

Sweetening your antibodies

IgG Fc afucosylation as molecular switch

Dr. Floris Loeff

Head of Biologics laboratory, Sanquin Health Solutions, Amsterdam

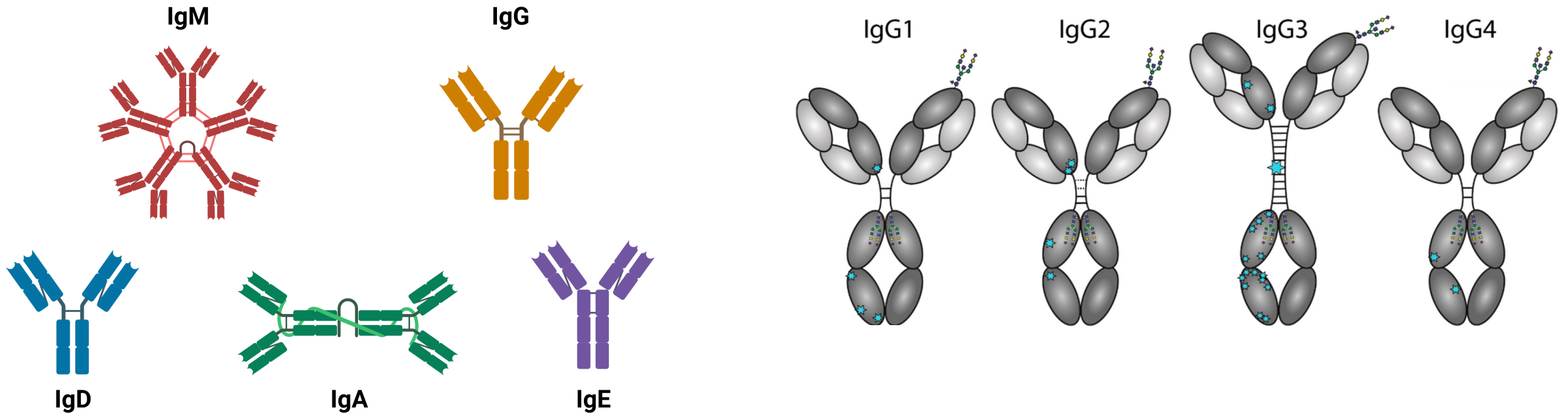
For Life.



The many flavours of antibodies

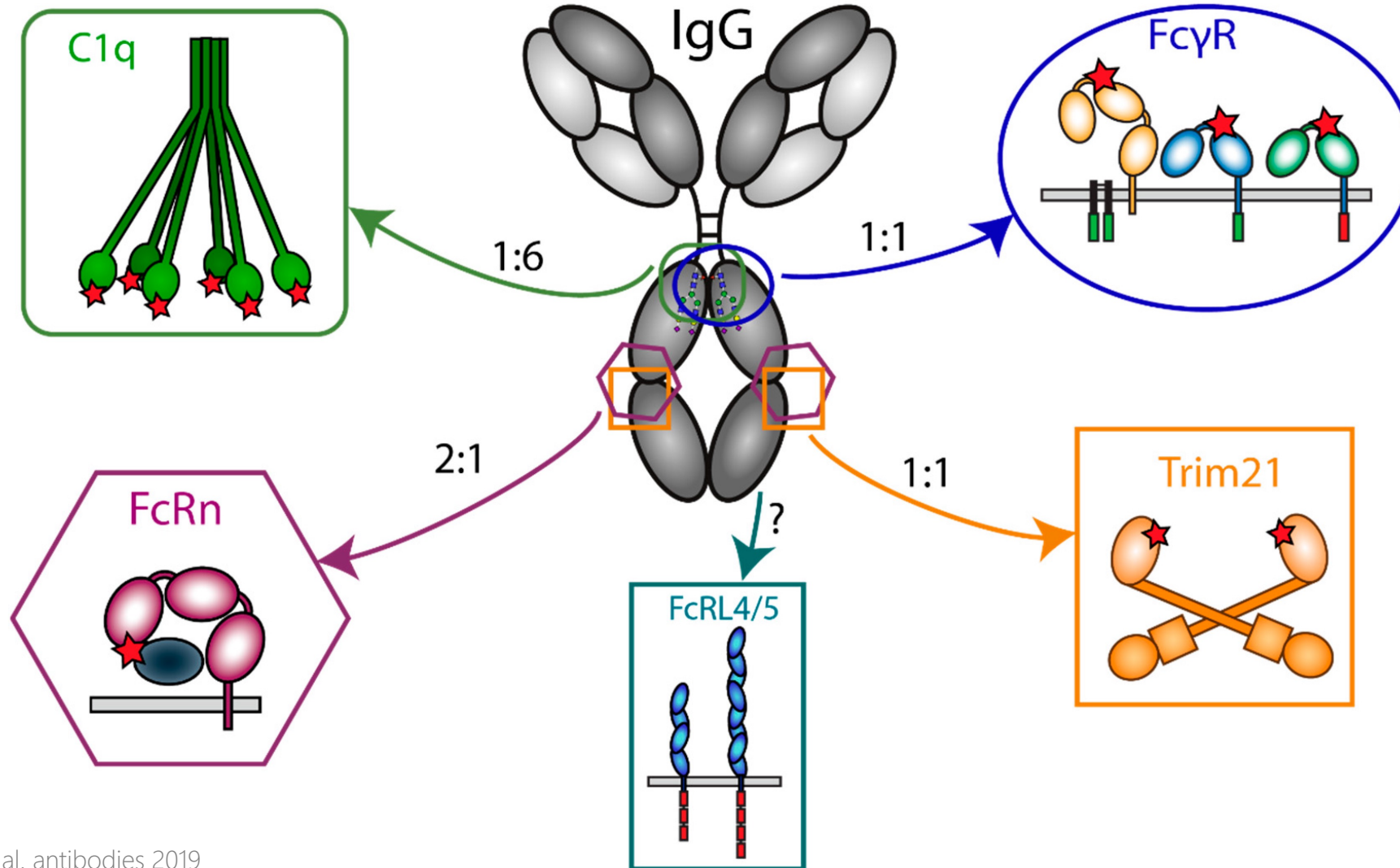
Isotypes

IgG subclasses



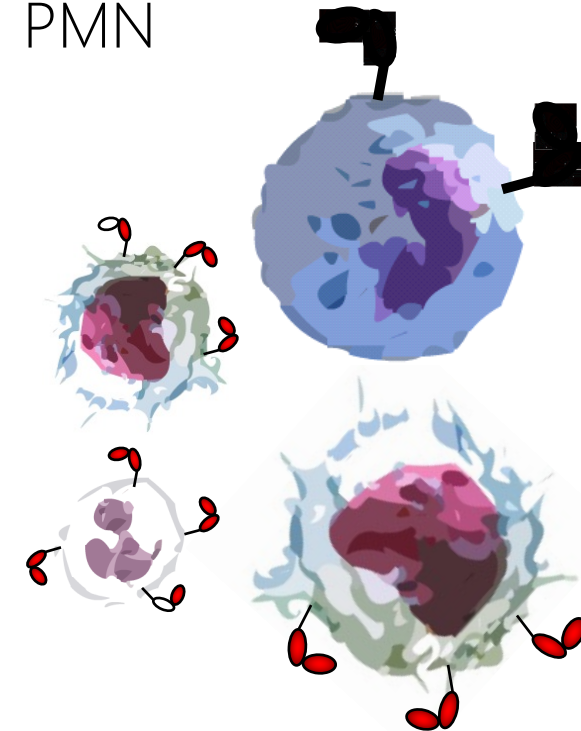


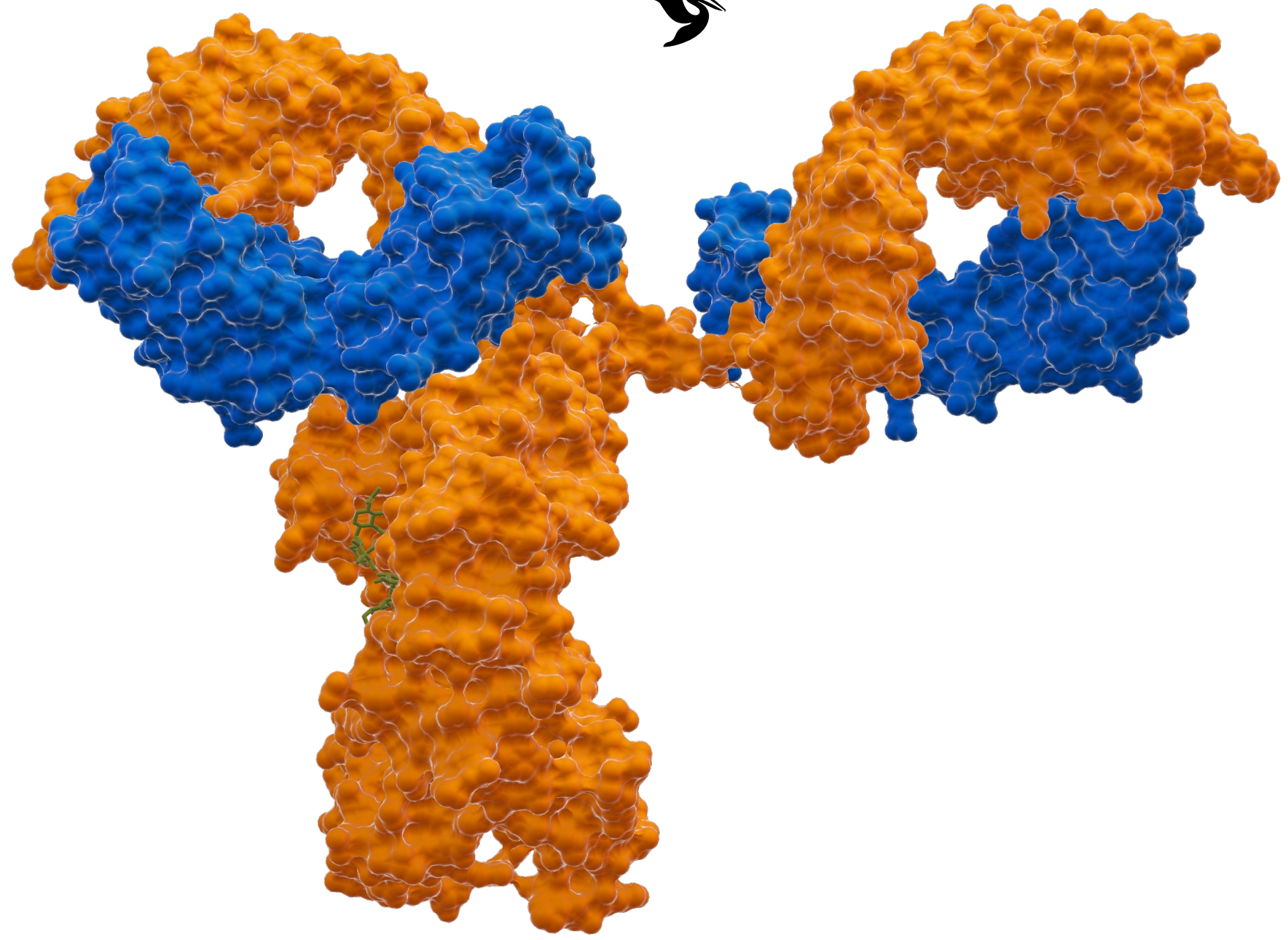
Interaction of IgG with Fc effector molecules

