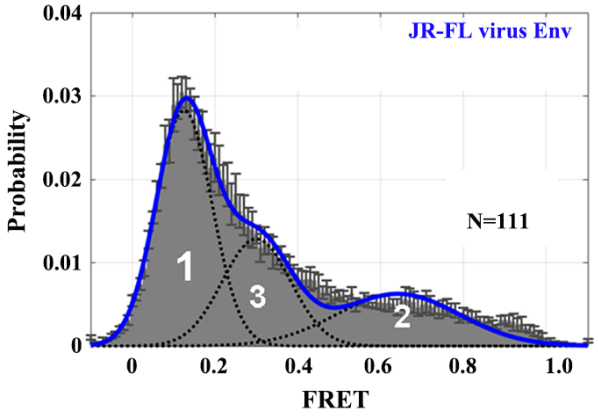


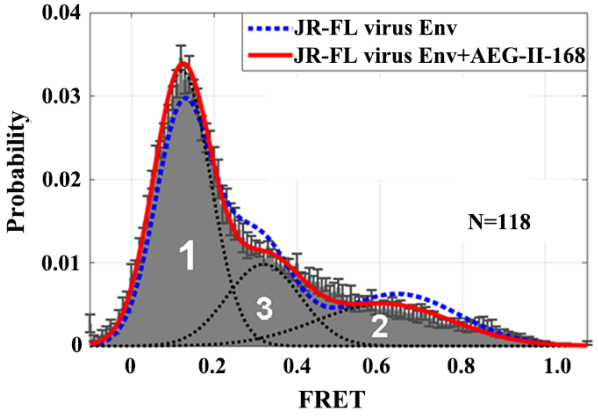
AEG-II-159

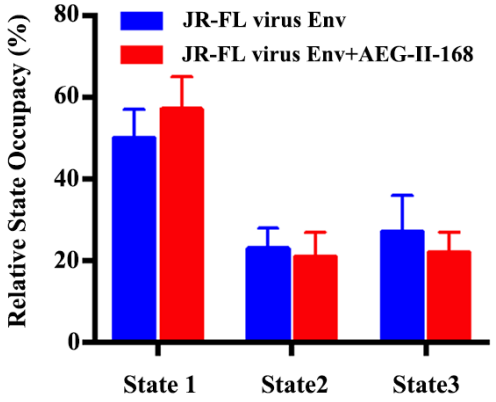
Retention of potent and specific antiviral activity!

