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Supporting Information

Molecularly Imprinted Polymer as Antibody Mimic with Affinity for Lysine Acetylated Peptides

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Content:

Synthesis of MIP with different functional monomers using bulk polymerization

Bulk imprinting polymerization was employed in the functional monomer selection. Three monomers: Am, MAA and TFMAA were used separately in the experiments. The KacA (template, 44 μmol), monomer (528 μmol), EBA (cross-linker, 1056 μmol) and APS (initiator, 15.8 μmol) were dissolved in 2 mL PBS and the solution was de-aerated by N_2 for 10 min. The polymerization was carried out at 45 °C for 24 h. The polymers (denoted as Mono-MIP) were ground and the fine particles were removed by sedimentation in acetone. The template molecules in Mono-MIPs were removed by Soxhlet extraction with H_2O/ACN/acetic acid (90/5/5, v/v). Non-imprinted polymers (Mono-NIPs) were synthesized with the same conditions as the correlated Mono-MIPs except the absence of template.

Silica modification with succinimide group

In order to graft succinimide groups on the silica, amino groups were introduced on the silica first by the following reaction procedures. Silica beads were activated with HCl solution (10%, v/v) and washed with water and dried subsequently. 3-Aminopropyltriethoxysilane (5 mL) reacted with activated silica (6 g) in 50 mL dry toluene for 12 h under nitrogen protection and reflux. The product (denoted as silica-NH_2) was washed with toluene and acetone successively and then dried under vacuum.

The silica-NH_2 particles (6.2 g) were suspended in 50 mL DMF containing 1.2 g succinic anhydride. The reaction was performed at 30 °C for 12 h. The product (denoted as silica-COOH) was washed with 95% ethanol and dried under vacuum. The silica-COOH particles (2 g) were suspended in 25 mL CH_2Cl_2 containing DCC (336.1 mg, 1.6 mmol) and NHS (182.7 mg, 1.6 mmol) and the reaction was carried out for 12 h at 30 °C under stirring. The product (denoted as silica-SI) was washed with DMF and ethanol and then dried under vacuum.

Equilibrium binding experiment for Mono-MIP evaluation

The absorption isotherms of KacA on the Mono-MIPs and Mono-NIPs were measured by batch binding experiment. The absorption solutions of KacA (0.2-2.0 mM) were prepared in PBS. 2.0 mL each KacA solution was added into a plastic vial

containing Mono-MIP or Mono-NIP particles (3 mg). The binding experiment was carried out for 24 h under gentle shaking at room temperature. After the incubation, the mixtures were centrifuged and the concentrations of KacA in the supernatant were determined by HPLC analysis. In the HPLC, a C_{18} analytic column (250 mm×4.6 mm i.d., Phenomenex) was used. The mobile phase was H_2O/ACN (93/7, v/v with 0.1% TFA) with a flow rate of 1.0 mL·min^{-1}. The analytes were detected at 205 nm.

Histone digestion

Histone (2 mg) was dissolved in 1.6 mL of denaturing buffer solution (100 mM ammonium bicarbonate, pH 8.5). A 400 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 the addition of 10 μL acetic acid.

The conditions in the HPLC and Mass analysis for the spiked histone digest

In the HPLC, the C_{18} column used was the same as that in the equilibrium binding experiment. A gradient elution was performed in which mobile phase B was from 1% to 95% in 50 min and the flow rate was 1.0 mL min^{-1}.The mobile phase A was 0.1% TFA in ACN and mobile phase B was 0.1% TFA in H_2O, respectively.

MALDI-TOF mass spectrometer (AutoflexIII LRF200-CID, Bruker Daltonics, Germany) and LC-MS mass spectrometer (LC-MS2020, Shimadzu Co., Ltd., Japan) were used for the analysis of three peptides spiked sample and KacA spiked sample respectively. 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 400 to 2000 were acquired.

