Supporting Information

An Adhesive Peptide From the C-Terminal Domain of α-Synuclein for Single-layer Adsorption of Nanoparticles onto Substrates

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1. Experimental Details

1.1. Synthesis of peptides

Peptides listed in Table S1 were synthesized by a manual method[1] or using an automated synthesizer (Liberty Lite, CEM). In brief, Rink Amide resin (0.71 mmol/g) was prepared by swelling in DMF (2 ml) for 20 min, and subjected to coupling cycles, in which the Fmoc protecting group was removed with a piperidine solution in DMF (20%, 2 ml) for 10 min, and the resin was subsequently washed with DMF (3 x 2 ml, 1 min). Then, a mixture in DMF of Fmoc-α-amino acid (4 equiv.), N-HBTU (4 equiv.) and DIEA (4 equiv.) was added to the resin. The coupling proceeded under nitrogen flow for 15 mins, and the resin was washed with DMF (3 x 2 ml, 1 min). For the automated synthesis, Rink Amide resin (0.05 mmol) was placed in the reaction vessel and swollen in DMF. Then, amino acid coupling cycles were performed under nitrogen atmosphere involving Fmoc removal with piperidine (20% in DMF), washing with DMF three times, addition of coupling mixture (5 equiv. of amino acid, 10 equiv. of DIC, and 10 equiv. of Oxyma), coupling reaction assisted with microwave radiation for 5 mins, and three washing cycles.

1.2 Purification and characterization of peptides.

1.2.1. αSP

Peptide was purified by preparative high-performance liquid chromatography (HPLC) (Waters 1525 with a binary pump and a dual wavelength Waters 2489 UV detector) using an Agilent Eclipse XDB-C18 column [A = H_2O + 0.1% TFA ; B = ACN + 0.1% TFA: 0 min (5% B) to 30 min (70% B), (88 mg, 45% isolated yield); uHPLC: gradient water-ACN (0.1 % TFA) 0 min (5% B) → 12 min (95% B). ^{1}H NMR (300 MHz, D_2O, 298 K) δ: 7.08 (m, 6 H), 6.81 (m, 6 H), 4.62 (m,

water overlapped), 4.52 (m, 3 H), 4.5 (m, 3 H, J_{HH} = 6 Hz), 4.38-4.15 (m, 11 H), 3.92-3.45 (m, 11 H), 3.15-1.80 (m, 48 H), 2.11 (s, 3 H), 1.37 (d, 3 H, J_{HH} = 6 Hz); HR-MS calculated for $C_{84}H_{112}N_{18}O_{33}S^{2-}$: 966.3685, found: 966.3679.

1.2.2. αSP_N

Peptide was purified by preparative HPLC using an Agilent Eclipse XDB-C18 column [A = H_2O + 0.1% TFA ; B = ACN + 0.1% TFA: 0 min (5% B) to 30 min (70% B), (85 mg, 41% isolated yield). uHPLC: gradient water-ACN (0.1 % TFA) 0 min (5% B) → 12 min (95% B); ^{1}H NMR (300 MHz, D_2O, 298 K) δ: 7.08 (m, 6 H), 6.79 (m, 6 H), 4.60-4.50 (m, water overlapped), 4.38-4.15 (m, 11 H), 3.92-3.45 (m, 11 H), 3.15-1.8 (m, 50 H), 2.11 (s, 3 H), 1.37 (d, 3 H, J_{HH} = 6 Hz); HR-MS calculated for $C_{87}H_{117}N_{19}O_{34}S_2^{2-}$, 1017.8731, found: 1017.8728.

1.2.3. αSP_C

Peptide was purified by preparative HPLC using an Agilent Eclipse XDB-C18 column [A = H_2O + 0.1% TFA ; B = ACN + 0.1% TFA: 0 min (5% B) to 30 min (70% B), (80 mg, 39% isolated yield). uHPLC: gradient water-ACN (0.1 % TFA) 0 min (5% B) → 12 min (95% B); ^{1}H NMR (300 MHz, D_2O, 298 K) δ: 7.08 (m, 6 H), 6.81 (m, 6 H), 4.75 (m, water overlapped), 4.61 (t, 2 H, $J_{H,H}$ = 6 Hz), 4.55-4.24 (m, 11 H), 4.23 (t, 2 H, $J_{H,H}$ = 6 Hz), 3.98-3.46 (m, 6 H), 3.20-1.80 (m, 43 H), 2.11 (s, 3 H), 1.37 (d, 3 H, J_{HH} = 6 Hz); HR-MS calculated for $C_{87}H_{117}N_{19}O_{34}S_2^{2-}$, 1017.8731, found: 1017.8728.

1.2.4. $6E_N$

Peptide was purified by preparative HPLC using an Agilent Eclipse XDB-C18 column [A = H_2O + 0.1% TFA; B = ACN + 0.1% TFA: 0 min (0% B) to 30 min (95% B), (18 mg, 20% isolated yield). uHPLC: gradient water-ACN (0.1 % TFA) 0 min (5% B) → 12 min (95% B); ^{1}H NMR (300 MHz, DMSO-d_6, 298 K) δ: 12.10 (bs, 5 H), 8.73 (bd, 1 H), 8.27-8.10 (m, 5 H), 7.85 (d, 2 H,

$J_{H,H}$ = 9 Hz), 7.32 (s, 1 H), 7.06 (s, 1 H), 4.38-4.11 (m, 7 H), 2.93 (b, 2 H), 2.25 (b, 12 H), 2.00-1.60 (m, 12 H).

1.2.5. $_{(4)}\alpha SP_N$, $_{(6)}\alpha SP_N$, $_{(8)}\alpha SP_N$, $_{(10)}\alpha SP_N$, $_{(12)}\alpha SP_N$, $_{(14)}\alpha SP_N$, $_{(tdm)}\alpha SP$, $_{(tdm)}\alpha SP_N$, $_{(tdm)}\alpha SP_C$, $_{(P\rightarrow A)}\alpha SP_N$, $_{(Y\rightarrow A)}\alpha SP_N$, and $_{(P/Y\rightarrow A)}\alpha SP_N$

Peptide was purified by preparative HPLC using an Agilent Eclipse XDB-C18 column [A = H_2O + 0.1% TFA; B = ACN + 0.1% TFA: 0 min (5% B) to 30 min (50% B); uHPLC: gradient water-ACN (0.1 % TFA) 0 min (5% B) → 12 min (95% B).

1.3. Preparation of αSP_N-AuNP

The αSP_N-AuNP conjugates were prepared by incubating the mixture of αSP_N (200 μM in deionized water, 20 μl) and 20-nm citrate-capped AuNP (Nanovex Biotechnologies, 0.05 mg/ml in 2 mM citrate at pH 6.5, 180 μl) overnight at 4°C and removal of excess peptides was performed by centrifugation at 16100g for 10 mins at 4°C three times.

1.4. Monolayer adsorption of αSP_N-AuNP onto various substrates

The prepared conjugates were centrifuged and resuspended with 2 μl of 50 mM citrate at pH 4.5, and then placed on the ultrasonically pre-cleaned substrates including Si, boron-doped p-type Si, SiO_2, Au, Ti, glass, PC, and PET. Following incubation at 40°C for 3 hours in a humid chamber, the substrates were washed with excess methanol (20%, 4°C) and purged with nitrogen gas. For the superlattice formation on mica, the incubation was carried out at either pH 4.75 (30°C), pH 4.50 (35°C), or pH 4.25 (40°C).

1.5. Atomic force microscopy (AFM) analysis

The αSP_N-AuNP-adsorbed substrates were examined with AFM (XE-100, Park Systems Corporation) under ambient atmosphere at room temperature in a non-contact mode using ACTA cantilevers. For analyzing thickness of the layer, each substrate was smoothly scratched with PET before the AFM measurement.

1.6. Scanning electron microscope (SEM) analysis

The conjugate-adsorbed substrates were sputter-coated with a 5-nm-thick layer of platinum, and then analyzed with SEM (Zeiss Ultra Plus) at 3.0 kV.

1.7. Circular dichroism (CD)

CD spectra were obtained with a Jasco J-1100 CD spectrometer at 20 ˚C in a 2 mm light path quartz cuvette after substraction of the solvent background signal.

1.8. Computational analysis for isoelectric point (pI) of αSP

Isoelectric point (pI) of αSP_N was obtained by calculation programs provided on the websites of INNOVAGEN (https://pepcalc.com).

1.9. NMR spectroscopy

Secondary chemical shift analysis and side-chain carboxyl pK_a determination were performed by analyzing a pH titration monitored by ^{1}H-^{13}C HSQC spectra obtained at natural isotope abundance. Unambiguous residue assignments were obtained by analyzing ^{1}H-^{1}H TOCSY and NOESY spectra at each titration data point, with 70 and 200 ms mixing times respectively. All peptide

samples were freshly dissolved independently from lyophilized powder in 5 mM citrate buffer containing 1.5 mM TCEP, pH 4.5. Final peptide concentration was adjusted to 1.5 mM and pH was adjusted to the desired value. Data were acquired at 310 K in a 600 MHz Bruker Avance spectrometer equipped with a cryoprobe. All samples contained 10 μM DSS (4,4-dimethyl-5-silapentane-1-sulfonic acid) for chemical shift referencing. Random coil Cα and Cβ chemical shifts were generated using the software POTENCI[2] at each of the indicated pH values, 310 K and solution ionic strength as calculated using the software Buffer Maker from ChemBuddy. For side-chain carboxyl pK_a determination, Cγ (Glu) or Cβ (Asp) chemical shifts were monitored in the same ^{1}H-^{13}C HSQC spectra series.[3] Data were normalized according to:

$$(\delta^{13}C_{pH=x} - \delta^{13}C_{pH=2.0})/(\delta^{13}C_{pH=6.0} - \delta^{13}C_{pH=2.0})$$

The resulting values were fitted to a four-parameter sigmoidal curve using the software Prism to obtain the pK_a values.

1.10. Dynamic light scattering (DLS)

Statistical distribution of the hydrodynamic diameters of peptide-AuNPs were obtained with a Zetasizer Nano ZSP (Malvern) in either MQ or 50 mM citrate buffer at pH 4.5.

1.11. Chaperone activity of peptides

Molecular chaperone activity of the peptides was examined according to the procedure previously reported.[4,5] Peptide solutions were co-incubated with either alcohol dehydrogenase (ADH) or lysozyme at 60ºC in 20 mM MES at pH 6.5, and their turbidity was monitored at 405 nm with Libra UV/Visible spectrophotometer (Biochrom). The ADH activity was accessed at 340 nm by NAD^+ reduction in the mixture of ADH and the peptides in 100 mM phosphate buffer at pH 7.0

containing 0.2 mM NAD^+ and 1 mM ethanol during the incubation at 60°C. For the control experiments, wild type human αS protein was prepared according to the protocol described in the previous report.[6]

2. Supporting figures

2.1. Conjugates preparation and adhesion experiments

Group	Name	Sequence
αSP	αSP	YEMPSEEGYQDYEPEA-NH_2
	αSP_N	CYEMPSEEGYQDYEPEA-NH_2
	αSP_C	YEMPSEEGYQDYEPEAC-NH_2
Tandem repeat of αSP	$_{(tdm)}\alpha SP$	QEGILEDMPVDPDNEA-NH_2
	$_{(tdm)}\alpha SP_N$	CQEGILEDMPVDPDNEA-NH_2
	$_{(tdm)}\alpha SP_C$	QEGILEDMPVDPDNEAC-NH_2
αSP_N mutants	$_{(P\rightarrow A)}\alpha SP_N$	CYEMASEEGYQDYEAEA-NH_2
	$_{(Y\rightarrow A)}\alpha SP_N$	CAEMPSEEGAQDAEPEA-NH_2
	$_{(P/Y\rightarrow A)}\alpha SP_N$	CAEMASEEGAQDAEAEA-NH_2
αSP_N truncation	$_{(14)}\alpha SP_N$	CMPSEEGYQDYEPEA-NH_2
	$_{(12)}\alpha SP_N$	CSEEGYQDYEPEA-NH_2
	$_{(10)}\alpha SP_N$	CEGYQDYEPEA-NH_2
	$_{(8)}\alpha SP_N$	CYQDYEPEA-NH_2
	$_{(6)}\alpha SP_N$	CDYEPEA-NH_2
	$_{(4)}\alpha SP_N$	CEPEA-NH_2
6E	$6E_N$	CEEEEEE-NH_2

Table S1. Nomenclature of the peptides.

