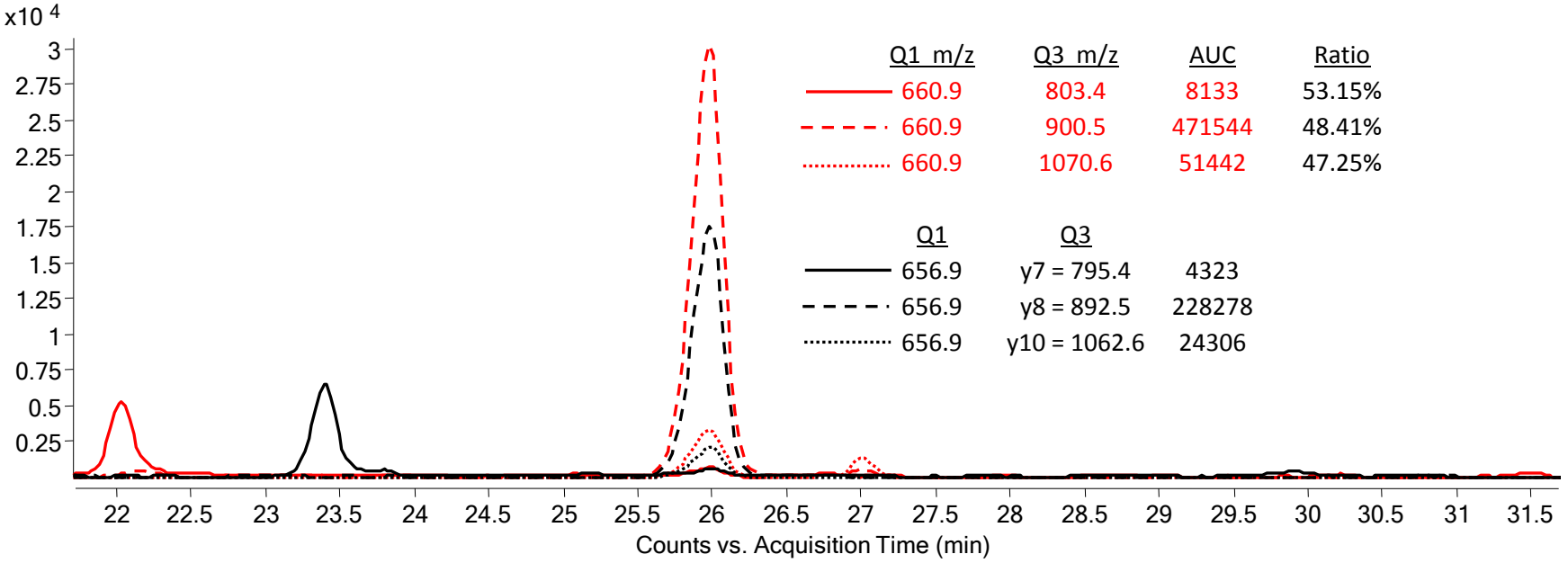
Supplemental SRM-MS Methods and Data

- Instrumentation
  - LC-SRM-MS (1200 Series HPLC-CHIP, 6410 Triple Quadrapole, Agilent Technologies). Peptides were eluted on-line to the MS-QQQ with a reverse phase linear gradient from 97 % A (0.1 % Formic acid in water) to 45 % B (0.1 % formic acid in acetonitrile).
  - Three transitions for each stably isotopically labeled peptide were developed empirically optimizing for both sensitivity and selectivity. Dwell time was constant at 45 *msec* for each transition and unit resolution was utilized for both Q1 and Q3.
  - Ion chromatograms for each transition were extracted with a symmetric expansion of 0.1 +/- *m/z*. Each extracted ion chromatogram (EIC) was then smoothed with a Gaussian Function of 15 points width. Peak areas for each transition were calculated utilizing default MS/MS integration. Peak areas were filtered to remove any peaks < 10 % of largest peak and a signal to noise ratio (height:peak-to-peak) of 5. Each transition passing the above filters and inside a retention time drift of 10% was manually verified.
  - EIC along with targeted transitions for each peptide measured are below.

