

Supporting Information

Real-Time Monitoring of ATP-Responsive Drug Release using Mesoporous-Silica-Coated Multicolor Upconversion Nanoparticles

Jinping Lai, Birju P. Shah, Yixiao Zhang, Letao Yang and Ki-Bum Lee*

[*] Department of Chemistry and Chemical Biology
Institute for Advanced Materials, Devices and Nanotechnology (IAMDN)
Rutgers University, Piscataway, NJ 08854
Fax: (+1)732-445-5312,
E-mail: kblee@rutgers.edu
<http://kblee.rutgers.edu>

MATERIALS

Erbium(III) acetate hydrate (99.9%), thulium(III) acetate hydrate (99.9%), ytterbium(III) acetate tetrahydrate (99.9%), yttrium(III) acetate hydrate (99.9%), oleic acid (technical grade, 90%), 1-octadecene (technical grade, 90%), ammonium fluoride (99.99+%), Boc-L-Tyr-OMe, H-Lys(Cbz)-OH, H-Asp(OBzl)-OH, 2,2'-dipicolylamine, paraformaldehyde, tris(2-aminoethyl)amine (TAEA), triphosgene, folic acid (FA), *N*-hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC·HCl), Tetraethylorthosilicate, 3-(triethoxysilyl)propyl isocyanate, *N*-cetyltrimethylammonium bromide (CTAB), ATP, ADP, AMP, GTP, UTP, CTP, RNA, DNA and 10 mM pH7.4 HEPES solution were purchased from Sigma-Aldrich or TCI Chemical and used as received.

CHARACTERIZATIONS

UV-vis absorption spectra were recorded on a Varian Cary 50 spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer with an external NIR laser with a wavelength at 980 nm as excitation light source (CNI high power fiber coupled diode laser system, FC-W-980). The total output power for the lasers is tunable from 1 mW to 10 W. The power density was detected by 1916-R handheld optical power meter with 818P thermophile detector (purchased from Newport corporation, USA). FT-IR spectra were collected on an Avatar Nicolet FT-IR330 spectrometer. ¹H NMR and ¹³C NMR were acquired on Varian 300/400 MHz NMR spectrometer. ESI-MS was collected on Finnigan LCQ™ DUO LC/MS spectrometer. MALDI-TOF spectrum was recorded on an ABI-MDS SCIEX 4800 MALDI-TOF/TOF mass spectrometer. The DLS size and zeta potential was collected on a Malvern Zetasizer nano S. Transmission electron microscopy (TEM) was performed on a Topcon 002B electron microscope at 200 kV. Sample preparation was carried out by placing a drop of the freshly prepared colloidal solution on a carbon-coated copper grid and allowing the solution to evaporate. Upconversion luminescent/fluorescence imaging of HeLa cell were performed on a Olympus IX83 inverted motorized microscope adapted with an additional external NIR laser with a wavelength at 980 nm as excitation light source (CNI high power fiber coupled diode laser system, FC-W-980, output power is set at 1W).

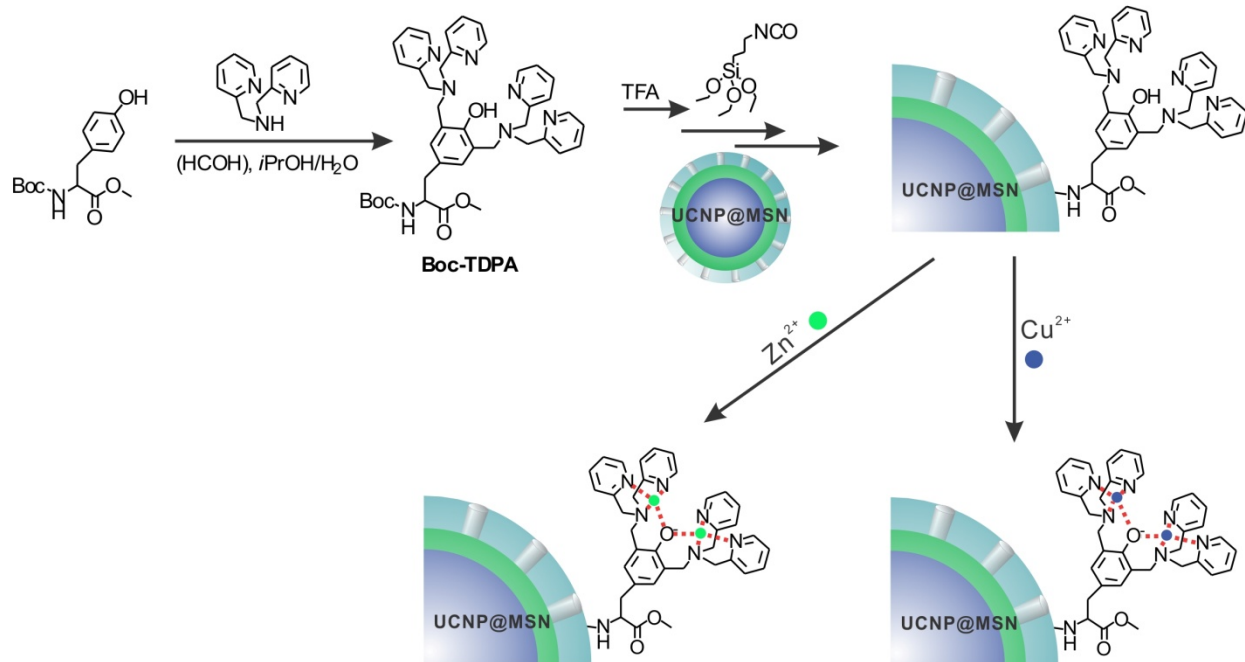


Figure S1. Synthesis of TDPA-Zn²⁺/Cu²⁺-UCNP@MSNs.

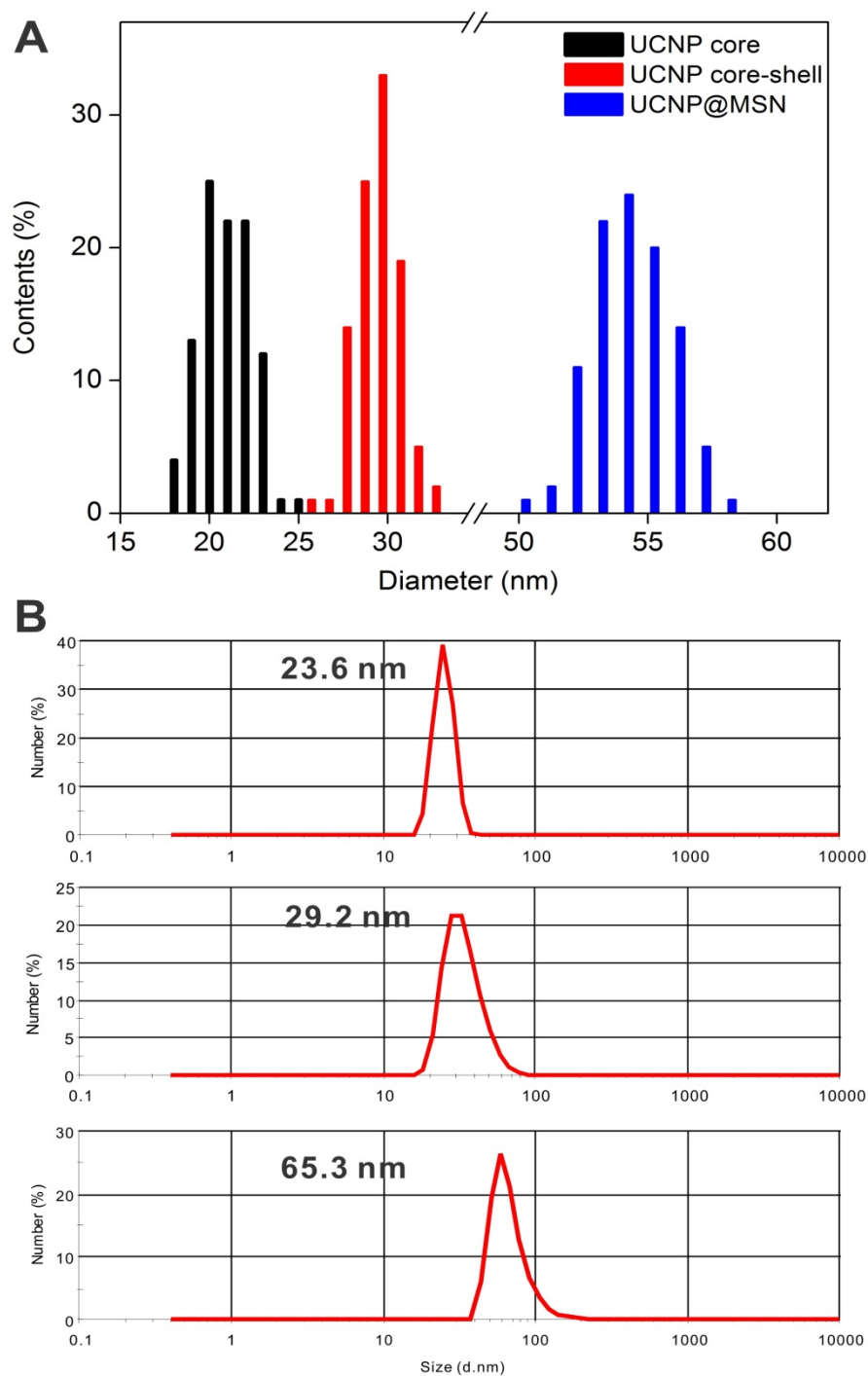


Figure S2. TEM (A) and DLS (B) size distribution of core, core-shell UCNP and metallic complex (TDPA-Zn²⁺) functionalized UCNP@MSN. TEM size distribution was counted based on 100 nanoparticles. The DLS size distribution was collected based on the dispersion of 2 wt% UCNP nanoparticles in hexane (B up, UCNP core; middle, UCNP core-shell), and 5 wt% UCNP@MSNs in aqueous solution (B bottom).

