Supplementary Information

Experimental Procedures

Preparation of MPS materials

MPS SBA-15 was prepared by dissolving 3g Pluronic P123 and 8.5g tetraethoxysilane (TEOS) in 120ml 1.5M HCl aqueous solution, with stirring at 35℃ for 20h. Subsequently, the solution was moved into autoclave and aged at 120℃ for 24h. The product was washed by water and ethanol, and dried at room temperature in air. The as-synthesized SBA-15 sample was calcined by slowly increasing temperature from ambient temperature to 500℃ for 8h and heating at 500℃ for 6h.

The MPS modified with thiol was fabricated by dispersing MPS SBA-15 (0.3g) in 30ml of toluene, followed by adding (3-mercaptopropyl)-trimethoxysilane (1.6mmol) into the solution. Then, the mixture was refluxed at 110℃ for 12h. The modified MPS was recovered by filtration, washed with ethanol three times, and dried under vacuum at room temperature.

Immobilization of enzyme

3mg MPS materials were dispersed in 1ml 50mM Tris-HCl buffer solution (pH=6.5) containing 0.3mg trypsin, incubated in shaker at 25℃ for 6h. Finally, the solids were separated from the mixture by centrifugation. The content of trypsin in the supernatant was examined by measuring absorbance at λ=280nm. Eventually, the amount of trypsin in supernatant decreased from 0.3mg to 0.112mg, the average of three parallel experiments.

Assessment of leaching

After rinsing the MPS materials immobilized with trypsin for several times until no absorption measurable at UV 280nm, hydrolysis of *N*-α-benzoyl-DL-arginine-4-nitroaniline (BAPNA) was adopted to assess leaching of immobilized trypsin. Firstly, 21.7mg BAPNA was dissolved in 1ml DMSO, and diluted to 50 ml with 50mM Tris-HCl (pH=8.2). 0.8mg MPS-trypsin solids were added into 3ml 1mM BAPNA in two vials, respectively. The suspensions were incubated in shaker at 37℃. After first 15min, MPS-trypsin solids were removed by centrifugation from one of the two vials, and the other vial stayed still. Supernatant from each vial was isolated every 15min and estimated by measuring absorbance at λ=405nm. The result is displayed in Figure S1, followed by the interpretation.

Adsorption of model proteins

Consistent to subsequent proteolysis conditions, concentrations of all the four proteins (Cyt *c*, lysozyme, myoglobin and BSA) were adjusted to 5nmol/ml and weight/volume concentration of SBA-15-SH was kept constant at 2mg/ml in pH=8.2 50mM Tris-HCl buffer. Each protein mixed with SBA-15-SH was shaken at 37℃. Kinetic experiment was conducted to determine the amount

adsorbed as a function of incubation time. At a given time, 1ml emulsion was taken from the bulk solution and centrifugated at 13000g for 2min. Amount of protein left in supernatant was calculated from UV absorbance at 280nm (lysozyme and BSA) and 410nm (Cyt *c* and myoglobin).

Digestion of model proteins

For the purpose of size selective proteolysis, we employed Cytochrome C, lysozyme and myoglobin as small proteins, and BSA as a large protein. To ensure fairness of size selectivity, we equalized the amount-of-substance concentrations for both proteins in the binary substrate systems. SBA-15-SH-trypsin particles were immersed in 200μl Tris-HCl (pH=8.2) buffer solution containing BSA and small protein (Cytochrome C/lysozyme/myoglobin) at the same concentration of 5 nmol/ml, with a substrate-to-enzyme ratio of circa 15:1 (w/w). Then the mixture was incubated in shaker at 37℃. For comparison, the traditional digestion was performed by adding equivalent free trypsin into the protein solution under the same incubating conditions. After the precipitation was removed through centrifugation at 18000g for 3min, the supernatant was desalted and lyophilized to dryness for MALDI-TOF and nano-LC-MS/MS analysis.

