Electronic Supplementary Information

Supramolecular hydrogel capsule showing prostate specific antigen-responsive function for sensing and targeting prostate cancer cells

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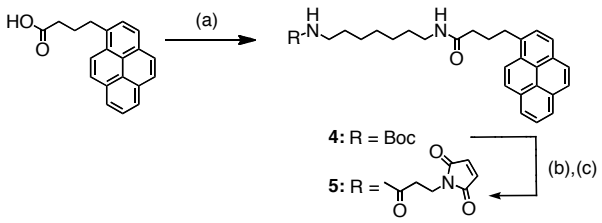
Synthesis: Compounds **1**[2a,19] and DUPA (DUPA(O^{t}Bu)-OH)[43] were synthesized according to the methods reported previously.

Synthesis of maleimide-appended hydrophobic tails (5)



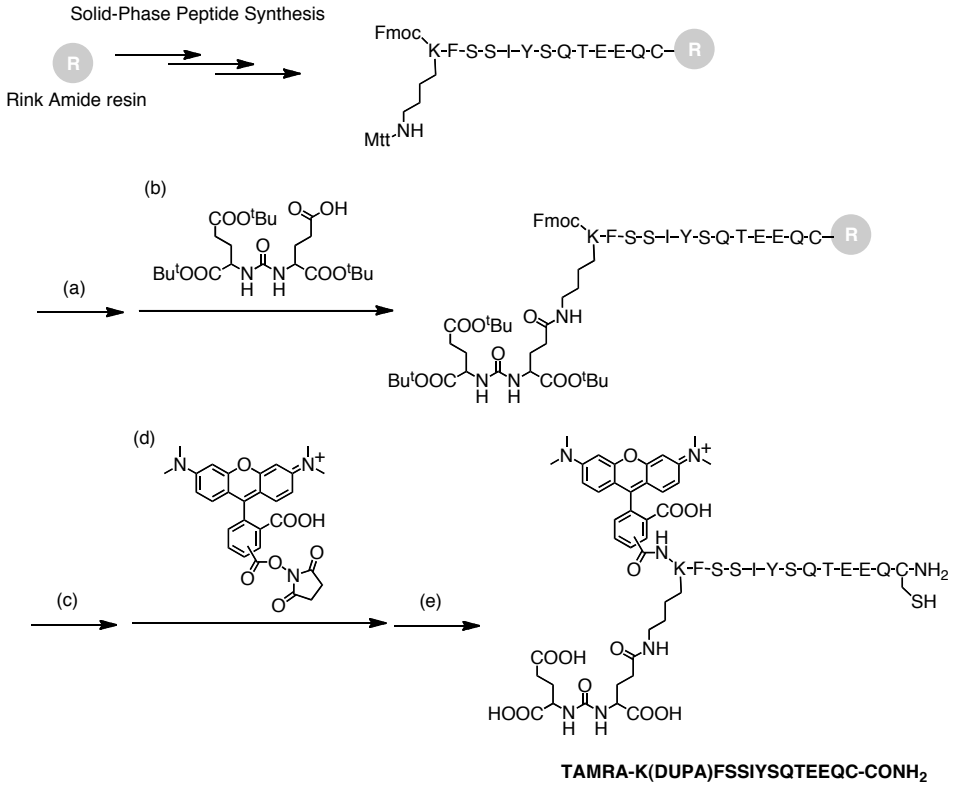
Scheme S1 Synthesis of **5**; (a) *N*-1-tertbutoxycarbonyl-1,7-diaminoheptane hydrochloride, WSC-HCl, DMF, DIEA, rt, 75%, (b) TFA, CH_2Cl_2, rt, (c) *N*-[β-Maleimidopropyloxy]-succinimide ester, DIEPA, DMF, rt, 62% for 2 steps.

Synthesis of 4: A solution of 1-pyrene butylic acid (920 mg, 3.2 mmol), *N*-1-tertbutoxycarbonyl-1,7-diaminoheptane hydrochloride (860 mg, 3.2 mmol), diisopropylethylamine (DIEA, 559 μL), and water soluble carbodiimide hydrochloride (WSC-HCl, 1-Ethyl-3-(3-dimethyl aminopropyl)carbodiimde hydrochloride, 669 mg, 3.5 mmol) in dry *N*,*N*-dimethylformamide (DMF, 25 mL) was stirred at room temperature overnight under Ar atmosphere. Then the solvent was removed under reduced pressure. The residue was dissolved in chloroform (50 mL) and the solution was washed with 5% aqueous citric acid (50 mL) and brine (50 mL). The organic layer was collected and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography (SiO_2, chloroform:methanol = 40:1) to afford compound **4** as a pale yellow solid (600 mg, 75%): ^{1}H NMR (400 MHz, $CDCl_3$, room temperature); δ = 1.24–1.28 (m, 6H), 1.43 (s, 9H), 1.50–1.52 (m, 6H), 2.17–2.29 (m, 2H), 3.06–3.12 (m, 2H), 3.23 (m, 2H), 3.40 (t, J = 7.6 Hz, 2H), 4.47 (br, 1H), 5.35 (br, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.97–8.18 (m, 7H), 8.31 ppm (d, J = 9.6 Hz, 1H). MALDI-MS (CHCA): Calcd for $[M + Na]^+$: m/z = 523.3; Found: 523.2.

