SUPPORTING INFORMATION FOR:

Strategy for Dual-Analyte Luciferin Imaging: *In Vivo* Bioluminescence Detection of Hydrogen Peroxide and Caspase Activity in a Murine Model of Acute Inflammation

Genevieve C. Van de Bittner,[1] Carolyn R. Bertozzi,[1-3] & Christopher J. Chang*[1-3]

[1]Departments of Chemistry and [2]Molecular and Cell Biology and the [3]Howard Hughes Medical Institute, University of California, Berkeley, California 94720, United States

chrischang@berkeley.edu

FIGURES AND FIGURE CAPTIONS

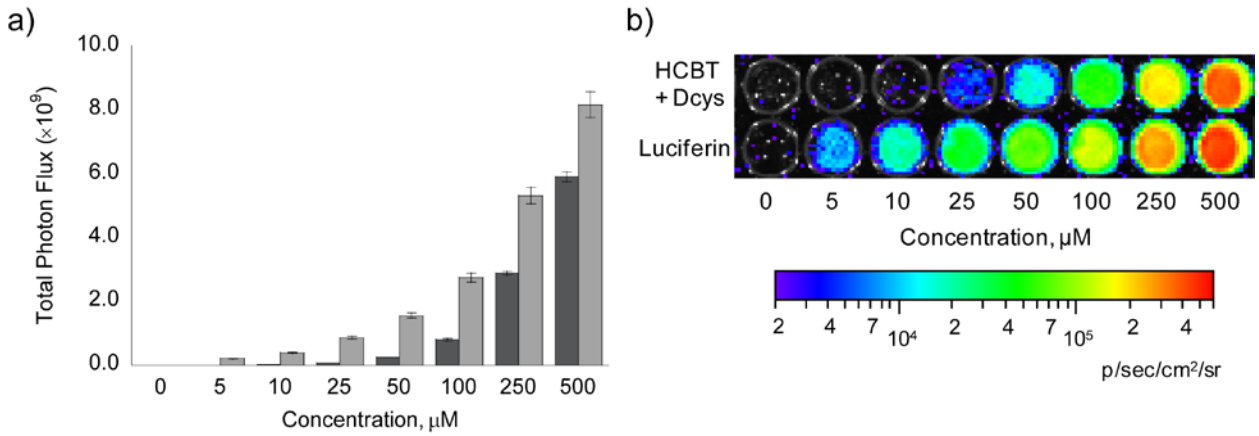

Figure S1. Comparison of HCBT/D-cysteine and luciferin in lysed PC3M-luc cells. (a) Total photon flux, integrated over 2 h, from lysed PC3M-luc cells with HCBT and D-cysteine (0–500 µM, dark grey bars) or luciferin (0–500 µM, light grey bars) in HBSS (25 mM glucose). (b) Representative image of lysed PC3M-luc cells with HCBT and D-cysteine or luciferin. Error bars are ±SEM.

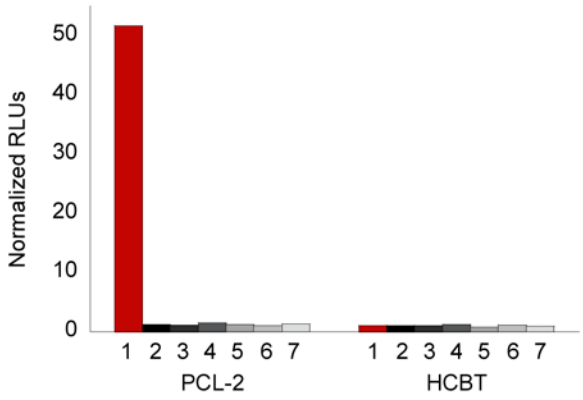

Figure S2. Comparison of bioluminescent signal from PCL-2 and HCBT incubated with various ROS. Relative total bioluminescent signal, integrated over 10 or 45 min, from PCL-2 and HCBT (5 µM) incubated with 100 µM of various ROS (1: H_2O_2, 2: TBHP, 3: $HOCl^-$, 4: $NO^{\cdot}$, 5: $^{\cdot}OH$, 6: $^{\cdot}O$*t*Bu, or 7: O_2^-) for 60 min. Signals normalized to signal from PCL-2 or HCBT in the absence of any ROS. For luciferin detection, PCL-2/HCBT solutions were incubated with D-cysteine (20 µM) for 15 min, prior to addition of 100 µg/mL luciferase in 50 mM Tris buffer with 10 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 2 mM ATP (pH 7.4).

