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Porous Polydimethylsiloxane Monolith for Protein Digestion

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The pore size distribution

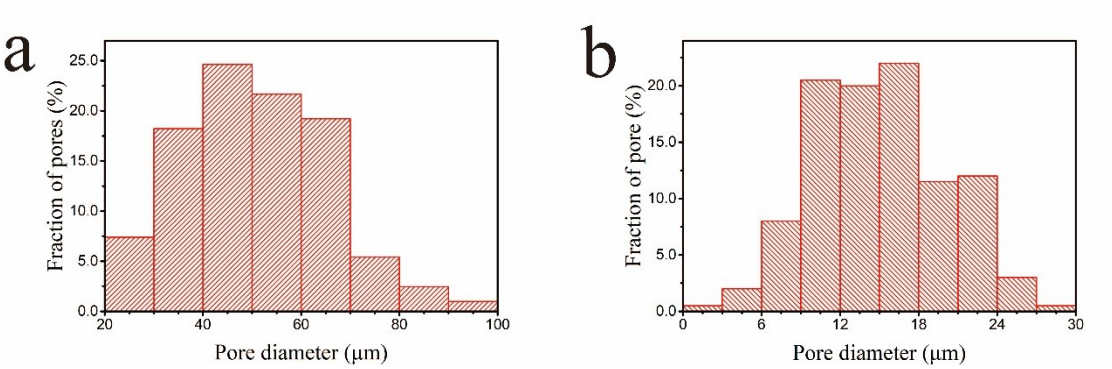

Figure S1. Histogram of pore size distribution: a) Macropore of porous PDMS monolith. b) Small interconnected pore.

The amount of trypsin immobilized on the porous PDMS monolith

The density of trypsin immobilized on the porous PDMS monolith was determined by measuring the total amount of the immobilized trypsin and the total volume of the pores insides the PDMS monolith. The amount of the trypsin was estimated through monitoring the absorbance of trypsin solution at 280 nm. Trypsin was dissolved in 50 mM Tris-HCl buffer containing 10 mM $CaCl_2$ (pH=8.5). Calibration curve was made through measuring the absorbance at 280 nm of different trypsin concentration. The amount of immobilized trypsin could be obtained by measuring the absorbance of trypsin solution at 280 nm before and after immobilization.

To estimate the total volume of the pores insides the PDMS monolith, DI water was infused into the porous PDMS monolith. The weight difference before and after the absorption of DI water was used to calculate the total volume of the pores insides the PDMS monolith.

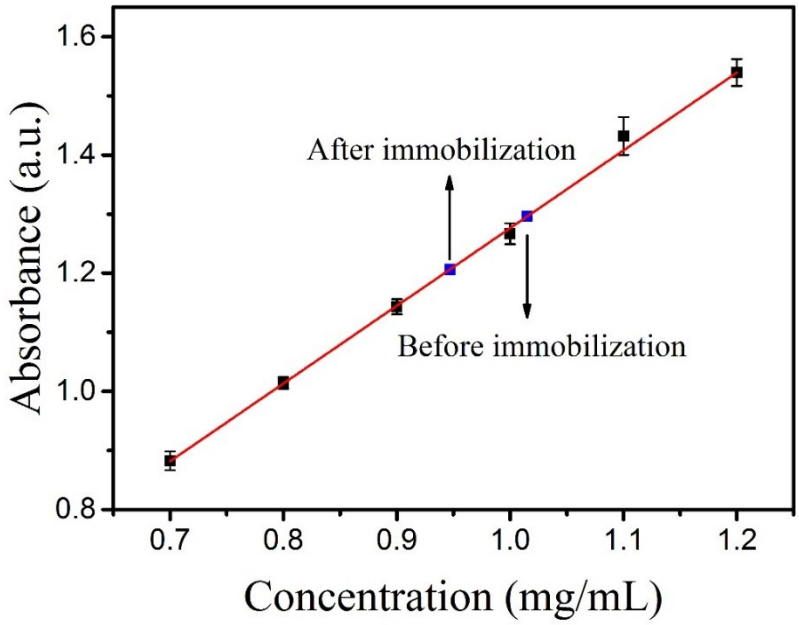

Figure S2. Calibration curve for measuring the concentration of trypsin solution. The blue data points were from the trypsin solution before and after immobilization.

