High-sensitivity measurements of multiple kinase activities in live single cells

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Increasing evidence has shown that population dynamics are qualitatively different from single cell behaviors. Reporters to probe dynamic, single cell behaviors are desirable, yet relatively scarce. Here we describe an easy-to-implement and generalizable technology to generate reporters of kinase activity for individual cells. Our technology converts phosphorylation into a nucleocytoplasmic shuttling event that can be measured by epifluorescence microscopy. Our reporters reproduce kinase activity for multiple types of kinases in a variety of cell lines, and allow for calculation of active kinase concentrations via a mathematical model. Using this technology, we made several experimental observations that had previously been technically unfeasible, including stimulus-dependent patterns of c-Jun N-Terminal Kinase (JNK) and Nuclear Factor kappa B (NF-κB) activation. We also measured JNK, p38 and ERK activities simultaneously, finding that p38 regulates the peak number, but not the intensity, of ERK fluctuations. Our approach opens the possibility of analyzing a wide range of kinase-mediated processes in individual cells.