

Supporting Information

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N/A – provided as separate Excel files

Figure S1

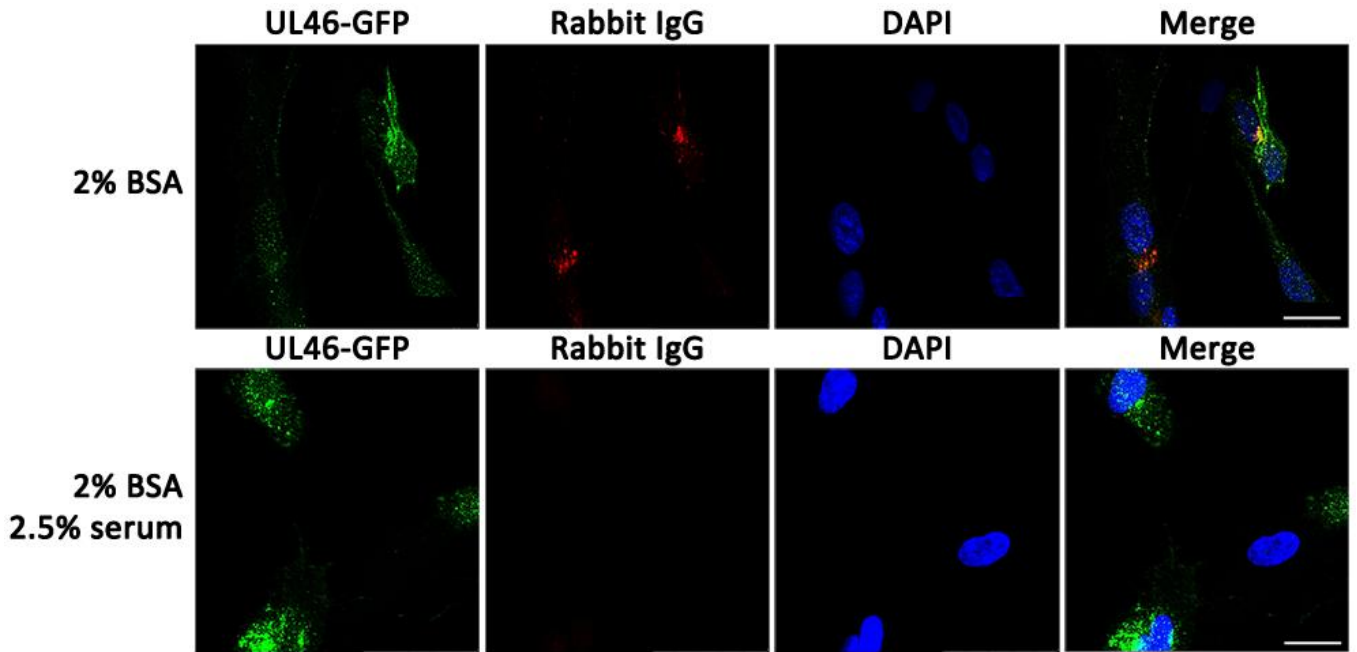


Figure S2

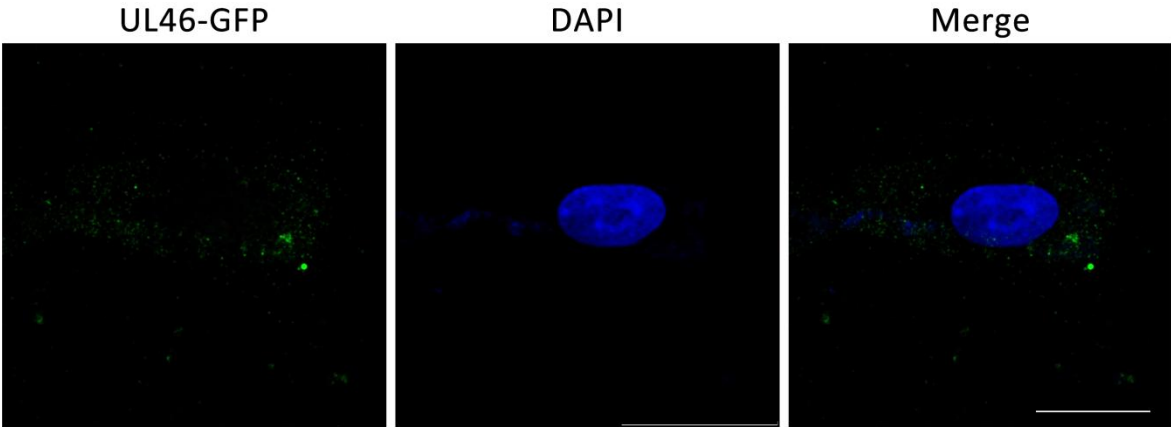


Figure S3

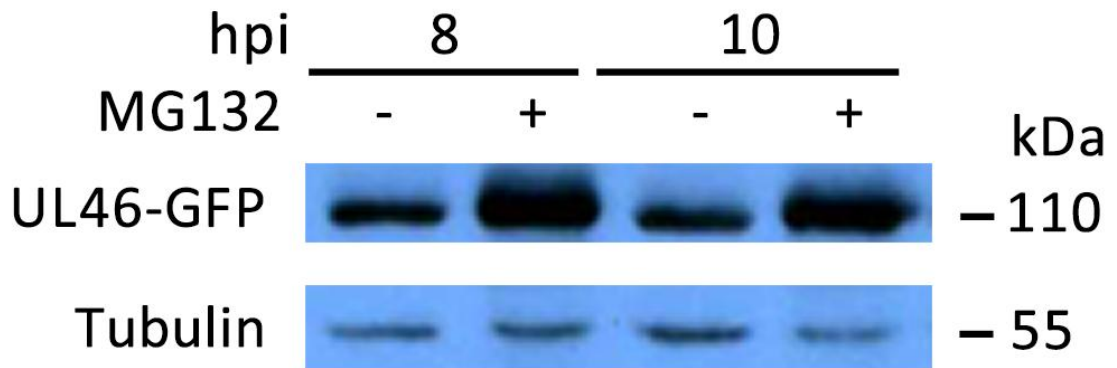
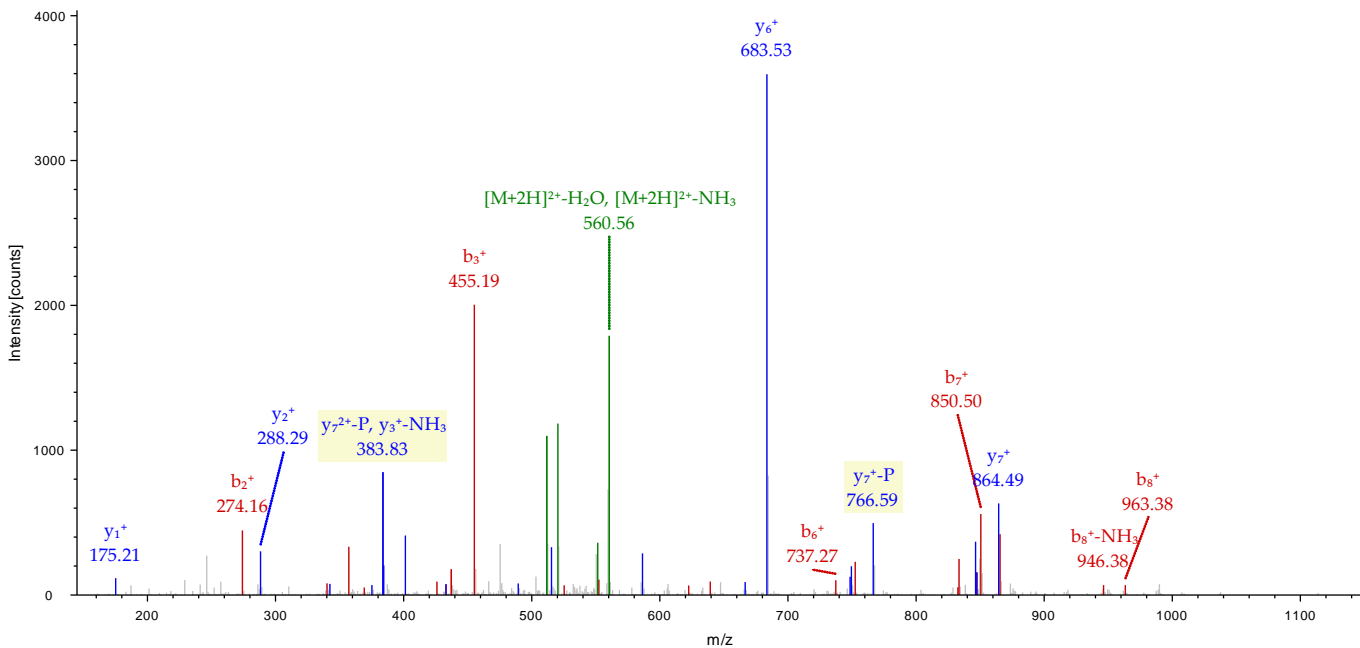
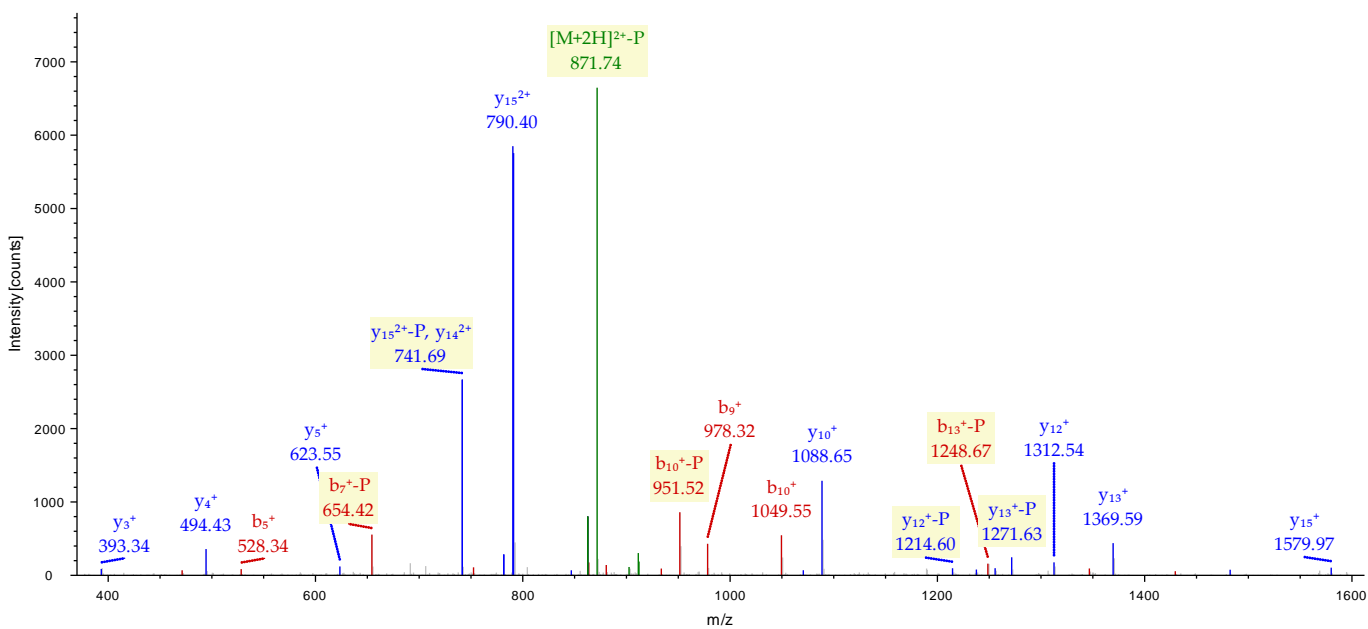