The myoglobin digestion at different flow rates

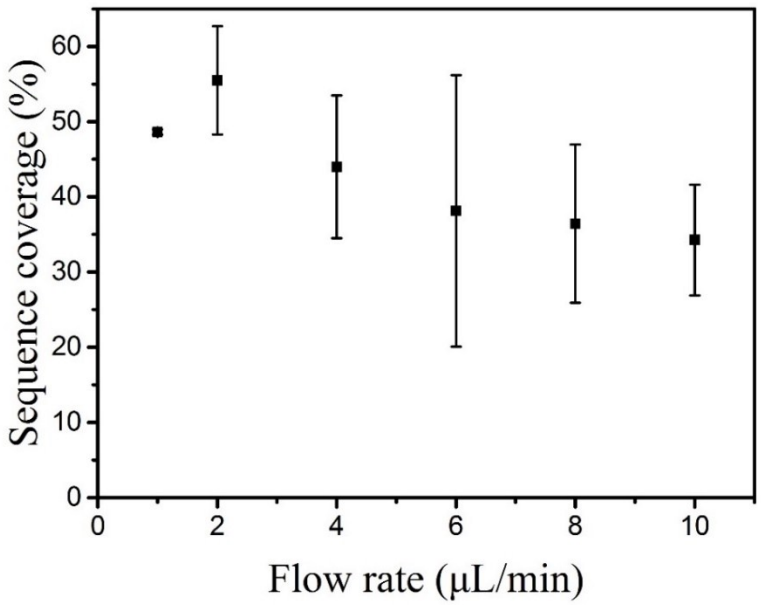

Figure S3. Protein sequence coverage of myoglobin digested by the porous PDMS monolith at different flow rates.

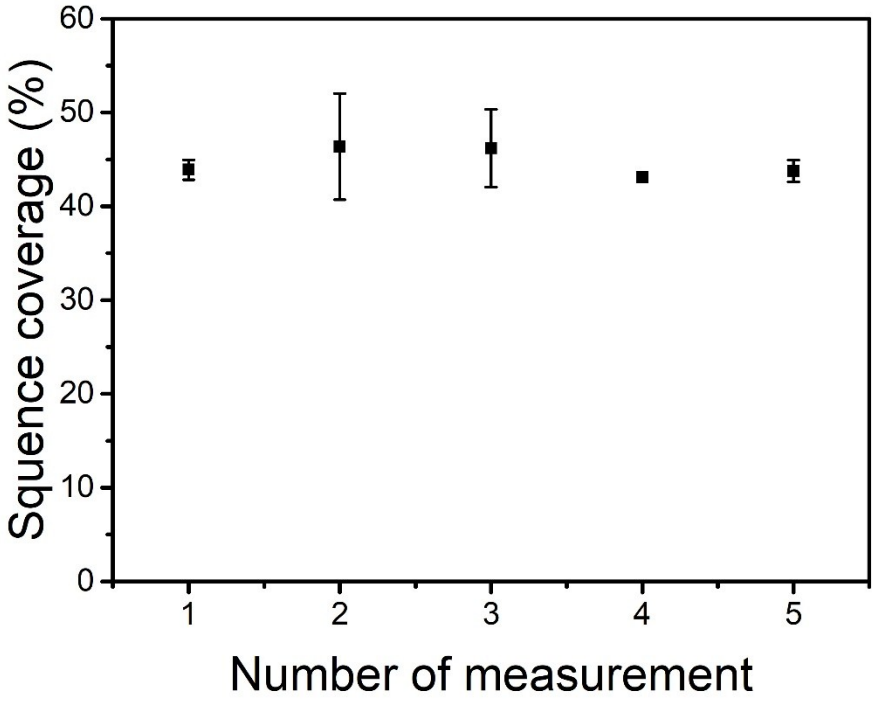

Figure S4. Protein sequence coverage of myoglobin digested by the porous PDMS monolith for five times.

