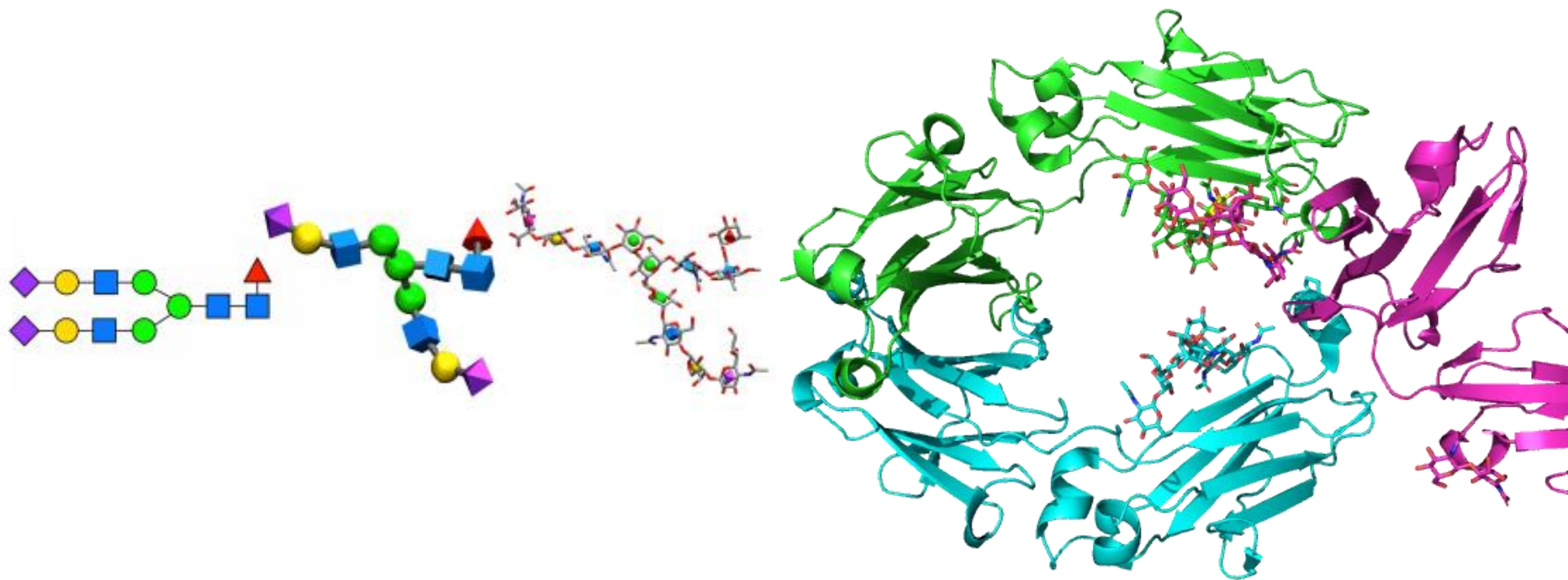


The Dynamics and Molecular Recognition of Complex Carbohydrates

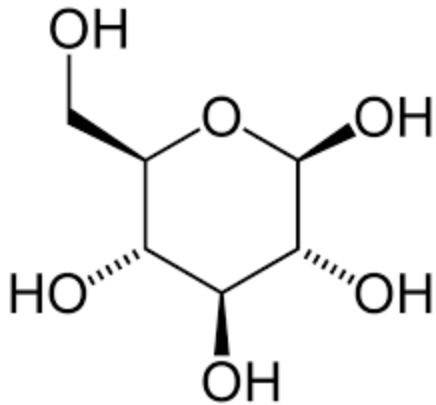
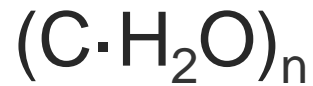


Aoife Harbison
Supervisor: Dr. Elisa Fadda

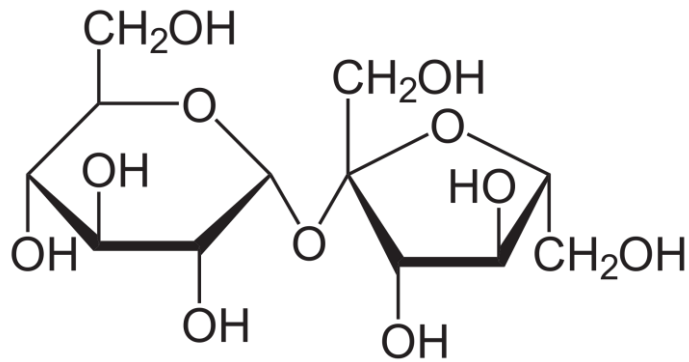
Overview

- Brief Introduction to carbohydrates and glycans
- Computational Chemistry
 - Molecular Dynamics
 - Methods for Enhanced Sampling
- Application to studying complex carbohydrates
 - Free N-glycans
 - N-glycans on IgG1 antibodies