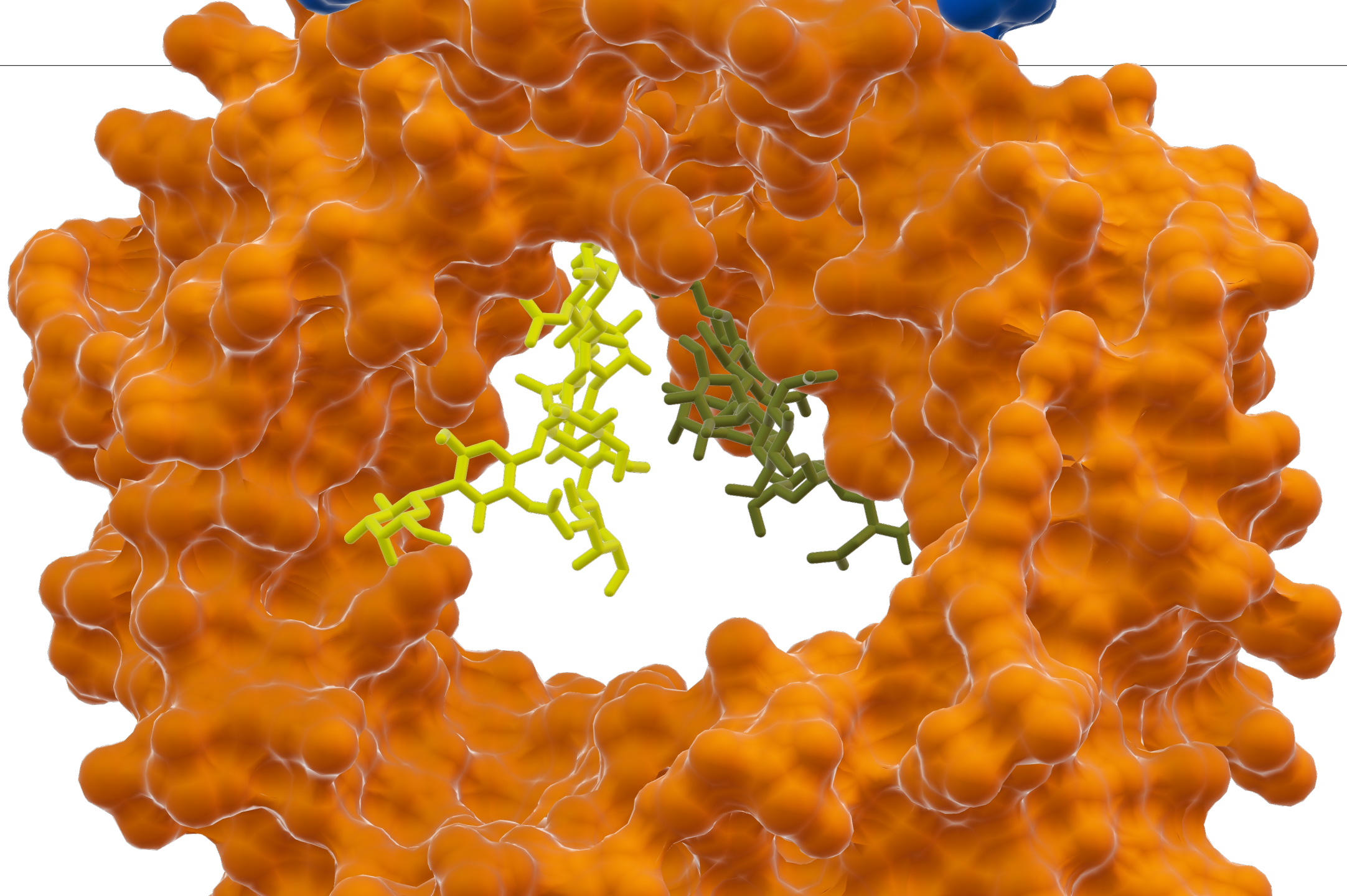


Fc γ RI, Fc γ RII, Fc γ RIII

- NK-cells
- Macrophages
- Monocytes
- PMN

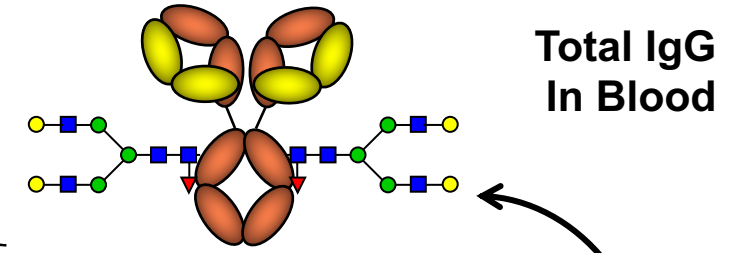
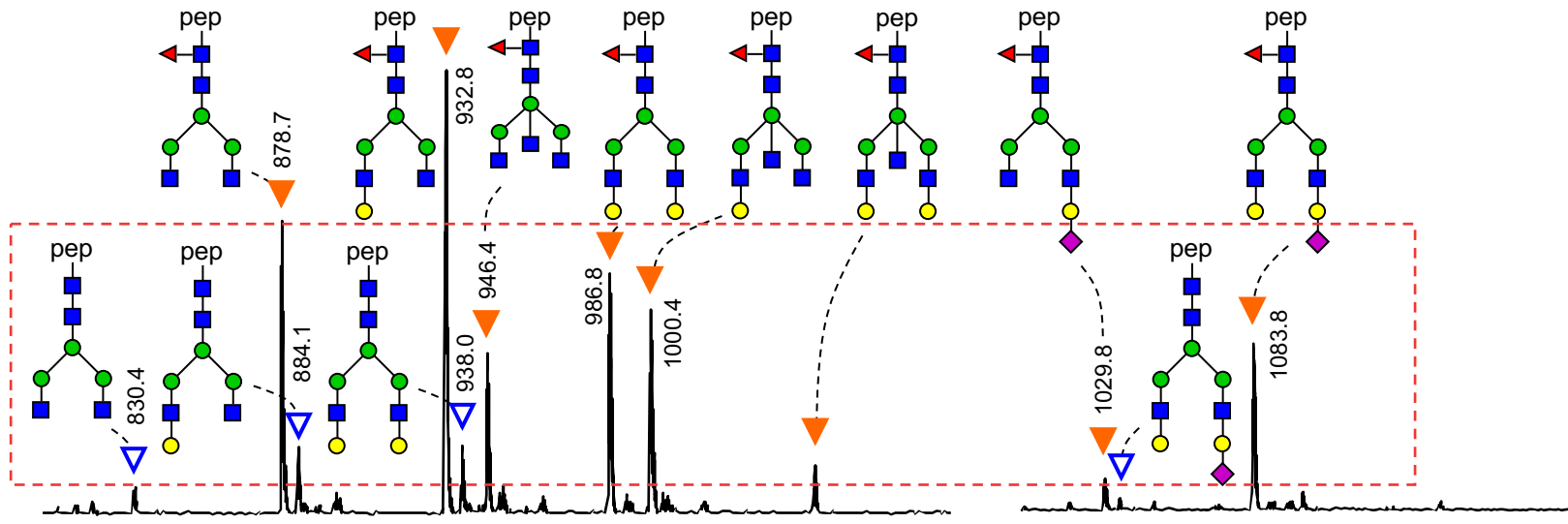






