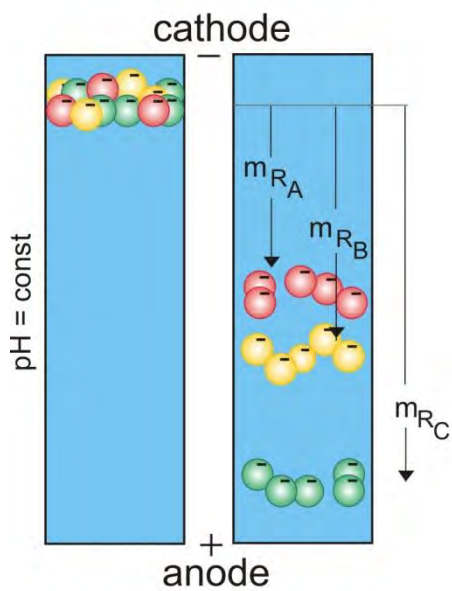
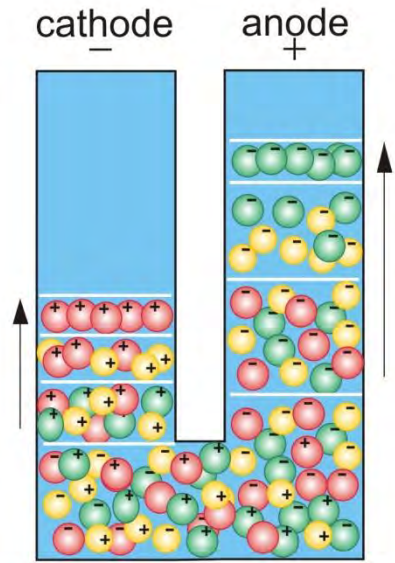