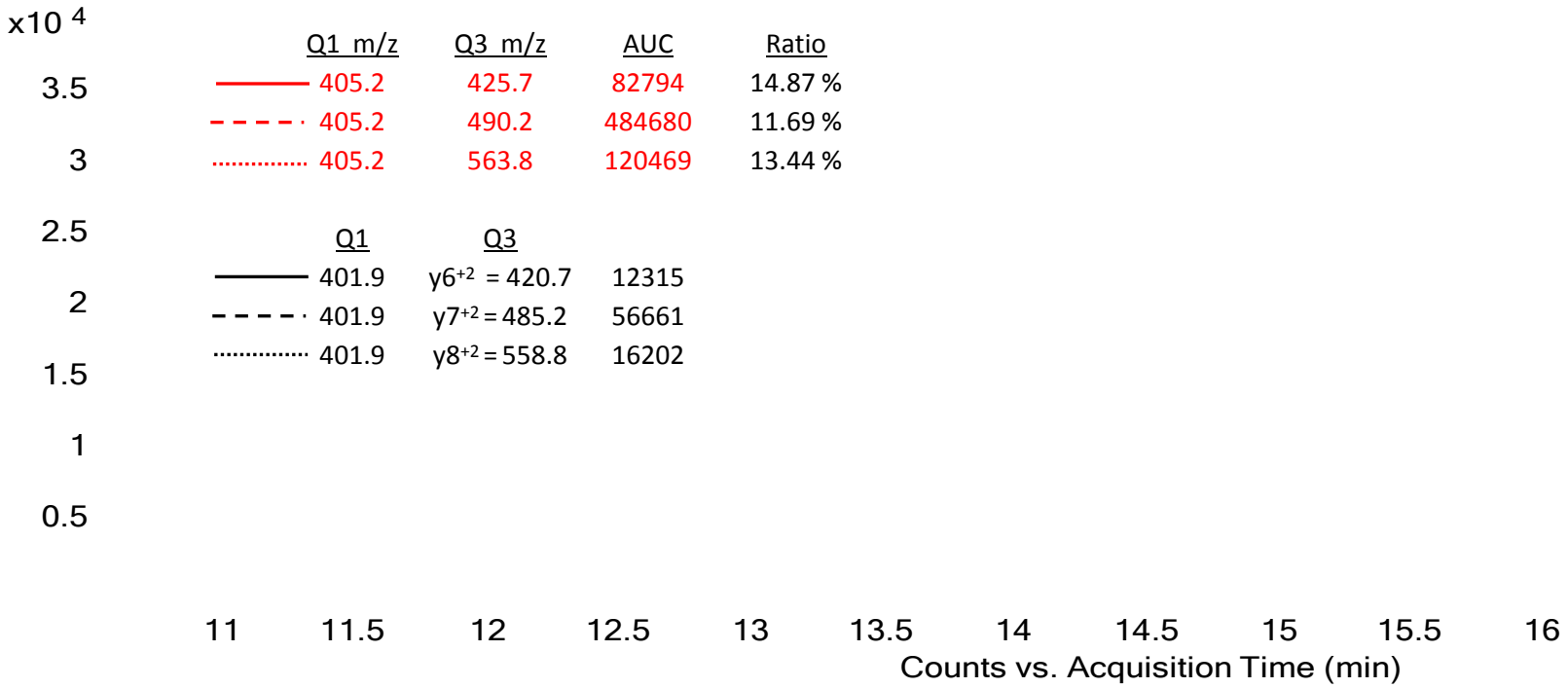
Fraction # 4
kRAS Peptide
SYGIPFIETSAK



SYGIPFIETSAK = $[M + 2H]^{+2}$ = 656.9 m/z

K = $^{13}C_6$ $^{15}N_2$

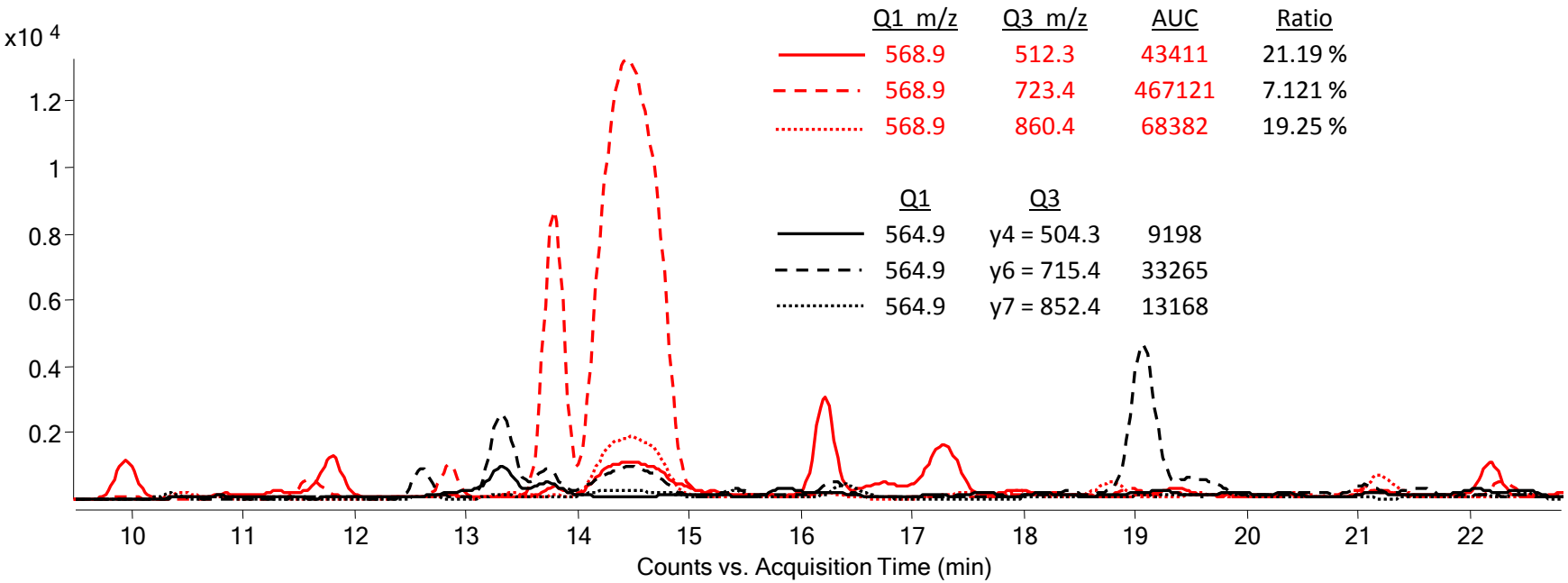
