Low back-pressure hierarchically structured multichannel microfluidic bioreactors for rapid protein digestion - proof of concept

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SUPPORTING INFORMATION

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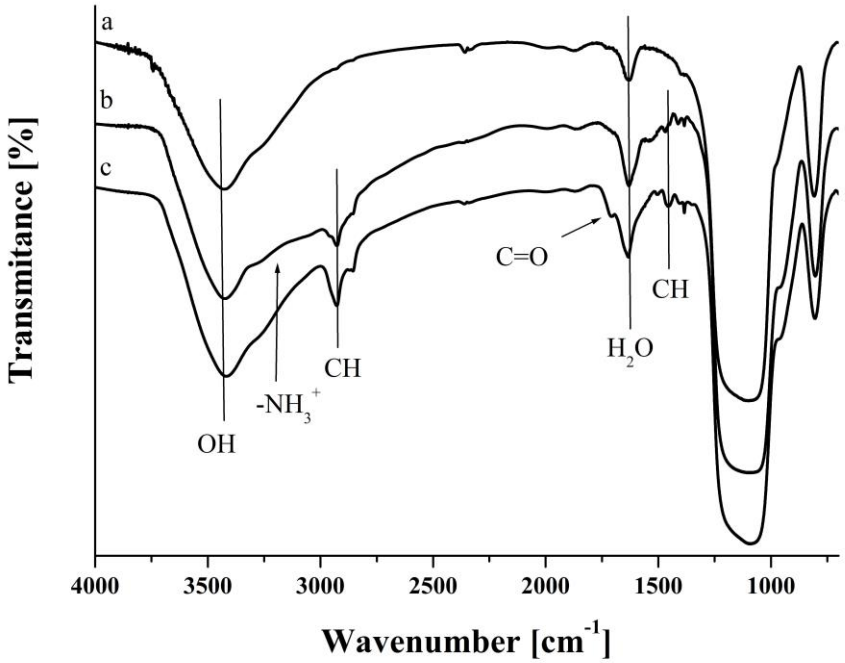

Figure S1. FT-IR spectra of powdered MH1: pristine (a), activated with APTS (b), and activated with APTS and GLA (c).

FTIR spectra show (Fig. S-1) that all samples possess bands at ca. 1220, 1070, 794, and 471 cm^{-1} that can be assigned to the typical Si–O–Si stretching and bending vibrations of condensed silica networks while peaks at 960 cm^{-1} correspond to non-condensed silanol groups [1, 2]. The broad band at 3400 cm^{-1} and the strong peak around 1630 cm^{-1} are due to stretching vibrations of silanol group and bending vibrations of adsorbed H_2O, respectively [2]. On the other hand, a broad absorbance at 2850–3000 cm^{-1} and a weak peak at 1450–1470 cm^{-1} seen in both modified samples (Fig. S-1 b, c) can be attributed to the stretching and bending vibrations of methylene groups [2], further confirming the incorporation of organic groups into the silicate framework. A broad band at 2700-3400 cm^{-1} can be attributed to the NH^{3+} stretching vibration [1]. GLA-activated silica (Fig. S-1 c) shows a peak at 1720 cm^{-1} which corresponds to the carbonyl groups of the free aldehyde end of GLA [3].

