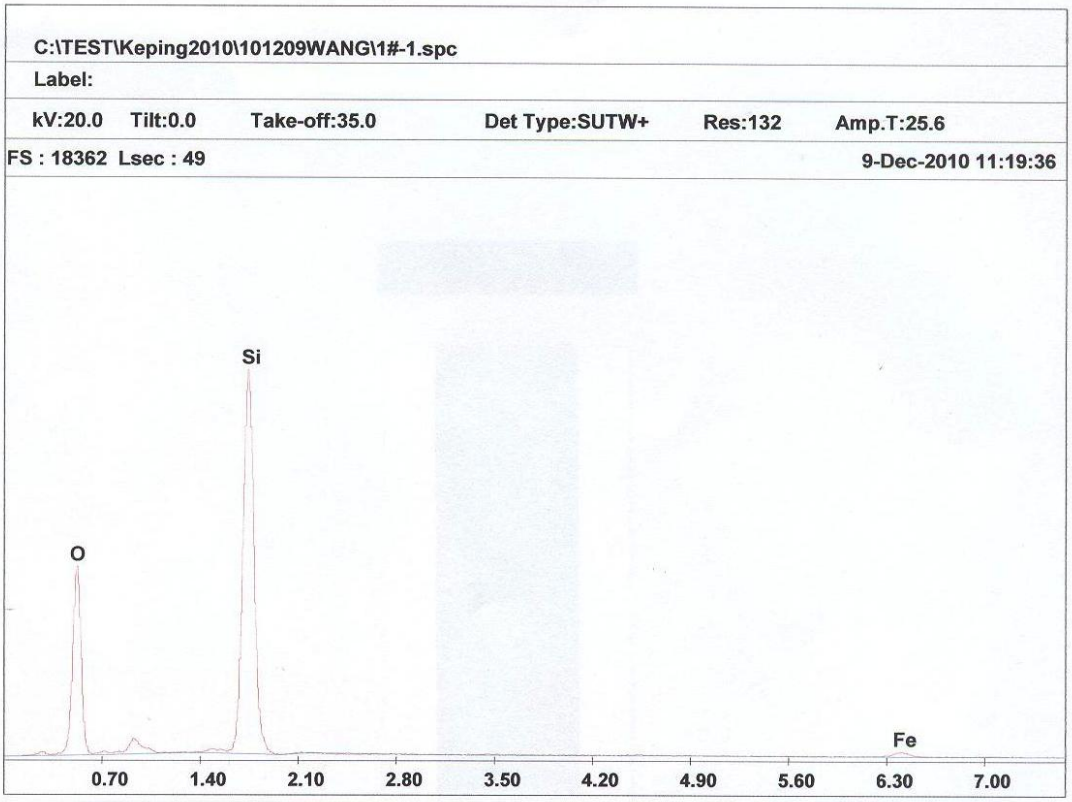
Electronic Supplementary Information

1. Synthesis protocol of Fe-ZSM-5 zeolite monolithic column

Hierarchical Fe-ZSM-5 zeolite monolithic affinity column were obtained *via* a steam assisted conversion (SAC) method. The initial precursor gel solutions were prepared from tetrapropyl ammonium hydroxide (TPAOH, 25 wt% in water, Aldrich), white carbon black (SiO_2), $FeCl_3.6H_2O$ and distilled water as the following molar composition: 150:390:8000:15(TPAOH: SiO_2: H_2O: Fe_2O_3).

Prior to fabrication of monolithic column, the fused silica capillary was rinsed with 1.0M NaOH for 1 h, and water for another hour. Also it was flushed with 0.1M HCl overnight at 120 ^oC. After the precursor gel solution was injected into the pretreated fused silica capillary column, the capillary was sealed and kept in an oven at 80 ^oC for drying process. Finally the column was placed on a stainless steel board placed horizontally in the middle of PTFE-lined stainless steel autoclave and distilled water was poured into the bottom of the autoclave. The drying gel inside the column is crystallized under autogenous water steam pressure at 140 ^oC for 48h. The autoclave was quenched and the resulted monolithic column was dried at 100 ^oC for 20 min without any additional purification.

