Supporting Information

Facile synthesis of the sulfotyrosine-containing α-Conotoxins

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Supporting data and figures

Table S1. Sequence of the synthesized α-Ctx in the current work. sY modification sites are red colored, the two disulfide bonds are highlighted yellow and blue, respectively.

Name	Sequence	Origin
PnIA	-GCCSLPPCA ANNPDYC 16	*Conus pennaceus*
PnIB	-GCCSLPPCA LSNPDYC 16	*Conus pennaceus*
EpI	-GCCSDPRCN MNNPDYC 16	*Conus episcopatus*
AnIA	--CCSHPACA ANNQDYC 15	*Conus anemone*
AnIB	GGCCSHPACA ANNQDYC 17	*Conus anemone*
AnIC	GGCCSHPACF ASNPDYC 17	*Conus anemone*
AuIA	-GCCSYPPCF ATNSDYC 16	*Conus aulicus*
PnMGMR-02	-GCCSRPPCA LSNPDYC 16	*Conus pennaceus*

1. Synthesis of the non-sY modified α-Ctx:

Table S2. Summarization of the synthesis of non-sY modified α-Ctx.

Conotoxins	AuIA	AnIA	AnIB	AnIC	PnIA	PnIB	EpI	PnMG MR-02
$[M+H]^+$ calculated	1725.6	1594.5	1708.6	1726.6	1637.7	1594.5	1787.6	1680.7
$[M+H]^+$ observed	1725.6	1594.3	1708.2	1726.6	1637.6	1594.3	1787.1	1680.6
Total yields/%	42	32	42	37	38	39	50	40

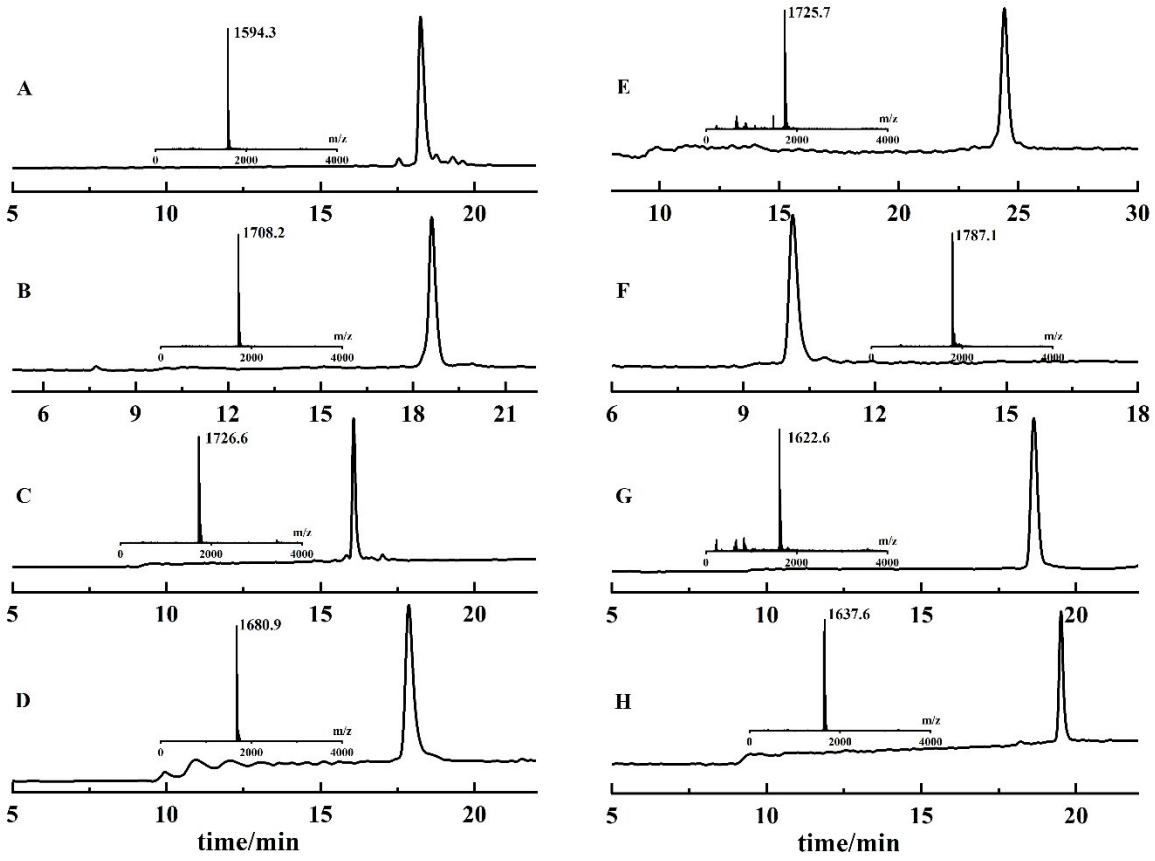

Fig. S1. HPLC analysis of the folded non-sY modified α-Ctx. **A**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of AnIA. MS: $[M+H]^+_{obs}$: 1594.3 Da, $[M+H]^+_{calc}$: 1594.5 Da. HPLC condition: 5%-15% MeCN (with 0.1% TFA) in 20 min and 60 °C. Total yield : 32%. **B**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of AnIB. MS: $[M+H]^+_{obs}$: 1708.2 Da, $[M+H]^+_{calc}$: 1708.6Da. HPLC condition: 5%-15% MeCN (with 0.1% TFA) in 20 min and 60 °C. Total yield: 42%. **C**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of AnIC. MS: $[M+H]^+_{obs}$: 1726.6 Da, $[M+H]^+_{calc}$: 1726.6 Da. HPLC condition: 10%-35% MeCN (with 0.1% TFA) in 15 min and 60 °C. Total yield: 37%. **D**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of PnMGMR-02. MS: $[M+H]^+_{obs}$: 1680.9 Da, $[M+H]^+_{calc}$: 1680.7 Da. HPLC condition: 0%-40% MeCN (with 0.1% TFA) in 15 min and 60 °C. Total yield: 40%. **E**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of AuIA. MS: $[M+H]^+_{obs}$: 1725.6 Da, $[M+H]^+_{calc}$: 1725.6 Da. HPLC condition: 10%-15%-25% MeCN (with 0.1% TFA) in 5 and 20 min, respectively, 60 °C. Total yield: 42%. **F**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of EpI. MS: $[M+H]^+_{obs}$: 1787.1 Da, $[M+H]^+_{calc}$: 1786.6 Da. HPLC condition: 10%-15% MeCN (with 0.1% TFA) in 10 min and 60 °C. Total yield: 50%. **G**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of PnIA. MS: $[M+H]^+_{obs}$: 1637.6 Da, $[M+H]^+_{calc}$: 1637.7 Da. HPLC condition: 10%-25% MeCN (with 0.1% TFA) in 15min and 60 °C. Total yields 38%. **H**. Analytical HPLC chromatogram (214nm) and MALDI-TOF MS of PnIB. MS: $[M+H]^+_{obs}$: 1594.3 Da, $[M+H]^+_{calc}$: 1594.5 Da. HPLC condition: 10%-40% MeCN (with 0.1% TFA) in 15min and 60 °C. Total yield: 39%.

2. Synthesis of the Fmoc-Tyr(OSO_3nP)-OH:

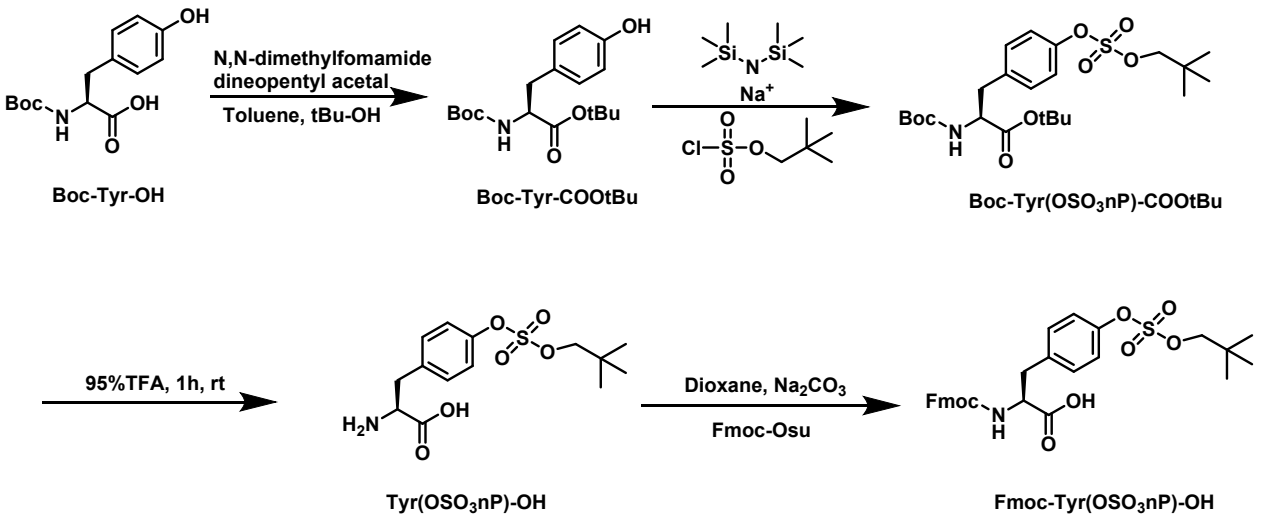

Fig. S2 Synthetic path of Fmoc-Tyr(OSO_3nP)-OH.[1,2]

Boc-Tyr-COOtBu (N-(tert-Butoxycarbonyl)-L-tert-butoxytyrosine)[1]: white solid, 62% yields; ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.01(d, J=8.4Hz, 2H), 6.73(d, J=8.2Hz, 2H), 5.02(d, J=7.4Hz, 2H), 4.37-4.41(m, 1H), 2.97(d, J=5.6Hz, 2H), 1.42, 1.41(2s, 18H).

