Electronic Supplementary Information:

Diffusion Behavior of Peptide Amphiphiles Containing Different Number of Alkyl Tails at a Hydrophobic Solid-Liquid Interface: Single Molecule Tracking Investigation

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Table of Contents

Additional experimental details:

Synthesis of the peptide

Standard Fmoc solid phase peptide synthesis of PA-A0. Rink amide AM resin (0.729 mmol/g loading) was swollen in DMF (10 mL) for 2 h. The first residue Fmoc-Ser(tBu)-OH (3 eq.) was attached to the resin at room temperature under argon atmosphere with PyBOP (3 eq.) and DIPEA (6 eq.) in DMF (10 mL) for 8 h and consequent washing with DMF (3 × 10 mL). Coupling and washing steps were repeated. The resin was filtered and washed with DMF (3 × 10 mL). The Fmoc protecting group was then removed by stirring the resin in 20% piperidine in DMF solution for 20 min (2 × 10 mL). After Fmoc deprotection the remaining solid was washed intensively with DMF (3 × 10 mL). A positive Kaiser test confirmed the cleavage of the Fmoc group and the formation of the free amino function. A solution of PyBOP (3 eq.) and Fmoc-Asp(OtBu)-OH (3 eq.) in DMF was added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 4 h and consequent washed with DMF. The reaction was repeated with fresh reagents. The resin was filtered and washed with DMF (3 × 10 mL). A negative Kaiser test confirmed the attachment of the amino acid. The Fmoc protecting group was then removed as described above, and the same procedure was done for the next four amino acids (e. g., Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH and Fmoc-Lys(Alloc)-OH, respectively). It should be noted that Fmoc on N-terminal end was not removed. Alloc removal was achieved by $Pd(PPh_3)_4$ (0.1 eq.), $PhSiH_3$ (24 eq.) in 10 mL DCM for 30 min at room temperature under argon atmosphere and consequent

washing with DCM (3 × 5 mL). The deprotection and washing process was repeated. Subsequently, a solution of PyBOP (3 eq.) and Rhodamine B (3 eq.) in DMF was added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 8 h and consequently washed with DMF. The reaction was repeated with fresh reagents. The Fmoc protecting group was then removed by stirring the resin in 20% piperidine in DMF solution for 20 min (2 × 10 mL). The resin was washed with DCM (3 × 5 mL), methanol (3 × 5 mL), and DCM (3 × 5 mL) and dried under reduced pressure for 1 h.

To cleave peptide, the resin was shaken under argon atmosphere in a mixture containing 95% TFA, 2.5 % water, and 2.5 % TIS for 3 h and washed twice with DCM. The filtrates were combined and concentrated in high vacuum at room temperature. Diethyl ether (20 mL) was added and the resulting suspension was centrifuged. The supernatant solvent was decanted and the solid was washed with diethyl ether and centrifuged again. After decanting, the product was dissolved in H_2O (20 mL), and lyophilized to give a purple solid. The crude product was purified by MPLC on C18 reversed-phase silica gel (10 % to 100 % methanol/water in 40 min, 0.05 % TFA) to obtain **PA-A0** as a purple solid.

Standard Fmoc solid phase peptide synthesis of PA-A1. The synthesis procedure of **PA-A1** is similar with that of **PA-A0**. The coupling sequence is as follows: Fmoc-Ser(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-Lys(Alloc)-OH and lauric acid. Subsequently, Alloc removal was achieved by $Pd(PPh_3)_4$ (0.1 eq.), $PhSiH_3$ (24 eq.) in 10 mL DCM for 30 min at room

temperature under argon atmosphere and consequent washing with DCM (3 × 5 mL). The deprotection and washing process was repeated. A solution of PyBOP (3 eq.) and Rhodamine B (3 eq.) in DMF was then added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 8 h and consequently washed with DMF. The reaction was repeated with fresh reagents. The resin was washed with DCM (3 × 5 mL), methanol (3 × 5 mL), and DCM (3 × 5 mL) and dried under reduced pressure for 1 h.