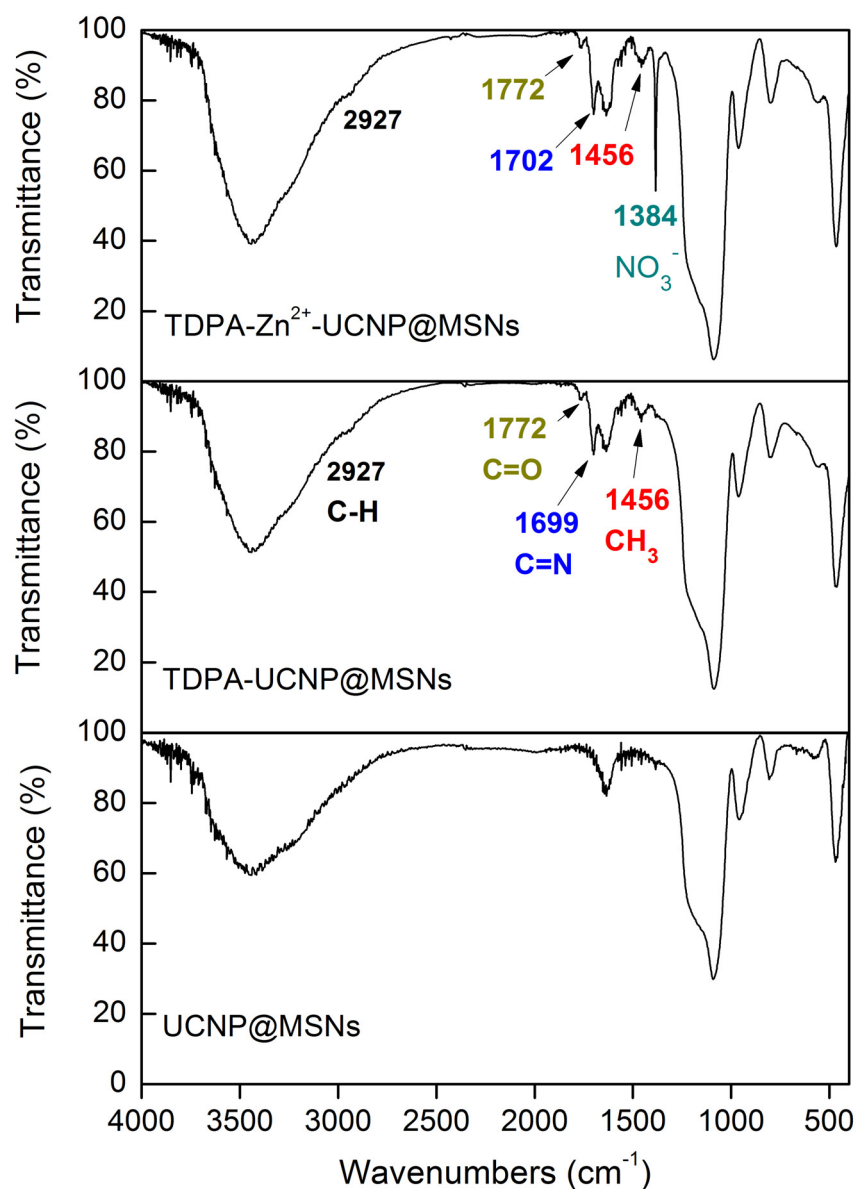


Figure S3. FTIR spectra characterizations of the UCNP@MSNs, TDPA-UCNP@MSNs and TDPA-Zn²⁺-UCNP@MSNs.

NOTE: Comparing to UCNP@MSNs, the TDPA-MSNs showed the absorption peaks at 2927 cm⁻¹ (C-H), 1772 cm⁻¹ (C=O, ester), 1699 cm⁻¹ (C=N) and 1456 cm⁻¹ (CH₃), indicating the successful conjugation of TDPA on the MSNs surface. The chelating with Zn²⁺ induced a new sharp peak at 1384 cm⁻¹, which may be due to the NO₃⁻ from the material Zn(NO₃)₂.

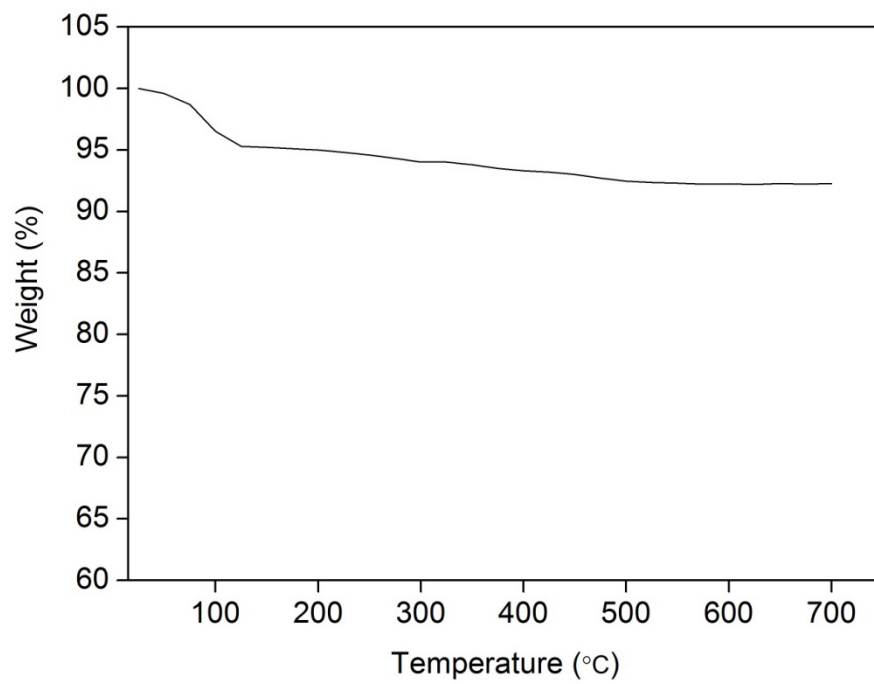


Figure S4. Thermogravimetric analysis of TDPA-UCNP@MSN.

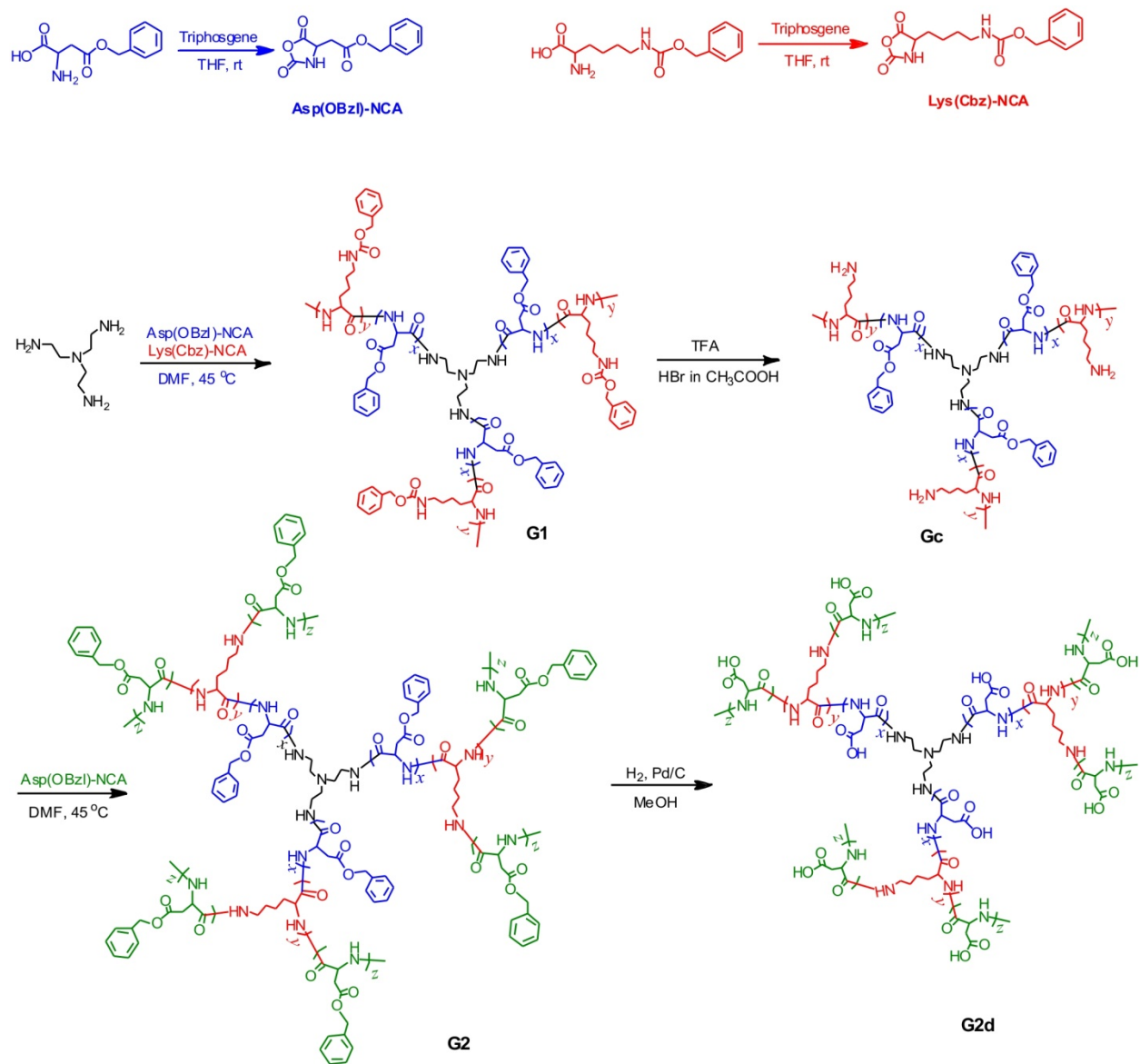


Figure S5. Synthesis of branched polypeptide peptide poly(Asp-Lys)-*b*-Asp via two generations ring opening metathesis polymerization (ROMP).

