Supporting Information

In vivo tailor-made protein corona of prodrug-based nanoassembly fabricated by redox dual-sensitive paclitaxel prodrug for the superselective treatment of breast cancer

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Experimental Section

Materials

Paclitaxel (PTX) (AR, 99%) was purchased from Dalian Meilun Biotech Co., Ltd (Liaoning, China). 6-maleimidocaproic acid (EMC) (AR, 98%) was supplied by Energy Chemical (Shanghai, China). Succinic anhydride (AR, 98%), 6-aminocaproic acid (AR, 99%), ethylene glycol (AR, 98%), 3, 3'-dithiodipropionic acid (AR, 99%) and 3, 3'-thiodipropionic acid (AR, 98%) were obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). p-toluenesulfonic acid (AR, 98%) was purchased from Tianjin Heowns Biochemical Technology Co., Ltd (Tianjin, China). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI) (AR, 98%), and 4-dimethylaminopyridine (DMAP) (AR, 99%) were obtained from Aladdin Biochemical Technology co. Ltd (Shanghai, China). Reagents of analytical grade such as dichloromethane were purchased from Shandong Fuyu Chemical Co., Ltd. (Shandong, China).

Synthesis of PTX prodrugs

(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((2R,3S)-3-benzamido-2-((6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)oxy)-3-phenylpropanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-3,4,4a,5,6,9,10,11,12,12a-decahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b(2aH)-diyl diacetate (**1**)

Compound **1** was an ester bond linked PTX prodrug with the maleimide group. EMC was conjugated directly to the C2’-hydroxyl (OH) position of PTX. Briefly, EMC (0.07 g, 0.35 mmol), EDCI (0.13 g, 0.70 mmol) and DMAP (0.01 g, 0.07 mmol) were dissolved in 15 mL dichloromethane and reacted with stirring at 0°C for 0.5 h under nitrogen. Then PTX (0.30 g, 0.35 mmol) dissolved in 10 ml dichloromethane was added to the mixture slowly and the reaction was further proceeded for 12 h at 25°C under nitrogen. Upon completion, the reaction mixture was washed with the saturated $NaHCO_3$ solution and saturated NaCl solution twice, respectively. The organic layers were collected and dried by anhydrous sodium sulfate (Na_2SO_4). After filtration, the solvent existed in the filtrate was removed by RE-52A rotary

evaporation (Shanghai Yarong Biochemical Instrumen Plant, China). The residue was then seperated and purified by column chromatography on silica gel (dichloromethane:methanol = 100:1, v/v). Finally, Compound **1** was obtained as a white solid (0.24 g, 65.5%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 8.13 (d, 2H), 7.75 (d, 2H), 7.61 (m, 1H), 7.52 (m, 3H), 7.42 (d, 4H), 7.40 (m, 3H), 7.00 (d, 1H, *J*=6.6 Hz, -NH-), 6.64 (s, 2H, -CO<u>CH</u>=<u>CH</u>CO-), 6.30 (t, 2H, 10-H, 13-H), 5.98 (dd, 1H, *J*=6.9 Hz, *J*=2.1 Hz, 3'-H), 5.69 (d, 1H, *J*=5.1 Hz, 2-H), 5.51 (d, 1H, *J*=2.4 Hz, 2'-H), 4.99 (d, 1H, J=7.2 Hz, 5-H), 4.46 (t, 1H, 7-H), 4.31 (d, 1H, *J*=6.3 Hz, 20α-H), 4.21 (d, 1H, *J*=6.6 Hz, 20β-H), 3.83 (d, 1H, *J*=5.1 Hz, 3-H), 3.45 (t, 2H, -<u>CH_2</u>-N (CO) CO), 2.52-2.60 (m, 1H, 6α-H), 2.47 (s, 3H, 4-CO<u>CH_3</u>), 2.39 (m, 2H, 14α-H, 14β-H), 2.35 (t, 2H, *J*=6.6 Hz, -<u>CH_2</u>CO-), 2.23 (s, 3H, 10-CO<u>CH_3</u>), 2.12 (t, 2H, *J*=6.6 Hz, -<u>CH_2</u>CO-), 1.94 (s, 3H, 18-H), 1.89 (t, 1H, 6β-H), 1.68 (s, 3H, 19-H), 1.51-1.64 (m, 4H, -<u>CH_2</u>CH_2<u>CH_2</u>-), 1.25-1.32 (m, 2H, -CH_2<u>CH_2</u>CH_2-), 1.24 (s, 3H, 17-H), 1.14 (s, 3H, 16-H).

MS (ESI) (m/z): calcd for $C_{57}H_{62}N_2O_{17}$: m/z 1069.4 $[M+Na]^+$.

(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((2R,3S)-3-benzamido-2-((6-(2,5-dioxopyrrolidin-1-yl)hexanoyl)oxy)-3-phenylpropanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-3,4,4a,5,6,9,10,11,12,12a-decahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b(2aH)-diyl diacetate (**2**)

Compound **2**, an analog of compound **1**, was also an ester bond linked PTX prodrug with the succinimide group. Compound **2** without the ability of albumin-binding was utilized as a negative control compound. Firstly, 6-succinimidylhexanoic acid (intermediate **1**) was synthesized based on the published report[1] with some modify. In brief, succinic anhydride (3.00 g, 30 mmol) and 6-aminocaproic acid (3.94 g, 30 mmol) were dissolved in glacial acetic acid (60 mL) and refluxed at 135°C for 8 h, then acetic anhydride (2.8 mL, 30 mmol) was added dropwise to the reaction mixture with another 2 h of reflux. After the reaction was over, remove the solvent with the assist of toluene under vacuum. The obtained oily crude product was purified by silica column chromatography

(dichloromethane:methanol = 100:1.2, v/v). Pure 6-succinimidylhexanoic acid was white powder with a yield of 57.6%.

