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Electrostatic Control of Peptide Side-Chain Reactivity Using Amphiphilic Homopolymer-Based Supramolecular Assemblies

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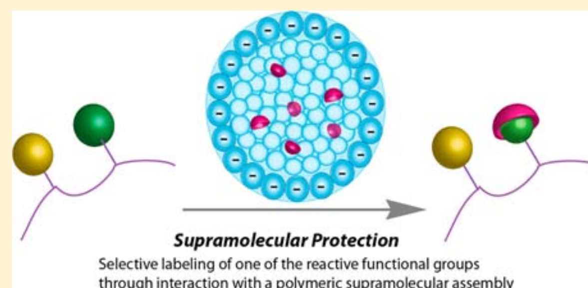
Supporting Information

ABSTRACT: Supramolecular assemblies formed by amphiphilic homopolymers with negatively charged groups in the hydrophilic segment have been designed to enable high labeling selectivity toward reactive side chain functional groups in peptides. The negatively charged interiors of the supramolecular assemblies are found to block the reactivity of protonated amines that would otherwise be reactive in aqueous solution, while maintaining the reactivity of nonprotonated amines. Simple changes to the pH of the assemblies' interiors allow control over the reactivity of different functional groups in a manner that is dependent on the pK_a of a given peptide functional group. The labeling studies carried out in positively charged supramolecular assemblies and free buffer solution show that, even when the amine is protonated, labeling selectivity exists only when complementary electrostatic interactions are present, thereby demonstrating the electrostatically controlled nature of these reactions.



INTRODUCTION

Reactions performed inside supramolecular assemblies have gained a lot of interest recently due to the unique features offered by their constricted space and organized construction. These assemblies can be exploited to enable different reactivity or regioselectivity, compared to those in bulk solution.[1] These molecular confinements have been investigated as a means of stabilizing intermediates or by preorganizing reagents to obtain regioselectivity.[2] Micelles formed by small surfactants and amphiphilic polymers have been shown to increase reactivity, change reaction pathways, or enhance regioselectivity by increasing local concentrations, stabilizing transition states, or incorporating a catalytic element.[3] Most of these systems have focused on increasing reactivity or enhancing regioselectivity.[4] Similarly, selective labeling in proteins has also been achieved using methods such as site-directed mutagenesis or chemical modification and incorporation of artificial amino acids.[5] However, development of supramolecular assemblies that can block specific reaction sites, while leaving others available, is rarely studied to our knowledge. We have been interested in utilizing self-assembling systems to selectively mask certain amino acid side chains, while leaving other reactive amino acids unmasked, to gain the capability to selectively label amino acids in peptides.

Given the ubiquity of charged functional groups in peptides and proteins, we focused on electrostatic interactions as a means of controlling reactivity. Amphiphilic homopolymers, which are capable of forming micelles in aqueous solutions and reverse micelles in nonpolar solvents,[6] were used as the supramolecular hosts. We have previously shown that the reverse micelles of these amphiphilic homopolymers can selectively enrich peptides from complex mixtures based solely on electrostatic interactions.[7] In the current work, we investigate whether these reverse micelle forming polymers can act as a "reaction flask" to selectively mask specific functional groups, while specifically labeling certain others in peptides using differences in electrostatic interactions between the functional groups in the peptide and those in the reverse micelle interiors (Scheme 1). In order to provide a robust new platform, we stipulated that our supramolecular approach should concurrently provide the following characteristics: (i) the reverse micelle-based polymeric assemblies be capable of selectively extracting peptides from the aqueous to the organic phase based on electrostatic complementarity; (ii) the interaction between specific amino acid residue within the peptide and the polymeric assembly is dictated by the pK_a's of the polymeric moieties and that of the peptide side chain functional group; (iii) this electrostatic binding masks the reactivity of the side chain functional groups, which provides the opportunity to specifically label functional groups; and (iv) since the pK_a's dictate the interaction, this interaction and therefore the reactivity of side chain functional groups can be modulated by variation of the aqueous phase pH. While the supramolecular system that exhibits such features could have broader implications in reaction chemistry, we focus on of improving our ability to detect peptides in complex mixtures by selectively enhancing the detection efficiency of desired peptides. Thus, development of the principles behind this supramolecular

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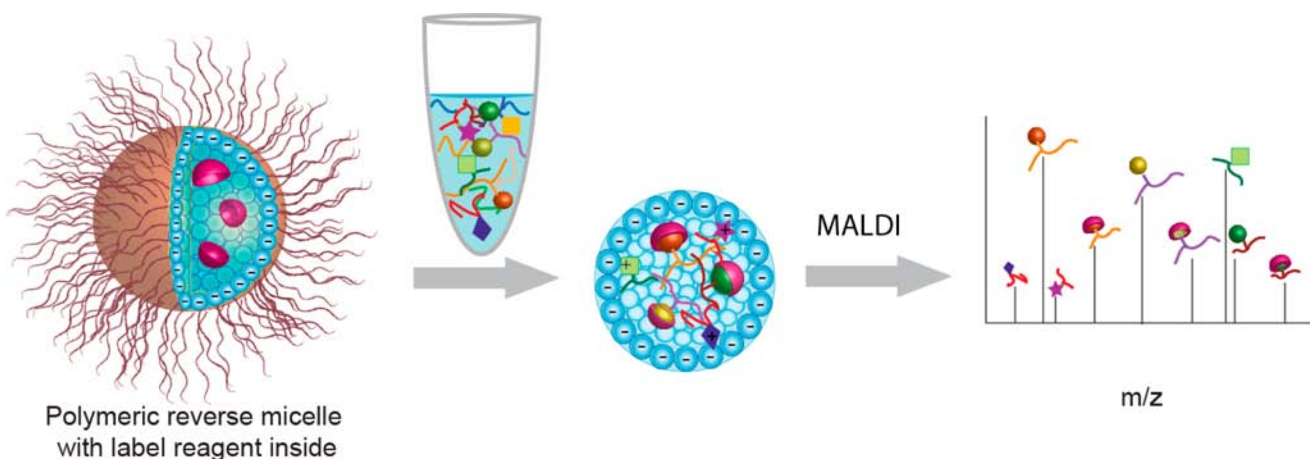
Scheme 1. Schematic Illustration of the Labeling of Extracted Peptides Inside Negatively Charged Reverse Micelles and Their Analysis by MALDI-MS



masking of functional groups in peptides could have implications in areas such as proteomics and biomarker detection.[8]

Peptides have been chosen as an initial model system, because they are smaller and simpler than proteins and because they are the products obtained in protein digestion during protein identification protocols. We use mass spectrometry (MS) as the detection method to analyze the versatility of our approach, because: (i) it is an information-rich detection method, i.e., it provides direct information regarding the m/z of a molecule instead of indirect and nonspecific information such as absorption or fluorescence spectral signatures; (ii) the extent of the chemical reaction between the peptide substrate and the labeling reagent can be ascertained using the shift in m/z; (iii) the specific labeling site in peptides can also be clearly ascertained using MS/MS; and (iv) MS is a popular method for proteomics and biomarker identification and therefore demonstrating our method using mass change would directly test its impact.

