Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Simultaneous Detection of Site-Specific Histone Methylations and Acetylation Assisted by Single Template Oriented Molecularly Imprinted Polymers

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Experimental

Chemicals and Reagents.

Unmethylated peptide EIAQDFK, methylated peptides (i.e., EIAQDFK$_{me1}$ TDLR, EIAQDFK$_{me2}$TDLR, EIAQDFK$_{me3}$TDLR), and acetylated peptide EIAQDFK$_{ac}$TDLR as well as the stable isotope-labeled internal standard were developed by ChinaPeptides Co., Ltd. (Shanghai, China). Purity of the peptides is over 95% provided by the manufacturer. Tetraethoxysilane (TEOS), methylmethacrylate (MAA), ethylene glycol dimethacrylate (EGDMA), and 2,2'-azobis(2-methylpropionitrile) (AIBN) were all obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). (3-Aminopropyl)triethoxysilane (APTES) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glutaraldehyde (GA) was obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). Toluene and dimethylformamide (DMF) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ammonium bicarbonate (NH_4HCO_3) was provided by Qiangshun Chemical Reagent Co., Ltd. (Shanghai, China). DL-dithiothreitol (DTT) and iodoacetamide (IAA) were both purchased from Sigma-Aldrich (St. Louis, MO, USA). Sequencing grade modified trypsin was obtained from Promega (Madison, WI, USA). Phosphate buffered saline (PBS) was provided by the Beyotime Institute of Biotechnology (Jiangsu, China). Methanol and acetonitrile (ACN) were supplied by Tedia Company, Inc. (Fairfield, OH, USA). Trifluoroacetic acid (TFA) and oxalic acid were provided by Aladdin Chemistry Co., Ltd. (Shanghai, China), and formic acid (FA) was purchased from Xilong Chemical Industrial Factory Co., Ltd. (Shantou,

China). Sulfuric acid (H_2SO_4) and acetone were supplied by Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Dulbecco's Modified Eagle Media (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, Leibovitz's L-15 medium and penicillin-streptomycin solution were obtained from Thermo Scientific HyClone (Logan, UT, USA). MEGM mammary epithelial cell growth medium was ordered from LONZA (Basel, Switzerland). Fetal bovine serum (FBS) was purchased from the Thermo Scientific HyClone & Gibco (Logan, UT). Trypan blue and sodium dodecyl sulfate (SDS) were supplied by Generay Biotech Co., Ltd. (Shanghai, China). Water was purified and deionized with a Milli-Q system manufactured by Millipore (Bedford, MA, USA).

Cell Culture.

MCF-7/WT (ATCC, Manassas, VA) cells were cultured in a DMEM media supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin at 37°C under a 5% CO_2 atmosphere. MCF-7/ADR (Keygen Biotech, Nanjing, China) cells were cultured in RPMI 1640 media (with L-glutamine and sodium pyruvate) supplemented with 10% fetal bovine serum. MDA-MB-231 cells were obtained from the Cell Resource Center of Chinese Academy of Medical Sciences (Shanghai, China) and cultured in L-15 supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C in a free gas exchange with atmospheric air, as instructed by the ATCC.[1] MCF-10A cells (ATCC, Manassas, VA) were non-tumorigenic breast epithelial cells and maintained routinely in MEGM media supplemented with 100 ng/mL cholera toxin and 1% penicillin/streptomycin at 37°C under a 5% CO_2 atmosphere. The cells were

split every 5-7 days by lifting cells with 0.25% trypsin, and feeding between splits was accomplished through the addition of fresh medium. To maintain a highly drug-resistant cell population, MCF-7/ADR cells were periodically reselected by growing them in the presence of 1000 ng/mL DOX. Experiments were performed using the cells cultured without DOX for 48 h. Cells were counted using a Scepter™ 2.0 handheld, automated Cell Counter (Millipore, Billerica, MA, USA). Cell viability was assessed by trypan blue (0.4%) exclusion, which was completed by mixing the cell suspension, trypan blue and 1 × PBS in a 2:5:3 ratio and counting the percentage of viable cells following a 5 min incubation at 37°C.

Preparation of Stock Solutions, Calibration Standards and Quality Controls (QCs).

Stock solutions (1 mM) of five surrogate peptides were first prepared by accurately weighing the unmethylated, monomethylated, dimethylated, trimethylated and acetylated synthetic peptides and dissolving them in deionized water. The solutions were stored at -20°C in amber glass tubes to protect them from light. The corresponding isotope-labeled synthetic peptides were used as internal standards (IS). The internal standards were also weighed, and 1 mM stock solutions were prepared in deionized water. Finally, a 100 nM internal standard solution containing all the internal standards was prepared by diluting the stock solutions with an ACN/water mixture (50:50, *v/v*) containing 0.1% FA.

The calibration standards of the surrogate peptides were prepared by sequentially diluting the stock solutions with H3 & H4-depleted cell extract. The experimental

details about immuno-depletion of cellular extract have been described in our previous work.[2] The concentrations of the standards were 0.5, 5, 10, 50, 100, 200, and 400 nM for each peptide in a mixture. Correspondingly, the QC samples for lower limit of quantification (LLOQ), low QC, mid QC, and high QC were prepared at 0.5, 1.5, 50, and 320 nM in the same matrix and frozen prior to use. Especially, all the solutions in following tests were prepared using H3 & H4-depleted cell extract if the condition was not defined.

Histone Protein Extraction.

Most of the available methods to extract histone depend on the good solubility of histone proteins in acidic solutions, where most other proteins in nuclear and nucleic acids would precipitate.[3] Briefly, cells (~10^6) were rinsed with ice-cold PBS twice and pelleted at 1,480 × g for 10 min, followed by resuspending in 2 mL of precooled lysis buffer (50 mM sodium bisulfite, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1% Triton X-100, and 8.6% sucrose, pH = 6.5) containing one protease inhibitor cocktail (Sigma-Aldrich, MO). Followed by dounce homogenization, the samples were centrifuged at 1,000 × g for 7 min. Next, the pellet was suspended in 0.4 mL of 0.4 N H_2SO_4 and underwent gentle shaking to isolate the histone proteins at 4°C overnight.[4] Then, the supernatant was collected by centrifugation at 12,000 × g for 10 min and subsequently mixed with 1 mL of acetone. The sample was incubated for 12 h at -20°C, and the coagulated proteins were gathered by spun at 15,000 × g for 10 min and air-dried. Finally, the gained histone proteins were dissolved in 100 μL of water and preserved at -20°C. The concentration of the extracted protein was determined using a BCA

protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA).

Characterization of the MIPs.

FT-IR analysis The composition of the particles was characterized using a FT-IR Tensor 27 spectrometer (Bruker, Germany) coupled with a liquid nitrogen-cooled mercury-cadmium-telluride detector (MCT), which had a resolution of 2 cm^{-1} and a spectral range of 4000-400 cm^{-1}. The particles were dried at 80°C for 12 h under vacuum prior to prepare the KBr pellet. In this context, a quantity of 2 mg of each sample was thoroughly ground with 100 mg of KBr for following pellet fabrication.

Evaluation of morphology and structure of the MIPs and the NIPs Morphology of the SiO_2-NPs and the MIPs were characterized with field emission scanning electron microscope (FE-SEM) (Carl Zeiss, Sigma 500, Germany). All of the samples were coated with gold with a thickness of 2-3 nm for ease of conduction. Transmission electron microscope (TEM; Tecnai G2 Spirit BioTwin, FEI, USA) was employed to examine the internal structure of these particles. The particles were suspended in distilled water at a suitable concentration and added to 300 mesh carbon coated copper grid, and then dried under vacuum.