Cell culture and preparation of cell lysate

Hela cells were cultured with modified RPMI-1640 medium (Thermo Fisher Scientific Inc., Beijing, ChinaThermo Fisher) supplemented with 10% fetal calf serum (FBS, Invitrogen, Carlsbad, California, USA) at 37 °C with 5% CO_2 in atmosphere. After being washed twice with ice-cold phosphate-buffered saline, cells were harvested and lysed by the use of Cell Lysis Kit (Sangon Biotech, Shanghai, China, containing both lysis buffer and protease inhibitors). The cell lysate was incubated on ice for 30 min and

centrifuged at 4 °C at 12000 g for 10 min. The supernatant was taken as extracted proteins and stored at -20 °C until further use.

MS analysis for peptide mixture

The enriched peptides was dissolved in 10 μL of HPLC mobile phase A (0.1% formic acid in water), and 5 μL was injected into a Nano-LC system Nano-LC (EASY-nLC 1000) coupled with Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Each sample was separated by a C_{18} column (15 cm×50 μmI.D. 2 μm C_{18}) using an HPLC-gradient in which mobile phase B (0.1% formic acid in ACN) was changed from 2% to 35% in75 min. The HPLC elute was electrosprayed directly into the mass spectrometer. The source was operated at 1.8 kV. For full MS survey scan, automatic gain control (AGC) target was 3e6, scan range was from 300 to 1750 with the resolution of 70,000. The MS2 spectra were acquired with 17, 500 resolution.All MS/MS spectra were searched against the Uniport-Human protein sequence database using the PD search engine (version 2.1.0, Thermo Fisher Scientific) with an overall false discovery rate (FDR) for peptides of less than 1%. Trypsin was specified as digesting enzyme. A maximum of 2 missing cleavages were allowed. Mass tolerances for precursor ions were set at ±10 ppm for precursor ions and ±0.02 Da for MS/MS. Oxidation of methionine and acetylation on protein N-terminal were fixed as variable modifications. Carbamidomethylation on Cys was specified as fixed modification. All MS/MS spectra were manually verified.

Table S1. The functional monomers used in the bulk imprinting polymerization and imprinting performance of the Mono-MIPs[a]

Imprinted polymer	Mono-MIP-1	Mono-MIP-2	Mono-MIP-3
Functional monomer used in the synthesis	Am	MAA	TFMAA
Imprinting performance	1.7	1.1	1.3

[a] The imprinting performance was evaluated by the ratio of binding capacity of the MIP with respect to that of correlated NIP.

Table S2 The surface density of immobilized groups on the silica after each modification step determined by elemental analysis[a]

Material	silica-NH_2	silica-COOH	silica-SI	silica-KacA	silica-Kac
Groups	-NH_2	-COOH	-NHS	KacA	Kac
Surface area density (*D*s), μmol·m^{-2}	4.1	2.2	1.7	0.3	0.1

[a] The *D*s of the amino groups on the silica-NH_2 were calculated based on the increment of nitrogen content by elemental analysis. The *D*s of other groups on the silica was estimated from the increment of the carbon content.

Table S3 Comparison of retention of the peptides on the KacA-MIP and NIP in the HPLC analysis[a]

Analyte	Retention factor, k		k_{MIP}/k_{NIP}
	KacA-MIP	NIP	
KacA	4.9	1.4	3.5
GGAKacR	4.9	2.0	2.5
GLGKacGGAK	4.0	1.5	2.7
$H2B_{101-116}$-K109Ac	4.5	1.7	2.6
KA	1.3	1.4	0.9
QA	1.0	1.1	0.9
GGAKR	2.6	1.6	1.6
GLGKGGAK	1.8	1.8	1.0

[a] The column was packed with KacA-MIP. Column size was 20 mm× 4.6 mm i.d. The mobile phase was PBS (20 mmol L^{-1} and pH 7) with a flow rate of 0.2 mL min^{-1}.

