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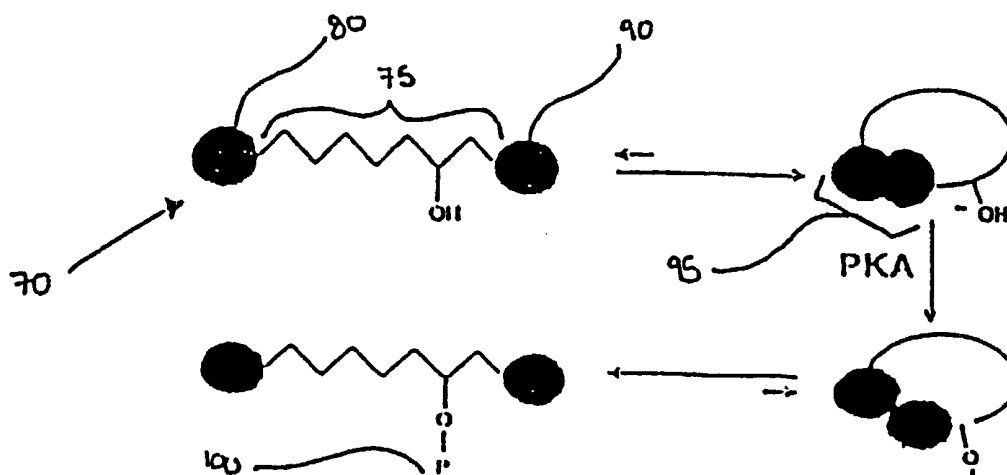
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(54) Title: **HOMOGENEOUS FLUORESCENCE METHOD FOR ASSAYING STRUCTURAL MODIFICATIONS OF BIOMOLECULES**



(57) Abstract: Double-labeled protein biomolecular substrates (70) and methods for the homogenous assay of processes which include covalent modification of the substrates (70) to form a detectable species are described. The biomolecular substrates (70) of the instant invention are labeled at two positions (80, 90) with two fluorescent dyes or with a fluorescent dye and a nonfluorescent dye. The two labeling dyes of the unmodified substrate (70) stack (95), thereby quenching the substrate's fluorescence. Upon covalent modification of the double-labeled substrate (70), however, the intramolecularly stacked dyes (95) dissociate and the fluorescence changes markedly. Examples are described for the preparation and use of substrates (70) for phosphorylation assays. Methods of invention do not require separation of the modified and unmodified substrates (70), nor do they require other special reagents or radioactive materials. Therefore the substrates can be used for monitoring intracellular processes of living cells.



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.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/53

US CL :435/4, 7.72, 15,21; 436/86, 89, 172

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. : 435/4, 7.72, 15,21; 436/86, 89, 172

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 practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	K. B. Lee et al, "A New Approach to Assay Endo-Type Carbohydrases: Bifluorescent-Labeled Substrates for Glycoamidases and Ceramide Glycanases" Analytical Biochemistry, 01 September 1995, Vol. 230, No. 1, pages 31-36, see entire document.	1-2,5-7,13, 15,21 ----- 3-4,8-12,14,16-20,22-27
X - Y	K. Matsuoka et al, "A Bi-Fluorescence-Labeled Substrate for Ceramide Glycanase Based on Fluorescence Energy Transfer" Carbohydrate Research, 16 October 1995, Vol. 276, No. 1, pages 31-42, see entire document.	1,2,5-7,13,15,21 ----- 3,4,8-12,14,16-20,22-27

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
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"O" document referring to an oral disclosure, use, exhibition or other means	
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International application No.
PCT/US00/40495

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	G. Harnischfeger "Effect of Chemical Modification of Amino Groups by Fluorescamine on Partial Reactions of Photosynthesis" <i>Biochimica et Biophysica Acta</i> , 7 September 1978, Vol. 503, No. 3, pages 473-479, see entire document.	1-27
Y	US 4,777,128 A (LIPPA) 11 October 1988, see entire document.	1-27
Y	Z. Zhang et al, "Amylase Substrate Based on Fluorescence Energy Transfer" <i>Analytica Chimica Acta</i> , 17 September 1990, Vol. 236, No. 2, pages 251-256, see entire document.	1-27
Y	A.-P. Wei et al, "Antibody-Mediated Fluorescence Enhancement Based on Shifting the Intramolecular Dimer/Monomer Equilibrium of Fluorescent Dyes" <i>Analytical Chemistry</i> , 01 May 1994, Vol. 66, No. 9, pages 1500-1506, see entire document.	1-27
Y	B. Pispisa et al, "Intramolecular Electronic Energy Transfer between Naphthalene and Protoporphyrin Chromophores Bound to Sequential Peptides" <i>Macromolecules</i> , 19 December 1994, Vol. 27, No. 26, pages 7800-7808, see entire document.	1-27
Y	K. M. Paarkhurst et al, "Kinetic Studies by Fluorescence Resonance Energy Transfer Employing a Double-Labeled Oligonucleotide: Hybridization to the Oligonucleotide Complement and to Single-Stranded DNA" <i>Biochemistry</i> , 10 January 1995, Vol. 34, No. 1, pages 285-292, see entire document.	1-27
Y	J. Ju et al, "Cassette Labeling for Facile Construction of Energy Transfer Fluorescent Primers" <i>Nucleic Acids Research</i> 15 March 1996, Vol. 24, No. 6, pages 1144-1148, see entire document.	1-27
Y	US 5,654,419 A (MATHIES ET AL) 05 August 1997, see entire document.	1-27
Y	M. P. Lillo et al, "Design and Characterization of a Multisite Fluorescence Energy-Transfer System for Protein Folding Studies: A Steady-State and Time-Resolved Study of Yeast Phosphoglycerate Kinase" <i>Biochemistry</i> 16 September 1997, Vol. 36, No. 37, pages 11261-11272, see entire document.	1-27
Y	US 5,707,804 A (MATHIES ET AL) 13 January 1998, see entire document.	1-27
Y	US 5,723,340 A (KARPF) 03 March 1998, see entire document.	1-27

INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	D. A. Pearce et al, "Peptidyl Chemosensors Incorporating a FRET Mechanism for Detection of Ni(II)" Bioorganic & Medicinal Chemistry Letters, 04 August 1998, Vol. 8, No. 15, pages 1963-1968, see entire document.	1-27
Y	L. A. Marcaurelle et al, "Direct Incorporation of Unprotected Ketone Groups into Peptides During Solid-Phase Synthesis: Application to the One-Step Modification of Peptides with Two Different Biophysical Probes for FRET" Tetrahedron Letters, 01 October 1998, Vol 39, No. 40, pages 7279-7282, see entire document.	1-27
Y	US 5,869,255 A (MATHIES ET AL) 09 February 1999, see entire document.	1-27
Y	FR 2768817 A (CIC BIO INTERNATIONAL SOCIETE ANONYME) 26 March 1999, see entire document.	1-27
A	C. G. Dos Remedios et al, "Fluorescence Resonance Energy Transfer Spectroscopy in a Reliable "Ruler" for Measuring Structural Changes in Proteins" Journal of Structural Biology, 1995, Vol. 115, pages 175-185.	1-27

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B. FIELDS SEARCHED

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CA, BIOSIS, and MEDLINE files in STN

search terms: kinase, fluoresc?, label?, absor?, resonant?, energy, transfer?, Exchang?, covalent?, double, di, bi, two, label, fluoroph?, modif?, bind?, bond?, complex?, bound?, immobil?