Support information

On-Plate Desalting and SALDI-MS Analysis of Peptides on Hydrophobic Silicate Nanofilms on a Gold Substrate

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Table S1. SPR angle for different substrates [a]

Surface	SPR angle	Angle shift	Standard Deviation
Blank Au	62.21±0.01	-	–
8Layer-flat	62.40±0.06	0.19	32%
4Layer-rough	63.00±0.12	0.79	15%
8Layer-rough	64.12±0.26	1.91	14%

[a] n = 5. Films on BK-7 glass.

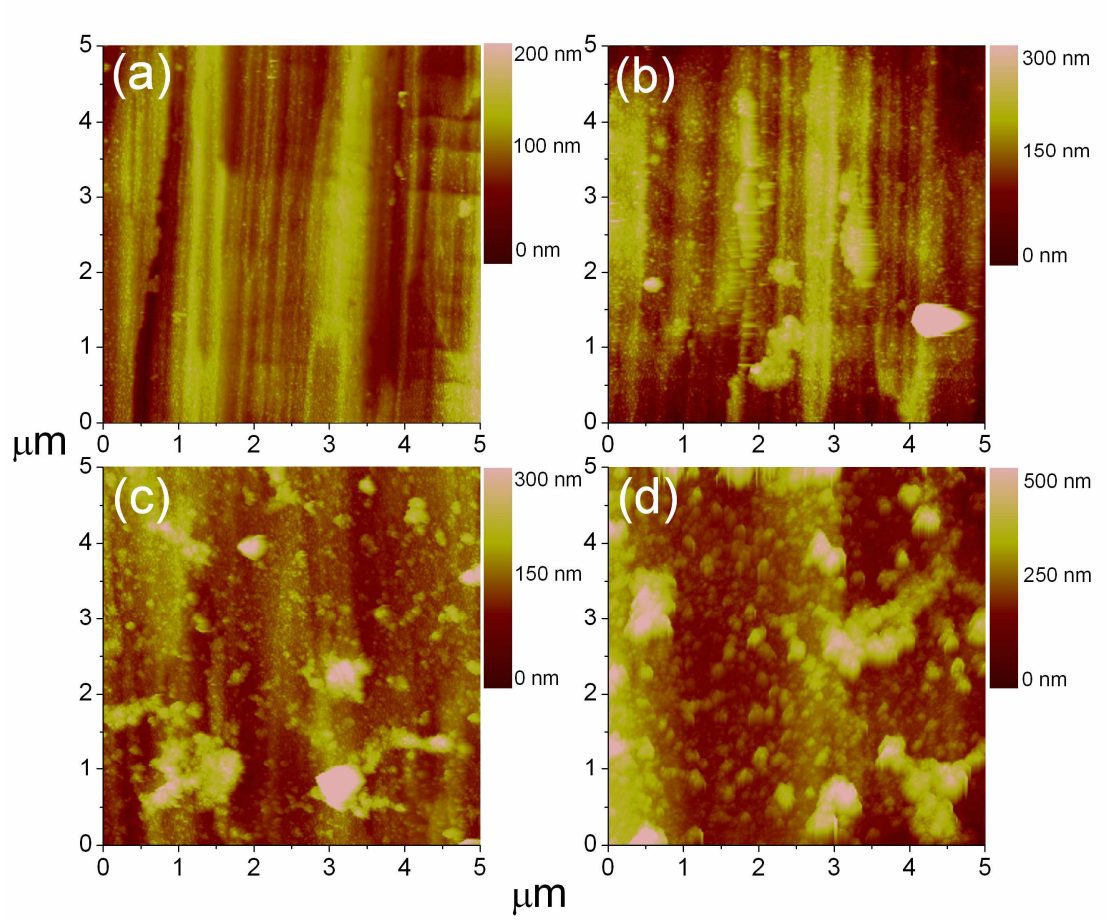

Figure S1. AFM images for calcinated substrates. (a) Au-covered steel substrate; (b) 8 layers of silicate fabricated with water washing between deposition of each polyelectrolytes; (c) 4 layers of silicate fabricated without water washing; (d) 8 layers of silicate fabricated without water washing. Bright streaks were observed across the images, corresponding to ridges and wrinkles on the SST substrate.