RESULTS AND DISCUSSION

Amphiphilic homopolymers are capable of forming environment-dependent amphiphilic assemblies, i.e., these polymers form micelle-like structures that are capable of sequestering hydrophobic guest molecules in water and form reverse micelle-like assemblies in apolar solvents.[9] Interestingly, when dispersed in a biphasic mixture of water and an apolar solvent, the polymers are kinetically trapped in the solvent in which they are initially assembled.[6] This observation formed the basis for utilizing the reverse micelles of these polymers to selectively bind and extract peptides from the aqueous phase into the polymeric interior in the apolar organic phase.[7,10] The driving force for this selectivity in binding is electrostatics, as it has been consistently observed that the polymer and the bound peptides were oppositely charged, i.e., reverse micelles based on a negatively charged polymer were able to selectively bind peptides with the pI >7, when the pH of the aqueous phase is ~7. It is likely that the electrostatic interaction between the charged peptide side chains and those of the polymer is responsible for the binding event. If this were correct, we hypothesized that the reactivity of the peptide side chain functionalities that are responsible for binding to the polymer will be significantly modulated (Scheme 2).

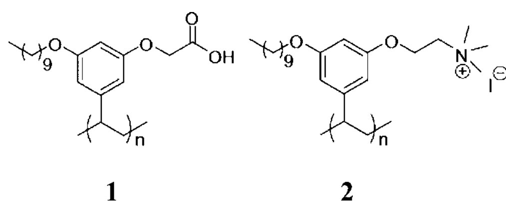
To test this hypothesis, we utilized the carboxylic acid based amphiphilic homopolymer shown as structure **1** (Chart 1). To form reverse micelles, 2 equiv of water per charged functional group were added to a solution of polymer **1** in toluene, and the mixture was sonicated for 4 h. Since this polymer is negatively charged, we used the positively charged peptide malantide (RTKRSGSVYEPLKI, pI = 10.3) as an initial test substrate. The lysine residues of malantide should be able to react with sulfo-*N*-hydroxysuccinimide acetate (sulfo-NHSA) to acetylate the amine side chains, as shown in Scheme 3. This labeling event can be conveniently monitored by MS, because the reaction should afford an increase in m/z of 42 per labeling event. Sulfo-NHSA was first added to the toluene solution containing the reverse micelle, and the resulting mixture was sonicated for ~15 min. An aqueous solution of the peptide was then immediately mixed with the toluene solution. After transfer of the peptide into the organic phase and subsequent analysis of this organic phase, the mass spectrum clearly shows the presence of malantide. Very little to no labeling by sulfo-NHSA, however, is observed (Figure 1a). It is possible that this reagent is simply unable to react with the amines in malantide at pH 7, as illustrated in Scheme 2a. When malantide is reacted with sulfo-NHSA in the aqueous phase, though, peaks that correspond to a shift in the m/z of 42 and 84 are indeed observed (Figure 1b), suggesting that the reaction occurs in at least two amine residues. In fact, there is no evidence for the presence of unreacted malantide under these conditions. Analysis of the location of the labeling sites on the peptide using MS/MS (Figure S2) indicates that the N-terminus and the two lysine residues can be labeled. Evidently, the peak with two acetyl groups corresponds to a mixture of three isomers, where the labels simultaneously react at the N-terminus and Lys3, the N-terminus and Lys13, and Lys3 and Lys13.

In order to determine that this reaction control was not just specific to this peptide, we also tested the effect of the reverse micelles with another positively charged peptide, apelin 13 (QRPRLSHKGPMPA, pI = 12.4). The difference in this peptide's reactivity in the aqueous phase and within the reverse micelles of polymer **1** is indeed similar to that observed for malantide (see Figure S3). These results are consistent with our hypothesis that the electrostatic interaction between the polymer and the peptide could mask the reactivity of certain amino acids.

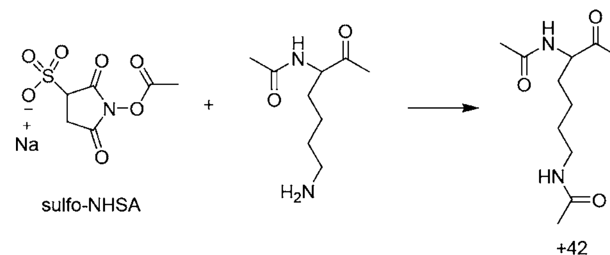
It is possible that the difference in reactivity observed for the two peptides above is simply due to the sterics offered by the reverse micelles or the inability of sulfo-NHSA to diffuse into the reverse micelle. To test these alternate possibilities, we used the reverse micelle formed by positively charged polymer **2** to carry out the binding and labeling. If the reactivity were under steric control, then the amine groups within this positively charged

Scheme 2. Schematic Illustration of the Electrostatic Interaction Driven Selectivity and the Detection Event

Chart 1. Structure of the Amphiphilic Homopolymer Based on Carboxylic Acid Moiety (negatively charged, 1) and Quaternary Ammonium Moiety (positively charge, 2)



Scheme 3. Reaction of Sulfo-NHSA with Lysine Residue



reverse micelle should remain unreactive. We used the anionic peptide PSTAIR (EGVPSTAIREISLLKE, pI = 4.6) to bind to the positively charged reverse micelle (Figure 2a). When treated with sulfo-NHSA, the amine residues of the peptide are indeed

