Novel Mass Spectrometric Method for Phosphorylation Quantification Using Cerium Oxide Nanoparticles and Tandem Mass Tags

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INTRODUCTION

Reversible protein phosphorylation/dephosphorylation plays a significant role in regulating a wide range of cellular processes.

Recently mass spectrometry based methods have been used to identify and determine stoichiometric level of phosphosites.

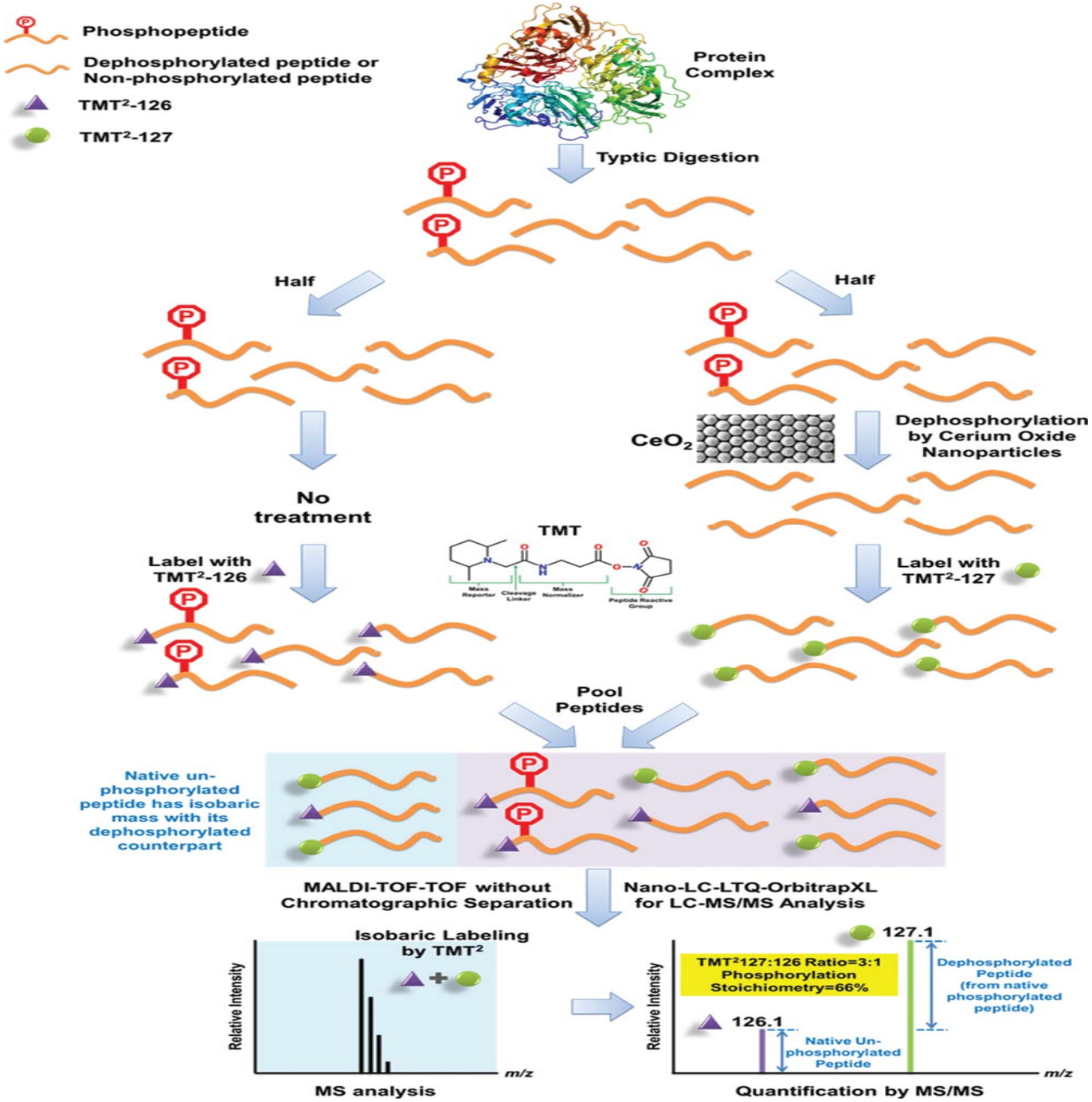
To date several recognized methods are used to quantify phosphorylation levels:
Stable isotope labeling with amino acids in cell culture (SILAC)
Isotope-coded affinity tag (iCAT)
Isotope tags for relative and absolute quantitation (iTRAQ)
mTRAQ (a developed variation of iTRAQ)
Tandem mass tagging (TMT)

These are much accurate for quantifying differences arising from varying biological conditions and are not directly configured to quantify absolute phosphorylation levels.

Biochemical methods such as radiolabeling of ^{32}P or Western blotting have been traditionally used to quantify phosphorylation levels. But both are time consuming.

