Supplementary Materials

Enzyme-Controlled Intracellular Self-Assembly of ^{18}F Nanoparticles for Enhanced MicroPET Imaging of Tumor

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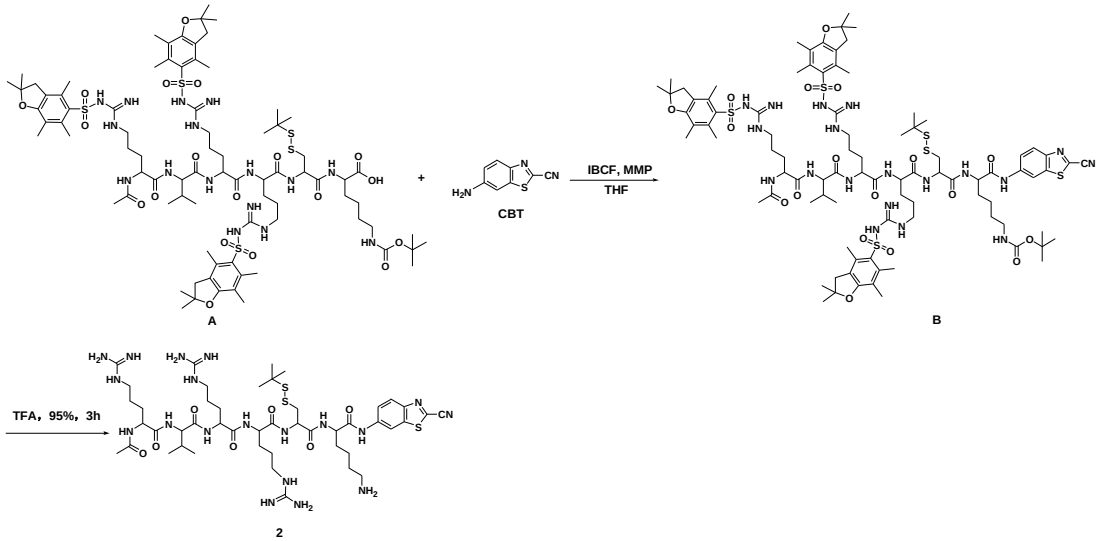
Contents:

1. Chemical syntheses and characterizations of 2, SFB, 1-Cold, ^{18}F-SFB, and 1

The preparations of compound **2**, N-succinimidyl- 4-fluorobenzoate (SFB), **1-Cold**, ^{18}F-SFB, and **1** were described as below；2-cyano-6-aminobenzothiazole (**CBT**) was synthesized following the literature method.[1]

*Preparation of Acetyl-Arg-Val-Arg-Arg-Cys(StBu)-Lys-CBT (**2**).*

Scheme S1. Synthetic route forcompound ***2***.



Peptide Ac-Arg-Val-Arg-Arg-Cys(StBu)-Lys-OH (**A**, 180 mg, 0.1 mmol) was prepared by solid phase peptide synthesis (SPPS). The isobutyl chloroformate (8.7 μL, 0.12 mmol) was added to a mixture of **A** (180 mg, 0.1 mmol) and MMP (4-methylmorpholine, 8.5 μL, 0.15 mmol) in THF (1.5 mL) at 0 °C under N_2 and the reaction mixture was stirred for 20 min. The solution of

2-cyano-6-aminobenzothiazole (CBT) (18 mg, 0.1 mmol) was added to the reaction mixture and further stirred for 1 h at 0 °C then overnight at room temperature. The pure product **B** (68 mg, 35%) was obtained after HPLC purification. The Boc and Pbf protecting groups were cleaved with 95% TFA in CH_2Cl_2 for 3 hrs in the presence of 1% triisopropylsilane. The pure product **2** (20 mg, 50%) was obtained after HPLC purification. ^{1}H NMR of compound **2** (d_4-CH_3OH, 400 MHz, δ, ppm) (Fig. S1): 8.68 (s，1 H), 8.12 (d，J = 9.0 Hz，1 H)，7.79 (d，J = 9.0 Hz，1 H), 4.56-4.65 (m，1 H), 4.46-4.54 (m, 1 H), 4.20-4.37 (m，3 H), 4.08 (d，J = 7.4 Hz，1 H), 3.04-3.26 (m，9 H), 2.93 (t, 2 H), 2.03(s, 3 H), 1.45-1.99 (br, 18 H), 1.31 (s, 9 H), 0.97 (t, 6 H); ^{13}C NMR of compound **2** (d_4-CH_3OH, 100 MHz, δ, ppm) (Fig. S2): 175.01, 174.69, 174.60, 174.42, 174.19, 172.73, 172.50, 158.73, 158.68, 150.01, 140.46, 138.02, 136.88, 125.98, 122.56, 119.37, 113.04, 61.49, 58.37, 55.76, 55.70, 55.35, 55.29, 55.00, 42.62, 42.03, 41.97, 40.56, 32.19, 31.32, 30.27, 29.65, 29.59, 28.07, 26.44, 26.41, 23.93, 22.60, 19.75, 19.26, 18.34; MS of **2**: calculated for $C_{46}H_{77}N_{19}O_7S_3$, $[(M+H)^+]$: 1104.54; obsvd. ESI-MS: m/z 1104.72.

