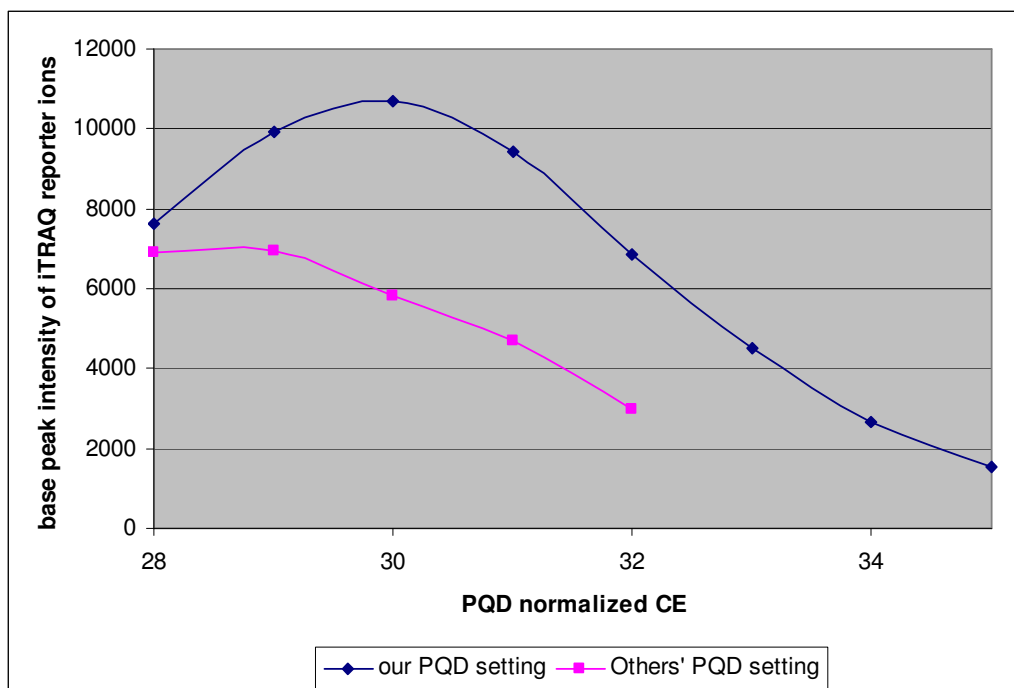


Supplementary material

PQD and ETD optimization

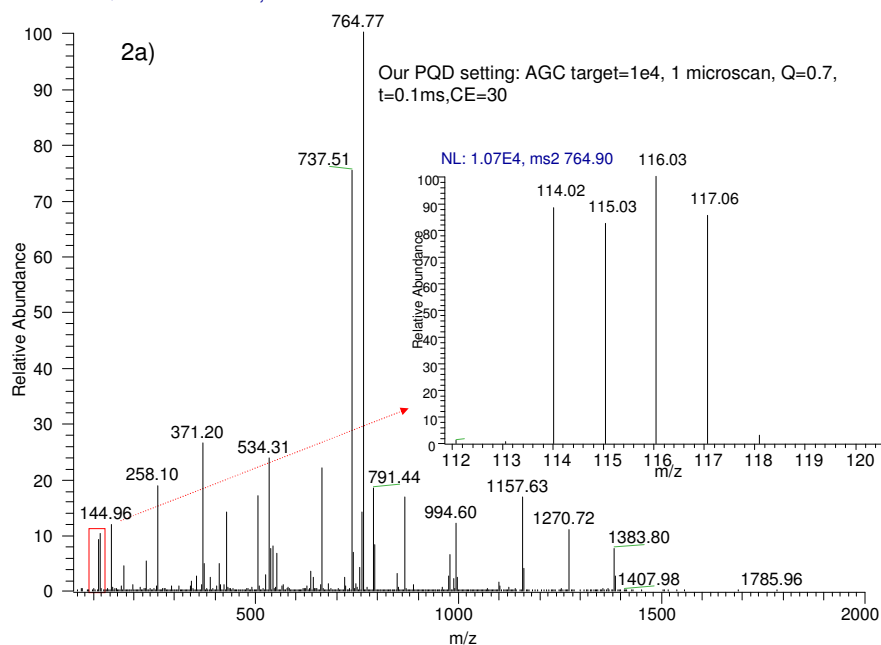
iTRAQ-labeled β -casein tryptic digest and the MassPREP™ Phosphopeptide Standards (Waters) were used to optimize instrument parameters for PQD. iTRAQ 114-, 115-, 116-, and 117-labeled β -casein tryptic digests (100 μ g each) were mixed at a ratio of 1:1:1:1 and desalted using a reversed phase C18 SPE. iTRAQ 114- and iTRAQ 117-labeled phosphopeptide standards were mixed at a ratio of 1:1 as well. Each infusion mixture (~ 20 pmol/ μ L total concentrations for the β -casein tryptic digests and ~ 15 pmol/ μ L total concentrations for the MassPREP™ Phosphopeptide Standards) was continuously infused into the mass spectrometer at 200 nL/min using a syringe pump.