To cleave peptide, the resin was shaken under argon atmosphere in a mixture containing 95% TFA, 2.5 % water, and 2.5 % TIS for 3 h and washed twice with DCM. The filtrates were combined and concentrated in high vacuum at room temperature. Diethyl ether (20 mL) was added and the resulting suspension was centrifuged. The supernatant solvent was decanted and the solid was washed with diethyl ether and centrifuged again. After decanting, the product was dissolved in H_2O (20 mL), and lyophilized to give a purple solid. The crude product was purified by MPLC on C18 reversed-phase silica gel (10 % to 100 % methanol/water in 40 min, 0.05 % TFA) to obtain **PA-A1** as a purple solid.

Standard Fmoc solid phase peptide synthesis of PA-A2. The synthesis procedure of **PA-A2** is similar with that of **PA-A0**. The coupling sequence is as follows: Fmoc-Ser(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Lys(Fmoc)-OH and lauric acid. Subsequently, Alloc removal was achieved by $Pd(PPh_3)_4$ (0.1 eq.), $PhSiH_3$ (24 eq.) in 10 mL DCM for 30 min at room temperature under argon atmosphere and consequent washing with

DCM (3 × 5 mL). The deprotection and washing process was repeated. A solution of PyBOP (3 eq.) and Rhodamine B (3 eq.) in DMF was then added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 8 h and consequently washed with DMF. The reaction was repeated with fresh reagents. The resin was washed with DCM (3 × 5 mL), methanol (3 × 5 mL), and DCM (3 × 5 mL) and dried under reduced pressure for 1 h.

To cleave peptide, the resin was shaken under argon atmosphere in a mixture containing 95% TFA, 2.5 % water, and 2.5 % TIS for 3 h and washed twice with DCM. The filtrates were combined and concentrated in high vacuum at room temperature. Diethyl ether (20 mL) was added and the resulting suspension was centrifuged. The supernatant solvent was decanted and the solid was washed with diethyl ether and centrifuged again. After decanting, the product was dissolved in H_2O (20 mL), and lyophilized to give a purple solid. The crude product was purified by MPLC on C18 reversed-phase silica gel (10 % to 100 % methanol/water in 40 min, 0.05 % TFA) to obtain **PA-A2** as a purple solid.

Standard Fmoc solid phase peptide synthesis of PA-A3. The synthesis procedure of **PA-A3** is similar with that of **PA-A0**. The coupling sequence is as follows: Fmoc-Ser(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH and Fmoc-Lys(Alloc)-OH. It should be noted that Fmoc on N-terminal end was not removed. Subsequently, Alloc removal was achieved by $Pd(PPh_3)_4$ (0.1 eq.), $PhSiH_3$ (24 eq.) in 10 mL DCM for 30 min at room temperature under argon atmosphere and consequent washing with DCM (3 × 5 mL). The deprotection and

washing process was repeated. A solution of PyBOP (3 eq.) and Rhodamine B (3 eq.) in DMF was then added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 8 h and consequently washed with DMF. The reaction was repeated with fresh reagents. The Fmoc protecting group was then removed by stirring the resin in 20% piperidine in DMF solution for 20 min (2 × 10 mL). After Fmoc deprotection, the remaining solid was washed intensively with DMF (3 × 10 mL). A positive Kaiser test confirmed the cleavage of the Fmoc group and the formation of the free amino function. A solution of PyBOP (3 eq.) and Fmoc-Lys(Alloc)-OH (3 eq.) in DMF was added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 4 h and consequently washed with DMF. The reaction was repeated with fresh reagents. The resin was filtered and washed with DMF (3 × 10 mL). A negative Kaiser test confirmed the attachment of the amino acid. The Fmoc protecting group was then removed by stirring the resin in 20% piperidine in DMF solution for 20 min (2 × 10 mL). After Fmoc deprotection, the remaining solid was washed intensively with DMF (3 × 10 mL). A positive Kaiser test confirmed the cleavage of the Fmoc group and the formation of the free amino function. A solution of PyBOP (3 eq.) and Fmoc-Lys(Fmoc)-OH (3 eq.) in DMF was added to the loaded resin, followed by the addition of DIPEA (6 eq.). The mixture was left to stir for 4 h and consequently washed with DMF. The reaction was repeated with fresh reagents. The resin was filtered and washed with DMF (3 × 10 mL). A negative Kaiser test confirmed the attachment of the amino acid. Alloc removal was achieved by $Pd(PPh_3)_4$ (0.1 eq.),

