Electronic Supplementary Material (ESI) for Analyst.

Supporting information for:

Improved mass spectrometric detection of acidic peptides by variations in the functional group pKa values of reverse micelle extraction agents

Bo Zhao[1], Mahalia A. C. Serrano[1], Meizhe Wang[1], Tianying Liu[1], Mallory R. Gordon[1], S. Thayumanavan[1,2,3]* and Richard W. Vachet[1,2,3]*

[1]Department of Chemistry, [2]Molecular and Cellular Biology Program, [3]Center for Bioactive Delivery – Institute for Applied Life Sciences, University of Massachusetts, Amherst, Massachusetts 01003, USA

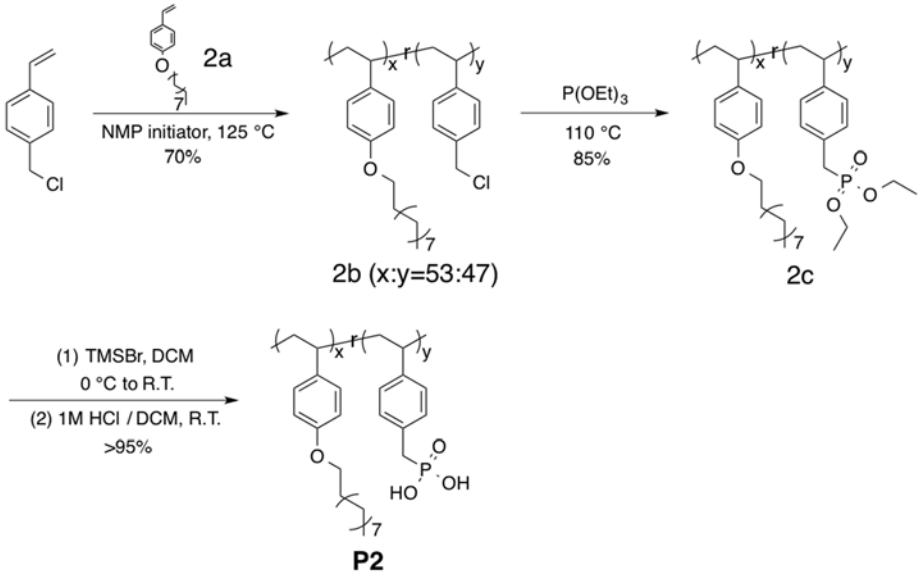
*thai@chem.umass.edu, *rwvachet@chem.umass.edu

Polymer synthesis and characterization

Synthesis of random copolymer P1

Synthesis of compound **P1** was mentioned elsewhere[1]

Synthesis of random copolymer P2

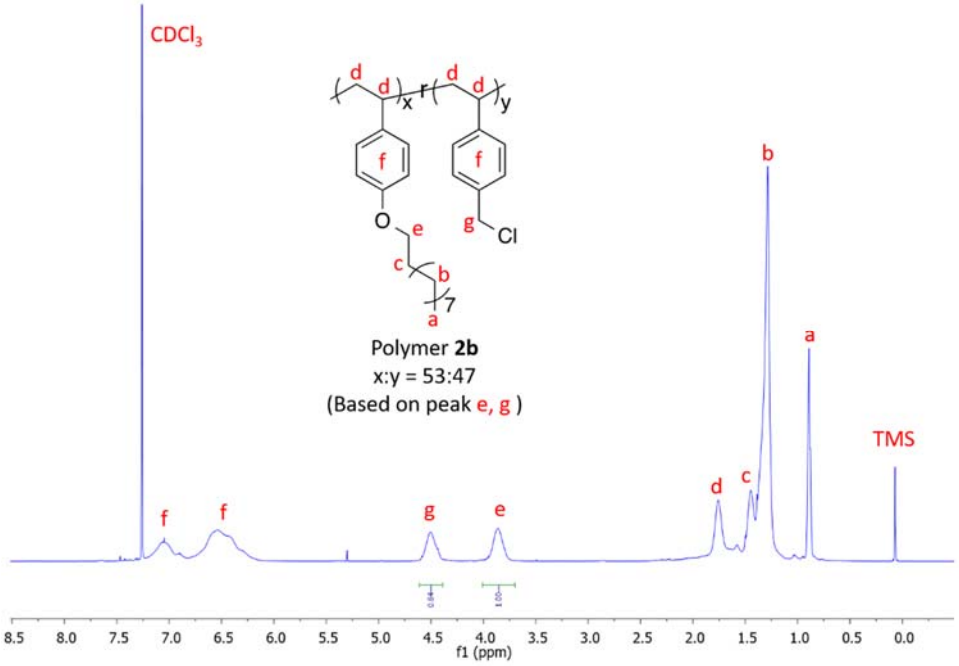


Synthesis of polymers **2a**:

Synthesis of compound **2a** was mentioned elsewhere[1]

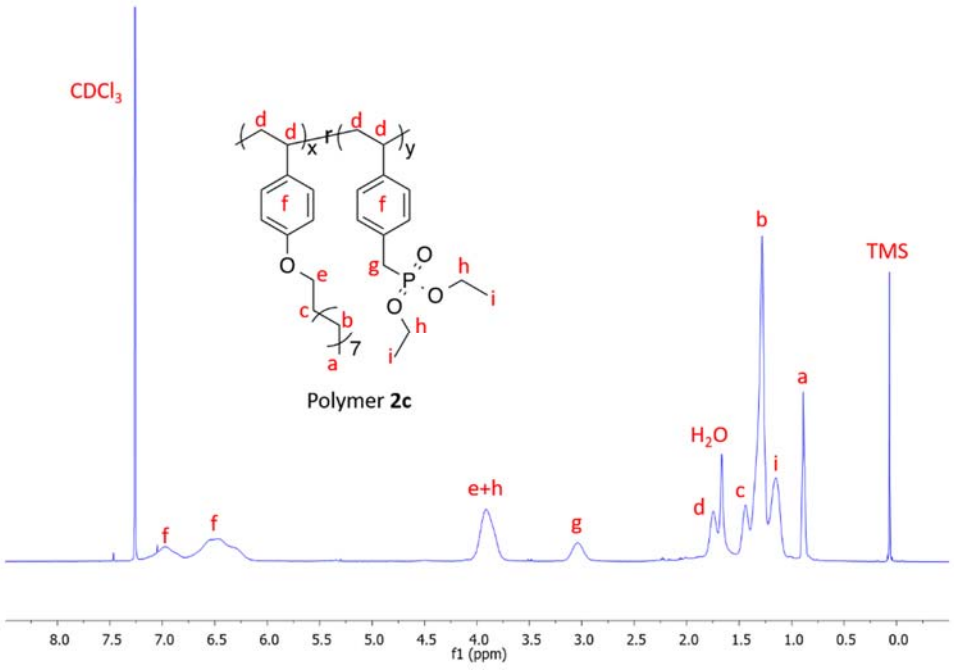
Synthesis of polymers **2b**:

