Supporting Information for

Visualizing Back Electron Transfer in Eosin Y Photoredox Catalysis

Kai Gu,[a] Wenqiao Zhou,[a] Chunming Liu[ab]*

[a] School of Polymer Science and Polymer Engineering, University of Akron, Akron, OH, United States, 44325

[b] Department of Chemistry, University of Akron, Akron, OH, United States, 44325

*Corresponding author: chunmingliu@uakron.edu (C.L.)

1. Experimental Methods

Materials. Diethyl 2-bromo-2-methylmalonate (DEBMM) (98%), ethyl α-bromophenyl-acetate (EBPA) (97%), methyl α-bromoisobutyrate (MBriB) (≥99%), triethylamine (TEA) (≥99.5%), 3-bromopropylamine hydrobromide (98%), 3-(triethoxysilyl) propyl isocyanate (95%), and butylamine (99.5%) were purchased from Sigma Aldrich. Eosin Y disodium salt (Eosin Y Certified, Ricca Chemical, 98.0%), and glass slides (Fisherbrand™ Economy Plain Glass Microscope Slides) were purchased from Fisher Scientific. N, N-dimethylformamide (DMF, 99.8%), diethyl ether (99%), anhydrous ethanol (EtOH, 200 proof), sodium hydroxide (NaOH) and No.1 glass coverslips (VWR® SuperSlips™ Micro Cover Glasses, Rectangular, No. 1) were purchased from VWR. Milli Q water (0.04μS/cm) was obtained from MilliporeSigma™ Direct-Q™ 3 water purification system.

EY functionalization and surface immobilization. EY-NH_2 was synthesized and immobilized on coverslips as previously reported.[1] Briefly, eosin Y disodium salt (3 mmol, 2.08 g), 3-bromopropylamine hydrobromide (4.5 mmol, 985 mg) and DMF (15 mL) were mixed in a 50 mL round bottom flask. The mixture was stirred vigorously in the dark at 80°C overnight and then quenched by immersing the flask in ice water bath. The product was obtained after precipitation in a mixture solution of diethyl ether and Milli Q water (v/v = 1:1). The EY-NH_2 product was collected after centrifugation and dried in vacuum for 24 hours.

Glass coverslips were cleaned by sonicating in detergent/H_2O/EtOH mixture (3/92/5 wt%) for ~3 hours and then sonicated in water for 5 min, 1 M NaOH aqueous solution for 1 min, water for 5 min and dried by air blow. The cleaned coverslips were functionalized with isocyanate groups by reacting with NCO-silane vapor for 2 hours. After that, the coverslips were cleaned by sonicating in EtOH for 5 min, detergent aqueous solution (3 wt%) for 15 min, and water for 5 min and dried by air blow. The isocyanate-functionalized coverslips were immersed in the DMF solution of EY-NH_2 and butylamine (Bu-NH_2) mixture (molar ratio 1:100) overnight. After the immobilization, the coverslips were cleaned again by sonicating in DMF for 5 min, detergent aqueous solution (3 wt%) for 15 min, and water for 5 min and dried by air blow. The EY immobilized coverslips were stored in the dark for future use.

Single-molecule fluorescence imaging. The imaging of EY molecules followed the same procedure as previously reported.[1] The EY immobilized coverslips were assembled into flow cell devices for TIRFM imaging by attaching the coverslips to glass slides using double-sided tape (Scotch double-sided tape) and sealing the coverslip edges with epoxy super glue (Devcon No. 14250). An objective-type total internal reflection fluorescence microscope (o-TIRFM), built based on an Olympus IX73 inverted microscope with TIRF objective (Olympus UAPON100XOTIRF), was used to image the EY molecules. A 2.5 mW 532 nm laser (CrystaLaser CL532-025-L filtered by Thorlabs NE03B ND filter) was used to excite the molecules on the glass surface. The photoemission of these molecules was filtered by a 542 nm longpass filter (Chroma ET542lp) and a 585/65 nm bandpass filter (Chroma ET585/65m) and collected by a sCMOS camera (Teledyne Photometrics Prime 95B). In each imaging experiment, 1000 consecutive images of the samples were taken, and the exposure time was 50 ms (frame rate: 20 fps).

