Novel protocols for protein purification by Free-Flow-Electrophoresis

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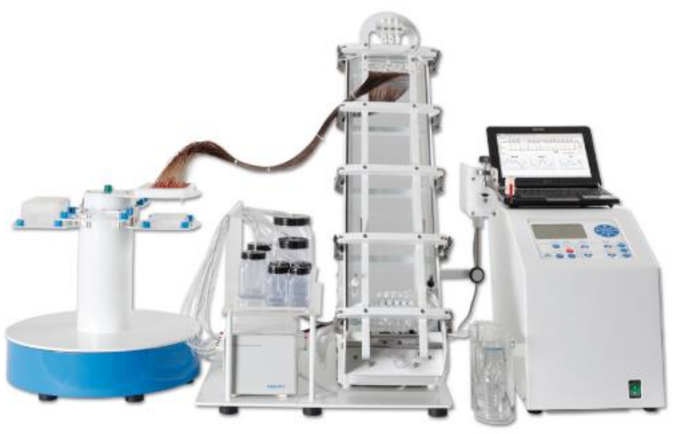
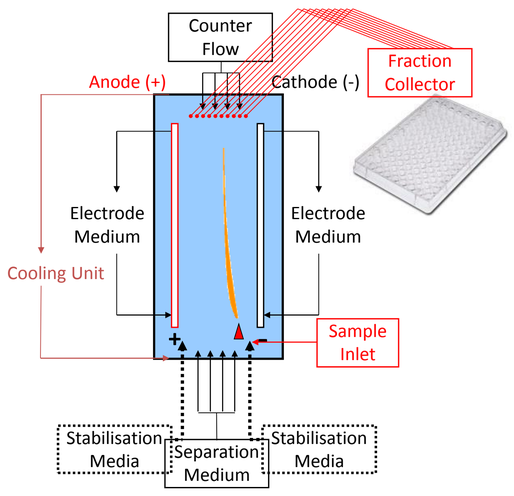
Introduction

Free-flow electrophoresis (FFE) is a **matrix-free** electrophoretic separation technique, which separates samples, ranging in size from metal ions to cells, according to minimal **differences in charge** and isoelectric point (pI). It can be used for scientific questions, where semi-preparative and **preparative amounts** of separated samples are needed. The corresponding resolution is less than Δ0.02 pH with a throughput of up to 3 mg/h.

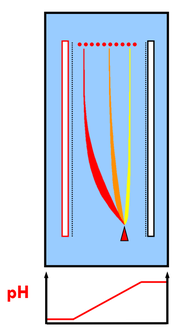
The FFE can operate under native or denaturing conditions and does not rely on a matrix for separation. Therefore it is ideal for separation problems, where the fragile three-dimensional structure of proteins and protein complexes have to be maintained. Because of the versatility of the technique, separation protocols can be tailored to fit the separation needs of the customer. There is already a wide range of protocols available for the separation of samples like rare metal ions, protein isoforms, liposomes, multiprotein complexes, peptides, cells, DNA origami, blood serum and nanoparticles.

