Electronic Supplementary Material (ESI) for Biomaterials Science.

Micelle carriers based on dendritic macromolecules containing bis-MPA and glycine for antimalarial drug delivery

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Supplementary Information

S1. Synthesis and chemical characterization of HDLDBC-bGMPA, bGMPA(NH_3^+)$_8$-Pluronic® F127-bGMPA(NH_3^+)$_8$.

S2. Self-assembly of nanocarriers in water and antimalarial drug encapsulation.

S3. Targeting experiments.

S1. Synthesis and chemical characterization of HDLDBC-bGMPA, bisGMPA(NH_3^+)$_8$-Pluronic® F127- bisGMPA(NH_3^+)$_8$

Synthesis of bisGMPA(NHBoc)$_8$- Pluronic® F127- bisGMPA(NHBoc)$_8$

Alkyne functionalized Pluronic® F127 (1.00 g, 7.74×10^{-2} mmol, 1.00 eq.) and t-Boc *bis*-GMPA dendron (514 mg, 2.01×10^{-1} mmol, 2.60 eq.) were dissolved into 8 mL of dimethylformamide (DMF) in a Schlenk flask and 3 vacuum-argon cycles were made to remove the air. The reaction mixture was stirred under argon atmosphere at 45 °C. $CuSO_4\cdot5H_2O$ (18.2 mg, 6.19×10^{-2} mmol, 0.80 eq), (*L*)-ascorbate (24.1 mg, 1.24×10^{-1}mmol, 1.60 eq.) and TBTA (32.8 mg, 6.19×10^{-2} mmol, 0.80 eq.) were dissolved into DMF (4 mL) in a second Schlenk flask and exposed to 3 vacuum-argon cycles. The copper solution was stirred under argon atmosphere at 45 °C for 15 min and was added through a cannula to the previous azide-alkyne reaction mixture. The resulting mixture was stirred under argon atmosphere at 45 °C for 2 days. Then, hot brine (100 mL) was added to the reaction mixture and the product was extracted with dichloromethane (3 × 100 mL). The organic phases were collected, washed with hot brine (2 × 100 mL), dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure to obtain a yellow solid. The crude product was precipitated into cold diethyl ether, recovered by filtration and washed with cold diethyl ether to yield a white powder. Finally, the product was dialyzed (cellulose membrane, 1000 Da cut-off, Spectra/Por®) against methanol for 24 hours to obtain a light yellow solid (855 mg, 61%).

^{1}H (400 MHz, $CDCl_3$) δ (ppm): 1.11 (m, 201H), 1.26 (m, 42H), 1.41 (m, 152H), 1.62 (m, 4H), 1.94 (m, 4H), 3.38 (m, 67H), 3.44-3.74 (m, ~1000H), 3.81 (t, 4H, J = 4.8 Hz), 3.88 (m, 32H), 3.94 (m, 8H), 3.88 (m, 16H), 4.11 (t, J = 6.4 Hz, 4H), 4.18-4.35 (m, 60H), 4.43 (t, J = 4.8 Hz, 4H) 5.29 (s, 4H), 5.35 (bs), 7.02 (d, J = 8.8 Hz, 4H), 7.17 (bs), 7.82 (s, 2H), 8.00 (d, J = 8.0 Hz, 4H). FTIR (ν_{max}/cm^{-1}): 3360 (N-H st), 2883 (C-H st), 1755 (C=O st ester), 1718 (C=O st carbamate), 1670 (C=O st amide), 1533 (N-H δ), 1468 (CH_2, -CH_3 δ), 1101 (C-O-C st).

SEC (ref PMMA): 2 populations; Mw 19745 g.mol^{-1}; Đ: 1.08.

Synthesis ofHDLDBC-BGMPA, bisGMPA(NH_3^+)$_8$-Pluronic® F127- bisGMPA(NH_3^+)$_8$

*Bis*GMPA(NHBoc)$_8$-Pluronic® F127-*bis*GMPA(NHBoc)$_8$ (653 mg, 3.62×10^{-2} mmol, 1.00 eq.) was dissolved into ethyl acetate (3 mL), and a saturated solution of $HCl_{(g)}$ (7 mL) in ethyl acetate was carefully added to it. The reaction mixture was stirred at room temperature for 45 min until a white gel appeared. The reaction mixture was diluted with ethyl acetate (40 mL) and was stirred for an additional 30 min. Then, it was stirred under vacuum to remove the hydrochloric acid and the solvent was evaporated under reduced pressure. The gel was washed firstly with pure ethyl acetate and subsequently with pure methanol. In each case, the solution was stirred for 15 min under vacuum in order to remove the residual hydrochloric acid traces and then, the solvent was evaporated under reduced pressure to obtain a solid.

