S-1 General Methods:

All reactions were performed in oven-dried (140°C) or flame-dried glassware under an atmosphere of dry N_2. Dichloromethane was distilled over calcium hydride and degassed by freeze-thaw cycle. Tetrahydrofuran and diethyl ether were distilled over sodium and benzophenone. HPLC grade hexane, ethyl acetate and ACS grade diethyl ether were used as solvents for chromatography and working-up reactions without further purification. Analytical thin-layer chromatography (TLC) was carried out using EM Science silica gel 60 F_{254} plates. The developed chromatogram was analyzed by UV lamp (254 nm), ethanolate phosphomolybdic acid, potassium permanganate ($KMnO_4$) or cerium ammonium molybdate (CAM). Liquid chromatography was performed using a forced flow (flash chromatography)[1] of the indicated system on silicyclic Silica Gel (230-400 mesh). ^{1}H NMR and ^{13}C NMR were recorded on a Varian 400 (400 MHz, ^{1}H; 100 MHz, ^{13}C) spectrometer. Spectra were referenced to residual chloroform (7.24 ppm, ^{1}H; 77.00 ppm, ^{13}C) or residual methanol (3.49 ppm, ^{1}H; 50.41 ppm, ^{13}C). Chemical shifts are reported in ppm, multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), comp (composite) and br (broad). Coupling constants, *J*, are reported in Hertz. ^{13}C NMR spectra were routinely run with broadband decoupling. High-resolution mass spectra (HRMS) were obtained on a Karatos MS9 and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion (M+1) or a suitable fragment ion. Ethidium bromide, rhodamine and cyanine dyes were purchased from Fluka/Aldrich and used without further purification. Sodium

1) W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923-2925.

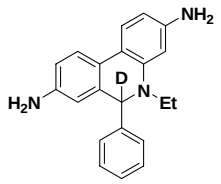
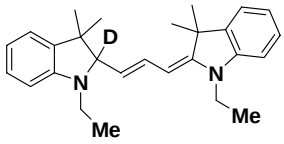
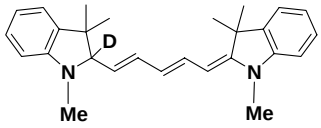
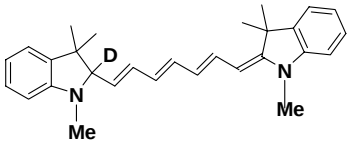
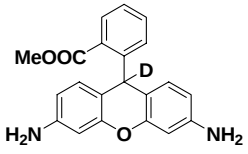
borohyride and TEMPOL were obtained from the Aldrich Chemical Company. Sodium borodeuteride was purchased from the Alfa Aesar (99% Isotopic). DHE was purchased from Life Sciences Inc. (Eugene, OR, USA) and used without further purification. Calf serum was purchased from Gibco BRL (Gaithersburg, MD, USA) and all other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA).

S-2 Synthetic Methods and Characterizations:

S-2.1 Synthesis of DDE, D-Cy3, D-Cy5, D-Cy7 and DDR (Table S-1):

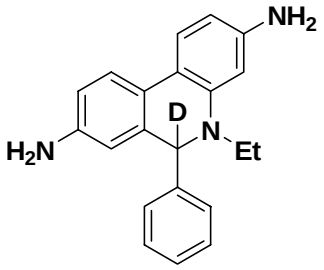
The procedure used to synthesize the deuterated probes **2**, **4**, **6**, **8** & **10** is described below, using DDE as a representative example. Ethidium bromide (**11**) (80 mg, 0.2 mmol) was dissolved in methanol (5 mL) and placed in a 4-dram vial covered with aluminum foil. $NaBD_4$ (4 mg, 0.1 mmol in 0.5 mL methanol) was added drop-wise to the red ethidium bromide solution, stirred for 10 min, generating a pale brownish solution. The reaction mixture was then stirred for an additional 10 min before removing the solvent under reduced pressure. The resulting solid was dissolved in dichloromethane (10 mL), followed by addition of water (5 mL) and the resulting mixture was vigorously shaken. The organic layer was extracted with additional dichloromethane (5 mL x 2), dried over anhyd. sodium sulfate and the solvent was removed under reduced pressure. The reduced dye thus obtained was used without further purification. D-Cy3, D-Cy5, D-Cy7 and DDR were synthesized in high chemical yields (Table S-1) following a similar reduction procedure. The detailed spectral characterization of DDE, D-Cy3, D-Cy5, D-Cy7 and DDR is listed in section S-2.2.

