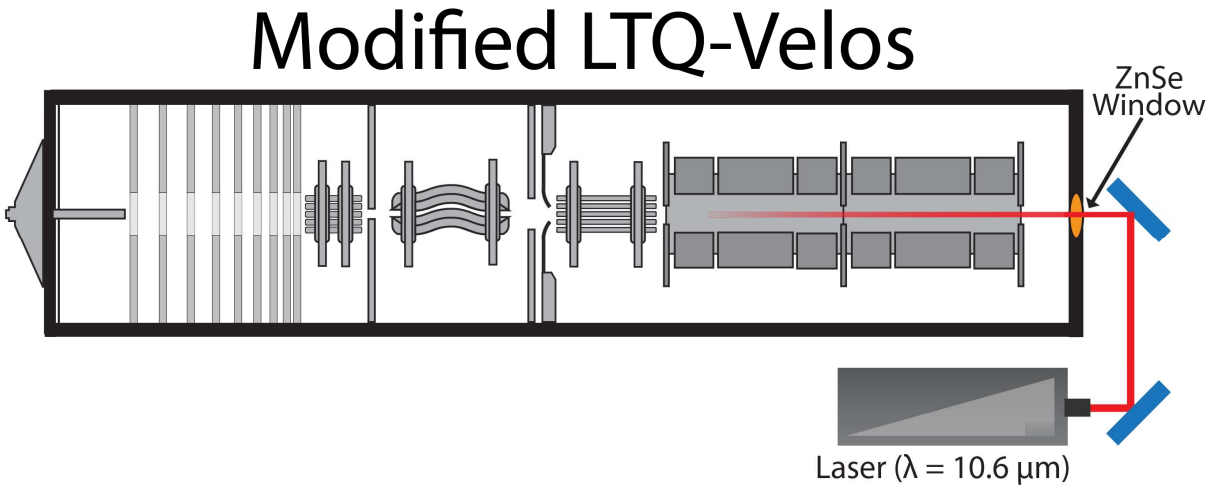
# Supplemental Information

## Experimental Section

**Sample Preparation**: A standard lab wild-type strain of yeast was grown in 1 L of YPD media.[2] Cells grown to mid-log phase were subsequently harvested by spinning down the culture at 10,000 rpm and washed with chilled water. The cell pellet was stored in –80 °C until lysis. Cells were lysed via Frech Press three times in standard lysis buffer (100 mMTris pH 8, 8 M urea, 75 mMNaCl, 1 mM DTT, 100 mM sodium butyrate, 1 mM sodium orthovanidate, Roche Protease inhibitors, and Roche PhosSTOP inhibitors). Lysate was centrifuged at 14,000 rpm for 10 min. Cysteine residues were reduced by incubating lysate with 5 mM DTT for 45 min at 37 °C followed by incubation in 15 mM IAA for 1 hour at room temperature in the dark. The alkylation reaction was capped by incubating the reaction with DTT for 15 min at room temperature. Proteins were digested overnight at 37 °C after the addition of 1mM $CaCl_2$, 50 mMTris (to decrease urea to 1 mM) and adjusting to pH 8 at an enzyme-to-substrate ratio of 1-to-50 of sequencing grade trypsin (Promega, Madison, WI). Each digest was quenched by the addition of TFA to a final concentration of 0.5% (pH ≤ 2), desalted via solid phase extraction on $tC_{18}$SepPak cartridge (Waters, Milford, MA), and the eluent lyophilized (stored in –80°C until further use).

**TMT Labeling:** Each TMT tag was resuspended in 41 uL of pure acetonitrile. Lyophilized peptides were re-suspended in nano-pure $H_2O$ to a final concentration of 2 µg/µL, with the addition of 200 mMtriethyl ammonium bicarbonate (TEAB) to a final volume of 132.5 µL. Resuspended peptides were mixed in two known ratios 1:5:2:1.5:1:3 for TMT 6-plex $TMT^{126}$, $TMT^{127}$, $TMT^{128}$, $TMT^{129}$, $TMT^{130}$, $TMT^{131}$, respectively. Each reaction was allowed to incubate at room temperature with intermittent mixing for one hour. Tagging was quenched with the addition of 8 µL 5% hydroxylamine for 15 min. The differentially labeled TMT samples were pooled in equal amounts, dried down, and stored in –80°C until further use.

