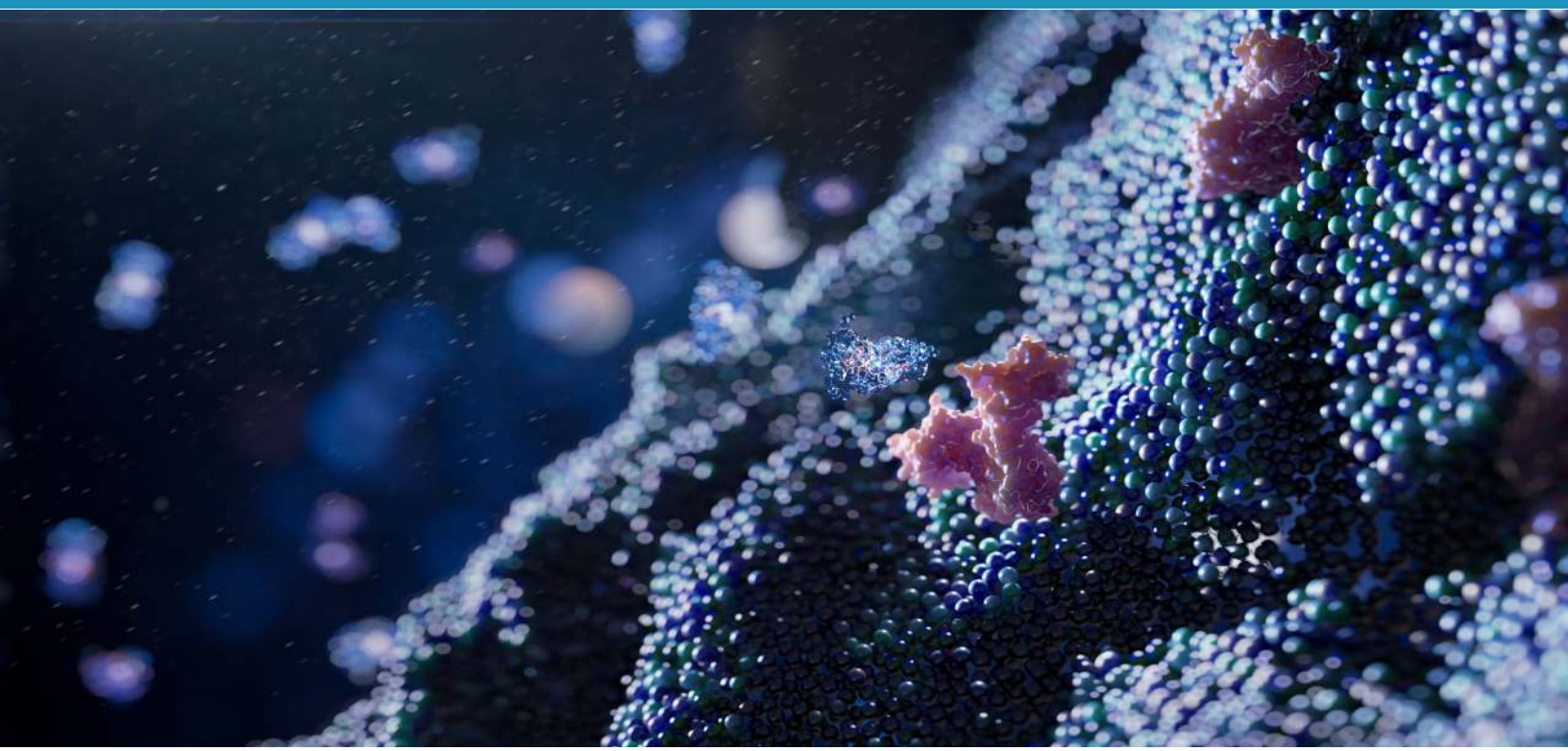
Bioinformatics Solutions Inc.



Identifying different glycan profiles of CD33 expressed in HEK293 and CHO cells

Kyle Hoffman, PhD, Applications Manager

Bioinformatics Solutions Inc., Waterloo, Canada

Abstract:

Glycoprotein biosynthesis is a complex process requiring multiple enzymes and cellular pathways. Since glycosyltransferases and glycoprotein biosynthetic pathways can differ between organisms, production of a recombinant glycoprotein in different host systems may result in undesirable glycoforms. In this study we characterize the differences in glycan profiles across six sites on CD33 expressed in HEK293 and CHO cells.

