Low dS/dN Does Not Correlate With High Variation of Amino Acid Sequences Along the gp120 Protein Structure

Zach Goldstein & Jordan Detamore
BIOL 368: Bioinformatics Laboratory
Department of Biology
Loyola Marymount University
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Outline

- **Low dS/dN ratios may correlate with high amino acid variations along the V3 loop of the gp120 protein**
- **Subjects selected from Markham et al. (1998) demonstrated high or low dS/dN values**
- **Amino acid sequence alignment using ClustalW and BoxShade showed conflicting results**
- **6 Non-consensus segments along gp120 are found along the V3 loop and on the exterior surface of the protein**
- **Further research would examine the impact of variations in these 6 non-consensus sites**

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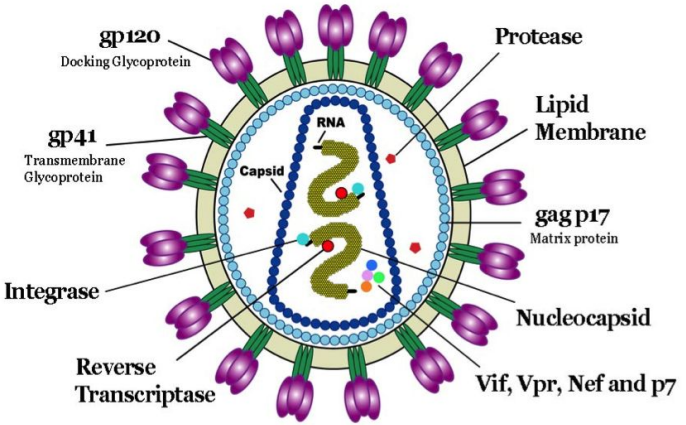
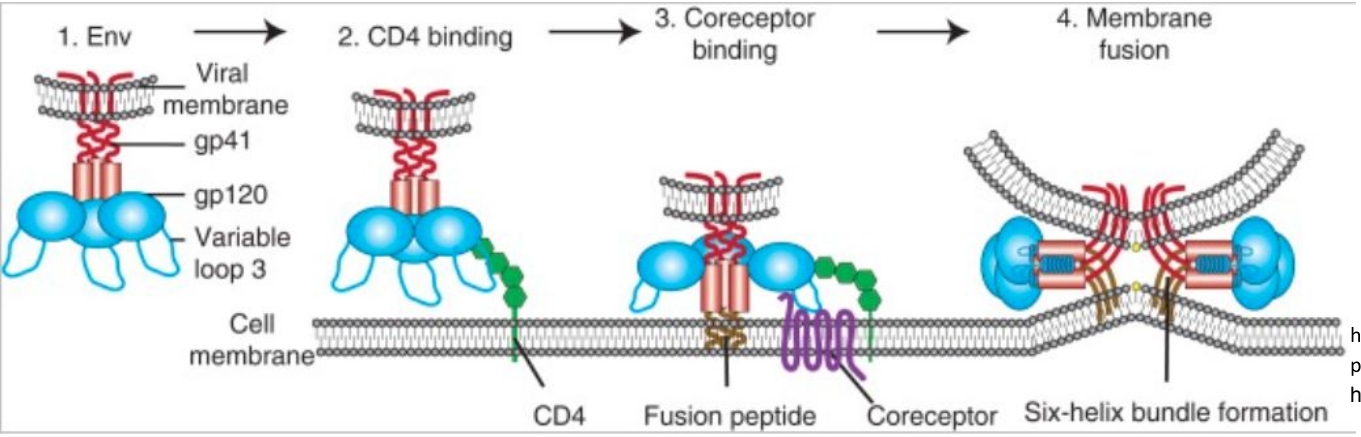
HIV is a Retrovirus With Many Different Structures

- **gp120: Facilitates HIV entry into host cell by binding to CD4**
- **gp41: Responsible for fusion of viral and target cell membranes**
- **V3 Region: Highly variable region of gp120 that facilitates coreceptor binding**





http://people.mpi-inf.mpg.de/~fmueller/thesis/thesis_mueller.pdf

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3405824/

Non-Synonymous Mutation Rates May Demonstrate Significant Changes in Amino Acid Sequence