Boc-Tyr(OSO_3nP)-COOtBu (N-(Tert-Butoxycarbonyl)-L-Tyrosine Tert-Butyl Ester Neopentyl Sulfate)[2]: yellow liquid, 86% yields; ^{1}H NMR (400 MHz, CD_3OD, ppm) δ7.35(d, J=8.1Hz, 2H), 7.29(d, J=7.7Hz, 2H), 4.27(dd, J=7.6, 8.8Hz, 1H), 4.14(s, 2H) 3.10(dd, J=6.1, 7.9Hz, 1H), 2.95(dd, J=4.6, 9.2Hz, 1H), 1.43(s, 9H), 1.41(s, 9H), 1.00(s, 9H).

Fmoc-Tyr(OSO_3nP)-OH (N-(9-Fluorenylmethoxycarbonyl) L-Tyrosine Neopentyl Sulfate)[2]: white solid, 53% yields; ^{1}H NMR (600 MHz, CD_3OD, ppm) δ7.79(d, J=7.3Hz, 2H), 7.60(m, 2H), 7.39-7.23(m, 8H), 4.42(m, 1H), 4.32-4.23(m, 2H), 4.15(t, J=6.9Hz, 1H), 4.05(s, 2H), 3.25(dd, J=4.7, 9.3Hz, 1H), 2.97(dd, J=4.3, 9.5Hz, 1H), 0.93(s, 9H); ^{13}C NMR (151 MHz, CD_3OD, ppm) δ 173.31, 156.93, 149.19, 143.82, 141.18, 136.92, 130.55, 127.38, 126.76, 124.84, 120.65, 119.51, 83.21, 66.45, 55.15, 47.60, 36.45, 31.30, 24.79. HRMS(ESI): $[M+H]^+_{obs}$: 554.1852 Da, $[M+H]^+_{calc}$: 554.1849 Da.

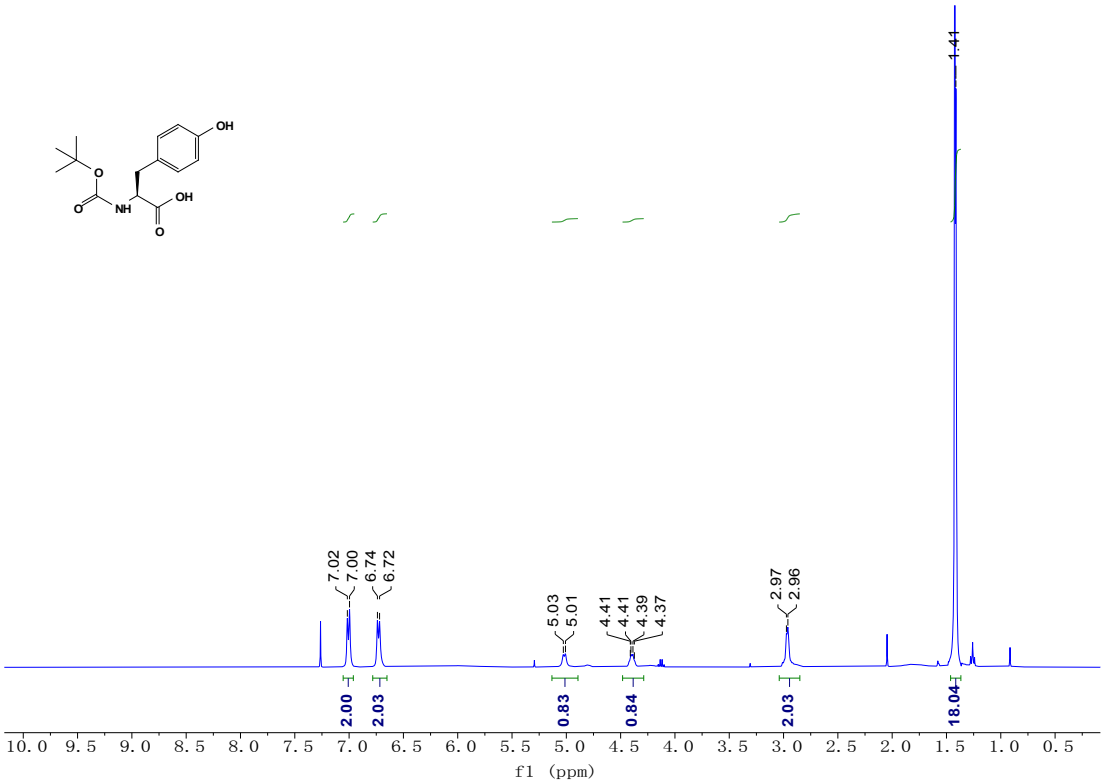

Fig. S3 ^{1}H-NMR spectrum of Boc-Tyr-COOtBu.

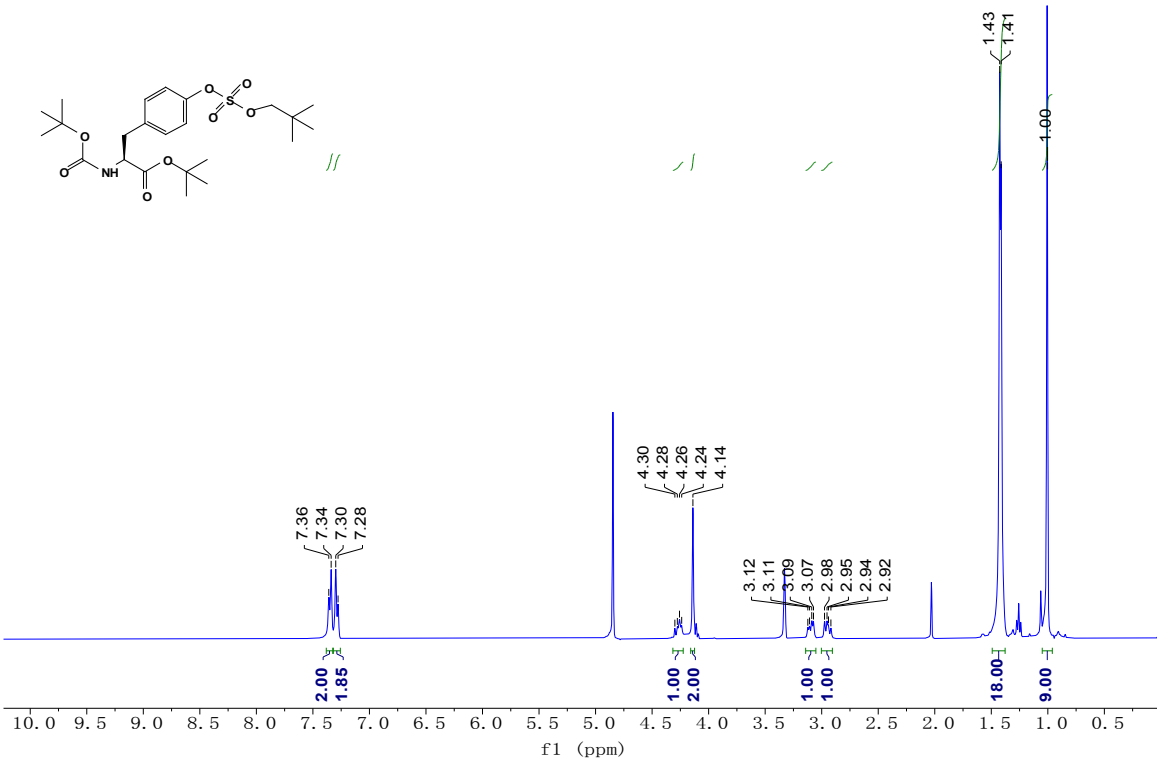

Fig. S4 ^{1}H-NMR spectrum of Boc-Tyr (OSO_3nP)-COOtBu.

