

Electronic Supporting Information

Metal ion-immobilized magnetic nanoparticles for global enrichment and identification of phosphopeptides by mass spectrometry

Rui Zhai,^{a,b} Fang Tian,^b Ruiqing Xue,^b Fenglong Jiao,^b Feiran Hao,^b Yangjun Zhang,^{*}
^b and Xiaohong Qian^{*,b}

^a. College of Life Science and Bio-Engineering, Beijing University of Technology, Beijing 100022, China;

^b. State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing 102206, China

Corresponding Authors

*E-mail: Xiaohong Qian: qianxh1@163.com. Phone&Fax: 86-01-80727777-1231.

*E-mail: Yangjun Zhang: 13683167093@163.com. Phone&Fax: 86-01-80727777-1142.

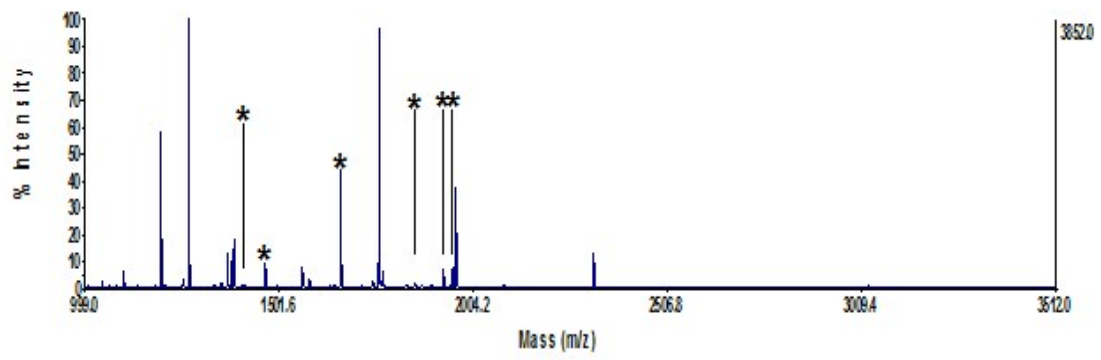


Figure S1. MALDI-TOF mass spectra of tryptic digest of 5 pmol of α -casein after direct analysis.

