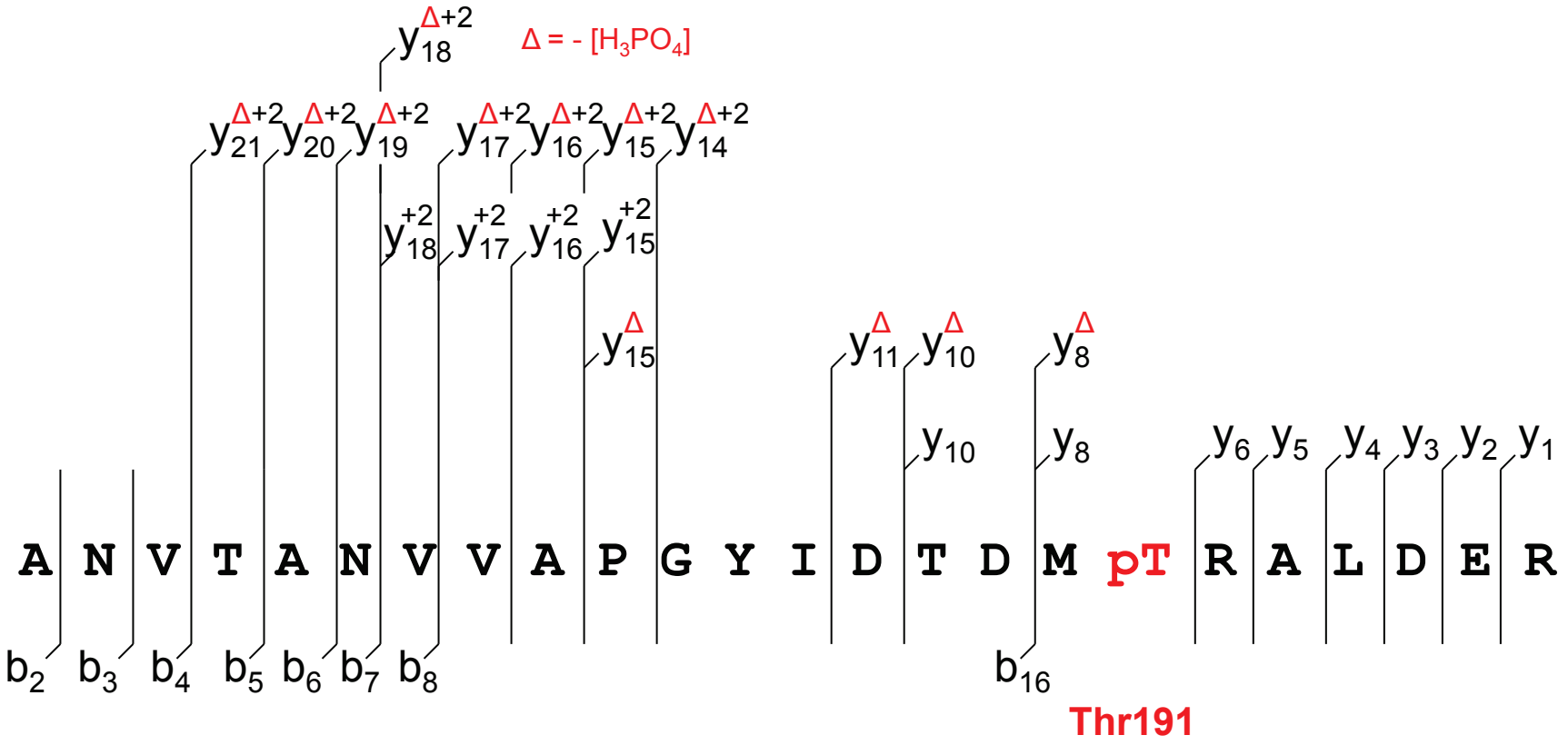