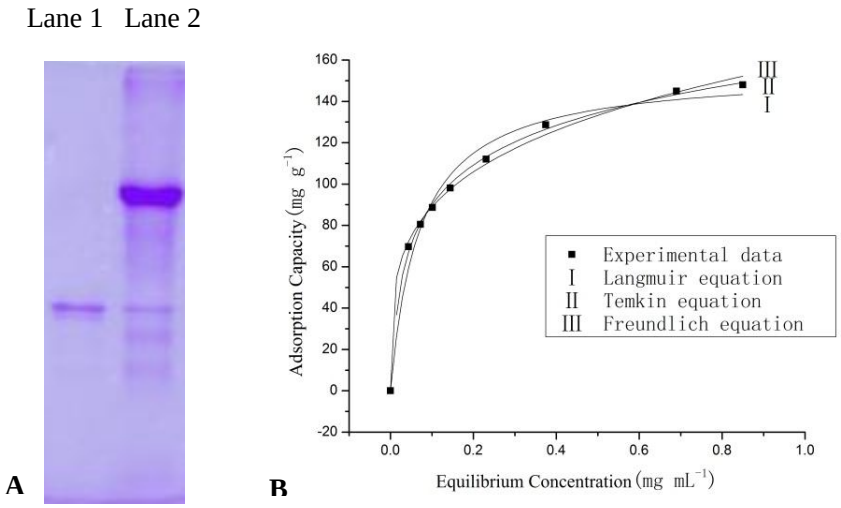
2. Detailed EDX report for Fe-ZSM-5 zeolite



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : User c:\edax32\eds\genuser.sec

Element	Wt %	At %	K-Ratio	Z	A	F
O K	55.85	69.54	0.2153	1.0207	0.3775	1.0005
SiK	41.71	29.59	0.3279	0.9783	0.8033	1.0001
FeK	2.43	0.87	0.0210	0.8621	1.0022	1.0000
Total	100.00	100.00				

