

Supporting Information

Enhanced Spatially Resolved Proteomics Using On-tissue Hydrogel-mediated Protein Digestion

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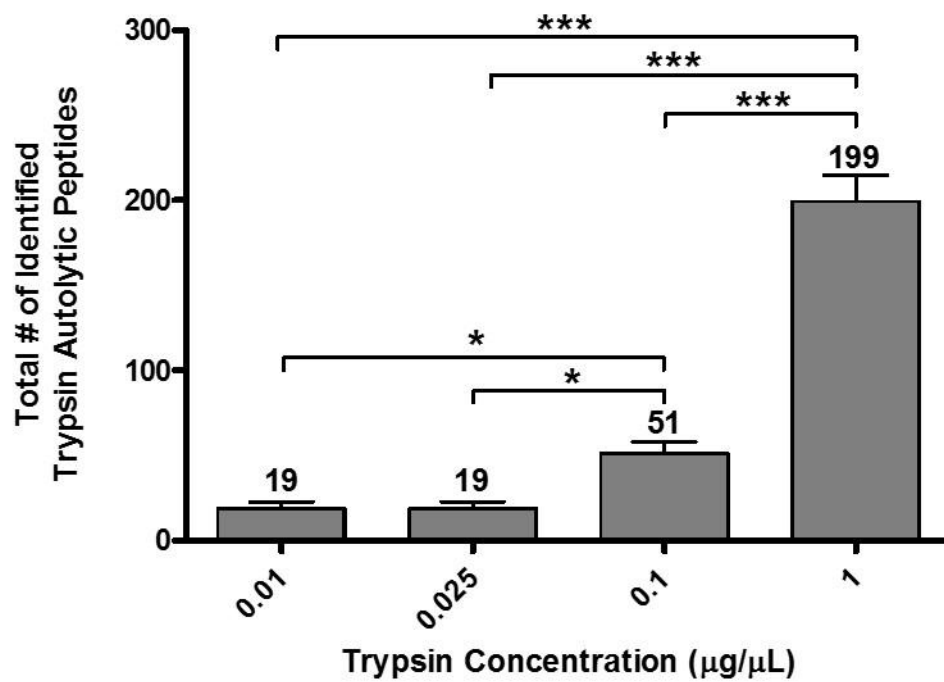
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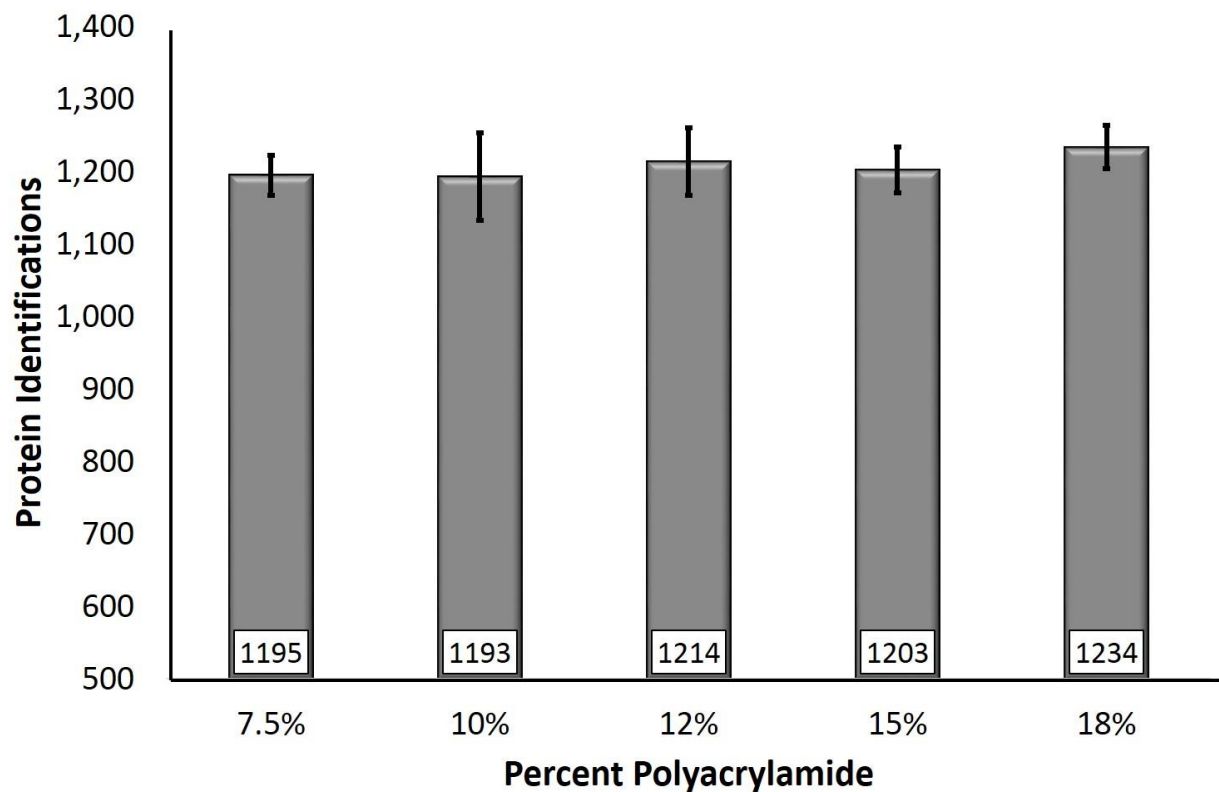
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<i>% Acrylamide</i>	7.5%	10%	12%	15%	18%
30% Acrylamide/Bis (mL)	1.25	1.67	2.00	2.50	3.00
1.5 M Tris, pH 8.7 (mL)	1.25	1.25	1.25	1.25	1.25
ddH ₂ O (ml)	2.48	2.06	1.73	1.23	0.73

