

Understanding PPI and drug-resistance profiles of HIV-1 protein variants using molecular dynamics



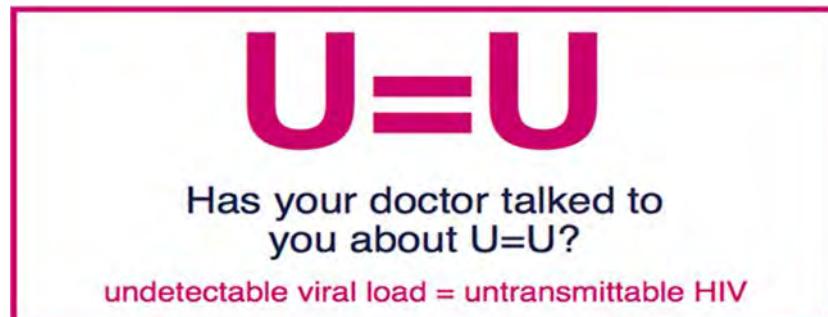
Natasha Wood
Computational Biology Group, UCT
Integrative Biomedical Sciences (IBMS)

HIV Background

- 90-90-90

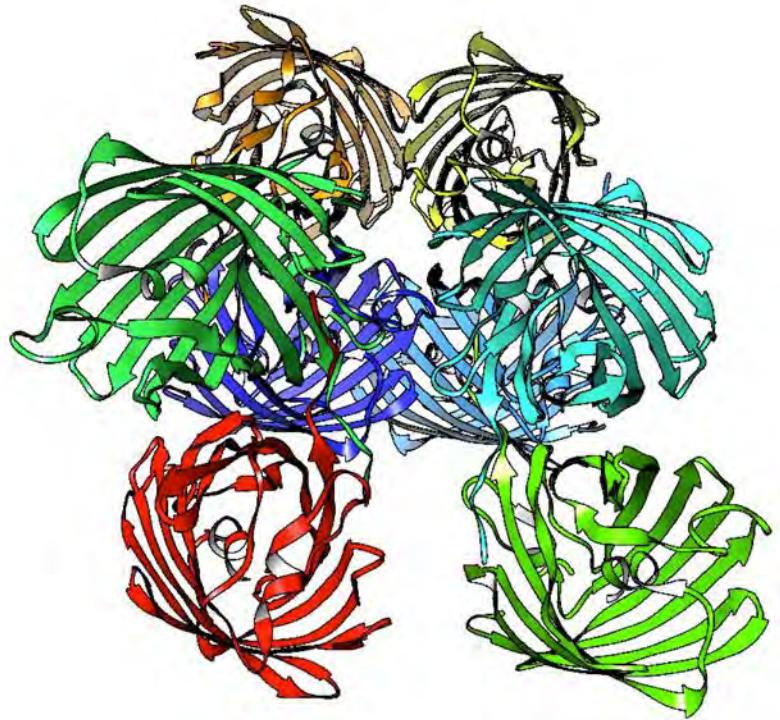


- $U = U$



HIV Background

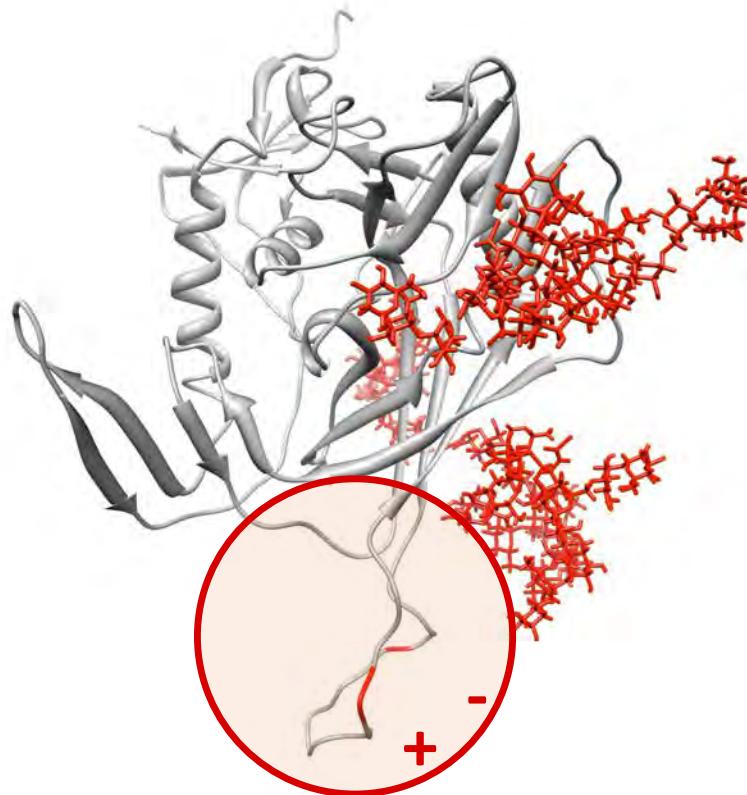
- 39 million people have died of AIDS
- 36.7 million people living with HIV/AIDS at the end of 2016
- 1.8 million people were newly infected with HIV in 2016
 - About 5000 new infections per day
- 2.1 million children worldwide are living with HIV



“You, too, can make your own
protein turn.”

Chemokine Receptors and the V3 loop

- Positions 11 / 24 / 25
- Net Charge
- N-linked Glycosylation



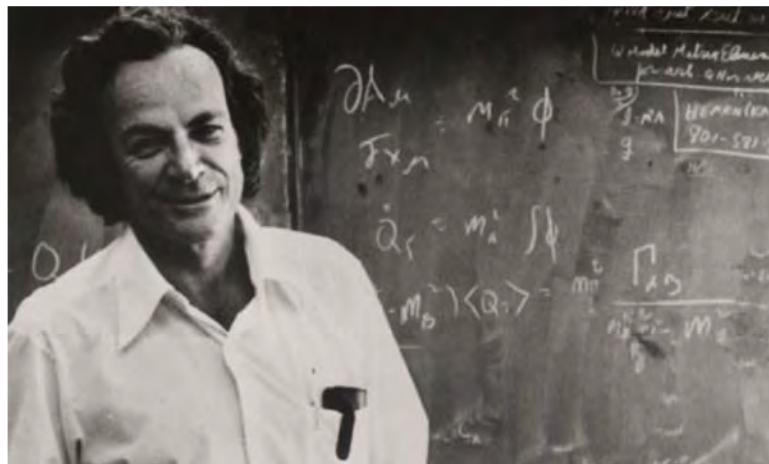




Introduction to Molecular Dynamics

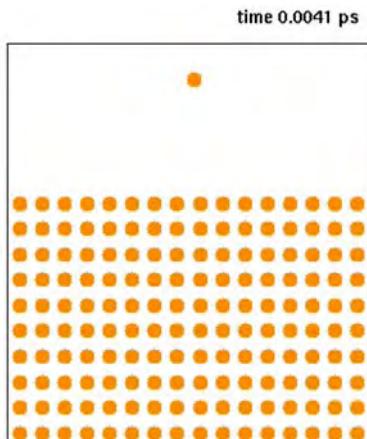
“everything that living things do can be understood in terms of the jiggling and wiggling of atoms.”

The Feynman Lectures in Physics vol. 1, 3-6 (1963)



Introduction to Molecular Dynamics

- Molecular dynamics is a technique for computer simulation of complex systems, modelled at the atomic level.

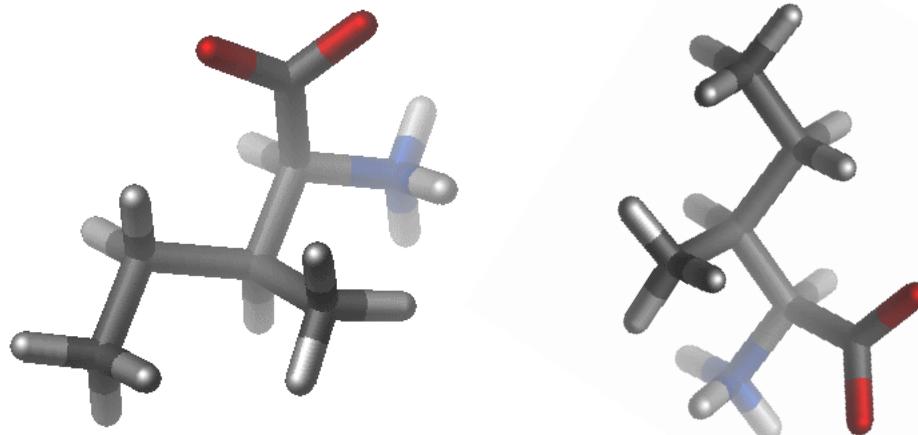


- Example of a molecular dynamics simulation in a simple system: deposition of a single Cu atom on a Cu surface.

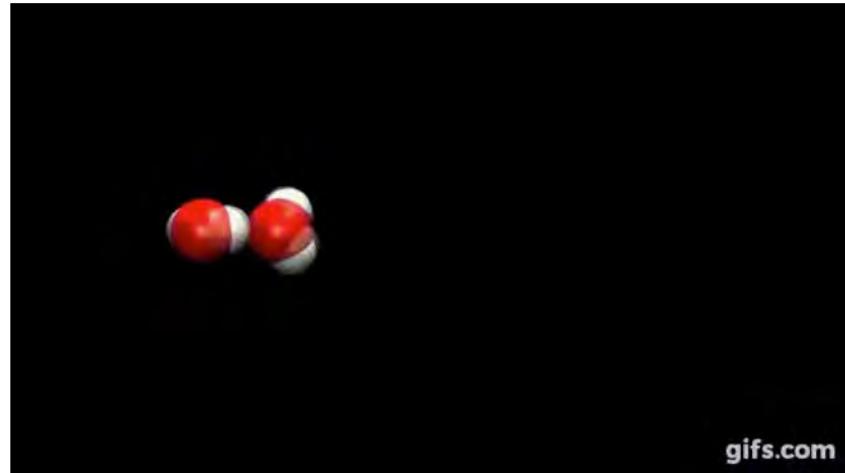
Introduction to Molecular Dynamics

A force field is made up by the contributions of many terms that represent the different types of interactions between the atoms of the protein molecule (energy function)

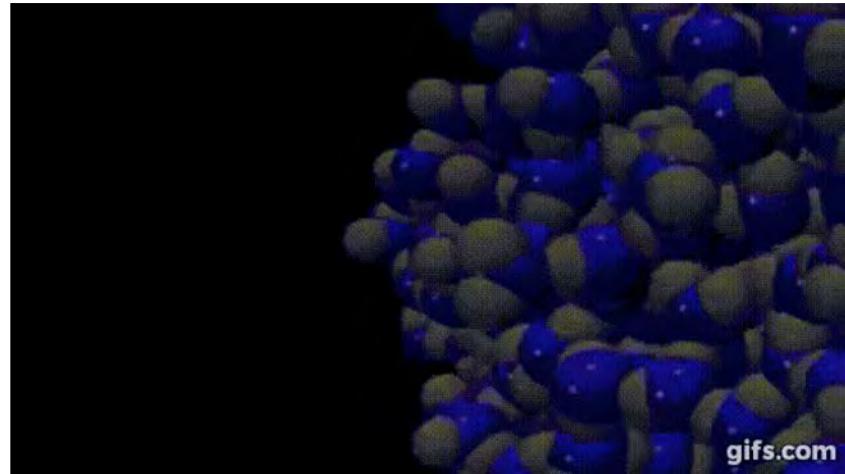
- van der Waals energy
- Electrostatic energy
- Hydrogen bond
- Bond energy
- Bond angle energy
- Dihedral angel energy



Introduction to Molecular Dynamics



Introduction to Molecular Dynamics



Chemokine Receptors and the V3 loop

- Positions 11 / 24 / 25
- Net Charge
- N-linked Glycosylation



Background

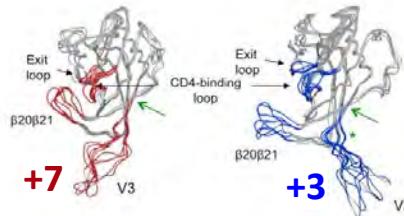
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PLOS ONE

Structural Dynamics of HIV-1 Envelope Gp120 Outer Domain with V3 Loop

Masaru Yokoyama^{1*}, Satoshi Naganawa², Kazuhisa Yoshimura³, Shuzo Matsushita³, Hironori Sato^{1*}

¹ Laboratory of Viral Genomics, Pathogen Genomics Center, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashi Murayama-shi, Tokyo, Japan, ² Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo, Japan, ³ Division of Clinical Retrovirology and Infectious Diseases, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto, Japan



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PLOS ONE

Insights into the Structure, Correlated Motions, and Electrostatic Properties of Two HIV-1 gp120 V3 Loops

Aliana López de Victoria^{1*}, Phanourios Tamamis¹, Chris A. Kieslich, Dimitrios Morikis¹

Department of Bioengineering, University of California Riverside, Riverside, California, United States of America



HIV-1 gp120 structures (2B4C)

Non-Glycosylated



Glycosylated^{5-glycans}



Non-Glycosylated and Glycosylated

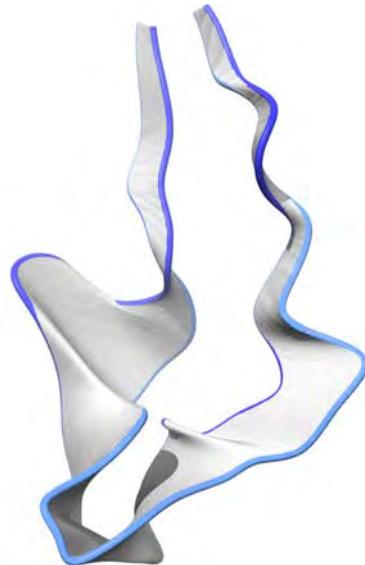
- MD Simulation, 30ns



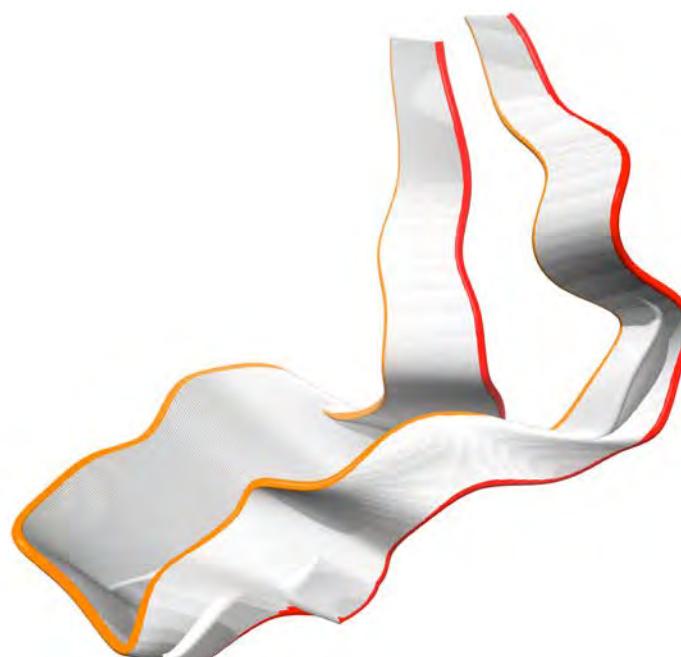
V3-loop Movement

- Principle Component Analysis

Non-Glycosylated



Glycosylated ^{5-glycans}



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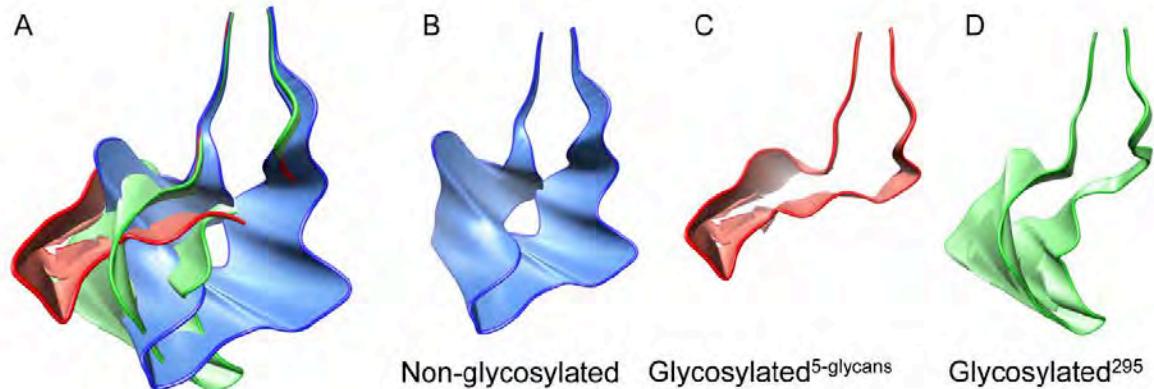
PLOS ONE

The Influence of N-Linked Glycans on the Molecular Dynamics of the HIV-1 gp120 V3 Loop

Natasha T. Wood¹, Elisa Fadda², Robert Davis³, Oliver C. Grant⁴, Joanne C. Martin⁴, Robert J. Woods^{3,4}, Simon A. Travers^{1*}

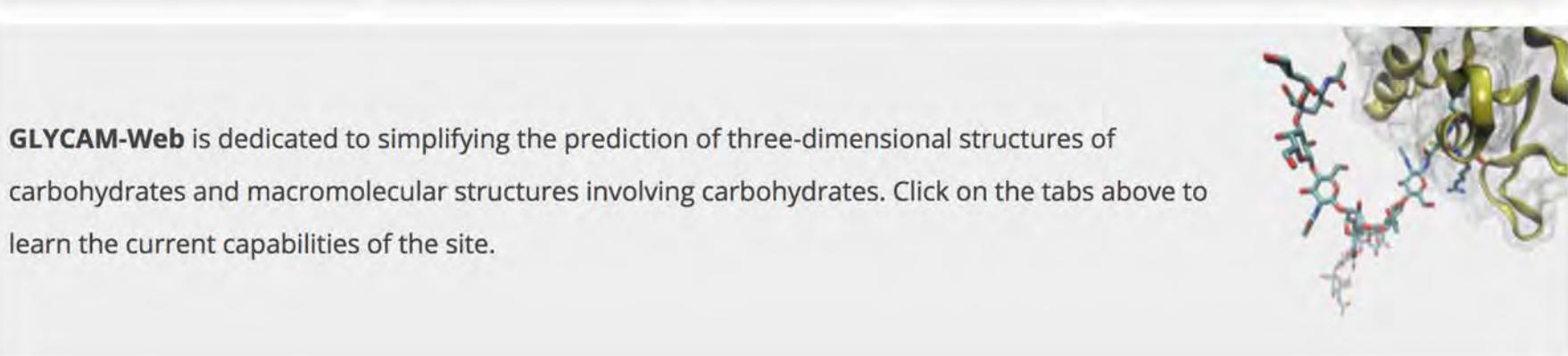
¹ South African National Bioinformatics Institute, South African Medical Research Council Bioinformatics Unit, University of the Western Cape, Cape Town, South Africa,

² Department of Chemistry, National University of Ireland, Maynooth, Maynooth, Ireland, ³Complex Carbohydrate Research Centre, University of Georgia, Athens, Georgia, United States of America, ⁴School of Chemistry, National University of Ireland, Galway, Galway, Ireland



- Monomer
- Glycosylation
- Glycan type

Glycosylation

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Currently available tools:

[Carbohydrate
Builder](#)[Glycoprotein
Builder](#)[Oligosaccharide
Libraries](#)[PDB
Preprocessor](#)[Build via Text](#)[Build via URL](#)[GAG Builder](#)[Auto Docking](#)[Gly-Spec
\(Grafting\)](#)[Carbohydrate
Visualization](#)

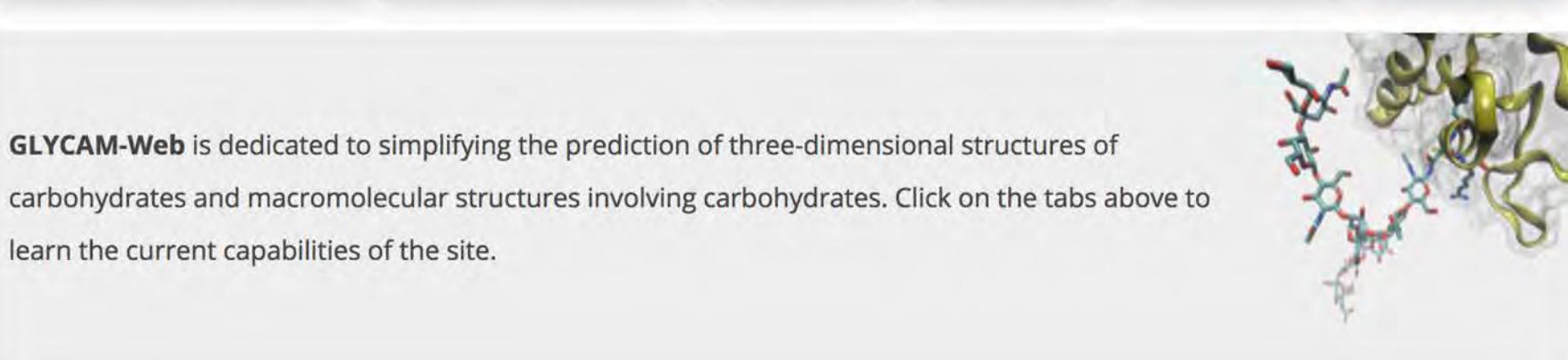
Coming Soon!

