SUPPORTING INFORMATION

Pepsin immobilized on high-strength hybrid particles for continuous flow online digestion at 10,000 psi

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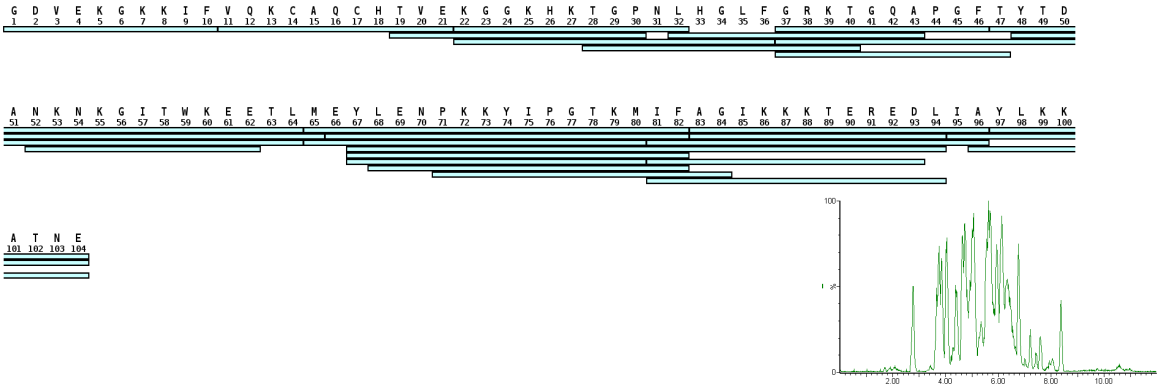
Six supporting figures, Figure S1-S6.

A. POROS Pepsin Column : 100 % coverage, 29 reproducible peptic peptides, N=3



B. BEH Pepsin Column : 100% coverage, 34 reproducible peptic peptides, N=3

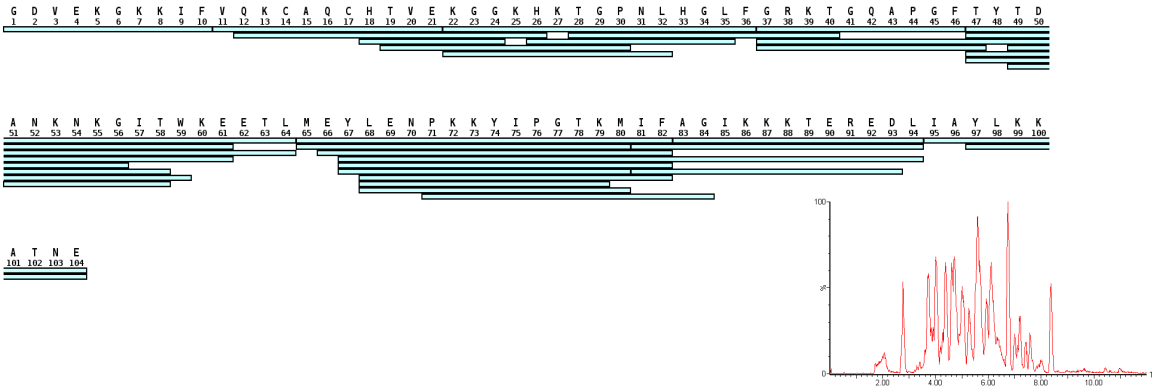
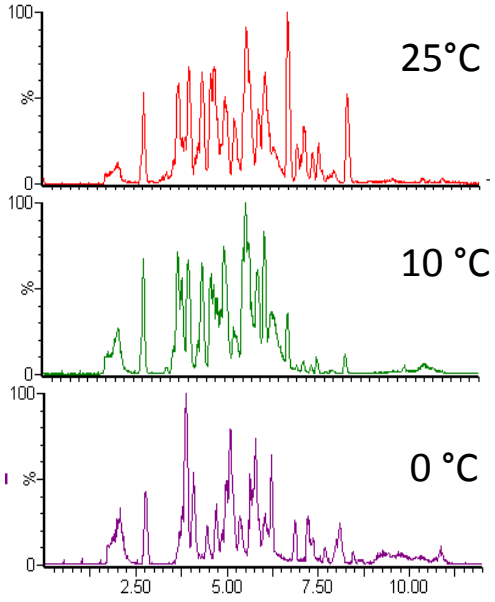


Figure S1. Comparison of cytochrome c peptide maps from online pepsin digestion using a POROS column (A) and BEH column (B) of the same column dimensions, 2.1x30 mm. 50 pmol of cytochrome c were injected at 25 °C and flowed through the column at a pressure of 950-1000 psi for 3 minutes at 100 µL/min. Reproducible peptides were found at least twice in triplicate runs. The chromatographic separations of each digestion are shown in the insets.

