Electronic Supplementary Information for

Limited Proteolysis in Porous Membrane Reactors Containing Immobilized Trypsin

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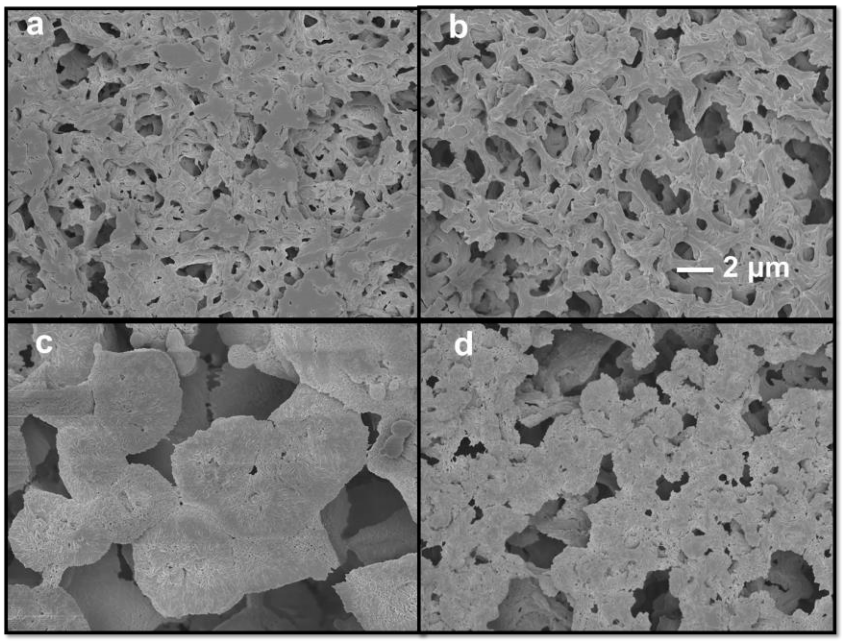

Figure S-1. SEM images (a-c) of membranes modified with trypsin using electrostatic adsorption. Nominal pore sizes prior to modification were (a) 0.45 µm, (b) 1.2 µm, and (c) 5.0 µm. Image (d) shows a 1.2 µm membrane modified with trypsin covalently immobilized to adsorbed PAA. The scale bar is the same for all SEM images.

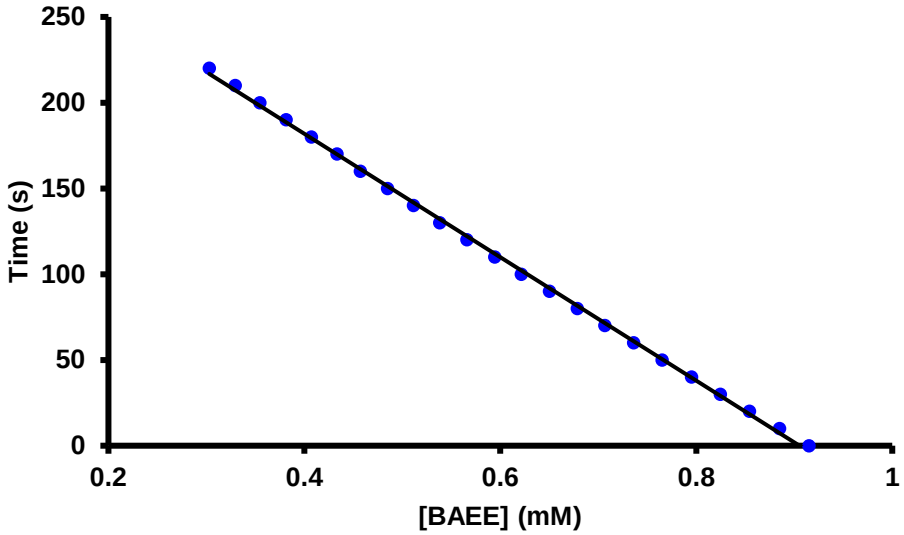

Figure S-2. In-solution digestion time as a function of the evolving (declining) BAEE concentration. The initial BAEE concentration in the solution was 1 mM, but this value decreased during the brief (5 s) mixing period. The line is a fit to the data using the equation below.

