Supporting Information for

"Chloroquine Transport in *Plasmodium falciparum* I: Influx and Efflux Kinetics for Live Trophozoite Parasites using a Novel Fluorescent Chloroquine Probe"

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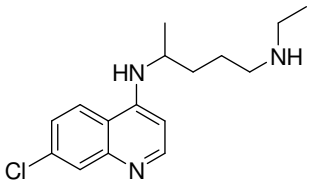
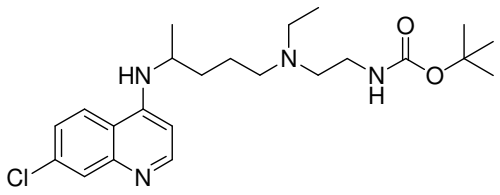
FIGURE S1: Synthetic methods

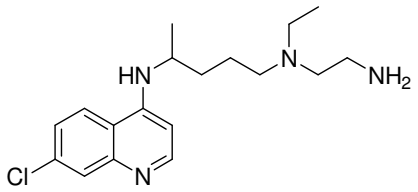
General. All reagents and solvents were commercially available and used without further purification. Flash chromatography was performed on Kieselgel 60, particle size 0.032-0.063 mm. NMR spectra were obtained on a 300 MHz (^{1}H-NMR) and 75 MHz (^{13}C-NMR) Varian FT-NMR spectrometer using $CDCl_3$ as solvent. Electrospray mass spectra (ESI-MS) were collected on a Thermo Finnigan LCQ instrument. Samples were dissolved in acetonitrile/water (1:1 v/v) containing 1% acetic acid (1 mg/mL) for MS analysis.



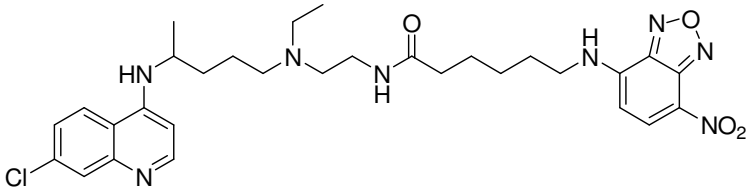
Monodesethylchloroquine (4). Monodesethylchloroquine was synthesized by following a procedure reported in the literature.[1] ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.13 (t, J = 6.9 Hz, 3H), 1.31 (d, J = 6.3 Hz, 3H), 1.50-1.86 (m, 4H), 2.60-2.74 (m, 4H), 3.72 (m, 1H), 5.52 (bd, J = 5.8 Hz, 1H), 6.39 (d, J = 5.4 Hz, 1H), 7.33 (dd, J = 2.4 Hz, 8.7 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.93 (d, J = 2.4 Hz, 1H), 8.50 (d, J = 5.4 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 15.0, 19.9, 26.3, 33.9, 44.0, 48.2, 49.1, 98.9, 117.3, 121.4, 124.7, 128.4, 134.5, 149.1, 149.2, 151.7.



***N*-(7-Chloro-4-quinolyl)-*N′*-ethyl-*N′*-(*N″*-*t*-Boc-aminoethyl)-1,4-diaminopentane (5).** A mixture of desethylchloroquine **4** (0.34 g, 1.15 mmol, 1 equiv.), *N*-*t*-Boc-glycinal **3** (0.55 g, 3.45 mmol, 3 equiv.) and a catalytic amount of AcOH was stirred in 10 mL of methanol at room temperature for 15 minutes (*N*-*t*-Boc-glycinal **3** was synthesized following a procedure reported in the literature).[2] Then, $NaCNBH_3$ (0.22 g, 3.45 mmol, 3 equiv.) was added and the reaction mixture was heated to reflux overnight. After cooling, 1 mL of water was added and methanol was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 and extracted with saturated $NaHCO_3$. The combined organic layers were evaporated under reduced pressure and the residue was purified by flash chromatography using MeOH/CH_2Cl_2 (1:9) as the mobile phase. After gradually increasing the concentration of MeOH to 30%, 0.33 g (0.76 mmol, 65% yield) of a yellow oil was obtained. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.00 (t, *J* = 6.9 Hz, 3H), 1.33 (d, *J* = 6.3 Hz, 3H), 1.42 (s, 9H), 1.50-1.82 (m, 4H), 2.40-2.62 (m, 6H), 3.16 (m, 2H), 3.73 (m, 1H), 4.99 (bs, 1H), 5.22 (m, 1H), 6.41 (d, *J* = 5.4 Hz, 1H), 7.34 (dd, *J* = 9.0 Hz, 1.8 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 8.49 (d, *J* = 5.4 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 11.3, 20.2, 23.7, 28.3, 34.2, 38.1, 47.4, 48.3, 52.5, 53.0, 79.1, 99.0, 117.1, 121.2, 125.1, 128.2, 134.9, 148.7, 149.2, 151.4, 156.0.