Proteolysis of human serum

Human serum used in this paper was collected from 100 healthy adults in The Second Affiliated Hospital of Dalian Medical University according to their standard clinical procedures. After collection, the serum samples were stored in -80℃ refrigerator before usage. Without any denaturation procedures, the crude serum was diluted to 1ml by 50mM Tris-HCl solution (pH=8.2), to reach the proteins concentration of circa 1mg/ml. SBA-15-SH-trypsin particles were mixed into 150μl of human serum solution, with a substrate-to-enzyme ratio of circa 15:1 (w/w). Meanwhile, equivalent free trypsin was added into a tube of same solution for the contrast experiment. Both mixtures were incubated in shaker at 37℃. After separated from the solid by centrifugation, the supernatant solutions were reduced by DTT, alkylated by IAA, desalted and lyophilized for the final nano-LC-MS/MS analysis.

Mass spectrometry analysis

MALDI-TOF MS experiments were carried on a Bruker Autoflex time-of-flight mass spectrometer (Bruker, Bremen, Germany), equipped with a delayed ion-extraction device and a pulsed nitrogen laser operated at 337nm. The range of laser energy was adjusted slightly to obtain good resolution and S/N. The instrument was operated in the positive ion linear mode. The MALDI uses a ground-steel sample target with 384 spots. Each spectrum was summed with 30 laser shots.

Nano-LC-MS/MS was performed on a LTQ linear ion trap mass spectrometer equipped with a Finnigan surveyor MS pump (ThermoFinnigan, San Jose, CA, USA). The pump flow rate was split by a cross to achieve 200nl/min. The mobile phase consisted of mobile phase A, 0.1% formic acid in H_2O, and mobile phase B, 0.1% formic acid in ACN. The LTQ instrument was operated in positive ion mode. A voltage of 1.8kV was applied to the cross. About 1μl of redissolved peptides

was loaded onto the C_{18} capillary column using a 75-μm-inner diameter × 220-mm-long capillary column as sample loop.

The model proteins and human serum samples were performed using 30min and 90min linear gradient elution from 5% to 35%, respectively. The mass spectrometer was set as one full MS scan followed by six MS/MS scans on the six highest peaks.

Database searching

The MS/MS spectra acquired from model proteins were submitted to SEQUEST for searching against a database composited with databases of both BSA and a small protein (Cyt *c*, lysozyme or myoglobin) from International Protein Index. The MS/MS spectra of human serum were searching against a complex database containing original and reversed human protein database of International Protein Index (ipi.human.3.17.fasta). The database searching parameters were set as follows. Trypsin was selected as the enzyme, with KR/P as the cleavage site. Enzyme limits was set fully enzymatic, cleaving at both ends. The tolerance of missed cleavage was 2. Carboxamidomethylation of cysteine was set as static modification and oxidation of methionine as dynamic modification. For the analysis of model proteins, the peptides were considered positively identified, if the Xcorr were higher than 1.9, 2.2, 3.75 for singly, doubly, triply charged peptides, and ΔCn were higher than 0.1. The database searching results of human serum samples were filtered with the criteria optimized by SFOER (version2.3)[1]. The false detection rate (FDR) was set less than 1%.

Reference

[1] X. N. Jiang, X. G. Jiang, G. H. Han, M. L. Ye, H. F. Zou, *Bmc Bioinformatics*, 2007, *8*.

Table S1. Peptide fragments observed in MALDI-TOF spectra in the protein pair of Cyt *c* and BSA matched with the peptide mass in UniProtKB database.

