Electronic Supplementary Material (ESI) for Analyst.


# Supporting Information

## Light Controlled Reversible Michael Addition of Cysteine: A New Tool for Dynamic Site-Specific Labeling of Proteins

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# Experimental Section

## A. Site-directed mutagenesis

All site directed mutagenesis were performed in the pET-17b vector containing hCRBPII-Q108K:K40L:T53A:R58L:Q38F:Q4F cloned between NdeI and XhoI.[1] Site directed mutagenesis was performed using polymerase chain reaction (PCR), with the following program (Table S1):

**Table S1.** PCR cycling conditions for site-directed mutagenesis

| | PCR Program | Time (min) |
|---|---|---|
| 1X | 94 °C | 3:00 |
| 20X | 94 °C | 0:20 |
| | 3 to 5 °C below $T_m$ | 0:55 |
| | 72 °C | 3:30 |
| 1X | 72 °C | 10:00 |
| 1X | 4 °C | 5:00 |

| Reactant | Volume |
|---|---|
| DNA template | 70 ng (x µL) |
| Forward primer | 20 pmol (y µL) |
| Reverse Primer | 20 pmol (z µL) |
| 10 mM dNTP | 1 µL |
| 50 mM $MgCl_2$ | 1 µL |
| DMSO | 5 µL |
| 10X Cloned Pfu Reaction buffer | 5 µL |
| Pfu Turbo DNA polymerase (2.5U/µL) | 1 µL |
| Nuclease free water | (50-x-y-z-7) uL |

The primers used for the site directed mutagenesis were purchased from Integrated DNA Technology (IDT), with melting temperature ($T_m$) from 55 °C to 65 °C. The sequences of the forward primers (5' to 3') are listed below. It should be noted that, in all cases, the reverse primer is the reverse complement of the forward primer.

**T51C:T53A** - GATAACTTCAAGTGCAAAGCCACTAGCACATTC

**Q4** - GAC GAG GGA CCA GAA TGG AAC C

The crude PCR product was digested with 20 units of DpnI enzyme (New England Biolabs) for 1 h at 37 °C. The digested product was then transformed into *E. coli* XL-1 Blue competent cells (Agilent) for 30 min on ice and then coated on LB-Amp/Tet agar plate. After incubation at 37 °C for 16 h, a single colony was cultured in LB media with antibiotics (100 µg/mL ampicillin and 12.5 µg/mL tetracycline) for 12 h. DNA purification was performed using Wizard Plus SV miniprep DNA purification system (Promega). The concentration of the isolated DNA was measured using Nanodrop® and sequenced at the Research Technology Support Facility at Michigan State University using a primer corresponding to the T7 promoter of the pET-17b plasmid.

**B. Protein expression and purification**

hCRBPII mutants in pET-17b vector were expressed in *E. Coli* BL21(DE3)pLyS competent cells (Promega). The target gene (100 ng of DNA for 50 µL of cell solution) was added to the cells on ice for 30 min and spread over LB-Amp/Chl agar plate. The plate was incubated at 37 °C for approximately 12 h and a single colony was picked to grow in a 1 L terrific broth (TB) media supplemented with antibiotics (100 µg/mL ampicillin and 27 µg/mL chloramphenicol). The cells were cultured for 8-10 h at 37 °C until the optical density (OD) reached approximately 1. Subsequently, isopropyl-*β*-D-thiogalactopyranoside (IPTG, Gold Biotechnology) was added to the culture at a final concentration of 1.0 mM and shaken at 37 °C for additional 16-20 h. The cells were then harvested by centrifugation (3000 rpm, 15 min, 4 °C), resuspended in Tris buffer (10

mM Tris, pH 7.8-8, 50 mL), lysed with ultrasonic homogenizer (Biologics, Inc, power 60%, 3 min), added with 500U of DNase I (recombinant, Roche Diagnostics) and kept at RT for 10 min. The solution was then centrifuged (3000 rpm, 40 min, 4 °C) to separate the pellet. The supernatant was loaded onto a FastQ anion exchange column (resin from GE Healthcare), equilibrated with Tris buffer (10 mM Tris, pH 7.8-8). The resin was subsequently washed with Tris buffer (2X50 mL) and eluted with Tris-elution buffer (10 mM Tris, 200 mM NaCl, pH 7.8-8.0, 50 mL). The eluent was desalted using Amicon® Ultra-15 Centrifugal Filter Units (MW cut-off: 10K), and further purified with a FPLC (NGC chromatography system, Biorad), equipped with a Source 15Q column (Q Sepharose Fast Flow, GE Healthcare) anion exchange resin using 50 mM Tris·HCl (solution A) and 2 M NaCl (solution B) at pH 8.1. All protein samples were collected with 4% of solution B and concentrated using Amicon Ultracentrifugation filter (MW cut-off: 10K) to a final volume of 1 mL. The sample (1 mL) was then loaded to size exclusion chromatography (Superdex 120 16/600 GL column, GE Healthcare) for further purification using a buffer containing solution A and 20% of solution B. Collected protein sample was concentrated (conc. 100-200 µM) and stored at 4 °C for optical studies.