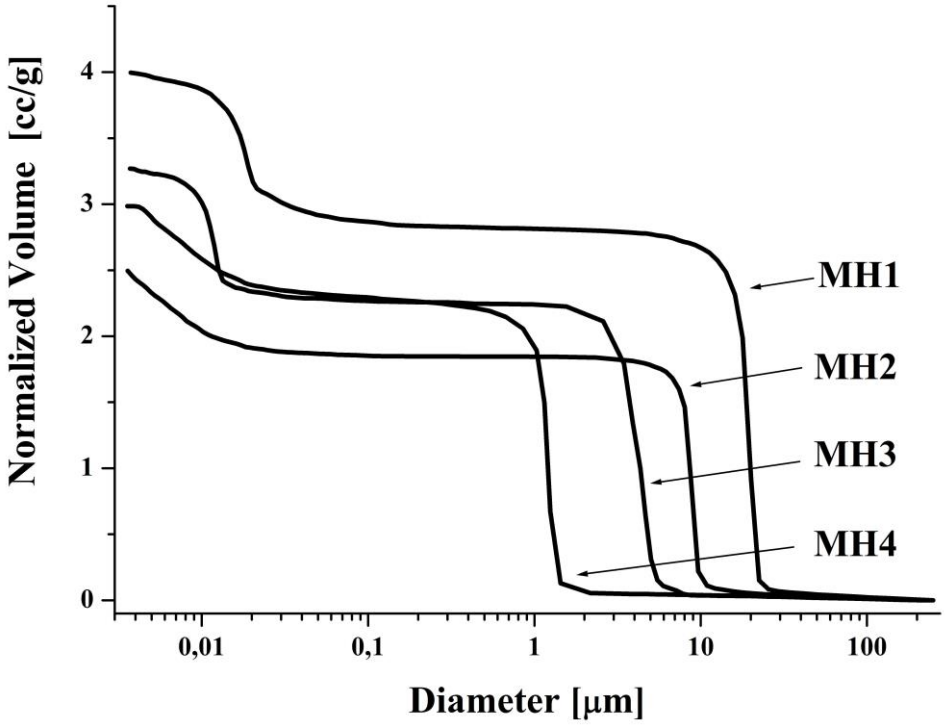

Figure S2. Cumulative pore volume distributions obtained from mercury intrusion measurements.

Table S1. Database searching results of myoglobin digested in a trypsin-functionalized MH1 reactor at different flow rates (digestion time of 24, 7.2 and 2.4 min).

Position	Mass (Da)	Peptide sequence	Flow rate [mL/min] 0.03	0.1	0.3
1 - 16	1816.08	-.GLSDGEWQQVLNVWGK.V		+	
1 - 31	3403.82	-.GLSDGEWQQVLNVWGKVEADIAGHGQEVLIR.L	+		+
17 - 31	1606.87	K.VEADIAGHGQEVLIR.L	+	+	+
32 – 42	1271.66	R.LFTGHPETLEK.F	+	+	
32 - 45	1661.97	R.LFTGHPETLEKFDK.F	+	+	
48 – 56	1086.55	K.HLKTEAEMK.A	+		
64 – 77	1378.86	K.HGTVVLTALGGILK.K	+	+	+
64 – 78	1506.95	K.HGTVVLTALGGILKK.K	+	+	
79 – 96	1982.09	K.KGHHEAELKPLAQSHATK.H	+	+	
80 – 96	1853.99	K.GHHEAELKPLAQSHATK.H	+	+	+
80 – 98	2119.20	K.GHHEAELKPLAQSHATKHK.I		+	
103 – 118	1885.04	K.YLEFISDAIIHVLHSK.H	+	+	
103 – 133	3368.76	K.YLEFISDAIIHVLHSKHPGDFGADAQGAMTK.A	+	+	
119 – 133	1502.68	K.HPGDFGADAQGAMTK.A	+		
134 – 145	1360.76	K.ALELFRNDIAAK.Y	+	+	+
146 - 153	941.49	K.YKELGFQG.-	+		+

Table S2. Database searching results of cytochrome c digested in a trypsin-functionalized MH1 reactor at different flow rates (digestion time of 24, 7.2 and 2.4 min).

Position	Mass (Da)	Peptide sequence	Flow rate [mL/min] 0.03	0.1	0.3
26 – 38	1433.76	K.HKTGPNLHGLFGR.K	+	+	+
28 – 38	1168.62	K.TGPNLHGLFGR.K	+	+	+
28 – 39	1296.69	K.TGPNLHGLFGRK.T	+	+	+
39 – 53	1584.75	R.KTGQAPGFSYTDANK.N	+	+	+
40 – 53	1456. 67	K.TGQAPGFSYTDANK.N	+	+	+
40 – 55	1698.78	K.TGQAPGFSYTDANKNK.G	+		+
56 – 72	2009.88	K.GITWGEETLMEYLENPK.K	+		
56 – 73	2137.95	K.GITWGEETLMEYLENPKK.Y	+	+	
73 – 79	806.47	K.KYIPGTK.M	+		
80 – 87	907.59	K.MIFAGIKK.K	+		
89 – 99	1306.69	K.GEREDLIAYLK.K	+		+
92 - 99	964.55	R.EDLIAYLK.K	+		

