

# FLIM indicators for quantitative measurement of pH

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## Introduction

- Most conventional fluorescent indicators are only capable of a relative assessment of changes in the studied parameter in the cell.
- In fluorescence biosensing, quantitative analyte detection capabilities are often associated with time domain analysis of fluorescent signal instead of spectral one
- In this project, we are testing promising pH-sensitive fluorophores with labile fluorescence lifetimes as fluorescent core for the previously described pH indicators and as independent pH indicators.

## Results

### EYFP-G65T

EYFP-G65T is spectrally much more sensitive to electrostatic interactions than the parental EYFP [1] and this feature of the chromophore allows us assume the lability of its fluorescence lifetime.

In the spectral domain, EYFP-G65T is sensitive to pH *in vitro* (Fig. 1 A).

In the time domain we found a nearly linear dependence of the EYFP-G65T fluorescence lifetime with a fourfold increase on pH within the range of 3.5-7.5 when it is excited at 500 nm. EYFP-G65T also has an excitation peak at 420 nm, but the fluorescence lifetime changes at this wavelength are insignificant (Fig. 1 B).

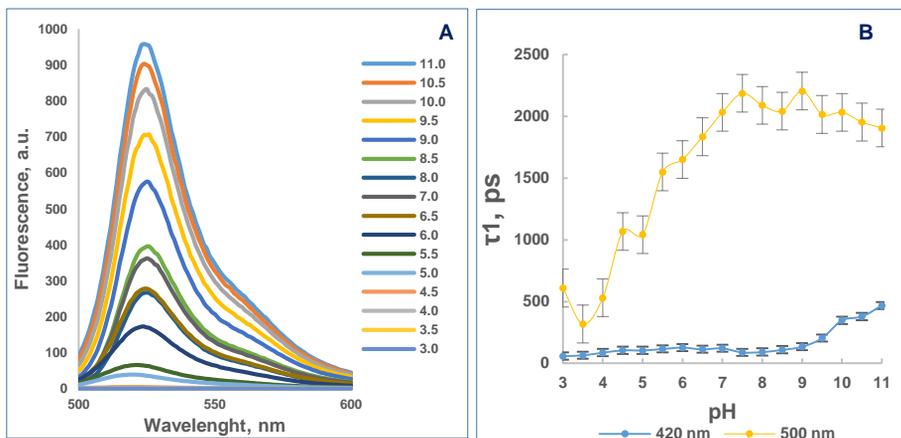


Fig.1. (A) Fluorescence spectra of EYFP-G65T at different pH (3.0–11.0) (B) Dependence of the EYFP-G65T fluorescence lifetime on pH

### SypHer3s and SypHer3s-G65T

Since cpEYFP is the fluorescent core of the pH indicator SypHer3s, we replaced its fluorescent core with cpEYFP-G65T and tested both indicators in spectral and time domains.

We didn't find any pH dependence of the fluorescence lifetime for both SypHer3s and SypHer3s-G65T.

However, in spectral domain, the total dynamic range of SypHer3s-G65T is two times higher than in the original indicator.

F500 / F420 SypHer3s-G65T ratio increases more than 135 times when the pH changes from 6.5 to 10.5 (Fig. 2).

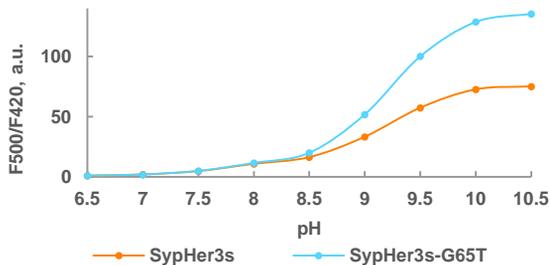


Fig.2. Comparison of SypHer3s and SypHer3s-G65T in spectral domain *in vitro*.

In addition, to determine pH sensitivity of the indicators in live cells, we measured the F488 / F458 ratio in the HEK293T cells within the pH range of 5.5-9.0 in a set of buffers containing nigericin and monensin ionophores in confocal microscopy experiments. The total dynamic range of SypHer3s-G65T in the spectral domain is 4 times greater than that of SypHer3s (Fig. 3).

