

The NISTmAb Disulfide-Linked Peptide Library v21.1

Release Note, March 8, 2021

1. Purpose of This Release Note:

It briefly describes major changes that were made to the first version of the library, The NISTmAb Disulfide Library v1, created on September 18, 2020 and released on February 8, 2021.

2. Issues Affecting the NISTmAb Disulfide Library Version 1 (v1)

2.1 Missing information on disulfide cleavage from consensus spectra

Extensive disulfide bond cleavage products were observed in varying portions of product ions generated by different disulfide-bonded (SS) peptides. The presence of these SS-containing fragments is the key to identify such peptides. However, these fragments are generally in low abundance, and may not be detectable. Hence, these small but critical fragments can be excluded during consensus spectra generation. To resolve this issue, a new algorithm was used to select the best spectrum for each SS-linked ion that contains more product ions arising from disulfide cleavage. This resulted in a set of selected spectra, which was incorporated into the library of consensus reference spectra. For distinguishing the two types of spectra, the keyword "Consensus" and "Selected" are used in the Comment section. Altogether, this change provides 596 selected reference spectra from Orbitrap Lumos and Q-Exactive runs.

2.2 Abundant unexpected fragments

These were found from the fragmentation of higher charged disulfide-linked peptide ions, such as 1+ and 2+ charged product ions resulting from the CO loss of double cleavage fragments. These ions were annotated in the new version.

2.3 Mixture spectra of modifications

Mixture spectra are often found from peptides with multiple sites of the same modification, for example, TPEVTCVVVDVSHEDPEVK_SS_CK contains multiple sites for an adduct Cation:Ca[II] such as E3, D10, E14, D15, and E17 in the first peptide. There is a phenomenon that there exists some degree of the co-existence of both modified and unmodified fragments at multiple sites in a peptide (oxidation and adducts). In this update, additional annotations of these fragments were manually added to the library reference.

2.4 Spectra with poor quality or insufficient information

Some low-quality spectra and spectra with insufficient information of the correct identifications were removed from this version.