Supporting Information

A PHOTOISOMERIZING RHODOPSIN MIMIC OBSERVED AT ATOMIC RESOLUTION

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A. MATERIALS AND METHODS

UV-vis spectra were recorded using a Cary 300 Bio WinUV, Varian spectrophotometer. All-*trans*-retinal was purchased from TRC and was used as received. All the other chemicals were purchased from Sigma-Aldrich unless otherwise specified. Fast Q anion exchange resin was purchased from GE Health Care. A BioLogic DuoFlow (BioRad) chromatography instrument was used for protein purifications. Source 15Q resin was purchased from GE Health Care. Sonication of bacterial cells was performed using an Ultrasonic Homogenizer from Biologics, Inc.

B. SITE-DIRECTED MUTAGENESIS

The CRABPII-pET17b plasmid described [1] was used for mutagenesis following the QuickChange Site-directed Mutagenesis Kit protocol (Agilent Technologies). PCR conditions for amplification of mutants are specified below:

Total Reaction Volume	**50 μL**
Template (DNA plasmid)	70ng (x μL)
Primer Forward	20 pmol (y μL)
Primer Reverse	20 pmol (z μL)
dNTP	1 μL
10x pfu Buffer	5 μL
Pfu Turbo (DNA Polymerase)	1 μL
DI water	50-x-y-z-7 μL

PCR Program		
1x	95 °C	30 min
	95 °C	30 sec
20x	Temprature 3-5 °C lower than primer melting temprature	1 min
	72 °C	4 min 30 sec
1x	72 °C	10 min
1x	25 °C	10 min

List of Primers

F3Q
Forward: 5'- CCAAAC**CAA**TCTGGCAACTGGAAA-3'
Reverse: 5'-TTTCCAGTTGCCAGA**TTG**GTTTGG-3'

A32Y
Forward: 5'-GAGGAAGATT**TAT**GTGGCTGC-3'
Reverse: 5'-GCAGCCAC**ATA**AATCTTCCTC-3'

A32W
Forward: 5'-GTGATGCTGAGGAAGATT**TGG**GTGGCTGC-3'
Reverse: 5'-GCAGCCAC**CCA**AATCTTCCTCAGCATCAC-3'

P39Y
For: 5' GCAGCGTCCAAG**TAT**GCAGTGG 3'
Rev: 5' CCACTGC**ATA**CTTGGACGCTGC 3'

P39Q
For: 5' GCAGCGTCCAAG**CAA**GCAGTGG 3'
Rev: 5' CCACTGC**TTG**CTTGGACGCTGC 3'

T54V
Forward: 5'-CTACATCAAA**GTC**TCCACCACCGTGCG -3'
Reverse: 5'- CGCACGGTGGTGGA**GAC**TTTGATGTAG -3'

R59Y
Forward: 5'- CCTCCACCACCGTG**TAC**ACCACAGAG -3'
Reverse: 5'- CTCTGTGGT**GTA**CACGGTGGTGGAGG -3'

R59W
Forward: 5'- CCTCCACCACCGTG**TGG**ACCACAGAG -3'
Reverse: 5'- CTCTGTGGT**CCA**CACGGTGGTGGAGG -3'

R111K
Forward: 5'-CCCAAGACCTCGTGGACC**AAA**GAACTGACCAACGATGGG-3'
Reverse: 5'-CCCATCGTTGGTCAGTTC**TTT**GGTCCACGAGGTCTTGGG-3

R132Q: Y134F
Forward: 5'- GTTGTGTGCACC**CAG**GTC**TTC**GTCCG-3'
Reverse: 5'-CGGAC**GAA**GAC**CTG**GGTGCACACAAC-3'

R132Y:Y134F
Forward: 5' GTTGTGTGCACC**TAT**GTC**TTC**GTCCGAGAG 3'
Reverse: 5' CTCTCGGAC**GAA**GAC**ATA**GGTGCACACAAC 3'

The PCR product (5 μL) was transformed into DH5α cells (50 μL) and grown on Luria-Bertani (LB)-agar plates supplemented with Ampicillin (75 μg/mL) for 15 h. A single colony was inoculated in 15 mL LB medium containing 100 μg/mL ampicillin and grown at 37 ºC while shaking, for 12 h. DNA purification was performed using a QIAGEN Miniprep DNA purification kit. The average isolated plasmid DNA concentration was 120 ng/μl in 60 μL solution. The DNA sequence was verified by the MSU gene sequencing facility using a primer on the T7 promoter.

C. Protein Expression and Purification of CRABPII/pET-17b Mutants

The target gene was transformed into E. coli BL21(DE3)pLysS competent cells (100 ng of DNA, for 100 μL of cell solution) following standard protocols and the cells were grown on Luria-Bertani (LB)-agar plates supplemented with antibiotics (Chloramphenicol: 27 μg/mL; Ampicillin: 100 μg/mL) at 37 ºC for 12 h. A single colony was used to inoculate 50 mL of LB medium containing 27 μg/mL chloramphenicol and 100 μg/mL ampicillin and was grown at 37 ºC, while shaking overnight. The resulting culture was used to inoculate 1 L of LB containing 27 μg/mL chloramphenicol and 100 μg/mL ampicillin and was grown at 37 ºC while shaking till OD_{600} reached 0.7-0.9. The expression was induced with addition of isopropyl-β-D-thiogalactopyranoside (IPTG, Gold Biotechnology, 1 mM) and the culture was shaken at 19 ºC for 36 h. The cells were harvested by centrifugation (5000 rpm, 20 min, 4 ºC) and resuspended in Tris-binding buffer (10 mM Tris, pH=8.0, 50 mL). The cells were lysed by sonication (VWR Scientific , power 70%, 3 x 1 min). The solution was spun down (12000 rpm, 30 min, 4 ºC) and the supernatant was loaded on a FastQ anion exchange column pre equilibrated with Tris-binding buffer. The column was washed twice with Tris-binding buffer (2 x 100 mL) and the protein was eluted with Tris-elution buffer (10 mM Tris, 150 mM sodium chloride, pH = 8.0, 70 mL). The eluent underwent buffer exchange with Tris-binding buffer using an ulltrafiltration cell under nitrogen pressure (~20 psi) (Millipore™, regenerated cellulose membrane YM10, NMWL: 10,000). The desired protein was further purified using a BioLogic DuoFlow system (BioRad) equipped with a 15Q anion exchange column (20 mL total column volume), according to the following protocol:

Description		Parameters
Isocratic flow	pH=8.1, 0% 2M NaCl	10.00 mL, 3.00 mL/min
Linear Gradient	pH=8.1, 0-4% 2M NaCl	20.00 mL, 3.00 mL/min
Isocratic flow	pH=8.1, 4% 2M NaCl	20.00 mL, 3.00 mL/min
Linear Gradient	pH=8.1, 4-8% 2M NaCl	10.00 mL, 3.00 mL/min
Isocratic flow	pH=8.1, 8% 2M NaCl	20.00 mL, 3.00 /min

The above protocol was optimized for CRABPII mutants. The buffer used for the above protocol is 50 mM Tris, pH is adjusted automatically. The proteins elute with 4% 2M NaCl.

D. SUMMARY OF MUTANTS

Table S1. Screening for hCRABPII mutants with fast kinetic PSB formation and slow PSB loss.

Mutant*	**Low pK_a** (λ_{max} nm)	**High pK_a** (λ_{max} nm)	**PSB formation (hours)**	**PSB loss (hours)**
M1-KFVQ:P39Q:R59Y	5.4 (530)	8.4 (556)	3	24
M2-KFVQ:P39Y:R59Y	3.4 (564)	8.15 (573)	0.2	10
M3-KFVQ:P39Q:R59Y:A32W:F3Q	9.4 (557)	-	5	50 % after 24h
M4-KFVQ: P39Q: R59W	5.1 (537)	7.8 (555)	3.5	24
M5-KFVQ:P39Q: R59Y:A32Y	5.6 (554)	8.4 (575)	4	24
M6-KFVQ:P39Q:R59Y:A32W	6.0 (563)	8.3 (584)	2	24
M7-KFVQ:P39Q:R59W:A32W	6.2 (582)	8.6 (598)	2	24
M8-KFVQ:P39Y: R59Y:A32Y	3.2 (587)	8.4 (588)	0.1	16
M9-KFVQ:P39Y:R59Y:A32W	2.5 (600)	8.3 (596)	0.2	12
M10-KFVQ:P39Y:R59W:A32W	3.1 (601)	8.3 (595)	0.3	10
M11-KFVQ:P39Q:R59Y:A32Y:F3Q	9.3 (545)	-	3	-
M12-KFVQ:P39Q:R59W:A32W:F3Q	9.0 (571)	-	3	-

* KFVQ is the abbreviation for R111K:Y134F:T54V:R132Q

E. EXTINCTION COEFFICIENT DETERMINATION

The absorption extinction coefficients (ε) for the various CRABPII mutants were determined according to the method described by Gill and von Hippel [2].

Extinction coefficients of the CRABPII mutants**:**

Protein	ε_{280nm} ($M^{-1}cm^{-1}$)
M1- KFVQ:P39Q:R59Y	20749
M2- KFVQ:P39Y:R59Y	22487
M3- KFVQ:P39Q:R59Y:A32W:F3Q	29004
M4- KFVQ:P39Q:R59W	25582
M5- KFVQ:P39Q:R59Y:A32Y	22441
M6- KFVQ:P39Q:R59Y:A32W	26681
M7- KFVQ:P39Q:R59W:A32W	29659
M8- KFVQ:P39Y:R59Y:A32Y	21881
M9- KFVQ:P39Y:R59Y:A32W	24768
M10- KFVQ:P39Y:R59W:A32W	30878
M11- KFVQ:P39Q:R59Y:A32Y:F3Q	21169
M12- KFVQ:P39Q:R:59W:A32W:F3Q	29697

KFVQ is the abbreviation for R132K:Y134F:T54V:R132Q

F. UV-VIS MEASUREMENTS

The CRABPII-PSB formation (λ_{max} > 450 nm) was followed by UV-vis in phosphate buffer (4 mM NaH_2PO_4, 16 mM Na_2HPO_4, 150 mM NaCl, pH=7.3). The experiment was performed with a final protein concentration of 50 μM upon addition of 0.5 equivalent of retinal (from a stock solution of 1 mM in ethanol). Absorptions with λ_{max} > 450 nm are due to formation of PSB, while deprotonated imine peaks (SB) appear at ~360 nm. Non-covalently bound retinal absorbs at ~380 nm.

