Supporting information for "A multiple reaction monitoring (MRM) method to detect Bcr-Abl kinase activity in CML using a peptide biosensor," Yang et al.

Figure S1. LC/MS characterization of Abl biosensor peptide. Used in assays and also spiked into K562 lysate for digestion and use as MRM calibration standard for EAIYAAPFAK fragment.

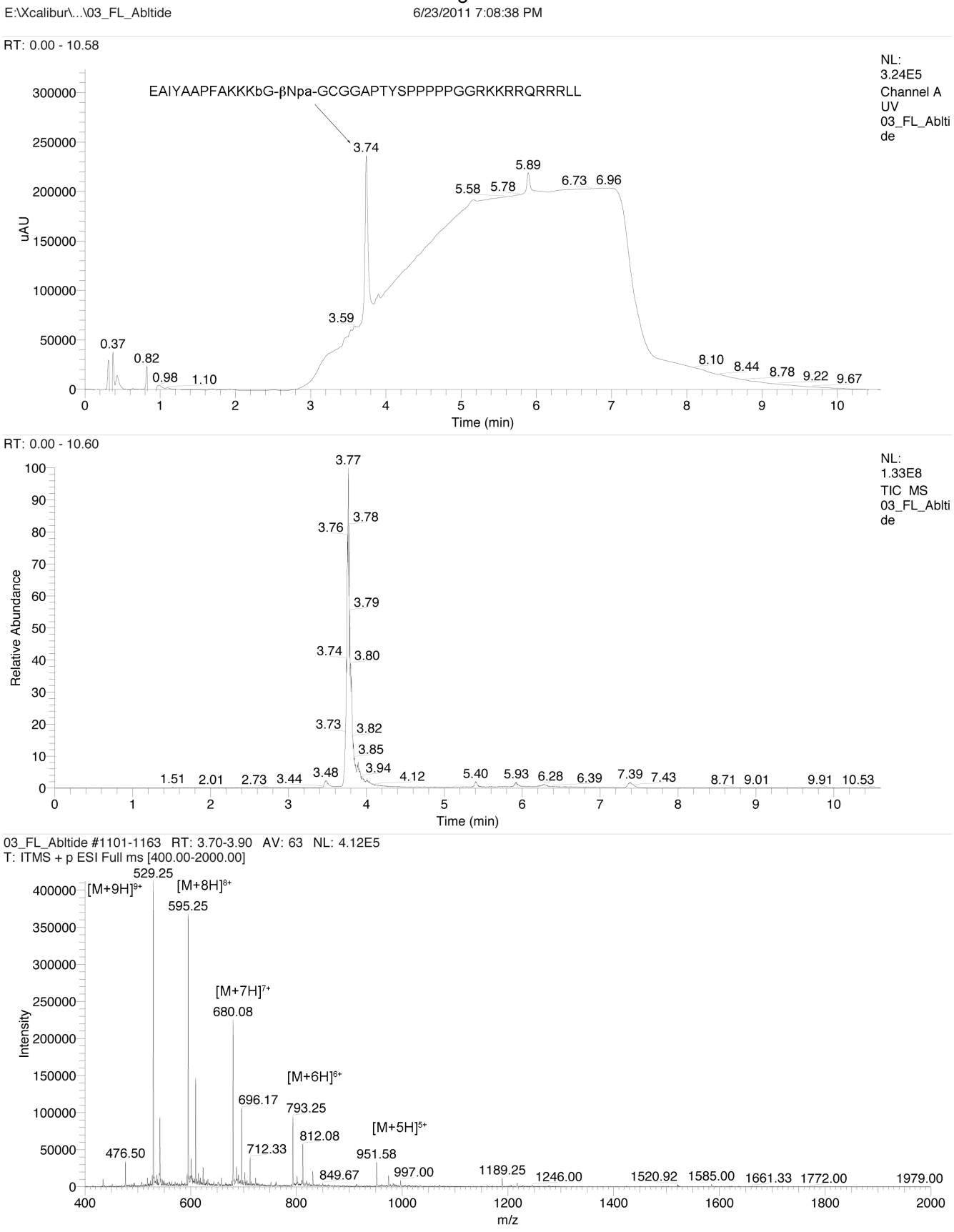
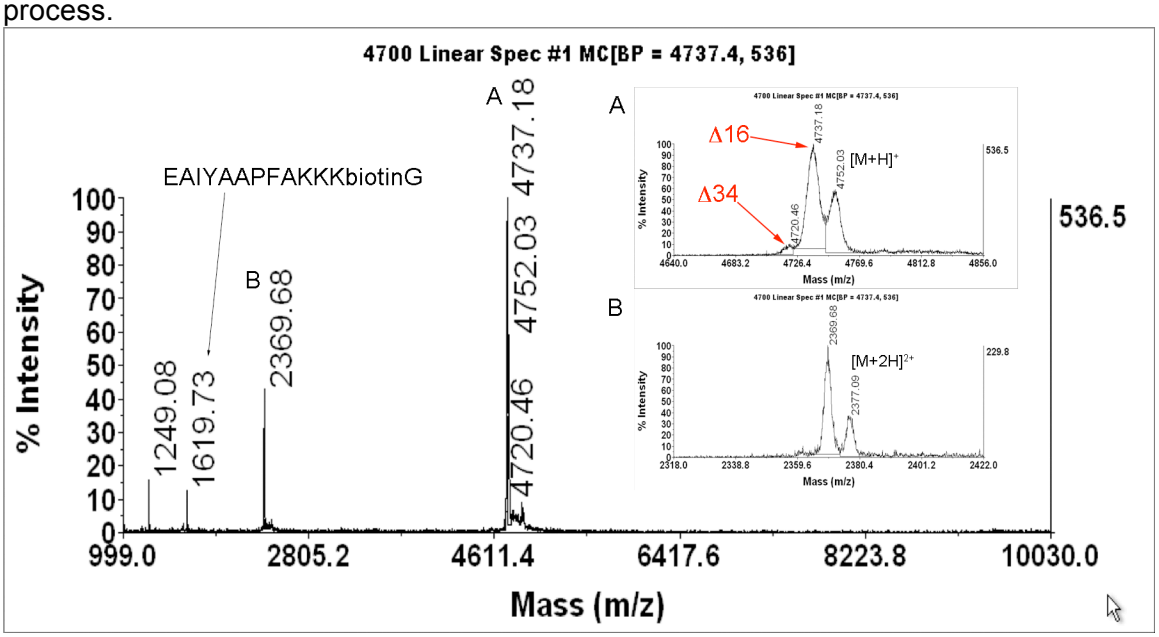