Adsorption tests The static adsorption experiments were conducted to investigate the adsorption capacity of the MIPs. Solutions of the template peptide and surrogate peptides with different concentrations were prepared. Then, 20 mg of MIPs or NIPs were dispersed in 1 mL of each sample. After incubation with gentle shaking for 24 h at room temperature, the mixtures were centrifuged for 10 min at 10,000 rpm. Then, the supernatant was preserved and the separated particles were treated with 200 μL of

methanol containing 10% FA to elute the adsorbed peptides. The collected supernatant after centrifugation was further determined by LC-MS/MS. The adsorption kinetics experiment was carried out using the same procedure mentioned above, while controlling the initial concentration at 1 μM and the adsorption time ranging from 0 to 60 min.

Selectivity test and competitive binding test To further determine the selectivity of the MIPs in oriented recognition, a competing peptide was selected. In addition, a series of mixed solutions consisting of one surrogate peptide with variable designated concentrations ranging from 0.5 to 400 nM and the other surrogate peptides at a constant high concentration (400 nM) were prepared. Moreover, five samples (S1 to S5) comprising five surrogate peptides with given concentrations randomly between 10 and 400 nM were also measured.

Immuno-Depletion of Cellular Extract.

Cellular extract was added at protein concentrations of 2.00 mg/mL to BioMagPlus Goat anti-rabbit IgG beads (Bangs Laboratories, Fisher, Indiana, USA) that had been pre-incubated with a rabbit polyclonal anti-H3 antibody (17168-1-AP, Proteintech Group, Inc., China) and anti-H3 antibody (16047-1-AP, Proteintech Group, Inc., China). Samples were incubated at 4°C for 60 min with gentle rotation, and then placed in a magnetic field (Magnetic separator; Bangs Laboratories, Fishers, Indiana, USA) for 2 min. Supernatants were collected and subjected to the depletion protocol a second time.

Instruments and Conditions.

For global proteomics analysis, unlabeled peptides were first fractionated by strong cation exchange (SCX) and then analyzed by LC-MS/MS. The SCX chromatography was performed on BioBasix SCX 150 mm × 3 mm, 5 μm, 300 Å column (Thermo, USA) with a flow rate of 0.3 mL/min. 200 μg of tryptic peptides were loaded on the column and eluted with buffer A (5 mM KH_2PO_4: 25% ACN, pH = 2.7) and buffer B (5 mM KH_2PO_4, 350 mM KCl: 25% ACN, pH = 2.7) in following gradient: B 0% (0 min) → 0% (5 min) → 60% (65 min) → 100% (70 min) → 100% (80 min) → 0% (90 min). The eluting fractions were collected from 0-25 min, 25-30 min, 30-34 min, then every 3 min intervals to 67 min, 67-85 min (15 in total). The fractions were lyophilized and stored at -80°C for further analysis. In LC-MS/MS, the peptide samples were separated using the Eksigent Ekspert™ nanoLC 415 System combined with the cHiPLC® system in Trap-Elute mode. The peptides were first loaded on the cHiPLC trap (150 μm × 300 μm ChromXP C18-CL, 3 μm, 120 Å) and washed for 10 min at 2 μL/min and eluted using a nano cHiPLC column (75 μm × 15 cm ChromXP C18-CL, 3 μm, 120 Å) in a 90 min linear gradient from 3-35% acetonitrile in water with 0.1% formic acid (*v/v*) at 300 nL/min. The eluting peptides were analyzed on a TripleTOF® 5600+ system (Sciex, Framingham, MA) equipped with a Nanospray-III® Source. MS1 spectra were collected in the range 350-1250 Da for 250 ms. The 20 most intense precursor ions in the mass range of 400-1250 Da with a charge state 2-5 were selected for fragmentation with a rolling collision energy and a collision energy spread of ± 15V, and MS/MS fragment spectra were collected in the range of 100-1500 Da for 50 ms. The data extraction of the SWATH runs was

performed by PeakView using the MS/MSALL with SWATH Acquisition MicroApp. Six fragments per peptide were selected and any shared peptides were excluded from the extraction. Protein quantification was employed the peptides with an FDR of less than 5%. The peak areas for peptides were obtained by summing the peak areas of the corresponding fragment ions.

For a simultaneous targeted analysis of five surrogate peptides, an Agilent Series 1290 UPLC system (Agilent Technologies, Waldbronn, Germany) coupled with a 6460 Triple Quad LC/MS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) were used. Chromatographic separation of the sample was performed on an Agilent SB-C18 column (2.1 mm × 30 mm, 2.7 μm) at room temperature. The mobile phase was consisted of solvent A (0.1% FA/water) and solvent B (0.1% FA/methanol). A gradient elution with a flow rate of 0.3 mL/min was performed in the following conditions: B 10% (0 min) → 10% (1 min) → 90% (4 min) → 90% (8 min) → 10% (9 min). The injection volume was 5 μL. The mass spectrometer was equipped with an electrospray ion source and operated in the positive MRM mode. Q1 and Q3 were both set at unit mass resolution. The flow of the drying gas was 10 L/min, and the drying gas temperature was kept at 350°C. The electrospray capillary voltage was optimized to 4000V. The nebulizer pressure was set to 35 psi. The data were collected and processed using the Agilent MassHunter Workstation Software (version B.06.00). The peptides were detected in MRM mode. In this study, three transitions that gave the best S/N (signal/noise) and LOQ (limit of quantification) were chosen for each surrogate peptide.

Supplementary Tables and Figures

Table S1. MRM transitions for the surrogate peptides of H3K79, H3K122, H4K31 and their corresponding internal standard peptides.

H3K79 Surrogate Peptides	MRM Transitions (*m/z*)		
74EIAQDFK80 (unmethylated)	425.8 → 294.0	425.8 → 408.9	425.8 → 608.2
EIAQDF*K (IS)	430.8 → 304.4	430.8 → 418.6	430.8 → 618.2
74EIAQDFK$_{me1}$TDLR84 (monomethylated)	450.6 → 397.2	450.6 → 454.7	450.6 → 554.3
EIAQDF*K$_{me1}$TDLR*(IS)	457.3 → 407.1	457.3 → 464.7	457.3 → 564.5
74EIAQDFK$_{me2}$TDLR84 (dimethylated)	455.4 → 404.3	455.4 → 461.9	455.4 → 561.4
EIAQDF*K$_{me2}$TDLR*(IS)	462.3 → 414.5	462.3 → 472.3	462.3 → 571.6
74EIAQDFK$_{me3}$TDLR84 (trimethylated)	460.0 → 468.8	460.0 → 532.7	460.0 → 568.3
EIAQDF*K$_{me3}$TDLR*(IS)	466.8 → 478.9	466.8 → 543.1	466.8 → 578.6
74EIAQDFK$_{ac}$TDLR84 (acetylated)	689.5 → 126.0	689.5 → 674.3	689.5 → 936.3
EIAQDF*K$_{ac}$TDLR*(IS)	699.7 → 126.0	699.7 → 694.3	699.7 → 956.5

* Stable isotope-labeled amino acids [$^{13}C_9$, ^{15}N]Phe and [$^{13}C_6$, $^{15}N_4$]Arg.