^{1}H (500 MHz, CD_3OD) δ (ppm): 1.14 (m, 201H), 1.27 (s, 6H), 1.32 (s, 20H), 1.39 (s, 24H), 1.64 (m, 4H), 1.95 (m, 4H), 3.47 (m, 67H), 3.51-3.73 (m, ~1000H), 3.84 (t, J = 4.8 Hz, 4H), 3.96 (s, 8H), 4.00 (s, 16H), 4.03 (s, 32H), 4.12 (t, J = 6.0 Hz, 4H), 4.31 (m, 24H), 4.45 (m, 40H) 5.30 (s, 4H), 7.38 (d, J = 8.8 Hz, 4H), 8.23 (d, J = 8.8 Hz, 4H), 8.19 (s, 2H).

^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 17.4-18.3, 26.3, 26.9, 29.3, 31.1, 41.5, 42.2, 47.3, 51.3, 62.6-76.8, 115.9, 124.2, 125.8, 132.8, 140.4, 163.7, 167.6, 168.8, 170.8-171.2, 174.0, 174.7-175.0.

FTIR (ν_{max}/cm^{-1}): 3600-3300 (bs N-H^+), 2885 (C-H st), 1751 (C=O st ester), 1663 (C=O st amide and N-H^+ δ), 1545 (N-H δ), 1468 (CH_2-, CH_3 δ), 1099 (C-O-C st).

