ChromaCon®

A new dimension in purification

Case Study

Use of the Contichrom® Platform in Discovery and Life Science Research

ChromaCon at a Glance

Company

- Labs and offices located in Zürich, Switzerland

Business

- Develop and market tools to enable drug discovery, improve and intensify development and manufacturing of chemical and biological medicines
- Commercialize disruptive technologies into high growth, profitable equipment and licensing business through marketing partnerships

Technology

- Broad IP on principle and manufacturability of product classes
- Develop Contichrom® discovery to production scale chromatography equipment

Strategy

- Reach fast break-even through sales of equipment and services
- Establish scalable business through licensing and project income

Discovery: The Challenge

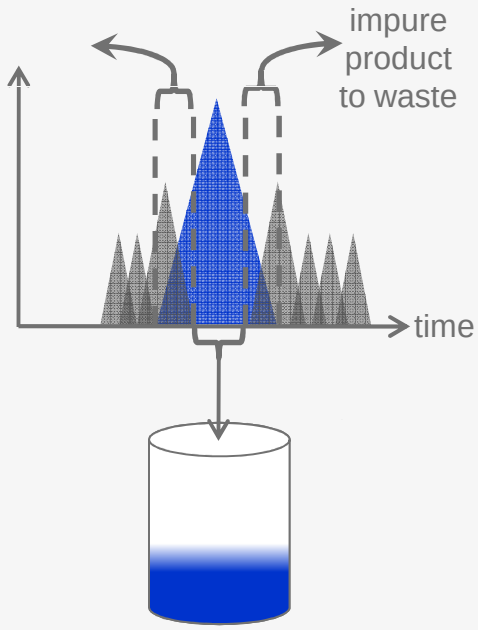
- In shotgun proteomics (bottom-up), due to the complexity of the samples, extensive front-end separation is essential to alleviate the problem of peptide undersampling, to maximize protein identifications and to tackle the vast dynamic range
- Current approaches are using multidimensional LC-MS/MS analysis of enzyme-digested proteins as a standard technique to reduce sample complexity
- Multidimensional sample fractionation is complex and slow and is usually customized for specific product classes as one-dimensional chromatography is usually not selective enough provide a general separation principle for all sample targets
- The problem is accentuated by the fact that in shotgun proteomics, current methods focus on acquiring MS/MS spectra on as many molecular species as possible without any care for whether those peptides are of interest or not
- The dynamic range in the context of a complex mixture is often limited because the ion trap can fill with co-eluting high abundance species as opposed to low abundance ions
- While the detection by MS has made great progress in the past years, leading to ever increasing sensitivity, sample fractionation progress has stalled

Discovery: The Solution

- An alternative to multidimensional chromatography is the use of a mass spectrometer with sufficient resolution and peak capacity to handle the complexity of the mixture with only a single dimension of chromatographic separation. Recent advances in commercially available hybrid Fourier transform mass spectrometers have facilitated the routine acquisition of mass spectra at >50,000 resolution, with accurate mass, and on a chromatographic timescale
- A downside to this approach is that the cost, complexity, and even footprint of these instruments limits the number of labs that have the resources available to purchase and operate these instruments
- The Contichrom® Discovery LC equipment uses a novel fractionation process allowing to selectively enrich any chosen species within a large excess of other interfering species thereby increasing the sensitivity of detection for a chosen species a 1000-fold
- The Contichrom® Discovery LC can be coupled online to a MS/MS allowing to automate the discovery process

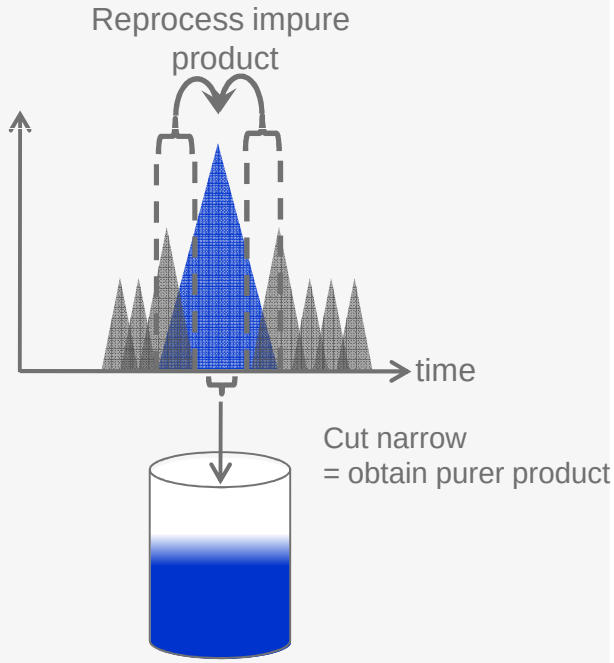
The process principle: recycle until it‘s pure