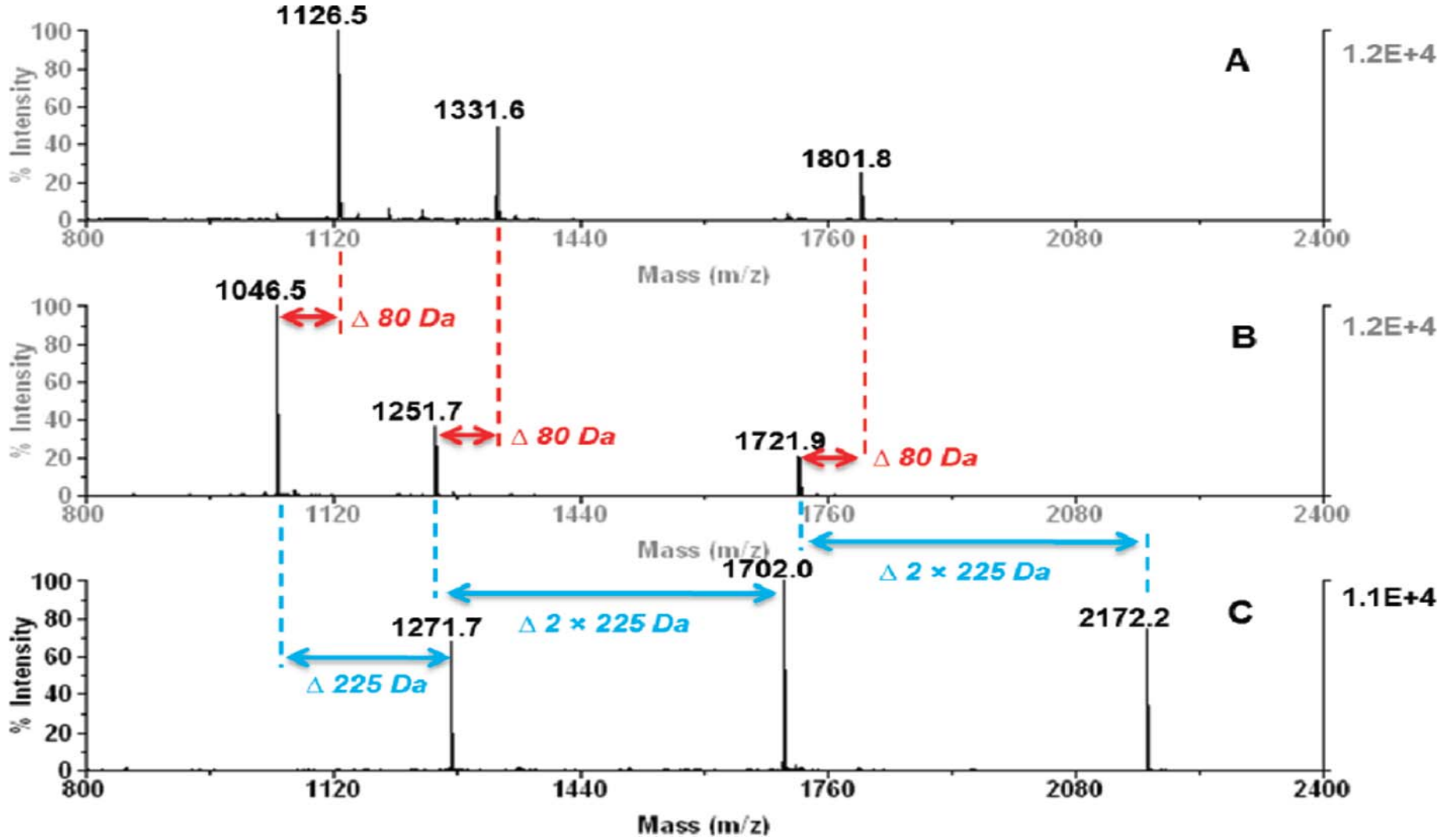
In this study, they have developed a method using CeO_2 nanoparticle and tandem mass tag to quantify the phosphorylation levels.