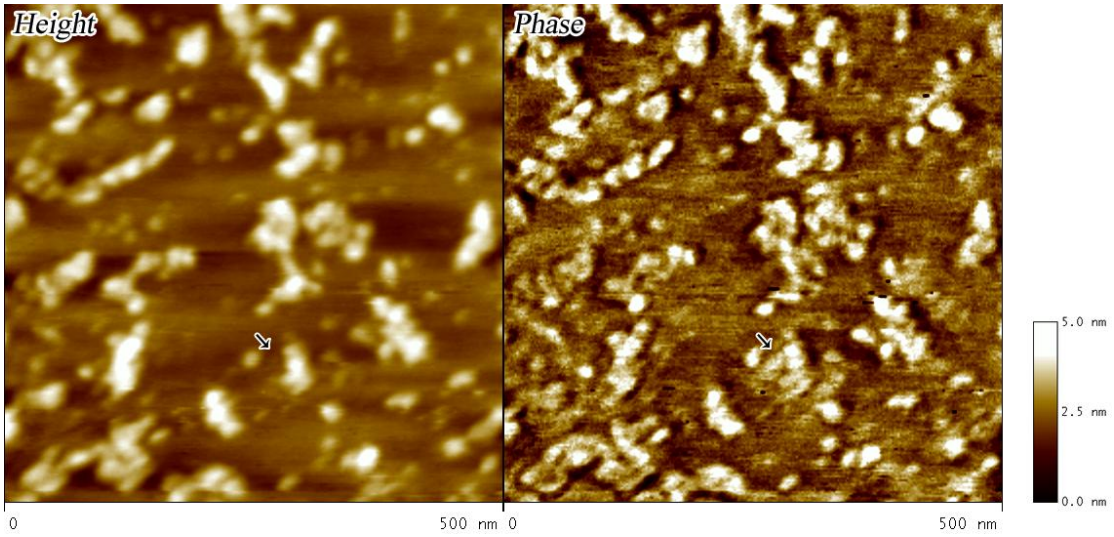
3. Model protein adsorption performance



S-Fig. 1 (A) Mixture of standard proteins including α-casein and BSA was applied to Fe-ZSM-5-zeolite monolith to reveal the selective enrichment of α-casein as phosphorylated protein. (B) Adsorption isothermal curve for a-casein on Fe-ZSM-5 zeolite monolith.

In our work, model proteins including α-casein with five phosphoserine residues and bovine serum albumin (BSA) as non-phosphorylated protein, were used to examine their respective performance. After the protein mixture solution containing 5 mL, 1.4 mg mL^{-1} α-casein and 5 mL, 4.2 mg mL^{-1} BSA were completely Vortex mixed with 10.0 mg zeolite monolith for 3 h at 2400 rpm, the filtered zeolite monolith was washed by 0.01 mol L^{-1} acetic acid solution. And then the zeolite monolith was Vortex mixed with 5 mL phosphate buffered saline pH 7.2 as eluting solution for 0.5 h at 2400 rpm under iced-bathing. Finally 10 μL the protein mixture solution, and the eluted solution were applied for 13% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). According to S-Fig. 1, there remained a single protein band on behalf of α-casein in lane 1, in comparison to lane 2 for the protein mixture solution. It demonstrated Fe-ZSM-5 zeolite monolith had high priority for coordination with phosphorylated α-casein. It had been also optimized that the bound α-casein could be recovered using 1.0 mol L^{-1} ammonium bicarbonate as the elution buffer solution. And Fe-ZSM-5-zeolite monolith could be regenerated for reuse with good efficiency, after Votex mixed with the elution solution at 2400 rpm for 30 min at room temperature. Furthermore the sorption isotherm for α-casein was measured in different protein concentrations. As illustrated in S-Fig. 1, it was fitted to Langmuir models, and the maximum sorption capacity for α-casein was 140 mg g^{-1}.

4. AFM images reveal the adsorption of phosvitin onto Fe-ZSM-5 zeolite



S-Fig. 2 AFM images of phosvitin adsorption onto Fe-ZSM-5 zeolite. Height and phase images were collected at the exact same point. The arrows in both images revealed the phosvitin attached to the Fe-ZSM-5 zeolite monolith.

As given in S-Fig. 2, in the phase image, the arrow pointing at a white color position, which reveals the different phase from the surroundings. However, in the height image, at the very same point, the height was nearly the same as the surroundings. It can be concluded that this very point was phosvitin, which was adsorbed by the surrounding Fe-ZSM-5 zeolite monolith.

5. T. *thermophila* culture conditions

T. thermophila, B2086, wild type, myting type II was provided by the Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan, China. The cells were cultured at 28 °C in SPP medium (1% tryptone OXOID, 0.2% glucose, Shanghai Boao Biotechnology Co., Ltd.). Erlenmeyer flasks containing 20 mL of SPP media were inoculated with T. thermophila cells at an initial population density of approximately 1.0×10^4-1.5×10^4 cells mL^{-1} during their exponential growth phase.

6. MS/MS protocols

a. In-gel tryptic digest.

The bands were manually excised from the CBB-stained gels and then transferred to V-bottom 96-well microplates loaded with 100 μl of 50% ACN/25 mM ammonium bicarbonate solution per well. After being destained for 1 h, gel bands were dehydrated with 100 μl of 100% ACN for 20 min and then thoroughly dried in a SpeedVac concentrator (Thermo Savant, USA) for 30 min. The dried gel particles were rehydrated at 4°C for 45 min with 2 μl/well trypsin (Promega, Madison, WI, USA) in 25 mM ammonium bicarbonate, and then incubated at 37°C for 12 h. After trypsin digestion, the peptide mixtures were extracted with 8 μl extraction solution (50% ACN/0.5% TFA) per well at 37°C for 1 h. Finally, the extracts were dried under the protection of N_2.

b. Nano-flow LC-MS/MS.