G. pKA DETERMINATION OF CRABPII MUTANTS

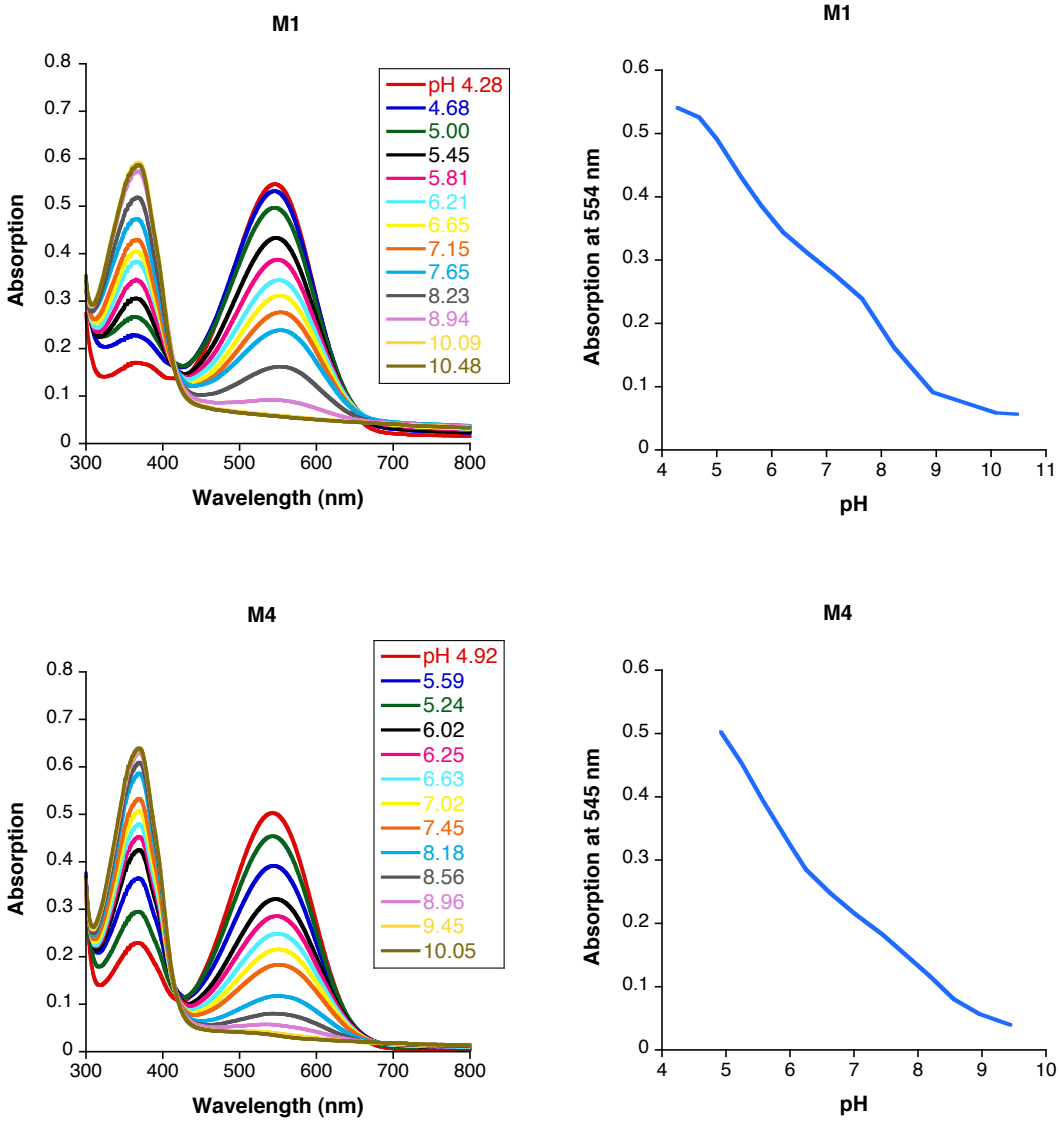
The pK_a values were determined based on the plot of the absorbance change vs pH by using the curve fitting protocol previously described for bacteriorhodopsin pK_a determination [3].

The total PSB absorbance change during each acidification or basification in comparison to the point with minimum PSB absorption (ΔA) is plotted against the pH.

$$\Delta A = \Delta A_0 / (1 + 10^{[pH - pKa]})$$

Base Titrations In Citrate Buffer

A 50 μM solution of various hCRABPII mutant proteins were incubated with 0.5 equivalent of retinal in 100 mM citrate buffer at pH=5.0. The UV-Visible spectrum was followed until PSB formation maximized. Titration of the PSB to SB was conducted by the addition of 1 M NaOH aliquots.



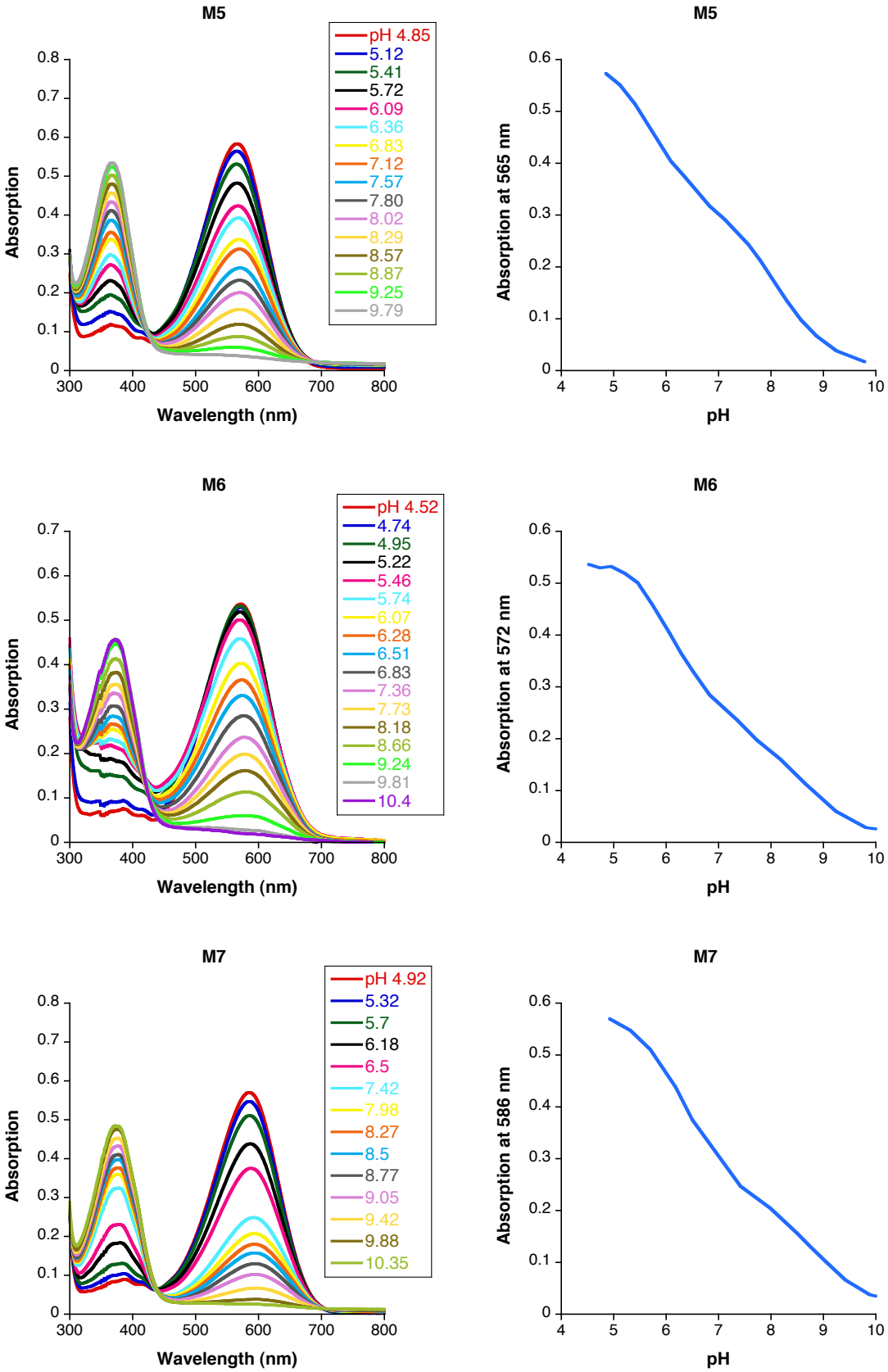
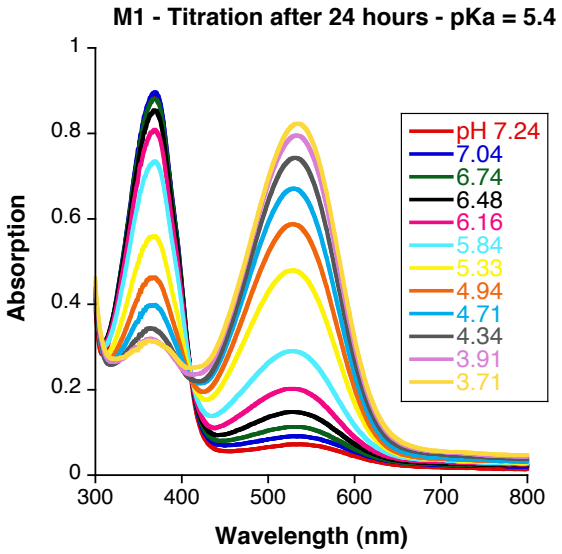
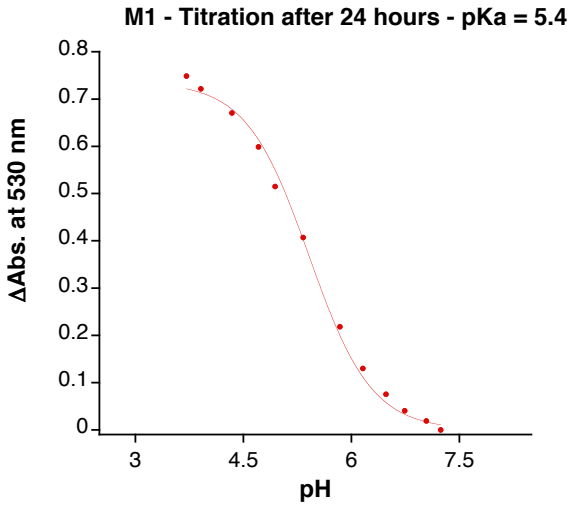


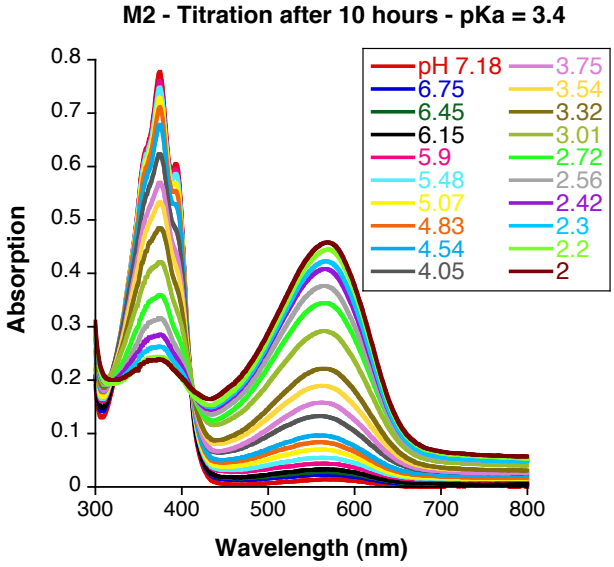
Figure S1. Base titrations in citrate buffer. Left panel: UV-visible spectra for each mutant shown; Right Panel: The plot of pH versus absorbance derived from the data shown to the left.

