Supporting Information for:

Polymer-Mediated Ternary Supramolecular Interactions for Sensitive Detection of Peptides

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EXPERIMENTAL METHODS

Reagents. The peptide bradykinin (RPPGFSPFR) was purchased from the Sigma-Aldrich. TMR-bradykinin (TAMRA dye conjugated at the N-terminal of the peptide) was obtained from Biopeptek Inc. The following peptides were purchased from the American Peptide Company: kinetensin (IARRHPYFL), malantide (RTKRSGSVYEPLKI), β-amyloid fragment 1-11 (DAEFRHDSGYE), preproenkephalin (SSEVAGEGDGDSMGHEDLY). Trifluoroacetic acid (TFA), α-cyano-4-hydroxy-cinnamic acid (CHCA) and α-cyano-4-chlorocinnamic acid (CClCA) were purchased from Sigma-Aldrich, while α-cyanocinnamic acid (CCA) was from Alfa Aesar. Tris(hydroxymethyl)aminomethane (Tris buffer), hydrochloric acid (HCl), ammonium hydroxide (NH_4OH), toluene, tetrahydrofuran (THF) and purified water were obtained from Fisher Scientific. THF was distilled over Na/Ph_2CO before use.

Preparation of Polymeric Reverse Micelles. To form reverse micellar assemblies, polymers were dissolved in toluene (0.5 mg/mL) and sonicated until a clear solution was obtained. Two equivalents of water per carboxylate moiety was added to the solution and sonicated again until the solution becomes optically clear.

Matrix Solutions. CHCA, CCA, and CClCA were all prepared at a concentration of 0.16 M in 350 μL THF:150 μL H_2O:6 μL TFA.

Peptide Extraction and Sample Preparation. Peptide solutions were prepared in 50 mM Tris buffer at the indicated pH. Extractions were done by adding 200 μL of the polymeric reverse micelle solution to 1 mL of the buffered peptide solution, mixing them by vortex for 2 hours, and separating the two phases by centrifugation at 12,500 rpm for 60 minutes. The aqueous phase was carefully drawn out and transferred to a separate tube. The remaining organic phase was dried under a stream of N_2 gas. The dried residue consisting of the polymer and the extracted peptides was reconstituted in 10 μL of THF and then 20 μL of the matrix solution. From this mixture, 1 μL was spotted on the MALDI target plate for MALDI-MS analysis or on indium tin oxide (ITO)-coated glass slides for fluorescence microscopy. Unextracted samples used for comparison were prepared such that they contained the same number of moles of the peptide and the matrix per μL spot, taking into consideration that the extraction process imparts a 100-fold enrichment (1 mL is reconstituted to 10 μL before addition of the matrix).

Fluorescence Microscopy. Fluorescence images were obtained using an Olympus FluoView FV1000 confocal fluorescence microscope. One microliter of sample spotted on ITO-coated glass slides was allowed to dry, and visualized under 10x magnification. The Cy3 channel (λ_{ex}=559 nm, λ_{em}=567 nm) and the ECFP channel (λ_{ex}=405 nm and λ_{em}=476 nm) were used to image TMR-bradykinin and CHCA matrix,

respectively. No TMR-bradykinin was visible using the ECFP channel and no CHCA was visible in the Cy3 channel. Images obtained were 1024 x 1024 pixels in size with a scale of 0.478 μm per pixel. Quantitative fluorescence intensity measurements were done using *ImageJ* software.

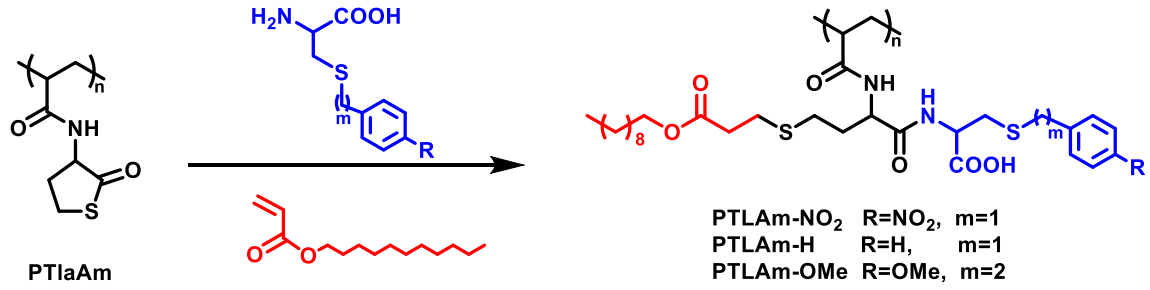
MALDI-MS Analysis. A Bruker Autoflex III time-of-flight mass spectrometer was used for the MALDI-MS analysis of all samples. Acquisition of all mass spectra was done in reflectron mode with an accelerating voltage of 19 kV. Each spectrum is the average of 300 laser shots at 25% laser power.

POLYMER SYNTHESIS AND CHARACTERIZATION

General methods: All reagents were commercially available and used as received unless stated otherwise. ^{1}H-NMR spectra were recorded on a 400 MHz or 500MHz NMR spectrometer using residual proton resonance of the solvents as internal standard. Chemical shifts are reported in parts per million (ppm). Mass spectra were obtained at the Molecular Weight Characterization facility at University of Massachusetts Amherst. The molecular weight of the polymers (1mg/mL) were determined by size exclusion chromatography on a single inject mode GPC using THF as eluent and 1μL of toluene was added as the internal reference. Poly(methylmethacrylate) (PMMA) standards were used for calibration and output was received and analyzed using a refractive index detector.

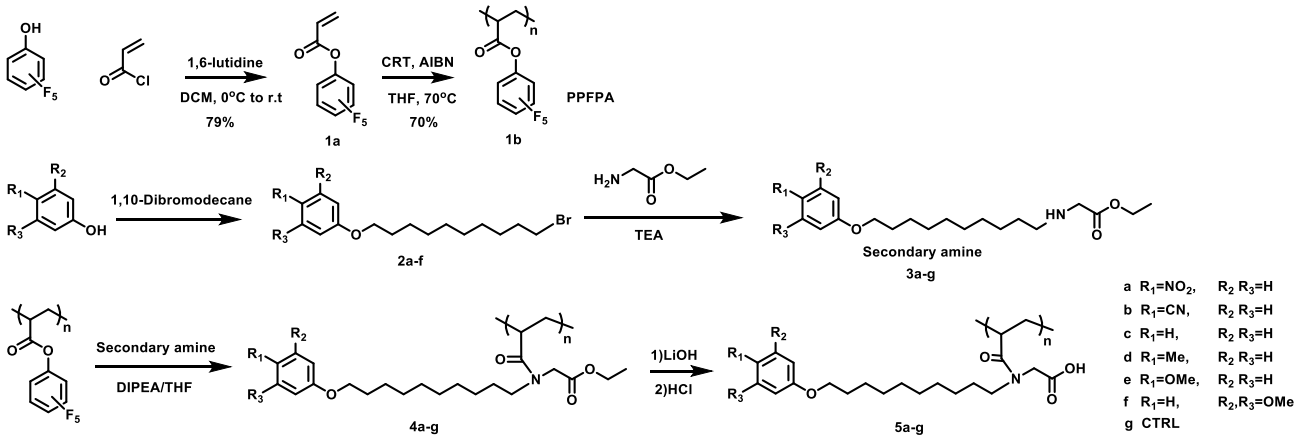
Synthesis of poly(dialkoxystyrene) (PDAS) amphiphilic polymer
The **PDAS** carboxylate polymer was synthesized as previously described elsewhere[1].

Synthesis of polythiolactone amide (PTLAm)-based amphiphilic polymers



The PTLAm polymer synthesis was initiated by RAFT polymerization of thiolactone acrylamide[2], followed by thiolactone ring opening with different amines (blue) and reaction with decyl acrylate (red) in a one pot reaction in DCM/MeOH solvent. GPC of precursor polymer PTlaAm: M_n=14.4K, M_w=18.5K, Đ=1.28. ^{1}H-NMR (500 MHz, Chloroform-d) δ 4.92 (br s, 1H), 3.27 (br s, 2H), 2.84 – 1.11 (m, 5H). Due to aggregation issues, NMR for final product is not available. IR spectrum peaks around 1699cm^{-1} belongs to the C=O of thiolactone disappeared after conjugation showed the completion of modification reaction.

Synthesis of polyacrylamide (PAm)-based amphiphilic polymers:



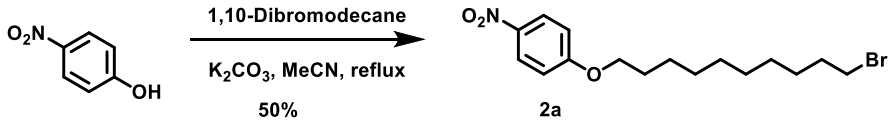
Synthesis of compound **1a**

The pentafluorophenylacrylate monomer (PFPA) **1a** was synthesized following the published paper[3] using acryloyl chloride and pentafluorophenol in the presence of 1,6-lutidine as the base.

Synthesis of polymer **1b**

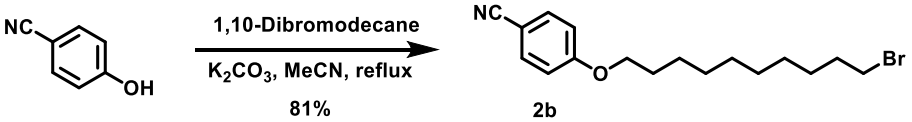
The precursor polymer PPFPA was synthesized by following the reported literature[4]. The PFPA monomer (1.0 g, 4.2 mmol) was added to a 10-mL Schlenk flask containing dry THF (1 mL), chain transfer agent cyanomethyl dodecyl trithiocarbonate (14 mg, 0.044 mmol), and AIBN (1.7 mg, 0.11 mmol). The resulting reaction mixture was degassed using three freeze-pump-thaw cycles for 10 minutes. This reaction mixture is stirred at 70 ^{o}C for 20 hours and then precipitated in MeOH to obtain the product as a white powder (60% yield). GPC (PMMA/THF): M_n=13K, M_w=18.8K, Đ=1.28. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 3.20-3.00 (br s, 1H), 2.20-1.90 (br s, 2H). ^{19}F-NMR (500 MHz; $CDCl_3$): 153.04 (s, 2F), 156.54 (s, 1F), -161.90 (s, 2F).

