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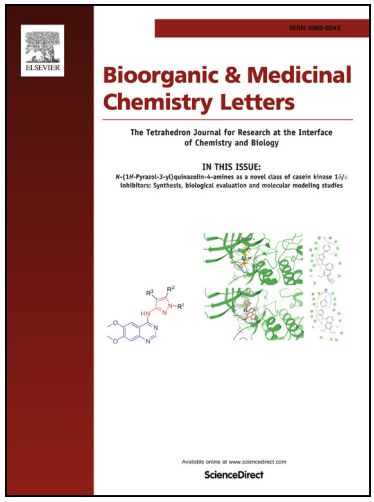

Efficient near infrared fluorescence detection of elastase enzyme using peptide-bound unsymmetrical squaraine dye

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Efficient near infrared fluorescence detection of elastase enzyme using peptide-bound unsymmetrical squaraine dye

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Abstract

Extended wavelength analyte-responsive fluorescent probes are highly desired for the imaging applications owing to their deep tissue penetration, minimum interference from autofluorescence by biomolecules. Near infra-red (NIR) sensitive and self-quenching fluorescent probe based on the dye-peptide conjugate (SQ 1 PC) was designed and synthesized by facile and efficient one-pot synthetic route for the detection of Elastase activity. In the phosphate buffer solution, there was an efficient quenching of fluorescence of SQ 1 PC (86 %) assisted by pronounced dye-dye interaction due to H-aggregate formation. Efficient and fast recovery of this quenched fluorescence of SQ 1 PC (> 50 % in 30 seconds) was observed on hydrolysis of this peptide-dye conjugate by elastase enzyme. Presently designed NIR sensitive self-quenching substrate offers the potential application for the detection of diseases related to proteases by efficient and fast detection of their activities.

Keywords: Far-red squaraine dye, aggregation, fluorescent peptide, self-quenching conjugate, Elastase detection, bio imaging.

Growing demand for point-of-care testing (POCT) devices for home diagnostic and facile health care monitoring, biosensors have attracted tremendous attentions owing to utilization of small amount of biological samples, ease of handling and user-friendliness [1, 2]. Although, biosensors are one of the strong contenders for POCT devices, due to the existing challenges like utilization of single analyte at a time, controlling the sensitivity in the presence of interfering agents, prior analyte processing for higher sensitivity and low throughput are needed to be considered for the further development of more efficient and versatile POCT devices. It is, therefore, necessary to develop biosensors that are sensitive and having the capability of high throughput detection along with their compatibility with imaging techniques for simultaneous multi-target analysis [3]. The advent of Microarray technology has led the possibility of rapid profiling of huge number of proteins in a single experiment [4, 5]. Working with protein arrays for bio-sensing applications is challenging and cumbersome to control polar and ionic interactions, unspecific adsorption of proteins and preservation of native form of protein along with their spatial orientations onto the surface after immobilization [6]. This is the reason why more attentions are being paid for the development of peptide arrays for rapid and high throughput screening of complex protein functions including qualitative as well as quantitative estimation of proteases [7]. Proteases are an important class of physiological enzymes which hydrolyse the amide bond at specific sites of the polypeptide chain thereby playing a vital role in the regulation of a large number of physiological processes such as cell proliferation/differentiation, apoptosis, DNA replication, haemostasis and immune responses [8, 9]. Human Neutrophil Elastase (HNE) belongs to serine class of proteases and is a proteolytic enzyme stored in the azurophilic granules of polymorphonuclear cells [10]. Although the proteolytic destruction of bacteria is one of the major roles of this enzyme, its hyperactivity leads to the pathogenesis of acute and chronic inflammations [11, 12]. Excess release of HNE cleaves the cellular receptors, activates protogenetic mediators and intrinsically involved in the epithelial as well as well as endothelial membrane damage [13, 14]. Therefore, knowledge of roles played by different serine protease along with their strict control and monitoring especially HNE activity has a great importance for therapeutic viewpoints.

In spite of fast growth and development of protease assay methods, methods based on immunoassay consisted of specific binding to the target protease with antibodies have been although