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One- and Two- photon Responsive Injectable Nano-Bundle Biomaterials from Co-Assembled Lipopeptides for Controlling Molecule Diffusion

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Supporting Information

The synthetic of C_4-Bhc-EE-NH_2

We have synthesized C_4-Bhc-EE-NH_2 molecule by multi-step reaction with coumarin carboxylic acid molecule C_4-Bhc-COOH and dipeptide EE as the basic units. The synthetic route was shown as follow.

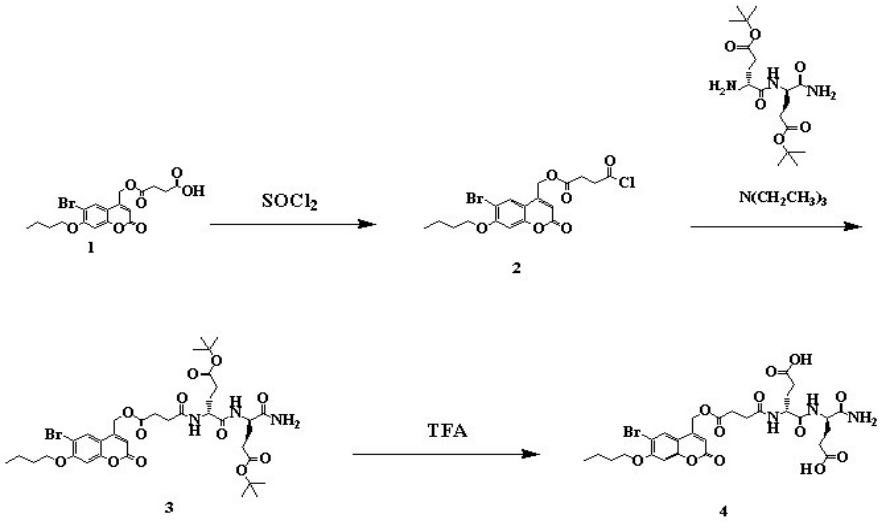

Fig. S1 The synthetic route of C_4-Bhc-EE-NH_2.

(1) The synthesis of compound 2

Compound 1 (coumarin carboxylic acid) (0.5 g, 1.17 mmol) and $SOCl_2$ (10 mL,

13.8 mmol) were heated in a sealed tube with stirring in an oil bath at 100 ° C for 2 hours. After the reaction completed, it was naturally cooled to room temperature. Then the product was transferred into a 100 mL flask and dried for use.

(2) The synthesis of compound 3

The dipeptide compound NH_2-EE(OtBu)-$CONH_2$ (0.453 g, 1.17 mmol) and triethylamine (3 mL, 15.56 mmol) were dissolved in 20 mL dichloromethane. And compound 2 was dissolved in dichloromethane, and dropped into the solution containing dipeptide. The solution was stirred at room temperature for 2 hours after the completion of the dropwise addition. A volume of 50 mL of H_2O was added to the solution for extraction and the organic phase was dried by anhydrous Na_2SO_4 for 2 hours. Then the organic solution was filtered and dried to get a crude product of compound 3. The impurities were separated by column chromatography using a solvent of dichloromethane: tetrahydrofuran = 30:1, 10:1 and 3:1 (v: v). And a yellow powdery solid product compound 3 of 0.325 g was finally obtained.

(3) The synthesis of compound 4

Compound 3 (0.325 g, 0.43 mmol) was dissolved in 10 mL dichloromethane. After stirring for 12 hours, trifluoroacetic acid (5 mL, 67.3 mmol) was added for the cleavage of protection group. Then, the product was rotary evaporated and dried. A volume of 30 mL ethyl acetate was added and dried again. The crude product was separated by column chromatography using a solvent mixture of ethyl acetate: THF: =30:1, 10:1 and 3:1 (v:v) to obtain a yellow powdery solid product compound 4 of 0.22 g finally.

The following was purity analysis of compound 4.

