

*Supporting Information for*

# **Enhanced Electrophoretic Depletion of Sodium Dodecyl Sulfate with Methanol for Membrane Proteome Analysis by Mass Spectrometry**

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## **Supplementary Materials:**

Figure S1: Flow scheme summarizing membrane proteome solubilization experiment with methanol

Figure S2: Transmembrane electrophoresis device

Figure S3: SDS PAGE image of proteins collected following SDS depletion by TME in water or in 40% methanol

Figure S4: Plot of conductivity vs SDS concentration indicating the CMC of SDS in water, as well as 10, 20 and 40% methanol

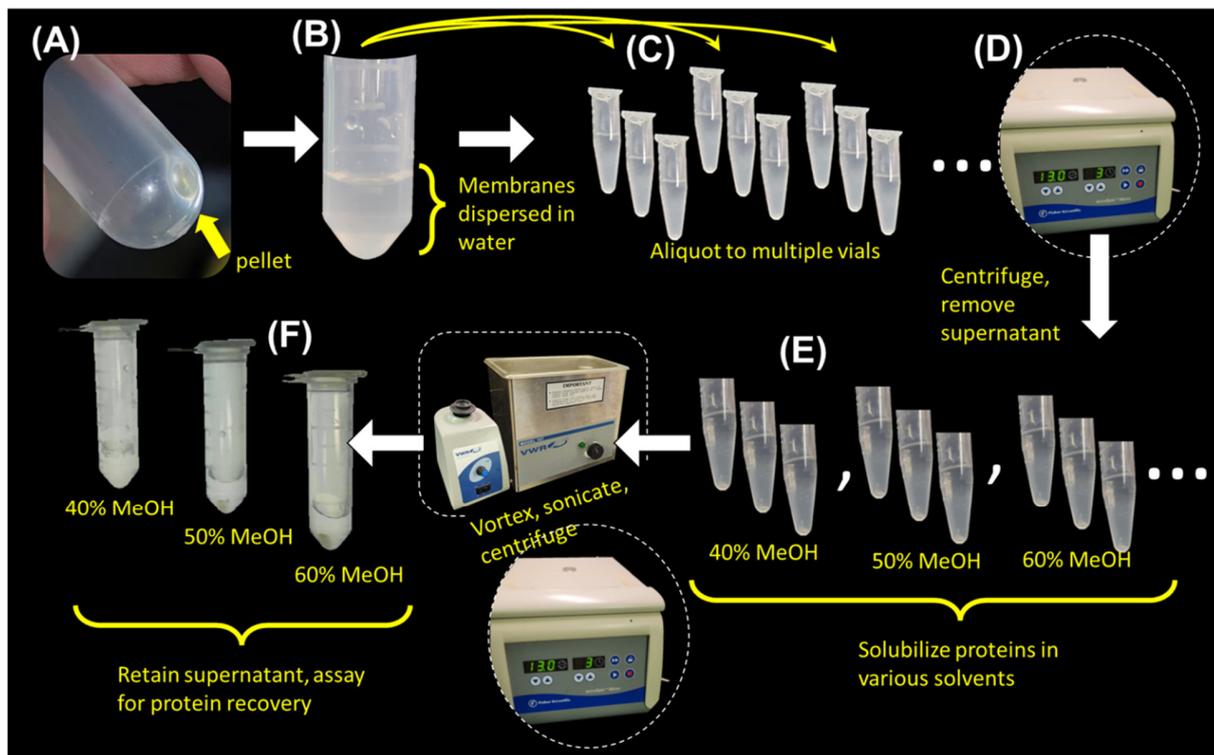
Figure S5: Histogram of the number of transmembrane segments for proteins enriched in methanol vs water

Figure S6: Histogram of isoelectric points for proteins enriched in 40% methanol vs water