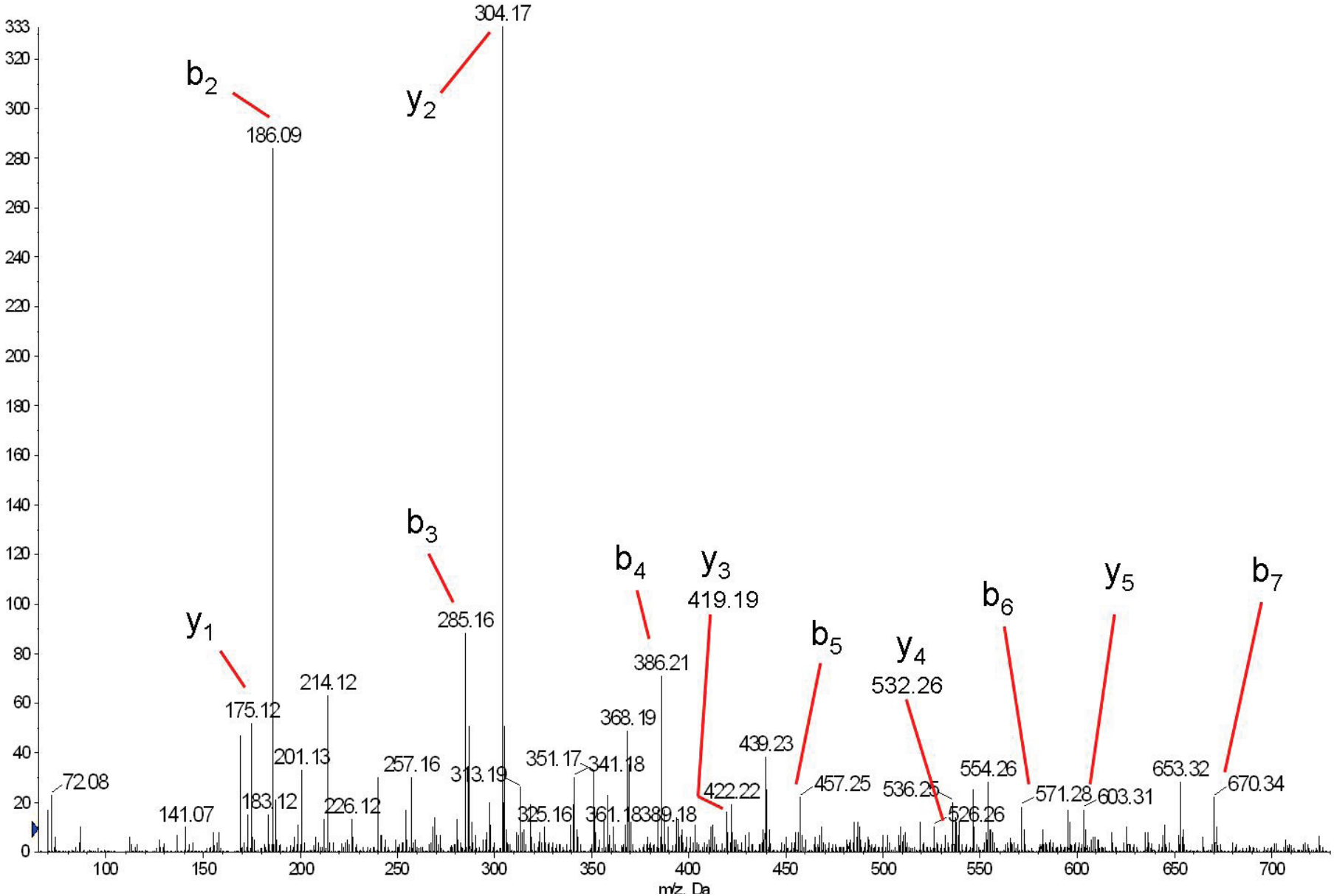
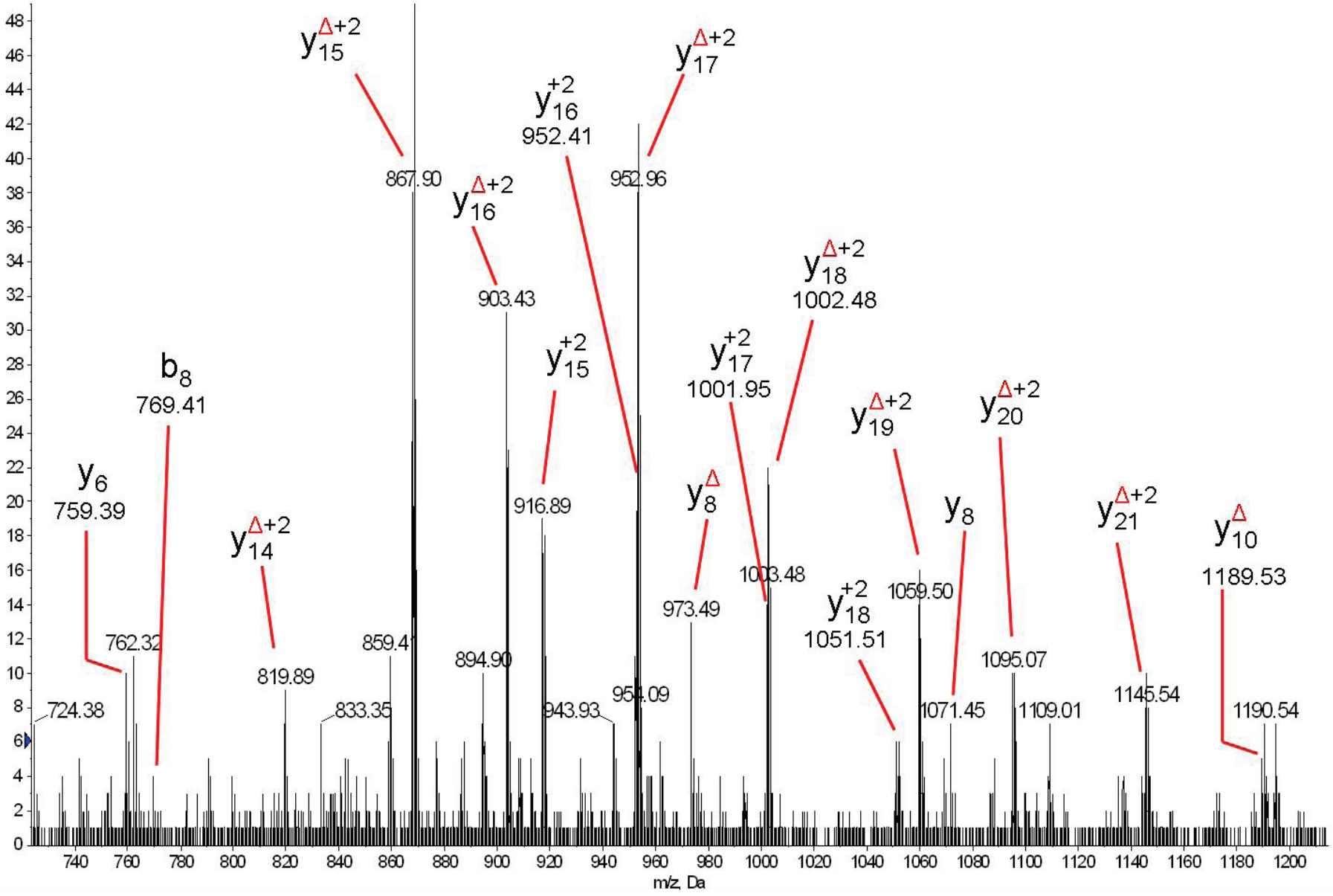
