REPO₄ (RE = La, Nd, Eu) affinity nanorods modified on a MALDI plate for rapid capture of target peptides from complex biosamples†

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A novel affinity MALDI target plate modified with rare-earth phosphate nanorods for rapid, direct and selective capture of phosphopeptides from complex biosamples.

Many biochemical and biomedical processes can be identified through accurate and reliable detection of biomolecules or biomarkers captured on the functionalized surfaces of devices.¹ Thus, nanostructured materials modified on surfaces of the devices for extraction of the low-abundant analytes from complex biosamples without laborious pretreatment have drawn considerable attention towards their research and applications.² For example, mesoporous silica with different surface functional groups modified on chips was designed to specifically fractionate and enrich the low molecular weight proteome from complex biological fluids.³ The graphene with high specific surface area, large loading capacity, unique π–π interaction modified on a plate was used as an ultrahigh efficiency preconcentration and detection platform for DNA oligomers.⁴ Furthermore, various metal affinity complexes or nanostructures functionalized on plates were developed for selective enrichment of specific peptides and proteins in proteomics research.⁵

Protein phosphorylation is one of the most important post-translated modifications because the majority of biological processes are regulated by reversible protein phosphorylation. Moreover, many phosphopeptides arising from the abnormal phosphorylation in tissues or biofluids are potential biomarkers with higher clinical sensitivity and specificity.⁶ Therefore, identification of the target phosphopeptides is a critical step for discovery and treatment of the diseases. Mass spectrometry (MS) can provide direct and intrinsic information of the target analytes and has become a powerful tool for peptide determination.⁷ Especially, matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF) MS is characterized by facile sample preparation and rapid detection. However, MS detection of low-abundant target analytes (e.g. phosphopeptides) from complex biosamples is always hindered by the suppression effect arising from the non-target impurities.⁸ Therefore, it is of great importance to develop an effective purification technique for improving target MS signals. Recently, some nanostructures were explored to purify and enrich selectively the target biomolecules, and they were then used for MS detection.⁹ Although some important advances in this field have been gained, the indispensable manipulation steps (e.g. repetitious washing and transfer of sample) inevitably result in sample loss and hamper the rapid MS detection.

Rare-earth materials with extensive application have been an active research field because of their catalytic activity, coordination, and luminescence.¹⁰ Recently, we have demonstrated that rare earth-based materials are very effective affinity materials in selective capture of phosphopeptides.¹¹ Herein, we report a novel affinity MALDI target plate modified with rare-earth phosphate (REPO₄, RE = La, Nd, Eu) nanorods (or affinity plate for short) for direct and selective capture of phosphopeptides from complex biosamples. As illustrated in Scheme 1a, the REPO₄ (RE = La, Nd, Eu) nanorods with good thermal and chemical stability were synthesized by a facile precipitation technique, and then they were immobilized on the MALDI target plate via a sintering process to form the array affinity spots. The target biomolecules can be specifically captured by affinity sites of the REPO₄ nanorods immobilized on the MALDI target plate, and the nonspecific biomolecules are removed. After purification by the selective capture and buffer washing, the MS signal of the target biomolecules can be effectively detected and identified, with a great improvement of the purification procedure and free of the interference of the nonspecific biomolecules.

Morphologies and structures of the as-prepared REPO₄ nanorods were characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM image of LaPO₄ nanorods (Fig. 1a) shows that the uniform LaPO₄ nanorods have a diameter of ca. 5 nm and a length of ca. 300 nm. The TEM image (Fig. 1b) reveals that some nanorods aggregated together to some extent, which can be attributed to the high surface energy and the large contact area of neighboring nanorods. Fig. 1c displays a representative high resolution TEM (HRTEM) image of a LaPO₄ nanorod,
indicating that the nanorod was composed of many primary nanocrystals. The lattice fringes of these nanocrystallites have a spacing of 0.305 nm, which is in accordance with the spacing of the (200) plane of the hexagonal LaPO₄. Fig. 1d–i gives the SEM and TEM images of NdPO₄ and EuPO₄ nanorods. The resulting NdPO₄ and EuPO₄ nanorods have similar morphologies to that of the LaPO₄ nanorods, and they also have the hexagonal structure with the lattice spacings of the 0.282 nm for plane (102) of hexagonal NdPO₄ and 0.438 nm for plane (101) of hexagonal EuPO₄. Above results demonstrate that the REPO₄ nanorods were successfully prepared.

As shown in Fig. S1a (ESI†), the EDX analysis confirms the elemental compositions of LaPO₄ (La, P and O), NdPO₄ (Nd, P and O) and EuPO₄ (Eu, P and O). The XRD patterns of the prepared REPO₄ nanorods (Fig. S1b, ESI†) indicate that they are isostructural compounds with the hexagonal phase indexed to LaPO₄, NdPO₄ and EuPO₄, respectively, which agree well with the TEM results. The FTIR spectra (Fig. S1c, ESI†) can further demonstrate the formation of the REPO₄ nanorods owing to the presence of the characteristic absorption peaks at around 1075 cm⁻¹ (v₀−p−o) and 522 cm⁻¹ (v₀−p−o).