Synthesis of compound **2a**



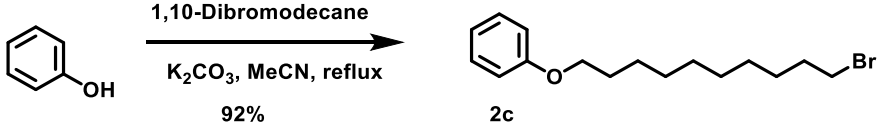
To a solution of acetonitrile (MeCN) mixed with K_2CO_3 (5.45 g, 39.49 mmol) and 18-crown-6 (0.52 g, 1.973 mmol), a solution of 4-nitrophenol (2.74 g, 19.73 mmol) was added. 1,10-dibromodecane (12.0 g, 39.47 mmol) in 150 mL was added via a funnel within 1hr. Then the system was heated under reflux for 24 h with a $CaCl_2$ guard. The reaction mixture was allowed to cool to room temperature and then was concentrated in vacuo (20 mL). Water (100 mL) was added and the aqueous layer was extracted thrice with 50-mL portions of ethyl acetate (EtOAc). The combined organic layers were washed with brine (20 mL), dried with $MgSO_4$, filtered and the solvent was removed in vacuo, yielding a white solid as the crude product. The solid was dissolved in minimal amounts of CH_2Cl_2 and absorbed onto silica followed by purification via silica gel column chromatography (gradient elution: 0 to 30% EtOAc in hexane, increase in increments of 5% EtOAc per 100 ml of eluent used). The solvent was removed in vacuo to yield a white solid (3.50 g, 50%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.16 – 8.14 (d, J = 9.2 Hz, 2H), 6.93 – 6.90 (d, J = 9.2 Hz, 2H), 4.02 (t, J = 6.5 Hz, 2H), 3.38 (t, J = 6.9 Hz, 2H), 1.80 (m, 4H), 1.50 – 1.25 (m, 12H).

Synthesis of compound **2b**



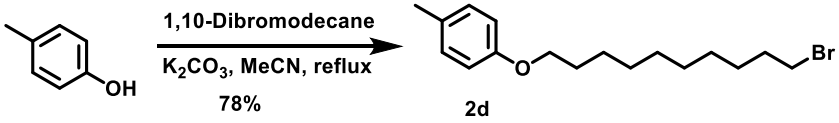
A suspension of K_2CO_3 (3.00 g, 21.71 mmol) in a solution of 4-cyanophenol (1.3 g, 10.91 mmol), 1,10-dibromodecane (12.75 g, 42.49 mmol) in MeCN (150 mL) was heated under reflux for 24 h under argon. The reaction progress was monitored by TLC. The reaction mixture was allowed to cool to room temperature, filtered through celite and concentrated in vacuo. Water (100 mL) was added and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried in $MgSO_4$, filtered and the solvent was removed in vacuo. The white solid was dissolved in minimal amounts of CH_2Cl_2 and absorbed onto silica followed by purification via silica gel column chromatography (gradient elution: 0 to 30% EtOAc in hexane, increase in increments of 5% EtOAc per 100 mL of eluent used). The solvent was removed in vacuo to yield a white solid (2.99 g, 81%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.56 – 7.54 (d, *J* = 8.7 Hz, 2H), 6.92 – 6.91 (d, *J* = 8.8 Hz, 2H), 3.97 (t, J = 6.5 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 1.88 – 1.73 (m, 4H), 1.48 – 1.24 (m, 12H).

Synthesis of compound **2c**



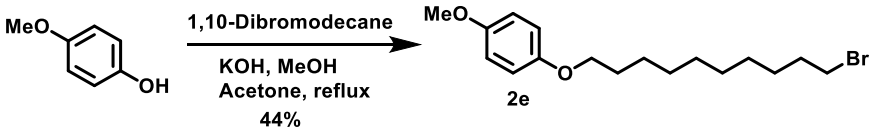
A suspension of K_2CO_3 (5.45 g, 39.49 mmol) in a solution of phenol (1.00 g, 10.6 mmol), 1,10-dibromodecane (12.75 g, 42.5 mmol) in MeCN (150 mL) was heated under reflux for 24 h under argon. The reaction mixture was run through celite pad and concentrated in vacuo. The white solid obtained was dissolved in minimal amounts of CH_2Cl_2 and absorbed onto silica followed by purification via silica gel column chromatography (gradient elution: 0 to 10% EtOAc in hexane, increase in increments of 1% EtOAc per 100 mL of eluent used). The solvent was removed in vacuo to yield a white solid (3.00 g, 92%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 6.92 (m, 3H), 3.96 (t, *J* = 6.4 Hz, 2H), 3.42 (t, *J* = 6.8Hz, 2H), 1.86 -1.78 (m, 4H), 1.53 – 1.28 (m, 12H).

Synthesis of compound **2d**



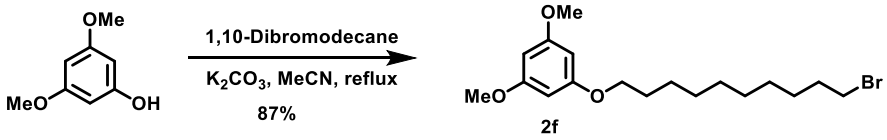
A solution of *p*-cresol (1.00 g, 9.24 mmol), 1,10-dibromodecane (11.10 g, 36.98 mmol) and K_2CO_3 (2.56 g, 18.5 mmol) in 100 mL of MeCN was refluxed overnight at 70 °C under argon. After the reaction was completed, the salt was filtered through celite pad, the MeCN was removed by evaporation. The reaction product was purified by column chromatography on silica gel with EtOAc/hexane (1:5) as the eluent. Yield: 78% (2.00 g). ^{1}H-NMR ($CDCl_3$): δ7.08 (d, *J*= 8.2 Hz, 2H,), 6.80 (d, *J*= 8.4 Hz, 2H,), 3.92 (t, *J*= 6.4 Hz, 2H,), 3.41 (t, *J*= 6.8 Hz 2H,), 2.28 (s, 3H), 1.82 (m, 4H), 1.30 (m, 12H).). FAB-MS (expected: 326.12m/z, obtained: 326.13, 328.12)

Synthesis of compound **2e**



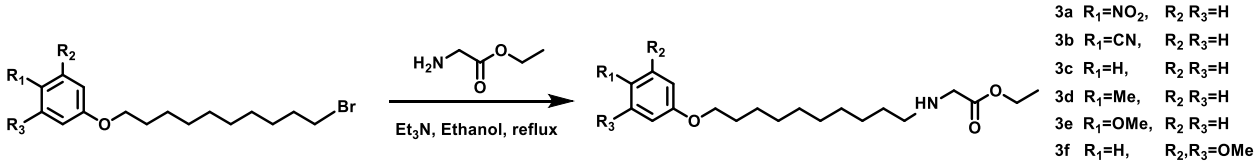
Potassium hydroxide (1.35 g, 23.9 mmol) in 100 mL of methanol was mixed with 4-methoxyphenol (2.48 g, 20.0 mmol) in 20 mL of methanol. The resulting solution was added using an addition funnel within 1.5 h to 1,10-dibromodecane (12.0 g, 40.0 mmol) in 200 mL of acetone. The mixture was refluxed overnight, concentrated, and diluted with water/diethyl ether. The ether extract was then filtered, washed with dilute water and brine, dried, condensed, and recrystallized from hexane, giving **2e** as a white crystal (3.0g, 44%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.76 (s, 4H), 3.82 (t, J = 6.6 Hz, 2H), 3.69 (s, 3H), 3.46 (t, J = 6.8 Hz, 2H), 1.69 (m, 4H), 1.41 –1.24 (m, 12H).

Synthesis of compound **2f**



A mixture of 3,5-dimethoxyphenol (1.02 g, 6.67 mmol), and K_2CO_3(1.84 g, 13.3 mmol) in MeCN (50mL) was added to 1,10-dibromodecane (8.0 g, 26.6 mmol) in MeCN (50 mL) solution dropwise and the solution was heated at reflux overnight. After filtration, the solvent was evaporated and the residue was extracted with EtOAc/H_2O twice. The combined organic layers was washed with brine and dried over Na_2SO_4, the filtrate was concentrated and purified by column chromatography (silica gel, hexane/ EtOAc, 10:1) to give **2f** as a white solid.(2.16 g, 87%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.08 (s, 3H), 3.91 (t, J = 6.6 Hz, 2H), 3.77 (s, 6H), 3.41 (t, J = 6.9 Hz, 2H), 1.85 (q, J = 6.9 Hz, 2H), 1.76 (q, J = 6.7 Hz, 2H), 1.47 – 1.31 (m, 12H).

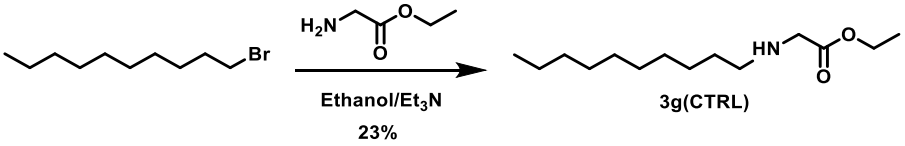
Synthesis of compound **3a-f**



Glycine ethyl ester hydrochloride (2 equiv.), triethylamine (4 equiv.), and the appropriate aliphatic methyl bromoacetate or aliphatic alkyl halide (1 equiv.) were mixed together in ethanol and the reaction mixture was refluxed for 24 h under argon. After the solvent was removed, the residue was added to water and extracted using CH_2Cl_2. Solvent was removed and the crude reaction mixture was purified through silica-gel column chromatography to get the monoalkylated glycine ester. Yield: 23%~45%.