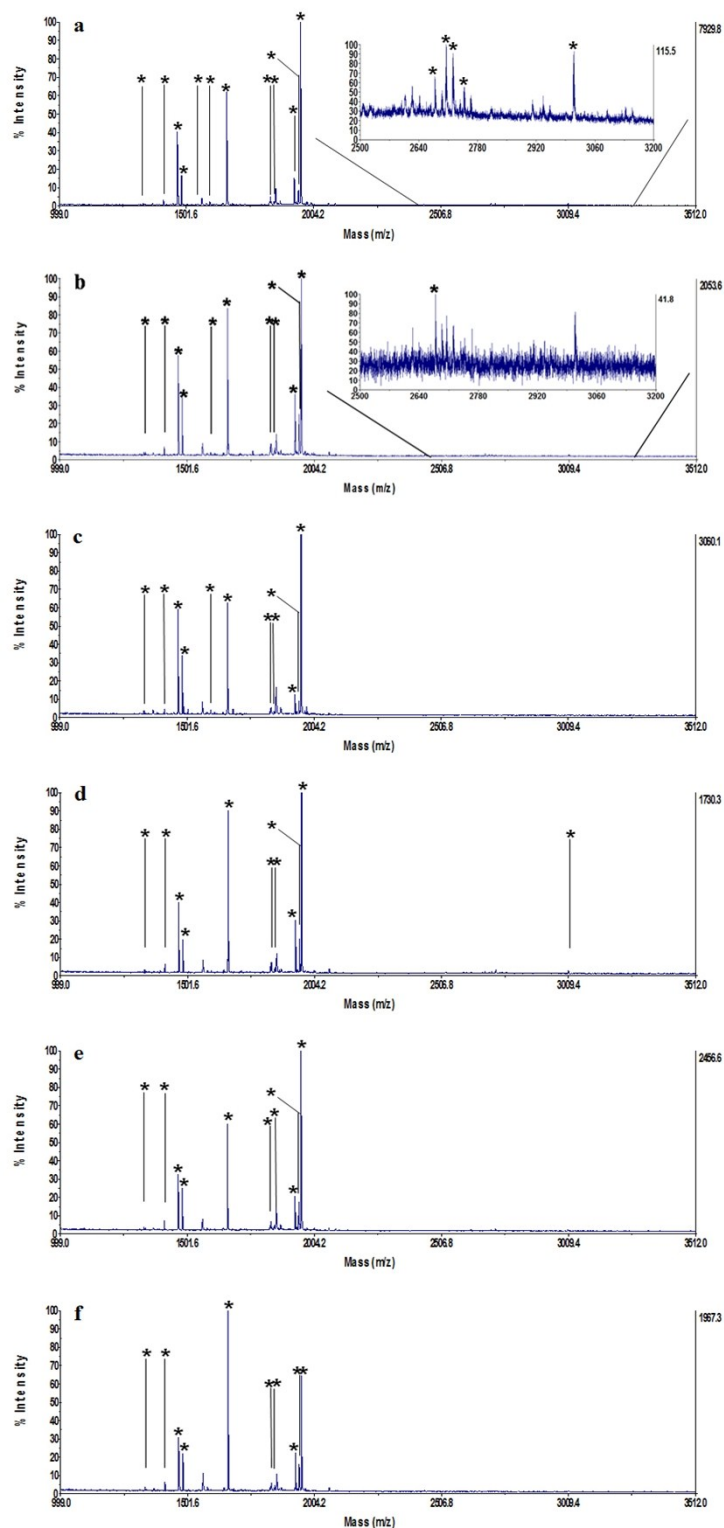


Figure S2. MALDI-TOF mass spectra of tryptic digest of 5 pmol of α -casein (a-f) after enrichment by $\text{Fe}_3\text{O}_4@\text{TCPP-DOTA-M}$ (Ti^{4+} , Zr^{4+} , Fe^{3+} , Tb^{3+} , Tm^{3+} , Ho^{3+}) for the first time.

