

**P013** Design of a FRET-based biosensor to visualize the spatio-temporal activation of Pak1 kinase in living cells  
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The protein kinase Pak1, an effector of the Rho family GTPases Cdc42 and Rac1, is an important regulator of the actin cytoskeleton, adhesion and motility. Moreover, it contributes to the oncogenic progression and invasiveness of mammalian cells.

We rationally designed a biosensor for Pak1 activation based on FRET (Fluorescence Energy Resonance Energy Transfer). The single molecule biosensor consists in an YFP-Pak1-CFP fusion protein that is expected to allow energy transfer from CFP to YFP only when Pak1 is in the closed inactive state. The cotransfection in Cos1 cells of the Pak1 biosensor with the physiological activator Cdc42V12 (mutationally blocked in the active form) induces a quantitative decrease of FRET due to the profound conformational changes upon GTPase binding and kinase activation. Accordingly, a biosensor encoding Pak1 constitutively activated by point mutation (L107F) or by membrane-localization (CAAX box at the C-terminus) also displayed a reduced FRET.

We validated the use of this novel Pak1 biosensor during cell spreading on fibronectin-coated dishes. The cytosolic biosensor did not display any obvious Pak1 activation, while the plasma membrane-localized version revealed dynamic Pak1 activity at the sites of active spreading, particularly evident when a wild-type Cdc42 is co-expressed.

In conclusion, we developed an original molecular tool which allowed us to visualize for the first time the spatio-temporal dynamics of Pak1 activity in living cells.