Theoretische Grundlagen von Elektrophorese-Techniken

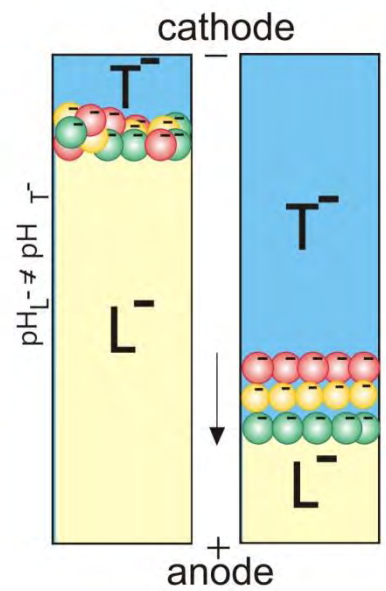
Zone Electrophoresis



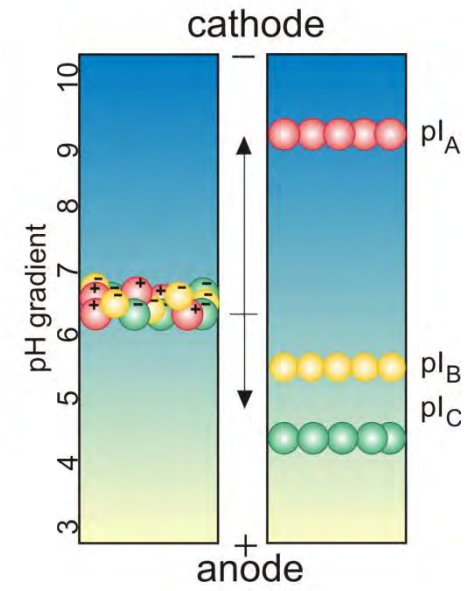
Moving Boundary Electrophoresis



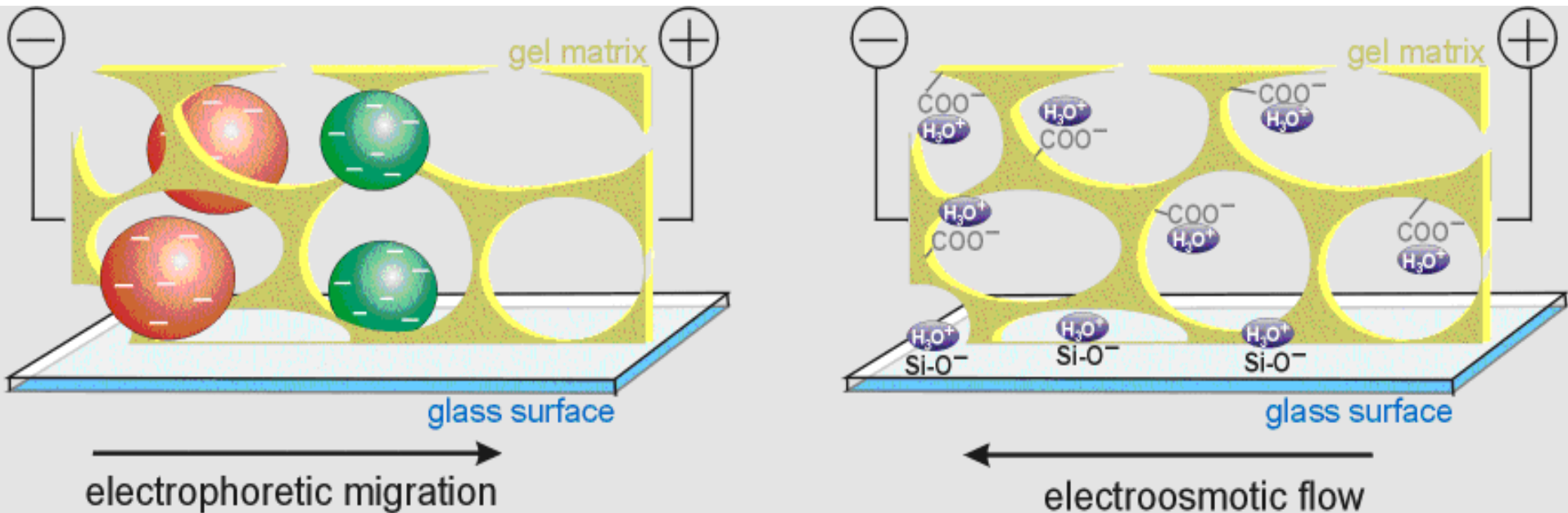
Isotachopheresis



Isoelectric Focusing