### C. Determination of extinction coefficient of protein

UV-vis spectra were measured with a Cary 300 Bio WinUV, Varian spectrophotometer using 1 cm, 1.0 mL quartz microcuvettes (Starna Cells). The extinction coefficients of the hCRBPII mutants were measured following the method described by Gill and von Hippel.[2] The theoretical extinction coefficient ($\varepsilon_{theo}$) is calculated using following equation:

$$\varepsilon_{theo} = a \times \varepsilon_{Trp} + b \times \varepsilon_{Tyr} + c \times \varepsilon_{cys}$$

where a, b and c are the number of tryptophan, tyrosine, and cysteine residues in the protein, respectively. $\varepsilon$ used for tryptophan, tyrosine, and cysteine are 5690 $M^{-1}cm^{-1}$, 1280 $M^{-1}cm^{-1}$ and 120 $M^{-1}cm^{-1}$, respectively. The absorbance of each protein was measured at 280 nm in 2XPBS and 6M guanidine hydrochloride solution. The absorbance value was used to calculate the experimental ε using the following equation:

$$\varepsilon_{exp} = \frac{A_{native}}{A_{denaturating}} \times \varepsilon_{theo}$$

**Table S2.** The values of the extinction coefficients

| Mutants | Proteins | $\varepsilon_{exp}$ ($M^{-1}cm^{-1}$) |
|---|---|---|
| M1 | Q108K:K40L:T53A:R58L:Q38F:Q4F | 28,000 |
| M2 | Q108K:K40L:**T51C**:T53A:R58L:Q38F:Q4F | 28,600 |
| M3 | Q108K:K40L:T51C:T53A:R58L:Q38F:**Q4** | 28, 520 |

### D. Determination of Extinction Coefficient of CM1V

The extinction coefficient of **CM1V** was measured by the combined use of $^1$H NMR and UV-vis spectroscopy. First, concentration of a **CM1V** stock solution in $CDCl_3$ was accurately measured by $^1$H NMR using triphenylphosphine as an internal standard. To illustrate, **CM1V** was dissolved in a known volume of $CDCl_3$ and mixed with a 1 M solution of triphenylphosphine (in $CDCl_3$) in a 4:1 volume ratio. The $^1$H-NMR of the mixture was collected and concentration of the **CM1V** was measured by comparing the area under the peaks (δ 9.65 for **CM1V** and δ 5.57 for triphenylphosphine). Subsequently 0.50, 0.75 and 1.0 µL of the mixture in $CDCl_3$ were dissolved separately in 1 mL of acetonitrile to measure the absorbance. Extinction coefficient was then calculated using Beer-Lambert's equation and the average value from three independent measurements was found to be 59,087 ± 5000 $M^{-1}$ $cm^{-1}$.

### E. UV-Vis measurement of hCRBPII/CM1V

For UV-vis measurement, a stock solution protein (100-200 μM) was prepared by concentrating the purified protein, collected from size-exclusion chromatography. In addition, a stock solution of **CM1V** (10 mM) was prepared in acetonitrile. For complex formation, 10-30 μM of protein was incubated with **CM1V** (5-15 μM, 0.5 equiv with respect to the protein) in PBS (pH 7.2) at room temperature, unless otherwise mentioned. The binding was followed by the disappearance of the **CM1V** ($\boldsymbol{\lambda}_{max}$ ~460 nm in PBS) and concomitant formation of complex. For **M1**-**M3**, the binding with **CM1V** is complete within 1 h at RT. For following the complexation of **M2**/**CM1V** at 4 °C, the protein in PBS was first cooled to 4 °C before addition of **CM1V** and the temperature was maintained throughout the process (Figure S7).

### F. p$K_a$ measurements of hCRBPII/CM1V

For p$K_a$ measurement of **M1**/**CM1V**, complex was prepared by incubating **M1** (20 μM) and **CM1V** (10 μM) PBS (pH 7.2) at RT. Titration was performed with acid (1 M citric acid solution), or base (1 M NaOH) and the absorption spectra was recorded (Figure S6). Absorbance at the $\boldsymbol{\lambda}_{max}$ of 550 nm was plotted as a function of pH of the solution. A curve fit to the following modified Henderson-Hassebalch equation was applied to determine the p$K_a$.

$$A = \frac{A_0}{1+10^{pH-pK_a}} + \text{constant}$$

where $A_0$ is the absorbance of the maximum PSB and the p$K_a$ is the mid-point of the titration. A constant is included for the zero absorbance of the deprotonated PSB.

For p$K_a$ measurement of **M2**/**CM1V**, first a cysteine bound complex (**M2/CM1V**-$C_{51}$) was prepared by incubating **M2** (30 μM) and **CM1V** (15 μM) PBS (pH 7.2) at RT. Then **M2**/**CM1V**-SB was formed by photo activation of the complex with UV (discussed in section H) and gradually

acidified to form the **M2**/**CM1V**-PSB (Figure S10). Absorbance at the $\lambda_{max}$ of 550 nm was plotted as a function of pH of the solution and p$K_a$ was as described for the **M1**/**CM1V** complex. All pH values were recorded with an accumet® Basic pH meter (Fisher Scientific) equipped with a PerpHect® ROSS® Micro Combination pH electrode (Thermo Scientific Orion).

**G. Fluorescence measurements**

Fluorescence spectra were recorded using a Fluorolog-3 spectrofluorometer (HORIBA, Ltd.) with a 1 cm, 3.5 mL quartz cuvette or 1 cm, 1.0 mL quartz microcuvette (Starna Cells). An entrance slit of 1 nm and exit slit of 12 nm was used for all measurements.

**H. Photo-irradiations**

Photo-irradiation of protein complexes in solution was performed with a Hg(Xe) Arc lamp (Oriel Instrument) attenuated with two neutral-density filters (Edmund Optics Inc.).[3] For photoactivation of **M2**/**CM1V**-$C_{51}$ and **M3**/**CM1V**-$C_{51}$, the respective complex in PBS was illuminated with a 300-400 bandpass filter (Edmund Optics Inc.) for 1 min at a desired pH (pH 7.2/4.2). Photoswitching between **M3**/**CM1V**-$C_{51}$ and **M3**/**CM1V**-PSB was performed with the use of both a 300-400 bandpass filter (1 min) and 500 nm long pass filter (5 min).