(1) ^{1}H-NMR spectrum of compound 4

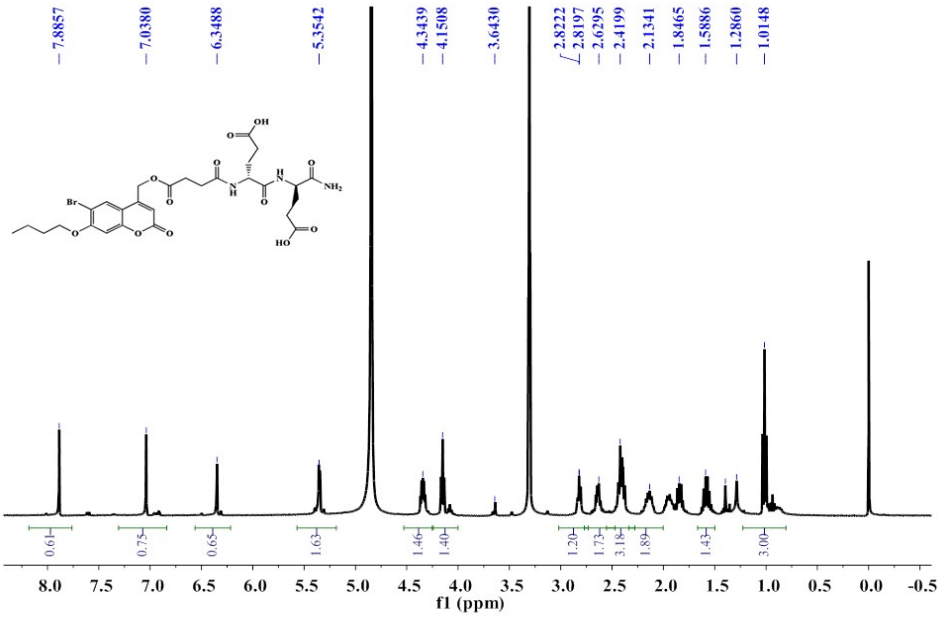

Fig. S2 The ^{1}H-NMR spectrum of compound 4.

^{1}H-NMR (MeOD, 400 MHz)：δ = 1.01(t,C*H*$_3$,3H)，1.28-1.58(m,CH_2,4H)，1.84-2.41(m,CH_2CH_2C=O,8H)，2.62-2.82(m,C=CH_2CH_2C=O,4H)，4.15 (m,CH_2-O-,2H)，4.34 (m,N-CH=O,2H)，5.35 (m,CH-O,2H)，6.34-7.88 (t,ArH,3H).

(2) Mass spectrum of compound 4

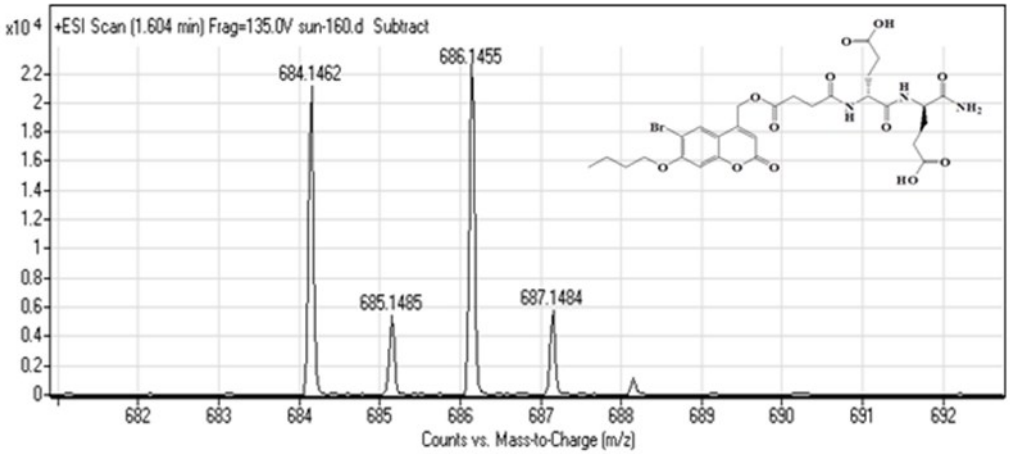

Fig. S3 The MS spectrum of compound 4.