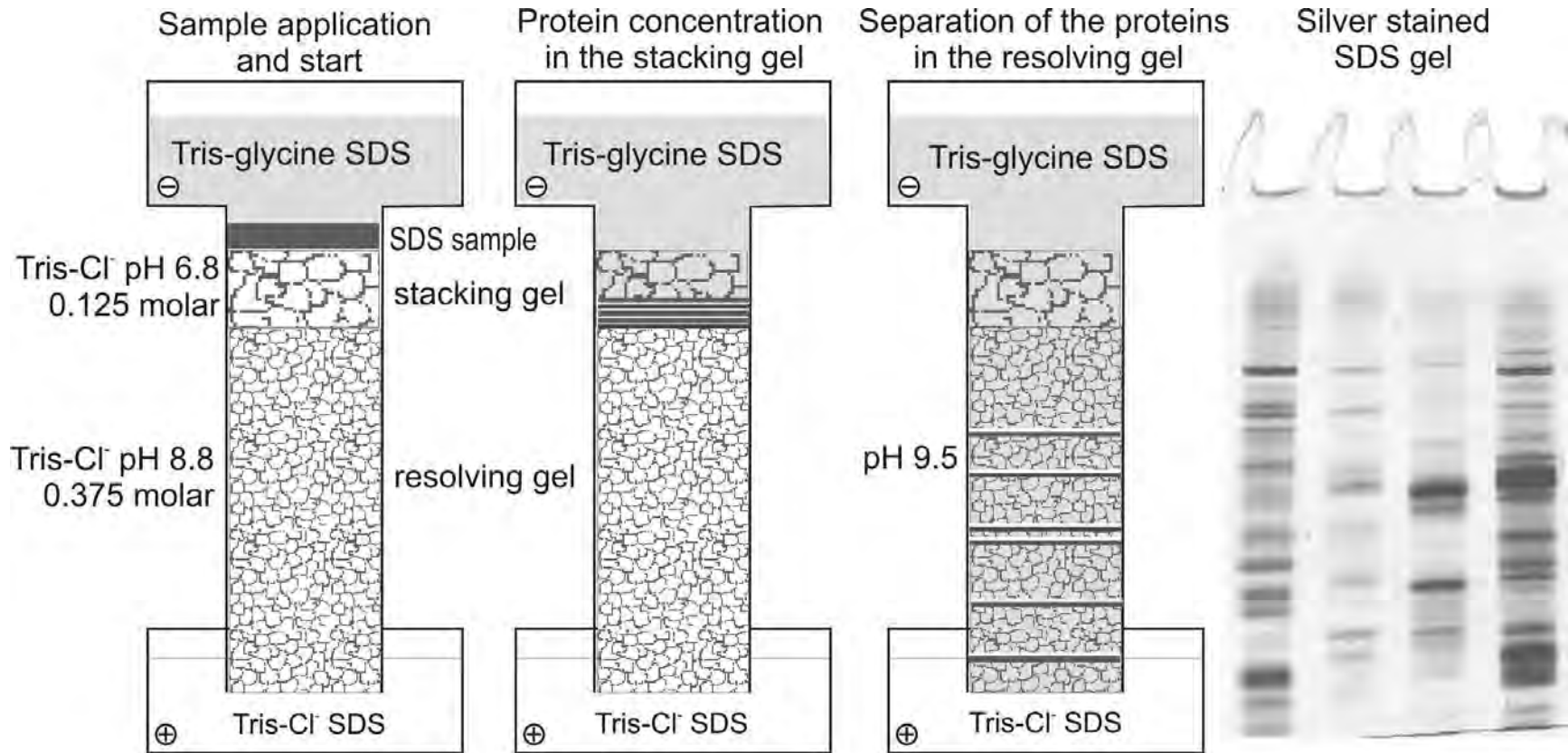


Electroendosmosis



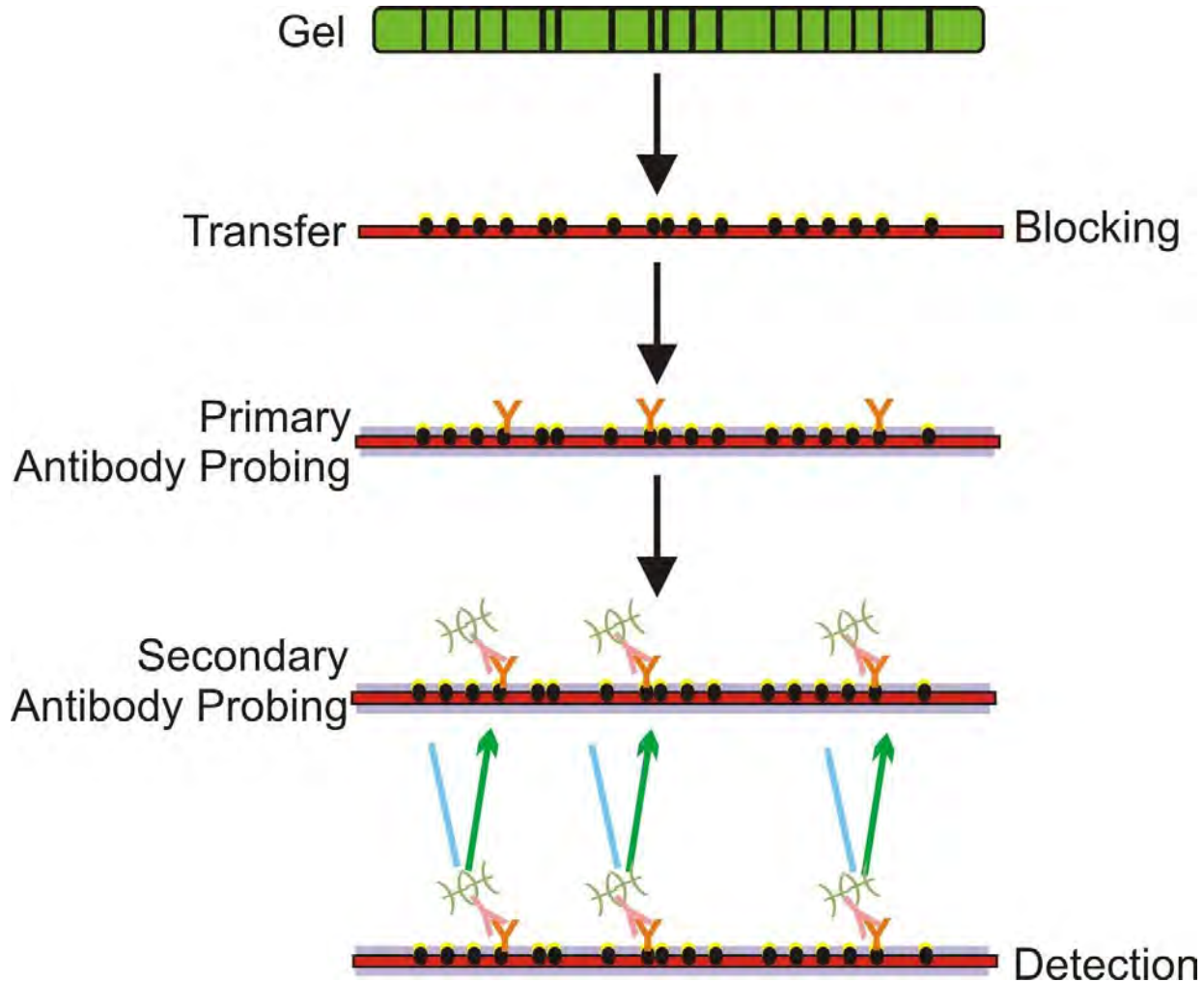
Fixed charges in the matrix and / or on a surface (e.g. glass) cause a bulk liquid flow.

SDS Disc electrophoresis



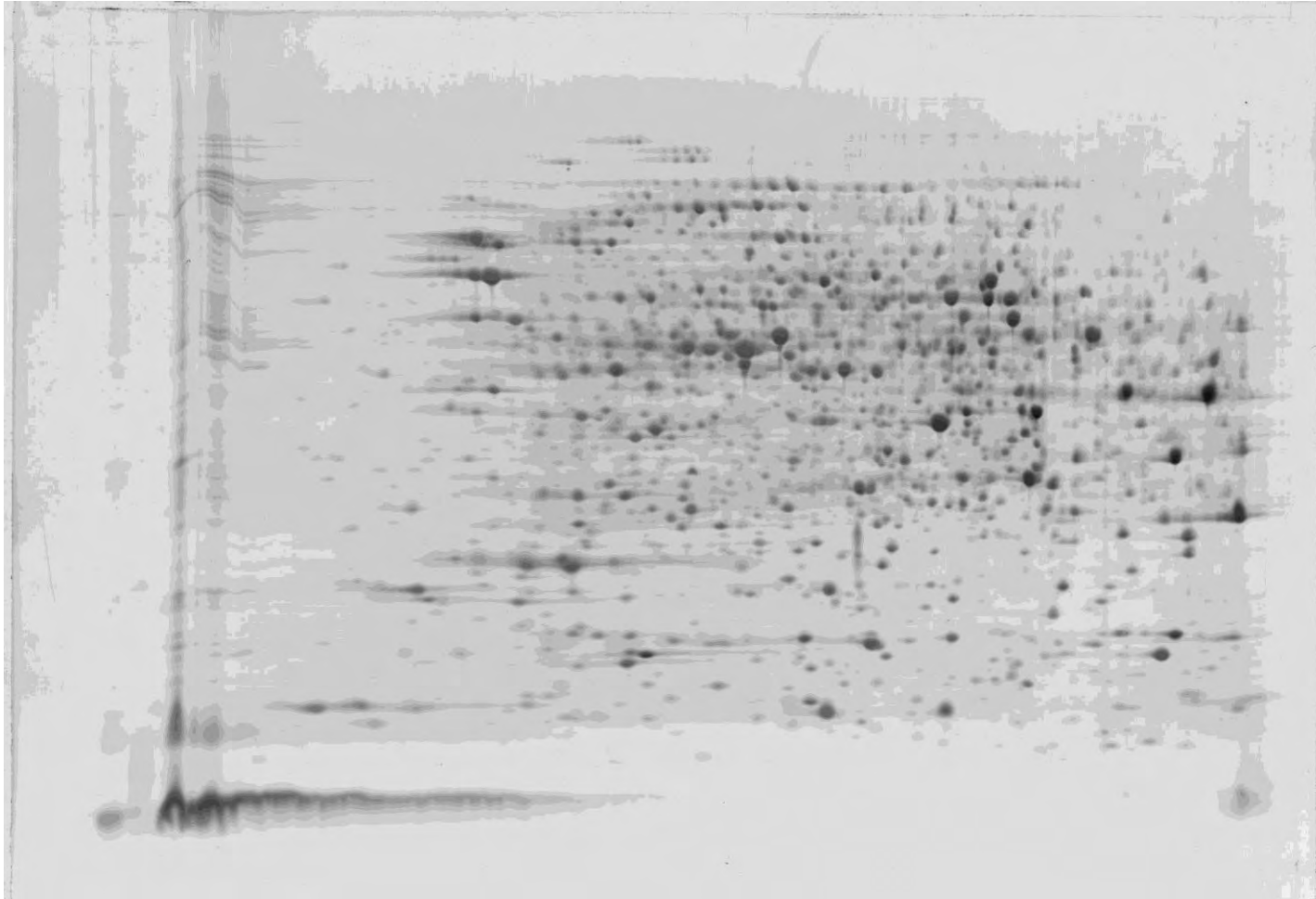
Adapted to SDS PAGE in the year 1970 by Laemmli