Conjugation of small functional molecules to polypeptide

The branched polypeptide **G2d** has abundant terminal amino groups (-NH₂) which can be used for further modification of polypeptide with functional molecules, such as targeting ligand folic acid (FA) and fluorescence dye TRITC. These amino groups can also be utilized to develop a fluorescamine based fluorescence assay for the calculation of the mean molecular weight and component ratio of the polypeptide. More details see below discussion.

(1) Labeling the polypeptide **G2d** with folic acid (FA), FA-**G2d**

To a 10 mL dry DMF in ice bath was added 44 mg of folic acid, 2 mg of DMAP, 12 mg of NHS and 20 mg of EDC·HCl. The solution was stirred at 0 °C for 30 min then was recovered to room temperature and stirred for another 3 h. Thereafter 200 mg of **G2d** was added and the mixture was further stirred overnight. After that, 20 mL acetone was added to precipitate the polypeptide. The polypeptide was collected by centrifugation and washed 3 times with methanol. After dried in vacuum, the **FA-G2d** was obtained as a slight yellow solid and characterized by UV-vis absorption spectrum, which showed the characteristic absorption peak of FA at 290 nm.

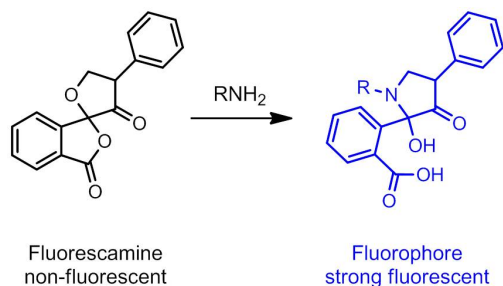
(2) Labeling the polypeptide **G2d** with TRITC

TRITC labeled polypeptide **G2d** was prepared by the conjugation of TRITC to the terminal NH₂ on polypeptide branches. Typically, to a 10 mL dry DMF solution was added of 1 mg of TRITC and 100 mg of **G2d**. The mixture was stirred overnight. After that, 20 mL acetone was added to precipitate the polypeptide. The polypeptide was collected by centrifugation and washed 3 times with methanol. After dried in vacuum, the **TRITC-G2d** was obtained as a slight pink solid and characterized by fluorescence emission spectrum, which showed the characteristic emission of TRITC at 570 nm.

Measurement of mean molecular weight (M_w) and calculation of the component ratio of the block polypeptide through fluorimetry combined with ¹H-NMR analysis

The polypeptide **G1** contains 3 main backbones and each backbone consist of x molecule of Asp and y molecule of Lys. The ratio of Asp to Lys ($x : y$) in **G1** can be calculated *via* ¹H-NMR analysis (See Figure S13 for an example). The signal at 1.25-1.78 ppm is due to the β , γ and δ -CH₂ for Lys (6H), while the signal at 4.65 ppm is due to the CH of Asp (1H). Thus using the integration area of these two peaks, the ratio $x : y$ can be calculated. However, due the overlap with the solvent (DMSO-*d*₆, δ 2.54 ppm) and water (δ 3.2 ppm), the signal for CH₂ in TAEA cannot be isolated, so the number of Asp and Lys cannot be quantified by using ¹H-NMR.

Herein, a fluorescence analysis method based on using fluorescamine was developed to calculate the mean molecular weight (M_w) of the synthetic polypeptide. Fluorescamine is a commercial and widely used chemical reagent for bioanalysis. Fluorescamine is non-fluorescent but its fluorescence can be turned on once it reacts with a primary amine group ($-NH_2$), as shown below. Thus the amount of amine group in the synthetic polypeptide can be quantified by using fluorescamine *via* fluorimetry.



(1) Measurement of the mean molecular weight of polypeptide **G1**, $M_w(\mathbf{G1})$

Polypeptide **G1** is tri-branched and there is a terminal NH_2 group in each branch, so each **G1** has 3 NH_2 groups. Thus

$$n_{G1} = n_{G1}(NH_2) / 3 \quad (1)$$

where n_{G1} is the molar amount of m_{G1} (g, weight) of polypeptide **G1** and $n_{G1}(NH_2)$ is the molar amount of NH_2 in polypeptide **G1**, which can be determined by using fluorescamine *via* fluorimetry. Based on it, the $M_w(\mathbf{G1})$ can be calculated by:

$$M_w(\mathbf{G1}) = m_{G1} / n_{G1} = 3 m_{G1} / n_{G1}(NH_2) \quad (2)$$

(2) Measurement of the mean molecular weight of polypeptide core, $M_w(\mathbf{Gc})$

The $M_w(\mathbf{Gc})$ can be directly achieved by the weight of polypeptide after deprotection of **G1**. For example, m_1 g of polypeptide **G1** was introduced to the HBr/CH_3COOH solution for removing the carbobenzyloxy protecting group (Cbz) on $\epsilon-NH_2$ groups in Lys. Thereafter, m_2 g of polypeptide core was recovered. The yield of the deprotection was assumed to be 100%, so there will be

$$n_{Gc} = n_{G1} = m_1 / M_w(\mathbf{G1}) \quad (3)$$

n_{Gc} is the molar amount of polypeptide core.

The $M_w(\mathbf{Gc})$ can be calculated by:

$$M_w(\mathbf{Gc}) = m_2 / n_{Gc} = M_w(\mathbf{G1}) \cdot m_2 / m_1 \quad (4)$$

(3) Calculation of the monomer ratio of Asp to Lys ($x : y$) in polypeptide **G1**

We can also calculate the Lys amount and the monomer ratio of Asp to Lys ($x : y$) in **G1** and **Gc** based on above information. The weight loss between $M_w(\text{Gc})$ and $M_w(\text{G1})$ is due to the removal of Cbz group from Lys. Removal of 1 mol Cbz group will induce a weight loss of 134 g. Thus, the number of Lys in the backbone of polypeptide **G1** (y) can be calculated by:

$$y = [M_w(\text{G1}) - M_w(\text{Gc})] / (134 \cdot 3) \quad (5)$$

Each **G1** is consist of 1 molecule of Tris(2-aminoethyl)amine (TAEA), $3x$ molecule of Asp(OBzl) residue and $3y$ molecule of Lys(Cbz) residue, the $M_w(\text{G1})$ can also be calculated by:

$$M_w(\text{G1}) = M_w(\text{TAEA}) + 3x \cdot M_w[\text{Asp}(\text{OBzl})] + 3y \cdot M_w[\text{Lys}(\text{Cbz})] - 3(x+y) \cdot M_w(\text{H}_2\text{O}) \quad (6)$$

The $M_w(\text{TAEA}) = 146.23 \text{ g} \cdot \text{mol}^{-1}$, $M_w[\text{Asp}(\text{OBzl})] = 223.23 \text{ g} \cdot \text{mol}^{-1}$, $M_w[\text{Lys}(\text{Cbz})] = 280.32 \text{ g} \cdot \text{mol}^{-1}$, $M_w(\text{H}_2\text{O}) = 18 \text{ g} \cdot \text{mol}^{-1}$, thus

$$x = [M_w(\text{G1}) - 146.23 - 894.96 \cdot y] / 723.69 \quad (7)$$

By using the value of $M_w(\text{G1})$ obtained in eq. 2, and y obtained in eq. 4, the number of Asp in each backbone of **G1** (x) can be obtained from eq. 7.

(4) Measurement of the mean molecular weight of polypeptide **G2**, $M_w(\text{G2})$

Each polypeptide **G2** has two types of terminal NH_2 , the 3 NH_2 on the tri-branched main backbone and $3y$ NH_2 in the side chains which initiated by Lys residues. So using the similar method for calculating $M_w(\text{G1})$, here we will have

$$M_w(\text{G2}) = m_{\text{G2}} / n_{\text{G2}} = (3 + 3y) m_{\text{G2}} / n_{\text{G2}}(\text{NH}_2) \quad (8)$$

Where m_{G2} is the weight of **G2** and $n_{\text{G2}}(\text{NH}_2)$ is the amount of NH_2 in **G2** which can be obtained *via* fluorescamine based fluorescence assay.

