Supporting Information:

GdF_3 as a promising phosphopeptide affinity probe and dephospho-labelling media: experiments and theoretically explain

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Experimental Details

Materials

β-casein, trypsin, 2,5-dihydroxybenzoic acid (2,5-DHB), 1-butyl-2-methylimidazolium tetrafluoroborate ($BMIMBF_4$) and gadolinium(III) chloride hexahydrate were purchased from Sigma-Aldrich. Ammonium hydroxide and ammonium bicarbonate were purchased from Fluka. Trifluoroacetic acid (TFA) was from J&K Technologies Inc. Acetonitrile (ACN) was obtained from Dikma Technologies Inc. Water used in MS was from Hangzhou Wahaha Group Co., Ltd. Other reagents were obtained from Beijing Chemical Works. Nonfat milk was obtained from a local supermarket. All the chemicals were of analytical grade except TFA and ACN, which are of HPLC grade.

Preparation of GdF_3 and $GdPO_4$

Synthesis of GdF_3

In a typical synthesis, 0.24 mmol gadolinium(III) chloride hexahydrate was dissolved in a mixed solvent containing 6 mL of water and 4 mL of $BMIMBF_4$, and the obtained solution was transferred to a 20 mL Teflon-lined stainless-steel autoclave, which was then heated at 120 °C for 15 h. After the autoclave cooled to room temperature, the product was collected by centrifugation, washed with deionized water and ethanol for several times, and dried at 60 °C.

Synthesis of $GdPO_4$

0.200 mmol $Gd(NO_3)_3 \cdot 6H_2O$ and 0.240 mmol $C_6H_5O_7 \cdot 2H_2O$ were dissolved in 10 mL water (solution A), and 0.200 mmol $(NaPO_3)_x$ was also dissolved in 10 mL water (solution B). Then

solution B was dropped into solution A, and the obtained mixture was heated at 180 °C for 24h in a 40 mL Teflon-lined stainless-steel autoclave. Finally the product was collected, washed and dried as mentioned above.

Enrichment of phosphopeptides.

In a typical experiment, the peptides were diluted to a certain concentration by binding buffer (50% ACN, 0.2% TFA, pH ~3). All the nanomaterials were washed in this binding buffer twice before use. Then the suspensions of those nanomaterials (typically 2 μg/μL) were added into 200 μL diluted peptides for selective enrichment. The mixed solutions were vibrated at room temperature for at least 30 min. After centrifugation (typically 10000 rpm, Eppendorf centrifuge 5417R), the supernatants were removed. After being washed with binding buffer for three times, the remained nanomaterials were redispersed in 5 μL 1.6 M ammonia aqueous solution under sonication for peptide release and dephosphorylation. After centrifugation the supernatants were mixed with matrix (DHB 20 mg/mL, 50% ACN, 1% H_3PO_4) for MALDI-MS analysis.

Instrumentation.

The products were characterized by scanning electron microscopy (SEM, Hitachi S4800, 5kV), X-ray diffraction (XRD, Rigaku Dmax-2000, Cu Kα radiation), transmission electron microscopy (TEM, FEI Tecnai T20, 200 kV), and high-resolution TEM (HRTEM, FEI Tecnai F30, 300 kV).

All MALDI-ToF MS spectra of the peptides were obtained by using a Bruker Daltonics ultraflex ToF mass spectrometer. The following voltage parameters were employed for MS analysis in our work: ion source 1, 25.00 kV; ion source 2, 22.35 kV; lens, 8.50 kV; reflector, 26.45 kV; reflector 2, 13.40 kV. The laser frequency was set on 200 Hz.

A mixture of 20 mg/mL 2, 5-DHB in 50% (v/v) ACN, 1% (v/v) phosphoric acid was introduced as the matrix. 0.5 μL of the washing buffer and 0.5 μL matrix solution were mixed on the p

The eluted peptides were also analyzed by LC-MS on a LTQ-Orbitrap Elite mass spectrometer (Thermal Fisher Scientific), using the following LC gradient:

Solvent A (97% H2O, 3% ACN, and 0.1% FA)
Solvent B (100% ACN and 0.1% FA)

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300	7
03:00	03:00	300	7
18:00	15:00	300	40
20:00	02:00	400	90
30:00	10:00	400	90

2 μL and 12 μL were set as the volume for sample pickup and sample loading, repectively.