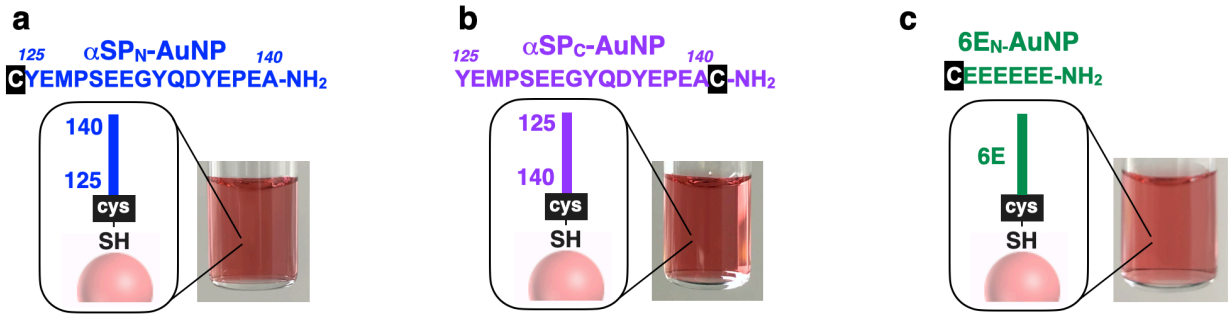

Figure S1. Optical photographs of colloidal (a) αSP_N-AuNP, (b) αSP_C-AuNP, and (c) $6E_N$-AuNP in 20 mM MES buffer at pH 6.5.

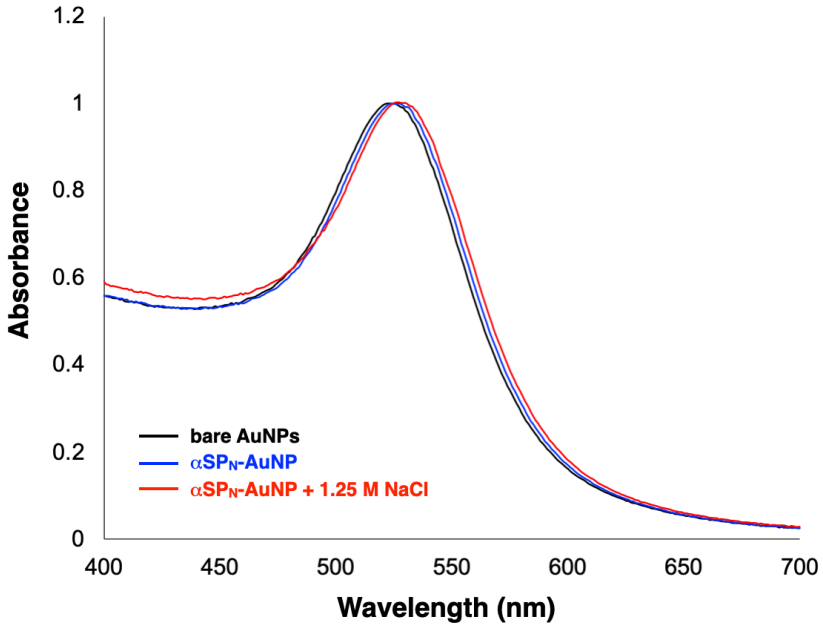

Figure S2. Absorption spectra of colloidal solutions of bare AuNPs (black line), αSP_N-AuNP (blue line) and αSP_N-AuNP with 1.25 M NaCl (red line).

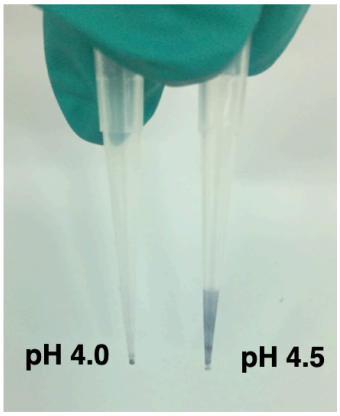

Figure S3. Photographs of tips used for pipetting αSP_N-AuNP in 50 mM citrate at either pH 4.0 or 4.5.

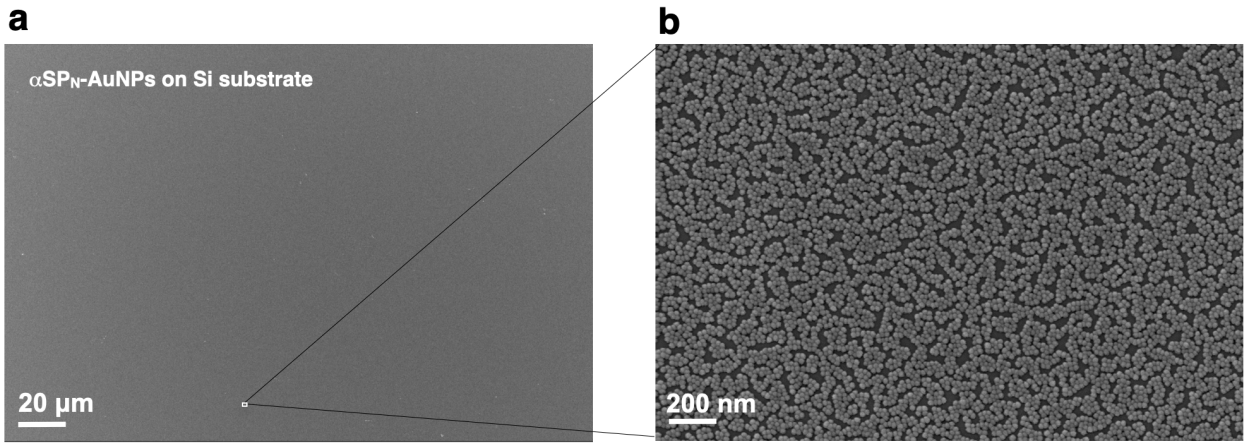

Figure S4. SEM images of αSP_N-AuNP adsorbed onto silicon substrate after incubation (50 mM citrate at pH 4.5) at 40°C for 3 hours. The images at (a) low and (b) high magnification are presented.

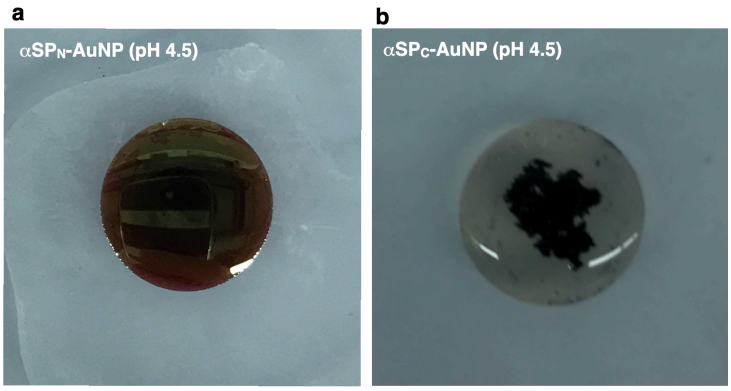

Figure S5. Photo images of (a) αSP_N-AuNP and (b) αSP_C-AuNP in 50 mM citrate at pH 4.5 on silicon surface after incubation at 40°C for 3 hours.

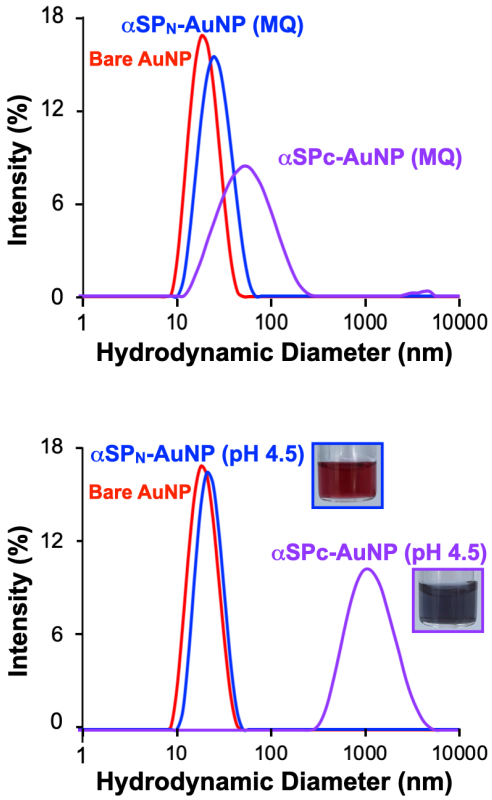

Figure S6. DLS spectra of αSP_N-AuNPs (blue line) and αSP_C-AuNPs (purple line) in either (top) MQ or (bottom) 50 mM citrate at pH 4.5. DLS spectra of bare AuNP (red line) in 2 mM citrate at pH 6.5 were provided for comparison. Optical images of the conjugate solutions showing different plasmonic colors of αSP_N-AuNPs (red) and αSP_C-AuNPs (blue) in 50 mM citrate at pH 4.5 are presented in insert.

