Supplementary Materials for

A bioactivated *in vivo* assembly nanotechnology fabricated NIR probe for small pancreatic tumor intraoperative imaging

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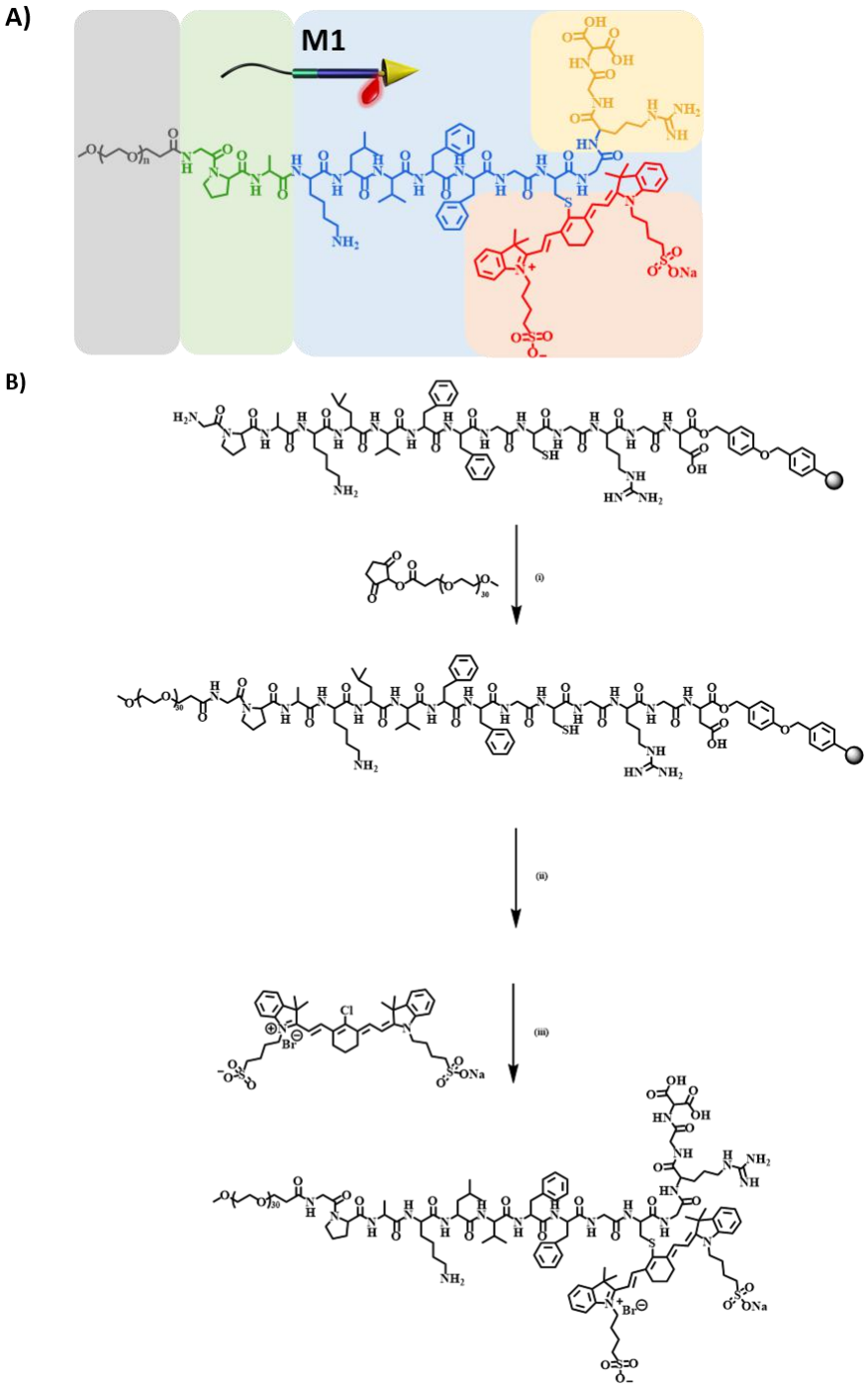
Supplementary Table 1 and Figs. 1-28

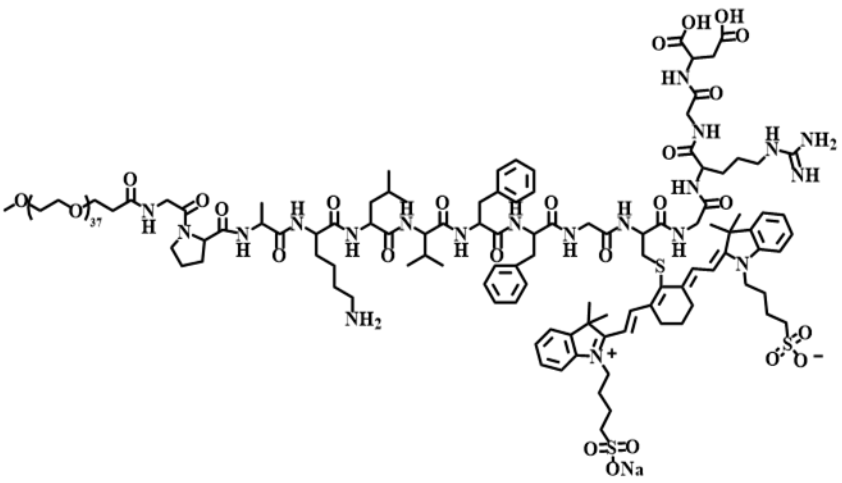
Content

Supplementary Table 1 | Secondary structure proportions of **M1**, **M3** and **R-M1** calculated by Reed's Reference based on CD spectra.

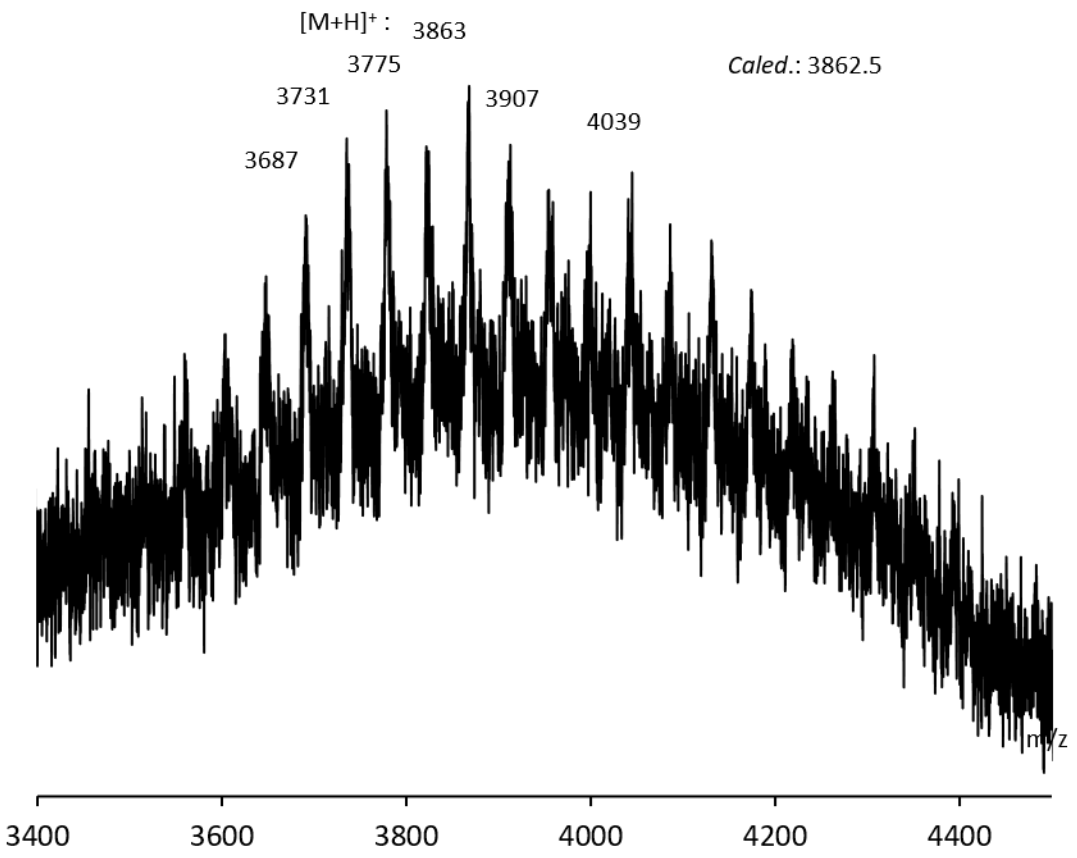
	M1	M3	R-M1
Helix	0	45	0.5
Beta	32.2	38.7	72
Turn	1.7	0	27.5
Random	66.1	16.3	0
RMS	14.093	5.66	23.908



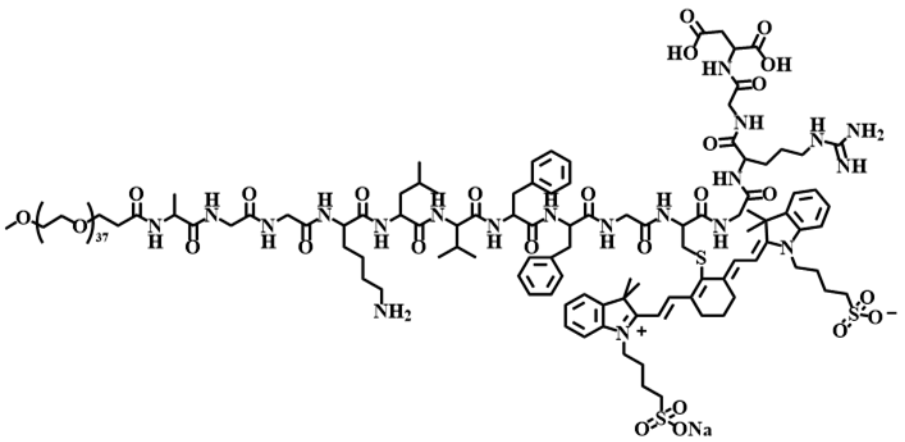
Supplementary Fig. 1 | A) Chemical structure of **M1**: mPEG-GPAKLVFFGC(IR783)GRGD. B) Synthesis of **M1**: (i). 5% NMM/DMF, 10equiv. HBTU, pH9.0, 24h. (ii). TFA/TIPS/1,2-ethanedithiol/Diwater (92.5%/2.5%/2.5%/2.5%, v/v), stirring, ice bath, 3h. (iii). Tris buffer, pH8.5, 12h.