Synthesis of 5: A solution **4** (150 mg, 0.30 mmol) in CH_2Cl_2/ trifluoroacetic acid (TFA) (8 mL/2 mL) was stirred at room temperature. After 1 h, the solvent was evaporated and the residual TFA was removed by azeotropy with toluene to afford brown oil. The obtained oil, *N*-[β-maleimidopropyloxy]-succinimide ester (99 mg, 0.38 mmol), and DIEA (156 μL, 0.90 mmol) in dry

DMF (7 mL) was stirred at room temperature for 11 h. Then the solvent was removed under reduced pressure. The residue was dissolved in chloroform (50 mL) and washed with 5% aqueous citric acid (50 mL), saturated aqueous sodium carbonate (50 mL), and brine (50 mL). The organic layer was collected and dried over anhydrous Na_2SO_4. The filtrate was concentrated to dryness, and the residue was purified by column chromatography (SiO_2, chloroform:methanol = 20:1) to afford compound **5** as a pale yellow solid (100 mg, 62% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, room temperature): δ = 1.27 (m, 6H), 2.20–2.24 (m, 2H), 2.43 (t, J = 7.2 Hz, 2H), 3.17–3.42 (m, 4H), 3.40 (t, J = 6.8, 2H), 3.76 (t, J = 7.2 Hz, 2H), 5.46 (br, 1H), 5.56 (br, 1H), 6.61 (s, 2H), 7.86 (d, J = 7.6 Hz, 1H), 7.98–8.18 (m, 7H), 8.30 ppm (d, J = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, room temperature): δ = 172.6, 170.4, 135.9, 134.1, 131.4, 130.9, 128.8, 127.5, 127.4, 126.7, 125.9, 124.9, 124.8, 123.4, 39.3, 36.1, 34.7, 34.2, 32.7, 29.4, 29.2, 28.4, 27.5, 26.5, 26.4 ppm. HRMS (FAB, NBA matrix): Calcd. for $[M(C_{34}H_{37}N_3O_4) + H]^+$: m/z = 552.2862; Found: 552.2859. Anal. Calcd. for $C_{34}H_{37}N_3O_4 \bullet (CHCl_3)_{0.1}$: C, 72.67; H, 6.63; N, 7.46; Found: C, 72.63; H, 6.71; N, 7.62 %.

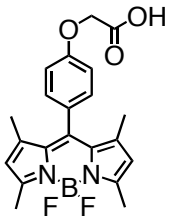
Synthesis of peptide (TAMRA-K(DUPA)FSSIYSQTEEQC-CONH$_2$)



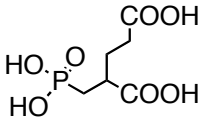
Scheme S2 Synthesis of TAMRA-K(DUPA)FSSIYSQTEEQC-CONH$_2$; (a) TFA/TIS/dichloromethane (1:4:95), (b) HBTU, HOBt, DIEA/NMP, (c) 20% piperidine/NMP, (d) DIEA/DMF, (e) TFA/TIS/H_2O (95:2.5:2.5)

Peptide was synthesized by conventional solid-phase peptide synthesis (SPPS) using commercially available Fmoc-Rink Amide resin (0.6 mmol/g, 50 mg, Nova biochem). Removal of the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group was achieved using 20% piperidine in 1-methyl-2-pyrrolidinone (NMP) at room temperature. The condensation reaction was carried out in the presence of Fmoc-amino acid (3.0 eq.), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 3.0 eq.), 1-hydroxybenzotriazole hydrate (HOBT·H_2O, 3.0 eq.), and DIEA (6.0 eq.) in NMP at room temperature. For incorporation of DUPA (DUPA(O^{t}Bu)-OH), after completion of the peptide elongation to N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ε}-4-methyltrityl-lysine (Fmoc-Lys(Mtt)-OH), the resin was treated with TFA/triisopropylsilan(TIS)/dichloromethane (1/4/95) for 2 min for three times to de-protect Mtt group. Then, the resin was washed with dichloromethane (5 × 2 mL). After swelling the resin in the NMP, a solution of DUPA(OtBu) (1.5 eq.) were incorporated to the resin with HBTU (1.5 eq.), HOBt (1.5 eq.), and DIEA (3.0 eq.) in NMP (250 μL) for 30 min × 2. After coupling reaction, the resin was washed with NMP (5 × 2 mL). After removal of the Fmoc group on the terminal amino group of the corresponding peptide, the resulting free amino group was allowed to react with 5/6-TAMRA SE (1.5 eq.) in DMF containing DIEA (3.0 eq.) for 3 h, and then treated with acid to release the peptide from the resin and remove the protecting groups with cleavage mixture (TFA/TIS/H_2O (95/2.5/2.5)) for 1.5 h. The resin was removed by filtration and washed with the cleavage mixture. The filtrate was concentrated under reduced pressure and the peptide was precipitated by the addition of tert-butylmethyl ether. The obtained crude peptide (63 mg, 55%) was used for coupling reaction with a maleimide-appended tail **5**. MALDI-MS (DHB): Calcd. for $[M]^+$: m/z = 2262.9; Found: 2262.7.

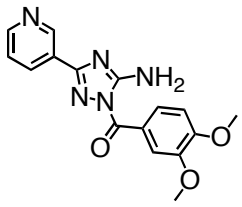
Scheme S3. Chemical structures of BODIPY, PMPA, and PSA inhibitor used in this study.



BODIPY



PMPA



PSA inhibitor

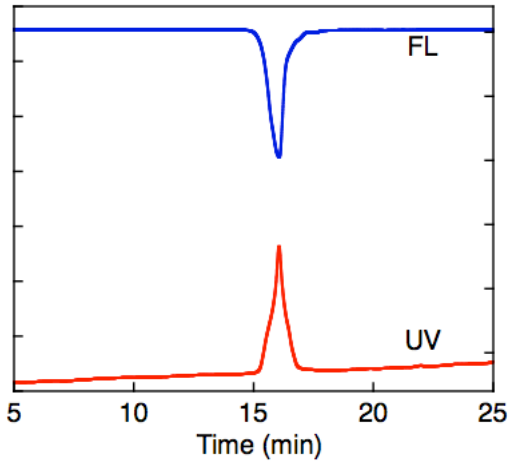


Figure S1. Analytical HPLC of purified **2**. (Eluent: A:B = 70:30 to 100:0 (A: Acetonitrile/0.1%TFA, B: H_2O/0.1%TFA), linear gradient over 40 min, flow rate = 1.0 mL/min, UV: 220 nm, FL: λ_{ex} = 552 nm, λ_{em} = 575 nm).