Figure S4

T18 CLtPANLIR

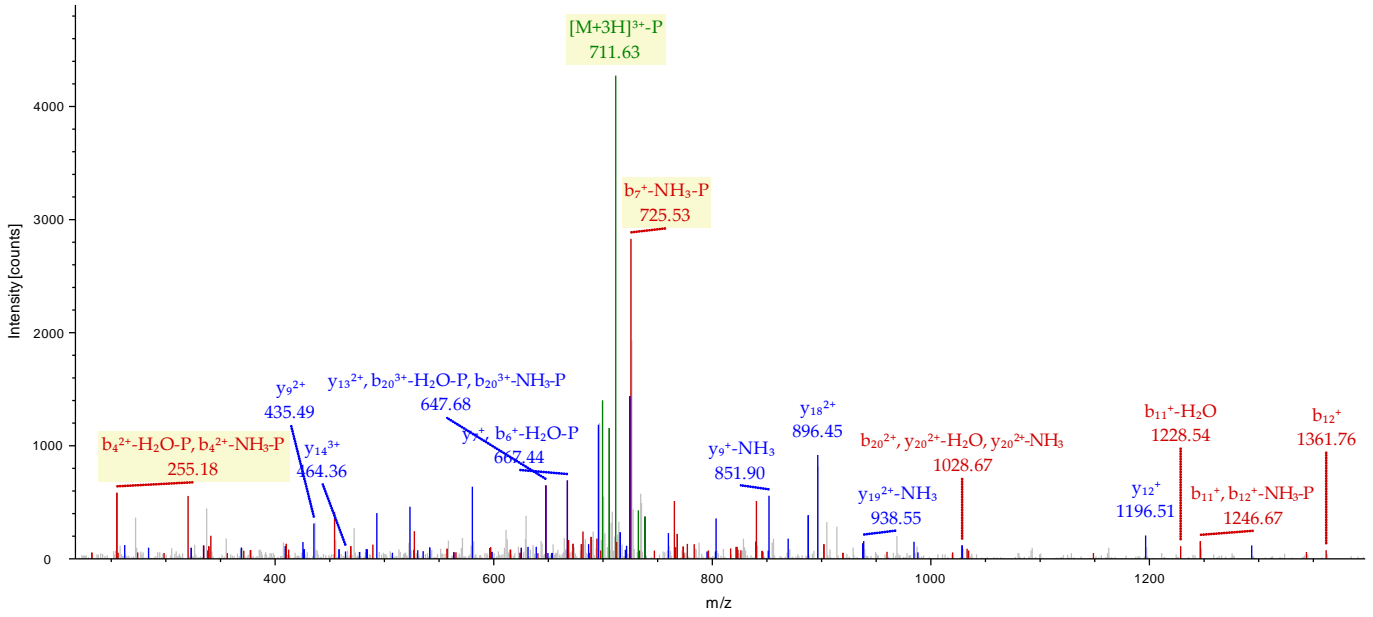


S263 FLPLGGsPEAPAETFAR



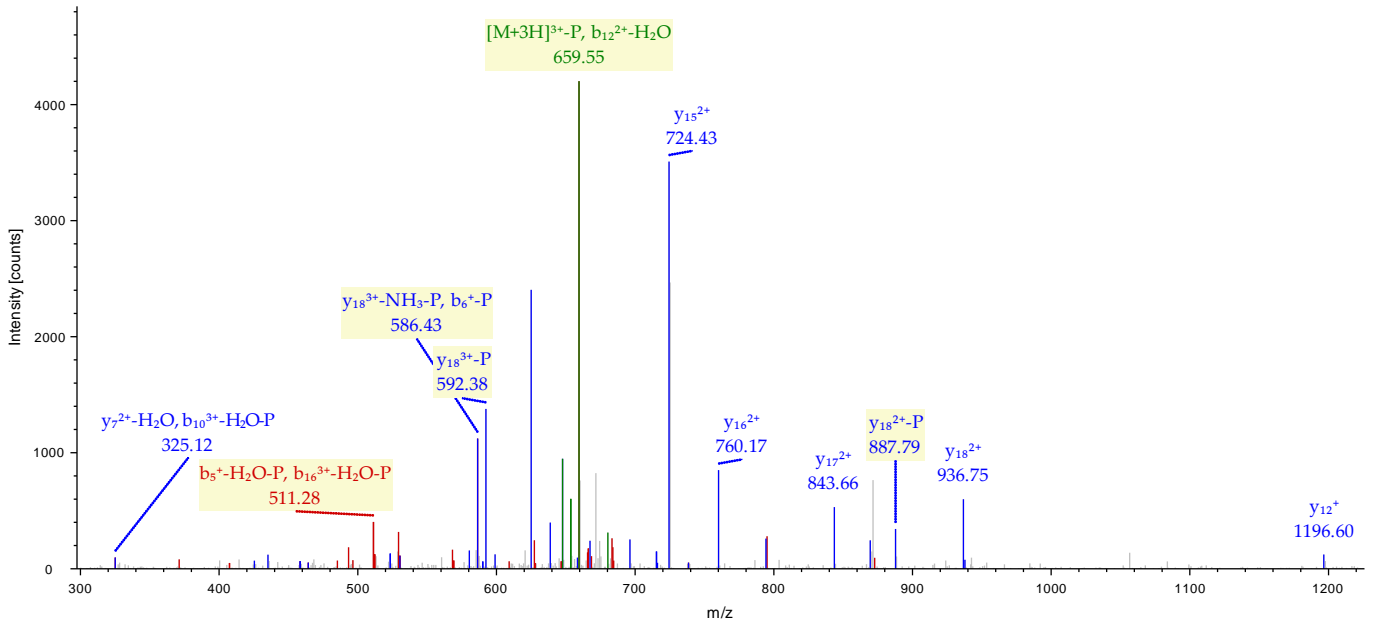
T431 (or T430)