* Glyco-
Complex
Processor

* GEMS * GMML

<http://glycam.org/>

Glycosylation

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Currently available tools:

[Carbohydrate Builder](#)[Glycoprotein Builder](#)[Oligosaccharide Libraries](#)[PDB Preprocessor](#)[Build via Text](#)[Build via URL](#)[GAG Builder](#)[Auto Docking](#)[Gly-Spec \(Grafting\)](#)[Carbohydrate Visualization](#)

Coming Soon!

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Processor

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<http://glycam.org/>

Glycosylation

GLYCAM

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What glycans bind to my protein?



Scenario
[Predict ligands \(I have a co-complex\)](#)
[Predict ligands \(I don't have a co-complex\)](#)

How does my glycan bind to its antibody?



Scenario
[Predict antibody binding](#)
 Determine the important residues (I have a co-complex)
 Determine the important residues (I don't have a co-complex)

What are the shapes of my glycan?



Scenario
[Generate 3D structure](#)
 Generate files for crystallography
[Calculate NMR observables](#)
[Generate files for an MD simulation](#)
[Let Glycam-Web perform my MD simulation](#)

What are the NMR properties of my glycan?



Scenario
[Calculate NMR observables](#)
 Generate files for an MD simulation

How does glycosylation affect my protein?



Scenario
 I don't have a 3D protein structure (Suggest Modeler)
[Predict 3D structure of glycoprotein](#)
[Predict shielding by glycans](#)
[Calculate antibody accessibility](#)

Search for glycans in the PDB.



Scenario
[glycoPDB search](#)

Check the quality of my 3D structure.

Glycosylation

Potential N-linked
glycosylation sites
(PNGS)

-----NXT|S-----

25-30 per monomer

Glycosylation

RESEARCH ARTICLE

Exploiting glycan topography for computational design of Env glycoprotein antigenicity

Wen-Han Yu^{1,2*}, Peng Zhao^{3*}, Monia Draghi^{1*}, Claudia Arevalo¹, Christina B. Karsten¹, Todd J. Suscovich¹, Bronwyn Gunn¹, Hendrik Streeck⁴, Abraham L. Brass⁵, Michael Tiemeyer³, Michael Seaman⁶, John R. Mascola⁷, Lance Wells^{3*}, Douglas A. Lauffenburger^{1,2*}, Galit Alter^{1*}

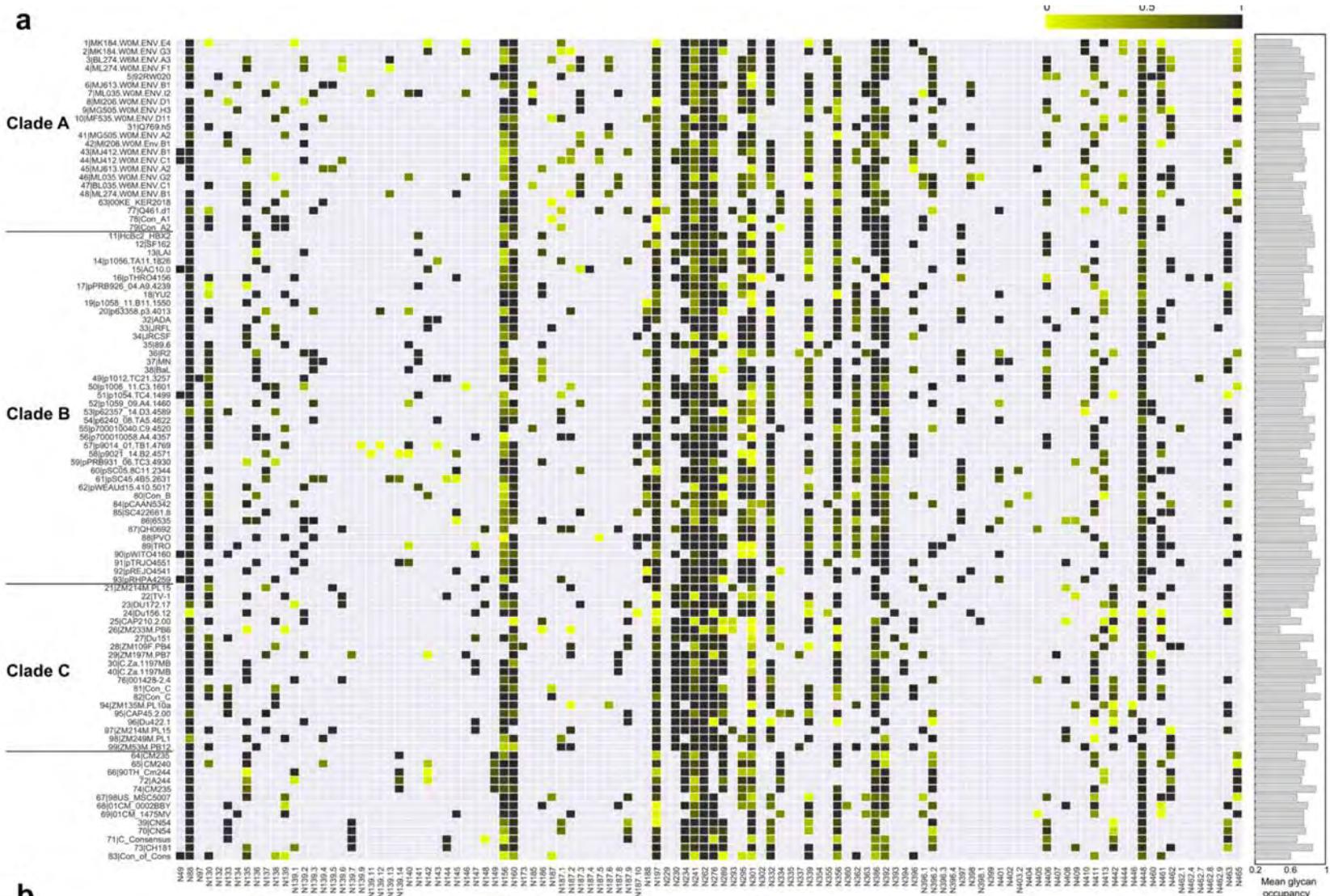
1 Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, Cambridge, MA, United States of America, **2** Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States of America, **3** Complex Carbohydrate Research Center, Department of Biochemistry and Molecular Biology, The University of Georgia, Athens, Georgia, United States of America, **4** Institute for HIV Research, University Hospital Essen, University Duisburg-Essen, Essen, Germany, **5** Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA, United States of America, **6** Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States of America, **7** Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States of America

* These authors contributed equally to this work.

* galter@mgh.harvard.edu (GA); lauffen@mit.edu (DAL); lwells@ccrc.uga.edu (LW)



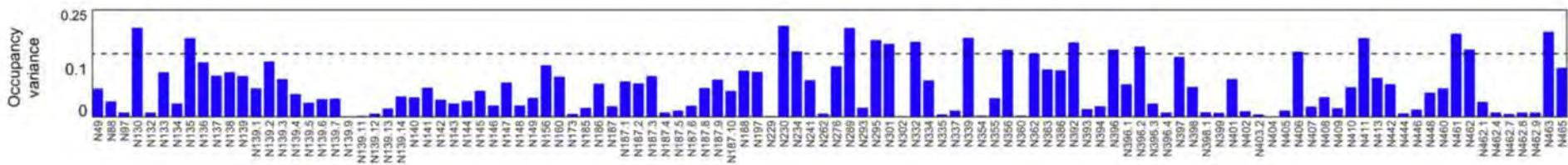
Glycosylation



Glycosylation

Potential N-linked glycosylation sites (PNGS)

-----NXT|S-----



Understanding PPI and drug-resistance profiles of HIV-1 protein variants using molecular dynamics

HIV-1 Env and escape from Abs

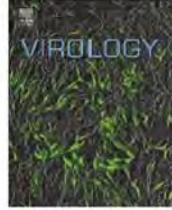
Virology 501 (2017) 12–24

 ELSEVIER

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Virology

journal homepage: www.elsevier.com/locate/yviro



Chinks in the armor of the HIV-1 Envelope glycan shield: Implications for immune escape from anti-glycan broadly neutralizing antibodies

Thandeka Moyo^{a,b}, Roux-Cil Ferreira^c, Reyaaz Davids^{a,b}, Zarinah Sonday^a, Penny L. Moore^d, Simon A. Travers^c, Natasha T. Wood^e, Jeffrey R. Dorfman^{a,b,*}

^a International Centre for Genetic Engineering and Biotechnology, Cape Town, South Africa
^b Division of Immunology, Department of Pathology, University of Cape Town, Cape Town, South Africa
^c South African National Bioinformatics Institute, University of the Western Cape, Cape Town, South Africa
^d University of the Witwatersrand and National Institute for Communicable Disease, Johannesburg, South Africa
^e Computational Biology Division, Department of Integrative Biomedical Sciences, University of Cape Town, Cape Town, South Africa

 CrossMark

Background

CAP45



Du156



Background

CAP45

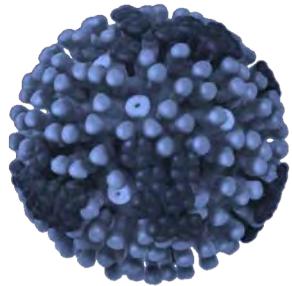


Du156



Remove
N301

CAP45^{N301}



Du156^{N301}



Background

CAP45



Du156



Remove
N301

CAP45^{N301}



Du156^{N301}



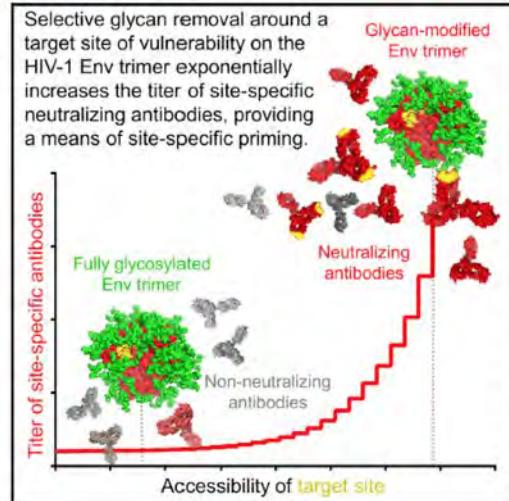
Glycan Deletion = Hole

Cell Reports

Article

Quantification of the Impact of the HIV-1-Glycan Shield on Antibody Elicitation

Graphical Abstract



Authors

Tongqing Zhou, Nicole A. Doria-Rose,
Cheng Cheng, ..., Adrian B. McDermott,
John R. Mascola, Peter D. Kwong

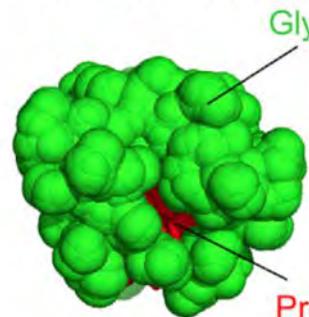
Correspondence

jmascola@nih.gov (J.R.M.),
pdkwong@nih.gov (P.D.K.)

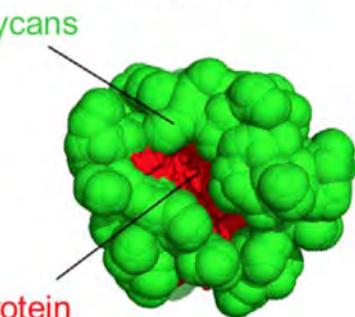
In Brief

Zhou et al. engineer diverse HIV-Env trimers with selective glycans removed around the CD4-receptor-binding site. They demonstrate the impact of the glycan shield on immunogenicity, uncover an exponential relationship between exposed antibody-accessible protein surface and Env immunogenicity, and achieve vaccine-elicited neutralization of glycan-deleted viruses.

Wild-type Env trimer



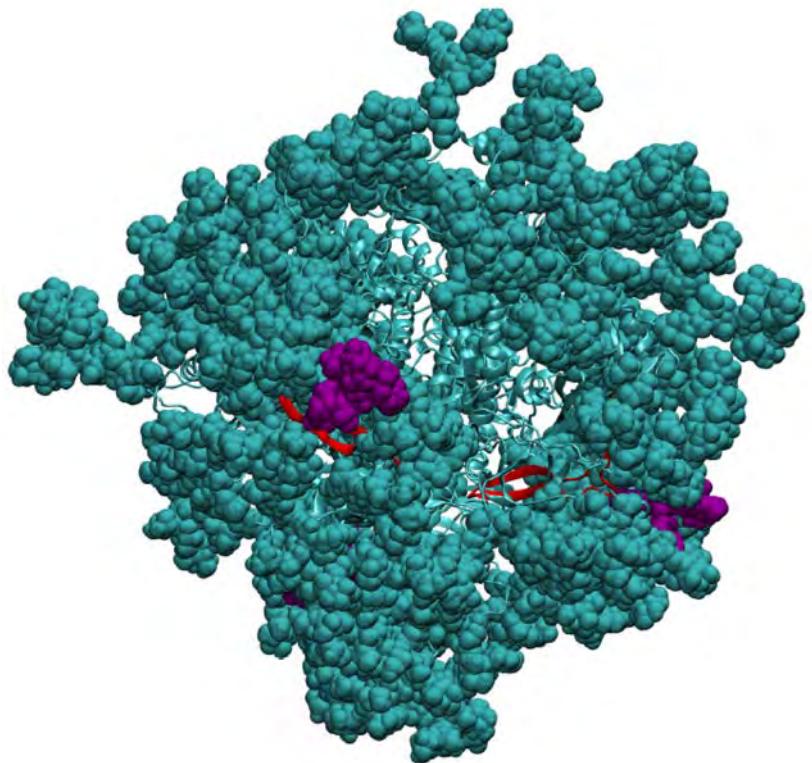
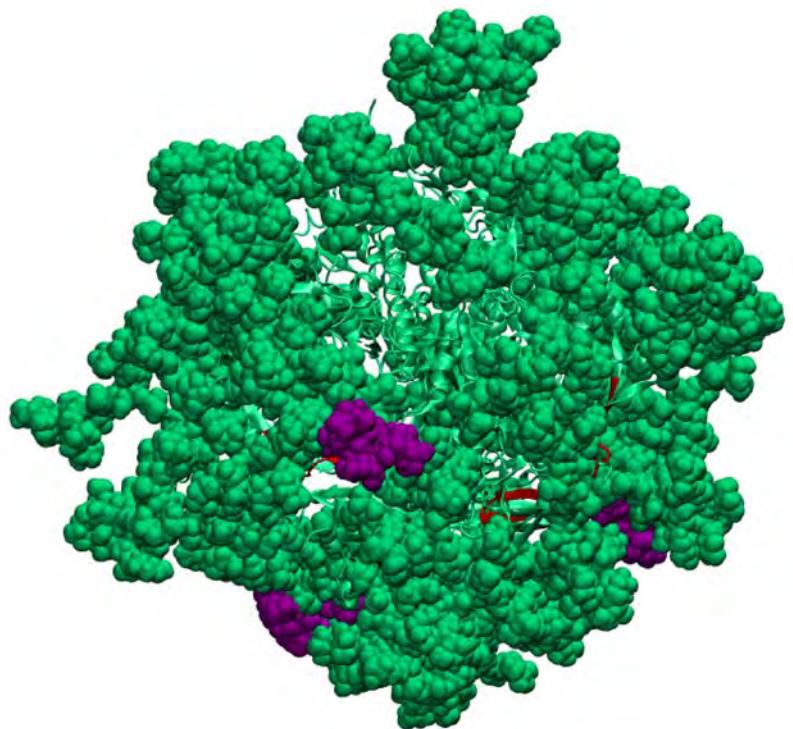
4-glycan-deleted



Glycans

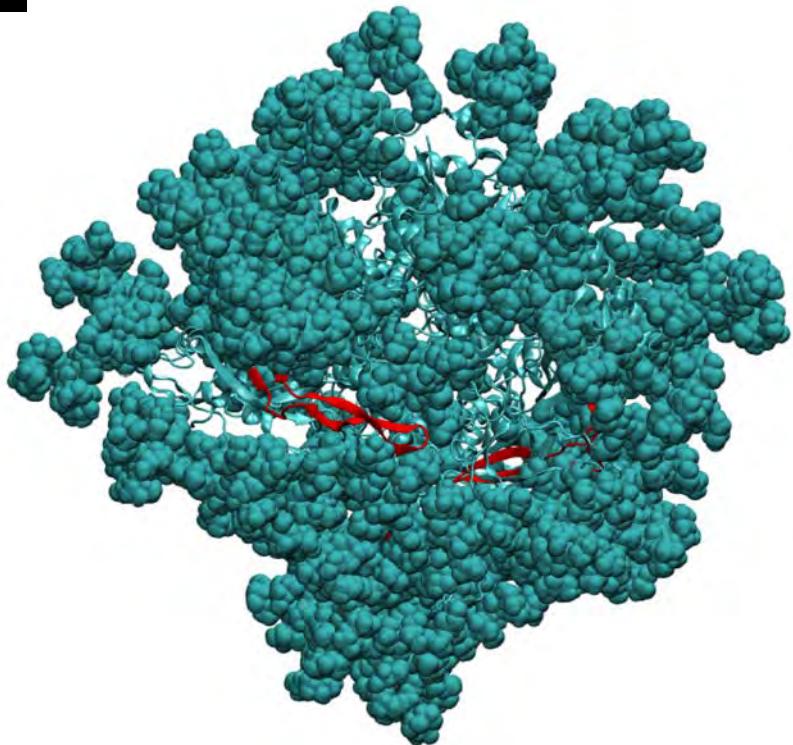
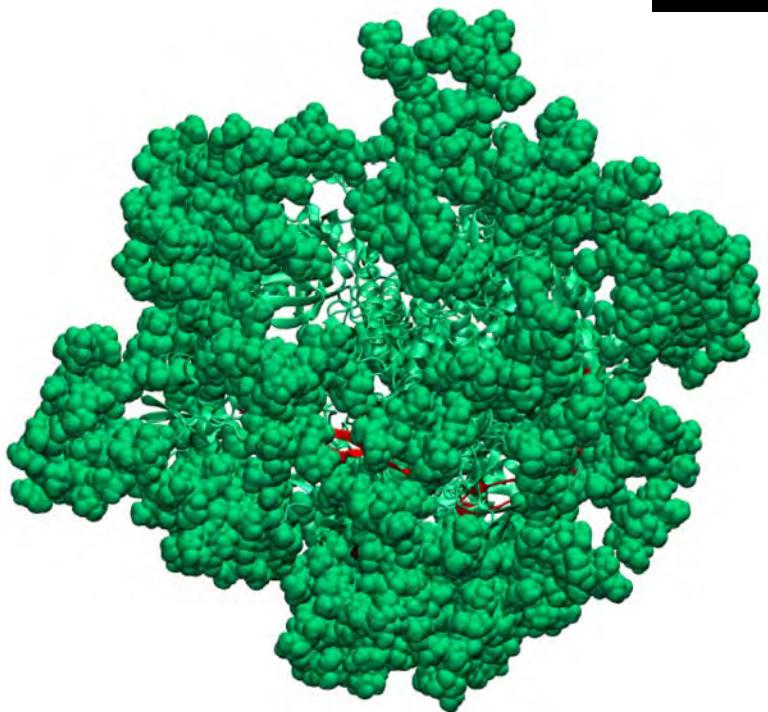
Protein surface

