Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Versatile fully-substituted triazoles functionalized polypeptides with stable α-helical conformation for gene delivery

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Experimental Section

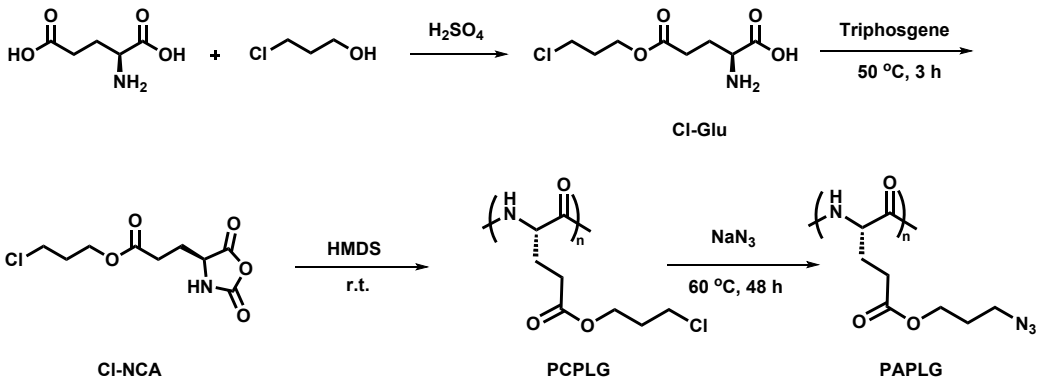
Materials, cell lines and methods

All chemicals were purchased from Energy Chemical and used as received unless otherwise specified. 3-Chloro-1-propanol was purchased from Macklin Chemical. Poly-$_L$-lysine (PLL), 1-dimethylamino-2-propyne and fluorescein isothiocyanate (FITC) were purchased from Aladdin Chemical. Polyethyleneimine (PEI-25k) was purchased from J&K Scientific company. Plasmid DNA encoding green fluorescence protein (pGFP) or luciferase (pLuc) were obtained using the endotoxin-free plasmid purification kit purchased from TIANGEN (Beijing, China). Hep1-6 cells (mouse liver cancer cells), 293T cells (human kidney epithelial cells) and HeLa cells (human cervix adenocarcinoma cells) were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) under a 5% CO_2-containing humidified atmosphere at 37 ^oC. ^1H NMR spectra were recorded on a Bruker AVANCE II 400 NMR spectrometer. The number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersities ($Đ$) of polymers were determined by gel permeation chromatography (GPC) equipped with an isocratic pump (model 515, Waters Corporation) (Standard: PMMA). DMF containing 0.01 M LiBr was used as the eluent at 45 °C at a flow rate of 1.0 mL·min^{-1}. Absolute M_n and $Đ$ of the polymers were measured by GPC analysis at 35 °C and a flow rate of 1.0 mL/min on an Agilent 1260 instrument with multi-angle laser light scattering (MALLS) detectors. THF was used as the eluent and the sample concentration was 5 mg/mL. The injection volume was 50 μL. The curve was calibrated using monodisperse polystyrene standards covering the molecular weight range from 580 to 460000 Da. Circular dichroism (CD) measurements were carried out on a JASCO J810 CD spectrometer. The polypeptides aqueous solution was placed in a quartz cell with a path length of 1.0 cm and measured at room temperature. Fourier transform infrared spectrometer (FT-IR) spectra were recorded on a Thermo Scientific Nicolet 6700 FTIR spectrometer. The expression of

GFP was tested by observing the green fluorescence using inverted fluorescence microscope (IX83, Olympus Corporation). The quantitative gene transection was evaluated using Luciferase assay kit purchased from Promega (Madison, WI, USA).

Synthesis of azide-bearing polypeptides (PAPLG)

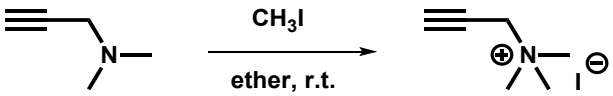
PAPLG with azide side chains was synthesized following the literature reported method.[1] (**Figures S1-S4**).



Scheme S1. Synthetic route of PAPLG.

Synthesis of *N,N,N*-trimethylpropargylammonium iodide (TPAI)

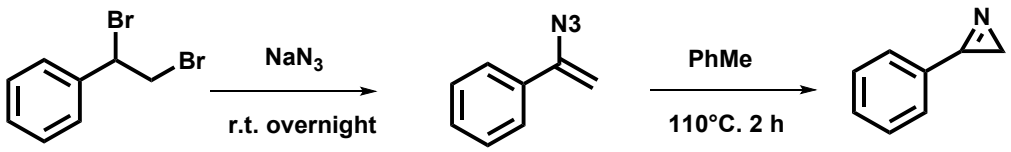
The alkyne-functionalized ammonium salts, TPAI, was synthesized through the methylation of 3-dimethylamino-1-propyne according to the literature reported method [2]**(Figure S5).**



Scheme S2. Synthetic route of TPAI.

Synthesis of 3-phenyl-2*H*-azirine

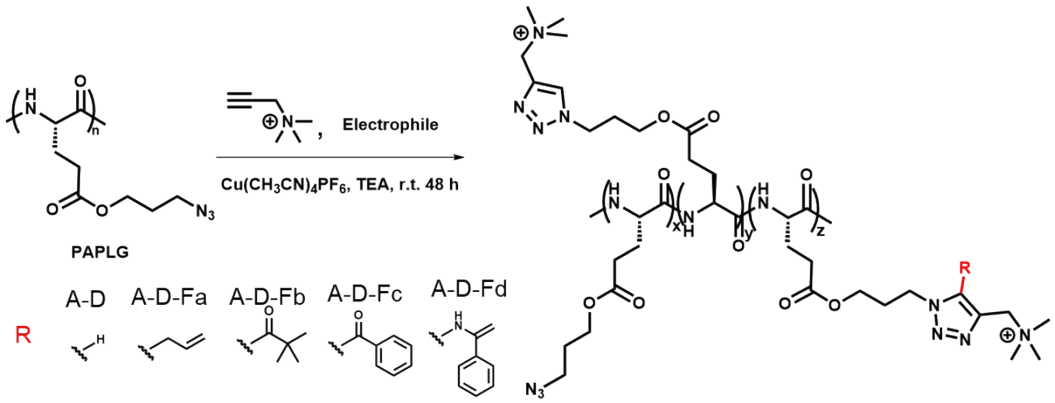
3-Phenyl-2H-azirine was synthesized following the literature.[3] **(Figure S6).**



Scheme S3. Synthetic route of 3-phenyl-2*H*-azirine.

Synthesis of polypeptides with triazole side chains.

1,4,5-fully substituted triazole polypeptides (A-D-Fa, A-D-Fb, A-D-Fc, and A-D-Fd) were synthesized *via* Cu-catalyzed interrupted click reaction starting from PAPLG bearing azide side chains, terminal alkyne (TPAI), and a library of electrophiles. Generally, PAPLG (10 mg, 0.047 mmol of side-chains azide groups, 1 equiv.), TPAI (11.7 mg, 0.052 mmol, 1.1 equiv.), various electrophiles (0.141 mmol, 3 equiv.), $Cu(MeCN)_4PF_6$ (17.5 mg, 0.047 mmol, 1 equiv.), and triethylamine (TEA) (7.8 uL, 0.0564 mmol, 1.2 equiv.) were dissolved in anhydrous DMF (600 μL) in a glove box. The solution was stirred at room temperature for 48 h. The resulting solution was precipitated in ether and washed for three times (6 mL × 3). The crude product was collected by centrifugation and the solvent was removed under vacuum. The resulting solid was dialyzed in a dialysis membrane (MWCO = 1 kDa) against EDTA/NaCl solution for 2 h to remove the copper salts and promote anion exchange, and then against deionized (DI) water for 2 days (water changed thrice every day). The final polypeptides containing 1,4,5-fully substituted triazoles were obtained as light yellow or light green powders after lyophilization (75-95% yield). The side chains of final polypeptides contain three components: unreacted azides, 1,4-disubstituted-1,2,3-triazoles (DT), and 1,4,5-fully-substituted-1,2,3-triazoles (FT). The general structure was shown in **Scheme S4** and x, y and z represented the percentage of corresponding side chains.



Scheme S4. Synthetic route of 1,4,5-fully substituted triazole polypeptides.

Polypeptides A-D-Fa was synthesized using 3-bromopropene as electrophile (86-92% yield), ^{1}H NMR (D_2O): Taking A50-D3-Fa47 as an example: δ 8.35 (s, *triazole*-, 1H), 5.81 (s, -CH_2CH=CH_2-, 1H), 5.12 and 4.90 (s, -CH=CH_2-, 2H), 4.56 (s, -$CH_2N^+(CH_3)_3$, 2H), 4.36 (s, -$CH_2CH_2CH_2$-, 2H), 4.05 (s, α-*H* and -$CH_2CH_2CH_2$-, 3H), 3.61 (s, -triazole-CH_2CH=CH_2, 2H), 3.30 (s, -$CH_2CH_2N_3$, 2H), 3.08 (s, -$CH_2N^+(CH_3)_3$, 9H), 2.56-1.83 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H). ^{1}H NMR of A50-D3-Fa47 with different component ratios were shown in **Figure S7-S14**.

Polypeptides A-D bearing 1,4-disubstituted-1,2,3-triazole was prepared following the literature[4] **(Figure S15-S16)**.

A0-D100, ^{1}H NMR (D_2O): δ 8.35 (s, -*triazole*-, 1H), 4.56 (s, -$CH_2N^+(CH_3)_3$, 2H), 4.50 (s, -$CH_2CH_2CH_2$-, 2H), 4.27 (s, α-*H*, 1H), 4.00 (s, -$CH_2CH_2CH_2$-, 2H), 3.06 (s, -$CH_2N^+(CH_3)_3$-, 9H), 2.39-1.92 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H).

A50-D50, ^{1}H NMR (D_2O): δ 8.35 (s, -*triazole*-, 1H), 4.56 (s, -$CH_2N^+(CH_3)_3$, 2H), 4.36 (s, -$CH_2CH_2CH_2$-, 2H), 4.05 (s, α-*H* and -$CH_2CH_2CH_2$-, 3H), 3.30 (s, -$CH_2CH_2N_3$, 2H), 3.08 (s, -$CH_2N^+(CH_3)_3$-, 9H), 2.56-1.83 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H).

Polypeptides A-D-Fb was synthesized using trimethylacetyl chloride as electrophile (75% yield), ^{1}H NMR (D_2O): δ 8.35 (s, -*triazole*-, 1H), 4.67 (s, -$CH_2N^+(CH_3)_3$, 2H), 4.48 (s, -$CH_2CH_2CH_2$-, 2H), 4.02 (s, α-*H* and -$CH_2CH_2CH_2$-, 3H), 3.30 (s, -$CH_2CH_2N_3$, 2H), 3.18 (s, -$CH_2N^+(CH_3)_3$, 9H), 2.59-1.84 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H), 1.15 (s, -$CO(CH_3)_3$, 9H). ^{1}H NMR of A-D-Fb was shown in

Figure S17.

Polypeptides A-D-Fc was synthesized using benzoyl chloride as electrophile (78% yield), ^{1}H NMR (D_2O): δ 8.35 (s, -*triazole*-, 1H), 7.73-7.53 (d, COC_6H_5, 5H), 4.53 (d, -$CH_2N^+(CH_3)_3$ and -$CH_2CH_2CH_2$-, 4H), 3.97 (d, α-*H* and -$CH_2CH_2CH_2$-, 3H), 3.27 (s, -$CH_2CH_2N_3$, 2H), 3.03 (d, -$CH_2N^+(CH_3)_3$, 9H), 2.52-1.80 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H). ^{1}H NMR of A-D-Fc was shown in **Figure S18**.

Polypeptides A-D-Fd was synthesized using 3-phenyl-2H-azirine as electrophile (90-95% yield), For A0-D0-Fd100, ^{1}H NMR (D_2O): δ 7.93-7.39 (m, -NHC_6H_5-, 5H), 4.52 (s, -$CH_2N^+(CH_3)_3$, 2H), 4.11 (s, -$CH_2CH_2CH_2$-, 2H), 3.82 (d, α-*H* and -$CH_2CH_2CH_2$-, 3H), 3.48 (s, -NH(C_6H_5)CCH_2, 2H), 3.06 (s, -$CH_2N^+(CH_3)_3$, 9H), 2.33-1.68 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H). For A50-D0-Fd50, ^{1}H NMR (TFA-*d*): δ 8.01-7.52. (m, -NHC_6H_5-, 5H), 4.86 (s, -$CH_2N^+(CH_3)_3$, 2H), 4.64 (s, -$CH_2CH_2CH_2$-, 2H), 4.47 (d, α-*H* and -$CH_2CH_2CH_2$-, 3H), 4.27 (s, -NH(C_6H_5)CCH_2, 2H), 3.22 (s, -$CH_2N^+(CH_3)_3$ and -N_3CH_2-, 11H), 3.22-2.07 (m, -CH_2CH_2COO- and -$CH_2CH_2CH_2$-, 6H). ^{1}H NMR of A-D-Fd was shown in Figure **S19-S20**.

Optimization of reaction conditions

To evaluate the modification efficiency, 3-bromopropene was selected to react with PAPLG and *N,N,N*-trimethylpropargylammonium iodide (TPAI) using $Cu(MeCN)_4PF_6$ as the catalyst and TEA as the base in DMF. Different Cu(I) catalysts were initially screened and $Cu(MeCN)_4PF_6$ showed best reactivity due to the excellent solubility in organic solvents compared to CuI or CuBr (**Table S1**). Therefore, the following side chain reactions were catalyzed by $Cu(MeCN)_4PF_6$ and the conjugation efficiency was furthermore evaluated under different feeding ratios. Increasing the amounts of electrophiles would lead to the increase of both FT side chains (α) and unreacted azides (x) (**Scheme S5a-b**). Increasing the feeding of TPAI would decrease the amounts of FT components without affecting the percentage of azides (**Scheme S5c**).

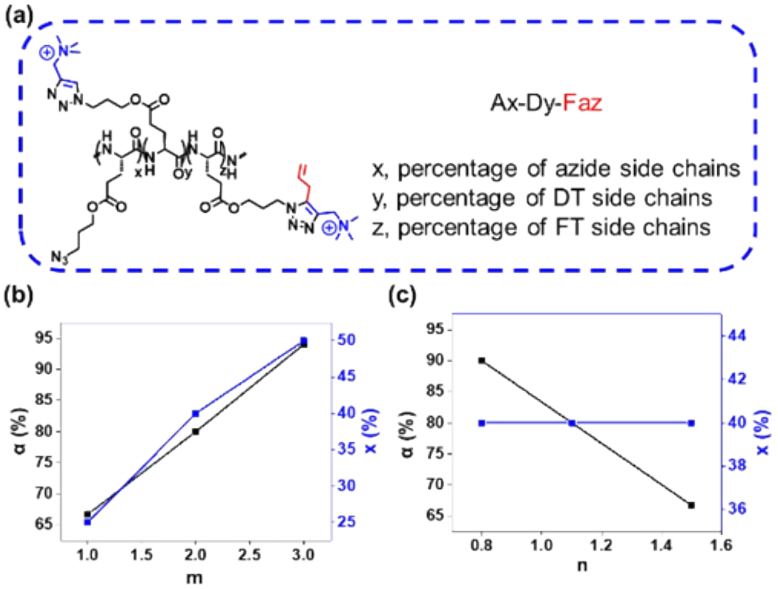
Since the cuprate-triazole intermediate trapped by suitable electrophiles was a key step in such reaction, the electrophilic reactivity could notably affect the modification

results as well as the final polypeptides' structures. It was noteworthy that 3-phenyl-*2H*-azirine was screened to be the most efficient electrophile with almost 100% FT on the side chains, which was also in accordance with the results demonstrated by other groups

Table S1 Effect of catalysts on the interrupted click reaction

Entry	Catalyst	Conjugation efficiency of Fa (%)
1	CuI	21
2	CuBr	13
3	$Cu(MeCN)_4PF_6$	47

Reaction condition: PAPLG = 1 eq. Cu[I]= 1 eq. TEA= 1.2 eq. 3-bromopropene = 3 eq. TPAI = 1.1 eq.