Conventional batch chromatography



pure product

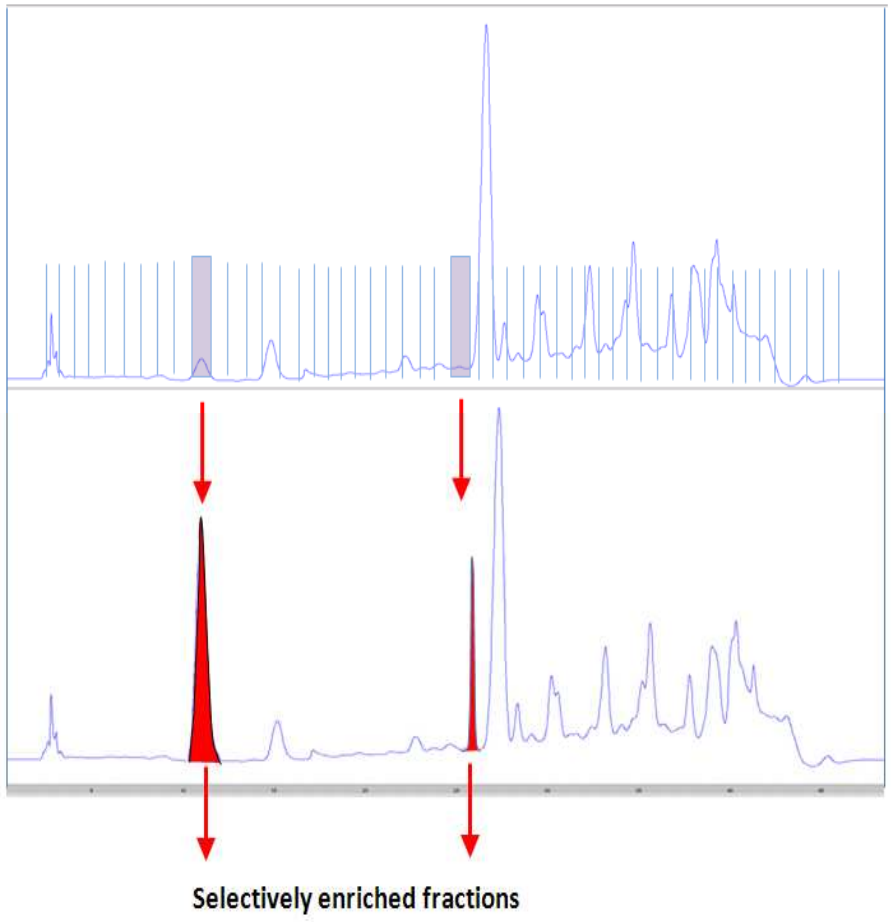
Chromacon‘s novel internal recycling chromatography (MCSGP)



more and purer product

Selective enrichment of target fractions

- The MCSGP process using the Contichrom® equipment allows to select a region of the chromatogram which is selectively enriched
- The enriched fraction is either directly analyzed by MS/MS (top-down) or subjected to tryptic digest before MS/MS analysis (bottom-up)
- For very complex samples, the enriched fraction can be also subjected to an orthogonal further fractionation step before subjected to MS/MS analysis



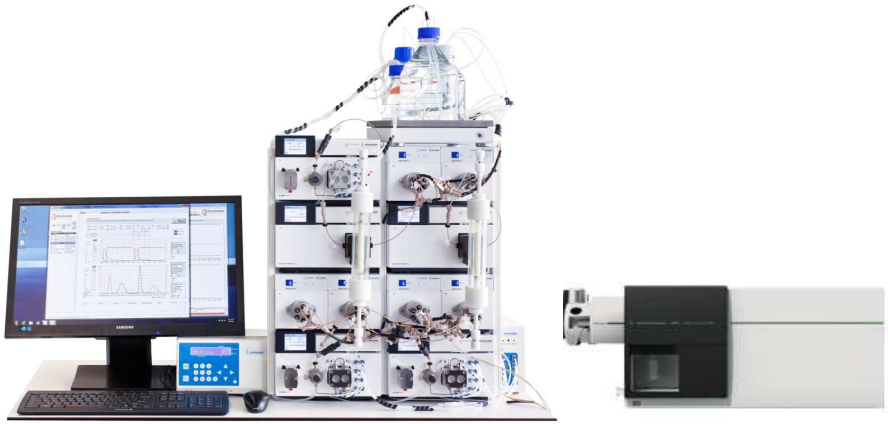
Contichrom®: Equipment for Discovery

Discovery

- Enabling on-line enrichment of lead targets for enhanced discovery via LC-MS/MS