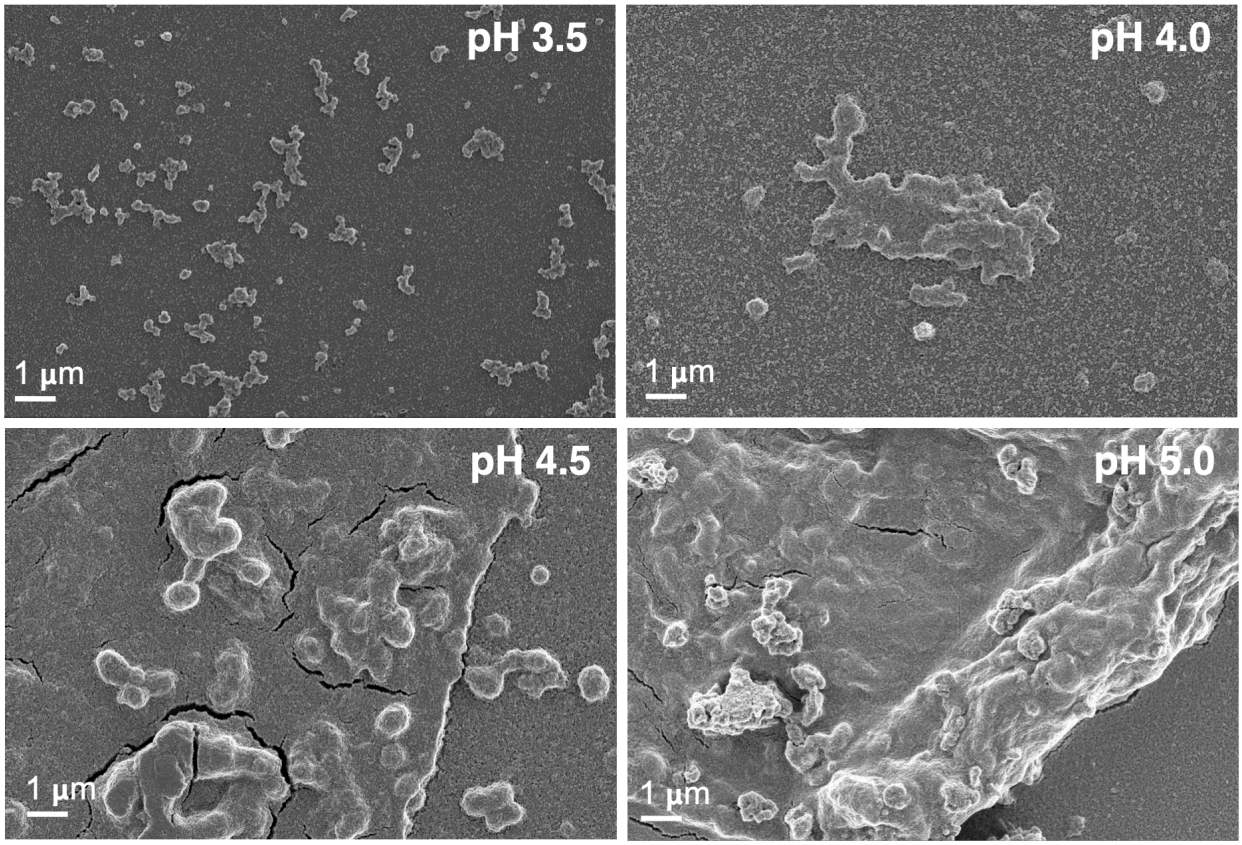

Fig S7. SEM images of $6E_N$-AuNP conjugates on silicon surface after incubation at 40°C for 3 hours in 50 mM citrate at various pHs of 3.5, 4.0, 4.5, and 5.0.

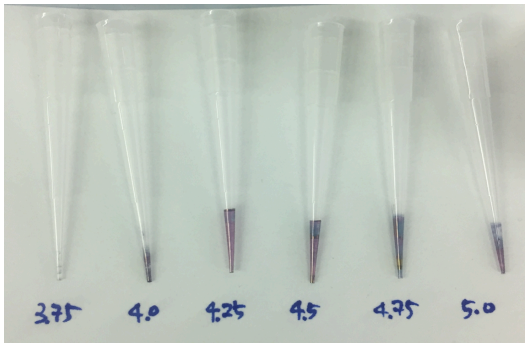

Figure S8. Images of tips used for pipetting αSP_N-AuNP solution in 50 mM citrate at different pHs ranging from 3.75 to 5.00 at 0.25 intervals.

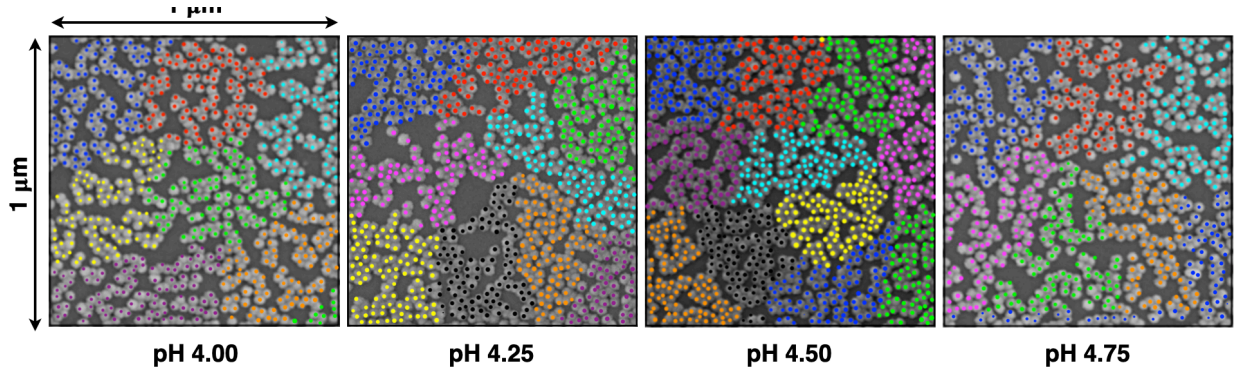

Figure S9. Manual counting of the adsorbed NPs on silicon surface (1 µm x 1 µm).

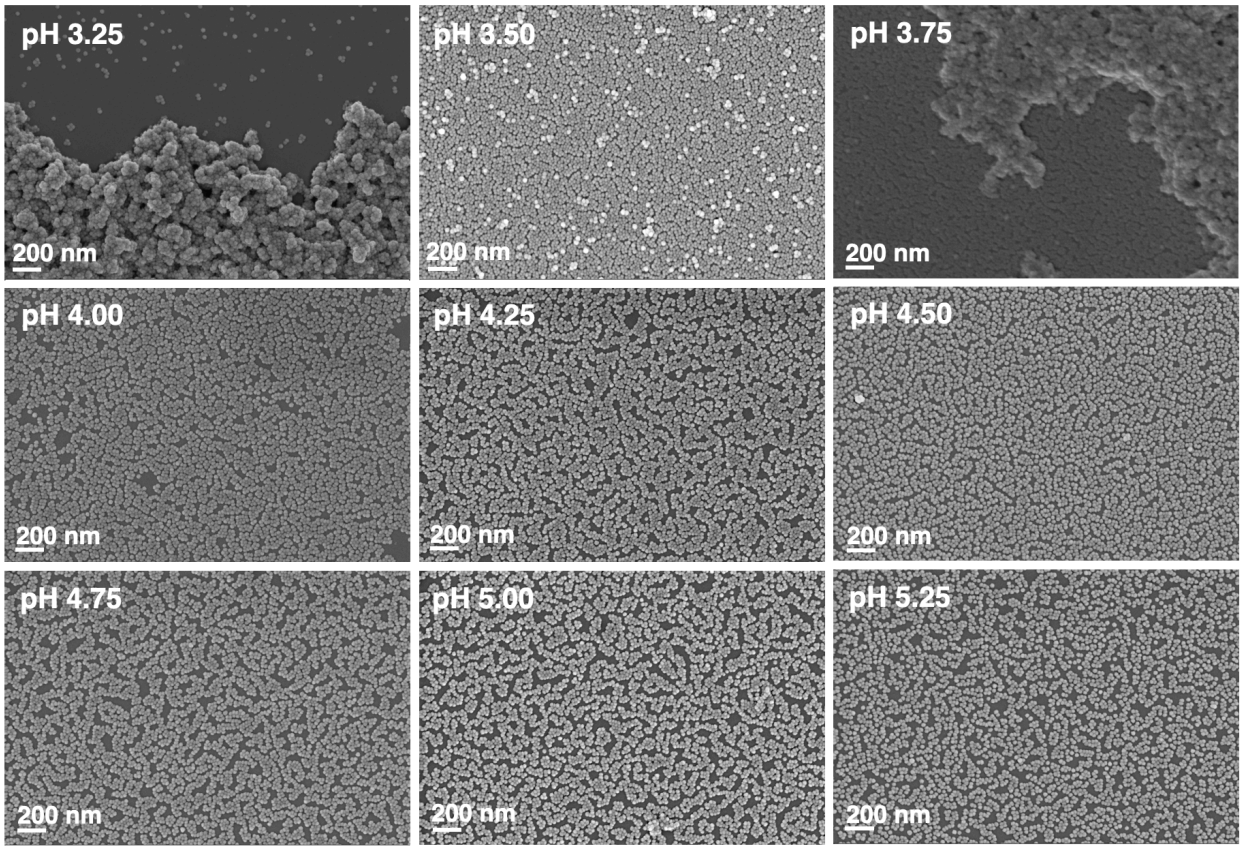

Figure S10. SEM images of αSP_N-AuNPs after incubation (40°C) on silicon substrate for 3 hours at different pHs varying between 3.25 and 5.25 in 0.25 intervals.

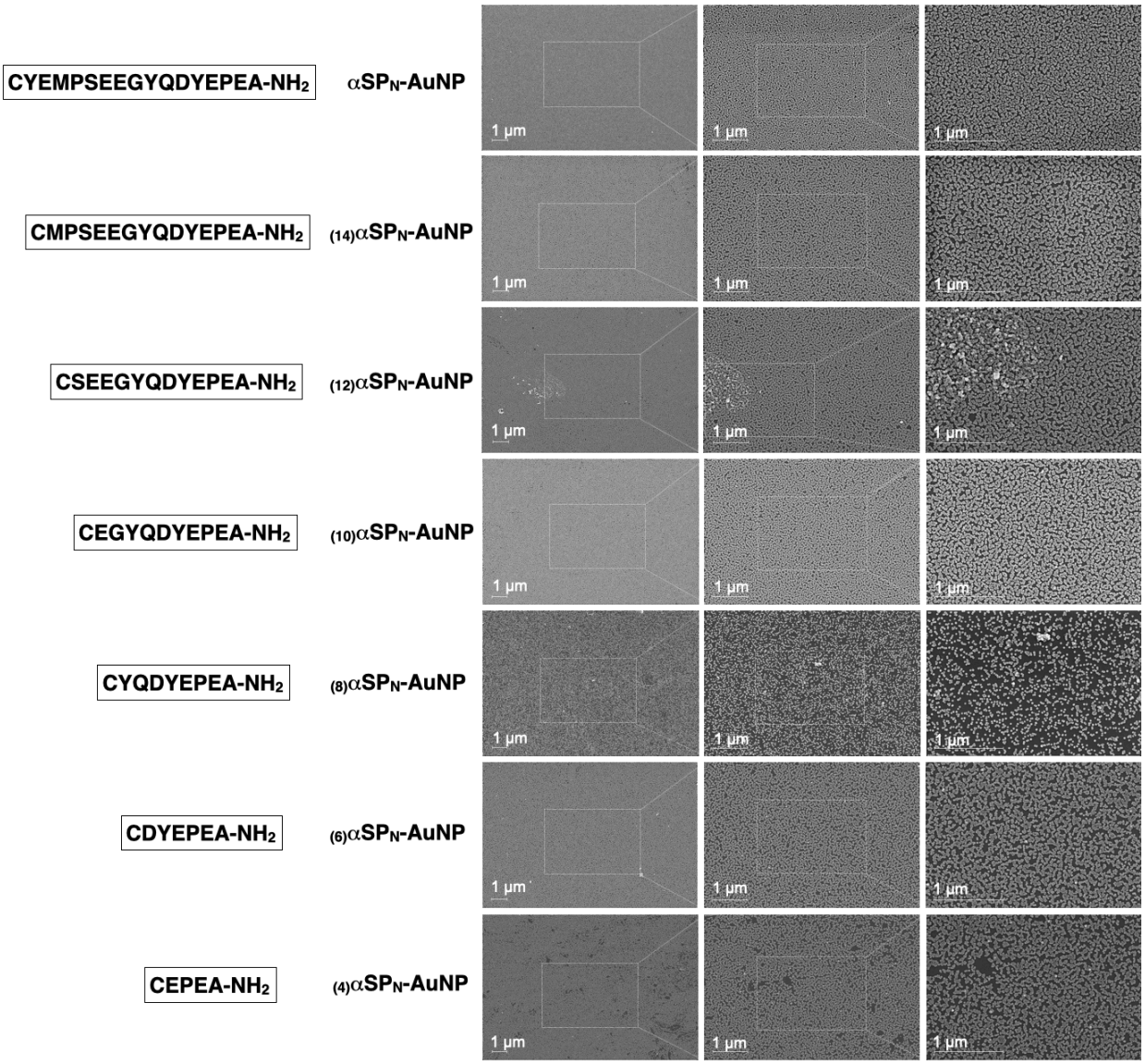

Figure S11. SEM images of serially truncated αSP_N-AuNPs adhered onto silicon surface following incubation at 40°C for 3 hours in 50 mM citrate buffer at pH 4.5.

