Electroblotting through a tryptic membrane for LC-MS/MS analysis of proteins separated in electrophoretic gels

Bickner, A. N.[a], Champion, M. M.[a], Hummon, A. B.[b], Bruening, M. L.[a,c]

a. Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, United States
b. Department of Chemistry and Biochemistry and the Comprehensive Cancer Center, The Ohio State University, Columbus OH 43210
c. Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, Indiana 46556, United States

Supporting Information

Table of Contents

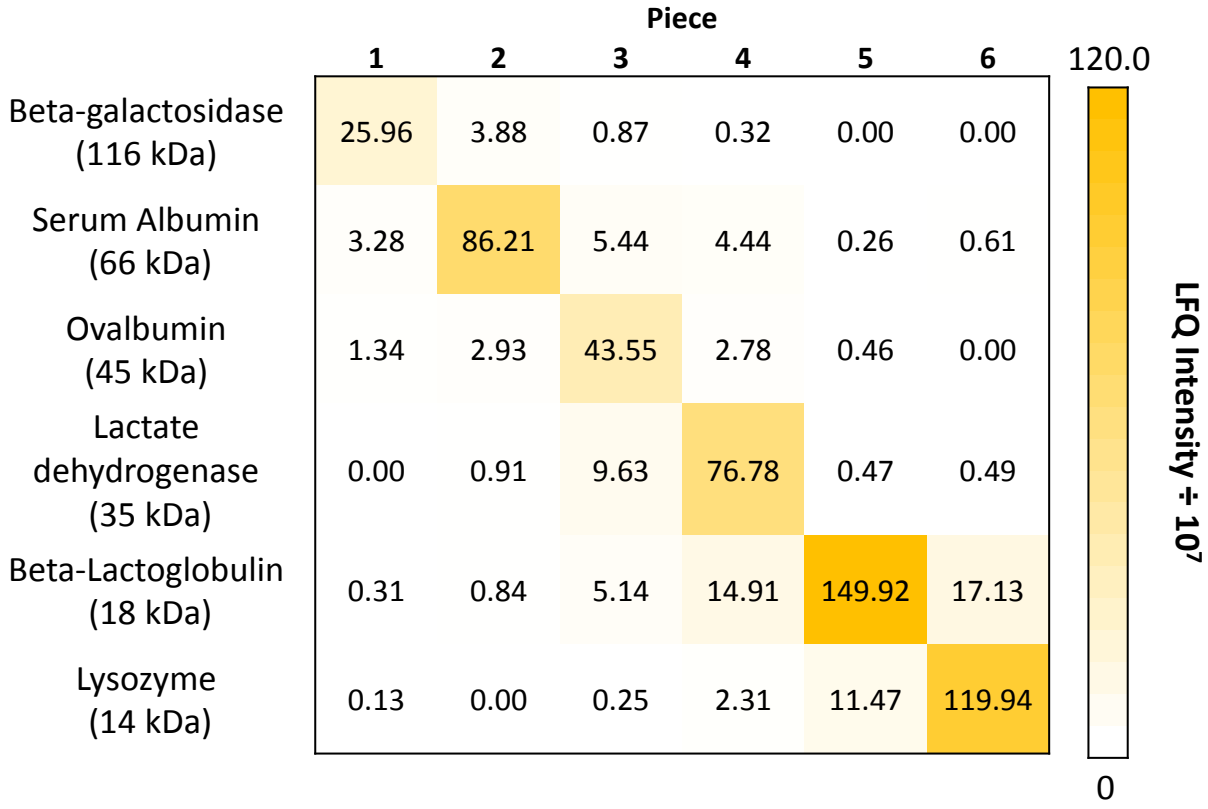

Figure S1. Average LFQ intensities (arbitrary units ÷ 10^7) of six standard proteins in sequential pieces of a PVDF capture membrane after electrodigestion. The x-axis shows pieces arranged in decreasing order of expected molecular weights. Intensities are the average of two experimental replicates.

