Spatially Defined Drug Targeting by *in situ* Host-Guest Chemistry in a Living Animal

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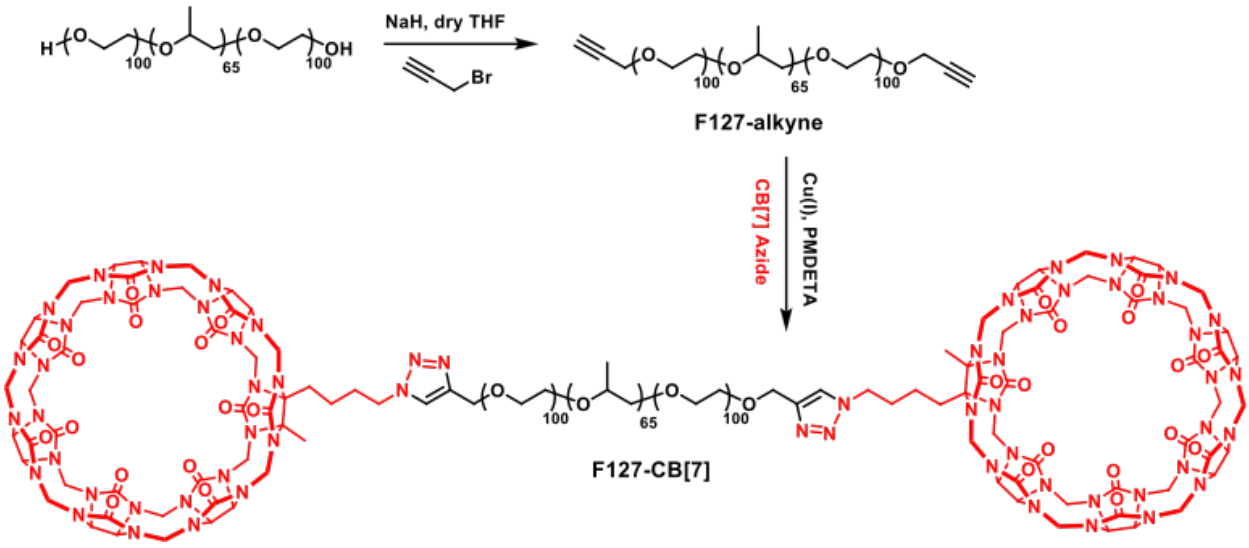
1- Synthetic and Experimental Methods

1.1 Synthesis of F127 End-Modified with Cucurbit[7]uril (F127-CB[7])



a. ***Synthesis of F127 terminated with alkyne group (F127-alkyne)*** - In a dry round-bottom flask, sodium hydride (0.27 g, 60% dispersion in mineral oil, *Beantown Chemical*) was slowly added to a solution of Pluronic **F127** (PEG-PPO-PEG, M_n = 12600, 2.20 g, *Sigma-Aldrich*) in dry THF (20 mL). The mixture was stirred for 20 minutes at room temperature until no hydrogen gas release was evident. Then, propargyl bromide (0.20 mL, 80 wt. % solution in toluene, *Beantown Chemical*) was added and the reaction mixture was stirred at room temperature for 100 hours. After quenching with a small volume of water, the reaction mixture was transferred to a 50 mL centrifuge tube. The supernatant was collected by centrifuge. The solid residue was washed with THF three times (20 mL each), combined with the supernatant, and the mixture was evaporated under reduced pressure. The residue was dissolved in 100 mL DCM and washed with brine once (100 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure into a small volume which was precipitated into cold diethyl ether. The pure product was obtained as light yellow solid (1.96 g, 86% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$, ***Figure S1***): δ (ppm) = 4.20 (d, J = 2.4 Hz, 3H), 3.80-3.27 (m, PPO main chain and PEG chain), 2.43 (t, J = 2.4 Hz, 1H), 1.17-1.08 (m, PPO methyl side chain).

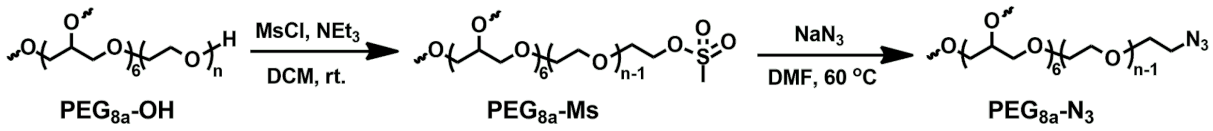
b. ***Synthesis of F127-CB[7]*** – CB[7]-azide (**CB[7]-N_3**) (101.84 mg) was synthesized according to previously published methods.[1,2] The pure **CB[7]-N_3** (127.3 mg) product was combined with *F127-alkyne* (633.8 mg), *p*-xylylenediamine (27.2 mg, 99%, TCI),

copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 2.5 mg, *BDH, ACS grade*) and *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA, 98%, 2 uL, *Acros*) and dissolved in 9 mL DMF/water (1/2, v/v) in a Schlenk flask. The flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (20.0 mg) into the flask before re-capping the flask. The flask was vacuumed and backfilled with N_2 for 5 cycles before immersion in a 50 ^oC oil bath to thaw the solution and initiate the 'click' reaction with stirring. After 48 h, the reaction was quenched by exposure to air. The reaction mixture was then transferred into dialysis tubing (MWCO = 3500, *Thermo Scientific*) and dialyzed against 3 L water over 24 h with water changed every 2 h. To remove residual *p*-xylylenediamine, the obtained solid after lyophilization was treated with excess acetic anhydride and triethylamine in 10 mL DMF for 24 h. Then the mixture was again transferred into dialysis tubing (MWCO = 3500, *Thermo Scientific*) and dialyzed against 3 L water over 24 h with water change for every 2 h. The pure product was obtained after lyophilization as light yellow solid (760.0 mg, 100% yield) and was determined to be fully substituted with **CB[7]**. ^{1}H-NMR (500 MHz Bruker, 25 ^oC, D_2O, with >1 equiv of *p*-xylylenediamine added as probe to quantify CB[7], ***Figure S2***): δ (ppm) = 8.07 (s, 1H), 7.52 (s, free probe), 6.62 (s, threaded probe), 5.83-5.62 (m, 14H), 5.61-5.42 (m, 12H), 4.70 (s, 2H), 4.49 (t, J = 6.5 Hz, 2H), 4.35−4.13 (m, 14H), 4.21 (s, free probe), 3.92 (s, threaded probe), 3.86-3.42 (m, PEG and PPO main chain), 2.33 (m, 2H), 2.04 (m, 2H), 1.78 (s, 3H), 1.22-1.08 (m, PPO-*Me*).

1.2 Thermal Transition and Micelle Formation of F127 vs. F127-CB[7]

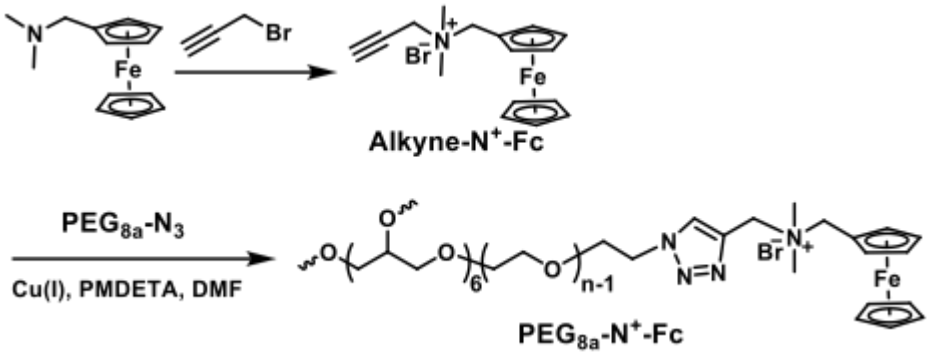
NMR samples in D_2O were prepared by dissolving **F127-CB[7]** at concentration of 100 mg/mL dissolved solids. A control of F127 was similarly prepared at an equivalent molar concentration, accounting for the molecular weight difference attributable to **CB[7]**. Temperature-dependent ^{1}H-NMR was conducted (500 MHz Bruker) in sealed tubes, beginning from 10 ^oC, with incremental heating and equilibration until a temperature of 40 ^oC was reached. At least 5 minutes of dwell time was provided after the set temperature was reached prior to collecting measurements. Data for **F127-CB[7]** and **F127** is included in ***Figure 2B*** *and* ***Figure S3***.

1.3 Synthesis of 8-arm PEG End-Modified with a Ferrocene Guest (PEG$_8$-Tz-N-Fc)



a. ***Synthesis of 8-arm PEG terminated with mesylate (PEG$_{8a}$-Ms)*** - In a dry round-bottom flask, hydroxyl-terminated 8-arm PEG (M_n = 20747, 4.00 g, *Creative PEGWorks*) was dissolved in 20 mL DCM with triethylamine (2.20 mL). The solution was cooled to 0 °C in an ice bath and methanesulfonyl chloride (MsCl, 1.20 mL, *Beantown Chemical*) was added slowly. The flask was then removed from the ice bath and the reaction mixture was stirred for 2 d at room temperature. After quenching with a small volume of water, the reaction mixture was diluted into 200 mL of DCM and washed with brine three times (200 mL each). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure into a small volume which was precipitated into cold diethyl ether. The product was obtained as a colorless powder (4.03 g, 98% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S4***): δ (ppm) = 4.37 (m, 2H), 3.83-3.43 (m, PEG chain), 3.07 (s, 3H).

b. ***Synthesis of 8-arm PEG terminated with azido group (PEG$_{8a}$-N$_3$)*** - Sodium azide (0.52 g, high purity, *VWR*) and PEG$_{8a}$-Ms (1.10 g) were suspended in DMF (10 mL) and stirred at 60 °C for 2 d. The reaction mixture was then diluted into 200 mL of DCM and washed with brine three times (200 mL each). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure into a small volume which was precipitated into cold diethyl ether. The product was obtained as a white powder (0.98 g, 91% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S4***): δ (ppm) = 3.81-3.43 (m, PEG chain), 3.38 (t, J = 10.1 Hz, 2H).

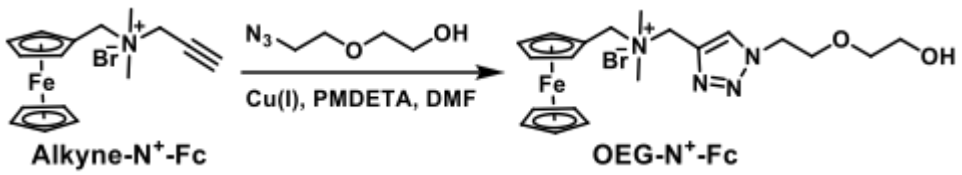


c. ***Synthesis of Alkyne-N-Fc*** – Propargyl bromide (186 ul, 80 wt. % solution in toluene, *Beantown Chemical*) was added slowly to a solution of (dimethylaminomethyl)ferrocene (0.405 g, 98+%, *Alfa Aesar*) in 2.5 mL acetonitrile and the reaction mixture was stirred at room temperature. After 30 min, the entire mixture was precipitated into 20 mL diethyl ether. Yellow solid (0.593 g, 99% yield) was collected by centrifuge and further washed twice with diethyl ether (10 mL). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$, ***Figure S5***): δ (ppm) = 4.92 (s, 2H), 4.56 (d, *J* = 2.3 Hz, 2H), 4.55 (t, *J* = 1.7 Hz, 2H), 4.37 (t, *J* = 1.7 Hz, 2H), 4.30 (s, 5H), 3.34 (s, 6H), 2.87 (t, *J* = 2.3 Hz, H).

d. ***Synthesis of PEG$_8$-Tz-N-Fc*** – **Alkyne-N-Fc** (0.1158 g), **PEG$_{8a}$-N$_3$** (0.8388 g), $CuSO_4 \cdot 5H_2O$ (4.0 mg) and PMDETA (3.2 uL) were dissolved in DMF (8 mL) in a Schlenk flask. The flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (20 mg) before re-capping. The flask was vacuumed and backfilled with N_2 over 5 cycles before immersion in a 40 ^{o}C oil bath to thaw the solution and initiate the 'click' reaction. After 24 h, the reaction was quenched by exposure to air. The reaction mixture was diluted with 20 mL of DCM and passed through a short Al_2O_3 column. The resulting liquid was evaporated under reduced pressure, following which the solution was precipitated into cold diethyl ether. The fully substituted product was obtained as white powder (0.42 g, 89% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$, ***Figure S5***): *δ* (ppm) = 8.70 (s, 1H), 5.02 (s, 2H), 4.69 (s, 2H), 4.61 (t, *J* = 5.3 Hz, 2H), 4.60 (s, 2H), 4.39 (t, *J* = 1.7 Hz, 2H), 4.27 (s, 5H), 3.92 (t, *J* = 5.3 Hz, 2H), 3.75-3.48 (m, PEG chain), 3.10 (s, 6H).

Using these same methods, a linear (2-arm) macromer, (PEG_2-Fc), was prepared from 6 kDa PEG. A 4-arm macromer (PEG_4-Fc) was also prepared from a 10 kDa 4-arm PEG starting material.

1.4 Synthesis of Model Guest Resembling PEG$_8$-Tz-N-Fc (OEG-Tz-N-Fc)



A model compound, **OEG-Tz-N-Fc**, was prepared by combining Alkyne-N-Fc (0.181 g), 2-(2-azidoethoxy)ethan-1-ol (0.066 g), $CuSO_4 \cdot 5H_2O$ (6.25 mg) and PMDETA (5 uL) in DMF (5 mL)

in a Schlenk flask. The flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (20 mg) before re-capping. The flask was vacuumed and backfilled with N_2 for 5 cycles before warming up to room temperature to thaw the solution and initiate the 'click' reaction. After 3 days, the reaction was quenched by exposure to air. The reaction mixture was diluted with 20 mL of DCM and passed through a short Al_2O_3 column. The combined fractions were evaporated under reduced pressure and precipitated into cold ether. The fully substituted product was obtained as yellow powder (0.150 g, 61% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, D_2O, ***Figure S6***): δ (ppm) = 8.33 (s, 1H), 4.68 (t, J = 5.0 Hz, 2H), 4.57 (t, J = 1.8 Hz, 2H), 4.52 (s, 2H), 4.50 (s, 2H), 4.46 (t, J = 1.8 Hz, 2H), 4.29 (s, 5H), 3.98 (t, J = 5.0 Hz, 2H), 3.66-3.62 (m, 2H),, 3.60-3.57 (m, 2H), 2.87 (s, 6H).