Tab. S1 The MS analysis of compound 4

molecule	Theoretical molecular weights	peaks	
Compound 4	683.13	684.1462	$[M(Br^{79})+2H]^+$
		685.1485	$[M+2H]^+$
		686.1455	$[M(Br^{81})+2H]^+$
		687.1484	$[M(Br^{81})+H]^+$

(4) HPLC of compound 4

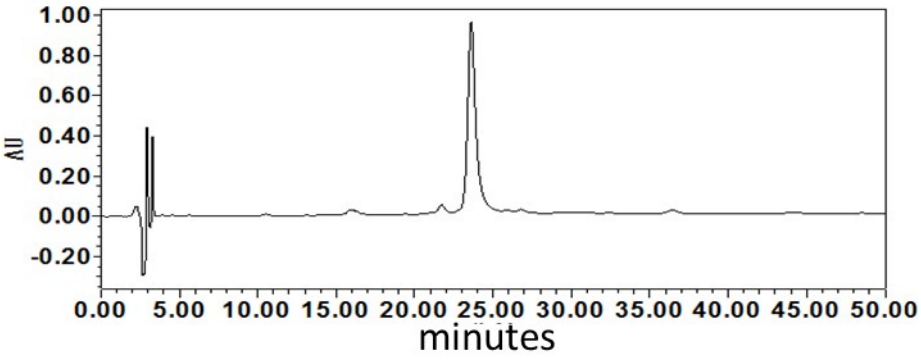

Fig. S4 RP-HPLC of compound 4

As shown in Tab. S1 the theoretical molecular weight of C_4-Bhc-EE-NH_2 corresponds to the detected value. In addition, the purity of the synthesized lipopeptide reached 94% according to the HPLC results.

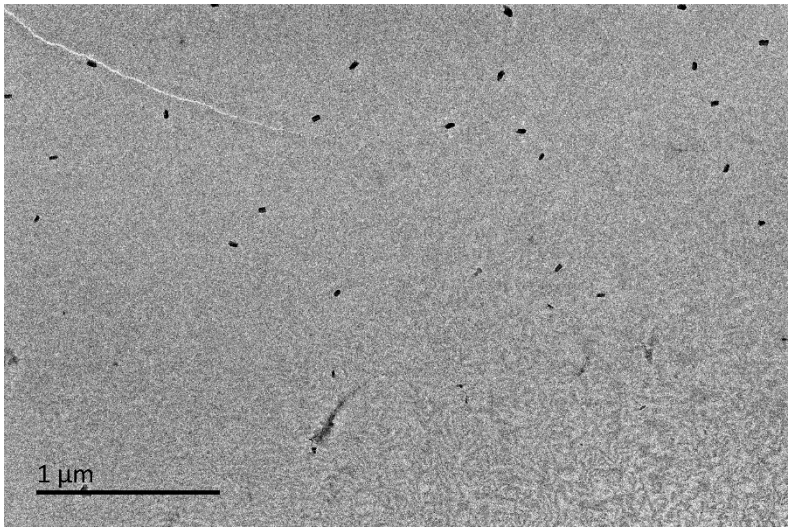

Fig. S5 TEM image of 2 mM C_4-Bhc-Glu-Glu-NH_2.

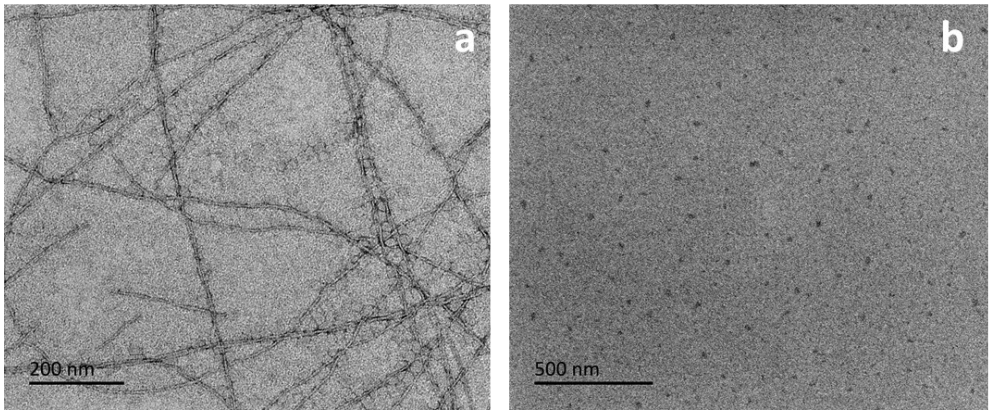

Fig. S6 TEM images of 6 mM C_{14}-Phe-Lys-Lys-NH_2 (a) and 6 mM C_4-Bhc-Glu-Glu-NH_2 (b).