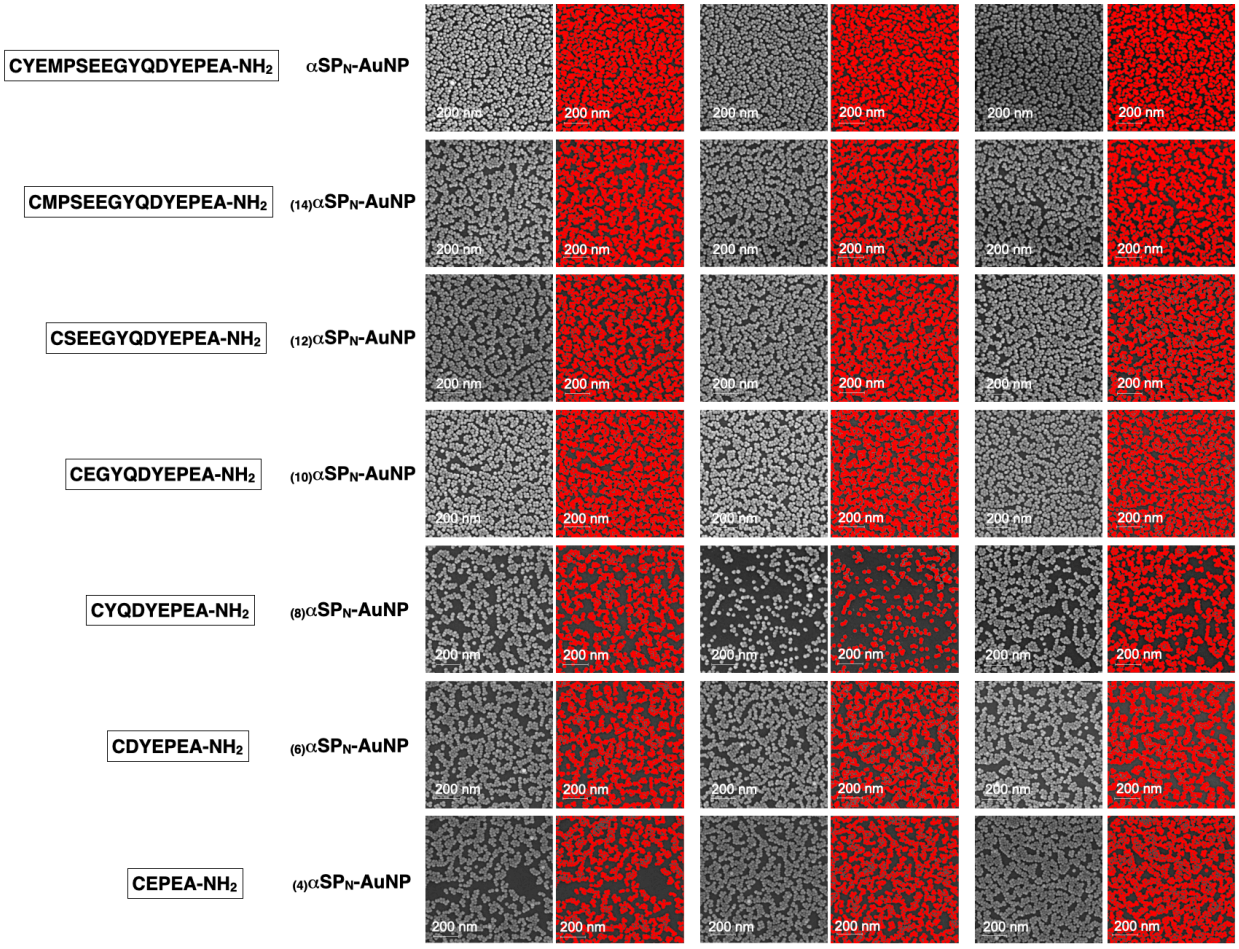

Figure S12. 3 sets of SEM images (1 **µ**m x 1 **µ**m) of serially truncated αSP_N-AuNPs adsorbed onto silicon substrate after incubating (40°C) for 3 hours in 50 mM citrate buffer at pH 4.5, and corresponding images of αSP_N-AuNP coverage (red color) analyzed by Fiji software.

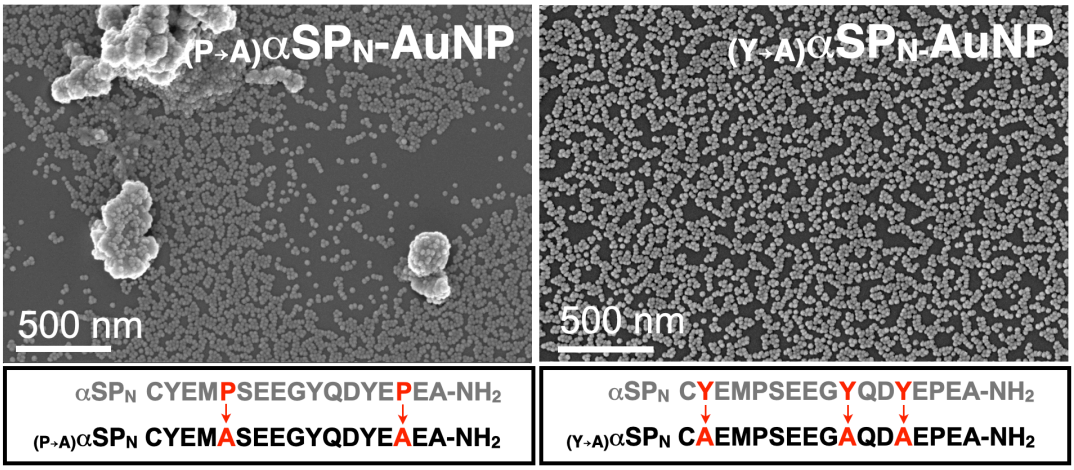

Figure S13. SEM images of $_{(P\rightarrow A)}$αSP$_N$-AuNP (left) and $_{(Y\rightarrow A)}$αSP$_N$-AuNP (right) deposited onto silicon surface after incubation at 40°C for 3 hours in 50 mM citrate (pH 4.5). Amino acids sequence information of each peptide is presented below the corresponding image.

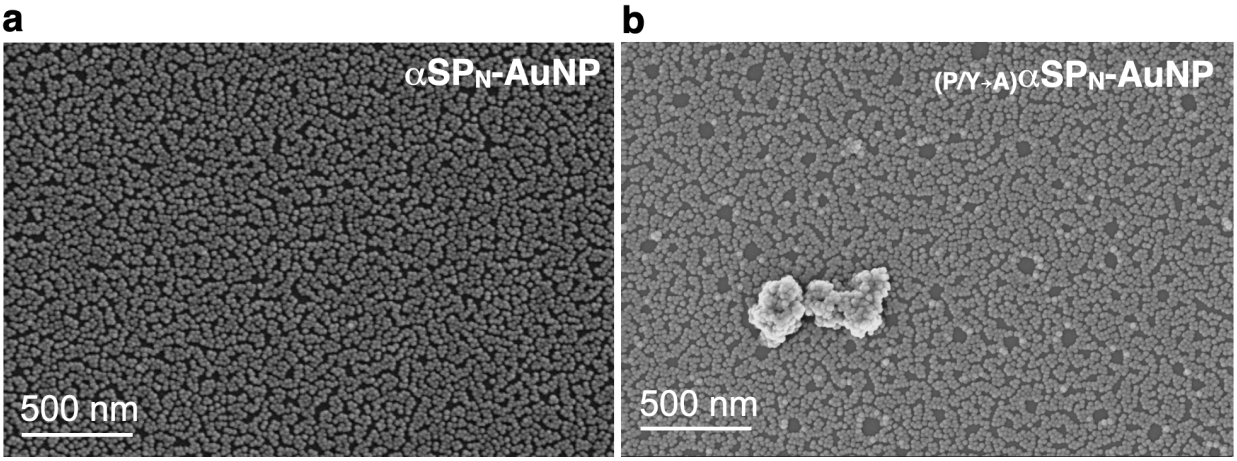

Figure S14. SEM images of (a) αSP$_N$-AuNPs and (b) $_{(P/Y\rightarrow A)}$αSP$_N$-AuNPs deposited onto silicon substrate following incubation for 3 hours at 40°C in 50 mM citrate buffer at pH 4.5.

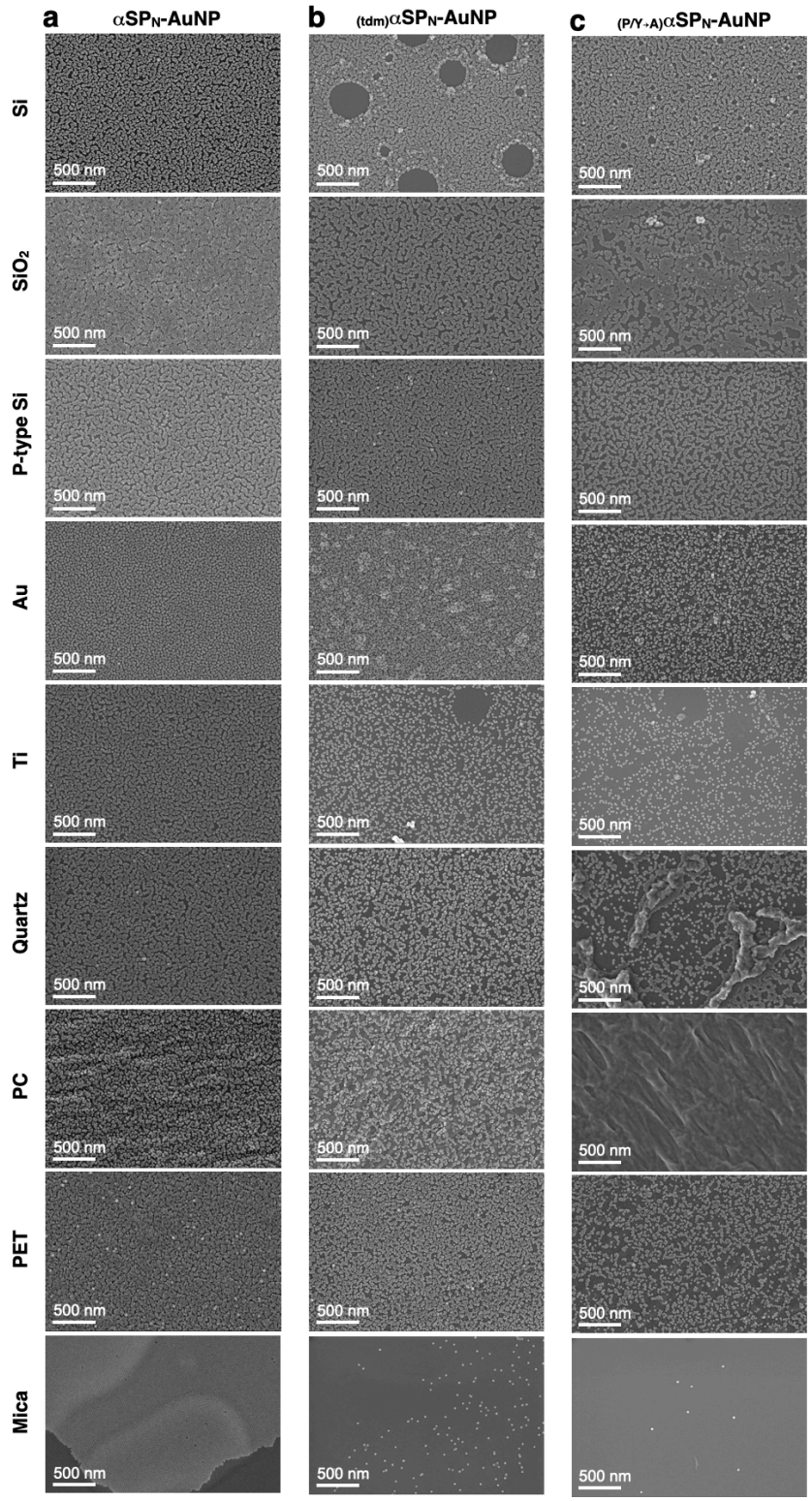

Figure S15. SEM images of (a) αSP_N-AuNPs, (b) $_{(tdm)}\alpha SP_N$-AuNPs, (c) $_{(P/Y\rightarrow A)}\alpha SP_N$-AuNPs adhered onto multiple substrates of Si, SiO_2, P-type Si, Au, Ti, quartz, PC, PET, or mica after incubation at 40°C (mica at 35°C) for 3 hours in 50 mM citrate buffer at pH 4.5.