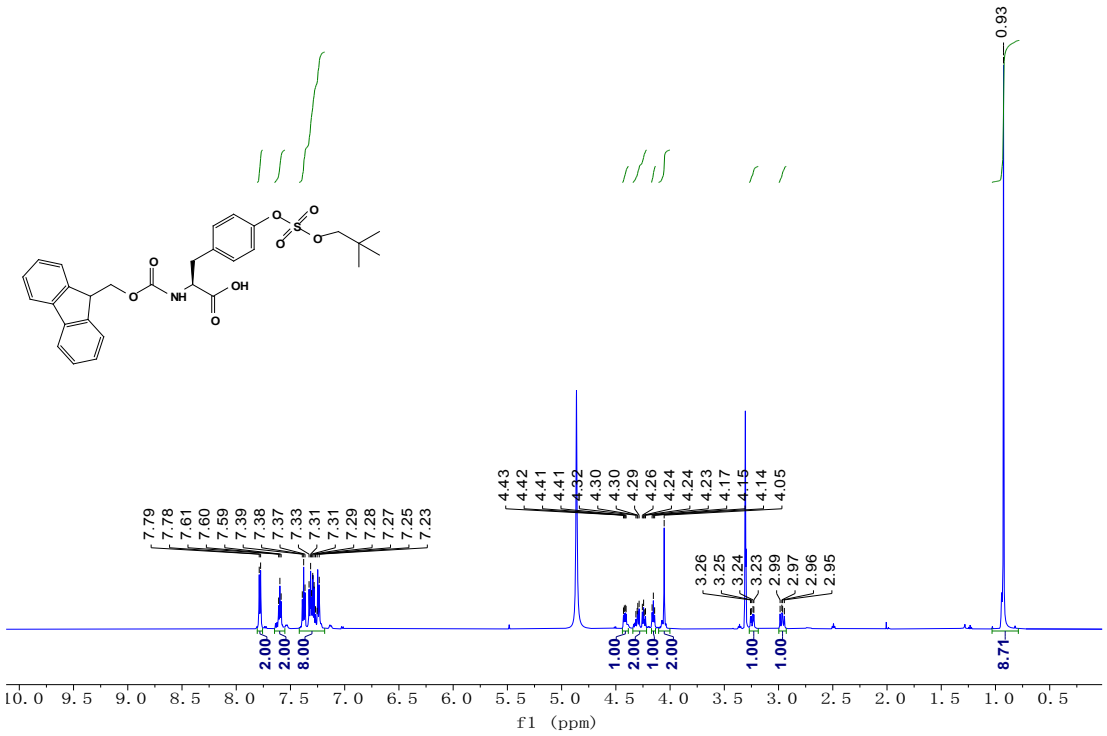

Fig. S5 ^{1}H-NMR spectrum of Fmoc-Tyr (OSO_3nP)-OH.

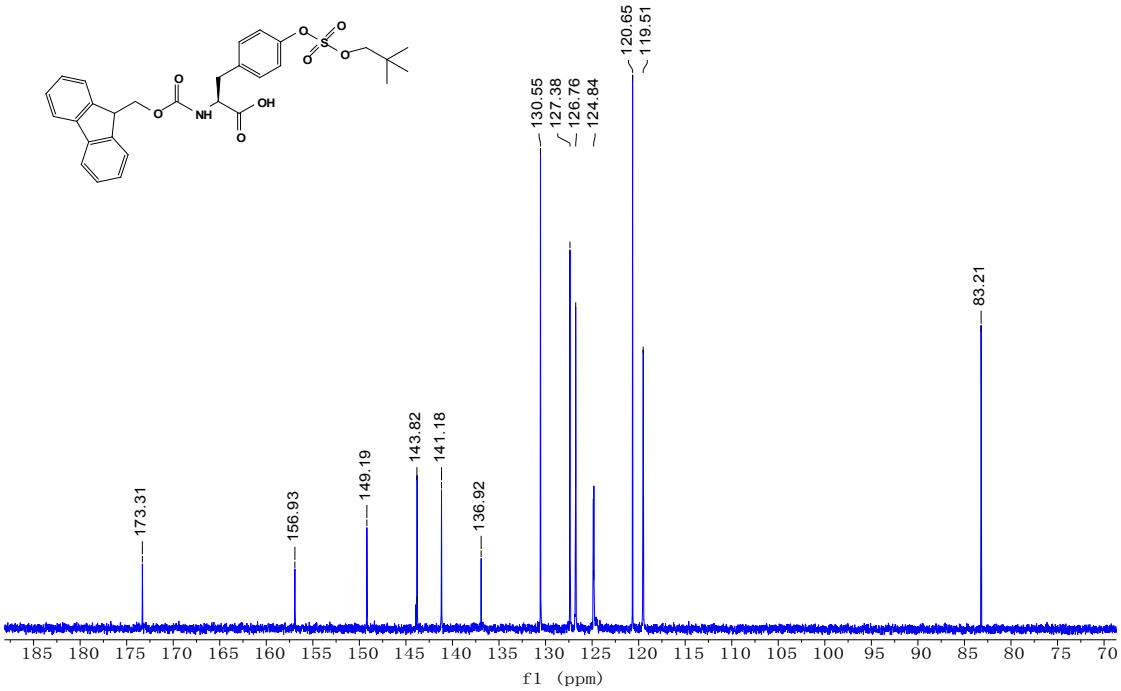

Fig. S6 ^{13}C-NMR spectrum of Fmoc-Tyr (OSO_3nP)-OH.

3. Deprotection of the nP-group:

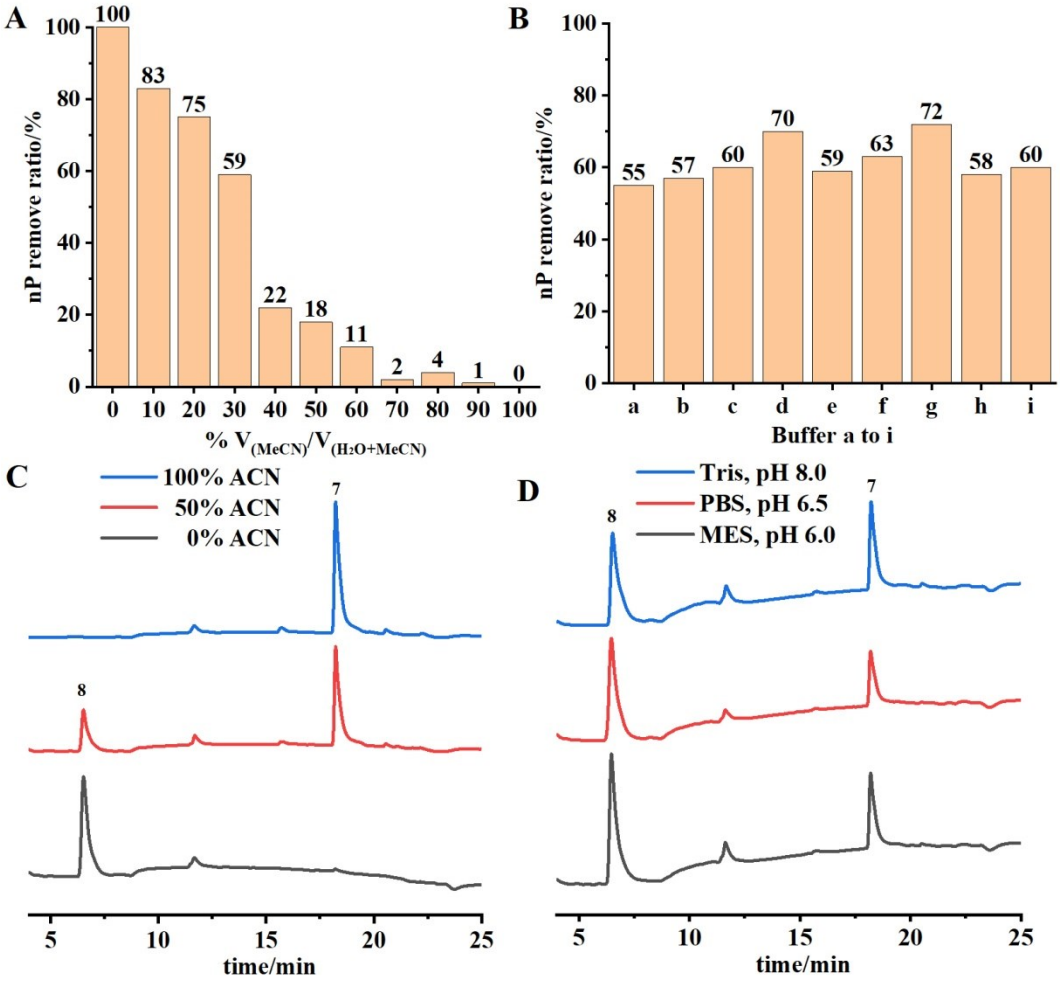

Fig. S7. The study nP removal on the Fmoc-Tyr(OSO_3nP)-OH monomer. **(A)**. The nP removal in with different ratio of MeCN/H_2O. **(B)**. The nP removal with different buffers (all with 20% MeCN for monomer solubilization). a): 0.1 mol/L NH_4HCO_3, pH 8.4; b): 10 mmol/L $K_3Fe(CN)_6$, 0.1 mol/L NH_4HCO_3, pH 8.4; c): 0.05 mol/L Tris, pH 8; d): 0.1 mol/L phosphate and NaCl, pH 7.5; e): 6 mol/L guanidine hydrochloride, 0.1 mol /L phosphate, pH 7.5; f): 1 mol/L ammonium acetate, pH 7; g): 0.1 mol/L phosphate, pH6.5; h): 0.05 mol/L Mes, pH 6; i): 2% glycerol, 0.1 mol/L NaCl and NaOAc, pH 5.5. **(C)**. Typical analytical HPLC (214nm) of nP removal from Fmoc-Tyr(OSO_3nP)-OH in MeCN/H_2O system. **(D)**. Typical analytical HPLC (214nm) of nP removal from Fmoc-Tyr(OSO_3nP)-OH in different buffer solutions. HPLC condition: 40%-95% MeCN (with 0.1% TFA) in 15min and 25 °C. **7** is the Fmoc-Tyr(OSO_3nP)-OH and **8** is product without nP.