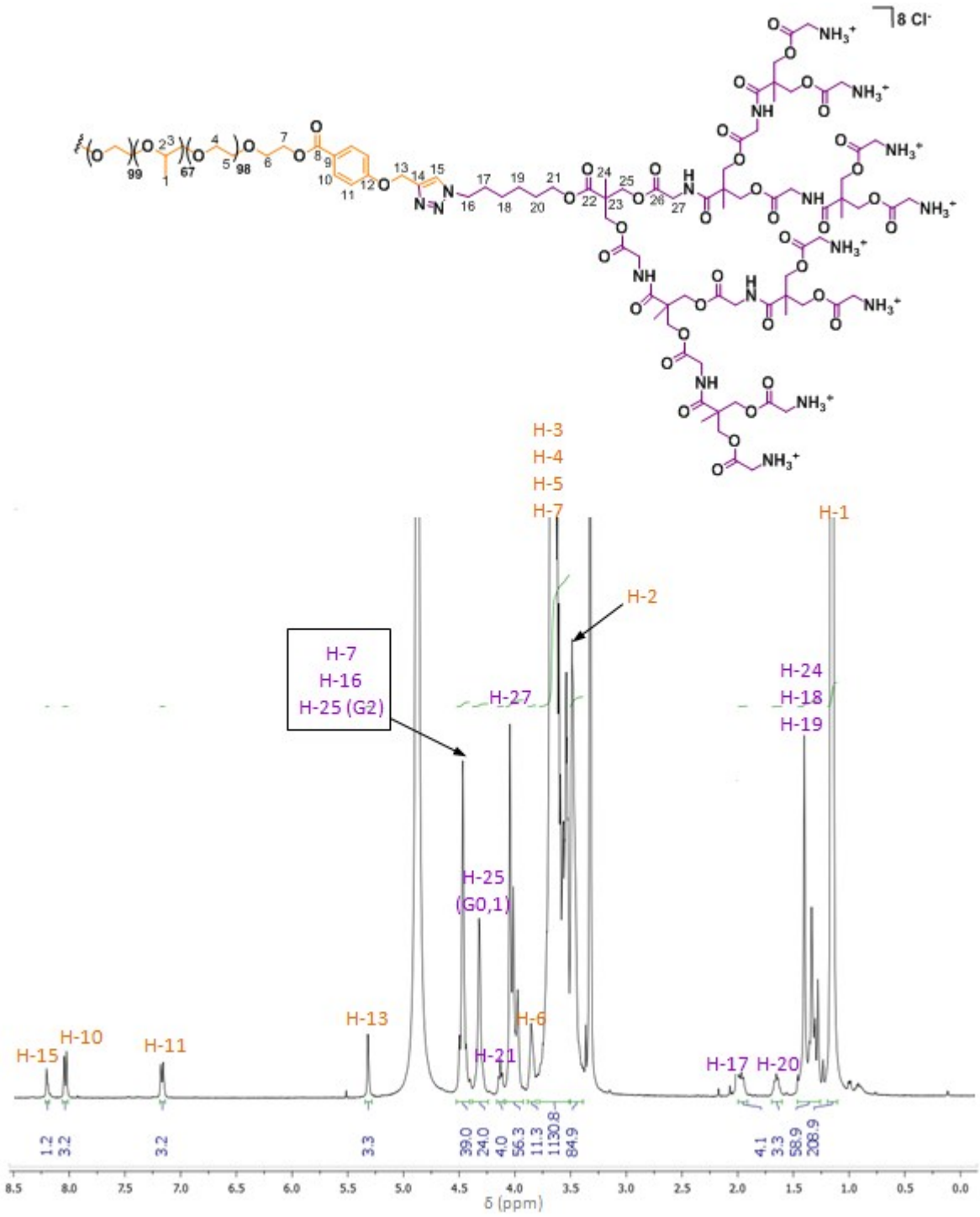


Fig. S1.1. ^{1}H NMR spectrum of $(NH_3^+)_8$-HDLDBC-$(NH_3^+)_8$ with signals relative integration recorded at 400 MHz in CD_3OD.

Table S1.1. Theoretical and measured signal integrations of ^{1}H NMR signals.

δ (ppm)	proton	theoretical signal integration	measured signal integration
1.14	H-1	201	208.9
1.27-1.39	H-18,19,24	50	58.9
1.64	H-20	4	3.3
1.95	H-17	4	4.1
3.47	H-2	67	84.9
3.51-3.73	H-3,4,5	~1000H	1131.8
3.84	H-6	4	undetermined[1]
3.96-4.03	H-27	56	56.3
4.12	H-21	4	4
4.31	H-25(G0,1)	24	24.0[2]
4.45	H-7,16,25(G2)	40	39.0
5.30	H-13	4	3.3
7.38	H-11	4	3.3
8.23	H-10	4	3.2
8.19	H-15	2	1.2

[1]Due to the overlapping of the signals at 3.51-3.73 and 3.84 ppm, the relative integration of this signal could not be determined experimentally. [2]Relative integration signal used as reference.

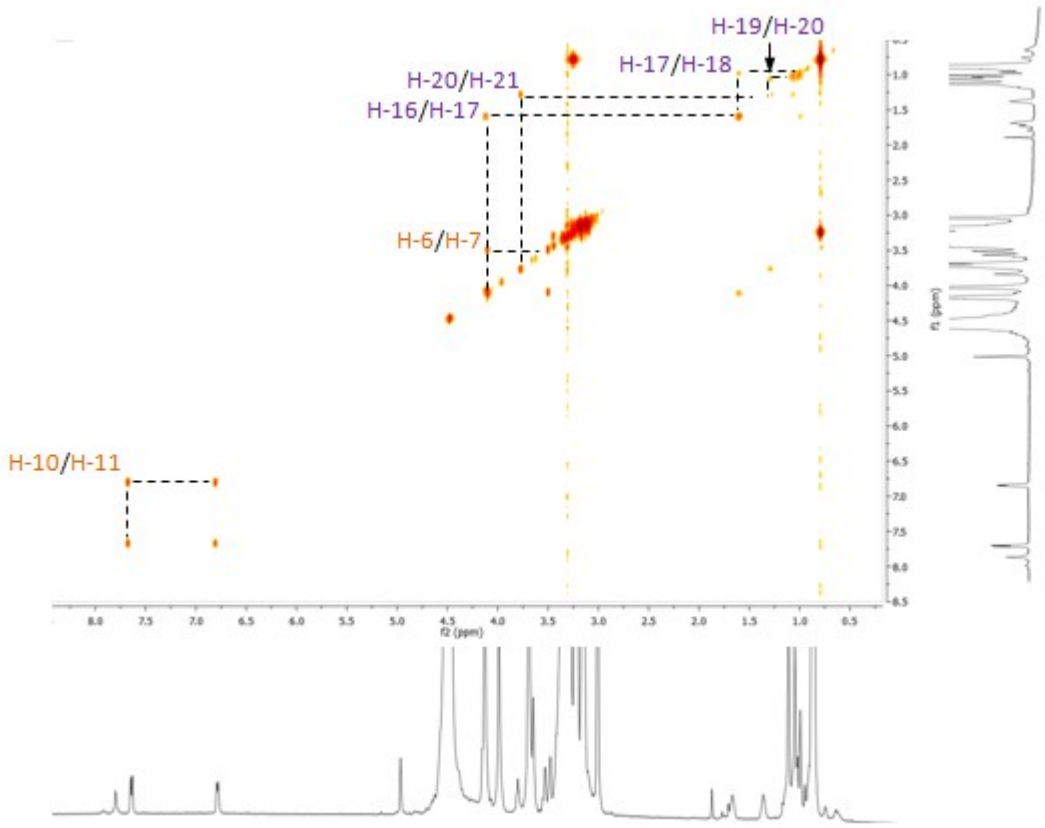


Fig. S1.2. ^{1}H-^{1}H COSY spectrum of bisGMPA(NH_3^+)$_8$-Pluronic® F127-bisGMPA(NH_3^+)$_8$ (HDLDBC-bGMPA) recorded at 500 MHz in CD_3OD.