RTtWSAGPPPDDMASGPGGHR



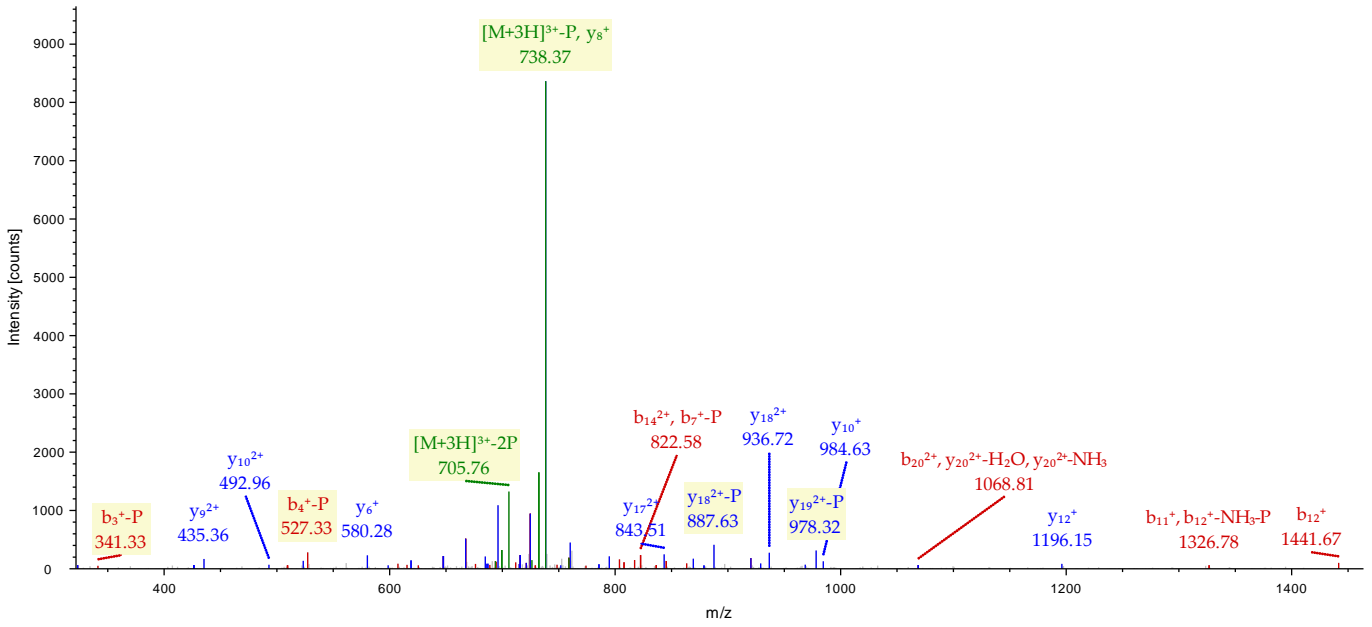
S433

TTW_sAGPPPDDMASGPGGHR



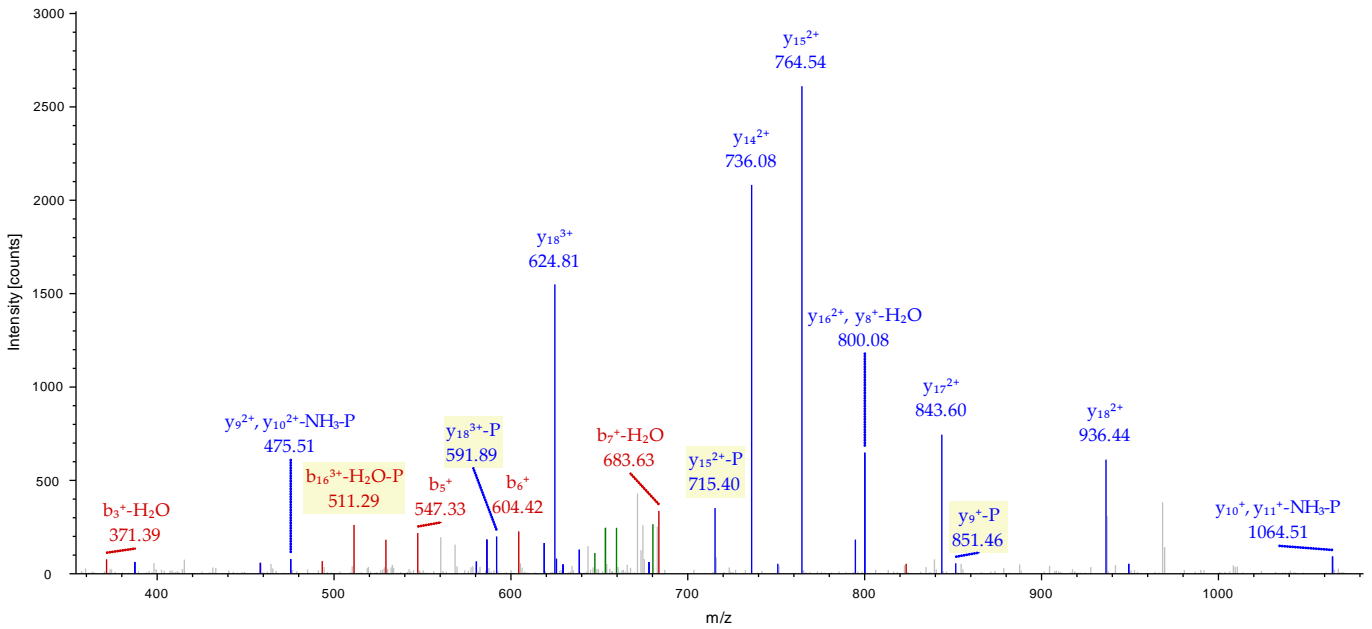
T431/S433

RTtW_sAGPPPDDMASGPGGHR



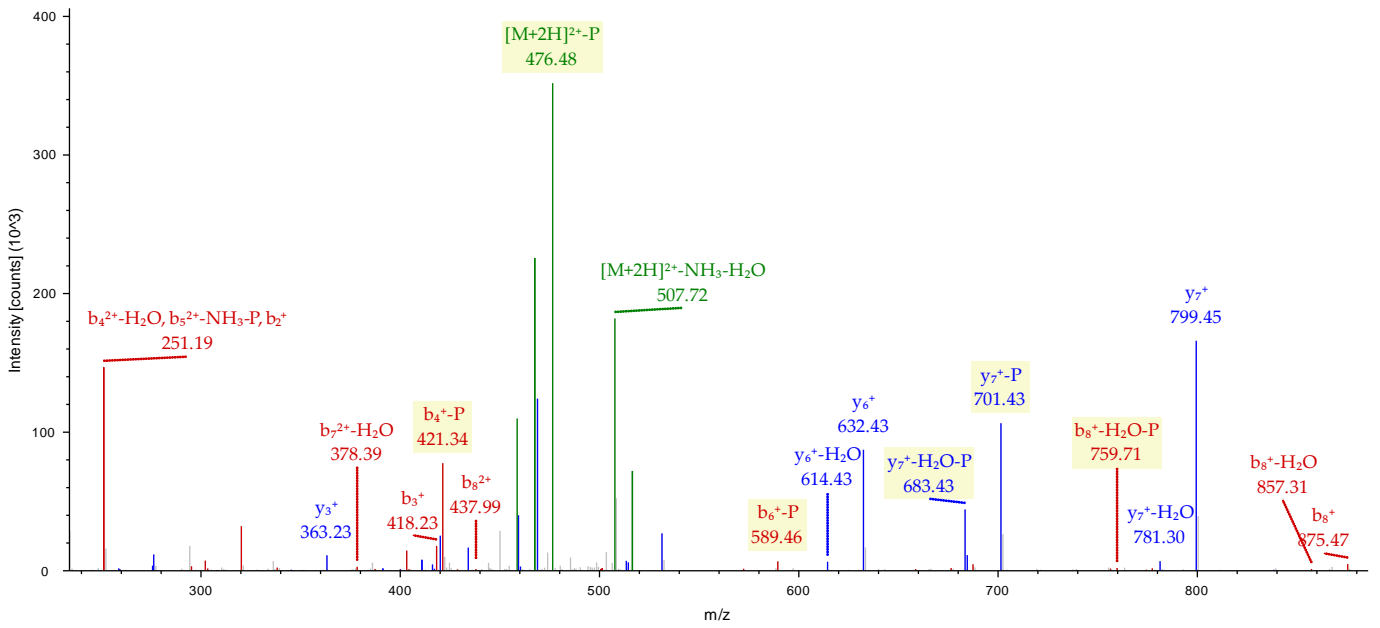
S443

TTWSAGPPPDDMAS_sGPGGHR



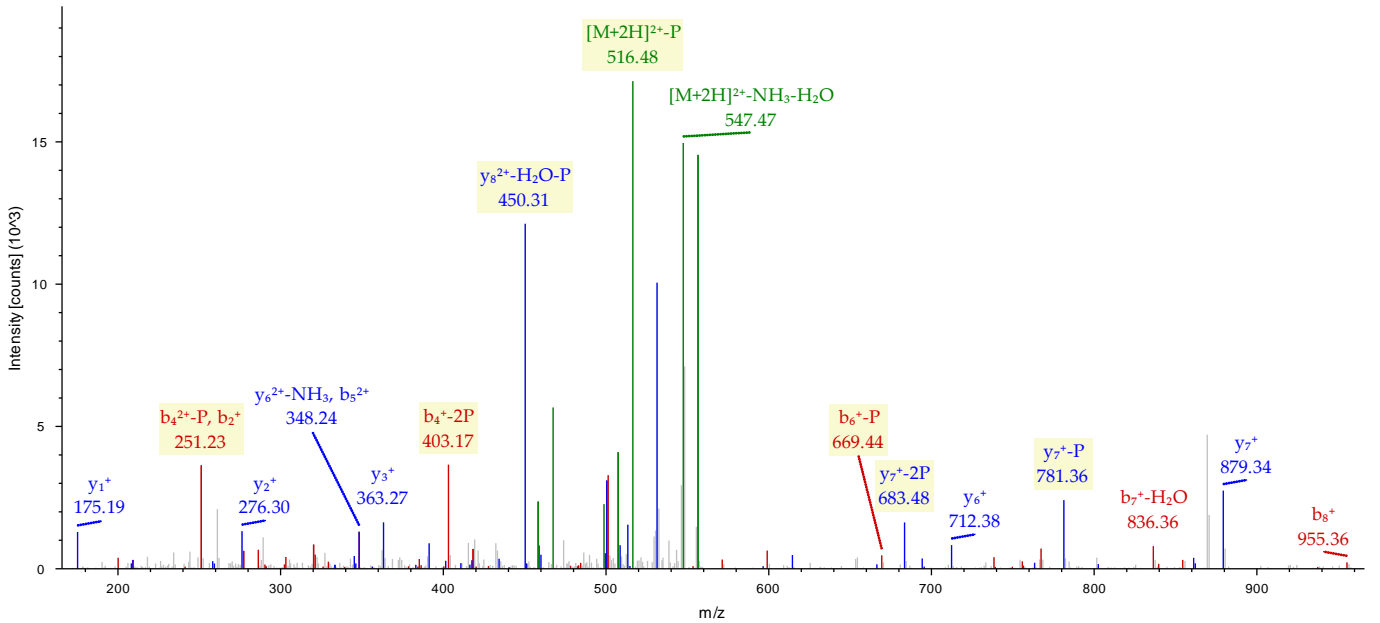
S478

LHsTPASTR



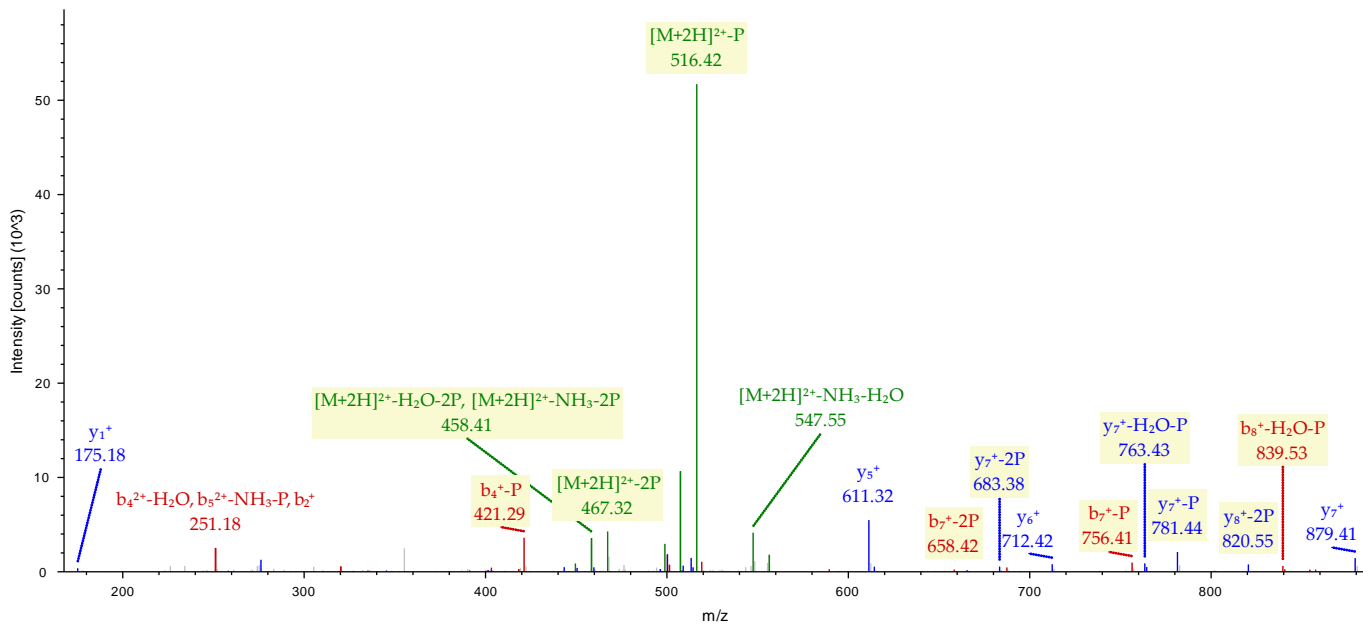