Effect of AEG-II-168 on the conformation of Env complexes on viruses

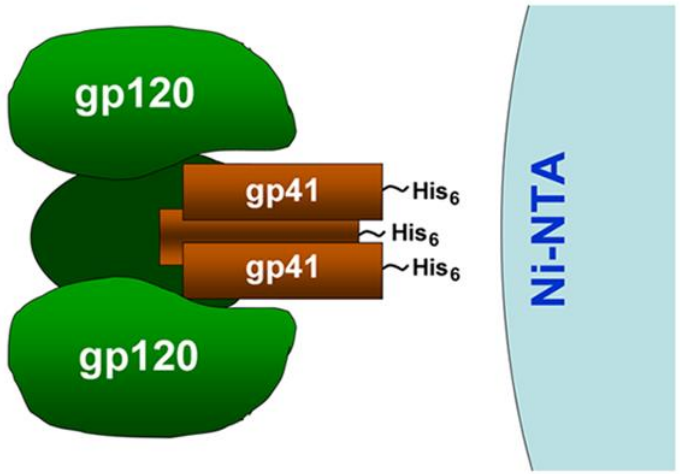


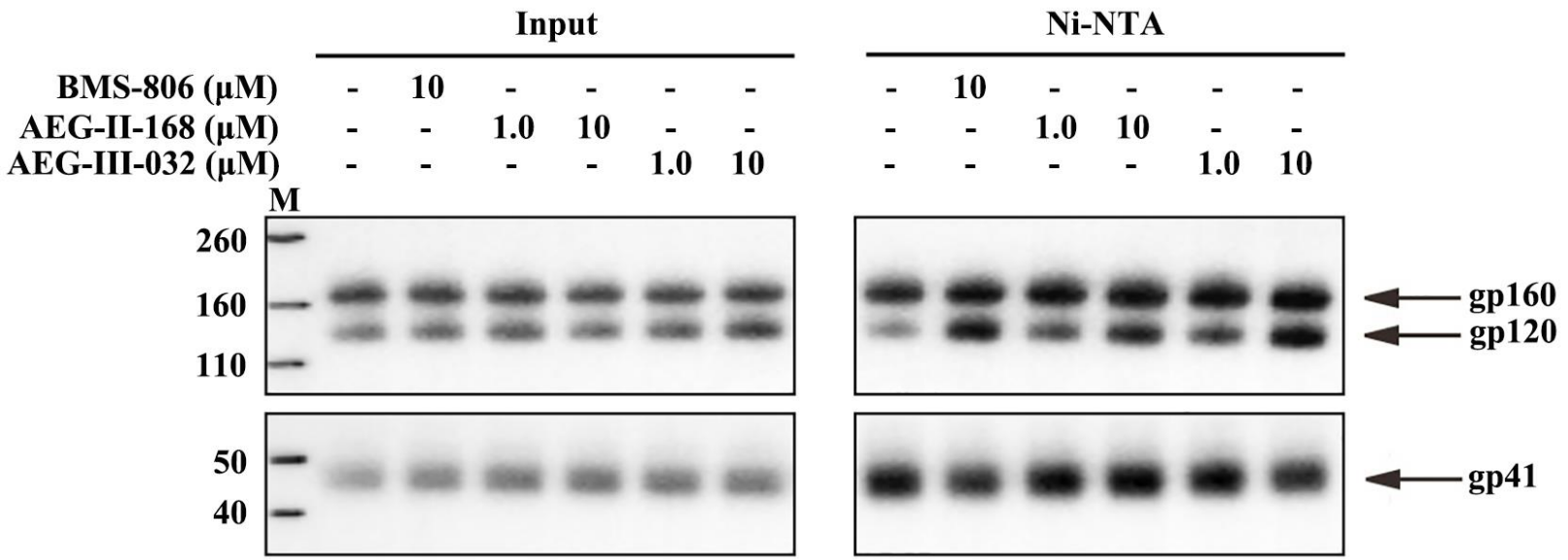




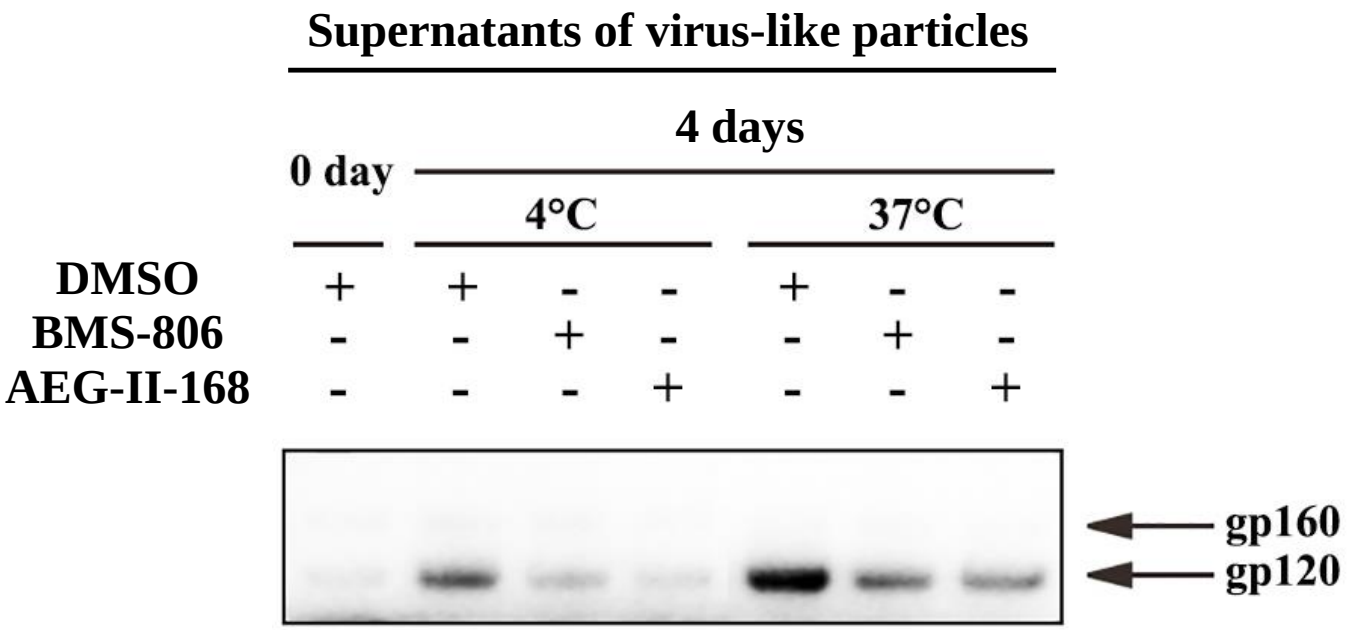


Effect of BMS-806 analogues on the association of gp120 with Env complexes in detergent-solubilized cell lysates





Effect of BMS-806 analogues on the association of gp120 with Env complexes on virus-like particles



Assay for binding of BMS-806 analogues to Env on HIV-1 virus-like particles

Incubate VLP with BMS analogue or DMSO

Irradiate with 100-W UV (365-nm) lamp x 10 min or mock irradiate

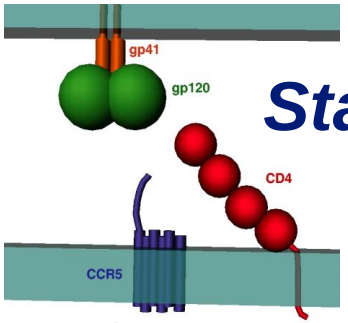
Pellet virus and wash x 2

Resuspend VLP in PBS for different lengths of time (0.5 hr - 2 weeks)