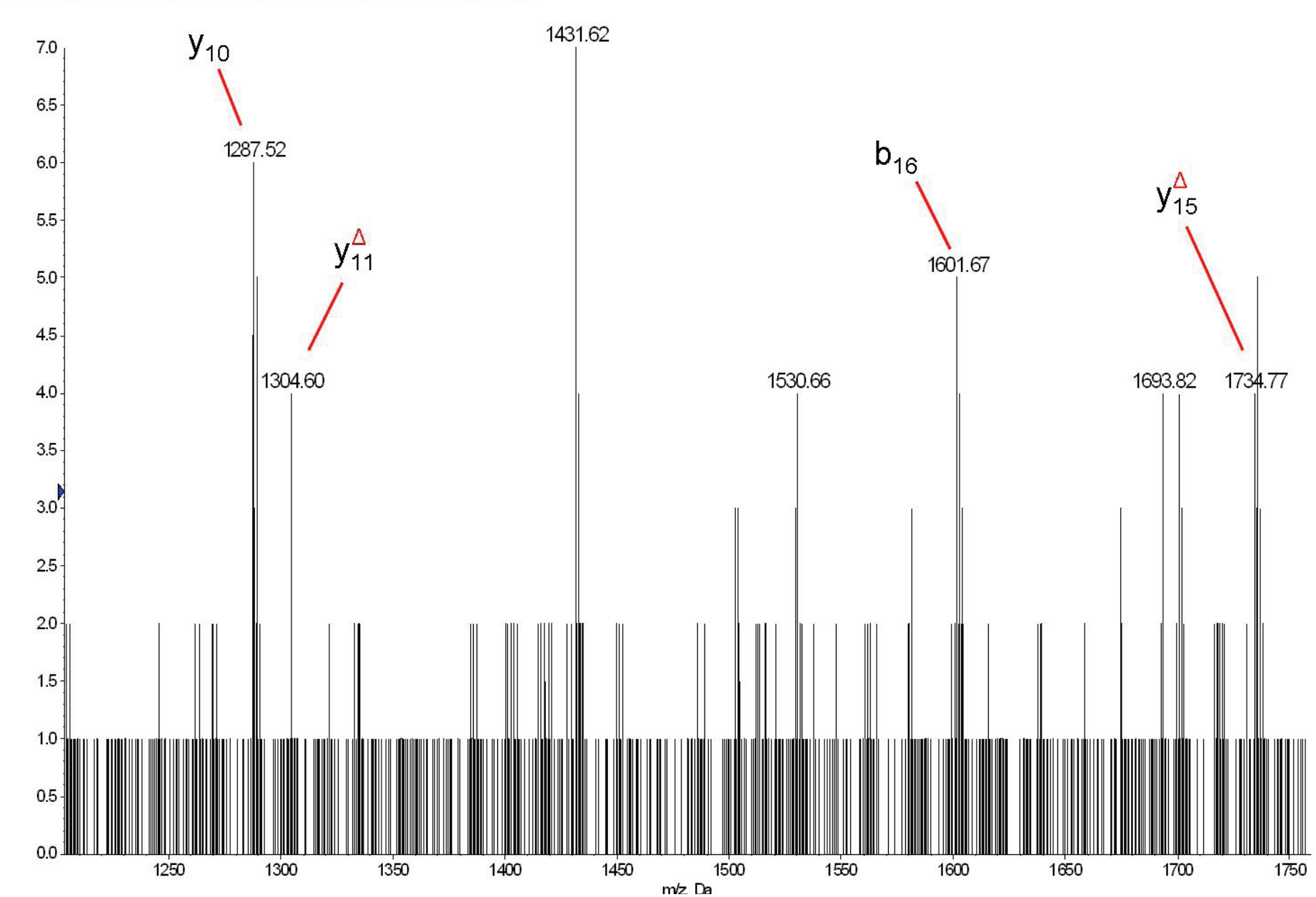
Figure S1

