Electronic Supplementary Information (ESI):

Spectral and Redox Properties of the GFP Synthetic Chromophores as a Function of pH in buffered media

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1. Chemicals

All compounds used in this experiment were ACS grade. The acetic acid, boric acid, phosphoric acid, KNO_3, $HClO_4$, NaOH, and phosphate buffered saline (PBS) were from Fisher Scientific. GFP chromophores were synthesized by Dong and Baldridge as described in refs. 1-4.

2. Buffer Solution

It was known that the redox properties of various organic molecules in aqueous solutions are strongly dependant on the ionic strength. In order to maintain proper ionic strength and proton exchangeability Britton-Robinson (BR) aqueous universal buffer solutions pH = 2-12 were prepared by mixing appropriate volumes of acids and basic buffer components.[5] The acidic buffer component comprises 0.04 M phosphoric acid, 0.04 M boric acid, and 0.04 M acetic acid. The basic buffer component is 0.2 M sodium hydroxide solution. For the electrochemical measurements and the absorption measurements, concentrated chromophore solutions in 0.5% methanol were added to the buffer solution in a 1:500 volume ratio to yield solution with 0.6 – 2.0 optical density at neutral pH. The buffer solution components are given in **Table S1** and **Table S2**. The initial pH of 1.81 was adjusted with 0.2 M NaOH.

3. Protein Sample Preparation

The genes encoding DsRed (amplified from DsRed2-1 plasmid, Clontech, Mountain View, CA), AcGFP (amplified from pAcGFP1-C1, Clontech, Mountain View, CA), KillerRed (GenBank accession number AAY40168.1), mRFP (GenBank accession number AAM54544), and EGFP

were cloned into pProTet between the *Sal*I and *Not*I restriction sites and contained an N-terminal 6xHN affinity tag. For protein expression, the plasmids were transformed into *E. Coli DH5-α*-pro. A 5 mL culture was inoculated into 1 L of Lysogeny Broth (LB, pH 7) containing 35 µg/mL of chloramphenicol, grown to an OD600 of 0.4 at 37 ºC (2-3 h), and then induced with anhydrotetracycline (1 mg/mL).

The gene encoding mKalama1 (GenBank accession number ABP88742.1) was purchased from AddGene (Cambridge, MA). The gene is inserted between the *Xho*I and *Eco*RI restriction sites in pBAD-His B and contained an N-terminal hexahistidine tag. The plasmid was transformed into E. coli DH5 αPro. A 5 mL culture was inoculated into 1 L of LB containing 50 µg/mL of ampicillin, grown to an OD600 of 0.5 at 37 °C (2-3 hrs), and then induced with 0.2 % arabinose.

The GFP-F99S/M153T/V163A/T203V/S205V (EGFP-T203V-S205V in the main text) gene was obtained as a gift from J. Remington (U. Oregon). The gene is inserted between BamHI and HindIII restriction sites in PQE-30 and contains an N-terminal hexahistidine tag. Mutagenesis of GFP-F99S/M153T/V163A/T203V/S205V to create the single point mutation H148Y was performed using the rapid-polymerase chain reaction (PCR) site-directed mutagenesis method.[6] The plasmids were transformed into *E. coli* M15 for expression. A 5-mL culture was inoculated into 1L of LB containing 50 µg/mL of ampicillin, grown to an OD600 of 0.5 at 37 ºC (2-3 hrs), and then induced with 1 mM IPTG.

After induction, the cultures were incubated with shaking at 30-34 ºC for 18-20 hours. Proteins were overexpressed to 10-20% of cell protein and purified via Ni^{2+}-nitrilotriacetic acid (NTA)-immobilized metal affinity chromatography (IMAC). All proteins were dialyzed with sodium phosphate buffer (PBS, 50 mM sodium phosphate, 250 mM NaCl, pH 7.2) after purification.

Dendra2, wt-GFP, wtGFP-D82N-K85M, and wt-GFP-S72A protein samples were provided as gifts from Konstantin Lukyanov at the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry in Moscow, Russia.

4. pH Measurements

The pH was measured with a VWR sympHony pH/ISE Meters 1145001 SB80PI Benchtop Meter with glass combination pH electrode (Radnor, PA). The pH meter was calibrated with standard buffer solutions with pH buffers 1, 4, 7, and 10 before each use.

