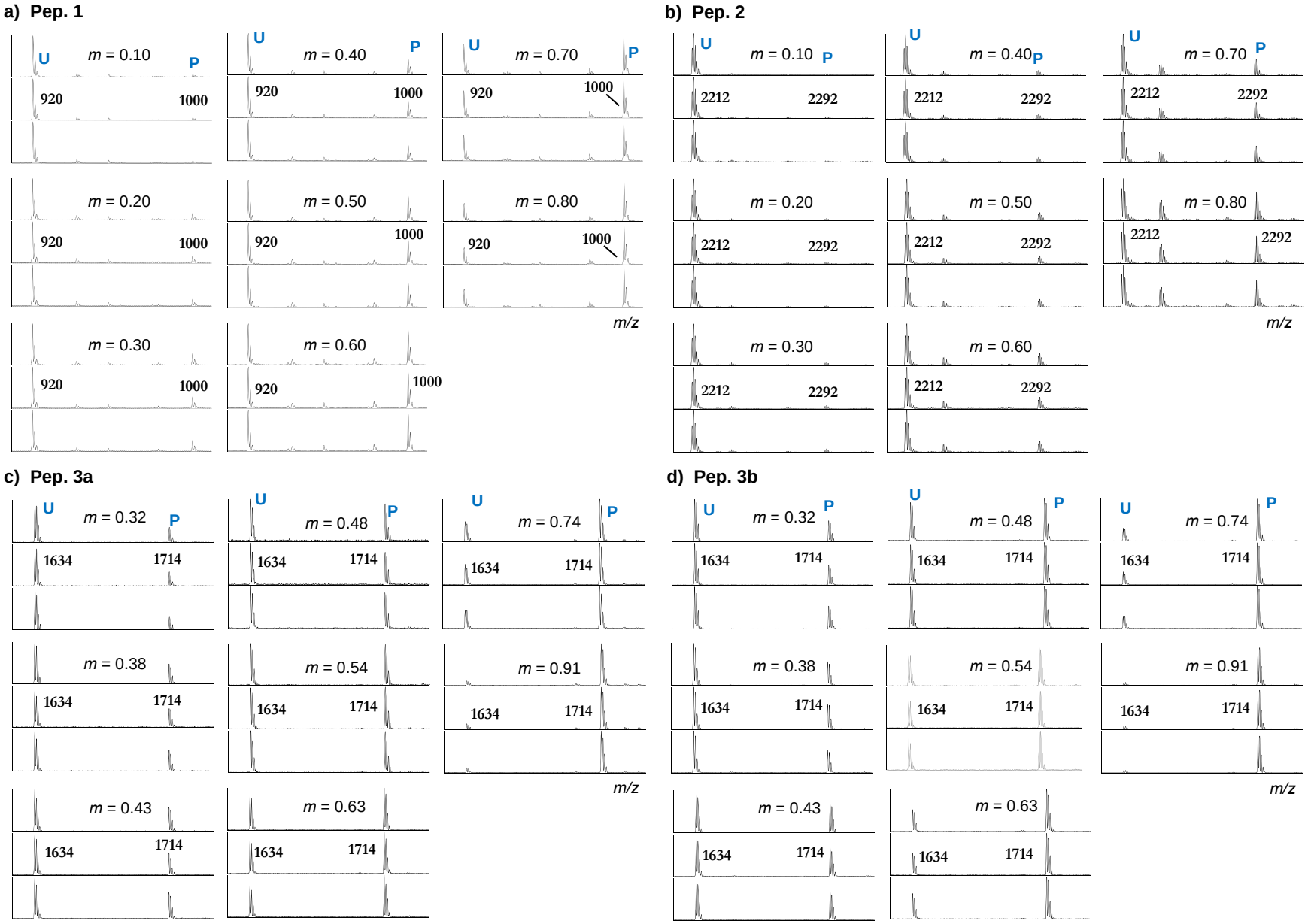
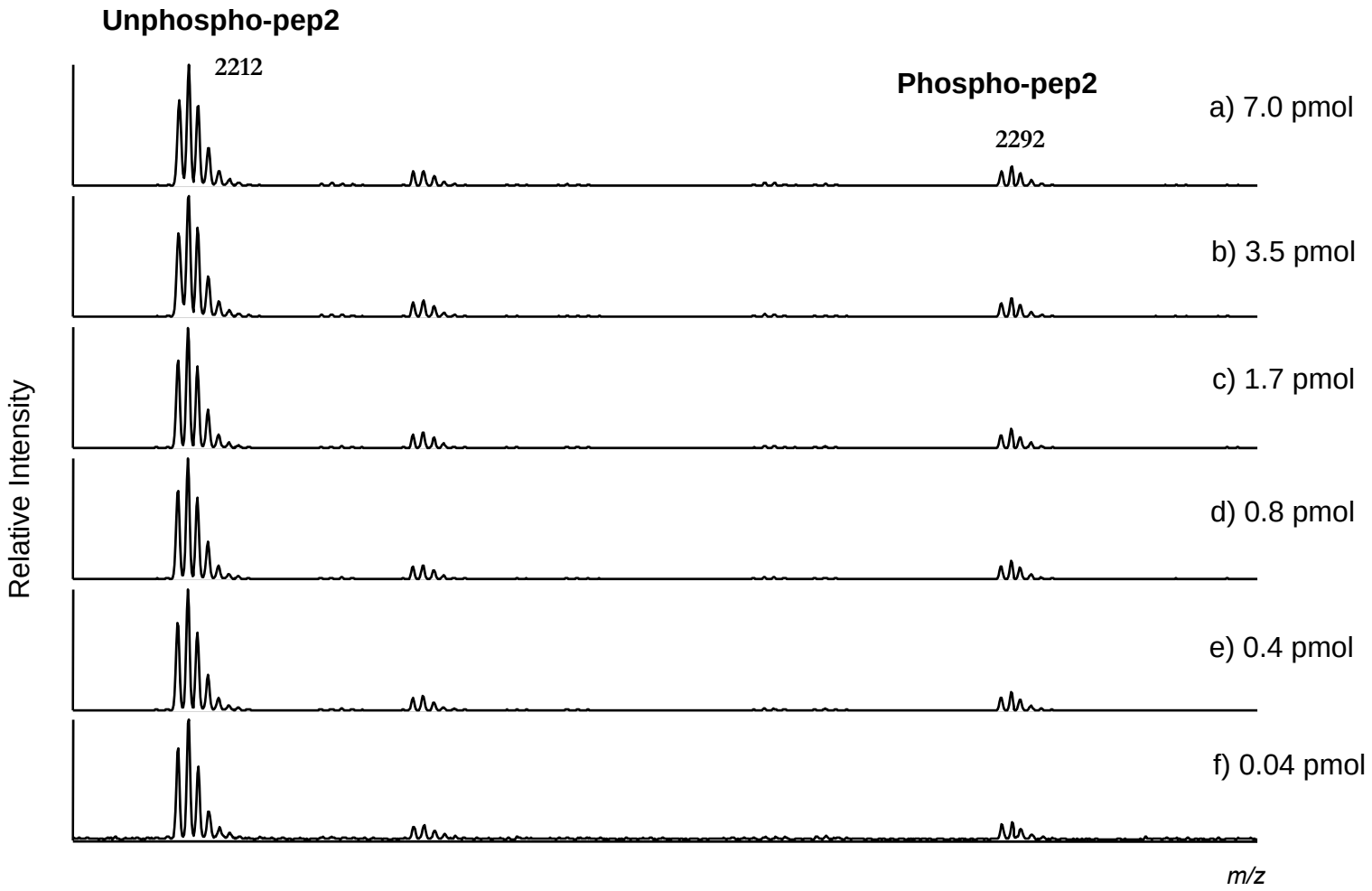
