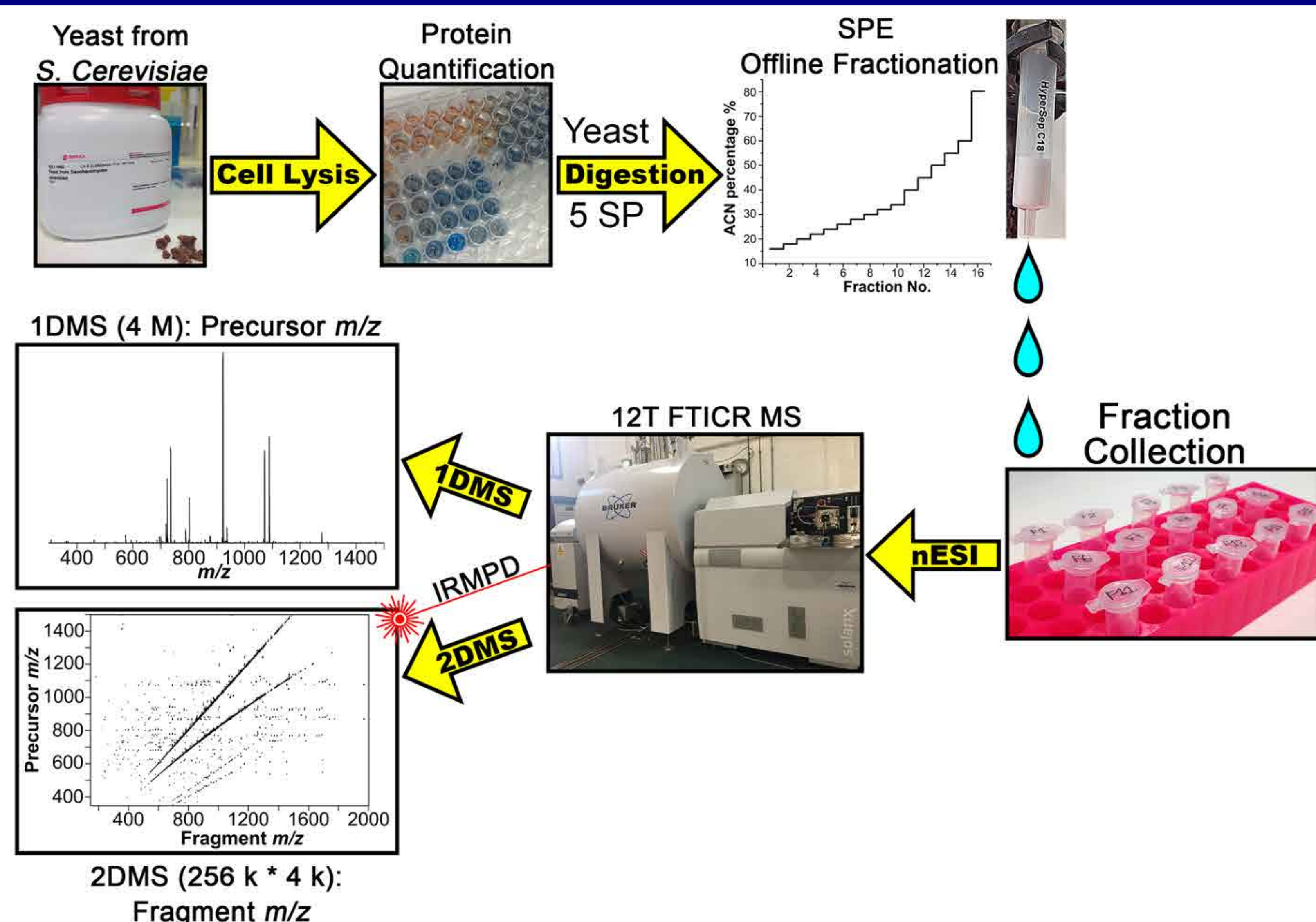


## Introduction

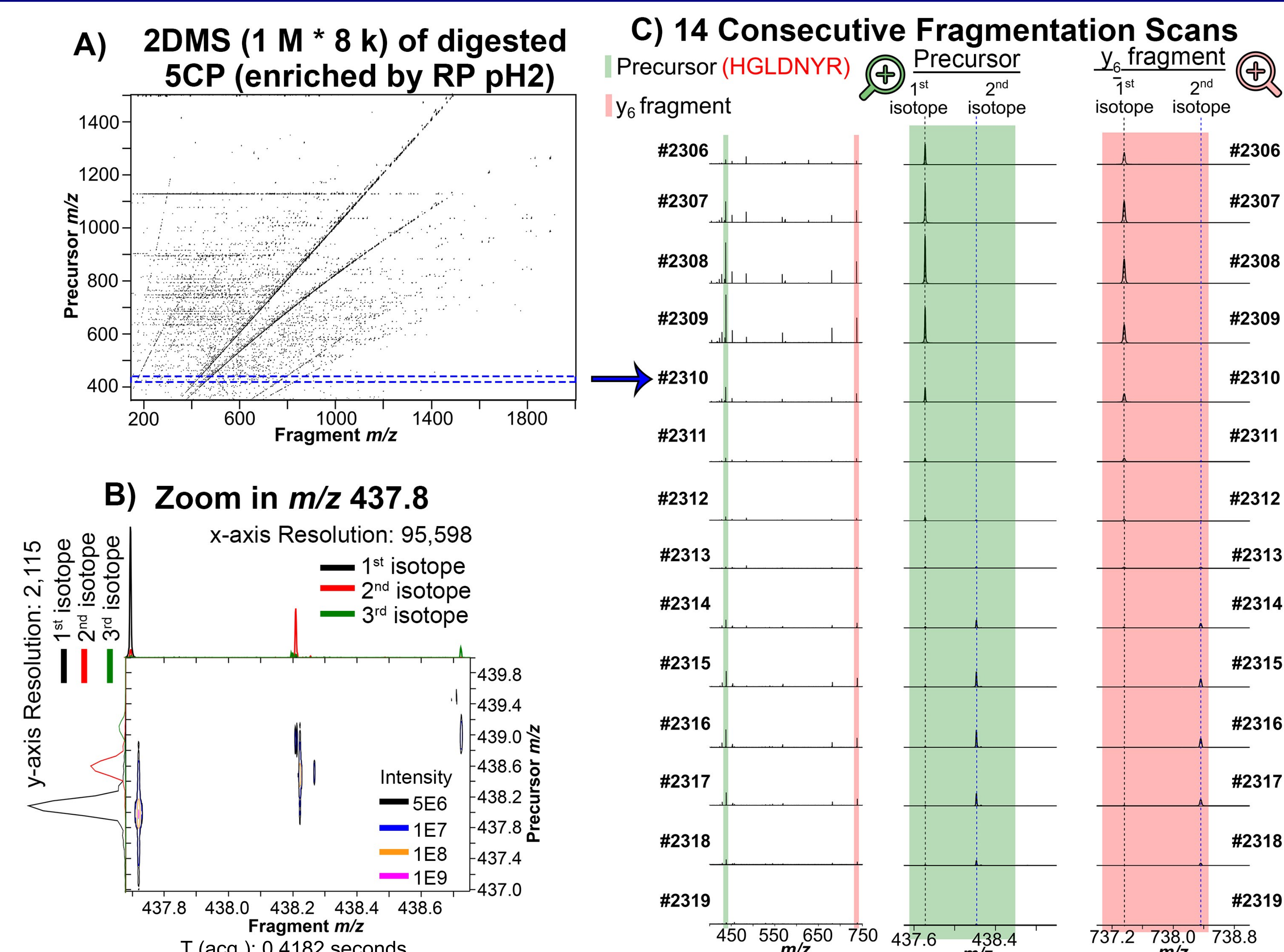
- Liquid chromatography (LC) is an essential tool for proteomics
- Suffers from:
  1. Bias of the chromatography columns
  2. Incompatibility with certain separation conditions
- 2DMS provides an extra separation dimension in the ICR cell<sup>1</sup>
- Applications for: bottom-up and top-down of standard protein;<sup>2</sup> small molecules;<sup>3</sup> polymers<sup>4</sup>
- Herein, we demonstrate the use of 2DMS for proteomics samples and compare the 2DMS results to LC tandem MS data