Nano-LC MS/MS experiment was performed on an HPLC system composed by two LC-20AD nano-flow LC pumps, an SIL-20 AC auto-sampler and an LC-20AB micro-flow LC pump (all Shimadzu, Tokyo, Japan) connected to an LTQ-Orbitrap mass spectrometer (ThermoFisher, San Jose, CA). Sample was loaded onto a CAPTRAP column (0.5 x 2 mm, MICHROM Bioresources, Auburn, CA) in 3 min at a flow rate of 50μL/min. The sample was subsequently separated by a C18 reverse-phase column (0.1 x 150 mm, packed with 3 μm Magic C18-AQ particles, MICHROM Bioresources, Auburn CA) at a flow rate of 500nL/min. The mobile phases were 5% acetonitrile with 0.1% formic acid (phase A and the loading phase) and 95% acenitrile with 0.1% formic acid (phase B). To achieve proper separation, a 90-min linear gradient from 5 to 45% phase B was employed. The separated sample was introduced into the mass spectrometer via an ADVANCE 30μm silica tip (MICHROM Bioresources, Auburn CA). The spray voltage was set at 1.0 kV and the heated capillary at 180℃. The mass spectrometer was operated in data-dependent mode and each cycle of duty consisted one full- MS survey scan at the mass range 400~2000 Da with resolution power of 100,000 using the Orbitrap section, followed by MS/MS experiments for 10 strongest peaks using the LTQ section. The AGC expectation during full-MS and MS/MS were 1000000 and 10000, respectively. Peptides were fragmented in the LTQ section using collision-induced dissociation with helium and the normalized collision energy value set at 35%. Only 2+ and 3+ peaks were selected for MS/MS run and previously fragmented peptides were excluded for 60s.

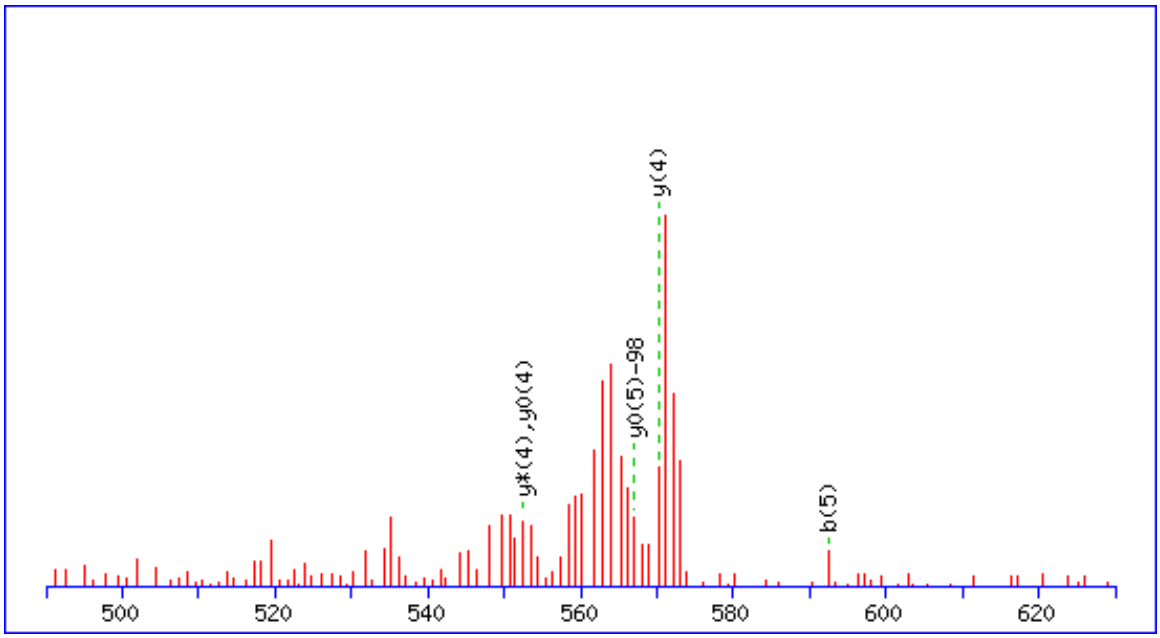
c. Database Search.