Western Blotting (Immunoblotting)



Two-dimensional Electrophoresis

pH 4 ← Isoelectric Focusing in 24 cm IPG Strip → pH 7

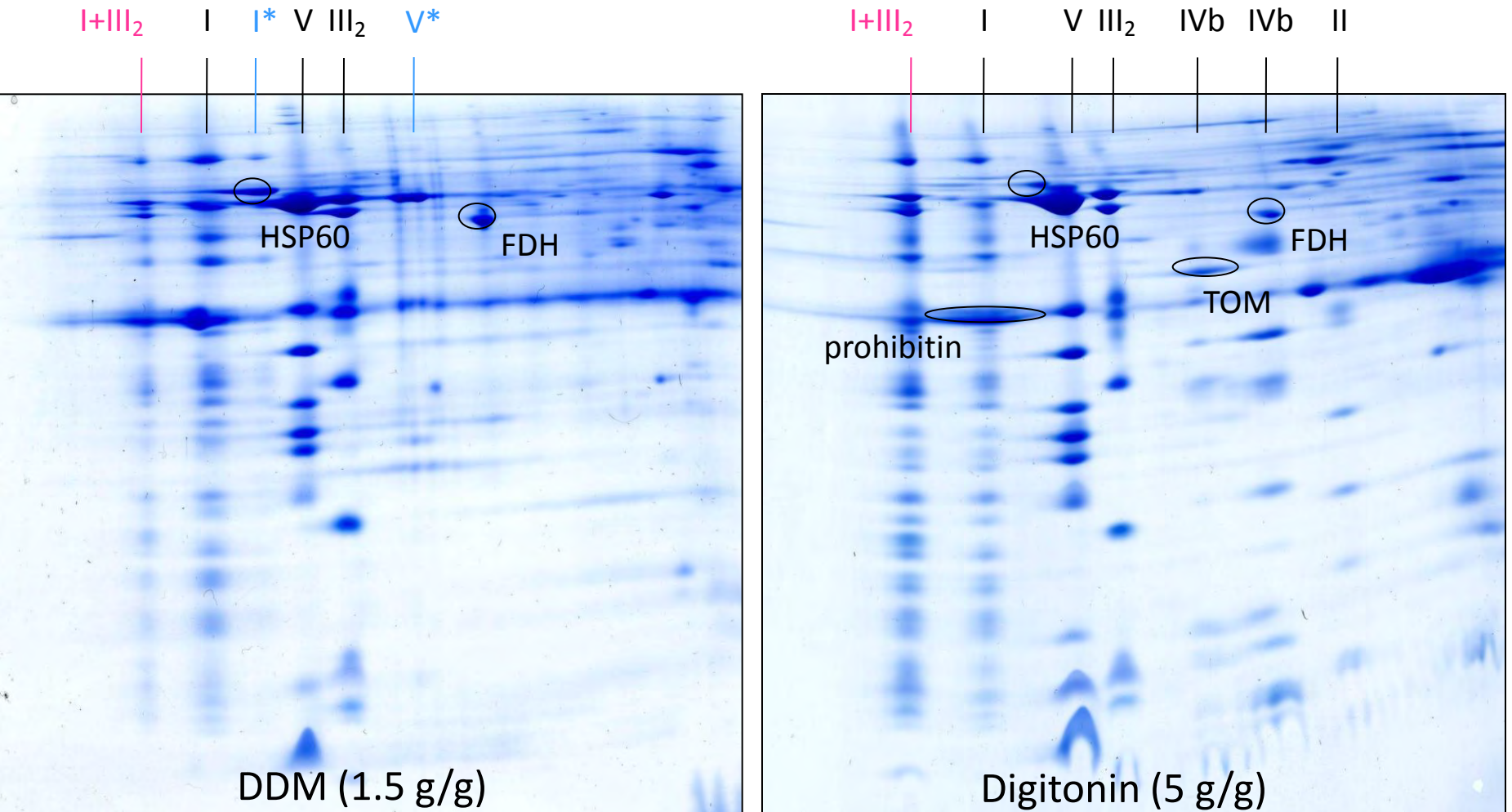


SDS
PAGE
in
12.5 % T
homogeneous
Gel



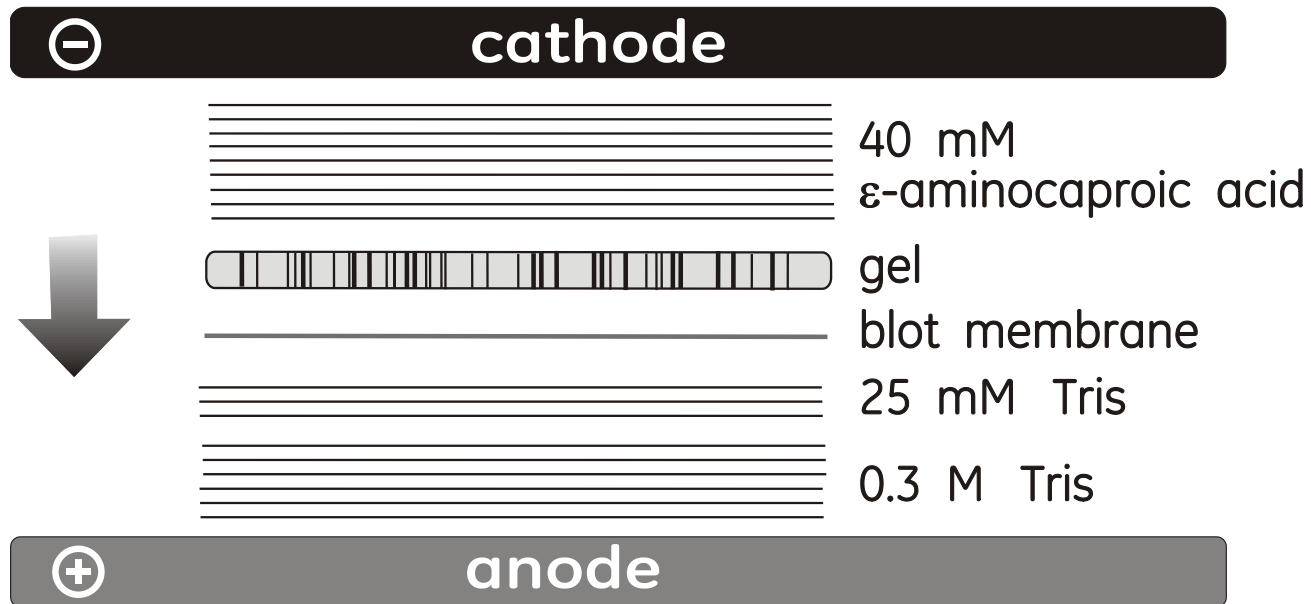
E. coli extract run in 2DGel flatbed large, fluorescent staining

Solubilization of mitochondrial proteins by Dodecyl maltoside (DDM) and Digitonin



From Prof. H.-P. Braun, Abteilung für angewandte Genetik, Universität Hannover

