Electronic Supplementary Information

Characterization of Efficient Proteolysis by Trypsin Loaded Macroporous Silica

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SI-1: Characterization of employed porous material

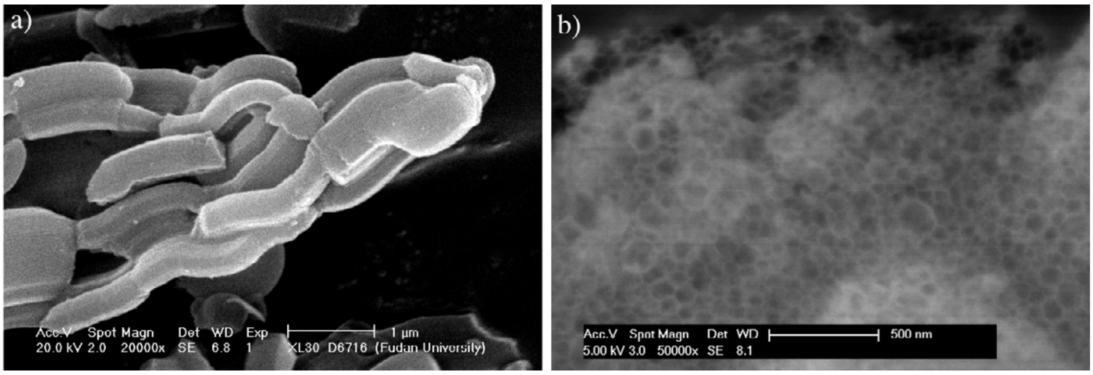

Figure SI-1. Scanning electron micrographs of SBA-15 a) and MOSF b), respectively

Table SI-1. Physical parameters of the chosen porous materials, calculated from nitrogen sorption data.

	SBA-15	MOSF
Cell/window size	~10nm pore size	~100nm in pore diameter
Pore volume	1.14cm^3/g	1.3cm^3/g
Surface area	503m^2/g	430m^2/g

SI-2: Electric double layer formation in the nanoporous silica

Assuming that the surface property of porous silica is similar as glass, the surface of silica inner pore should be negatively charged with a coulomb intensity around $1\text{mC}\cdot\text{m}^{-2}$, when the silica is separated in a buffer with pH 8. (Analytical and Physical Electrochemistry, Hubert H. Girault, Presses Polytechniques Universitaires Romandes, 2004) To compensate these negative charges, positive ions could be confined in the nanopores with a concentration calculated as $qA/(FV)$, where q is the surface charge intensity ($\text{C}\cdot\text{m}^{-2}$), A is the specific surface area of porous silica, V is the total pore volume of porous silica and F is the Faraday constant. For MOSF and SBA-15, the specific surface areas are both around $500\text{m}^2/\text{g}$, and the total pore volumes are both around $1\text{cm}^3/\text{g}$. The positive ion concentration in the nanopores of both MSOF and SBA-15 can be roughly calculated as ~5mM, indicating an electric double layer thickness of ~10nm generated on the surface of the inner pores of the porous silica (Analytical and Physical Electrochemistry, Hubert H. Girault, Presses Polytechniques Universitaires Romandes, 2004).

SI-3: Interference of SDS and urea on the reactor catalyzed digestion.

