### Introduction

Identification of proteins in a complex biological matrix presents a formidable challenge for analysis. This challenge is addressed by the development of specific separation methods to achieve resolution and high recovery results for the downstream analyses of these samples. Numerous techniques are employed for resolving protein mixtures including gel electrophoresis, liquid chromatography, and immunoaffinity capture. Although chromatographic separations are particularly well understood, comprehensive coverage of the complex biological samples has been slow. The key to addressing the complexity of these issues is to develop advanced chromatographic systems that enable the efficient separation of intact proteins as well as their subsequent recovery. Here, we present a novel approach for resolving protein mixtures with high efficiency and reproducibility.

### Experimental

**Flowchart:**

- **Biological Sample:** Membranes, Lipid rafts, Cellular extract, Serum proteins
- **Sample Prep:** Extraction, Subcellular, Denaturation
- **Intact BP Protein Separation:** Workflow, Top-down or Bottom-up
- **Analysis:** In-solution tryptic digestion, Digestion, Reproducible Fractionation

**Workflow:**

1. **Membrane and Soluble Proteins**
   - Membranes, Lipid rafts
   - Cellular extract, Serum proteins

2. **Sample Prep:**
   - Extraction, Subcellular, Denaturation

3. **Intact BP Protein Separation:**
   - Workflow, Top-down or Bottom-up

4. **Analysis:**
   - In-solution tryptic digestion, Digestion, Reproducible Fractionation

**Protein Recovery**

- **HPLC-Chip LC/MS**

**SDS-PAGE for Protein Adsorption Analysis**

- Column: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26

**Lipid Rafts & On-Column Delipidation**

- Lipid Extractions

### Immunodepleted Serum Proteins

- nA: Superficially Parous

**Table:**

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### Protein Recovery

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### Conclusions

We have developed a superimporuporllp macroscopic HPLC column (mRP) and optimized sample specific separation parameters and co-column adsorption strategies, that are effective as an alternative technique to SDS-PAGE separation of highly complex and hydrophobic protein matrices. We have detailed the separation advantages of performing mass spectrometric analysis directly following HPLC separation of intact proteins without the need for downstream protein separations by gel-based methods. Employing an mRP fragmentation ‘only’ strategy allows many of the traditional gel constraints such as time consumption, irreproducibility, poor recovery, limited detection and detection of only a few high abundant proteins. In our profiling study of human brain membrane proteins, the gel-free (in-solution tube digestion) approach saved 4 days of analysis time and enabled approximately 40% more protein identity when compared to an SDS PAGE separation approach.

With use of the superimporuporllp macroscopic mRP column, sample specific conditions and conditions specific conditions, temperature, we have achieved enhanced selectivity and reduced bead fouling, and have potentially reduced and reproducible separations. The multi-dimensional separation techniques described, when combined with our intermediate MALDI-TOF analysis, have expanded the dynamic range for protein analysis. In addition, intact protein separation on the mRP column was more superior to identical separations performed on standard gel column matrices. In complex protein mixtures, the mRP gave a higher degree of reproducibility, permitted greater peak resolution and enabled higher protein recovery.

### References


**Figure:**

- **Lipid Extractions**
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**Image:**

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- Lipid Extractions

### References