A.



B.

Digestion Temperature	25 °C	10 °C	0 °C
Cytochrome c % coverage	100 %	99 %	63 %
# of reproducible peptides	34	34	8

Figure S2. Comparison of cytochrome c sequence coverage (%) from online pepsin digestions using a BEH column at various digestion temperatures. (A) Chromatographic separations of cytochrome c peptides are shown at digestion temperatures of 0, 10 and 25 °C. The % coverage and the number of reproducible peptides found in each cytochrome c digestion are summarized in the table (B). Reproducible peptides were found at least twice in triplicate runs.

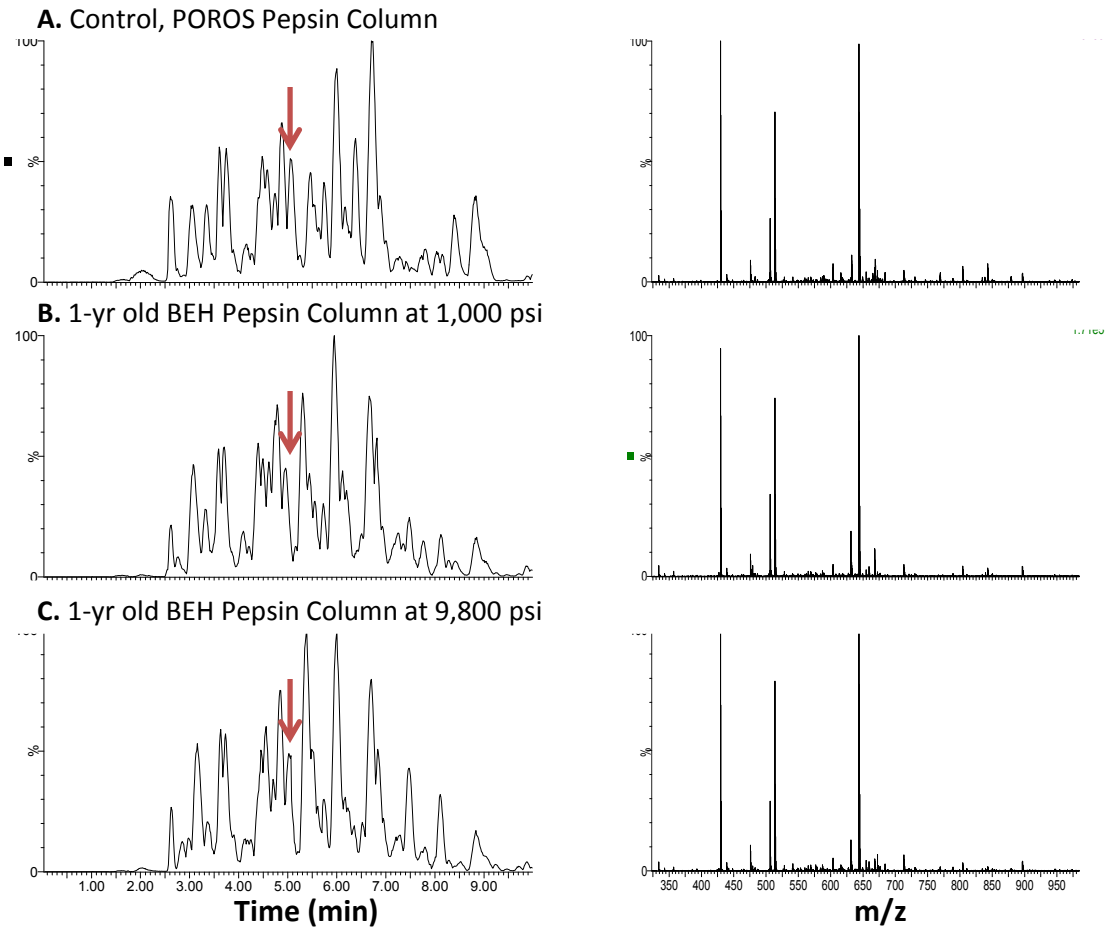
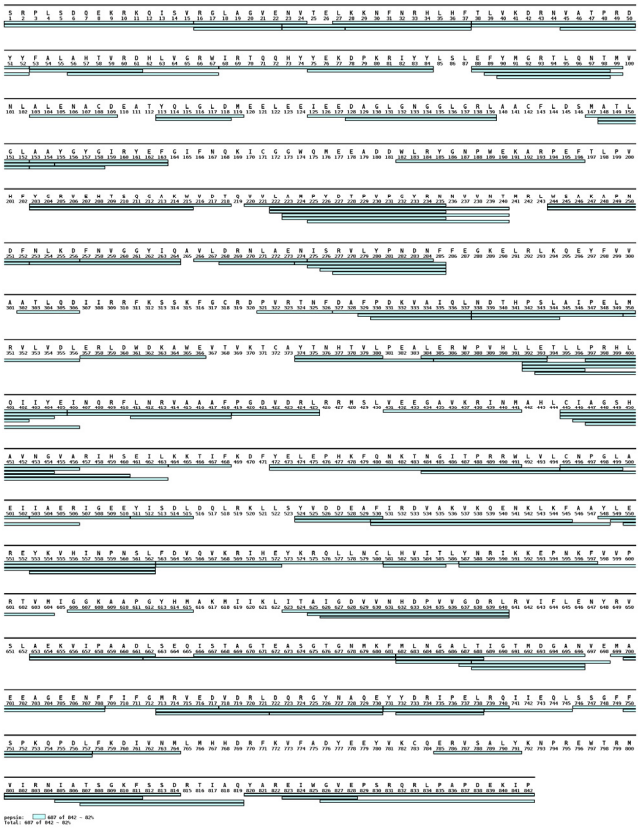