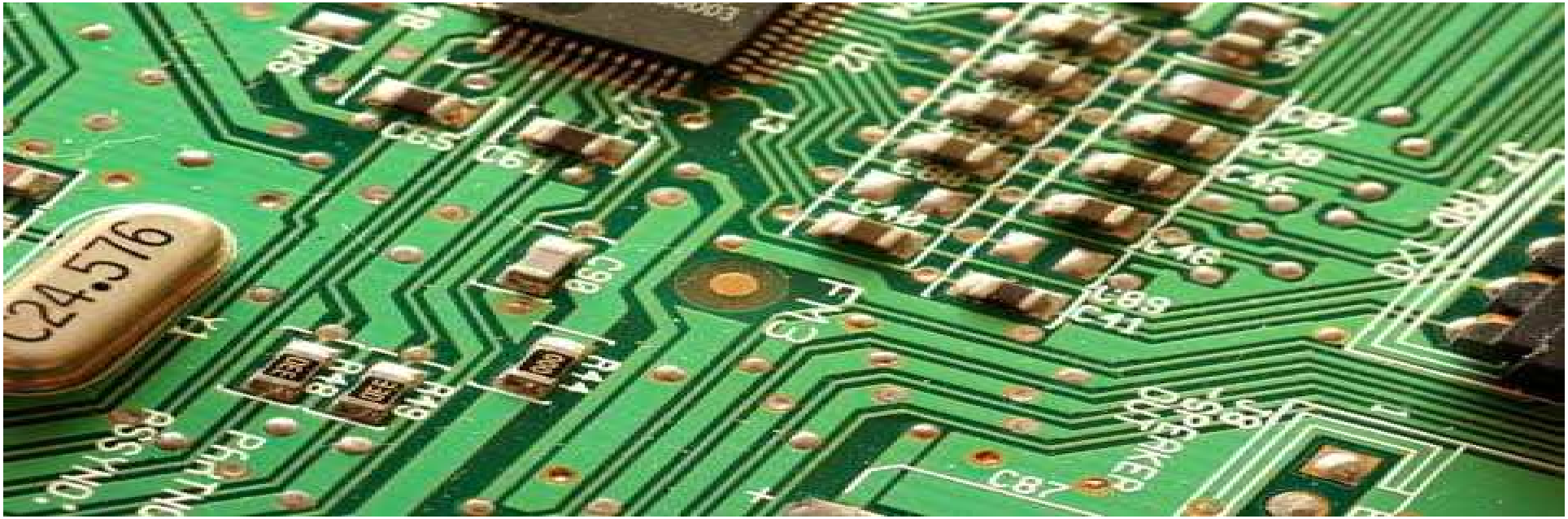
Semi-preparative Labscale

- Cost-competitive, all-in-one process capabilities for discovery and purification



MCSGP coupled to MS/MS (in development)

Semi-preparative Lab-scale equipment **launched**



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Contichrom® Software

Contichrom® software

Fast and secure process development

- Wizards with graphical user interface for easy method programming
- Automated conversion from batch to MCSGP process
- Extensive library of pre-defined methods for all standard operations

Easy to operate

- Intuitive software for operation of batch and MCSGP
- Active flow path highlighted in flowsheet
- Pause/continue functionality, even for continuous chromatographic operations