Fraction # 4
kRAS Peptide
SFEDIHHYR



SFEDIHHYR = $[M + 2H]^{+2}$ =401.9 m/z

R = $^{13}C_8$ $^{15}N_2$

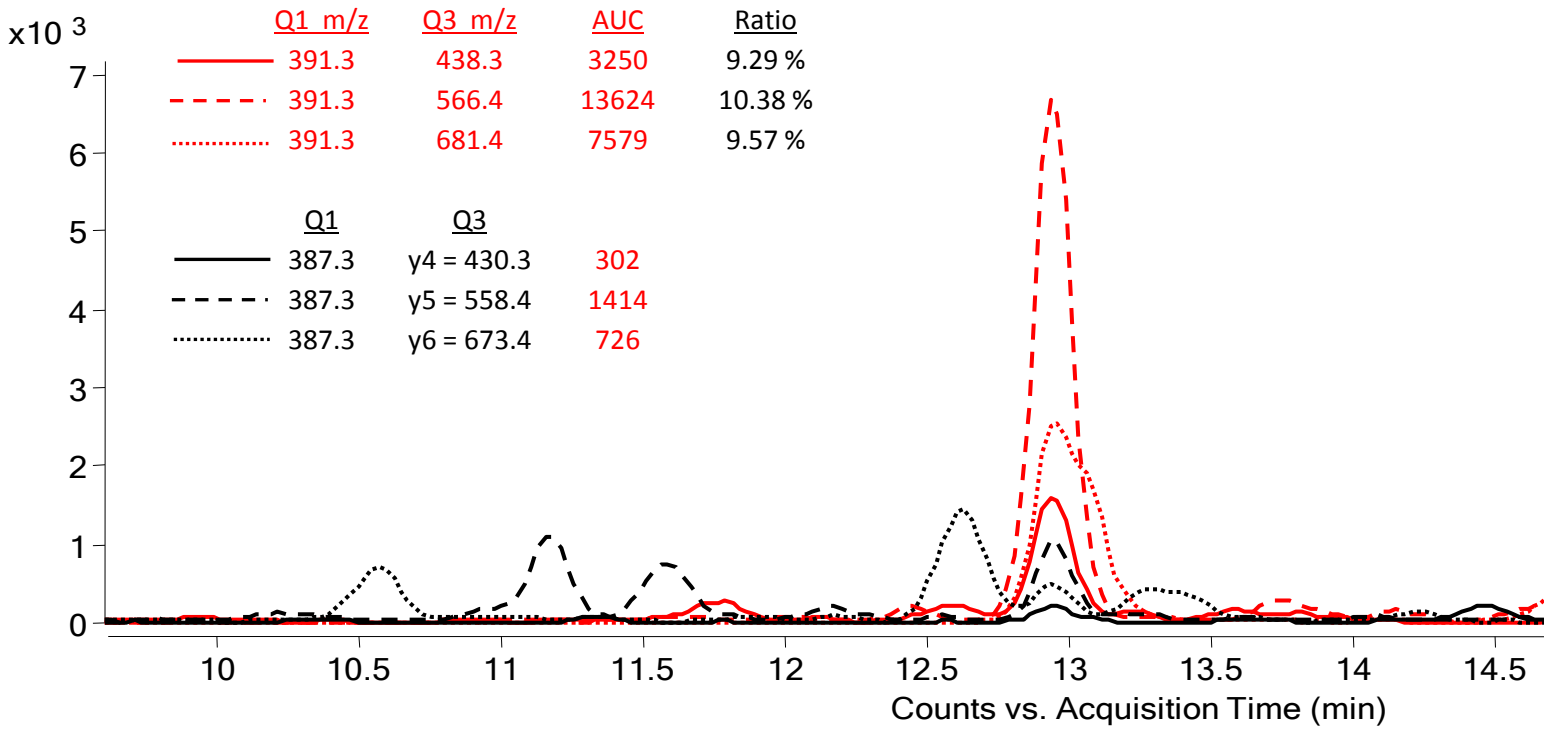
Fraction # 4
UBE Peptide