SCHEMATIC OVERVIEW OF THE PROTOCOL



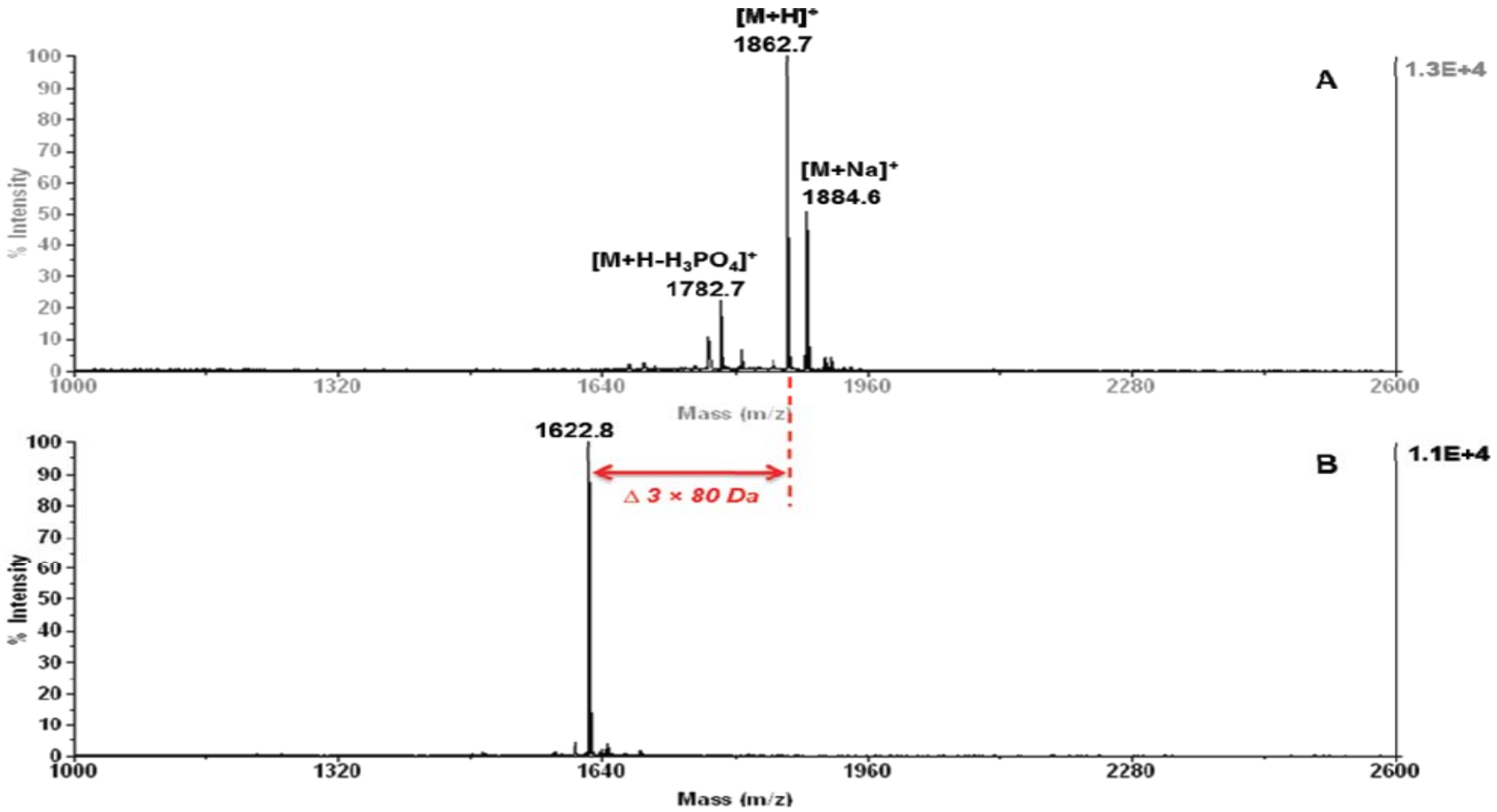
Overall level of phosphorylation:
(1-1/r) x 100%
R= TMT^{127}/TMT^{126}

Evaluation of Cerium Oxide Nanoparticle Directed Dephosphorylation Efficiency and Assessment of TMT Labeling Efficiency