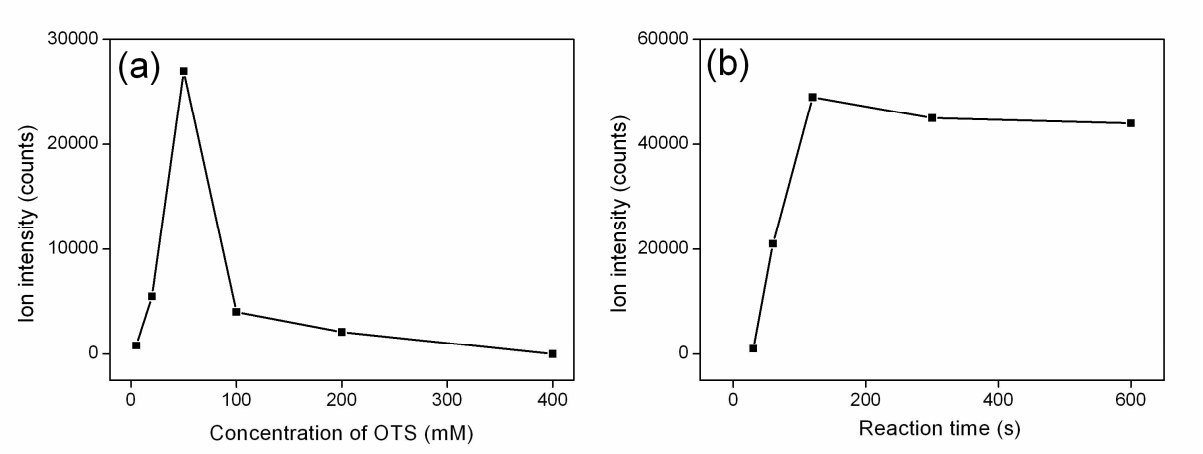

Figure S2. Effect of OTS modification on LDI performance of calcinated substrate. (a) Effect of OTS concentration, reaction time was 30 s; (b) effect of reaction time, OTS concentration was 10 mM. The analyte was 20 pmol of [Sar1, Thr8]-angiotensin Ⅱ.

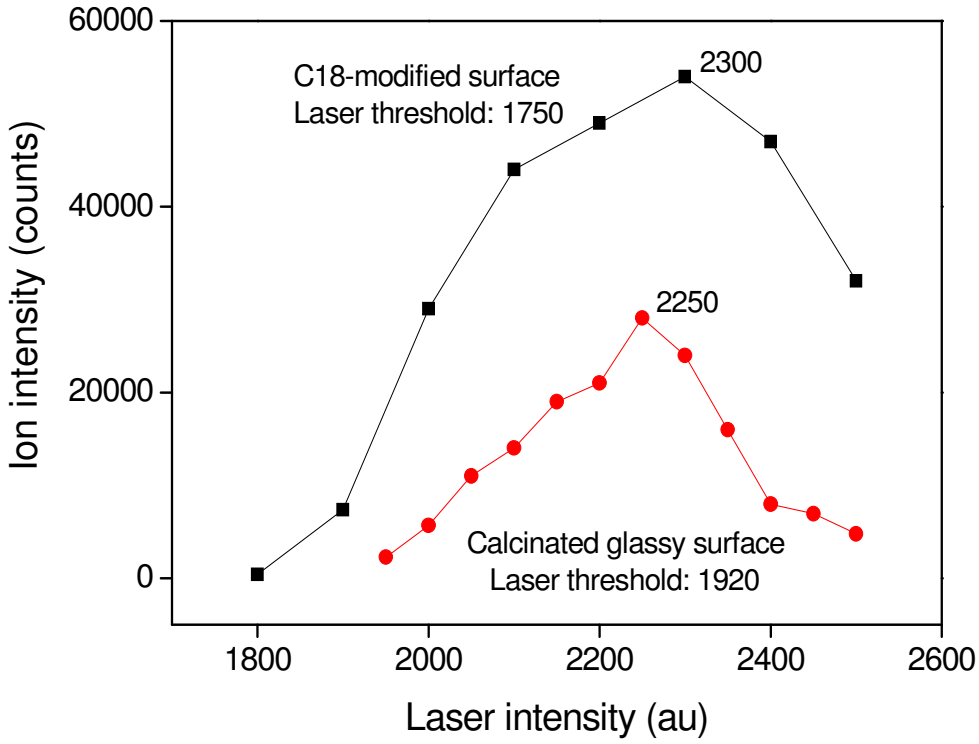

Figure S3. Dependency of signal-to-noise ratio for peptide protonated ions on laser intensity in SALDI-MS detection with C18-modified (square) and unmodified (circle) glassy substrates. Substrates were covered by 5 layers of silicate. The analyte was 20 pmol of [Sar1, Thr8]-angiotensin Ⅱ. Error bars are omitted for simplicity.

