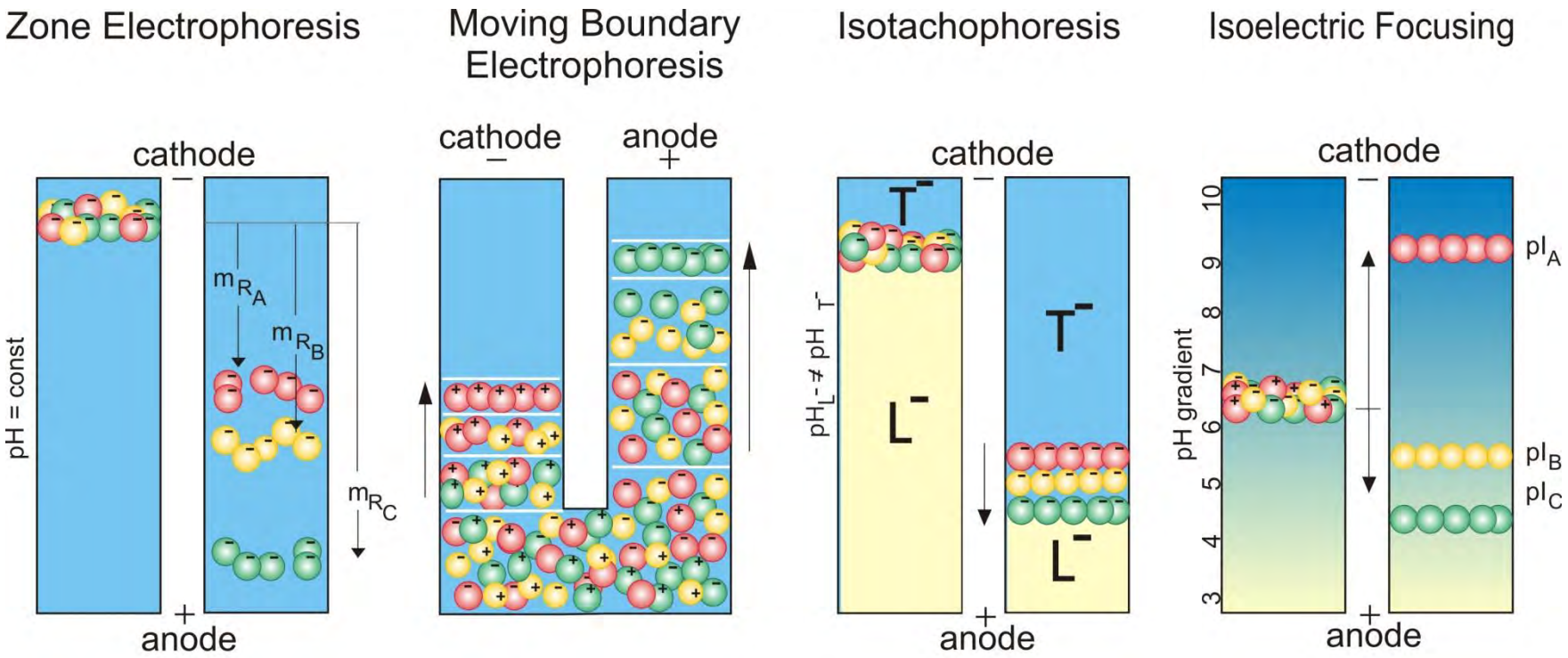
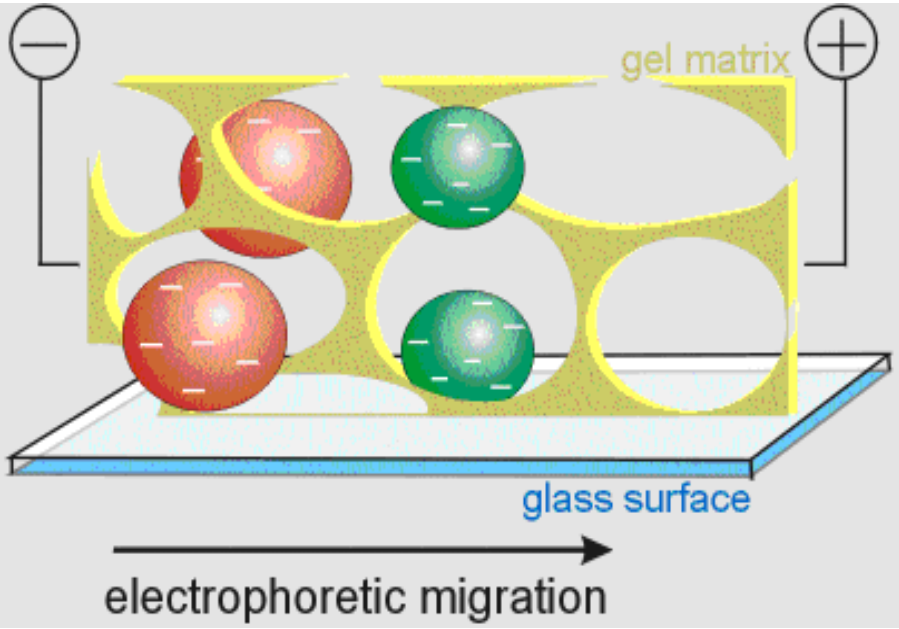
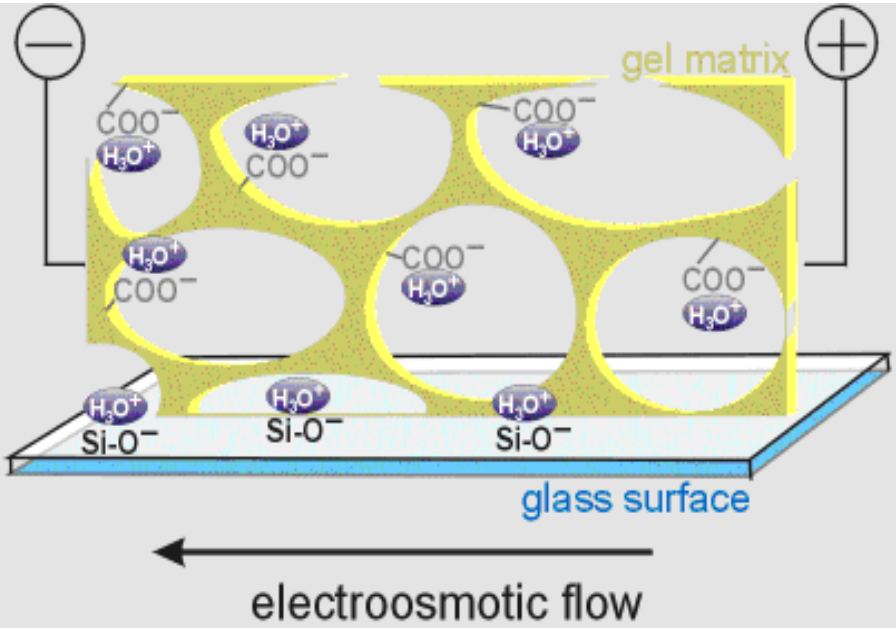
Theoretische Grundlagen von Elektrophorese-Techniken