Principle of Free Flow Electrophoresis

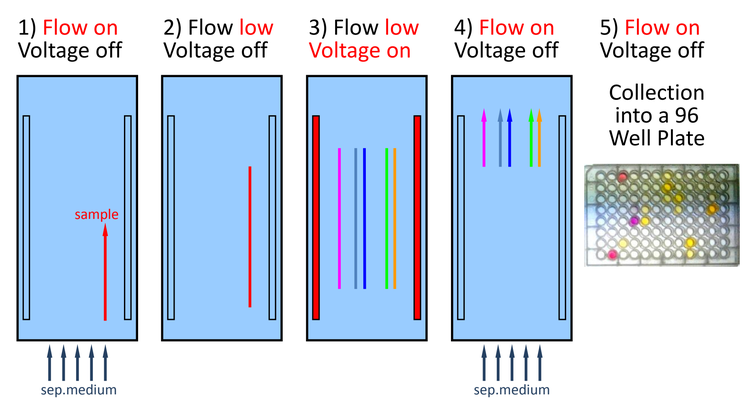




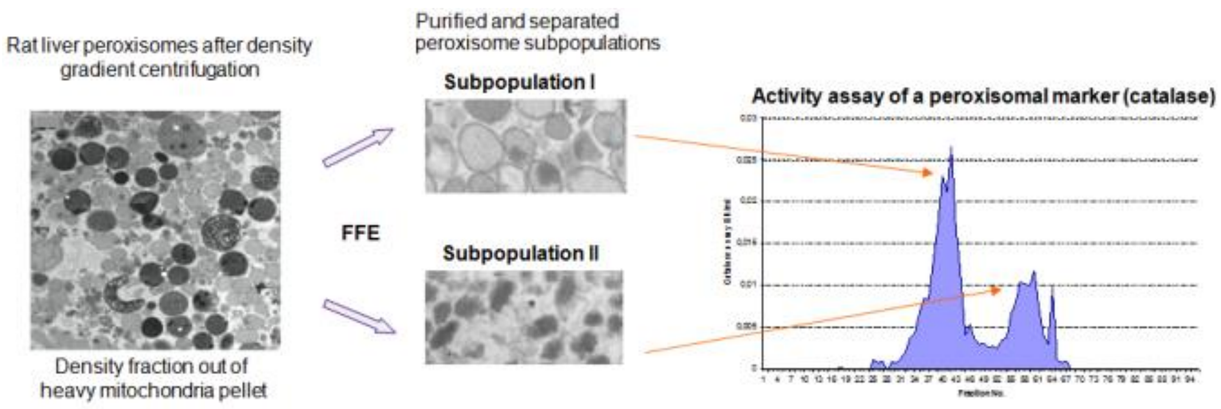
IEF-FFE



iZE-FFE

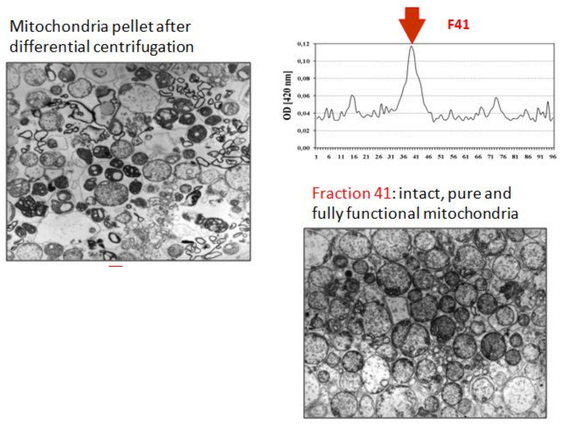


Identification of Subpopulations of Rat Liver Peroxisomes



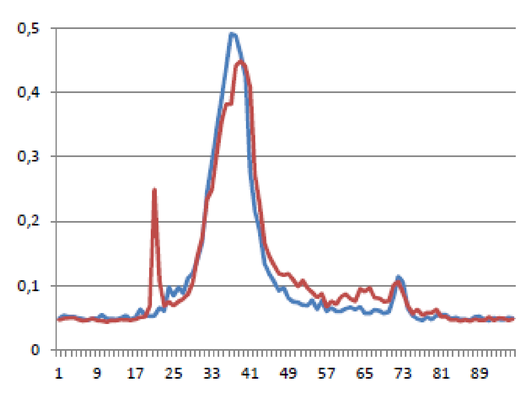
Two different subpopulations of Peroxisomes derived from rat liver homogenates. They are documented by electron microscopy analysis and enzymatic (Catalase) activity tests.
The rat liver homogenate was pre-purified by density gradient centrifugation and the pelleted sample was solubilized in FFE buffer.
The FFE separation was performed in zone electrophoresis mode and two different subpopulations of peroxisomes could be identified.