1.5 Determination of K_{eq} by ^{1}H-NMR competition experiments

^{1}H-NMR competition experiments were performed on a 500 MHz Bruker NMR spectrometer, according to the method described by Isaacs and colleagues.[3,4] Briefly, NMR samples were prepared at three different ratios of *a)* a model compound for the PEG-appended guest, *b)* a competitive guest with a previously reported binding constant, and *c)* free unmodified **CB[7]**. Samples were prepared in D_2O and allowed to reach equilibrium for times ranging from ~30 minutes for the weakest guest to 4 days for the strongest guests. Equilibrium was verified by tracking the measured ^{1}H-NMR resonances until the spectra became constant. ^{1}H-NMR spectra were then acquired for each sample. The resonances which could be clearly assigned to the bound (marked as ***'** in ***Figures S7, S15, S20, S25***) and the free (marked as ***** in ***Figures S7, S15, S20, S25***) guest and did not overlap with any other signals were integrated. The concentrations of the bound and free guest were obtained by this method, also yielding the concentration for the bound and the free form of the competing guest as well. In these equilibrium mixture, the K_{rel} values were calculated using the relationships below.[3,4] K_{rel} values for 3 independent experiments with varying and known ratios of each of three components were averaged to obtain the final estimated K_{eq} values reported in the paper.

$$K_{rel,\,test/known} = \frac{[test]_{bound}[known]_{free}}{[test]_{free}[known]_{bound}}$$

K_{rel} can alternatively be expressed as following:

$$K_{rel,\,test/known} = \frac{K_{eq,\,test}}{K_{eq,\,known}}$$

Rearranging yields the following expression for K_{eq}:

$$K_{eq,\,test} = K_{rel,\,test/known} \times K_{eq,\,known}$$

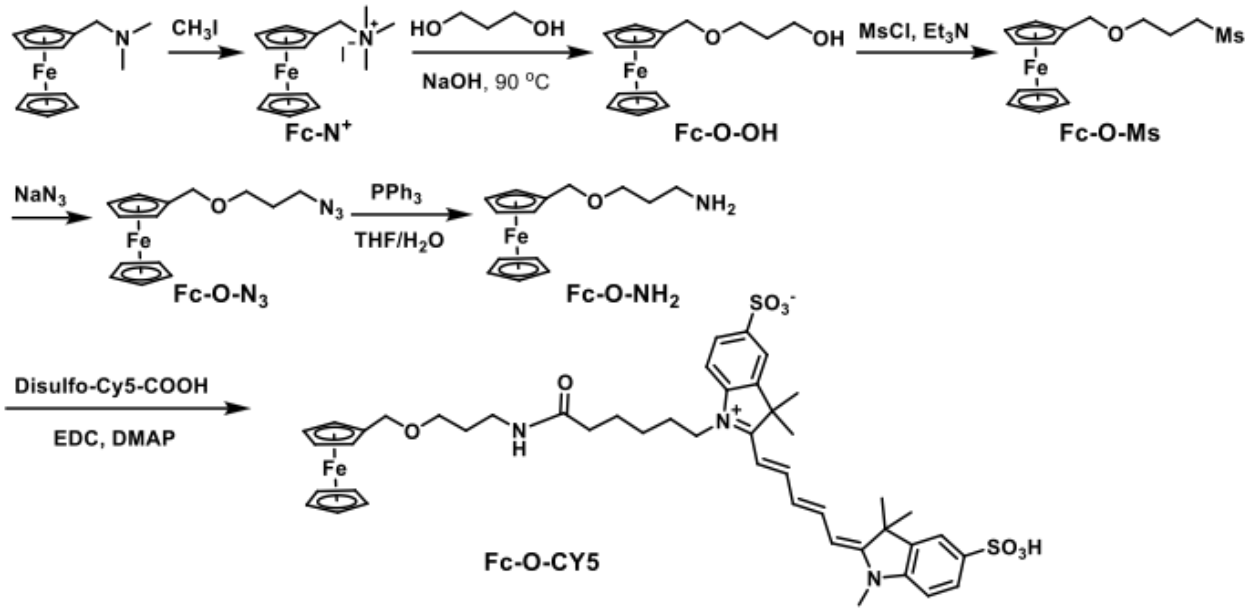
1.6 Rheological Testing

Viscoelastic properties of the hydrogels were studied using TA Instruments Discovery HR-2 rheometer fitted with a Peltier stage. All hydrogels were prepared as described at a consistent 10 w/v% solids, altering the molar ratio of CB[7]:Fc to be 1:0, 1:1, 2:1, and 3:1. Oscillatory frequency sweep measurements from 0.1 rad/s to 100 rad/s were conducted at 2% strain, a value verified to be in the linear viscoelastic region by a strain sweep conducted for all hydrogels. Thermal ramps were conducted, first raising the temperature slowly (0.5°C per minute) from 20°C to 45°C, and then cooling at the same rate over the same range. To study self-healing, step-strain experiments were conducted at 10 rad/s, cycling between 2% and 200% strain. All measurements were performed using a 20 mm 1.988° cone plate and the solvent trap accessory was used to minimize sample drying during testing.

1.7 Synthesis of diSulfo-Cy5-COOH

The fluorescent cyanine dye, **diSulfo-Cy5-COOH**, was synthesized according to published methods as a starting material.[5] The recorded ^{1}H-NMR spectrum (***Figure S10***) was identical to that of the authentic commercial sample. ^{1}H-NMR (500 MHz Bruker, 25 °C, DMSO-d_6): δ (ppm) = 8.36 (t, J = 13.1 Hz, 2H), 7.79 (s, 2H), 7.61 (m, 2H), 7.29 (d, J = 8.1 Hz, 2H), 6.55 (t, J = 12.3 Hz, 1H), 6.27 (m, 2H), 4.07 (t, J = 7.1 Hz, 2H), 3.58 (s, 3H), 2.17 (t, J = 7.2 Hz, 2H), 1.67 (s, 14H), 1.53 (m, 2H), 1.35 (m, 2H).

1.8 Synthesis of "Weak" Model Prodrug, Fc-O-Cy5



a. ***Synthesis of compound Fc-N$^+$*** **–** A solution of (dimethylaminomethyl)ferrocene, (2.43 g) in 20 mL of ether stirred in a vial on ice was combined with iodomethane (2.00 g, 99%, *Alfa Aesar*) in 5 ml of ether by dropwise addition. After the addition, the reaction mixture was stirred for 30 min at room temperature. The precipitate product Fc-N$^+$ (3.65 g, 95% yield) was collected by filtration, washed with ether (10 mL) three times, and dried. ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, D_2O): δ (ppm) = 4.56 (t, J = 3.6 Hz, 2H), 4.48 (t, J = 3.6 Hz, 2H) 4.44 (s, 2H), 4.33 (s, 5H), 3.0 (s, 9H).

b. ***Synthesis of compound Fc-O-OH*** – A mixture of compound **Fc-N$^+$** (0.475 g) and NaOH (0.90 g) in 10 mL of 1,3-propandiol was stirred at 90 ^{o}C for 24 hours. After cooling to room temperature, the mixture was partitioned between DCM (200 mL) and water (200 mL). The DCM layer was further washed with water five times (100 mL each), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give an orange oil (0.344 g, 100% yield) that crystalized upon cooling. ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$, ***Figure S11***): δ (ppm) = 4.40 (s, 2H), 4.33 (t, J = 1.7 Hz, 2H), 4.26 (t, J = 1.7 Hz, 2H), 4.25 (s, 5H), 3.86 (td, J_1 = 5.7 Hz, J_2 = 5.4 Hz, 2H), 3.74 (t, J = 5.7 Hz, 2H), 2.52 (t, J = 5.4 Hz, 1H), 1.92 (m, 2H).

c. ***Synthesis of compound Fc-O-Ms*** – Methanesulfonyl chloride (MsCl, 0.25 mL) was added slowly into a solution of compound **Fc-O-OH** (0.34 g) and triethylamine (0.45 mL) in 20 mL DCM on an ice bath. The flask was then removed from the ice bath and the reaction

mixture was stirred for 24 hours at room temperature. After quenching with a small volume of water, the reaction mixture was partitioned between DCM/diethyl ether (1/1 (v/v), 200 mL) and water (200 mL). The organic layer was further washed with water five times (100 mL each), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure into an orange solid. The product (0.38 g, 91% yield) was used for next step without further purification. ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$, ***Figure S11***): δ (ppm) = 4.29 (t, J = 5.8 Hz, 2H), 4.28 (s, 2H), 4.21 (m, 2H), 4.15 (m, 2H), 4.13 (s, 5H), 3.52 (t, J = 5.9 Hz, 2H), 2.94 (s, 3H), 1.97 (m, 2H).

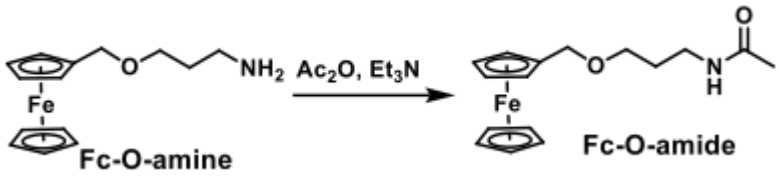
d. ***Synthesis of compound Fc-O-N$_3$*** – Sodium azide (0.70 g, high purity, *VWR*) and compound **Fc-O-Ms** (0.38 g) were suspended in DMF (20 mL) and stirred at 50 ^{o}C for 2 d. After cooling to room temperature, the reaction mixture was then partitioned between DCM/diethyl ether (1/1 (v/v), 200 mL) and water (200 mL). The organic layer was further washed with water at least five times (100 mL each), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give orange solid (0.33 g, 98% yield) which was used for next step without further purification. ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$, ***Figure S11***): δ (ppm) = 4.28 (s, 2H), 4.22 (m, 2H), 4.15 (m, 2H), 4.13 (s, 5H), 3.49 (t, J = 6.0 Hz, 2H), 3.36 (t, J = 6.7 Hz, 2H), 1.81 (m, 2H).

e. Synthesis of compound **Fc-O-NH$_2$** – A reaction mixture of compound **Fc-O-N$_3$** (0.33 g), triphenylphosphine (0.35 g, 99%, *Sigma-Aldrich*) and water (1 mL) in 9 mL of THF was stirred at room temperature overnight. The solvent was then removed under reduced pressure and the residue was purified on a silica column, eluting with a mixture of DCM/MeOH/Et_3N (10/1/0.1, v/v/v). The target product was obtained as an orange oil (0.24 g, 80% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$**,** ***Figure S11***): δ (ppm) = 4.27 (s, 2H), 4.22 (m, 2H), 4.14 (m, 2H), 4.13 (s, 5H), 3.50 (t, J = 6.2 Hz, 2H), 2.77 (t, J = 6.7 Hz, 2H), 1.70 (m, 2H).

f. ***Synthesis of Fc-O-Cy5*** – A mixture of compound **Fc-O-NH$_2$** (29.8 mg), disulfo-Cy5-COOH (63.2 mg), EDC·HCl (60.1 mg, 95+%, *Matrix Scientific*) and DMAP (3.2 mg, 95+%, *Matrix Scientific*) in 1.5 mL of DMF was stirred at room temperature for 36 h. After 36 h, most of the solvent was removed under reduced pressure and the residue was purified on a silica column, eluting with a mixture of DCM/MeOH (3/1, v/v). The target product was obtained as blue solid (72.0 mg, 81% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C,

DMSO-d_6, ***Figure S12***): δ (ppm) = 8.34 (t, J = 13.0 Hz, 2H), 7.80 (s, 2H), 7.62 (m, 2H), 7.30 (t, J = 6.6 Hz, 2H), 6.55 (t, J = 12.4 Hz, 2H), 6.25 (dd, J_1 = 13.0 Hz, J_2 = 12.4 Hz, 2H), 4.19 (m, 2H), 4.16 (s, 2H), 4.11 (m, 5H), 4.10 (m, 2H), 4.06 (t, J = 7.5 Hz, 2H), 3.57 (s, 3H), 3.01 (m, 2H), 2.97 (m, 2H), 1.99 (m, 2H), 1.67 (m, 16H), 1.53 (m, 2H), 1.30 (m, 2H).

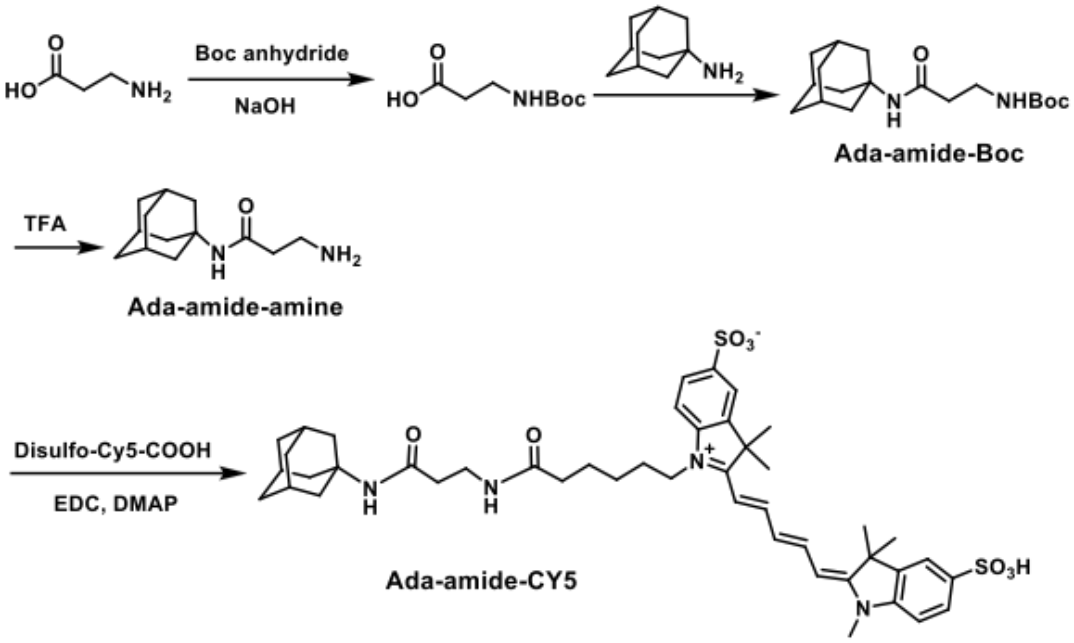
Model "Weak" Guest for Determination of Binding Constant



Acetic anhydride (33 μL) was slowly added into a solution of compound **Fc-O-Amine** (95 mg) and triethylamine (59 μL) in 2 mL of DCM. The reaction mixture was stirred for 1 hour, then diluted with 5 mL DCM and washed with a 5% K_2CO_3 aqueous solution four times (5 mL each). The DCM layer was passed through a short Al_2O_3 column and evaporated under reduced pressure to give orange crystals.^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, D_2O, ***Figure S14***): δ (ppm) = 4.36 (s, 2H), 4.31 (m, 2H), 4.25 (m, 2H), 4.22 (s, 5H), 3.52 (t, J = 6.4 Hz, 2H), 3.15 (t, J = 6.7 Hz, 2H), 1.70 (m, 2H).

The binding affinity for this model agent to **CB[7]** was then determined using competition ^{1}H-NMR as described above (***Figure S15***).

1.9 Synthesis of "Medium" Model Prodrug, Ada-Am-Cy5



a. ***Synthesis of Boc protected β-alanine*** – Boc anhydride (16.00 g, 99%, *Sigma-Aldrich*) was slowly added to a mixture of β-alanine (6.10g, 98%, *BeanTown Chemical*) and NaOH (3.00 g) in dioxane/water (100 mL, 1/1, v/v). The reaction mixture was stirred overnight. Most of the dioxane was then evaporated under reduced pressure and 200 mL of water was added. The pH value of the aqueous mixture was adjusted to around 3 by the addition of formic acid and was then washed twice with hexane (100 mL each). The mixture was extracted by DCM twice (100 mL each). The DCM extracts were combined, washed by water (100 mL) once, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give colorless sticky liquid which crystalized slowly (13.55 g, 100% yield). The product was used for next step without further purification. ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$): δ (ppm) = 6.21(bs) and 5.07 (bs) (1H), 3.40 (bs, 2H), 2.56 (bs, 2H), 1.46 (bs, 9H).