$PhSiH_3$ (24 eq.) in 10 mL DCM for 30 min at room temperature under argon atmosphere and consequent washing with DCM (3 × 5 mL). The Fmoc protecting group was then removed by stirring the resin in 20% piperidine in DMF solution (2 × 10 mL) for 20 min. After Fmoc deprotection, the remaining solid was washed intensively with DMF (3 × 10 mL). A positive Kaiser test confirmed the cleavage of the Fmoc group and the formation of the free amino function. A solution of PyBOP (9 eq.) and lauric acid (9 eq.) in DMF was added to the loaded resin, followed by the addition of DIPEA (18 eq.). The mixture was left to stir for 8 h and consequently washed with DMF. The reaction was repeated with fresh reagents. A negative Kaiser test confirmed the attachment of lauric acid. The resin was washed with DCM (3 × 5 mL), methanol (3 × 5 mL), and DCM (3 × 5 mL) and dried under reduced pressure for 1 h.

To cleave peptide, the resin was shaken under argon atmosphere in a mixture containing 95% TFA, 2.5 % water, and 2.5 % TIS for 3 h and washed twice with DCM. The filtrates were combined and concentrated in high vacuum at room temperature. Diethyl ether (20 mL) was added and the resulting suspension was centrifuged. The supernatant solvent was decanted and the solid was washed with diethyl ether and centrifuged again. After decanting, the product was dissolved in H_2O (20 mL), and lyophilized to give a purple solid. The crude product was purified by MPLC on C18 reversed-phase silica gel (10 % to 100 % methanol/water in 40 min, 0.05 % TFA) to obtain **PA-A3** as a purple solid.

Supplementary Figures:

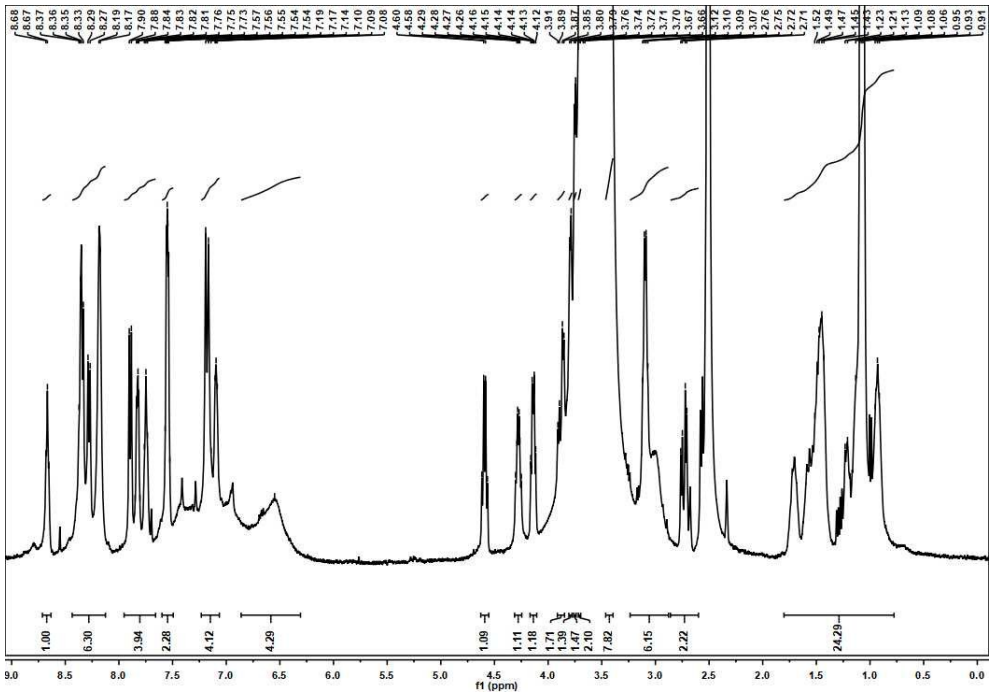

Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) spectrum of compound **PA-A0**.

