Supporting Information

Balancing the Intermolecular Forces in Peptide Amphiphiles for Controlling Self-Assembly Transitions

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(i): Methods

Synthesis of Peptide Amphiphiles (PA):

All amino acids and coupling agents were purchased from ChemImpex. The peptides were synthesized via solid-phase Fmoc chemistry. The peptide sequences were built C-Terminus to N-Terminus using a Seiber Amide Resin (AAPPTEC) on typically a 0.5 mmol scale. All of the prepared PAs were synthesized manually as outlined in the following paragraph.

The resin was placed in a shaker vessel and then swollen with dichloromethane (DCM) for 30 minutes. The DCM was removed, and then N, N-Dimethylformamide (DMF) was added to the shaker vessel to swell the resin for 30 minutes. 20% piperidine in DMF was added for the deprotection of the Fmoc protecting group and allowed to shake for 15 minutes. The liquid was removed, and replenished to shake for another 15 minute interval. The piperidine/DMF solution was removed and washed with DCM, DMF (2x) and DCM (2x) sequentially. The removal of the Fmoc protecting group was confirmed via a Kaiser Test. The coupling solution for each amino acid contained 4 eq. of O-Benzotriazole N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) or 2-(7-Aza-1H-benzotriazole-1-ly)-1,1,3,3- tetramethyluronium hexafluorophosphate (HATU), 3.95 eq. of amino acid, 6 eq. of N,N- diisopropylethylamine (DIPEA) and 3 drops of Triton X-100 in approximately 10mL of DMF. This solution was added to the shaker vessel and allowed to couple for 3 hours to the deprotected species (amino acid or resin). The solution was then removed, and the resin was washed with DMF (3x) followed by DCM (2x). The coupling of the amino acid was confirmed by another Kaiser Test. This process was repeated for each amino acid as well as the palmitic or pentadecanoic acid tail.

Resin Cleavage:

A 120 mL cleavage mixture was made containing 1% Trifluoroacetic acid (TFA), 2% Anisole and 97% DCM. The cleavage of the peptide from the resin was achieved by adding 20-25 mL portions of the cleavage mixture to the shaker vessel, and allowing it to shake for 25 minutes. After the allotted time, the solution was drained to a round-bottom flask, and another portion of the cleavage mixture was added to shake. Once all of the cleavage mixture was used,

the TFA/DCM solution in the round-bottom flask was then neutralized with DIPEA. The excess solvent was removed under vacuum and the PA was precipitated out with cold water and cooled at 10 °C for 45-60 minutes. The resulting solid was isolated via filtration.

Attachment of DO3A to PAs:

The solid PA was placed in a round bottom flask, along with 25 mL of pyridine. The mixture was shaken, sonicated, and then stirred in an oil bath at 60 °C for 60 minutes to ensure full dissolution of the PA. A coupling solution containing 2 eq. HATU, 2 eq. 1,4,7-tris(carboxymethylaza)cyclododecane-10-azaacetylamide (DO3A), 4.4 eq. DIPEA (relative to the PA) and a small portion (2-4 mL) of pyridine were added to the PA. The mixture was then set to stir for 18-24 hours at room temperature. To ensure maximal coupling, excess solvent was removed under vacuum, until only 10-15 mL of the mixture remained. A second coupling mixture of 2 eq. HATU, 4.4 eq. DIPEA and 1 mL of pyridine was added to the PA and stirred for 18-24 hours. The PA-DO3A was then crashed out with cold water and cooled at 10 °C for 45-60 minutes. The resulting PA-DO3A solid was isolated via filtration.

Removal of tert-butyl groups on PA:

A 20 mL solution containing 95% TFA, 3% water and 2% Anisole was prepared and added to the PA-DO3A. The mixture was set to stir for 20-24 hours at room temperature in order to remove tert-butyl groups from the glutamic acid, tyrosine and DO3A species. Excess TFA was removed under vacuum and the PA-DO3A was crashed out with cold diethyl ether. The mixture was cooled at -5°C for 45 minutes and the resulting solid was isolated via filtration.

Purification of PA-DO3A:

The crude PA-DO3A (150-250 mg) was dissolved in a 10 mL solution consisting of 9 mL of water, 1 mL of acetonitrile and a few drops of NH_4OH. This solution was, shaken, sonicated and vortexed to ensure full dissociation. The resulting solution was then filtered through a 0.45 µm PVDF filter. Purification of the PA-DO3A was carried out using a Shimadzu preparative High Performance Liquid Chromatograph (HPLC) duel pump system controlled by LC-MS solution software, with an Agilent PLRP-S polymer column (Model No. PL1212.3100 150 mm x 25 mm). The two solvents that were used for the mobile phase were water with 0.1% NH_4OH (v/v) and acetonitrile with 0.1%NH_4OH (v/v). The product was eluted via a linear gradient from 10% acetonitrile to 20% acetonitrile over 22.5 minutes, and then from 20% acetonitrile to 40% acetonitrile over an additional 67.5 minutes. The desired product was collected in fractions of 10-15 mL. The presence of the product in the fractions was confirmed by a Bruker Electrospray Ionization Time of Flight Mass Spectrometry (ESI-TOF MS) and the purity by Shimadzu analytical HPLC. Fractions $\geq$ 95% in purity were added together, acetonitrile was removed under vacuum and the liquid fraction were freeze-dried yield a white powder.

Incorporation of Gd^{3+} in PA-DO3A:

The pure PA-DO3A was dissolved in 4-6 mL of water. 2 eq. of 0.01M $GdCl_3$ in 0.01M HCl were added to the PA-DO3A solution. Diluted NaOH(aq) was added to this solution to set the pH at 5.0 - 5.1. The solution was placed in an oil bath to stir at 60°C. After 60 minutes, the solution was removed from the oil bath, the pH was readjusted to a value of 5.0 – 5.1, and then

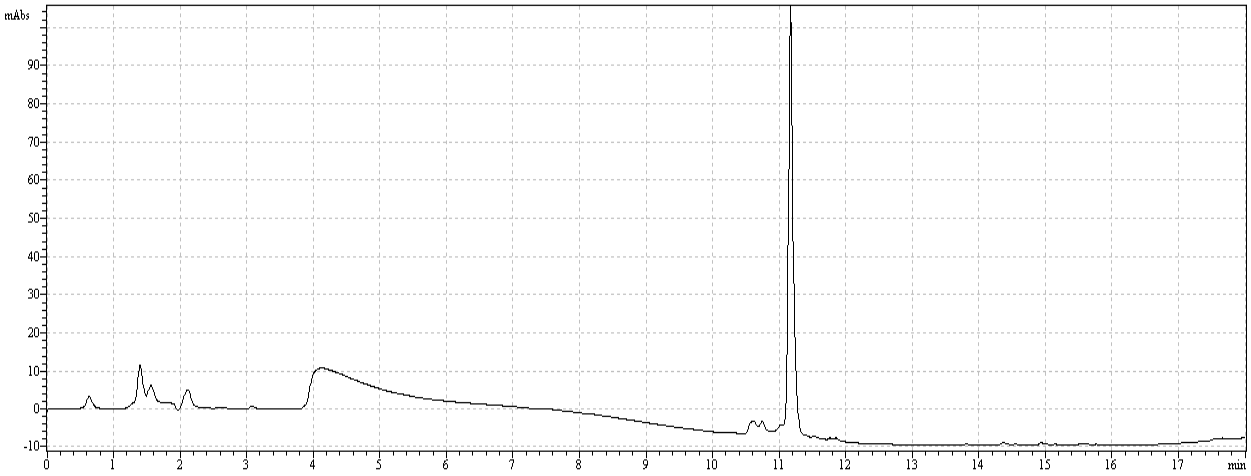
placed back in the hot oil bath to stir for 12-18 hours. The solution was then returned to room temperature and the pH was raised to a value greater than 10 using 1 M NaOH to precipitate out all non-chelated Gd^{3+} to produce solid $Gd(OH)_3$. The solution was filtered with a 0.45 µm PVDF filter and the pH was lowered to a neutral value of approximately 7.