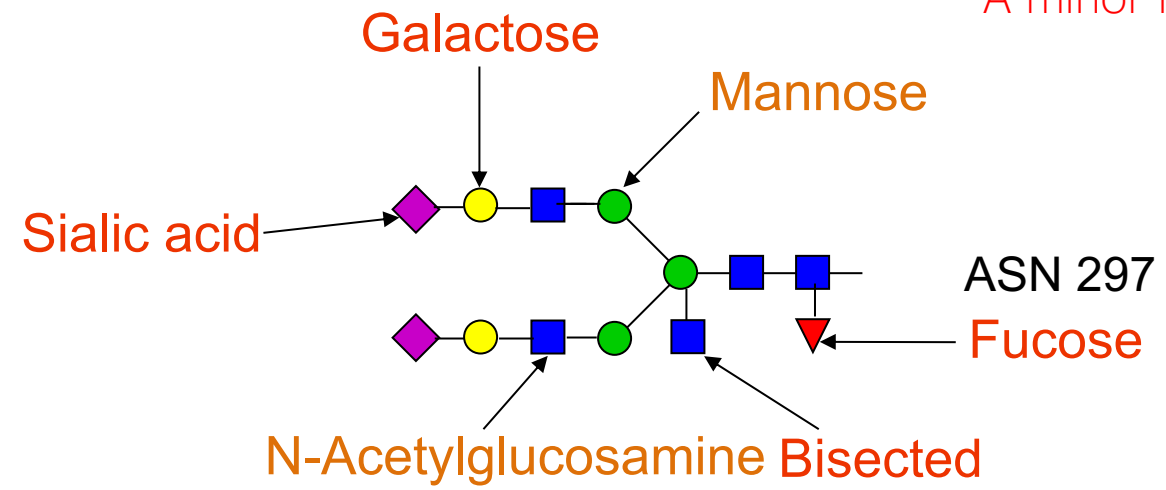


IgG fucosylation of conserved N297-linked Fc glycan



Total IgG In Blood

Blood

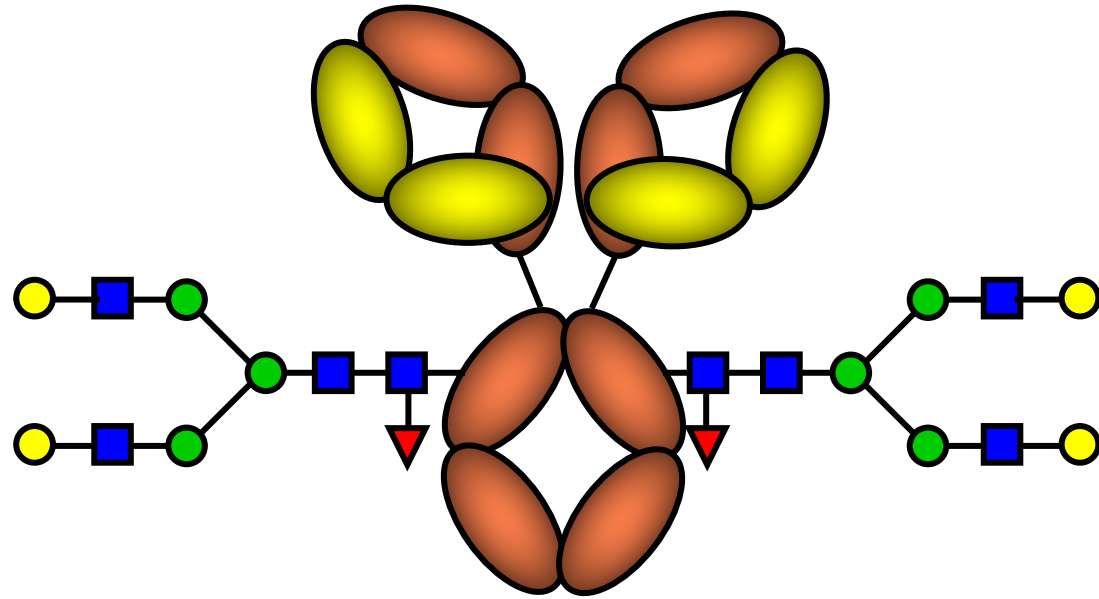


Afucosylated IgG
A minor fraction of total

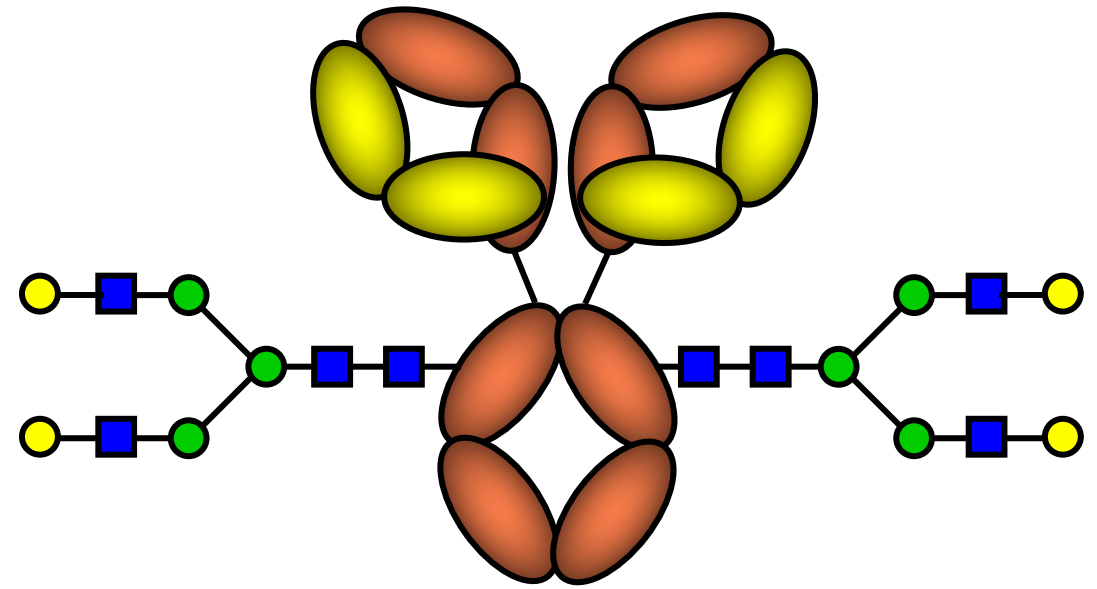


Fucosylated IgG is the norm

~94%



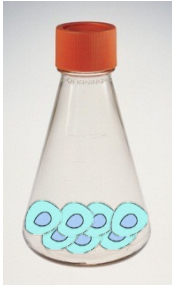
~6%



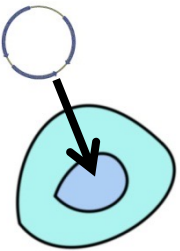


In-house workflow producing glycosylation variants of recombinant antibodies

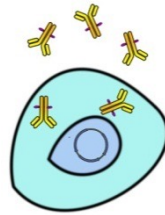
Culture cells



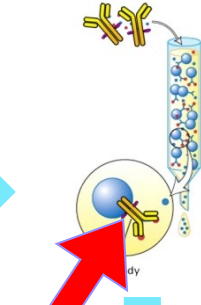
Transfection



IgG production



IgG isolation

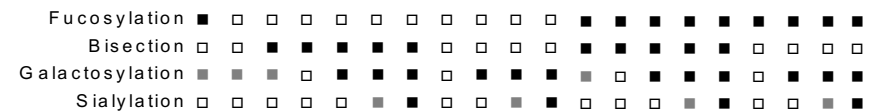
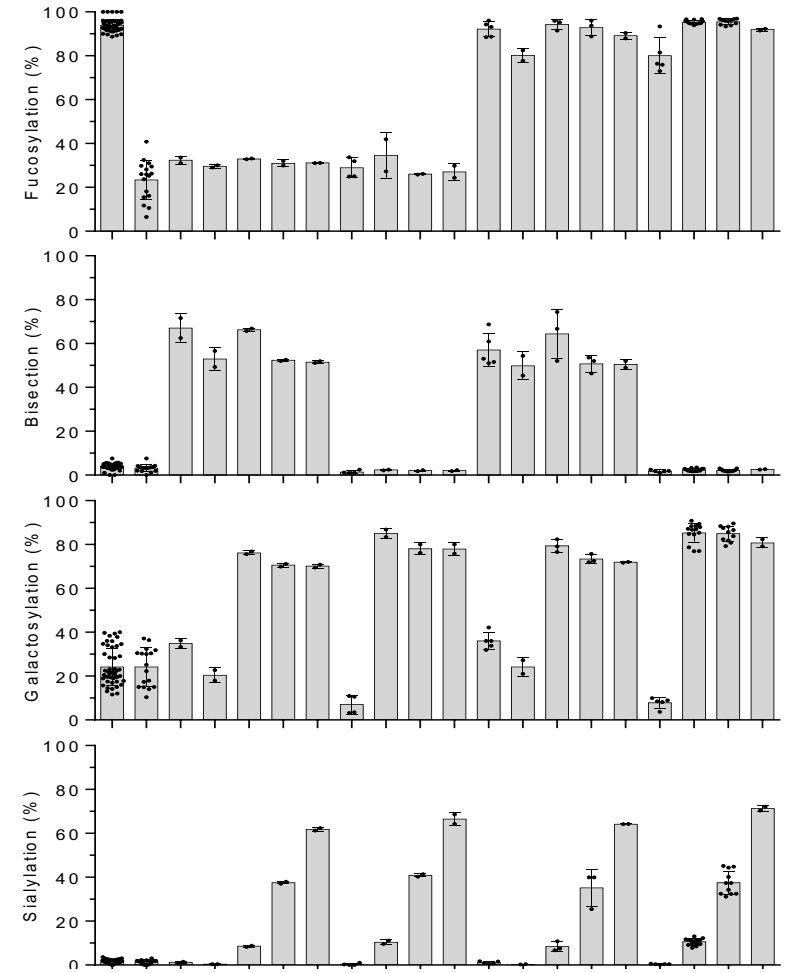
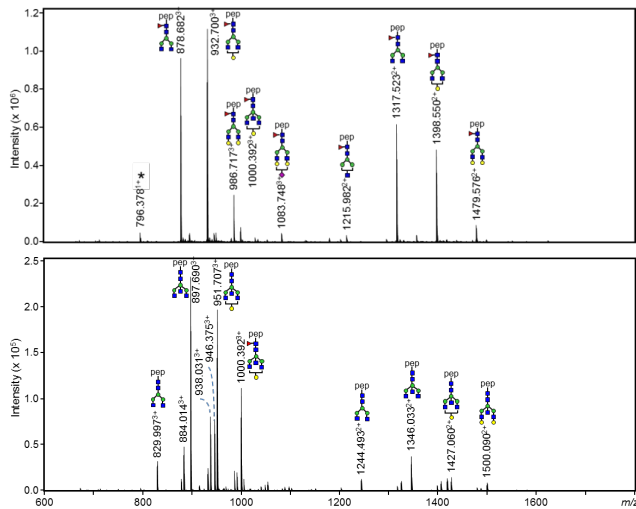


Ex vivo glycosylation

Glycan analysis in Mass Spec

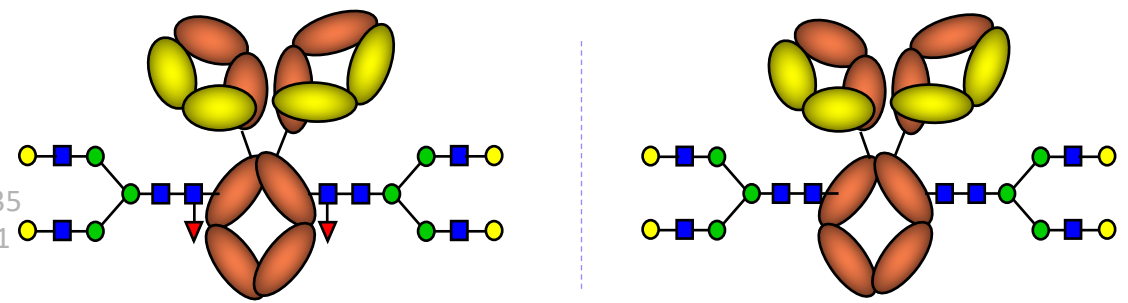
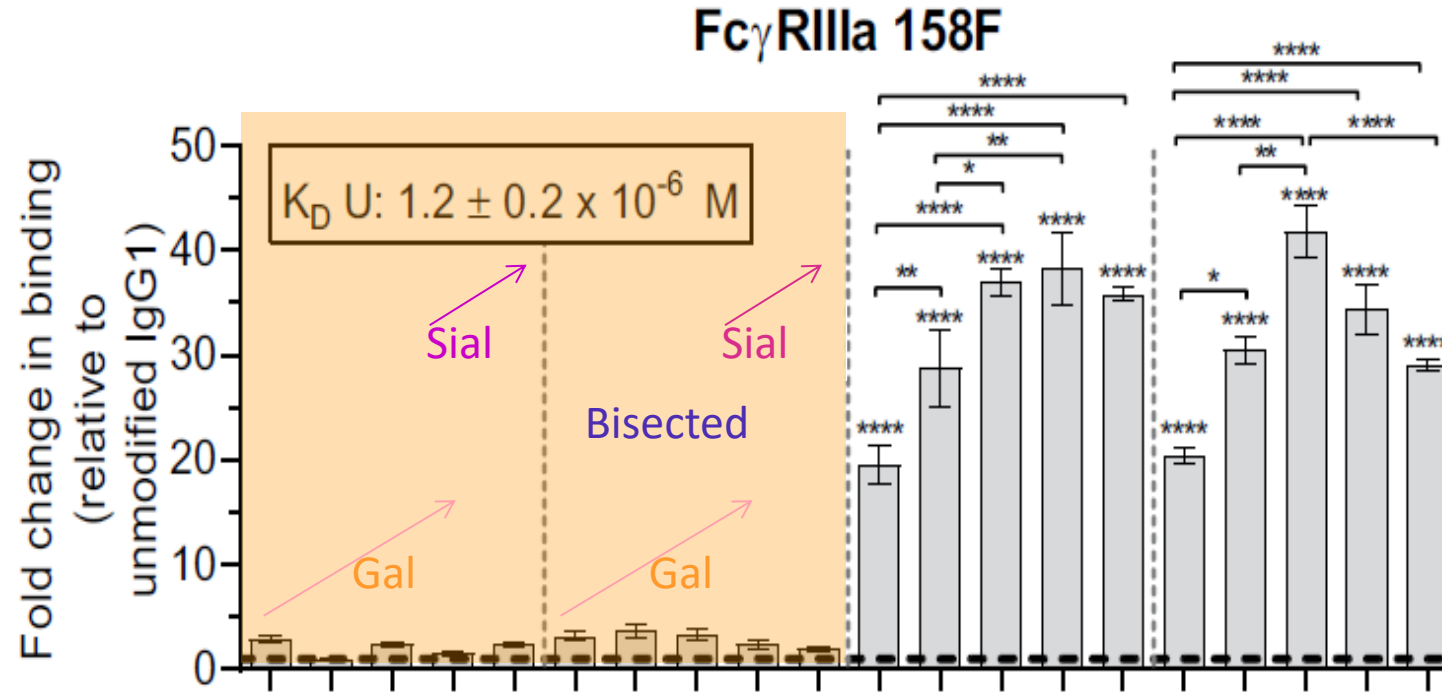


Data analysis





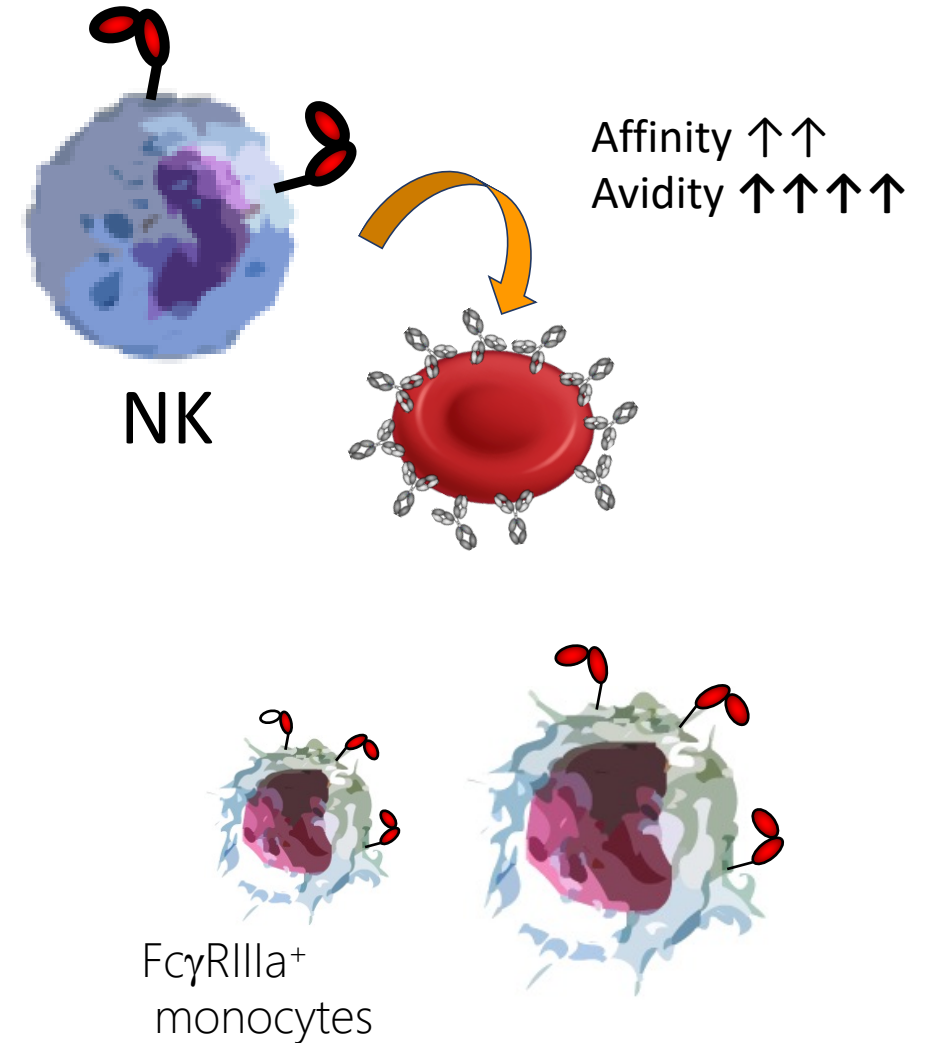
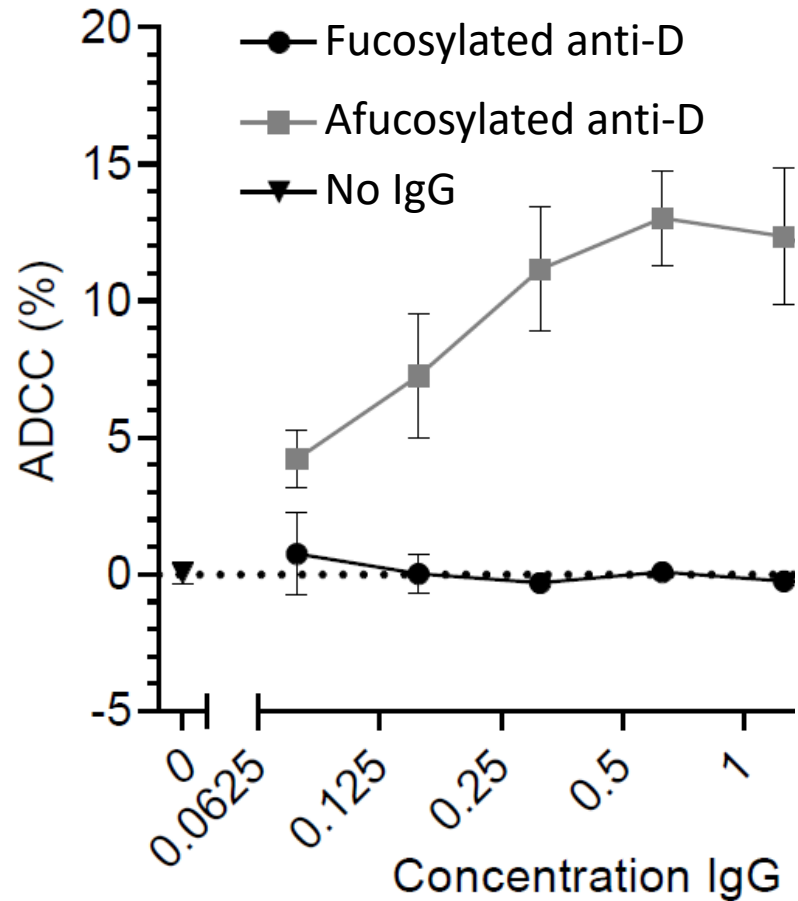
Afucosylated IgG has elevated binding for the human FcγRIII family