Pellet virus and lyse in Triton X-100

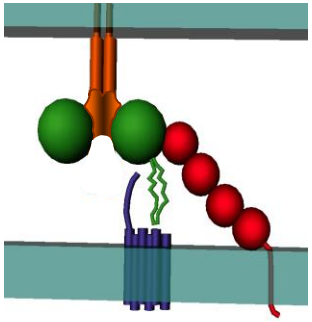
Incubate solubilized Env trimers with antibodies and Protein-A Sepharose beads

Analyze precipitated Env proteins by Western blot

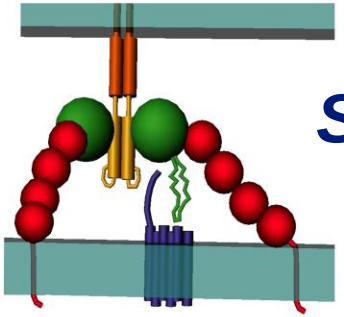


State 1

BMS-806 analogues



State 2

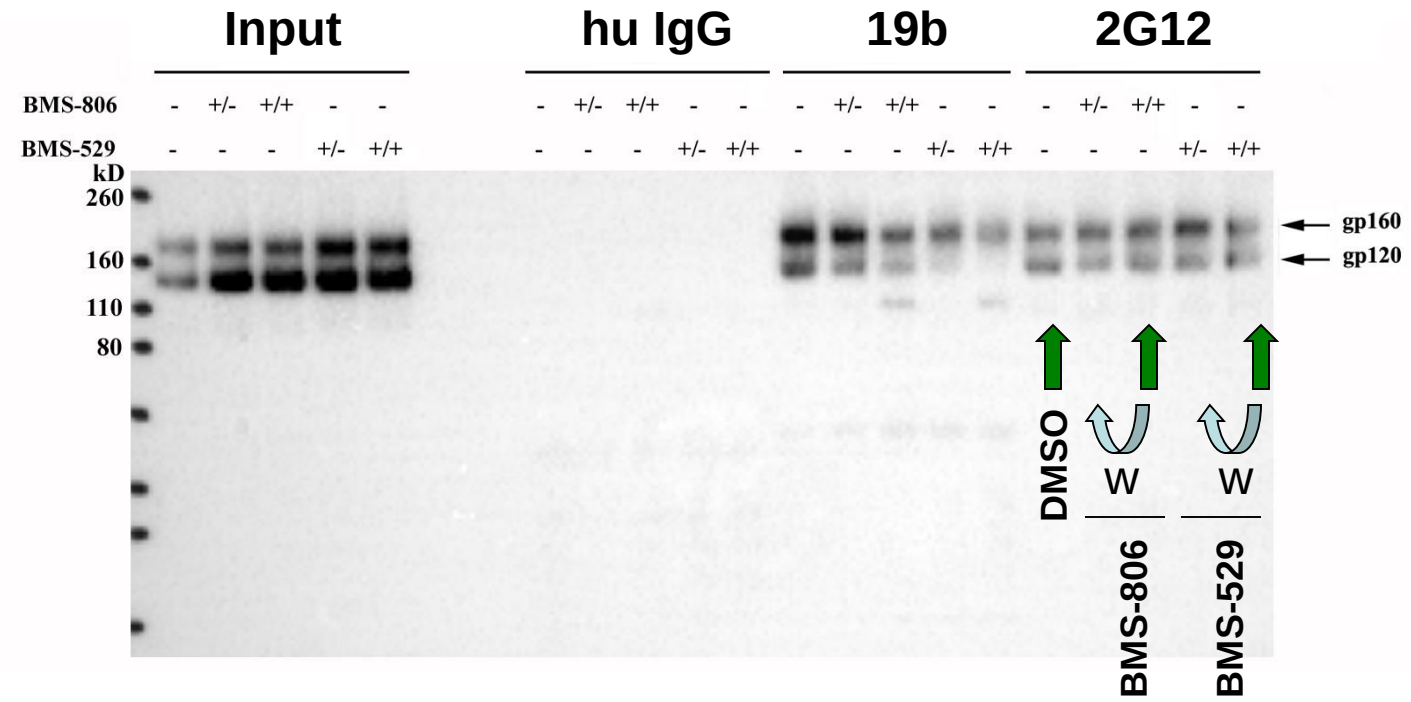


State 3

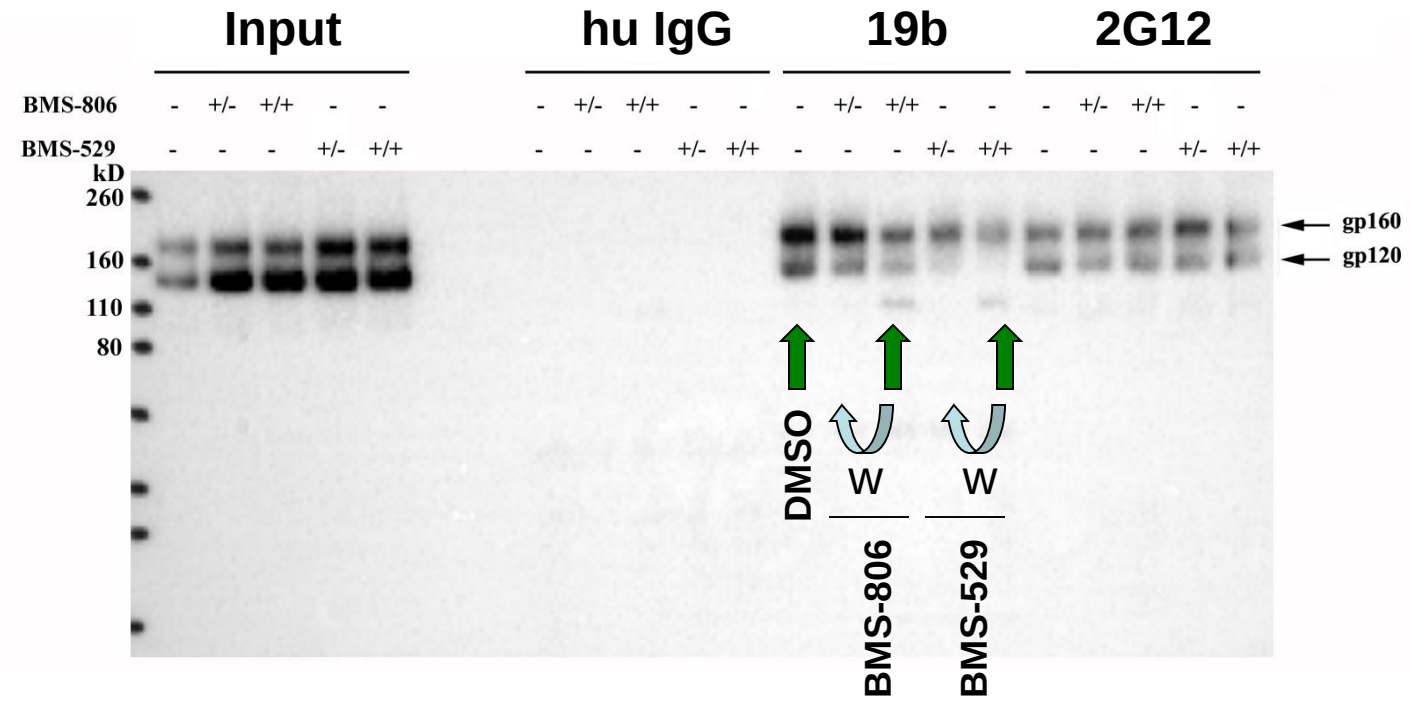
19b

2G12

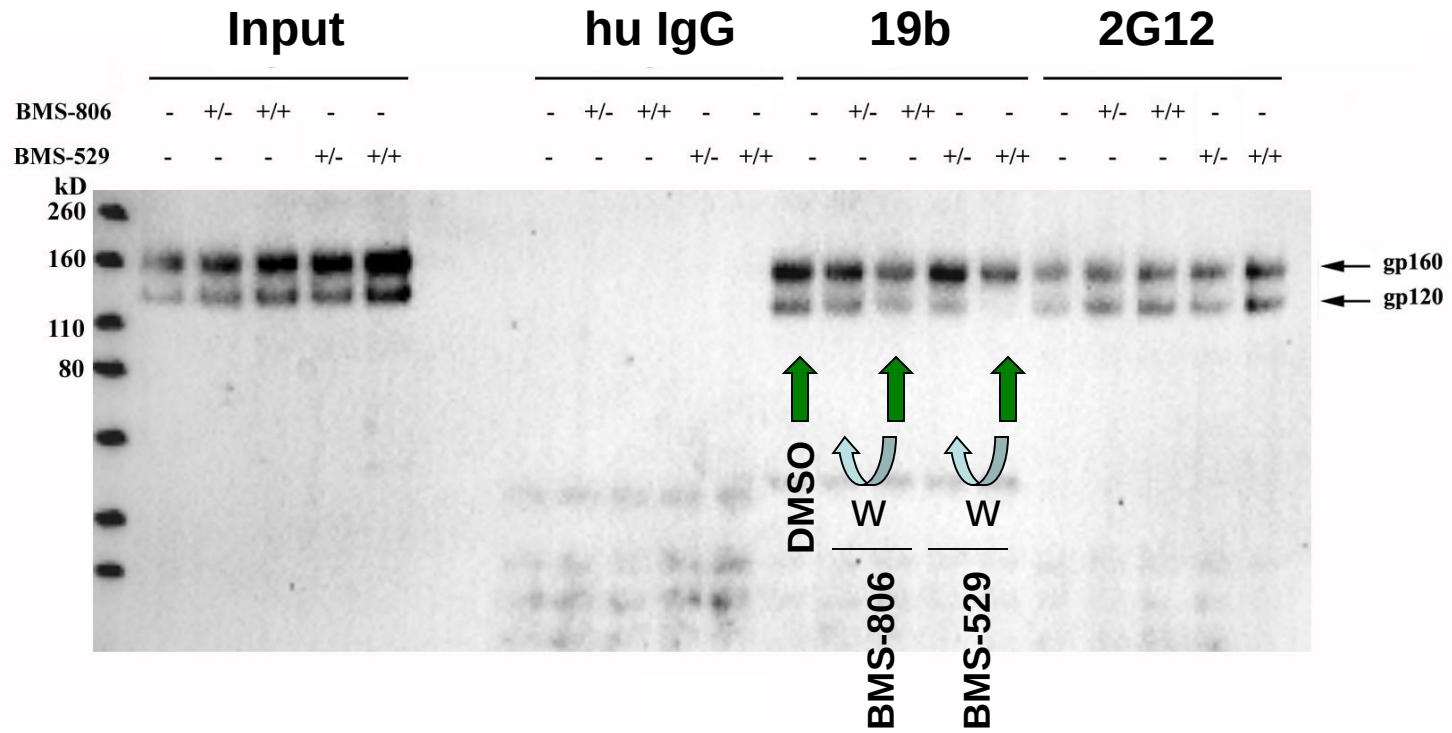
20-hour wash


Input
hu IgG