Figure S3. Comparison of IgG digestions using a POROS pepsin column (A) and a 1 year-old BEH column at 1,000 psi (B) and 9,800 psi (C). The same peptic peptides eluting at 5.2 min (indicated by the red arrow) were repeatedly produced in all of the conditions and confirmed with their mass spectra (panels on the right).

A. 9,800 psi



B. 950 psi

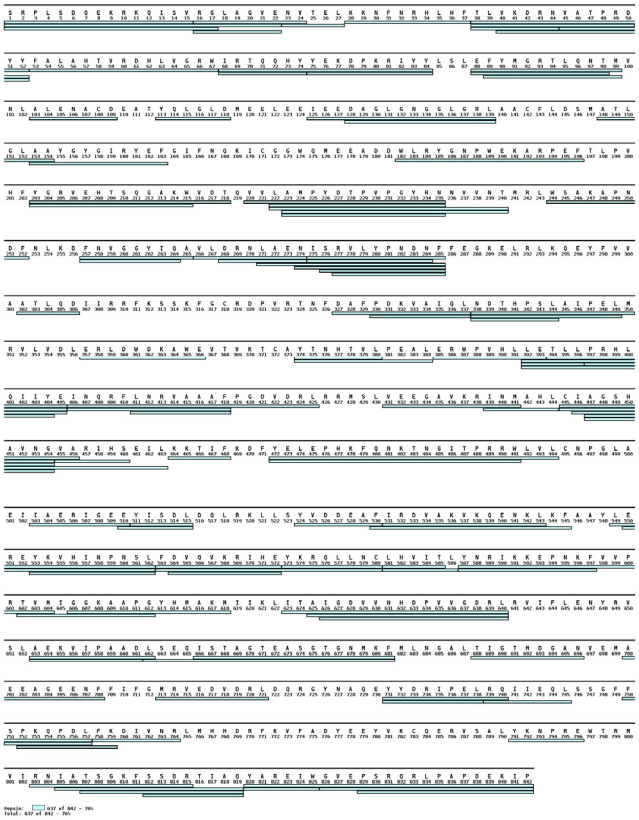


Figure S4. (A) Entire phosphorylase b map comparison at high (A) and low (B) pressure. These maps were made with MSTools, Kavan, D. and Man, P. (2011) *Int. J. Mass Spectrom.* **302**: 53-58.

