Phosphorylation and Glycosylation by ECD

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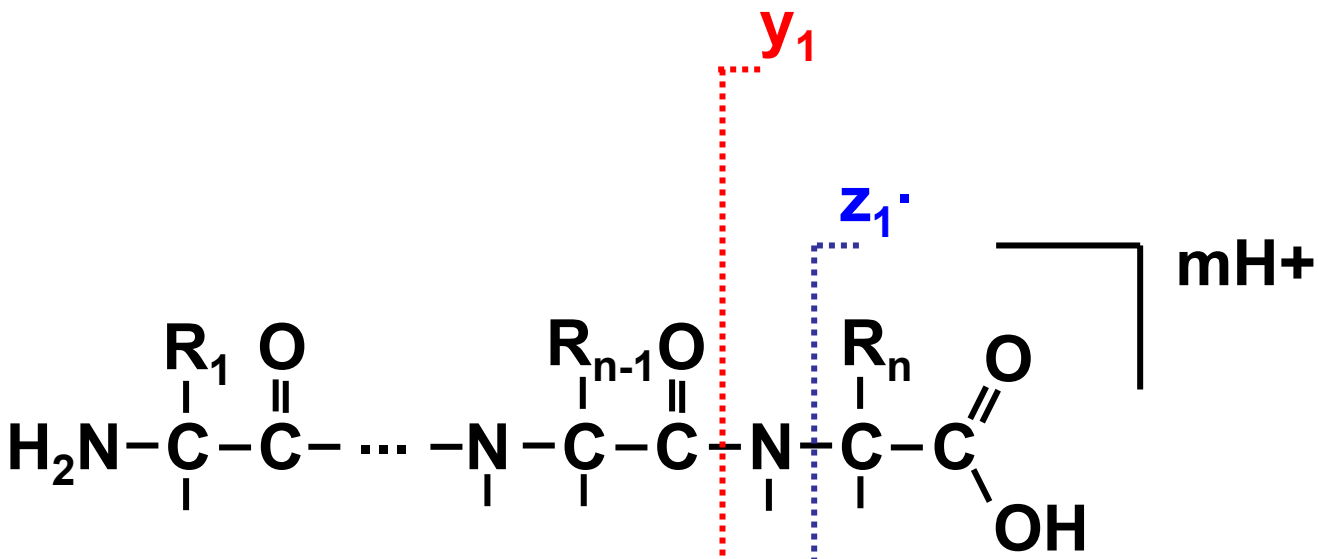
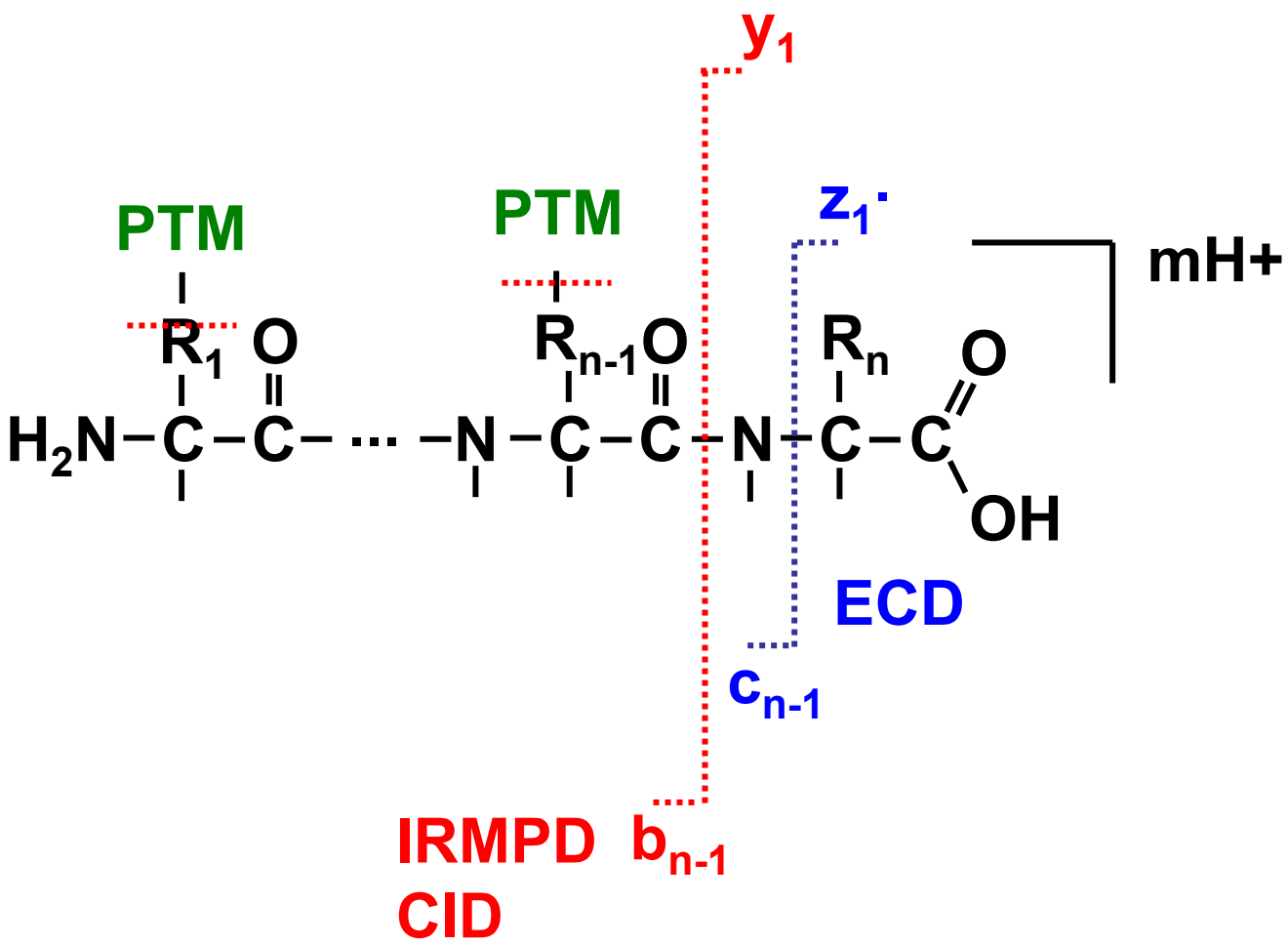
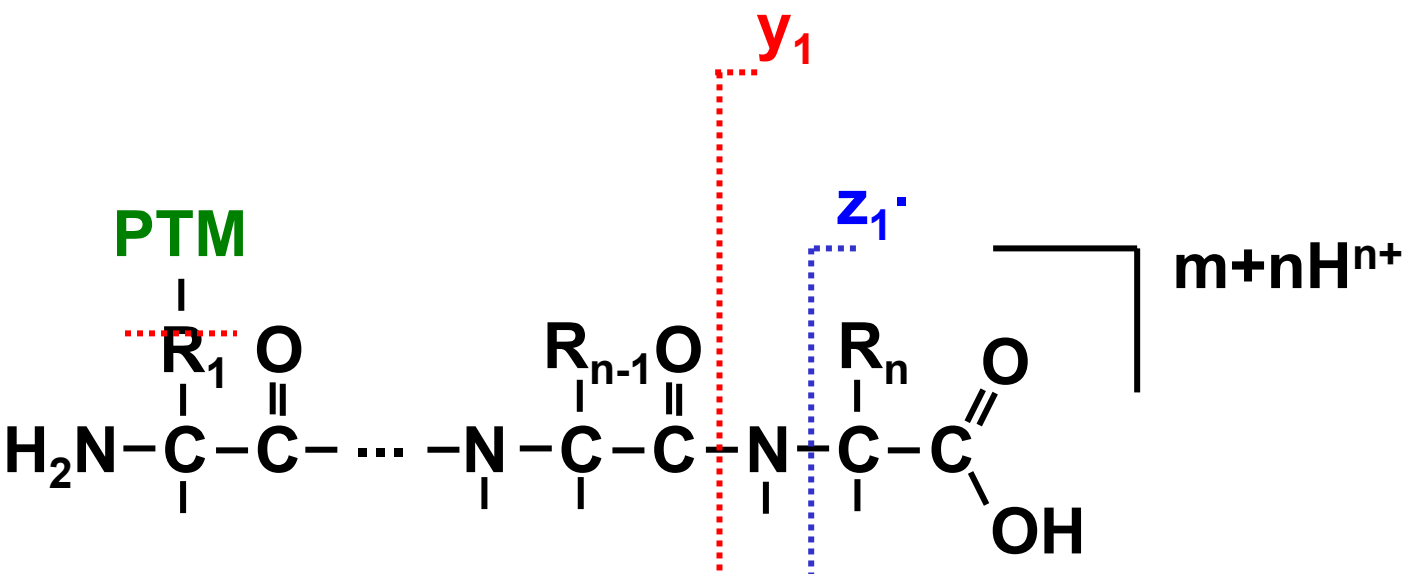
UAB DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR GENETICS

How do Peptides Cleave in the gas phase?