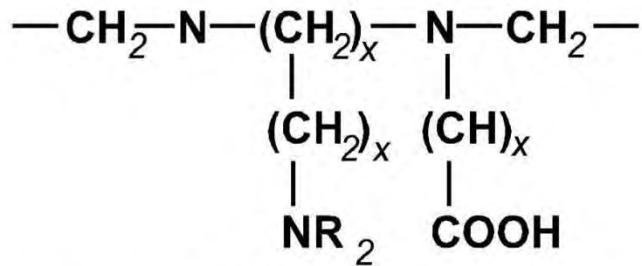
Discontinuous blotting buffer



all buffers contain 20% methanol

IEF with carrier ampholytes

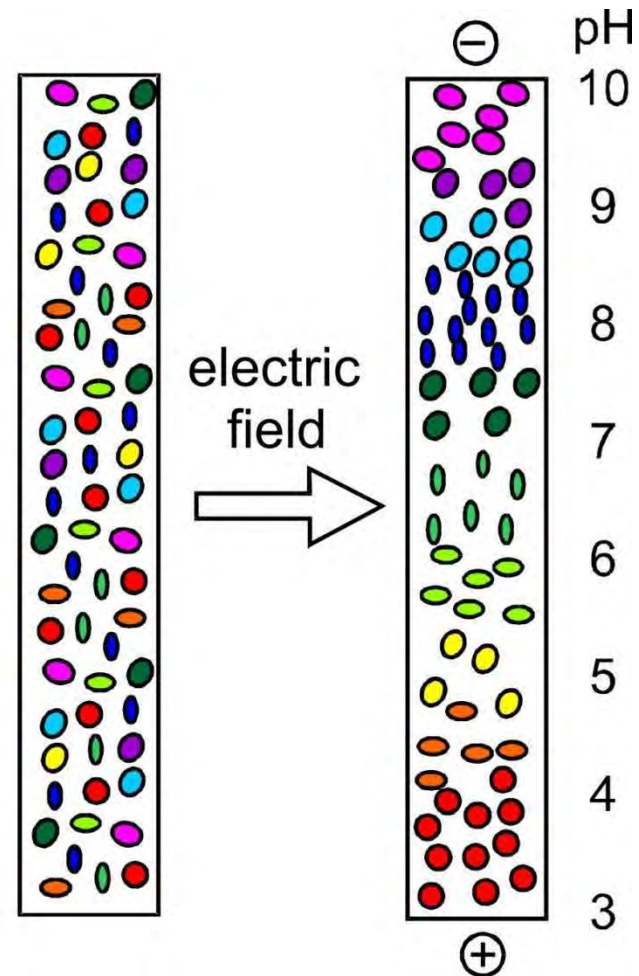
Carrier Ampholytes (SERVALYTE™)



where R = H

or - (CH₂)_x- COOH,

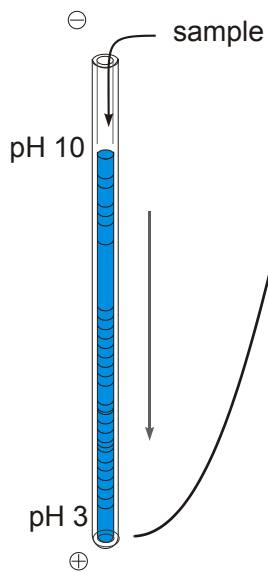
x = 2 or 3



High-resolution 2-D Electrophoresis

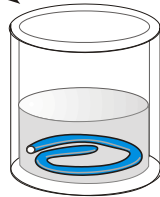
First Dimension:

Denaturing isoelectric focusing in presence of urea, Nonidet NP-40 in vertical gel rod