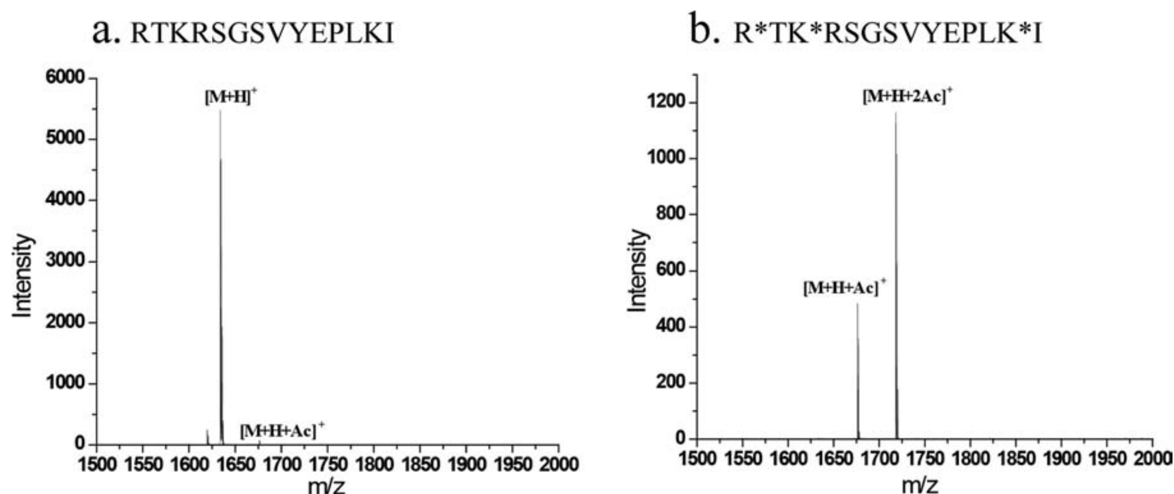


Figure 1. Mass spectra of malantide reacted with sulfo-NHSA (a) inside the negatively charged polymer reverse micelle at pH 7.0 and (b) in free aqueous solution at pH 7.0.

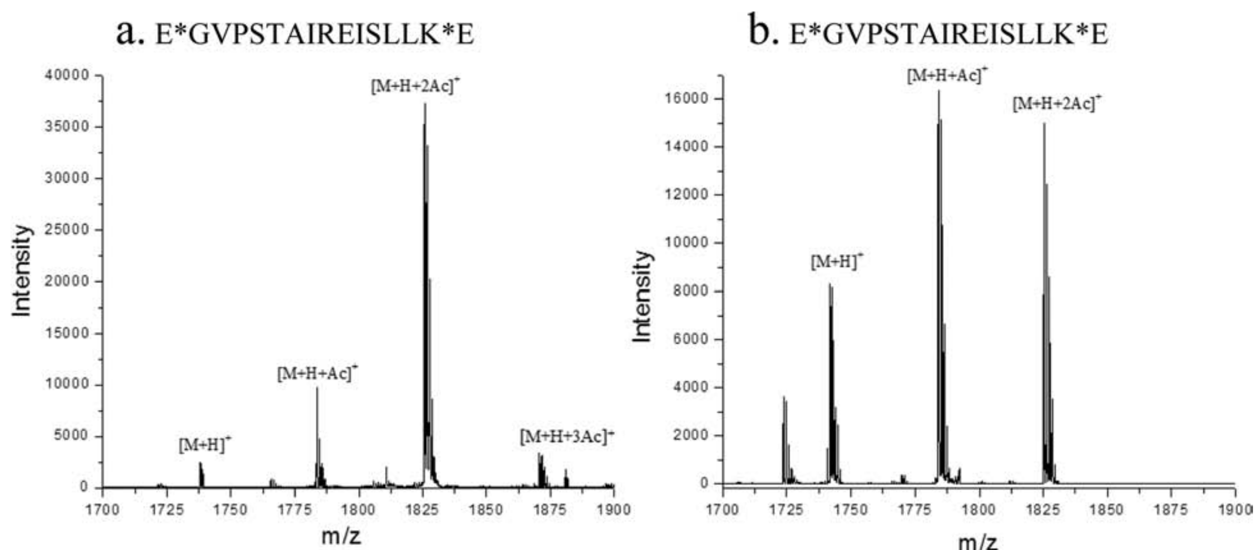


Figure 2. Mass spectra of PSTAIR reacted with sulfo-NHSA (a) inside the positively charged polymer reverse micelle at pH 7.0 and (b) in free aqueous solution at pH 7.0.

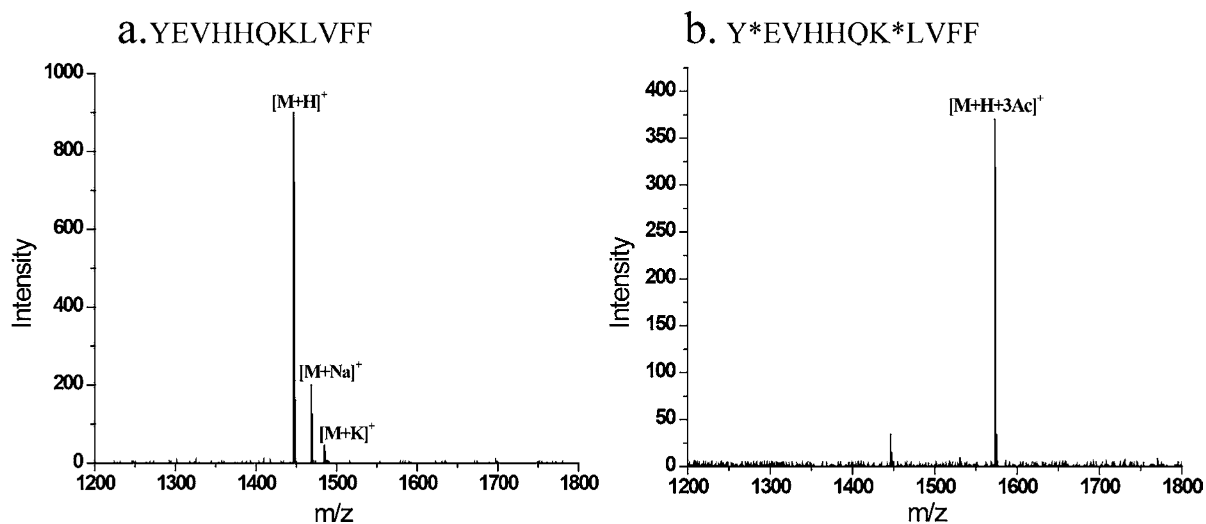


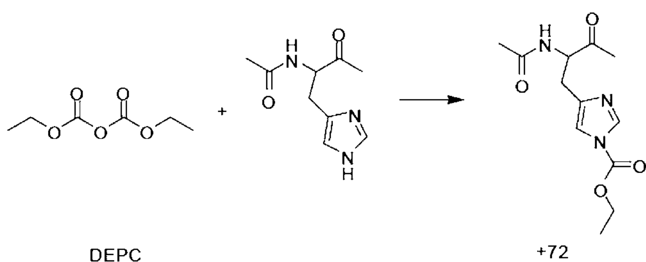
Figure 3. Mass spectra of β-Amyloid 10−20 reacted with sulfo-NHSA (a) inside the negatively charged polymer reverse micelle at pH 7.0 and (b) inside the positively charged polymer reverse micelle at pH 7.0.