Table S-1: Synthesis of the Deuterated Probes **2**, **4**, **6**, **8** and **10**.

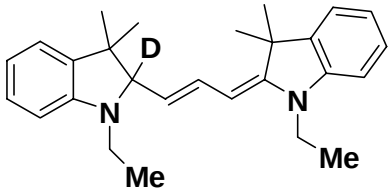
Entry	Product	Yield [%][a]
i	 **DDE (2)**	**94**
ii	 **D-Cy3 (4)**	**96**
iii	 **D-Cy5 (6)**	**96**
iv	 **D-Cy7 (8)**	**95**
v	 **DDR (10)**	**93**

[a] Isolated yield

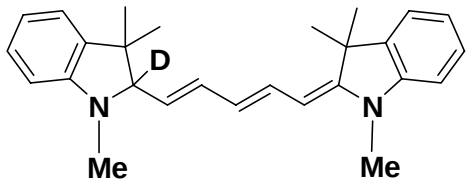
S-2.2 Spectral Data for DDE, Deuterocyanines and DDR:



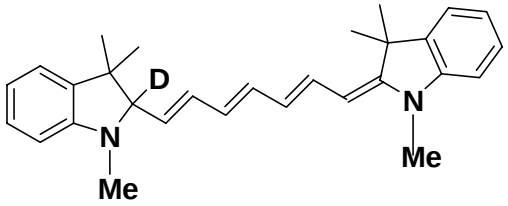
DDE (2): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.17 (comp, 5H), 6.59 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 6.16 (d, *J* = 8.0, 2.4 Hz, 1H), 6.05 (d, *J* = 2.4 Hz, 1H), 3.55 (bs, 4H), 3.37 (comp, 1H), 3.18 (comp, 1H), 1.16 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): 155.1, 148.2, 141.4, 139.1, 139.6, 138.8, 137.6, 137.1, 135.5, 136.3, 136.6, 135.2, 134.7, 134.5, 134.0, 56.2, 45.1, 10.3; HRMS (EI) for $C_{21}H_{20}DN_3$ [M+] calcd: 316.18130; found 316.18127.



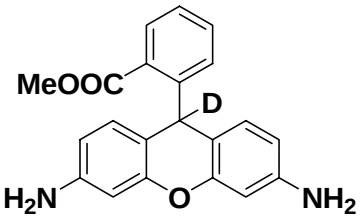
D-Cy3 (4): ^{1}H NMR (400 MHz, CD_3OD): δ 7.10 (t, *J* = 7.4 Hz, 2H), 6.99 (t, *J* = 6.4 Hz, 1H), 6.96 (d, *J* = 6.4 Hz, 1H), 6.85 (dd, *J* = 14.8, 11.4 Hz, 1H), 6.73 (t, *J* = 7.4 Hz, 1H), 6.61 (t, *J* = 7.8 Hz, 2H), 6.44 (d, *J* = 7.8 Hz, 1H), 5.38 (d, *J* = 11.4 Hz, 1H), 5.33 (d, *J* = 14.8, 1H), 3.62 (comp, 4H), 1.53 (s, 3H), 1.52 (s, 3H), 1.27 (s, 3H), 1.17 (t, *J* = 6.8 Hz, 3H), 1.16 (s, 3H), 1.04 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): 154.2, 149.7, 144.7, 139.2, 138.6, 130.8, 127.6, 127.0, 121.7, 121.3, 120.5, 118.8, 117.7, 107.5, 105.7, 94.5, 44.8, 43.9, 39.3, 36.1, 27.5, 27.4, 25.0, 24.2, 23.4, 10.3, 9.1. HRMS (EI) for $C_{27}H_{33}DN_2$ [M+] calcd: 387.27693; found: 387.27722.



