Materials and Methods

Cyanocobalamin was purchased from Lalilab Inc. Alexa Fluor 700 carboxylic acid succinimidyl ester was purchased from Life Technologies. All other chemical reagents were purchased from Sigma Aldrich. 8x10 Color Film Gels were manufactured by MacNan and supplied by Amazon. 525 nm and 660 nm LEDs were purchased from LED Supply. 725 nm and 780 nm LEDs were purchased from Techmark. Erythrocytes were purchased from Allcells. HRMS data was acquired on a Thermo Scientific electrospray linear trap quadrupole fourier transform mass spectrometer (LT-QFT).

C_{18}-Cbl (1, Scheme S2). Cyanocobalamin (CN-Cbl, 200 mg, 148 µmol) was dissolved in 10 mL anhydrous dimethylsulfoxide (DMSO) and carbonylditriazole (CDT, 121 mg, 740 µmol) was added. The solution was rapidly stirred for 45 min. To this solution octadecylamine (ODA, 398 mg, 1.48 mmol) was added. The resulting mixture was stirred for 1 h before being added to 90 mL ether/chloroform. The resulting precipitate was collected by centrifugation and decantation. The pellet was dried under vacuum and 10 mL EtOH was added. A CDT-mediated ODA dimer formed as a white precipitate, and was removed by centrifugation. The Cbl product was precipitated in 40 mL ether/chloroform (1:1) and collected by centrifugation and decantation. The pellet was dissolved in EtOH and purified on a 100 g Biotage KP-C18-HS flash column with a linear gradient of H_2O:MeOH from 100% H_2O to 100% MeOH in 8 column volumes. **1** eluted at 100% MeOH and, upon removal of MeOH furnished a red solid in 75% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 7.50 - 7.75 (m, 4 H), 7.35 (br. s., 1 H), 7.27 (br. s., 1 H), 7.17 (d, *J*=17.6 Hz, 2 H), 7.04 (d, *J*=10.6 Hz, 2 H), 6.77 (br. s., 1 H), 6.71 (br. s., 1 H), 6.51 (br. s., 1 H), 6.46 (s, 1 H), 6.24 (br. s., 2 H), 5.92 (s, 1 H), 4.62 - 4.80 (m, 2 H), 4.33 - 4.42 (m, 1 H), 4.18 - 4.30 (m, 1 H), 3.90 - 4.15 (m, 4 H), 3.67 - 3.76 (m, 2 H), 3.08 (d, *J*=11.0 Hz, 1 H), 2.95 (d, *J*=6.3 Hz, 2 H), 2.72 - 2.86 (m, 2 H), 2.54 (s, 2 H), 2.48 (br. s., 2 H), 2.42 (s, 2 H), 2.25 (d, *J*=5.5 Hz, 2 H), 2.17 (br. s., 4 H), 2.07 (br. s., 3 H), 1.58 - 1.84 (m, 6 H), 1.46 - 1.57 (m, 2 H), 1.38 (d, *J*=5.9 Hz, 2 H), 1.33 (br. s., 2 H), 1.20 - 1.29 (m, 27 H), 1.18 (br. s., 1 H), 1.15 (d, *J*=5.9 Hz, 2 H), 1.06 (s, 2 H), 0.85 (t, *J*=7.3 Hz, 3 H), 0.33 ppm (br. s., 2 H). ESI MS calcd. for $C_{82}H_{125}CoN_{15}O_{15}P + H^+$ (M^{1+}): m/z = 1650.8627, found 1650.7273; $C_{82}H_{125}CoN_{15}O_{15}P + 2H^+$ (M^{2+}): m/z = 825.9353, found 825.9091.

C_{18}-Cbl-propylamine (2a). 1 (100 mg, 61 µmol) was dissolved in 10 mL of EtOH under N_2. NH_4Br (500 mg, 5% w/v) and Zn powder (200 mg, 3 mmol) were added and the solution stirred for 20 min. To this slurry, 3-chloropropylamine hydrochloride (40 mg, 305 µmol) was added. The resulting mixture was stirred for 3 h under continuous N_2 flow. A color change from red to orange was observed. Zn was removed by centrifugation, and the Cbl product recrystallized twice in 50 mL ether/chloroform (1:1). The resulting precipitate was collected by centrifugation and decantation. The pellet was

dried under vacuum and 10 mL EtOH was added. UV-Vis analysis revealed that alkylation had gone to completion. **2a** was purified on a 100 g Biotage KP-C18-HS flash column with a linear H_2O:MeOH (0.1% TFA) gradient from 100% H_2O – 100% MeOH 8 column volumes. **2a** eluted at 100% MeOH and, upon removal of MeOH furnished an orange solid in quantitative yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ= 7.76 (s, 1H), 7.67 (s, 1H), 7.55 - 7.63 (m, 2 H), 7.51 (s, 1H), 7.37 – 7.44 (m, 3 H), 7.34 (s, 1H), 7.29 (s, 1H), 7.10 – 7.19 (m, 3 H), 7.00 (s, 1H), 6.93 (s, 1H), 6.88 (s, 1H), 6.77 (s, 1H), 6.48 - 6.65 (br. m, 2 H), 6.25 (s, 1H), 6.12 (s, 1H), 4.71 (q, 2H, *J* = 7.04 Hz), 4.36 (d, *J* = 8.61 Hz, 2H), 4.15 – 4.33 (br. m, 7H), 3.97 – 4.09 (m, 4H), 3.71 (t, *J* = 6.62 Hz, 2H), 3.51 (br. s, 2H), 3.12 (d, *J* = 8.61 Hz, 1H), 2.92 - 3.07 (br. m, 3H), 2.75 (br. m, 1H), 2.44 – 2.48 (br. m, 2H), 2.42 (s, 3H), 2.37 (s, 3H), 2.15 – 2.24 (m, 7H), 1.98 – 2.08 (m, 2H), 1.69 – 1.95 (br. m, 8H), 1.64 (s, 3H), 1.37 (br. s, 3H), 1.19 – 1.29 (m, 28 H), 1.15 (d, *J* = 6.26 Hz, 3H), 1.06 (s, 2H), 0.85 (t, *J* = 6.65 Hz, 3H), 0.47 (br. m, 3H), 0.32 (br. m, 2H), 0.07 (br. m, 2H),-0.40 (br. m, 2H). ESI MS calcd. for $C_{84}H_{133}CoN_{15}O_{15}P + H^+$ (M^{1+}): m/z = 1682.9253, found 1682.9276; $C_{84}H_{133}CoN_{15}O_{15}P + 2H^+$ (M^{2+}): m/z = 841.9666, found 841.9669.

C_{18}-Cbl-butyrate (**2b**). **1** (100 mg, 61 µmol) was dissolved in 10 mL of EtOH under N_2. NH_4Br (500 mg, 5% w/v) and Zn powder (200 mg, 3 mmol) were added and the solution stirred for 20 min under N_2. To this slurry, 4-chlorobutryic acid (30 µL, 305 µmol, mw = 122, d = 1.24) was added. The resulting mixture was stirred for 3 h under continuous N_2 flow. A color change from red to orange was observed. Zn was removed by centrifugation, and the Cbl was recrystallized twice in ether/chloroform (50 mL). The resulting precipitate was collected by centrifugation and decantation. The pellet was dried under vacuum and 10 mL EtOH was added. **2b** was purified on a 100 g Biotage KP-C18-HS flash column with a linear gradient from 100% H_2O – 100% MeOH 8 column volumes. **2b** eluted at 100% MeOH and, upon removal of MeOH furnished an orange solid in 82% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 7.58 - 7.73 (m, 3 H), 7.47 - 7.57 (m, 2 H), 7.31 - 7.40 (m, 2 H), 7.13 - 7.26 (m, 3 H), 7.04 - 7.12 (m, 2 H), 6.92 - 7.00 (m, 2 H), 6.74 - 6.88 (m, 3 H), 6.59 - 6.69 (m, 2 H), 6.04 - 6.43 (m, 5 H), 4.50 - 4.75 (m, 3 H), 4.32 - 4.46 (m, 2 H), 4.14 - 4.33 (m, 4 H), 3.90 - 4.09 (m, 3 H), 3.72 - 3.87 (m, 2 H), 2.91 - 3.01 (m, 3 H), 2.08 - 2.46 (m, 16 H), 1.75 - 2.00 (m, 8 H), 1.60 - 1.73 (m, 5 H), 1.32 - 1.52 (m, 7 H), 1.15 - 1.32 (m, 26 H), 1.02 - 1.14 (m, 4 H), 0.93 - 1.02 (m, 2 H), 0.85 (t, *J*=7.1 Hz, 1 H), 0.50 - 0.76 (m, 4 H), 0.30 - 0.36 (m, 1 H), 0.01 - 0.24 (m, 3 H), -0.66 - -0.40 ppm (m, 3 H). ESI MS calcd. for $C_{85}H_{132}CoN_{14}O_{17}P + H^+$ (M^{1+}): m/z = 1711.9042, found 1711.9075; $C_{85}H_{132}CoN_{14}O_{17}P + 2H^+$ (M^{2+}): m/z = 856.4560, found 856.4571.

C_{18}-Cbl-MTX (Scheme S3). MTX (30 mg, 66 µmol), *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 25 mg, 66 µmol), and *N,N*-diisopropylethylamine (DIPEA, 58 µL, 332 µmol) were dissolved in 5 mL of dimethylformamide (DMF) and stirred for 5 min. **2a** (120 mg, 71 µmol) was added and the solution was stirred overnight. We were unable to separate **2a** and **C_{18}-Cbl-MTX** via

HPLC and thus the mixture was exposed to 4,5,6,7,7-hexachloro-5-norbornene-2,3-dicarboxylic anhydride (37 mg, 185 µmol). The solution was stirred for 30 min and then frozen at -80 °C being careful not to thaw until just before purification. **C_{18}-Cbl-MTX** was purified on a 5 µm, 250 x 21.2 mm Viva C4 preparative column from Restek, using a H_2O:CH_3CN, 0.1% TFA gradient (elution time 46 min, Table S1). Orange solid, 37% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 8.71 (s, 1 H), 8.19 - 8.26 (m, 1 H), 7.82 (d, *J*=12.9 Hz, 2 H), 7.61 - 7.76 (m, 4 H), 7.58 (d, *J*=5.1 Hz, 3 H), 7.36 (d, *J*=9.8 Hz, 3 H), 7.05 - 7.21 (m, 3 H), 6.92 (br. s., 2 H), 6.73 - 6.87 (m, 4 H), 6.61 (br. s., 2 H), 6.28 (d, *J*=16.4 Hz, 4 H), 4.87 (br. s., 2 H), 4.71 (d, *J*=4.7 Hz, 2 H), 4.21 - 4.41 (m, 8 H), 4.04 (d, *J*=9.0 Hz, 3 H), 3.72 (br. s., 2 H), 3.30 - 3.42 (m, 2 H), 2.88 - 3.18 (m, 5 H), 2.78 (br. s., 2 H), 1.38 (br. s., 4 H), 1.17 - 1.31 (m, 27 H), 1.14 (d, *J*=5.9 Hz, 3 H), 0.96 (d, *J*=7.4 Hz, 3 H), 0.85 (t, *J*=6.7 Hz, 4 H), 0.56 (br. s., 2 H), 0.24 (br. s., 2 H), -0.02 (s, 2 H), -0.53 ppm (s, 2 H). ESI MS calcd. for $C_{104}H_{153}CoN_{23}O_{19}P + 2H^+$ (M^{2+}): m/z = 1060.0469, found 1060.0476; $C_{104}H_{153}CoN_{23}O_{19}P + 3H^+$ (M^{3+}): m/z = 707.0339, found 707.0341.

C_{18}-Cbl-COL (Scheme S4). 4 was synthesized as previously reported[1] (Scheme S4). **2b** (63 mg, 37 µmol, mw = 1681), HBTU (10 mg, 26 µmol, mw = 379), and DIPEA (15 µL, 86 µmol) were dissolved in 2 mL of DMF and stirred for 5 min. **4** (10 mg, 28 µmol, mw = 1386) was added to the solution and the mixture stirred overnight. **C_{18}-Cbl-COL** was purified on a 5 µm, 250 x 21.2 mm, Viva C4 preparative column using a H_2O:CH_3CN, 0.1% TFA, gradient (elution time 35 min, Table S3). Orange solid, 43% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 8.23 (d, *J*=7.4 Hz, 1 H), 7.78 (br. s., 1 H), 7.49 - 7.70 (m, 3 H), 7.42 (br. s., 1 H), 7.35 (br. s., 2 H), 7.13 - 7.26 (m, 2 H), 7.02 - 7.13 (m, 3 H), 6.93 - 7.02 (m, 2 H), 6.88 (d, *J*=7.4 Hz, 2 H), 6.77 (s, 2 H), 6.59 (br. s., 1 H), 6.13 - 6.35 (m, 3 H), 4.71 (dd, *J*=11.2, 6.5 Hz, 2 H), 4.23 - 4.41 (m, 6 H), 4.14 (dd, *J*=17.8, 6.8 Hz, 3 H), 4.04 (d, *J*=7.0 Hz, 3 H), 3.88 (d, *J*=4.3 Hz, 3 H), 3.79 (s, 2 H), 3.61 (br. s., 1 H), 3.46 - 3.53 (m, 7 H), 3.30 - 3.43 (m, 2 H), 3.14 - 3.21 (m, 1 H), 3.00 - 3.13 (m, 2 H), 2.96 (d, *J*=5.9 Hz, 2 H), 2.75 (s, 1 H), 2.31 - 2.41 (m, 5 H), 2.20 - 2.29 (m, 6 H), 2.07 (s, 1 H), 1.73 - 1.95 (m, 6 H), 1.59 - 1.71 (m, 5 H), 1.32 - 1.46 (m, 4 H), 1.17 - 1.31 (m, 25 H), 1.05 - 1.16 (m, 6 H), 0.81 - 0.90 (m, 3 H), 0.42 - 0.63 (m, 4 H), 0.19 (br. s., 2 H), -0.34 ppm (s, 2 H). ESI MS calcd. for $C_{105}H_{153}CoN_{15}O_{21}P + 2H^+$ (M^{2+}): m/z = 1026.0295, found 1026.0299.

C_{18}-Cbl-DEX (Scheme S5). 5 was synthesized as previously reported[2]. **5** (6 mg, 12 µmol), HBTU (5 mg, 12 µmol), and DIPEA (10 µL, 57 µmol) were dissolved in 1 mL of DMF and stirred for 5 min. **2a** (30 mg, 18 µmol) was added and the solution stirred overnight. **C_{18}-Cbl-DEX** was purified on a 5 µm 250 x 21.2 mm a Viva C4 preparative column using a H_2O:CH_3CN, 0.1% TFA gradient (elution time 62 min, Table S1). Orange solid, 70% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 8.09 - 8.20 (m, 2 H), 8.02 - 8.07 (m, *J*=5.1 Hz, 1 H), 7.92 (s, 1 H), 7.78 (s, 2 H), 7.60 - 7.69 (m, 2 H), 7.47 - 7.59 (m, 3 H), 7.21 - 7.37 (m, 4 H), 7.09 - 7.20 (m, 3 H), 6.94 (br. s., 2 H), 6.77 (br. s., 3 H), 6.58 (br. s., 1 H), 6.22 (dd, *J*=10.2, 2.0 Hz, 2 H), 6.08 - 6.16 (m, 2 H), 6.01 (s, 1 H), 5.38 (d, *J*=4.3 Hz, 1 H), 5.14 (s, 1 H), 5.04 (br. s., 1 H), 4.99 (s, 1 H), 4.76 (br. s., 1 H), 4.66 -

4.74 (m, 2 H), 4.20 - 4.34 (m, 4 H), 4.07 - 4.19 (m, 3 H), 4.03 - 4.07 (m, 1 H), 3.96 - 4.02 (m, 2 H), 3.80 - 3.89 (m, 2 H), 3.64 - 3.71 (m, 2 H), 3.54 - 3.60 (m, 3 H), 3.05 - 3.21 (m, 6 H), 2.92 - 3.04 (m, 4 H), 2.40 - 2.47 (m, 5 H), 2.38 (br. s., 2 H), 2.16 - 2.24 (m, 5 H), 1.65 - 1.84 (m, 7 H), 1.48 (s, 5 H), 1.38 (br. s., 4 H), 1.23 (s, 27 H), 1.15 (d, *J*=6.3 Hz, 2 H), 0.97 - 1.11 (m, 4 H), 0.85 (t, *J*=7.3 Hz, 3 H), 0.78 (d, *J*=7.4 Hz, 2 H), 0.46 (br. s., 3 H), 0.27 - 0.38 (m, 2 H), -0.02 - 0.12 (m, 3 H), -0.50 - -0.37 ppm (m, 2 H). ESI MS calcd. for $C_{110}H_{164}CoFN_{15}O_{22}P + 2H^+$ (M^{2+}): m/z = 1079.0693, found 1079.0707.

C_{18}-CbI-TAM (Scheme S6). 5-Tetramethylrhodamine (TAM) (5 mg, 12 µmol), HBTU (4.5 mg, 12 µmol), and DIPEA (8.3 µL, 48 µmol) were dissolved in 5 mL of DMF and stirred for 5 min. **2a** (20 mg, 12 µmol) was added and the solution stirred overnight. **C_{18}-CbI-TAM** was purified on a 5 µm 250 x 21.2 mm a Viva C4 preparative column using a H_2O:CH_3CN, 0.1% TFA gradient (elution time 46 min, Table S1). Red solid, 75% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 8.56 - 8.63 (m, 1 H), 8.42 - 8.47 (m, 1 H), 8.01 - 8.06 (m, 1 H), 7.95 - 8.00 (m, 1 H), 7.74 - 7.81 (m, 1 H), 7.65 - 7.70 (m, 1 H), 7.53 (br. s., 3 H), 7.32 - 7.36 (m, 1 H), 7.24 - 7.29 (m, 1 H), 7.03 - 7.21 (m, 4 H), 6.96 (br. s., 4 H), 6.73 - 6.81 (m, 2 H), 6.54 - 6.60 (m, 1 H), 6.19 - 6.26 (m, 1 H), 6.09 - 6.17 (m, 1 H), 4.66 - 4.74 (m, 1 H), 4.13 - 4.39 (m, 5 H), 3.96 - 4.08 (m, 2 H), 3.51 (s, 4 H), 3.27 (br. s., 5 H), 2.43 (br. s., 2 H), 2.30 - 2.36 (m, 2 H), 2.16 - 2.27 (m, 5 H), 2.05 - 2.09 (m, 2 H), 1.70 (br. s., 3 H), 1.37 (br. s., 3 H), 1.18 - 1.31 (m, 28 H), 1.15 (d, *J*=6.3 Hz, 2 H), 1.03 (br. s., 1 H), 0.85 (t, *J*=6.8 Hz, 3 H), 0.47 (br. s., 3 H), 0.15 - 0.29 (m, 2 H), -0.27 - -0.10 ppm (m, 2 H). ESI MS calcd. for $C_{109}H_{154}CoN_{17}O_{19}P^+ + 1H^+$ (M^{2+}): m/z = 1048.0377, found 1048.0386

C_{18}-CbI-FAM (Scheme S7). 5-carboxyfluorecein (5-FAM, 5 mg, 12 µmol), O-(N-succinimidyl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TSTU, 3.6 mg, 12 µmol), and DIPEA (8.3 µL, 48 µmol) were dissolved in 5 mL of DMF and stirred for 5 min. **2a** (20 mg, 12 µmol) was added and the solution stirred overnight. **C_{18}-CbI-FAM** was purified on a 5 µm 250 x 21.2 mm a Viva C4 preparative column using a H_2O:CH_3CN, 0.1% TFA gradient (elution time 46 min, Table S1). Orange solid, 90% yield. ^{1}H NMR (DMSO-d_6 ,400MHz): δ = 8.53 (br. s., 1 H), 8.18 (s, 1 H), 7.99 (d, *J*=7.8 Hz, 1 H), 7.79 (br. s., 1 H), 7.48 - 7.69 (m, 5 H), 7.28 - 7.46 (m, 5 H), 7.08 - 7.21 (m, 3 H), 6.96 (br. s., 2 H), 6.87 (br. s., 2 H), 6.78 (br. s., 2 H), 6.67 - 6.71 (m, 2 H), 6.50 - 6.63 (m, 5 H), 6.31 (br. s., 3 H), 4.71 (dd, *J*=11.2, 6.5 Hz, 3 H), 4.24 - 4.42 (m, 7 H), 4.00 - 4.16 (m, 4 H), 3.73 (br. s., 2 H), 3.36 (d, *J*=7.0 Hz, 2 H), 3.17 (br. s., 2 H), 3.02 - 3.11 (m, 2 H), 2.91 - 3.01 (m, 3 H), 2.69 - 2.85 (m, 4 H), 2.32 - 2.41 (m, 7 H), 2.21 - 2.29 (m, 6 H), 2.07 (s, 1 H), 1.72 - 1.97 (m, 9 H), 1.69 (br. s., 3 H), 1.33 - 1.44 (m, 6 H), 1.26 (br. s., 5 H), 1.22 (s, 27 H), 1.14 (d, *J*=6.3 Hz, 2 H), 0.94 (s, 2 H), 0.85 (t, *J*=6.7 Hz, 3 H), 0.57 (br. s., 3 H), 0.29 - 0.40 (m, 1 H), 0.13 - 0.28 (m, 2 H), -0.38 - -0.20 ppm (m, 2 H). ESI MS calcd. for $C_{105}H_{143}CoN_{15}O_{21}P + 2H^+$ (M^{2+}): m/z = 1020.9905, found 1021.9900.