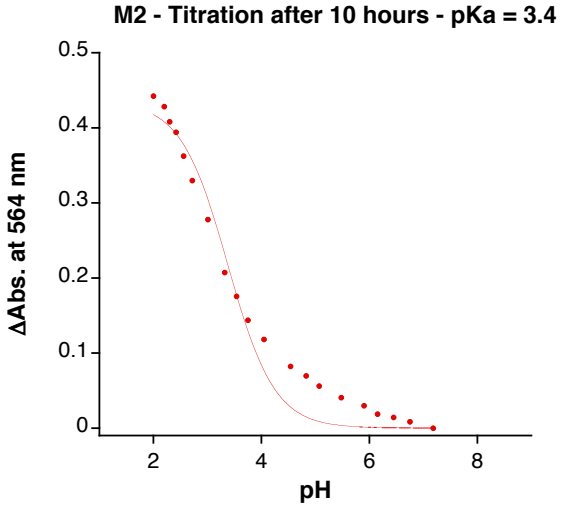
Acid Titrations

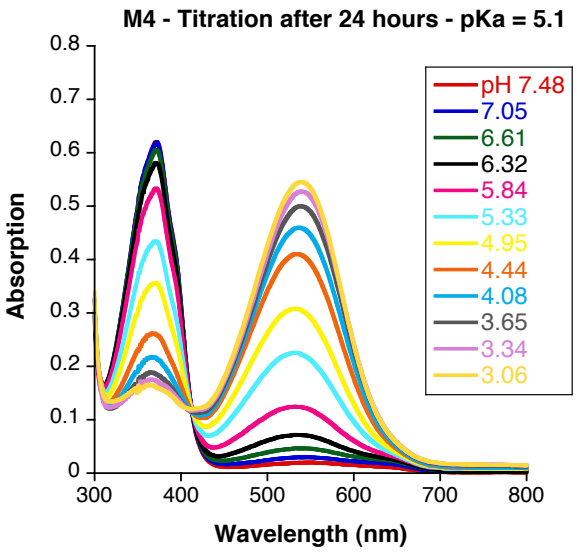
A 50 μM solution of the various hCRABPII mutant proteins were incubated with 0.5 equivalent of retinal in PBS buffer. The UV-Visible spectrum was followed until PSB loss plateaued (12-24 h). A saturated citric acid buffer solution was used to acidify the protein for the first few points. Further acidification with a 6 M HCl solution completed the SB to PSB conversion.

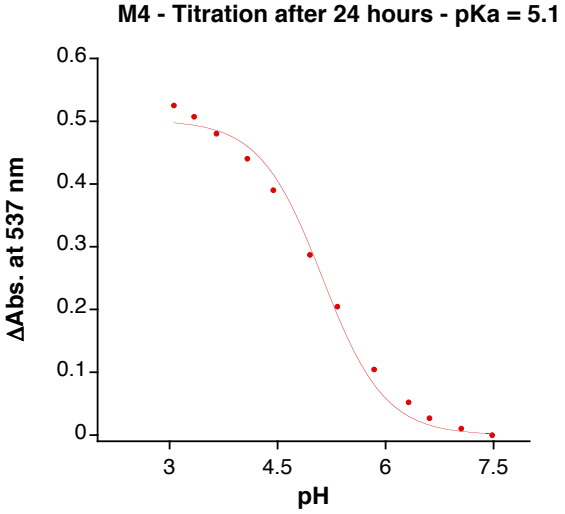


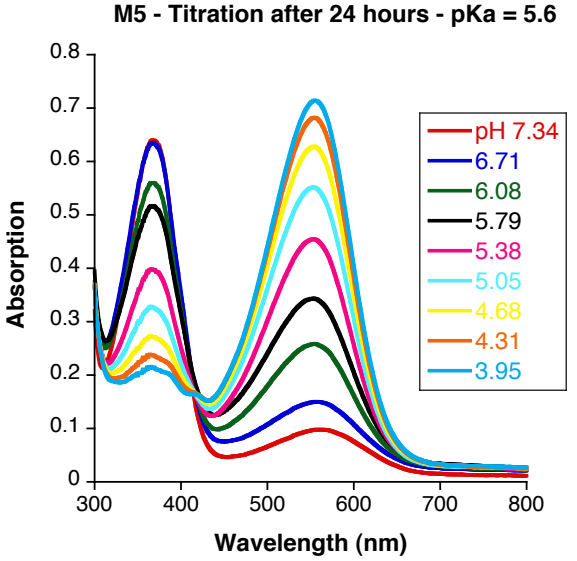


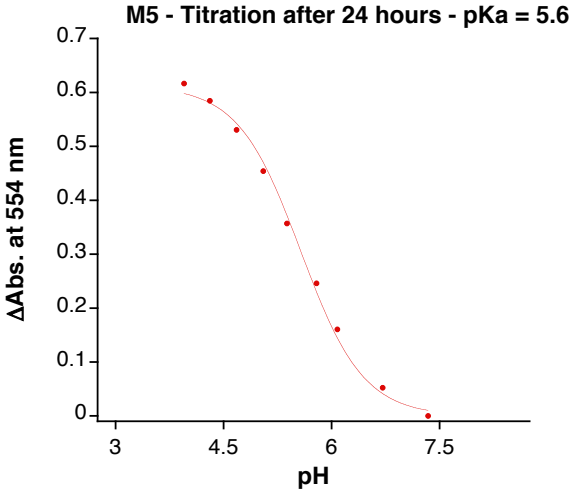


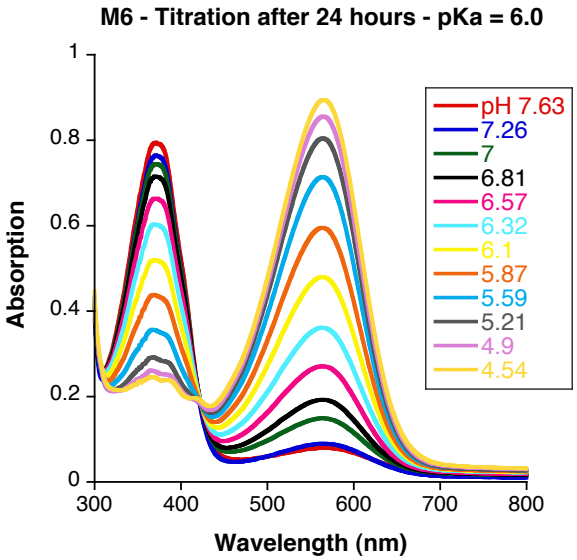


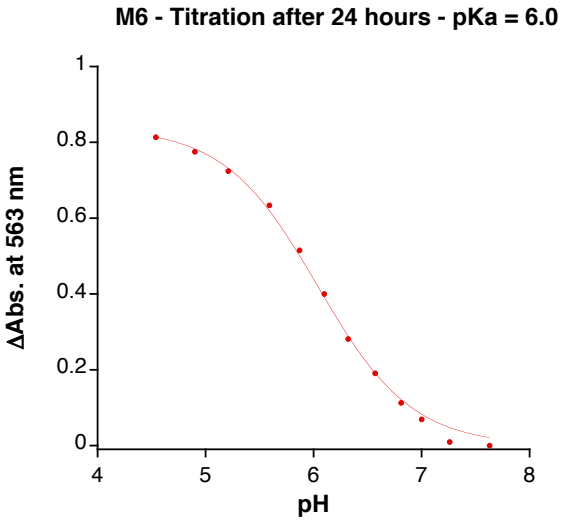

M4 - Titration after 24 hours - pKa = 5.1
Absorption
Wavelength (nm)
pH 7.48
7.05
6.61
6.32
5.84
5.33
4.95
4.44
4.08
3.65
3.34
3.06

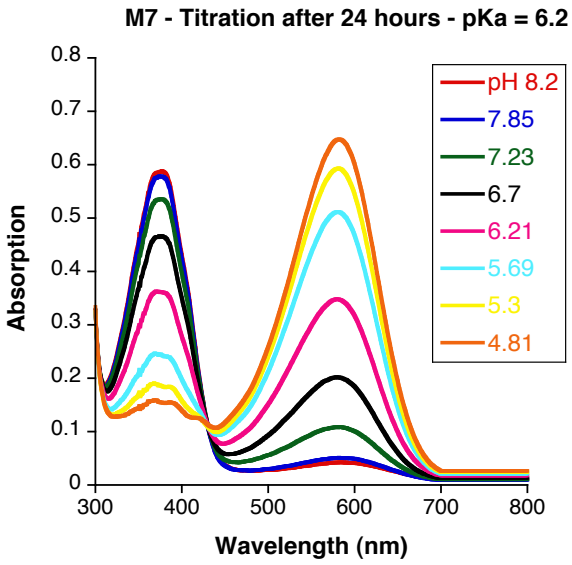

M4 - Titration after 24 hours - pKa = 5.1
ΔAbs. at 537 nm
pH

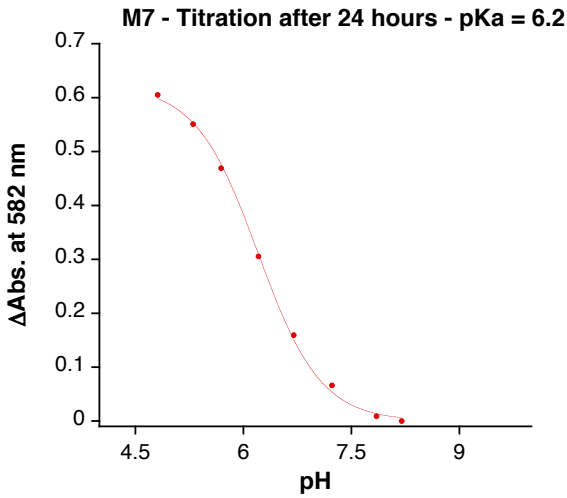

M5 - Titration after 24 hours - pKa = 5.6
Absorption
Wavelength (nm)
pH 7.34
6.71
6.08
5.79
5.38
5.05
4.68
4.31
3.95

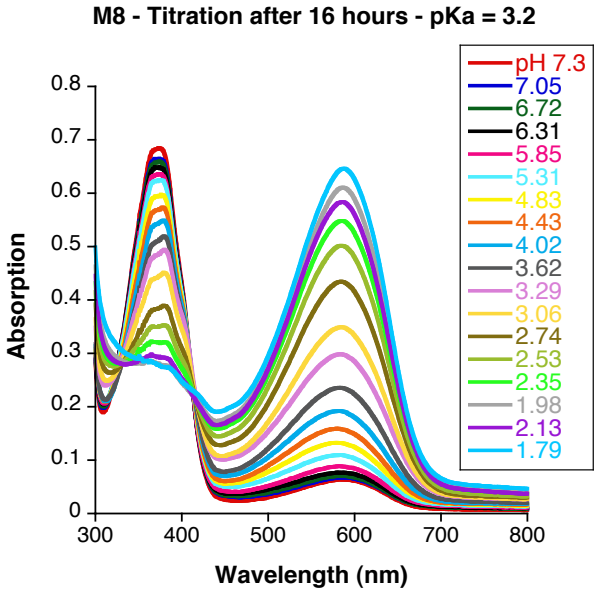

M5 - Titration after 24 hours - pKa = 5.6
ΔAbs. at 554 nm
pH

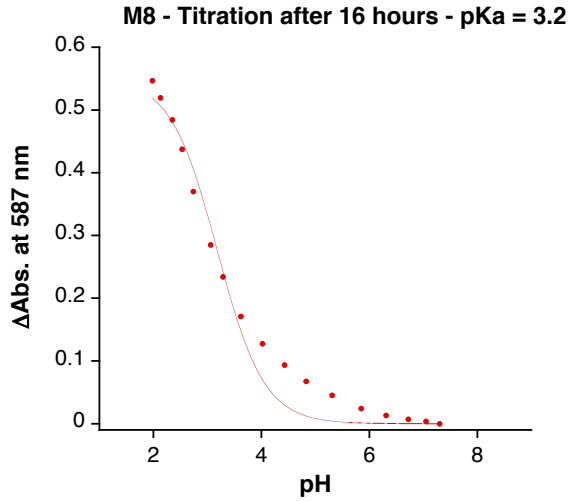

M6 - Titration after 24 hours - pKa = 6.0
Absorption
Wavelength (nm)
pH 7.63
7.26
7
6.81
6.57
6.32
6.1
5.87
5.59
5.21
4.9
4.54