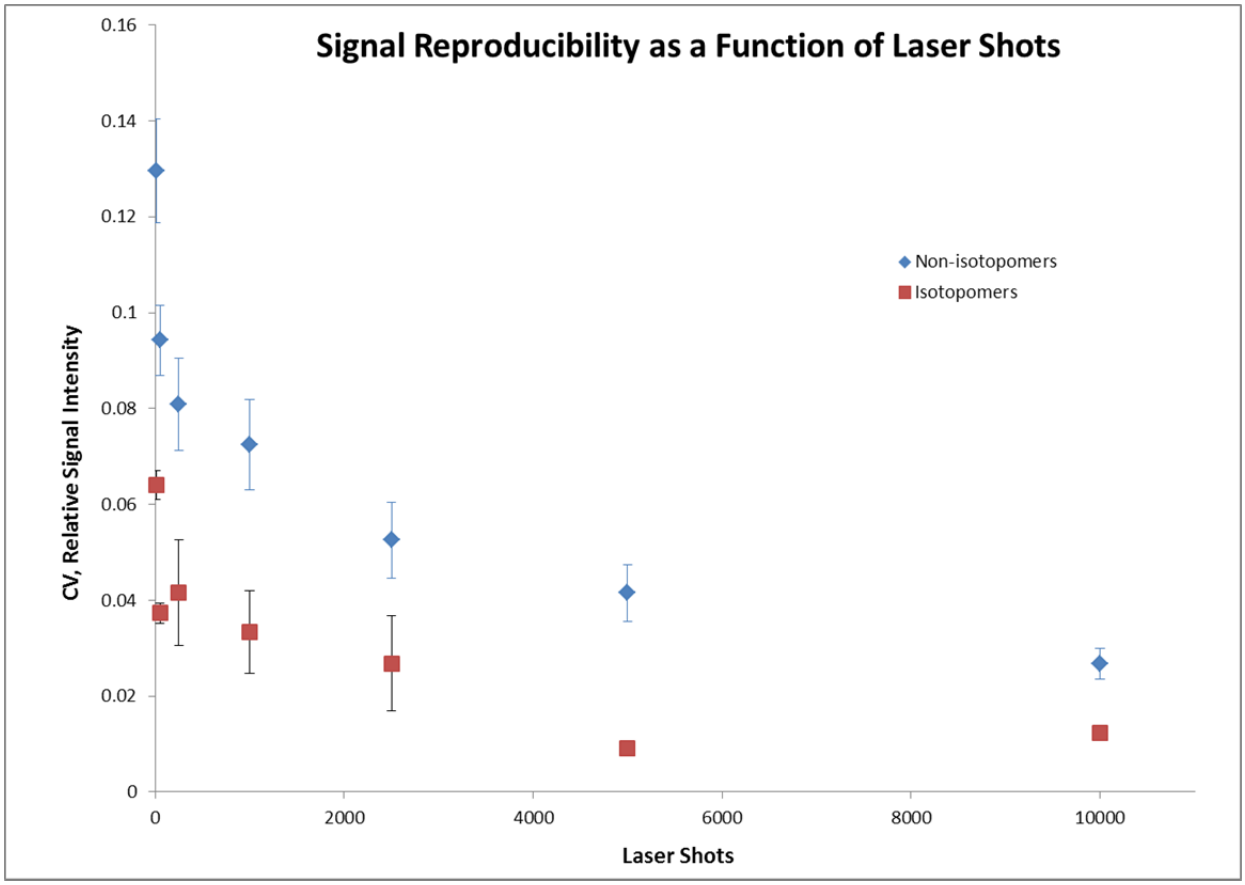