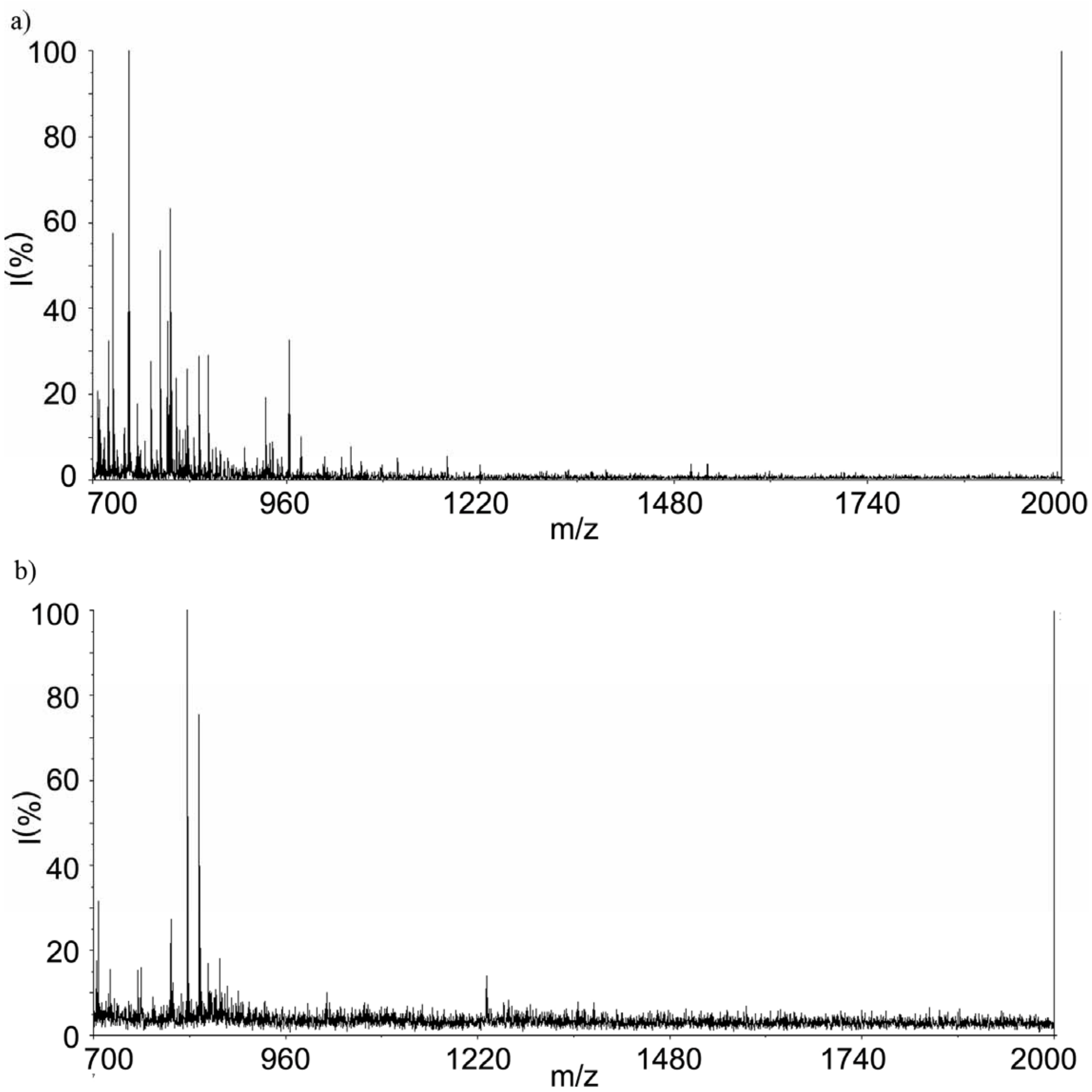

Figure SI-2. Mass spectra of proteolysis products from 20min trypsin-MOSF catalyzed digestion of myoglobin (20ng/μL) that is dissolved with a) 0.1% SDS and b) 500mM urea. The SDS and urea were removed by using SCX Zip Tips before MS identification.