Scheme S5. (a) General structure of A-D-Fa polypeptide. (b) Contents of side chains (α, x) as a function of m. Reaction condition: PAPLG: TPAI: 3-bromopropene = 1: 1.1 : m. (c) Contents of side chains (α, x) as a function of n. Reaction condition: PAPLG: TPAI: 3-bromopropene = 1: n: 2. α was calculated using the equation: α= z/(y+z)*100%.

Calculation of modification efficiency

The integration of peaks at 1.8-2.6 ppm representing the same side-chains moieties was defined as 6 (6H). For polypeptides A-D and A-D-Fa, the values of y, z was calculated according to the integration of peaks at 8.36 and 5.86 ppm, respectively. The values of y, z for A-D-Fb was calculated according to the integration of peaks at 8.35 and 1.15 ppm, respectively. The value of y, z for A-D-Fc was calculated according to the integration of peaks at 8.36 and 7.53-7.73 ppm, respectively. The values of y, z for A-D-Fd was calculated according to the integration of peaks at 7.93-7.39 and 2.33-1.68 ppm (tested in D_2O) or 8.01-7.52 and 3.22 ppm (tested in TFA-*d*), respectively. The value of x was calculated using the equation: x=1-(y+z).

Synthesis of polypeptides with hydroxyl groups

A-D-Fd-OH was synthesized by the thiol−ene reaction of mercaptopropanol and A0-D0-Fd100. In a glove box, A0-D0-Fd100 (10 mg, 0.022 mmol of repeating units, 1 equiv.), mercaptopropanol (3 mg, 0.032 mmol, 1.5 equiv.) and azobisisobutyronitrile (AIBN) (1.1 mg, 0.0064 mmol, 0.3 equiv.) were dissolved in DMF, and reacted at 50 ℃ for 24 h. The resulting solution was precipitated in ether and washed for three times (6 mL × 3). The crude product was collected by centrifugation and the solvent was removed under vacuum. The resulting solid was dialyzed in a dialysis membrane (MWCO = 1 kDa) against DI water for 1 day. The final polypeptides containing hydroxyl groups were obtained as light-yellow powder after lyophilization. The ^{1}H NMR was shown in **Figure S21** and the modified ratio was calculated above 90% according to the ^{1}H NMR.

CD spectroscopy

Unless otherwise specified, polypeptides were dissolved in DI water at a concentration of 0.05 mg/mL. The pH value was adjusted by adding a specific volume of concentrated HCl or NaOH, and measured with a pH meter (Lichen-BX Instrument Technology CO. Ltd). After the pH was adjusted to a desired value, the solution was transferred into a quartz cuvette for CD testing. The salt concentration was adjusted by

adding a specific volume of NaCl, NH_4Cl, or Na_2SO_4 solution in the quartz cuvette. The concentration of polypeptides was recalculated after adding the salt solution. The molar ellipticities were calculated in deg ×cm^2×$dmol^{-1}$. The helicity was calculated using the equation: $[\theta]_\lambda$ =(MRW×θ_λ)/(d×c), helicity= (−$[\theta]_{222}$+3000)/39000[5]. MRW is the mean residue weight, θ_λ is the observed ellipticity at wavelength λ (i.e. 222 nm), d is the path length (mm) and c is the concentration (mg/mL), where the concentration was 0.05 mg/mL. CD spectra of polypeptides with different component ratios were shown in **Figures S24-S39**.

Membrane activity

The membrane activity of the polypeptides was evaluated by measuring the cellular uptake level of a membrane-impermeable dye, fluorescein isothiocyanate (FITC)[6]. First, FITC-Tris as a non-penetrating fluorescent marker was co-incubation with the polypeptides. FITC uptake level was evaluated to demonstrate the pore formation level on cell membranes[6-8]. Briefly, cells were seeded on 96-well plates at 1.5×10^4 cells/well and cultured for 15 h. The medium was replaced by serum-free medium, into which polypeptides and FITC were added at 2 μg/well and 1 μg/well, respectively. Free FITC without the addition of polypeptides was as a control. After incubation at 37 °C for 2 h, cells were washed with PBS solution for 3 times and then lysed with RIPA lysis buffer (100 μL/well). The amount of FITC in the cell lysate was quantified by spectrofluorimetry (λ_{ex}=488 nm, λ_{em}=530 nm), and the protein content was determined by BCA kit. The FITC uptake level was represented as ng FITC associated with 1 mg of cellular protein (ng FITC/mg protein). Cells incubated with free FITC in the absence of polypeptides were included as a negative control.

Cytotoxicity experiments

Cells were seed on 96-well plates at a density of 10^4 cells/well and cultured for 15 h. The medium was replaced by serum-free medium, into which 10 uL of polypeptides with different concentration was added. After being cultured for 4 h, the medium was

replaced by serum-containing medium and the cells were further incubated for 20 h. Cell viability was then evaluated by the MTT assay following the manufacturer's procedure.

Gel retardation assay

The polypeptides (0.2 mg/mL) were added to pGFP solutions (0.2 mg/mL) dropwise at various N/P ratios, and the mixtures were incubated at room temperature for 20 min to form the polyplexes. The polyplexes were loaded on a 1% agarose gel at 200 ng DNA/well followed by electrophoresis at 110 V for 30 min.

DNA transfection

HeLa cells were seeded on a 96-well plate at the original density of 10^4 cells/well and incubated overnight. The polypeptides (0.2 mg/mL) were added to pGFP solutions (0.2 mg/mL) dropwise at a weight ratio (polypeptides/pGFP) of 10, followed by incubation at room temperature for 20 min to form the polyplexes. The cell culture medium was changed to serum-free DMEM, followed by the addition of polyplexes at the dosages of 0.2 μg pGFP /well. The medium was refreshed by DMEM containing 10% FBS after 4 h incubation. The green fluorescent protein expression level was evaluated after 24 h using an inverted fluorescence microscope. PEI-25k and PLL was selected as the positive control at the optimal weight ratios of 1.5:1 and 10:1, respectively.

HeLa cells were seeded on 96-well plates at 10^4 cells/well and incubated for another 24 h until the cells reached 70% confluence. The cell culture medium was replaced by serum-free DMEM (100 μl/well), into which polypeptides/pLuc complexes at different weight ratios (1, 5, 10 and 15) were added at 0.2 μg DNA/well. After incubation for 4 h, the medium was replaced by fresh medium containing 10% FBS. Cells were further cultured for another 20 h before quantification of luciferase expression level using a Bright-Glo Luciferase assay kit (Promega, Madison, USA) and cellular protein level using a BCA kit. In order to evaluate the transfection efficiency

of polyplexes in the presence of serum, cells were incubated with polyplexes in DMEM supplemented with 10% FBS for 4 h. The weight ratio of the A-D-F100-OH to pLuc was changed to 10. The result was expressed as relative luminescence unit (RLU) associated with 1 mg of cellular protein.

Supplementary Figures

^{1}H NMR characterization

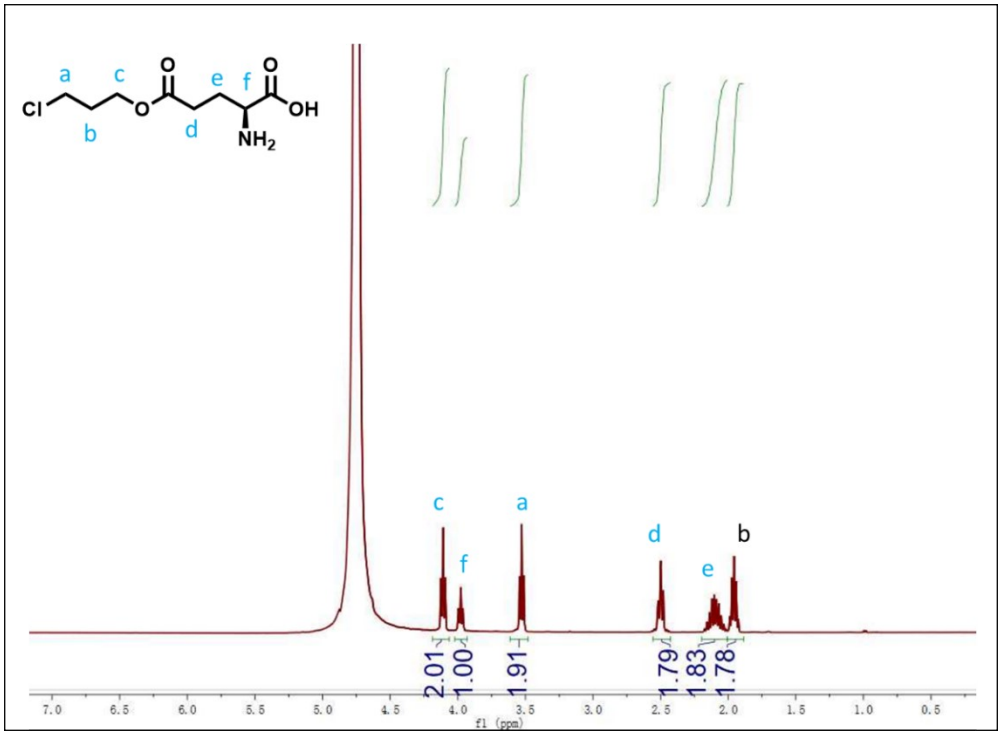


Figure S1. ^{1}H NMR spectrum of Cl-Glu in D_2O.

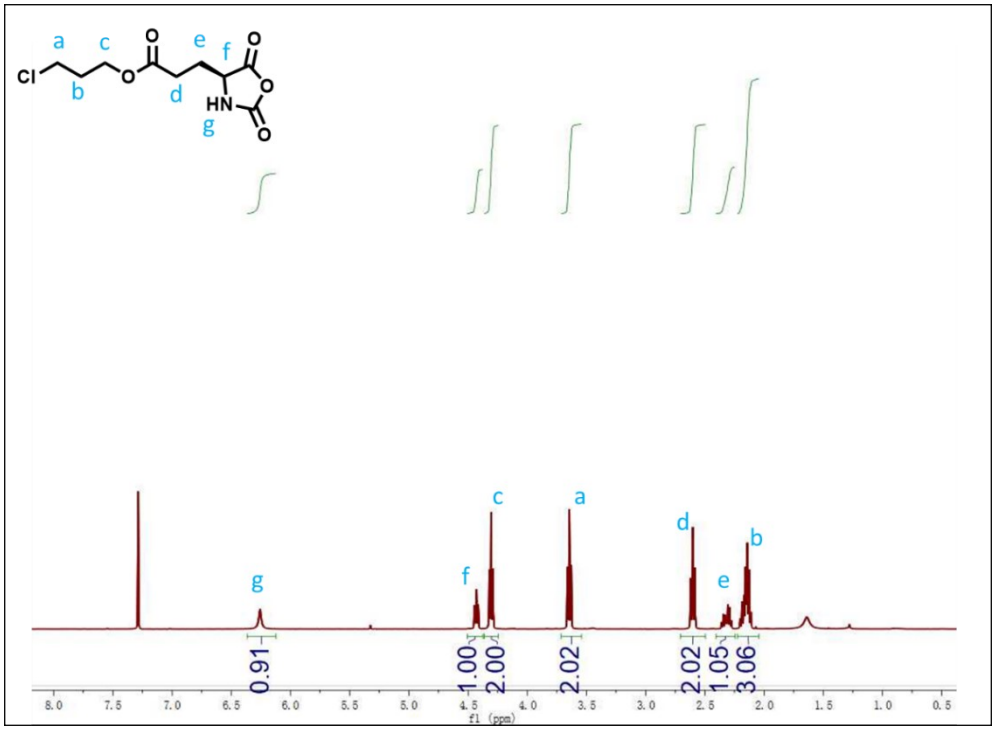


Figure S2. ^{1}H NMR spectrum of Cl-NCA in $CDCl_3$.

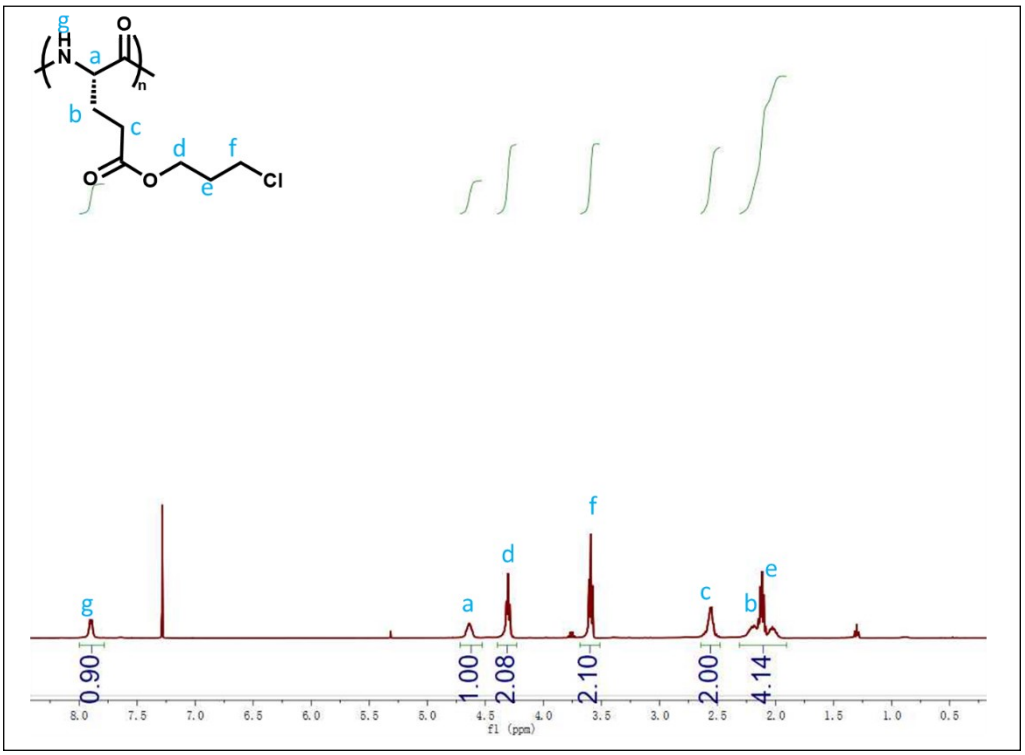


Figure S3. ^{1}H NMR spectrum of PCPLG in $CDCl_3$.

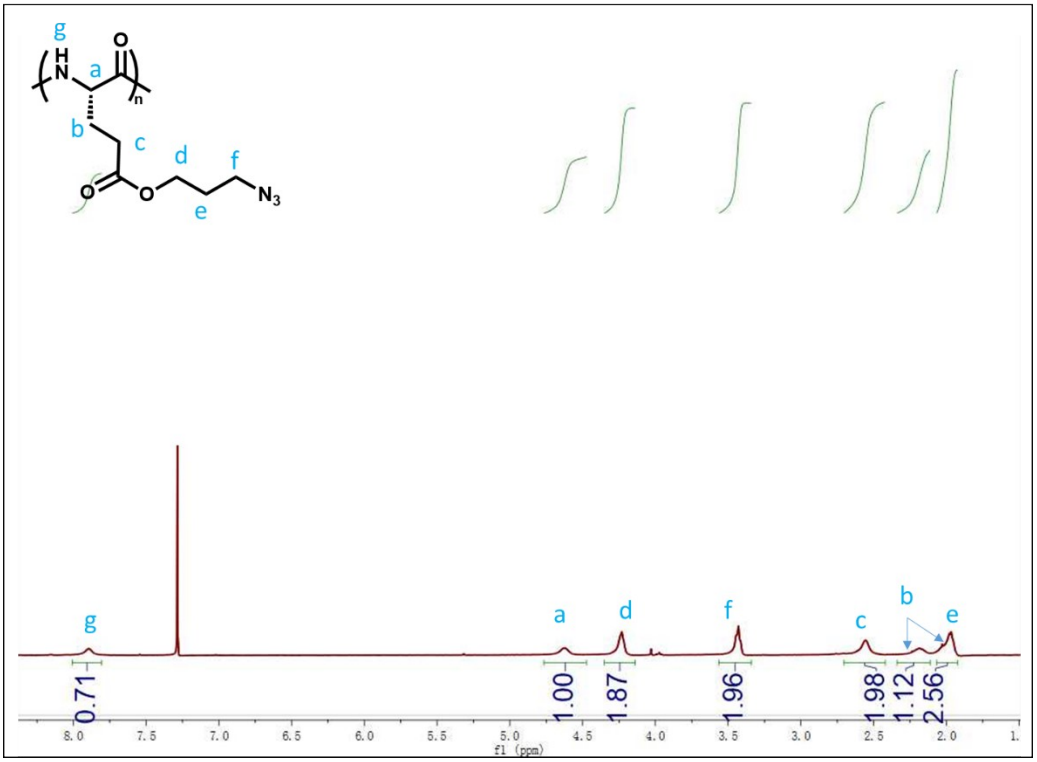


Figure S4. ^{1}H NMR spectrum of PAPLG in $CDCl_3$.