**Supplemental Figure S1.** Mass spectra of the experiments in which response factor ratio *α* was determined for each phospho – and unphosphopeptide mixtures from the observed mass spectrometric signal intensity ratio *S* for three models in table 1, pep.1 AAAAYRAAR / AAAApYRAA, pep.2 LRWGFTTPDKKHQKEPPF / RWGFTpTPDKKHQKEPPF, pep. 3a RQSVELHSPQSLPR / RQpSVELHSPQSLPR), and pep. 3b RQSVELHSPQSLPR / RQSVELHpSPQSLPR. Nominal *m/z* values are shown.



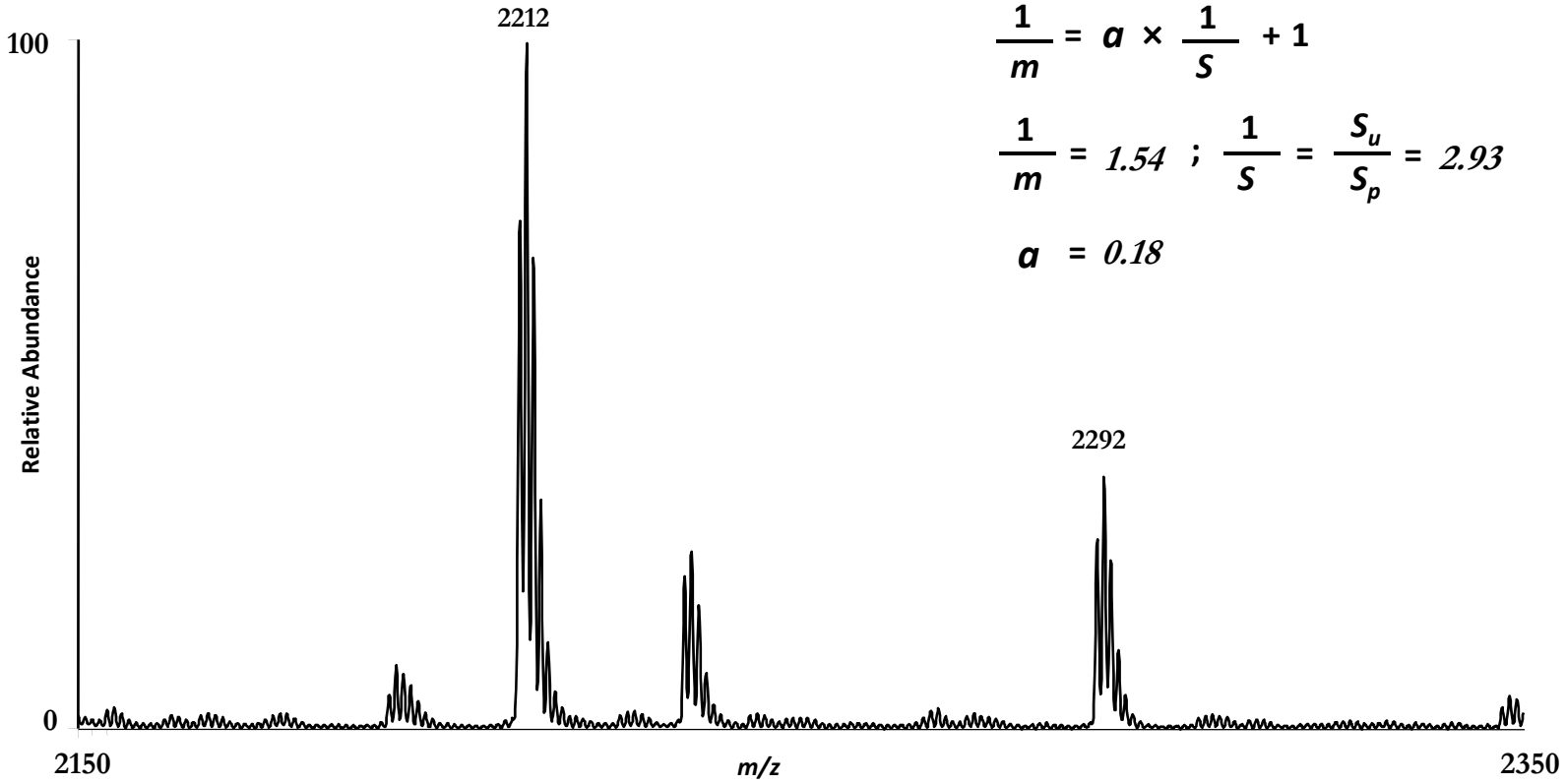

**Supplemental Figure S2.** Mass spectra of the experiments for dynamic range test in Table 3 in which pep 2/ p-pep 2 mixtures (LRWGFTTPDKKHQKEPPF/RWGFTpTPDKKHQKEPPF) with phosphorylation fraction (*m* = 0.5) in an amount range of 7.0 – 0.04 pmol were analyzed by MALDI-MS.




**Supplemental Figure S3.** Variability in MALDI-TOF MS signal, as measured by the coefficient of variance of the signal intensity ratios for either a non-isotopomeric (blue) or isotopomeric (red) peptide pair decreases as a function of increased laser shots for both isotopomeric and non-isotopomeric pairs. Both types of pairs show an increase in reproducibility with the number of laser shots. Isotopomers showing a consistent ~2-3 fold advantage in reproducibility over non-isotopomers. Because both plots appear to asymptote, the data suggests that sufficiently increasing laser shots may make MALDI a reasonable method for quantification either with or without isotope label.