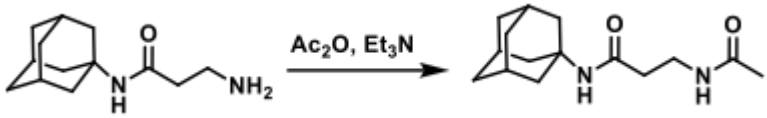
b. ***Synthesis of compound Ada-Am-Boc*** – A mixture of Boc protected β-alanine (1.89 g), 1-adamantylamine (1.51 g, *TCI*), EDC·HCl (3.01 g) and DMAP (30 mg) in 50 mL of DCM was stirred at room temperature for 24 h. The reaction mixture was diluted in 200 mL of DCM, washed with water (100 mL) five times, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a colorless solid (3.20 g, 100% yield). The product was used for next step without further purification. ^{1}H-NMR (500 MHz Bruker,

25 °C, $CDCl_3$, ***Figure S16***): δ (ppm) = 5.24 (s, 1H), 5.21 (s, 1H),3.36 (m, 2H), 2.31 (m, 2H), 2.07 (m, 3H), 1.98 (m, 6H), 1.67 (m, 6H), 1.43 (bs, 9H).

c. ***Synthesis of compound Ada-Am-amine*** – Compound **Ada-Am-Boc** (3.20 g) was dissolved in 30% TFA in DCM (50 mL) and stirred for 3 hours at room temperature. Then, most of the contents were evaporated under reduced pressure to give a sticky residue. The residue was dissolved in 200 mL DCM, washed with aqueous NaOH (100 mL, pH=12) three times, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give colorless liquid (1.75 g, 79% yield). The product was used for next step without further purification. ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S16***): δ (ppm) = 6.26 (s, 1H), 2.95 (t, J = 5.9 Hz, 2H), 2.21 (t, J = 5.9 Hz, 2H), 2.05 (m, 3H), 1.99 (m, 6H), 1.66 (m, 6H).

d. ***Synthesis of Ada-Am-Cy5*** – A mixture of compound **Ada-Am-amine** (32.0 mg), disulfo-CY5-COOH (64.2 mg), EDC·HCl (60.0 mg) and DMAP (3.0 mg) in 2 mL of DMF was stirred at room temperature for 24 h. After 24 h, most of the solvent was removed under reduced pressure and the residue was purified on a silica column, eluting with a mixture of ethyl acetate/MeOH (3/2, v/v). The target product was obtained as blue solid (62.0 mg, 73% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, DMSO-d_6**,** ***Figure S17***): δ (ppm) = 8.34 (t, J = 13.0 Hz, 2H), 7.80 (s, 2H), 7.62 (m, 2H), 7.30 (t, J = 6.6 Hz, 2H), 6.54 (t, J = 12.4 Hz, 2H), 6.26 (dd, J_1 = 13.0 Hz, J_2 = 12.4 Hz, 2H), 4.06 (m, 2H), 3.57 (s, 3H), 3.13 (m, 2H), 2.11 (t, J = 7.1 Hz, 2H), 2.00 (t, J = 7.2 Hz, 2H), 1.94 (m, 3H), 1.87 (m, 6H), 1.67 (m, 16H), 1.56 (m, 6H), 1.51 (m, 2H), 1.30 (m, 2H).

Model "Medium" Guest for Determination of Binding Constant

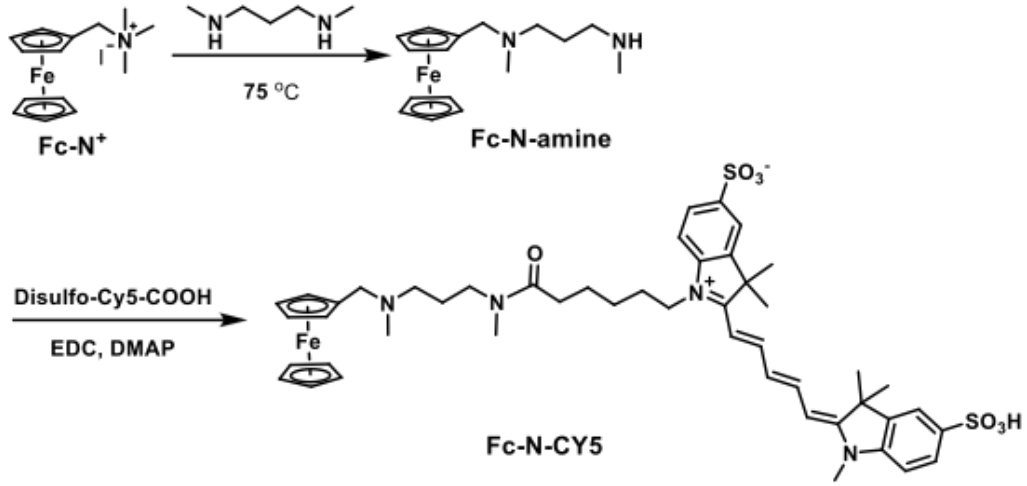


Acetic anhydride (0.14 mL) was slowly added into a solution of Ada-amide-amine (0.20 g) and triethylamine (0.20 mL) in 5 mL of DCM. The reaction mixture was stirred for 3 days, then diluted with 10 mL of DCM and washed with 5% K_2CO_3 aqueous solution for four times (15 mL each). The DCM layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give white crystal (0.21 g, 88% yield).^{1}H-NMR (500 MHz Bruker, 25 °C, D_2O, ***Figure S19***): δ (ppm)

= 6.43 (s, 1H), 5.27 (s, 1H), 3.47 (m, 2H), 2.32 (t, *J* = 5.9 Hz, 2H), 2.07 (m, 3H), 1.98 (m, 6H), 1.67 (m, 6H).

The binding affinity for this model agent to **CB[7]** was then determined using competition ^{1}H-NMR as described above (***Figure S20***).

1.10 Synthesis of "Strong" Model Prodrug, Fc-N-Cy5

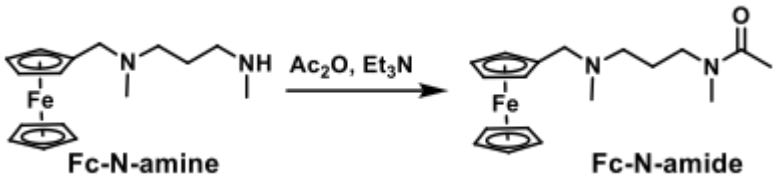


a. ***Synthesis of compound Fc-N-amine*** – A mixture of compound **Fc-N$^+$** (1.22 g), N,N'-dimethyl-1,3-propanediamine (1.26 g, 97%, *Alfa Aesar*) and K_2CO_3 (0.43 g) in 10 mL of acetonitrile was stirred at 75 °C for 4 days. After cooling to room temperature, 20 mL DCM was added to the mixture and the solid was removed by centrifuge. The supernatant was passed through a short Al_2O_3 column, evaporated under reduced pressure, and resuspended in 20 mL DCM. The insoluble solid was removed by centrifuge and the DCM solution was evaporated under reduced pressure to give orange oil (0.81 g, 85% yield). The product was used for next step without further purification. ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S21***): δ (ppm) = 4.15 (m, 2H), 4.10 (m, 7H), 4.38 (s, 2H), 2.58 (t, *J* = 6.8 Hz, 2H), 2.40 (s, 3H), 2.34 (t, *J* = 7.2 Hz, 2H), 2.15 (s, 3H), 1.66 (m, 2H).

b. ***Synthesis of Fc-N-Cy5*** – A mixture of compound **Fc-N-amine** (45.0 mg), disulfo-Cy5-COOH (66.4 mg), EDC·HCl (40.0 mg) and DMAP (2.0 mg) in 1.5 mL of DMF was stirred at room temperature for 24 h. After 24 h, the solvent was removed under reduced pressure and the residue was purified on a C_{18} column, eluting with a gradient mixture of water/MeOH (7/3 to 4/6, v/v). The target product was obtained as blue solid (60.0 mg, 62%

yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, DMSO-d_6, ***Figure S22***): δ (ppm) = 8.35 (t, J = 13.0 Hz, 2H), 7.80 (s, 2H), 7.62 (m, 2H), 7.30 (t, J = 6.6 Hz, 2H), 6.53 (t, J = 12.4 Hz, 2H), 6.27 (dd, J_1 = 13.0 Hz, J_2 = 12.4 Hz, 2H), 4.38 (s, 2H), 4.26 (s, 2H), 4.26 (s, 2H), 4.20 (s, 5H), 4.16-4.0 (m, 4H), 3.58 (s, 3H), 3.21 (m) and 3.16 (m) (2H), 2.90-2.68 (m, 6H), 2.55 (m, 2H), 2.20 (m) and 2.15 (m) (2H), 1.67 (m, 16H), 1.47 (m, 2H), 1.29 (M, 2H). ESI-MS: 925.33 (expected $[M]^+$), 925.20 (observed $[M]^+$).

Model "Strong" Guest for Determination of Binding Constant



A reaction mixture of **Fc-N-amine** (60.0 mg), acetic anhydride (19 µL) and triethylamine (28 µL) in 3 mL of DCM was stirred at room temperature for 3 hours. After 3 hours, the reaction mixture was diluted with 3 mL of DCM and washed with 5% K_2CO_3 aqueous solution four times (5 mL each). The DCM layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by C18 column, eluting with a gradient mixture of water/MeOH (8/2 to 2/8, v/v). The target product was obtained as orange solid (17.0 mg, 25% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, D_2O, ***Figure S24***): δ (ppm) = 4.29-4.26 (m, 2H), 4.24-4.18 (m, 7H), 3.52 (s) and 3.47 (s) (2H), 3.34-3.27 (m, 2H), 2.96 (s) and 2.82 (s) (3H), 2.37-2.30 (m, 2H), 2.18 (s) and 2.15 (s) (3H), 2.04 (s) and 2.03 (s) (3H), 1.77-1.64 (m, 2H).

The binding affinity for this model agent to **CB[7]** was then determined using competition ^{1}H-NMR as described above (***Figure S25***).

1.11 In vivo Implantation and Imaging

F127-CB[7]:PEG$_8$-Fc (0.1 mL, 10 wt% solid content, 3:1 ratio of CB[7]:Fc) was injected subcutaneously into the dorsal region, between the scapulae, of 7-8 week old female mice. For analysis by histology, C57BL/6J mice were used. For *in vivo* imaging, hairless but immunocompetent SKH1-E mice were used. Transcutaneous injections were performed using a 25G needle attached to a 1 mL syringe. For histology studies, animals were euthanized at 3, 7, 14, 30, 45, and 60 days following implantation, animals were euthanized and the implantation bed was

excised, analyzed by gross necropsy for gel content, and then fixed in formalin and subjected to routine histological processing, sectioning, and staining with H&E. Tissue sections were imaged for gel content and cellular infiltration and clearance. For imaging studies, 48 hours after gel administration mice were intraperitoneally injected with 200 μL guest-linked dye in PBS at a concentration of 1 mM. Equimolar concentration in these dye samples was further verified by measuring the fluorescent emission intensity (Tecan M200Pro Plate Reader) to ensure fluorescence levels were equal between all dye solutions. At serial timepoints following administration, mice were imaged under inhaled anesthesia using an IVIS Lumina Imager with a Cy5 filter set. For studies exploring repeated dosing, Fc-N-Cy5 was administered every 12 hours, with images collected immediately prior to the next dose administration for a total of 9 dye administrations. All *in vivo* fluorescence images were processed, analyzed, and quantified using ImageJ. These studies were approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Notre Dame.

1.12 Dye Extraction and Quantification

F127-CB[7]:PEG$_8$-Fc (0.1 mL or 0.2 mL, 10 wt% solid content, 3:1 ratio of CB[7]:Fc) was injected subcutaneously into the dorsal region, between the scapulae, of 7-8 week old female SKH1-E mice. Fc-N-Cy5 was intraperitoneally injected in a volume of 200 μL in PBS at a concentration of 1 mM. After 24 hours, animals were euthanized and the hydrogels were explanted. The hydrogels were dissolved and dye was extracted into PBS (15 mL) by sonication on ice for approximately 2 hours and fluorescence of the extract was measured using a Tecan M200Pro Plate Reader with the readings converted to concentrations using a standard curve of Fc-N-Cy5 dissolved in PBS.

1.13 Surface Modification of Glass Beads

Borosilicate glass beads (2.00 g, D = 45-53 μm, *Cospheric*) were treated with Piranha solution at 80 °C for 1 hour. The glass beads were sequentially washed with water (20 mL × 4), ethanol (20 mL × 4), and acetone (20 mL × 4) and then dried with a stream of N_2. A mixture of 3-aminopropyl)triethoxysilane (APTES, 0.3 mL, 99%, Sigma-Aldrich), 95% ethanol aqueous solution (15 mL), and acetic acid (0.3 mL) was stirred for 5 min and transferred to the vial

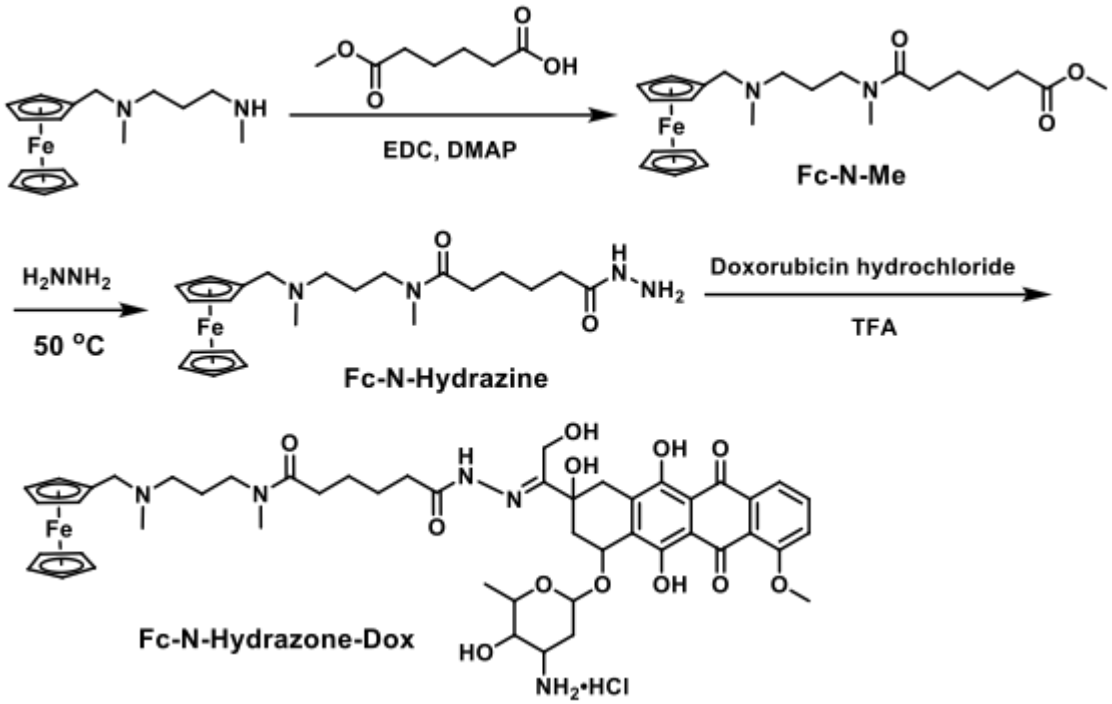
containing the Piranha-treated beads. The reaction mixture was gently stirred for 10 min at room temperature. The supernatant was decanted and the beads were sequentially washed with ethanol (20 mL × 4) and acetone (20 mL × 4). The beads were then dried with a stream of N_2 and heated at 110 °C for 10 min. Then, the beads were gently stirred in a mixture of 4-pentynoicacid (0.20 g), EDC (0.50 g), and DMAP (40.0 mg) in 5 mL of DMF for 24 hours. The beads were sequentially washed with ethanol (20 mL × 4) and acetone (20 mL × 4) and then dried with a stream of N_2. Finally, the beads (1.00 g), CB[7]-azide (28.0 mg), $CuSO_4 \cdot 5H_2O$ (2.0 mg) and PMDETA (98%, 1.6 μL) were suspended in DMF/water (1/1, v/v, 10 mL) in a Schlenk flask. The flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (10 mg) before re-capping. The flask was vacuumed and backfilled with N_2 for 5 cycles before warming up to room temperature to thaw the solution and initiate the 'click' reaction with gentle stirring. After 3 days, the reaction was quenched by exposure to air. The beads were sequentially washed with water (20 mL × 4), ethanol (20 mL × 4), and acetone (20 mL × 4) and then dried with a stream of N_2. To verify **CB[7]** surface functionalization, 10.0 mg beads were soaked an aqueous solution of Fc-N-Cy5 (0.01 mg/mL) for 5 minutes and washed with large amount of water at least 10 times until the supernatant was colorless. By naked eyes, the beads were blue. These beads were further analyzed by fluorescence microscopy and compared to control beads which had undergone all other processing steps apart from **CB[7]** functionalization. These control beads did not have a blue color and showed no signal by fluorescence microscopy. A schematic of functionalization and example data from fluorescence microscopy are shown in ***Figure S28***. Subsequently, these beads were injected subcutaneously into SKH1-E mice, and systemic homing studies with Fc-N-Cy5 were performed as before with the use of *in vivo* imaging.