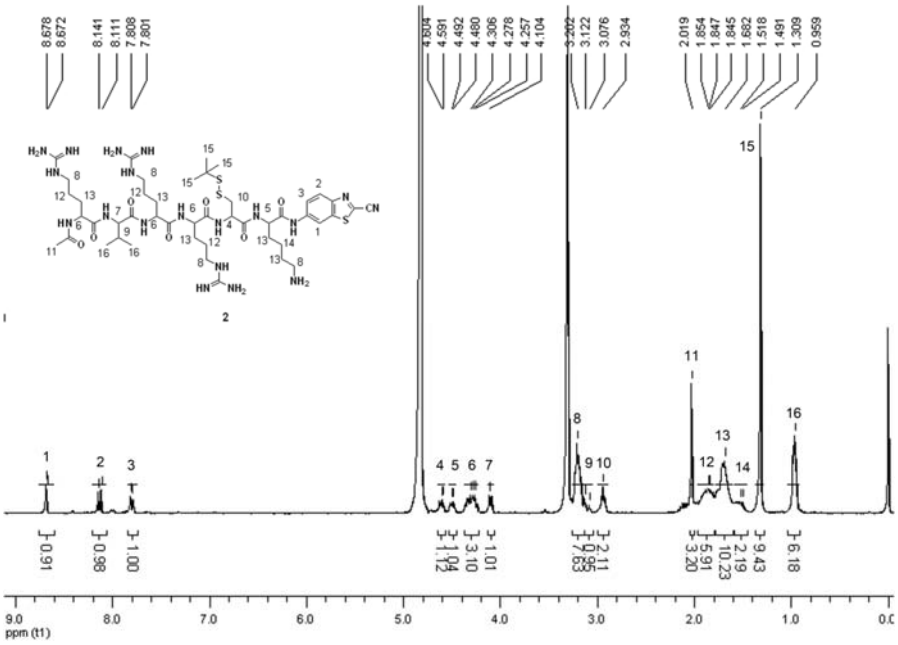


Figure S1.^{1}H NMR spectrum of compound **2**.

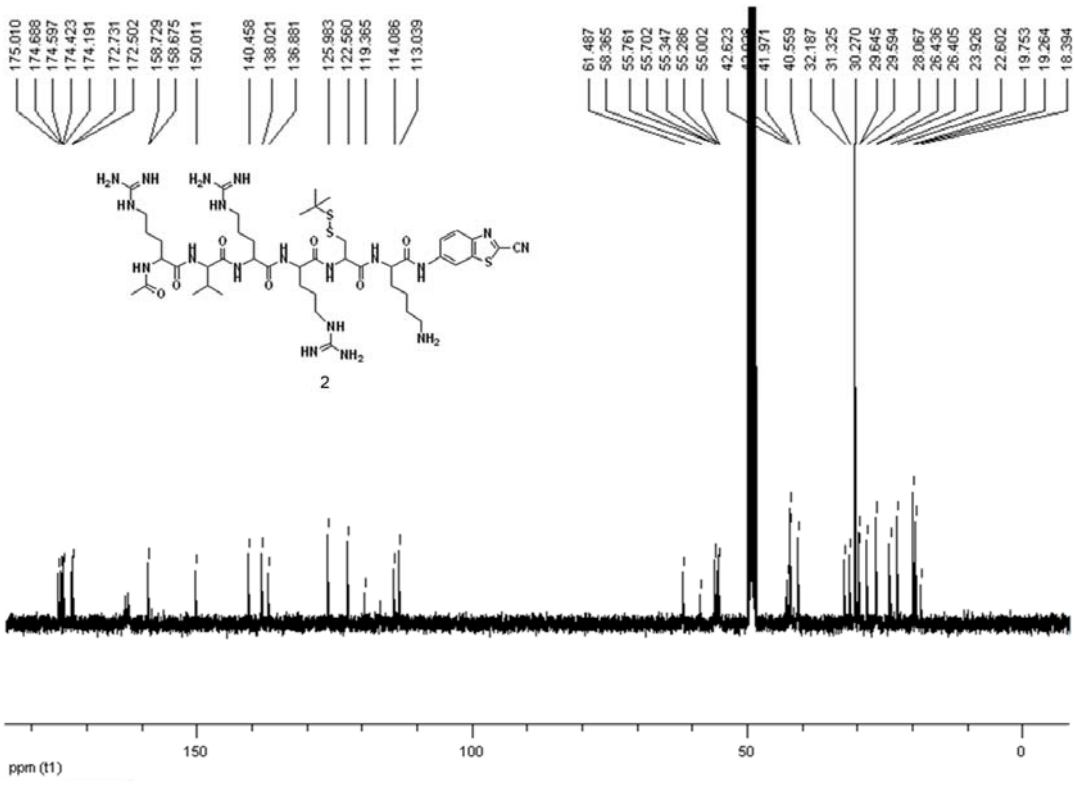


Figure S2. ^{13}C NMR spectrum of compound **2**.

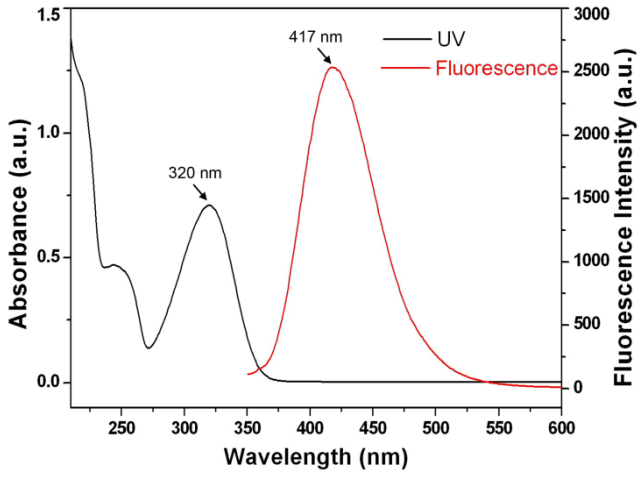
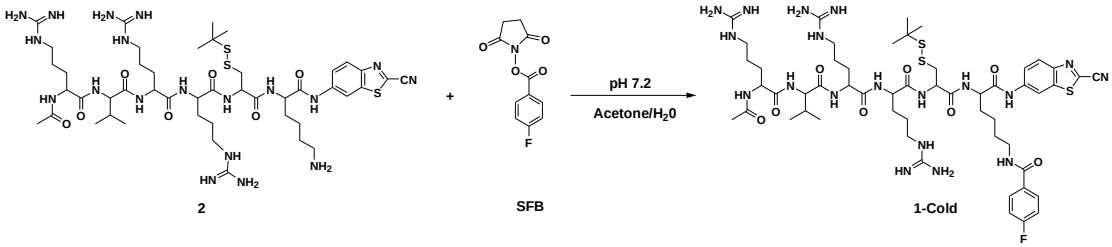


Figure S3. UV-vis spectrum (black), and fluorescence emission spectrum (red) of **2** in distilled water, repectively.

