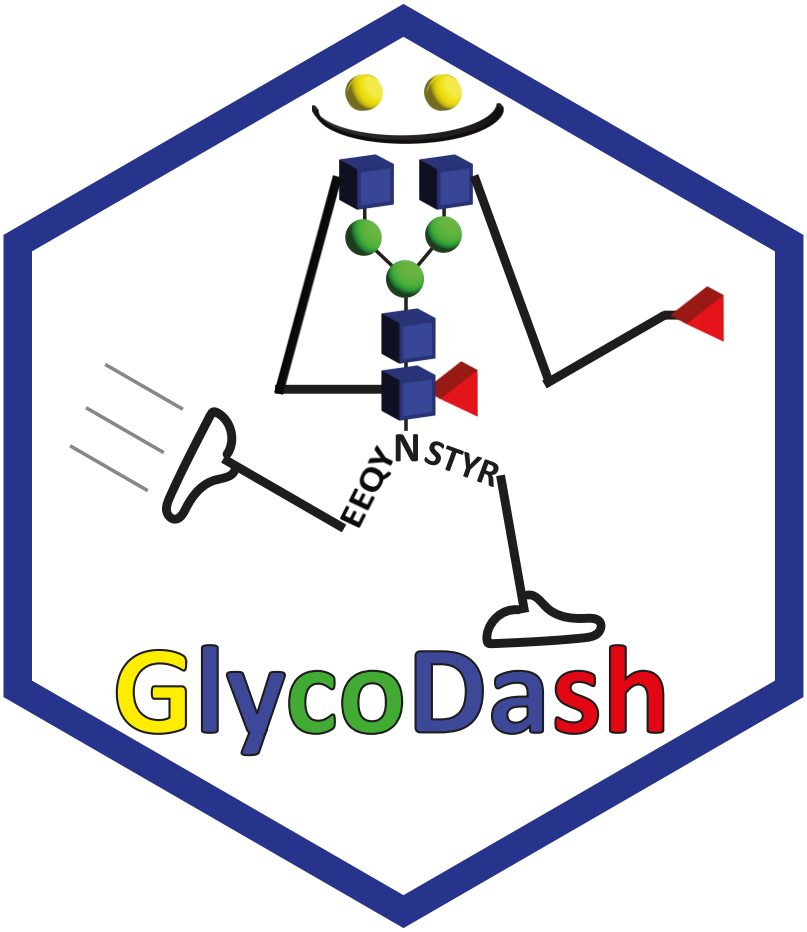
# GlycoDash

## *User Guide*




*Version 1.9.1*

# Contents

# Installation and requirements

*Note: when connected to the LUMC network within Leiden University Medical Center, GlycoDash can be accessed via [http://cpmtools:8888/](http://cpmtools:8888/) in your browser. This is a beta version where new features can be tested before they are implemented in an official release.*

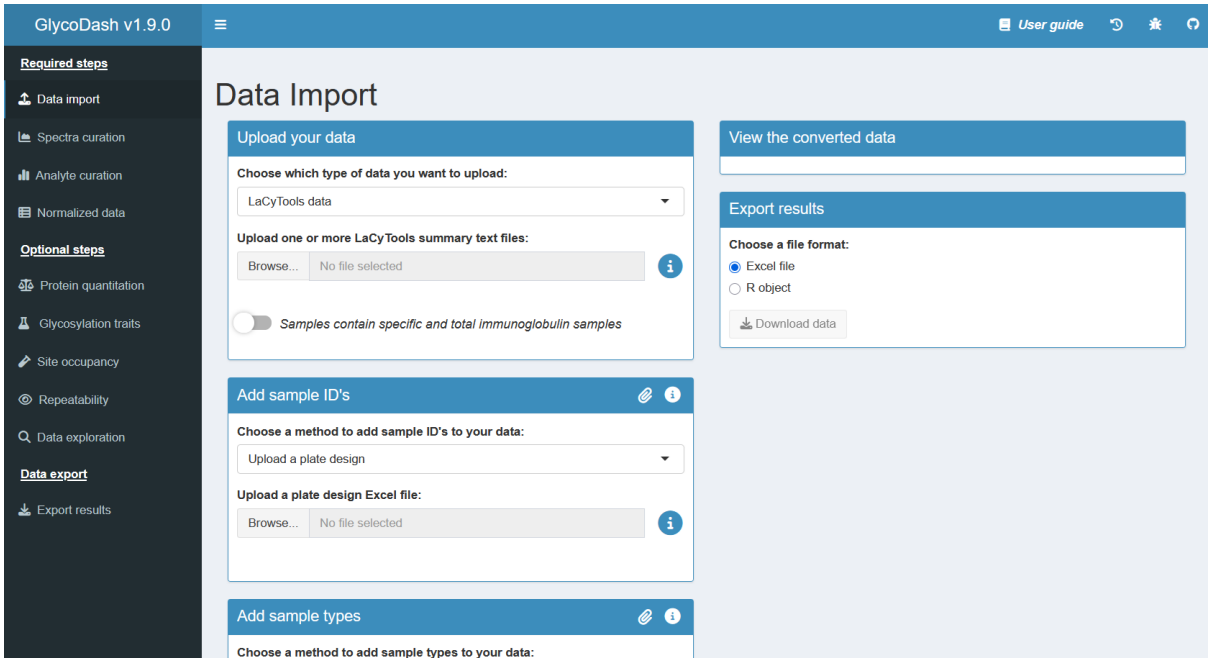
To install the latest official release of GlycoDash on your computer, follow the steps listed in the README section on GitHub: [https://github.com/Center-for-Proteomics-and-Metabolomics/GlycoDash](https://github.com/Center-for-Proteomics-and-Metabolomics/GlycoDash)

We recommend using Docker to install and run GlycoDash, as this will automatically take care of package and version dependencies (available at [https://docker.com/](https://docker.com/)). Alternatively, you can run GlycoDash within RStudio (available at [https://posit.co/downloads/](https://posit.co/downloads/)). For the development of GlycoDash v1.9.0, R version 4.5.0 is used. The required packages are listed in Appendix 1.

When running GlycoDash in RStudio, having different R and package versions installed may cause GlycoDash to malfunction.

# User interface overview

When launching GlycoDash, you will be presented with the page shown below.




The top-left corner displays the version number of GlycoDash. The left column contains various tabs. The first four tabs (Data Import, Spectra Curation, Analyte Curation and Normalized Data) should be completed in the listed order, as detailed in subsequent sections. Later tabs are optional and can be skipped.

The buttons in the top-right corner can be used to download the latest version of the user guide, to download a changelog, to view a list of known bugs and issues, or to visit the GitHub page containing the source code of GlycoDash.

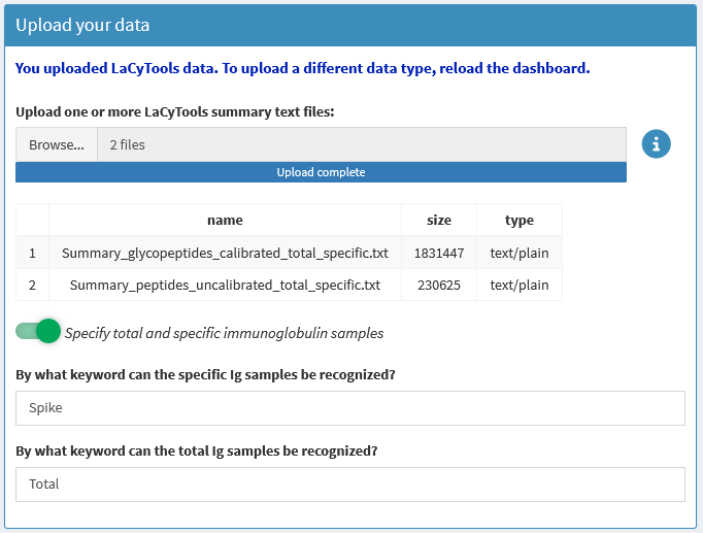
# Data import

## Raw data input

In the "Upload your data" box, choose the type of raw data that you want to upload: LaCyTools data or Skyline data. Hovering over the blue info icon will show information about the formatting requirements. See the sections below for more details.

The uploaded data is converted into a clean data frame, with a column for each variable in the data. The converted data is displayed in the top-right box "View the converted data" in the dashboard.

**Optional:** when applicable, specify keywords by which total and specific immunoglobulin samples can be recognized in the sample names. The specification of keywords is case sensitive. The table with converted data will be given an additional column called "group" which specifies whether a sample contains total or specific glycosylation data. Total and specific samples will be separately curated in later steps.



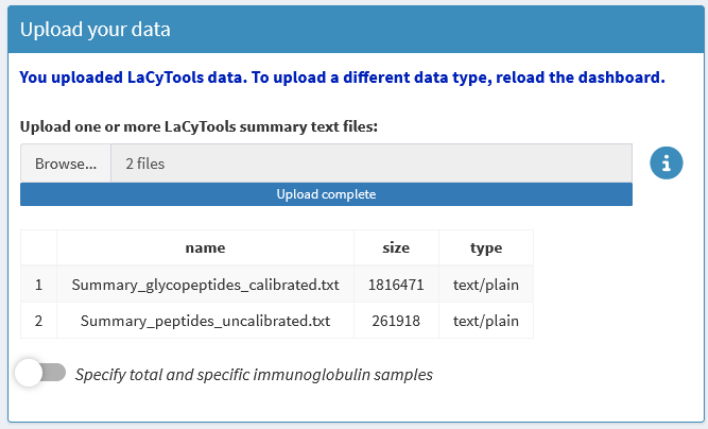

### Formatting of LaCyTools data

You can upload one or more summary text files created by LaCyTools. The names and sizes of the uploaded files will be displayed in a table (see screenshot below).

Each LaCyTools summary text file must contain at least the following outputs:

1. Background subtracted absolute intensity
2. Mass accuracy (ppm)
3. Isotopic pattern quality (IPQ)
4. Signal-to-noise ratio (S/N)

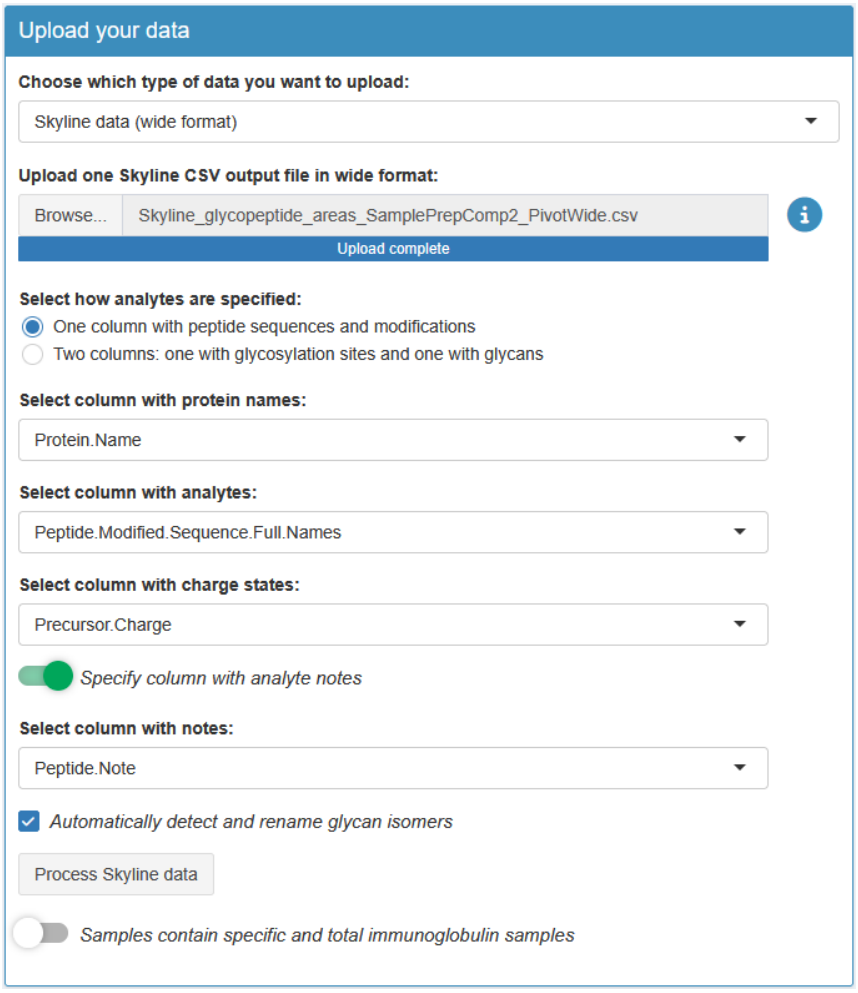
These outputs should be present for each analyte, per charge state.



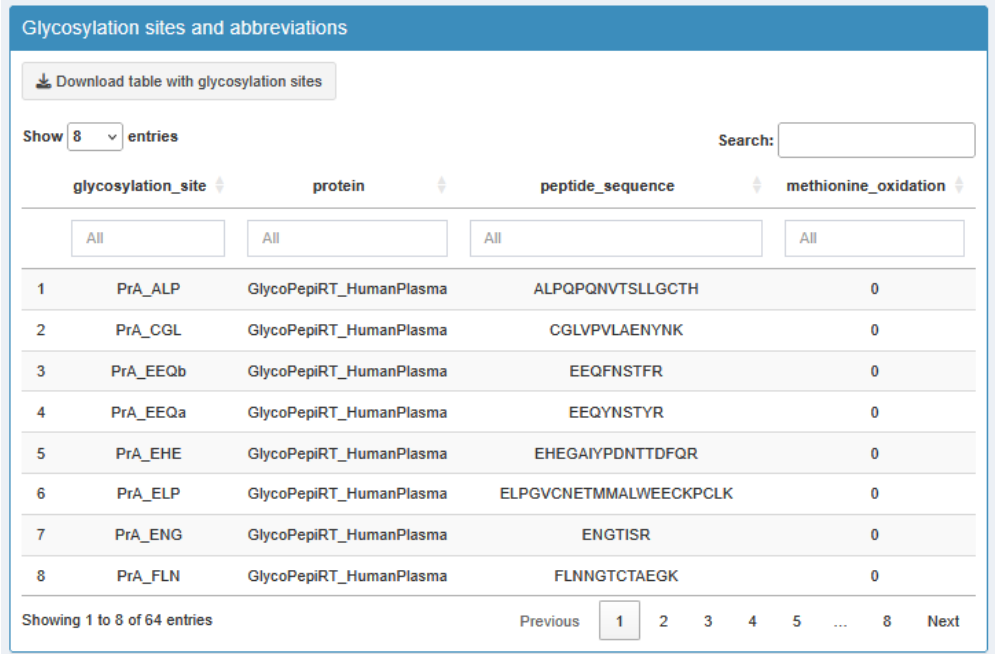
**Formatting of Skyline data (wide format)**

You can upload one CSV output file from Skyline. The required formatting of the file depends on the way in which analytes are specified. Analytes (glycopeptides) in the file must be specified in one of two ways:

1. ***One column*** where each entry contains both a peptide sequence and a glycan composition, e.g. "EEQYN[H3N4F1]STYR". A sequence in this column may also contain methionine oxidation and cysteine carbamidomethyl (CAM) modifications. These modifications may be fully written out ("[Oxidation (M)]", "[Carbamidomethyl (C)]"), or they can be specified using a three letter abbreviation ("[Oxi]", "[CAM]"). Other modifications are currently not supported.
   The column will likely be called either "*Peptide Modified Sequence Full Names*" or "*Peptide Modified Sequence Three Letter Codes*".
   When analytes are specified this way, there must also be a column containing protein names and a column specifying the charge states of the analytes. These columns will likely be called "*Protein Name*" and *"Precursor Charge"*, respectively. Additionally, the file should contain columns with *Total Area MS1, Isotope Dot Product* and *Average Mass Error PPM*, **for each sample name.** This means that the data is structured in wide format.
   In the user interface, select the required columns and press the "Process Skyline data" button.

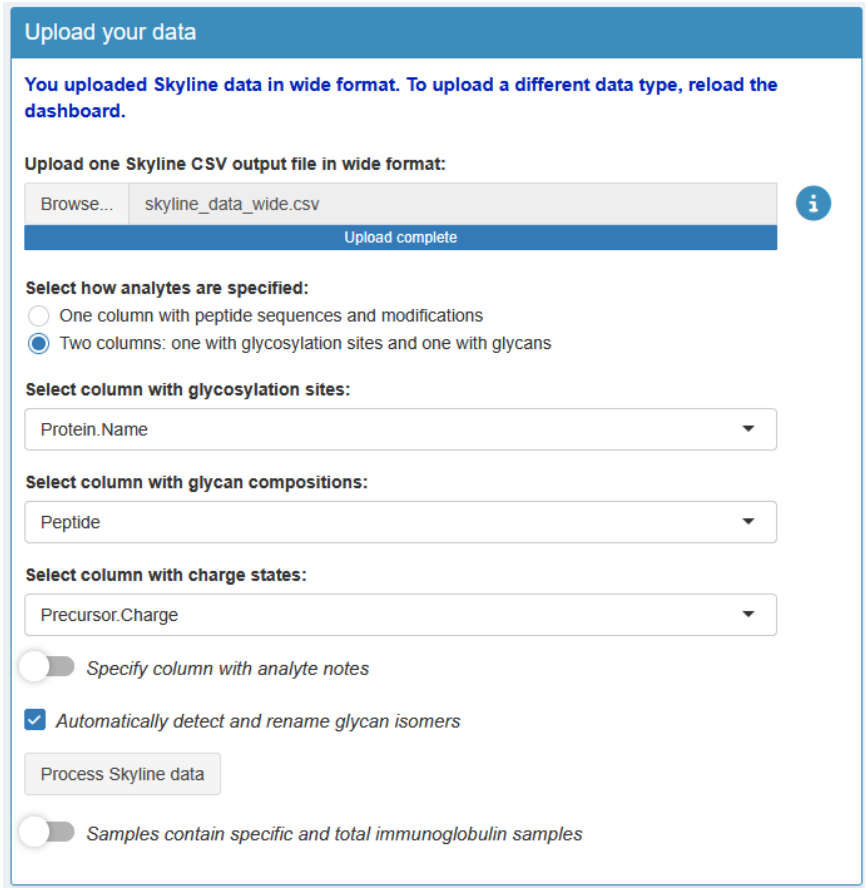


Each unique combination of protein name and peptide sequence will be assigned a unique glycosylation site. The protein names will be converted to "PrA", "PrB", etc. in alphabetical order. Peptide sequences will be abbreviated using the first three letters of the sequence. When two or more peptides in a protein share the first three sequence letters, suffices "_a", "_b", "_c", etc. are added to the sequence abbreviation. Finally, the protein and sequence abbreviations are pasted together with an underscore separating them, to form a string representing a unique glycosylation site. A downloadable table is generated where the protein names and peptide sequences can be linked to the assigned glycosylation sites.