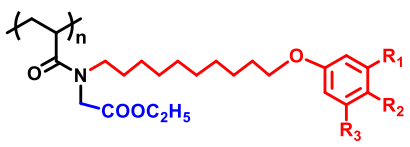
3a: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.15 – 8.13 (d, *J* = 9.2 Hz, 2H), 6.90 – 6.88 (d, *J* = 9.3 Hz, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.01 (t, *J* = 6.5 Hz, 2H), 3.36 (s, 2H), 2.56 (t, *J* = 7.2 Hz, 2H), 1.78 (m, 2H), 1.52 (s, 1H), 1.43 (m, 4H), 1.36 – 1.21 (m, 13H); ESI-MS: expected 380.23, obtained 381.24. **3b**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.97 (t, *J* = 6.5 Hz, 2H), 3.37 (s, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.82 – 1.72 (m, 2H), 1.46 (m, 4H), 1.27 (m, 13H). **3c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.22 (m, 2H), 6.89 (dd, *J* = 16.2, 7.8 Hz, 3H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.92 (t, *J* = 6.6 Hz, 2H), 3.37 (s, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.76 (p, *J* = 6.8 Hz, 2H), 1.55 – 1.39 (m, 4H), 1.38 – 1.21 (m, 13H); ESI-MS expected 335.25, obtained 335.25. **3d**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.2 Hz, 2H), 6.83 – 6.76 (d, *J* = 8.5 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.92 (t, *J* = 6.6 Hz, 2H), 3.39 (s, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.28 (s, 3H), 1.80 – 1.71 (m, 2H), 1.58 – 1.39 (m, 4H), 1.38 – 1.25 (m, 13H). **3e**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.83 (s, 4H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.90 (t, *J* = 6.6 Hz, 2H), 3.77 (s, 3H), 3.39 (s, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 1.80 – 1.70 (m, 2H), 1.46 (m, 4H), 1.37 – 1.24 (m, 13H); FAB-MS expected 365.26, obtained 366.2. **3f**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.10 (s, 3H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.93 (t, *J* = 6.6 Hz, 2H), 3.79 (s, 6H), 3.42 (s, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.82 – 1.74 (m, 2H), 1.48 (m, 4H), 1.32 (m, 13H).

Synthesis of compound **3g** (CTRL)



Glycine ethyl ester hydrochloride (12.4 g, 96 mmol), trimethylamine (16.2 mL, 192 mmol), and 1-bromodecane (10.62 g, 48 mmol) were mixed together in ethanol (200 mL) and the reaction mixture was refluxed for 36 h under argon. After solvent removal, the residue was added to water and extracted using EtOAc. Solvent was removed and the crude product was purified through silica-gel column chromatography to get the monoalkylated glycine ester **3g**. Yield: 23%. The low yield is due to the possible bis-alkylated byproduct. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.19 (q, J = 7.1 Hz, 2H), 3.40 (s, 2H), 2.59 (t, J = 7.2 Hz, 2H), 1.87 (s, 1H), 1.49 (m, 2H), 1.35 – 1.23 (m, 17H), 0.87 (t, J = 6.7 Hz, 3H).

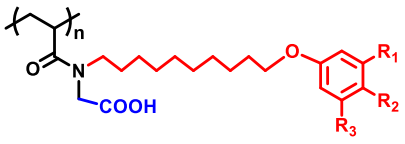
Synthesis of polymer **4a-g**



		R_1	R_2	R_3
4a	PAm-NO_2	H	NO_2	H
4b	PAm-CN	H	CN	H
4c	PAm-H	H	H	H
4d	PAm-Me	H	Me	H
4e	PAm-OMe	H	OMe	H
4f	PAm-di-OMe	OMe	H	OMe
4g	PAm-CTRL	no aromatic ring		

50 mg (0.21 mmol) of polypentafluorophenylacrylate (PPFPA), 146.3 µL (0.84 mmol) of DIPEA and 4 equivalents of secondary amine with respect to PFPA unit, were dissolved in freshly distilled THF (500 mg polymer/mL). The mixture was stirred overnight at 65 °C. The resulting polymer was isolated by precipitation in n-hexane and was then dried in vacuo. Yield: ~70%. ^{1}H-NMR shows corresponding peaks with the same chemical shift as the secondary amine. ^{19}F NMR shows no peak left from the fluorinated precursor polymer, indicating 100% conversion of PPFPA polymer to polyacrylamide polymers **4a-g**.

Synthesis of compound **5a-g**



		R_1	R_2	R_3
5a	PAm-NO_2	H	NO_2	H
5b	PAm-CN	H	CN	H
5c	PAm-H	H	H	H
5d	PAm-Me	H	Me	H
5e	PAm-OMe	H	OMe	H
5f	PAm-di-OMe	OMe	H	OMe
5g	PAm-CTRL	no aromatic ring		

The final polymer was obtained by deprotection of the carboxylate group. Lithium hydroxide was added to each polymer dissolved in THF, methanol and water (8 mL: 2 mL: 1 mL). The reaction was then allowed to take place overnight at room temperature. The solvents were evaporated and 10 mL of water was then added, followed by neutralization with 3 M HCl solution to precipitate the polymer. The polymer was filtered and vacuum-dried. Molecular weight cannot be obtained by GPC due to solubility issues. Conversion of the ester to the free carboxylic acid was confirmed by IR (vibration at 3100 cm^{-1} for O-H and 1730 cm^{-1} for C=O of carboxylic acid).

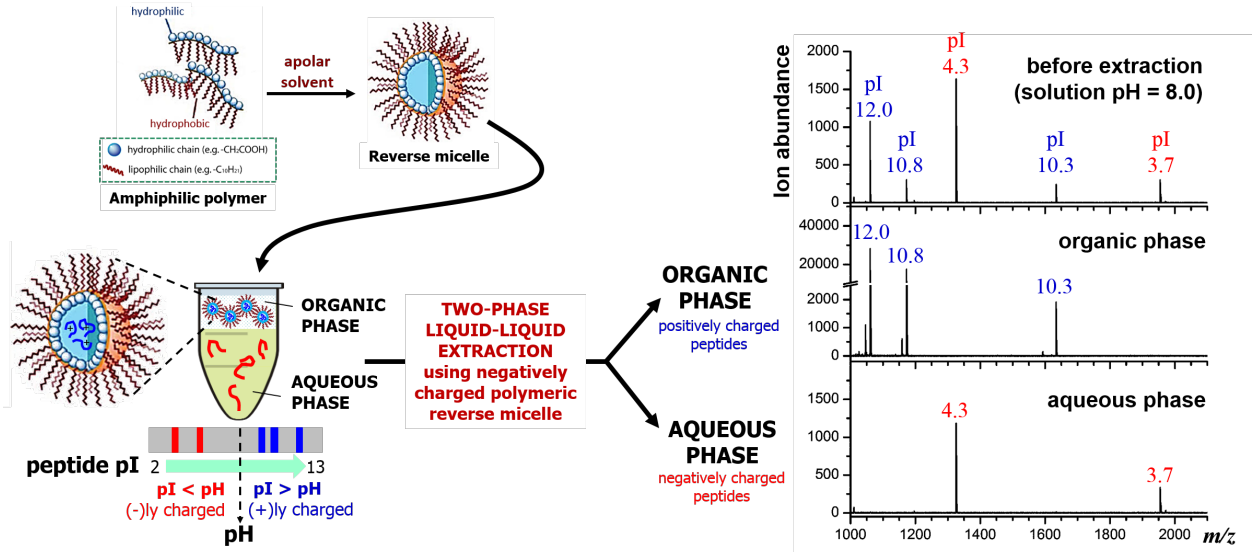


Figure S1. General scheme for the selective extraction and MALDI-MS analysis of peptides using reverse micelles of amphiphilic polymers.

Amphiphilic polymers self-assemble into reverse micelles in non-polar solvents such as toluene and are used to selectively extract peptides that bear a complementary charge. Negatively charged reverse micelles such as those from carboxylate-functionalized polymers will thus extract positively charged peptides. Peptides with isoelectric point (pI) greater than the pH have net positive charges and get extracted by the reverse micelles into the organic phase. On the other hand, peptides with pI lower than the pH of the solution have net negative charges and are therefore not extracted but rather remain in the aqueous phase. This pI-based extraction is used to selectively enrich biomarkers of interest from complex biological mixtures.

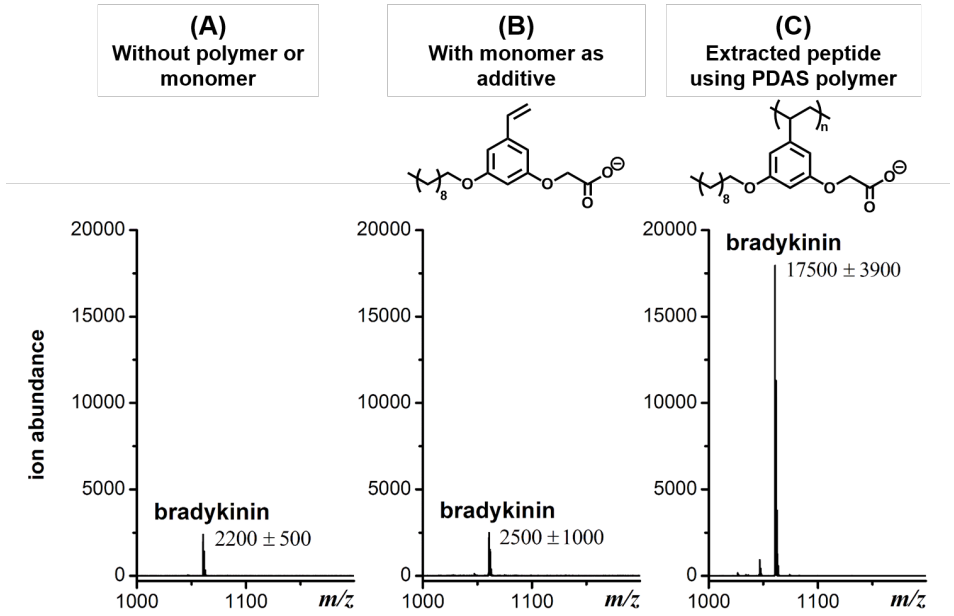


Figure S2. Comparison of MALDI-MS signal for bradykinin peptide (**A**) in the absence of the polymer or monomer, (**B**) with the monomer as matrix additive, and (**C**) extracted using the PDAS polymer.