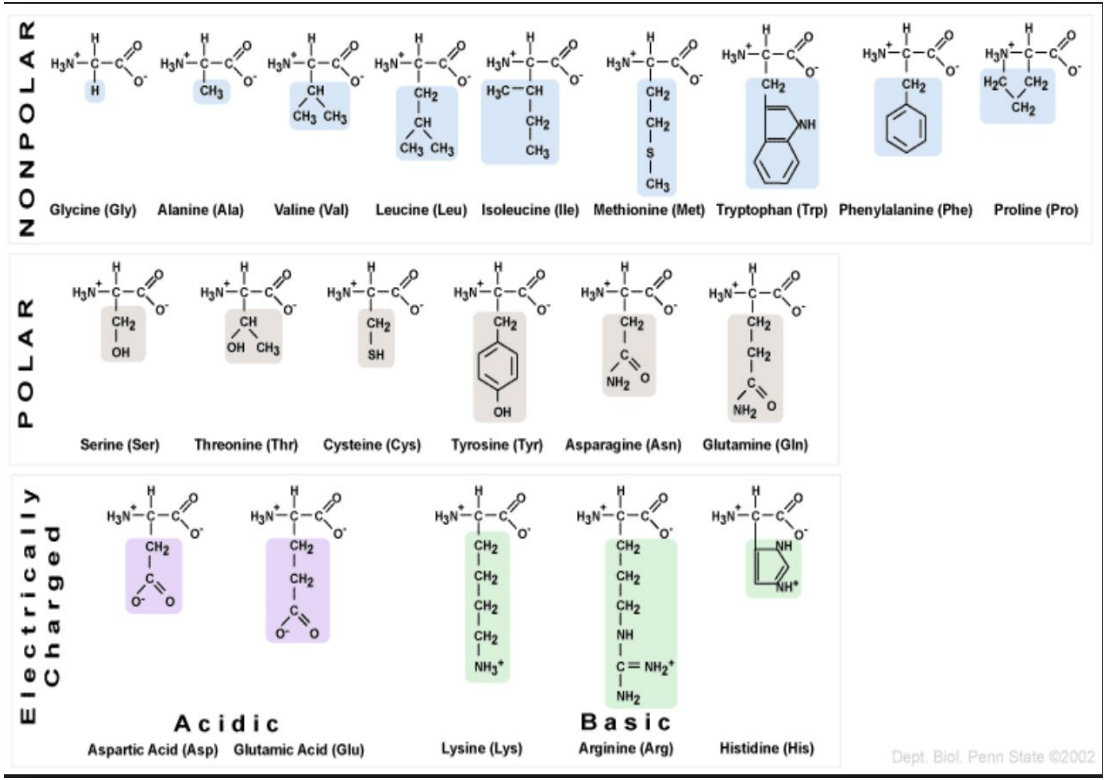
- Non synonymous mutations (dN) are nucleotide changes that lead to the coding of a different amino acid.
- Synonymous mutations (dS) are nucleotide changes that do not lead to the coding of a different amino acid.
- These ratios were formed for each consensus strain for all subjects.
- The smaller the ratio, the larger amount of non synonymous mutations, the more potential for differences in amino acid sequences

20 Standard Amino Acids Vary in Structure and Properties

4 Groups of Amino Acids
- Acidic
- Basic
- Uncharged Polar
- Nonpolar

Position affects function

Size affects function



Do Mutation Rates Affect Variation in Amino Acid Sequences?

Questions:

Does a low dS/dN ratio value result in high amino acid variation?
Do subjects with low dS/dN ratios vary from each other?
Do the sites of variation have nonconservative mutations?

Hypotheses:

Low dS/dN ratio values will result in high amino acid variation.
Subjects with low dS/dN ratios will vary greatly from each other.
The sites of high variation will result in nonconservative mutations.