Spectra were acquired using a Waters MaldiMX MALDI-TOF mass spectrometer in positive reflectron mode using unphosphorylated $^{13}C_3$ labeled and unlabeled peptide 1 for the isotopomeric pair, and unphosphorylated and phosphorylated unlabeled peptide 1 for the non-isotopomeric pair. MALDI-TOF is utilized in this case because it involves the least post-ionization ion manipulation of all major analyzers that are coupled to MALDI, and therefore we surmise that any effects are entirely a function of the ionization/extraction process, isolated from ion transport. MALDI samples were prepared using the dried droplet method as previously described using a 1 pmol 1:1:1 mixture of all three peptides (labeled, unlabeled and phosphorylated) in a saturated solution (solvent 70:29.9:0.1 acetonitrle:water:TFA v/v/v) of alpha-cyano, 4-hydroxycinnamic acid matrix. Spectra were acquired in manual mode using an $N_2$ laser firing at 60 Hz.

## Pep. 2 mixtures of phospho- and unphosphopeptides ($m = 0.65$)




**Supplemental Figure S4. Mass spectra of mixture of phospho – and unphosphopeptide 2 (0.14 pmol) in a Cdk kinase buffer.** The response factor ratio $\alpha$ was determined from the observed mass spectrometric signal intensity ratio, $S = 2.93$, and the known phosphorylation fraction, $m = 0.65$ for the measurements of *in vitro* inhibition of Cdk2 phosphorylation of Pep. 2.