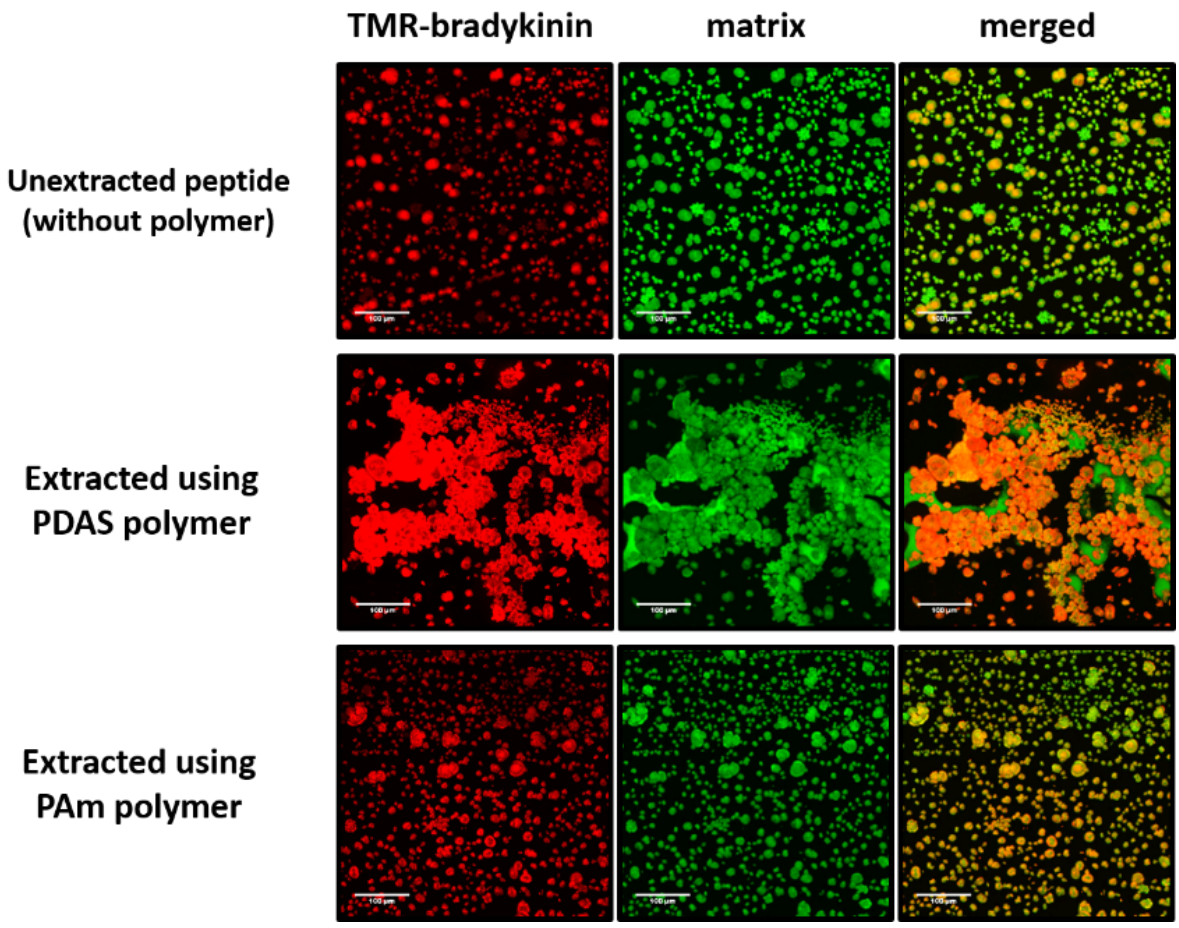


Figure S3. Fluorescence microscopy images showing the degree of clustering and co-localization of TMR-bradykinin and CHCA matrix in unextracted (5μM) and extracted (50 nM) samples using the PDAS and PAm amphiphilic polymers. Scale bar = 100 μm.

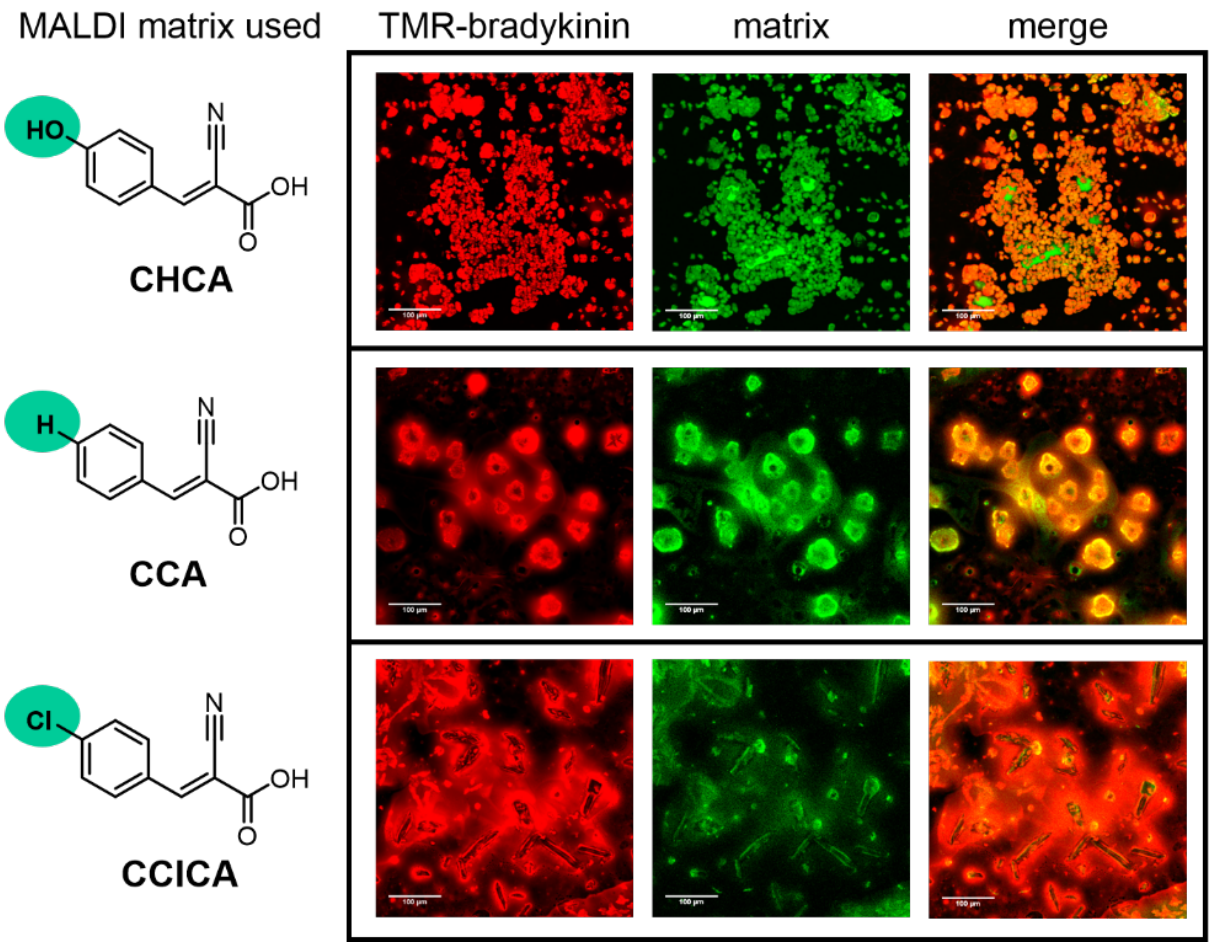


Figure S4. Fluorescence microscopy images of extracted TMR-bradykinin (50 nM) with different MALDI matrices: **(A)** CHCA matrix; **(B)** CCA matrix; **(C)** CClCA matrix. Scale bar = 100 μm.

