

Supporting Information

1 **Analysis of phosphorylation site occupancy of peptides based on a**
2 **chemical derivatization method**

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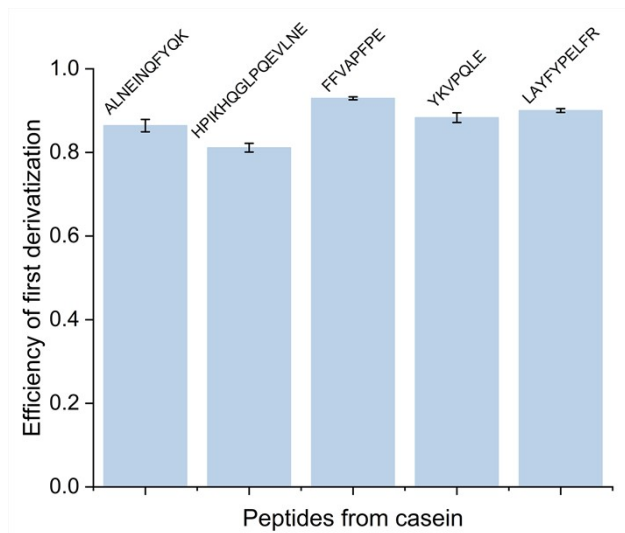
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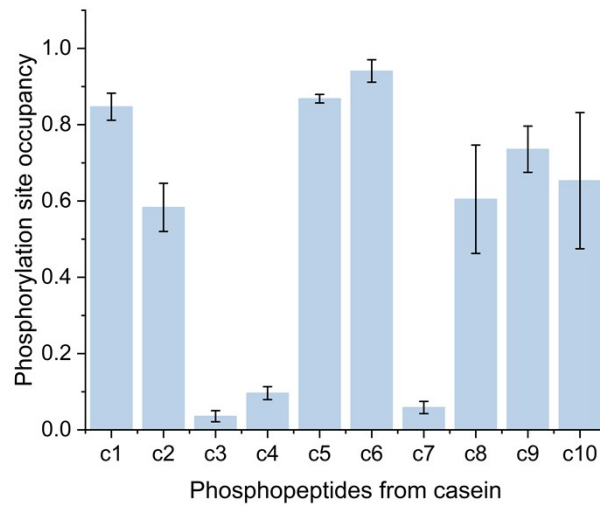
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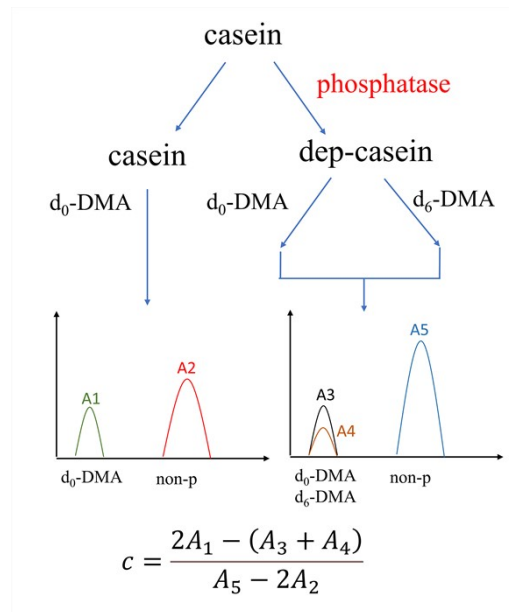
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2 **Fig.S1** The initial derivatization efficiency for the five peptides derived from casein ranges from
 3 81.14% to 92.95% (n=3).



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5 **Fig.S2** The phosphorylation site occupancies of 10 peptides derived from bovine casein exhibit a
 6 range of 3.58% to 94.09% (n = 3). For further information regarding peptides, please refer to Table
 7 S2.



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2 **Fig.S3** The evaluation strategy for the investigation of the difference in ionization efficiency
 3 between DMA-labeled phosphopeptides and their non-phosphorylated forms. The c values for three
 4 typical peptides derived from casein were calculated according to the aforementioned formulae,
 5 yielding the following results: IVPNSAEER, 7.7; DIGSESTEDAQMEDIK, 0.28; and
 6 APSFSDIPNPIGSE, -4.2 ($A_5 < 2A_2$).