Reiner Westermeier,
SERVA Electrophoresis GmbH
Heidelberg

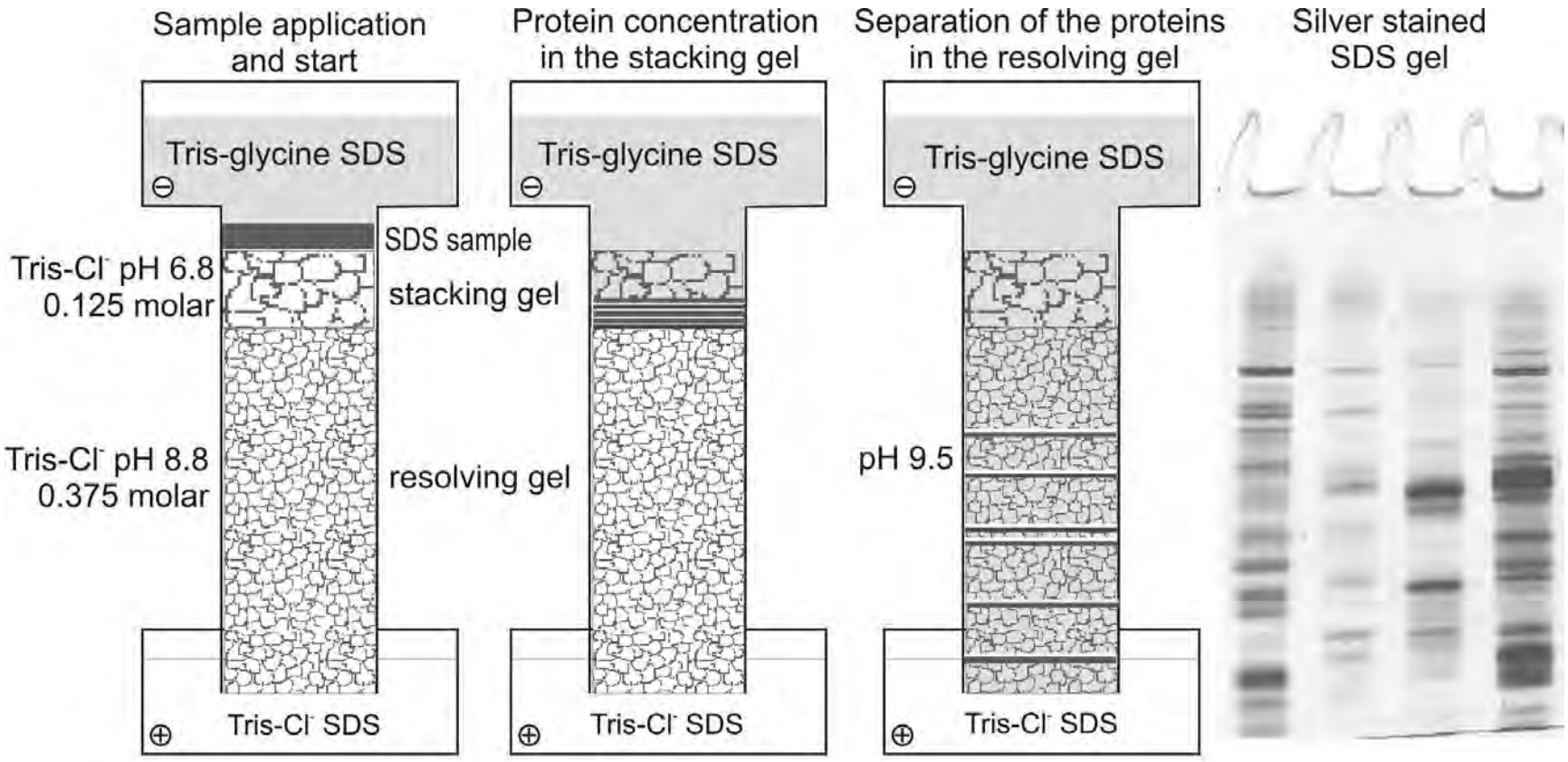
Electroendosmosis





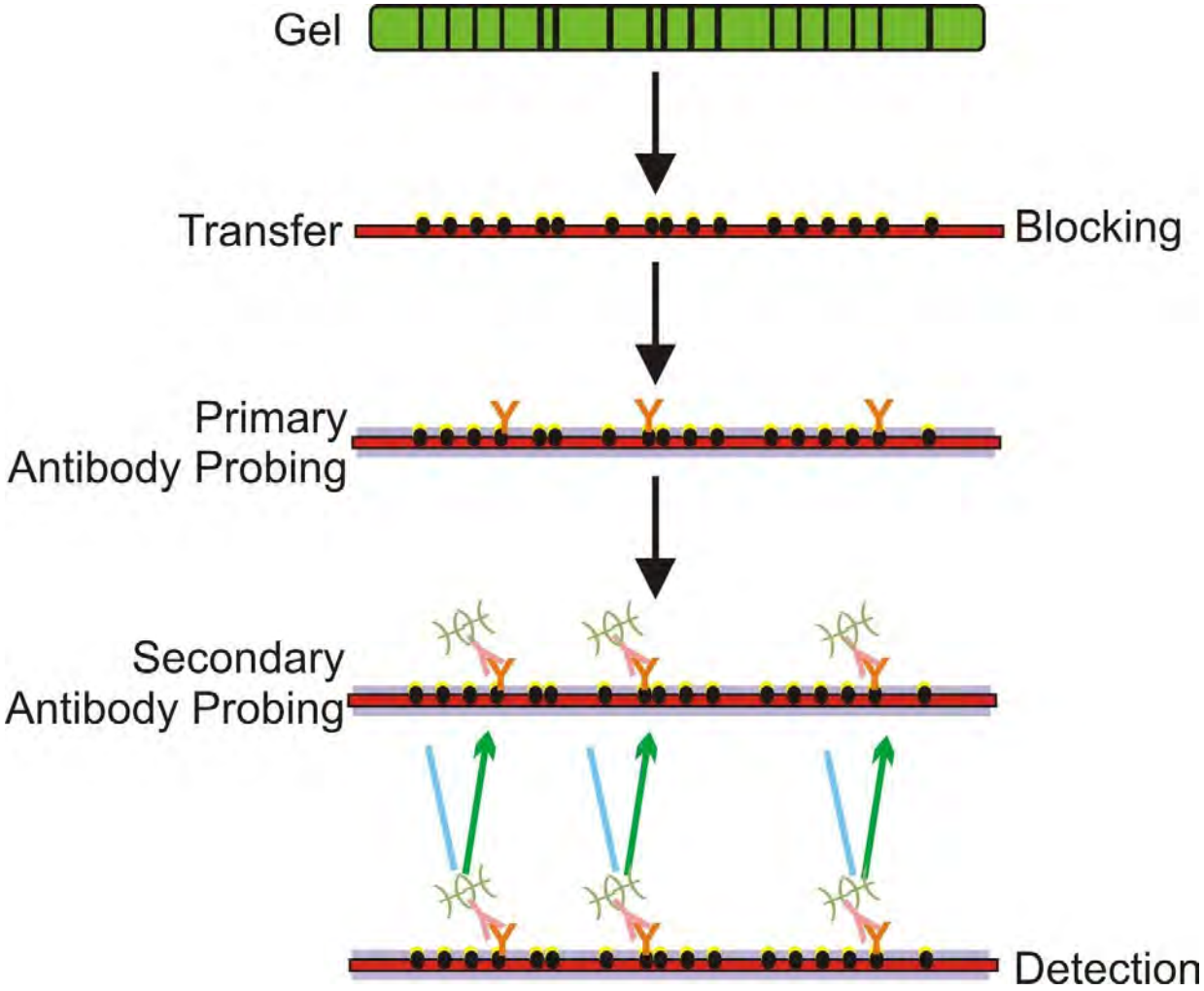