Tryptic peptide with no missed cleavage with the characteristic phosphorylation on the Threonine 191 residue from *in vitro* phosphorylated MabA(Monoisotopic mass : 2671.28 Da, MS/MS of the triply charged ion at m/z 891.7).



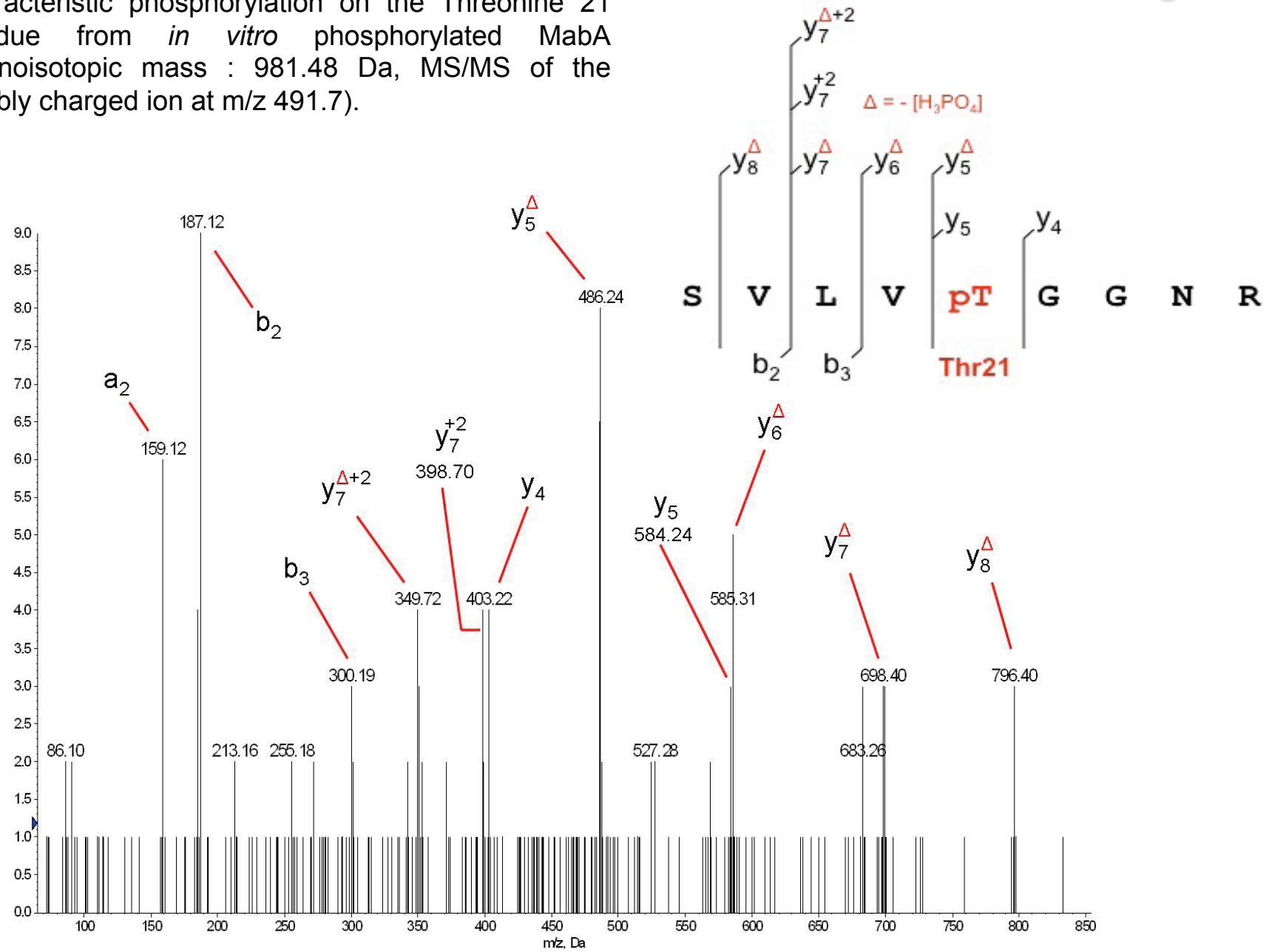

304.17
b2
y2
186.09
b3
b4
y3
419.19
y1
285.16
b5
y4
b6
y5
b7
386.21
532.26
214.12
175.12
368.19
439.23
201.13
257.16
313.19
351.17
341.18
554.26
653.32
72.08
422.22
457.25
536.25
571.28
670.34
183.12
226.12
603.31
141.07
325.16
361.18
389.18
526.26
333
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
100
150
200
250
300
350
400
450
500
550
600
650
700
m/z, Da


y15 Δ+2
867.90
y16 Δ+2
903.43
y16 +2
952.41
y17 Δ+2
952.96
y15 +2
916.89
b8
769.41
y6
759.39
y14 Δ+2
819.89
762.32
724.38
859.41
833.35
894.90
943.93
954.09
y8 Δ
973.49
y17 +2
1001.95
1003.48
y18 Δ+2
1002.48
y18 +2
1051.51
y19 Δ+2
1059.50
y8
1071.45
y20 Δ+2
1095.07
1109.01
y21 Δ+2
1145.54
y10 Δ
1189.53
1190.54
m/z, Da


y10
1287.52
1431.62
y11Δ
1304.60
b16
1601.67
y15Δ
1530.66
1693.82
1734.77
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1250
1300
1350
1400
1450
1500
1550
1600
1650
1700
1750
m/z, Da

Tryptic peptide with no missed cleavage with the characteristic phosphorylation on the Threonine 21 residue from *in vitro* phosphorylated MabA (Monoisotopic mass : 981.48 Da, MS/MS of the doubly charged ion at m/z 491.7).

Figure S2



Tryptic peptide with no missed cleavage with the characteristic phosphorylation on the Threonine 114 residue from *in vitro* phosphorylated MabA(Monoisotopic mass : 1254.62 Da, MS/MS of the doubly charged ion at m/z 628.3).