The column "methionine_oxidation" lists the number of oxidized methione residues in the corresponding peptide sequence. For each oxidized methionine, the glycosylation_site entry is given an extra suffix "Ox".

2. ***Two columns***, one with glycosylation sites and one with glycan compositions. The glycosylation sites and glycans will be combined into a single analyte name (e.g., glycosylation site "PepI" and glycan "H3N4F1" will be combined into analyte "PepI1H3N4F1"). When analytes are specified this way, there must also be a column specifying the charge states of the analytes. This column will likely be called "*Precursor Charge*".
   Additionally, the file should contain columns with *Total Area MS1, Isotope Dot Product* and *Average Mass Error PPM*, **for each sample name.** This means that the data is structured in wide format.
   In the user interface, select the required columns and press the "Process Skyline data" button.



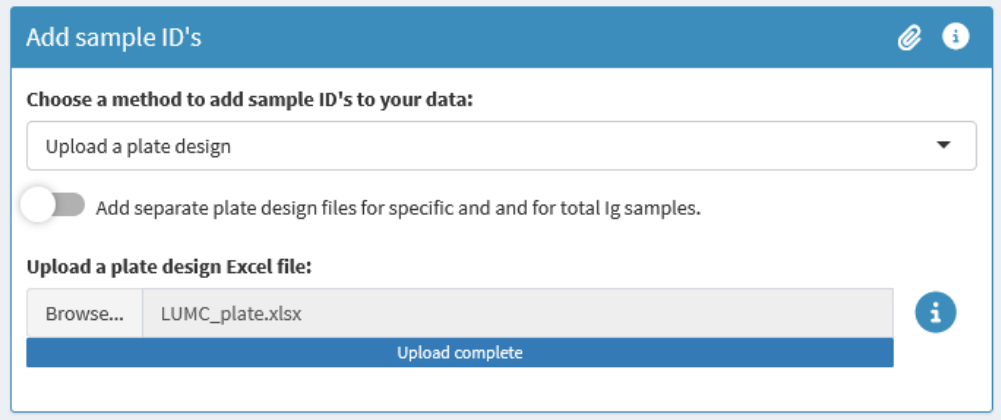

Optionally, Skyline data may contain a column with notes for each analyte. This column is likely called "*Peptide Note*". When toggling the option "Specify column with analyte notes" selecting the corresponding column, an extra tab with notes for each analyte will be added to the output Excel file when exporting the processed data.

By default, analytes with isomeric glycan compositions are automatically detected and renamed when processing Skyline data. For example, when "PepIH5N2" is present twice per sample (in each charge state), they are renamed to "PepIH5N2_a" and "PepIH5N2_b". This renaming of isomers can be prevent by unchecking the checkbox.

## Adding sample ID's

1. Choose a method to add sample ID's:
    - *Upload a plate design* – This method requires each sample name to include a plate number and well position separated by an underscore in the correct format. Examples of correctly formatted samples names are:
        - "MS_**PL01_B09**_01_2020.raw" → *plate 1, well B09*
        - "IM1_**PL02_A11**_03_measurement.raw" → *plate 2, well A11*

      The plate design should be an Excel file, with different plates separated by an empty line. The plates in the plate design are automatically numbered starting at 1. Each cell in a plate layout should contain a sample ID for the corresponding sample. If a plate well was not measured, you can leave the corresponding cell empty. Duplicate sample ID's are allowed.
      An example plate design can be downloaded in GlycoDash by clicking the paperclip button.




      **Optional**: when applicable, you can upload separate plate designs for total and specific immunoglobulin samples.

    - *Upload a sample list* – Use this method when your sample names do not follow the previously mentioned format. The sample list should be an Excel file with two columns:
        - *sample_name* – This column should contain all the sample names that are present in your data (matching the names in the first column of the converted data).
        - *sample_id* – This column should contain the corresponding sample ID's corresponding to each sample name (e.g. "blank", "pool", "plasma").

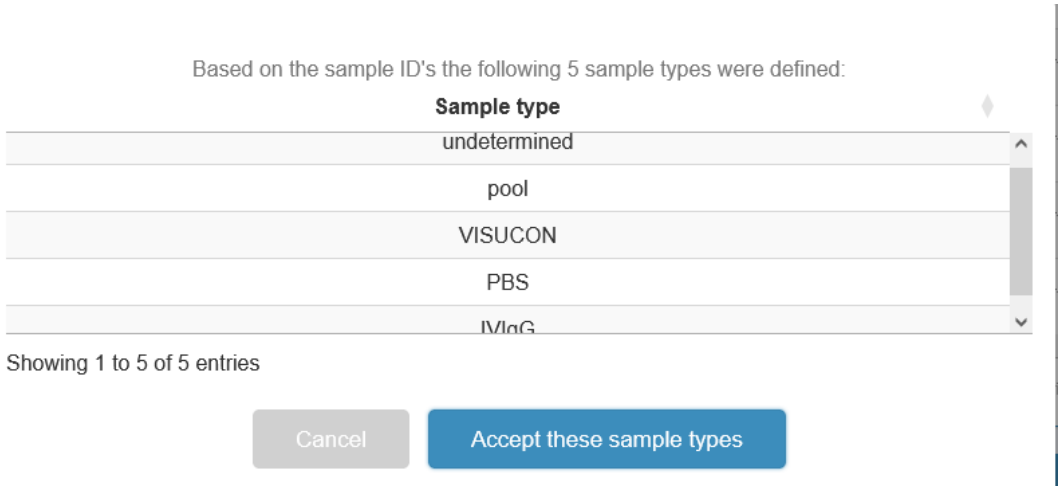
      An example sample list can be downloaded by clicking the paperclip button.

2. Upload the required Excel file. A "sample_id" column is automatically created and shown in the box with the converted data.

## Adding sample types

1. Choose a method to add sample types.
    - *Automatically* – Sample types will be detected based on shared substrings of letters in sample ID's. For example, sample ID's "standard_1" and "standard_2" would both be assigned the sample type "standard".
    - *Upload a sample type list* – The list should be an Excel file with two columns:
        - *sample_id* – This column should contain each sample ID once (even if a sample ID appears multiple times in your data).
        - *sample_type* – This column should specify the sample type corresponding to each sample ID. For instance, sample ID "PBS" could be assigned the sample type "Blank". Sample ID's "standard_1", "standard_2" and "standard_3" could all be assigned the sample type "standard".
          An example sample type list can be downloaded by clicking the paperclip button.'

2. Depending on your choice in the previous step:
    - *Automatically* – Click the "Determine the sample types" button. A popup is shown with the detected sample types. To use these detected sample types, click the blue "Accept these sample types" button. If you change your mind and want to use the other method instead, click "Cancel".




    - *Sample type list* – Upload your Excel file. The sample types are added to your data automatically.

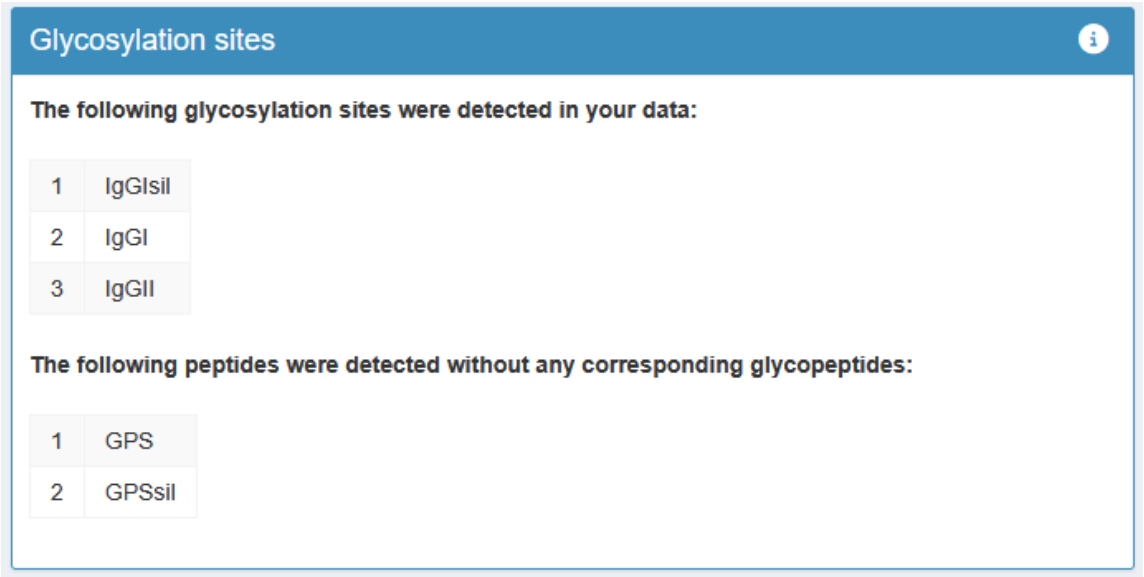
## Detection of glycosylation sites

Glycopeptides in your data will automatically be assigned to a glycosylation site based on their names. For example, two analytes "IgGI1H3N4F1" and "IgGI1H4N4F1" would both be assigned to the same glycosylation site "IgGI". Spectra curation and analyte curation will later be performed per glycosylation site.

**Optional:** your data may contain heavy isotope labeled glycopeptides, e.g. SILuMAb IgG1 glycopeptides. These will be assigned their own glycosylation site (e.g., "IgGIsil" as in the screenshot below) and can later be used for protein quantitation.

Non-glycosylated peptides in your data are automatically detected. Two situations can be distinguished:

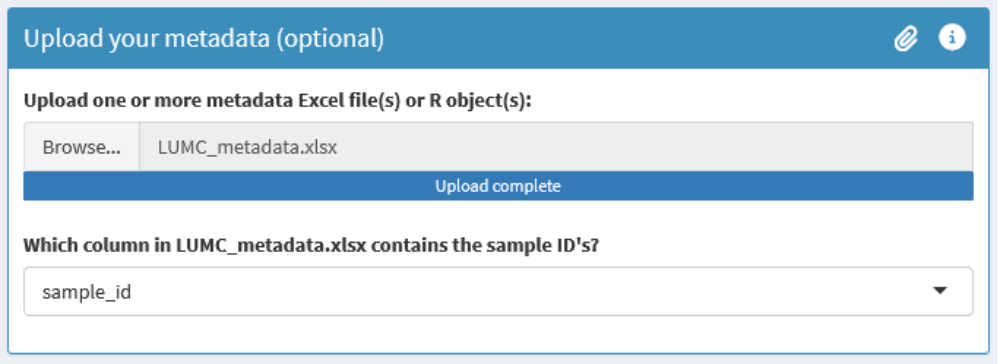
- When there are glycopeptides in your data that correspond to the non-glycosylated peptide in their glycosylation site, then this peptide can later be used for calculating site occupancies. For example, if the analyte "IgGI1" is present in your data, then this could be used to calculate the occupancy of glycosylation site "IgGI".
- When no corresponding glycopeptides exist in your data, then the non-glycosylated peptide is assumed to not belong to a glycosylation site. These peptides can later be used for protein quantitation.



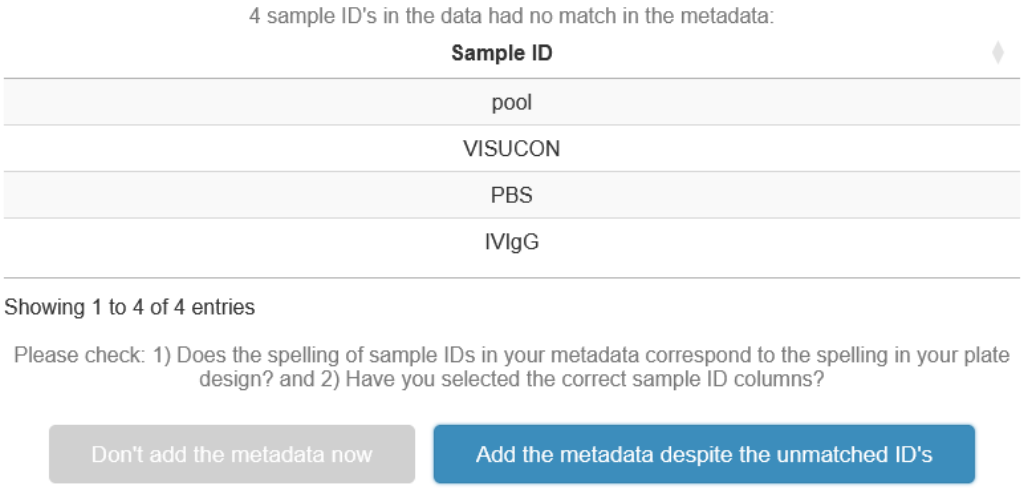
## Adding metadata (optional)

You can upload an Excel file containing metadata (e.g. age, sex, disease status), which will be merged with the glycosylation data. This integration ensures that the final output data is ready for immediate further data analysis in a software program of your choice. The metadata can later also be used to perform analyte curation per biological group.

1. Prepare an Excel file with metadata. The file should contain a column with sample ID's (the name of this column does not matter in this case), and additional columns with metadata.
2. Upload the metadata file. In the dropdown box, select the name of the column that contains the sample ID's.




3. One of two things will happen:
    - If all sample ID's in your data have a match in the metadata, then the metadata will immediately be combined with your data and displayed in the box with converted data.
    - If there are sample ID's in your data that do not have a match in the metadata, a warning popup will appear. You can then decide whether you want to add the metadata or not.
      For example, in the example screenshot below, the sample ID's "pool", "VISUCON", "PBS" and "IVIgG" do not have a match in the metadata. This is expected these sample ID's belong to standards. In this case it would be appropriate to add the metadata despite the unmatched ID's.

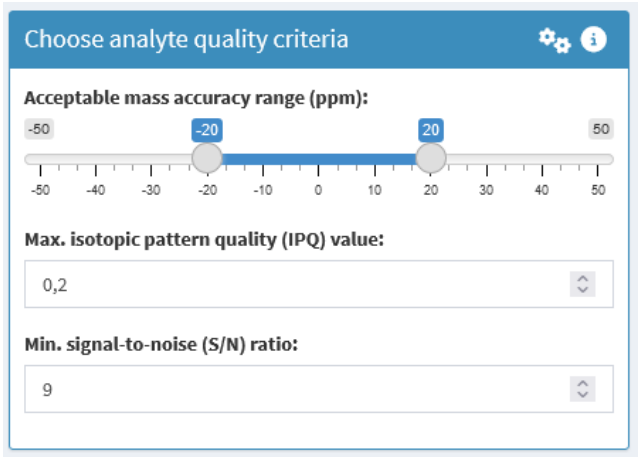


# Spectra curation

## Choosing analyte quality criteria

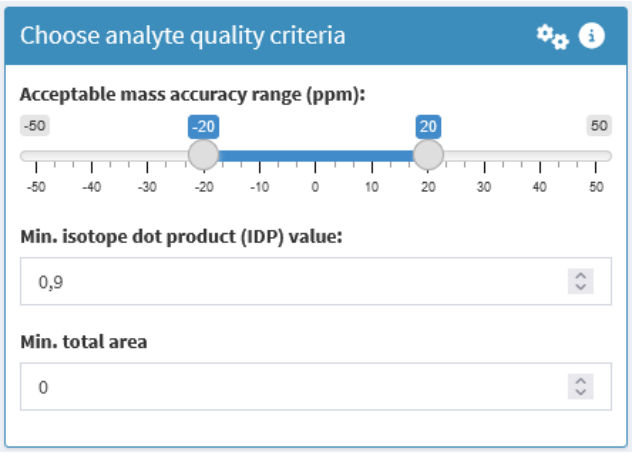
The analyte quality criteria are used to judge whether the signal of an analyte is of sufficient quality in a given sample to be reliably quantified. It is important to note that different charge states for the same glycopeptide are treated as distinct analytes. This information is used for spectra curation and for the subsequent analyte curation step. Depending on the type of data uploaded, there are three distinct quality criteria for which boundaries can be configured. An analyte is deemed to be of sufficient quality when it meets all three criteria. Only in very specific cases, one or two of the listed quality criteria may be used, by clicking the gears icon and deselecting the criteria that you wish to ignore.