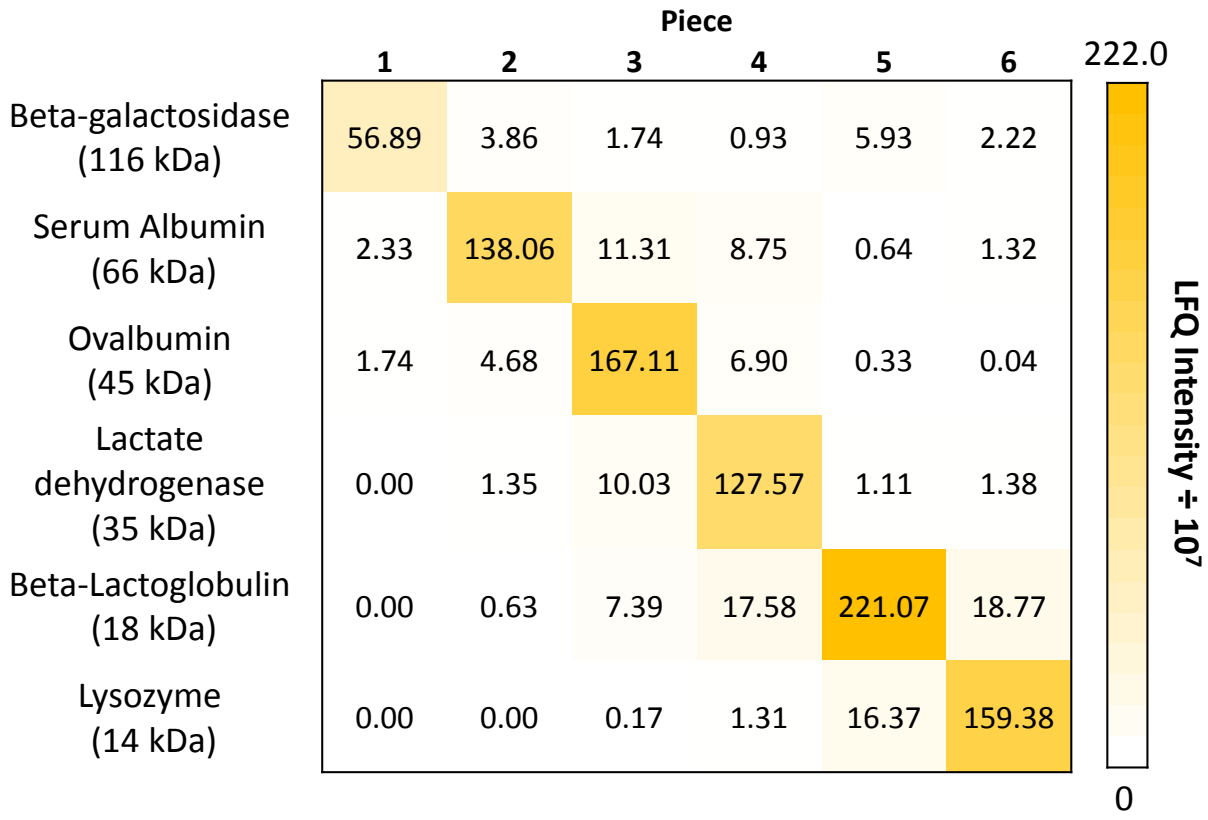

Figure S2. Average LFQ intensities (arbitrary units ÷ 10^7) of six standard proteins in sequential pieces of polyacrylamide gel after in-gel digestion. The x-axis shows pieces arranged in decreasing order of expected molecular weights. Intensities are the average of two experimental replicates.