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Table. S1 The phosphorylation site occupancies of peptides from casein.

Peptide No.	Sequence	Occupancy-1	Occupancy-2	Occupancy-3	Average±SD
c1	KENLCpSTFCK	80.63%	86.96%	86.53%	84.71±3.54%
c2	TVDMEpSTEVFTK	57.26%	52.63%	65.12%	58.34±6.31%
c3	SpSSEESITR	4.70%	4.12%	1.93%	3.58±1.46%
c4	EKFQpSEE	9.63%	11.31%	7.95%	9.63±1.68%
c5	PFLQPEVMGVpSKVK	86.29%	88.13%	86.08%	86.83±1.12%
c6	DIGpSEpSTEDQAMEDIK	95.82%	95.74%	90.69%	94.09±2.94%
c7	HIQKEDVpSER	7.36%	6.07%	4.17%	5.87±1.61%
c8	IVPNpSAEER	62.01%	73.83%	45.54%	60.46±14.21%
c9	EERLHpSMK	82.30%	79.53%	67.40%	73.58±6.07%
c10	APpSFSDIPNPIGSE	82.61%	80.26%	70.15%	65.32±17.86%

1 **Table. S2** The phosphorylation site occupancies of peptides from goldfish tissue samples.

2 Peptides with an identical No. were identified in both the liver and kidney tissues.

Tissue	Peptide No.	Sequence	Occupancy-1	Occupancy-2	Occupancy-3	Average±SD
Liver	p1	IMITQTpTDTVNE	91.41%	92.94%	89.99%	91.44±1.47%
	p2	EFpSPSFQHRGNR	86.99%	86.63%	87.58%	87.07±0.48%
	p3	QIDQpTQNQLNK	73.27%	88.71%	85.38%	82.46±8.13%
	p4	FYNpSIMWVKE	72.36%	76.45%	78.45%	75.75±3.11%
	p5	MYpSKGASE	65.67%	69.07%	65.88%	66.88±1.91%
	p6	pTIVSIGDPK	14.26%	15.33%	16.62%	15.40±1.18%
	p7	DLpTGDLSETFAE	20.54%	7.28%	7.36%	11.73±7.63%
	p8	IQGLDGFLKMQGpTR		N.N.D.*		100.00%
	p9	ESQVMGpTTMSNVK		N.N.D.		100.00%
	p10	pTTPAVTSGGGK		N.N.D.		100.00%
	p11	LLWGSRGpSE	85.28%	88.69%	37.29%	70.42±28.74%
Kidney	p1	IMITQTpTDTVNE	83.67%	90.43%	85.97%	86.69±3.44%
	p2	EFpSPSFQHRGNR	88.69%	84.88%	88.74%	87.44±2.21%
	p3	QIDQpTQNQLNK		N.N.D.		100.00%
	p4	FYNpSIMWVKE		N.N.D.		100.00%
	p5	MYpSKGASE	73.19%	76.37%	79.82%	76.46±3.32%
	p6	pTIVSIGDPK	74.35%	79.37%	84.90%	79.54±5.28%
	p7	DLpTGDLSETFAE	95.87%	94.30%	95.92%	95.36±0.92%
	p8	IQGLDGFLKMQGpTR	70.27%	77.90%	77.00%	75.06±4.17%
	p9	ESQVMGpTTMSNVK		N.N.D.		100.00%
	p12	LSpTENVR	67.76%	73.87%	71.40%	71.01±3.07%
	p13	ALLASLpSR	49.51%	49.45%	42.63%	47.20±3.96%
	p14	AAPEpTVVK	32.49%	15.10%	66.13%	37.91±25.95%
	p15	EpTDLGNYTCpTVE	83.67%	90.43%	85.97%	86.69±3.44%
	p16	DDEIAALVVDNGpSGMC		N.N.D.		100.00%

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3 *: Non-phosphorylated peptide was not detected.