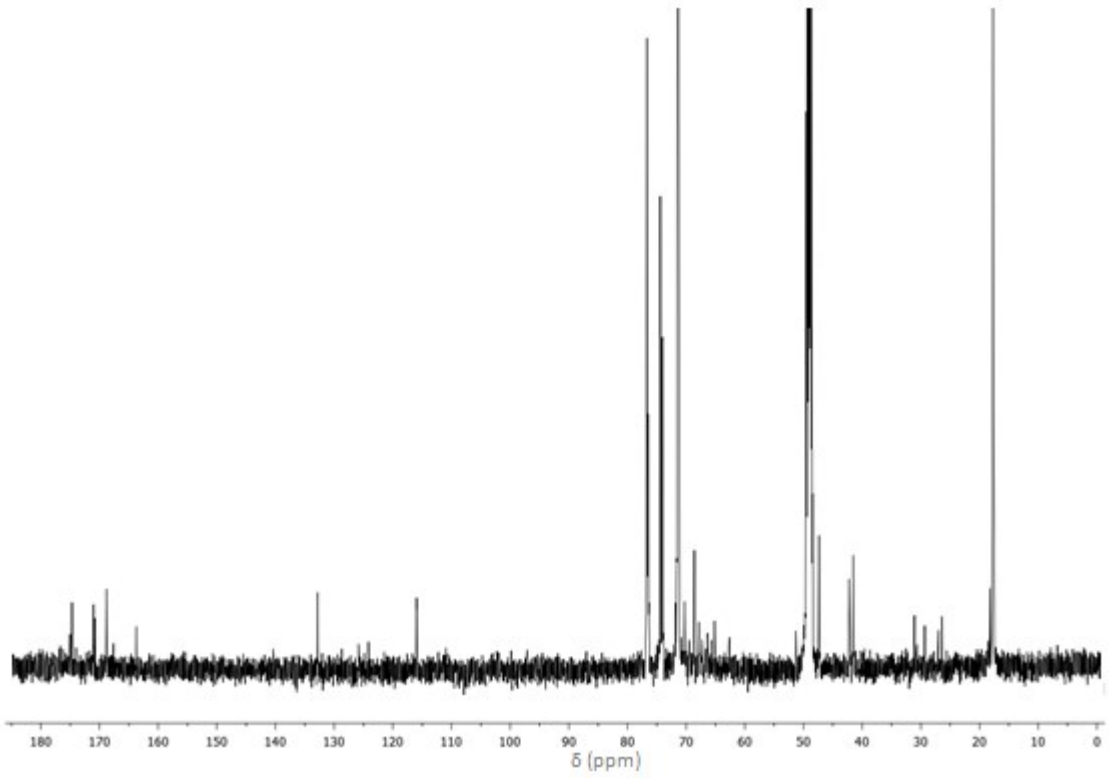
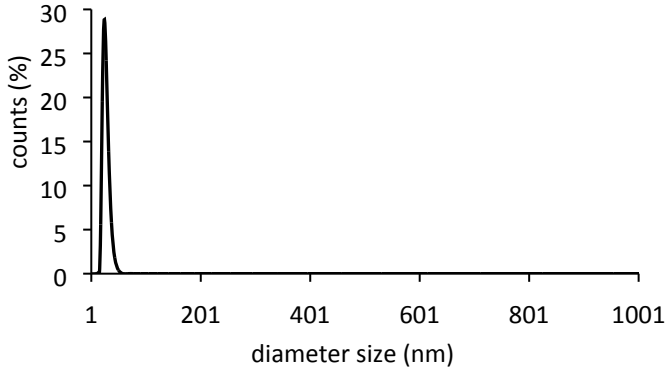
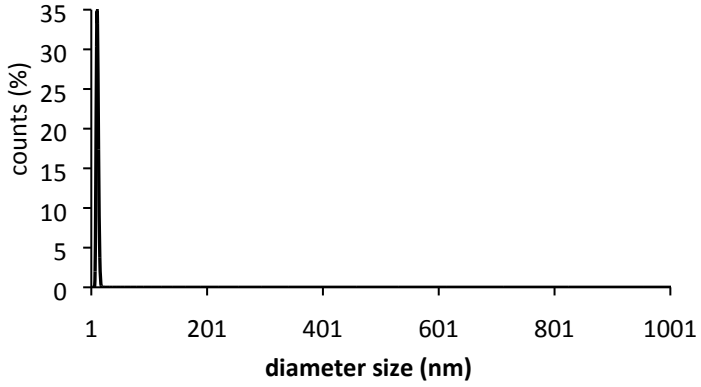