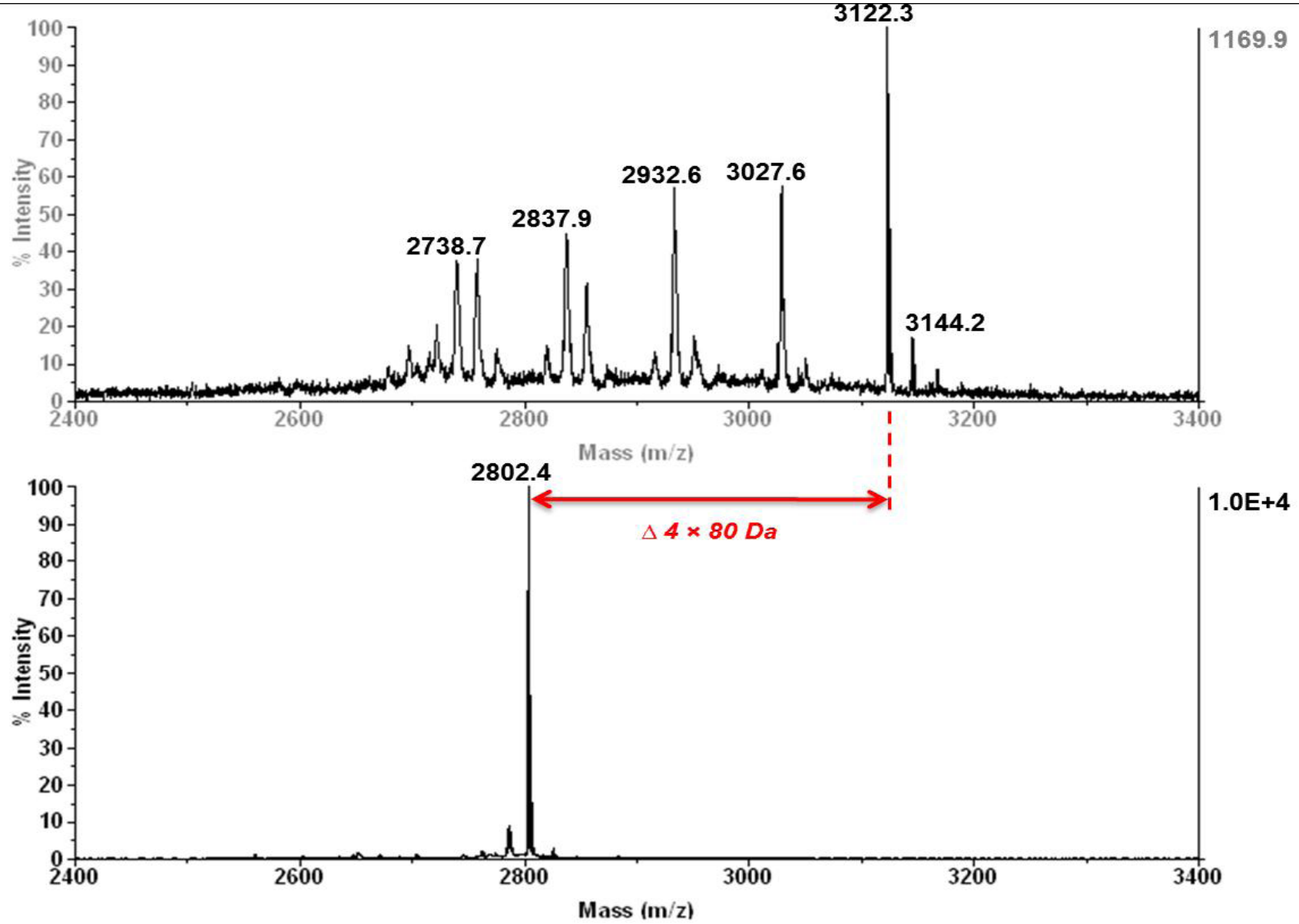


MALDI-TOF mass spectra of 1 pmol phosphopeptide mixture (DRVpYIHPF $[M + H]^+$ 1126.5 *m/z*, IKNLQpSLDPSH $[M + H]^+$ 1331.6 *m/z*, and DFNKFHpTFPQTAIGV $[M + H]^+$ 1801.8 *m/z*)