Glycosylated trimers



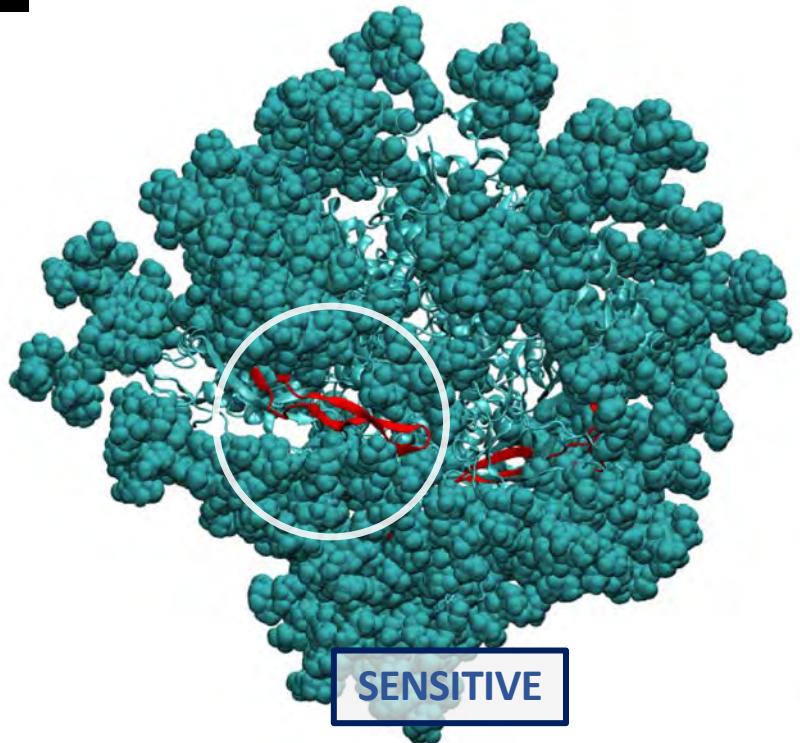
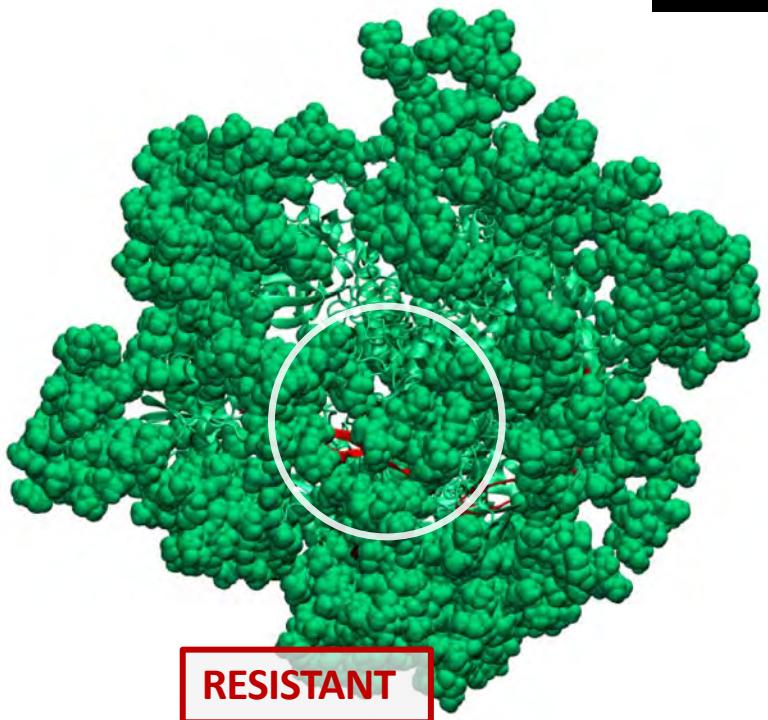
Glycosylated trimers

**Remove
N301**



Glycosylated trimers

Remove
N301



Samples – PNGS comparison

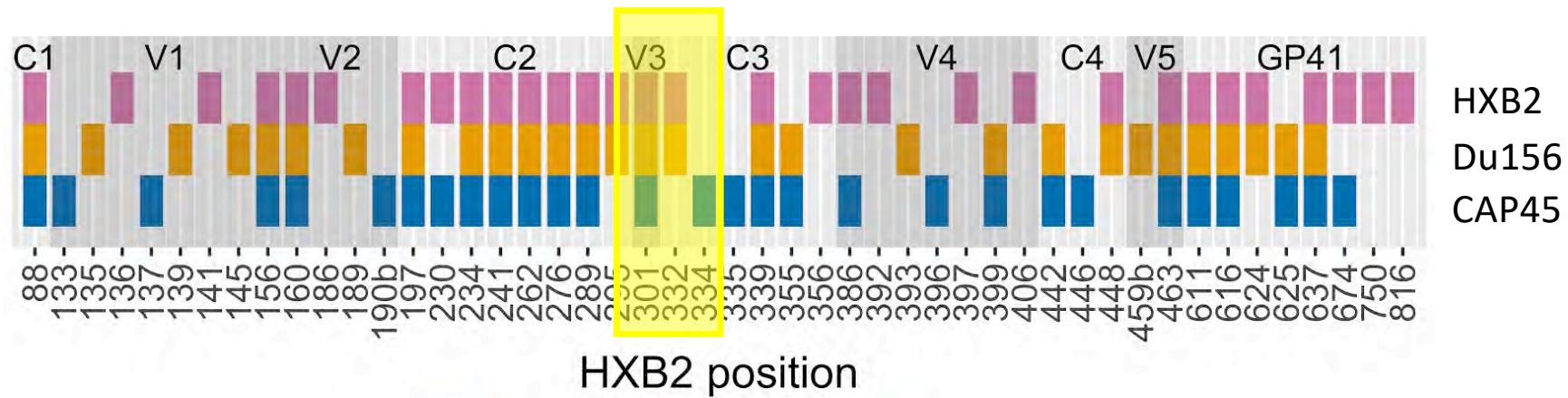
N X S/T

(X any amino acid apart from Pro)

Samples – PNGS comparison

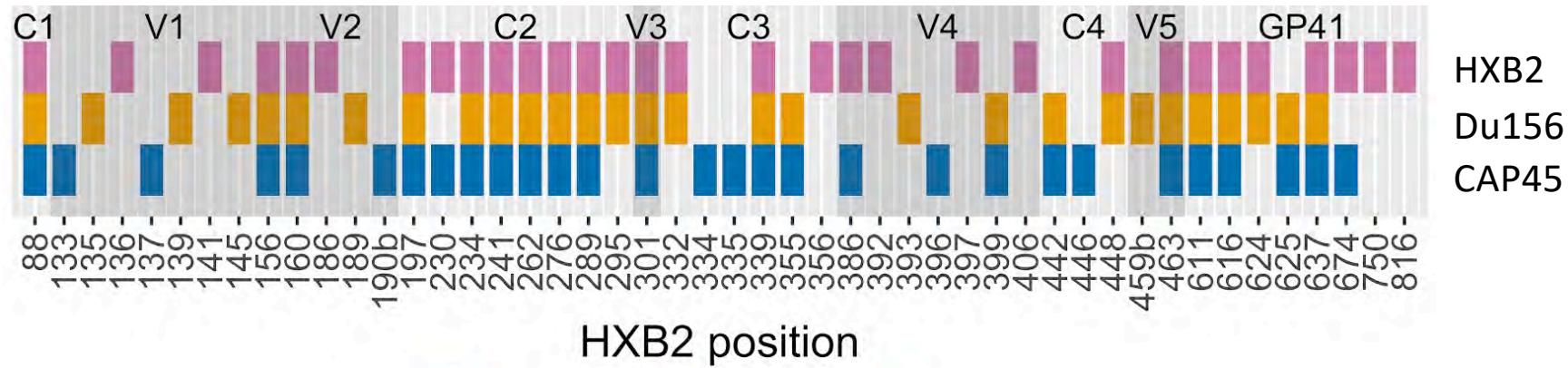
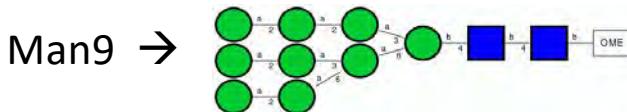
N X S/T

(X any amino acid apart from Pro)



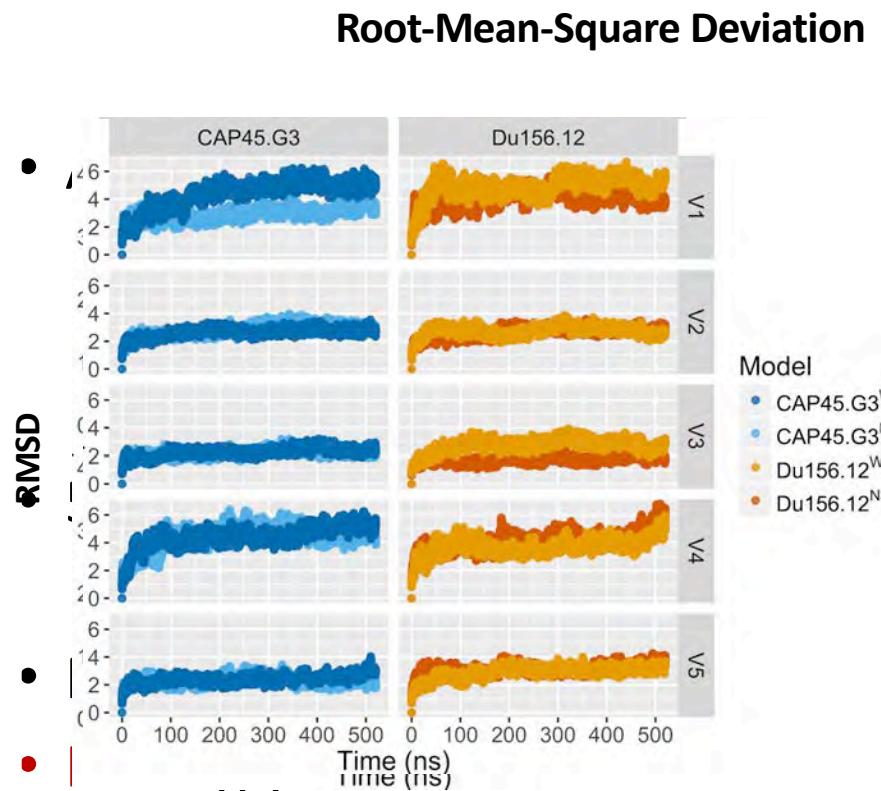
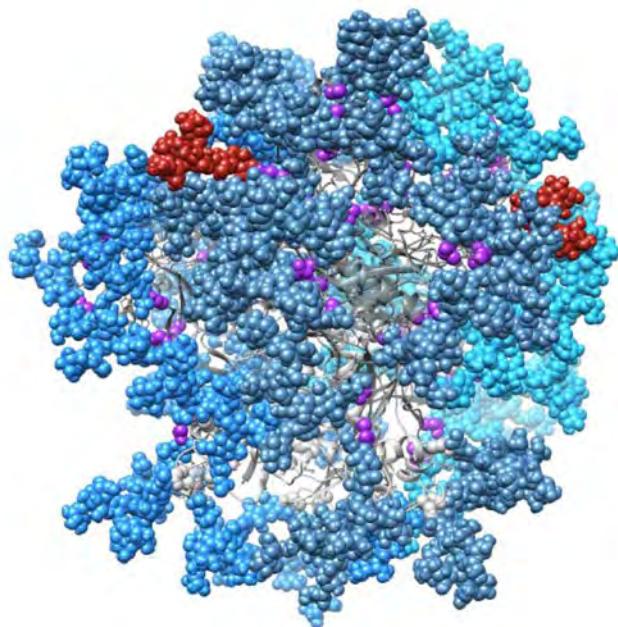
- 90% sequence identity
- 29 PNGS's

After modelling



- CAP45: 160C and 399B, and 335 on all 3 monomers
 - Du156: 160B, and 339 and 393 on all 3 monomers

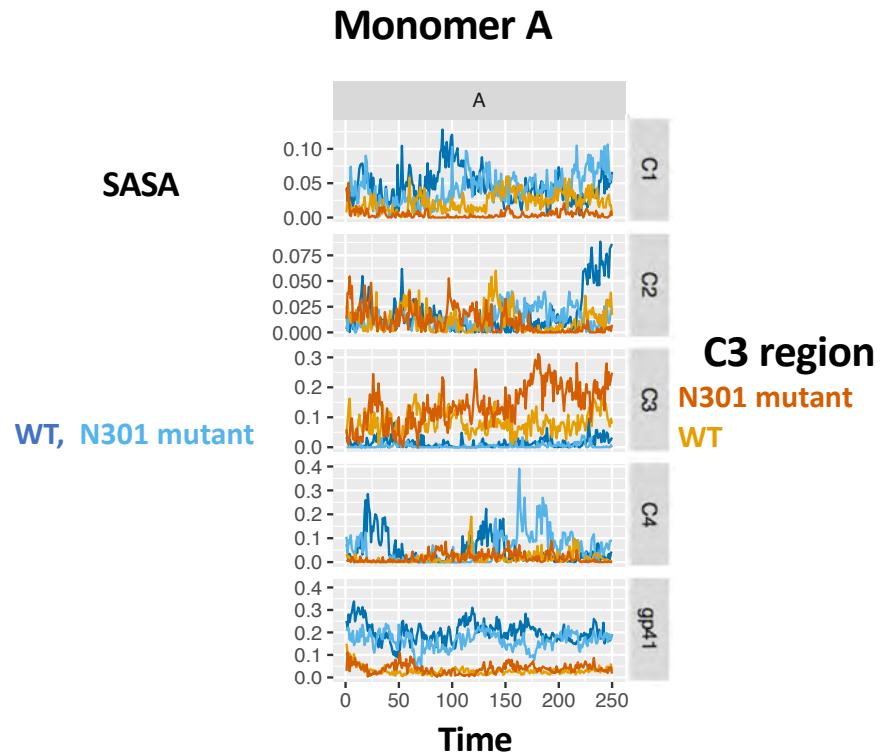
MD Simulation



Solvent Accessible Surface Area (SASA)

- Divide protein into different sections, eg C1, C2 etc
- Define the total SASA per area as the SASA with no glycans
- Then add the glycans back
- Calculate the change in SASA per area
- Do this for each time point
- For a 10Å probe

Solvent Accessible Surface Area (SASA)

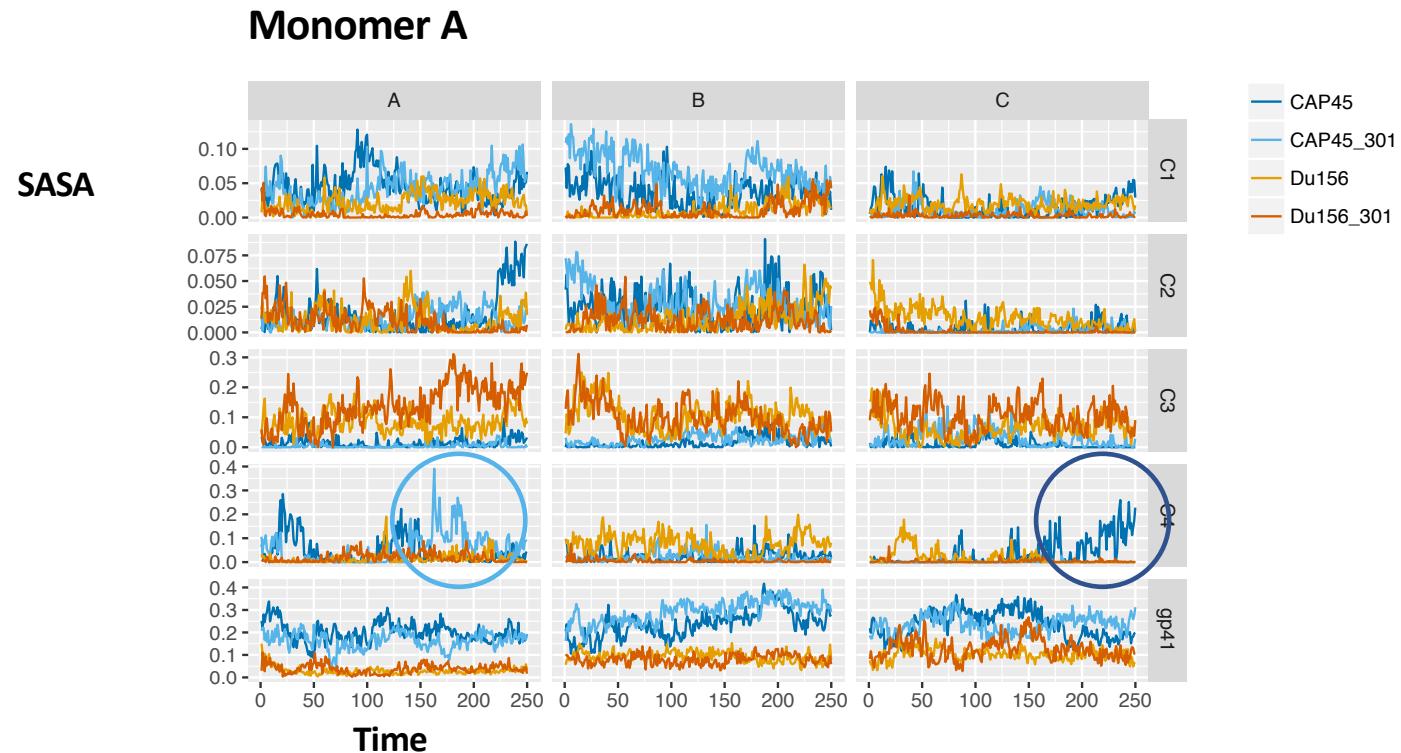


No change in SASA between WT and mutant

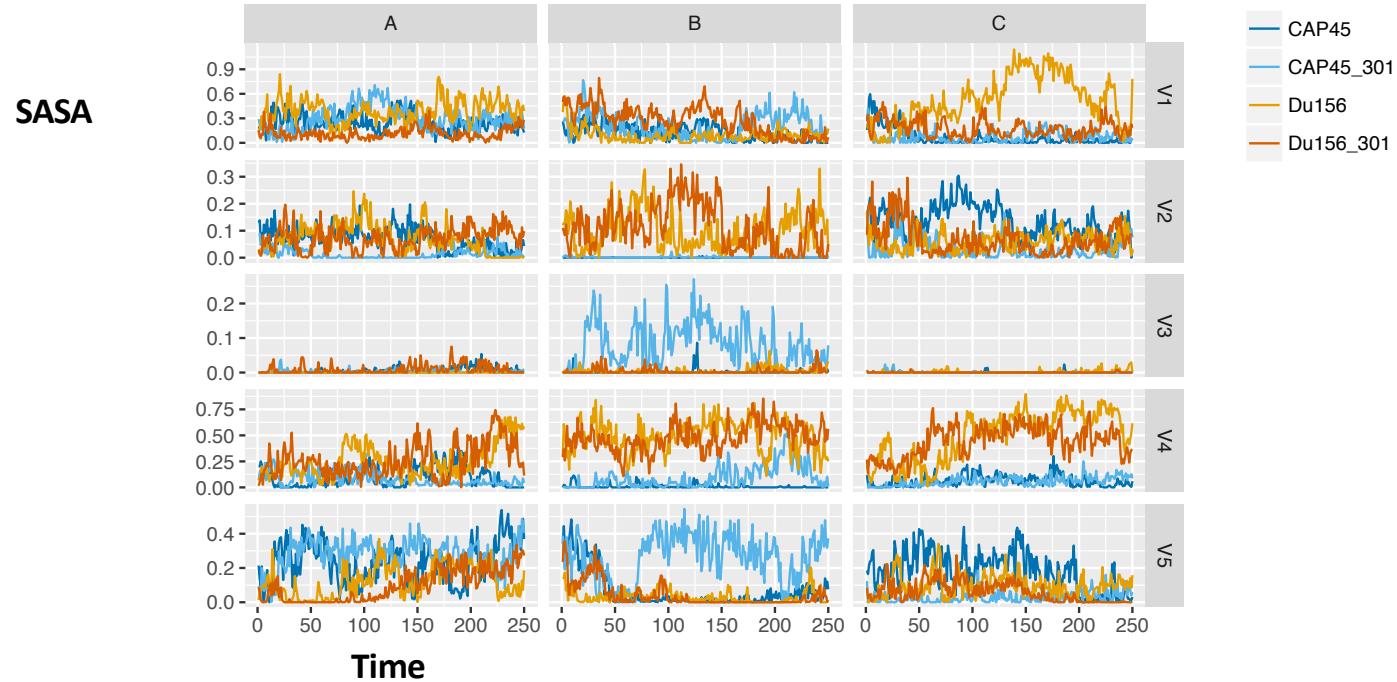


Some variation in SASA between WT and Mutant

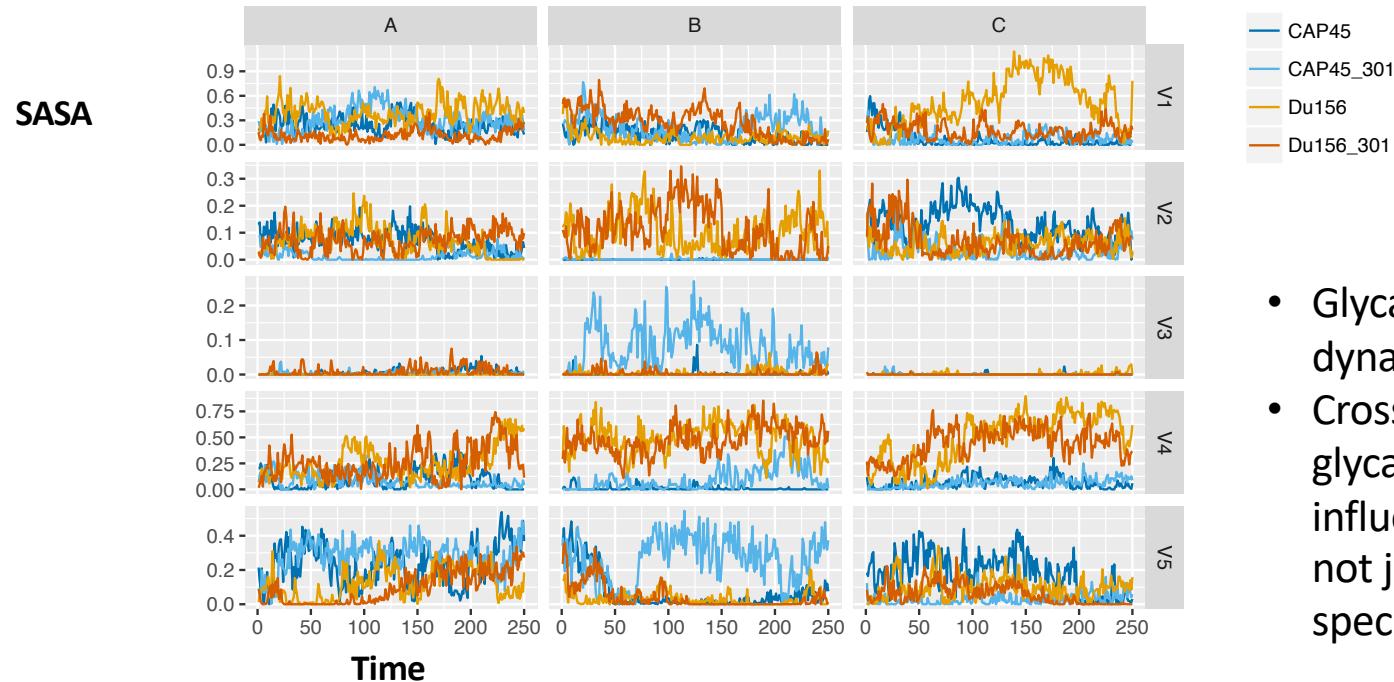
Solvent Accessible Surface Area (SASA)