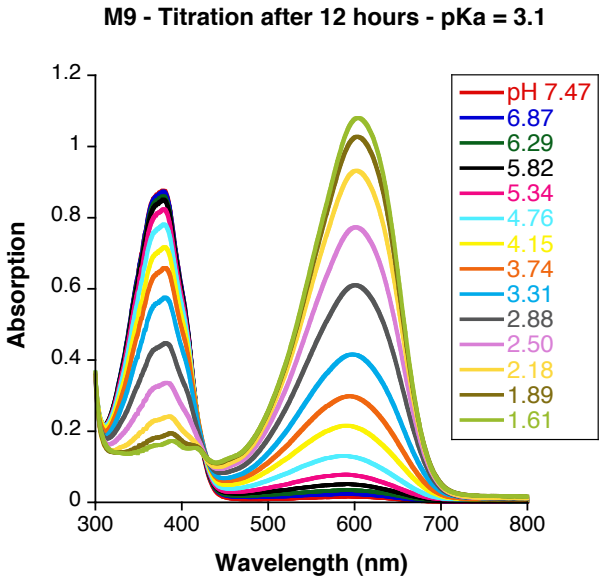

M6 - Titration after 24 hours - pKa = 6.0
ΔAbs. at 563 nm
pH

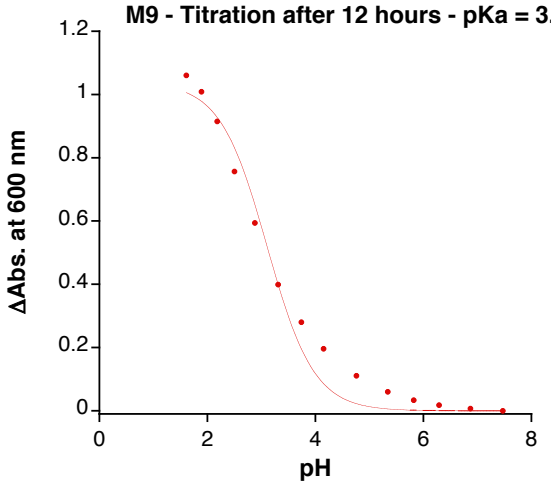

M7 - Titration after 24 hours - pKa = 6.2
Absorption
Wavelength (nm)
pH 8.2
7.85
7.23
6.7
6.21
5.69
5.3
4.81


M7 - Titration after 24 hours - pKa = 6.2
ΔAbs. at 582 nm
pH


M8 - Titration after 16 hours - pKa = 3.2
Absorption
Wavelength (nm)
pH 7.3
7.05
6.72
6.31
5.85
5.31
4.83
4.43
4.02
3.62
3.29
3.06
2.74
2.53
2.35
1.98
2.13
1.79


M8 - Titration after 16 hours - pKa = 3.2
ΔAbs. at 587 nm
pH


M9 - Titration after 12 hours - pKa = 3.1
Absorption
Wavelength (nm)
pH 7.47
6.87
6.29
5.82
5.34
4.76
4.15
3.74
3.31
2.88
2.50
2.18
1.89
1.61


M9 - Titration after 12 hours - pKa = 3.1
ΔAbs. at 600 nm
pH

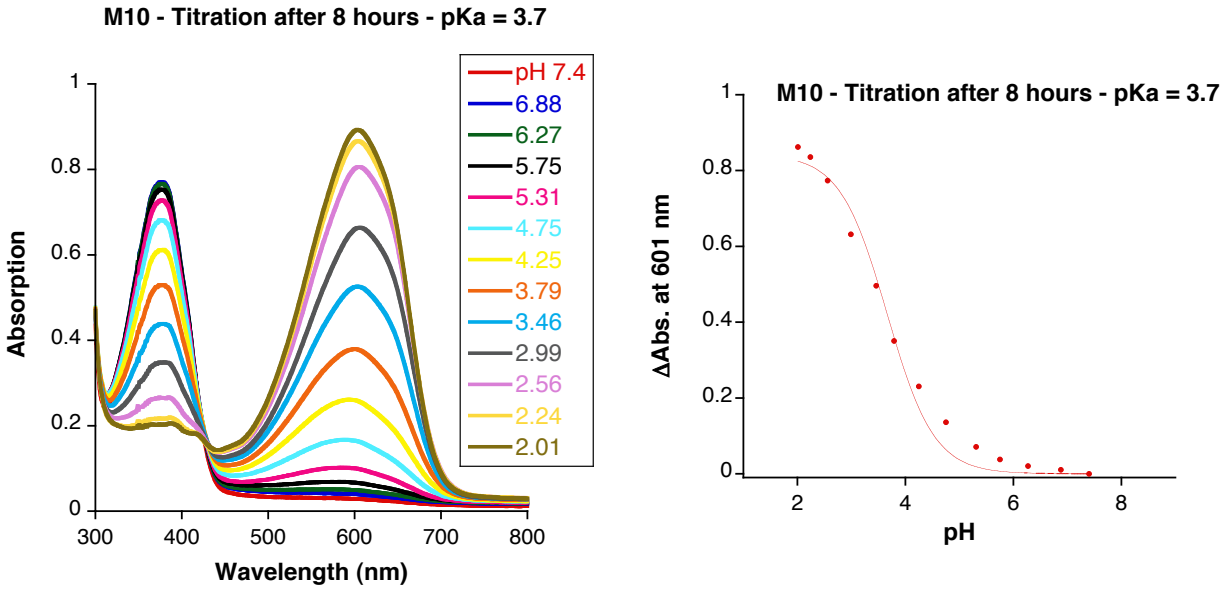
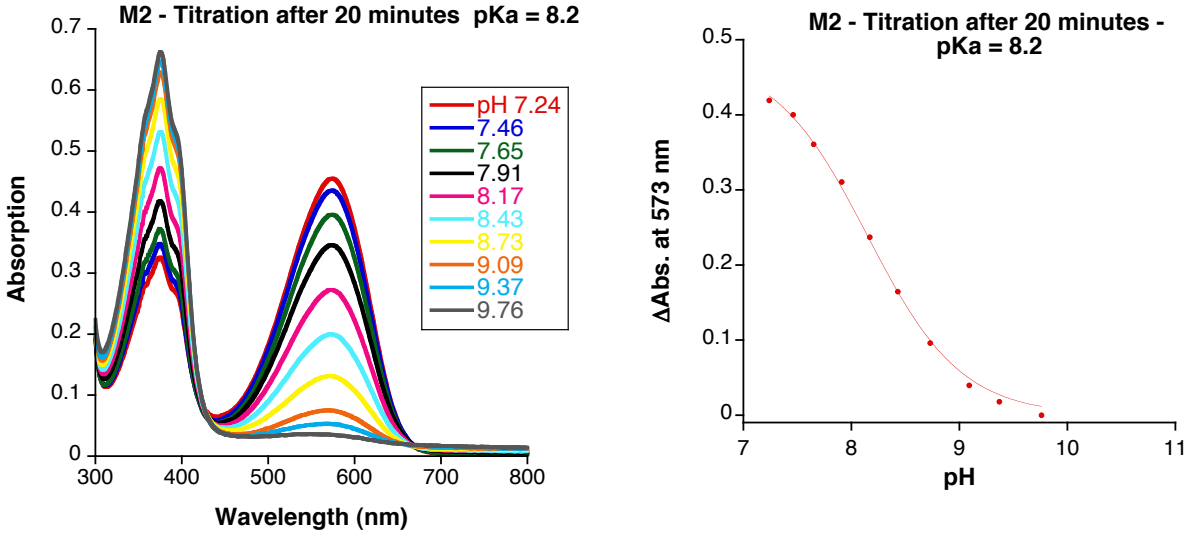
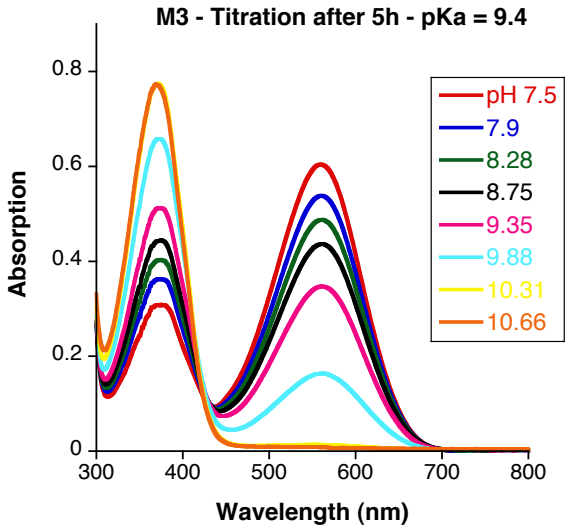


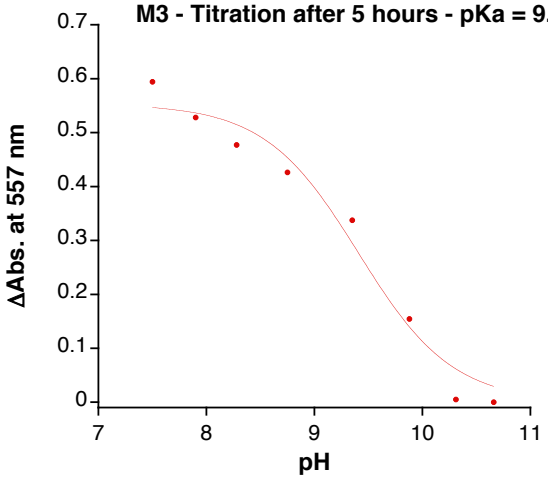
Figure S2. Acid titrations after PSB: Left panel: UV-visible graphs. Right panel: The plot of pH versus absorbance difference (ΔA).