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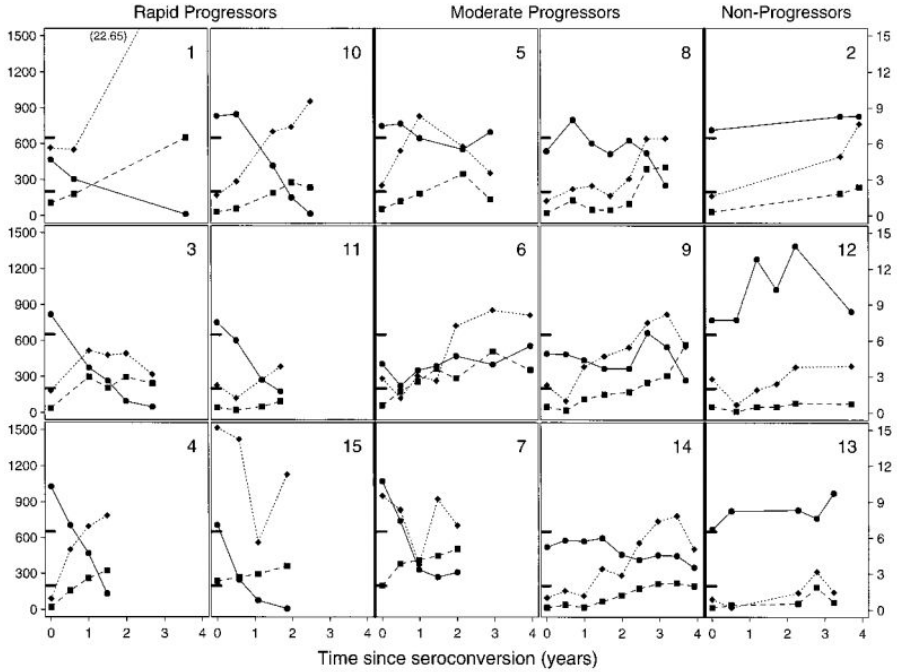
Subjects Selected Based on Differences in dS/dN Ratios



- Four subjects with highest dS/dN ratio and four subjects with lowest dS/dN ratio were chosen
- Three random clones were selected from the first and last visit
- Subjects had different numbers of visits spread over the four years
    - Unable to chose subjects from same visit date

New Groups Formed Based on Highest and Lowest dS/dN Ratios

Rapid Progressor (Subject #)	Moderate Progressor (Subject #)	Non-progressor (Subject #)
4	7	2
10	8	12
11	14	13
15	5	
3	9	
1	6	

Subjects demonstrating highest dS/dN ratios	Subjects demonstrating lowest dS/dN ratios
7	4
5	9
2	11
13	14