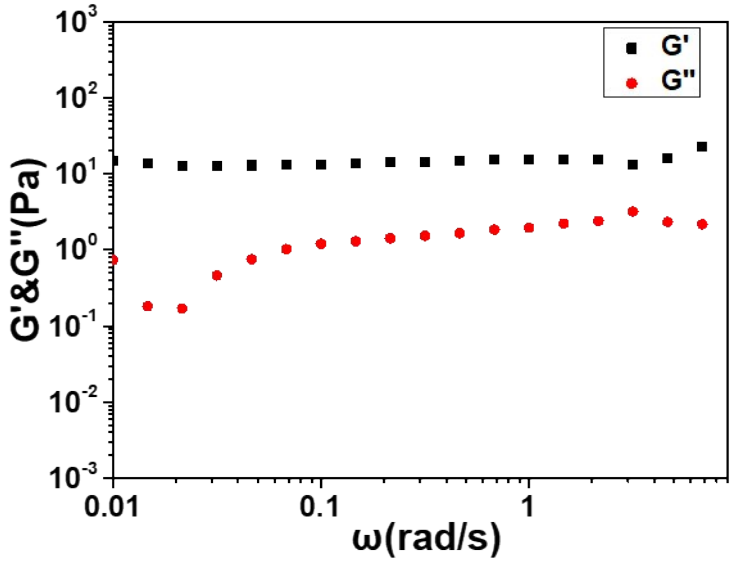

Fig. S7 Viscoelasticity of self-healed 2 mM C_4-Bhc-Glu-Glu-NH_2 / 4 mM C_{14}-Phe-Lys-Lys-NH_2.

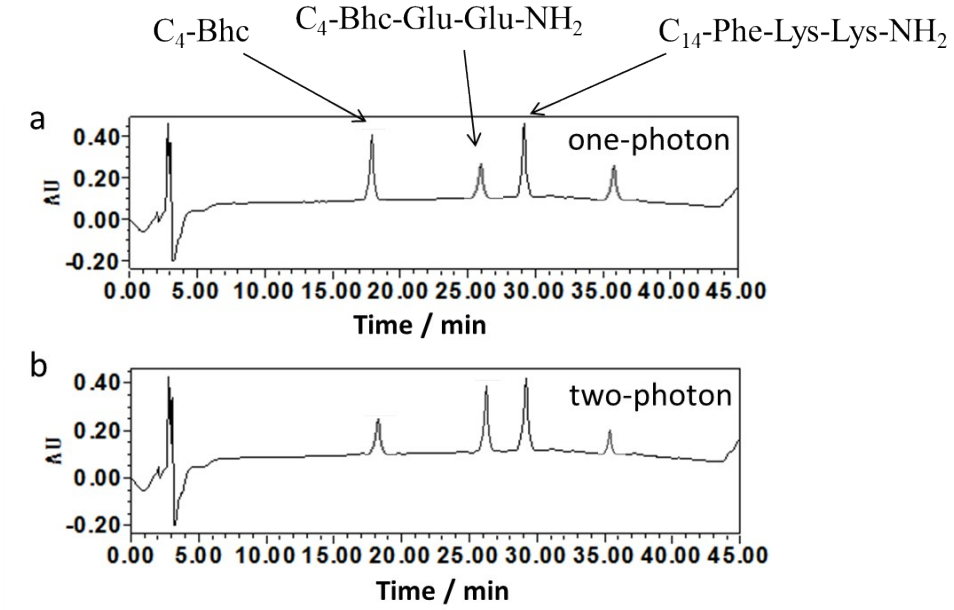

Fig. S8 HPLC measurements of NB after one- and two- photon irradiation

Tab. S2 DOX release amount per unit area at different time

Time (h)	$Time^{0.5}$ ($h^{0.5}$)	Q_{free} ($g \cdot cm^{-2}$)	Q_{NB} ($g \cdot cm^{-2}$)	Q_{NB+UV} ($g \cdot cm^{-2}$)	Q_{NB+NIR} ($g \cdot cm^{-2}$)
0.5	0.71	--	--	--	--
1	1	1.7	0.48	--	--
2	1.41	2.3	0.75	0.96	1.19
3	1.73	2.9	0.92	1.32	1.45
4	2	3.2	1.16	1.61	1.78
6	2.45	--	1.3	2.01	2.15

