

Supplementary Materials

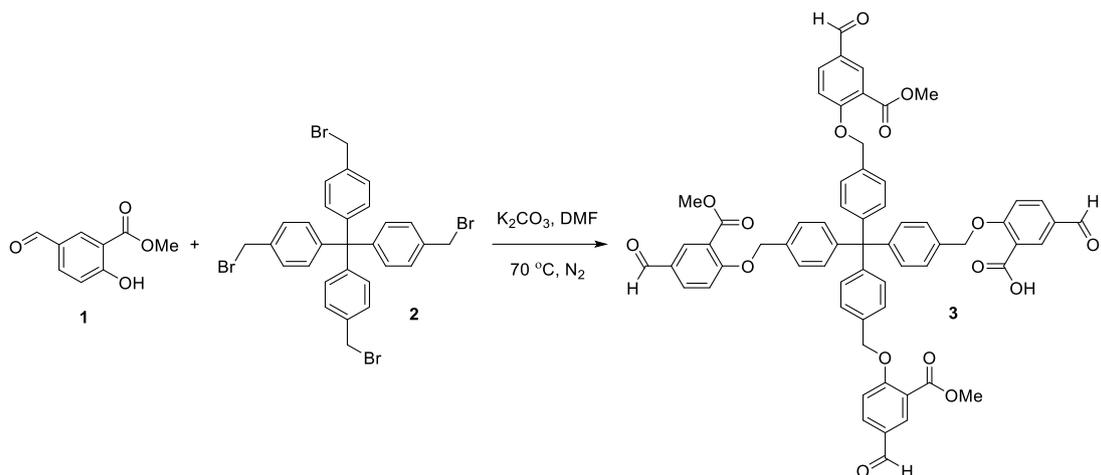
Synthesis and intracellular basic protein delivery of a polyanionic flexible organic framework

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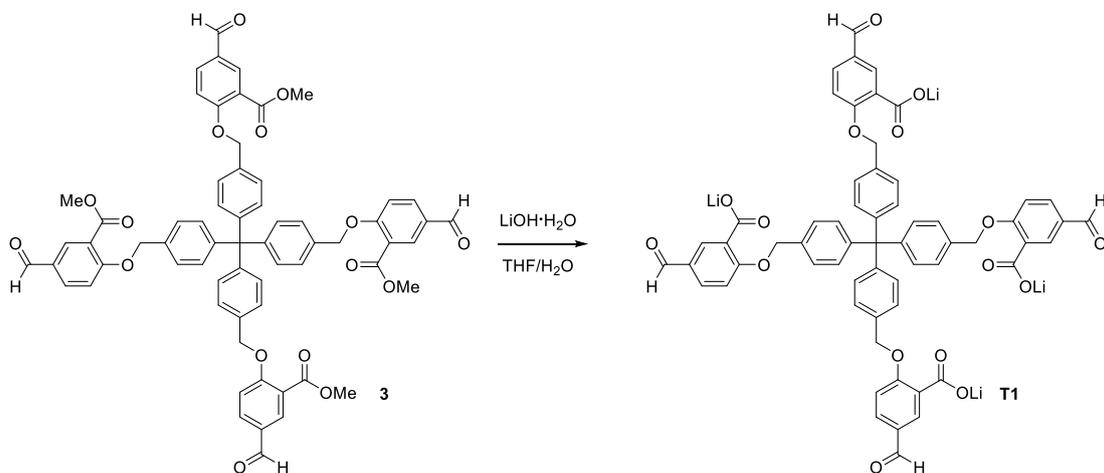
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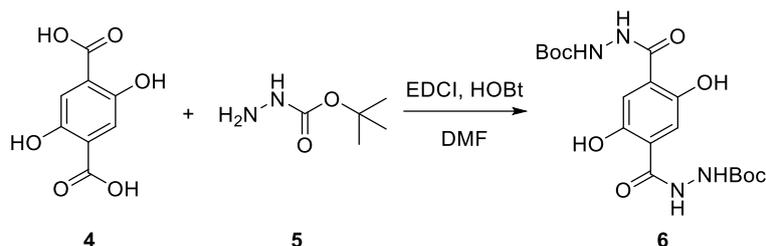
* **Correspondence to:** Prof. Zhan-Ting Li, Dr. Yue-Yang Liu, Department of Chemistry, Fudan University, 2205 Songhu Road, Shanghai 200438, China. E-mail: ztli@fudan.edu.cn; 19110220110@fudan.edu.cn



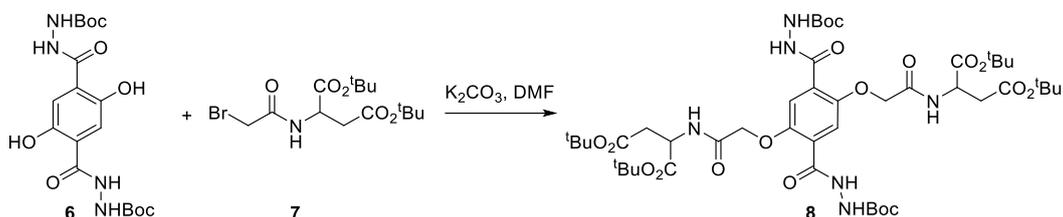
Compound 3. To a round-bottom flask (250 mL) containing anhydrous *N,N*-dimethylformamide (DMF, 150 mL) were added compound **1** (3.0 g, 17 mmol) and K_2CO_3 (18.8 g, 136 mmol). Then, compound **2** (2.3 g, 3.3 mmol) was added, the mixture was stirred at 70 °C overnight under ambient protection and then water (300 mL) added. The precipitate formed was filtered and washed with water (50 mL \times 3) and then dried to afford compound **3** as a white solid (3.6 g, 67%). 1H NMR (400 MHz, $CDCl_3$) δ 9.93 (d, $J = 2.0$ Hz, 4H), 8.38 (t, $J = 2.0$ Hz, 4H), 8.02 (d, $J = 8.4$ Hz, 4H), 7.41 (d, $J = 7.2$ Hz, 10H), 7.17 (d, $J = 8.8$ Hz, 6H), 5.28 (s, 8H), 3.92 (d, $J = 1.8$ Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.2, 165.4, 162.8, 146.4, 134.8, 134.5, 133.7, 129.5, 126.1, 121.1, 113.8, 77.2, 70.6, 52.4. HRMS (ESI): Calcd for $C_{65}H_{52}O_{16}Na$: 1111.3148 $[M+Na]^+$. Found: 1111.3143.



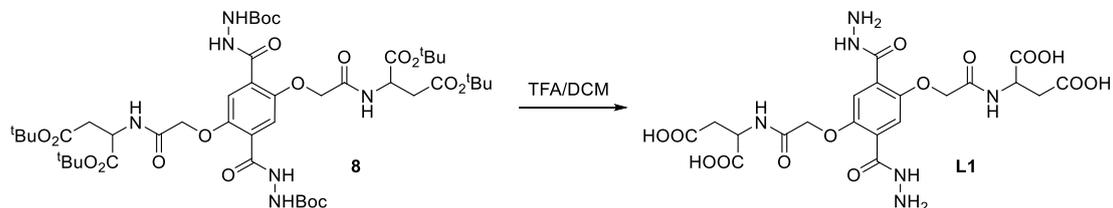
Compound T1. To a solution of compound **3** (100 mg, 0.09 mmol) and $LiOH \cdot H_2O$ (23 mg, 0.54 mmol) was added a solution of THF and H_2O (5 mL). The solution was stirred at room temperature for 12 hours. Then 1 M HCl (5 mL) was added and filtered to afford a white solid (81 mg, 85%). 1H NMR (400 MHz, Deuterium Oxide + Dioxane) δ 9.56 (s, 4H), 7.86 (d, $J = 2.2$ Hz, 4H), 7.40 (d, $J = 8.4$ Hz, 4H), 6.97-6.91 (m, 12H), 6.60 (s, 8H), 5.24 (s, 8H), 3.74 (s, 8H). ^{13}C NMR (100 MHz, Deuterium Oxide) δ 174.2, 160.5, 145.7, 134.2, 133.2, 130.7, 130.5, 130.0, 128.4, 126.3, 113.8, 69.4. HRMS (ESI): Calcd for $C_{61}H_{40}Li_4O_{16}$: 1042.2647 $[M-2Li]^{2+}$. Found: 1042.3879.