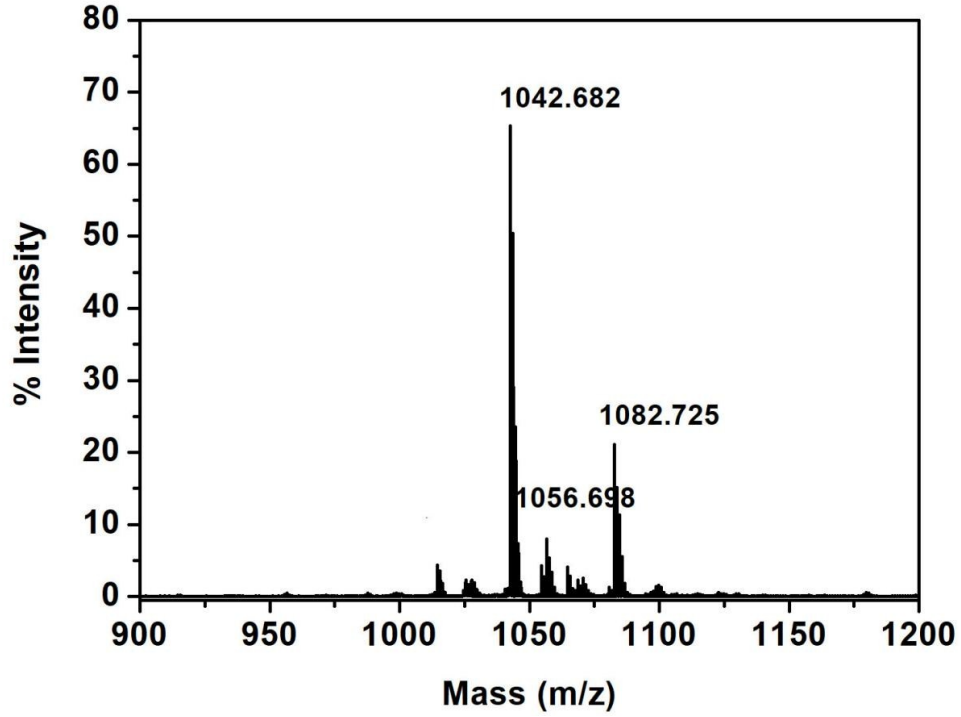

Figure S2. MALDI-TOF-MS spectrum of **PA-A0** m/z calculated for $C_{51}H_{72}N_{13}O_{11}^{+}$ 1042.55, found $[M]^{+}$ 1042.68.

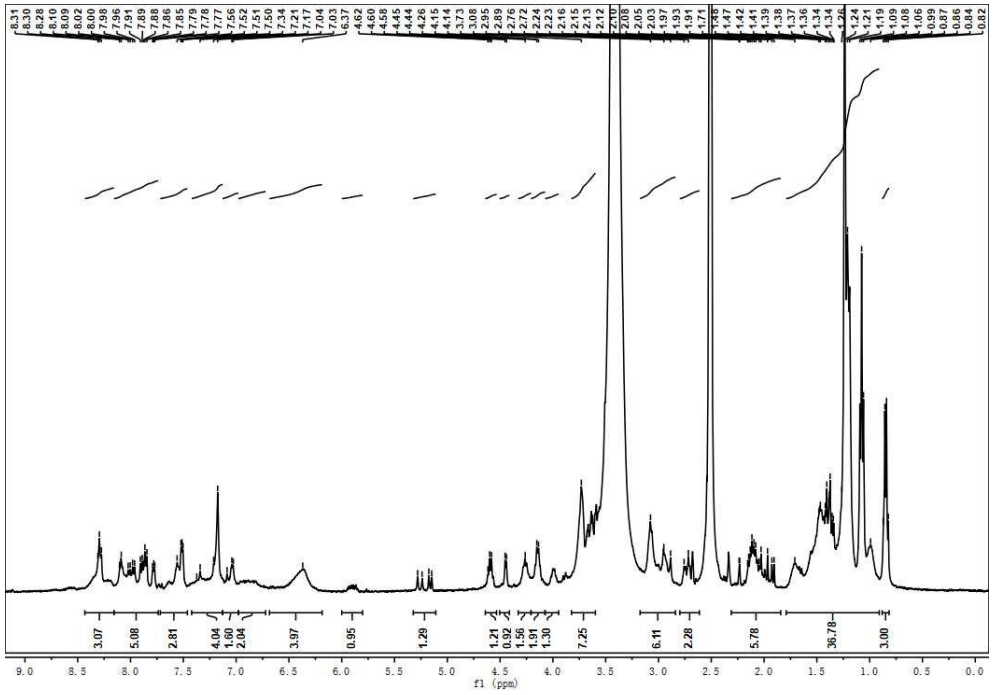

Figure S3. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) spectrum of compound **PA-A1**.

