

TITLE: Excitation-Dependent pKa Extends the Sensing Range of Fluorescence Lifetime
pH Sensors

AUTHORS: Emily P. Haynes
 Mary Canzano
 Mathew Tantama, mt4@wellesley.edu

SUPPLEMENTARY MATERIALS

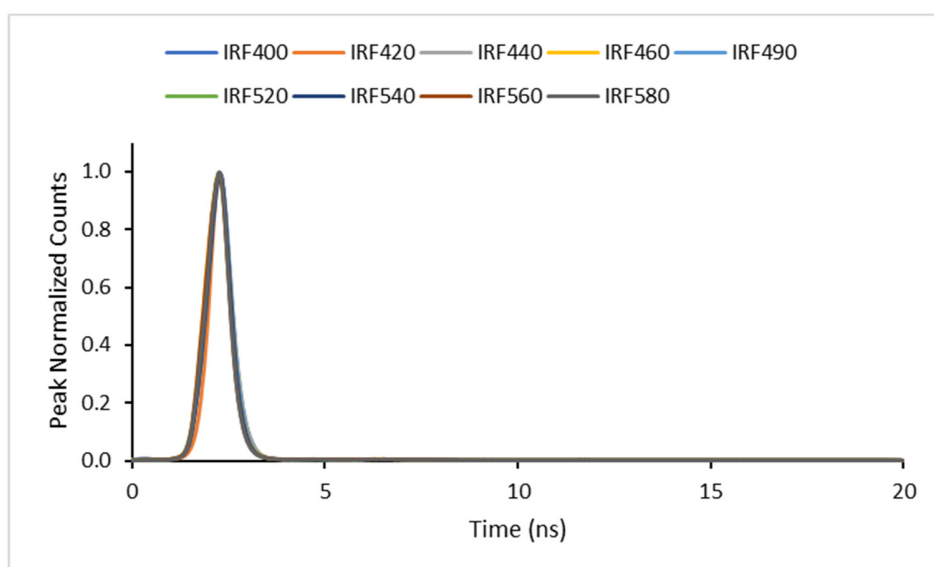
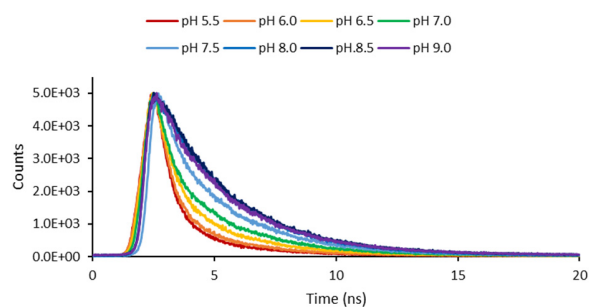
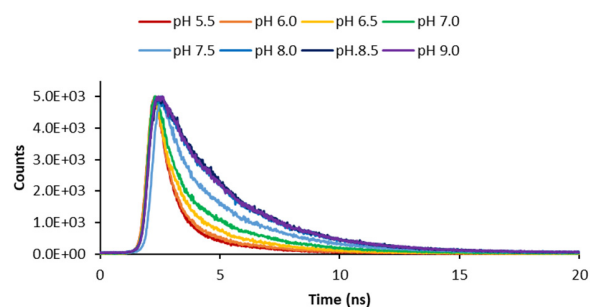


Figure S1. Instrument response functions did not differ significantly across wavelength settings.