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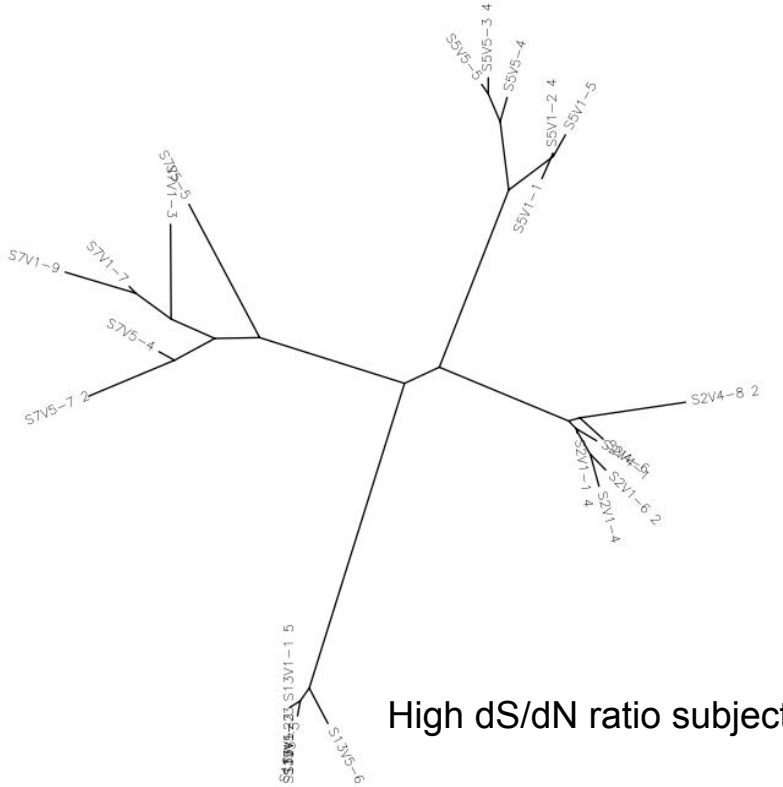
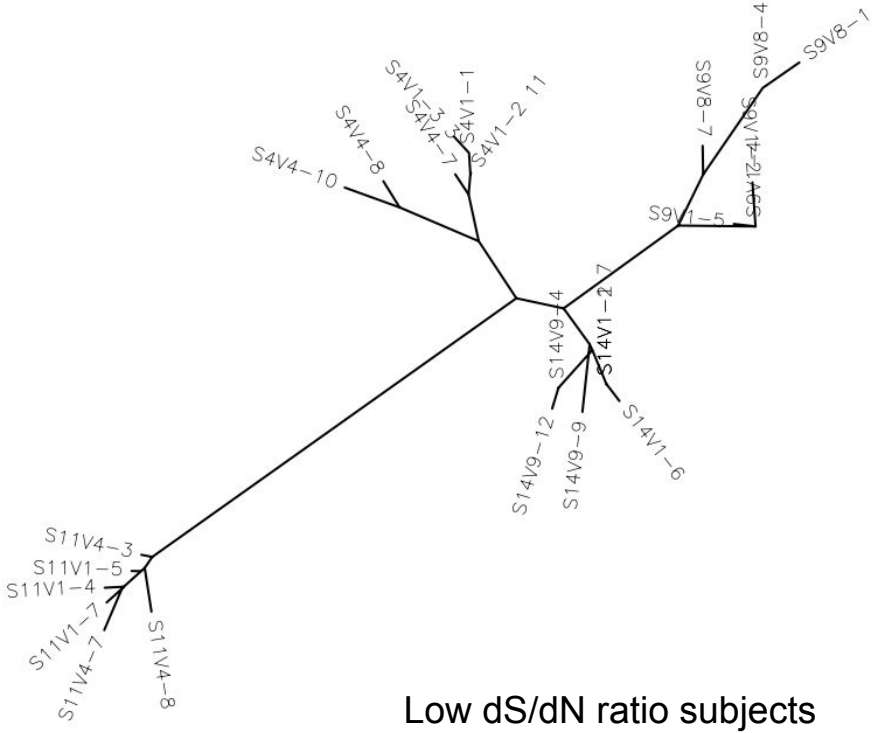
Phylogenetic Trees Show No Clear Difference in Variation Between Low dS/dN Subjects and High dS/dN Subjects



High dS/dN ratio subjects



Low dS/dN ratio subjects

Biology Workbench: ClustalW

BoxShade and ClustalW Were Used to Analyze Amino Acid Sequences

Boxshade default similarities	**Individual Similarities:** D: E F: YW G: A I: LVM L: VMI M: ILV N: Q R: K T: S V: MIL W: FY Y: WF **Groups:** FYW IVLM RK DE GA TS NQ
ClustalW 1.60 similarities	**Individual Similarities:** A: GS D: EN E: DQ F: YW G: A H: RK I: LVM K: HR L: VMI N: QD Q: NE R: KH S: TA T: S V: MIL W: FY Y: WF **Groups:** ACST NT EQ NQ ED KRQH GN NH FY IVLM

