



Novel Fret Method

Tech ID: 30331 / UC Case 2008-629-2

BACKGROUND

Fluorescence Resonance Energy Transfer (FRET) occurs between two adjacent fluorophores when energy transfer from excited donor to acceptor results in quenching of donor and excitation of acceptor. Due to its sensitivity to the distance between fluorophores, FRET has been used to study molecular interactions. FRET-based techniques have been extensively used in biological research including identification of protein interactions, real-time monitoring of intracellular signaling activities, and high-throughput screening of bioactive molecules such as those involved in the SUMO pathway.

Small ubiquitin-related modifiers (SUMO) are post-translational protein modifiers involved in immune signal transduction, transcriptional regulation and neurodegenerative diseases. SUMO undergoes reversible conjugation to the target protein via the help of SUMO ligases (Figure 1). Identifying small chemical inhibitors of SUMO ligases is important because small chemicals offer better spatial and temporal control of SUMOylation process compared with traditional methods such as gene knockout studies. Currently there is no available small chemical compound specific for SUMOylation pathways, which presents a need for developing high-throughput screening assays for these small molecule inhibitors.

BRIEF DESCRIPTION

Dr. Jiayu Liao and colleagues at the University of California, Riverside have developed a FRET assay using nitrobenzoxadiazole (NBD) and coumarin (CUM) amino acid analogs as a FRET pair. These fluorophores are genetically encoded into peptides and proteins surrounding a protease cleavage site or ligand binding site and used for FRET-based high throughput screening for enzymes or small molecule inhibitors involved in pathways such as SUMOylation. Researchers have demonstrated FRET for peptides encoded with NBD and CUM separated by 4 and 6 amino acids and excited at 340 nm (Figure 1).

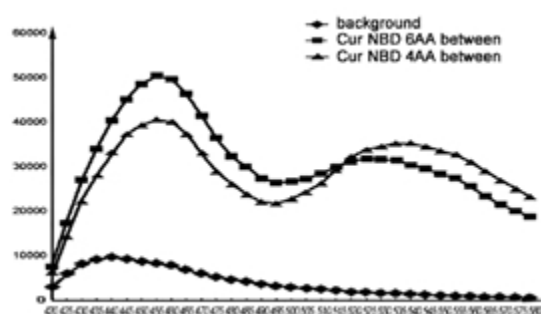


Figure 1. Fluorescent intensity of peptide I (6 amino acids between CUM and NBD) and II (4 amino acids between CUM and NBD) excited at 340 nm.

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OTHER INFORMATION

KEYWORDS

FRET, SUMO, SUMOylation,
 fluorophore, high-throughput screen

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SUGGESTED USES

These new FRET pairs may be used for high-throughput screening assays to measure protein-protein interactions.

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	8,940,506	01/27/2015	2008-629

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