C_{18}-AF700. Alexa Fluor 700 NHS ester (1 mg, 1 µmol), DIPEA (5 µL, 29 µmol), and octadecylamine (5 mg, 19 µmol) were dissolved in 500 µL DMF and mixed by agitation

overnight. The resulting mixture was added to 5 mL H_2O:CH_2Cl_2 mixture (5:1). The CH_2Cl_2 layer was washed three times with 4 mL H_2O, the CH_2Cl_2 removed under reduced pressure and the product purified by flash chromatography silica column (30 g) using a binary solvent system (A: CH_2Cl_2, B: MeOH) with an initial A:B ratio of 100:0, gradually increasing to 80:20. The purified lipidated fluorophore was concentrated by rotary evaporation. Note: the structure of AF700 has not been released.

Cy5 and Cy7. Cy5 and **Cy7** were synthesized as previously described.[3]

C_{18}-Cy5 (Scheme S8). Cy5 (6.2 mg, 12.8 µmol) and diisopropylcarbodiimide (DIC, 2.5 mg, 19.2 µmol) were dissolved in CH_2Cl_2 (320 µL) and mixed for 5 min. A solution of ODA (6.9 mg) in chloroform (100 µL) was then added and the solution was allowed to react for 1 h. The solution was diluted with additional CH_2Cl_2 (2 mL) and purified via silica gel elution using a binary solvent system (A: CH_2Cl_2, B: MeOH) with an initial A:B ratio of 90:10, gradually increasing to 1:4. Removal of solvent by rotary evaporation yielded a blue-viscous oil (74%, calculated from absorption, λ_{max}: 646 nm). ^{1}H NMR (CD_2Cl_2, 400 MHz): δ = 7.85 - 7.95 (m, 2 H), 7.37 - 7.46 (m, 4 H), 7.28 (q, *J* = 7.3 Hz, 2 H), 7.11 - 7.18 (m, 2 H), 6.63 (t, *J* = 12.5 Hz, 1 H), 6.12 - 6.25 (m, 2 H), 4.01 (t, *J* = 7.6 Hz, 2 H), 3.57 (s, 3 H), 3.12 - 3.21 (m, 2 H), 2.20 (t, *J* = 7.0 Hz, 2 H), 1.84 (br m, 6 H), 1.70 (s, 12 H), 1.42 - 1.55 (m, 4 H), 1.26 (m, 28 H), 0.88 (t, *J* = 7.5 Hz, 3 H). ESI MS calc. for $C_{50}H_{76}N_3O^+$ (M^{1+}): m/z = 734.5988, found 734.5983.

C_{18}-Cy7 (Scheme S8). Cy 7 (5.0 mg, 9.8 µmol) was coupled to ODA as described for **C_{18}-Cy5**. Silica gel chromatography afforded a blue-green oil (95.2%, calculated from absorption**,** λ_{max}: 760 nm). ^{1}H NMR (CD_2Cl_2, 400 MHz): δ = 7.69 - 7.83 (m, 2 H), 7.35 - 7.45 (m, 4 H), 7.26 (m, 2 H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 7.8 Hz, 1H), 6.45 - 6.63 (m, 2 H), 6.19 (d, *J* = 13.7 Hz, 1 H), 6.08 (br m, 2 H), 3.95 - 4.03 (m, 2 H), 3.52 (s, 2 H), 3.17 (d, *J* = 6.3 Hz, 2 H), 2.19 (t, *J* = 7.2 Hz, 2 H), 1.67 - 1.82 (br m, 21 H), 1.44 - 1.52 (br m, 4 H), 1.22 - 1.34 (m, 26 H), 0.88 (t, *J* = 7.4 Hz, 3 H). ESI MS calc. for $C_{52}H_{78}N_3O^+$ (M^{1+}): m/z =760.6145, found 760.6324.

Cl-DY800 (Scheme S9). N-(5-carboxypentyl)-2,3,3-trimethyl indolium-5-sulfonate (300 mg, 850 µmol) and 3-chloro-2,4-trimethyleneglutacondianil hydrochloride (305 mg, 850 µmol) were refluxed in a solution of acetic acid/acetic anhydride (4 mL, 1:1 ratio) for 2 h. The solvent was then removed under reduced pressure and the residue re-dissolved in a mixture of pyridine and acetic acid (4 mL, 1:1 ratio). N-(3-sulfopropyl)-2,3,3-trimethyl indolium-5-sulfonate (325 mg, 850 µmol) was added and the solution was heated to 80 °C for 1 h. The product was then precipitated and washed 3x with ethyl acetate (100 mL). The blue precipitate was dissolved in water and eluted on a Biotage KP-C18-HS column using a binary solvent A:B solvent system (A: H_2O .1 v/v% TFA, B: CH_3CN .1 v/v% TFA). The A:B ratio was gradually increased from an initial ratio of 19:1 to 2:3. Removal of the solvent by lyophilization yielded a green solid (180 mg, 25% yield, λ_{max}: 794 nm). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.25 (dd, *J* = 16.8 Hz, 14.5 Hz, 2 H), 7.79

(d, *J* = 9.0 Hz, 2 H), 7.64 - 7.69 (m, 2 H), 7.47 (d, *J* = 8.6 Hz, 1 H), 7.36 (d, *J* = 8.2 Hz, 1 H), 6.59 (d, *J* = 14.1 Hz, 1 H), 6.30 (d, *J* = 14.1 Hz, 1 H), 4.39 (br s, 2 H), 4.18 (br s, 2H), 2.67 – 2.79 (m, 4 H), 2.64 (t, *J* = 6.6 Hz, 2 H), 2.20 (t, 7.1 Hz, 2 H), 1.98 – 2.09 (br m, 2 H), 1.79 – 1.89 (br m, 2 H), 1.78 – 1.62 (m, 12 H), 1.61 – 1.48 (m, 3 H), 1.31 – 1.44 (m, 3 H). ESI MS calc. for $C_{39}H_{45}ClN_2O_{11}S_3^{2-}$ + $3H^+$ (M^{1+}): m/z = 851.2109, found 851.2115.

DY800 (Scheme S9). Chloro-DY800 (100 mg, 120 µmol) was dissolved in anhydrous DMF (18 mL) to which a solution of sodium phenoxide (139 mg, 1.2 mmol) in DMF (1mL) was added. The solution was stirred for 1 h, and then poured into a vigorously stirring solution of diethyl ether (250 mL). The precipitate was collected and then purified with a Biotage KP-C18-HS column using a binary solvent A:B solvent system (A: H_2O .1 v/v% TFA, B: CH_3CN .1 v/v% TFA). The A:B ratio was gradually increased from an initial ratio of 19:1 to 2:3. Lyophilization provided the product as a green solid (86 mg, 79% yield, λ_{max}: 774 nm). ^1H NMR (DMSO-d_6 ,400MHz): d = 7.74 - 7.88 (m, 3 H), 7.56 - 7.65 (m, 3 H), 7.38 – 7.46 (m, 3 H), 7.25 (d, *J* = 8.2 Hz, 1 H), 7.16 (d, *J* = 8.2 Hz, 2 H), 7.05 (t, *J* = 7.2 Hz, 1 H), 6.49 (d, *J* = 14.5 Hz, 1 H), 6.13 (d, *J* = 14.1 Hz, 1 H), 4.33 (br s, 2 H), 4.08 (br s, 2 H), 2.68 - 2.80 (m, 4 H), 2.56 (t, *J* = 6.5 Hz, 2 H), 2.19 (t, *J* = 7.2 Hz, 2 H), 1.88 - 2.02 (m, 4 H), 1.61 - 1.73 (m, 3 H), 1.50 - 1.59 (m, 3 H), 1.21 - 1.40 (m, 12 H). ESI MS calc. for $C_{45}H_{50}N_2O_{12}S_3^{2-}$ + $3H^+$ (M^{1+}): m/z = 909.2761, found 909.2764.

C_{18}-DY800 (Scheme S10). DY800 (2.0 mg, 2.2 µmol) and HCTU (1.4 mg, 3.3 µmol) were dissolved in anhydrous DMF (500 µL) and allowed to react for 5 min. A 500 µL solution of ODA in chloroform (1 mg/mL) was added and the solution mixed for 1 h. The product was extracted into aqueous solution by washing of the organic solution with water (2 x 1 mL). The aqueous phase was then purified by HPLC using a stationary C_4 phase with a A:B solvent system (A: H_2O .1 v/v% TFA, B: CH_3CN .1 v/v% TFA). The A:B ratio was gradually increased from 10:1 to 1:19. The solvent was removed by rotary evaporation to give a blue-green oil (35% yield, λ_{max}: 776 nm in ethanol). ^1H NMR (DMSO-d_6 ,400M Hz): δ = 7.72 – 7.87 (m, 3 H), 7.56 - 7.65 (m, 3 H), 7.37 - 7.45 (m, 3 H), 7.19 - 7.26 (m, 1 H), 7.12 - 7.18 (m, 2 H), 7.02 - 7.09 (m, 1 H), 6.50 (d, *J* = 14.2 Hz, 1 H), 6.12 (d, *J* = 14.5 Hz, 1 H), 4.29 - 4.37 (m, 2 H), 4.02 - 4.11 (m, 2 H), 2.98 (dd, *J* = 12.7, 6.8 Hz, 3 H), 2.74 - 2.80 (m, 2 H), 2.65 - 2.74 (m, 2 H), 2.31 - 2.35 (m, 2 H), 2.02 (t, *J* = 7.4 Hz, 3 H), 1.89 - 1.99 (m, 4 H), 1.60 - 1.72 (m, 3 H), 1.47 - 1.58 (m, 3 H), 1.26 - 1.40 (m, 12 H), 1.22 (s, 30 H), 0.85 ppm (t, *J* = 6.8 Hz, 3 H). ESI MS calc. for $C_{63}H_{87}N_3O_{11}S_3^{2-}$ + $3H^+$ (M^{1+}): m/z = 1160.5737, found 1160.5738.

MTX LC-MS Assay. The following experiments were analyzed using the MTX LC-MS Assay: Octanol/Water Partition MTX, RBC Drug Loading Capacity, and MTX Photolysis from Erythrocyte Membranes. Samples of 75 µL were injected onto an 1200 series Agilent HPLC with a UV-Vis detector, 1260 infinity fluorescent detector, and 6110 quadrapole mass spectrometer from a 384 well plate. The mobile phase consisted of

H_2O:CH_3CN 0.1% FA (Formic Acid) (gradient provided in Table S6). The column used was a Viva C4 analytical column 5 µm, 50 x 21.2 mm from Restek. Concentrations were determined by taking the area under UV absorbance trace at 300 nm from 3.1 - 3.7 min where the **C_{18}-Cbl-MTX** cleavage products were shown to elute and this integration was compared to known standards (**Figure S10b**). Spectrofluorimetry (λ_{ex}: 365 nm λ_{em}: 470 nm) was used to detect the possible presence of MTX by-products due to light degradation[4] (none were observed), undamaged MTX is non-fluorescent.

COL LC-MS Assay. The following experiments were analyzed using the COL LC-MS Assay: RBC Drug Loading Capacity Samples and Photo-release of COL and DEX from C_{18}-Cbl-COL- and C_{18}-Cbl-DEX-embedded Erythrocytes**.** Samples of 75 µL were injected onto an 1200 series Agilent HPLC with a UV-Vis detector, 1260 infinity fluorescent detector, and 6110 quadrapole mass spectrometer from a 384 well plate. The mobile phase consisted of H_2O:CH_3CN (0.1% FA) (gradient provided in Table S6). The column used was a Viva C4 analytical column 5 µm, 50 x 21.2 mm. Concentrations were determined by taking the area under UV absorbance trace at 365 nm from 4.3 - 4.8 min where the **C_{18}-Cbl-COL** cleavage products were shown to elute and this integration was compared to known standards (**Figure S10c**). LC gradient is given in the MTX LC-MS Assay.

DEX LC-MS Assay. The following experiments were analyzed using the DEX LC-MS Assay: RBC Drug Loading Capacity Samples and Photo-release of COL and DEX from **C_{18}-Cbl-COL- and C_{18}-Cbl-DEX**-embedded Erythrocytes**.** Samples of no less than 120 µL were boiled for 10 min to denature and precipitate any protein and lipids present in the erythrocytes samples. Protein was then pelleted by centrifugation at 21,000 g. Samples of 75 µL were injected onto an 1200 series Agilent HPLC with a UV-Vis detector, 1260 infinity fluorescent detector, and 6110 quadrapole mass spectrometer from a 384 well plate. The mobile phase consisted of H_2O:CH_3CN (0.1% FA) (gradient provided in Table S6). The column used was a Viva C4 analytical column 5 µm, 50 x 21.2 mm. Concentrations were determined by taking the area under UV absorbance trace at 239 nm from 4.5 - 4.9 min (if erythrocytes were present in the experiment) where DEX was shown to elute. If no RBCs were present, integration occurred from 5.3 - 5.7 min. Integrations were compared to known standards of DEX (**Figure S10d**).

Octanol/Water Partition MTX. Octanol (100 µL) containing **C_{18}-Cbl-MTX** (5 µM) was thoroughly mixed with phosphate buffered saline (PBS, 500 µL) in a 1.5 mL clear centrifuge tube and allowed to equilibrate for 30 min in the dark before undergoing centrifugation for 10 min at 21,000 g. Samples were photolyzed with a 525 nm LED for 0 min, 1 min, 2 min, 5 min, 10 min, and 20 min before being mixed by shaking and allowed to equilibrate for 15 min. This was followed by a 10 min centrifugation at 21,000 g. The octanol layer was removed and 100 µL aliquots were taken from the remaining aqueous layer and loaded onto a 384 well plate. The concentration of MTX was determined by the MTX LC-MS assay.

Octanol/Water Partition TAM. Octanol (100 µL) containing the **C_{18}-Cbl-TAM** (10 µM) was thoroughly mixed with PBS (300 µL) in a 1.5 mL clear centrifuge tube and allowed to equilibrate for 30 min in the dark before undergoing centrifugation for 10 min at 21,000 g in a quartz fluorescence cuvette with a 200 µL viewing window. The fluorescence of the aqueous layer was measured using a PTI Model 710 LPS-220 (λ_{ex}: 555 nm λ_{em}: 570 - 600 nm). The solution was transferred to a clear 1.5 mL centrifuge tube and photolyzed using a 525 nm centered LED array for 0 min, 1 min, 2 min, 3 min, and 10 min. The solutions were transferred back to the cuvette for 10 min centrifugation at 21,000 g and the fluorescence was measured again. All samples were measured in triplicate.

Erythrocyte Storage. Erythrocytes were washed 3x with RBC buffer (PBS, 1 mM $MgCl_2$) and diluted to 10% hematocrit. Erythrocytes were stored for no more than one month at 4 °C.

Erythrocyte Loading Procedure. Lipidated compounds were added at various loading concentrations to erythrocytes (10% hematocrit). The erythrocytes were then incubated at RT for 20 min and subsequently washed 3x and stored at 10% hematocrit in RBC buffer. RBCs were allowed to sit for up to 48 h before use.

Widefield Microscopy with Cbl-TAM Loaded Erythrocytes. 10% hematocrit erythrocytes were loaded with 10 µM **C_{18}-Cbl-TAM** using the Erythrocyte Loading Procedure. 2 µL of loaded erythrocytes were then added to 200 µL RBC buffer and imaged using widefield microscopy. Images were taken using 20 µs exposure times and a Cy3 filter cube 333 ms apart.

Hemolysis Assay. To a 1.5 mL Eppendorf tube containing 100 µL of a Cbl drug conjugate (**C_{18}-Cbl-MTX**, **C_{18}-Cbl-COL**, or **C_{18}-Cbl-DEX**) in PBS at various concentrations (5 µM, 10 µM, 20 µM, and 40 µM) was added 100 µL RBCs in RBC buffer (10% hematocrit). Two controls were performed: 100 µL of RBCs treated with PBS buffer and RBCs treated with SDS (100% hemolysis). Final concentrations were 0.05% SDS; 0 µM, 2.5 µM, 5 µM, 10 µM, and 20 µM lipidated complex. Cells were mixed by flicking before centrifugation at 300 g for 30 min. Samples were re-homogenized and allowed to incubate at 4 °C overnight. Samples were pelleted at 1000 g for 5 min. 150 µL of the supernatant was plated in a 96 well plate and analyzed at 550 nm by UV Vis. SDS samples were diluted 10 fold in order to accurately measure heme concentration. SDS absorbance multiplied by 10 was considered to be complete hemolysis and PBS treated blood was considered to be completely intact and the absorbance from those samples was subtracted from the background of the rest.

$$\%\ Hemolysis = \frac{sample\ absorbance}{100\%\ Abs\ of\ Lysate\ *\ 10}\ *100\%$$

RBC Drug Loading Capacity. 10% hematocrit RBCs were loaded with varying concentrations of **C_{18}-Cbl-MTX**, **C_{18}-Cbl-COL**, and **C_{18}-Cbl-DEX** using the **Erythrocyte Loading Procedure**. **C_{18}-Cbl-MTX** was loaded at 6 μM, 4 μM, 2 μM, and 1 μM. **C_{18}-Cbl-COL** was loaded at 10 μM, 8 μM, 6 μM, 4 μM, and 2 μM. The cells were diluted to 5% hematocrit and photolyzed for 2 h using a board containing 525 nm centered LEDs. The cells were then spun down at 1000 g. Concentration was determined by the respective LC-MS assays.

Dihydrofolate Reductase (DHFR) Inhibition Assay. DHFR activity was monitored using the Sigma Dihydrofolate Reductase Assay Kit. This kit was used to monitor conversion of NADPH to $NADP^+$. Briefly, assay buffer was prepared containing 1.5 mU DHFR, 100 μM NADPH, and 1x assay buffer (provided with kit). Inhibition of DHFR activity at various MTX concentrations or various photolyzed **C_{18}-Cbl-MTX** concentrations (100 nM - 5 μM) was monitored using a fluorescent plate reader (λ_{ex}: 340 nm λ_{em}: 450 nm).