Dialysis of the PA-(DO3A:Gd):

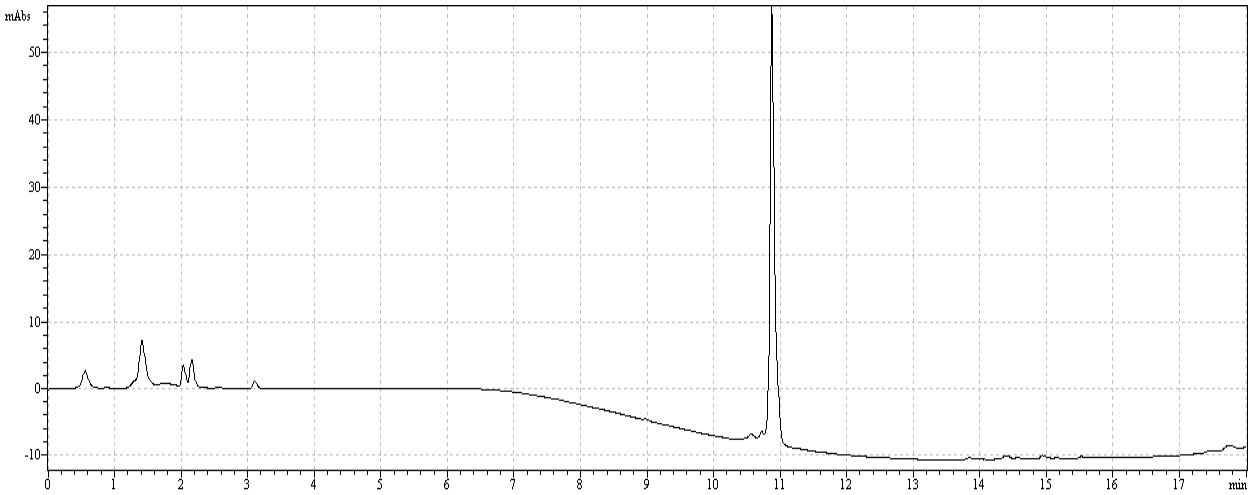
The PA-(DO3A:Gd) solution was pipetted into a Spectra/Por® Biotech Cellulose Ester dialysis membrane (molecular weight cut off: 500 g/mol) and placed in 4 L of Millipore water. The water was changed 10-12 times over a 72 hour period. The dialyzed solution was then freeze-dried. The resulting solid yielded the pure PA-(DO3A:Gd) powder which was confirmed through ESI- TOF MS and analytical HPLC.

Fig. S1. HPLC of purified PA samples.

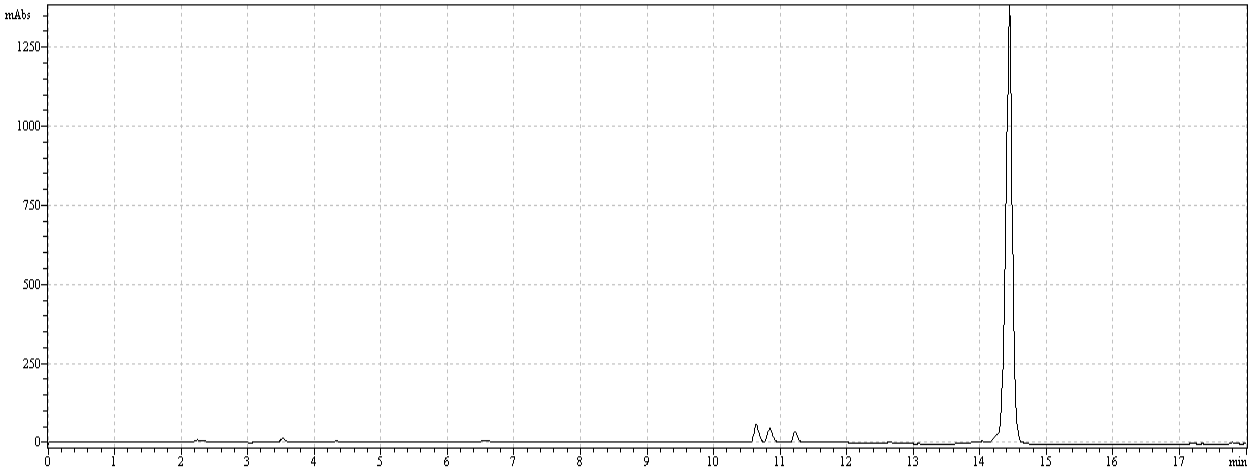
palmitoyl-YYAAEEEEK(DO3A:Gd)-NH_2 (PA1)



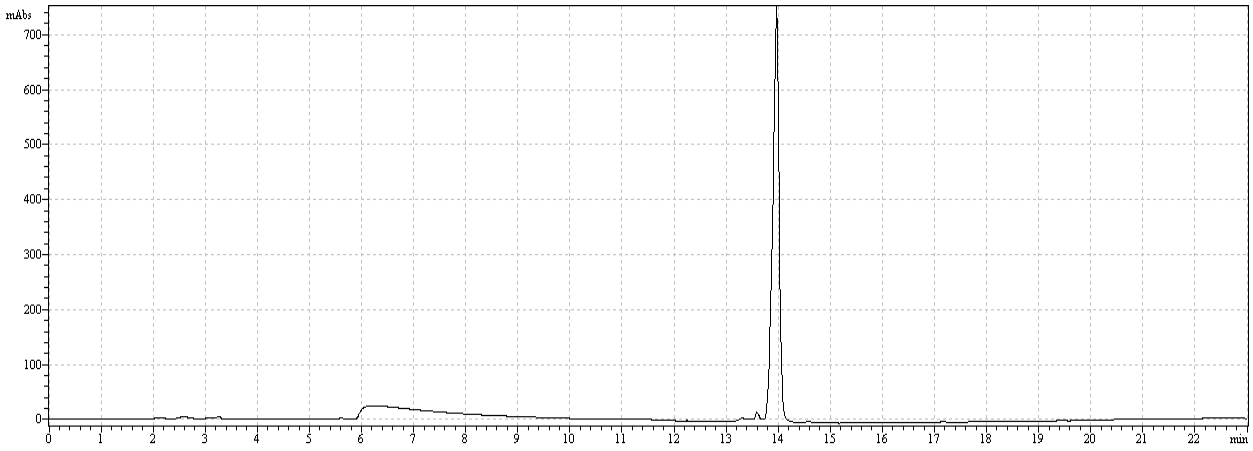
palmitoyl-YYAAEEEEEK(DO3A:Gd)-NH_2 (PA2)



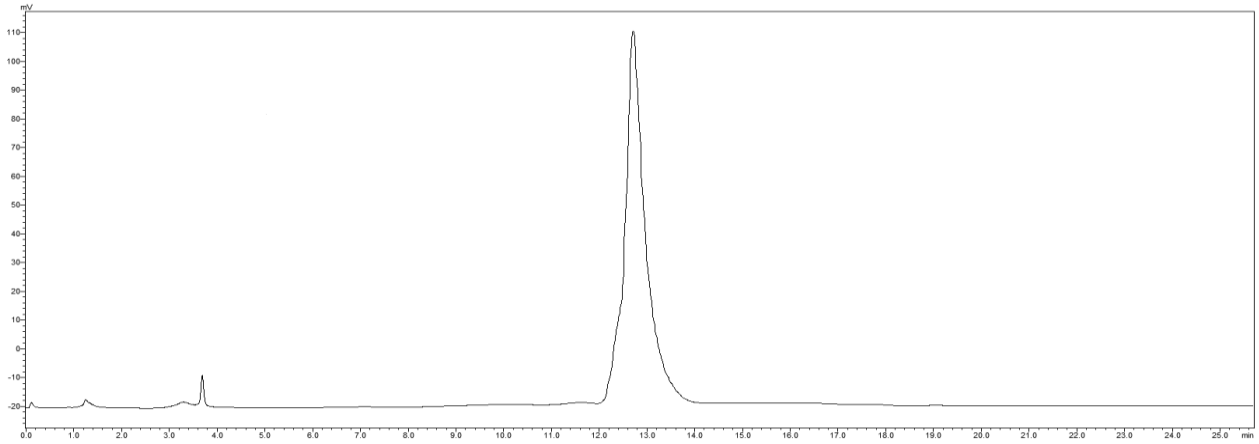
pentadecyl-YYAAEEEEK(DO3A:Gd)-NH_2 (PA3)



palmitoyl-YAAAEEEEK(DO3A:Gd)-NH_2 (PA4)



palmitoyl-YAAAEEEEEK(DO3A:Gd)-NH_2 (PA5)



pentadecyl-YAAAEEEEK(DO3A:Gd)-NH_2 (PA6)

