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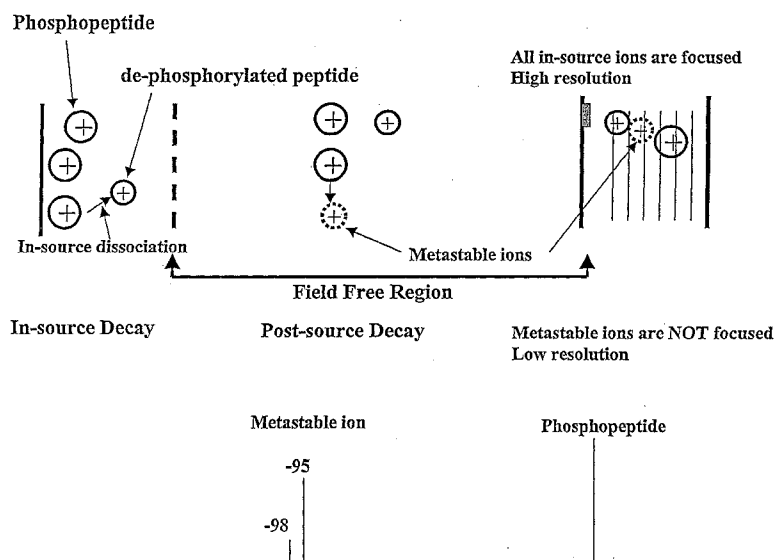
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[Continued on next page]

(54) Title: IDENTIFICATION OF MODIFIED PEPTIDES BY MASS SPECTROMETRY



(57) Abstract: In various aspects, provided are methods for identification of the presence of modified peptides using at least in part mass spectrometric signals from metastable ions that are formed in the field free region of the analyzer. For example, in various embodiments where the modified peptides are phosphopeptides, such metastable ions can appear approximately 95 u lower than the precursor ion m/z value from which they were formed when the peptide contains a phosphate group. In various embodiments, detection of such metastable ions in a MALDI-TOF mass spectrum can identify the modified peptide. In various aspects, provided are methods for identification of the presence of modified peptides using at least in part MS/MS data from two or more of modified peptides, demodified peptides and non-modified peptides by comparing the sequence and/or site of modification information for two or more of these three forms of a peptide.



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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER INV. G01N33/68		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, EMBASE, INSPEC, COMPENDEX, FSTA, WPI Data, PAJ		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MULLER D R ET AL: "Mass spectrometric characterization of stathmin isoforms separated by 2D PAGE" JOURNAL OF MASS SPECTROMETRY, vol. 34, no. 4, April 1999 (1999-04), pages 336-345, XP002392101 ISSN: 1076-5174	1-4
Y	abstract	8, 11
A	page 338, right-hand column, paragraph 2 - page 345, right-hand column, last paragraph; figures 2,4 ----- -/--	15-19
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
A document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
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International application No
PCT/US2005/012347

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WIRTH URS ET AL: "Post-translational modification detection using metastable ions in reflector matrix-assisted laser desorption/ionization-time of flight mass spectrometry." PROTEOMICS, vol. 2, no. 10, October 2002 (2002-10), pages 1445-1451, XP002392102 ISSN: 1615-9853</p>	1-4
Y A	<p>abstract page 1446, left-hand column, paragraph 4 - right-hand column, paragraph 3; figures 1,2,7-9 page 1448, right-hand column, last paragraph - page 1449, left-hand column, last paragraph</p>	8,11 15-19
A	<p>----- HARVEY D J ET AL: "Relationship between in-source and post-source fragment ions in the matrix-assisted laser desorption (ionization) mass spectra of carbohydrates recorded with reflectron time-of-flight mass spectrometers" INTERNATIONAL JOURNAL OF MASS SPECTROMETRY, vol. 188, no. 1-2, 17 May 1999 (1999-05-17), pages 131-146, XP004165680 ISSN: 1387-3806 abstract page 134, right-hand column, last paragraph - page 140, right-hand column, paragraph 1</p>	15,16
A	<p>----- MANN M ET AL: "Analysis of protein phosphorylation using mass spectrometry: deciphering the phosphoproteome" TRENDS IN BIOTECHNOLOGY, vol. 20, no. 6, 1 June 2002 (2002-06-01), pages 261-268, XP004352765 ISSN: 0167-7799 the whole document</p>	1-4, 15-19
A	<p>----- ANNAN R S ET AL: "Phosphopeptide analysis by matrix-assisted laser desorption time-of-flight mass spectrometry." ANALYTICAL CHEMISTRY, vol. 68, no. 19, 1 October 1996 (1996-10-01), pages 3413-3421, XP002392103 ISSN: 0003-2700 the whole document</p> <p>----- -/--</p>	1-4, 6-12, 15-20