**LC Gradient**: Separations were carried out using a 165 minute gradient of 2% to 5% B (0.2% formic acid in ACN) over 3 minutes followed by a linear gradient increasing buffer B to 35% over 120 minutes, followed by a ramp up to 70% B over 10 minutes and held for 5 minutes. The gradient was dropped back to 98% A (0.2% formic acid in H2O) over a period of 5 minutes and allowed to re-equilibrate for 20 minutes.



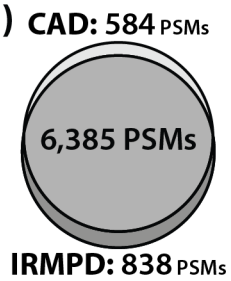

**Supplementary Figure 1:**Modified dual-cell QLT ion trap. The addition of a ZnSe window enables IRMPD to be readily performed in the low pressure ion trap.

A)

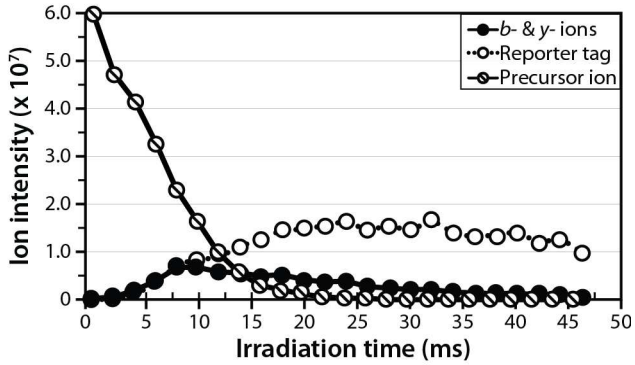
| | IRMPD | CAD |
|---|---|---|
| Trial #1 | 7,223 | 6,969 |
| Trial #2 | 6,617 | 6,432 |
| Trial #3 | 6,909 | 6,747 |

Peptide Spectral Matches (PSMs)

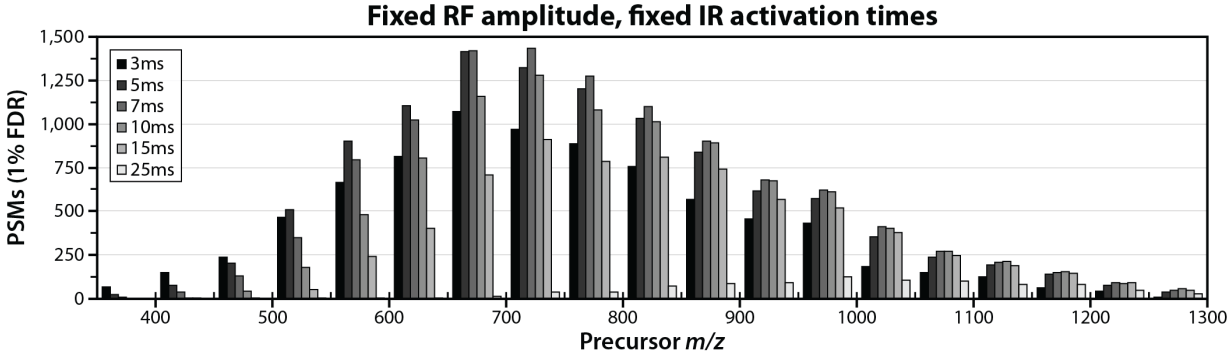
B)