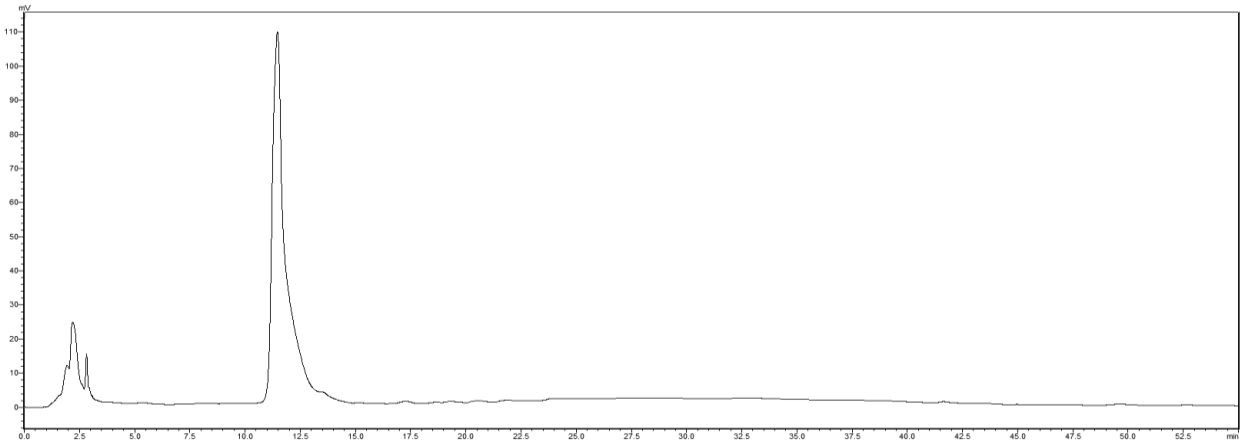
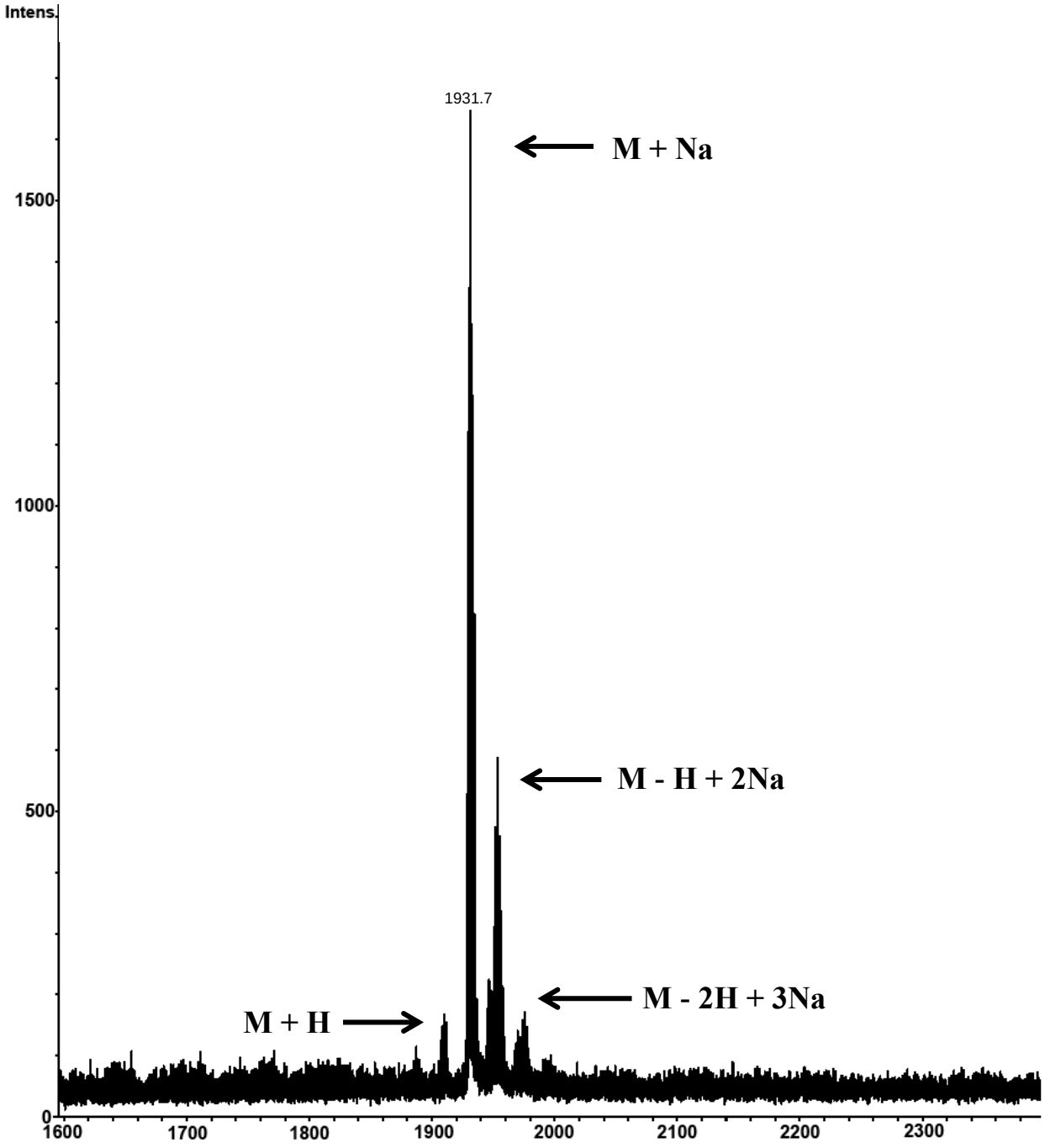
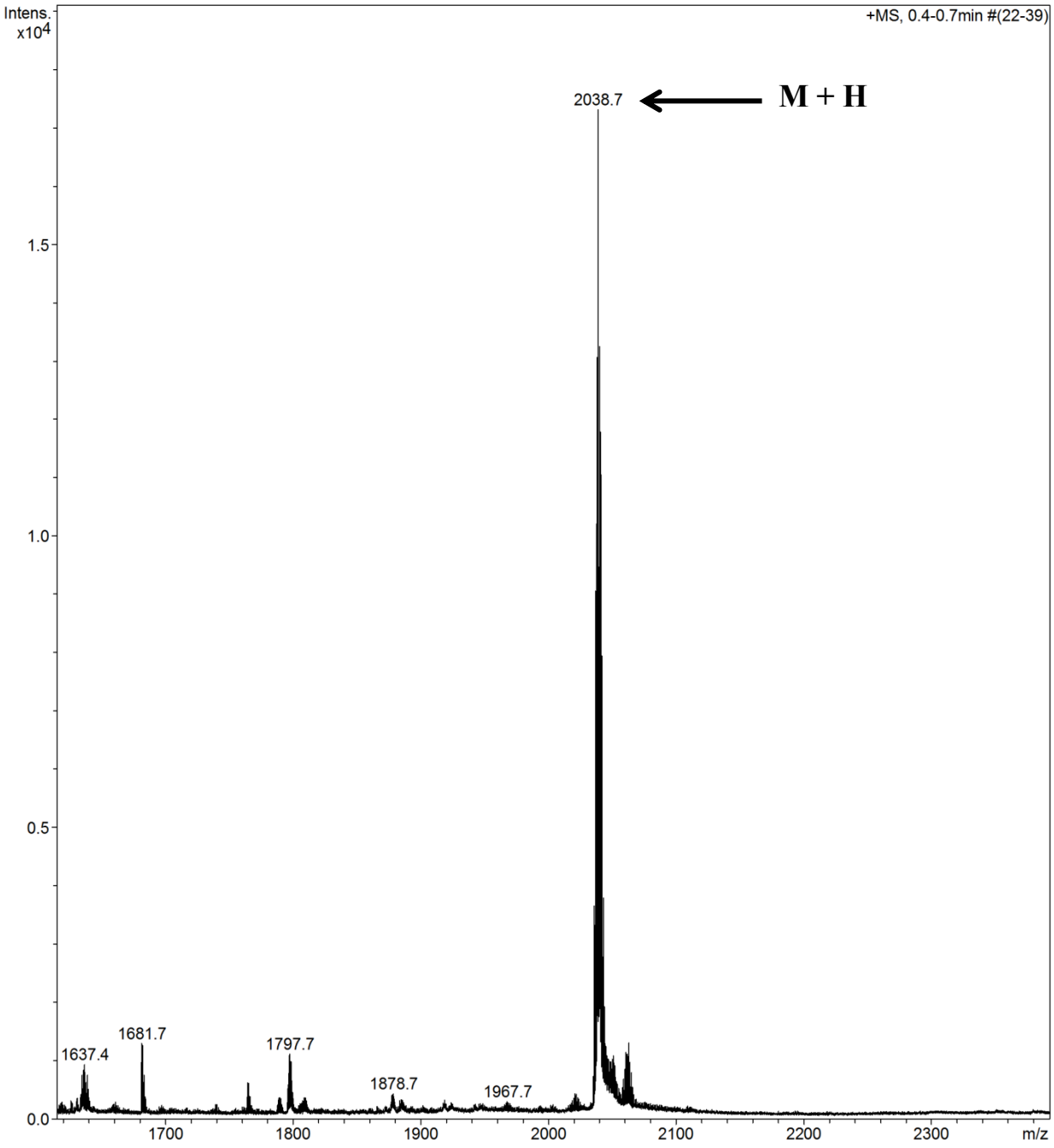


Fig. S2. ESI-TOF MS for all PAs. All measurements were made in positive mode, except PA3 which was measured using negative mode.