H3K122 Surrogate Peptides	MRM Transitions (*m/z*)		
116VTIMPK123 (unmethylated)	344.9 → 375.2	344.9 → 488.2	344.9 → 589.5
VTI*MPK (IS)	349.7 → 375.2	349.7 → 498.3	349.7 → 599.3
116VTIMPK$_{me1}$DIQLAR127 (monomethylated)	467.0 → 477.6	467.0 → 599.6	467.0 → 650.2
VTI*MPK$_{me1}$DIQLAR*(IS)	473.8 → 482.7	473.8 → 609.8	473.8 → 660.4
116VTIMPK$_{me2}$DIQLAR127(dimethylated)	471.8 → 484.5	471.8 → 606.8	471.8 → 657.3
VTI*MPK$_{me2}$DIQLAR*(IS)	478.4 → 489.6	478.4 → 616.7	478.4 → 667.4
116VTIMPK$_{me3}$DIQLAR127(trimethylated)	476.6 → 491.3	476.6 → 613.9	476.6 → 664.4
VTI*MPK$_{me3}$DIQLAR*(IS)	483.1 → 496.5	483.1 → 623.7	483.1 → 674.4
116VTIMPK$_{ac}$DIQLAR127(acetylated)	714.1 → 126.0	714.1 → 600.4	714.1 → 885.5
VTI*MPK$_{ac}$DIQLAR*(IS)	724.3 → 126.0	724.3 → 610.3	724.3 → 895.5

* Stable isotope-labeled amino acids [D_{10}]Ile and [$^{13}C_6$, $^{15}N_4$]Arg.

H4K31 Surrogate Peptides	MRM Transitions (*m/z*)		
23DNIQGITKPAIR36 (unmethylated)	442.5 → 456.2	442.5 → 584.8	442.5 → 685.4
DNIQGI*TKPAIR* (IS)	449.6 → 466.3	449.6 → 594.4	449.6 → 695.3
23DNIQGITK$_{me1}$PAIR36 (monomethylated)	447.3 → 435.2	447.3 → 499.0	447.3 → 555.6
DNIQGI*TK$_{me1}$PAIR*(IS)	454.2 → 445.3	454.2 → 509.2	454.2 → 565.7
23DNIQGITK$_{me2}$PAIR36 (dimethylated)	452.2 → 442.3	452.2 → 506.0	452.2 → 561.5
DNIQGI*TK$_{me2}$PAIR*(IS)	458.8 → 452.4	458.8 → 516.2	458.8 → 572.3
23DNIQGITK$_{me3}$PAIR36 (trimethylated)	456.9 → 449.3	456.9 → 513.1	456.9 → 569.4
DNIQGI*TK$_{me3}$PAIR*(IS)	463.4 → 459.2	463.4 → 523.4	463.4 → 579.6
23DNIQGITK$_{ac}$PAIR36 (acetylated)	684.5 → 126.0	684.5 → 626.3	684.5 → 840.5
DNIQGI*TK$_{ac}$PAIR*(IS)	694.8 → 126.0	694.8 → 636.4	694.8 → 860.4

* Stable isotope-labeled amino acids [D_{10}]Ile and [$^{13}C_6$, $^{15}N_4$]Arg.

Table S2. Digestion efficiency for the substrate peptides.

Substrate Peptides	Surrogate Peptides	Digestion Efficiency (%)
VREIAQDFKTD	EIAQDFK	98.4
VREIAQDFK$_{me1}$TDLRFQ	EIAQDFK$_{me1}$TDLR	87.3
VREIAQDFK$_{me2}$TDLRFQ	EIAQDFK$_{me2}$TDLR	96.6
VREIAQDFK$_{me3}$TDLRFQ	EIAQDFK$_{me3}$TDLR	95.2
VREIAQDFK$_{ac}$TDLRFQ	EIAQDFK$_{ac}$TDLR	90.6

Table S3. Imprinting factors and cross-reactivity values of the MIPs for adsorption of the surrogate peptides of H3K79, H3K122 and H4K31.

(A)

Surrogate Peptides	Adsorption Amount (μmol/g)		*IF*	*CR*
	MIPs	NIPs		
EIAQDF (template)	0.91 ± 0.03	0.37 ± 0.03	2.43 ± 0.37	-
EIAQDFK	0.63 ± 0.02	0.29 ± 0.02	2.14 ± 0.22	0.93 ± 0.17
EIAQDFK$_{me1}$TDLR	0.56 ± 0.03	0.25 ± 0.04	2.21 ± 0.20	0.97 ± 0.16
EIAQDFK$_{me2}$TDLR	0.54 ± 0.02	0.23 ± 0.03	2.36 ± 0.24	0.96 ± 0.10
EIAQDFK$_{me3}$TDLR	0.48 ± 0.02	0.23 ± 0.02	2.10 ± 0.10	0.92 ± 0.10
EIAQDFK$_{ac}$ TDLR	0.52 ± 0.03	0.25 ± 0.02	2.18 ± 0.30	0.95 ± 0.20

(B)

Surrogate Peptides	Adsorption Amount (μmol/g)		*IF*	*CR*
	MIPs	NIPs		
VTIMP (template)	0.96 ± 0.01	0.33 ± 0.03	2.93 ± 0.24	-
VTIMPK	0.70 ± 0.02	0.24 ± 0.01	2.89 ± 0.03	0.99 ± 0.08
VTIMPK$_{me1}$DIQLAR	0.64 ± 0.01	0.23 ± 0.03	2.74 ± 0.43	0.94 ± 0.07
VTIMPK$_{me2}$DIQLAR	0.60 ± 0.01	0.22 ± 0.03	2.71 ± 0.40	0.92 ± 0.04
VTIMPK$_{me3}$DIQLAR	0.60 ± 0.02	0.22 ± 0.01	2.73 ± 0.12	0.93 ± 0.09
VTIMPK$_{ac}$ DIQLAR	0.62 ± 0.04	0.22 ± 0.02	2.85 ± 0.06	0.97 ± 0.05

(C)

Surrogate Peptides	Adsorption Amount (μmol/g)		*IF*	*CR*
	MIPs	NIPs		
DNIQGIT (template)	0.93 ± 0.03	0.34 ± 0.02	2.77 ± 0.09	-
DNIQGITKPAIR	0.67 ± 0.03	0.24 ± 0.04	2.77 ± 0.36	0.99 ± 0.09
DNIQGITK$_{me1}$PAIR	0.62 ± 0.06	0.23 ± 0.01	2.69 ± 0.20	0.97 ± 0.04
DNIQGITK$_{me2}$PAIR	0.59 ± 0.05	0.23 ± 0.01	2.59 ± 0.24	0.93 ± 0.07
DNIQGITK$_{me3}$PAIR	0.60 ± 0.06	0.23 ± 0.02	2.65 ± 0.17	0.96 ± 0.06
DNIQGITK$_{ac}$PAIR	0.62 ± 0.07	0.23 ± 0.02	2.72 ± 0.19	0.98 ± 0.07

Table S4. Comparison of the slopes of the calibrations curves measured with (*w*) and without (*w/o*) other surrogate peptides at high concentration.

	Slope of Calibration Curve				
	H3K79	**H3K79$_{me1}$**	**H3K79$_{me2}$**	**H3K79$_{me3}$**	**H3K79$_{ac}$**
***w/o* Other Surrogate Peptides (×10^{-2})**	4.66 ± 0.06	4.62 ± 0.08	4.44 ± 0.23	4.58 ± 0.06	4.52 ± 0.14
***w* Other Surrogate Peptides (×10^{-2})**	4.69 ± 0.17	4.59 ± 0.16	4.48 ± 0.11	4.55 ± 0.09	4.45 ± 0.02
p value*	0.810	0.811	0.764	0.702	0.427

*$p < 0.05$ is considered statistically different.