4. Synthesis of the sY modified α-Ctx:

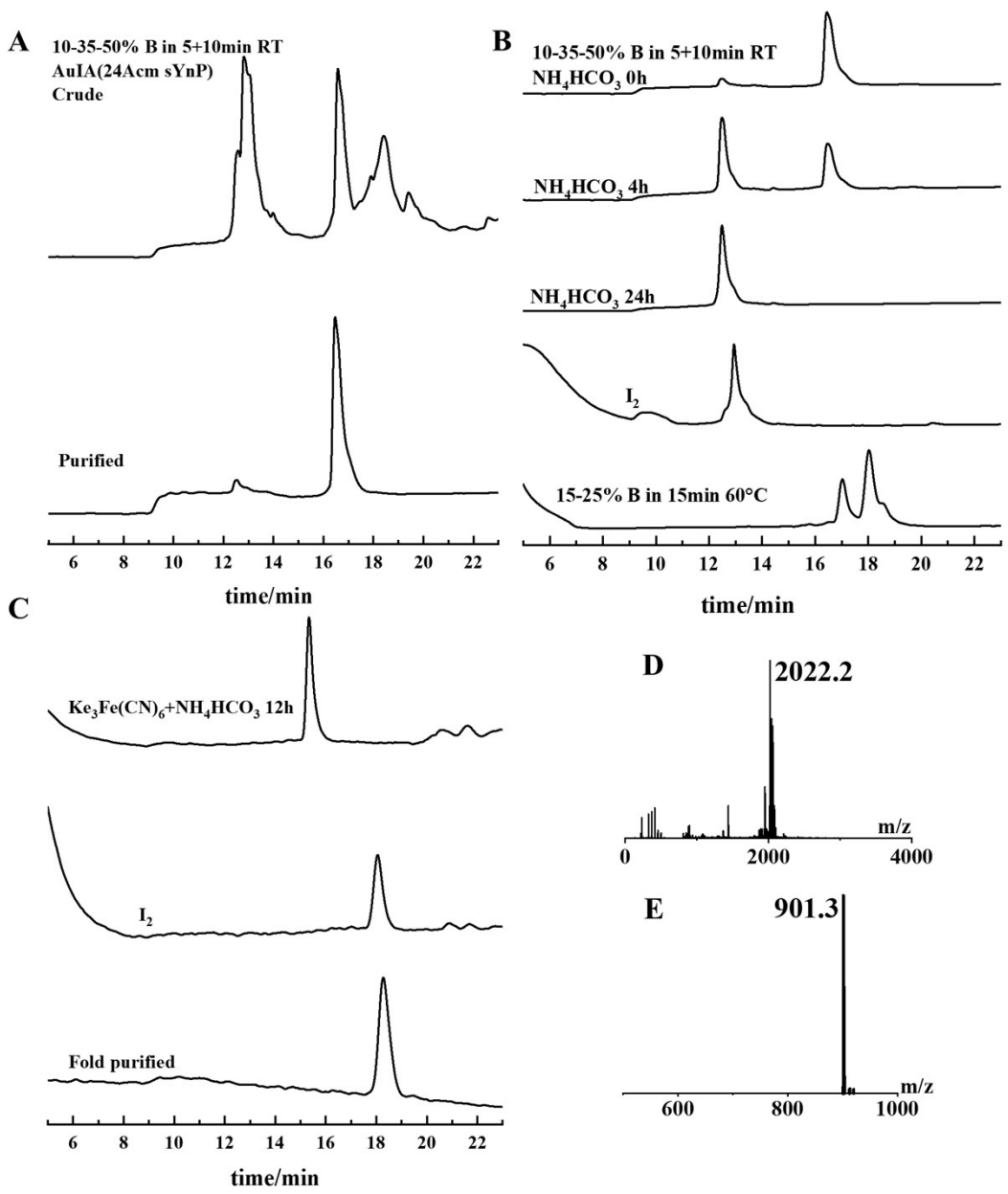

Fig. S8. HPLC trace and MS of AuIA[Y15sY] by two-step folding strategy. **A.** HPLC of linear AuIA(24Acm sYnP). **B.** HPLC trace for AuIA[Y15sY] folding by NH_4HCO_3 and I_2. **C.** HPLC trace for AuIA[Y15sY] folding by NH_4HCO_3, addition of $K_3Fe(CN)_6$ and then I_2. **D.** MALDI-TOF-MS of linear AuIA(24Acm sYnP). MS: $[M+H]^+_{obs}$: 2022.2 Da, $[M+H]^+_{calc}$: 2021.6 Da. **E.** ESI-MS of folded AuIA[Y15sY]. MS: $[M-2H]^{2-}_{obs}$: 901.3 Da, $[M-2H]^{2-}_{calc}$: 901.3 Da.

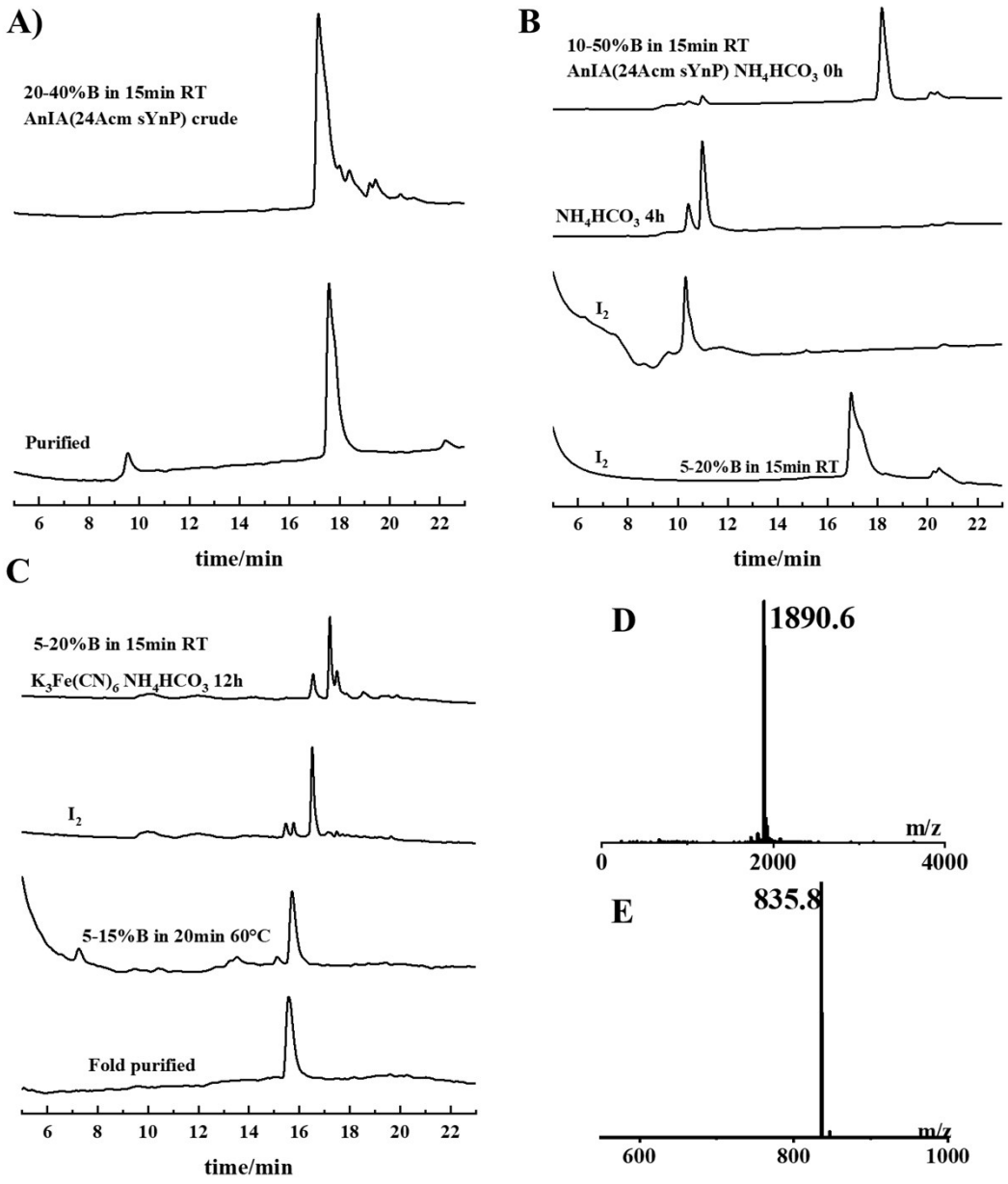

Fig. S9. HPLC trace and MS of AnIA[Y14sY] by two-step folding strategy. **A.** HPLC of linear AnIA(24Acm sYnP). **B.** HPLC trace for AnIA[Y14sY] folding by NH_4HCO_3 and I_2. **C.** HPLC trace for AnIA[Y14sY] folding by NH_4HCO_3, addition of $K_3Fe(CN)_6$ and then I_2. **D.** MALDI-TOF-MS of linear AnIA(24Acm sYnP). MS: $[M+H]^+_{obs}$: 1890.6 Da, $[M+H]^+_{calc}$: 1890.5 Da. **E.** ESI-MS of folded AnIA[Y14sY]. MS: $[M-2H]^{2-}_{obs}$: 835.8 Da, $[M-2H]^{2-}_{calc}$: 835.8 Da.