S478/T479

LHstPASTR



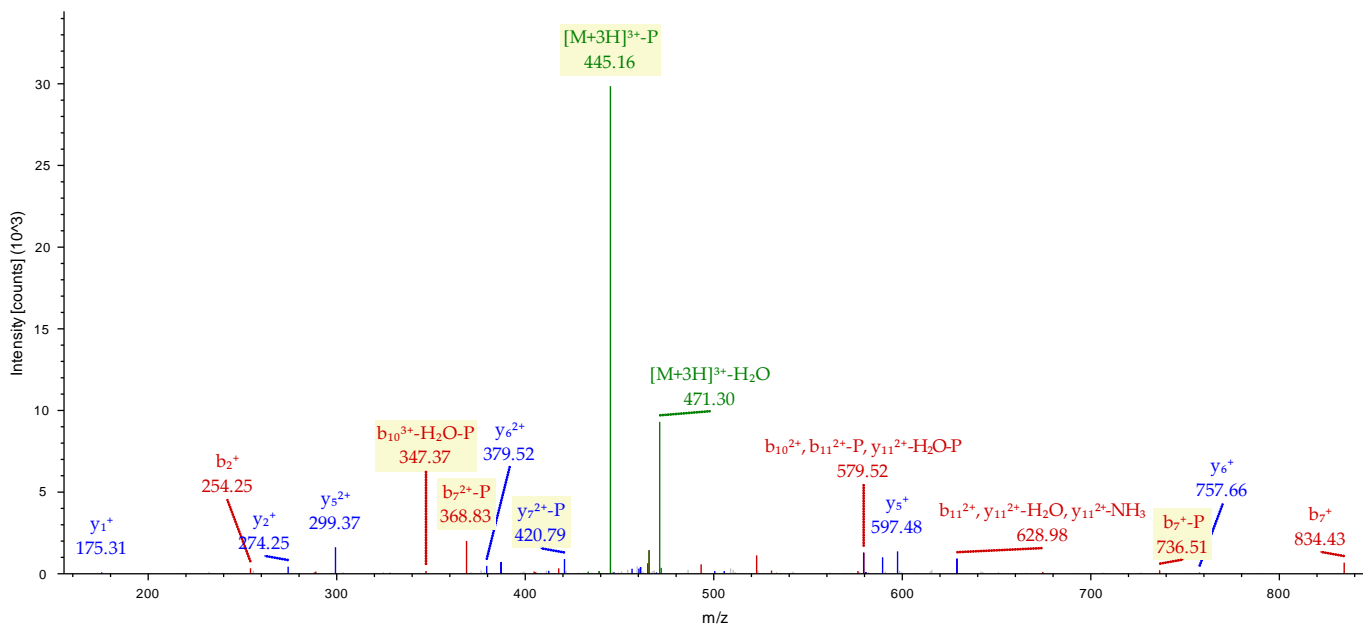
S478/S481

LHsTPAsTR

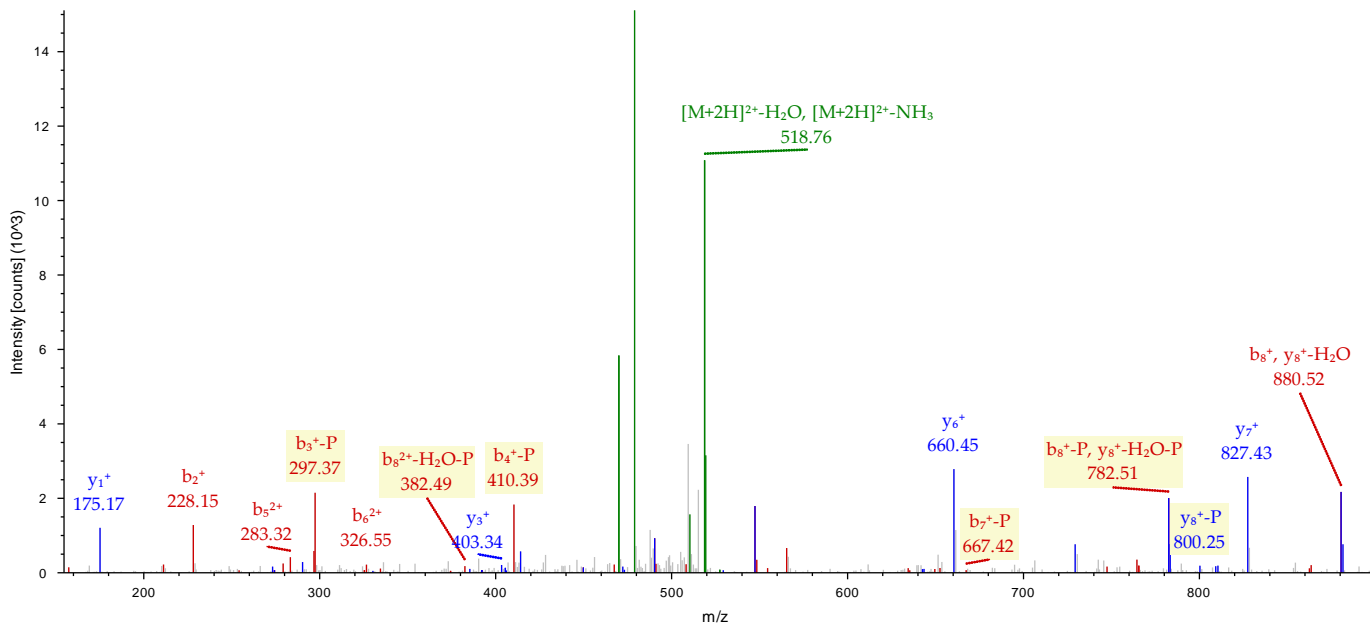


T545

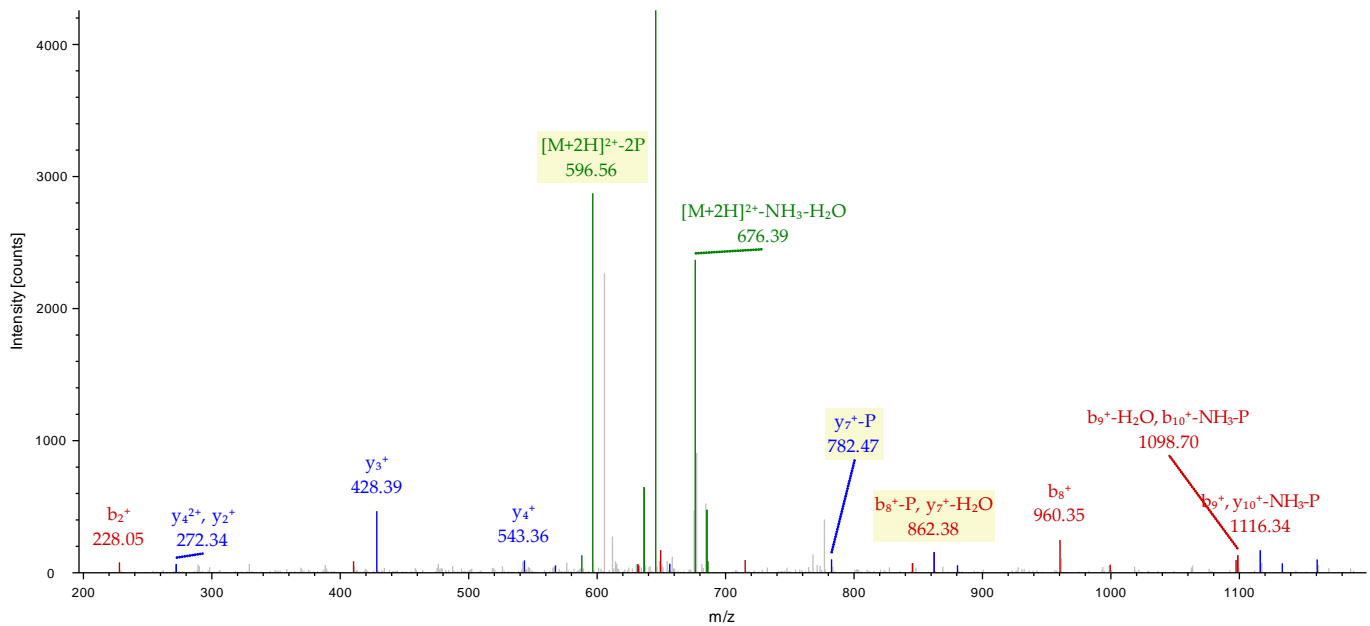
RPPAAAtCPLLVR



S554

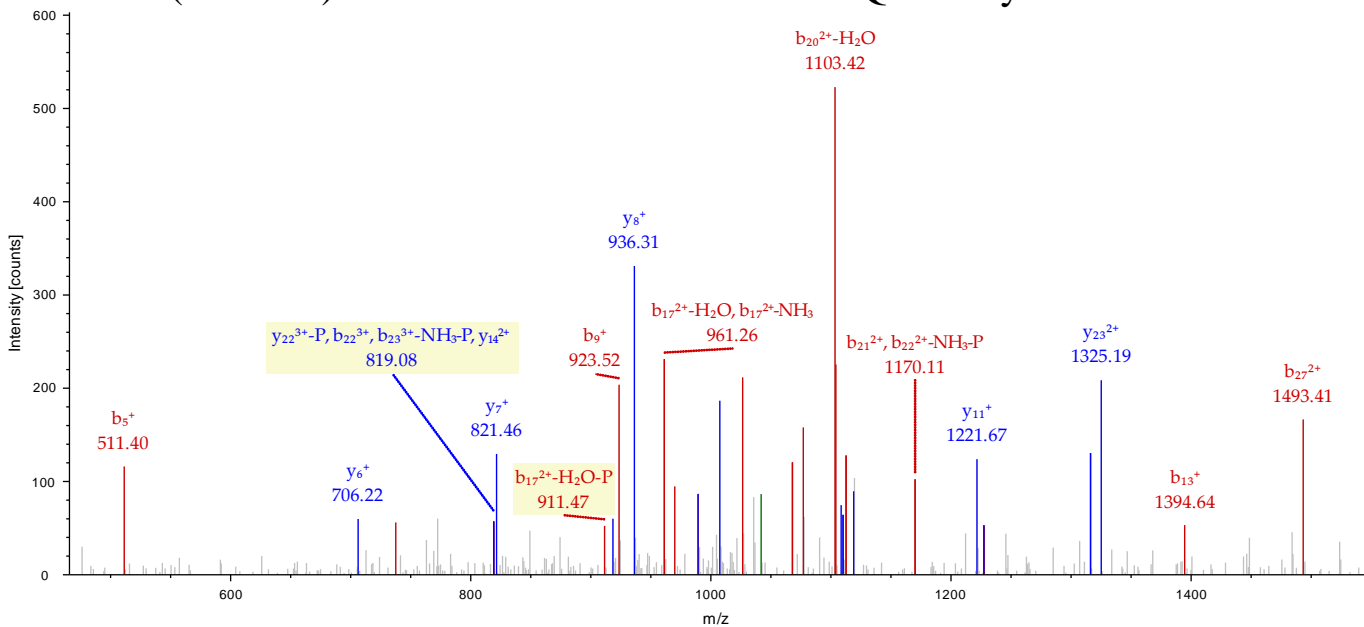
RA_sLGSLDRPR

S554/S557

RA_sLG_sLDRPR

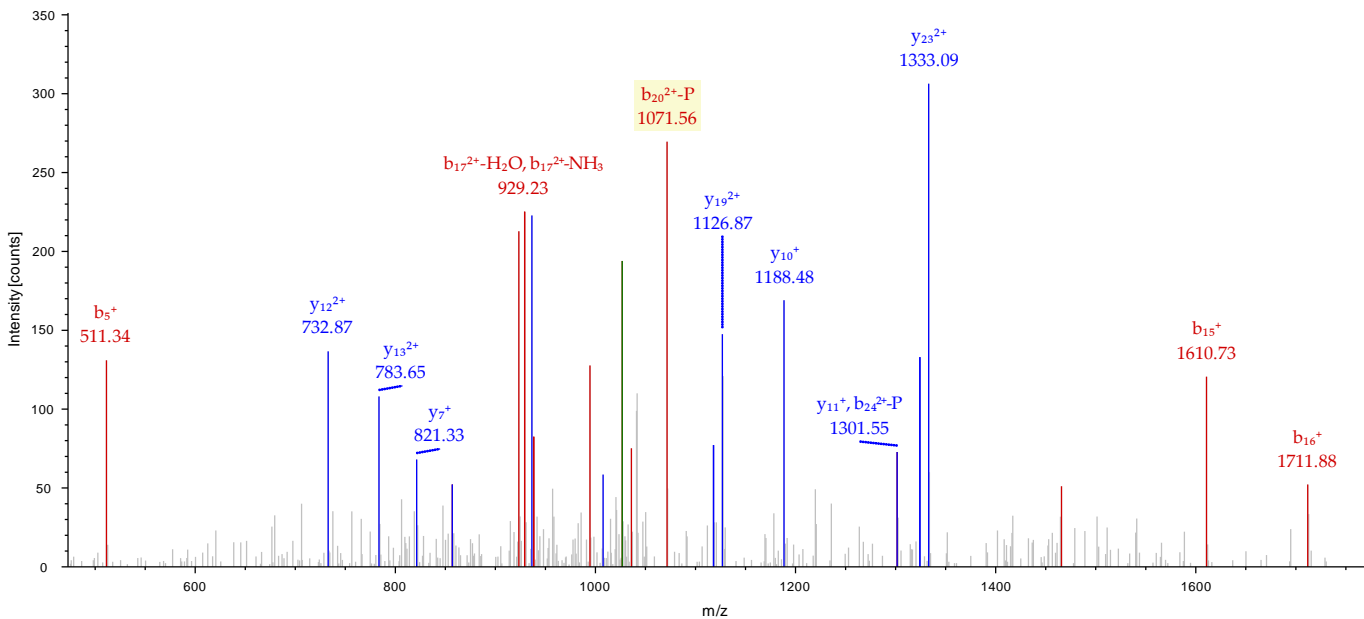
Y579 (or T578)

