

Electronic Supplementary Information

for

Unveiling the photophysical mechanistic mysteries of tetrazine-functionalized fluorogenic labels

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Supplementary figures and tables

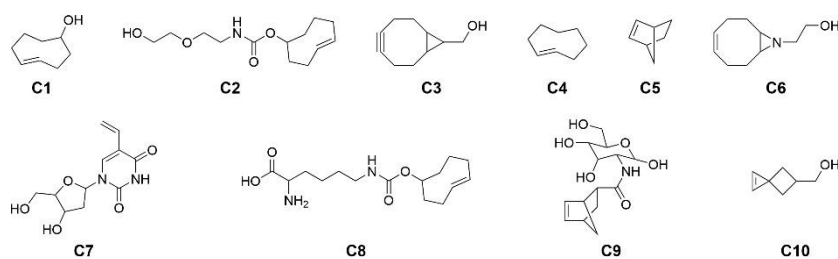


Fig. S1 Molecular structures of different counterparts.

Table S1 Reaction conditions of different tetrazine-isolated labels dominated by energy transfer to a dark state (ETDS).

Label	Counterpart	Reaction condition	Reference
1-1a,b	C1	PBS 7.4	Ref. S1 ¹
1-2a-c	C1	Acetonitrile (ACN)	Ref. S2 ²
1-3a-c	C1	PBS 7.4	Ref. S3 ³
1-4a-e	C2	PBS 7.4	Ref. S4 ⁴
1-5a	C3	PBS 7.4	Ref. S5 ⁵
1-6a-e	C1	PBS 7.4	Ref. S6 ⁶
1-7a-c	C4	ACN/Water = 1:1, v/v	Ref. S7 ⁷
1-8a,b	C5	ACN	Ref. S8 ⁸
1-9a-d	C1	ACN/Water = 1:1, v/v	Ref. S9 ⁹
1-10a-d	C3	PBS 7.4	Ref. S10 ¹⁰
1-11a-c	C3	PBS 7.4	Ref. S11 ¹¹
1-12a-d	C3	Ethanol (EtOH)	Ref. S12 ¹²
1-13a,b	C3	PBS 7.4	Ref. S13 ¹³
1-14a	C3	PBS 7.4	Ref. S14 ¹⁴
1-15a-f	C3	ACN	Ref. S15 ¹⁵

Table S2 Reaction conditions of different tetrazine-isolated labels dominated by internal conversion to a dark state (ICDS).

Label	Counterpart	Reaction condition	Reference
2-1a-e	C6	Methanol (MeOH)	Ref. S16 ¹⁶
2-1f-i	C3	ACN	Ref. S17 ¹⁷
2-2a-d	C4	ACN/Water = 1:1, v/v	Ref. S7 ⁷
2-3a-d	C3	ACN/HEPES = 2/1, v/v	Ref. S18 ¹⁸
2-4a-c	C4	Spin-coated solid film	Ref. S19 ¹⁹
2-5a,b	C3	EtOH	Ref. S20 ²⁰
2-6a	C7	ACN	Ref. S21 ²¹
2-7a	C3	MeOH	Ref. S22 ²²
2-7b-e	C3	Dimethylformamide (DMF)/Water = 1/1, v/v	Ref. S22 ²²
2-8a,b	C3	PBS 7.4	Ref. S13 ¹³
2-9a,b	C3	Water/dimethyl sulfoxide (DMSO) = 99/1, v/v	Ref. S23 ²³

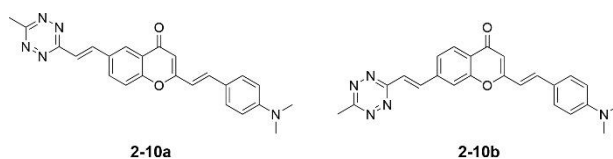


Fig. S2 Molecular structures of 2-10a (left) and 2-10b (right).