For measuring the half-life of thermal decay, the **M3**/**CM1V**-$C_{51}$ was first illuminated for 1 min and absorbance was followed at 550 nm over time. On the other hand, the rate of photochemical decay was measured under constant irradiation through a 500 nm long pass filter and intermittent collection of the absorbance at 550 nm. The half-life of decay was calculated by fitting the values with a mono-exponential decay function.

## I. Protein Crystallization and data collection

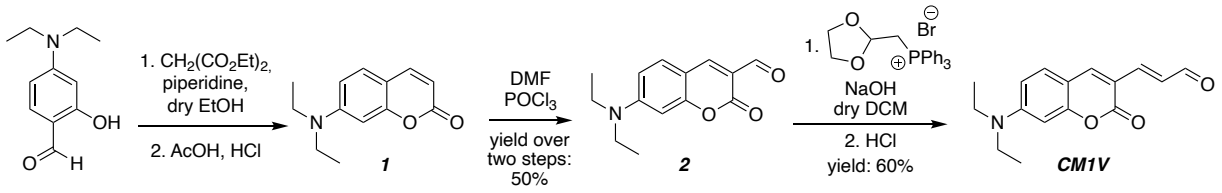
Pure protein samples were concentrated to 7-10 mg/mL using Amicon® Ultra centrifugal units (5 mL, 10 kDa cutoff), and approximately 4 equivalents of **CM1V** were added and the solution was allowed to incubate overnight at RT. Crystallization was done by vapor diffusion using 24 well plates (Hampton Research) with 1 mL reservoir volume. For each well, 1 µL of protein was added to 1 µL of well solution. Crystals typically appeared within 1-3 days in well solutions containing 25-40% PEG 4000, 0.1 M ammonium acetate, and 0.1 M sodium acetate with a pH range of 4.0 to 4.8. All UV irradiation studies were done using a TLC hand lamp. Crystals were flash frozen in a solution containing the mother liquor and 30% glycerol. Diffraction data was collected at the Advanced Photon Source (APS) (Argonne IL) LS-CAT, (sector 21-ID-D,F, and G) at 1.00 Å wavelength radiation at 100 K, using either an Eiger 9M, MAR300 or MAR350 detector. Data reduction and scaling were performed using the HKL2000 program package.[4] All structures were solved by Molecular Replacement using PHASER and refined using the PHENIX program package.[5, 6] Three cycles of refinement were implemented for each run. Placement of the **CM1V** ligand and all ordered water molecules was done using COOT (0.8.9.1).[7]

**Table S3.** List of crystallographic data

| | **M1** | **M2** (unirradiated) | **M2** (UV irradiated) | **M3** | **M2** at pH 7.2 |
|---|---|---|---|---|---|
| Wavelength | 1.1272 Å | 1.1272 Å | 1.1272 Å | 1.54184 Å | 1.1272 Å |
| Resolution Range | 29.71 - 1.42 (1.471 - 1.42) | 33.63 - 1.689 (1.749 - 1.689) | 33.49 - 1.601 (1.658 - 1.601) | 17.59 - 1.5 (1.554 - 1.5) | 29.59-1.322 (1.369-1.322) |
| Space group | C 1 2 1 | C 1 2 1 | C 1 2 2 | C 1 2 1 | C 1 2 1 |
| a (Å) | 29.612 | 29.613 | 29.698 | 29.07 | 29.222 |
| b (Å) | 67.106 | 67.25 | 66.972 | 66.29 | 66.833 |
| c (Å) | 63.903 | 63.687 | 63.817 | 63.62 | 63.704 |
| a (°) | 90 | 90 | 90 | 90 | 90 |
| b (°) | 90.108 | 92.275 | 92.102 | 90 | 90.926 |
| γ (°) | 90 | 90 | 90 | 90 | 90 |
| Molecules per Asymmetric Unit | 1 | 1 | 1 | 1 | 1 |
| Total reflections | 468357 | 470858 | 622275 | 36796 | 798011 |
| Unique Reflection | 22948 (2284) | 13067 (1152) | 14890 (1476) | 18253 (1604) | 28092 (2805) |
| Multiplicity | 6.4 | 9.8 | 6.6 | 2.3 | 11.9 |
| Completeness (%) | 97.28 (96.21) | 93.01 (81.70) | 90.18 (88.56) | 94.20 (82.71) | 98.13 (98.42) |
| Average I/ σ | 49.1 | 50.7 | 65.4 | 14.9 | 30.8 |
| $R_{meas}$ (%) | 3.6 | 4.1 | 8.0 | 3.5 | 8.7 |
| $R_{pim}$ (%) | 1.4 | 1.3 | 3.4 | 2.2 | 2.4 |
| Reflections used in refinement | 22934 (2283) | 13058 (1152) | 14878 (1471) | 18241 (1603) | 28092 (2805) |
| Reflections used for $R_{free}$ | 1987 (201) | 1296 (117) | 1468 (147) | 1807 (152) | 2009 (201) |
| Rwork (%) | 22.61 | 22.09 | 20.37 | 22.87 | 18.72 |
| Rfree (%) | 22.85 | 27.16 | 26.21 | 27.98 | 21.59 |
| RMSD from ideal values | | | | | |
| Bond Angle | 1.045 | 1.71 | 1.84 | 1.38 | 0.096 |
| Bond Length (Å) | 0.013 | 0.022 | 0.048 | 1.6 | 0.010 |
| Average B factor | 21.51 | 46.95 | 35.42 | 26.59 | 19.81 |
| Number of water molecules | 53 | 94 | 77 | 76 | 156 |
| PDB IDs | 8D6N | 8D6L | 8D6H | 8DB2 | 8DN1 |