Fixed charges in the matrix and / or on a surface(e.g. glass) cause a bulk liquid flow.

SDS Disc electrophoresis



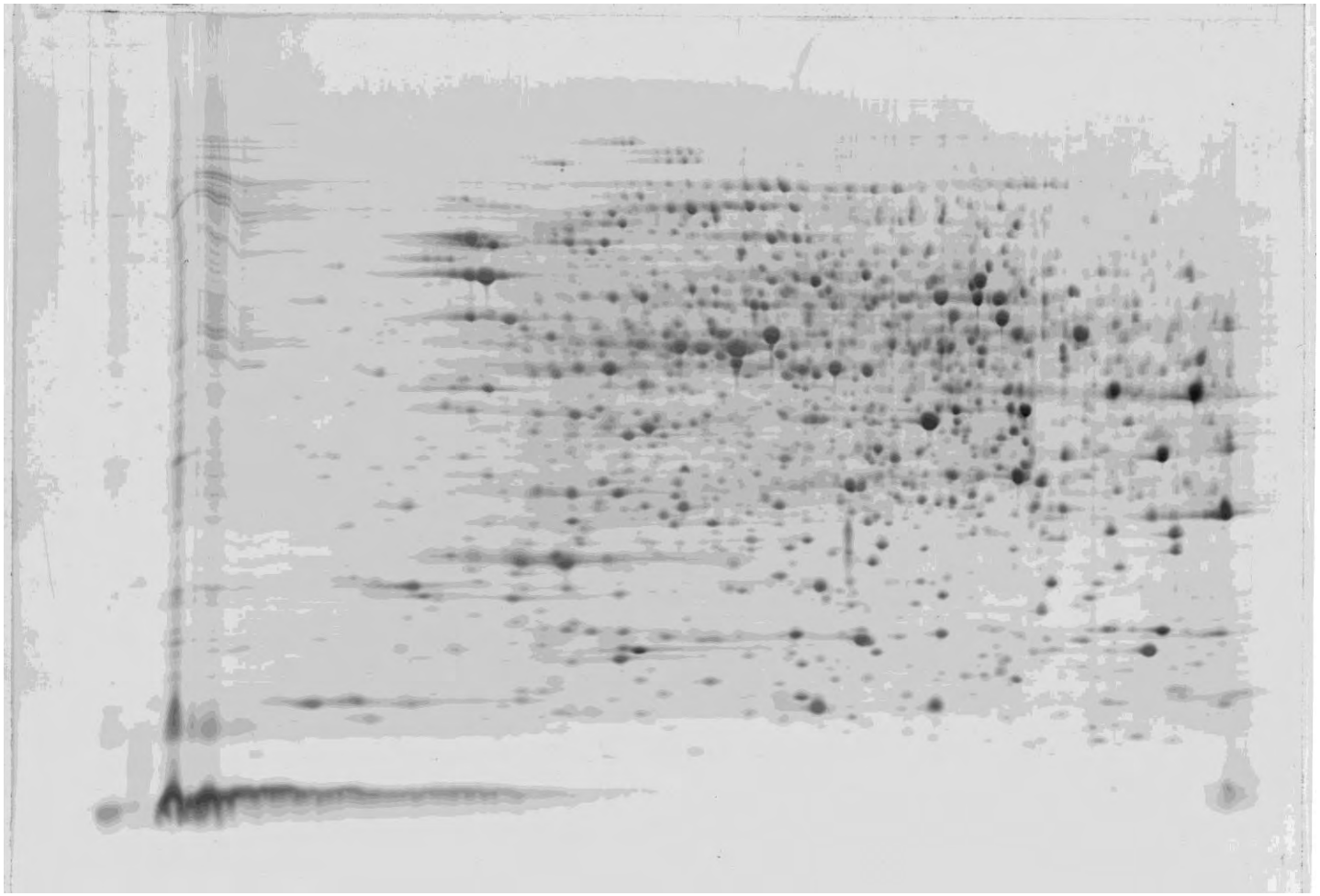
Adapted to SDS PAGE in the year 1970 by Laemmli