quite extensively labeled once the peptide is inside the reverse micelles, as illustrated in Scheme 2b. MS/MS data confirm that both the lysine and N-terminus are acetylated by sulfo-NHSA (Figure S4). In fact, the MS results indicate that PSTAIR is more reactive with sulfo-NHSA inside the positively charged reverse micelle than in free solution (Figures 2b and S5). This could be attributed to the high local peptide concentration inside the reverse micelles or the favorable positioning of sulfo-NHSA in the positively charged interiors.

In the examples above, the polymer and the peptide were simultaneously varied to test whether electrostatics is the main reason for the observed difference in reactivity. The support for our hypothesis would be stronger, if we were to use the same peptide with two different polymers. This could be tested, because we have previously shown that peptides can be extracted by both negatively and positively charged polymers if the pH of the solution is close to the peptide's pI.[8b,10a] Accordingly, β-amyloid 10−20 (YEVHHQKLVFF, pI = 7.9) was reacted with sulfo-NHSA in both negatively and positively charged reverse micelles at pH 7.0. As shown in Figure 3a, there is no reactivity of the peptide toward sulfo-NHSA inside the negatively charged reverse micelle (akin to Scheme 2a). Inside the positively charged reverse micelle, however, the peptide is very reactive as shown in Figure 3b (akin to Scheme 2b). MS/MS confirms that both lysine and the N-terminus are labeled (Figure S6). Interestingly, a third labeling site is observed in the presence of the positively charged polymers, which could be due to the labeling of the glutamine or tyrosine side chains. MS/MS experiments are not able to unambiguously confirm the location of the third label. It is important to note that the reaction of the peptide in free aqueous solution at pH 7.0 shows a labeling pattern that is similar to that of the positively charged reverse micelle (Figure S7 and S8), but the extent of labeling in the positively charged reverse micelle is significantly greater.

An alternate explanation for all the observed reactions is that sulfo-NHSA is less reactive in the presence of negatively charged polymers, because of the electrostatic repulsion between the reverse micellar interior and the inherent charge of the reagent. To test this possibility, we studied a charge neutral reagent, diethylpyrocarbonate (DEPC). In addition to testing the above-mentioned possibility, DEPC's reactivity toward functional groups beyond amines provides the opportunity to further test the versatility of this approach. DEPC can also react with histidines to provide an increase in m/z of 72 per label (Scheme 4). We were interested in testing whether selective labeling of different peptide sites can be achieved based on pK_a differences of the side chain functionalities. DEPC can react with the N-terminus, histidine, and lysine residues; the reactive moieties in these residues exhibit pK_a values of about 8, 6, and 10, respectively. To illustrate the reaction selectivity that can be achieved with the negatively charged reverse micelles, three different types of peptides were examined: (i) a peptide with lysine and a free N-terminus; (ii) a peptide with histidine and a free N-terminus; and (iii) a peptide with histidine, lysine, and a free N-terminus.

Scheme 4. DEPC Labeling of Histidine Residue Increases m/z by 72 Units



Malantide (RTKRSGSVYEPLKI, pI = 10.3) contains two lysine residues and obviously an N-terminus but does not have a histidine residue. When this peptide is reacted with DEPC in free solution and inside the reverse micelle at pH 7.0 (Figure 4), both the lysine residues and the N-terminus are labeled in free solution (aqueous phase), as evidenced by the three DEPC additions to the peptide (Figure 4a). Note that the lysines are indeed protonated at pH 7.0 and therefore might be less available for reaction in the aqueous phase. It seems, though, that the equilibrium between the protonated and unprotonated forms is sufficient for this reaction to occur in the aqueous phase. In contrast, when the peptide is extracted and reacted inside the negatively charged reverse micelle at pH 7.0, the lysines no longer react with DEPC (Figure 4b). These results are consistent with our hypothesis that lysine is protected from the reaction through electrostatic interactions within the reverse micelle interior, as illustrated in Scheme 2c. Most of the N-termini should be protonated (90% in theory); however, a percentage of these functionalities are presumably available for reaction. Indeed, only the N-terminus is labeled at pH 7.0 inside the reverse micelles (see Figure S9 in for MS/MS data). These results with DEPC rule out electrostatic repulsion between a negatively charged sulfo-NHSA and the negatively charged reverse micelle interiors as the reason for the observed reactivity differences in Figure 1. It should be noted, however, that sulfo-NHSA did not react with the N-terminus of malantide in the reverse micelles at this pH (Figure 1). This is most likely due to the fact that DPEC is much more reactive than sulfo-NHSA.

We then tested the reactivity of malantide at pH 11.2, a condition under which the lysine residues and the N-terminus should not be protonated and thus should not be available for electrostatic interactions with the polymer, as illustrated in Scheme 2d. In this case, we find that all these residues react with DEPC both in free solution and inside the reverse micelles (Figure 4c,d). In addition to the lysine residues and the N-terminus, MS/MS data also show that Tyr9 is also labeled at this pH in both media (Figures S11 and S12). This is understandable, because tyrosine's pK_a is ∼10; as such, it should be deprotonated at pH 11.2 and becomes a good nucleophile to react with DEPC.

Our prior work has shown that the nature of the interaction between the polymer-based reverse micelles and the extracted molecules is based on electrostatics, not only using mass spectrometry as the analytical technique but also using absorption and fluorescence spectroscopy.[6,10a] To test whether the interaction between the peptide and polymer assembly is really due to electrostatics and more importantly to test whether the reactivity variations are due to the electrostatic interactions, we carried out DEPC modification of malantide in the presence of the carboxylate polymer **1** with varying salt concentrations (100, 250, and 500 mM and 1 M) at pH 7.0. The degree of lysine reactivity masking by the polymer gradually decreases with increasing ionic strength (Figure S13). In addition, when the DEPC labeling of malantide was carried out in the presence of a charge-neutral oligoethylene glycol-based reverse micelle (Chart S1), the peptide was not extracted from the aqueous solution, and there was no selectivity in peptide labeling (Figure S14). All these results clearly demonstrate the electrostatic nature of the reactivity control.

