

Protease finger printing for biomarker discovery

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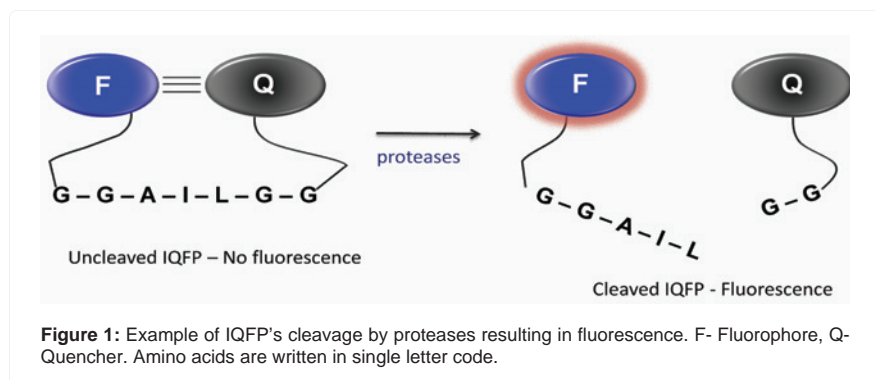
Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between two chromophores in which excitation is transferred from a donor molecule (fluorophore) to an acceptor molecule (quencher) without photon emission. Removal of the acceptor molecule from donor's proximity results in quantifiable fluorescence (Figure 1). FRET is an important technique and can be utilized in monitoring peptide substrate cleavage by proteases in different biological samples.

We report a simple, sensitive, specific FRET based platform for detection of proteases profiles in different biological samples by screening against a combinatorial library of internally quenched peptide probes (IQFP's). The library was utilized to determine the proteolytic profile of two clinically relevant biological fluids, serum and bronchoalveolar lavage fluid. Both fluids displayed a distinct and quantifiable proteolytic signature.

The library was further utilized to distinguish between the protease profiles of different *Aspergillus* species and also to identify peptide probes that are indicative of invasive aspergillosis (IA) in a guinea pig model.

Substrate specificity of biologically relevant recombinant enzymes such as prolyl oligopeptidase (POP) and fibroblast activation protein (FAP) both of which are implicated in number of human diseases was also determined using the IQFP library screen.

The approach provides a comprehensive finger print of the proteolytic activity of complex biological fluids as well as individual proteases. The technology is currently being applied to identify proteases as biomarkers in a variety of disease states for subsequent development of in vitro diagnostics.



Biography

Kalyani Jambunathan is a researcher at SRI International's Center for Advanced Drug Research (CADRE), at present working on NIAID funded project to develop FRET based assay to detect fungal derived proteolytic activity during invasive Aspergillosis. She graduated with a BSc in chemistry from Madras Christian College (MCC, India), MSc in chemistry from Indian Institute of Technology-Madras (IIT-M) and PhD in chemistry under the guidance of Dr. H. Mario Geysen in Department of Chemistry at University of Virginia. Her areas of specialization include but not limited to peptide science, molecular biology, assay design and development.