Solvent Accessible Surface Area (SASA)

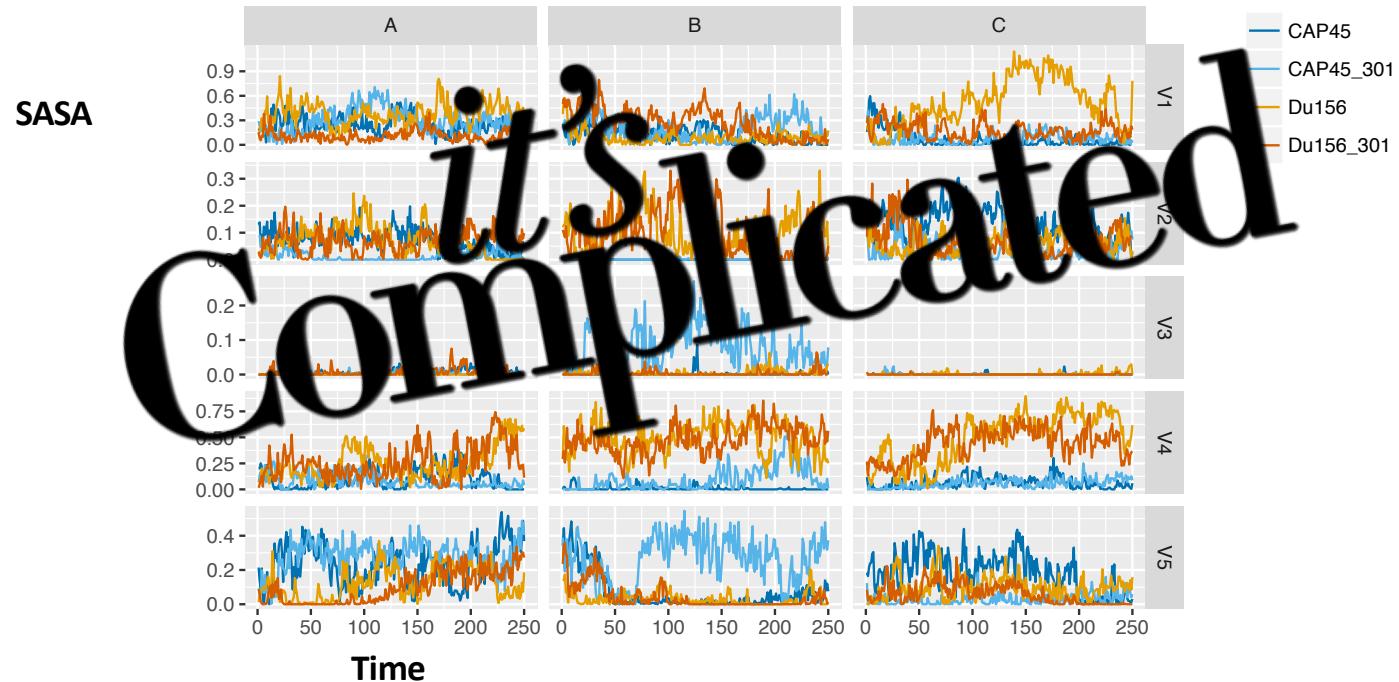


Solvent Accessible Surface Area (SASA)

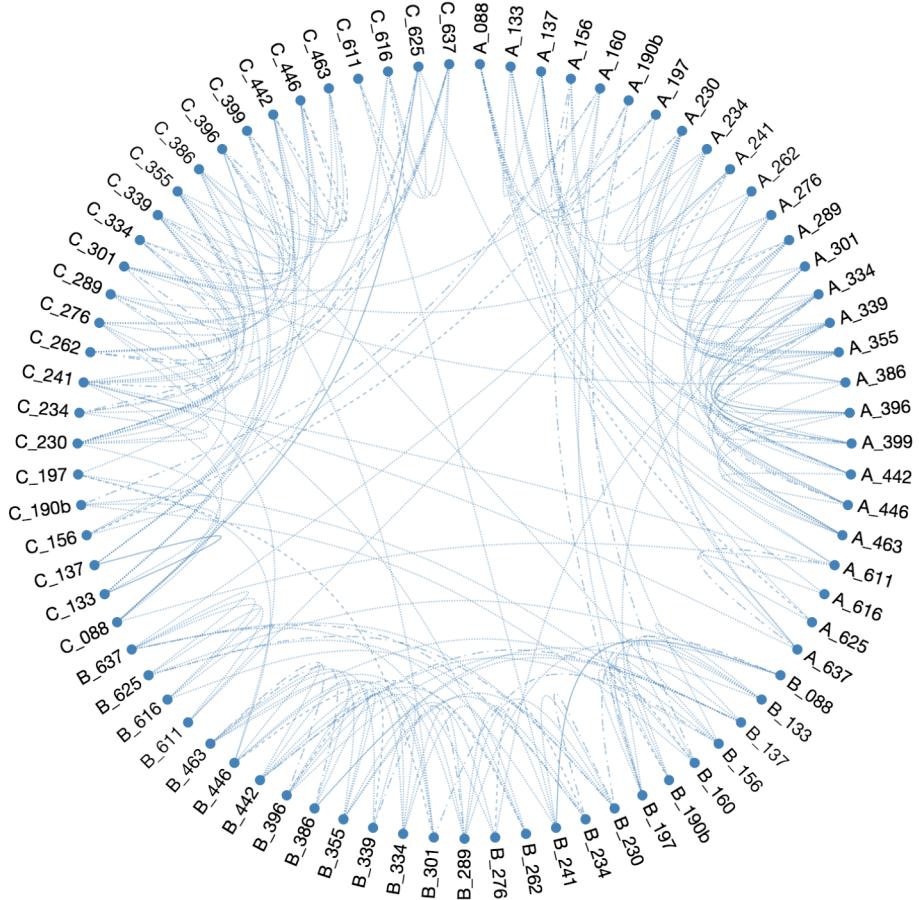
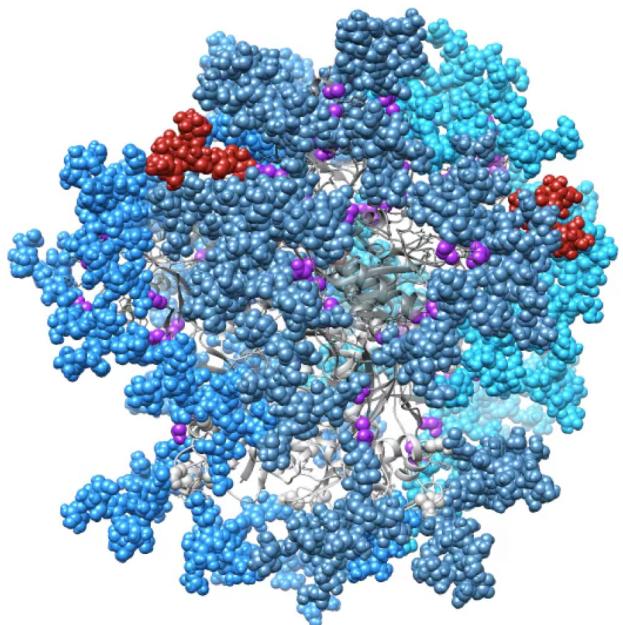


- Glycans are very dynamic
- Cross-monomer glycan dynamics influence SASA, not just glycans in specific sections

Solvent Accessible Surface Area (SASA)