Figure S2. MALDI-TOF characterization of Abl biosensor; shows what appear to be laser-induced photolytic degradation peaks that may represent the intermediates proposed in Scheme S1.[1-2] While the resolution at this mass range makes the m/z differences appear to be 15-17 and 32-34, similar peaks in spectra from other fragments (including Fig. S5 and Fig. S6) show that these losses are more likely to be 16 and 34, and these values are consistent with a plausible proposed mechanism for their formation (Scheme 1). Photolysis peaks are not observed in the LC/ESI-MS spectrum given in S1, indicating that this is a laser desorption mediated process.





1. Gaplovsky, M.; Il'ichev, Y. V.; Kamdzhilov, Y.; Kombarova, S. V.; Mac, M.; Schworer, M. A.; Wirz, J., Photochemical reaction mechanisms of 2-nitrobenzyl compounds: 2-Nitrobenzyl alcohols form 2-nitroso hydrates by dual proton transfer. *Photoch Photobio Sci* **2005,** *4* (1), 33-42.
2. Il'ichev, Y. V.; Schworer, M. A.; Wirz, J., Photochemical reaction mechanisms of 2-nitrobenzyl compounds: Methyl ethers and caged ATP. *Journal Of The American Chemical Society* **2004,** *126* (14), 4581-4595.


Scheme S1. Proposed potential photodegradation intermediates (as observed in MALDI-TOF spectra). The observed ions exhibit m/z values of 16 amu (major) and 34 amu (minor) less than the expected m/z. The major species is likely formed via gas phase ion chemistry involving protonation of one of the oxygens in the nitro group by initial photo-tautomerization (which is well-characterized in the literature, see 1. and 2. above), additional protonation by acid and elimination as water. For the minor product, the second loss of water (18 amu) to give a total net loss of 34 amu overall could occur—however, the mechanism for this is not clear and while it is very interesting, its absolute determination would be outside the scope of this work. However, this phenomenon suggests that MALDI-MS might be a useful way to study gas phase photochemistry, and this could be the subject of future investigation.