Fig. S1.3. ^{13}C NMR spectrum of bisGMPA(NH_3^+)$_8$-Pluronic® F127-bisGMPA(NH_3^+)$_8$ (HDLDBC-bGMPA) recorded at 125 MHz in CD_3OD.

A. HDLDBC-bGMPA



B.DHP-bMPA (n=4)



C.

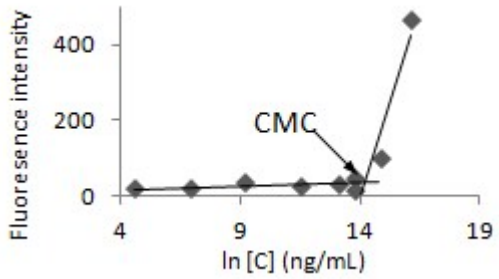


Fig. S2.1. A. DLS curve of HDLDBC-bGMPA and B. DLS curve of DHP-bMPA (n =4). DLS measurements were performed with a Malvern Instruments Nano ZS at 1mg/mL at 25°C ; results are given as size distribution in number. C. CMC determination for naked Pluronic® F-127 at 25 °C according to the Nile Red fluorescence determination.

Table S2.1. Drug loading within the smallest DHP-bMPA (n=3 in Fig. 1B)

drug	mg drug/mg carrier	EE[1] (%)
CQ	0.318	65
PQ	0.127	26
QN	0.233	46

[1]EE refers to encapsulation efficiency.