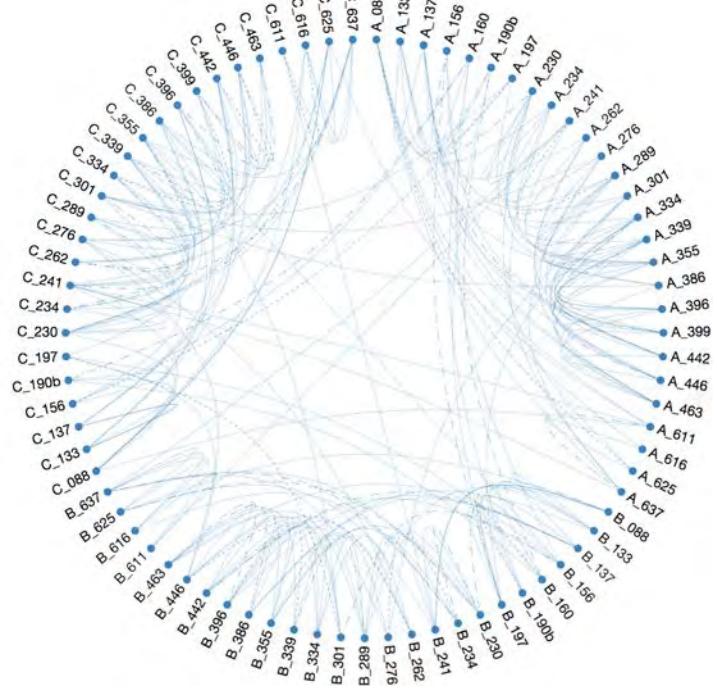


MD Simulation: H-bond Analysis

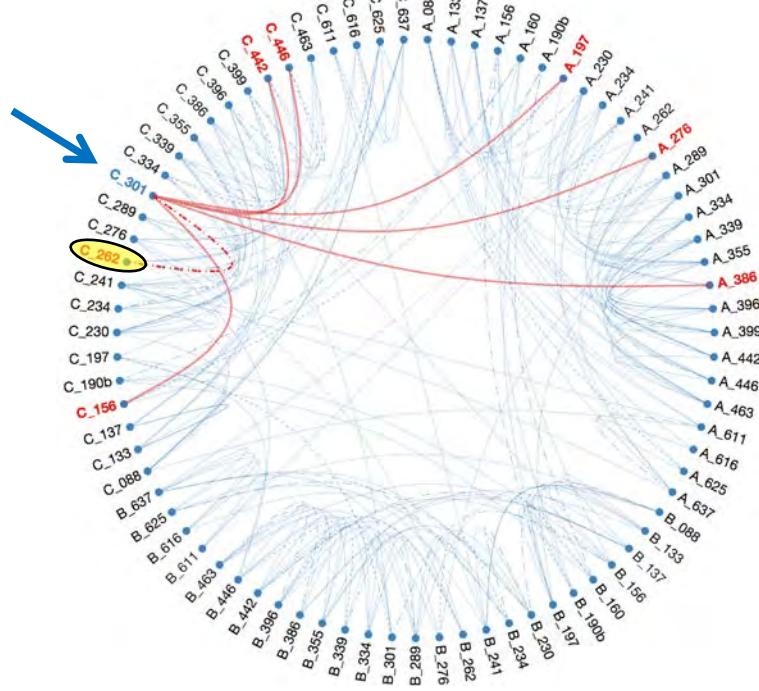


H-bond interactions

CAP45

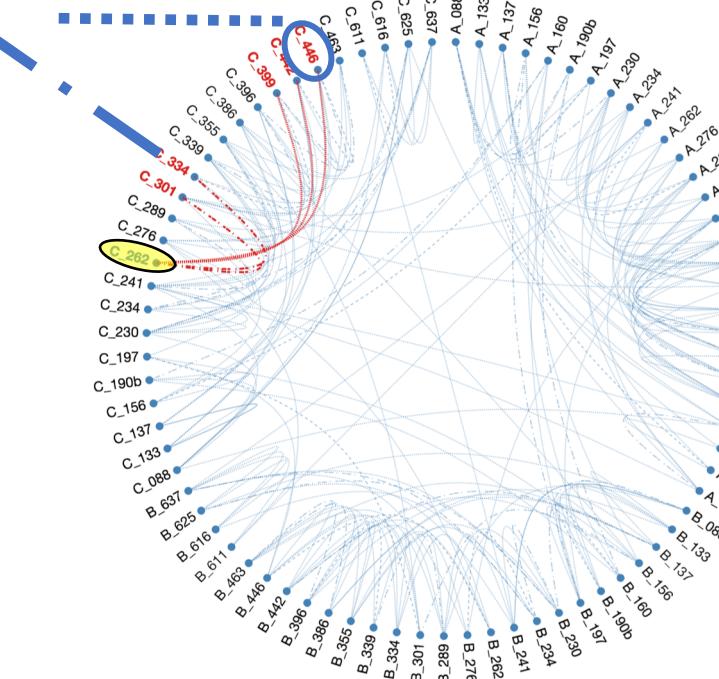


CAP45: Monomer C: N301

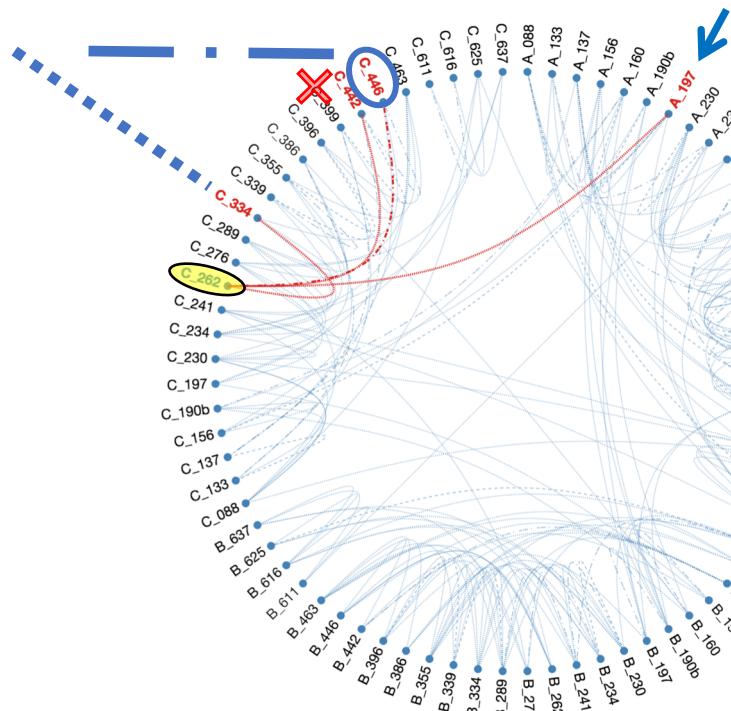


H-bond interactions

CAP45: C _ **N301 – N262**

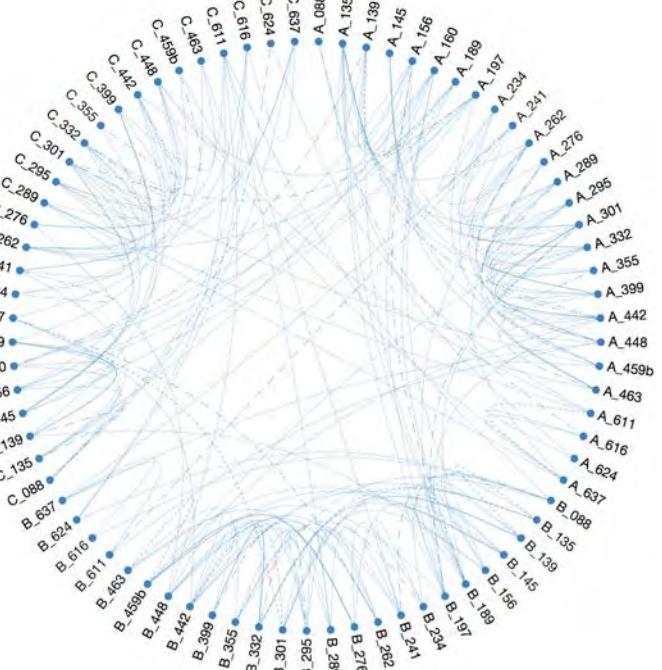


CAP45^{N301}: C _ **N262**

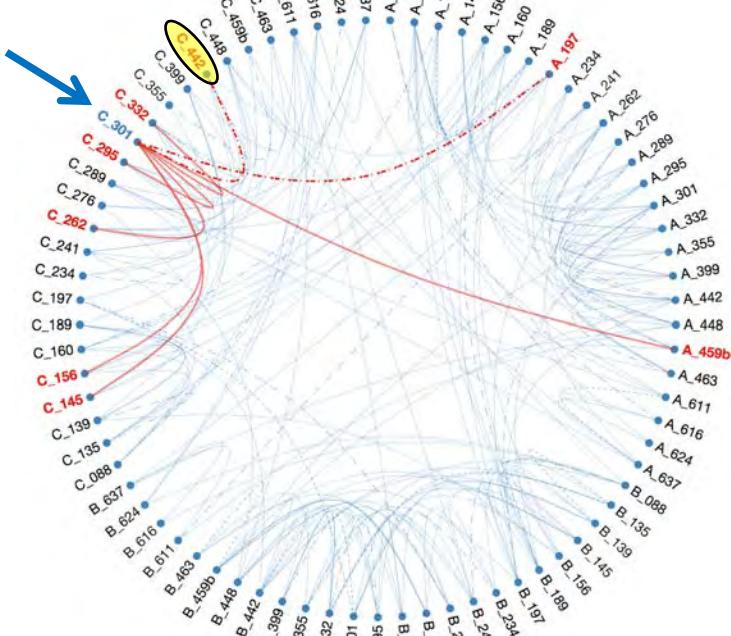


H-bond interactions

Du156

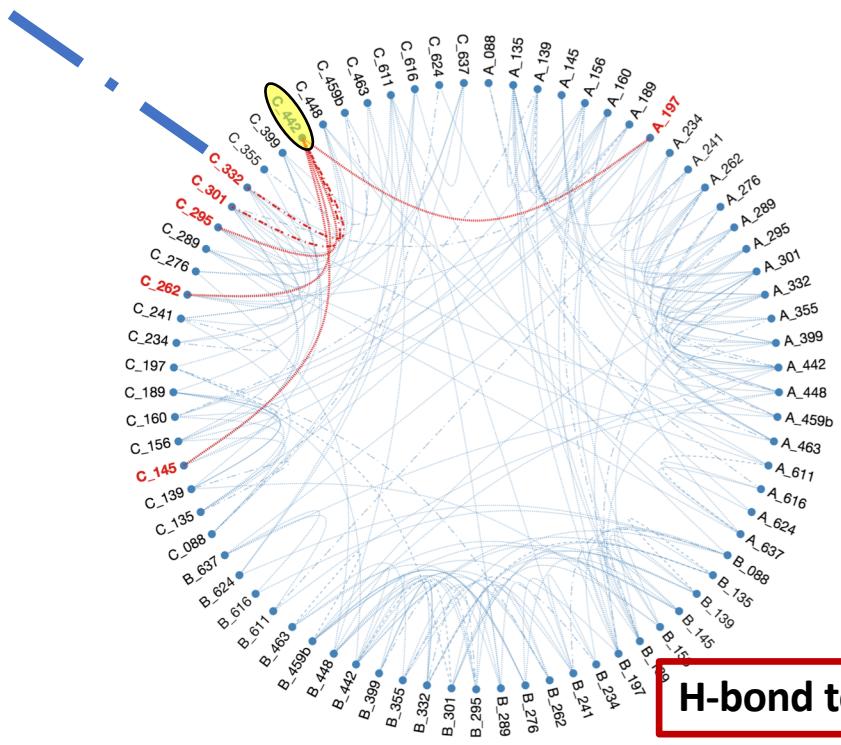


Du156: Monomer C: N301

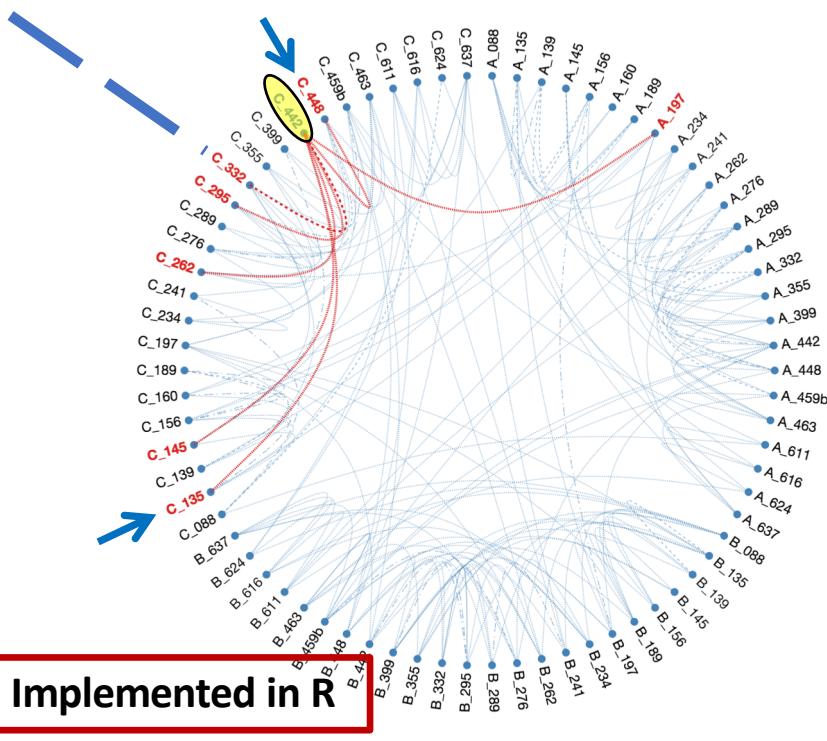


H-bond interactions

Du156 C _ N301 – N442



Du156^{N301}: C _ N442



H-bond tool: Implemented in R

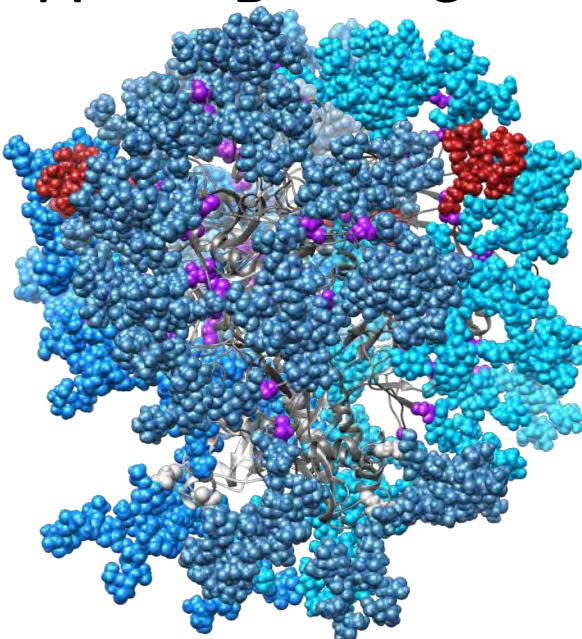
Principal Component Analysis

- Which glycans contribute most to the differences between WT and N301 mutant

PCA on monomers

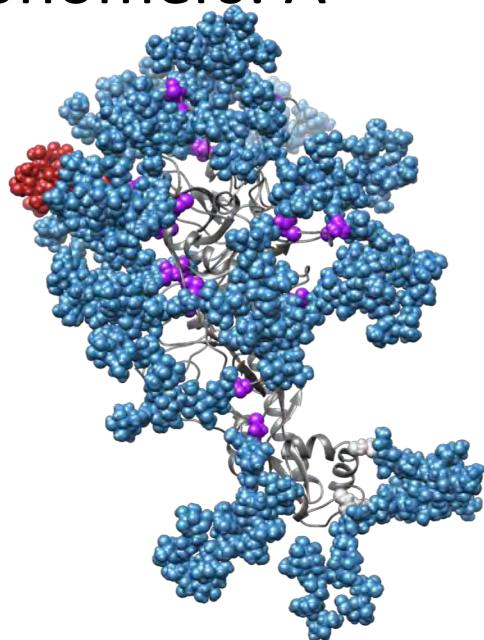
Monomers:

A B C

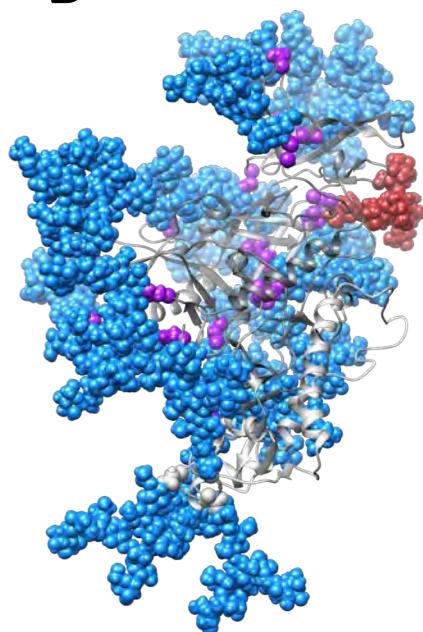


PCA on monomers

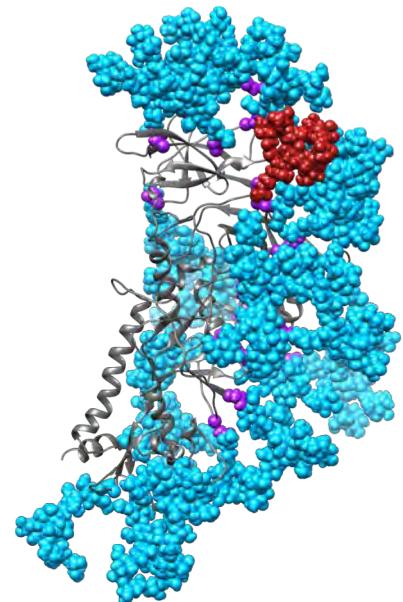
Monomers: A



B

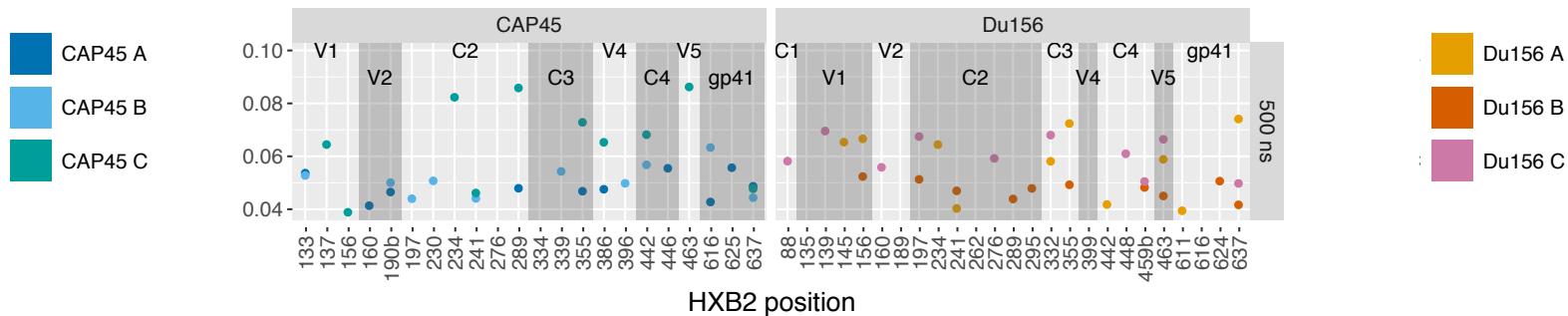


C

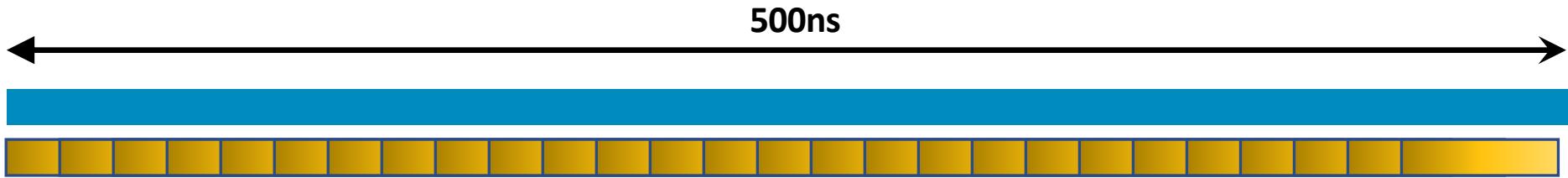


Differences between WT and N301 mutants

- monomer A^{WT} + monomer A^{N301mutant} = pseudo-trajectory
 - Bootstrap – want to find those sites that are repeatedly top contributors
 - But, our observations are not independent
 - Moving block bootstrap

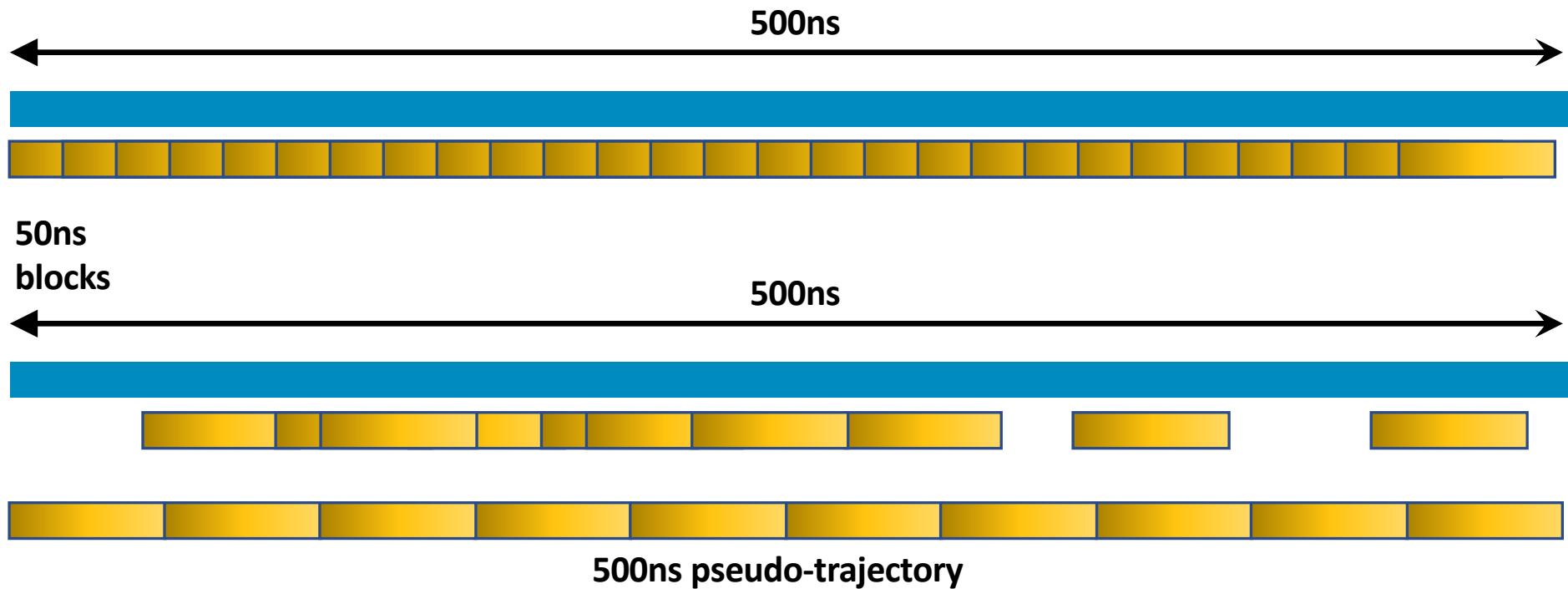


Moving block bootstrap



- 50ns blocks
- Randomly select 10 of these blocks
- To create a 500ns pseudo-trajectory