Treatment of HeLa Cells with COL. HeLa cells were plated in a 6-well glass bottom plate (Mattek) at a density of 1.5 x 10^5 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were treated with COL (Sigma C9754; 1 mM stock in DMSO) or DMSO for either 30 min or 1 h at 37 ºC in a humidity-controlled incubator. At the conclusion of the incubation period, cells were fixed with 1 mL of methanol at room temperature for 10 min. Cells were washed 2 x 1 mL with PBS and blocked for 1 h in 5% donkey serum. Blocking was followed by overnight incubation at 4 ºC with mouse anti-tubulin antibody (Cell Signaling 3873S) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Cells were then washed with PBS (3 x 5 min) before incubation with anti-mouse AlexaFluor 488 secondary antibody (Life Technologies A21202) at 1:500 dilution in antibody dilution buffer. After washing cells with PBS (3 x 5 min), images were acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 40X phase contrast objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

Treatment of HeLa Cells with C_{18}-Cbl-COL-Loaded RBCs. HeLa cells were plated in 24-well glass bottom plates (Mattek) at a density of 3.3 x 10^4 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were washed 2x with PBS, followed by the addition of 100 μL of L-15 media. Cells were then treated with 250 μL of a suspension of **C_{18}-Cbl-COL** loaded erythrocytes in PBS (6 μM loading concentration at 5% hematocrit) or 250 μL PBS (control cells). Cells were then either kept in the dark at 37 ºC in a humidity-controlled incubator, or exposed to 530 nm LED flood light (PAR38; 500 – 570 nm emission; 5 mW power) for 5, 10, or 20 min at room temperature. All cells incubated for 1 h in a 37 ºC in a humidity-controlled incubator post-photolysis. At the conclusion of the incubation period, cells were washed 3 x 1 mL

with PBS and then fixed with 1 mL of methanol at room temperature for 10 min. Cells were washed 2 x 1 mL with PBS and blocked for 1 h in 5% Donkey Serum. Blocking was followed by overnight incubation at 4 ºC with mouse anti-tubulin antibody (Cell Signaling 3873S) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Cells were then washed with PBS (3 x 5 min) before incubation with anti-mouse AlexaFluor 488 secondary antibody (Life Technologies A21202) at 1:500 dilution in antibody dilution buffer. After washing cells with PBS (3 x 5 min), images were acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 40X phase contrast objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

Treatment of HeLa Cells with DEX. HeLa cells were plated in a 6-well glass bottom plate (Mattek) at a density of 7.5 x 10^4 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were treated with varying concentrations of DEX (1 mM stock in DMSO) or DMSO for 1 h at 37 ºC in a humidity-controlled incubator. At the conclusion of the incubation period, cells were fixed with 4% PFA in PBS for 10 min at room temperature, then washed 1x with PBS, and then treated with 1 mL of methanol at room temperature for 5 min. Cells were washed 2 x 1 mL with PBS and subsequently incubated overnight at 4 ºC with rabbit anti-GRα antibody (abcam 3580) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Cells were then washed with PBS (3 x 5 min) before incubation with anti-rabbit AlexaFluor 488 secondary antibody (Life Technologies A21206) at 1:500 dilution in antibody dilution buffer for 1 h at room temperature. Cells were washed with PBS (3 x 5 min) and Hoescht 33342 (100 µg/mL in PBS) applied for 30 min before an additional wash with PBS. Images were subsequently acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 40X phase contrast objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

Treatment of HeLa Cells with C_{18}-Cbl-DEX Loaded RBCs. HeLa cells were plated in 12-well glass bottom plates (Mattek) at a density of 2.5 x 10^4 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were washed 2x with PBS, then treated with 500 µL of a suspension of **C_{18}-Cbl-DEX** loaded red blood cells in L-15 media (1 µM loading concentration at 5% hematocrit) or 500 µL L-15 (control cells). Cells were then either kept in the dark at 37 ºC in a humidity-controlled incubator, or exposed to 530 nm LED flood light (PAR38; 500 – 570 nm emission; 5 mW power) for 10, 20, or 30 min at room temperature. All cells incubated for 1 h in a 37 ºC in a humidity-controlled incubator post-photolysis. At the conclusion of the incubation period, cells were washed 3 x 1 mL with PBS and then fixed with 4% PFA in PBS for 10 min at room temperature, then washed 1x with PBS and treated with 1 mL of methanol at room temperature for 5 min. Cells were subsequently washed 2 x 1 mL with PBS and then incubated overnight at 4 ºC with rabbit anti-GRα antibody (abcam 3580) at 1:100

dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Next, cells were washed with PBS (3 x 5 min) before incubation with anti-rabbit AlexaFluor 488 secondary antibody (Life Technologies A21206) at 1:500 dilution in antibody dilution buffer for 1 h at room temperature. Cells were finally washed with PBS (3 x 5 min). Images were subsequently acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 40X phase contrast objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

Photolytic Release of TAM and FAM from C_{18}-Cbl-TAM- and C_{18}-Cbl-FAM-Embedded Erythrocytes. Erythrocytes were taken from 10% hematocrit stock solutions. RBCs were loaded with 1 μM **C_{18}-Cbl-TAM** or **C_{18}-Cbl-FAM** as described in the Erythrocyte Loading Procedure. For those experiments requiring lipidated fluorophores, 5 μM were also loaded from stock solutions in DMSO. Erythrocytes were resuspended to 10% hematocrit and exposed to LEDs centered at varying wavelengths for set time points. After photolysis, the erythrocyte solution was centrifuged at 1,000 g and the supernatant was analyzed for TAM (λ_{ex}: 550 nm λ_{em}: 580 nm) or FAM (λ_{ex}: 492 nm λ_{em}: 519 nm) release using a fluorescent plate reader.

Cbl-DEX Light Independent Translocation Test. HeLa cells were plated in 6 well glass bottom plates (Mattek) at a density of 8.8 x 10^4 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were washed 2x with PBS, then treated with 250 μL of a suspension of **C_{18}-Cbl-DEX** loaded erythrocytes in L-15 media (1 μM loading concentration at 5% hematocrit) or 250 μL L-15 (control cells). HeLa cells were then incubated in the dark for 1 h at 37 ºC in a humidity-controlled incubator. After the 1 h pre-incubation, HeLa cells were washed 3 x 1 mL with PBS (dark room; red safe light) to remove the erythrocytes and 2 mL of L-15 added to each well. The washed HeLa cells were then exposed to a green LED light source (PAR38; 500 – 570 nm emission; 5 mW power) or kept in the dark for 15 min at room temperature. All cells were incubated for 1 h in a 37 ºC in a humidity-controlled incubator post-photolysis. At the conclusion of the second incubation period, cells were washed 3 x 1 mL with PBS and then fixed with 4% PFA in PBS for 10 min at room temperature, then washed 1x with PBS and treated with 1 mL of methanol at room temperature for 5 min. Cells were subsequently washed 2 x 1 mL with PBS and then incubated overnight at 4 ºC with rabbit anti-GRα antibody (abcam 3580) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Next, cells were washed with PBS (3 x 5 min) before incubation with anti-rabbit AlexaFluor 488 secondary antibody (Life Technologies A21206) at 1:500 dilution in antibody dilution buffer for 1 h at room temperature. Cells were finally washed with PBS (3 x 5 min). Images were subsequently acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 40X phase contrast objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

Assessment of the C_{18}-Cbl-TAM:C_{18}-Cy5 Ratio for Optimal TAM Release. To 10% hematocrit erythrocytes, various concentrations of **C_{18}-Cbl-TAM** (0 to 10 μM) and various concentrations of **C_{18}-C_{18}-Cy5** were added (0 to 20 μM). Erythrocytes were photolyzed using the 660 nm LED board for 30 min. After photolysis, erythrocytes were spun down at 1,000 g for 3 min and the supernatant was analyzed for TAM (λ_{ex}: 550 nm λ_{em}: 580 nm) release using a fluorescent plate reader.

$$photon\ flux\ \left(\frac{\mu mol\ photons}{m^2 s}\right) = \frac{\frac{P}{m^2}}{hv * 6.022 * 10^{17}}$$

Light Power Measurements. All light measurements were recorded with a Coherent Field Max II detector. Each measurement is reported as the average of 13 readings and the error is reported as the standard deviation. The light was measured in a fashion consistent with photolytic release of drugs erythrocytes.

Confocal Images of Erythrocytes Loaded with C_{18}-Cy5. C_{18}-Cy5 was loaded onto erythrocytes (10% hematocrit) at a concentration of 5 μM using the Erythrocyte Loading Procedure. 2 μL of loaded erythrocytes were then added to 200 μL RBC buffer and imaged using confocal microscopy. Images were taken using 4% laser power, and 10 μs/pixel in line scan mode with a 635 nm laser.

MTX Photolysis from Erythrocyte Membranes. C_{18}-Cbl-MTX and either **C_{18}-Cy5, C_{18}-AF700, C_{18}-Cy7, C_{18}-DY800** were loaded onto erythrocytes (Erythrocyte Loading Procedure) using a concentration of 1 μM (**C_{18}-Cbl-MTX**) and 5 μM (**C_{18}-fluorophore**), respectively. After loading, erythrocytes were resuspended to 5% hematocrit and exposed to 660 nm, 725 nm, or 780 nm centered LEDs for 30 min. Other loaded erythrocytes were photolyzed for 2 h using 525 nm LEDs to free all bound MTX to assess the total amount of drug loaded. After photolysis, the erythrocyte solution was centrifuged at 1,000 g and the supernatant analyzed for MTX using the MTX LC-MS assay.

Photo-release of COL and DEX from C_{18}-Cbl-COL- and **C_{18}-Cbl-DEX-loaded Erythrocytes. C_{18}-Cbl-COL** (5 μM) or **C_{18}-Cbl-DEX** (0.5 μM) were loaded with either **C_{18}-DY800** or **C_{18}-Cy5** fluorophore according to the Erythrocyte Loading Procedure. **C_{18}-DY800** was added to a final concentration of 5 and 2.5 μM for **C_{18}-Cbl-COL** and **C_{18}-Cbl-DEX** erythrocytes, respectively. **C_{18}-Cy5** was added to a final concentration of 25 and 2.5 μM for **C_{18}-Cbl-COL** and **C_{18}-Cbl-DEX** erythrocytes, respectively. Erythrocytes were resuspended to 5% hematocrit. The **C_{18}-DY800** samples were exposed to 525 nm for 2 h or 780 nm for 0 and 30 min and the **C_{18}-Cy5**-containing samples were exposed to 525 nm and 660 nm in similar fashion. After photolysis, the erythrocyte solution was centrifuged at 1,000 g and the supernatant analyzed for COL or DEX release by LC/MS. Baseline was determined by analyzing erythrocyte samples lacking the Cbl bound drugs.

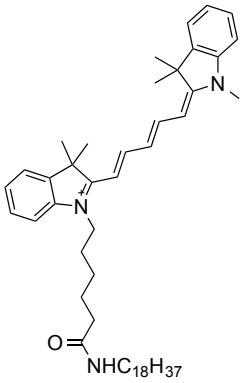
Treatment of HeLa Cells with C_{18}-Cbl-DEX/C_{18}-Cy5-loaded Erythrocytes. HeLa cells were plated in 35 mm glass bottom dishes (Mattek) at a density of 1.0 x 10^5 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, the HeLa cells were washed 3x with PBS, then treated with 200 µL of a suspension of **C_{18}-Cbl-DEX/C_{18}-Cy5** (0.5 µM/2.5 µM) loaded erythrocytes in L-15 media (5% hematocrit) or 200 µL L-15 (control cells). Samples were either kept in the dark or exposed to a 660 nm LED array for 10, 20, or 30 min using the appropriate filter sets. All samples were placed in a 37 ºC in a humidity-controlled incubator post-photolysis until harvest 1 h after the end of the last photolysis interval. HeLa cells were washed 3 x 1 mL with PBS and then fixed with 4% PFA in PBS for 10 min at room temperature, then washed 1x with PBS and treated with 1 mL of methanol at room temperature for 5 min. Cells were subsequently washed 2 x 1 mL with PBS and then incubated overnight at 4 ºC with rabbit anti-GRα antibody (abcam 3580) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Next, cells were washed with PBS (3 x 5 min) before incubation with anti-rabbit AlexaFluor 488 secondary antibody (Life Technologies A21206) at 1:500 dilution in antibody dilution buffer for 1 h at room temperature. Cells were finally washed with PBS (3 x 5 min). Images were subsequently acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 60X oil objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

Treatment of HeLa Cells with Cbl-COL/C_{18}-Cy5-loaded Erythrocytes. HeLa cells were plated in 35 mm glass bottom dishes (Mattek) at a density of 1.0 x 10^5 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, HeLa cells were washed 3x with PBS, then treated with 200 µL of a suspension of **C_{18}-Cbl-COL/C_{18}-Cy5** (5 µM/25 µM) loaded erythrocytes in L-15 (5% hematocrit) or 200 µL L-15 (control cells). Samples were then either kept in the dark at 37 ºC in a humidity-controlled incubator, or exposed to a 660 nm LED array for 10, 20, or 30 min using the appropriate filter sets. All samples were incubated for 1 h in a 37 ºC in a humidity-controlled incubator post-photolysis. At the conclusion of the incubation period, HeLa cells were washed 3 x 1 mL with PBS and then fixed with 1 mL of methanol at room temperature for 10 min. Cells were washed 2 x 1 mL with PBS and blocked for 1 h in 5% Donkey Serum. Blocking was followed by overnight incubation at 4 ºC with mouse anti-tubulin antibody (Cell Signaling 3873S) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Cells were then washed with PBS (3 x 5 min) before incubation with anti-mouse AlexaFluor 488 secondary antibody (Life Technologies A21202) at 1:500 dilution in antibody dilution buffer. After washing cells with PBS (3 x 5 min), images were acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 60X oil objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

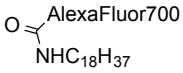
Treatment of HeLa Cells with C_{18}-Cbl-COL/C_{18}-DY800-loaded Erythrocytes. HeLa cells were plated in 35 mm glass bottom dishes (Mattek) at a density of 1.0 x 10^5 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were washed 3x with PBS, then treated with 200 µL of a suspension of **C_{18}-Cbl-COL/C_{18}-DY800** (5 µM/5 µM) loaded erythrocytes in L-15 (5% hematocrit) or 200 µL L-15 (control cells). Samples were then either kept in the dark at 37 ºC in a humidity-controlled incubator, or exposed to a 780 nm LED array for 10, 20, or 30 min using the appropriate filter sets. All samples were incubated for 1 h in a 37 ºC in a humidity-controlled incubator post-photolysis. At the conclusion of the incubation period, HeLa cells were washed 3 x 1 mL with PBS and then fixed with 1 mL of methanol at room temperature for 10 min. Cells were washed 2 x 1 mL with PBS and blocked for 1 h in 5% Donkey Serum. Blocking was followed by overnight incubation at 4 ºC with mouse anti-tubulin antibody (Cell Signaling 3873S) at 1:100 dilution in antibody dilution buffer (1% BSA; 0.3% Triton-X-100; PBS). Cells were then washed with PBS (3 x 5 min) before incubation with anti-mouse AlexaFluor 488 secondary antibody (Life Technologies A21202) at 1:500 dilution in antibody dilution buffer. After washing cells with PBS (3 x 5 min), images were acquired with an inverted Olympus IX81 microscope equipped with a Hamamatsu C8484 camera, 60X oil objective and a FITC filter cube (Semrock). Metamorph software was employed for image analysis.

CETSA Assay of HeLa Cells Treated with C_{18}-Cbl-MTX-loaded RBCs. HeLa cells were plated in 12-well tissue culture plates at a density of 9.4 x 10^4 cells per well and maintained at 37 ºC in a humidity-controlled incubator with a 5% CO_2 atmosphere in DMEM (10% FBS, 1% Pen-Strep). The following day, cells were washed 3x with PBS, then treated with 300 µL of a suspension of **C_{18}-Cbl-MTX**-loaded erythrocytes in L-15 (5 µM MTX loading concentration at 5% hematocrit), 300 µL L-15 (control cells), or 300 µL L-15 with 10 µM MTX (positive control). Samples were then either kept in the dark at 37 ºC in a humidity-controlled incubator, or exposed to a green LED light source (PAR38; 500 – 570 nm emission; 5 mW power) for 10, 20, or 30 min. At the end of the photolysis interval, all samples were incubated for 1 h in a 37 ºC in a humidity-controlled incubator. The cells were then washed with 3 x 1 mL with PBS and trypsinized with 300 µL 0.05% Trypsin (Gibco) for 5 min at 37 ºC. Trypsinized cells were then pelleted (5 min, 2000 rpm, 4 ºC), washed 1 x 300 µL PBS, and then pelleted again. After removal of the supernatant, cell pellets were heated at 52 ºC in a temperature controlled heat block for 3 min, and then cooled for 3 min at room temperature. 30 µL of lysis buffer (25 mM Tris HCl/2 mM DTT/1X Pierce HALT protease and phosphatase inhibitor) was added to each pellet, which were then subjected to 2 freeze-thaw cycles in liquid N_2. The resulting solutions were then spun at 17000 g for 20 min at 4 ºC. The resulting supernatants were removed and combined with 6X LSB-BME and boiled for 4 min at 95 ºC, then analyzed by western blot (overnight incubation at 4 ºC with Santa Cruz anti-DHFR E18 primary antibody (1:1000 in TBST/5%BSA) and Cell Signaling anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:2000), followed by incubation with the appropriate secondary antibodies.

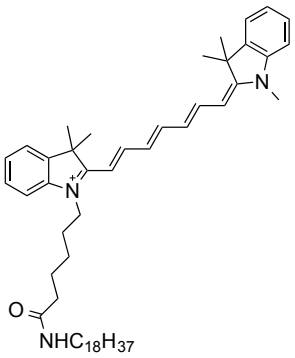
Scheme S1. Structure of C_{18} conjugated fluorophores used in this study.