## J. Synthesis of CM1V

**Scheme S1.** Synthesis of CM1V




Compounds **1** and **2** were synthesized following previous literature reported procedures.[8] For synthesis of **CM1V**, compound **2** (73 mg, 0.34 mmol, 1 equiv), ((1,3-dioxolan-2-yl)methyl)triphenylphosphonium bromide (175 mg, 0.41 mmol, 1.2 eqiuv) were dissolved in dry dichloromethane (5 mL) and stirred for 30 min. Powdered NaOH (15 mg, 0.38 mmol, 1.1 equiv) was added to the mixture and stirred until the spot for the starting material disappeared on TLC. Concentrated HCl (1 mL) was added and stirred at RT for 30 min. Subsequently, deionized water (10 mL) and dichloromethane (10 mL) were added, and the organic layer was separated and dried over anhydrous $Na_2SO_4$. After concentrating under reduced pressure, the product was purified using flash chromatography on silica gel column with a mixture of ethyl acetate in hexane (10-50%) affording compound **CM1V** as a salmon pink solid (55 mg, 0.20 mmol, yield 60%). The $^1H$ and $^{13}C$ of the pure compound match with the compound prepared previously.[9] $^1H$ NMR (500 MHz, DMSO-$d_6$) δ = 9.58 (d, *J*=8.0 Hz, 1H), 8.34 (s, 1H), 7.57 – 7.48 (m, 2H), 6.93 (dd, *J*=15.7 Hz, 8.0 Hz, 1H), 6.79 (dd, *J*=9.0 Hz, 2.5 Hz, 1H), 6.58 (d, *J*=2.4 Hz, 1H), 3.48 (q, *J*=7.0 Hz, 4H), 1.14 (t, *J*=7.0 Hz, 6H). $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 194.91, 159.93, 157.18, 152.68, 148.86, 146.55, 131.51, 128.22, 112.96, 110.48, 108.65, 96.65, 44.83, 12.82.

## K. Synthesis of CM1V-SB and PSB with *n*-butylamine

For synthesis of SB, **CM1V** (1mg, 3.7 µmol) was dissolved in ethanol (0.25 mL) and *n*-butylamine (18 µL, 50 equiv) and stirred until the bright color of the aldehyde completely disappeared (~30 min). Subsequently, solvent was completely evaporated under $N_2$ flow, and the

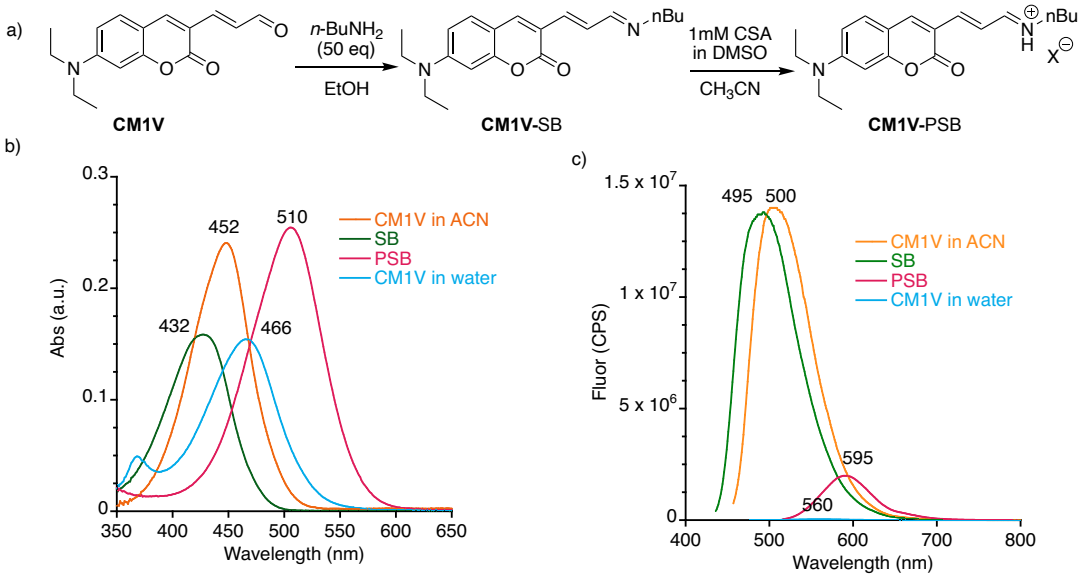



**Figure S1.** a) Synthesis of SB and PSB from **CM1V** using *n*-butylamine b) Normalized absorption and c) emission spectrum of **CM1V** aldehyde, SB and PSB in acetonitrile.

material was redissolved in acetonitrile to make stock of **CM1V**-SB (10 mM). To collect the absorption spectrum, 1 µL of the stock was dissolved in acetonitrile (1 mL). Acidification of the solution with 1 mM camphor sulfonic acid (10 µL) in DMSO resulted in the formation of PSB.

