

Supporting Information

Free-standing Lamellar 3D Architectures Assembled from Chitosan as Reusable Titanium-immobilized Affinity Membrane for Efficiently Capturing Phosphopeptides

Lei Pan^{a, b}, Shujuan Ma^b, Ruizhi Tang^b, Wenrui Wu^{a, b}, Junjie Ou^{b, c*}, Cong Li^{a*}, Yehua Shen^a

^a Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry and Materials Science, National Demonstration Center for Experimental Chemistry Education, Northwest University, Xi'an, 710127, China

^b Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences(CAS), Dalian, 116023, China

^c University of Chinese Academy of Sciences, Beijing, 100049, China

To whom correspondence should be addressed:

(J.J. Ou) Tel: +86-411-84379576. Fax: +86-411-84379620.

E-mail: junjieou@dicp.ac.cn

(C. Li) Tel: +86-13571961479. Fax: +86-29-88302635.

E-mail: licong@nwu.edu.cn

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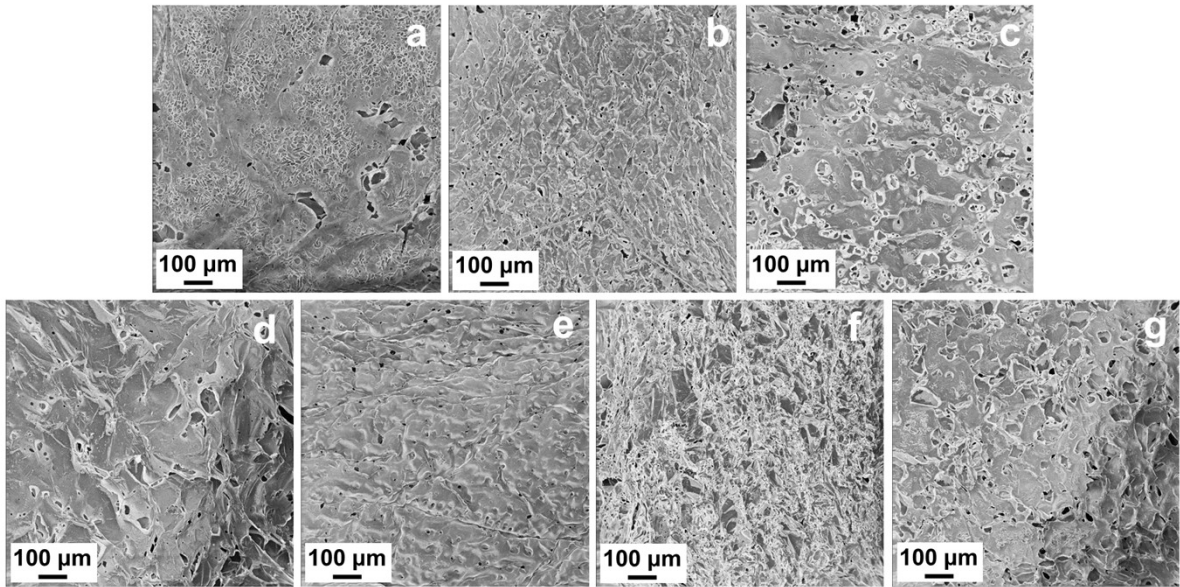


Figure S1. HIM images of the top surface microstructure of (a-g) CM-I-VII.