D-Cy5 (6): ^{1}H NMR (400 MHz, CD_3OD): 7.07-7.26 (comp, 2H), 7.00 (d, *J* = 7.2 Hz, 1H), 6.79 (comp, 1H), 6.73 (t, *J* = 7.4 Hz, 1H), 6.56 (d, *J* = 8.0 Hz, 1H), 6.52 (d, *J* =8.0 Hz, 1H), 6.34 (comp, 2H), 6.18 (dd, *J* = 14.4, 11.6 Hz, 1H), 6.30 (dd, *J* = 15.2, 8.8 Hz, 1H), 5.61 (d, *J* = 14.4 Hz, 1H), 5.31 (d, *J* = 11.6 Hz, 1H), 3.08 (s, 3H), 2.63 (s, 3H), 1.55 (s, 6H), 1.26 (s, 3H), 1.03(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 156.7, 151.8, 145.5, 139.5, 139.0, 135.8, 130.4, 128.3, 127.7, 127.6, 125.1, 121.8, 121.7, 119.4, 118.6, 108.1, 105.9, 96.8, 45.6, 44.5, 34.6, 29.2, 28.5, 25.8, 24.3; HRMS (EI) for $C_{27}H_{31}DN_2$ [M+] calcd: 385.26283; found: 385.26231.



D-Cy7 (8): ^{1}H NMR (400 MHz, CD_3OD): δ 7.10-7.17 (comp, 3H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.79 (comp, 3H), 6.58 (d, *J* = 8.0 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 6.36 (comp, 2H), 6.21 (dd, *J* = 14.4, 10.4 Hz, 1H), 6.07 (dd, *J* = 14.4, 11.2 Hz, 1H), 5.66 (d, *J* = 15.2, 1H), 5.33 (d, *J* = 12.0 Hz, 1H), 3.10 (s, 3H), 2.65 (s, 3H), 1.58 (s, 6H), 1.29 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 156.7, 152.2, 145.5, 139.5, 139.0, 135.9, 134.7, 130.4, 128.3, 127.9, 127.7, 127.6, 125.1, 121.9, 121.7, 119.4, 118.6, 108.1, 105.9, 96.7, 45.5, 44.5, 34.6, 29.2, 28.5, 25.8, 24.3; HRMS (EI) for $C_{29}H_{33}DN_2$ [M+] calcd: 411.28002; found: 411.27959.



DDR (10): ^{1}H NMR (400 MHz, CD_3OD): δ 7.52 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.12 (ddd, *J* = 7.8, 7.6, 1.6 Hz, 1H), 6.98 (ddd, *J* = 8.0, 7.6, 1.6 Hz, 1H), 6.84 (ddd, *J* = 7.8, 7.6, 1.6, 1H), 6.51 (d, *J* = 8.4 Hz, 2H), 6.24 (d, *J* = 2.6 Hz, 2H), 6.13 (dd, *J* = 8.4, 2.6 Hz, 2H), 3.76 (s, 3H), 3.12 (bs, 4H); ^{13}C NMR (100 MHz, CD_3OD): 164.2, 151.7, 149.3, 147.5, 131.9, 131.4, 130.1, 129.2, 129.0, 125.6, 114.8, 114.6, 111.1, 109.3, 68.4, 51.6; HRMS (ESI) for $C_{21}H_{17}DO_3N_2$ [M+Na] calcd: 370.1215; found 370.1219.

S-3 Determination of the kinetic isotope effect (k_H/k_D) for reaction with superoxide (Table 1):

The rate constants for the reaction of **1-10** with superoxide were determined via a competitive inhibition assay with superoxide dismutase (SOD), following the procedure reported by Finkelstein et al.[2] Superoxide was generated by the enzymatic reaction between xanthine and xanthine oxidase (50 μM / 50 mU). The data were plotted according to the equation $[(V_o/V)-1] = k_{SOD}[SOD] / k_{H(D)}[Probe]$, where V_o and V are the rates at which **1-10** react with superoxide in the absence and presence of SOD respectively. The k_H/k_D values were determined by taking the ratio of the slopes of the regression lines generated from $[(V_o/V)-1] = k_{SOD}[SOD] / k_{H(D)}[Probe]$. The plots for **1-10** are shown in figures S-1 - S-5 and the k_H/k_D values are listed in Table-1.

2) E. Finkelstein, G. M. Rosen, E. J. Rauckman, *J. Am. Chem. Soc.* **1980**, *102*, 4994-4999.

