# CONTROLLING SAMPLE DISPERSION IN MULTIDIMENSIONAL CAPILLARY LC-MS

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## INTRODUCTION

A common technique in proteomics is to digest the whole protein fraction of a tissue sample and analyse the resulting mix of peptides. Samples generated this way tend to be very complex and due to a number of required sample preparation steps can be dilute.
By using a multidimensional approach one can resolve a number of issues. The methods can be divided in two groups: sample preparation and improvement in resolution.

## SAMPLE PREPARATION INCLUDES:

**• Preconcentration/desalting**
A column with a lower affinity (e.g. C4) can be positioned in front of an analytical C18 column. The column would be short with a relative large ID and is used to load a large, dilute sample fast onto the trap column while at the same time remove salts and other contaminations originating from the digest solution. The sample is then eluted in a concentrated and purified form onto the analytical column.

**• Depletion/Enrichment columns**
These columns utilise a specific interaction to either remove or to capture certain parts of the sample. Any specific interaction or biorecognition can be utilised. Common examples are immuno affinity columns for the depletion of high abundance serum proteins (**Figure 1**), immobilised lectins for binding of carbohydrate structures in carboxylated peptides or immobilised metal ions (Fe(III) or Ga(III)) to bind phosphorylated peptides.

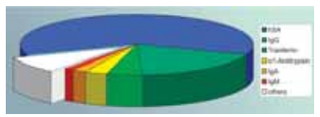

*Figure 1. Abundance of proteins in human serum. Only a small portion of the "others" fraction is of any interest for proteomic research. Depletion of the 6 highest abundant proteins simplifies the analysis by reducing the masking effect of these proteins. Drawback is the possible co-precipitation of some proteins.*

## IMPROVEMENT OF RESOLUTION:

By employing a precolumn with opposite selectivity one can subdivide the sample in a number of fractions. The most commonly used combination is a strong cation exchanger followed by an analytical C18 column. The sample is loaded onto the SCX column and eluted with increasing salt concentrations in a step-wise fashion onto the analytical column (or a prior desalting column) (**Figure 2**).

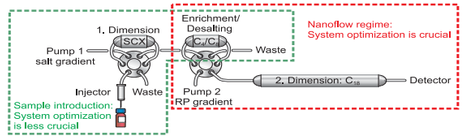


*Figure 2. Possible setup for a multidimensional separation in proteomic science.*

## IMPORTANCE OF HIGH RESOLUTION

A mammalian cell expresses about 2000 proteins which when digested can form >10,000 peptides. **Figure 3** shows the increase in complexity of the tryptic digests of Cytochrome C (a – Mw:12,200) and a monoclonal antibody (b – Mw: 150,000).

The task of separating the individual peptides becomes significantly more complex when mixtures of proteins are analysed. When using an automated MS/MS detector, the software has to decide which masses (peptides) should be further investigated by the second dimension MS (sequence determination). To limit the amount of data the number of signals further analysed are limited to, for example, 10. If there are more than ten peptides overlapping in one peak, the peptides from the lowest abundant protein are not analysed and therefore lost. However, in a lot of cases the low abundant proteins are of higher scientific interest.

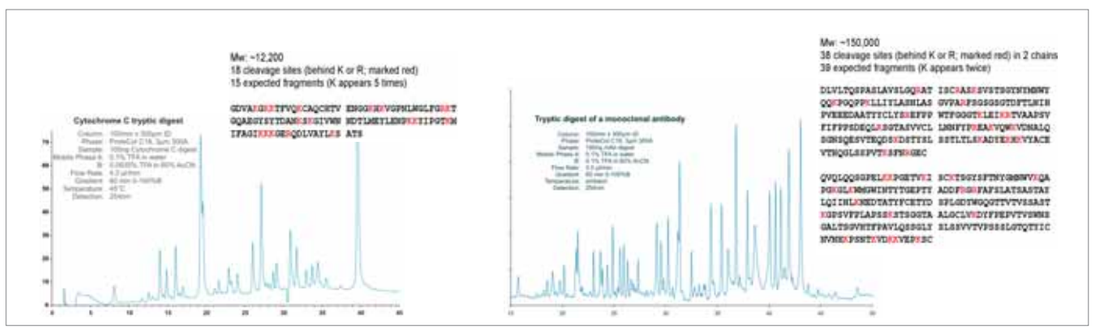


*Figure 3. Chromatogram and sequence of Cytochrome C and IgG antibody*

## FACTORS CAUSING BAND BROADENING

There are a number of factors causing band broadening in Capillary LC:

**• Void volumes**
Void volumes can be introduced by non-ideal connections and excessive tubing length. The influence of tubing length on the chromatographic performance is shown in **Figure 4**.

**• Disturbance in the laminar flow**
Under normal chromatographic conditions, the flow inside capillaries is laminar. Each connection can disturb the laminar flow and cause turbulence by having a gap between the connecting capillary ends or by misalignment of the bore caused by the tolerance on concentricity of capillary tubing (**Figure 5**).

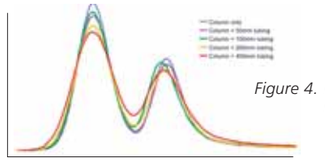

*Figure 4. Effect of tubing length on resolution (tubing ID 50µm.*

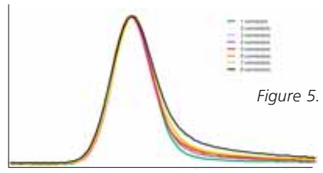

*Figure 5. Effect of the number of connections on peak symmetry.*

**• Nonspecific interactions**
A sample has the opportunity to interact with any surface within the flow path. If the surface chemistry differs from the selectivity of the stationary phase these interactions may lead to peak tailing and a reduction in chromatographic performance (**Table 1**).

| SOURCE OF NON-SPECIFIC INTERACTION | REMEDY |
|---|---|
| Exposed parts of the packing matrix | End-capping |
| Polymeric tubing material can be active for some molecules | Change tubing |
| Fused silica (Si-OH interacts with bases) | Chemical deactivation |
| Ferrous metal (tubing, frits) binds phosphate | Avoid Fe, coat element |

*Table 1. Origins on non-specific binding in a chromatographic system and method to minimize the effect.*

## THE PROTECOL™ APPROACH TO CAPILLARY LC

Unique features of the ProteCol range:

- Integrated connection tubing: reduced the amount of connections thus reduced chance of user-introduced voids
- Zero volume butt connections: no additional volumes through connections
- Minimal volume filters and pre-columns
- All wetted surfaces are deactivated: minimisation of nonspecific binding

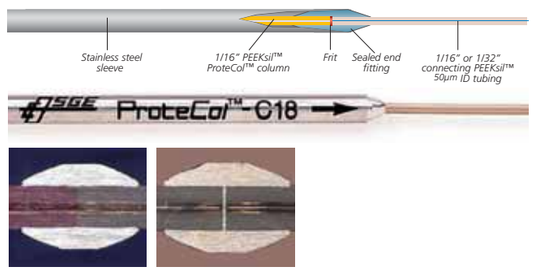


*Figure 6. ProteCol column design and close up of cross-section of the column end.*

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