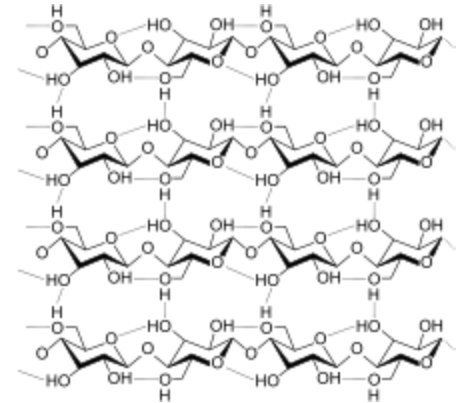
Carbohydrates



Monosaccharide



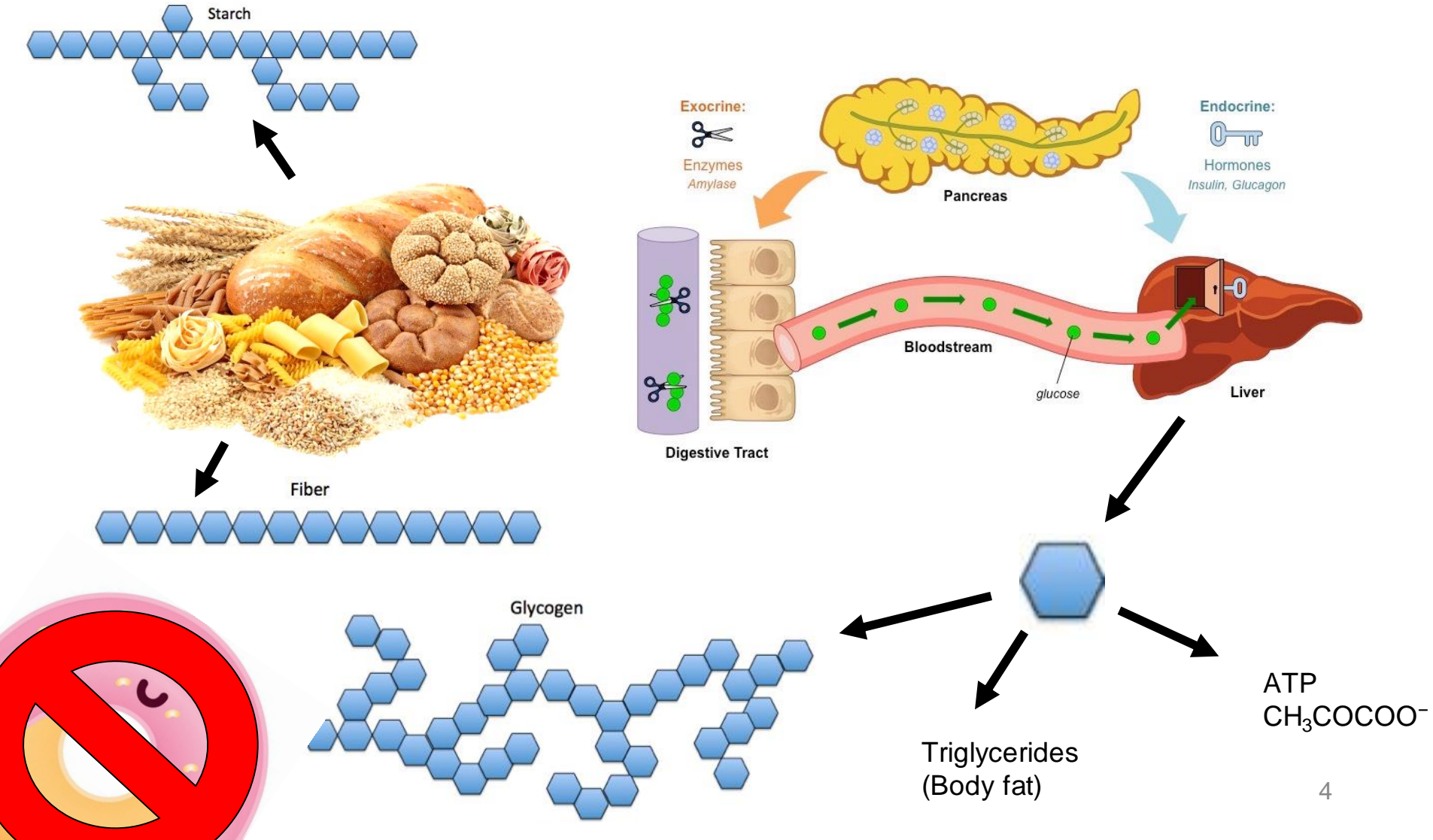
Disaccharide



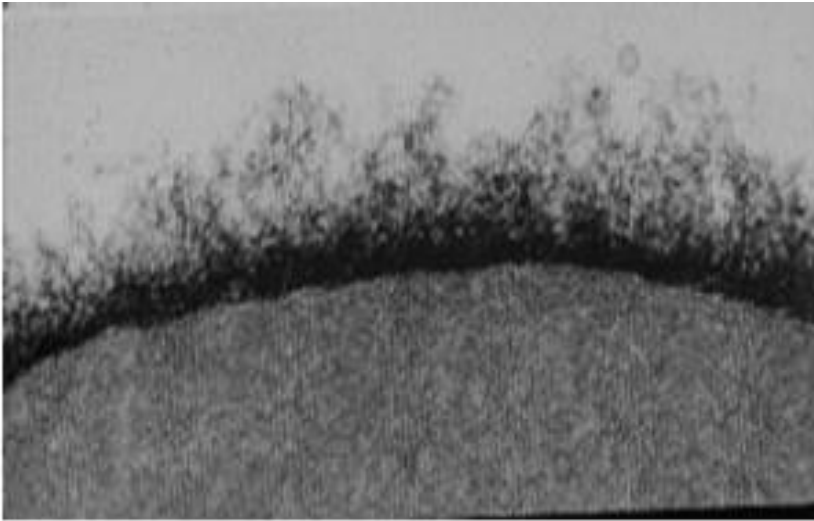
Polysaccharide



Carbohydrates



Complex Carbohydrates: Glycans

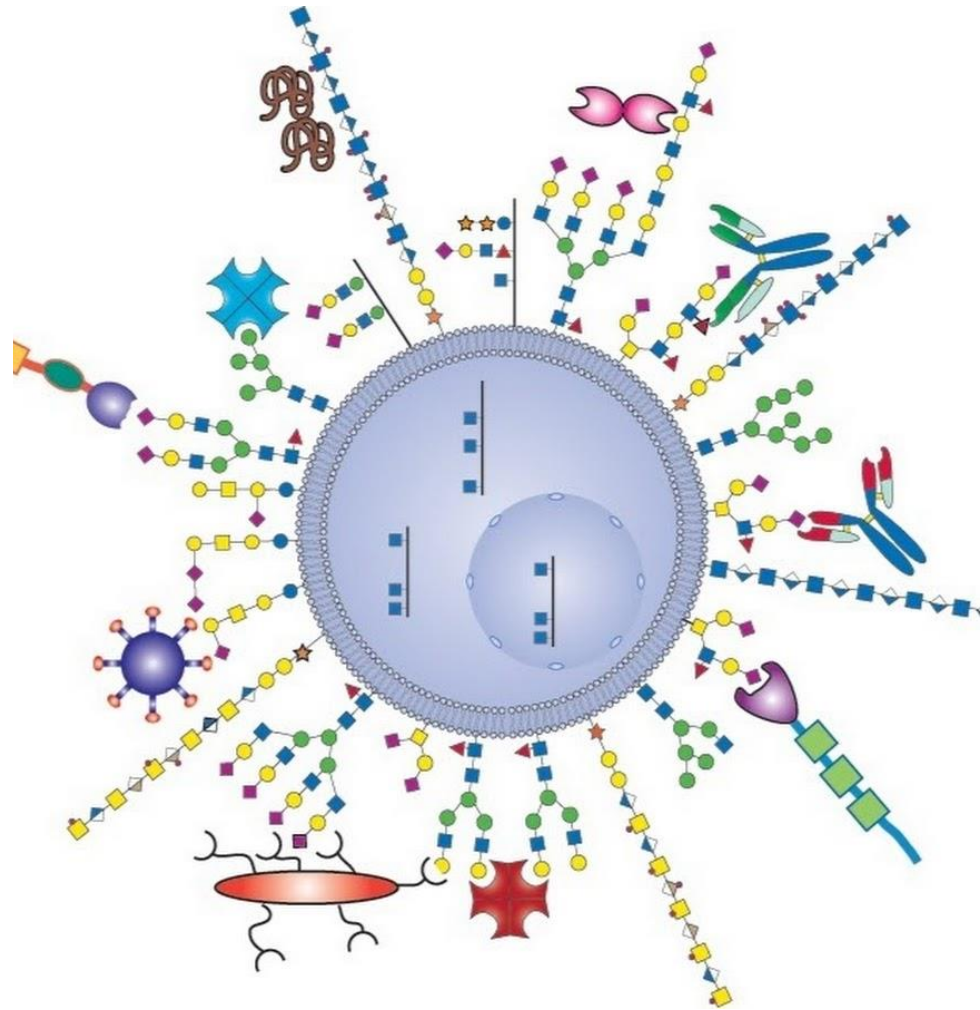


Allon Weiner, The Weizmann Institute of Science, Rehovot, Israel. 2006.

Glycans are post translational modifications of proteins and lipids

They cover the cell membrane constituting what is called glycocalyx















































































Glycans modulate protein or other biomolecules' activity and function



Richard Cummings, NCFG (2013)

<https://www.youtube.com/watch?v=sT1qghJZdW8>

Complex Carbohydrates: Glycans

White (Generic)	Blue	Green	Yellow	Orange	Pink	Purple	Light Blue	Brown	Red
Hexose 	Glc 	Man 	Gal 	Gul 	Alt 	All 	Tal 	Ido 	
HexNAc 	GlcNAc 	ManNAc 	GalNAc 	GulNAc 	AltNAc 	AllNAc 	TalNAc 	IdoNAc 	
Hexos amine 	GlcN 	ManN 	GalN 	GulN 	AltN 	AllN 	TalN 	IdoN 	
Hexuronate 	GlcA 	ManA 	GalA 	GulA 	AltA 	AllA 	TalA 	IdoA 	
Deoxyhexose 	Qui 	Rha 			6dAlt 		6dTal 		Fuc 
DeoxyhexNAc 	QuiNAc 	RhaNAc 							FucNAc 
Di-deoxyhexose 	Oli 	Tyv 		Abe 	Par 	Dig 	Col 		
Pentose 		Ara 	Lyx 	Xyl 	Rib 				
Nomulosonate 		Kdn 				Neu5Ac 	Neu5Gc 	Neu 	
Unknown 	Bac 	LDManHep 	Kdo 	Dha 	DDManHep 	MurNAc 	MurNGc 	Mur 	
Assigned 	Api 	Fru 	Tag 	Sor 	Psi 				

Complex Carbohydrates: Glycans



University of Utrecht, "Illuminating the Secret World of your Glycans".


Interest In Glycobiology


By modifying the structure and content of a glycan, the functional properties of the glycoprotein can change, and so enable or disable particular desired/undesired interactions with the protein and other biomolecules, making them attractive as biomarkers and for therapeutic targeting.

**nature
REVIEWS** RHEUMATOLOGY

Review Article | Published: 14 September 2017

Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity?

Michaela Seeling, Christin Brückner & Falk Nimmerjahn 

Nature Reviews Rheumatology **13**, 621–630 (2017) | [Download Citation](#) 

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Abstract
Background
Methods
Results
Discussion
Conclusions
Declarations
References

Research **Open Access**

Glycan analysis of colorectal cancer samples reveals stage-dependent changes in CEA glycosylation patterns

Qianqian Zhao ¹, Tiancheng Zhen ¹, Zaijun Deng, Qianqian Li, Yaming Liu, Shaojie Yang, Dengbo Ji and Yan Li 

¹Contributed equally

Clinical Proteomics 2018 **15**:9

<https://doi.org/10.1186/s12014-018-0182-8> | © The Author(s) 2018

Received: 12 June 2017 Accepted: 23 January 2018 Published: 2 March 2018

**Molecular
Oncology**

Research Article **Open Access**  




N-glycan signatures identified in tumor interstitial fluid and serum of breast cancer patients: association with tumor biology and clinical outcome

Thilde Terkelsen, Vilde D. Haakensen, Radka Saldova, Pavel Gromov, Merete Kjær Hansen, Henning Stöckmann, Ole Christian Lingjærde, Anne-Lise Børresen-Dale, ... [See all authors](#) 

First published: 26 April 2018 | <https://doi.org/10.1002/1878-0261.12312>

Search for full-text at Maynooth University [Go here for SFX](#)

[Read the full text](#) >

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Gastroenterology

Volume 154, Issue 5, April 2018, Pages 1320–1333.e10



Original Research

Full Report: Clinical—Alimentary Tract

Glycosylation of Immunoglobulin G Associates With Clinical Features of Inflammatory Bowel Diseases

Mirna Simurina ^{1,*,}, Noortje de Haan ^{2,*,}, Frano Vučković ^{3,*,}, Nicholas A. Kennedy ⁴, Jerko Stambuk ³, David Falick ², Irena Trbojević-Akmačić ³, Florent Clerc ², Genadij Razdorov ³, Anna Khon ⁵, Anna Latiano ⁶, Renata Džinca ², Silvio Danese ⁶, Stephan Targan ⁹, Carol Landers ⁹, Maria Dubinsky ⁹

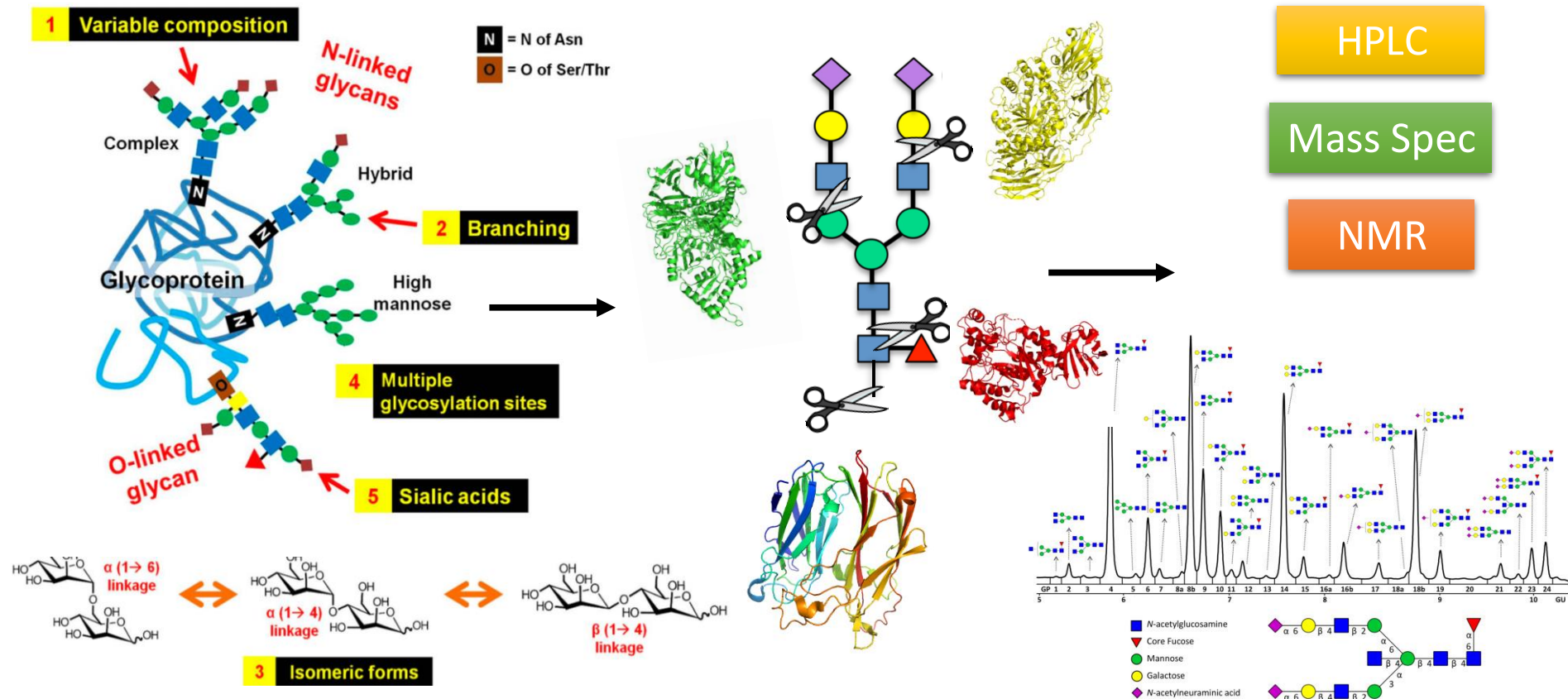
The Inflammatory Bowel Disease Biomarkers Consortium

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<https://doi.org/10.1053/j.gastro.2018.01.002>

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Structural Analysis of Glycans