19b
2G12
BMS-806
BMS-529
kD
260
160
110
80
gp160
gp120
DMSO
W
W
BMS-806
BMS-529

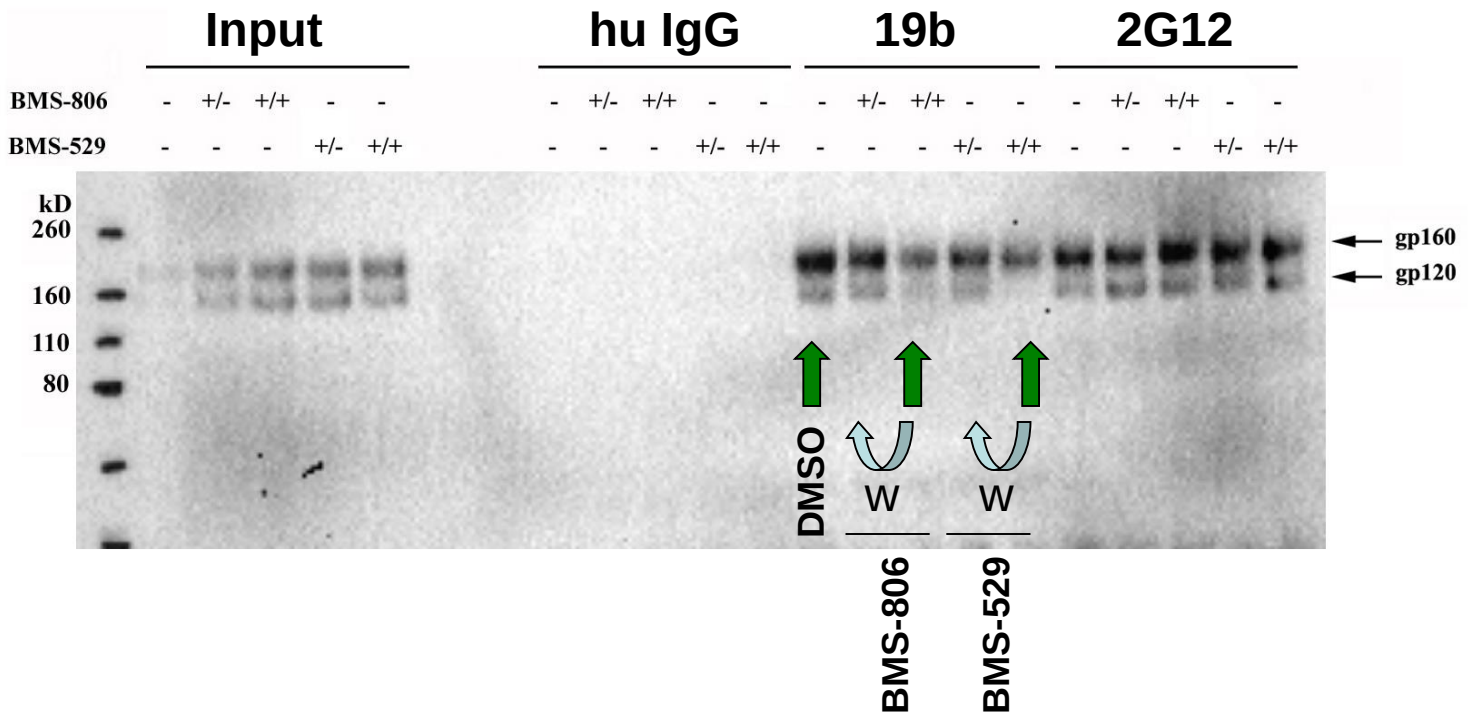
20-hour wash


Input
hu IgG
19b
2G12
BMS-806
BMS-529
kD
260
160
110
80
gp160
gp120
DMSO
W
W
BMS-806
BMS-529