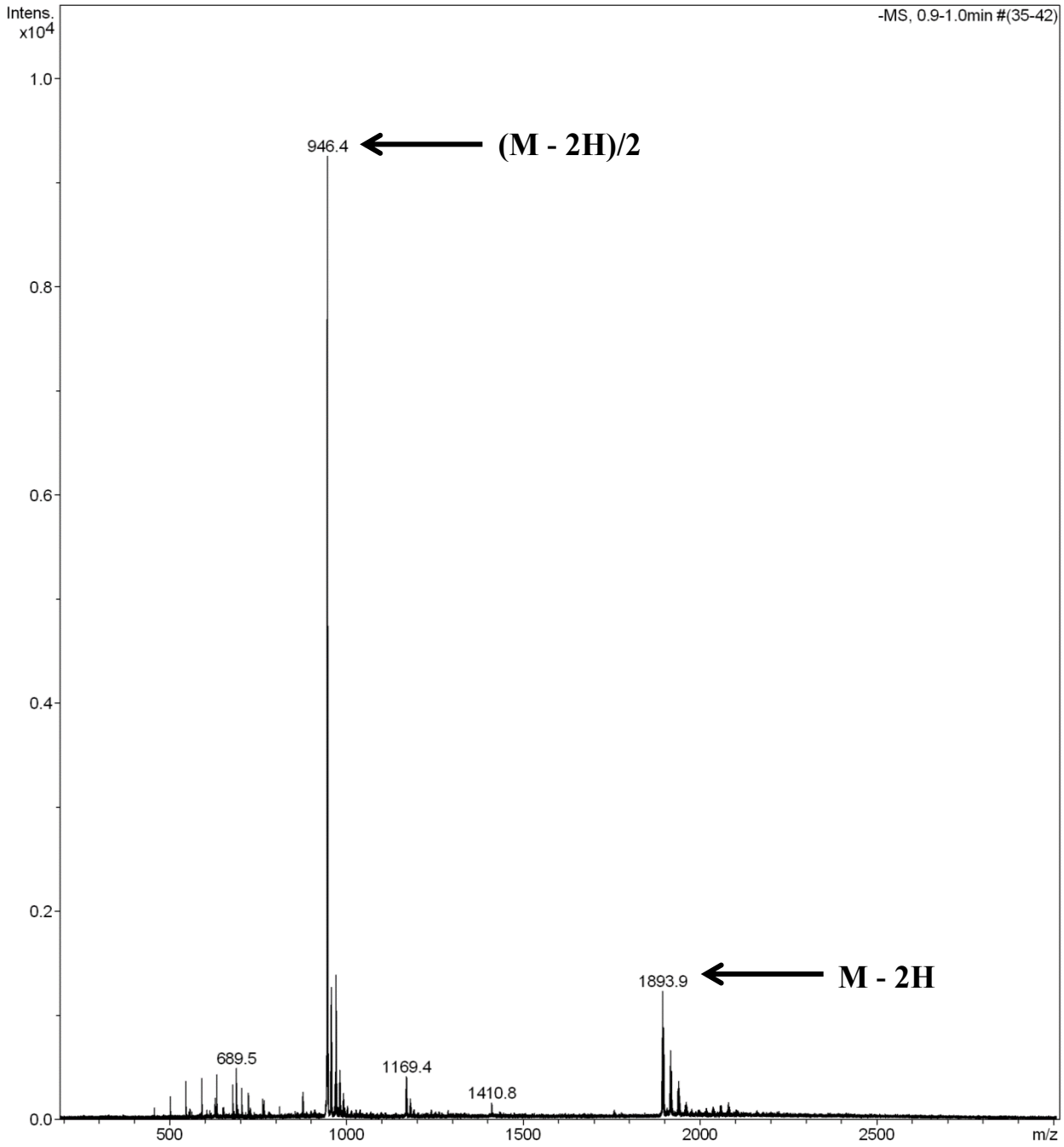
palmitoyl-YYAAEEEEK(DO3A:Gd)-NH_2 (MW: 1908.8 g/mol)



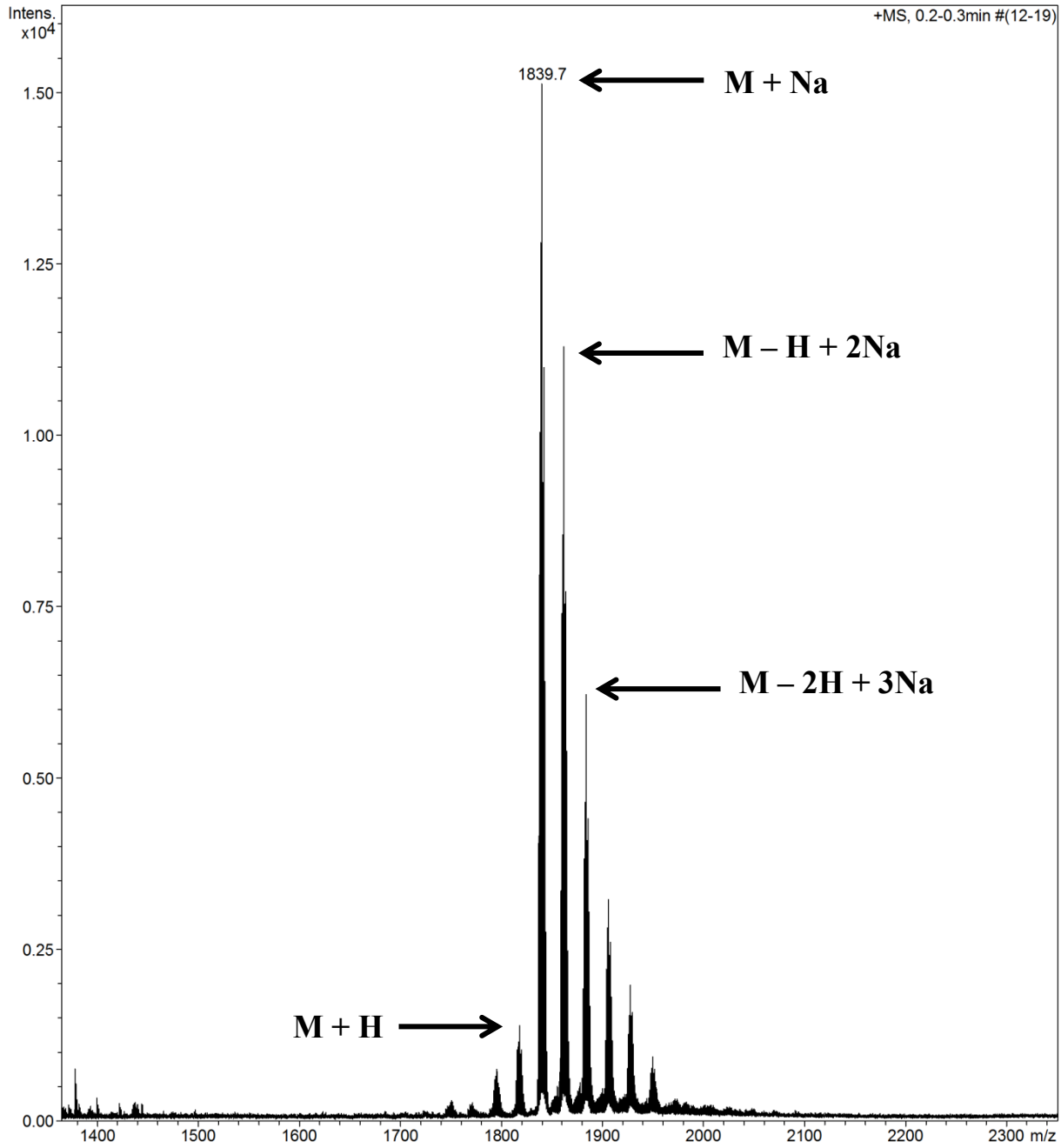
palmitoyl-YYAAEEEEEK(DO3A:Gd)-NH_2 (MW: 2038 g/mol)



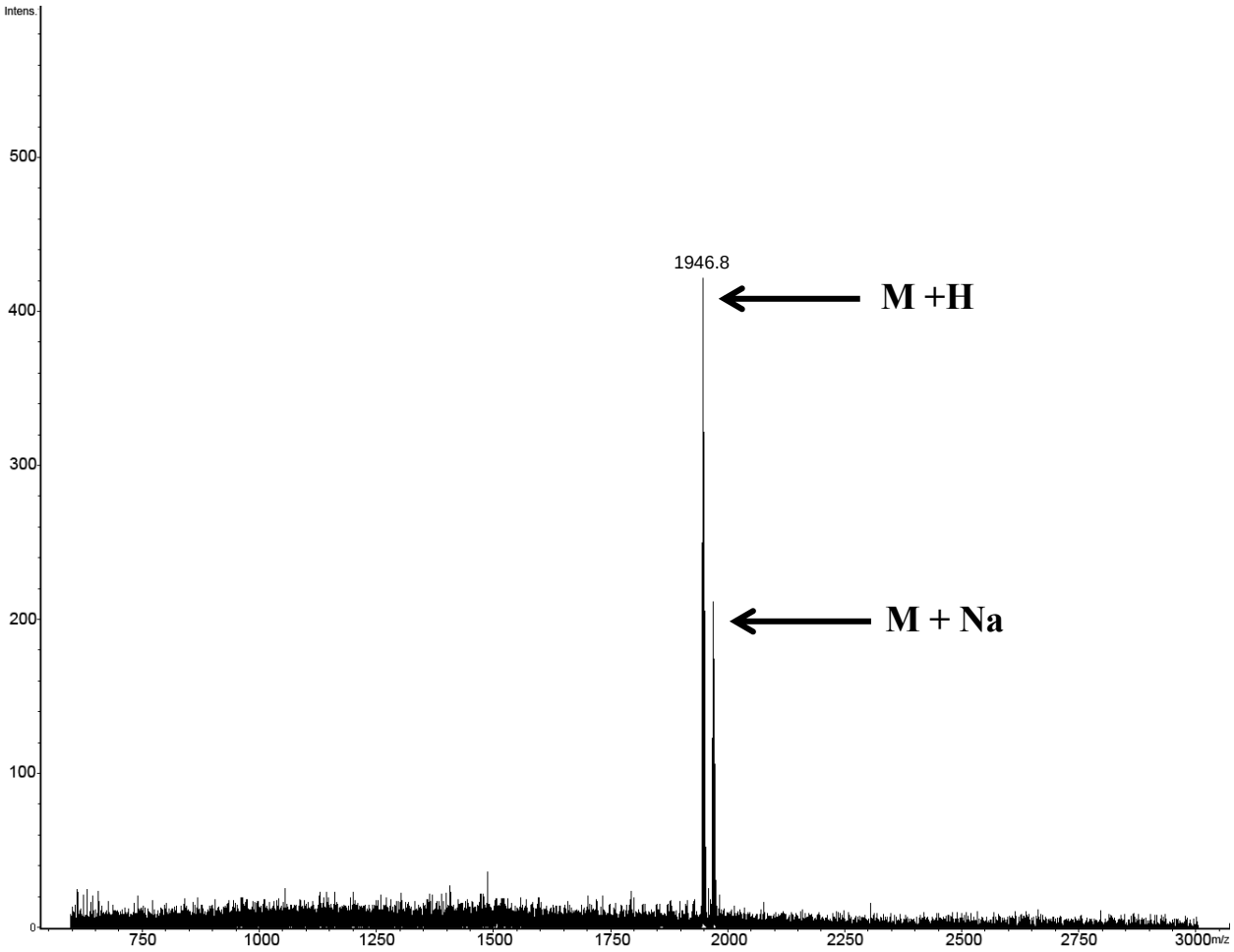
pentadecyl-YYAAEEEEK(DO3A:Gd)-NH_2 (MW: 1894.8 g/mol) (negative mode)