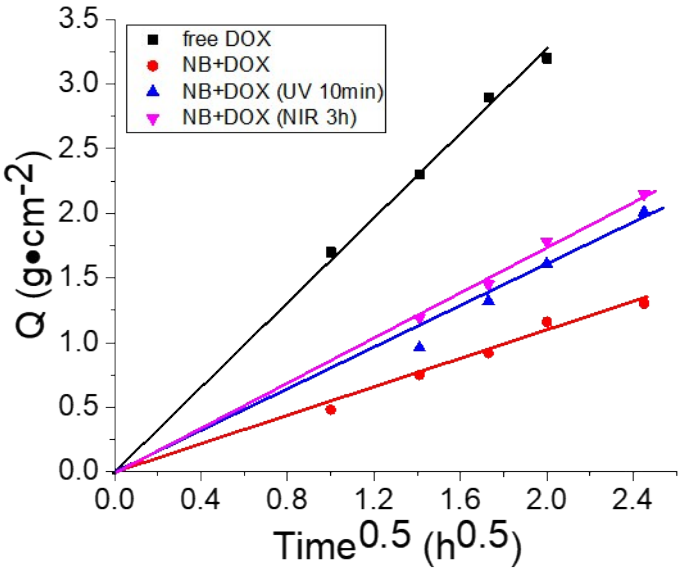

Fig. S9 DOX released per unit area versus $t^{0.5}$.

Tab. S3 BSA release amount per unit area at different time

Time (h)	$Time^{0.5}$ ($h^{0.5}$)	Q_{free} ($g \cdot cm^{-2}$)	Q_{NB+UV} ($g \cdot cm^{-2}$)	Q_{NB+NIR} ($g \cdot cm^{-2}$)
0.5	0.71	--	--	--
1	1	2.03	--	--
2	1.41	2.96	2.46	2.36
3	1.73	3.69	3.03	2.84
4	2	4.47	3.73	3.06
6	2.45	5.32	4.36	4.06
8	2.83	--	5.05	4.85

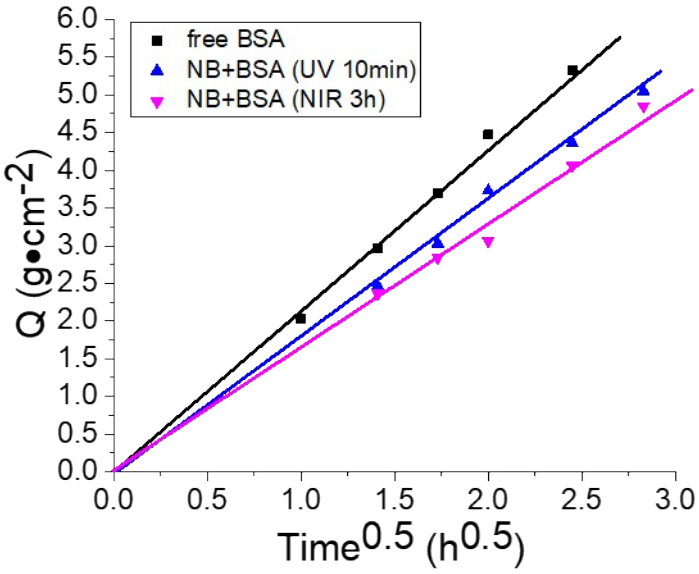

Fig. S10 BSA released per unit area versus $t^{0.5}$.