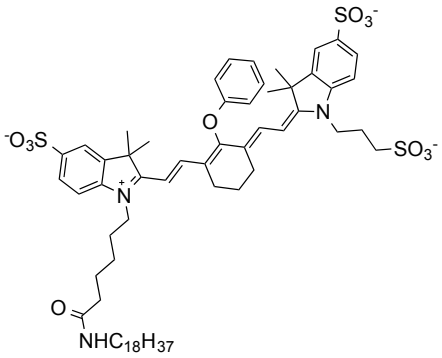
C_{18}-Cy5
λ_{ex} = 646 nm



C_{18}-AF700
λ_{ex} = 700 nm

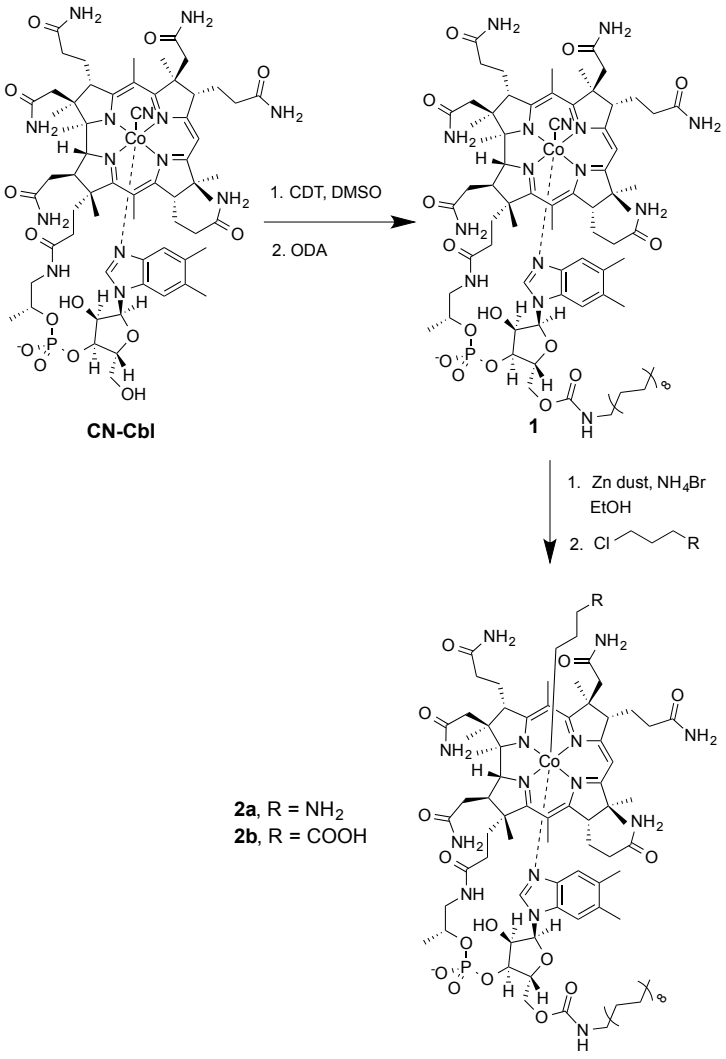


C_{18}-Cy7
λ_{ex} = 747 nm

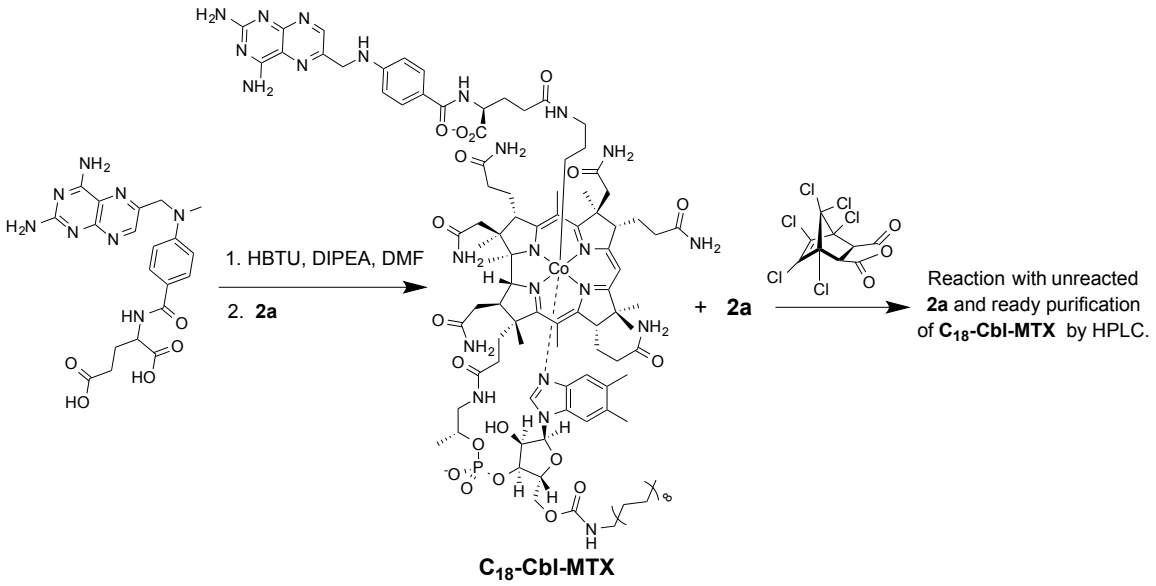


C_{18}-DY800
λ_{ex} = 784 nm

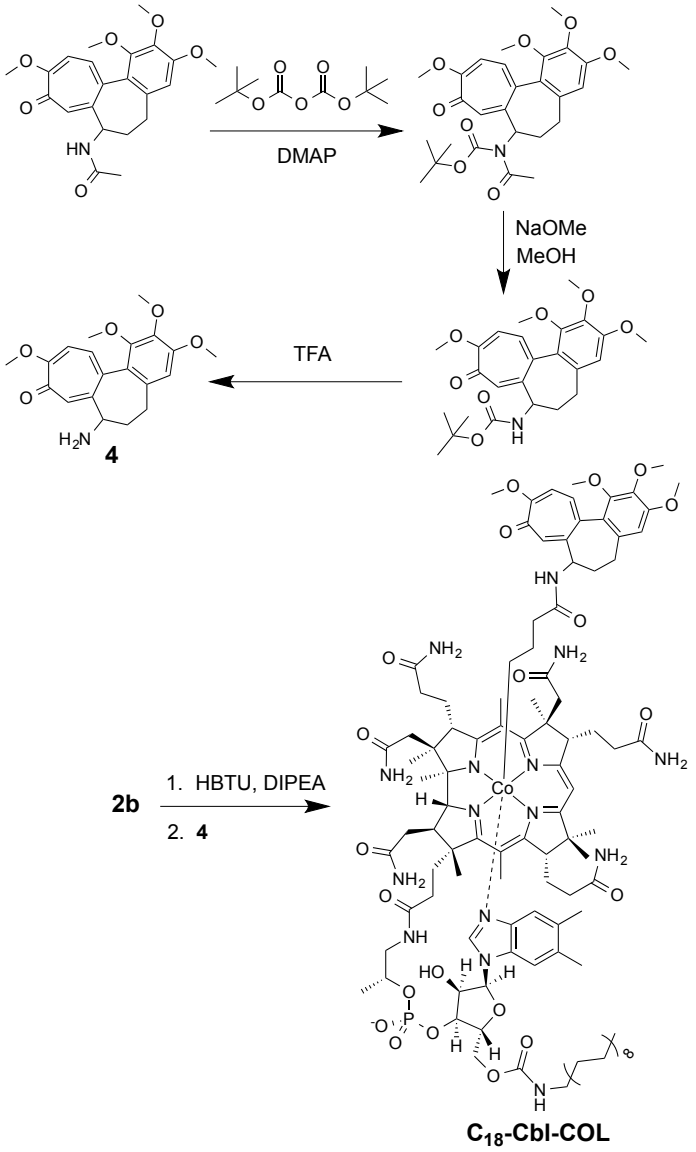
Scheme S2. Synthesis of C_{18}-modified Cbls**.**



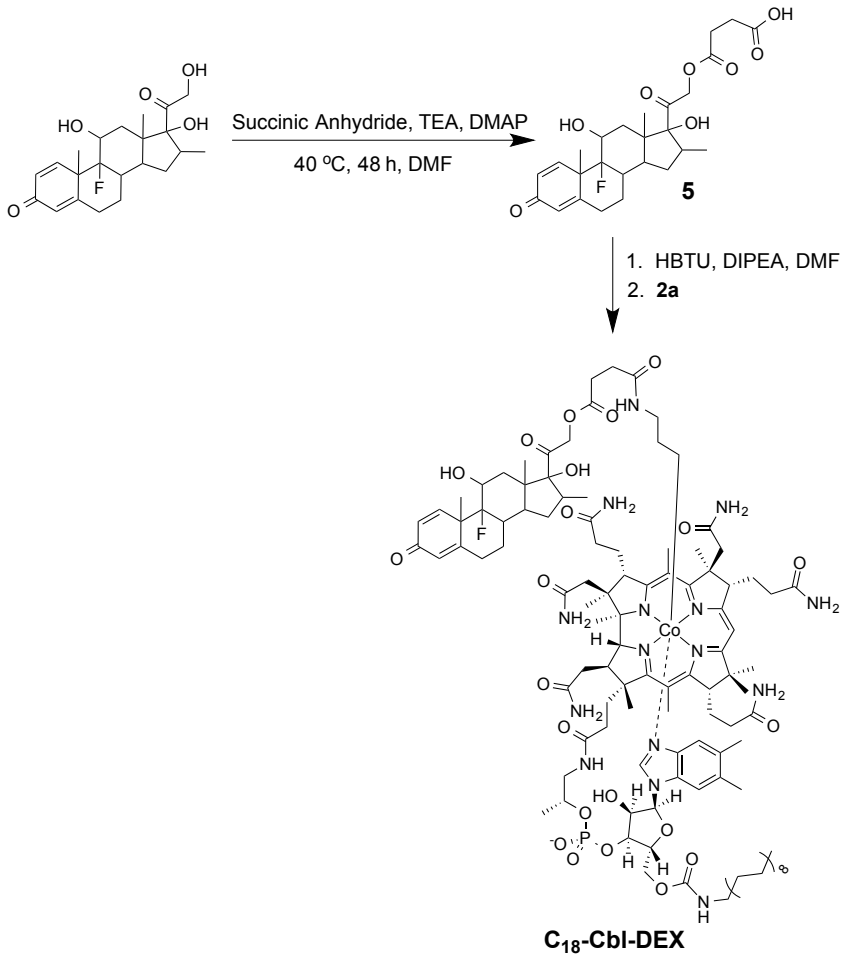
Scheme S3. Synthesis and purification of **C_{18}-Cbl-MTX.** Note: Due to the expense of MTX, it was employed as the limiting reagent.



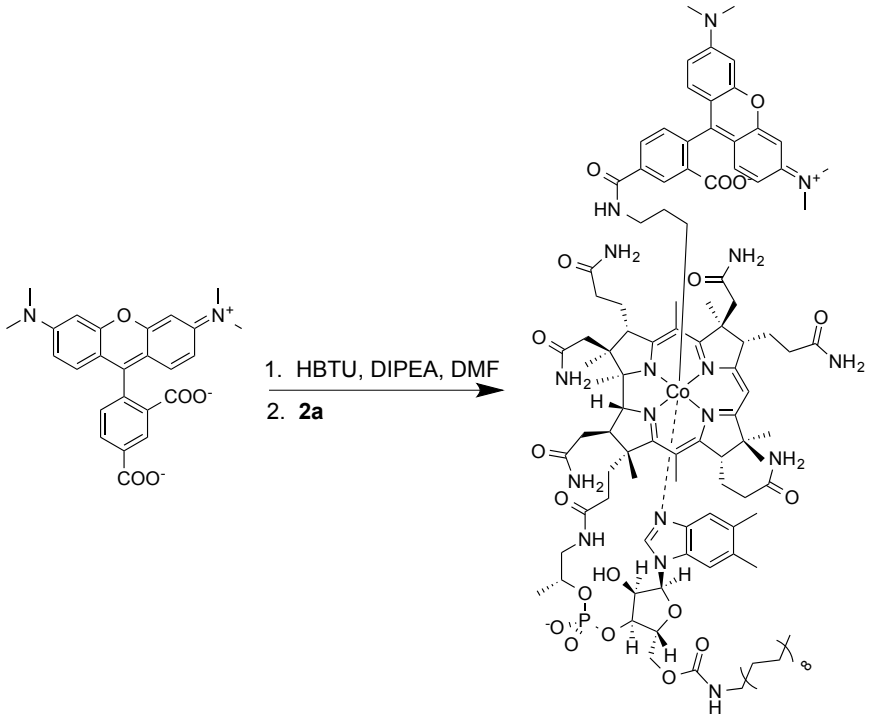
Scheme S4. Synthesis of **C_{18}-CbI-COL.**



Scheme S5. Synthesis of **C_{18}-CbI-DEX.**

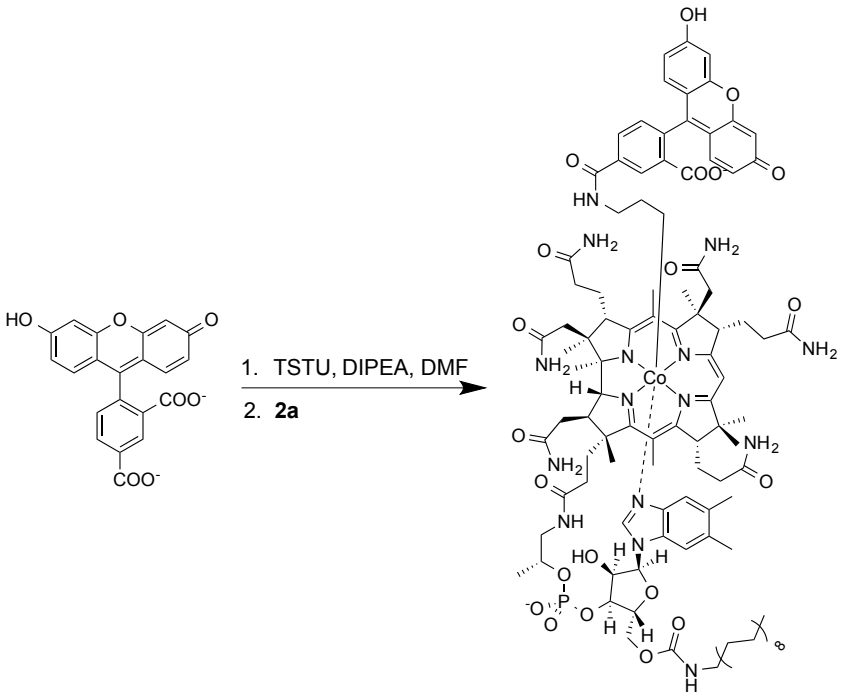


Scheme S6. Synthesis of **C_{18}-Cbl-TAM.**



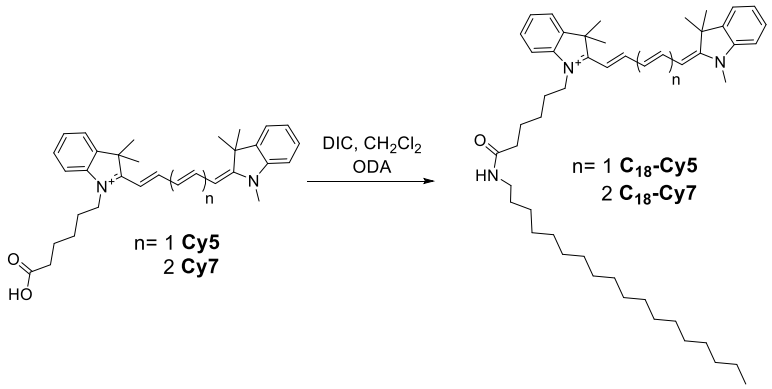
C_{18}-Cbl-TAM

Scheme S7. Synthesis of **C_{18}-CbI-FAM.**

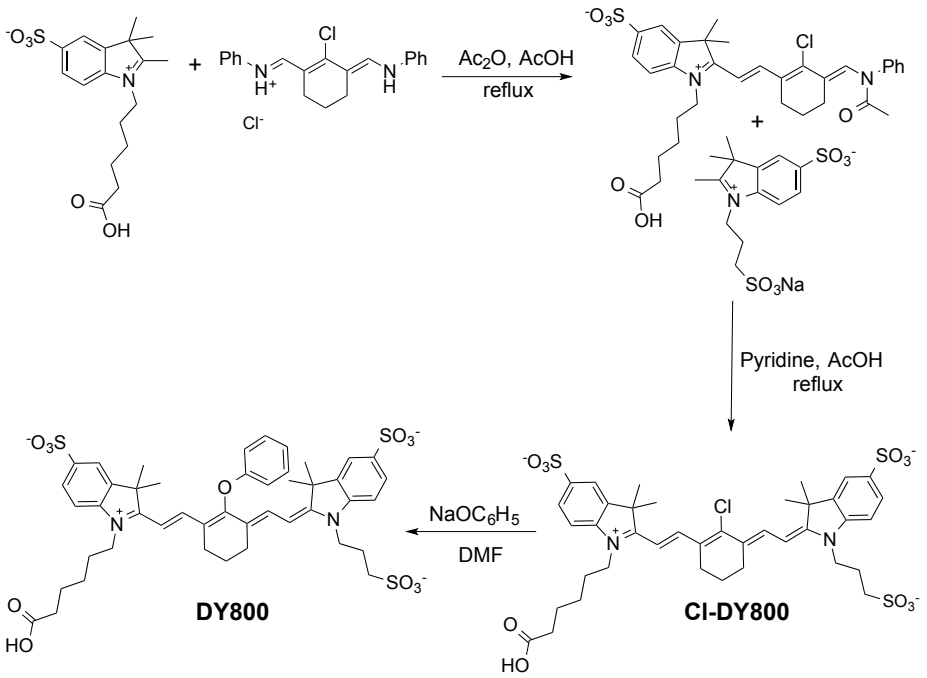


C_{18}-CbI-FAM

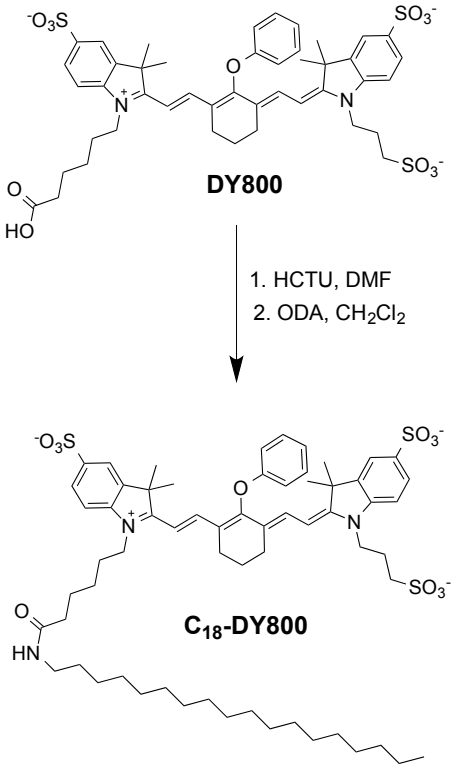
Scheme S8. Synthesis of **C_{18}-Cy5** and **C_{18}-Cy7.**



Scheme S9. Synthesis of **DY800** fluorophore.



Scheme S10. Synthesis of **C_{18}-DY800.**



Scheme S11. Photolysis of Cbl drug conjugates. **C_{18}-Cbl-MTX, C_{18}-Cbl-COL, C_{18}-Cbl-DEX.** MTX, COL, and DEX are represented as "DRUG". See Tables S5 and S8.

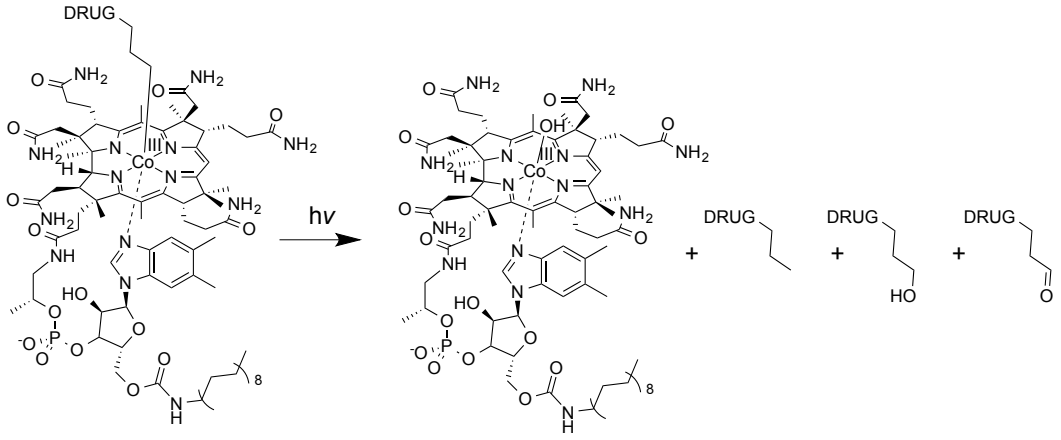


Figure S1. LC-MS UV/Vis chromatograms of purified **chloro-DY800** collected with a reverse phase C_{18} at (a) 300 nm and (b) 600 nm. **DY800** is also shown with monitoring at (c) 300 nm and (d) 600 nm. Water was used as the loading solvent.

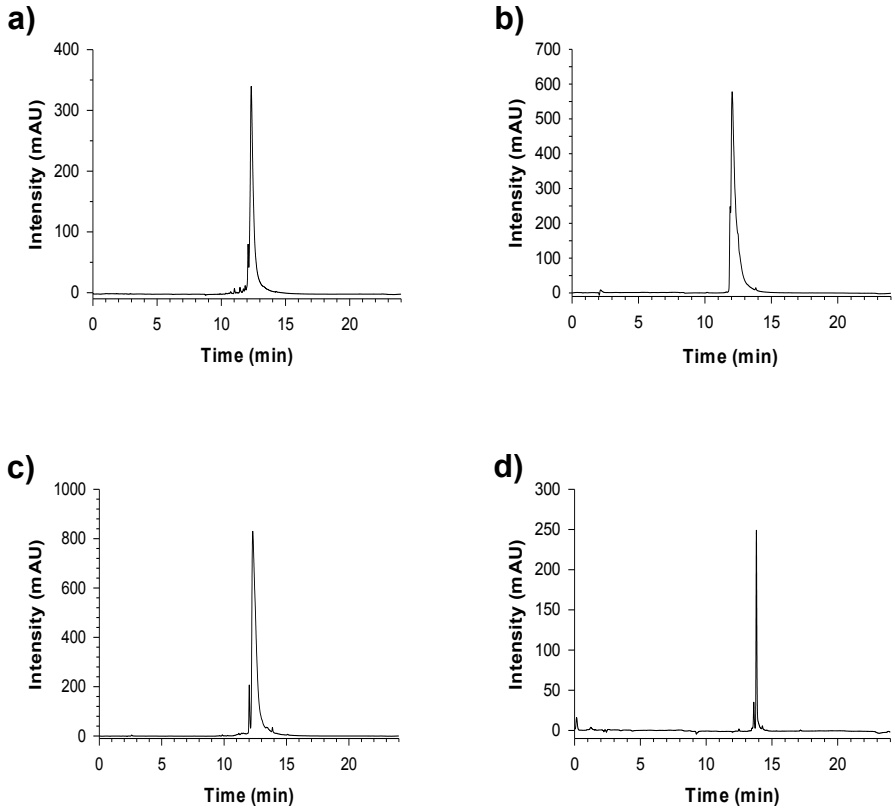


Figure S2. LC-MS UV/Vis chromatogram of purified **C_{18}-DY800** collected using a reverse phase C_{18} column and monitored at (a) 300 nm and (b) 600 nm. Water was used as the loading solvent.

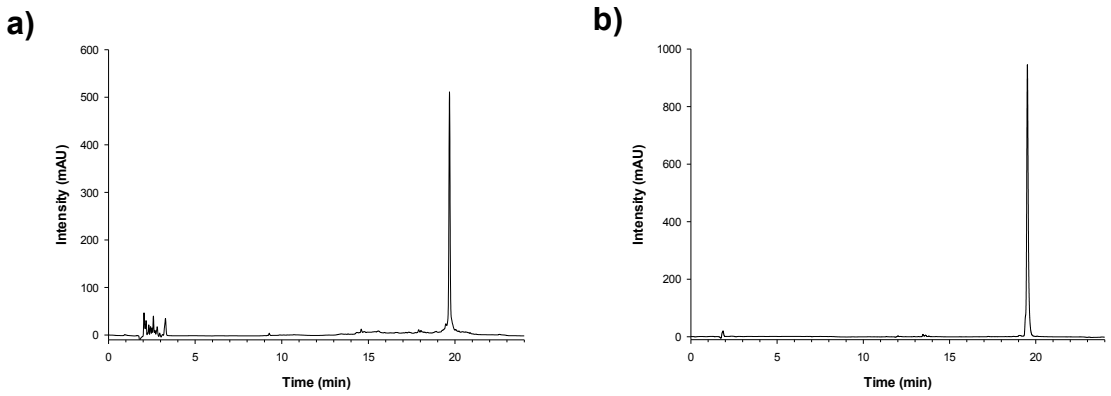


Figure S3. LC-MS UV/Vis chromatogram of purified **C_{18}-Cy5** collected using a C_4 column and monitored at (a) 300 nm and (b) 600 nm. Pure DMSO was used as the loading solvent.