## L. Studying the formation of AD-1 and AD-2

To study the formation of **AD-1** and **AD-2** by NMR, the 1.1 mg (0.0041 mmol, 1 eq) of **CM1V**-SB was dissolved in $CD_3CN$ and *β*-ME (8.6 µL, 0.12 mmol, 30 eq) was added. $^1$H NMR spectrum of the mixture was collected every 5 minutes until all the starting SB is consumed (after 20 mins). With lower equivalent of *β*-ME, a single product could not be obtained, and the reaction becomes

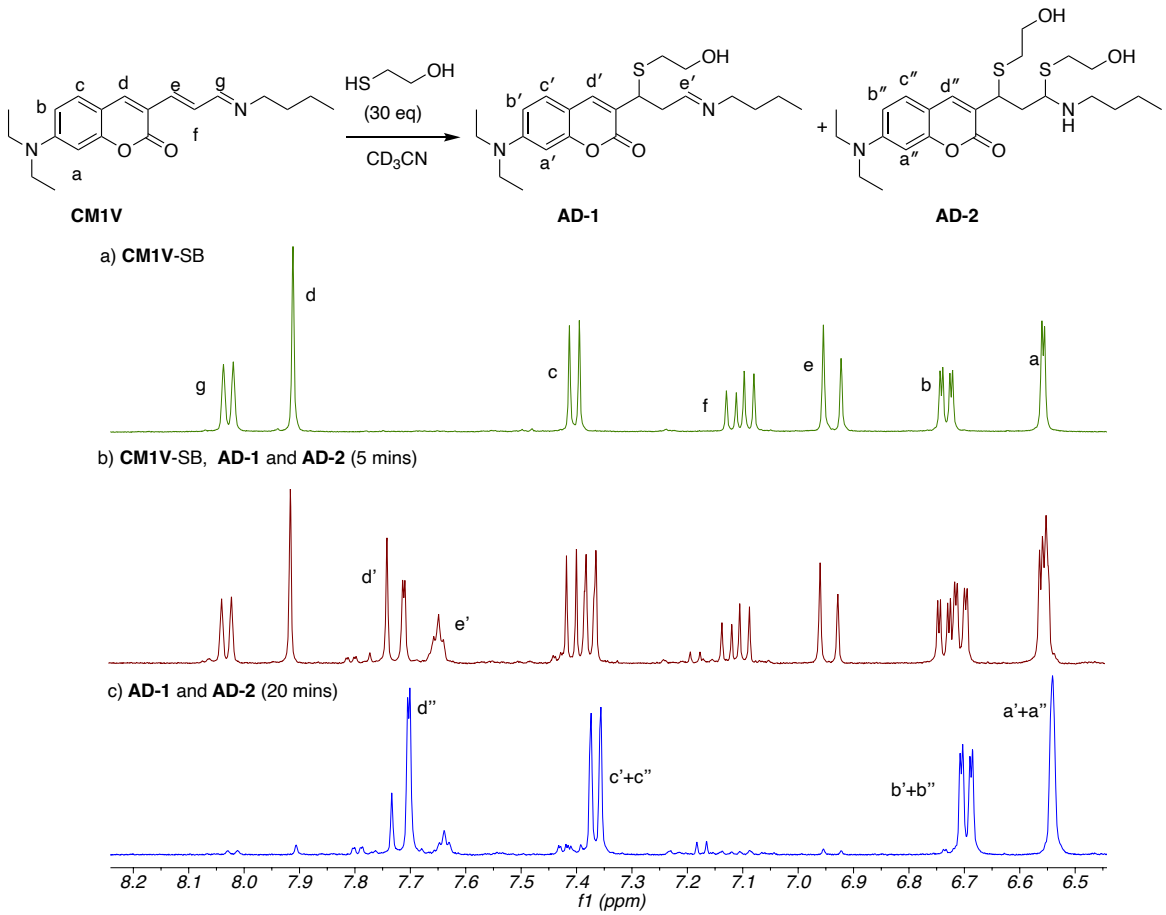



**Figure S2.** Study of the reaction between **CM1V**-SB and β-ME (30 eq) using $^{1}$H-NMR a) **CM1V**-SB only b) mixture of starting SB and the products (**AD-1** and **AD-2**) after 5 mins c) **AD-1** and **AD-2** after 20 mins.

slower. For UV-vis study, a solution of **CM1V**-SB was prepared in acetonitrile (10 µM) and *β*-ME was gradually added, and the absorption spectrum was collected after each addition. Alternatively, **CM1V**-PSB was first formed with addition of CSA (5 µL of 1 mM solution in DMSO) to **CM1V**-SB (5 µM) and then *β*-ME was mixed until the absorption for PSB disappears with concomitant formation absorption maximum around 390 nm. **CM1V**-PSB requires a larger excess of *β*-ME for the reaction to be complete, possibly because of the lower reactivity of *β*-ME under acidic condition required to form PSB.