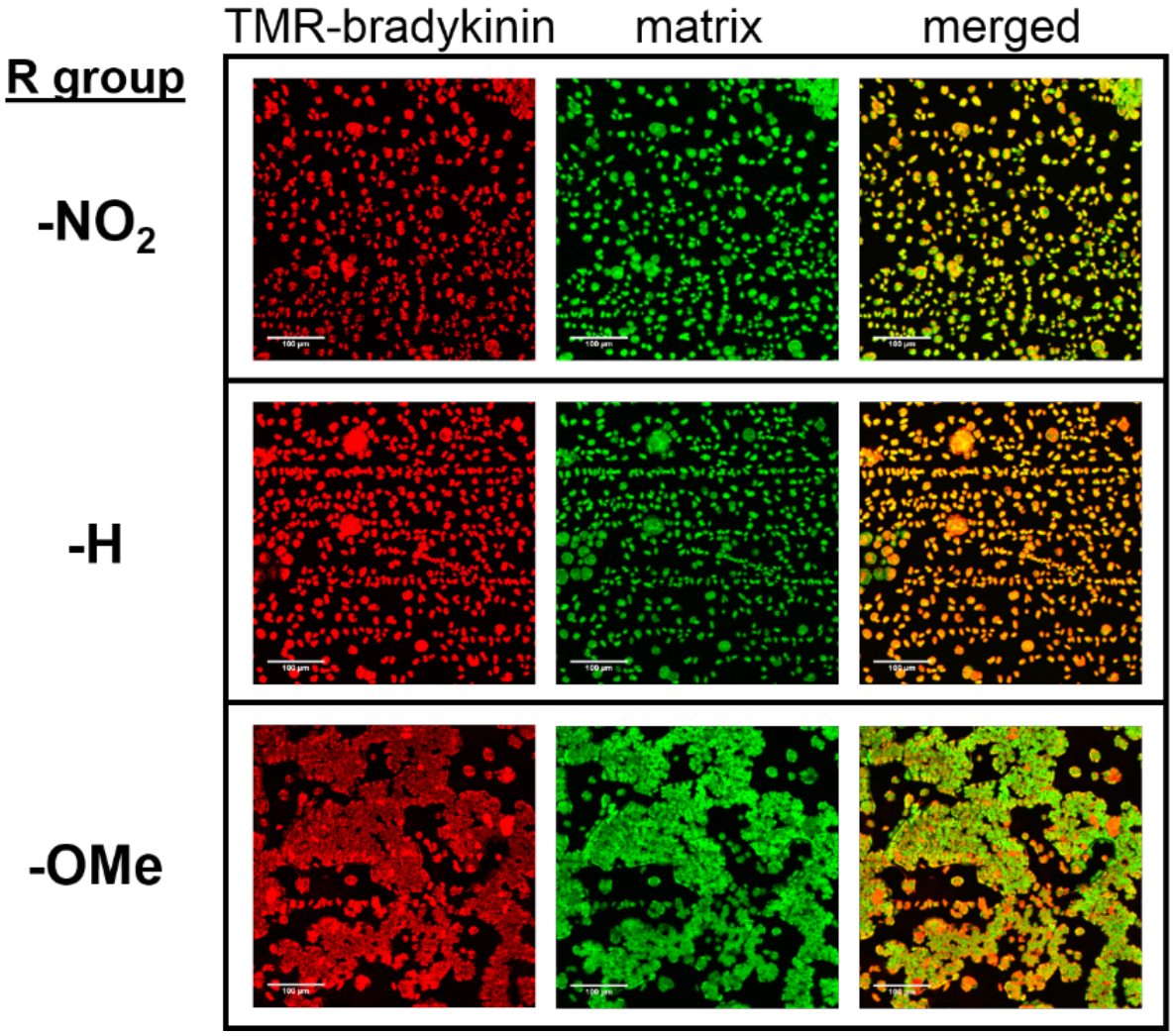


Figure S5. Fluorescence microscopy images showing the degree of clustering and co-localization of TMR-bradykinin and CHCA matrix in extracted samples (50 nM peptide) using the PTLAm amphiphilic polymers with different R groups. Scale bar = 100 μm.

Characterization of the polymers:

A) PTLAm-based amphiphilic polymers

Dynamic Light Scattering

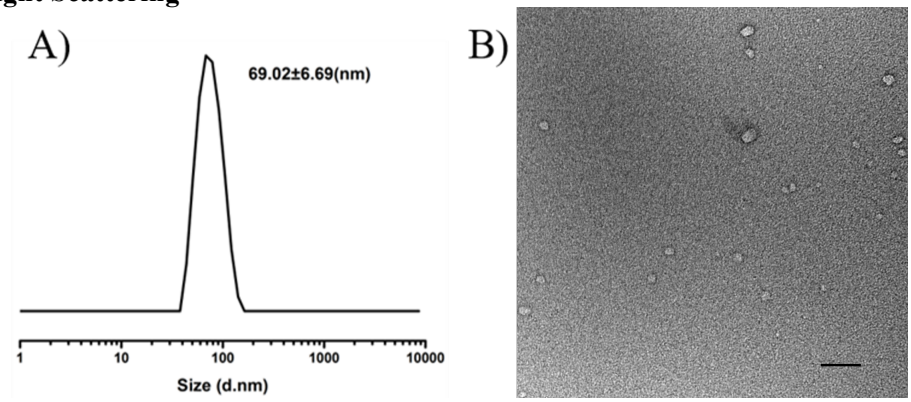


Figure S6. A) DLS data and B) TEM image for the reverse micelles prepared from polymer **PTLAm-H** at a concentration of 0.5 mg/mL. Scale bar = 50 nm.

Selective Extraction of Peptides

Table S1. Peptides used for determining the selectivity of extraction.

Peptide	Sequence	m/z	Calculated pI*	Net charge at pH 8
Bradykinin	RPPGFSPFR	1060.6	12.00	(+)
Kinetensin	IARRHPYFL	1172.7	10.84	(+)
Malantide	RTKRSGSVYEPLKI	1633.9	10.28	(+)
β-amyloid (1-11)	DAEFRHDSGYE	1325.5	4.31	(-)
Preproenkephalin	SSEVAGEGDGDSMGHEDLY	1954.8	3.71	(-)
*pI calculated from ExPASy Bioinformatics Resource Portal (http://web.expasy.org/compute_pi/)				

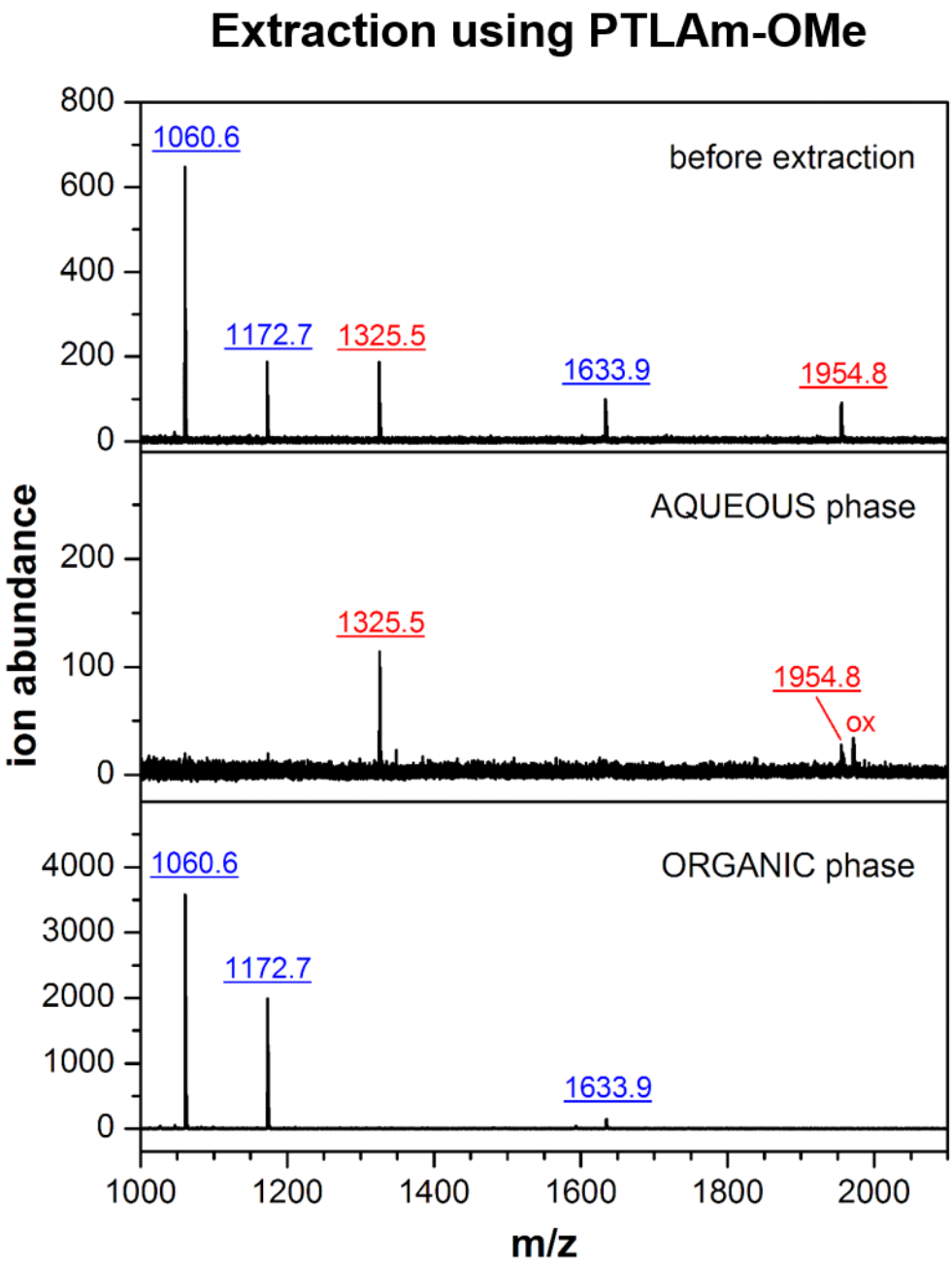


Figure S7-A. Example MALDI mass spectra of a mixture of peptides before and after extraction at pH 8.0 using reverse micelles of the PTLAm-based carboxylate polymers. Only peptides that are positively charged (blue) at pH 8.0 are extracted into the organic phase while peptides that are negatively charged (red) remain in the aqueous phase.