DHE/DDE:

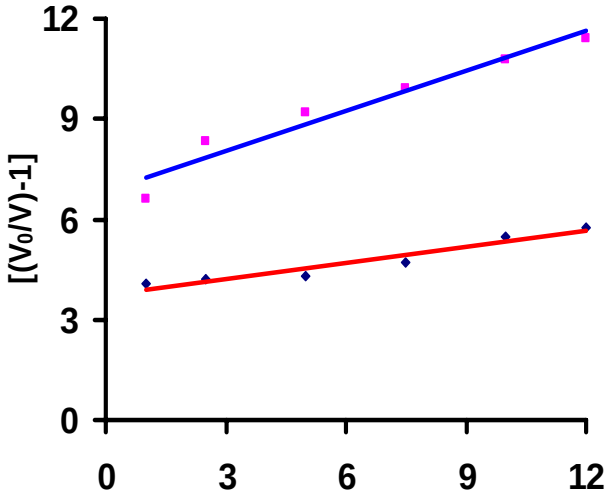


Figure S-1: Inhibition of oxidation of DHE and DDE with superoxide by SOD. The concentrations of DHE and DDE used in these experiments were 50 μM.

H-Cy3/D-Cy3:

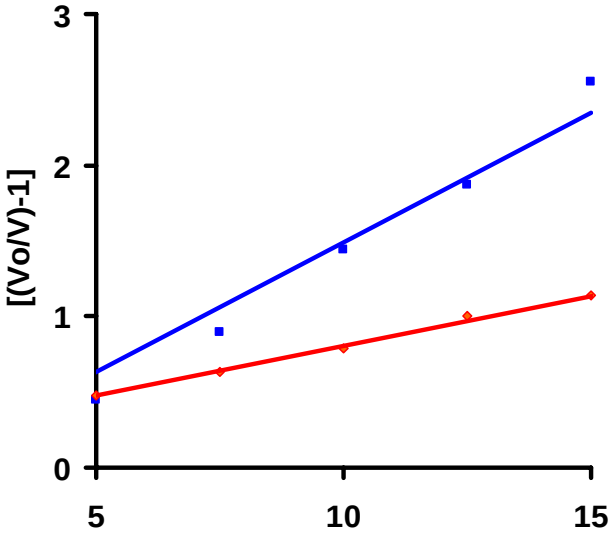


Figure S-2: Inhibition of oxidation of H-Cy3 and D-Cy3 with superoxide by SOD. The concentrations of H-Cy3 and D-Cy3 used in these experiments were 20 μM.

H-Cy5/D-Cy5:

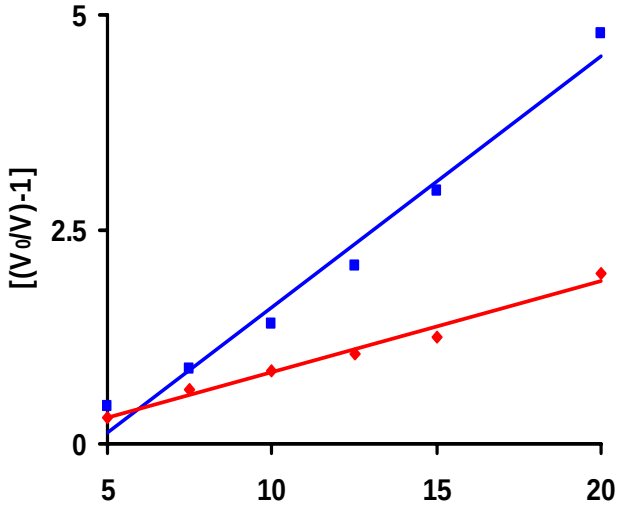


Figure S-3: **Inhibition of oxidation of H-Cy5 and D-Cy5 with superoxide by SOD.** The concentrations of H-Cy5 and D-Cy5 used in these experiments were 20 μM.

