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Supplementary Material

An Artificial Receptor Synthesized by Surface-confined Imprinting for the Recognition of Acetylation on Histone H4 K16

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1. Experimental section

Materials. The peptides with sequences: GGAKacR, GGAK, GGAKR, GGVKacR, GLGKacGGAKacR [H4-K12/16Ac], GKacGGKacGLGKacGGAKacR [H4-K5/8/12/16Ac] GGAK(boc)R(pbf) and GGAKacR(pbf) were purchased from ChinaPeptides Co., Ltd. (Shanghai, China). The purities of the peptides were >95%. Histone was from MAYA Reagent (Zhejiang, China) and trypsin was from Beijing Hua Lishi Scientific Co. Ltd. (Beijing, China). Silica gel (surface area: 232 $m^2 \cdot g^{-1}$, pore volume: 0.89 $cm^3 \cdot g^{-1}$, pore size: 170 nm and average particle diameter: 20 μm) were purchased from Nano-Micro Co. Ltd. (Suzhou, China). The 3-aminopropyltriethoxysilane (APTES, 99%), methylacrylic acid (MAA, 99%) and *N,N*-ethylenebis(acrylamide) (EBA, 96%) were purchased from Tianjin Heowns Biochemical Technology Co., Ltd (Tianjin, China). MAA was purified prior to use via distillation under reduced pressure. The *N,N*-methylenebis(acrylamide) (MBA, 98%) and 2-(trifluoromethyl)acrylic acid (TFMAA, 98%) were obtained from J&K chemical (Beijing, China). Hydrofluoric acid and glutaraldehyde were purchased from the Tianjin Chemical Reagent Research Institute (Tianjin, China). Ammonium persulfate (APS, >98%) and acetic acid were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All chemicals were of analytical grade unless noted other-wise. Ultra-pure water, obtained from a NanopureII system (Barnstead, USA) was used in the experiments unless other-wise stated. In the manuscript, PBS without the specification of the concentration and pH refers to the phosphate buffer solution with 20 $mmol \cdot L^{-1}$ and pH 7. It was prepared with NaH_2PO_4/Na_2HPO_4 according to the *Handbook of Biochemistry and Molecular Biology*.[1]

Synthesis of surface imprinted microspheres using silica as sacrificial support

The surface imprinted microspheres were synthesized by three major steps: immobilization of template peptide on the silica surface, imprinting polymerization and removal of the template-bound silica.

Immobilization of aminopropyl groups on the silica surface

The silica gels were activated by hydroxylation in 10% (v/v) hydrochloric acid and then washed and dried under vacuum. The activated silica beads (5 g) were suspended in 50 mL dry toluene. After addition of 6.5 mL 3-aminopropyltriethoxysilane, the suspension was

refluxed for 12 h under nitrogen protection. The silica grafted with aminopropyl group (denoted as silica-NH_2) was washed with toluene, acetone and dried under vacuum.

Immobilization of aldehyde groups on the surface of silica

The freshly obtained silica-NH_2 particles (5 g) were immersed in 25 mL PBS containing 6.0 mmol glutaraldehyde. The reaction was performed for 6 h at room temperature under stirring. The resulting aldehyde-modified silica, denoted as silica-CHO, was then rinsed with deionized water, ethanol and dried under vacuum at 45 °C for 12 h.

Coupling of GGAKacR on the surface of silica

The aldehyde-modified silica beads (2 g) were added into 20 mL PBS containing the GGAKacR(pbf), the template peptide with arginine side chain protection by 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (pbf). The concentration of the peptide was 40 mg mL^{-1}. The mixture was incubated for 24 h at room temperature. The peptide modified silica, denoted as GGAKacR(pbf)-Si, was washed with PBS and ethanol and then dried for 12 h at 45 °C under vacuum. The arginine side chain was de-protected by mixing the GGAKacR(pbf)-Si (2 g) with 30 mL 80% TFA in ethanol and shaking for 30 min at room temperature. The product, denoted as GGAKacR-Si, was washed with ethanol and then dried under vacuum at 60 °C for 24 h.

Coupling of GGAKR on the surface of silica

The procedure of coupling GGAKR on the silica was the same as coupling of GGAKacR. The peptide GGAK(boc)R(pbf) with Boc and pdf protections for side chains of Lys and Arg respectively was used in the coupling reaction. The product was denoted as GGAKR-Si.

Synthesis of MIP beads with surface-confined binding sites.

The peptide-modified silica beads (GGAKacR-Si or GGAKR-Si, 0.5 g) were immersed in 1.5 mL of phosphate buffer solution (PBS, 20 mM, pH 7.0) containing functional monomer, cross-linker and initiator (APS). The concentration of the functional monomer (TFMAA) and cross-linker (EBA) was 0.6 $mol \cdot L^{-1}$ and 0.9 $mol \cdot L^{-1}$, respectively. The ratio of APS to the total monomers was 1:100 (w/w). The volume of the pre-polymerization solution is two times larger than the nominal total pore volume of silica in the reaction. After incubation for 4 h at room temperature, ethanol (about 10 mL) was added into the mixture. The liquid outside the particles was removed by a quick vacuum suction with a sand core funnel. Then

the particles were transferred into a glass vial. After deaerated with N_2, the vial was sealed and polymerization was performed for 24 h at 45 °C. Different literatures[2-5] have been referred in establishing the method of soaking the polymerization solution into silica.

In a screw-capped polypropylene tube, the obtained composite material was suspended in 10 mL acetone. Then 1.5 mL of 40% aqueous HF was added slowly into the tube under shaking in an ice water bath. After incubation overnight at room temperature on a rocking table, the suspension was diluted with 100 mL deionized H_2O, filtered and then washed extensively with H_2O/acetone (80/20, v/v) until the solution was neutral. The non-imprinted polymers (NIPs) were synthesized with the same method except using silica-CHO instead of GGAKacR-Si in the polymerization. The yield of polymer was about 85%-90% for imprinted and non-imprinted polymers, calculated by the mass ratio of the obtained polymer and total monomers added into nominal total pore volume of silica in the reaction. The GGAKR imprinted polymer was denoted as GGAKR-MIP. The MIP refers to the GGAKacR imprinted MIP in this manuscript.

The reaction of peptide immobilization, formation of the imprinted binding sites and rebinding of the template and longer peptide by epitope approach are demonstrated in the Fig. S1.