Introduction:

Protein glycosylation is one of the most common and complex post-translational modifications (PTMs) and plays a critical role in protein folding, stability, localization, function, and interactions (1). Expression of a recombinant glycoprotein in different host cell lines can result in different glycoforms, altering the properties and function of the protein (2). For this reason, careful consideration must be made when producing glycoproteins for research or therapeutic use. Here, we use our new PEAKS Glycan Module software add-on to identify major differences in the glycan profiles at six positions in myeloid cell surface antigen CD33 (Figure 1).

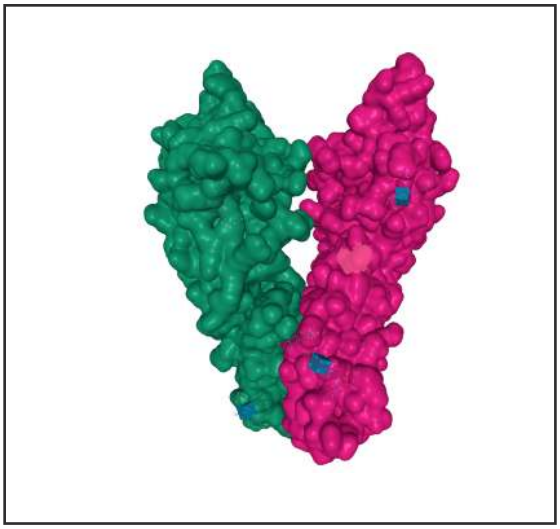


Fig 1. CD33 or Siglec-3 (sialic acid binding Ig-like lectin 3, SIGLEC3, SIGLEC-3, gp67, p67) is a transmembrane receptor expressed on cells of myeloid lineage.(3) It is usually considered myeloid-specific, but it can also be found on some lymphoid cells (4).

Methods:

CD33 was purchased from R&D Systems and SinoBiological. Samples were reduced and alkylated then acidified with phosphoric acid to lower pH to 1. Then 6X volume of the binding/washing buffer (100 mM TEAB in 90% methanol) was added to the sample. Samples were loaded on the S-trap column, eluted by centrifugation, then washed 3 times with binding/washing buffer. Then 2 ug was digested with trypsin in 50 mM TEAB buffer. The digested products were eluted and separated using a nanoElute® UHPLC then analyzed on a timsTOF Pro (Bruker) instrument. All raw data was analyzed using PEAKS Glycan Module (Figure 2) with the following parameters. Precursor, glycan, and peptide fragment ion mass errors were set to 15 PPM, 20 PPM, and 0.05 Da, respectively. Carbamidomethylation was set as a fixed PTM and oxidation (M) and deamidation (NQ) were set as variable PTMs. For protein/peptide identification the human Uniprot database was used for the CD33 sample from SinoBiological and Chinese hamster ovary Uniprot database was used for CD33 from R&D Systems. Only peptide and glycopeptides below a 1% false discovery rate were considered.

Sample	Percent Coverage	# of unique glycans	Glycosites
CD33 – R&D Systems	60.16%	209	N99, N100, N113, N160, N209, N230
CD33 - SinoBiological	57.69%	207	N99, N100, N113, N160, N209, N230

Table 1. Number of glycans and glycosites identified in CD33.