2. Determining photobleaching rate of photobleaching EYs

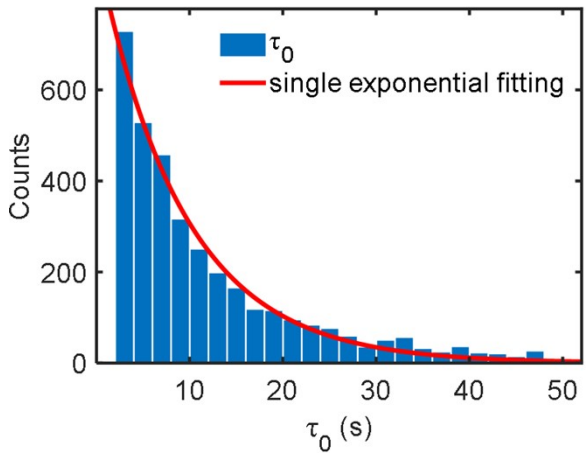

Figure s1. The distribution of photobleaching time τ_0 in 0.02 M DEBMM.

The distribution of photobleaching time τ_0 is fitted with single exponential decay function:

$$f(\tau_0) = A\exp\left(-\frac{\tau_0}{T_0}\right)$$

where A is prefactor, T_0 is the decay constant of τ_0. The T_0 values in different conditions are summarized in the table below, all errors are fitting errors.

DEBMM		**EBPA**		**MBriB**		**TEA**	
Conc. (M)	T_0 (s)	Conc. (M)	T_0 (s)	Conc. (M)	T_0 (s)	Conc. (M)	T_0 (s)
0.01	10.76±0.73	0.001	9.06±0.28	0.1	8.11±0.61	0.005	5.81±0.38
0.02	9.19±0.79	0.004	5.27±0.22	0.2	7.59±0.24	0.01	5.40±0.27
0.04	6.25±0.52	0.006	4.39±0.16	0.4	5.84±0.13	0.02	4.46±0.09
0.06	5.24±0.16	0.01	2.81±0.06	-	-	-	-

3. Determining the decay constants of τ_{on} of photoblinking EYs

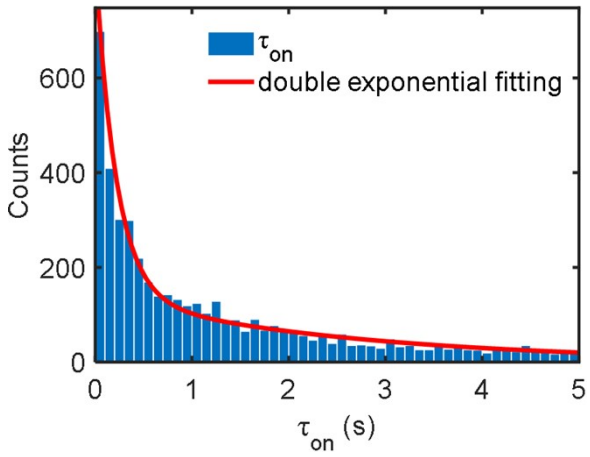

Figure s2. The distribution of τ_{on} of photoblinking EYs in 0.02 M DEBMM.

The distribution of photobleaching time τ_{on} is fitted with double exponential decay function:

$$f(\tau_{on}) = A_1 \exp\left(-\frac{\tau_{on}}{T_{on1}}\right) + A_2 \exp\left(-\frac{\tau_{on}}{T_{on2}}\right)$$

where A_1 and A_2 are prefactors, T_{on1} and T_{on2} are the decay constant of τ_{on}. The T_{on} values in different conditions are summarized in the table below, all errors are fitting errors.