VWGPAPEGEPDQMEATyLTADDDDDDDAR

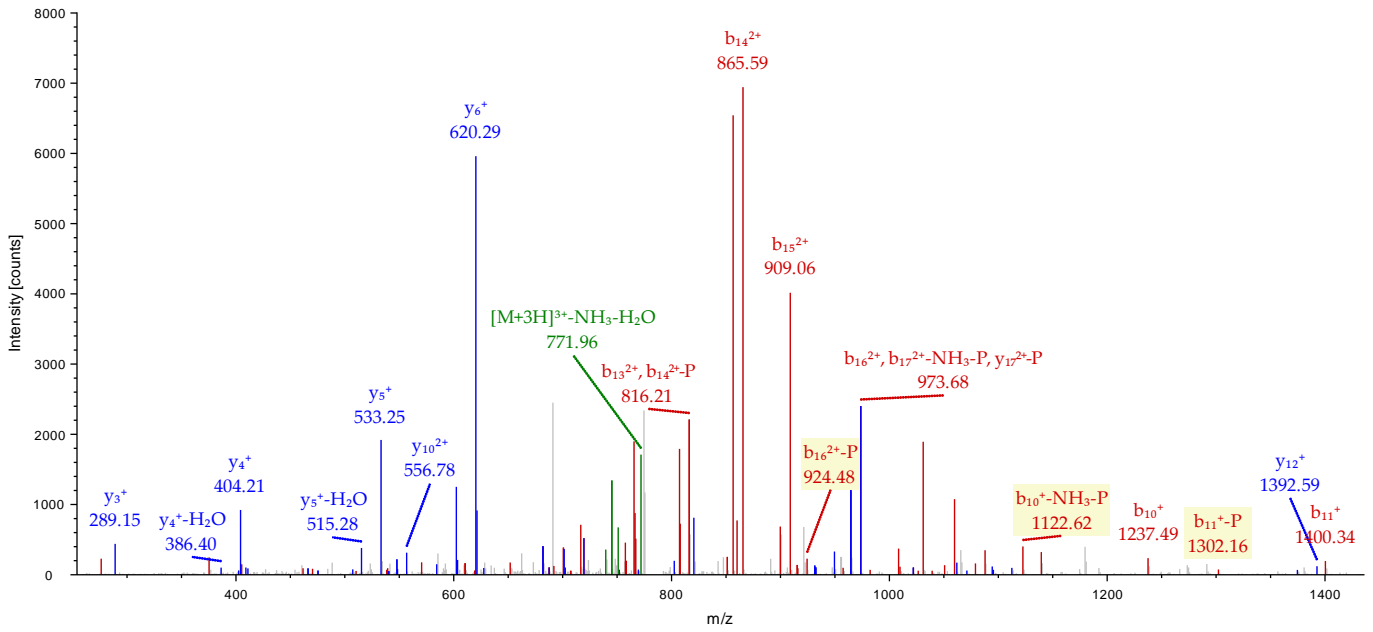


T581

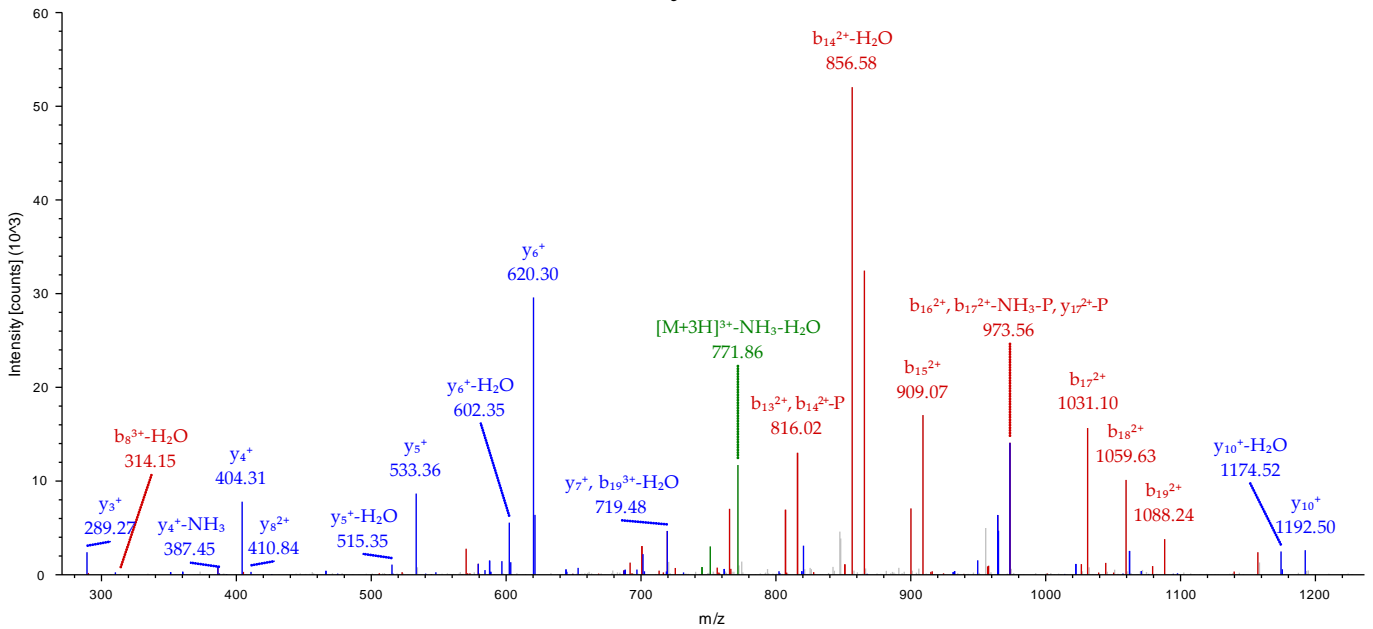
VWGPAPEGEPDQMEATyLtADDDDDDDAR



S611

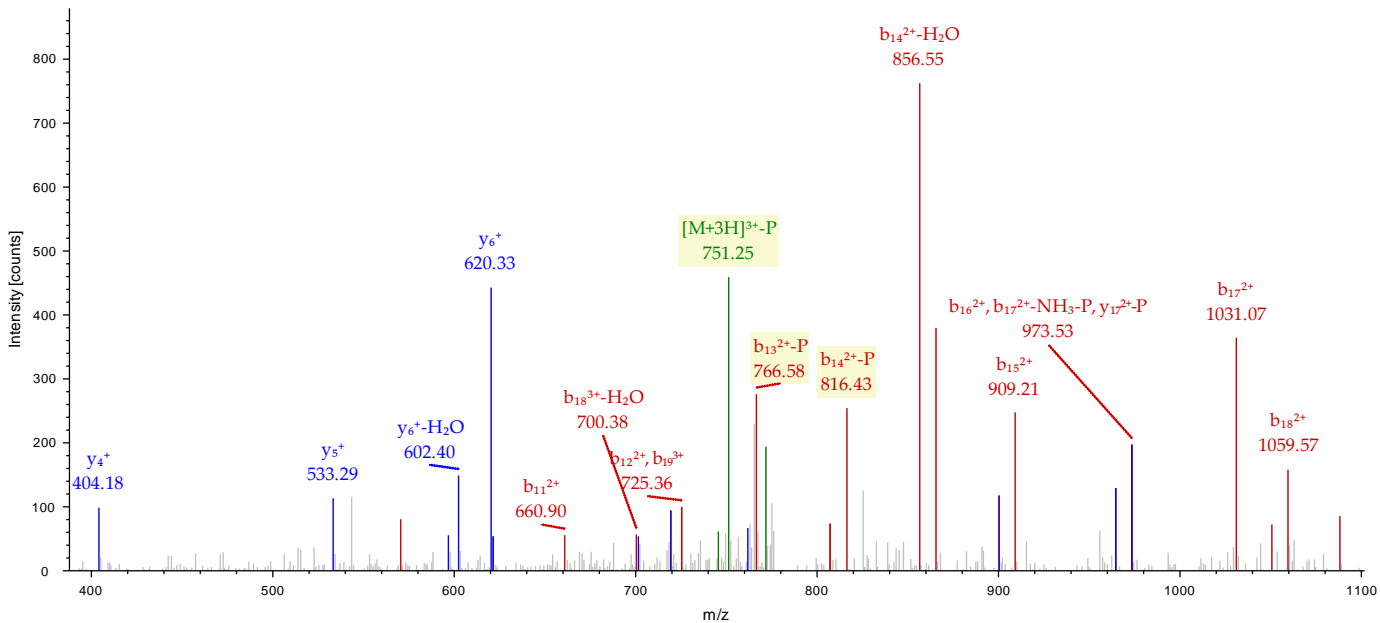
HAPYEDDES_iIYETVSEDGGR

Y613

HAPYEDDES_iyETVSEDGGR

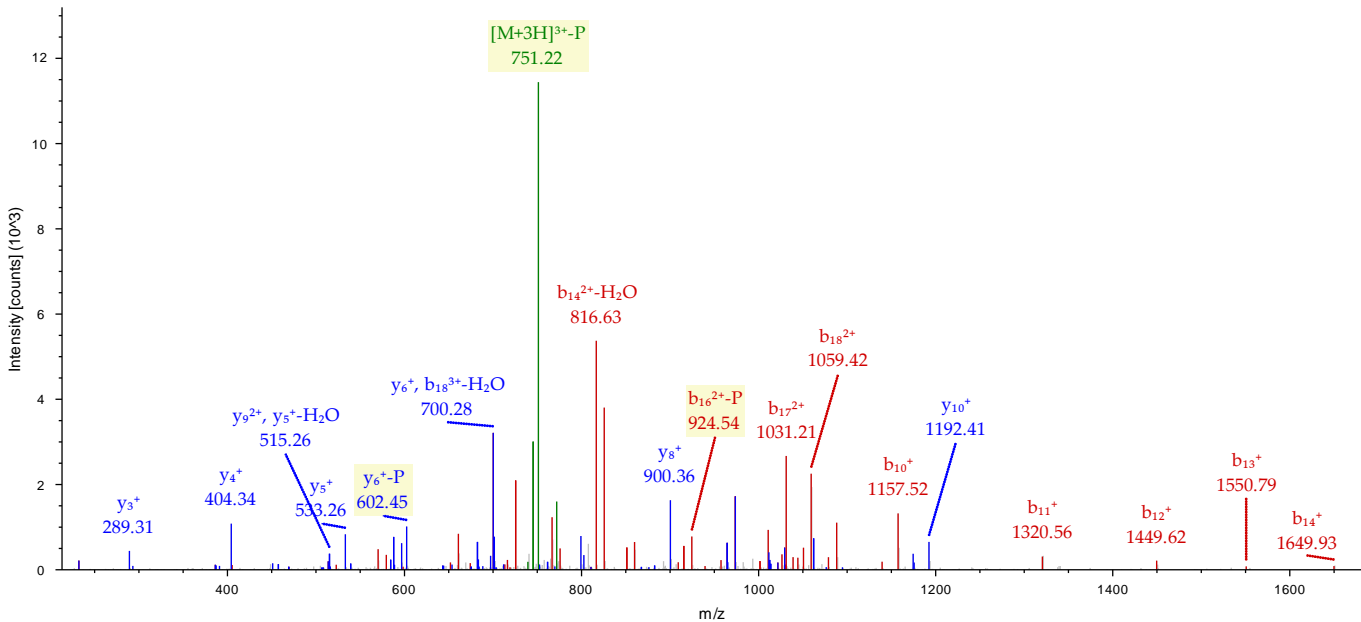
T615

HAPYEDDESIYEtVSEDGGR



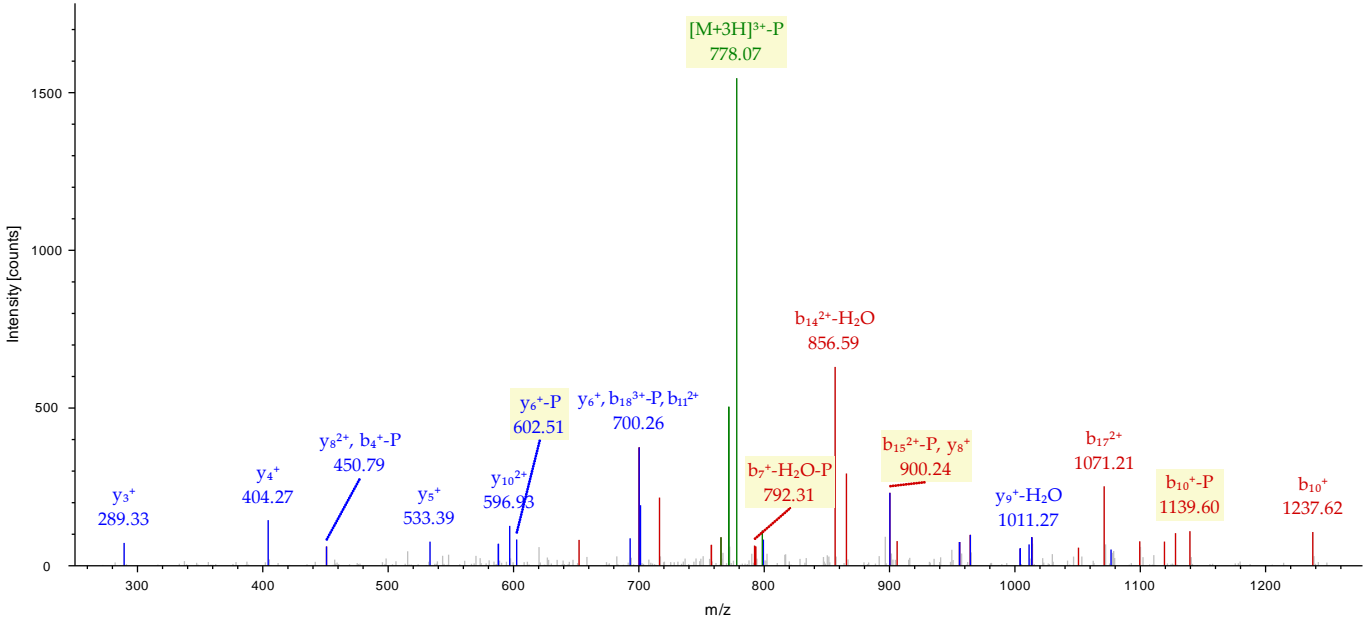