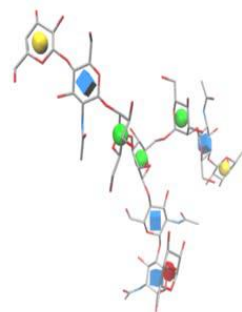
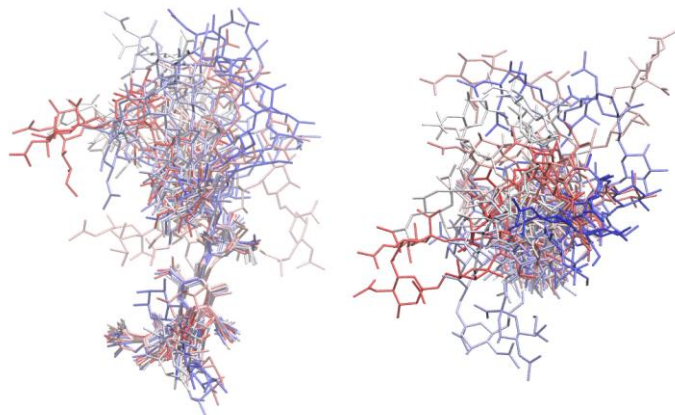
Limitations: not able to account for 3D structure, difficult to differentiate isomers, expensive and time consuming

Computational Chemistry

(Computational Biophysics)



- Allows us to understand the significance of the 3D structure of molecules
- Able to examine and explore the physical properties of a molecular system
- Easy (enough) to implement and inexpensive relative to experimental analysis



Alignment of N-glycan snapshots, side and above

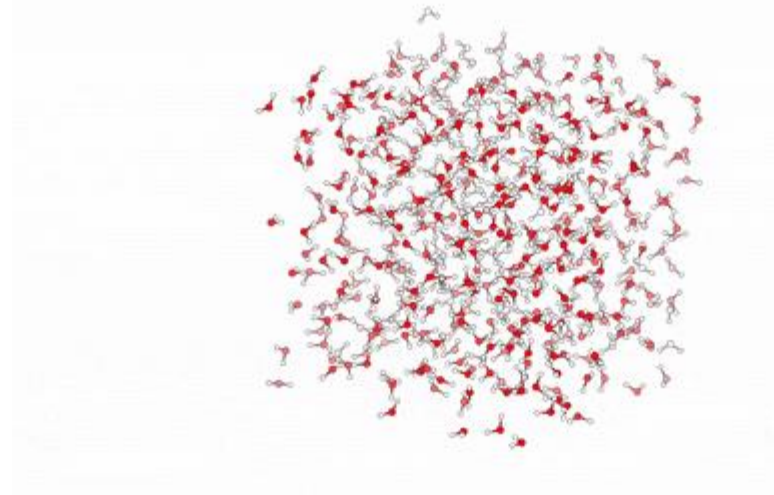
Molecular Dynamics

Representing the **motion** of the atoms and molecules within a specified system **over time**, using classical Newtonian equations of motions

The initial state of the molecule/system is taken from crystal structure, homology model, built using modelling software

Atoms are represented as single point masses inside Van der Waals potentials
→ behave as hard spheres

Bond and angle restraints are treated as simple harmonic oscillators



Molecular Dynamics

Then interaction potentials are applied to the atoms and molecules using **force fields**, which are composed of:

a functional form

&

parameter sets

Potential energy of the system can be calculated by terms:

$$E_{\text{total}} = E_{\text{bonded}} + E_{\text{nonbonded}}$$

$$E_{\text{bonded}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{dihedral}}$$

$$E_{\text{nonbonded}} = E_{\text{electrostatics}} + E_{\text{van der Waals}}$$

Empirical set of data for each atom type and molecule type

Atom: atomic mass, Van der Waals radius, partial charge

Molecule: equilibrium values for bond lengths, bond angles, dihedrals angles

Selecting the right force field is imperative, have to choose wisely!

Molecular Dynamics

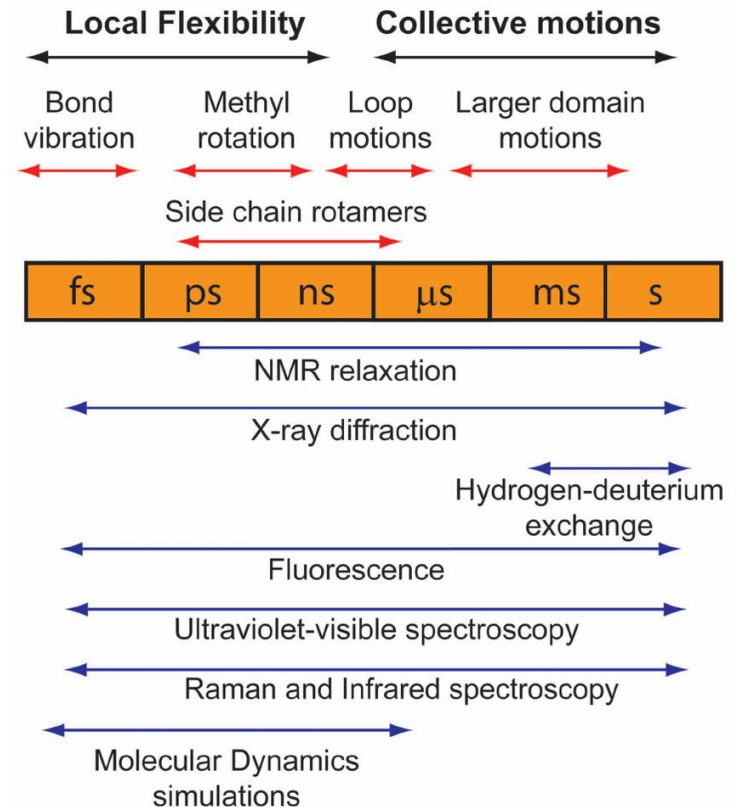
Starting the simulation, velocities from the Boltzmann distribution @ 300K randomly allocated
Then for each time step:

- Compute forces on each particle
- Solve Newton's 2nd Law of motion for each atom to calculate the new coordinates and new velocity for each atom position
- Repeat for a specified number of steps

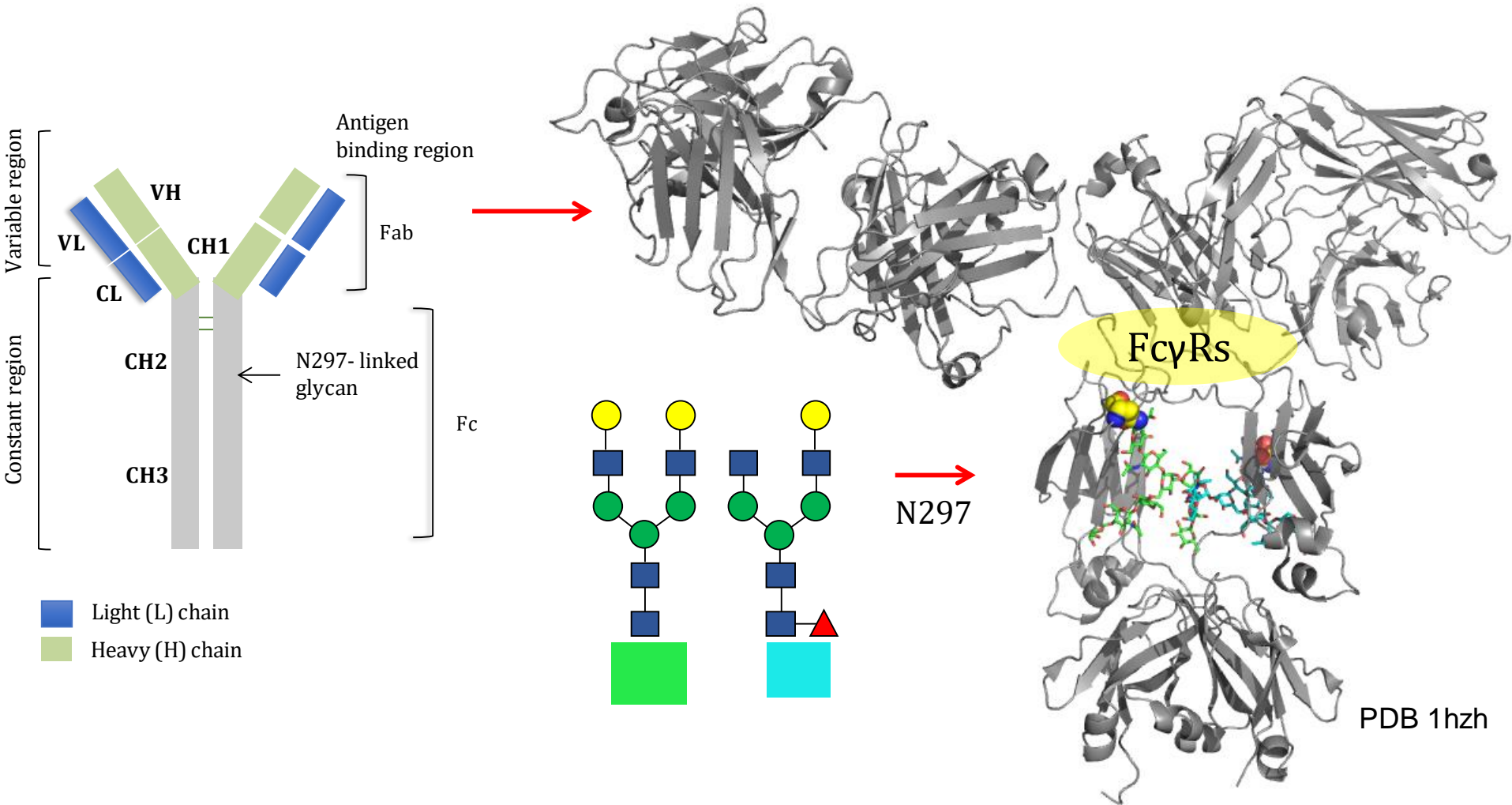
A stepwise numerical integration method is used to solve the equation
Usually a time step of 1 or 2fs is used, which gives a reasonable approximation to the solution