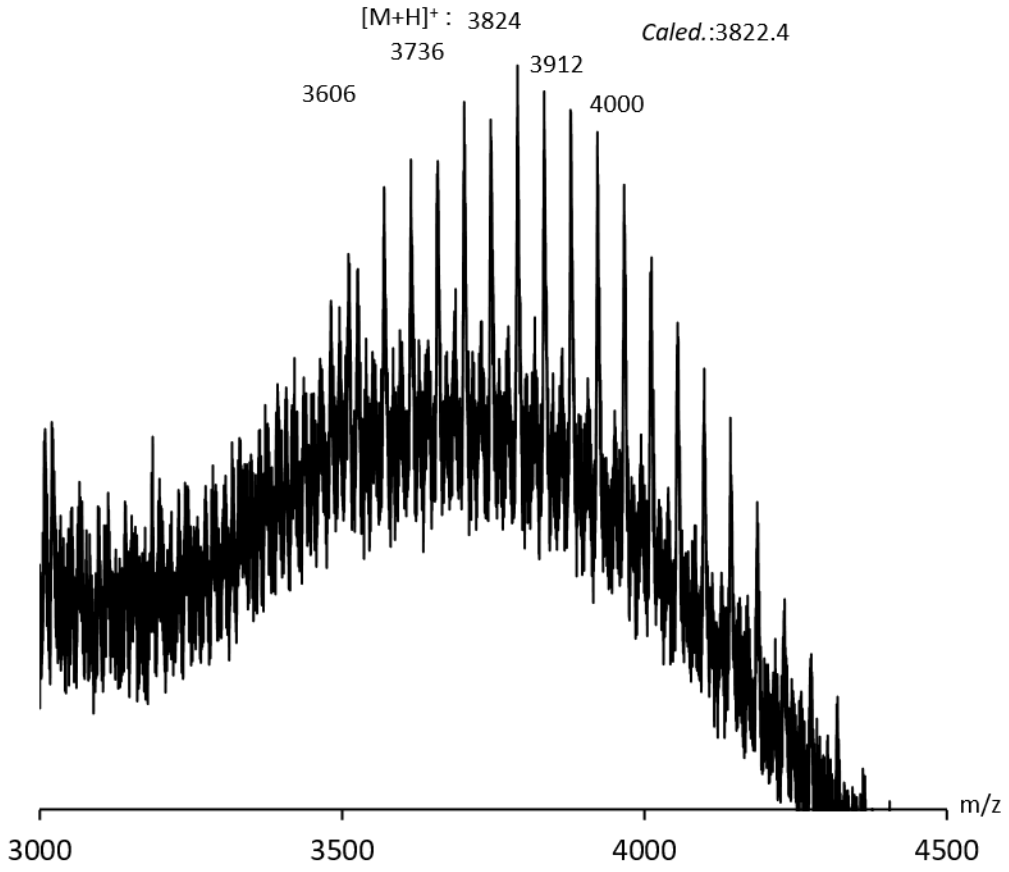
M1 : mPEG-GPAKLVFFGC(IR783)GRGD



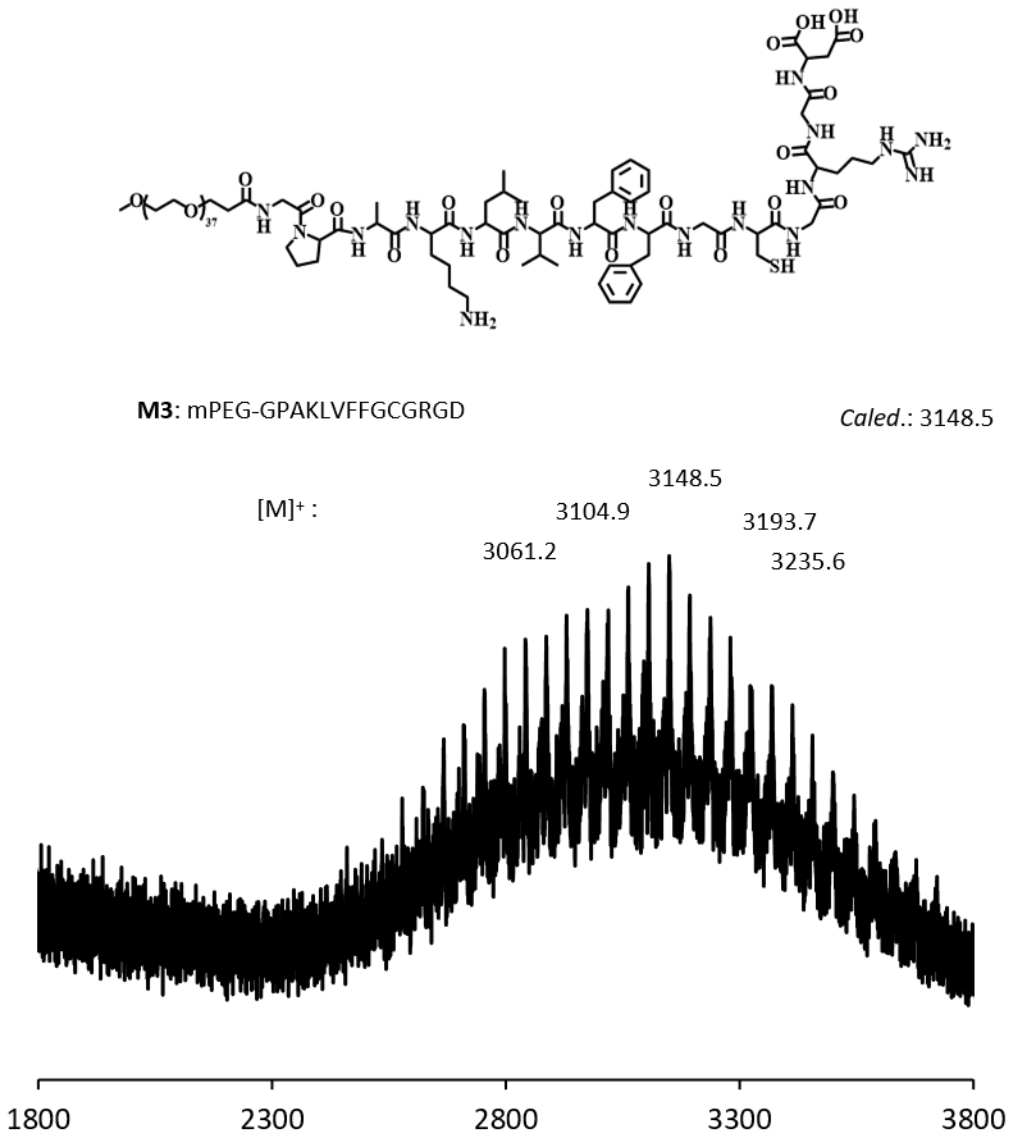
Supplementary Fig. 2 | Chemical structure and MALDI-TOF mass spectra of **M1**: mPEG-GPAKLVFFGC(IR_{783})GRGD.



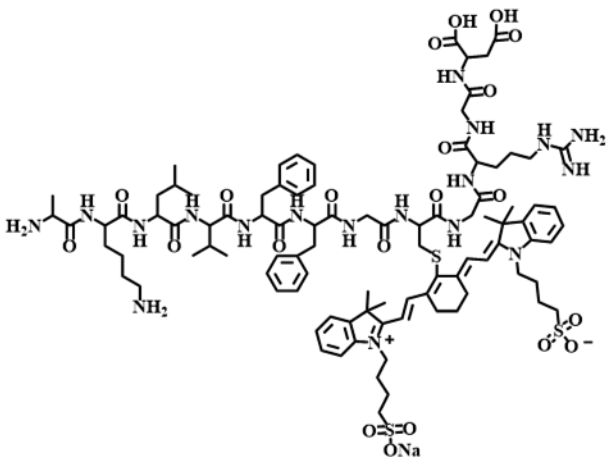
M2: mPEG-AGGKLVFFGC(IR783)GRGD

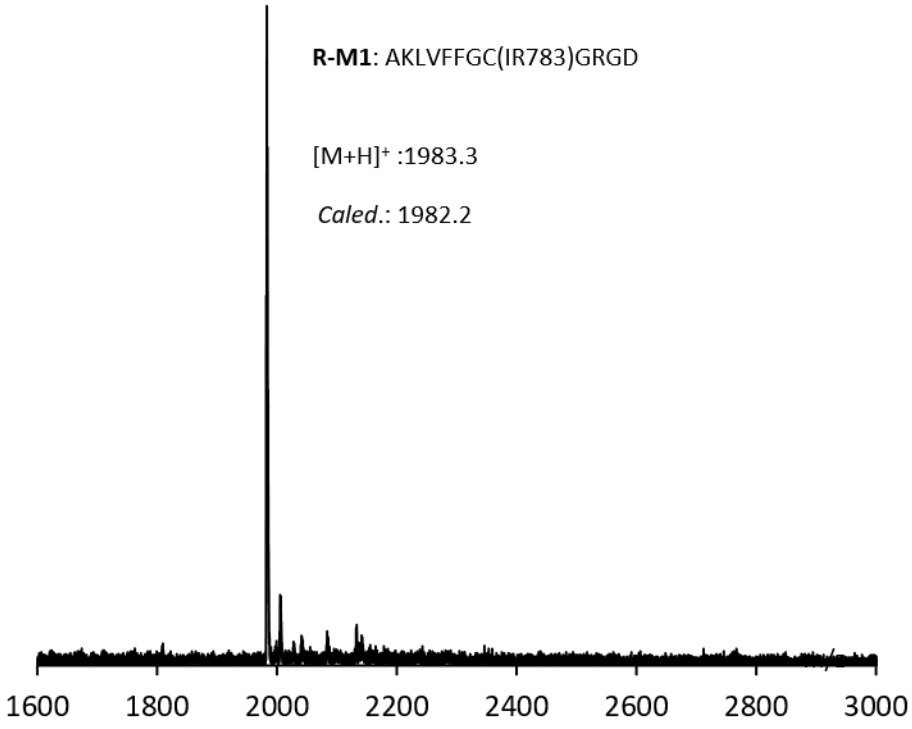


Supplementary Fig. 3 | Chemical structure and MALDI-TOF mass spectra of **M2**: mPEG-AGGKLVFFGC(IR_{783})GRGD.

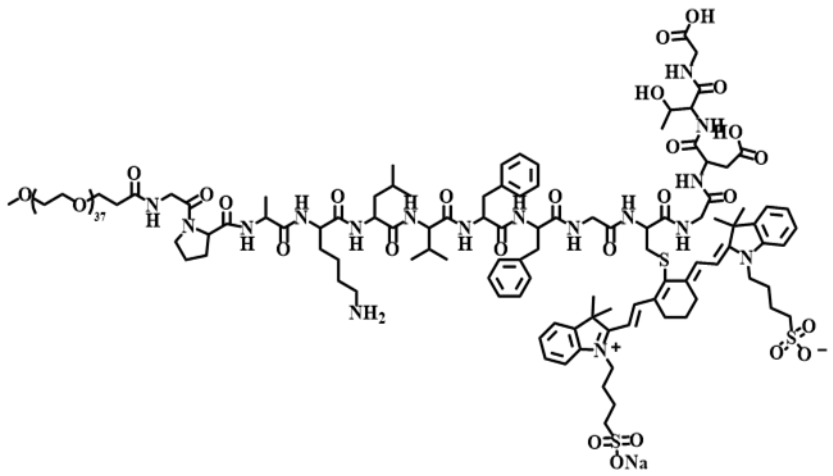


Supplementary Fig. 4 | **Chemical** structure and MALDI-TOF mass spectra of **M3**: mPEG-GPAKLVFFGCGRGD.