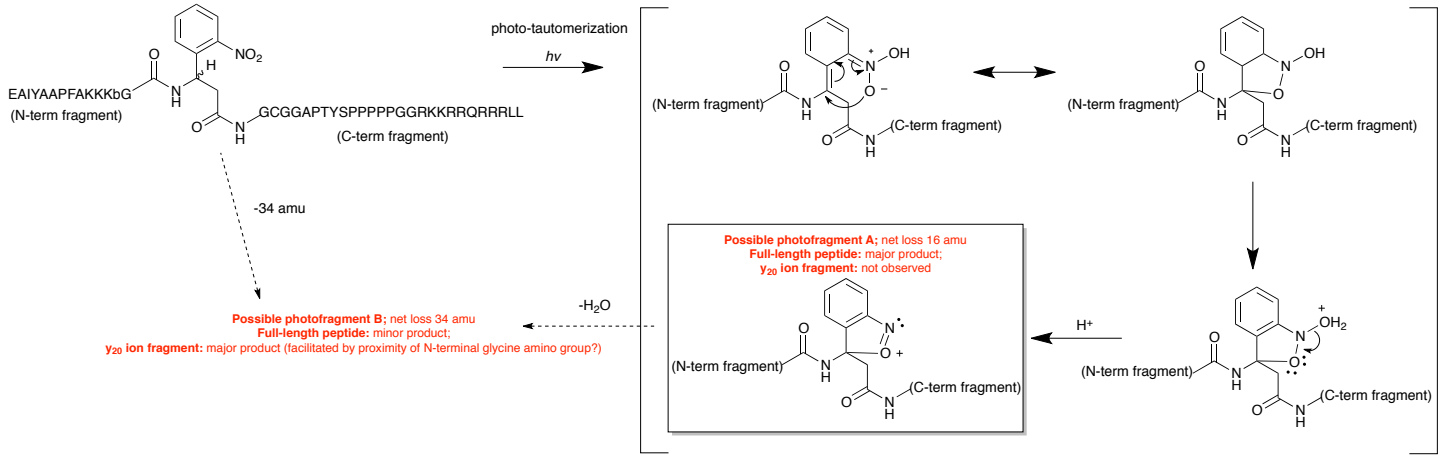


Figure S3. Synthetic phosphorylated Abl biosensor reporter module. Added to K562 lysate followed by digestion and use as MRM calibration standard.

