SUPPLEMENTARY INFORMATION

Isolation and quantification N-glycans from Immunoglobulin G antibodies for quantitative glycosylation analysis

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MATERIALS AND METHODS

Cell culture and antibodies: A monoclonal antibody (aIL8-hFc), an IgG-1 type full length humanized monoclonal antibody of 146 KDa, was produced from a Chinese hamster ovary cell line (CHO-DP12, ATCC # CRL-12445). Another monoclonal antibody (EG2-hFc), a chimeric (llama-human) heavy chain antibody of 79.7 KDa, was produced from a Chinese hamster ovary cell line (CHO-EG2). Both cell lines were cultured in BIOGRO-CHO serum free medium (BioGro Technologies Inc., Winnipeg, Canada). Cells were seeded at a density of ~2.5 x 10^5 cells/mL and cultured for 4 days. The supernatant was separated from cells by centrifuging the culture harvest at 350g for 5 min and filtered through 0.2 µm filter. The supernatants were concentrated by 10-fold by using 30 KDa cut-off centrifugal concentrators (EMD Millipore) and stored -20^{o}C for later use.

Gel electrophoresis: SDS-PAGE of all the samples in this work was performed by reducing the antibody. Briefly, the antibody samples were mixed with equal volumes of 2x Laemmli buffer containing 5% (v/v) β-mercaptoethanol and reduced by heating at 95^{o}C for 5 min. The samples were run on Criterion^{TM} TGX^{TM} 12-well 8-16% gels (Bio-Rad, Cat#P5671103) in SDS buffer using a standard protocol from the manufacturer.

Macro-heterogeneity linearity: Solutions of glycosylated and deglycosylated EG2-hFc were mixed at pre-determined proportions to produced mAb samples with different % glycosylated mAb. Glycosylation analysis of samples was performed as per the protocol with 50 µg of total mAb, 100 units/mL PNGase F and 2 nmoles of DP4. The antibody was eluted from Protein-A columns and quantified with absorbance at 280 nm using nanodrop spectrophotometer.

Macro-heterogeneity analysis of mAb samples: Exponentially growing CHO-EG2 cells were seeded in to medium containing 5 mM glucose at a density of 2.6 x 10^6 cells/mL. The cells were cultured for 72 h and the culture supernatant was harvested every 24 h. The supernatants were concentrated 10-fold and 1 mL of sample from each condition was used for glycan analysis. N-glycosylation analysis and quantification of loaded mAb were performed as described in the protocol. A separate set of samples were purified for quantification and SDS-PAGE analysis.

RESULTS

The response of glycan yield as a function of mAb concentration from the mixture of glycosylated and non-glycosylated mAbs was investigated. As seen from the Fig. S1, the glycan yield was linearly proportional to the glycosylated mAb fraction. This indicates that the presence of non-glycosylated antibody in mAb sample does not alter either the binding of total antibody or the N-glycan digestion by PNGase F.

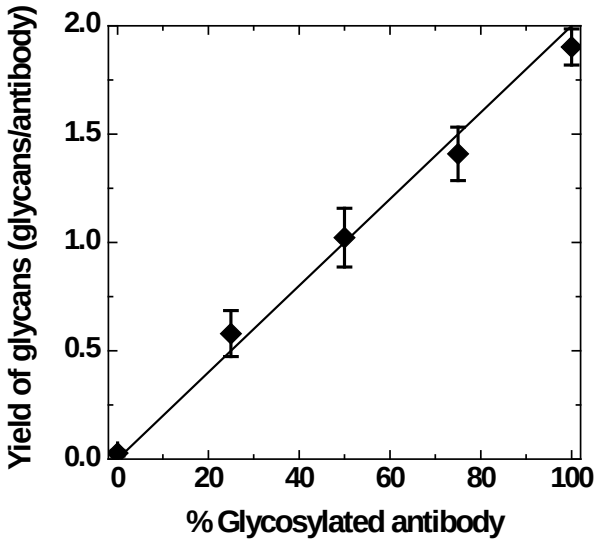

Figure S1: Linear response of glycan yield as a function of % glycosylated antibody in the antibody sample mixture (n=4).

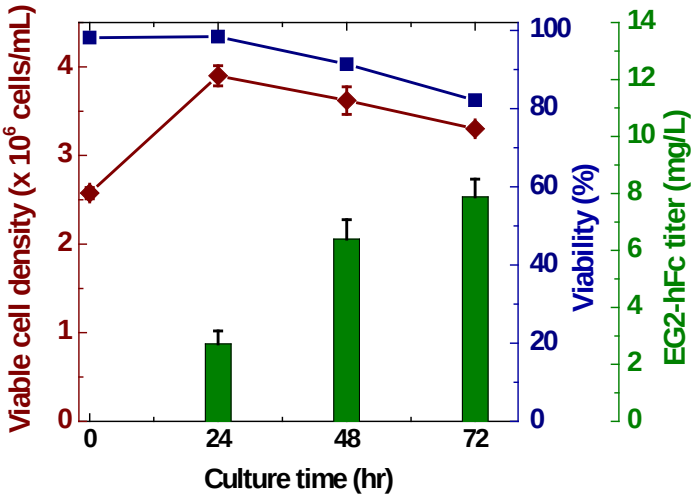

Figure S2: CHO-EG2 cells culture performance and EG2-hFc production profiles after seeding into BioGro-CHO medium containing only 5 mM glucose (n=2).

Other affinity ligand columns like mini Protein-G columns can also be used for isolating N-glycans from antibodies. Similar N-glycan composition of an EG2-hFc sample was obtained when Protein-G columns were used instead of Protein-A (Table S1). This method was also used to isolated N-glycans from human serum polyclonal IgGs. No significant difference in the composition of N-glycans obtained by using Protein-A or Protein-G columns (Table S2). In addition, no glycans were detected by in-solution glycan analysis of reduced serum IgGs that were eluted from Protein-A/Protein-G after PNGase F digestion of immobilized intact IgGs by the current method. This indicates that the N-glycans can be isolated from all the IgG isotypes with affinity-ligand method using protein-A or protein-G columns. Protein-A has high affinity to IgG-1, IgG-2 and IgG-4 type antibodies, whereas Protein-G has affinity towards all IgG types. From the serum sample analysis, the efficiency of isolating N-glycans from different IgG isotypes cannot be distinguished. All the mAbs used in our study are IgG-1 based antibodies and is the case with majority of therapeutic mAbs. The efficiency of isolating N-glycans from other IgG isotype mAbs need to be tested individually.

Table S1: Composition of N-glycans of EG2-hFc sample obtained by using either protein-A or Protein-G columns for isolating glycans (n=2)

Glycan	Protein A	Protein G
FA2	18.9±0.1	19.4±0.1
M5	2.70±0.01	2.47±0.04
FG[6]1	18.1±0.1	18.7±0.1
FG[3]1	8.44±0.11	8.52±0.05
FG2	31.7±0.4	31.9±0.2
FS(3)1	11.4±0.1	10.9±0.1
FS(3)2	5.59±0.52	4.98±0.05

Table S2: Composition of N-glycans of IgGs from human serum (Sigma-Aldrich Cat# H4522) obtained by current method using either protein-A or Protein-G columns (n=2)

Glycan	Protein A	Protein G
FA2	28.9±0.5	31.4±1.6
M5	4.32±0.35	4.91±0.44
FG[6]1	21.8±0.2	24.2±0.6
FG[3]1	14.1±0.1	15.6±0.1
FG2	13.7±0.1	12.8±1.6
FG1S(6)1	2.69±0.03	2.58±0.02
FS(6)1	9.05±0.07	6.21±0.96