$$F(X) = -\nabla E(X) = -\frac{\partial E}{\partial X}$$

$$M \ddot{X} = F(X)$$



IgG1 Fc N-glycosylation



N-Glycosylation of IgG1 @N297

94% of the glycoforms in Fc are fucosylated

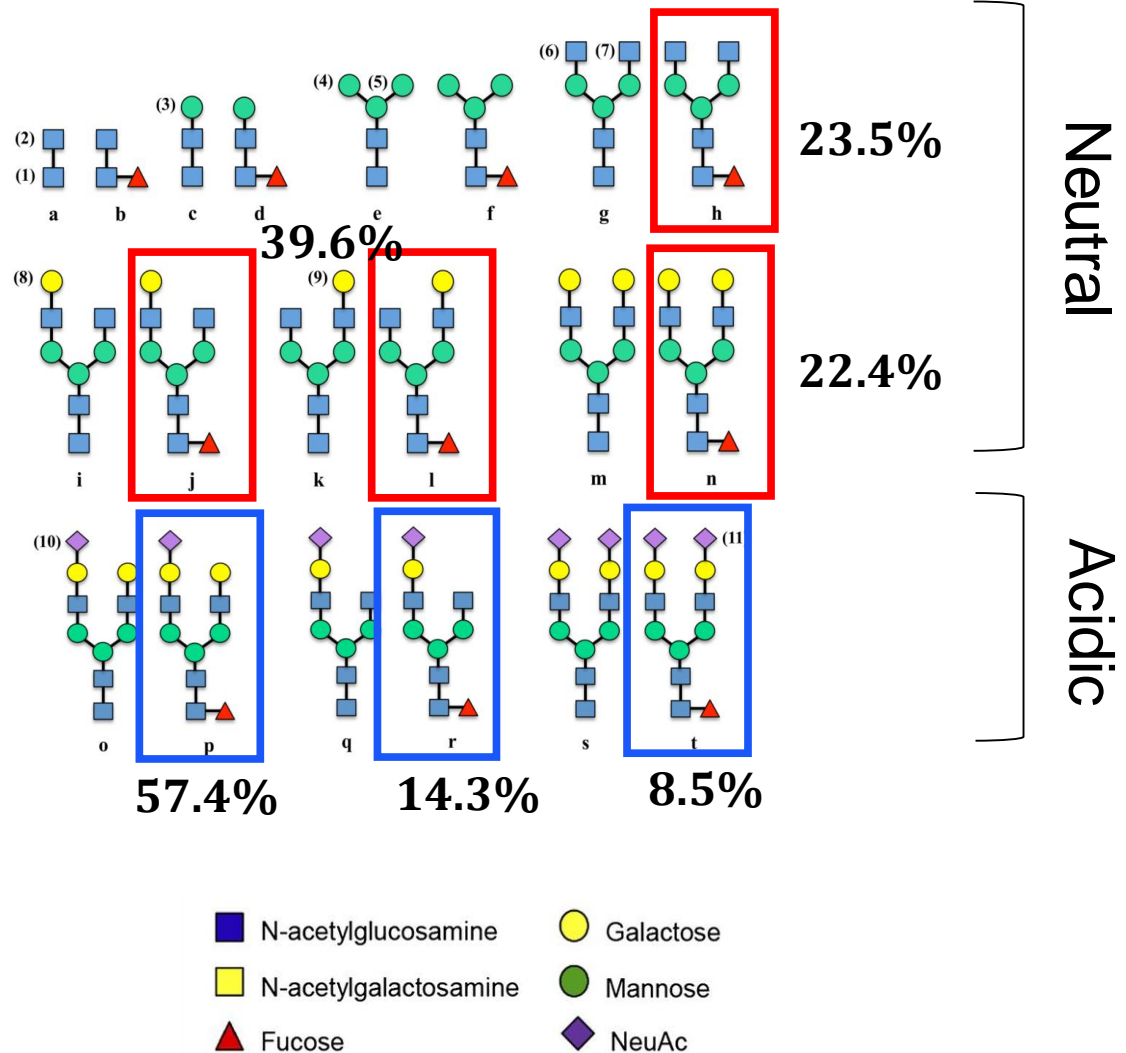
Raju *et al. Glycobiology* (2000),
10, 477-486

Gal and Sia levels are lower in RA patients

Pucic *et al, Mol. Cell Proteomics*
(2011)

Core-Fuc reduces ADCC

Parekh *et al, Nature* (1985),
316: 452-457



MD analysis of N-glycan series

Structures built with
glycam-WEB
(*glycam.org*)

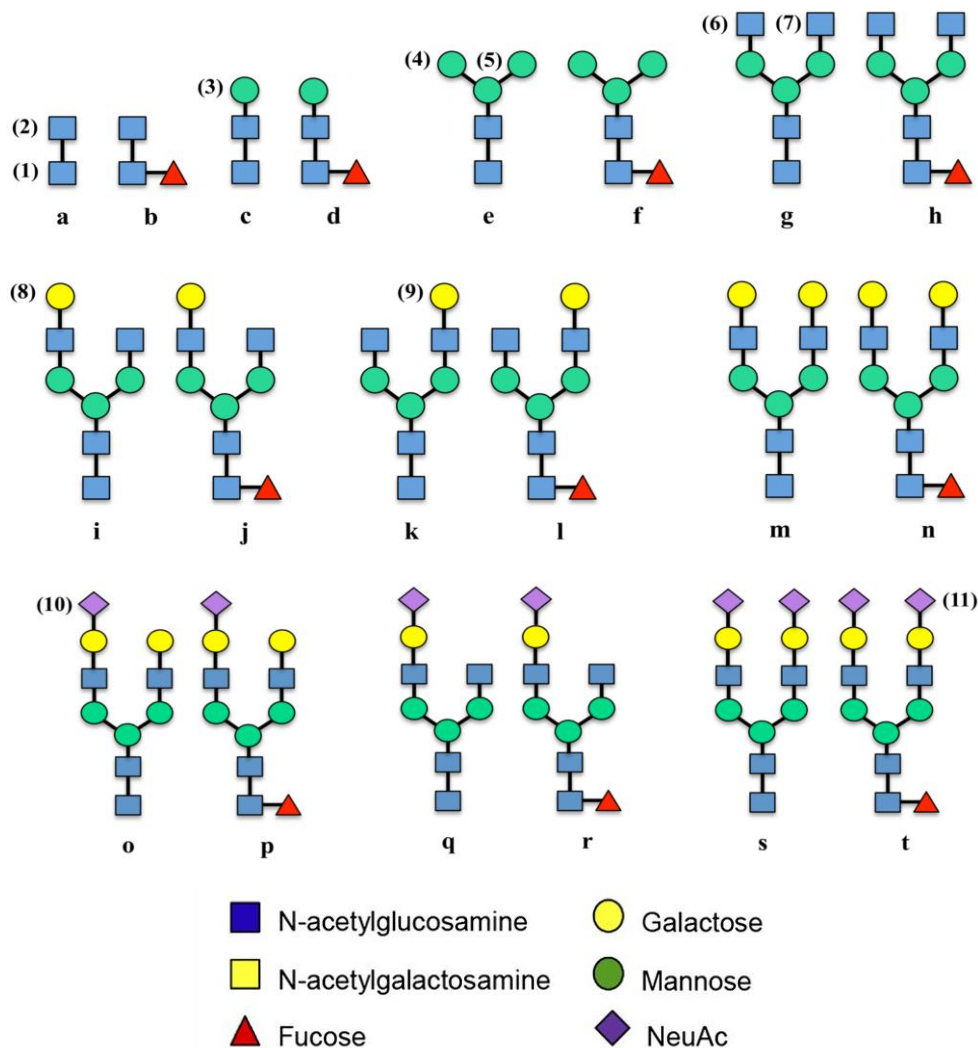
Forcefields:

Glycam06h-
12SB/TIP3P

AMBER v.12/16

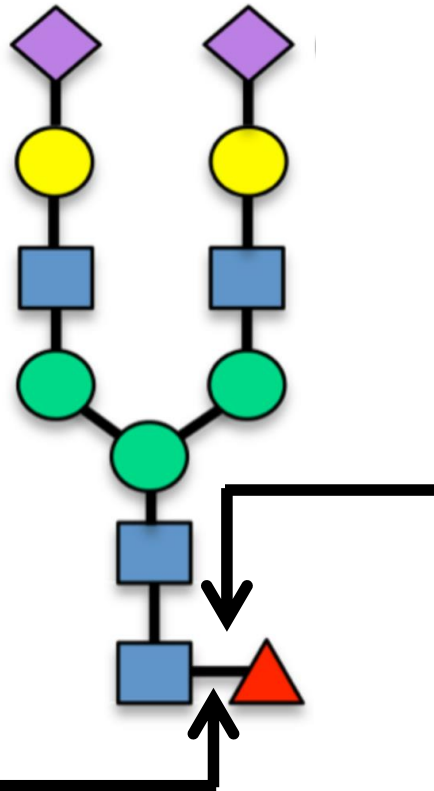
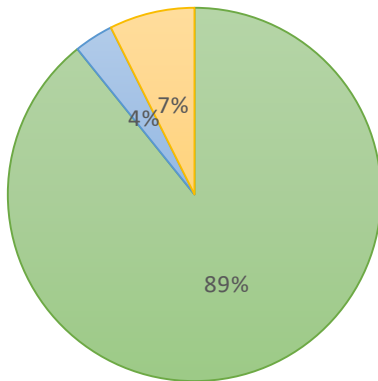
62 μ s of cumulative
simulation time

1,785,600 CPU hours
on Fionn
(2 nodes, 24ppn,
60 hrs per 100ns)

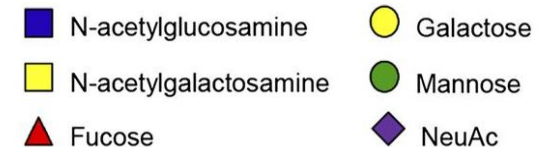
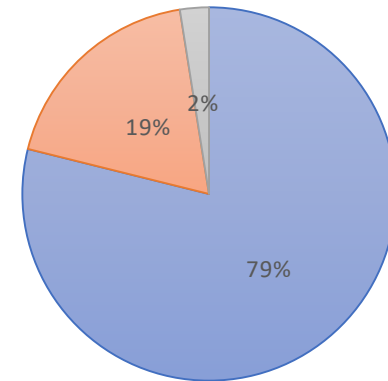