palmitoyl-YAAAEEEEK(DO3A:Gd)-NH_2 (MW: 1817 g/mol)



palmitoyl-YAAAEEEEEK(DO3A:Gd)-NH_2 (MW: 1946 g/mol)



pentadecyl-YAAAEEEEK(DO3A:Gd)-NH_2 (MW: 1803 g/mol)

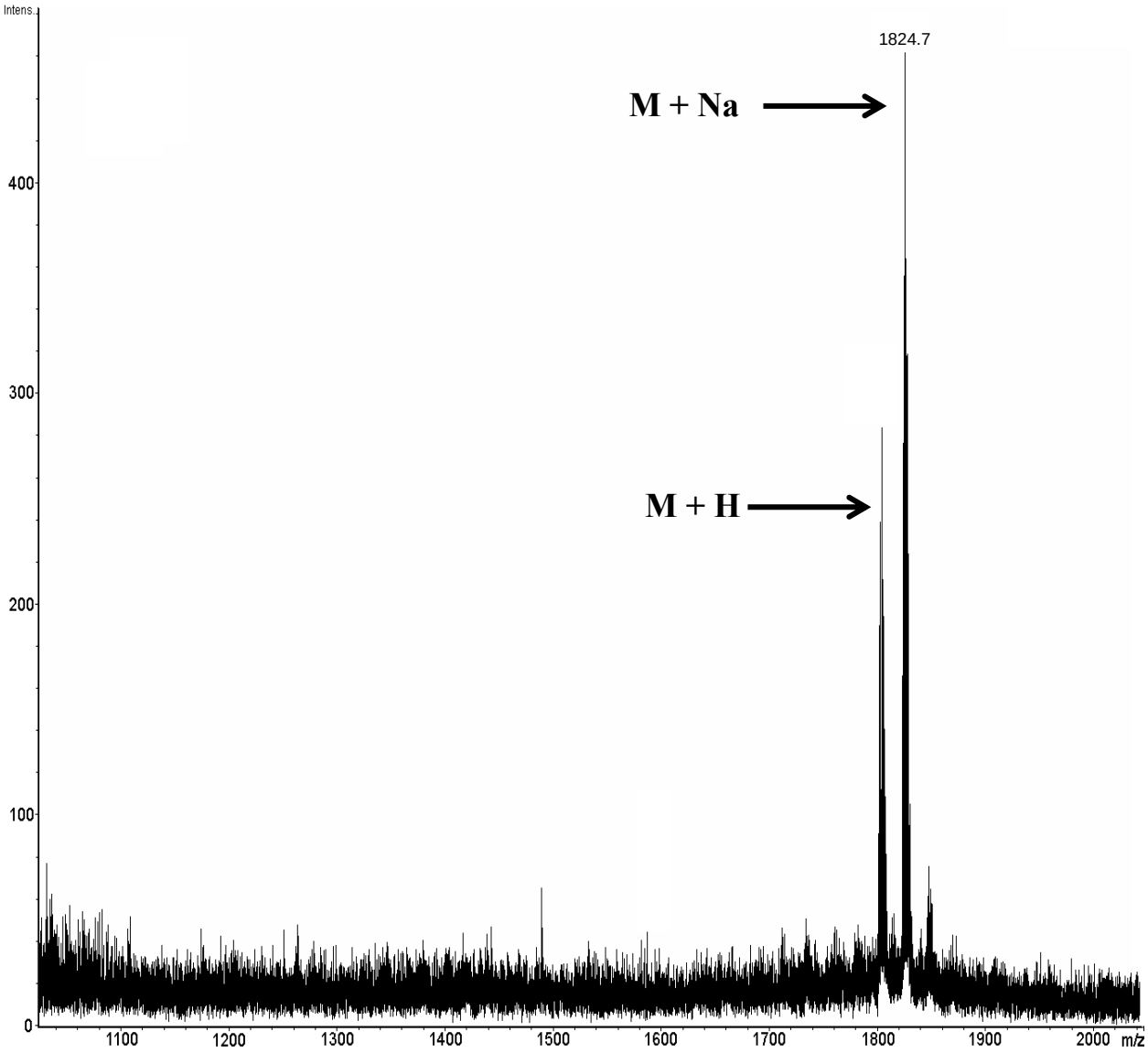
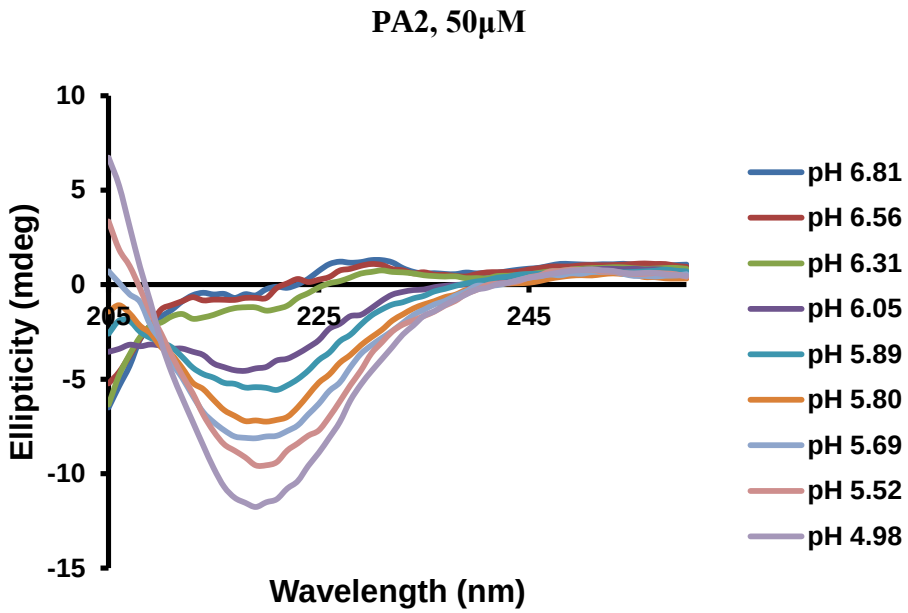
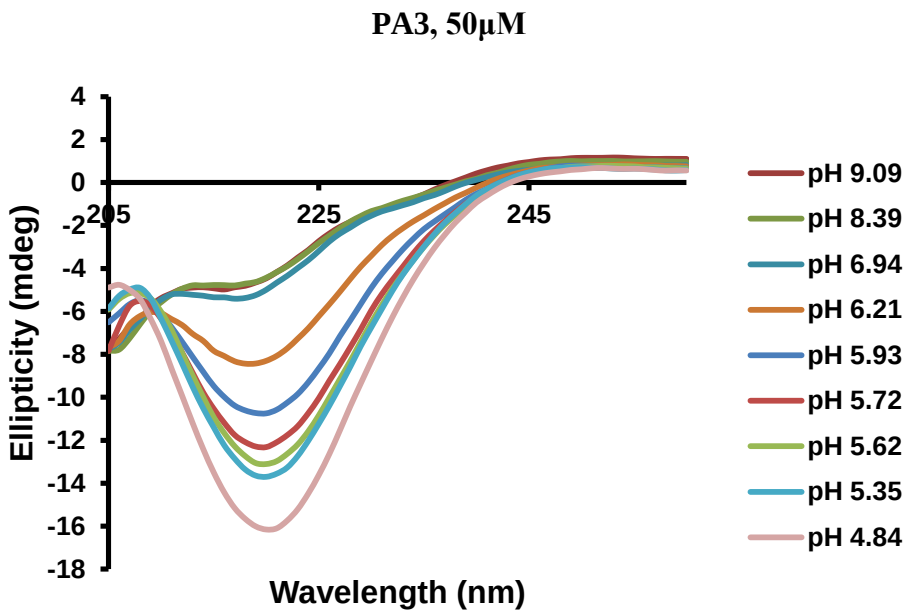
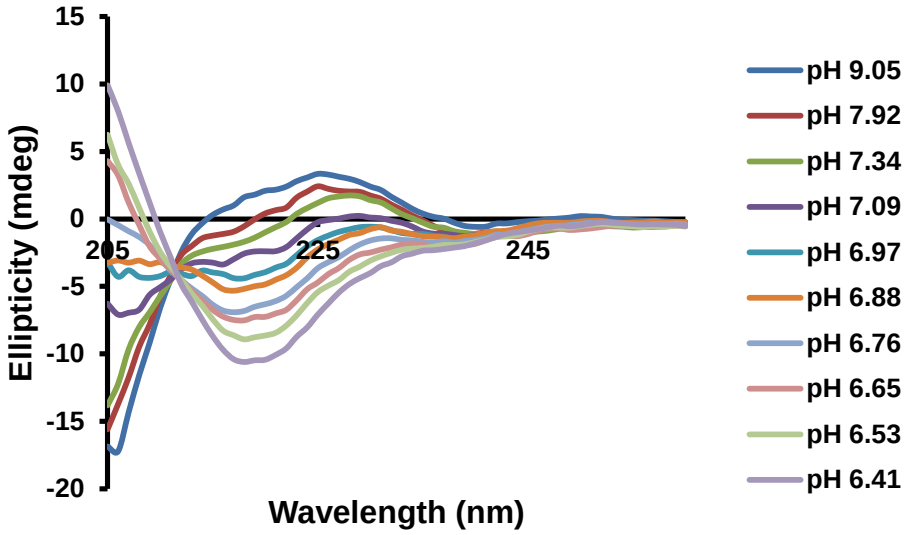