IYHPNIDEK



K = $^{13}C_6$ $^{15}N_2$

IYHPNIDEK = $[M + 2H]^{+2}$ = 564.9 *m/z*

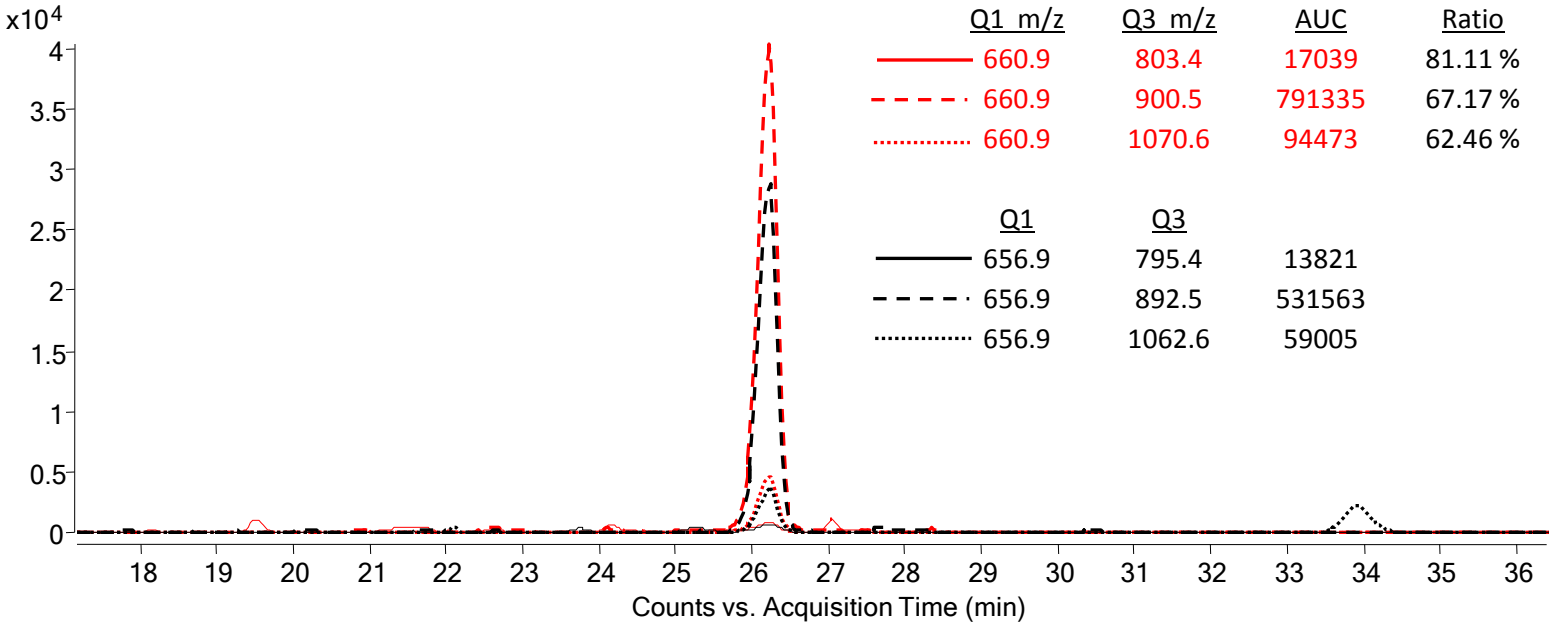
Fraction # 4
Fusion Break Point Junction
TDQGLLK