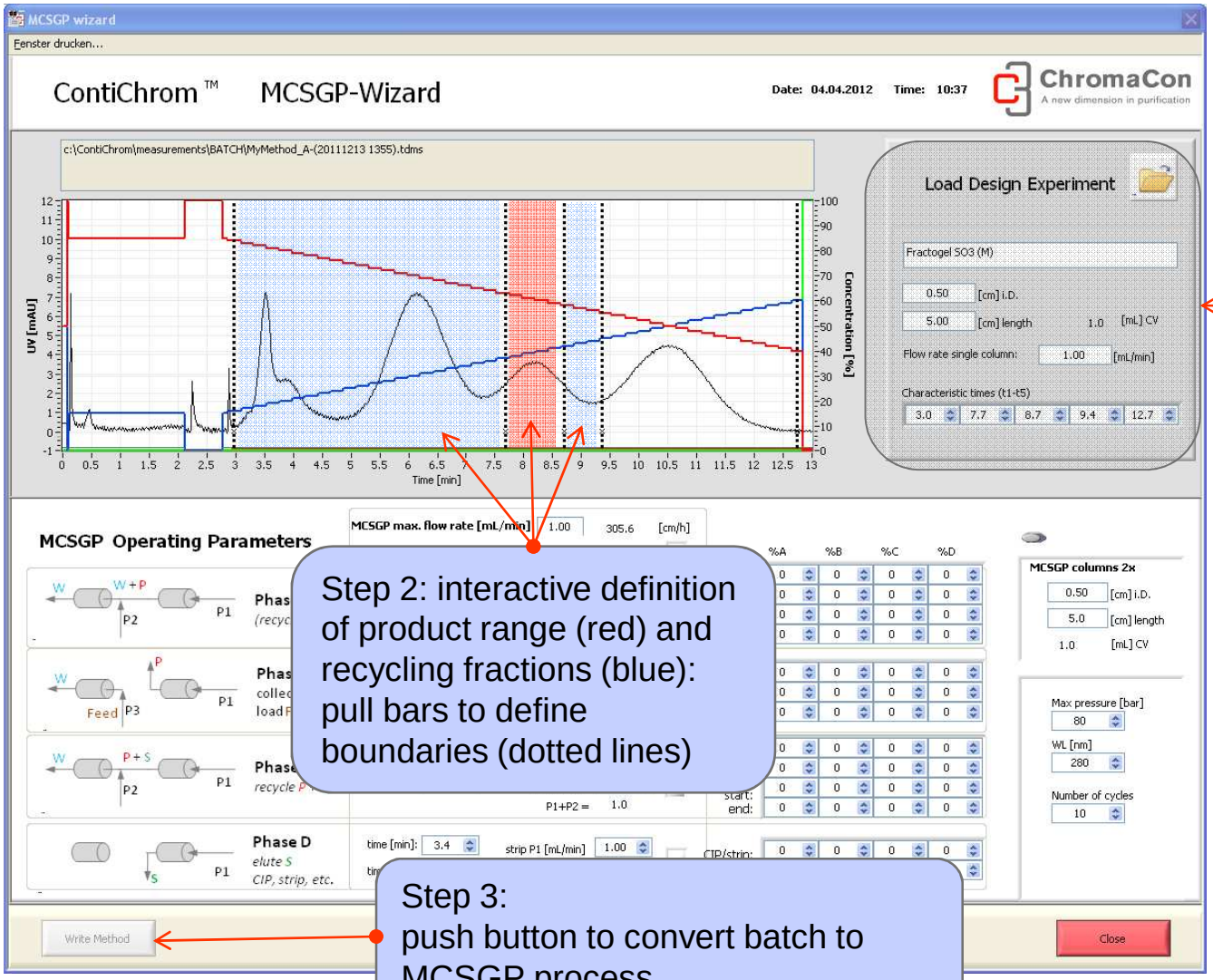
Integrated evaluation and reporting

- Detailed evaluation capabilities with standardized PDF reports
- Data export functions

Full data security and traceability

- Full audit trail and change control
- User management hierarchy provides high operational and data security
- FDA 21 CFR Part 11 compliant

Automated conversion of batch to MCSGP method



Step 1:
retrieve chosen chromatogram of batch run from database

Step 2: interactive definition of product range (red) and recycling fractions (blue): pull bars to define boundaries (dotted lines)

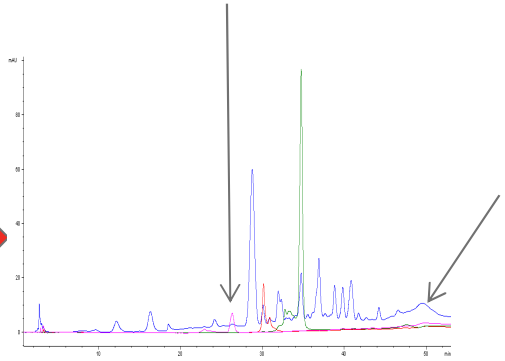
Step 3:
push button to convert batch to MCSGP process