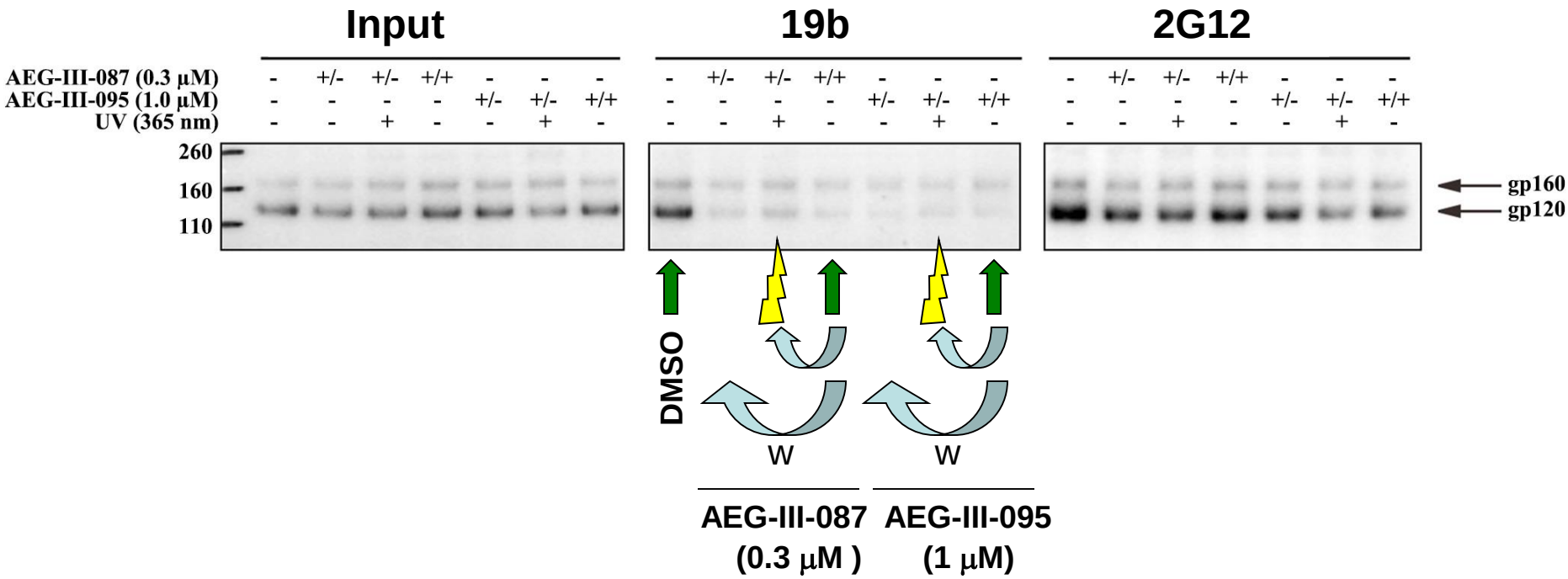
2-day wash


Input
hu IgG
19b
2G12
BMS-806
BMS-529
kD
260
160
110
80
gp160
gp120
DMSO
W
W
BMS-806
BMS-529

