FLIM-BASED SCREENING OF DYNAMICS OF SIGNAL TRANSDUCTION: PHOSPHODIESTERASES AND cAMP BREAKDOWN

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We present our genetic screening methodology for real time monitoring of dynamics of cellular cAMP (cyclic adenosine monophosphate) breakdown. Changes in cAMP levels are detected with the dedicated FRET (Fluorescence Resonance Energy Transfer) biosensor developed in our lab [1]. For quantitative measurements of FRET changes, FLIM (Fluorescence Lifetime Imaging) is the top choice.

In this study, 22 genes for discrete phosphodiesterases (PDEs) were individually silenced using siRNAs. cAMP levels were elevated in two ways: 1) cells were loaded with DMNB-caged cAMP analogue that was converted to cAMP by controlled automated UV irradiation; 2) cAMP concentration was elevated by transient β-adrenergic receptor activation with Isoproterenol. The screen was performed in a multiwell format in HeLa cells stably expressing the FRET biosensor and cAMP breakdown was measured in real time with 2-second resolution. Both approaches yielded highly reproducible results, each identifying PDE3A and PDE10A as the most active enzymes in breaking down cAMP.

Automated data analysis workflow was developed using custom Python scripts. Fluorescence lifetimes were measured by time-correlated single photon counting using the Leica FALCON system. Cell segmentation was performed by a deep-learning algorithm, Cellpose [2]. The lifetime time traces for all individual cells were fitted to a logistic function to obtain the cAMP breakdown rate corresponding to the cellular PDE activity.

Currently we are expanding this technology in two directions. 1) For exploring of potential influence of hypoxic conditions (relevant conditions in cancer progression) on cellular PDE activity, we have equipped the FLIM setup with custom designed gas control chamber (O_2 and CO_2). Here, the use of caged nucleotides and/or receptor agonists is again very useful by enabling controlled cellular stimulation without the need of pipetting under hypoxia. 2) With adoption of the pooled format and CRISPR/Cas9 gene editing, our combination of high-end fluorescence lifetime imaging with deep-learning based cell segmentation and automated analysis pipeline will contribute to performing genome-wide screens focused on dynamics of signal transduction under physiologically relevant conditions.

[1] Klarenbeek, J., Goedhart, J., van Batenburg, A., Groenewald, D. and Jalink, K. "Fourth-Generation Epac-Based FRET Sensors for cAMP Feature Exceptional Brightness, Photostability and Dynamic Range: Characterization of Dedicated Sensors for FLIM, for Ratiometry and with High Affinity." *PLoS ONE* 10, (2015).
[2] Stringer, C., Wang, T., Michaelos, M. & Pachitariu, M. Cellpose: a generalist algorithm for cellular segmentation. Nat. Methods 18, 100–106 (2021).