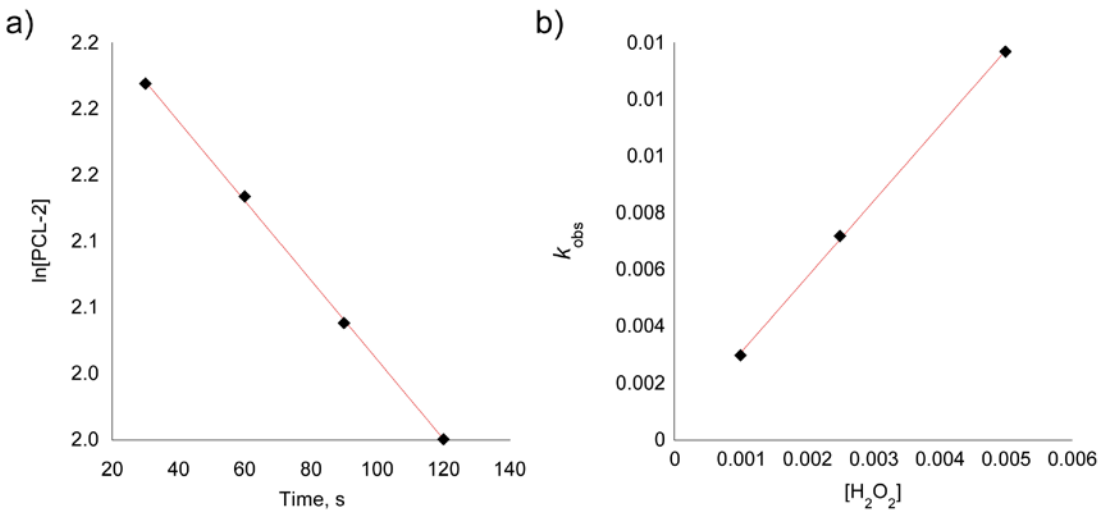

Figure S3. Kinetic studies for determination of the second-order rate constant for the reaction between PCL-2 and H_2O_2. (a) Representative plot of ln[PCL-2] versus time for the pseudo-first-order reaction between PCL-2 and H_2O_2 for determination of k_{obs}. (b) Plot of k_{obs} versus $[H_2O_2]$ for determination of the second-order rate constant.

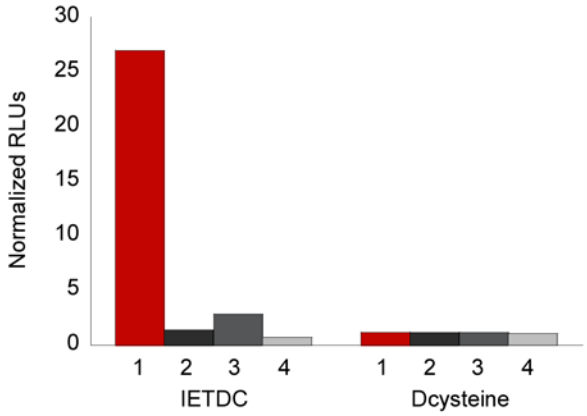

Figure S4. Comparison of bioluminescent signal from IETDC and D-cysteine incubated with various caspase enzymes. Relative total bioluminescent signal, integrated over 10 min, from IETDC and D-cysteine (5 µM) incubated with various caspase enzymes and caspase 8 plus Q-VD-OPh for 60 min (1: caspase 8, 2: caspase 8 + inhibitor, 3: caspase 3, 4: caspase 9). Signals normalized to signal from IETDC or D-cysteine in the absence of any caspase enzymes or Q-VD-OPh. For luciferin detection, 100 µg/mL luciferase in 50 mM Tris buffer with 10 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 2 mM ATP (pH 7.4) was added to the IETDC and D-cysteine solutions, which also contained HCBT (5 µM).