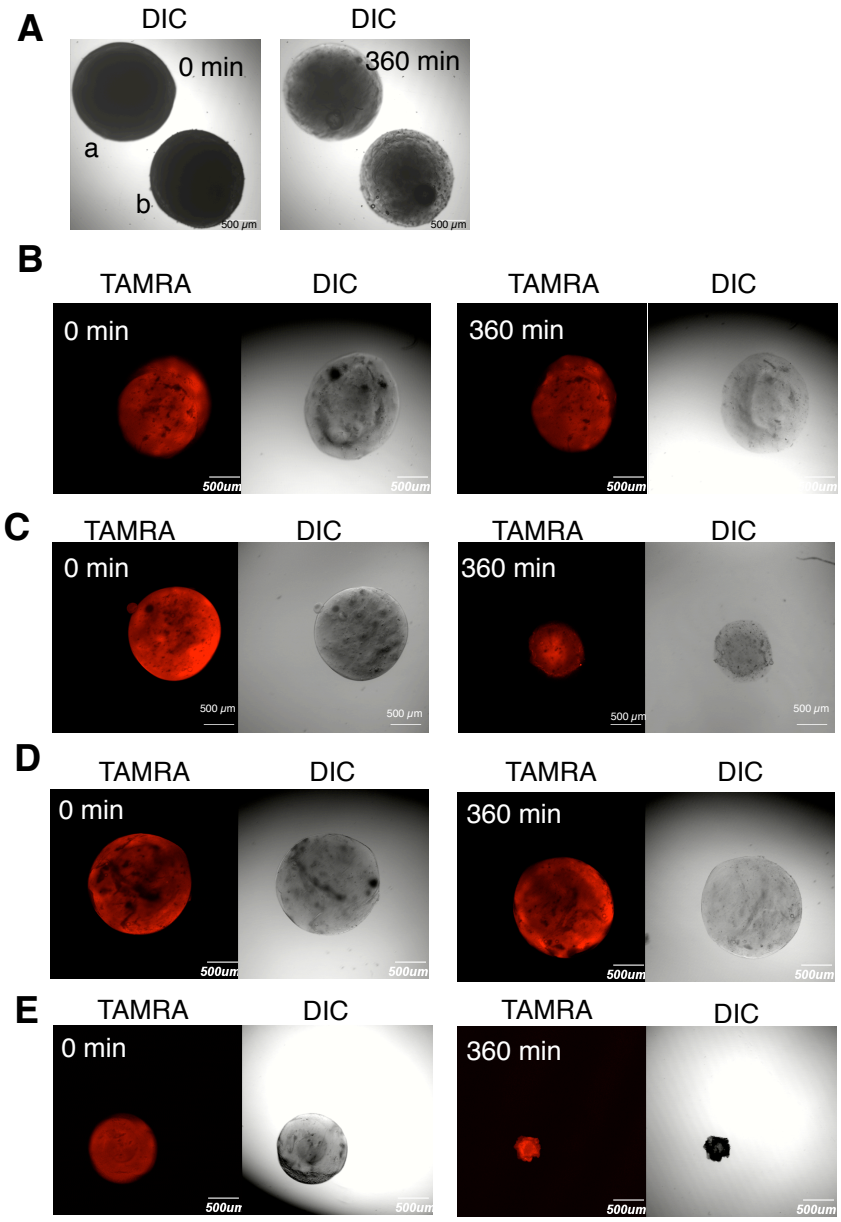


Figure S2. Stability of SH-capsule 1 and 1/2 in aqueous media. (**A**) CLSM images of SH-capsule **1** (a: [**1**] = 10 wt% in 2.0 μL, b: [**1**] = 10 wt% in 2.0 μL (containing 10 vol% of DMSO when the capsule was prepared)) in distilled water, (**B**, **C**) CLSM images of SH-capsule **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 2.0 μL (containing 10 vol% of DMSO when the capsule was prepared)) in (**B**) distilled water and (**C**) 50 mM Tris-HCl (pH 8.3), 25 mM NaCl, (**D**) RPMI 1640 supplemented with 10% FBS, and (**E**) CLSM images of SH-capsule **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 1.0 μL (containing 10 vol% of DMSO when the capsule was prepared)) in distilled water at room temperature. SH capeules (2 μL) shrunk in Tris-HCl buffer (**C**).