A mixture of the compound **2a** (200 mg, 0.77 mmol), commercial available compound 4-Vinylbenzyl chloride (117 mg, 0.77 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 10 mg, 0.031 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 ºC under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in DCM, and dialyzed against DCM/MeOH (v/v= 6/1) for 2 days. The solution was collected and dried under vacuum to yield 220 mg (70% yield) of **2b**. GPC (PMMA/THF): M_n= 12K Da, Đ=1.2;



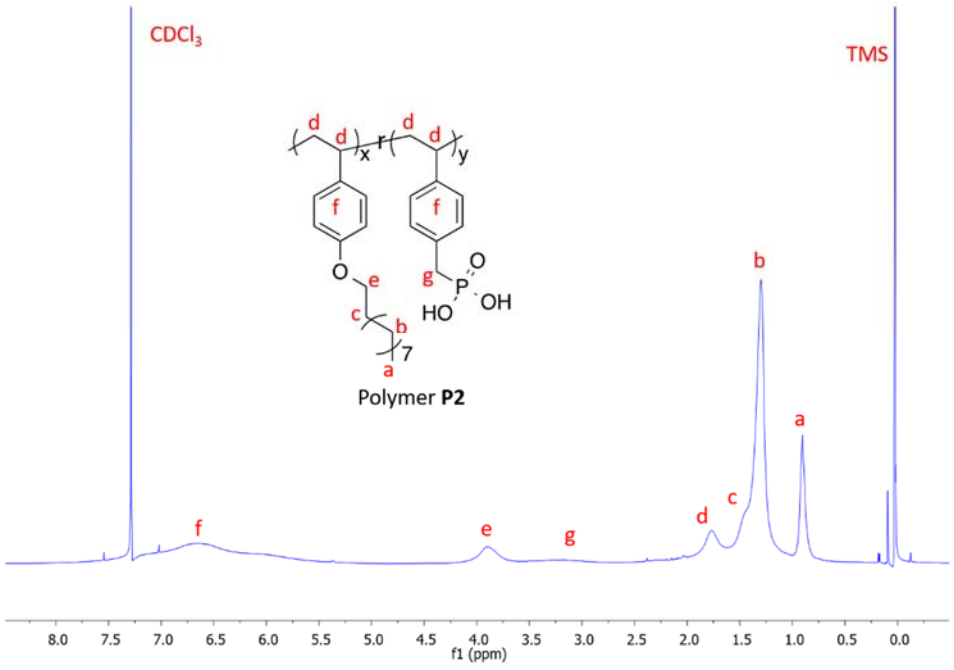
Synthesis of polymers **2c**:

Polymer precursor **2b** was added to 2mL of Triethylphosphite in a round bottom flask and stirred with reflux at 110 ºC for 24 h. The reaction mixture was then cooled to room temperature and dialyzed against DCM/MeOH (v/v= 6/1) for 2 days to remove excess Triethylphosphite. The solution was collected and dried under vacuum to yield 235 mg (85% yield) of **2c**. ^{1}H NMR indicates that there is a quantitative conversion from benzyl chloride to benzyl phosphonate functional group (based on the chemical shift of peak “g” and the emerging peaks “h” and “i”).

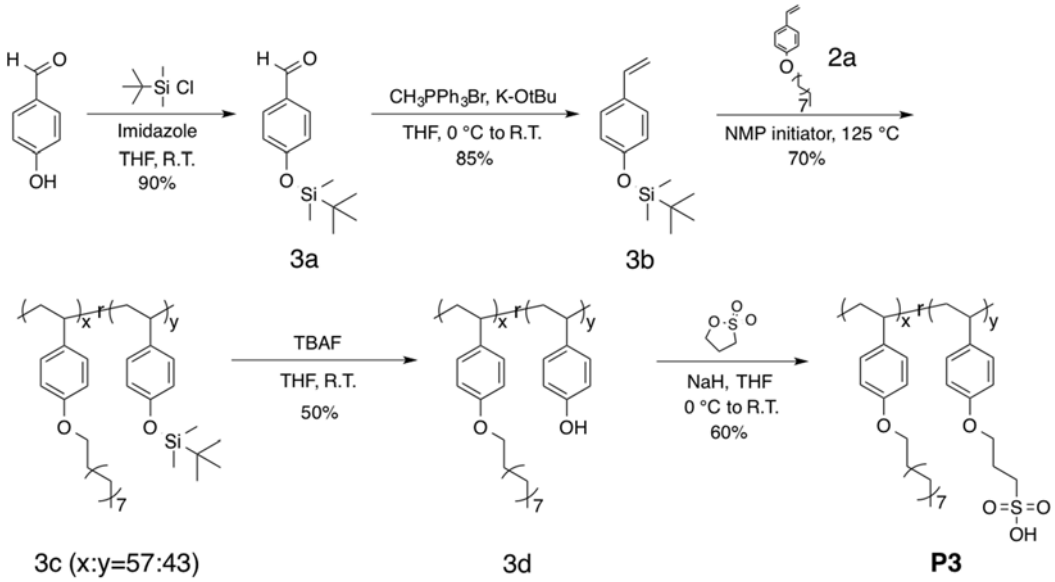


Synthesis of polymers **P2**:

Polymer **2c** was dissolved in 3 mL of DCM in a round bottom flask and stirred in an ice bath for 15 min. Bromotrimethylsilane (0.17 mL, 1.54 mmol) was slowly added to the solution. The reaction mixture was further stirred for 12 h. After the reaction, the solvent and excess Bromotrimethylsilane was evaporated to obtain dark yellow solids. 3 mL DCM was added to re-dissolve the compounds and 1M HCl aqueous solution (1 mL) was added. The reaction mixture was stirred at room temperature for 1 hour. After the reaction, DCM was evaporated and water was removed, and the residues are lyophilized to obtain 220 mg of the final polymer **P2**. The disappearance of peaks "h" and "i" in the precursor indicates that the ethyl groups were deprotected. ^{1}H NMR (400 MHz, $CDCl_3$) was shown below.