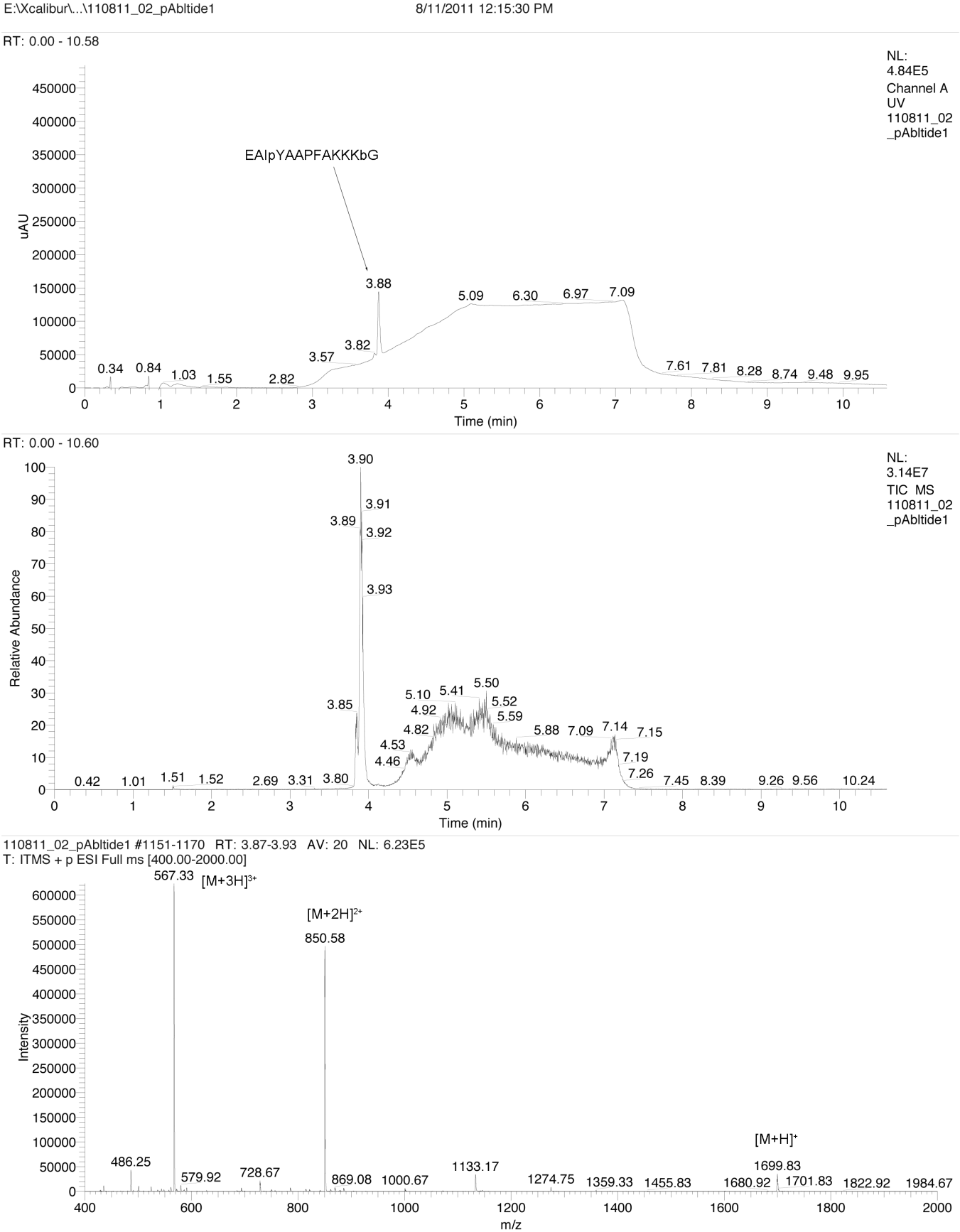
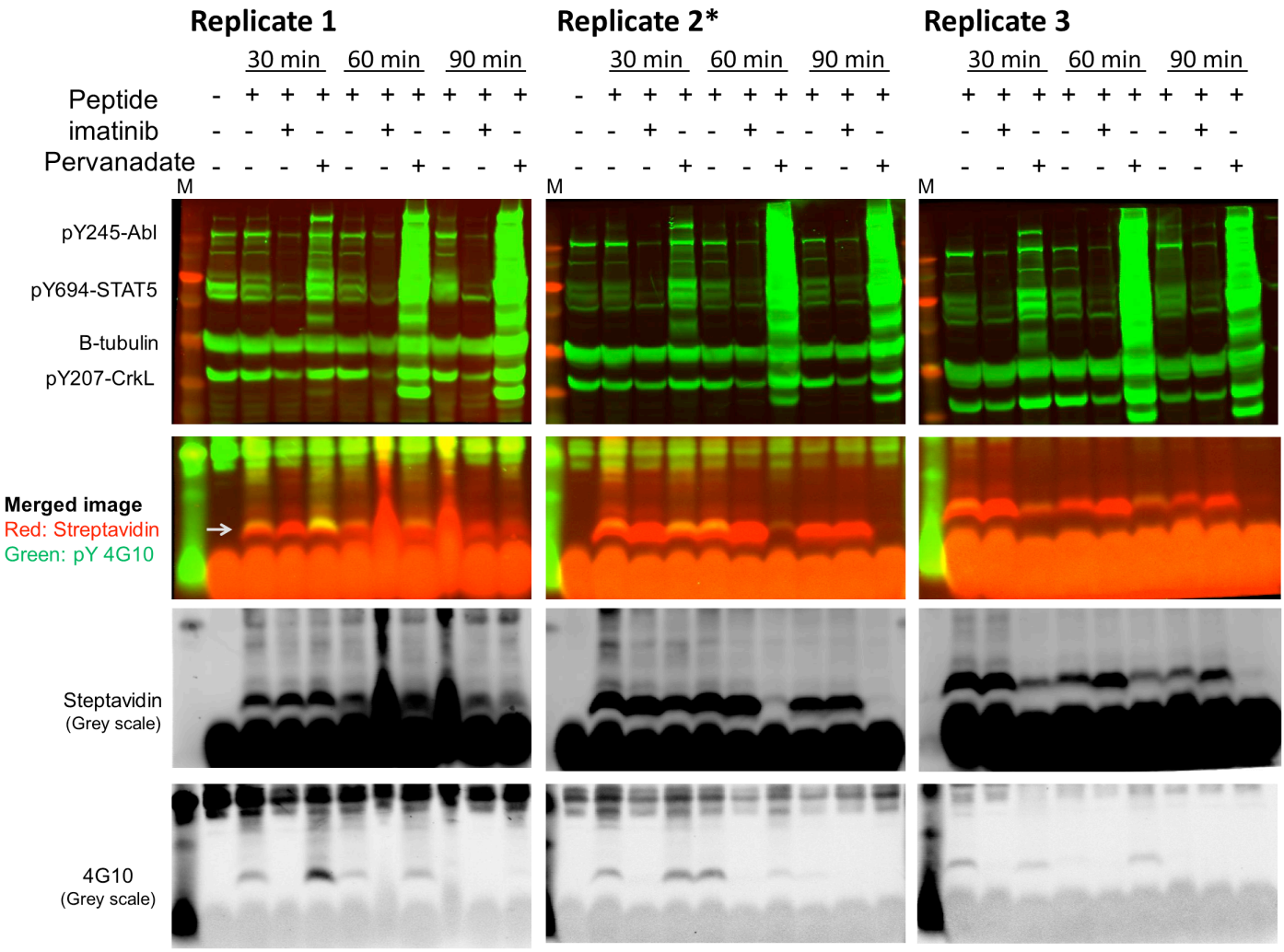


Figure S4. Additional Western blots from all replicates for data shown in Figure 2 in the main manuscript.