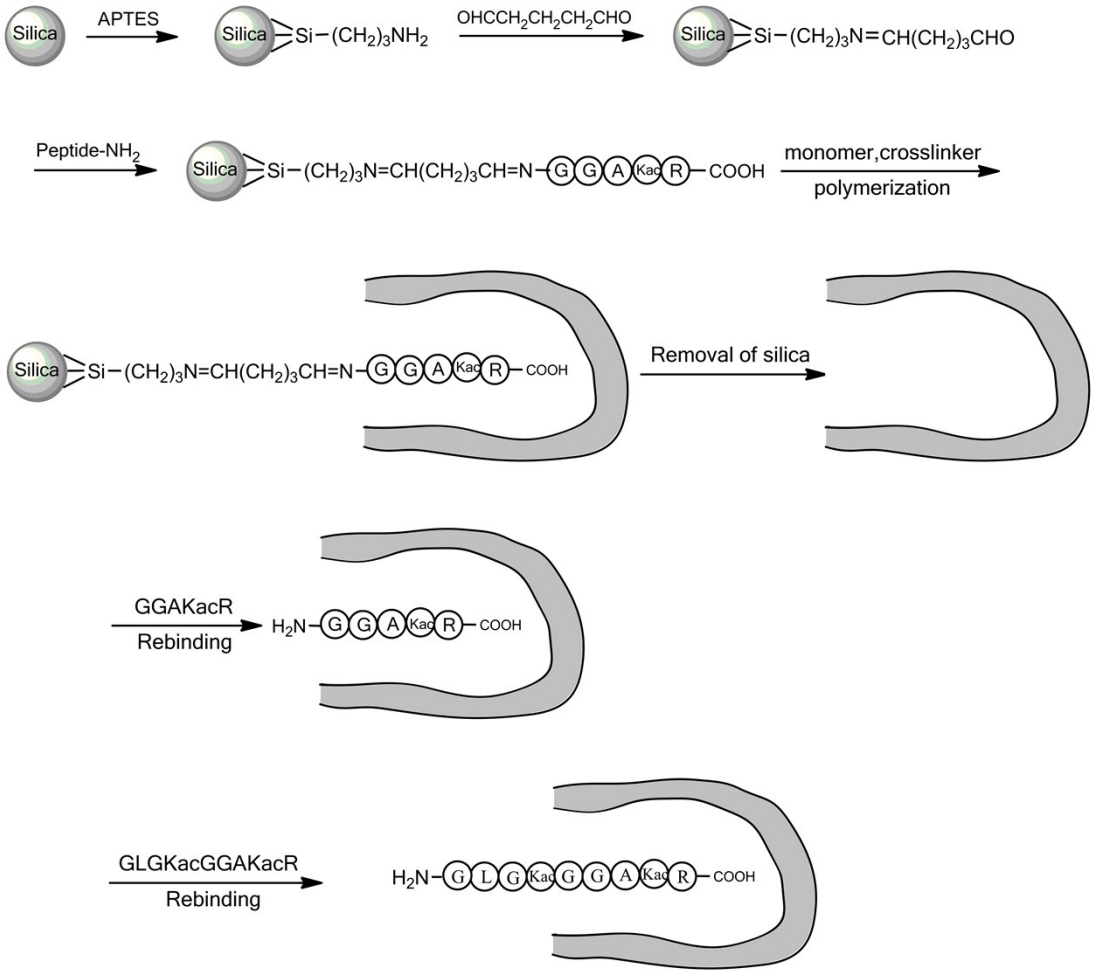

Fig. S1 The reaction of peptide immobilization, formation of the imprinted binding sites and rebinding of the template and longer peptide by epitope approach

Characterization of the MIP and modified silica

The morphology of the MIP beads was assessed by the scanning electron microscope (SEM). After each grafting reaction, the elemental analysis was performed using an elemental analyzer (Vario EL CUBE, Elementar, Germany). Fourier Transform Infrared Spectrometer (AVATAR-360, Nicolet, USA) was employed to study the composition of the materials. A HPLC instrument (Shimadzu Co., Ltd., Japan) equipped with two 20AD pumps and an SPD-20A UV detector was used for the chromatographic analysis**.** MALDI-TOF mass spectrometer (AutoflexIII LRF200-CID, Bruker Daltonics, Germany) was used for the analysis of peptides in the histone digestion extraction experiment. The MALDI-TOF MS analysis was performed with positive ion mode and a pulsed nitrogen laser operating at 337 nm. The α-cyano-4-hydroxycinnamic acid was used as the matrix in the MALDI-TOF. The

mass spectra with m/z ranging from 500 to 2200 was acquired. The Flexanalysis (ver. 3.0) software was used for analysis and post process. Circular dichroism (CD) spectra were obtained at room temperature on a JASCO model J-715 spectropolarimeter. The cylindrical fused quartz cell with an optical path length of 1.0 cm was used in the measurement. In the experiment, the solvent baseline was subtracted, and the spectra were normalized in units of molar ellipticity [θ] (deg $cm^2 \cdot dmol^{-1}$). The swelling property of the polymer was investigated by using a lab-made graduation glass tube with graduation. The swelling ratio was determined by the ratio of volume difference between the swollen polymer and the dry polymer. The swelling ratio for the MIP-3 was 28% in PBS (20 mM, pH 7.0).

Equilibrium binding study

Equilibrium binding experiment for GGAKacR and Scatchard analysis

The binding isotherms of peptides on MIP and NIP were determined by batch binding experiment. MIP or NIP particles (5 mg) were incubated in 2.0 mL of GGAKacR solution for 24 h at room temperature under gentle shaking. The GGAKacR solutions with concentrations ranged from 0.1 to 2.0 $mmol \cdot L^{-1}$ were prepared in PBS (20 $mmol \cdot L^{-1}$ and pH 7) and used in the experiment. After incubation, the mixture was centrifuged and the concentration of peptide in the supernatant was determined by HPLC analysis using a C_{18} analytic column. ACN/H_2O (7/93, v/v, 0.1% TFA) with a flow rate of 1.0 $mL \cdot min^{-1}$ was used as mobile phase and the analytes were detected at 205 nm. The amount of the bound peptide in unit mass of dry polymer (Q, $\mu mol \cdot g^{-1}$) was calculated according to Equation 1.

$$Q = \frac{(C_0 - C_t)V}{w} \qquad (1)$$

Where C_0 and C_t ($mmol \cdot L^{-1}$) are the concentrations of peptides in the initial solution and in the supernatant respectively. The V (mL) is the volume of solution and w (g) is the mass of the polymers.

To have a further analysis of the binding property of the MIP, the Scatchard analysis was performed. The equation: $B/F = (B_{max} - B)K_a$ was used to calculate the association constant and apparent maximum number of binding sites. In the equation, B and F are the concentrations of bound analytes and free analyte respectively. K_a is the association constant

and B_{max} is the apparent maximum number of binding sites.

Equilibrium binding experiment for selectivity evaluation

To study the imprinted selectivity of the MIP, the binding affinity of the MIP for different peptides were compared. The PBS solution with peptide concentration of 1.0 mmol·L^{-1} was used in the binding experiments. The same procedure in section "*Equilibrium binding experiment for GGAKacR and Scatchard analysis*" was used in the batch binding experiment. The correlated binding affinity of NIP for theses peptides were also determined to evaluate the imprinting effect.

The imprinted selectivity (IS) was calculated by

$$IS = \frac{Q_{MIP}}{Q_{NIP}} \qquad (2)$$

Where Q_{MIP} and Q_{NIP} (μmol·g^{-1}) are the bound amount of peptide on the MIPs and on the corresponding NIPs, respectively.

Competitive binding experiment

The PBS (20 mM, pH 7.0) containing GGAKacR and five other peptides with equal concentration (1.0 mmol·L^{-1}) were prepared. To 2.0 mL aliquot of peptide mixture solution, MIP or NIP particles (5 mg) were added. The suspension was incubated for 24 h at room temperature under gentle shaking. Thereafter, the mixtures were centrifuged and the free peptides were determined by HPLC analysis. The amount of each peptide bound to the polymer was calculated by Equation 1.

HPLC evaluation

The surface imprinted and non-imprinted microspheres were soaked in PBS and slurry packed into two stainless steel HPLC tubes (20 mm × 4.6 mm i.d.) separately. The columns were washed with phosphate buffer (20 mM, pH 7.0) until a stable baseline was obtained. The chromatographic analysis was performed by using phosphate buffer solutions (20 mM, pH 7.0) as the mobile phase at a flow rate of 0.1 mL·min^{-1}. Different peptide samples with concentration of 1.0 mmol·L^{-1} in PBS were analyzed and detected by UV detection at 205

nm. The retention factor k was calculated by the equation: $k = (t_r - t_0)/t_0$, where t_r is the retention time of the analytes and t_0 is the dead time measured by acetone. The selectivity factor α is defined as $\alpha = k_t/k_a$, where k_t and k_a are the retention factors of the template and its analogues, respectively.

Extraction of GGAKacR and GLGKacGGAKacR from spiked histone digest by MIP

Histone (1 mg) was dissolved in 800 μL of denaturing buffer solution (100 mM ammonium bicarbonate, pH 8.5). A 200 μL aliquot of 0.1 mg·mL^{-1} trypsin solution was added to obtain a trypsin-to-protein ratio of 1:50 (w/w). The digestion was carried out overnight at 37 °C. The reaction was stopped by adding 5 μL acetic acid. The GGAKacR or two peptides (GGAKacR and GLGKacGGAKacR) spiked digest solutions were prepared by mixing 400 μL of digested histone solution with 10 μL peptide stock solution and then diluted with PBS. The molar ratios of GGAKacR/histone and GLGKacGGAKacR/histone were 1/5 in the spiked solution.

In the extraction, the spiked histone digest (2.0 mL) was added into a vial containing 10 mg imprinted polymers. The mixture was shaken at room temperature for 12 h. After centrifugation, the supernatant was withdrawn. The imprinted polymers were washed with 500 μL PBS for three times. In the wash step, the MIP particles suspended in PBS were shaken for 20 min and then the supernatant was decanted subsequently. After the washing step, the peptides bound on the MIP particles were eluted by shaking in HAc/ACN/H_2O (5/5/90, v/v) for 2 h. The elution processes were performed three times. The eluted fractions were combined and lyophilized. The residues were dissolved in 2.0 mL PBS and analyzed by MALDI-TOF MS and HPLC analysis. In the HPLC, the same C_{18} column as in the equilibrium binding experiment section was used. The mobile phase A was 0.1% TFA in ACN and mobile phase B was 0.1% TFA in H_2O. The column was conditioned in 1% B. The gradient elution: 1-50 min (1% -95% B) was employed. The flow rate was 1.0 mL/min. The analytes were detected at 205 nm.

