Electronic Supplementary Information
for

A hollow silica bubble based immobilized trypsin for high efficient proteome digestion and buoyant separation

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Measurement of the trypsin loading amount on GMA@SiB

To determine the loading amount of trypsin on GMA@SiB, a series of trypsin solution with different concentrations were prepared and utilized to make a calibration curve by measuring the UV absorption value at 280 nm. Then, trypsin solution (PBS pH 7.4) with an initial concentration of 4mg/mL was mixed with GMA@SiB for

trypsin immobilization overnight at 4°C. After immobilization, the trypsin@SiBwere

collected and the UV value of solution at 280 nm was measured to calculate the amount of trypsin loaded on the GMA@SiB particles.
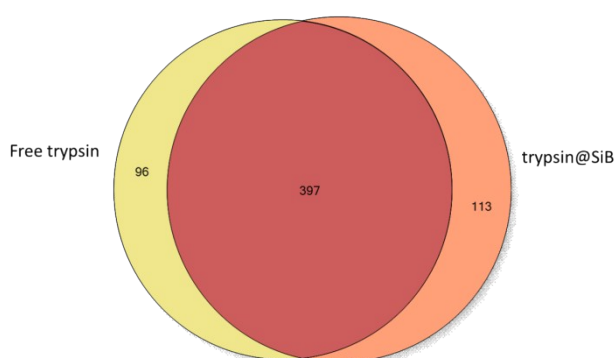
Figure S1

Fig. S1 Protein groupsidentified by trypsin@SiB and free trypsin
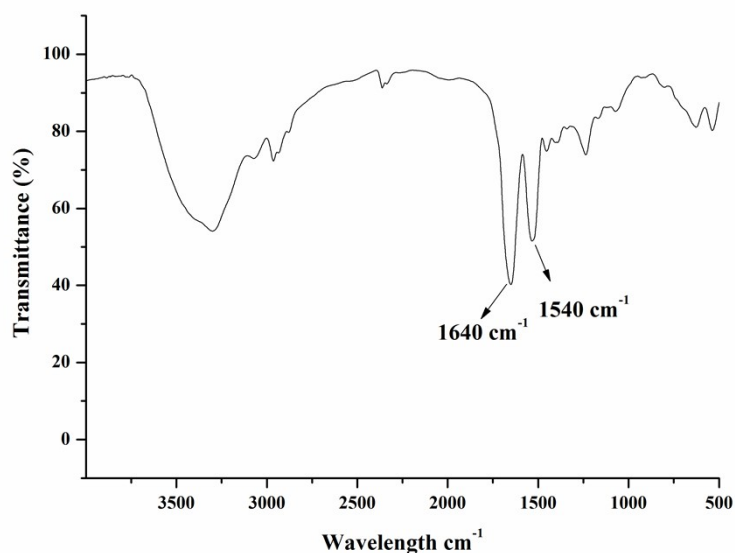
Figure S2
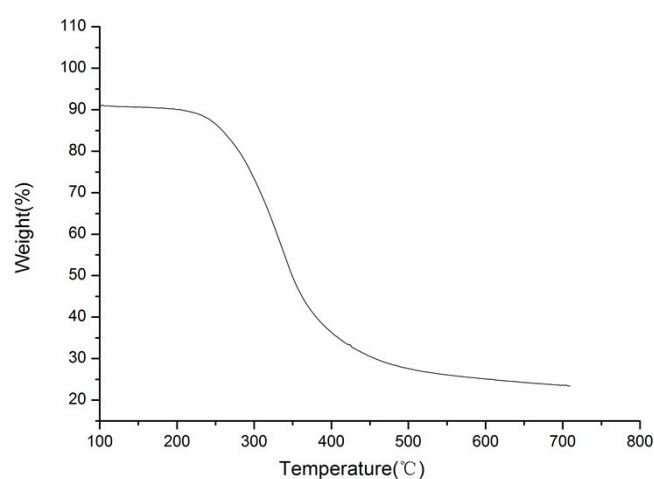

Fig. S2 FT-IR spectra of trypsin

Fig. S3 TGA curves of pristine trypsin

Table S1 Amino acid sequence coverage of BSA obtained from (a) trypsin@SiB for 1min digestion and (b) free trypsin for 12h digestion. Matched amino acids in the identified peptides (allowing 2 miss cleavages) are shown in red

(a)

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  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLTSSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA
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(b)

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  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLTSSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
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451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC

501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT

551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV

601 STQTALA
```

Table S2 Detailed information of the observed peptides in BSA tryptic digest by free trypsin and trypsin@SiB

Peptide sequence (BSA)	m/z	free trypsin	trypsin@SiB
-.MKWVTFISLLLLFSSAYSR.G	2277.0901	N	Y
R.DTHKSEIAHR.F	1192.6269	Y	Y
R.DTHKSEIAHRFK.D	1467.6757	Y	N
R.FKDLGEEHFK.G	1248.6415	Y	Y
K.DLGEEHFK.G	973.4676	Y	Y
K.LVNELTEFAK.T	1162.6438	Y	Y
K.TCVADESHAGCEK.S	1462.6224	Y	Y
K.SLHTLFGDELCK.V	1418.7123	Y	Y
K.VASLRETYGDMADCCEK.Q	2019.8857	Y	N
R.ETYGDMADCCEK.Q	1477.5665	Y	Y
K.QEPERNECFLSHK.D	1672.7962	Y	Y
K.QEPERNECFLSHKDDSPDLPK.L	2540.1299	Y	Y
R.NECFLSHK.D	1033.5088	Y	Y
R.NECFLSHKDDSPDLPK.L	1900.8647	Y	Y
K.DDSPDLPK.L	885.4123	Y	Y
K.LKPDPNTLCDEFK.A	1575.7873	Y	Y
K.LKPDPNTLCDEFKADEK.K	2018.9770	Y	Y
K.LKPDPNTLCDEFKADEKK.F	2147.0811	N	Y
K.ADEKKFWGK.Y	1107.5250	N	Y
K.FWGKYLYEIARR.H	1600.7553	N	Y
K.YLYEIAR.R	926.5005	Y	Y
R.RHPYFYAPELLYYANK.Y	2044.0610	Y	N
R.HPYFYAPELLYYANK.Y	1887.8986	N	Y
K.YNGVFQECCQAEDK.G	1746.7334	Y	Y
K.GACLLPKIETMR.E	1403.6637	Y	Y
K.GACLLPKIETMREK.V	1660.9488	N	Y
K.IETMREK.V	905.4736	N	Y
K.IETMREKVLTSSAR.Q	1619.9804	N	Y
R.EKVLTSSAR.Q	989.5807	Y	Y
K.VLTSSARQR.L	1016.5914	N	Y
K.VLTSSARQRLR.C	1285.6448	N	Y
R.CASIQKFGER.A	1194.5723	Y	Y
R.ALKAWSVARLSQK.F	1456.7145	N	Y
R.LSQKFPK.A	846.4971	N	Y

K.AEFVEVTK.L	921.4849	Y	Y
K.LVTDLTKVHK.E	1152.5881	N	Y
K.LVTDLTKVHKECCHGDLLECADDR.A	2883.4988	N	Y
K.VHKECCHGDLLECADDR.A	2112.8990	N	Y
K.VHKECCHGDLLECADDRADLAK.Y	2611.1578	N	Y
K.ECCHGDLLECADDR.A	1748.7048	Y	Y
K.ECCHGDLLECADDRADLAK.Y	2246.9512	Y	Y
K.YICDNQDTISSK.L	1442.6716	Y	Y
K.LKECCDKPLLEK.S	1531.7986	Y	Y
K.ECCDKPLLEK.S	1290.6241	Y	Y
K.SHCIAEVEK.D	1071.5313	Y	Y
K.SHCIAEVEKDAIPENLPPLTADFAEDK.D	3008.5062	N	Y
K.SHCIAEVEKDAIPENLPPLTADFAEDKDVCK.N	3510.5774	N	Y
K.DAIPENLPPLTADFAEDK.D	1954.8334	N	Y
K.DAIPENLPPLTADFAEDKDVCK.N	2457.1202	N	Y
K.DAIPENLPPLTADFAEDKDVCKNYQEAK.D	3190.4048	N	Y
K.DAFLGSFLYEYSRR.H	1722.8423	N	Y
R.RHPEYAVSVLLR.L	1438.8492	Y	Y
R.HPEYAVSVLLR.L	1282.7214	Y	Y
R.HPEYAVSVLLRLAK.E	1594.8230	Y	Y
K.EYEATLEECCAK.D	1501.6407	Y	Y
K.DDPHACYSTVFDK.L	1553.7724	Y	Y
K.HLVDEPQNLIK.Q	1304.7403	Y	Y
K.QNCDQFEK.L	1067.4657	Y	Y
K.LGEYGFQNALIVR.Y	1478.8200	Y	Y
K.LGEYGFQNALIVRYTRK.V	2027.2441	N	Y
R.KVPQVSTPTLVEVSR.S	1638.9762	Y	Y
K.VPQVSTPTLVEVSRSLGK.V	1895.9649	Y	N
K.VPQVSTPTLVEVSR.S	1510.8538	N	Y
R.SLGKVGTRCCTKPESER.M	1963.9523	Y	Y
R.CCTKPESER.M	1165.5204	Y	Y
R.MPCTEDYLSLILNR.L	1723.9057	Y	Y
R.MPCTEDYLSLILNRLCVLHEKTPVSEK.V	3244.5970	N	Y
R.LCVLHEK.T	897.4778	Y	Y
K.CCTESLVNR.R	1137.5247	Y	Y
R.RPCFSALTPDETYVPK.A	1879.9523	Y	Y
K.LFTFHADICTLPDTEK.Q	1906.9447	Y	Y
K.QIKKQTALVELLK.H	1510.8981	Y	N
K.KQTALVELLK.H	1141.7372	Y	Y
K.QTALVELLK.H	1013.6217	Y	Y
K.ATEEQLK.T	817.4267	Y	Y
K.TVMENFVAFVDK.C	1398.6965	Y	Y
K.CCAADDKEACFAVEGPK.L	1926.8371	Y	Y

| K.EACFAVEGPK.L | 1106.5430 | Y | Y |
| K.LVVSTQTALA.- | 1001.5842 | Y | Y |

Table S3 Elemental analysis of SiB, GMA@SiB and trypsin@SiB

	N[%]	C[%]	H[%]	S[%]
SiB	0.06	0	0.336	0.258
GMA@SiB	0.08	10.08	1.316	0.188
trypsin@SiB	1.96	5.66	1.896	0.065