Compound 6. To the solution of compound **4** (5.0 g, 25 mmol) and compound **5** (7.3 g, 55 mmol) in 50 mL DMF was added EDCI (10.5 g, 55 mmol) and HOBT (7.4 g, 55 mmol). The reaction was stirred for 12 hours at room temperature. Then water (100 mL) was added and filtered to afford a yellow solid (9.9 g, 93%). ^1H NMR (400 MHz, DMSO- d_6) δ 11.09 (s, 2H), 10.25 (s, 2H), 9.06 (s, 2H), 7.42 (s, 2H), 1.43 (s, 18H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.5, 155.1, 150.1, 120.0, 116.4, 79.5, 28.1. HRMS (ESI): Calcd for $\text{C}_{18}\text{H}_{26}\text{N}_4\text{O}_8\text{Na}$: 449.1643 $[\text{M}+\text{Na}]^+$. Found: 449.1656.

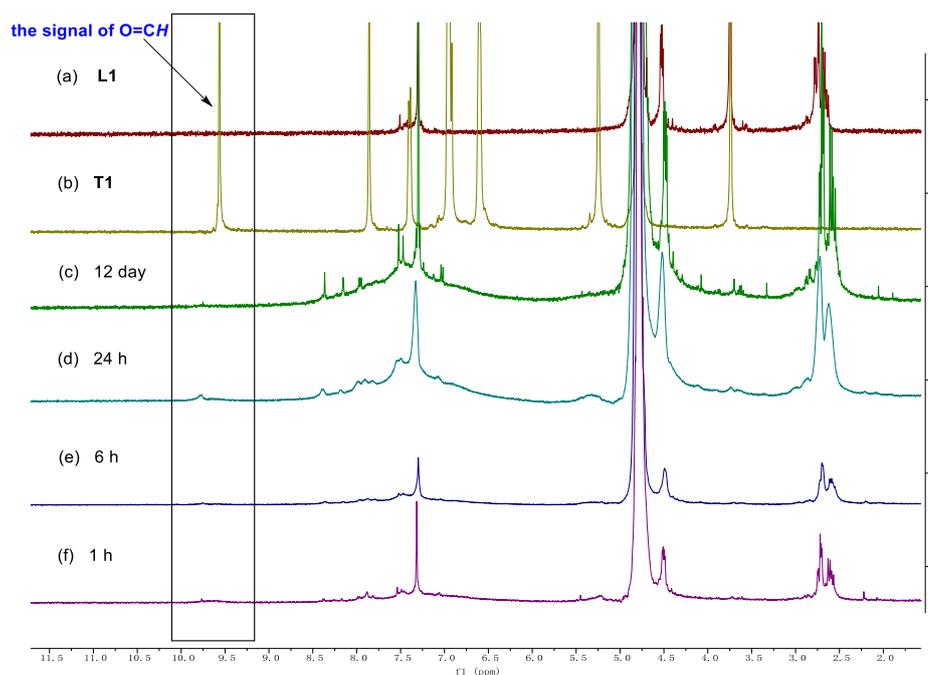


Compound 8. To a round-bottom flask (250 mL) containing anhydrous DMF (20 mL) were added compound **6** (3.0 g, 7.0 mmol) and K_2CO_3 (3.9 g, 28 mmol). Then, compound **7** (7.7 g, 21 mmol) was added, the mixture was stirred at room temperature under ambient protection for 12 hours 80%. The residue was dissolved in ethyl acetate and washed with brine, dried over anhydrous Na_2SO_4 . After removing of the solvent, the crude product was purified by column chromatography on silica gel to yield **8** as white solid (Yield: 68%). ^1H NMR (400 MHz, DMSO- d_6) δ 10.23 (s, 2H), 8.99 (s, 2H), 8.58 (d, $J = 8.0$ Hz, 2H), 7.36 (s, 2H), 4.71 (s, 4H), 4.58 (d, $J = 7.6$ Hz, 2H), 2.73 (dd, $J = 16.4$ Hz, 6.8 Hz, 2H), 2.58 (dd, $J = 16.4$ Hz, 6.4 Hz, 2H), 1.43-1.37 (m, 54H). ^{13}C NMR (100 MHz, CDCl_3) δ 170.8, 169.7, 166.9, 164.2, 155.4, 149.9, 83.0, 82.2, 81.6, 68.5, 49.0, 37.5, 29.8, 28.4, 28.2, 28.0. HRMS (ESI): Calcd for $\text{C}_{46}\text{H}_{73}\text{N}_6\text{O}_{18}$: 997.4976 $[\text{M}+\text{H}]^+$. Found: 997.4969.

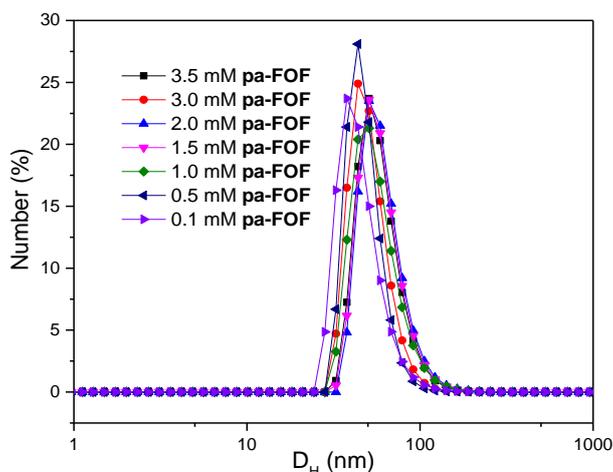


Compound L1. The compound **8** (100 mg, 0.1 mmol) was dissolved in a mixture of trifluoroacetic acid (TFA) and dichloromethane (DCM, volume ratio 20:1) at room temperature for one day, during which time nuclear magnetic monitoring was used to detect whether all the protective groups were removed. After the reaction was finished, acetonitrile was added and obtained white solid. The acetonitrile suspension was centrifuged at 10000 rpm for 5 minutes and the supernatant was sucked out. The solid

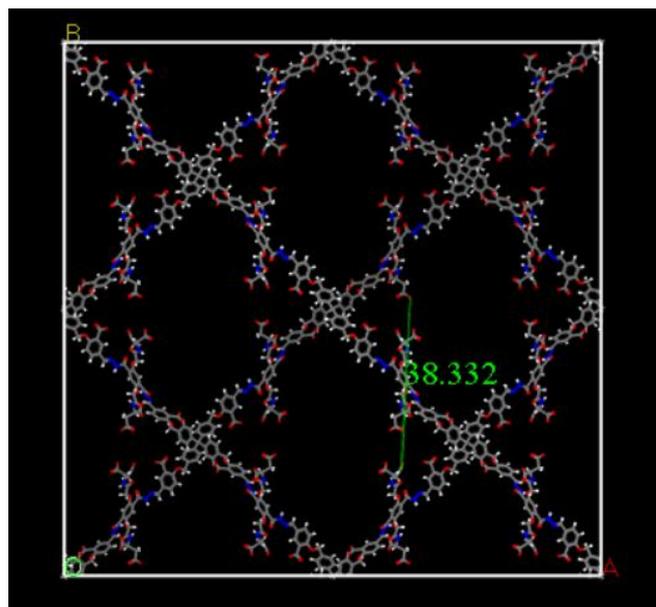
was collected and added to ammonia water to spin dry again, and the white solid was obtained as the target compound with a yield of 80%. ^1H NMR (400 MHz, Deuterium Oxide + Dioxane) δ 7.30 (s, 2H), 4.76-4.73 (m, 4H), 4.54-4.50 (m, 2H), 2.78 (d, $J = 4.0$ Hz, 1H), 2.74 (d, $J = 4.0$ Hz, 1H), 2.67 (d, $J = 8.0$ Hz, 1H), 2.63 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (100 MHz, Deuterium Oxide) δ 177.3, 177.2, 169.8, 166.4, 149.4, 125.5, 115.2, 68.1, 51.8, 38.2. HRMS (ESI): Calcd for $\text{C}_{20}\text{H}_{24}\text{N}_6\text{O}_{14}\text{Na}$: 595.1243 $[\text{M}+\text{Na}]^+$. Found: 595.1220.