*LaCyTools data:*




- **Acceptable mass accuracy range (ppm)** – Sets the acceptable mass accuracy in ppm. The maximum range is −50 to +50 ppm, with the default being −20 to +20 ppm.
- **Maximum isotopic pattern quality (IPQ) value** – Sets the maximum acceptable IPQ value for an analyte. The better the observed isotopic pattern of an analyte matches its theoretical isotopic pattern, the lower the IPQ will be. The default value is 0.2.
- **Minimum signal-to-noise (S/N) ratio** – The minimum S/N ratio that an analyte should have to be of sufficient quality. The default value is set to 9.

*Skyline data:*



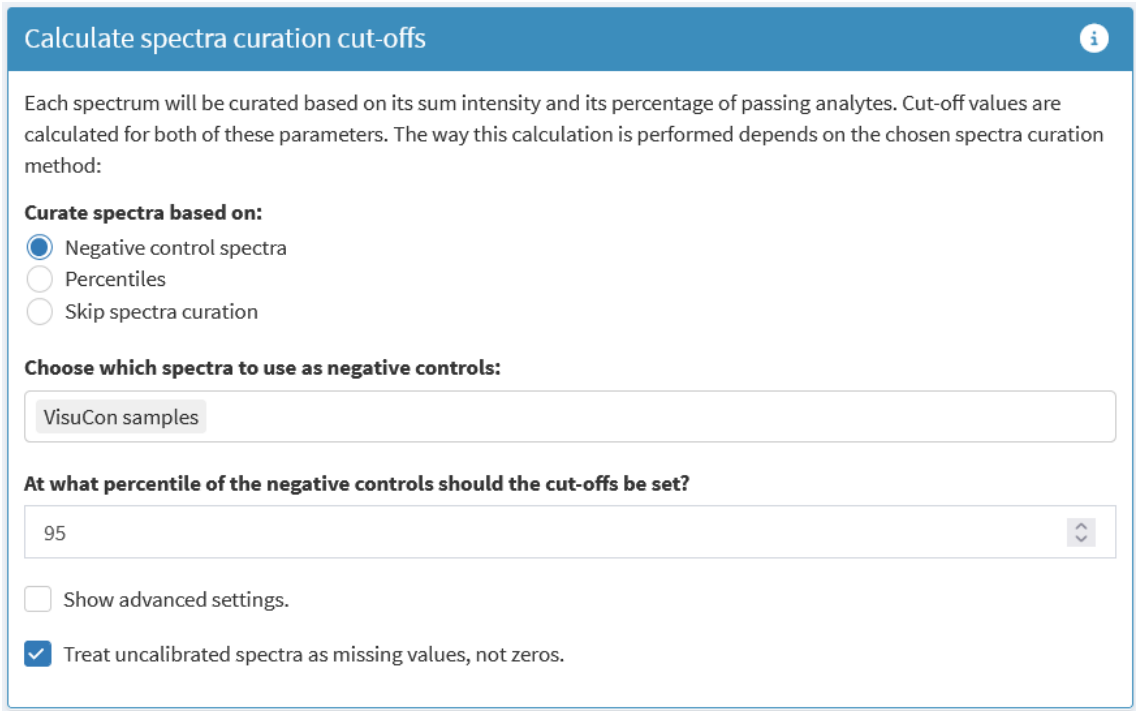

- **Acceptable mass accuracy range (ppm)** – Same as for LaCyTools data.
- **Minimum isotope dot product (IDP) value** – The minimum IDP value that an analyte should have. A higher IDP value means that the observed isotopic pattern fits the theoretical isotopic pattern better, with a maximum value of 1. The default value is set to 0.9.
- **Minimum total area** – As Skyline does not output S/N, the total area can be used as an alternative. By default this value is set to 0 (i.e. it is ignored).

## Spectra curation cut-offs

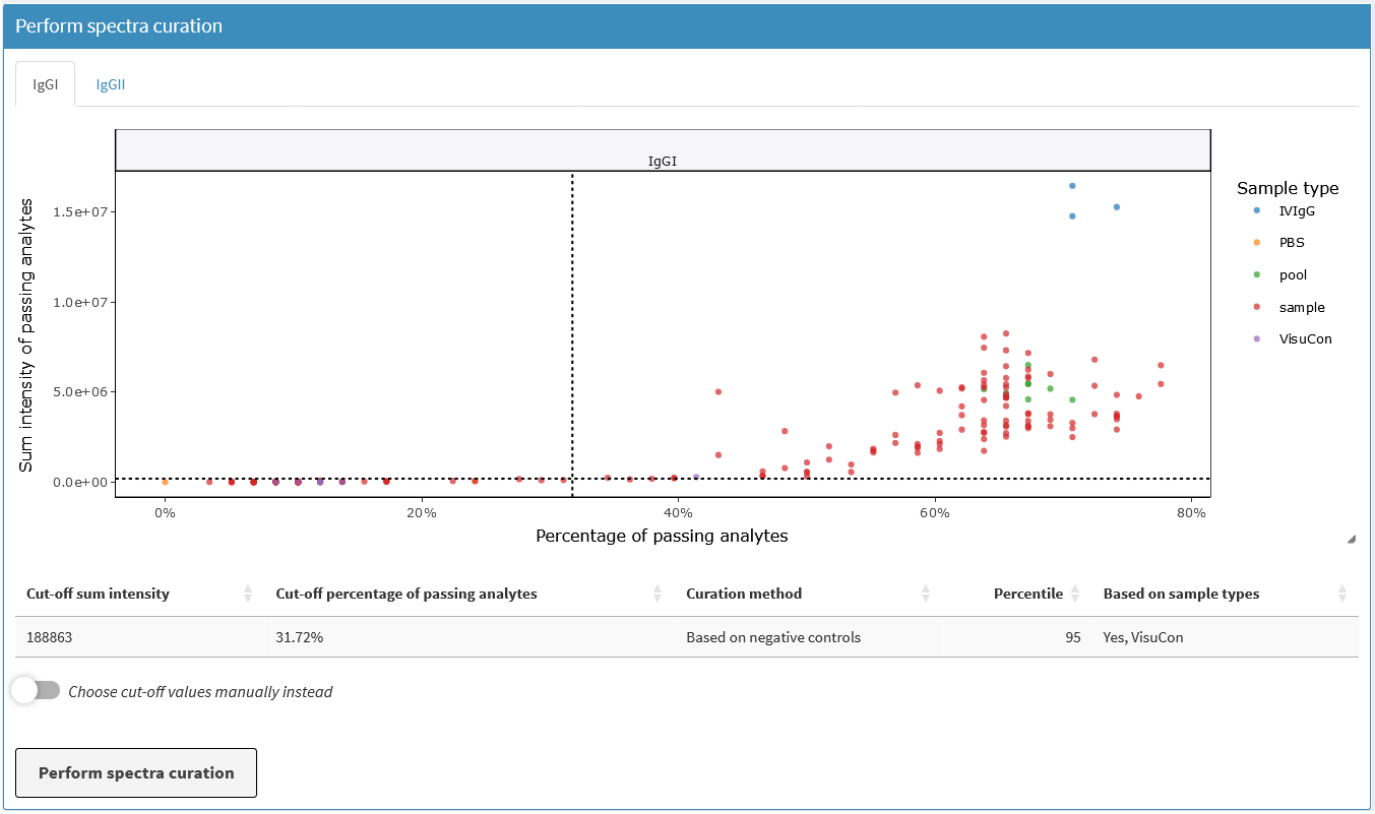
For each sample and glycosylation site in your data, the intensities of all passing glycopeptide analytes are summed, and the percentage of passing analytes out of all targeted analytes is calculated. Each spectrum will be curated based on these values. The two available methods for curating the spectra are described below. Alternatively, you have the option to skip spectra curation, in which case all spectra will be utilized in subsequent processing steps.

*Curate spectra based on negative controls:*

1. Choose which sample types should be used as negative controls.
2. Set a percentile of negative control measurements for calculating cut-off values. The default percentile is 95. GlycoDash will automatically compute cut-off values for both the percentage and sum intensity of passing analytes. These cut-offs are calculated separately for each glycosylation site.
    - By default, uncalibrated spectra are treated as missing values and are excluded from the calculations. You have the option to include them with a value of zero instead by unchecking the checkbox "Treat uncalibrated spectra as missing values, not zeros".




During curation, spectra whose percentage of passing analytes and sum intensity exceed or equal the corresponding cut-off values will pass curation, and will be used for further data processing. In the "Perform spectra curation box", all spectra are displayed per glycosylation site in an interactive plot, color-coded by sample type. The cut-offs are indicated by dashed bars, and the exact cut-off values are displayed below the plot for reference.

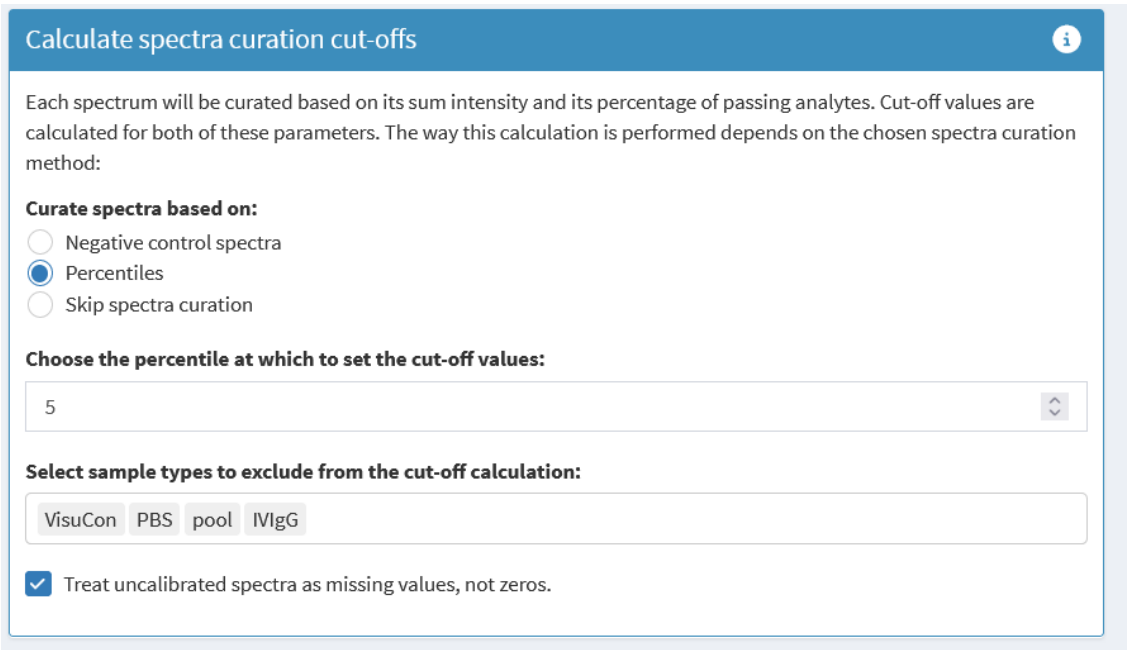



3. If you are satisfied with the cut-offs, click the “Perform spectra curation” button.

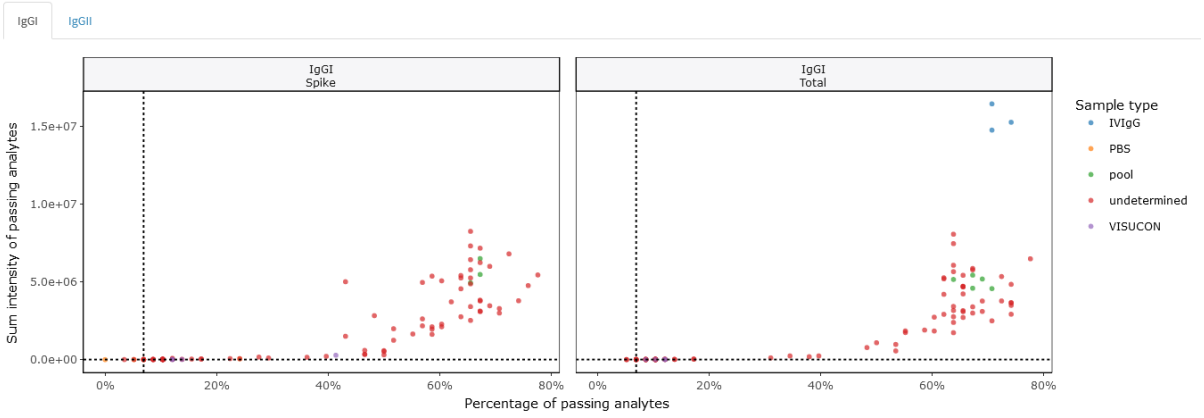
*Curate spectra based on percentiles*

In this scenario, cut-off values are determined at a specific percentile based on all measurements associated with a glycosylation site, with the option to exclude certain sample types from the calculation. This means a fixed percentile of all data, that with the lowest data quality, is excluded from further processing.

1. Choose the percentile at which to set the cut-off values. The default value is 5.
2. Optional: exclude sample types from the calculations. We recommend to exclude all types of controls. Excluded types will be curated based on the calculated cut-off.
3. Review the cut-offs in the “Perform spectra curation” box.
4. Once satisfied with the cut-offs, click the “Perform spectra curation” button.



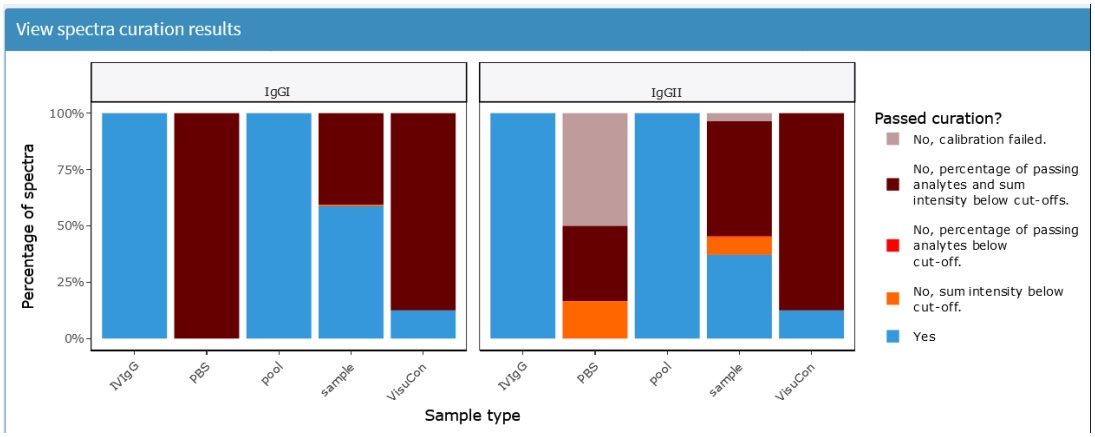
**Note**: in cases where your data includes both total and specific immunoglobulin samples, cut-off values will be set independently for total and specific spectra. When curating based on negative control spectra, which sample types to use as negative controls must be selected separately for total and specific spectra.




| Group | Cut-off sum intensity | Cut-off percentage of passing analytes | Curation method | Percentile | Based on sample types |
|---|---|---|---|---|---|
| Spike | 5272 | 6.90% | Based on percentiles | 5 | Yes, undetermined, VISUCON, PBS and pool |
| Total | 6161 | 6.90% | Based on percentiles | 5 | Yes, undetermined, pool, VISUCON, IVIgG and PBS |

## Spectra curation results

After performing spectra curation, an interactive plot displaying the results is generated. The plot shows the percentage of passing and failing spectra for each sample type per glycosylation site. The reasons for failing curation are indicated by different colors. For LaCyTools input, the percentage of uncalibrated spectra is also displayed (first pass spectra curation).




Below the plot, three tables are shown with:

- Details of passing spectra per analyte.
- Overview of failed spectra.
- Details of failed spectra per analyte.

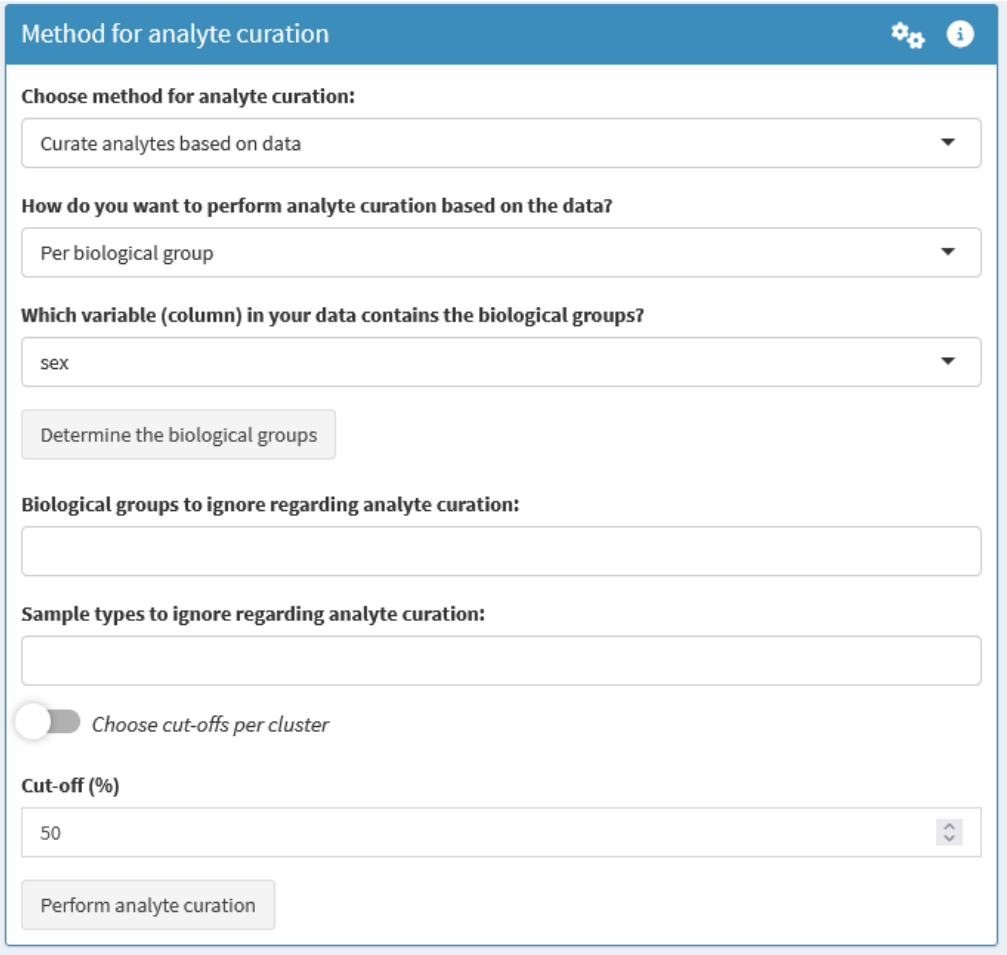
There is an option to download each of these tables as an Excel file at the bottom of the page, in the “Export results” box.