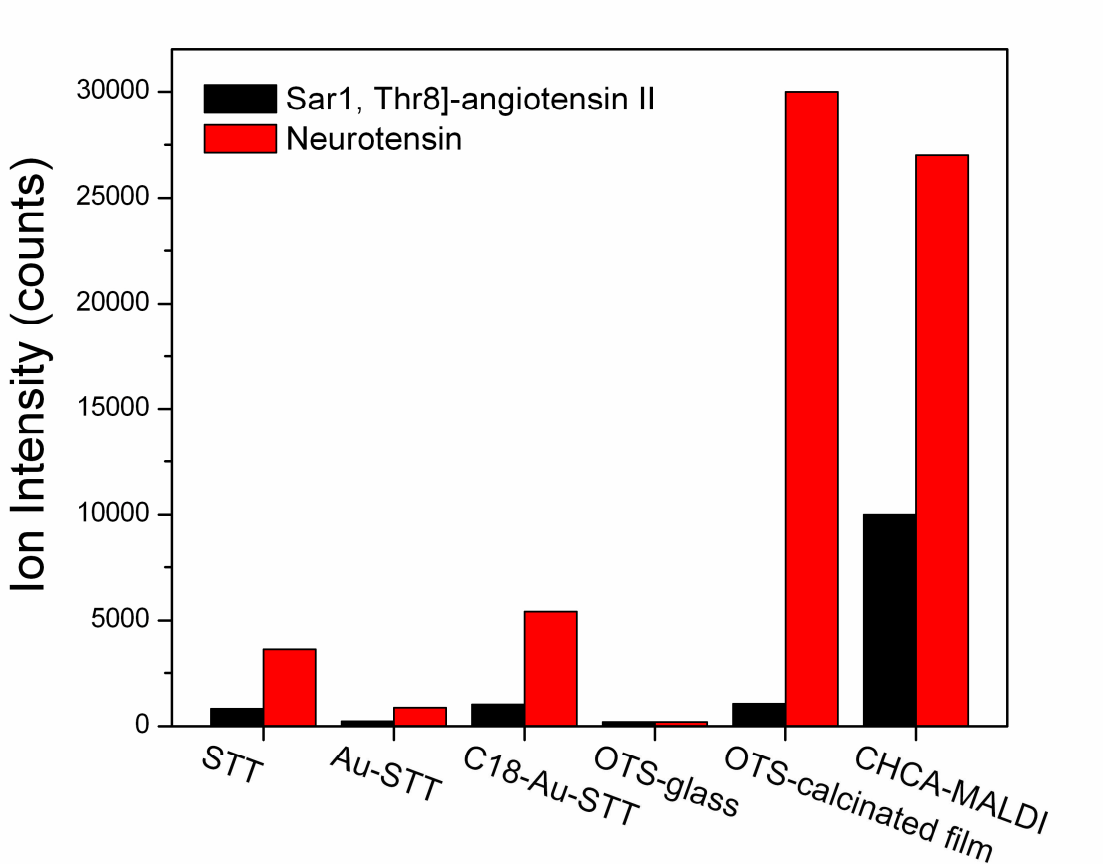

Figure S4. Comparison of SALDI performance with different substrates. STT: stainless steel tape; Au-STT: gold-coated stainless steel tape; C18-Au-STT: gold-coated steel substrate treated with 1-octadecanethiol; OTS-glass: OTS modified glass substrate; OTS-calcinated film: OTS-modified calcinated substrate (8-layer smooth surface); CHCA-MALDI: MALDI with CHCA matrix. The analyte was mixture of [Sar1, Thr8]-angiotensin Ⅱ and neurotensin, 20 pmol each.

