Optimization of Fraction Collection in Comprehensive Two-Dimensional Liquid Chromatography Bottom-up Proteomics

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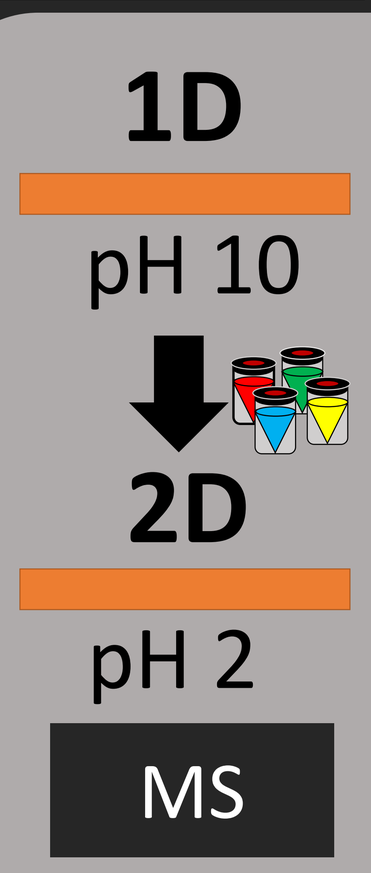
Goal

Optimizing 2D-LC fractions to maximize the number of protein identifications

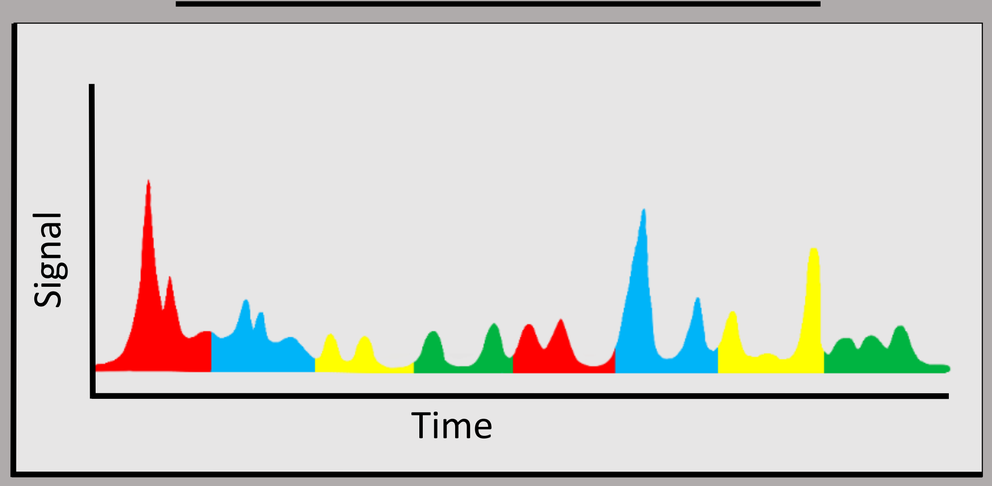
2D-LC is great for global proteomics

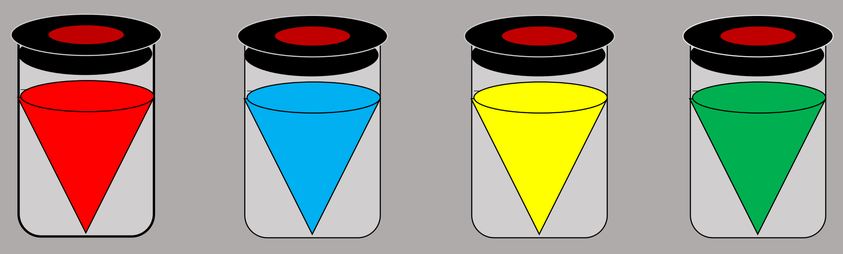
In the world of global bottom-up proteomics, the analyst will often encounter complex samples in small amounts. To take on these tiny-yet-vast samples, powerful tools are needed, and when it comes to separation, chromatography is king.

In this study, two-dimensional liquid chromatography (2D-LC) was used to achieve maximal peak capacity (i.e. the theoretical number of peaks that can be separated) for a peptide sample (e.g. digested HeLa). 2D-LC increases peak capacity dramatically, as peak capacity is multiplicative when the column dimensions have significantly different selectivity [1]. Reversed phase (RP) is the most efficient and repeatable chromatographic principle. As such, low pH RP is usually used in the second dimension (2D) because it can be directly combined with electrospray ionization-mass spectrometry (ESI-MS). For the first dimension (1D), high pH RP is great for peptides, as different pH in the two dimensions significantly alter their selectivity [1]. The setup is also within the realm of nano-LC, sporting narrow columns that increase sensitivity, allowing for ultra-low sample amounts [2].



4-vial fractionation scheme





Fractionation Schemes

The output from the 1D column is collected in fractions, which are subsequently injected onto the 2D column. To improve peak capacity and sensitivity, specific fractionation strategies are employed, where several fractions are collected in the same vial. In the **4-vial fractionation scheme** (left) the 1st, 5th, 9th, … fractions are collected in the first vial (red), the 2nd, 6th, 10th, … in the second (blue), etc [3].

The number of vials the fractions are collected in, the fraction collection time and the total number of fractions, are important parameters that collectively comprise the concatenation scheme. In order to maximize the peak capacity, **this fractionation scheme needs to be optimized**.

The Spider Fractionator

As the gradient run time for the 1D chromatography is **75** minutes, demanding the full attention of the operator at all times, automatization is appreciated, if not neccessary in the long run.

Automatization is achieved with a **spider fractionator** (right), a simple valve switching system taking 1D column output and directing it to the correct vial using programmed time intervals [**4**].

End-Goal

Without optimizing the fractionation scheme, Reubsaet *et al*. managed to identify approx. 7000 proteins from a very limited amount of HeLa lysate (2 µg) [3].
An improved fractionation scheme will unlock the method's true potential.

References

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[2] Wilson, S.R., T. Vehus, H.S. Berg, and E. Lundanes, *Nano-LC in proteomics: recent advances and approaches.* Bioanalysis, 7 (**2015**) 1799-1815.
[3] Reubsaet, L., M.J. Sweredoski, A. Moradian, B. Lomenick, R. Eggleston-Rangel, and S.D. Garbis, *Nano volume fractionation strategy for dilute-and-shoot injections in off-line loss-less proteomic workflows for extensive protein identifications of ultra-low sample amounts.* Journal of Chromatography A, 1609 (**2020**) 460507.
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