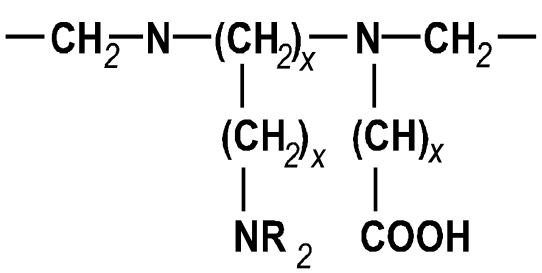
2-D Electrophoresis with Immobilized pH Gradients: Principle and Methodology

Reiner Westermeier, GE Healthcare Bio-sciences

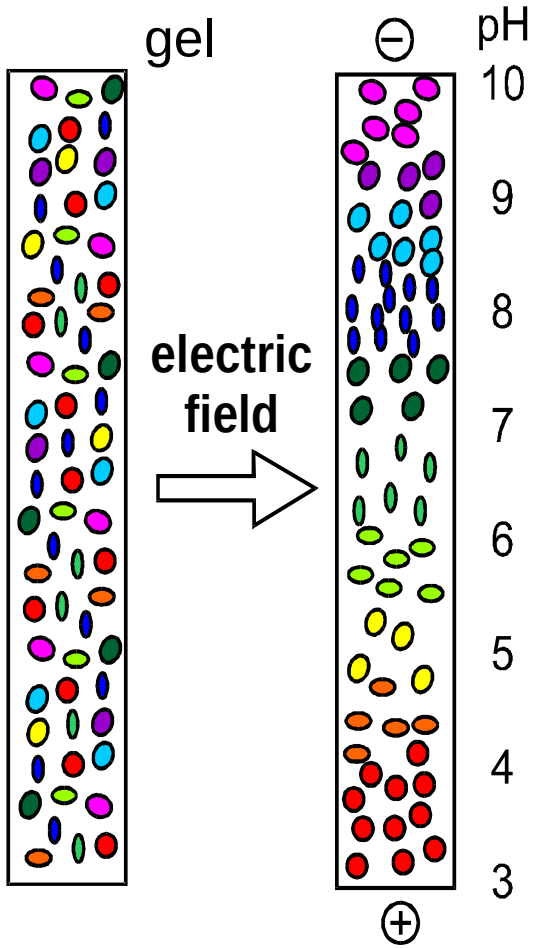