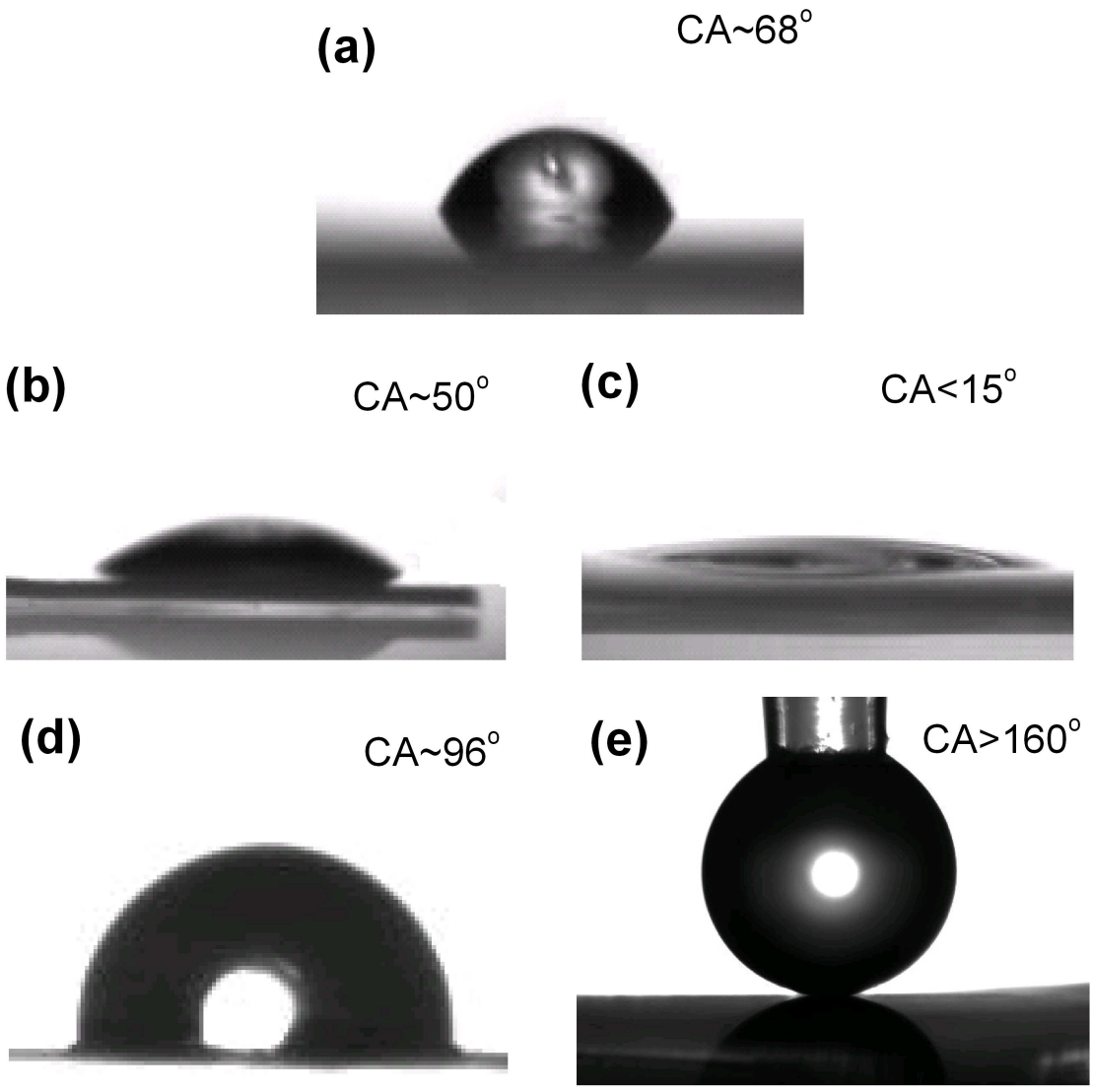

Figure S5. Contact angle measurement on different substrates. (a) Bare gold substrate; (b) substrate coated with 8-layer S-film (with washing); (c) substrate coated with 8-layer R-film (without washing); (d) substrate coated with 8-layer S-film after OTS modification; (e) substrate coated with 8-layer R-film after OTS modification. CA: contact angle.