1.14 Surface Modification of Polystyrene Beads

Carboxy-modified solid polystyrene beads (0.10 g, D = 45-53 μm, *Spherotech*) were gently stirred in a mixture of propargylamine (50 uL), EDC (0.20 g), and DMAP (18.0 mg) in 10 mL of THF for 24 hours. The beads were sequentially washed with methanol (15 mL × 4) and acetone (15 mL × 4) and dried under a stream of air. Beads (80.0 mg), CB[7]-azide (6.0 mg), $CuSO_4 \cdot 5H_2O$ (1.0 mg), and PMDETA (98%, 0.8 μL) were suspended in DMF/water (1/1, v/v, 4 mL) in a Schlenk flask. The flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (5.0 mg) before re-capping. The flask was vacuumed and

backfilled with N_2 for 5 cycles before warming up to room temperature to thaw the solution and initiate the 'click' reaction with gentle stirring. After 3 days, the reaction was quenched by exposure to air. The beads were sequentially washed with water (5 mL × 4), ethanol (5 mL × 4), and acetone (5 mL × 4) and dried under a stream of air. The successful functionalization of **CB[7]** on the surface was verified by loading Fc-N-Cy5 and observed by naked eye and by fluorescence microscopy compared to controls, as described above for glass beads. A schematic of functionalization and example data from fluorescence microscopy are shown in ***Figure S28***. Subsequently, these beads were injected subcutaneously into SKH1-E mice, and systemic homing studies with Fc-N-Cy5 were performed as before with the use of *in vivo* imaging.

1.15 Synthesis of Fc-Hdz-Dox



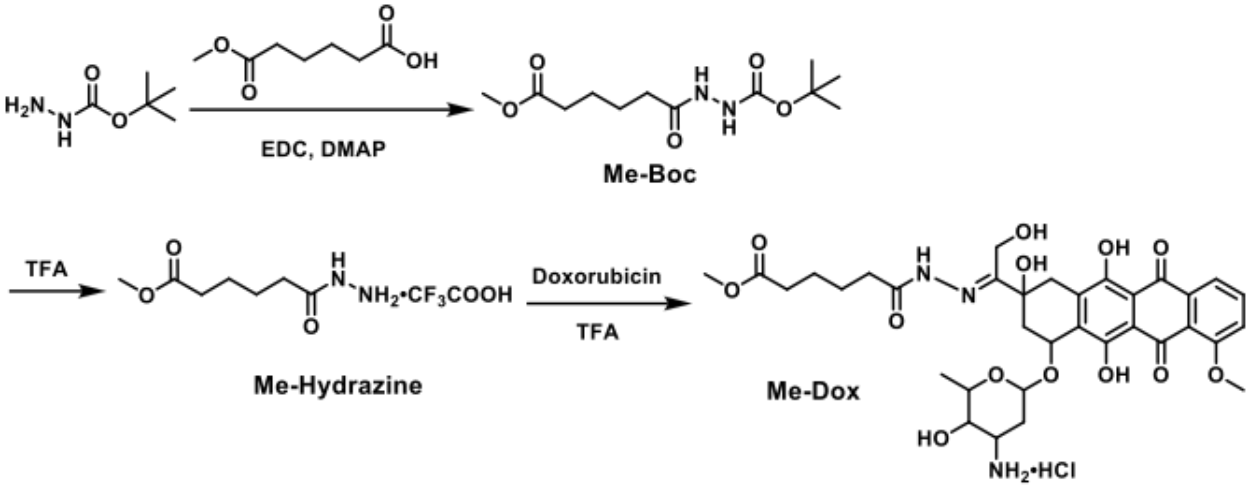
a. ***Synthesis of compound Fc-N-Me*** – A mixture of compound **Fc-N-amine** (300.0 mg), monomethyl adipate (200.0 mg, 98%, *Ark Pharm*), EDC·HCl (300.0 mg), and DMAP (5.0 mg) in 10 mL of DCM was stirred at room temperature for 36 h. After 36 h, the reaction mixture was diluted with 10 mL of DCM and washed with 5% K_2CO_3 aqueous solution four times (10 mL each). The DCM layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified on a C_{18} column, eluting with a gradient

mixture of water/MeOH (6/4 to 0/100, v/v). The target product was obtained as orange solid (300.0 mg, 69% yield). ^{1}H-NMR (500 MHz Bruker, 25°C, $CDCl_3$, ***Figure S29***): δ (ppm) = 4.15-4.08 (m, 9H), 3.66 (s) (3H), 3.36 (s) and 3.35 (s) (2H), 3.33(t, *J* = 7.3 Hz) and 3.26(t, *J* = 7.3 Hz) (2H), 2.93 (s) and 2.88 (s) (3H), 2.38-2.30 (m, 3H), 2.30-2.22 (m, 3H), 2.13 (s) and 2.12 (s) (3H), 1.70-1.62 (m, 6H).

b. ***Synthesis of compound Fc-N-Hydrazine*** – A mixture of compound **Fc-N-Me** (300.0 mg) and hydrazine monohydrate (0.66 mL, 98%, *BeanTown Chemical*) in 10 mL of MeOH was stirred at 50 °C for 24 hours. After cooling to room temperature, the sample was evaporated under reduced pressure and the residue was purified on a C_{18} column, eluting with a gradient mixture of water/MeOH (8/2 to 2/8, v/v). The target product was obtained as orange solid (300.0 mg, 100% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$**,** ***Figure S29***): *δ* (ppm) = 7.60 (s) and 7.51 (s) (1H), 4.16-4.06 (m, 9H), 3.36 (s) and 3.37 (s) (2H), 3.35 (t, *J* = 7.3 Hz) and 3.26 (t, *J* = 7.3 Hz) (2H), 2.93 (s) and 2.89 (s) (3H), 2.37-2.19 (m, 6H), 2.13 (s) and 2.12 (s) (3H), 1.71-1.61 (m, 6H).

c. ***Synthesis of Fc-Hydrazone-Dox (Fc-Hdz-Dox)*** **–** Compound **Fc-N-Hydrazine** (75.0 mg) and Doxorubicin hydrochloride (14.0 mg, 98%, *Bide Pharmatech*) were dissolved in 1 mL of dry MeOH. Then trifluoroacetic acid (40 μL) was added to the solution and the reaction mixture was stirred for 2 days in the dark. The entire reaction solution was precipitated into 40 mL of dry ethyl acetate. The dark red precipitate was collected by centrifugation and washed with dry ethyl acetate two times (5 mL each). The product (24 mg, 100% yield) was obtained as dark red solid after dried under high vacuum. ^{1}H-NMR (500 MHz Bruker, 25 °C, DMSO**,** ***Figure S30***): *δ* (ppm) = 10.31 (s, 1H), 7.92-7.90 (m, 4H), 7.82 (m, 6H), 7.65 (m, 2H), 5.77 (m, 1H), 5.49-5.43 (m, 3H), 5.32-5.27 (m, 2H), 4.95 (m, 1H), 4.44-4.35 (m, 6H), 4.31-4.23 (m, 4H), 4.22-4.02 (m), 3.97 (s, 3H), 3.49 (s, 3H), 3.28-3.14 (m), 2.30-2.67 (m), 2.63-2.53 (m), 2.32-2.01 (m), 1.91-1.25 (m), 1.15 (d, *J* = 6.5 Hz, 3H). <u>*ESI-MS*</u>: 968.37 (expected $[M]^+$), 968.28 (observed $[M]^+$).

1.16 Synthesis of Me-Hdz-Dox



a. ***Synthesis of compound Me-Boc*** – A mixture of *tert*-Butyl carbazate (1.32 g, 95+%, *Matrix Scientific*), monomethyl adipate (1.66 g), EDC·HCl (2.00 g), and DMAP (20.0 mg) in 20 mL of DCM was stirred at room temperature for 12 h. The reaction mixture was then diluted with 20 mL of DCM and washed with 5% K_2CO_3 aqueous solution four times (40 mL each). The DCM layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified with silica column, eluting with DCM/ethyl acetate (9/1, v/v). The target product was obtained as colorless sticky liquid (2.51 g, 92% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$**,** ***Figure S33***): δ (ppm) = 7.59 (s, 1H), 6.58 (s, 1H), 3.66 (s, 3H), 2.34 (m, 2H), 2.24 (m, 2H), 1.79-1.63 (m, 4H), 1.46 (s, 9H).

b. ***Synthesis of compound Me-Hydrazine*** – A solution of compound **Me-Boc** (1.00 g) in 5 mL of 20% TFA in DCM was stirred at room temperature for 2 hours. After 2 hours, the volatiles were evaporated under reduced pressure to give colorless sticky liquid (100% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, $CDCl_3$**,** ***Figure S33***): δ (ppm) = 9.30 (s, 3H), 3.66 (s, 3H), 2.38-2.31 (m, 4H), 1.69-1.56 (m, 4H).

c. ***Synthesis of Me-Dox*** – Compound **Me-Hydrazine** (144.0 mg) and Doxorubicin hydrochloride (29.0 mg) were dissolved in 2 mL of dry MeOH. Trifluoroacetic acid (30 μL) was added to the solution and the reaction mixture was stirred for 2 days in the dark. The reaction solution was then precipitated into 40 mL of dry ethyl acetate. The dark red precipitate was collected by centrifugation and washed with dry ethyl acetate two times (5 mL each). The product was dried under high vacuum and obtained as a dark red solid (21 mg, 57% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, DMSO**,** ***Figure S34***): δ (ppm) = 10.31

(s, 1H), 7.92-7.90 (m, 4H), 7.82 (m, 6H), 7.65 (m, 2H), 5.75 (m, 1H), 5.48 (m, 1H), 5.45 (s, 1H), 5.44 (s, 1H), 5.30-5.26 (m, 2H), 4.95 (t, J = 6.6 Hz, 1H), 4.42-4.33 (m, 2H), 4.05-3.95 (m, 2H), 3.96 (s, 3H), 3.56 (m, 4H), 3.49 (s, 3H), 3.30 (d, J = 17.0 Hz, 1H), 2.76 (d, J = 17.0 Hz, 1H), 2.37-2.05 (m, 6H), 1.87 (m, 1H), 1.70 (m, 1H), 1.63-1.45 (m), 1.33 (m), 1.15 (d, J = 6.5 Hz, 3H). *ESI-MS*: 700.10 (expected $[M]^+$), 700.15 (observed $[M]^+$).

1.17 Fc-Hdz-Dox Release Studies

F127-CB[7]:PEG$_8$-Fc hydrogels (0.56 mL, 10 w/v% solid content, 3:1 molar ratio of **CB[7]** and guest) were preloaded with 0.933 mg Fc-N-Hdz-Dox by mixing at 4 °C. Then the hydrogel was divided into mini-dialysis kit and dialyzed against 45 mL PBS at pH = 7.4 or 5.5 and 37 °C. At serial times, 0.5 mL of the dialysis solution was samples and replaced with fresh buffer. The doxorubicin content was examined by measuring the fluorescence on a TECAN Infinite M200 PRO microplate reader, fitting to a standard curve established from pure doxorubicin in buffer.

1.18 In Vitro Drug Functional Assay

A standard MTT assay was used to measure and compare *in vitro* cytotoxiciy of Fc-N-Hdz-Dox and Me-Hdz-Dox. Briefly, MDA-MB-231 cells were seeded in 96 well plates at a density of 20,000 cells per well in 200 μL complete growth media (DMEM with 10% FBS) and cultured for 24 hrs. Assorted concentrations of Fc-Hdz-Dox, Me-Hdz-Dox and free doxorubicin were prepared in fresh growth media were prepared and added to each well. The cells were incubated for 72 hrs. Controls consisted of cells grown in the same volume of normal media, and background was collected from wells with media only. The media in each well were replaced with 100 uL of *3*-(*4,5*-Dimethylthiazol-*2*-yl)-*2,5*-Diphenyltetrazolium Bromide (MTT) salt in PBS solution at a concentration of 2 mg/mL. After incubation at 37 °C for 1 hr, the supernatant was replaced with 100 μL of dimethyl sulfoxide (DMSO) and plates were agitated for 15 minutes at room temperature. Absorbance readings were collected on a TECAN Infinite M200 PRO microplate reader at 570 nm. Results were expressed as viability relative to the cell number in the untreated wells. Samples were fit to a standard dose-response function (GraphPad Prism v8) to extract the IC_{50} for each compound.

1.19 In Vivo Tumor Model

Luciferase-expressing MDA-MB-231 cells (*Gen Target Inc*) were received and passaged 3 times under sterile conditions in standard growth media (DMEM with 10% FBS). Tumors were induced by the injection of 10^6 cells suspended in 50% growth factor-reduced Matrigel (BD Biosciences) to a total volume of 200 μL (100 μL PBS and 100 μL Matrigel) subcutaneously onto the shaved right flank of NSG mice under sterile conditions. After 14 days, mice without palpable tumors were culled from the study. Remaining mice were treated by injection of **F127-CB[7]:PEG$_8$-Fc** hydrogels (0.1 mL, 10 w/v% solid content, 3:1 molar ratio of **CB[7]** and guest) immediately adjacent to the tumor using a 22G syringe. This day was considered as 'day 0' throughout the study. One day after gel injection (day 1), tumors started being tracked using *in vivo* bioluminescence imaging using an IVIS Lumina Imager. Mice were intraperitoneally injected with 0.1 ml of 30 mg/ml *D*-Luciferin in 1x DPBS 10 min before imaging, determined from control experiments to be sufficient to capture luminescent signal at its plateau. Mice were randomized into groups. Treated animals received Fc-Hdz-Dox or Me-Hdz-Dox (6 mg/kg Dox-equivalent dose) by i.p. injection in 0.1 ml saline 1, 2, and 3 days after hydrogel application. PBS was injected in the same way into control animals. Throughout the study, *in vivo* bioluminescence imaging and body weight was collected for treated mice every three days. At day 28, mice were euthanized and tumors were excised and weighed. These studies were approved the the University of Notre Dame IACUC and completed with consultation from veterinary staff.

1.20 Safety Statement

MDA-MB-2321 human breast cancer cells were handled according to common BSL-2 procedures for the handling of patient-derived human cell lines. Extra care should be taken in the handling of sodium azide, including avoiding use of metal spatula and using respiratory barriers. Care should be taken to avoid direct contact with doxorubicin.

2-Supporting Data and Characterization

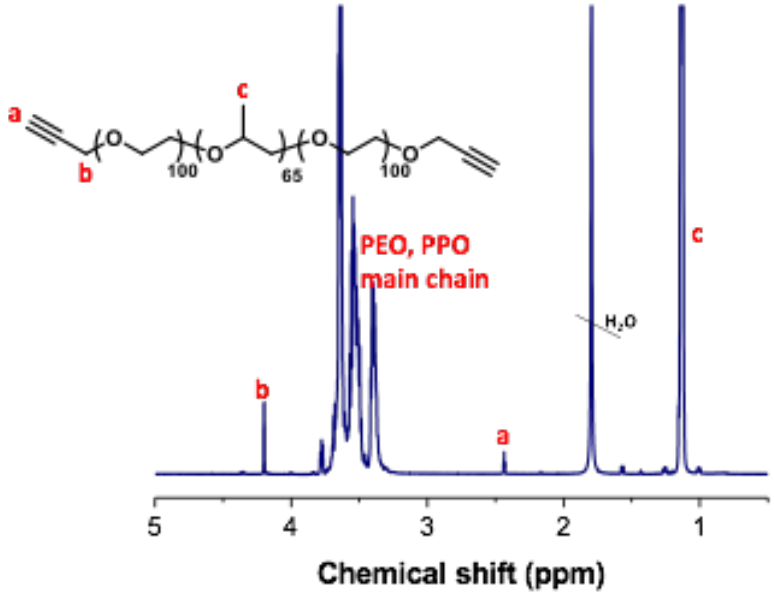


Figure S1: ^{1}H-NMR of F127 terminated with alkyne groups (F127-alkyne) in $CDCl_3$ used in the synthesis of the **F127-CB[7]**.