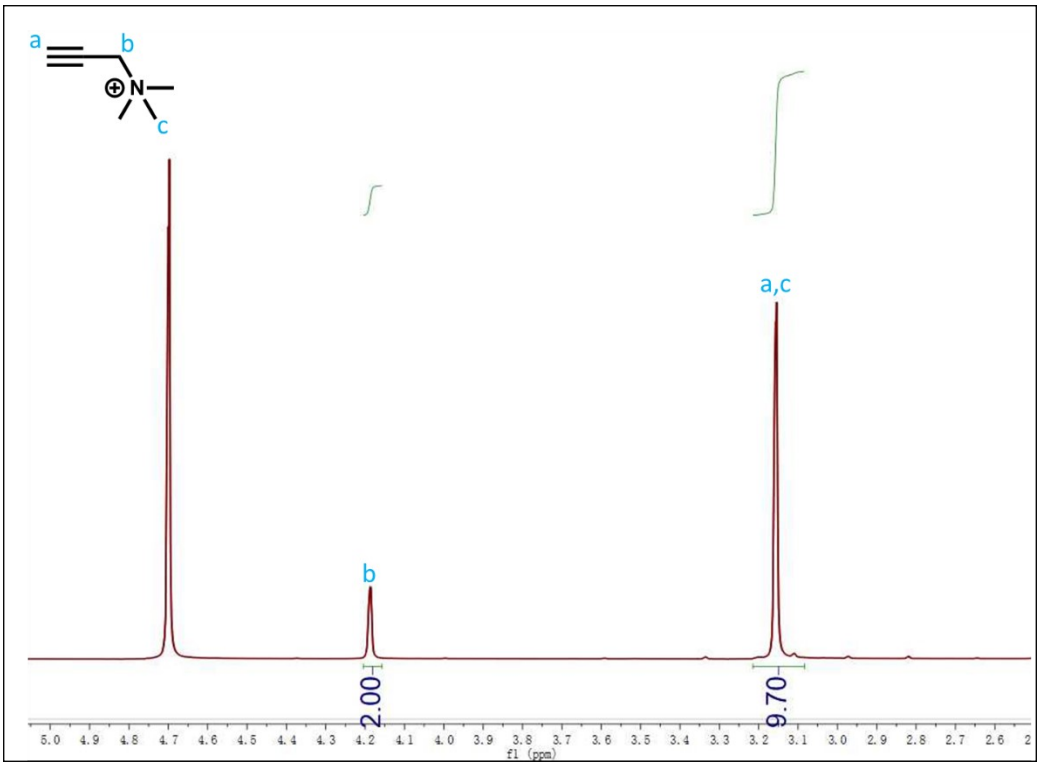


Figure S5. ^{1}H NMR spectrum of TPAI in D_2O.

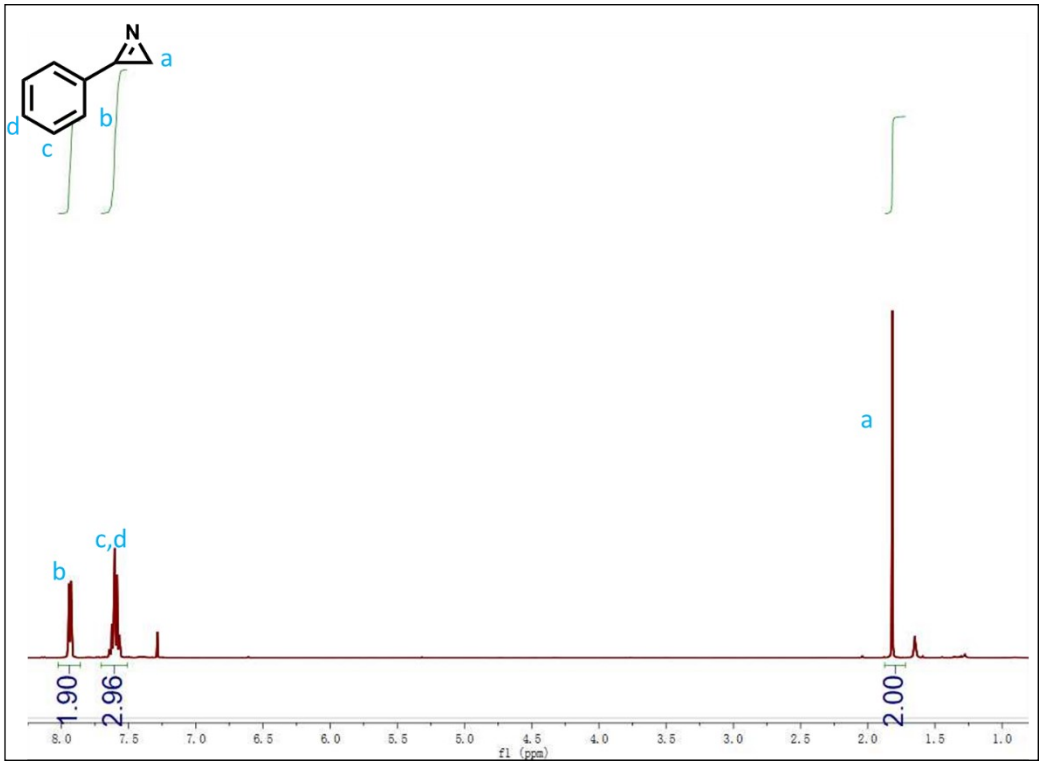


Figure S6. ^{1}H NMR spectrum of 3-phenyl-2*H*-azirine in $CDCl_3$.

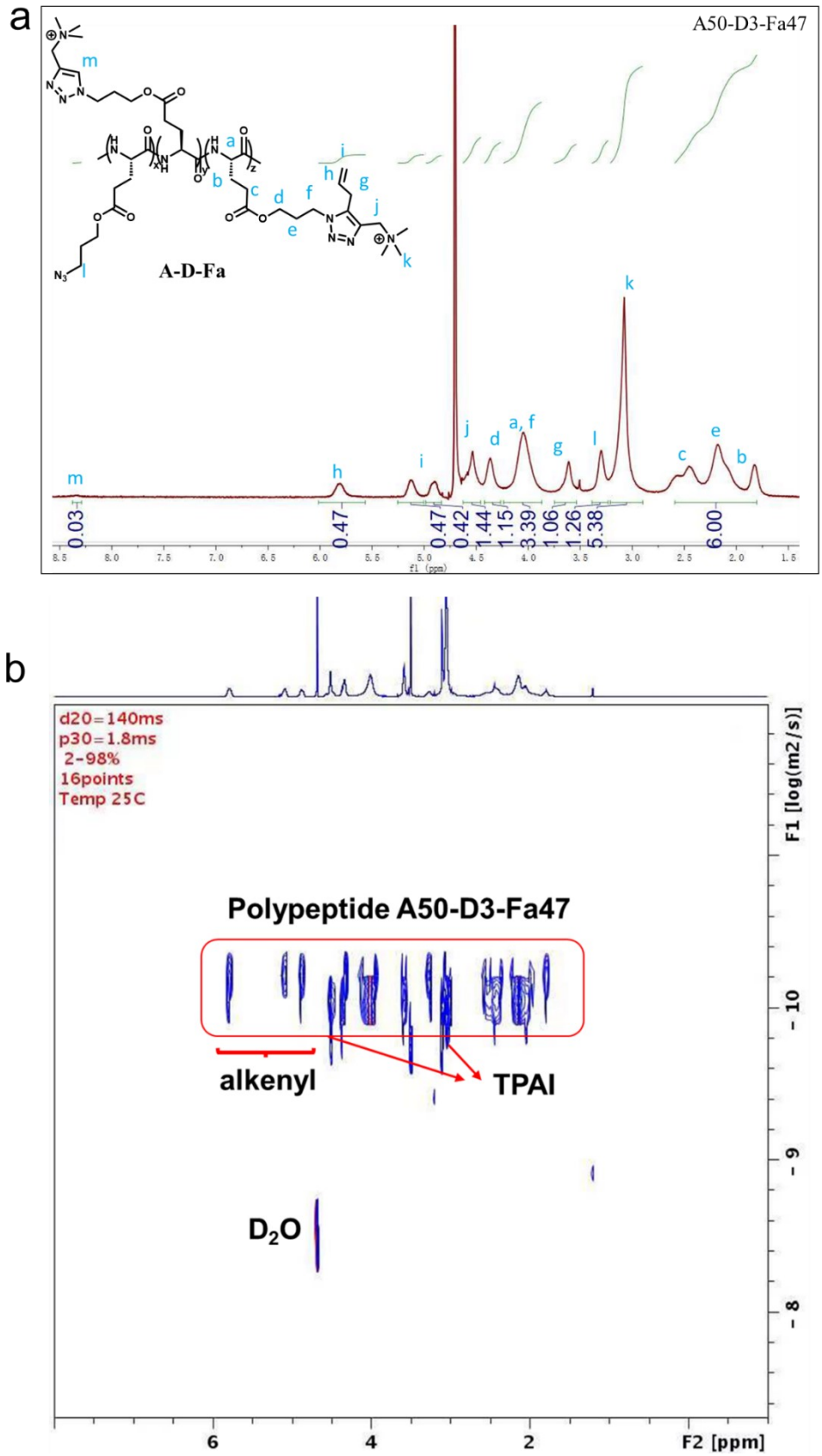


Figure S7. ^{1}H NMR spectrum of A50-D3-Fa47 in D_2O (a). DOSY NMR spectrum of the representative polypeptide A50-D3-Fa47 (b).

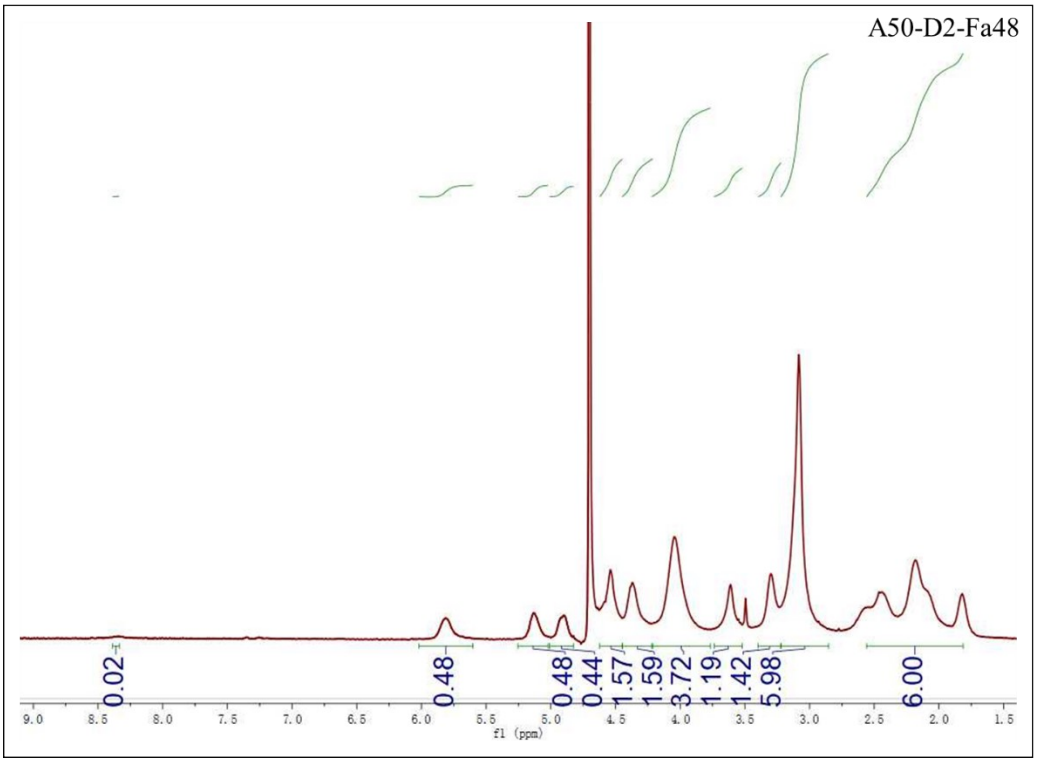


Figure S8. ^{1}H NMR spectrum of A50-D2-Fa48 in D_2O (DP=36).

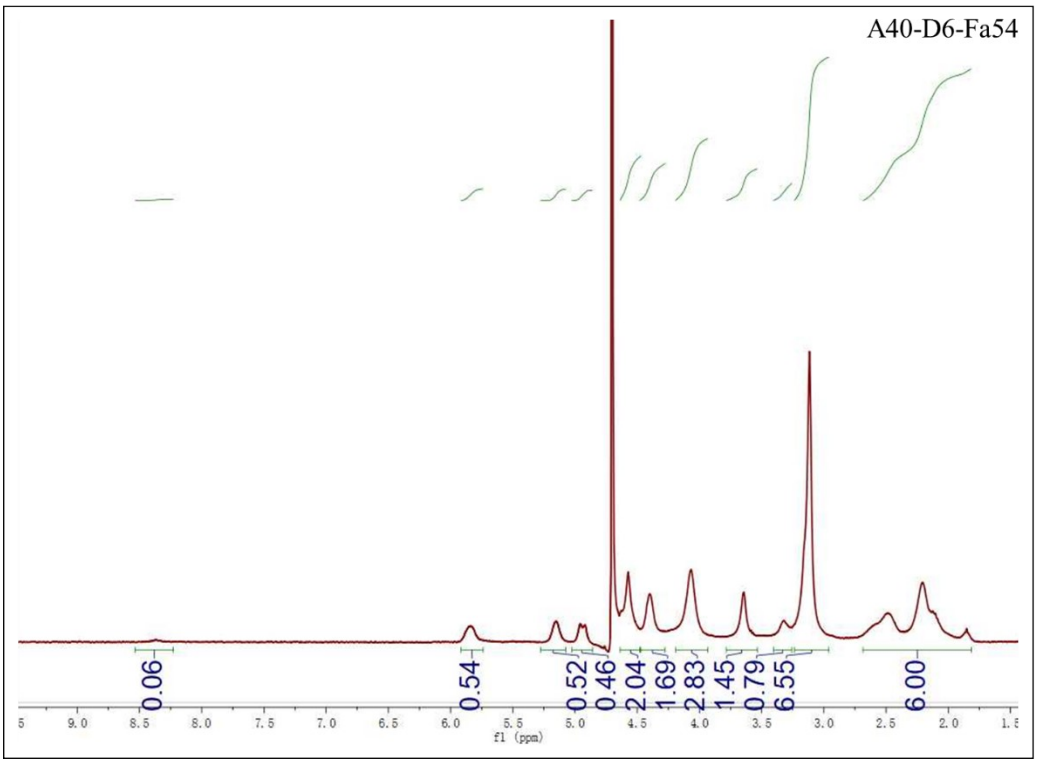


Figure S9. ^{1}H NMR spectrum of A40-D6-Fa54 in D_2O.