5. Electrochemical Set-up

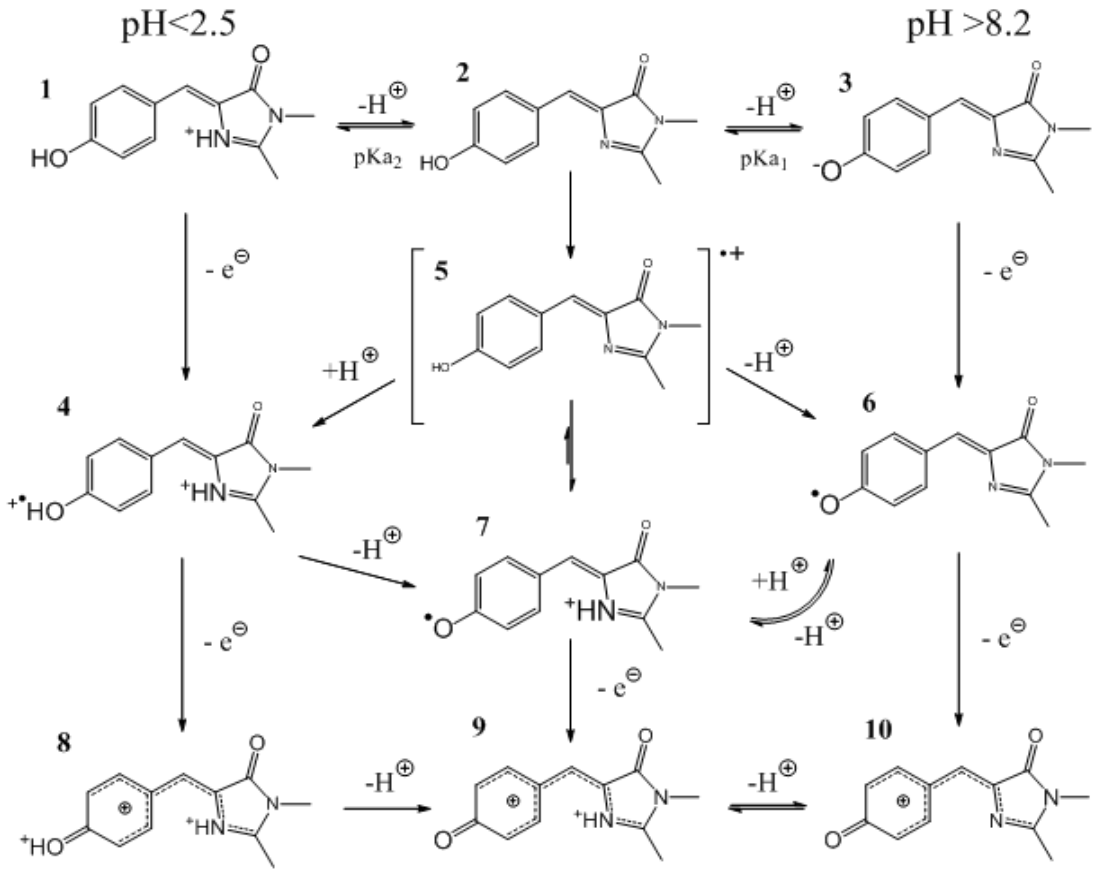
The voltammetric measurements were conducted at room temperature with the CHI 660 potentiostat manufactured by CH Instruments, Inc. using a three-electrode setup. The working electrode (WE) was a glassy carbon electrode (GCE) (d=3 mm), a platinum foil was the counter electrode (CE) and the Ag/AgCl in 0.1 M KCl // 0.1 M KNO_3 was the reference electrode. The glassy carbon electrode (GCE) was always polished before each experiment using 0.05 μ alumina and sonicated in MeOH for 5 minutes. After this treatment, the GCE was placed in the buffer solution and differential pulse (DP) voltammograms were recorded until a steady state. The standard potential of the Ag/AgCl reference electrode is 0.288 V vs. the SHE.[7] Cyclic voltammograms and DP voltammograms were taken under a nitrogen atmosphere for each sample after the electrolytes were purged with nitrogen for at least five minutes before each experiment. Cyclic voltammograms of the chromophores were recorded with scan rate of 0.5 V/s, while the proteins were recorded at 0.01 V/s. It is generally accepted that higher scan rates enhances the peak current. The 0.5 V/s scan rate for the chromophore solutions was selected in order to be able to observe unstable reaction products on the reverse scan. That was not the case. In order to enhance the adsorption of the proteins at the electrode the slower scan rate for the CV was applied. The observed oxidation peaks at the slower scan rate were well defined. The DP voltammograms were obtained using the following parameters: pulse amplitude of 0.05 V, pulse width of 0.2 s, sample width of 0.0167 s, and a pulse period of 0.5 s. The current of oxidation peak for all the tested compounds was always decreasing with the number of scans due to adsorption of oxidation products on the electrode. It was observed that all compounds investigated here showed much higher oxidation peak currents using DP than the CV method.