S617

HAPYEDDESIYETVsEDGGR



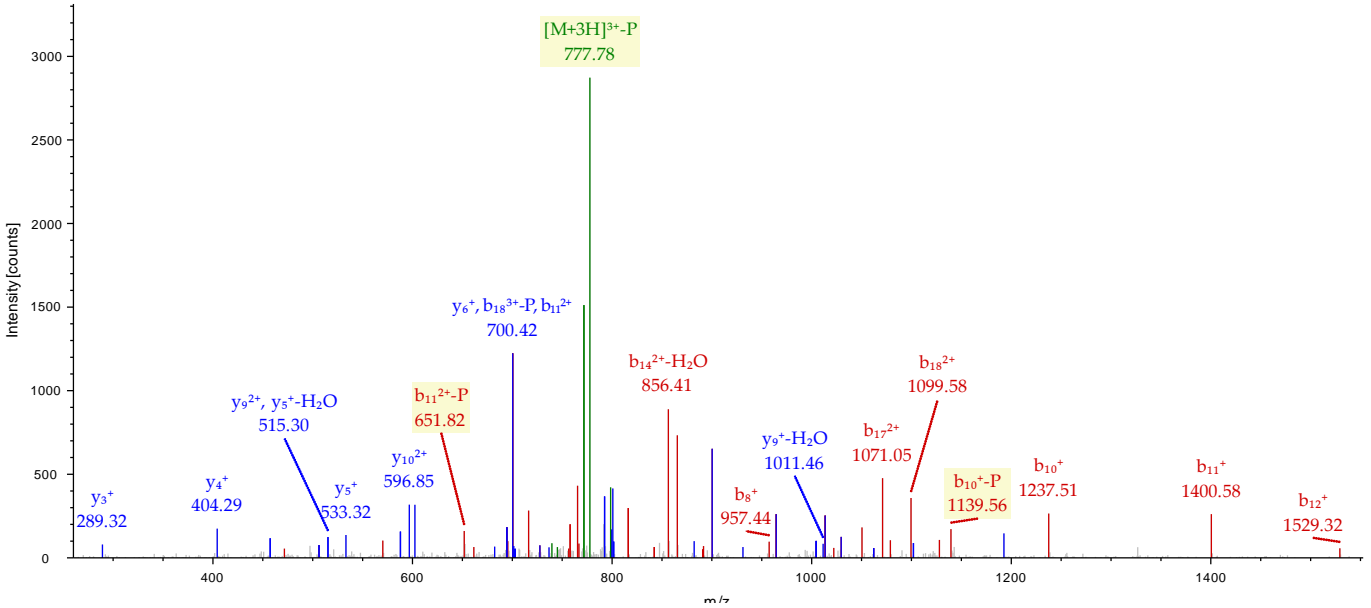
Y606/S617

HAPyEDDESIYETV_sEDGGR



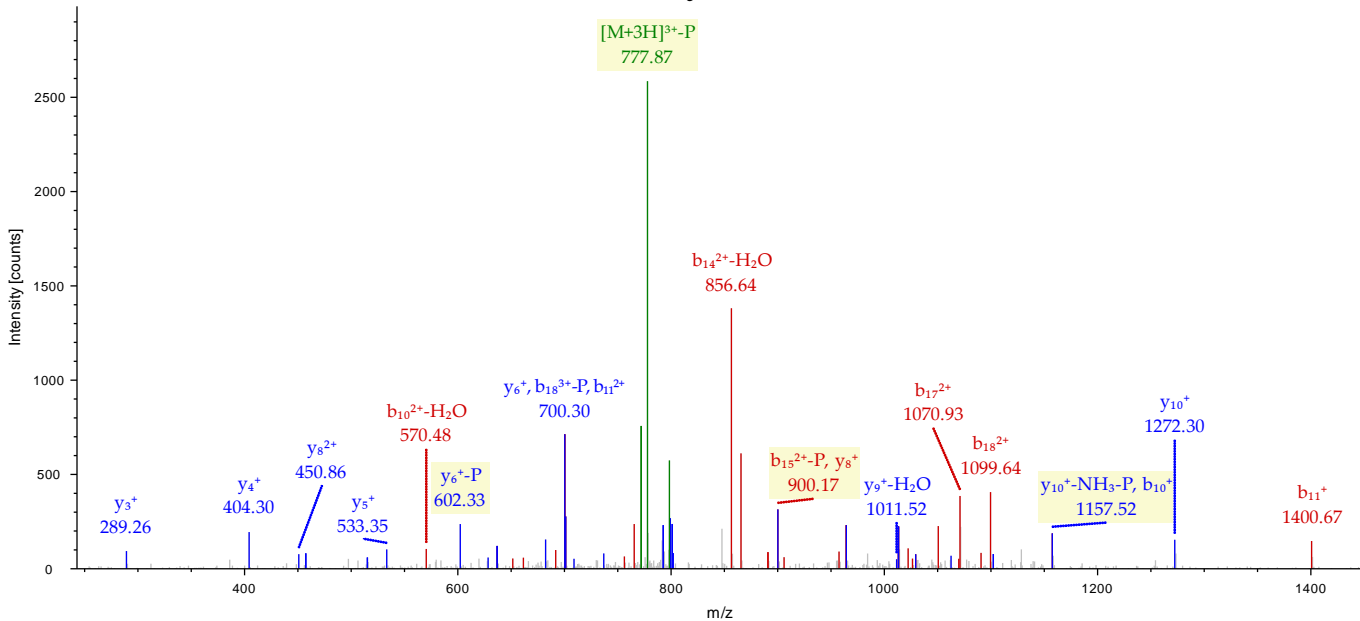
S611/S617

HAPyEDDE_sIYETV_sEDGGR



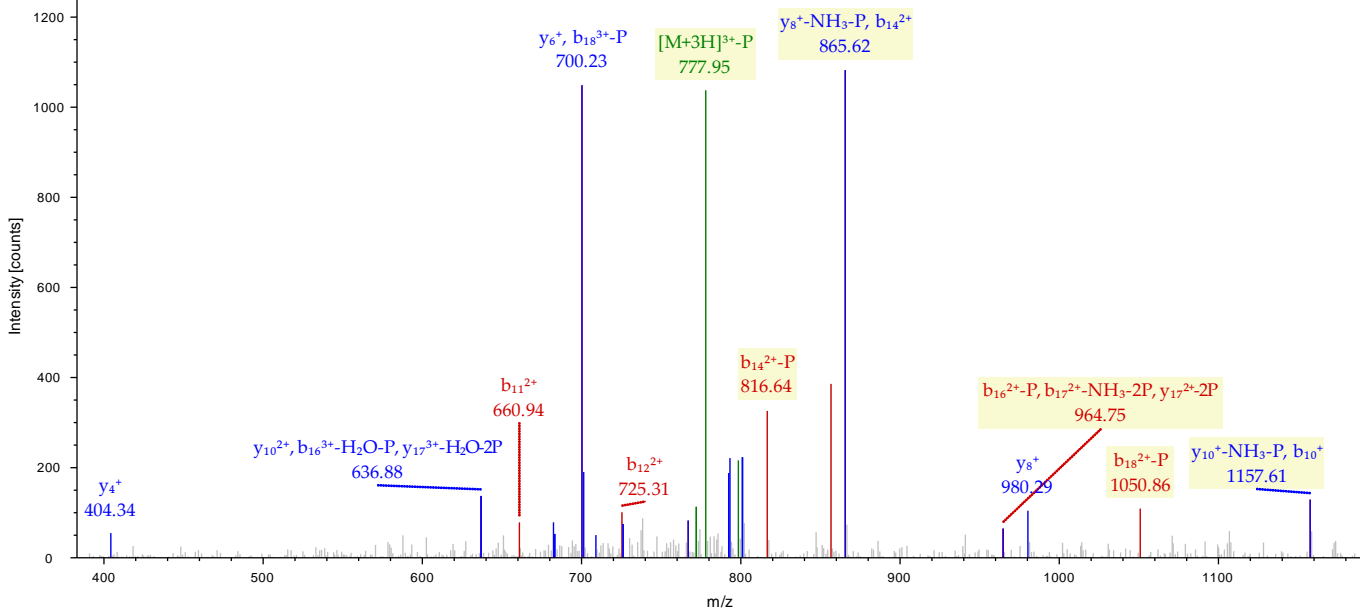
Y613/S617

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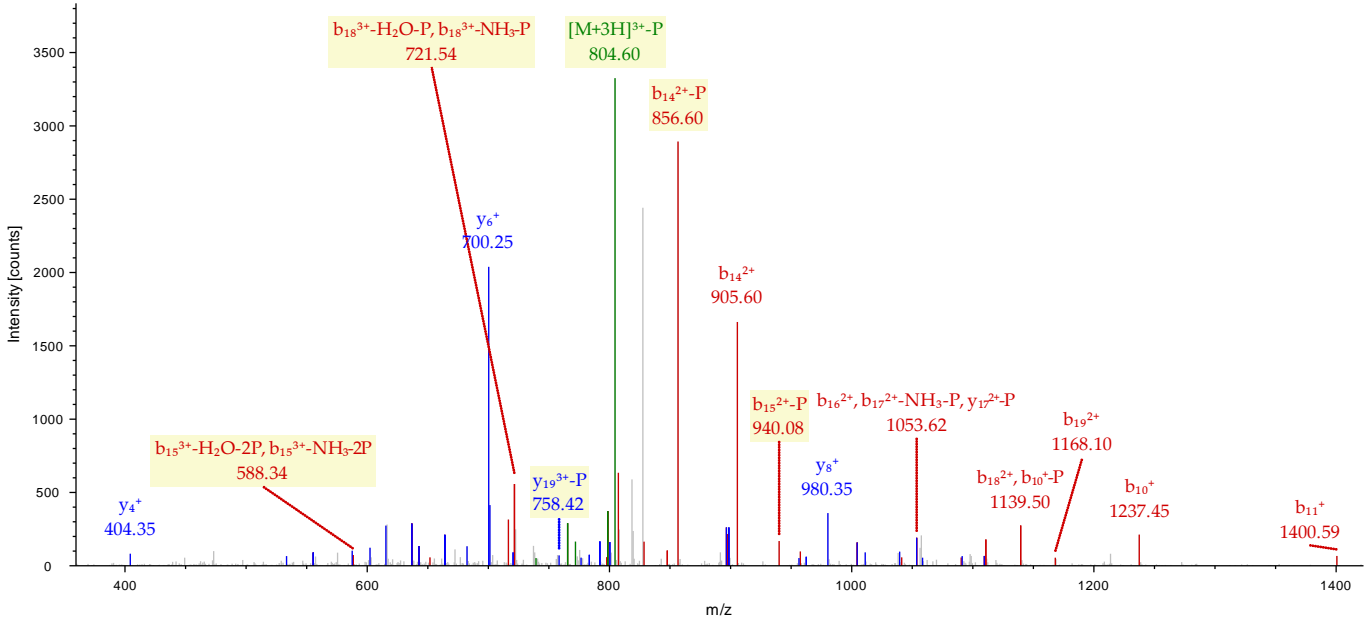