(5) Measurement of the mean molecular weight of polypeptide **G2d**, $M_w(\text{G2d})$

Similar to the measurement of $M_w(\text{Gc})$, the $M_w(\text{G2d})$ can be directly achieved by the weight of polypeptide after deprotection of **G2**. For example, m_1 g of polypeptide **G2** was introduced to remove the benzyl protecting group (OBzl) on D -COOH groups in Asp. After that, m_2 g of final polypeptide was recovered. The $M_w(\text{G2d})$ can be calculated by:

$$M_w(\text{G2d}) = m_2 / n_{\text{G2d}} \quad (9)$$

n_{G2d} is the molar amount of polypeptide **G2d**. As the yield of the deprotection can be achieved at 100%, which can be confirmed by $^1\text{H-NMR}$ spectrum where the characteristic signal OBzl group (δ 7.2ppm, 5.1 ppm) disappear after de-protection. Thus there will be

$$n_{\text{G2d}} = n_{\text{G2}} = m_1 / M_w(\text{G2}) \quad (10)$$

thus,

$$M_w(\text{G2d}) = m_2 / n_{\text{G2d}} = M_w(\text{G2}) \cdot m_2 / m_1 \quad (11)$$

(6) Calculation of the number of Asp in each side chain (z) in polypeptide G2d

We can calculate the Asp amount in each side chain (z) in **G2d** based on a similar method to the calculation of x, y in procedure 3. The difference in mean molecular weight of **G2** and **G2d** is due to the removal of OBzl groups on all Asp. Each G2 contains 2 types of Asp, the $3(x+z)$ molecule of Asp in the backbones and the $3yz$ molecule of Asp in the side chains. Thus, each polypeptide **G2d** has $3[x+(y+1)z]$ molecule of Asp. Removal of 1 mol OBzl group in H-Asp(OBzl)-OH will induce a weight loss of 90 g. Thus the total number of Asp in **G2d** can be calculated by:

$$3[x+(y+1)z] = [M_w(\text{G2}) - M_w(\text{G2d})] / 90 \quad (12)$$

with the x obtained from eq. 7, and the y obtained from eq. 5., the Asp amount in each side chain (z) can be obtained by using eq. 12.

Four polypeptide G2di ($i = 1, 2, 3$ and 4) were prepared in this project and the main calculations are listed in Table 1.

Calculation of drug loading amount of the UCNP@MSN

The loading efficiency of drugs loaded into the pores of UCNP@MSNs can be measured by the following method. To measure the loading efficiency, TDPA-Zn²⁺-UCNP@MSNs (a mg) and drug (b mg) were dispersed in 5 mL pH7.4 HEPES solution and stirred at room temperature for 2 h. Then the nanoparticles were collected by centrifugation (supernatant also was collected) and were re-dispersed into a HEPES solution of polypeptide (G2di) with sonication. The drug-loaded nanoparticles were isolated by centrifugation and was washed with pH7.4 HEPES. The supernatants were combined and the amount of unloaded drug was determined to be c mg by UV-vis absorption spectrum. Thus the amount of drug loaded into the pores of UCNP@MSN can be calculated by $(b-c)$ mg drug / a mg of particle. The solvent using for loading CPT is methanol.

Based on this approach, the fluorescein loading amount using our UCNP@MSN with a total size of 54 nm and a thickness of 12 nm MSN shell was calculated to be 13.2 mg fluorescein /g of UCNP@MSN. And the loading amounts for DOX and CPT was calculated to be 27.6 mg DOX/ g of UCNP@MSN and 19.5 mg CPT/ g of UCNP@MSN.

Calculation of the number of drug loaded in single UCNP@MSNs

We assumed the UCNP@MSN used in this work has spherical structure and has a UCNP core with diameter of 30 nm and a MSN shell with thickness of 12 nm. The density (d) for hexagonal NaYF₄ is 4.2 g/cm³, and for MSN is around 0.9 g/cm³. Thus a single UCNP@MSN in this work will have a weight of

1.21×10^{-16} g ($m = m_{\text{UCNP}} + m_{\text{MSN}} = d_{\text{UCNP}} \cdot V_{\text{UCNP}} + d_{\text{MSN}} \cdot V_{\text{MSN}}$, where V is the volume), and there will be 0.82×10^{16} particles per gram. Thus,

(1) for fluorescein, the loading amount per particle is $13.2 \text{ mg} / (0.82 \times 10^{16})$, which is 1.61×10^{-18} g, 4.84×10^{-21} mol, 2900 molecules.

(2) for Dox, the loading amount per particle is $27.6 \text{ mg} / (0.82 \times 10^{16})$, which is 3.34×10^{-18} g, 5.76×10^{-21} mol, 3400 molecules.

(3) for CPT, the loading amount per particle is $19.5 \text{ mg} / (0.82 \times 10^{16})$, which is 2.38×10^{-18} g, 6.8×10^{-21} mol, 4100 molecules.

Measurement of ATP responsive drug release from polypeptide wrapped nanoparticles

100 mg polypeptide wrapped drug loaded UCNP@MSNs were dispersed in 50 mL HEPES solution (0.2 wt%). Later, different amounts of ATP (0.1mM, 1mM, 5 mM and 10 mM) were added to the suspension under stirring. After a set time point, a portion (1 mL) of the solution was took out and the upconversion luminescence (UCL) of the solution was collected directly using fluorometer. Thereafter, the solution (1 mL) was treated with centrifugation and the supernatant was collected and measured with fluorometer (for DOX and fluorescein) or absorption spectrometer (for CPT) to determine the concentration of released drugs.

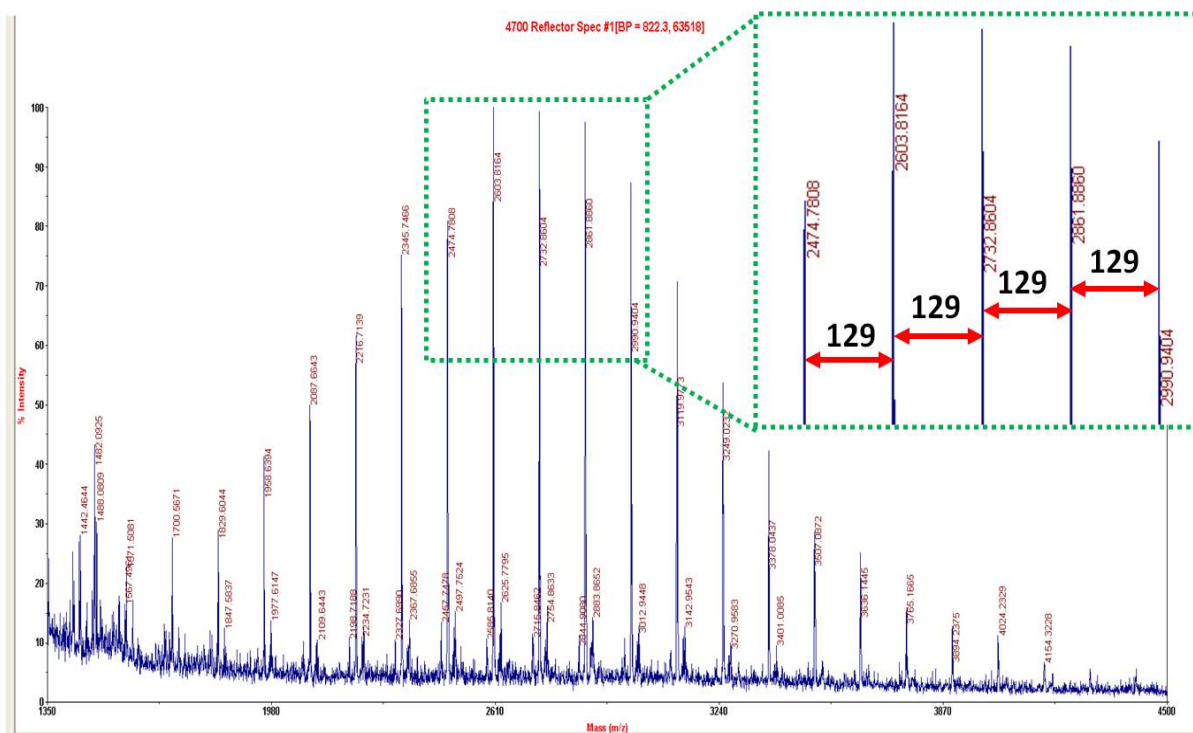


Figure S6. MALDI-TOF characterizations of polypeptides core, Gc.

NOTE: The mass difference between two peaks is 129, which clearly indicates the loss of Lys residue from the polypeptide under MALDI condition. This result demonstrated that the polypeptide core indeed has lysine residue with activated NH_2 group.

Table S1. DLS size and ζ -potential characterizations of the nanoparticles.*

Nanoparticles	DLS Size (nm)	ζ potential (mV)
UCNP@MSNs	56 \pm 3	-18.6
TDPA-UCNP@MSNs	63 \pm 3	-8.7
TDPA-Zn ²⁺ -UCNP@MSNs	65 \pm 4	+22.5
TDPA-Zn ²⁺ -UCNP@MSN@G2d2	87 \pm 6	-25.4
TDPA-Zn ²⁺ -UCNP@MSN@G2d4	91 \pm 7	-32.1

* Nanoparticles were dispersed in aqueous solution for DLS size distribution (number) and ζ potential measurement.