For comparison, the same procedure was used for the extraction of GGAKacR and GLGKacGGAKacR from spiked histone digest by NIP.

2. Selection of imprinting condition in the imprinting polymerization

Different imprinting conditions have been studied. The two monomers and cross-linker used in the research are listed in the Table S1.

Table S1. Different synthetic conditions used in the imprinting process [a]

Polymer	Monomer	Cross-linker	Cross-linker/monomer (molar ratio)
MIP-1	MAA	MBA	0.23
MIP-2	MAA	EBA	1.5
MIP-3	TFMAA	EBA	1.5

[a] Total GGAKacR in the reaction was 0.01 mmol calculated by the product of density of the GGAKacR on silica and mass of GGAKacR-Si. The molar ratio of monomer/template was 30/1. The initiator was APS and solvent was phosphate buffer. The other conditions were shown in the experimental section.

The equilibrium binding study was used to evaluate the binding affinity of the resulting MIPs. The concentration of the GGAKacR were ranged from 0.2-2.0 $mmol \cdot L^{-1}$. The imprinting factor (IF) was used to compare the imprinting effect of the MIPs (Fig. S2). The IF was calculated by $IF=Q_{s(MIP)}/Q_{s(NIP)}$, in which $Q_{s(MIP)}$ and $Q_{s(NIP)}$ are the saturated bound amount of GGAKacR on the MIP and NIP respectively.

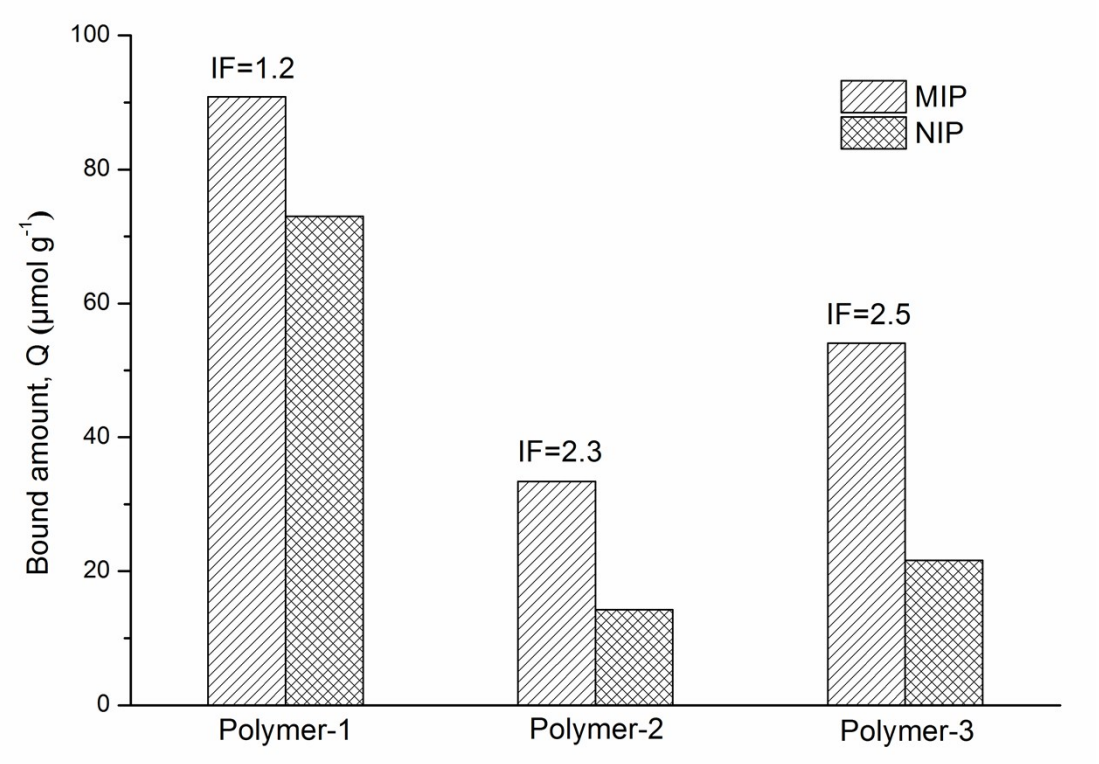

Fig. S2 The binding capacity and imprinted factors of MIPs for the GGAKacR from the equilibrium binding experiments. The experimental condition is in the experimental section. The Polymer-1 represents the MIP-1 and NIP-1, Polymer-2 for the MIP-2 and NIP-2 and Polymer-3 represents the MIP-3 and NIP-3, respectively.

3. Characterization of the derivertized silica and imprinted polymer

The density of the grafted groups on the derivertized silica

After each silica modification, the surface area density (*D*s) of the coupled ligands was estimated by elemental analysis. The results of the elemental analysis are shown in the Table S2.

Table S2 The results of elemental analysis for derivatized silica

Material	Silica gel	silica-NH_2	silica-CHO	GGAKacR-Si
C%	-	4.01	9.07	10.11
H%	0.83	1.31	2.01	2.32
N%	-	1.43	1.23	1.57

The *D*s of the aminopropyl groups on the silica-NH_2 were 5.11 μmol·m^{-2} calculated by the increment of nitrogen content. The surface area densities of aldehyde groups on the Si-CHO and GGAKacR on the GGAKacR–silica were 3.58 and 0.1 μmol·m^{-2} respectively, estimated by increment of the carbon content.

FT-IR analysis

Fourier Transform Infrared Spectroscopy was employed to study the composition of the materials (Fig. S3).

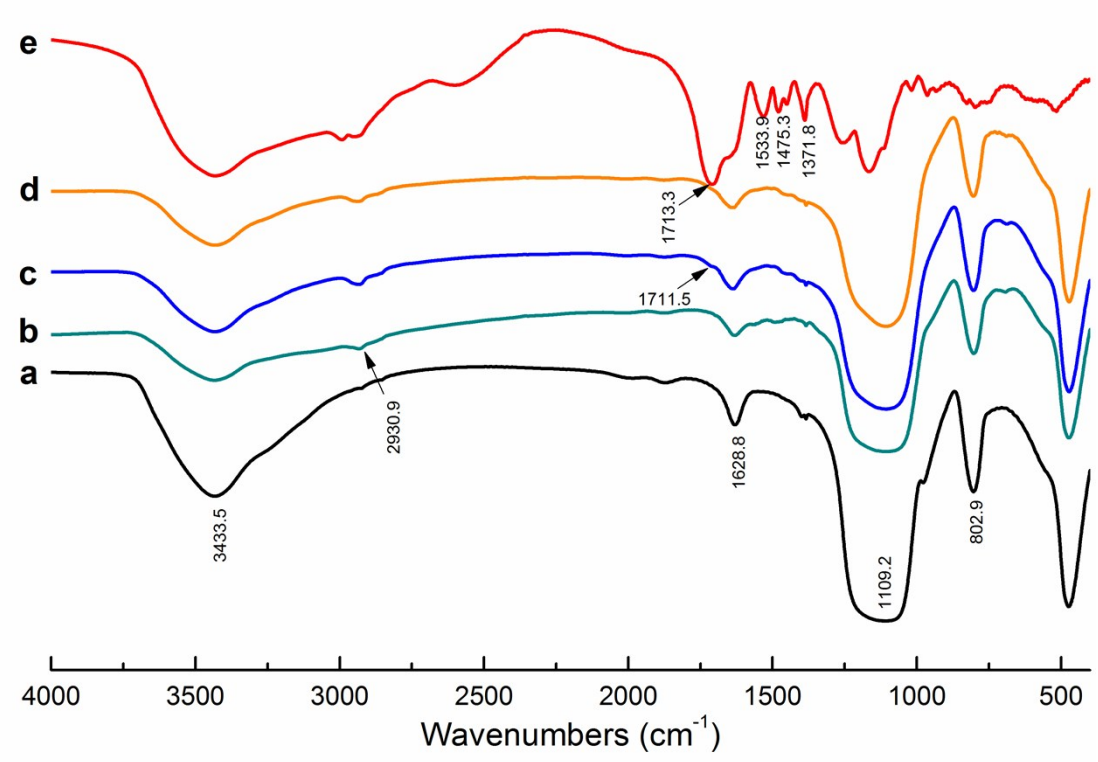

Fig. S3 FT-IR spectra of silica gel (a), Si-NH_2 (b), Si-CHO (c), Si-GGAKacR (d) and MIP-3 (e)

In the FT-IR spectrum of blank silica, the absorptions at 1109.2 cm^{-1} and 802.9 cm^{-1} (the Si–O–Si asymmetric and symmetric stretching, respectively) have shown the characteristic silica bands. After modification with aminopropyl groups, the peak at 2930.9 cm^{-1} corresponding to C–H stretch of methylene, appeared in the FT-IR of Si-NH_2. In the FT-IR of Si-CHO, the peak at 1711.5 cm^{-1} from C=O stretching appeared, which verified the

successful grafting of aldehyde group. In the FT-IR of MIP-3, strong peak at 1713.3 cm^{-1} due to the C=O stretching was observed. The absorptions at 1533.9 cm^{-1} (N–H bending), 1475.3 cm^{-1} and 1371.8 cm^{-1} (C–H bending) from the compositions of TFMAA and EBA also appeared. Furthermore, the Si–O–Si asymmetric and symmetric stretching at 1109.2 cm^{-1} and 802.9 cm^{-1} reduced considerably in the FT-IR of MIP-3, which has shown the successful removal of the silica.