***N*-(7-Chloro-4-quinolyl)-*N′*-ethyl-*N′*-(*N″*-aminoethyl)-1,4-diaminopentane (6).** To a solution of *N*-(7-chloro-4-quinolyl)-*N′*-ethyl-*N′*-(*N″*-*t*-Boc-aminoethyl)-1,4-diaminopentane **5** (0.17 g, 0.39 mmol, 1 equiv.) in MeOH, 2M HCl in Et_2O (1.5 mL, 3.0 mmol, 7.7 equiv.) was added and allowed to stir overnight at room temperature. Methanol was removed *in vacuo* and the residue was dissolved in CH_2Cl_2, extracted with 1N NaOH solution and the combined organic layers were removed under reduced pressure. Flash chromatography (2% Et_3N/MeOH) gave 0.1 g (0.3 mmol, 78% yield) of a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$/CD_3OD) δ = 0.95 (t, *J* = 6.9 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 3H), 1.42-1.76 (m, 4H), 2.35-2.54 (m, 6H), 2.55-2.75 (m, 5H), 3.66 (m, 1H), 6.36 (d, *J* = 5.7 Hz, 1H), 7.31 (dd, *J* = 9.0 Hz, 2.1 Hz, 1H), 7.81 (d, *J* = 9.0 Hz), 7.84 (d, *J* = 2.1 Hz, 1H), 8.37 (d, *J* = 5.7 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 11.5, 20.1, 23.8, 34.0, 39.4, 47.4, 48.1, 53.1, 55.9, 98.9, 117.1, 121.2, 124.7, 128.4, 134.4, 149.0, 149.1, 151.7.



***N*-(7-Chloro-4-quinolyl)-*N′*-ethyl-*N′*-(*N″*-6′-(7-nitro-2,1,3-benzoxadiazol-4-amino) hexanamidoethyl-1,4-diaminopentane (1).** A mixture *N*-(7-Chloro-4-quinolyl)-*N′*-ethyl-*N′*-(*N″*-aminoethyl)-1,4-diaminopentane **6** (0.055 g, 0.164 mmol, 1.28 equiv.) and succinimidyl 6-*N*-[4-(7-nitrobenz-2-oxa-1,3- diazol)]aminohexanoate **7** (0.050 g, 0.128