Dekkers et al *Frontiers in Immunology* 2017;8:877
 Temming et al *Journal of Immunology* 2019;203(12):3126-3135
 Bruggeman et al *Journal of Immunology* 2017;199(1):204-211
 Shields et al *J Biol Chem* 2002;277:26733-40
 Shinkawa *J Biol Chem* 2003;278:3466-73



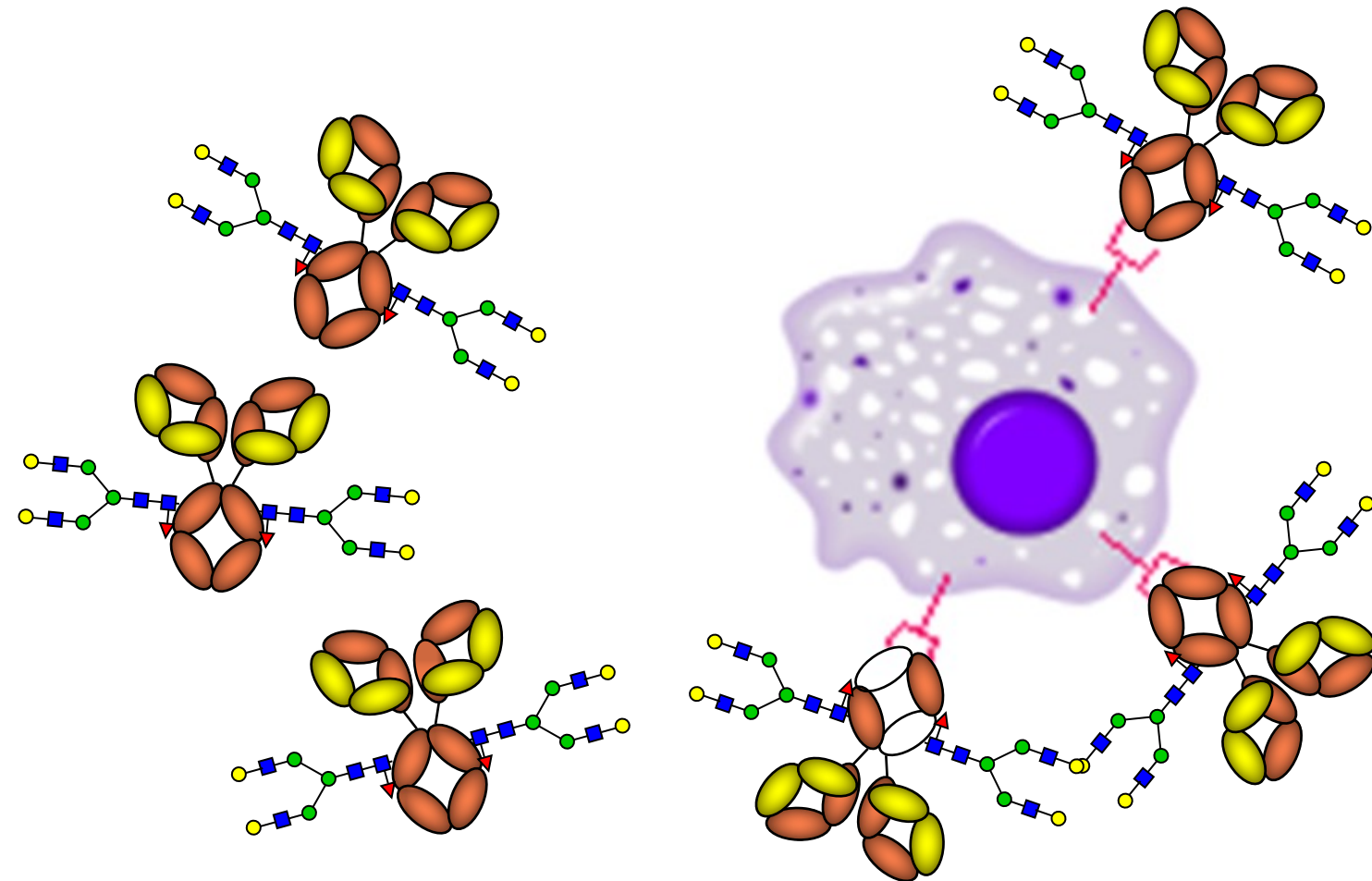
Afucosylation enhances NK-cell mediated ADCC





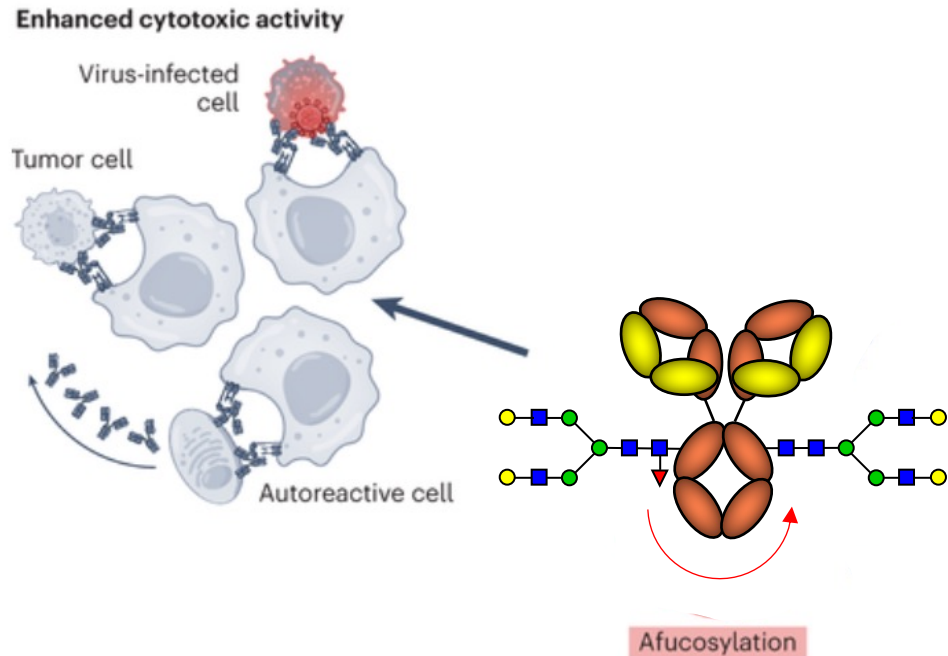
Fucosylation as molecular switch

Afucosylated IgG outcompetes fucosylated IgG for FcγR binding





Afucosylation enhanced cytotoxicity



Tumor killing

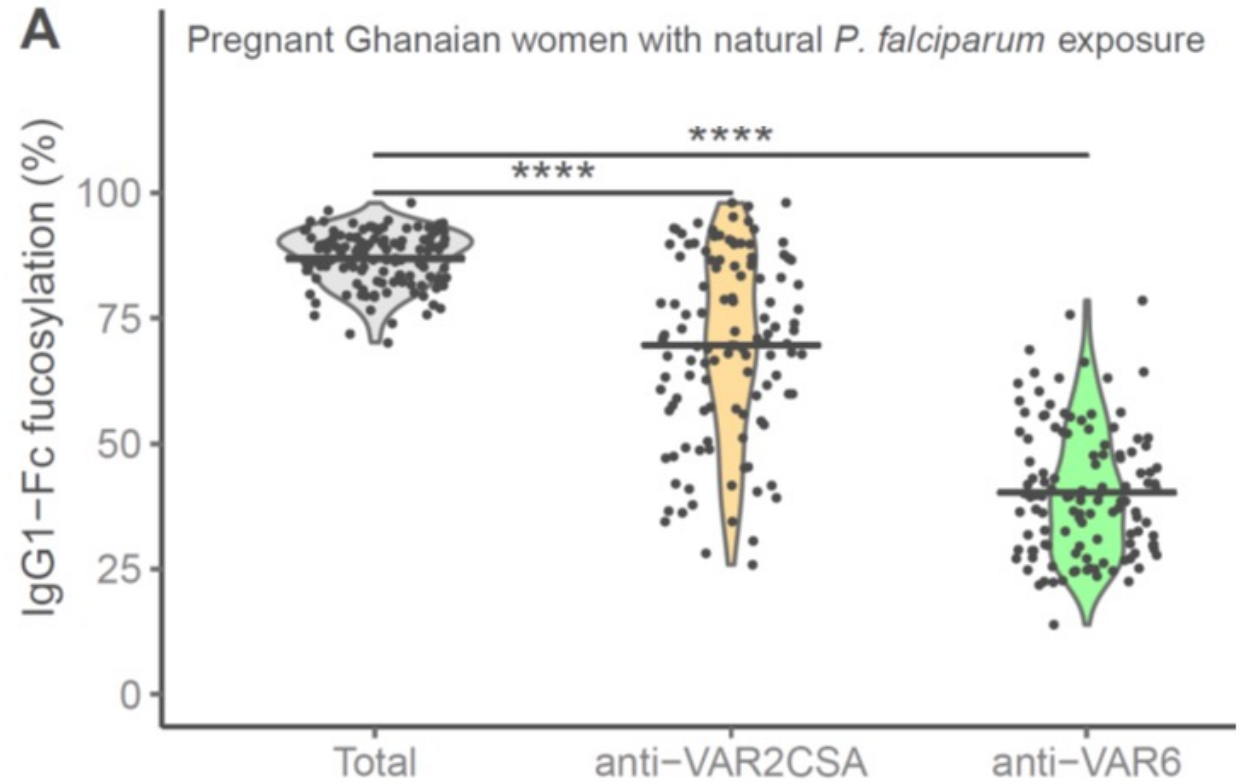
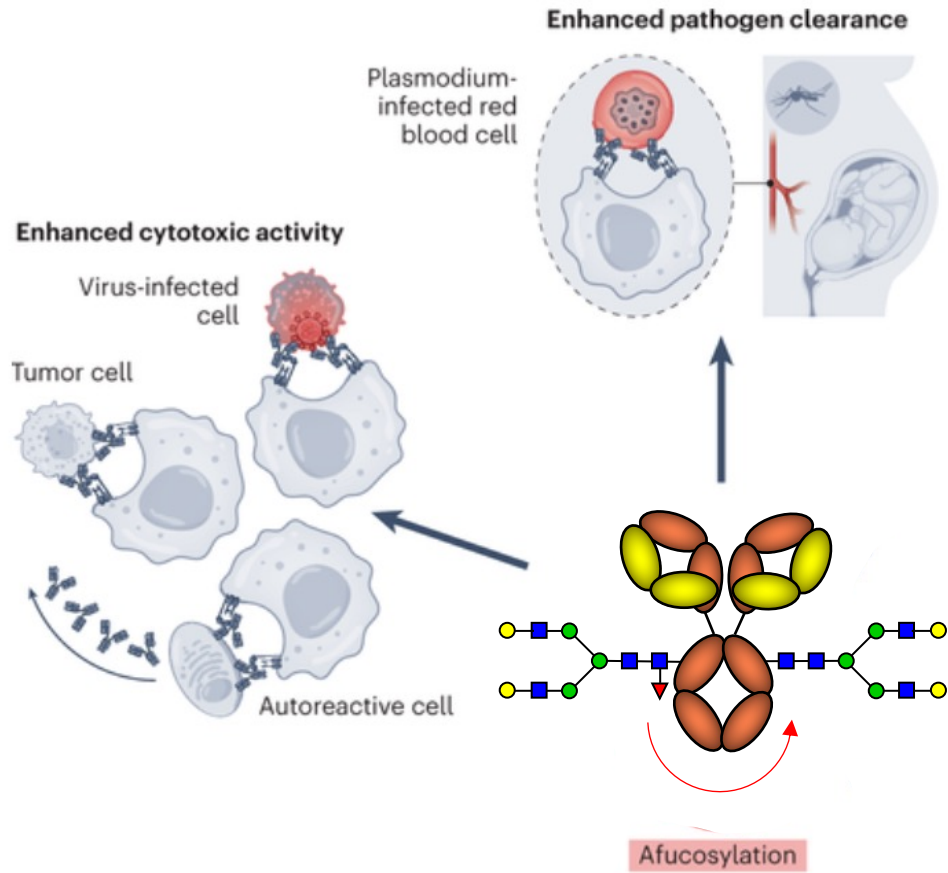
Obinutuzumab
 Mogamulizumab
 Margetuximab
 Belantamab
 Amivantamab
 Ublituximab
 Tomuzotuximab
 Imgatuzumab
 Cusatuzumab
 Bemarituzumab