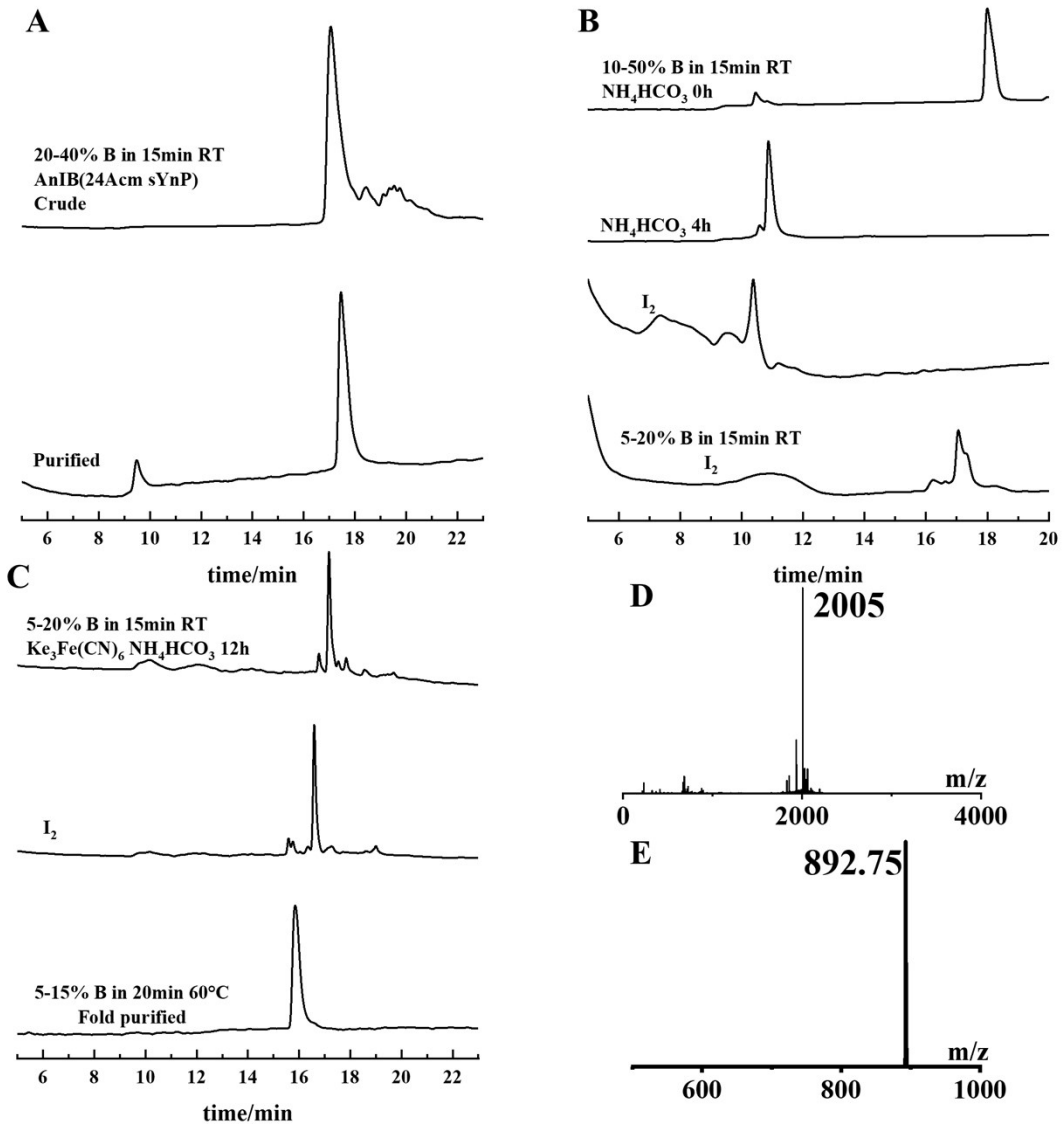

Fig. S10. HPLC trace and MS of AnIB[Y16sY] by two-step folding strategy. **A.** HPLC of linear AnIB(24Acm sYnP). **B.** HPLC trace for AnIB[Y16sY] folding by NH_4HCO_3 and I_2. **C.** HPLC trace for AnIB[Y16sY] folding by NH_4HCO_3, addition of $K_3Fe(CN)_6$ and then I_2. **D.** MALDI-TOF-MS of linear AnIB(24Acm sYnP). MS: $[M+H]^+_{obs}$: 2005 Da, $[M+H]^+_{calc}$: 2004.6 Da. **E.** ESI-MS of folded AnIB[Y16sY]. MS: $[M-2H]^{2-}_{obs}$: 892.75 Da, $[M-2H]^{2-}_{calc}$: 892.8 Da.

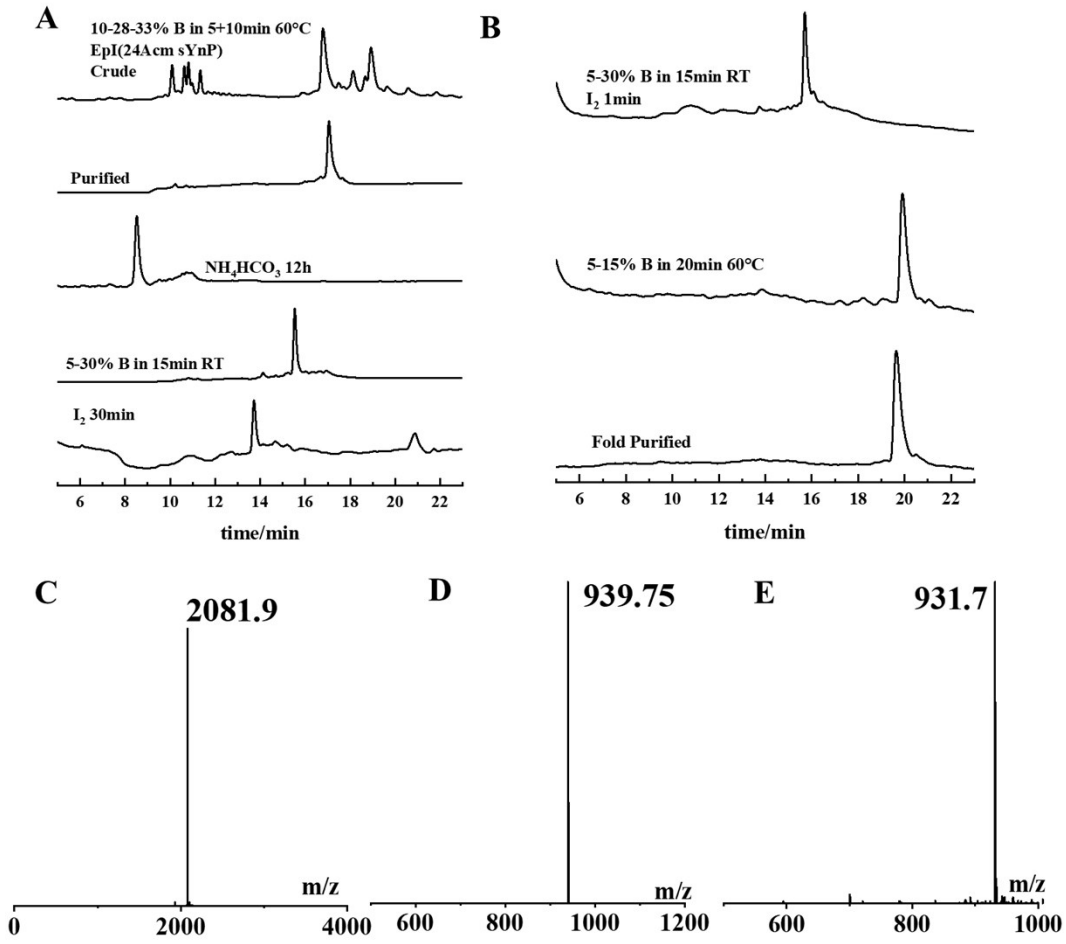

Fig. S11. HPLC trace and MS of EpI[Y15sY] by two-step folding strategy. **A.** HPLC trace for EpI[Y15sY] folding by treatment with I_2 for 30 minutes. **B.** HPLC trace for EpI[Y15sY] folding by treatment with I_2 for 1 minute. **C.** MALDI-TOF-MS of linear EpI(24Acm sYnP). MS: $[M+H]^+_{obs}$: 2081.9 Da, $[M+H]^+_{calc}$: 2082.6 Da. **D.** ESI-MS of folded EpI[Y15sY] with oxidation of Met to sulfoxide. MS: $[M-2H]^{2-}_{obs}$: 939.75 Da, $[M-2H]^{2-}_{calc}$: 939.8 Da. **E.** ESI-MS of folded EpI[Y15sY]. MS: $[M-2H]^{2-}_{obs}$: 931.7 Da, $[M-2H]^{2-}_{calc}$: 931.8 Da.