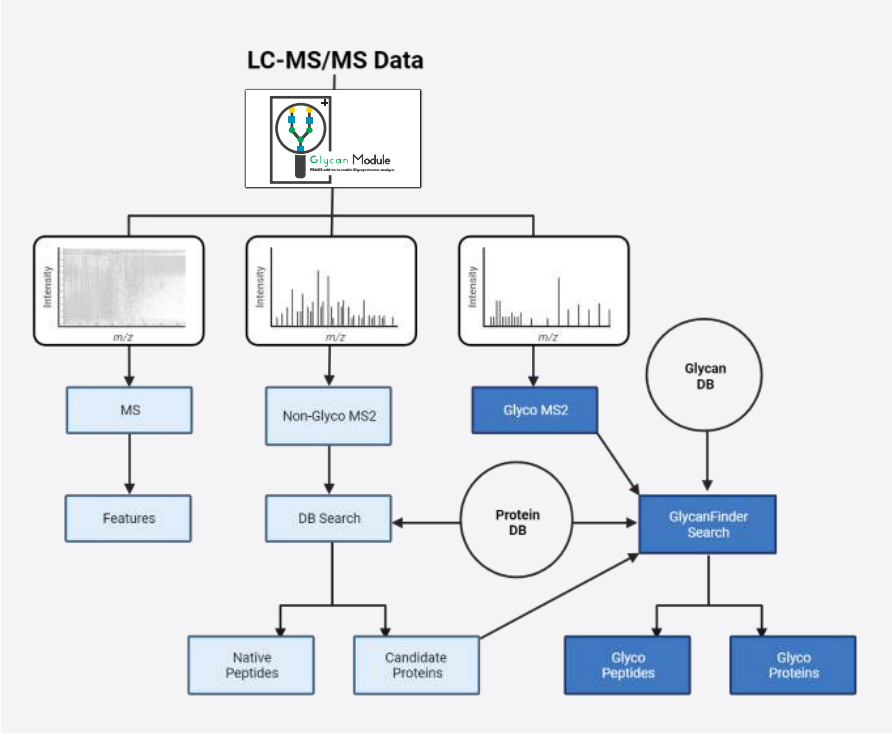
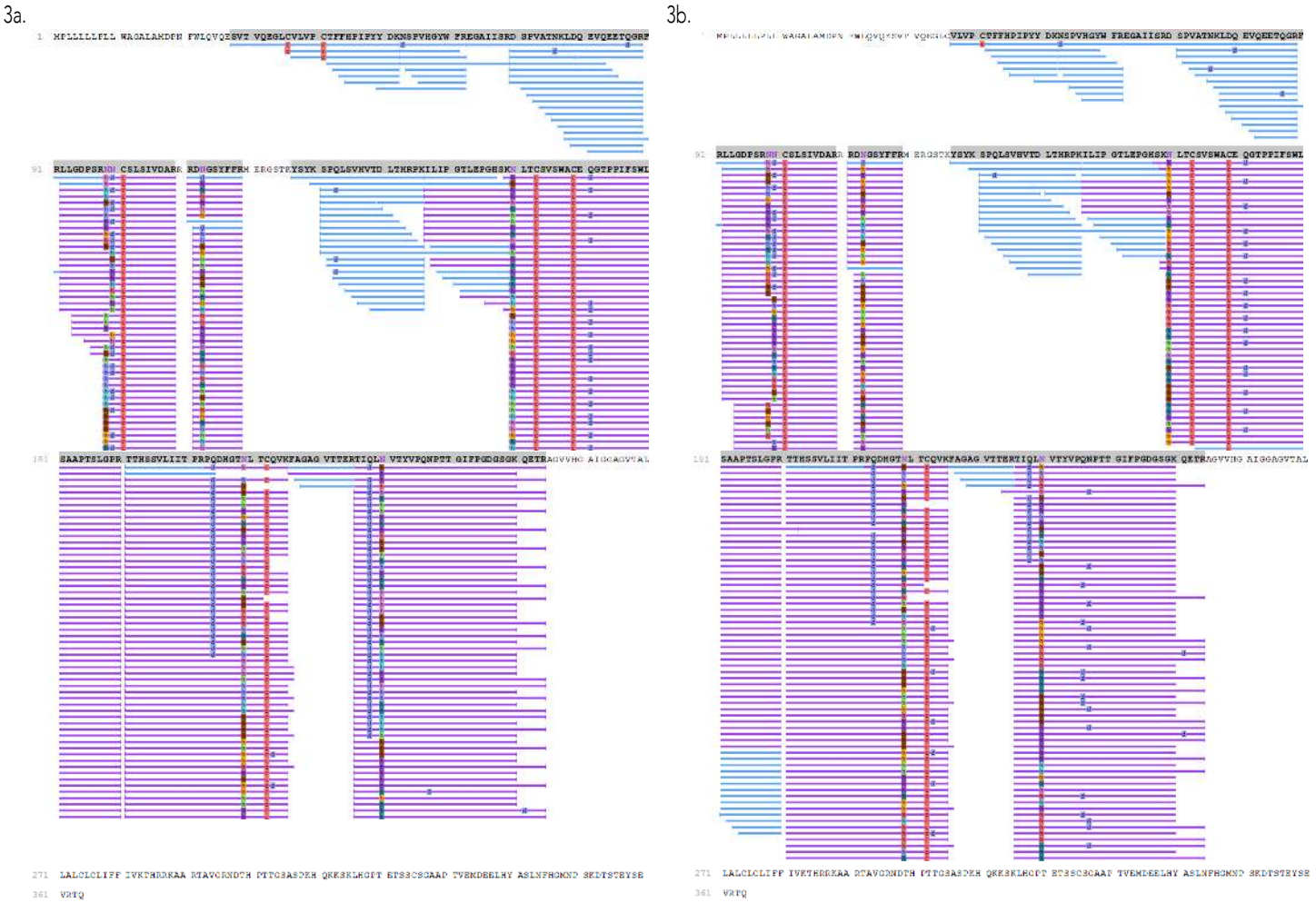