Table S4 The acetylated peptides identified by MIP enrichment

Sequence	Gene Name	Protein Description	Modifications	q-Value	PEP
kQLATkAAR	H3F3B	Histone H3 (Fragment) OS=Homo sapiens GN=H3F3B PE=1 SV=1 - [K7EK07_HUMAN]	K1(Acetyl); K6(Acetyl)	0	0.02435
GkGGkGLGkGGAkR	HIST1H4A	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]	K2(Acetyl); K5(Acetyl); K9(Acetyl); K13(Acetyl)	0	0.006212
HVMTNLGEkLTDEEVDEMIR	CALM1	Calmodulin (Fragment) OS=Homo sapiens GN=CALM1 PE=1 SV=1 - [G3V361_HUMAN]	K9(Acetyl)	0	9.401E-08
dGCEYSLGLTPTGILIFEGANkIGLFFWPK	EPB41L4B	Isoform 2 of Band 4.1-like protein 4B OS=Homo sapiens GN=EPB41L4B - [E41LB_HUMAN]	N-Term(Acetyl); K22(Acetyl)	0	0.03549
lkEKAPKHVGFSVLLcWIVcMLVNIIFPMYVTGK	VN1R4	Vomeronasal type-1 receptor 4 OS=Homo sapiens GN=VN1R4 PE=2 SV=2 - [VN1R4_HUMAN]	N-Term(Acetyl); K2(Acetyl); C16(Carbamidomethyl); C20(Carbamidomethyl)	0.005	0.2603
vkEHGTHQQLLAQK	ABCB4	H7C0M2_HUMAN Multidrug resistance protein 3 (Fragment) OS=Homo sapiens GN=ABCB4	N-Term(Acetyl); K2(Acetyl)	0.001	0.112
hVLIHTGQRPFkCVLCTkSFR	ZNF770	Zinc finger protein 770 OS=Homo sapiens GN=ZNF770 PE=2 SV=1 - [ZN770_HUMAN]	N-Term(Acetyl); K12(Acetyl); K18(Acetyl)	0.003	0.1771
ELPDYLmkILTER	POTEE	POTE ankyrin domain family member E OS=Homo sapiens GN=POTEE PE=1 SV=3 - [POTEE_HUMAN]	M7(Oxidation); K8(Acetyl)	0.006	0.3097
VQESGIVkDASIIHR	DENND3	Isoform 2 of DENN domain-containing protein 3 OS=Homo sapiens GN=DENND3 - [DEND3_HUMAN]	K8(Acetyl)	0.002	0.129
VVQNPPkPVmTTRPTAVK	FNBP4	Formin-binding protein 4 (Fragment) OS=Homo sapiens GN=FNBP4 PE=1 SV=2 - [F5GXL1_HUMAN]	K7(Acetyl); M10(Oxidation)	0	0.02246