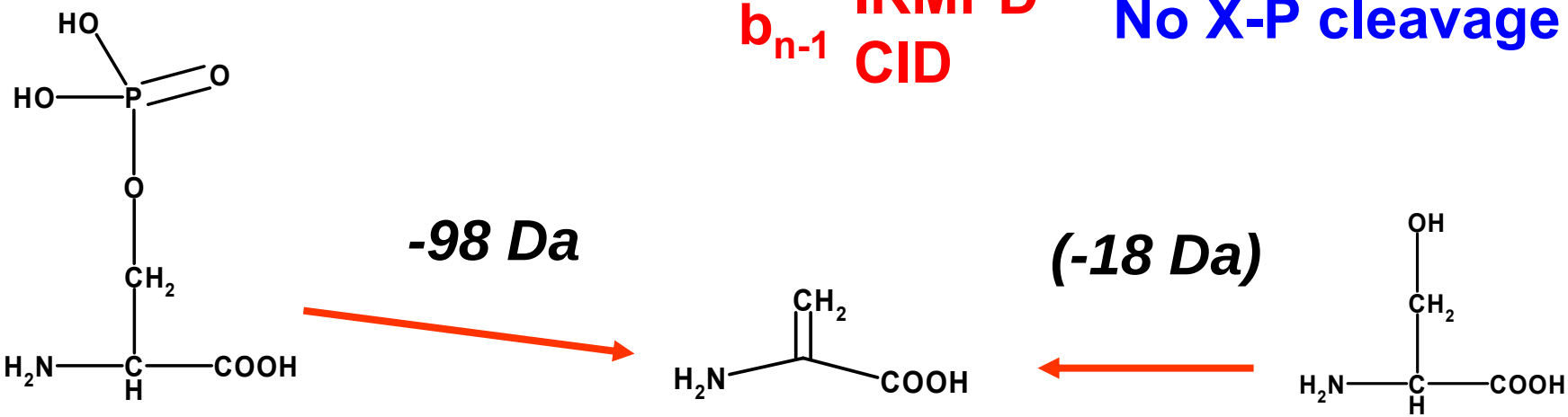
How do Peptides With Labile PTMs Cleave?

