Supporting Information for

Covalent Labeling with Isotopically Encoded Reagents for

Faster Surface Structural Analysis of Proteins by Mass Spectrometry

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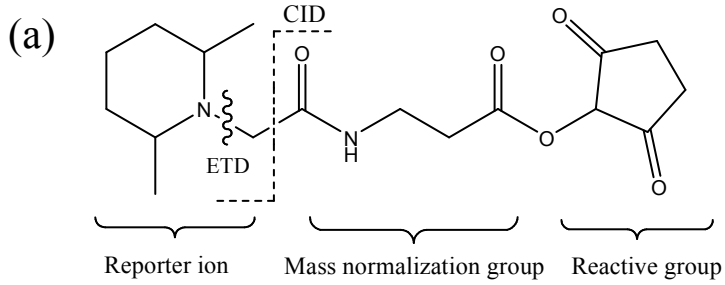
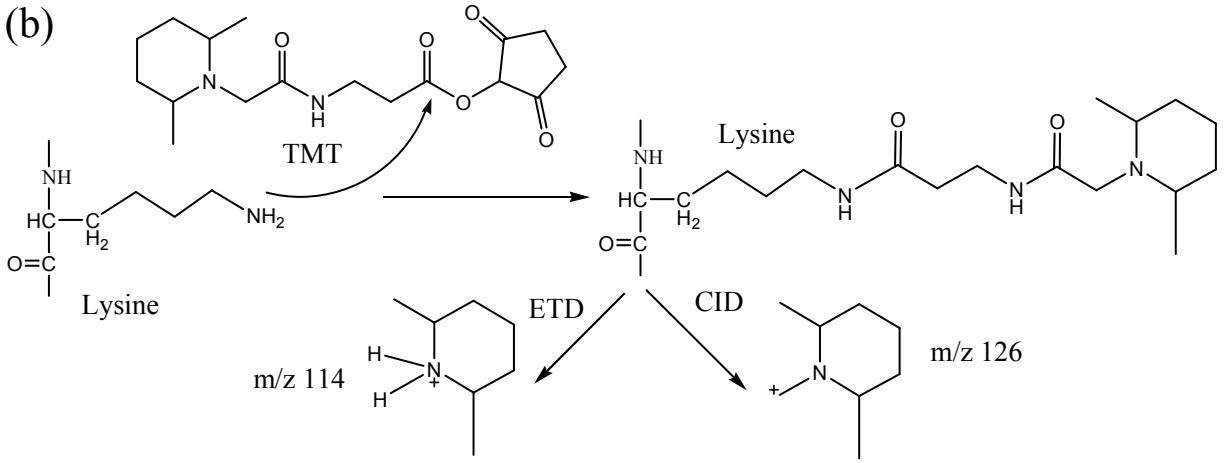
Table S1. The TMT[6] concentration and the $\ln\left[1-6\times\frac{I_{modi}^{total}}{I_{unmodi}^{total}+I_{modi}^{total}}\times\frac{i_x}{(i_1+i_2+i_3+i_4+i_5+i_6)}\right]$ value for peptide fragment (SRHPAENGKSNF) from β2m under PAN fragmentation. This data is used to fit the pseudo-first order reaction. All the experiments were repeated three times, and the means and standard deviations are reported.