IEF with carrier ampholytes

Pharmalytes
Ampholines

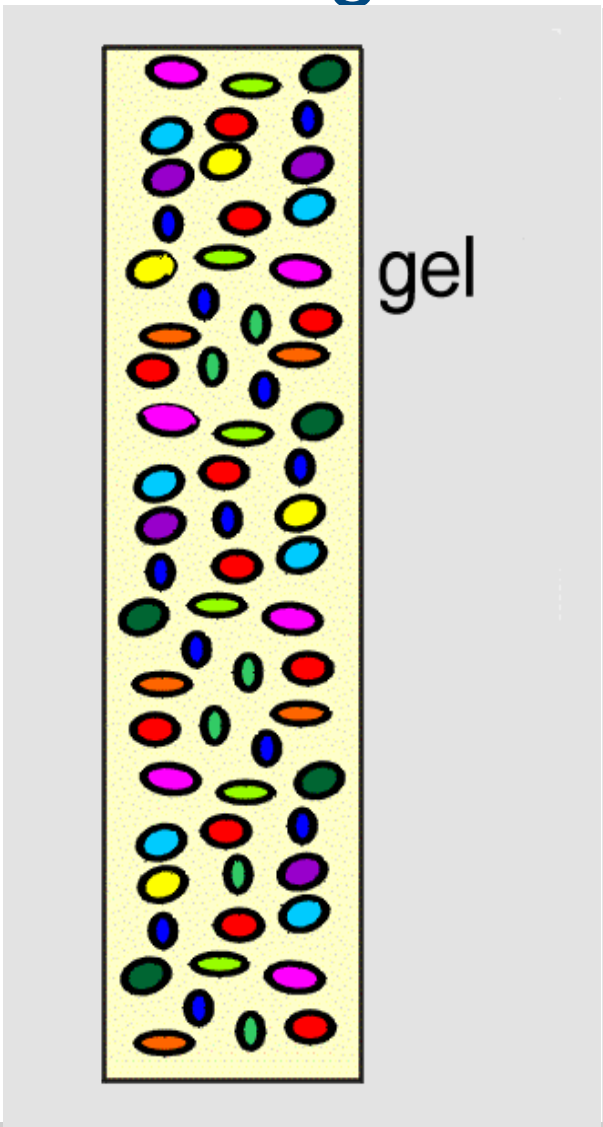


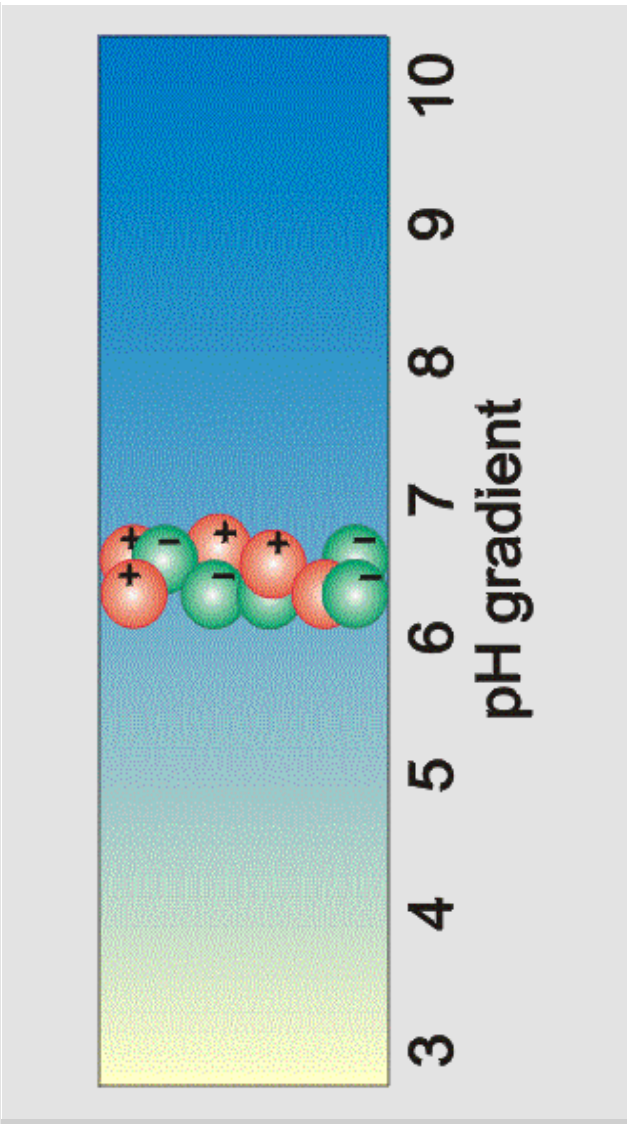
where R = H
or - $(CH_2)_x$ - COOH,
x = 2 or 3