*A grey scale version of replicate 2 was shown in fig 2A.

Figure S5. Expanded view of MALDI-TOF spectrum for degradation products.

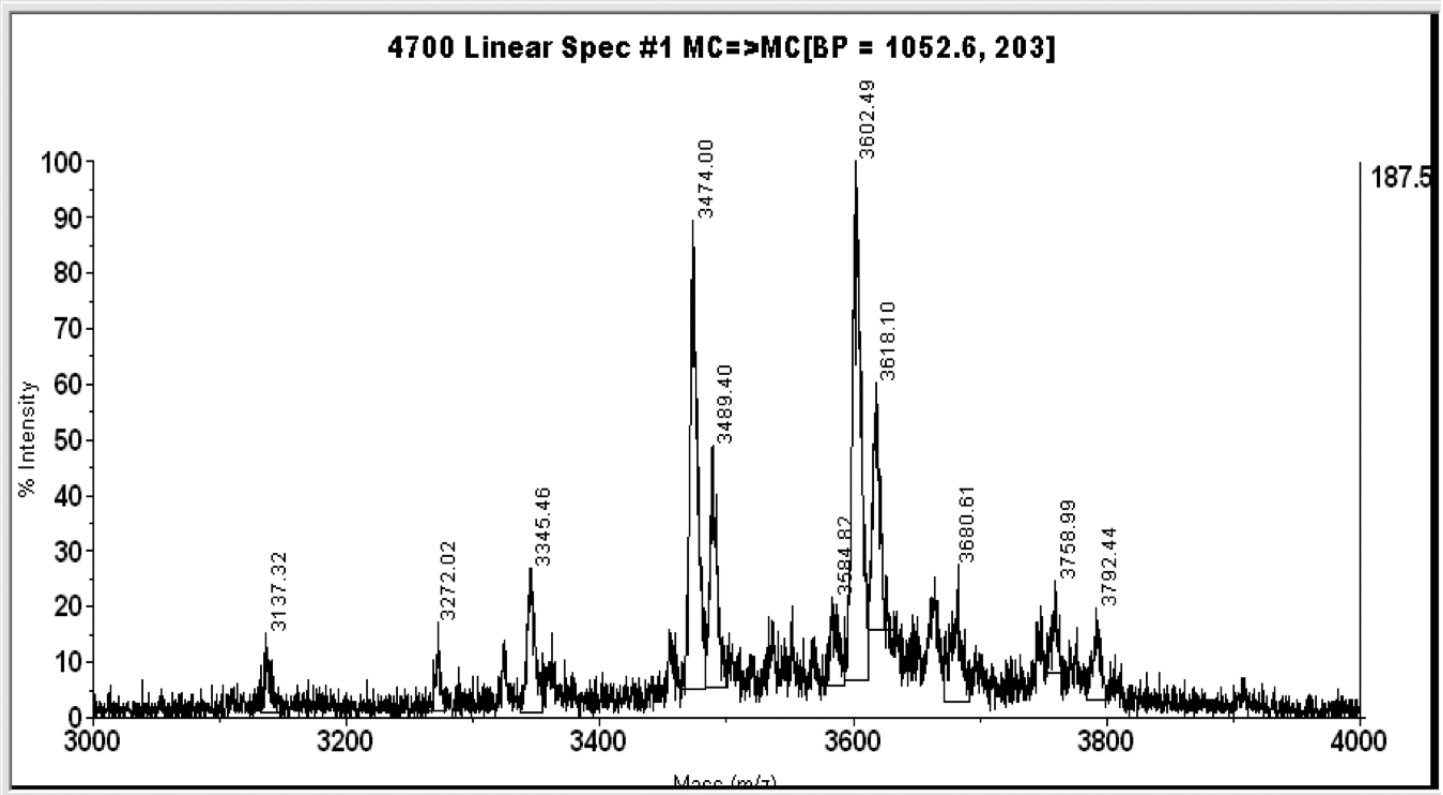
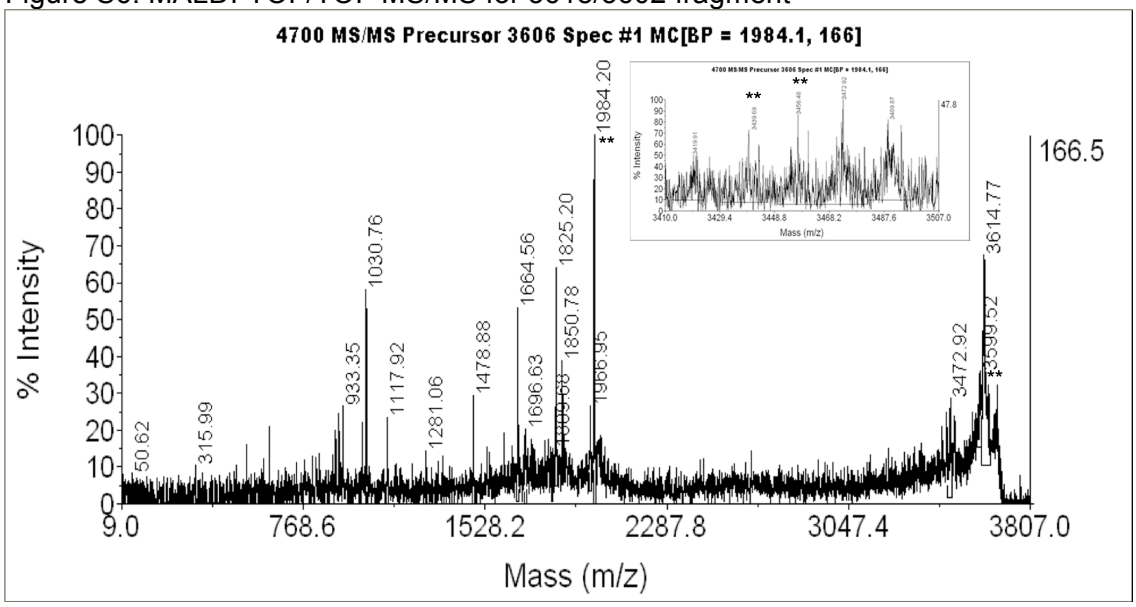