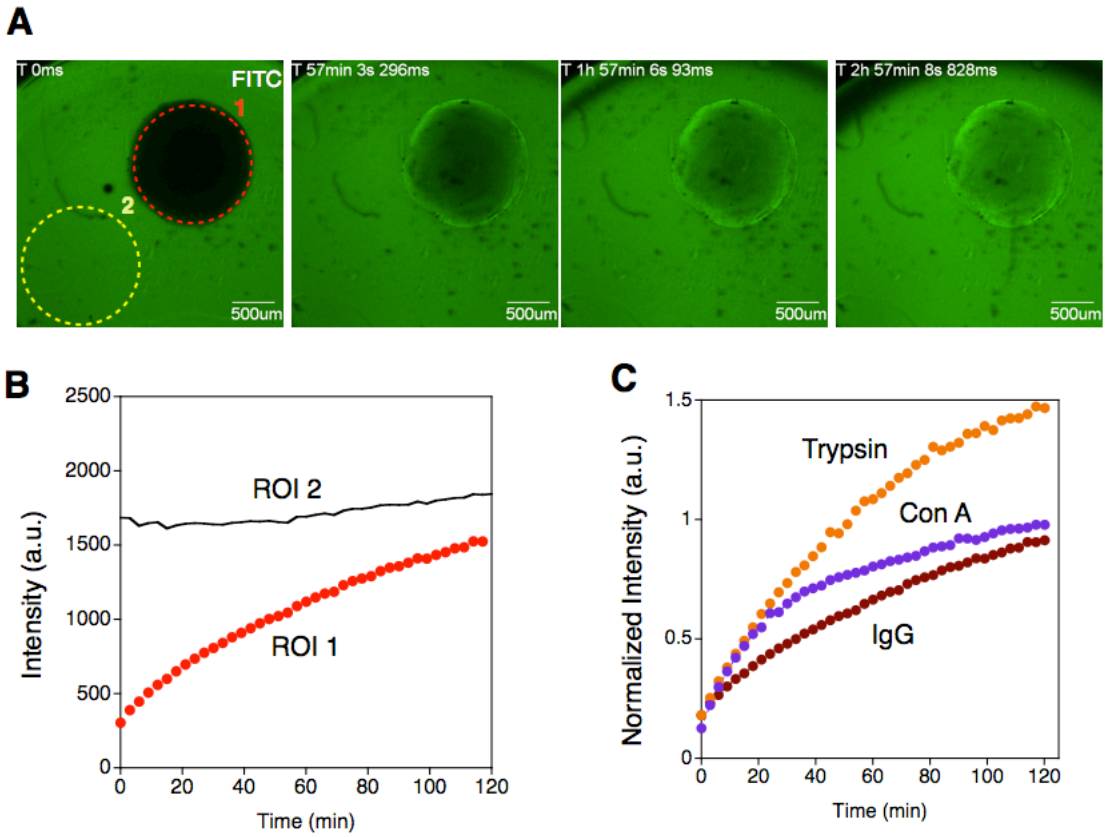


Figure S3. Diffusion of proteins into SH-capsule 1. (**A**) Typical CLSM images of SH-capsule **1**, showing diffusion of FITC-labeled IgG into the capsule after 57, 117, 177 min and (**B**) corresponding fluorescence intensity of ROIs (indicated in the left image of panel (**A**)) as a function of time. (**C**) Fluorescence intensity change inside gel capsule (intensity inside SH-capsule **1** was normalized against that outside) as a function of time for three different proteins ([Protein] = 2.0 μM).

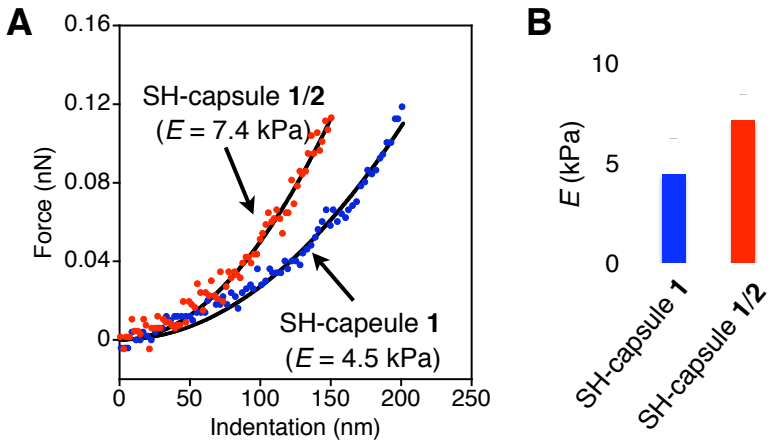


Figure S4. Evaluating mechanical property of SH-capsule 1 and 1/2 using AFM. (**A**) Typical force indentation curves of SH-capsule **1** and **1/2** ([**1**] = 10 wt%, [**2**] = 0 or 50 μM, distilled water for SH-capsule **1** and distilled water contining DMSO (10 vol%) for preparing SH-capsule **1/2**). The solid lines represent the fit by a conical tip model. (**B**) Averaged Young's modulus *E* (kPa) for all data collected from three different samples of SH-capsule **1** and **1/2** (n = 33 for SH-capsule **1** (4.4±1.8 kPa); n = 16 for SH-capsule **1/2** (7.1±1.3 kPa)).