Other

Inebilizumab
 Benralizumab

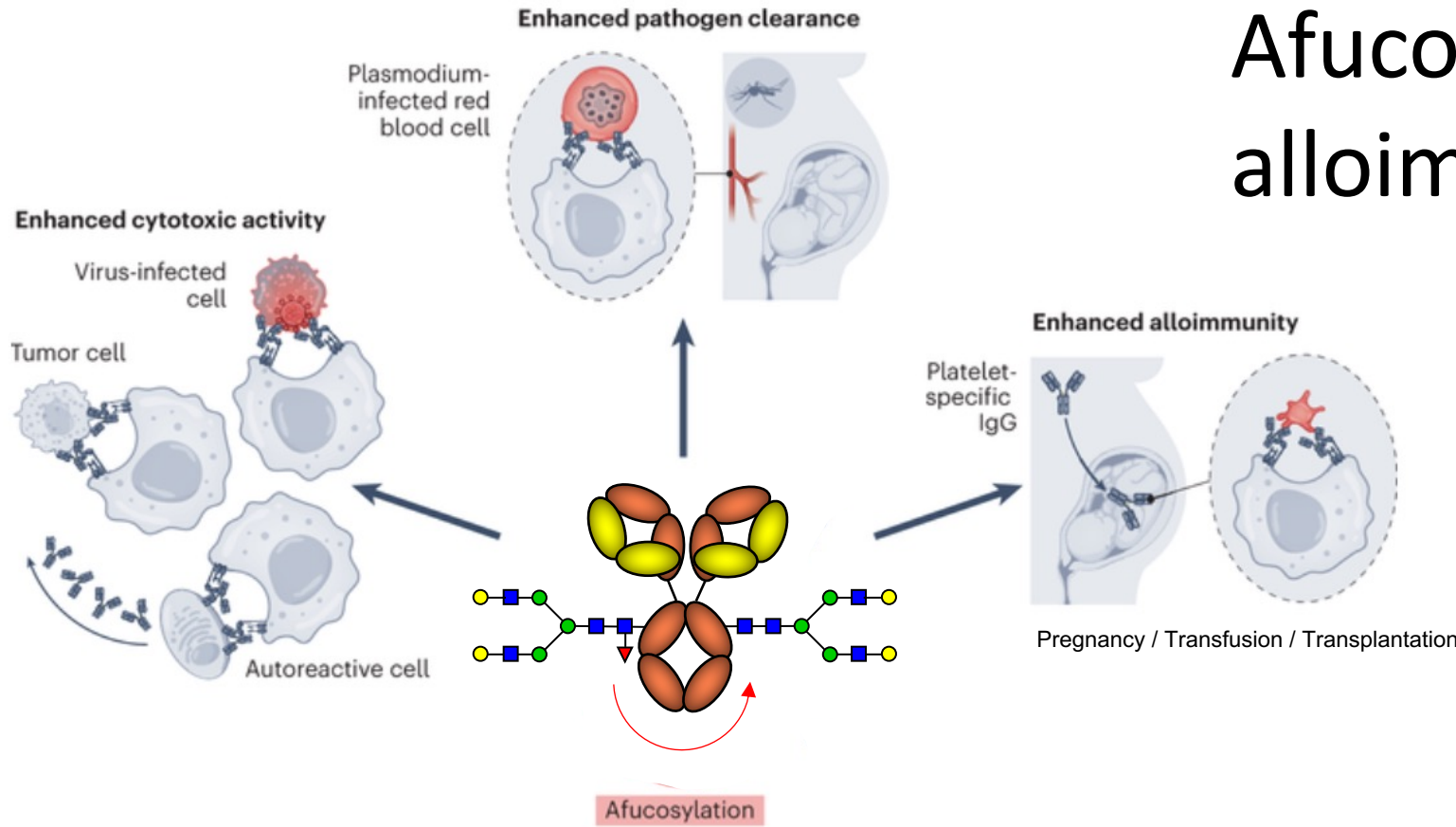


Afucosylation enhanced pathogen clearance

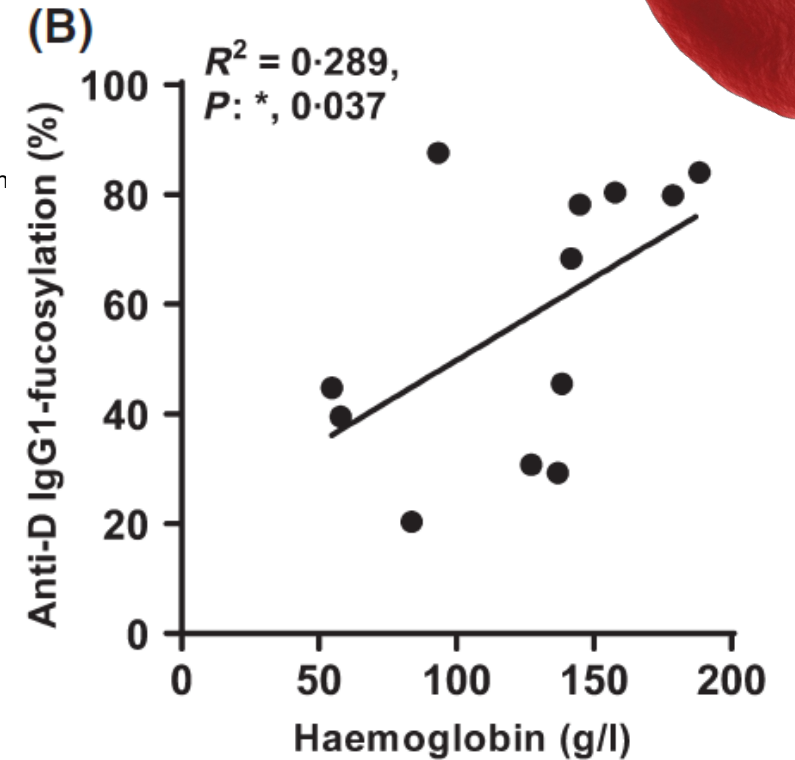




Afucosylation enhanced alloimmunity



anti-Rhesus D in pregnancy

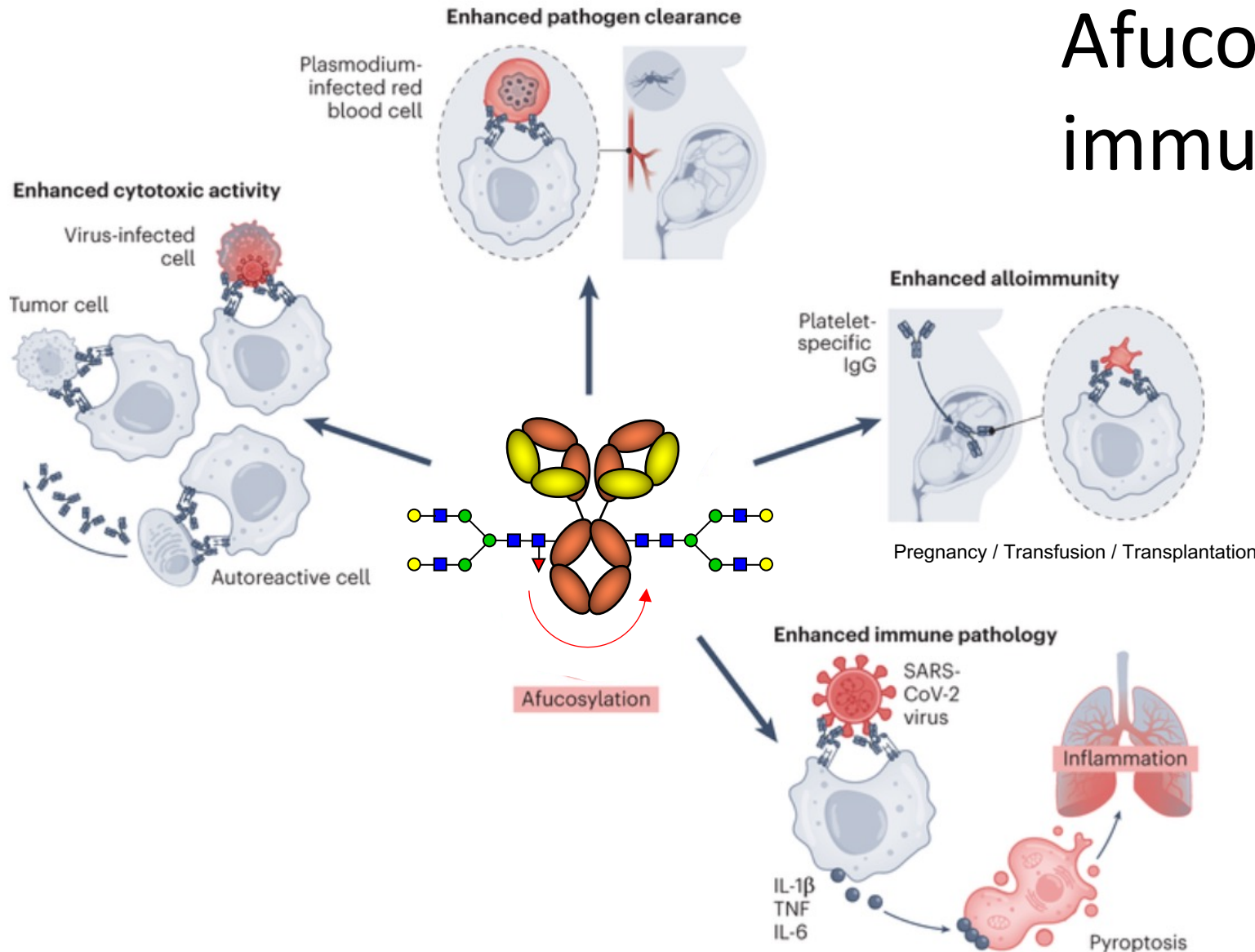


Sonneveld et al *Br J Haematol.* 2017 Feb;176(4):651-660.
 Kapur et al, *Transfusion*, 2015 ;55(3):553-62.
 Kapur et al, *Br J Haematol.* 2014, 166(6):936-45.