Cyt *c*

Mass	Position	MC*	Peptide sequence	Immobilized trypsin	Free trypsin
2209.1209	57-74	2	GITWKEETLMEYLENPKK	√	√
2081.0259	57-73	1	GITWKEETLMEYLENPK	√	
1633.8189	10-23	1	IFVQKCAQCHTVEK	√	√
1606.9162	89-101	3	KTEREDLIAYLKK	√	
1598.7809	40-54	1	KTGQAPGFTYTDANK	√	
1495.6985	62-73	0	EETLMEYLENPK	√	√
1478.8213	90-101	2	TEREDLIAYLKK	√	√
1433.7760	27-39	1	HKTGPNLHGLFGR	√	
1350.7263	90-100	1	TEREDLIAYLK	√	
1168.6221	29-39	0	TGPNLHGLFGR	√	√

BSA

Mass	Position	MC*	Peptide sequence	Immobilized trypsin	Free trypsin
2867.1844	375-399	1	EYEATLEECCAKDDPHACYSTVFDK		√
2387.1435	131-151	1	DDSPDLPKLKPDPNTLCDEF K		√
2301.0822	341-359	1	NYQEAKDAFLGSFLYEYSR		√
2060.1498	434-451	2	YTRKVPQVSTPTLVEVSR		√
2045.0279	168-183	1	RHPYFYAPELLYYANK		√
1942.9769	29-44	2	SEIAHRFKDLGEEHFK		√
1942.8204	264-280	1	VHKECCHGDLLECADDR		√
1844.8483	123-138	1	NECFLSHKDDSPDLPK		√
1823.8996	508-523	0	RPCFSALTPDETYVPK		√
1723.8438	347-360	1	DAFLGSFLYEYSRR		√
1639.9377	437-451	1	KVPQVSTPTLVEVSR	√	√
1633.6621	184-197	0	YNGVFQECCQAEDK		√
1633.0159	548-561	2	KQTALVELLKHKPK		√
1616.7485	118-130	1	QEPERNECFLSHK		√
1578.5981	267-280	0	ECCHGDLLECADDR		√
1519.7461	139-151	0	LKPDPNTLCDEFK		√
1511.9519	545-557	2	QIKKQTALVELLK		√
1511.8427	438-451	0	VPQVSTPTLVEVSR		√
1479.7954	421-433	0	LGEYGFQNALIVR		√
1468.7655	25-36	2	DTHKSEIAHRFK		√
1439.8117	360-371	1	RHPEYAVSVLLR	√	√
1362.6722	89-100	0	SLHTLFGDELCK		√
1305.7161	402-412	0	HLVDEPQNLIK		√
1283.7106	361-371	0	HPEYAVSVLLR		√
1163.6306	66-75	0	LVNELTEFAK		√
1052.4499	460-468	0	CCTKPESER		√
1015.4877	310-318	0	SHCIAEVEK		√
977.4509	123-130	0	NECFLSHK		√

* Number of missed cleavage.

Table S2. Peptide fragments observed in MALDI-TOF spectra in the protein pair of lysozyme and BSA matched with the peptide mass in UniProtKB database.

lysozyme

Mass	Position	MC*	Peptide sequence	Immobilized trypsin	Free trypsin
2671.1954	64-86	1	NTDGSTDYGILQINSRWWCN DGR	√	
1753.8351	64-79	0	NTDGSTDYGILQINSR	√	√
1675.8009	116-130	0	IVSDGNGMNAWVAWR	√	√
1451.7609	20-32	2	VFGRCELAAAMKR	√	√
1428.6502	52-63	0	FESNFNTQATNR	√	√
1268.6092	40-51	0	GYSLGNWVCAAK	√	
1045.5425	135-143	0	GTDVQAWIR	√	√
1030.5177	32-39	1	RHGLDNYR	√	
936.3781	80-86	0	WWCNDGR	√	√
874.4166	33-39	0	HGLDNYR	√	√