Western Blotting (Immunoblotting)



Two-dimensional Electrophoresis

pH 4 ⟵ Isoelectric Focusing in 24 cm IPG Strip ⟶ pH 7

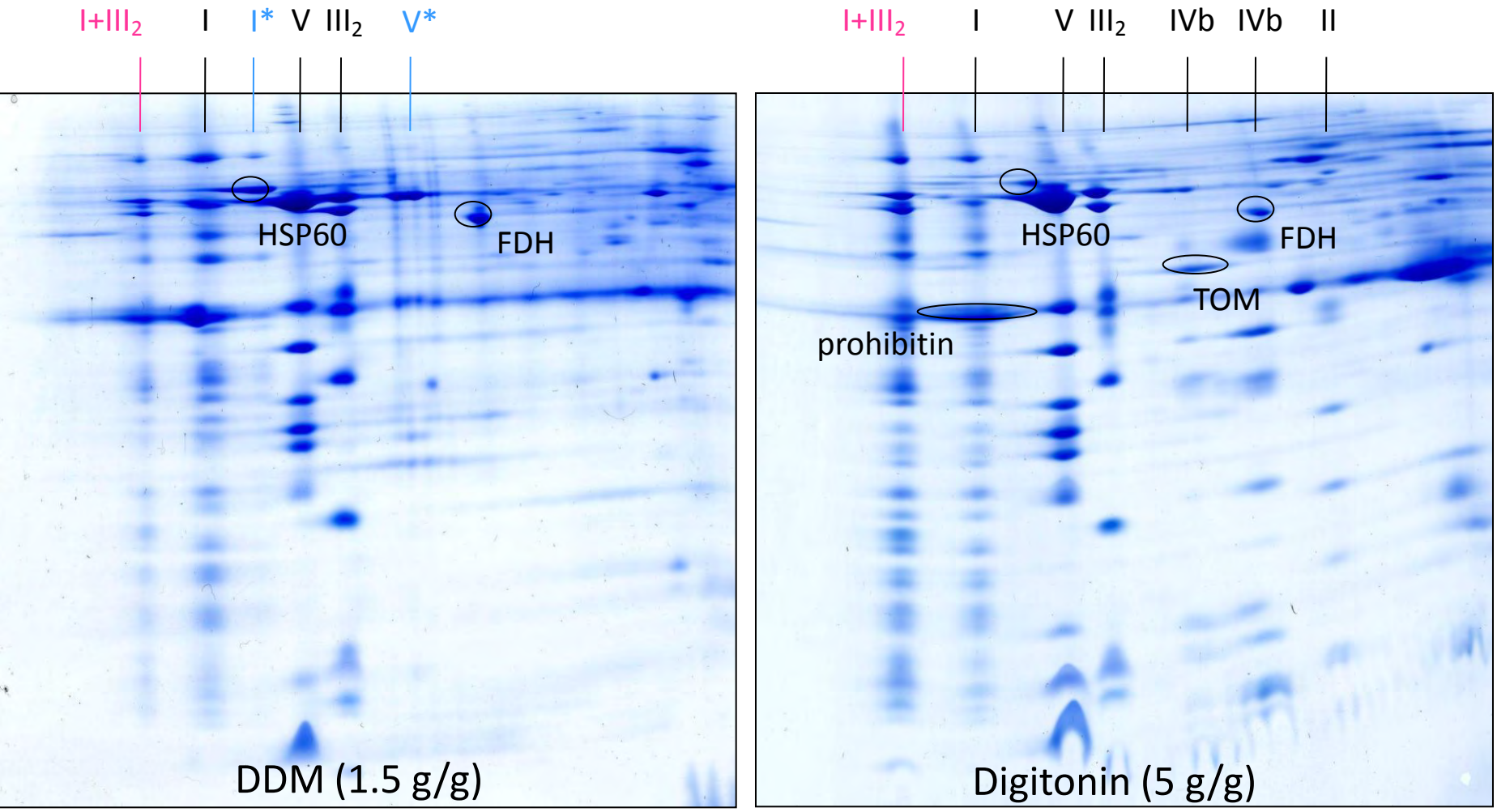


SDS
PAGE
in
12.5 % T
homogeneous
Gel

↓

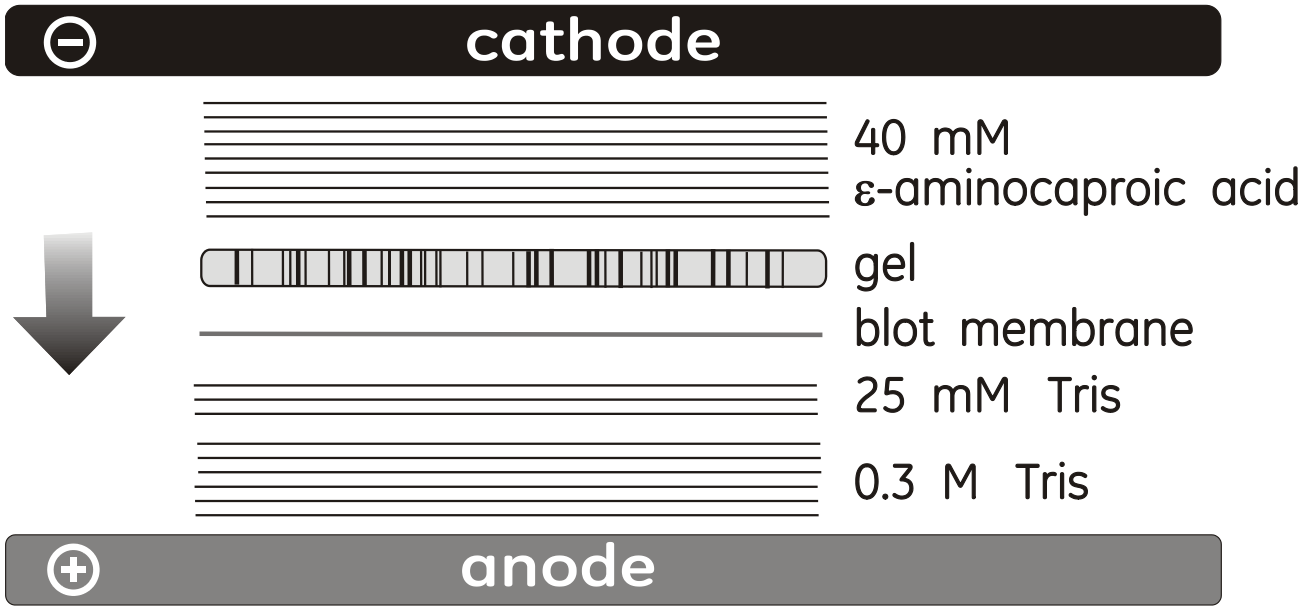
E. coli extract run in 2DGel flatbed large, fluorescent staining

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



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

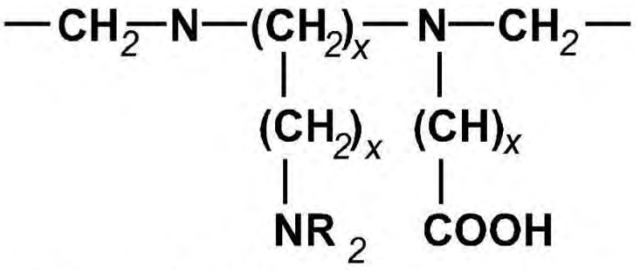
Discontinuous blotting buffer



all buffers contain 20% methanol

IEF with carrier ampholytes

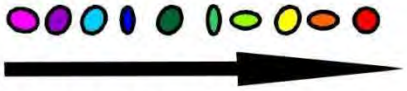
Carrier Ampholytes
(SERVALYTE™)



