Details about GRable

GRable is a software to estimate site-specific glycan compositions of glycopeptides, using an MS1-based glycoproteomic method named “Glyco-RIDGE” (Glycan heterogeneity-based Relational Identification of Glycopeptide signals on Elution profile). First, this method identifies glycopeptide signals based on the chromatographic properties of glycopeptides and mass differences due to the glycan heterogeneity. That is, glycopeptides having the same core peptide but different glycans elute within a narrow range of elution time (**Fig. 1A**). Accordingly, glycopeptide signals with a similar elution time and mass differences corresponding to glycan units (e.g. Hex, HexNAc, and dHex) can be assigned as a cluster, without MS2 spectrum analyses (**Fig. 1B**). In parallel, core peptides present actually in the glycopeptide sample are identified by PNGase-mediated deglycosylation followed by LC/MS (**Fig. 1C**). The mass value of glycopeptide is sum of those of core peptide and glycan. Glycan mass is presumable from the glycan compositions. Therefore, the combination of peptide and glycan is searchable from these three lists, i.e., the masses of glycopeptides, peptides, and glycans. After the comprehensive matching, the most plausible matches are selected by multi-evidence including MS2 evaluation.

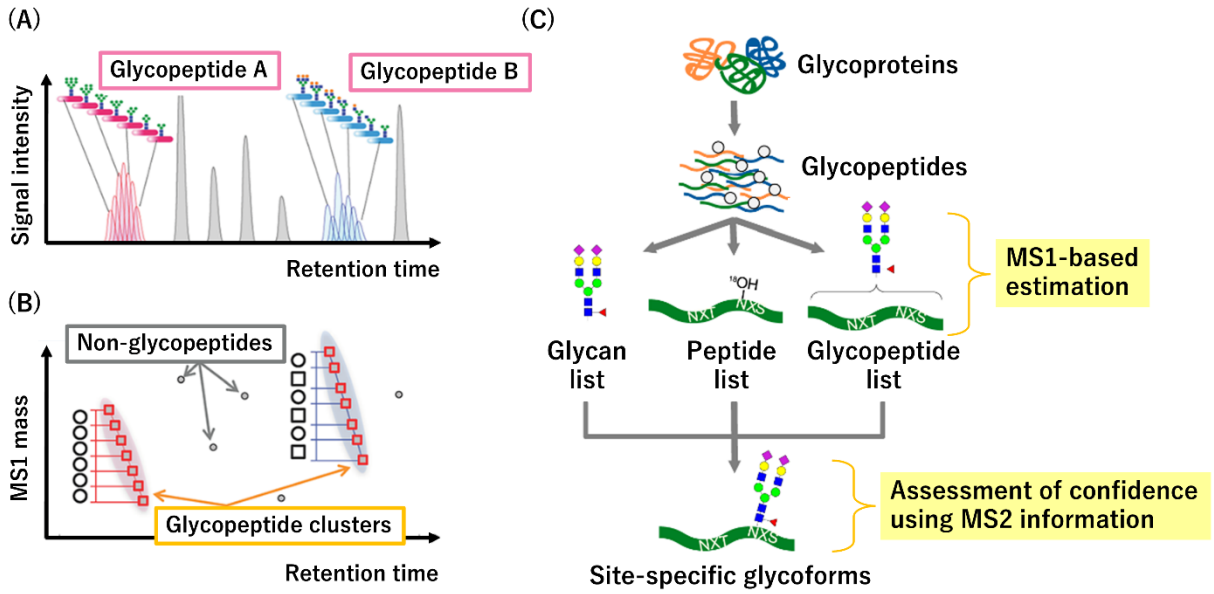

Fig. 1. Principle of the Glyco-RIDGE method

GRable proceeds in seven steps using the four files (**Fig. 2**). In step 1, GRable allows the registration of the LC/MS data of glycopeptides in the mzML format, which ensures great versatility and ease of data processing. In the current version, the deconvolution function (Step 2) is not applicable although it is visible in the user interface for future implementation. Step 3 was designed to set the RT range, mass range, and minimum signal intensity (threshold) over which the analysis was performed. Step 4 was intended to find and group signals of an identical ion based on three parameters: time (scan), mass (MH^+), and intensity and to obtain the monoisotopic mass of each signal group at the peak time, using our unique algorithms. In step 5, a series of signals from the glycopeptide group were found as a cluster based on the elution behavior and mass difference of its members. In step 6, GRable searches for a combination of core peptides and glycan compositions that match the mass of the putative glycopeptide within the allowed mass error (user setting) according to the following equation: Observed M(glycopeptide) = calculated M(core peptide identified) + M(Hex)∗i + M(HexNAc)∗j + M(dHex)∗k + M(NeuAc)∗l (M is a mass value, and i, j, k, and l are integers). In step 7, the most plausible combination among multiple candidate combinations suggested for one glycopeptide cluster was selected. Subsequently, their reliability at the cluster

level as well as that at the single glycopeptide level was evaluated using information from the results and additional MS2 information. The results of steps 3 to 5 were visually confirmed using a viewer in the main window of the software. The detailed results of steps 4 to 7 can be exported as an Excel file with each setting.

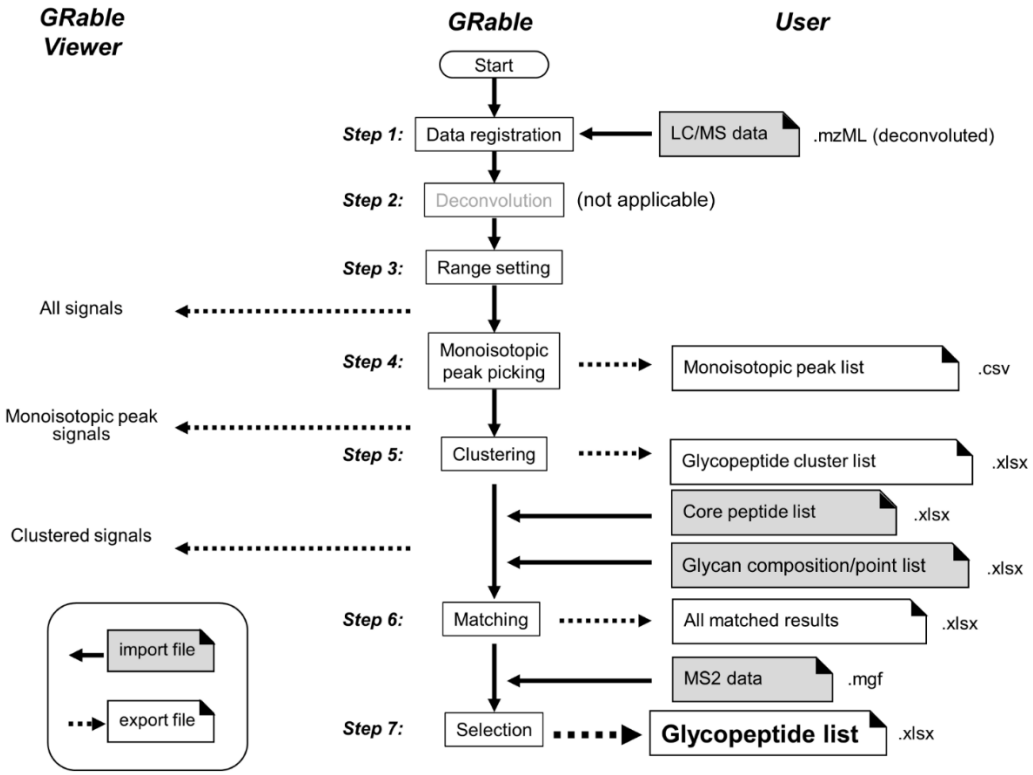

Fig. 2. Overview of data processing of GRable

Contact

If you belong to an academic research institute, you can use the full version without functional limitations by concluding a joint research agreement between your organization and AIST. Please contact us: M-grable-inquiry@aist.go.jp

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GRable software

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Applications of the Glyco-RIDGE method

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