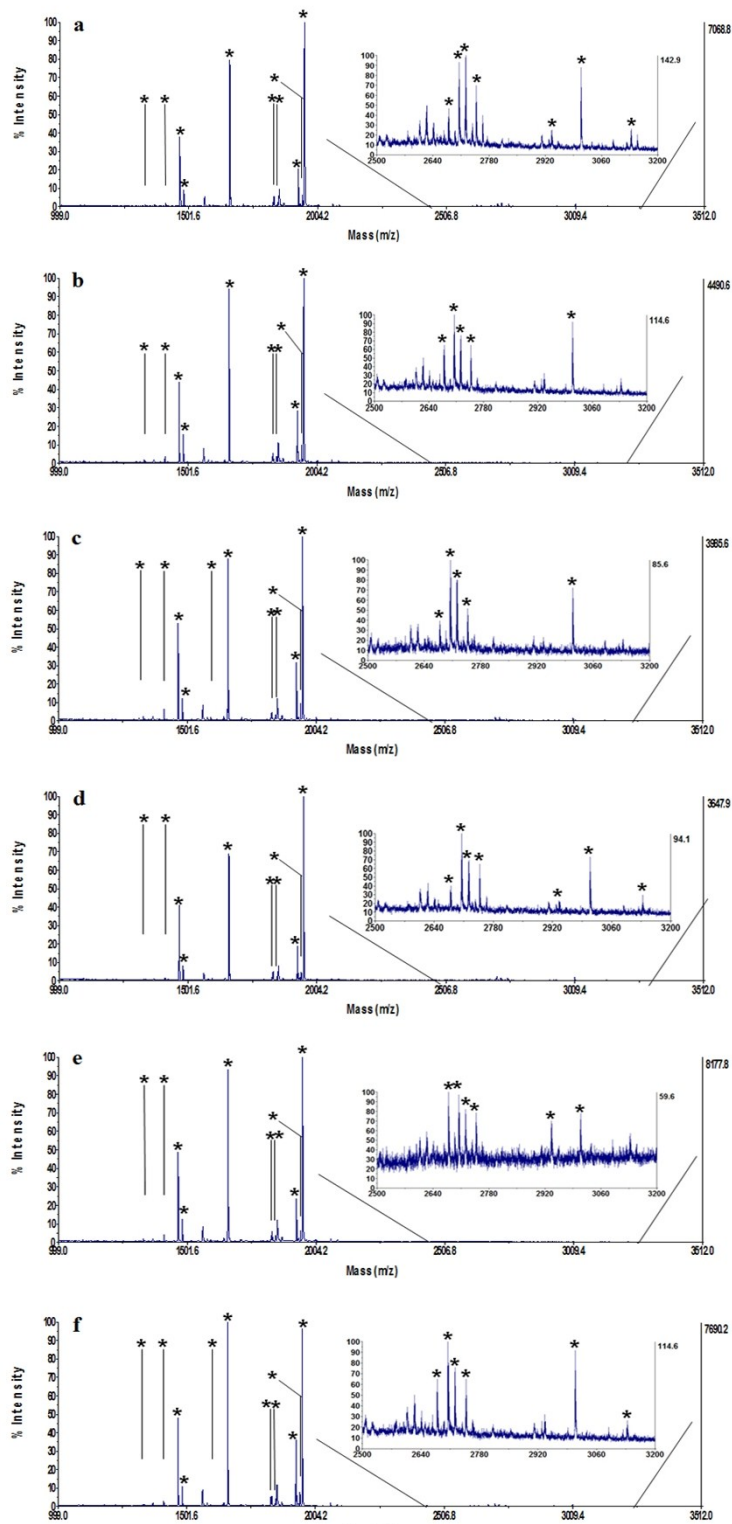


Figure S3. MALDI-TOF mass spectra of tryptic digest of 5 pmol of α -casein (a-f) after enrichment by $\text{Fe}_3\text{O}_4@\text{TCPP-DOTA-M}$ (Ti⁴⁺, Zr⁴⁺, Fe³⁺, Tb³⁺, Tm³⁺, Ho³⁺) for the fifth time.

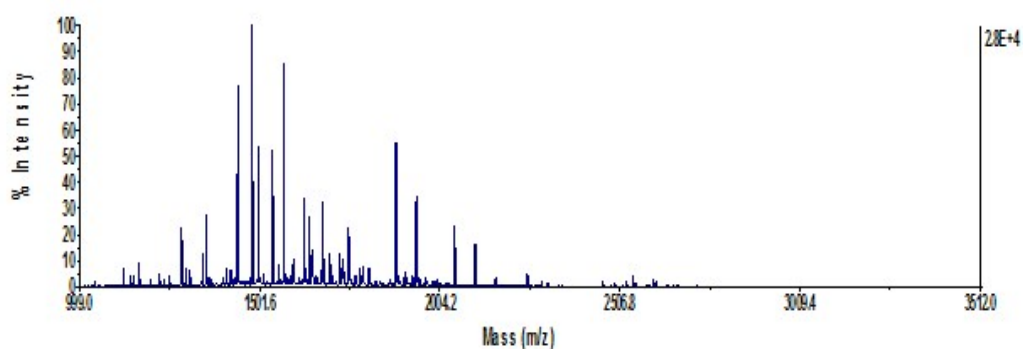


Figure S4. MALDI-TOF mass spectra of the tryptic digest mixture of α -casein and BSA at a molar ratio of 1:50 after direct analysis.