LQGLHkPVYHALSDCGDHVVImNTR	MRPL13	39S ribosomal protein L13, mitochondrial (Fragment) OS=Homo sapiens GN=MRPL13 PE=1 SV=2 - [E5RJ17_HUMAN]	K6(Acetyl); M22(Oxidation)	0.003	0.155
VkLAYDIAVGLSYLHFK	TESK2	Dual specificity testis-specific protein kinase 2 OS=Homo sapiens GN=TESK2	K2(Acetyl)	0.008	0.4282
QSLPQEDPDVVVIDSSkHSDDSVAmK	KCNH7	Isoform 2 of Potassium voltage-gated channel subfamily H member 7 OS=Homo sapiens GN=KCNH7 - [KCNH7_HUMAN]	K17(Acetyl); M25(Oxidation)	0.008	0.3932
QANLVHFPPGFQPIPCkPLFFDLALNHVAFPPLEDK	SRP68	Isoform 3 of Signal recognition particle subunit SRP68 OS=Homo sapiens GN=SRP68 - [SRP68_HUMAN]	K17(Acetyl)	0	0.0003731
ALSDHHIYLEGTLLkPNmVTPGHACTQK	ALDOA	Fructose-bisphosphate aldolase OS=Homo sapiens GN=ALDOA PE=1 SV=1 - [H3BQN4_HUMAN]	K15(Acetyl); M18(Oxidation)	0	0.0000717
LRPVPPYQHEEIIGkVcELINEVK	ZNF618	Isoform 2 of Zinc finger protein 618 OS=Homo sapiens GN=ZNF618 - [ZN618_HUMAN]	K15(Acetyl); C17(Carbamidomethyl)	0.008	0.4265
TTSTLASSLPGHAAkTLPGGAGK	EHMT1	Isoform 4 of Histone-lysine N-methyltransferase EHMT1 OS=Homo sapiens GN=EHMT1 - [EHMT1_HUMAN]	K15(Acetyl)	0	0.02136
LGFPQILLEk	ARHGAP39	Rho GTPase-activating protein 39 OS=Homo sapiens GN=ARHGAP39 PE=1 SV=2 - [RHG39_HUMAN]	K10(Acetyl)	0.007	0.3571
rPPPHPGkcPENGWDESTLELFLHELAImDSNNFLGNCGVGER	SEPSECS	Isoform 3 of O-phosphoseryl-tRNA(Sec) selenium transferase OS=Homo sapiens GN=SEPSECS - [SPCS_HUMAN]	N-Term(Acetyl); K8(Acetyl); C9(Carbamidomethyl); M29(Oxidation)	0.006	0.3009
kGSkkAVTkAQK	HIST1H2BK	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]	K1(Acetyl); K4(Acetyl); K5(Acetyl); K9(Acetyl)	0	0.003027
sGRGKGGkGLGkGGAkR	HIST1H4A	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]	N-Term(Acetyl); K8(Acetyl); K12(Acetyl); K16(Acetyl)	0	0.06343