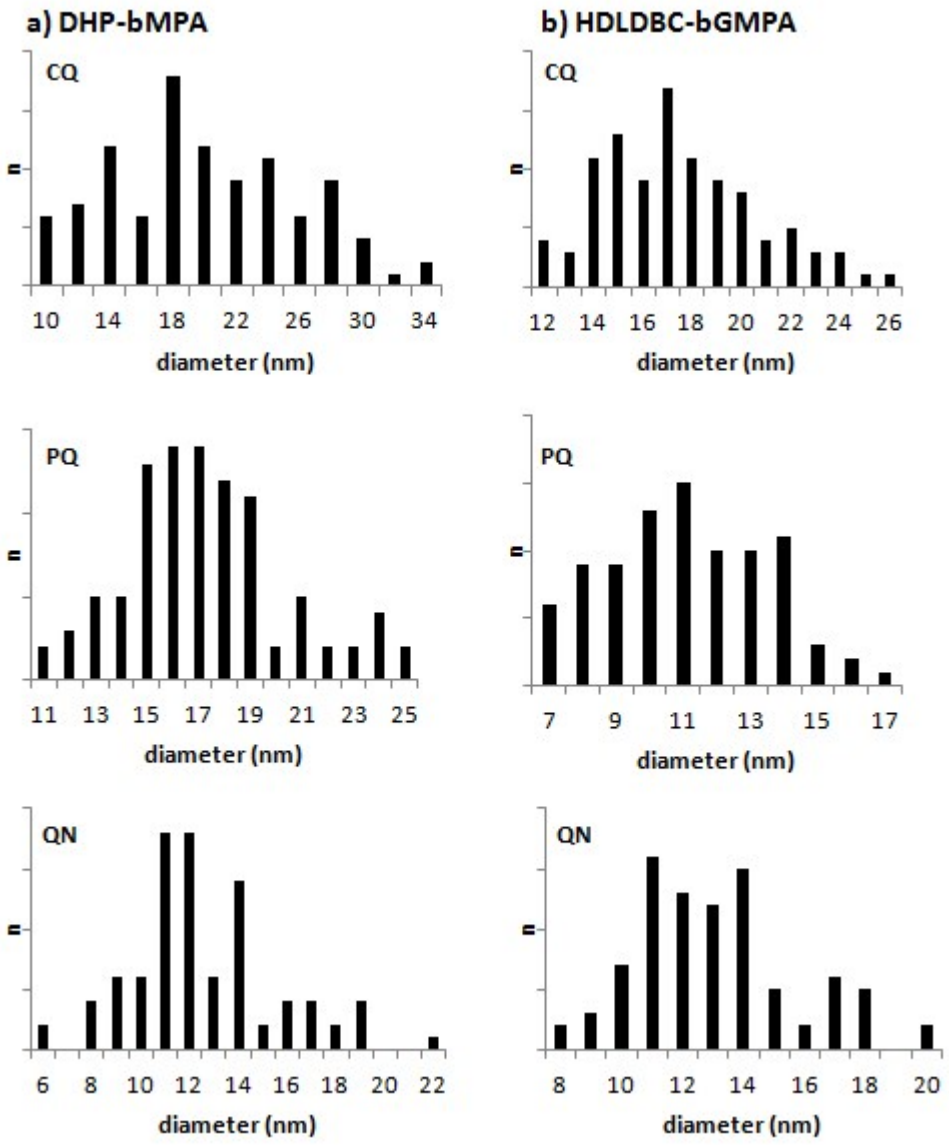


Fig. S2.2. Size distribution histograms of DHP-bMPA- and HDLDBC-bGMPA-drug conjugates.