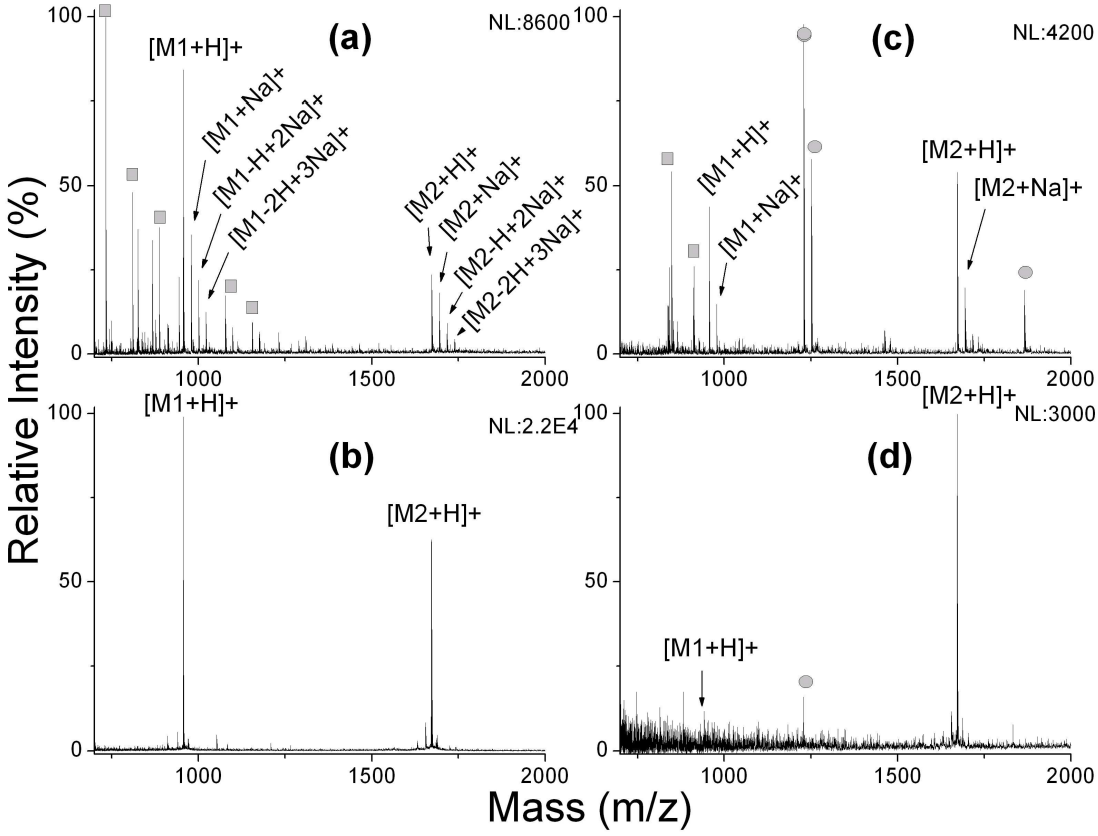

Figure S6. MALDI and SALDI mass spectra of peptide mixtures in the presence of detergents. (a) 0.2% SDS, CHCA matrix; (b) 0.2% SDS, OPD/SALDI on an OTS-modified calcinated surface; (c) 0.1% CHAPs, CHCA matrix; (d) 0.1% CHAPs, OPD/SALDI on an OTS-modified calcinated surface. The analyte was the mixture of [Sar1, Thr8]-angiotensin Ⅱ (M1) and neurotensin (M2), 2 pmol each. Squares (□) represent peaks from CHCA related ions; Circles (○) represent peaks from detergents. NL: normalized level.