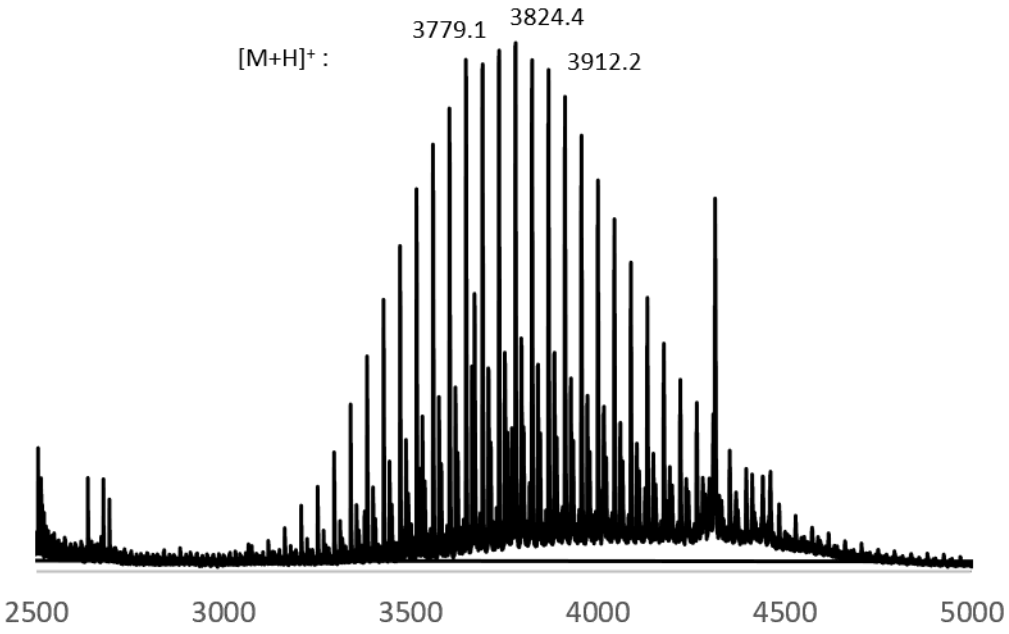




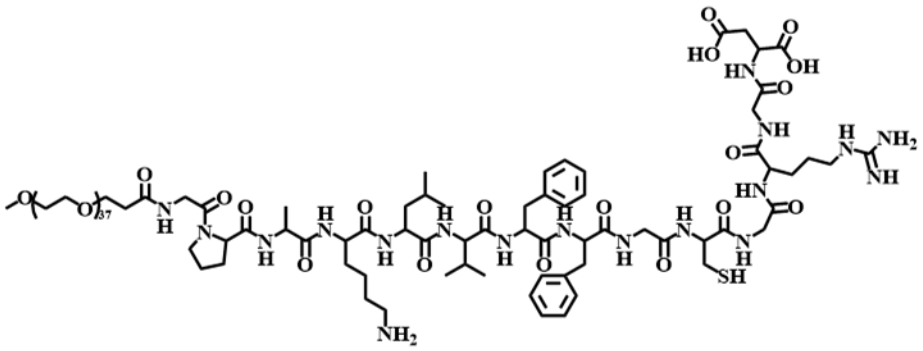
Supplementary Fig. 5 | **Chemical** structure and MALDI-TOF mass spectra of **R-M1**: AKLVFFGC(IR_{783})GRGD.



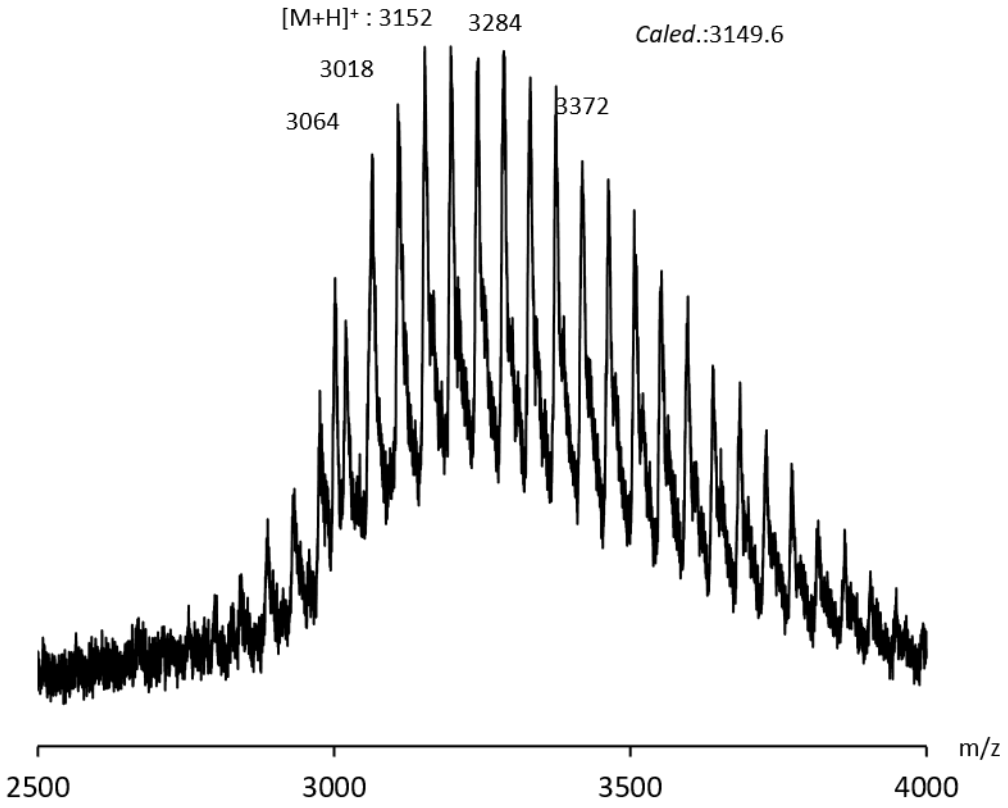
M4: mPEG-GPAKLVFFGC(IR783)GDTG *Caled*.: 3823.1



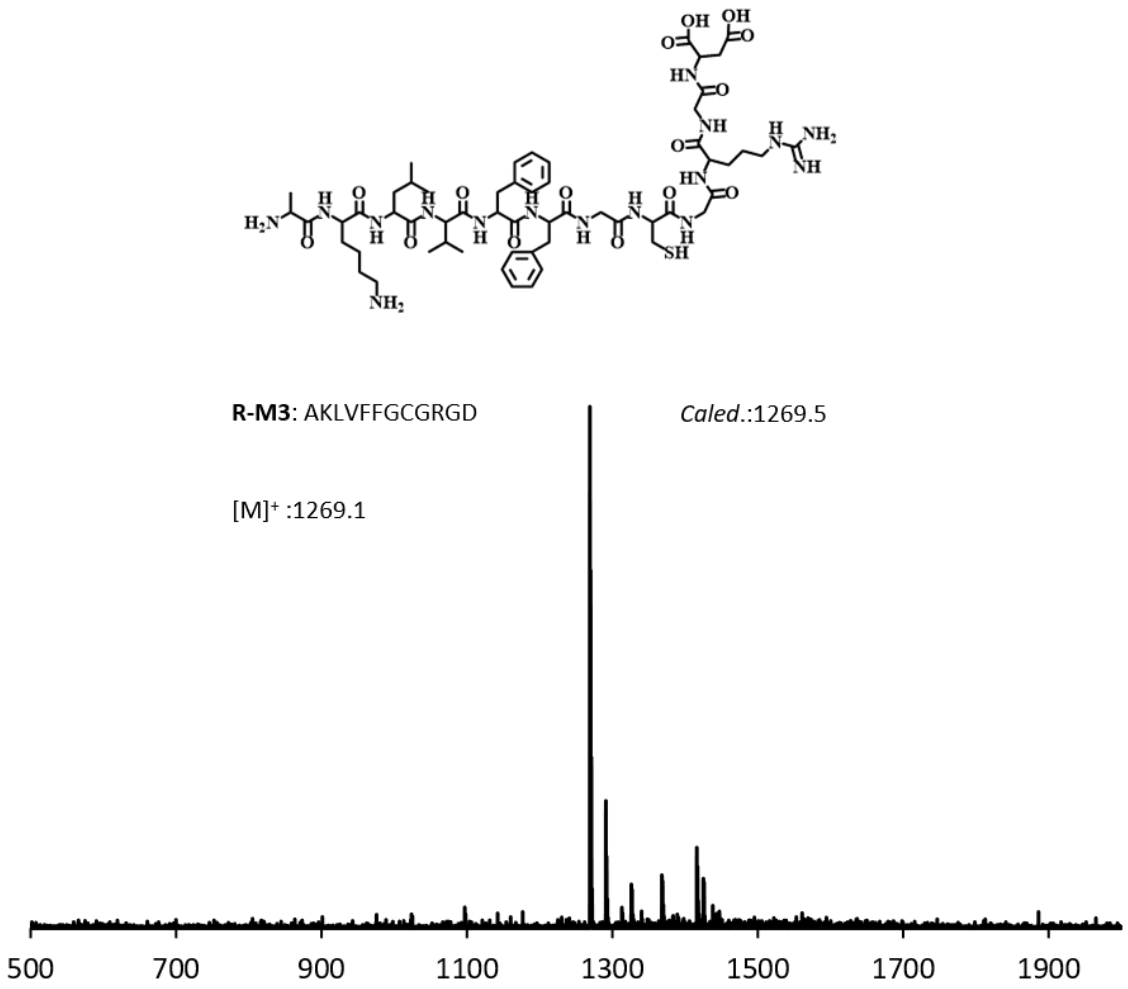
Supplementary Fig. 6 | Chemical structure and MALDI-TOF mass spectra of **M4**: mPEG-GPAKLVFFGC(IR_{783})GDTG.



