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Abstract

FLIM Indicators for Quantitative Measurement of pH †

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Abstract: Monitoring of intracellular pH changes in situ can provide valuable information about cellular metabolism and a deeper understanding of physiological processes. Most traditional fluorescent indicators are only capable of a relative assessment of changes in the studied parameter in the cell. We associate the possibility of measuring the absolute values that characterize the analyte with the detection of the indicator signal in the time domain, where its quantitative measure is the fluorescence lifetime (tau). In this project, we test promising pH-sensitive fluorophores with labile fluorescence lifetimes—EYFP-G65T and EGFP-Y145L/S205V—both as fluorescent cores for the previously described pH indicators and as independent pH indicators. Measurement of the fluorescence attenuation kinetics of four structures (EYFP-G65T, EGFP-Y145L/S205V, SypHer3s, and SypHer3s-G65T) over a wide pH range revealed areas where tau is linearly dependent on pH. The differences in the fluorescence excitation modes of these molecules makes it possible to use them in one experimental system to assess pH changes in a wide range, 4.0–9.0. We showed that under the conditions of traditional fluorescence microscopy (in the cytoplasm of HEK293 cells), the SypHer3s-G65T indicator shows a dynamic response range approximately 3 times wider than the original SypHer3s.

Keywords: fluorescence lifetime; time-resolved microscopy; fluorescence lifetime imaging microscopy; FLIM; fluorescence imaging; fluorescent proteins; fluorescent biosensors; genetically encoded indicators; small molecules visualization

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