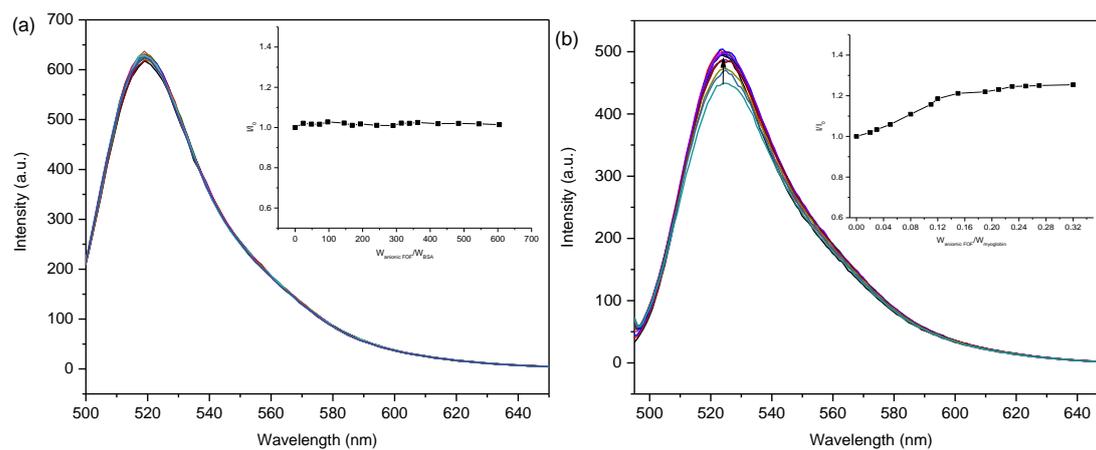
Supplementary Figure 1. ^1H NMR spectrum (400 MHz) of the mixture of L1 (10 mM), T1 (5.0 mM) in D_2O (pD = 6.5, DCl) at 25°C at different times, highlighting the quantitative formation of pa-FOF after standing at 80°C for 24 hours.



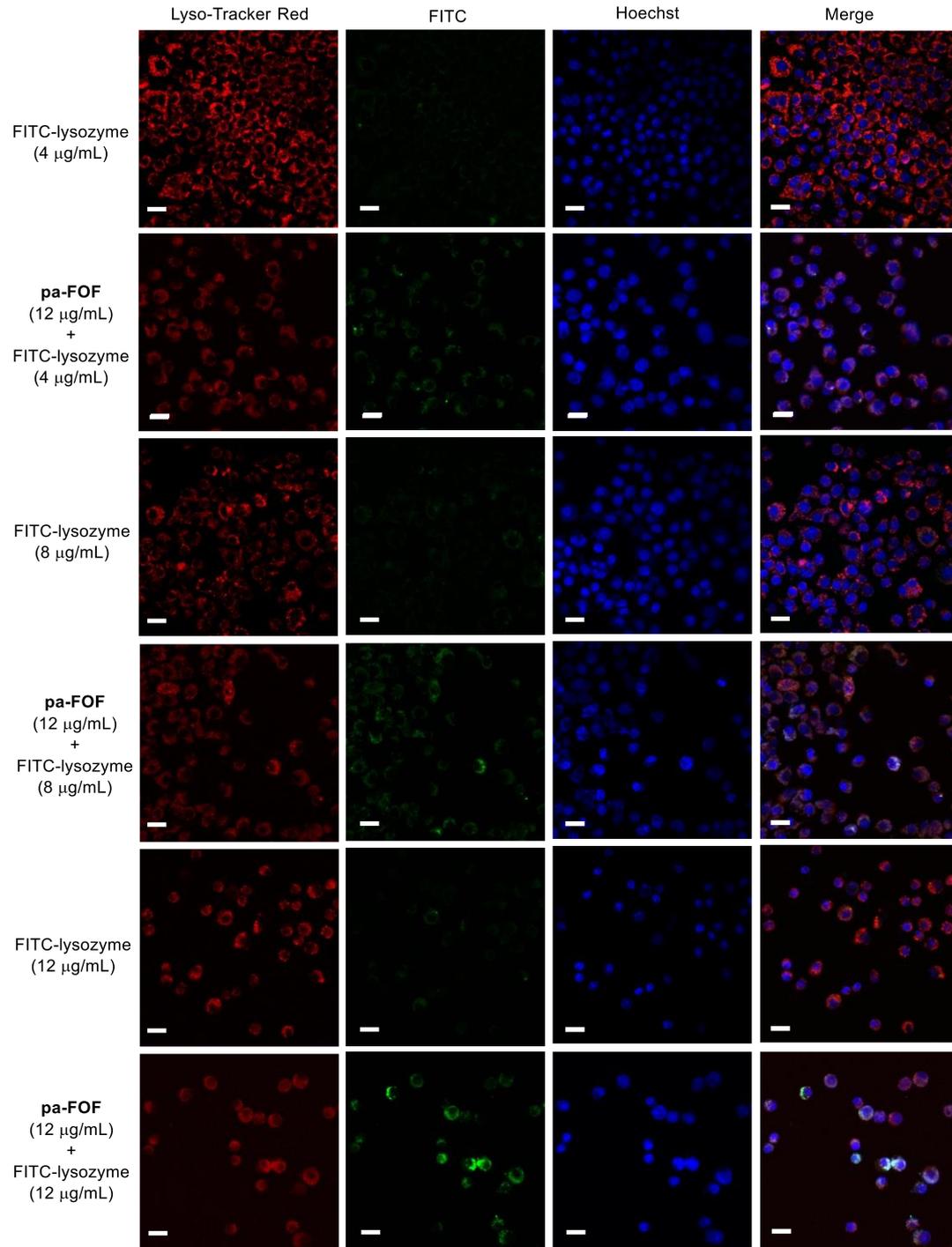
Supplementary Figure 2. DLS profile of pa-FOF of different concentrations in water recorded after the solutions were left at room temperature for 1 week. The profile was comparable with that recorded after the preparation of the solutions.



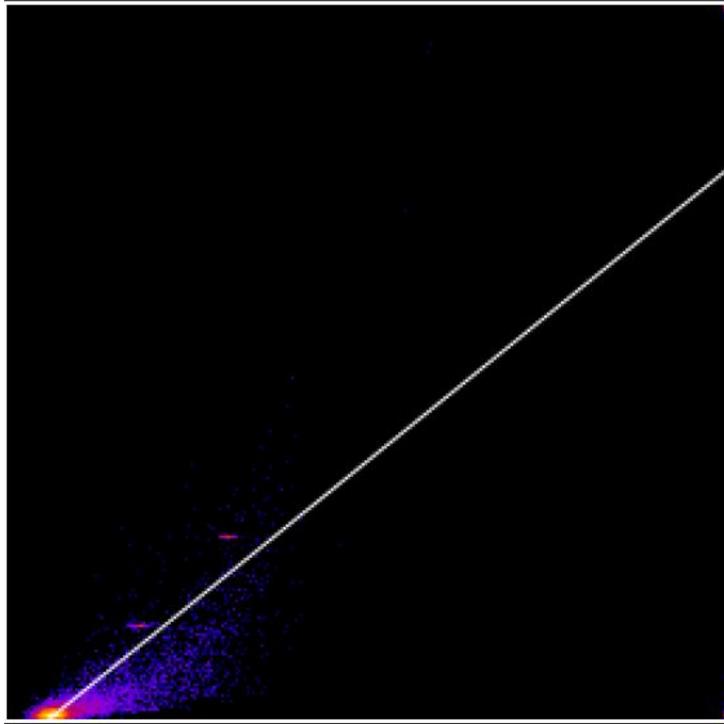
Supplementary Figure 3. Molecular modeling of **pa-FOF**.