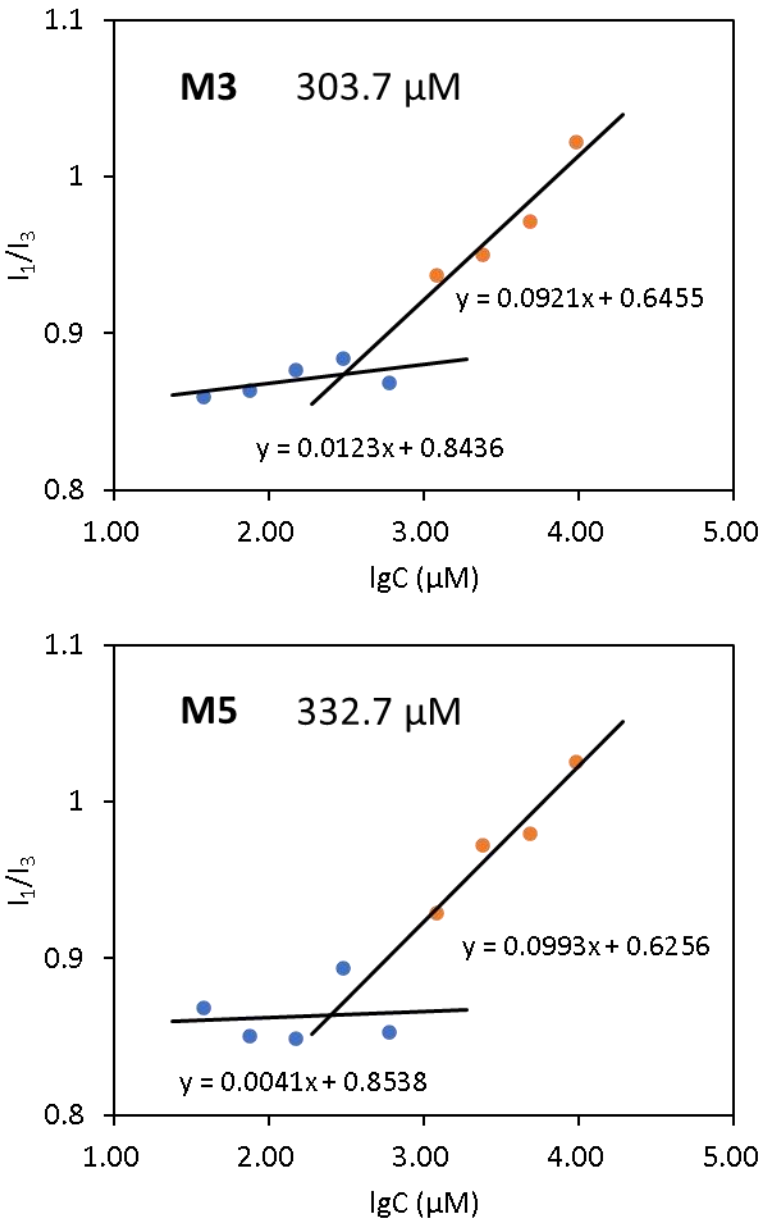
M5: : mPEG-AGGKLVFFGCGRGD



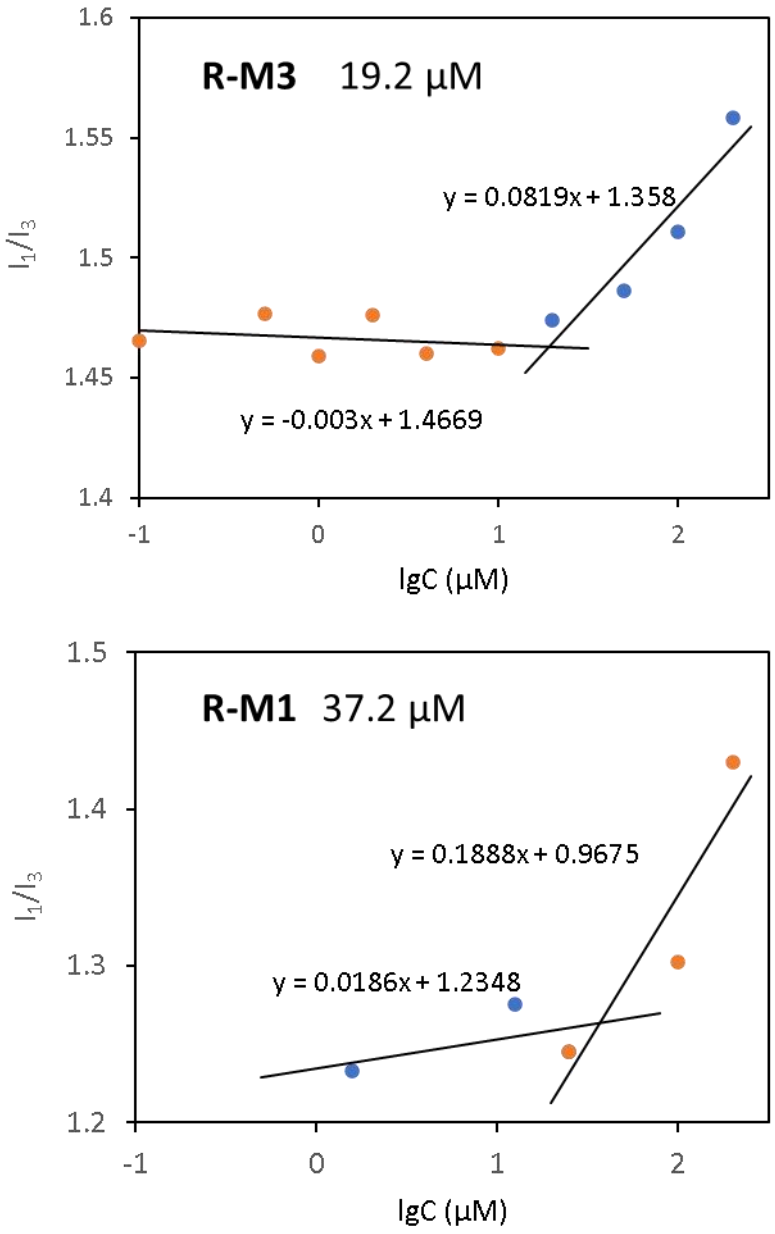
Supplementary Fig. 7 | Chemical structure and MALDI-TOF mass spectra of **M5**: mPEG-AGGKLVFFGCGRGD.



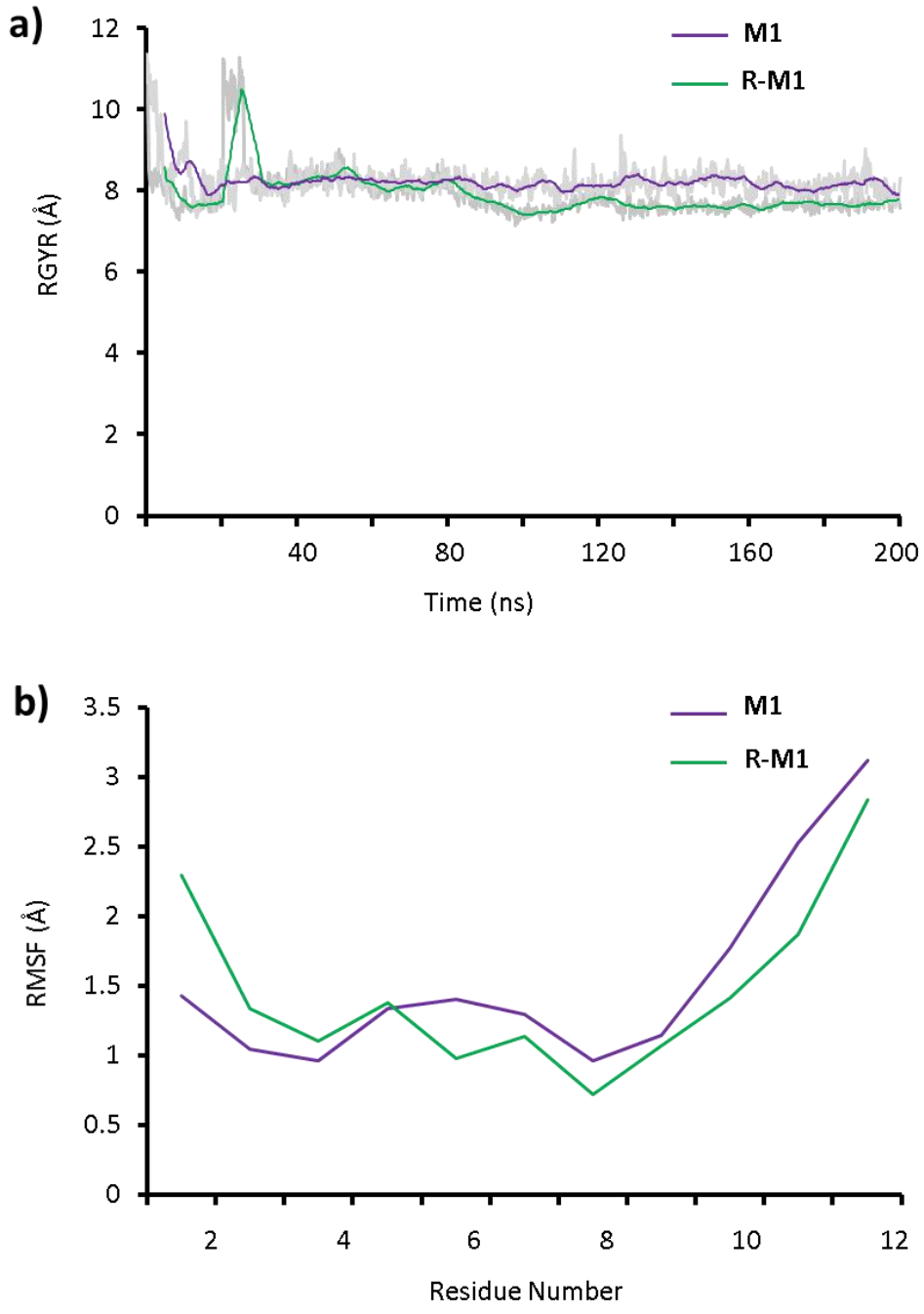
Supplementary Fig. 8 | Chemical structure and MALDI-TOF mass spectra of **R-M3**: AKLVFFGCGRGD.



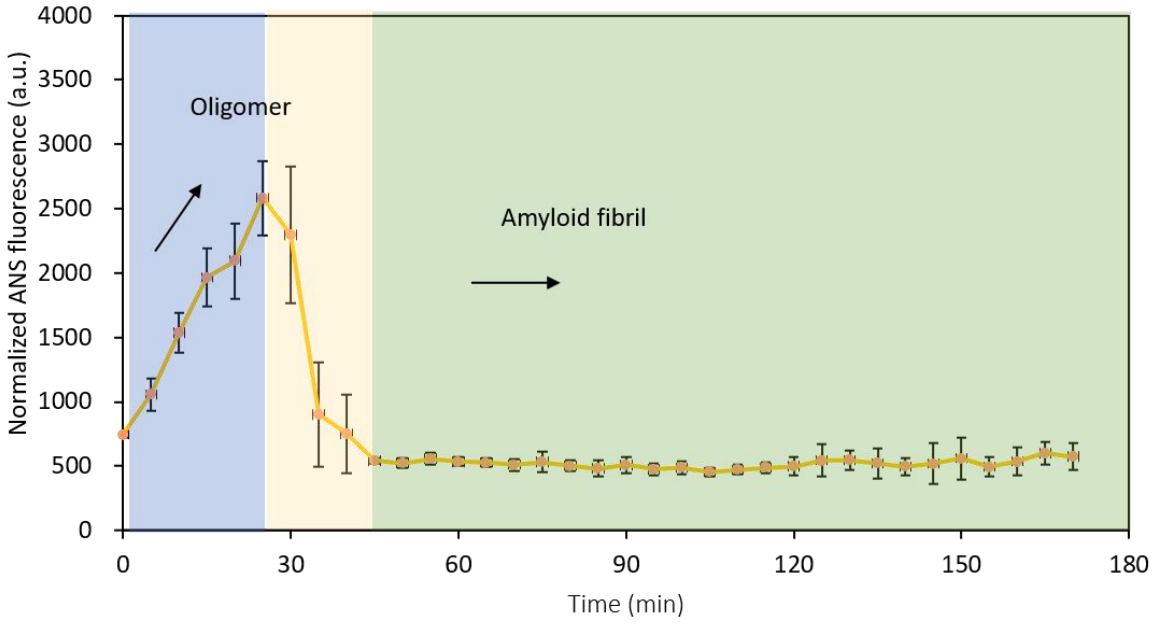
Supplementary Fig. 9 | The critical aggregation concentration (CAC) of **M3** and **M5** was measured by using pyrene as a probe. The ratio of peak I_1 to peak I_3 can be obtained by measuring the fluorescence of pyrene in different concentrations of molecules. Experiments were repeated three times. The x axis is logarithm concentration and the y axis is the ratio of peak intensities of 374 nm and 383 nm. Ex= 334 nm.