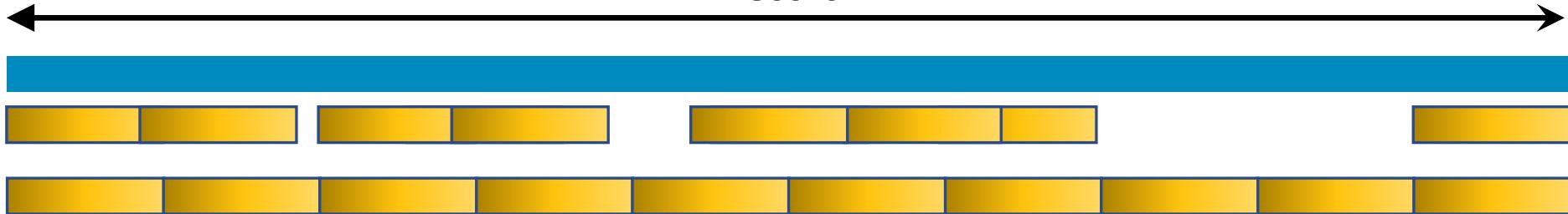
Moving block bootstrap



Moving block bootstrap: WT vs N301 mutant

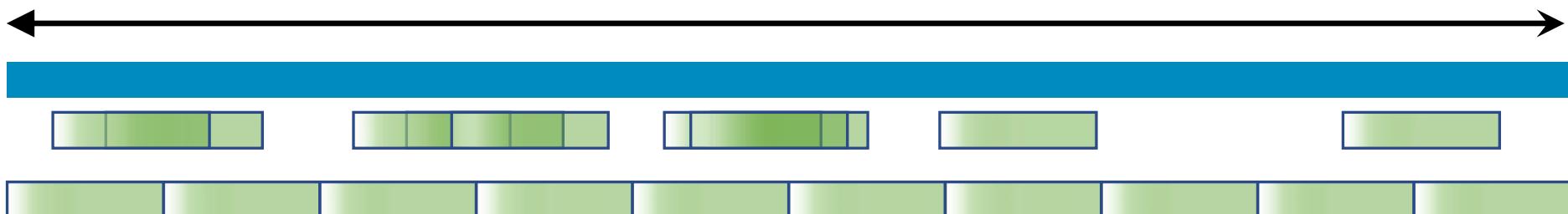
CAP45

500ns



CAP45^{N301}

500ns



Moving block bootstrap

CAP45

500ns



CAP45^{N301}

500ns



→ Bootstrap pseudo-trajectory replicate

Moving block bootstrap

CAP45

500ns



CAP45^{N301}

500ns

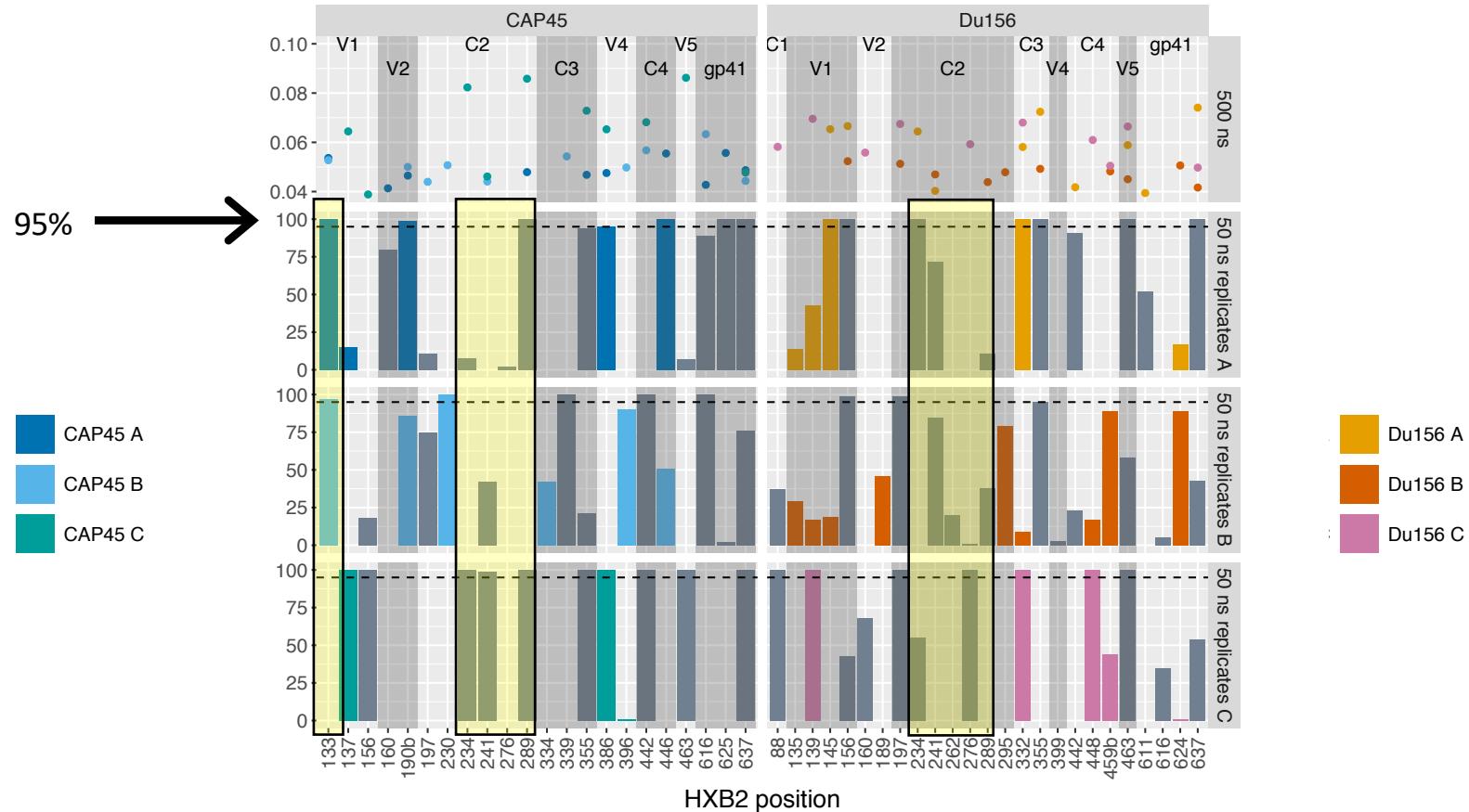


→ Bootstrap pseudo-trajectory replicate

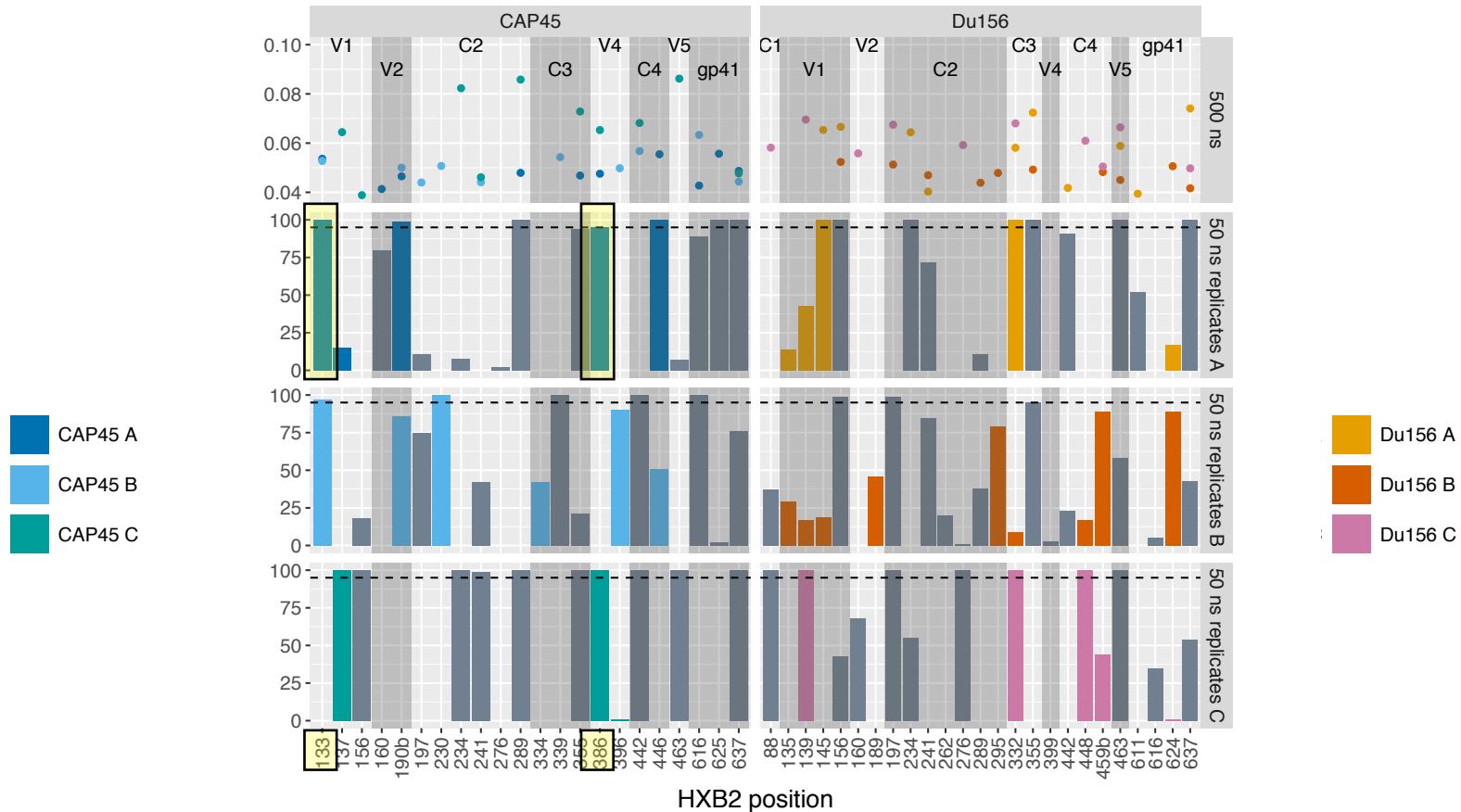
Repeat 100 times

PCA on each trajectory

Differences between WT and N301 mutants

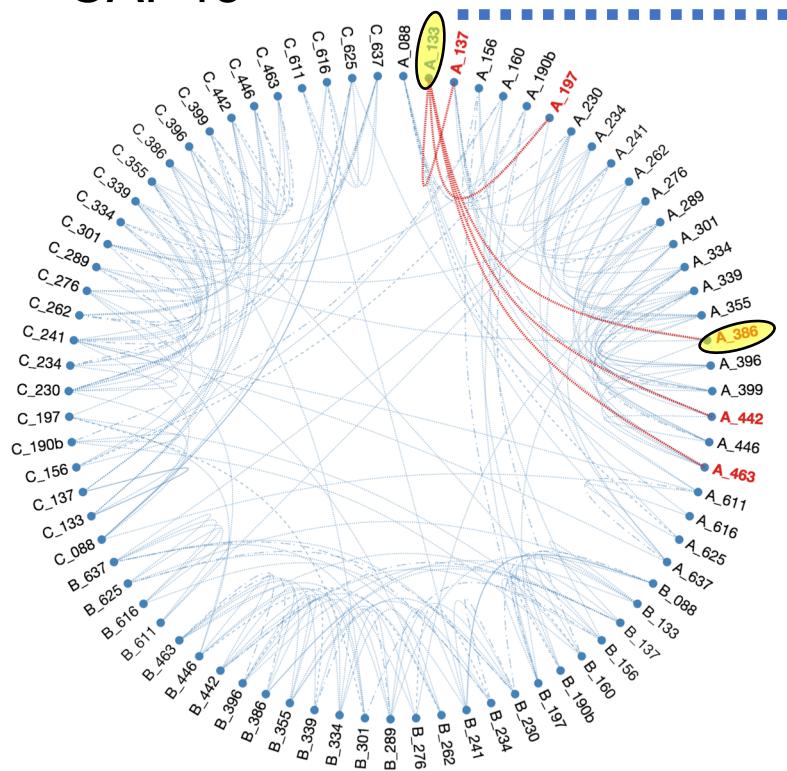


Differences between WT and N301 mutants

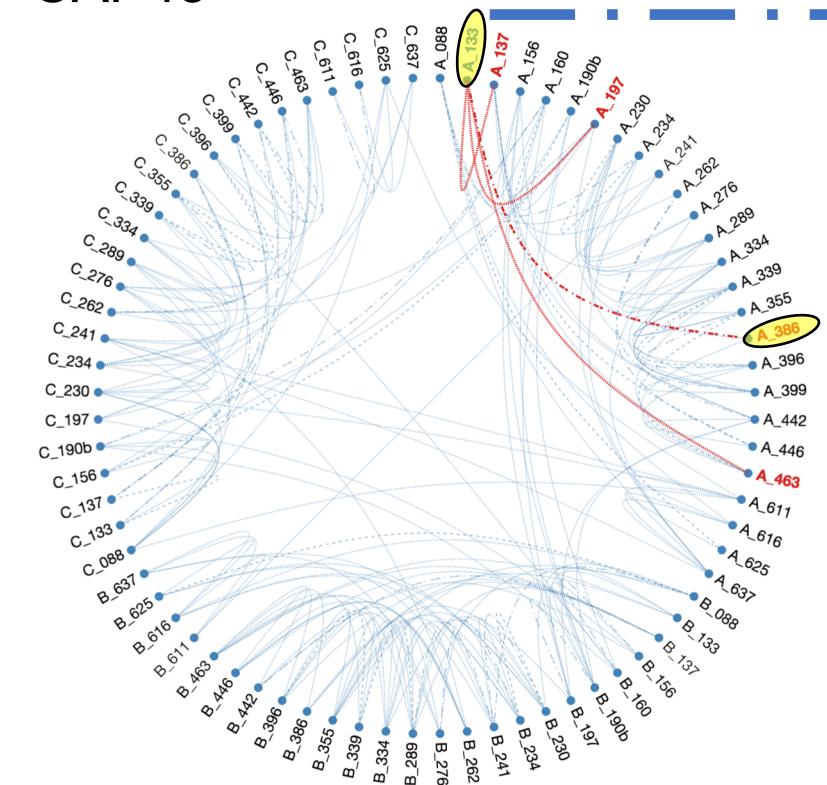


CAP45: N133 and N386 – monomer A

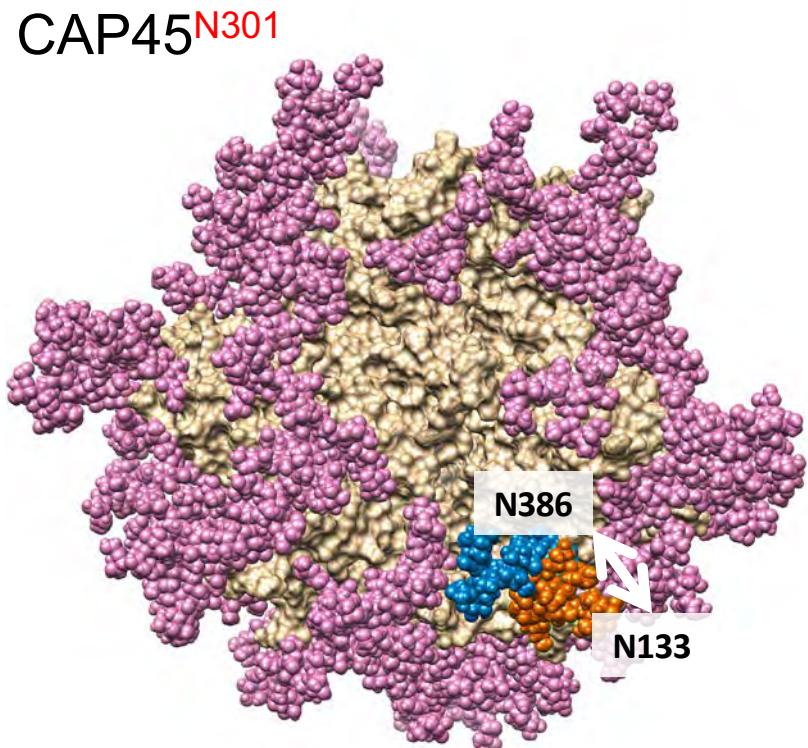
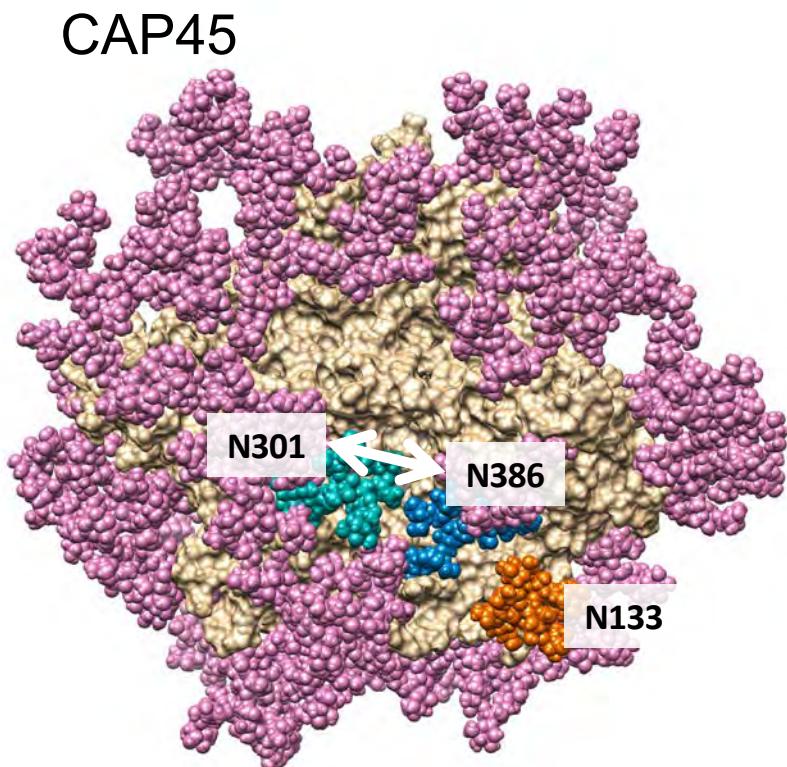
CAP45



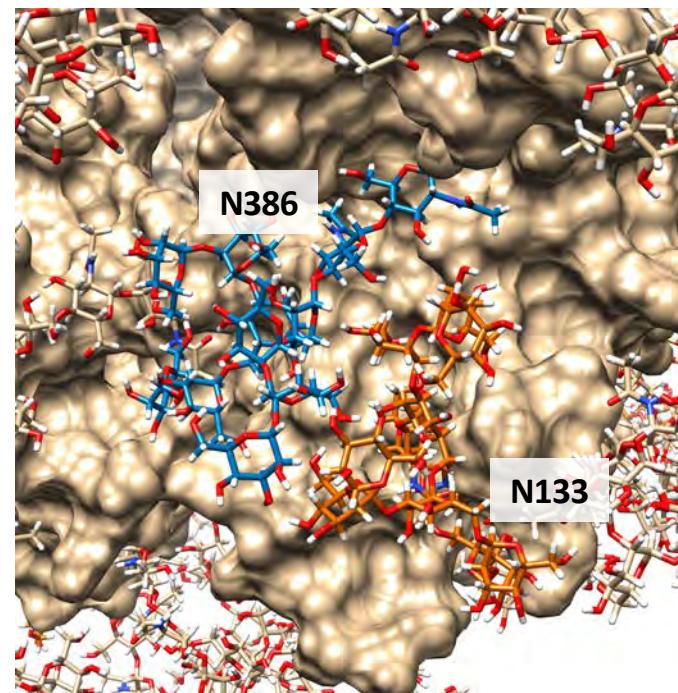
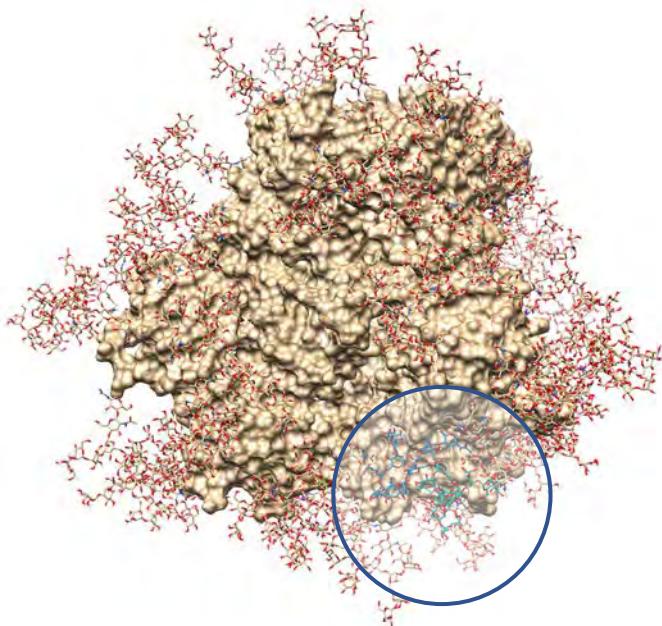
CAP45N301