Efficiency towards multiphosphopeptides

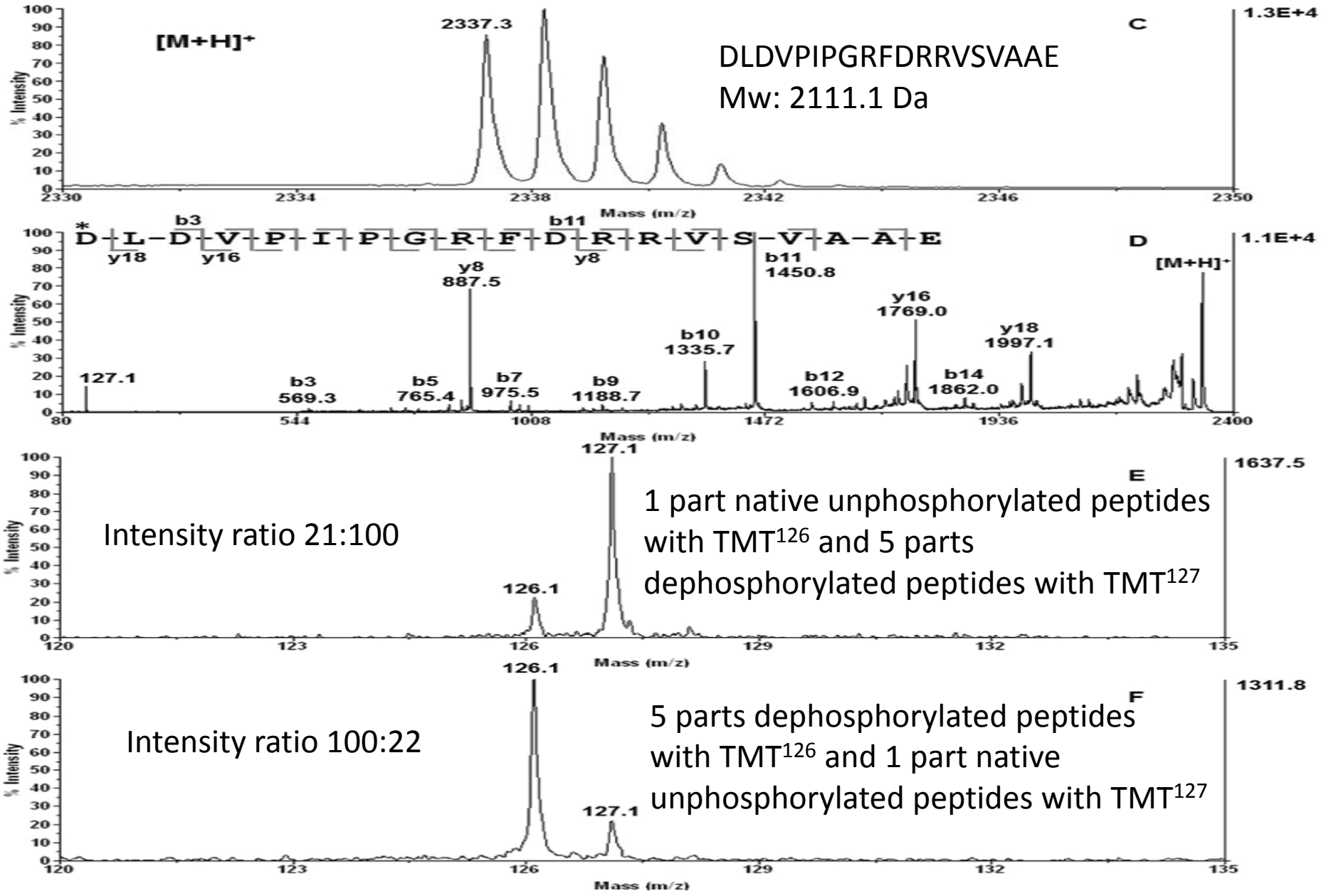


MALDI-TOF mass spectra of 1 pmol triphosphopeptide (TRDI-pY-ETD-pY-pY-RK, $[M + H]^+$ 1862.7 *m/z*)

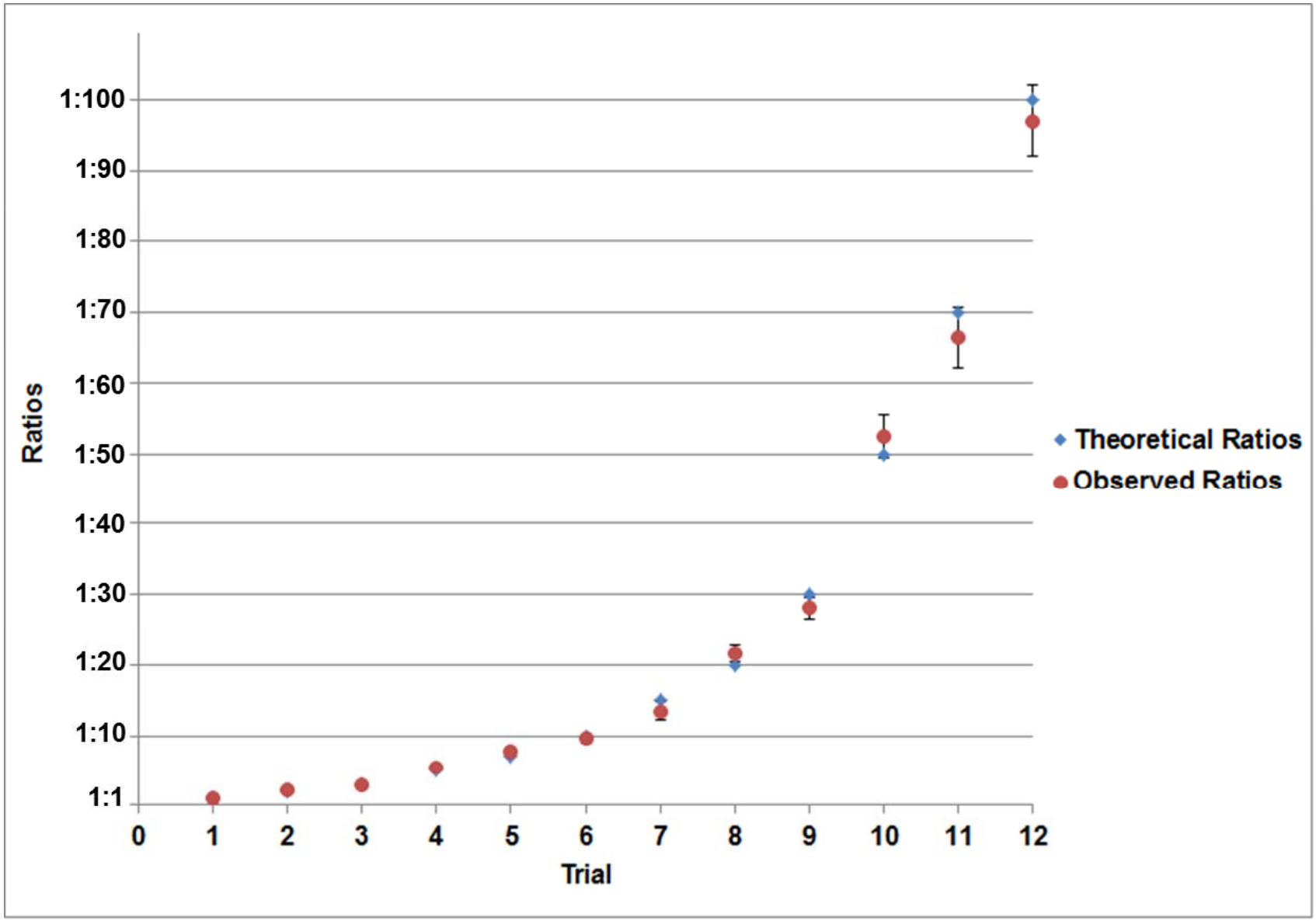


MALDI-TOF mass spectra of 1 pmol tetraphosphopeptide (RELEELNVPGEIVE-pS-L-pS-pS-pS-EESITR, $[M+H]^+$ 3122.3 *m/z)*

Competency of TMT-Duplex Labeling on Either Dephosphorylated Peptides or Native Unphosphorylated Peptides