*Preparation of Acetyl-Arg-Val-Arg-Arg-Cys(StBu)-Lys(FB)-CBT (**1-Cold**).*

Scheme S2. Synthetic route for ***1-Cold***.



*Synthesis of N-succinimidyl- 4-fluorobenzoate (**SFB**):*

4-fluorobenzoic acid (1.40 g, 10 mmol) was mixed with N-hydroxysuccinimide (1.38 g, 12 mmol), then 30 mL of chloroform was added to obtain a well-dispersed solution. After N,N'-Dicylcohexylcarbodiimide (2.47 g, 12 mmol) was added into the mixture, the solution was stirred overnight at room temperature. The chloroform was evaporated and the N-succinimidyl 4-fluorobenzoate was purified by chromatography with ethyl acetate-petroleum ether (1:2) as the eluent to yield 1.92 g (80%) of white solid. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm) (Fig. S4): 8.16 (m, 2 H), 7.19 (m, 2 H), 2.89 (s, 4 H).

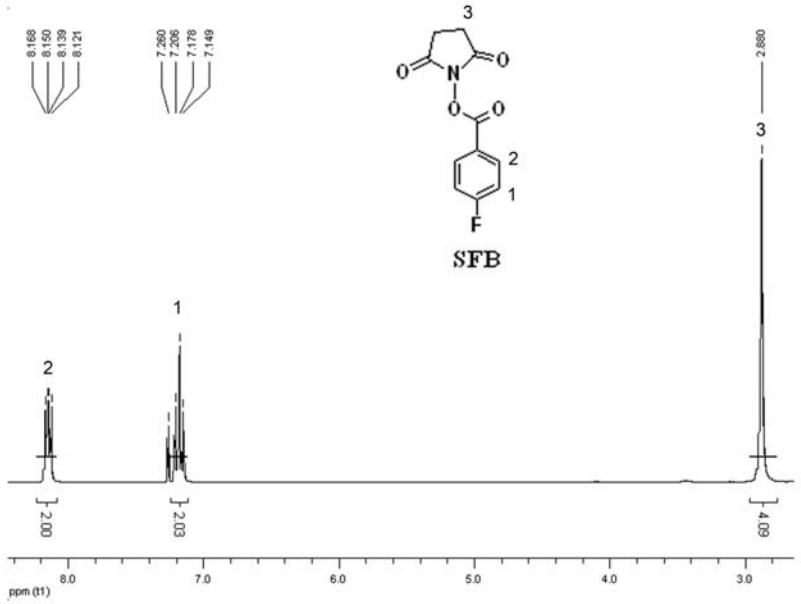


Figure S4. ^{1}H NMR spectrum of SFB.

*Synthesis of **1-Cold**:*

Compound **2** (20 mg, 0.018mmol) was dissolved in 5 mL of water, and the pH of the solution was adjusted to 8.5 with sodium carbonate. N-succinimidyl 4-fluorobenzoate (25 mg, 0.11 mmol) was dissolved in 10 mL of acetone, and then added into the water solution dropwise. The ratio of water/acetone was adjusted to keep the reaction mixture clear. The mixture was stirred at room temperature for 6 hrs. The reaction mixture was subjected to HPLC purification to yield pure compound **1-Cold** (15 mg, 0.012 mmol, 67%). The method was referred from the literature.[2] ^{1}H NMR of compound **1-Cold** (d_4-CH_3OH, 300 MHz, δ, ppm) (Fig. S5): 8.63 (s, 1 H), 8.10 (d, J = 9.0 Hz, 1 H), 7.70-7.85 (m, 3 H), 7.11 (t，2 H)，4.57-4.67 (m, 1 H), 4.44-4.53 (m, 1 H), 4.24-4.38 (m, 3 H), 4.12 (d, J = 7.1 Hz,1 H), 3.33-3.43 (m，2 H), 3.01-3.24 (br, 9 H), 2.01(s, 3 H), 1.42-1.96 (br, 18 H), 1.30 (s, 9 H), 0.94 (d, 6 H). MS of **1-Cold**: calculated for $C_{53}H_{80}FN_{19}O_8S_3$,[$(M+H)^+$]: 1226.56; obsvd. Maldi-MS: m/z 1226.56.