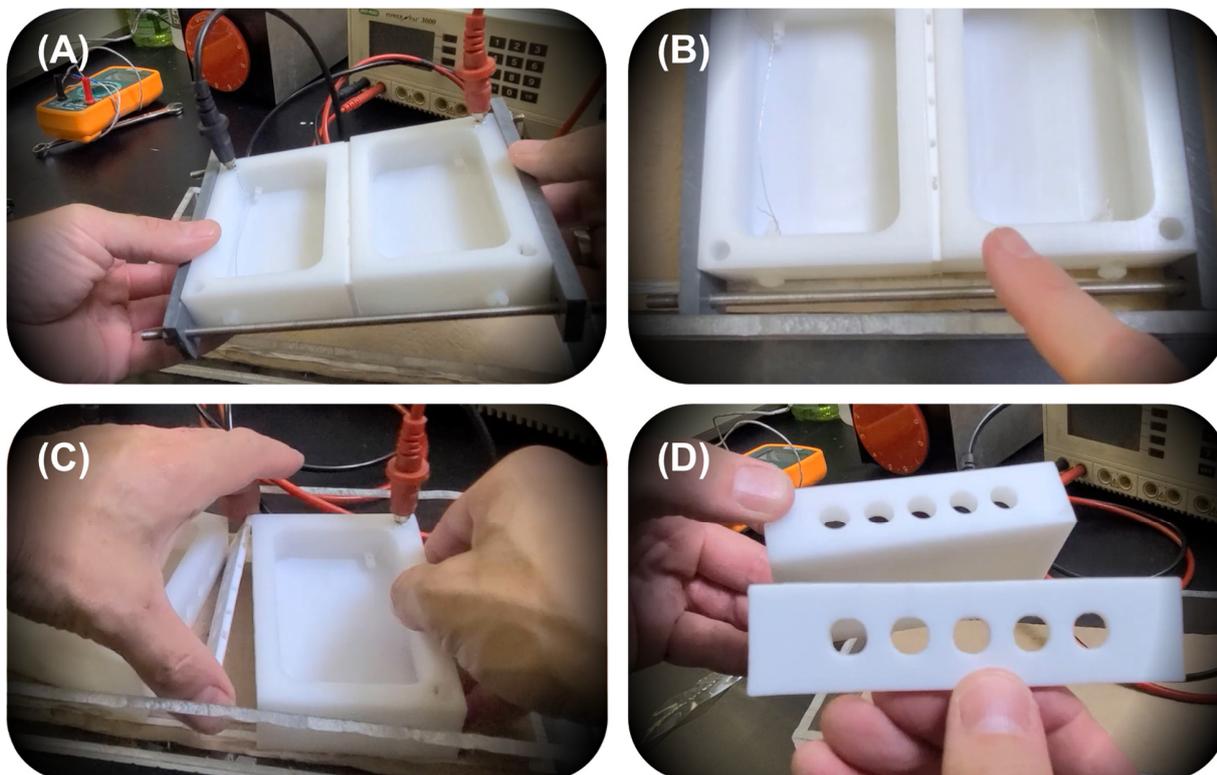
## **Also available:**

Table S1: List of proteins identified along with their relative abundance;