H-Cy7/ D-Cy7:

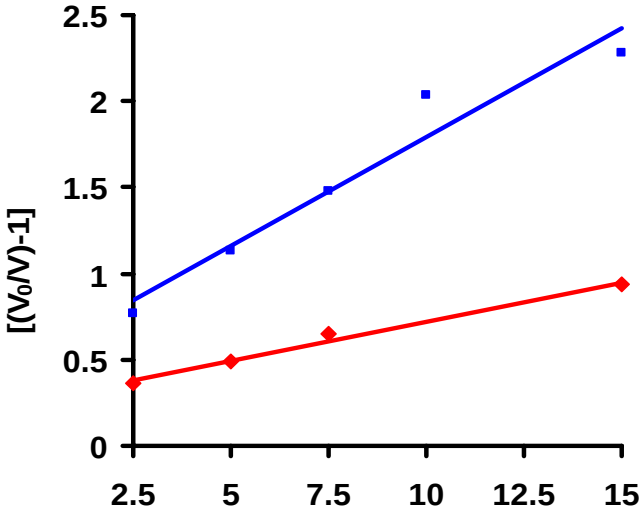


Figure S-4: **Inhibition of oxidation of H-Cy7 and D-Cy7 with superoxide by SOD.** The concentrations of H-Cy7 and D-Cy7 used in these experiments were 20 μM.

DHR/DDR:

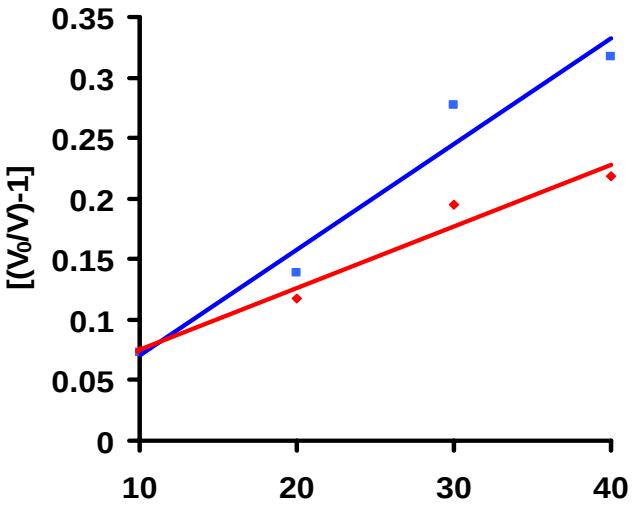


Figure S-5: Inhibition of oxidation of DHR and DDR with superoxide by SOD. The concentrations of DHR and DDR used in these experiments were 10 μM.

S-4 Accuracy of DHE and DDE towards detecting the hydroxyl radical (Figure 1a):

The hydroxyl radical was generated *in situ* by reacting hydrogen peroxide with Fe^{2+}. To a 1 mL solution of DDE or DHE in methanol (50 μM), various quantities of a hydrogen peroxide stock solution were added, to generate hydrogen peroxide concentrations ranging from 1-60 nM. Aqueous Fe^{2+} (1 μM) was then added to the DDE/DHE solutions to generate a 200 nM concentration. The resulting solution was kept at ambient temperature for 5 min and the fluorescence intensity was measured ($\lambda_{ex}/\lambda_{em}$ = 515/560 nm) against a reagent blank at the same time. The experiments were done in triplicate and the average value and standard deviation were plotted.

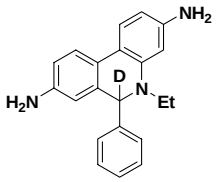
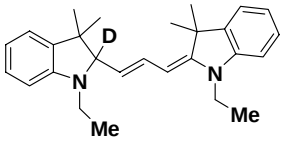
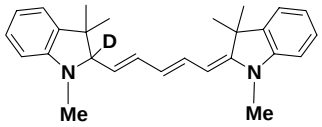
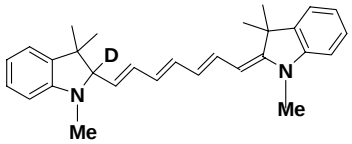
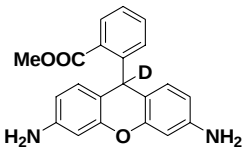
S-5 Stability of DHE and DDE to auto-oxidation during solid storage (Figure 1b):

Solid DDE (10.0 mg) and DHE (10.0 mg) were added into two separate 4-dram vials, covered with aluminum foil, and stored at room temperature. At various time points, the DDE and DHE were weighed out from the corresponding vials, dissolved in PBS, generating a 78 μM concentration, using methanol as a co-solvent (1%). The fluorescence intensity of this solution was measured, using an excitation at 515 nm and an emission of 559 nm, and divided by the fluorescence intensity of a 78 μM ethidium bromide solution, to obtain the percent oxidized.