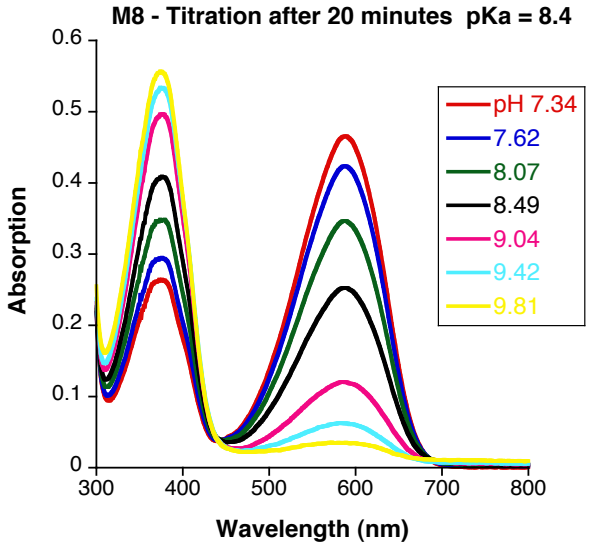
Base Titrations In PBS Buffer

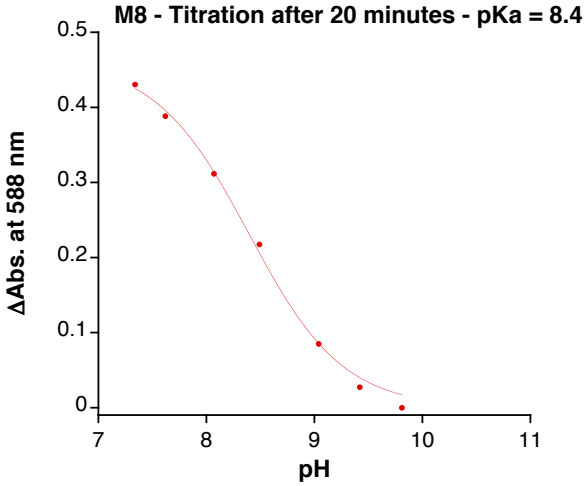
A 50 μM solution of hCRABPII proteins was incubated with 0.5 equivalent of retinal in PBS buffer. The UV-Visible spectrum was followed until PSB formation was at a maximum. 1 M NaOH solution was used to basify the protein solution until the complete conversion of PSB to SB was achieved.

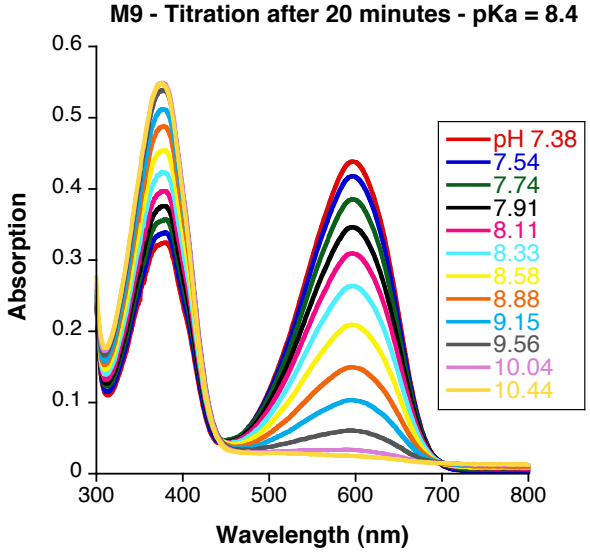


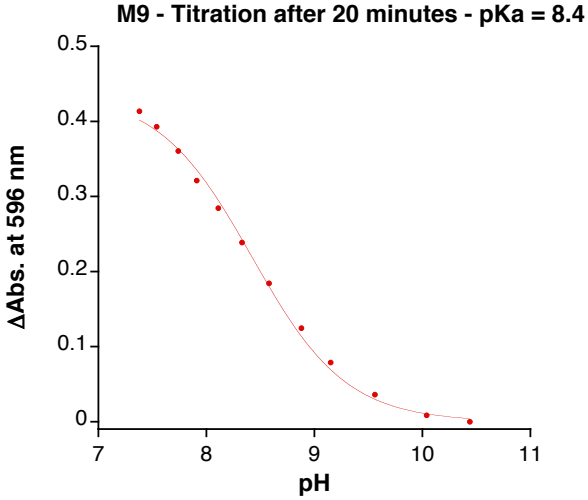

M3 - Titration after 5h - pKa = 9.4
Absorption
Wavelength (nm)
pH 7.5
7.9
8.28
8.75
9.35
9.88
10.31
10.66


M3 - Titration after 5 hours - pKa = 9.4
ΔAbs. at 557 nm
pH


M8 - Titration after 20 minutes pKa = 8.4
Absorption
Wavelength (nm)
pH 7.34
7.62
8.07
8.49
9.04
9.42
9.81


M8 - Titration after 20 minutes - pKa = 8.4
ΔAbs. at 588 nm
pH


M9 - Titration after 20 minutes - pKa = 8.4
Absorption
Wavelength (nm)
pH 7.38
7.54
7.74
7.91
8.11
8.33
8.58
8.88
9.15
9.56
10.04
10.44


M9 - Titration after 20 minutes - pKa = 8.4
ΔAbs. at 596 nm
pH

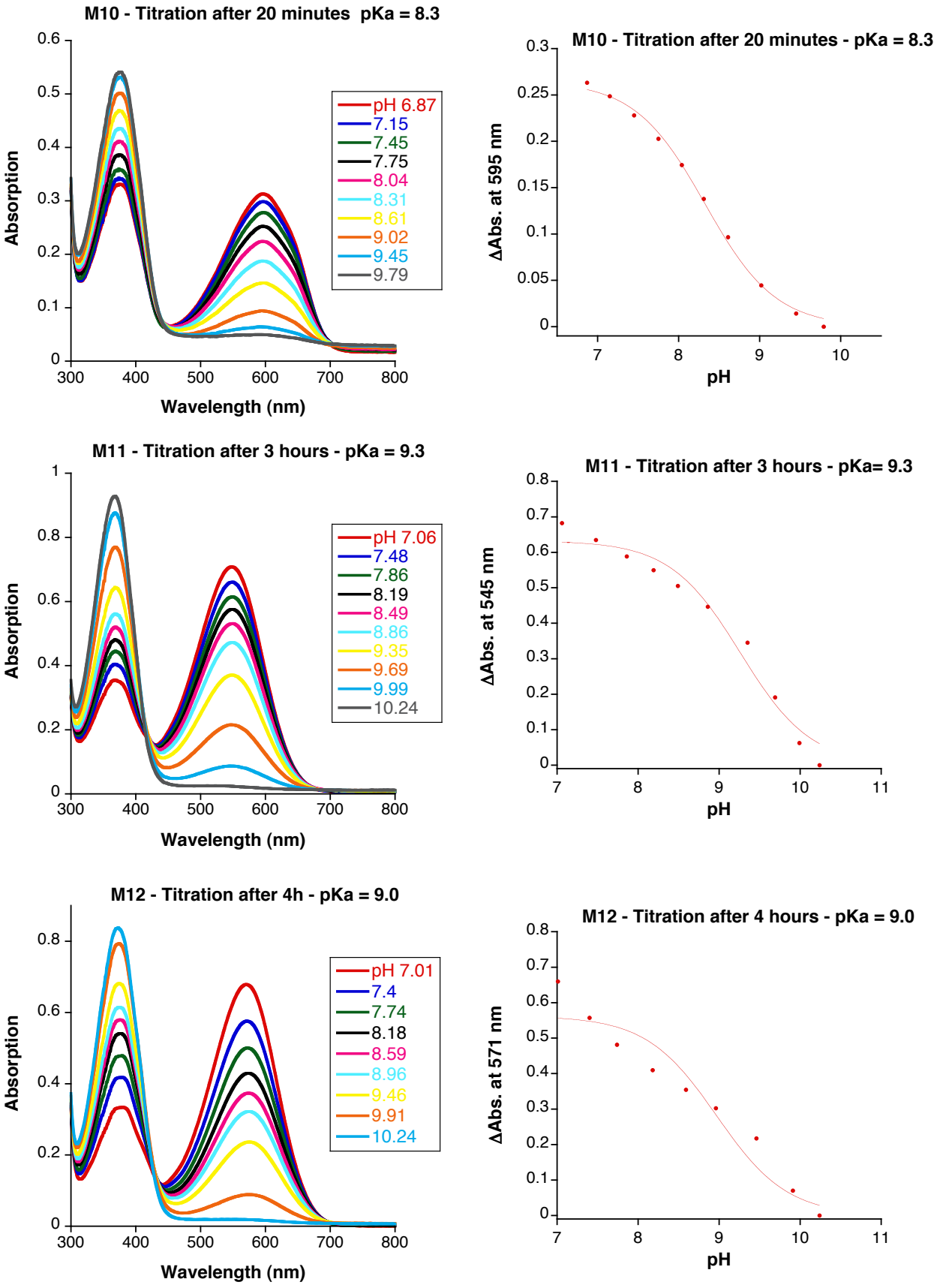


Figure S3. Base titrations after maximum PSB formation : Left panel: UV-visible graphs. Right panel: The plot of pH versus absorbance difference (ΔA).

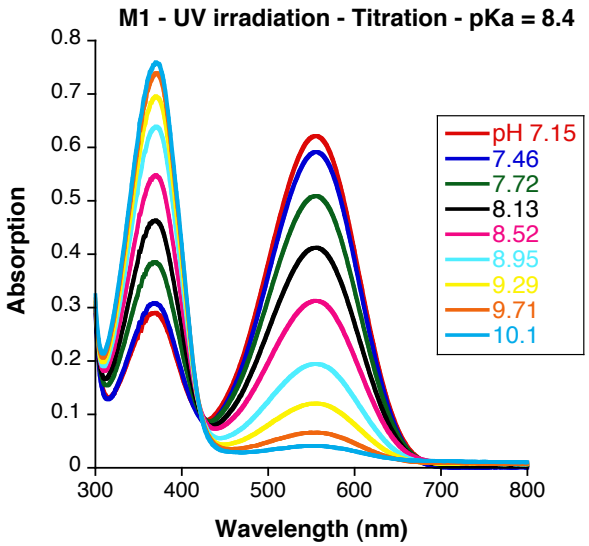
H. Light Irradiations and Titrations

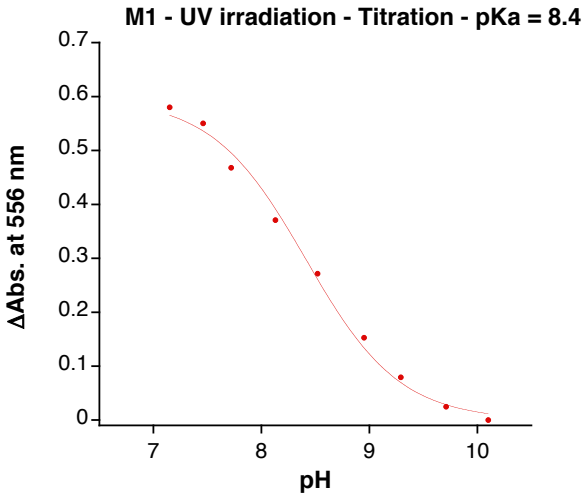
Light Irradiation Protocols

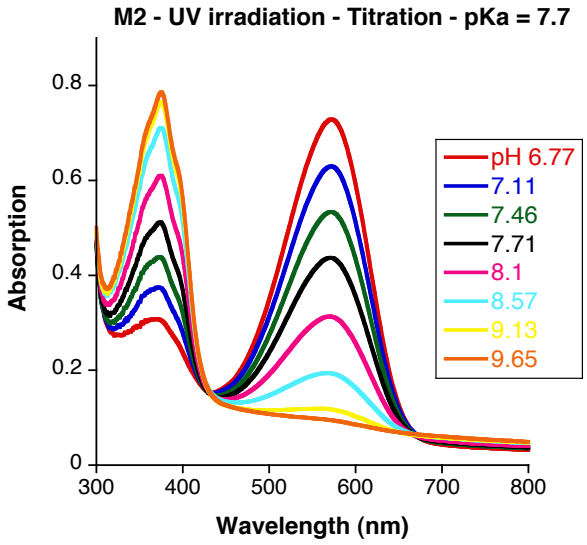
An Oriel Illuminator (Model 66142, Oriel Instruments) connected to a power supply (Model 668820, Oriel Instruments, 500 W Mercury (Xenon) lamp) was used for all light irradiations. For both UV and visible irradiations, a combination of two filters was used.
UV irradiations: Glass filter (6 mm thickness) to filter UV light below ~ 320 nm; U-360 (UV) 2" square band-pass filter (Center Wavelength CWL = 360 nm, Full Width-Half Max FWHM = 45 nm, purchased from Edmund Optics, Figure S1a).
Visible light yellow irradiations: Glass filter (6 mm thickness) to filter UV light below ~320 nm; Y-50 2" square long-pass filter (cut-off position = 500 ± 6 nm, purchased from Edmund Optics).