Table S1. Composition of the Britton-Robinson Buffer.[5]

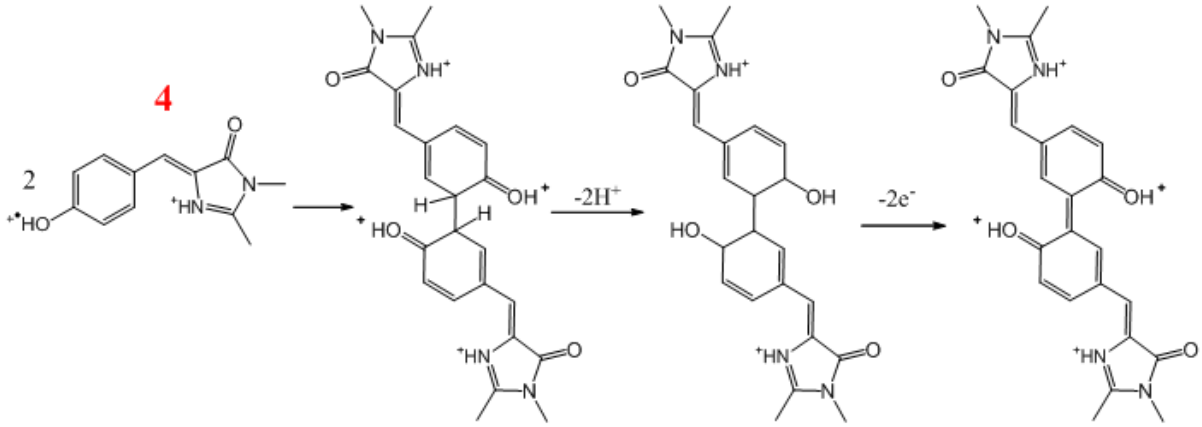
Compound	**Molarity (M) (Stock Solution)**	**Amount Used For 1.00 L**	**Concentration in solution (M)**
Acetic Acid	17.4	2.30 mL	0.04
H_3PO_4	14.7	2.72 mL	0.04
Boric Acid	-	2.4761 g	0.04

Table S2. Various Buffer Solutions titrated using 0.2M NaOH with a pH range 1.81-9.94.

Sample	**pH**	**Buffer Volume**	**NaOH added (mL)**
1	1.81	10 mL	0
2	2.47	10 mL	1.25
3	2.76	10 mL	1.75
4	2.96	10 mL	1.90
5	3.22	10 mL	2.00
6	3.24	10 mL	2.30
7	4.46	10 mL	3.00
8	5.89	10 mL	4.30
9	6.41	10 mL	4.75
10	6.98	10 mL	5.35
11	7.33	10 mL	5.70
12	7.82	10 mL	6.10
13	8.27	10 mL	6.30
14	8.78	10 mL	6.70
15	9.44	10 mL	7.40
16	9.94	10 mL	8.00



Scheme S1. Combined acid-base and redox behaviours of *p*-HOBDI including its possible single- and two-electron oxidation products. Adapted from Ref. 8.



Scheme S2. Possible dimerization of *p*-HOBDI after first oxidation in an acidic buffer (pH < 2.7). Adapted from Ref. 8.

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