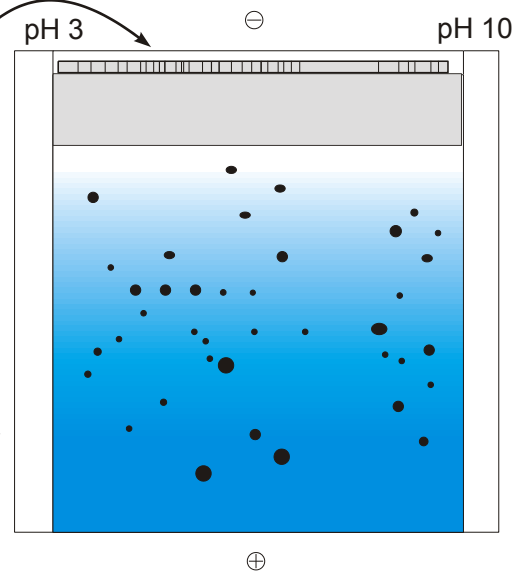
Separation acc. to Isoelectric Points (charge)

gel rod rebuffered in SDS buffer



Second Dimension:

SDS polyacrylamide gel electrophoresis in discontinuous gradient gel



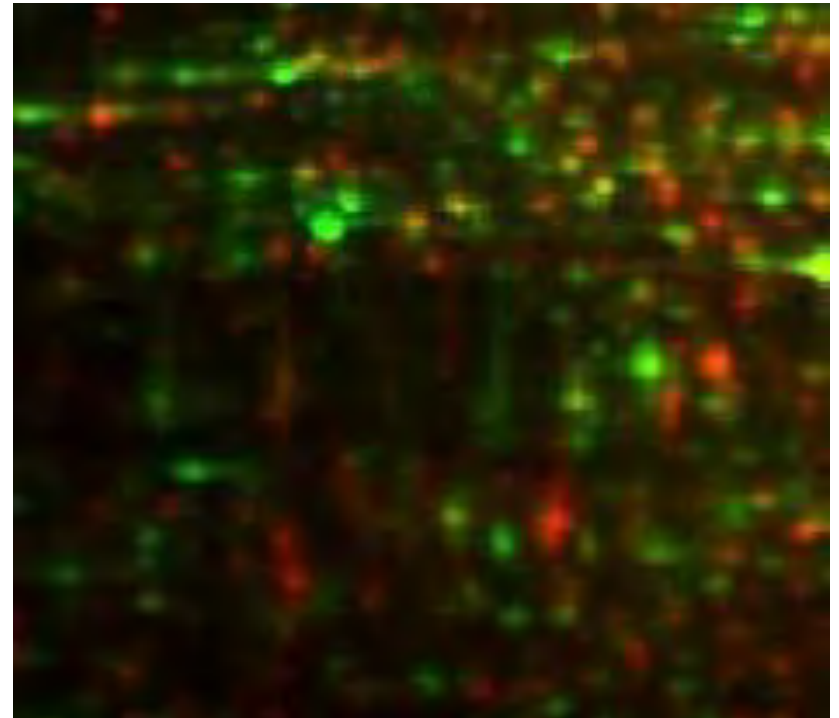
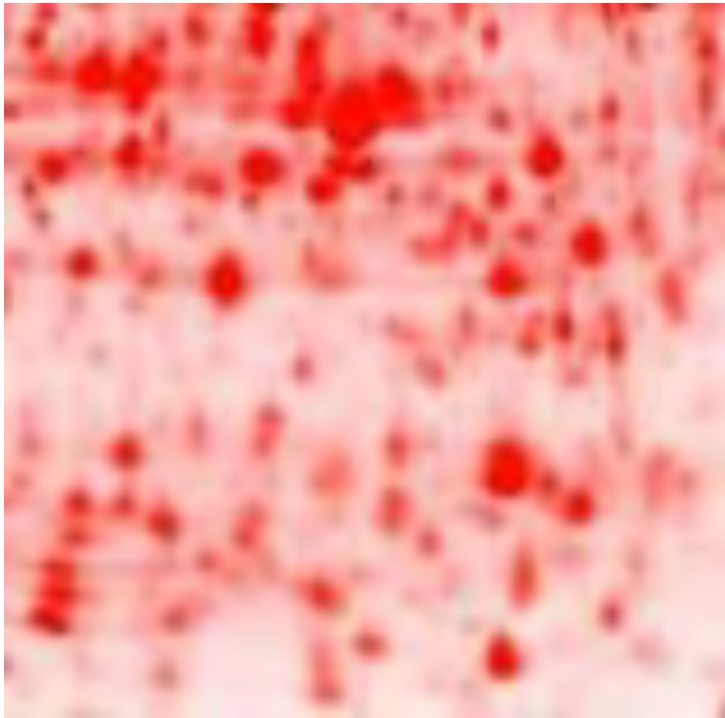
Separation acc. to Molecular Weight (mass)

Principle according to P.H. O'Farrell (1975)
O'Farrell PH.