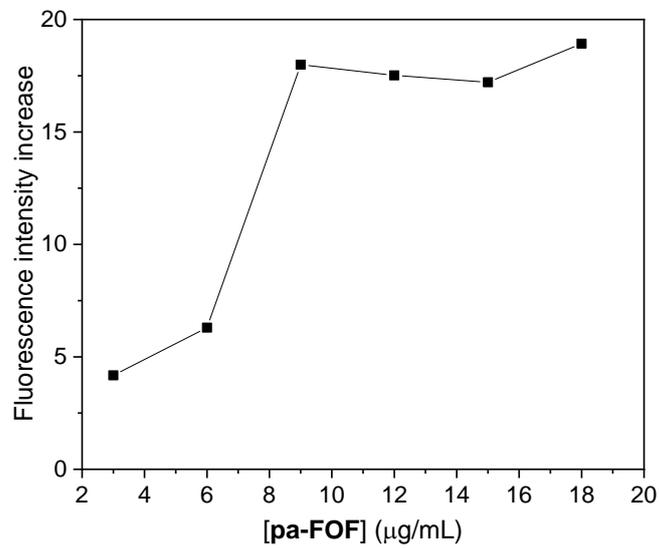
Supplementary Figure 4. (a) Fluorescence spectra ($\lambda_{\text{ex}} = 490 \text{ nm}$) of FITC-BSA ($50 \mu\text{g/mL}$) in the presence of the increasing amount of **pa-FOF** ($0\text{-}64 \mu\text{g/mL}$) in water (Inset: I/I_0 versus $W_{\text{pa-FOF}}/W_{\text{BSA}}$ ($\lambda_{\text{em}} = 520 \text{ nm}$)). (b) Fluorescence spectra ($\lambda_{\text{ex}} = 490 \text{ nm}$) of FITC-myoglobin ($400 \mu\text{g/mL}$) in the presence of the increasing amount of **pa-FOF** ($0\text{-}58 \mu\text{g/mL}$) in water (Inset: I/I_0 versus $W_{\text{pa-FOF}}/W_{\text{myoglobin}}$ ($\lambda_{\text{em}} = 520 \text{ nm}$)).



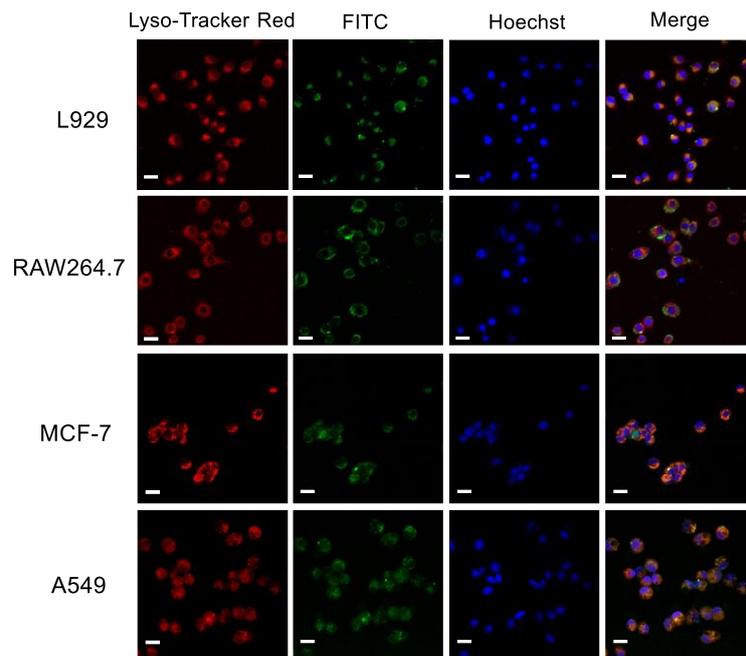
Supplementary Figure 5. Confocal laser microscopic images of ana-1 cells after incubation for 16 hours with FITC-lysozyme (4, 8, and 12 $\mu\text{g}/\text{mL}$) in the presence or absence of **pa-FOF** (12 $\mu\text{g}/\text{mL}$). The lysosomes and nuclei were stained with Lyso-Tracker Red (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .



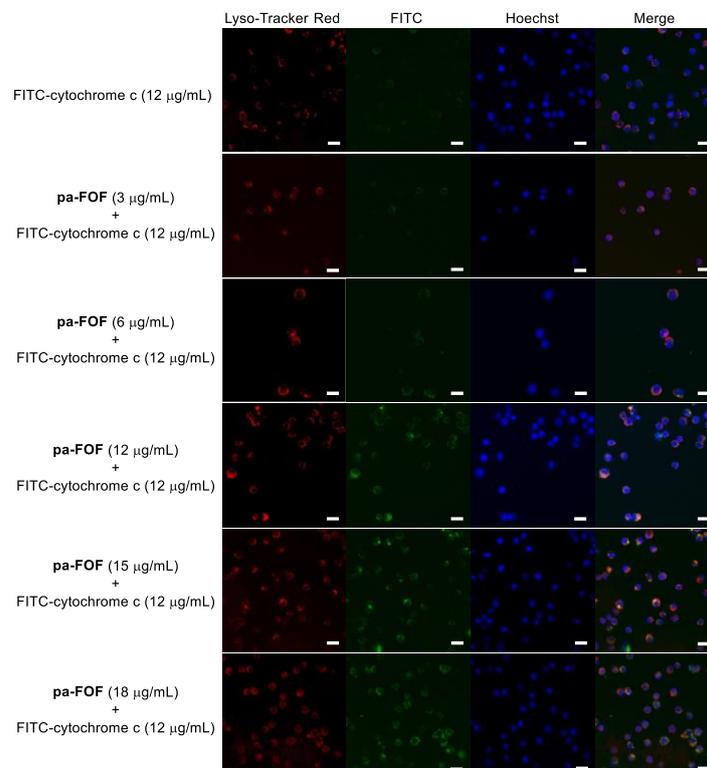
Supplementary Figure 6. Calculation of Pearson's correlation coefficients between the signals from the FITC-lysozyme and Lyso-Tracker Red after co-incubation of ana-1 cells with **pa-FOF** (12 $\mu\text{g/mL}$) and FITC-lysozyme (12 $\mu\text{g/mL}$).



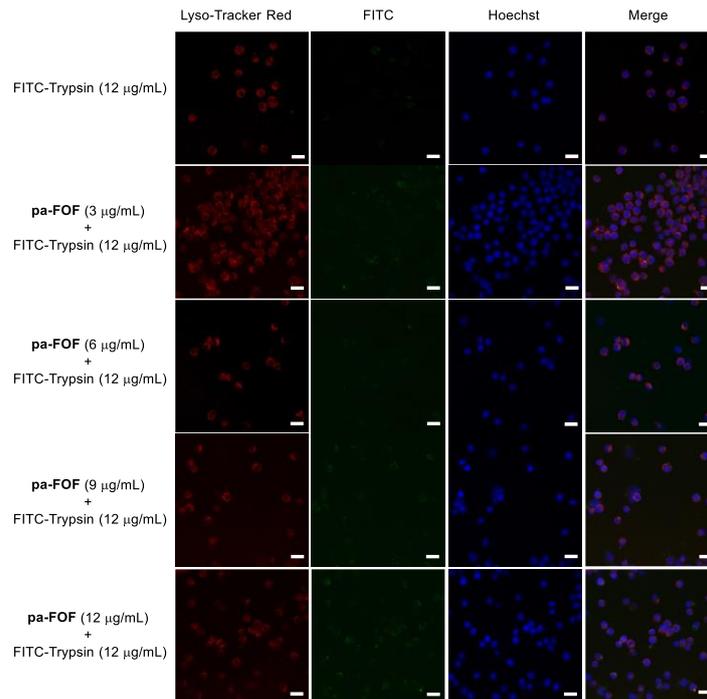
Supplementary Figure 7. Fluorescence intensity analysis of FITC channels using ImageJ software.



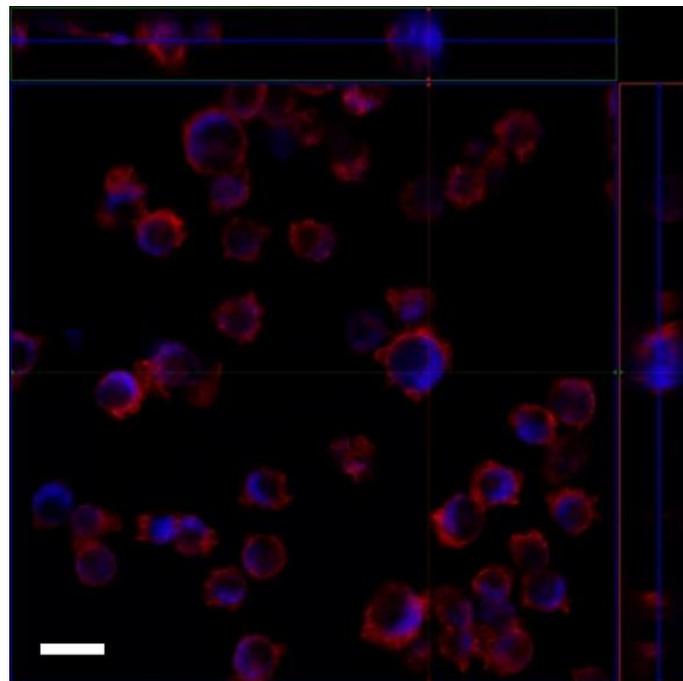
Supplementary Figure 8. Confocal laser microscopic images of L929, RAW264.7, MCF-7 and A549 cells after incubation for 16 hours with FITC-lysozyme (12 $\mu\text{g}/\text{mL}$) in the presence of **pa-FOF** (9 $\mu\text{g}/\text{mL}$). The lysosomes and nuclei were stained with Lyso-Tracker Red (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .



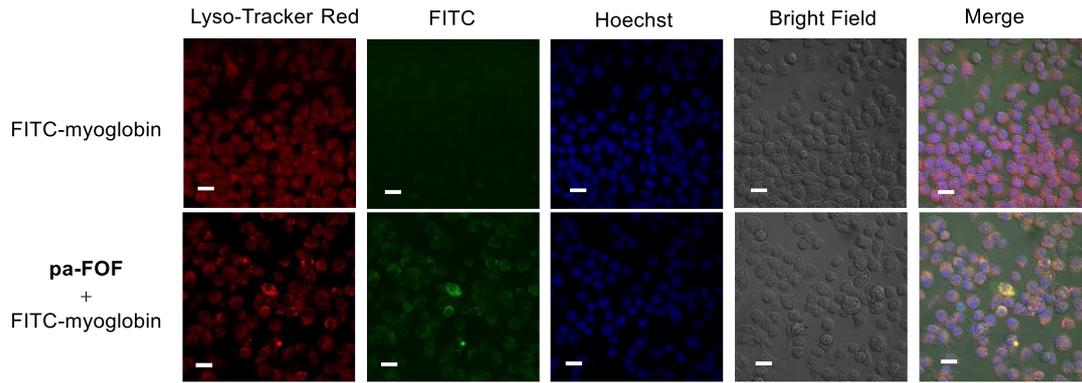
Supplementary Figure 9. Confocal laser microscopic images of ana-1 cells after incubation for 16 hours with FITC-cytochrome c (12 $\mu\text{g}/\text{mL}$) in the presence of **pa-FOF** (0-18 $\mu\text{g}/\text{mL}$). The lysosomes and nuclei were stained with Lyso-Tracker Red (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .



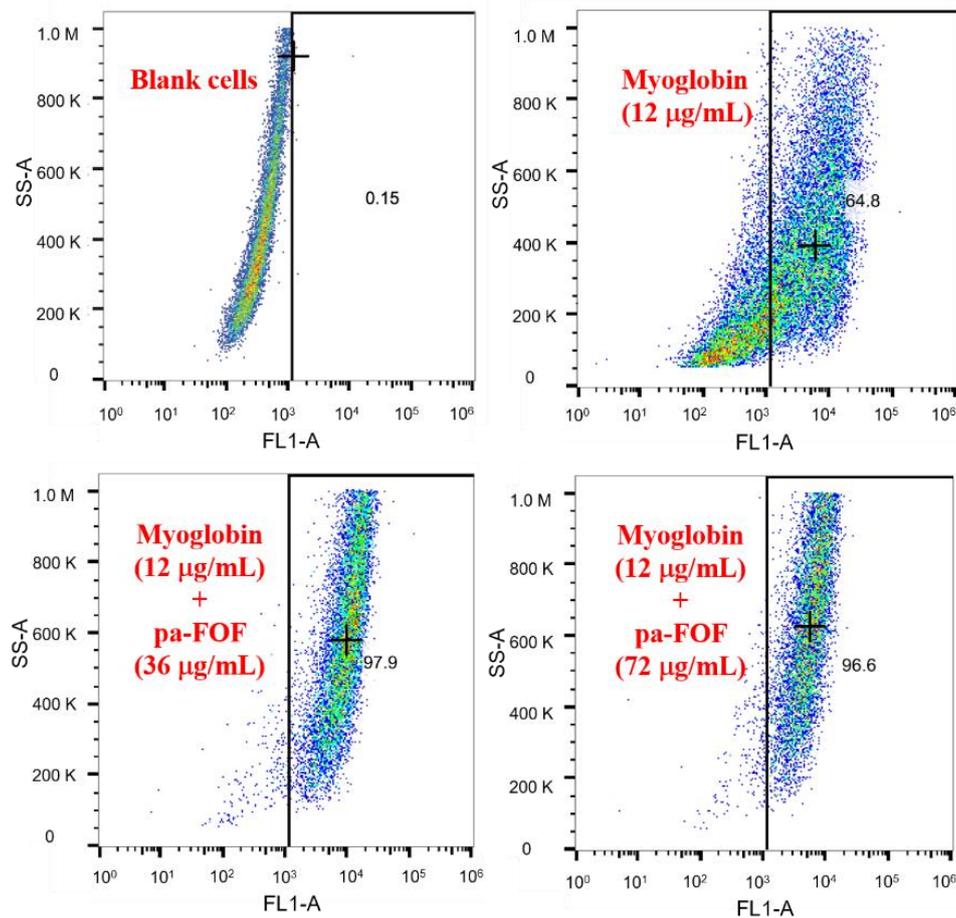
Supplementary Figure 10. Confocal laser microscopic images of ana-1 cells after incubation for 16 hours with FITC-Trypsin (12 $\mu\text{g/mL}$) in the presence of **pa-FOF** (0-12 $\mu\text{g/mL}$). The lysosomes and nuclei were stained with Lyso-Tracker Red (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .



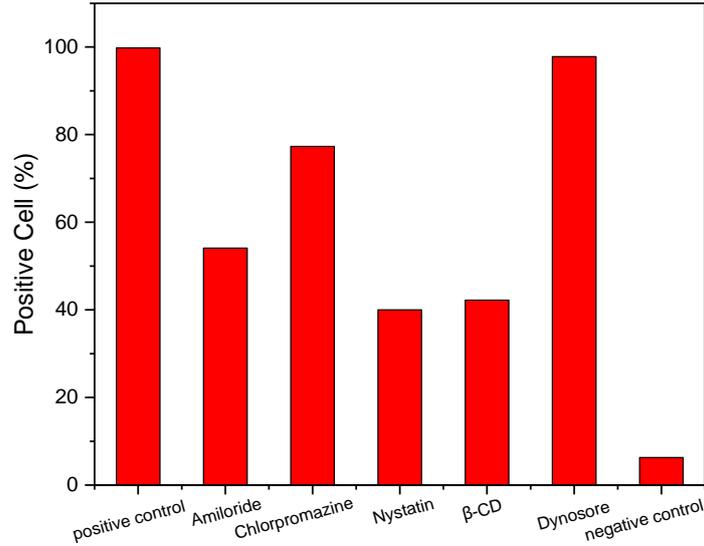
Supplementary Figure 11. Representative Z-stack images (images have x and y projections of 3-dimensional Z-stack images below and to the right of each image, respectively) of ana-1 cells after incubation with **pa-FOF** (blue) for 16 hours. The cytoskeleton was stained with RhB-phalloidin (red). Scale bar: 20 μm .