Table S5. Accuracy and precision for the QC samples. The precision and accuracy of this assay were estimated by QC samples of each target peptides at four concentrations in three validation runs. The intra- and inter-day precisions were expressed as the percent coefficient of variation (%CV). The accuracy was estimated by comparing the calculated mean concentrations to their nominal values (%bias). Both accuracy and precision were ≤ ± 15% (LLOQ, ≤ ± 20%).

Nominal Concentration	0.500 nM	1.50 nM	50.0 nM	320 nM
EIAQDFK				
Mean	0.544	1.55	53	339
%Bias	8.8	3.1	6.1	5.9
Intra-day Precision (%CV)	4.2	3.1	4.1	1.6
Inter-day Precision (%CV)	13.2	9	8.7	6.2
EIAQDFK$_{me1}$TDLR				
Mean	0.454	1.61	46.9	341
%Bias	-9.2	7.3	-6.3	6.6
Intra-day Precision (%CV)	10.4	7.6	7.6	3.3
Inter-day Precision (%CV)	13.6	4.7	9.1	4.3
EIAQDFK$_{me2}$TDLR				
Mean	0.56	1.44	45	311
%Bias	11.9	-3.8	-10	-2.9
Intra-day Precision (%CV)	5.3	3.2	2.7	1.4
Inter-day Precision (%CV)	12.7	8	8.3	3.1
EIAQDFK$_{me3}$TDLR				
Mean	0.524	1.43	54.1	315
%Bias	4.7	-4.4	8.2	-1.4
Intra-day Precision (%CV)	6.8	5.3	8.9	2.8
Inter-day Precision (%CV)	13	6.9	6.1	3.7
EIAQDFK$_{ac}$TDLR				
Mean	0.563	1.54	52.7	325
%Bias	12.6	2.4	5.5	1.6
Intra-day Precision (%CV)	6.3	3.2	7.5	3.7
Inter-day Precision (%CV)	11.7	2.3	2.4	3.4
n	18	18	18	18
Number of Runs	3	3	3	3

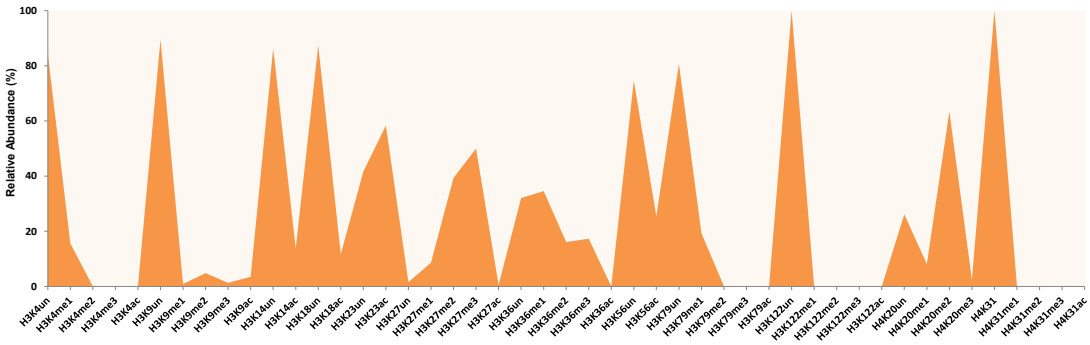
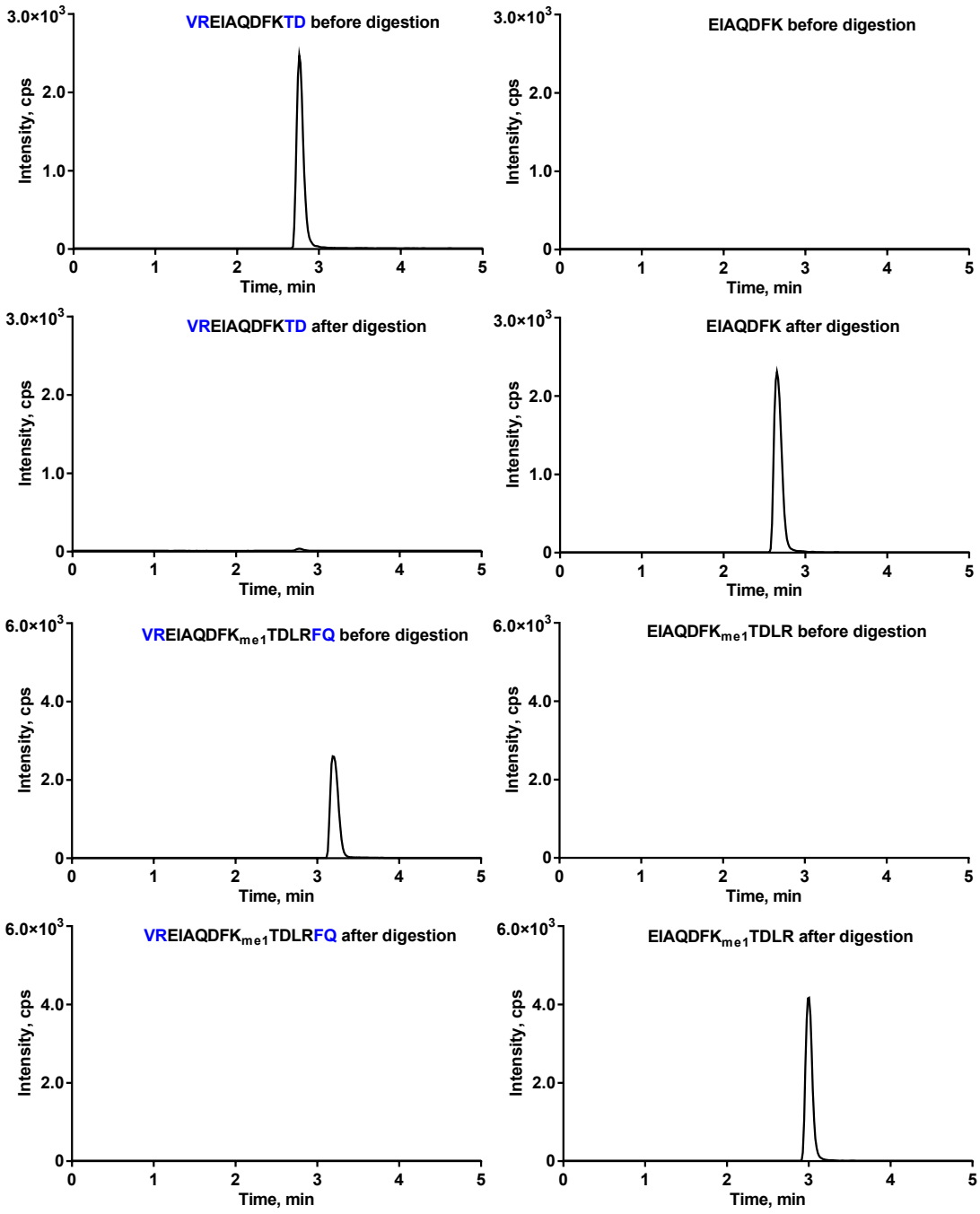


Figure S1. Relative abundance of partial detected histone modifications in MCF-7 cells. Although most of the reported modified peptides have been detected, some potential modifications with low abundance are missing.


