

Monday-0320 Development of a Detection Method for Alpha-Synuclein Fibrils Using MALDI-TOF MS

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1. Background

α -Synuclein (α -syn) is normally monomeric, but in α -synucleinopathies such as Parkinson's disease and dementia with Lewy bodies, it forms pathological fibrils in the brain that seed further aggregation of monomeric α -syn. Based on this seeding activity, seed amplification assay has shown that α -syn fibrils can serve as biomarkers for α -synucleinopathies in clinical studies [1]. Here, we are developing a new method for specifically detecting α -syn fibrils by combining matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a cross-linking reaction.

2. Methods

Recombinant α -syn monomers and fibrils from Cosmo Bio Co., Ltd. were processed separately.

- Each sample was reacted with the cross-linker Sulfo-NHS-SS-Diazirine (SDAD) to allow the NHS ester to react with primary amines ($-\text{NH}_2$) on Lys (K) and the N-terminus of α -syn, followed by photoactivation of the diazirine groups, enabling non-specific linking to α -syn.
- The cross-linked proteins were digested with Glu-C for 18 h.
- Disulfide bonds in SDAD were cleaved by reduction, and the resulting thiols were followed by alkylation.
- Samples were analyzed with MALDI-TOF MS using the AXIMA PerformanceTM (Shimadzu/Kratos, UK).

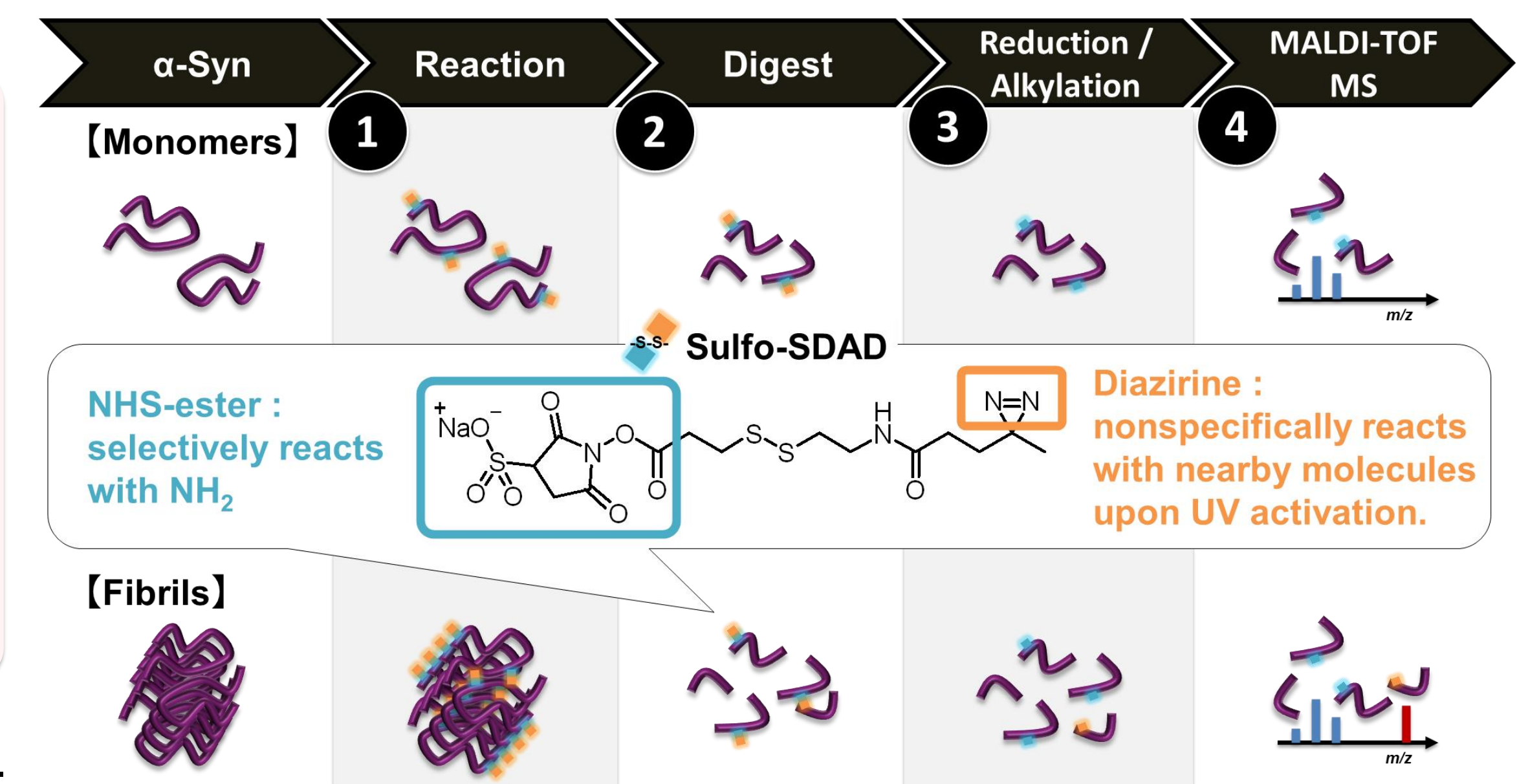


Figure 1. Cross-linking MS (XL-MS) methodology for α -Syn.