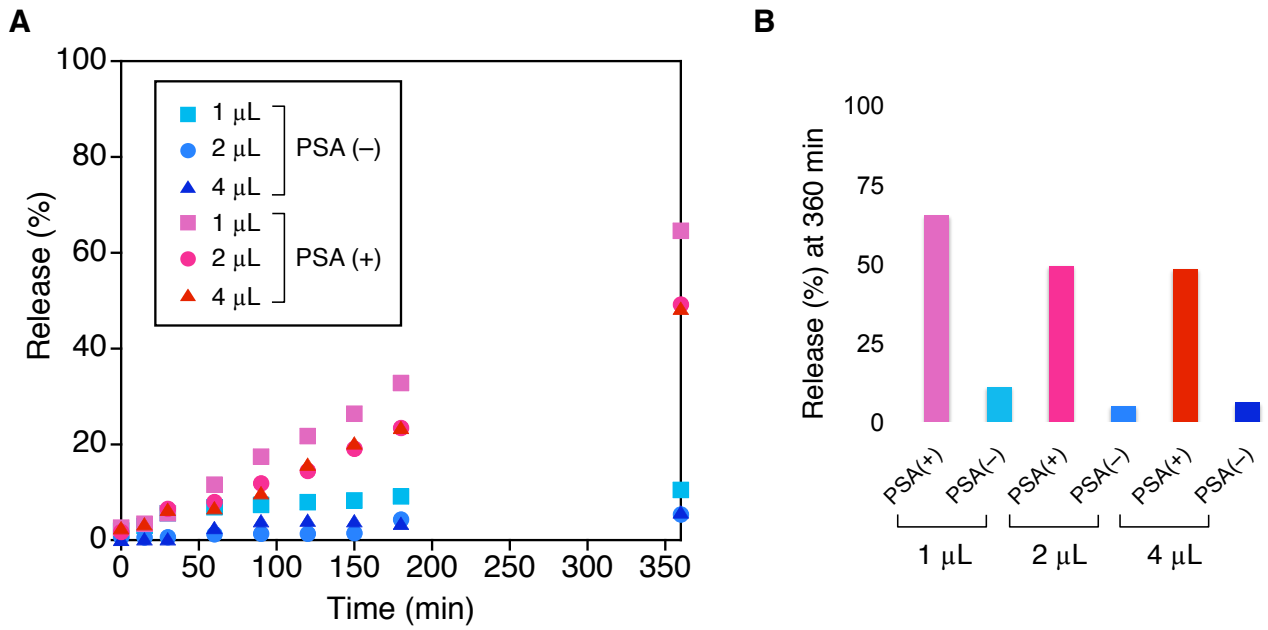


Figure S5. Size dependence of PSA-responsive release of fluorescent drug model from SH-capsule 1/2. **(A)** Release profile and (**B**) release % at 360 min estimated from fluorescence spectral changes of supernatant of solutions containing SH-capsule **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 1.0 μL, 2.0 μL, and 4.0 μL) at the bottom (Solution; **PSA(–):** [PSA] = 0 nM, 50 mM Tris-HCl pH 8.3, 25 mM NaCl, **PSA(+)**: [PSA] = 100 nM, 50 mM Tris-HCl pH 8.3, 25 mM NaCl, room temperature)

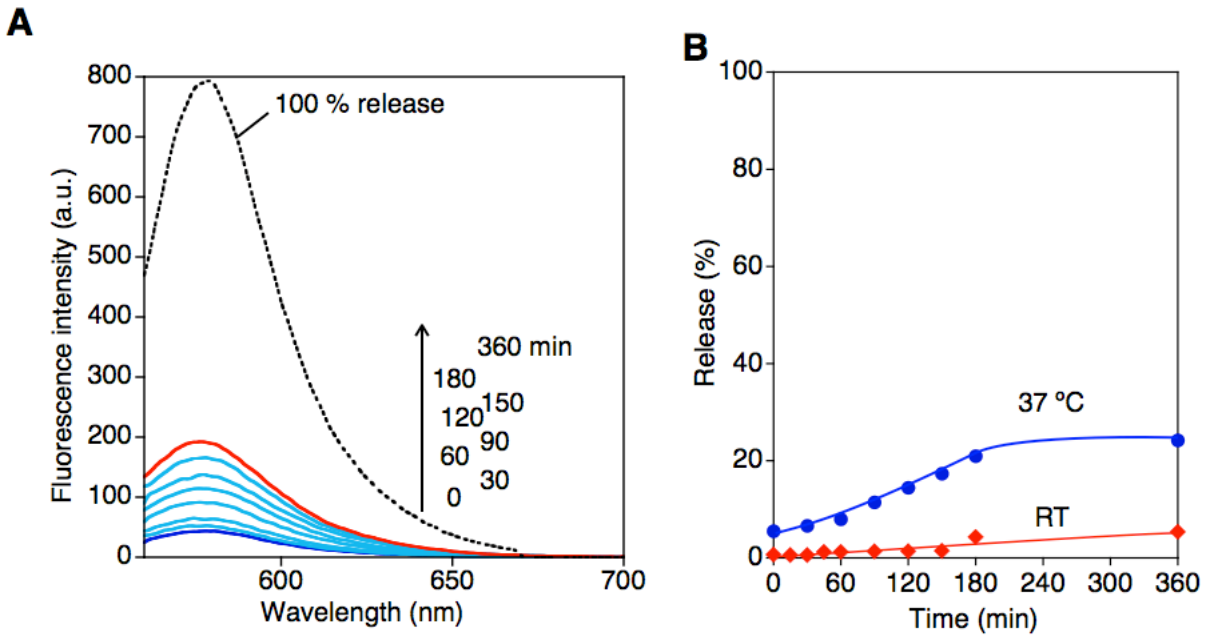


Figure S6. Evaluating encapsulation efficiency of 2 inside SH-capsule 1/2. (**A**) Fluorescence spectral changes of supernatant of aqueous solution containing SH-capsule **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 2.0 μL) at the bottom at 37ºC (50 mM Tris-HCl pH 8.3, 25 mM NaCl). (**B**) Release profile by tracing TAMRA fluorescence from SH-capsule **1/2** at room temperature and 37ºC.

