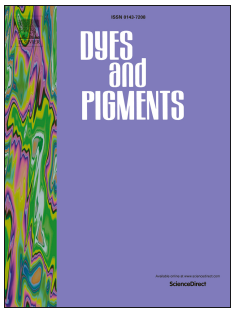
Accepted Manuscript



Novel ratiometric xanthene-based probes for protease detection

Yana Okorochenkova, Jan Hlaváč

PII: S0143-7208(17)30585-5

DOI: 10.1016/j.dyepig.2017.04.039

Reference: DYPI 5936

To appear in: *Dyes and Pigments*

Received Date: 22 March 2017

Revised Date: 18 April 2017

Accepted Date: 19 April 2017

Please cite this article as: Okorochenkova Y, Hlaváč J, Novel ratiometric xanthene-based probes for protease detection, *Dyes and Pigments* (2017), doi: 10.1016/j.dyepig.2017.04.039.

Novel ratiometric xanthene-based probes for protease detection

Yana Okorochenkova[a], Jan Hlaváč[b*]

[a]*Institute of Molecular and Translation Medicine, Faculty of Medicine and Dentistry, Palacký University, Hnevotinska 5, 779 00 Olomouc, Czech Republic.*

[b]*Department of Organic Chemistry, Faculty of Science, Palacký University, 771 46 Olomouc, Czech Republic*

jan.hlavac@upol.cz

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 mechanisms [1].

Förster resonance energy transfer (FRET) is among the most relevant methods for the monitoring of protease activity [2]. In this strategy, a peptide is tagged with a pair of