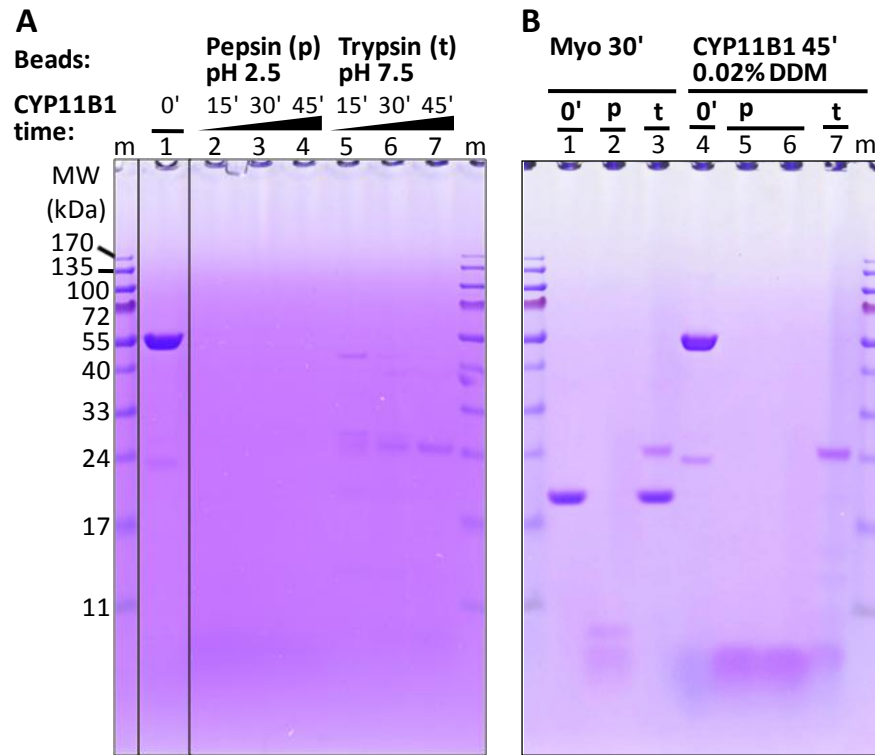


**Supplemental Materials for**

**Less is more: Membrane protein digestion beyond urea-trypsin solution for next-level proteomics**, by Xi Zhang, Ph.D.

**Supplemental Figure S1.**



**Supplemental Figure S1.** Comparison of digestions by immobilized trypsin beads and pepsin beads, using a multi-helix protein cytochrome P450 11B1 (CYP11B1) and SDS-PAGE. **(A)** CYP11B1 (4  $\mu$ M) digested by POROS trypsin (pH 7.5) and pepsin (pH 2.5) beads (each 20  $\mu$ L neat beads, equivalent to column volume of 2 mm i.d. x20 mm) after 15, 30 and 45 min at 22  $^{\circ}$ C in 0.02% DDM digestion buffer. **(B)** Comparison of trypsin (pH 7.5) and pepsin (pH 2.5) beads (20  $\mu$ L each) digestion of myoglobin and CYP11B1 (0.02% DDM) after 30 and 45 min respectively. Purified overexpressed human CYP11B1 (provided by Dr. Keith W. Miller in 1% cholate and 0.1% Tween 20) was diluted by 11 fold to 4  $\mu$ M with 0.02% DDM buffers before digestion. A 30 min incubation with one column volume of bead slurry is routinely used to predict the digestion completion (not peptide reproducibility) of column mode.