Figure S6. MALDI-TOF/TOF MS/MS for 3618/3602 fragment




| y ion | sequence | m/z | b ion | sequence | m/z |
|---|---|---|---|---|---|
| 32 | EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 3490.96111 | 1 | H2N-E | 130.12317 |
| 31 | AIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 3419.88251 | 2 | H2N-EA | 201.20177 |
| 30 | IYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 3306.72351 | 3 | H2N-EAI | 314.36077 |
| 29 | YAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 3143.54791 | 4 | H2N-EAIY | 477.53637 |
| 28 | AAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 3072.46931 | 5 | H2N-EAIYA | 548.61497 |
| 27 | APFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 3001.39071 | 6 | H2N-EAIYAA | 619.69357 |
| 26 | PFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 2904.27431 | 7 | H2N-EAIYAAP | 716.80997 |
| 25 | FAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 2757.09811 | 8 | H2N-EAIYAAPF | 863.98617 |
| 24 | AKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 2686.01951 | 9 | H2N-EAIYAAPFA | 935.06477 |
| 23 | KKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 2557.84591 | 10 | H2N-EAIYAAPFAK | 1063.23837 |
| 22 | KK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 2429.67231 | 11 | H2N-EAIYAAPFAKK | 1191.41197 |
| 21 | K$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRKK-OH | 2075.20231 | 12 | H2N-EAIYAAPFAKKK$_{biotin}$ | 1545.88197 |
| 20 | GβNpaGCGGAPTYSPPPPPGGRKK-OH** (observed m/z: 1984.20) | 2018.15051 | 13 | H2N-EAIYAAPFAKKK$_{biotin}$G | 1602.93377 |
| 19 | bNpaGCGGAPTYSPPPPPGGRKK-OH | 1825.09051 | 14 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpa | 1795.99377 |
| 18 | GCGGAPTYSPPPPPGGRKK-OH | 1768.03871 | 15 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaG | 1853.04557 |
| 17 | CGGAPTYSPPPPPGGRKK-OH | 1664.90011 | 16 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGC | 1956.18417 |