# Analyte curation

## Analyte curation methods

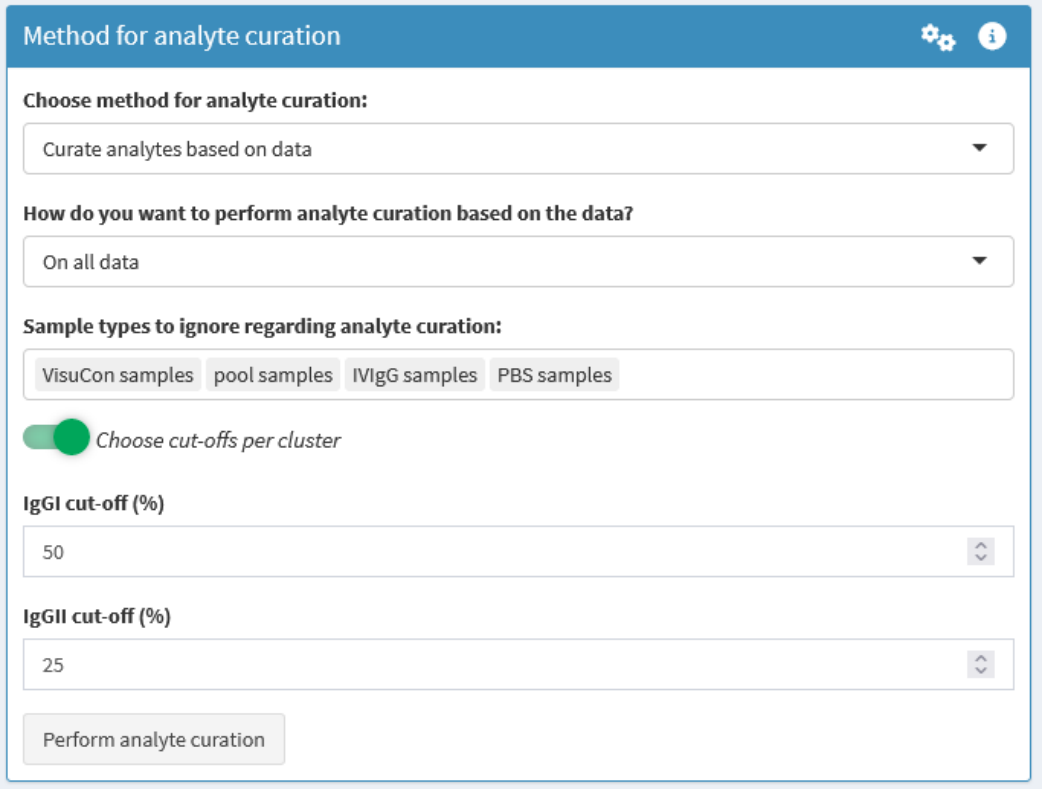
There are two main methods for analyte curation. The preferred method is to curate the analytes based on the data.* There are three different ways to do so:

1. *Per biological group* – An analyte passes curation if it meets the quality criteria in a percentage of spectra exceeding the chosen cut-off in at least one biological group (e.g. disease status or treatment). The analyte is then used for total area normalization in all samples, irrespective of the biological group.
   - Choose a variable (column) in your data that contains the biological groups.
   - Click the "Determine the biological groups" button.
   - A popup, displaying detected biological groups, appears for verification.
   - Optional: select biological groups to ignore (e.g. when dealing with a small number of samples in a specific group).
   - Optional: select sample types to ignore. Sample types without a biological group assigned (e.g. blanks) are automatically excluded, even if not selected here.
   - Choose a cut-off or different cut-offs per cluster.
   - Click the "Perform analyte curation" button.




* Alternatively, you can choose to supply an analyte list (as an Excel file or R object) that contains the names of all the analytes that should be kept. All charge states of these analytes will pass curation. This method should only be used if you performed analyte curation based on your data outside of GlycoDash.

2. *On all data* – When an analyte in a given charge state fulfills the quality criteria (as defined in the spectra curation step) in a percentage of spectra exceeding a chosen cut-off percentage, then it passes curation and is used for total area normalization during the next step in all samples.
    - There is an option to ignore certain sample types in the assessment. For instance, we recommend to exclude all controls.
    - Choose a cut-off, the default is 50%, which is applied to all glycosylation sites. To choose different cut-off percentages per cluster, toggle the *"Choose cut-offs per cluster"* switch.

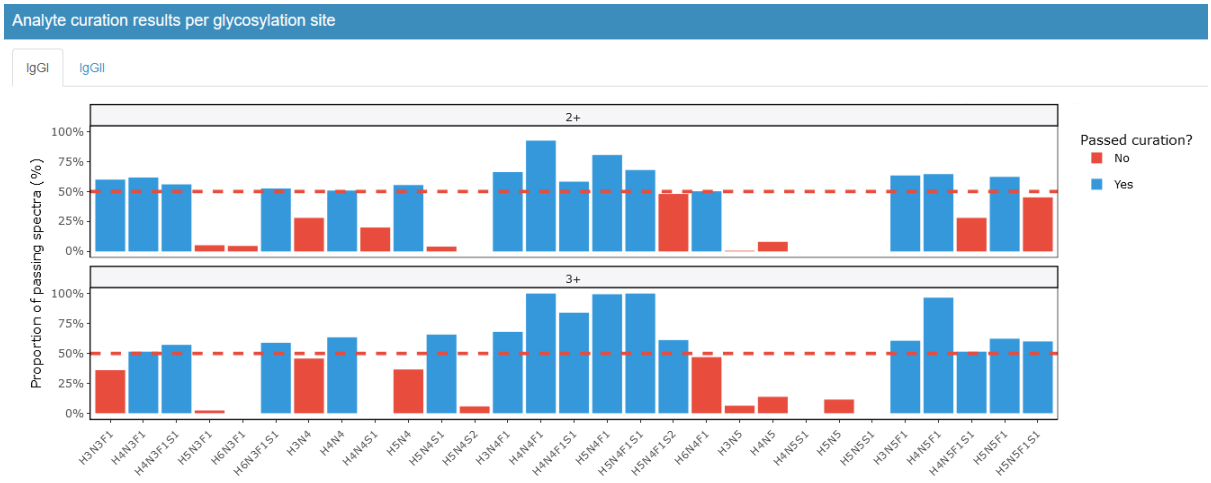



3. *Per sample* – The data is curated per sample. If an analyte meets the quality criteria in one sample, it is used for total area normalization in that sample. However, if the analyte does not meet the quality criteria in another sample, it will not be used for normalization in that particular sample.

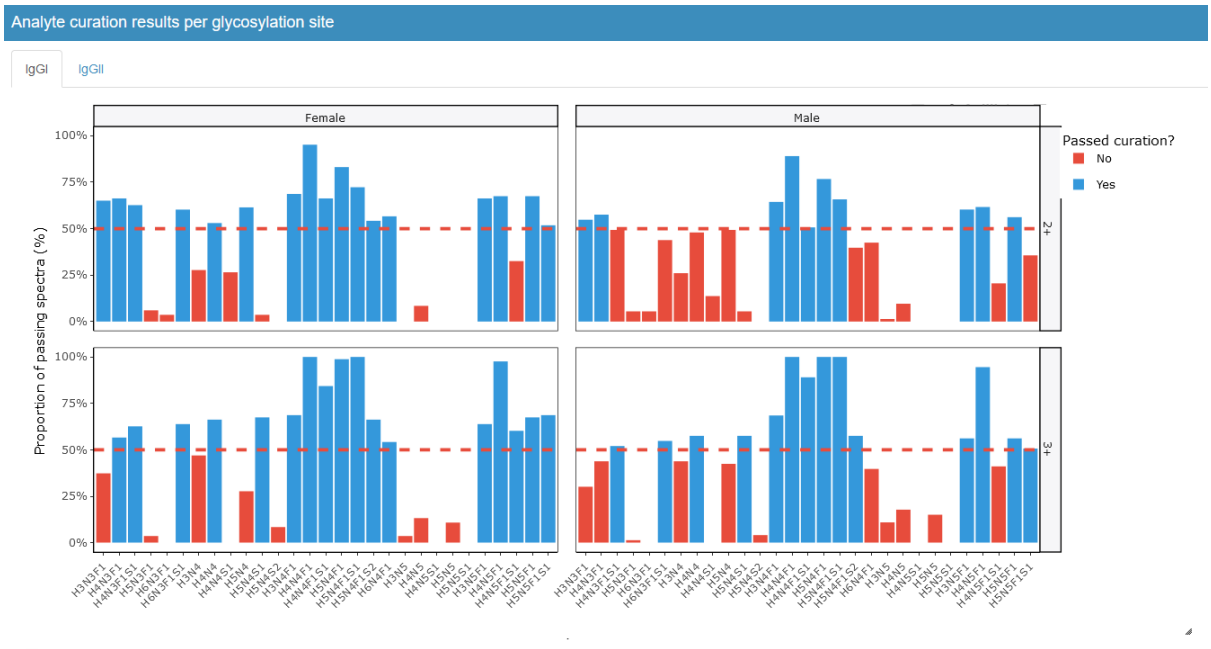
## Analyte curation results

When performing analyte curation per sample, no plots or results will be displayed.

For analytes that were curated based on all data, an interactive plot is generated for each glycosylation site. Each analyte and charge state is represented by a bar in the plot, color-coded to indicate whether it passed curation.

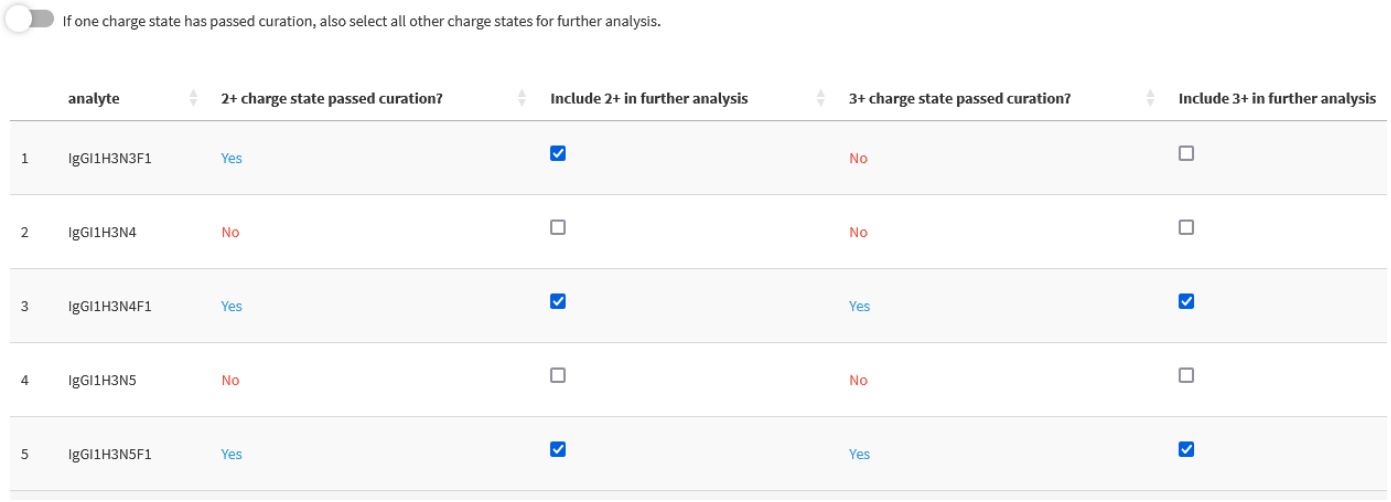



If analyte curation is performed per biological group, similar plots are generated for each biological group.




Following the curation of analytes based on all data or per biological group, a table is generated below the plots. This table lists all analyte names and includes two columns for each charge state: one indicating whether the analyte passed curation ("Yes" or "No"), and another with a checkbox to include the analyte in that charge state for further analysis.

Charge states of analytes that passed curation are pre-selected. Via the checkboxes, any analyte and charge state can de excluded or included for further analysis, if external information warrants this. Additionally, there is an option to automatically select all charge states of an analyte when it passes in at least one charge state. Strong interferences should be excluded in this case, e.g. by manual inspection of raw data or LaCyTools/Skyline output. Then, this likely increases accuracy at the expense of precision.




**Note:** if your data contains total and specific immunoglobulins, you have the option to exclude either one as a sample type during analyte curation. It is advisable to exclude total samples and base the curation solely on specific samples.

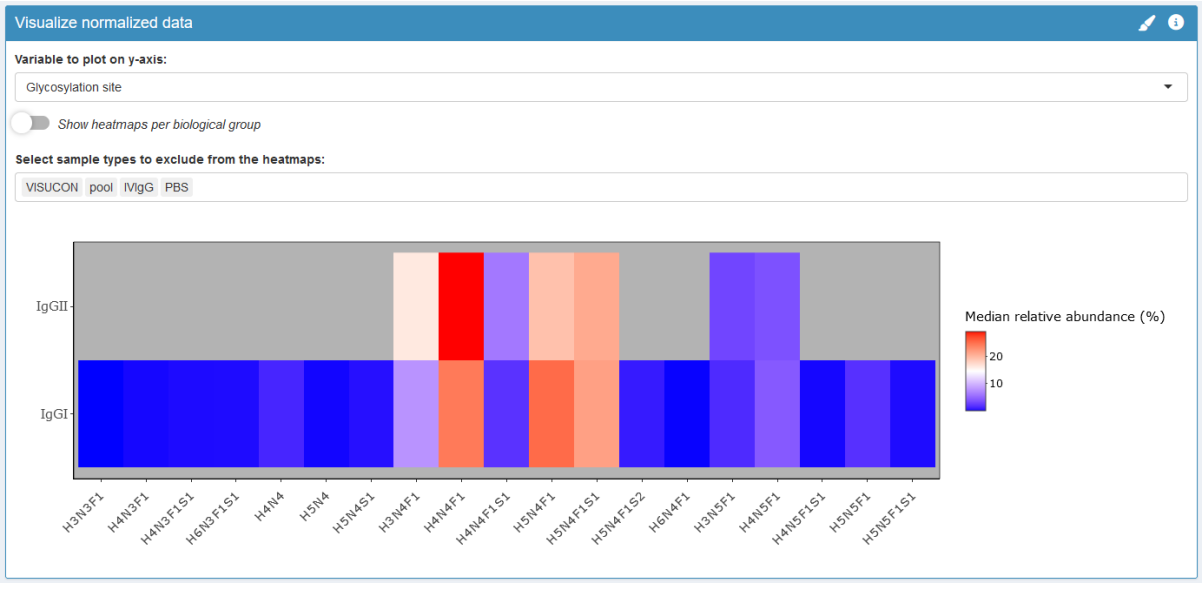
## Normalized data

Following analyte curation, total area normalization is automatically conducted on the data per glycosylation site. A table displaying the normalized data is generated, including the following information:

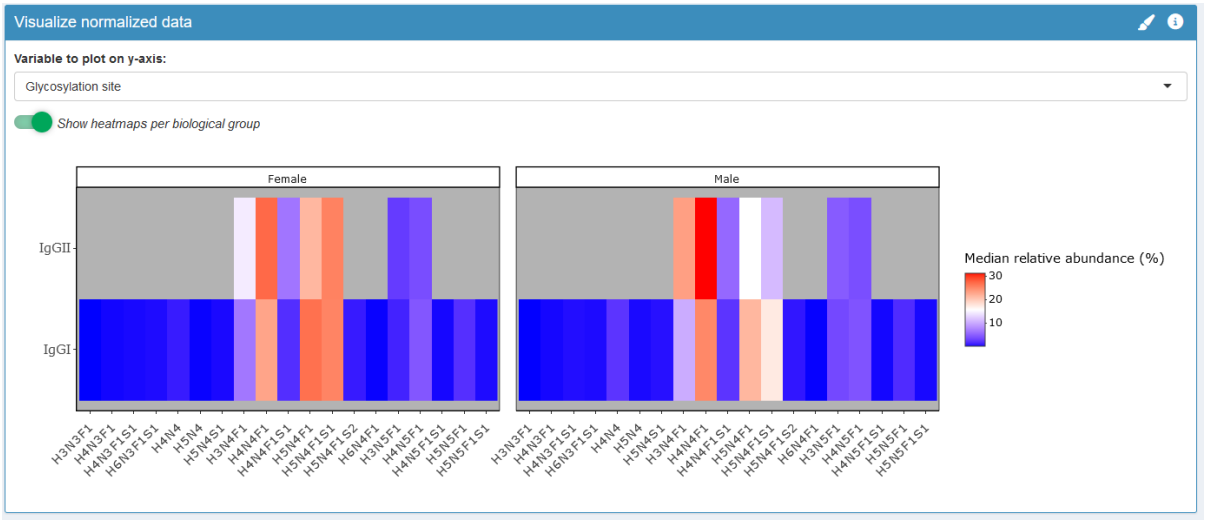
- Sample names
- Sample types
- Sample ID's
- Plate well positions, if applicable.
- The number of replicates for each sample ID (e.g. if there are ten pools in your data all labeled with the same sample ID "pool", then these will all have a value of 10 in the "number_of_replicates" column.
- Metadata, if applicable.
- Per glycosylation site: a column showing sum intensities.
- For each analyte: a column displaying relative abundances in percentages. The analytes associated with a specific glycosylation site will collectively sum to 100%.