Morphology of the silica and MIP studied by scanning electron microscopy

The scanning electron micrographs of silica and MIP-3 are shown in the Fig. S4. The result demonstrated that the dispersive MIP particles with similar size as silica were obtained.

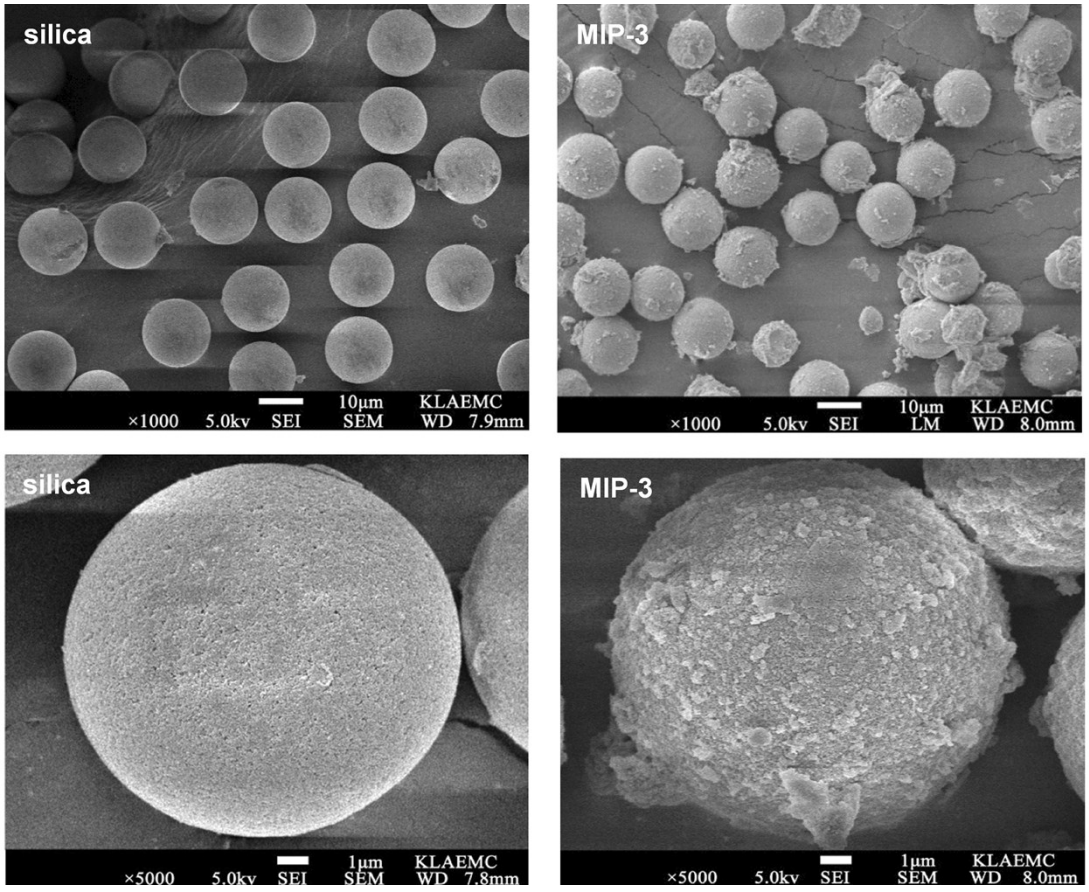

Fig. S4 Scanning electron micrographs of silica and MIP-3

4. Evaluation of binding affinity of GGAKacR imprinted polymer by equilibrium binding study

The equilibrium binding isotherms of GGAKacR on the MIP and NIP (Fig. S5) were plotted with the concentration of analyte bound to a polymer (*B*) versus the concentration of free analyte remaining in solution (*F*). The result demonstrated that MIP has much higher binding affinity than the NIP. The imprinting factor was 2.5, calculated by IF=$Q_{s(MIP)}/Q_{s(NIP)}$.

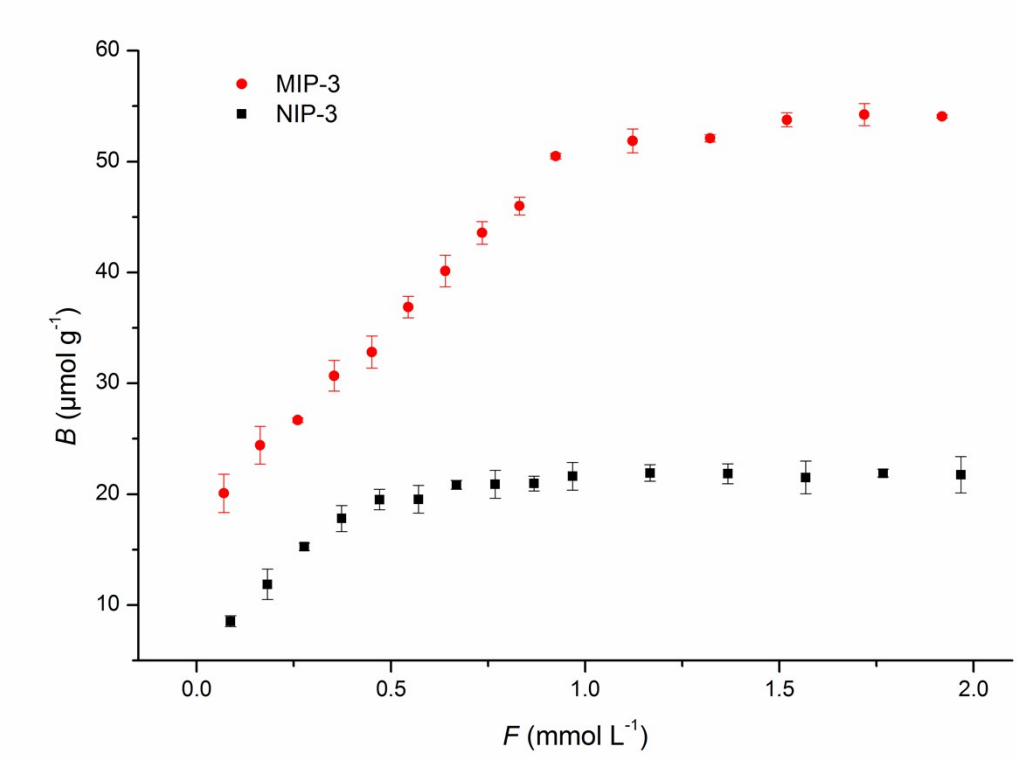

Fig. S5 Equilibrium binding isotherms of GGAKacR on the MIP and NIP

The bi-Langmuir isotherms was used to evaluate the binding sites parameters.[6] The Scatchard analysis was performed for MIP-3. In the Scatchard analysis, the association constants K_a and the apparent maximum number of binding sites B_{max} were determined from the slope and the intercept respectively in the plot of B/F versus B. We found that two straight lines fitting the Scatchard equation can be drawn (Fig. S6). It is indicated that the affinities of the binding sites are heterogeneous and can be approximately classified in two types.[7] The binding sites parameters from the Scatchard analysis are shown in the Table S3.