## Experimental Section

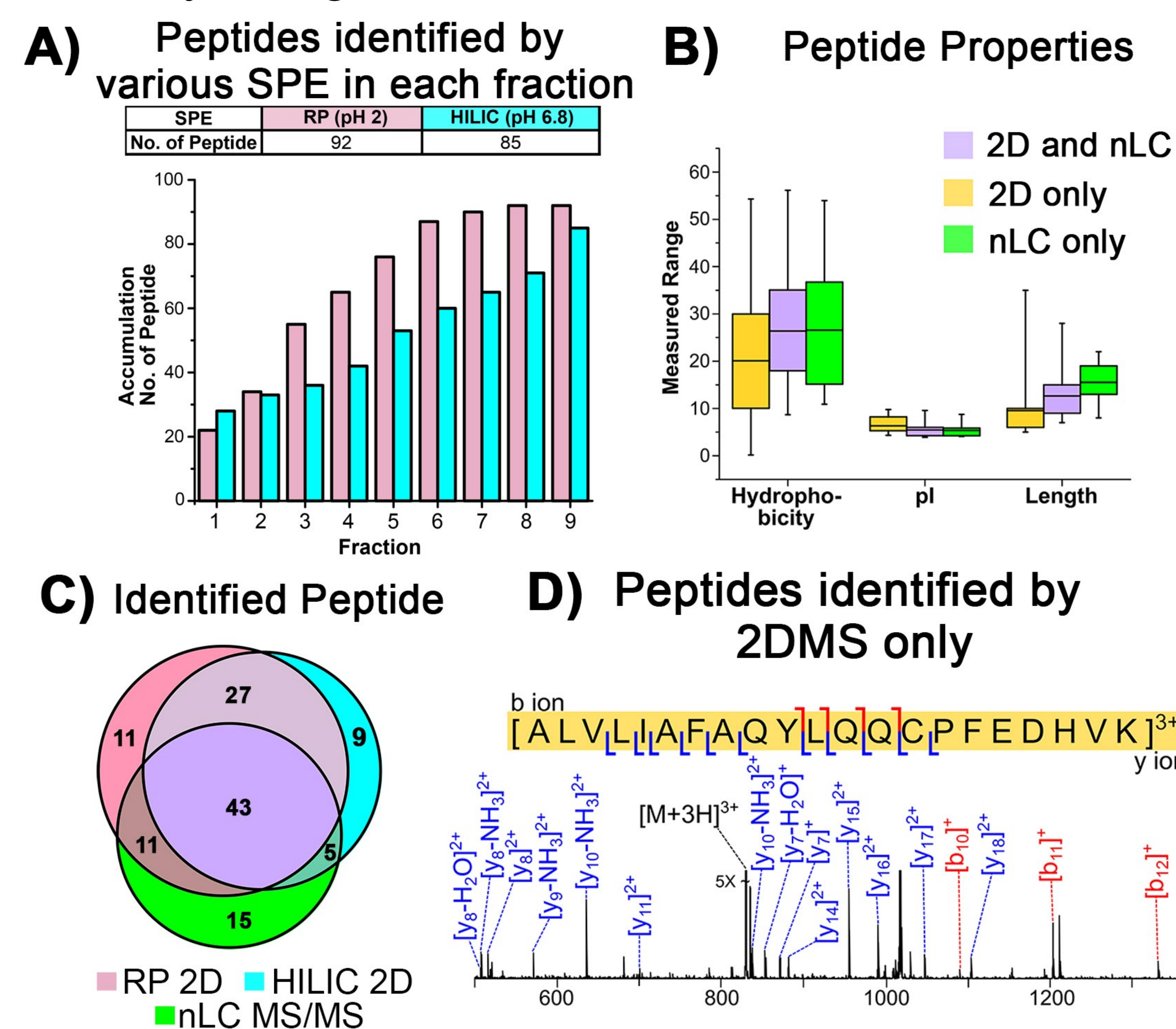


**Fig 1.** A summarised workflow of the sample preparation and 2DMS data acquisition of the offline fractionated yeast as well as 5 standard proteins (SP)

## Result - 5 Standard Proteins



**Fig 2.** High resolution 2DMS with resolution 1 M \* 8 k data-points of tryptic digested 5SP



**Fig 3.** Proteomic results of tryptic digested 5SP using 2DMS with SPE fractionation and nLC MS/MS

## Result - Yeast Proteome

**Fig 4.** Summarised results of digested yeast using 2DMS and nLC MS/MS

## Conclusion

- 2DMS is a viable alternative technique for proteomic studies
- Complementary peptide and protein assignments between 2DMS and nLC-MS/MS allows deeper proteome coverage
- 2DMS has a higher efficiency in observing hydrophilic, basic, or short peptides, which may be beneficial for assignment of hydrophilic PTMs, i.e. phosphopeptides and glycopeptides

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## References

1. van Agthoven, Maria A., et al. *EUR. BIOPHYS. J.* 48.3 (2019): 213-229.
2. Floris, Federico, et al., *JASMS*, 29.1 (2017): 207-210.
3. van Agthoven, Maria A., et al. *JASMS* 26.12 (2015): 2105-2114.
4. Floris, Federico, et al. *Anal. Chem.* 89.18 (2017): 9892-9899.