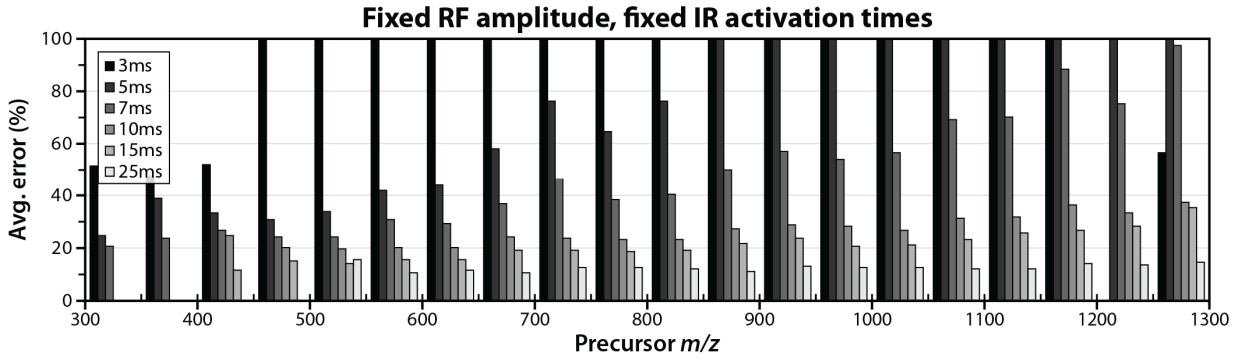

**Supplementary Figure 2:**Replicate test for IRMPD vs. CAD. IRMPD conditions were 10 ms irradiation time, *q*-value of 0.10, and laser power of 48 W. Using these IRMPD conditions we were able to consistently produce a greater number of peptide-spectral matches (PSMs) at a 1% FDR than CAD (A). Comparing IMRPD PSMs with corresponding CAD PSMs indicates very high overlap between peptides identified by CAD and IRMPD (B).



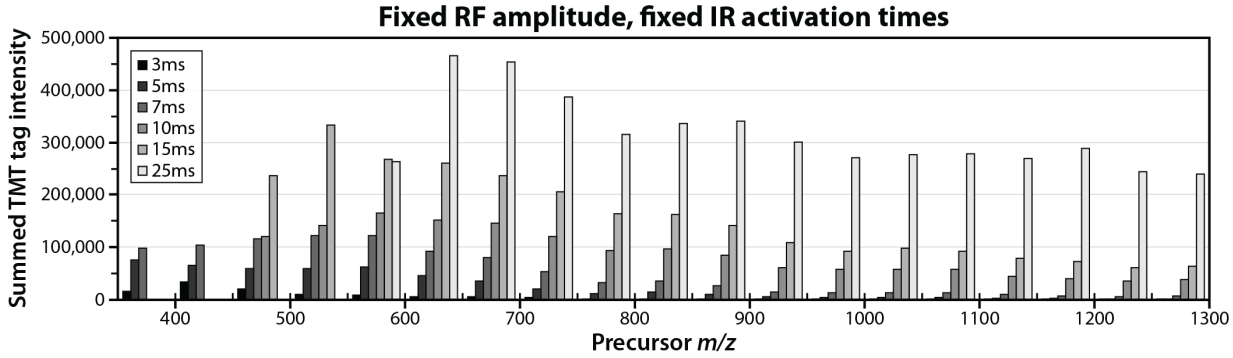

**Supplementary Figure 3:**IRMPD time-resolved plot of precursor, b- / y- type, and isobaric reporter tag intensities shown in absolute quantity. The maximum yield of b- and y- type product ions occurs and a shorter time than the maximum yield of TMT reporter tag.




**Supplemental Figure 4:**PSMs binned by precursor m/z value for IRMPD experiments. For each experiment, the RF amplitude was held constant, resulting in a fixed low-mass cutoff (LMCO) and dynamic precursor *q*- value. IRMPD activation times were varied from 3 to 25 ms. The resulting data indicate that setting the RF in this manner results in optimal IRMPD activation times varying strongly as a function of precursor *m/z* value.