S-6 Deuterium/Hydrogen (D/H) exchange in aqueous solution (Table S-2):

The D/H exchange of the deuterated probes DDE (**2**), D-Cy3 (**4**), D-Cy5 (**6**), D-Cy7 (**8**) and DDR (**10**) was determined by ^{1}H-NMR analysis of the α-amine C—H bond generated from D/H exchange. The procedure used to study the D/H exchange in the deuterated probes **2**, **4**, **6**, **8** and **10** is described below, using DDE (**2**) as a representative example. DDE (5.4 mg, 0.017 mmol) was dissolved in methanol (2 mL) and added to 2 mL PBS (pH 7.4), covered with aluminum foil and kept at room temperature for 4h. The solution was concentrated under reduced pressure, extracted with dichloromethane (3 x 2 mL) and dried over anhyd. sodium sulfate. The resulting dichloromethane was concentrated under reduced pressure and analyzed by ^{1}H-NMR (in $CDCl_3$). The percent D/H-exchange was quantified by integrating the α-amine C—H bond generated from D/H exchange at δ = 5.31 ppm (s) with respect to the aromatic peak at δ = 6.59 ppm (dd, J = 8.4, 2.4 Hz, 1H). The experiments were performed in triplicate. The percent D/H-exchange of DDE, D-Cy3, D-Cy5, D-Cy7 and DDR are listed in Table S-2.

Table S-2: D/H Exchange for the deuterated probes **2**, **4**, **6**, **8** and **10** in methanol/PBS mixture

Radical Oxidant Probes	Solvent System	Time (h)	D/H Exchange	σ	Exchangeable Peak In NMR Spectrum (In $CDCl_3$)
 **DDE (2)**	2 mL Methanol + 2 mL PBS	4	0.33%	0.47%	δ = 5.31 ppm (s, 1H) (α-amine C—H)
 **D-Cy3 (4)**	2.5 mL Methanol + 0.5 mL PBS	4	2.00%	1.41%	δ = 3.59 ppm (d, *J* = 9.2 Hz, 1H) (α-amine C—H)
 **D-Cy5 (6)**	4 mL Methanol + 0.5 mL PBS	4	1.00%	0.00%	δ = 3.29 ppm (d, *J* = 8.8 Hz, 1H) (α-amine C—H)
 **D-Cy7 (8)**	4 mL Methanol + 0.5 mL PBS	4	1.67%	0.94%	δ = 3.28 ppm (d, *J* = 9.2 Hz, 1H) (α-amine C—H)
 **DDR (10)**	2 mL Methanol + 2 mL PBS	4	6.67%	1.25%	δ = 5.76 ppm (s, 1H) (ε-amine C—H)

S-7 Cell culture with DHE and DDE (Figure 2):

Rat aortic smooth muscle cells (RASMs) were harvested from rat thoracic aortas as described previously[3]. Cells were cultured in Dulbeco's modified eagle's medium (DMEM) supplemented with 10% heat-inactivated calf serum, 2 mM L-glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin (Invitrogen, USA). Passage 9 cells were seeded onto glass-bottomed 35 mm dishes and cultured for 24 h in serum free DMEM. Dishes of cells were treated with either 100 nM Angiotensin II or PBS for 4 h. Cells were then washed and the DMEM was replaced with phosphate buffered saline (PBS). The cells were brought to a final concentration of 10 μM DHE or 10 μM DDE, by adding a stock solution of DDE or DHE in dimethyl sulfoxide (DMSO). The cells and was incubated for 30 min and then imaged using a confocal microscope (Zeiss LSM 510 META laser scanning confocal microscope) at a fixed exposure time. A Z-stack image of the cells was taken, ten images of slices of equal thickness were taken and the intensities were integrated using Zeiss Zen software. The mean fluorescence intensity of the images containing equal numbers of cells was quantified using Image Pro software. One way ANOVA analysis was used to measure statistical significance of the results, taking p values of <0.05 as statistically significant.