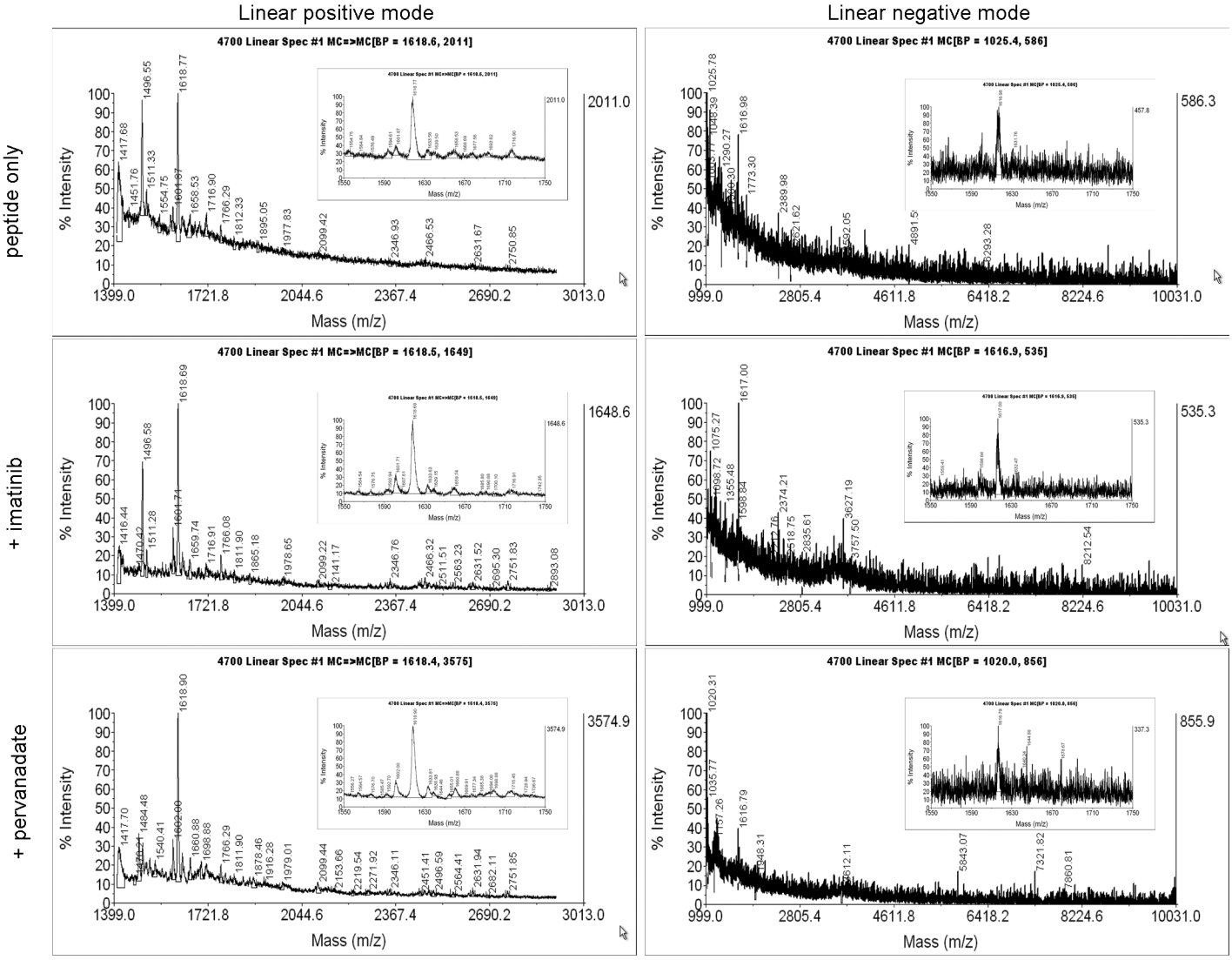
| | | | | | |
|---|---|---|---|---|---|
| 16 | GGAPTYSPPPPPGGRKK-OH | 1607.84831 | 17 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCG | 2013.23597 |
| 15 | GAPTYSPPPPPGGRKK-OH | 1550.79651 | 18 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGG | 2070.28777 |
| 14 | APTYSPPPPPGGRKK-OH | 1479.71791 | 19 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGA | 2141.36637 |
| 13 | PTYSPPPPPGGRKK-OH | 1382.60151 | 20 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAP | 2238.48277 |
| 12 | TYSPPPPPGGRKK-OH | 1281.49671 | 21 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPT | 2339.58757 |
| 11 | YSPPPPPGGRKK-OH | 1118.32111 | 22 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTY | 2502.76317 |
| 10 | SPPPPPGGRKK-OH | 1031.24311 | 23 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYS | 2589.84117 |
| 9 | PPPPPGGRKK-OH | 934.12671 | 24 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSP | 2686.95757 |
| 8 | PPPPGGRKK-OH | 837.01031 | 25 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPP | 2784.07397 |
| 7 | PPPGGRKK-OH | 739.89391 | 26 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPP | 2881.19037 |
| 6 | PPGGRKK-OH | 642.77751 | 27 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPP | 2978.30677 |
| 5 | PGGRKK-OH | 545.66111 | 28 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPP | 3075.42317 |
| 4 | GGRKK-OH | 488.60931 | 29 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPG | 3132.47497 |
| 3 | GRKK-OH | 431.55751 | 30 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGG | 3189.52677 |
| 2 | RKK-OH | 275.37051 | 31 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGR | 3345.71377 |
| 1 | KK-OH | 147.19691 | 32 | H2N-EAIYAAPFAKKK$_{biotin}$GβNpaGCGGAPTYSPPPPPGGRK | 3473.88737 |

Predicted fragmentation for 3618/3602 degradation product

*Observed/tentatively assigned

**Ion observed (m/z 1984.2) has m/z consistent with MS/MS fragment $y_{20}$ exhibiting loss of 34 amu, which may predominate due to some effect of the proximity of the N-terminal glycine in this particular fragment (which, when protonated, could act as a proton donor); other potential photodegradation peaks also marked as ** in spectrum. For further discussion, see Fig. S2 and Scheme S1.