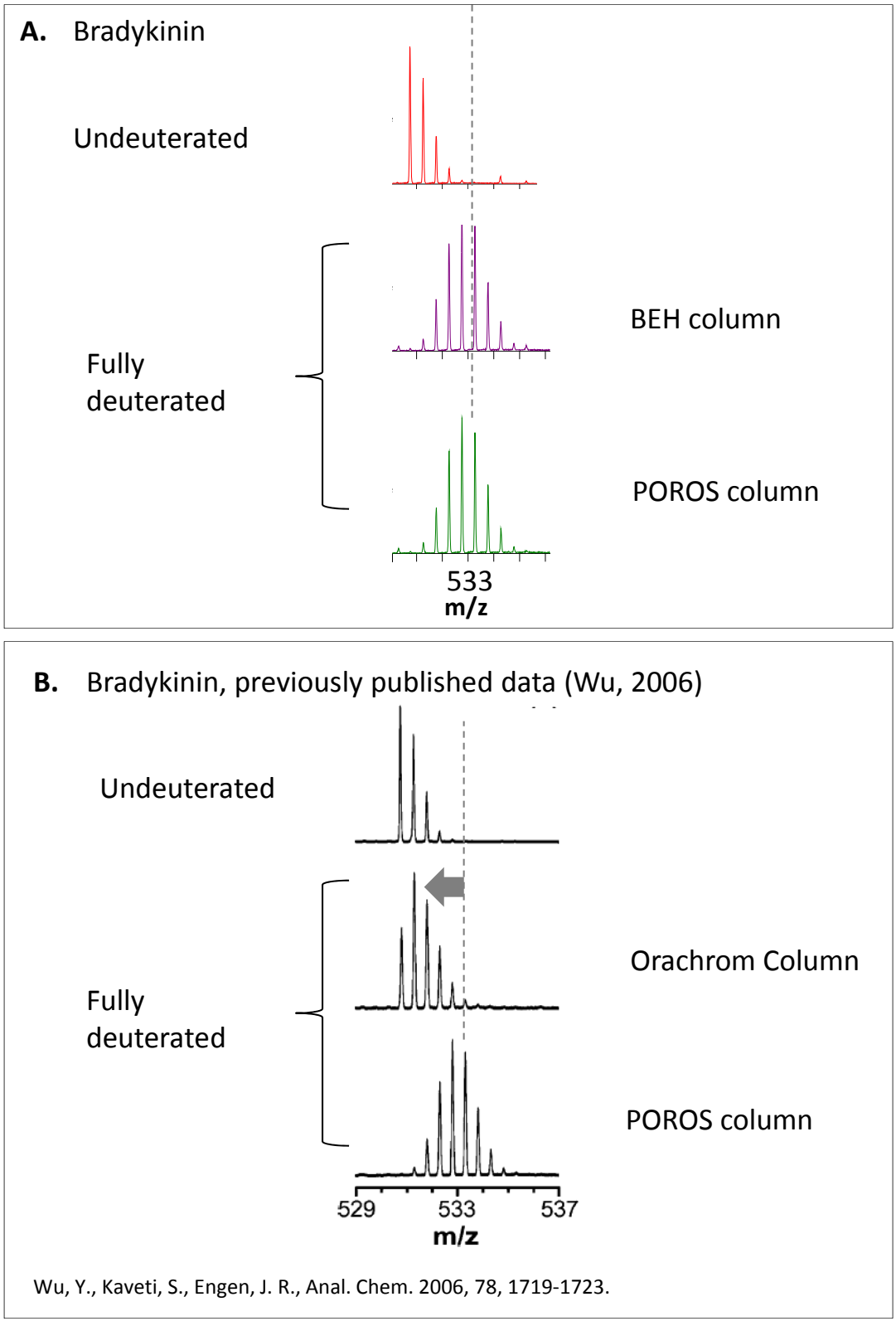


Figure S5. (A) Comparison of deuterium loss from fully deuterated bradykinin peptide using POROS and BEH pepsin columns. The results in (A) are compared with previously published data (B) of the same peptide under the same experimental conditions.