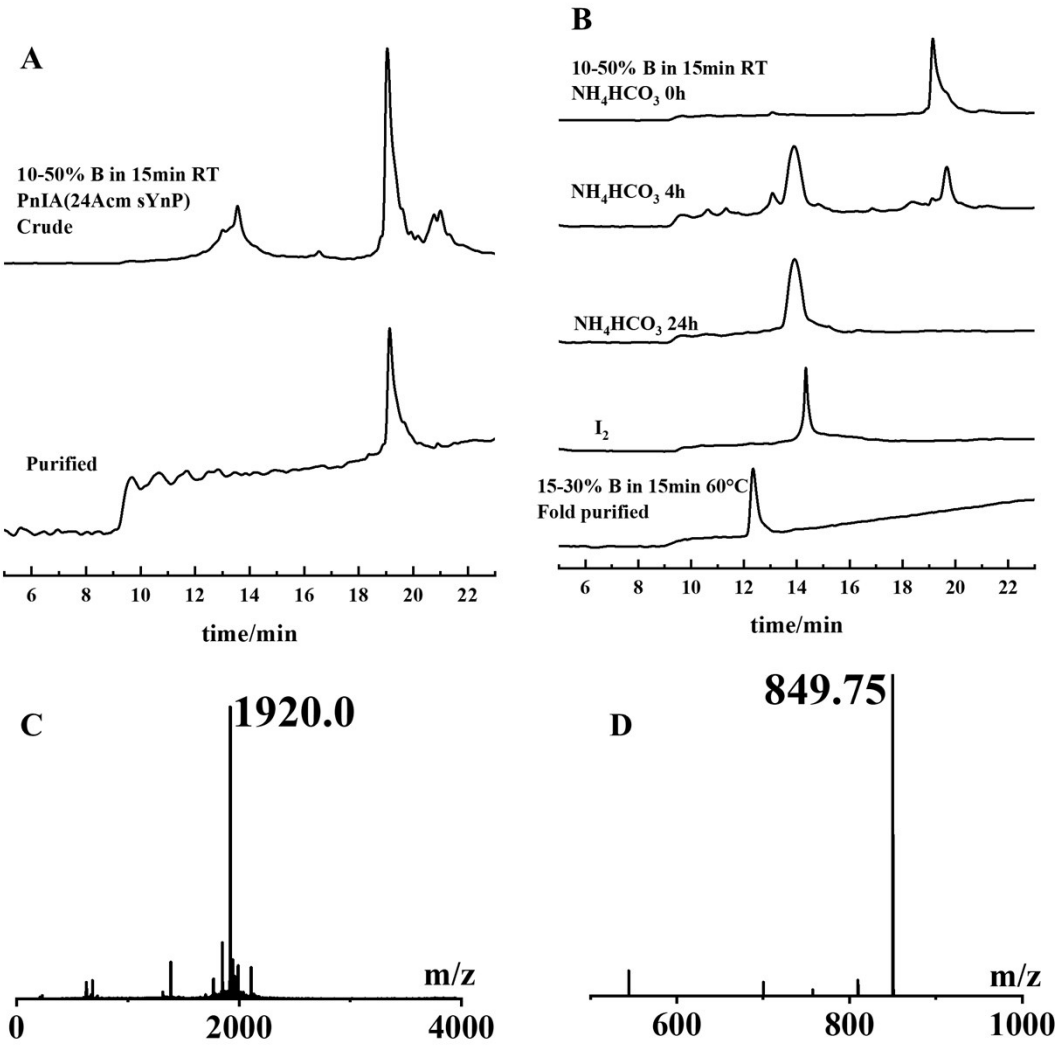

Fig. S12. HPLC trace and MS of PnIA[Y15sY] by two-step folding strategy. **A.** HPLC of linear PnIA(24Acm sYnP). **B.** HPLC trace for PnIA[Y15sY] folding by NH_4HCO_3 and I_2. **C.** MALDI-TOF-MS of linear PnIA(24Acm sYnP). MS: $[M+H]^+_{obs}$: 1920.0 Da, $[M+H]^+_{calc}$: 1918.6 Da. **D.** ESI-MS of folded PnIA[Y15sY]. MS: $[M-2H]^{2-}_{obs}$: 849.75 Da, $[M-2H]^{2-}_{calc}$: 849.8 Da.

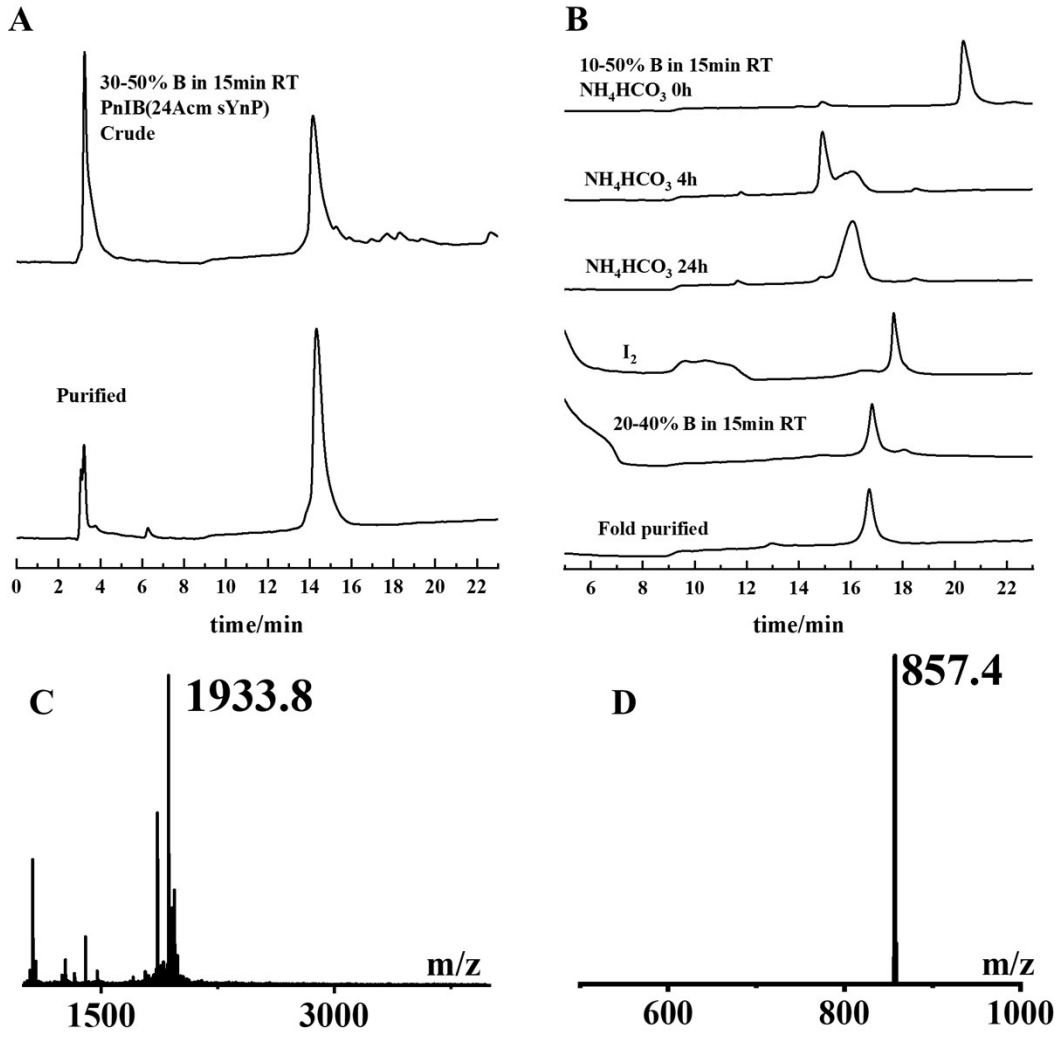

Fig. S13. HPLC trace and MS of PnIB[Y15sY] by two-step folding strategy. **A.** HPLC of linear PnIB(24Acm sYnP). **B.** HPLC trace for PnIB[Y15sY] folding by NH_4HCO_3 and I_2. **C.** MALDI-TOF-MS of linear PnIB(24Acm sYnP). MS: $[M+H]^+_{obs}$: 1933.8 Da, $[M+H]^+_{calc}$: 1933.7 Da. **D.** ESI-MS of folded PnIB[Y15sY]. MS: $[M-2H]^{2-}_{obs}$: 857.4 Da, $[M-2H]^{2-}_{calc}$: 857.4 Da.