It can be seen from the photo of the affinity plate modified with the REPO₄ nanorods in Fig. S2a (ESI†) that the affinity REPO₄ nanorods have been accurately immobilized on the target spots for affinity capture and MALDI-TOF detection. As shown in the SEM images (Fig. S2b–d), the dense affinity layers were constructed by the assembled REPO₄ nanorods and there are many nanopores on the affinity layers. The Lewis base of phosphate moieties in phosphopeptides can bind with positively charged affinity REPO₄ nanorods (Table S1, ESI†) to form an electrostatic and/or coordinate bond. After removing unbound peptides and depositing the matrix, the captured peptides were used for MS detection (Scheme 1b).

The tryptic digest of β-casein (standard model phosphoprotein) was used to investigate selective capture and identification of phosphopeptides on the affinity plate. Fig. S3a (ESI†) shows the mass spectrum of 1 μL β-casein (1 pmol) digest before capture. The nonphosphopeptides dominate the spectrum and only one phosphopeptide can be directly detected due to the low ionization efficiency and the suppression of the nonphosphopeptides. However, three expected phosphopeptides with strong intensities and a clean background were evidently detected in the mass spectrum of the β-casein digest deposited on the LaPO₄ nanorods modified MALDI target plate (Fig. S3b and Table S2, ESI†), and similar results can also be obtained from the affinity MALDI plate modified with NdPO₄ or EuPO₄ nanorods (Fig. S3c and d, ESI†).

To further evaluate the selectivity of the affinity plate, 1 μL of the tryptic digest of β-casein (standard model phosphoprotein, 1 pmol) and BSA (nonphosphoprotein, 25 pmol) in a ratio of 1:25 were used. No phosphopeptides can be identified in the MS of direct detection because of the interference from the abundant nonphosphopeptides (Fig. 2a). For comparison, commercial TiO₂ microspheres were first used to enrich the target peptides from peptide mixtures. Although the enrichment is effective, many nonphosphopeptides were also detected besides the repeated operation of time-consuming centrifugalization and elution (Fig. 2b). In contrast, after facile online capture, three phosphopeptides with strong intensities, high S/N ratios and clean background were easily detected (Fig. 2c), and similar results can also be obtained from the affinity MALDI plate modified with NdPO₄ or EuPO₄ nanorods (Fig. S4, ESI†), demonstrating their high selectivity for phosphopeptides.

To verify the sensitivity of the online capture of phosphopeptides via the affinity plate, β-casein digest with different concentrations (10⁻⁷ M, 5 × 10⁻⁸ M, 10⁻⁸ M) were used. As shown in Fig. S5 (ESI†), the intensities and S/N ratios of target peptides decrease with the decrease of concentration. More importantly, after treatment with the affinity plate, target peptides can still be detected in amounts as low as 10 fmol (Fig. S6a–c, ESI†), indicating the high sensitivity and S/N ratios of our strategy. However, no effective signal can be observed for the commercial TiO₂ beads, which may be ascribed to the loss of samples during the repeated steps of centrifugalization and elution (Fig. S6d, ESI†). In addition, the affinity plate was easily regenerated for recycle, and it was reused up to 4 times. As shown in Fig. S7 (ESI†), the selective affinity of the fourth regenerated affinity plate is still effective.

To investigate the effectiveness of the online capture of phosphopeptides via the affinity plate in practical bioapplication, a tryptic digest (i.e. peptide mixture) of nonfat milk was first applied as a real complex sample. Only one weak phosphopeptide can be detected from the untreated peptide mixture (Fig. 3a). After enrichment using commercial TiO₂ beads, 10 phosphopeptides can be detected, while many nonphosphopeptides were also...
and the purified phosphopeptides deposited on the plate can be directly detected using MS (Scheme 1b). Fig. S9b and c (ESI†) shows the results of online capturing phosphopeptides via the affinity plate. Three phosphopeptides (Table S4, ESI†) within a mass range of 1000–3500 Da were successfully detected. In contrast, it is difficult to detect the target phosphopeptides directly (Fig. S9a, ESI†) due to the serious interferences of many salts, abundant proteins and nonphosphopeptides. Above results demonstrate that the affinity plate has great potential for the selective capture and fast analysis of phosphopeptides from complex biological samples.

In summary, the affinity MALDI plates modified with REPO₄ (RE = La, Nd, Eu) nanorods were explored to selectively capture and purify trace phosphopeptides from the complex biosamples without using a time-consuming and complicated separation process. After deposition of the matrix, the enriched phosphopeptides on the affinity plate can be directly detected using MALDI-TOF MS. The whole process can be completed in a few minutes and the required sample loading amount is extremely low (e.g. fmol). Therefore, this work would be highly beneficial for future applications in rapid and high throughput capture and identification of target biomolecules, in particular, trace phosphopeptide biomarkers from complex biosamples.

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Notes and references