Conformational Propensities: Fucose

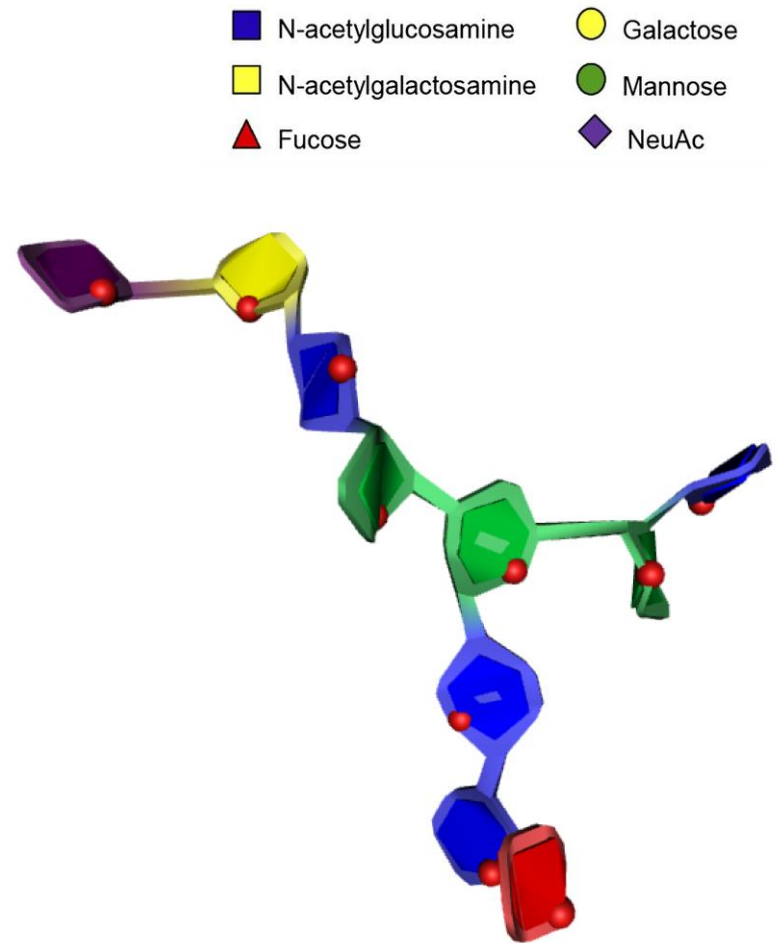
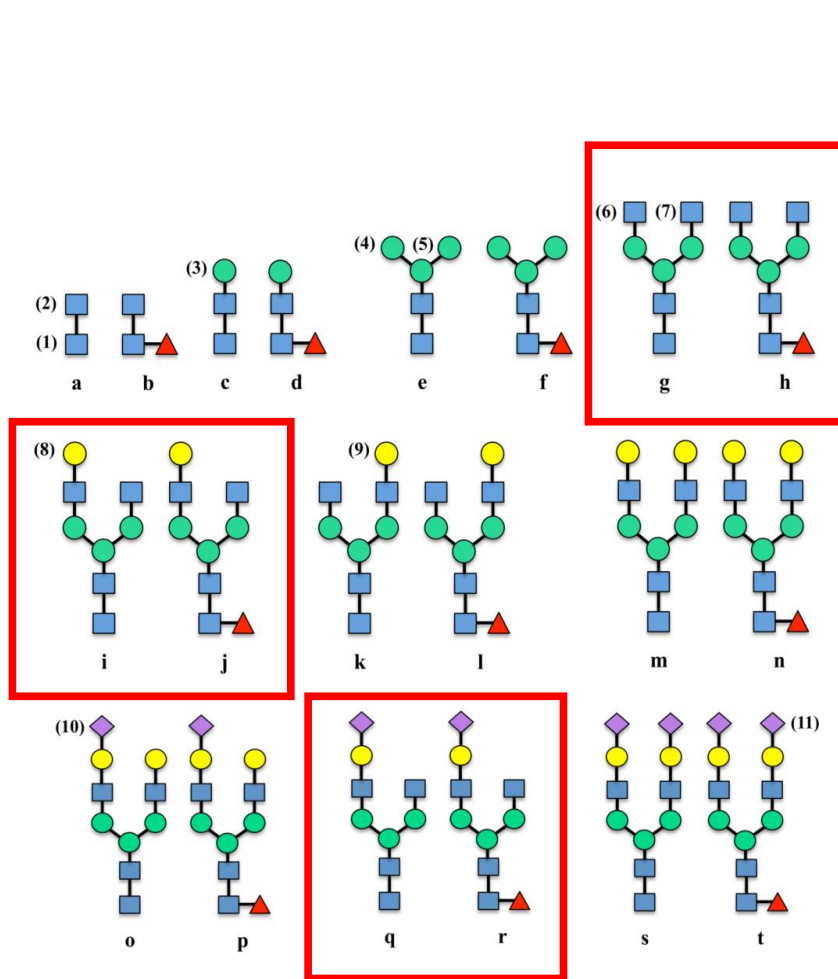
ω_1 (89%) = 47°
 ω_2 (7%) = -169°
 ω_3 (4%) = -55°



ψ_1 (79%) = -185°
 ψ_2 (19%) = 95°
 ψ_3 (3%) = -95°

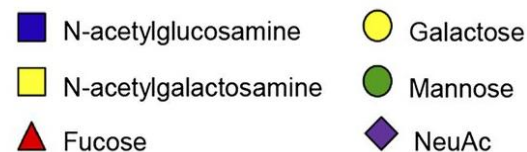
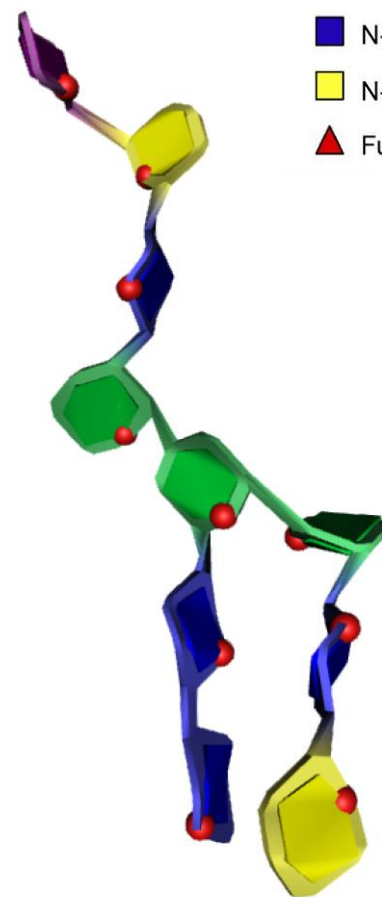
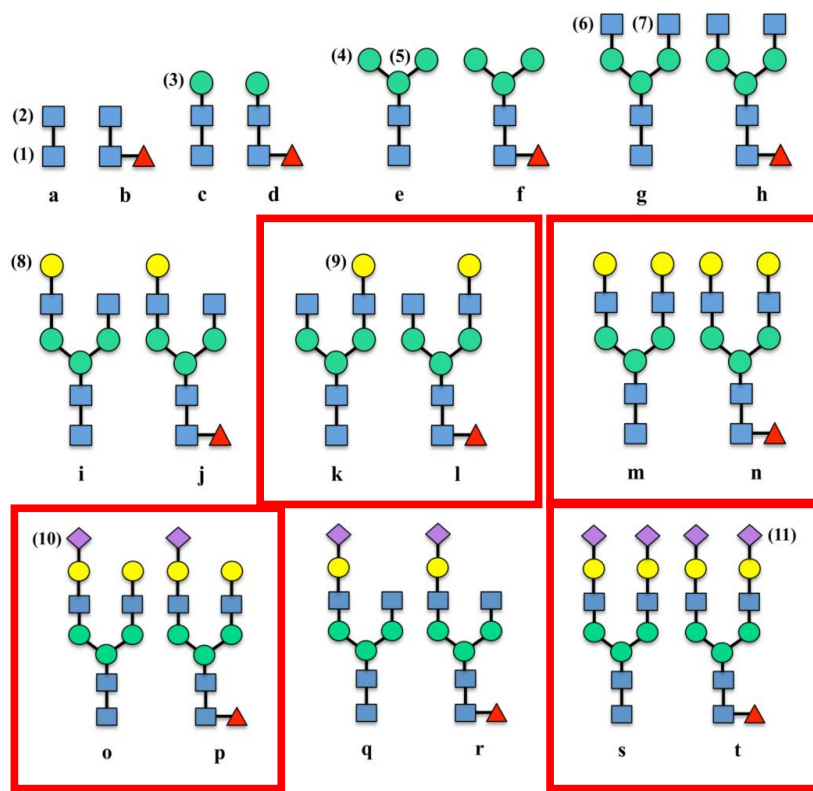


Conformational Propensities: (1-6) Arm

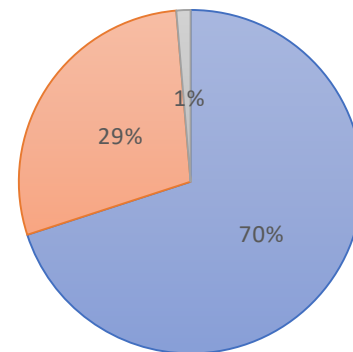


"OPEN"

Conformational Propensities: (1-6) Arm



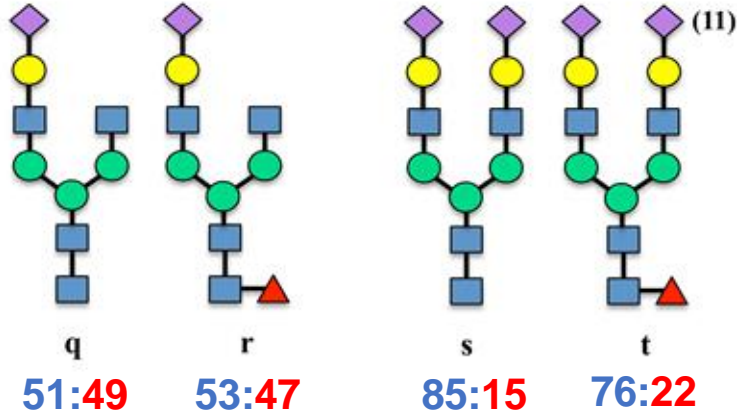
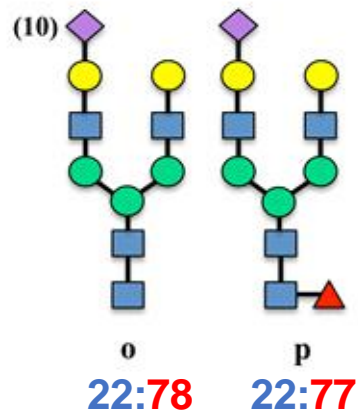
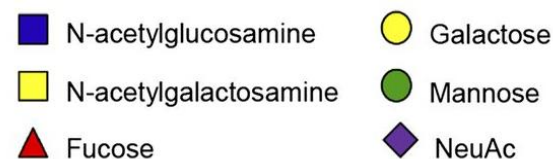
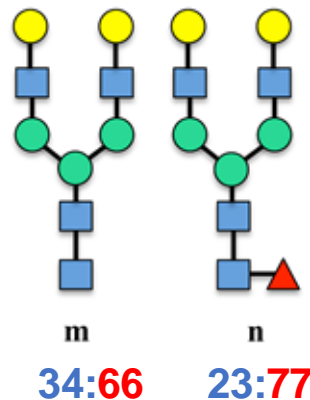
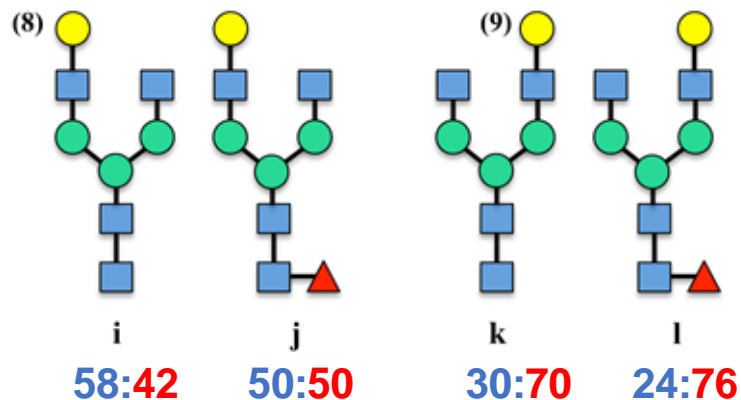
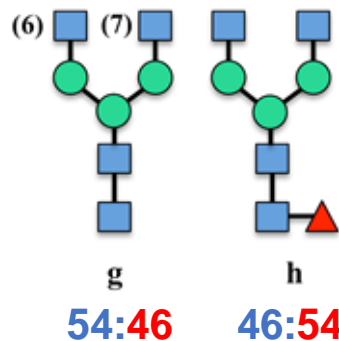
$$\begin{aligned}\psi_1 (70\%) &= 82^\circ \\ \psi_2 (29\%) &= -178^\circ \\ \psi_3 (1\%) &= -95^\circ\end{aligned}$$



"CLOSED"