BSA

Mass	Position	MC*	Peptide sequence	Immobilized trypsin	Free trypsin
2484.1459	118-138	2	QEPERNECFLSHKDDSPDLP K		√
2472.1976	413-433	1	QNCDQFEKLGEYGFQNALIV R		√
2414.1696	508-528	1	RPCFSALTPDETYVPKAFDE K		√
2060.1498	434-451	2	YTRKVPQVSTPTLVEVSR		√
1823.8996	508-523	0	RPCFSALTPDETYVPK		√
1723.8438	347-360	1	DAFLGSFLYEYSRR	√	√
1639.9377	437-451	1	KVPQVSTPTLVEVSR	√	√
1616.7485	118-130	1	QEPERNECFLSHK		√
1517.8587	236-248	2	AWSVARLSQKFPK		√
1511.9519	545-557	2	QIKKQTALVELLK		√
1511.8427	438-451	0	VPQVSTPTLVEVSR		√
1479.7954	421-433	0	LGEYGFQNALIVR	√	√
1439.8117	360-371	1	RHPEYAVSVLLR		√
1305.7161	402-412	0	HLVDEPQNLIK		√
1283.7106	361-371	0	HPEYAVSVLLR		√
1163.6306	66-75	0	LVNELTEFAK		√

* Number of missed cleavage.

Table S3. Peptide fragments observed in MALDI-TOF spectra in the protein pair of myoglobin and BSA matched with the peptide mass in UniProtKB database.

myoglobin

Mass	Position	MC*	Peptide sequence	Immobilzed trypsin	Free trypsin
2859.4998	18-43	1	VEADIAGHGQEVLIRLFTGH PETLEK	√	
2601.4915	98-119	2	HKIPIKYLEFISDAIIHVLH SK	√	
2336.3376	100-119	1	IPIKYLEFISDAIIHVLHSK	√	
2150.2543	58-78	2	ASEDLKKHGTVVLTALGGIL K	√	
2110.1515	79-97	2	KKGHHEAELKPLAQSHATK	√	
1982.0566	80-97	1	KGHHEAELKPLAQSHATK	√	
1937.0167	33-48	2	LFTGHPETLEKFDKFK	√	
1885.0218	104-119	0	YLEFISDAIIHVLHSK	√	√
1853.9616	81-97	0	GHHEAELKPLAQSHATK	√	
1815.9024	2-17	0	GLSDGEWQQVLNVWGK	√	
1729.8789	49-63	2	HLKTEAEMKASEDLK	√	
1661.8533	33-46	1	LFTGHPETLEKFDK	√	
1606.8547	18-32	0	VEADIAGHGQEVLIR	√	√
1502.6692	120-134	0	HPGDFGADAQGAMTK	√	√
1378.8416	65-78	0	HGTVVLTALGGILK	√	
1360.7583	135-146	1	ALELFRNDIAAK	√	
1271.6630	33-43	0	LFTGHPETLEK	√	
748.4352	135-140	0	ALELFR	√	√

BSA

Mass	Position	MC*	Peptide sequence	Immobilized trypsin	Free trypsin
2867.1844	375-399	1	EYEATLEECCAKDDPHACYS TVFDK		√
2472.1976	413-433	1	QNCDQFEKLGEYGFQNALIV R		√
2415.208	298-318	2	LKECCDKPLLEKSHCIAEVE K		√
2301.0822	341-359	1	NYQEAKDAFLGSFLYEYSR		√
2060.1498	434-451	2	YTRKVPQVSTPTLVEVSR		√
2045.0279	168-183	1	RHPYFYAPELLYYANK		√
1994.0045	490-507	2	TPVSEKVTKCCTESLVNR	√	√
1942.9769	29-44	2	SEIAHRFKDLGEEHFK		√
1942.8204	264-280	1	VHKECCHGDLLECADDR		√
1823.8996	508-523	0	RPCFSALTPDETYVPK		√
1639.9377	437-451	1	KVPQVSTPTLVEVSR	√	√
1627.7996	286-299	1	YICDNQDTISSKLK	√	
1517.8587	236-248	2	AWSVARLSQKFPK	√	√
1511.9519	545-557	2	QIKKQTALVELLK	√	√
1511.8427	438-451	0	VPQVSTPTLVEVSR	√	√
1479.7954	421-433	0	LGEYGFQNALIVR	√	√
1439.8117	360-371	1	RHPEYAVSVLLR	√	√
1305.7161	402-412	0	HLVDEPQNLIK	√	√
1294.7041	246-256	1	FPKAEFVEVTK	√	
1283.7106	361-371	0	HPEYAVSVLLR	√	√
1249.6211	35-44	1	FKDLGEEHFK	√	
1163.6306	66-75	0	LVNELTEFAK	√	√
1052.4499	460-468	0	CCTKPESER		√
1015.4877	310-318	0	SHCIAEVEK	√	√
977.4509	123-130	0	NECFLSHK		√