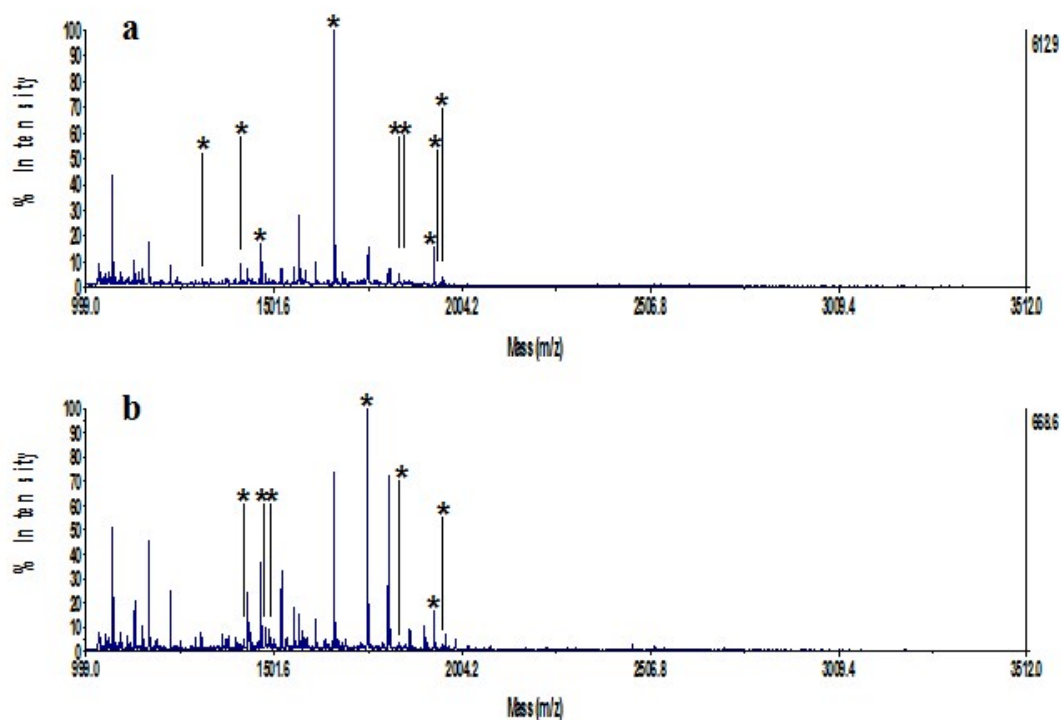


Figure S5. MALDI-TOF mass spectra of the mixture of α -casein tryptic digest and BSA tryptic digest at a molar ratio of 1:50 analyses after enrichment by (a) Ti^{4+} -immobilized commercial IMAC materials and (b) Zr^{4+} -immobilized commercial IMAC materials.

Table S1. Enriched phosphopeptides from tryptic digest of α -casein by $\text{Fe}_3\text{O}_4@\text{TCPP-DOTA-Ti}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|---|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 6 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 7 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 9 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 10 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 11 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 12 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 13 | Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2704.25 |
| 14 | NTMEHV[pS] [pS] [pS]EE[pS]IISQETYKQ S2-(17-37) | 4 | 2747.23 |
| 15 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S2. Enriched phosphopeptides from tryptic digest of α -casein by $\text{Fe}_3\text{O}_4@\text{TCPP-DOTA-Zr}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|--------------------------------------|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 6 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 7 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 9 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 10 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 11 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |

| | | | |
|----|---|---|---------|
| 12 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 13 | Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2704.25 |
| 14 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S3. Enriched phosphopeptides from tryptic digest of α -casein by $\text{Fe}_3\text{O}_4@T\text{CPP-DOTA-Fe}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|---|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 2 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 3 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 4 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 5 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 6 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 7 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 9 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 10 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 11 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S4. Enriched phosphopeptides from tryptic digest of α -casein by $\text{Fe}_3\text{O}_4@T\text{CPP-DOTA-Tb}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|-----------------------------------|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 6 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 7 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 9 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |

| | | | |
|----|---|---|---------|
| 10 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 11 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 12 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 13 | Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2704.25 |
| 14 | NTMEHV[pS] [pS] [pS]EE[pS]IISQETYKQ S2-(17-37) | 4 | 2747.23 |
| 15 | NANEEEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S5. Enriched phosphopeptides from tryptic digest of α -casein by $\text{Fe}_3\text{O}_4@T\text{CPP-DOTA-Tm}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|---|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 2 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 3 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 4 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 5 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 6 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 7 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 8 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 9 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 10 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 11 | NANEEEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S6. Enriched phosphopeptides from tryptic digest of α -casein by $\text{Fe}_3\text{O}_4@T\text{CPP-DOTA-Ho}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|---------------------------------|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |

| | | | |
|----|---|---|---------|
| 6 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 7 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 9 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 10 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 11 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 12 | Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2704.25 |
| 13 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S7. Enriched phosphopeptides from tryptic digest of α -casein and BSA at a molar ratio of 1:50 by $\text{Fe}_3\text{O}_4@T\text{CPP-DOTA-Ti}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|--|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 6 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 7 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 9 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 10 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 11 | NTMEHV[pS] [pS] [pS]EE[pS]IISQETYK S2-(17-36) | 4 | 2619.15 |
| 12 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 13 | QMEAE[pS]I[pS] [pS] [pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2721.16 |
| 14 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S8. Enriched phosphopeptides from tryptic digest of α -casein and BSA at a molar ratio of 1:50 by $\text{Fe}_3\text{O}_4@T\text{CPP-DOTA-Zr}$.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|--------------------------------|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |

| | | | |
|----|---|---|---------|
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 6 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 7 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 9 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 10 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 11 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 12 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S9. Enriched phosphopeptides from tryptic digest of α -casein and BSA at a molar ratio of 1:50 by Fe₃O₄@TCPP-DOTA-Fe.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|---|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | EQL[pS]T[pS]EENSKK S2-(141-152) | 2 | 1539.7 |
| 6 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 7 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 8 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 9 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 10 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 11 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 12 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S10. Enriched phosphopeptides from tryptic digest of α -casein and BSA at a molar ratio of 1:50 by Fe₃O₄@TCPP-DOTA-Tb.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|--------------------------------|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |

| | | | |
|----|--|---|---------|
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 6 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 7 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 9 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 10 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 11 | NTMEHV[pS] [pS] [pS]EE[pS]IISQETYK S2-(17-36) | 4 | 2619.15 |
| 12 | VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK S1-(52-73) | 3 | 2678.24 |
| 13 | Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2704.25 |
| 14 | QMEAE[pS]I[pS] [pS] [pS]EEIVPN[pS]VEAQK S1-(74-94) | 5 | 2721.16 |
| 15 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S11. Enriched phosphopeptides from tryptic digest of α -casein and BSA at a molar ratio of 1:50 by Fe₃O₄@TCPP-DOTA-Tm.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|---|----------------------------|---------|
| 1 | EQL[pS]T[pS]EENSK S2-(141-151) | 1 | 1331.62 |
| 2 | EQL[pS]T[pS]EENSK S2-(141-151) | 2 | 1411.59 |
| 3 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 4 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 5 | TVDME[pS]TEVFTKK S2-(153-165) | 1 | 1594.82 |
| 6 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 7 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 8 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 1 | 1847.86 |
| 9 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 10 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 11 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |
| 12 | NANEEYSIG[pS] [pS] [pS]EE[pS]AEVATEEVK S2-(61-85) | 4 | 3008.28 |

Table S12. Enriched phosphopeptides from tryptic digest of α -casein and BSA at a molar ratio of 1:50 by Fe₃O₄@TCPP-DOTA-Ho.

| NO. | peptide sequence | Number of Phosphate groups | (M+H) |
|-----|--------------------------------------|----------------------------|---------|
| 1 | TVDME[pS]TEVFTK S2-(153-164) | 1 | 1466.72 |
| 2 | TVD[Mo]E[pS]TEVFTK S2-(153-164) | 1 | 1482.7 |
| 3 | VPQLEIVPN[pS]AEER S1-(121-134) | 1 | 1660.92 |
| 4 | YLGEYLIVPN[pS]AEER S1-(104-119) | 1 | 1832.83 |
| 5 | DIG[pS]E[pS]TEDQAMEDIK S1-(58-73) | 2 | 1927.84 |
| 6 | DIG[pS]E[pS]TEDQA[Mo]EDIK S1-(58-73) | 2 | 1943.81 |
| 7 | YKVPQLEIVPN[pS]AEER S1-(119-134) | 1 | 1952.12 |