CAP45: N301, N133 and N386



CAP45^{N301}: N133 and N386 – monomer A



Background

CAP45



Du156



Remove
N301

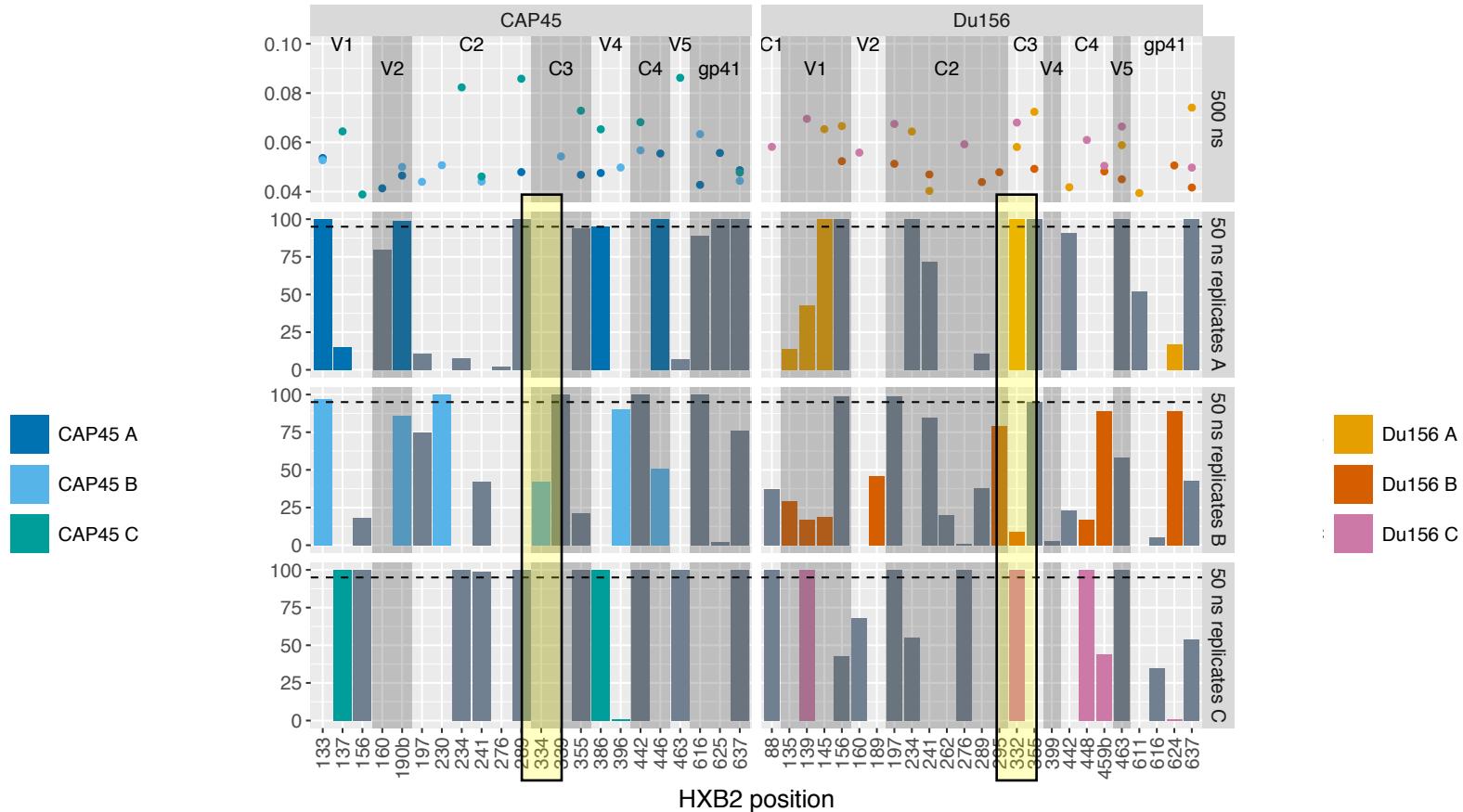
CAP45^{N301}



Du156^{N301}

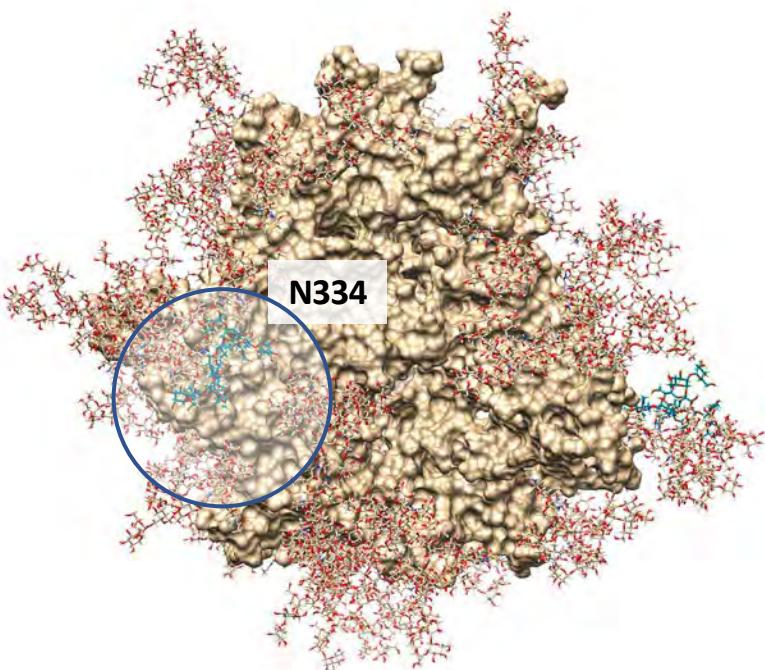


Differences between WT and N301 mutants

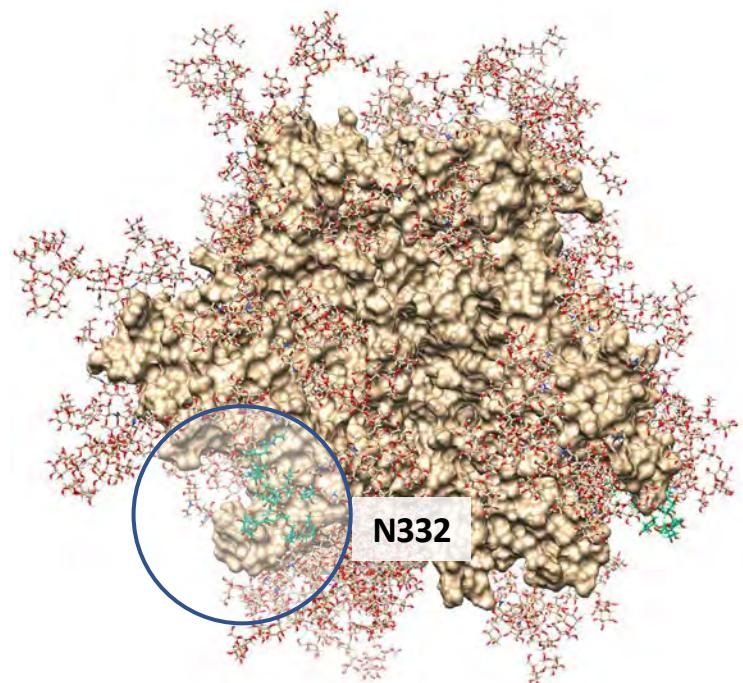


N334 vs N332

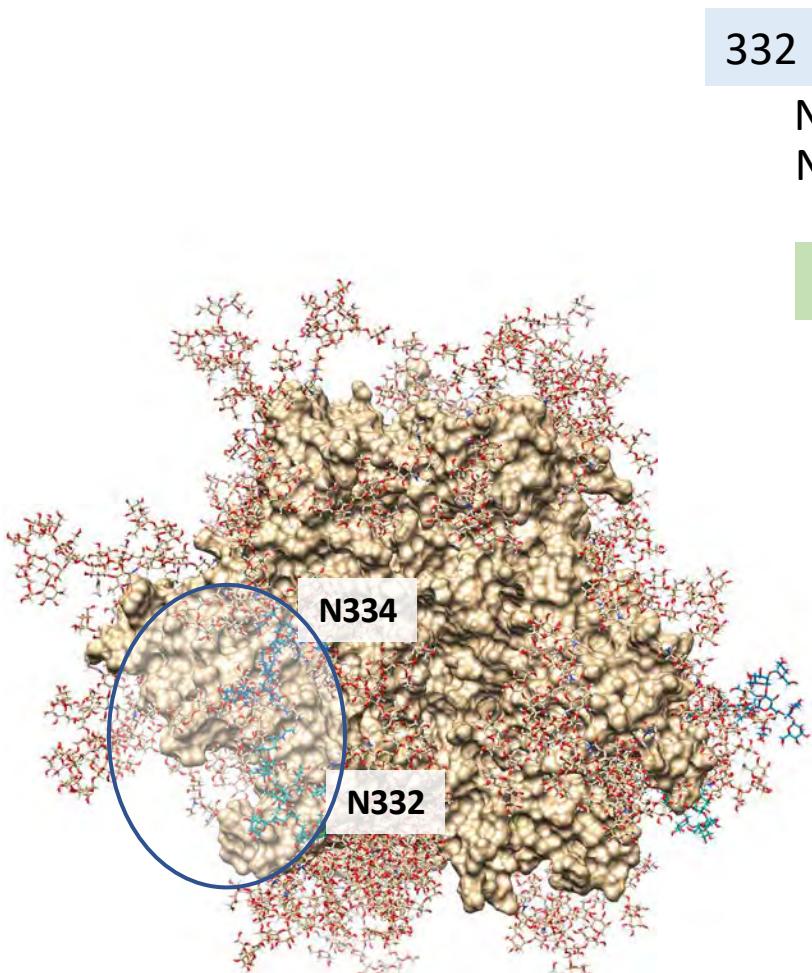
CAP45 – N334



Du156 – N332

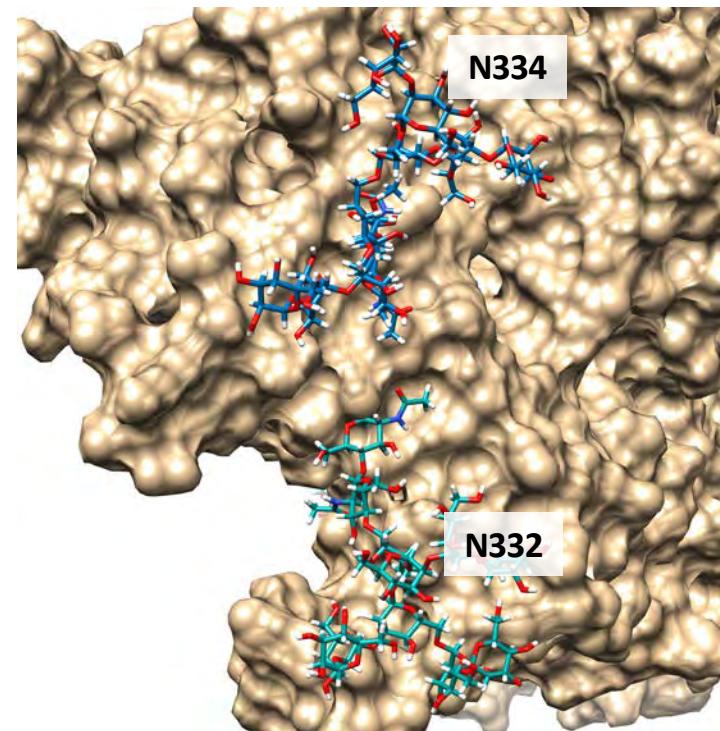


N334 vs N332

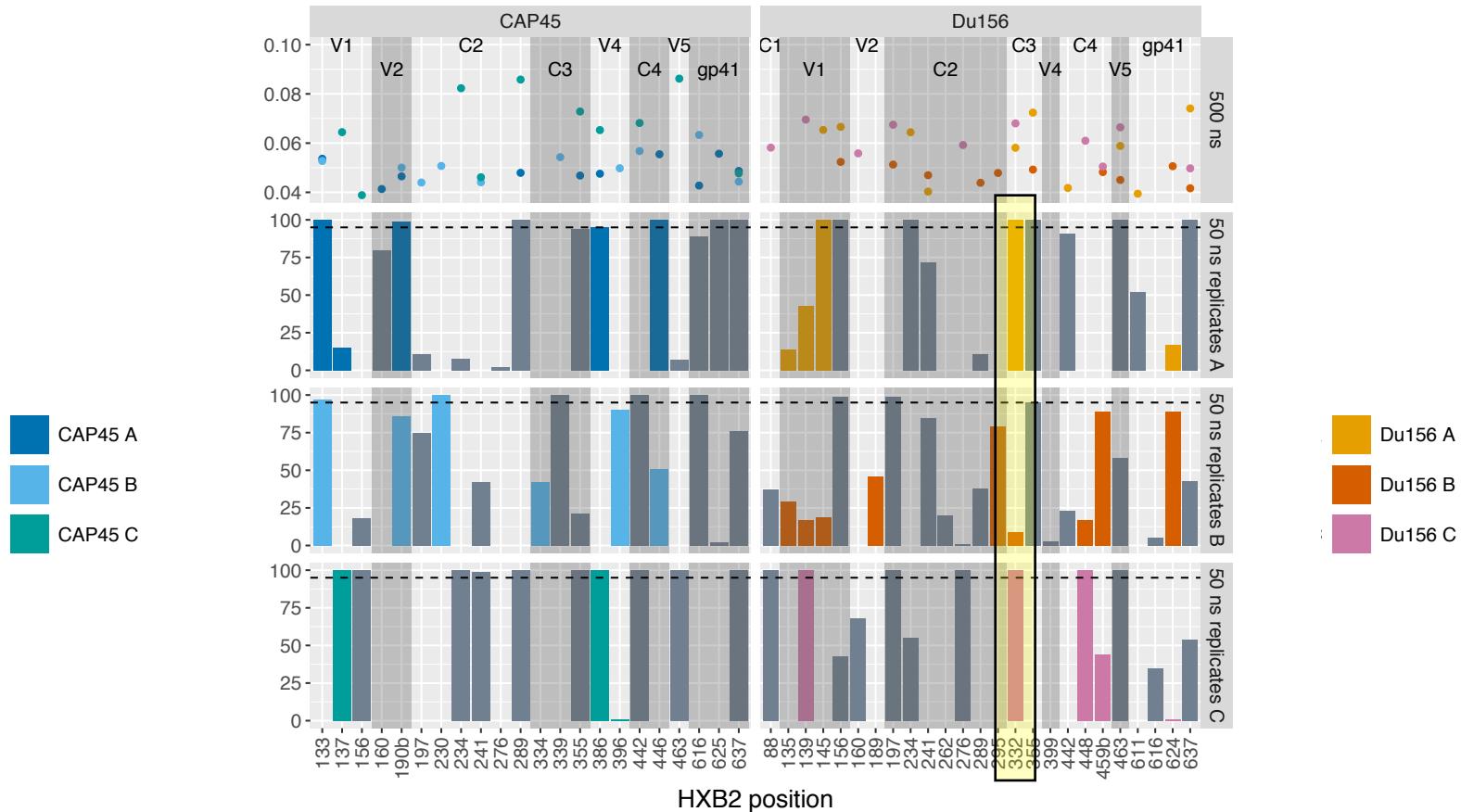


334

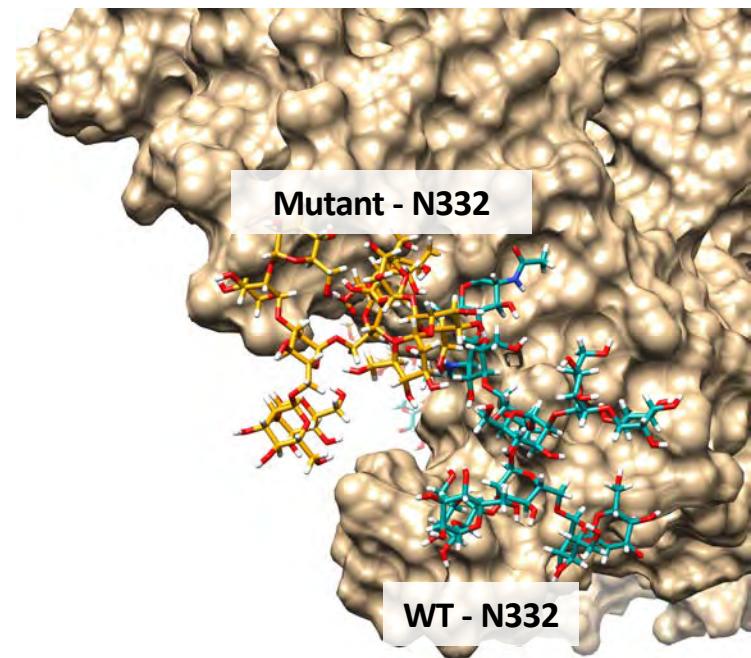
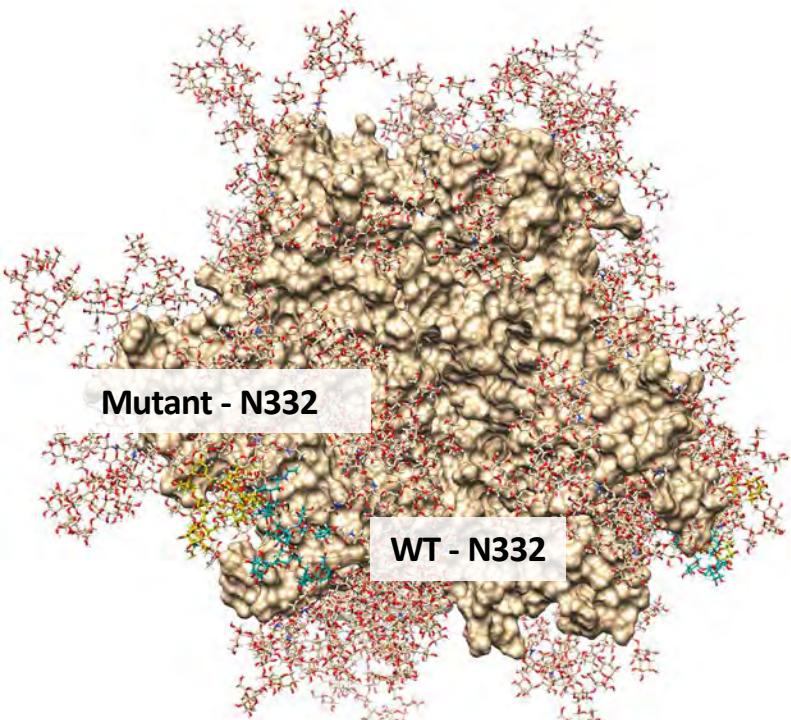
NXS|T



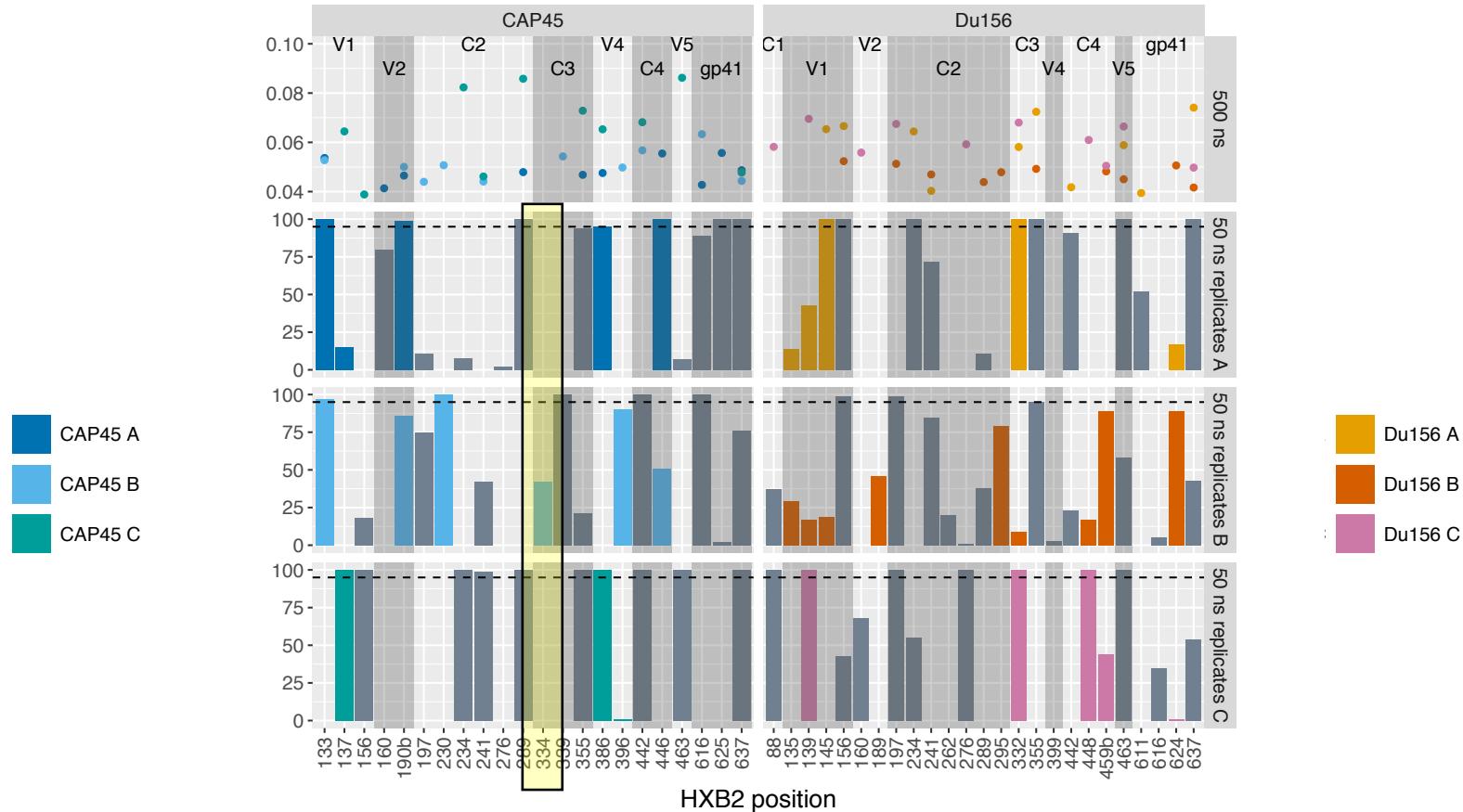
Differences between WT and N301 mutants



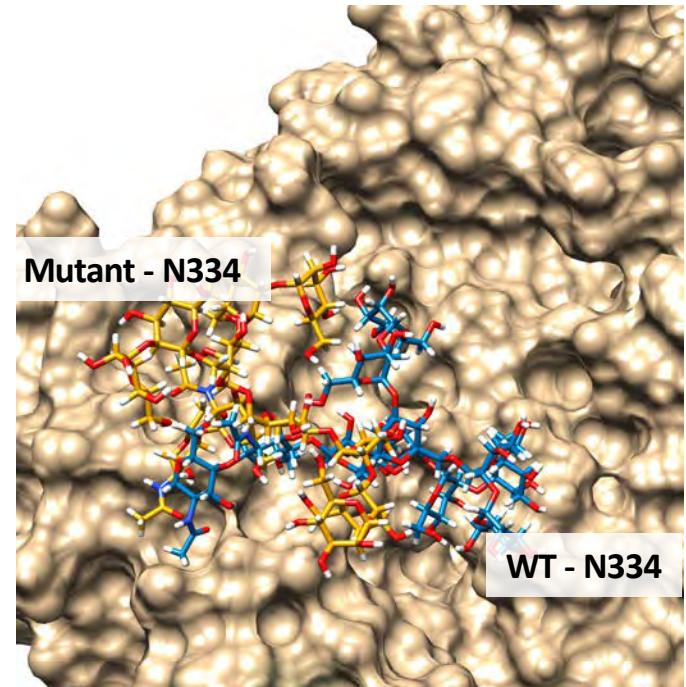
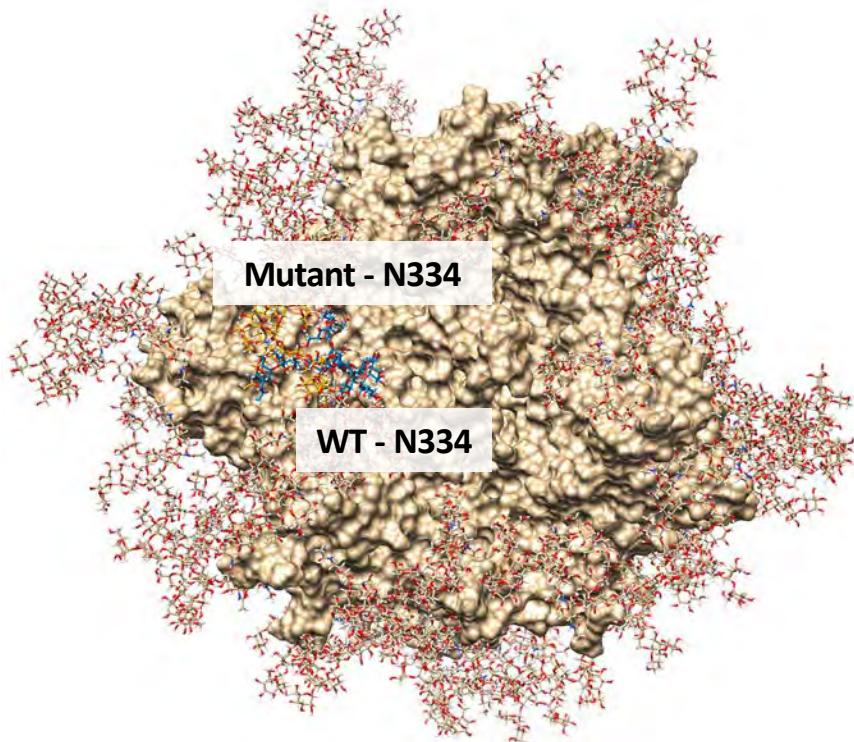
Du156 N332 comparison WT and mutant



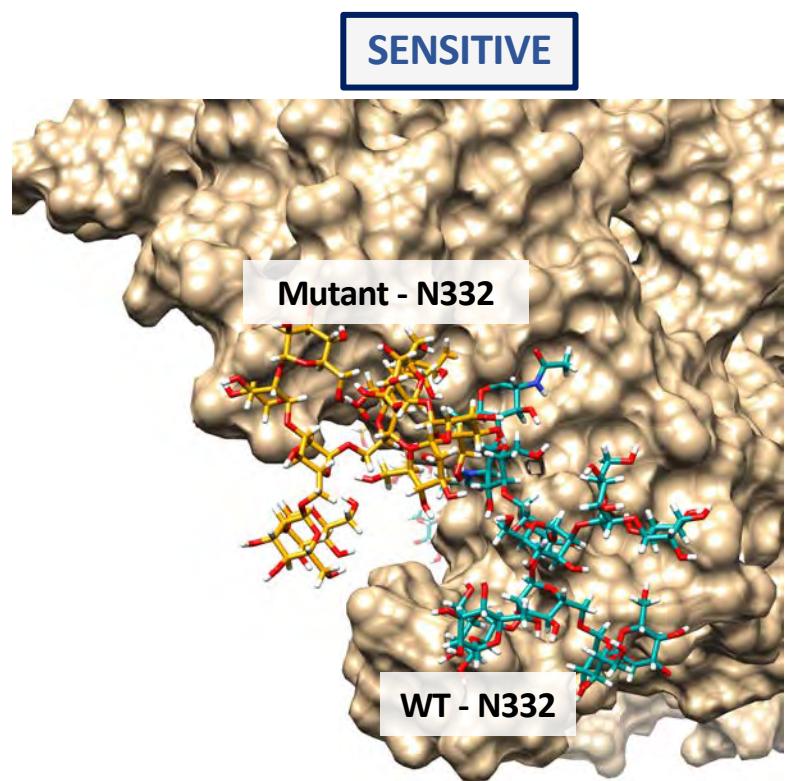
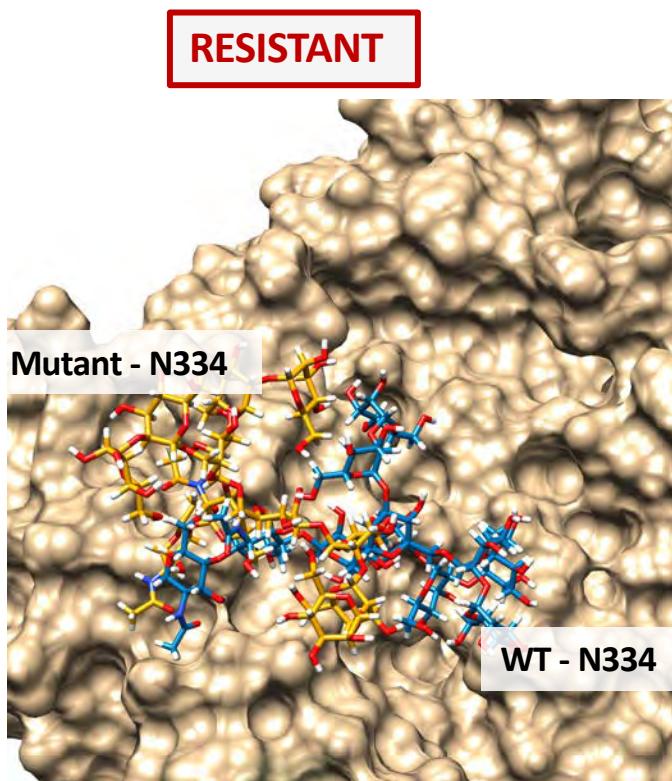
Differences between WT and N301 mutants

