# The HIV Env Signal Peptide Impacts the Glycosylation and Antigenicity of gp120


Jason Yolitz[1,2], Catherine Schwing[1], Claudia Cicala[1], Danlan Wei[1], Fatima Nawaz[1,3], Katija Jelicic[1], Donald Van Ryk[1], Anthony Fauci[1], and James Arthos[1].

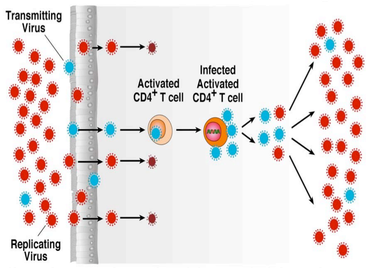
[1]National Institute of Allergy and Infectious Disease, National Institutes of Health, [2]Johns Hopkins University, [3]Sackler Institute of Biomedical Research, New York University


## Abstract

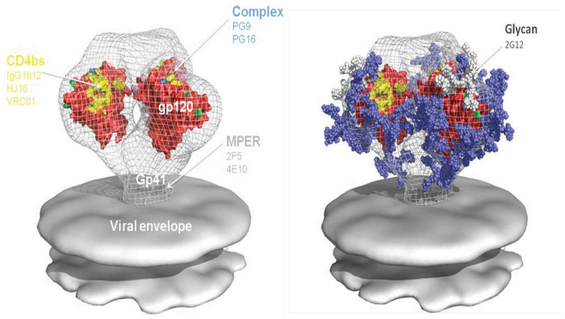

Transmission of HIV across mucosal tissues involves a complex yet inefficient series of events that are not yet fully understood. The HIV-1 envelope protein (Env) of early-transmitting viruses has been found to present distinct transmission signatures. One such signature involves a reduced number of potential N-linked glycosylation sites, underscoring the importance of post-translational modifications in transmission fitness. Recently, an additional transmission signature has been identified in the signal peptide of Env. This signature involves the over-representation of basic residues at a specific position in the signal peptide. We investigated the potential impact of this signal peptide signature on gp120 glycosylation and antigenicity. Two recombinant Envs were constructed, one derived from a chronic isolate that lacks the signal peptide signature and a second from a transmitting isolate that includes the signal peptide signature. Chimeric Envs were also constructed in which the two signal peptides were swapped. All four gp120s were probed with glycan-, structure- and interaction-specific probes in a surface plasmon resonance binding assay. The signature found in the signal peptide of Env influences qualitative aspects of Env glycosylation that in turn affect the structure and antigenicity of Env in a major way. The signal peptide of a transmitting isolate increases Env's affinity for DC-SIGN, a lectin receptor expressed on dendritic cells that is believed to be involved in mucosal transmission. Additionally, affinity for the monoclonal antibody 17b, which recognizes the CD4-induced (open) conformation of Env is also increased by the transmitting isolate's signal peptide. We conclude that the HIV Env signal peptide is an important determinant of Env processing, folding and antigenicity. These observations may aid in the development of Env based HIV vaccines.


## Introduction

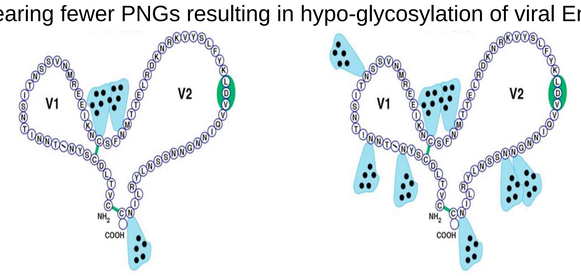
Mucosal HIV transmission is an inefficient process and is commonly the result of a single "founder" virus. This bottleneck-produced "founder" virus replicates in an activated CD4⁺ T cell and produces replicating virus.