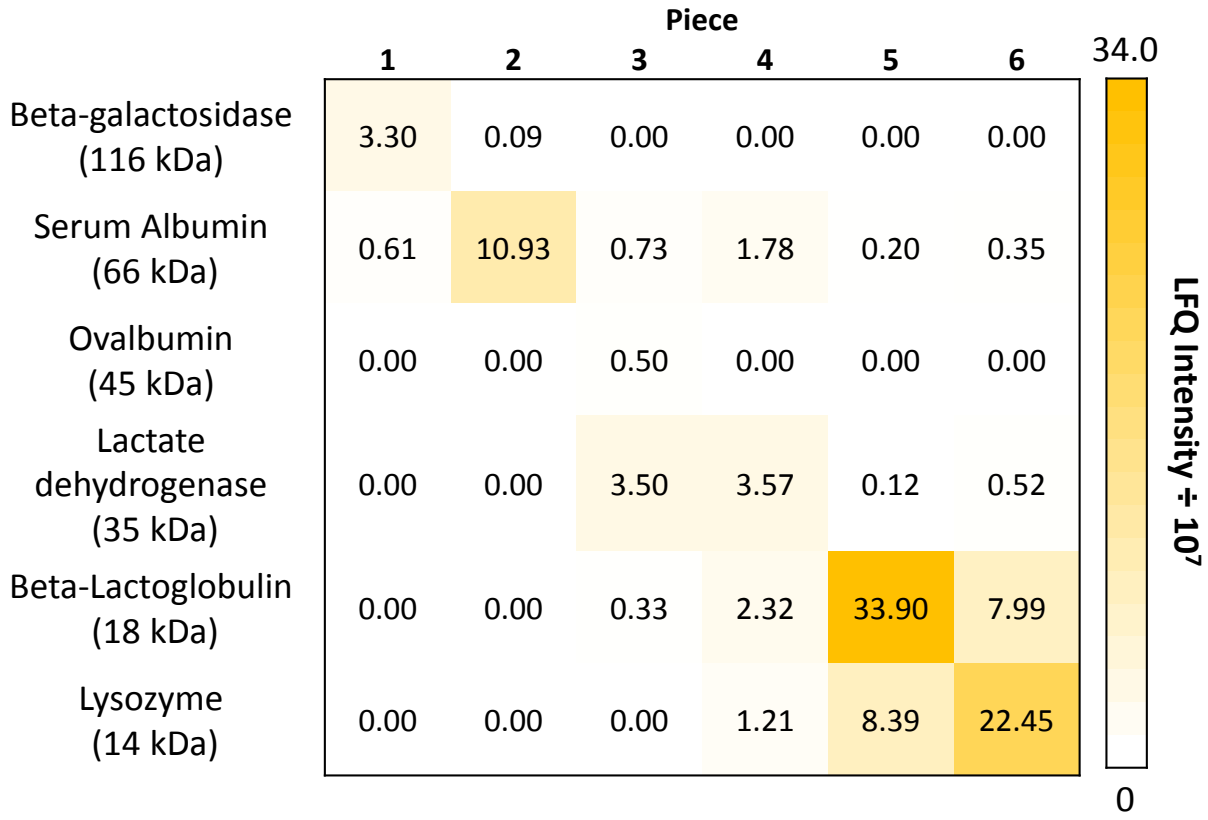

Figure S3. Average LFQ intensities (arbitrary units ÷ 10^7) of six standard proteins in sequential pieces of PVDF capture membrane after electroblotting and on-membrane digestion. The x-axis shows pieces arranged in decreasing order of expected molecular weights. Intensities are the average of two experimental replicates.

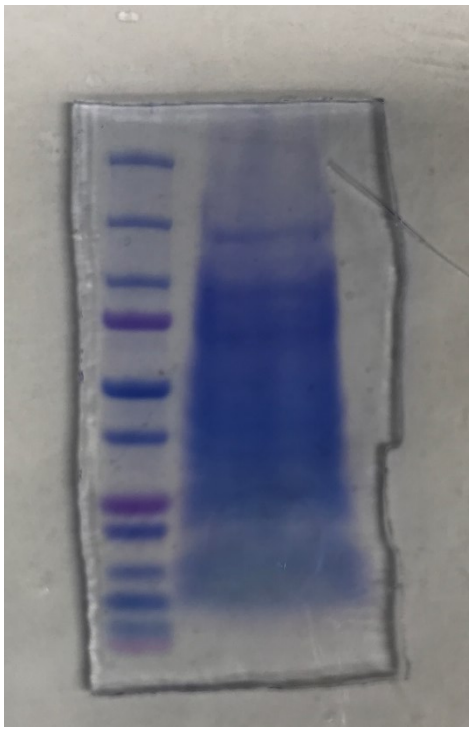

Figure S4. SDS-PAGE separation of 100 µg of E. coli whole cell lysate (right lane).

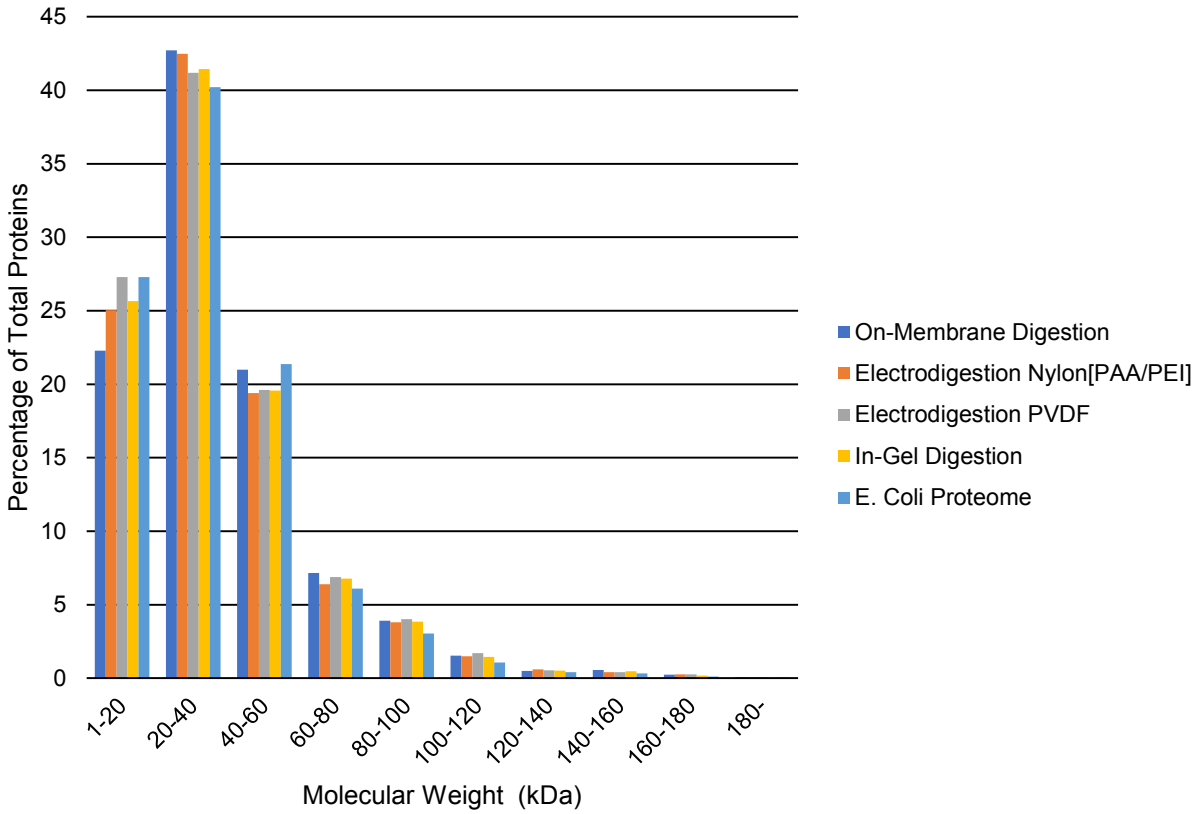

Figure S5. Distribution of molecular weights of proteins identified in *E. coli* cell lysate analyzed using the four methods in **Fig. 1**. For comparison, the figure also shows the molecular weight distribution in the E. coli proteome.