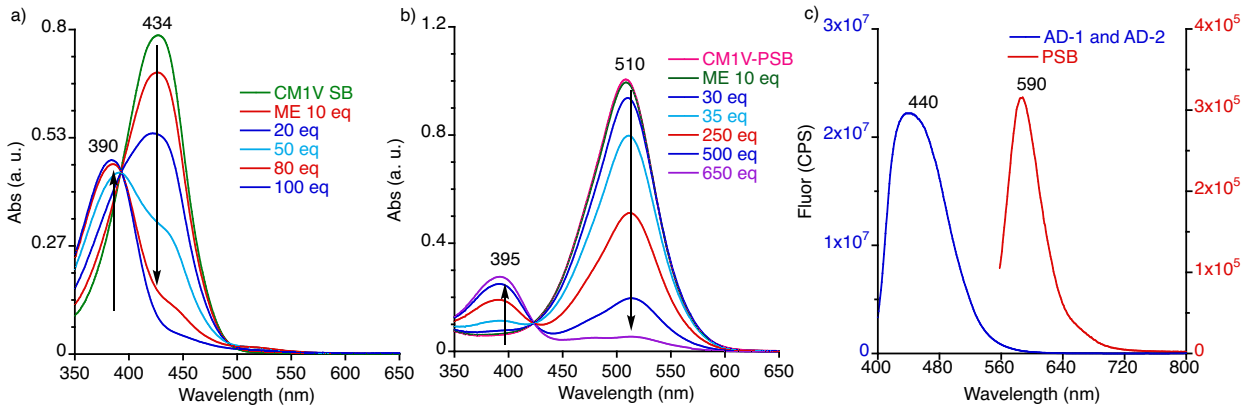



**Figure S3.** Change of absorption with addition of *β*-ME for a) **CM1V**-SB and b) **CM1V**-PSB. c) Emission spectrum of thiol bound species (**AD-1** and **AD-2**) and PSB.

## M. Supporting Information Figures

| | **1** | **10** | **20** | **30** | **40** | **50** |
|---|---|---|---|---|---|---|
| **wt-hCRBPII** | TRDQNGTWEM | | ESNENFEGYM | KALDIDFATR | KIAVRLTQTK | VIDQDGDNFK |
| **M1** | TRDFNGTWEM | | ESNENFEGYM | KALDIDFATR | KIAVRLTFTL | VIDQDGDNFK |
| **M2** | TRDFNGTWEM | | ESNENFEGYM | KALDIDFATR | KIAVRLTFTL | VIDQDGDNFK |
| **M3** | TRDQNGTWEM | | ESNENFEGYM | KALDIDFATR | KIAVRLTFTL | VIDQDGDNFK |
| | | 60 | 70 | 80 | 90 | 100 |
| **wt-hCRBPII** | TKTTSTFRNY | | DVDFTVGVEF | DEYTKSLDNR | HVKALVTWEG | DVLVCVQKGE |
| **M1** | TKATSTFLNY | | DVDFTVGVEF | DEYTKSLDNR | HVKALVTWEG | DVLVCVQKGE |
| **M2** | CKATSTFLNY | | DVDFTVGVEF | DEYTKSLDNR | HVKALVTWEG | DVLVCVQKGE |
| **M3** | CKATSTFLNY | | DVDFTVGVEF | DEYTKSLDNR | HVKALVTWEG | DVLVCVQKGE |
| **wt-hCRBPII** | 110 | | 120 | 130 | 133 | |
| **M1** | KENRGWKQWI | | EGDKLYLELT | CGDQVCRQVF | KKK | |
| **M2** | KENRGWKKWI | | EGDKLYLELT | CGDQVCRQVF | KKK | |
| **M3** | KENRGWKKWI | | EGDKLYLELT | CGDQVCRQVF | KKK | |
| | KENRGWKKWI | | EGDKLYLELT | CGDQVCRQVF | KKK | |

**Figure S4.** Sequence alignment of **M1, M2** and **M3** with wild type hCRBPII**.**

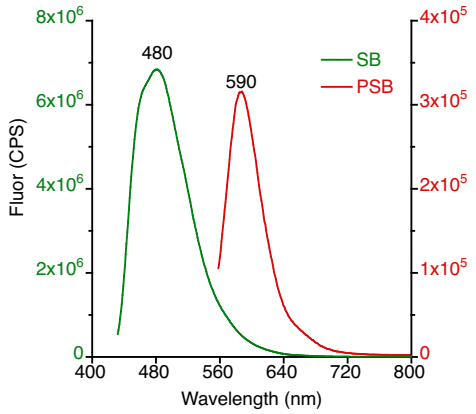



**Figure S5.** Emission spectrum of **M1**/**CM1V**, showing the emission maximum of SB (in green) and PSB (in red). The complex was excited at the respective absorption maximum to collect the emission.

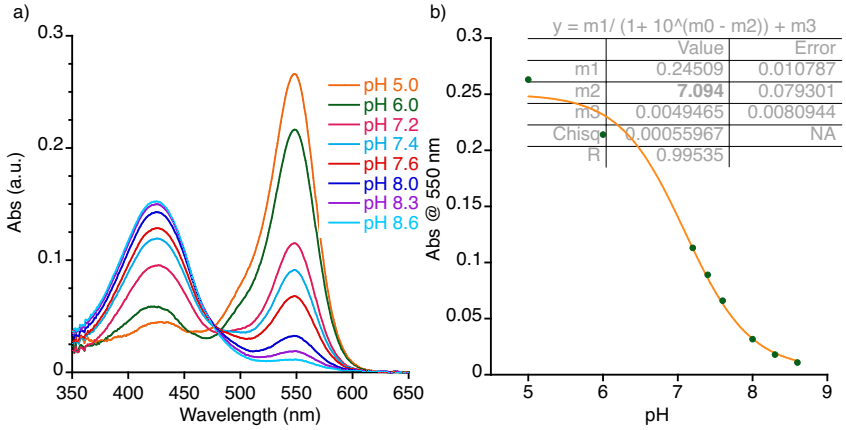



**Figure S6.** Determination of p$K_a$ for **M1**/**CM1V**: a) titration of complex in PBS buffer b) The plot of pH versus absorbance derived from the data in panel a, fitted to the Henderson-Hasselbalch equation, revealing a p$K_a$ of 7.1.