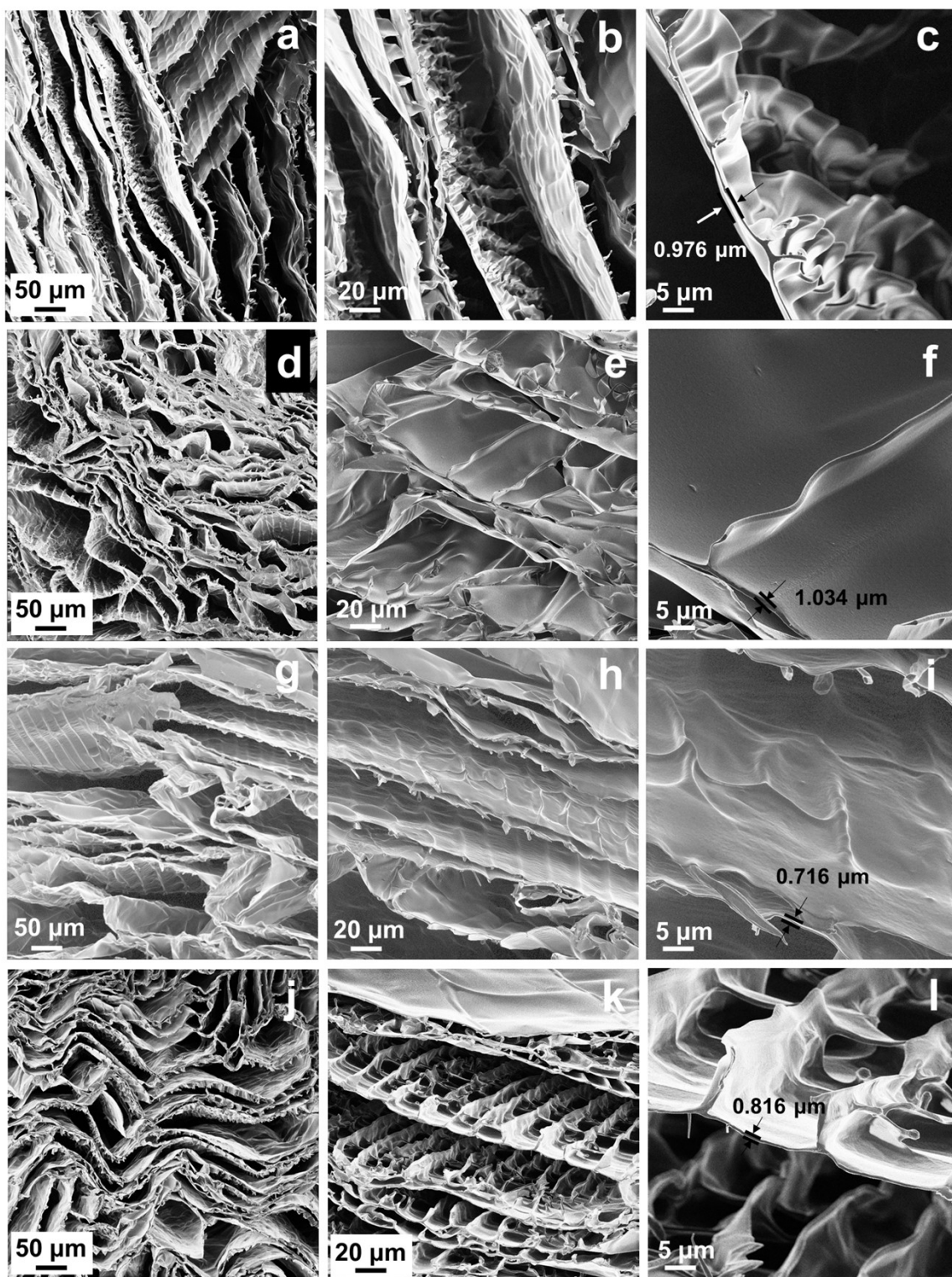


Figure S2. HIM images of cross section microstructure of (a-c) CM-IV, (d-f) CM-V, (g-i) CM-VI and (j-l) CM-VII.