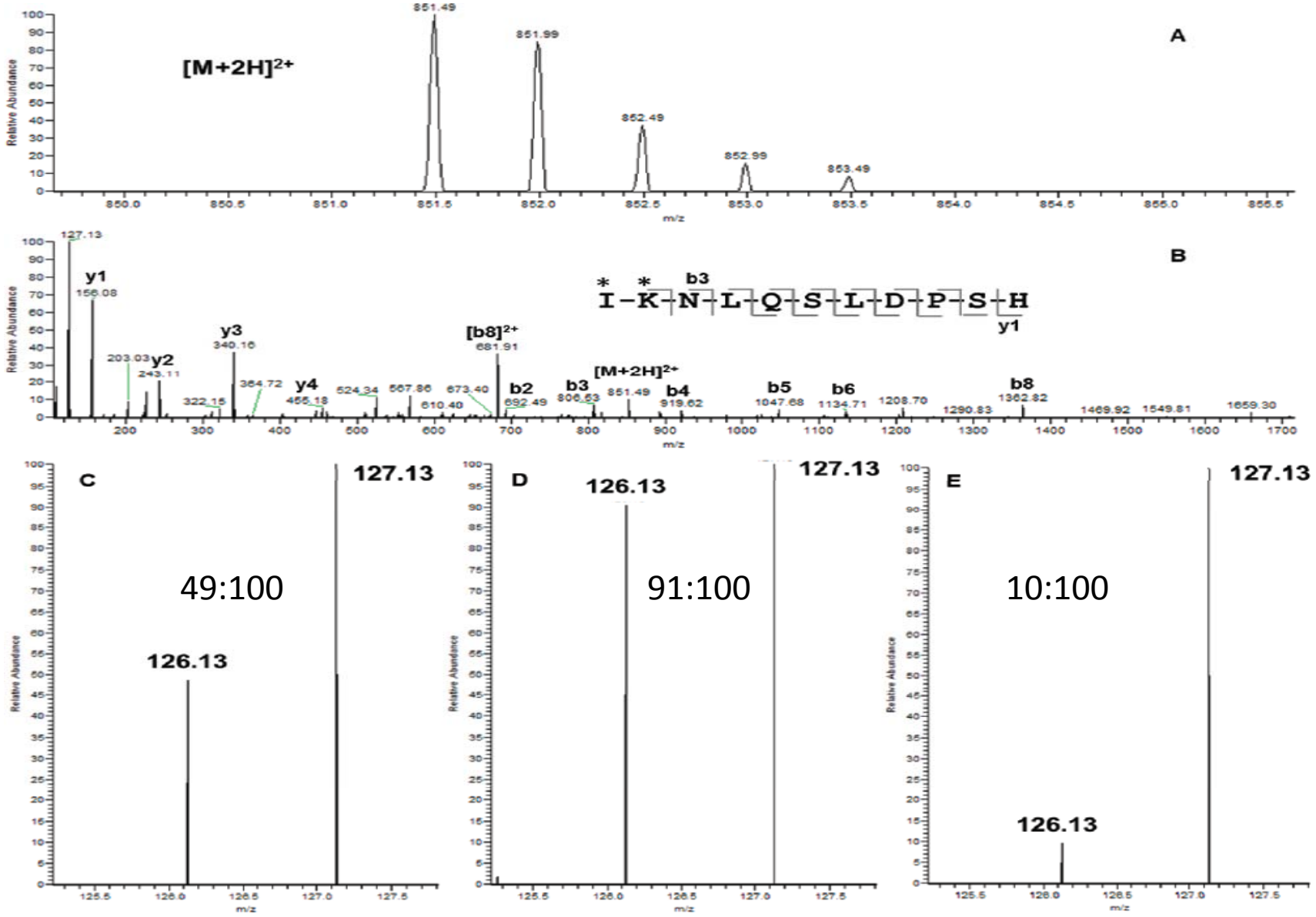
Evaluation of TMT-Duplex Labeling Efficiency Using the Nano-LC-LTQ-OrbitrapXL