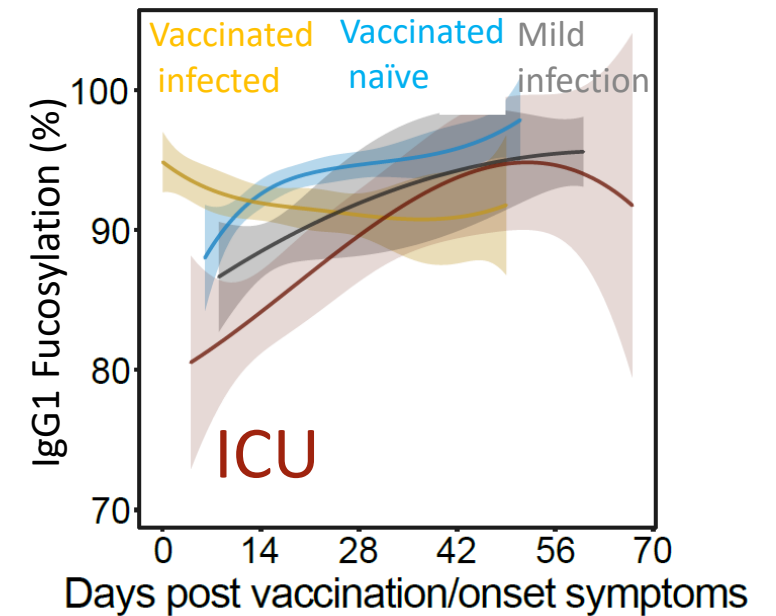
Sonnefeld et al, *Br J Haematol* 2016;174(2):310-20.
 Kapur et al, *Blood* 2014;123:471-80
 Wuhrer et al *J Proteome Res.* 2009;8:450-6,



Afucosylation enhanced immune pathology



COVID-19

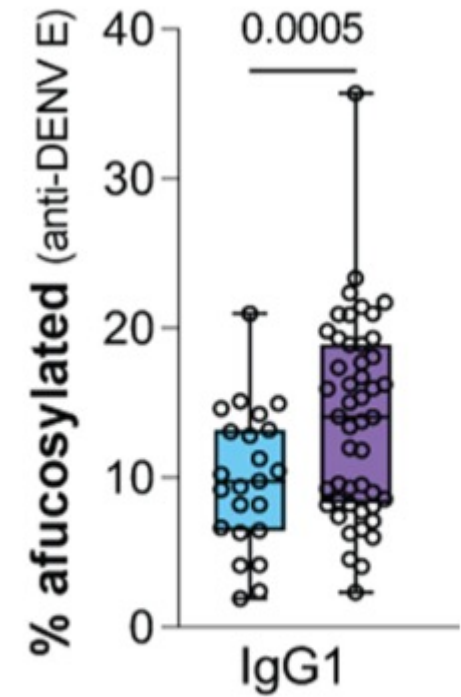
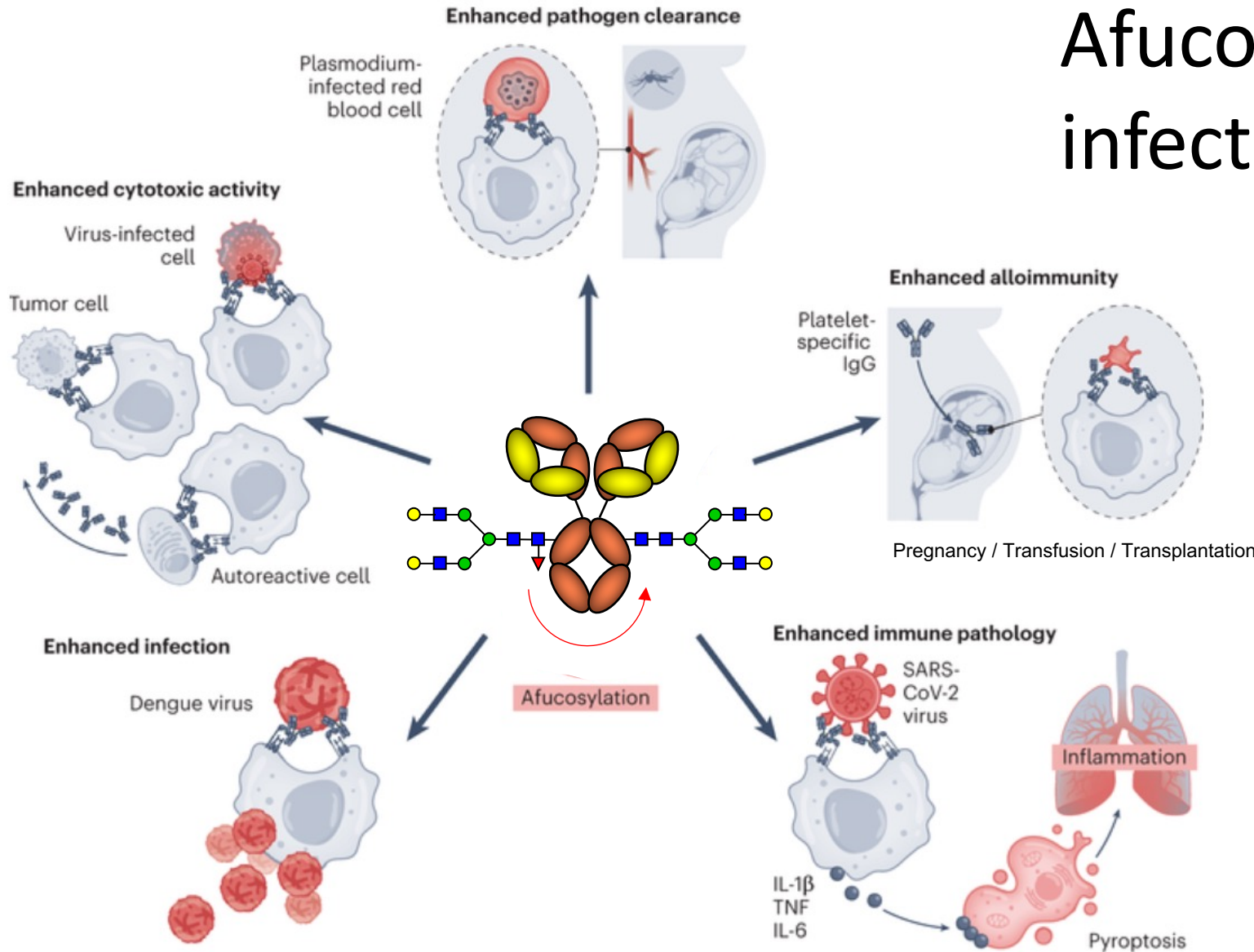


Larsen et al Science 2021;371(6532):eabc8378



Afucosylation enhanced infection

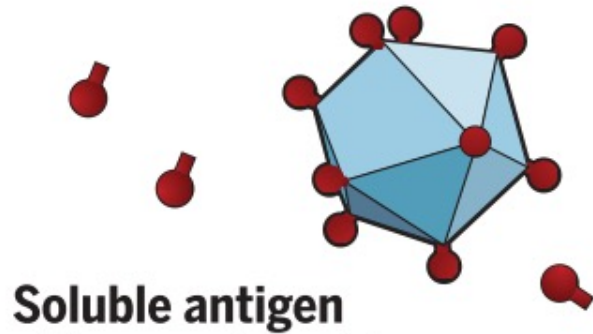
Enhanced FcγR mediated entry



■ Inapparent Dengue
■ Hospitalized Dengue

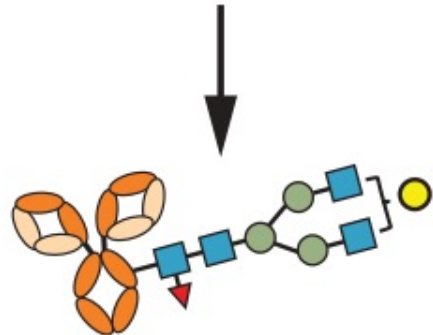


Self-membrane associated display of antigen required to generate afucosylated IgG response