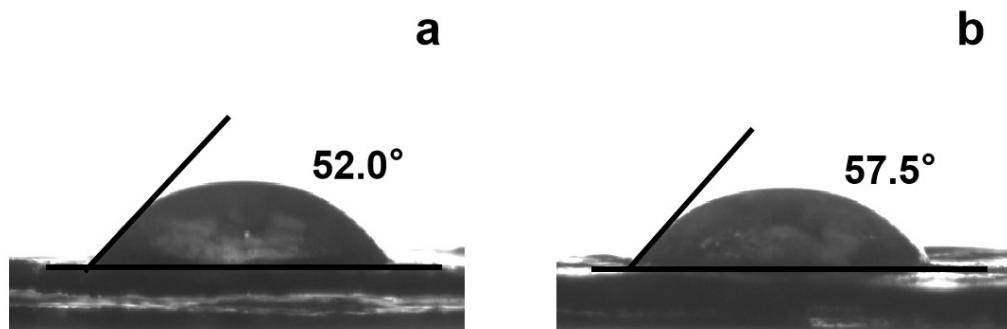


Figure S3. Water contact angles of (a) CM-I and (b) CM-II.

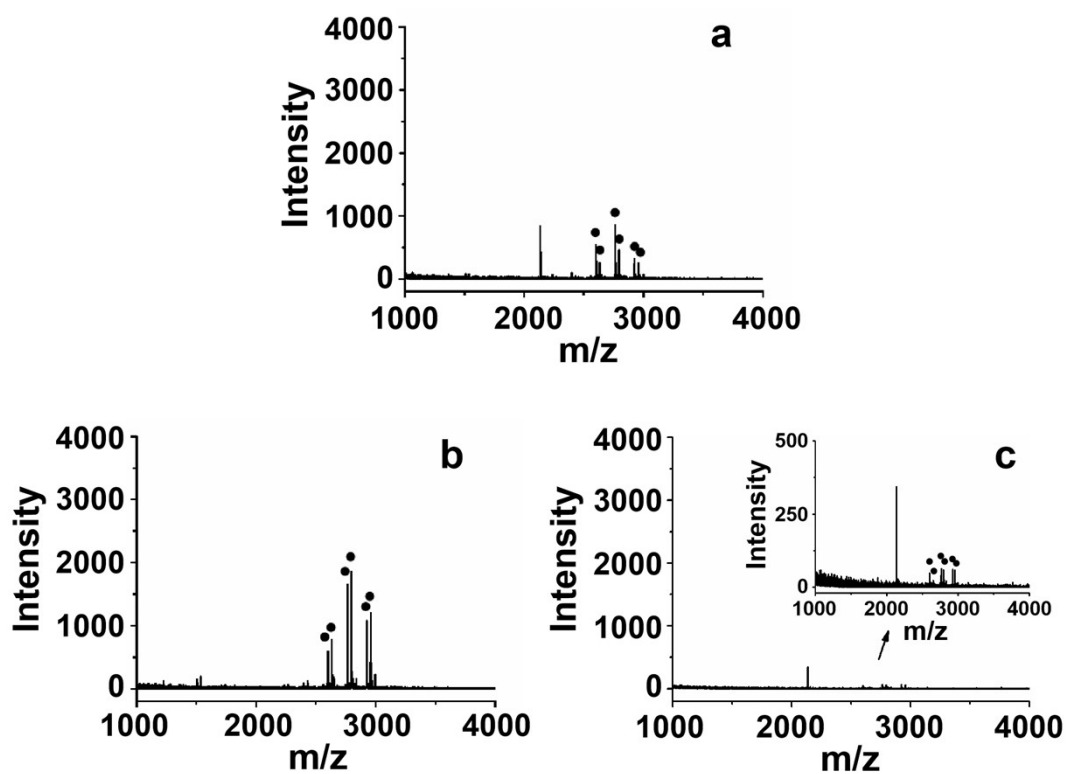


Figure S4. MALDI-TOF MS spectra of tryptic digest of IgG after enrichment by CM-III using the loading solution of ACN/H₂O/TFA of (a) 85/14/1, (b) 87/12/1 and (c) 91/8/1 (v/v/v). The dot (•) indicates glycopeptides.