Conformational Propensities: (1-6) Arm

Comparing the % of
open and **closed**
conformers



GLYCOBIOLOGY

Official Journal of the Society for Glycobiology

Volume 29 Number 1 January 2019

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Structural Biology

Sequence-to-structure dependence of isolated IgG Fc complex biantennary N-glycans: a molecular dynamics study

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Received 29 August 2018; Revised 28 September 2018; Editorial decision 12 October 2018; Accepted 15 October 2018

Abstract

Fc glycosylation of human immunoglobulins G (IgGs) is essential for their structural integrity and activity. Interestingly, the specific nature of the Fc glycoforms is known to modulate the IgG effector function and inflammatory properties. Indeed, while core-fucosylation of IgG Fc-glycans greatly affects the antibody-dependent cell-mediated cytotoxicity function, with obvious repercussions in the design of therapeutic antibodies, sialylation can reverse the antibody inflammatory response, and galactosylation levels have been linked to aging, to the onset of inflammation, and to the predisposition to rheumatoid arthritis. Within the framework of a structure-to-function relationship, we have studied the role of the N-glycan sequence on its intrinsic conformational propensity. Here we report the results of a systematic study, based on extensive molecular dynamics simulations in excess of 62 μ s of cumulative simulation time, on the effect of sequence on the structure and dynamics of increasingly larger, complex biantennary N-glycoforms isolated from the protein, i.e. from chitobiose to the larger N-glycan species commonly found in the Fc region of human IgGs. Our results show that while core fucosylation and sialylation do not affect the intrinsic dynamics of the unlinked N-glycans, galactosylation of the α (1–6) arm shifts dramatically its conformational equilibrium from an outstretched to a folded conformation. These findings are in agreement with and can help rationalize recent experimental evidence showing a differential recognition of positional isomers in glycan array data and also the preference of sialyltransferase for the more accessible, outstretched α (1–3) arm in both isolated, and Fc-bound N-glycans.

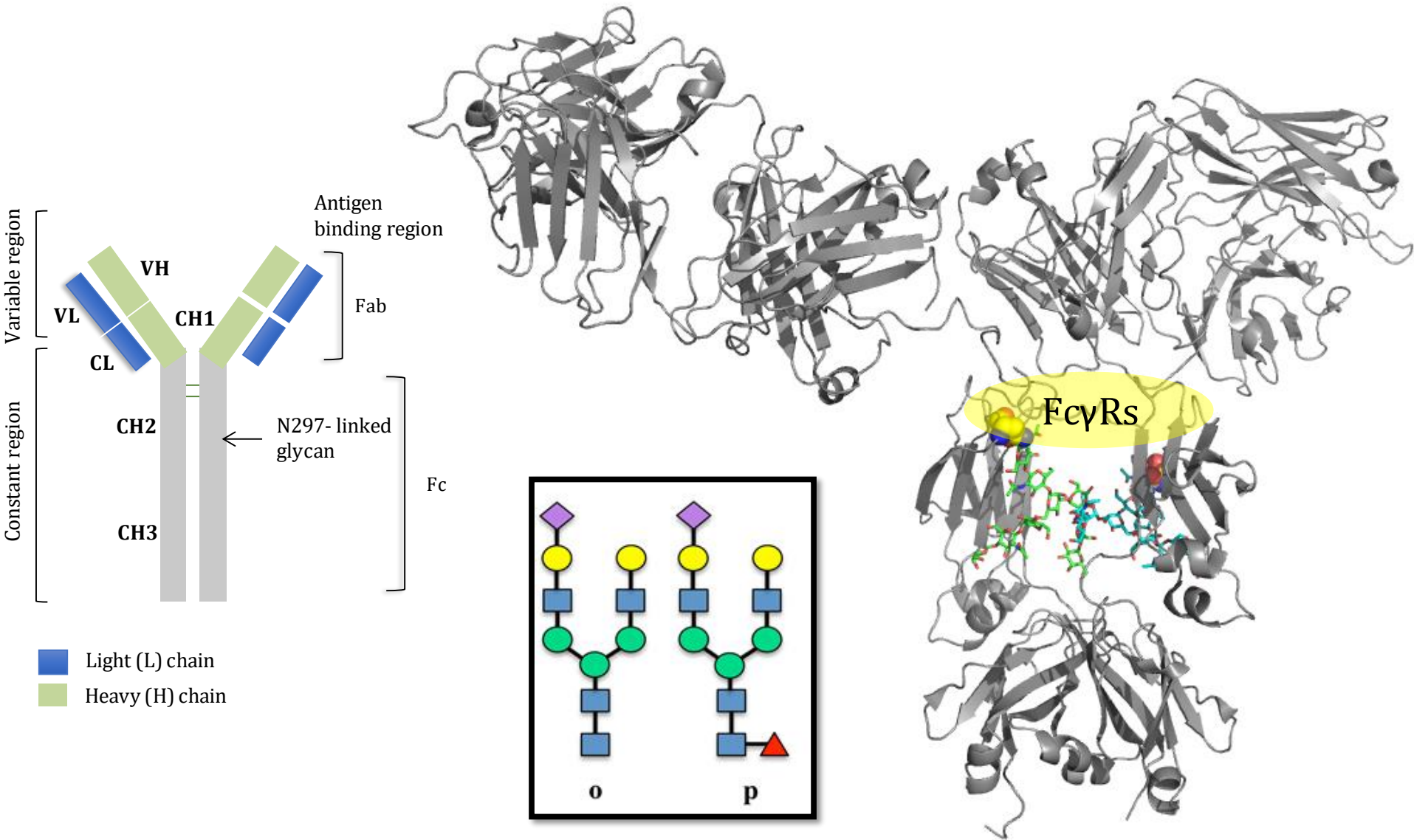
Key words: Fc-glycosylation, glycoinformatics, IgG, molecular dynamics, N-glycans

Introduction

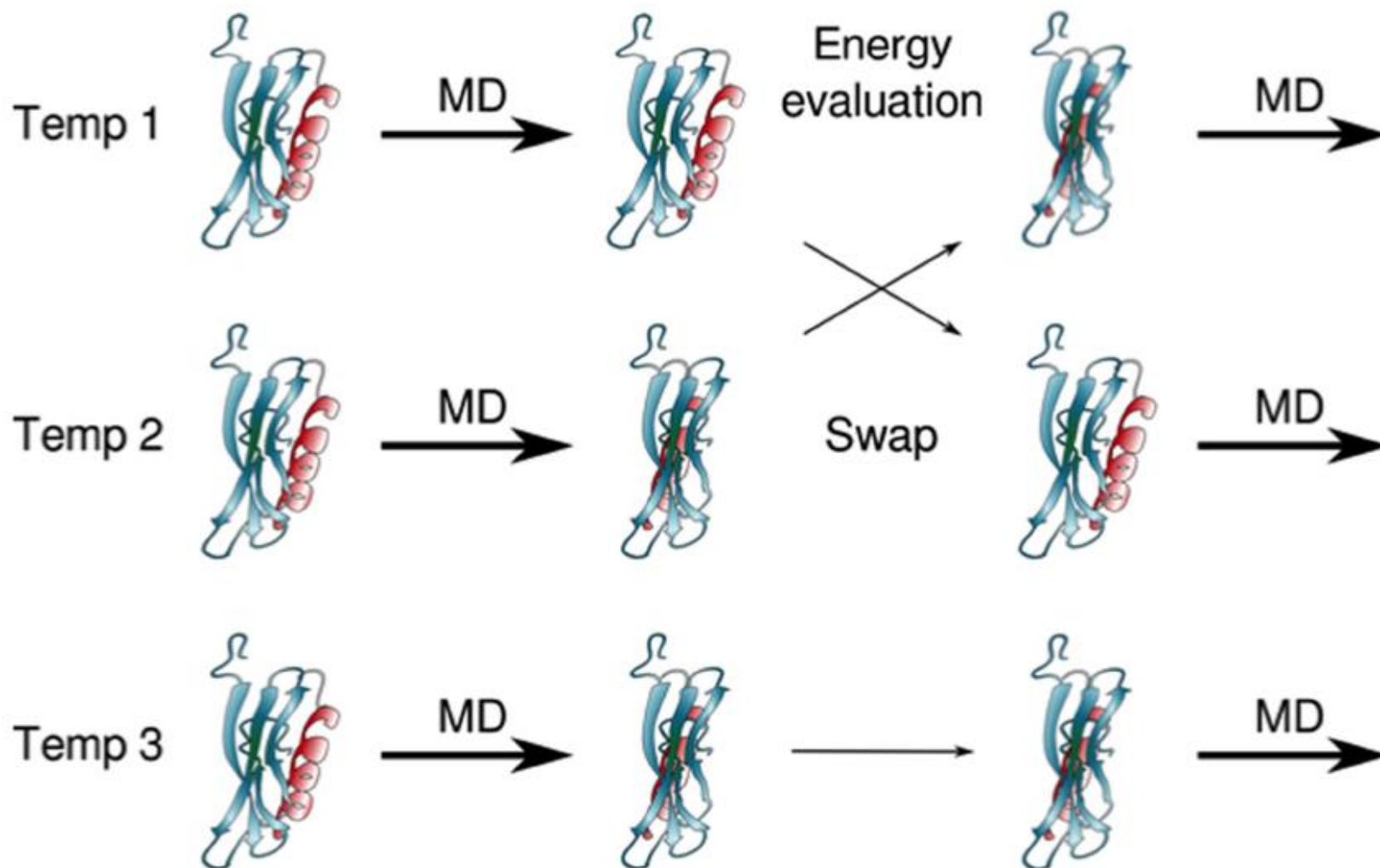
N-glycosylation of the immunoglobulin G (IgG) fragment crystallizable (Fc) region is essential for its structural stability and function (Krapp, Minura, et al. 2003; Arnold, Wormald, et al. 2007; Kobata 2008; Fang, Richardson, et al. 2016). The sequence and branching of the Fc N-glycoforms, bound at the highly conserved Asn 297 in both CH₂ domains of the Fc region, strongly affect the antibody-mediated effector function (Raju 2008; Sabedi and Barb 2016; Hayes, Frostell, et al. 2017) by modulating the binding to the immune cells' Fc receptors, thus the antibody-mediated immune

response (Tao and Morrison 1989; Shields, Namenuk, et al. 2001). In this context the effects of core-fucosylation, sialylation and galactosylation are particularly interesting. Between 81 and 98.7% of the Fc N-glycans in human IgGs are core-fucosylated (Puck, Knezevic, et al. 2011). Core fucosylation, where fucose is α (1–6) linked to the chitobiose core, greatly affects the IgG antibody-dependent cell-mediated cytotoxicity (ADCC) function. More specifically, a strongly enhanced ADCC corresponds to nonfucosylated Fc N-glycan species (Satoh, Iida, et al. 2006; Kanda, Yamada, et al. 2007; Matsumiya, Yamaguchi, et al. 2007; Strohl 2009; Ratner