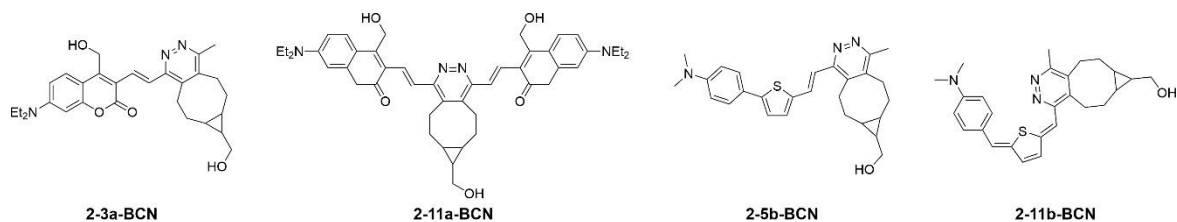


Fig. S3 Molecular structures of 2-3a-BCN, 2-11a-BCN, 2-5b-BCN, 2-11b-BCN.

Table S3 Reaction conditions of different tetrazine-isolated labels dominated by photoinduced electron transfer (PET) or photoinduced charge centralization (PCC).

Label	Counterpart	Reaction condition	Reference
3-1a-c	C3	Chloroform (CF)	Ref. S24 ²⁴
3-2a-j	C8	PBS 7.4	Ref. S25 ²⁵
3-3a-e	C1	DMF/PBS = 1/1, v/v	Ref. S26 ²⁶
3-4a	C9	DMF/PBS = 1/1, v/v	Ref. S27 ²⁷
3-5a-d	C10	PBS 7.4	Ref. S28 ²⁸
3-5e	C3	PBS 7.4	Ref. S28 ²⁸
4-1a,b	C3	DMF/Water = 1/1, v/v	Ref. S22 ²²

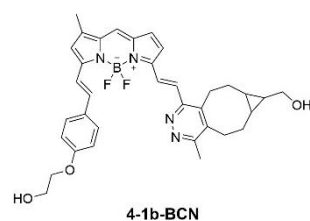


Fig. S4 Molecular structure of **4-1b-BCN**.

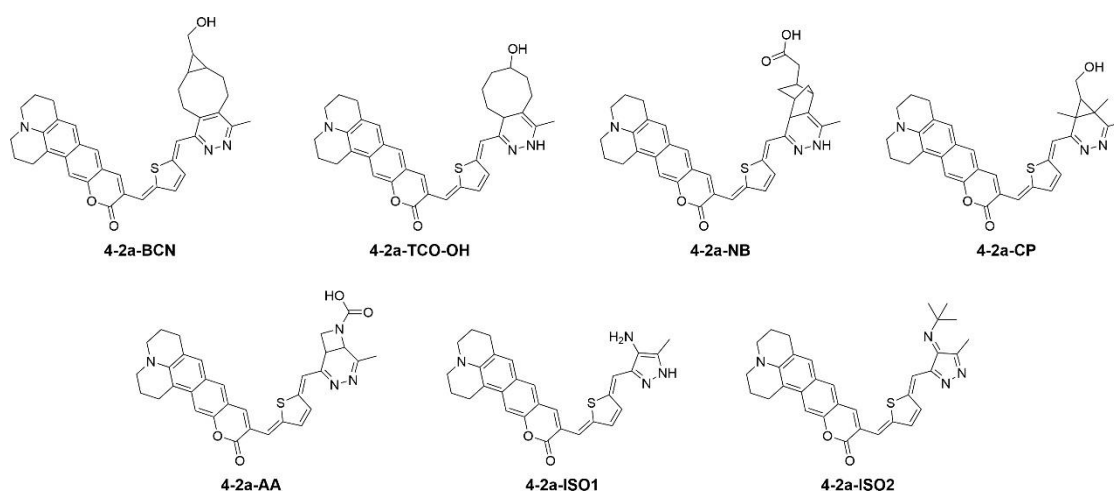


Fig. S5 Molecular structures of **4-2a** after reacting with different counterparts.

Table S4 Reaction conditions of different tetrazine-isolated labels with “Matthew Effect” in aggregation feature.

Label	Counterpart	Reaction condition	Reference
5-1a-l	C3	Water	Ref. S29 ²⁹
5-2a-f	C3	Water	Ref. S30 ³⁰
5-3a-c	C3	Water	Ref. S31 ³¹

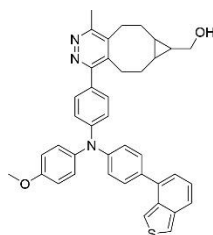


Fig. S6 Molecular structure of 5-3c-BCN.

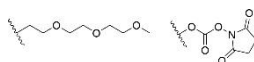


Fig. S7 Molecular structure of two R₁ (left) and R₂ (right) in Fig. 12.

Supplementary references

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