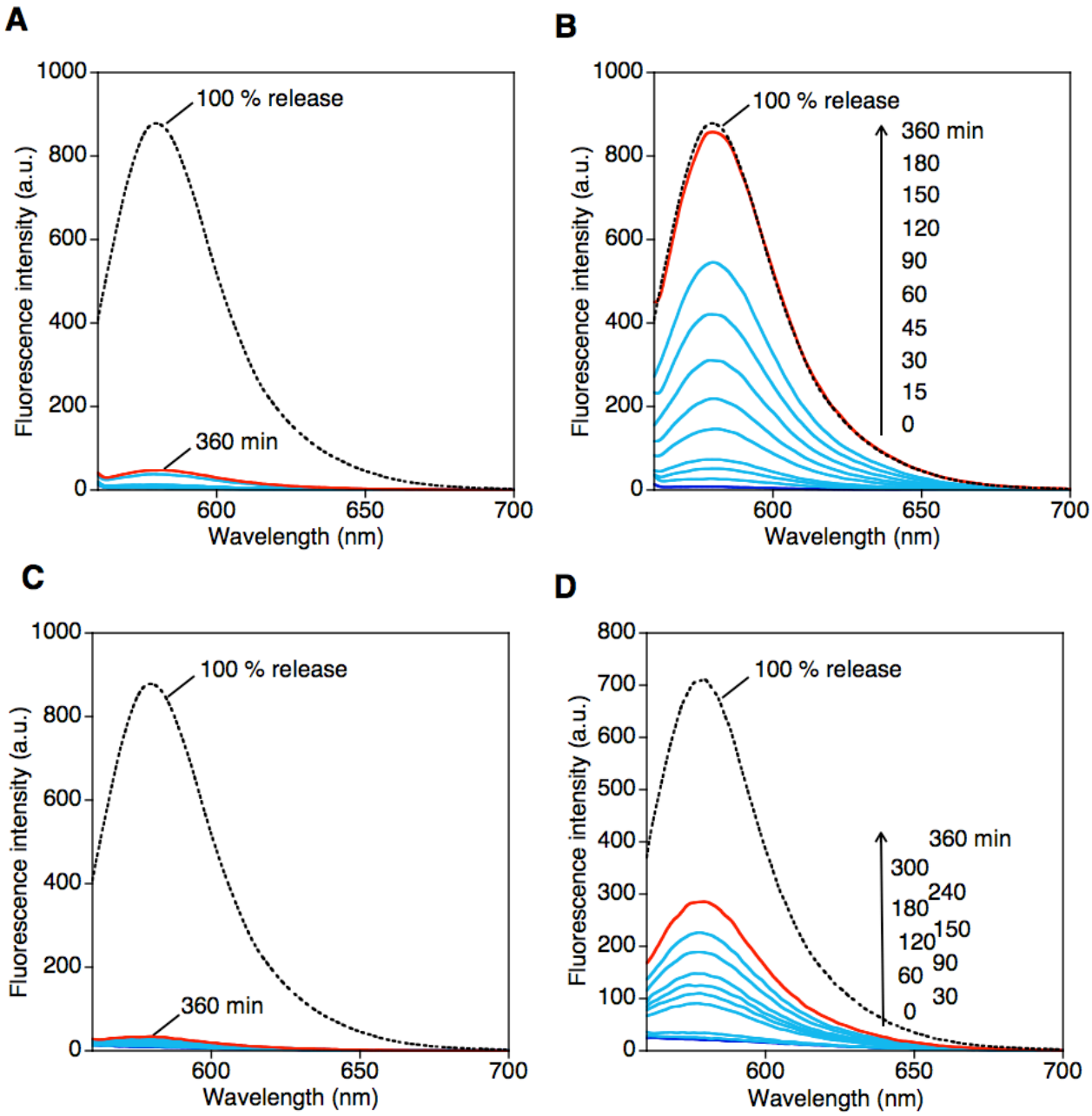


Figure S7. PSA-responsive release of fluorescent drug model from SH-capsule 1/2. Fluorescence spectral changes of supernatant of solution containing SH-capsule **1**/**2** ([**1**] = 10 wt%, [**2**] = 50 μM in 2.0 μL) at the bottom (**A**: [PSA] = 0 nM, 50 mM Tris-HCl pH 8.3, 25 mM NaCl, **B**: [PSA] = 500 nM, 50 mM Tris-HCl pH 8.3, 25 mM NaCl, **C**: RPMI 1640 with 10% FBS, 25 mM HEPES, pH 7.4, **D**: RPMI 1640 with 10% FBS, cultured LNCaP for 4 days, 25 mM HEPES, pH 7.4, room temperature)

