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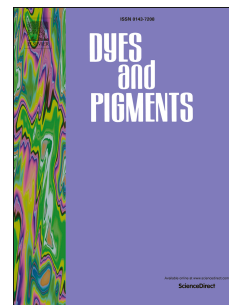
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Novel ratiometric xanthene-based probes for protease detection

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Abstract

Novel FRET and donor-fluorescent quencher systems based on fluorescein-rhodamine B fluorophores have been developed as protease detectors and their applicability to chymotrypsin as a model protease sensing has been studied. The designed probes demonstrated long-term photostability and very good sensitivity in a chymotrypsin assay, allowing quantitative enzyme determination with the advantage of ratiometric measurement. The detection limit as well as linearity range for enzyme detection is tunable by the type of a linker between the dyes. The solid-phase synthetic protocol enables fast and easy adaptation of the system for various protease assays as well as its properties regulation.

Keywords

FRET, enzymes, fluorescein, rhodamine B, solid-phase synthesis

1. Introduction

Proteolytic enzymes play essential roles in the cell physiological processes related to protein degradation and can therefore be considered as signalling molecules for possible pathological mechanisms¹.

Förster resonance energy transfer (FRET) is among the most relevant methods for the monitoring of protease activity². In this strategy, a peptide is tagged with a pair of

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