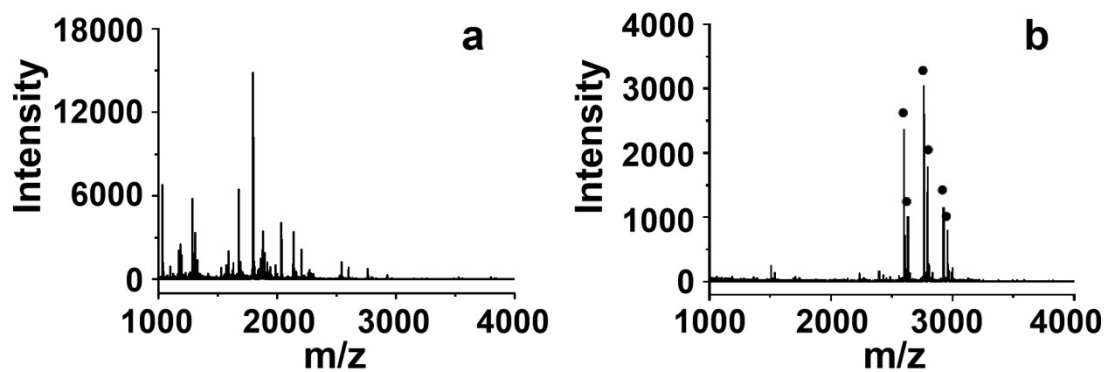


Figure S5. MALDI-TOF MS spectra of tryptic digest of IgG. (a) Before enrichment and (b) after enrichment with CM-III by HILIC using ACN/H₂O/TFA (89/10/1, v/v/v) as loading solution. The dot (•) indicates glycopeptides.

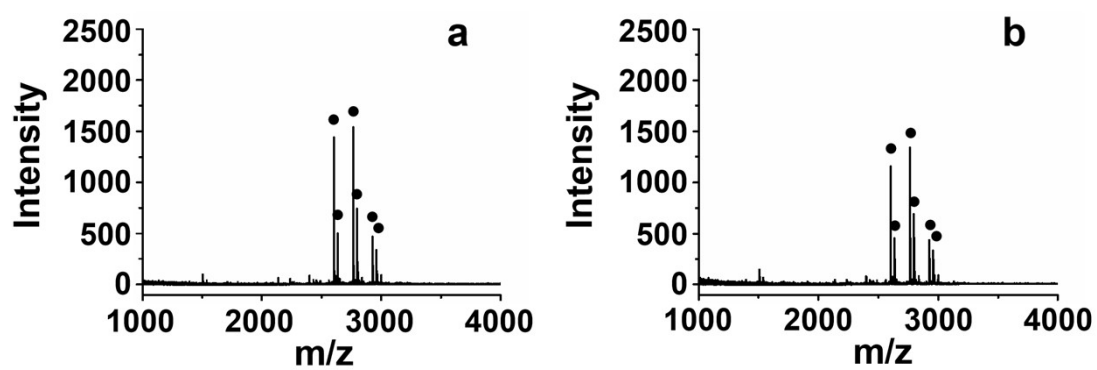


Figure S6. MALDI-TOF MS spectra of tryptic digest of 10 μg of IgG after enrichment by (a) CM-I and (b) CM-II using the loading solution of ACN/H₂O/TFA of 89/10/1 (v/v/v). The dot (•) indicates glycopeptides.