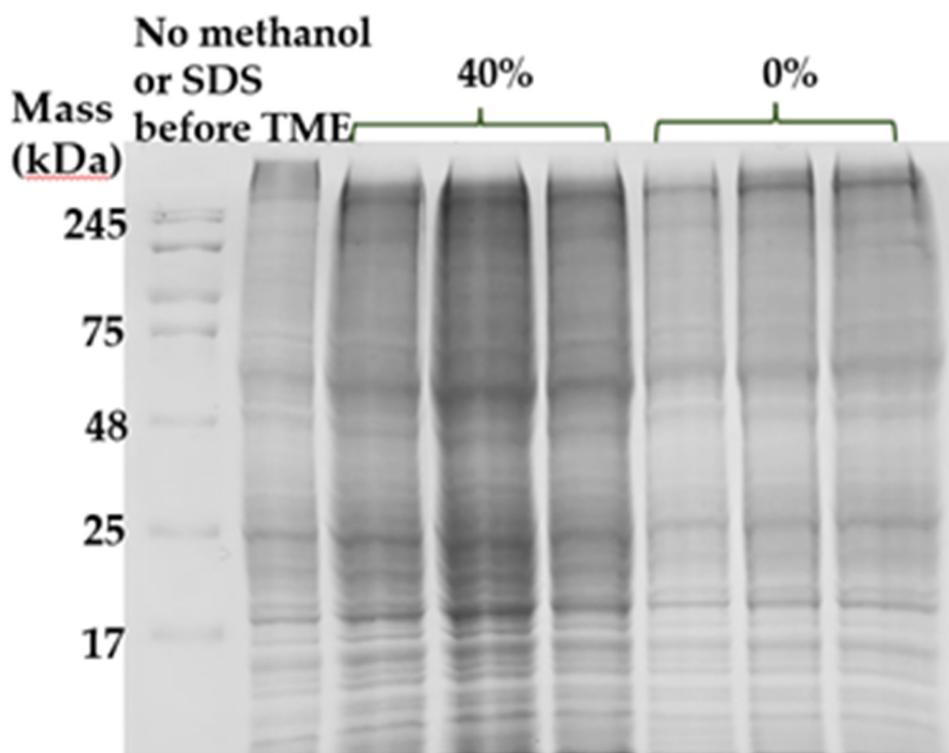
Table S2: Gene Ontology Data for proteins enriched in 40% methanol and in water.



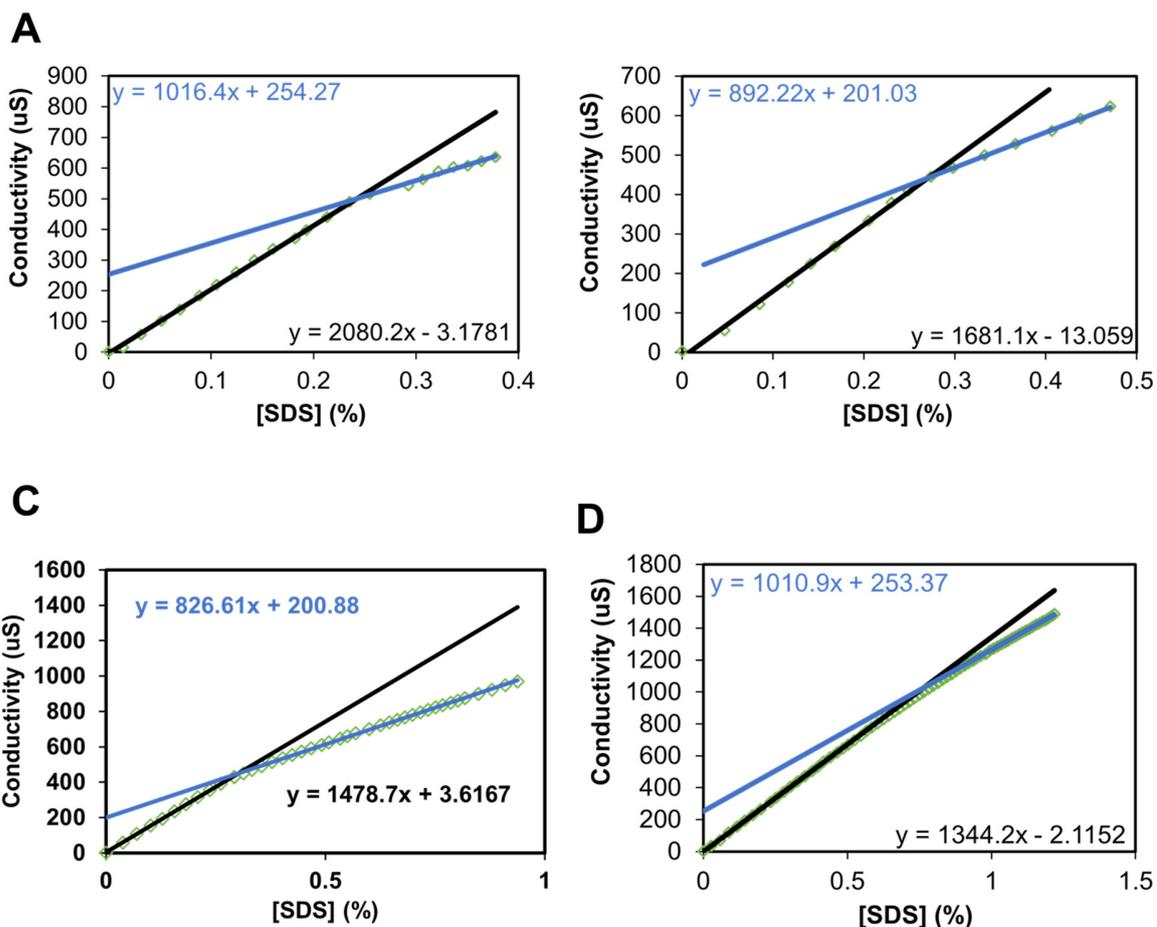
**Figure S1.** Flow chart summarizing membrane proteome extraction in methanol/water. **(A)** An enriched membrane fraction was prepared by ultracentrifugation of lysed yeast cells; **(B)** the pellet was agitated to form a homogeneous suspension of undissolved membranes in water; **(C)** Equal volumes of the suspension were aliquoted to multiple vials, thus transferring an equivalent quantity of membranes to test protein solubilization efficiency; **(D)** the suspension was centrifuged, and the supernatant discarded, resulting in the final pellet to test proteome solubilization efficiency; **(E)** The appropriate solvent system (ie 0.5% SDS in water, or varying ratios of methanol/ water) were added to the vials, testing three replicates per condition; **(F)** Following vortex and sonication, solubilized proteins were collected following centrifugation to remove the undissolved proteins in the membrane pellet.