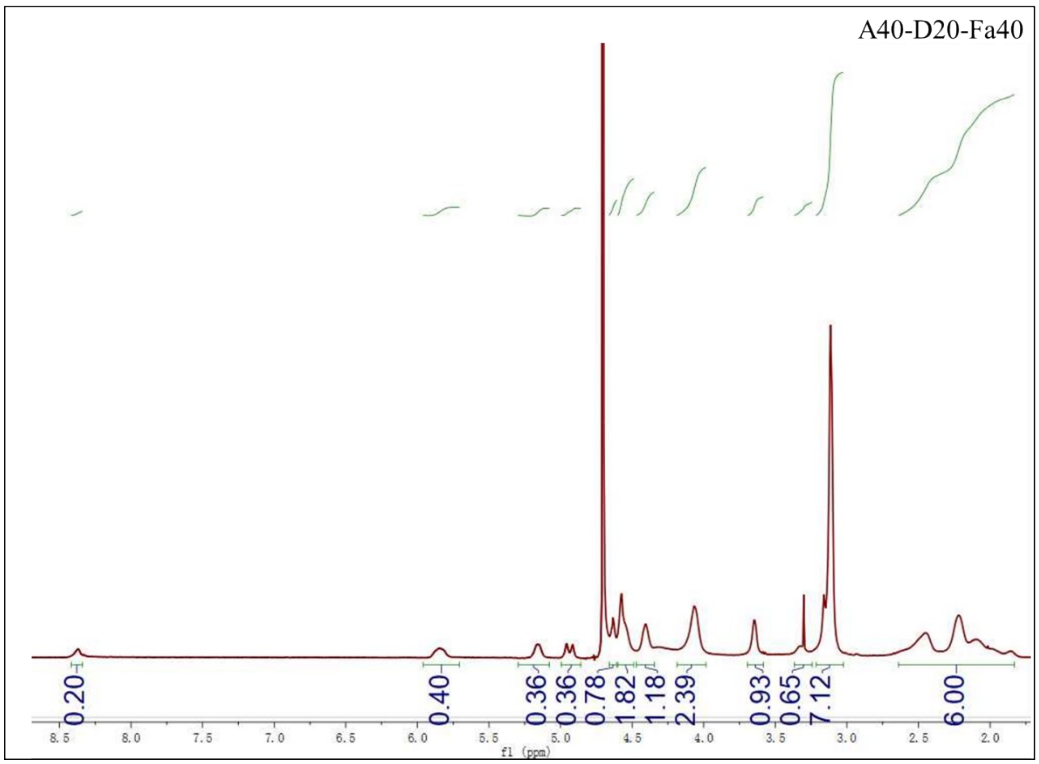


Figure S10. ^{1}H NMR spectrum of A40-D20-Fa40 in D_2O.

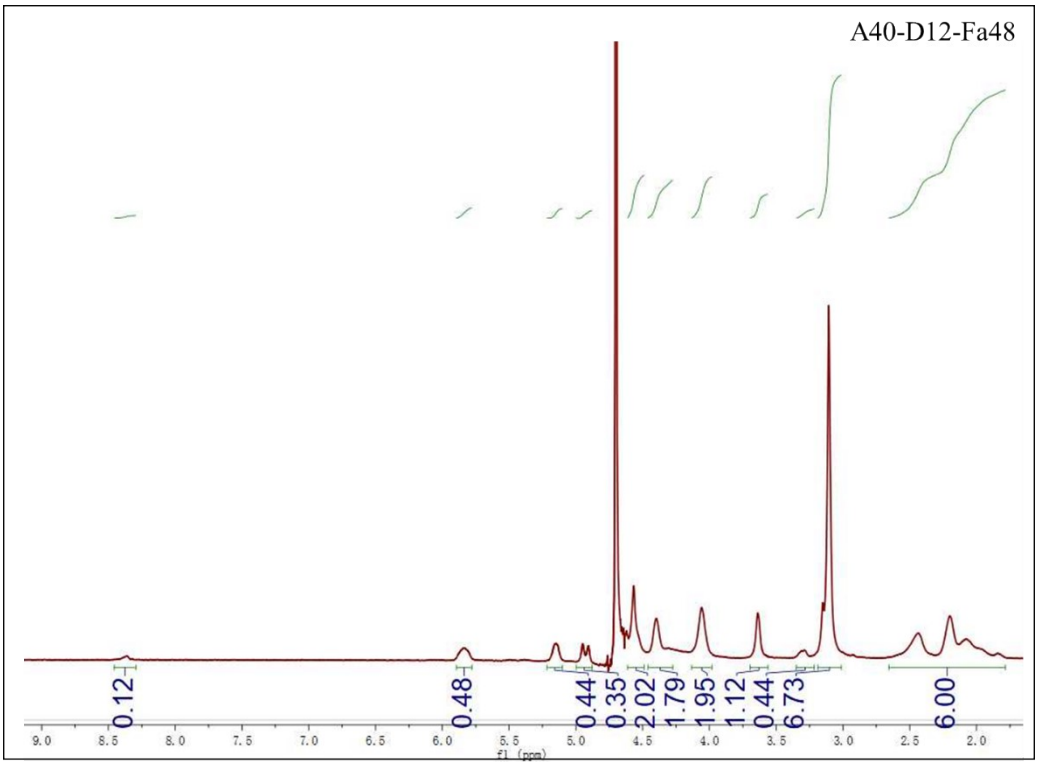


Figure S11. ^{1}H NMR spectrum of A40-D12-Fa48 in D_2O.

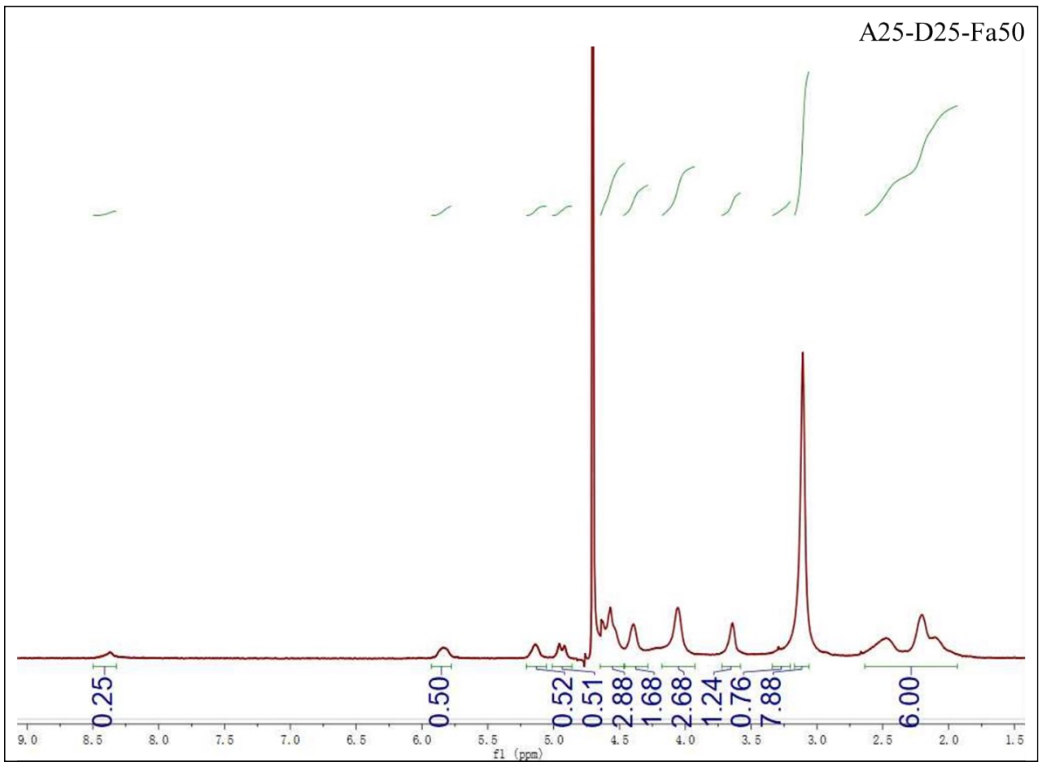


Figure S12. ^{1}H NMR spectrum of A25-D25-Fa50 in D_2O.

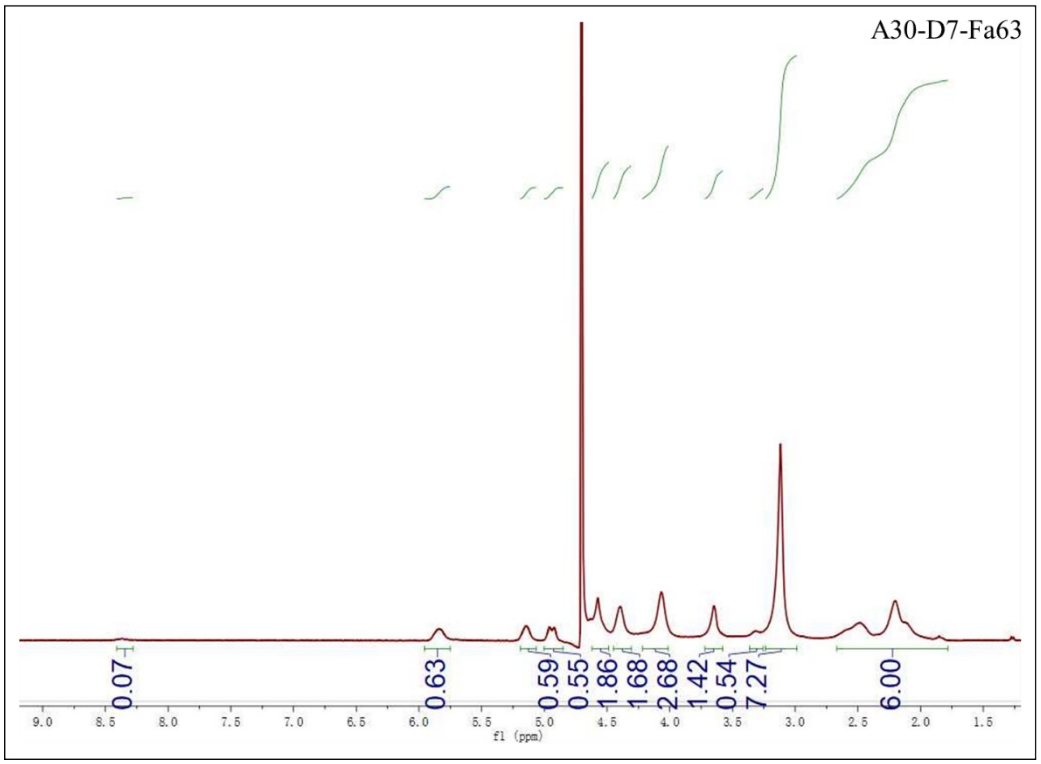


Figure S13. ^{1}H NMR spectrum of A30-D7-Fa63 in D_2O.

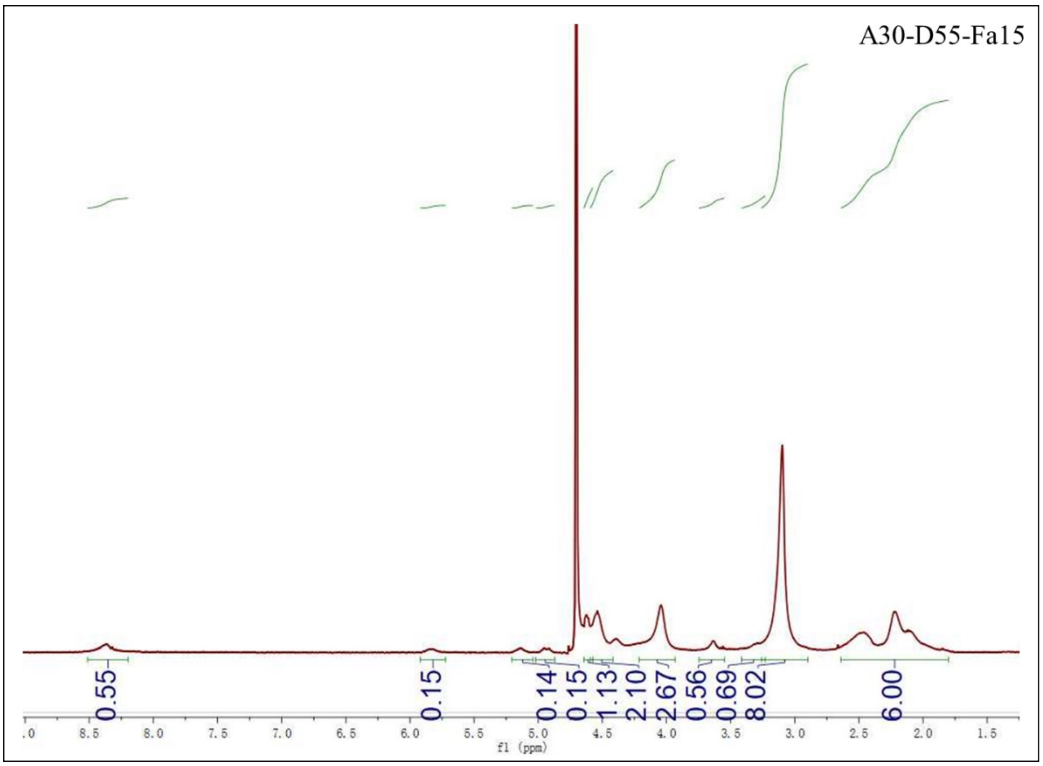


Figure S14. ^{1}H NMR spectrum of A30-D55-Fa15 in D_2O.

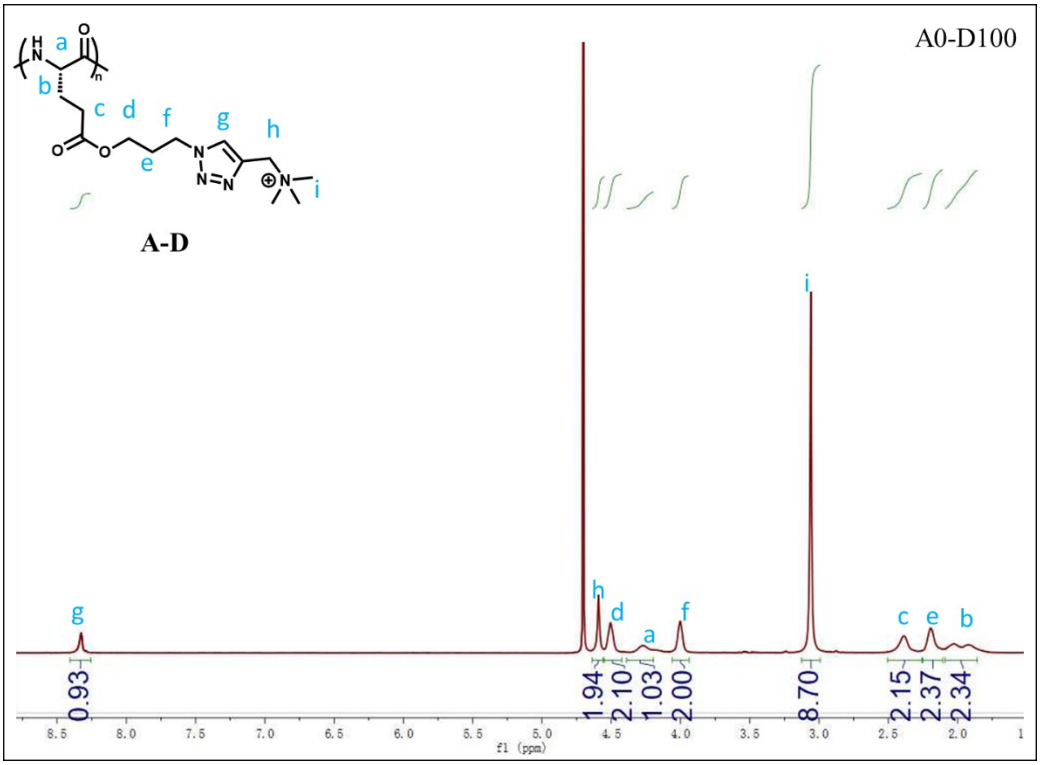


Figure S15. ^{1}H NMR spectrum of A0-D100 in D_2O.