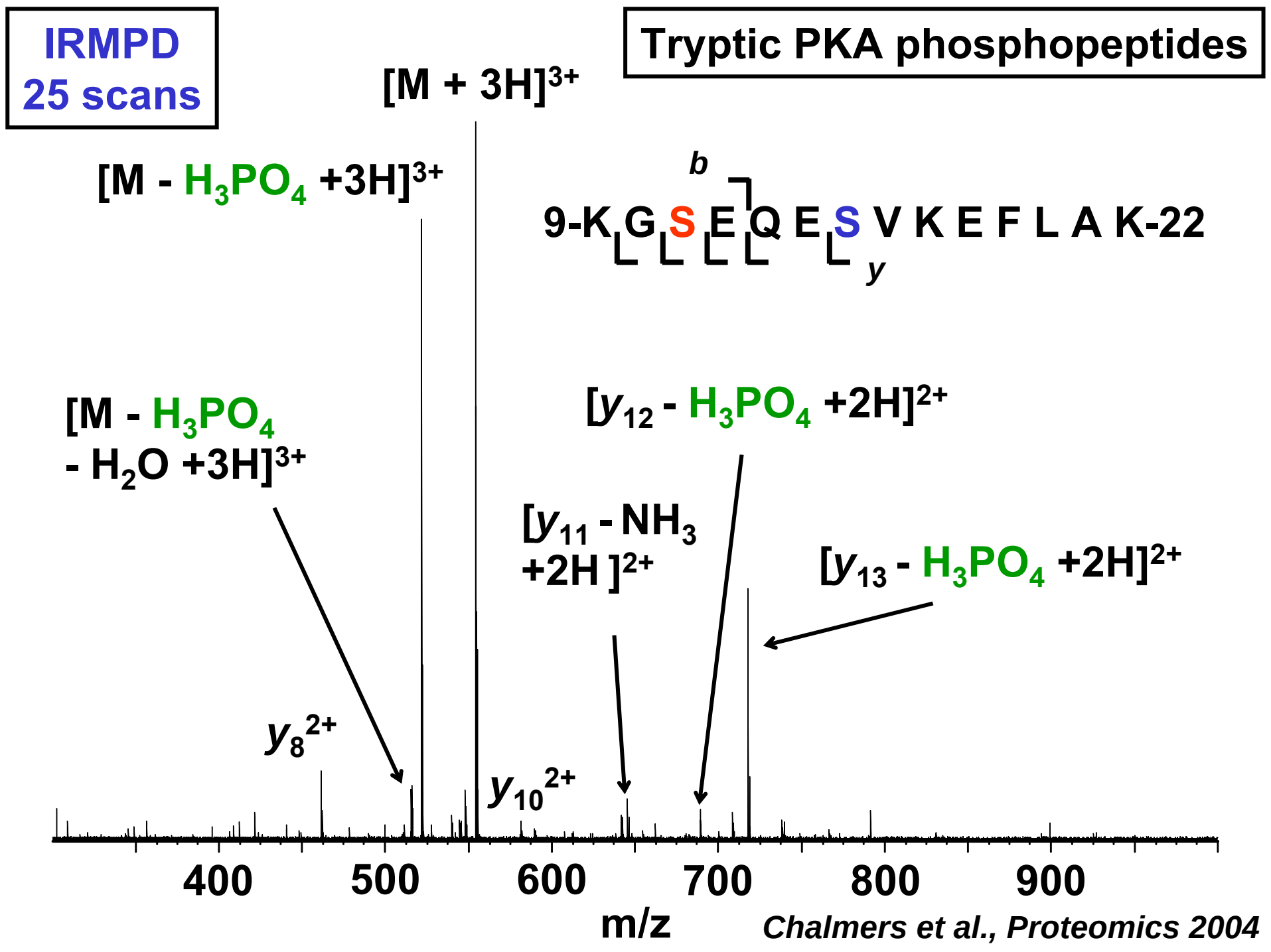


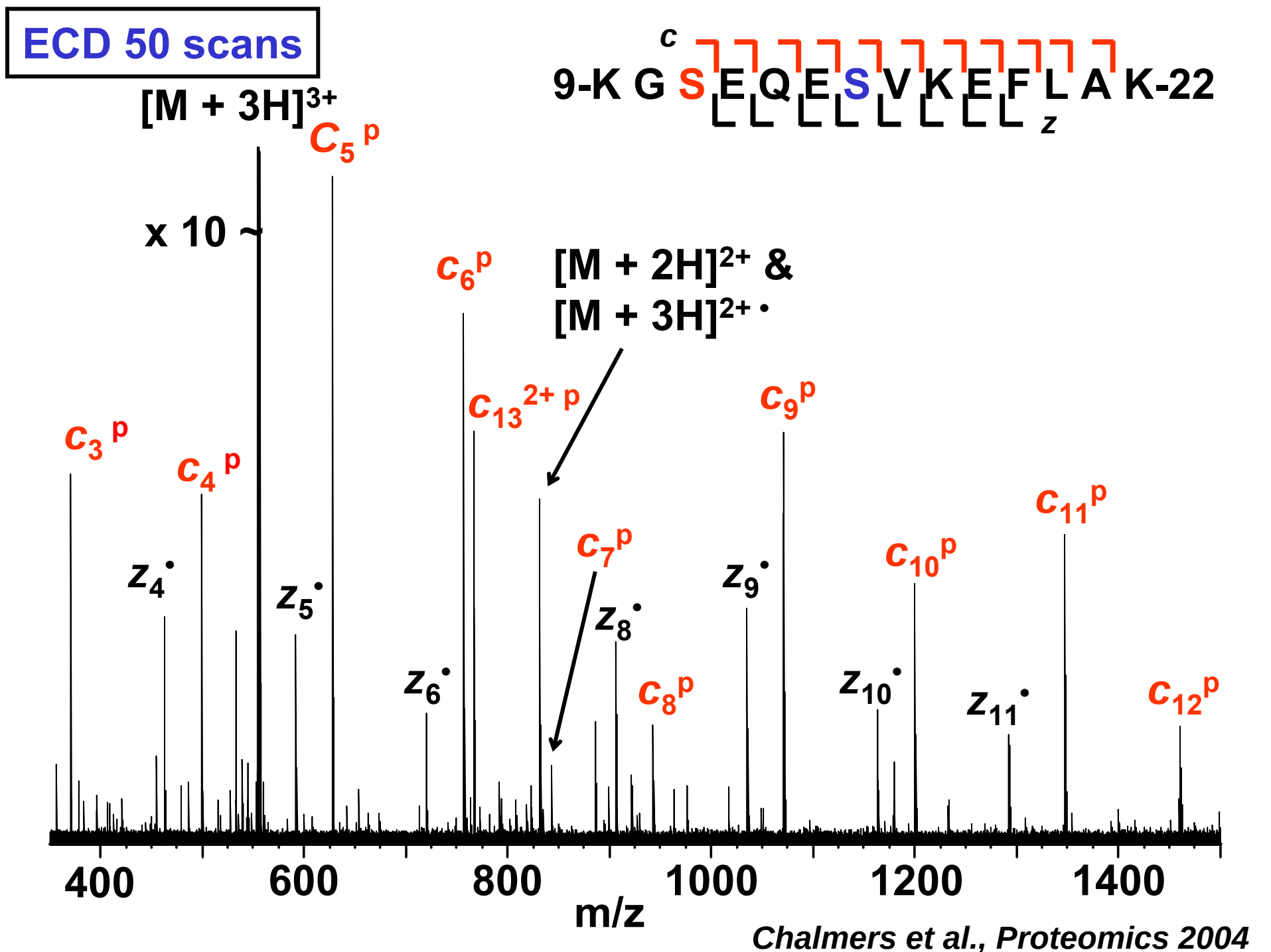


Facile loss of H_3PO_4
X-P cleavage preferred

Retention of labile modifications
No X-P cleavage

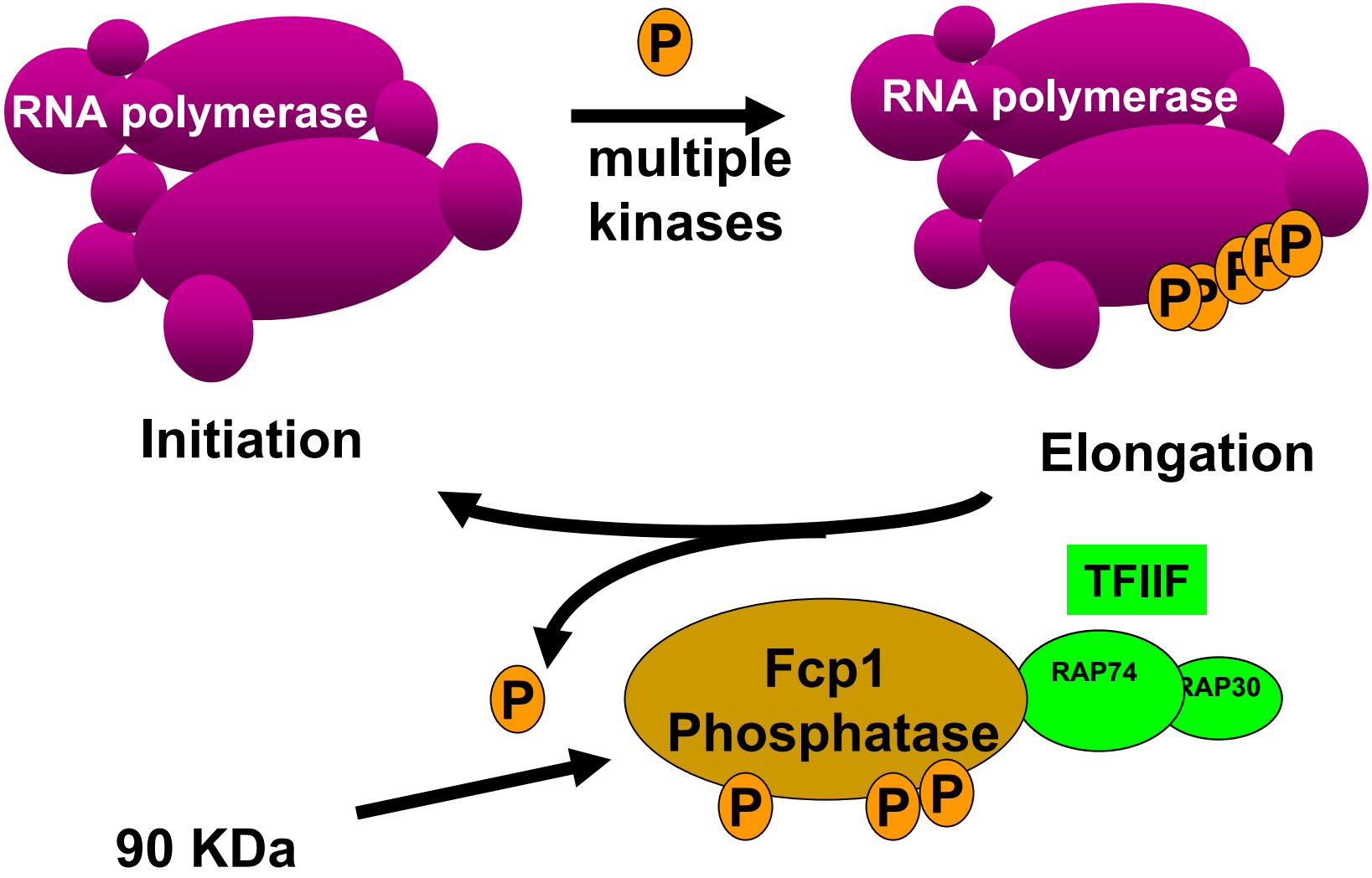



IRMPD
25 scans
Tryptic PKA phosphopeptides
[M + 3H]3+
[M - H3PO4 +3H]3+
b
9-K G S E Q E S V K E F L A K-22
y
[M - H3PO4
- H2O +3H]3+
[y12 - H3PO4 +2H]2+
[y11 - NH3
+2H]2+
[y13 - H3PO4 +2H]2+
y8 2+
y10 2+
400
500
600
700
800
900
m/z
Chalmers et al., Proteomics 2004