Fig. S3. CD spectra of PAs 2-6 measured at different pH values, with PAs dissolved in 150 mM NaCl and 2.2 mM $CaCl_2$.

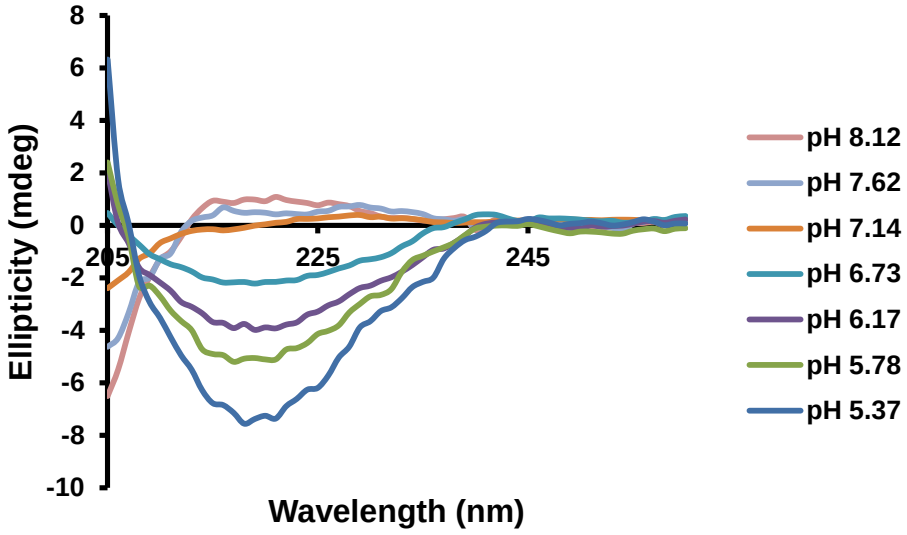




PA4, 50µM



PA5, 50µM



PA6, 50µM

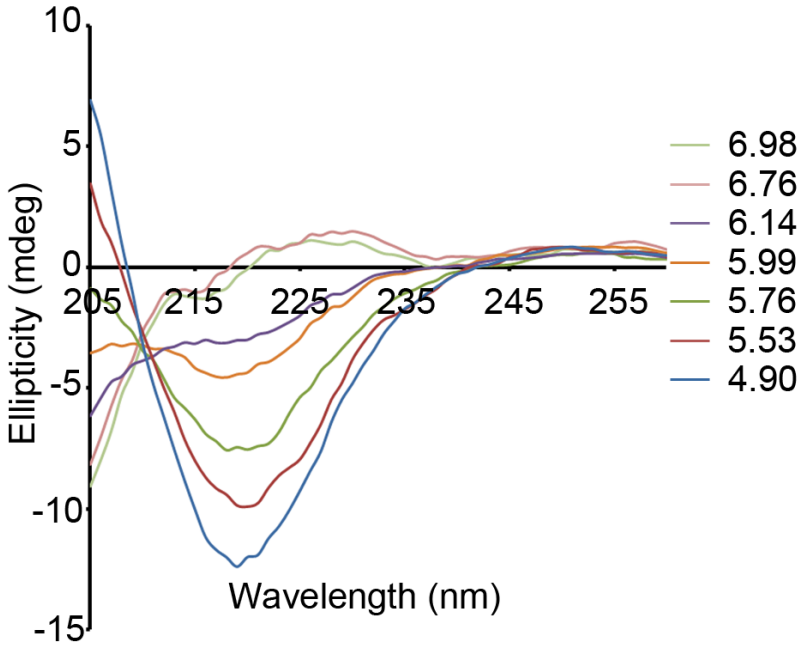
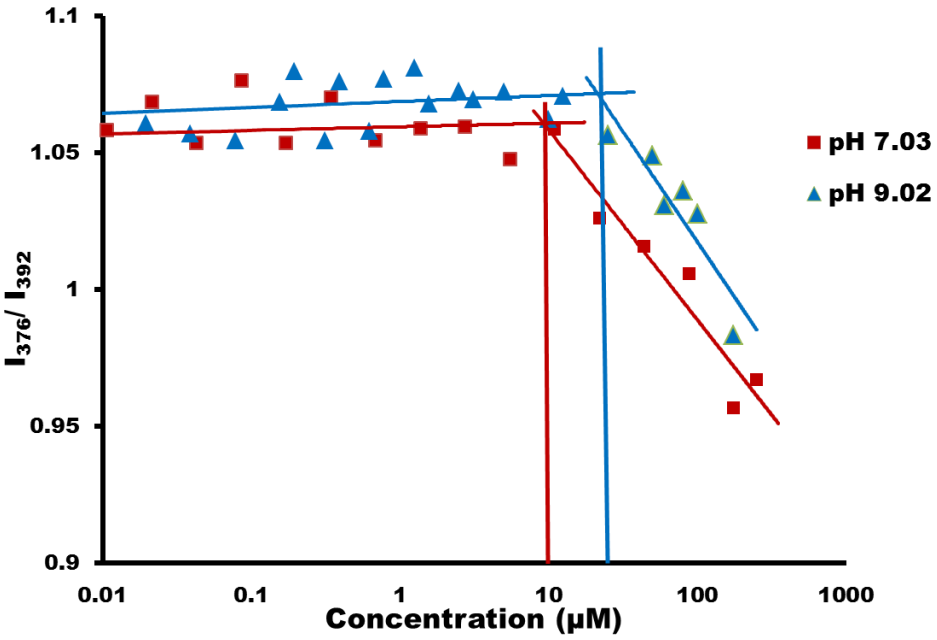
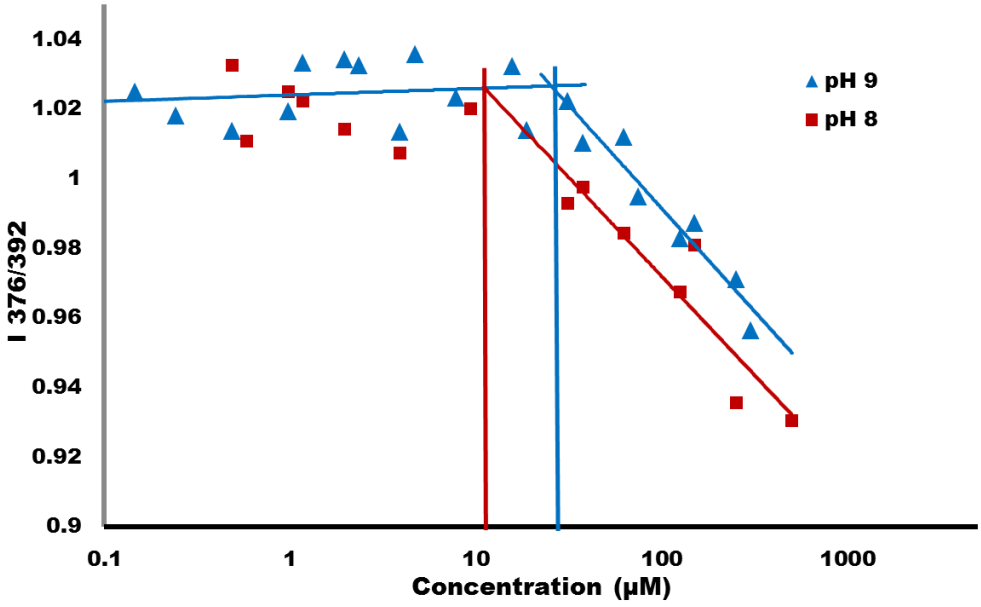

10
5
0
-5
-10
-15
205
215
225
235
245
255
Ellipticity (mdeg)
Wavelength (nm)
6.98
6.76
6.14
5.99
5.76
5.53
4.90

Fig. S4: CAC measurements of PAs 2-6 at different pH values. The Intensity of the 376 and 392 nm pyrene fluorescence is compared with different amounts of PA concentration, at different pH values.