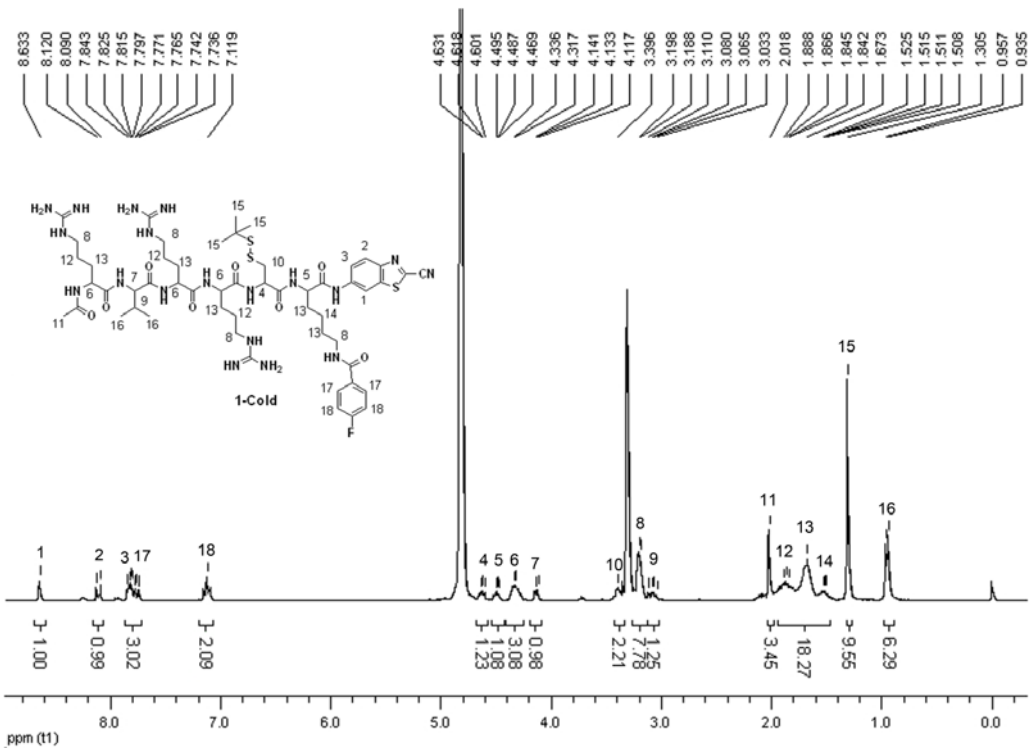


Figure S5. ^{1}H NMR spectrum of compound **1-Cold**.

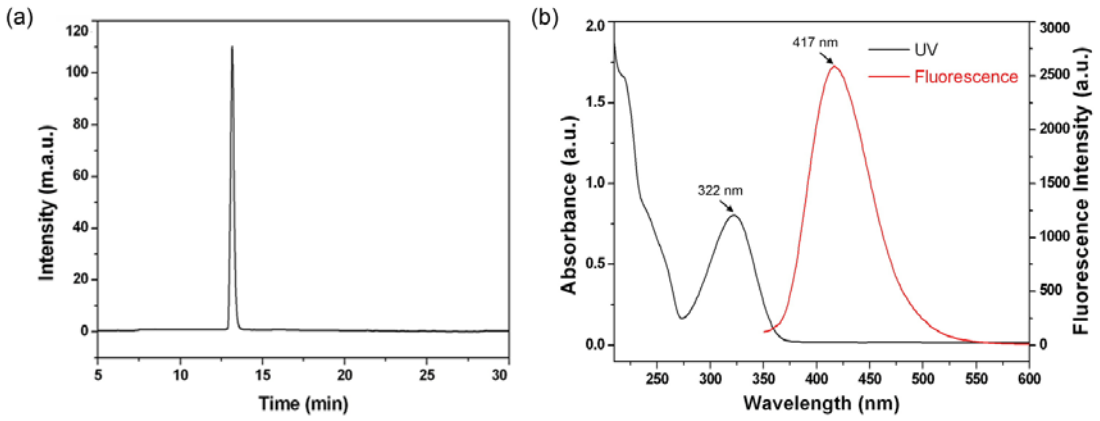
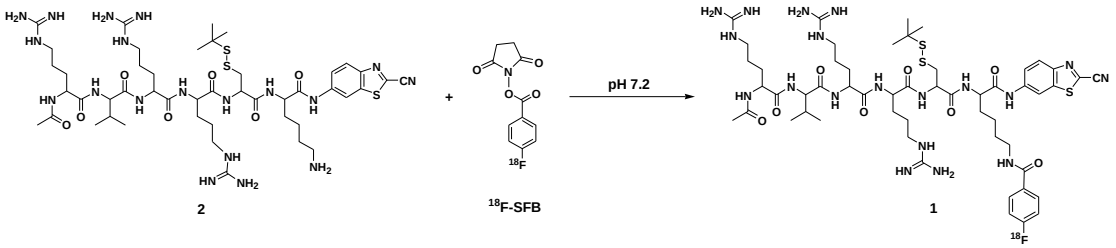


Figure S6. HPLC trace (a) and UV-vis spectrum (black) and fluorescence emission spectrum (red) (b) of **1-Cold** in distilled water, respectively.

*Preparation of Acetyl-Arg-Val-Arg-Arg-Cys(StBu)-Lys(^{18}F-FB)-CBT (**1**).*

Scheme S3. Synthetic route for ***1.***



Radiosynthesis of ^{18}F-SFB. [^{18}F]-fluoride(1000 mCi) was prepared by proton bombardment of 2.5 mL [^{18}O] enriched water target via the ^{18}O (p,n) ^{18}F nuclear reaction. The [^{18}F]-fluoride produced from cyclotron was then trapped onto a Sep-Pak QMA cartridge. The [^{18}F]fluoride was then released into tube 1 from the cartridge by using the K_{222}(4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8] hexacosane) (15.0 mg/mL) / K_2CO_3 solution (2.75 mg/mL) (1.5 mL, 10:1 CH_3CN/H_2O). After solvent evaporated to dryness under a stream of N_2 at 120 °C, the azeotropic drying was repeated once with 1.2 mL of CH_3CN to yield the anhydrous K_{222}/K[^{18}F]F complex. After ethyl 4-(trimethylammonium) benzoate trifluormethane sulfonate (10 mg) solution (CH_3CN) was transferred into tube 1, the mixture was heated at 90 °C for 12 min to produce ethyl 4-[^{18}F]

fluorobenzoate. The NaOH solution (0.5 M, 0.5 mL) was added after cooling and then heated at 90 °C for 6 min. The mixture of solution (1.5 mL, 0.1 M HCl, 7.5 mL CH_3CN) was added to neutralize and then trapped in the C18 Sep-Pak cartridge. The cartridge was eluted with CH_3CN and collected into tube 2 added with TPAOH (40 μL). Then the mixture was dried at 100 °C, subsequently, TSTU (O-(N-succimidyl)-N,N,N',N'-tetramethyl uronium Tetrafluoroborate) (15 mg) dissolved in CH_3CN (2 mL) was added to the reaction mixture and then the mixture was heated at 90 °C for 6 min to afford ^{18}F-SFB. After acidifying with 1.5 mL of HCl (0.1 M), the final solution was passed through a C18 Sep-Pak cartridge and the crude ^{18}F-SFB was eluted with 2 mL of CH_3CN. The radiochemical yield (RCY) (decay-corrected to the end of synthesis) of ^{18}F-SFB is 40% with radiochemical purity above 90% analyzed by radio-HPLC and radio-TLC analysis. Figure S7 shows that the retention time of ^{18}F-SFB (red line) is corresponding to that of SFB (black line).