The analytes was scanned by MS in positive mode with the range of 350.00-1800.00. “Data Dependent Neutral Loss MS3” was selected as experiment type for monitoring the neutral loss of H3PO4 or HPO3 moiety from phosphopeptides, and fragment with the following neutral loss under MS/MS was further collected for MS3 analysis: 26.6554, 32.6589, 39.9831, 48.9884, 79.9663, 97.9769. A minimal signal intensity of 5000 for parent ions was required for MS/MS and MS3 analysis, and 30 % was selected as normalized collision energy.

Data was acquired for 25 min after 2 min delay.

For calculation, the geometries of all molecules are optimized with unrestricted density functional theory using hybrid functional of B3LYP. The Stuttgart effective core potential was used for Gadolinium and 6-31G(d) basis set was used for rest of light element atoms. Optimized geometries were confirmed by frequency calculation with no imaginary frequency. All calculations were performed with Gaussian09 package.

Figures and Tables

1 Characterization of the synthesized materials

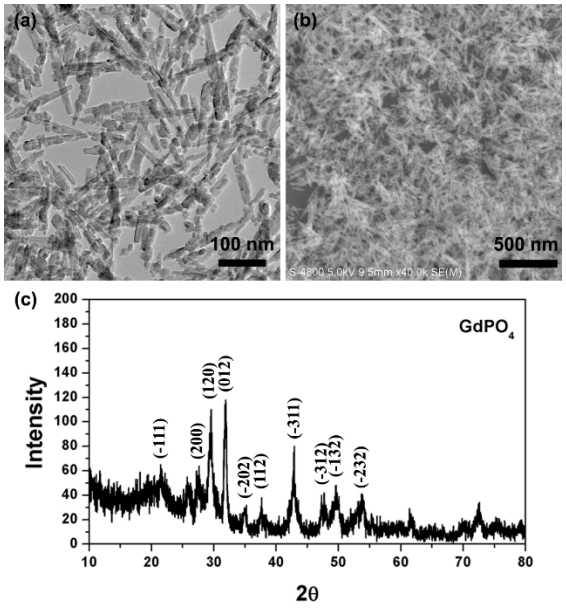

Figure S1 TEM image (a), SEM image (b) and XRD pattern (c) of $GdPO_4$

2 Molecular geometries

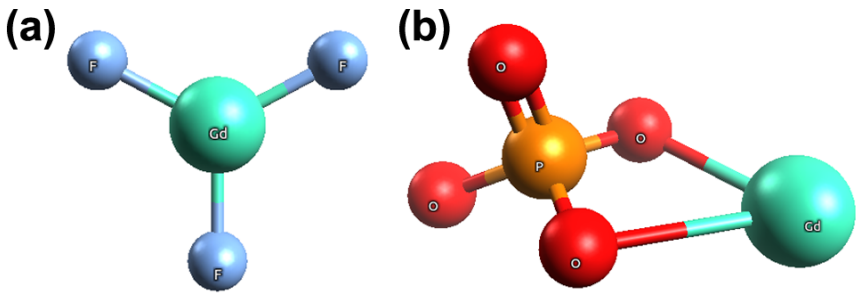

Figure S2 Optimized molecular geometries of GdF_3 (a) and $GdPO_4$ (b)

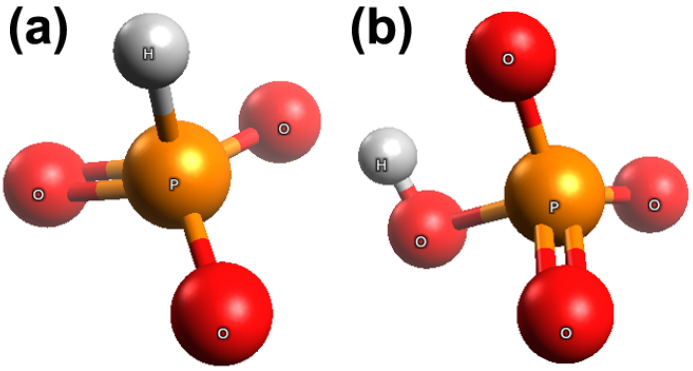

Figure S3 Optimized molecular geometries of HPO_3 (a) and HPO_4 (b)