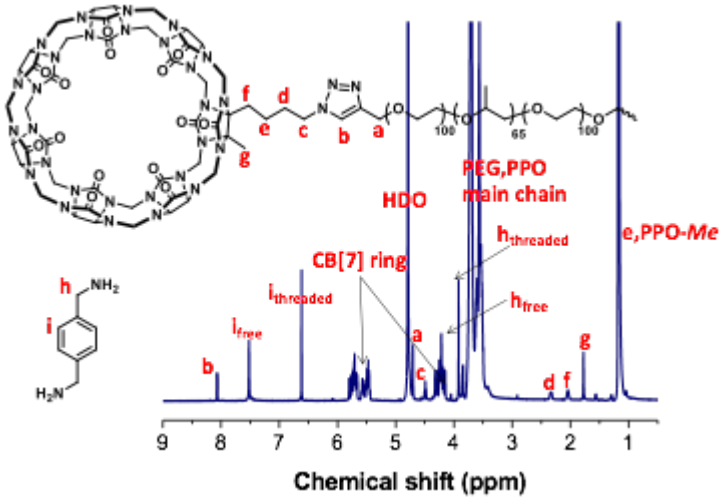


Figure S2: ^{1}H-NMR of F127 terminated with **CB[7]** (**F127-CB[7]**) with >1 equivalent p-xylylenediamine added as a visualization probe in D_2O.

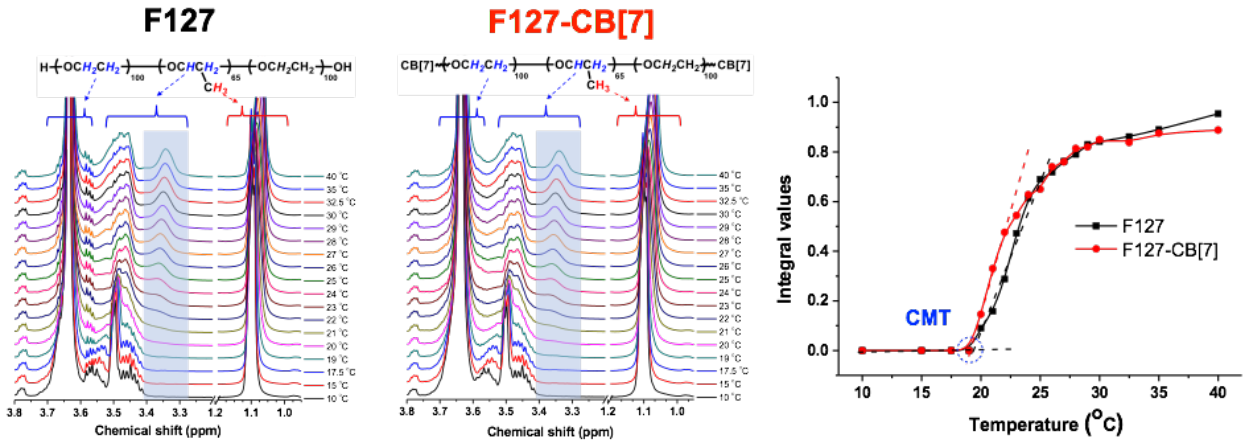


Figure S3: Temperature-dependent ^{1}H-NMR in D_2O of **F127** and **F127-CB[7]** to determine the critical micelle temperature (CMT).

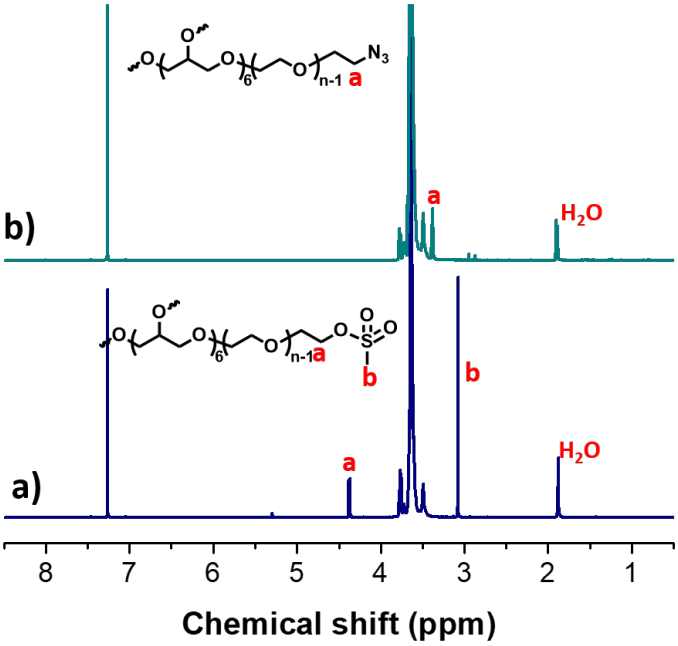


Figure S4: ^{1}H-NMR spectra of (a) mesylate-modified and (b) azide-modified 8-arm PEG used in the synthesis of the CB[7]-guest-terminated PEG macromer.

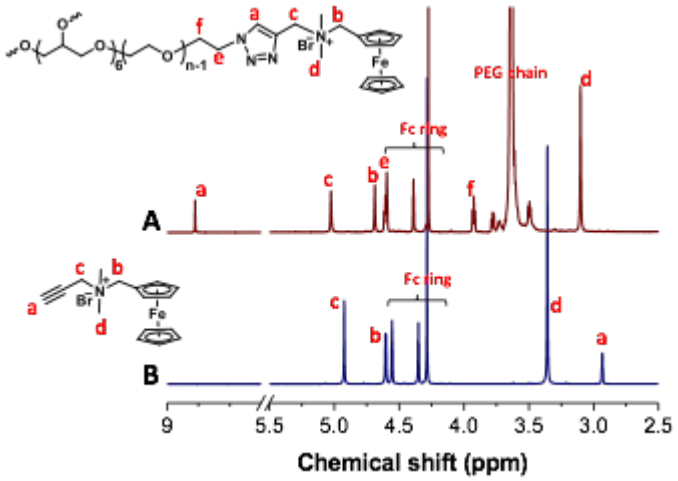


Figure S5: ^{1}H-NMR spectra of 8-arm PEG terminated with Fc guest (PEG_8-Tz-N-Fc, A) as well as its alkyne-N-Fc precursor (B), both in $CDCl_3$.

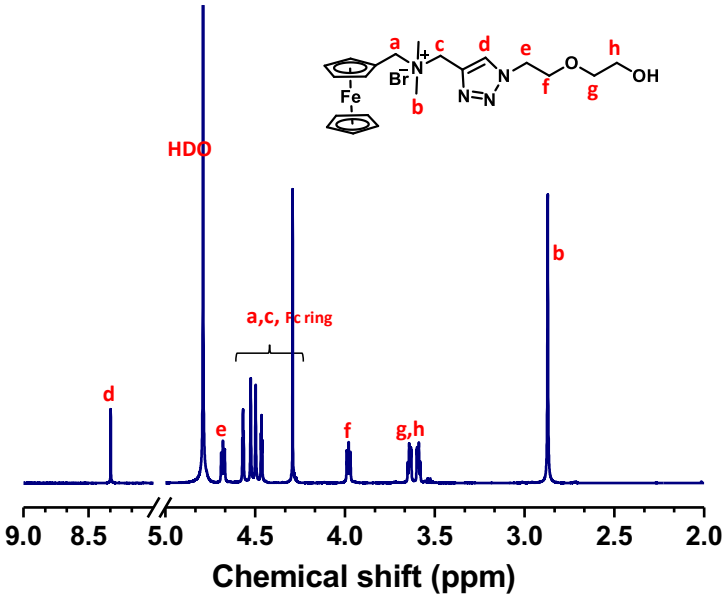


Figure S6: ^{1}H-NMR spectra of model guest (**OEG-Tz-N-Fc**) for use in determining binding affinity to **CB[7]**, in D_2O.

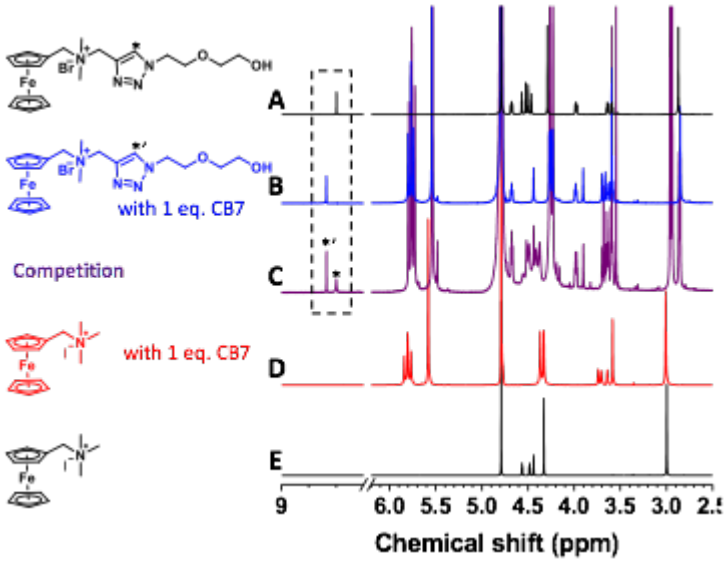


Figure S7: Example ^{1}H-NMR spectra from guest competition studies to determine K_{eq} for **PEG$_{8a}$-Fc**. (a) Model guest **OEG-Tz-N-Fc**, (b) model guest **OEG-Tz-N-Fc** with 1 eq. **CB[7]**, (c) model guest **OEG-Tz-N-Fc** (1.356 mM) and competitive guest (2.887 mM) with **CB[7]** (1.911 mM), (d) competitive guest with **CB[7]** and (e) competitive guest in D_2O. K_{rel} value of 0.87 was obtained from averaging three different competition experiments with different ratios of guest, competitor, and **CB[7]**. Comparing to the literature-reported value of K_{eq} of 3.98×10^{12} M^{-1} for the competitor,[6] the K_{eq} for this guest **OEG-Tz-N-Fc** was determined at 3.46×10^{12} M^{-1}. (*'-bound signal, *-free signal used for the calculation of bound and free guest species)

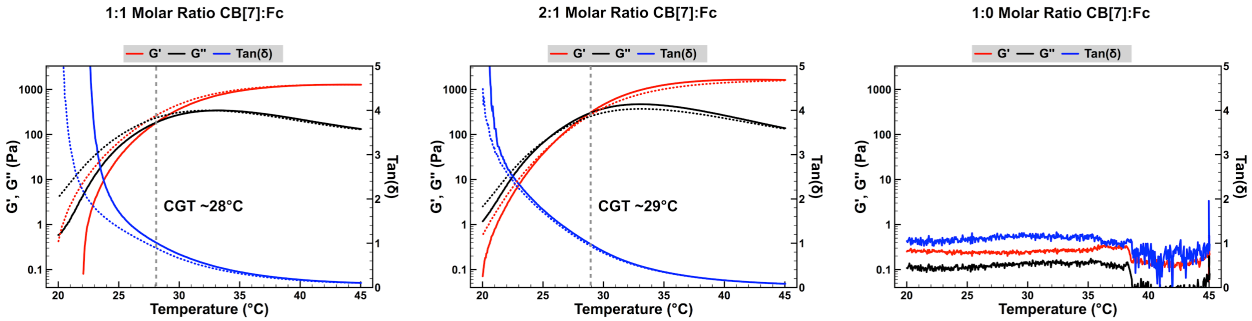


Figure S8: Control variable temperature rheology experiments with **PEG$_8$-Fc** and **F127-CB[7]** altering the CB[7]:Fc ratio while keeping the total dissolved solids constant. A thermal sweep was performed, with solid lines (heating) and dashed lines (cool) plotted.

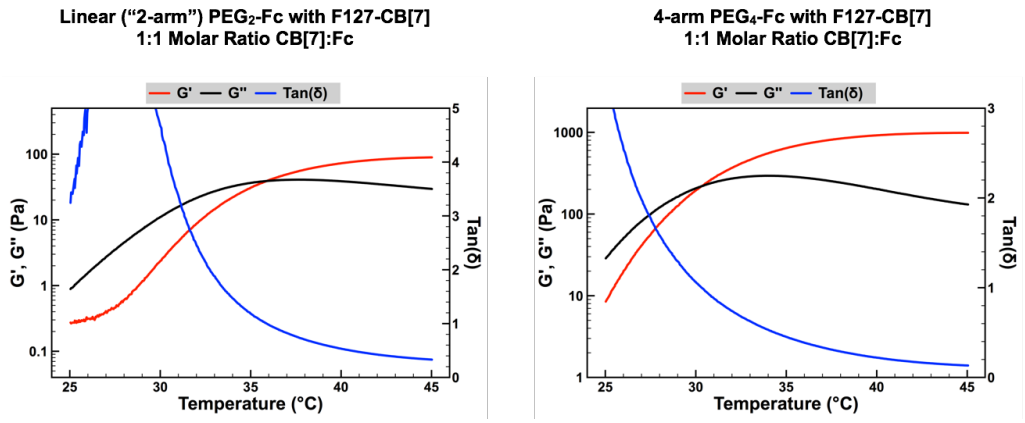


Figure S9: Control rheology experiments altering the valency of the Fc guest macromer, using instead a linear (2-arm) macromer, **PEG$_2$-Fc** (left), as well as a 4-arm macromer **PEG$_4$-Fc** (right) to gel the **F127-CB[7]** starting material.

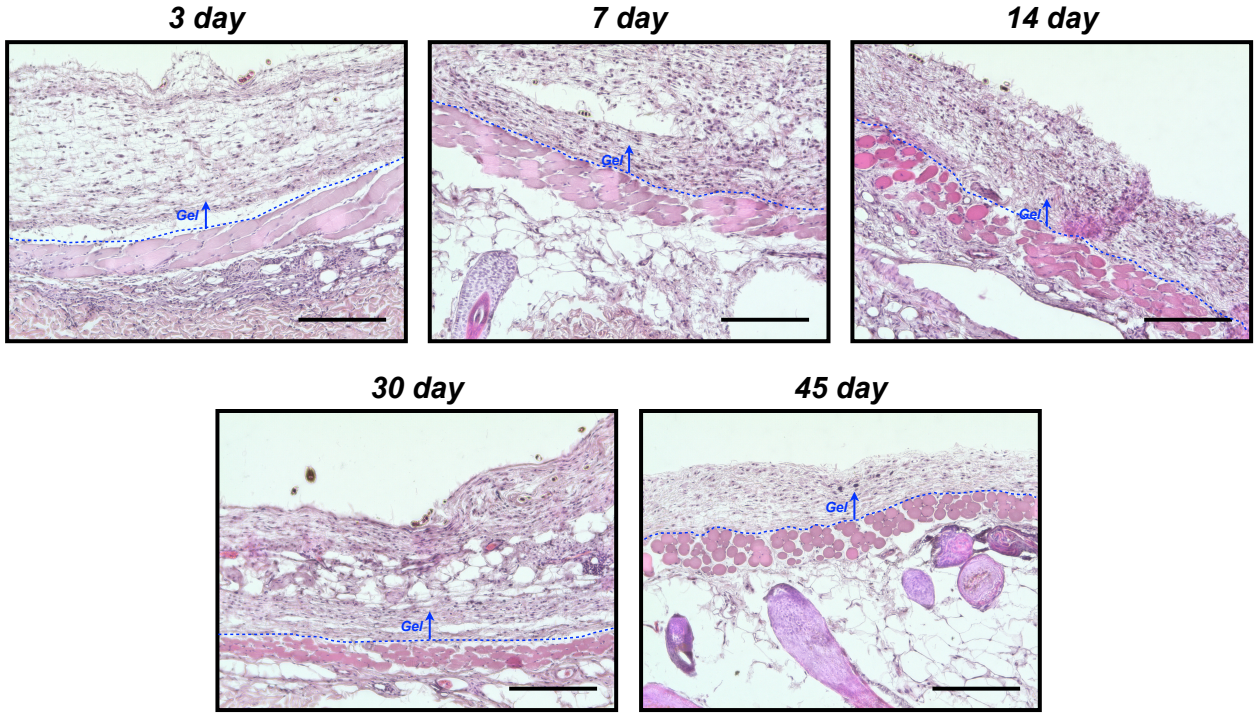


Figure S10: Representative histology images (20x) stained with H&E evaluating the tissue reaction, cell infiltration, and clearance of F127-CB[7]:PEG$_8$-Fc hydrogels at days 3, 7, 14, 30, and 45 following implantation. No hydrogel could be found or recovered for histology by day 60. The gel/tissue margin is noted with the blue dotted line and the arrow indicates the position of the hydrogel. Uninfiltrated hydrogel apparently dissolved in the course of histology processing. All studies were performed in n=3 mice per timepoint. Scale bar for all images is 200 μm.