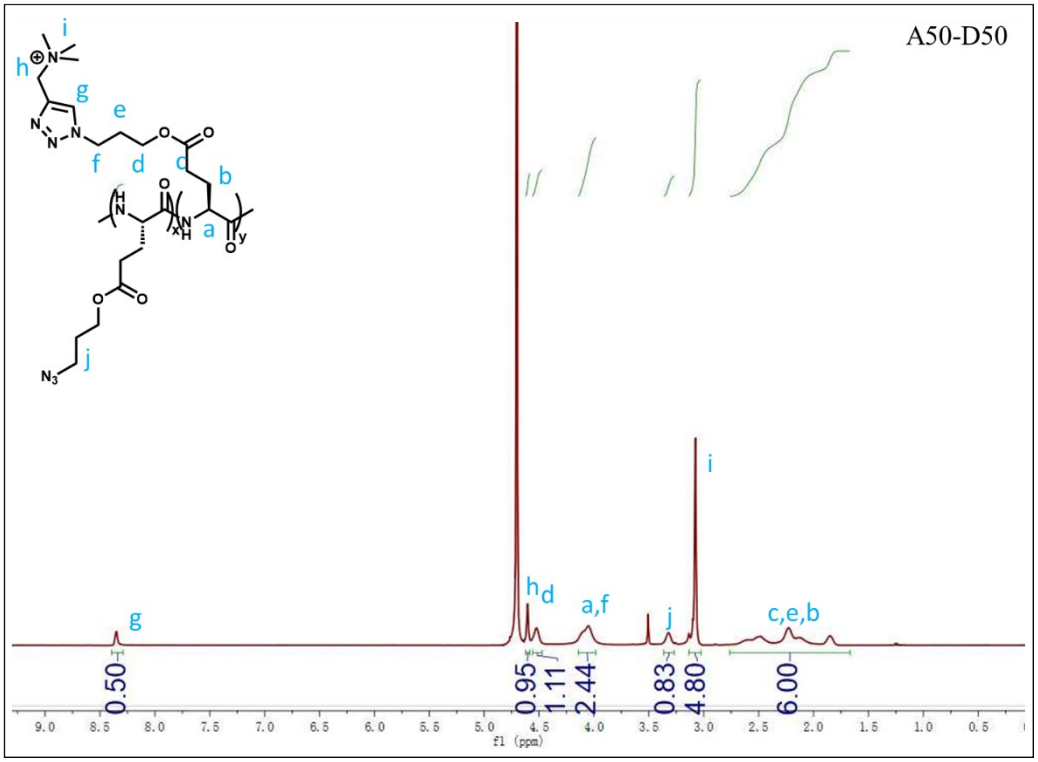


Figure S16. ^{1}H NMR spectrum of A50-D50 in D_2O.

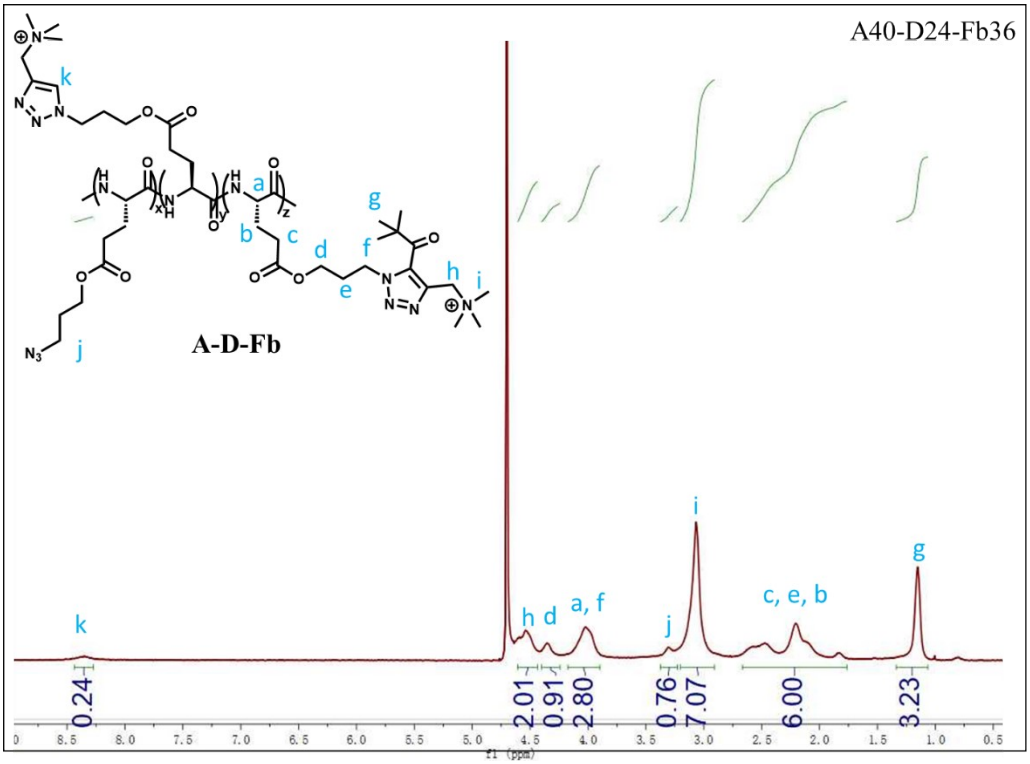


Figure S17. ^{1}H NMR spectrum of A40-D24-Fb36 in D_2O.

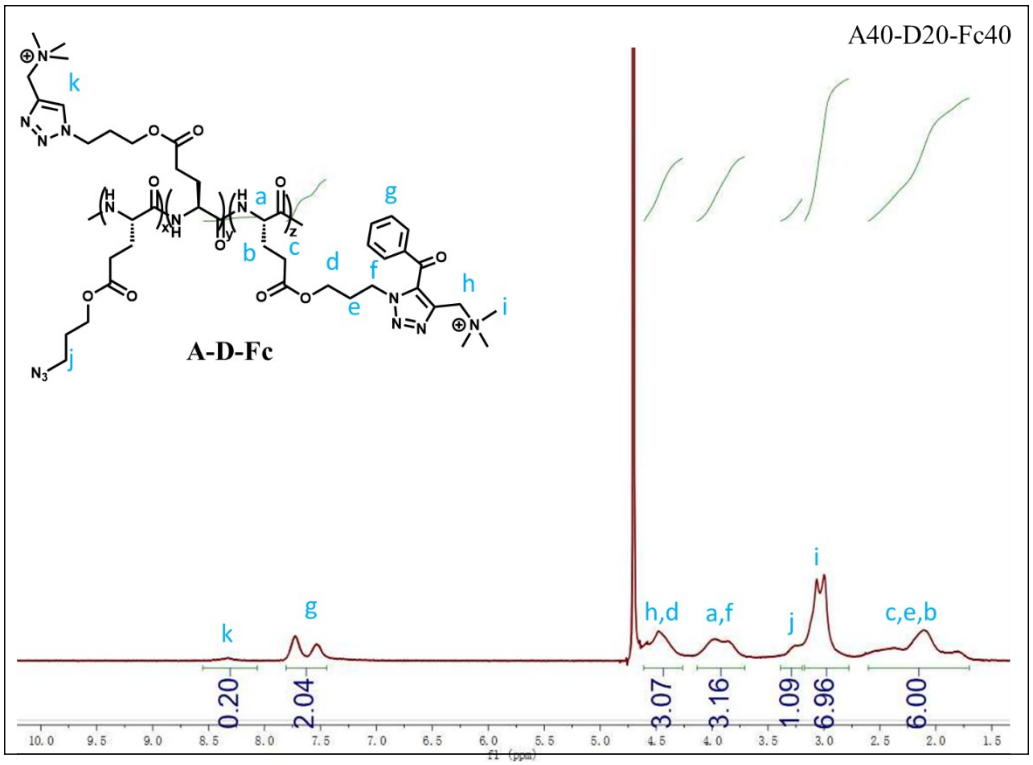


Figure S18. ^{1}H NMR spectrum of A40-D20-Fc40 in D_2O.

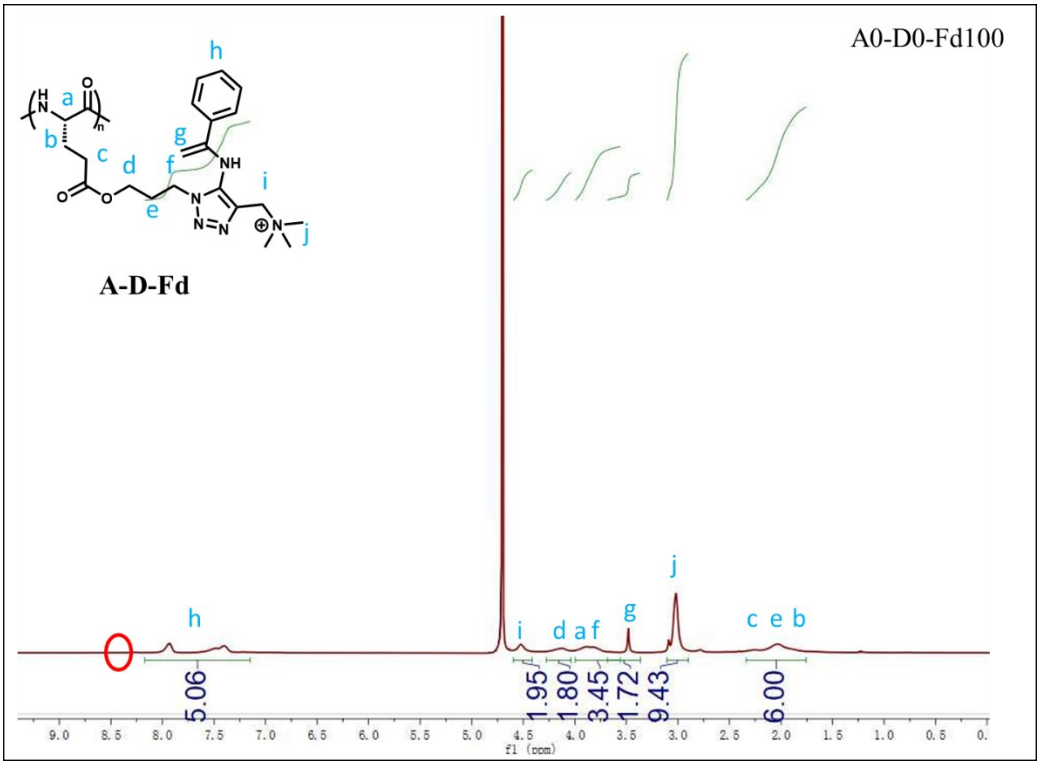


Figure S19. ^{1}H NMR spectrum of A0-D0-Fd100 in D_2O. The H in C5 of triazole at 8.40 ppm is disappeared.

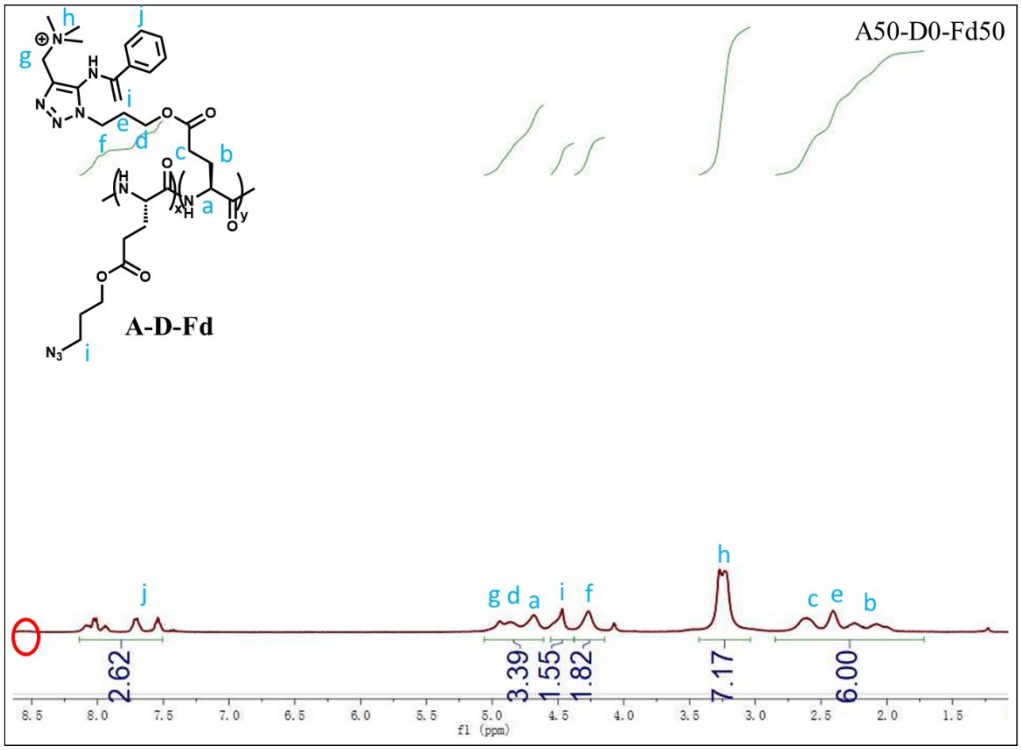


Figure S20. ^{1}H NMR spectrum of A50-D0-Fd50 in TFA-*d*. The H in C5 of triazole at 8.40 ppm is disappeared.

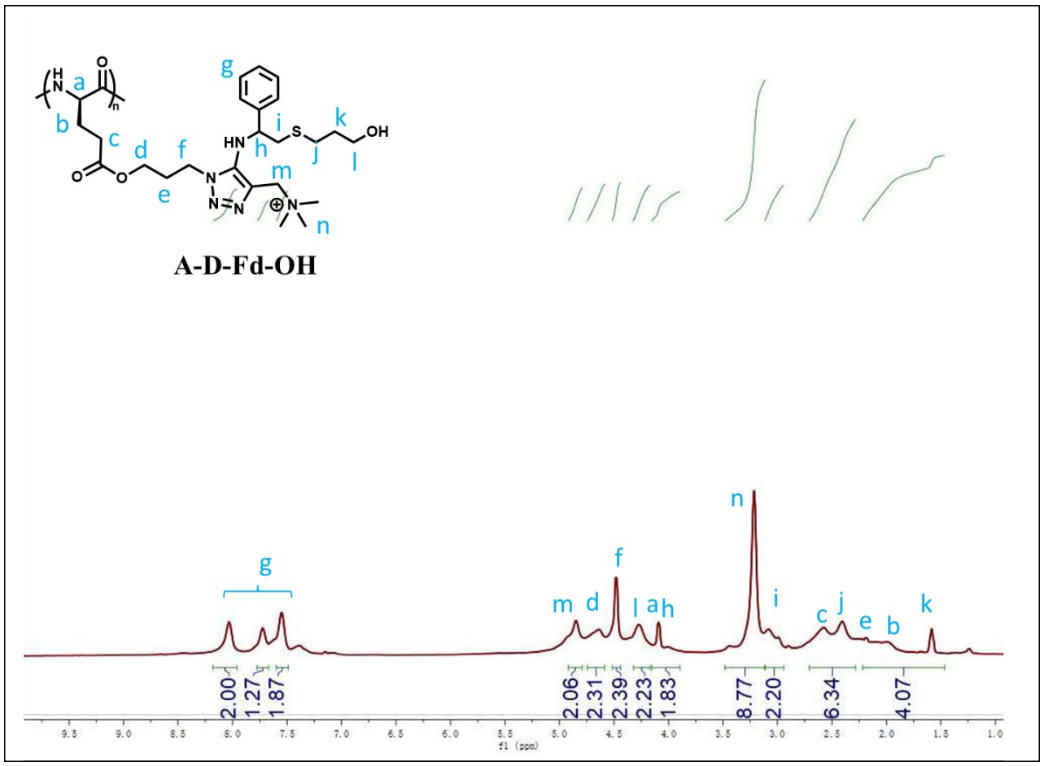


Figure S21. ^{1}H NMR spectrum of A-D-Fd-OH in TFA-*d*.