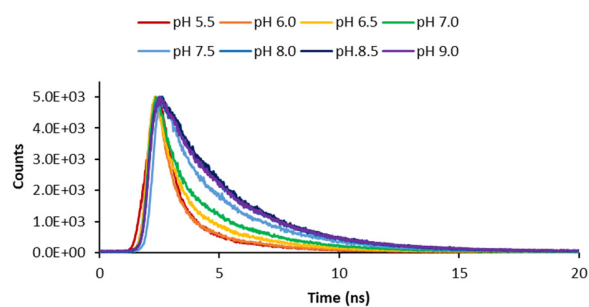
(A) 483 nm



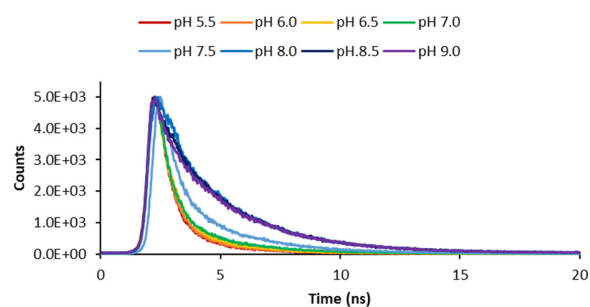
(D) 543 nm



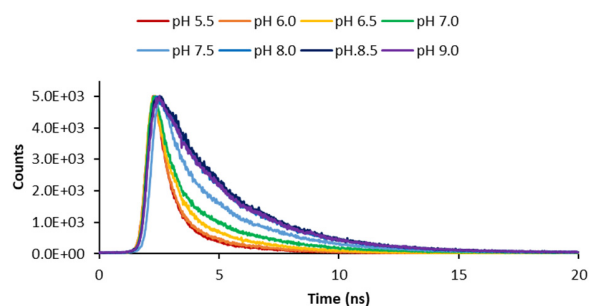
(B) 503 nm



(E) 563 nm



(C) 523 nm



(F) 583 nm

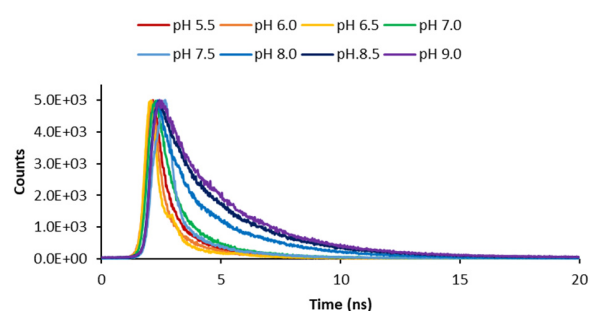
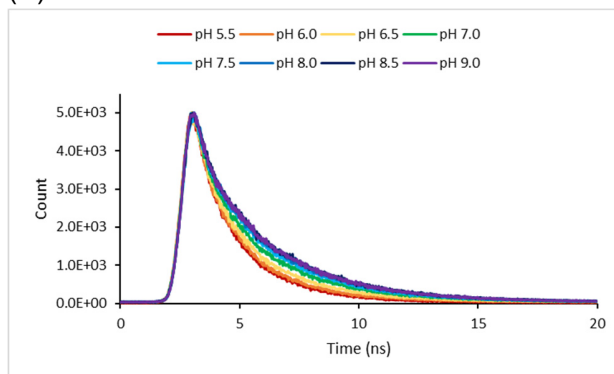
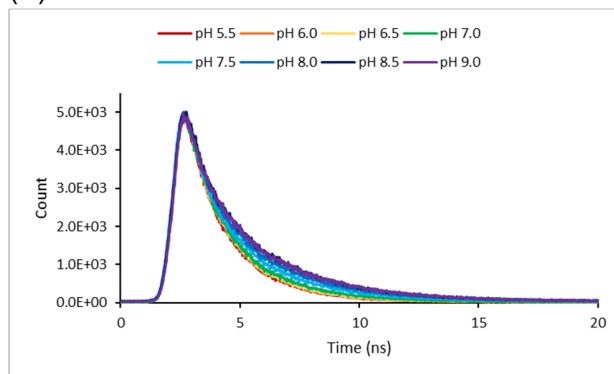


Figure S2. Excitation wavelength and pH dependent fluorescence lifetime decays of mCherryTYG (mCherryM66T) purified protein.