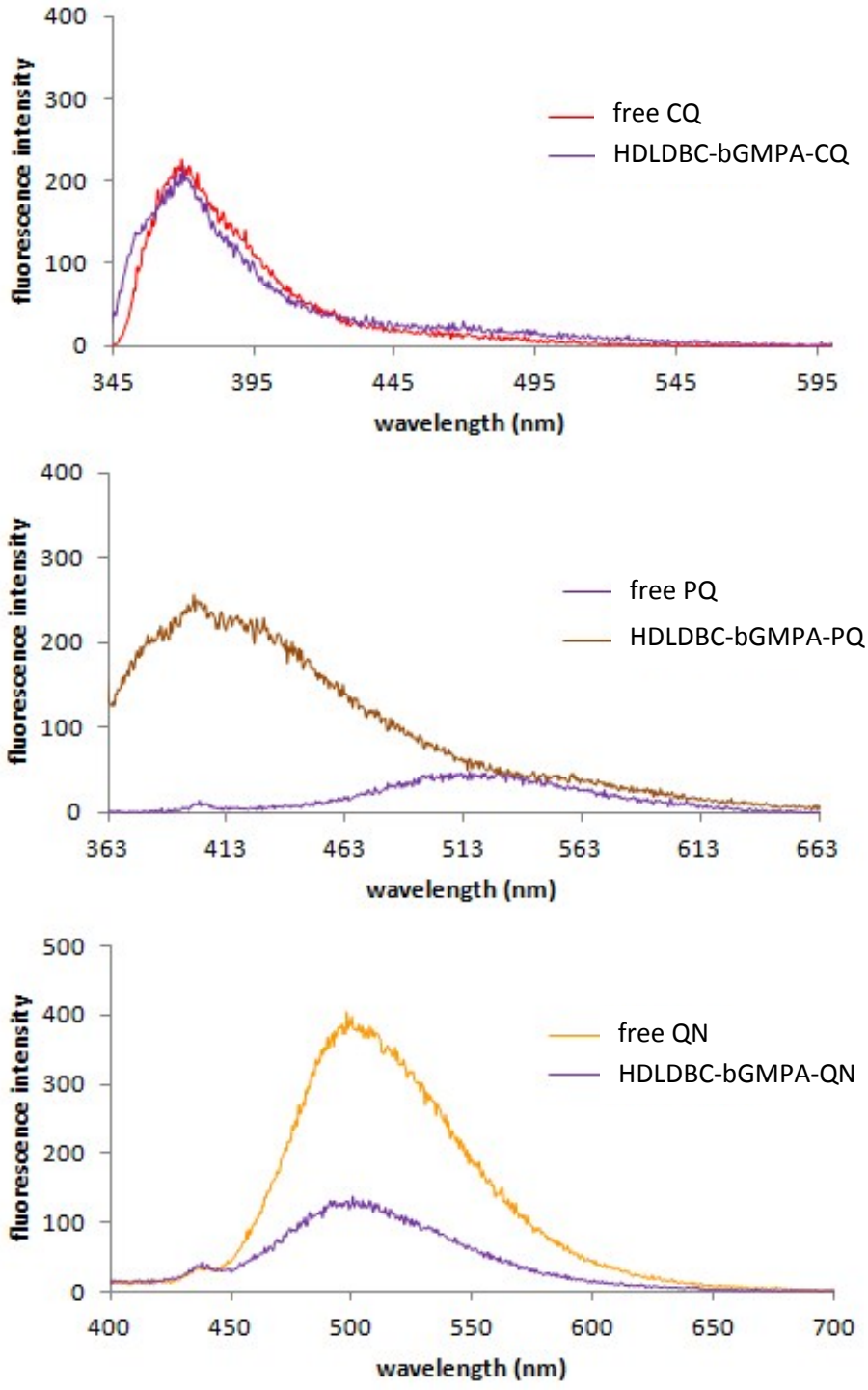


Fig. S2.3. Fluorescence spectra in water of equal concentrations of antimalarial drugs either free or in HDLDBC-bGMPA conjugates.

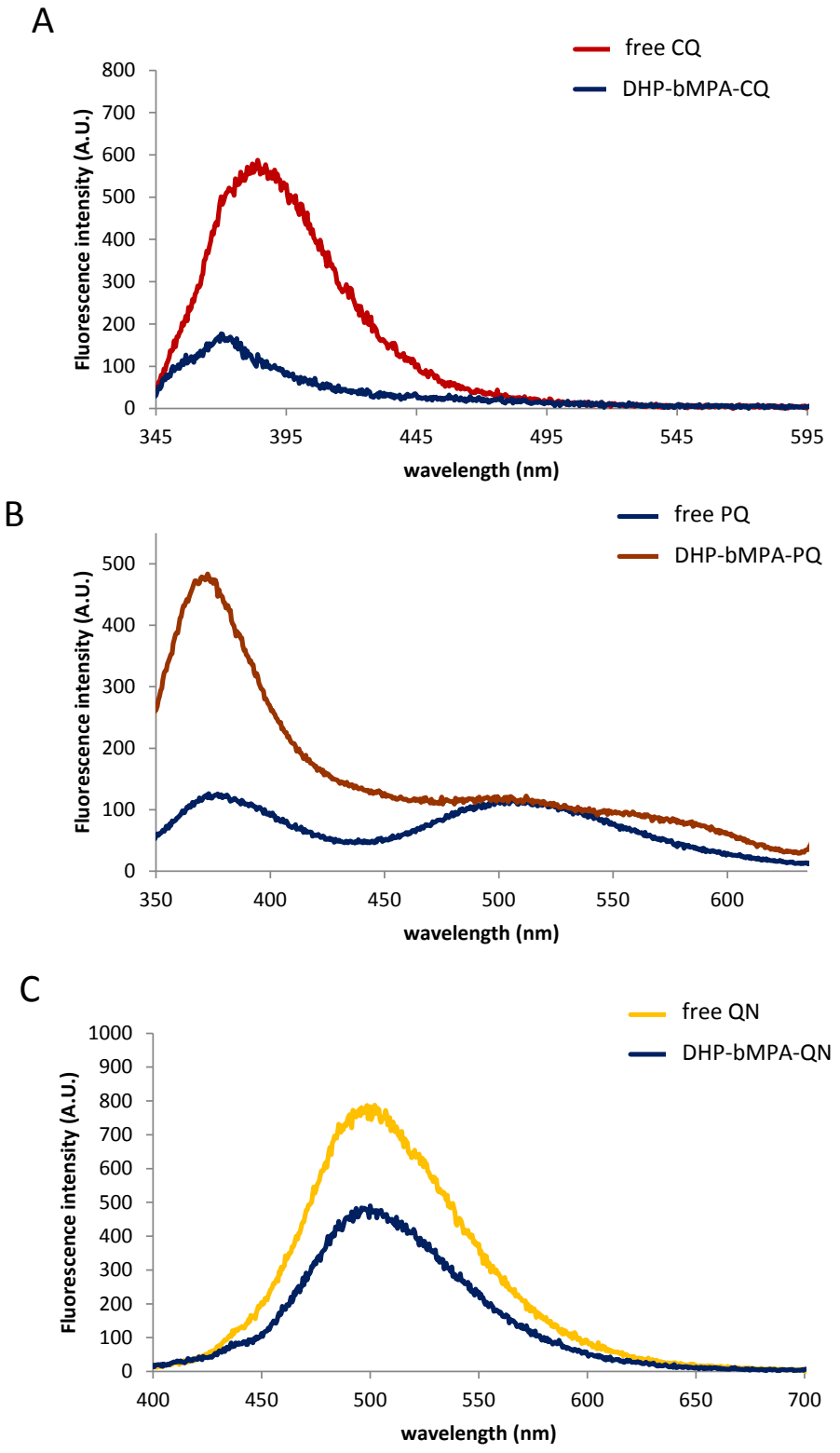


Fig. S2.4. Fluorescence spectra in water of equal concentrations of antimalarial drugs either free or in DHP-bMPA conjugates.