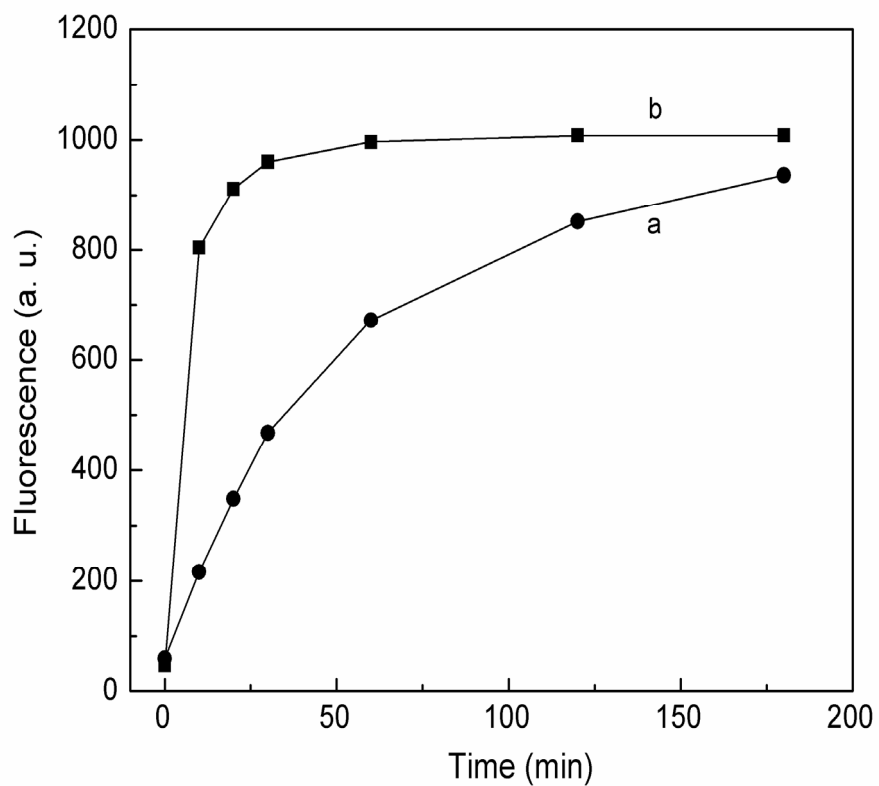


Figure S7. Release profiles of fluorescein (a) and surface bond polypeptide (b).

NOTE: The rapid release curve shows that polypeptide release precedes the release of the fluorescein. These results further demonstrate that the triggered release of fluorescein is due to uncapping entrances by ATP through the replacement of polypeptide on outer surface of TDPA-Zn²⁺-UCNP@MSNs.

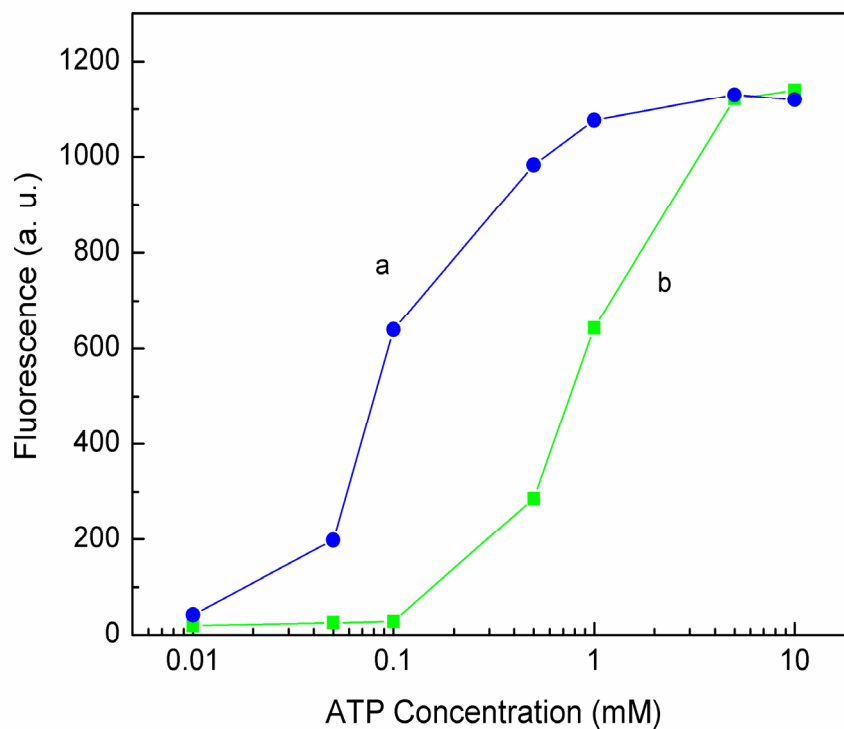


Figure S8. ATP concentration-dependent fluorescein release properties of polypeptide G2d2-wrapped TDPA-Cu²⁺-UCNP@MSNs (a) and TDPA-Zn²⁺-UCNP@MSNs (b), respectively.

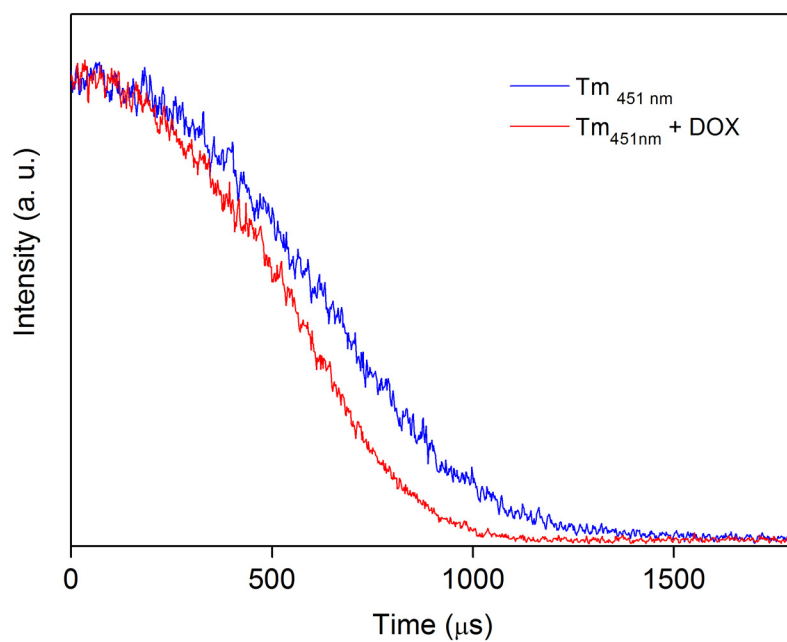


Figure S9. Upconversion luminescence decay of Tm (451 nm) in the UCNP@MSN before and after loading with DOX (1 wt% dispersion of nanoparticle in 10 mM pH7.4 HEPES solution). Excitation at 980 nm, $80 \text{ W}\cdot\text{cm}^{-2}$.

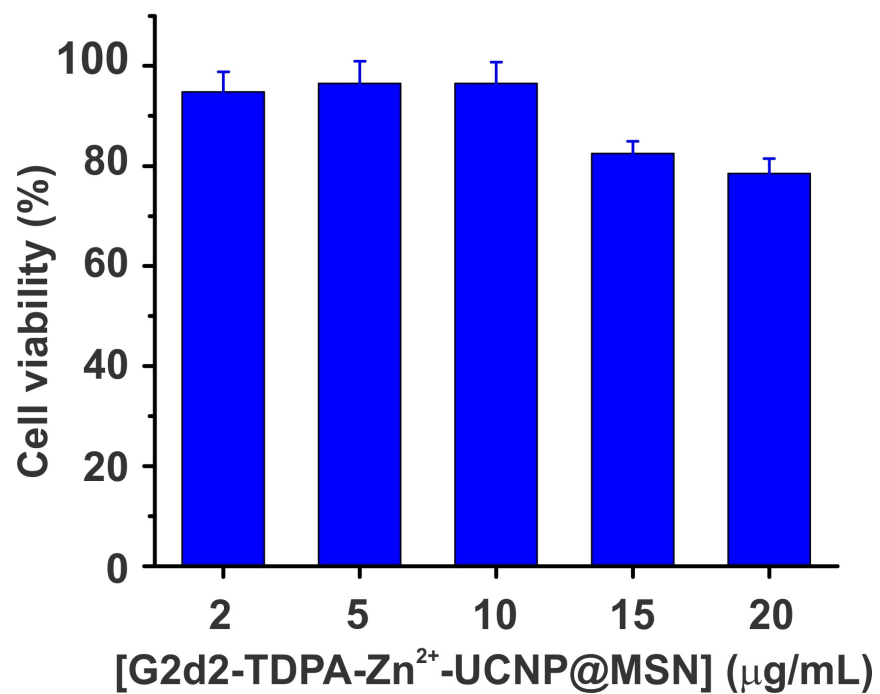


Figure S10. (A) Measurement of polypeptide-wrapped nanoparticles concentration-dependent HeLa cell viability using MTS assay (G2d2-TDPA-Zn²⁺-UCNP@MSNs).