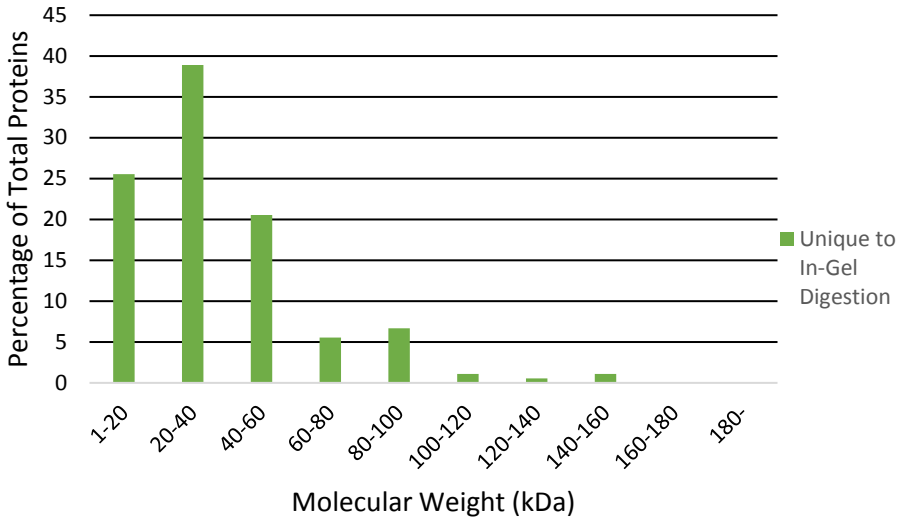

Figure S6. Distribution of the molecular weights of proteins uniquely identified after in-gel digestion of *E. coli* cell lysate.

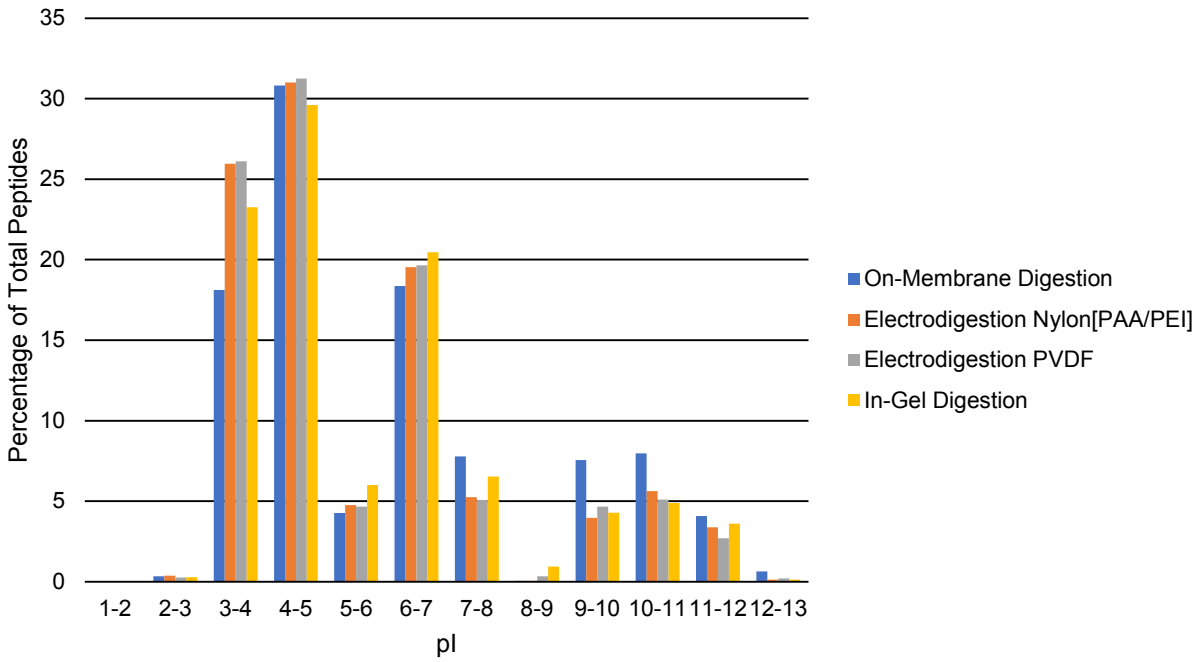

Figure S7. Distribution of isoelectric points of peptides identified in *E. coli* cell lysate analysed using the four methods in **Fig. 1**.