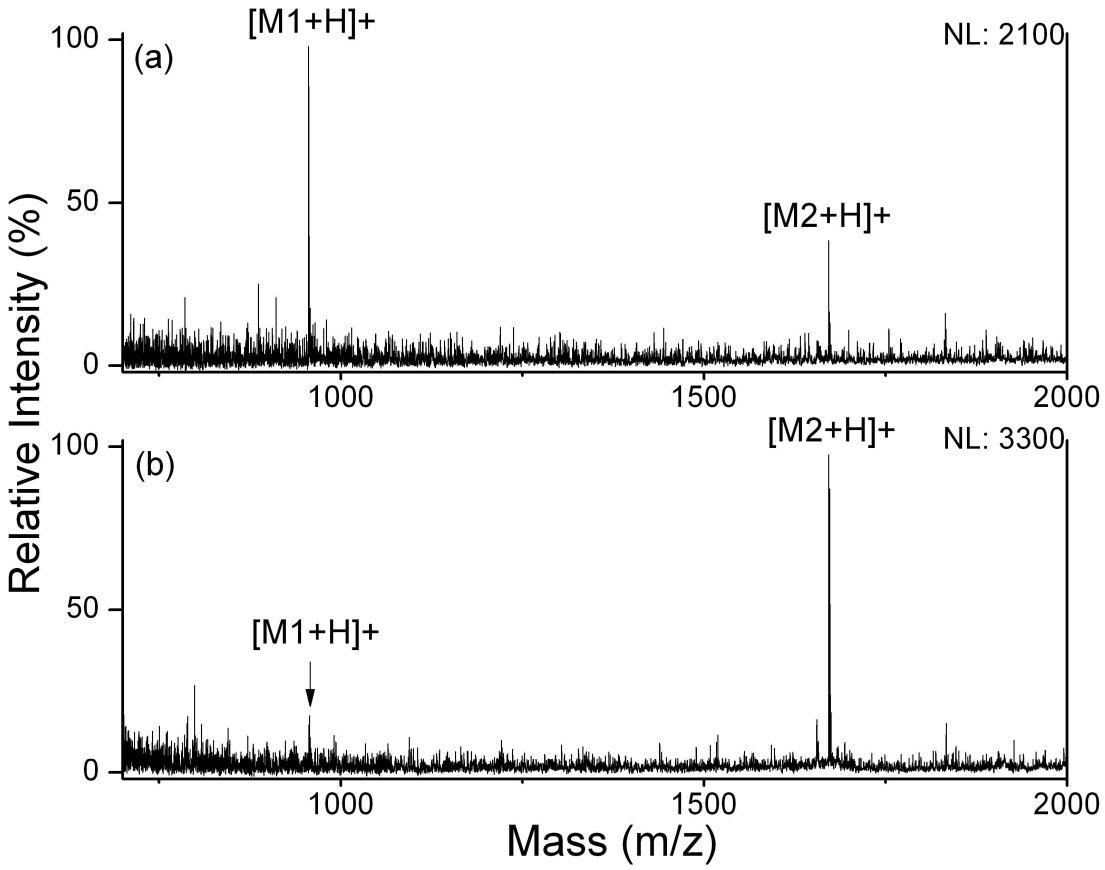

Figure S7. SALDI-MS analysis of peptides treated by different desalting methods: (a) off-probe desalting with ZipTip-C18 pipette tip; (b) on-plate desalting with OTS-modified 8-layer R-film. The analyte was the mixture of [Sar1, Thr8]-angiotensin Ⅱ (M1) and neurotensin (M2) in 200 mM NaAc, 100 fmol each.

Table S2. Detected peptides in 40fmol cytochrome c digest in the presence of 50 mM NH_4HCO_3

Peaks	Calculated m/z	Sequence	Position	pI[a]	GRAVY[a]	MALDI-MS	SALDI-MS
CT1	779.448	MIFAGIK	81-87	8.50	1.600	*	*
CT 2	790.405	TDANKNK	50-56	8.26	-2.457		*
CT 3	955.500	TGPNLHGLF	29-37	6.40	0.067	*	*
CT 4	1065.521	GEREDLIAY	90-98	4.14	-0.733		*
CT 5	1193.616	KGEREDLIAY	89-98	4.68	-1.050	*	*
CT 6	1220.653	HKTGPNLHGLF	27-37	8.76	-0.591	*	*
CT 7	1321.711	KKGEREDLIAY	88-98	6.18	-1.309	*	*
CT 8	1456.670	TGQAPGFSYTDANK	41-54	5.50	-0.993	*	
CT 9	1584.765	KTGQAPGFSYTDANK	40-54	8.50	-1.187	*	
CT 10	1633.819	IFVQKCAQCHTVEK	10-23	8.06	0.021	*	*
CT 11	1880.858	VQKCAQCHTVEKGGKHK	12-28	9.31	-1.106	*	
CT 12	2060.223	AGIKKKGEREDLIAYLKK	84-101	9.70	-0.911		*
CT 13	2138.047	GITWGEETLMEYLENPKK	57-74	4.49	-0.961	*	
		Matched Peptides				10	9
		Sequence Coverage (%)				77.1	50.5

[a]pI and Grand average of hydropathicity index (GRAVY) were calculated by using the ProtParam tool (http: //ca.expasy.org/tools/protparam.html). Asterisks (*) represents the identified peptides in MS detection.