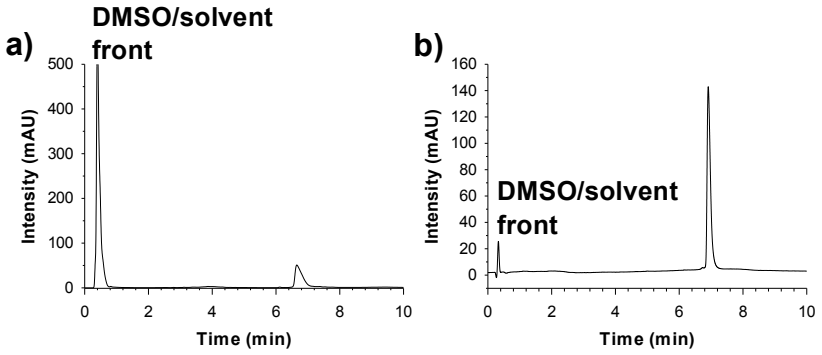


Figure S4. LC-MS UV/Vis chromatogram of purified **C_{18}-Cy7** collected using a C_4 column and monitored at (a) 300 nm and (b) 600 nm. Pure DMSO was used as the loading solvent

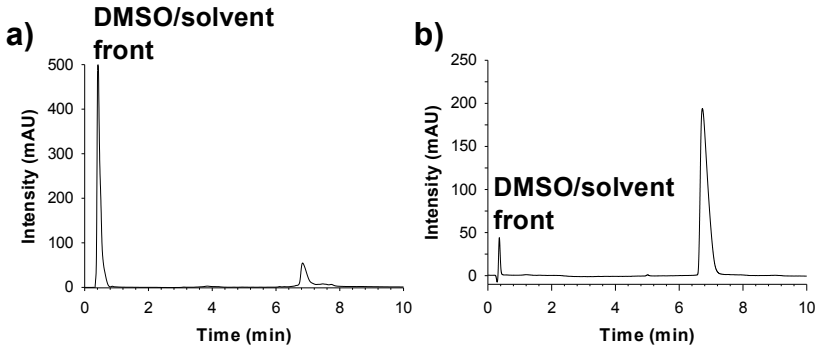


Figure S5. LC-MS UV/Vis chromatograms of purified (a) **C_{18}-CbI-MTX** monitored at 300 nm (b) **C_{18}-CbI-COL** monitored at 365 nm and (c) **C_{18}-CbI-DEX** monitored at 239 nm. The chromatogram of (c) is solvent background corrected. All peaks are normalized to **C_{18}-CbI-COL.**

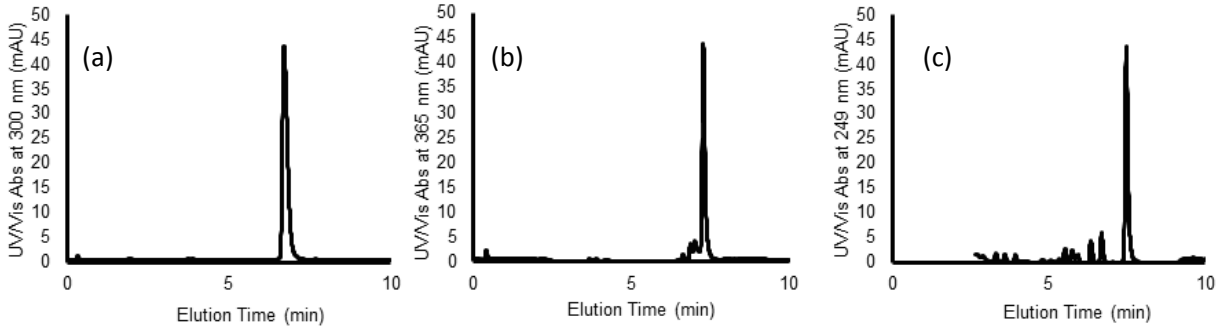


Figure S6. Light induced migration of TAM from octanol to water monitored by fluorescence of the aqueous layer. **C_{18}-CbI-TAM** (primarily soluble in the octanol layer) in a water/octanol partition was photolyzed at 525 nm. Fluorescence of TAM in the water layer λ_{ex}: 555 nm λ_{em}: 585 nm. Photolysis time: 0 (red), 1 (orange), 2 (yellow), 3 (green), and 10 min (blue). The inset shows the emission spectrum of aqueous TAM (570 nm – 600 nm). Data are represented as averages with standard errors of three independent experiments.

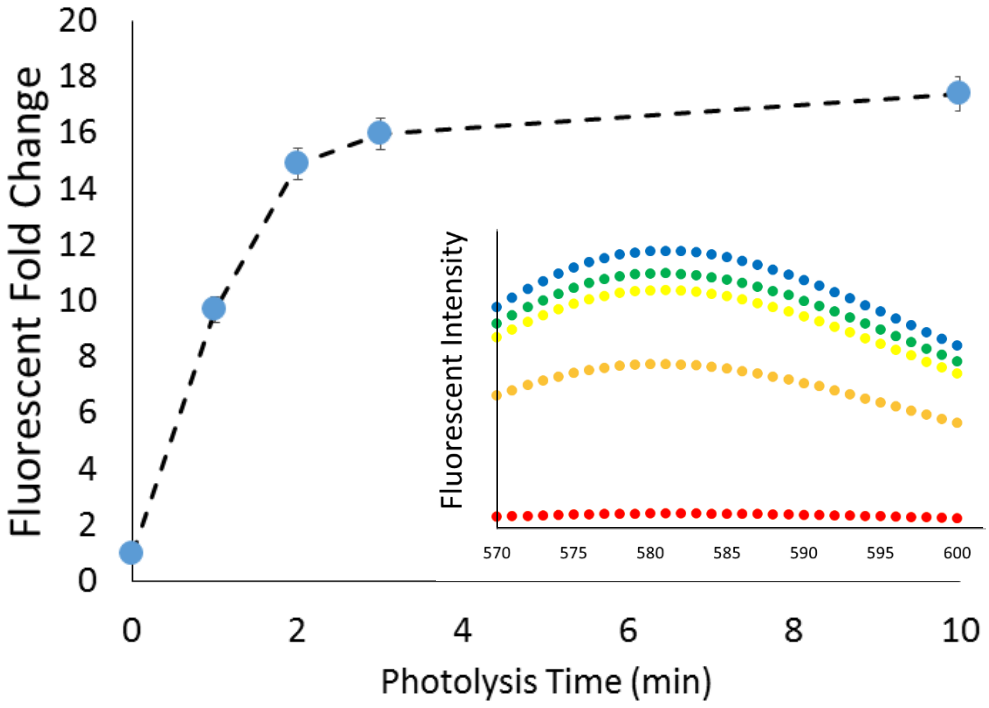


Figure S7. Light induced migration of MTX from octanol to water as quantified by the MTX LC-MS assay. **C_{18}-CbI-MTX** (primarily soluble in the octanol layer) was photolyzed using 525 nm LEDs. Migration is represented as the mole percent of total amount of MTX found in the aqueous layer. Data are represented as averages with standard errors of three independent experiments.

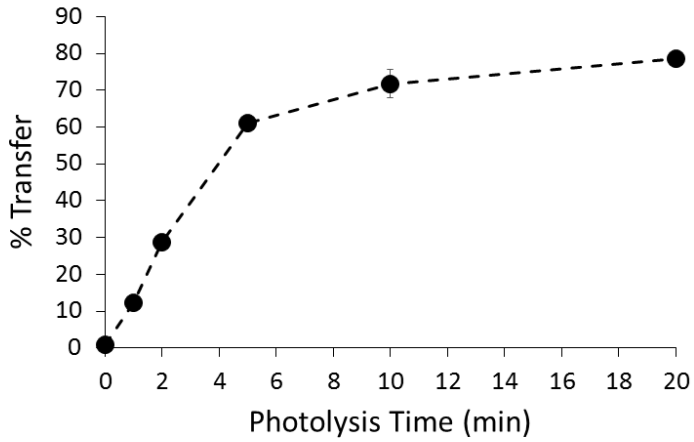


Figure S8. Erythrocytes loaded with 10 μM **C_{18}-CbI-TAM** and visualized at (a) long wavelength (λ_{ex} = 650 nm, λ_{em} = 684 nm) to prevent photo-cleavage of the Co-TAM bond. Visualization and photolysis of **C_{18}-CbI-TAM** at (b) λ_{ex} = 570 nm, λ_{em} = 590 nm, with 20 ms exposure times. Photolysis occurred within the first time frame. Images were taken every 1/3 s. After the first exposure, the fluorophore is nearly completely dissociated from the erythrocyte membrane and only present in solution. All images were acquired using an Olympus IX-81 widefield fluorescence microscope with a 60x oil immersion objective.

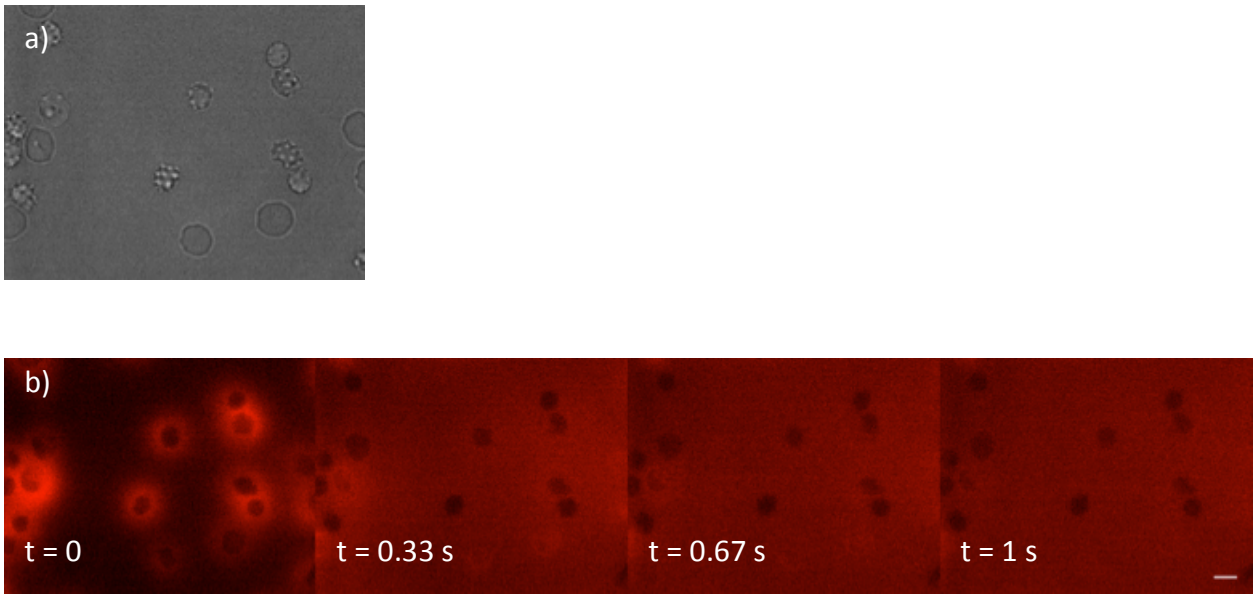


Figure S9. Structural integrity of erythrocytes exposed to various concentrations of lipidated drug conjugates for 14 h. Leaked heme was measured by optical density at 550 nm minus the optical density of a DMSO control (7 ± 1%) and divided by the absorbance of a 100% lysed control (0.05% SDS). In this way, only hemolysis due to compounds was measured. Data are represented as averages with standard errors of three independent experiments. **C_{18}-Cbl-MTX** (blue), **C_{18}-Cbl-COL** (orange), and **C_{18}-Cbl-DEX** (grey).

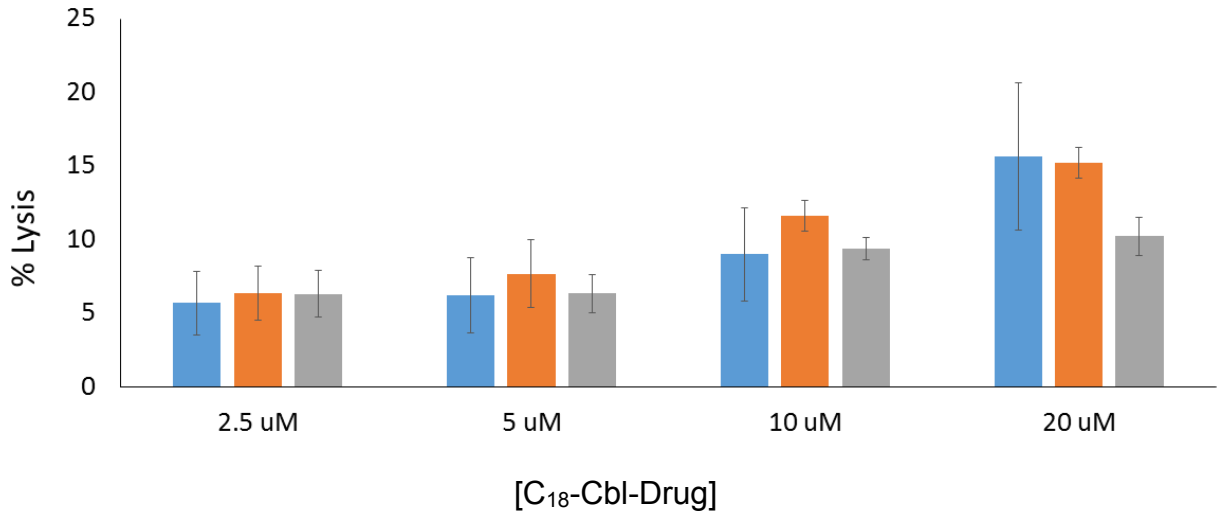
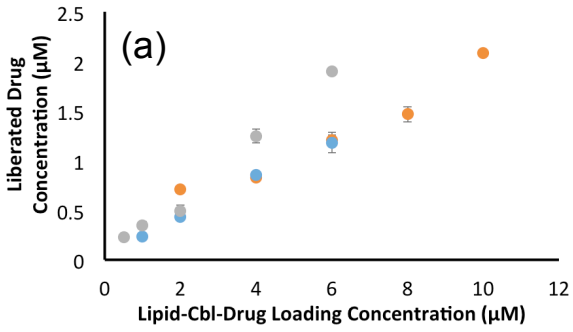
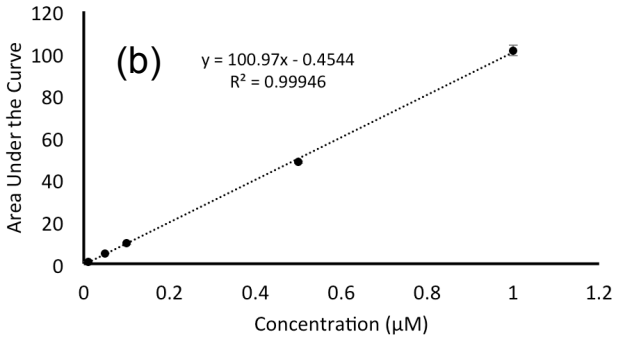
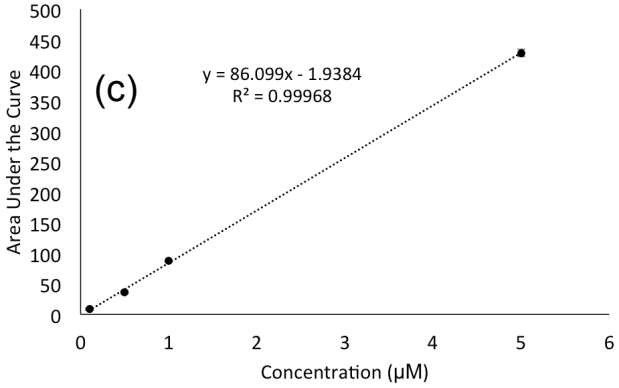
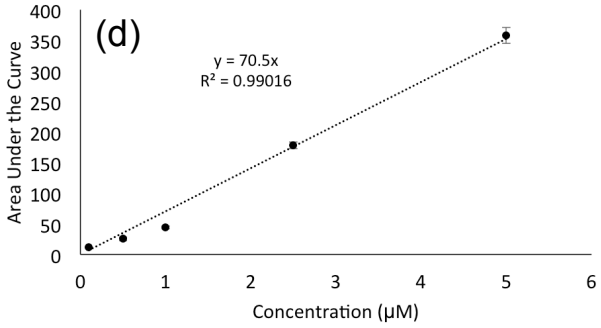


Figure S10. Drug loading on and release from human erythrocytes. (a) Amount of drug photo-released from erythrocytes as a function of C_{18}-Cbl-Drug loading concentration. Samples were photolyzed to completion using 525 nm LEDs exposure for 2 h. Concentrations were determined by LC-MS assays described for each drug. Data are represented as averages with standard errors of three independent experiments. **C_{18}-Cbl-MTX** (blue), **C_{18}-Cbl-COL** (orange), and **C_{18}-Cbl-DEX** (grey). Drug release quantity determined by comparison to standards and standard curves as follows: (b) standard curve used with MTX LC-MS assay was generated by titration of known amounts of commercially available MTX and subsequent analysis of the area under the curve by UV-Vis (300 nm) via LC-MS; concentrations used were 1.0 μM, 0.5 μM, 0.1 μM, 0.05 μM, and 0.01 μM. Data are represented as averages of three independent experiments; (c) standard curve used with COL LC-MS assay was generated by titration of known amounts of commercially available COL and subsequent analysis of the area under the curve by UV-Vis (365 nm) via LC-MS. Concentrations used were 5 μM, 1 μM, 0.5 μM, 0.1 μM. Data are represented as averages of three independent experiments; (d) standard curve used with DEX LC-MS assay was generated by titration of known amounts of commercially available DEX and subsequent analysis of the area under the curve by UV-Vis (239 nm) via LC-MS; concentrations used were 5.0 μM, 2.5 μM, 1 μM, 0.5 μM, and 0.1 μM. Data are represented as averages of three independent experiments. (e) Quantification of drug loading and photo-release. Erythrocytes (200 μL, 5% hematocrit) were incubated with 4 μM of each Cbl drug conjugate for 30 min. At the end of this time, three washes were performed in which the erythrocytes were spun down at 1000 g and the PBS buffer was exchanged. Supernatants were collected, pooled, photolyzed for 30 min at 525 nm, and then analyzed by the various LC-MS assays described for each drug. This provided a measure of unbound C_{18}-Cbl-drug. The erythrocytes were resuspended at 200 μL and photolyzed for 1 h at 525 nm [as in (a)] and the supernatant analyzed by the drug specific LC-MS assays. Black bars represent the total amount of drug loaded onto erythrocytes at 5% hematocrit in 200 μL. Conversion of the y-axis values to fmol of C_{18}Cbl-Drug/RBC was calculated from the experimentally derived value of 2.3 ± 0.1 x 10^7 RBC/mL (5% hematocrit): 0.28 ± 0.04 fmol of **C_{18}-Cbl-DEX**/RBC, 1.03 ± 0.03 fmol of **C_{18}-Cbl-COL**/RBC, and 0.57 ± 0.01 fmol of **C_{18}-Cbl-MTX**/RBC. White bars represent the amount of drug liberated from photolysis into the supernatant. Data are represented as averages of three independent experiments.




(b)
y = 100.97x - 0.4544
R² = 0.99946
Area Under the Curve
Concentration (μM)


(c)
y = 86.099x - 1.9384
R² = 0.99968
Area Under the Curve
Concentration (μM)


(d)
y = 70.5x
R² = 0.99016
Area Under the Curve
Concentration (μM)

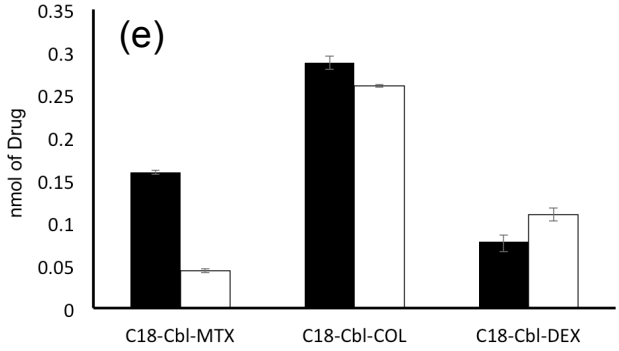

(e)
nmol of Drug
C18-Cbl-MTX
C18-Cbl-COL
C18-Cbl-DEX

Figure S11. Inhibition of DHFR by MTX (circles) and photolyzed erythrocyte-bound **C_{18}-Cbl-MTX** (triangles).