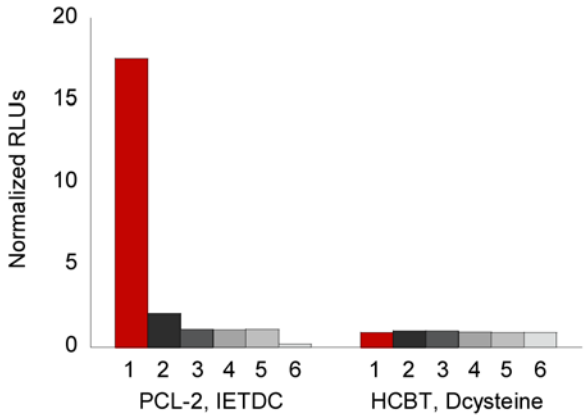

Figure S5. Comparison of bioluminescent signal from PCL-2/IETDC and HCBT/D-cysteine for *in vitro* dual-analyte detection. On left, total bioluminescent signal, integrated over 10 min, from PCL-2 (10 µM) and IETDC (10 µM) alone or incubated with H_2O_2 (250 µM) and caspase 8 (1 unit) in the presence or absence of catalase (1 unit) and/or Q-VD-OPh (10 µM). From left to right, 1: PCL-2, IETDC, H_2O_2, and caspase 8; 2: PCL-2, IETDC, and H_2O_2; 3: PCL-2, IETDC, and caspase 8; 4: PCL-2, IETDC, H_2O_2, caspase 8, and catalase; 5: PCL-2, IETDC, H_2O_2, caspase 8, and Q-VD-OPh; 6: PCL-2, IETDC, H_2O_2, caspase 8, catalase, and Q-VD-OPh. On right, total bioluminescent signal, integrated over 45 min, from HCBT (5 µM) and D-cysteine (5 µM) alone or incubated with H_2O_2 (250 µM) and/or caspase 8 (1 unit) in the presence or absence of catalase (1 unit) and/or Q-VD-OPh (10 µM). From left to right, 1: HCBT, D-cysteine, H_2O_2, and caspase 8; 2: HCBT, D-cysteine, and H_2O_2; 3: HCBT, D-cysteine, and caspase 8; 4: HCBT, D-cysteine, H_2O_2, caspase 8, and catalase; 5: HCBT, D-cysteine, H_2O_2, caspase 8, and Q-VD-OPh; 6: HCBT, D-cysteine, H_2O_2, caspase 8, catalase, and Q-VD-OPh. Signals normalized to signal from PCL-2/IETDC or HCBT/D-cysteine alone. To quantify luciferin formation, 100 µg/mL luciferase in 50 mM Tris buffer with 10 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 2 mM ATP (pH 7.4) was added to the PCL-2/IETDC or HCBT/D-cysteine solutions.