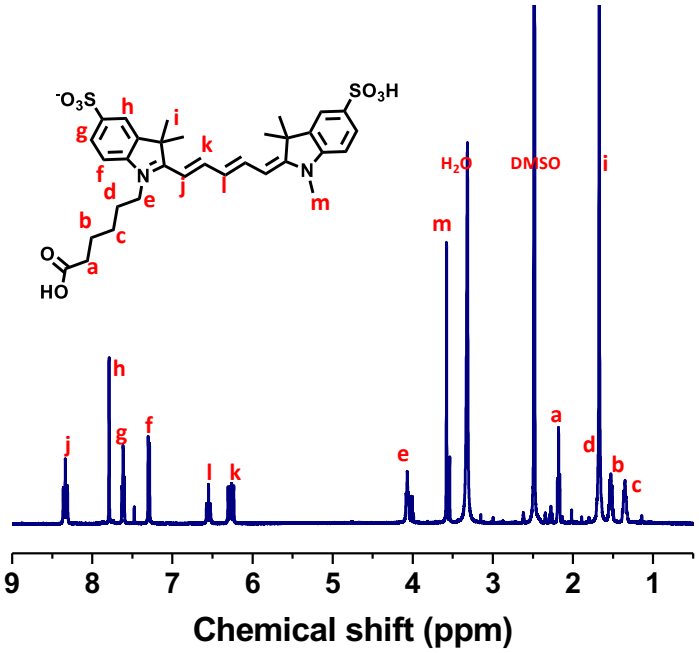


Figure S11: ^{1}H-NMR of disulfo-Cy5-COOH in DMSO-d_6.

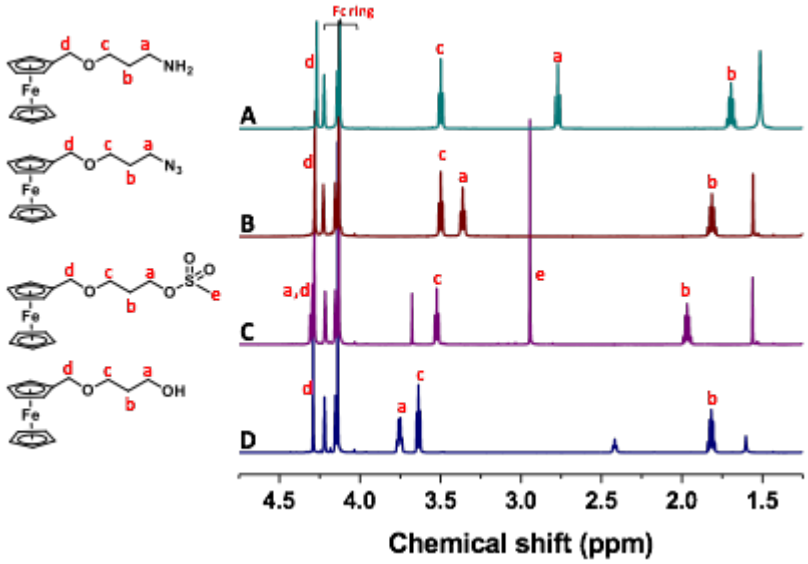


Figure S12: ^{1}H-NMR spectra of compound (A) **Fc-O-NH$_2$**, (B) **Fc-O-N$_3$**, (C) **Fc-O-Ms**, and (D) **Fc-O-OH** in $CDCl_3$.

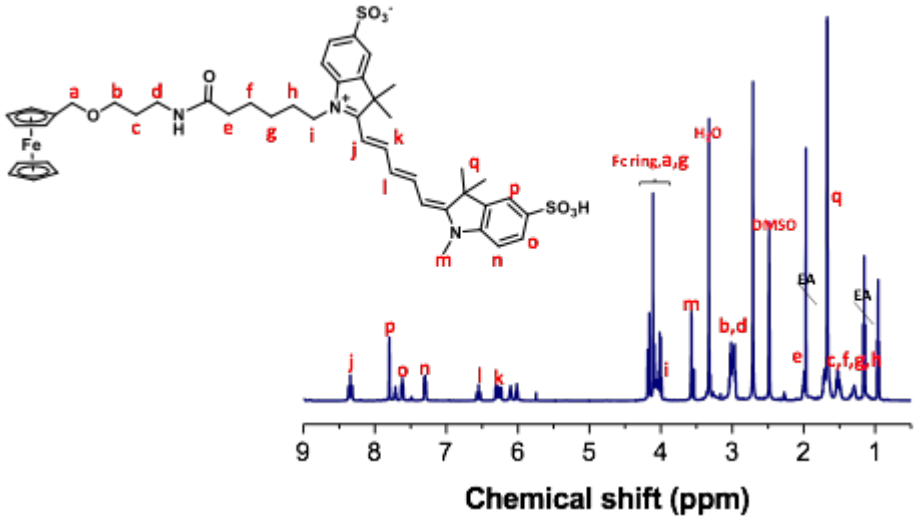


Figure S13: ^{1}H-NMR spectrum of **Fc-O-Cy5** in DMSO-d_6.

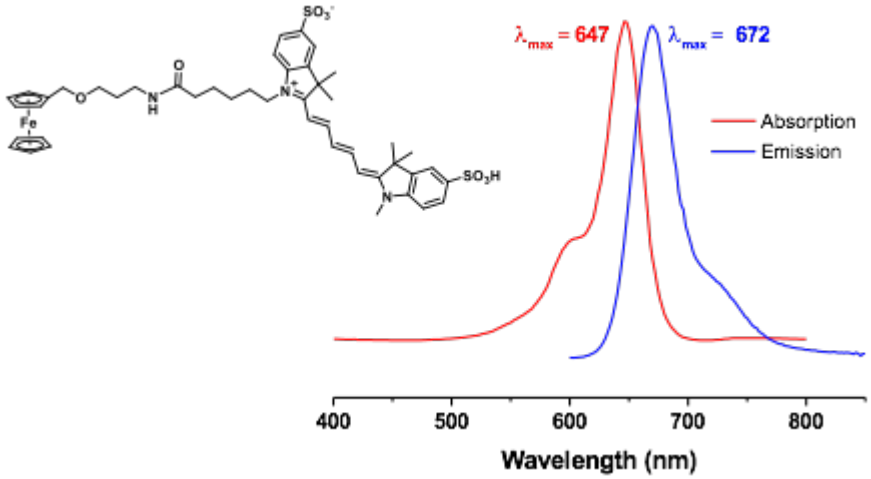


Figure S14: Absorbance spectra (red) and emission spectra (blue, @ ex:620 nm) of **Fc-O-Cy5** in PBS.

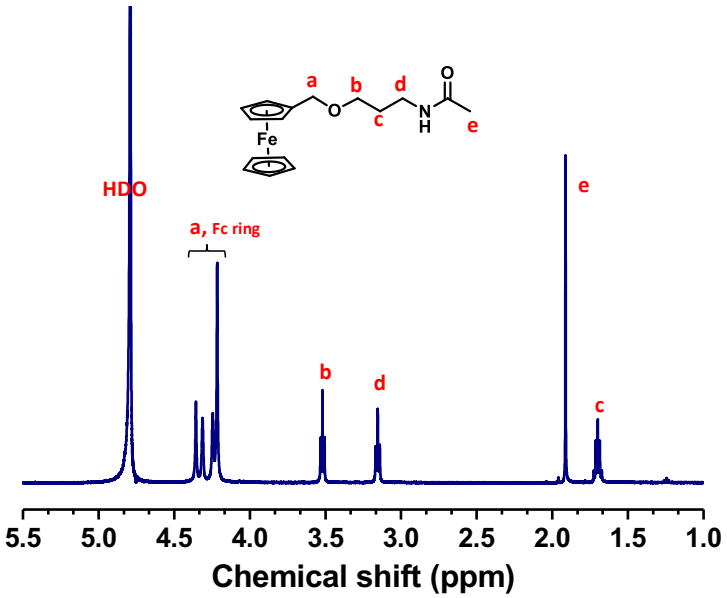


Figure S15: ^{1}H-NMR of model guest (**Fc-O-Ac**) for use in determining binding affinity to **CB[7]** of Fc-O-Cy5, in D_2O.

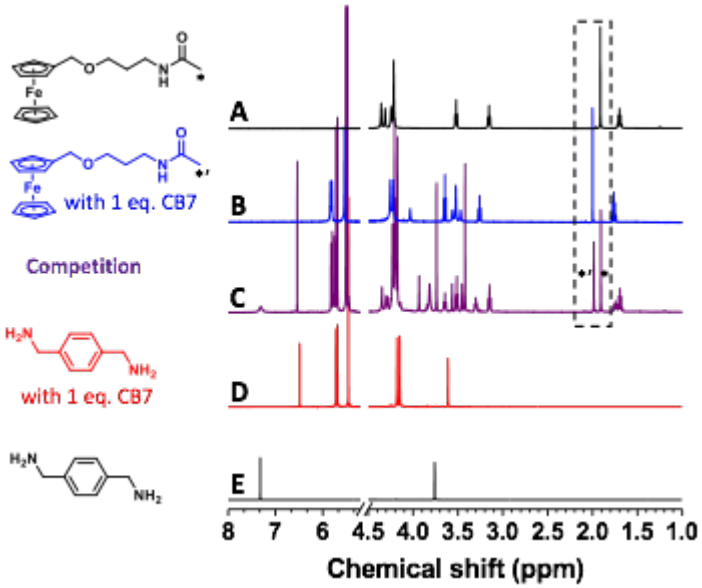


Figure S16: Example ^{1}H-NMR spectra from guest competition studies to determine K_{eq} for **Fc-O-Cy5**. (a) model guest **Fc-O-Ac**, (b) model guest **Fc-O-Ac** with **CB[7]**, (c) model guest **Fc-O-Ac** (1.904 mM) and competitive guest p-xylylenediamine (1.96 mM) with **CB[7]** (2.18 mM), (d) competitive guest p-xylylenediamine with **CB[7]** and (e) competitive guest p-xylylenediamine in D_2O. K_{rel} value of 0.52 was obtained from averaging three different competition experiments with different ratios of guest, competitor, and **CB[7]**. Comparing to the literature-reported value of K_{eq} of 1.84×10^{9} M^{-1} for the competitor p-xylylenediamine,[6] the K_{eq} for guest moieties **Fc-O-Cy5** was determined at 9.5×10^{8} M^{-1}. (*'-bound signal, *-free signal for the calculation of bound and free guest species).

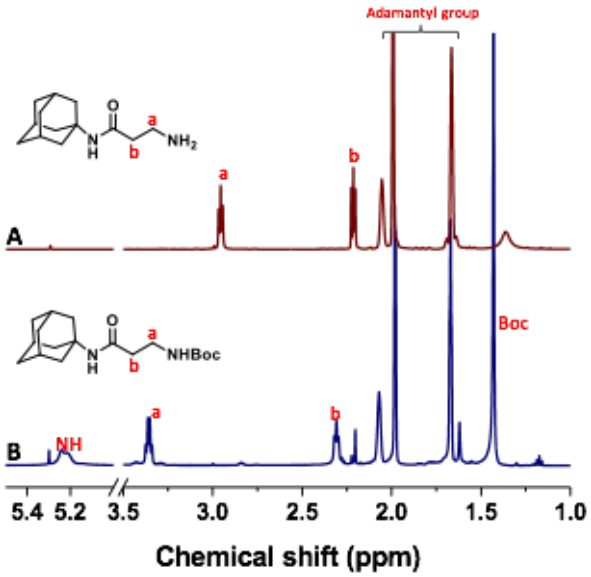


Figure S17: ^{1}H-NMR spectra of (A) Ada-Am-Amine and (B) Ada-Am-Boc precursors to **Ada-Am-Cy5**, in $CDCl_3$.

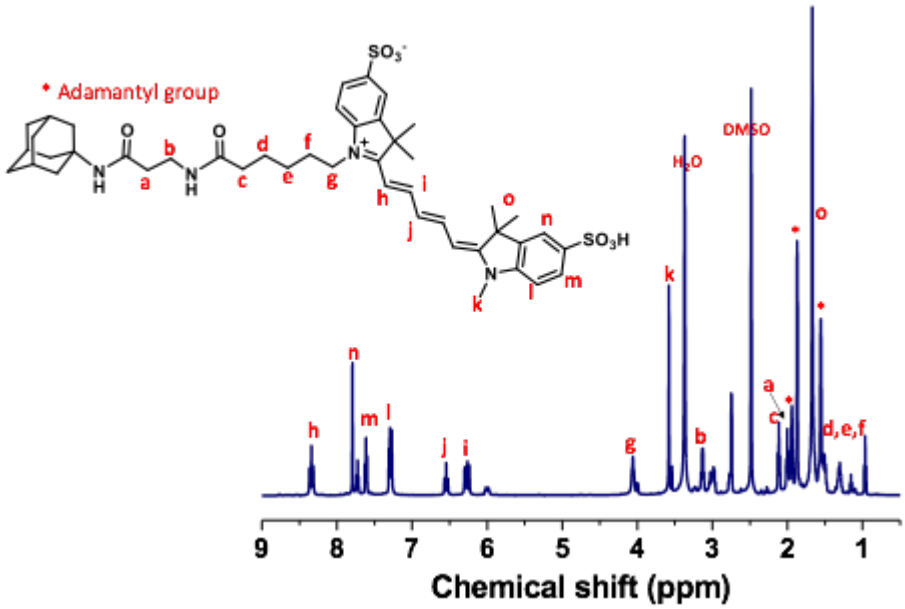


Figure S18: ^{1}H-NMR spectrum of **Ada-Am-Cy5** in DMSO-d_6.

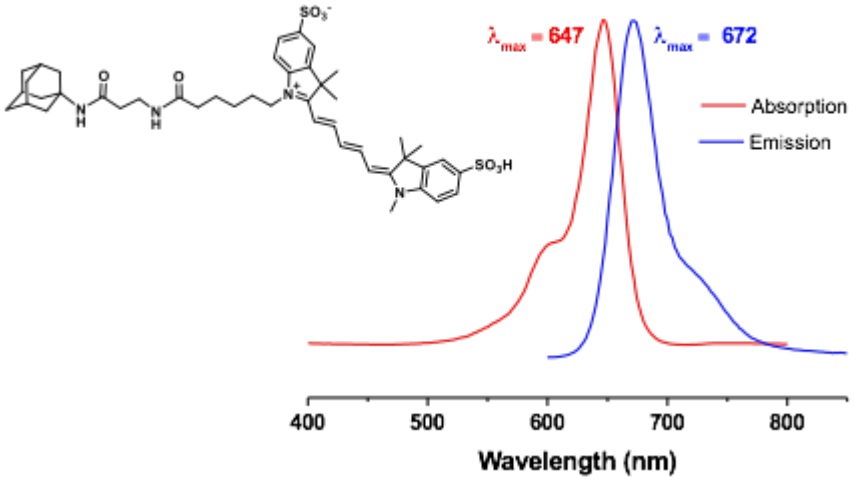


Figure S19: Absorbance spectra (red) and emission spectra (blue, @ ex:620 nm) of Ada-Am-Cy5 in PBS.

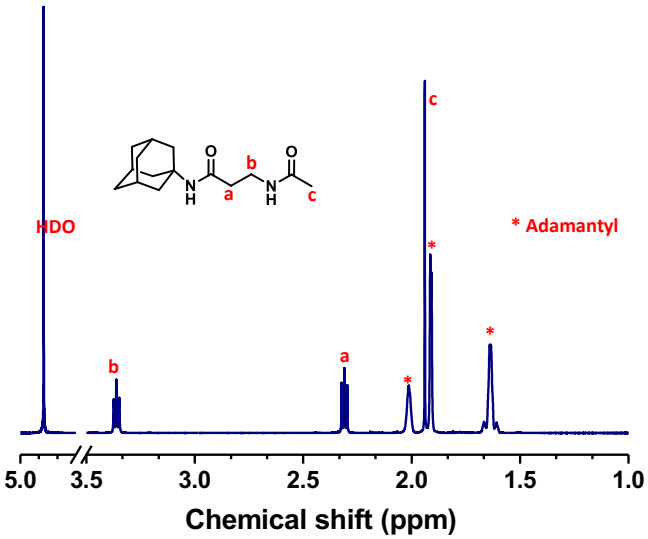


Figure S20: ^{1}H-NMR of model guest (**Ada-Am-Ac**) for use in determining binding affinity to **CB[7]** of **Ada-Am-Cy5**, in D_2O.