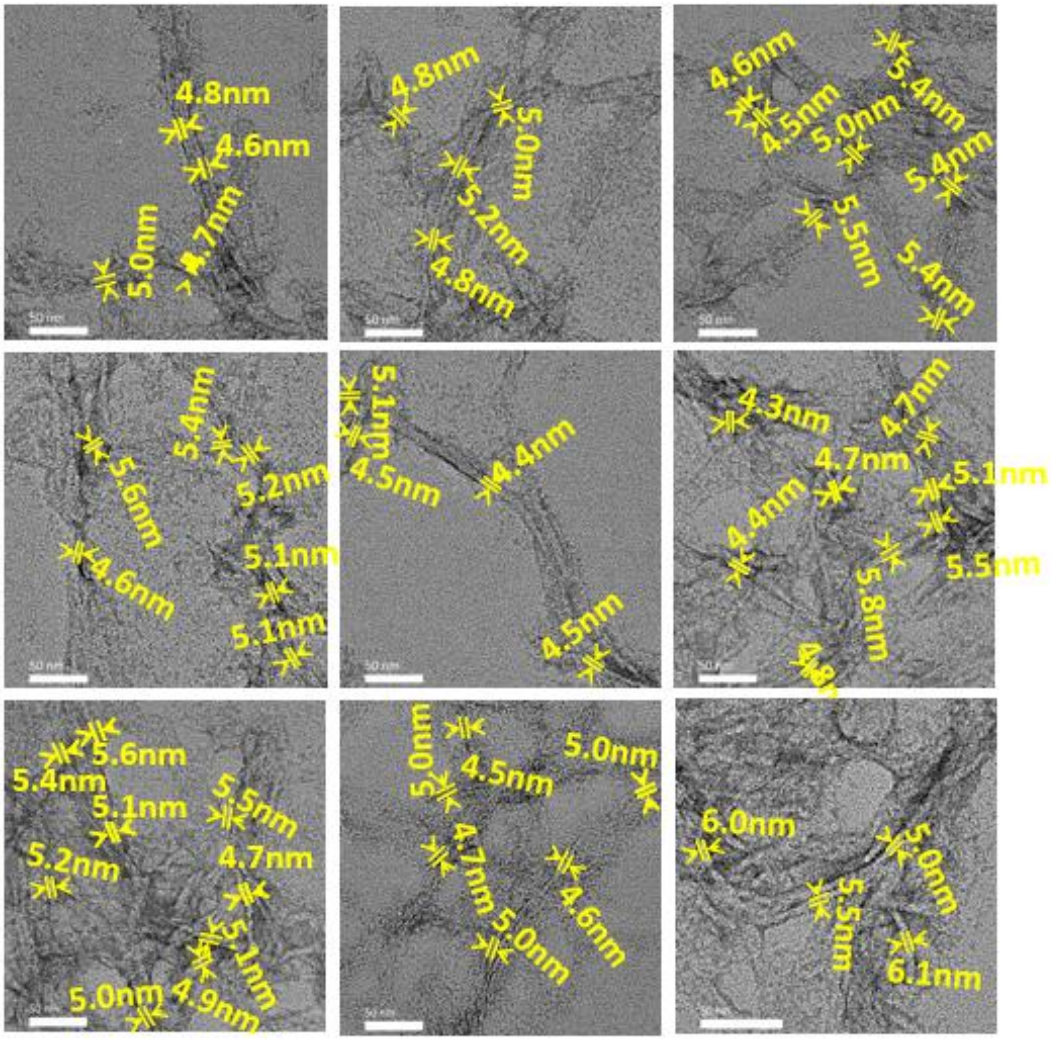
Supplementary Fig. 10 | The critical aggregation concentration (CAC) of **R-M3** and **R-M1** was measured by using pyrene as a probe. The ratio of peak I_1 to peak I_3 can be obtained by measuring the fluorescence of pyrene in different concentrations of molecules. To figure out the CAC of the molecule. Experiments were repeated three times. The x axis is logarithm concentration and the y axis is the ratio of peak intensities of 374 nm and 383 nm. Ex= 334 nm.



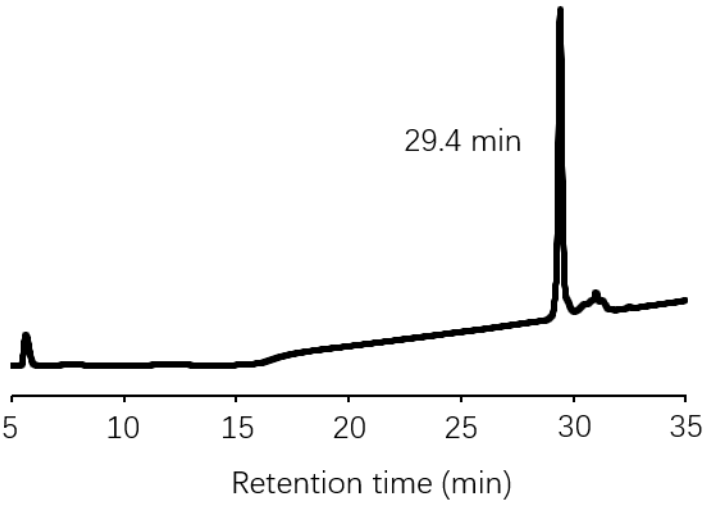
Supplementary Fig. 11 | The radius of gyration (RGYR) (a) and root mean square fluctuation (RMSF) (b) of AKLVFFGCGRGD sequences of **M1** and **R-M1**.



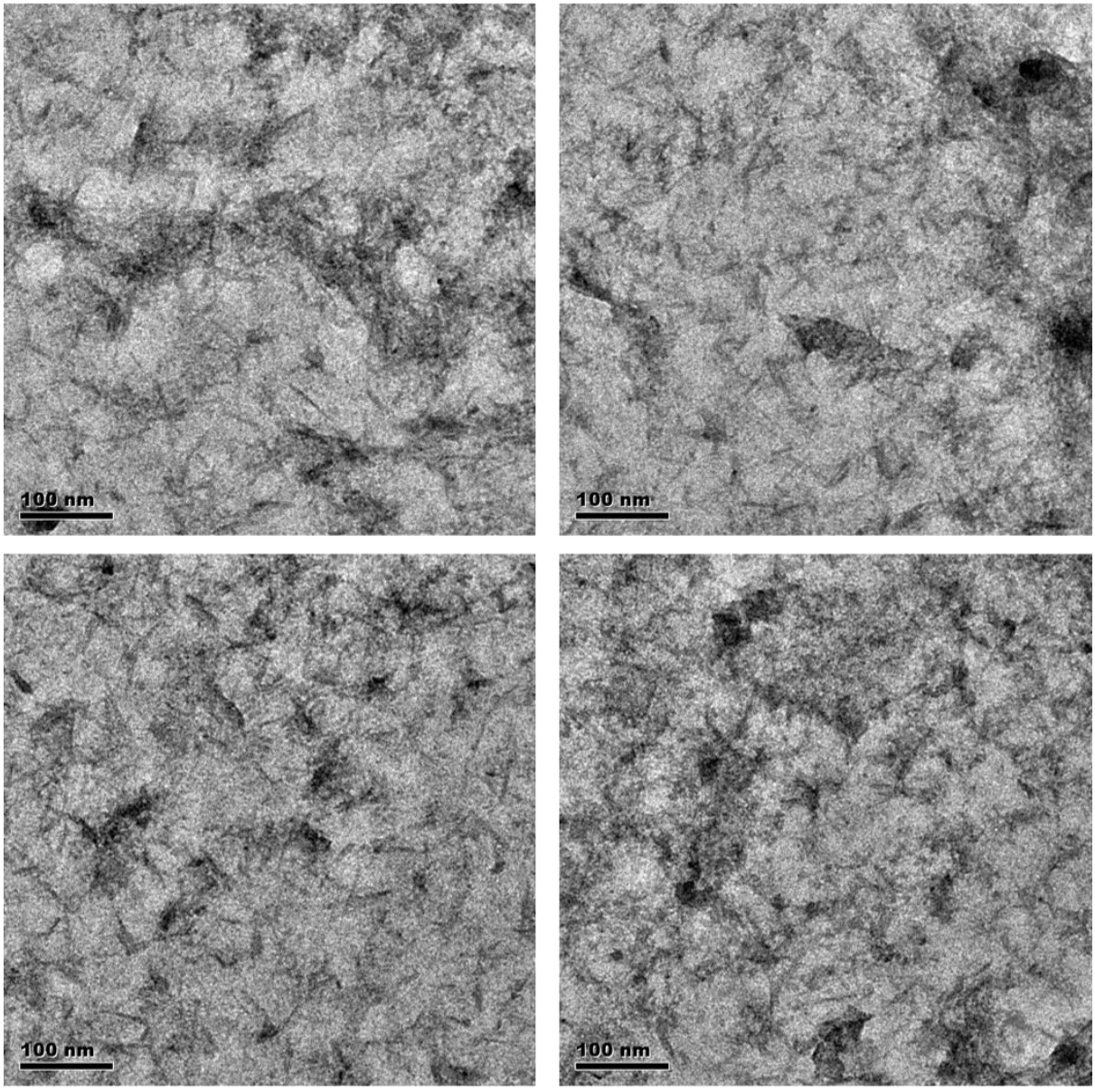
Supplementary Fig. 12 | The ANS staining dynamic fibril growth curve of **R-M1**. ANS was added to an aqueous solution of **R-M1** (100 μM) and fluorescence was measured immediately. The same experiment was carried out four times. The mean of results of four parallel experiments is shown and data were expressed as mean ± SD (n=4).



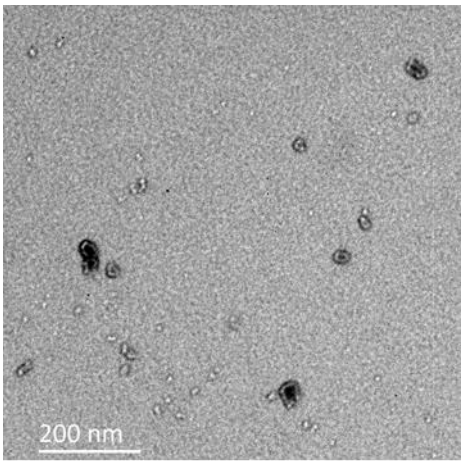
Supplementary Fig. 13 | The TEM image of self-assembled β-sheet nanofibers of **M1** (100 μM) after tailoring by FAP-α (50 μM) in buffer for 12 h. Scale bar = 50 nm.



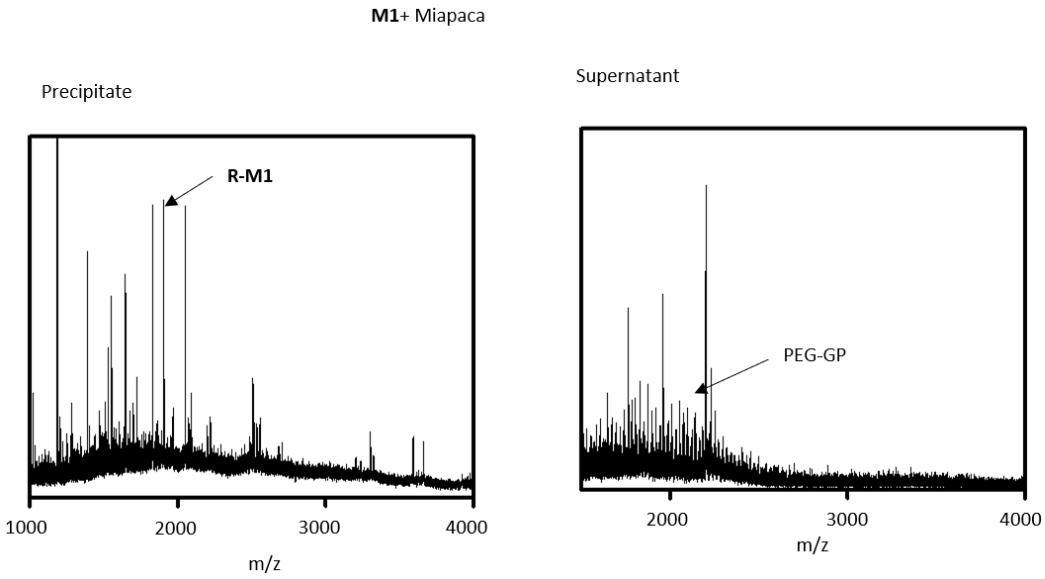
Supplementary Fig. 14 | HPLC curve of **M3** co-incubated with inactivated FAP-α for 12h. The FAP-α was heat to 1 h to inactivate in advanced. HPLC: C18 column and a linear gradient of acetonitrile/water with 0.1% TFA from 10 %/ 90 % to 50 %/ 50 % and a flow speed of 1 mL/min in 35 min at 25 °C.