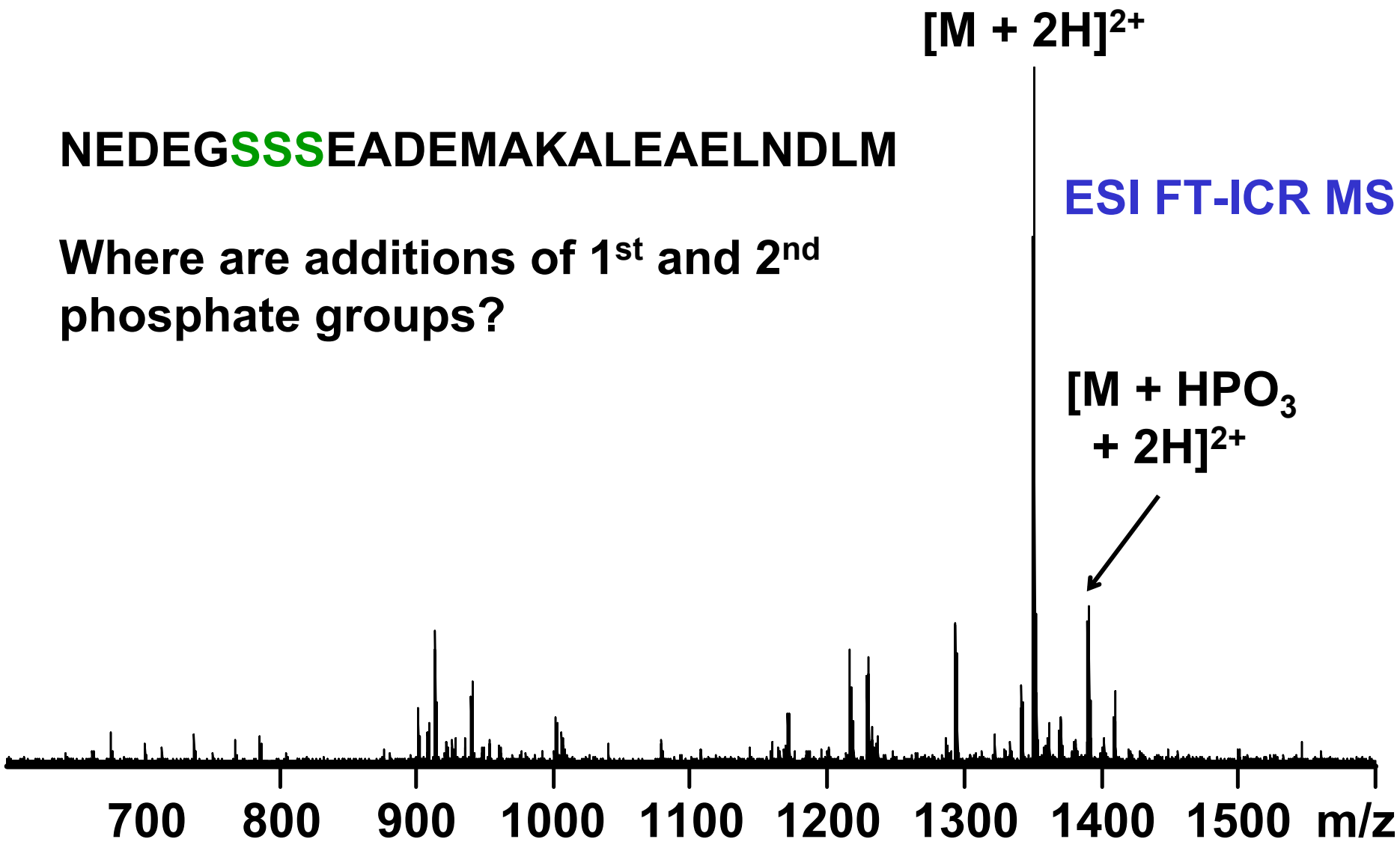


Chalmers et al., Proteomics 2004

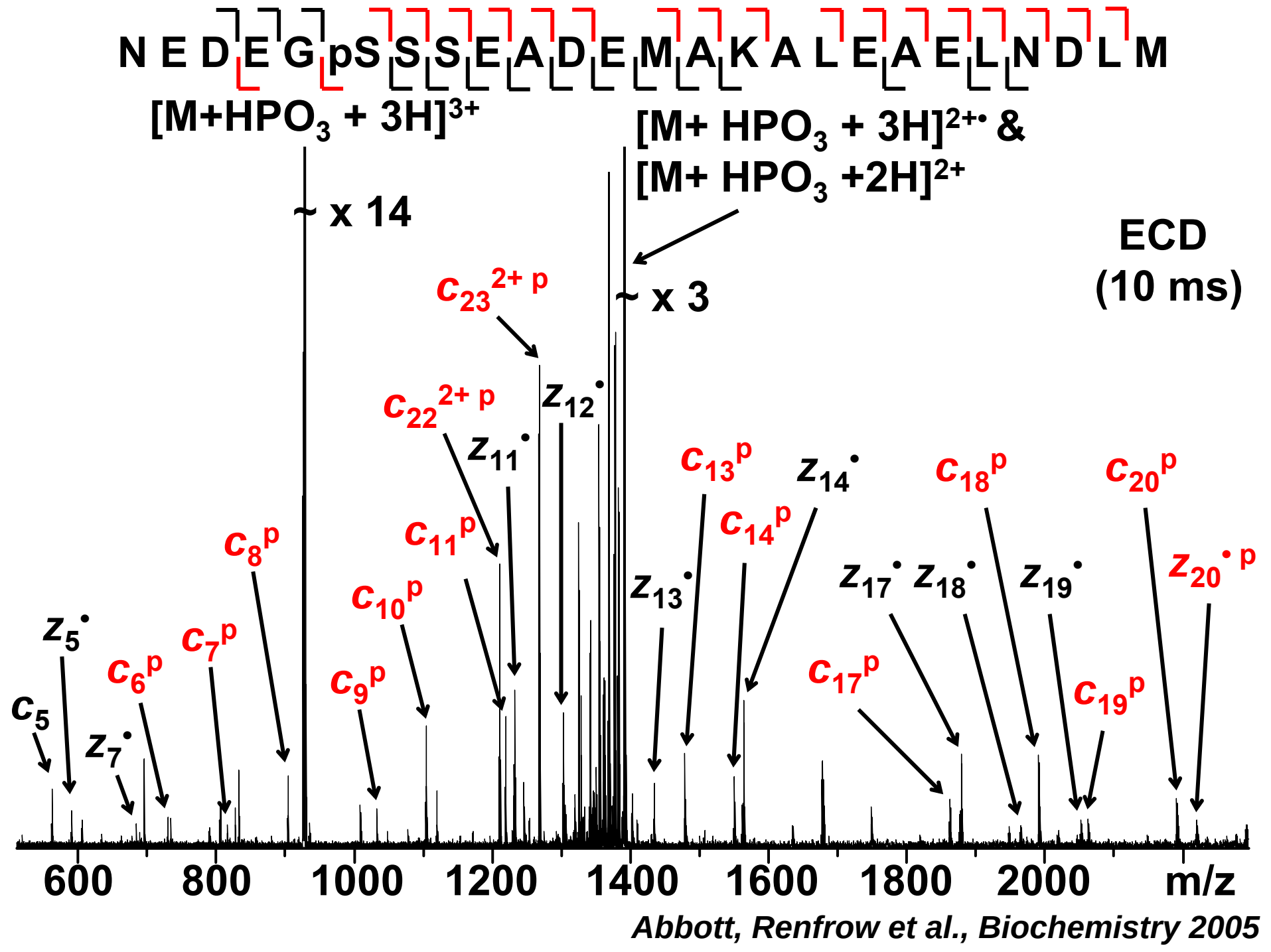
Dephosphorylation of RNA polymerase largest subunit