Soluble antigen

- Non-enveloped viruses
- Protein based vaccines

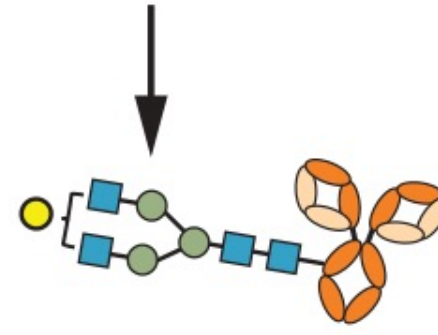


Fucosylated IgG



Membrane bound foreign protein

- Alloantigen on blood cells
- Enveloped viral infected cells

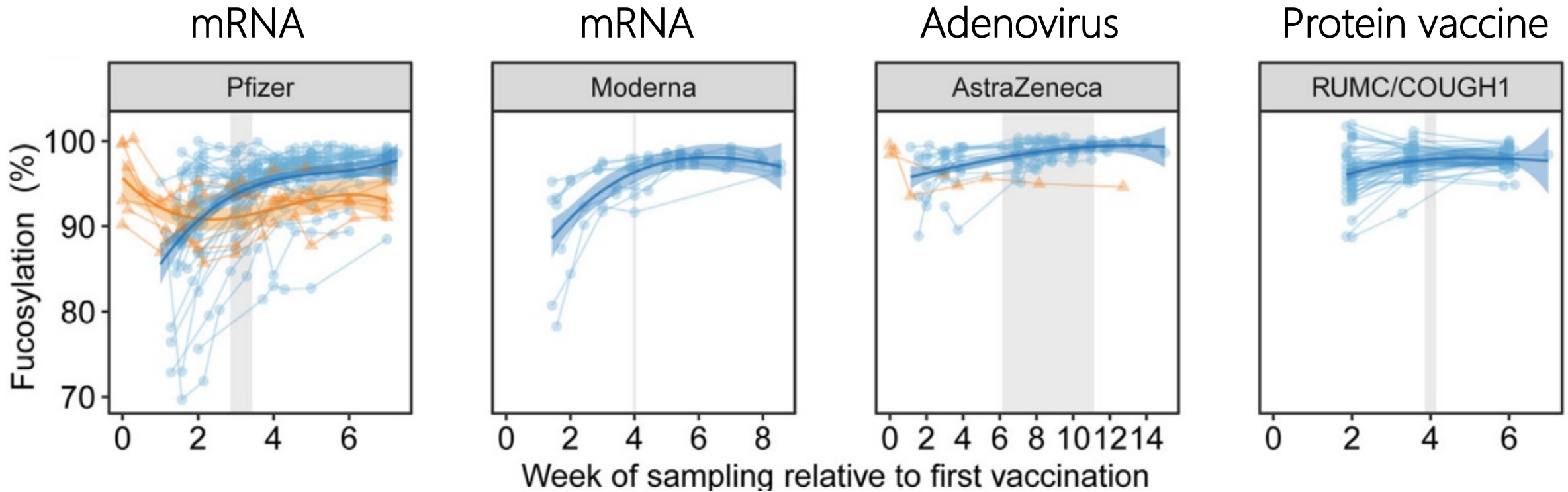


Afucosylated IgG



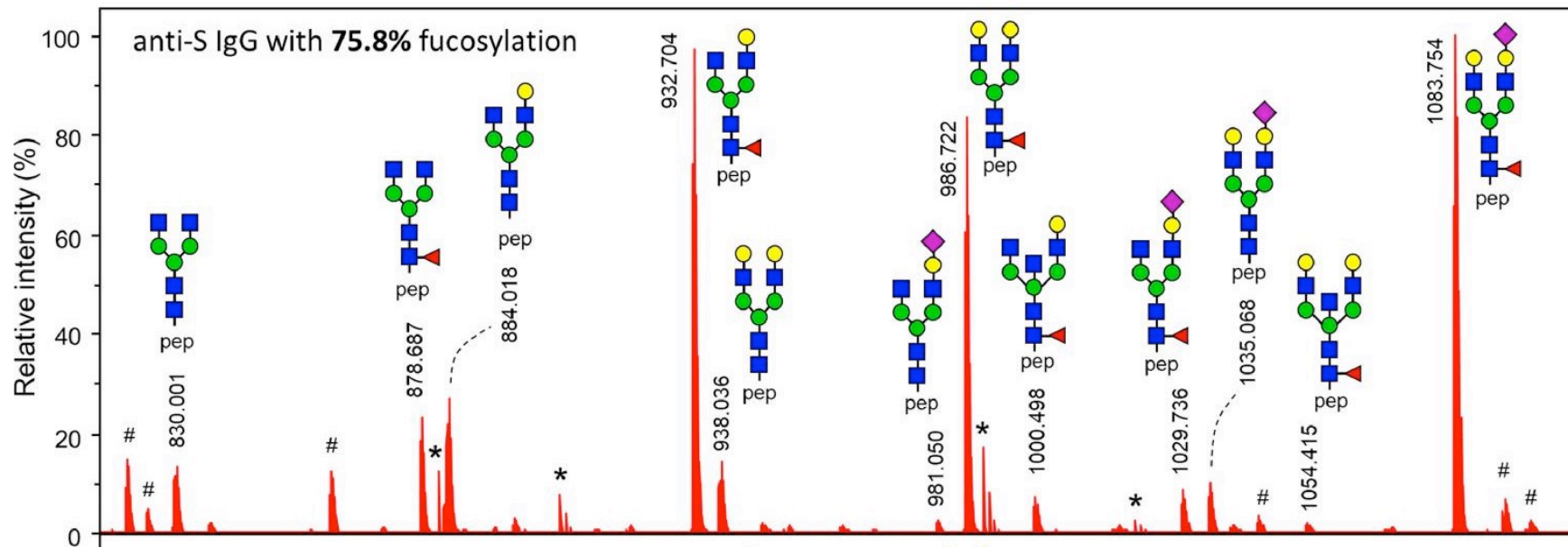
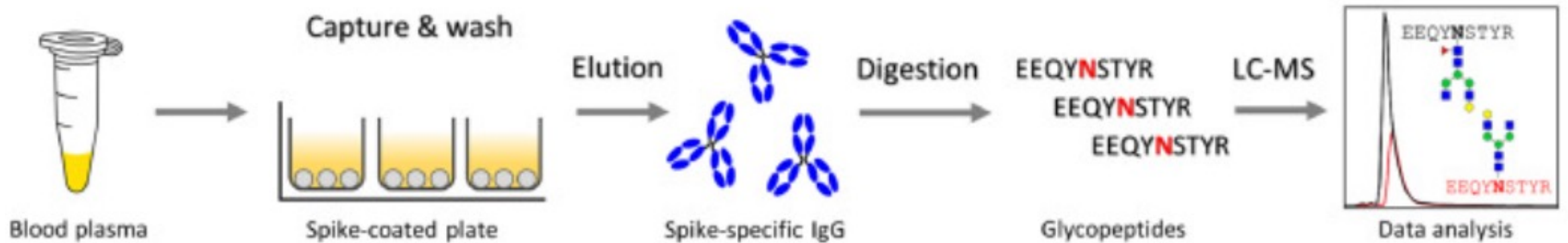
Vaccine platform impacts fucosylation of specific antibody response

Fucosylation state dynamic over time



Antigen-experienced
Naive

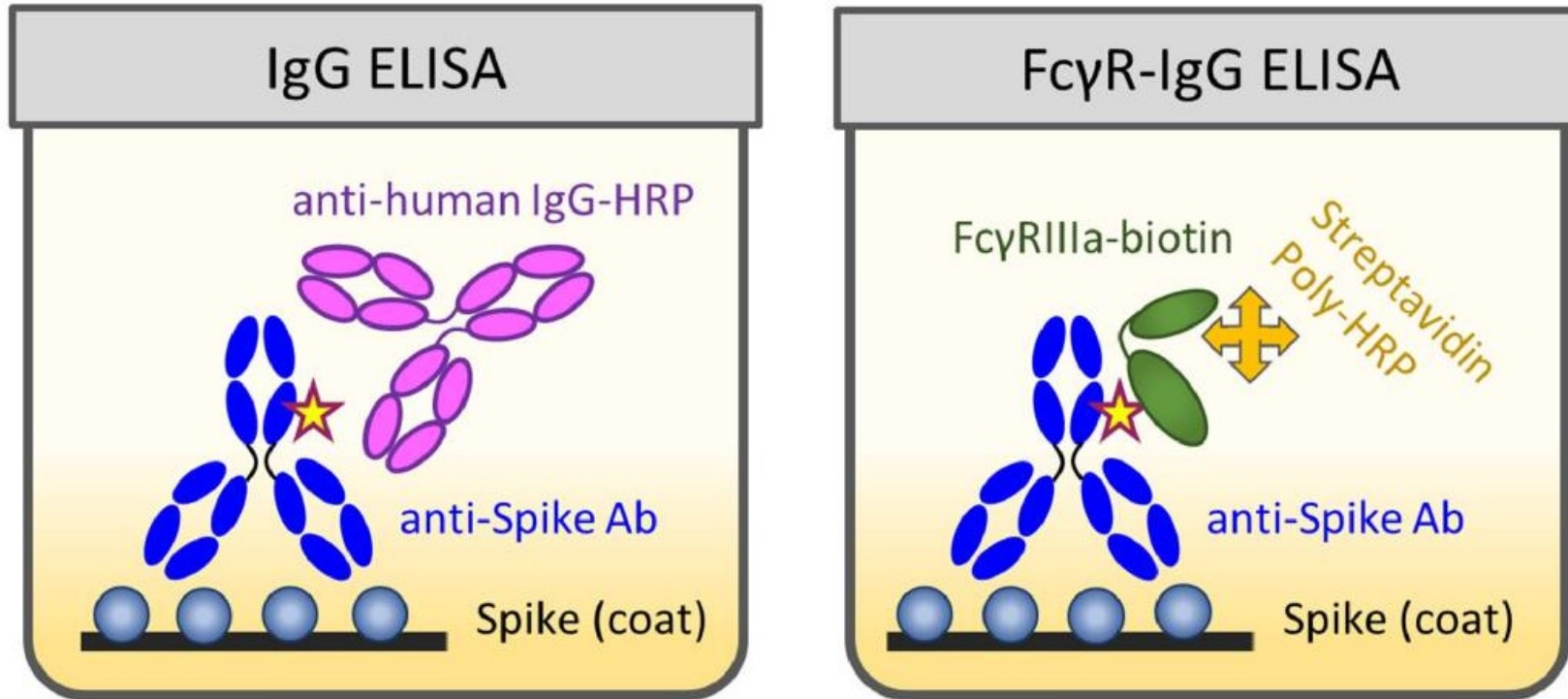
LC-MS method for glycosylation profiling





Fucose-sensitive ELISA (FEASI) for quantification of fucosylation

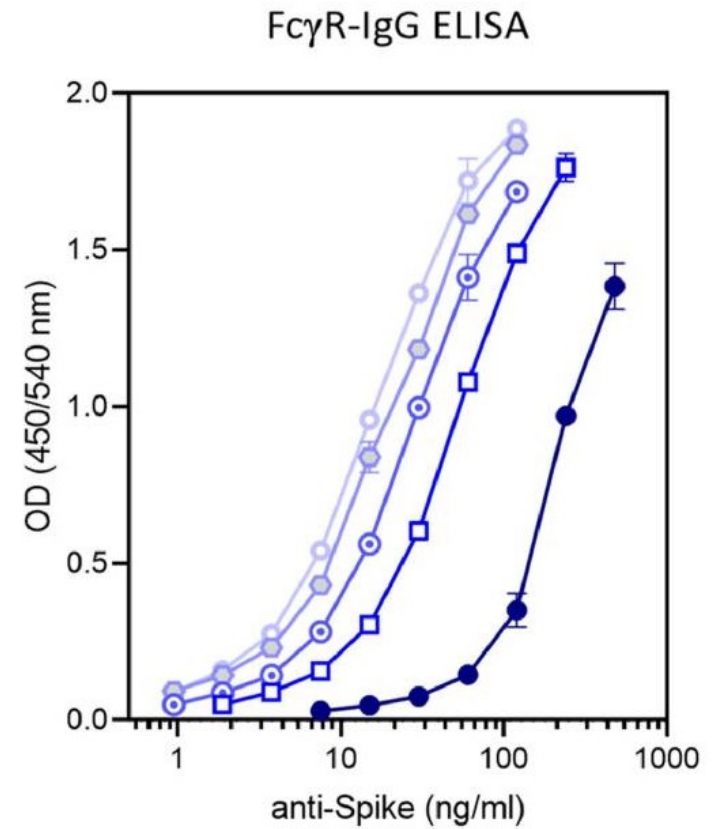
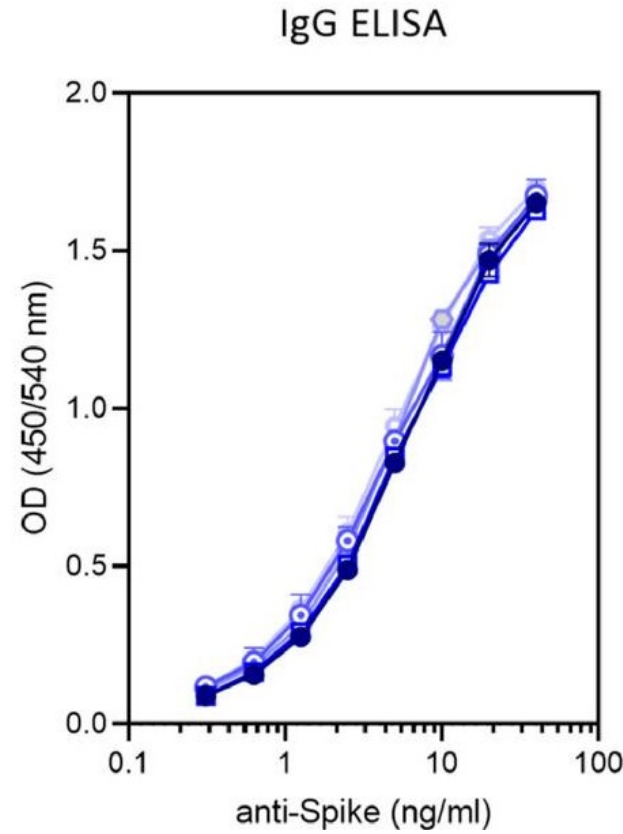
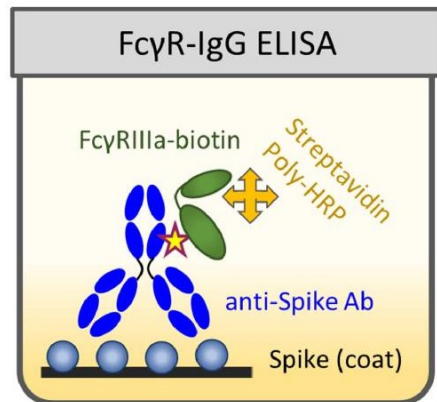
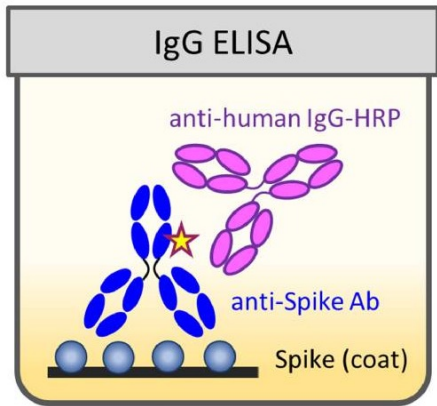
★ N297-linked glycan