Table S5 The acetylated peptides identified without enrichment

Sequence	Gene Name	Protein Description	Modifications	q-Value	PEP
GGkGLGkGGAkR	HIST1H4A	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]	K3(Acetyl); K7(Acetyl); K11(Acetyl)	0	0.008123
kTTGEENGVEAEEWGK	DNM1L	Dynamin-1-like protein OS=Homo sapiens GN=DNM1L PE=1 SV=1 - [B4DPZ9_HUMAN]	K1(Acetyl)	0.006	0.1022
GkGGkGLGkGGAkR	HIST1H4A	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]	K2(Acetyl); K5(Acetyl); K9(Acetyl); K13(Acetyl)	0	0.0002344
kGSkkAVTkAQK	HIST1H2BK	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]	K1(Acetyl); K4(Acetyl); K5(Acetyl); K9(Acetyl)	0	0.002527
kQLATkAAR	H3F3B	Histone H3 (Fragment) OS=Homo sapiens GN=H3F3B PE=1 SV=1 - [K7EK07_HUMAN]	K1(Acetyl); K6(Acetyl)	0	0.005753

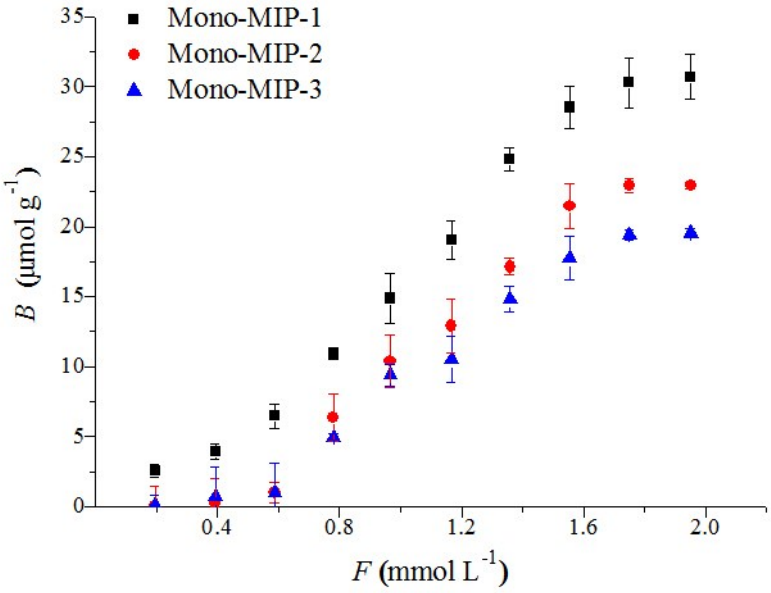

Fig. S1. Equilibrium binding isotherms of KacA on different Mono-MIPs

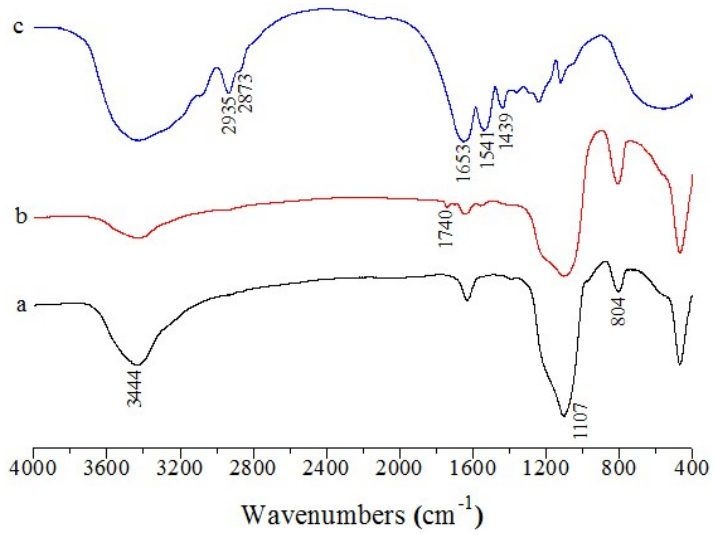

Fig. S2. FT-IR spectra of silica gel (a), silica-SI (b) and KacA-MIP(c)

In the FT-IR spectrum of blank silica (a), the absorption peaks at 1107 cm^{-1} and 804 cm^{-1} are from the Si–O–Si asymmetric and symmetric stretching, respectively. The peak from C=O stretching (at 1740 cm^{-1}) in internal amide appeared in the FT-IR of silica-SI (b), which proved the successful modification of succinimide group. In the

FT-IR of KacA-MIP (c), the absorptions at 1653 cm^{-1} (C=O stretching), 1541 cm^{-1} (N-H bending) from amide appeared which are from the composition of the Am and EBA. The peaks at 2873 and 2935 cm^{-1} are from the C-H stretching of the methylene groups. The Si–O–Si asymmetric and symmetric stretching at 1107 cm^{-1} and 804 cm^{-1} reduced considerably, indicating the successful removal of the silica. For the silica-NH_2 and silica-COOH, because the amount of the grafted groups were much less compared to total composition of the material, the corresponding IR peaks of grafted groups cannot be observed in the FT-IR analysis.