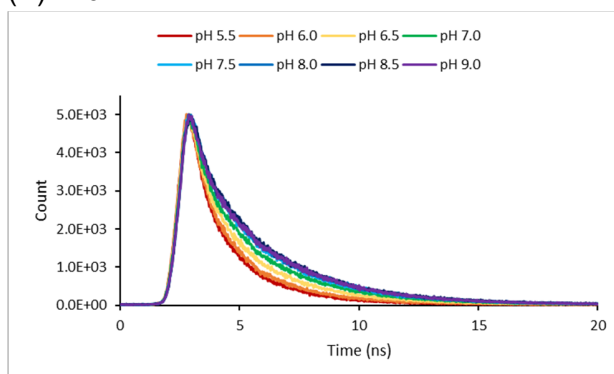
(A) 400 nm



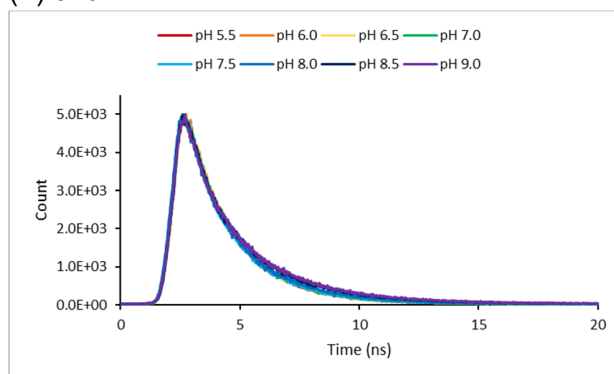
(E) 490 nm



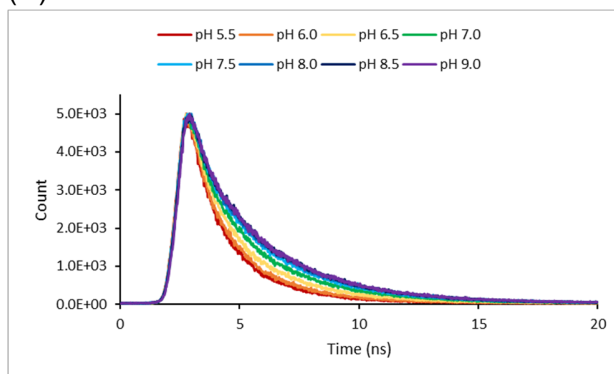
(B) 420 nm



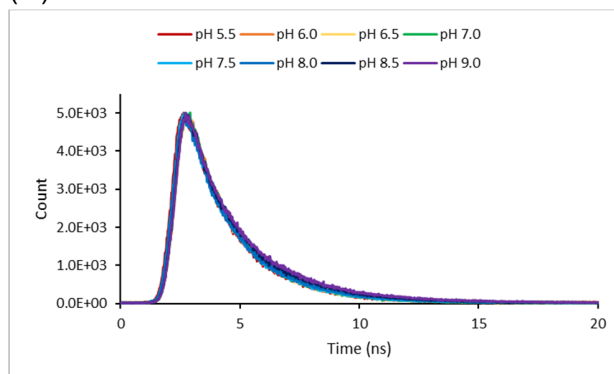
(F) 520 nm



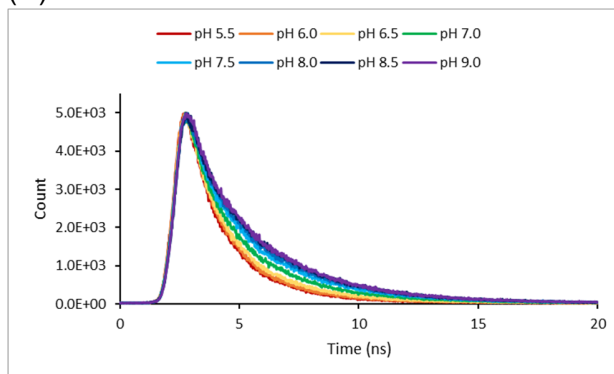
(C) 440 nm



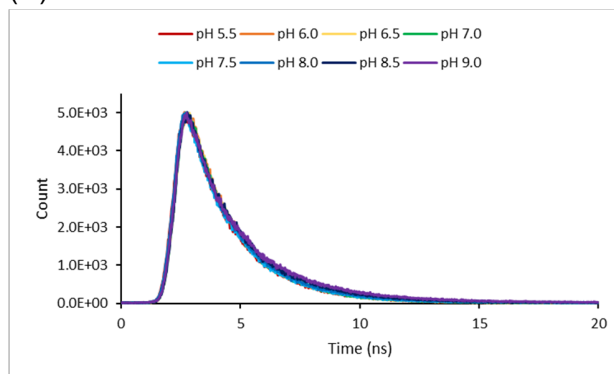
(G) 540 nm



(D) 460 nm



(H) 560 nm



(I) 580 nm

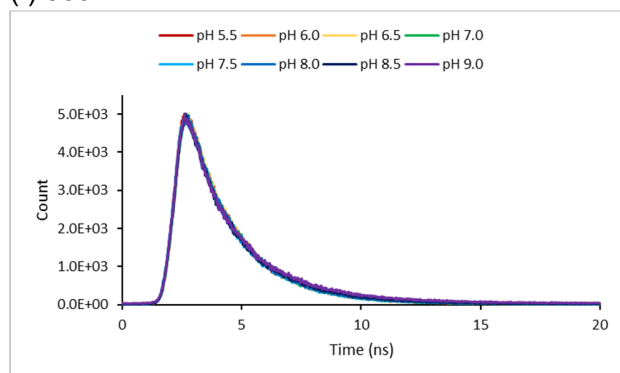
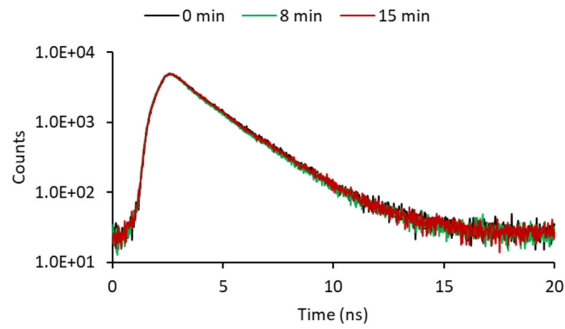


Figure S3. Excitation wavelength and pH dependent fluorescence lifetime decays of pHRed purified protein.

(A) 575 nm



(B) 440 nm

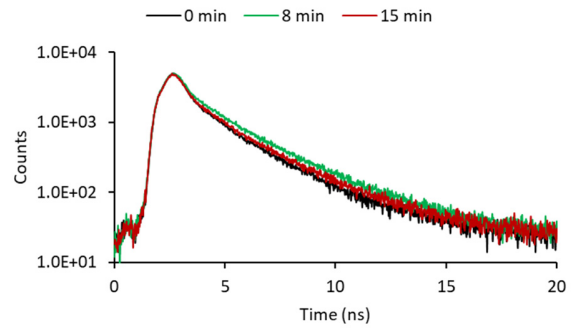


Figure S4. Fluorescence lifetime decays of pHRed expressed in live bacteria during metabolic challenge. *E. coli* were pre-starved at time 0 min. They were then fed glucose, causing cytosolic alkalinization at 8 minutes, after which cyanide was added to block metabolism and cause re-acidification by 15 minutes. (A) Excitation at 575 nm is predicted exhibit a $pK_a^{app} \sim 8.5$ that is insensitive to these metabolic pH changes. Indeed, the representative fluorescence decays do not show any differences over time. (B) In contrast, excitation at 440 nm is predicted to exhibit a $pK_a^{app} \sim 6.5$, which is sensitive to these metabolic pH changes. Indeed, the representative lifetime decays show an increase in lifetime with glucose at 8 minutes that is reversed by cyanide at 15 minutes.