Figure S3

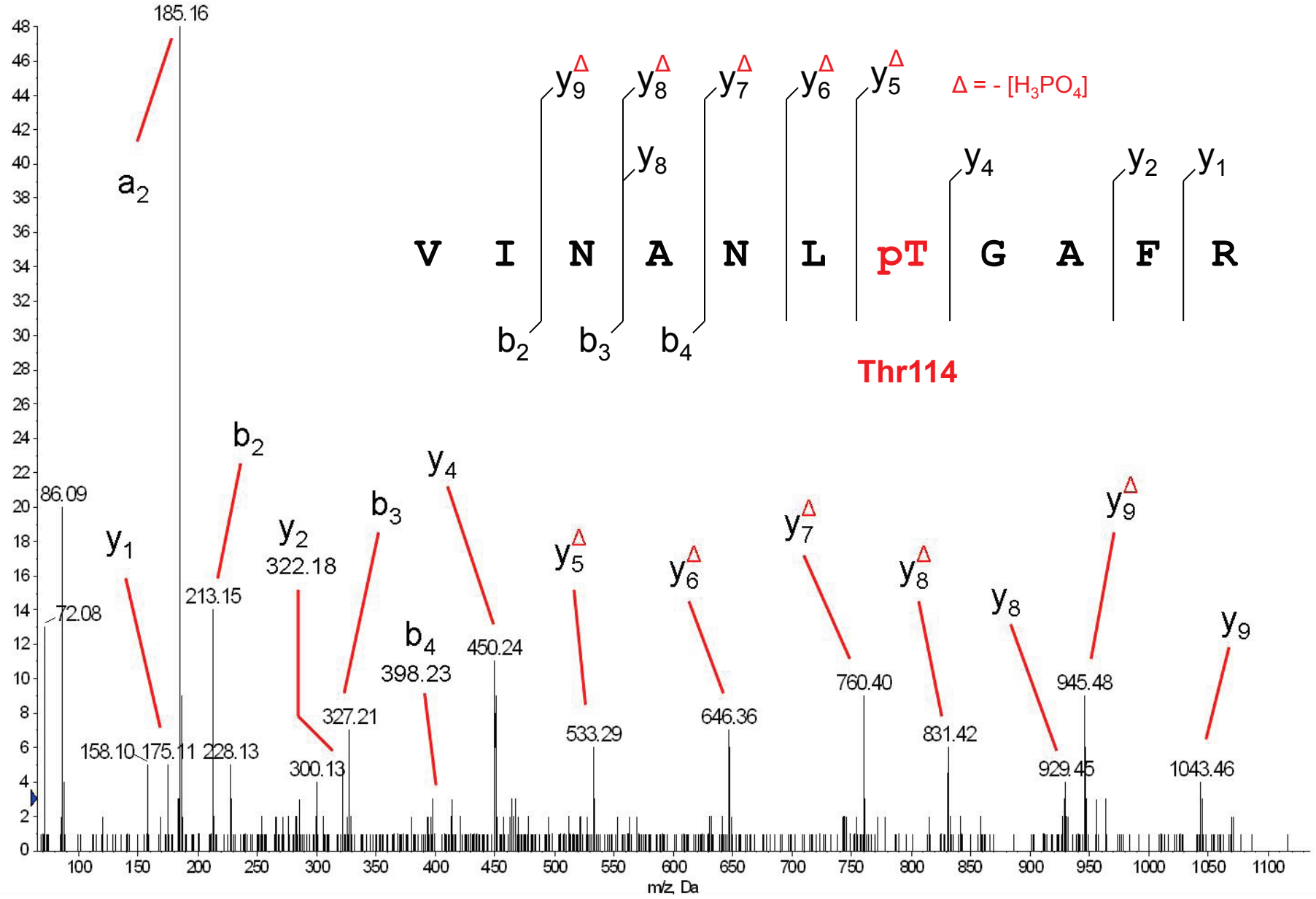