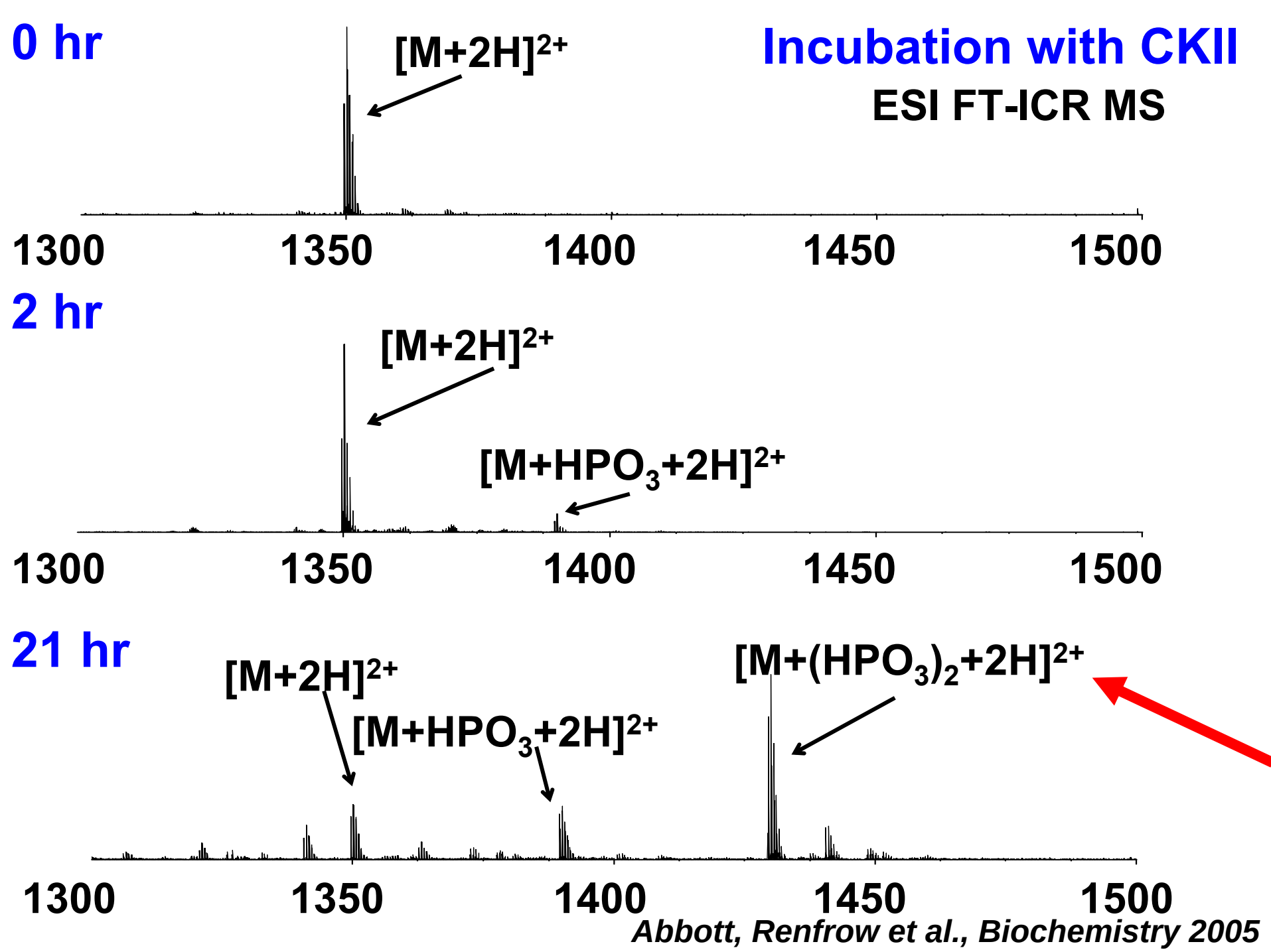
Fcp1 C-terminal fragment



Abbott, Renfrow et al., Biochemistry 2005

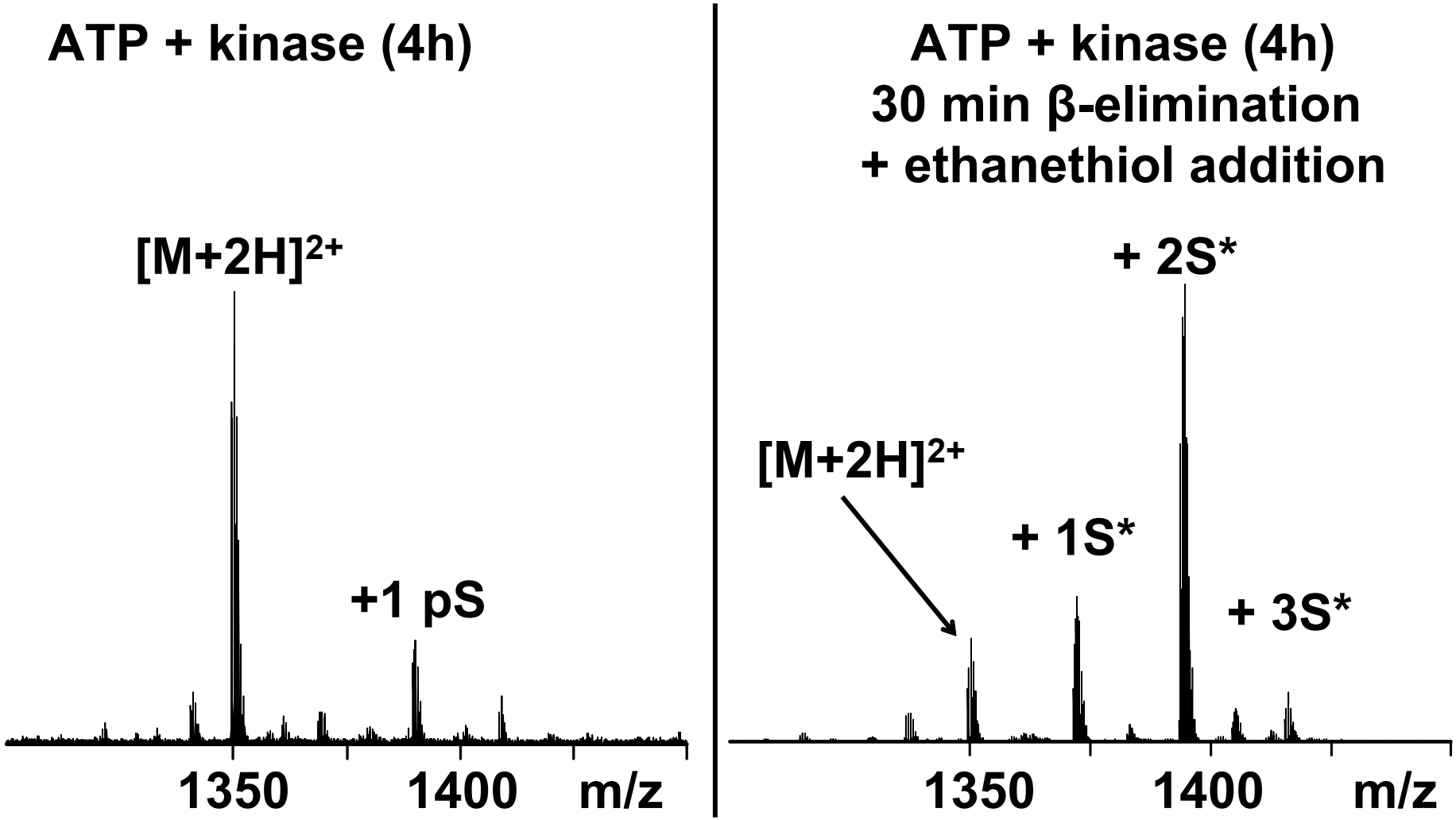


Abbott, Renfrow et al., Biochemistry 2005



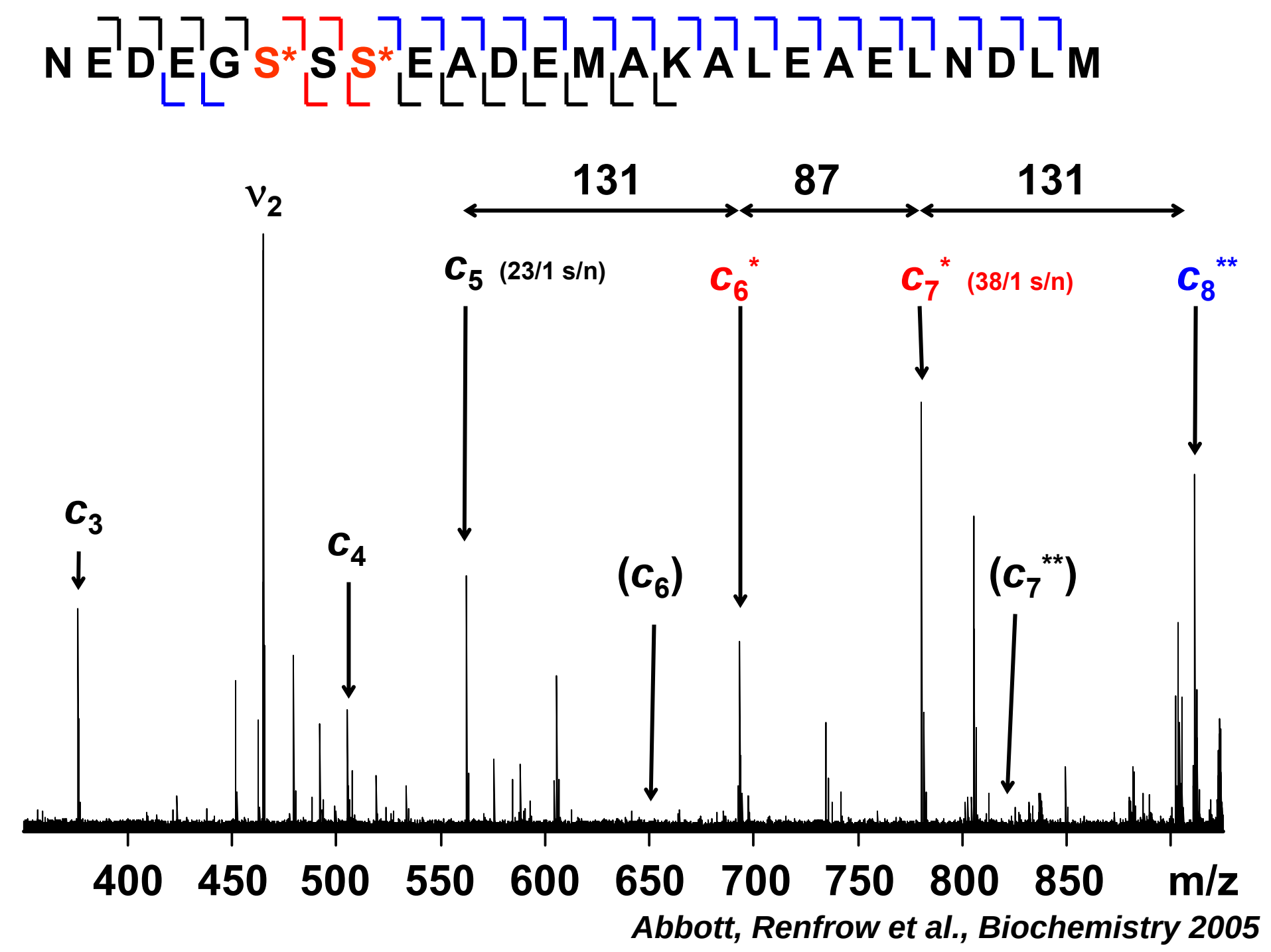
Abbott, Renfrow et al., Biochemistry 2005

Positive ion mode ESI MS



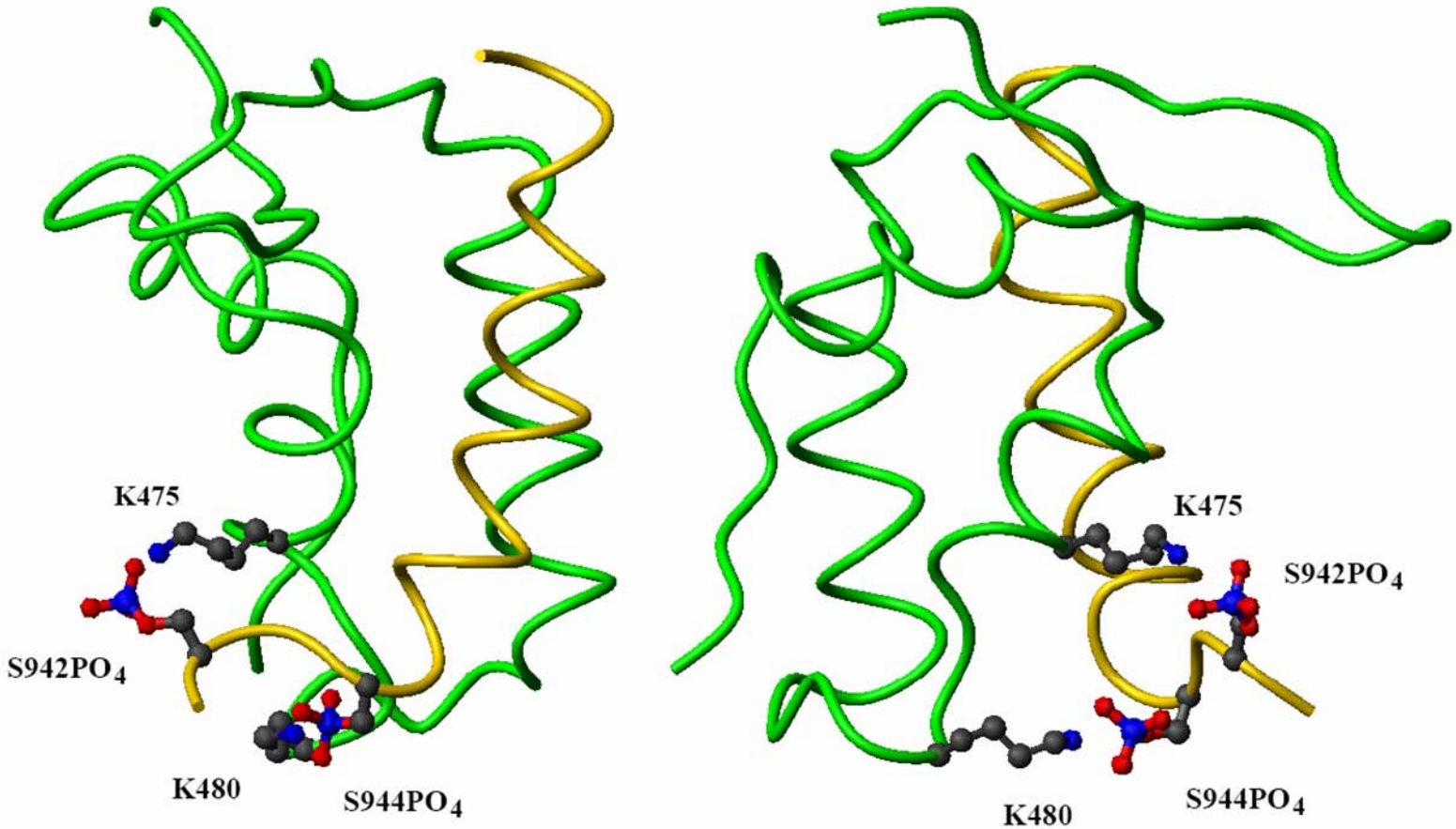
Molloy, M.P., Andrews, P.C. **Phosphopeptide derivatization signatures to identify serine and threonine phosphorylated peptides by mass spectrometry**. Anal. Chem. 2001, 73, 22, 5387-5394

Abbott, Renfrow et al., Biochemistry 2005

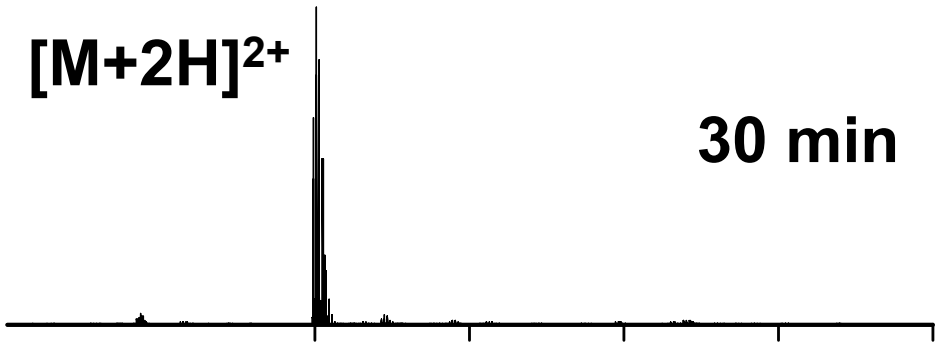


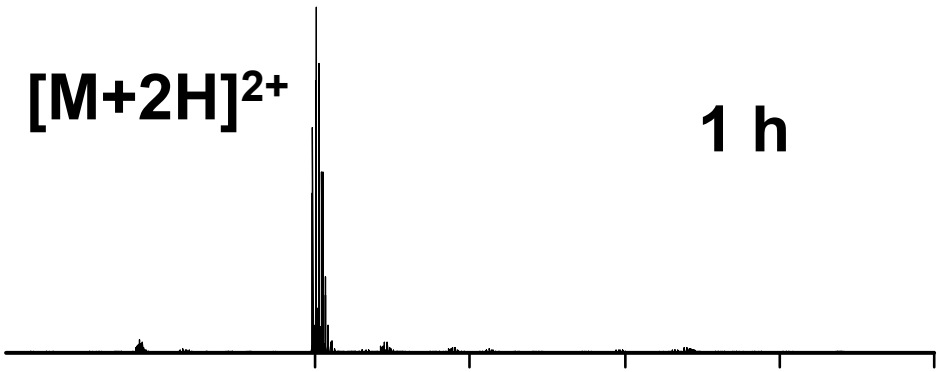
Abbott, Renfrow et al., Biochemistry 2005

