

FRET-FLIM ASSESSMENT OF CONFORMATIONAL CHANGES OF A dJUN FRET BIOSENSOR IN RESPONSE TO STRESS ACTIVATION OF THE JNK PATHWAY

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Cells use multi-protein kinase cascades to signal information from the cell membrane to the nucleus as a response to a variety of external cues. These responses are linked to regulation of gene expression, as transcription factors are often the targets of regulatory phosphorylation events. One example of a signaling network dedicated to maintaining cell, tissue, and organism fidelity in the face of cellular stress is the Jun amino (N)-terminal kinase (JNK) cascade. Jun is a target of JNK phosphorylation that mediates transcriptional regulation of gene expression when in complex with Fos, forming the transcription factor *i.e* activator protein-1 (AP1). This study aims to develop an unambiguous method to measure in real-time the activity of the JNK signaling pathway in *Drosophila* cells by evaluating the level of dJun phosphorylation using an intramolecular FRET sensor (Fluorescence Resonance Energy Transfer). The dJun-FRET chimeric polypeptide is composed of a modified *Drosophila* Jun phosphorylation domain joined to a FHA2 phosphothreonine-binding module by a flexible linker and flanked by cyan fluorescent protein (CFP) donor and yellow fluorescent protein (YFP) acceptor modules (Figure 1).

The activity of the pathway was analyzed in different conditions by assessing conformational changes in the dJun-FRET module by using a combination of FRET and Fluorescence Lifetime Imaging Microscopy (FLIM). By measuring the donor (CFP) lifetime in the presence and the absence of regulators of the JNK pathway, the distance between the donor and acceptor can be estimated (Figure 2) and robust quantitative information about the level of activity of the pathway can be obtained in real-time. This FRET-FLIM study demonstrates that following the dynamics of the JNK signaling cascade in real-time *in vivo* is technically feasible, allowing future analysis targeting stress-mediated JNK-signaling responses.

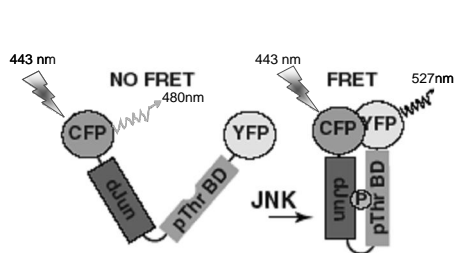


Figure 1. dJUN Intramolecular FRET

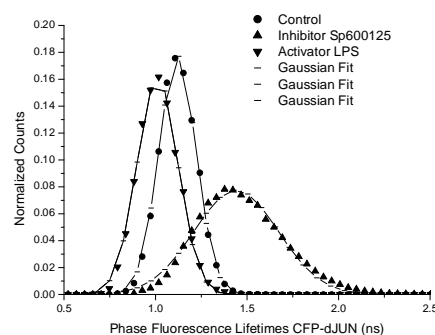


Figure 2. FRET-FLIM results - Histogram of averaged donor fluorescence lifetimes of CFP- dJUN in *Drosophila* cells in selected regions of interest (ROI). The fluorescence lifetime decreases in the presence of the activator and increases in the presence of the inhibitor. These lifetime changes are related to a conformational change (the distance between the N- and C-terminus of the protein) induced by these regulators

Reference:

Bakal *et al.* Phosphorylation Networks Regulating JNK Activity in Diverse Genetic Backgrounds. *Science* **322**, 453 (2008).

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