Contichrom® discovery process

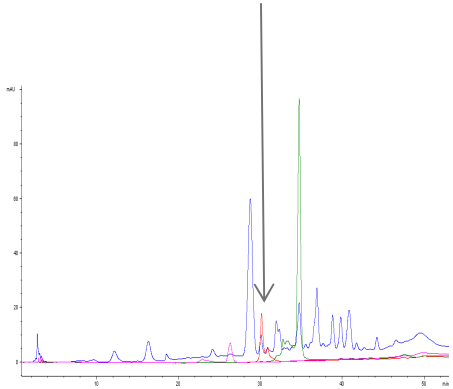
Red: 1D Contichrom™ LC with online selective enrichment of a rare species

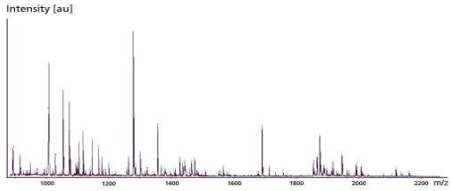
Protein mixture

Peptide fragments



Blue: original chromatogram

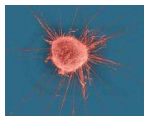




MS/MS Spectrum

Repeat 1D LC with online selective enrichment of another rare species until all peaks sequenced

Match against database



Isolate rare species For functional characterization

Contichrom® in Discovery case I

Target identified

Hits identified

Lead identified

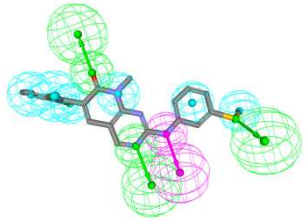
Lead isolated

Lead characterized

Lead to development

1D-Analytics with Contichrom® MS/MS

Contichrom® semi-preparative chromatography w/o MS-MS



Having sufficient feed material

Identifies lead by structure and RRT

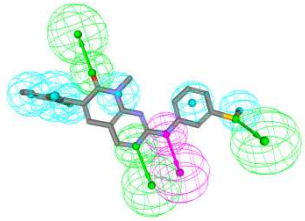
Enriches & isolates lead for functional characterization

Contichrom® in Discovery case II

Target identified
Hits identified
Lead identified
Lead isolated
Lead characterized
Lead to development

2D-Analytics with MS/MS

Contichrom® semi-preparative chromatography w/o MS-MS



feed material is limited

Identifies lead by structure and RRT

Enriches & isolates lead for functional characterization

The need for target enrichment

- After discovery of a new lead by analytical chromatography, there is a major problem to purify a chosen lead with sufficient purity and in sufficient quantities, so that this specific lead can be first identified and then studied in the absence of other interfering substances
- With classical HPLC systems, several hundred runs may be needed to isolate sufficient target lead from a complex mixture.
- In contrast, using the Contichrom® platform allows to enrich and then isolate the target lead continuously by internal recycling and continuous addition of feed.
- Contichrom® can be coupled to a MS system for lead identification
- A Contichrom® Discovery equipment platform is currently in development