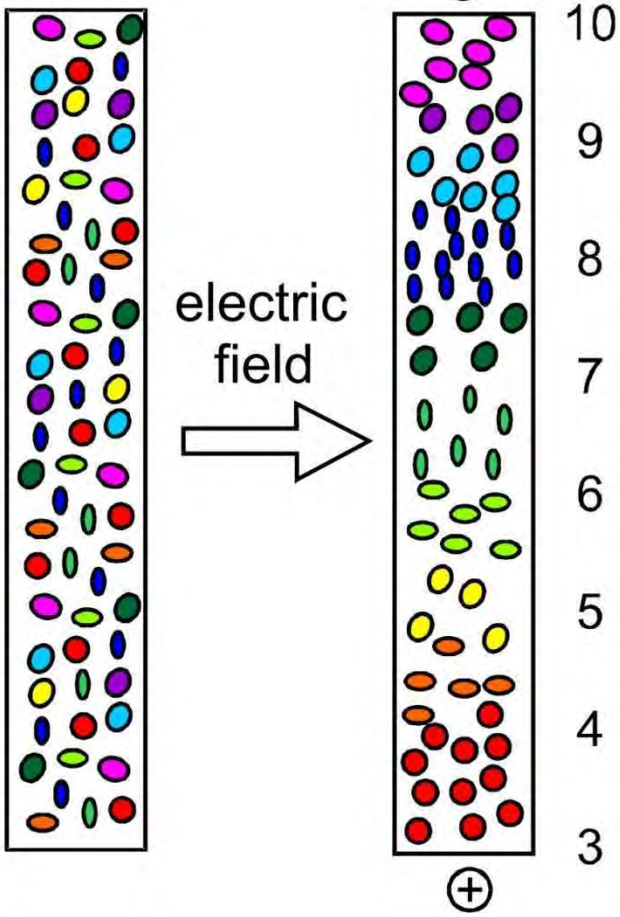
where R = H

or - $(CH_2)_x$- COOH,

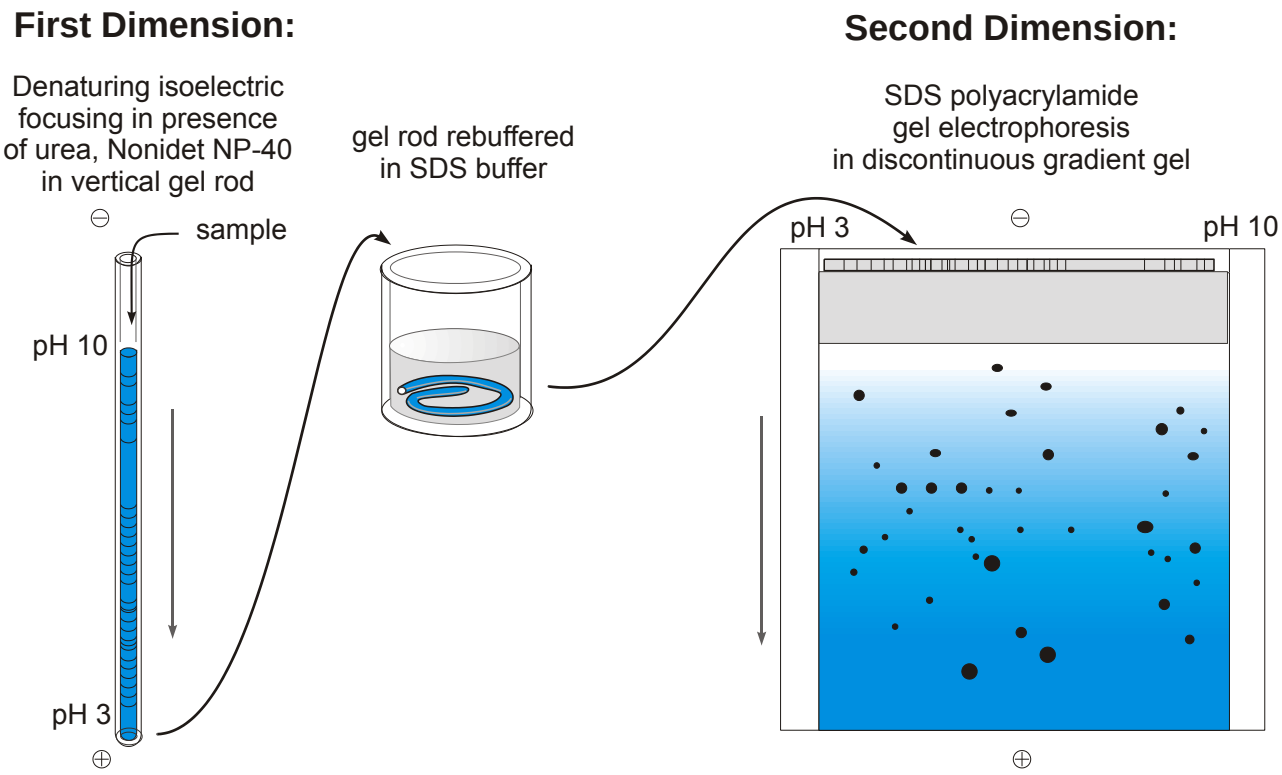
x = 2 or 3



decreasing pI



High-resolution 2-D Electrophoresis