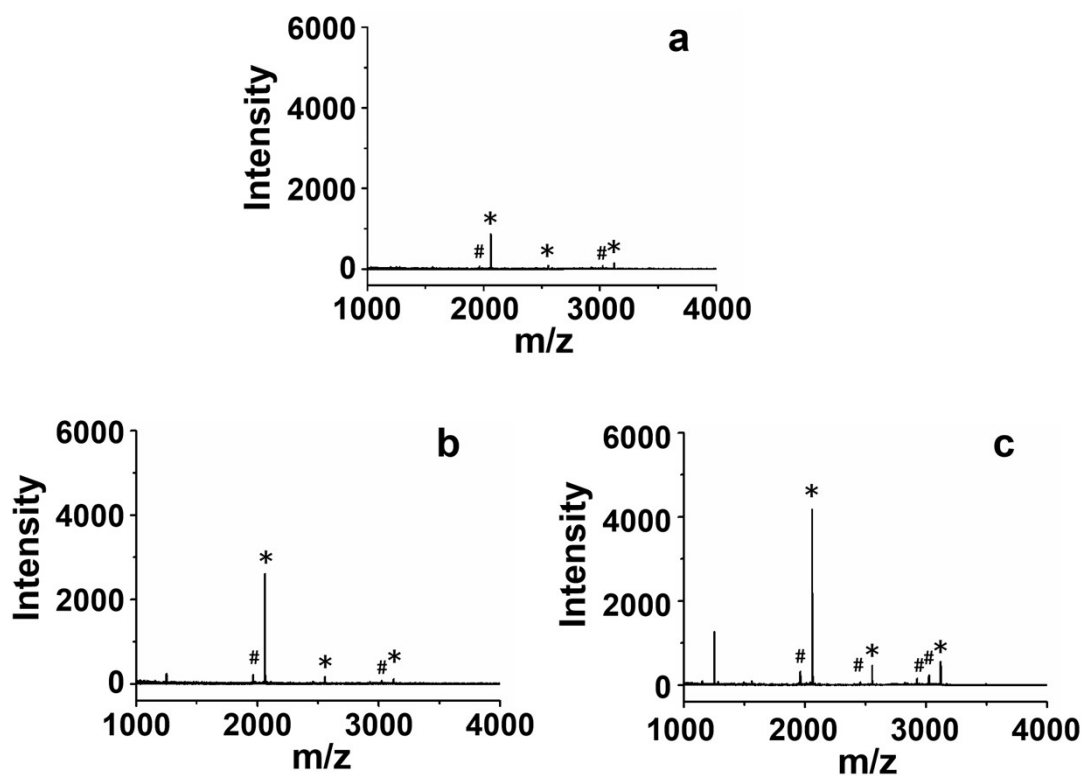


Figure S7. MALDI TOF-MS spectra of tryptic digest of 10 μg of β -casein after enrichment by (a) Ti^{4+} -CM-I, (b) -II and (c) -III. The asterisk (*) indicates phosphopeptides and (#) indicates dephosphorylated fragments.