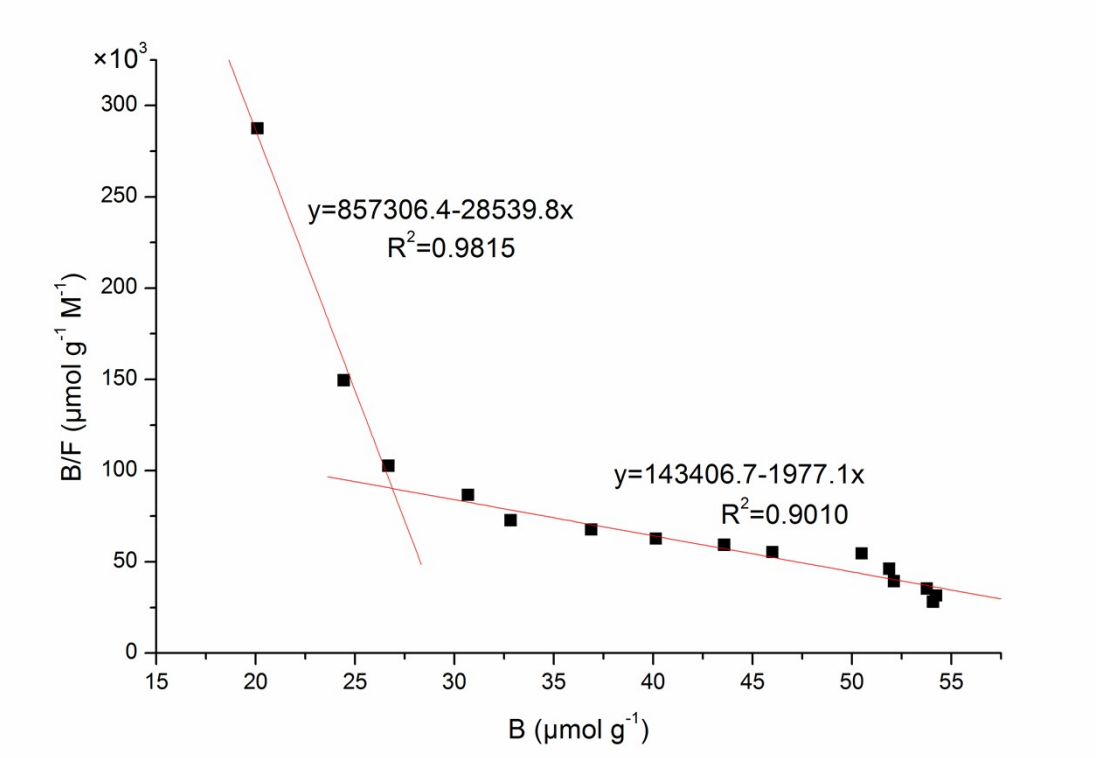

Fig. S6 Scatchard analysis of binding of GGAKacR on the MIP-3

Table S3 The association constants and the apparent maximum number of binding sites of MIP-3 determined from Scatchard analysis

High affinity			Low affinity		
K_a ($\times 10^4$ M^{-1})	B_{max} (μmol g^{-1})	R^2	K_a ($\times 10^3$ M^{-1})	B_{max} (μmol g^{-1})	R^2
2.85±0.28	30.04±5.76	0.9815	1.98±0.20	72.53±10.75	0.9010

For the NIP-3, binding isotherm was also evaluated by Langmuir model. The results from Langmuir fitting are listed in the Table S4. An associate constant K_a of (5.69±0.39) mM^{-1} and N_t of (25.14±2.89) μmol g^{-1} respectively were obtained from Schachard plot with fitting R^2 of 0.9364.

Table S4 The Langmuir fitting coefficients for NIP-3 binding isotherm

N_t	24.51±0.46 μmol g^{-1}

a	6.42±0.59 mM^{-1}
R^2	0.9670

5. Evaluation of the selectivity of the MIP

Selectivity of the MIP evaluated by competitive binding experiment

The binding affinities of the MIP-3 for the template and for the analogue peptides were compared by the equilibrium binding experiment. In the competitive binding experiment, a solution with peptide mixture was used. Each peptide had same concentration (1.0 $mmol \cdot L^{-1}$). The imprinted selectivity (IS), based on the ratios of bound amount of the peptides on the MIP-3 to that on the NIP-3, were also calculated. The results from the competitive binding experiment are shown in the Fig. S7.

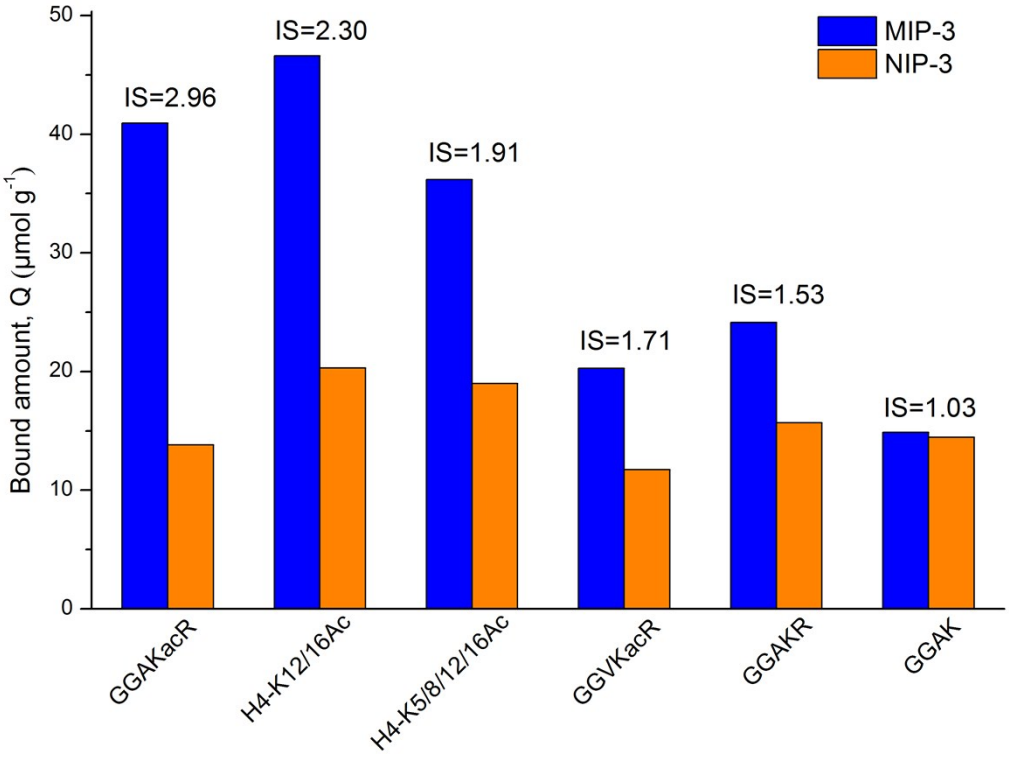

Fig. S7 Competitive binding of GGAKacR and its analogues on MIP-3 and NIP-3. The concentration for each peptide was 1.0 $mmol \cdot L^{-1}$ in the binding solution.

The competitive binding has shown similar order of binding affinity as the individual binding experiment. The MIP has highest selectivity for GGAKacR followed by H4-K12/16Ac and H4-K5/8/12/16Ac carrying epitope. Although the bound amounts of GGAKacR, H4-K12/16Ac and H4-K5/8/12/16Ac were less than that in the individual binding experiment due to the competition, good affinity and selectivity were observed for these peptides.

Selectivity of the MIP evaluated by HPLC analysis

The retention of the peptides and imprinted selectivity and separation factors of MIP-3 in the HPLC analysis is shown in the Table S5.

Table S5. Retention of the peptides and imprinted selectivity and separation factors in the HPLC analysis [a]

Analyte	Retention factor		k_{MIP}/k_{NIP}	Separation factor, α
	MIP-3	NIP-3		
GGAKacR	5.9	2.3	2.6	-
H4-K12/16Ac	3.4	1.7	2.0	1.7
H4-K5/8/12/16Ac	2.6	1.2	2.2	2.3
GGVKacR	1.6	1.7	0.9	3.7
GGAKR	1.0	0.7	1.4	5.9
GGAK	1.6	3.5	0.5	3.7

[a] The separation factor (α) was calculated by $k_{template}/k_{analogues}$.

The results also demonstrated that MIP has good imprinted selectivity and separation ability.