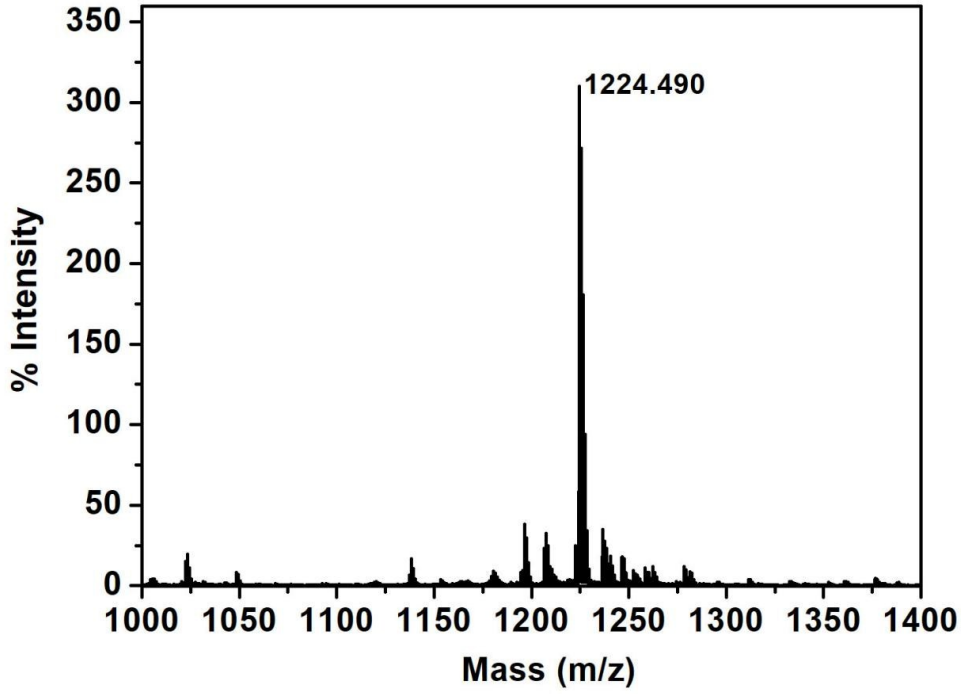

Figure S4. MALDI-TOF-MS spectrum of **PA-A1** m/z calculated for $C_{63}H_{94}N_{13}O_{12}^{+}$ 1224.71, found $[M]^{+}$ 1224.49.