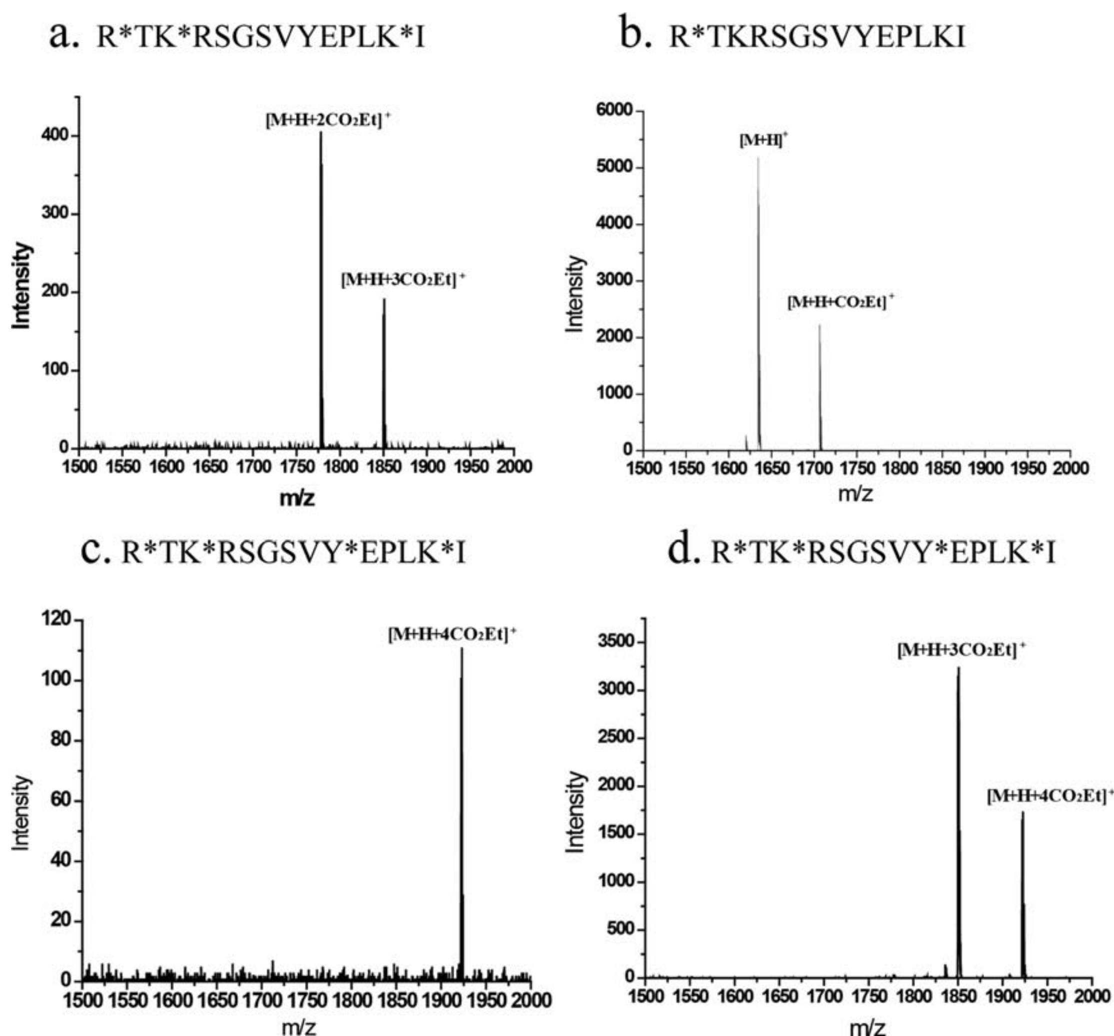


Figure 4. Mass spectra of malantide reacted with DEPC (a) in free aqueous solution at pH 7.0, (b) inside the negatively charged reverse micelles at pH 7.0, (c) in free solution at pH 11.2, and (d) inside the negatively charged reverse micelles at pH 11.2.

Next, we investigated a peptide containing a reactive N-terminus and a histidine residue. Kinetensin (IARRHPYFL, pI = 11.0) was reacted with DEPC at pH 5.5 and 7.0 to investigate the pH selectivity of this reaction (Figure 5). At a pH of 5.5, the side chain of histidine and the N-terminus should be protonated. Nonetheless, these functional groups still react with DEPC in free solution as evidenced by the two DEPC additions to the peptide (Figures 5a and S15). When the peptide is extracted and reacted inside the negatively charged reverse micelle at pH 5.5, however, both of these sites should be blocked for reactivity at this pH. Indeed, we find that only the unreacted peptide is found in the presence of the negatively charged reverse micelles (Figure 5b). If this failure to react is indeed due to electrostatic blocking, then the peptide should be more reactive at pH 7.0 because the histidine residue should not be protonated. When the reaction in free solution is conducted at pH 7.0, we again find the addition of two DEPC molecules in free solution (Figures 5c and S16) with the MS/MS data confirming that both His5 and the N-terminus are labeled. In contrast, we find the addition of only one DEPC molecule when the peptide is reacted inside the reverse micelle at pH 7.0 (Figure 5d). MS/MS data indicate that the peptide ions with one DEPC added are a mixture of two isomers, a predominant one in which His5 is labeled and another in which the N-terminus is labeled (Figure S17). His5 is the predominantly labeled site because at this pH most of the histidine residues in the peptides are not protonated. With a typical pK_a around 8, a small fraction (~10% in theory) of the N-termini in the peptides should also not be protonated and could therefore be reactive, but not to the same extent as the His residues. Together these data suggest that simple changes to pH can enable selective labeling of amino acid side chains inside the reverse micelles of these amphiphilic homopolymers. As with malantide, we also carried out DEPC labeling of kinetensin inside negatively charged reverse micelle at different solution ionic strengths at pH 5.5 (Figure S18). While the interaction between malantide's lysine and the polymer was fully screened at much higher salt concentrations, the anticipated weaker interaction between the histidine units of kinetensin and the carboxylate polymer at pH 5.5 was screened even at 100 mM salt concentration. As with malantide, kinetensin also was not extracted into the organic phase, and no selectivity in labeling was observed with the charge-neutral oligoethylene glycol-based reverse micelle (Figure S19).