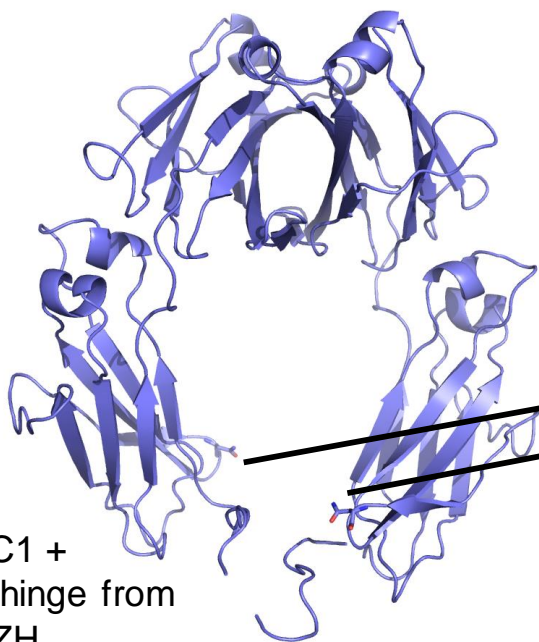
IgG1 Fc N-glycosylation



Replica Exchange MD



IgG1 Fc + N-glycans simulations



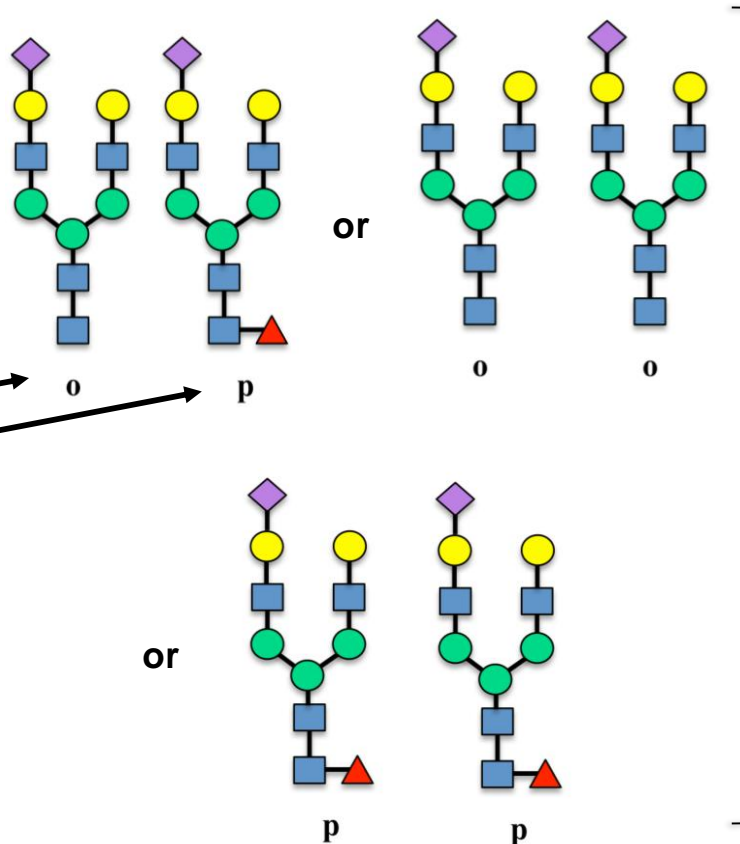
PDB 1FC1 +
Fc IgG1 hinge from
PDB 1HZH

Glycans built with glycam-WEB
(glycam.org)

MD simulation

Glycam06j-1/TIP3P

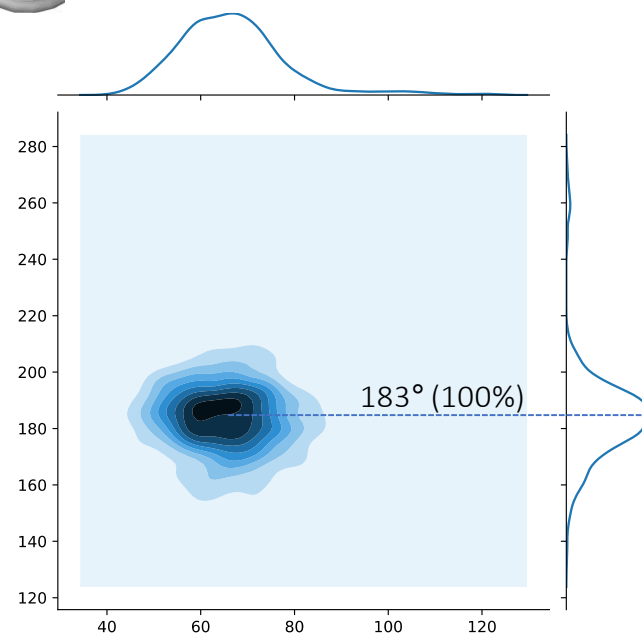
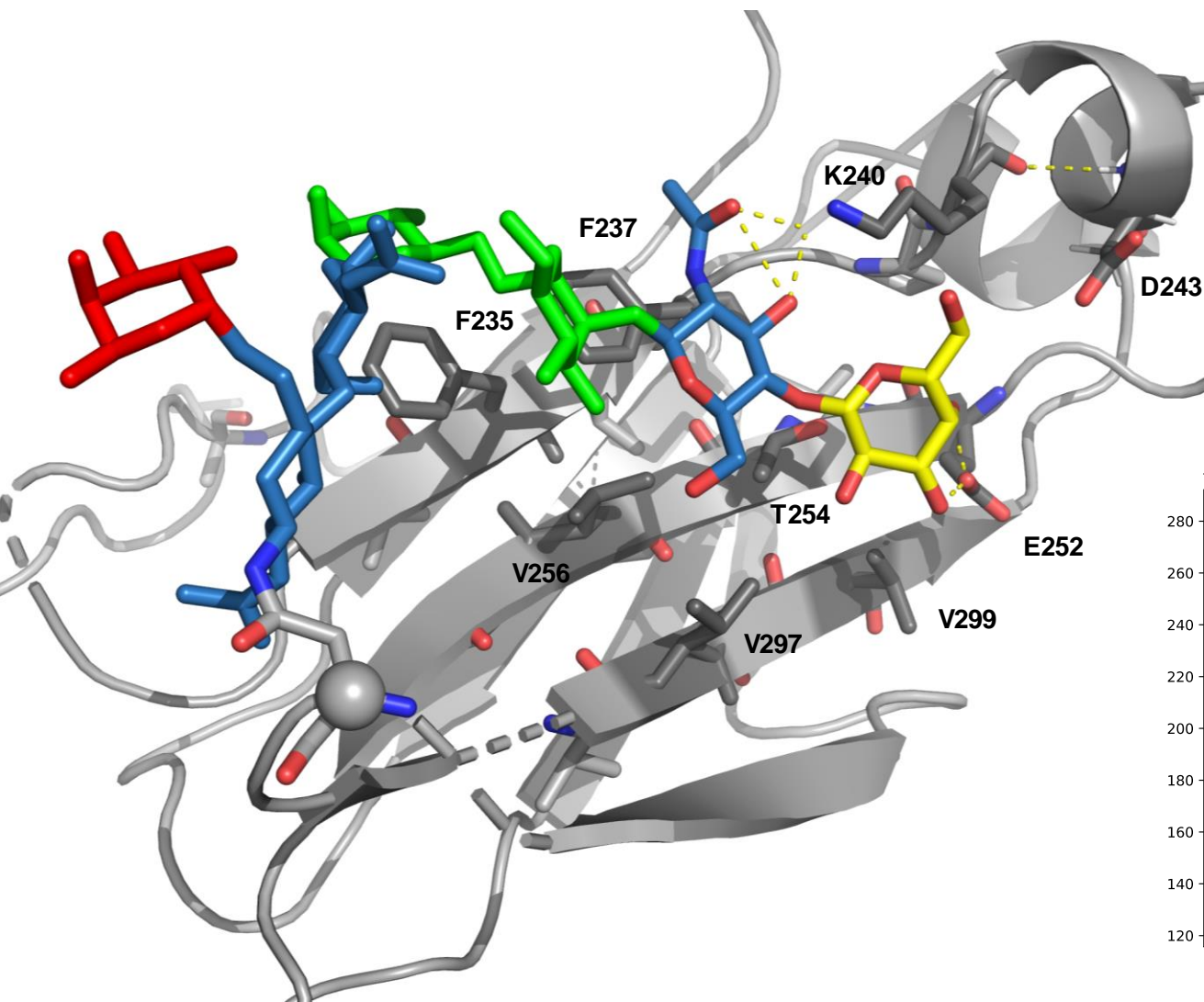
AMBER AMBER12SB with NAMD 2.31b1



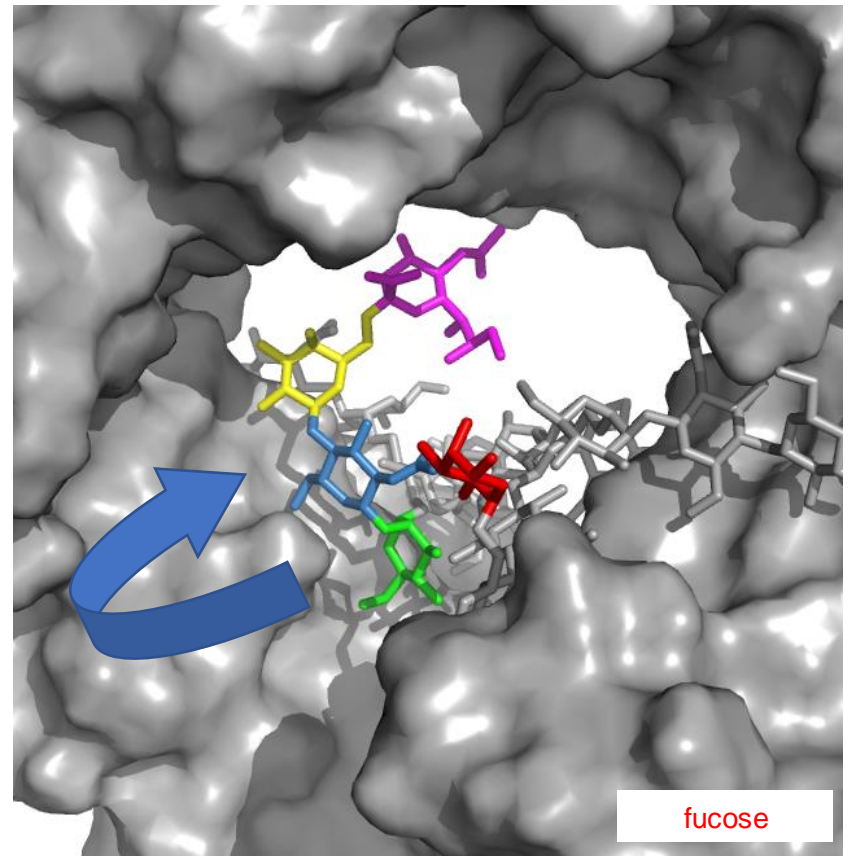
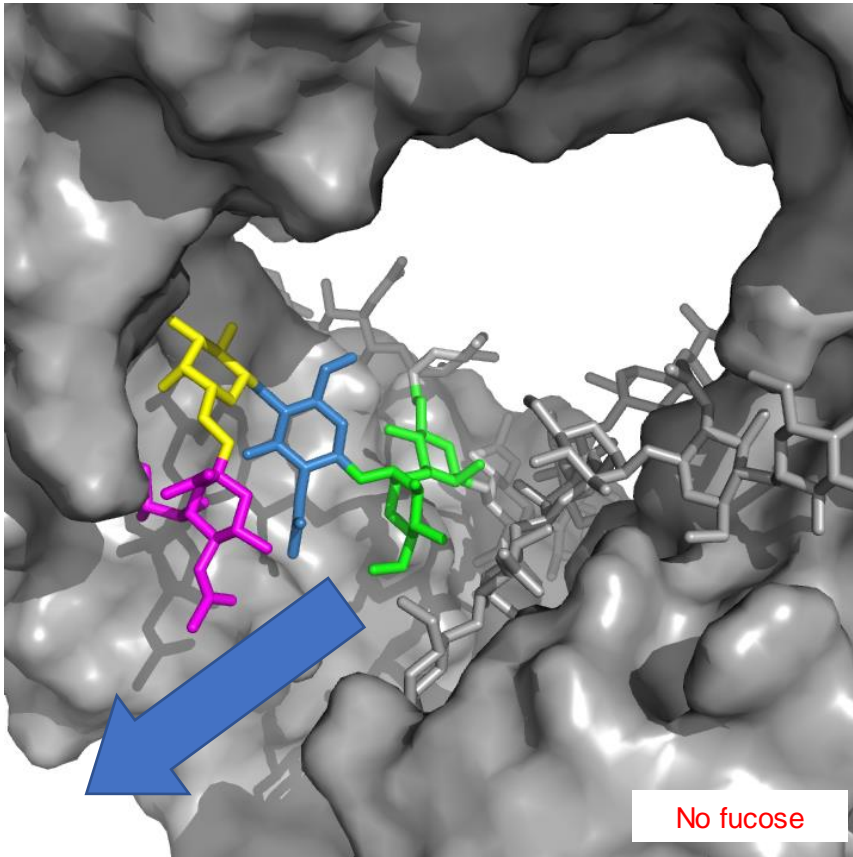
"OPEN" &
"CLOSED"
Starting
conformation