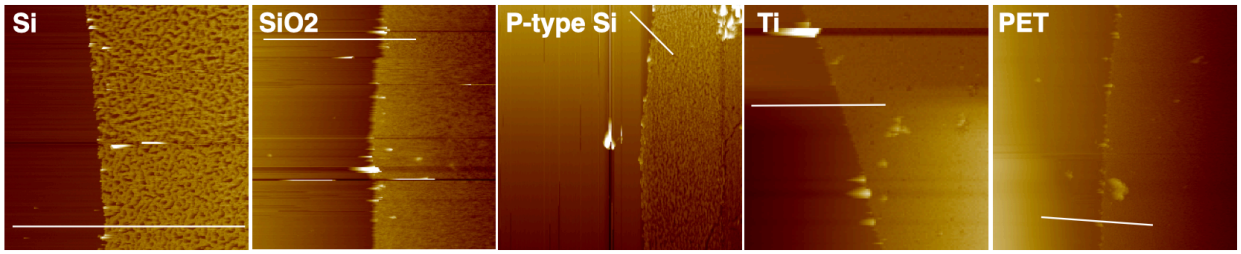

Figure S16. AFM 2-D images (10 **µ**m x 10 **µ**m) of αSP_N-AuNPs monolayer on different substrates of Si, SiO_2, boron-doped p-type Si, Ti, and PET. The white lines on the images indicate the location of height profile in Figure 4c.

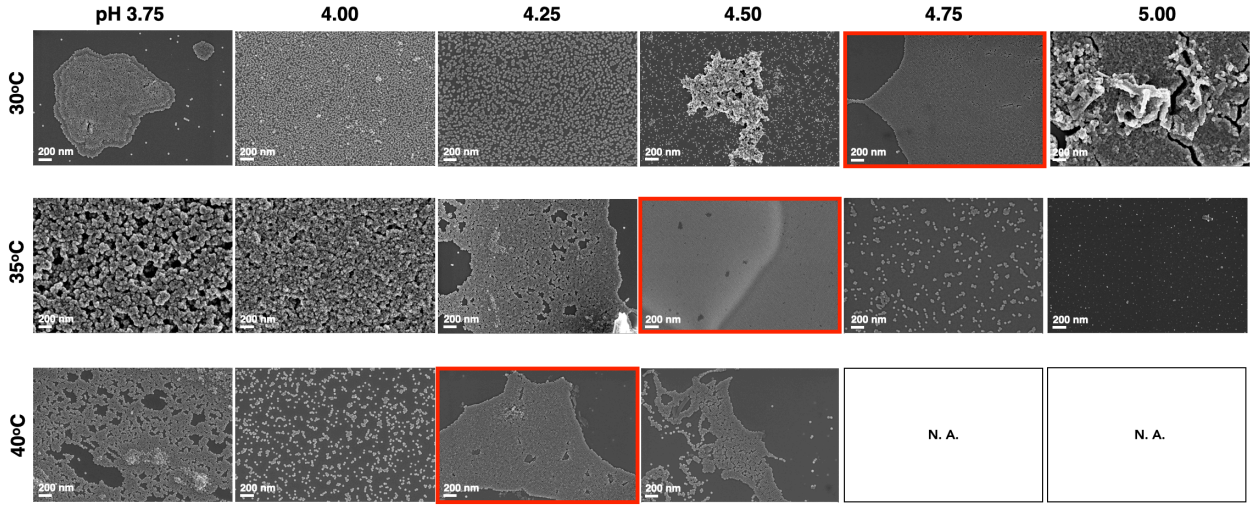

Figure S17. pH-Dependent superlattice formation of αSP_N-AuNPs single layer on mica substrate. SEM images of αSP_N-AuNPs deposited onto the mica surface after incubation for 3 hours at various pHs varying between 3.75 and 5.00 in 0.25 intervals at different temperatures of 30°C (top row), 35°C (middle row), and 40°C (bottom row). The images in a red-lined box show the best superlattice at each temperature.

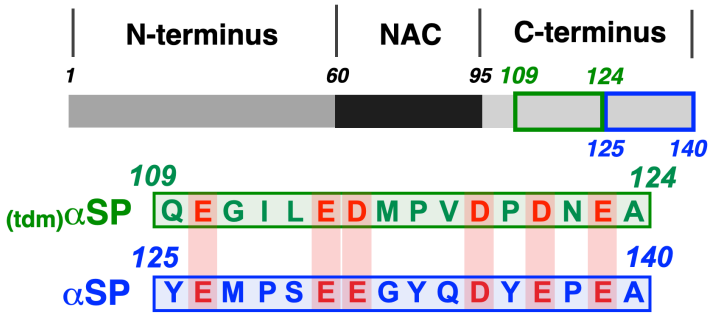

Figure S18. Sequence of $_{(tdm)}$αSP (green) and αSP (blue) located in the C-terminal region of αS protein. Anionic amino acids are indicated in red.

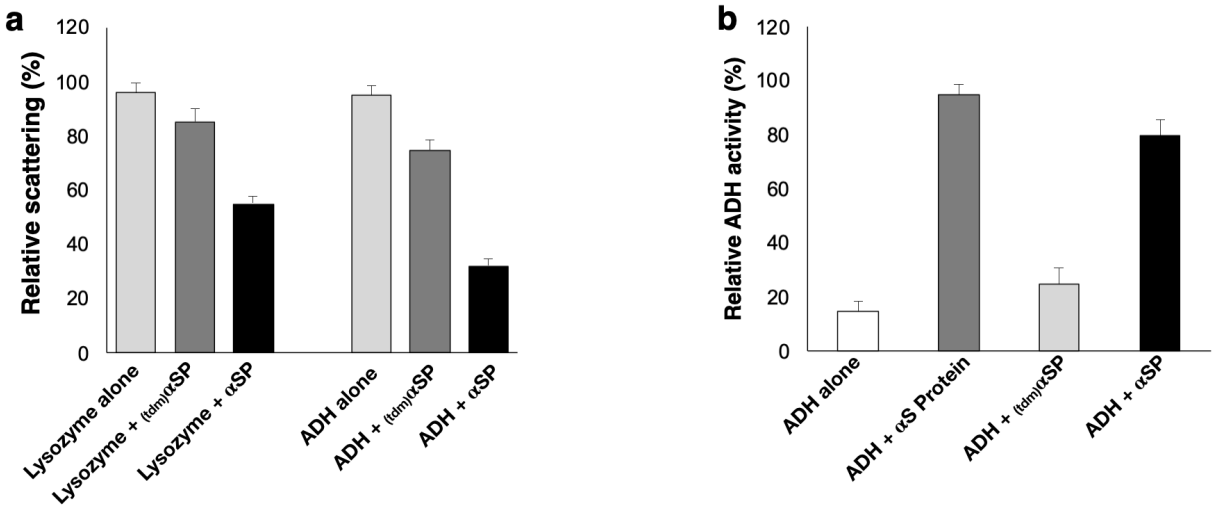

Figure S19. Chaperone activity of αSP. (a) Relative scattering of thermal aggregation of lysozyme and ADH in the presence or absence of either $_{(tdm)}$αSP or αSP accessed with a turbidity assay. (b) Relative ADH activity monitored at 340 nm after co-incubating (60°C) with either αS protein, $_{(tdm)}$αSP, or αSP.

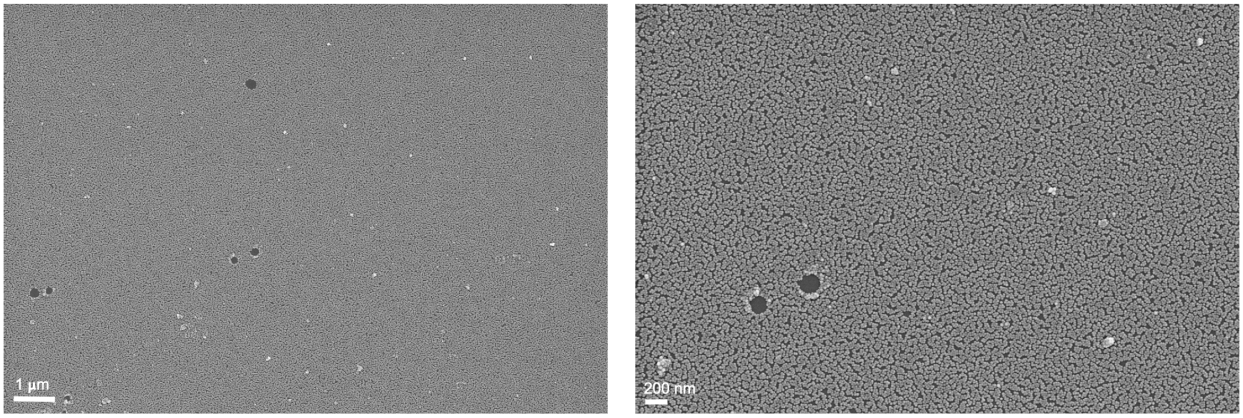

Figure S20. SEM images of $_{(tdm)}$αSP$_N$-AuNPs deposited onto silicon surface after incubating for 3 hours at 40ºC in 50 mM citrate buffer at pH 4.5.

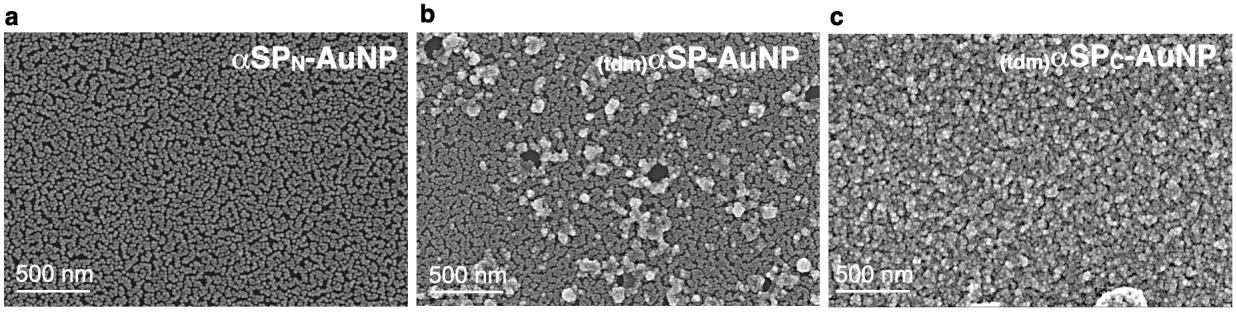

Figure S21. SEM images of (a) αSP$_N$-AuNP, (b) $_{(tdm)}$αSP-AuNP, and (c) $_{(tdm)}$αSP$_C$-AuNP adsorbed onto silicon substrate after incubation for 3 hours (40ºC) in 50 mM citrate at pH 4.5.