Completely Conserved Residues
Background Color: Green

Identical Residues
Background Color: Yellow

Similar Residues
Background Color: Cyan

Different Residues
Background Color: Red

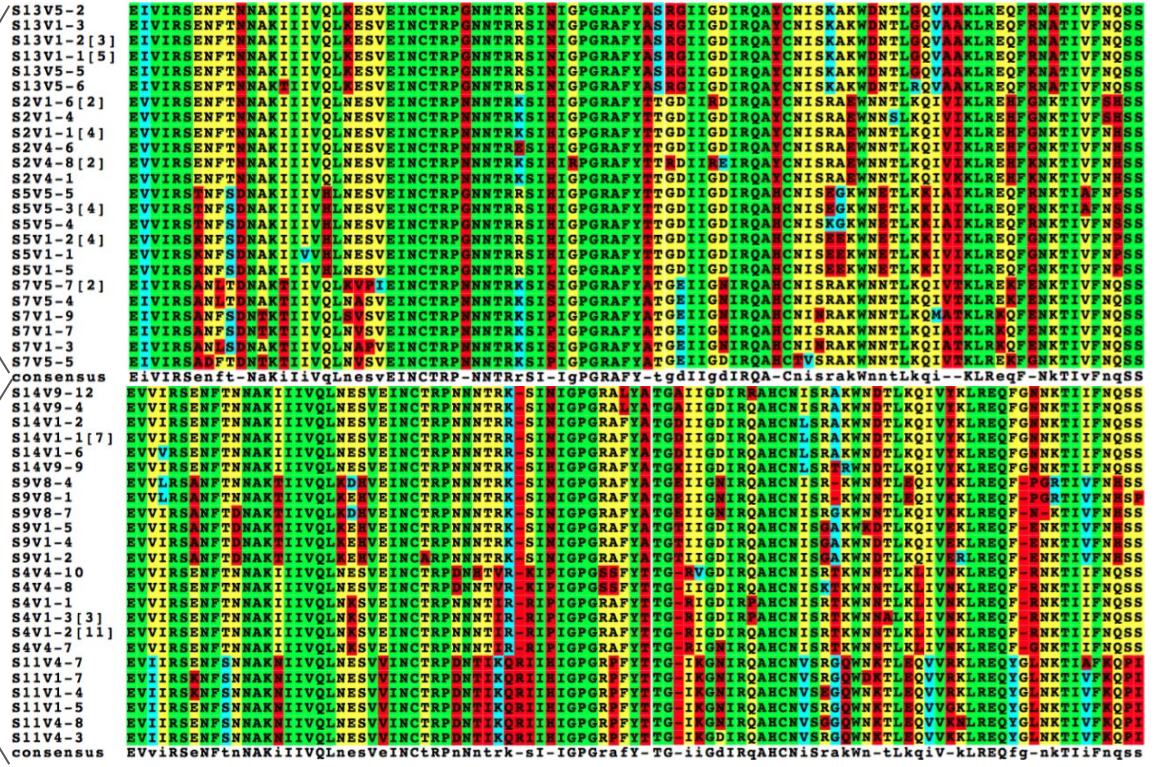
Low Ratio Subjects Had More Non-Consensus Sites than High Ratio Subjects

	High dS/dN Ratio Subjects	Low dS/dN Ratio Subjects	All Subjects
Fully Conserved Residue	49	43	30
Conservation of Strong Group	22	20	23
Conservation of Weak Group	10	6	13
No Consensus	14	24	31

BoxShade Highlights a Higher Frequency of Non-Consensus Sites in Subjects From the High dS/dN Group