VREIAQDFKTD before digestion
EIAQDFK before digestion
VREIAQDFKTD after digestion
EIAQDFK after digestion
VREIAQDFKme1TDLRFQ before digestion
EIAQDFKme1TDLR before digestion
VREIAQDFKme1TDLRFQ after digestion
EIAQDFKme1TDLR after digestion
Intensity, cps
Time, min
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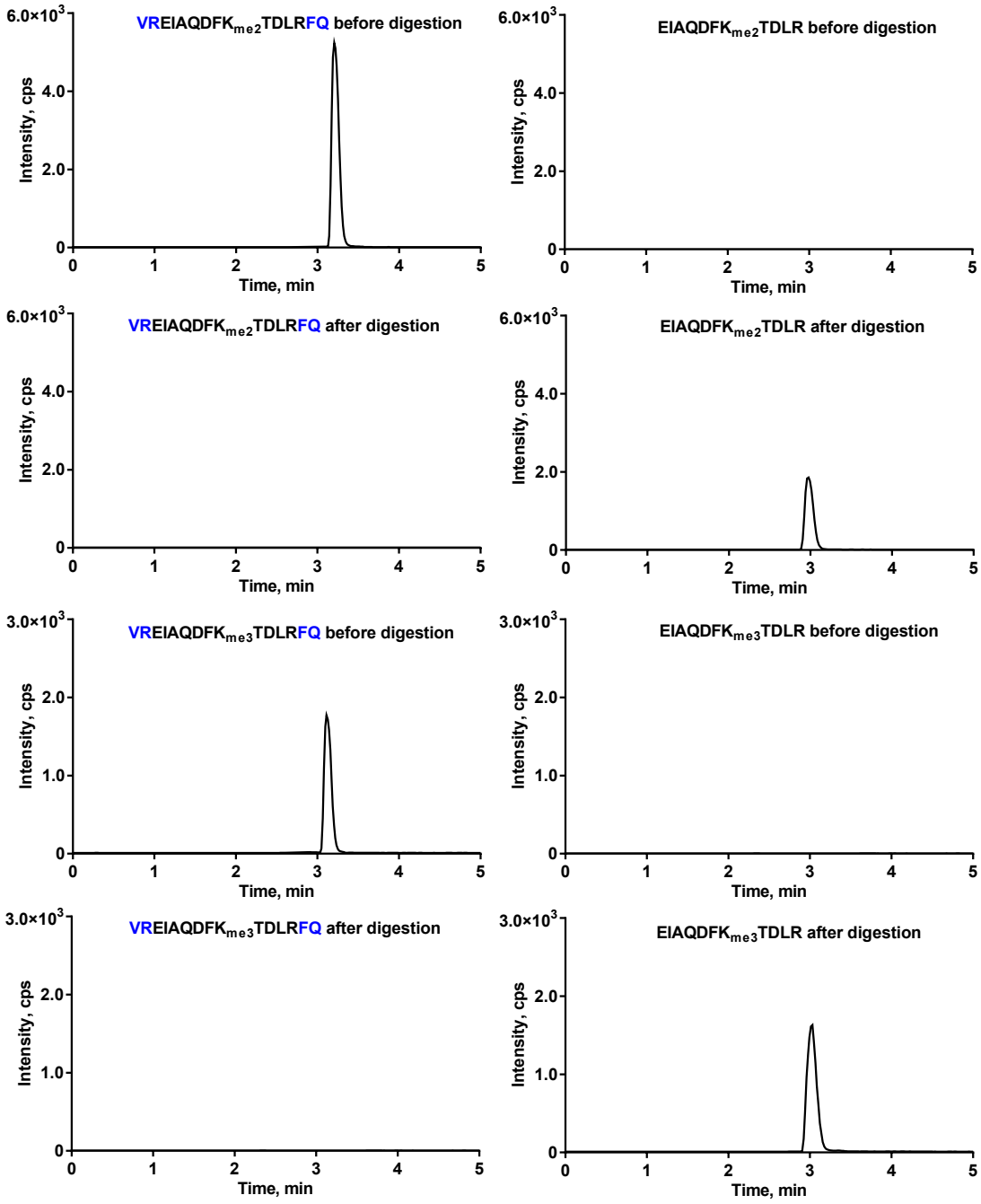
6.0×10^3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EIAQDFK$_{me2}$TDLR before digestion
VREIAQDFK$_{me2}$TDLRFQ after digestion
EIAQDFK$_{me2}$TDLR after digestion
VREIAQDFK$_{me3}$TDLRFQ before digestion
EIAQDFK$_{me3}$TDLR before digestion
VREIAQDFK$_{me3}$TDLRFQ after digestion
EIAQDFK$_{me3}$TDLR after digestion
Intensity, cps
Time, min
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2.0
0
3.0×10^3
1.0
1
2
3
4
5

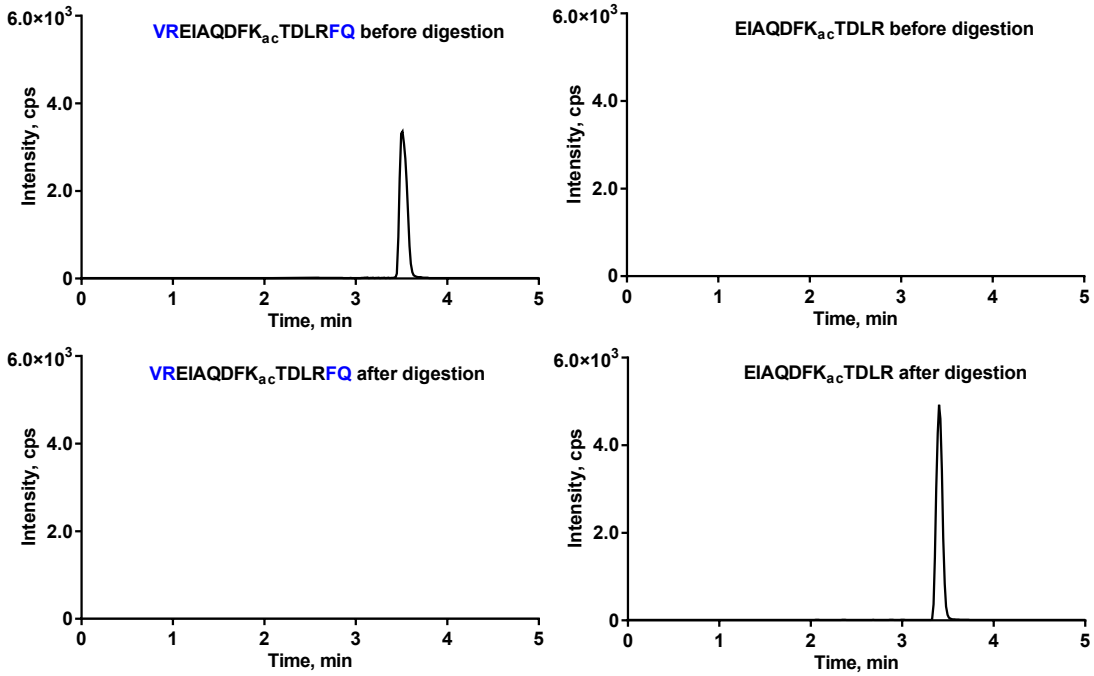
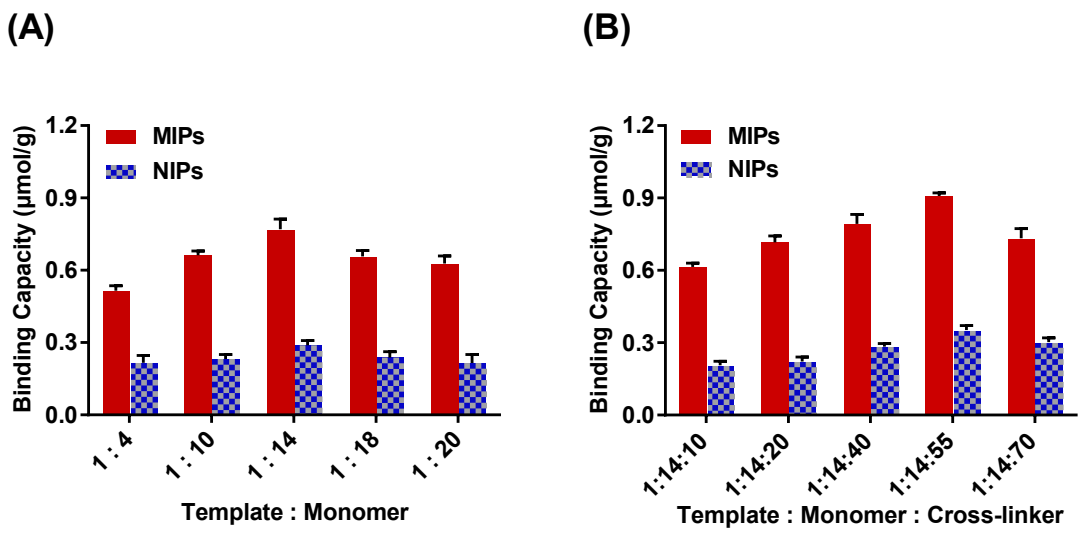