2.2. Circular dichroism (CD) of prepared peptides

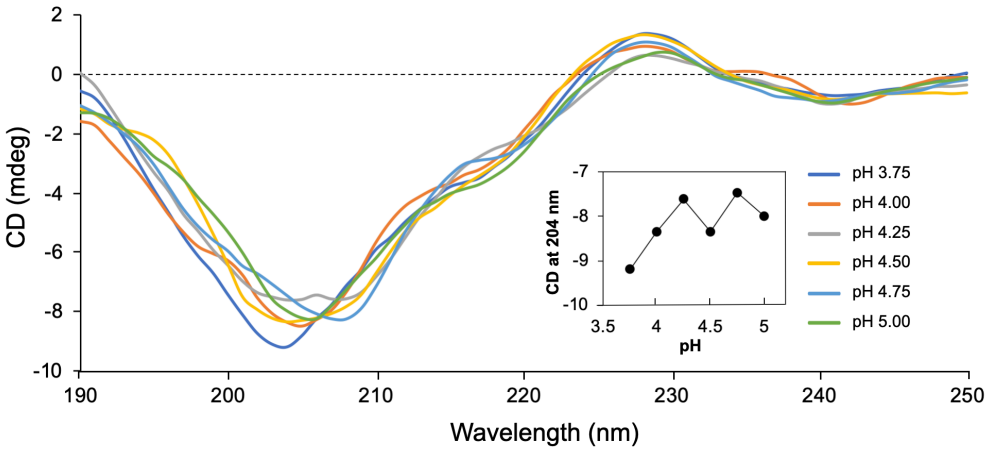

Figure S22. CD spectra of free αSP_N (50 μM) in 5 mM citrate at different pH of 3.75 (blue), 4.00 (orange), 4.25 (gray), 4.50 (yellow), 4.75 (light blue), and 4.50 (green). CD values at 204 nm versus pHs are presented in inset.

2.3. Characterization of αSP by NMR

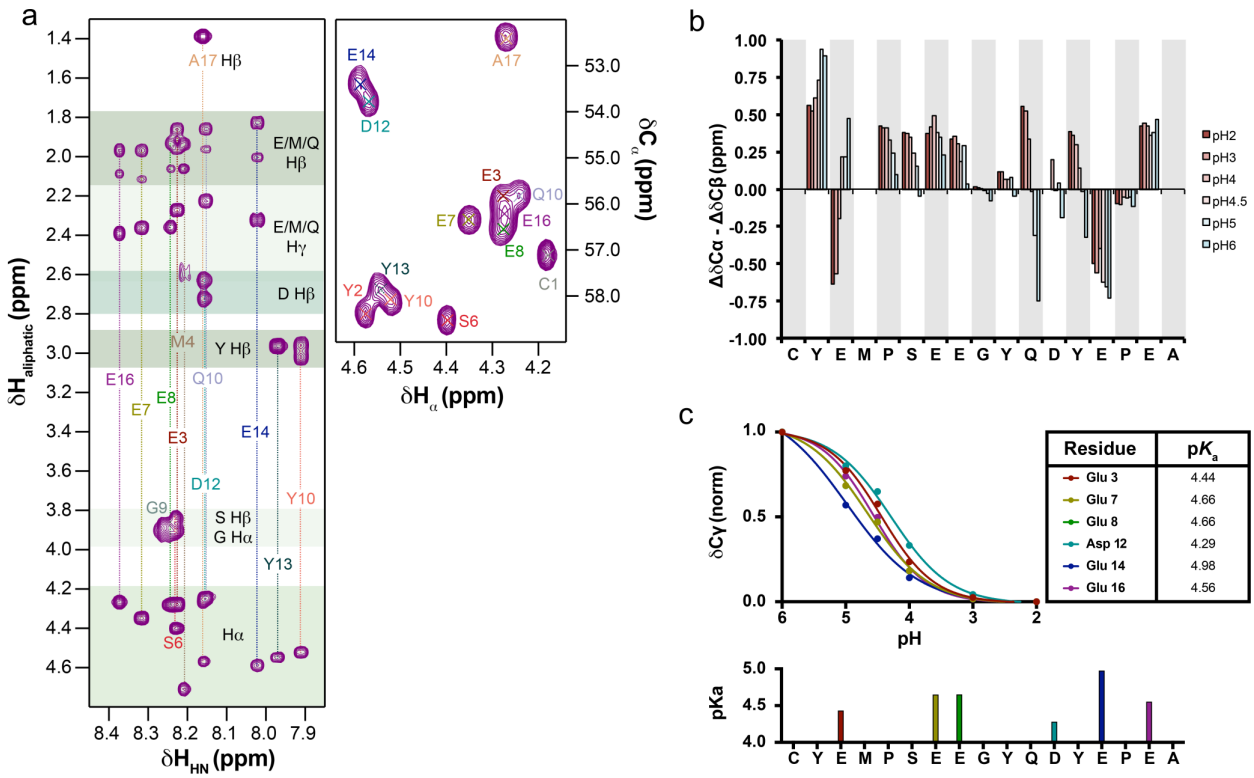

Figure S23. (a) NMR spectra acquired at pH 4.5 and 310 K, with αSP_N resonance assignments. On the left panel, a region of the ^{1}H-^{1}H TOCSY spectrum is shown corresponding to the correlations between H^N and side-chain aliphatic protons. Vertical lines and different colors indicate aliphatic resonances corresponding to the same residue, correlated to their own H^N. Assignments (in black) were obtained with the help of a ^{1}H-^{1}H NOESY spectrum acquired in analogous conditions. Horizontal bands indicate regions of the spectrum gathering particular resonance types. On the right panel, a region of the ^{1}H-^{13}C HSQC spectrum is shown displaying the Hα-Cα region, including assignments. (b) ^{13}C secondary chemical shifts were determined through the assignment of Cα and Cβ resonances in a series of ^{1}H-^{13}C HSQC spectra at the indicated pH values, with assignments assisted by the ^{1}H-^{1}H TOCSY and NOESY pairs at each pH value. Small $\Delta\delta C^{\alpha} - \Delta\delta C^{\beta}$ in the ±1 ppm range are compatible with a polyproline II secondary structure. A random coil-α-helix-β-sheet equilibrium, which would yield similar values, can be ruled out considering CD data. Methionine 3 Cα chemical shifts could not be measured as the signal is masked behind the water signal along the titration. (c) The pK_a values of the carboxylic groups at the side-chains of Glu and Asp residues in the peptide were determined by monitoring the Cγ (Glu) or Cβ (Asp) chemical shifts in the same series of pH-varying ^{1}H-^{13}C HSQC spectra.[3]

2.4. Characterization of peptides synthesized

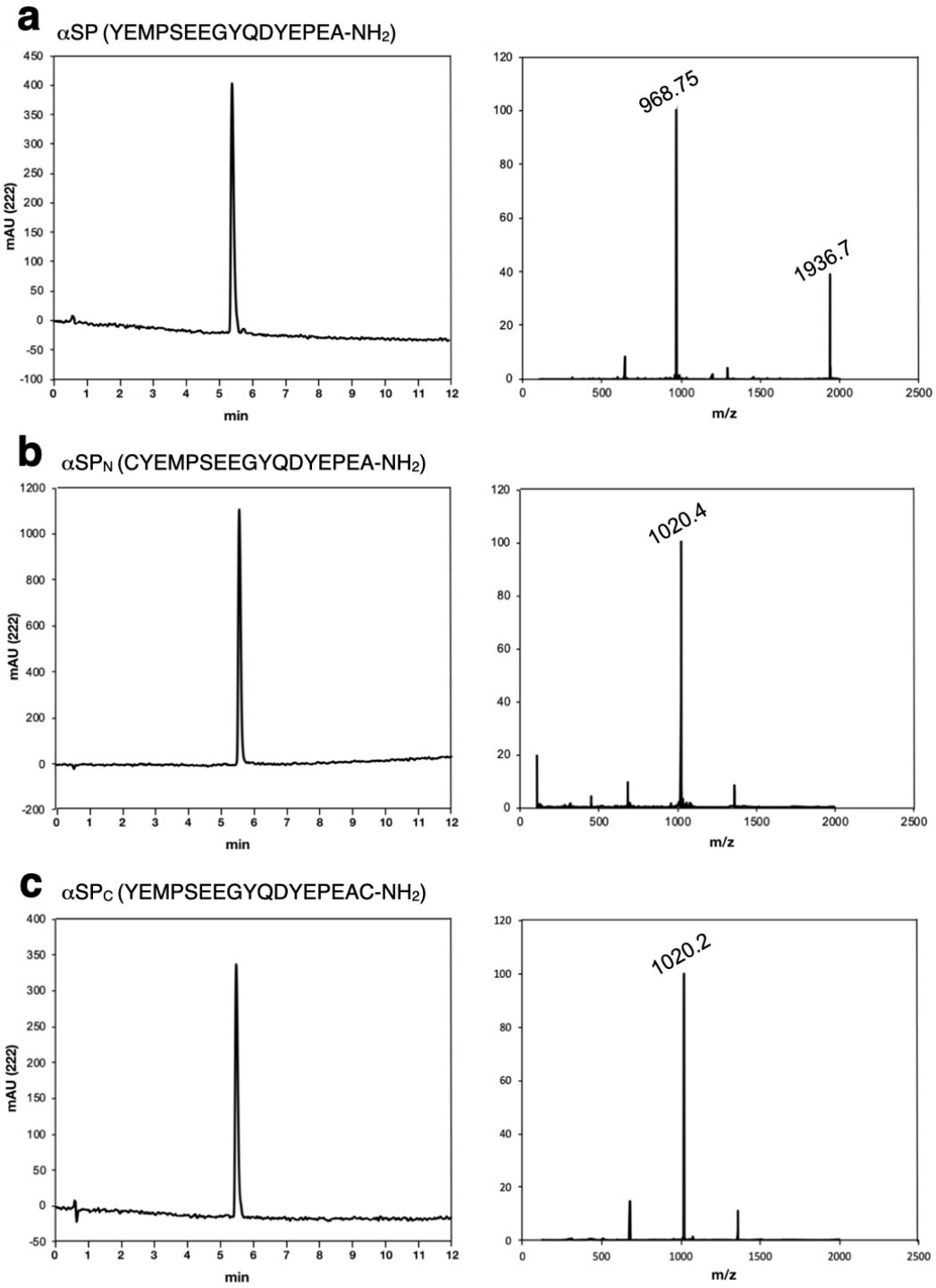

Figure S25. HPLC chromatograms (left) of (a) αSP, (b) $αSP_N$, and (c) $αSP_C$ and their MS spectra (right) obtained from the main peak.