S-8 *In Vivo* imaging of ROS with H-Cy7 (7) and D-Cy7 (8) (Figure 3):

Sixteen BALB/c mice were divided into two groups (8 per group), one group was given an intraperitoneal (I.P.) injection of LPS (1 mg in 400 μL saline) and the second group was given saline (400 μL). Four hours later, animals were anesthetized with isoflurane and their abdominal fur was removed using hair removal cream. Four mice from the LPS and saline

3) A. M. Zafari, M. Ushio-Fukai, M. Akers, Q. Yin, A. Shah, D. G. Harrison, W. R. Taylor, K. K. Griendling, *Hypertension* **1998**, *32*, 488-490.

treated groups were injected I.P. with H-Cy7 (25 nanomoles in 50 μL methanol), and similarly four mice from the LPS and saline treated groups were injected I.P. with D-Cy7 (25 nanomoles in 50 μL methanol). The mice were imaged at the same time after H-Cy7 or D-Cy7 injection (5-8 min after the injection), using an IVIS® Lumina Imaging System (Xenogen Corporation). Fluorescent images were taken with an excitation wavelength of 710 nm and an emission wavelength of 760 nm. The mean fluorescence intensity from the I.P. cavity of each mouse was integrated with Living Image® 3.0 software, (Caliper Life Sciences, Inc.) and compared using analysis of variance (ANOVA) with Student-Newman-Keuls post-hoc corrections. Values are presented as means ± standard error.

S-9 Mechanistic Rational:

The high KIE values for aerial oxidation of the radical oxidant probes (**1-10**) suggest that they oxidize via a mechanism involving an exciplex intermediate (**16**). In contrast, the low KIE values observed for the oxidation of **1-10** with superoxide suggest that this oxidation occurs via either a ET-PT-ET or HAT-ET pathway (see Figure S-6).

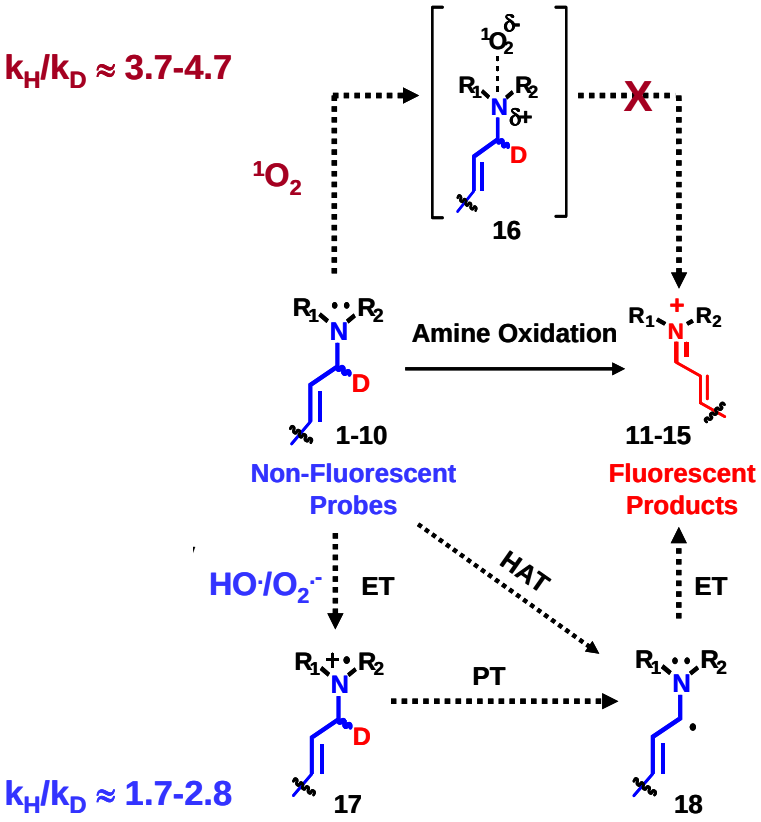


Figure S-6: The oxidation of radical oxidant probes by air/light (singlet oxygen) and superoxide occur by different mechanistic pathways. Radical oxidant probes (1-10) have high KIEs for aerial oxidation suggesting a pathway involving the exciplex (**16**). In contrast, 1-10 have low KIEs for oxidation with radicals, suggesting a ET-PT-ET or HAT-ET pathway.