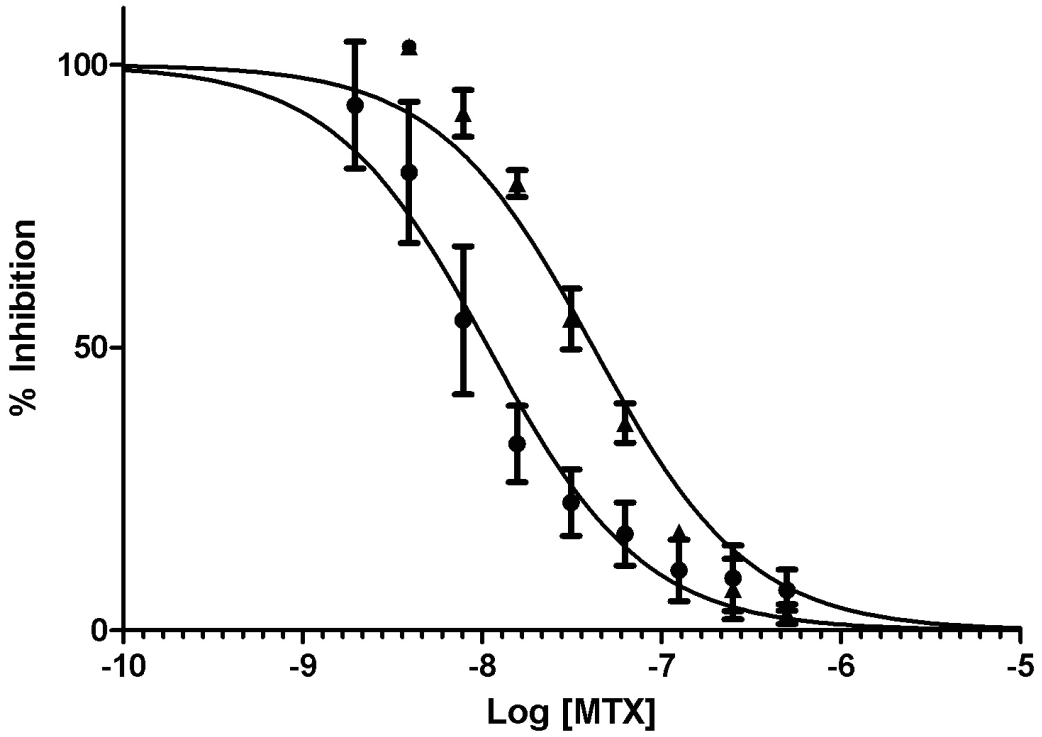


Figure S12. Assessment of MTX photo-release (@ 525 nm) from **C_{18}-Cbl-MTX**-loaded erythrocytes via the cellular thermal shift assay (CETSA). In the absence of MTX ("Dark", "No Treatment", and "'-'MTX") no DHFR is detected from HeLa cells following incubation of HeLa cells at 52 °C. By contrast, exposure of HeLa cells to **C_{18}-Cbl-MTX**-loaded erythrocytes with increasing illumination times (10, 20, and 30 min) leads to increasing amounts of DHFR stable to 52 °C. Loading control: GAPDH.

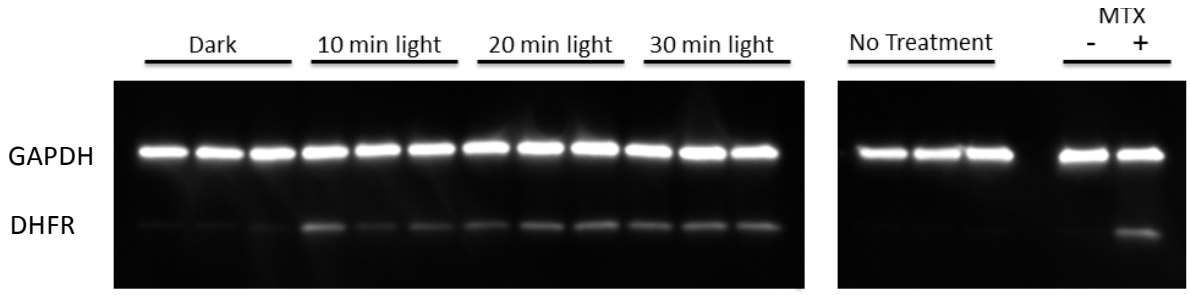


Figure S13. Effect of COL on microtubules in HeLa cells. COL was added to HeLa cell culture and allowed to incubate for 1 h before the cells were fixed and stained for tubulin to visualize microtubule networks. (a) DMSO only (b) 250 nM (c) 500 nM (d) 1 μM (e) 2 μM (f) 4 μM. At 2 μM COL well structured microtubules disappear and pools of depolymerized tubulin are visible at the cell periphery.

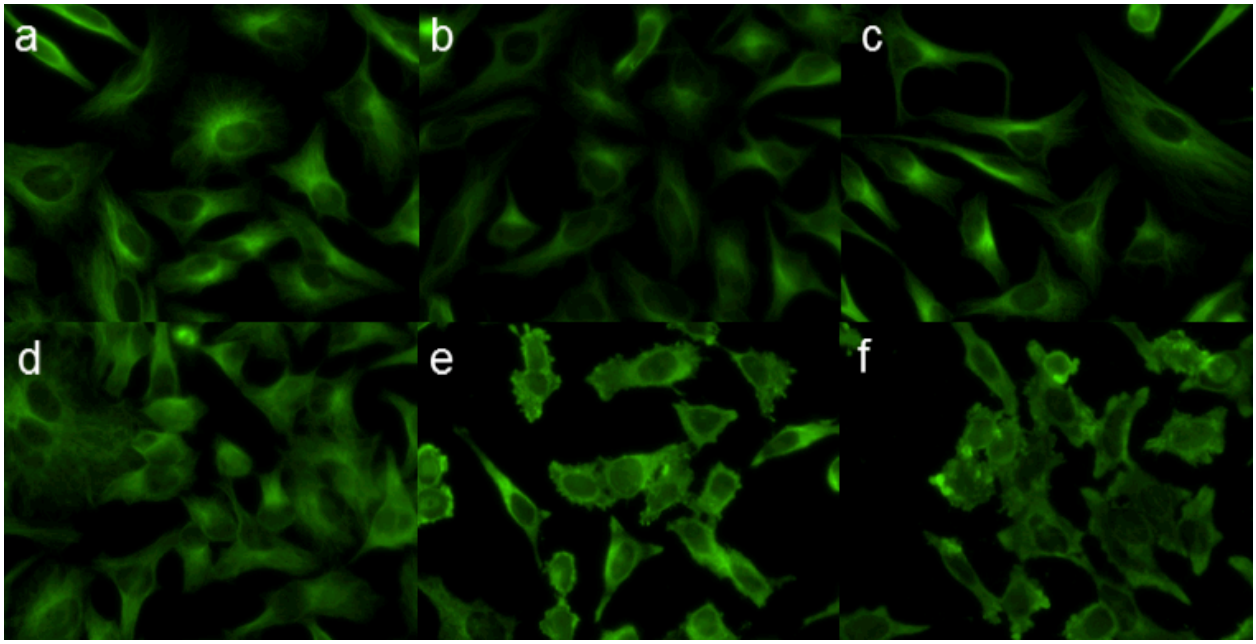


Figure S14. Effect of photo-released (@ 525 nm) COL from erythrocyte-bound **C_{18}-CbI-COL** on microtubules in HeLa cells. Human erythrocytes were loaded with **C_{18}-CbI-COL** (5% hematocrit, 6 μM). (a) 20 min photolysis at 525 nm, (b) dark, (c) no erythrocytes and dark, (d) no erythrocytes and 20 min photolysis at 525 nm. Disruption of microtubule networks from photolyzed **C_{18}-CbI-COL** (1.2 μM release according to Figure S6) is shown in panel a.

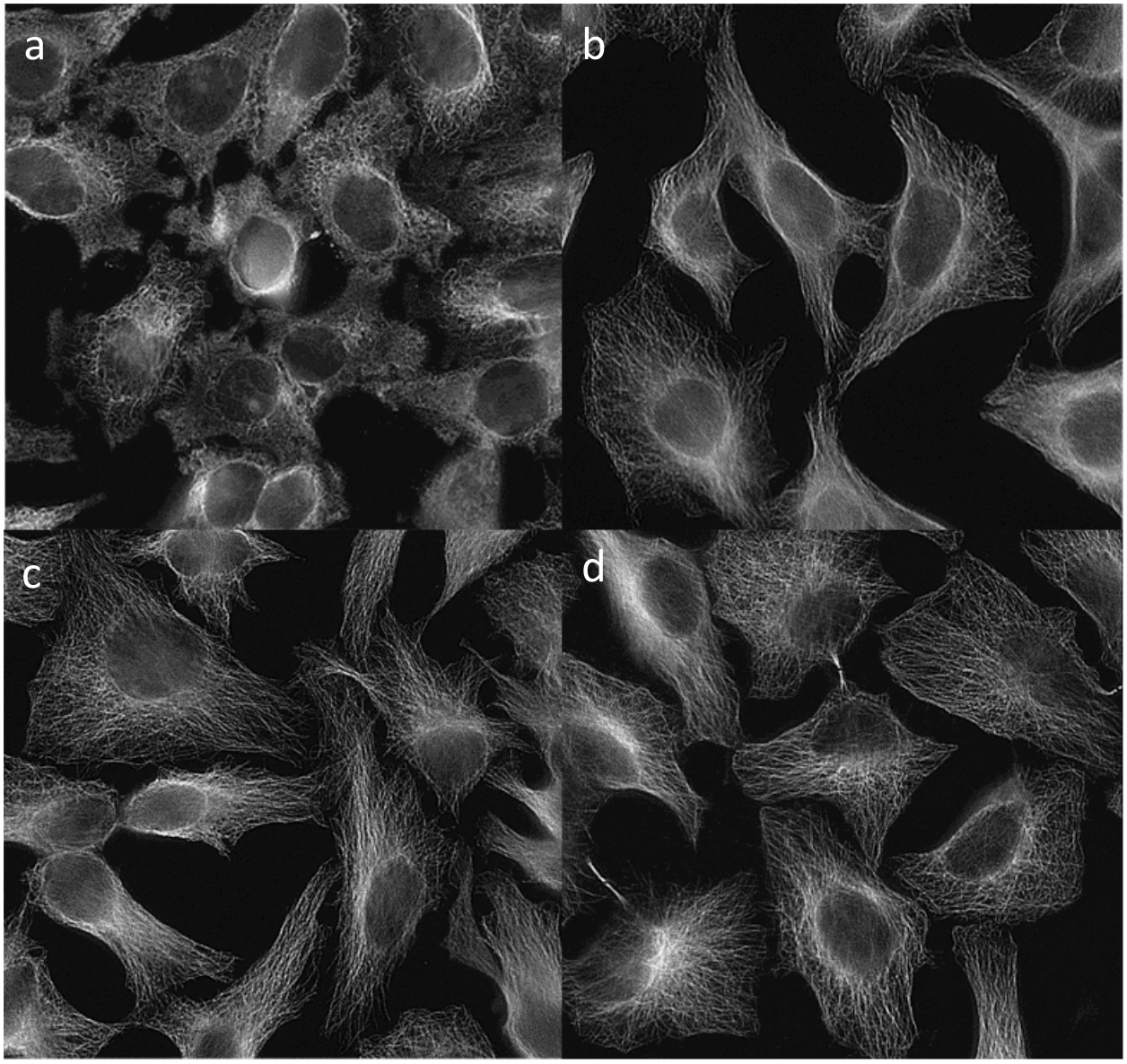


Figure S15. Effect of DEX on the subcellular location of GRα in HeLa cells. HeLa cells were stained with Alexa488 antiRabbit/anti-GRα (green) and HOESCHT 33342 (blue). (a) In HeLa cells treated with DMSO, GRα is largely cytosolic and does not co-localize with (b) nuclear HOESCHT 33342 stain. (c) In HeLa cells treated with 250 nM DEX, GRα migrates to the nucleus and (d) co-stains with HOESCHT 33342.

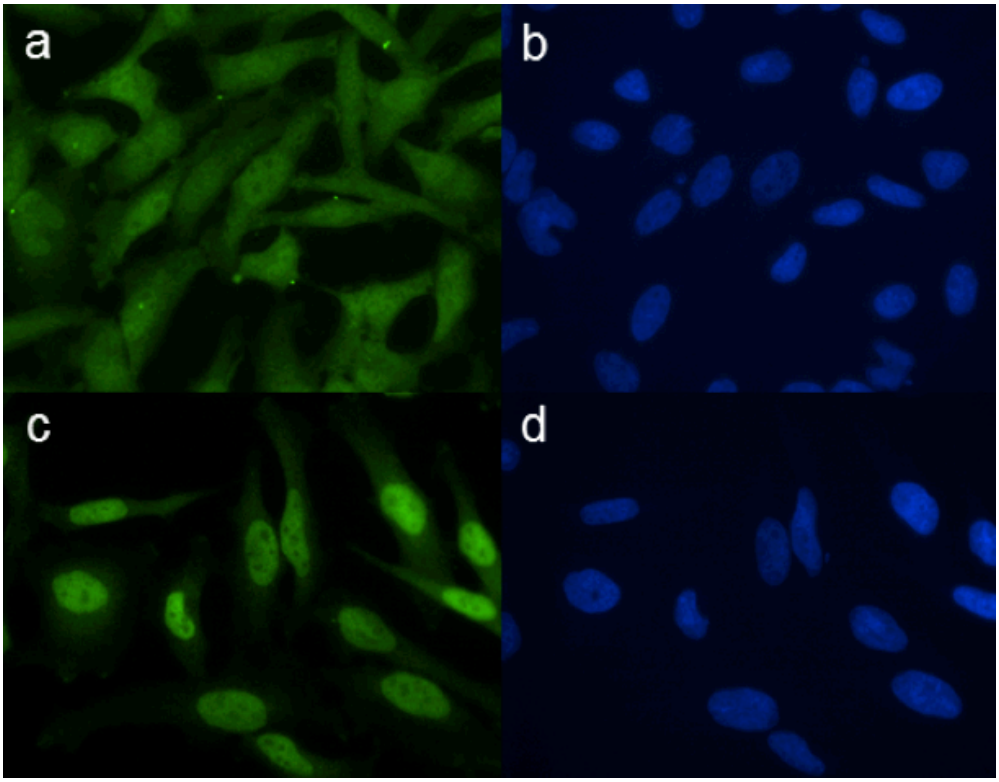


Figure S16. Effect of photo-released (@ 525 nm) DEX from erythrocyte-bound **C_{18}-Cbl-DEX** on the subcellular location of GRα in HeLa cells. (a) HeLa cells were treated with erythrocytes loaded with 1 μM **C_{18}-Cbl-DEX** without photolysis. (b) HeLa cells were treated with erythrocytes loaded with 1 μM **C_{18}-Cbl-DEX** with 10 min photolysis at 525 nm. HeLa cells were stained with Alexa488 antiRabbit/anti-GRα.

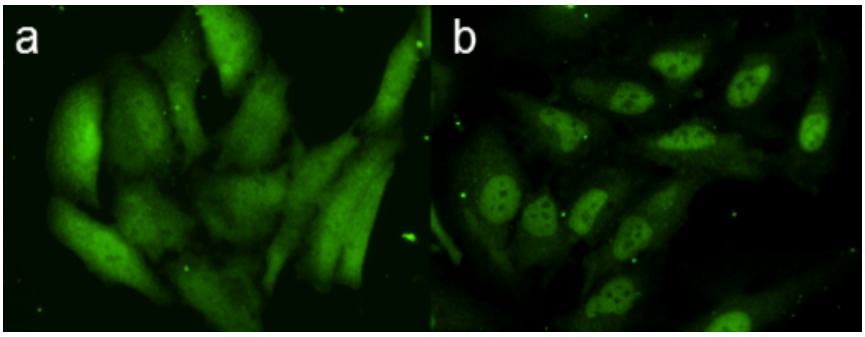


Figure S17. Assessment of **C_{18}-CbI-DEX** transfer between the erythrocyte and HeLa cell membranes. Erythrocytes were loaded with **C_{18}-CbI-DEX** (1 μM at 5% hematocrit). A 525 nm LED light source was used for all illumination experiments. (a) HeLa cells were exposed to **C_{18}-CbI-DEX** loaded erythrocytes in the dark for 1 h followed by removal of the erythrocytes via washing prior to photolysis and then fixed and stained for GRα. (b) HeLa cells were exposed to **C_{18}-CbI-DEX** loaded erythrocytes in the dark for 1 h followed by removal of the erythrocytes via washing; without photolysis; fixed and stained for GRα. (c) HeLa cells were not exposed to erythrocytes but were washed and illuminated; and then fixed and stained for GRα. In all instances, GRα remained cytosolic, indicating that DEX was not released from the erythrocytes.

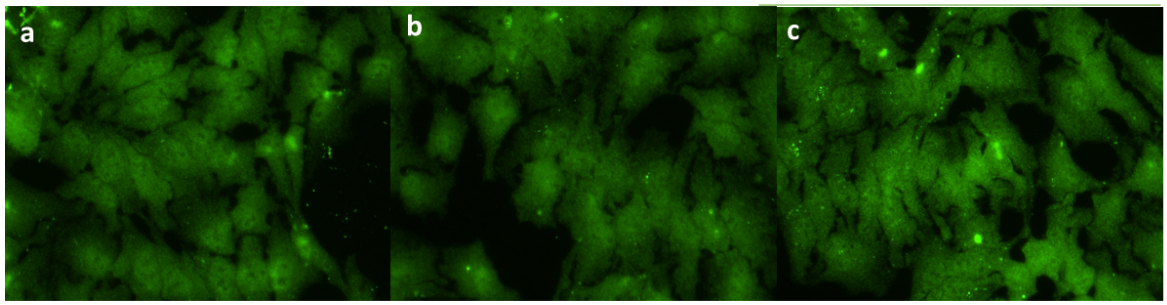


Figure S18. LED boards. All boards were powered with a 12V DC power supply. LEDs were wired in units of 4 LEDs in series with a 100 Ω resistor in parallel.

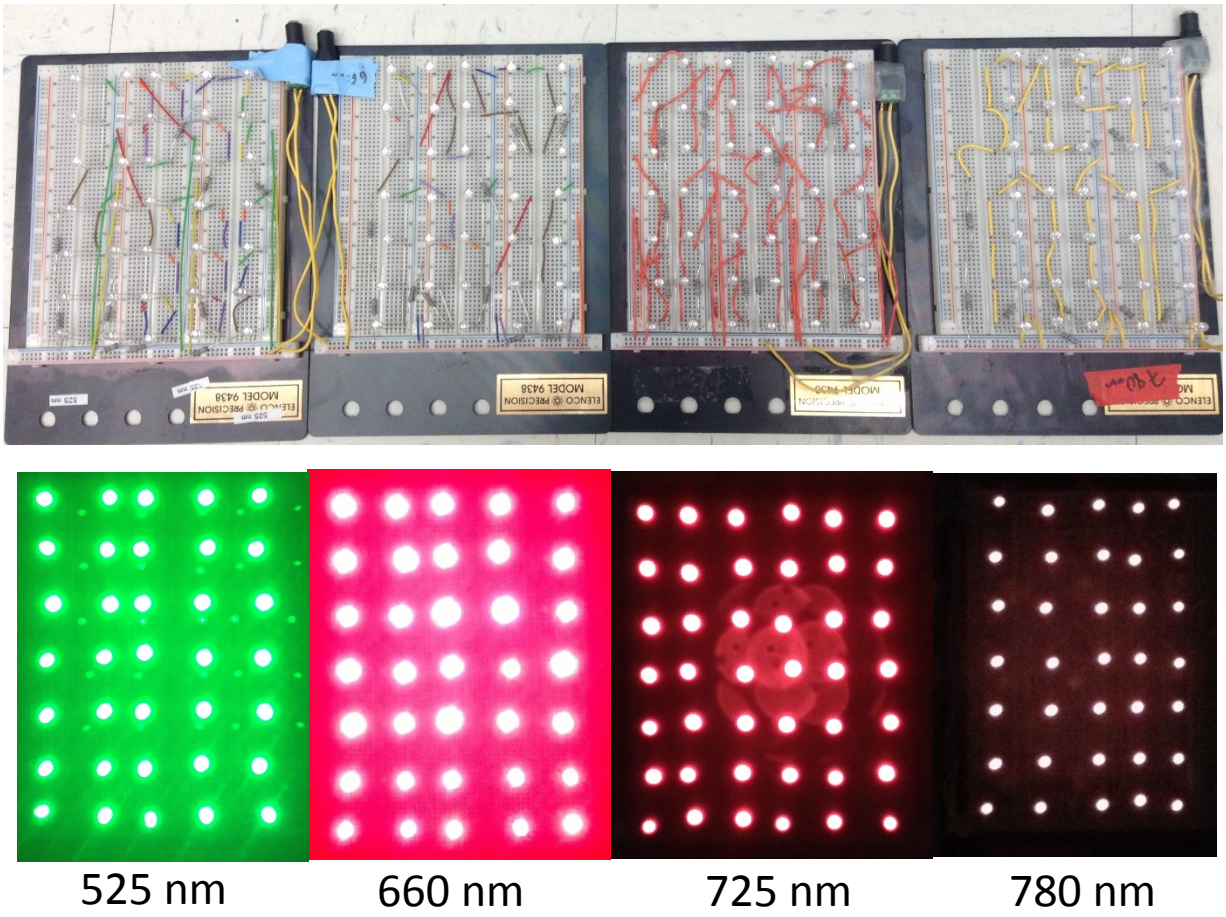


Figure S19. Heat map of TAM release from erythrocytes (5% hematocrit) exposed to variable concentrations of **C_{18}-Cbl-TAM** and **C_{18}-Cy5** followed by photolysis (30 min with 660 nm). The most efficient release (yellow/orange/red) is observed at low **C_{18}-Cbl-TAM**:**C_{18}-Cy5** ratios. Given that lipid concentrations >10 μM begin to compromise erythrocyte structural integrity according to the Hemolysis Assay, we chose to employ 1 μM:5 μM **Cbl-Drug**:**C_{18}-fluorophore** ratios in our initial studies.

