FIGURE 1. MALDI-ISD mass spectrum of the DTT-reduced IgG1 mAb. Amino acid residues underlined were identified after phase correction by c or z+2 ions with a mass error threshold of 50ppm, covering (A) 37.6% of the light chain and (B) 14.3% of the heavy chain.

FIGURE 2. LC-MS/MS mass spectrum of IdeS/DDT treated IgG mAb. The charge state distribution of light chain (LC) and Fd domain are shown. Inset shows the full mass spectrum collected after accumulation of 20 scans over an m/z range 600-3500 at a resolving power of 120,000.

FIGURE 3A. ETD mass spectrum of precursor m/z 1446 (charge 16+) was searched against IgG1 mAb light chain sequence with mass tolerance of 100ppm. ETD mass spectrum was produced from accumulation of 49 scans. Identified c- and z- fragment ion series are indicated in diamonds in the spectrum and relevant sequence identification is shown in the sequence.

FIGURE 3B. ETD mass spectrum of precursor m/z 1512 (charge 17+) was searched against Fd sequence of IgG1 mAb with mass tolerance of 100ppm. ETD mass spectrum was produced from accumulation of 50 scans. Identified c- and z- fragment ion series are indicated in diamonds in the spectrum and relevant sequence identification is shown in the sequence. N-terminal glutamine (Q) to pyroglutamate (pyroGlu) conversion was confirmed with precursor mass error 3.9 ppm (0.10Da).

FIGURE 4. Fourier transform ion cyclotron resonance (FT-ICR) mass spectrum of the intact fusion protein peaks (charge 38+). The mass spectrum was produced from accumulation of 1650 scans collected in a time domain of 128K words data points. Major peaks demonstrate a heterogeneous combination of glycosylation, predominantly by sialic acids (NANA). Additional information regarding the glycan nomenclature and structure are provided in Figure 5.

FIGURE 5. The glycan nomenclature and structure.

FIGURE 6. MALDI-ISD mass spectrum of the DTT reduced fusion protein. Underlined sequence indicates the amino acid residues identified by phase-corrected c and/or z+2 ions, which showed an overall sequence coverage of 25.3% of the full sequence.

FIGURE 7. LC-MS mass spectrum of IdeS/DDT treated fusion protein. Charge state distribution of glycosylated protein species from of C-terminal fragment is shown. The mass spectrum was produced from accumulation of 20 scans over an m/z range 600-3500 at a resolving power of 120,000.

FIGURE 8. LC-MS/MS ETD mass spectra of (A) precursor m/z 1266, and (B and C) m/z 1055 were searched against C-terminal fragment of DTT/IdeS treated fusion protein. Identified c- and z- fragment ion series are indicated in diamonds in the mass spectra and relevant sequence identifications are shown in the associated sequences to the right of each mass spectrum. Detection of glycosylated protein species, G0f and G1f was confirmed on N207 (in green) with C-terminal lysine truncation.