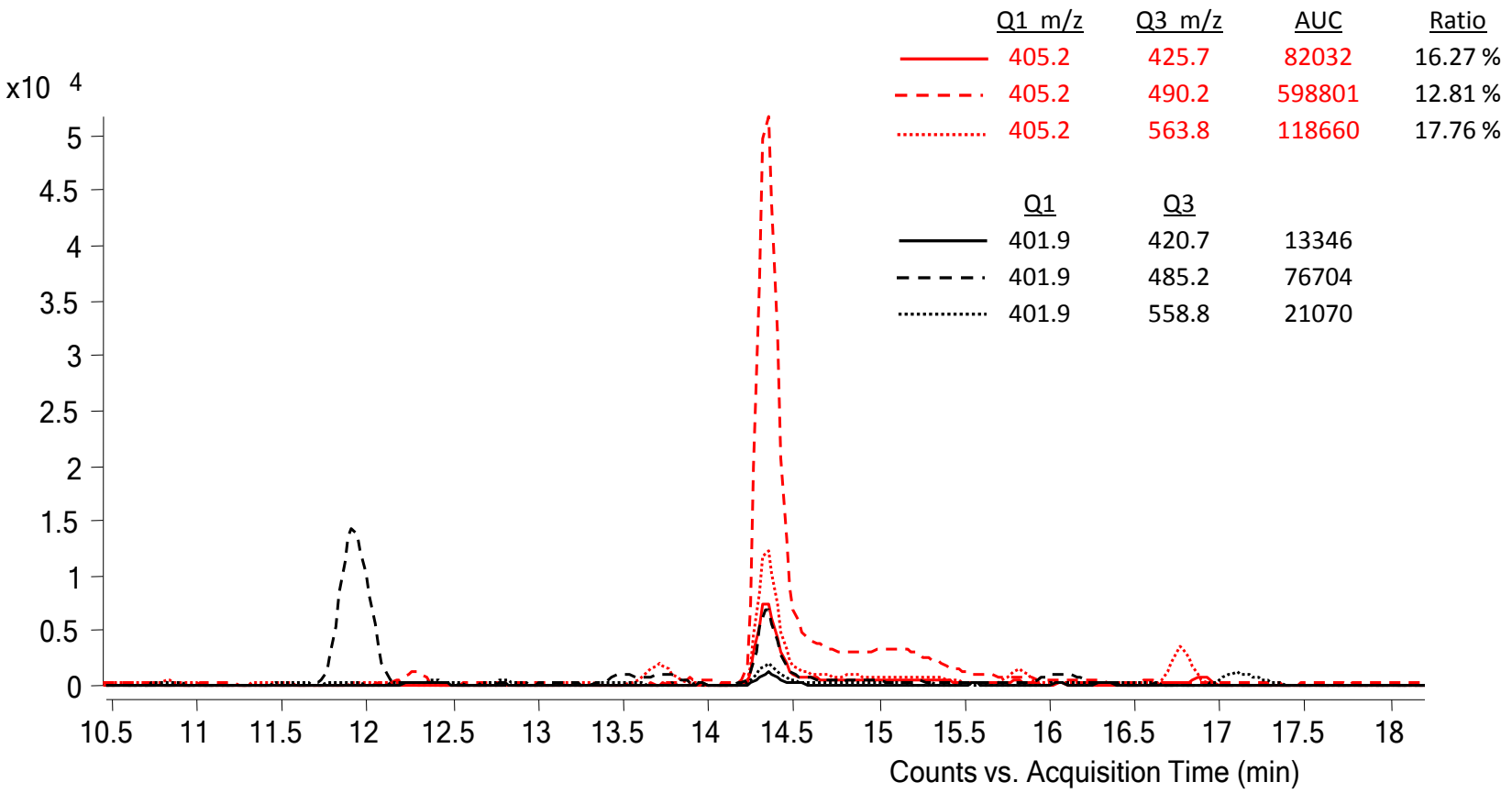
TDQGLLK = $[M + 2H]^{+2}$ = 387.3 *m/z*

K = $^{13}C_6$ $^{15}N_2$

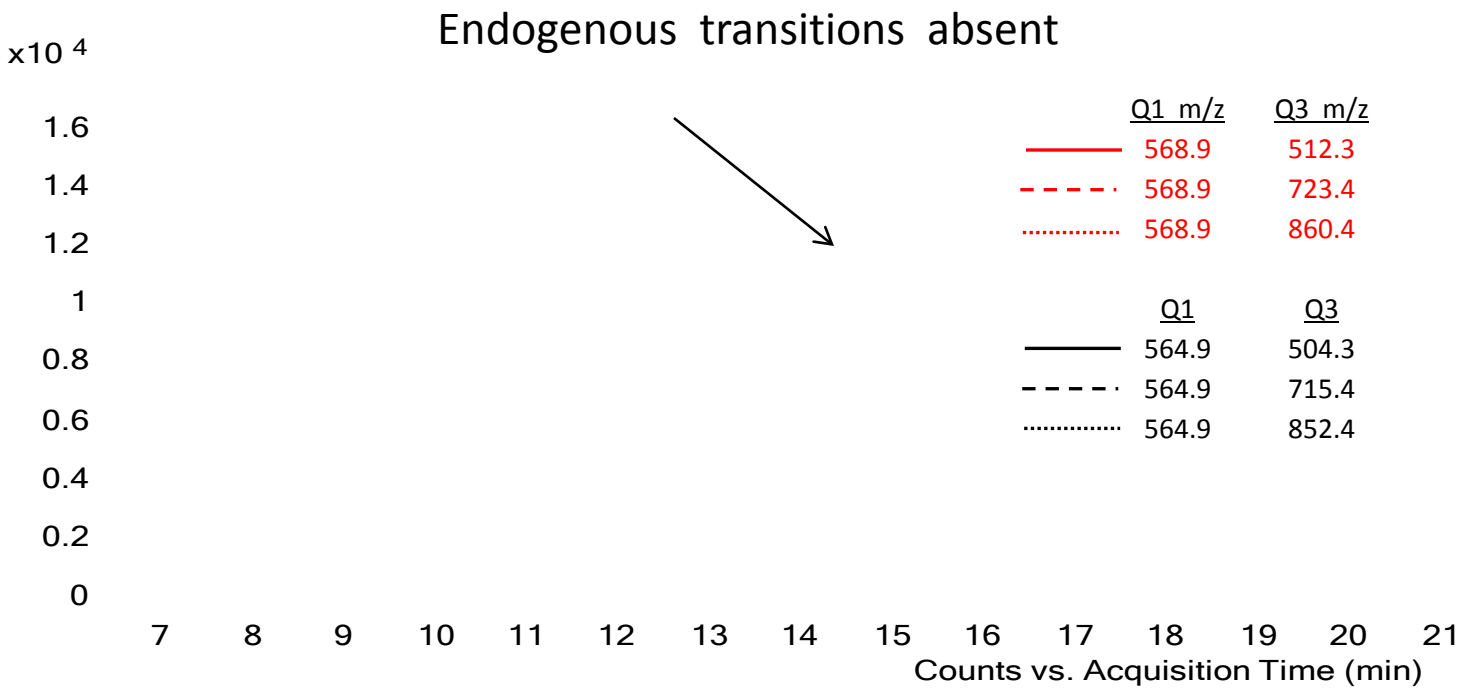