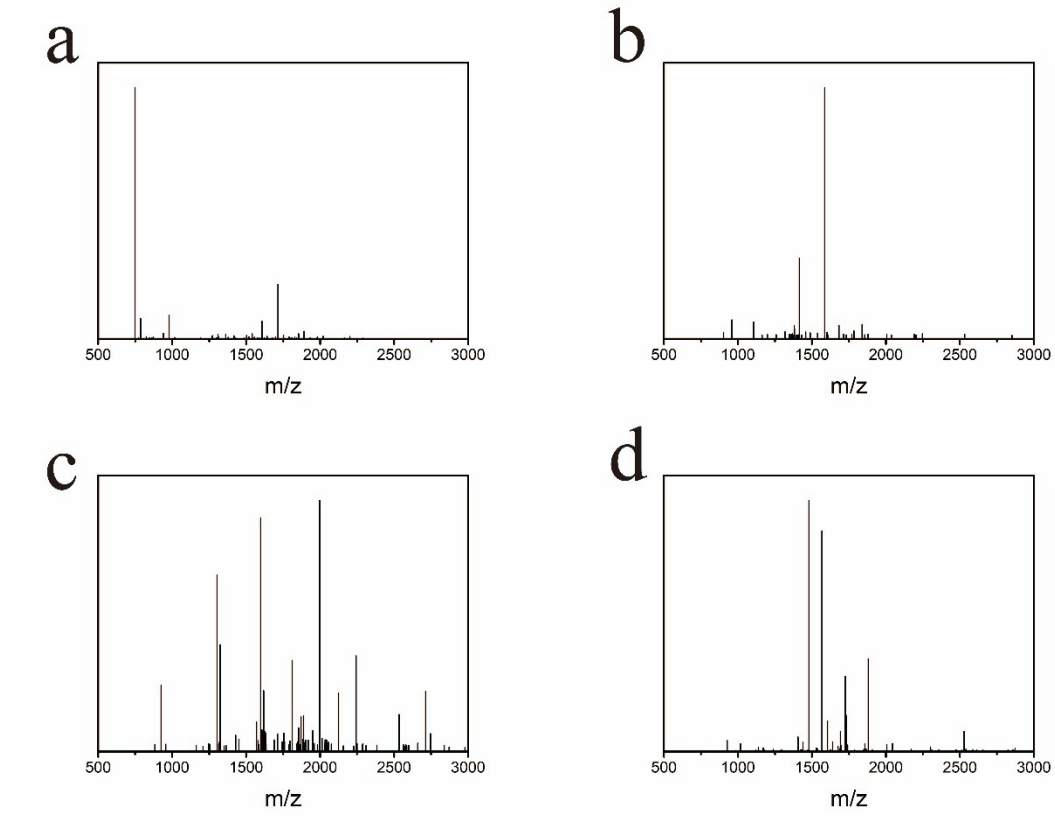

Figure S5. Mass spectra of model proteins digested by the porous PDMS monolith at batch-to-batch mode: a) Myoglobin, b) HRP, c) Amylase, d) BSA.

Database Searching

The data were searched by ProteinScape software 3.0 using MASCOT (Matrix Sciences, London, United Kingdom) as a search engine against NCBI database. Only significant hits which were defined by Mascot probability analysis and with at least one matched peptide were accepted. Target proteins can also be identified by combined (MS+MS/MS) analysis. The searches were performed with a peptide mass tolerance of 50-100 ppm, MS/MS ion mass tolerance of 0.5 Da.

Table S1. The identified peptides of myoglobin digested by the trypsin-immobilized porous PDMS monolith.

Range	m/z	Sequence	Number of miss-cleavage sites
17-31	1606.8766	K.VEADIAGHGQEVLIR.L	0
32-42	1271.6920	R.LFTGHPETLEK.F	0
64-77	1378.8639	K.HGTVVLTALGGILK.K	0
80-96	1853.9987	K.GHHEAELKPLAQSHATK.H	1
103-118	1885.0243	K.YLEFISDAIIHVLHSK.H	0
119-133	1502.6946	K.HPGDFGADAQGAMTK.A	0
134-139	748.4379	K.ALELFR.N	0
134-145	1360.7856	K.ALELFRNDIAAK.Y	1

Table S2. The identified peptides of myoglobin digested in solution for 30 minutes.

Range	m/z	Sequence	Number of miss-cleavage sites
80-97	1982.0033	K.KGHHEAEIKPLAQSHATK.H	2
81-97	1853.8823	K.GHHEAEIKPLAQSHATK.H	1
135-148	1651.7812	K.ALELFRNDIAAQYK.E	1

Table S3. The identified peptides of myoglobin digested in solution for 12 hours.