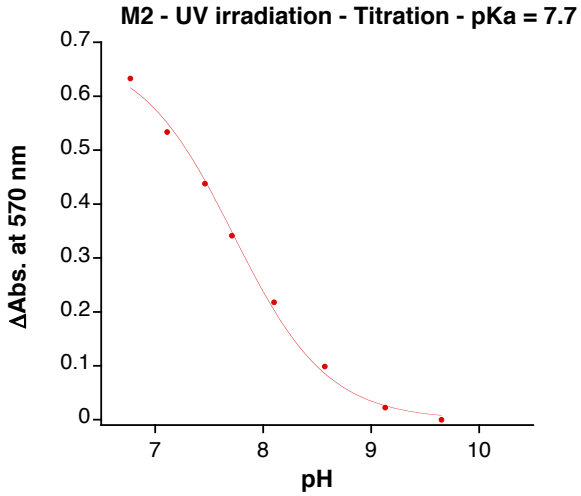
UV Irradiation - Titrations In PBS Buffer

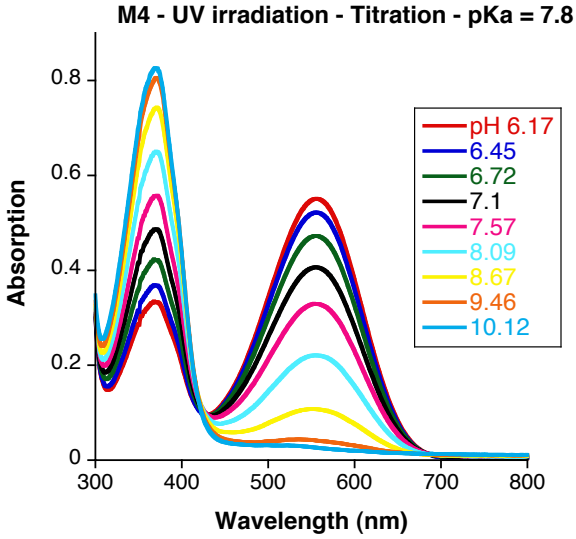
A 50 μM solution of hCRABPII proteins was incubated with 0.5 equivalent of retinal in PBS buffer. The UV-Visible spectrum was followed until PSB formation was at a maximum. UV irradiation (1-2 min) as described above was used to convert the remaining SB to PSB. A 1 M NaOH solution was used to basify the protein solution to achieve complete conversion of the PSB to SB.

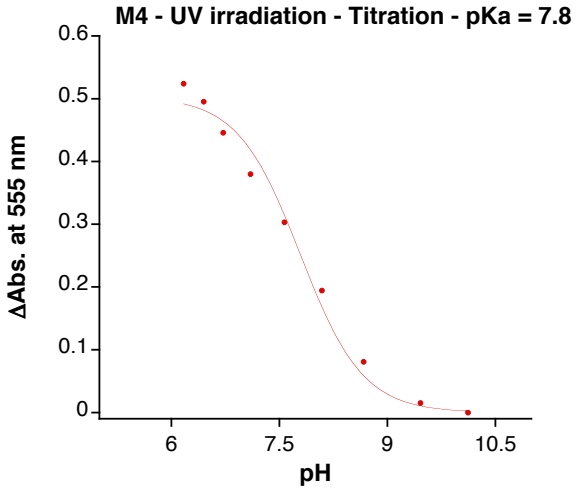


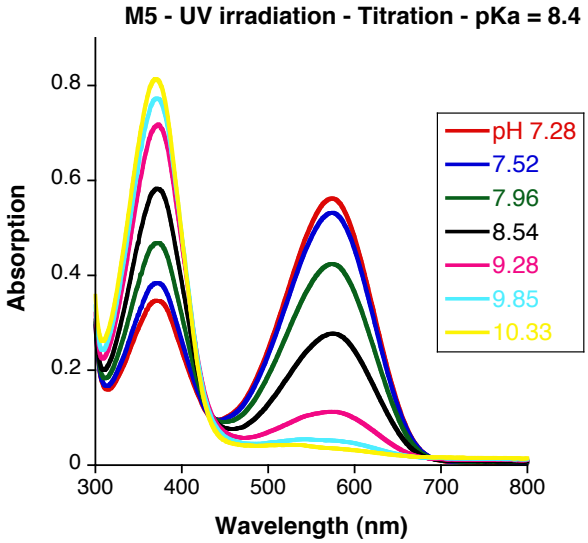


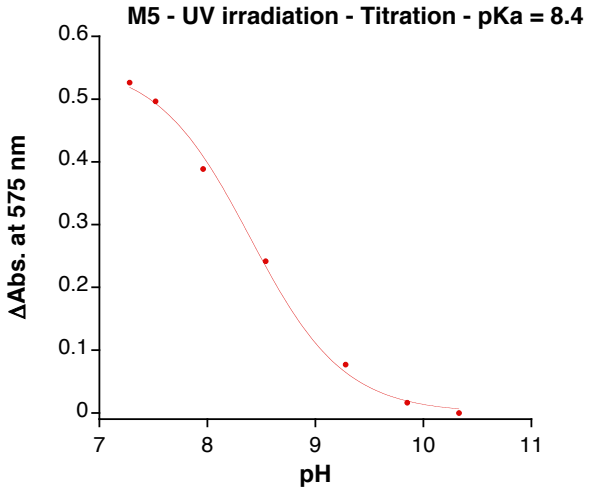

M2 - UV irradiation - Titration - pKa = 7.7
Absorption
Wavelength (nm)
pH 6.77
7.11
7.46
7.71
8.1
8.57
9.13
9.65


M2 - UV irradiation - Titration - pKa = 7.7
ΔAbs. at 570 nm
pH


M4 - UV irradiation - Titration - pKa = 7.8
Absorption
Wavelength (nm)
pH 6.17
6.45
6.72
7.1
7.57
8.09
8.67
9.46
10.12


M4 - UV irradiation - Titration - pKa = 7.8
ΔAbs. at 555 nm
pH


M5 - UV irradiation - Titration - pKa = 8.4
Absorption
Wavelength (nm)
pH 7.28
7.52
7.96
8.54
9.28
9.85
10.33


M5 - UV irradiation - Titration - pKa = 8.4
ΔAbs. at 575 nm
pH

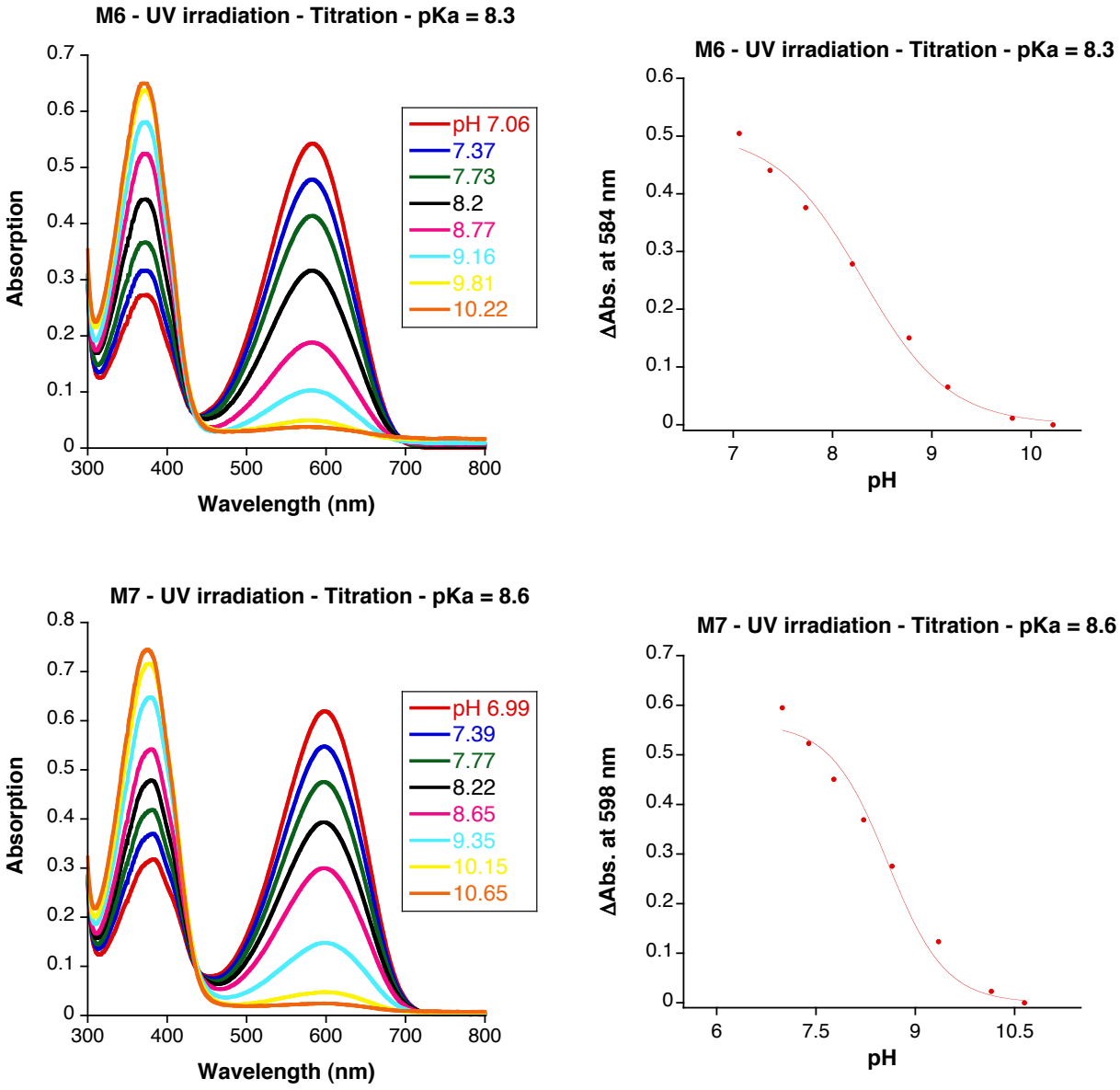


Figure S4. Base titrations after UV irradiation. Left panel: UV-visible spectra. Right panel: The plot of pH versus absorbance difference.