SI-4: RPLC spectrum of the rat liver cytoplasm sample

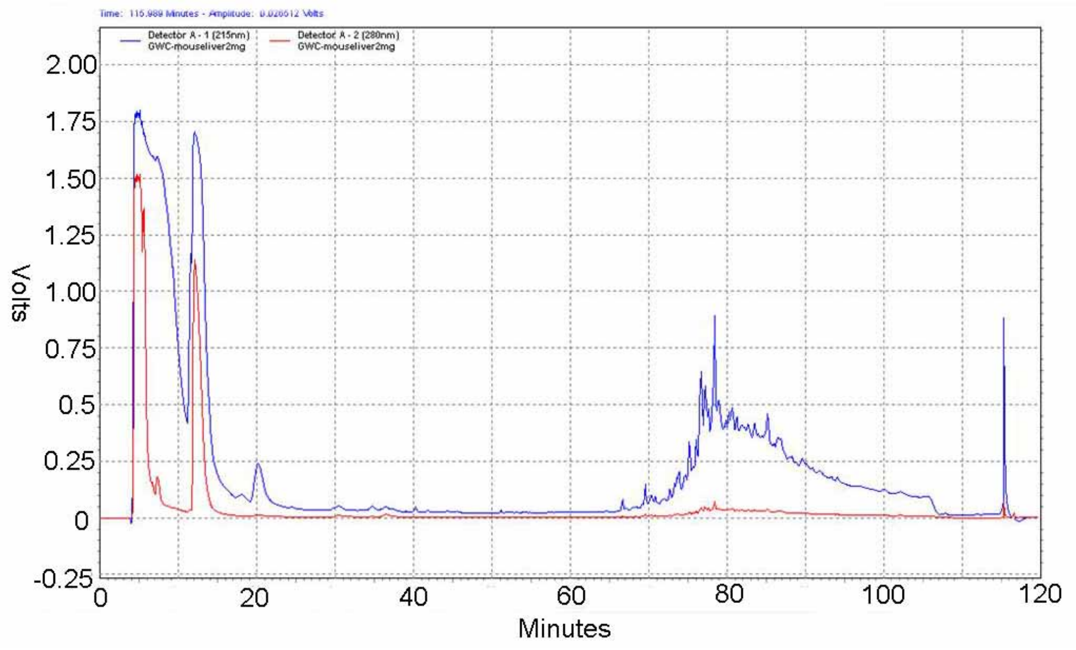

Figure SI-3. RPLC spectrum of the rat liver cytoplasm sample carried out on Agilent ZORBAX SB-C18 column (4.6 ×250 mm, 5 μm, 300A$^{\circ}$, C18, Hypersil, EliteHPLC, China) for 120 min by using 0.05%(v/v) TFA in water as mobile phase A and 0.05%(v/v) TFA in acetonitrile as mobile phase B on a Shimadzu LC-20AD capillary pumping system. The fraction separated from 77min to 78min was chosen to be digested. The blue curve was recorded by detecting the UV light absorption of separated fractions under 215nm wavelength; while the red curve was recorded by detecting the UV light absorption of separated fractions under 280nm wavelength.

SI-5: Identified proteins from rat liver tissue RPLC fraction 77-78min

Table SI-2. The proteins identified from the tryptic peptides generated after 3h of digestion with free tripysin.