Compound **2** was synthesized from intermediate **1** and PTX, and was purified as described for compound **1** to obtain a white solid (0.22 g, 59.9%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 8.16 (d, 2H), 7.76 (d, 2H), 7.61 (m, 1H), 7.52 (m, 3H), 7.42 (d, 4H), 7.40 (m, 3H), 7.08 (d, 1H, *J*=6.9 Hz, -NH-), 6.30 (t, 2H, 10-H, 13-H), 5.98 (dd, 1H, *J*=6.9 Hz, *J*=1.2 Hz, 3'-H), 5.69 (d, 1H, *J*=5.4 Hz, 2-H), 5.51 (d, 1H, *J*=2.4 Hz, 2'-H), 5.00 (d, 1H, J=6.9 Hz, 5-H), 4.46 (t, 1H, 7-H), 4.31 (d, 1H, *J*=6.3 Hz, 20α-H), 4.21 (d, 1H, *J*=6.6 Hz, 20β-H), 3.83 (d, 1H, *J*=5.4 Hz, 3-H), 3.45 (t, 2H, -$\underline{CH_2}$-N (CO) CO), 2.63 (s, 4H, -CO$\underline{CH_2CH_2}$CO-), 2.52-2.59 (m, 1H, 6α-H), 2.48 (s, 3H, 4-CO$\underline{CH_3}$), 2.40 (m, 2H, 14α-H, 14β-H), 2.34 (t, 2H, *J*=6.6 Hz, -$\underline{CH_2}$CO-), 2.23 (s, 3H, 10-CO$\underline{CH_3}$), 2.15 (t, 2H, *J*=6.9 Hz, -$\underline{CH_2}$CO-), 1.94 (s, 3H, 18-H), 1.89 (t, 1H, 6β-H), 1.68 (s, 3H, 19-H), 1.52-1.65 (m, 4H, -$\underline{CH_2}CH_2\underline{CH_2}$-), 1.25-1.31 (m, 2H, -$CH_2\underline{CH_2}CH_2$-), 1.23 (s, 3H, 17-H), 1.14 (s, 3H, 16-H).

MS (ESI) (m/z): calcd for $C_{57}H_{64}N_2O_{17}$: m/z 1071.2 $[M+Na]^+$.

(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((R)-2-((S)-benzamido(phenyl)methyl)-20-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,10,15-trioxo-3,11,14-trioxa-7-thiaicosanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-3,4,4a,5,6,9,10,11,12,12a-decahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b(2aH)-diyl diacetate (**3**)

Compound **3**, a redox-sensitive PTX prodrug, was obtained by inserting a single thioether bond between PTX and the maleimide group. The specific synthesis process was as follows:

P-toluenesulfonic acid (pTsOH, 0.48 g, 2.79 mmol) was firstly dissolved in ethylene glycol (35 mL, 0.59 mol). Then EMC (2.00 g, 9.48 mmol) was dissolved in a mixture of dichloromethane and toluene (40 mL, 1:1, v/v) and added to the above solution. The reaction was carried out at 110°C under reflux with nitrogen protection for 2 h. When the reaction mixture was cooled to room temperature, the product was extracted with dichloromethane four times and washed with the saturated $NaHCO_3$

solution twice. Dry and filter the organic layers. Crude product was obtained by the rotary evaporation and purified by silica column chromatography (petroleum ether:ethyl acetate = 2:1, v/v). The pure 6-maleimidocaproic acid 2-hydroxyethyl ester (intermediate **2**) was a light yellow oily liquid (1.12 g, 46.3%).

3, 3'-thiodipropionic acid (0.42 g, 2.36 mmol) was dissolved in 10 mL acetic anhydride with stirring under nitrogen protection. After reaction at 30° C for 4 h, the solvent was removed. Dissolve the residue with dichloromethane and add a solution of intermediate **2** (0.40 g, 1.57 mmol) and DMAP (0.04g, 0.31 mmol) in 20 mL of dichloromethane. The reaction was continued at ambient temperature overnight under nitrogen protection. Remove the solvent and purify the crude product by silica column chromatography (dichloromethane:methanol = 100:1.2, v/v). Then intermediate **3** was obtained as a white solid (0.11 g, 46.3%).

Compound **3** was synthesized from intermediate **3** and PTX, and was purified as described for compound **1** to obtain a white solid (0.12 g, 38.4%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 8.15 (d, 2H), 7.77 (d, 2H), 7.62 (m, 1H), 7.52 (m, 3H), 7.42 (d, 4H), 7.40 (m, 3H), 7.05 (d, 1H, *J*=6.9 Hz, -NH-), 6.66 (s, 2H, -CO<u>CH</u>=<u>CH</u>CO-), 6.25 (t, 2H, 10-H, 13-H), 5.99 (dd, 1H, *J*=5.7 Hz, *J*=2.1 Hz, 3'-H), 5.69 (d, 1H, *J*=5.4 Hz, 2-H), 5.51 (d, 1H, *J*=2.4 Hz, 2'-H), 4.99 (d, 1H, J=6.9 Hz, 5-H), 4.46 (t, 1H, 7-H), 4.33 (d, 1H, *J*=6.0 Hz, 20α-H), 4.26 (s, 4H, -O<u>CH_2CH_2</u>O-), 4.21 (d, 1H, *J*=6.3 Hz, 20β-H), 3.82 (d, 1H, *J*=5.1 Hz, 3-H), 3.49 (t, 2H, -<u>CH_2</u>-N (CO) CO), 2.69-2.77 (m, 6H, -CO<u>CH_2CH_2</u>S<u>CH_2</u>CH_2CO-), 2.59 (t, 2H, *J*=5.4 Hz, -CO$CH_2CH_2SCH_2$<u>CH_2</u>CO-), 2.52-2.56 (m, 1H, 6α-H), 2.46 (s, 3H, 4-CO<u>CH_3</u>), 2.37 (m, 2H, 14α-H, 14β-H), 2.31 (t, 2H, *J*=5.7 Hz, -<u>CH_2</u>CO-), 2.23 (s, 3H, 10-CO<u>CH_3</u>), 2.15 (t, 2H, *J*=6.9 Hz, -<u>CH_2</u>CO-), 1.94 (s, 3H, 18-H), 1.89 (t, 1H, 6β-H), 1.68 (s, 3H, 19-H), 1.57-1.65 (m, 4H, -<u>CH_2</u>CH_2<u>CH_2</u>-), 1.30-1.34 (m, 2H, -CH_2<u>CH_2</u>CH_2-), 1.23 (s, 3H, 17-H), 1.14 (s, 3H, 16-H).