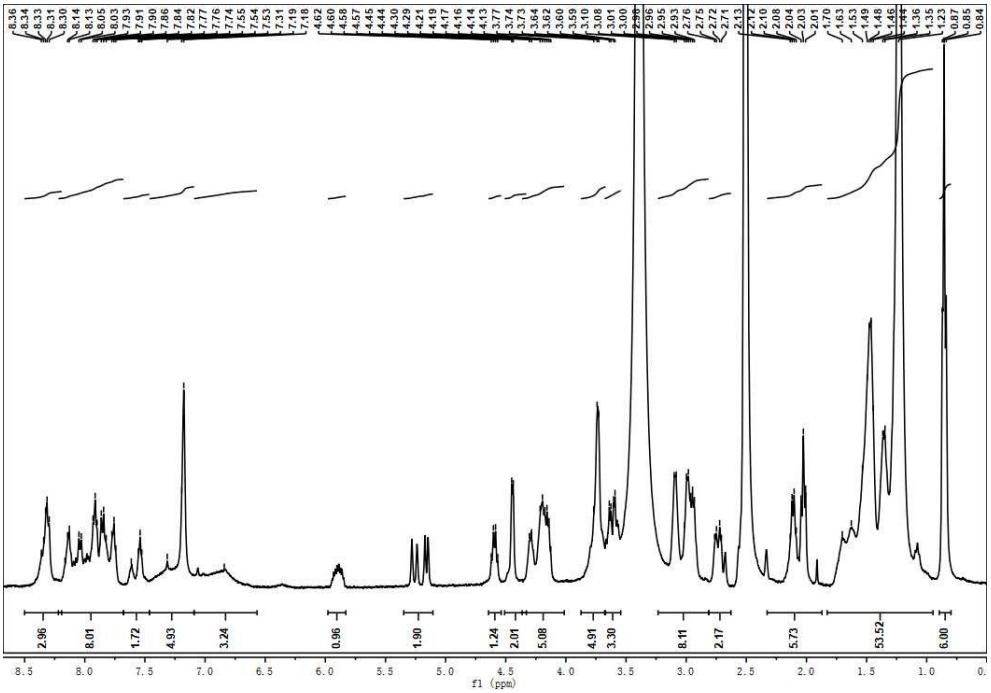

Figure S5. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) spectrum of compound **PA-A2**.

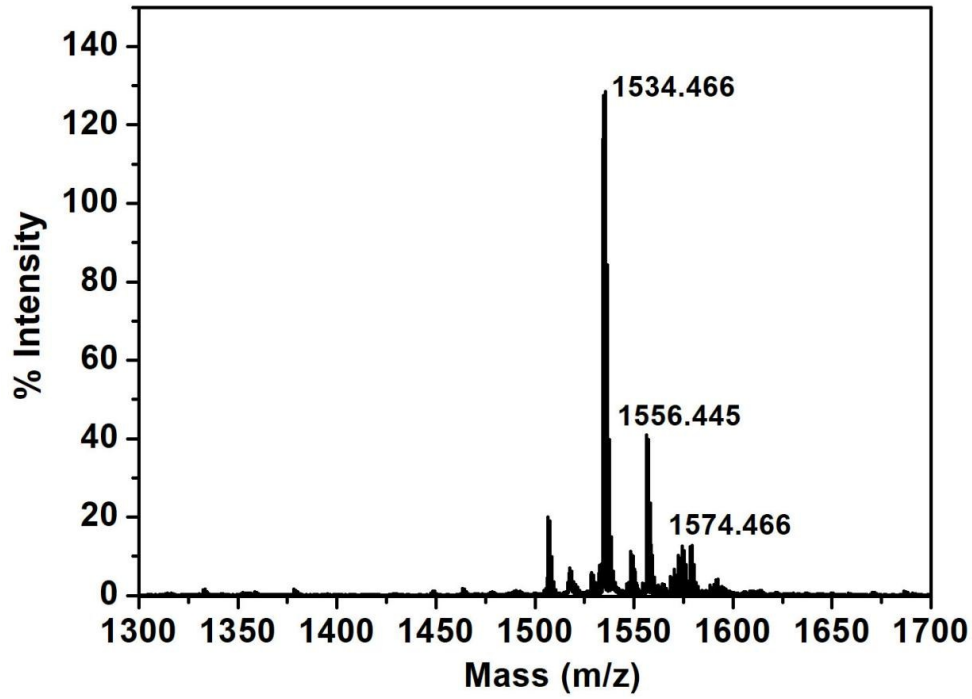

Figure S6. MALDI-TOF-MS spectrum of **PA-A2** m/z calculated for $C_{81}H_{128}N_{15}O_{14}^{+}$ 1534.98, found $[M]^{+}$ 1534.47.