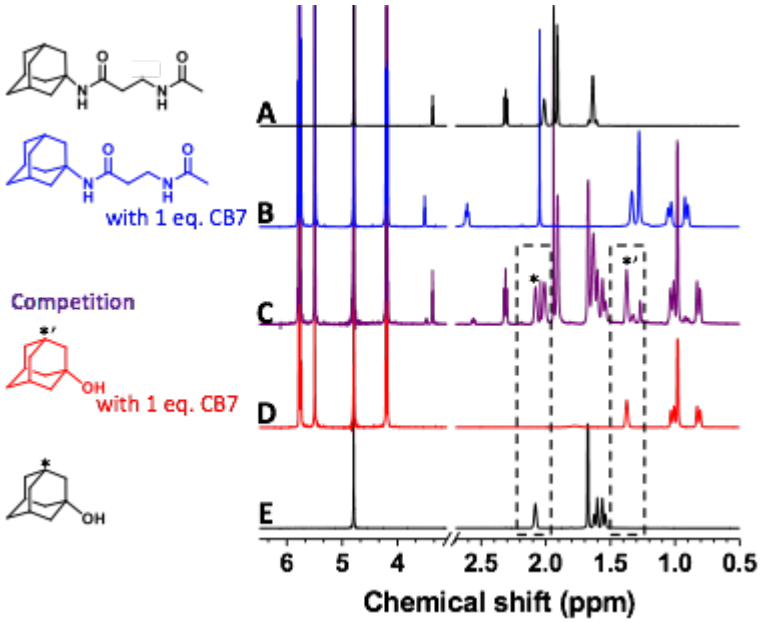


Figure S21: Example ^{1}H-NMR spectra from guest competition studies to determine K_{eq} for **Ada-Am-Cy5**. (a) model guest **Ada-Am-Ac**, (b) model guest **Ada-Am-Ac** with **CB[7]**, (c) model guest **Ada-Am-Ac** (1.893 mM) and competitive guest 1-adamantanol (1.88 mM) with **CB[7]** (2.595 mM), (d) competitive guest 1-adamantanol with **CB[7]** and (e) competitive guest 1-adamantanol in D_2O. K_{rel} value of 0.89 was obtained from averaging three different competition experiments with different ratios of guest, competitor, and **CB[7]**. Comparing to the literature-reported value of K_{eq} of 2.3 $\times 10^{10}$ M^{-1} for the competitor,[6] the K_{eq} for guest moieties Ada-amide-CY5 was determined at 2.05×10^{10} M^{-1}. (*'-bound signal, *-free signal for the calculation of bound and free guest species)

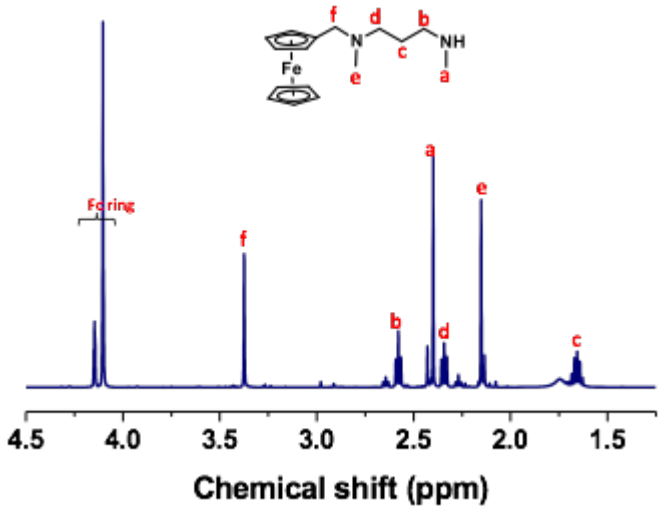


Figure S22: ^{1}H-NMR spectrum of **Fc-N-Amine**, precursor to **Fc-N-Cy5**, in $CDCl_3$.

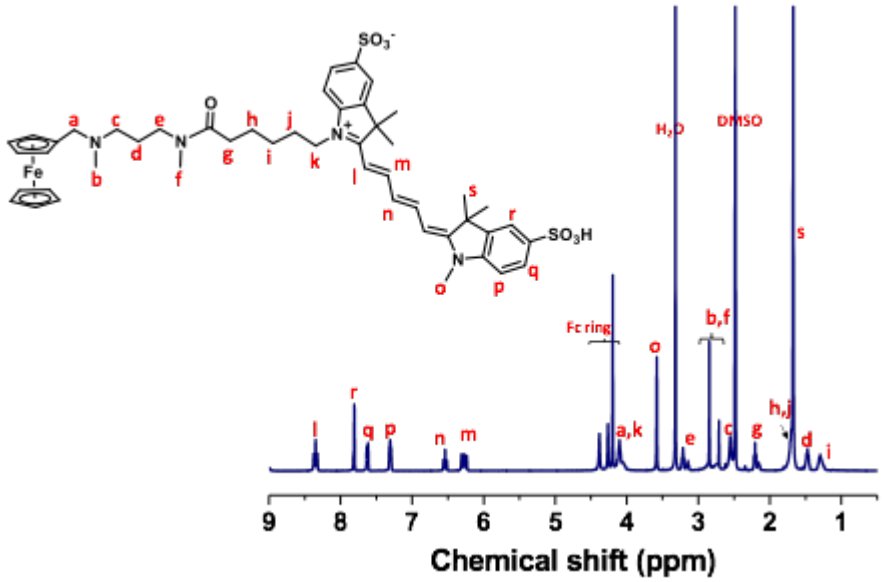


Figure S23: ^{1}H-NMR spectrum of **Fc-N-Cy5** in DMSO-d_6.

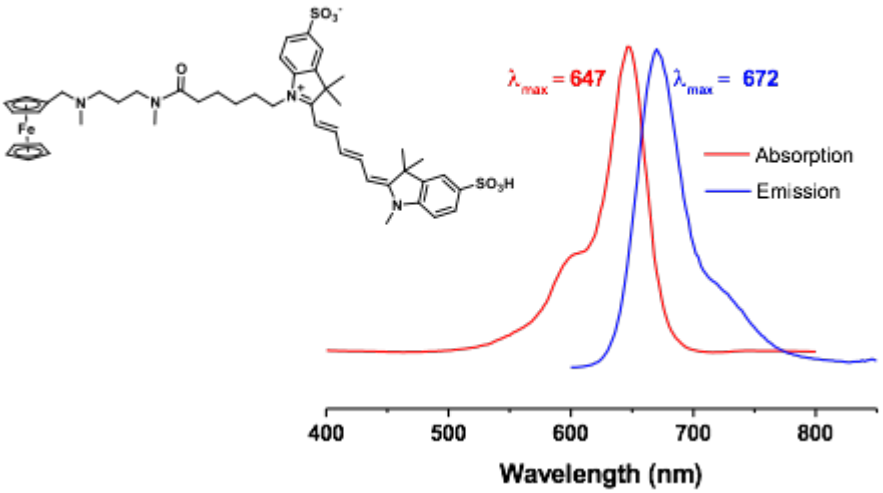


Figure S24: Absorbance spectra (red) and emission spectra (blue, @ ex:620 nm) of **Fc-N-Cy5** in PBS.

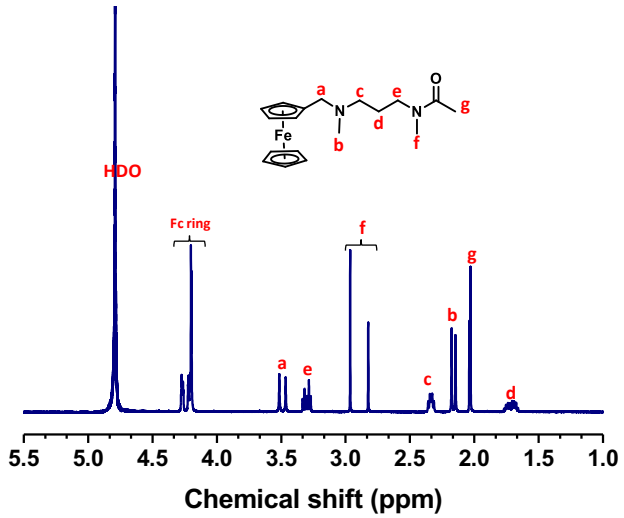


Figure S25: ^{1}H-NMR of model guest (**Fc-N-amide**) for use in determining binding affinity to **CB[7]** of **Fc-N-Cy5**, in D_2O.

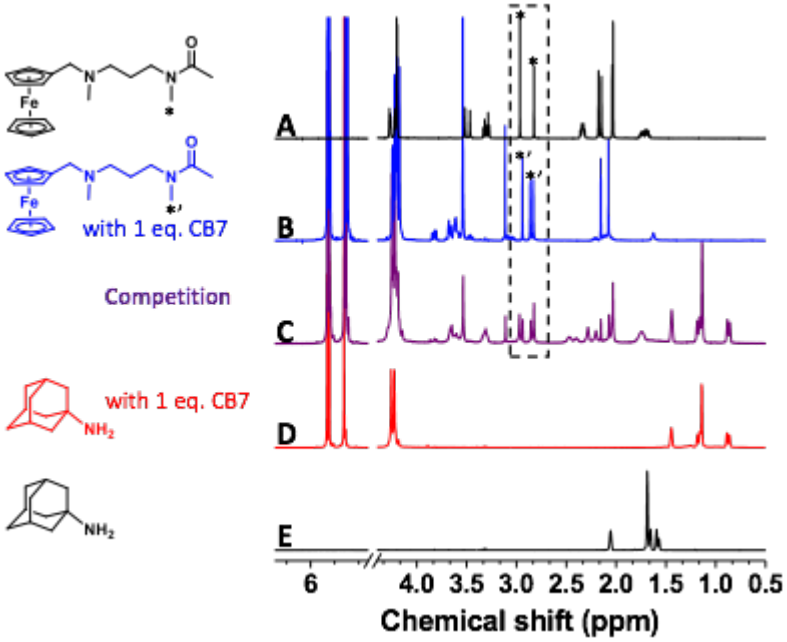


Figure S26: Example ^{1}H-NMR spectra from guest competition studies to determine K_{eq} for Fc-N-Cy5. (a) model guest Fc-N-Ac, (b) model guest Fc-N-Ac with CB[7], (c) model guest Fc-N-Ac (2.0 mM) and competitive guest (1.257 mM) with CB[7] (2.178 mM), (d) competitive guest 1-adamantylamine with CB[7] and (e) competitive guest 1-adamantylamine in D_2O. K_{rel} value of 0.36 was obtained from averaging three different competition experiments with different ratios of guest, competitor 1-adamantylamine, and CB[7]. Comparing to the literature-reported value of K_{eq} of 4.17×10^{12} M^{-1} for the competitor 1-adamantylamine,[6] the K_{eq} for guest moieties Fc-N-Cy5 was determined at 1.5×10^{12} M^{-1}. (*'-bound signal, *-free signal for the calculation of bound and free guest species)

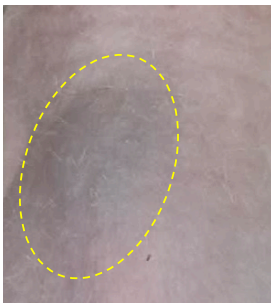


Figure S27: Photograph of subcutaneous F127-CB[7]:PEG$_8$-Fc gel, demonstrating the blue color that emerges following system administration of Fc-N-Cy5.

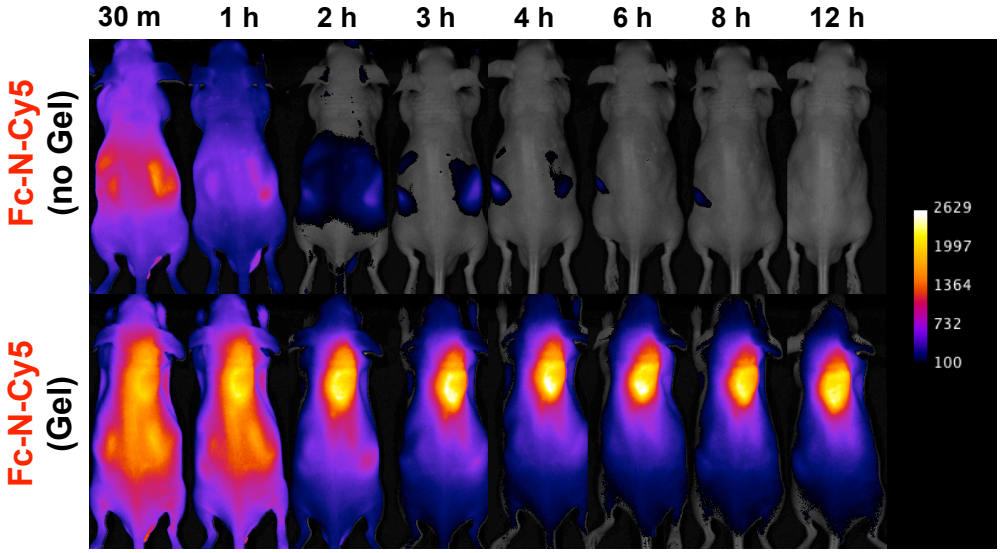


Figure S28: Optical clearance profile for Fc-N-Cy5 in animals without (top) compared to with (bottom) the F127-CB[7]:PEG8-Fc hydrogel.

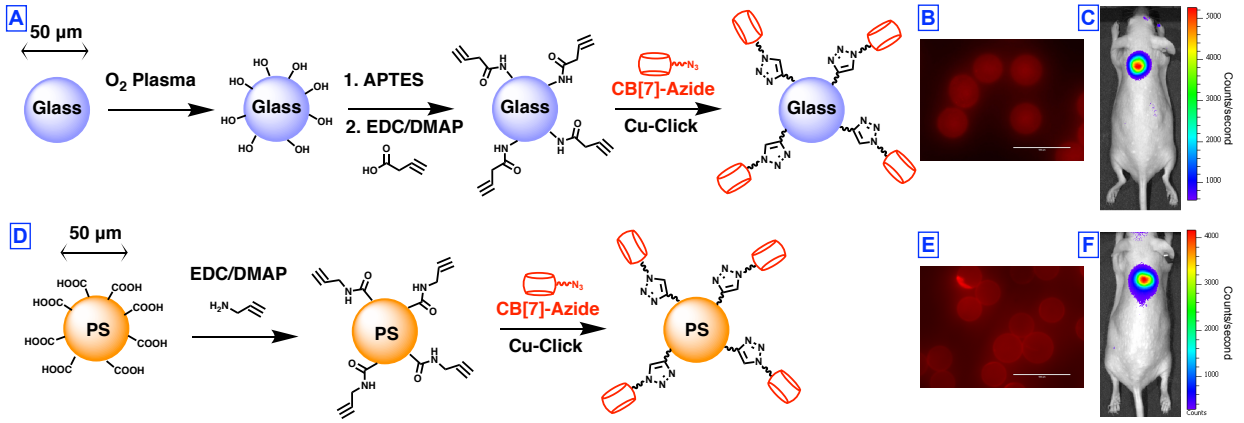


Figure S29: (A) Scheme for **CB[7]** modification of glass beads. (B) Fluorescence microscopy showing **Fc-N-Cy5** bound to the surface of glass beads. (C) In vivo imaging demonstrating homing of **Fc-N-Cy5** to a subcutaneous site where glass beads have been injected following systemic administration of the dye. (D) Scheme for **CB[7]** modification of polystyrene beads. (E) Fluorescence microscopy showing **Fc-N-Cy5** bound to the surface of polystyrene beads. (F) In vivo imaging demonstrating homing of **Fc-N-Cy5** to a subcutaneous site where polystyrene beads have been injected following systemic administration of the dye.