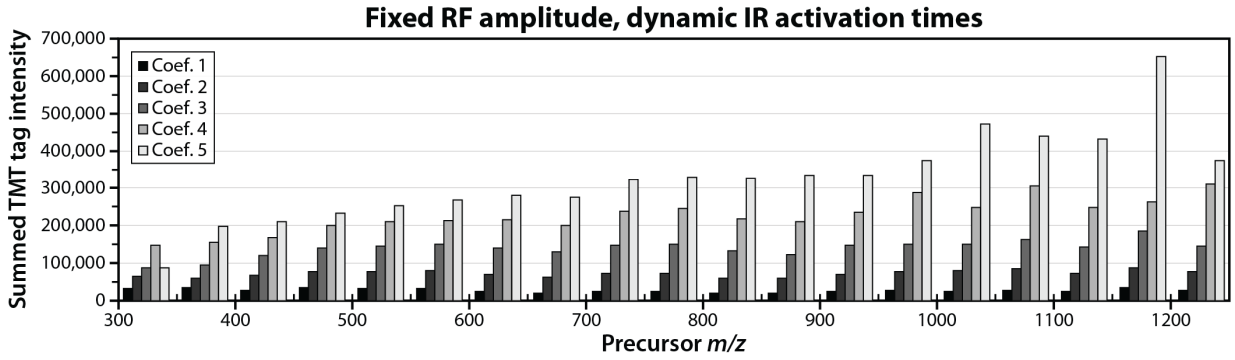

**Supplemental Figure 5:**Average quantitative accuracy for PSMs binned by precursor m/z value for IRMPD experiments. For each experiment, the RF amplitude was held constant, resulting in a fixed low-mass cutoff (LMCO) and dynamic precursor *q*- value. IRMPD activation times were varied from 3 to 25 ms. At a given precursor m/z value, increasing the irradiation time decreases the metric score (reflecting better quantitative accuracy). At a given IRMPD irradiation time, quantitative accuracy is the best for precursors having a low m/z value.



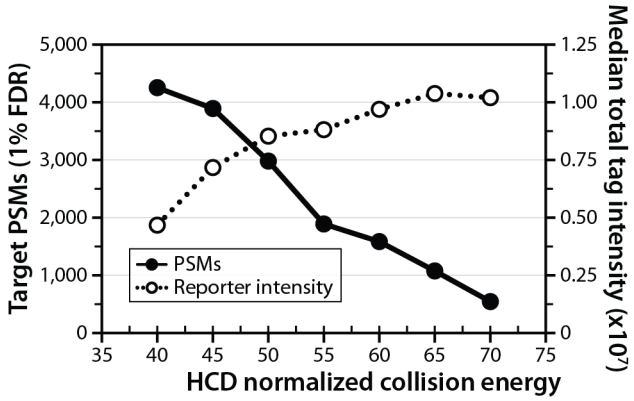

**Supplementary Figure 6:** Average **s**ummed TMT reporter tag intensity grouped by precursor m/z value. Using fixed RF amplitude and fixed IRMPD activation time results in highly uneven TMT reporter tag intensity, with the reporter tags intensity dropping off rapidly with increasing precursor m/z value. This in turn correlates to uneven quantitative accuracy.

## Supplementary Figures 4, 5, and 6:

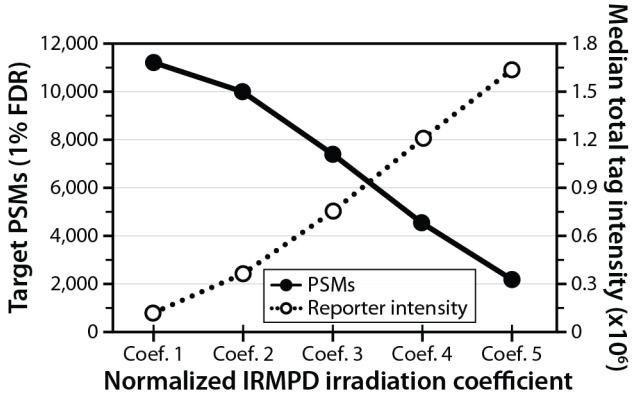
For each experiment, we searched the resulting data using OMSSA, with resulting peptide spectral matches (PSMs) within a 1% FDR binned according to precursor m/z value (**Fig. 5**). By setting the QLT RF to a fixed amplitude during IRMPD, the optimal irradiation time becomes strongly dependent upon precursor m/z value. For precursor peptides having low m/z values (*e.g.*, m/z< 500), short activation times produce the most favorable results. Similarly, high m/z peptides (e.g., m/z> 1000) are best identified using long irradiation times. For peptides having intermediate precursor m/z values, the best results are obtained by utilizing intermediate irradiation times.