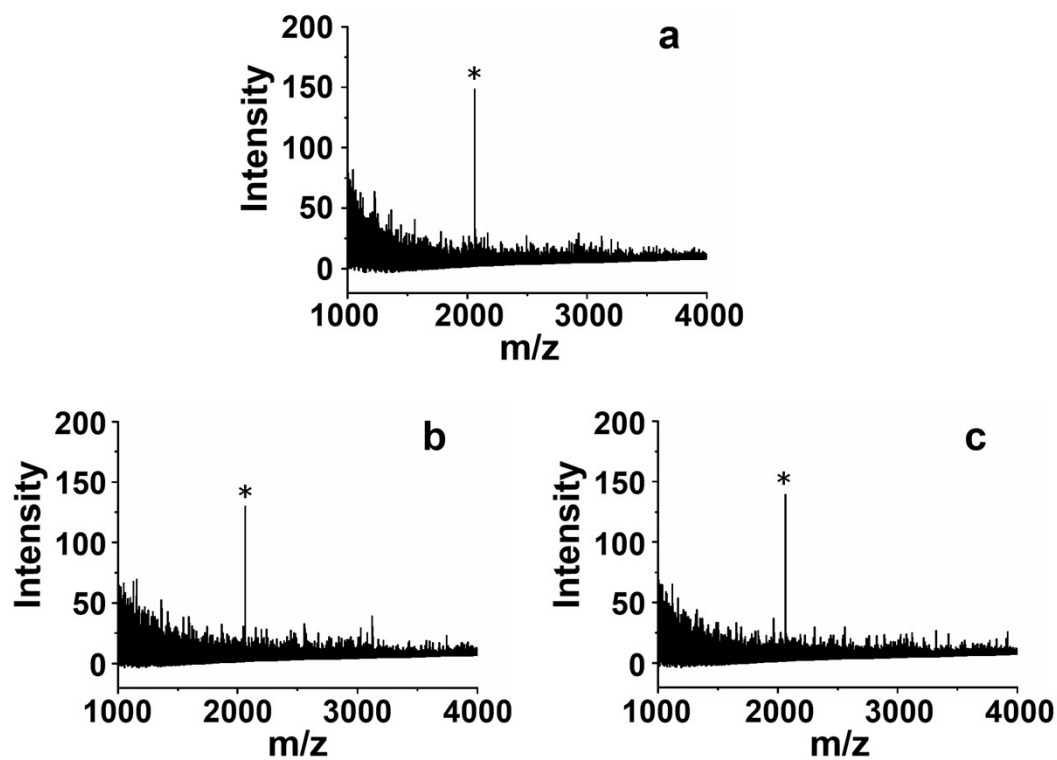


Figure S8. MALDI TOF-MS spectra of tryptic digest of 100 fmol of β -casein after enrichment by (a) Ti^{4+} -CM-I, (b) Ti^{4+} -CM-II and (c) Ti^{4+} -CM-III. The asterisk (*) indicates phosphopeptides.