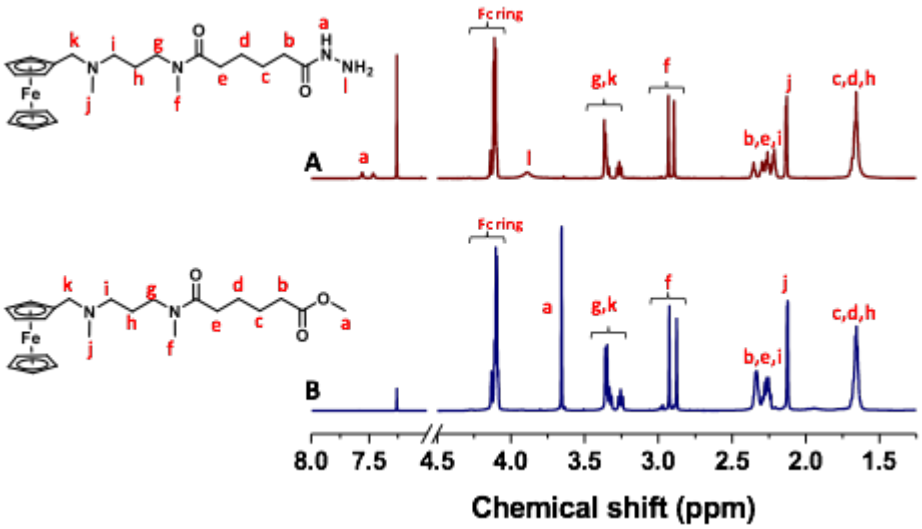


Figure S30: ^{1}H-NMR spectra of precursors to **Fc-Hdz-Dox** in $CDCl_3$.

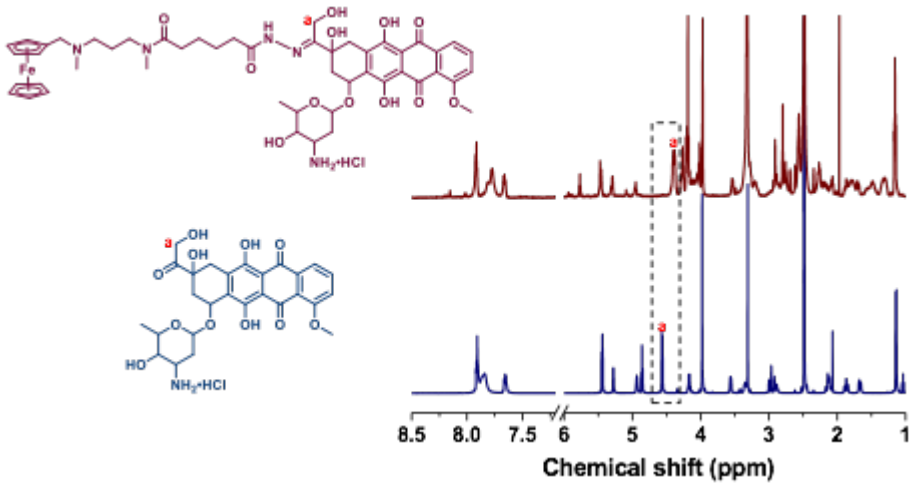


Figure S31: ^{1}H-NMR spectra of **Fc-Hdz-Dox** in DMSO-d_6.

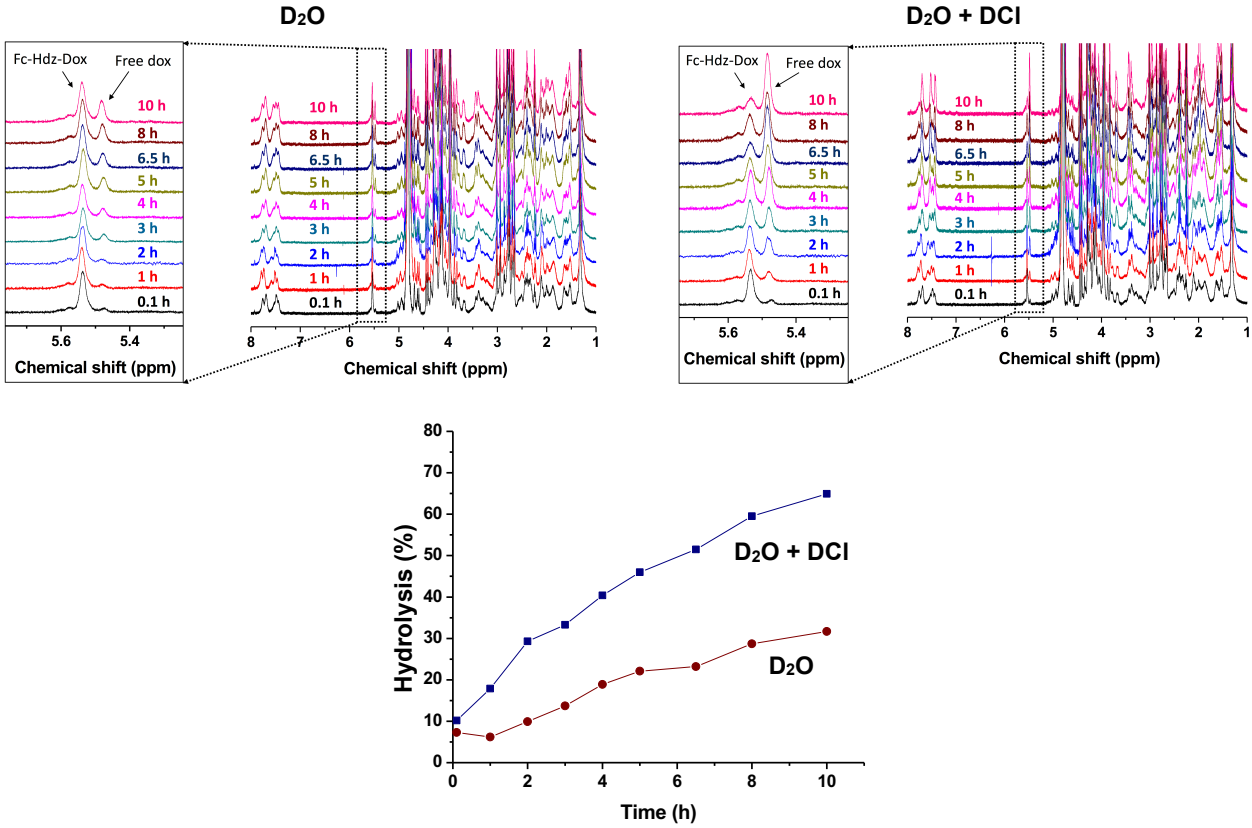


Figure S32: ^{1}H-NMR studies performed in D_2O (pH ~7) and in D_2O + DCl (pH ~5) to monitor the rate of hydrolysis of the hydrazone bond.

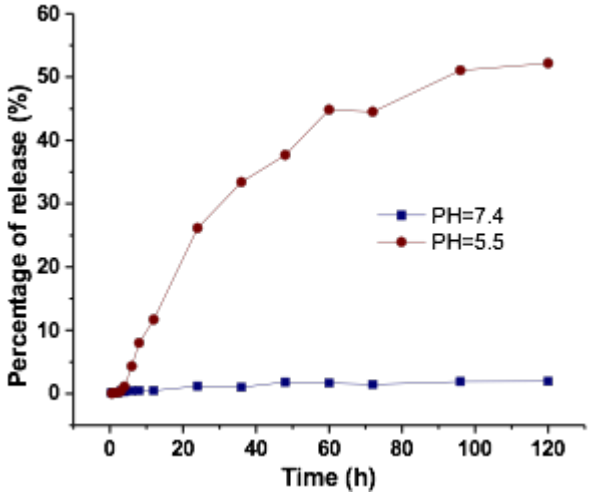


Figure S33: Doxorubicin release from **F127-CB[7]:PEG$_8$-Fc** hydrogels loaded with **Fc-Hdz-Dox** over time at pH 5.5 (red) and 7.4 (blue).

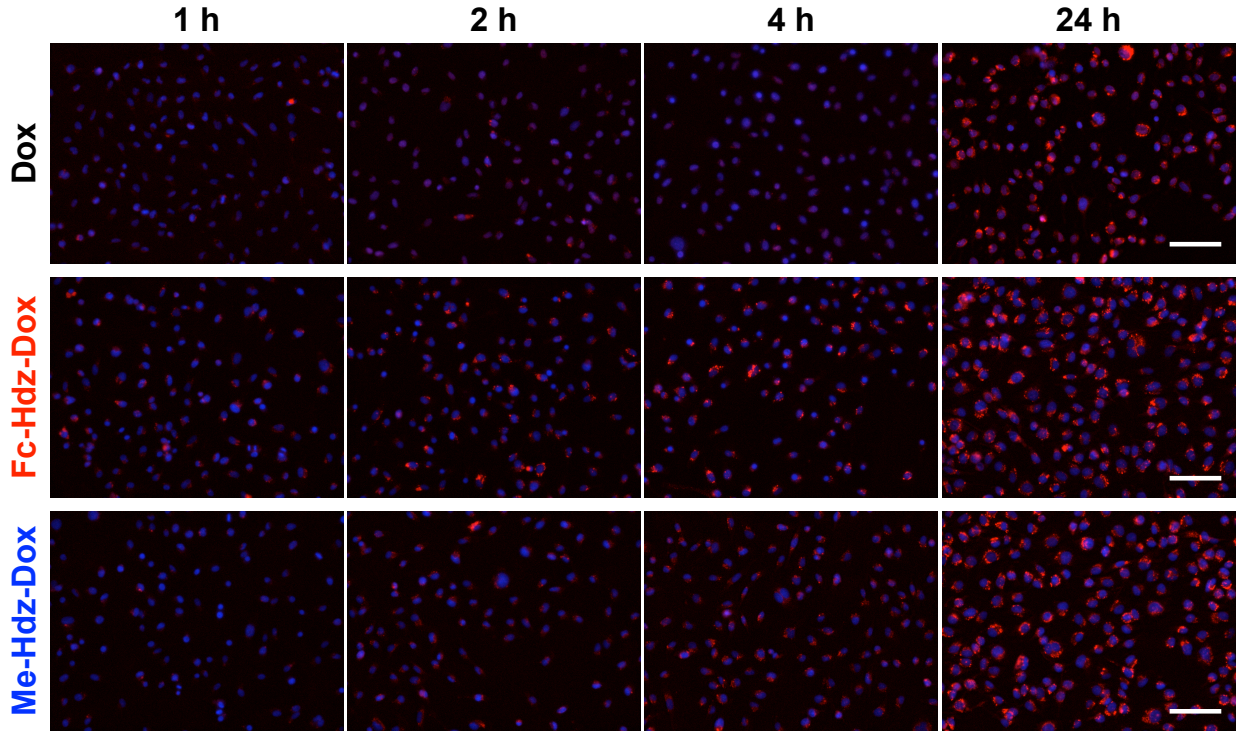


Figure S34: Fluorescence time-course experiment on MDA-MB-231 cells treated with 0.25 μM doxorubicin or doxorubicin equivalent prodrug (red fluorescence) along with a DAPI nuclear stain (blue). The nucleus of cells treated with doxorubicin shows greater overlay of red and blue signal (purple) in the nucleus at each time-point, though drugs of all types enter cells at seemingly comparable rate. The scales bars correspond to 100 μm for all images.

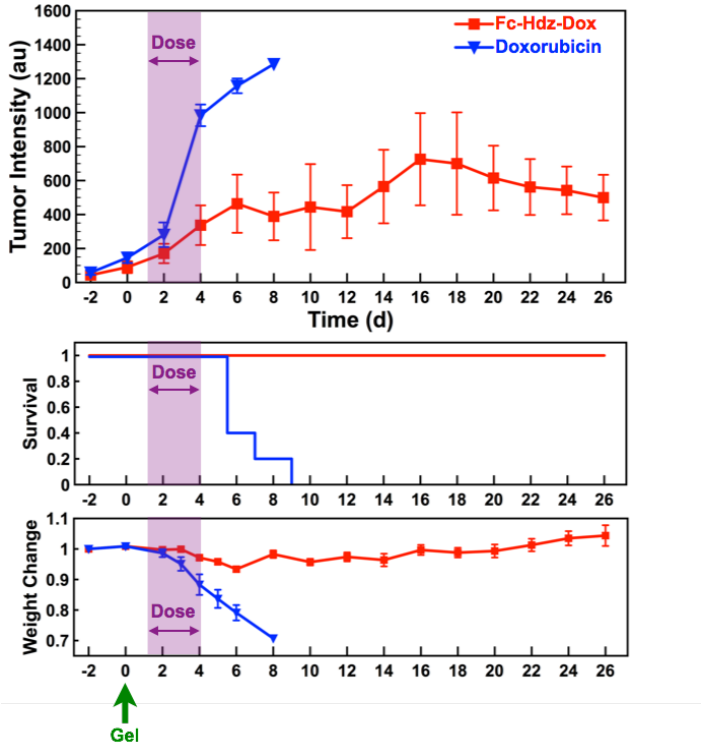


Figure S35: Preliminary studies comparing **Fc-Hdz-Dox** to Doxorubicin in MDA-MB-231 tumor models, dosed at 3 mg/kg doxorubicin equivalent on days 1, 2, and 3.

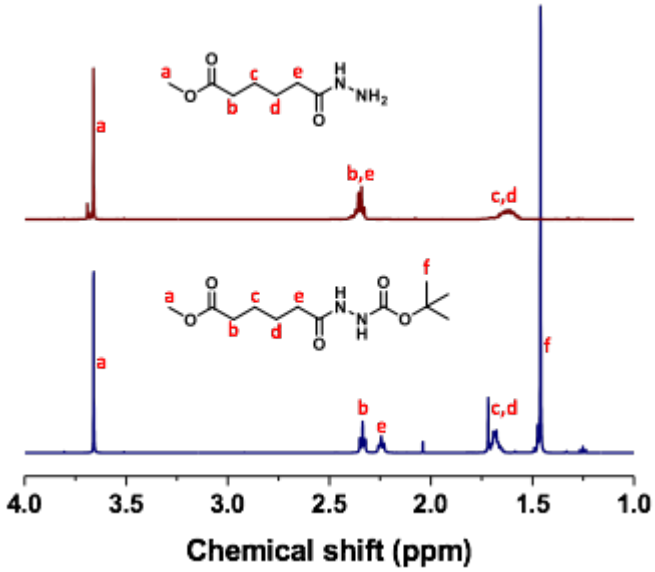


Figure S36: ^{1}H-NMR spectra of precursors to **Me-Hdz-Dox** in $CDCl_3$.

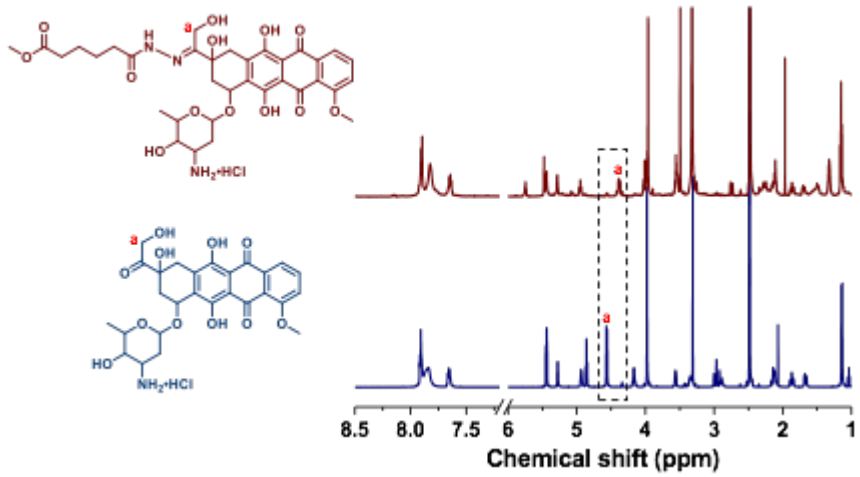


Figure S37: ^{1}H-NMR spectrum of **Me-Hdz-Dox** in DMSO-d_6.

3-Supporting References:

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