Title (en)

HOMOGENEOUS FLUORESCENCE METHOD FOR ASSAYING STRUCTURAL MODIFICATIONS OF BIOMOLECULES

Title (de)

HOMOGENES FLUORESZENZVERFAHREN ZUM BESTIMMEN STRUKTURELLER MODIFIKATIONEN VON BIOMOLEKÜLEN

Title (fr)

PROCEDE HOMOGENE BASE SUR LA FLUORESCENCE ET SERVANT A ANALYSER DES MODIFICATIONS STRUCTURELLES DE BIOMOLECULES

Publication

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Application

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Abstract (en)

[origin: WO0107638A2] Double-labeled protein biomolecular substrates and methods for the homogenous assay of processes by which biomolecules are covalently modified are described. The methods of the present invention utilize biomolecular substrates labeled at two positions with two fluorescent dyes or with a fluorescent dye and a nonfluorescent dye. The two labeling dyes of the unmodified biomolecular substrates stack, thereby quenching the substrate's fluorescence. Upon covalent modification of the double-labeled substrate, however, the intramolecularly stacked dyes dissociate and the fluorescence of the phosphorylated substrate changes markedly. Methods utilizing the double-labeled substrates of the present invention do not require physical separation of modified and unmodified substrate molecules, nor do they require other special reagents or radioactive materials. Methods for preparing and characterizing the substrates used in the assay procedure are described, as are methods utilizing the substrates of the present invention for high-throughput screening, for monitoring intracellular processes of covalent biomolecular modification in living cells, for diagnostic and therapeutic applications for diseases involving dysfunctional processes of covalent biomolecular modification, and for discovering novel enzymatic substrates.

[origin: WO0107638A2] 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.

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