To optimize collision energy settings for iTRAQ quantification at different activation Q and delay time combinations (Q=0.55, t=0.4 ms and Q=0.7, t=0.1 ms), we selected several peptides with different charge states and monitored the iTRAQ reporter ion signals for their intensities and their ratios at different normalized collision energies. For comparison between our PQD settings (Q=0.7, t=0.1 ms, AGC target=1e4 and 1 microscan) with others' PQD settings (Q=0.55, t=0.4 ms, AGC target=5e4 and 2 microscans),¹⁶ we changed the AGC target value and the number of microscans at the optimized CE setting. To optimize the ETD setting for quantification of iTRAQ-labeled peptides, we changed different cation and anion AGC values with different activation times. Specifically, we compared our ETD settings (cation AGC target=1e4, anion AGC target= 1e5, activation time=125 ms for ETD) with others' PQD settings (cation AGC target= 4e4, anion AGC target=3e5, activation time=100 ms for ETD).^{21, 22}

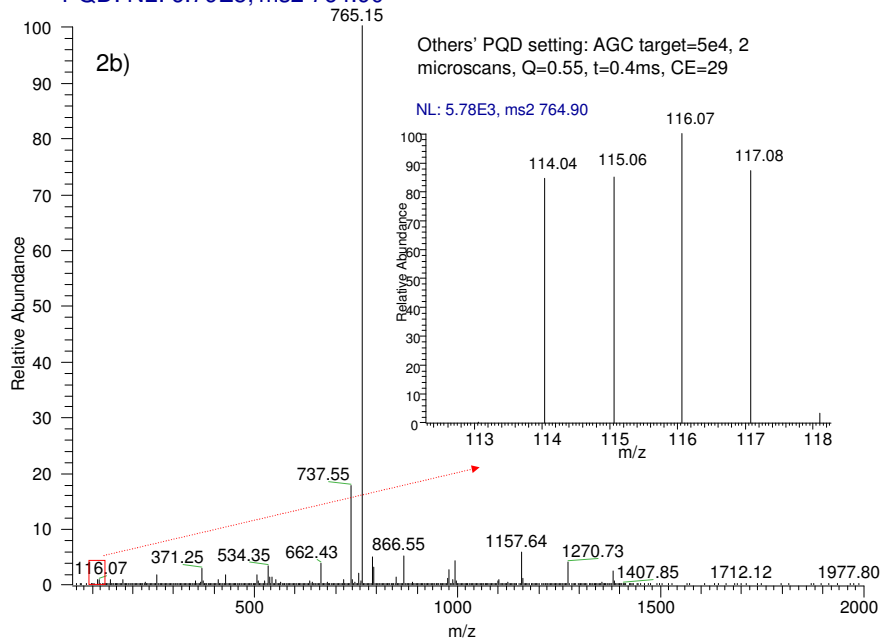


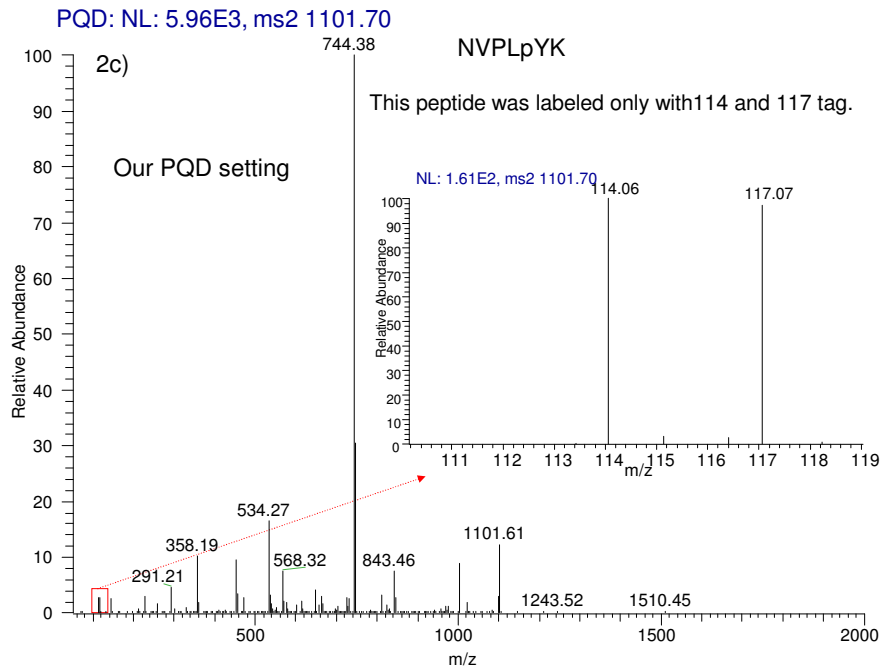
Supplementary Figure 1. Optimizing the CE at different PQD settings using a 2+ peptide from iTRAQ-labeled β -casein tryptic digest with $[M+2H]^{2+}=764.90$. Our setting: $Q=0.7$, $t=0.1$ ms, $AGC=1e4$ and 1 microscan; others' settings: $Q=0.55$, $t=0.4$ ms, AGC target= $5e4$ and 2 microscans.