Cycling Of The M2 And M3 Mutants In Solution

A 50 μM solution of protein was incubated with 0.5 equivalent of retinal in PBS buffer for 20 min. Following the protocol described above, visible yellow light irradiation for 1 min was performed followed by 3 min UV irradiation. After each step UV-Vis spectra were collected. The process was repeated for 5 cycles.

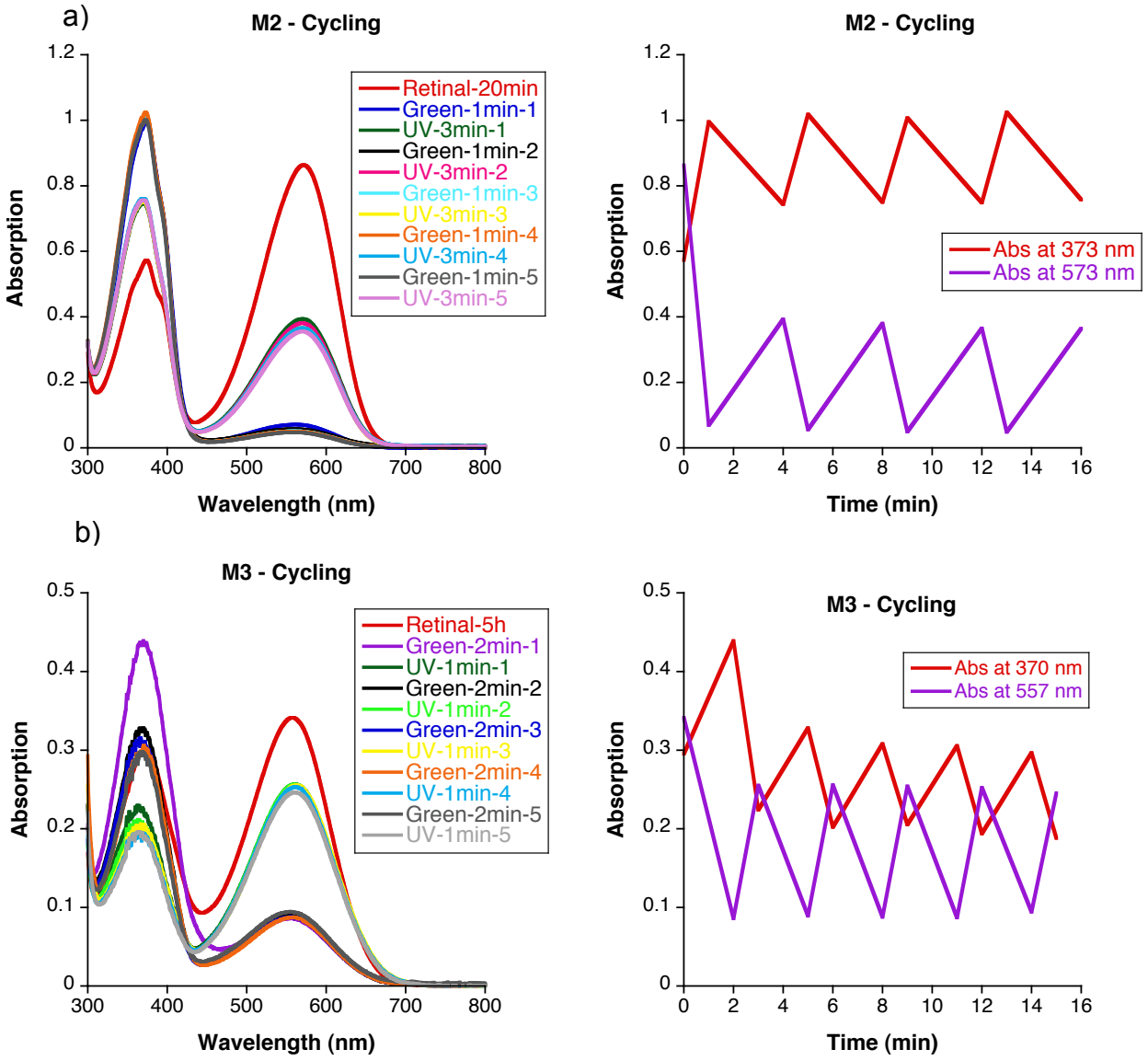


Figure S5. Cycling of the hCRABPII proteins in solution with UV and yellow light for mutant M2 (a) and M3 (b).

PSB Recovery after Green Light Irradiation

A 50 μM solution of each hCRABPII mutant was incubated with 0.5 equivalent of retinal in PBS buffer until maximum PSB formation was achieved. The protein solution was irradiated with visible yellow light until the PSB absorption was at its minimum. The recovery of the PSB was then monitored by UV-vis spectroscopy.

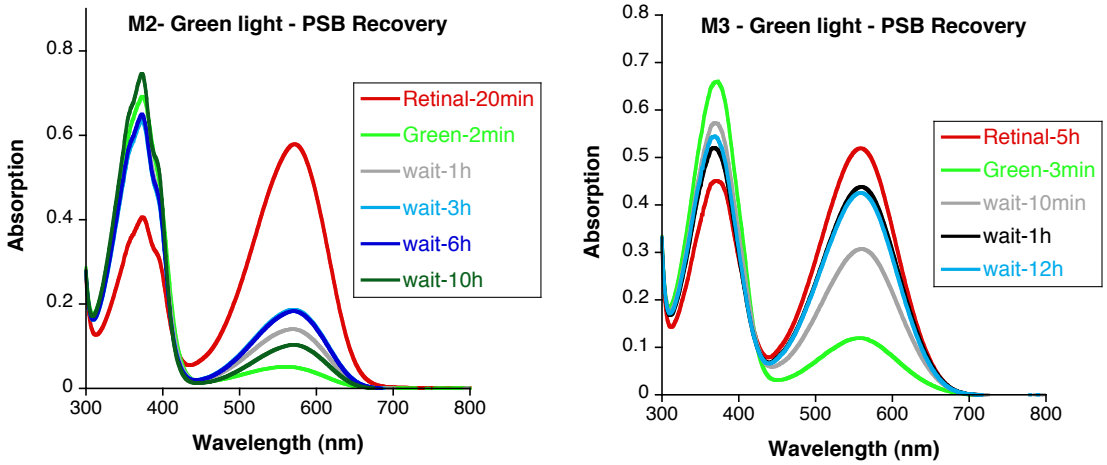


Figure S6. Mutants M2 and M3 are irradiated with visible light until maximum PSB loss. PSB recovery was then monitored. In 1 h mutant M3 has significant PSB recovery, while M2 much less.

I. Protein Crystallization

M2 Mutant

High pK_a form

To the concentrated protein solution at 20 mg/mL was added four equivalents of retinal and incubated for 20 minutes. A 24 well crystallization plate was used for vapor diffusion crystallization with the reservoir solution of 1 mL, containing 12% PEG3350 and 0.1 M malonate at pH = 6.0. 1 μL of protein solution was mixed with 1 μL reservoir solution. Crystals appeared and grew after 24 h at 4 °C. Crystals were flash frozen in a solution containing the mother liquor and 20% glycerol.

Low pK_a form

To a protein solution at 1.1 mg/mL was added four equivalents of retinal. The spectra of the protein-ligand mixture were tracked until complete loss of PSB was obtained. The mixture was concentrated to 20 mg/mL using concentrator cells. A twenty-four well crystallization plate was used for vapor diffusion crystallization with the reservoir solution containing 12% PEG 3350 and 0.1 M malonate at pH = 6.0. 1 μL of protein

solution was mixed with 1 µL reservoir solution. Crystals appeared and grew after 24 h. These crystals had a light blue color indicating the presence of PSB in the crystals. In order to deprotonate the PSB in the crystals, they were soaked in 12% PEG 3350 and 0.1 M malonate at pH = 7.5 for 30 minutes until crystals became colorless. The soaked crystals were flash frozen in the same solution containing 20% glycerol.

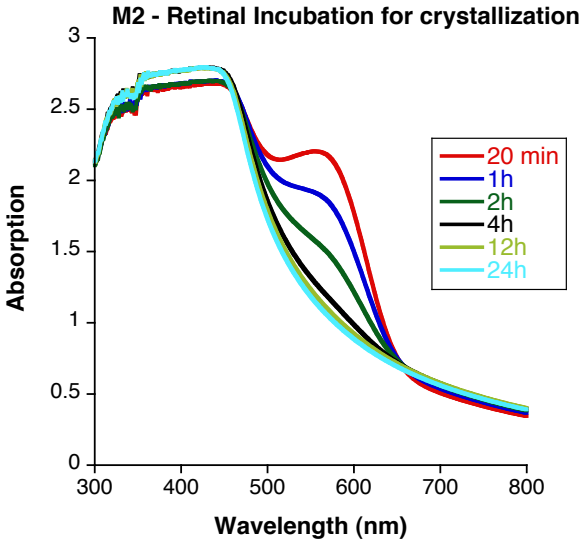


Figure S7. M2 mutant PSB loss overtime in the solution used for crystallization.

For UV Irradiated PSB Product

Soaked crystals described in the previous section were UV irradiated with a hand held TLC UV lamp for 30 minutes in the dark. The UV irradiated crystals showed a dark blue color at the end of the irradiation period. These crystals were transfered to a solution of 12% PEG 3350, 0.1M malonate at pH = 7.5 and 20% glycerol and flash frozen immediately under dim microscope light, while still being irradiated with the UV light.

M1 mutant

Low pK_a form

To the protein solution at 1 mg/mL was added four equivalents of retinal. The spectra of the protein-ligand mixture were tracked until complete loss of the PSB was obtained. Then the mixture was concentrated to 20 mg/mL using concentrator cells. A twenty-four well crystallization plate was used for vapor diffusion crystallization with the reservoir solution containing 12% PEG 3350 and 8% Tacsimate at pH = 6.0. 1 µL of protein solution was mixed with 1 µL reservoir solution. Crystals appeared and grew after 24 h. These crystals had a light red color indicating the presence of PSB in the crystals. In

order to deprotonate the PSB in the crystals, they were soaked in 12% PEG 3350 and 8% Tacsimate at pH = 7.5 for 45 minutes until crystals became colorless. The soaked crystals were flash frozen in the same solution containing 20% glycerol.

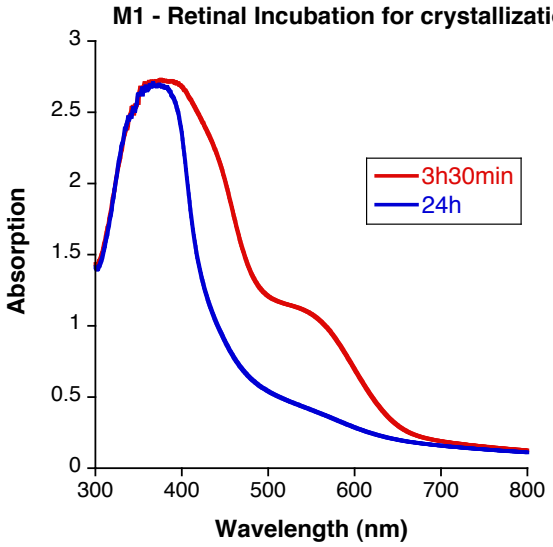


Figure S8. M1 mutant PSB loss overtime in the solution used for crystallization.