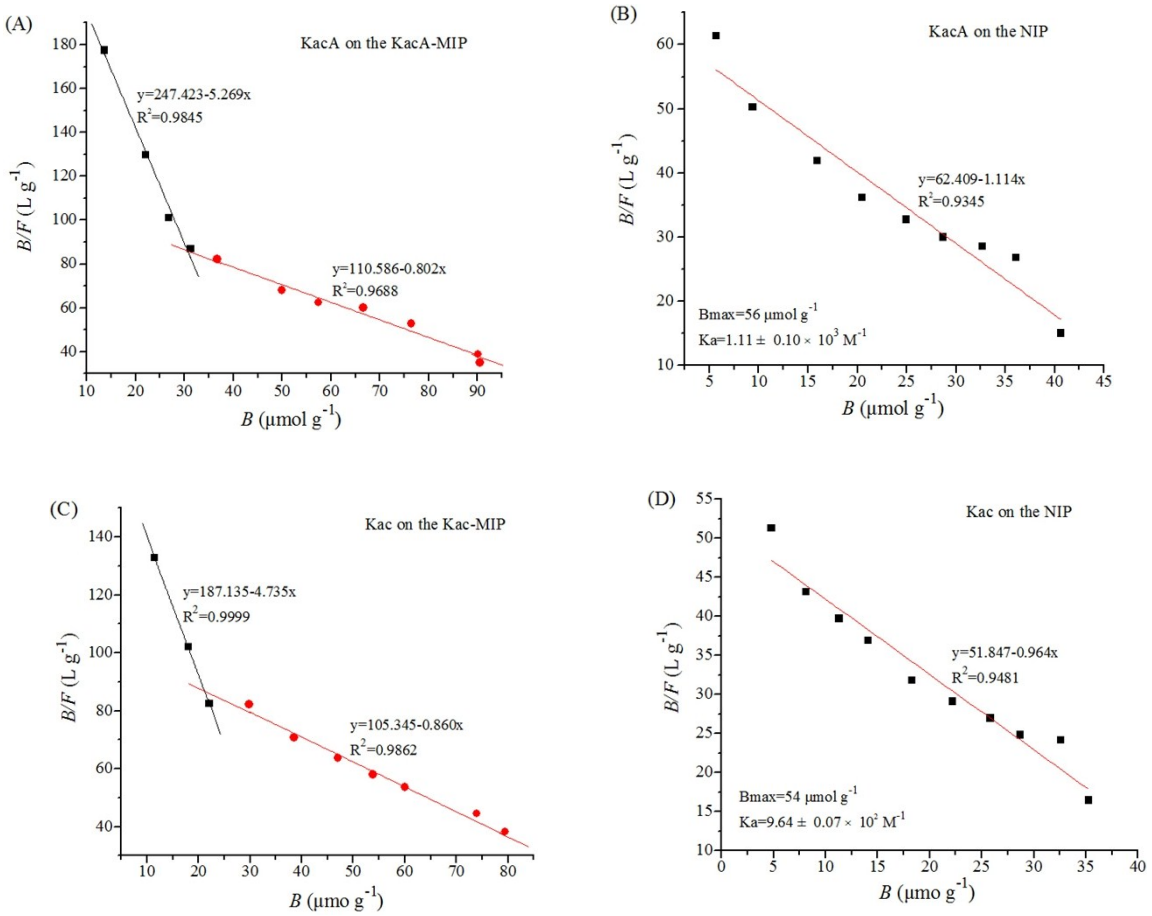

Fig. S3. Scatchard analysis of binding of template molecules on the KacA-MIP, Kac-MIP and NIP

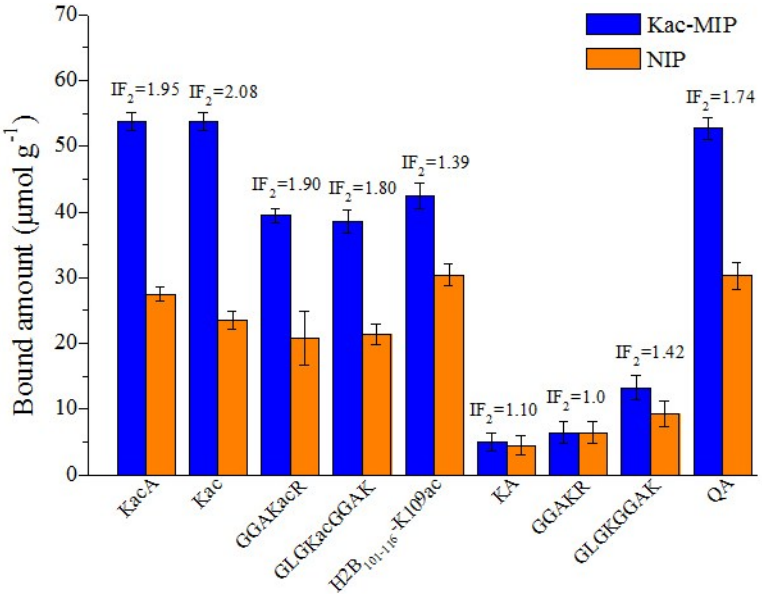

Fig. S4. Bound amount of different peptideson Kac-MIP and NIP from the individual binding experiment. The peptide concentration of every binding solution was 1.0 mmol·L^{-1}.