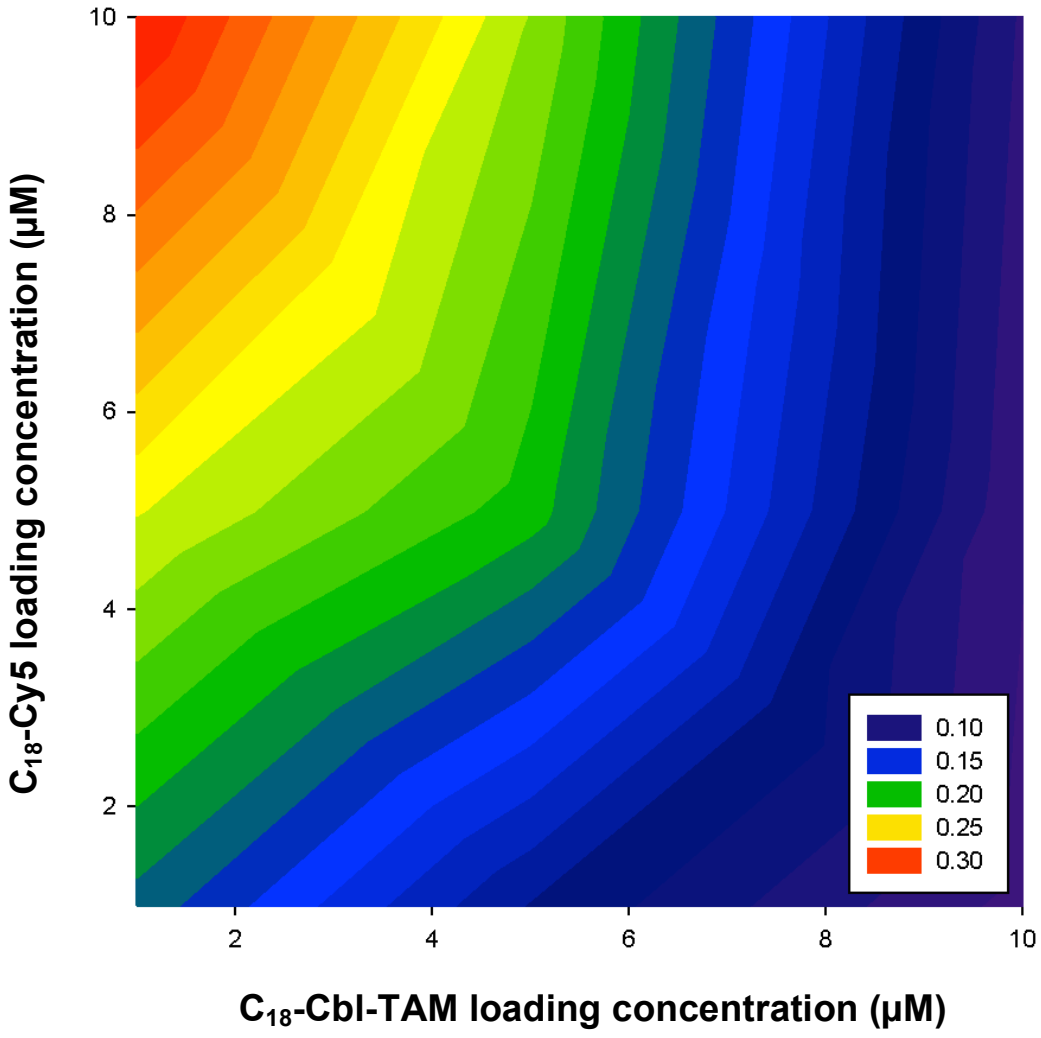


Figure S20. Release of FAM from erythrocytes loaded with **C_{18}-Cbl-FAM**/C_{18}-fluorophores (1 μM/5 μM) after 30 min illumination. Release is displayed as the fraction of FAM liberated out of the total amount of FAM bound. Total FAM bound was determined by measuring the concentration of FAM in the supernatant after 3 h of photolysis (@ 525 nm). The light sources employed were arrays of LEDs (shown in Figure S18) centered at 660 nm (Cy5), 725 nm (AF700 and Cy7), and 780 nm (DY800). The fraction of FAM released (white), the fraction of FAM release normalized by the power of the different light sources (black), and the fraction of FAM released normalized by photon density produced by the various light sources (grey). Power was normalized to **C_{18}-Cy5** by multiplying the fraction released (white bar) by a ratio of the power output of the LED board used to the output of the 660 board used for **C_{18}-Cy5** (Table S7). Photon density normalization was carried out in a similar fashion with photon flux values reported in Table S7. Data are represented as averages with standard errors of three independent experiments.

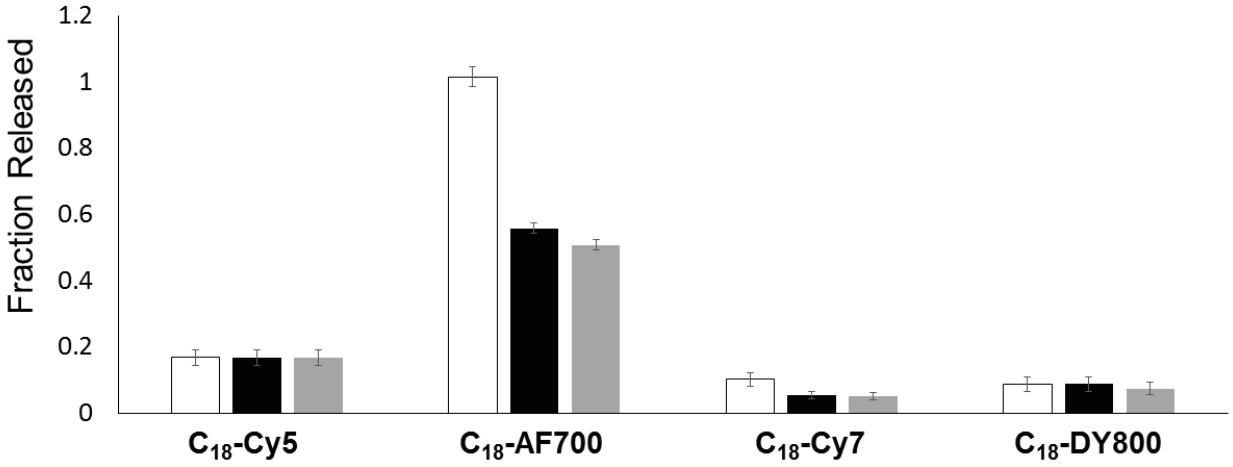


Figure S21. Release of TAM from erythrocytes loaded with **C_{18}-Cbl-TAM**/C_{18}-fluorophores (1 μM/5 μM) after 30 min of illumination. Release is displayed as the fraction of TAM liberated. The light sources employed are the LED arrays shown in Figure S18. In the absence of a long wavelength fluorescent antenna, illumination at 660, 725, or 780 nm fails to release membrane-tethered TAM. Data are represented as averages with standard errors of three independent experiments.

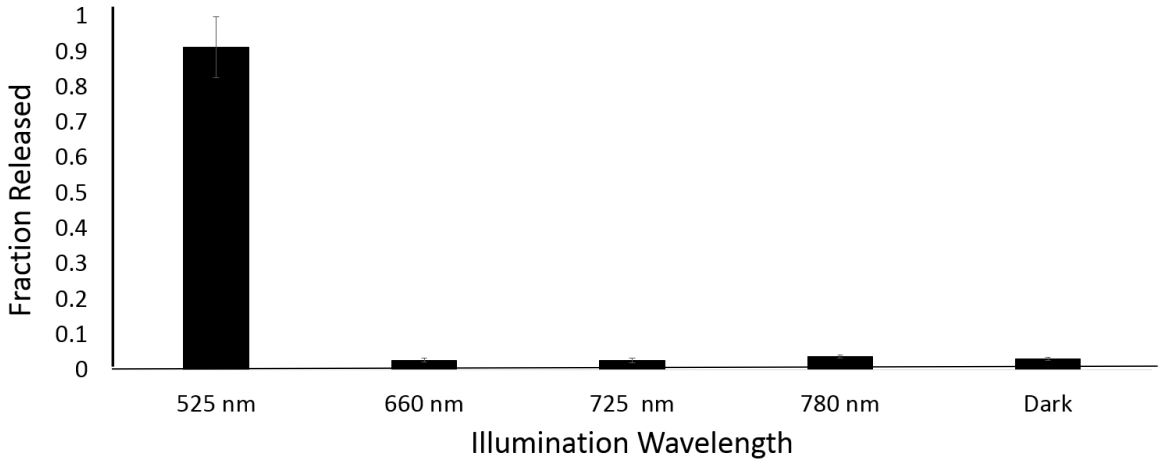


Figure S22. Release of TAM from erythrocytes loaded with **C_{18}-CbI-TAM** (1 μM) as a function of photolysis time. Release is displayed as the fraction of TAM liberated. All samples were analyzed at one hour. The light sources employed are the LED arrays (shown in Figure S18) centered at 525 nm (circles) and 660 nm (triangles). Data are represented as averages with standard errors of three independent experiments.

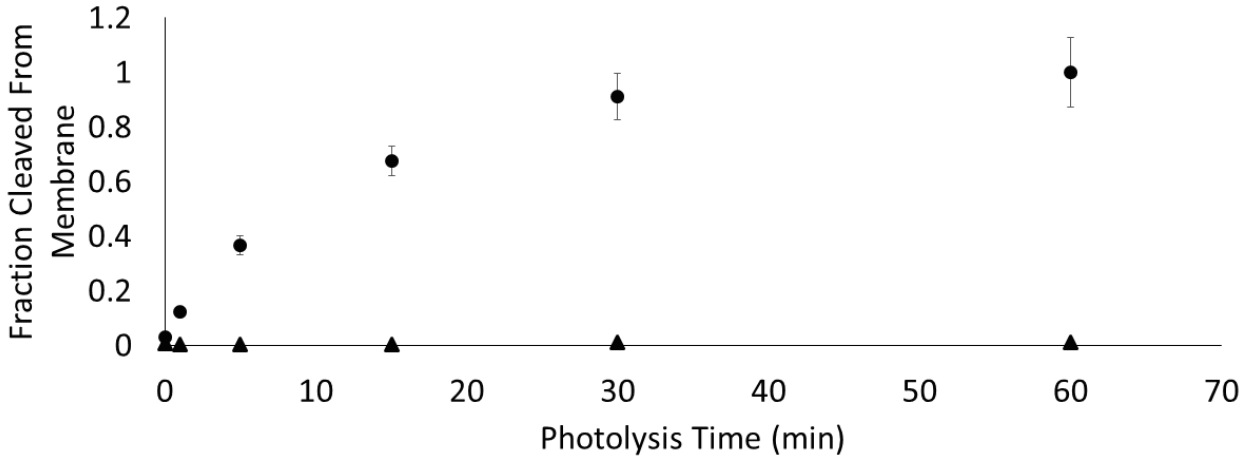


Figure S23. Release of FAM from erythrocytes loaded with **C_{18}-CbI-FAM** (1 μM) as a function of photolysis time. Release is displayed as the fraction of FAM liberated. All samples were analyzed at one hour. The light sources employed were arrays of LEDs (shown in Figure S18) centered at 525 nm (circle) and 660 nm (triangle). Data are represented as averages with standard errors of three independent experiments.

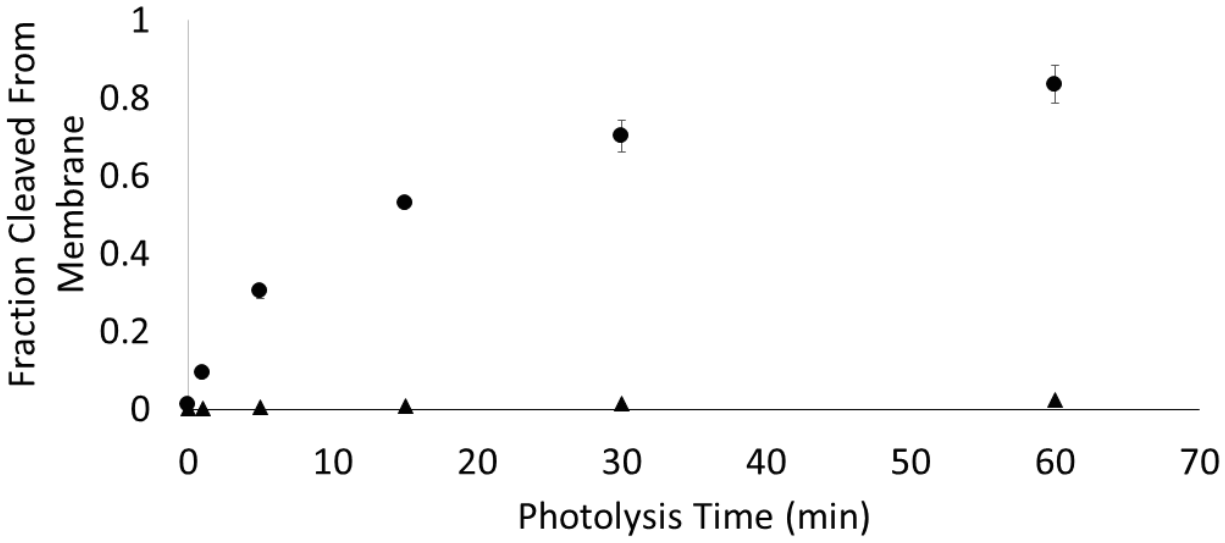


Figure S24. Confocal images of erythrocytes loaded with **C_{18}-Cy5**. **C_{18}-Cy5** (5 μM) was added to 10% hematocrit erythrocytes. Following incubation, erythrocytes were washed, plated at 0.1% hematocrit, and imaged by confocal microscopy. DIC (left) Cy-5 fluorescence (middle), and overlay (right). Bar length = 5 μm.

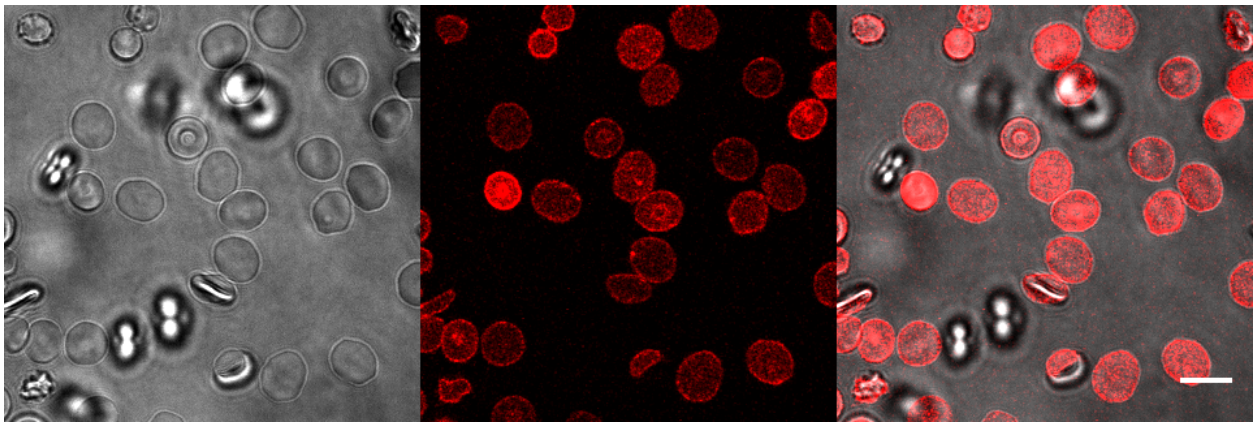


Figure S25. Release of MTX from **C_{18}-CbI-MTX**/C_{18}-fluorophore-loaded erythrocytes. (a) MTX release was measured by the LC-MS MTX Assay following photolysis at the λ_{ex} of each fluorophore. LED arrays (Figure S18) were centered at 660 nm (Cy5), 725 nm (AF700 and Cy7), and 780 nm (DY800). Data are represented as averages with standard errors of three independent experiments. In the presence (white column) and absence of illumination (black columns). (b) The relative efficiency of MTX release. The total nmols of MTX released from photolyzed RBC MTX mixtures in (a) was divided by the total number of photons that fell on the cross sectional surface area of those mixtures during 30 min of illumination time. The relative efficiency of the release of MTX for each of the various fluorescent antennae is given in nmol MTX released/μmol photons-cm^2. LED arrays (Figure S18) were centered at 660 nm (**C_{18}-Cy5**), 725 nm (**C_{18}-AF700** and **C_{18}-Cy7**), and 780 nm (**C_{18}-DY800**).

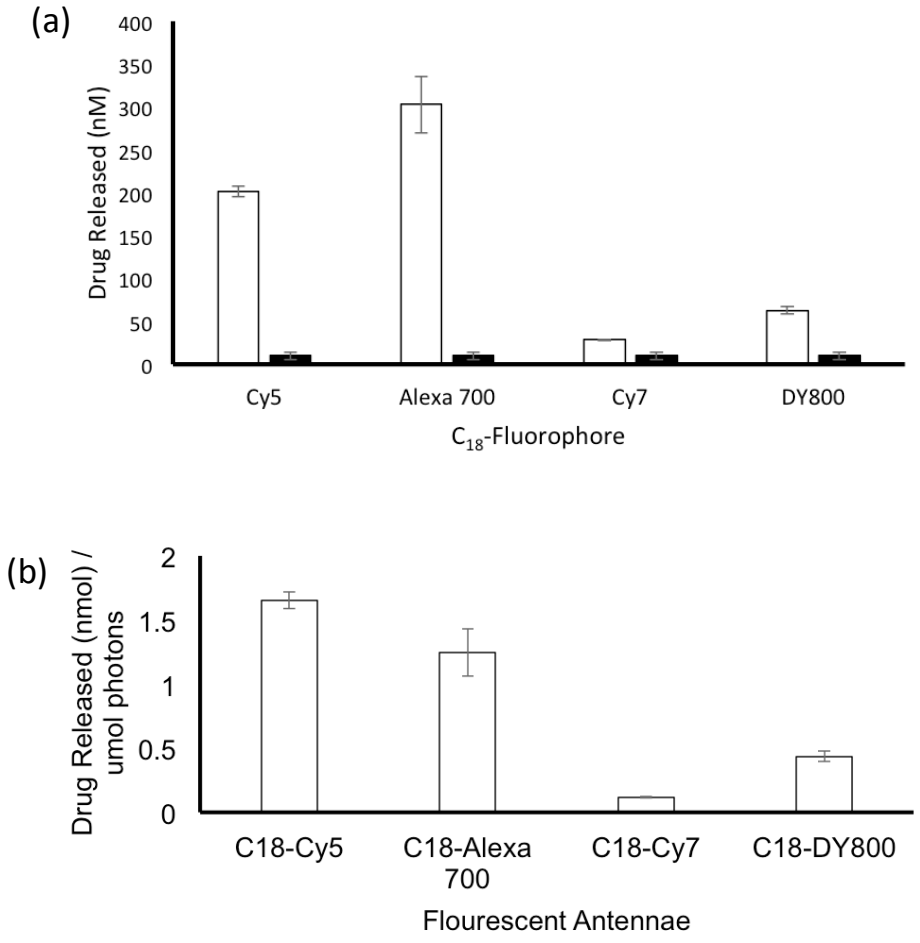


Figure S26. (a) Release of COL and (b) DEX from **C_{18}-Cbl-COL**/C_{18}-fluorophore-loaded erythrocytes and **C_{18}-Cbl-DEX**/C_{18}-fluorophore-loaded erythrocytes, respectively. Drug release was measured by the LC-MS COL or DEX Assays (white columns) following illumination at 660 nm (**C_{18}-Cy5**) or 780 nm (**C_{18}-DY800**). Control experiments in the dark were performed in parallel (black columns). Data are represented as averages with standard errors of three independent experiments. (c) The relative efficiency of drug release. The total nmols of drug released from photolyzed RBC drug mixtures in (a) and (b) was divided by the total number of photons that fell on the cross sectional surface area of those mixtures during 30 min of illumination time. The relative efficiency of drug release for each of the various fluorescent antennae is given in nmol drug released/μmol photons-cm^2. LED arrays (Figure S18) were centered at 660 nm (**C_{18}-Cy5**).