$$t \cong \frac{[S]_0 - [S]}{V_{max}}$$

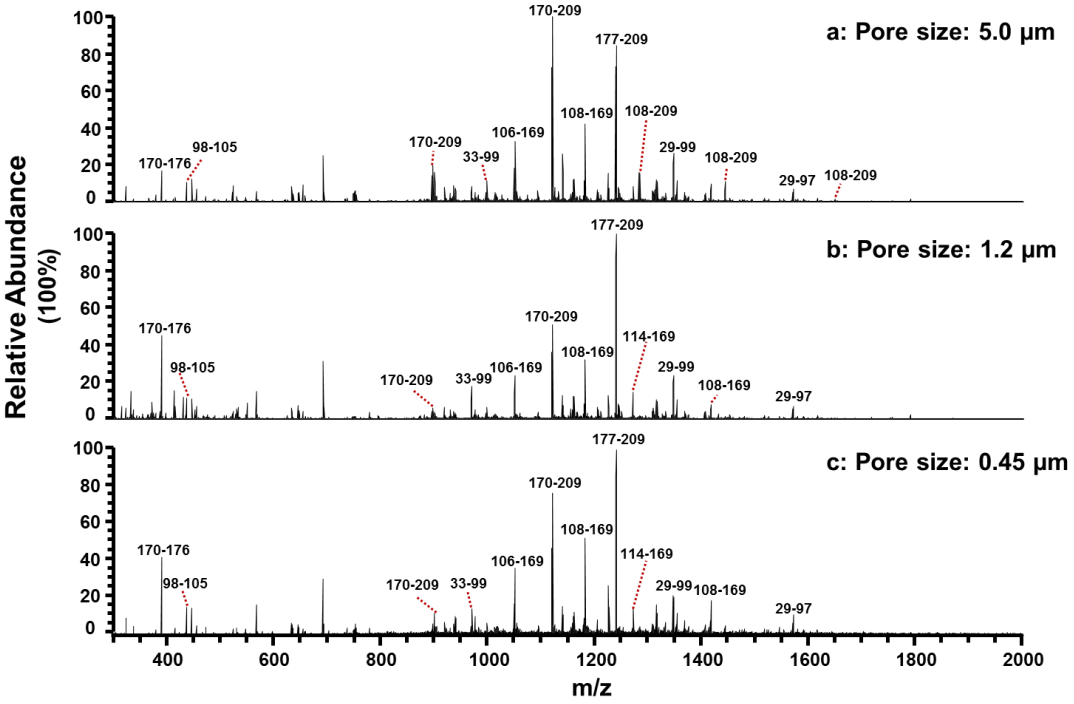

Figure S-3. ESI-Orbitrap mass spectra of β-casein digested in trypsin-containing membranes (electrostatic immobilization) with pore sizes of (a) 5.0 µm, (b) 1.2 µm and (c) 0.45 µm. The flow rate through the membranes was 120 mL/h (residence time of 3.3 ms).