Synthesis of random co-polymer P3



Synthesis of compound **3a**:

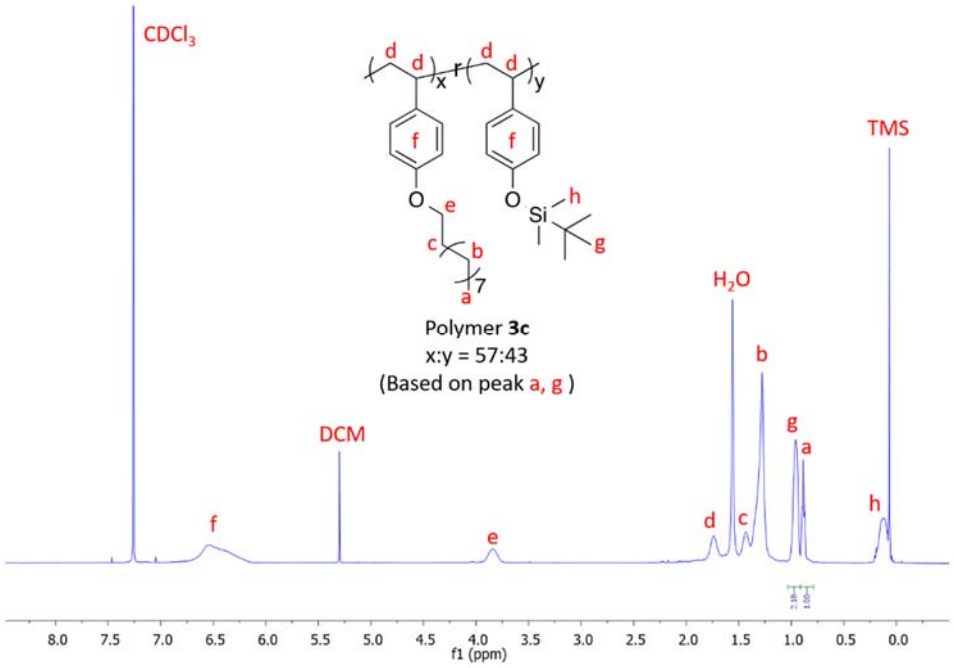
To a solution of THF mixed with 4-Hydroxybenzaldehyde (5.16 g, 42.22 mmol) and Imidazole (4.02 g, 59.11 mmol), tert-Butyldimethylsilyl chloride (8.91 g, 59.11 mmol) was added and stirred for 12 hours at room temperature. After the reaction, NaCl saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline 3 times. The solvent was evaporated to dryness and purified by silica gel column chromatography (2-3% ethyl acetate in hexanes) to obtain 9.0 g (90% yield) of **3a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.89 (s, 1H), δ 7.77-7.79 (d, 2H), δ 6.93-6.94 (d, 2H), δ 0.98 (s, 9H), δ 0.24 (s, 6H). ESI-MS (expected: $[m+H]^+$= 237.1, obtained: $[m+Na]^+$= 259.1)

Synthesis of compound **3b**:

Methyltriphenylphosphonium bromide (12.41 g, 34.75 mmol) and Potassium tert-butoxide (3.90 g, 34.75 mmol) were mixed in a round bottom flask, and dry THF (20 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield the bright yellow solution. **3a** (5.47 g, 23.17 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline 3 times. The organic layer was evaporated to dryness and purified by silica gel column chromatography (0.5% ethyl acetate in hexanes) to afford 4.6 g (85% yield) of **3b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.28-7.29 (d, 2H), δ 6.78-6.80 (d, 2H), δ 6.62-6.68 (q, 1H), δ 5.59-5.62 (d, 1H), δ 5.11-5.13 (d, 1H), δ 0.98 (s, 9H), δ 0.20 (s, 6H).

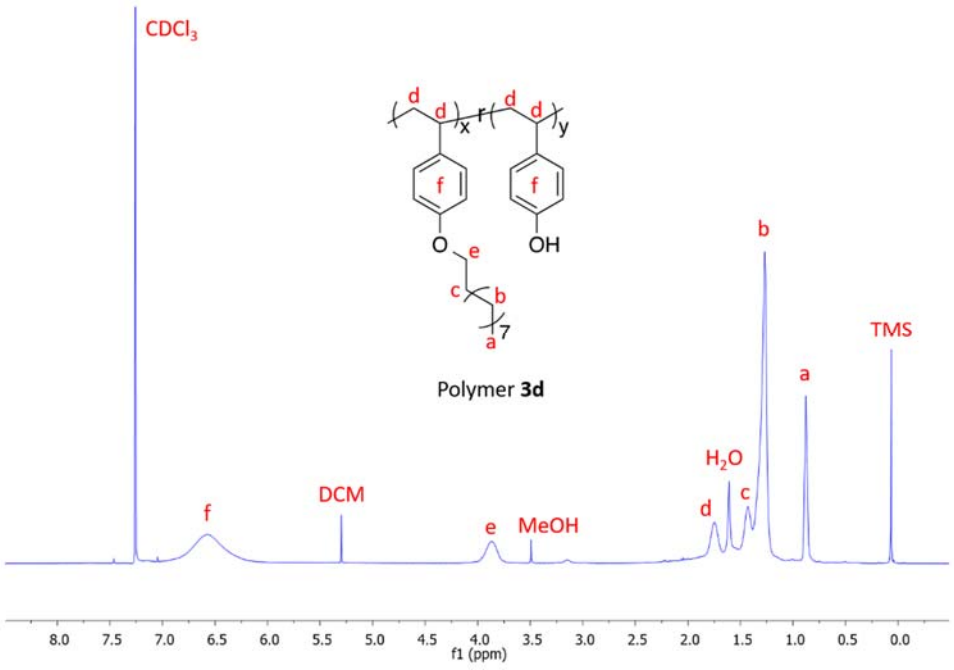
Synthesis of random co-polymer **3c**:

A mixture of the compound **3b** (269 mg, 1.15 mmol), **2a** (300 mg, 1.15 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 15 mg, 0.046 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 °C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 430 mg (75% yield) of **3c**. GPC (PMMA/THF): M_n= 12K Da, Đ= 1.1. ^{1}H NMR (400MHz, $CDCl_3$) was shown below.