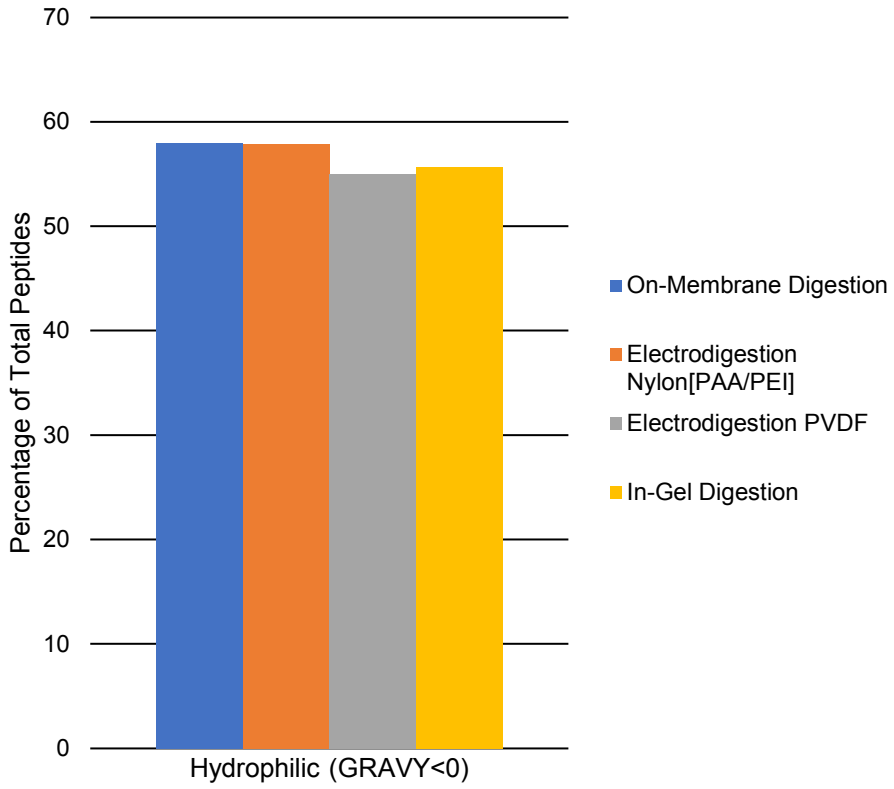

Figure S8. Hydrophilicity (percentage of peptides with GRAVY score < 0) of peptides identified in E. coli cell lysate analyzed using the four methods in **Fig. 1**.

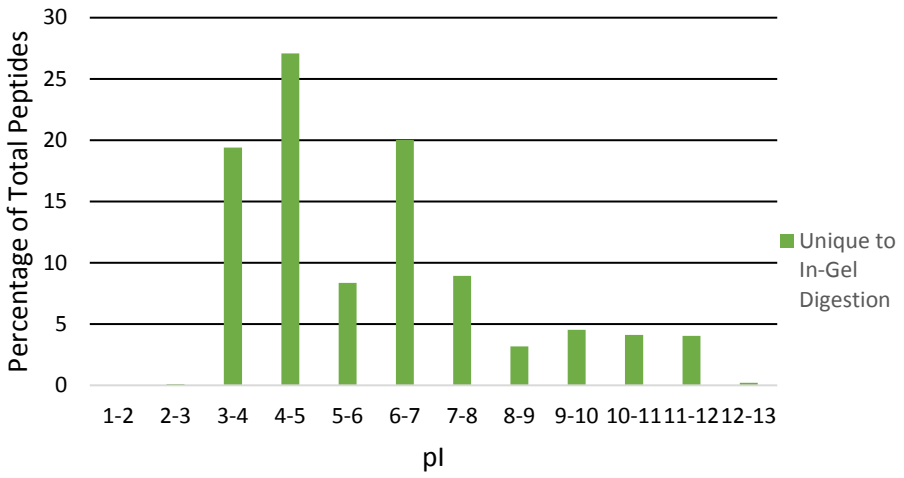

Figure S9. Distribution of isoelectric points of peptides uniquely identified after in-gel digestion of E. coli cell lysate.

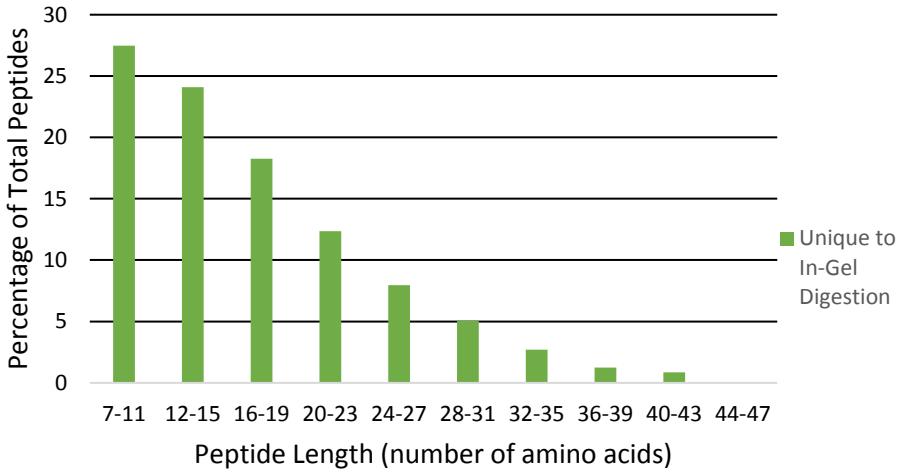

Figure S10. Distribution of the lengths (number of amino acids) of peptides uniquely identified after in-gel digestion of *E. coli* cell lysate. Figure S15 shows a similar distribution of all peptides identified using different methods.