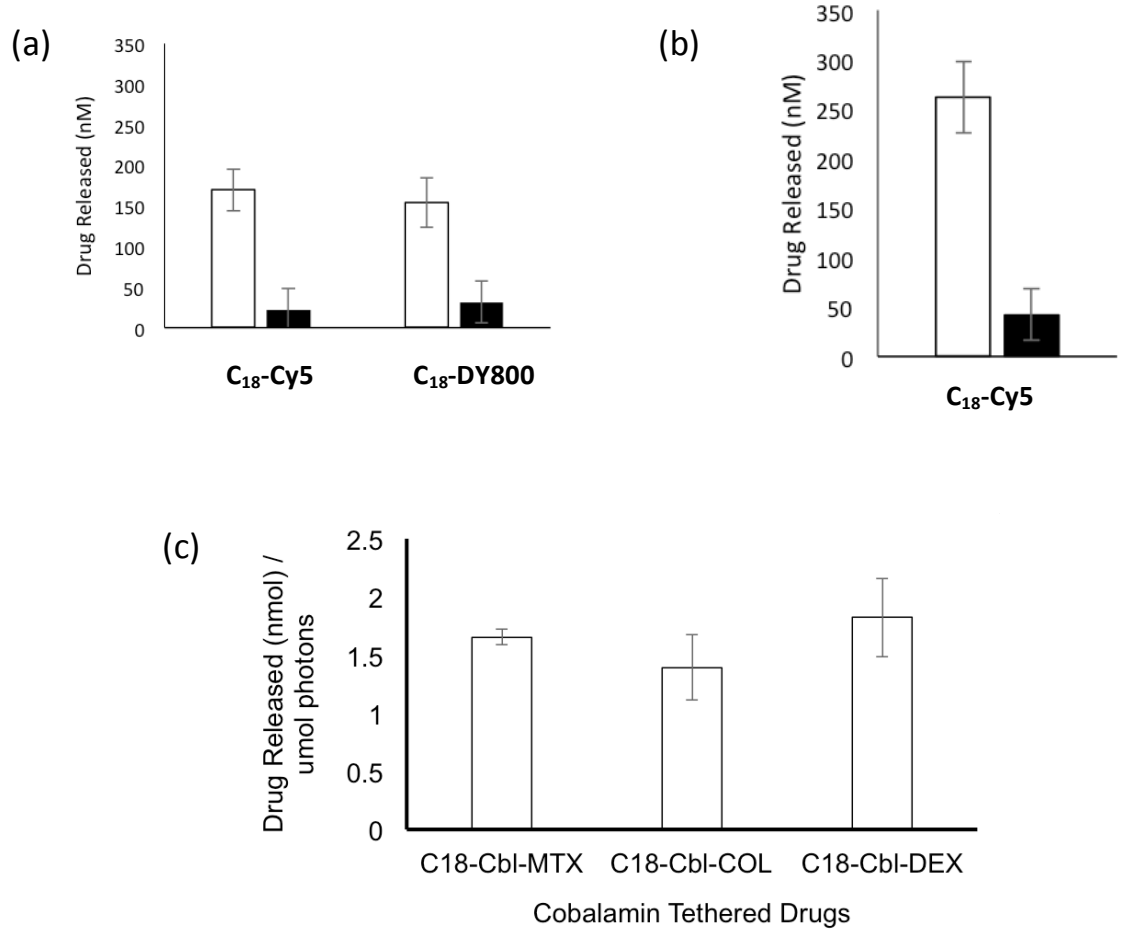


Figure S27. Wavelength selective release of DEX from erythrocyte-bound **C_{18}-CbI-DEX** and the effect on GRα subcellular location. (a) HeLa cells treated with DMSO. (b) HeLa cells treated with erythrocyte-bound **C_{18}-CbI-DEX** in the dark. (c) HeLa cells treated with erythrocyte-bound **C_{18}-CbI-DEX** and illuminated at 780 nm. (d) HeLa cells treated with erythrocyte-bound **C_{18}-CbI-DEX** and illuminated at 525 nm. GRα in HeLa cells was visualized using Alexa488 antiRabbit/anti-GRα.

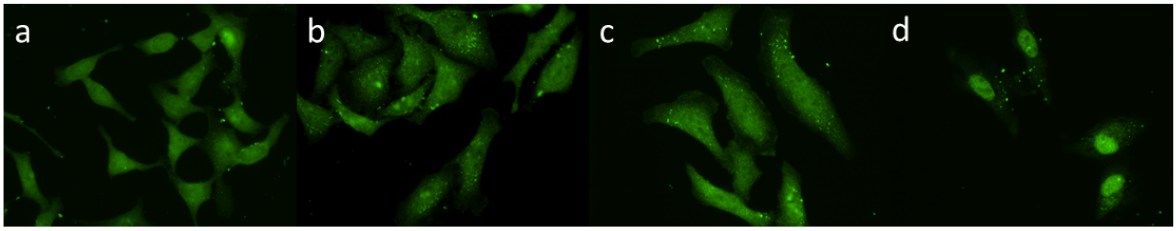


Figure S28. Quantitation of the effect of DEX release from **C_{18}-CbI-DEX/C_{18}-Cy5**-loaded erythrocytes on GRα subcellular location in HeLa cells. Analysis of data from Figure 5. GRα is shown in green and HOESCHT 33342 nuclear staining in blue. (a) HeLa cells treated with **C_{18}-CbI-DEX/C_{18}-Cy5**-loaded erythrocytes and illuminated at 660 nm. (b) HeLa cells treated with **C_{18}-CbI-DEX/C_{18}-Cy5**-loaded erythrocytes in the dark. Lines indicate where cross-sectional fluorescent intensity is measured. A plot of two of these cross sections showing fluorescent intensities of GRα (green) and Hoescht (blue) is shown in (c, light) and (d, dark). (e) The correlation between HOESCHT and FITC fluorescence from (c). (f) The absence of correlation between HOESCHT and FITC fluorescence from (d). R^2 values for HOESCHT/FITC correlations for light (0.90 ± 0.01, n = 6) and dark (0.49 ± 0.09, n = 6) samples.

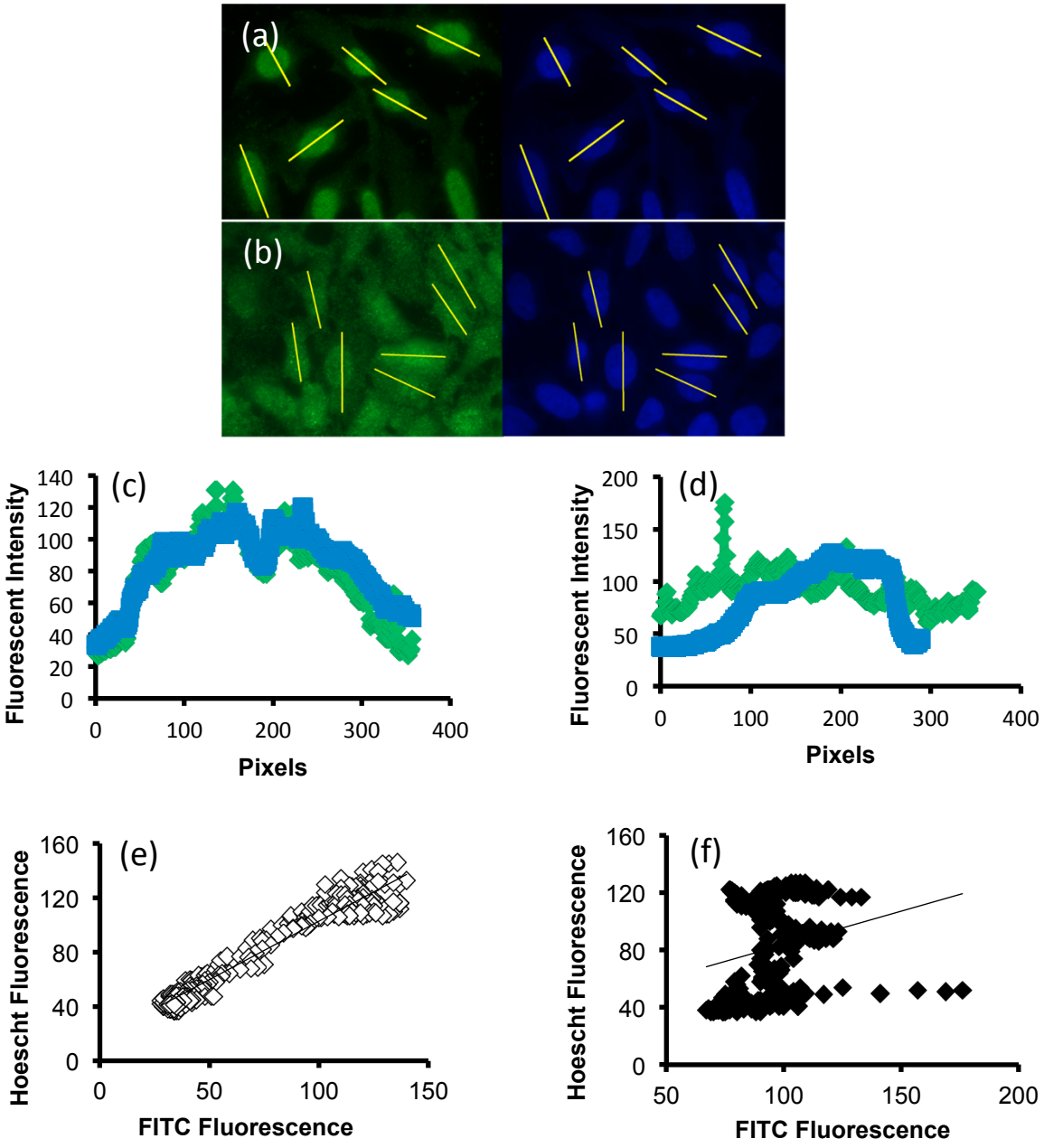


Figure S29. Long wavelength release of COL from erythrocytes. (a) Untreated HeLa cells, (b) HeLa cells treated with **C_{18}-CbI-COL/C_{18}-Cy5**-loaded erythrocytes in the dark, (c) HeLa cells treated with **C_{18}-CbI-COL/C_{18}-Cy5**-loaded erythrocytes and illuminated at 660 nm.

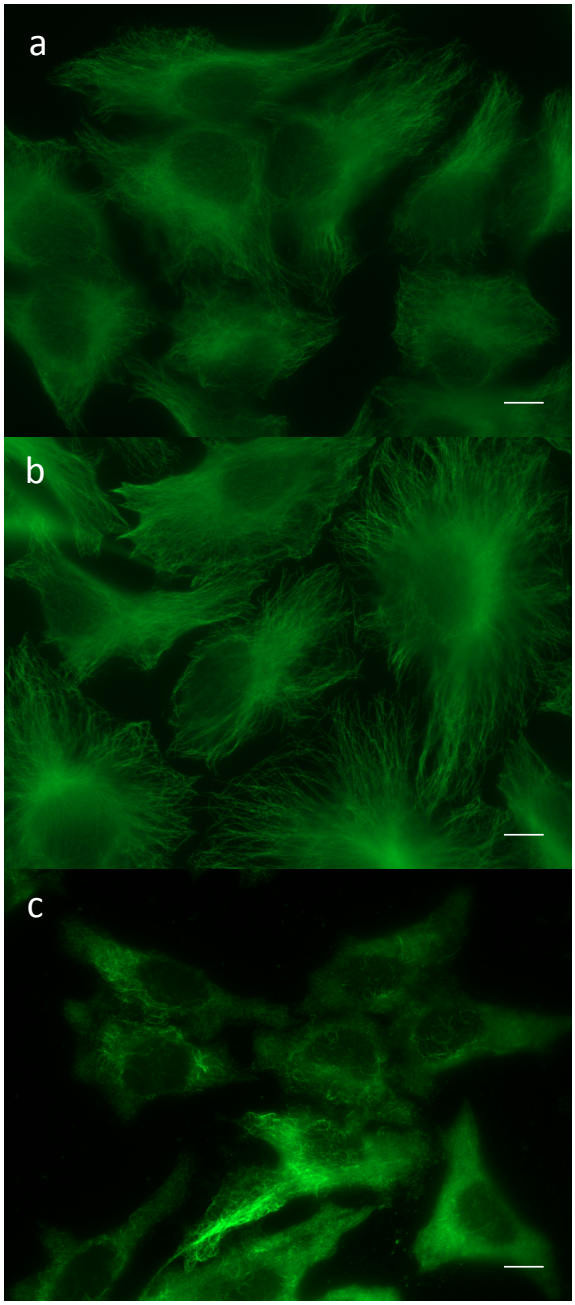


Figure S30. MTX release from **C_{18}-CbI-MTX/C_{18}-Cy7**-loaded erythrocytes at 725 nm shifts the thermal stability of DHFR where left 3 lanes (dark) and right 3 lanes (725 nm). GAPDH used as loading control.

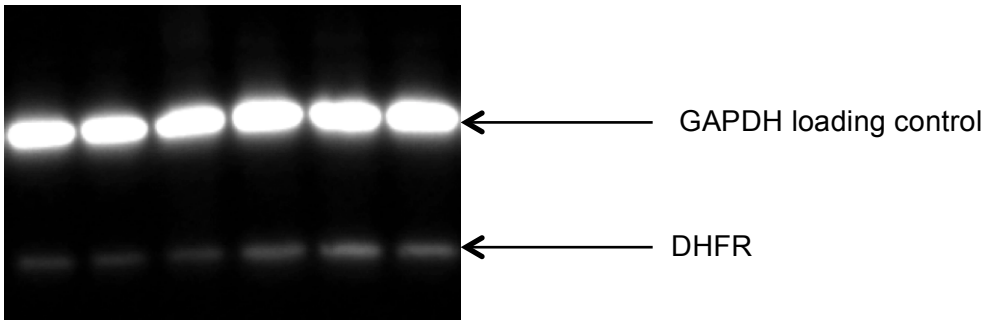


Table S1. Solvent gradient used for preparative HPLC to purify **C_{18}-Cbl-MTX**, **C_{18}-Cbl-DEX**, **C_{18}-Cbl-COL**, **C_{18}-Cbl-TAM**, and **C_{18}-Cbl-FAM**. All solvents contained 0.1% v/v TFA.

Time (min)	Flow (mL/min)	H_2O (%)	CH_3CN (%)
0.01	10.00	90.0	10.0
10.00	10.00	90.0	10.0
30.00	10.00	45.0	55.0
45.00	10.00	35.0	65.0
60.00	10.00	25.0	75.0
65.00	10.00	0.0	100.0
75.00	10.00	0.0	100.0
80.00	10.00	90.0	10.0
90.00	10.00	90.0	10.0
91.00	0.00	90.0	10.0

Table S2. Gradient used for LC-MS analysis of **C_{18}-DY800** and intermediates using an Agilent Eclipse Plus C_{18} column (3.5 µm, 4.6 mm x 150 mm). All solvents contained 0.1% v/v FA. Product elution was detected using absorbance at 600 nm.

Time (min)	Flow (mL/min)	Water (%)	CH_3CN (%)
0	1	97	3
5	1	97	3
18	1	3	97
20	1	3	97
24	1	97	3

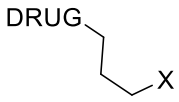
Table S3. Gradient used for LC-MS analysis of **C_{18}-Cy5** and **C_{18}-Cy7** using a Viva C_4 column (5 µm, 50 mm x 2.1 mm). Product elution was detected using absorption at 600 nm. All solvents contained 0.1% v/v FA.

Time (min)	Flow (mL/min)	Water (%)	CH_3CN (%)
0	1	90	10
1	1	90	10
7	1	5	95
8	1	0	100
10	1	0	100
11	1	90	10
16	1	90	10

Table S4. Elution times and predicted/detected masses of intact **C_{18}-Cbl-MTX**, **C_{18}-Cbl-COL**, and **C_{18}-Cbl-DEX** when analyzed by their various LC-MS assays using the gradient from Table S2.

C_{18}-Cbl-Drug	Elution Time (min)	Predicted Masses ($M + 2H^+$) z=2 m/z	Detected Masses ($M + 2H^+$) z=2 m/z
C_{18}-Cbl-MTX	6.8	1060.0	1060.3
C_{18}-Cbl-COL	7.3	1026.0	1026.5
C_{18}-Cbl-DEX	7.5	1079.1	1079.3

Table S5. Elution times and detected masses of the photocleavage products for **C_{18}-Cbl-MTX**, **C_{18}-Cbl-COL**, and **C_{18}-Cbl-DEX** after illumination at 525 nm light for 20 min in PBS buffer, pH 7.4. These products are described in detail in Scheme S11. Detection was performed using the MTX, COL, and DEX LC-MS assays. The solvent gradient is described in Table 2. Note that the aldehyde (X=O) byproducts are detected in the hydrated form for cleaved MTX and COL but not for DEX.



	Elution Time (min)				Yield (mole fraction)	
	X = H	**X = OH**	**X = O**	**C_{18}-Cbl**	**X = H**	**Other Products**
C_{18}-Cbl-MTX	3.3	2.9	3.1	6.8	0.83	0.17
Mass	494.2	510.2	528	812.8		
C_{18}-Cbl-COL	4.7	4.4	4.4	6.7	0.20	0.80
Mass	426.1	442	460	812.7		
C_{18}-Cbl-DEX	5.5	5.5	5.5	6.7	n/a	n/a
Mass	514.3	533	532	812.7		

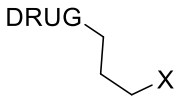
Table S6. The solvent gradient used for analytical MTX, COL, and DEX LC-MS assays. All solvents contained 0.1% v/v FA.

Time (min)	Flow (mL/min)	Water (%)	CH_3CN (%)
0	1	97	3
1	1	97	3
8	1	20	80
10	1	20	80
11	1	97	3
15	1	97	3

Table S7. Power output of each of the LED arrays (Figure S17) after filters were applied to cut off wavelengths shorter than those specified. MacNan colored film gels (Aqua, Blue, Green, Magenta, and Red) were used in combination as filters for the following LEDs: 525 nm (Green), 660 nm (Red and Magenta), 725 nm (Red and Magenta), and 780 nm (Blue, Green, and Aqua).

LED Wavelength (nm)	**Power (mW/cm^2)**	**Photon Flux (µmol photon/s-m^2)**
525	1.50 ± 0.03	66 ± 1
660	3.30 ± 0.02	183 ± 1
725	6.00 ± 0.05	365 ± 3
780	3.30 ± 0.02	216 ± 1

Table S8. Elution times for the products of **C_{18}-Cbl-MTX** and **C_{18}-Cbl-COL** after fluorophore assisted, photocleavage from erythrocytes. **C_{18}-Cbl-MTX** was loaded with a 1:5 **C_{18}-Cbl-Drug**:C_{18}-fluorophore ratio. **C_{18}-Cbl-COL** and **C_{18}-Cbl-DEX** were loaded with a 1:1 C_{18}-Cbl-Drug:C_{18}-fluorophore. *Cbl-DEX is not listed in the table as the ester of the cleaved product is hydrolyzed upon photo-release (possibly due to the presence of esterases) and only DEX was recovered*. Photolysis times were all 30 min at the wavelengths indicated. These products are described in detail in Scheme S11. Detection was performed using the MTX, COL, and DEX LC-MS assays. The solvent gradient is described in Table 2.



	Elution Times (min)				Yield (mole fraction)	
	X = H	**X = OH**	**X = O**	**C_{18}-Cbl**	**X = H**	**Other Products**
C_{18}-Cbl-MTX						
525 nm	3.4	2.9	3	n/a	0.76	0.24
660 nm (Cy5)	3.4	2.9	3	n/a	0.80	0.20
725 nm (AF700)	3.3	2.9	3	n/a	0.80	0.20
725 nm (Cy7)	3.4	n/a	3	n/a	0.80	0.20
780 nm (DY800)	3.4	2.9	n/a	n/a	0.80	0.20
C_{18}-Cbl-COL						
525 nm	4.6	4.3	4.3	n/a	0.91	0.09
660 nm (Cy5)	4.5	n/a	n/a	n/a	<0.94	
780 nm (DY800)	4.5	n/a	n/a	n/a	<0.90	

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