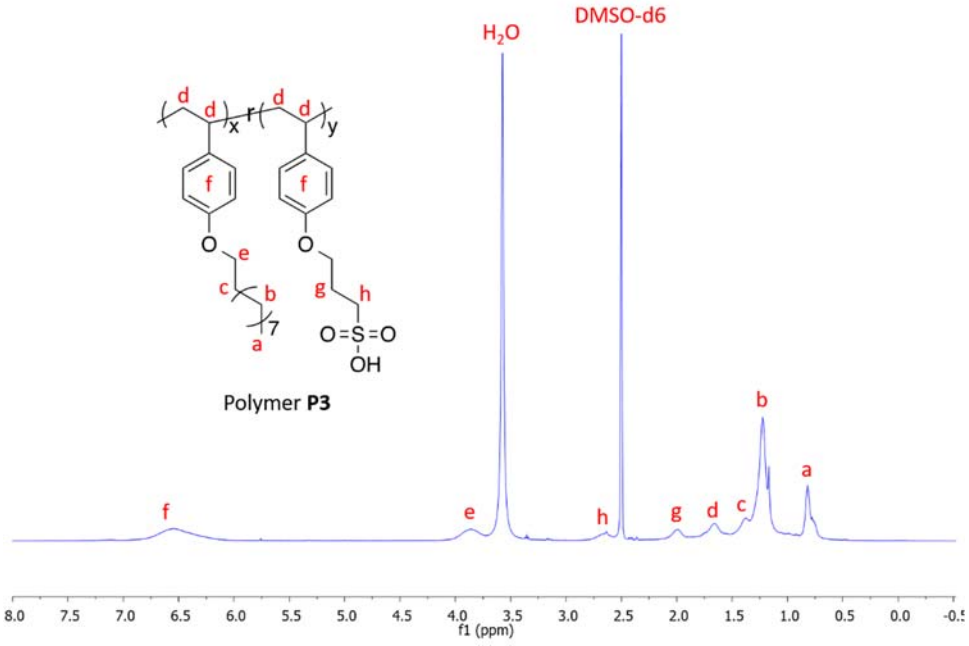
Synthesis of random co-polymer **3d**:

THF (3 mL) was added to dissolve the dried random co-polymer **3c**. Tetrabutylammonium fluoride (5.75 mL, 1M in THF) was added to the reaction in an ice bath, and stirred for 12 h. The reaction mixture was evaporated and re-dissolved with minimal amount of DCM. Then MeOH was used to precipitate polymers 3 times. The product was collected and dried under vacuum to yield 200 mg (50% yield) of **3d**. The disappreance of peaks "h" and "g" in the precursor indicates that the TBS was deprotected. ^{1}H NMR (400MHz, $CDCl_3$) was shown below.



Synthesis of random co-polymer **P3**:

THF (5 mL) was added to dissolve the dried random co-polymer **3d**. Sodium hydride (0.13 g, 5.75 mmol) was added to the reaction in an ice bath, and stirred for 15 min. The reaction mixture was then added the 1,3-Propanesultone (0.70 g, 5.75 mmol) and stirred for 12 hours. After the reaction, 2 mL of H_2O was added dropwise to the reaction to quench the NaH in the mixture in an ice bath. Then mixture was dried, and the water was removed. The dried sample was dialyzed against DCM/MeOH (v/v= 6/1) for 1 day. The solution was evaporated and dried under vacuum to obtain 130 mg of the final product **P3**. ^{1}H NMR (400MHz, $CDCl_3$) was shown below.



Extraction for the peptides mixture

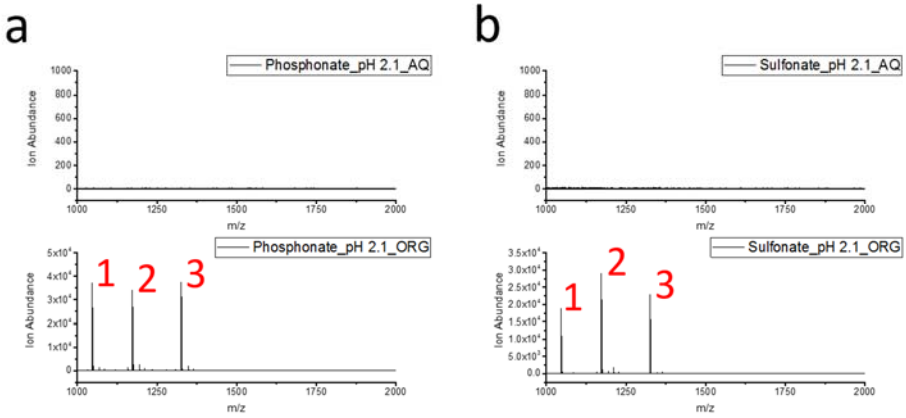


Figure S1. (a) MALDI mass spectrum of aqueous phase (AQ) and organic phase (ORG) after extraction using reverse micelles of polymer **P2** at pH 2.1. (b) MALDI mass spectrum of aqueous phase (AQ) and organic phase (ORG) after extraction using reverse micelles of polymer **P3** at pH 2.1.

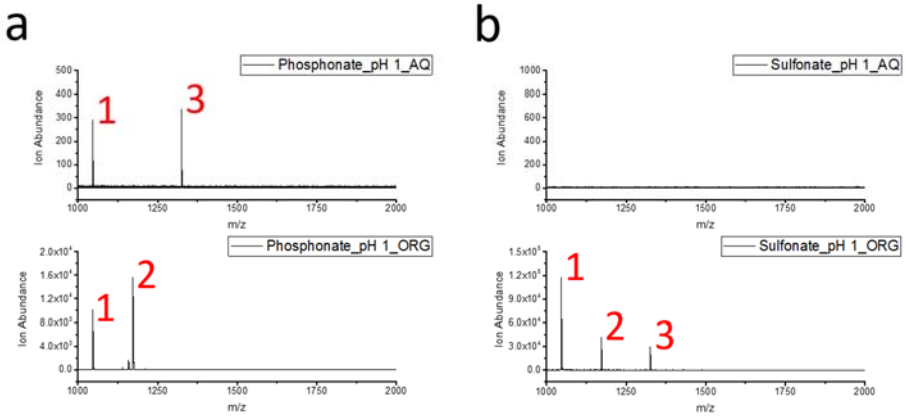


Figure S2. (a) MALDI mass spectrum of aqueous phase (AQ) and organic phase (ORG) after extraction using reverse micelles of polymer **P2** at pH 1.0. (b) MALDI mass spectrum of aqueous phase (AQ) and organic phase (ORG) after extraction using reverse micelles of polymer **P3** at pH 1.0.