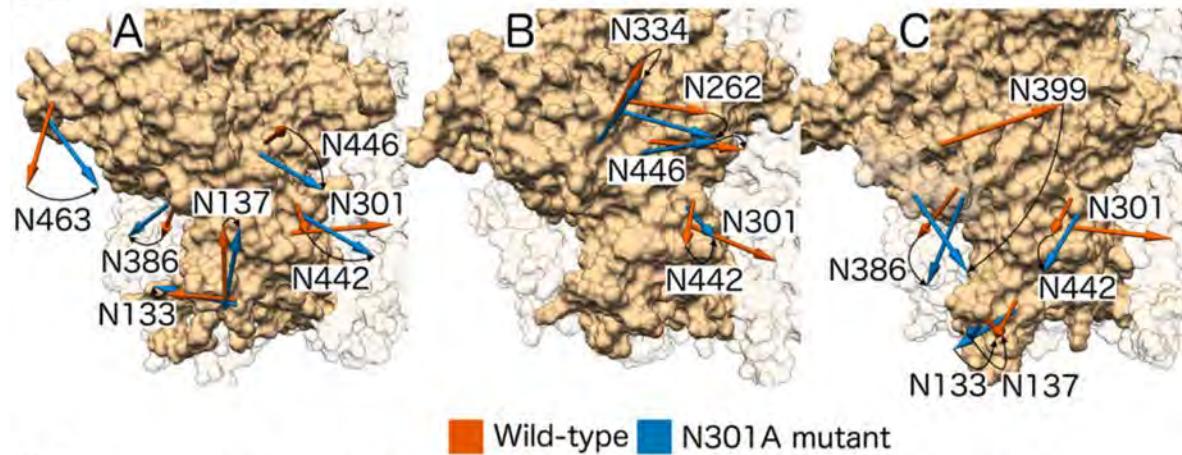
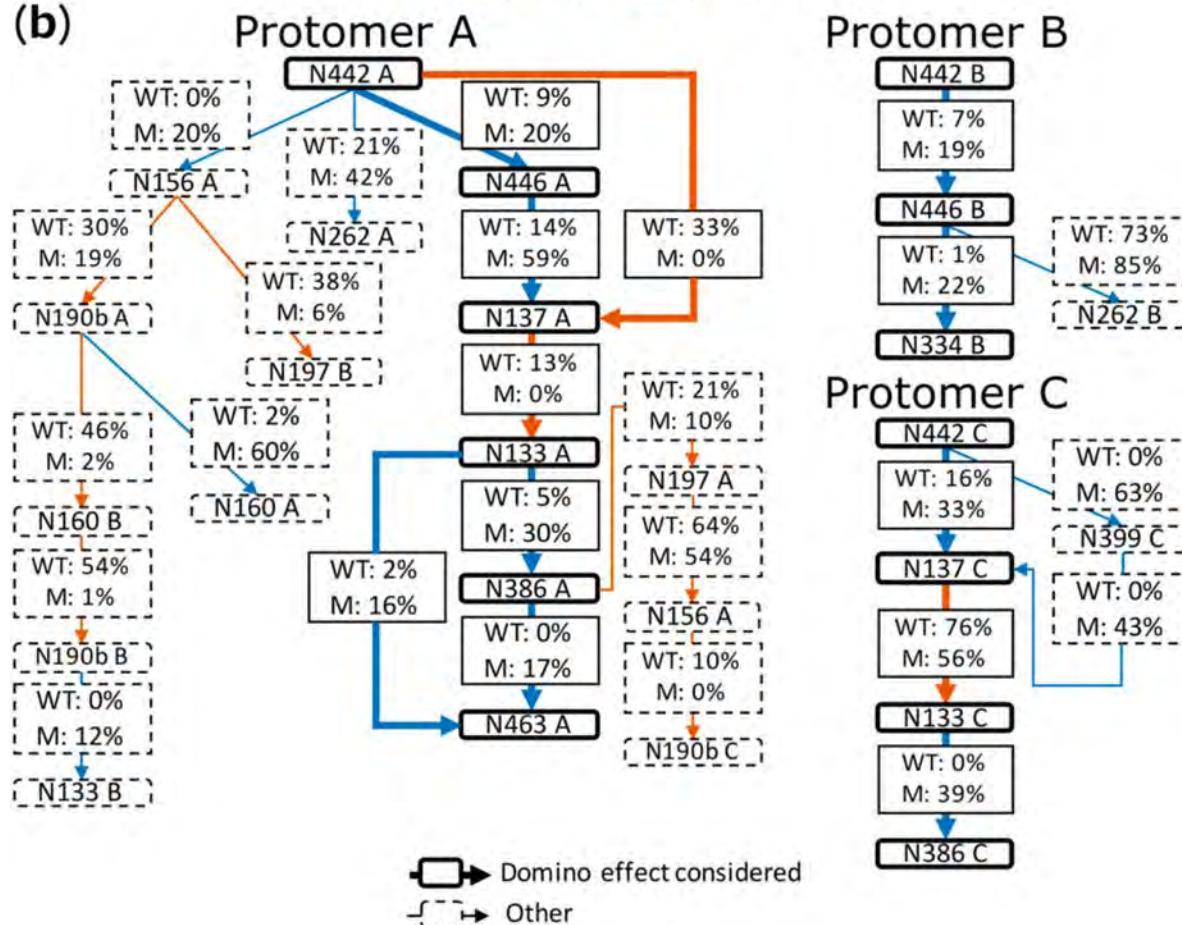


CAP45: N334 comparison WT and mutant



CAP45 vs Du156



(a)**(b)**

SCIENTIFIC REPORTS



OPEN

Structural Rearrangements Maintain the Glycan Shield of an HIV-1 Envelope Trimer After the Loss of a Glycan

Received: 11 May 2018

Accepted: 26 September 2018

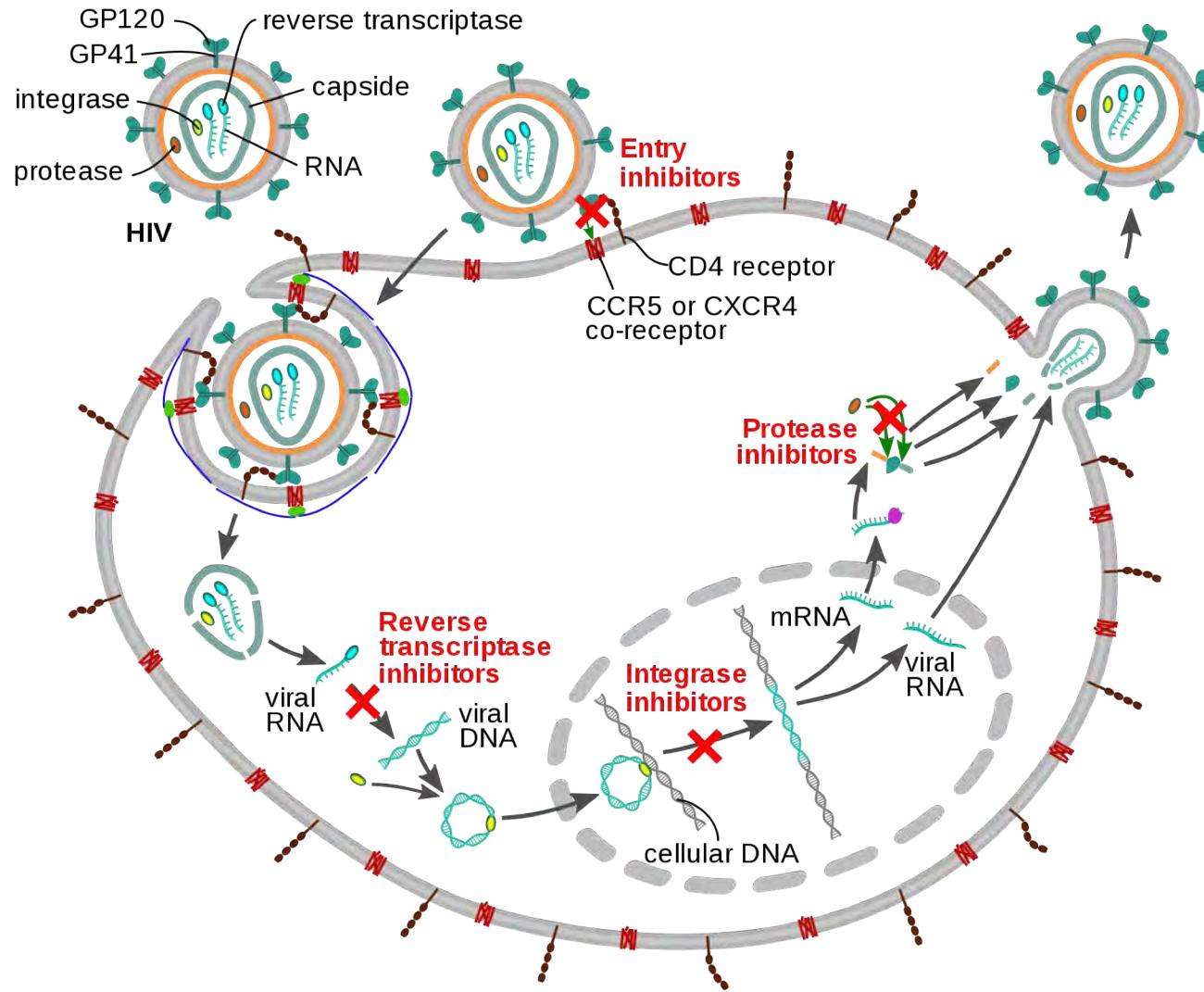
Published online: 09 October 2018

Roux-Cil Ferreira¹, Oliver C. Grant², Thandeka Moyo¹, Jeffrey R. Dorfman¹, Robert J. Woods², Simon A. Travers¹ & Natasha T. Wood⁵

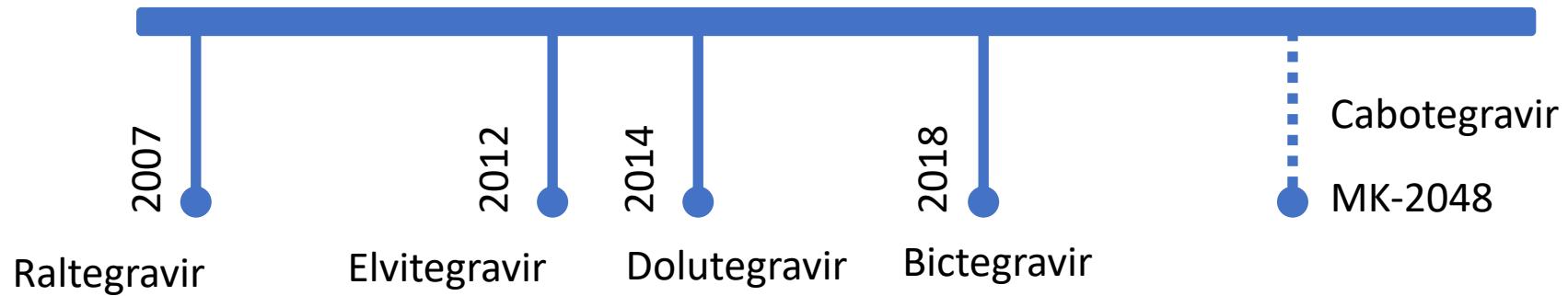
The HIV-1 envelope (Env) glycoprotein is the primary target of the humoral immune response and a critical vaccine candidate. However, Env is densely glycosylated and thereby substantially protected from neutralisation. Importantly, glycan N301 shields V3 loop and CD4 binding site epitopes from neutralising antibodies. Here, we use molecular dynamics techniques to evaluate the structural rearrangements that maintain the protective qualities of the glycan shield after the loss of glycan N301. We examined a naturally occurring subtype C isolate and its N301A mutant; the mutant not only remained protected against neutralising antibodies targeting underlying epitopes, but also exhibited an increased resistance to the VRC01 class of broadly neutralising antibodies. Analysis of this mutant revealed several glycans that were responsible, independently or through synergy, for the neutralisation resistance of the mutant. These data provide detailed insight into the glycan shield's ability to compensate for the loss of a glycan, as well as the cascade of glycan movements on a protomer, starting at the point mutation, that affects the integrity of an antibody epitope located at the edge of the diminishing effect. These results present key, previously overlooked, considerations for HIV-1 Env glycan research and related vaccine studies.

Understanding PPI and drug-resistance profiles of HIV-1 protein variants using molecular dynamics

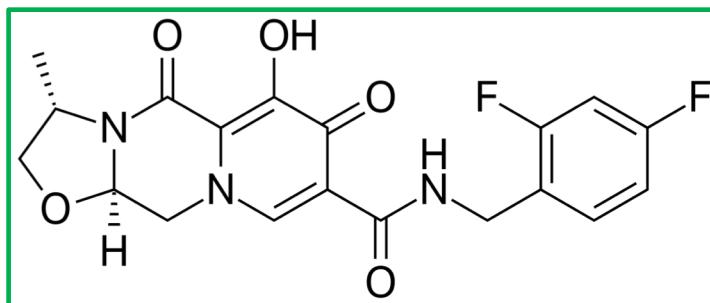
HIV-1 Drug targets



Integrase inhibitors



Integrase inhibitors



Raltegravir

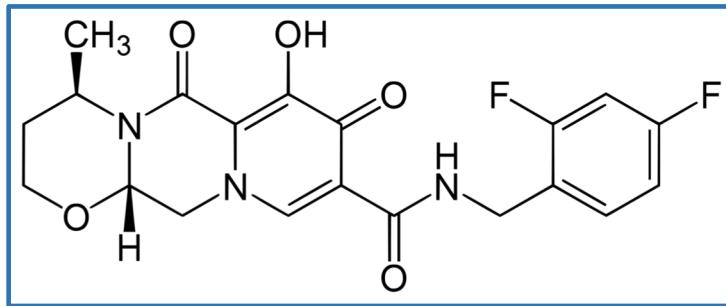
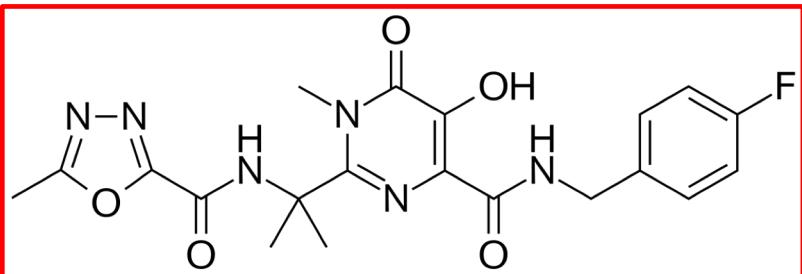
Elvitegravir

Dolutegravir

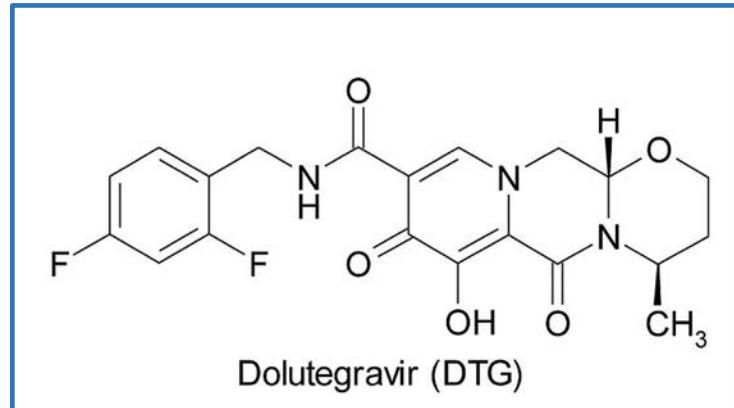
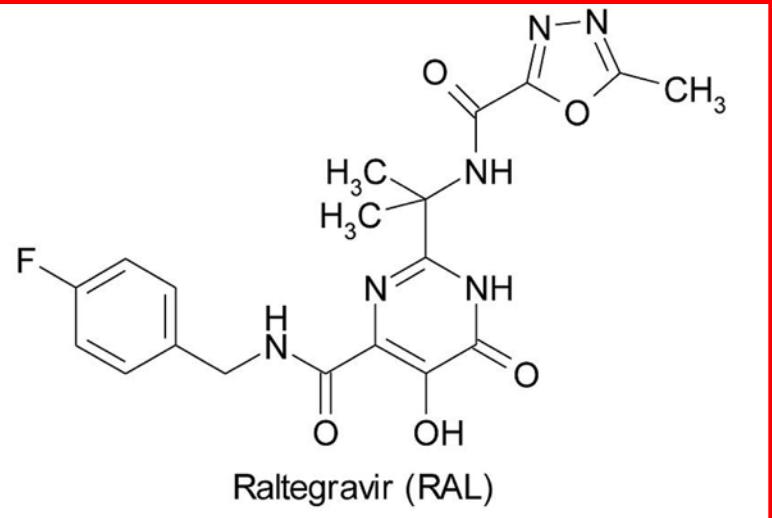
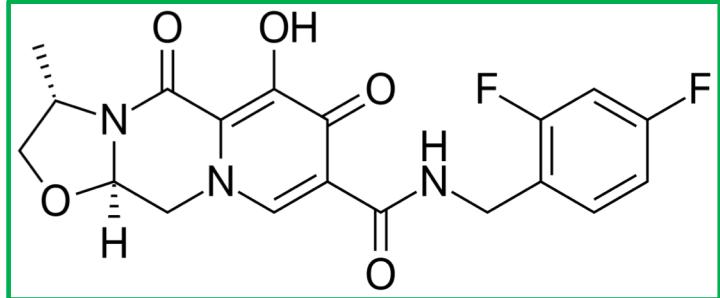
Bictegravir

Cabotegravir

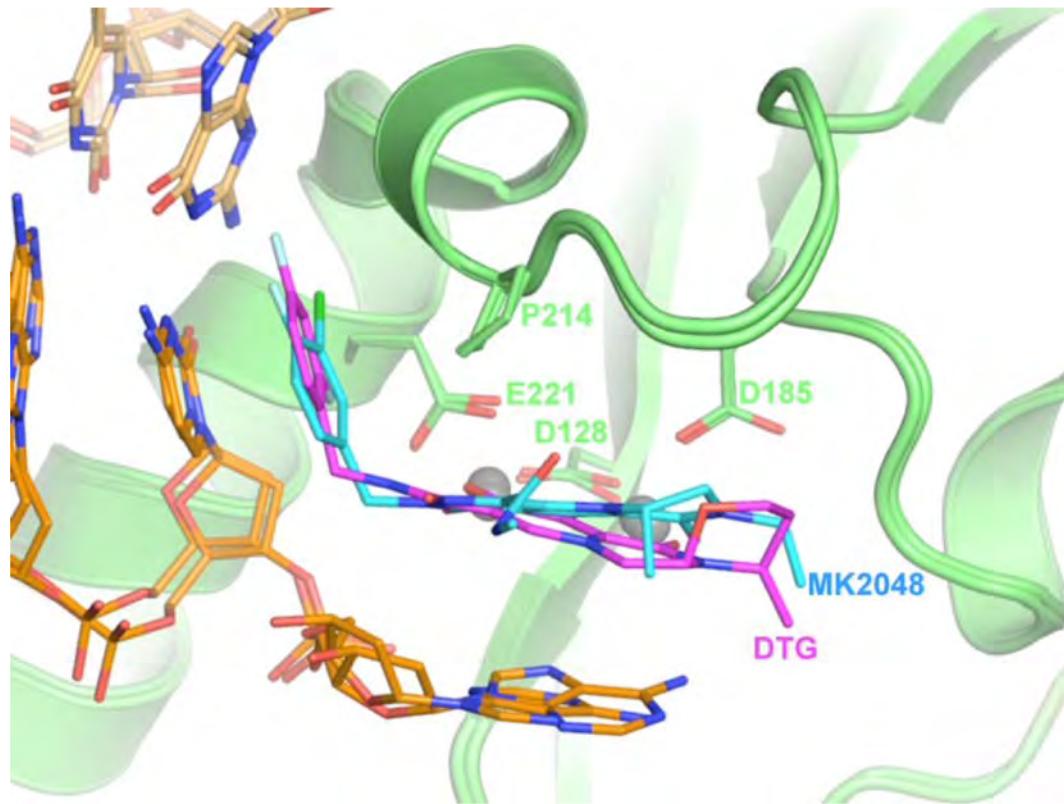
MK-2048



Integrase inhibitors



Integrase inhibitors



Comparison of **Dolutegravir** and **MK-2048**

Summary

- Difficult to compare MD simulations
- By combining analysis methods, we can generate hypotheses for the different phenotypic observations
- The removal of a glycan can have a significant effect
- The impact of the removal of a glycan depends on the rest of the glycan landscape
- MD and glycan tools/websites will get better
- Drug resistance is not absolute
- But, “you can’t out-adhere drug resistance”

Thanks!

- Roux-Cil Ferreira (UWC)
- David Matten (UCT)
- Clare Garrard (UCT)
- Oliver Grant (Complex Carbohydrate Research Center, Atlanta)
- Simon Travers (University of the Western Cape)
- Darren Martin (UCT)
- Nicky Mulder (UCT)