Range	m/z	Sequence	Number of miss-cleavage sites
17-31	1606.8555	K.VEADIAGHGQEVLIR.L	0
32-42	1271.6597	R.LFTGHPETLEK.F	0
64-77	1378.8365	K.HGTVVLTALGGILK.K	0
103-118	1885.0326	K.YLEFISDAIIHVLHSK.H	0
119-133	1502.6625	K.HPGDFGADAQGAMTK.A	0
134-145	1360.7539	K.ALELFRNDIAAK.Y	1

Table S4. The identified peptides of HRP digested by the trypsin-immobilized porous PDMS monolith.

Range	m/z	Sequence	Number of miss-cleavage sites
20-27	959.5091	R.DTIVNELR.S	0
76-82	803.4253	R.GFPVIDR.M	0
284-298	1586.8251	R.MGNITPLTGTQGQIR.L	0

Table S5. The identified peptides of HRP digested in solution for 12 hours.

Range	m/z	Sequence	Number of miss-cleavage sites
20-27	959.5177	R.DTIVNELR.S	0
76.82	803.4334	R.GFPVIDR.M	0
284-298	1586.8343	R.MGNITPLTGTQGQIR.L	0

Table S6. The identified peptides of Amylase digested by the trypsin-immobilized porous PDMS monolith.

Range	m/z	Sequence	Number of miss-cleavage sites
24-47	2713.4597	K.RLQNDSAYLAEHGITAVWIPPAYK.G	1
215-229	1855.9052	R.WGTWYANELQLDGFR.L	0
243-249	925.4805	R.DWVNHVR.E	0
414-437	2243.2215	R.EGDSSVANSGLAALITDGPGGAKR.M	1
443-456	1598.7819	R.QNAGETWHDITGNR.S	0

Table S7. The identified peptides of Amylase digested in solution for 12 hours.

Range	m/z	Sequence	Number of miss-cleavage sites
24-47	2713.4260	K.RLQNDSAYLAEHGITAVWIPPAYK.G	1
155-169	2036.8379	K.WHWYHFDGTDWDESR.K	0
215-229	1855.8725	R.WGTWYANELQLDGFR.L	0
243-249	925.4551	R.DWVNHVR.E	0
414-437	2243.1253	R.EGDSSVANSGLAALITDGPGGAKR.M	1
443-456	1598.7212	R.QNAGETWHDITGNR.S	0

Table S8. The identified peptides of BSA digested by the trypsin-immobilized porous PDMS monolith.

Range	m/z	Sequence	Number of miss-cleavage sites
144-159	2045.0237	R.RHPYFYAPELLYYANK.Y	1
323-335	1567.7444	K.DAFLGSFLYEYSR.R	0
336-347	1439.8107	R.RHPEYAVSVLLR.L	1
389-409	2529.2208	K.QNCDQFEKLGEYGFQNALIVR.Y	1
397-409	1479.7919	K.LGEYGFQNALIVR.Y	0
413-427	1639.9385	R.KVPQVSTPTLVEVSR.S	1
445-458	1724.8302	R.MPCTEDYLSLILNR.L	0
484-499	1880.9247	R.RPCFSALTPDETYVPK.A	1

Table S9. The identified peptides of BSA digested in solution for 12 hours.

Range	m/z	Sequence	Number of miss-cleavage sites
137-143	927.4848	K.YLYEIAR.R	0
323-335	1567.7387	K.DAFLGSFLYEYSR.R	0
336-347	1439.8015	R.RHPEYAVSVLLR.L	1
397-409	1479.7934	K.LGEYGFQNALIVR.Y	0
413-427	1639.9361	R.KVPQVSTPTLVEVSR.S	1
445-458	1724.8358	R.MPCTEDYLSLILNR.L	0
484-499	1880.9215	R.RPCFSALTPDETYVPK.A	1

Table S10. Protein digestion results by the porous PDMS monolith and in-solution digestion

	Porous PDMS monolith digestion			In-solution digestion		
	Sequence Coverage (%)	Identified peptides numbers	Miss-cleavage peptides proportion	Sequence Coverage (%)	Identified peptides numbers	Miss-cleavage peptides proportion
Myoglobin	55.5	8	0.25	48.0	6	0.16
HRP	9.8	3	0	9.7	3	0
Amylase	17.4	5	0.40	20.5	6	0.33
BSA	18.2	8	0.62	15.4	7	0.42