MS (ESI) (m/z): calcd for $C_{65}H_{74}N_2O_{21}S$: m/z 1273.1 $[M+Na]^+$.

(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((R)-2-((S)-benzamido(phenyl)methyl)-2 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,11,16-trioxo-3,12,15-trioxa-7,8-dithiaheni

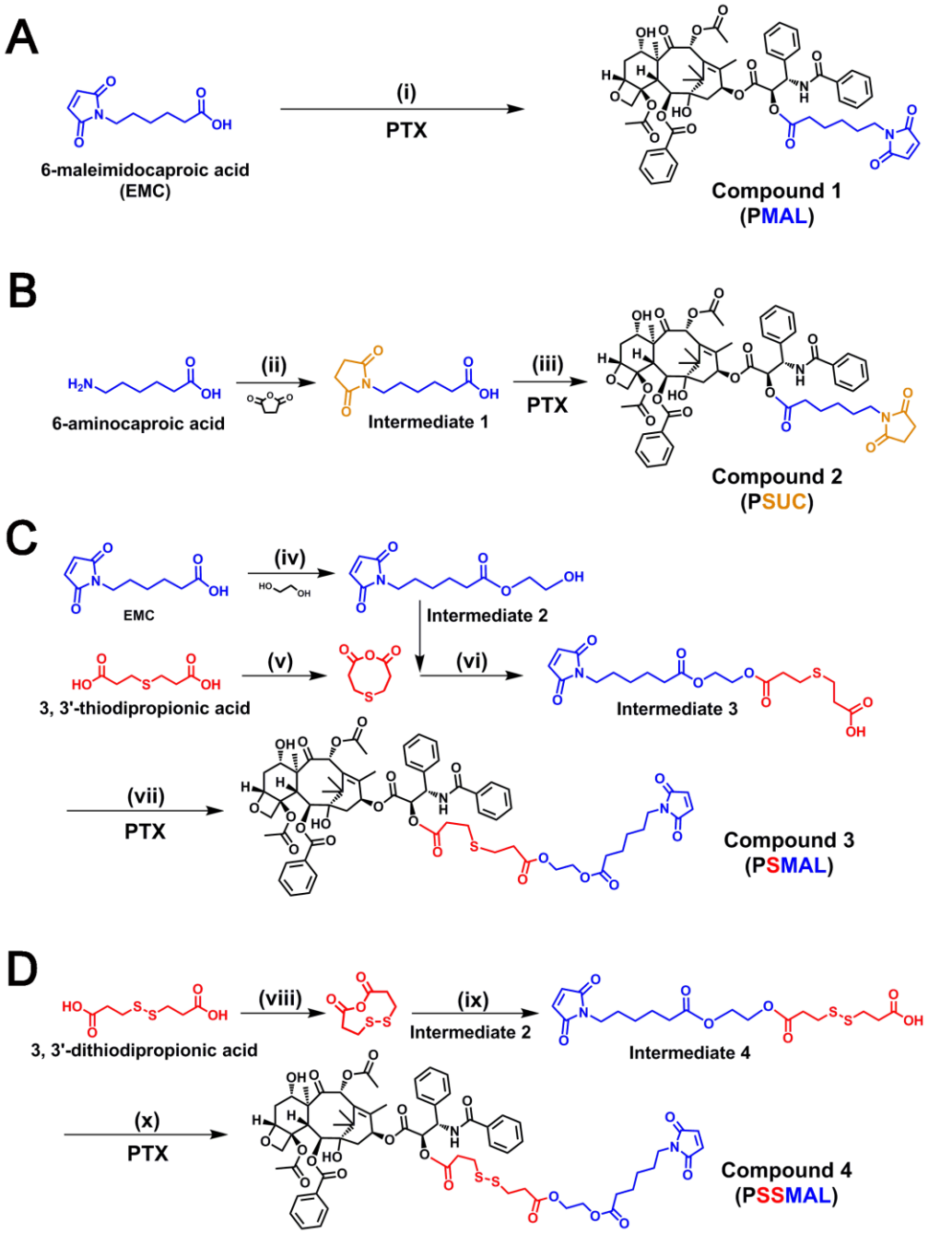
cosanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-3,4,4a,5,6,9,10,11,12,12a-decahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b(2aH)-diyl diacetate (**4**)