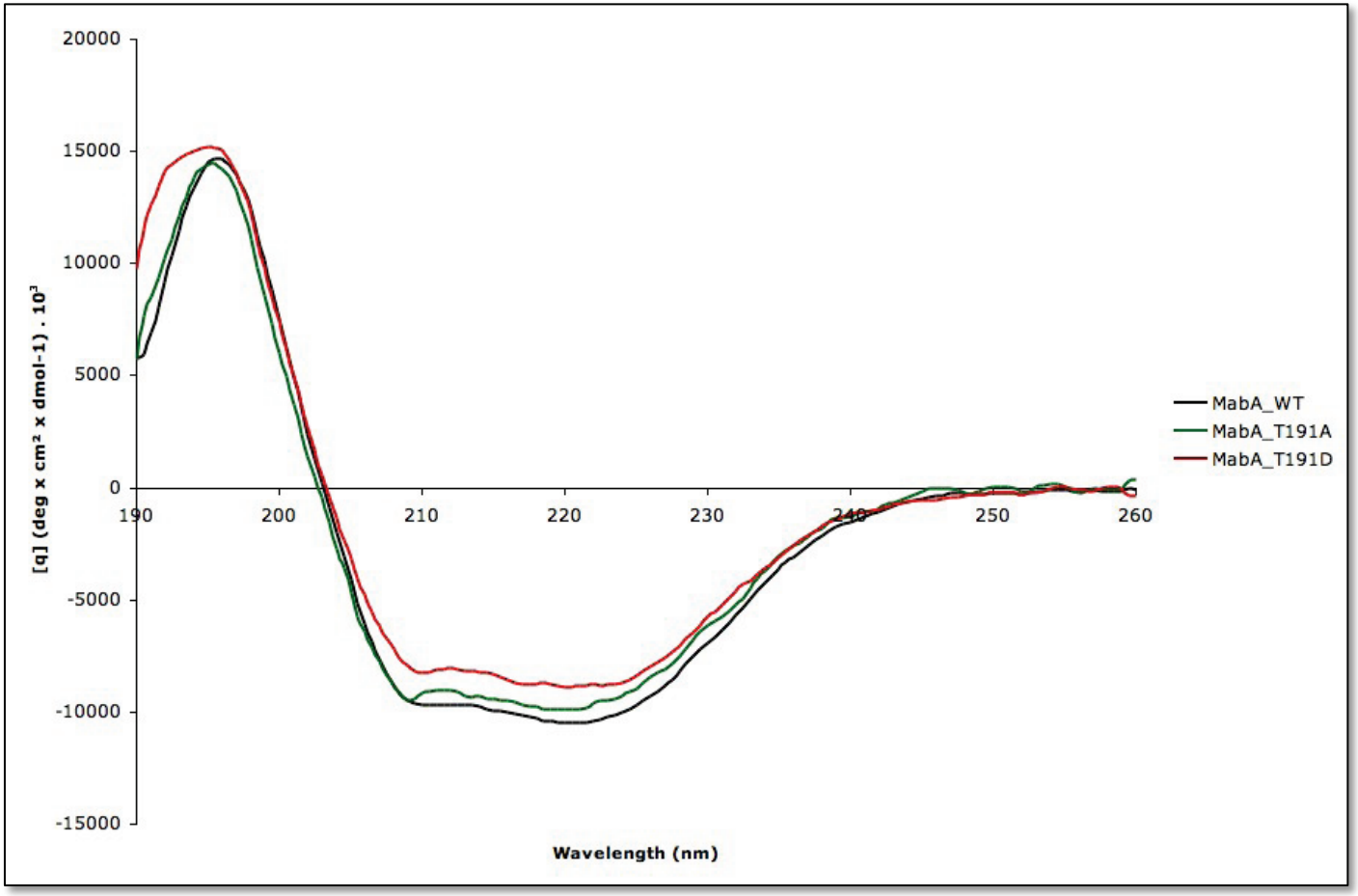