Above the table, there is an option to normalize the charge states separately instead of combining them. At the bottom of the page, there is an option to download the normalized data as an Excel file or R object.

The normalized data is visually represented in one or more heat maps, with glycan compositions on the $x$-axis and glycosylation sites on the $y$-axis by default. Each color in the heat map corresponds to the median relative abundance for a specific peptide/glycan combination, calculated across all samples. It is possible to exclude certain sample types from the calculations.

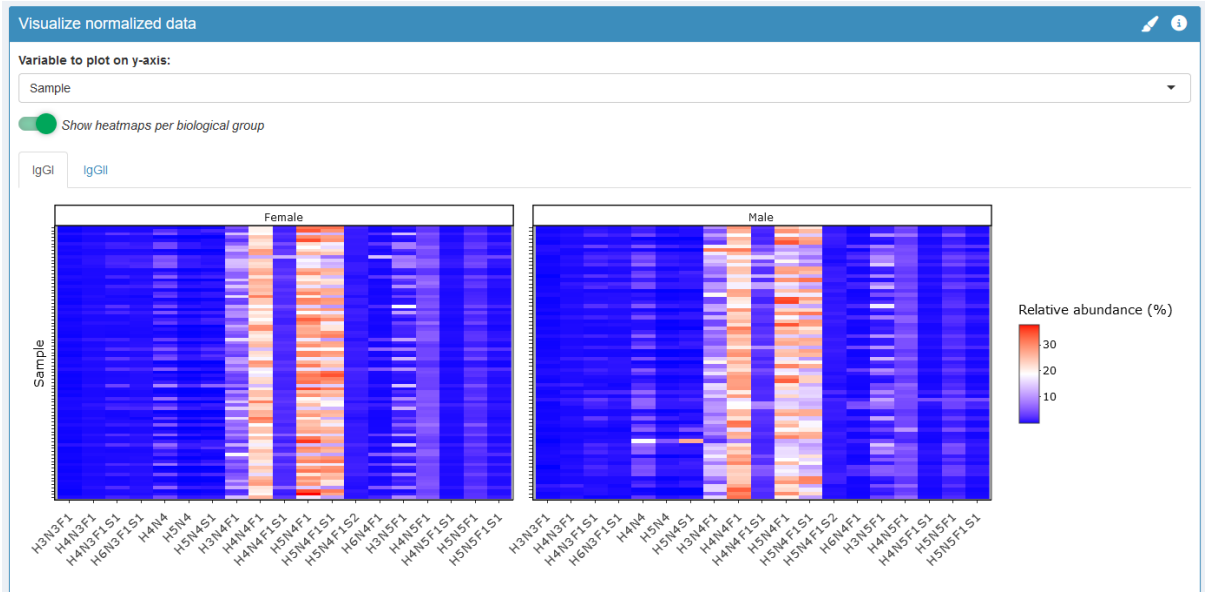


If analyte curation was performed per biological group, there will be an option to separate heat maps per biological group (this option is enabled by default).




Alternatively, the individual samples can be plotted on the $y$-axis. In this case, a heat map is generated for each glycosylation site, with the ability to exclude sample types.

When plotting samples on the $y$-axis, there is also an option to create separate heat maps per biological group, if applicable.




The colors in the heat maps can be customized by clicking the paintbrush icon in the top-right corner of the box.

## Protein quantitation (optional)

This option can be used when your samples contains one or more stable isotope labeled standards, such as SILuMAb for quantitation of antigen-specific IgG1.* You can quantify multiple proteins simultaneously, using glycopeptides and/or non-glycosylated peptides.

An Excel file is required with the following columns:

- *protein* – Name of protein to be quantified using the natural/labeled pair.
- *natural* – Name of the natural glycosylation site (when quantifying based on glycopeptides), or the name of a natural non-glycosylated peptide, **as detected by GlycoDash** (see Data import).
- *labeled* – Name of the stable isotope labeled glycosylation site (when quantifying based on glycopeptides), or of the labeled non-glycosylated peptide, **as detected by GlycoDash** (see Data import).
- *standard_ng* – The absolute amount of stable isotope labeled standard that was added to each sample, in nanograms (ng).
- *sample_ul* – The volume of sample from which proteins were purified, in microliters (µL).

***Example*** **–** Below is a screenshot of an example Excel file. Here two proteins were quantified:

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | **protein** | **natural** | **labeled** | **standard_ng** | **sample_ul** |
| 2 | IgG1 | IgGI | IgGIsil | 2 | 20 |
| 3 | IgG1 | GPS | GPSsil | 2 | 20 |
| 4 | IgG4 | IgGIV | IgGIVsil | 2 | 20 |

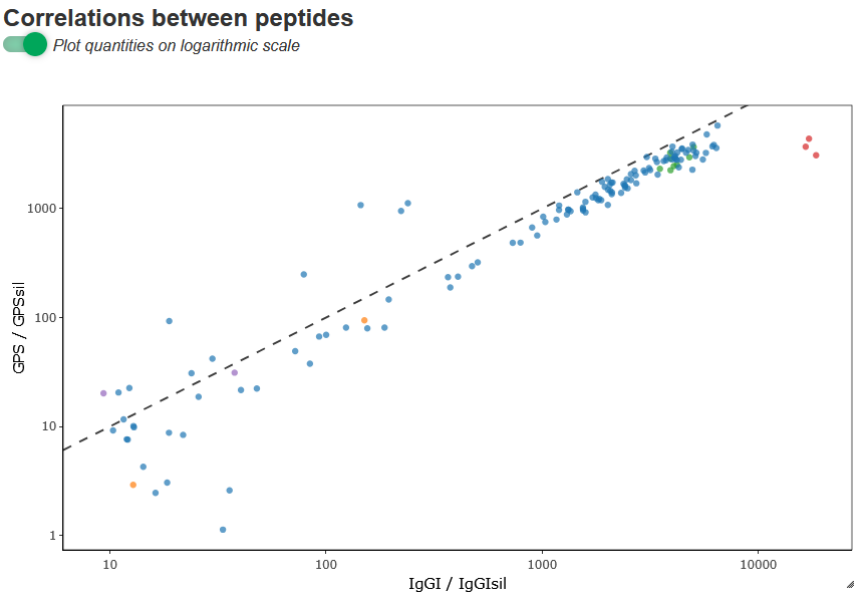
- IgG1 was quantified based on glycopeptides and a non-glycosylated peptide.
    - "IgGI" was the glycosylation site of natural IgG1 glycopeptides, "IgGIsil" was the name of the glycosylation site with the labeled IgG1 glycopeptides.
    - "GPS" was the name a natural non-glycosylated peptide, "GPSsil" was the name of the corresponding labeled peptide.
- IgG4 was quantified based only on glycopeptides.

Quantitation using glycopeptides is based on the sum intensity ratio of the natural glycopeptides and the labeled glycopeptides. Quantitation using non-glycosylated peptides is based on the intensity ratio between natural peptide and the labeled peptide,

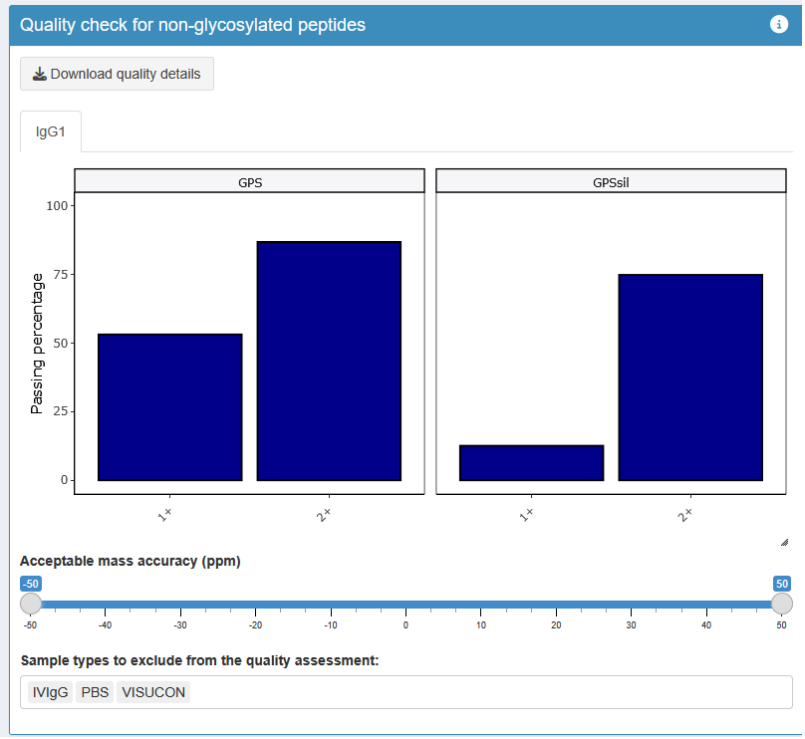
Quantities are reported as concentrations in the original sample in nanograms per millimeter (ng/mL). *Note: this may be a systematic underestimation if the standard is not added to the original sample but later in the sample preparation.*

* See our technical note for details: https://pubs.acs.org/doi/full/10.1021/acs.jproteome.4c00538

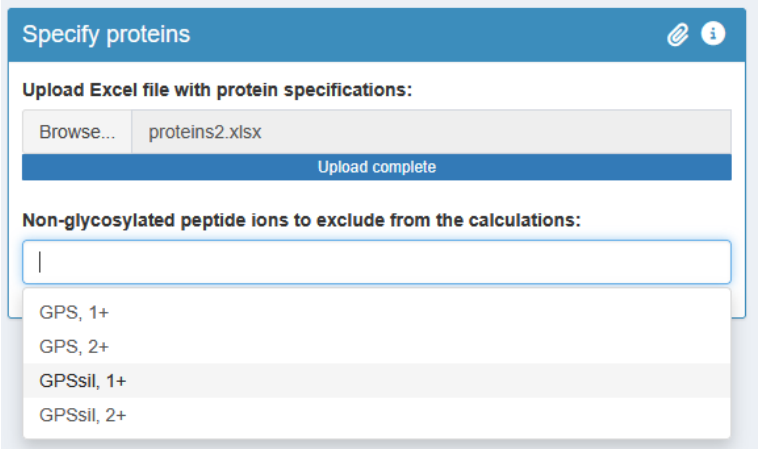
When more than one natural/labeled pair is specified for a protein, the median quantity is reported. For example, in the Excel file above, IgG1 was quantified based on both IgGI/IgGIsil and GPS/GPSsil, which gave two different quantities for each sample. The reported quantity is the median of these two quantities. Correlation plots between the quantities based on the different pairs are generated in addition. The dashed line is a line of equality ($y = x$). The closer the data points are to this lines, the more the two quantity calculations are in agreement. Ideally, there should be a good correlation. Otherwise, we suggest to investigate the validity of each natural/labeled pair.




Non-glycosylated peptides are not considered during spectral curation and analyte curation. When non-glycosylated peptides are used for quantitation of a protein, the percentage of samples in which the peptide ions fulfill the three quality criteria is plotted. For S/N and IPQ (in the case of LaCyTools data), or total area and IDP (in the case of Skyline data), the same criteria that were used for spectral and analyte curation are applied. Because non-glycosylated peptides are often integrated without calibration, you may want to be more lenient when it comes to the acceptable mass error. This value can therefore be set manually here. There is an option to exclude certain sample types (e.g., blanks and negative controls) from the quality assessment, which should be handled in the same way as for the glycopeptide analyte curation.



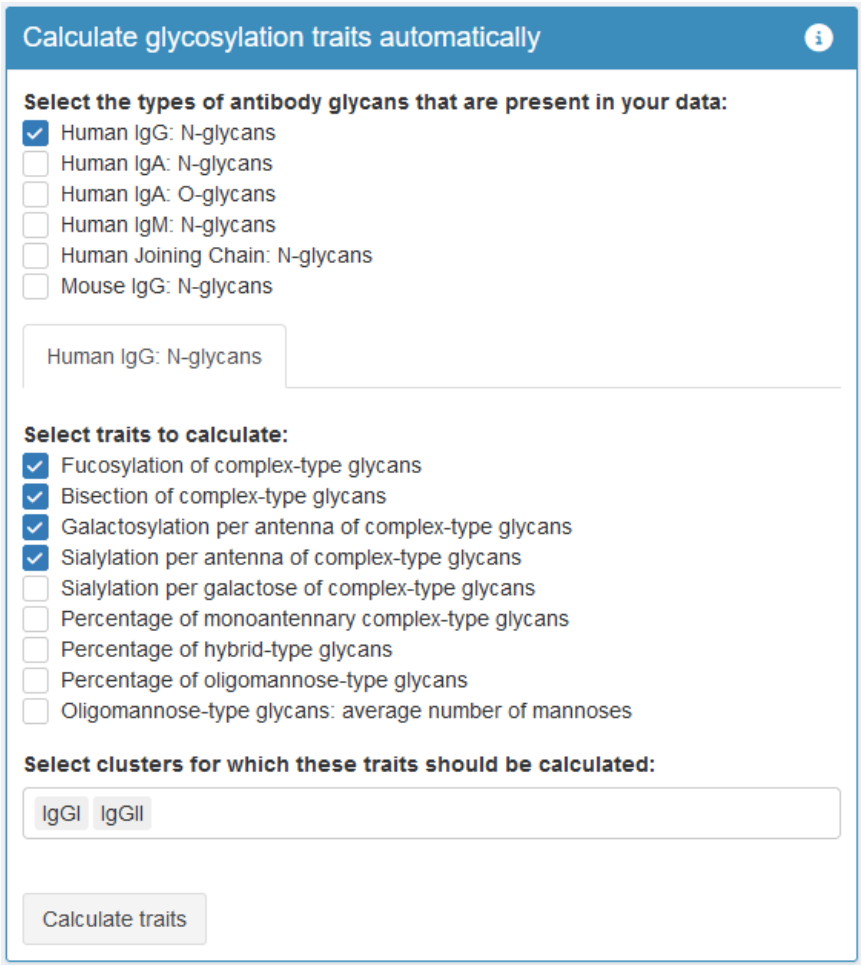

If you conclude that a certain peptide or one of its charge states is of insufficient quality, you may exclude it from being used for quantitation:



## Glycosylation traits (optional)

You can automatically calculate glycosylation traits for human IgG, IgA and IgM (including Joining Chain), and for mouse IgG. These calculations rely on a reference list containing *N*-glycan compositions with known structures, listed in Appendix 2. If your data includes glycan compositions not listed there, a warning message will be displayed when using this option.

1. Select the types of glycans that are present in your data.
2. Choose the traits you wish to calculate.
3. Select the glycosylation sites in your data for which you want to calculate the traits. Then push the "Calculate traits" button.




A table displaying the formulas used for calculating the traits will be generated. This table can be downloaded as an Excel file, which can be easily modified and used for calculating custom glycosylation traits (described below). In some cases a message is shown and a trait is not reported, namely when:

- A trait equals 100 for all samples;
- A trait equals 0 for all samples;
- A trait would be calculated using only one glycan.

In the example screenshot below, the trait "IgGII_fucosylation" is not reported because it is 100 for all samples.