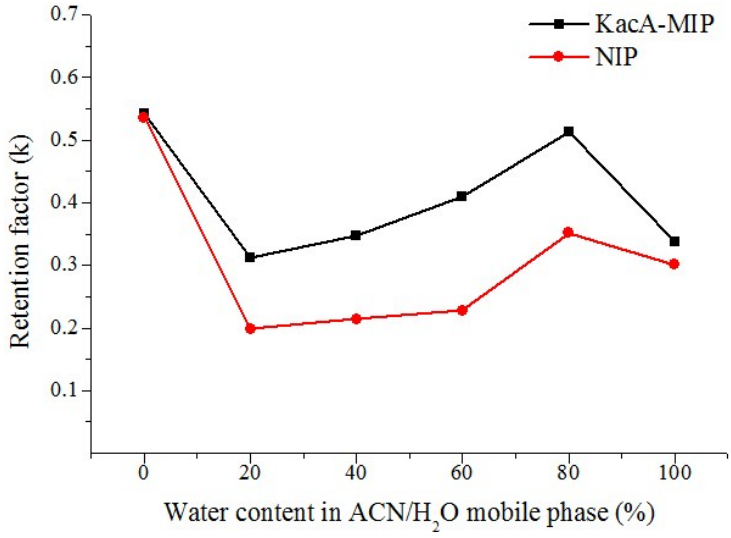

Fig. S5. Retention factors of the KacA on the KacA-MIP and NIP column as a function of the water content in ACN/H_2O mobile phase. The column size was 20 mm × 4.6 mm i.d. The mobile phase flow rate was 0.2 mL min^{-1}. The detection wavelength was 205 nm.

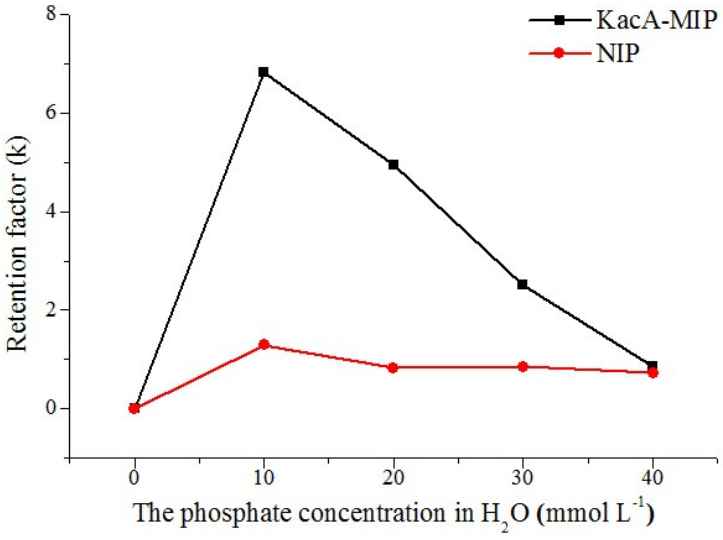

Fig. S6. Influence of mobile phase ionic strength on the retention of the peptide on the KacA-MIP and NIP. The mobile phase was 100% H_2O or phosphate buffer at pH 7.0 with the specified salt concentration.

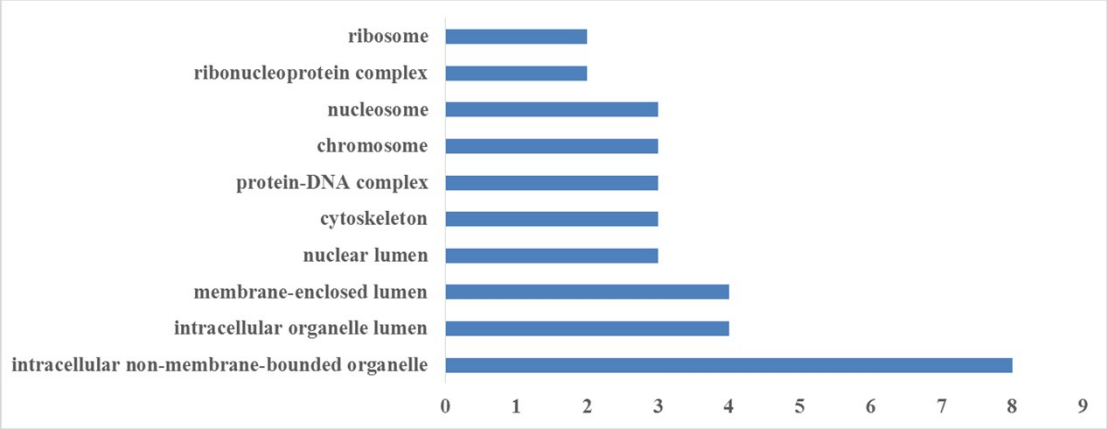

Fig. S7. Analysis of cellular component on the acetylated proteins enriched by KacA-MIP