We then studied a peptide containing lysine, histidine, and N-terminal functional groups. Apelin 13 (QRPRLSHKGPMPA, pI = 12.4) was reacted with DEPC at pH 5.5, 7.0, and 10.0 (Figure 6). At pH 5.5, the peptide reacts in free solution (Figures 6a and S20), but no reactivity is observed inside the reverse micelles

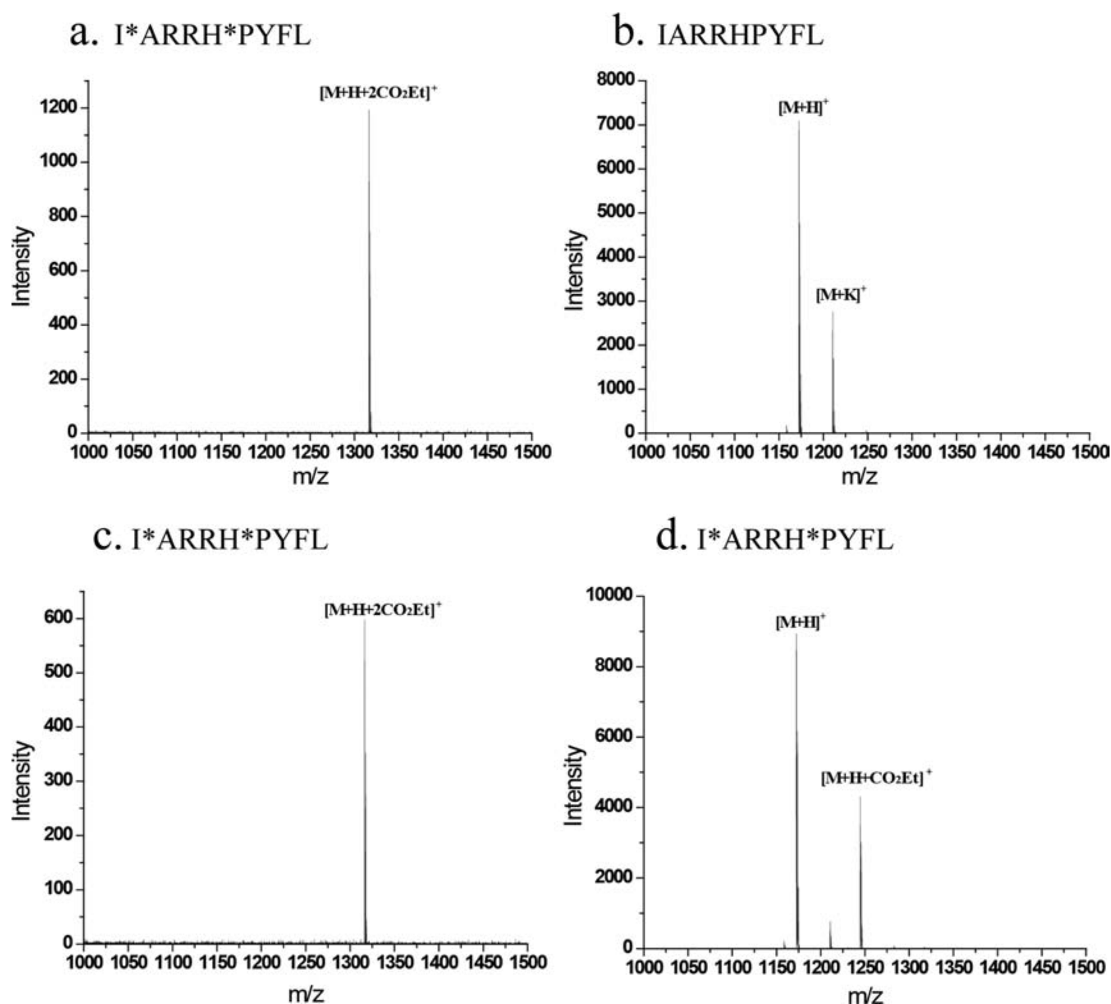


Figure 5. Mass spectra of kinetensin reacted with DEPC (a) in free aqueous solution at pH 5.5, (b) inside the negatively charged reverse micelles at pH 5.5, (c) in free solution at pH 7.0, and (d) inside the negatively charged reverse micelles at pH 7.0.

(Figure 6b). This observation is consistent with the expectation that N-terminus, histidine, and lysine groups are all protonated and protected from reaction at this pH (akin to Scheme 2a). At pH 7.0 however, the peptide reacts with DEPC inside the reverse micelles (Figure 6c), but only histidine and the N-terminus are labeled (akin to Scheme 2c). The lysine moiety is blocked, because it is still protonated at this pH (see Figure S21 for MS/MS data). When the pH is increased to 10.0, the peptide is even more reactive (Figure 6d and Scheme 2d), and a small degree of labeling of the lysine residue can indeed be found (see Figure S22 for MS/MS data). Again, these results are consistent with our hypothesis that electrostatic interactions within the reverse micelles control reactivity and also that this reactivity can be tuned by varying aqueous solution pH. As a general control for the DEPC labeling experiments, we also investigated the reactivity of peptides in positively charged polymers and found again that reactive functional groups were not protected from reaction inside the positively charged reverse micelles (Figures S23−S25).

We were interested in finding whether we can concurrently perform selective binding between the two phases and execute the selective labeling of the peptides based on the electrostatic masking. In this case, we anticipated that we would be able to achieve selectivity based on both the pI of the peptide and the pK_a of the specific side chain. If the pI of the peptide is such that it is not extracted, the side chains in that peptide might not be protected even though the pK_a of the side chain is suitable for electrostatic masking. To test this possibility, we studied the sulfo-NHSA labeling of malantide (pI 10.3) and PSTAIR (pI 4.6) inside negatively charged supramolecular assemblies at pH 7.0. Considering the negative charge of the reverse micelle assembly, the positively charged malantide should be selectively extracted inside, while PSTAIR should be left behind in the aqueous phase. Indeed, as seen in Figure 7, the lysine residue in malantide is blocked from reacting with sulfo-NHSA, while the lysine in PSTAIR was labeled by the reagent (Figures S26 and S27). This demonstrates that both the pI of the peptide and the pK_a of the functional groups in the peptide can be used to control labeling selectivity in the presence of the appropriately charged amphiphilic homopolymer-based supramolecular assemblies. The versatility of this approach was further confirmed by testing the phenomenon with DEPC as the labeling agent and by using different peptide mixtures of PSTAIR and apelin 13 (Figure S28−S33).