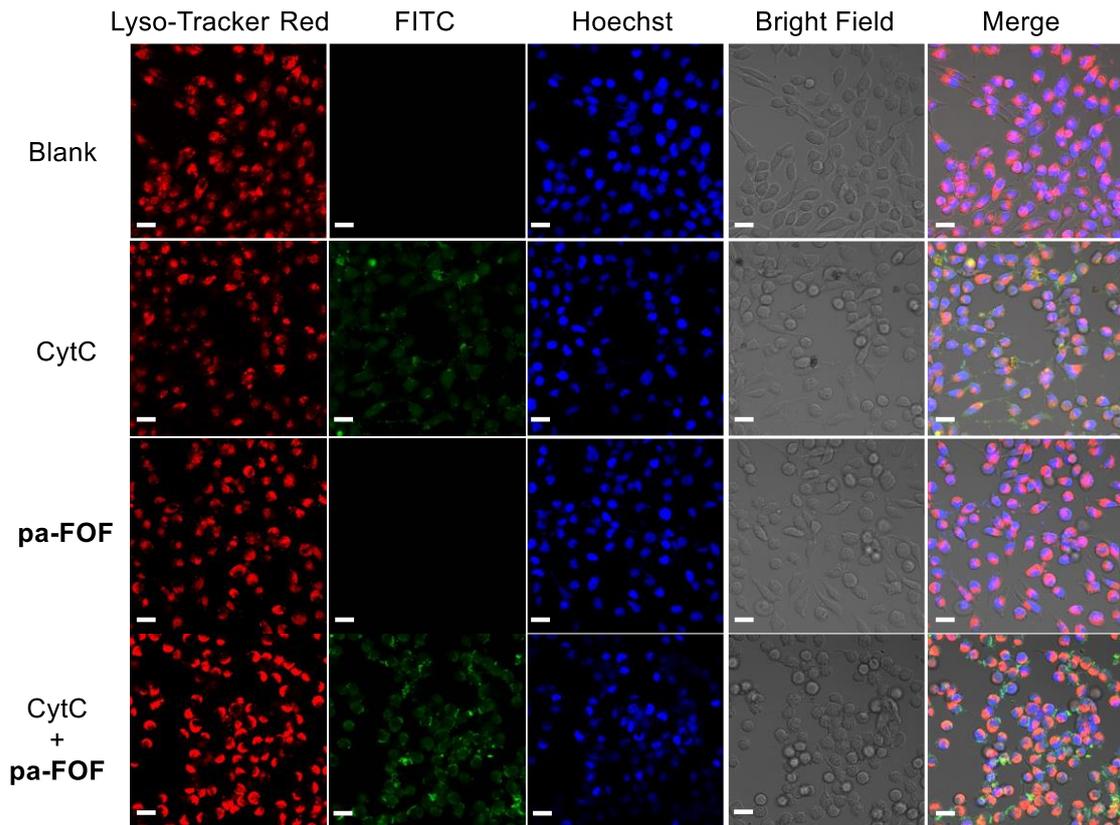
Supplementary Figure 12. Confocal laser microscopic images of ana-1 cells after incubation for 16 hours with FITC-myoglobin (12 $\mu\text{g}/\text{mL}$) and FITC-myoglobin (12 $\mu\text{g}/\text{mL}$) in the presence of **pa-FOF** (9 $\mu\text{g}/\text{mL}$). The lysosomes and nuclei were stained with Lyso-Tracker Red (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .



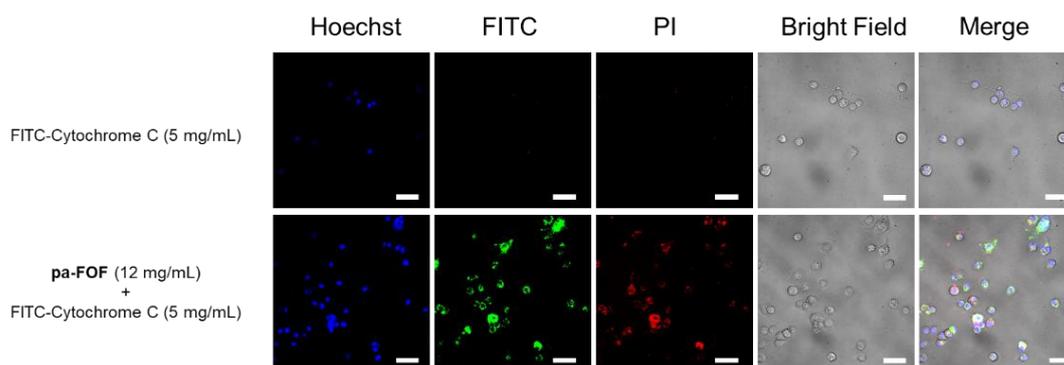
Supplementary Figure 13. The delivery of FITC-myoglobin (12 $\mu\text{g}/\text{mL}$) by **pa-FOF** (0-72 $\mu\text{g}/\text{mL}$) for ana-1 cell lines. The cells were tested after incubation in F12/DMEM medium for 16 hours.



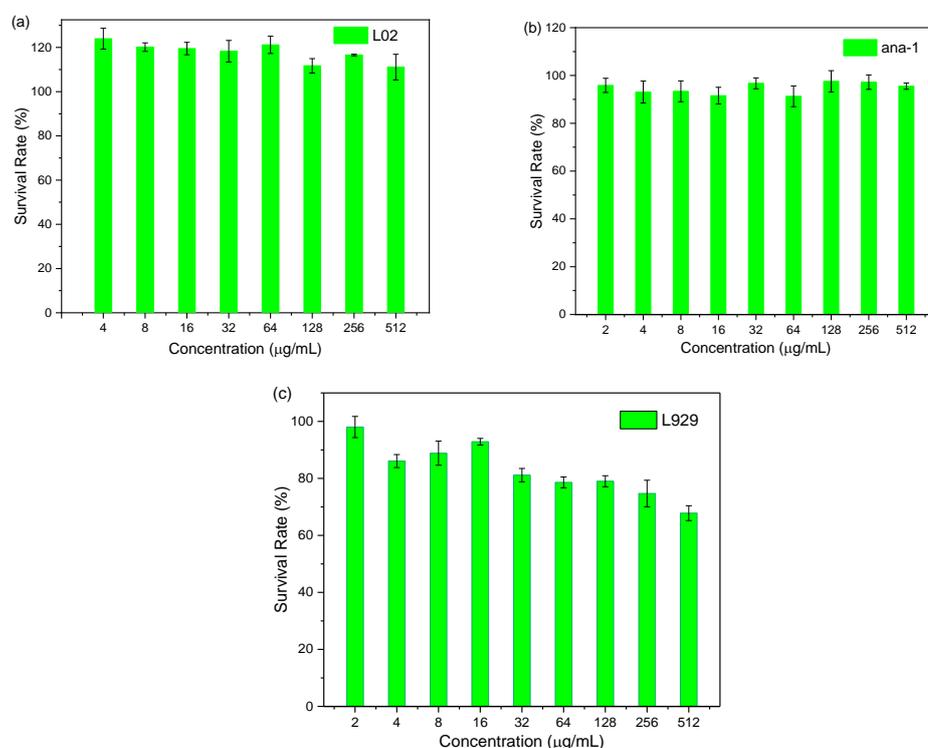
Supplementary Figure 14. Delivery (internalization) experiments of FITC-lysozyme (12 $\mu\text{g}/\text{mL}$) into ana-1 cells by **pa-FOF** (9.0 $\mu\text{g}/\text{mL}$) after incubation for 16 hours in the absence and presence of endocytosis inhibitors amiloride, chlorpromazine, nystatin, β -CD, and dynosore.