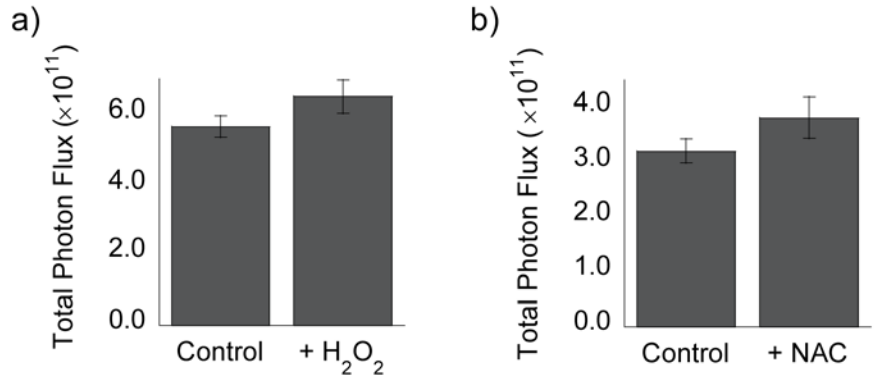

Figure S6. Bioluminescent signal from HCBT and D-cysteine with H_2O_2 and NAC in FVB-luc$^+$ mice. (a) Total photon fluxes, 0–15 min post-injection, for mice (n = 4–5) injected with a mixture of HCBT and D-cysteine (i.p., 0.01 µmol each, in 50 µL of 1:1 DMSO:PBS) immediately prior to injection of H_2O_2 (i.p., 4.5 µmol in 100 µL PBS) or vehicle (i.p., 100 µL PBS). (b) Total photon fluxes, 0–15 min post-injection, for mice (n = 3) injected with a mixture of HCBT and D-cysteine (i.p., 0.01 µmol each, in 50 µL of 1:1 DMSO:PBS) immediately prior to injection of NAC (i.p., 10 mg/kg in 100 µL PBS) or vehicle (i.p., 100 µL PBS). Error bars are ±SEM.

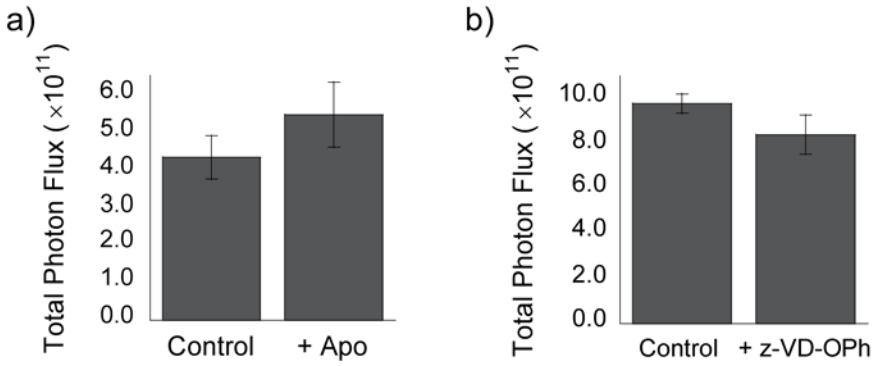

Figure S7. Bioluminescent signal from HCBT and D-cysteine with apocynin and z-VD(OMe)-OPh in FVB-luc^{+} mice. (a) Total photon fluxes, 0–15 min post-injection, for mice (n = 3) injected with apocynin (i.p., 10 mg/kg in 20 µL DMSO) or vehicle (i.p., 20 µL DMSO) two min prior to injections of a mixture of HCBT and D-cysteine (i.p., 0.01 µmol each, in 50 µL of 1:1 DMSO:PBS). (b) Total photon fluxes, 15–45 min post-injection, for mice (n = 3) injected with z-VD(OMe)-OPh (i.p., 1 µmol in 20 µL DMSO) or vehicle (i.p., 20 µL DMSO) 30 min prior to injections of a mixture of HCBT and D-cysteine (i.p., 0.01 µmol each, in 50 µL of 1:1 DMSO:PBS). Error bars are ±SEM.

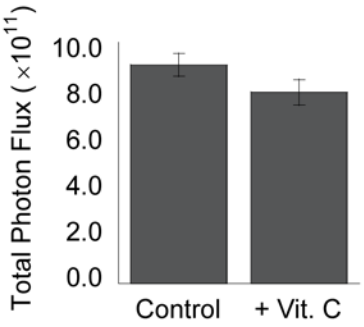

Figure S8. Bioluminescent signal from HCBT and D-cysteine with ascorbic acid in FVB-luc^{+} mice. Total photon fluxes, 30–60 min post-injection, for mice (n = 3–4) injected with ascorbic acid (i.p., 200 mg/kg in 30 µL saline) or vehicle (i.p., 30 µL saline) 4.5 h prior to injections of a mixture of HCBT and D-cysteine (i.p., 0.01 µmol each, in 50 µL of 1:1 DMSO:PBS). Error bars are ±SEM.

Table S1. Comparison the response of PCL-1 and PCL-2 to H_2O_2 *in vivo*.

H_2O_2, μmol	Turn-on, PCL-2 (0.05 μmol)	Turn-on, PCL-1 (0.5 μmol)
0.037	N/A	1.5-fold
0.15	N/A	2.0-fold
0.5	2.8-fold	N/A
0.6	N/A	2.7-fold
1.5	6.4-fold	N/A
2.4	N/A	3.6-fold
4.5	10.3-fold	N/A