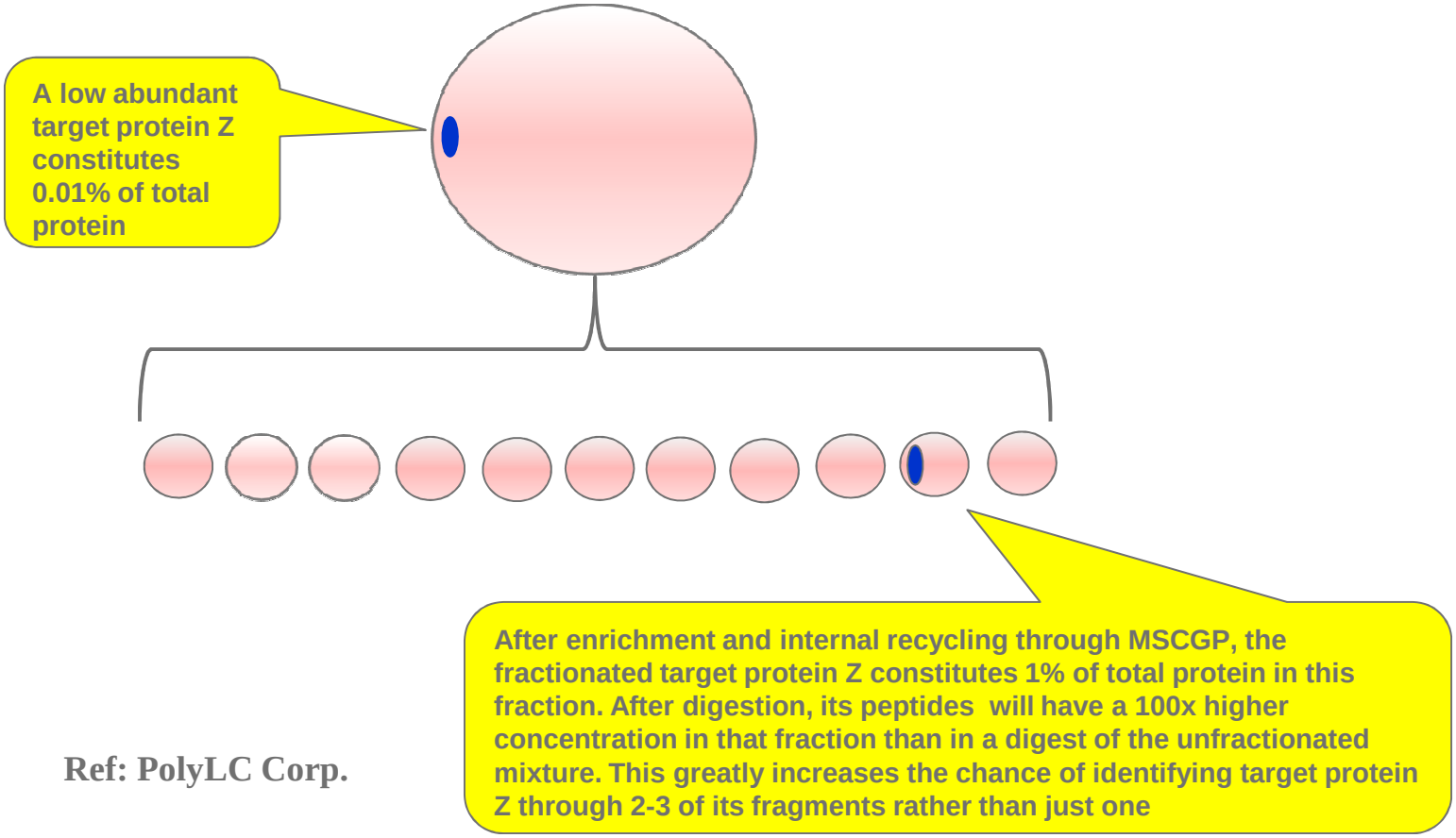
Contichrom® enabling features in Discovery

- The Contichrom® Discovery platform is enabling the discovery and isolation of novel leads due to the synergy of some technology features:

  - The use of small-particle size, high resolution analytical columns for semi-preparative isolation of leads
  - The use of the MCSGP process principle in countercurrent mode allowing to define high purity and yield as fixed parameters
  - The automated operations mode allowing first the continuous enrichment through internal recycling of a target and then its isolation amidst a vast excess of other proteins
  - No other Discovery technology has the capability of selective enrichment of an unknown target before identification and isolation

Example: Fractionation of a proteome

- The fractionation of intact proteins of a proteome is necessary because it results in an increased likeliness of detection of proteins of low abundance



Ref: PolyLC Corp.

Example: Fractionation of a proteome

ASSUMPTIONS:

A: 5000 proteins in the mixture yielding 40'000 tryptic fragments

B: the average Mw of the proteins is 30kDa

C: at least 15 fmol are needed to obtain a peptide sequence by MS/MS

- This means 450 pg of each protein x 5000 proteins = 2.25 µg of total protein needed
- Unfortunately proteins differ by 10^6 in relative abundance and few proteins dominate the sample. Therefore in order to get 15 fmol of the least abundant protein you need to have at least 10^5 more sample
- 2.25 µg x 10^5 = **225 mg TOTAL PROTEIN NEEDED**
- This means that you need to collect and process 500+ fractions at the SCX step

Ref: Andrew J Alpert PolyLC Inc.