Compound **4**, another redox-sensitive PTX prodrug, was obtained by inserting a disulfide bond between PTX and the maleimide group. The synthesis process was similar to compound **3**, except that 3, 3'-thiodipropionic acid was substituted with 3, 3'-dithiodipropionic acid to obtain intermediate **4**. Compound **4** was synthesized from intermediate **4** and PTX as a white solid (0.13 g, 40.5%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 8.16 (d, 2H), 7.76 (d, 2H), 7.61 (m, 1H), 7.52 (m, 3H), 7.42 (d, 4H), 7.40 (m, 3H), 7.08 (d, 1H, *J*=7.2 Hz, -NH-), 6.66 (s, 2H, -CO<u>CH</u>=<u>CH</u>CO-), 6.25 (t, 2H, 10-H, 13-H), 6.00 (dd, 1H, *J*=6.3 Hz, *J*=2.4 Hz, 3'-H), 5.70 (d, 1H, *J*=5.4 Hz, 2-H), 5.53 (d, 1H, *J*=2.4 Hz, 2'-H), 4.99 (d, 1H, J=7.8 Hz, 5-H), 4.46 (t, 1H, 7-H), 4.33 (d, 1H, *J*=6.6 Hz, 20α-H), 4.25 (s, 4H, -O<u>CH_2CH_2</u>O-), 4.21 (d, 1H, *J*=6.6 Hz, 20β-H), 3.83 (d, 1H, *J*=5.4 Hz, 3-H), 3.50 (t, 2H, -<u>CH_2</u>-N (CO) CO), 2.79-2.91 (m, 6H, -CO<u>CH_2CH_2</u>SS<u>CH_2</u>CH_2CO-), 2.72 (t, 2H, *J*=5.1 Hz, -COCH$_2$CH$_2$SSCH$_2$<u>CH_2</u>CO-), 2.53-2.60 (m, 1H, 6α-H), 2.46 (s, 3H, 4-CO<u>CH_3</u>), 2.36 (m, 2H, 14α-H, 14β-H), 2.31 (t, 2H, *J*=5.7 Hz, -<u>CH_2</u>CO-), 2.23 (s, 3H, 10-CO<u>CH_3</u>), 2.17 (t, 2H, *J*=6.6 Hz, -<u>CH_2</u>CO-), 1.94 (s, 3H, 18-H), 1.89 (t, 1H, 6β-H), 1.68 (s, 3H, 19-H), 1.57-1.65 (m, 4H, -<u>CH_2</u>CH_2<u>CH_2</u>-), 1.28-1.34 (m, 2H, -CH_2<u>CH_2</u>CH_2-), 1.24 (s, 3H, 17-H), 1.14 (s, 3H, 16-H).

MS (ESI) (m/z): calcd for $C_{65}H_{74}N_2O_{21}S_2$: m/z 1305.4 $[M+Na]^+$.

References

1. R. M. de Figueiredo, P. Oczipka, R. Fröhlich and M. Christmann, *Synthesis*, 2008, **8**, 1316-1318.



(i) EDCI, DMAP, N_2, r.t.; **(ii)** Reflux, N_2; **(iii)** EDCI, DMAP, N_2, r.t.; **(iv)** pTsOH, reflux,N_2; **(v)** Acetic anhydride, N_2, 30°C; **(vi)** DMAP, N_2, r.t.; **(vii)** EDCI, DMAP, N_2, r.t.; **(viii)** Acetic anhydride, N_2, 30°C; **(ix)** DMAP, N_2, r.t.; **(x)** EDCI, DMAP, N_2, r.t.

173

174 **Scheme S1** Synthetic routes of (A) compound **1** (PMAL), (B) compound **2** (PSUC), compound **3**

175 (PSMAL) and compound **4** (PSSMAL).