DEBMM		**EBPA**		**MBriB**		**TEA**	
Conc. (M)	T_{on1} (s)	Conc. (M)	T_{on1} (s)	Conc. (M)	T_{on1} (s)	Conc. (M)	T_{on1} (s)
0.01	0.29±0.00	0.001	0.20±0.00	0.1	0.13±0.00	0.005	-
0.02	0.22±0.00	0.004	0.19±0.00	0.2	0.14±0.00	0.01	-
0.04	0.19±0.00	0.006	0.17±0.00	0.4	0.14±0.00	0.02	-
0.06	0.30±0.00	0.01	0.12±0.00	-	-	-	-

DEBMM		**EBPA**		**MBriB**		**TEA**	
Conc. (M)	T_{on2} (s)	Conc. (M)	T_{on2} (s)	Conc. (M)	T_{on2} (s)	Conc. (M)	T_{on2} (s)
0.01	3.13±0.03	0.001	2.49±0.02	0.1	2.50±0.03	0.005	-
0.02	2.57±0.03	0.004	2.06±0.01	0.2	2.36±0.02	0.01	-
0.04	2.32±0.01	0.006	1.81±0.01	0.4	2.07±0.01	0.02	-
0.06	2.02±0.02	0.01	1.04±0.01	-	-	-	-

4. Determining the decay constant of τ_{off} of photoblinking EYs

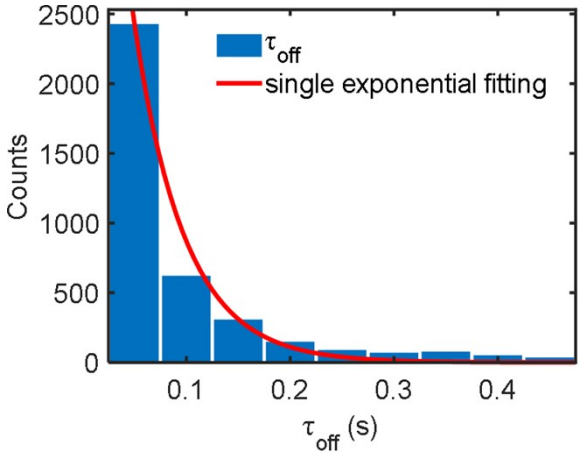

Figure s3. The distribution of τ_{off} of photoblinking EYs in 0.02 M DEBMM.

The distribution of photobleaching time τ_{off} is fitted with single exponential decay function:

$$f(\tau_{off}) = A\exp\left(-\frac{\tau_{off}}{T_{off}}\right)$$

where A is prefactor, T_{off} is the decay constant of τ_{off}. The T_{off} values in different conditions are summarized in the table below, all errors are fitting errors.

DEBMM		**EBPA**		**MBriB**		**TEA**	
Conc. (M)	T_{off} (s)	Conc. (M)	T_{off} (s)	Conc. (M)	T_{off} (s)	Conc. (M)	T_{off} (s)
0.01	0.049±0.00	0.001	0.058±0.00	0.1	0.051±0.00	0.005	-
0.02	0.045±0.00	0.004	0.058±0.00	0.2	0.048±0.00	0.01	-
0.04	0.046±0.00	0.006	0.061±0.00	0.4	0.049±0.00	0.02	-
0.06	0.049±0.00	0.01	0.061±0.00	-	-	-	-

5. P_{blink} values

DEBMM		EBPA		MBriB		TEA	
Conc. (M)	P_{blink} (%)	Conc. (M)	P_{blink} (%)	Conc. (M)	P_{blink} (%)	Conc. (M)	P_{blink} (%)
0.01	11	0.001	16	0.1	14	0.005	7
0.02	16	0.004	32	0.2	17	0.01	7
0.04	28	0.006	33	0.4	23	0.02	9
0.06	34	0.01	43	-	-	-	-

References

1. K. Gu, S. Liu and C. Liu, *Langmuir*, 2022, **38**, 15848-15857.