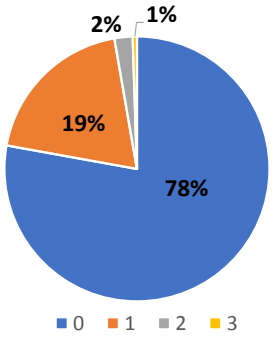

Figure S11. Percentages of species with 0, 1, 2, and 3 missed cleavages in peptides uniquely identified after in-gel digestion of *E. coli* cell lysate.

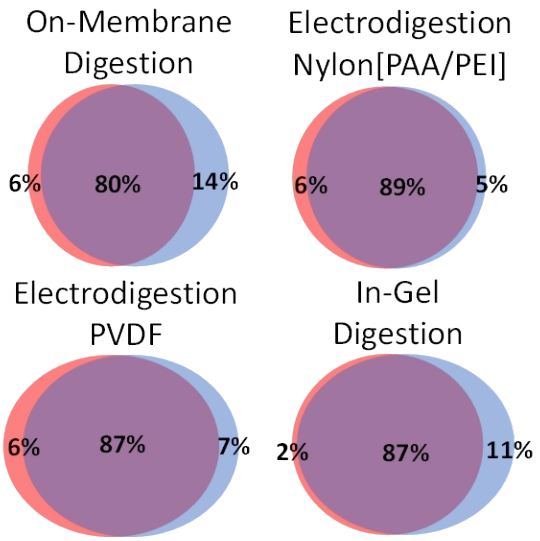

Figure S12. Experimental repeatability of *E. coli* cell lysate protein identifications using different digestion methods. The diagrams represent percentages of unique and shared proteins between experimental replicates.