3. Results

3-1. Digestion with Glu-C

In the absence of the SDAD cross-linker, α -syn was digested with Glu-C, and peptides with 0-2 missed cleavages (MC) were predominantly detected. This indicates that α -syn fibrils, as well as monomers, are susceptible to proteolytic digestion.

MDVFMKGLSK AKEGVVAAAE KTKQGVAAEA GKTKEGVLYV GSKTKEGVVH GVATVAEKT
EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP
DNEAYEMPSE EGYQDYEPEA

Figure 2. Sequence of α -Syn. Glu-C cleaves on the C-terminal side of Asp (D) or Glu (E) basically (Gray marker position). The underlined peptides were detected by MS.

3-2. Mass spectra of Glu-C-digested, SDAD-cross-linked α -Syn

Mass spectra showed peaks corresponding to Glu-C-digested peptides modified with SDAD. NHS-linked peaks were observed in both α -Syn monomers and fibrils, whereas UV-cross-linked peaks were observed only in α -Syn fibrils. This may be due to the short distances between α -Syn molecules in fibrils. These results suggest that UV-cross-linked peaks can distinguish α -Syn fibrils from monomers.

Table 1. Digested α -syn peptides and corresponding SDAD-modified peaks detected by MALDI-TOF MS.

Residues	m/z	Sequence	NHS-linked (+145 Da)	UV-cross-linked (+216 Da)
132 -140	1072.1	GYQDYEPEA	—	—
	1288.4		—	1 molecule
47 - 105	5809.6	GVVHGVATVA	—	—
	6025.9	EKTKEQVTNV	—	1 molecule
	6170.9	GGAVVTGVTA	1 molecule	1 molecule
	6315.9	VAQKTVEGAG	2 molecules	1 molecule
		SIAAATGFVK		
		KDQLGKNEE		