Figure S2. LC-MS/MS chromatograms of the five surrogate peptides and their corresponding substrate peptides before and after digestion. The sequence of the substrate peptides different from the surrogate peptides is highlighted in blue. For clarity, only one MRM transition for each peptide is presented. The result shows the absence of the substrate peptides and the presence of the surrogate peptides after digestion.



Polymer	T (μmol)	M (μmol)	C (μmol)	Ratio T : M : C	Binding Capacity (μmol/g)
1	16	224	160	1:14:10	0.61 ± 0.02
2	16	224	320	1:14:20	0.72 ± 0.03
3	16	224	640	1:14:40	0.79 ± 0.04
4	16	224	880	1:14:55	0.93 ± 0.02
5	16	224	1120	1:14:70	0.73 ± 0.04

*T = template, M = monomer, C = cross-linker

Figure S3. Optimization of MIPs preparation conditions to maximize binding capacity, including (A) different ratios of template and functional monomer (i.e., MAA) where the template/cross-linker molar ratio was 1:40 and (B) different ratios of template and cross-linker (i.e., EGDMA).

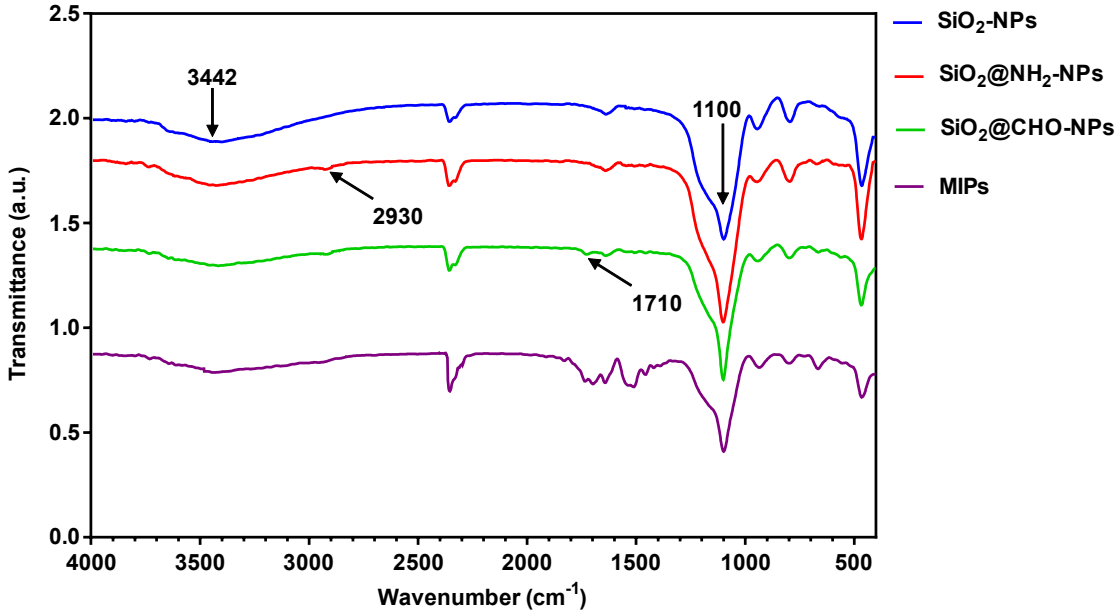
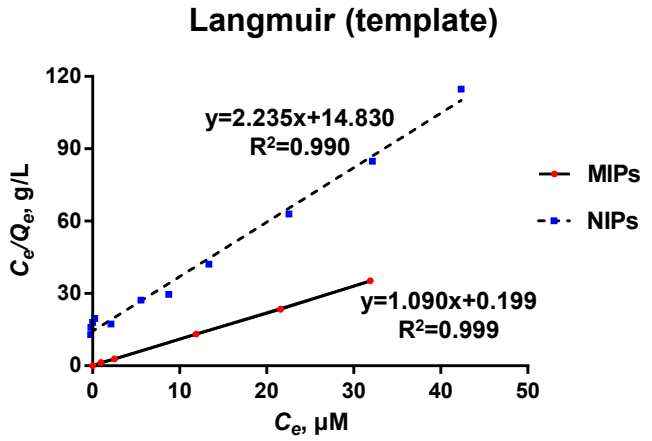


Figure S4. FT-IR spectra of SiO_2-NPs, SiO_2@NH_2-NPs, SiO_2@CHO-NPs and the MIPs. The strong absorption peaks near 1100 cm^{-1} and 3442 cm^{-1} suggest the formation of SiO_2-NPs. After the treatment with APTES, the peak near 2930 cm^{-1} due to C-H stretch of methylene is observed. In the FT-IR spectrum of SiO_2@CHO-NPs, the peak at approximately 1710 cm^{-1} indicates that aldehyde group has been successfully grafted to SiO_2@NH_2-NPs. Finally, the peak around 1710 cm^{-1} of the MIPs suggested the existence of EGDMA, demonstrating that the MIPs have been successfully prepared.



Nanoparticles	Q_{max} (μmol/g)[a]	K_L (L/μmol)[b]	R^2[c]
MIPs	0.917	5.463	0.999
NIPs	0.447	0.152	0.990

[a] Theoretical maximum adsorption capacity
[b] Langmuir constant
[c] Correlation coefficient

Figure S5. The best fit adsorption isotherm of Langmuir model and the estimated adsorption parameters.