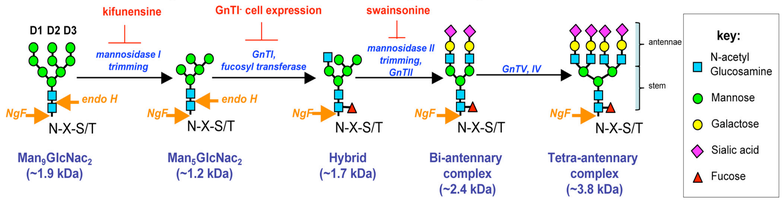

HIV-1 Env is heavily glycosylated with variable glycan forms (high-mannose, complex, and hybrid glycans). The number of potential N-linked glycosylation sites (PNGs) varies from 18 to 28 depending on the virus clade and maturation level.




Signature 1 of early transmitting viruses is demonstrated by transmitting HIV isolates bearing fewer PNGs resulting in hypo-glycosylation of viral Env.




Differences in glycan processing and maturation can have a profound impact on protein folding, structuring and binding to target receptors.

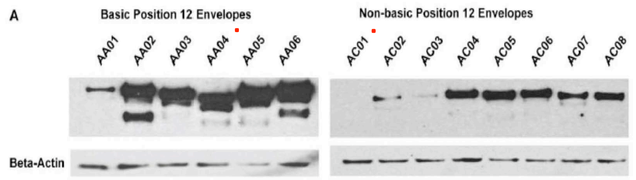



## Introduction (cont'd)

Signature 2 is demonstrated by transmitting HIV isolate sequences bearing an over-represented basic residue at position 12 of the Env leader sequence.

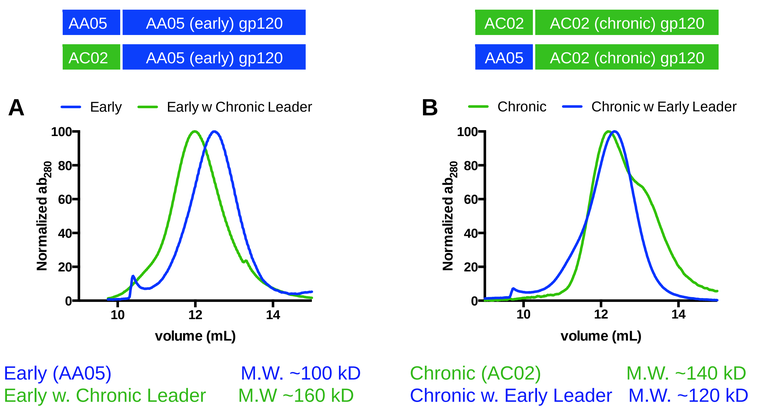
**Transmitting (early)** MRVKGIRKNYQ**H**LWRWGIMLLGMLMICSEEDKLWVTVY
**Chronic (late)** MRVKGIRKNYW**W**KWGTL--LLGMLMICSAEEQLWVTVY
**********  *  ******** *  ******

Env containing a basic residue at position 12 of the leader sequence is associated with higher expression levels of HIV-1 Env.




Asmal et al. 2010

## Results




Early (AA05) M.W. ~100 kD
Early w. Chronic Leader M.W ~160 kD
Chronic (AC02) M.W. ~140 kD
Chronic w. Early Leader M.W. ~120 kD

**Figure 1.** Addition of early transmitting leader peptide decreases the molecular weight of chronic gp120 as demonstrated by size-exclusion chromatography.