T615/S617

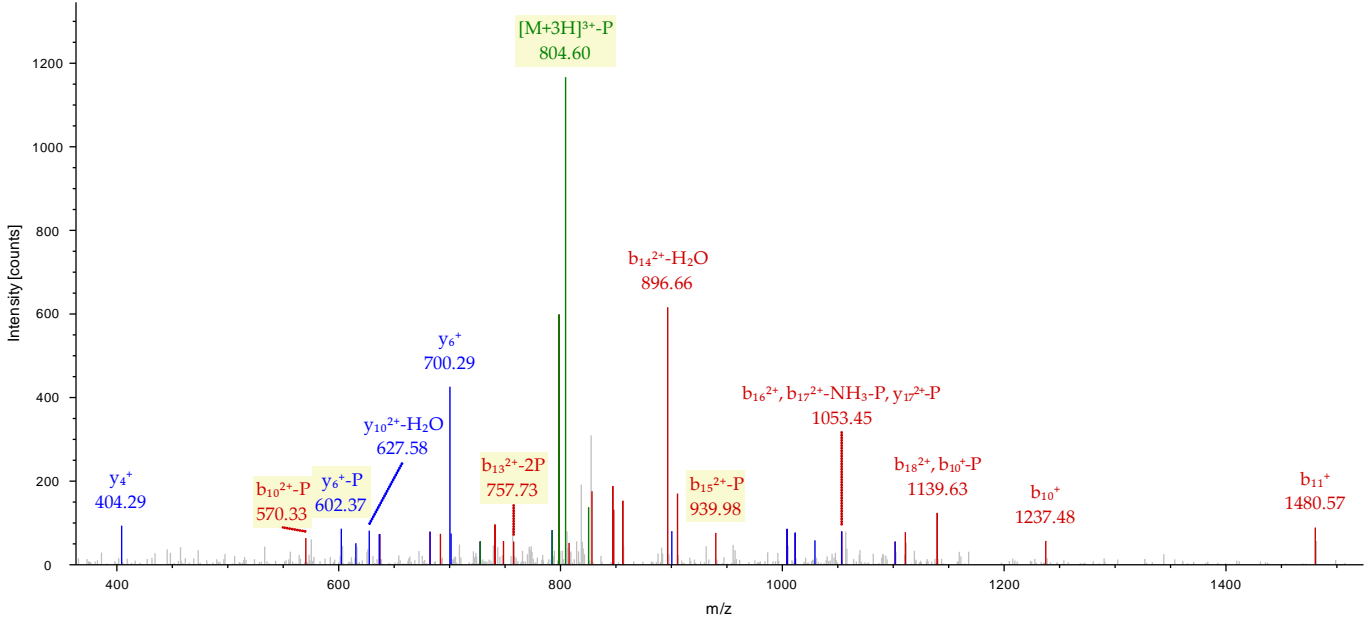
HAPYEDDES_IyEtV_sEDGGR



Y606/T615/S617 HAPyEDDESIYEtVsEDGGR

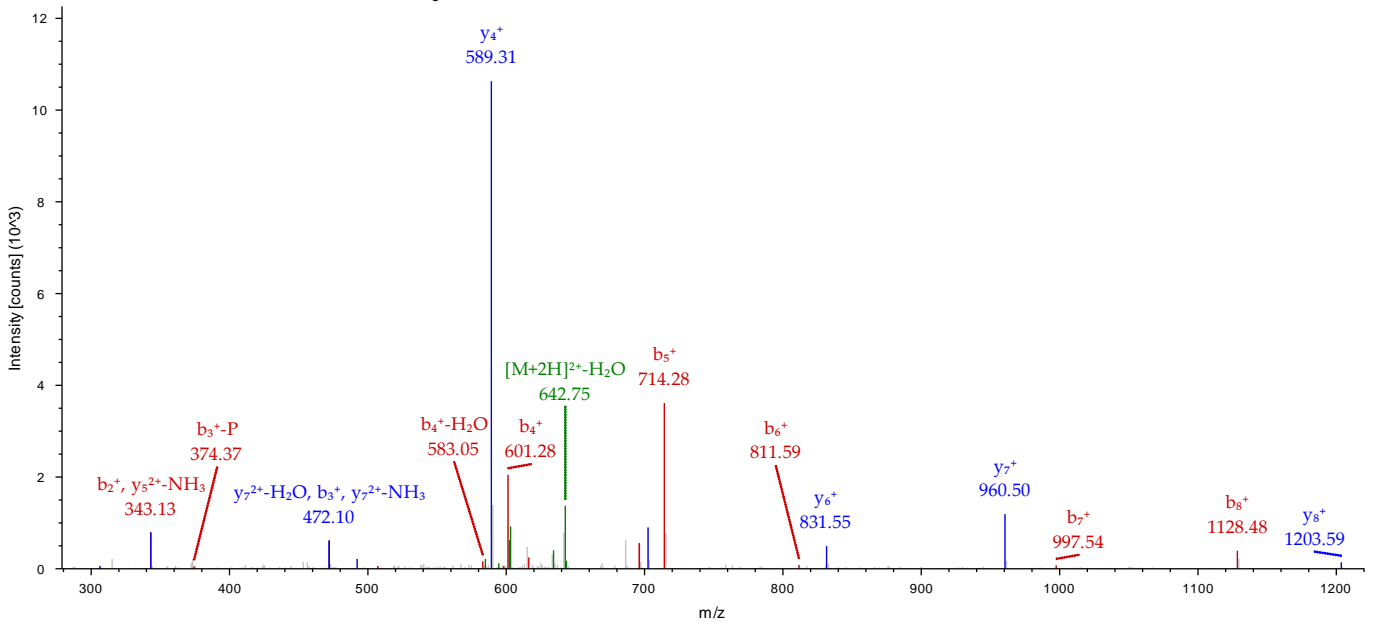


S611/Y613/S617 HAPYEDDEsIyETVsEDGGR



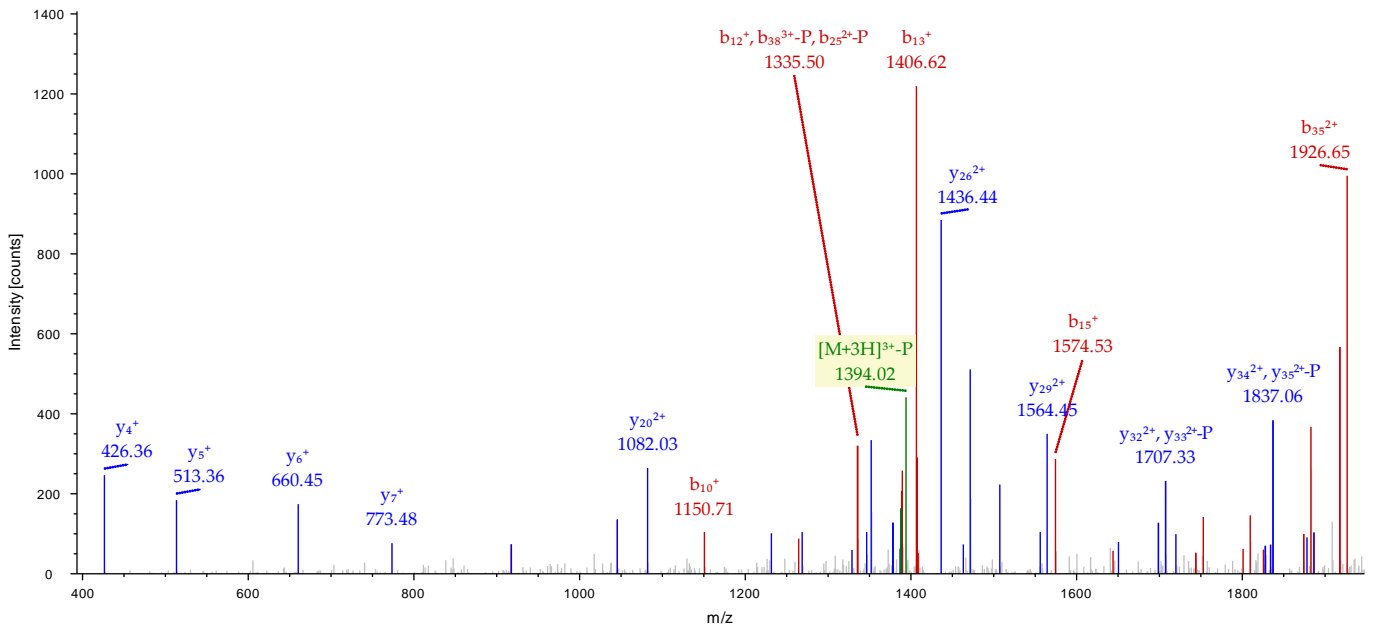
Y624

VyEEIPWMR



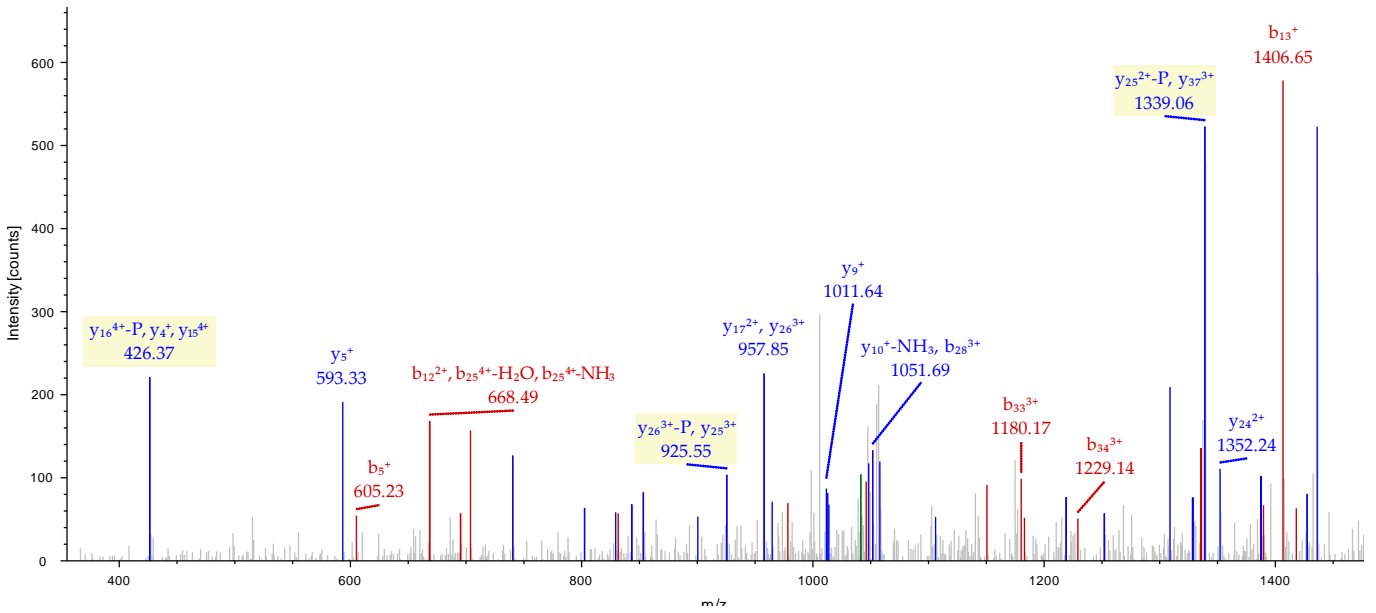
S647

VYENVCVNTANAAPAsPYIEAENPLYDWGGsALFSPpGR



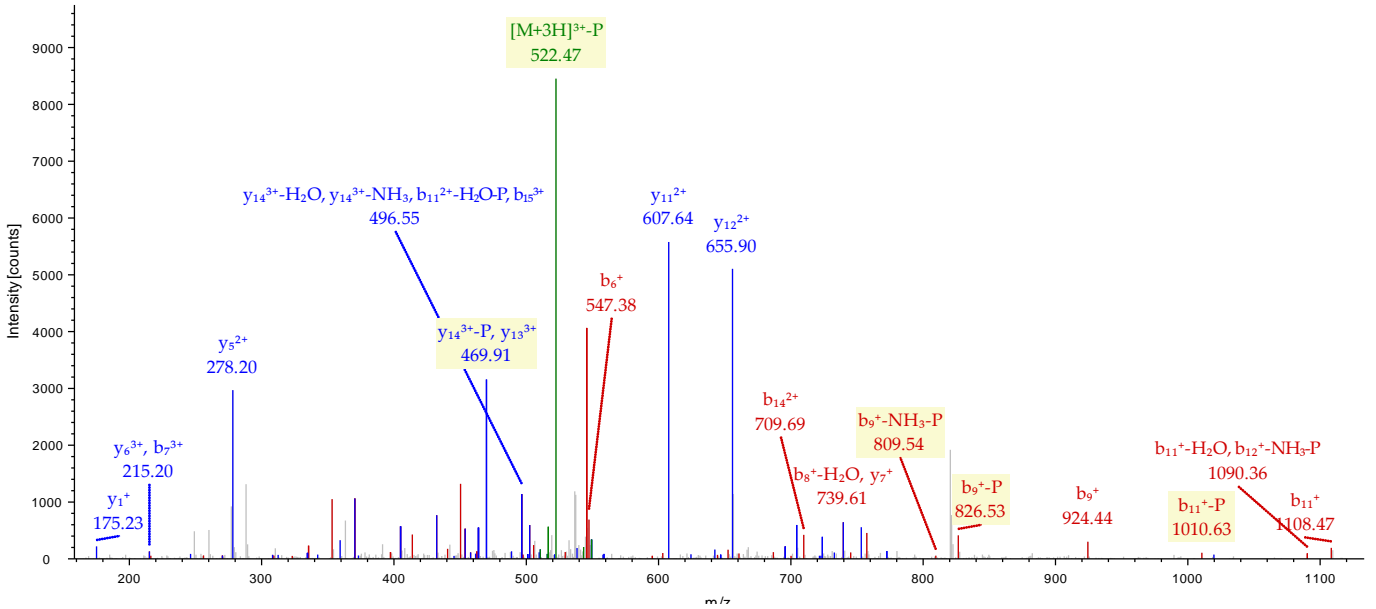
S666

VYENVCVNTANAAPASPYIEAENPLYDWGGSA_LF_SPPGR



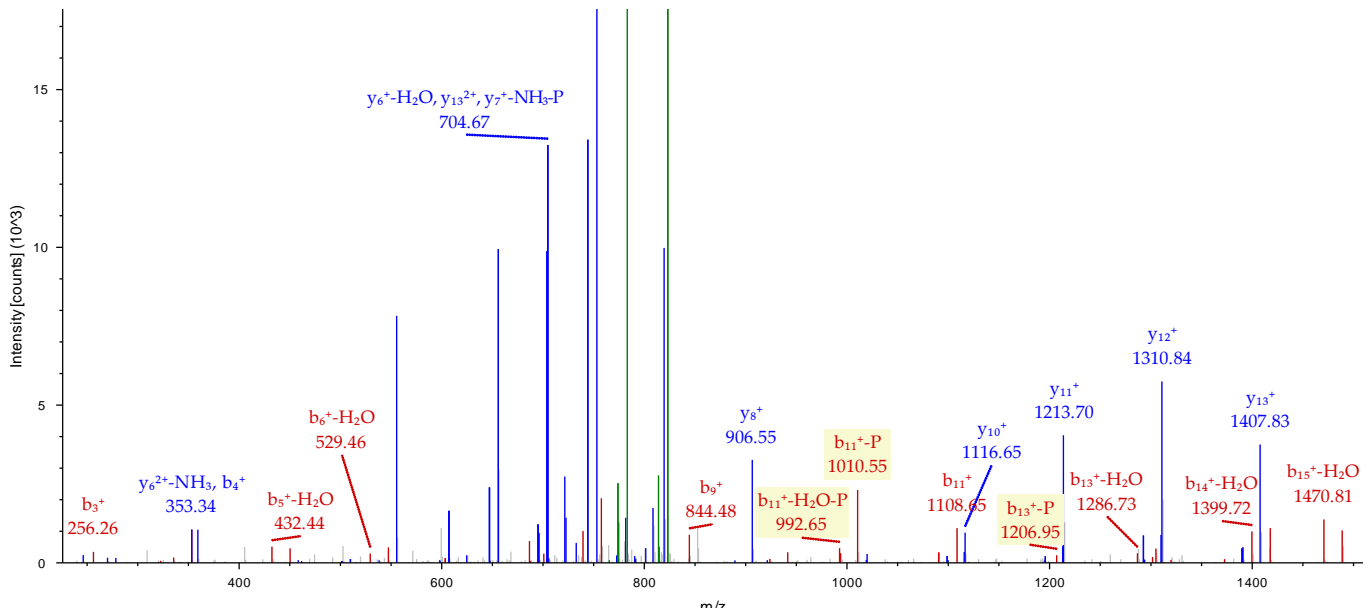
S679

TGPPPPPL_SPSPVLAR



S681

TGPPPPPLSPsPVLAR



S679/S681

TGPPPPPLsPSPVLAR

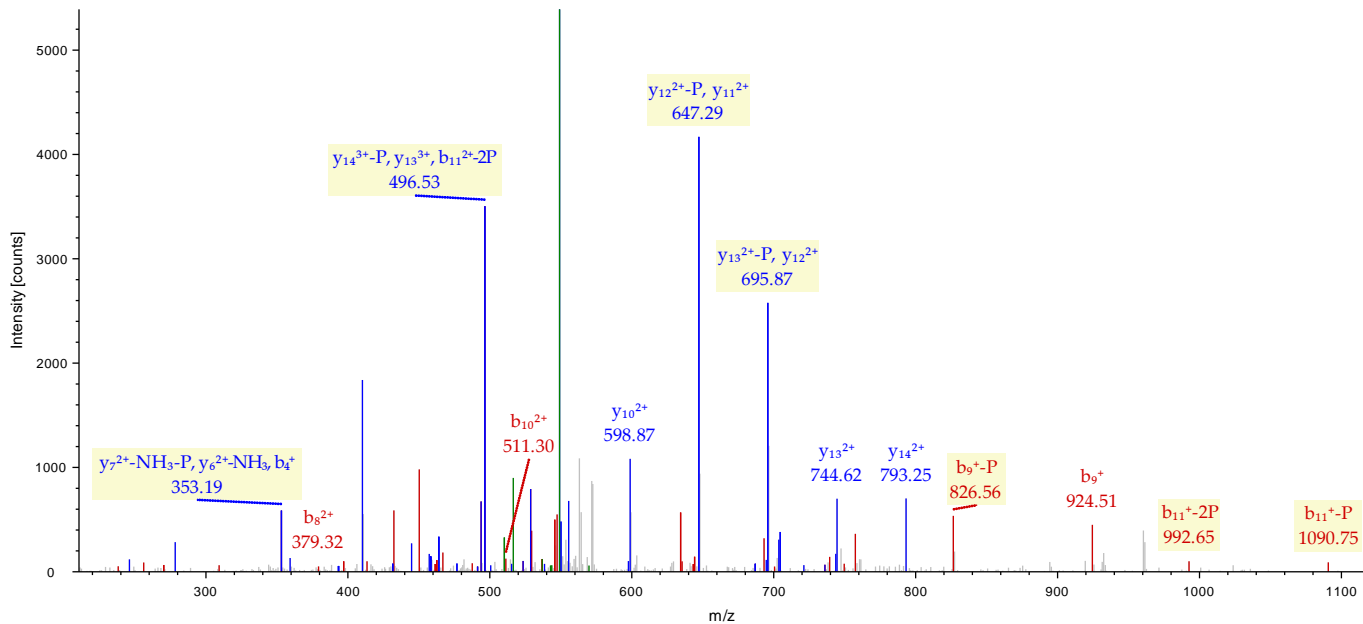


Figure S1. Addition of human serum eliminates non-specific immunofluorescence signals. HFFs were infected at an MOI of 0.25 and fixed at 6 hpi for imaging. Cells were blocked and stained in PBS-T supplemented with either 2% BSA or 2% BSA plus 2.5% human serum. Scale bars demarcate 25 μm .

Figure S2. Imaging of pUL46-GFP from incoming virions at high MOI. Vero cells were infected at an MOI of 100 and fixed at 2 hpi for direct fluorescence imaging. Scale bar demarcates 25 μm .

Figure S3. Proteasome-mediated degradation of pUL46 at late stages of infection. HFFs were infected with pUL46-GFP HSV-1 at an MOI of 10, treated with mock or 10 μM MG132 at 1 hpi, and harvested at 8 and 10 hpi. Whole cell lysates were separated by SDS-PAGE.

Figure S4. Representative MS/MS spectra of pUL46 phosphopeptides. CID MS/MS spectra are shown, accompanying the identification of phosphopeptides in Table 1.