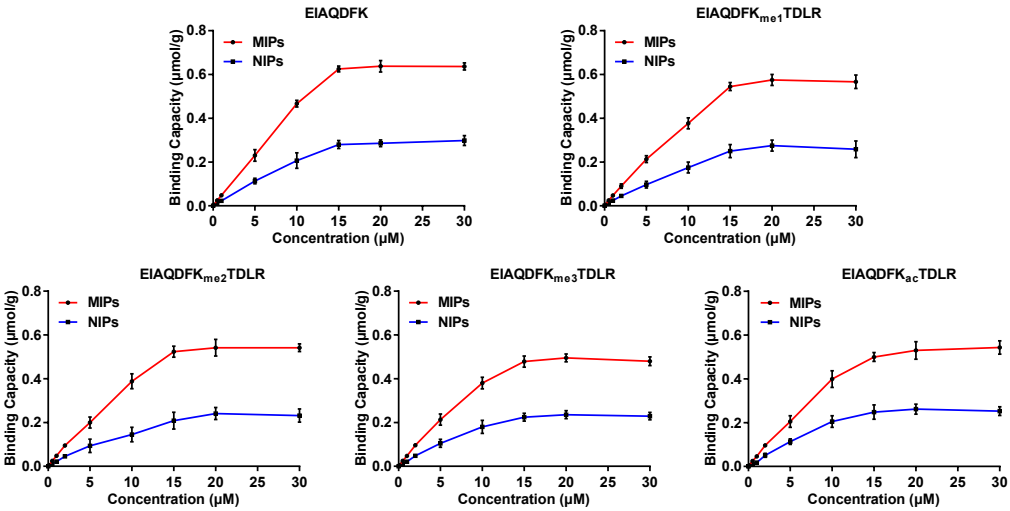


Figure S6. Adsorption capacity of the MIPs and the NIPs to the five H3K79 surrogate peptides. The static adsorption test was performed in 1 mL of the solution with different initial concentration of the template peptide ranging from 0 to 30 μM and 20 mg of the MIPs or the NIPs. The solution was incubated at the room temperature for 24 h.

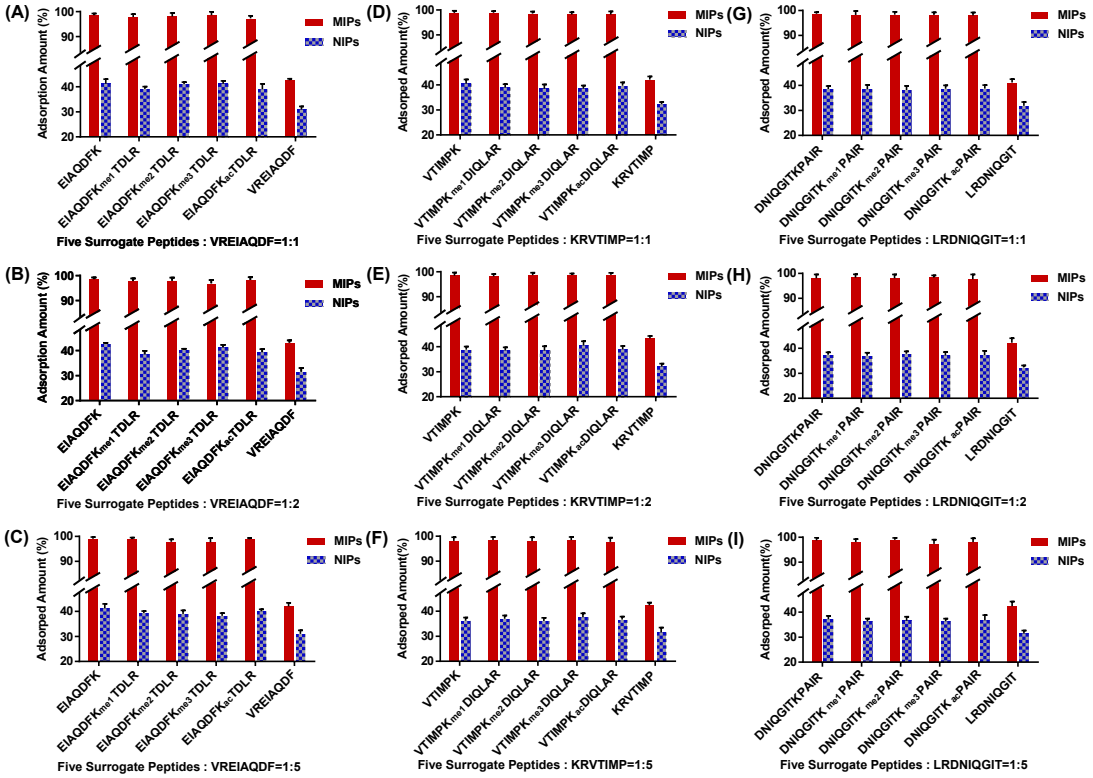


Figure S7. Oriented selectivity of the MIPs at different concentration ratios of the surrogate peptides and the competing peptide.

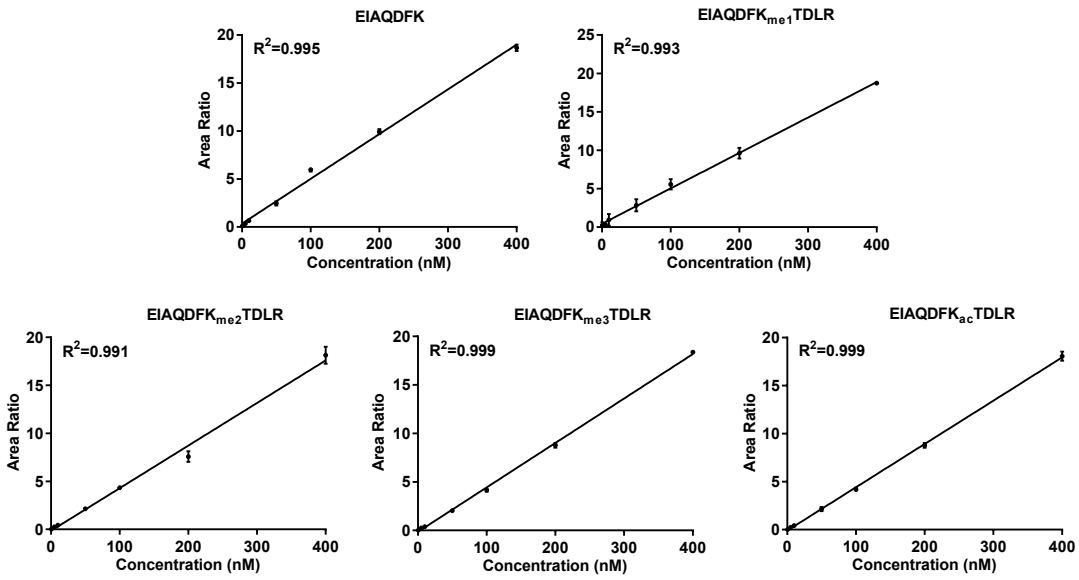


Figure S8. Calibration curves of the five H3K79 surrogate peptides.