6. Supplementary figures in the sections of influence of HPLC mobile phase on the retention of GGAKacR and extraction of GGAKacR from histone digest

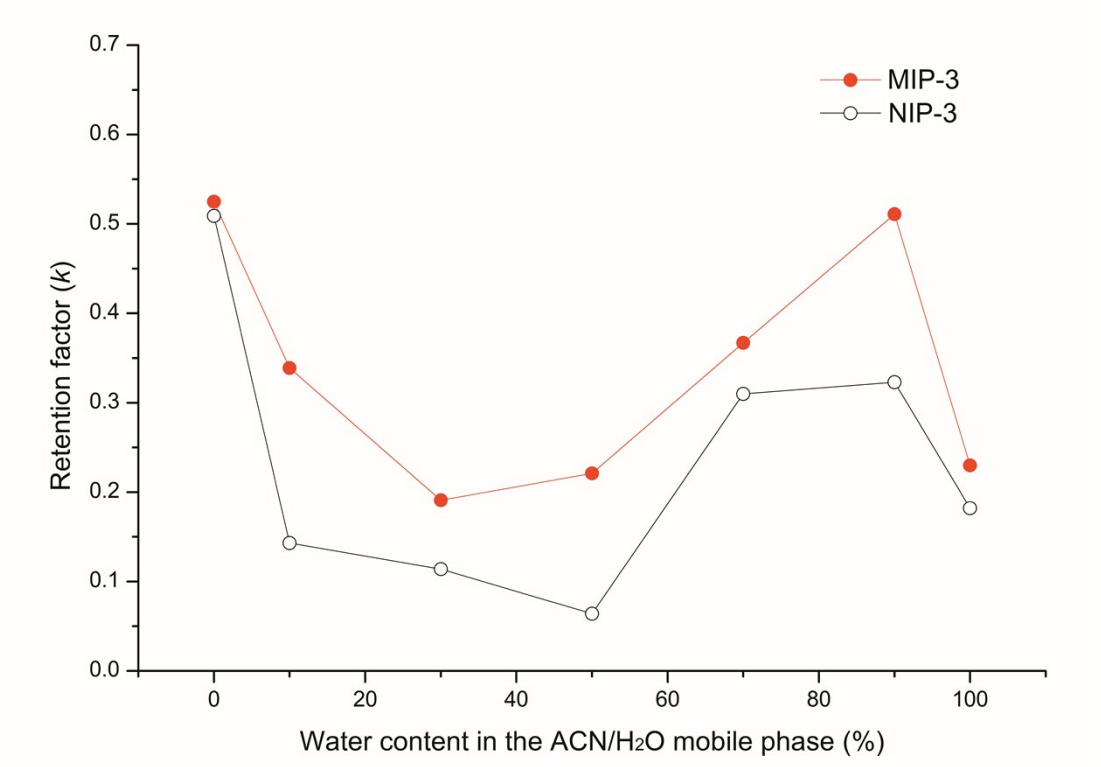

Fig. S8 Retention factors of the GGAKacR on the MIP and NIP column as a function of the water content in ACN/H_2O mobile phase. The column size was 20 mm × 4.6 mm i.d.. The mobile phase flow rate was 0.1 mL min^{-1}. The detection wavelength was 205 nm.

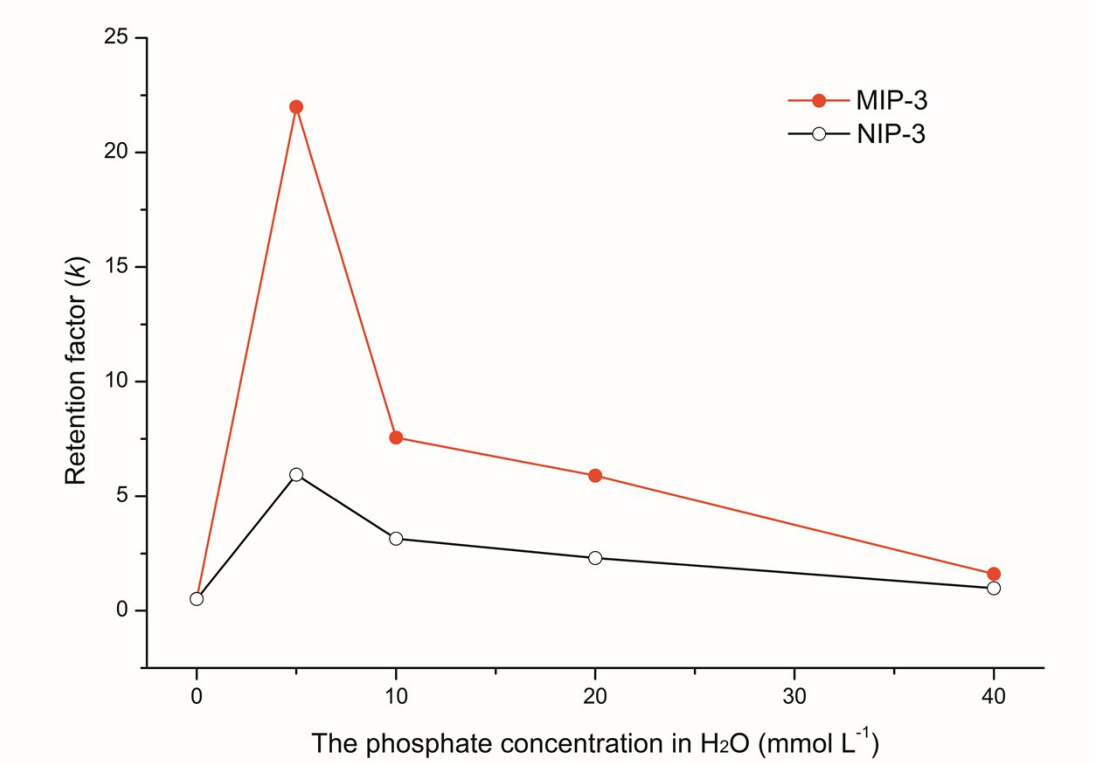

Fig. S9 Influence of mobile phase ionic strength on the retention of the analytes on the MIP-3 and NIP-3. The mobile phase was 100% H_2O or phosphate buffer at pH 7.0 with the specified salt concentration.

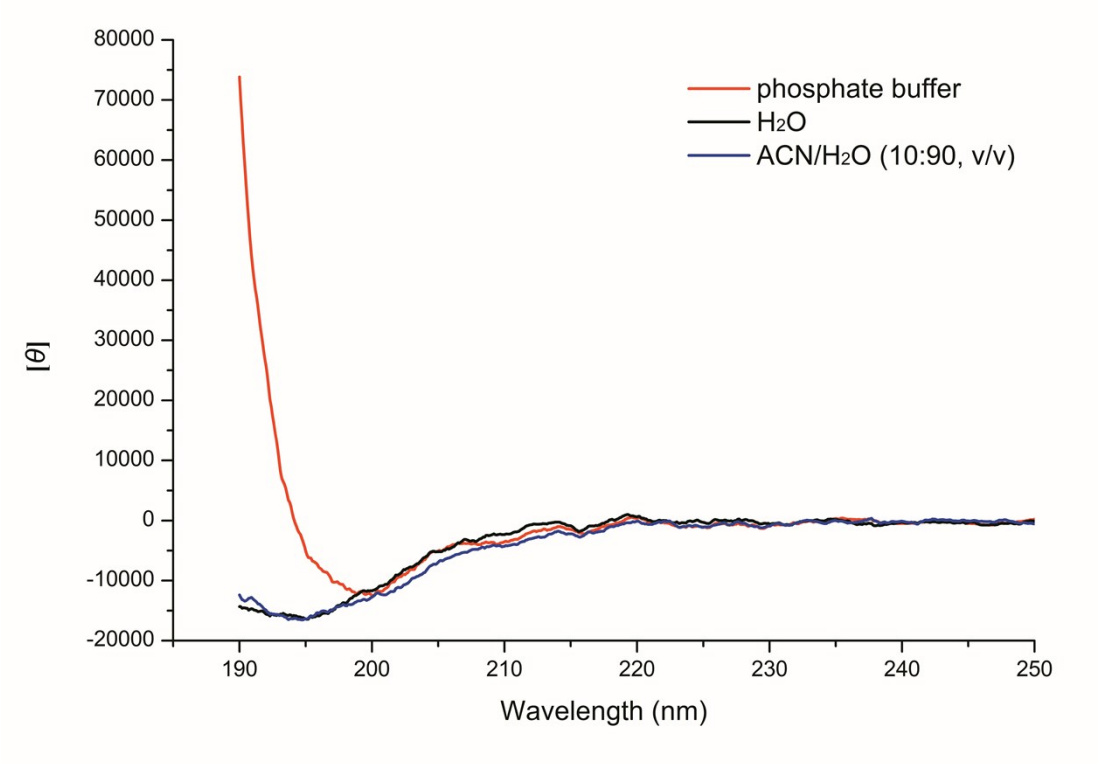

Fig. S10 Circular dichroism spectra of GGAKacR in different solvents

The peptide samples with the concentration of 0.01 mmol·L^{-1} prepared in different solvents (PBS, H_2O, or ACN/H_2O) were used in the measurement. The phosphate buffer was 20 mmol·L^{-1} and pH 7.