Protein searches were performed with the Mascot 2.2.07 software (www.matrixscience.com) against the database containing the NCBI nr protein database (downloaded from NCBI website at Jun. 2008) with the following criteria: 2 possible missed cleavage sites with enzyme set to trypsin, peptide mass tolerance of 20 ppm, fragment mass tolerance of 0.80 Da, Acetylated protein N-term and oxidized Met were considered as possible modifications. The acceptance

criterion for peptide identifications was the rate of false positive identification less than 5%.

7. MS/MS Data for verifying protein phosphorylation

MS/MS Fragmentation of HLDLLTNQK



S-Fig. 3 MS/MS spectrum for fragmentation of peptide HLDLLTNQK, m/z ranges from 490 to 630.

Monoisotopic mass of neutral peptide Mr(calc): 1160.5591

Variable modifications:

T6: Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769

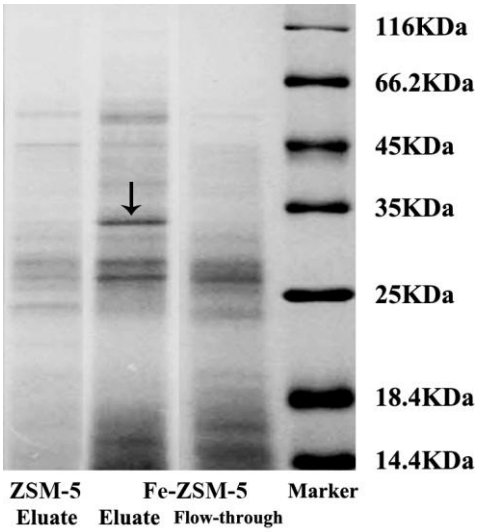
Ions Score: 31

Expect: 10

Matches (Bold Red): 15/120 fragment ions using 29 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	138.0662	69.5367					H							9
2	251.1503	126.0788					L	1024.5074	512.7574	1007.4809	504.2441	1006.4969	503.7521	8
3	366.1772	183.5922			348.1666	174.5870	D	911.4234	456.2153	894.3968	447.7021	893.4128	447.2100	7
4	479.2613	240.1343			461.2507	231.1290	L	796.3964	398.7019	779.3699	390.1886	778.3859	389.6966	6
5	592.3453	296.6763			574.3348	287.6710	L	683.3124	342.1598	666.2858	333.6466	665.3018	333.1545	5
6	773.3593	387.1833			755.3488	378.1780	T	570.2283	285.6178	553.2018	277.1045	552.2177	276.6125	4
7	887.4023	444.2048	870.3757	435.6915	869.3917	435.1995	N	389.2143	195.1108	372.1878	186.5975			3
8	1015.4608	508.2341	998.4343	499.7208	997.4503	499.2288	Q	275.1714	138.0893	258.1448	129.5761			2
9							K	147.1128	74.0600	130.0863	65.5468			1

8. Entire Gel for Fig. 4 in the manuscript



S-Fig. 3 SDS-PAGE profiles for the eluate and flow-through solution of *T. thermophila* intracellular protein mixtures after protein phosphorylation immobilization by Fe-ZSM-5 zeolite monolithic column compared to eluate solution immobilized by ZSM-5 zeolite monolithic column.