mmol, 1 equiv.) in CH_2Cl_2 were stirred at room temperature for 1 h. The reaction mixture was extracted with water and CH_2Cl_2 and the combined organic layers were evaporated under reduced pressure. Flash chromatography (1:1 MeOH/CH_2Cl_2) gave 0.066 g (0.11 mmol, 85% yield) of a dark red oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.00 (t, *J* = 6.9 Hz, 3H), 1.26-1.40 (m, 5H), 1.50-1.80 (m, 8H), 2.02 (t, *J* = 7.2 Hz, 2H), 2.44-2.60 (m, 6H), 3.29 (m, 2H), 3.46 (m, 2H), 3.73 (m, 1H), 5.06 (d, *J* = 7.5 Hz, 1H), 6.05 (bs, 1H), 6.12 (d, *J* = 8.7 Hz, 1H), 6.42 (d, *J* = 5.7 Hz, 1H), 7.34 (dd, *J* = 9.0 Hz, 2.4 Hz, 1H), 7.71 (d, *J* = 9.0 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 8.45 (d, *J* = 8.7 Hz, 1H), 8.50 (d, *J* = 5.7 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 11.7, 20.6, 24.0, 24.8, 26.5, 28.0, 34.4, 36.1, 37.1, 43.9, 47.7, 48.6, 52.3, 53.2, 98.8, 99.3, 117.4, 121.4, 123.4, 125.4, 128.6, 135.2, 136.9, 144.2, 144.5, 144.7, 149.3, 152.0, 172.9; MS (ESI) *m*/*z* calcd for $C_{30}H_{39}ClN_8O_4$ 610.3. Found $(M + H)^+$: 611.2.

References:

1) Ansari, A. M.; Craig, J. C. A Convenient, Short Synthesis of Desthylchloroquine [7-Chloro-4-(4'-ethylamino-1'-methyl-butylamino)quinoline]. *Synthesis* **1995**, 147-149.

2) Myers, M. C.; Pokorski, J. K.; Appella D. H. Peptide Nucleic Acids with a Flexible Secondary Amine in the Backbone Maintain Oligonucleotide Binding Affinity. *Org. Lett.* **2004**, *6*, 4699-4702.

FIGURE S2: NMR Spectra of NBD-CQ and intermediates
^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) of *N*-(7-chloro-4-quinolyl)-*N*′-ethyl-*N*′-(*N*″-6′-(7-nitro-2,1,3-benzooxadiazol-4-amino)hexanamidoethyl-1,4-diaminopentane (**1**)