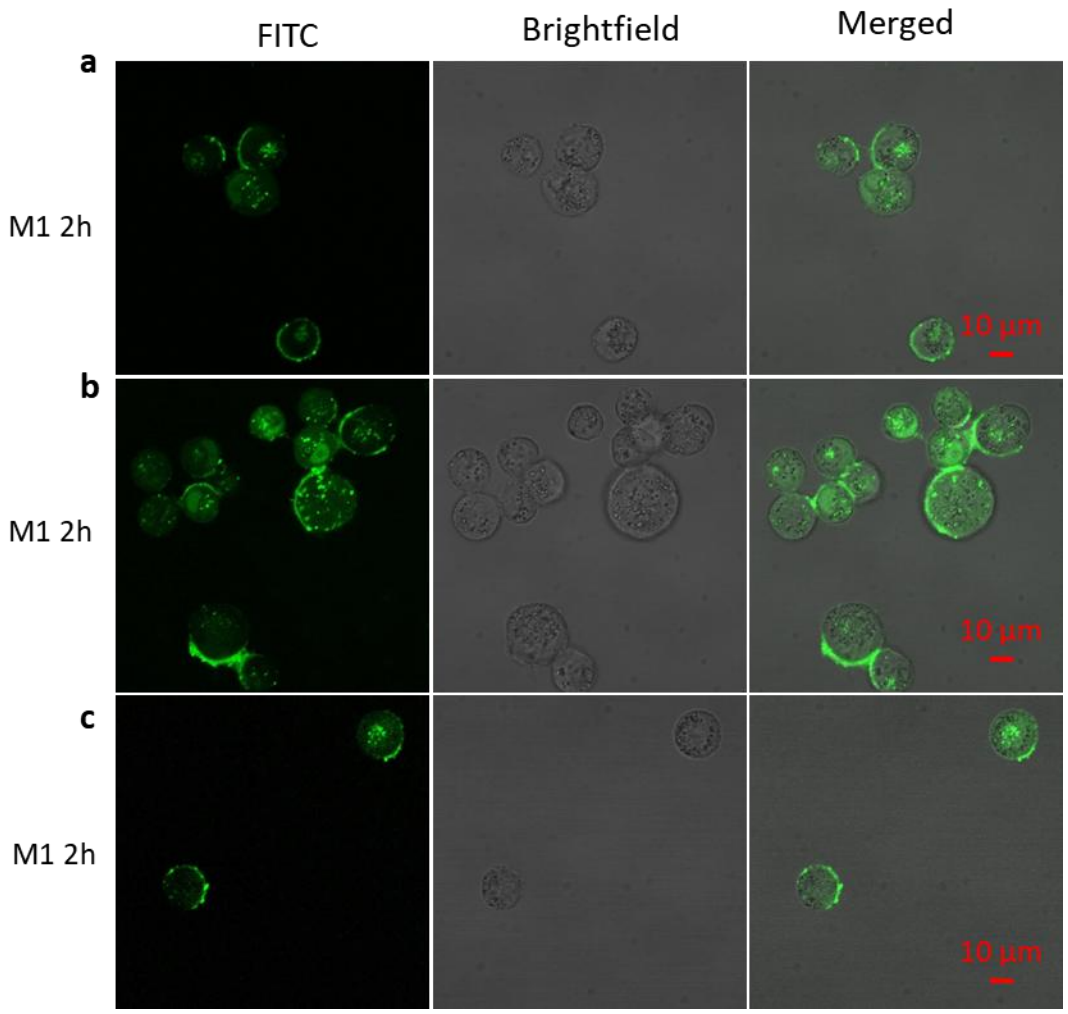
Supplementary Fig. 15 | Representative TEM image of four independ experiment of **M1** (100 μM) co-incubated with Miapaca-2 cell lysis for 12 h. Scale bar: 100 nm.



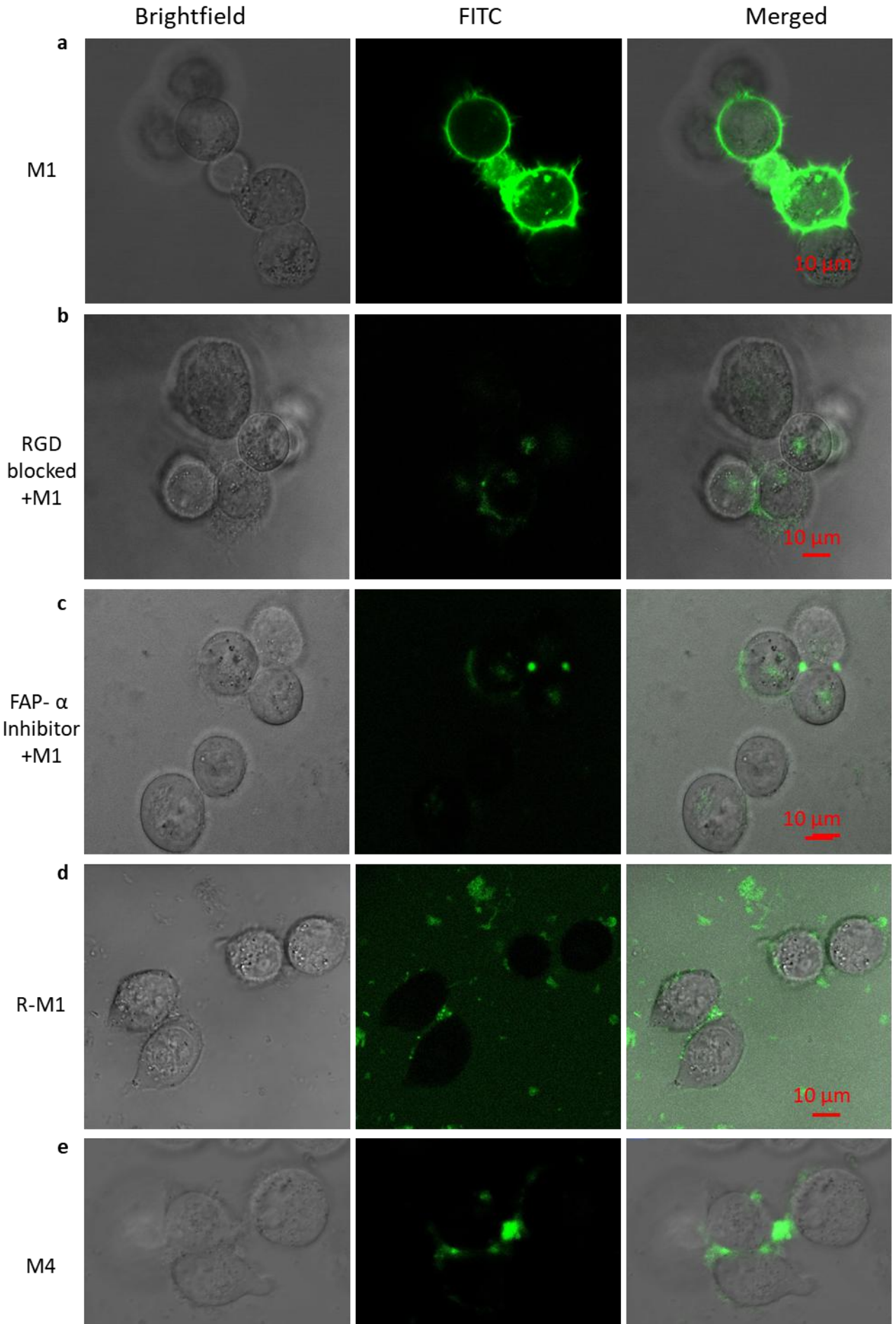
Supplementary Fig. 16 | TEM image of **M1** (100 μM) co-incubated with inactivated FAP-α (50 μM) for 12h. The FAP-α was heat to 1 h to inactivate in advanced. Scale bar: 200 nm.



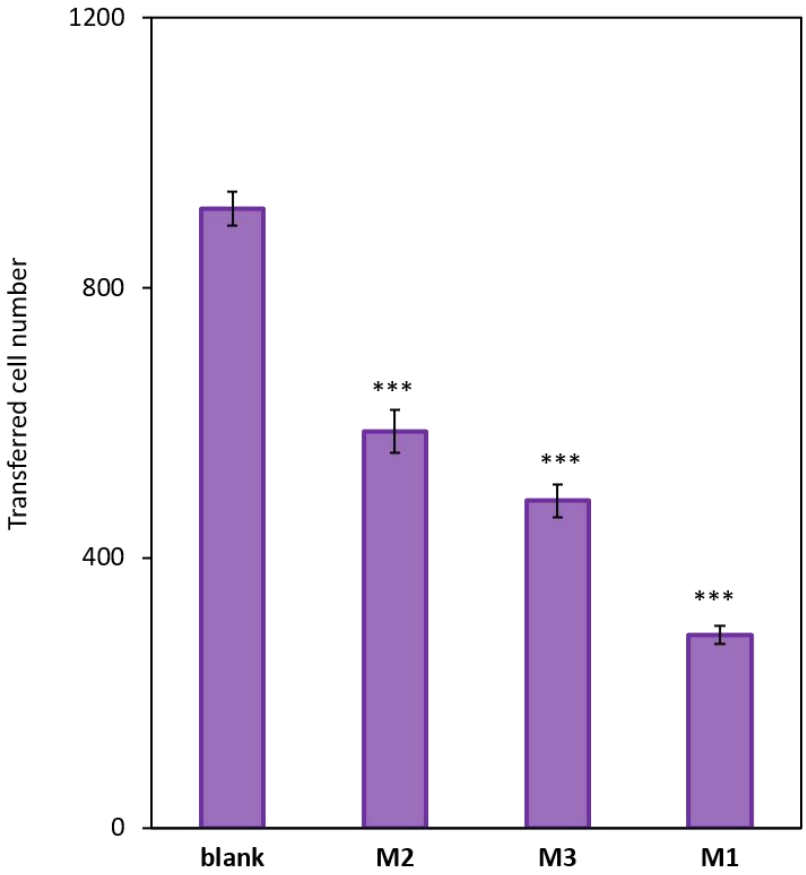
Supplementary Fig. 17 | MALDI-TOF mass spectra of **M1** co-incubated with Miapaca-2 cell lysis for 2 h.



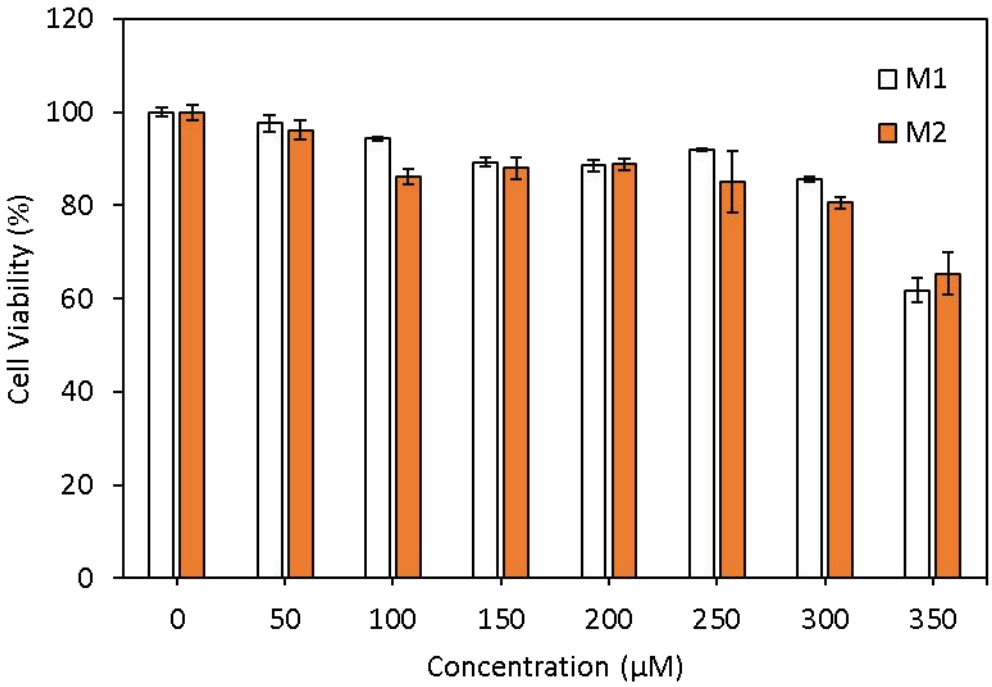
Supplementary Fig. 18 | Representative confocal images in three independ experiment of **M1** (100 μM) with BIVA effect on Miapaca-2 cells after incubation for 2 h. Scale bar: 10 μm.