High dS/dN Subjects

Low dS/dN Subjects



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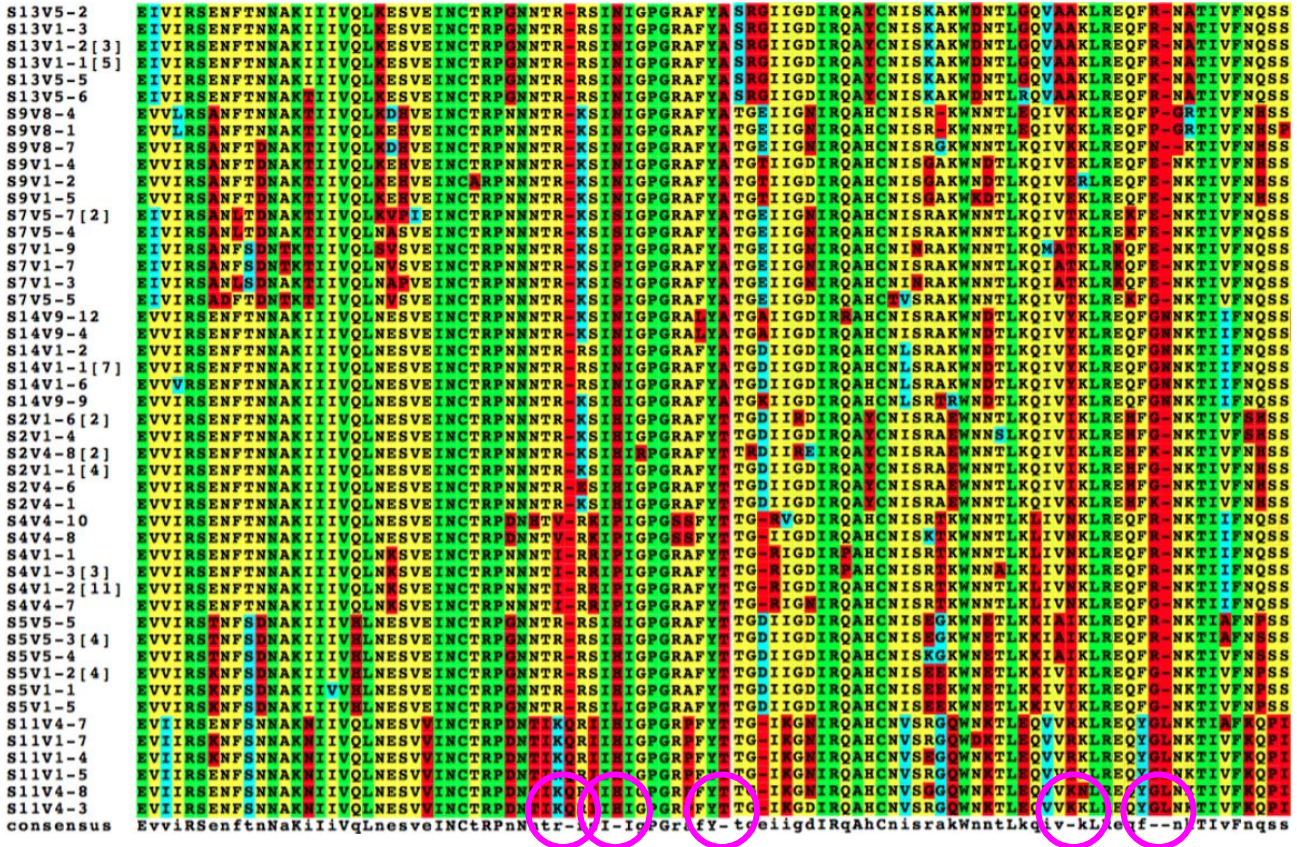
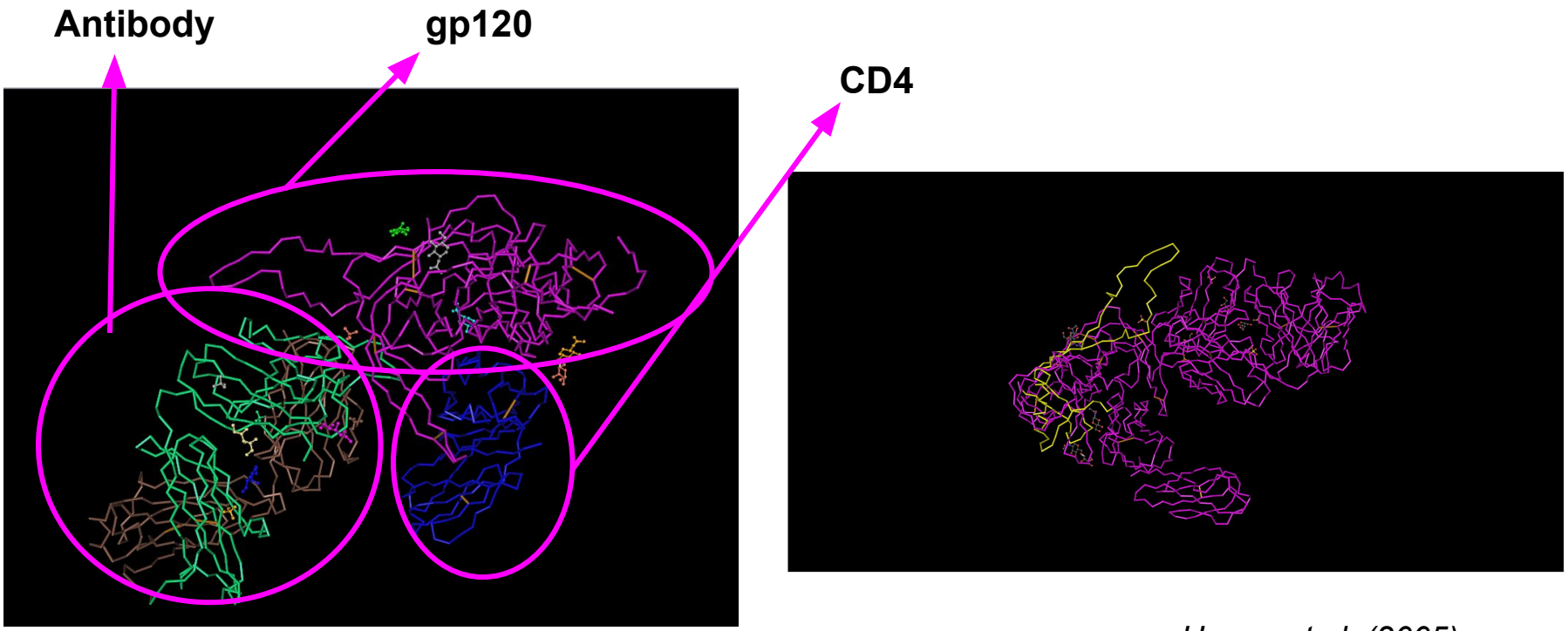
Comparison of all Subjects Show 6 Sites With no Amino Acid Consensus