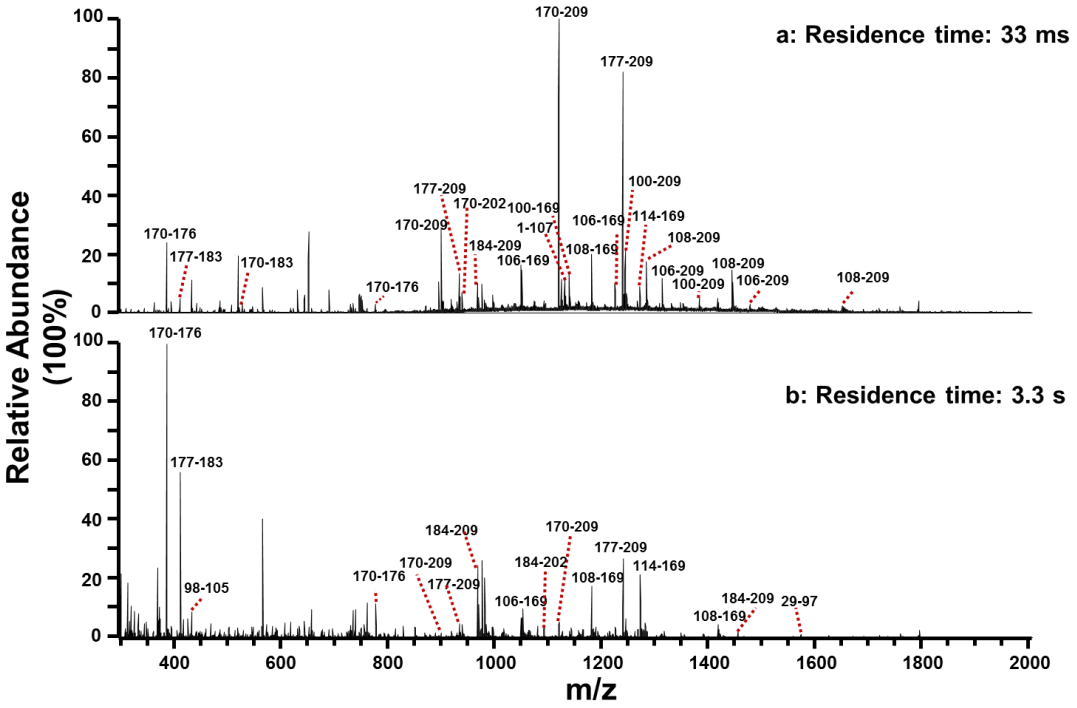

Figure S-4. ESI-Orbitrap mass spectra of β-casein digested in trypsin-containing membranes (covalent immobilization to PAA, pore sizes of 1.2 µm) using residence times of (a) 33 ms and (b) 3.3 s.

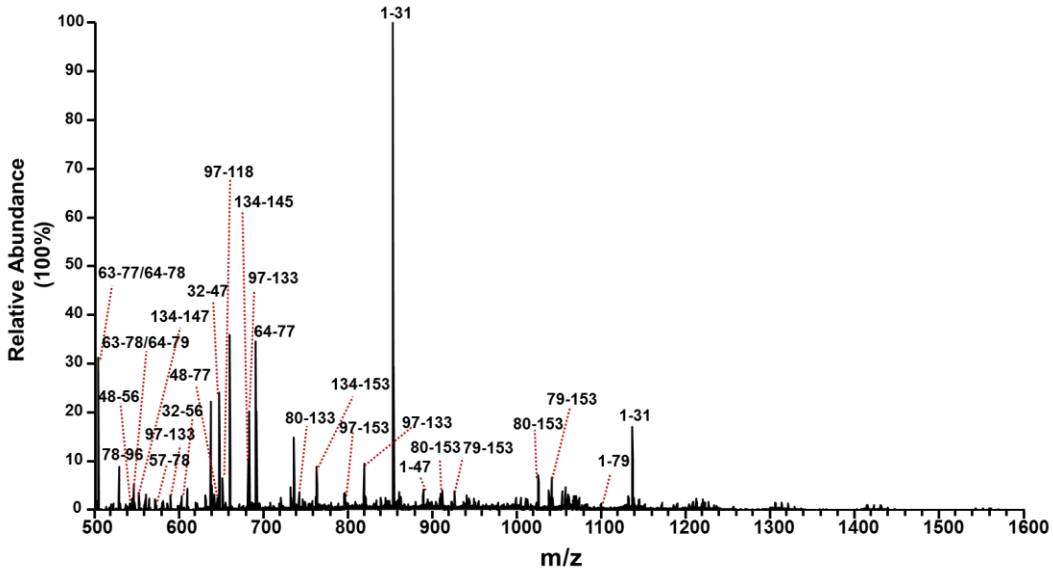

Figure S-5. Electrospray ionization mass spectrum of apomyoglobin digested in a membrane (1.2 μm pores) containing electrostatically immobilized trypsin. The flow rate through the membrane was 120 mL/h (residence time of 3.3 ms).

Table S-1. Masses and sequences of peptides detected during MS analysis of an in-membrane digest of apomyoglobin. Digestion employed a residence time of 3.3 ms, and a membrane (1.2 μm pores) containing electrostatically immobilized trypsin.

$[M+H]^+$	Sequence
8777.77	1-79
8312.36	79-153
8184.28	80-153
6349.34	97-153
5920.09	80-133
5321.77	1-47
4085.14	97-133
3776.00	32-56
3403.76	1-31
3345.89	48-78
3004.60	32-56
2283.00	134-153
2278.35	57-78
2110.16	78-96
1982.08	79-96
1937.02	32-47
1857.97	48-63
1853.96	80-96
1651.93	134-147
1635.03	63-78/64-79
1553.80	140-153
1506.94	63-77/64-78
1378.84	64-77
1360.76	134-145
1271.68	32-42
1086.58	48-56
941.48	146-153
790.44	57-63
748.44	134-139

