Supplemental Information

Phosphopeptide Enrichment on Functionalized Polymer Microspots for MALDI-MS Analysis

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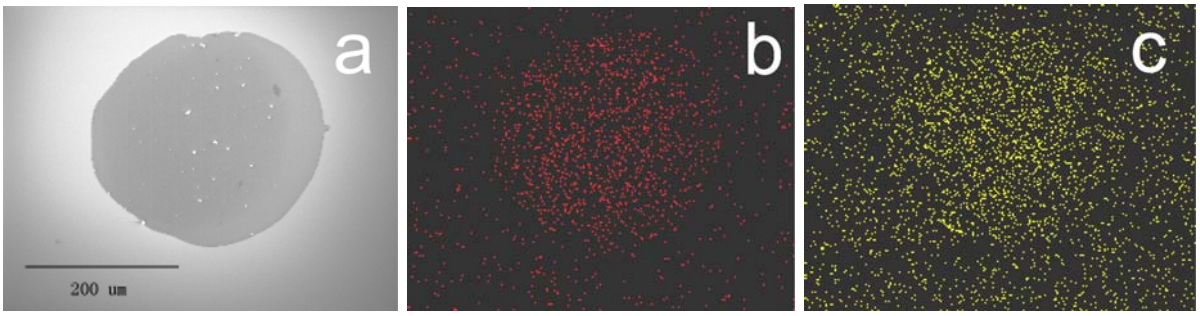

Fig. S1 (a) SEM image of a microspot of PHEMA-NTA-Fe(III) brushes surrounded by a residual PDMS layer on Si, (b) EDS carbon mapping of the spot, and (c) EDS oxygen mapping of the spot.

Table S1 Phosphopeptides that result from tryptic digestion of *β*-casein and ovalbumin. The amino acids in parentheses represent cleavage sites and are not part of the peptide sequence.

Phosphoprotein	$[M+H]^+$ m/z	Phosphorylation sites	Amino acid sequence
β-casein	2062	1	(K)FQpSEEQQQTEDELQDK
β-casein	2966	4	(R)ELEELNVPGEIVEpSLpSpSpSEESITR
β-casein	3122	4	(A)RELEELNVPGEIVEpSLpSpSpSEESITR*
ovalbumin	2089	1	(R)EVVGpSAEAGVDAASVSEEFR
ovalbumin	2511	1	(K)LPGFGDpSIEAQCGTSVNVHSSLR
ovalbumin	2901	1	(R)FDKLPGFGDpSIEAQCGTSVNVHSSLR*

* $4P_{3122}$ and $1P_{2901}$ are generated by unspecific cleavage and missed cleavage, respectively, of the phosphoprotein by trypsin.