## Formulas used to automatically calculate the glycosylation traits

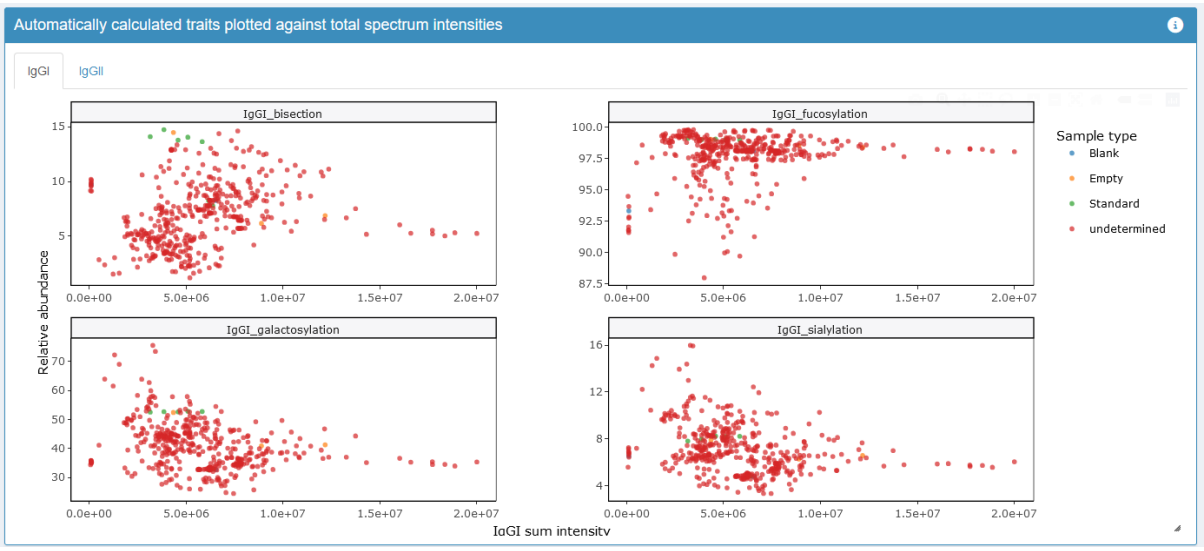
Download as Excel file

Show 10 entries  Search:

| trait | formula |
|---|---|
| All | All |
| IgGI_fucosylation | 1 * (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H4N5F1S1) |
| IgGI_bisection | 1 * (IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H4N5F1S1) |
| IgGI_galactosylation | 0.5 * (IgGI1H4N4F1 + IgGI1H4N4F1S1 + IgGI1H4N5F1 + IgGI1H4N5F1S1) + 1 * (IgGI1H5N4F1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2) |
| IgGI_sialylation | 0.5 * (IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H4N5F1S1) + 1 * IgGI1H5N4F1S2 |
| IgGII_fucosylation | Not reported: 100 for all samples |
| IgGII_bisection | 1 * (IgGII1H3N5F1 + IgGII1H4N5F1 + IgGII1H5N5F1) |
| IgGII_galactosylation | 0.5 * (IgGII1H4N4F1 + IgGII1H4N4F1S1 + IgGII1H4N5F1) + 1 * (IgGII1H5N4F1 + IgGII1H5N4F1S1 + IgGII1H5N5F1) |
| IgGII_sialylation | 0.5 * (IgGII1H4N4F1S1 + IgGII1H5N4F1S1) |

Showing 1 to 8 of 8 entries  Previous 1 Next

For each glycosylation site, the automatically calculated traits are plotted against total spectrum intensities as a sanity check. Correlations between traits and total spectrum intensities for standards (e.g. Pools or VisuCon) indicate that differences in traits between samples are (at least partly) a technical artefact, rather than a biological effect.



If your data is not suitable for automatic glycosylation trait calculation, there is an option to calculate them manually.

1. Create an Excel file with formulas specifying how traits should be calculated. An example Excel file can be download by clicking the paperclip icon. The excel should consist of two columns:
   - *"trait"* – Containing the names of all the glycosylation traits you wish to calculate. Trait names should not include spaces.
   - *"formula"* – Containing the formulas for the glycosylation traits. Analyte names in the formulas should include both the peptide and glycan composition, e.g. "IgGI1H4N4F1".

2. Upload the Excel file in the "Calculate custom glycosylation traits" box.

The traits will be calculated, and a table listing the trait names and formulas will be provided as a sanity check.

**Formulas used to calculate the custom glycosylation traits**

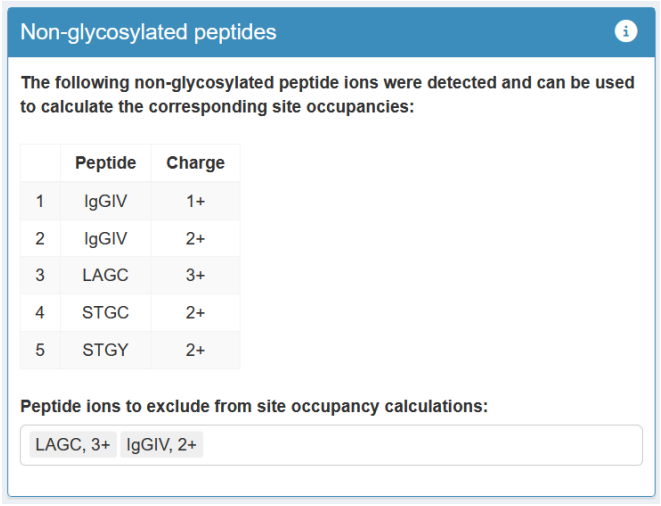
| trait | formula |
|---|---|
| GLT_fucosylation | GLT1H4N5F1S1 + GLT1H5N4F1S1 + GLT1H5N4F1S2 + GLT1H5N5F1S1 + GLT1H5N5F1S2 |
| GLT_bisection | GLT1H5N5F1S1 + GLT1H5N5F1S2 |
| GLT_sialylation | 0.5 * (GLT1H4N5F1S1 + GLT1H5N4F1S1 + GLT1H5N4S1 + GLT1H5N5F1S1) + GLT1H5N4F1S2 + GLT1H5N4S2 + GLT1H5N5F1S2 |

Showing 1 to 3 of 3 entries

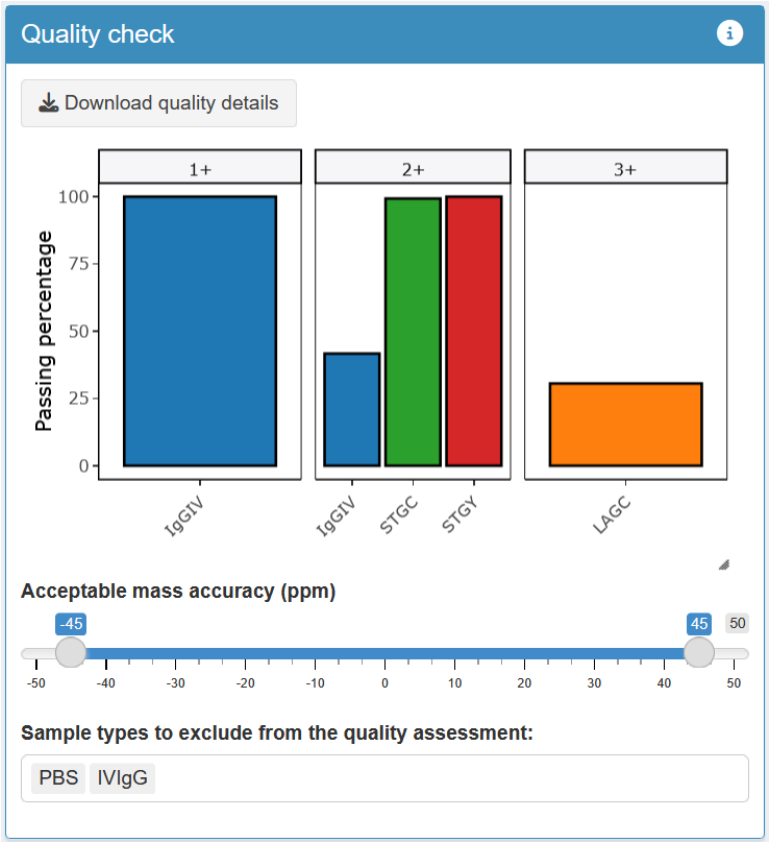
## Site occupancy (optional)

GlycoDash will automatically detect the presence of non-glycosylated peptides in your data. These are ignored during spectral and analyte curation. When a non-glycosylated peptide is present for a glycosylation site, this peptide can be used to calculate the corresponding site occupancy (e.g., when there are analytes IgGI1H3N4F1, IgGI1H4N4F1, etc. in your data, the analyte IgGI1 without a glycan composition can be used).

The detected non-glycosylated peptides and their charge states are listed in a table, and corresponding site occupancies are automatically calculated. You have the option to exclude ions from the site occupancy calculations, e.g. when the quality is insufficient (see below). When you exclude all charge states for a peptide, the corresponding site occupancy is not calculated.



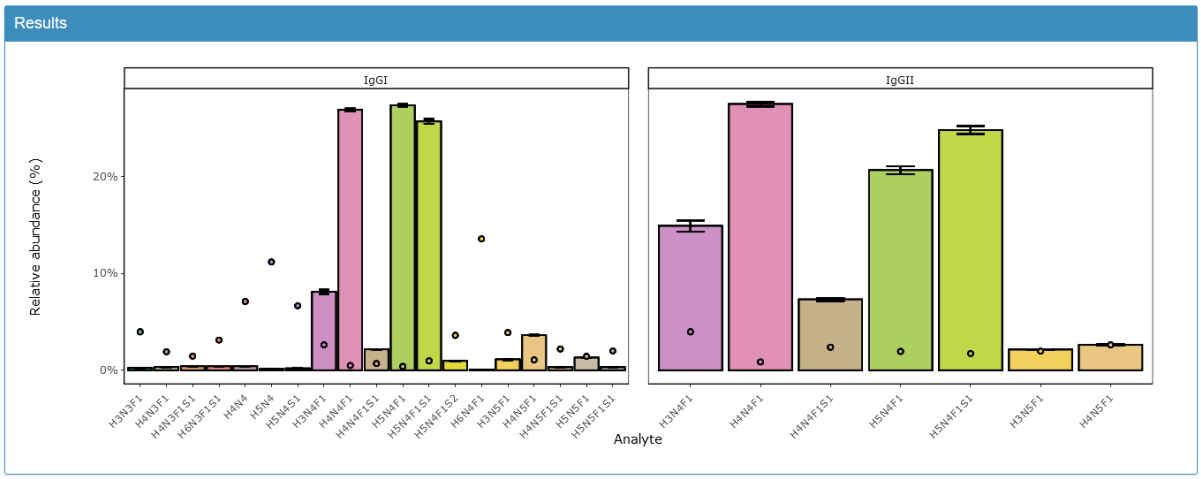

In the "Quality check" box, the quality of each detected peptide ion is visualized. The percentage of samples in which the ion fulfills three quality criteria is plotted, with the option to exclude certain sample types from the assessment. For S/N and IPQ (in the case of LaCyTools data), or total area and IDP (in the case of Skyline data), the same criteria that were used for spectral and analyte curation are applied. Because non-glycosylated peptides are often integrated without calibration, you may want to be more lenient when it comes to the acceptable mass error. This value can therefore be set manually here.



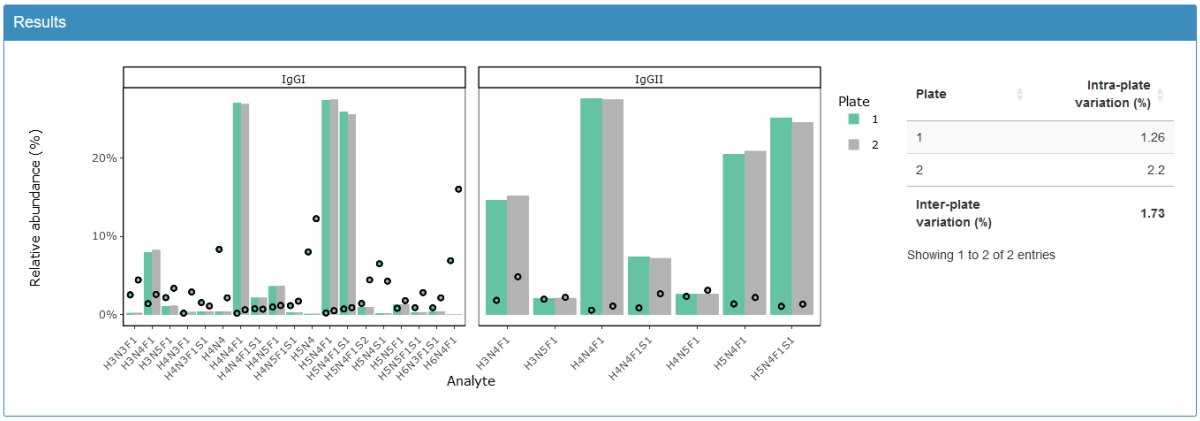
# Repeatability (optional)

For sample ID's that appear more than once in the dataset (e.g. "pool"), the repeatability of analyte relative abundances can be visualized. Repeatability of multiple sample ID's you can be assessed by creating separate tabs using the "Add a tab +" button in the top-right corner of the box.

1. Select the sample ID for which repeatability should be visualized.
2. Optional: toggle the switch to assess repeatability per plate.
3. Click "Assess repeatability". An interactive bar plot is generated displaying the relative abundances of all analytes. Error bars on each bar represent the (mean ± standard deviation) for the corresponding analyte. A dot over each bar indicates the relative standard deviation (RSD = SD/mean × 100%).



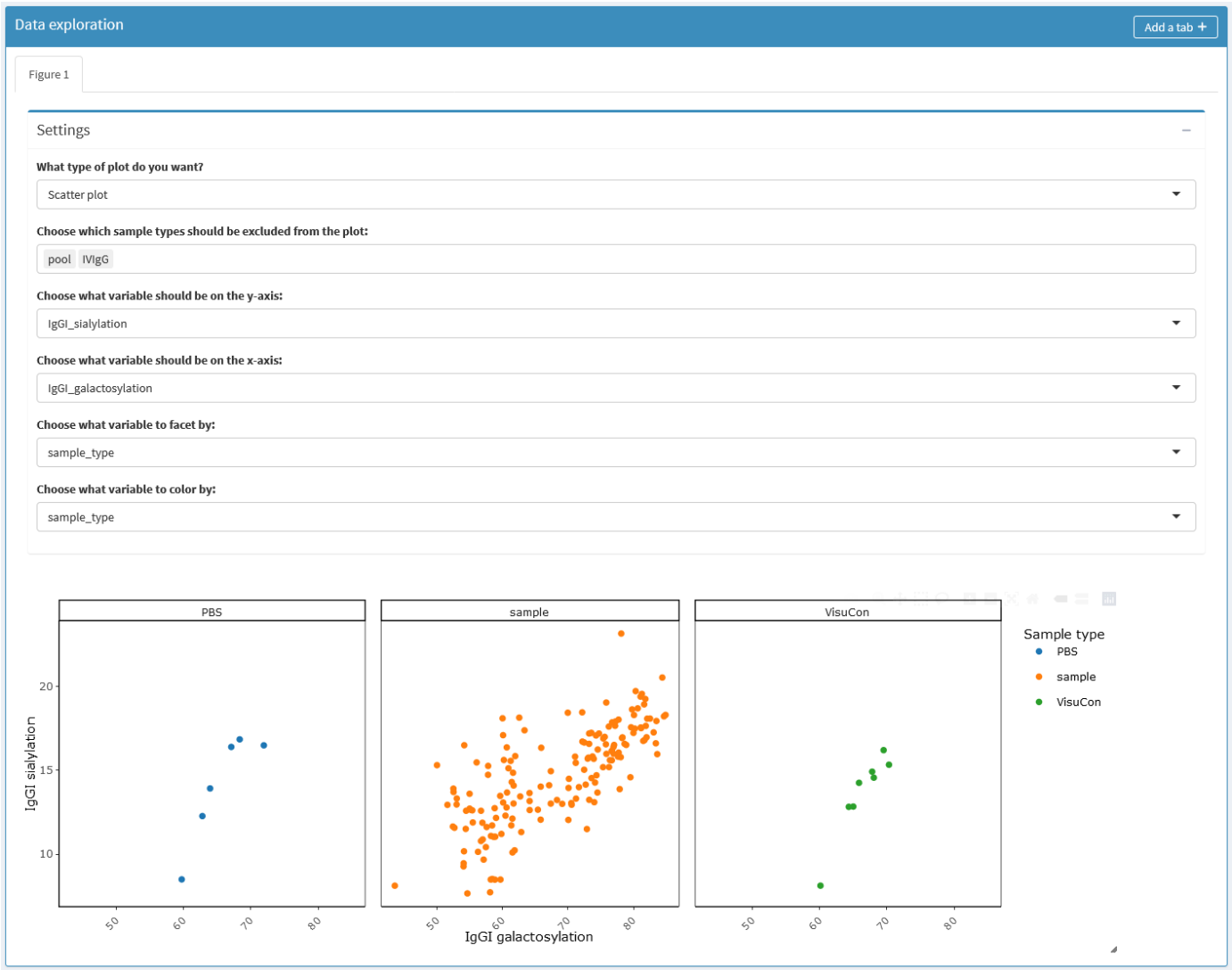

- When assessing repeatability per plate, a table is presented, showing median intra-plate variations calculated as the median of the analyte RSDs on a plate. At the bottom of the table, the inter-plate variation is displayed, calculated as the mean of the intra-plate variations.



# Data exploration (optional)

The data exploration tab offers various options for simple data visualization. To create multiple plots, click the “Add a tab +” button in the top-right corner of the box.

1. Choose the type of plot to create: boxplot, scatter plot, or histogram.
2. Optional: select sample types to exclude from the plot.
3. Select the variable to be plotted on the $x$-axis.
4. Select the variable to be plotted on the $y$-axis (not applicable for histograms).
5. Optional: choose a variable to facet by.
6. Optional: choose a variable to color by.



## Data export

In the data export tab you can download the processed data as an Excel file or as an R object. The processed data includes:

- Sample names, sample types and sample ID's.
- Plate wells, if applicable.
- Metadata, if applicable.
- Sum intensities of all glycosylation sites.
- Relative abundances of all analytes.
- Calculated glycosylation traits, if applicable.
- IgG1 quantities, if applicable.

