

Supplemental material:

Table S1 Spectral characteristics of conventional fluorescent dyes used as pH in vivo indicators. The real isosbestic point is derived from normalized spectra whereas the apparent isosbestic point is from the raw data. The Stokes-shifts are defined here by the difference between the wavelength of the major (and the minor) absorption peak and the wavelength of the emission peak. Index 'x' of S designates sensitivity calculated from excitation spectra, and index 'm' values are calculated from emission spectra. 'em', 'ex', and 'abs' designate wavelengths in the emission spectrum, the excitation spectrum, and in the absorption spectrum, respectively. All data are derived from 10 kDa dextran derivatives:

dextran-BCECF = 2',7'-Bis(2-carboxyethyl)-5,6-carboxyfluorescein (#D1878, Invitrogen-Molecular Probes); dextran-FITC = Fluorescein-Isothiocyanate (#FD10S; Sigma); dextran-SNARF[®] = (#D3303, Invitrogen); dextran-NERF[®] = (#D3319, Invitrogen)

dye-species parameter	BCECF	SNARF [®]	FITC	NERF [®]
real isosbestic point λ_{iso}	489 nm(ex)	610 nm(em)	476 nm (ex)	502 nm (ex)
apparent isosbestic point	439 nm(ex)	626 nm(em)	427 nm (ex)	451 nm (ex)
left peak WL ($\lambda_1 < \lambda_{iso}$)	460nm(ex)	587 nm(em)	454 nm (ex)	472 nm (ex)
right peak WL ($\lambda_2 > \lambda_{iso}$)	506nm(ex, abs)	632 nm(em)	495 nm (ex)	514 nm (ex)
Stokes-shift ($\lambda_{em} - \lambda_{abs}$)	18 nm	2 nm	21 nm	23 nm
maximum WL (λ_{max})	524 nm(em)	585 nm(ex)	516nm(em)	537 nm (em)
$R_{max} (F(\lambda_1)/F(\lambda_2))$	4.6	4.9	3.6	3.5
$R_{min} (F(\lambda_1)/F(\lambda_2))$	0.5	0.25	0.4	0.8
$R_{max} - R_{min}$	4.1	4.6	3.1	2.7
R_{max} / R_{min}	9.6	19	8.1	4.3
apparent pK ($\lambda_1; \lambda_2$)	6.9	8.7	6.3	5.2
Responsiveness ($\Delta \log(R) \cdot \Delta pH$)	2.2	2.9	1.6	1.2
pH sensitivity (S_x)	0.9	0.94	0.84	0.65
pH sensitivity (S_m)	0.3	1.0	0.36	0.36

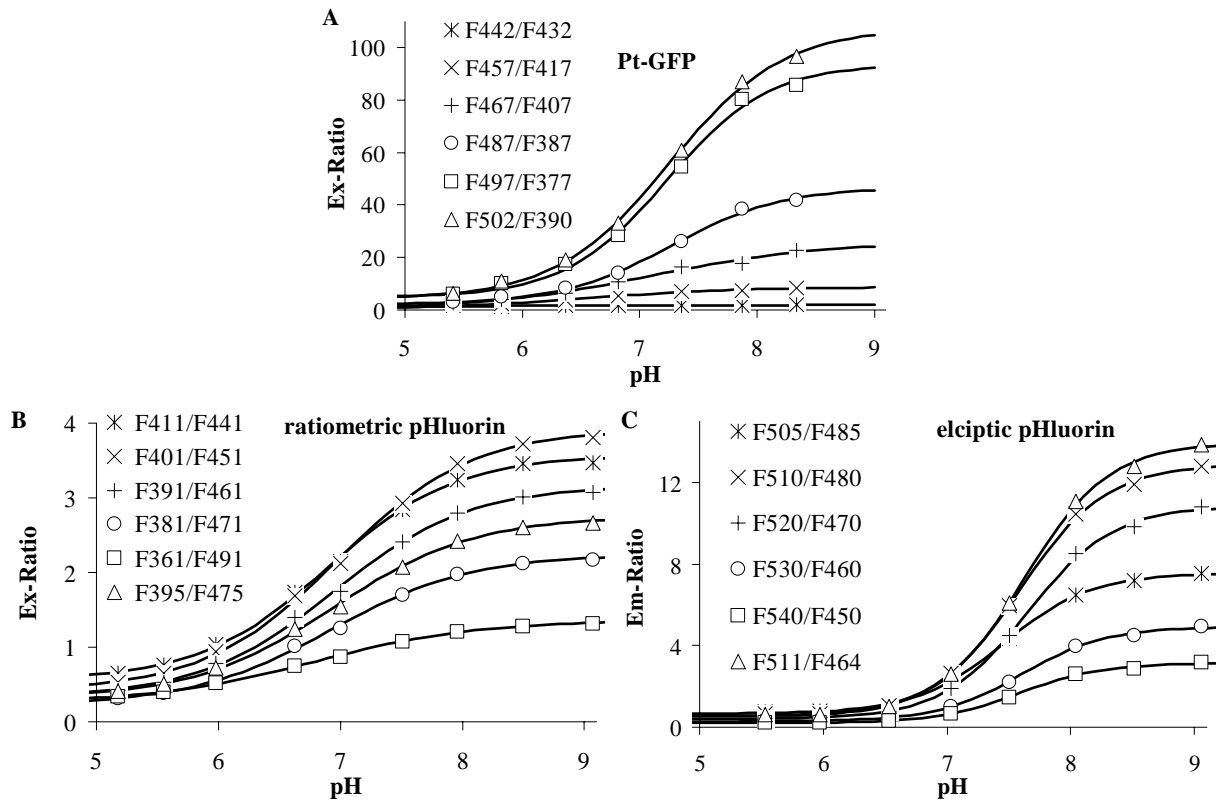


Figure S1 The dependency of ratio calibration curves on the used pair of wavelengths.

Table S2 The dependency of the apparent pK_a on the used pair of excitation wavelengths.

GFP	Symbol:	✱	✕	+	○	□	△
Pt-GFP	$\lambda_{\text{ex-pair}}$	F442/F432	F457/F417	F467/F407	F487/F387	F497/F377	F502/F390
	apparent pK	5.0	6.6	7.1	7.3	7.2	7.3
ratiometric pHluorin	$\lambda_{\text{ex-pair}}$	F411/F441	F401/F451	F391/F461	F381/F471	F361/F491	F395/F475
	apparent pK	6.9	7.0	6.9	6.9	6.8	6.9
ecliptic pHluorin	$\lambda_{\text{em-pair}}$	F505/F485	F510/F480	F520/F470	F530/F460	F540/F450	F511/F464
	apparent pK	7.5	7.6	7.6	7.6	7.6	7.6