Movie S1. Dynamic pUL46 localization in live cells using time-lapse microscopy. HFF cells were infected with pUL46-GFP HSV-1 at an MOI of 10, and imaging by time-lapse microscopy was started at 2 hpi; Zero acquisition time in the movie is equivalent to 2hpi.

Table S1. Putative specific pUL46 interactions at 6 hpi. For proteins receiving average SAINT probability scores of ≥ 0.9 in either stringent or mild conditions, a ratio of stringent:mild normalized spectral counts was calculated and reported.

Table S2. Non-specific proteins removed following SAINT analysis. Raw data are shown for all proteins that received average SAINT probability scores < 0.9 in both mild and stringent conditions.

Table S3. Putative specific pUL46 interactions at 14 hpi. For proteins receiving four fold enrichment over GFP control, a ratio of 14hpi : 6hpi normalized spectral counts was calculated and reported.

Table S4. Non-specific proteins removed following fold enrichment. Raw data are shown for all proteins that received below four fold enrichment versus GFP control.

Table S5. Putative specific pUL46 interactions in MG132-treated cells. pUL46 interacting proteins with average SAINT probability scores ≥ 0.9 following MG132 treatment are listed.

Table S6. Non-specific proteins removed by SAINT analysis in MG132-treated cells. Raw data are shown for all proteins that received average SAINT probability scores < 0.9 in MG132-treated cells.