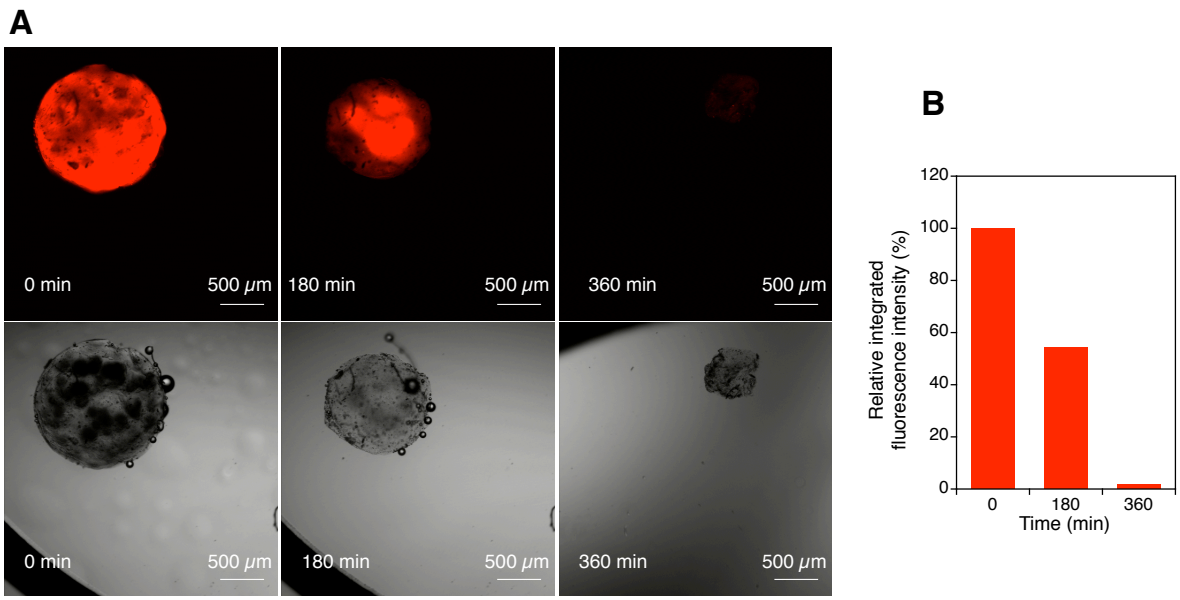


Figure S8. SH-capsule 1/2 after PSA treatment. (**A**) Time lapse CLSM images of SH-capsule **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 2.0 μL) after PSA treatment ([PSA] = 500 nM) in 50 mM Tris-HCl (pH 8.3), 25 mM NaCl at room temperature (See Figure S2C for without PSA). (**B**) Change in integrated fluorescence intensity inside the SH capsule **1/2** as a function of time.

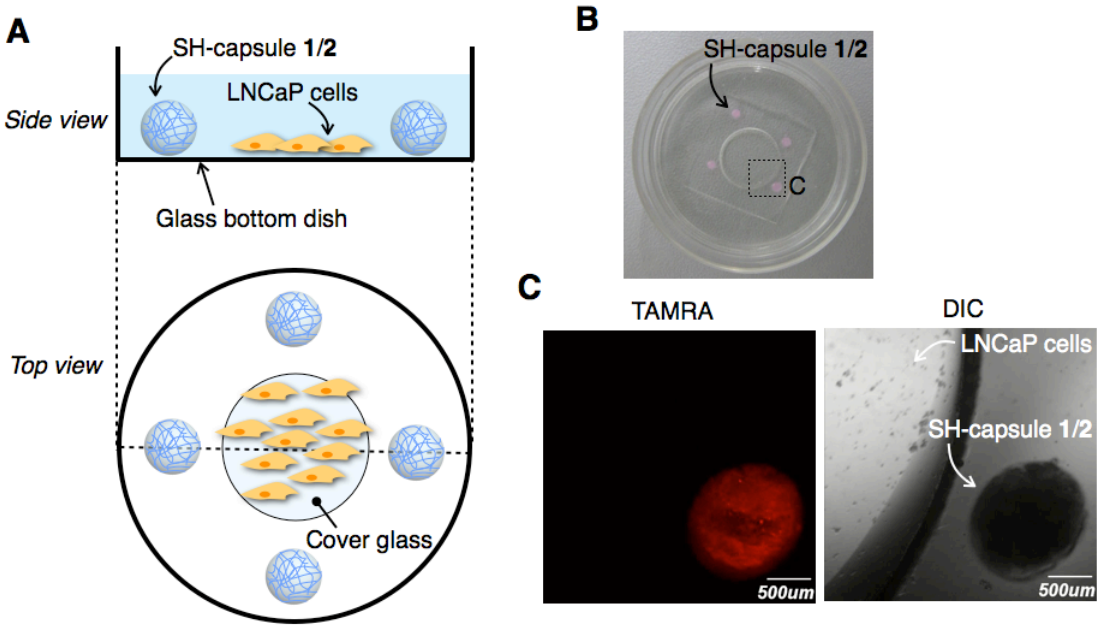


Figure S9. Evaluating PSA-responsive release and PSMS-specific targeting of SH-capsule 1/2 (**A**) Schematic illustration of the experimental setup. (**B**) Photograph of glass-bottom dish cultivated LNCaP cells, in which four SH-capsules **1/2** were dropped. (**C**) CLSM images of the area enclosed in a square in panel **B**.