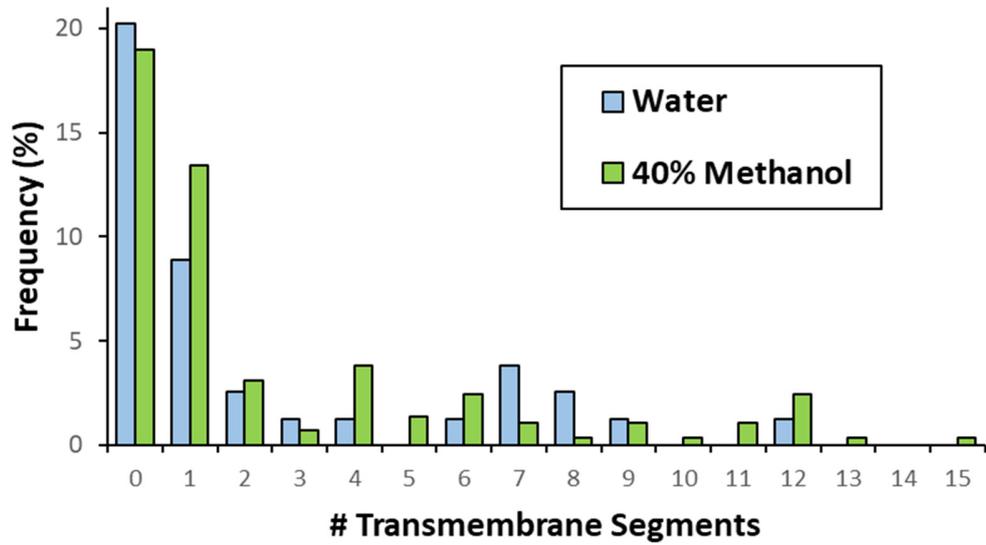
**Figure S2.** Transmembrane electrophoresis system (A) Shown is the fully assembled system, prior to depositing buffer; (B) samples are deposited into holes of the central sample plate, sandwiched by dialysis membranes, with the cathode and anode buffers to either side; (C) the sample cell is only 3 mm thick; (D) holes are machined into the central plate to form three sample cells, each capable of retaining up to 250  $\mu\text{L}$  of sample.