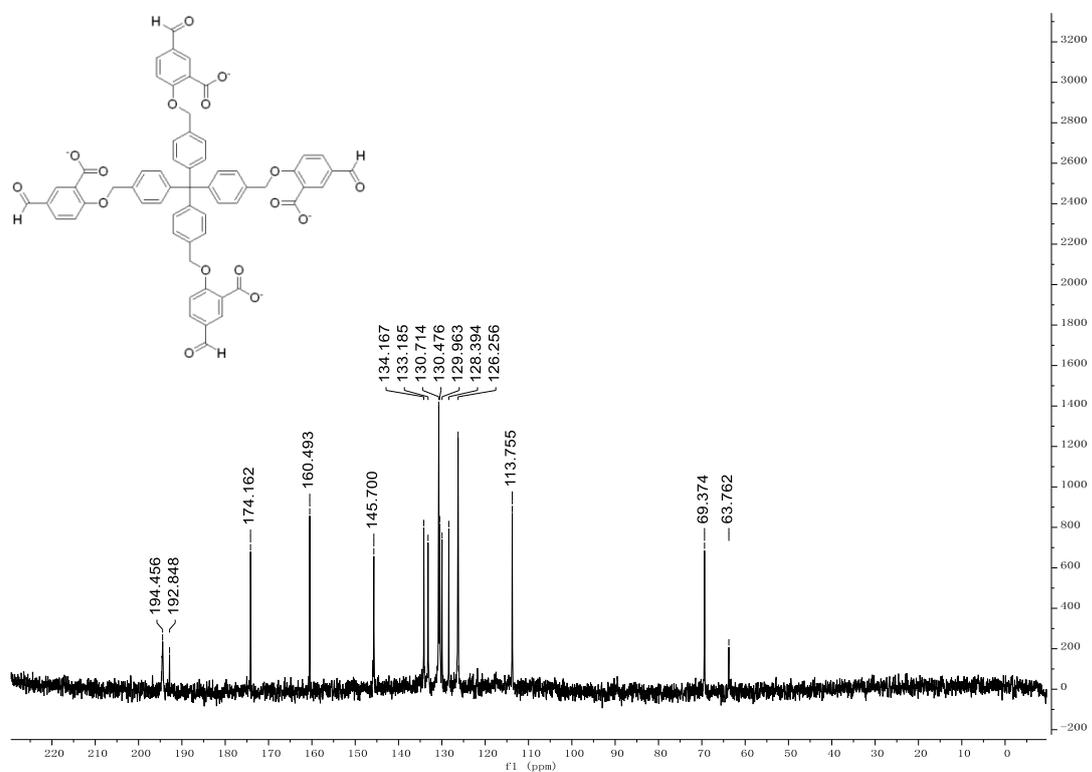
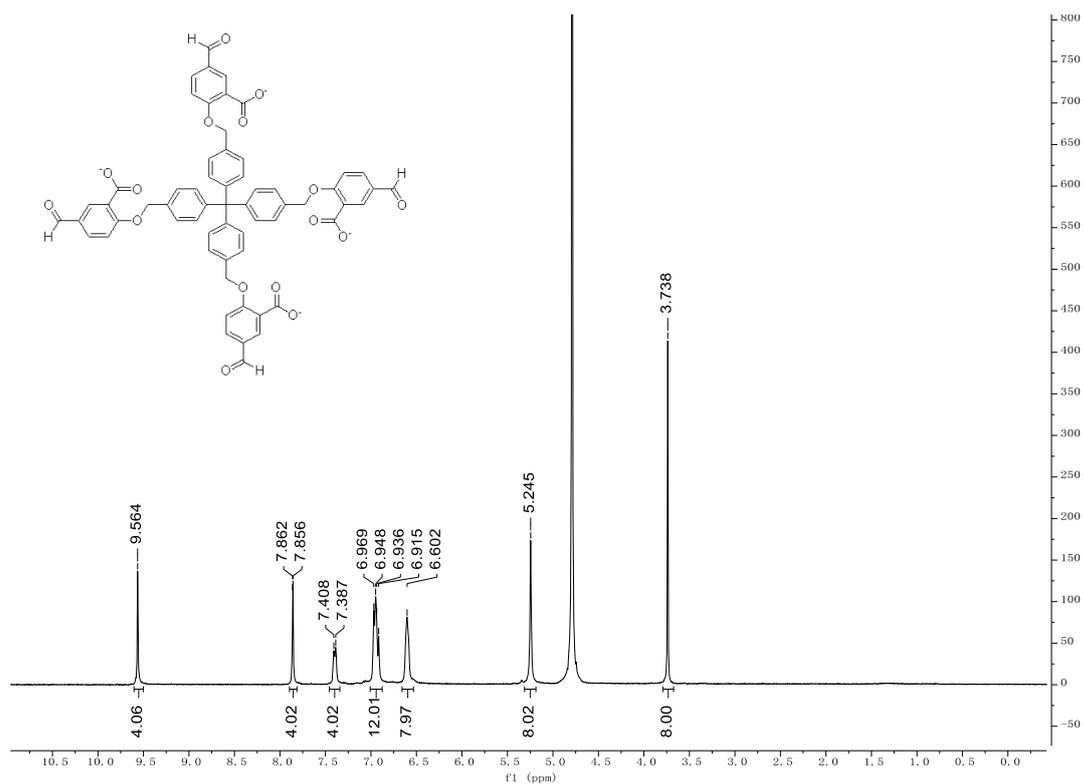
Supplementary Figure 15. CLSM images of L929 cells after incubation for 16 hours with FITC-CytC (5 $\mu\text{g}/\text{mL}$), **pa-FOF** (12 $\mu\text{g}/\text{mL}$), and FITC-CytC (5 $\mu\text{g}/\text{mL}$)/**pa-FOF** (12 $\mu\text{g}/\text{mL}$). The lysosomes and nuclei were stained with Lyso-Tracker Red (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .

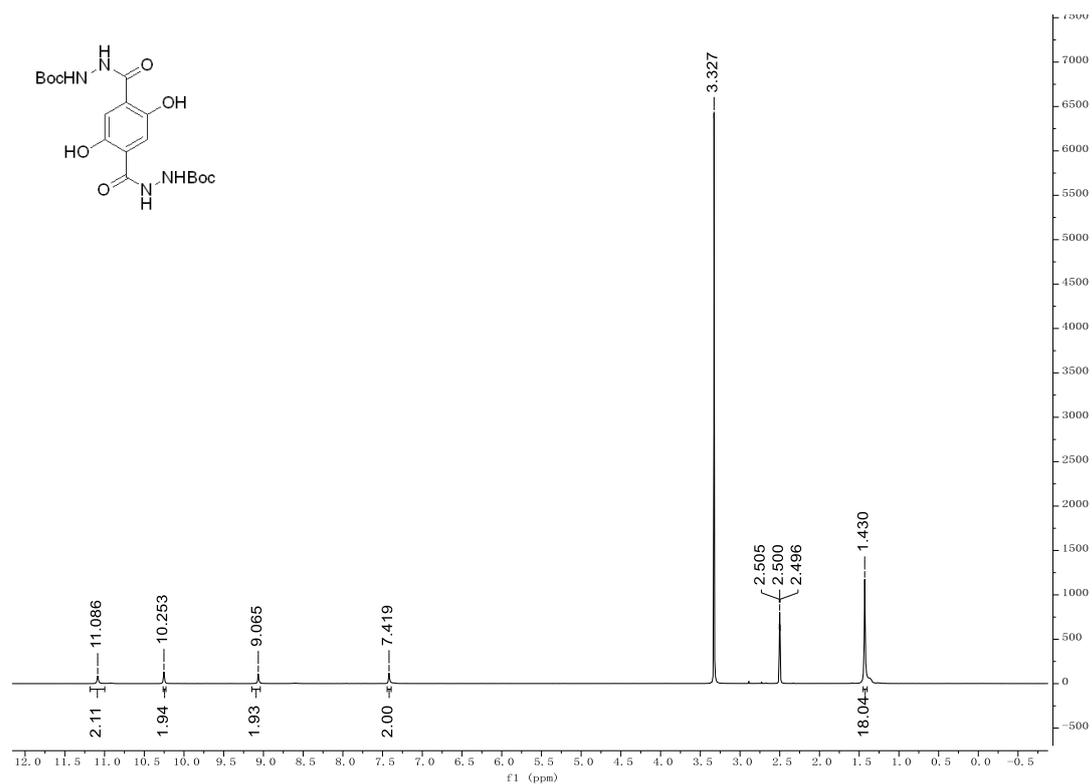


Supplementary Figure 16. CLSM images of ana-1 cells after incubation for 16 hours with FITC-cytochrome c (5 $\mu\text{g}/\text{mL}$) and FITC-cytochrome c (5 $\mu\text{g}/\text{mL}$)/**pa-FOF** (12 $\mu\text{g}/\text{mL}$). The nuclei were stained with PI (red) and Hoechst 33342 (blue), respectively. Scale bar: 20 μm .