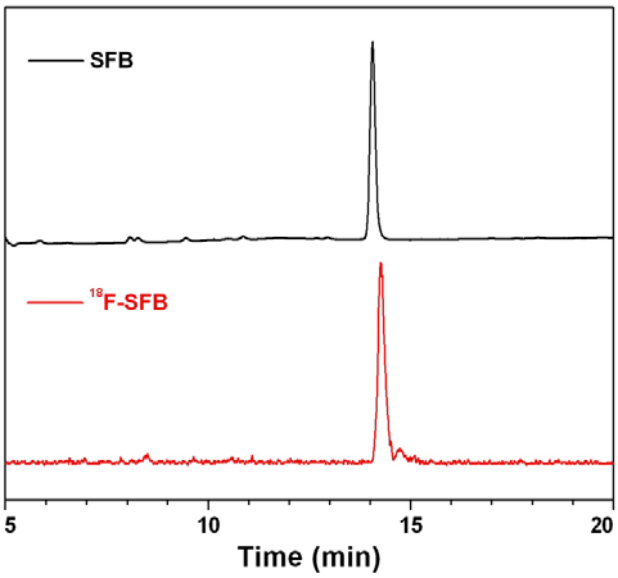


Figure S7. Radio-HPLC traces of **SFB** (black, absorbance at 254 nm) and **^{18}F-SFB** (red, radiochromatograph).

*Synthesis of **1***:

^{18}F-SFB (20 μL, 5 mCi) was added into the 150 μL solution of **2** (100 μg, 90 nmol) dissolved in phosphate buffered saline (PBS) at pH 7.2, heated at 50 °C for 0.5 h. Then the reaction mixture was injected into the radio-HPLC for purification and analysis. the HPLC peak at 15.9 min on the raio-HPLC trace, which has a same retention time of that of **1-Cold**, was collected and dried at 100 °C by N_2 to remove the acetonitrile as **1** with 50% radiochemical yield (RCY) (decay-corrected to the end of synthesis, EOS) and specific radioactivity of 0.6 ± 0.4 Ci μmol^{-1} (EOS) (Figure S8).

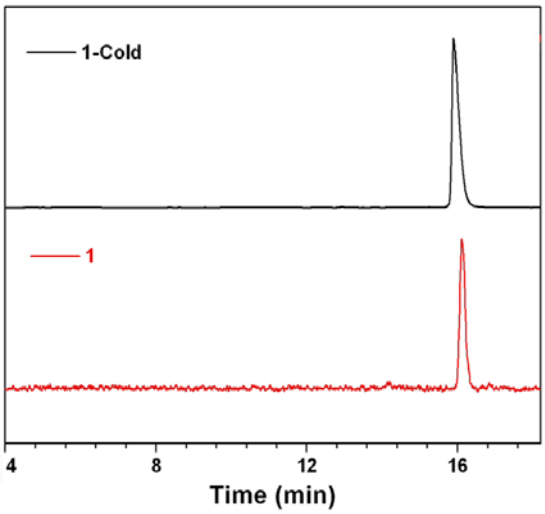


Figure S8. Radio-HPLC traces of **1-Cold** (black, absorbance at 320 nm) and **1** (red, radiochromatograph).

2. Supplementary figures and tables

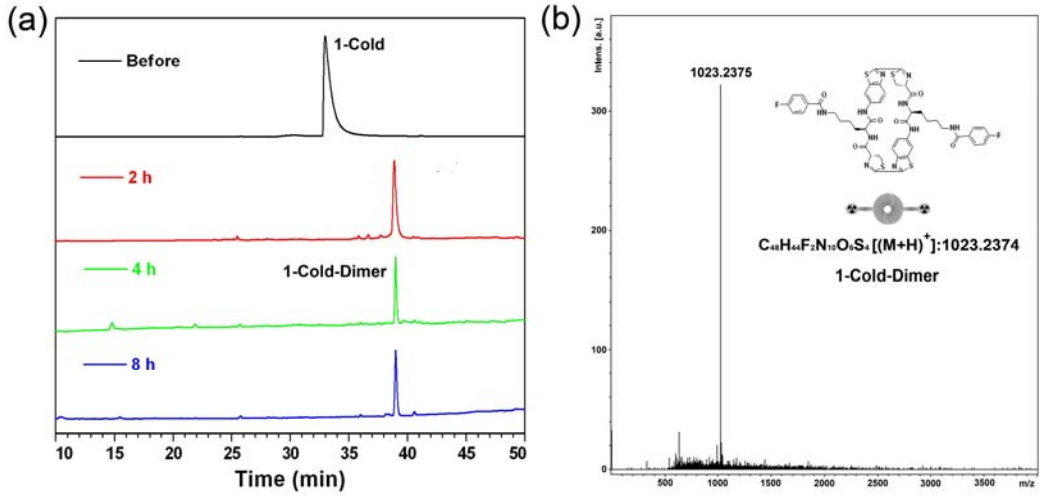


Figure S9. (a) HPLC traces of the incubation mixture of **1-Cold** at 100 μM after 2 h (red), 4 h (green), 8 h (blue) incubation with 1 nmol/U of furin at 37 °C , and HPLC trace of **1-Cold** in water (black). (b) High resolution MALDI mass spectrum of HPLC peak at 38.9 min in figure a.