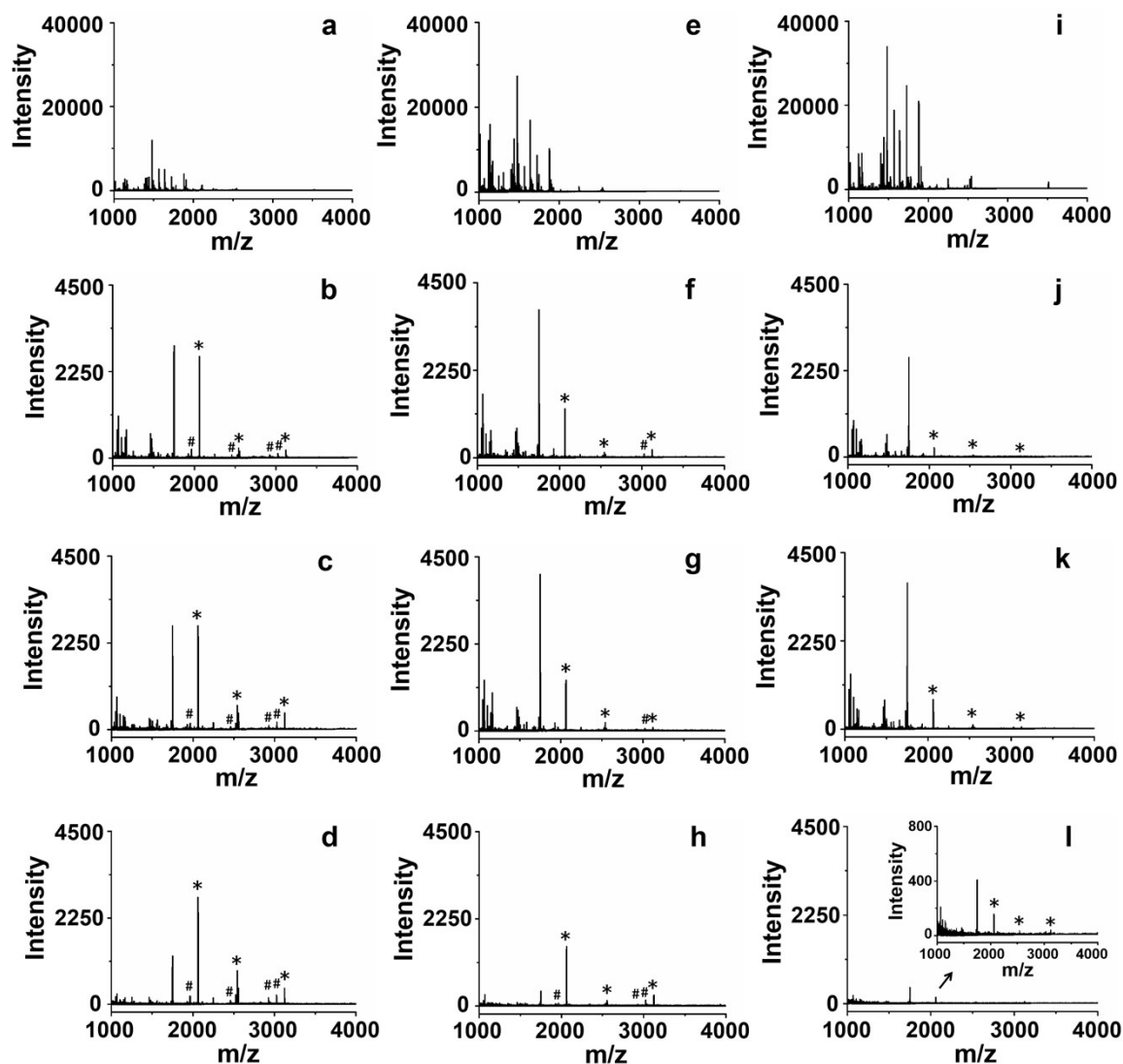


Figure S9. MALDI-TOF MS spectra of mixed tryptic digest of β -casein and BSA after enrichment by (b, f, j) Ti^{4+} -CM-I, (c, g, k) Ti^{4+} -CM-II and (d, h, l) Ti^{4+} -CM-III. The mass ratio of BSA to β -casein in the digest were (a-d) 10:1, (e-h) 50:1, (i-l) 100:1. The asterisk (*) indicates phosphopeptides and (#) indicates dephosphorylated fragments.