FEASI for quantification of fucosylation

★ N297-linked glycan



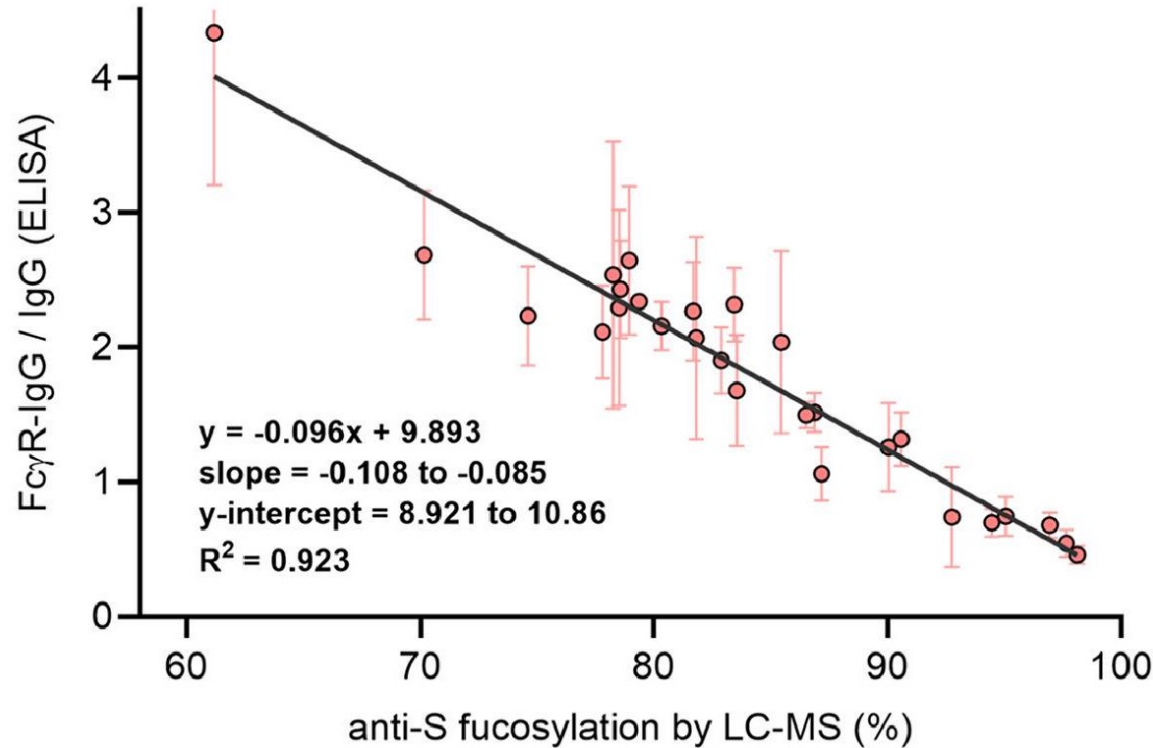
anti-S mAb with

- 96% fuc
- 75% fuc
- 55% fuc
- ⊖ 35% fuc
- ◇ 14% fuc

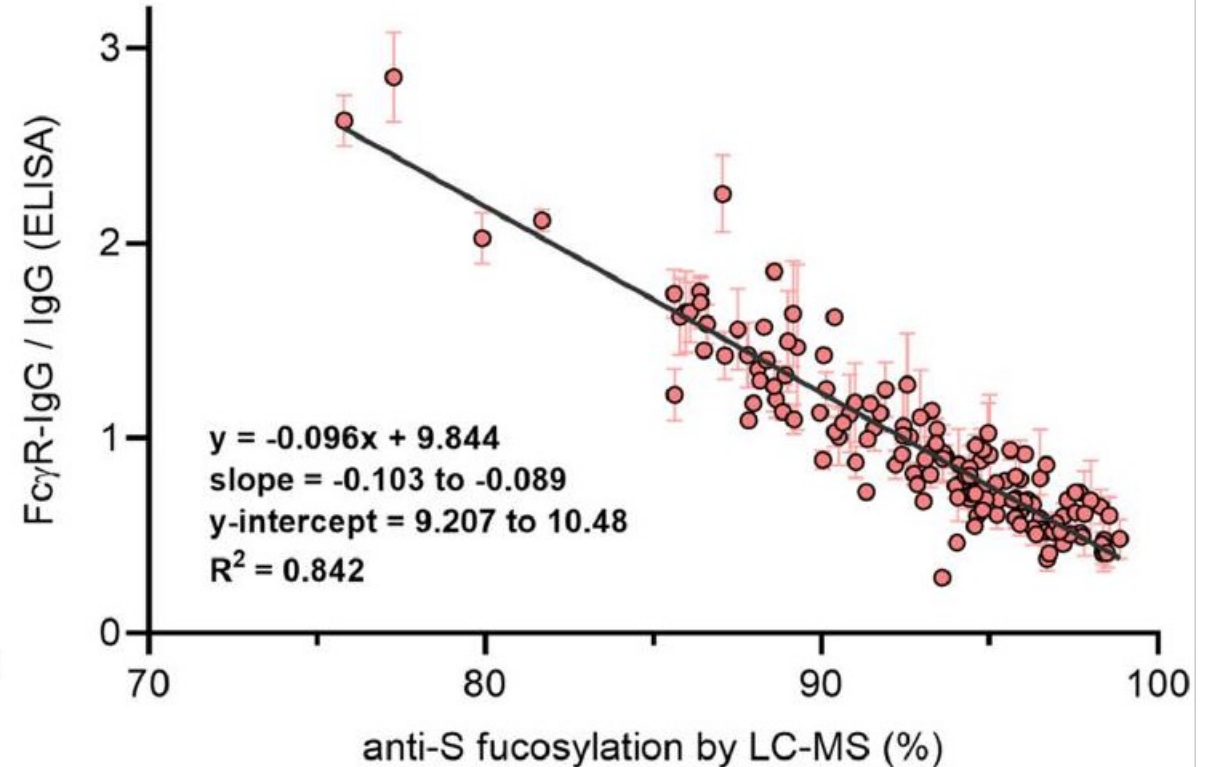


FEASI screen of fucosylation of antigen-specific IgG

ICU COVID-19 patient plasma samples

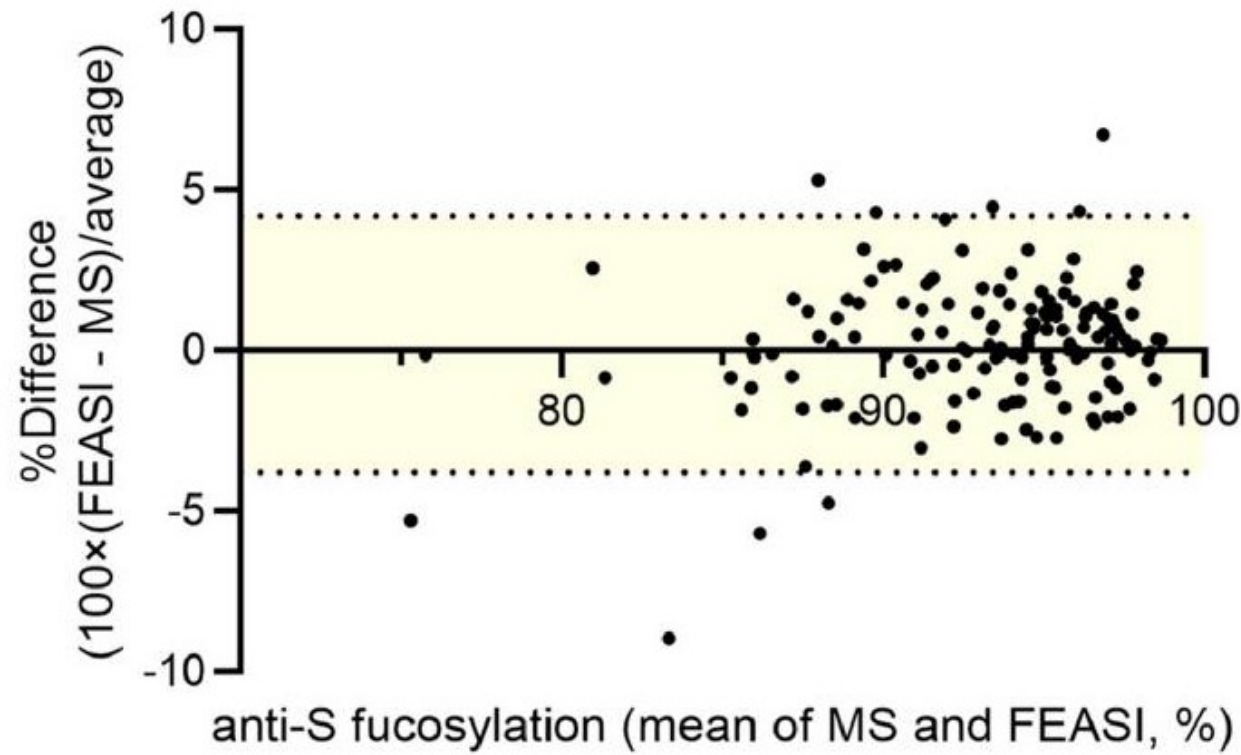


Convalescent COVID-19 plasma donors

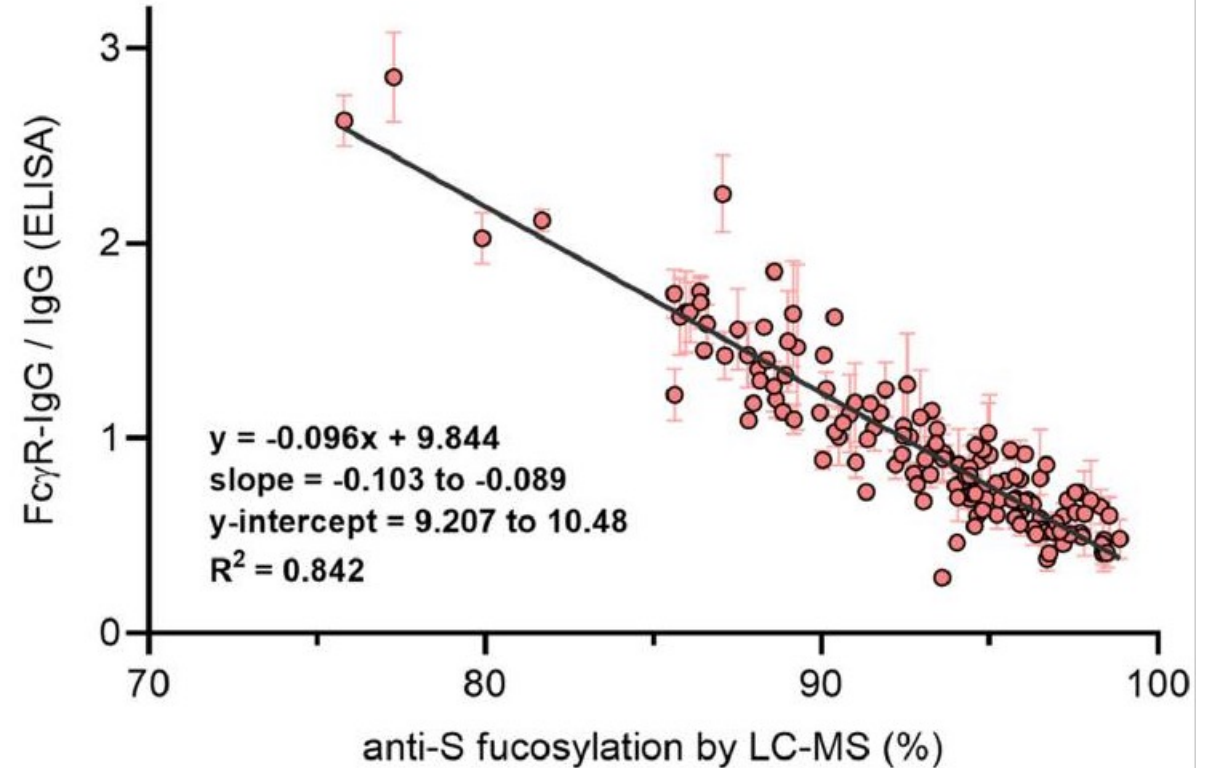




FEASI screen of fucosylation of antigen-specific IgG



Convalescent COVID-19 plasma donors





Conclusions and implications

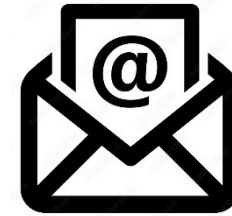
- Response to natural infection may provide clue what vaccination response is required
- IgG glycosylation analysis helps to better understand patient specific vaccination in immune response and of side effects
- FEASI can quickly and reliably screen for fucosylation of antigen-specific IgG



Acknowledgements

Sanquin Diagnostic Services

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f.loeff@sanquin.nl

Sanquin Research

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Sanquin

For Life.