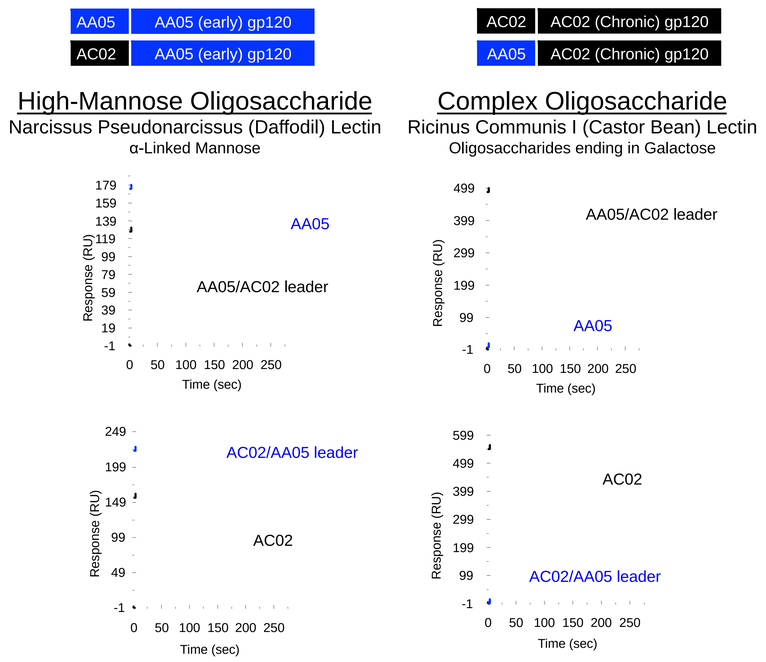
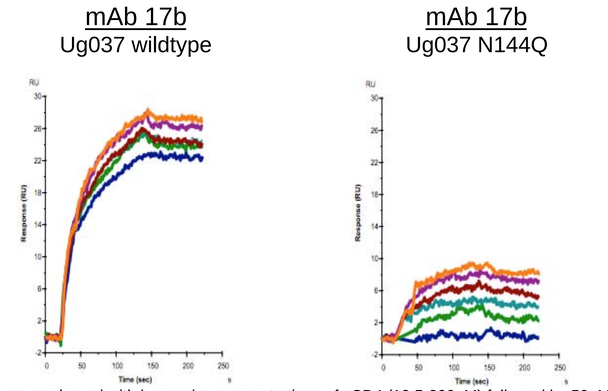



**Figure 2.** Swapping Env leader peptide sequences between transmitting and chronic gp120s induces alternate glycosylation patterns as shown by Surface Plasmon Resonance (SPR) Assay (Biacore).

## Results (cont'd)




**Figure 3.** Swapping Env leader peptides between transmitting and chronic gp120s induces alternate affinities for DC-SIGN, a C-type lectin expressed on dendritic cells. HIV Env binds to DC-SIGN and some reports suggest a relevant interaction between virus and DC-SIGN at the time of transmission.




gp120 was pre-bound with increasing concentrations of sCD4 (12.5-200nM) followed by 50nM 17b.

**Figure 4.** Removing two PNGs from a subtype A chronic gp120 changes the structure of mature gp120 as shown by alternate binding to mAb 17b, a conformational sensitive probe of gp120 structure.

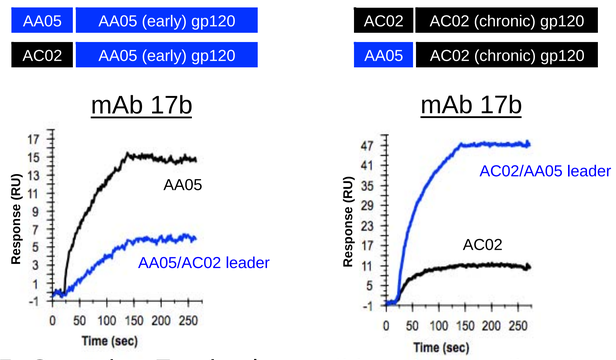



**Figure 5.** Swapping Env leader peptides changes the structure of mature gp120 as shown by alternate binding to mAb 17b.

## Conclusions

- Two genetic signatures of transmitting HIV-1 impact N-linked glycosylation of viral Env.
- Signature 2, an over-represented basic residue at position 12 of the Env leader sequence, biases Env to present high mannose glycans.
- Signature 2 also alters the interaction between gp120 and DC-SIGN, which may play a key role in early transmission.

## References


Asmal M, Hellmann I, Liu W, Keele BF, Perelson AS, et al. (2011) A Signature in HIV-1 Envelope Leader Peptide Associated with Transition from Acute to Chronic Infection Impacts Envelope Processing and Infectivity. PLoS ONE 6(8): e23673.

Binley J, Ban YA, Crooks ET, Eggink D, Osawa K, et al. (2010) Role of Complex Carbohydrates in Human Immunodeficiency Virus Type 1 Infection and Resistance to Antibody Neutralization. Journal of Virology 84(11): 5637-55.



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