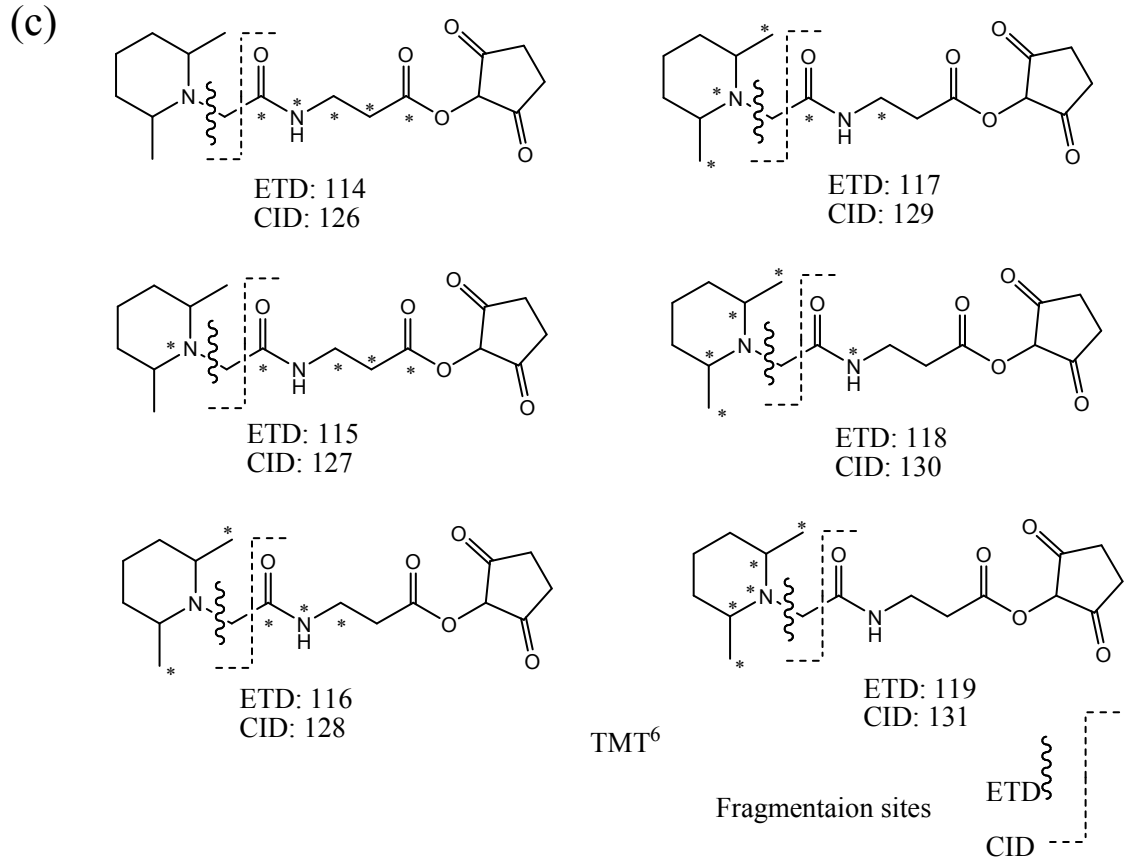
[TMT] mM	$\ln\left[1-6\times\frac{I_{modi}^{total}}{I_{unmodi}^{total}+I_{modi}^{total}}\times\frac{i_x}{(i_1+i_2+i_3+i_4+i_5+i_6)}\right]$
0.04	-0.11 ± 0.06
0.06	-0.24 ± 0.04
0.08	-0.40 ± 0.05
0.1	-0.6 ± 0.1
0.12	-0.6 ± 0.1
0.16	-0.7 ± 0.1

Table S2. The TMT[0] concentration and the $ln\frac{I_{unmodi}}{I_{unmodi}+I_{modi}}$ value for peptide fragment (SRHPAENGKSNF) from β2m. This data is used to fit the pseudo-first order reaction. All the experiments were repeated three times, and the means and standard deviations are reported.