No.	protein	pI	Mw	No.	protein	pI	Mw
1	sp|O08709|PRDX6_MOUSE	5.71	24870.64648	34	sp|P19639|GSTM4_MOUSE	7.62008	25701.607
2	sp|O35490|BHMT1_MOUSE	8.01	45020.53125	35	sp|O70591|PFD2_MOUSE	6.190047	16533.701
3	sp|P00329|ADH1_MOUSE	8.43	39771.45703	36	sp|P08228|SODC_MOUSE	6.020043	15942.667
4	sp|P01942|HBA_MOUSE	7.96	15085.1709	37	sp|P09671|SODM_MOUSE	8.790106	24602.928
5	sp|P02088|HBB1_MOUSE	7.12	15840.17383	38	sp|P10648|GSTA2_MOUSE	8.880108	25532.957
6	sp|P02089|HBB2_MOUSE	7.84	15878.25	39	sp|P11352|GPX1_MOUSE	6.730059	22179.254
7	sp|P07309|TTHY_MOUSE	5.76	15775.92481	40	sp|P15532|NDKA_MOUSE	6.840062	17207.795
8	sp|P07724|ALBU_MOUSE	5.74	68692.60156	41	sp|P18760|COF1_MOUSE	8.210093	18559.545
9	sp|P10649|GSTM1_MOUSE	7.71	25969.98047	42	sp|P20029|GRP78_MOUSE	5.060021	72422.164
10	sp|P10853|H2B1F_MOUSE	10.3	13936.16309	43	sp|P20152|VIME_MOUSE	5.060021	53687.738
11	sp|P12710|FABPL_MOUSE	8.58	14245.55078	44	sp|P25688|URIC_MOUSE	8.480099	35039.191
12	sp|P16015|CAH3_MOUSE	6.89	29366.27539	45	sp|P35979|RL12_MOUSE	9.480122	17804.557
13	sp|P17742|PPIA_MOUSE	7.73	17971.33789	46	sp|P56480|ATPB_MOUSE	5.190024	56300.539
14	sp|P19221|THRB_MOUSE	6.03	70268.86719	47	sp|P99027|RLA2_MOUSE	4.420006	11650.907
15	sp|P24270|CATA_MOUSE	7.72	59765.35547	48	sp|P99029|PRDX5_MOUSE	9.090113	21897.473
16	sp|P25444|RS2_MOUSE	10.3	31231.32031	49	sp|Q60668|HNRPD_MOUSE	7.610079	38354.117
17	sp|P26443|DHE3_MOUSE	8.05	61336.86719	50	sp|Q64433|CH10_MOUSE	7.900086	10962.703
18	sp|P27773|PDIA3_MOUSE	5.88	56678.46484	51	sp|Q8BIQ5|CSTF2_MOUSE	6.35005	61341.168
19	sp|P32020|NLTP_MOUSE	7.15	59125.73047	52	sp|Q99J08|S14L2_MOUSE	6.680058	46300.316
20	sp|P62908|RS3_MOUSE	9.68	26674.30469	53	sp|Q9CQ48|NUDC2_MOUSE	4.980019	17659.871
21	sp|Q5EBG8|CA050_MOUSE	5.29	21862.71094	54	sp|Q9CXI5|ARMET_MOUSE	8.340096	20373.684
22	sp|Q64523|H2A2C_MOUSE	10.9	13988.36719	55	sp|Q9CY58|PAIRB_MOUSE	8.600101	44714.145
23	sp|Q8QZT1|THIL_MOUSE	8.7	44816.14063	56	sp|Q9DCX2|ATP5H_MOUSE	5.510031	18749.449
24	sp|Q8VBW8|TTC36_MOUSE	5.12	20132.50195	57	sp|Q9JII6|AK1A1_MOUSE	6.890063	36586.938
25	sp|Q91X83|METK1_MOUSE	5.51	43508.66406	58	sp|P10126|EF1A1_MOUSE	9.100113	50113.898
26	sp|Q91Y97|ALDOB_MOUSE	8.52	39506.98047	59	sp|Q99020|ROAA_MOUSE	7.680081	30831.271
27	sp|Q99PL5|RRBP1_MOUSE	9.35	172878.1875	60	sp|P49429|HPPD_MOUSE	6.580056	45054.254
28	sp|Q9CPU0|LGUL_MOUSE	5.24	20809.59961	61	sp|P56391|CX6B1_MOUSE	8.96011	10071.45
29	sp|Q9CZX8|RS19_MOUSE	10.4	16085.49121	62	sp|P0C0S6|H2AZ_MOUSE	10.580147	13552.732
30	sp|Q9D6J6|NDUV2_MOUSE	6.99	27285.36328	63	sp|P10639|THIO_MOUSE	4.790015	11675.441
31	sp|Q9DCW4|ETFB_MOUSE	8.24	27623.30273	64	sp|Q99KB8|GLO2_MOUSE	6.490054	28901.016
32	sp|Q9R257|HEBP1_MOUSE	5.18	21052.82422	65	sp|P46656|ADX_MOUSE	5.370028	20122.869
33	sp|Q9WVA2|TIM8A_MOUSE	5.1	11042.44336	66	sp|P62991|UBIQ_MOUSE	6.560055	8564.8428

Table SI-3. The isoelectric point and molecular weight of proteins identified from the tryptic peptides generated after 3h digestion with MOSF-trypsin nanoreactor (right, black characters) and from standard overnight in-solution digestion method (left, red characters). The proteins existing in both lists are characterized as bold and italic type.