Brightfield
FITC
Merged
a
M1
10 µm
b
RGD blocked +M1
10 µm
c
FAP- α Inhibitor +M1
10 µm
d
R-M1
10 µm
e
M4

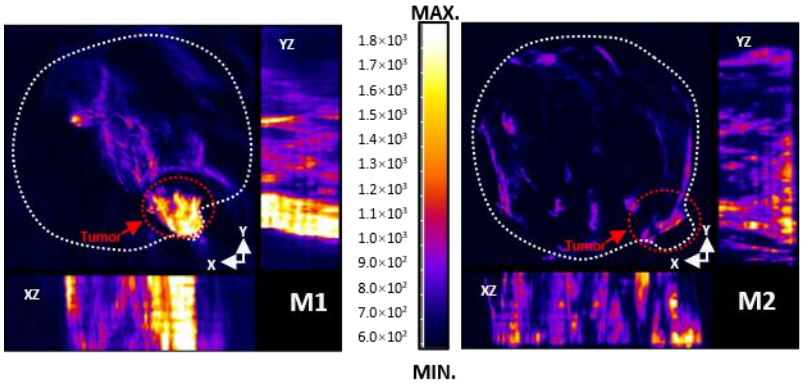
Supplementary Fig. 19 | CLSM images of Miapaca-2 cells treated with different molecules or **M1** under different conditions. All the molecules are labelled with FITC. **a,** CLSM images of **M1** with BIVA effect on Miapaca-2 cells after incubation for 1 h. **b, M1** incubated av integrins blocked Miapaca-2 cells for 1 h and washed by fresh DMEM for three times (Miapaca-2 cells ware treated by RGD for 1h at 50 μM and washed by fresh DMEM for three times before adding **M1**). **c, M1** incubated Fap-α inhibited Miapaca-2 cells for 1h and washed by fresh DMEM for three times (Miapaca-2 cells ware treated by Fap-α inhibitor Ac-Gly-BoroPro for 1h at 50 nM and wahed by fresh DMEM for three times before adding **M1**). d, **R-M1** incubated Miapaca-2 cells after incubation for 1 h without washing. **e, M4** incubated Miapaca-2 cells after incubation for 1 h and washed by fresh DMEM for three times. Green: FITC labelled molecules. Scale bar: 10 μm.



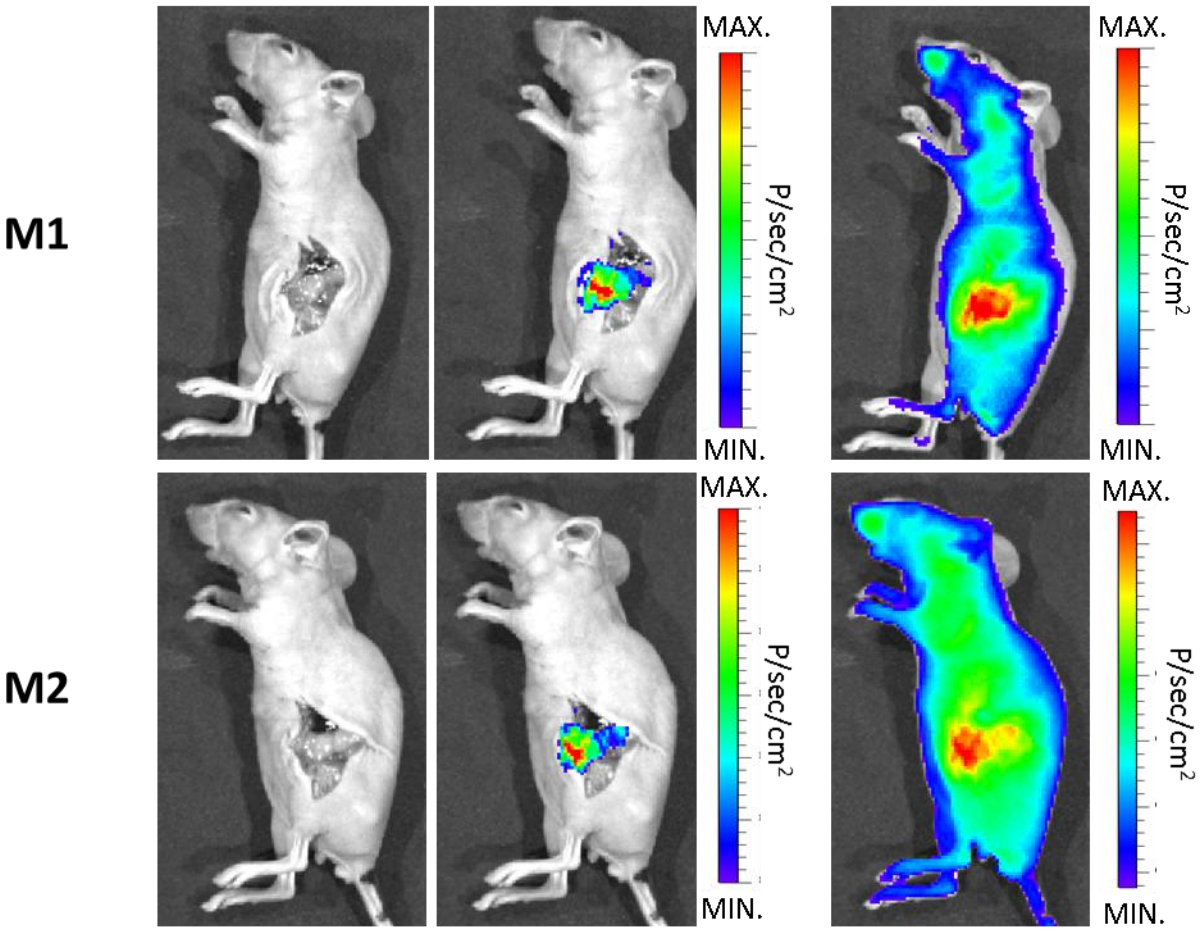
Supplementary Fig. 20 | The quantitative results of the migrated cells after treatment of PBS (blank), **M2**, **M3** and **M1** (Fig 5g). The mean of results of five parallel experiments is shown and data were expressed as mean ± SD (n=5). p_{M1} = 5.85E-06, p_{M2} = 5.94E-05, p_{M4} = 3.16 E-04, Statistical analysis: one-way ANOVA followed by post hoc Tukey's test, ***p<0.001.



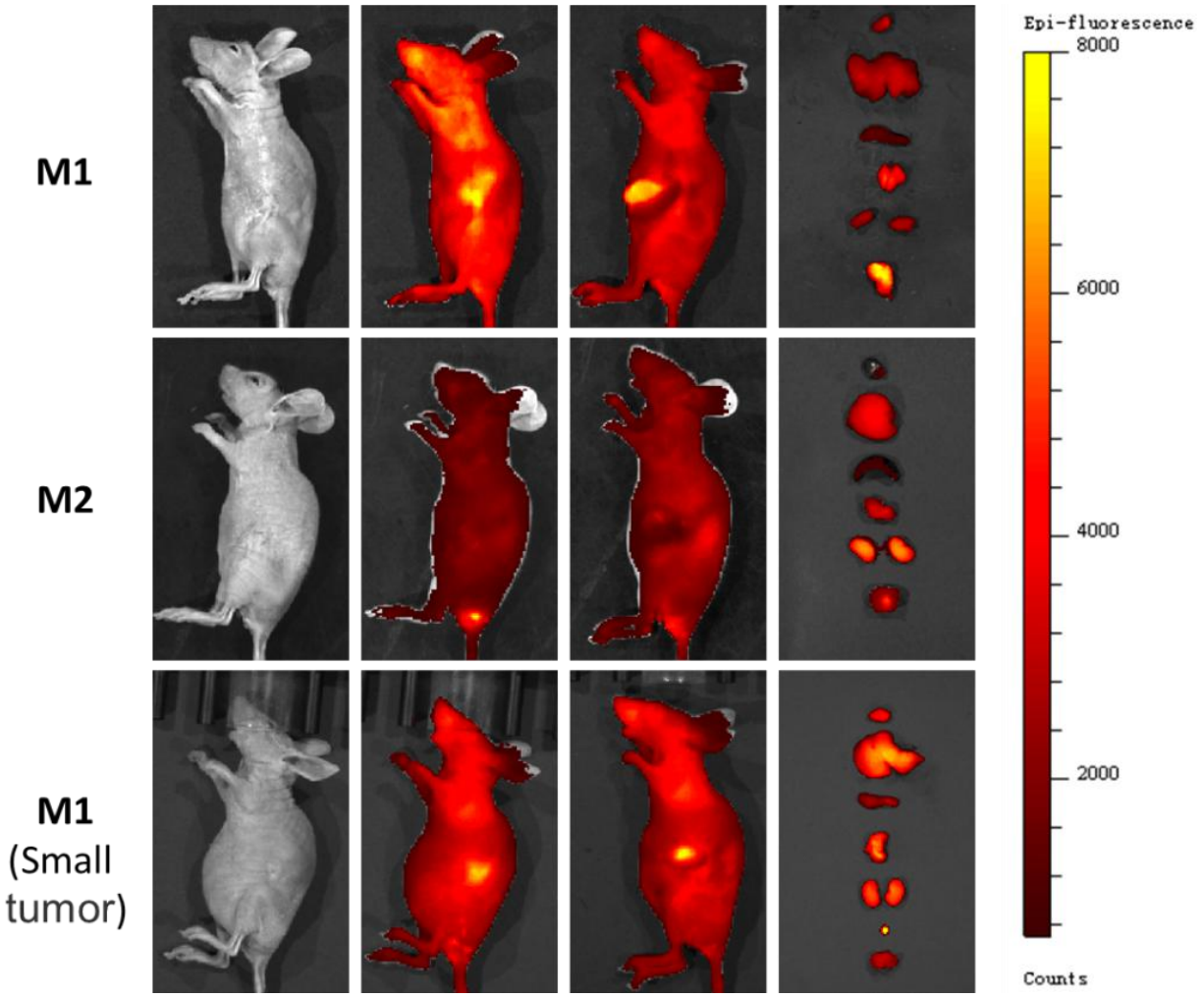
Supplementary Fig. 21 | Cell viability assay of Miapaca-2 cells treated with a series concentration of **M1** and **M2** for 24 h. Tested by Cell Counting Kit-8 (Beyotime, C0039) The mean of results of six parallel experiments is shown and data were expressed as mean ± SD (n=6).