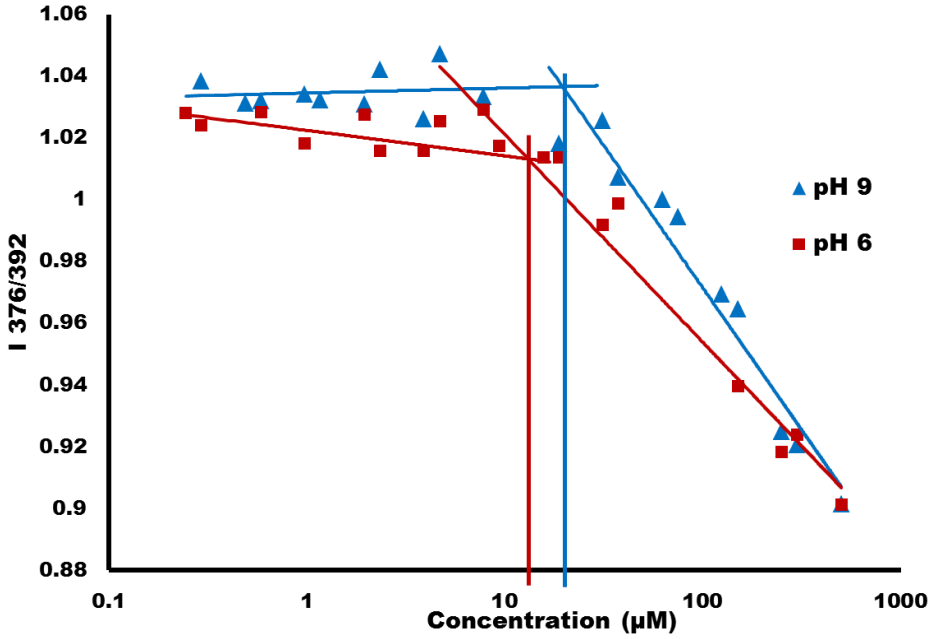
PA2



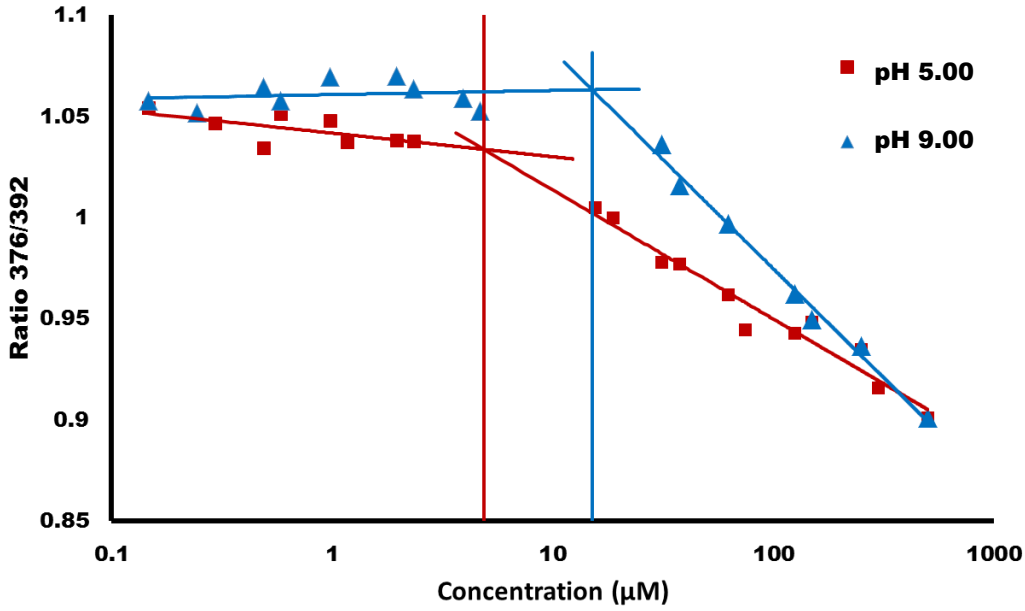
PA3



PA4


1.06
1.04
1.02
1
0.98
0.96
0.94
0.92
0.9
0.88
I 376/392
0.1
1
10
100
1000
Concentration (µM)
pH 9
pH 6

PA5


1.1
1.05
1
0.95
0.9
0.85
Ratio 376/392
0.1
1
10
100
1000
Concentration (µM)
pH 5.00
pH 9.00

PA6

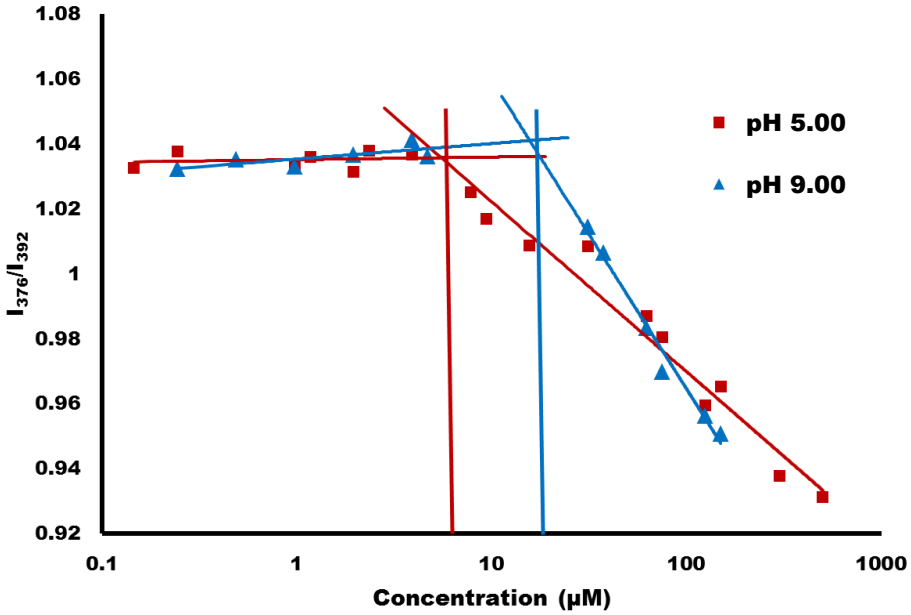
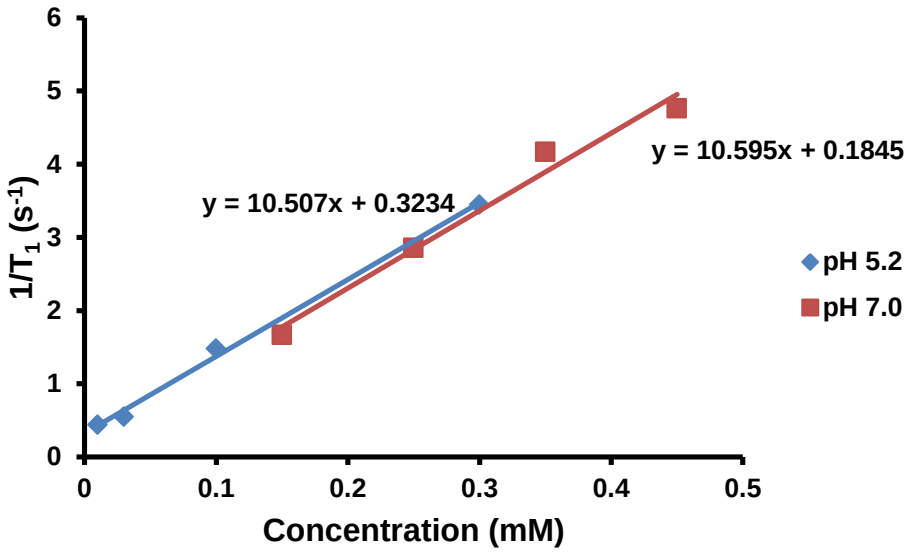
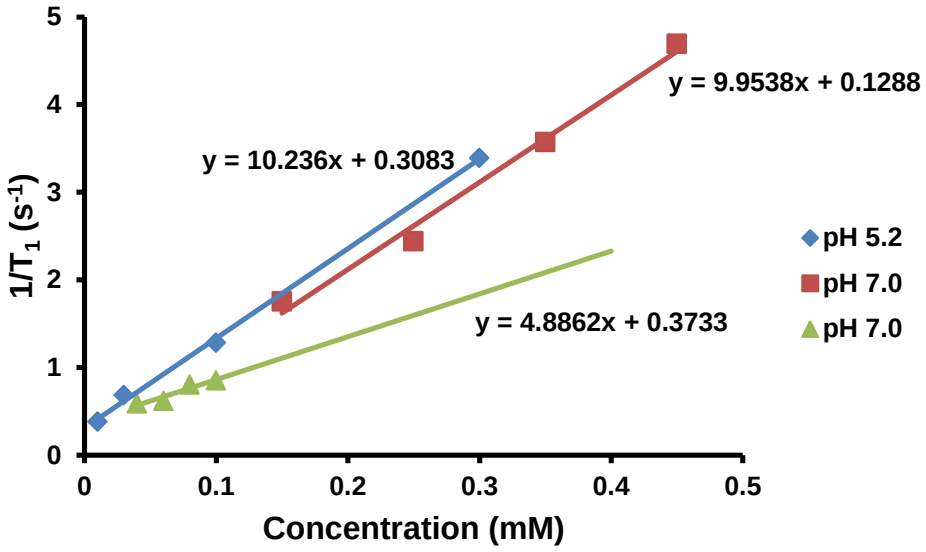

