

## OVERVIEW

- A multiplexed GELFrEE device is reported for proteome prefractionation
- 8-fold increase in loading capacity & throughput
- LC-MS/MS of 16 resulting fractions gave 420 unique yeast proteins
- Separation is highly correlated to mass of identified proteins

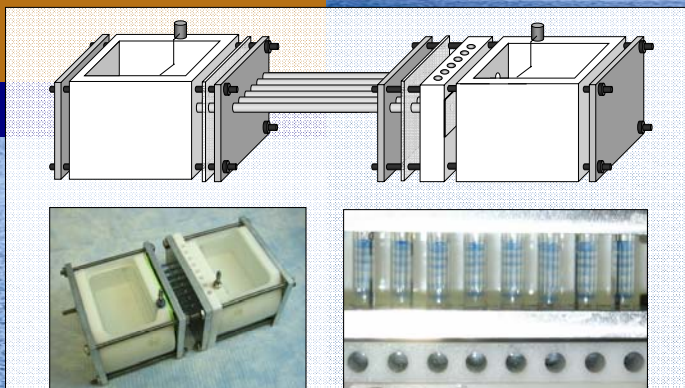
## INTRODUCTION

GELFrEE<sup>1</sup> affords rapid mass-based protein separation over a range 10-150 kDa. Here, we demonstrate a multiplexed design enabling increased loading capacity and throughput. We demonstrate comprehensive analysis of the yeast proteome using GELFrEE coupled to LC-MS/MS analysis.

<sup>1</sup>Tran & Doucette, *Anal. Chem.* **2008**, *80*, 1568-1573.

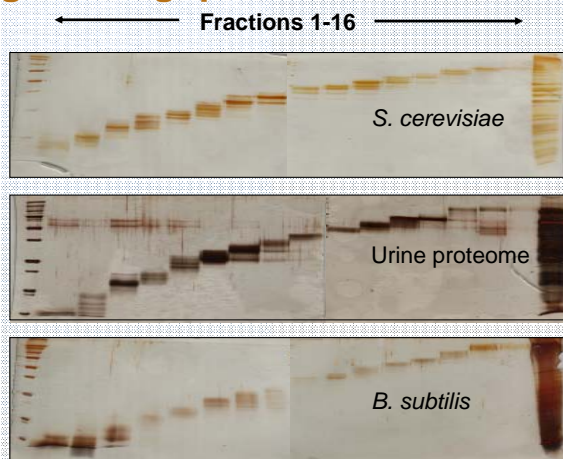
## METHODS

- Instrument design: 8 gel columns are coupled in parallel to independent collection chambers
- All buffers and gels were prepared according to Laemmli protocol
- 150 µL of sample is loaded per gel column
- Fractions were analyzed by 1D gels or precipitated, digested and subject to LC-MS/MS (1.5 hr gradient 5-40% ACN / 0.1% formic acid) on an LTQ linear ion trap MS



A schematic and photo of the GELFrEE device, showing separation of stained protein markers in the gel columns (right).

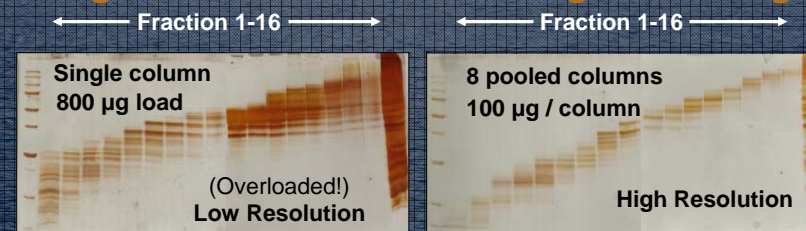
## High throughput mass fractionation



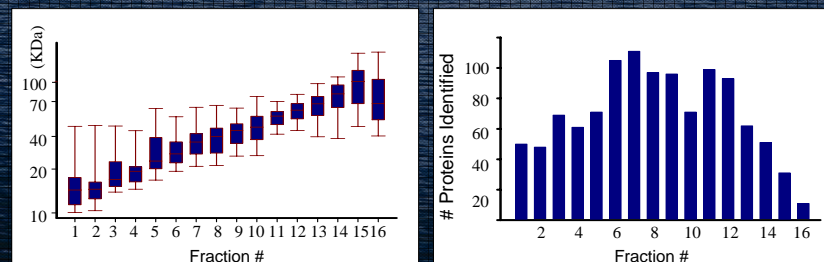
## ACKNOWLEDGEMENTS

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## Higher resolution at 8-fold higher loading

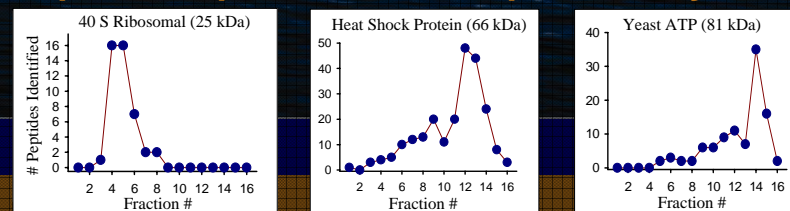


## Fractions give high mass correlation in MS



Fractions correlate highly with MW. 1126 yeast proteins (420 unique) were identified using LC MS/MS analysis of the 16 fractions.

## Separation profile of select proteins by MS



## CONCLUSIONS

- Multiplexed GELFrEE affords high throughput & loading
- Compatible with LC MS/MS analysis
- Collected fractions highly correlate to molecular weight in MS