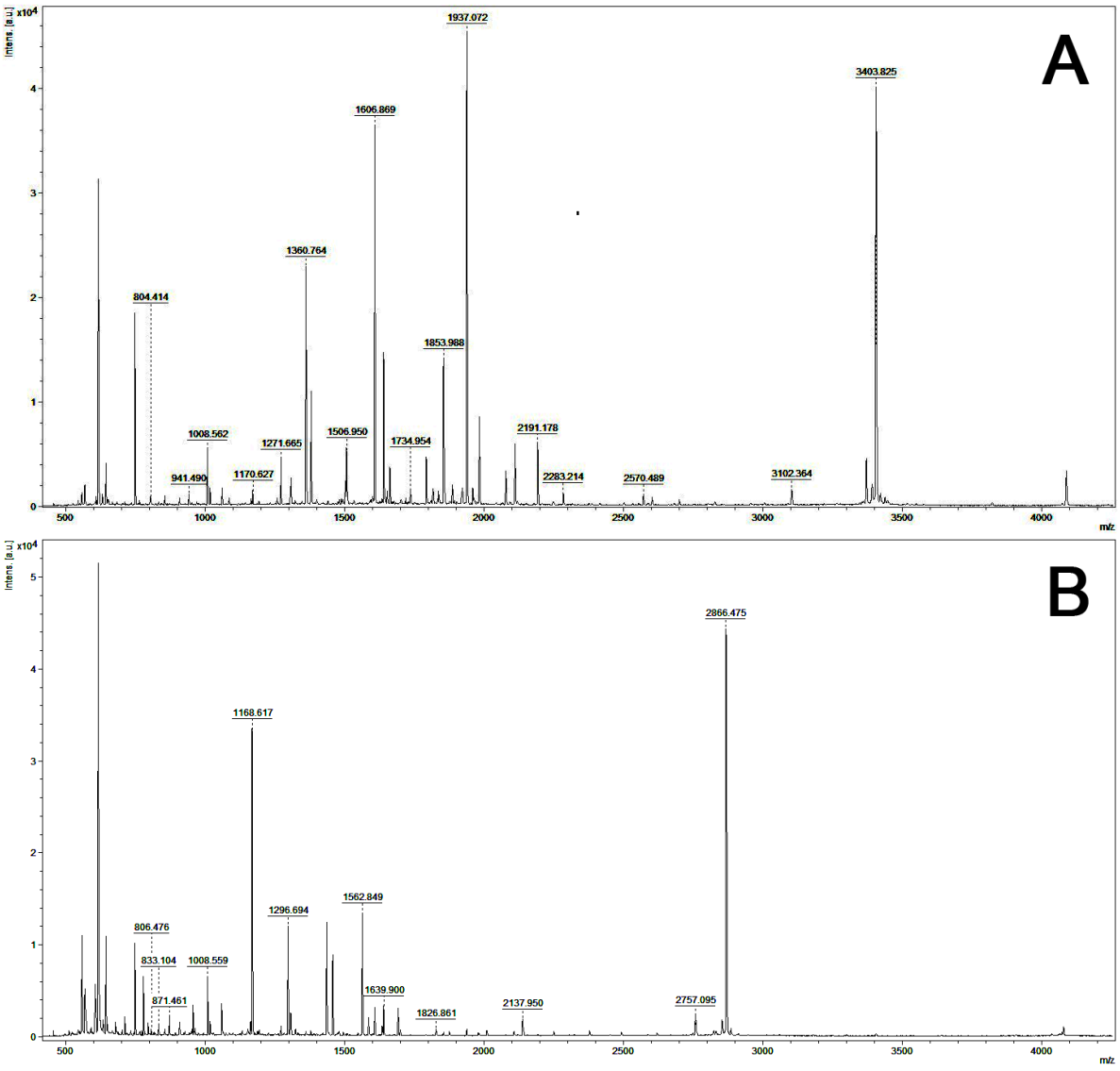

Figure S3. MALDI-TOF/TOF mass spectra of the digest of (A) myoglobin and (B) cytochrome c obtained using a MH1 bioreactor at flow rate of 0.03 mL/min.

Ma et al. [4] observed a decrease in sequence coverage with an increase in the molecular weight of the protein for the in-solution digestion of cytochrome c (51 %) and myoglobin (42 %), but this trend was reversed for on-column digestion using hybrid silica (coverage of 47 % and 52 % for cytochrom c and myoglobin, respectively). In their view, this trend is not valid for all proteins, and it can be ascribed to interactions between the resulting peptides in the digest mixture. In our opinion, of critical importance are the values of isoelectric point of the protein to be digested, that of trypsin and also of the siliceous support, which either facilitate or impede their contact. The isoelectric point of functionalized sol-gel silica is in the range of

2-5 [5], and 10.5, 9.6 and 7.4 for trypsin, cytochrome c and myoglobin, respectively [4, 6]. The continuous protein digestions were carried out at pH 7.8. Thus, in this situation both silica and myoglobin were negatively charged, whereas trypsin and cytochrome c possessed positive charges. Therefore, from the electrostatic interactions, a much larger affinity could be expected between trypsin and myoglobin than between trypsin and cytochrome c. Additionally, the positively charged cytochrome c was likely to bind to the negatively charged surface of silica monolith [7], while myoglobin was not likely to undergo significant electrostatic binding. The complex interactions, which followed had a pronounced effect on the efficacy of the protein digestion.

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