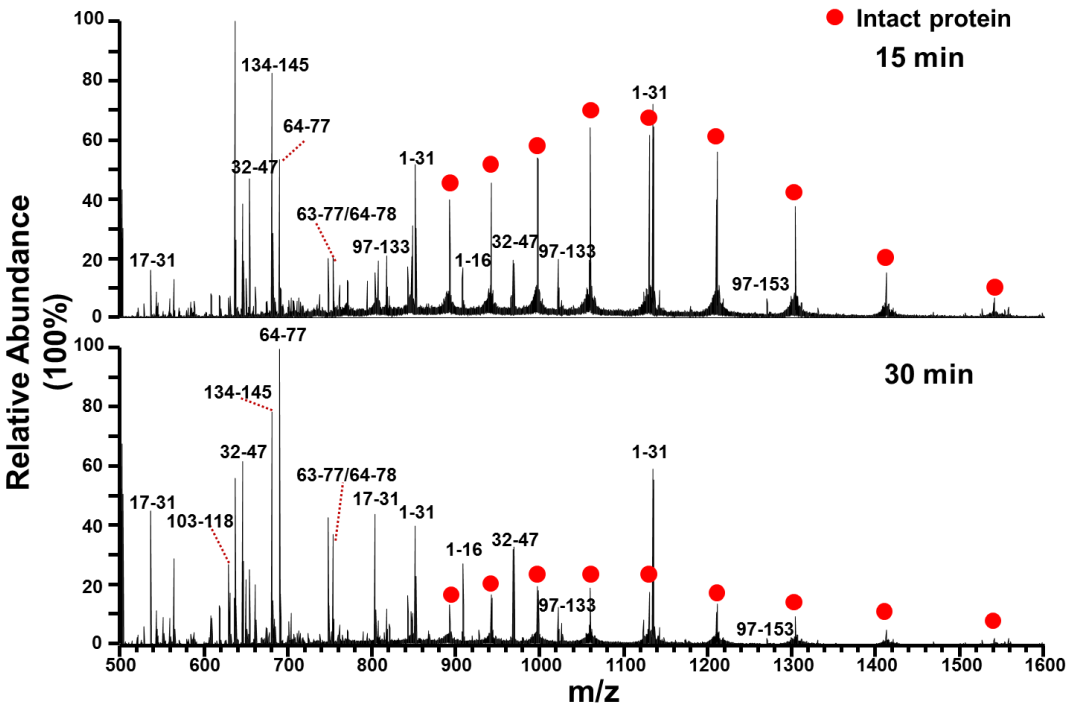

Figure S-6. Electrospray ionization mass spectra of apomyoglobin digested in solution for (a) 15 min and (b) 30 min with a trypsin to apomyoglobin ratio of 1:20. Red circles indicate signals from intact apomyoglobin.

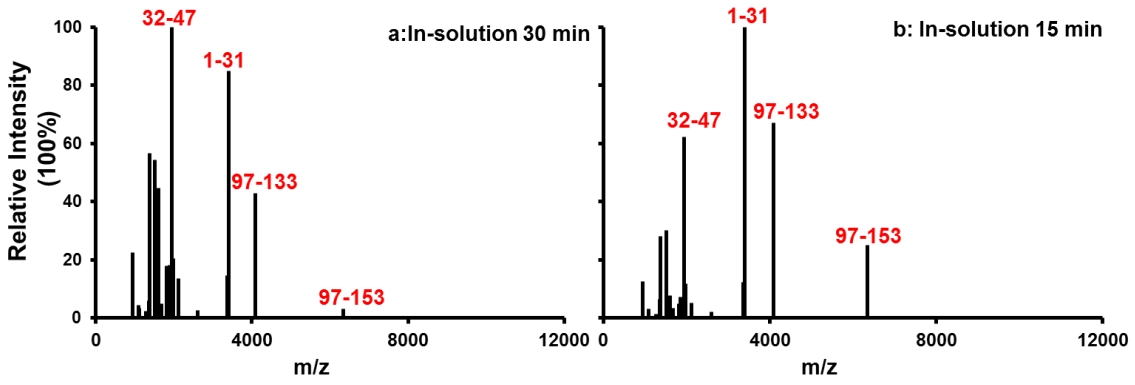

Figure S-7. Manually deconvoluted mass spectra of apomyoglobin digested in solution for (a) 30 min and (b) 15 min with a trypsin to protein ratio of 1:20. Figure S-6 shows the original spectra. The mass of the intact protein lies outside the plotted range.