Fig 2. PEAKS Glycan Module Workflow

Results and Discussion:

We identified six highly glycosylated sites across CD33, decorated with over 200 different N-linked glycans in both the SinoBiological and R&D Systems samples (Table 1). Deep coverage of glycopeptides was obtained for each of the six glycosites (Figure 3), whereas the lack of peptide mapping to the N- and C-terminal regions of both CD33 samples suggests the protein has been processed or truncated. We next compared glycan compositions between CD33 samples expressed in HEK293 and Chinese hamster ovary (CHO) cells. While each protein had the same glycosites and a similar number of glycans, only 53% of glycan were shared (Figure 4). Moreover, the glycan profiles at each of the six sites significantly varied between samples. An example of the glycan diversity between each protein is shown in Figure 5 for site N113 in CD33. There was a higher representation of fucosylated glycans in the CD33 from SinoBiological, likely a result of expression in HEK293 cells. Each of the glycopeptides were supported by good quality spectra that were below a 1% FDR for both glycan and peptide identification. Representative glycopeptide spectra at each site are displayed in Figure 6, showing b- and y-ions that support the peptide sequence and glycan fragmentation ions determining the glycan compositions. In conclusion, we identified significant differences in glycoforms of CD33 when prepared in HEK293 and CHO cell cultures and careful considerations should be made when purchasing protein for research use that were expressed in different host cells.



	ΔM	PTM
D	+0.98	Deamidation (NQ)
C	+57.02	Carbamidomethylation

Fig 3. Peptide mapping of CD33 from SinoBiological and R&D Systems. A) Peptide coverage of CD33 from R&D Systems. B) Peptide coverage of CD33 from SinoBiological. Blue and purple bars represent non-glycopeptides and glycopeptides, respectively. Coloured squares on each peptide represent different glycans, carbamidomethylation (red), and deamidation (blue).

4.

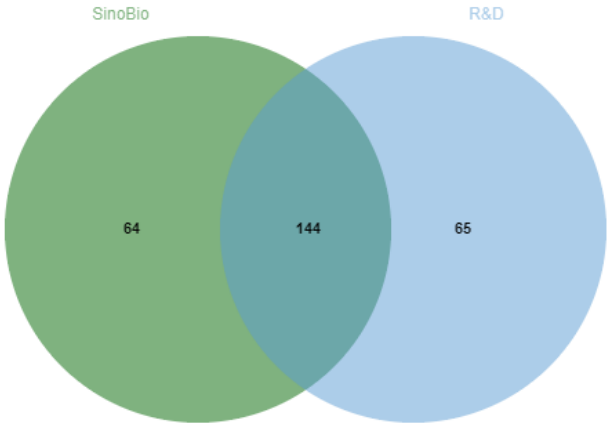
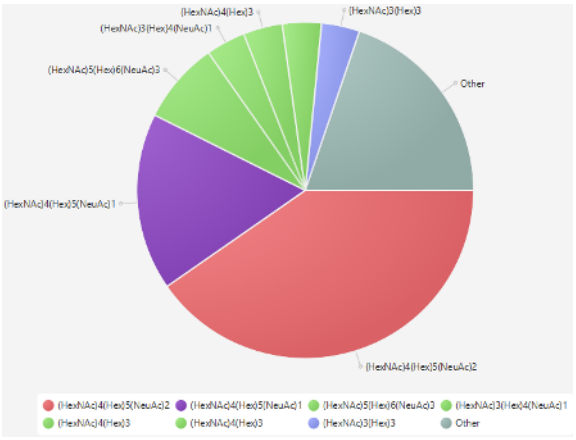


Fig 4. Comparison of glycans identified in CD33 from R&D Systems and SinoBiological.