Determination of extraction capacity

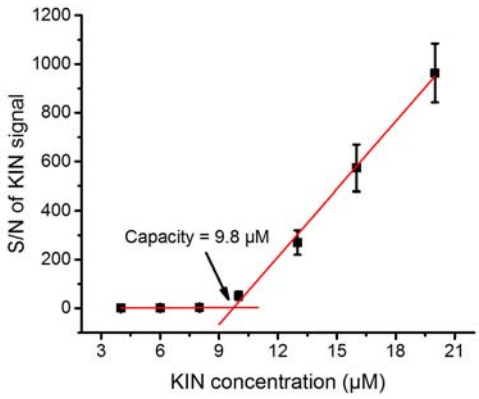


Figure S3. Example of kinetensin (KIN) peptide extraction capacity measurement using reverse micelles of polymer **P1** at pH 5.4. Signal-to-noise (S/N) is used as a measure of peptide signal to account for well-known spot-to-spot variations in the noise levels in MALDI-MS measurements. In addition, we used a S/N ratio of > 3 to confirm that a peptide ion was truly measured. Linear regression fitting was used to obtain the capacity.

Stability of reverse micelles

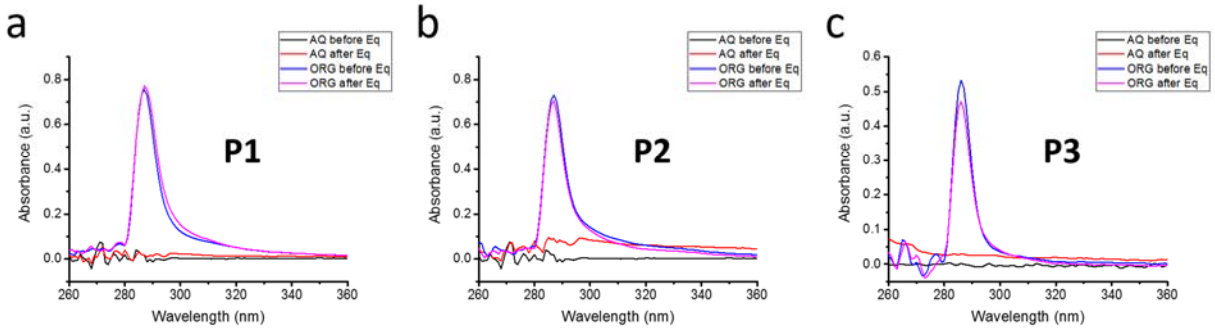


Figure S4. UV-Vis absorption measurements with reverse micelles starting in toluene (ORG), before and after equilibration (Eq) with an aqueous Tris buffer (AQ). (a) **P1** at pH 8.7. (b) **P2** at pH 8.7. (c) **P3** at pH 9.8.

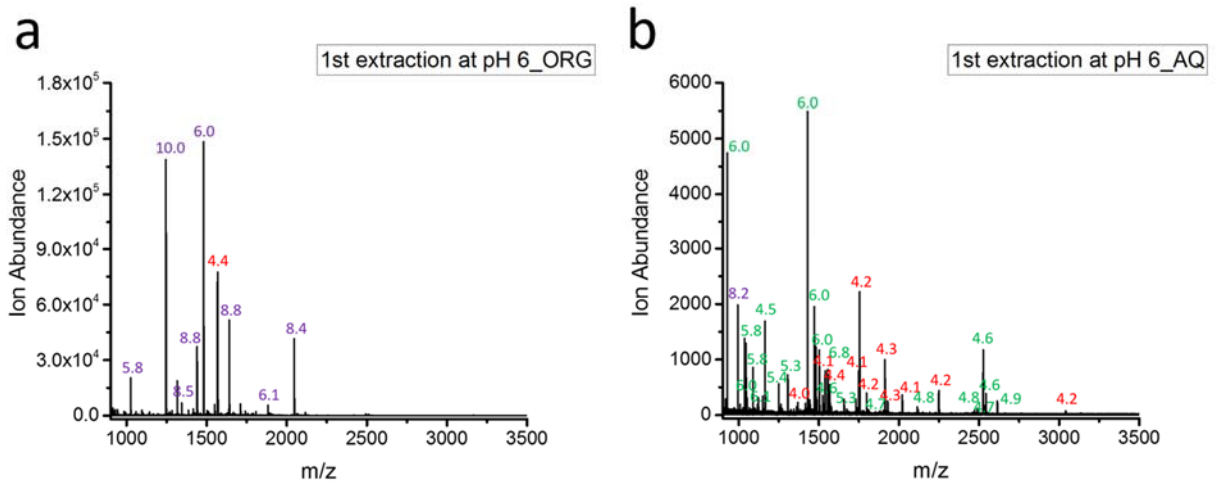


Figure S5. The MALDI spectra of (a) the organic phase after the first extraction using 400 μL of 2.0×10^{-5} M of **P3** at pH 6. (b) the remaining aqueous phase after the first extraction using **P3** at pH 6. (The number above the peaks correspond to the calculated peptide pI values.)