Additionally, you can download a data processing report in HTML format that can be opened in any browser. This report contains the GlycoDash version that was used, the names of all uploaded files, all choices that were made during each processing step in GlycoDash, and all data visualizations. The generated plots retain their interactivity in this HTML document.

# Appendix 1: R packages

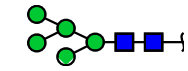
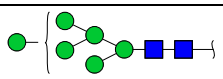
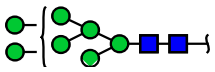
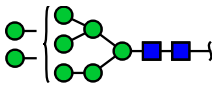
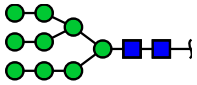
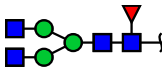
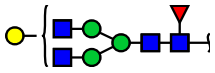
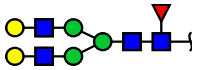
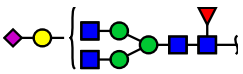
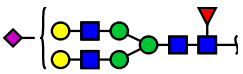
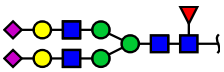
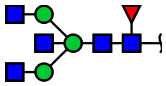
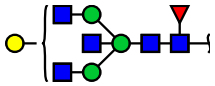
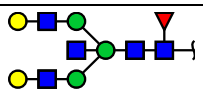
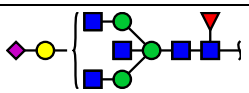
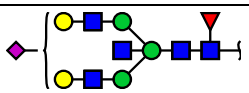
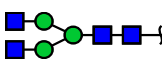
The following R packages are currently used in GlycoDash:

- bsplus (version 0.1.5)
- config (version 0.3.2)
- DT (version 0.33)
- dplyr (version 1.1.4)
- ggplot2 (version 3.5.2)
- golem (version 0.5.1)
- htmltools (version 0.5.8.1)
- kableExtra (version 1.4.0)
- knitr (version 1.50)
- magrittr (version 2.0.3)
- plater (version 1.0.5)
- plotly (version 4.10.4)
- processx (version 3.8.6)
- purrr (version 1.0.4)
- readxl (version 1.4.5)
- rlang (version 1.1.6)
- RLumShiny (version 0.2.3)
- rmarkdown (version 2.29)
- RColorBrewer (version 1.1-3)
- shiny (version 1.10.0)
- shinyalert (version 3.1.0)
- shinyBS (version 0.61.1)
- shinybusy (version 0.3.3)
- shinycssloaders (version 1.1.0)
- shinydashboard (version 0.7.3)
- shinydashboardPlus (version 2.0.5)
- shinyFeedback (version 0.4.0)
- shinyjqui (version 0.4.1)
- shinyjs (version 2.1.0)
- shinyWidgets (version 0.9.0)
- snakecase (version 0.11.1)
- spelling (version 2.3.1)
- testthat (version 3.2.3)
- tidyr (version 1.3.1)
- tidyselect (version 1.2.1)
- writexl (version 1.5.4)

# Appendix 2: Glycan compositions for automatically calculating glycosylation traits.

Below are lists of glycan compositions, and their assumed structures, that can be used for automatically calculating glycosylation traits.

## Human IgG

| | |
|---|---|
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H3N4F1 |  |
| H4N4F1 |  |
| H5N4F1 |  |
| H4N4F1S1 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H3N5F1 |  |
| H4N5F1 |  |
| H5N5F1 |  |
| H4N5F1S1 |  |
| H5N5F1S1 |  |
| H3N4 |  |

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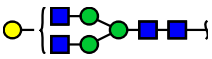
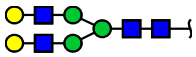
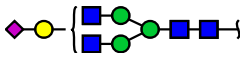
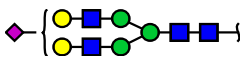
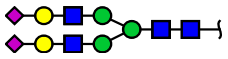
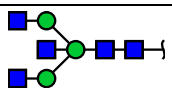
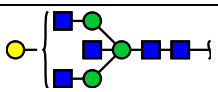
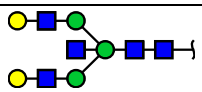
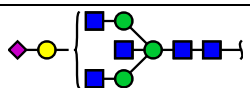
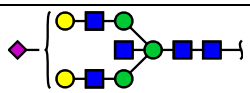
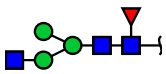
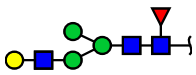
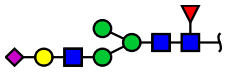
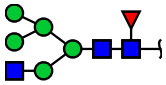
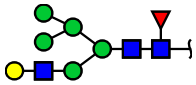
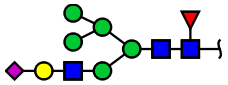
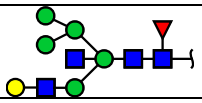
| H4N4 |  |
| H5N4 |  |
| H4N4S1 |  |
| H5N4S1 |  |
| H5N4S2 |  |
| H3N5 |  |
| H4N5 |  |
| H5N5 |  |
| H4N5S1 |  |
| H5N5S1 |  |
| H3N3F1 |  |
| H4N3F1 |  |
| H4N3F1S1 |  |
| H5N3F1 |  |
| H6N3F1 |  |
| H6N3F1S1 |  |
| H6N4F1 |  |

## Human IgA

### IgA2 N47

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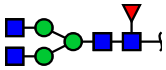
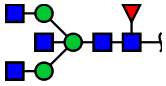
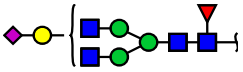
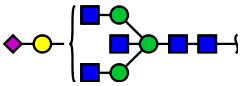
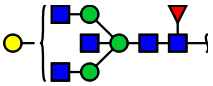
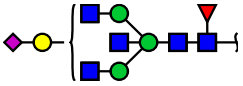
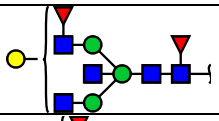
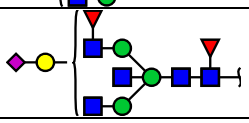
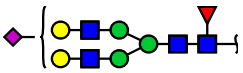
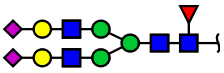
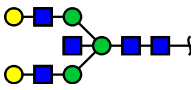
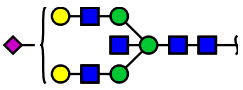
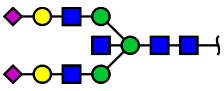
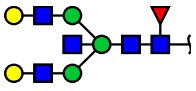
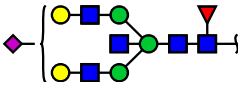
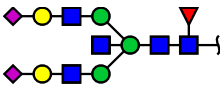
| H3N4F1 |  |
| H3N5F1 |  |
| H4N4F1S1 |  |
| H4N5S1 |  |
| H4N5F1 |  |
| H4N5F1S1 |  |
| H4N5F2 |  |
| H4N5F2S1 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H5N5 |  |
| H5N5S1 |  |
| H5N5S2 |  |
| H5N5F1 |  |
| H5N5F1S1 |  |
| H5N5F1S2 |  |

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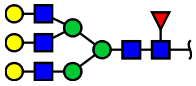
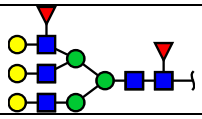
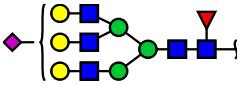
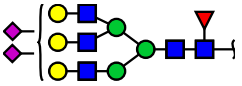
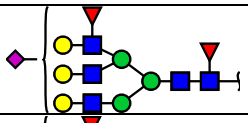
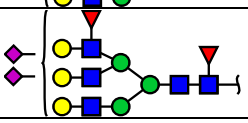
| H5N6F1 |  |
| H5N6F2 |  |
| H5N6F1S1 |  |
| H5N6F1S2 |  |
| H5N6F2S1 |  |
| H5N6F2S2 |  |

**IgA1/2 N144/131**

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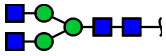
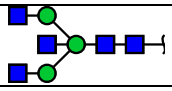
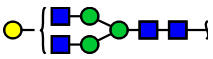
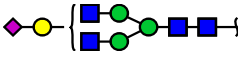
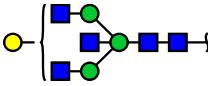
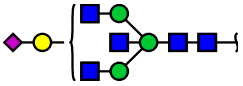
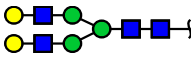
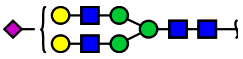
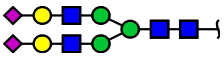
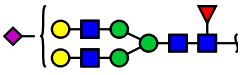
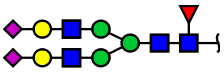
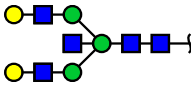
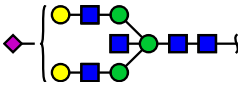
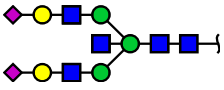
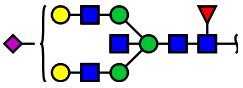
| H3N4 |  |
| H3N5 |  |
| H4N4 |  |
| H4N4S1 |  |
| H4N5 |  |
| H4N5S1 |  |
| H5N4 |  |
| H5N4S1 |  |
| H5N4S2 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H5N5 |  |
| H5N5S1 |  |
| H5N5S2 |  |
| H5N5F1S1 |  |

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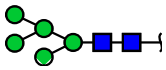
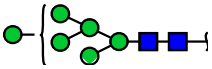
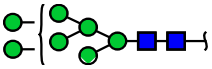
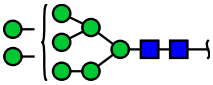
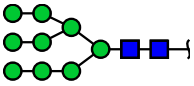
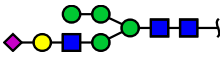
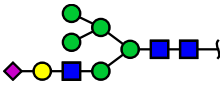
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H5N3S1 |  |
| H6N3S1 |  |
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**IgA2 N205**

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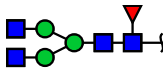
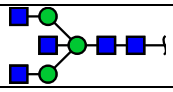
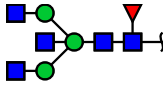
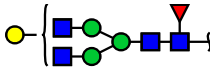
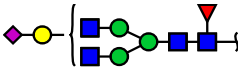
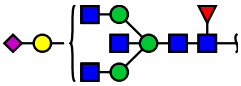
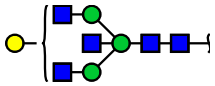
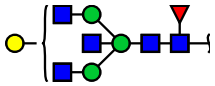
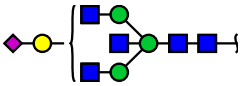
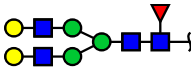
| H3N4F1 |  |
| H3N5 |  |
| H3N5F1 |  |
| H4N4F1 |  |
| H4N4F1S1 |  |
| H4N5F1S1 |  |
| H4N5 |  |
| H4N5F1 |  |
| H4N5S1 |  |
| H5N4F1 |  |

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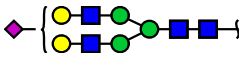
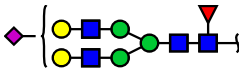
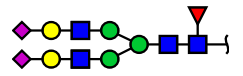
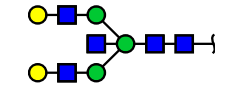
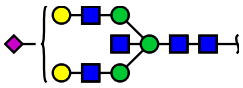
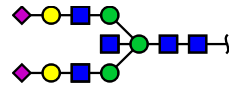
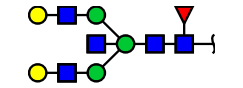
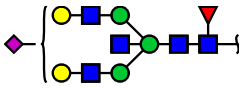
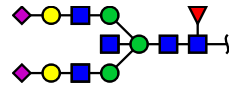
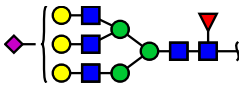
| H5N4S1 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H5N5 |  |
| H5N5S1 |  |
| H5N5S2 |  |
| H5N5F1 |  |
| H5N5F1S1 |  |
| H5N5F1S2 |  |
| H6N5F1S1 |  |

**IgA1/2 N340/327**

The glycans below may carry one sulphate group ("Su1" at the end of the composition).

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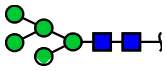
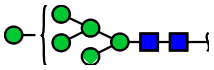
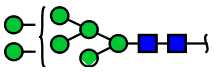
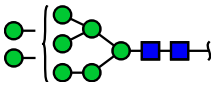
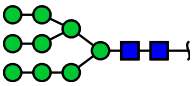
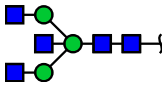
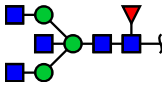
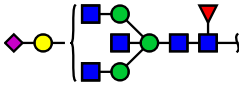
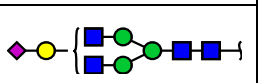
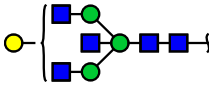
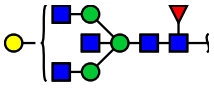
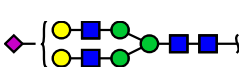
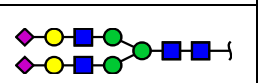
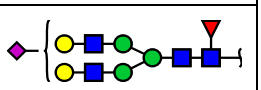
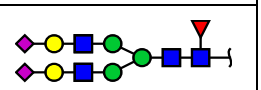
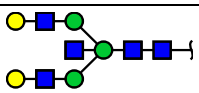
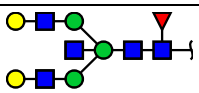
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H3N5 |  |
| H3N5F1 |  |
| H4N5F1S1 |  |
| H4N4S1 |  |
| H4N5 |  |
| H4N5F1 |  |
| H5N4S1 |  |
| H5N4S2 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H5N5 |  |
| H5N5F1 |  |

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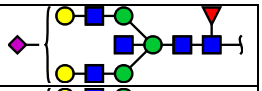
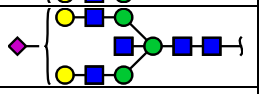
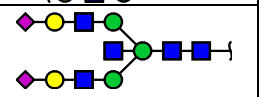
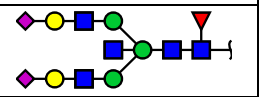
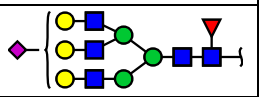
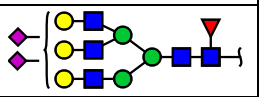
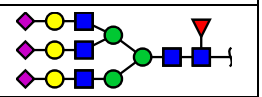
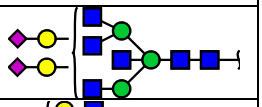
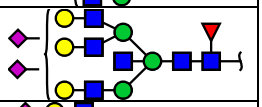
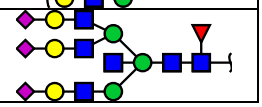
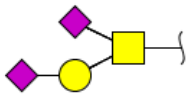
| H5N5F1S1 |  |
| H5N5S1 |  |
| H5N5S2 |  |
| H5N5F1S2 |  |
| H6N5F1S1 |  |
| H6N5F1S2 |  |
| H6N5F1S3 |  |
| H5N6S2 |  |
| H6N6F1S2 |  |
| H6N6F1S3 |  |

**IgA1 *O*-glycans**

This IgA1 glycopeptide can carry up to six *O*-glycans. Each *O*-glycan has a core GalNAc, which can carry one galactose. In the compositions below, each "N" is therefore a separate *O*-glycan. Sialic acids are assumed to be bonded to a galactose. The exception to this are compositions where there are more sialic acids than galactoses. In that case, any extra sialic acids are assumed to be bonded to the core GalNAc.