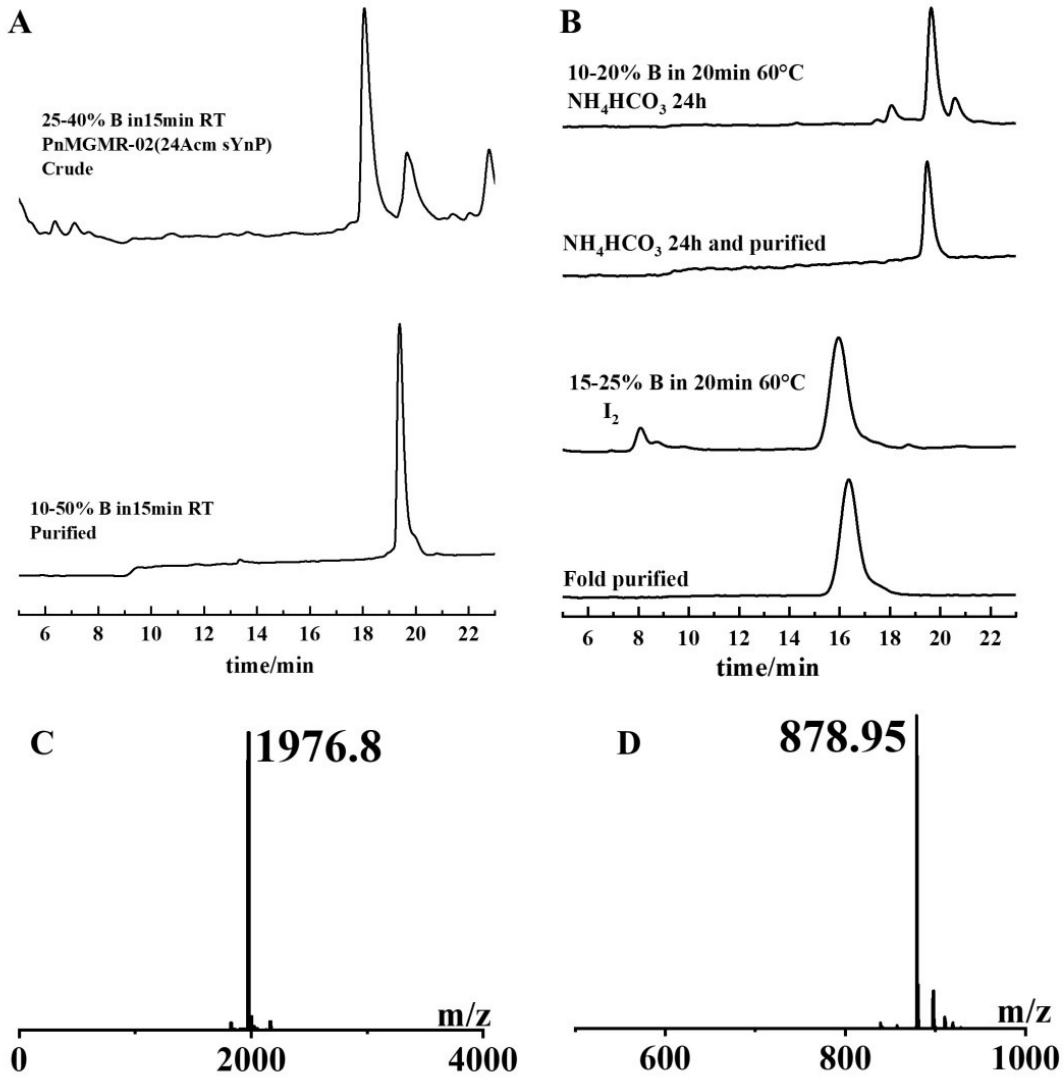

Fig. S14. HPLC trace and MS of PnMGMR-02[Y15sY] by two-step folding strategy. **A.** HPLC of linear PnMGMR-02 (24Acm sYnP). **B.** HPLC trace for PnMGMR-02 [Y15sY] folding by NH_4HCO_3 and I_2. **C.** MALDI-TOF-MS of linear PnMGMR-02 (24Acm sYnP). MS: $[M+H]^+_{obs}$: 1976.8 Da, $[M+H]^+_{calc}$: 1976.7 Da. **D.** ESI-MS of folded PnMGMR-02 [Y15sY]. MS: $[M-2H]^{2-}_{obs}$: 878.95 Da, $[M-2H]^{2-}_{calc}$: 878.9 Da.

Table S3. Summarization of the synthesis of sY modified α-Ctx.

Conotoxins	AuIA	AnIA	AnIB	AnIC	PnIA	PnIB	EpI	PnMGMR-02
$[M-2H]^{2-}$ calculated	901.3	835.8	892.8	901.8	849.8	857.4	931.8	878.9
$[M-2H]^{2-}$ observed	901.3	835.8	892.8	901.8	849.8	857.4	931.7	879.0
Total yields/%	32	43	36	72	34	37	33	45

5. Raw HPLC traces for serum stability test

Note: *indicates impurities from serum.

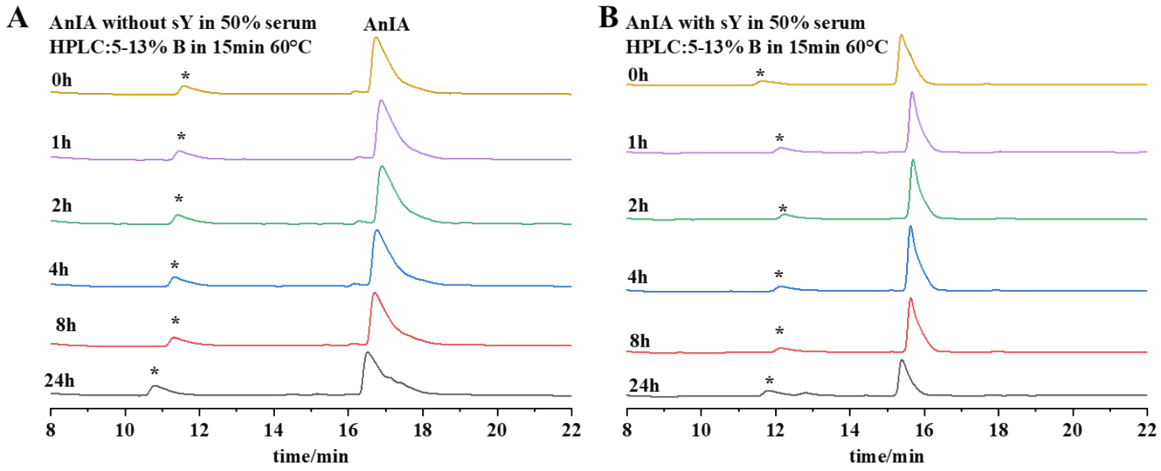

Fig. S15. HPLC of serum stability of AnIA. **A.** AnIA without sY. **B.** AnIA[Y14sY].

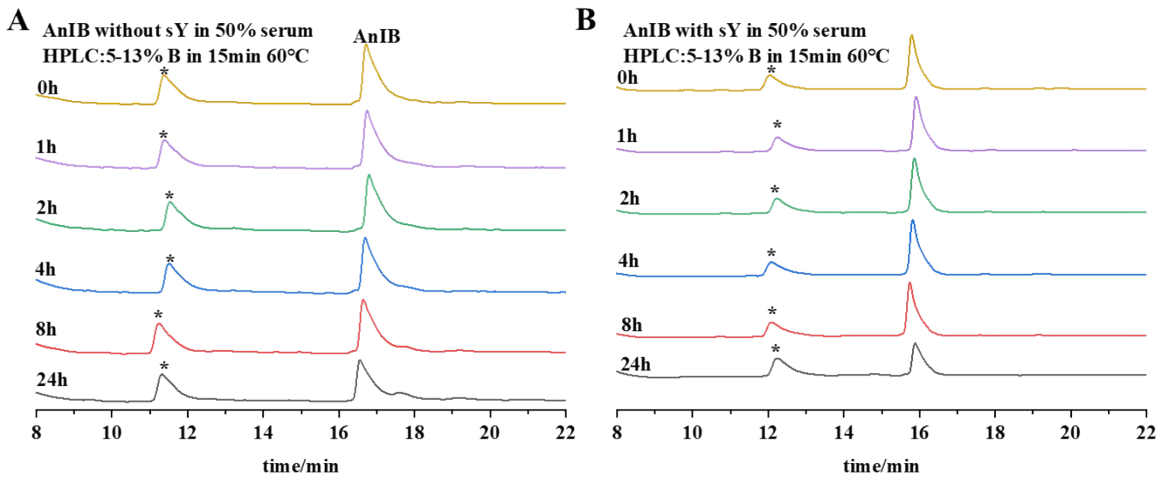

Fig. S16. HPLC of serum stability of AnIB. **A.** AnIB without sY. **B.** AnIB[Y16sY].

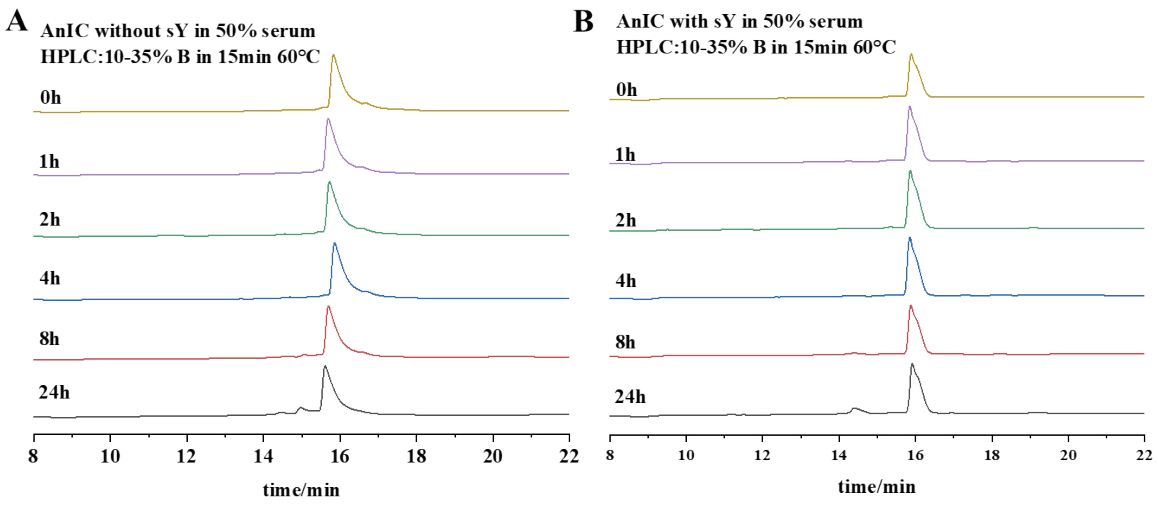

Fig. S17. HPLC of serum stability of AnIC. **A.** AnIC without sY. **B.** AnIC[Y14sY].