For UV irradiated PSB product:

Soaked crystals described in the previous section were UV irradiated with a hand held TLC UV lamp for 1 h in dark. The UV irradiated crystals showed a red color at the end of the irradiation period, and were flash frozen immediately under dim microscope light in 12% PEG 3350, 8% Tacsimate at pH = 7.5 and 20% glycerol.

M10 mutant

To the concentrated protein solution at 20 mg/mL was added four equivalents of retinal and incubated for three hours. A 24 well crystallization plate was used for vapor diffusion crystallization with the reservoir solution of 1 mL, containing 20% PEG3350 and 0.1 M malonate at pH = 6.0. 1 µL of protein solution was mixed with 1 µL reservoir solution. Crystals appeared after 24 h and grew in 5 days at 4 °C. Crystals were flash frozen in a solution containing the mother liquor and 20% glycerol.

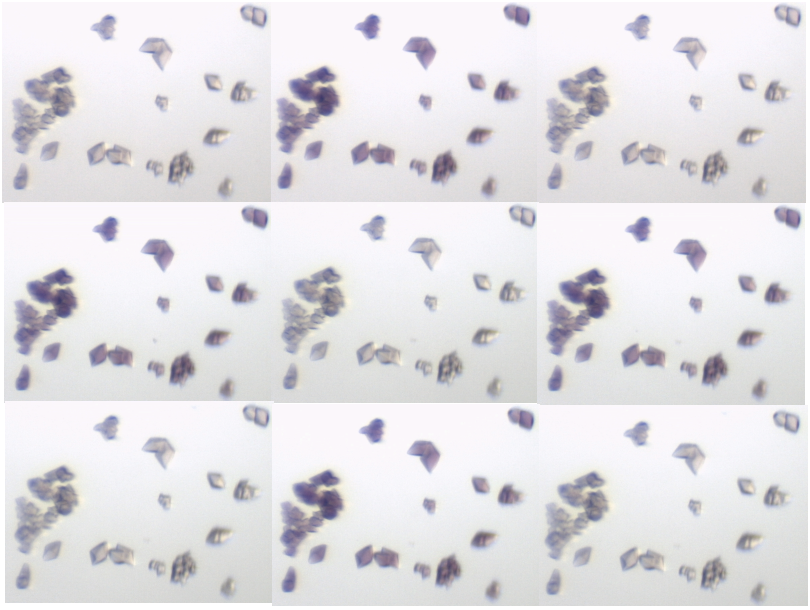


Figure S9. M1 crystals during UV light and visible light irradiation after soaking to pH 7.5, as described above. Each cycle is exposed for 10 minutes with UV light and for 10 minutes with Green light to induce the color change.

M3 mutant

To the concentrated protein solution at 20 mg/mL was added four equivalents of retinal and incubated for three hours. A 24 well crystallization plate was used for vapor diffusion crystallization with the reservoir solution of 1 mL, containing 20% PEG3350 and 0.1 M bis Tris Propane at pH = 6.0, 0.2 M sodium fluoride. 1 µL of protein solution was mixed with 1 µL reservoir solution. Crystals appeared after 24 h and grew in 5 days at 4 °C. Crystals were flash frozen in a solution containing the mother liquor and 20% glycerol.

J. Data Collection and Refinement

Diffraction data were collected at the Advanced Photon Source (APS) (Argonne IL) at the LSCAT (sector 21) at 1.00 Å wavelength radiation at 100 K. Data reduction and scaling were performed using the HKL2000 program package [4]. The structures were solved using Molecular Replacement using the CCP4 program [5] suite, and refined using the PHENIX program package [6]. The search model was R132K:R111L:L121E mutant of Cellular Retinoic Acid Binding Protein II (protein data bank code 2G7B). Three cycles of refinement was implemented for each run and placement of ordered water molecules was done in COOT (0.6.1) [7]. The chromophore was manually fitted in the electron density at the end of the refinement.

Table S2. X-ray Crystallography Data and Refinement Statistics.

Protein	**M1**-SB-cycle1	**M1**-UV-cycle1	**M1**-SB-cycle2	**M1**-UV-cycle2	**M1**-SB-cycle3	**M1**-UV-cycle3
Space group	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$
a (Å)	58.6	58.3	58.6	58.5	58.5	58.7
b (Å)	58.6	58.3	58.6	58.5	58.5	58.7
c (Å)	99.4	99.9	99.9	100.4	99.7	100.4
α (°)	90	90	90	90	90	90
β (°)	90	90	90	90	90	90
γ (°)	120	120	120	120	120	120
Molecules per Asymmetric Unit	1	1	1	1	1	1
Total reflection	189007	163428	161415	161005	155486	145510
Unique Reflection	17990	15647	14985	14868	14966	13508
Completeness (%)	99.6 (99.6)[a]	99.4 (85.3)	99.5 (99.3)	100 (100)	99.4 (99)	100 (99.8)
Average I/σ	36.1 (3.78)	34.86 (3.65)	33.94 (4.69)	39.06 (4.38)	26.69 (2.00)	38.81 (4.38)
R_{merge} (%)	7.1 (52.3)	8.3 (40.5)	8.9 (44.6)	8 (54.4)	9.8 (57.3)	6.7 (55.7)
Resolution (Å) (Last Shell)	50-1.83 (1.86-1.83)	50-1.92 (1.95-1.92)	50-1.95 (1.98-1.95)	50-1.96 (1.99-1.96)	50-1.95 (1.98-1.95)	50-2.03 (2.07-2.03)
R_{cryst}/R_{free} (%)	18.57/23.84	19.74/24.45	18.99 /23.80	20.52/24.93	19.16/24.05	21.02/25.73
RMSD From Ideal Values						
Bond Length (Å)	0.007	0.008	0.007	0.008	0.008	0.008
Bond Angle (°)	1.192	1.228	1.106	1.104	1.201	1.152
Average B factor	26.90	29.8	29.76	34.71	29.99	36.24
Number of water molecules	199	141	161	123	147	106
PDB IDs	4YBP	4YBU	4YCE	4YCH	4YDA	4YDB

[a] Values in the parenthesis refer to the last resolution shell.

Table S3. X-ray Crystallography Data and Refinement Statistics.

Protein	**M2**- PSB	**M2**-SB- cycle1	**M2**-UV-cycle1	**M2**-SB- cycle2	**M2**-UV-cycle2	**M2**-SB-cycle3	**M2**-UV-cycle3
Space group	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$
a (Å)	58.9	58.9	58.9	59.2	58.779	59.0	59.0
b (Å)	58.9	58.9	58.9	59.2	58.779	59.0	59.0
c (Å)	99.95	99.7	100.4	100.0	101.2	100.4	101.1
α (°)	90	90	90	90	90	90	90
β (°)	90	90	90	90	90	90	90
γ (°)	120	120	120	120	120	120	120
Molecules per Asymmetric Unit	1	1	1	1	1	1	1
Total reflection	164293	281303	163969	179720	132920	140437	126296
Unique Reflection	15102	25940	15237	16619	12329	13048	11730
Completeness (%)	99.5 (99.4)[a]	99.4 (98.8)	99.6 (99.6)	99.8 (99.2)	99.8 (99.3)	99.9 (99.8)	100 (99.9)
Average I/σ	34.6 (3.0)	62 (4.44)	49.5 (4.41)	48.04 (4.53)	41.94 (4.62)	40.72 (5.12)	38.92 (4.1)
R_{merge} (%)	9.4 (63.9)	4.7 (47.9)	6 (52.6)	5.9 (53)	6.5 (51.6)	6.8 (53.3)	7.5 (54.2)
Resolution (Å) (Last Shell)	50-1.95 (1.98-1.95)	50-1.62 (1.65-1.62)	50-1.95 (1.98-1.95)	50-1.90 (1.93-1.90)	50-2.10 (2.14-2.10)	50-2.06 (2.10-2.06)	50-2.14 (2.18-2.14)
R_{cryst}/R_{free} (%)	19.34/24.07	19.14/22.52	20.01 /26.53	20.43/23.81	20.38/23.65	20.16/25.45	19.87/23.46
			RMSD From Ideal Values				
Bond Length (Å)	0.007	0.006	0.007	0.007	0.007	0.009	0.009
Bond Angle (°)	1.239	1.167	1.144	1.123	1.097	1.228	1.167
Average B factor	34.85	29.04	35.97	32.94	39.96	36.02	40.63
Number of water molecules	110	210	116	135	90	123	92
PDB IDs	4YFP	4YFQ	4YFR	4YGG	4YGH	4YGZ	4YH0

[a] Values in the parenthesis refer to the last resolution shell.

Table S4. X-ray Crystallography Data and Refinement Statistics.

Protein	M3	M11
Space group	P1	P1
a (Å)	36.574	36.737
b (Å)	39.54	39.21
c (Å)	58.183	58.402
α (°)	87.078	86.775
β (°)	80.016	79.881
γ (°)	70.511	70.831
Molecules per Asymmetric Unit	2	2
Total reflection	156111	135505
Unique Reflection	41716	42116
Completeness (%)	95.9 (88.4)	93.3 (70.2)
Average I/σ	31.21 (2.60)	31 (2.55)
R_{merge} (%)	6.4 (46.1)	6.9 (28.8)
Resolution (Å) (Last Shell)	50-1.58 (1.61-1.58)	50-1.57 (1.60-1.57)
R_{cryst}/R_{free} (%)	18.17/21.62	19.63 /22.53
Bond Length (Å)	0.007	0.006
Bond Angle (°)	1.120	1.118
Average B factor	21.26	25.56
Number of water molecules	376	299
PDB IDs	4YKM	4YKO

[a] Values in the parenthesis refer to the last resolution shell.

K. THE STRUCTURAL REPRODUCIBILITY OF M2 PHOTOCYCLING

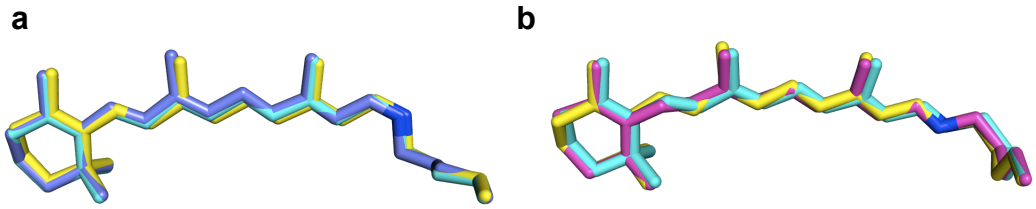


Figure S10. Cycling of the **M2** mutant crystals: a. Overlay of the retinal for the first three cycles of UV light irradiated crystals of the **M2** mutant for 30 minutes; b. Overlay of the retinal for the first three cycles of visible light irradiated crystals of the **M2** mutant for 30 minutes.

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