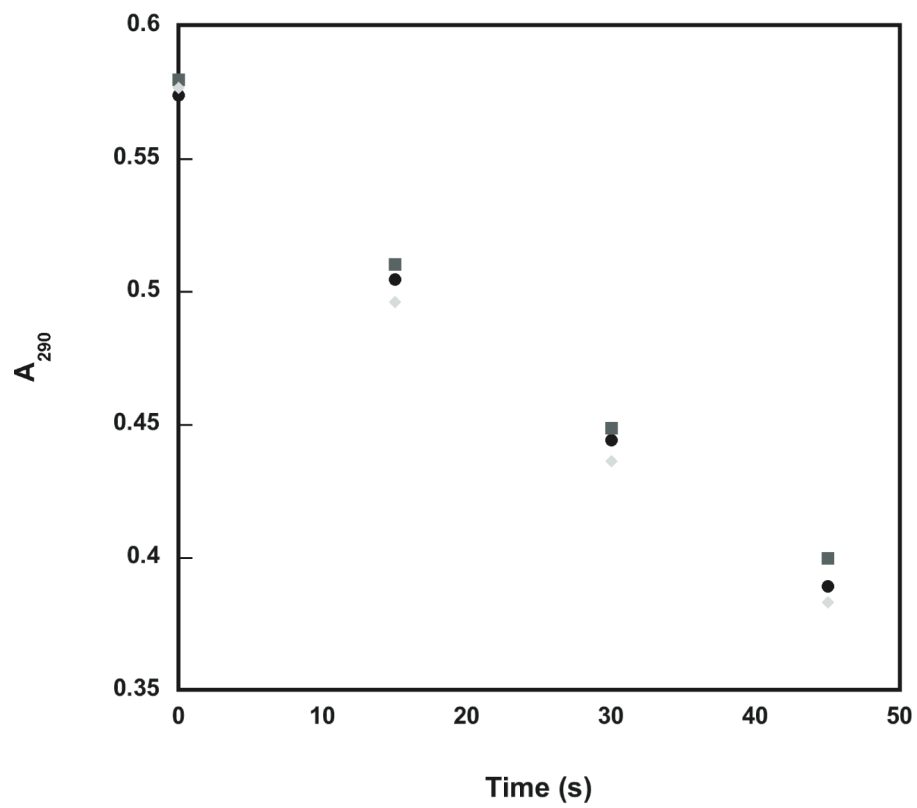


Supplemental Figure 1

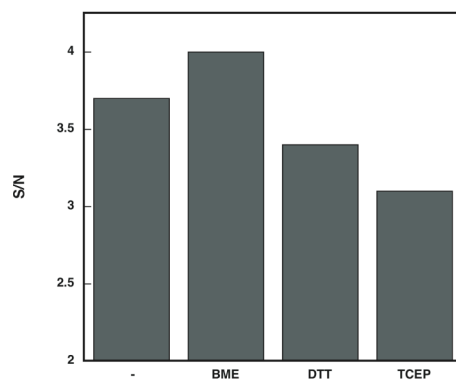


Supplemental Figure 2

A

	No reductant		BME		DTT		TCEP	
	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)
<b>Initial</b>	.92	37.0 ± 1.1	.92	45.7 ± 1.8	.91	54.3 ± 2.1	.90	27.1 ± 1.1
<b>On-blink</b>	.28	13.3 ± 0.7	.11	22.5 ± 2.5	.34	29.1 ± 1.9	.38	13.1 ± 0.8
<b>Off-blink</b>	.29	18.2 ± 0.9	.13	37.8 ± 3.9	.41	11.9 ± 0.7	.40	44.6 ± 2.7

B

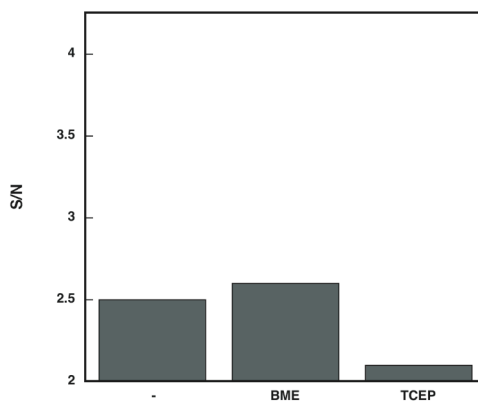


Supplemental Figure 3

**A**

	No reductant		BME		DTT		TCEP	
	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)
<b>Initial</b>	.89	14.5 ± 0.4	.87	16.4 ± 0.5	---	---	.81	4.6 ± 0.3
<b>On-blink</b>	1.24	7.0 ± 0.2	1.87	6.7 ± 0.1	---	---	2.36	2.0 ± 0.1
<b>Off-blink</b>	1.27	11.4 ± 0.3	1.92	8.4 ± 0.2	---	---	2.41	9.4 ± 0.3

**B**



Supplemental Tables 1-3

**Supplemental Table 1: Cy3 stability in the presence of additives**

	No additive		MEA		DABCO		Ascorbic Acid		nPG	
	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)
<b>Initial</b>	.92	37.0 ± 1.1	.81	40.1 ± 1.6	.84	35.8 ± 1.3	.91	43.3 ± 1.2	.94	48.7 ± 1.3
<b>On-blink</b>	.28	13.3 ± 0.7	.48	14.0 ± 0.7	.39	13.4 ± 0.7	.24	18.6 ± 1.9	.24	20.4 ± 2.0
<b>Off-blink</b>	.29	18.2 ± 0.9	.51	35.0 ± 1.8	.42	34.1 ± 1.7	.27	19.3 ± 1.0	.27	13.7 ± 1.4

