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- (71) Applicant (for all designated States except US): **UNIVERSITY OF PITTSBURGH OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION** [US/US]; 200 Gardner Steel Conference Center, Pittsburgh, PA 15260 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **ROBBINS, Paul, D.** [US/US]; 191 Main Entrance Drive, Mt. Lebanon, PA 15228 (US). **MI, Zhibao** [CN/US]; 221 Buchanan Place, Apt. C2, Pittsburgh, PA 15228 (US). **FRIZZEL, Raymond** [US/US]; 5304 Forbes Avenue, Pittsburgh, PA 15217 (US). **GLORIOSO, Joseph, C.** [US/US]; 202 Scarborough Drive, Cheswick, PA 15024 (US). **GAMBOTTO, Andrea** [IT/US]; 1250 South Negley Avenue, Pittsburgh, PA 15217 (US). **MAI, Jeffrey, C.** [US/US]; 6112 Alder Street, Apt. E3, Pittsburgh, PA 15206 (US).
- (74) Agents: **SEIDE, Rochelle, K.** et al.; Baker Botts, L.L.P., 30 Rockefeller Plaza, New York, NY 10112-4498 (US).
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- Published:**
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IDENTIFICATION OF PEPTIDES THAT FACILITATE UPTAKE AND CYTOPLASMIC AND/OR NUCLEAR TRANSPORT OF PROTEINS, DNA AND VIRUSES

(57) Abstract: The present invention relates to internalizing peptides which facilitate the uptake and transport of cargo into the cytoplasm and nuclei of cells as well as methods for the identification of such peptides. The internalizing peptides of the present invention are selected for their ability to efficiently internalize cargo into a wide variety of cell types both *in vivo* and *in vitro*. The method for identification of the internalizing peptides of the present invention comprises incubating a target cell with a peptide display library, isolating peptides with internalization characteristics and determining the ability of said peptide to internalize cargo into a cell.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/04632

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/00, 38/04; C07K 16/00
 US CL : 424/185.1; 530/327

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)
 U.S. : 424/185.1; 530/327

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	MAI, Jeffrey C. et al. Efficiency of Protein Transduction Is Cell Type-dependent and is Enhanced by Dextran Sulfate. The Journal Of Biological Chemistry. 16 August 2002, Vol. 277, No. 33, pages 30208-30218.	1-3, 15, 6-7, 11 (elected SEQ ID NO: 76 and 99 only)

Further documents are listed in the continuation of Box C. 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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"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
"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	"&"	document member of the same patent family

Date of the actual completion of the international search Date of mailing of the international search report

10 November 2003 (10.11.2003)

17 MAY 2004

Name and mailing address of the ISA/US
 Mail Stop PCT, Attn: ISA/US
 Commissioner for Patents
 P.O. Box 1450
 Alexandria, Virginia 22313-1450
 Facsimile No. (703)305-3230

Authorized officer

Padmashri Ponnaluri
 Telephone No. 703-308-0916

Janice Ford
for

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/04632

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

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

1. Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim 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. Claim 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:
Please See Continuation 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.: 1-3, 15, 6-7, 11 (seq id NO: 76 and 99 only)

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.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

The inventions listed as Groups I-47 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of groups 1-14 is the specific peptide sequence;
The special technical feature of group 15 is method of identifying a peptide;
The special technical feature of groups 16-29 is a fusion protein;
The special technical feature of groups 30-43 is a method of inducing white blood cells;
The special technical feature of groups 44-57 is a method of inhibiting apoptosis;
The special technical feature of groups 58-71 is a method of delivering anti-oxidant;
The special technical feature of group 72 is a method of internalization;
The special technical feature of groups 73-85 is a method of eliciting an immune response;
The special technical feature of 86 is a peptide which does not have same amino acid sequence of groups 1-14;
The special technical feature of group 87 is a peptide-cargo complex.

According to the guidelines in Section (f)(i)(a) of Annex B of the PCT Administrative Instructions, the special technical feature as defined by PCT Rule 13.2 shall be considered to be met when all the alternatives of a Markush-group are of similar nature. For chemical alternatives, such as the claimed sequences, the Markush group shall be regarded as being of similar nature when

- (A) all alternatives have a common property or activity and
- (B)(1) a common structure is preset, i.e., a significant structure is shared by all of the alternatives or
- (B)(2) in cases where the common structure cannot be the unifying criteria, all alternatives belong to an art recognized class of compounds in the art to which the invention pertains.

The groups listed above are considered to be each separate inventions for the following reasons:

It is noted that each of the peptides of groups 1-14 has been presented in an improper Markush format, as distinct products. For example, in the instant claim each peptide sequence is structurally and functionally distinct from each other and, as a result, has a different special technical feature. As the products encompassed by the claims do not share a special technical feature, the distinct products may not be properly presented in the alternative. Consequently, the special technical feature as defined by PCT Rule 13.2 has NOT been met because the requirements for both part (a) and part (b) above have NOT been met.

Thus the inventions of groups 1-87 do not share a special technical feature and the invention lacks unity.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups 1-14, claim(s) 1-3, 15, and 6-7, 11, each group is drawn to a peptide having one specific amino acid sequence of SEQ ID NO: 76-86, SEQ ID NOs 97-99 and the peptide composition and the immunogen and method of inducing synovial cell death using the peptide.

Group 15 II, claim(s) 4, drawn to a method of identifying peptides capable of cellular internalization of cargo linked thereto.

Groups 16-29, claim(s) 5, 11, drawn to an expression cassette comprising a DNA encoding a fusion protein comprising a leader sequence, a protein of interest and an internalizing peptide having an amino acid sequence (each group has a different amino acid sequence).

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Groups 30-43, claim(s) 8, 11, drawn to a method of reducing white blood cells in arthritis joints by administering a peptide-cargo complex to said cells, wherein the peptide has amino acid sequence of SEQ ID NOS 76-86 and SEQ ID NOs: 97-99 (each group has a different amino acid sequence).

Groups 44-57, claim(s) 9, 11, drawn to a method of inhibiting apoptosis in an islet cell comprising administering a peptide-cargo complex to said islet cell, wherein the peptide has an amino acid sequence of SEQ ID NOs: 76-86, and 97-99 (each group has a different amino acid sequence).

Groups 58-71, claim(s) 10, , drawn to a method of delivering anti-oxidant and anti-inflammatory agents to lung epithelial cells comprising administering a peptide-cargo complex, wherein the peptide has amino acid sequence of SEQ ID NOs: 76-86 and 97-99 (each group has a different amino acid sequence).

Groups 72, claim(s) 12-14, drawn to a method of internalization into a peptide-cargo complex into a cell, and a kit comprising a peptide-cargo complex.

Groups 73-85, claim(s) 16, 30-34, drawn to a method of eliciting an immune response in a subject comprising administering to a target cell comprising a peptide-cargo complex wherein the peptide has an amino acid sequence of SEQ ID NOs: 76-86 and 97-99 (each group has a different amino acid sequence).

Group 86, claim(s) 17-29, drawn to a peptide and peptide-cargo complex, expression cassette and a method of use of the peptide.

Group 87, claim(s) 35, drawn to a peptide-cargo complex.

Continuation of B. FIELDS SEARCHED Item 3:
WEST, DERWENT, CAPLUS, REGISTRY