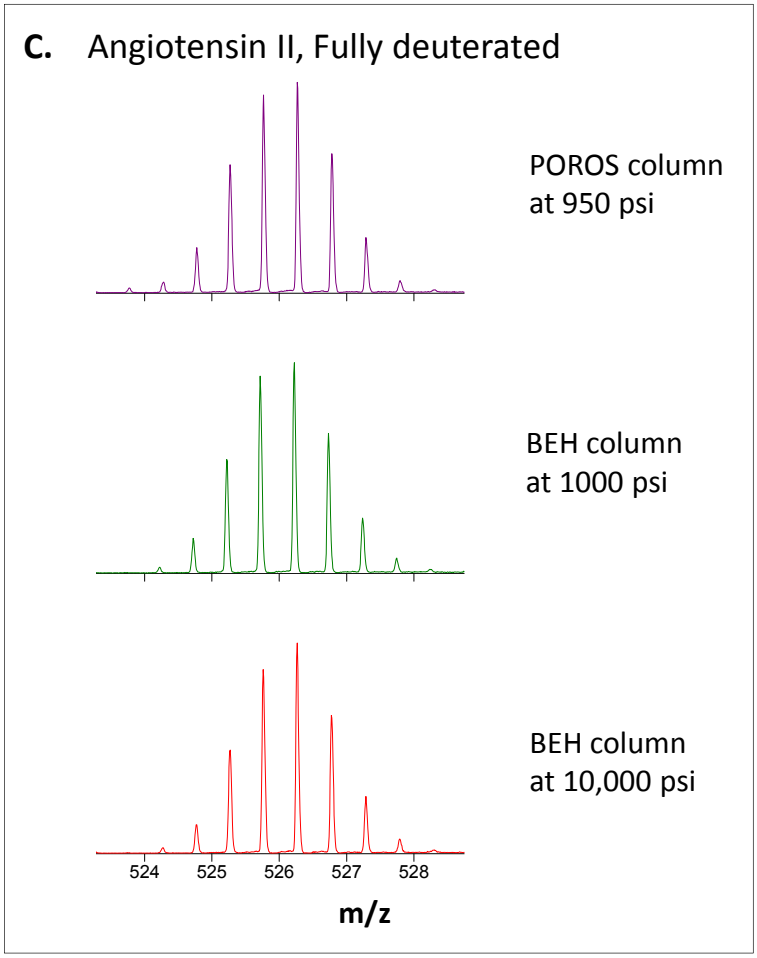


Figure S5 (continued). (C) Comparison of deuterium loss from fully deuterated angiotensin II peptide using POROS and BEH pepsin columns at low and elevated pressure. The results shown that there is no significant difference in back-exchange between low and elevated pressure digestion with BEH pepsin particles.

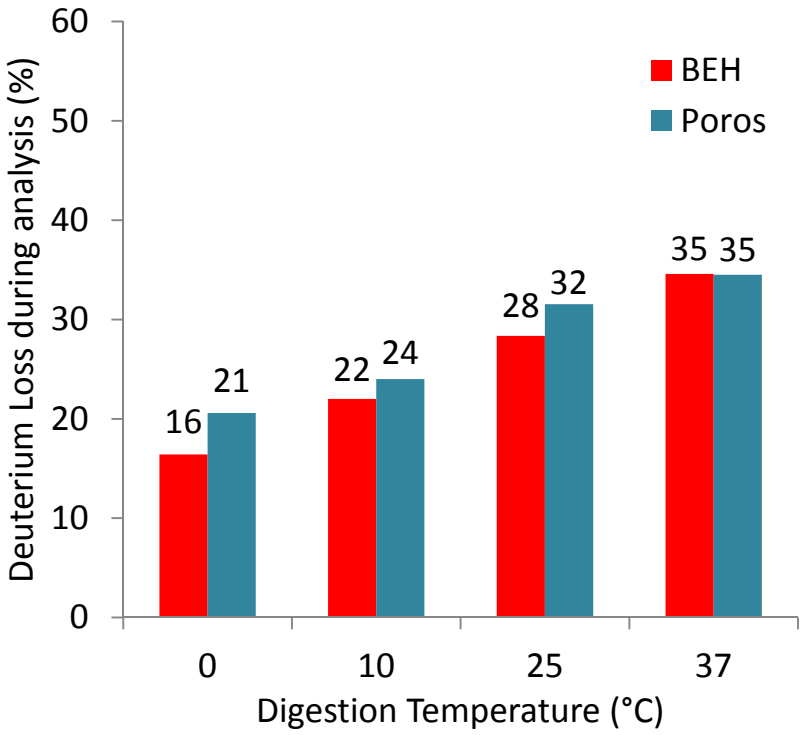


Figure S6. Comparison of deuterium loss of totally deuterated bradykinin for BEH and POROS columns a different digestion temperatures. All conditions other than digestion temperature were held constant.