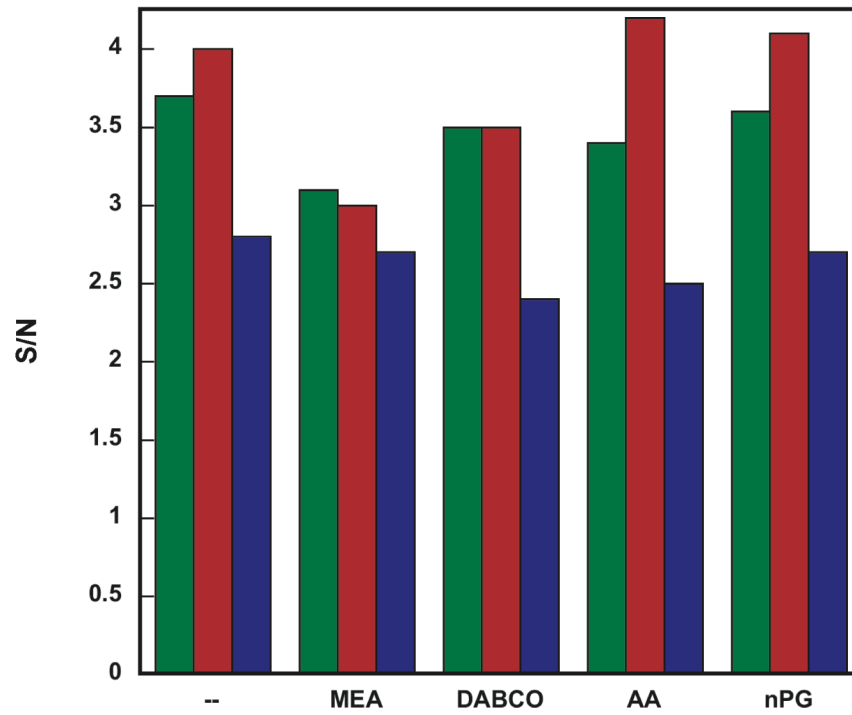
**Supplemental Table 2: Cy5 stability in the presence of additives**

	No additive		MEA		DABCO		Ascorbic Acid		nPG	
	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)
<b>Initial</b>	.96	34.1 ± 0.9	.64	5.3 ± 0.2	.92	34.3 ± 1.3	.97	28.5 ± 0.7	.99	24.1 ± 0.5
<b>On-blink</b>	.11	15.1 ± 0.7	2.36	4.5 ± 0.1	.19	14.2 ± 1.2	.20	10.6 ± 0.6	.07	15.0 ± 1.1
<b>Off-blink</b>	.12	21.4 ± 1.6	2.40	30.6 ± 0.5	.20	18.1 ± 1.5	.21	10.8 ± 0.5	.07	24.7 ± 1.8

**Supplemental Table 3: Alexa488 stability in the presence of additives**

	No additive		MEA		DABCO		Ascorbic Acid		nPG	
	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)	Events per mol.	Lifetime (s)
<b>Initial</b>	.89	15.5 ± 0.4	.90	12.5 ± 0.4	.87	15.6 ± 0.6	.89	22.2 ± 0.7	.90	19.3 ± 0.7
<b>On-blink</b>	1.75	5.3 ± 0.1	1.41	4.2 ± 0.1	1.86	5.5 ± 0.1	2.16	4.6 ± 0.1	1.79	5.4 ± 0.1
<b>Off-blink</b>	1.78	7.9 ± 0.1	1.44	9.3 ± 0.3	1.90	9.2 ± 0.2	2.22	7.4 ± 0.2	1.84	10.4 ± 0.3

Supplemental Figure 4



SUPPLEMENTAL FIGURE 1 Standardizing activity of PCD from various organisms. Initial rate timecourses of PCA consumption as monitored by absorbance at 290-nm for commercially available PCD (Sigma-Aldrich) (black circles), PCD purified from *Burkholderia cepacia* courtesy of David Ballou (dark gray squares), PCD purified from *Pseudomonas putida* courtesy of Douglas Ohlendorf (light gray diamonds). Experimental error as measured by the standard deviation of the absorbance at 290-nm from three separate experiments is less than 5% for each point. Error bars are not shown for clarity.

SUPPLEMENTAL FIGURE 2 Cy3 dye stability in the presence of biological reducing agents. (a) Table of frequencies (per molecule) and lifetimes for initial, on-blink, and off-blink events in the absence or presence of 10mM BME, DTT, and TCEP. Lifetime distributions are fit as described in Materials & Methods. (b) Bar plot of signal to noise ratio (S/N) for Cy3, determined on a per molecule basis

SUPPLEMENTAL FIGURE 3 Alexa488 dye stability in the presence of biological reducing agents. (a) Table of frequencies (per molecule) and lifetimes for initial, on-blink, and off-blink events in the absence or presence of 10mM BME, DTT, and TCEP. Values for DTT were not determined due to the inability to accurately assign on and off states due to low signal to noise. Lifetime distributions are fit as described in Materials & Methods. (b) Bar plot of signal to noise ratio (S/N) for Alexa488, determined on a per molecule basis

SUPPLEMENTAL TABLES 1-3 Cy3, Cy5, and Alexa488 stability in the presence of various additives. Tables of frequencies (per molecule) and lifetimes for initial, on-blink, and off-blink events in the absence or presence of MEA, DABCO, ascorbic acid, nPG for all three dyes tested.

SUPPLEMENTAL FIGURE 4 Bar plot of signal to noise for Cy3 (green), Cy5 (red), and Alexa488 (blue) in the presence of additives, determined on a per molecule basis.