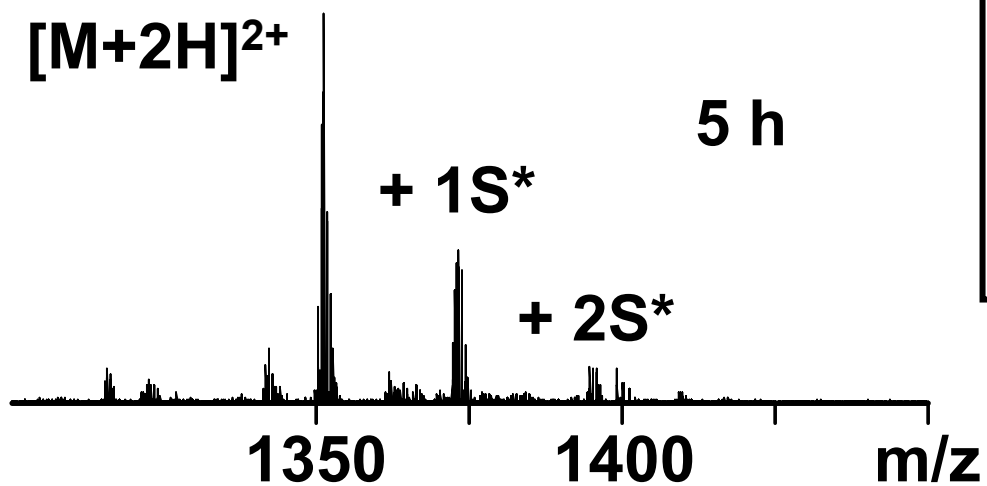
Fcp1A phosphatase CTD phosphorylation

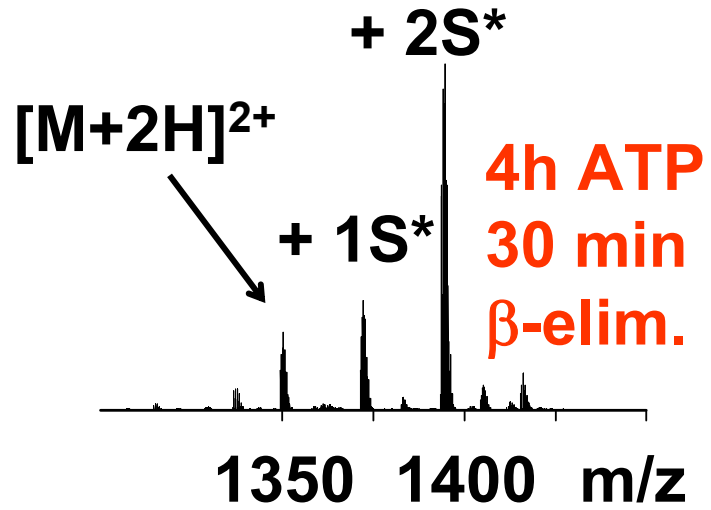


Abbott, K.L; Renfrow, M.B.; Chalmers, M.J.; Nguyen, B.D.; Marshall, A.G., Legault, P.; Omichinski, J.G. "Enhanced Binding of RNAP II CTDPhosphatase FCP1 to RAP74 following CK2 phosphorylation" *Biochemistry*, submitted