Figure S4



Comparison of CD spectra for MabA_WT, MabA_T191A and MabA_T191D. CD spectra were obtained using a Chirascan spectrophotometer (Applied Photophysics) at a protein concentration of approximately 1mg/ml in buffer 50 mM Tris/HCl pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM EDTA.

Table S1. Bacterial Strains and Plasmids used in this study

Strains or Plasmids	**Genotype or Description**	**Source or Reference**
E. coli TOP10	F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lacZΔM15* Δ*lacX74 deoR recA1 araD139* Δ(*ara-leu*)7697 *galU galK rpsL endA1 nupG*; used for general cloning	Invitrogen
E. coli BL21(DE3)Star	F2 *ompT hsdSB*(rB2 mB2) *gal dcm* (DE3); used to express recombinant proteins in *E. coli*	Stratagene
M. bovis BCG 1173P2	Vaccine strain	WHO, Stockholm
pETPhos	pET15b (Novagen) derivative including the replacement of the thrombin site coding sequence with a tobacco etch virus (TEV) protease site and Ser to Gly mutagenesis in the N-term His-tag	(1)
pETPhos_*mabA_WT*	pETPhos derivative used to express His-tagged fusion of MabA in *E. coli*	This work
pETPhos_*mabA_T21A*	pETPhos derivative used to express His-tagged fusion of MabA_T21A in *E. coli*	This work
pETPhos_*mabA_T114A*	pETPhos derivative used to express His-tagged fusion of MabA_T114A in *E. coli*	This work
pETPhos_*mabA_T191A*	pETPhos derivative used to express His-tagged fusion of MabA_T191A in *E. coli*	This work
pETPhos_*mabA*_T21A/T191A	pETPhos derivative used to express His-tagged fusion of MabA_T21A/191A in *E. coli*	This work
pETPhos_*mabA*_T21A/T114A/T191A	pETPhos derivative used to express His-tagged fusion of MabA_T21A/191A in *E. coli*	This work
pETPhos_*mabA_T21D*	pETPhos derivative used to express His-tagged fusion of MabA_T21D in *E. coli*	This work
pETPhos_*mabA_T114D*	pETPhos derivative used to express His-tagged fusion of MabA_T114D in *E. coli*	This work
pETPhos_*mabA_T191D*	pETPhos derivative used to express His-tagged fusion of MabA_T191D in *E. coli*	This work
pMK1	*E. coli*/mycobacterial shuttle vector, allows expression of N-term His-tagged proteins, derived from pMV261 (2) and containing the pET28a polylinker	This work
pMK1_*mabA_WT*	pMK1 derivative used to express His-tagged fusion of MabA in mycobacteria	This work
pMK1_*mabA_T21A*	pMK1 derivative used to express His-tagged fusion of MabA_T21A in mycobacteria	This work
pMK1_*mabA_T114A*	pMK1 derivative used to express His-tagged fusion of MabA_T114A in mycobacteria	This work