Figure S7. MALDI-TOF to analyze enriched reporter segment.
Replicate 1:



Replicate 2:

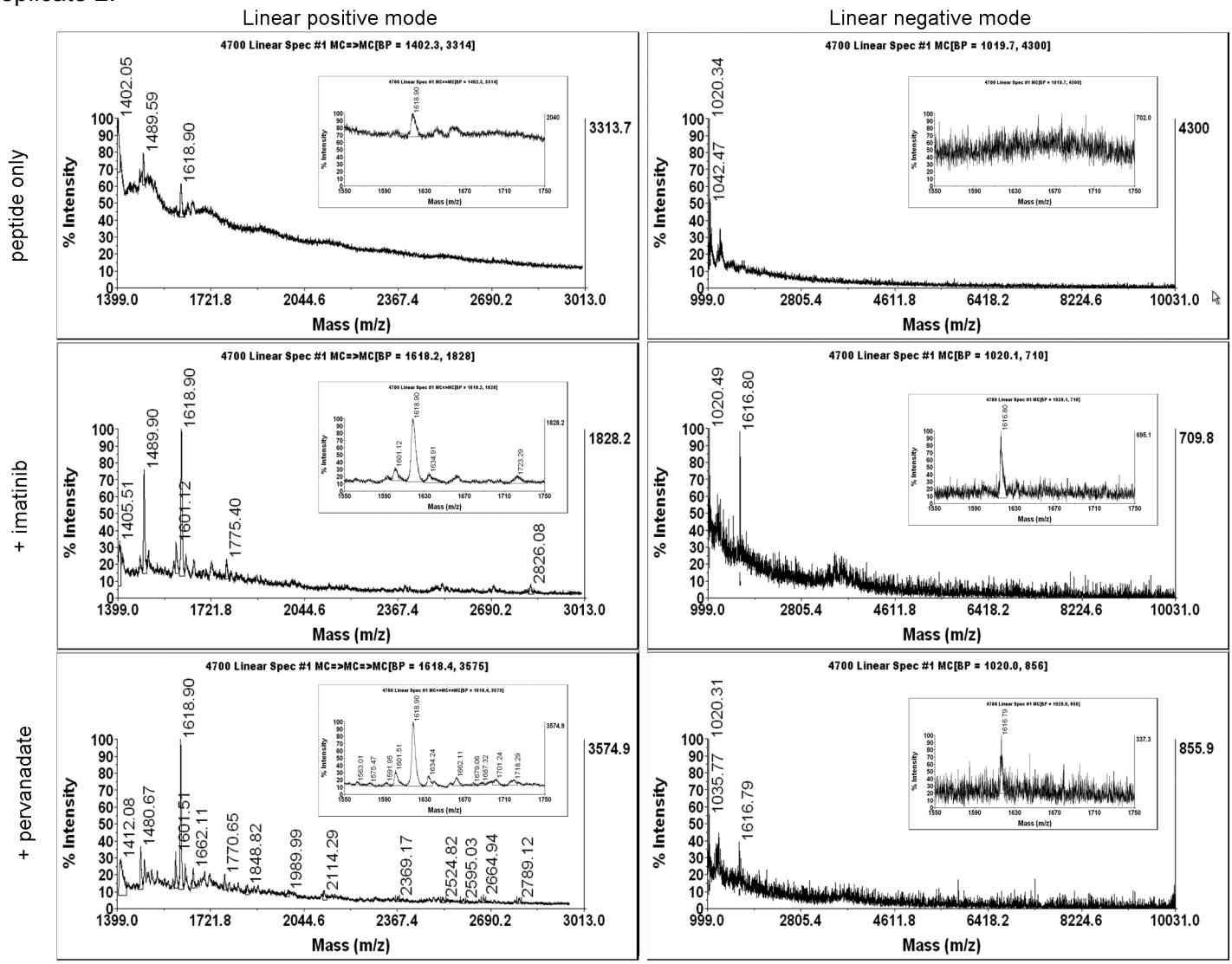



Table S8. Table of raw MRM data. See additional .csv file.

Table S9. Table of peak integrations and calibration. See attached Excel file.