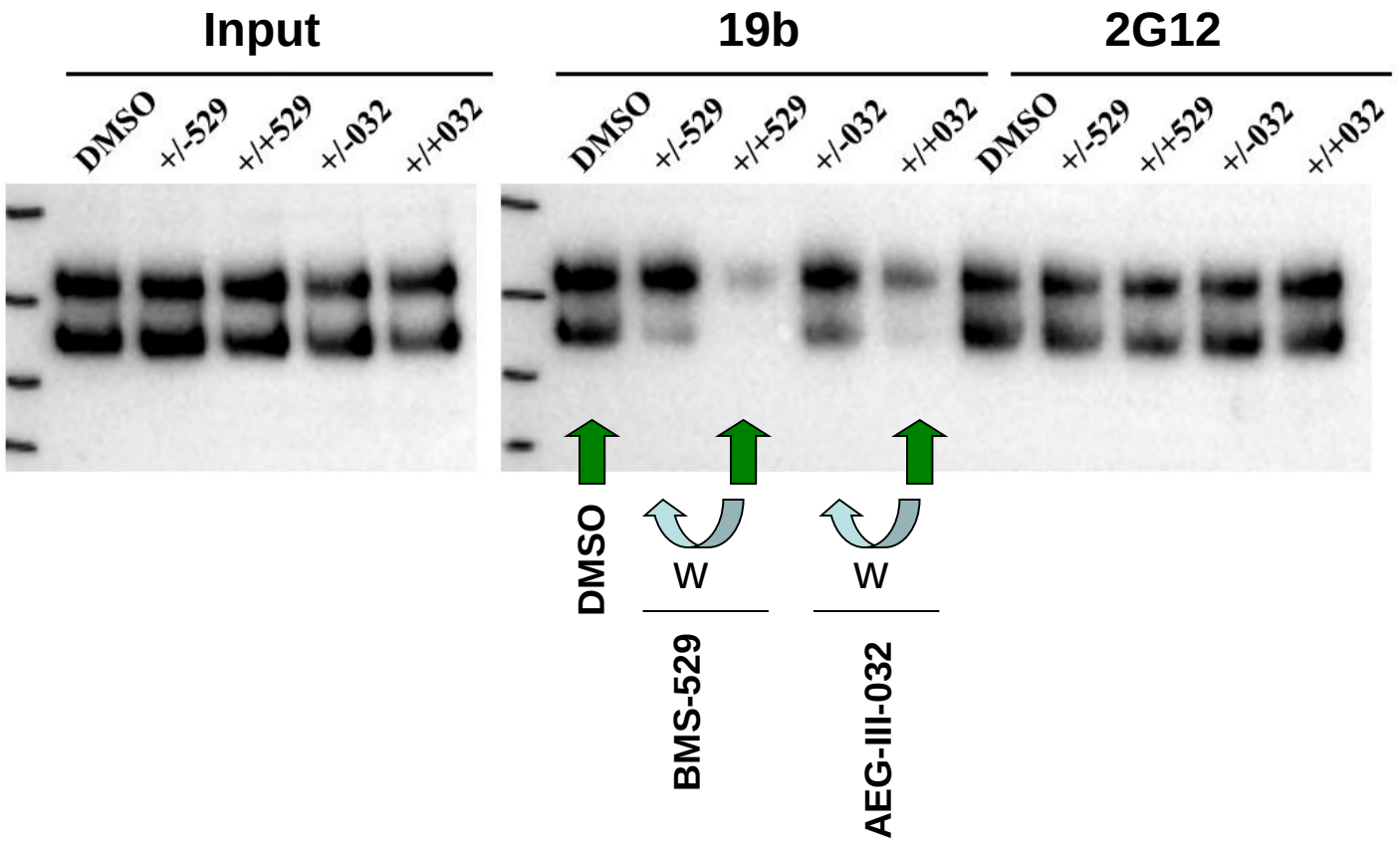
4-day wash


Input
hu IgG
19b
2G12
BMS-806
BMS-529
kD
260
160
110
80
gp160
gp120
DMSO
W
W
BMS-806
BMS-529

AEG-III-087 and AEG-III-095 specifically decrease 19b recognition of Env on the surface of virus-like particles for at least 3 weeks