Fraction # 7
kRAS Peptide
SYGIPFIETSAK



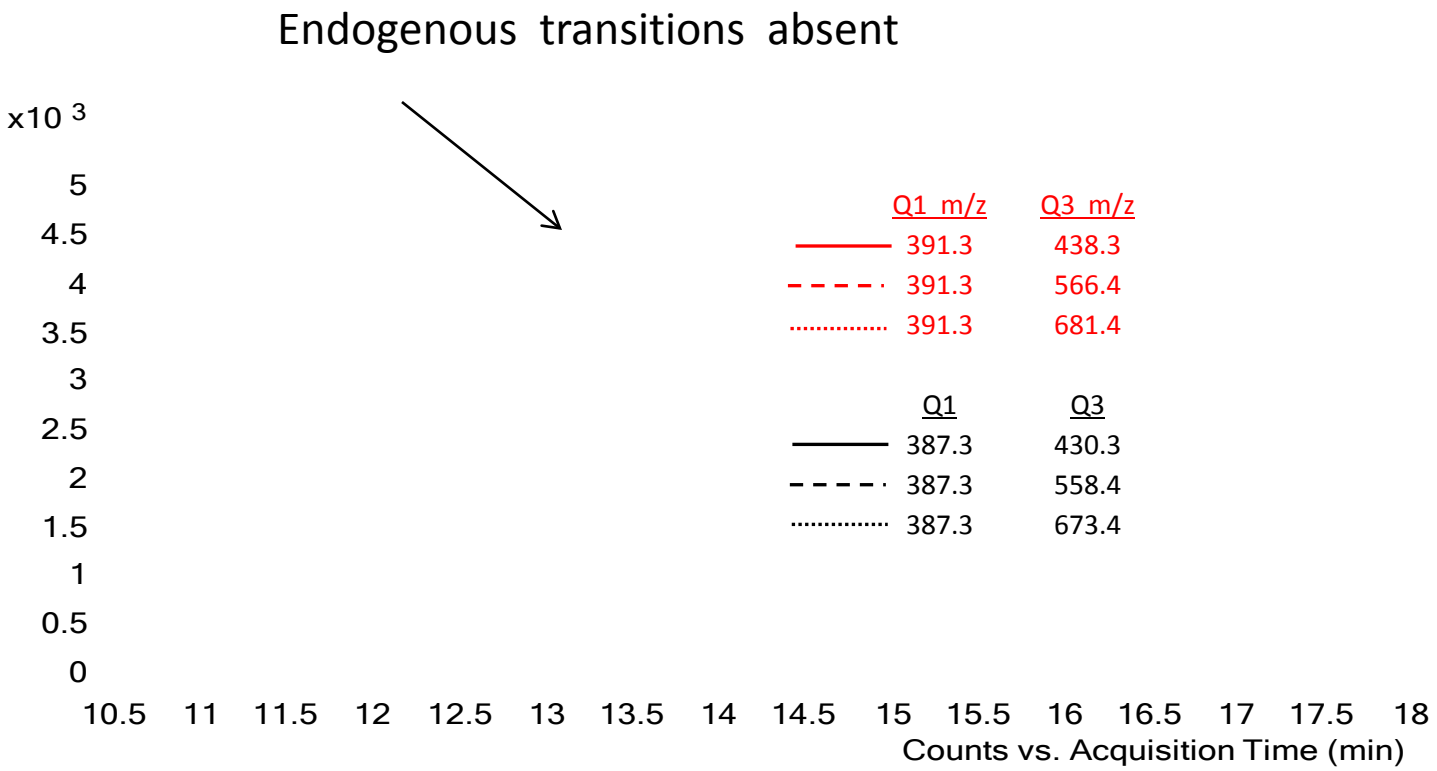
Fraction # 7
kRAS Peptide
SFEDIHHYR



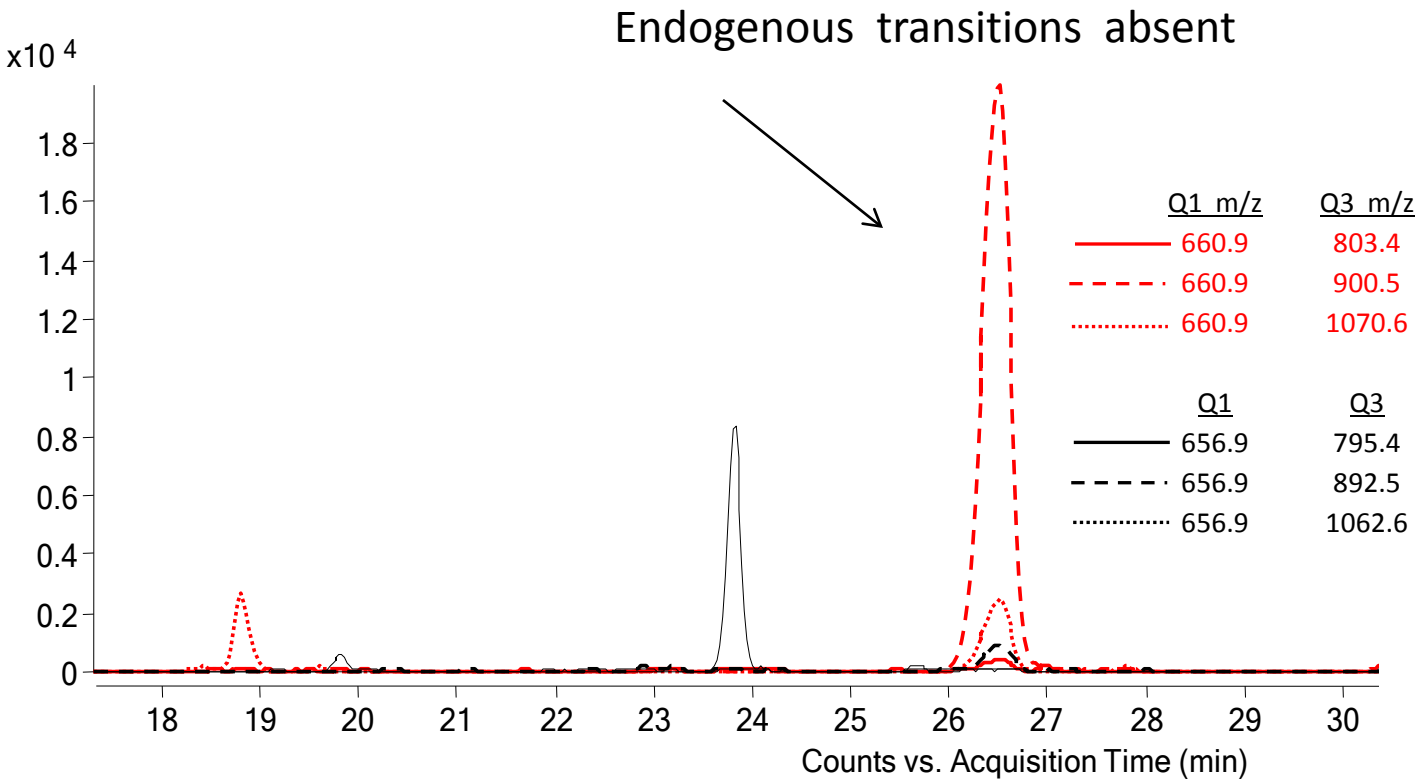