No.	pI	Mw	No.	PI	Mw	No.	pI	Mw	No.	PI	Mw
1	***4.330004***	47994.6	57	***7.340073***	68091.02	1	3.979997	28537.53	83	7.070067	102088.2
2	***4.420006***	11650.91	58	***7.62008***	25701.61	2	***4.330004***	47994.6	84	***7.090067***	32675.22
3	4.910017	34384.79	59	***7.65008***	34108.37	3	***4.420006***	11650.91	85	***7.120068***	15840.17
4	***5.060021***	72422.16	60	***7.700081***	33465.91	4	4.610011	32560.05	86	***7.150069***	59125.73
5	***5.060021***	53687.74	61	***7.710082***	25969.98	5	4.680012	32862.82	87	***7.340073***	68091.02
6	***5.100022***	11042.44	62	***7.720082***	59765.36	6	4.740014	92475.85	88	***7.62008***	25701.61
7	***5.120022***	20132.5	63	***7.730082***	17971.34	7	4.790015	32838.08	89	7.63008	44729.57
8	5.130023	71973.5	64	***7.840085***	15878.25	8	4.890017	48699.59	90	***7.65008***	34108.37
9	***5.150023***	33406.85	65	***7.960087***	15085.17	9	4.900017	31293.03	91	7.670081	21984.06
10	***5.180024***	21052.82	66	***8.010088***	45020.53	10	4.980019	17659.87	92	7.700081	33465.91
11	***5.190024***	56300.54	67	***8.030088***	35490.41	11	5.00002	50006.88	93	***7.700081***	13559.46
12	***5.190024***	20830.44	68	***8.030088***	59335.16	12	5.01002	26791.2	94	***7.710082***	25969.98
13	***5.240025***	20809.6	69	***8.050089***	61336.87	13	5.02002	52572.75	95	***7.720082***	59765.36
14	***5.350028***	48968.5	70	***8.210093***	18559.54	14	***5.060021***	72422.16	96	***7.730082***	17971.34
15	***5.380028***	53600.41	71	***8.240093***	27623.3	15	***5.060021***	53687.74	97	7.750082	18235.85
16	***5.510031***	43508.66	72	8.250093	70922.81	16	5.060021	61422.24	98	***7.840085***	15878.25
17	5.510031	18749.45	73	***8.320095***	41857.93	17	***5.100022***	11042.44	99	7.900086	10962.7
18	5.630034	31380.98	74	***8.350096***	16665.11	18	***5.100022***	61172.12	100	7.910086	51340.84
19	***5.680035***	22165.33	75	***8.360096***	30048.64	19	5.120022	20132.5	101	***7.960087***	15085.17
20	***5.690035***	54565.35	76	***8.430098***	39771.46	20	***5.150023***	33406.85	102	8.000088	48297.27
21	***5.710036***	24870.65	77	8.430098	35810.03	21	***5.180024***	21052.82	103	***8.010088***	45020.53
22	***5.740036***	68692.6	78	8.460098	14957.21	22	***5.190024***	20830.44	104	***8.030088***	35490.41
23	***5.760037***	15775.92	79	***8.480099***	35039.19	23	***5.190024***	56300.54	105	***8.030088***	59335.16
24	***5.840039***	68600.97	80	***8.490099***	37047.68	24	5.230025	11777.88	106	***8.050089***	61336.87
25	5.870039	52514.07	81	***8.5201***	39506.98	25	***5.240025***	20809.6	107	8.070089	85463.54
26	***5.88004***	56678.46	82	***8.580101***	14245.55	26	5.290026	21862.71	108	***8.210093***	18559.54
27	5.91004	34216.42	83	***8.700104***	44816.14	27	5.290026	41736.76	109	***8.240093***	27623.3
28	***5.950041***	17270.54	84	***8.790106***	24602.93	28	***5.350028***	48968.5	110	***8.320095***	41857.93
29	***5.990042***	30448.64	85	***8.850107***	35000.2	29	5.370028	32667.05	111	8.340096	32314.11
30	***5.990042***	29546.54	86	***8.870108***	50906.22	30	***5.380028***	53600.41	112	***8.350096***	16665.11
31	***6.020043***	15942.67	87	***8.880108***	25532.96	31	5.47003	16131.43	113	***8.360096***	30048.64
32	***6.090044***	32804.31	88	***8.97011***	37402.68	32	5.500031	46415.53	114	***8.430098***	35810.03
33	***6.100045***	33023.19	89	***9.000111***	54540.88	33	***5.510031***	43508.66	115	***8.480099***	35039.19
34	6.100045	41525.52	90	***9.090113***	39652	34	***5.510031***	18749.45	116	***8.490099***	37047.68
35	***6.130045***	15137.4	91	***9.100113***	50113.9	35	5.510031	88847.04	117	***8.5201***	39506.98

