Glycosylation analysis of recombinant and vaccine-derived SARS-CoV-2 spike glycoproteins

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My Background

DPhil in Biochemistry, Crispin Lab
2016 – Glycobiology Institute, Oxford
2017 – Division of Structural Biology, Oxford
2018 – University of Southampton

Projects:
Glycobiology of viruses (Lassa, Corona, Influenza, Zika etc.)
Antibody glycosylation (Bats, bispecifics, tandem Fcs, Follicular Lymphoma)
HIV vaccine development (NIH/BMGF/CHAVD)
Antibody engineering (ABC)
Viral envelope proteins can be extensively glycosylated

Why do we care about glycans?
- Multiple roles during the viral life-cycle
- Immune evasion by glycan shielding ("self")
- Inform upon immunogen integrity
- Presentation of glycan-based epitopes

Watanabe et al., 2019 BBA Gen Subj
Mammalian N-linked glycan pathway

N-X-S/T

ER

Nascent Peptide
Oligosaccharyltransferase
Glucosidase I&II
Calnexin/Calreticulin Folding Cycle
ER α-Mannosidase I

Cis-Golgi
Oligomannose-type
GlcNAcT-I
Golgi Mannosidase IA-C

Medial-Golgi
Fucosyltransferase
α-Mannosidase II
Fucosyltransferase

Trans-Golgi
Hybrid-type
Complex-type

Watanabe et al., 2019 BBA Gen Subj
Roles of glycosylation in viral pathobiology
Preparing samples for site specific analysis

+6M urea

Unfolded protein

+DTT reducing agent

Unfolded protein with no disulphide bonds

+iodoacetamide (IAA)

Unfolded protein with unpaired capped cysteines (alkylated)

+DTT reducing agent

Removes excess IAA

nanoLC-MS

Protease Digest

GluC (E/D)

Trypsin (R/K)

Chymotrypsin (F/Y/W)

Alpha-lytic protease (T/A/S/V)

Singly occupied N-glycopeptides
Recombinant SARS-CoV-2 Spike (2P-stabilised)
Site-specific glycosylation analysis of the SARS-CoV-2 Spike

Watanabe et al. Science 2020
Site-specific glycosylation analysis of the SARS-CoV-2 Spike

Watanabe et al. Science 2020
Glycan epitopes on SARS-CoV-2 Spike

Pinto et al. Nature 2020
ChAdOx1 nCoV-19/AZD1222 produces membrane associated S glycoproteins in native conformations

Watanabe et al. ACS Cen Sci 2021
CryoET and subtomogram average of ChAdOx1 nCoV-19 derived spike

Watanabe et al. ACS Cen Sci 2021
Site-specific glycan processing of S proteins upon infection with ChAdOx1 nCoV-19

Cao et al. Nature Comms 2017
Site-specific glycan processing of S proteins upon infection with ChAdOx1 nCoV-19
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...and many more
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