Generated REMD:
17ns * 90 replicas for
each starting structure

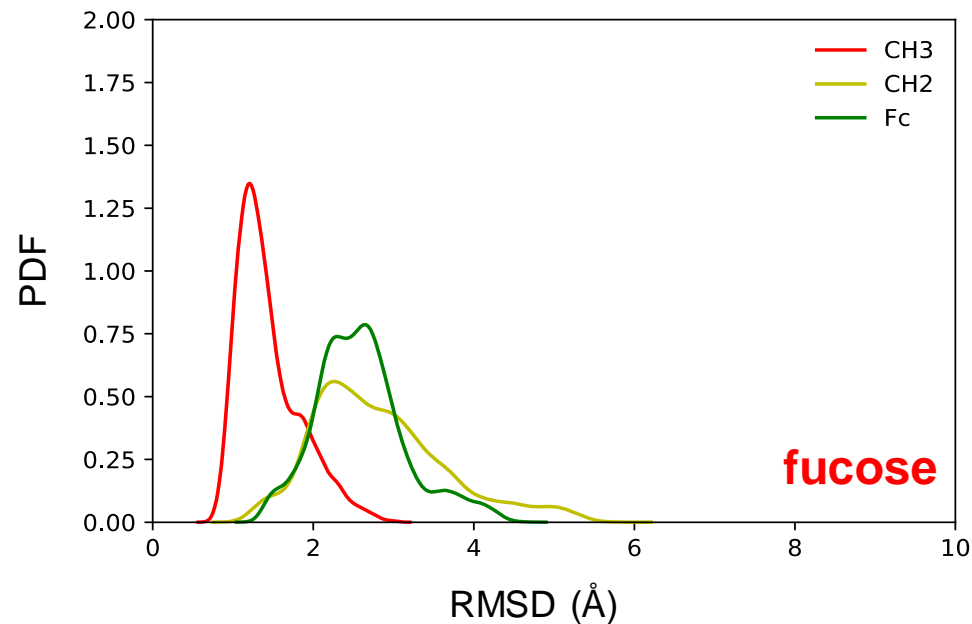
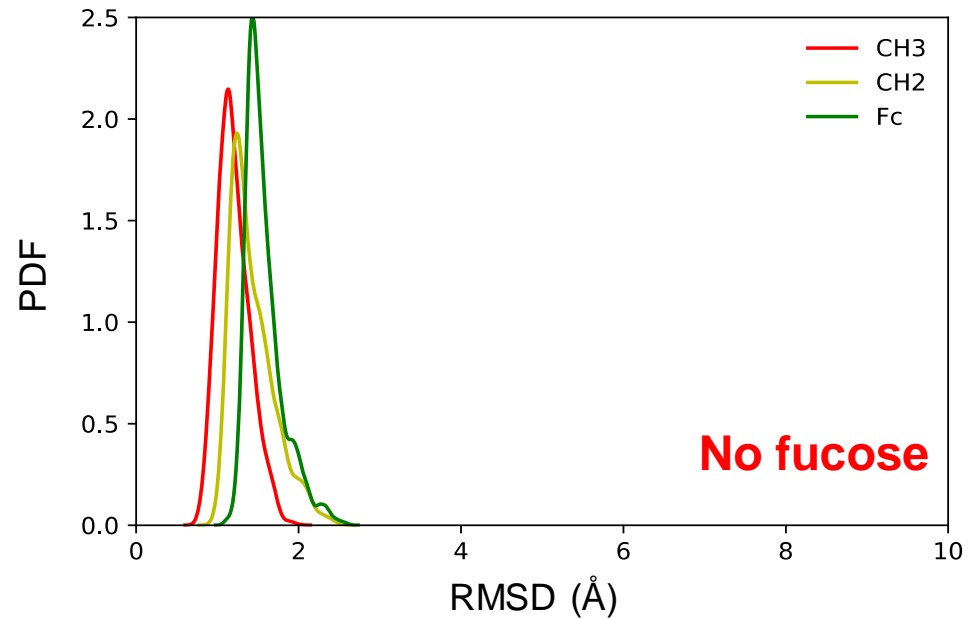
Restriction of the $\alpha(1-6)$ arm

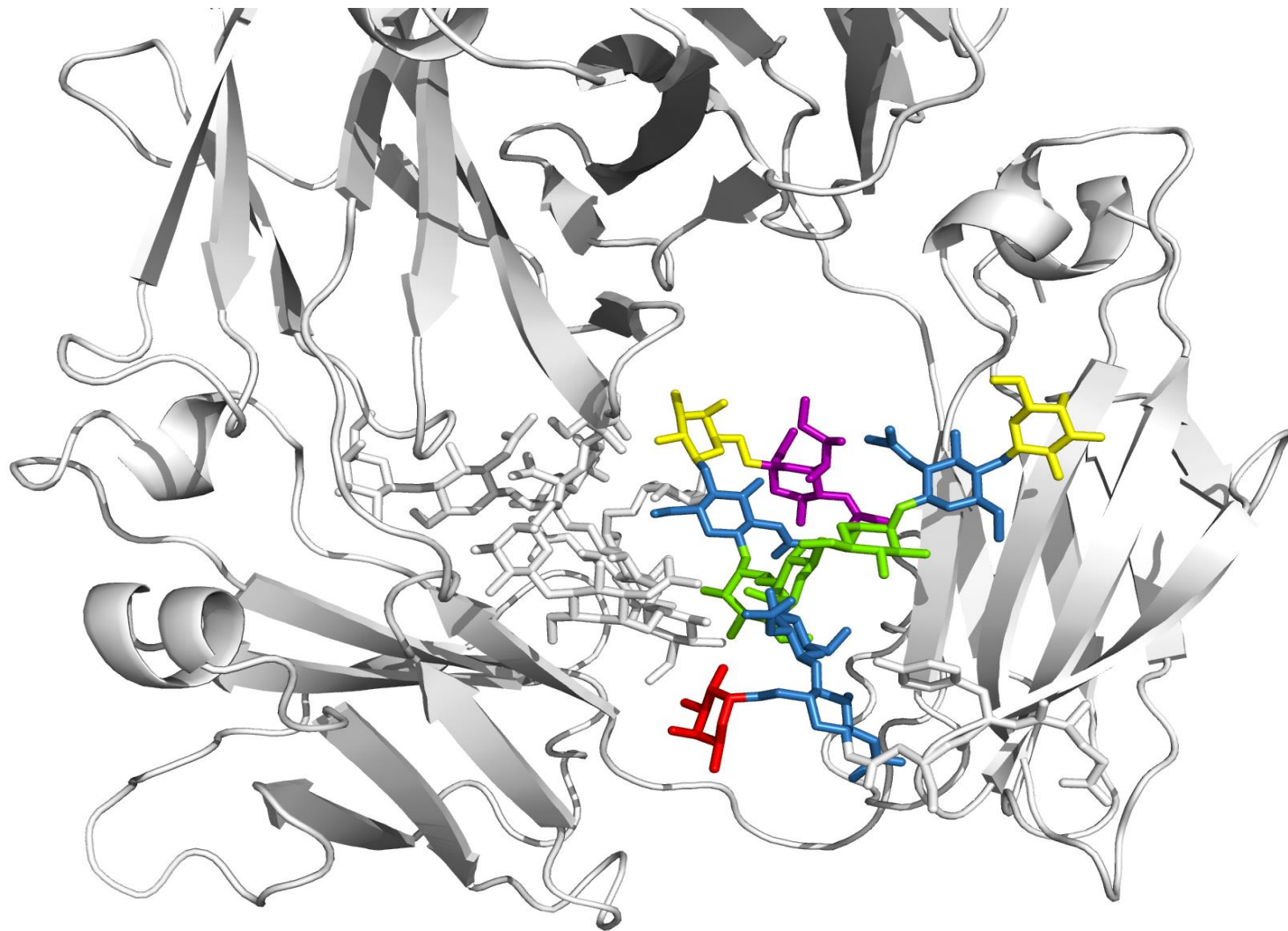


Core fucose regulates the $\alpha(1-3)$ arm



Dynamics of the CH2 domain (protein)







New Results

Comment on this paper

Previous

An atomistic perspective on ADCC quenching by core-fucosylation of IgG1 Fc N-glycans from enhanced sampling molecular dynamics

 Aoife Harbison,  Elisa Fadda

doi: <https://doi.org/10.1101/701896>

This article is a preprint and has not been certified by peer review [what does this mean?].


Posted July 15, 2019.

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 Supplementary Material


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Abstract

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Abstract

The immunoglobulin type G (IgG) Fc N-glycans are known to modulate the interaction with membrane-bound Fc γ receptors (Fc γ Rs), fine-tuning the antibody's effector function in a sequence-dependent manner. Particularly interesting in this respect are the roles of galactosylation, which levels are linked to autoimmune conditions and aging, of core fucosylation, which is known to reduce significantly the antibody-dependent cellular cytotoxicity (ADCC), and of sialylation, which also reduces ADCC but only in the context of core-fucosylation. In this work we provide an atomistic level perspective through enhanced sampling computer simulations, based on replica

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Thank you for your attention!