Application of the Contichrom® Platform

- General mining of proteomes, automation possible
- Mining for plasma proteome and isolation of biomarkers and targets
- Mining and isolation of natural products with therapeutic potential
- Fractionation and isolation of leads or product-related impurities for further analysis and validation

Proteomics of Complex Mixtures

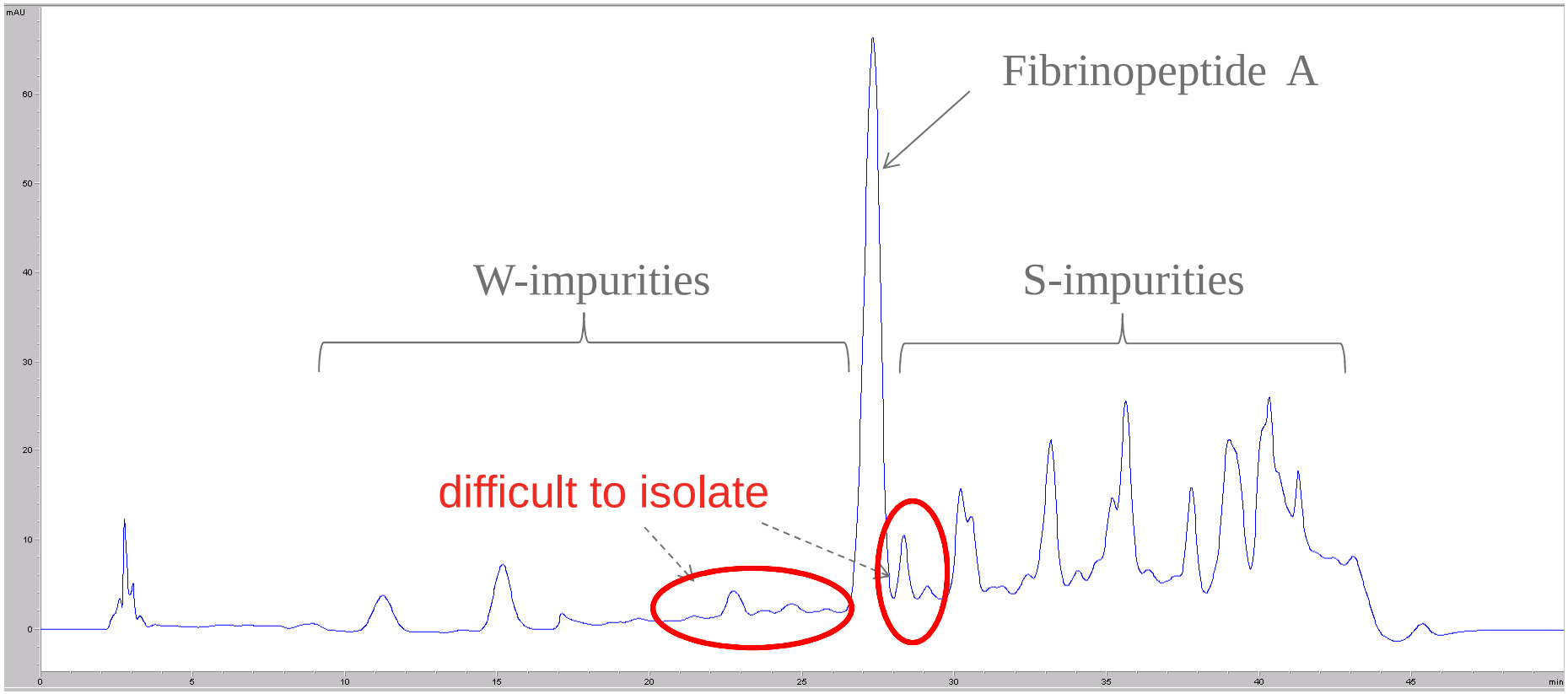
- To process several hundred fractions by conventional chromatography is time and resource intense and often not feasible

- The Contichrom™ platform allows to automatically enrich isolate lead compounds rapidly for further analysis