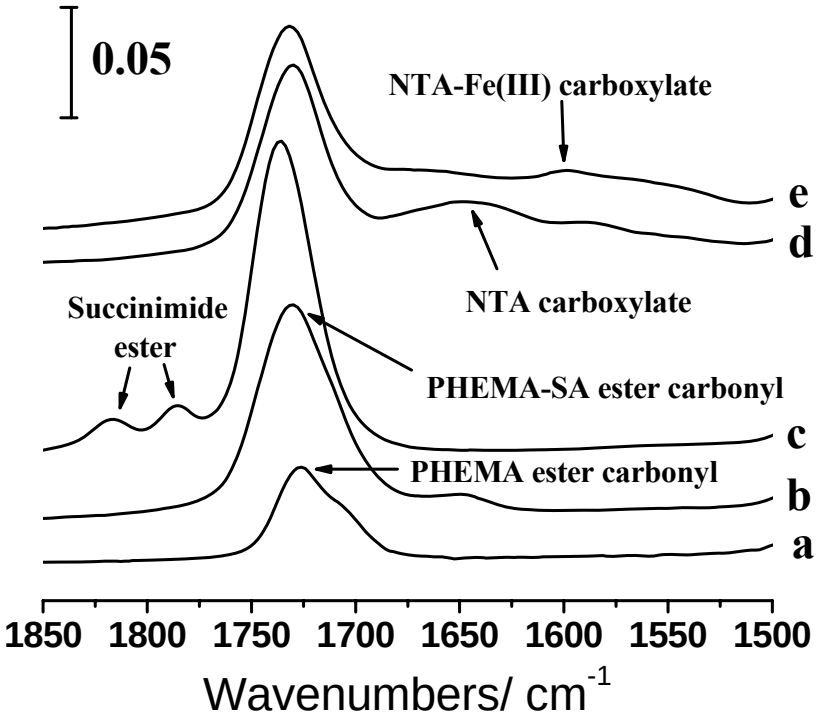

Fig. S2 Transmission FTIR absorbance spectrum of a double-side polished Si wafer coated with PHEMA brushes on both sides (a), and absorbance spectra of the same sample after the following modification steps: (b) reaction with succinic anhydride (SA), (c) activation with 0.1 M *N*-hydroxysuccinimide/ *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (NHS/EDC), (d) derivatization with aminobutyl NTA and (e) immersion in 0.1 M $FeCl_3$ followed by rinsing with 250 mM HAc/30% acetonitrile/70% water. Reference 13 provides a detailed explanation of similar spectra of PHEMA films on Au.