Details of peptides in the MALDI spectra

Table S1. Detailed information of peptides in Figure 5a.

m/z	Sequence	Modification	Protein	pI
927.89	YLYEIAR		BSA	6.0
1026.04	WWCNDGR	1Carbamidomethyl 2Oxidation	Lyz	5.8
1036.83	CELAAAMKR	1Acetyl	Lyz	8.2
1045.98	GTDVQAWIR		Lyz	5.8
1051.83	QNCDQFEK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.4
1089.00	GTDVQAWIR	1Acetyl	Lyz	5.8
1121.93	CCTESLVNR	1Carbamidomethyl 1Pyro-carbamidomethyl	BSA	6.0
1149.92	CCTKPESER	1Carbamidomethyl 1Pyro-carbamidomethyl	BSA	6.1
1164.11	LVNELTEFAK		BSA	4.5
1246.24	IQRTPKIQVY		b2m	10.0
1250.12	FKDLGEEHFK		BSA	5.5
1306.24	HLVDEPQNLIK		BSA	5.3
1360.16	TEFTPTEKDEY		b2m	4.0
1400.25	TVMENFVAFVDK		BSA	4.4
1416.25	TVMENFVAFVDK	1Oxidation	BSA	4.4
1420.25	SLHTLFGDELCK	1Carbamidomethyl	BSA	5.3
1429.23	FESNFNTQATNR		Lyz	6.0
1438.22	ETYGDMADCCEK	1Carbamidomethyl	BSA	3.9

		1Oxidation		
1440.38	RHPEYAVSVLLR		BSA	8.8
1472.25	FESNFNTQATNR	Acetyl	Lyz	6.0
1479.11	ETYGDMADCCEK	1Carbamidomethyl 1Oxidation	BSA	3.9
1480.39	LGEYGFQNALIVR		BSA	6.0
1486.26	FESNFNTQATNR	Carbamidomethyl	Lyz	6.0
1503.21	EYEATLEECCAK	2Carbamidomethyl	BSA	4.1
1523.29	YTEFTPTEKDEY		b2m	4.0
1533.37	LKECCDKPLLEK	2Carbamidomethyl	BSA	6.2
1538.40	LCVLHEKTPVSEK	1Carbamidomethyl	BSA	6.8
1555.27	DDPHACYSTVFDK	1Carbamidomethyl	BSA	4.4
1568.36	DAFLGSFLYEYSR		BSA	4.4
1579.34	ECCHGDLLECADDR		BSA	4.1
1629.44	YICDNQDTISSKLK		BSA	6.0
1640.59	KVPQVSTPTLVEVSR		BSA	8.8
1661.44	NRCKGTDVQAWIR	1Carbamidomethyl 1Oxidation	Lyz	9.5
1676.45	IVSDGNGMNAWVAWR		Lyz	5.8
1708.46	IVSDGNGMNAWVAWR	2Oxidation	Lyz	5.8
1725.50	MPCTEDYLSLILNR	1Carbamidomethyl	BSA	4.4
1731.35	ECCHGDLLECADDR	3Carbamidomethyl 1Glu->pyro-Glu	BSA	4.1
1741.49	MPCTEDYLSLILNR	1Carbamidomethyl 1Oxidation	BSA	4.4
1748.38	YNGVFQECCQAEDK	2Carbamidomethyl	BSA	4.1
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
1797.53	CCAADDKEACFAVEGPK	1Pyro-carbamidomethyl	BSA	4.3
1881.65	RPCFSALTPDETYVPK	1Carbamidomethyl	BSA	6.1
1902.62	NECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.7
1908.66	LFTFHADICTLPDTEK	1Carbamidomethyl	BSA	4.5
1911.52	CCAADDKEACFAVEGPK	2Carbamidomethyl 1Pyro-carbamidomethyl	BSA	4.3
2020.74	ETYGDMADCCEKQEPER / LKPDPNTLCDEFKADEK / VASLRETYGDMADCCEK	1Oxidation / 1Carbamidomethyl / 2Carbamidomethyl 1Oxidation	BSA	4.1 / 4.4 / 4.3
2045.82	RHPYFYAPELLYYANK		BSA	8.4
2248.79	ECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.2
2471.01	RPCFSALTPDETYVPKAFDEK	1Carbamidomethyl	BSA	4.8
2488.04	YNGVFQECCQAEDKGACLLPK	3Carbamidomethyl	BSA	4.7
2525.10	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.6
2542.13	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.6
2613.15	VHKECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.9
3039.38	EYEATLEECCAKDDPHACYSTVFDK	3Carbamidomethyl	BSA	4.2

Table S2. Detailed information of peptides in Figure 5b.

m/z	Sequence	Modification	Protein	pI
927.89	YLYEIAR		BSA	6.0
993.81	WWCNDGR	1Carbamidomethyl	Lyz	5.8
1246.24	IQRTPKIQVY		b2m	10.0
1365.18	ETYGDMADCCEK		BSA	3.9
1429.23	FESNFNTQATNR		Lyz	6.0
1472.25	FESNFNTQATNR	Acetyl	Lyz	6.0
1479.11	ETYGDMADCCEK	1Carbamidomethyl 1Oxidation	BSA	3.9
1503.21	EYEATLEECCAK	2Carbamidomethyl	BSA	4.1
1523.29	YTEFTPTEKDEY		b2m	4.0
1533.37	LKECCDKPLLEK	2Carbamidomethyl	BSA	6.2
1629.44	YICDNQDTISSKLK		BSA	6.0
1640.59	KVPQVSTPTLVEVSR		BSA	8.8
1657.39	QEPERNECFLSHK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	5.5
1676.45	IVSDGNGMNAWVAWR		Lyz	5.8
1731.35	ECCHGDLLECADDR	3Carbamidomethyl 1Glu->pyro-Glu	BSA	4.1
1748.38	YNGVFQECCQAEDK	2Carbamidomethyl	BSA	4.1
1751.63	LSQKFPKAEFVEVTK		BSA	8.5
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
1797.53	CCAADDKEACFAVEGPK	1Pyro-carbamidomethyl	BSA	4.3
1881.65	RPCFSALTPDETYVPK	1Carbamidomethyl	BSA	6.1
1902.62	NECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.7
1911.52	CCAADDKEACFAVEGPK	2Carbamidomethyl 1Pyro-carbamidomethyl	BSA	4.3
1928.54	CCAADDKEACFAVEGPK	3Carbamidomethyl	BSA	4.3
2020.74	ETYGDMADCCEKQEPER / LKPDPNTLCDEFKADEK / VASLRETYGDMADCCEK	1Oxidation / 1Carbamidomethyl / 2Carbamidomethyl 1Oxidation	BSA	4.1 / 4.4 / 4.3
2045.82	RHPYFYAPELLYYANK		BSA	8.4
2114.70	VHKECCHGDLLECADDR	3Carbamidomethyl	BSA	4.8
2118.66	ETYGDMADCCEKQEPER	2Carbamidomethyl	BSA	4.1
2248.79	ECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.2
2525.10	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.6
2542.13	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.6
2613.15	VHKECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.9