Purificiation of Mitochondria from *S. Cerevisiae*



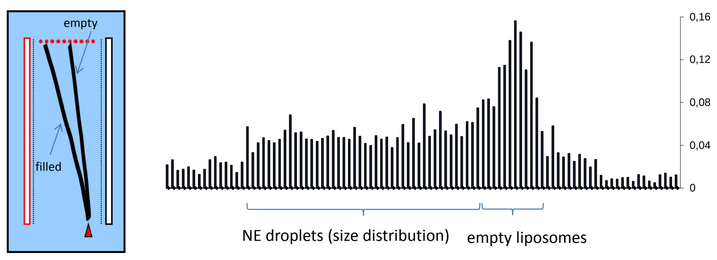
The example shows the homogeneity and overall structural integrity of the ZE-FFE purified mitochondria. It is assessed by electron microscopy analysis of ultra thin slices of pelleted mitochondrial preparations

Comparison of commercial Liposomes



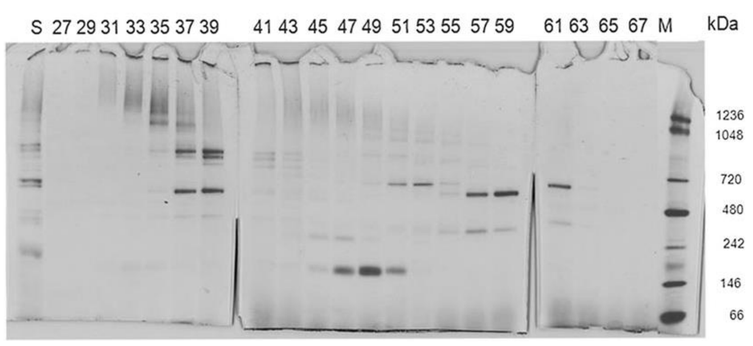
The example shows the comparison of two commercial liposome mixtures with the help of iZE-FFE.

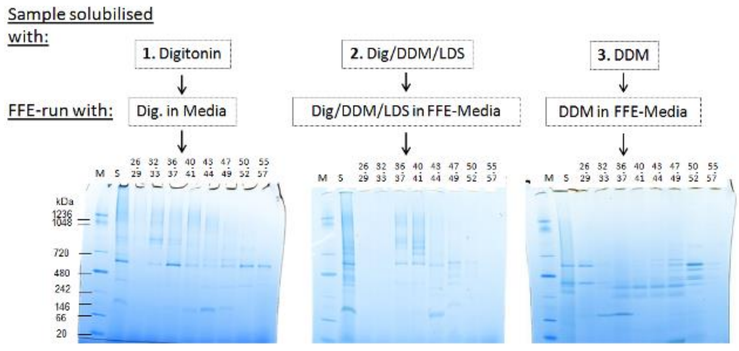
ZE Separation of Nanoemulsion-Liposome-Mix by FFE-ZE



Particles with high density sediment into a low velocity stream of the parabolic flow profile. These particles are exposed to the electric field for a longer time and therefore migrate a larger distance along the electric field.
Liposomes (Ø 230nm) are filled with buffer, NE droplets are filled with perfluorooctyl bromide (ρ = 1.93 g/mL).

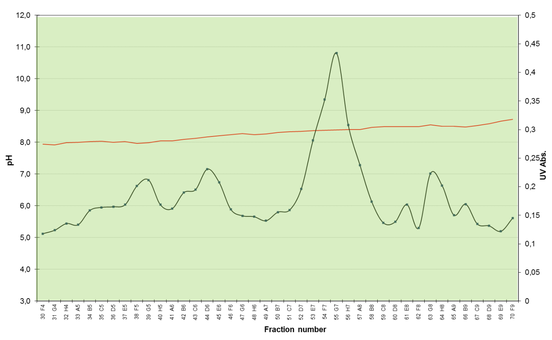
Separation of protein complexes by FFE-IZE