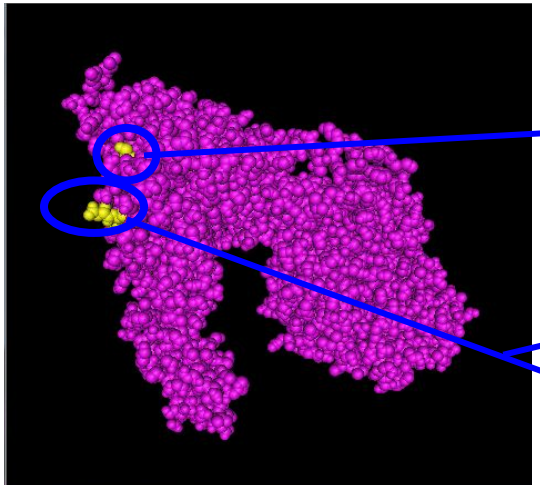
The V3 Region Sticks Out of gp120 Facing the Antibody



Huang et al. (2005)

Three Sites Along the V3 Region Show Variability Along the Outside of the Protein

Key
A: Acidic
B: Basic
P: Polar
NP: Nonpolar



Lysine (B)→ Glycine (NP), Arginine (B), Isoleucine (NP), Asparagine (P), Tyrosine (P), Glutamic Acid (A), Threonine (P), Alanine (NP)