FT-IR and GPC characterization

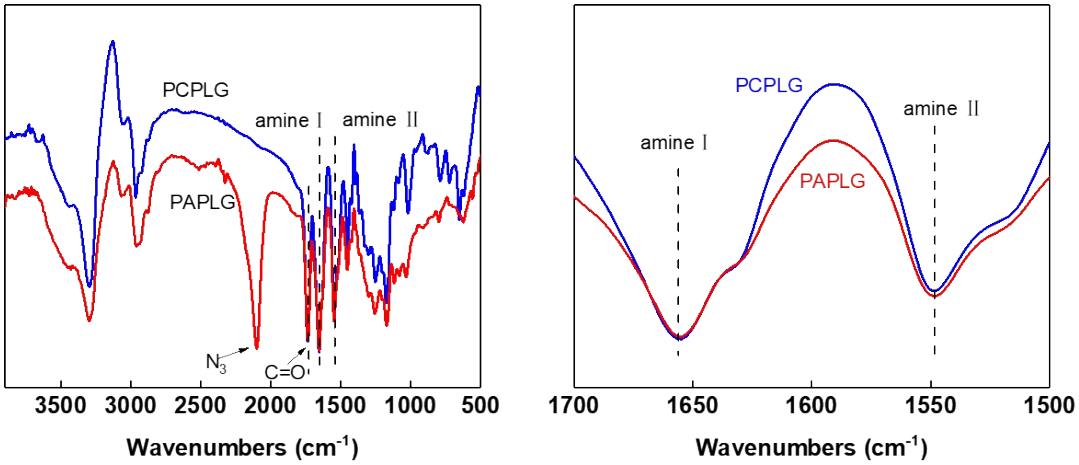


Figure S22. FT-IR spectra of PCPLG and PAPLG.

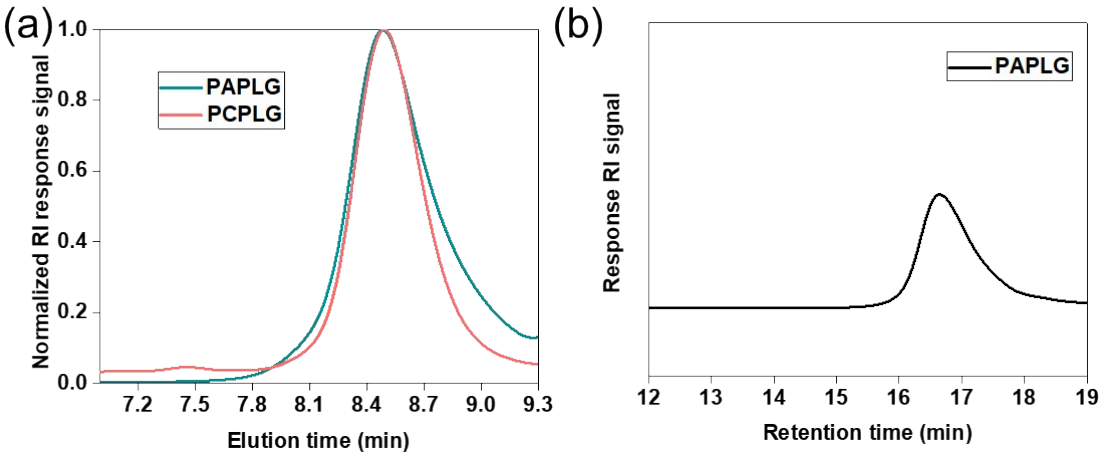


Figure S23. GPC curves of PCPLG and PAPLG with DMF containing 0.01 M LiBr as the eluent at 45 °C at a flow rate of 1.0 mL·min^{-1} (standard: PMMA) (a). GPC curve of PAPLG (precursor of ionic polypeptides) with THF as the eluent at 35 °C at a flow rate of 1.0 mL·min^{-1} (standard: PS) (b).

Table S2. Molecular weights of PCPLG and PAPLG (designed DP = 50)[a].

Polymer	M_n	M_w	$Đ$	Calculated DP
PCPLG	10330	12293	1.19	50
PAPLG	10343 (10734)	12412 (12505)	1.2 (1.18)	50 (50)
PAPLG[b]	7712	8714	1.13	36[b]

[a] The values in brackets meaning absolute molecular weight determined by MALLS detectors.

[b] Designed DP = 36.

Analysis of polypeptides by IR and GPC:

^{1}H NMR analysis reveals a quantitative conversion of PCPLG to PAPLG. The α-helical conformations of PCPLG and PAPLG in the solid state have also been verified as evidenced by the presence of characteristic amide I (1650 cm^{-1}) and amide II bands

Gel permeation chromatography (GPC) gave monomodal peaks for both the PCPLG and PAPLG with low polydispersity indices (*Đ*) (**Figure S23**) and their M_n information was summarized in **Table S2**. As shown in Table S2, the absolute M_n of PAPLG was also tested by MALLS detector and the result was in accordance to the designed M_n.

CD characterization

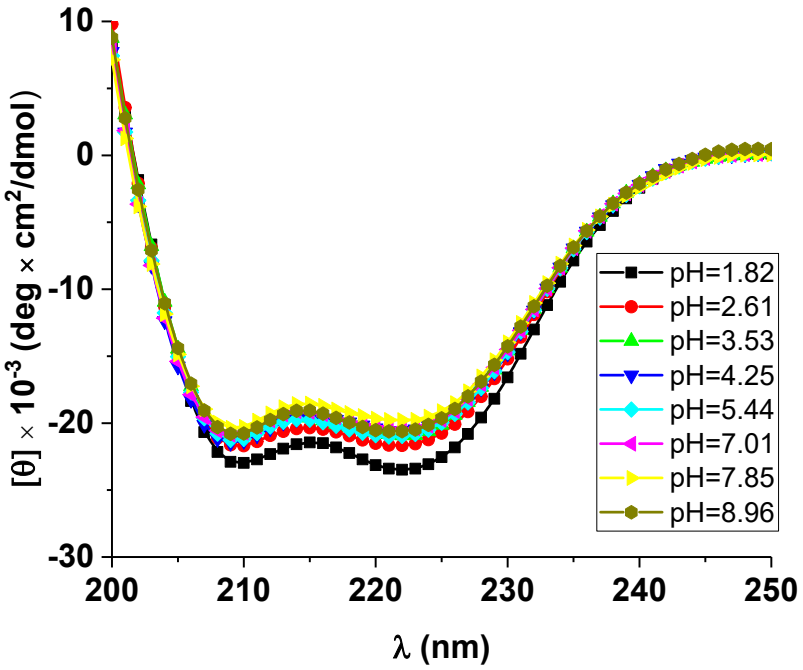


Figure S24. CD spectra of A50-D3-Fa47 tested at 0.05 mg/mL.

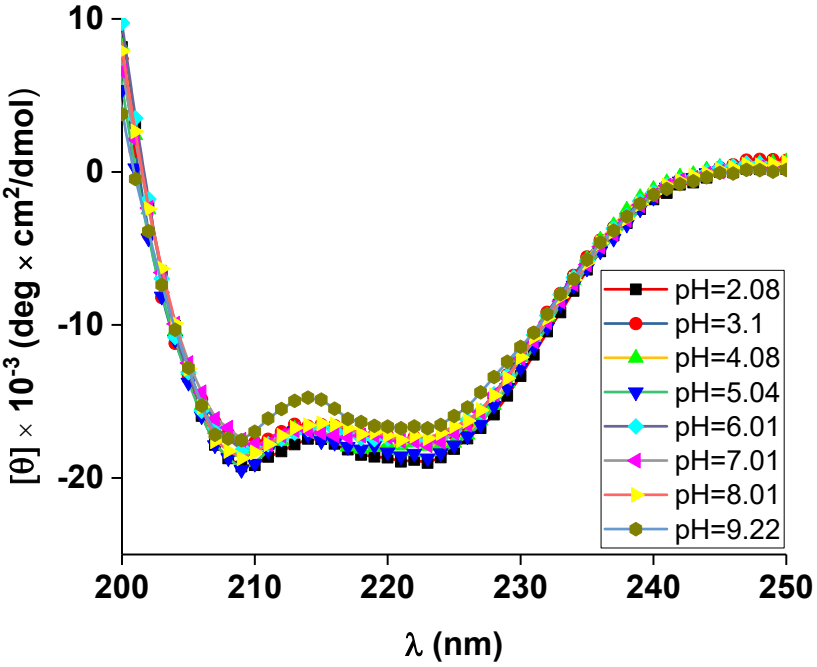


Figure S25. CD spectra of A50-D2-Fa48 tested at 0.05 mg/mL.

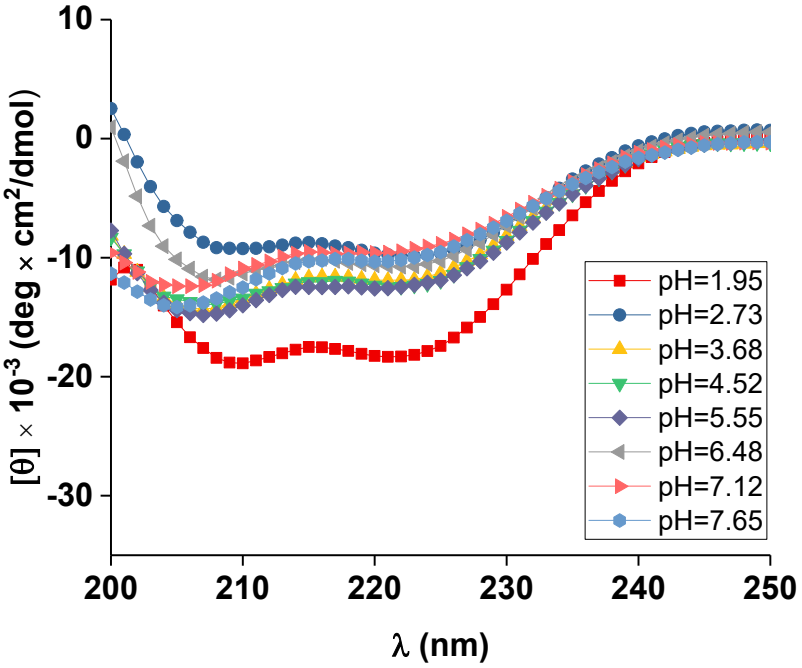


Figure S26. CD spectra of A40-D6-Fa54 tested at 0.05 mg/mL.

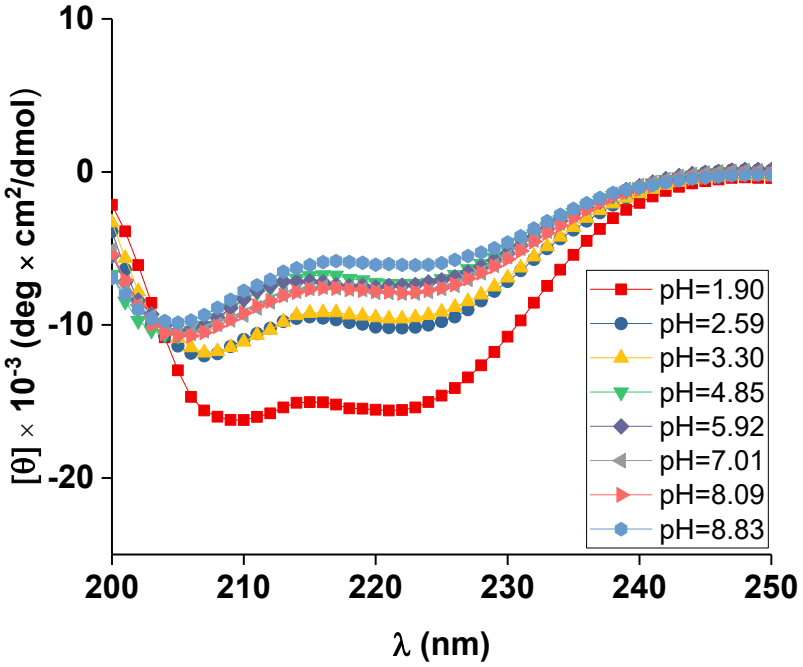


Figure S27. CD spectra of A40-D20-Fa40 tested at 0.05 mg/mL.

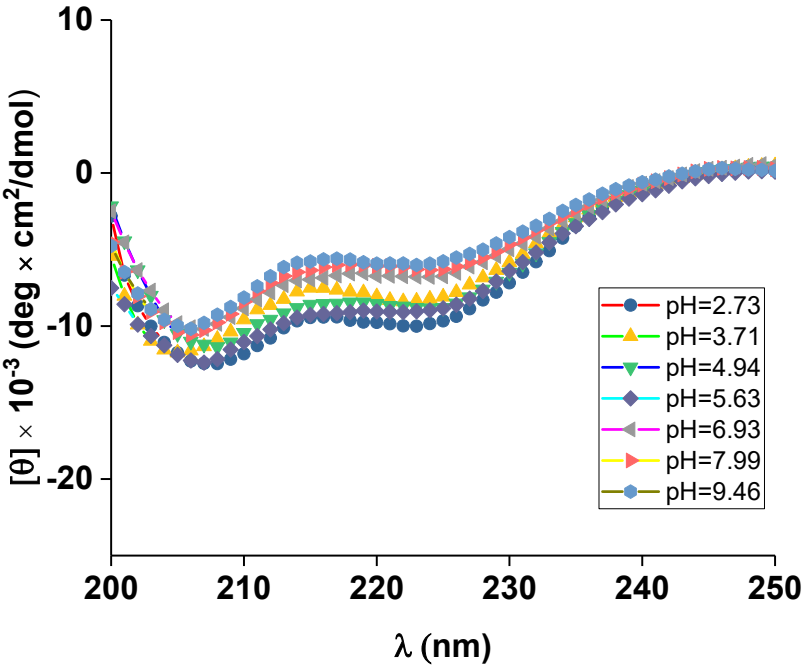


Figure S28. CD spectra of A30-D7-Fa63 tested at 0.05 mg/mL.

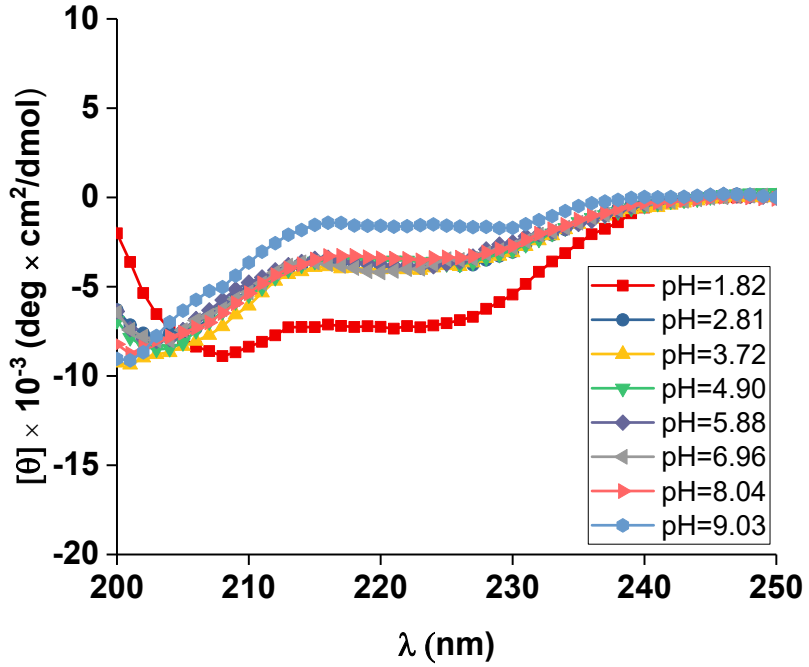


Figure S29. CD spectra of A30-D55-Fa15 tested at 0.05 mg/mL.