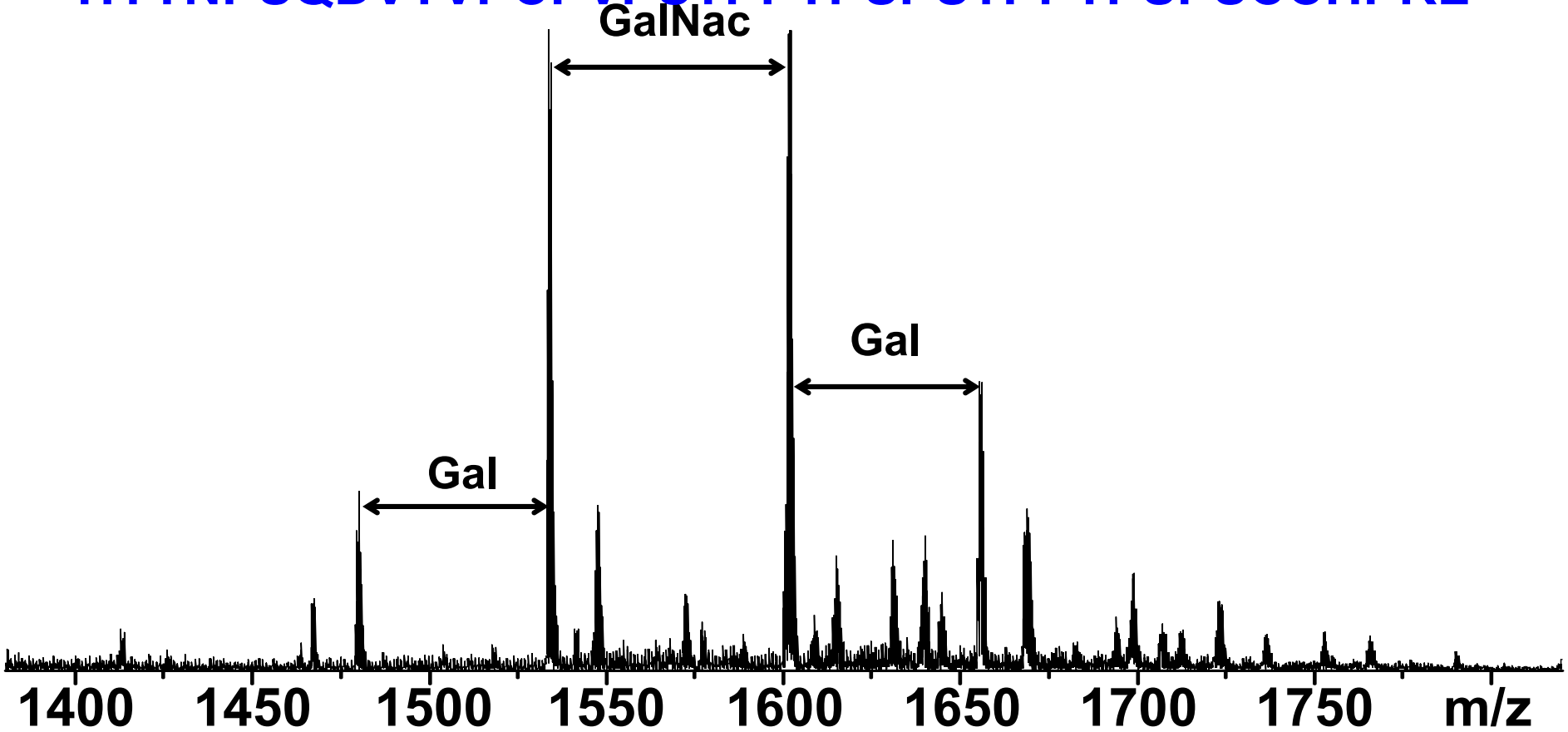


Control
No kinase or ATP

Beta elimination
+ ethanethiol addition
time course

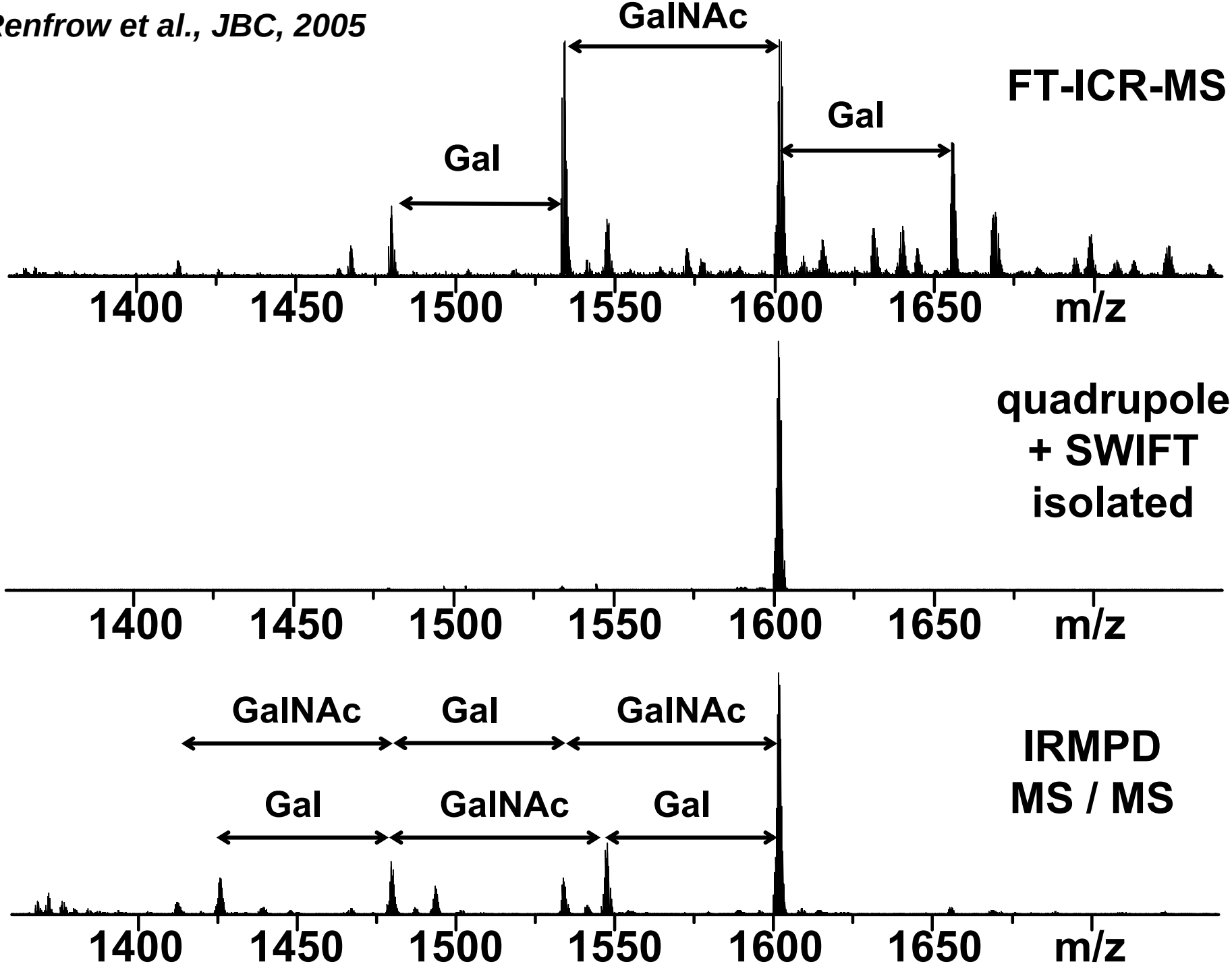
ESI FT-ICR MS of (Mce) IgA1 HR isolated from trypsin-pepsin digest