Example: Enrichment of a peptide related impurity

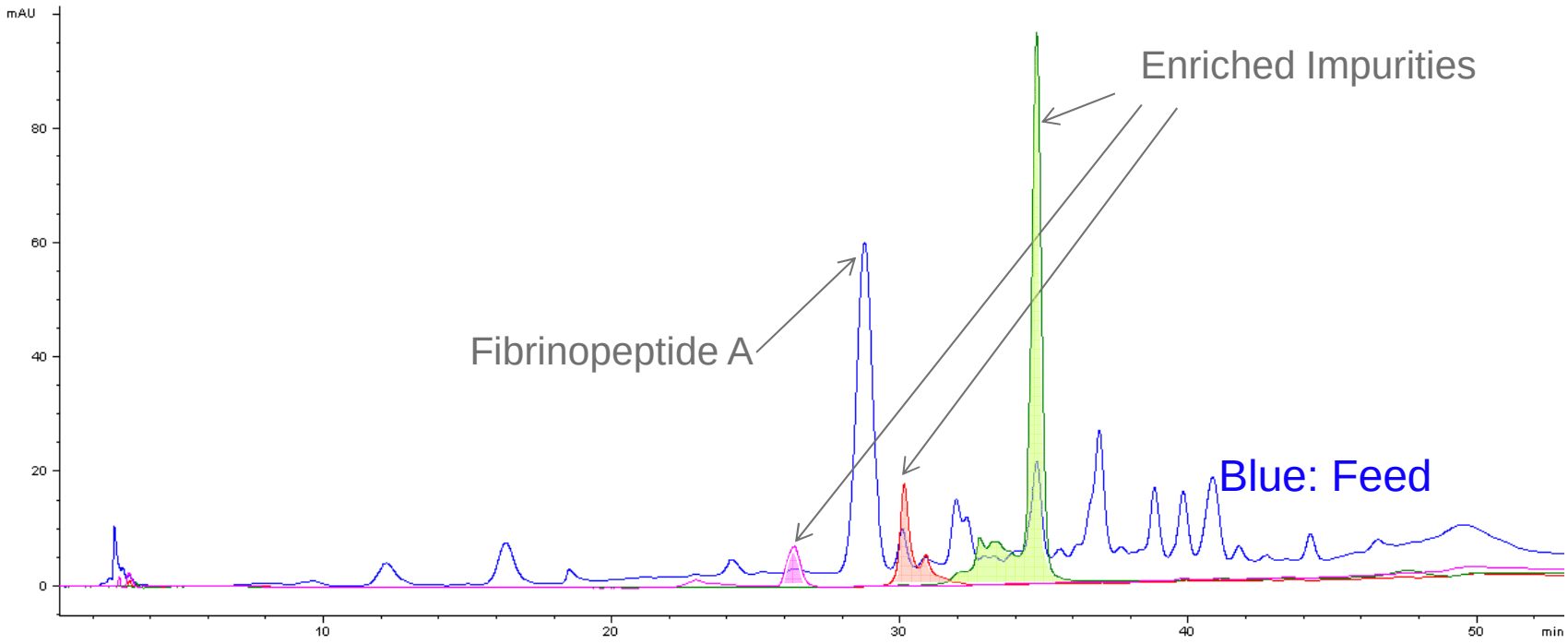
Example: Related Impurity Isolation

- Fibrinopeptide A: Analytical injection 100 uL of Feed (3.0 g/L Fibrinopeptide) onto preparative column



Enrichment and isolation of impurities by Contichrom®

- Overlay of chromatogram of final gradient elutions (1min/ frac) showing W-component fractions and close S-component fractions



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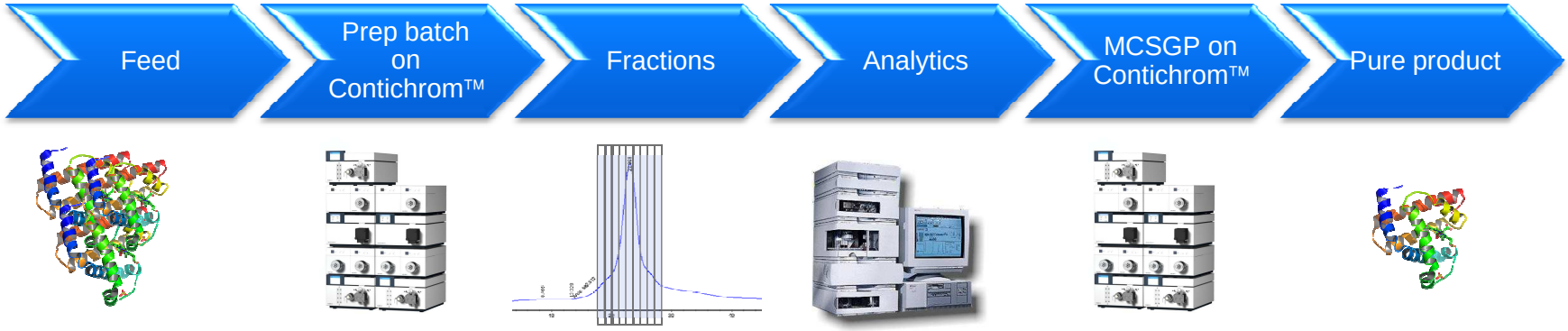
Appendix

Prep chromatography with Contichrom®



- Advantages:
  - no waste of product due to high yields
  - avoids tedious collection of prep fractions
  - Single run
  - High throughput

Target isolation by batch chromatography