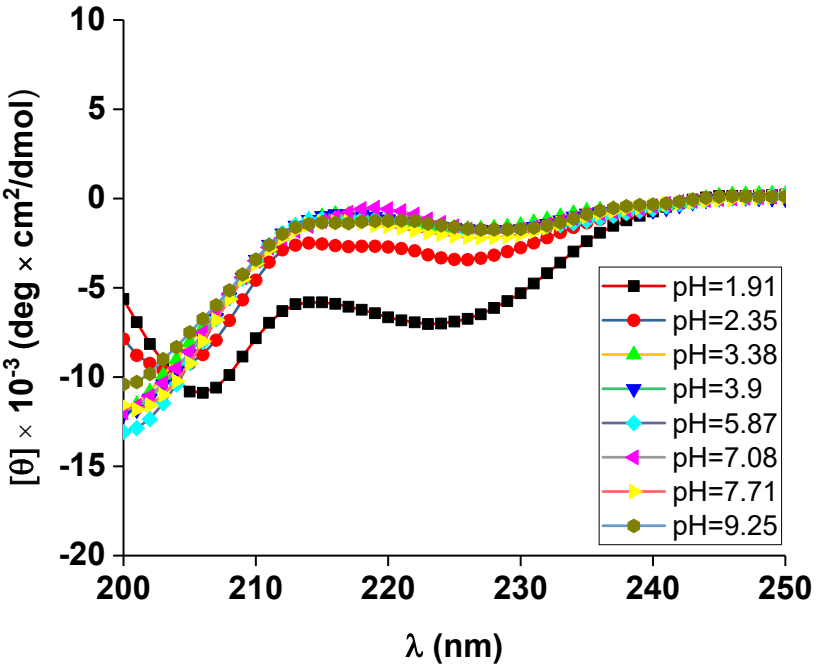


Figure S30. CD spectra of A0-D100 tested at 0.05 mg/mL.

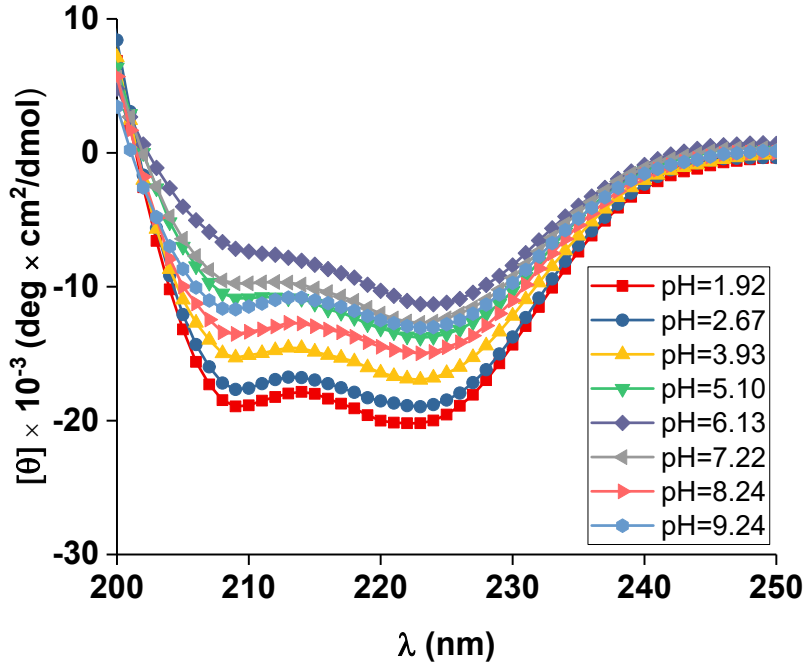


Figure S31. CD spectra of A50-D50 tested at 0.05 mg/mL.

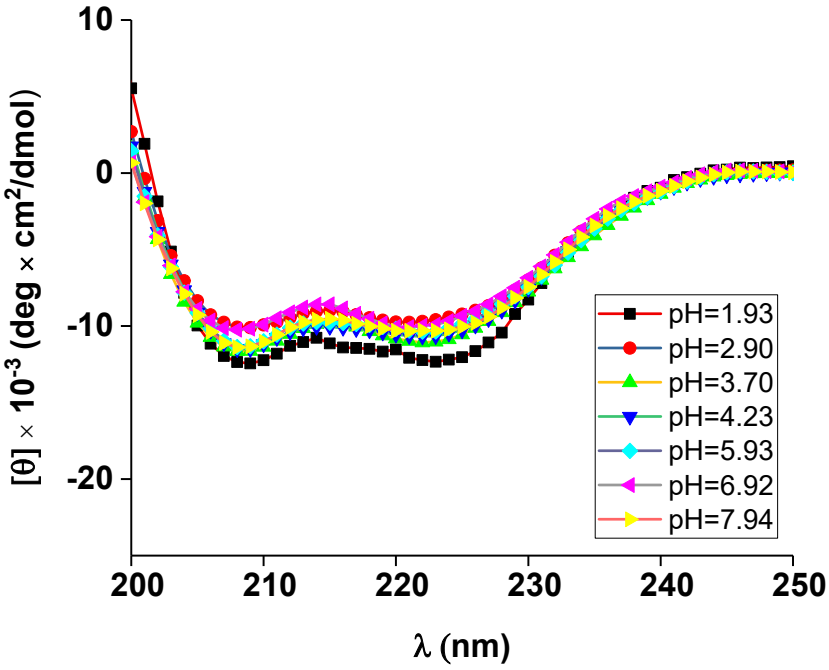


Figure S32. CD spectra of A40-D24-Fb36 tested at 0.05 mg/mL.

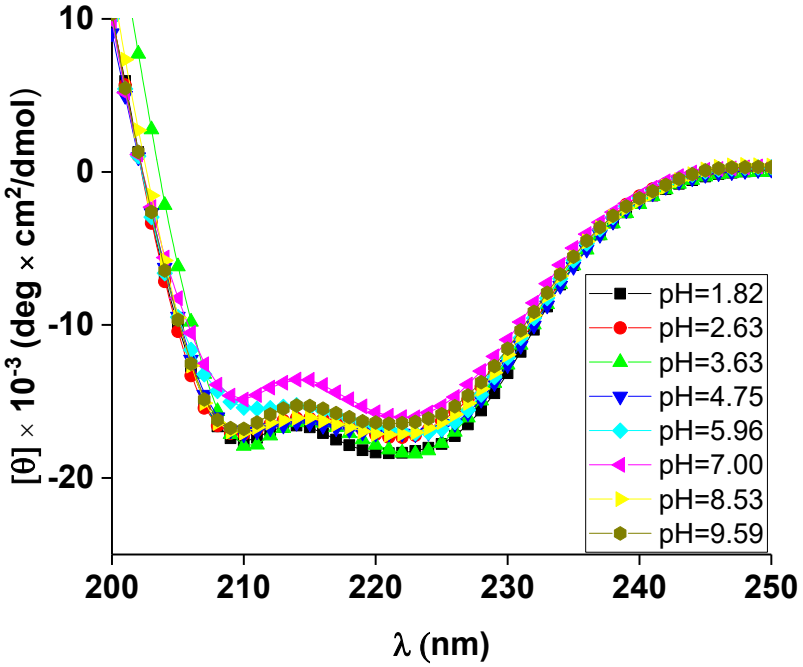


Figure S33. CD spectra of A40-D20-Fc40 tested at 0.05 mg/mL.

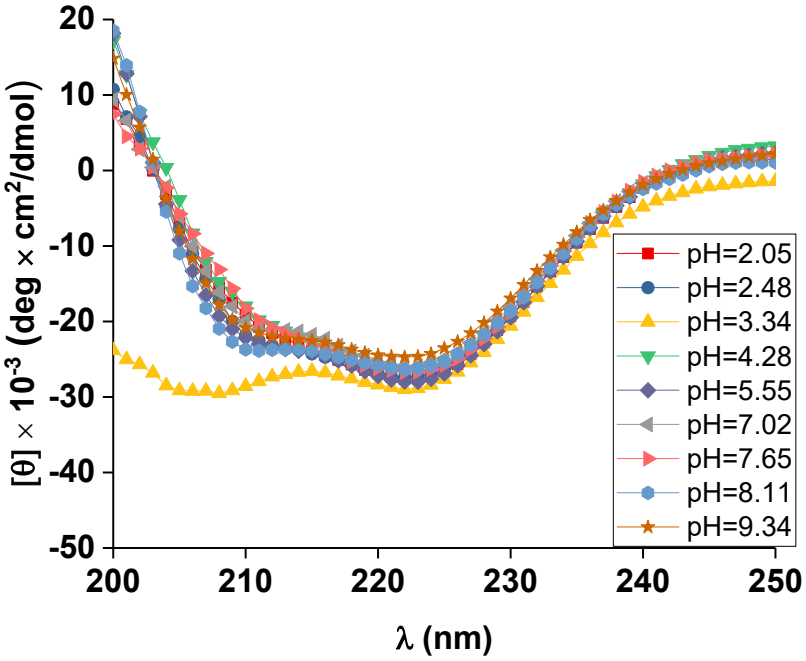


Figure S34. CD spectra of A0-D0-Fd100 tested at 0.05 mg/mL.

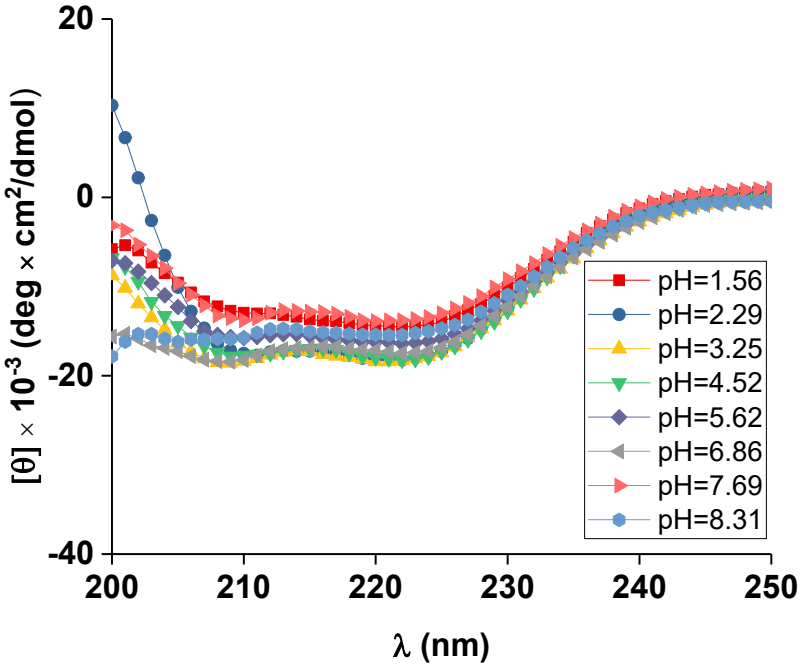


Figure S35. CD spectra of A50-D0-Fd50 tested at 0.05 mg/mL.

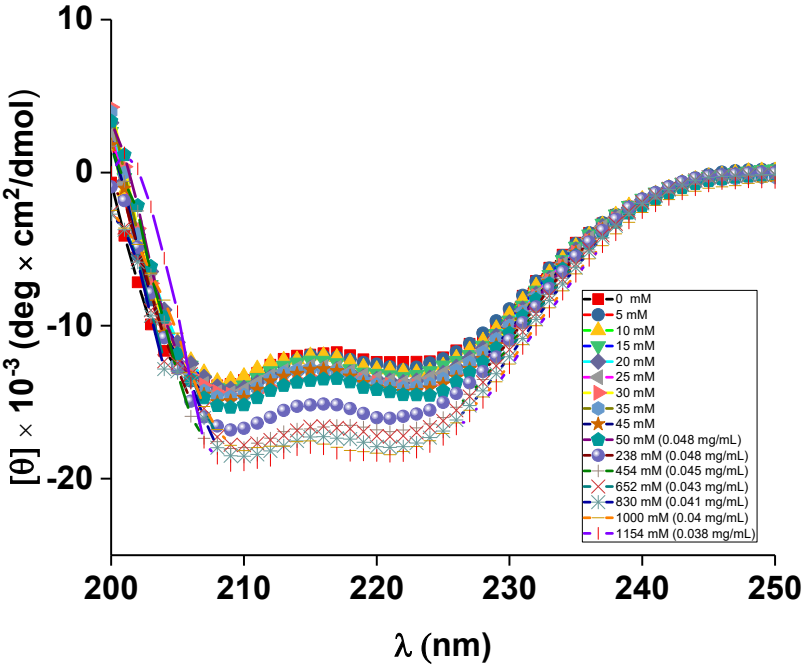


Figure S36. CD spectra of A40-D20-Fa40 upon stepwise addition of 1 M or 5 M NaCl solution. The data in parentheses indicated the recalculated concentration of polypeptides upon the addition of salts.

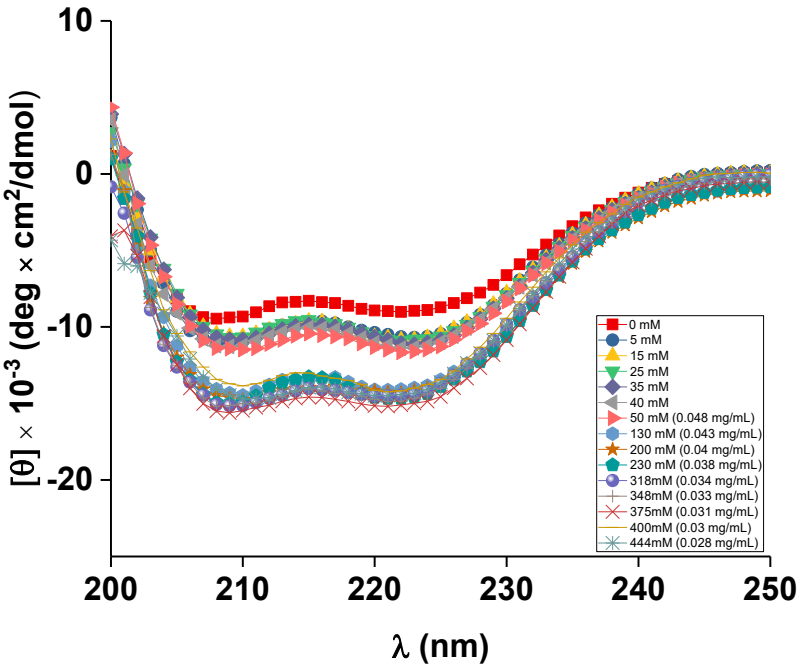


Figure S37. CD spectra of A40-D20-Fa40 upon stepwise addition of 1 M Na_2SO_4 solution tested at 0.05 mg/mL. The data in parentheses indicated the recalculated concentration of polypeptides upon the addition of salts.

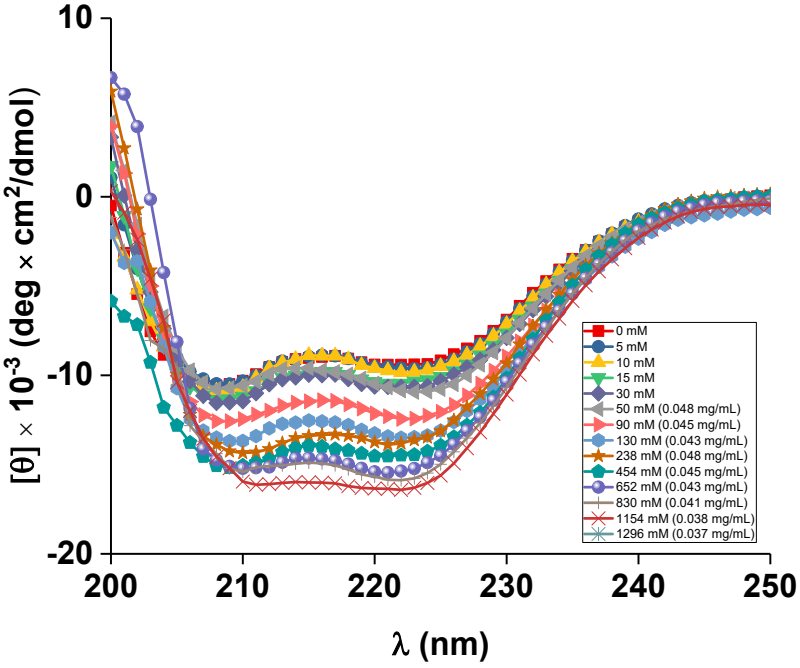


Figure S38. CD spectra of A40-D20-Fa40 upon stepwise addition of 1 M or 5 M NH_4Cl solution tested at 0.05 mg/mL. The data in parentheses indicated the recalculated concentration of polypeptides upon the addition of salts.