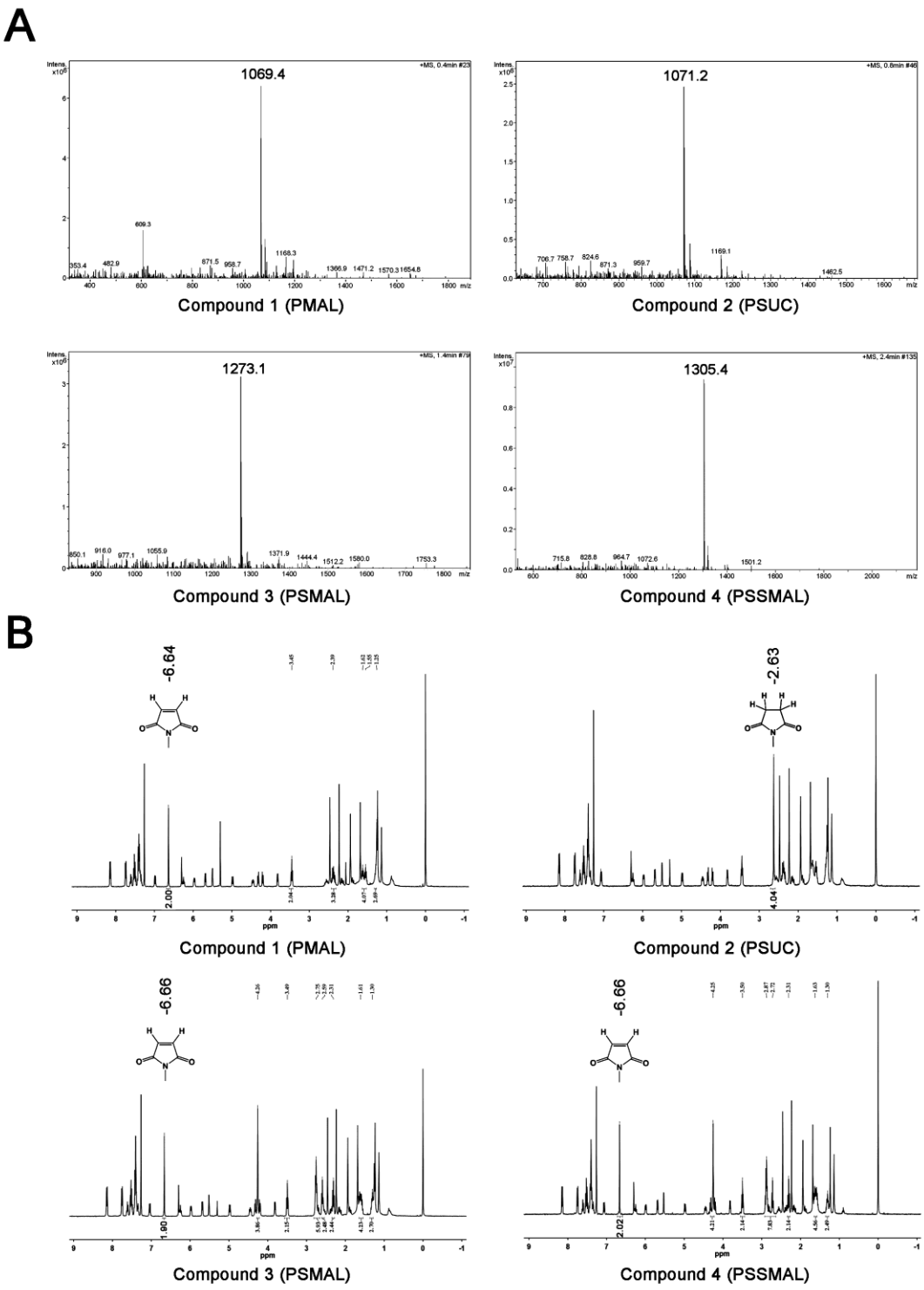
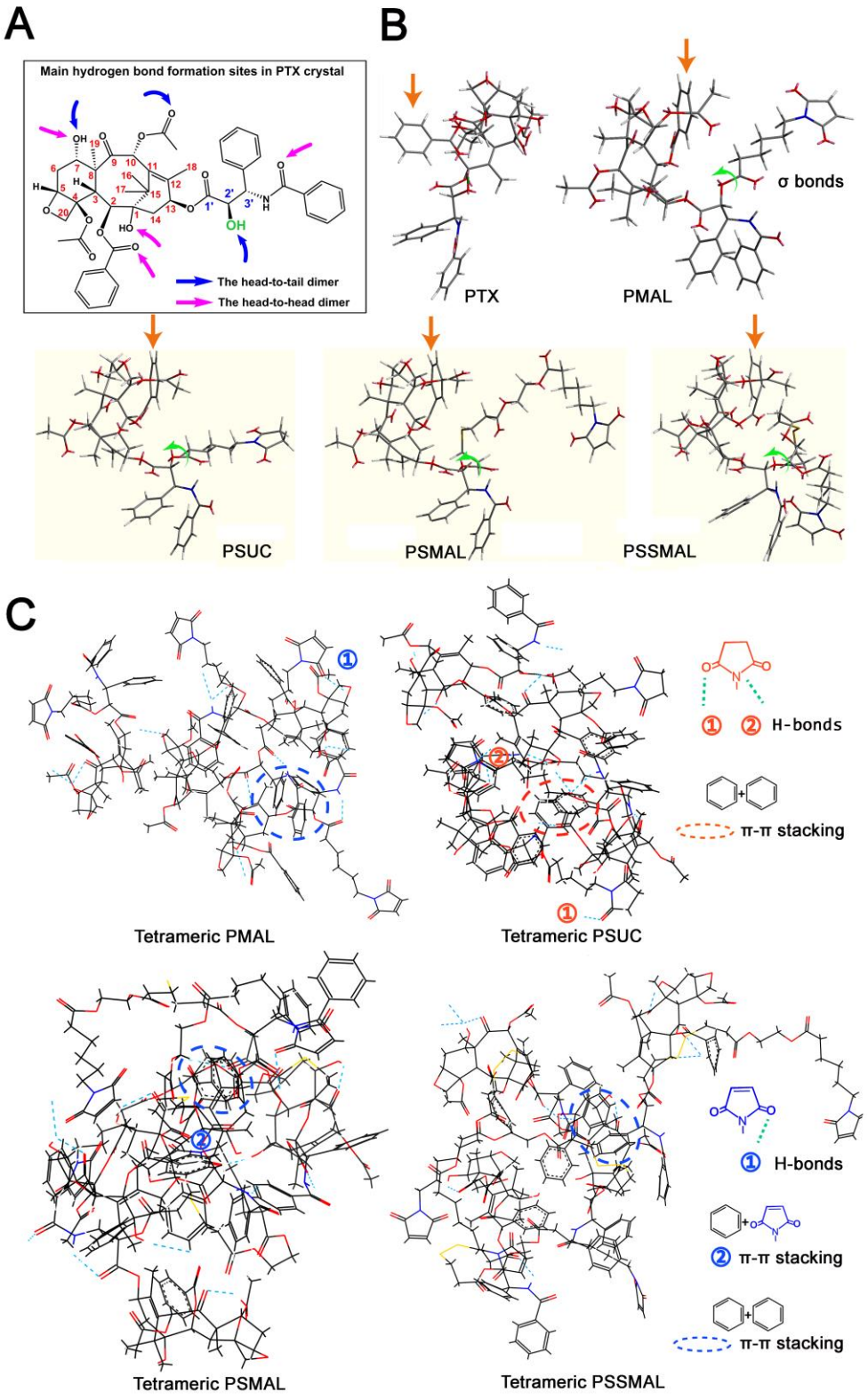


Fig. S1 (A) Mass spectra and (B) ^{1}H-NMR spectra in $CDCl_3$ of four PTX-prodrugs.



178

179 **Fig. S2** (A) Main hydrogen bond formation sites in PTX crystal; (B) 3D chemical structures of
180 PTX and four PTX-prodrugs (green arrows indicate the newly formed σ bonds; orange arrows
181 indicate the site change of 2-benzoyl groups in prodrug molecules); (C) MD simulations of
182 tetrameric prodrugs with the indication of main intermolecular forces.

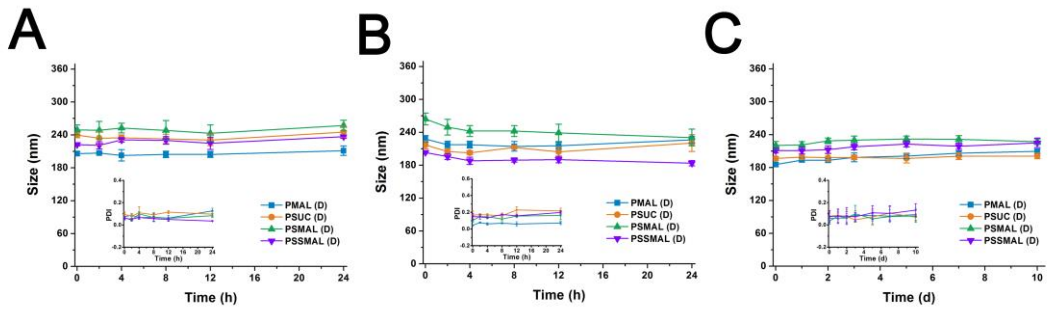


Fig. S3 Stability of prodrug nanoassemblies. (A) After incubation in PBS (pH 7.4) supplemented with 10% FBS at 37 °C for 24 h; (B) After incubation in BSA solution (20 mg/mL in PBS) at 37 °C for 24 h; (C) After store at 4 °C for 10 d (mean ± SD, n = 3).