PQD:NL: 1.04E5, ms2 764.90

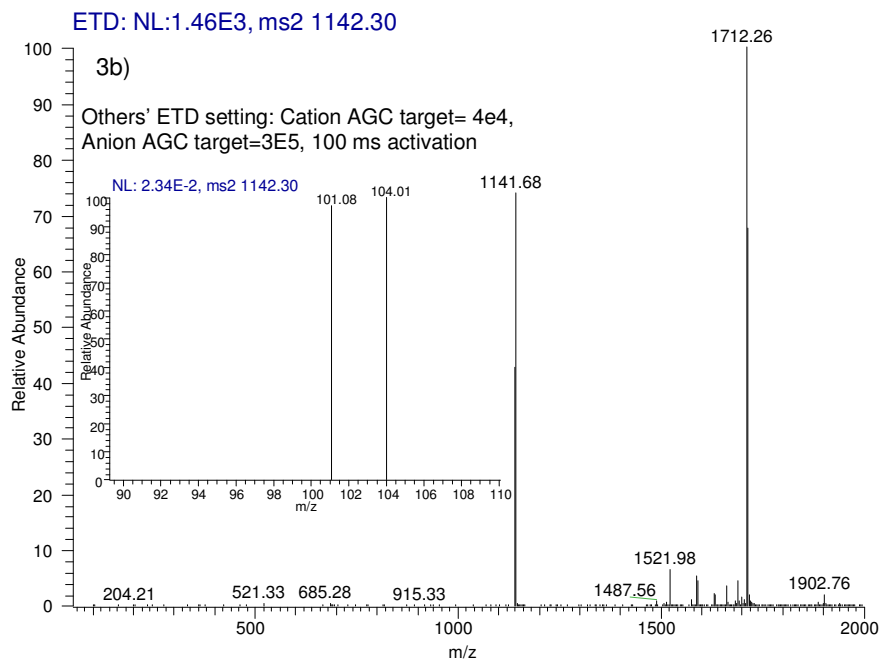
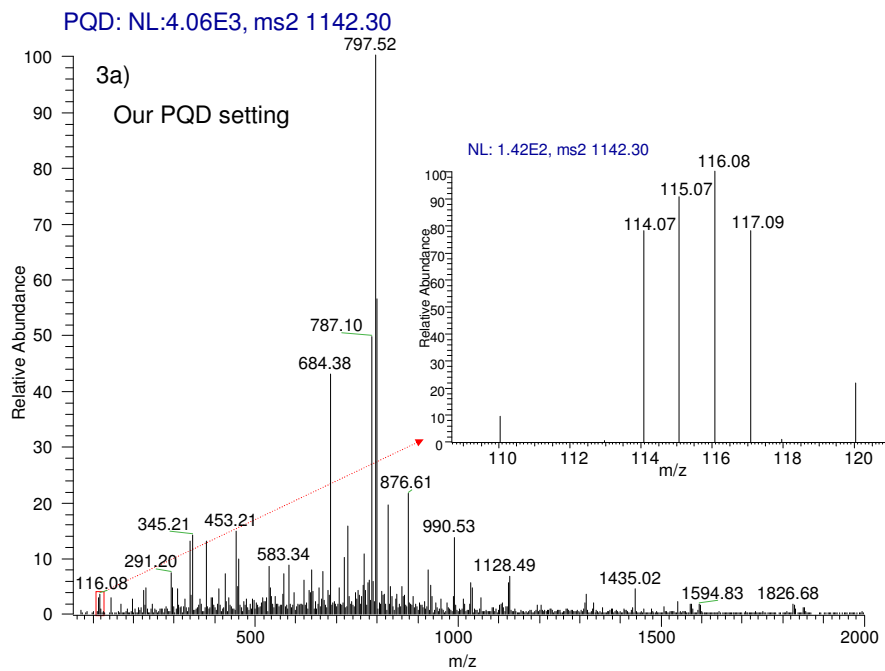


PQD: NL: 6.79E5, ms2 764.90





Supplementary Figure 2. PQD spectra of the peptide indicated in Supplementary Figure 1 at 2a) our setting, 2b) others' setting. 2c) shows the PQD spectra at our setting for phosphopeptide NVPLpYK.



Supplementary Figure 3. PQD and ETD spectra for a 3+ peptide from iTRAQ-labeled β -casein tryptic digest with $[M+3H]^{3+}=1142.30$ using 3a) our PQD setting, and 3b) others' ETD setting.