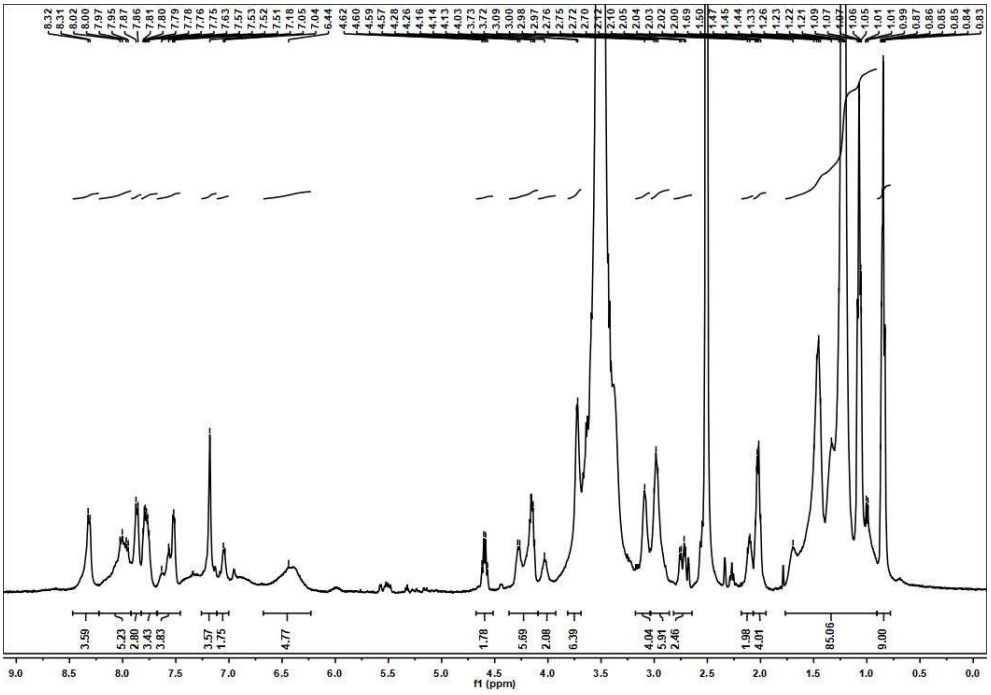

Figure S7. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) spectrum of compound **PA-A3**.

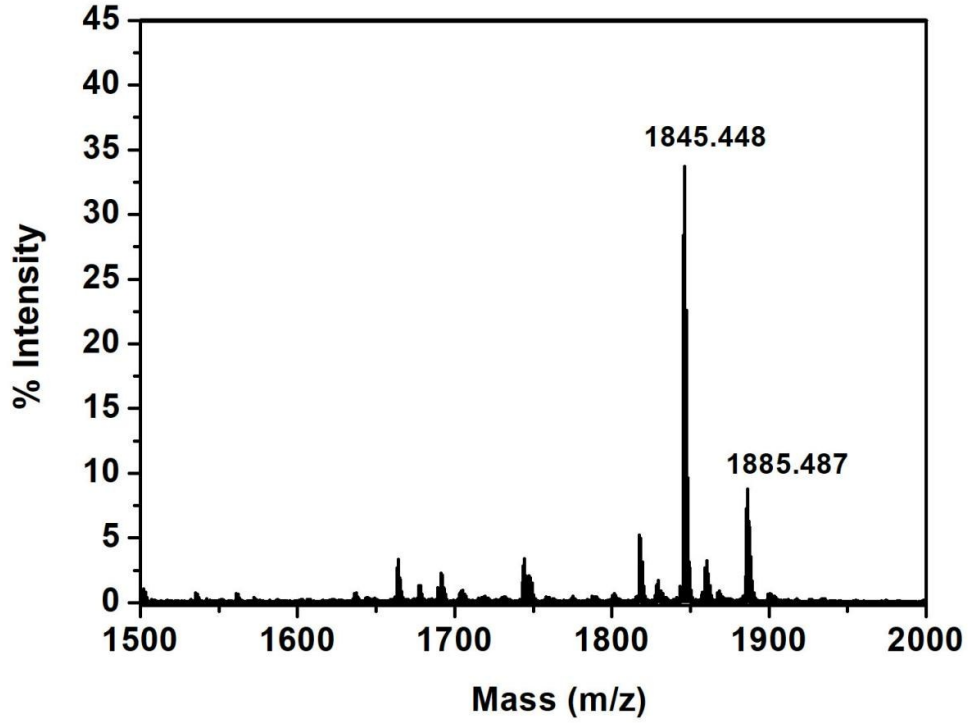

Figure S8. MALDI-TOF-MS spectrum of **PA-A3** m/z calculated for $C_{99}H_{162}N_{17}O_{16}^{+}$ 1846.24, found $[M]^{+}$ 1845.45.

Supplementary Tables:

Table S1. Parameters for CTRW simulation of each PAs.

Samples	α	β	f_{im}	f_m
PA-A0	2.2	2.5	0.05	0.95
PA-A1	2	2.5	0.3	0.7
PA-A2	1.4	2.5	0.6	0.4
PA-A3	1.2	2.5	0.8	0.2

Table S2. Diffusion coefficient for PAs evaluated from experiments and simulations, respectively.

Samples	*D (experiment data)*	*D (simulative data)*
PA-A0	1.47	1.45
PA-A1	1.25	1.20
PA-A2	1.02	1.00
PA-A3	0.75	0.81