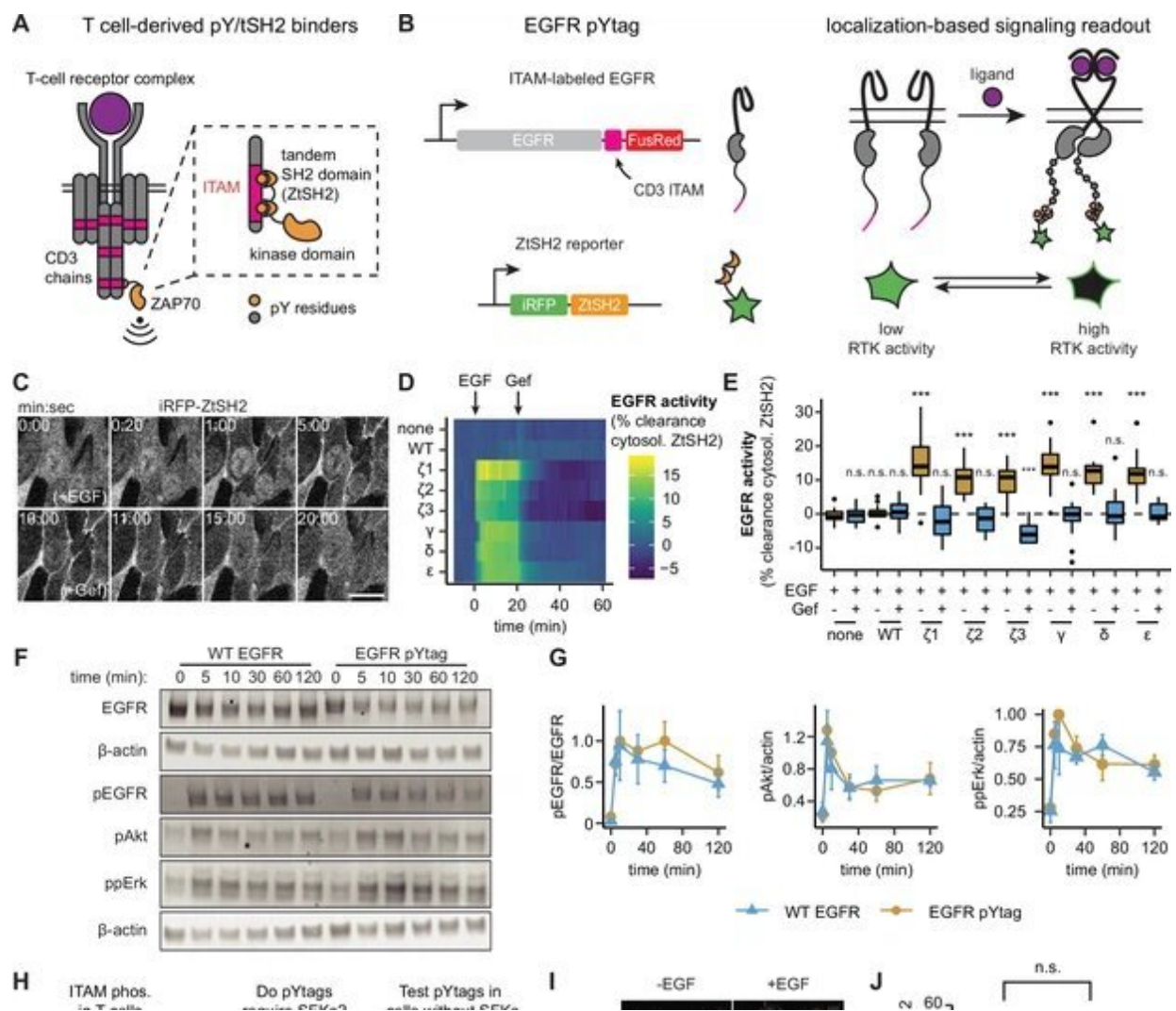


Fluorescent tags allow live monitoring of growth factor signaling proteins inside living cells