5a.



5b.

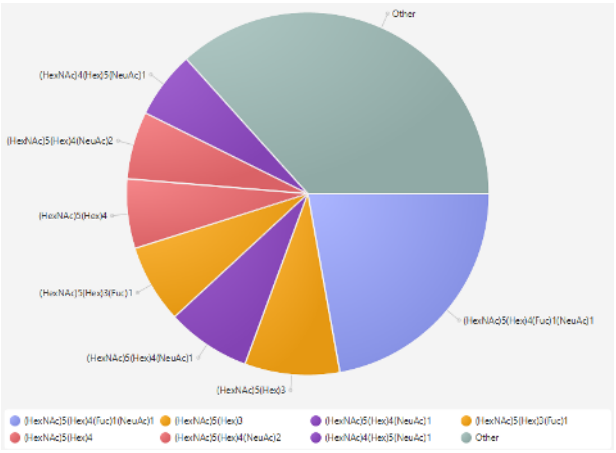


Fig 5. Glycan profiles of CD33 at N113. A) Glycan profiling at N113 of CD33 from R&D System. B) Glycan profiling at N113 of CD33 from SinoBiological.

6.

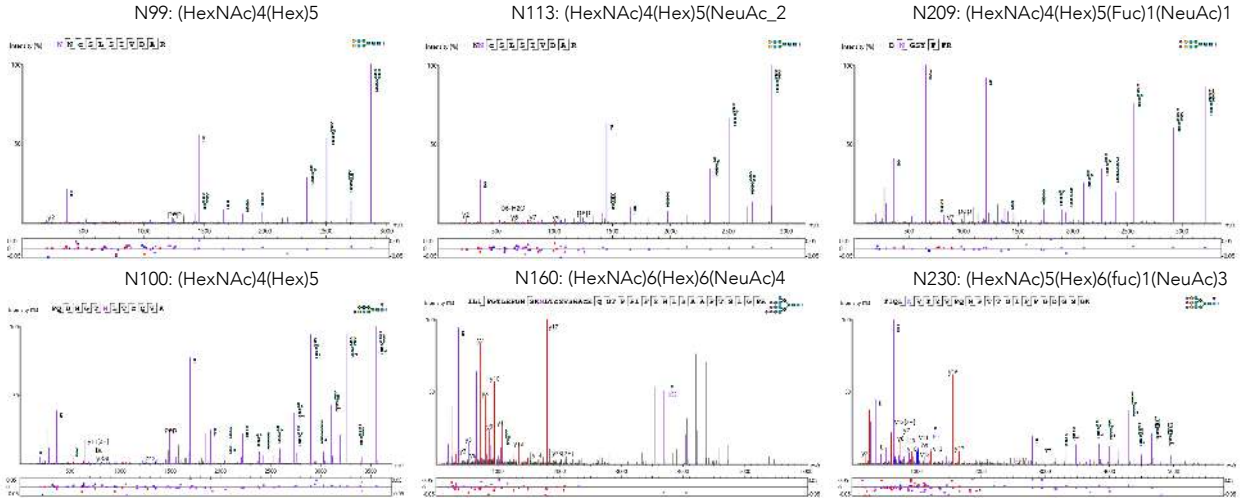


Fig 6. Glycopeptide spectra at each glycosite in CD33. B- and y-ions are highlighted in blue and red, respectively. Glycan fragment ions are highlighted in purple. The composition of each glycan is displayed above each spectrum. Structure annotation is also provided for each glycan and displayed at the top right of each spectrum.

References:

1. Eichler, J. Protein glycosylation. In Current Biology Cell Press. 2019;29:229–231.
2. Swiech K, de Freitas MC, Covas DT, Picanço-Castro V. Recombinant glycoprotein production in human cell lines. Methods Mol Biol. 2015;1258:223-40.
3. Garnache-Ottou F, Chaperot L, Biichle S, Ferrand C, Remy-Martin JP, Deconinck E, et al. (February 2005). "Expression of the myeloid-associated marker CD33 is not an exclusive factor for leukemic plasmacytoid dendritic cells". Blood. 105 (3): 1256–1264.
4. FDA approves Mylotarg for treatment of acute myeloid leukemia". Food and Drug Administration. 24 March 2020.