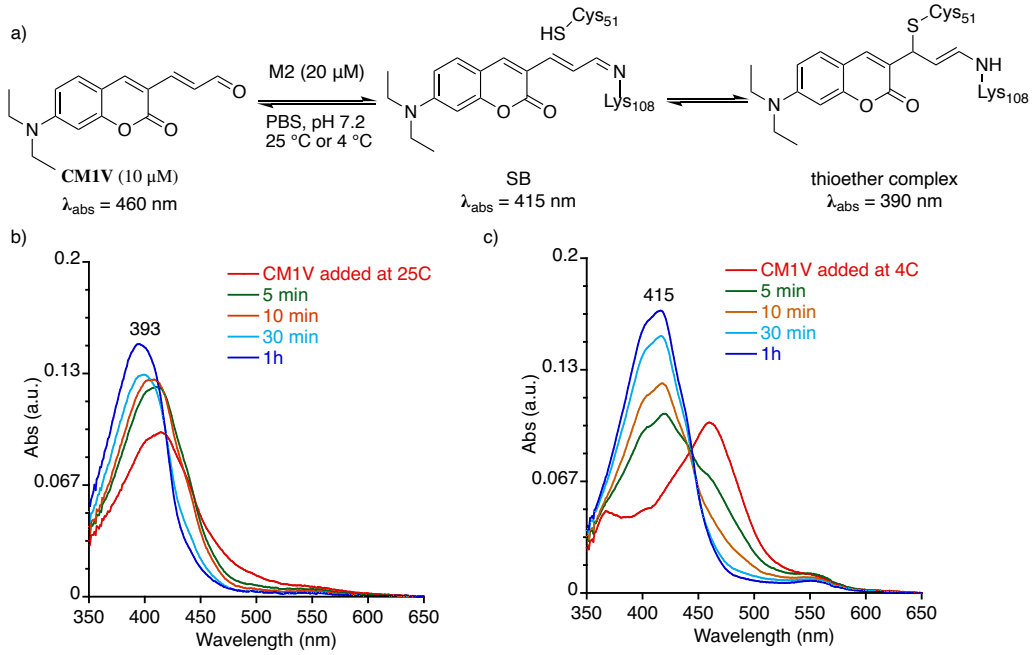



**Figure S7.** a) The steps of dual binding for **M2**/**CM1V**:SB formation is followed by the addition of cysteine b) Change of absorption spectrum with the addition of **CM1V** (10 µM) with **M2** (20 µM), at 25 °C showing the large blue shift within 1h c) the reaction can be slowed by lowering the temperature to 4 °C, showing the formation intermediate at $\lambda_{max}$ = 415 nm, close to the absorption of SB of **M1**/**CM1V** ($\lambda_{max}$ = 425 nm).

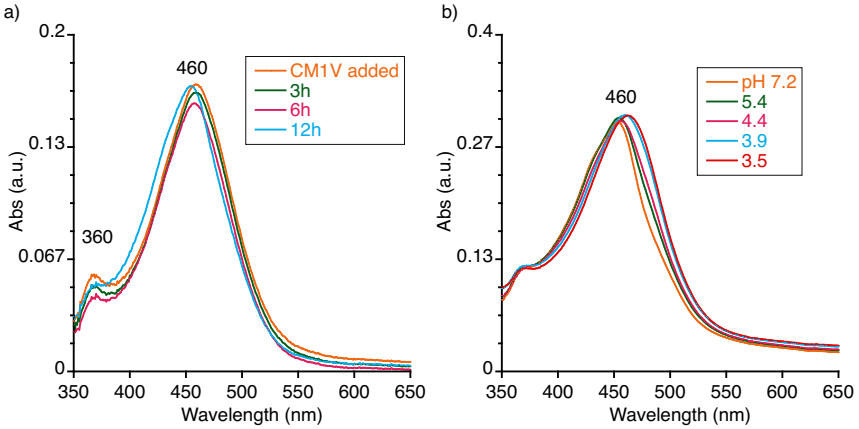



**Figure S8** Upon incubation of **Q108A**:K40L:T51C:T53A:R58L:Q38F:Q4F with **CM1V**, no complex formation was observed as apparent by the lack of significant spectral change with a) time and b) acidification of the medium.

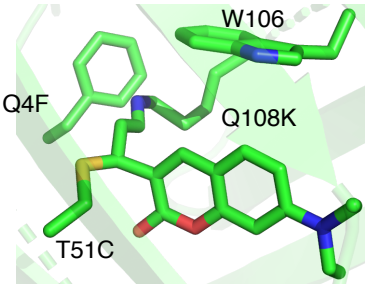



**Figure S9.** X-Ray crystal structure of **M2**/**CM1V**-$C_{51}$ after photo illumination at pH 7.2. The structure shows intact C-S bond.