July 10 2023, by Jade Boyd



pYtags: a biosensing strategy to monitor receptor tyrosine kinase (RTK) activity

in living cells. (A) The T-cell receptor complex contains six immunoreceptor tyrosine-based activation motifs (ITAMs) from CD3 chains that, when phosphorylated, bind to the tSH2 domain of ZAP70 (ZtSH2). (B) Three repeats of CD3 ITAMs were appended to the C-terminus of epidermal growth factor receptor (EGFR) and clearance of ZtSH2 from the cytosol was assessed. (C) Timelapse images of NIH3T3 cells expressing EGFR pYtag (CD3 ϵ variant), treated first with EGF (100 ng/mL) and then with gefitinib (10 μ M). Scale bar, 20 μ m. (D) Mean clearance of cytosolic ZtSH2 in cells co-expressing iRFP-ZtSH2 and EGFR C-terminally labeled with one of six CD3 ITAMs. EGF (100 ng/mL) and gefitinib (10 μ M) were sequentially added at times denoted by arrows. $n = 2$ independent experiments. (E) Clearance of cytosolic ZtSH2 10 min post-EGF treatment and 40 min post-gefitinib treatment from (D). Lines denote mean values, boxes denote 25–75th percentiles, and whiskers denote minima and maxima. $n \geq 14$ cells from two independent experiments. n.s., not significant, *** $p < 0.001$ 20 cells from three independent experiments. Credit: *eLife* (2023). DOI: 10.7554/eLife.82863

Synthetic biologists from Rice University and Princeton University have demonstrated "live reporter" technology that can reveal the workings of networks of signaling proteins in living cells with far greater precision than current methods. The first-of-its-kind reporting tool can show, for example, how quickly signaling networks respond and how their responses vary from cell to cell in both time and space.

Researchers created the tool using unobtrusive proteins that piggyback on an essential signaling mechanism [human cells](#) use to regulate growth, differentiation, migration, inflammation and other processes.

In the study, recently published in the open-access journal *eLife*, the Rice-Princeton team demonstrated its modular approach for tagging [receptor tyrosine kinases](#) (RTKs) with reporter proteins that activate [green fluorescent proteins](#) whenever their RTK partners become

phosphorylated.

Kinases are enzymes that can alter the behavior of other proteins by attaching or detaching [phosphate groups](#), a process called phosphorylation. RTKs are specialized kinases that themselves become phosphorylated when they detect incoming signals or stimuli outside the cell, and then regulate vital cell functions.

The team showed the "live reporter" system could be used with a microscope to produce a video record of signaling network activity in living cells. Where cells glow and how brightly, reveals the location and intensity of signal network response, said Caleb Bashor, co-corresponding author of the study and an assistant professor of both bioengineering and biosciences at Rice.

"Most of the time, when you're studying stuff that happens inside of cells, like signaling networks or gene networks, you have to destroy the cells in order to look at their contents," Bashor said. "Anytime you can build something where the cells stay alive, and you can watch how the signaling [network](#) works in real time, inside of the cell, it's a great advantage."

The researchers dubbed the reporters pYtags, in reference to biochemical nomenclature where tyrosine is denoted as "Y" and as "pY" when phosphorylated.

Bashor and Xiaoyu Yang, a Ph.D. student in Bashor's research group, developed pYtags in collaboration with the research groups of Princeton's Jared Toettcher and Celeste Nelson. The study showed the system could record the activity of RTKs called [growth factor receptors](#) in human fibroblast cells.

"We take an engineered [protein](#) that's part of a different system—it's

actually part of immune signaling—and we put it into this new context, which is fibroblast cells that Jared works with in his lab at Princeton," Bashor said. "We think it's probably not interacting with anything else in the cell because it's from a completely different cell type. So, it just kind of hangs out on the end of the growth factor receptor."

The pYtag reporter is designed to co-activate with its RTK partner and trigger a proportional amount of fluorescence. So the stronger the RTK response, the brighter the cell glows when viewed through a microscope.

"It can receive that phosphorylation signal from the growth factor receptor," Bashor said. "So, when the receptor gets activated, the [green fluorescent protein](#) comes in, binds to something close to the membrane, and you get what looks like this green ring around the outside of the cell. That tells you, in real time, when the cells are seeing the growth factor and how fast the pathway is turning on."

Bashor said pYtags could be used to monitor many types of tyrosine kinase receptors.

"We show in the paper that this reporter could be put onto multiple different growth-factor receptor types, and that could be used as a reporter for all of them," he said. "This is a window into the dynamics of cellular signaling that we really didn't have before."

More information: Payam E Farahani et al, pYtags enable spatiotemporal measurements of receptor tyrosine kinase signaling in living cells, *eLife* (2023). [DOI: 10.7554/eLife.82863](https://doi.org/10.7554/eLife.82863)

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