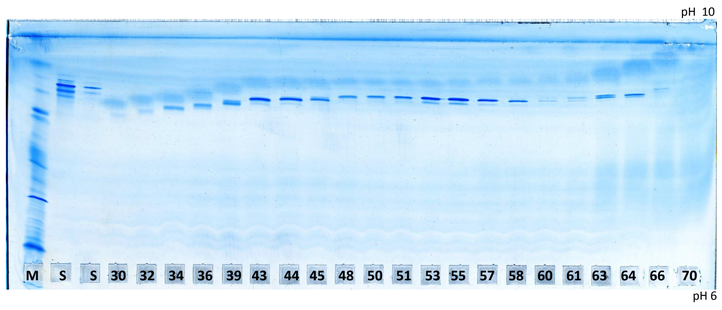


Blue Native Gels of FFE-separated thylacoid membrane protein complexes, after solubilization by different dergents.

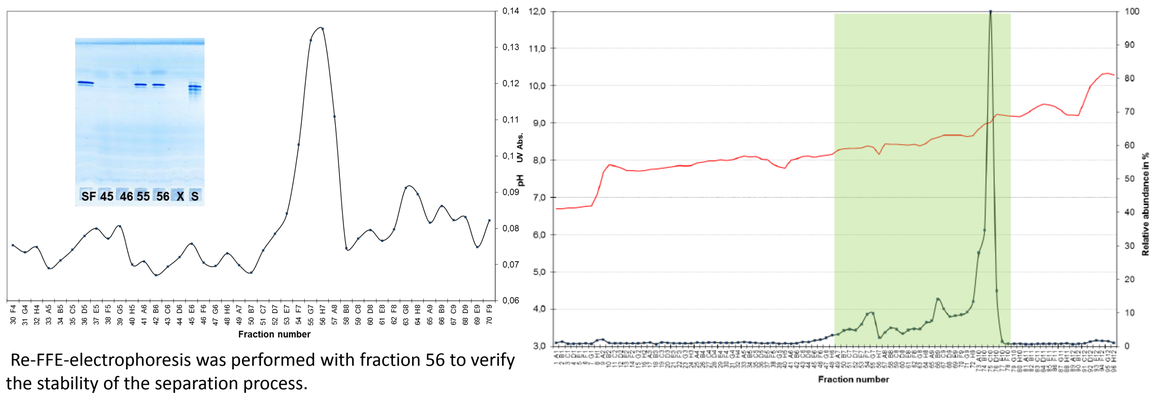
Separation of protein isoforms (mAB) by FFE-IEF



IEF-FFE separation of crude mAB sample on pH with an ultraflat pH-gradient from 8 to 8.5. The fractions within the green area were applied to an IEF gel (Figure 2).



IEF-PAGE (pH 6-10) of the crude sample (S) and selected fractions of IEF-FFE separated mAB sample. M: Serva IEF Marker.



Re-FFE-electrophoresis was performed with fraction 56 to verify the stability of the separation process.

Key Features of Free-Flow Electrophoresis

- Matrix free separation, ideal for conserving protein activity/protein complexes
- High resolution separation of protein complexes, membrane proteins, protein isoforms, cells, subcellular compartments (like organelles, ribosomes, etc.),
- Separation size range from ions to cells
- Sample recovery up to 99 %, depending on sample and mode of operation
- High reproducibility of individual runs
- Separation in several minutes
- Protocols for Isoelectric Focusing separation with different commercial ampholytes and atoxic small molecular ProLytes™ for direct clinical application
- Fast and sensitive detection of separation quality via UV, IEF-gels
- Compatible with many other downstream techniques, e.g. HPLC, MS, SDS-, IEF- and 2D-GE
- Suited for separation of unstable proteins due to cooling of samples and separation chamber down to 4 °C
- Reduction of the complexity of the proteome before further proteomic analysis
- Enriching low-abundant proteins by removing excess of unwanted proteins
- Compatible with large and small sample volumes from around 50 µL up to several milliliters.
- Preparative as well as analytical operation modes
- Supports all electrophoretic separation modes (IEF, IZE, ZE, ITP)
- Can be run under native or denaturing conditions