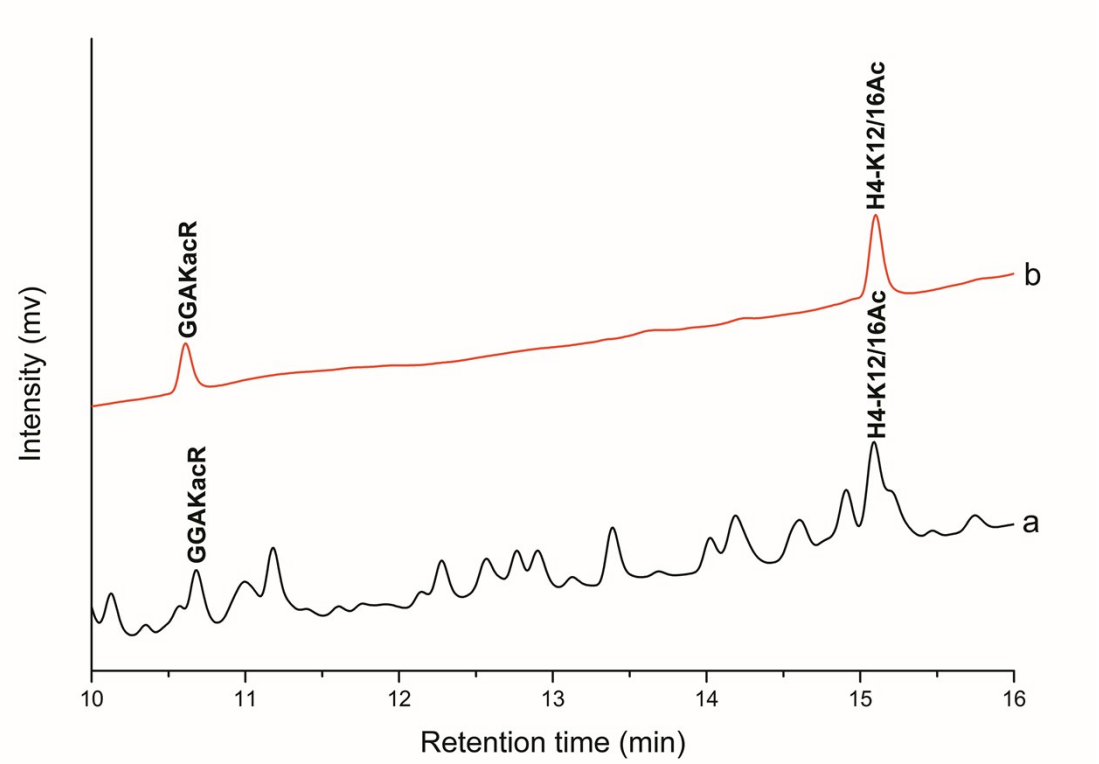

Fig. S11 Reversed-phase HPLC profiles of the GGAKacR and H4-K12/16Ac spiked histone digests before and after the extraction with MIP-3. (a) GGAKacR and H4-K12/16Ac spiked histone digest, (b) extracted fraction from the MIP-3. In the HPLC analysis, a C_{18} analytic column (250 × 4.6 mm i.d., Phenomenex) was used. The mobile phase A was 0.1% TFA in ACN and B was 0.1% TFA in H_2O. The gradient elution: 1-50 min (1% -95% B) was employed. The analytes was detected at 205 nm.

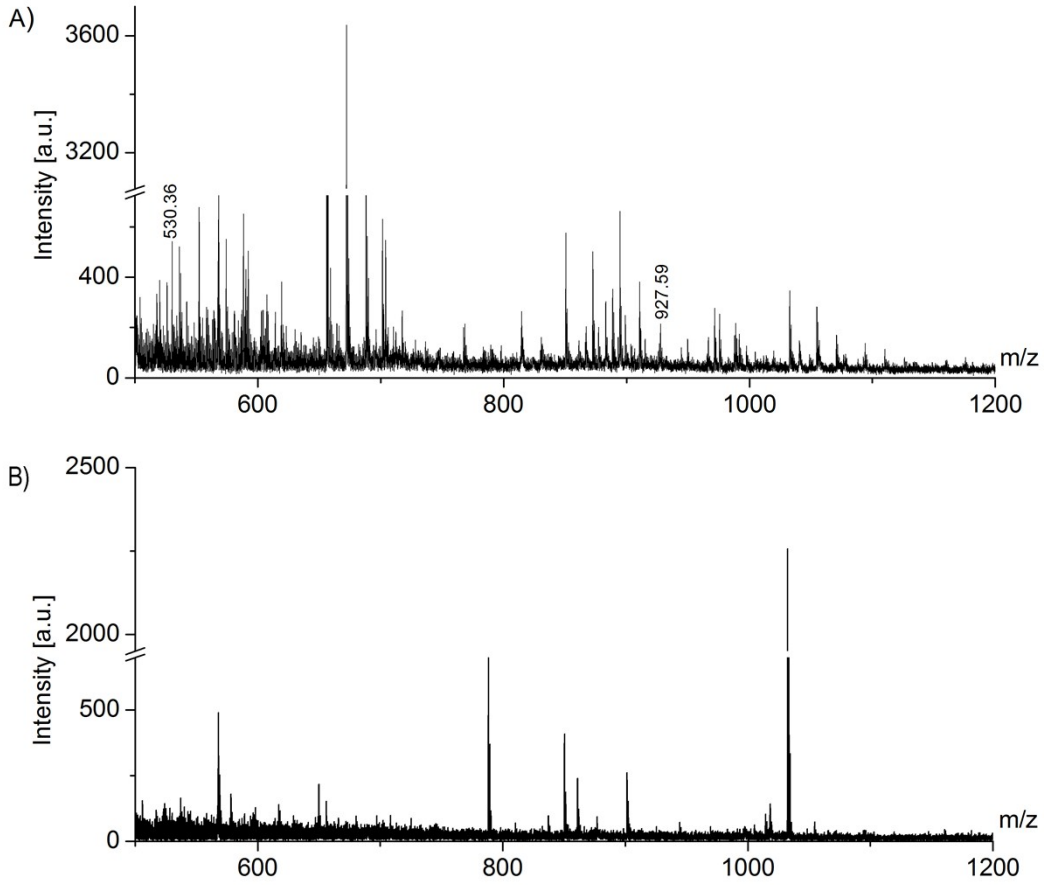

Fig. S12 MALDI-TOF MS spectra of the GGAKacR and H4-K12/16Ac spiked histone digest before and after the extraction with NIP-3. (A) GGAKacR and H4-K12/16Ac spiked histone digest, (B) extracted fractions from NIP-3. The m/z 530.36 and 927.59 are signals of the GGAKacR+H and H4-K12/16Ac+H, respectively.