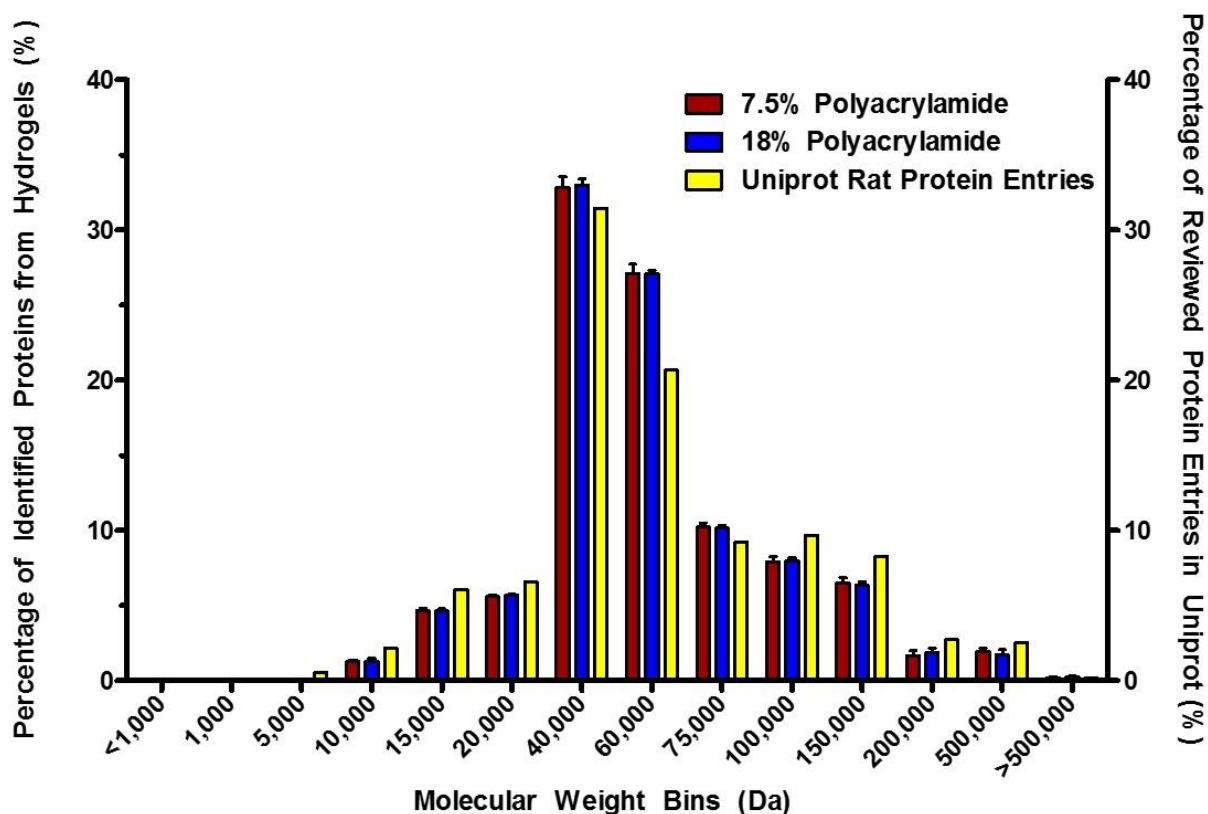
Supplemental Figure S-1: Gel formulation protocols for each percentage polyacrylamide. These solutions were degassed prior to polymerization with 50 μ L 10% ammonium persulfate and 10 μ L TEMED.



Supplemental Figure S-2: Average numbers of identified trypsin autolytic peptides from 7.5% polyacrylamide hydrogels (~2.6 mm diameter) containing varying concentrations of trypsin. Each hydrogel was placed on a 12 µm thick section of rat liver (n=3 for each concentration, *: $p \leq 0.05$, * $p \leq 0.001$).**



Supplemental Figure S-3: Protein identifications are unchanged for ~2.6 mm diameter hydrogels fabricated using different percentages of polyacrylamide. Each hydrogel was placed on a 12 μ m thick section of rat liver and contained 0.1 μ g/ μ L trypsin ($n \geq 3$ for each percentage).



Supplemental Figure S-4: Molecular weight distributions of protein identifications from 7.5% and 18% polyacrylamide hydrogels and the reviewed protein identifications in the Uniprot rat protein database. Masses of identified proteins were binned for the 7.5% (red) and 18% (blue) polyacrylamide hydrogels (left axis), as well as the UniProt rat proteome (yellow – right axis). n=5 for both of the hydrogel conditions.

Supplemental Figure S-5: Complete list of proteins identified from the experiment described in Figure 5 (see Excel file).