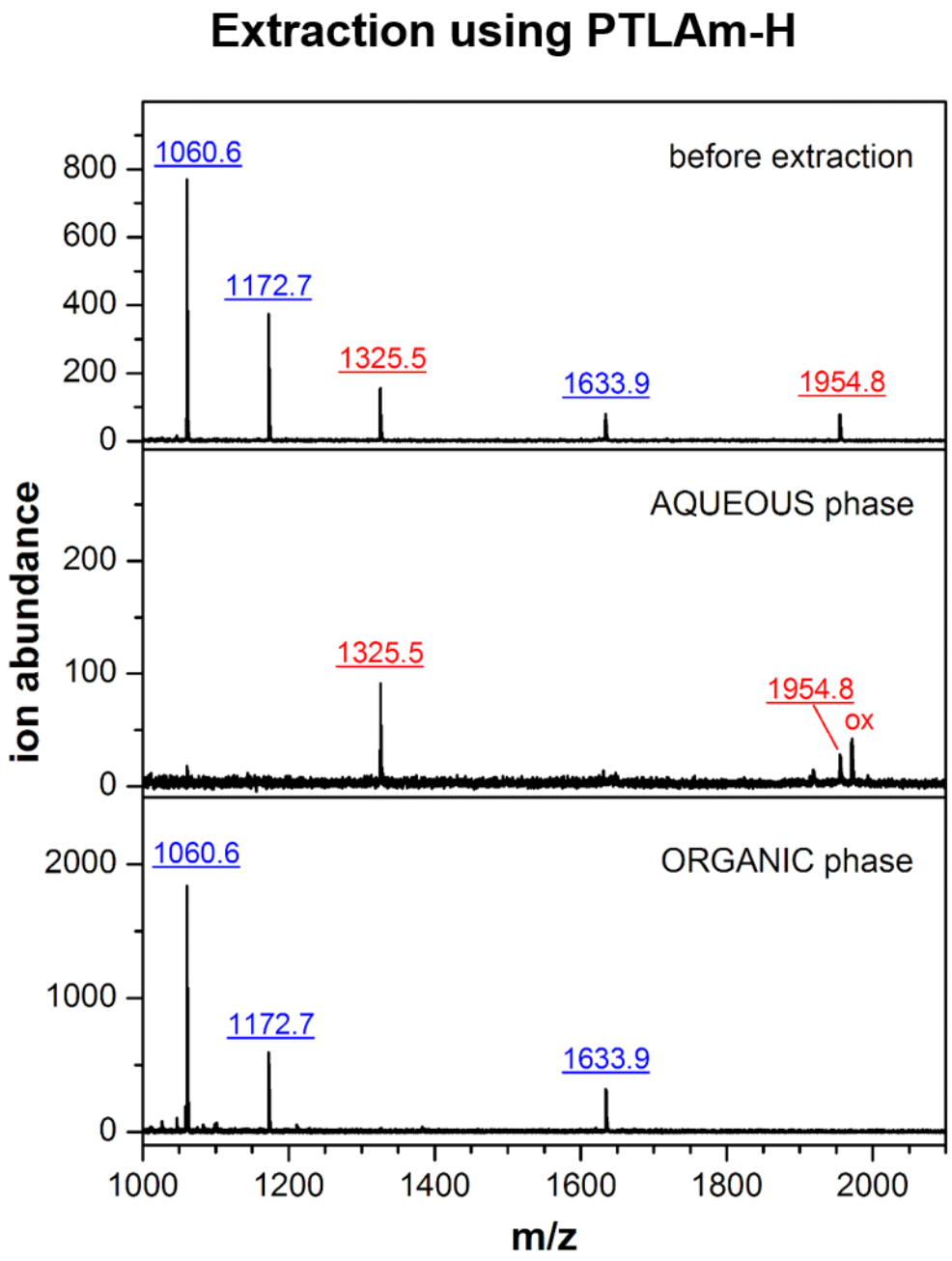


Figure S7-B. Example MALDI mass spectra of a mixture of peptides before and after extraction at pH 8.0 using reverse micelles of the PTLAm-based carboxylate polymers. Only peptides that are positively charged (blue) at pH 8.0 are extracted into the organic phase while peptides that are negatively charged (red) remain in the aqueous phase.

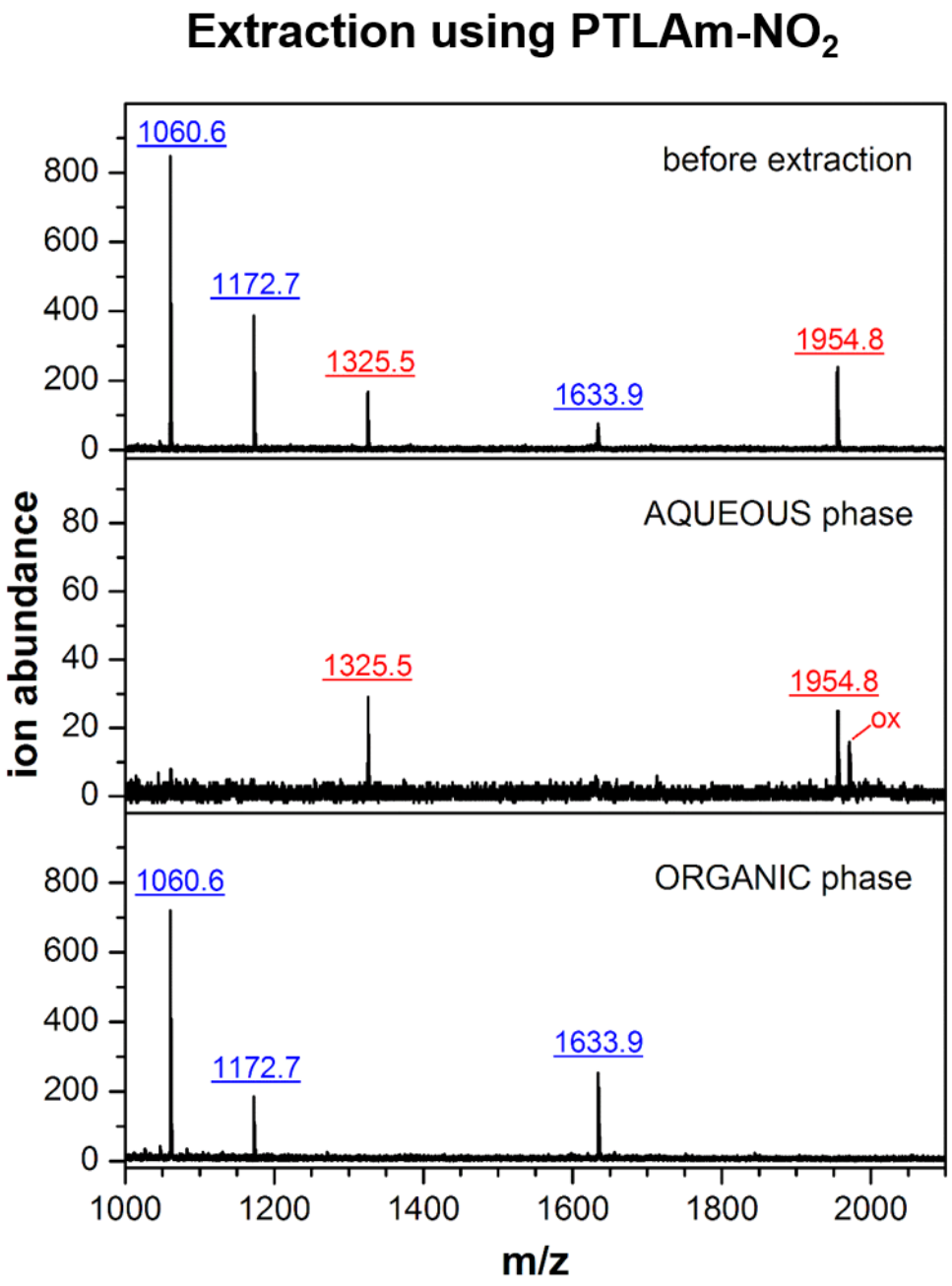


Figure S7-C. Example MALDI mass spectra of a mixture of peptides before and after extraction at pH 8.0 using reverse micelles of the PTLAm-based carboxylate polymers. Only peptides that are positively charged (blue) at pH 8.0 are extracted into the organic phase while peptides that are negatively charged (red) remain in the aqueous phase.

B) PAm-based amphiphilic polymers

Dynamic Light Scattering

The size distributions of PAm-based amphiphilic homopolymer reverse micelles were determined by DLS measurements. The polymers were dissolved in toluene and sonicated until a clear solution was obtained. Two equivalents of water (aqueous NaOH) per carboxylate unit were added to form the water pool inside the reverse micelles. DLS measurements were carried out in a quartz cuvette The particle sizes obtained for all reverse micelles were between 150 nm and 300 nm based on an average of 3 correlations of 10 minutes each.

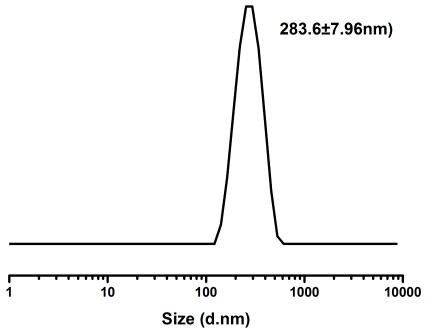


Figure S8. Example DLS data for the reverse micelles prepared from polymer **PAm-H** at a concentration of 0.5 mg/mL.

Transmission Electron Microscopy

TEM measurements were performed using a JEOL 2000FX 100K TEM. Samples were prepared by dipping the copper EM grid into the reverse micelle solution and air drying overnight.

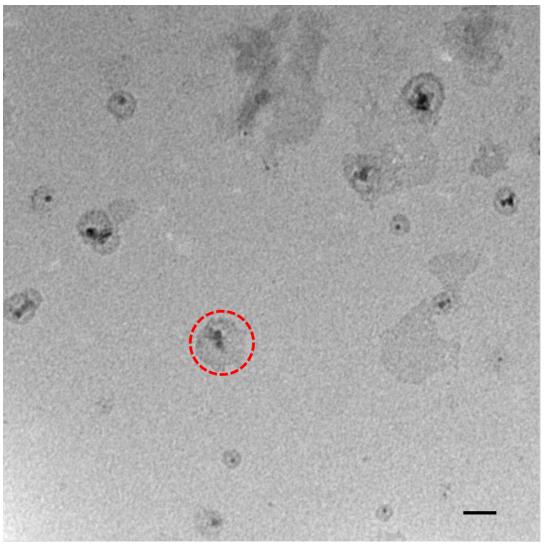


Figure S9. TEM image of the reverse micelles prepared from polymer **PAm-H** at concentration of 0.5mg/mL. The black dot in each reverse micelle is due to the crystallization of NaOH that was added to form the water pool. Scale bar = 100 nm.