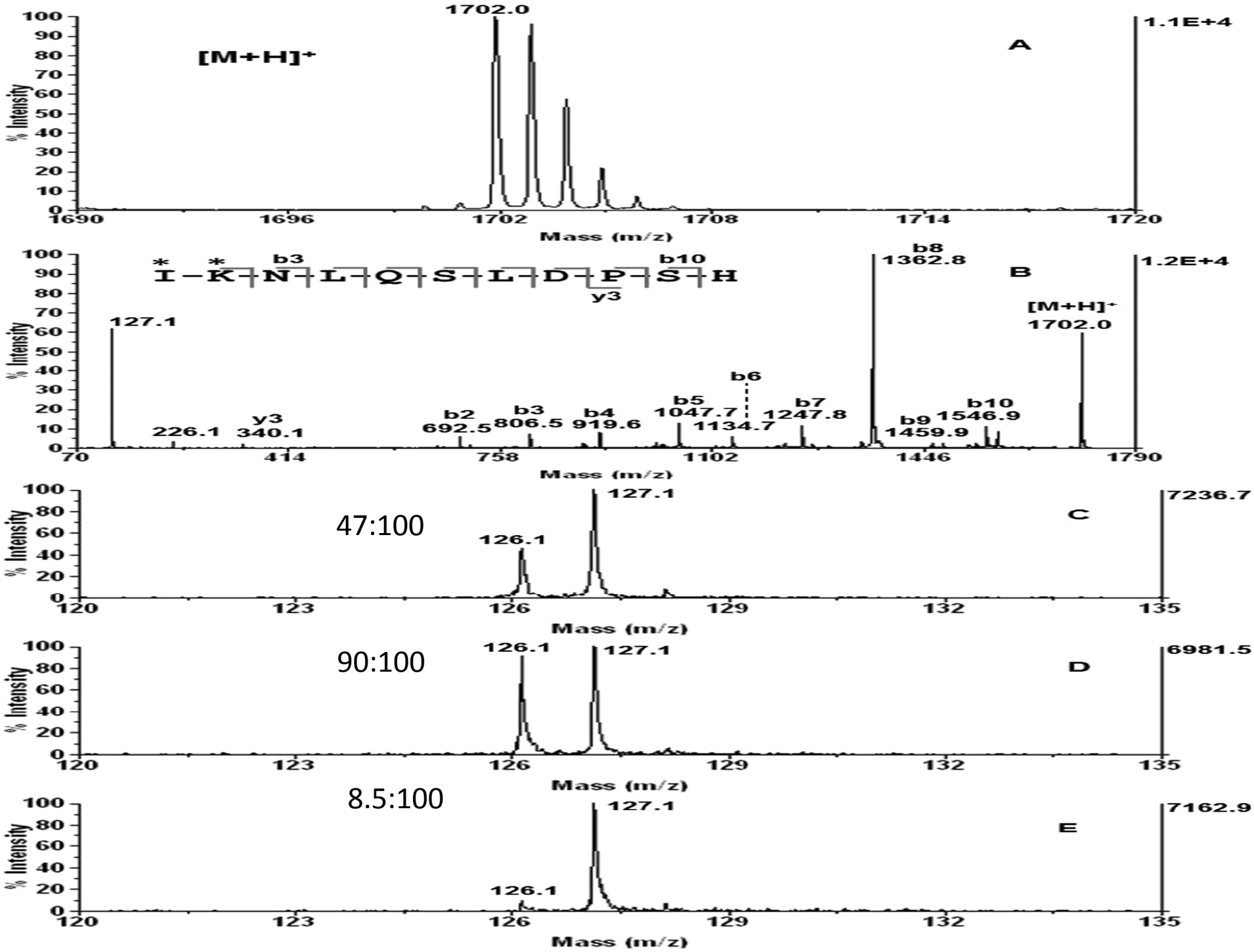


Trypsin digested BSA have been used for the labellng with TMT^{126} and TMT^{127}

Analysis of Premixed Phosphopeptides Mixture on MALDI-TOF-TOF and LTQ-OrbitrapXL

The phosphopeptides DRVpYIHPF, IKNLQpSLDPSH, and DFNKFHpTFPQTAIGV, along with their nonphosphorylated counterparts, were mixed in varying amounts (1:9, 1:1, and 9:1)