Table S-2. Masses and sequences of peptides detected during MS analysis of a 15-min in-solution digest of apomyoglobin.

$[M+H]^+$	Sequence
6348.35	97-153
4085.16	97-133
3403.75	1-31
3368.68	103-133
2601.5	97-118
2110.16	78-96
1982.08	79-96
1937.04	32-47
1885.03	103-118
1853.90	80-96
1815.90	1-16
1635.04	63-78/64-79
1606.87	17-31
1506.94	63-77/64-78
1378.84	64-77
1360.78	134-145
1271.68	32-42
1086.56	48-56
941.48	146-153

Table S-3. Masses and sequences of peptides detected during MS analysis of a 30-min in-solution digest of apomyoglobin.

$[M+H]^+$	Sequence
6348.35	97-153
4085.16	97-133
3403.75	1-31
3368.68	103-133
2601.5	97-118
2110.16	78-96
1982.08	79-96
1937.04	32-47
1885.03	103-118
1853.90	80-96
1815.90	1-16
1635.04	63-78/64-79
1606.87	17-31
1506.94	63-77/64-78
1378.84	64-77
1360.78	134-145
1271.68	32-42
1102.56	48-56 ^{55}M Oxidation
1086.56	48-56
941.48	146-153

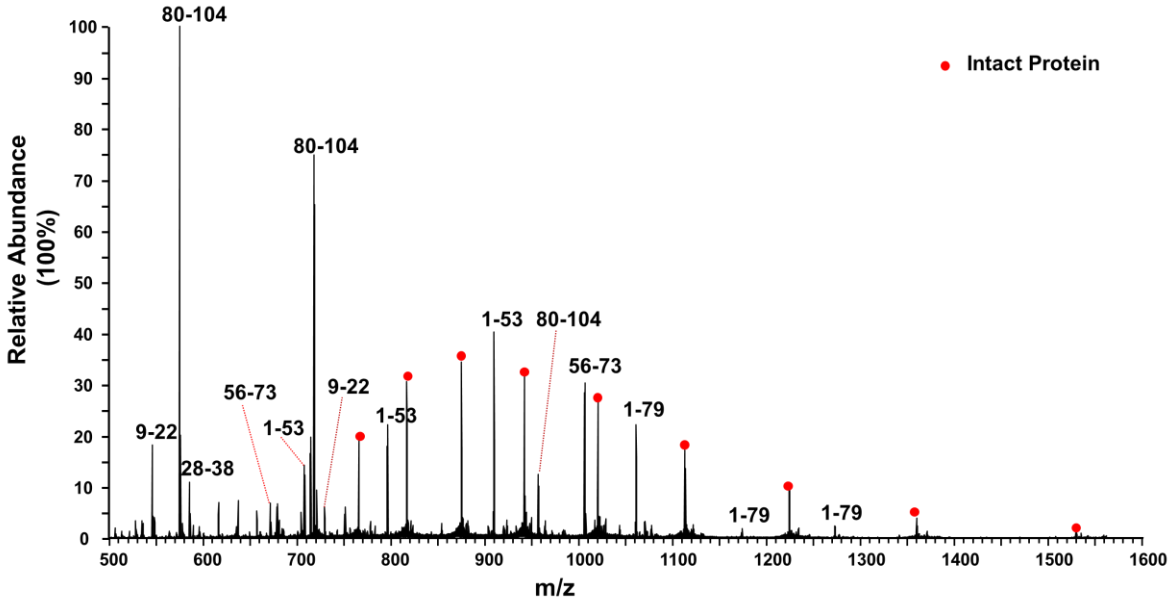

Figure S-8. Electrospray ionization mass spectrum of cytochrome-c digested in a membrane (1.2 μm pores) containing electrostatically immobilized trypsin. The flow rate through the membrane was 12 mL/h (residence time of 33 ms). Red circles indicate signals from intact cytochrome-c.

Table S-4. Masses and sequences of peptides detected during MS analysis of an in-membrane digest of cytochrome-c. Digestion employed a residence time of 33 ms, and a membrane (1.2 μm pores) containing electrostatically immobilized trypsin.

$[M+H]^+$	Amino Acids
2866.60	80-104
9376.72	1-79
1168.63	28-38
1633.63	9-22
4909.33	1-39
6364.12	1-53
779.46	80-86
2138.06	56-73
964.54	92-99
678.38	74-79
634.40	9-13