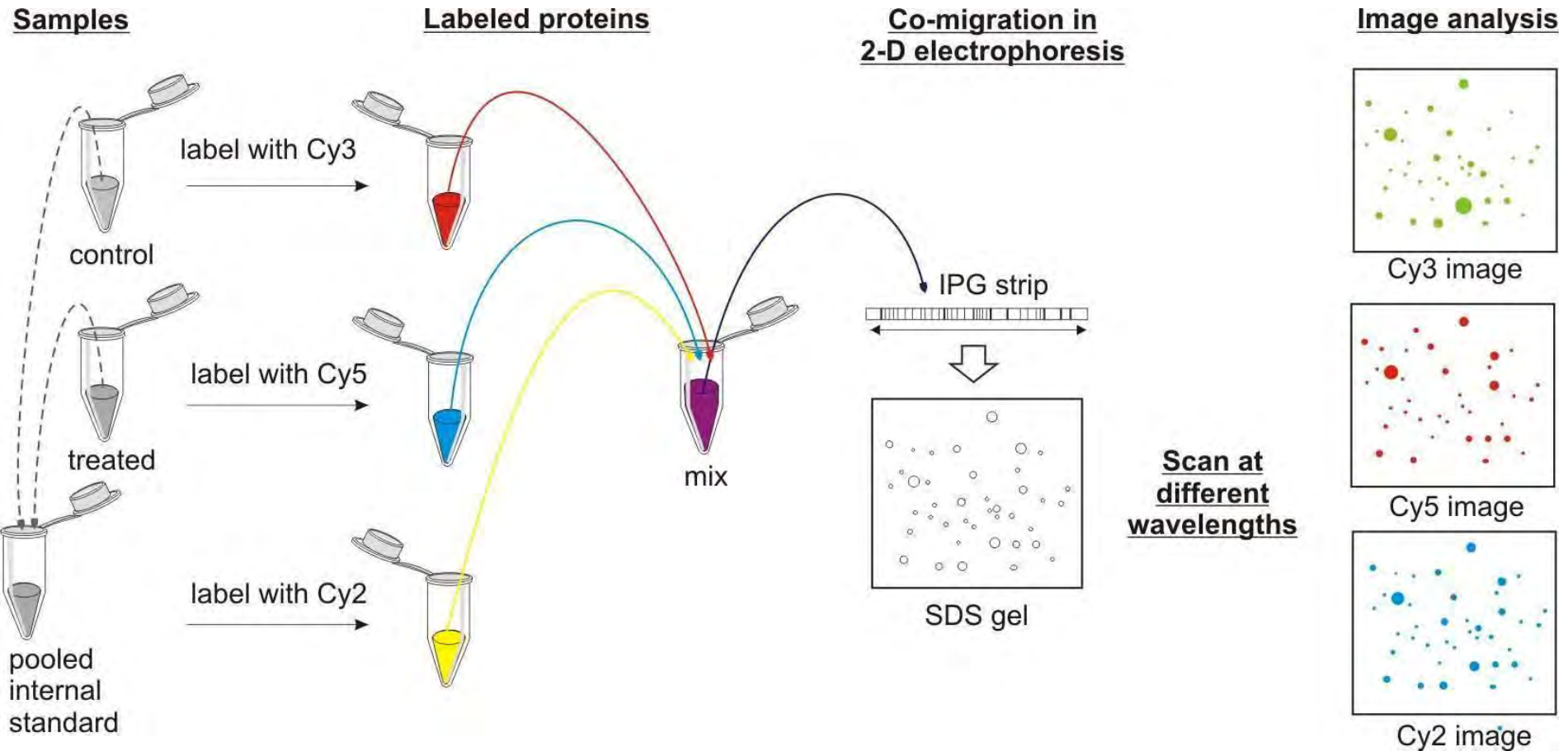
High-resolution two-dimensional electrophoresis of proteins.
J Biol Chem. 250 (1975) 4007-4021.

Protein Detektion:

Fluoreszenz-Markierung - Färbung –
Scannen – ImageAnalyse

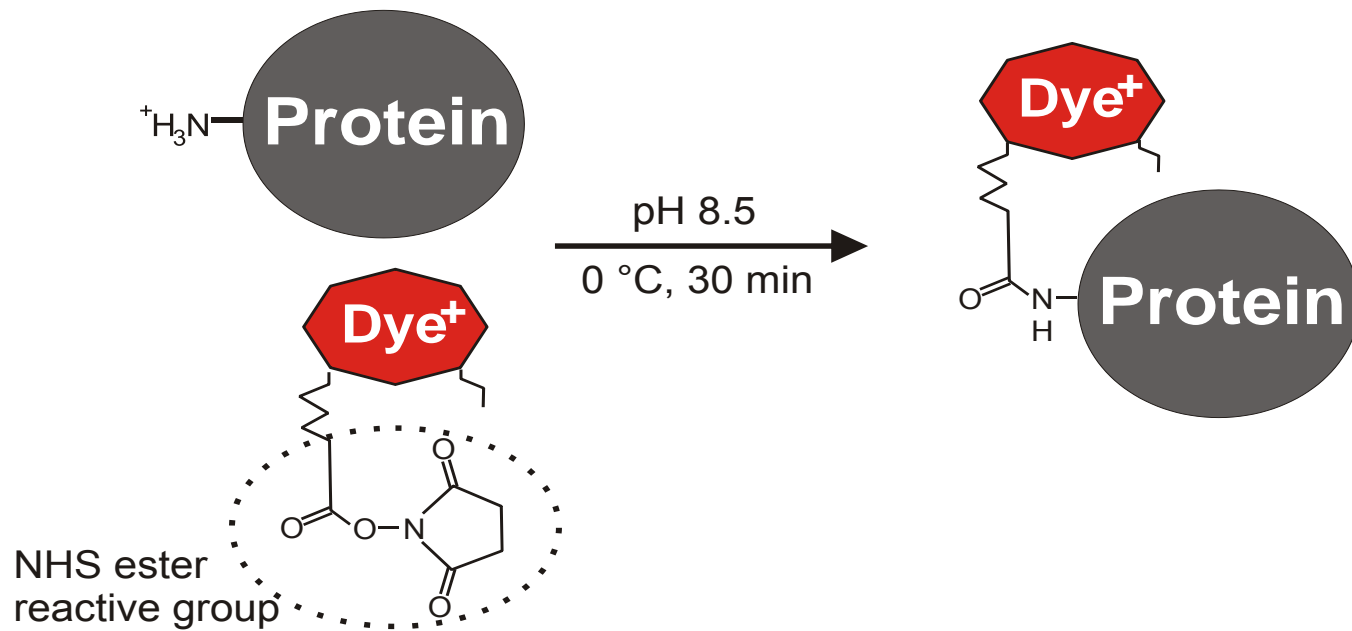


Difference gel electrophoresis (DIGE)