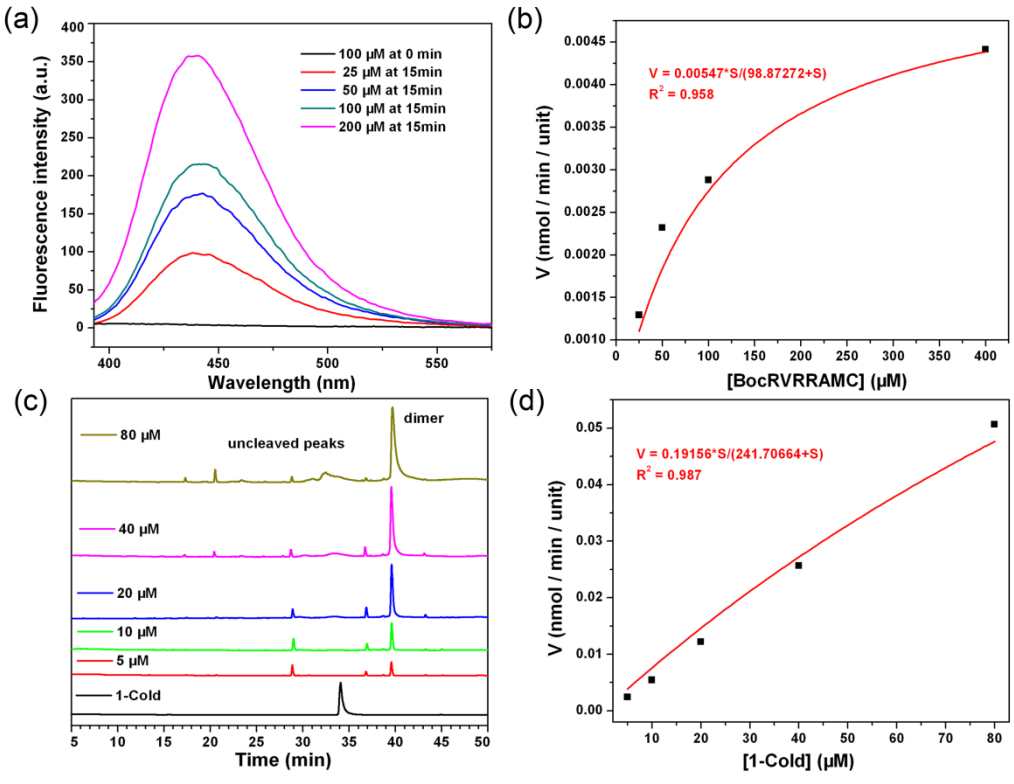


Figure S10. (a) Fluorescence spectra of Boc-Arg-Val-Arg-Arg-AMC at different concentrations

after 15 min incubation with furin (10 unit/100 μL) at 37 °C and fluorescence spectrum of Boc-Arg-Val-Arg-Arg-AMC at 100 μM without furin (black). Excitation: 370 nm. (b) Non-linear regression analysis of furin cleavage rate V (nmol/min/unit) as a function of Boc-Arg-Val-Arg-Arg-AMC concentration to give formula: V = 0.00547*S/(98.87272+S). [S] = concentration of substrate used. The K_M (99 μM) and V_{max} (0.00547 nmol/min/unit) were obtained from the formula above. (c) HPLC traces of the incubation mixture of **1-Cold** at different concentrations after 15 min incubation with furin (10 unit/100 μL) at 37 °C, and HPLC trace of **1-Cold** in water (black). Absorbance: 320 nm. (d) Non-linear regression analysis of furin cleavage rate V (nmol/min/unit) as a function of **1-Cold** concentration to give formula: V = 0.19156*S/(241.70664+S). [S] = concentration of substrate used. The K_M (242 μM) and V_{max} (0.19156 nmol/min/unit) were obtained from the formula above.

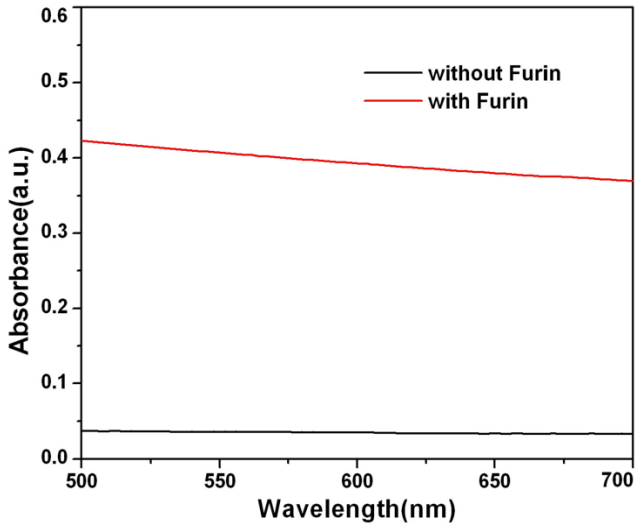


Figure S11. Absorption spectra (500-700 nm due to the light scattering) of **1-Cold** at 100 μM without furin (black) or incubated with furin at 1 nmol/U, pH 7.4, and 37 ℃ for 4 h (red).

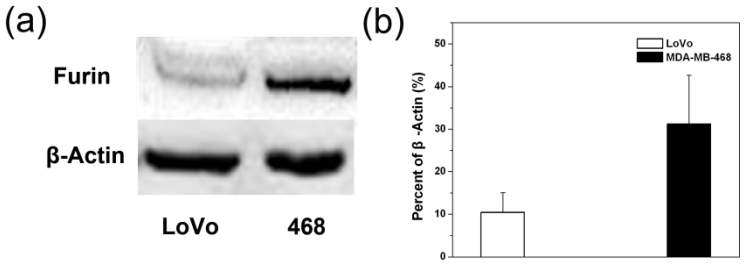


Figure S12. Western blot analysis (a) and quantification (b) of furin in human breast cancer MDA-MB-468 cells and human colon carcinoma LoVo cells.