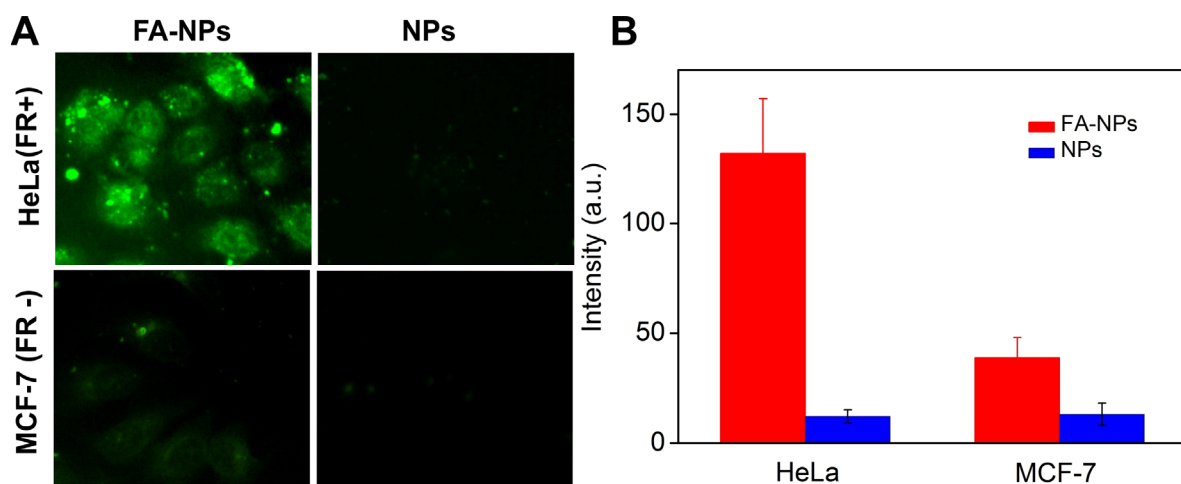


Figure S11. Targeting capability of folic-acid modified and unmodified TDPA-Zn²⁺-UCNP@MSNs (load with fluorescein) in HeLa (folate receptor-positive) and MCF-7 (folate receptor-negative) cells. The fluorescence imaging (A) and the quantification of the fluorescence intensity of the cells (B).

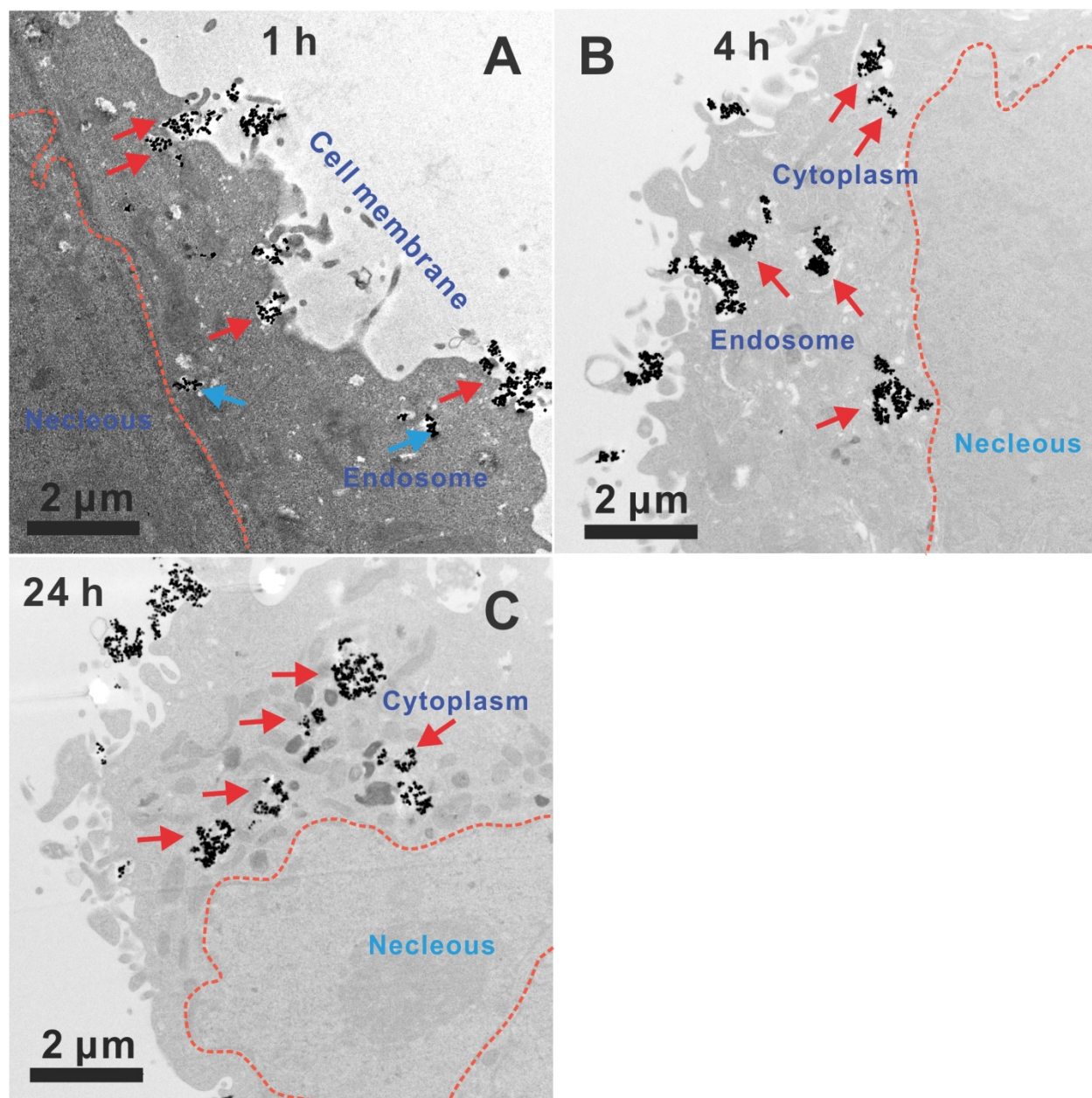


Figure S12. TEM images characterizing the cellular uptake and distribution of our nanoparticles at different time points (1h, 4h, 24h).

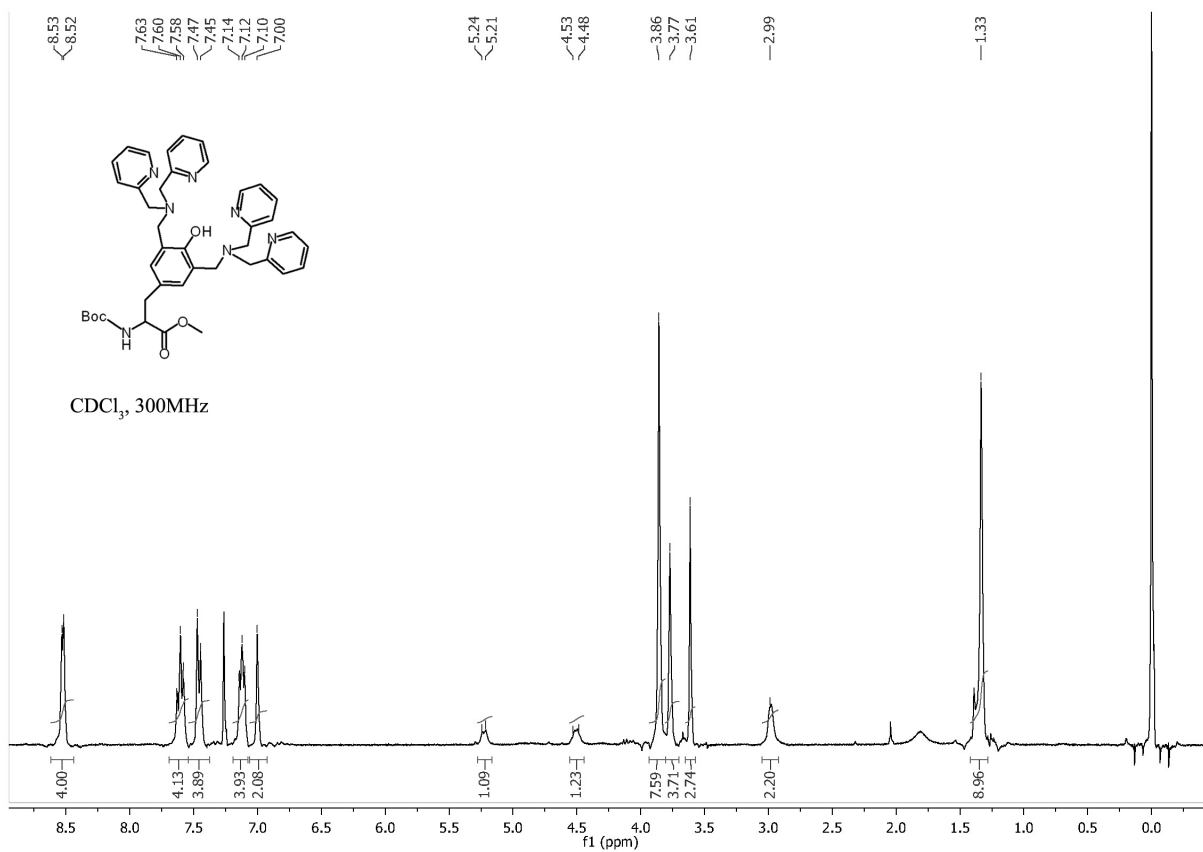


Figure S13. ¹H-NMR spectrum of Boc-TDPA (CDCl₃, 300 MHz).

