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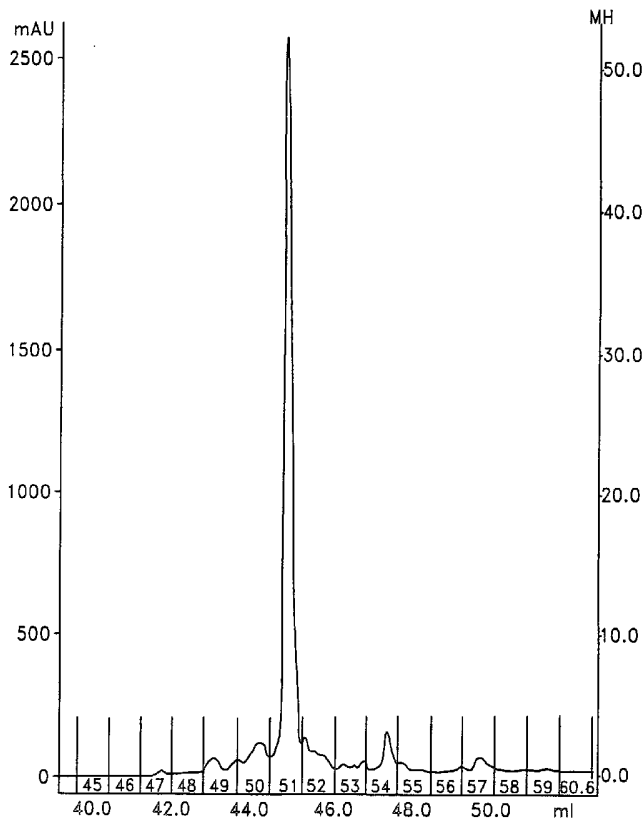
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[Continued on next page]

(54) Title: DIFFERENTIAL LABELING FOR QUANTITATIVE ANALYSIS OF COMPLEX PROTEIN MIXTURES



(57) Abstract: The present invention relates to a method of simultaneously identifying and determining the levels of expression of cysteine-containing proteins in normal and perturbed cells, a method for proteomic analysis, a process for preparing fusion proteins, and compounds and reagents related thereto.



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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 02/02487

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K7/04 C07K1/00 G01N33/68 C12Q1/37

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)

IPC 7 C07K G01N C12Q

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)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| A          | HIGASHIURA K ET AL: "THE CHEMICAL<br>CONVERSION OF CARBOXYL-TERMINAL GLYCINES<br>IN PEPTIDES INTO TAURINE"<br>JOURNAL OF THE CHEMICAL SOCIETY CHEMICAL<br>COMMUNICATIONS,<br>no. 9, 1989, pages 521-522, XP009004781<br>ISSN: 0022-4936<br>page 521; figure 1<br>--- | 25-54                 |
| A          | VINGIELLO, F. A. ET AL:<br>"4-(7-Benz'a!anthracenyl)-N-(2-substituted<br>ethyl)benzamides"<br>ORGANIC PREPARATIONS AND PROCEDURES<br>INTERNATIONAL (1972), 41, 43-7, 1972,<br>XP009004771<br>page 43; figure III<br>page 44, paragraph 3<br>---<br>-/--              | 25-54                 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*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
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 02/02487

## 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>WO 00 11208 A (UNIV WASHINGTON)<br/>2 March 2000 (2000-03-02)</p> <p>page 6, line 10 - line 13<br/>page 7, line 20 - line 21<br/>page 29, line 30 - page 30, line 5<br/>claims 1-48</p> <p>---</p>   | 1-37,<br>47-53,<br>55-84 |
| X          | <p>WO 97 35887 A (AMRAD OPERATIONS PTY LTD<br/>;KOENTGEN FRANK (AU); SUESS GABRIELE MARI)<br/>2 October 1997 (1997-10-02)<br/>claims 1,10,12<br/>page 17, line 12 - line 19<br/>page 18, line 1 - line 6<br/>page 18, line 14 - line 19</p> <p>---</p>  | 1-37,<br>47-53           |
| P,X        | <p>WO 01 96539 A (DOUDNA JENNIFER A ;UNIV<br/>YALE (US); BATEY ROBERT T (US); LUCAST<br/>LOU) 20 December 2001 (2001-12-20)<br/>page 41, line 18 - line 20</p> <p>---</p>   | 1-37,<br>47-53           |
| A          | <p>GYGI S P ET AL: "Quantitative analysis of<br/>complex protein mixtures using<br/>isotope-coded affinity tags."<br/>NATURE BIOTECHNOLOGY. UNITED STATES OCT<br/>1999,<br/>vol. 17, no. 10, October 1999 (1999-10),<br/>pages 994-999, XP001010578<br/>ISSN: 1087-0156<br/>abstract; figure 1<br/>page 1, column 2, line 12 - line 24</p> <p>---</p> | 1-37,<br>47-53,<br>55-84 |
| P,A        | <p>CAI, YUPING A. ET AL: "Recombinant<br/>Phycobiliproteins"<br/>ANALYTICAL BIOCHEMISTRY (2001), 290(2),<br/>186-204,2001, XP002185513<br/>page 188, column 2, line 16 - line 21;<br/>figures 1,2</p> <p>---</p>  | 1-37,<br>47-53,<br>55-84 |
| A          | <p>KAPUST, RACHEL B. ET AL: "Controlled<br/>Intracellular Processing of Fusion<br/>Proteins by TEV Protease"<br/>PROTEIN EXPRESSION AND PURIFICATION<br/>(2000), 19(2), 312-318,2000, XP002233787<br/>abstract<br/>page 313, column 2, line 18 - line 23</p> <p>-----</p>   | 1-37,<br>47-53,<br>55-84 |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 02/02487