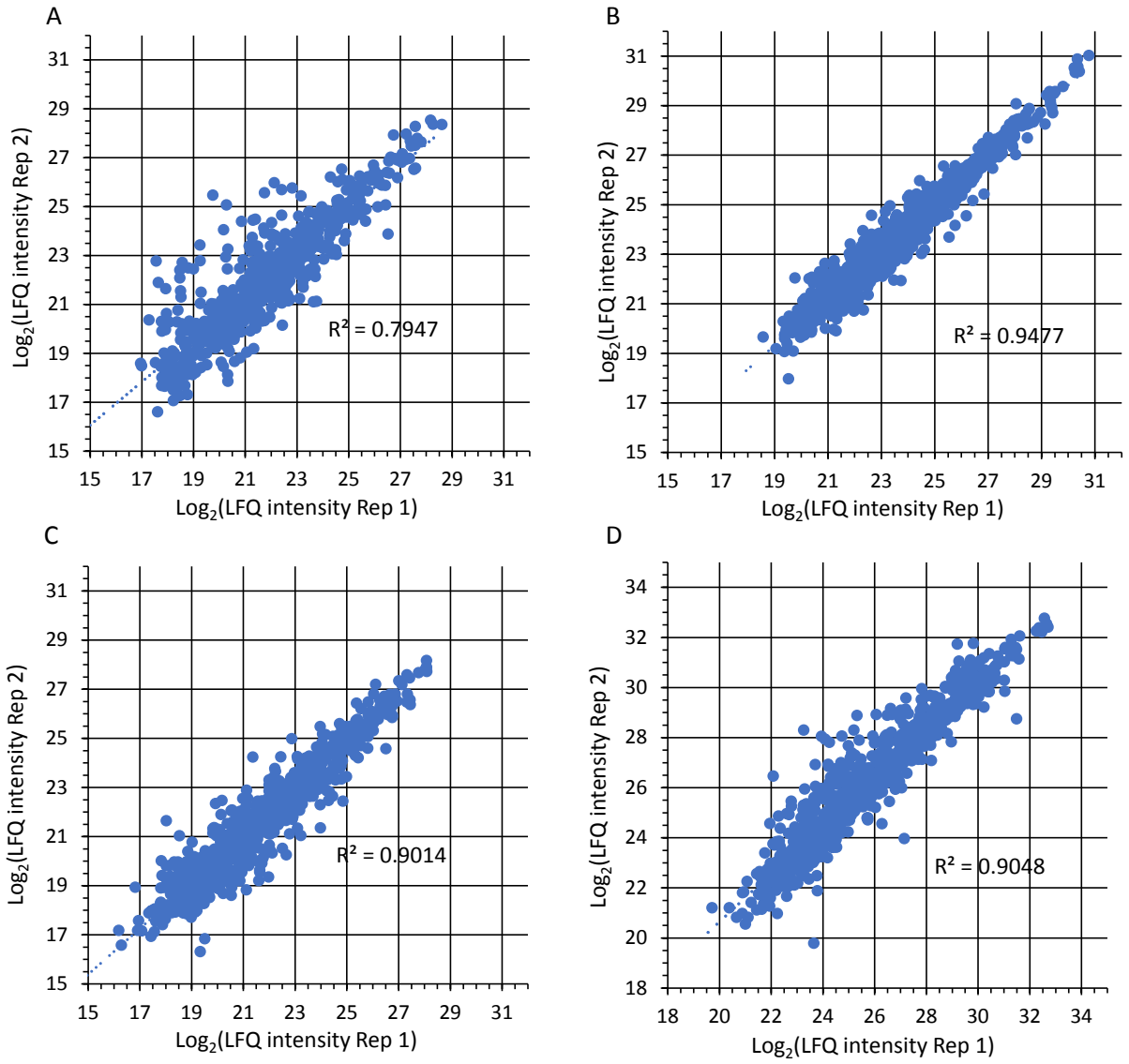

Figure S13. Plots of log_2(LFQ intensity) of identified proteins for replicate 1 versus replicate 2 in A) on-membrane digestion after electroblotting, B) electrodigestion using PAA/PEI nylon capture membranes, C) electrodigestion using PVDF capture membranes, and D) in-gel digestion.

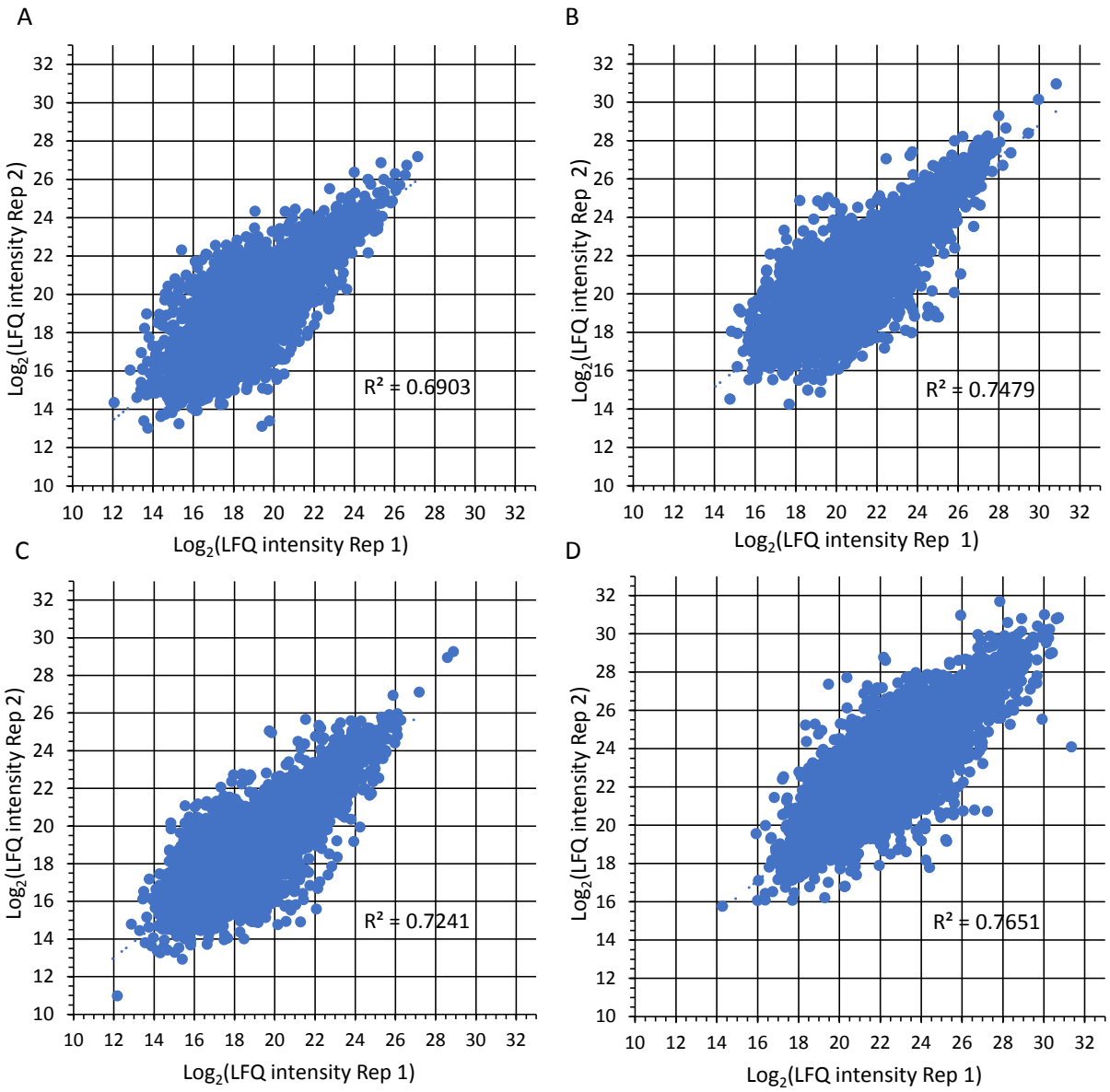

Figure S14. Plots of log_2(LFQ intensity) of identified peptides for replicate 1 versus replicate 2 in A) on-membrane digestion after electroblotting, B) electrodigestion using PAA/PEI nylon capture membranes, C) electrodigestion using PVDF capture membranes, and D) in-gel digestion.