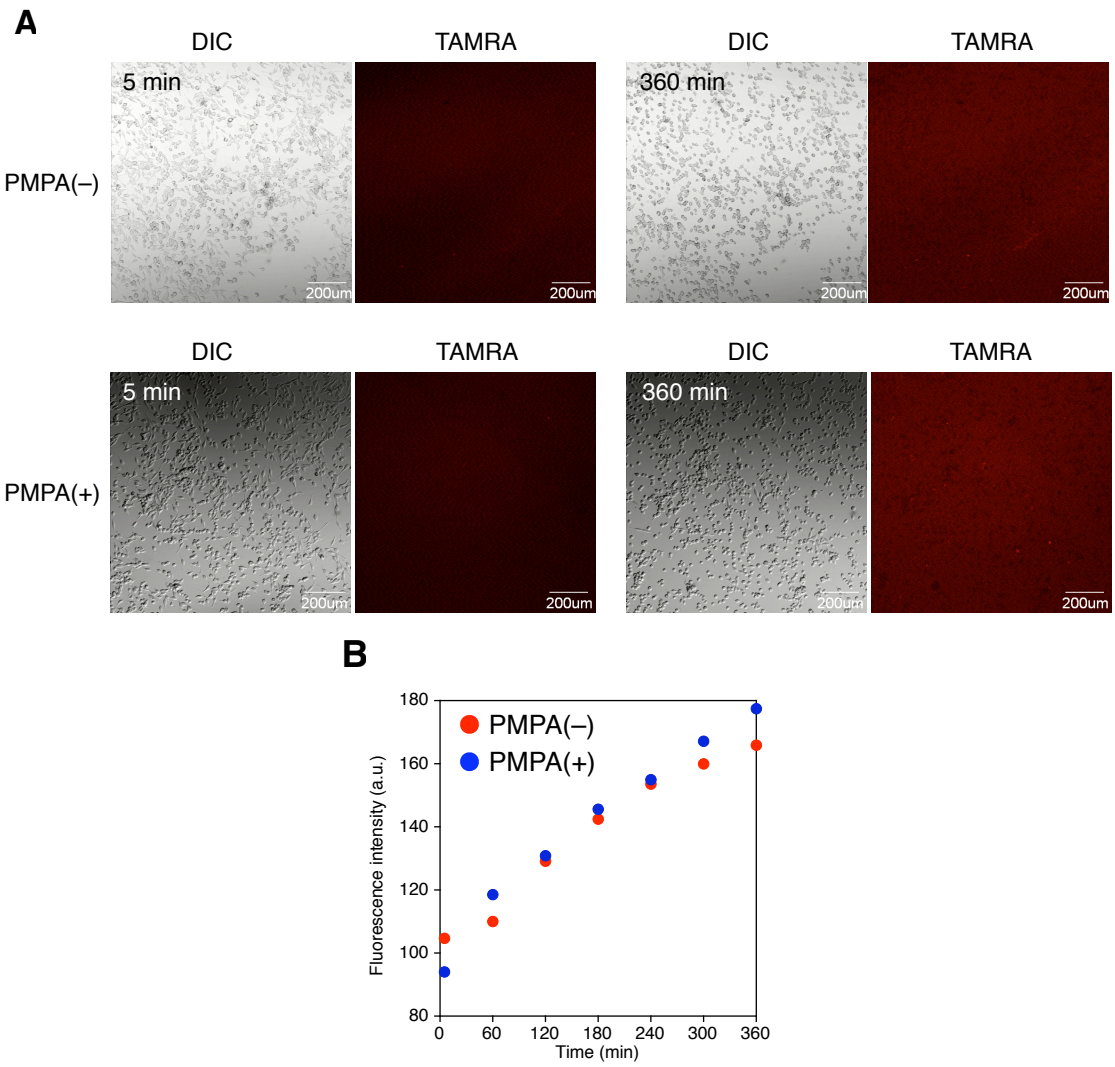


Figure S10. Evaluating PSA-responsive release of SH-capsule 1/2. (**A**) CLSM images of LNCaP prostate cancer cells incubated with SH-capsules **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 2.0 μL) after (a) 5 min and (b) 6 h in the presence and absence of PMPA ([PMPA] = 5.0 μM), (**B**) Change in fluorescence intensity around LNCaP as a function of time evaluated by time lapse CLSM images of LNCaP incubated with SH-capsules **1/2**.

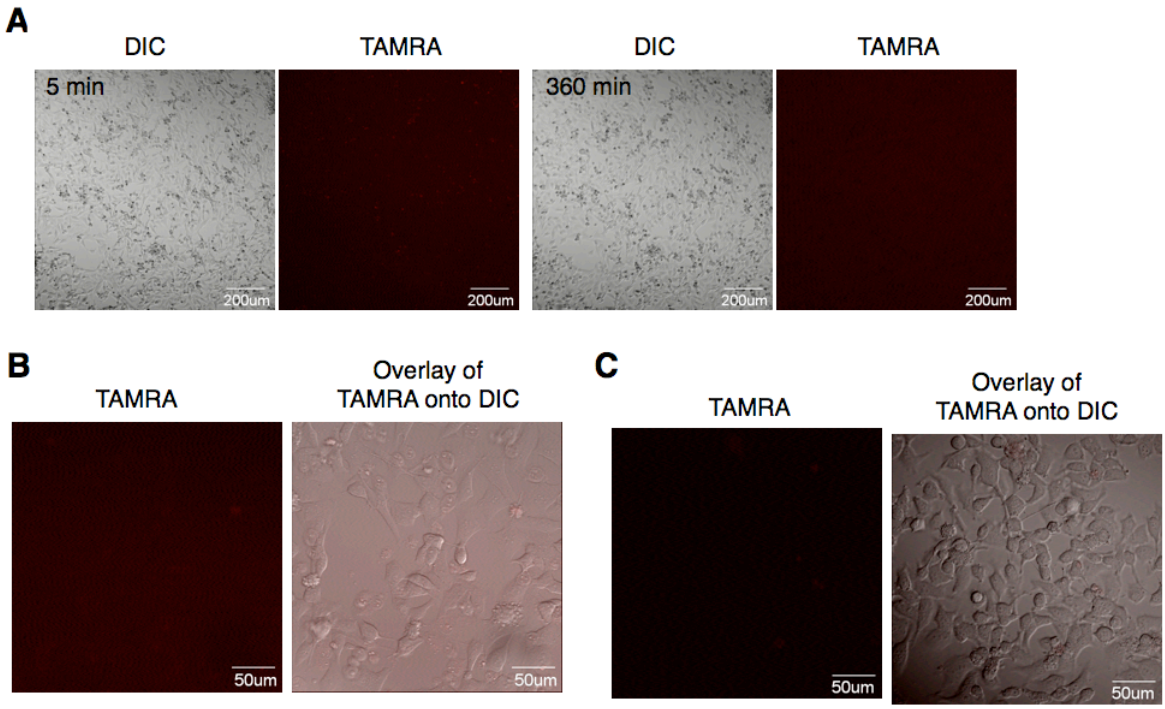


Figure S11. Evaluating PSA-responsive release and PSMS-specific targeting of SH-capsule 1/2 (Negative control experiments). (**A**) CLSM images of PSMA-negative HeLa cells incubated with SH-capsules **1/2** ([**1**] = 10 wt%, [**2**] = 50 μM in 2.0 μL) after (a) 5 min and (b) 6 h, (**B**) Magnified CLSM images of HeLa cells after incubation with SH-capsules **1/2** after 6 h. (**C**) Magnified CLSM images of HeLa cells after incubation with **3** for 3 h followed by medium exchange.