FLIPs: Novel genetically encoded biosensors for polarization microscopy

Paul Miclea[1], Vendula Nagy Markova[1], Alexey Bondar[1,2,3], Alina Sakhi[4], **Dr. Josef Lazar**[1]
[1]First Faculty of Medicine, Charles University in Prague, Prague, Czechia, [2]Biological Center CAS, Budweis, Czechia, [3]University of South Bohemia, Budweis, Czechia, [4]Inst. of Organic Chemistry and Biochemistry CAS, Prague, Czechia

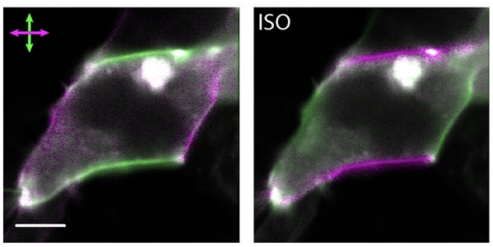
Background
Genetically encoded fluorescent biosensors convert specific biomolecular events into optically detectable signals. Typically, such probes utilize a suitable fluorescence quenching process in order to modulate the absorption or emission spectrum the fluorophore, or the fluorophore's lifetime. However, an unrelated detection principle, directionality of optical properties of fluorescent molecules, should also allow development of genetically encoded biosensors. Remarkably, despite numerous potential advantages of such biosensors (ratiometric readout, resistance to bleaching artifacts, information about protein structure, possibility of multiplexing, orthogonality to other approaches), optical directionality has remained largely unexploited as a detection principle. Our goal was to make optical directionality of fluorescent molecules widely applicable to imaging of biomolecular processes of cell signaling.

Methods
Using techniques of molecular biology, we set out to develop a series of genetically encoded biosensors suitable for observations of molecular processes of cell signaling by polarization-resolved fluorescence microscopy.

Results
We have now identified a novel design of genetically encoded fluorescent biosensors, which we term FLIPs(1,2,3) (Fluorescence anisotropy and LInear dichroism Probes). FLIPs offer an extremely simple design, high sensitivity, multiplexing capability, ratiometric output and resilience to bleaching artifacts. Importantly, FLIPs allow their targets to remain non-modified. The probes are applicable to imaging cellular activity of G protein coupled receptors, G proteins, arrestins, receptor tyrosine kinases and other signaling proteins, using simple microscopy instrumentation. The sensitivity of the probes allows even imaging of endogenously expressed targets.

Conclusion
The simplicity, modularity and versatility of the FLIP design allows rapid creation of biosensors of desirable properties. By exploiting a simple, universal, yet long overlooked detection principle, FLIPs open a wide new area of development both in genetically encoded fluorescent biosensors and in optical instrumentation.

Graphic:



Keywords:

Polarization microscopy, biosensors, GPCR signaling

Reference:

1) Miclea P., Nagy Markova V., Bondar A., Sakhi A., Lazar J.; Novel Genetically Encoded Biosensors for Functional Imaging of Cell Signaling by Polarization Microscopy; https://www.biorxiv.org/content/10.1101/2024.02.23.581811v2
2) Lazar J., Bondar A., EPO patent application 21209717.4
3) 'FLIP' and 'FLIPs' are are trademarks used by Innovative Bioimaging, s.r.o.