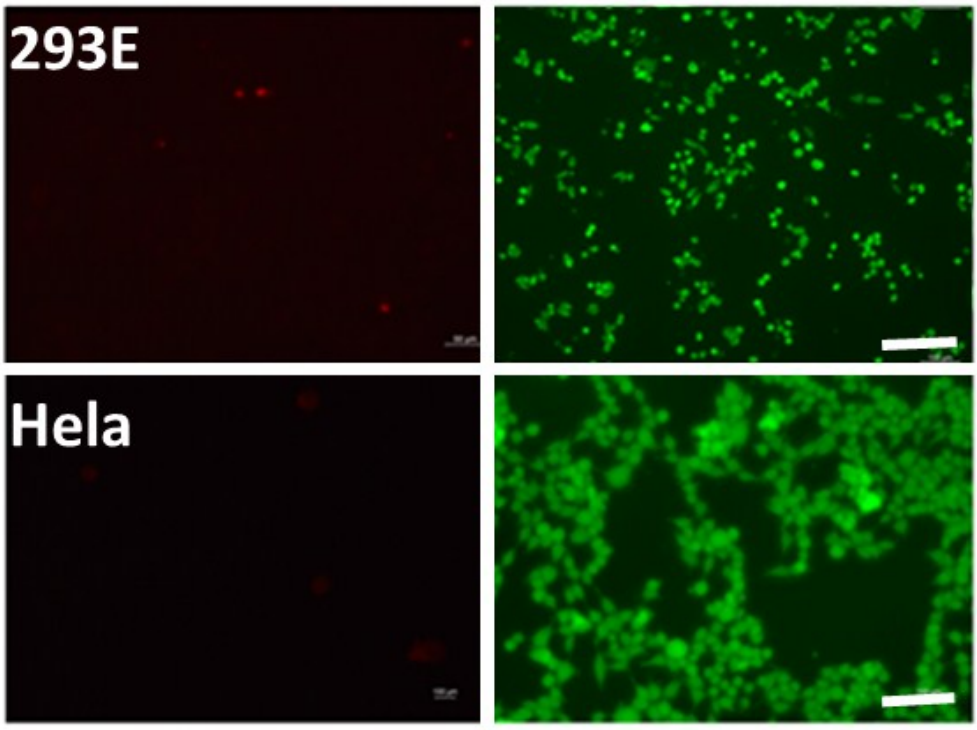

Fig. S11 Living/dead cell dual-staining assay for 2 mM C_4-Bhc-Glu-Glu-NH_2 / 4 mM C_{16}-Phe-Lys-Lys-NH_2 NB biomaterial without DOX after 48 h. Scale bar is 200 μm.