Table S1. Percent of total proteins and peptides with LFQ intensities in both replicates, and average percent differences between replicate LFQ intensities for proteins and peptides.

Method	% of total proteins with LFQ intensities in both replicates[a]	Average %Difference between protein LFQ intensities in two replicates[b]	% of total peptides with LFQ intensities in both replicates[a]	Average %Difference between peptide LFQ intensities in two replicates[b]
On-Membrane Digestion	51	48 ± 41 %	56	57 ± 45 %
Electrodigestion PAA/PEI	57	28 ± 24 %	62	52 ± 43 %
Electrodigestion PVDF	56	36 ± 31 %	60	55 ± 43 %
In-Gel Digestion	63	38 ± 35 %	64	49 ± 43 %

[a]In some cases MaxQuant identifies proteins and peptides without giving an LFQ intensity.
[b]Calculated for proteins and peptides with LFQ intensities in both replicates. The uncertainty is the standard deviation.

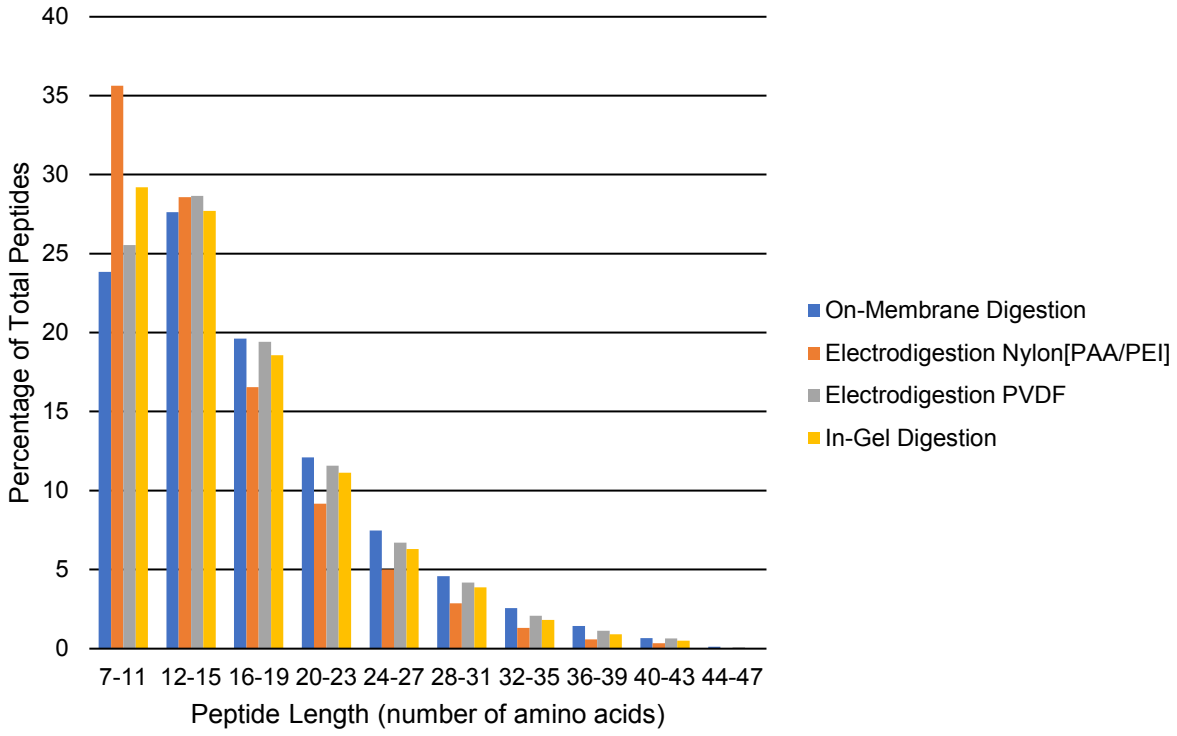

Figure S15. Distribution of peptide lengths identified in *E. coli* cell lysate analyzed using the four methods in **Fig. 1**.

Data File Submission

Raw data files, and MaxQuant protein and peptide matches were submitted to the MassIVE database. Private access can be found using the URL ftp://MSV000086010@massive.ucsd.edu and username; MSV000086010_reviewer, password; Electrodigestion. Upon public submission the URL will change to ftp://massive.ucsd.edu/MSV000086010/.