Asparagine (P)→ Glycine (NP), Arginine (B), Lysine (B), Glutamic Acid (A), Proline (NP)

Lysine (B)→ Leucine (NP), Asparagine (P), Insertion

Cn3D macromolecular structure viewer

Three Sites Along the immunogenetic tip of the V3 Region Show High Variability

Lysine (B)→ Glutamine (P) or insertion

Isoleucine (NP)→ Asparagine (P), Proline (NP),
Serine (P), Histidine (B) , Leucine (NP)

Threonine (P)→ Alanine (NP)

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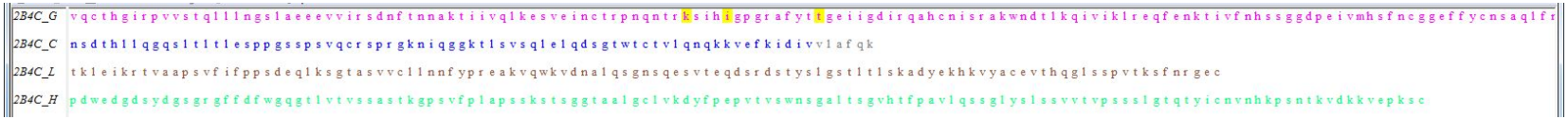
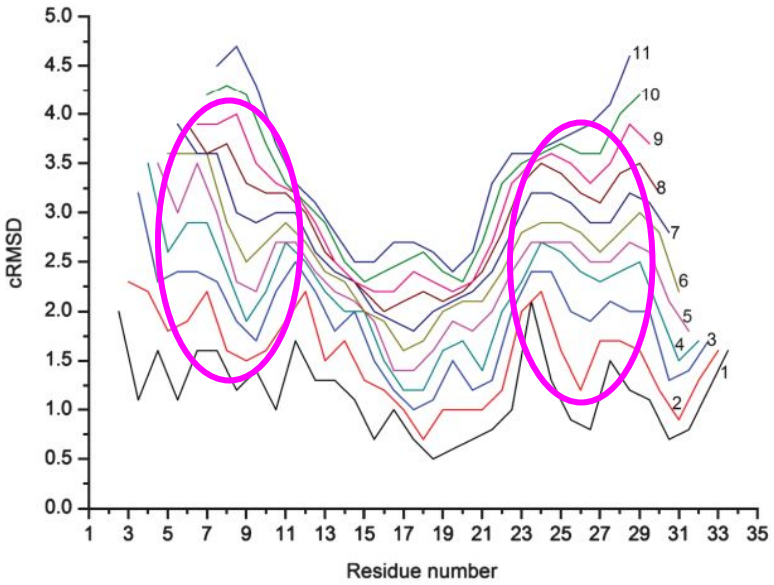
Cn3D macromolecular structure viewer



Non-Consensus Regions of gp120 Match Variable Segments Studied by Andrianov & Anishchenko (2012)

- A 35-residue-long segment of the V3 loop shows high structure variability
- 3 segments along the same V3 region found to be highly variable
- Conservation of amino acid composition found at “pinch” sites are targets for anti-AIDS medication



Low dS/dN Ratios Demonstrate no Clear Relationship to Higher Rates of Non-Consensus Segments Along the V3 Loop

- The sites of high variation result in nonconservative mutations, causing changes in protein structure and function
- Interactions and functions of protein may be affected by variations in primary sequence
- Non-Consensus segments found along V3 loop and on the outside region of gp120 using BoxShade were not consistent with ClustalW

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Functions of Amino Acid Variations Must be Studied to Observe the Effect of Non-Consensus Sites

- Low dS/dN ratio subjects show trend towards significant rates of mutations causing variable amino acid structure using BoxShade
- Specific functions of amino acid substitutions along the V3 loop should be studied
- V3 region should be targeted for anti-AIDS medications due to rigidity in structure, studying variations in structure may prove helpful
- Data can be used to identify HIV-1 immune system evasion

Summary

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Acknowledgements

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References

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Biology Workbench