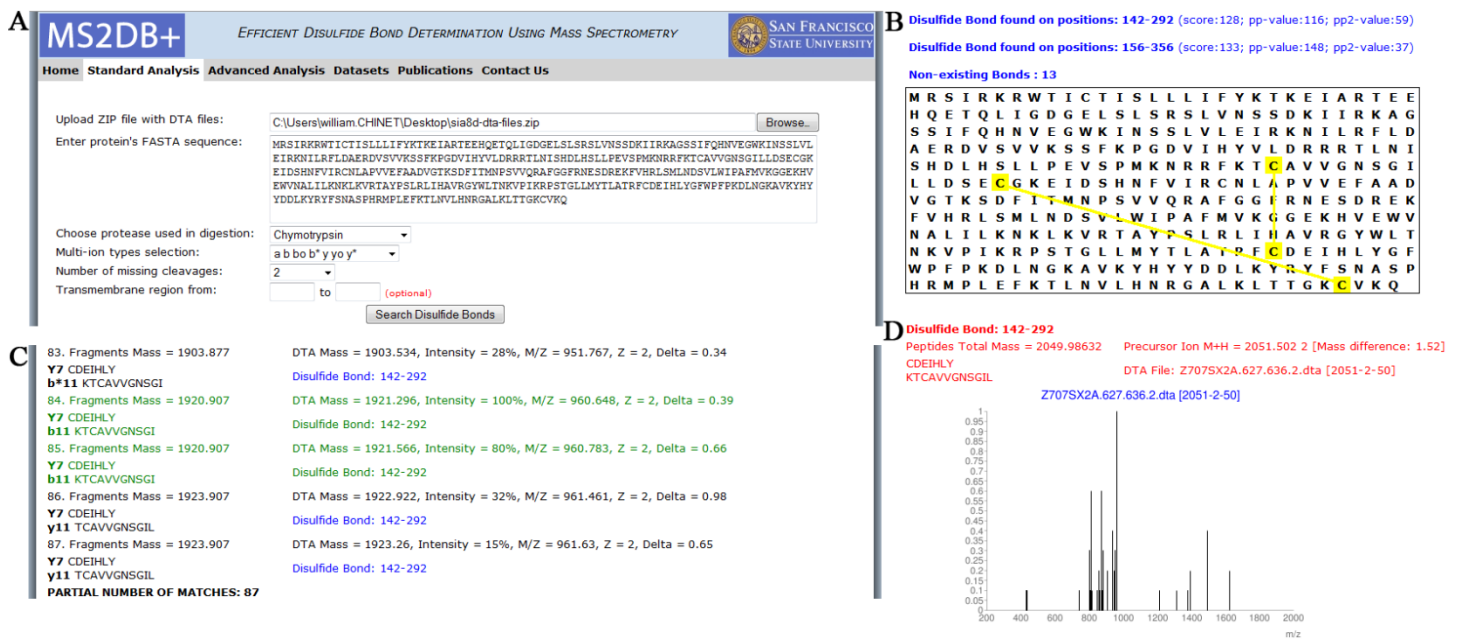# Supplemental Data I: Usage Example - ST8Sia IV

Figure 1. (A) The standard analysis interface of MS2DB+ showing the key inputs for the molecule ST8SiaIV. (B) Disulfide bonds (in yellow) found for ST8SiaIV. (C) Some of the confirmatory matches found for the S-S bond between cysteines C^{142}-C^{292}. Matches whose MS/MS fragment's normalized intensity equal or exceed 50% are marked in green). (D) The confirmatory spectrum for an initial match (in red) containing the most abundant confirmatory peaks found while determining the S-S bond between cysteines C^{142}-C^{292}.

Here, we describe a walkthrough demonstrating the use of MS2DB+ using the protein Syalyltransferase ST8Sia IV (UniProt ID Q92187). This example is one of nine Glycosyltransferases with varying S-S bonding topologies, the MS/MS data for which have been made available in the *Datasets* page of the MS2DB+ site.

As a first step, in the standard analysis page (Fig.1.A), the user needs to upload a ZIP file containing the corresponding set of DTA files, enter the protein's FASTA sequence and select the protease used during digestion. Next, MS2DB+ analyzes the data and lists the disulfide bonds found with their empirical and statistical scores calculated. Each bond is shown graphically, as in Fig.1.B where two bonds (C^{142}-C^{292} and C^{156}-C^{356}) are shown. MS2DB+ also provides other relevant information for each disulfide bond, including (1) the disulfide-bonded peptide structure and the DTA file involved in the initial match, (2) a graph containing the most abundant spectral matches (Fig.1.D), (3) the list of confirmatory matches along with the fragment ions matched (theoretical and experimental), (4) their respective mass values and mass difference (both measured in Daltons), and (5) the fragments charge state (*z*) and the peaks normalized intensity (abundance) (Fig.1.C). Each confirmatory match whose normalized intensity (abundance) is found to be equal or greater than 50% is highlighted in green to facilitate rapid identification.