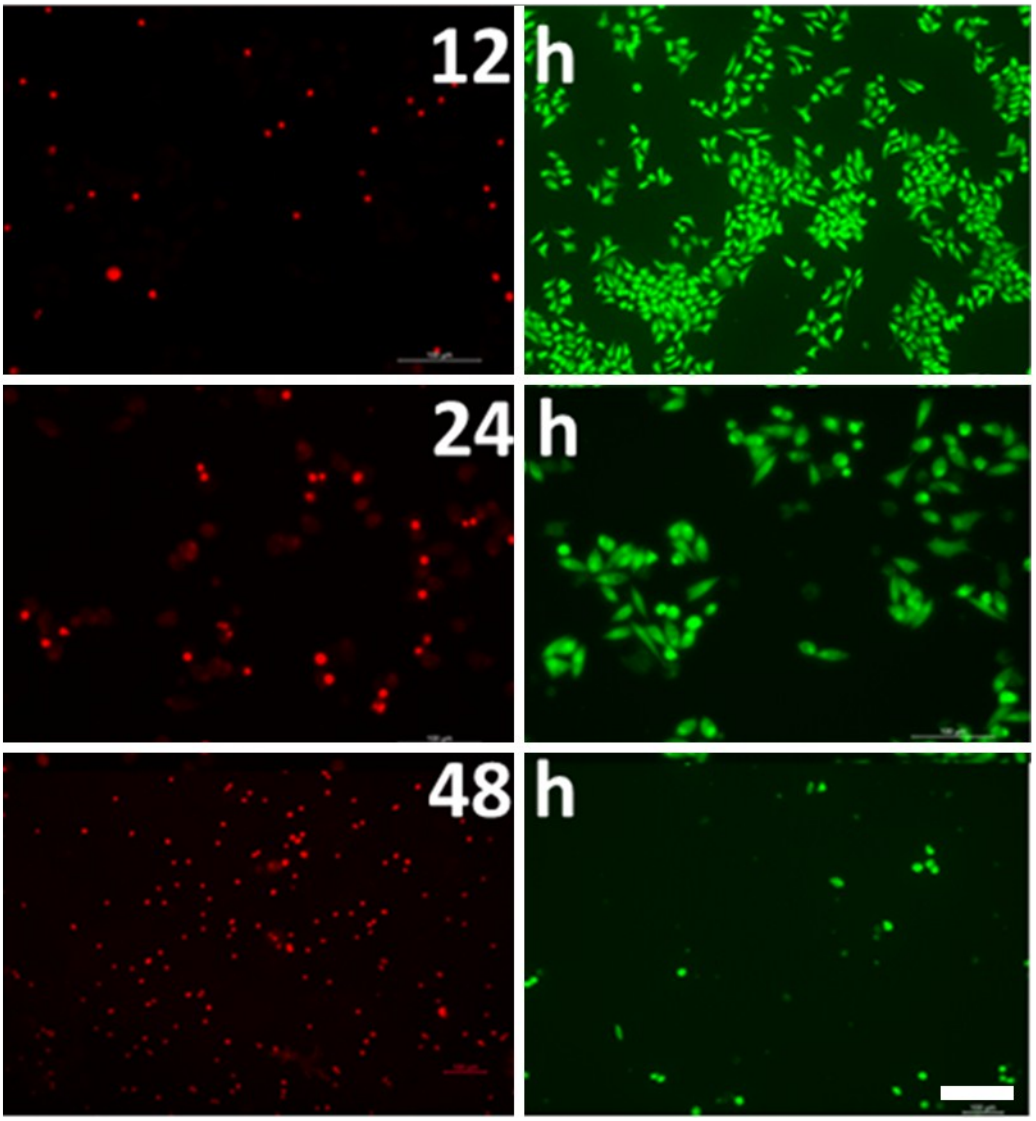

Fig. S12 Living/dead cell dual-staining assay for 2 mM C_4-Bhc-Glu-Glu-NH_2 / 4 mM C_{16}-Phe-Lys-Lys-NH_2 NB biomaterial with DOX. Scale bar is 200 μm.

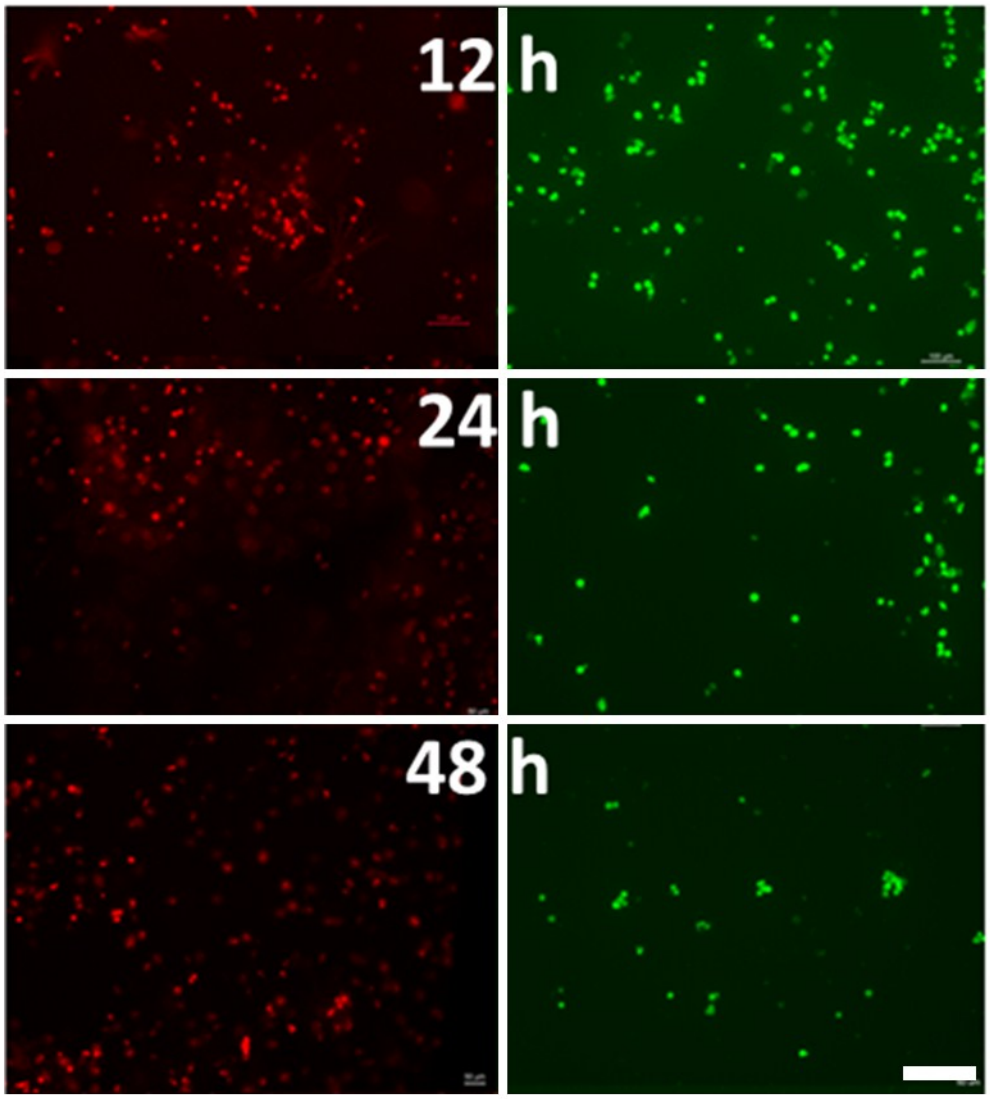

Fig. S13 Living/dead cell dual-staining assay for 2 mM C_4-Bhc-Glu-Glu-NH_2 / 4 mM C_{16}-Phe-Lys-Lys-NH_2 NB biomaterial with DOX after one-photon irradiation. Scale bar is 200 μm.