Table S3. Detailed information of peptides in Figure 5c.

m/z	Sequence	Modification	Protein	pI
927.89	YLYEIAR		BSA	6.0
993.81	WWCNDGR	1Carbamidomethyl	Lyz	5.8
1026.04	WWCNDGR	1Carbamidomethyl 2Oxidation	Lyz	5.8
1036.83	CELAAAMKR	1Acetyl	Lyz	8.2
1045.98	GTDVQAWIR		Lyz	5.8
1051.83	QNCDQFEK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.4
1246.24	IQRTPKIQVY		b2m	10.0
1429.23	FESNFNTQATNR		Lyz	6.0
1472.25	FESNFNTQATNR	Acetyl	Lyz	6.0
1479.11	ETYGDMADCCEK	1Carbamidomethyl 1Oxidation	BSA	3.9
1503.21	EYEATLEECCAK	2Carbamidomethyl	BSA	4.1
1523.29	YTEFTPTEKDEY		b2m	4.0
1555.27	DDPHACYSTVFDK	1Carbamidomethyl	BSA	4.4
1640.59	KVPQVSTPTLVEVSR		BSA	8.8
1657.39	QEPERNECFLSHK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	5.5
1731.35	ECCHGDLLECADDR	3Carbamidomethyl 1Glu->pyro-Glu	BSA	4.1
1748.38	YNGVFQECCQAEDK	2Carbamidomethyl	BSA	4.1
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
1797.53	CCAADDKEACFAVEGPK	1Pyro-carbamidomethyl	BSA	4.3
1881.65	RPCFSALTPDETYVPK	1Carbamidomethyl	BSA	6.1
1911.52	CCAADDKEACFAVEGPK	2Carbamidomethyl 1Pyro-carbamidomethyl	BSA	4.3
1928.54	CCAADDKEACFAVEGPK	3Carbamidomethyl	BSA	4.3
2020.74	ETYGDMADCCEKQEPER / LKPDPNTLCDEFKADEK / VASLRETYGDMADCCEK	1Oxidation / 1Carbamidomethyl / 2Carbamidomethyl 1Oxidation	BSA	4.1 / 4.4 / 4.3
2118.66	ETYGDMADCCEKQEPER	2Carbamidomethyl	BSA	4.1
2248.79	ECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.2
2525.10	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.6
2542.13	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.6

Table S4. Detailed information of peptides in Figure 5d.

m/z	Sequence	Modification	Protein	pI
927.89	YLYEIAR		BSA	6.0
993.81	WWCNDGR	1Carbamidomethyl	Lyz	5.8

1365.18	ETYGDMADCCEK		BSA	3.9
1429.23	FESNFNTQATNR		Lyz	6.0
1479.11	ETYGDMADCCEK	2Carbamidomethyl	BSA	3.9
1503.21	EYEATLEECCAK	2Carbamidomethyl	BSA	4.1
1555.27	DDPHACYSTVFDK	1Carbamidomethyl	BSA	4.4
1731.35	ECCHGDLLECADDR	3Carbamidomethyl 1Glu->pyro-Glu	BSA	4.1
1748.38	YNGVFQECCQAEDK	2Carbamidomethyl	BSA	4.1
1750.38	ECCHGDLLECADDR	3Carbamidomethyl	BSA	4.1
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
1911.52	CCAADDKEACFAVEGPK	2Carbamidomethyl 1Pyro-carbamidomethyl	BSA	4.3
1928.54	CCAADDKEACFAVEGPK	3Carbamidomethyl	BSA	4.3
2117.78	ETYGDMADCCEKQEPER	2Carbamidomethyl	BSA	4.1
2248.79	ECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.2
2525.10	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.6

Table S5. Detailed information of peptides in Figure S5a.

m/z	Sequence	Modification	Protein	pI
1026.04	WWCNDGR	1Carbamidomethyl 2Oxidation	Lyz	5.8
1246.24	IQRTPKIQVY		b2m	10.0
1344.19	SRHPAENGKSNF		b2m	8.5
1440.38	RHPEYAVSVLLR		BSA	8.8
1480.39	LGEYGFQNALIVR		BSA	6.0
1568.36	DAFLGSFLYEYSR		BSA	4.4
1640.59	KVPQVSTPTLVEVSR		BSA	8.8
1881.65	RPCFSALTPDETYVPK	1Carbamidomethyl	BSA	6.1
2045.82	RHPYFYAPELLYYANK		BSA	8.4

Table S6. Detailed information of peptides in Figure S5b. Peptides with asterisks are peptides that were not detected in the original digests mixtures.

m/z	Sequence	Modification	Protein	pI
927.89	YLYEIAR		BSA	6.0
1036.83	CELAAAMKR	1Acetyl	Lyz	8.2
1045.98	GTDVQAWIR		Lyz	5.8
1051.83	QNCDQFEK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.4
1089.00	GTDVQAWIR	1Acetyl	Lyz	5.8
1121.93	CCTESLVNR	1Carbamidomethyl 1Pyro-carbamidomethyl	BSA	6.0
1149.92	CCTKPESER	1Carbamidomethyl	BSA	6.1