1.08
1.06
1.04
1.02
1
0.98
0.96
0.94
0.92
I_{376}/I_{392}
0.1
1
10
100
1000
Concentration (μM)
pH 5.00
pH 9.00

Fig. S5. $1/T_1$ vs. Concentration graphs showing the relaxivity (r_1) of PAs 1-6 at pH 5.2 and pH 7.0. At pH 7.0, the spherical micelle morphologies are plotted as green, and the nanofiber morphologies are plotted as red. The pH 5.2 nanofiber morphologies are plotted in blue.

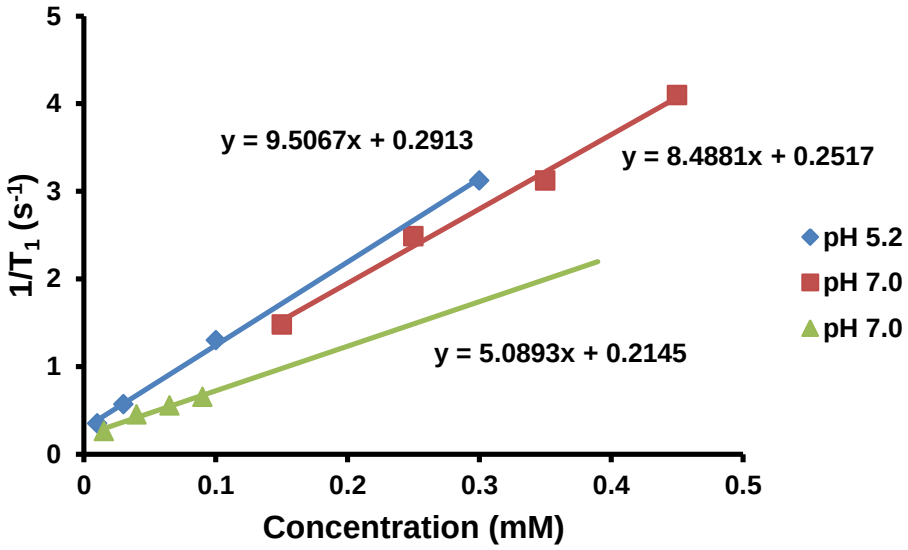
PA1



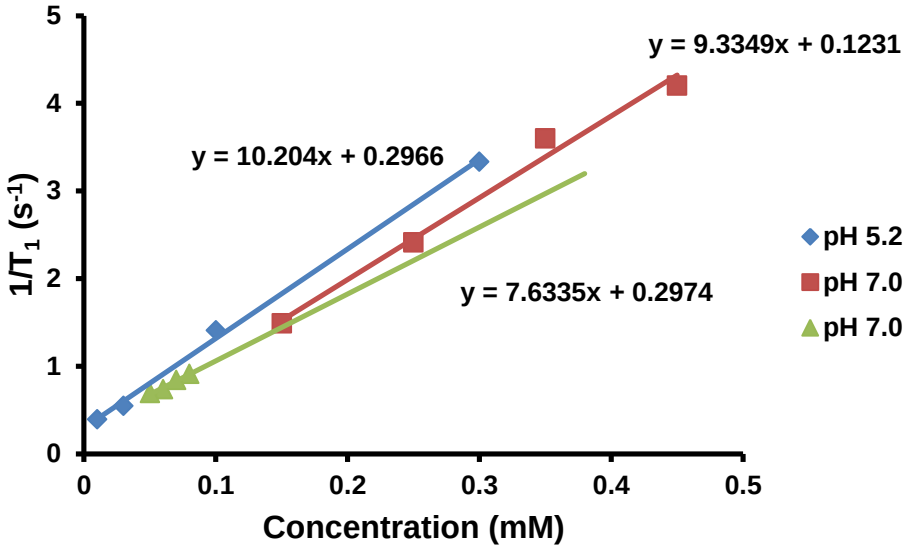
PA2



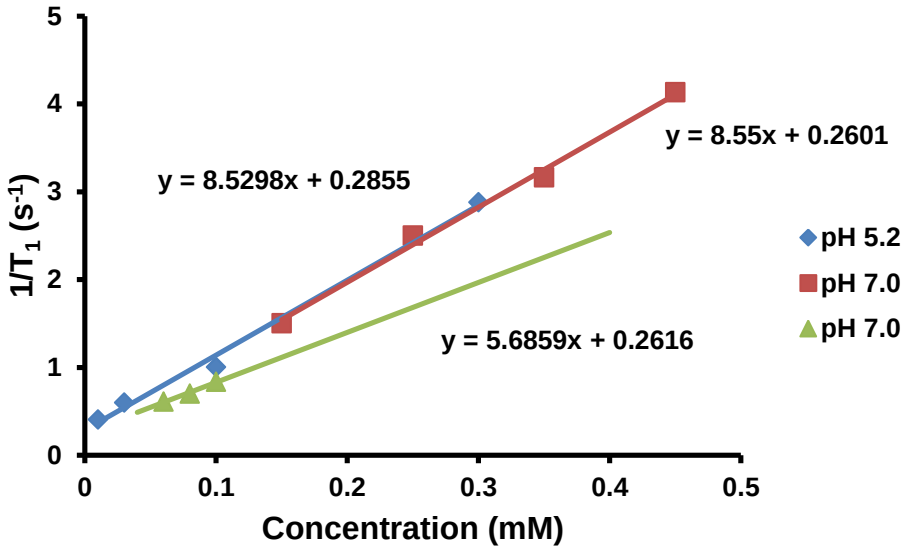
PA3


y = 9.5067x + 0.2913
y = 8.4881x + 0.2517
y = 5.0893x + 0.2145
1/T1 (s-1)
Concentration (mM)
pH 5.2
pH 7.0
pH 7.0

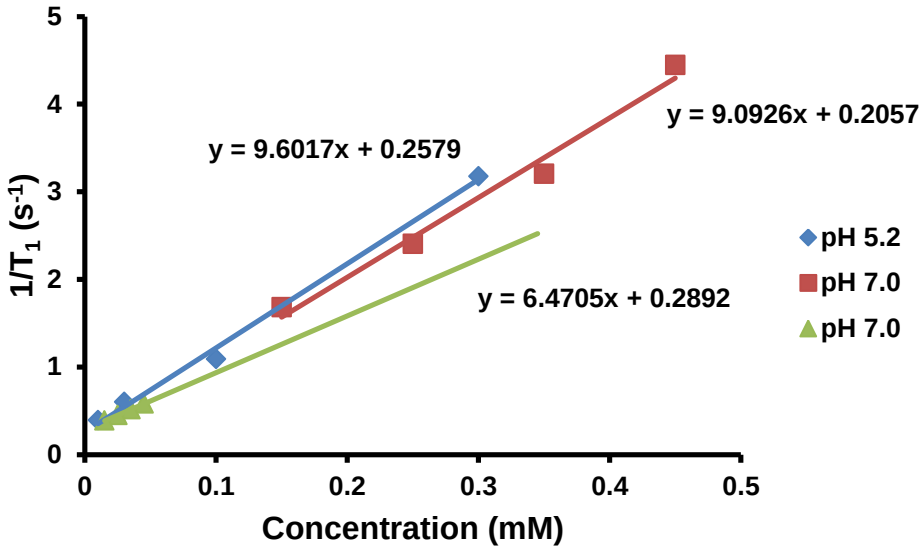
PA4


y = 9.3349x + 0.1231
y = 10.204x + 0.2966
y = 7.6335x + 0.2974
1/T1 (s-1)
Concentration (mM)
pH 5.2
pH 7.0
pH 7.0

PA5


5
4
3
2
1
0
0
0.1
0.2
0.3
0.4
0.5
1/T1 (s-1)
Concentration (mM)
y = 8.5298x + 0.2855
y = 8.55x + 0.2601
y = 5.6859x + 0.2616
pH 5.2
pH 7.0
pH 7.0

PA6


5
4
3
2
1
0
0
0.1
0.2
0.3
0.4
0.5
1/T1 (s-1)
Concentration (mM)
y = 9.6017x + 0.2579
y = 9.0926x + 0.2057
y = 6.4705x + 0.2892
pH 5.2
pH 7.0
pH 7.0