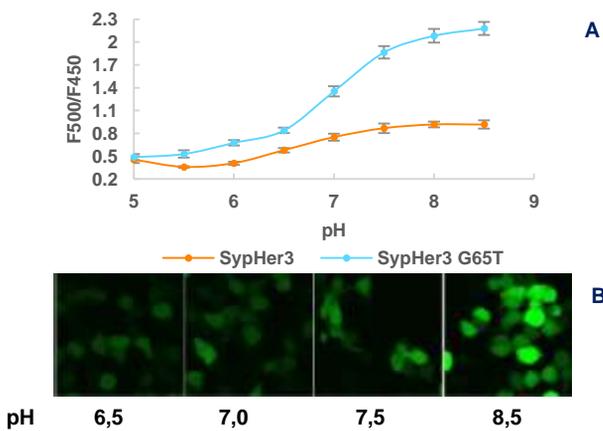


Fig.3. (A) Comparison of SypHer3s and SypHer3s-G65T in spectral domain *in cellulo*. (B) SypHer3s-G65T in HEK 293T

## References

1. Sen et al., 2019
2. Mamontova et al., 2020

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### EGFP-Y145L-S205V

EGFP-Y145L/S205V, a promising probe for pH measurement within the alkaline range, is an EGFP mutant designed in our lab and possessing high chromophore pKa [2].

The feature of the GFP chromophore is its ability to be either in protonated or deprotonated state (Fig. 4). This makes it sensitive to changes in pH. And we can assume that this sensitivity will affect the lifetime.

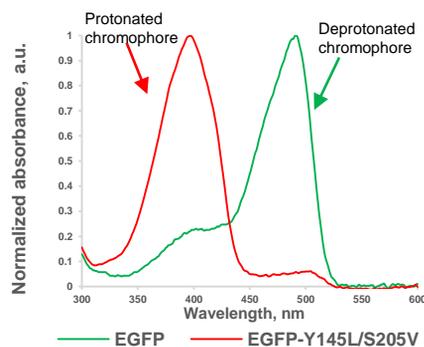


Fig. 4. Absorption spectra of EGFP-Y145L-S205V

Considering the high pKa of the mutant (~10), we expected that it would be especially sensitive within the alkaline pH region. However, our time-resolved experiments at two excitation wavelengths (400 and 500 nm) revealed that EGFP-Y145L/S205V has a linear dependence of the fluorescence lifetime on pH in the range of 6.0-7.5 at 400 nm excitation (the lifetime shows a 2,5-increase).

The fluorescence lifetime at Ex 500 nm remains unchanged.

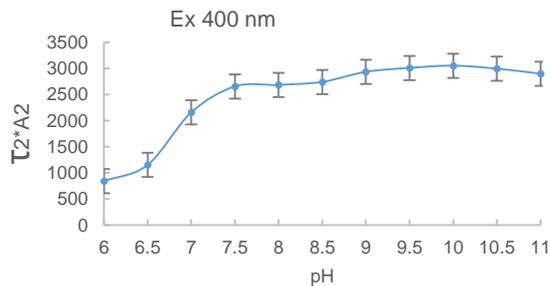


Fig. 5. Dependence of the EGFP-Y145L-S205V fluorescence lifetime on pH

## Conclusions and perspectives

- ✓ The total dynamic range of SypHer3s-G65T's (i.e., fluorescence response to pH) in the spectral domain *in vitro* is two times wider *in vitro* and four times wider *in cellulo* than those of SypHer3s
- ✓ EYFP-G65T fluorescence lifetime changes linearly from 300 to 2000 ps within the pH range of 3.5-7.5
- ✓ The pH range 6.0-7.5 can be additionally studied using EGFP-Y145L/S205V with fluorescence lifetime changes linearly from 850 to 2500 ps.
- Difference in excitation mode makes it possible to use EYFP-G65T and EGFP-Y145L/S205V in one experimental system to assess pH changes in a wide range of 3.5-7.5
- Possibility to assess pH in different cellular compartments