Critical Aggregation Concentration (CAC) measurements

Different concentrations of the polymeric reverse micelles were prepared by dissolving polymer in toluene and sonicating for 2hrs to obtain clear solutions. The surface tensions of these solutions were recorded using a tensiometer and plotted against the reverse micelle concentration. As an example, the CAC measurement of polymer **PAm-H** is shown below.

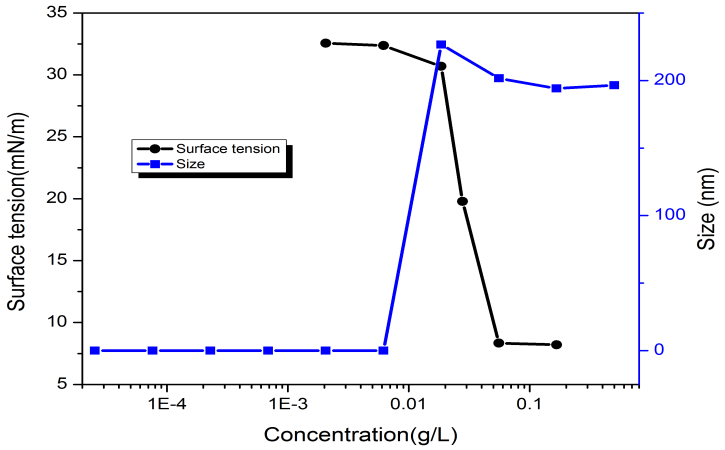


Figure S10. Example surface tension (black dots) and particle size (blue square) measurements vs. concentration of polymer **PAm-H**. A CAC of ~0.055mg/mL was identified as the concentration at which the surface tension and size changes.

Extraction Capacity Determination

Increasing concentrations of the peptide bradykinin was extracted using 200 µL of each polymer and the peptide peak in the aqueous phase was monitored. The extraction capacity is taken as the concentration of peptide at which a sudden increase in signal is seen in the aqueous phase, indicating that the reverse micelles are saturated and can no longer accommodate more peptides in the organic phase.

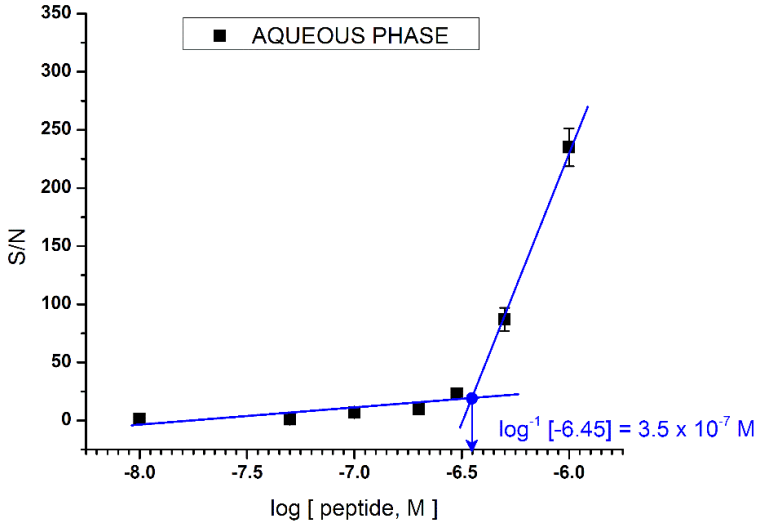


Figure S11. Example of peptide extraction capacity measurement for polymer **PAm-H** at pH 8.0.

Selective Extraction of Peptides

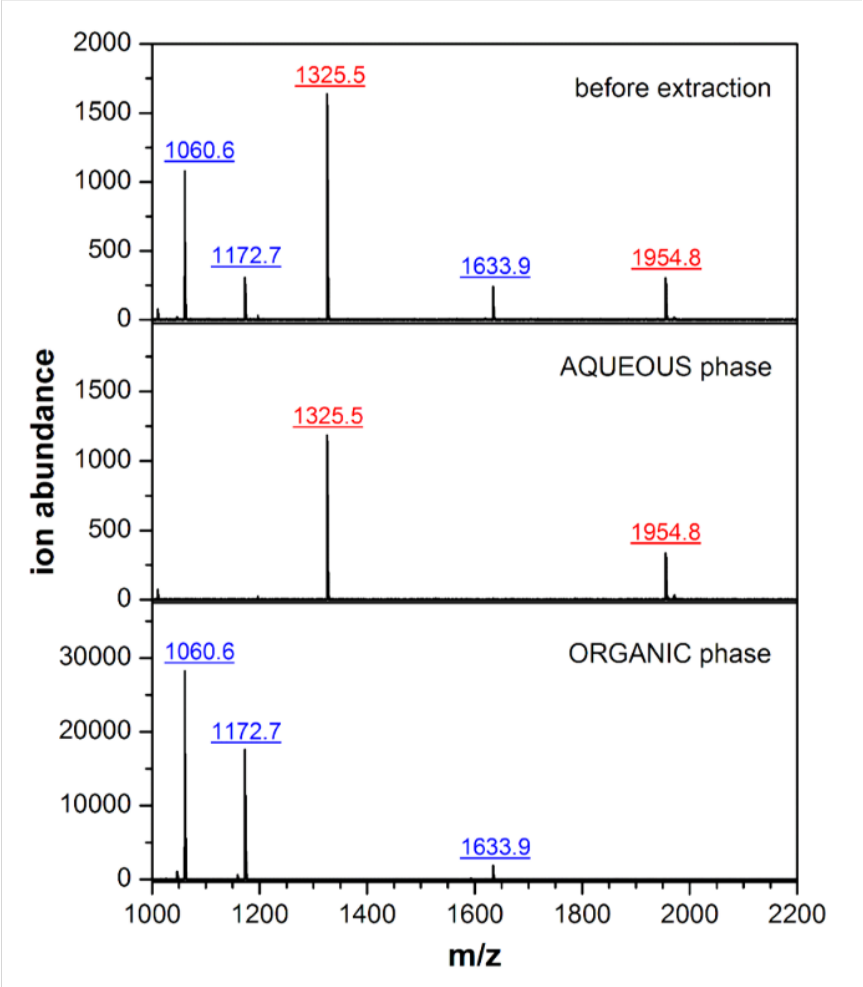


Figure S12. Example MALDI mass spectra of a mixture of peptides (see Table S1 for peptide sequence and pI) before and after extraction at pH 8.0 using reverse micelles of the polyacrylamide-based polymer **PAm-H**. Only peptides that are positively charged (blue) at pH 8.0 are extracted into the organic phase while negatively charged peptides (red) remain in the aqueous phase.

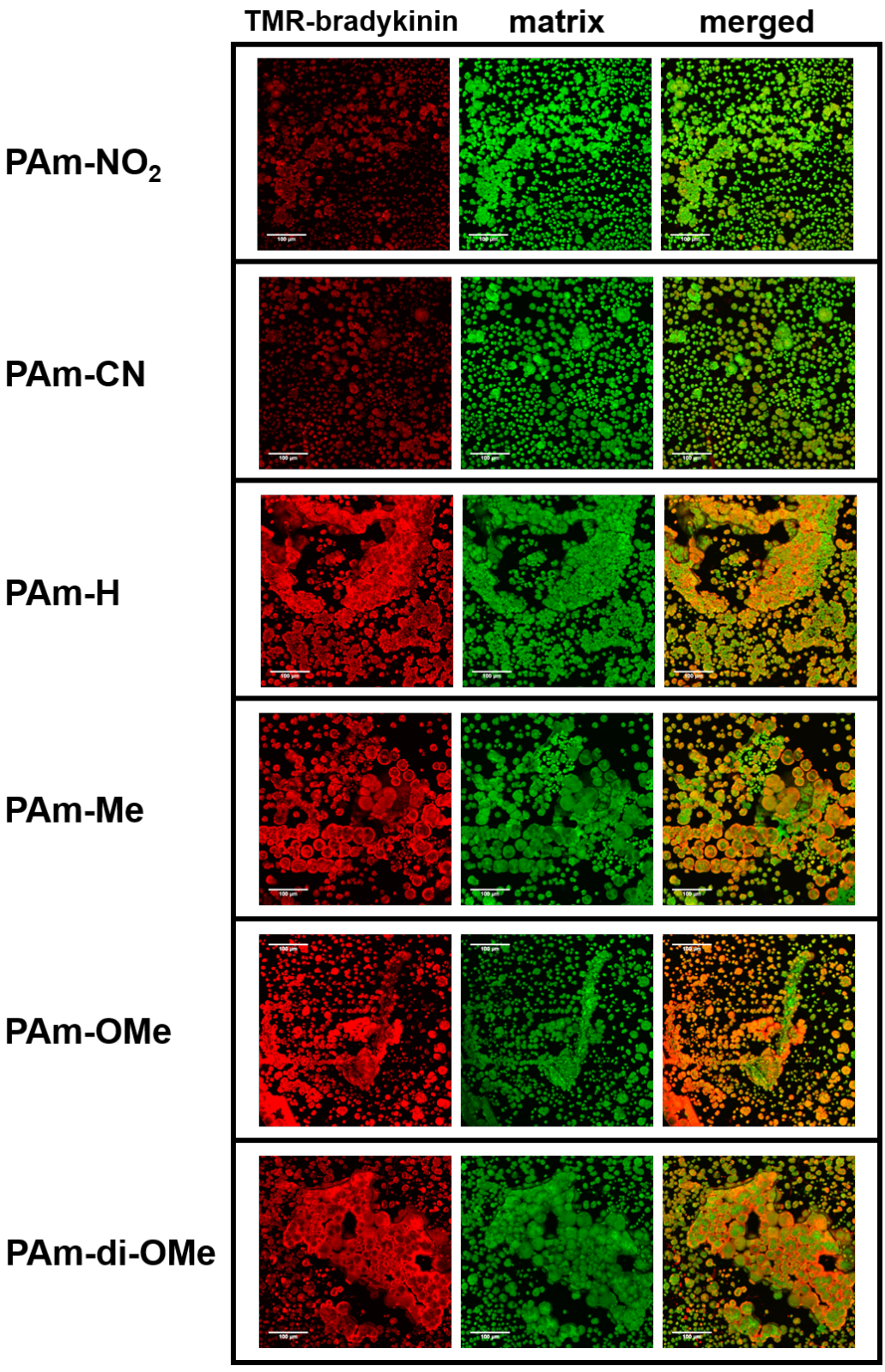


Figure S13. Fluorescence microscopy images showing the degree of clustering and co-localization of TMR-bradykinin peptide (50 nM) and CHCA matrix extracted using the PAm polymers indicated in each panel. Scale bar = 100 μm.