HYTNPSQDVTVPCPVPSTPPTPSPSTPPTPSPSCCHPRL

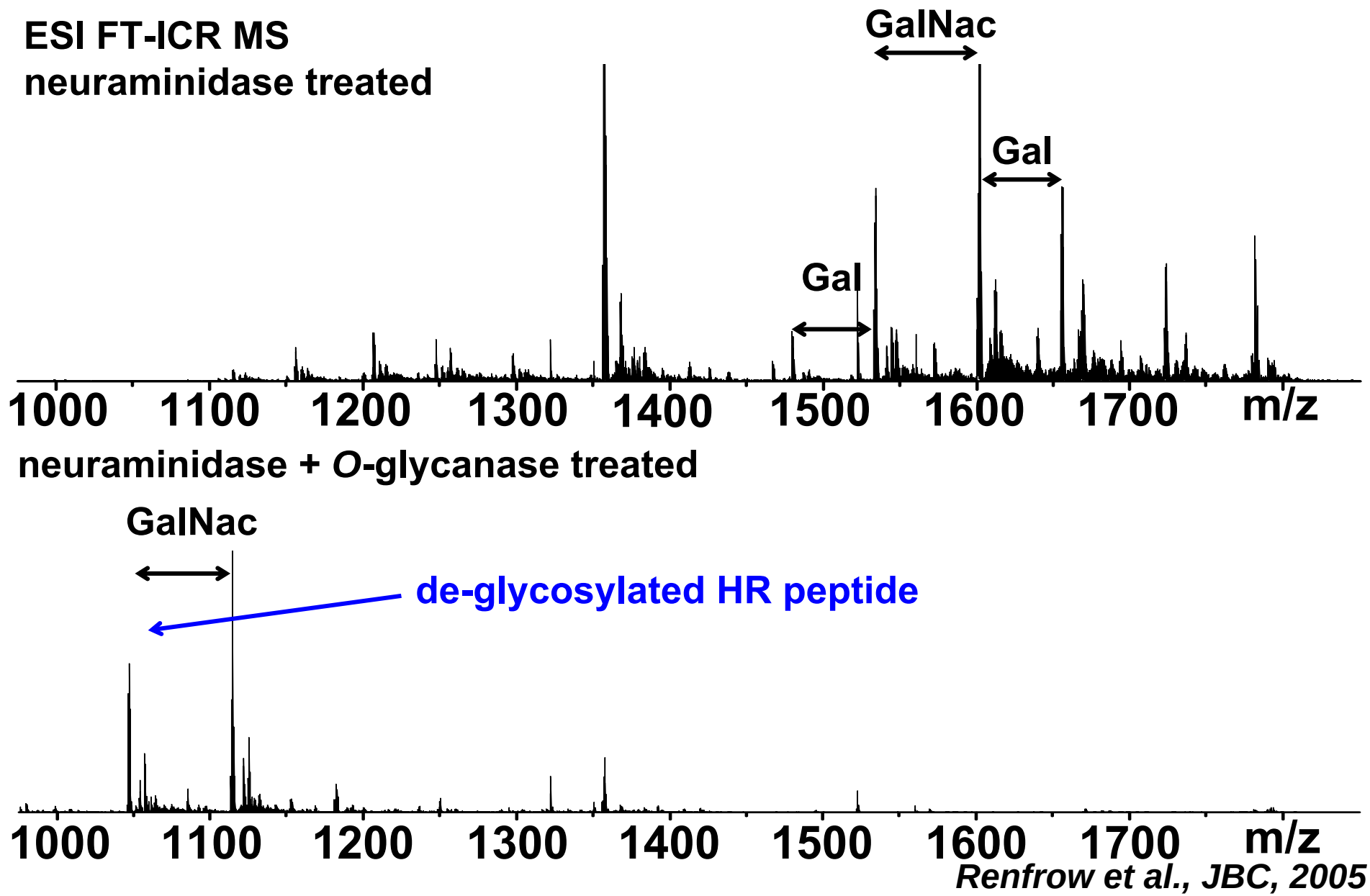


Renfrow et al., JBC, 2005

Renfrow et al., JBC, 2005



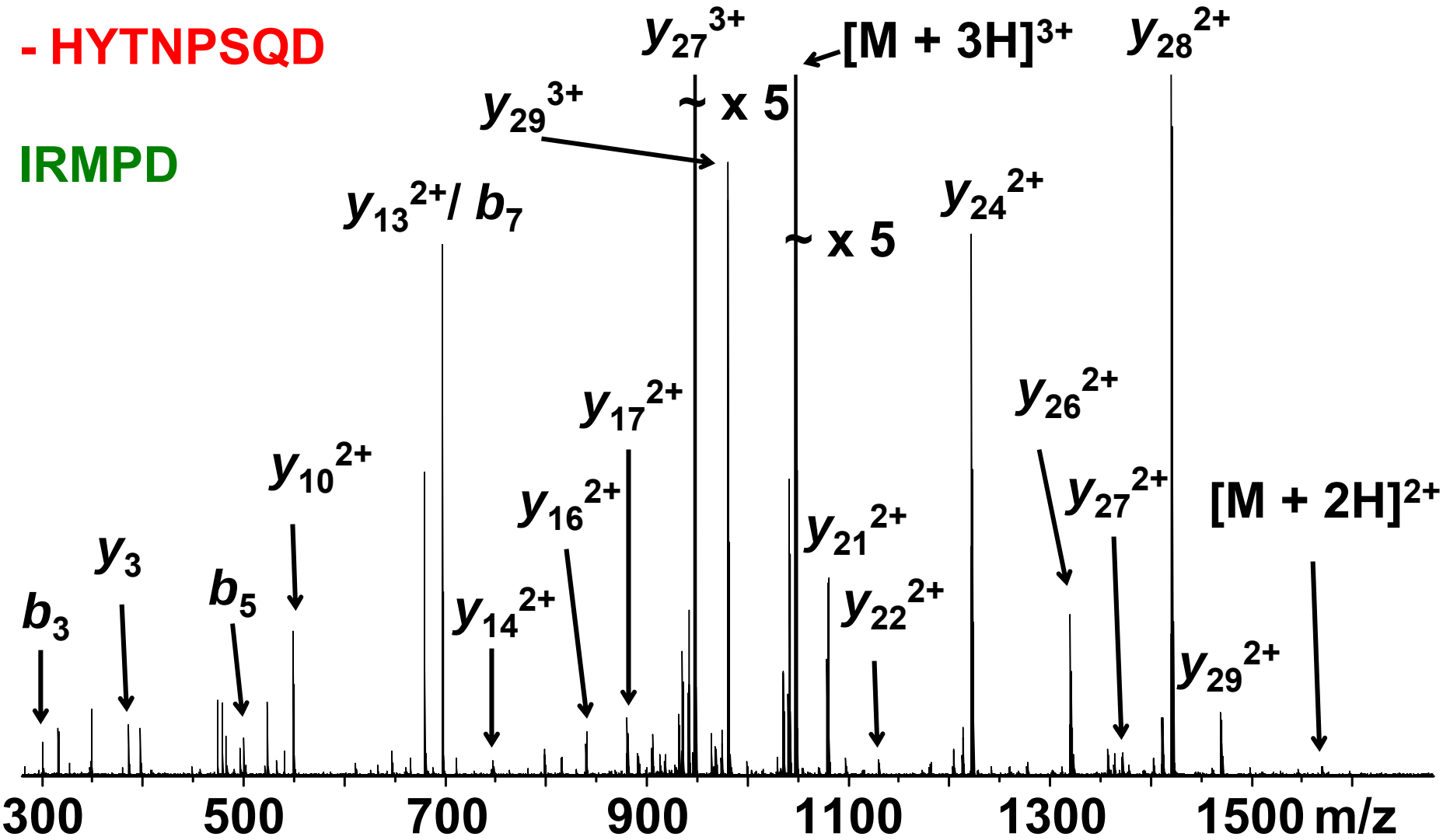
De-glycosylated IgA1 Hinge Region



Renfrow et al., JBC, 2005

Confirmed IgA1 Hinge Region sequence

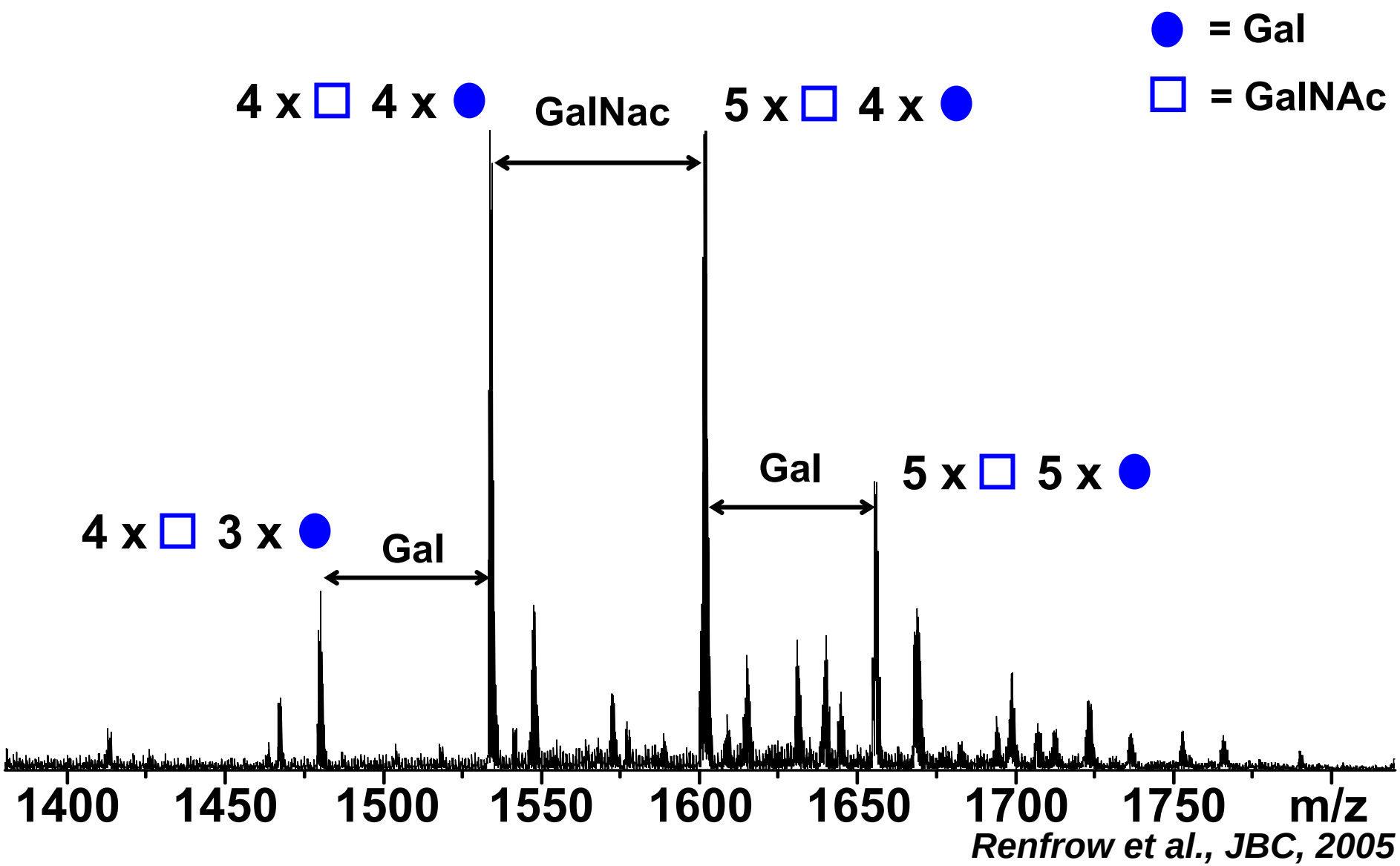
V T V P C P V P S T P P T P S P S T P P T P S P S C C H P R L



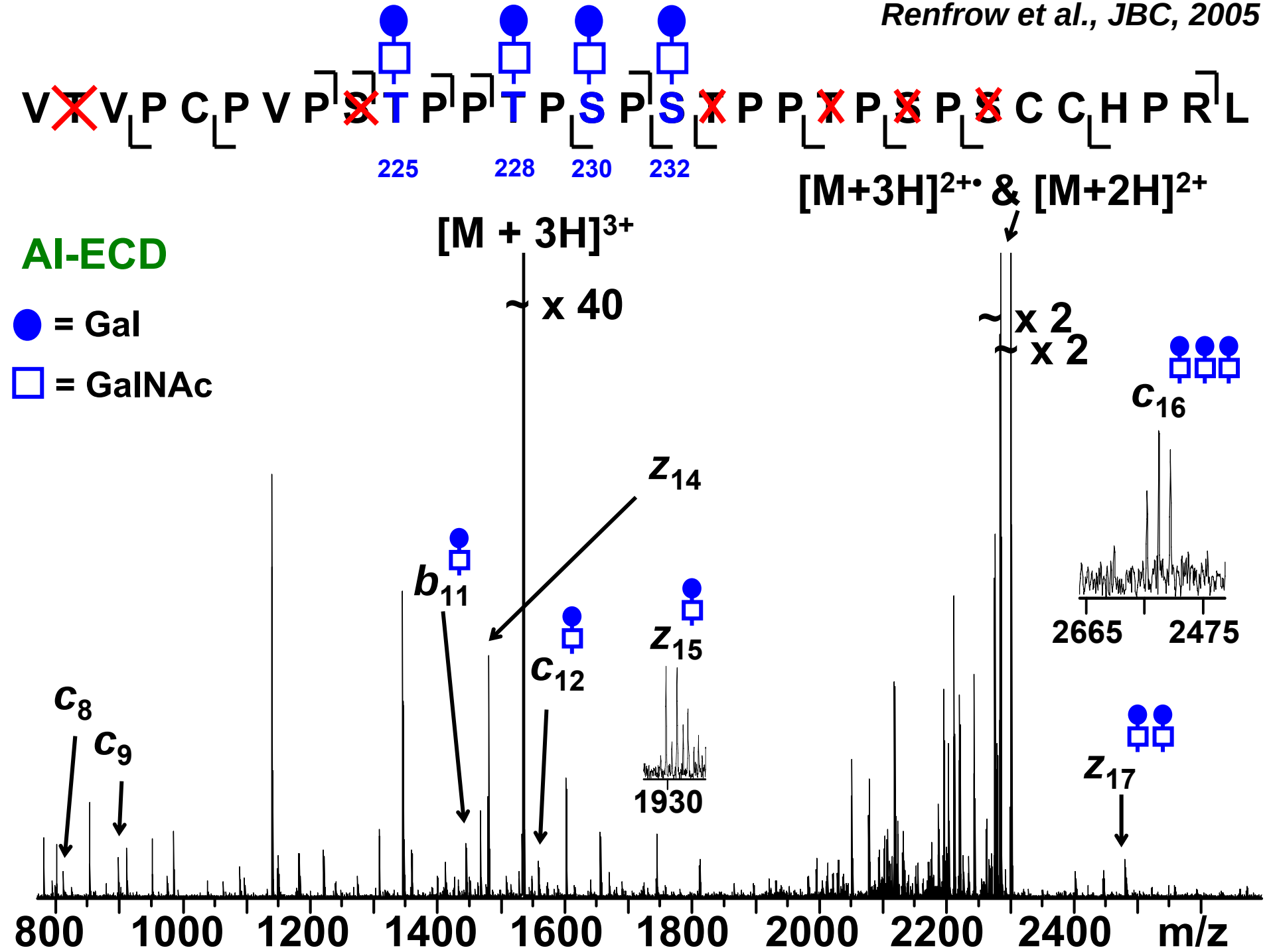
Renfrow et al., JBC, 2005

GlycoMod assigned *O*-glycans

V T V P C P V P S T P P T P S P S T P P T P S P S C C H P R L



Renfrow et al., JBC, 2005


Renfrow et al., JBC, 2005
V T V P C P V P S T P P T P S P S X P P X P S P S C C H P R L
225
228
230
232
[M+3H]2+• & [M+2H]2+
[M + 3H]3+
AI-ECD
= Gal
= GalNAc
~ x 40
~ x 2
~ x 2
c16
z14
b11
z15
c12
c8
c9
2665
2475
1930
z17
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z

Analyzing Phosphorylation and Glycosylation by ECD FT-ICR MS

- **Some PTM's can be so labile they are the dominant fragment in a CID MS/MS (*i.e.* no useful information).**

- **Electron Capture Dissociation (ECD) fragments peptides and proteins by a different mechanism, leaving labile PTM's intact**

- **While ECD is performed in an FT-ICR MS, a new method, Electron Transfer Dissociation (ETD) is performed in a 2D and 3D ion trap (as quickly as CID in an ion trap).**