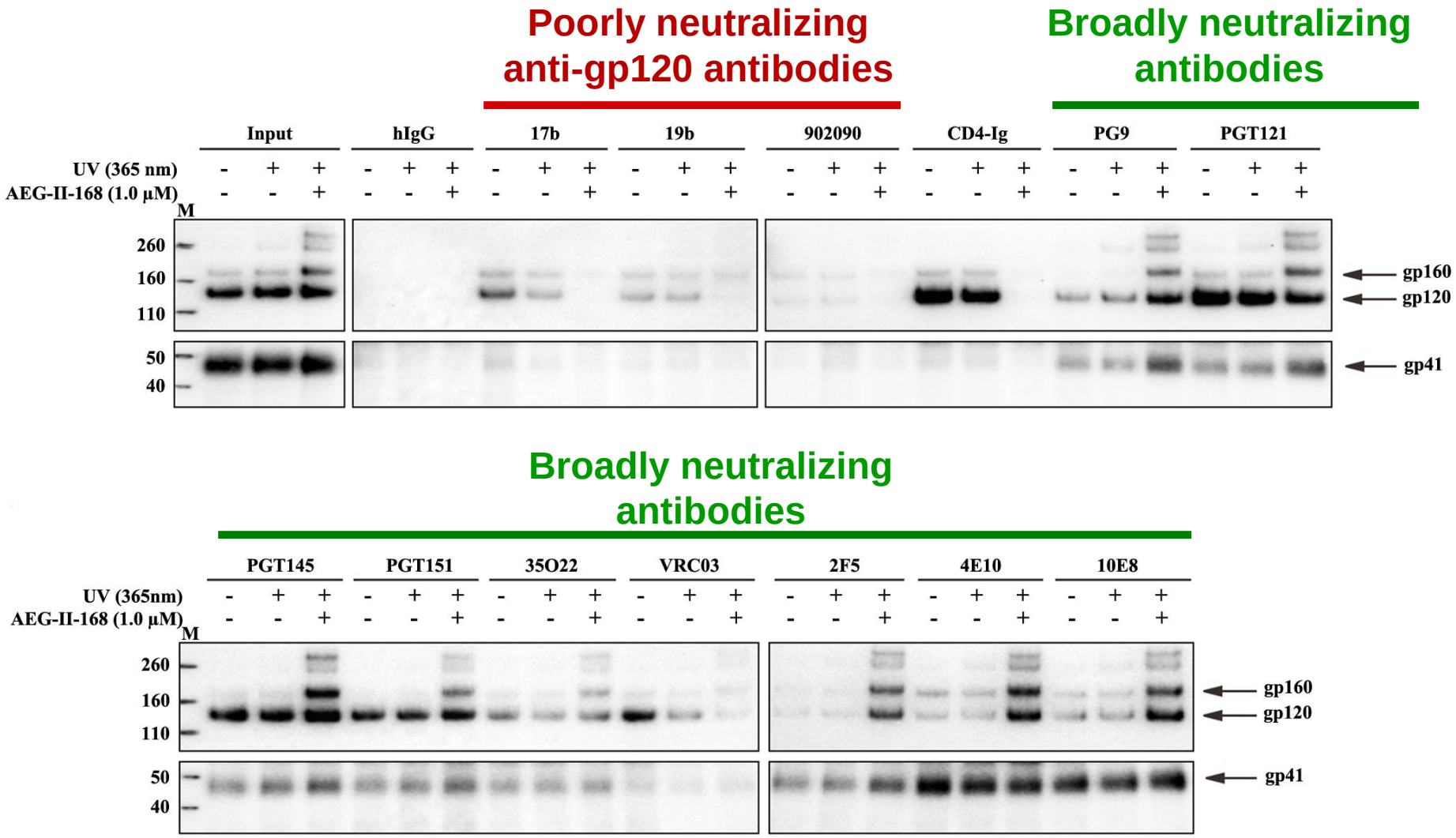


The decrease of 19b recognition of Env by AEG-III-032 (without a photoactivatable group) is reversed by 7 days



Concentration of compounds: 1 uM

Long-term effect of AEG-II-168 + UV on the conformation of Env complexes on the surface of virus-like particles (2 weeks)



Conclusions

- BMS-806 analogues stabilize a State-1-like HIV-1 Env conformation (ie., reducing exposure of epitopes for poorly neutralizing antibodies while maintaining most bNab epitopes)
- Photoactivatable BMS-806 analogues induce conformational changes in Env similar to those induced by BMS-806 and BMS-529, with little reversibility 2-3 weeks after a single exposure!
- The photoactivatable group contributes to the low reversibility of the analogues' effects
- The long-term effects of photoactivatable BMS-806 analogues on Env conformation do not depend on, but may be enhanced by, exposure to UV light

Relevance to Vaccine Development

- Long-acting BMS-806 analogues could enrich State-1-like conformations in HIV-1 Env preparations, allowing the following hypothesis to be tested:
  Envs enriched in State 1 will more effectively elicit broadly neutralizing antibodies.

Acknowledgements

Dana-Farber Cancer Institute

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University of Alabama, Birmingham

Haitao Ding
John Kappes

St. Jude Children's Research Hospital

Scott Blanchard

Yale University

Maolin Lu
Walther Mothes

Drexel University

Mohammadjavad Mohammadi
Cameron Abrams

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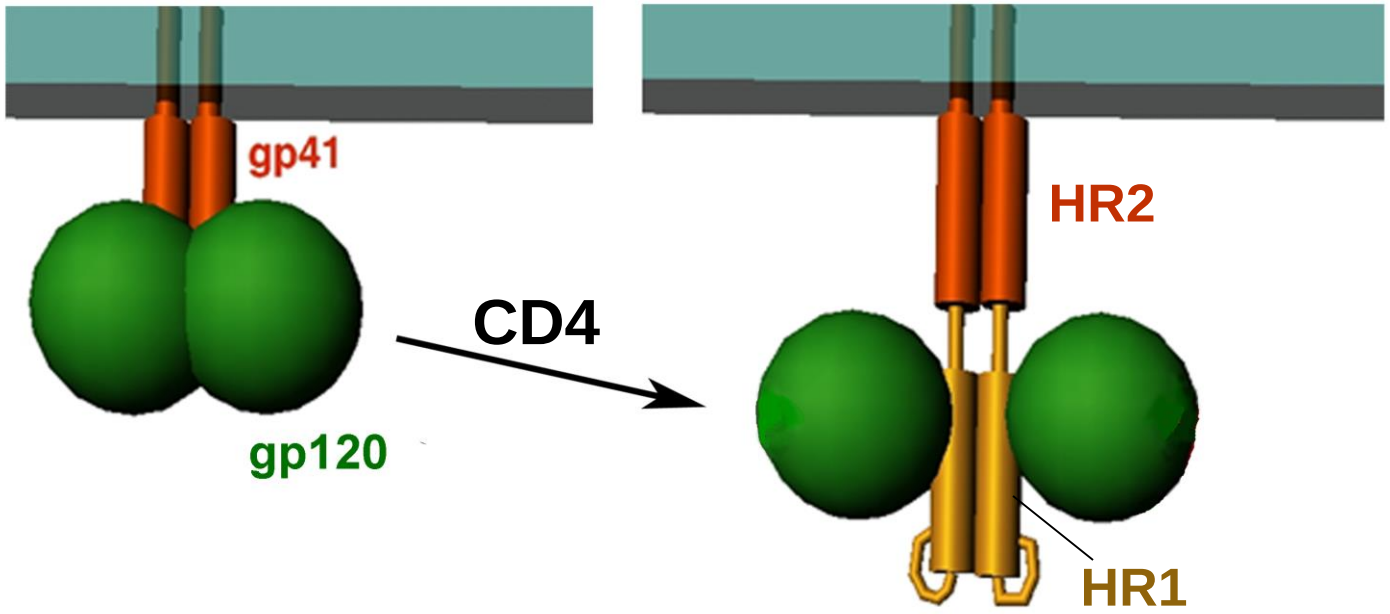
gp41
gp120
CD4
HR2
HR1

Effect of BMS-806 on the sCD4-induced exposure of the gp41 HR1 coiled coil on cell-surface Env