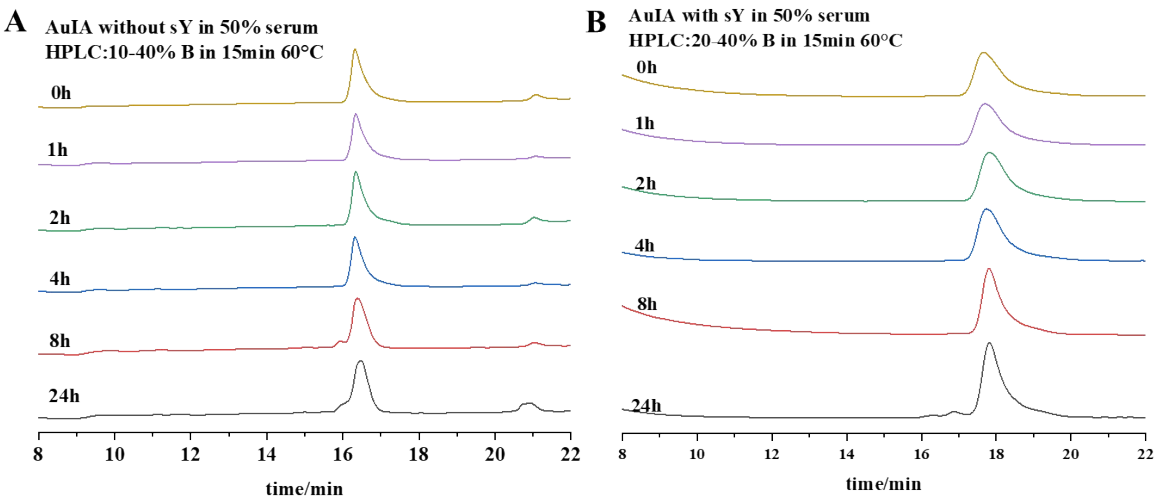

Fig. S18. HPLC of serum stability of AuIA. **A.** AuIA without sY. **B.** AuIA[Y16sY].

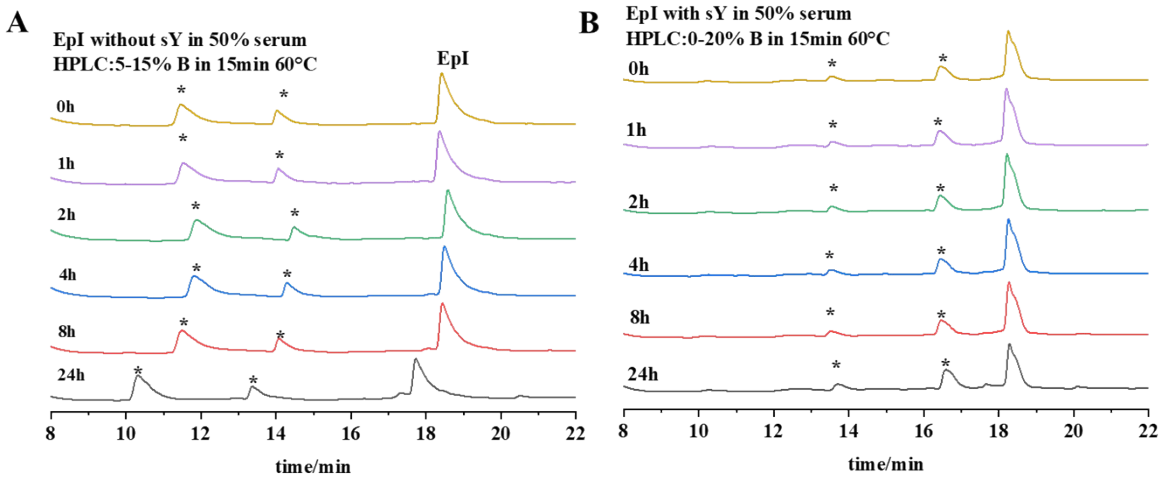

Fig. S19. HPLC of serum stability of EpI. **A.** EpI without sY. **B.** EpI[Y15sY].

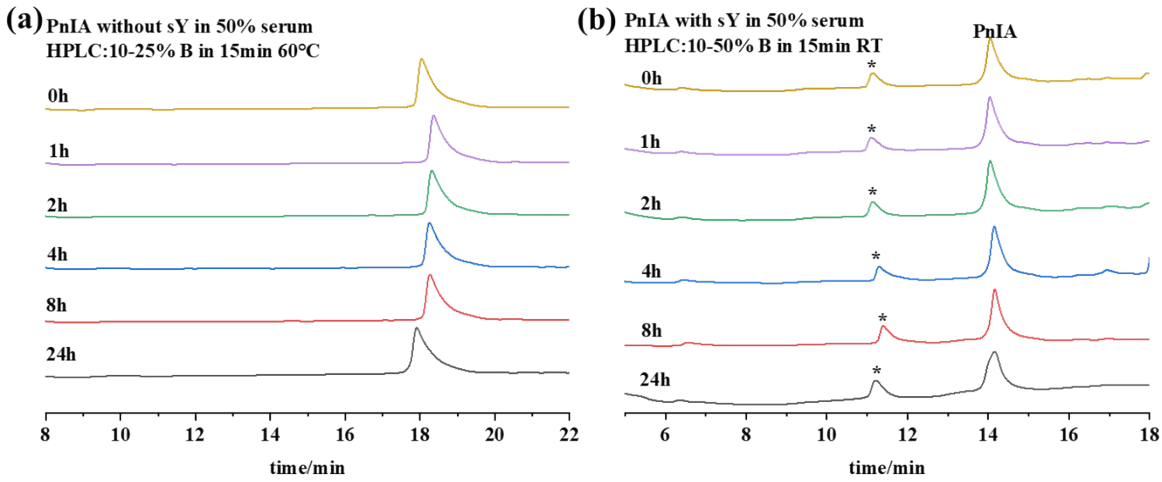

Fig. S120. HPLC of serum stability of PnIA. **A.** PnIA without sY. **B.** PnIA[Y15sY].

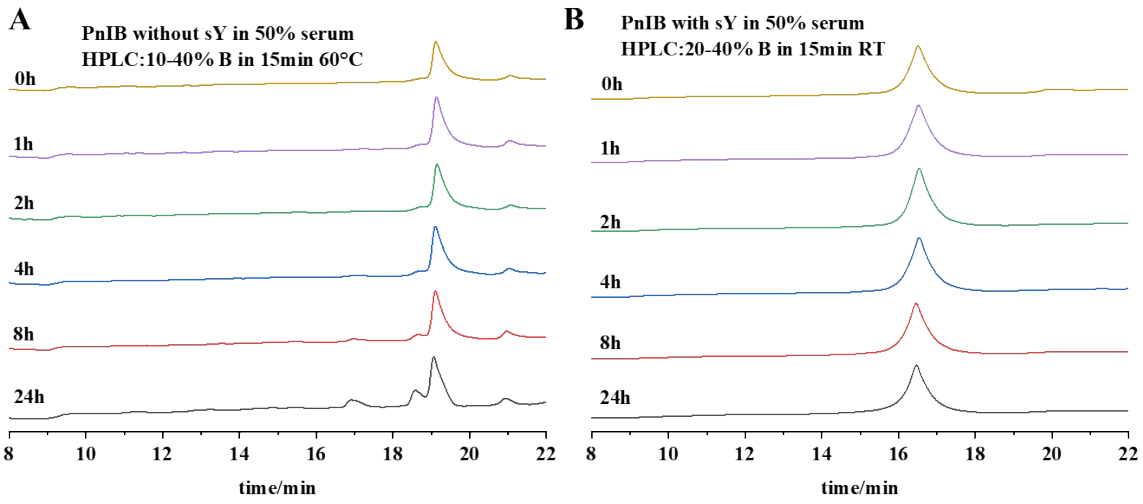

Fig. S21. HPLC of serum stability of PnIB. **A.** PnIB without sY. **B.** PnIB[Y15sY].

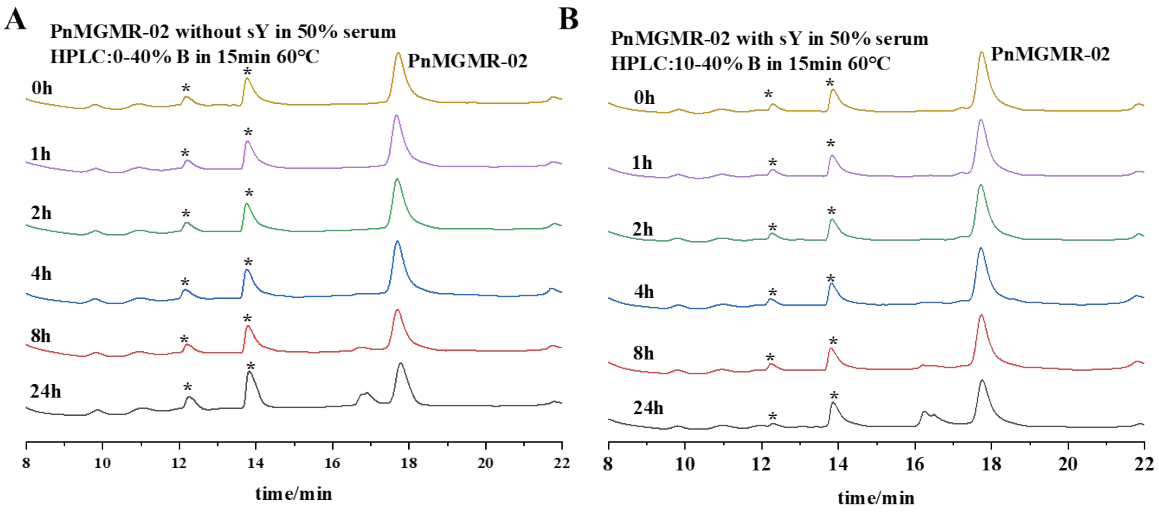

Fig. S22. HPLC of serum stability of PnMGMR-02. **A.** PnMGMR-02 without sY. **B.** PnMGMR-02[Y15sY].

Reference:

1. N. Schlienger, S. Peyrottes, T. Kassem, J.-L. Imbach, G. Gosselin, A. M. Aubertin and C. Pe´rigaud, *J. Med. Chem.,* 2000, **43**, 4570-4574.
2. L. S. Simpson, J. Z. Zhu, T. S. Widlanski and M. J. Stone, *Chem. Biol.*, 2009, **16**, 153-161.