Fraction # 7
UBE Peptide
IYHPNIDEK



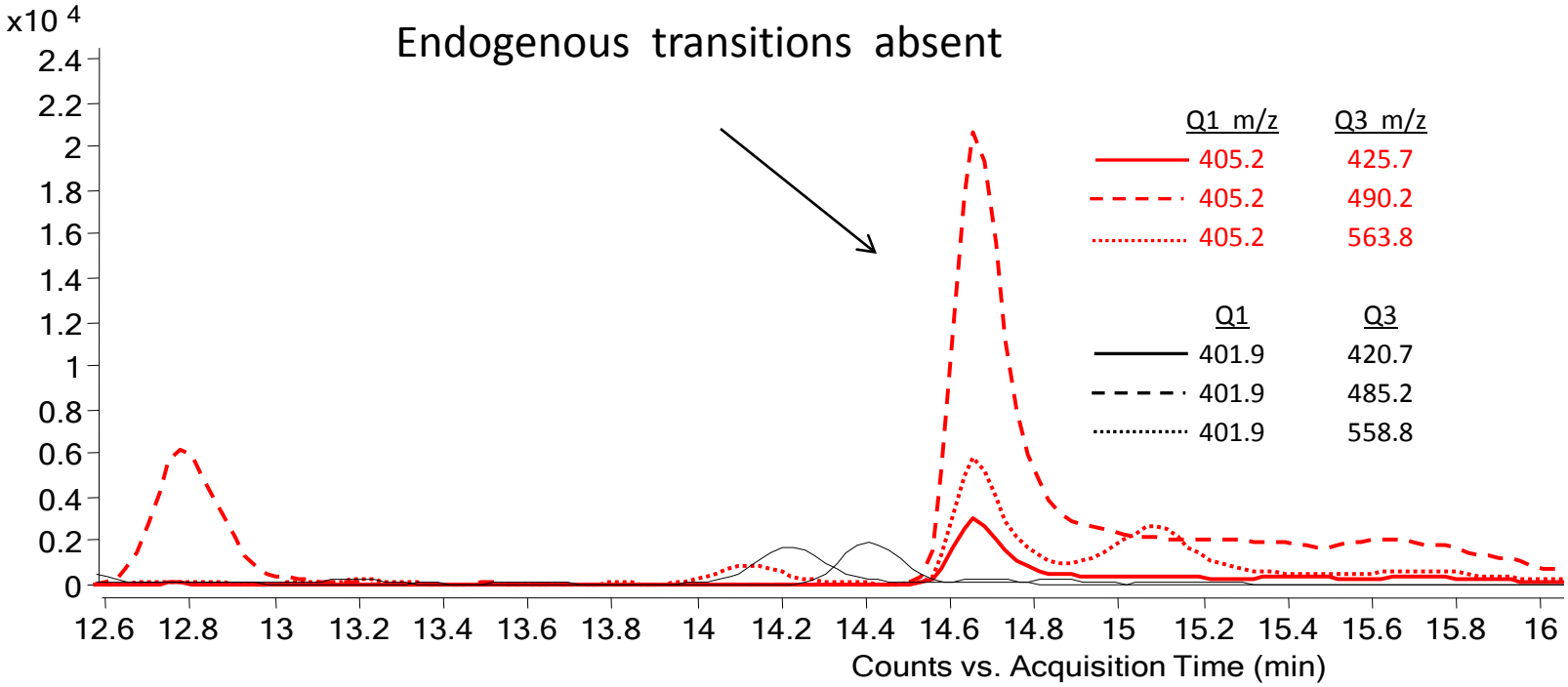
Fraction # 7
Fusion Break Point Junction
TDQGLLK