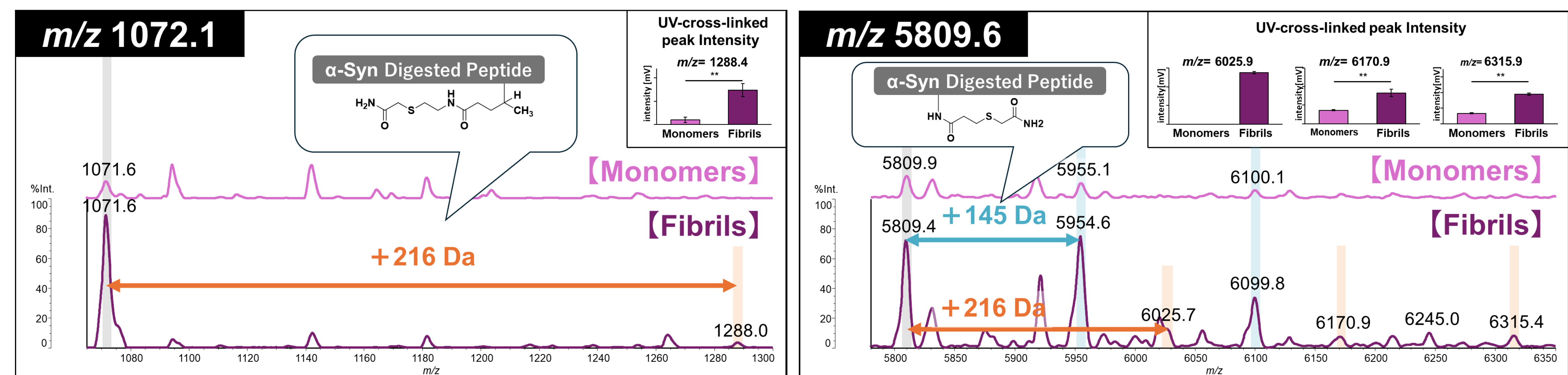
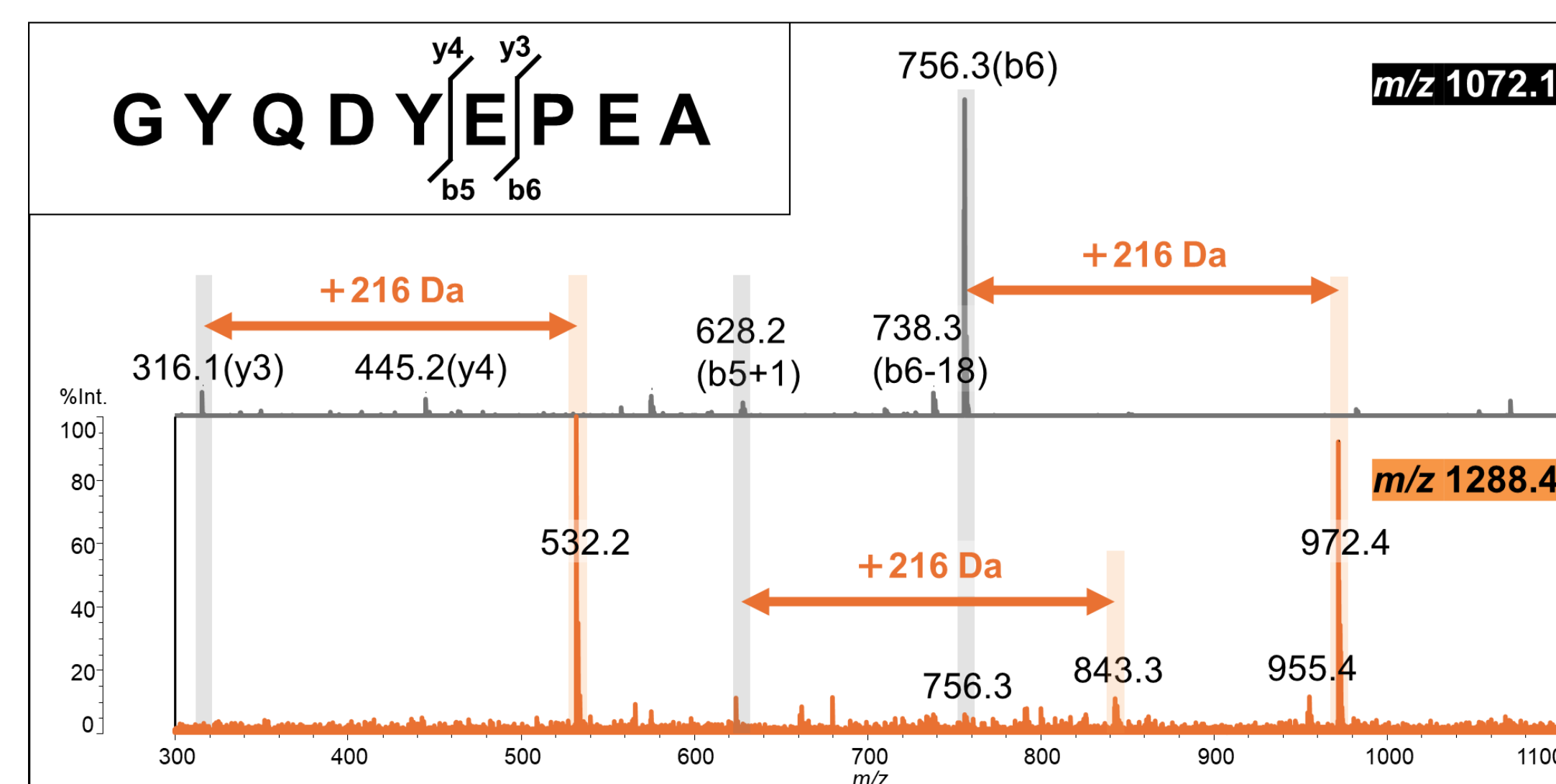


Figure 3. Peptide peaks detected by MALDI-TOF MS (*: $p < 0.05$, **: $p < 0.01$). Grey: α -Syn digested peptides; Blue: NHS-linked peaks; Orange: UV-cross-linked peaks. The bar graph shows the intensity analysis of UV-cross-linked peaks.

3-3. MS/MS analysis of UV-cross-linked peptide

These peaks were identified by MS/MS analysis. For example, the MS/MS spectrum of the UV-cross-linked peak at m/z 1288.4, corresponding to the digested α -syn peptide at m/z 1072.1, showed multiple fragment ions with a +216 Da mass shift.

Figure 4. MS/MS spectra of the ions at m/z 1072.1 (top) and m/z 1288.4 (bottom)



4. Conclusions

- This XL-MS approach may provide a promising tool for identifying distinct α -syn Fibril structures in α -synucleinopathies.
- Further studies are needed to improve detection sensitivity by reducing peptide loss during the workflow.

[1] G. Fairfoul, et al., *Ann Clin Transl Neurol*, 3 (10) (2016), pp. 812-818.

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