S3. Targeting experiments

Complexation of DHP-bRho with heparin-FITC

In order to study the possible benefits of the conjugation of heparin to the polymers for their pRBC targeting specificity[1], DHP-bMPA-Rho was complexed to heparin-FITC through electrostatic interactions between the positive charges of the ammonium groups of the dendrimer and the negatively charged sulfate groups of heparin. The formation of the complexes between heparin and DHP-bMPA-Rho was studied by UV/Vis spectroscopy with the Methylene Blue competition assay. Free Methylene Blue absorbance (λ_{max} = 665 nm) shifts when complexed to heparin (λ_{max} = 565 nm). For this assay, samples containing heparin (10 µg/mL) and Methylene Blue 50 µM were dissolved in 10 mM TRIS·HCl, pH 7.4, and placed in a 96-well culture plate. They were gently rotationally stirred at room temperature for 15 min to allow Methylene Blue to fully complex heparin. Then, the DHPs were added to the samples at different (w_{DHP}/w_{Hep}) ratios and the final volume of the samples was adjusted to 150 µL. They were newly stirred for another 30 min. UV/Vis spectra were recorded between 400 and 800 nm with an EPOCH UV/Vis spectrophotometer. The maximum A_{665}/A_{565} ratio was calculated to determine the w_{DHP}/w_{Hep} ratio at which 100% of Methylene Blue is displaced. This ratio corresponds to the ratio at which the maximum DHP/heparin association is achieved. The experiments were made in triplicate.

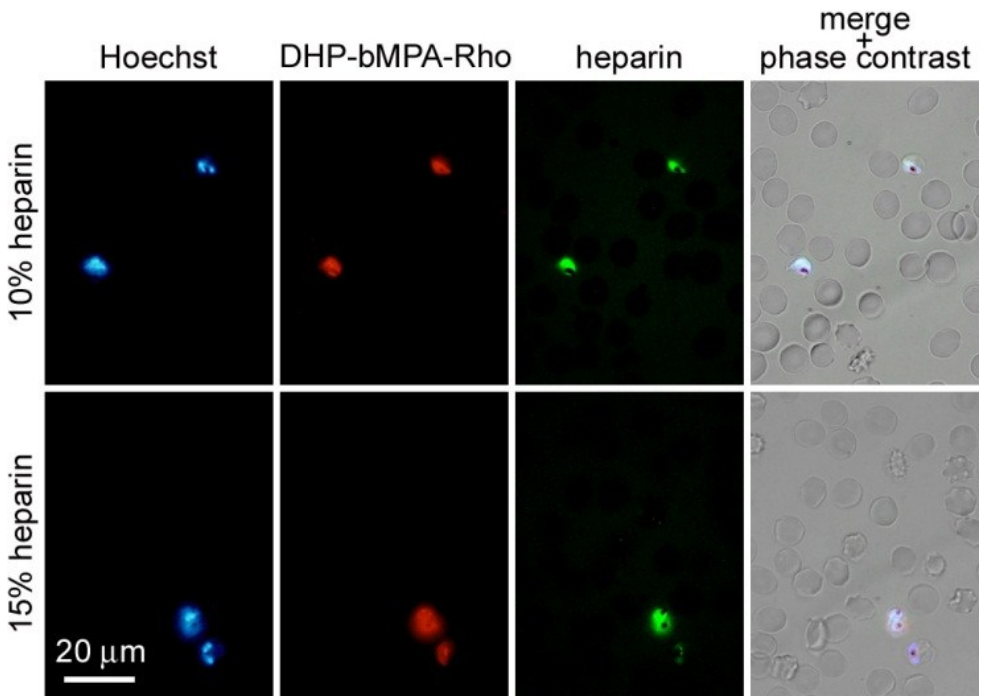


Fig. S3.1. Fluorescence microscopy cell targeting analysis to RBCs and pRBCs of rhodamine-labeled DHP-bMPA coated with FITC-labeled heparin.

[1] Marques J., Moles E., Urbán P., Prohens P., Busquets M.A., Sevrin C., Grandfils C., Fernàndez-Busquets X. Application of heparin as a dual agent with antimalarial and liposome targeting activities towards *Plasmodium*-infected red blood cells. *Nanomedicine* 2014 ;10:1719-1728.