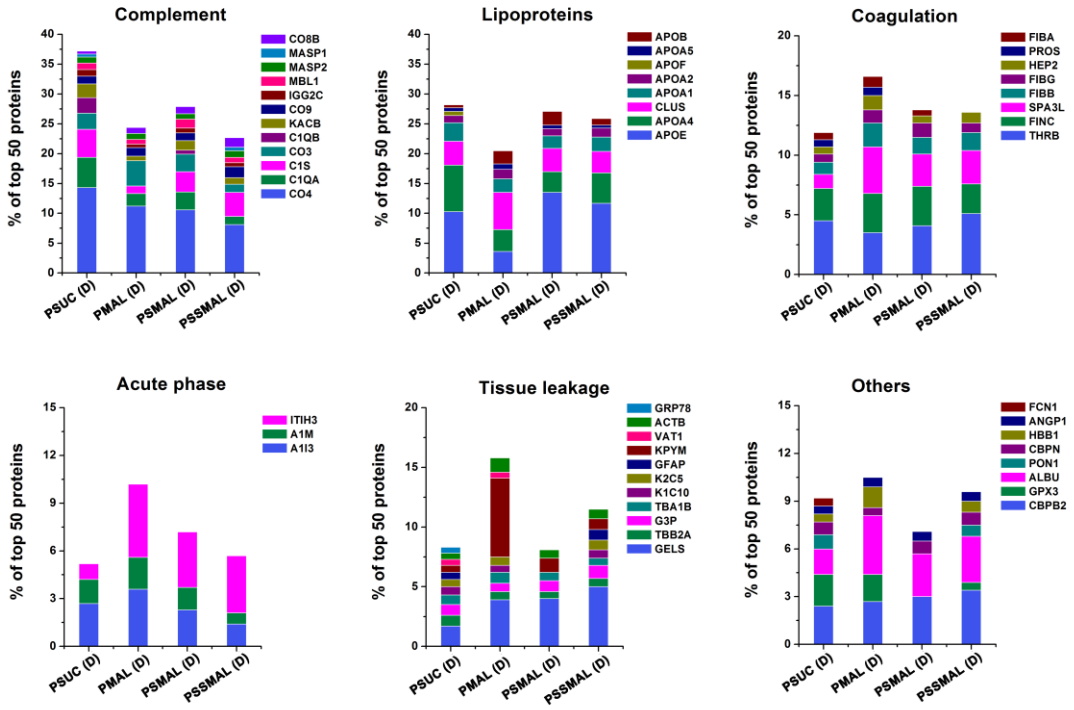
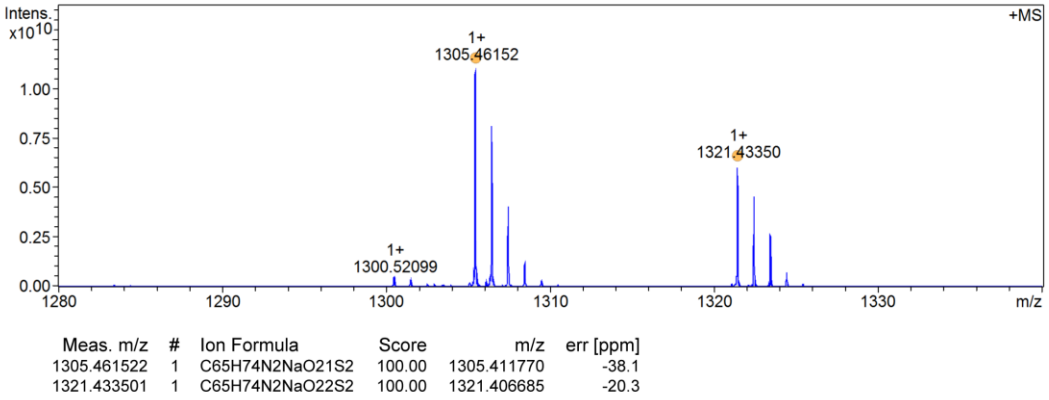


Fig. S4 Bioinformatic classification of top 50 identified proteins in the coronas of prodrug nanoassemblies.



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193 **Fig. S5** The MS spectra of oxidation product of PSSMAL treated with 10 mM H_2O_2.

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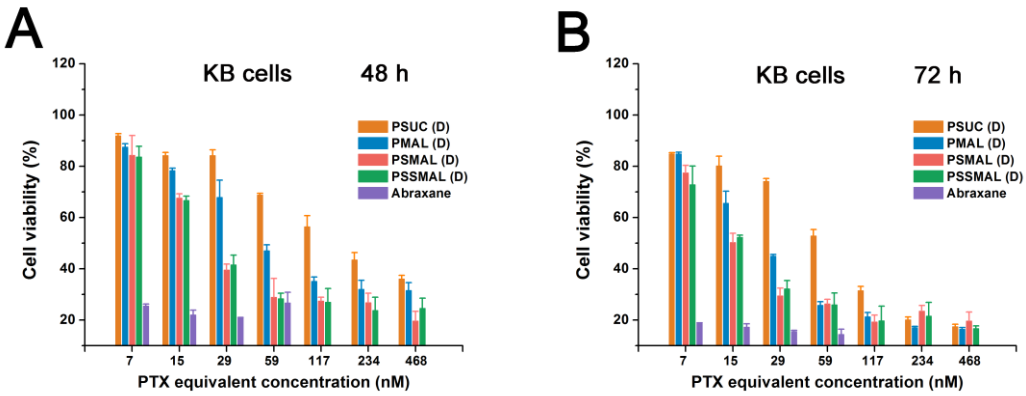


196

197 **Fig. S6** Cell viability of KB cells treated with various concentrations of Abraxane® and PTX

198 prodrug nanoassemblies after (A) 48 h and (B) 72 h (mean + SD, n = 3).