Separation acc. to Isoelectric Points (charge)

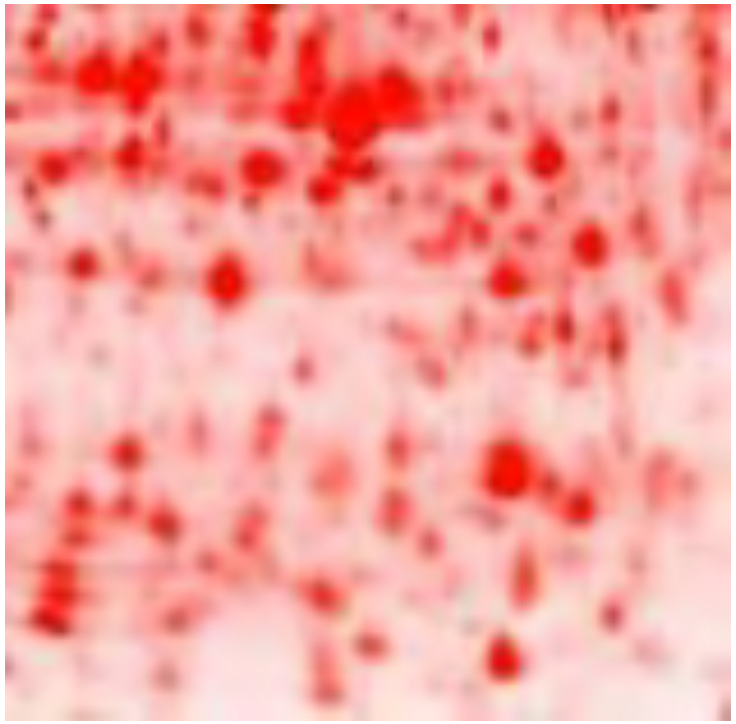
Separation acc. to Molecular Weight (mass)

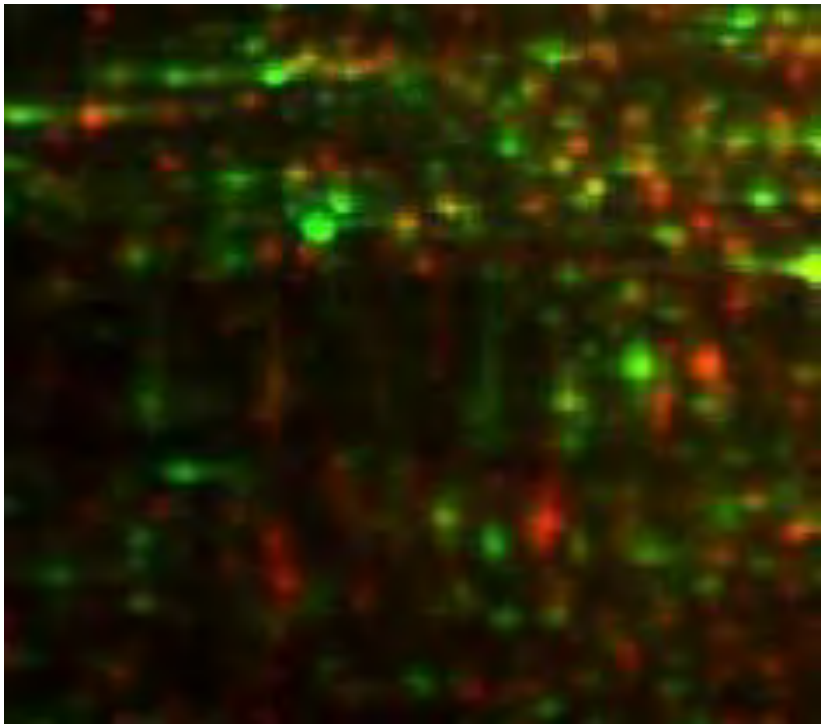
Principle according to P.H. O'Farrell (1975)