TDPA_1008_3#43 RT: 1.08 AV: 1 NL: 2.77E5
T: +c Full ms [150.00-2000.00]

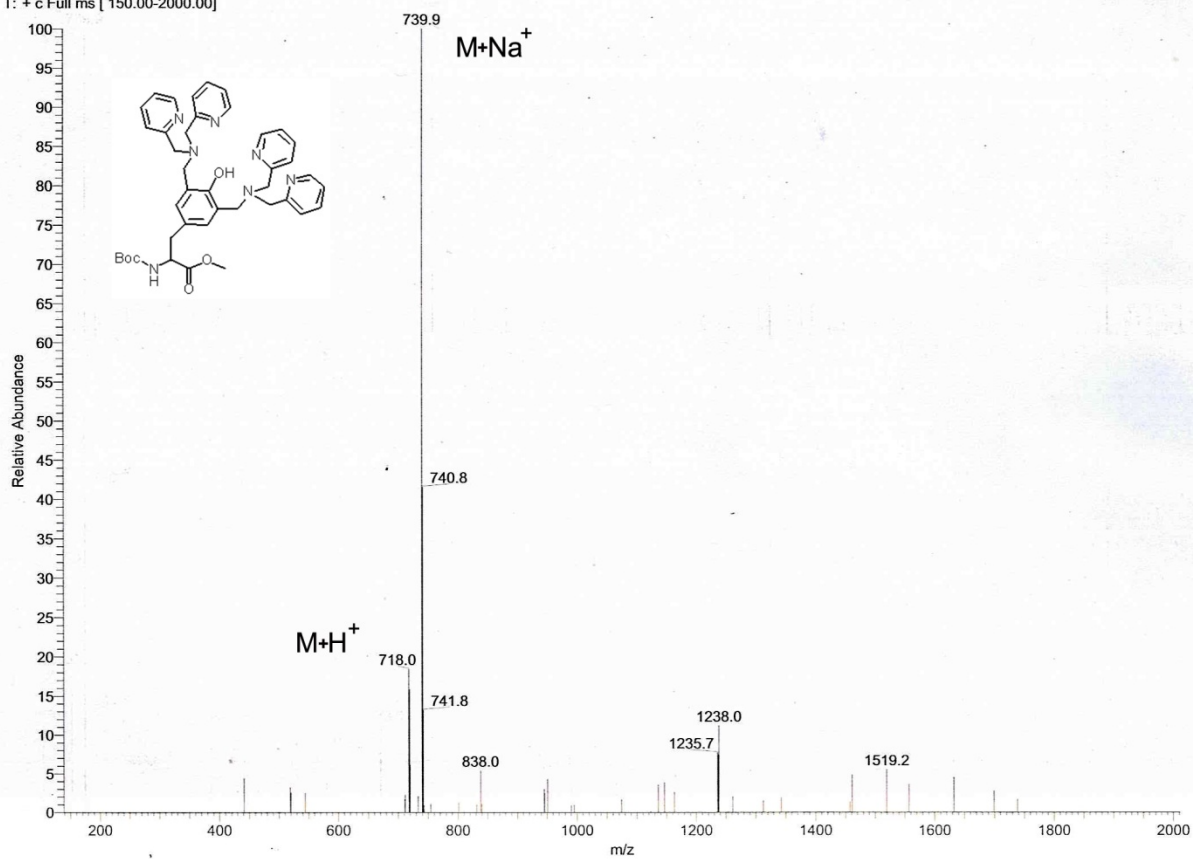


Figure S14. ESI-MS spectrum of Boc-TDPA.

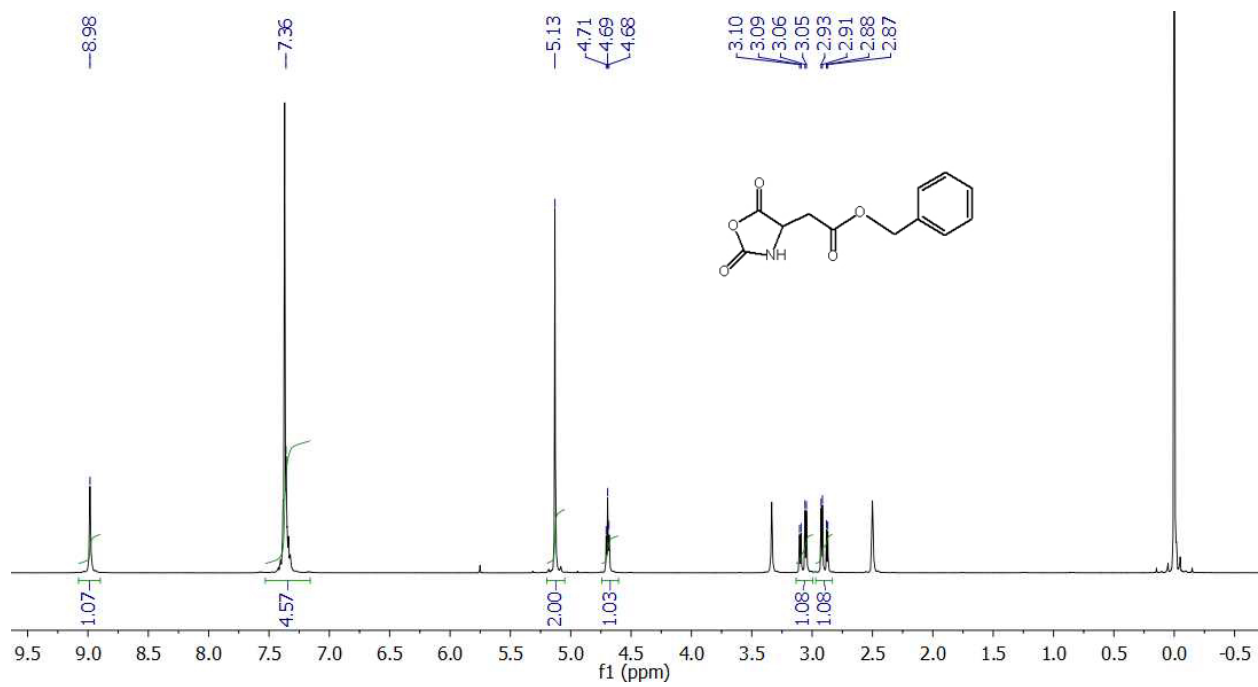


Figure S15. $^1\text{H-NMR}$ spectrum of L-Asp(OBzl)-NCA ($\text{DMSO-}d_6$, 400 MHz).

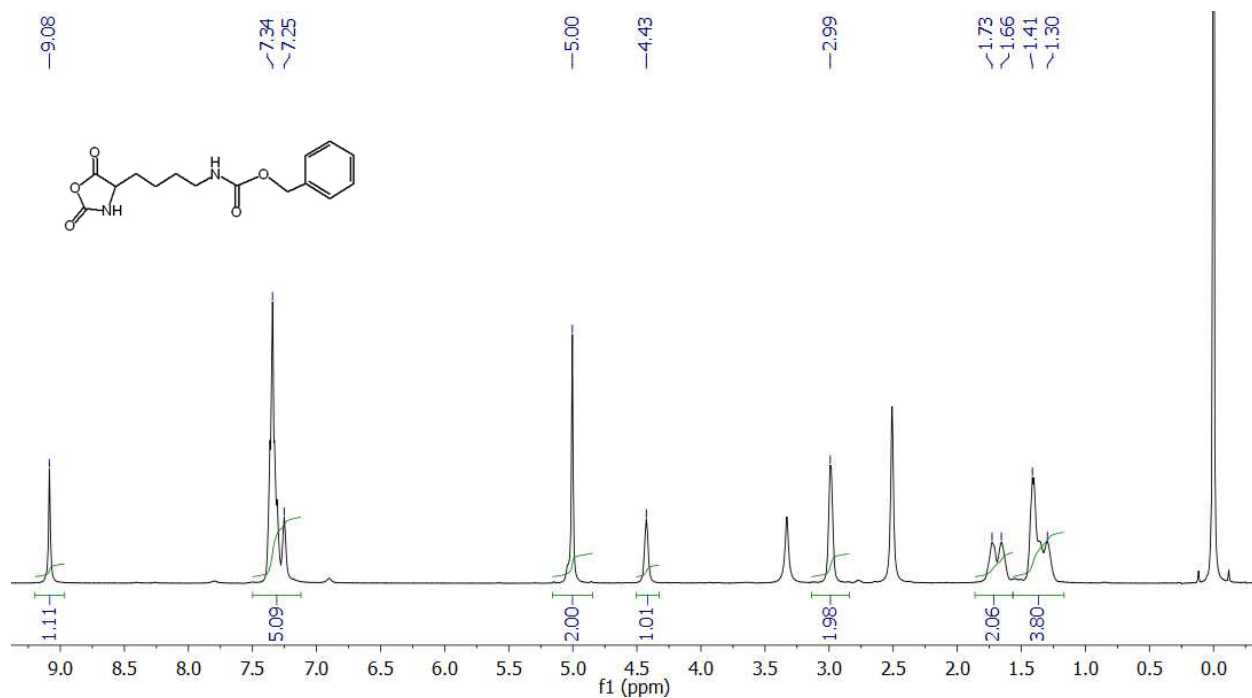


Figure S16. ¹H-NMR spectrum of L-Lys(Cbz)-NCA, (DMSO-d₆, 500 MHz).