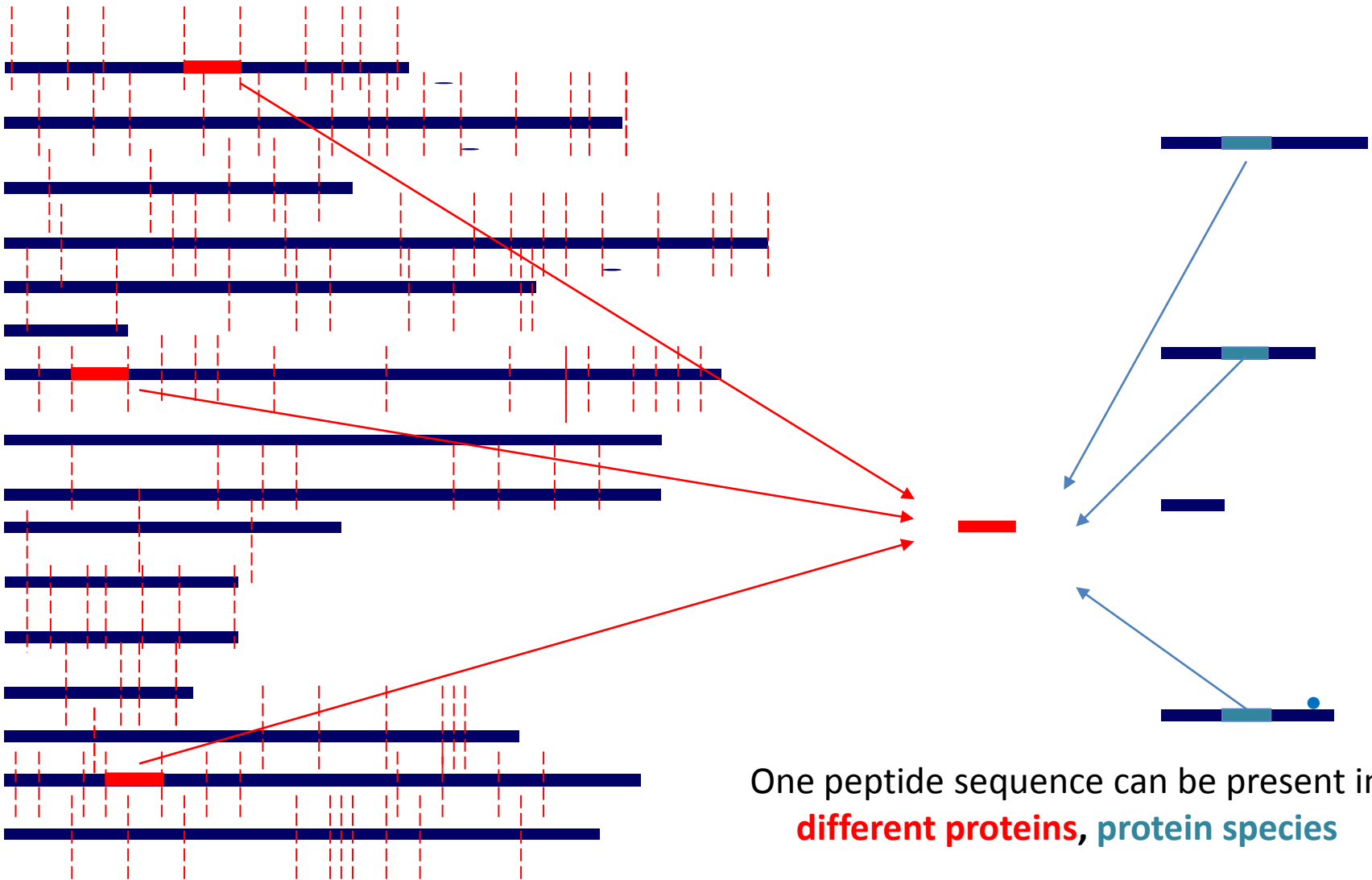


DIGE Labeling Chemistry

DIGE CyDye minimal labeling fluors

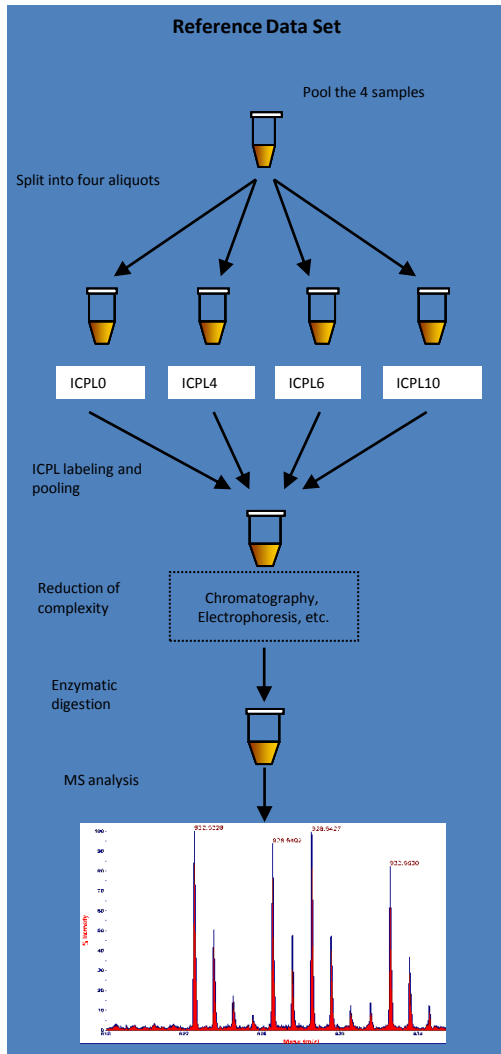


Peptide information is ambiguous



One peptide sequence can be present in
different proteins, **protein species**

ICPL Strategy



Each theoretical quadruplet position is covered by isotopic labeled peptides with a ratio of 1:1:1:1, even if a peptide is present only in one proteomic state

The experimental dataset does not necessarily contain each protein in all proteomic states, resulting in "incomplete" quadruplet pattern

Each peptide mass pattern is compared with the reference database and matched to its reference quadruplet.