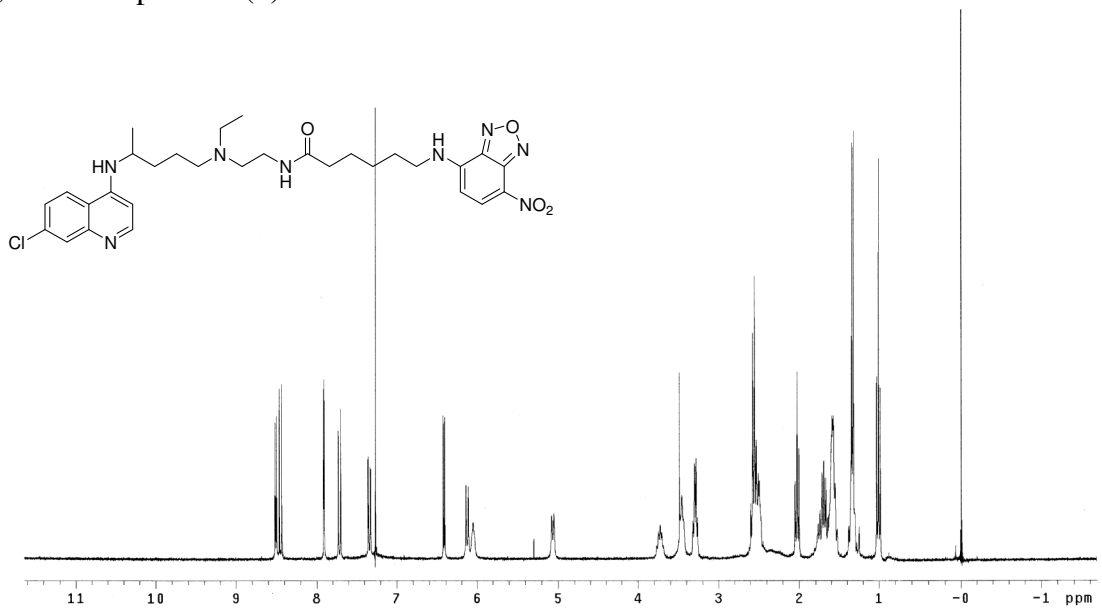
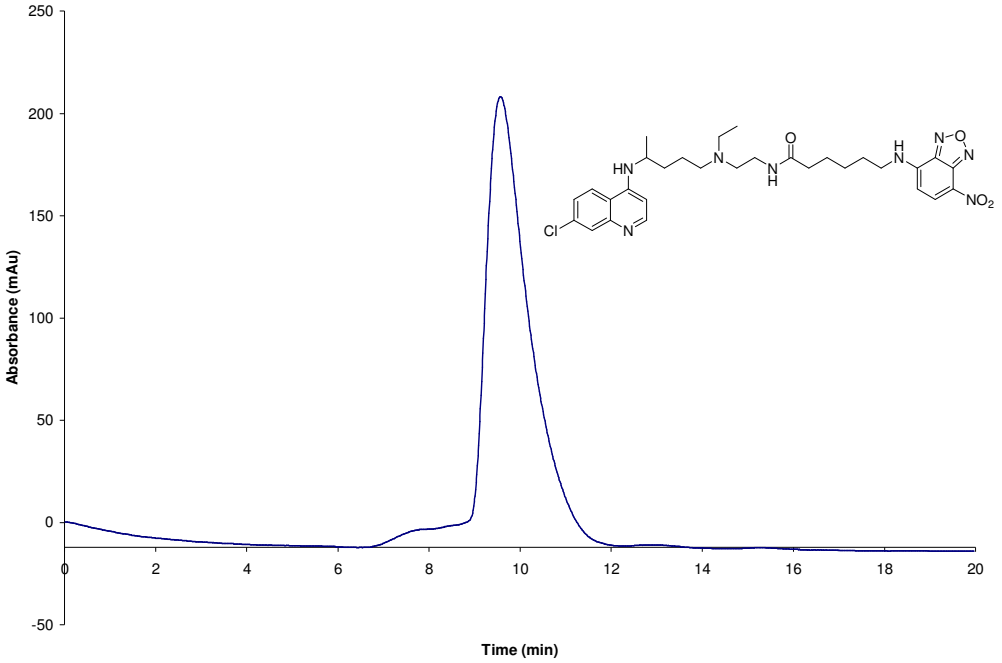
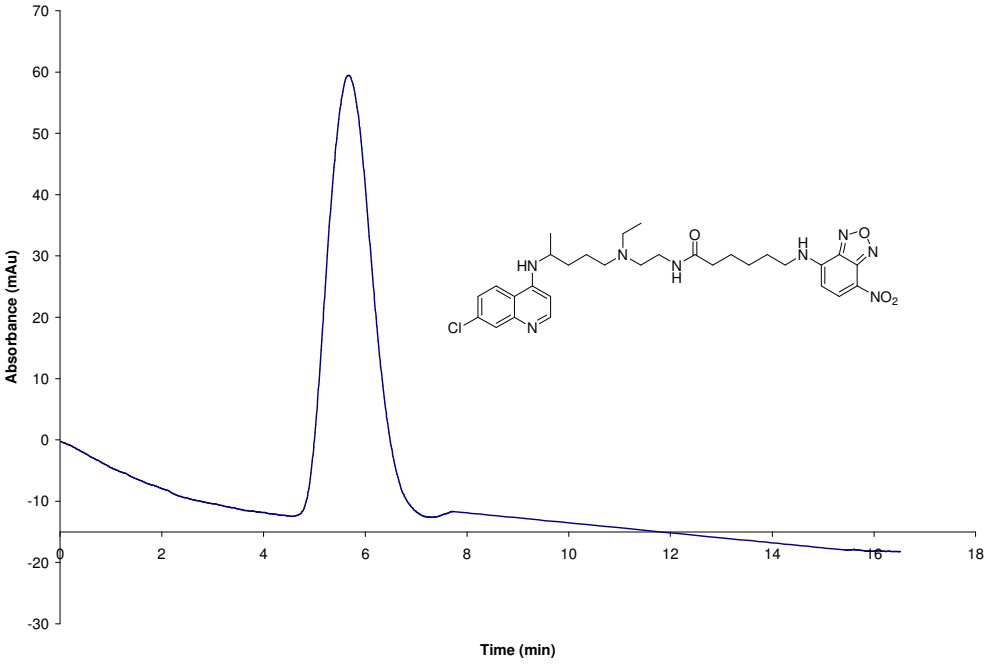