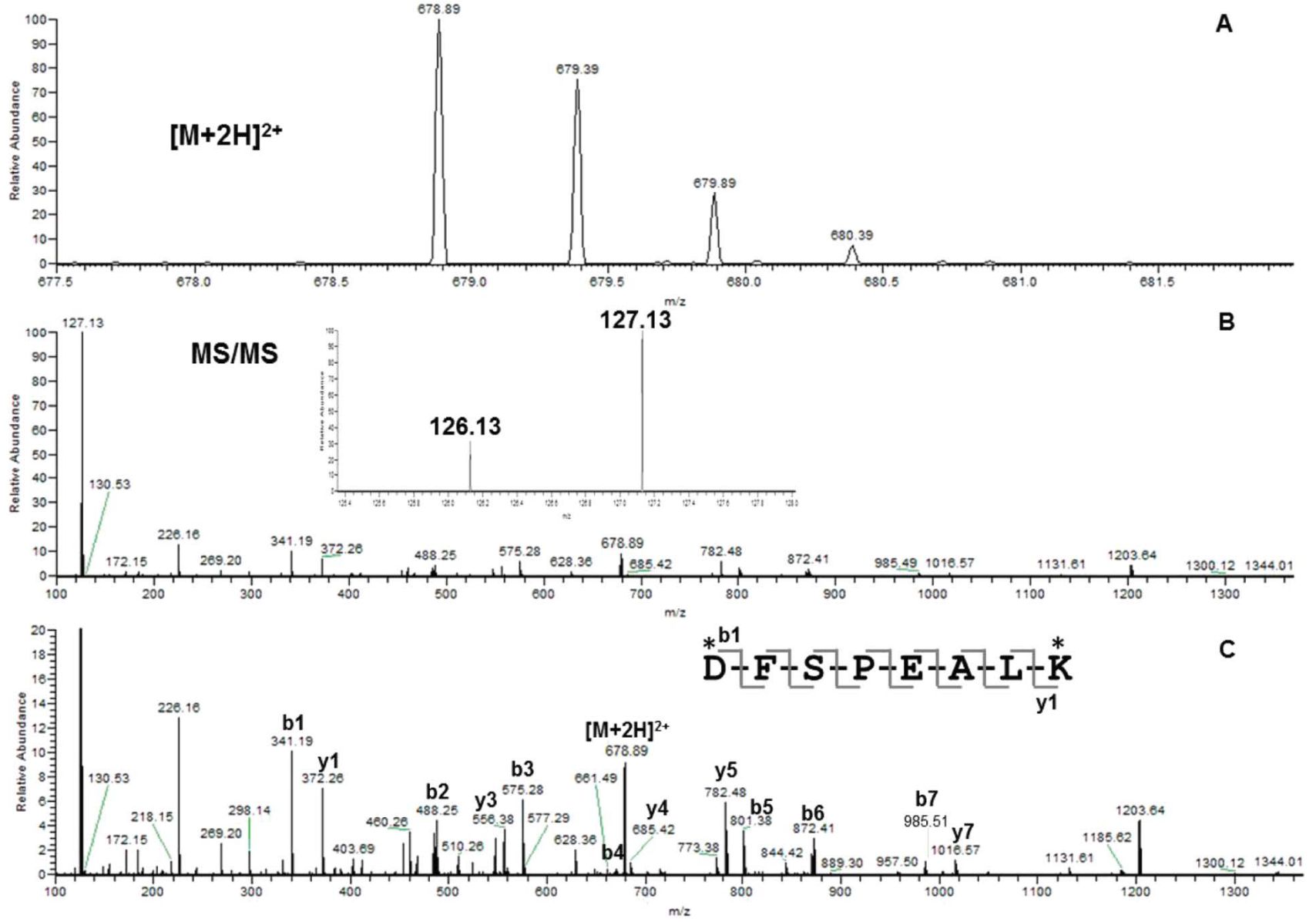

A
[M+H]+
1702.0
1.1E+4
% Intensity
Mass (m/z)
B
I–K–N–L–Q–S–L–D–P–S–H
b3
b10
y3
b8
1362.8
1.2E+4
[M+H]+
1702.0
127.1
b6
b5
1047.7
1134.7
b7
1247.8
b2
692.5
b3
806.5
b4
919.6
y3
340.1
226.1
b9
1459.9
b10
1546.9
C
47:100
127.1
126.1
7236.7
D
90:100
126.1
127.1
6981.5
E
8.5:100
127.1
126.1
7162.9

	IKNLQpSLDPSH : IKNLQSLDPSH mixed in 1:1		IKNLQpSLDPSH : IKNLQSLDPSH mixed in 1:9		IKNLQpSLDPSH : IKNLQSLDPSH mixed in 9:1	
Replica	MALDI-TOF-TOF	LTQ-Orbitrap	MALDI-TOF-TOF	LTQ-Orbitrap	MALDI-TOF-TOF	LTQ-Orbitrap
1	47:100	49:100	90:100	91:100	8.5:100	10:100
2	51:100	50:100	92:100	89:100	9:100	11:100
3	47:100	47:100	87:100	89:100	11:100	9:100

	DRVpYIHPF : DRVYIHPF mixed in 1:1		DRVpYIHPF : DRVYIHPF mixed in 1:9		DRVpYIHPF : DRVYIHPF mixed in 9:1	
Replica	MALDI-TOF-TOF	LTQ-Orbitrap	MALDI-TOF-TOF	LTQ-Orbitrap	MALDI-TOF-TOF	LTQ-Orbitrap
1	48:100	49:100	91:100	88:100	11:100	11:100
2	52:100	49:100	90:100	92:100	8.5:100	10:100
3	49:100	47:100	92:100	91:100	10:100	9:100

	DFNKFHpTFPQTAIGV : DFNKFHTFPQTAIGV mixed in 1:1		DFNKFHpTFPQTAIGV : DFNKFHTFPQTAIGV mixed in 1:9		DFNKFHpTFPQTAIGV : DFNKFHTFPQTAIGV mixed in 9:1	
Replica	MALDI-TOF-TOF	LTQ-Orbitrap	MALDI-TOF-TOF	LTQ-Orbitrap	MALDI-TOF-TOF	LTQ-Orbitrap
1	50:100	50:100	91:100	88:100	10:100	10:100
2	51:100	49:100	90:100	92:100	9:100	11:100
3	52:100	49:100	89:100	91:100	11:100	11:100

Quantification of Phosphorylation of eIF3H



Summary and Conclusion

A new protocol have been described to measure the absolute abundance of phosphorylation.

CeO_2 nanoparticles have been used for complete dephosphorylation.

Absolute phosphorylation level of Ser183 of human eIF3H derived from cancer cell have been determined for the first time as 70%.

But this method is not suited to quantify phosphorylation stoichiometries on multiphosphorylated sites within a single peptide.

Future Possibilities

The concept can be extended towards other biological systems. Not only phosphorylation, other biologically relevant reactions can be studied using the TMT concept where the reaction is not complete. In such cases we can find the extent of reactions.

Various nanosystems can also be studied to replace costly enzymes for specific biologically relevant reactions.

THANK YOU