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 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.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 1-28, 30, 31, 34-37, 47-53, 55-84  
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:  
see FURTHER INFORMATION sheet PCT/ISA/210
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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

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.:  
1-25(part), 26-30, 31-32(part), 33, 34-37(part), 47-53(part), 55-70(part), 71-82, 83(part), 84

### 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

1. Claims: 1-25 (part), 26-30, 31-32(part), 33, 34-37(part), 47-53(part), 55-70 (part), 71-82, 83 (part), 84

A compound of formula (II); a method for simultaneously identifying and determining the levels of expression of cystein-containing proteins in normal and perturbed cells comprising reacting said proteins with a reagent of formula (II) only; a method for proteomic analysis comprising reacting a protein sample with a reagent of formula (II); a process for preparing a fusion protein, wherein the protein is fused to a reagent of formula (II).

2. Claims: 1-25 (part), 31-32 (part), 34-37 (part), 38-46, 47-53 (part), 54, 55-70(part), 83(part)

A compound of formula (III); a method for simultaneously identifying and determining the levels of expression of cystein-containing proteins in normal and perturbed cells comprising reacting one set of said proteins with a reagent of formula (III) and reacting the other set of said proteins with a reagent of formula (III) or with a reagent of formula (II); a process for preparing a fusion protein, wherein the protein is fused to a reagent of formula (III).

3. Claims: 1-24 (all in part).

A compound of formula (I), excluding the compounds of formulae (II) and (III).

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.1

The SEQ ID Nos: 2-16 are compounds of formula (II) (see page 20 paragraph 66), and not examples of the "Link", therefore the subject-matter of claim 32 is unclear.

It is to be noted that the definition of Ph for the compounds of formula (III) in claims 45 and 54 (the electron withdrawing groups NO<sub>2</sub> and OMe are in positions ortho and META to the -CH<sub>2</sub>- group) is different to the definition of Ph for the compounds of formula (III) in claim 25, 55, 56 and 57 (the electron withdrawing groups are ortho or PARA to the -CH<sub>2</sub>- group).

Furthermore, the subject-matter of claim 56 is the same as the subject-matter of claim 55.

Moreover, the description discloses that the functional group designated as "link" reacts selectively with certain protein functional groups (page 18, paragraph 56 and page 19, paragraph 64); and the description discloses that "Acyl" is a chemical substituent of formula R-C(O)-, where R is alkyl, aryl or heteroaryl (page 16, paragraph 48). According to the description, a fusion protein will therefore occur, wherein the protein is linked to a compound of formula (II) via the "link" functional group, which is not the case in the embodiments of claims 83 and 84 wherein said protein is linked to (II) via the "acyl" functional group. Therefore, these statements in the description imply that the subject-matter for which protection is sought may be different to that defined by the claims 83 and 84, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them.

## Continuation of Box I.2

Claims Nos.: 1-28,30,31,34-37,47-53,55-84

Present claims 1-28, 30, 31, 34-37, 47-53 and 55-84 relate to an extremely large number of possible compounds/methods/process. In fact, the claims contain so many options, variables and possible permutations and provisos that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely:

- (1) the compounds as disclosed in claims 29, 32 and 33 and in the description on page 20, paragraphs 66 and 67; on page 35, paragraph 113; on page 38, paragraph 126; on page 41, paragraph 138; and on page 42, paragraph 139;
- (2) the use of said compounds in a method for identifying and determining the levels of expression of cysteine-containing proteins in normal and perturbed cells (see claims 55-70 all in part, and pages 26-27, paragraphs 77 and 78);
- (3) a method for proteomic analysis comprising the use of said compounds which react with the reactive side-chains of cysteine on the proteins to be analyzed (claims 71-82 all in part; examples 2-4, 8, 9 and 11 and page

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

3, paragraph 8) and  
(4) a process for preparing a fusion protein comprising said compounds (claims 83-84 in part).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

**INTERNATIONAL SEARCH REPORT**  
information on patent family members

International Application No  
PCT/US 02/02487

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)  | Publication<br>date  |
|---|---------------------|---|--|
| WO 0011208 A                              | 02-03-2000          | AU 755334 B<br>AU 5691399 A<br>EP 1105517 A<br>JP 3345401 B<br>JP 2002523058 T<br>JP 2003107066 A<br>US 2002076739 A  | 12-12-2002<br>14-03-2000<br>13-06-2001<br>18-11-2002<br>30-07-2002<br>09-04-2003<br>20-06-2002   |
| WO 9735887 A                              | 02-10-1997          | AU 731580 B<br>AU 2143497 A<br>CA 2249455 A<br>EP 0935612 A<br>GB 2326643 A, B<br>JP 2000507105 T<br>US 6326179 B<br>US 6521741 B<br>US 6043069 A<br>ZA 9702620 A | 05-04-2001<br>17-10-1997<br>02-10-1997<br>18-08-1999<br>30-12-1998<br>13-06-2000<br>04-12-2001<br>18-02-2003<br>28-03-2000<br>20-11-1997 |
| WO 0196539 A                              | 20-12-2001          | AU 6827301 A  | 24-12-2001   |