**Supplementary Figure 7:** Average **s**ummed TMT reporter tag intensity grouped by precursor m/z value. Using fixed RF amplitude and setting IRMPD activation time dynamically results in mostly even TMT reporter tag intensity over a wide range of precursor m/z values.



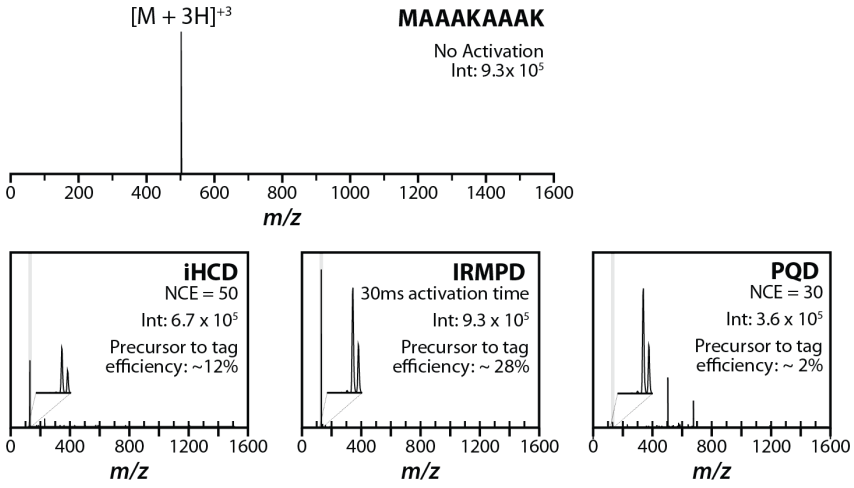

**Supplementary Figure 8[1]:** Peptide spectral matches (PSMs) and median reporter tag intensity as a function of HCD Collision energy. Optimal conditions for reporter tag generation are substantially higher than optimal conditions for PSM production.




**Supplementary Figure 9:** Peptide spectral matches (PSMs) and median reporter tag intensity as a function of Normalized IRMPD irradiation coefficient. Optimal irradiation times for reporter tag generation are substantially longer than optimal conditions for PSM production.

## Supplementary Figures 8 and 9:

Both IRMPD and HCD show similar trends; for each dissociation method, activation conditions can be optimized for PSM production (1% FDR) or tag generation, but not both simultaneously. While there is a difference in the Median total Tag intensity, this is due to different normalization factors associated with the different mass spectrometers used to collect the two plots.




**Supplementary Figure 10:** IRMPD, PQD, and iHCD (beam-type activation of precursor peptides using ESI injection optics as the collision cell) activation of peptide precursor MAAAKAAAK. For PQD,iHCD and IRMPD, fragmentation conditions were optimized for the production of TMT reporter ions. We find that the use of IRMPD results in a greater proportion of precursor charges being converted into TMT reporter tags as compared to iHCD or PQD.

## Supplementary Figure10:

IRMPD, PQD, and iHCD were each optimized for the production of TMT reporter tag ions.


1 Wenger, C. D. *et al.* Gas-phase purification enables accurate, multiplexed proteome quantification with isobaric tagging. *Nat. Methods***8**, 933-935, doi:10.1038/nmeth.1716 (2011).

2 Lee, M. V. *et al.* A dynamic model of proteome changes reveals new roles for transcript alteration in yeast. *Mol. Syst. Biol.***7**, doi:514

10.1038/msb.2011.48 (2011).