* Number of missed cleavage.

Table S4. Unique peptides and sequence coverage of each protein in the three protein pairs analyzed by nano-LC-MS/MS

	Immobilized trypsin		Free trypsin	
	Unique peptides	Sequence coverage (%)	Unique peptides	Sequence coverage (%)
Cyt *c*	15	71.43	10	62.86
BSA	29	42.67	55	75.62
Lysozyme	14	80.27	8	51.02
BSA	17	30.81	40	67.87
Myoglobin	18	100	18	100
BSA	33	54.53	44	68.53

Table S5. Unique peptides and sequence coverage of Cyt *c* and BSA with various mole ratios treated by immobilized trypsin.

Cyt *c*:BSA (mole ratio)	Cyt *c*		BSA	
	Unique peptides	Sequence coverage(%)	Unique peptides	Sequence coverage(%)
1:10	14	64.76	11	17.13
1:100	4	25.71	18	32.78
1:300	2	23.81	15	24.88
1:1000	1	13.33	15	21.25
1:3000	2	23.81	10	15.65
1:10000	1	11.43	11	16.80
1:30000	0	0	12	16.64

Table S6. Unique peptides and sequence coverage of Cyt *c* and BSA with various mole ratios treated by free trypsin.

Cyt *c*:BSA (mole ratio)	Cyt *c*		BSA	
	Unique peptides	Sequence coverage(%)	Unique peptides	Sequence coverage(%)
1:10	9	59.05	66	88.63
1:100	3	22.86	69	81.71
1:300	2	23.81	70	80.40
1:1000	0	0	65	82.54

Table S7. Unique peptides and sequence coverage of the protein pair of BSA and Cyt *c* for a series of incubation times.

Immobilized trypsin	Cyt *c*		BSA	
	Unique peptides	Sequence coverage (%)	Unique peptides	Sequence coverage (%)
10min	8	45.71	3	5.27
20min	12	67.62	9	17.13
40min	11	62.86	10	16.31
1h	17	74.29	12	16.64
4h	16	65.71	19	29.98
12h	15	71.43	29	42.67
24h	18	79.05	42	70.18

Free trypsin	Cyt *c*		BSA	
	Unique peptides	Sequence coverage (%)	Unique peptides	Sequence coverage (%)
10min	16	65.71	26	41.52
20min	14	64.76	25	41.35
40min	14	64.76	27	44.32
1h	8	56.19	34	57.83
4h	7	58.10	39	66.56
12h	10	62.86	55	75.62
24h	7	46.67	42	67.71

Table S8. Comparison of proteins identified in human serum digested by immobilized and free trypsin for 1h incubation (sorted by MW).*