pMK1_*mabA_T191A*	pMK1 derivative used to express His-tagged fusion of MabA_T191A in mycobacteria	This work
pMK1_*mabA_T21D*	pMK1 derivative used to express His-tagged fusion of MabA_T21D in mycobacteria	This work
pMK1_*mabA_T114D*	pMK1 derivative used to express His-tagged fusion of MabA_T114D in mycobacteria	This work
pMK1_*mabA_T191D*	pMK1 derivative used to express His-tagged fusion of MabA_T191D in mycobacteria	This work
pSD26	Mycobacterial acetamide-inducible expression vector	(3)
pSD26_*mabA_WT*	pSD26 derivative used to induce His-tagged fusion of MabA in mycobacteria	This work
pSD26_*mabA_T191A*	pSD26 derivative used to induce His-tagged fusion of MabA_T191A in mycobacteria	This work
pSD26_*mabA_T191D*	pSD26 derivative used to induce His-tagged fusion of MabA_T191D in mycobacteria	This work

Table S2. Primers used in this study

Primers	**5' to 3' Sequence**[ab]
pMK1 MabA dir	accact<u>catatg</u>actgccacagccactgaag (NdeI)
pMK1 MabA rev	c<u>gaattc</u>tgcagcgtccttgtgttgtgtcagt (EcoRI)
pSD26 MabA dir	gtgactgccacagccactgaag
pSD26 MabA rev	gtggcccatacccatgccgc
MabA T21A dir	cccgttcagtcctggtt**g**ccggaggaaaccgggggatcgg
MabA T21A rev	ccgatcccccggtttcctccgg**c**aaccaggactgaacggg
MabA T114A dir	aggtcatcaacgccaacctc**g**ccggggcgttccgggtggc
MabA T114A rev	gccacccggaacgccccgg**c**gaggttggcgttgatgacct
MabA T191A dir	gctacatcgacaccgatatg**g**cccgcgcgctggatgagcg
MabA T191A rev	cgctcatccagcgcgcggg**c**catatcggtgtcgatgtagc
MabA T21D dir	atcccgttcagtcctggtt**ga**cggaggaaaccgggggatc
MabA T21D rev	gatcccccggtttcctccg**tc**aaccaggactgaacgggat
MabA T114D dir	ggtcatcaacgccaacctc**ga**cggggcgttccgggtggct
MabA T114D rev	agccacccggaacgccccg**tc**gaggttggcgttgatgacc
MabA T191D dir	ctacatcgacaccgatatg**ga**ccgcgcgctggatgagcgg
MabA T191D rev	ccgctcatccagcgcgcgg**tc**catatcggtgtcgatgtag

[a] Restriction sites are underlined and specified into brackets.
[b] Mutagenized bases are shown in bold.

References

1. Canova, M. J., Kremer, L., and Molle, V. (2008) *Plasmid* **60**(2), 149-153
2. Stover, C. K., de la Cruz, V. F., Fuerst, T. R., Burlein, J. E., Benson, L. A., Bennett, L. T., Bansal, G. P., Young, J. F., Lee, M. H., Hatfull, G. F., and et al. (1991) *Nature* **351**(6326), 456-460
3. Daugelat, S., Kowall, J., Mattow, J., Bumann, D., Winter, R., Hurwitz, R., and Kaufmann, S. H. (2003) *Microbes Infect* **5**(12), 1082-1095