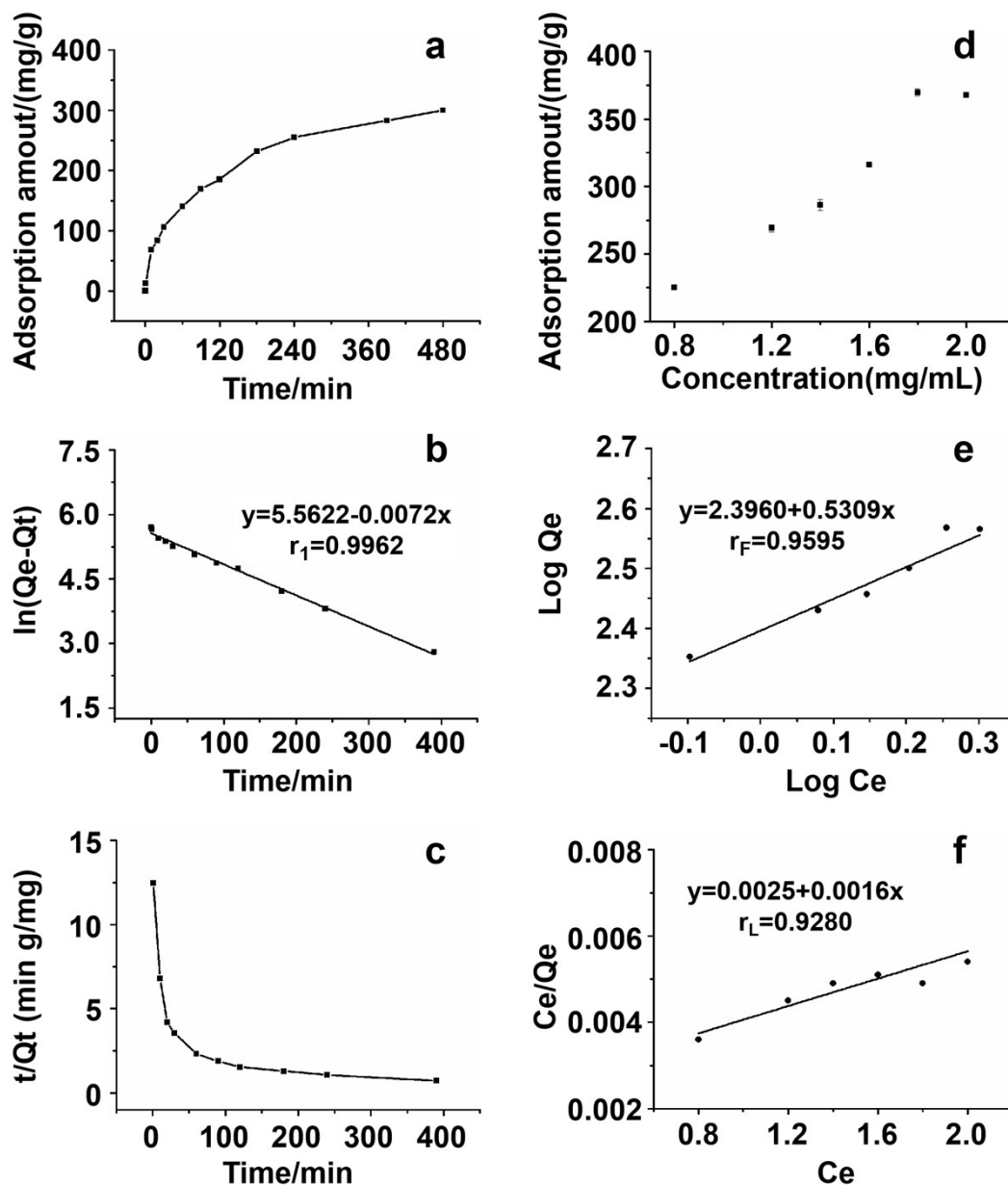


Figure S10. The dynamic and static adsorption experiments for pyridoxal 5'-phosphate by Ti⁴⁺-CM-III at room temperature. (a) Adsorption curve and (b) pseudo-first-order and (c) pseudo-second-order kinetic plots. (d) Adsorption isotherms and the linear fitting by (e) Freundlich model and (f) Langmuir model.

Table S1. Kinetic parameters of pyridoxal 5'-phosphate adsorption on Ti⁴⁺-CM-III by pseudo-first-order and pseudo-second-order models.

C ₀ (mg/mL)	Q _e (mg/g)	pseudo-first-order			pseudo-second-order		
		k ₁ (1/min)	Q _{1cal} (mg/g)	r ₁	k ₂ (g/mg·min)	Q _{2cal} (mg/g)	r ₂
1.08	299	0.0072	260	0.9962	-	-	0.5660

Table S2. Adsorption isotherm parameters by Freundlich and Langmuir models of pyridoxal 5'-phosphate on Ti⁴⁺-CM-III.

Freundlich isotherm model			Langmuir isotherm model		
K _F (mg/g)	1/n	r _F	Q _m (mg/g)	K _L (L/g)	r _L
249	0.5309	0.9595	625	0.64	0.9280

Table S3. List of phosphopeptides enriched by Ti⁴⁺-CM-III from human serum.

No.	Observed m/z	Peptides sequence	Number of phosphorylation
H1	1389.38	D[pS]GEGDFLAEGGGV	1
H2	1460.41	AD[pS]GEGDFLAEGGGV	1
H3	1545.47	D[pS]GEGDFLAEGGGVR	1
H4	1616.49	AD[pS]GEGDFLAEGGGVR	1

[pS] phosphorylated site.