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

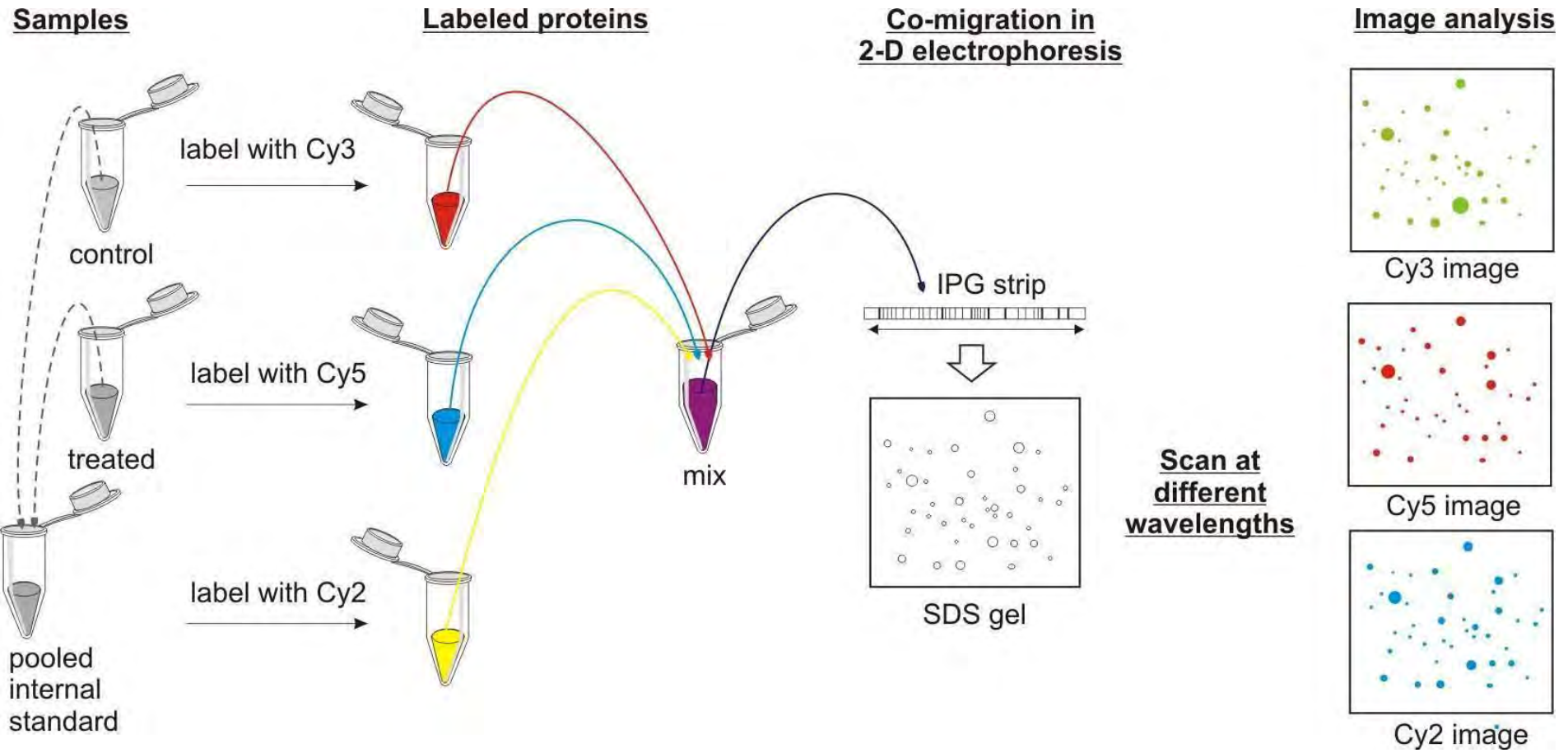
Protein Detektion:

Fluoreszenz-Markierung - Färbung – Scannen – ImageAnalyse



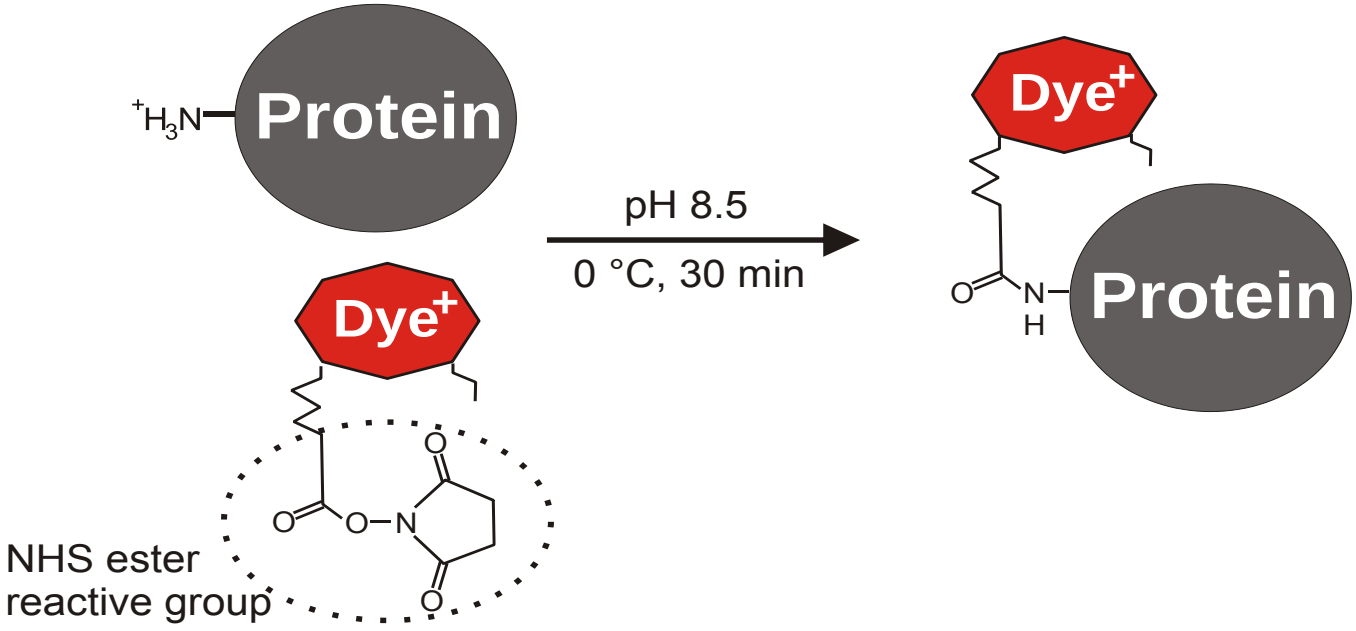


Difference gel electrophoresis (DIGE)

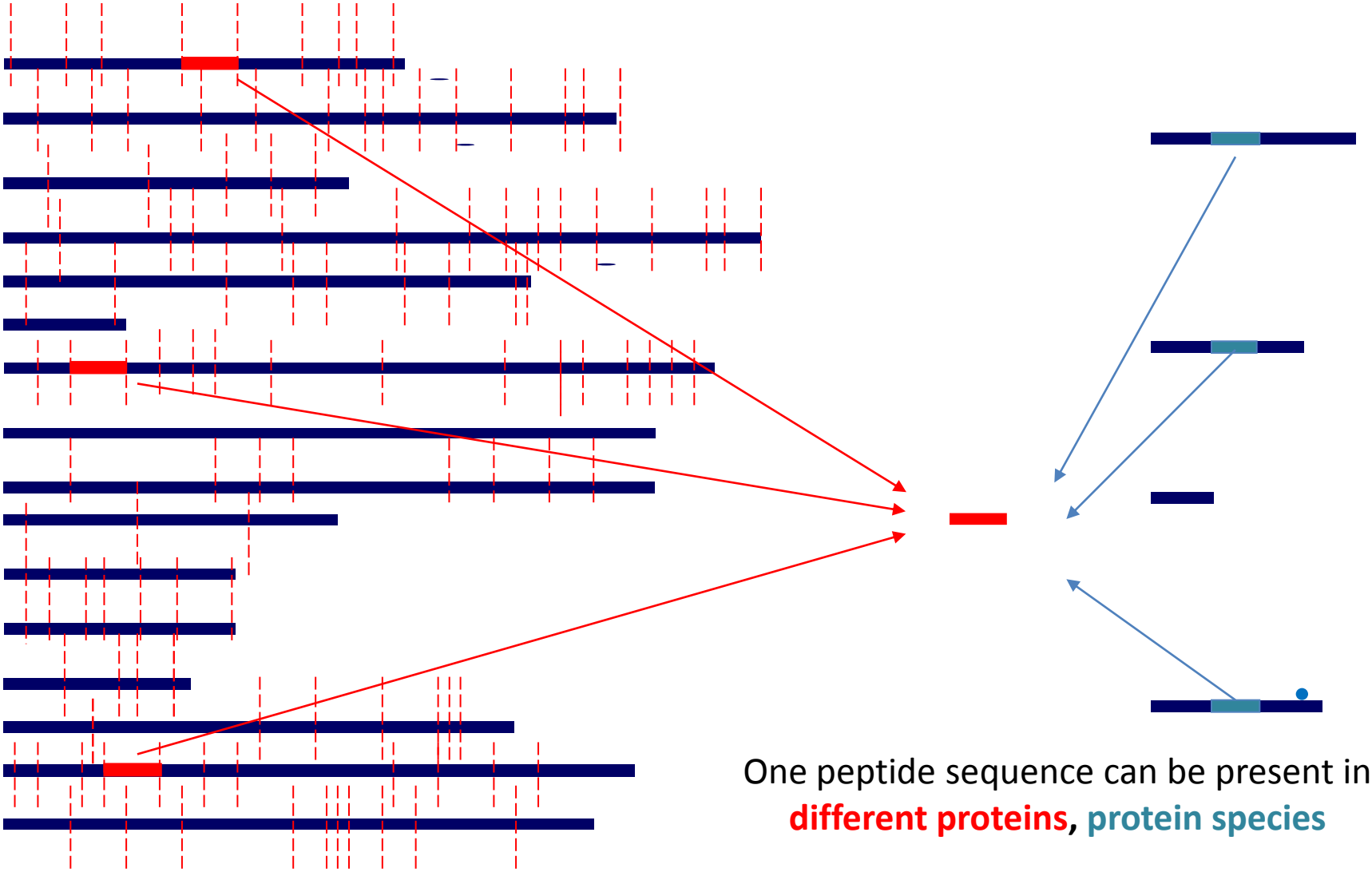


DIGE Labeling Chemistry

DIGE CyDye minimal labeling fluors



Peptide information is ambiguous



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

ICPL Strategy