36	6.170046	24324.58	92	***9.140114***	38284.98	36	5.530032	21066.6	118	8.5301	30641.21
37	***6.35005***	13776.89	93	9.170115	12096.96	37	5.620034	31875.64	119	***8.580101***	14245.55
38	***6.420052***	141742.5	94	***9.220116***	59752.64	38	5.680035	22165.33	120	8.640102	33880.5
39	***6.430052***	44698.85	95	***9.230116***	66765.57	39	***5.690035***	54565.35	121	8.640102	56822.82
40	***6.480053***	164617.2	96	9.230116	82669.94	40	***5.710036***	24870.65	122	8.640102	74634.73
41	***6.490054***	28901.02	97	9.330118	52443.5	41	***5.740036***	68692.6	123	***8.700104***	44816.14
42	6.500054	13770.28	98	***9.350119***	172878.2	42	***5.760037***	15775.92	124	8.770105	10000.43
43	***6.580056***	45054.25	99	***9.350119***	9117.577	43	***5.840039***	68600.97	125	8.770105	66414.57
44	6.690058	31319.58	100	9.39012	220599	44	***5.88004***	56678.46	126	***8.790106***	24602.93
45	***6.730059***	22179.25	101	***9.42012***	51386.37	45	5.88004	38752.32	127	***8.850107***	35000.2
46	***6.840062***	17207.79	102	9.460121	48136.83	46	***5.950041***	17270.54	128	***8.870108***	50906.22
47	***6.890063***	29366.28	103	***9.480122***	17804.56	47	***5.990042***	29546.54	129	***8.880108***	25532.96
48	***6.890063***	36586.94	104	***9.650126***	12784.05	48	***5.990042***	30448.64	130	8.890108	46589.65
49	***6.910063***	27497.62	105	***9.680126***	26674.3	49	6.020043	15942.67	131	8.96011	10071.45
50	***6.940064***	76723.88	106	9.960133	21881.43	50	6.020043	38988.21	132	***8.97011***	37402.68
51	***6.990065***	27285.36	107	***10.13014***	8469	51	6.070044	16101.47	133	***9.000111***	54540.88
52	***7.060067***	72585.46	108	***10.25014***	31231.32	52	***6.090044***	32804.31	134	***9.090113***	39652
53	***7.090067***	32675.22	109	***10.31014***	13936.16	53	6.090044	52003.64	135	***9.100113***	50113.9
54	***7.120068***	15840.17	110	10.40014	16085.49	54	***6.100045***	33023.19	136	***9.140114***	38284.98
55	***7.150069***	59125.73	111	10.58015	13552.73	55	***6.130045***	15137.4	137	9.140114	31994.32
56	7.170069	85004.53	112	11.04016	14135.49	56	6.160046	36511.18	138	***9.220116***	59752.64
						57	6.250048	129684.5	139	***9.230116***	66765.57
						58	6.260048	37798.63	140	9.340118	8235.602
						59	***6.35005***	61341.17	141	9.350119	172878.2
						60	6.360051	47140.86	142	9.350119	9117.577
						61	***6.420052***	141742.5	143	9.41012	40035.92
						62	***6.430052***	44698.85	144	***9.42012***	51386.37
						63	***6.480053***	164617.2	145	9.450121	75441.9
						64	***6.490054***	28901.02	146	***9.480122***	33363.22
						65	***6.580056***	45054.25	147	9.480122	17804.56
						66	6.610056	35334.47	148	9.480122	23274.81
						67	6.630057	42119.68	149	9.510122	22030.8
						68	6.640057	13048.85	150	9.550123	23713.48
						69	6.660058	37057.8	151	***9.650126***	12784.05
						70	6.660058	32951.46	152	***9.680126***	26674.3
						71	6.700058	27901.84	153	9.780128	34400.63
						72	6.710059	52227.2	154	9.84013	15524.08
						73	6.730059	22179.25	155	9.870131	35729.97
						74	6.840062	34357.07	156	9.890131	15081.29

75	6.860062	34460.75	157	9.950132	10344.23
76	6.890063	29366.28	158	***10.13014***	8469
77	6.890063	36586.94	159	10.14014	14839.5
78	6.910063	46103.74	160	***10.25014***	31231.32
79	6.940064	76723.88	161	***10.31014***	13936.16
80	6.970065	17363.06	162	***10.58015***	13552.73
81	6.990065	27285.36	163	10.90015	13988.37
82	7.060067	72585.46			