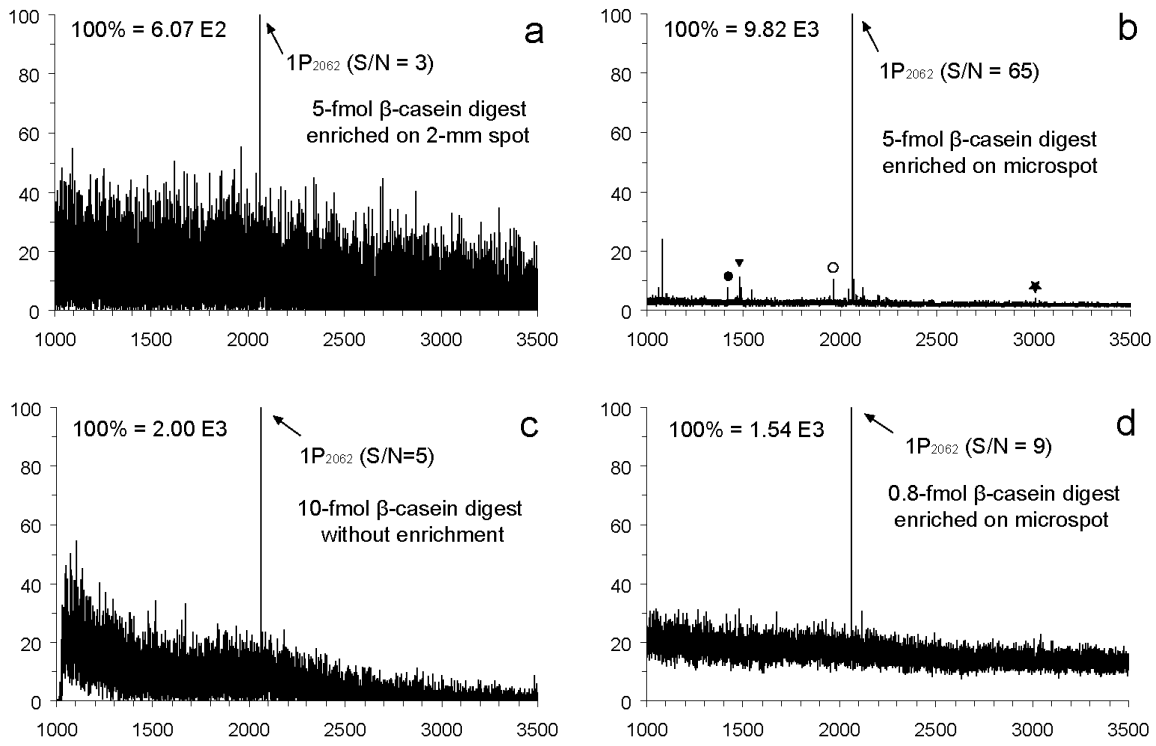

Fig. S3. MALDI mass spectra of β-casein digests. The spectra were obtained (a) after enrichment on the 2-mm PHEMA-NTA-Fe(III) spot, (b, d) after enrichment on the PHEMA-NTA-Fe(III) microspot, and (c) using a conventional stainless steel MALDI plate without enrichment. The amount of β-casein digest is noted in each mass spectrum. The monophosphopeptides detected are labeled as 1P, with their S/N ratios in parentheses. Other phosphopeptide-related peaks are labeled with different symbols: ▼ doubly protonated phosphopeptide, ● α-S1-casein phosphopeptide, ★ α-S2-casein phosphopeptide, ○ phosphopeptide signal after loss of 98 Da (H_3PO_4). Peak identification was facilitated by CID-MS/MS.