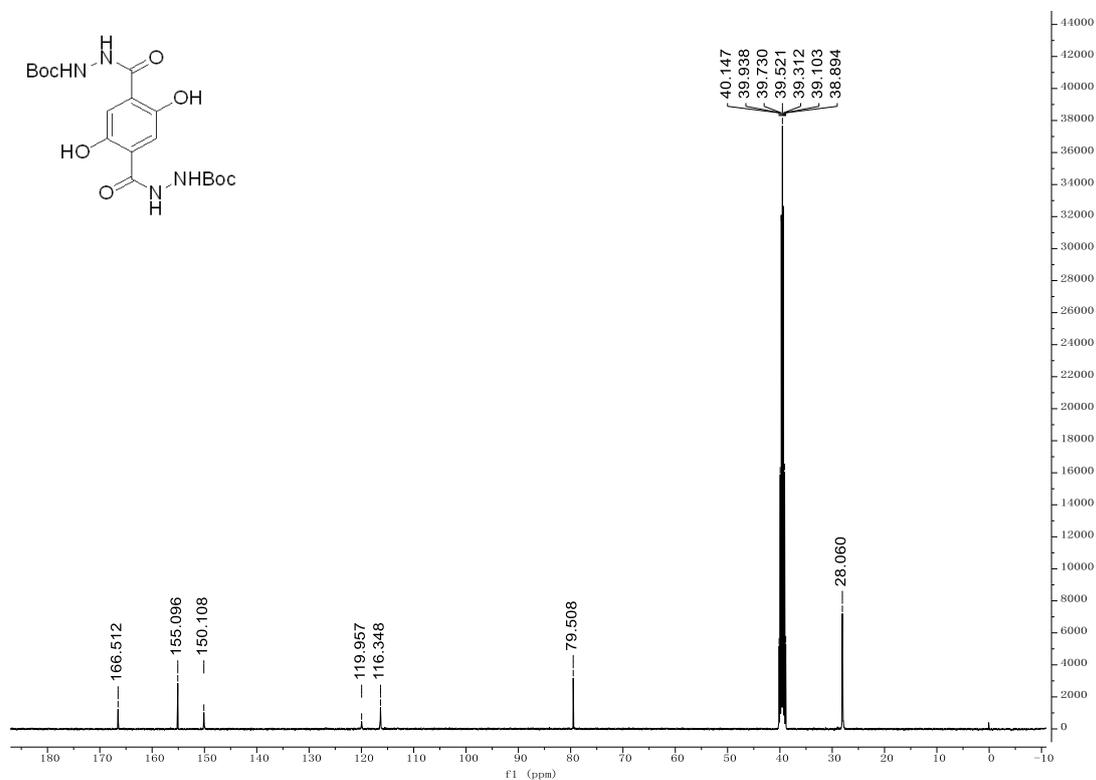


Supplementary Figure 17. Cell viability values (%) of (a) L02, (b) ana-1, and (c) L929 cell lines estimated by CCK-8 proliferation tests versus incubation concentration of **pa-FOF** represented by [T1]. The cells ($\sim 2 \times 10^4$ per well) were incubated with the **pa-FOF** at 37 $^\circ\text{C}$ for 24 h. Error bars represent the s.d. of uncertainty for each point.

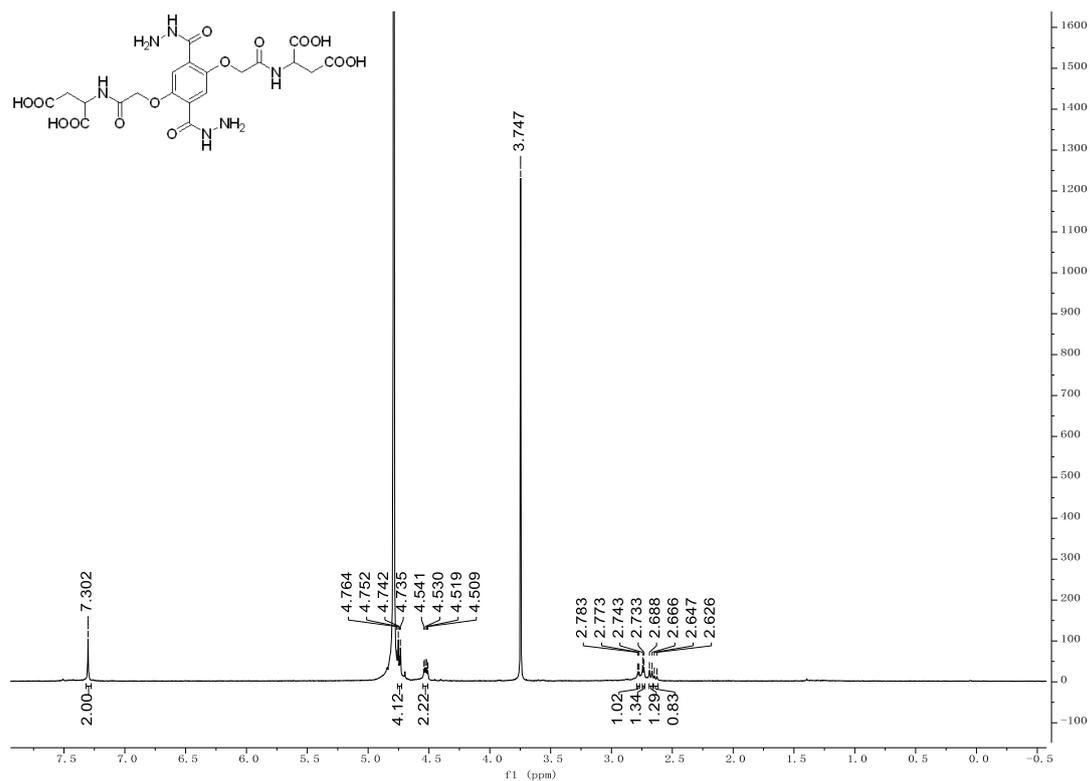




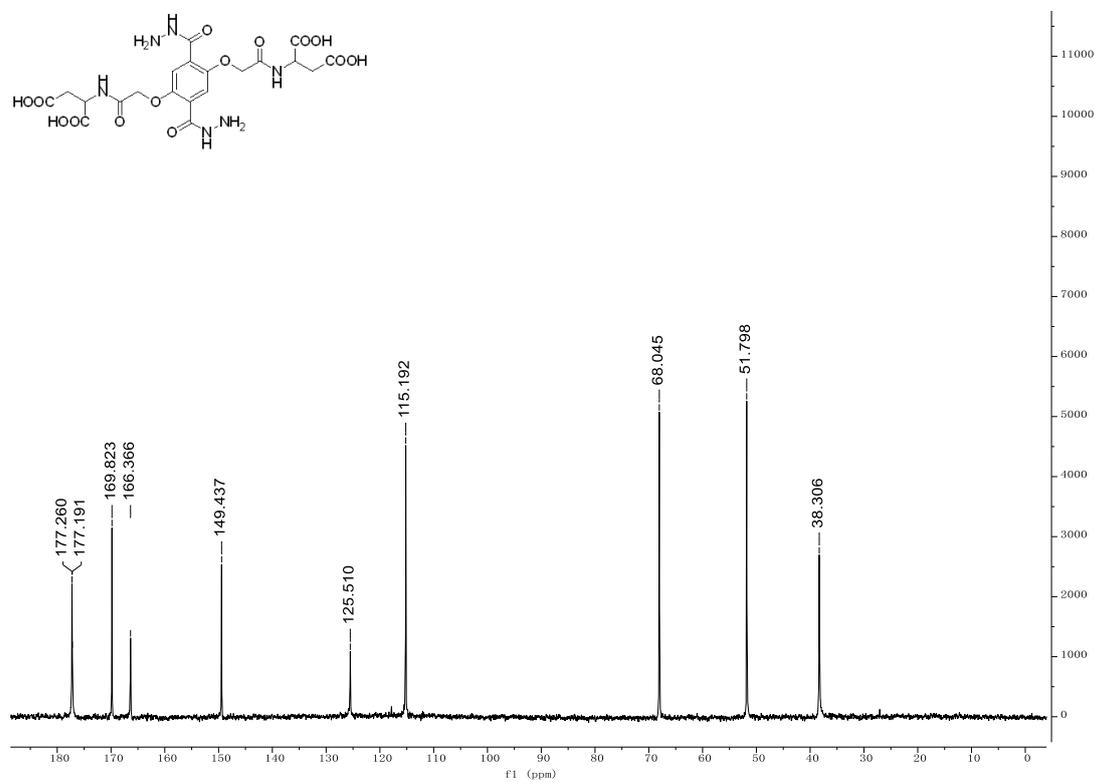
Supplementary Figure 22. ¹H NMR spectra of compound **6** in DMSO (400 MHz, 25 °C).



Supplementary Figure 23. ¹³C NMR spectra of compound **6** in DMSO (400 MHz, 25 °C).



Supplementary Figure 26. ¹H NMR spectra of compound L1 in D₂O (400 MHz, 25 °C).



Supplementary Figure 27. ¹³C NMR spectra of compound L1 in D₂O (400 MHz, 25 °C).