CONCLUSIONS

We have developed an amphiphilic homopolymer-based supramolecular assembly that allows selective labeling of side chain functionalities in peptides. We have shown that: (i) peptide side chain functional groups are masked for reaction when the peptide

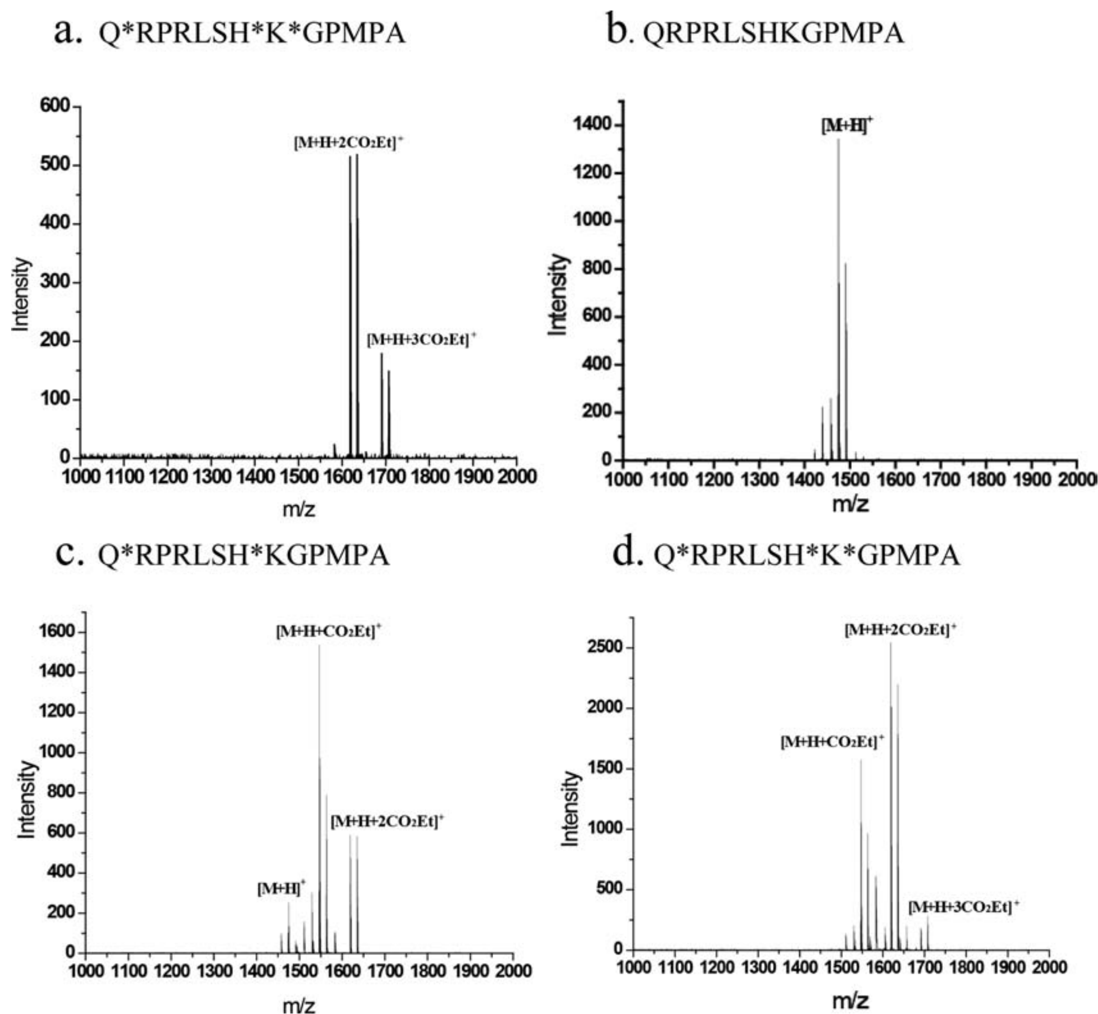


Figure 6. Mass spectra of apelin 13 reacted with DEPC (a) in free aqueous solution at pH 5.5, inside negatively charged reverse micelles at (b) pH 5.5, (c) pH 7.0, and (d) pH 10.0. Note: The unlabeled peaks in each spectrum correspond to oxidative modifications to the side chain of methionine. These oxidative modifications are also observed in control experiments, indicating that the peptide is oxidized in the pure sample.

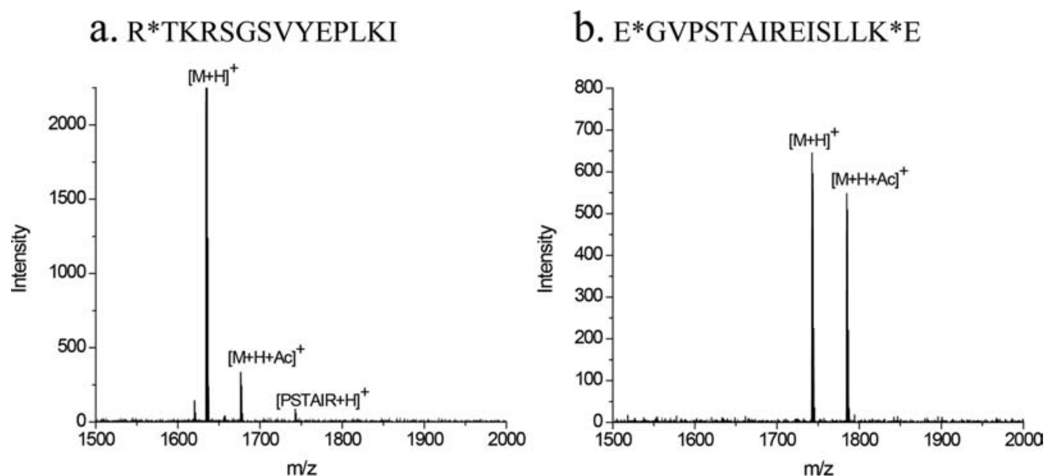


Figure 7. Mass spectra of malantide and PSTAIR reacted with sulfo-NHSA in negatively charged assemblies at pH 7.0 (a) malantide and (b) PSTAIR.