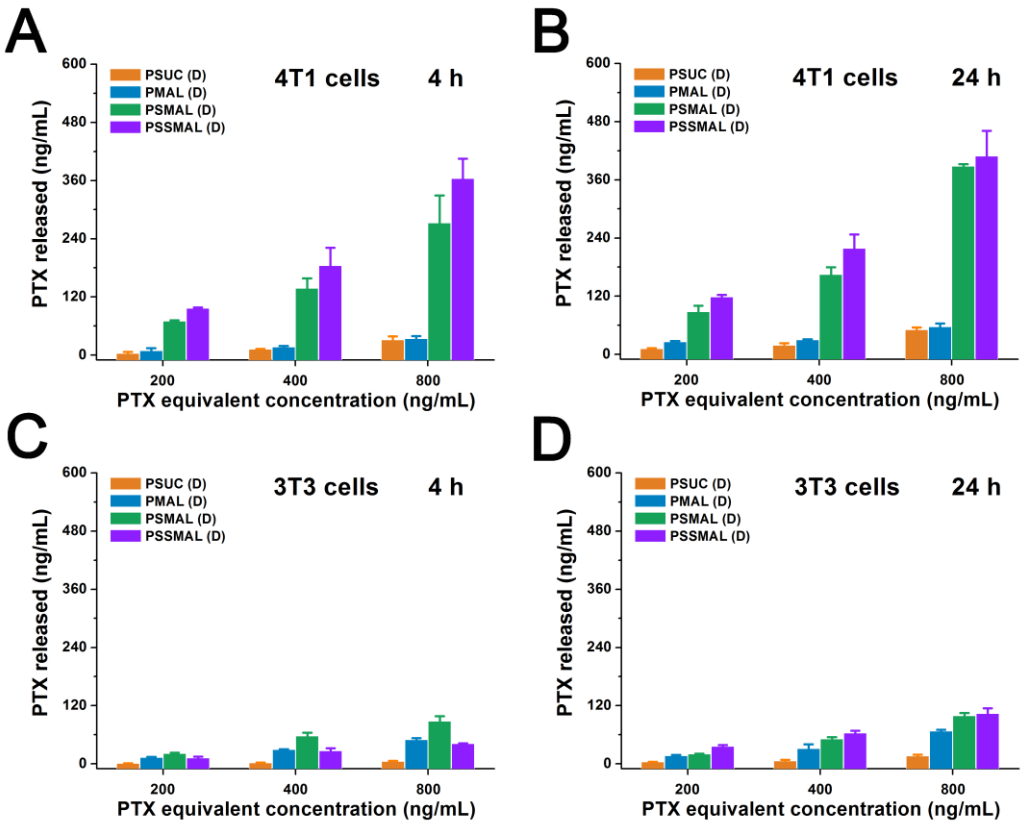


Fig. S7 Free PTX released from prodrug nanoassemblies after incubation with (A, B) 4T1 cells or (C, D) NIH/3T3 cells for 4 h and 24 h (mean + SD, n = 3).

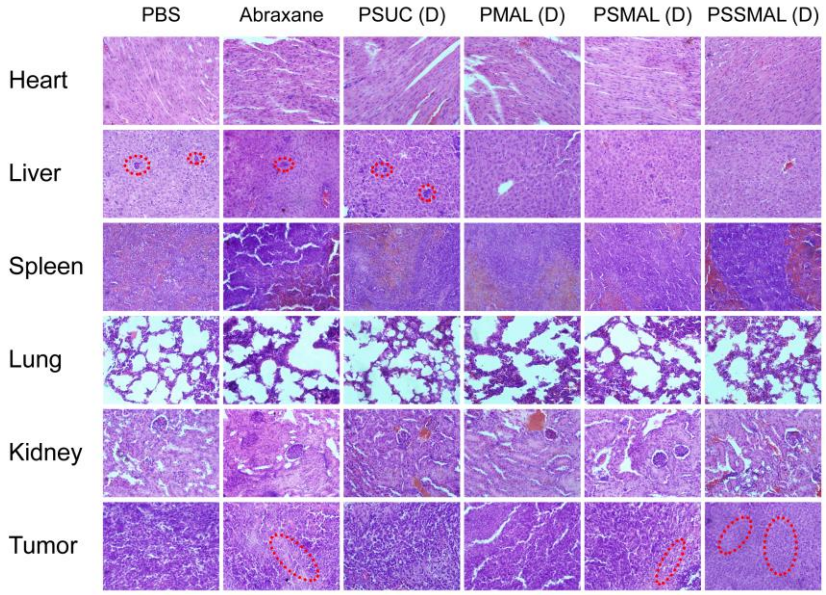
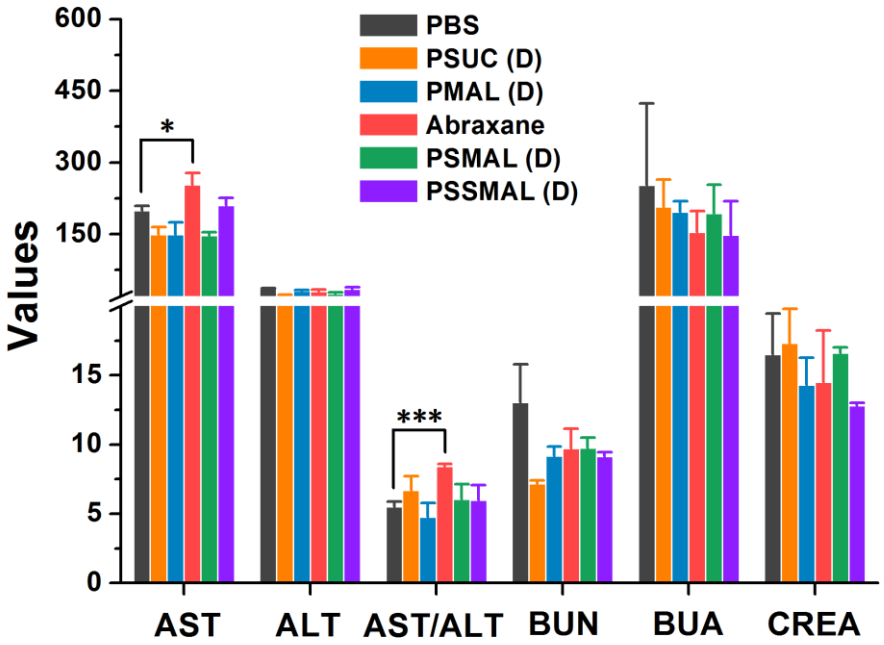