Quantitative measurement of fluorescence intensity by *ImageJ*

To quantitatively assess how densely the peptides are clustering in a given sample, the *ImageJ* software [5] was used to measure the fluorescence intensity per area. For each fluorescence image, five circular regions with 100 μm diameter, which is similar to the spot size of the laser used in the MALDI experiments, were selected and the integrated fluorescence for each region was obtained using the 'Measure' command under the 'Analyze' menu of the *ImageJ* software. Raw values were corrected by accounting for any background fluorescence in the black region of the image.

$$Corrected\ Fluorescence = (Integrated\ Density, I) - (Area\ of\ selected\ region, \square m^2)(Mean\ fluorescence\ of\ background, \frac{I}{\square m^2})$$

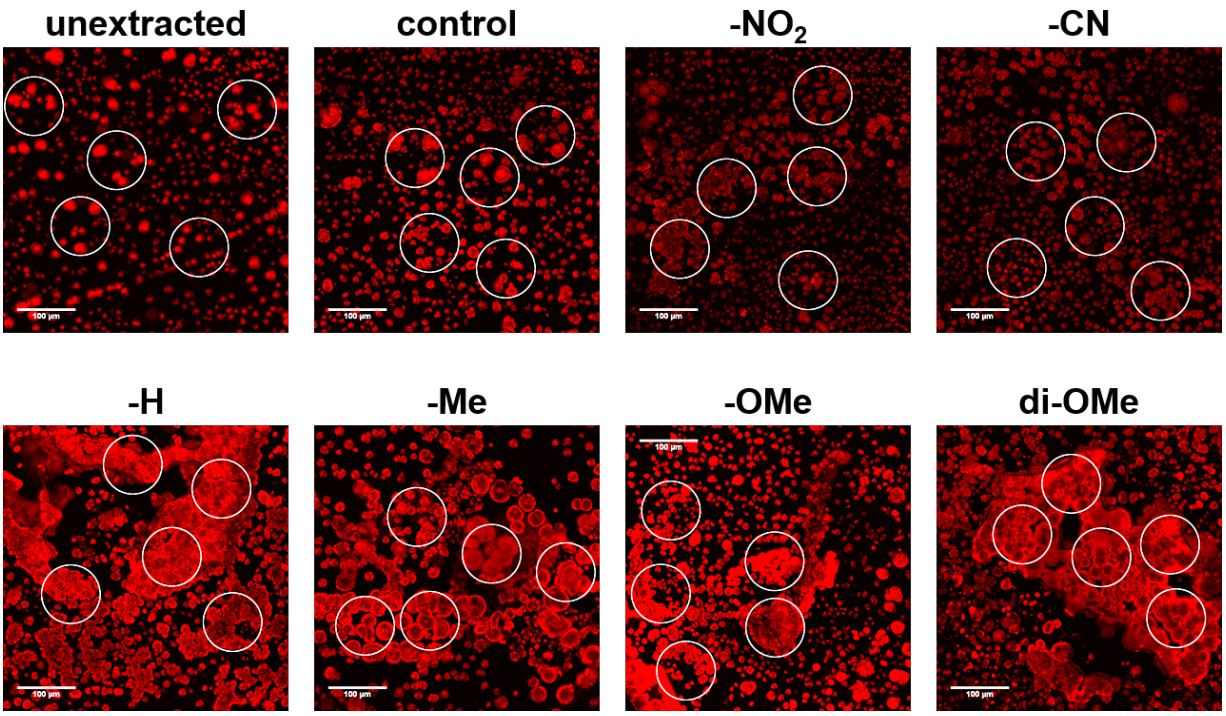


Figure S14. Five circular regions selected for each image to quantify the degree of clustering of the peptide in the polymer-matrix-peptide mixture in the organic phase after extraction using the PAm polymers (**5a-g**).

Co-localization analysis

The co-localization of the TAMRA-bradykinin peptide with the MALDI matrix in each fluorescence micrograph was analyzed using the *Colocalization Colormap* plugin (https://sites.google.com/site/colocalizationcolormap/) for *Fiji* [6], an image analysis distribution by *ImageJ*. An index of correlation (I_{corr}) is calculated for a pair of images (two different channels representing the peptide and the matrix fluorescence signals). Normalized mean deviation product (nMDP) values ranging from -1 (exclusion) to +1 (co-localization) is calculated between a pair of pixels and the fraction of positively correlated pixels is given by the index of correlation (I_{corr}). A colormap is generated to visualize the co-localization of the two fluorescent species.

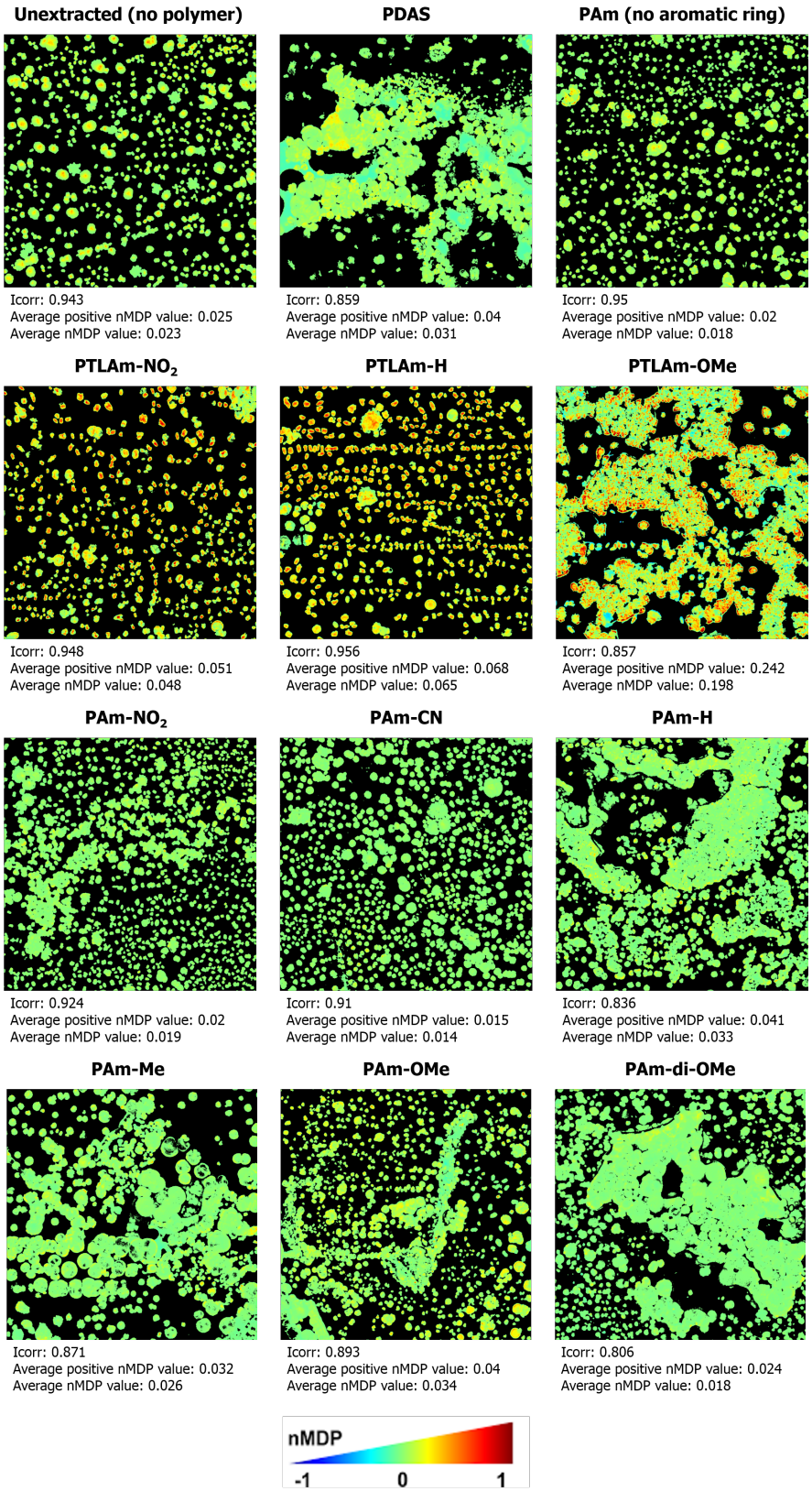


Figure S15. Co-localization analysis with the calculated index of correlation, average positive nMDP value, and average nMDP value for each fluorescence micrograph. Color scale used to represent the negative nMDP (exclusion) and positive nMDP values (co-localization) on the images is given.

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