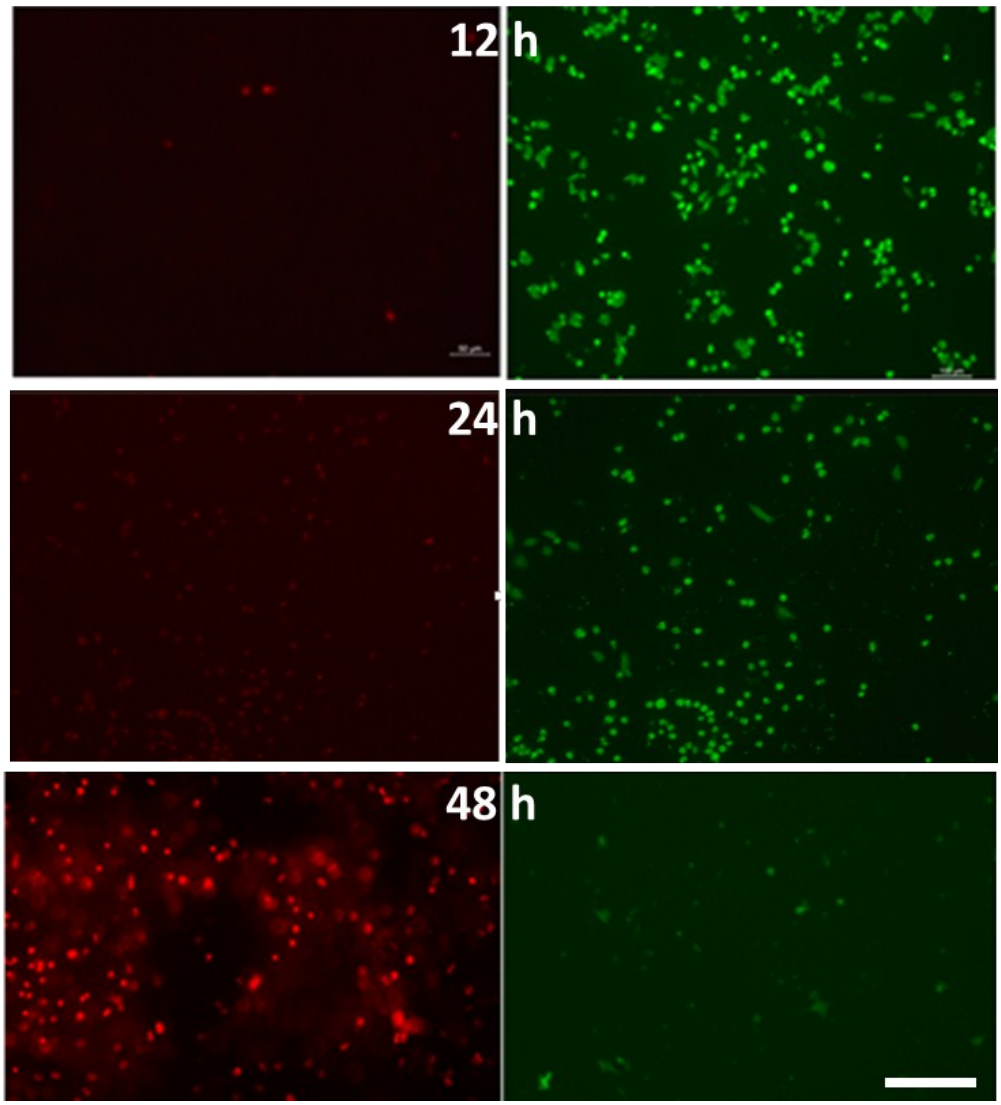

Fig. S14 Living/dead cell dual-staining assay for 2 mM C_4-Bhc-Glu-Glu-NH_2 / 4 mM C_{16}-Phe-Lys-Lys-NH_2 NB biomaterial with DOX after two-photon irradiation. Scale bar is 200 μm.