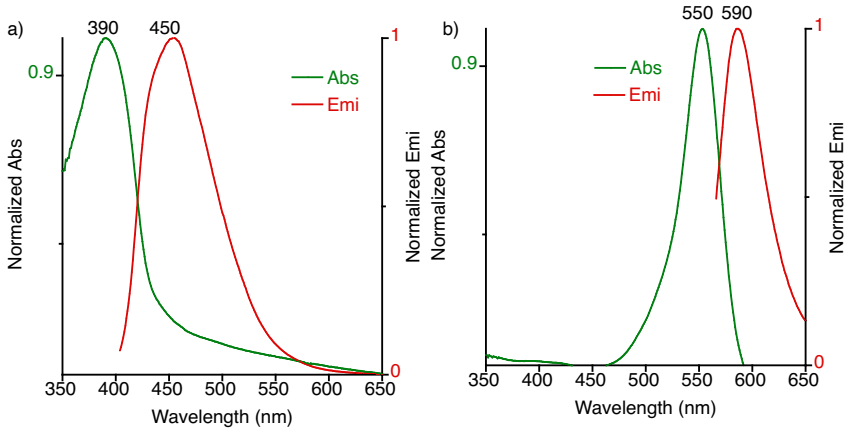



**Figure S10.** a) Normalized absorption and emission spectrum of **M2**/**CM1V** OFF state and b) ON state.

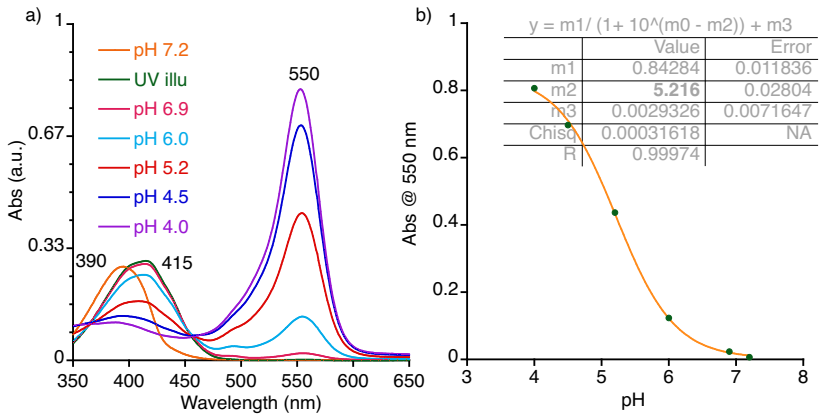



**Figure S11.** Determination of p$K_a$ for **M2**/**CM1V**: a) titration of complex in PBS buffer b) The plot of pH versus absorbance derived from the data in panel a, fitted to the Henderson-Hasselbalch equation, revealing a p$K_a$ of 5.2.

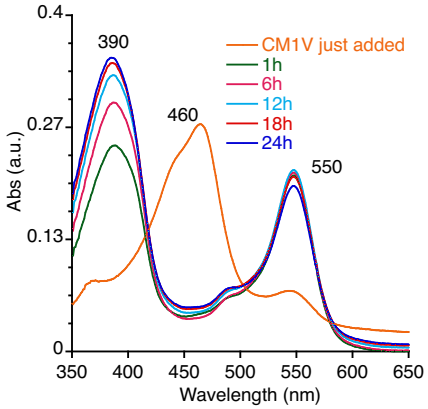



**Figure S12.** Time course study of **M3**/**CM1V** binding: Upon incubation of **CM1V** (15 μM) with **M3** (30 μM), cysteine bound complex at $\lambda_{max}$ = 390 nm was observed. A non-interconvertible PSB was also observed at $\lambda_{max}$ = 550 nm.

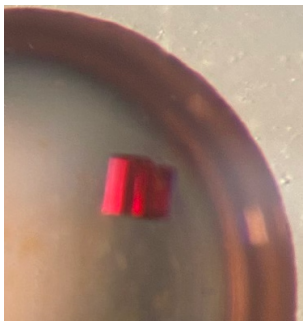



**Figure S13.** Red colored crystal of **M3**/**CM1V**, representing the PSB.

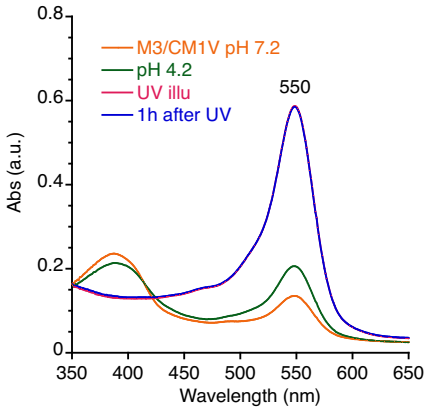



**Figure S14.** Photo illumination study of **M3/CM1V** at pH 4.2. Complexation of **M3** (20 μM) and **CM1V** (10 μM) leads to a mixture of cysteine bound complex and PSB (in orange). Acidification to pH 4.3 (in green), followed by photoexcitation of the complex (in red) leads to PSB which does not convert back to the cysteine bound form even after 1 h (in blue).

## N. References


1. E. M. Santos, W. Sheng, R. Esmatpour Salmani, S. Tahmasebi Nick, A. Ghanbarpour, H. Gholami, C. Vasileiou, J. H. Geiger and B. Borhan, *J. Am. Chem. Soc.*, 2021, **143**, 15091-15102.
2. S. C. Gill and P. H. von Hippel, *Anal. Biochem.*, 1989, **182**, 319-326.
3. W. Sheng, S. T. Nick, E. M. Santos, X. Ding, J. Zhang, C. Vasileiou, J. H. Geiger and B. Borhan, *Angew. Chem. Int. Ed. Engl.*, 2018, **57**, 16083-16087.
4. Z. Otwinowski and W. Minor, in *Methods Enzymol.*, Academic Press, 1997, vol. 276, pp. 307-326.
5. M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson, *Acta. Crystallogr. D. Biol. Crystallogr.*, 2011, **67**, 235-242.
6. P. D. Adams, P. V. Afonine, G. Bunkoczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart, *Acta. Crystallogr. D. Biol. Crystallogr.*, 2010, **66**, 213-221.
7. P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta. Crystallogr. D. Biol. Crystallogr.*, 2010, **66**, 486-501.
8. X. Jiang, Y. Yu, J. Chen, M. Zhao, H. Chen, X. Song, A. J. Matzuk, S. L. Carroll, X. Tan, A. Sizovs, N. Cheng, M. C. Wang and J. Wang, *ACS Chem. Biol.*, 2015, **10**, 864-874.
9. L. Yuan, W. Lin and Y. Yang, *Chem. Comm.*, 2011, **47**, 6275-6277.