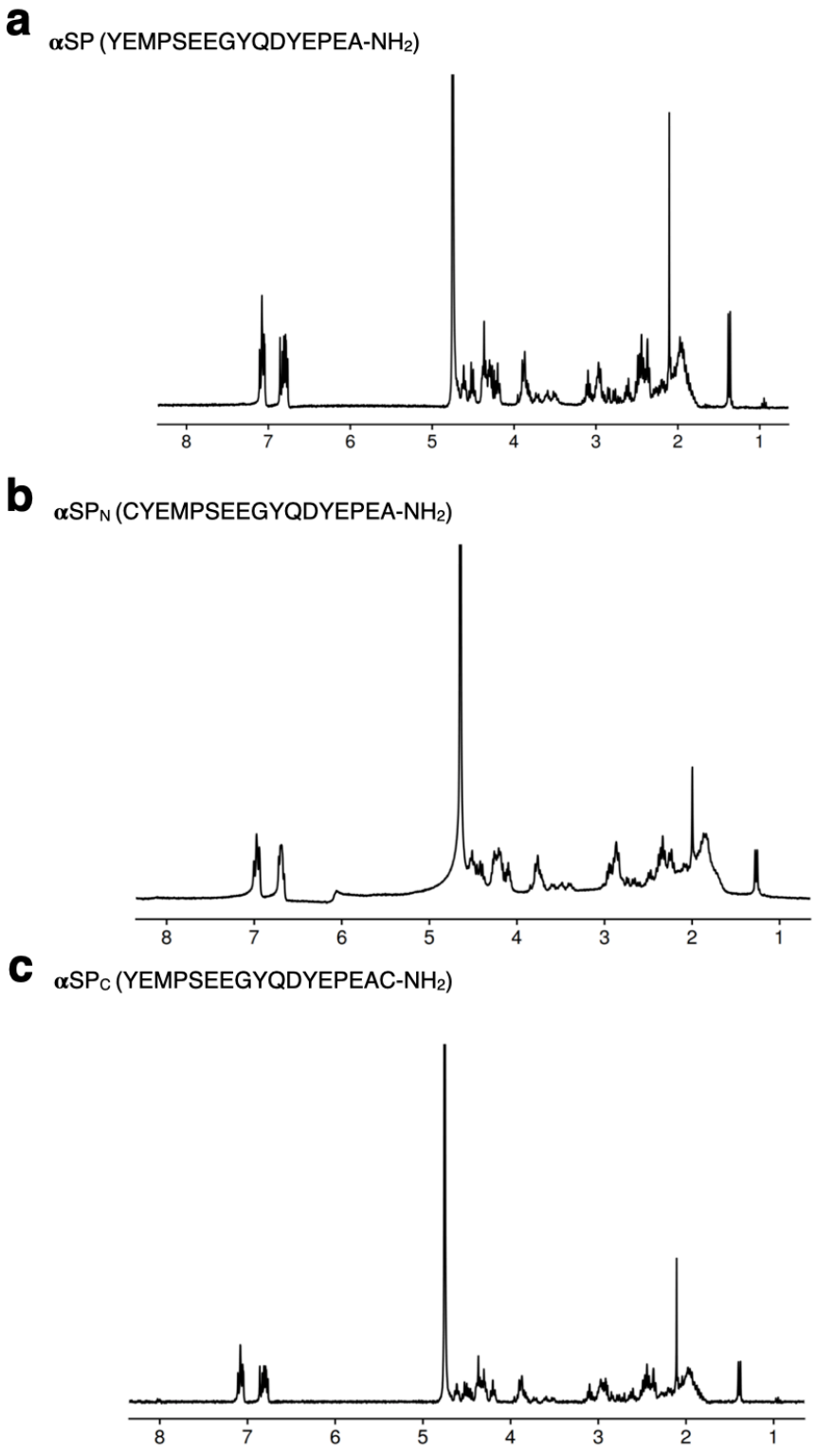

Figure S26. ^{1}H NMR (300 MHz, D_2O) of (a) αSP, (b) $αSP_N$, and (c) $αSP_C$.

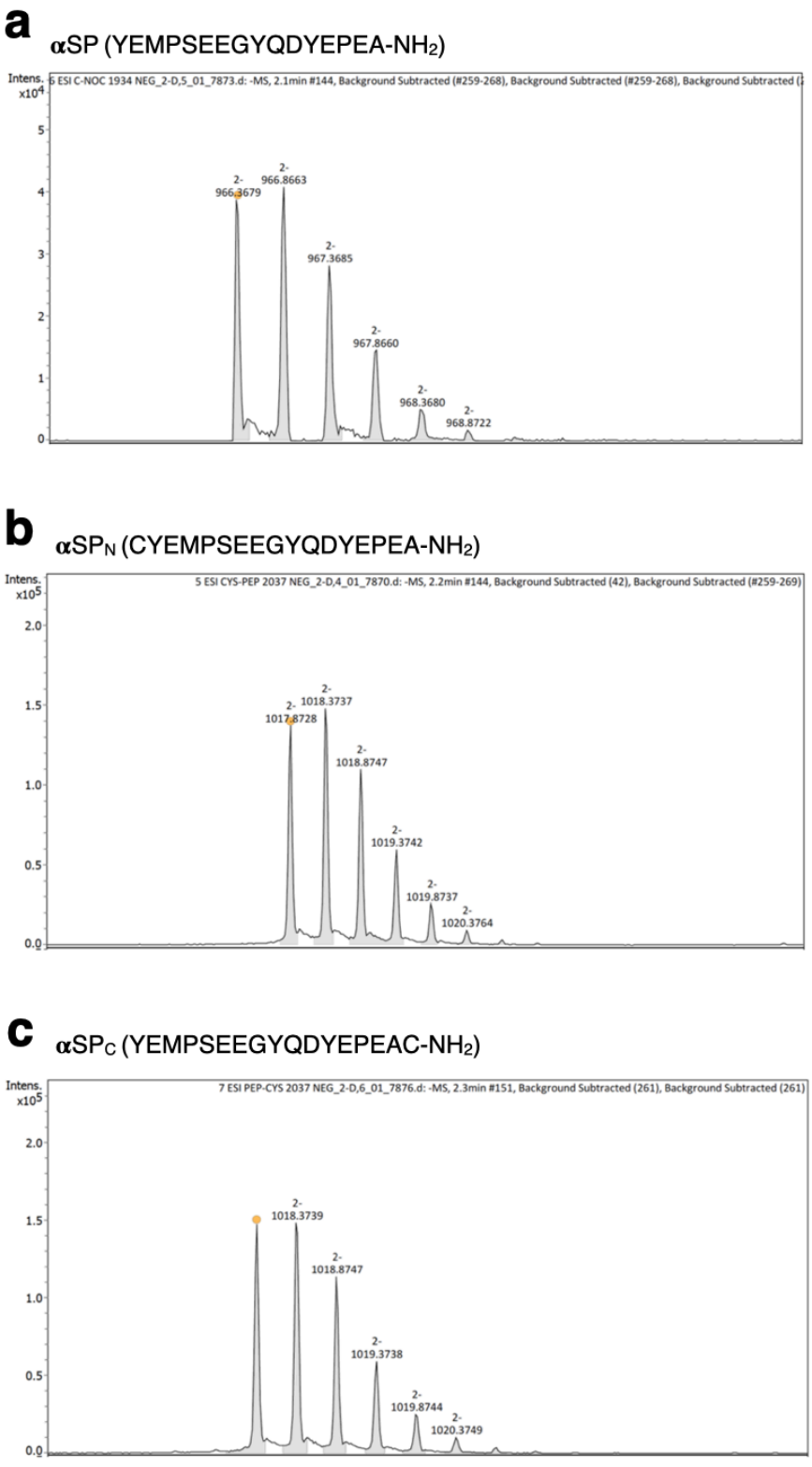

Figure S27. HR-MS of (a) αSP, (b) $αSP_N$, and (c) $αSP_C$.

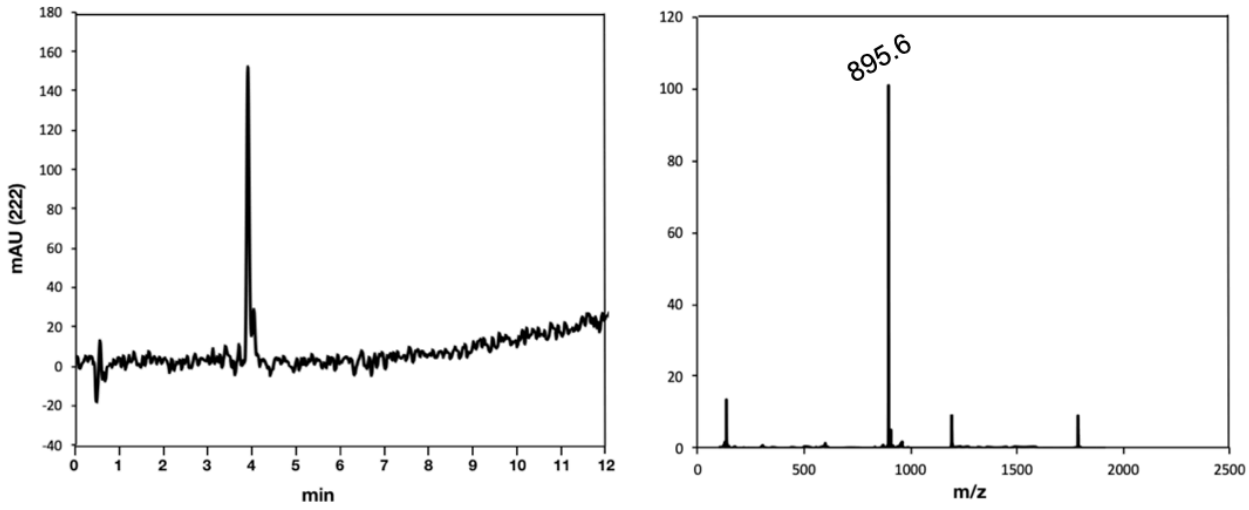

Figure S28. HPLC chromatogram (left) of $6E_N$ (CEEEEEE-NH_2) and its MS spectra (right) obtained from the main peak.

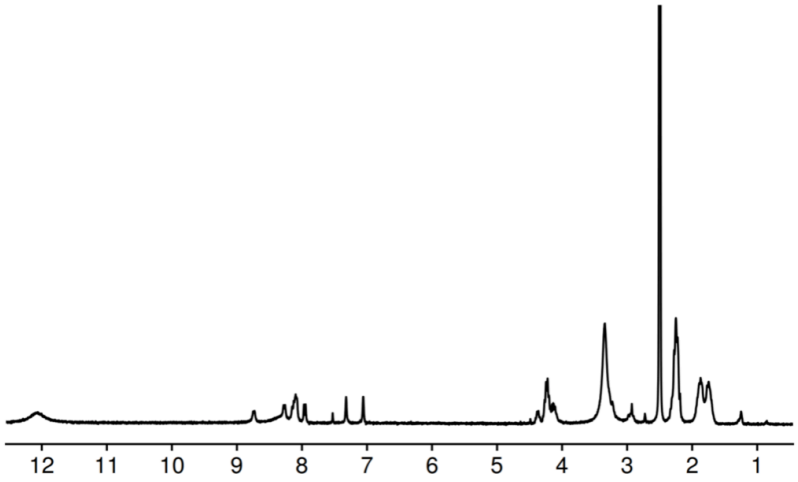

Figure S29. ^{1}H NMR (300 MHz, DMSO-d_6) of $6E_N$ (CEEEEEE-NH_2)