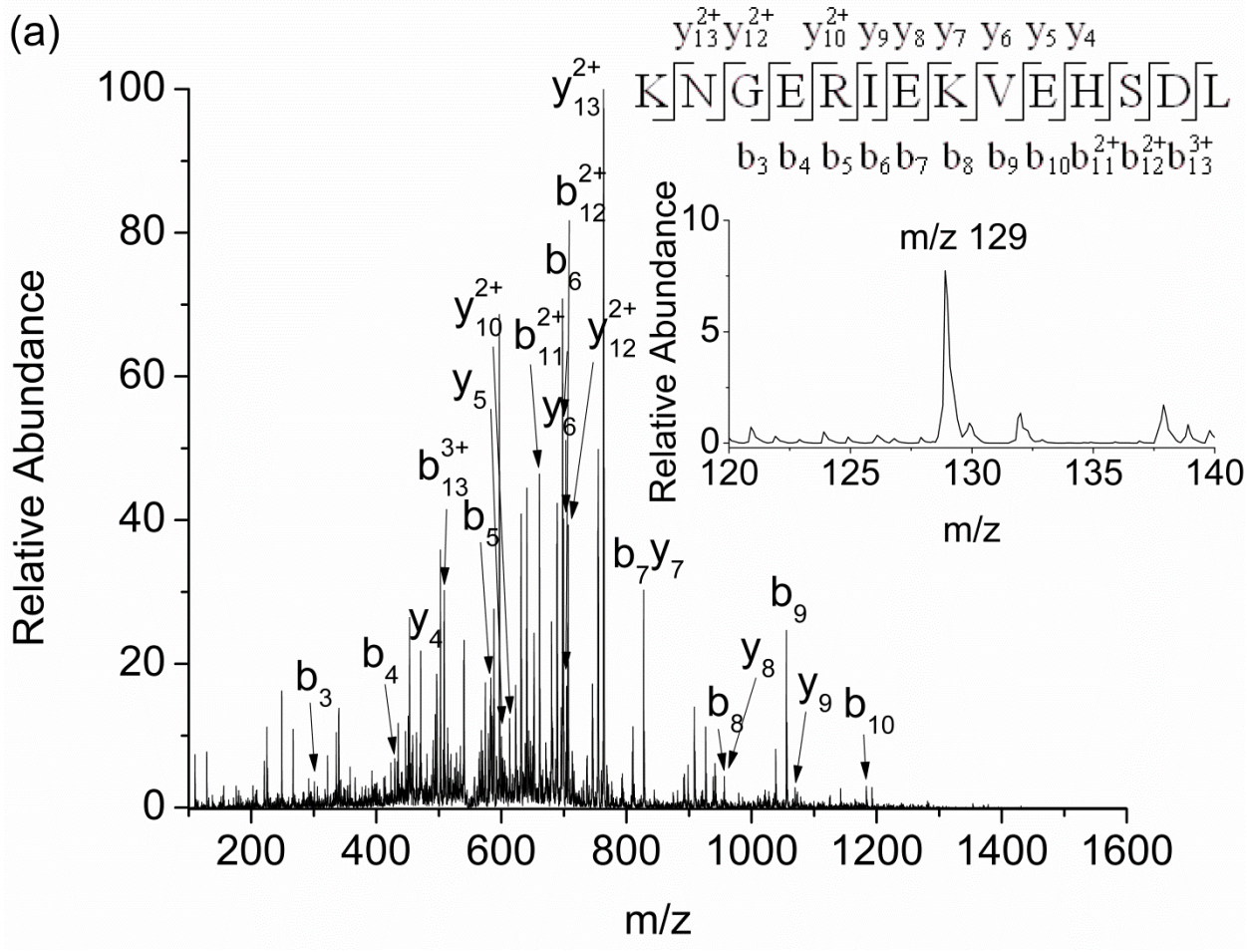
[TMT] mM	$ln\frac{I_{unmodi}}{I_{unmodi}+I_{modi}}$
0.04	-0.4 ± 0.1
0.06	-0.49 ± 0.04
0.08	-0.7 ± 0.1
0.1	-0.8 ± 0.1
0.12	-0.77 ± 0.08
0.16	-1.1 ± 0.2


(a)
CID
ETD
Reporter ion
Mass normalization group
Reactive group


(b)
TMT
Lysine
Lysine
ETD
CID
m/z 114
m/z 126


(c)
ETD: 114
CID: 126
ETD: 117
CID: 129
ETD: 115
CID: 127
ETD: 118
CID: 130
ETD: 116
CID: 128
ETD: 119
CID: 131
TMT6
Fragmentaion sites
ETD
CID

Scheme S1. (a) General structure of the TMT reagents. (b) Reaction of TMT with lysine and the reporter ions that result after MS/MS, by ETD or CID. (c) Structure, isotope position, MS/MS fragmentation sites and CID/ETD reporter ions for TMT[6].