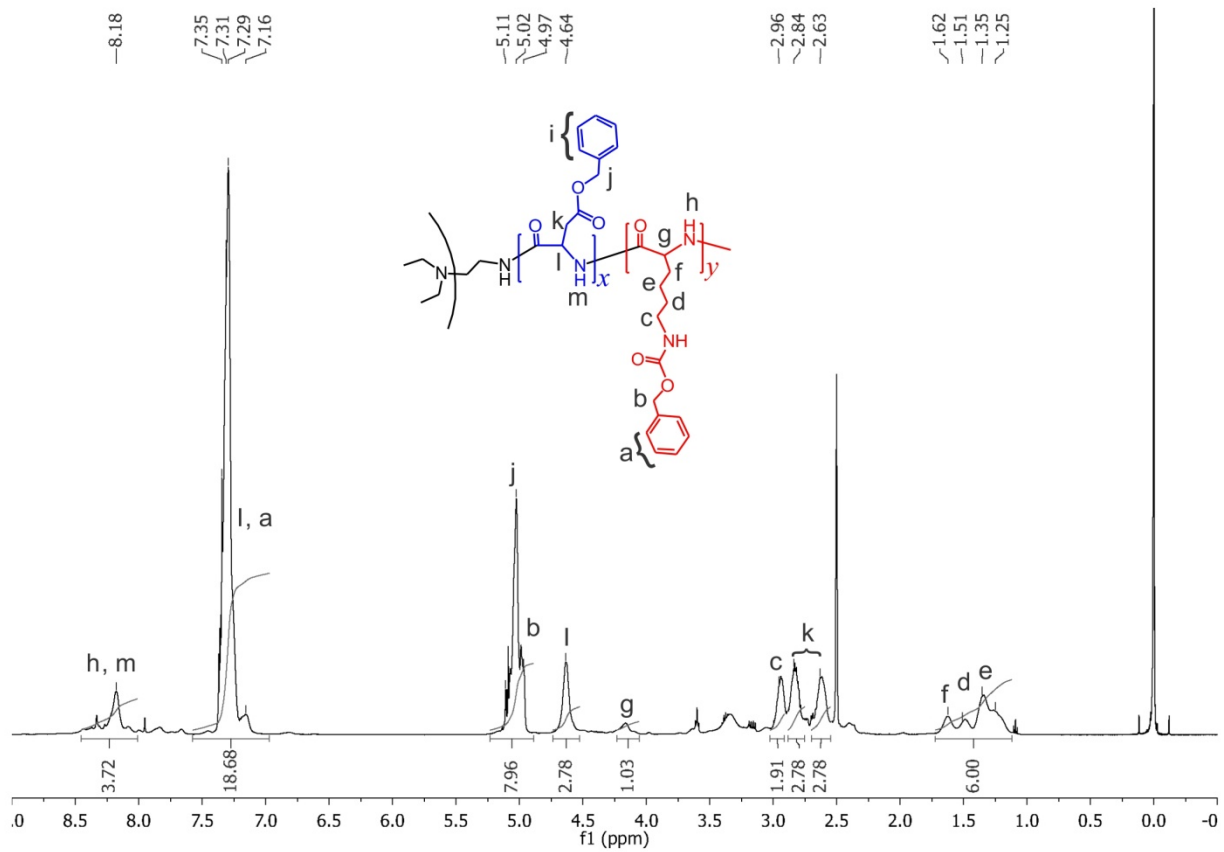


Figure S17. $^1\text{H-NMR}$ spectrum of poly[Asp(Obzl)-Lys(Cbz)] (G1), (DMSO- d_6 , 500 MHz).

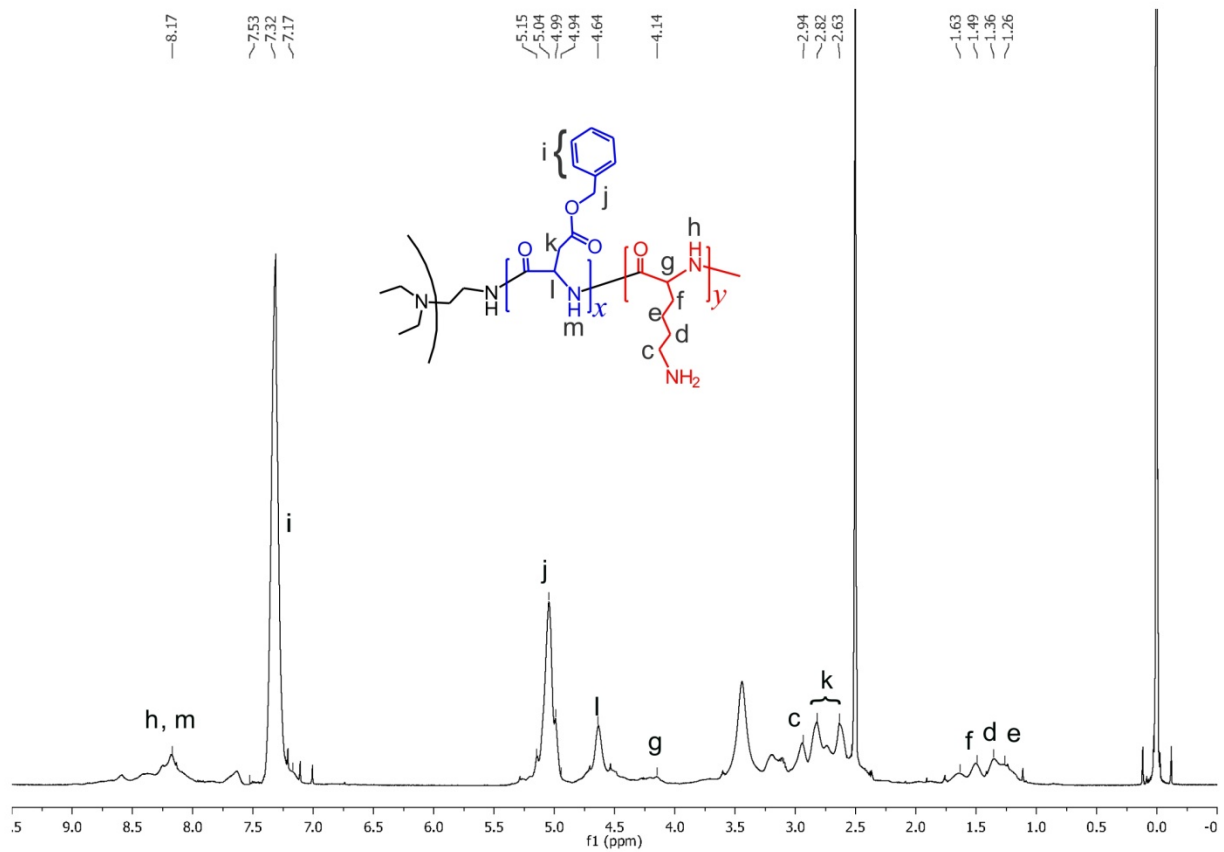


Figure S18. $^1\text{H-NMR}$ spectrum of poly[Asp(Obzl)-Lys] (Gc), ($\text{DMSO-}d_6$, 500 MHz).

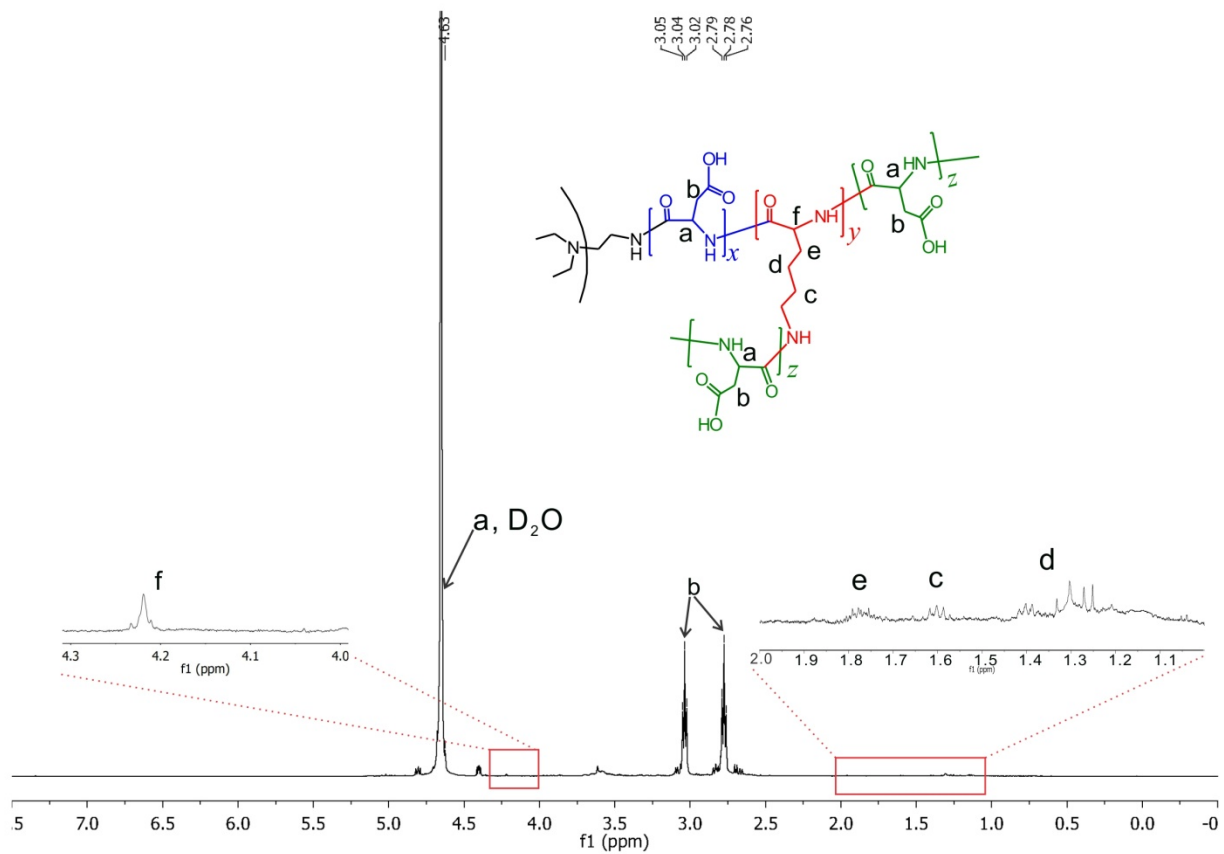


Figure S20. ¹H-NMR spectrum of poly(Asp-Lys)-*b*-Asp (G2d), (D₂O, 500 MHz).