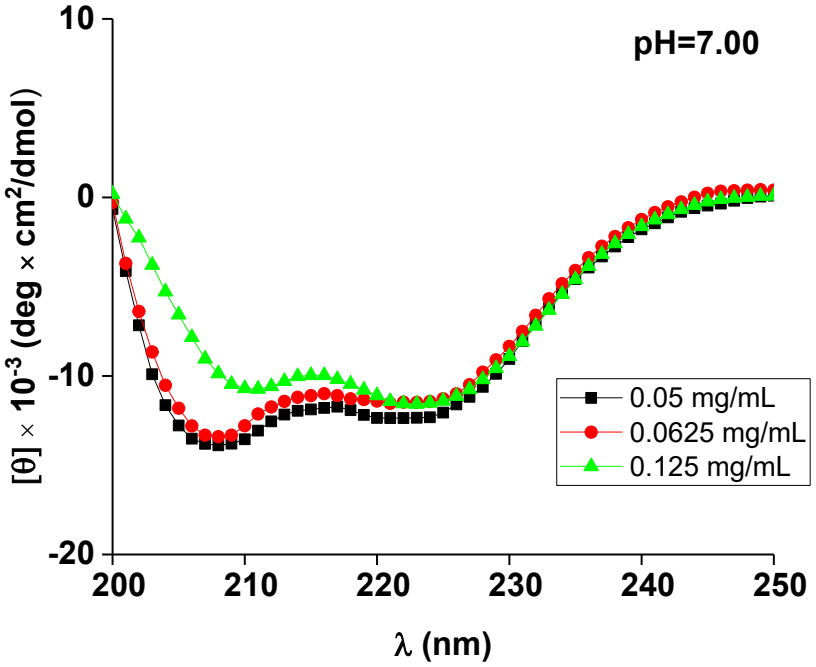


Figure S39. CD spectra of A40-D20-Fa40 at different concentrations.

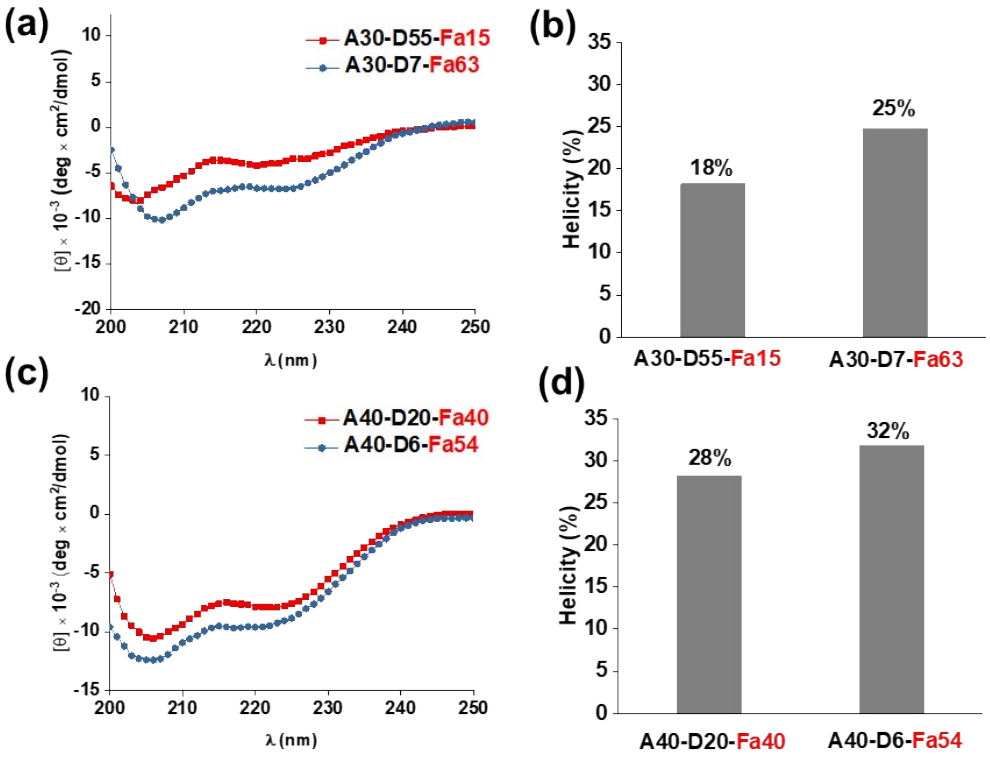


Figure S40. CD spectra (a, c) and calculated helicities (b, d) of polypeptides at pH=7.0.

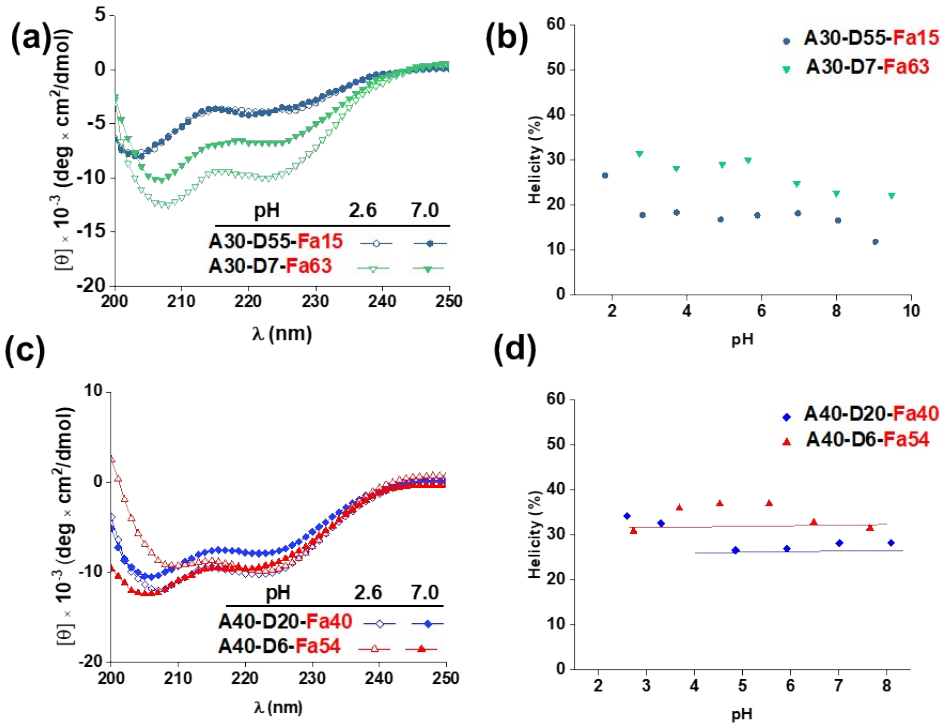


Figure S41. CD spectra of polypeptides at pH=2.6 and 7.0 (a, c) and plots of calculated helicities as a function of pH (b,d).

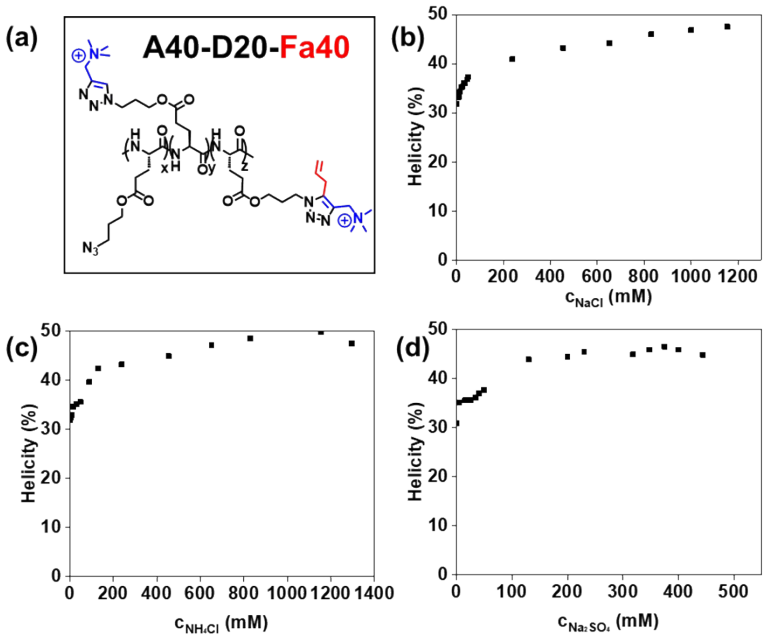


Figure S42. Salt-enhanced helix formation. Helicity of A40-D20-Fa40 as a function of salt concentration at pH=7.0 (a, b, c).

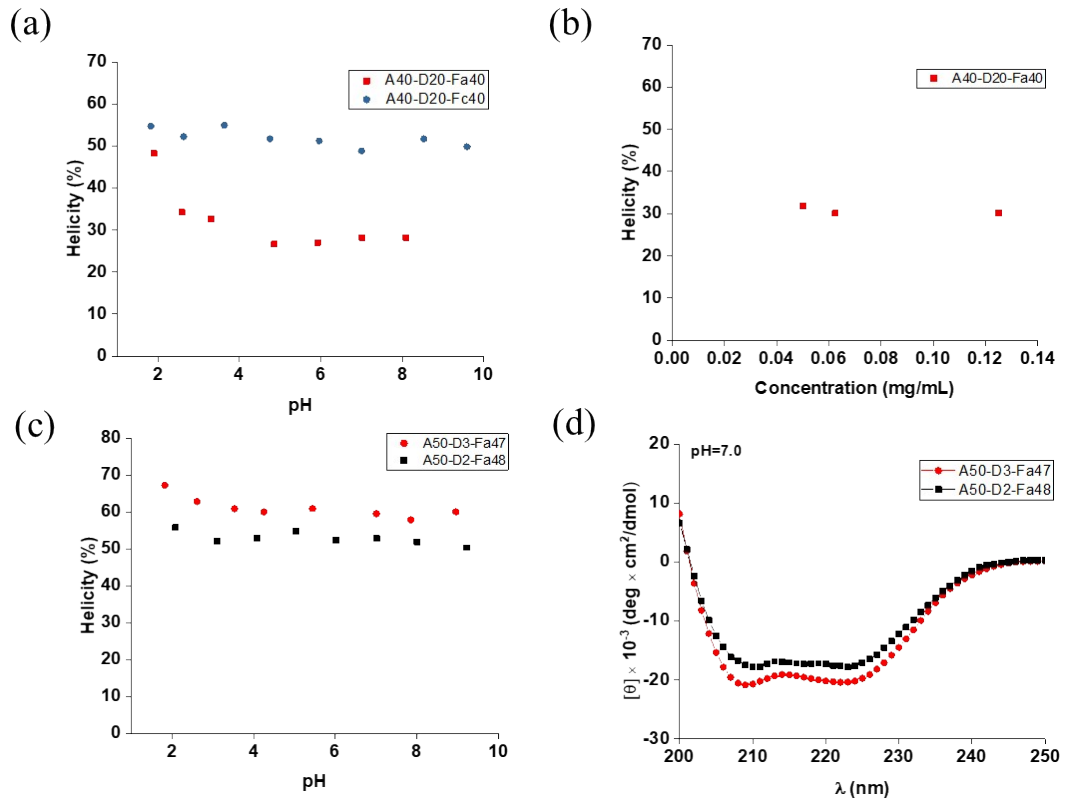


Figure S43. (a) Helicity of A40-D20-Fa40 and A40-D20-Fc40 as a function of pH. (b) Helicity of A40-D20-Fa40 as a function of concentration. (c) Helicities of A50-D3-Fa47 and A50-D2-Fa48 as a function of pH. (d) CD spectra of A50-D3-Fa47 and A50-D2-Fa48 at pH=7.0.

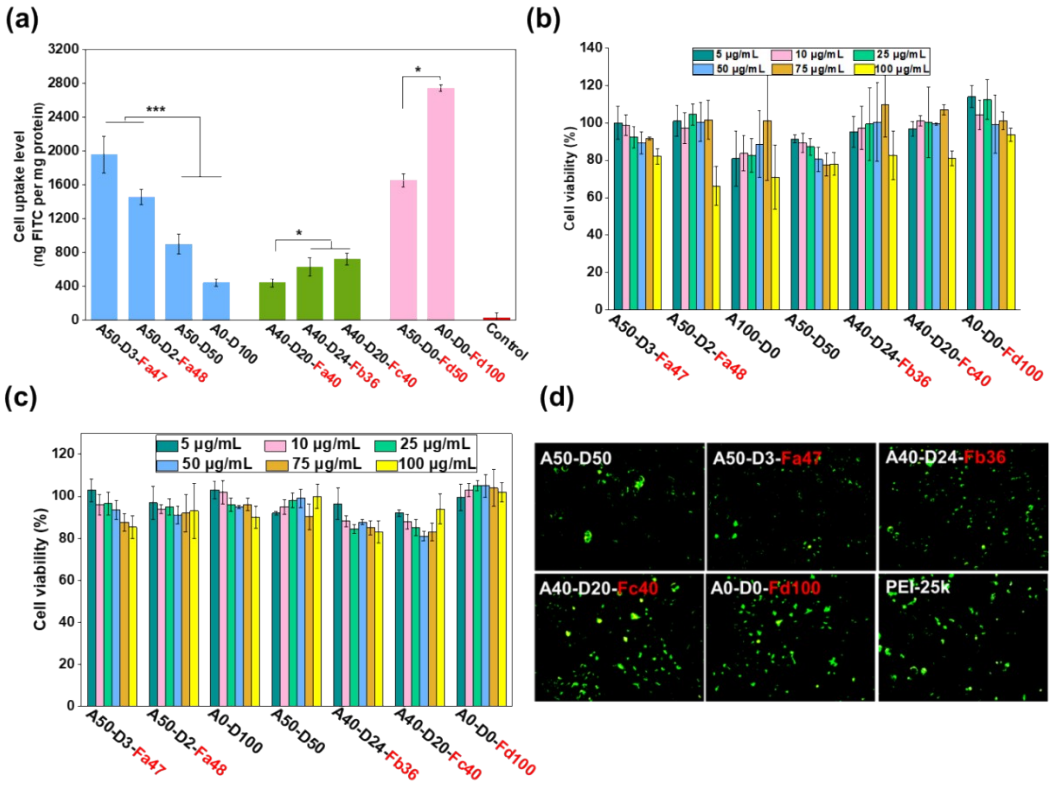


Figure S44. Cell-penetrating properties of polypeptides in Hep1-6 cells (a). Cell viability of polypeptides in Hep1-6 (b) and 293T (c) cells measured by the MTT assay (n=3). *In vitro* transfection efficiencies of polyplexes evaluated by GFP expression in Hela cells under serum-free conditions (d).

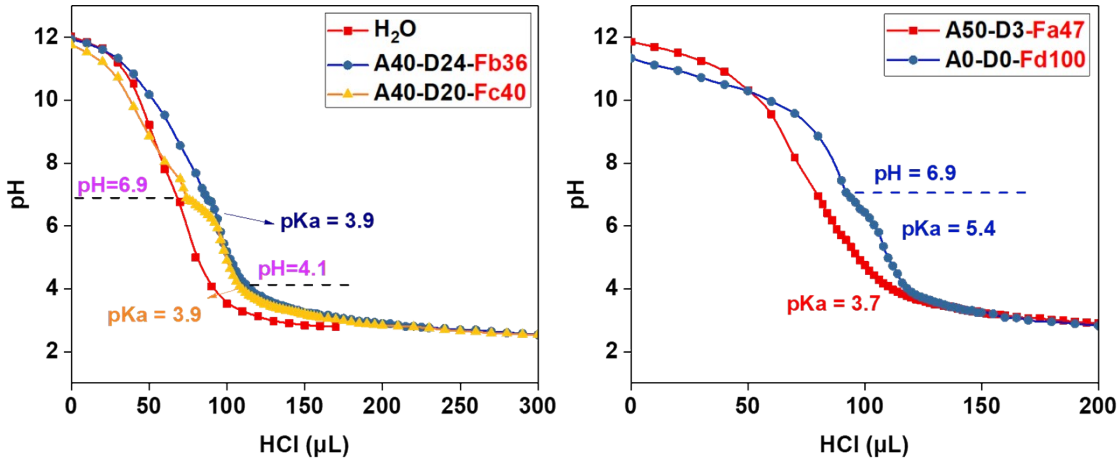


Figure S45. The pH titration curves of A50-D3-Fa47, A40-D24-Fb36, A40-D20-Fc40 and A0-D0-Fd100.

Supporting Reference

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