Table S3. Identified β-casein peptides in MS detection

Peaks	Calculated m/z	Sequence	Position	pI[a]	GRAVY[a]	MALDI-MS	SALDI-MS
BT1	830.452	AVPYPQR	192-198	8.79	-0.929	*	
BT2	1138.640	VKEAMAPKHK	113-122	9.70	-1.030	*	
BT3	1383.799	LLYQEPVLGPVR	206-217	6.00	0.283	*	*
BT4	2061.828	FQpSEEQQQTEDELQDK[b]	48-63	3.77	-2.331	*	
BT5	2107.231	LLYQEPVLGPVRGPFPIIV	206-224	6.00	0.832	*	*
BT6	2556.093	FQpSEEQQQTEDELQDKIHPF[b]	48-67	4.08	-1.740	*	
		Matched Peptides				6	2
		Sequence Coverage (%)				25.0	8.5

[a] see Table S2. [b] phosphorylation at serine position. Asterisks (*) represents the identified peptides in MS detection.

Table S4. Identified α-casein peptides in MS detection

Peaks	Calculated m/z	Sequence	Position	pI[a]	GRAVY[a]	MALDI-MS	SALDI-MS
AT1	824.430	YPELFR	161-166	6.00	-0.717	*	*
AT2	971.498	FYPELFR	160-166	6.00	-0.214	*	*
AT3	1267.704	YLGYLEQLLR	106-115	6.00	0.070	*	*
AT4	1337.681	HIQKEDVPSER	95-105	5.45	-1.755	*	
AT5	1759.945	HQGLPQEVLNENLLR	23-37	5.40	-0.753	*	*
AT6	1951.952	YKVPQLEIVPNpSAEER[b]	119-134	4.79	-0.794	*	*
		Matched Peptides				6	5
		Sequence Coverage (%)				27.6	22.4

[a] see Table S2. [b] Phosphorylation at serine position. Asterisks (*) represents the identified peptides in MS detection.

Table S5. Detected peptides of 40 pmol BSA digests in the presence of 800 mM urea

	Calculated m/z	Sequence	Position	Modification	pI[a]	GRAVY[a]	MALD-MS	SALDI-MS
1	789.472	LVTDLTK	257-263		5.84	0.429		*
2	853.424	CASIQKF	223-229	CYS_CAM (233)[b]	8.22	0.486		*
3	1017.584	GFQNALIVR	425-433		9.75	0.578	*	*
4	1075.521	YANKYNGVF	180-188		8.50	-0.567	*	*
5	1170.525	SQYLQQCPF	52-60	CYS_CAM (58)	5.24	-0.567	*	*
6	1180.547	GFQNALIVRY	425-434		8.75	0.390	*	*
7	1226.703	ISLLLLFSSAY	7-17		5.52	1.873		*
8	1351.685	LSHKDDSPDLPK	127-138		5.30	-1.558		*
9	1439.812	RHPEYAVSVLLR	360-371		8.75	-0.133		*
10	1479.795	LGEYGFQNALIVR	421-433		6.00	0.292		*
11	1639.938	KVPQVSTPTLVEVSR	437-451		8.75	-0.067		*
12	1673.77	QEPERNECFLSHK	118-130	CYS_CAM (125)	5.50	-1.723		*
13	1880.921	RPCFSALTPDETYVPK	508-523	CYS_CAM (510)	6.06	-0.537		*
			Matched Peptides				4	13
			Sequence Coverage (%)				4.6	16.8

[a] see Table S2. [b] Cysteine was treated with iodoacetamide to form carbamidomethyl-cysteine (CYS_CAM). The modified position is shown in parentheses. Asterisks (*) represents the identified peptides in MS detection.