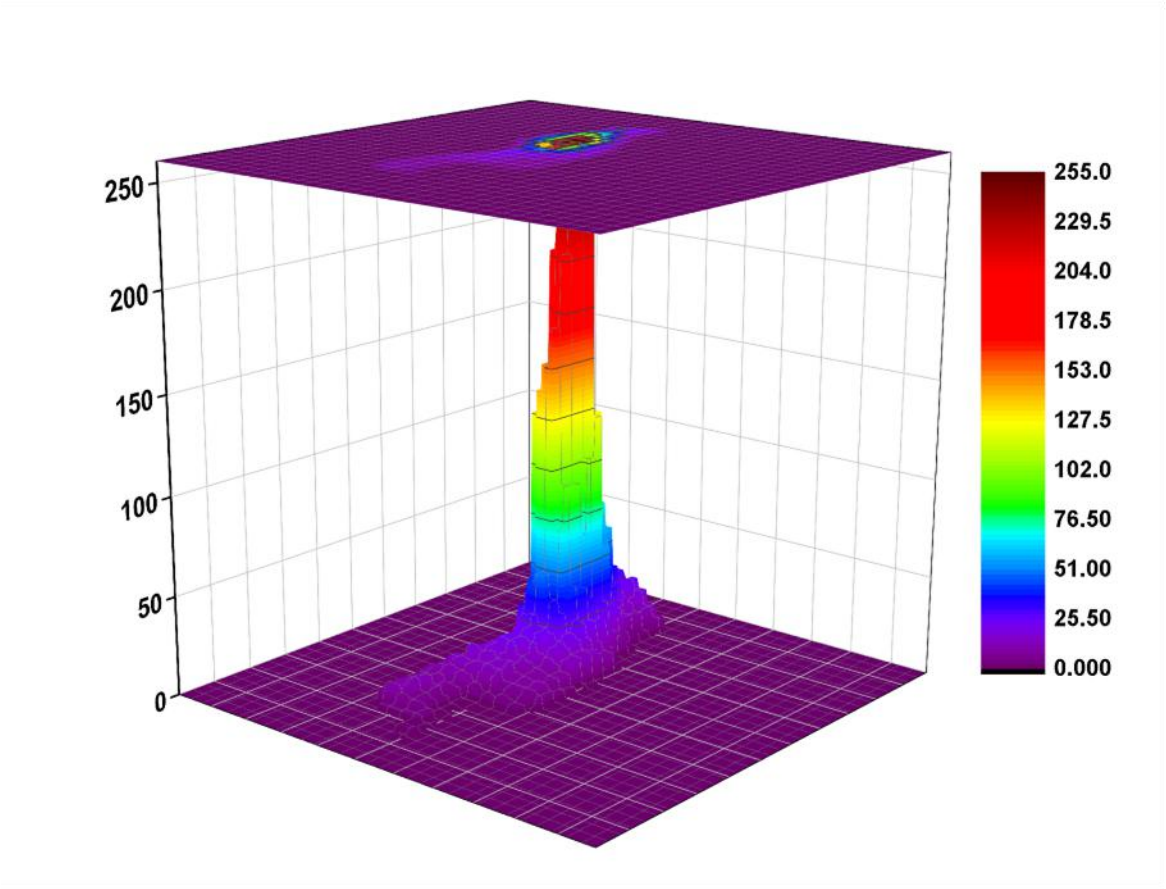
Supplementary Fig. 22 | 3D reconstruction PA images of tumor-bearing mice injected with **M1** (16 mg/kg) through tail vein. PA images of mice were acquired with MOST (mode: MOST 128) at 12 h post injection.



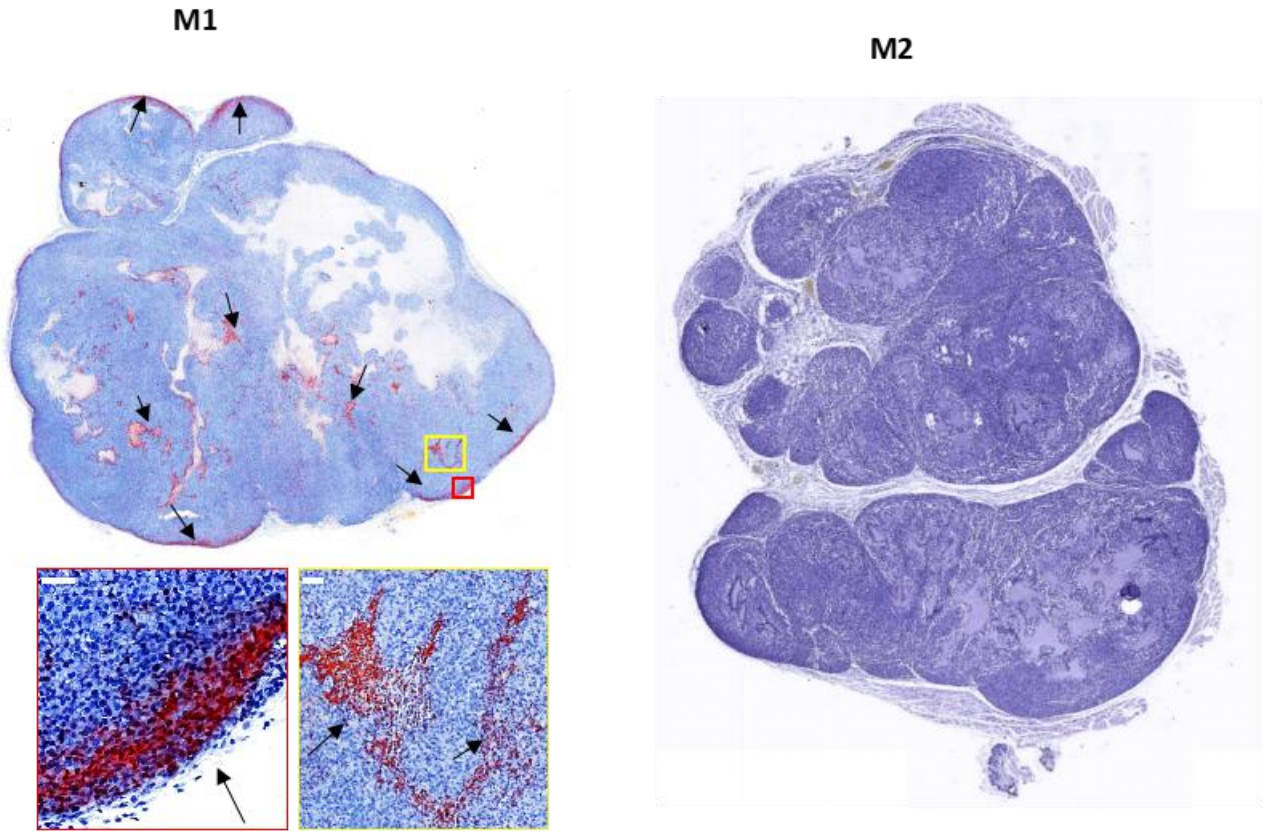
Supplementary Fig. 23 | NIR fluorescence image of mice bearing MIA PaCa-2 cells after intravenous administration of ICG, M2 and M1 with a dose of 16 mg/kg (Fig. 7a).



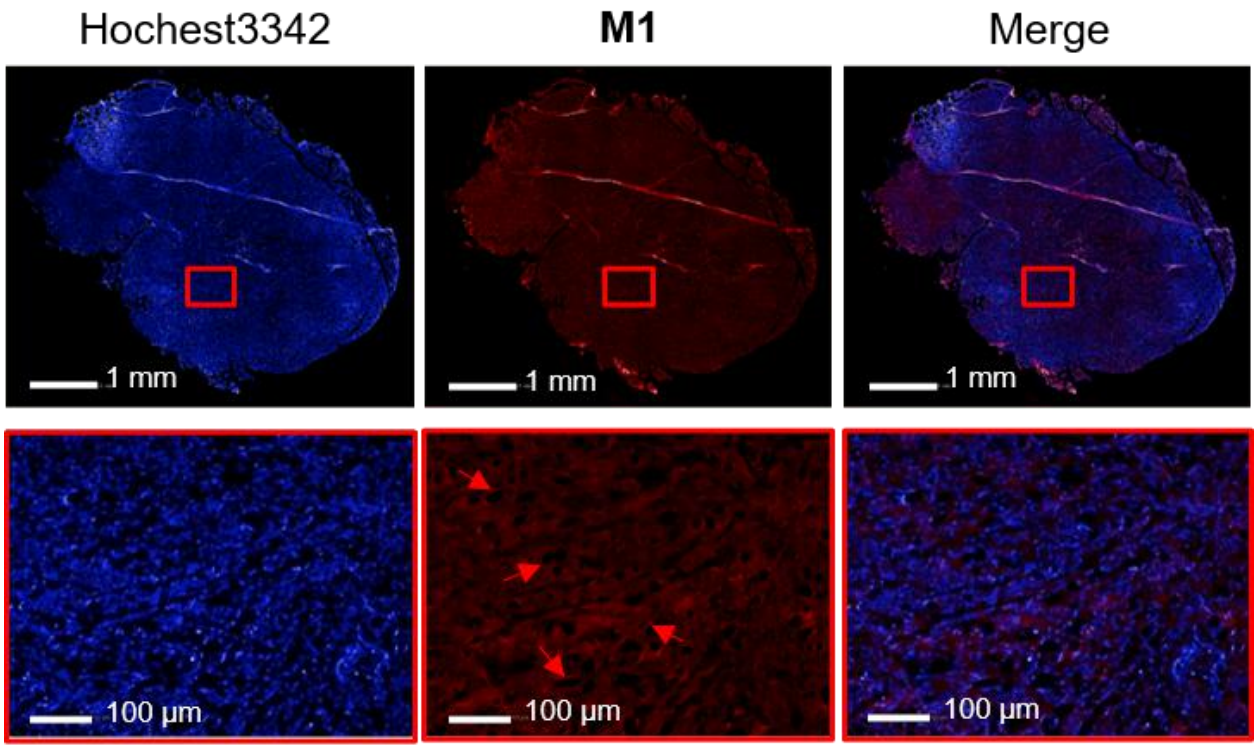
Supplementary Fig. 24 | The *in vivo* and *in situ* NIR images of orthotopic pancreatic tumors by **M1** and **M2**, and the *ex vivo* of organ biodistribution including heart, liver, spleen, lung, kidney, and tumor after vein injection at 16mg/kg for 48 h.



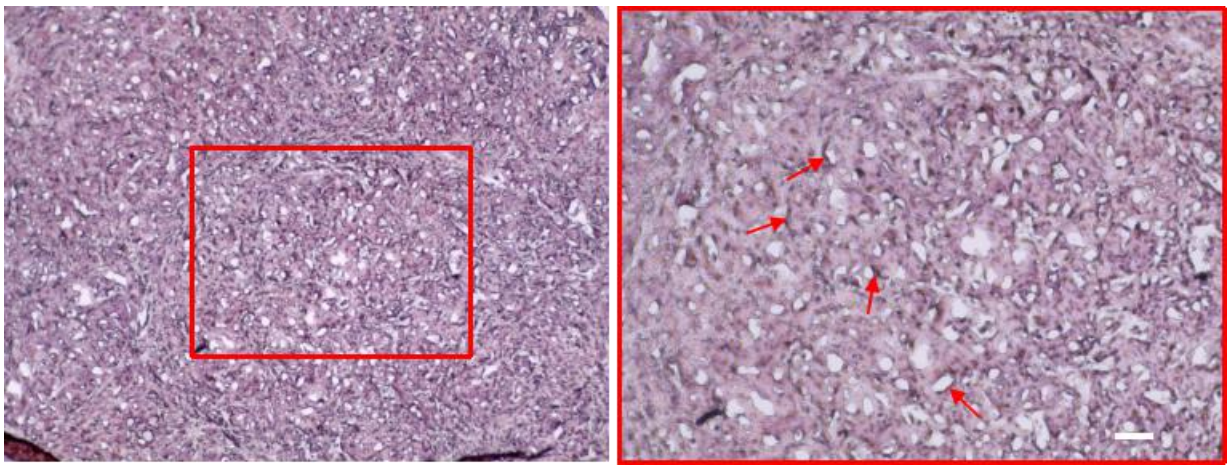
Supplementary Fig. 25 | The 3D quantitative fluorescence intensity distribution of small size (~ 2 mm diameter) *ex vivo* orthotopic pancreatic tumor images of **M1** (Fig. 7d).



Supplementary Fig. 26 | The whole tumor histologic section in mice treated with **M1** and **M2** (16 mg/kg) with congo red (red) stained assembled fibrils. Two enlarged images were respectively corresponded to the red and yellow box. Scale bar= 50 μm.



Supplementary Fig. 27 | Fluorescence images of tumor histologic section treated with **M1** (16 mg/kg) for 48 h. Blue: Hochest 33342, Red: Cy3 labeled **M1**. Scale bar: shown in figure.



Supplementary Fig. 28 | The tumor histologic section in mice treated with **M1** (16 mg/kg) for 48 h with congo red (red) stained assembled fibrils. A enlarged images were respectively corresponded to the red box. Scale bar: 100 μm