is electrostatically bound to the polymer; (ii) even if a peptide is extracted into the reverse micelle, reactivity masking of a functional group occurs only when they are electrostatically engaged in a binding interaction with the complementary polymer functional groups; (iii) by varying the pH of the solution, site selective labeling of amino acid residues can be achieved by taking advantage of the inherent differences in their pK_a; and (iv) the combination of pI of the peptide and the pK_a of the functional group can be used to achieve labeling selectivity with respect to both the functional group and the peptide. We

Table 1. Summary of the Selective Labeling of Peptides in the Presence of Polymeric Reverse Micelles

peptide	labeling reagent	charge of reverse micelle	pH	labeling result[a]
malantide	sulfo-NHSA	negative	7.0	RTKRSGSVYEPLKI
		aqueous phase	7.0	**R**T**K**RSGSVYEPL**K**I
	DEPC	negative	7.0	**R**TKRSGSVYEPLKI
		aqueous phase	7.0	**R**T**K**RSGSVYEPL**K**I
		negative	11.2	**R**T**K**RSGSV**Y**EPL**K**I
		aqueous phase	11.2	**R**T**K**RSGSV**Y**EPL**K**I
PSTAIR	sulfo-NHSA	positive	7.0	**E**GVPSTAIREISLL**K**E
		aqueous phase	7.0	**E**GVPSTAIREISLL**K**E
	DEPC	positive	5.5	EGVPSTAIREISLL**K**E
		positive	7.0	EGVPSTAIREISLL**K**E
β-amyloid 10–20	sulfo-NHSA	negative	7.0	YEVHHQKLVFF
		positive	7.0	**Y**EVHHQ**K**LVFF
kinetensin	DEPC	negative	5.5	IARRHPYFL
		aqueous phase	5.5	**I**ARR**H**PYFL
		negative	7.0	**I**ARR**H**PYFL
		aqueous phase	7.0	**I**ARR**H**PYFL
apelin 13	sulfo-NHSA	negative	7.0	QRPRLSHKGPMPA
		aqueous phase	7.0	**Q**RPRLSH**K**GPMPA
	DEPC	negative	5.5	QRPRLSHKGPMPA
		aqueous phase	5.5	**Q**RPRLS**HK**GPMPA
		negative	7.0	**Q**RPRLS**H**KGPMPA
		aqueous phase	7.0	**Q**RPRLS**HK**GPMPA
		negative	10.0	**Q**RPRLS**HK**GPMPA
		aqueous phase	10.0	**Q**RPRLS**HK**GPMPA

[a]The labeled sites, identified through MS/MS, are in bold and underlined.

have summarized the experimental findings that led to these conclusions in Table 1. Selective labeling of specific functionalities among a multitude of available sites using our system could open a potentially new and convenient approach for structural elucidation studies and functional investigations of biomolecules. Our polymeric supramolecular assembly could also have the potential to improve targeted proteomics or biomarker studies by selectively labeling peptides of interest in complex mixtures with functional groups that enhance their detection efficiency by MS.

■ EXPERIMENTAL METHODS

Reagents. Kinetensin, malantide, apelin 13, PSTAIR, and β-amyloid 10–20 were obtained from the American Peptide Company. Sulfo-*N*-hydroxy succinimideacetate (sulfo-NHSA) was purchased from Thermo Scientific. 3-(*N*-morpholino)propanesulfonic acid (MOPS), diethylpyrocarbonate (DEPC), α-cyano-hydroxycinnamic acid (α-CHCA), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich. Water, used in these experiments, was purified first using a Milli-Q water purification system (Millipore, Bedford, MA). Tetrahydrofuran (THF), toluene, ammonium hydroxide (NH_4OH), and sodium hydroxide (NaOH) were purchased from Fisher Scientific. THF was distilled over Na/Ph_2CO before use. All other chemicals were used as obtained from commercial sources.

Instrumentation. MALDI-MS analyses were performed on a Bruker Autoflex III time-of-flight mass spectrometer. All mass spectra were acquired in the reflectron mode, and an average of 200 laser shots at an optimized power (20–60%) was used. Collision-induced dissociation (CID) via the fragmentation analysis and structural TOF (FAST) mode was used for the tandem MS (MS/MS) experiments. In the FAST mode, a specific precursor ion mass of interest was chosen, and the settings were modified so that the acquisition of at least 10 segments of product ions was possible. Argon gas was used for CID, and the pressure was adjusted to obtain optimum results. BioTools was used to facilitate the analysis of the tandem mass spectra. Immonium ions in the low m/z range were found to be the most diagnostic ions for determining the amino acids that were labeled.

Peptide Extraction. Aqueous solutions of the peptides were prepared using a peptide concentration of 5.0×10^{-7} M in 25 mM MOPS, and NH_4OH or NaOH was added to achieve the desired aqueous phase pH. The carboxylate polymer at a concentration of 1.0×10^{-4} M and the quaternary ammonium polymer at a concentration of 3.3×10^{-5} M were prepared separately in toluene. The critical micelle concentrations (cmc) of the carboxylate and quaternary ammonium polymer are 0.34 mg/mL (1.5×10^{-5} M) and 0.78 mg/mL (7.3×10^{-6} M), respectively (see Figure S1), and so the concentrations used in the experiments exceed the cmc's for both polymers. The concentrations of the peptide in the aqueous phase and polymer in the toluene phase were also chosen to ensure complete transfer of the peptide into the reverse micelle. These concentrations were chosen based on previous reverse micelle capacity measurements, as described previously.[10a] A two-phase extraction was performed by first mixing 1 mL of the peptide-containing aqueous solution with 200 μL of the toluene solution containing the reverse micelles. The mixture was vortexed for 2 h before centrifuging the sample to separate the two phases. The toluene phase was dried with flowing N_2 gas, redissolved in 10 μL THF, mixed with 20 μL of an α-CHCA matrix solution (22.5 mg in 350 μL THF/150 μL H_2O/6 μL TFA), spotted on a MALDI target, and then readied for analysis by MALDI-MS. For the control experiments in free aqueous phase, a solution containing the peptide at a concentration of 5×10^{-7} M in 25 mM MOPS at the desired pH was reacted with the labeling reagent for 1–2 h. The amount of labeling reagent added was the same molar ratio added to the reverse micelle solutions. After reaction, these control solutions were immediately analyzed by MALDI-MS after 10 μL this solution was mixed with 10 μL matrix solution and then spotted it on a MALDI target.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis, other experimental details, and tandem mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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