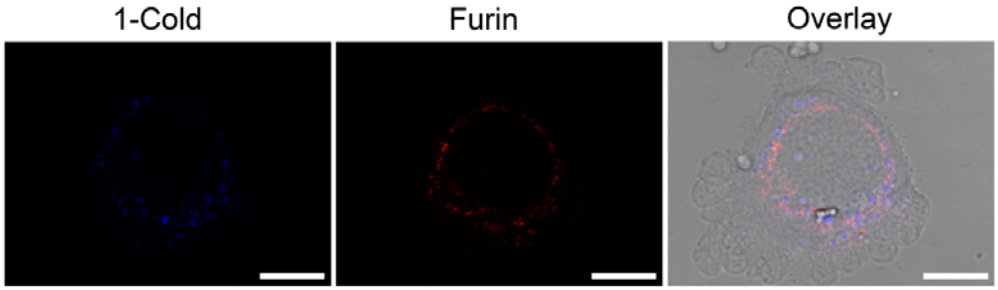


Figure S13. Left, Confocal fluorescence image (λ_{ex} = 405 nm, λ_{em} = 414-530 nm) of MDA-MB-468 cells incubated with **1-Cold** at 100 μM and 37 °C for 1 h. Middle, Immunostaining of MDA-MB-468 cells with rhodamine-labeled antibody against furin after incubation with **1-Cold** at 100 μM and 37 °C for 1 h (λ_{ex} = 543 nm, λ_{em} = 554-683 nm). Right, An overlay of the fluorescence images with the white field image of MDA-MB-468 cells. Scale bar: 10 μm.

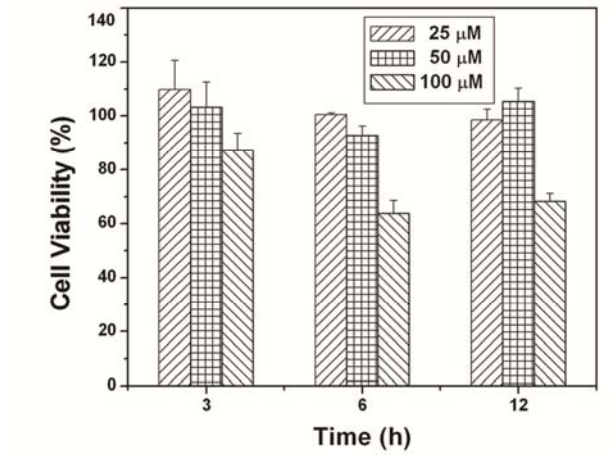


Figure S14. MTT assay of **1-Cold** on MDA-MB-468 cells. Cell viability values (%) estimated by

MTT proliferation test at concentrations of 25, 50 and 100 μM of **1-Cold**. MDA-MB-468 cells were cultured in the presence of **1-Cold** for 3, 6 and 12 h at 37 °C under 5% CO_2. These experiments were performed in triplicate. Results are representative of three independent experiments. Error bars represent standard deviations.

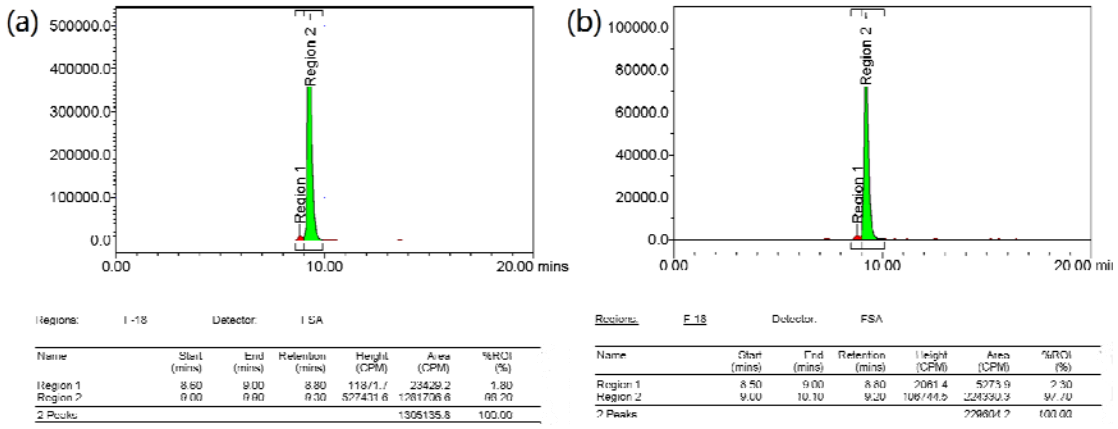


Figure S15. HPLC traces of **1** (a, radiochromatograph) and **1** incubated with fetal bovine serum at 37 °C for 5 hours (b, radiochromatograph), respectively.

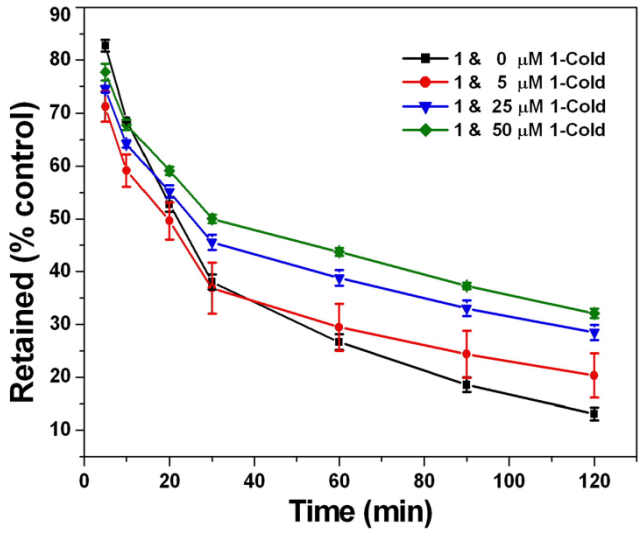
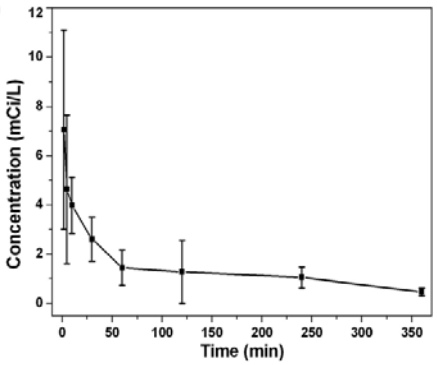