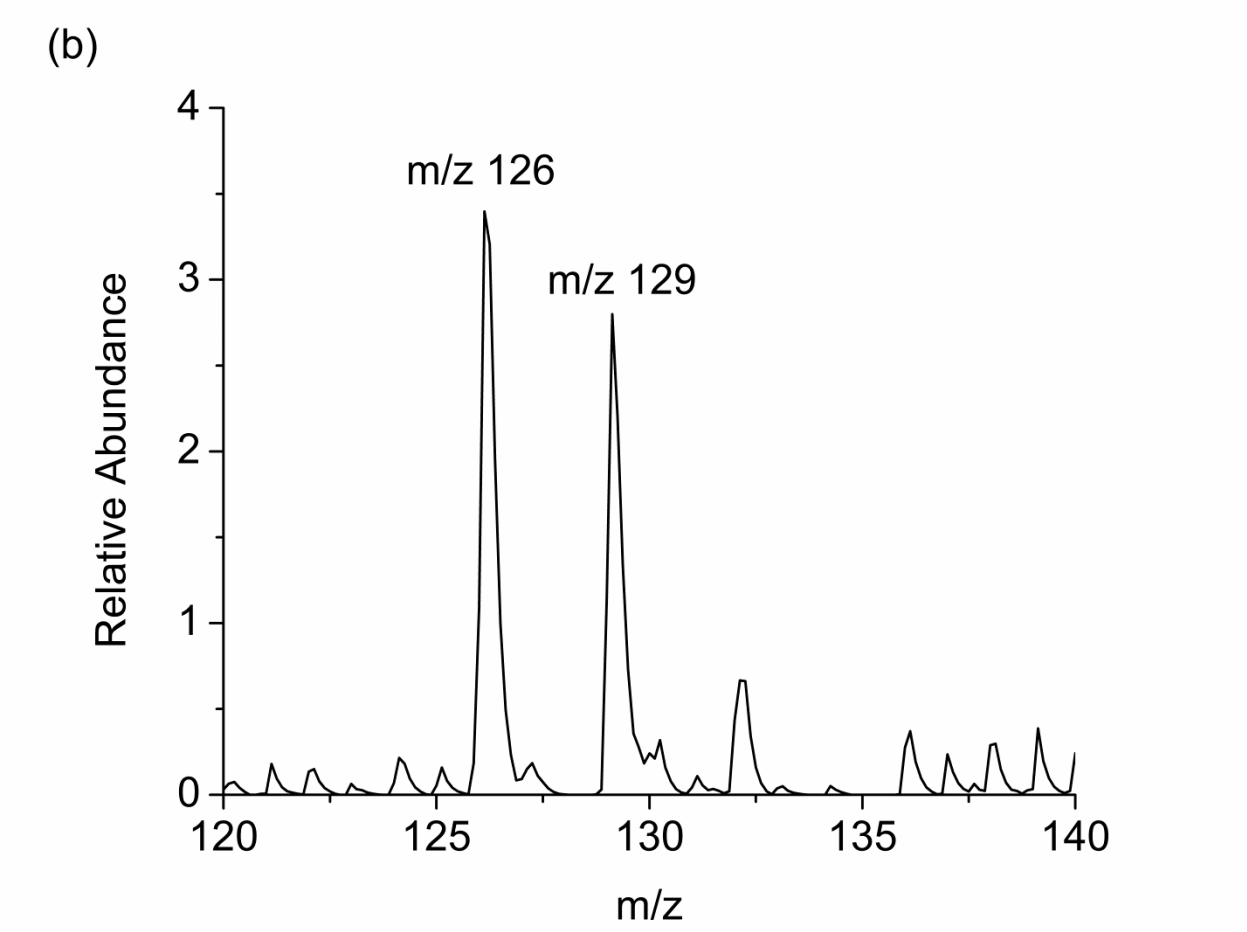


Figure S1: (a) CID tandem mass spectrum of the $[M+4H]^{4+}$ ion of the peptide fragment KNGERIEKVEHSDL from β2m, acquired using the PAN mode (see experimental section for details). A series of b and y ions confirmed the sequence of the peptide fragment. (b) CID mass spectrum of the $[M+4H+TMT^{0}]^{4+}$ ion of the peptide fragment KNGERIEKVEHSDL in the m/z range around the reporter ions. This spectrum was acquired using the PAN mode.