FIGURE S3: HPLC analysis of *N*-(7-chloro-4-quinolyl)-*N′*-ethyl-*N′*-(*N″*-6′-(7-nitro-2,1,3-benzooxadiazol-4-amino)hexanamidoethyl-1,4-diaminopentane (**1**)



Conditions: C18 column (YMC-ODS-AQ), mobile phase: 20% acetonitrile, 70% water containing 0.1% trifluoroacetic acid, injection volume: 20 µl, concentration: 0.5 mg/mL, UV detection: 254 nm, flow rate: 1 mL/min



Conditions: Nucleosil NH2 column (Alltech applied science), mobile phase: 80% acetonitrile, 20% water containing 0.1% trifluoroacetic acid, injection volume: 10 µl, concentration: 0.5 mg/mL, UV detection: 254 nm, flow rate: 1 mL/min

FIGURE S4: Fluorescence spectra
(Left) 1 uM NBD-CQ excitation scan from 415 nm to 530 nm (dashed line), and emission scan from 485 nm to 650 nm (solid line). Excitation λ_{max}= 470 nm and emission λ_{max}= 550 nm. 3 ml of a 1 uM NBD-CQ in 10 mM MES-Tris pH 6.5 buffer was used for scanning excitation and emission using a PTI Alphascan Fluorometer.
(Right) 100 nM NBD-CQ in pH 5.5 (□), 6.5 (Δ) and 8.5 (○). Inset is an expansion for NBD-CQ concentrations less than 1 µM.

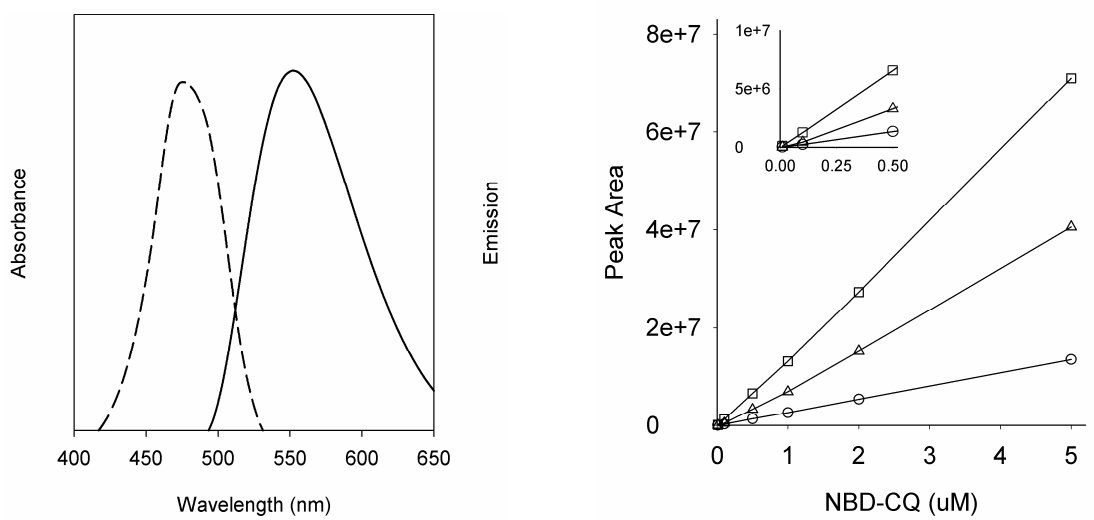


FIGURE S5: Thin layer calibration of NBD-CQ
Thin layer NBD-CQ calibration in 25 mM MES-Tris pH 5.2 (▼) and 5.6 (Δ).