*Supported compositions for automatic traits:*

| | |
|---|---|
| H2N3S2 | H4N4S5 |
| H2N4S2 | H4N5 |
| H2N5S1 | H4N5S1 |
| H3N3S1 | H4N5S2 |
| H3N3S2 | H4N5S3 |
| H3N3S3 | H4N5S4 |
| H3N3S4 | H4N6S2 |
| H3N4 | H4N6S3 |
| H3N4S1 | H4N6S4 |
| H3N4S2 | H5N5 |
| H3N4S3 | H5N5S1 |
| H3N4S4 | H5N5S2 |
| H3N5 | H5N5S3 |
| H3N5S1 | H5N5S4 |
| H3N5S2 | H5N6S3 |
| H3N5S3 | H5N6S4 |
| H3N6 | H4N6 |
| H3N6S1 | H2N5S2 |
| H3N6S2 | H5N5S5 |
| H3N6S3 | H3N5S4 |
| H4N4 | H4N5S5 |
| H4N4S1 | H5N6 |
| H4N4S2 | H5N6S2 |
| H4N4S3 | H4N4S5 |
| H4N4S4 | H4N5 |

## Human IgM

### N46

| | |
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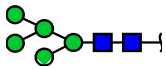
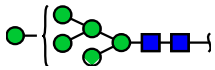
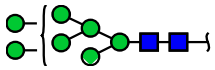
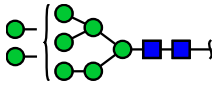
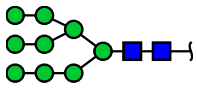
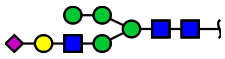
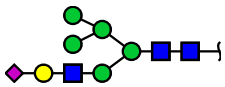
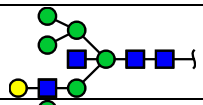
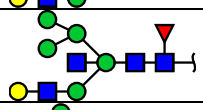
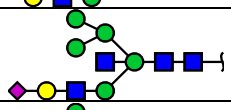
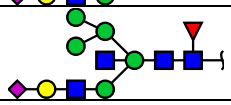
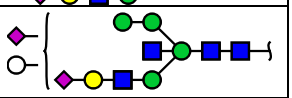
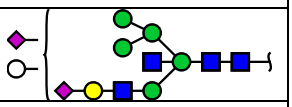
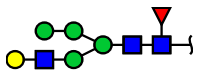
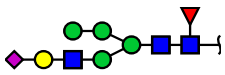
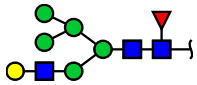
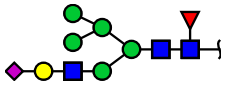
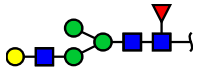
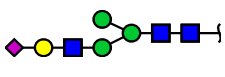
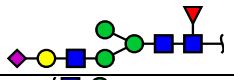
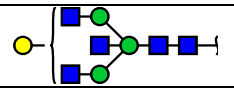
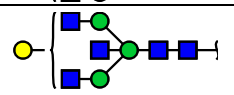
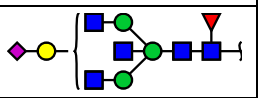
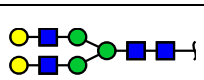
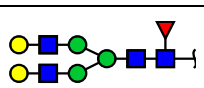
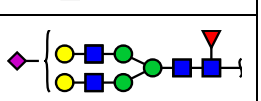
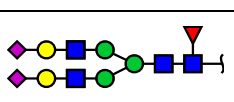
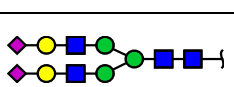
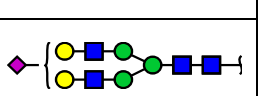
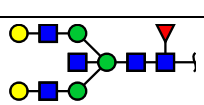
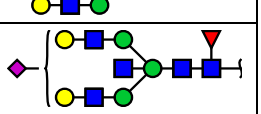
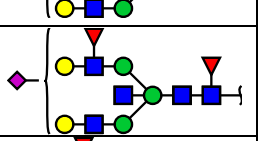
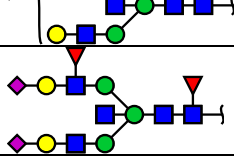
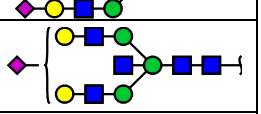
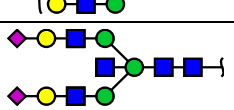
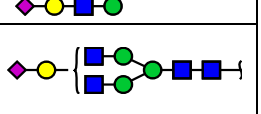
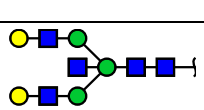
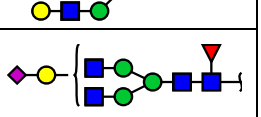
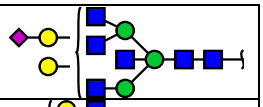
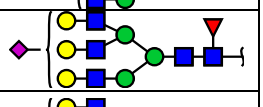
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H5N3S1 |  |
| H6N3S1 |  |
| H6N4 |  |
| H6N4F1 |  |
| H6N4S1 |  |
| H6N4F1S1 |  |
| H6N4S2 |  |
| H7N4S2 |  |
| H5N3F1 |  |
| H5N3F1S1 |  |
| H6N3F1 |  |
| H6N3F1S1 |  |
| H4N3F1 |  |
| H4N3S1 |  |

| | |
|---|---|
| H4N3F1S1 |  |
| H4N5 |  |
| H4N5F1 |  |
| H4N5F1S1 |  |
| H5N4 |  |
| H5N4F1 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H5N4S2 |  |
| H5N4S1 |  |
| H5N5F1 |  |
| H5N5F1S1 |  |
| H5N5F2S1 |  |
| H5N5F1S2 |  |
| H5N5S1 |  |
| H5N5S2 |  |
| H4N4S1 |  |
| H5N5 |  |
| H4N4F1S1 |  |

| | |
|---|---|
| H5N6S1 |  |
| H6N5F1S1 |  |
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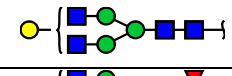
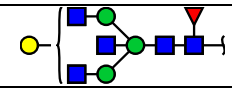
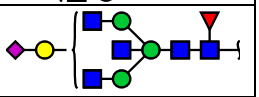
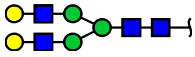
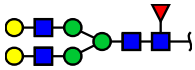
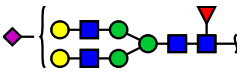
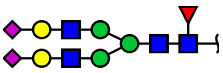
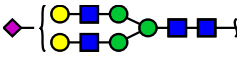
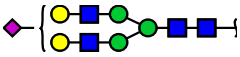
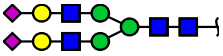
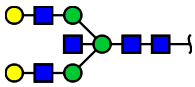
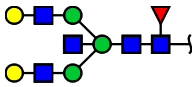
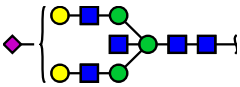
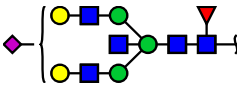
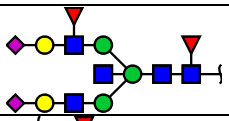
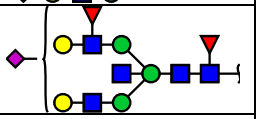
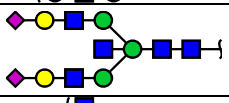
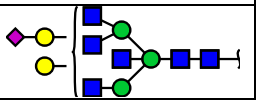
**N209**

| | |
|---|---|
| H4N4 |  |
| H4N5F1 |  |
| H4N5F1S1 |  |
| H5N4 |  |
| H5N4F1 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |
| H5N4F2S2 |  |
| H5N4S1 |  |
| H5N4S2 |  |
| H5N5 |  |
| H5N5F1 |  |
| H5N5S1 |  |
| H5N5F1S1 |  |
| H5N5F1S2 |  |
| H5N5F2S2 |  |
| H5N5S2 |  |
| H5N6S1 |  |

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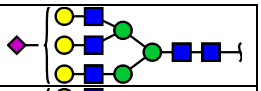
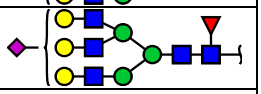
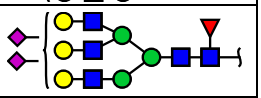
| H6N5S1 |  |
| H6N5F1S1 |  |
| H6N5F1S2 |  |

**N272**

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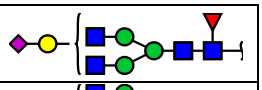
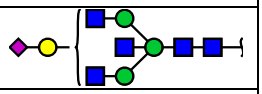
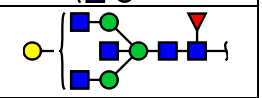
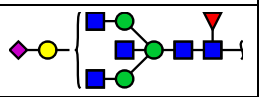
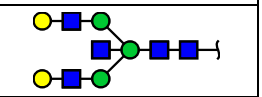
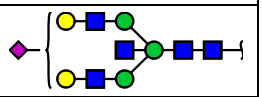
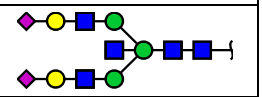
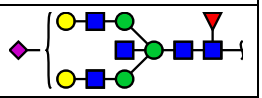
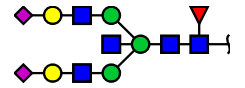
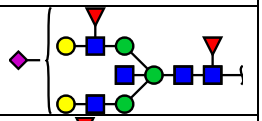
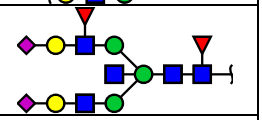
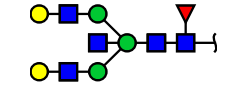
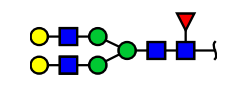
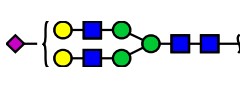
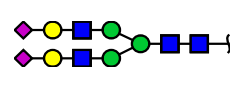
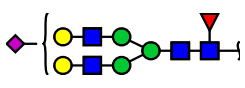
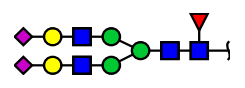
| H4N4F1S1 |  |
| H4N5S1 |  |
| H4N5F1 |  |
| H4N5F1S1 |  |
| H5N5 |  |
| H5N5S1 |  |
| H5N5S2 |  |
| H5N5F1S1 |  |
| H5N5F1S2 |  |
| H5N5F2S1 |  |
| H5N5F2S2 |  |
| H5N5F1 |  |
| H5N4F1 |  |
| H5N4S1 |  |
| H5N4S2 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |

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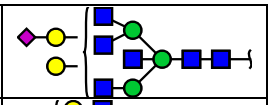
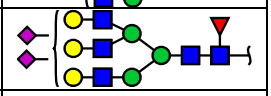
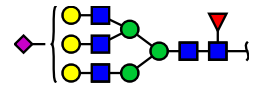
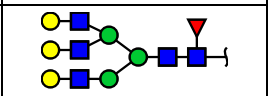
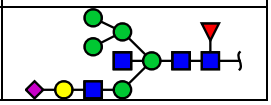
| H5N6S1 |  |
| H6N5F1S2 |  |
| H6N5F1S1 |  |
| H6N5F1 |  |
| H6N4F1S1 |  |

**N279**

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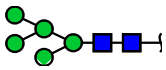
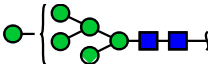
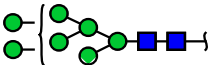
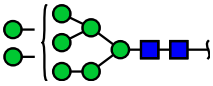
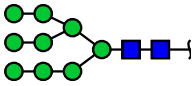
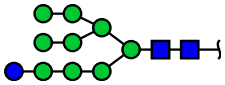
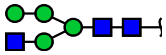
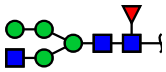
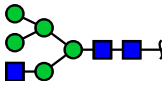
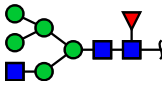
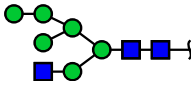
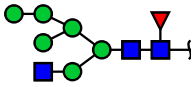
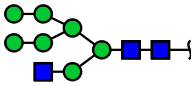
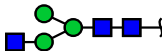
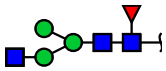
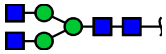
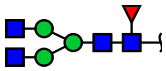
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H10N2 |  |
| H4N3 |  |
| H4N3F1 |  |
| H5N3 |  |
| H5N3F1 |  |
| H6N3 |  |
| H6N3F1 |  |
| H7N3 |  |
| H3N3 |  |
| H3N3F1 |  |
| H3N4 |  |
| H3N4F1 |  |

**N440**

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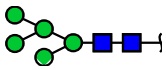
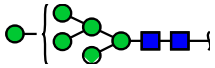
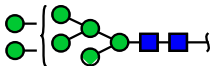
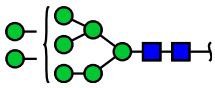
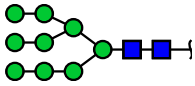
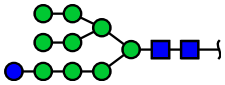
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H10N2 |  |

## Human Joining Chain (JC)

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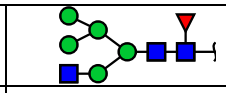
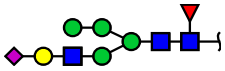
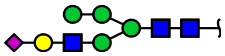
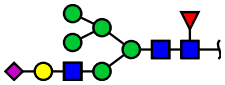
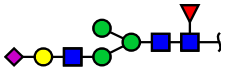
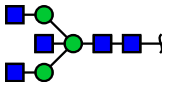
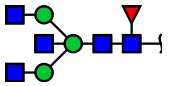
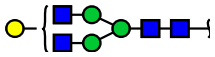
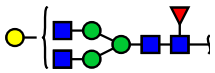
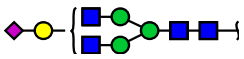
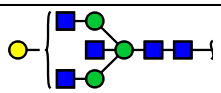
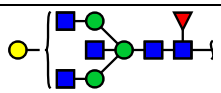
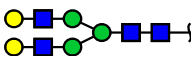
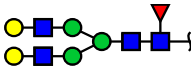
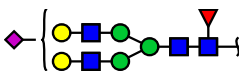
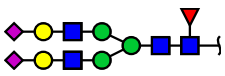
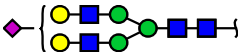
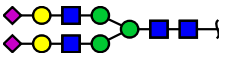
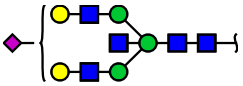
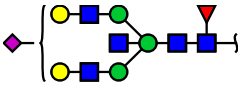
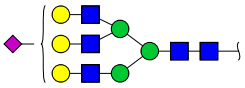
| H5N3F1 |  |
| H5N3F1S1 |  |
| H5N3S1 |  |
| H6N3F1S1 |  |
| H4N3S1 |  |
| H4N3F1S1 |  |
| H3N5 |  |
| H3N5F1 |  |
| H4N4 |  |
| H4N4F1 |  |
| H4N4S1 |  |
| H4N5 |  |
| H4N5F1 |  |
| H5N4 |  |
| H5N4F1 |  |
| H5N4F1S1 |  |
| H5N4F1S2 |  |

| | |
|---|---|
| H5N4S1 |  |
| H5N4S2 |  |
| H5N5S1 |  |
| H5N5F1S1 |  |
| H6N5S1 |  |

## Mouse IgG

| | |
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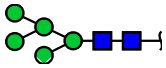
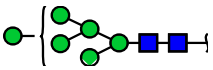
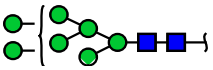
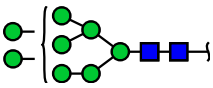
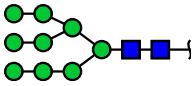
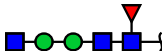
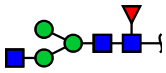
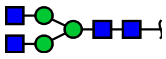
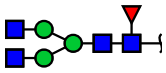
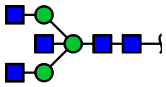
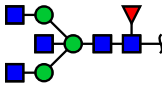
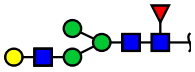
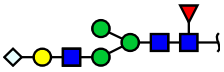
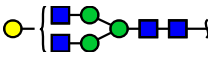
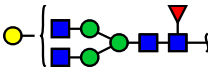
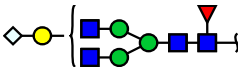
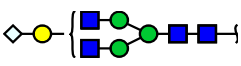
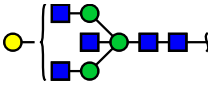
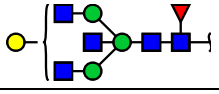
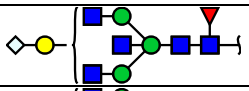
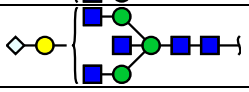
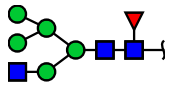
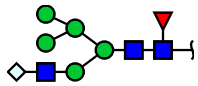
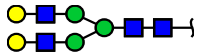
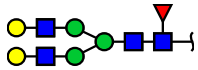
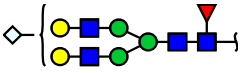
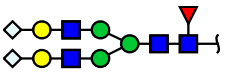
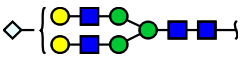
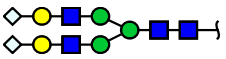
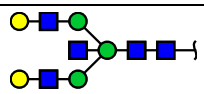
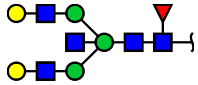
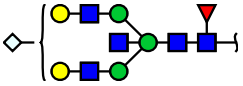
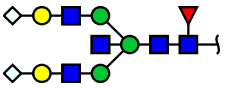
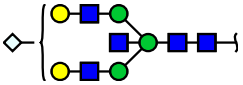
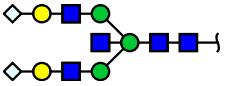
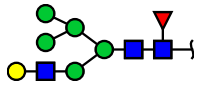
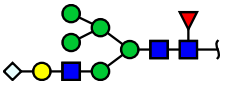
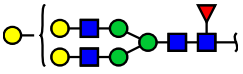
| H5N2 |  |
| H6N2 |  |
| H7N2 |  |
| H8N2 |  |
| H9N2 |  |
| H2N3F1 |  |
| H3N3F1 |  |
| H3N4 |  |
| H3N4F1 |  |
| H3N5 |  |
| H3N5F1 |  |
| H4N3F1 |  |
| H4N3F1G1 |  |
| H4N4 |  |
| H4N4F1 |  |
| H4N4F1G1 |  |
| H4N4G1 |  |
| H4N5 |  |
| H4N5F1 |  |
| H4N5F1G1 |  |
| H4N5G1 |  |
| H5N3F1 |  |
| H5N3F1G1 |  |
| H5N4 |  |
| H5N4F1 |  |
| H5N4F1G1 |  |
| H5N4F1G2 |  |
| H5N4G1 |  |
| H5N4G2 |  |
| H5N5 |  |
| H5N5F1 |  |
| H5N5F1G1 |  |
| H5N5F1G2 |  |
| H5N5G1 |  |
| H5N5G2 |  |
| H6N3F1 |  |
| H6N3F1G1 |  |
| H6N4F1 |  |

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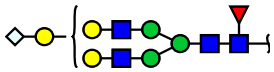
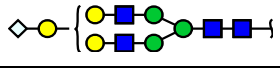
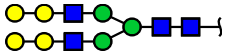
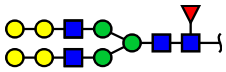
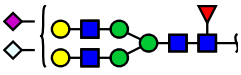
| H6N4F1G1 |  |
| H6N4G1 |  |
| H7N4 |  |
| H7N4F1 |  |
| H5N4F1G1S1 |  |