		1Pyro-carbamidomethyl		
1164.11	LVNELTEFAK		BSA	4.5
1250.12	FKDLGEEHFK		BSA	5.5
1306.24	HLVDEPQNLIK		BSA	5.3
1360.16	TEFTPTEKDEY		b2m	4.0
1400.25	TVMENFVAFVDK		BSA	4.4
1416.25	TVMENFVAFVDK	1Oxidation	BSA	4.4
1429.23	FESNFNTQATNR		Lyz	6.0
1438.22	ETYGDMADCCEK	1Carbamidomethyl 1Oxidation	BSA	3.9
1440.38	RHPEYAVSVLLR		BSA	8.8
*1444.22	YICDNQDTISSK	1Carbamidomethyl	BSA	4.2
*1464.18	TCVADESHAGCEK	2Carbamidomethyl	BSA	4.7
1472.25	FESNFNTQATNR	Acetyl	Lyz	6.0
1479.11	ETYGDMADCCEK	1Carbamidomethyl 1Oxidation	BSA	3.9
1480.39	LGEYGFQNALIVR		BSA	6.0
1486.26	FESNFNTQATNR	Carbamidomethyl	Lyz	6.0
1503.21	EYEATLEECCAK	2Carbamidomethyl	BSA	4.1
1523.29	YTEFTPTEKDEY		b2m	4.0
1533.37	LKECCDKPLLEK	2Carbamidomethyl	BSA	6.2
1538.40	LCVLHEKTPVSEK	1Carbamidomethyl	BSA	6.8
1555.27	DDPHACYSTVFDK	1Carbamidomethyl	BSA	4.4
1568.36	DAFLGSFLYEYSR		BSA	4.4
*1577.40	LKPDPNTLCDEFK	1Carbamidomethyl	BSA	4.6
*1674.45	ECCHGDLLECADDR	2Carbamidomethyl 1Glu->pyro-Glu	BSA	4.1
*1739.58	DDPHACYSTVFDKLK		BSA	5.3
1748.38	YNGVFQECCQAEDK	2Carbamidomethyl	BSA	4.1
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
1797.53	CCAADDKEACFAVEGPK	1Pyro-carbamidomethyl	BSA	4.3
1902.62	NECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.7
1911.52	CCAADDKEACFAVEGPK	2Carbamidomethyl 1Pyro-carbamidomethyl	BSA	4.3
*1928.53	CCAADDKEACFAVEGPK	3Carbamidomethyl	BSA	4.3
2020.74	ETYGDMADCCEKQEPER / LKPDPNTLCDEFKADEK / VASLRETYGDMADCCEK	1Oxidation / 1Carbamidomethyl / 2Carbamidomethyl 1Oxidation	BSA	4.1 / 4.4 / 4.3
*2114.72	VHKECCHGDLLECADDR	3Carbamidomethyl	BSA	4.8
2248.79	ECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.2
2471.01	RPCFSALTPDETYVPKAFDEK	1Carbamidomethyl	BSA	4.8
2488.04	YNGVFQECCQAEDKGACLLPK	3Carbamidomethyl	BSA	4.7
2525.10	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.6

2542.13	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl	BSA	4.6
2613.15	VHKECCHGDLLECADDRADLAK	3Carbamidomethyl	BSA	4.9
3039.38	EYEATLEECCAKDDPHACYSTVFDK	3Carbamidomethyl	BSA	4.2

Table S7. Detailed information of peptides in Figure 7a. Peptides with asterisks are peptides that were not detected in the original digests mixtures.

m/z	Sequence	Modification	Protein	pI
1045.98	GTDVQAWIR		Lyz	5.8
*1077.98	GTDVQAWIR	2Oxidation	Lyz	5.8
1164.11	LVNELTEFAK		BSA	4.5
*1202.08	KNGERIEKVE		b2m	6.2
1250.12	FKDLGEEHFK		BSA	5.5
1306.24	HLVDEPQNLIK		BSA	5.3
*1344.19	SRHPAENGKSNF		b2m	
1429.23	FESNFNTQATNR		Lyz	6.0
1472.25	FESNFNTQATNR	Acetyl	Lyz	6.0
1480.39	LGEYGFQNALIVR		BSA	6.0
1538.40	LCVLHEKTPVSEK	1Carbamidomethyl	BSA	6.8
1568.36	DAFLGSFLYEYSR		BSA	4.4
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
*1824.59	RPCFSALTPDETYVPK		BSA	6.1
2525.10	QEPERNECFLSHKDDSPDLPK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.6

Table S8. Detailed information of peptides in Figure 7b. Peptides with asterisks are peptides that were not detected in the original digests mixtures.

m/z	Sequence	Modification	Protein	pI
927.89	YLYEIAR		BSA	6.0
1036.83	CELAAAMKR	1Acetyl	Lyz	8.2
1045.98	GTDVQAWIR		Lyz	5.8
1051.83	QNCDQFEK	1Carbamidomethyl 1Gln->pyro-Glu	BSA	4.4
1089.00	GTDVQAWIR	1Acetyl	Lyz	5.8
1121.93	CCTESLVNR	1Carbamidomethyl 1Pyro-carbamidomethyl	BSA	6.0
1149.92	CCTKPESER	1Carbamidomethyl 1Pyro-carbamidomethyl	BSA	6.1
1164.11	LVNELTEFAK		BSA	4.5
1306.24	HLVDEPQNLIK		BSA	5.3
1360.16	TEFTPTEKDEY		b2m	4.0
*1421.08	ETYGDMADCCEK	1Carbamidomethyl	BSA	3.9
1429.23	FESNFNTQATNR		Lyz	6.0
*1444.22	YICDNQDTISSK	1Carbamidomethyl	BSA	4.2
*1464.18	TCVADESHAGCEK	2Carbamidomethyl	BSA	4.7

1472.25	FESNFNTQATNR	Acetyl	Lyz	6.0
1479.11	ETYGDMADCCEK	1Carbamidomethyl 1Oxidation	BSA	3.9
1486.26	FESNFNTQATNR	Carbamidomethyl	Lyz	6.0
1503.21	EYEATLEECCAK	2Carbamidomethyl	BSA	4.1
1523.29	YTEFTPTEKDEY		b2m	4.0
*1541.18	LCVLHEKTPVSEK	1Carbamidomethyl	BSA	6.8
1555.27	DDPHACYSTVFDK	1Carbamidomethyl	BSA	4.4
1731.35	ECCHGDLLECADDR	3Carbamidomethyl 1Glu->pyro-Glu	BSA	4.1
1748.38	YNGVFQECCQAEDK	2Carbamidomethyl	BSA	4.1
1754.52	NTDGSTDYGILQINSR		Lyz	4.2
*1786.37	NTDGSTDYGILQINSR	2Oxidation	Lyz	4.2
1797.53	CCAADDKEACFAVEGPK	1Pyro-carbamidomethyl	BSA	4.3
1911.52	CCAADDKEACFAVEGPK	2Carbamidomethyl 1Pyro-carbamidomethyl	BSA	4.3
*1928.53	CCAADDKEACFAVEGPK	3Carbamidomethyl	BSA	4.3
*1949.49	VASLRETYGDMADCCEK	1Carbamidomethyl	BSA	4.3

Reference

[1] B. Zhao, J. Zhuang, M. A. C. Serrano, R. W. Vachet and S. Thayumanavan, *Macromolecules*, 2017, **50**, 9734-9741.