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2005/012347

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEE C H ET AL: "On-membrane digestion of beta-casein for determination of phosphorylation sites by matrix-assisted laser desorption/ionization quadrupole/time-of-flight mass spectrometry" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 15, no. 3, 2001, pages 191-202, XP002405270 ISSN: 0951-4198	6,7,9,10
Y	the whole document in particular abstract; p.195, col. 2, last par. to p. 200, col. 1, last par.; fig. 3+5	8,11,12
X	----- PETERS E C ET AL: "EXPLORING THE PHOSPHOPROTEOME WITH MASS SPECTROMETRY" MINI REVIEWS IN MEDICINAL CHEMISTRY, vol. 4, March 2004 (2004-03), pages 313-324, XP008066970 ISSN: 1389-5575	6,7,9,10
Y	the whole document in particular p.317, col. 2, l. 37-47 and fig. 3.	8,11,12
X	----- SALEK MOGJIBORAHMAN ET AL: "Analysis of protein tyrosine phosphorylation by nanoelectrospray ionization high-resolution tandem mass spectrometry and tyrosine-targeted product ion scanning." ANALYTICAL CHEMISTRY, vol. 75, no. 11, 1 June 2003 (2003-06-01), pages 2724-2729, XP002405271 ISSN: 0003-2700	20
Y	the whole document in particular p. 2727, col. 1, par. 2 to p. 2729, last line; fig. 3+5.	12
A	----- RESING K A ET AL: "Mass spectrometric analysis of 21 phosphorylation sites in the internal repeat of rat profilaggrin, precursor of an intermediate filament associated protein" BIOCHEMISTRY, vol. 34, 1995, pages 9477-9487, XP002090544 ISSN: 0006-2960 the whole document	6-11
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2005/012347

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HOGAN JASON M ET AL: "Phosphorylation site identification via ion trap tandem mass spectrometry of whole protein and peptide ions: bovine alpha-crystallin A chain." ANALYTICAL CHEMISTRY, vol. 75, no. 23, 1 December 2003 (2003-12-01), pages 6509-6516, XP002405272 ISSN: 0003-2700 the whole document in particular fig. 4+5.</p>	12,20
A	<p>DEGNORE J P ET AL: "Fragmentation of phosphopeptides in an ion trap mass spectrometer." JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, vol. 9, no. 11, November 1998 (1998-11), pages 1175-1188, XP004142532 ISSN: 1044-0305 the whole document</p>	6-11
P,X	<p>JIN WEN-HAI ET AL: "Phosphoproteome analysis of mouse liver using immobilized metal affinity purification and linear ion trap mass spectrometry." RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 18, no. 18, 16 August 2004 (2004-08-16), pages 2169-2176, XP002405273 ISSN: 0951-4198 the whole document</p>	20
P,Y	<p>in particular abstract; p. 2173, col. 1, 1. 4 to col. 2, l. 9; fig. 1.</p>	12

INTERNATIONAL SEARCH REPORT

International application No.
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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 5, 13, 14, 21
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(v) PCT - Presentation of information
2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4,15-19

Methods of identifying the presence of a modified peptide in a mixture of peptides by a mass spectrometry instrument being based on the observation that metastable fragment ions have a lower resolution than precursor ions and the observation that the mass difference between the metastable fragment ion and the respective parent ion is smaller than expected for stable fragments.

2. claims: 6-12,20

Methods of identifying the presence of a modified peptide in a mixture of peptides by a mass spectrometry instrument being based on the comparison of MS/MS spectra of modified peptides with MS/MS spectra of de-modified and/or non-modified peptides.