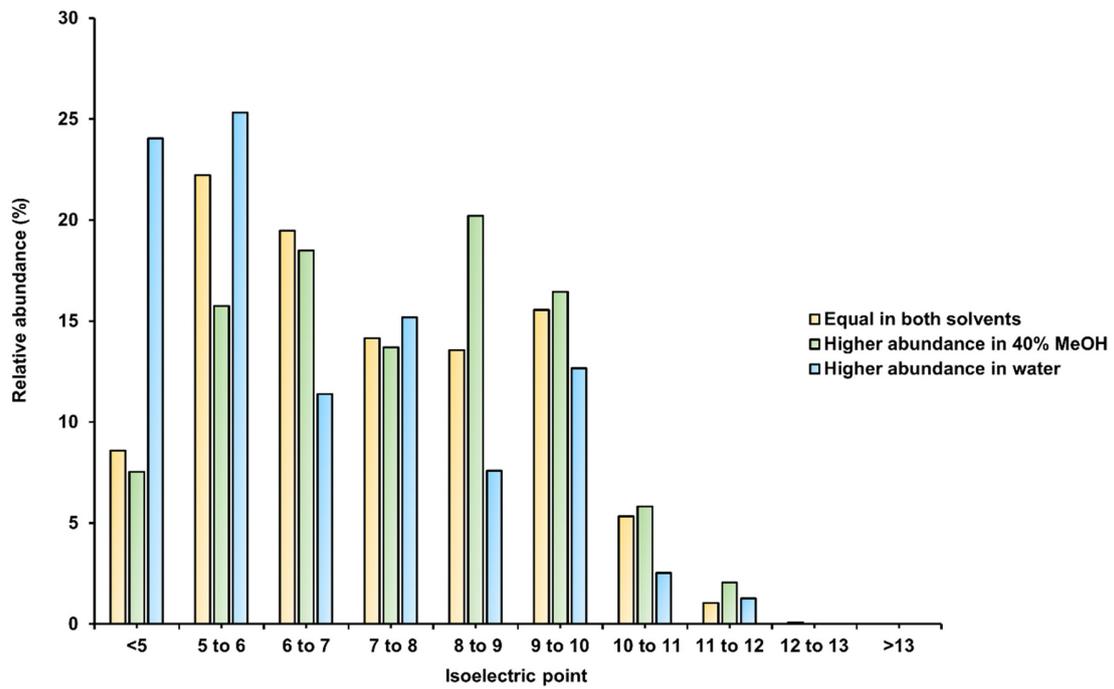
**Figure S3.** SDS PAGE of membrane proteome fractions initially extracted with 0.5% SDS and depleted of the surfactant using TME. It required 9 minutes for SDS to be reduced below 100 pm in the water preparation, while only 3 minutes for the sample containing 40% methanol. Shown are the proteins recovered in the soluble fraction following TME depletion.



**Figure S4.** Plot of conductivity vs SDS concentration, whereby the breaks in the linear trendlines indicate the CMC. **A)** 0% methanol. **B)** 10% methanol. **C)** 20% methanol. **D)** 40% methanol. The intersection points correspond to SDS concentrations of 8.4 mM (pure water); 9.4 mM (10% methanol); 10.5 mM (20% methanol); and 26.5 mM (40% methanol).



**Figure S5.** Histogram summarizing the number of transmembrane segments contained in membrane proteins determined to be enriched from the TME preparation with 40% methanol (green), or enriched in the water-containing TME preparation.



**Figure S6.** Histogram demonstrating the distribution of isoelectric point extracted in each condition. Proteins that are statistically enriched in the 40% MeOH preparation show a larger fraction of proteins with pI close to the TME operating buffer (pH 8.1).