IPI number	Protein	Molecular weight/Da	Spectral Count	
			Immobilized trypsin	Free trypsin
IPI00022229.1	Apolipoprotein B-100 precursor	515556.6	0	74
IPI00414283.3	Fibronectin 1 isoform 4 preproprotein	256509.1	1	14
IPI00164623.4	Complement C3 precursor	187304.2	30	52
IPI00478003.1	Alpha-2-macroglobulin precursor	163276.1	0	15
IPI00029739.4	Splice Isoform 1 of Complement factor H precursor	139069.1	0	14
IPI00017601.1	Ceruloplasmin precursor	122203.8	0	16
IPI00305461.2	Inter-alpha-trypsin inhibitor heavy chain H2 precursor	106435.2	1	33
IPI00292530.1	Inter-alpha-trypsin inhibitor heavy chain H1 precursor	101388	5	27
IPI00218192.1	Inter-alpha-trypsin inhibitor heavy chain H4 precursor	101240.9	0	16
IPI00019580.1	Plasminogen precursor	90568.2	0	11
IPI00026314.1	Gelsolin precursor	85696.54	1	22
IPI00019591.1	Complement factor B precursor	85532	9	14
IPI00022463.1	**Serotransferrin precursor**	77049.18	0	39
IPI00032328.1	Kininogen-1 precursor	71944.57	0	21
IPI00745872.1	**Serum albumin precursor**	69366.04	14	1076
IPI00448925.3	IGHG1 protein	60101.51	0	12
IPI00022371.1	Histidine-rich glycoprotein precursor	59577.68	0	6
IPI00745089.1	Alpha 1B-glycoprotein	54252.9	0	7
IPI00742696.1	vitamin D-binding protein precursor	52917.08	0	29
IPI00291262.3	Clusterin precursor	52494.07	3	28
IPI00022488.1	Hemopexin precursor	51675.82	0	44
IPI00553177.1	**Alpha-1-antitrypsin precursor**	46736.02	0	11

IPI00641737.1	**Haptoglobin precursor**	46722.58	0	45
IPI00304273.2	Apolipoprotein A-IV precursor	45398.53	27	20
IPI00022431.1	Alpha-2-HS-glycoprotein precursor	39324.27	8	28
IPI00020986.2	Lumican precursor	38428.57	0	6
IPI00298828.3	Beta-2-glycoprotein 1 precursor	38297.82	0	3
IPI00021842.1	Apolipoprotein E precursor	36153.69	25	23
IPI00021841.1	Apolipoprotein A-I precursor	30777.46	72	115
IPI00022432.1	Transthyretin precursor	15886.84	9	1
IPI00019399.1	Serum amyloid A-4 protein precursor	14806.53	7	2
IPI00552578.2	Serum amyloid A protein precursor	13531.87	3	0
IPI00027547.2	Dermcidin precursor	11283.75	3	0
IPI00021856.3	Apolipoprotein C-II precursor	11283.74	16	20
IPI00021854.1	Apolipoprotein A-II precursor	11174.9	4	35
IPI00021857.1	Apolipoprotein C-III precursor	10852.19	20	11
IPI00021855.1	Apolipoprotein C-I precursor	9331.815	2	5

*High-abundance proteins in human serum are in bold type.

Table S9. Absorbance of BAPNA solution treated by no enzyme, SBA-15 and SBA-15-SH for various incubation times.

t/min	0	15	30	45	60
No enzyme	0.0783	0.078	0.0776	0.0788	0.0813
SBA-15	0.0783	0.0741	0.0734	0.0718	0.0731
SBA-15-SH	0.0783	0.0744	0.0767	0.0746	0.0753