Fraction # 9
kRAS Peptide
SYGIPFIETSAK



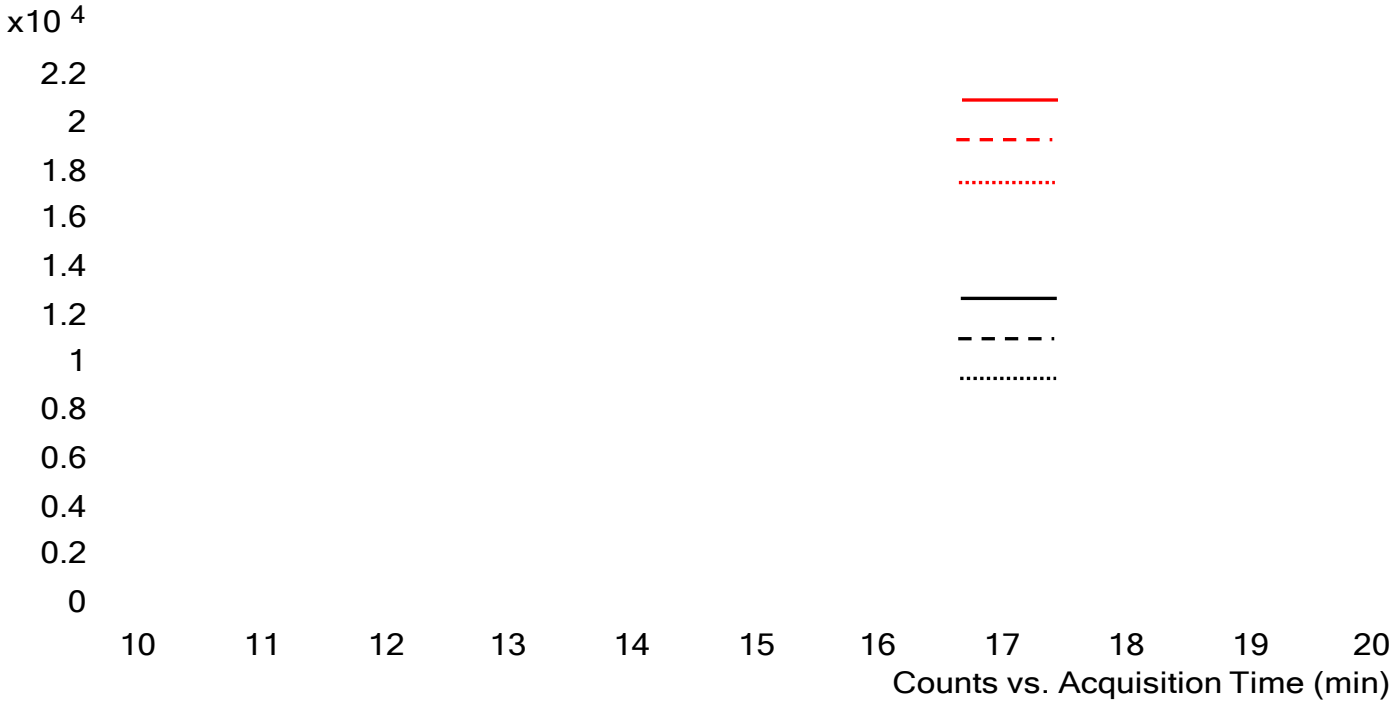
Fraction # 9
kRAS Peptide
SFEDIHHYR



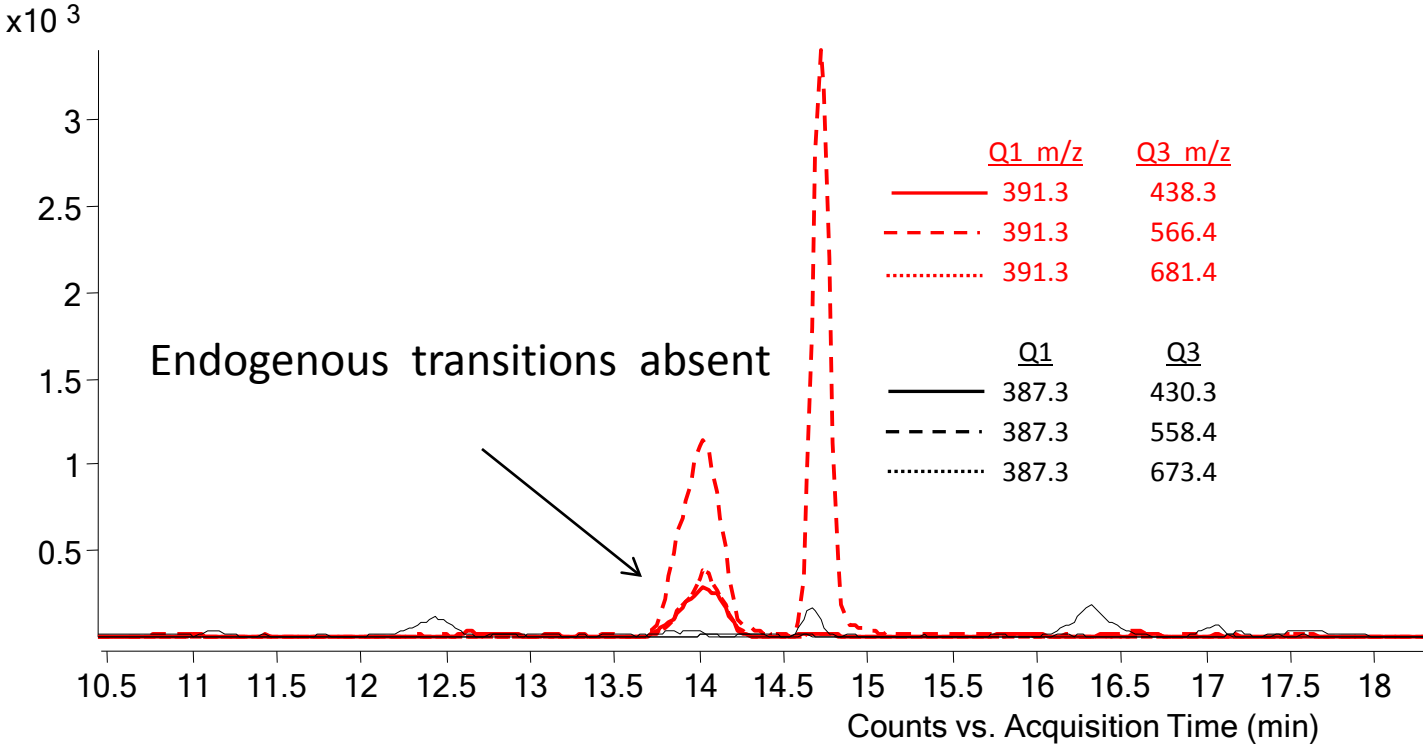
Fraction # 9
UBE Peptide
IYHPNIDEK

Q1 m/z	Q3 m/z	AUC	Ratio
568.9	512.3	51491	10.46 %
568.9	723.4	596737	21.19 %
568.9	860.4	89761	54.51 %

Q1	Q3	
564.9	504.3	5389
564.9	715.4	126454
564.9	852.4	48836



Fraction # 9
Fusion Break Point Junction
TDQGLLK



Summary of Attached Slides

- The relative ratio of each transition is in agreement btw light (black) and heavy (red).
- We did not set out to strictly quantitate either the canonical and chimeric proteins, as we did not complete the rigors of generating standard curves, etc. However, we utilized stably isotopically labeled internal standards to ensure transitions overlapped in chromatographic space reproducibly and that each of the transitions were in relative quantitative agreement.
- In combination with 1D-SDS PAGE, using the three transitions gave us a great degree of confidence that we were indeed measuring what we believe to be the fusion protein and the endogenous species.
- Only chromatogram figures for DU145 + 500 nM of Velcade are shown above however; we completed 0, 10, 100, 500, 1000, and 2000 nM dose-response curve. The findings are the same (positive finding for the chimeric protein in faction 4), although there was no apparent dose-response.