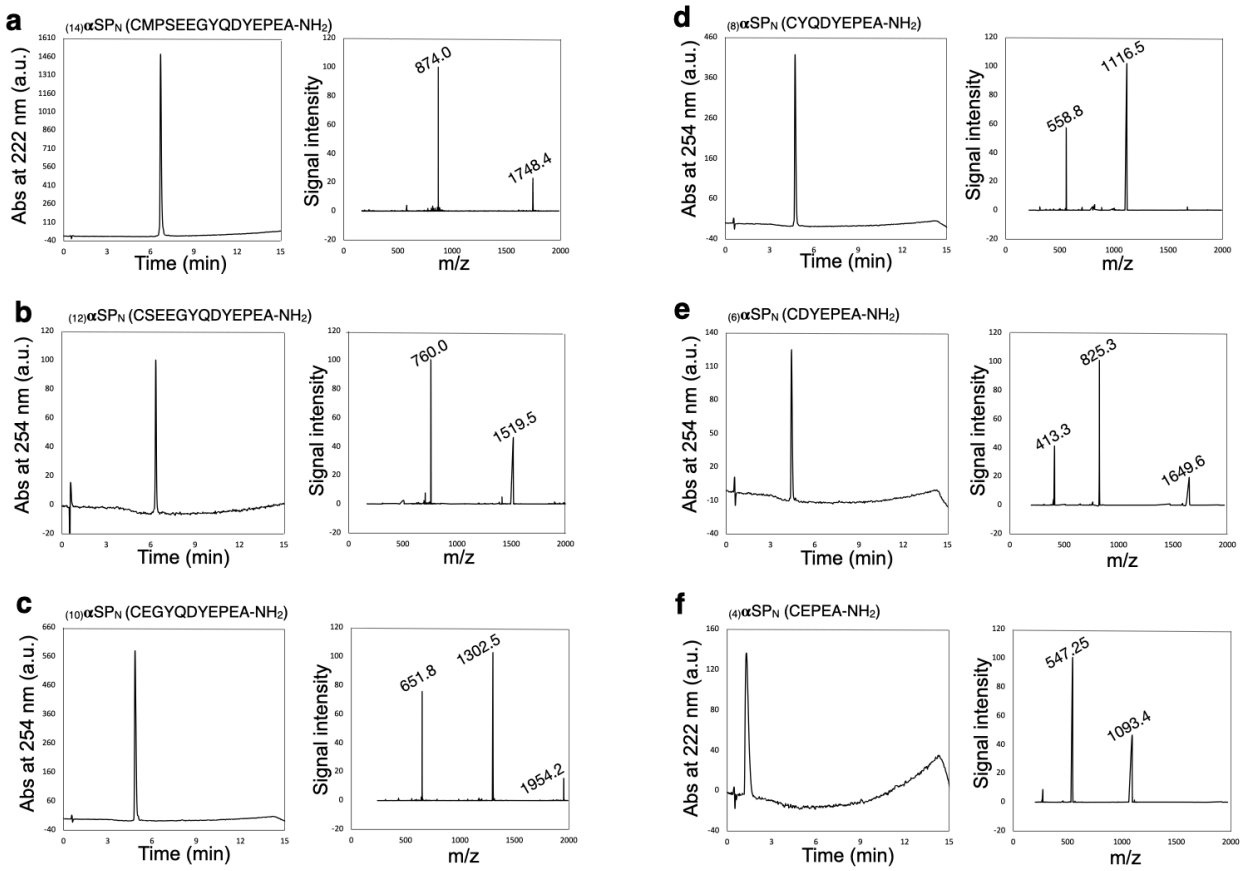

Figure S30. HPLC chromatograms (left) and MS spectra (right) obtained from the main peak of (a) $_{(14)}\alpha SP_N$, (b) $_{(12)}\alpha SP_N$, (c) $_{(10)}\alpha SP_N$, (d) $_{(8)}\alpha SP_N$, (e) $_{(6)}\alpha SP_N$, and (f) $_{(4)}\alpha SP_N$.

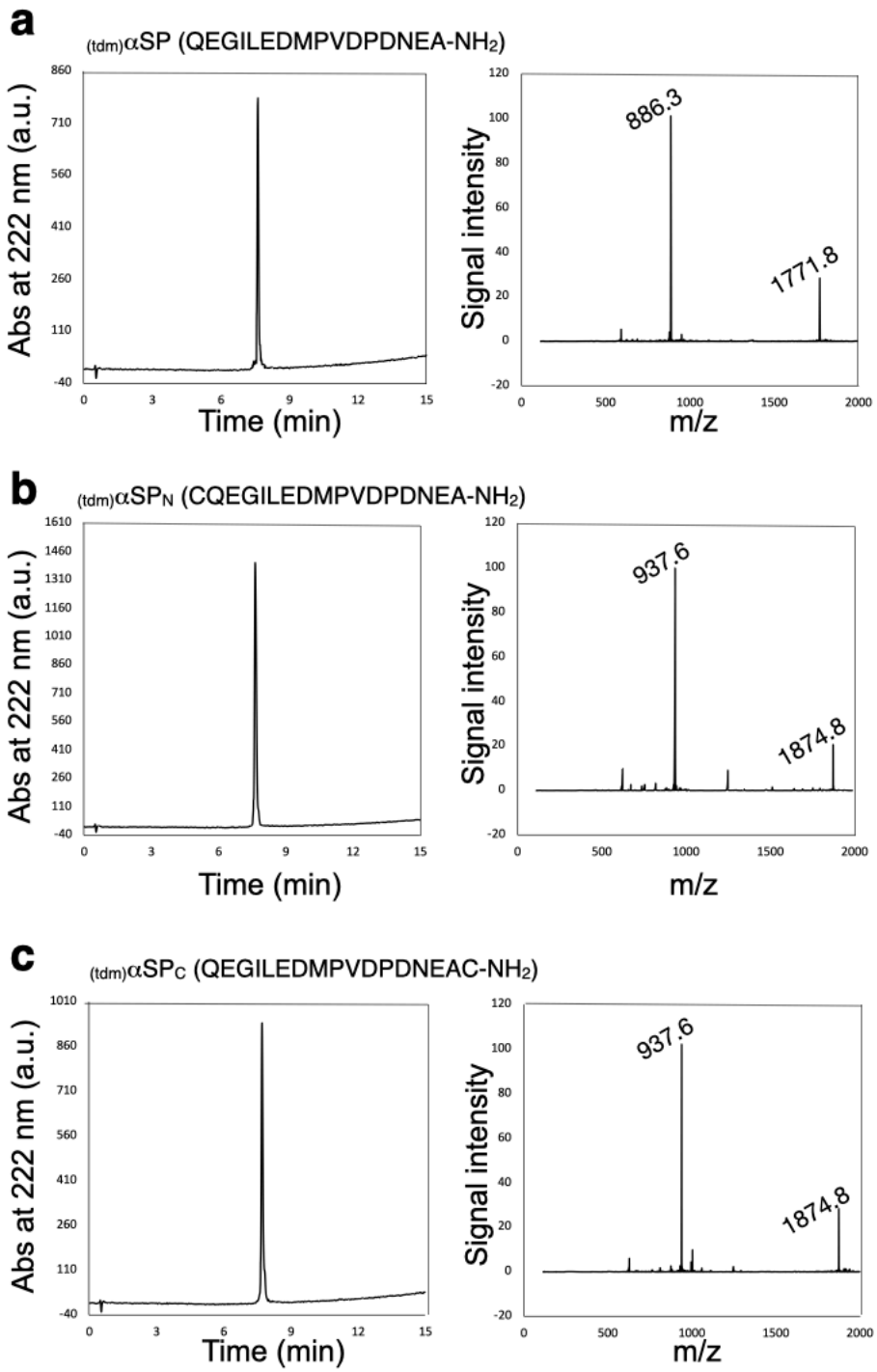

Figure S31. HPLC chromatograms (left) of (a) $_{(tdm)}\alpha SP$, (b) $_{(tdm)}\alpha SP_N$, and (c) $_{(tdm)}\alpha SP_C$ and their MS spectra (right) obtained from the main peak.

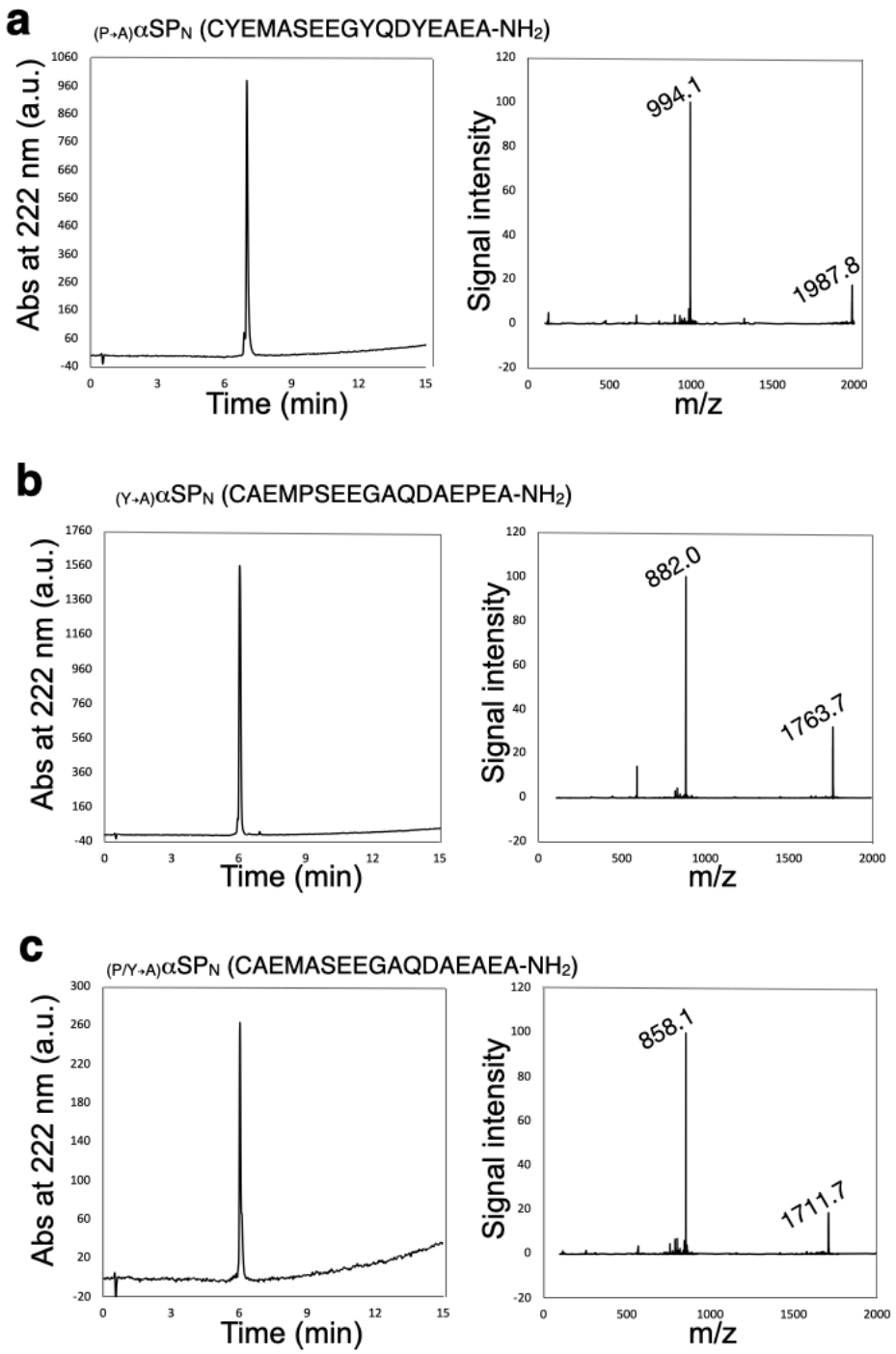

Figure S32. HPLC chromatograms (left) of (a) $_{(P\rightarrow A)}\alpha SP_N$, (b)$_{(Y\rightarrow A)}\alpha SP_N$, and (c) $_{(P/Y\rightarrow A)}\alpha SP_N$ and their MS spectra (right) obtained from the main peak.

3. References

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