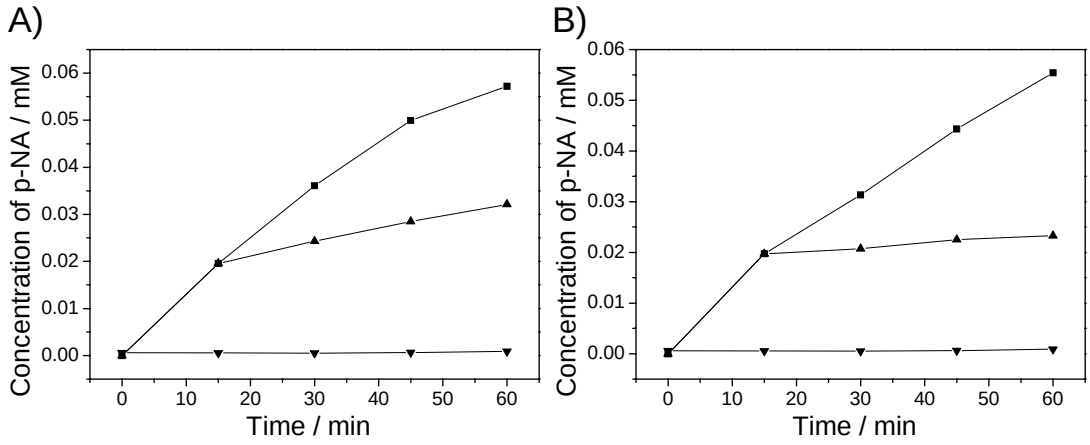

Figure S1. Concentration of hydrolysis product p-nitroaniline (p-NA) versus time catalyzed by immobilized trypsin on (A) SBA-15 and (B) SBA-15-SH. In each figure, "■" was used to label the reaction in existence of immobilized trypsin all through the incubation; "▲" was used to label the reaction in absence of immobilized trypsin after initial 15min; "▾" was used to label the reaction in absence of immobilized trypsin all through the incubation.

Interpretation: We explored the absorbance of BAPNA solution treated with no enzyme to estimate its autolysis. As listed in Table S9, the absorbance exhibited tiny change, indicating that the substrate was considered stable during 1h incubation at 37□ and the autolysis of BAPNA could be ignored in this experiment. For SBA-15 and SBA-15-SH, the minor decrease was attributed to the adsorption of BAPNA on mesoporous silica.

After removal of SBA-15-trypsin particles at 15min from beginning, the concentration of p-NA increased continuously (with slope of 2.78×10^{-4} mM·min^{-1}), suggesting that trypsin leaching from the support hydrolyzed the substrate BAPNA (the lower branch in Figure S1A). Differently, concentration of p-NA exhibited little change (with slope of 8.34×10^{-5} mM·min^{-1}) in absence of

immobilized trypsin on SBA-15-SH (the lower branch in Figure S1B). This difference indicated that SBA-15-SH performed well in overcoming the drawback of trypsin leaching. The better behavior of SBA-15-SH mainly benefited from the interaction between -S-S- exposed on the surface of trypsin molecule and thiol groups modified on mesoporous silica[1].

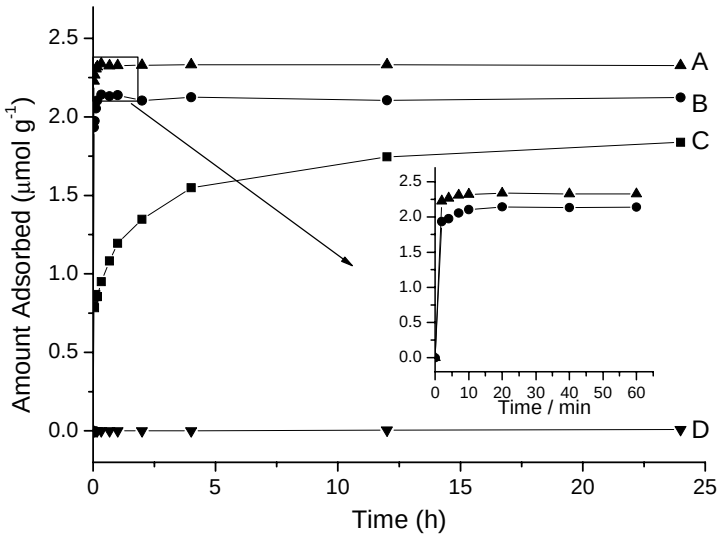

Figure S2. Amount of (A) Cyt *c*, (B) lysozyme, (C) myoglobin and (D) BSA absorbed on SBA-15-SH as a function of time. Inset: Cyt *c* and lysozyme adsorption curves in the initial 1h.

Reference

[1] H. H. P. Yiu, P. A. Wright, N. P. Botting, *Journal of Molecular Catalysis B-Enzymatic*, 2001, **15**, 81.