Figure S16. Concentration-dependent intracellular self-assembly: Time course of cellular efflux of **1** after 60 min incubation with 1 million MDA-MB-468 cells at 4 μCi and co-incubated with **1-Cold** at

different concentrations.

(a)



(b)

Parameter	Mean Value
$t_{1/2\alpha}$/min	1.364 ±1.644
$t_{1/2\beta}$/min	84.74 ± 57.463
AUC_{0-t}/min mCi L^{-1}	452.154 ± 221.603
$AUC_{0-\infty}$/min mCi L^{-1}	560.443 ± 287.400
MRT_{0-t} /min	107.151 ± 22.820
$MRT_{0-\infty}$/min	182.744 ± 43.866
CL_{tot}/Lmin^{-1} kg^{-1}	0.006 ± 0.003
V_d/L kg^{-1}	1.042 ± 0.003
C_{max}/mCi L^{-1}	7.471 ± 0.250

Figure S17. (a) Profiles of mean plasma concentration of **1** vs. time after i.v. administration of 3.0 mCi/kg of **1** to mice. (b) Mean values of the pharmacokinetic parameters of **1** after i.v. administration of 3.0 mCi/kg of **1** to mice (n = 5).

Table S1. HPLC condition for the purification of compounds **2** and **1-Cold**.

Time (minute)	Flow (mL/min.)	H_2O % (0.1%TFA)	CH_3CN% (0.1%TFA)
0	3.0	30	70
3	3.0	30	70
35	3.0	0	100
37	3.0	0	100
38	3.0	30	70
40	3.0	30	70

Table S2. HPLC condition for the analysis and purification of the enzymatic products of **1-Cold** incubated with furin.

Time (minute)	Flow (mL/min.)	H_2O % (0.1%TFA)	CH_3CN % (0.1%TFA)
0	1.0	90	10
3	1.0	90	10
55	1.0	30	70
57	1.0	30	70
58	1.0	90	10
60	1.0	90	10

Table S3. HPLC condition for the purification of compound ^{18}F-SFB and **1**.

Time (minute)	Flow (mL/min.)	H_2O % (0.1%TFA)	CH_3CN% (0.1%TFA)
0	3.0	70	30
1	3.0	70	30
35	3.0	30	70
37	3.0	30	70
38	3.0	70	30
40	3.0	70	30

Table S4. Biodistribution of **1** in mice derived from PET quantification (% ID/g, n = 4)

Organ	Time (min)	Co-injected with **1** and **1-Cold**	Injected with **1**	Ratio (Co-injected/**1** alone)
heart	10	13.68 ± 6.27	6.14 ± 5.01	2.2
	30	7.08 ± 0.16	1.74 ± 0.00	4.0
	60	4.97 ± 2.77	0.53 ± 0.08	9.5
	120	2.09 ± 2.96	0.00 ± 0.00	
	240	0.00 ± 0.00	0.00 ± 0.00	
	360	0.00 ± 0.00	0.00 ± 0.00	
liver	10	15.16 ± 8.37	19.63 ± 7.83	0.78
	30	12.43 ± 1.74	7.04 ± 1.31	1.77
	60	6.05 ± 3.15	1.38 ± 0.13	4.40
	120	3.17 ± 3.59	0.38 ± 0.19	8.45
	240	1.11 ± 1.34	0.19 ± 0.01	5.84
	360	0.75 ± 0.87	0.15 ± 0.02	5.14
kidney	10	18.13 ± 2.58	18.43 ± 1.30	0.98
	30	57.88 ± 30.49	13.08 ± 0.88	4.43
	60	62.00 ± 59.75	3.23 ± 0.33	19.20
	120	52.11 ± 67.05	1.09 ± 0.84	48.03
	240	45.45 ± 63.77	0.56 ± 0.55	81.89
	360	29.80 ± 42.14	0.00 ± 0.00	
gall bladder	10	0.00 ± 0.00	0.00 ± 0.00	
	30	14.48 ± 20.47	7.04 ± 9.96	2.06
	60	28.48 ± 3.39	6.26 ± 2.11	4.55
	120	29.95 ± 24.95	4.69 ± 0.81	6.38
	240	20.47 ± 17.13	4.13 ± 0.19	4.96
	360	15.82 ± 17.52	2.86 ± 0.25	5.53
tumor	10	2.52 ± 0.95	1.02 ± 0.19	2.46
	30	4.08 ± 0.48	1.12 ± 0.08	3.62
	60	3.31 ± 1.22	0.52 ± 0.18	6.37
	120	2.27 ± 1.86	0.29 ± 0.02	7.95
	240	1.55 ± 1.75	0.19 ± 0.03	8.16
	360	1.28 ± 1.07	0.19 ± 0.05	6.54

3. References

1 E. H. White, H. Worther, H. H. Seliger and W. D. Mcelroy, *J. Am. Chem. Soc.* , 1966, **88**, 2015-2019.

2 Y. Gao, Y. Kuang, Z. F. Guo, Z. H. Guo, I. J. Krauss and B. Xu, *J. Am. Chem. Soc.* , 2009, **131**, 13576-13577.