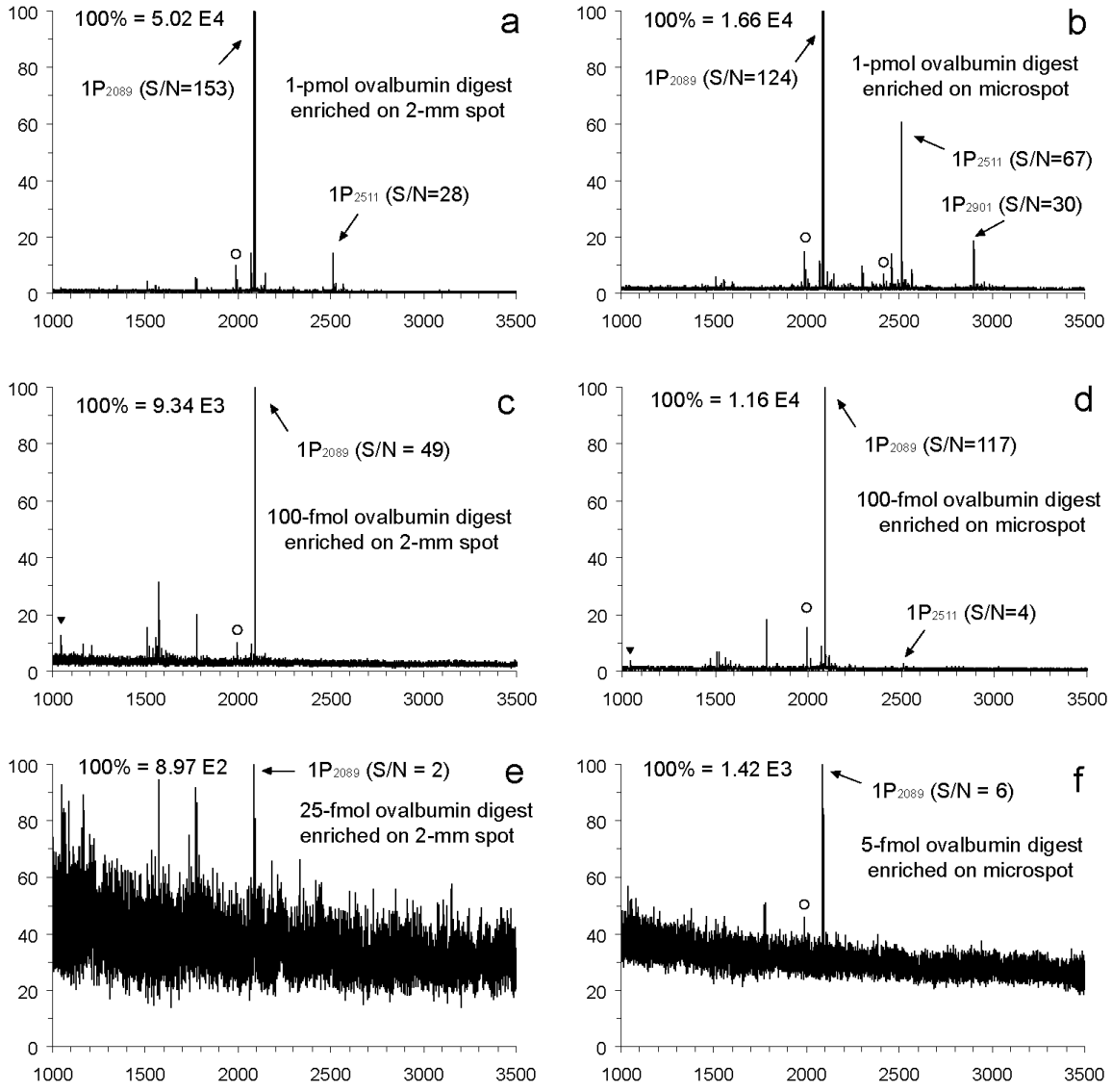

Fig. S4 MALDI mass spectra of ovalbumin digests after enrichment on (a, c, e) 2-mm spots of PHEMA-NTA-Fe(III) brushes, and (b, d, f) microspots of PHEMA-NTA-Fe(III) brushes. The amount of ovalbumin digest is noted in each mass spectrum. The monophosphopeptides are labeled as 1P, with their S/N ratios in parentheses. Phosphopeptides formed by loss of 98 Da (H_3PO_4) are labeled as ○; doubly protonated phosphopeptides are labeled as ▼. Peak identification was facilitated by CID-MS/MS.

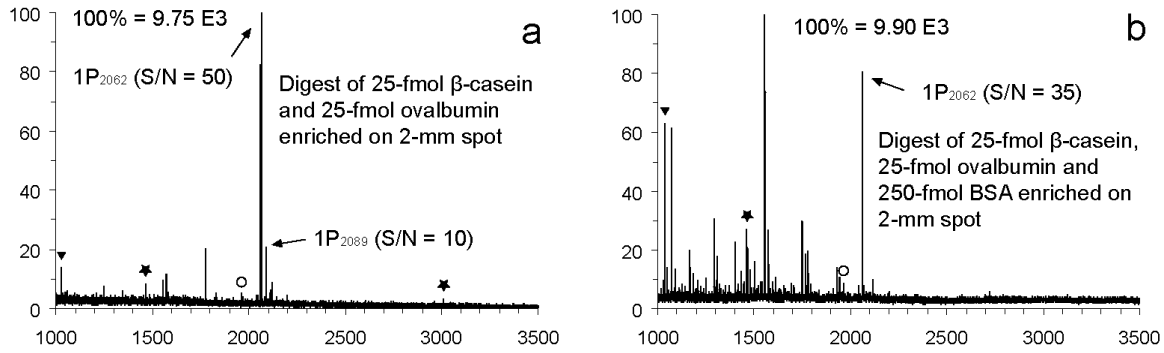

Fig. S5 MALDI mass spectra of mixtures of protein digests. The spectra were obtained using enrichment on 2-mm spots of PHEMA-NTA-Fe(III) brushes using a wash-bottle rinse before matrix was added. The compositions of the digest mixtures are noted in each mass spectrum. Monophosphopeptides are labeled as 1P, with their S/N ratios in parentheses. Other phosphopeptide-related peaks are labeled with different symbols: ▼ doubly protonated phosphopeptide, ★ α-S2-casein phosphopeptide, and ○ phosphopeptide signal after loss of 98 Da (H_3PO_4). Peak identification was facilitated by CID-MS/MS.

Determination of the amount of Fe(III) chelated in PHEMA-NTA-Fe(III) brushes:

Fe(III) ions were eluted from five single-side polished silicon wafers (2.4 × 1.1 cm dimensions) coated with PHEMA-NTA-Fe(III) brushes (average thickness 78 nm) by sequentially immersing the wafers in the same solution of 7.0 mL of 50 mM EDTA (pH 7.2) for 5 h with stirring (one wafer was taken out before the new one was put in). The absorbance of Fe(III) in the stripping solution was determined using a Varian Spectra AA-200 atomic absorption spectrophotometer, and the amount of Fe(III) was calculated from this absorbance using a calibration curve, which was determined by measuring the absorbance of $FeCl_3$ standard solutions in 50 mM EDTA.