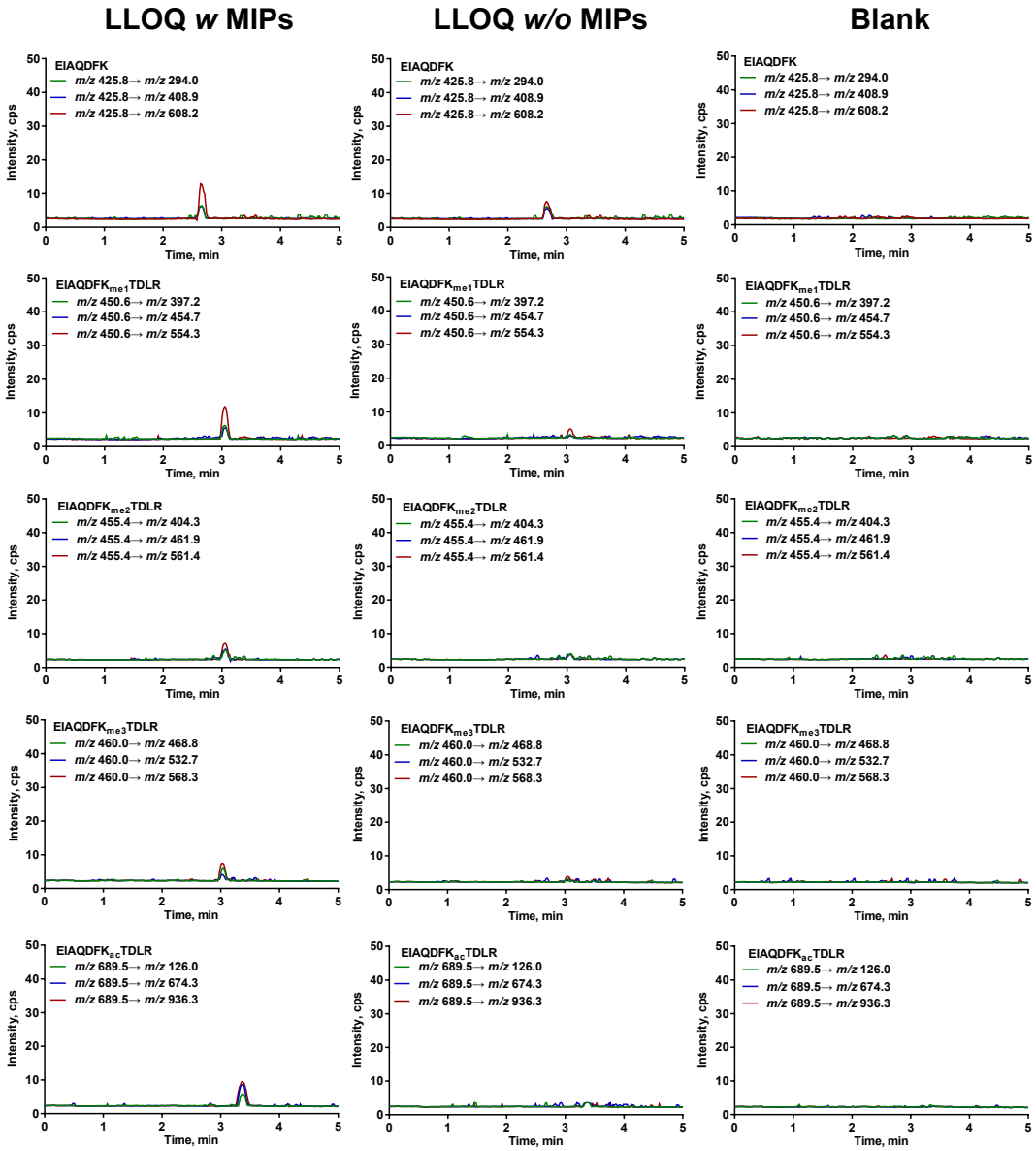


Figure S9. LC-MS/MS chromatograms of 0.5 nM surrogate peptides (i.e., LLOQ in this study) with (*w*) and without (*w/o*) the MIPs, and the corresponding blanks.

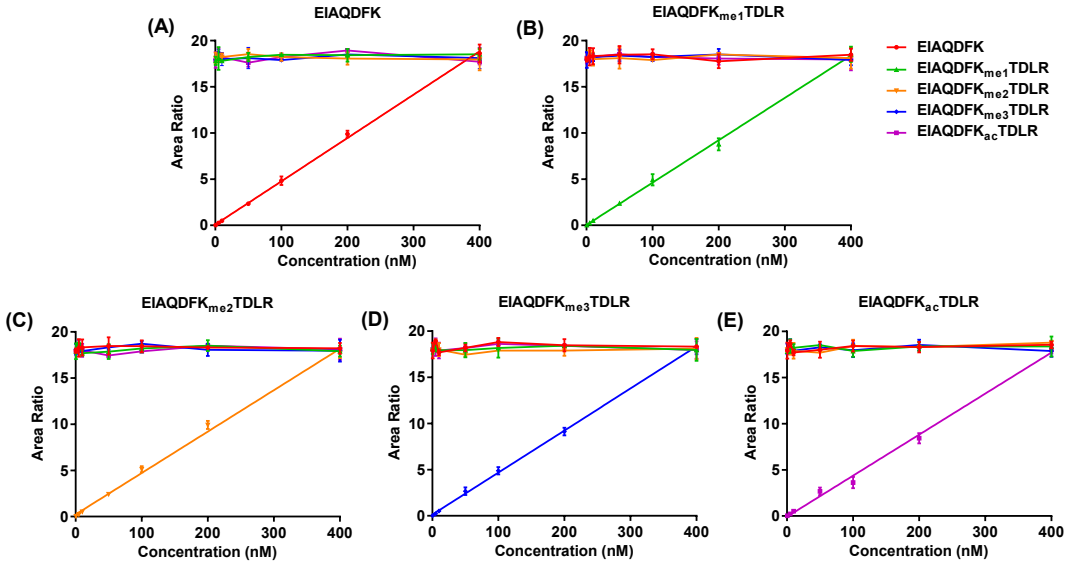
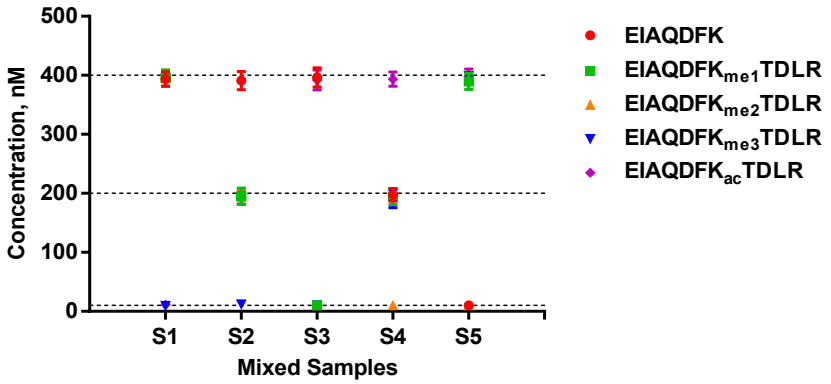


Figure S10. Calibration curves contain all five surrogate peptides, with one peptide with increasing concentration ((A) unmethylated, (B) monomethylated, (C) dimethylated, (D) trimethylated and (E) acetylated) and the other four with a constant concentration of 400 nM each.



Mixed Samples	Concentration (nM)				
	H3K79	H3K79$_{me1}$	H3K79$_{me2}$	H3K79$_{me3}$	H3K79$_{ac}$
S1	400	400	400	10	10
S2	400	200	200	10	200
S3	400	10	10	10	400
S4	200	200	10	200	400
S5	10	400	10	400	400

Figure S11. Simultaneous quantification of the five H3K79 surrogate peptides at varying concentrations. Five samples (S1 to S5) comprising five surrogate peptides with given concentrations randomly between 10 and 400 nM were measured and the calculated concentrations (filled symbols and bars) and theoretical concentrations (dashed lines) are shown.

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