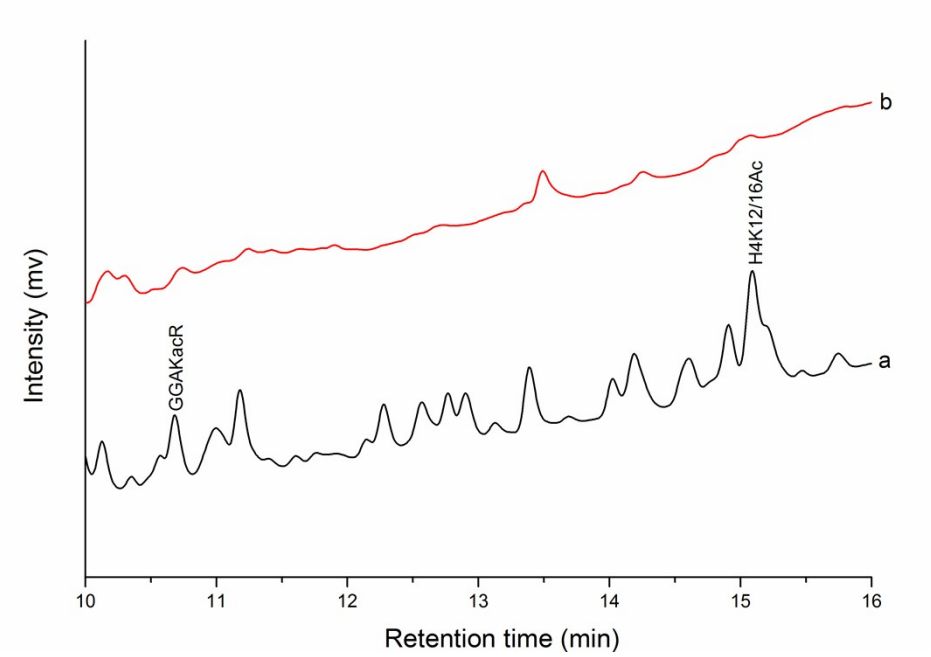

Fig. S13 Reversed-phase HPLC profiles of the GGAKacR and H4-K12/16Ac spiked histone digests before and after the extraction with NIP-3. (a) GGAKacR and H4-K12/16Ac spiked histone digest, (b) extracted fraction from the NIP-3. In the HPLC analysis, a C_{18} analytic column (250 × 4.6 mm i.d., Phenomenex) was used. The mobile phase A was 0.1% TFA in ACN and B was 0.1% TFA in H_2O. The gradient elution: 1-50 min (1% -95% B) was employed. The analytes was detected at 205 nm.

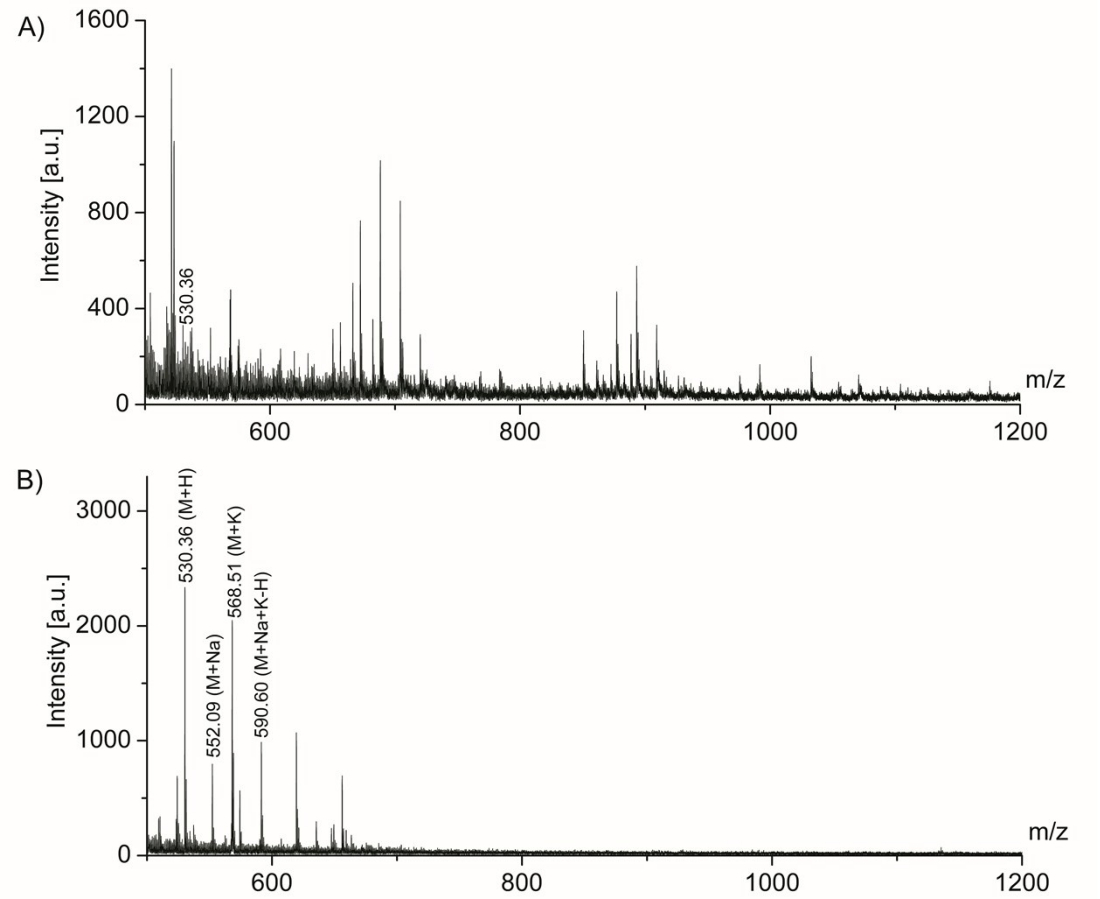

Fig. S14 MALDI-TOF MS spectra of the GGAKacR spiked histone digests before and after solid-phase extraction by MIP-3. (A) GGAKacR spiked histone digest, (B) eluted fractions from MIP-3. The m/z 530.36 is the signal of the GGAKacR+H (M+H). The m/z 552.09, 568.01 and 590.60 are the signals of M+Na, M+K and M+Na+K respectively produced in the positive-ion mode.

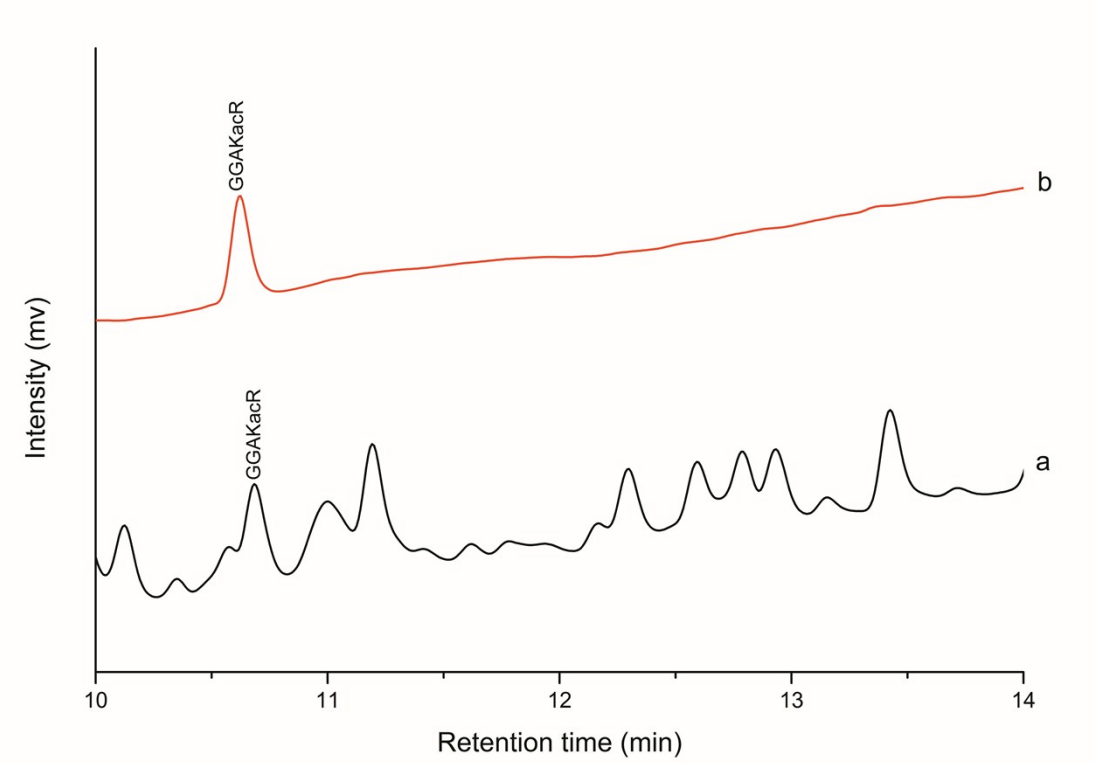

Fig. S15 Reversed-phase HPLC profiles of the GGAKacR spiked histone digests before and after solid-phase extraction by MIP-3. (a) GGAKacR spiked histone digestion, (b) extracted fraction from the MIP-3. In the HPLC analysis, a C_{18} analytic column (250×4.6 mm i.d., Phenomenex) was used. The gradient elution was illustrated in the extraction section. The analytes was detected at 205 nm.

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