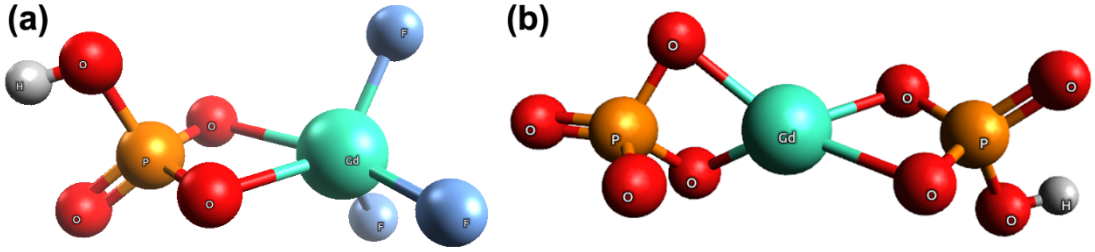

Figure S4 Optimized molecular geometries of GdF_3 phosphate complex (a) and $GdPO_4$ phosphate complex (b)

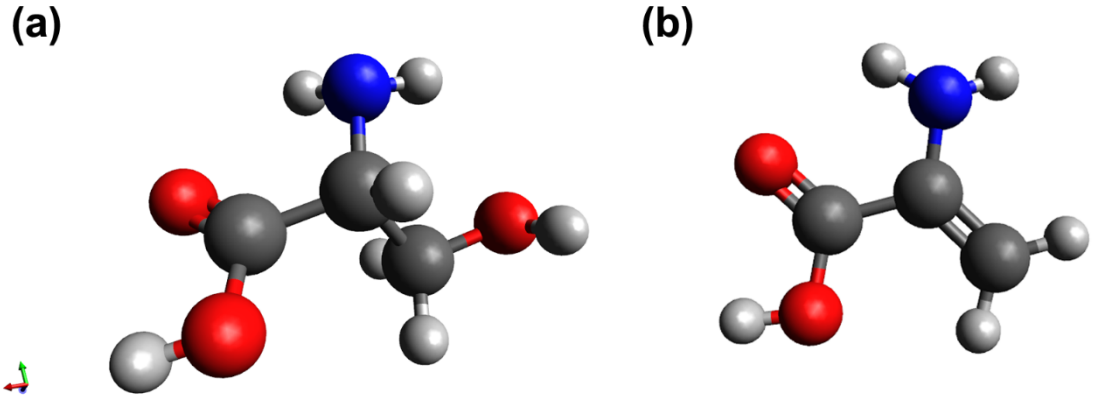

Figure S5 Optimized molecular geometries of dephosphorylation products, HOCH2CHNH2COOH (a) and CH_2=CNH_2COOH (b)

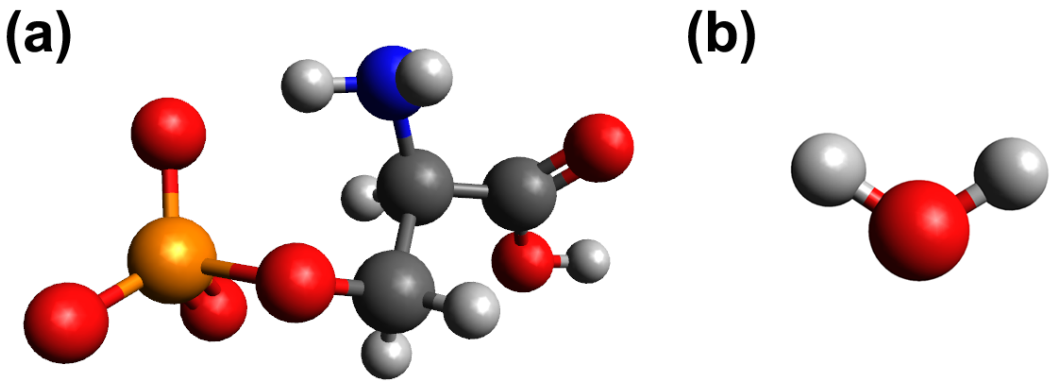

Figure S5 Optimized molecular geometries of Simplified phosphopeptide-S^p (a) and H_2O (b)

3 Phosphopeptide sequences

Table S1 Sequences of the detected phosphopeptides from β-casein digests

AA	sequence	m/z	Phosphorylation sites
33-48	FQ[pS]EEQQQTEDELQDK	**2061.76**	**1**
33-52	FQ[pS]EEQQQTEDELQDKIHPF	**2555.96**	**1**
1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	**3122.17**	**4**