Fig. S8 H&E staining images of heart, liver, spleen, lung, kidney and tumor after the last treatment.



206

207 **Fig. S9** Liver and kidney function parameters after the last treatment (mean + SD, n = 3), * P <

208 0.05, *** P < 0.001.

209

Table S1. Characterization of prodrug nanoassemblies.

Formulations	Size (nm)	PDI	Zeta potential (mV)	DL (%)
PMAL (D)	216.0 ± 2.5	0.075 ± 0.005	-30.3 ± 0.8	68.0
PSUC (D)	244.9 ± 0.6	0.067 ± 0.001	-31.3 ± 0.7	67.8
PSMAL (D)	244.3 ± 4.3	0.050 ± 0.013	-24.6 ± 0.6	56.9
PSSMAL (D)	214.4 ± 1.8	0.031 ± 0.002	-19.8 ± 0.8	55.4

Table S2. Top 50 identified proteins in the coronas.

NO.	Protein name	Abbreviation
1	Complement C4	CO4
2	Clusterin	CLUS
3	Inter-alpha-trypsin inhibitor heavy chain H3	ITIH3
4	Complement C3	CO3
5	Gelsolin	GELS
6	Serine protease inhibitor A3L	SPA3L
7	Apolipoprotein A-IV	APOA4
8	**Serum albumin**	**ALBU**
9	Alpha-1-inhibitor 3	A1I3
10	Apolipoprotein E	APOE
11	Prothrombin	THRB
12	Fibronectin	FINC
13	Carboxypeptidase B2	CBPB2
14	Apolipoprotein A-I	APOA1
15	Apolipoprotein B-100	APOB
16	Serine protease inhibitor A3N	SPA3N
17	Complement C1q subcomponent subunit A	C1QA
18	Fibrinogen beta chain	FIBB
19	Alpha-1-macroglobulin	A1M
20	Glutathione peroxidase 3	GPX3
21	Apolipoprotein A-II	APOA2
22	Complement component C9	CO9
23	Pyruvate kinase PKM	KPYM
24	Histidine-rich glycoprotein	HRG
25	Complement C1s subcomponent	C1S

26	Hemoglobin subunit beta-1	HBB1
27	Actin, cytoplasmic 1	ACTB
28	Heparin cofactor 2	HEP2
29	Fibrinogen gamma chain	FIBG
30	Mannan-binding lectin serine protease 2	MASP2
31	Complement component C8 beta chain	CO8B
32	Tubulin alpha-1B chain	TBA1B
33	Apolipoprotein A-V	APOA5
34	Fibrinogen alpha chain	FIBA
35	Fructose-bisphosphate aldolase A	ALDOA
36	Ig kappa chain C region, B allele	KACB
37	Hemoglobin subunit alpha-1/2	HBA
38	Mannose-binding protein A	MBL1
39	Glyceraldehyde-3-phosphate dehydrogenase	G3P
40	Myosin-9	MYH9
41	Tubulin beta-2A chain	TBB2A
42	Extracellular matrix protein 1	ECM1
43	Vitamin K-dependent protein S	PROS
44	Keratin, type II cytoskeletal 5	K2C5
45	Ig gamma-2C chain C region	IGG2C
46	Keratin, type I cytoskeletal 10	K1C10
47	Keratin, type II cytoskeletal 1	K2C1
48	Angiopoietin-1	ANGP1
49	Carboxypeptidase N catalytic chain	CBPN
50	Synaptic vesicle membrane protein VAT-1 homolog	VAT1

Table S3. IC_{50} values of Abraxane® and prodrug nanoassemblies to three cell lines.

Formulations	4T1 cells (nM)		KB cells (nM)		NIH/3T3 cells (nM)	
	48 h	72 h	48 h	72 h	48 h	72 h
Abraxane®	22.7 ± 1.5	21.3 ± 2.3	<1	<1	382 ± 33	74 ± 26
PMAL (D)	555 ± 72	297 ± 27	83.3 ± 3.1	31.7 ± 1.5	5023 ± 1915	2119 ± 326
PSUC (D)	752 ± 41	406 ± 68	188 ± 20	64.0 ± 2.0	10648 ± 2963	2220 ± 214
PSMAL (D)	281 ± 69	319 ± 30	18.7 ± 1.5	17.7 ± 2.5	2475 ± 455	894 ± 18
PSSMAL (D)	280 ± 37	262 ± 14	19.0 ± 5.3	17.0 ± 2.6	2075± 650	970 ± 94

Table S4. $T_{1/2}$ values of prodrug nanoassemblies in rat plasma.

Formulations	$T_{1/2}$ (min)
PMAL (D)	46.4 ± 8.2
PSUC (D)	30.2 ± 6.7
PSMAL (D)	18.2 ± 0.5
PSSMAL (D)	59.7 ± 12.2