decreasing pI



Isoelectric Focusing

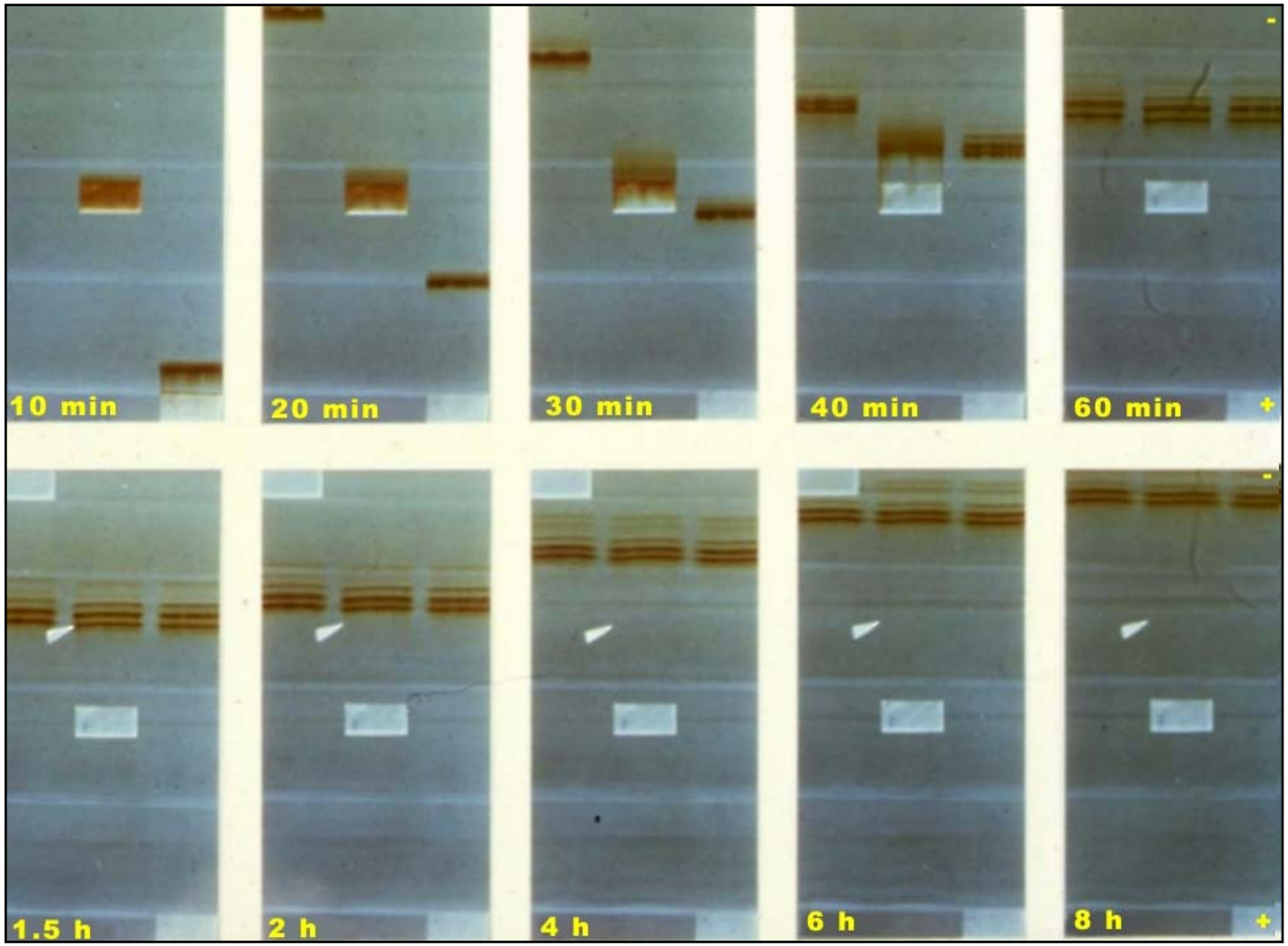




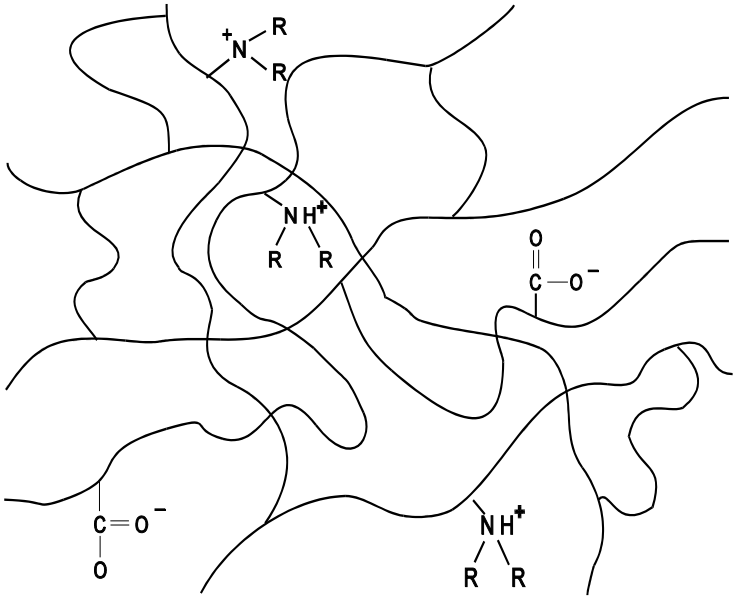
IEF of oligoclonal IgG

Cerebrospinal fluid and Serum,

The Cathodal Drift



Immobilized pH gradients (IPG)



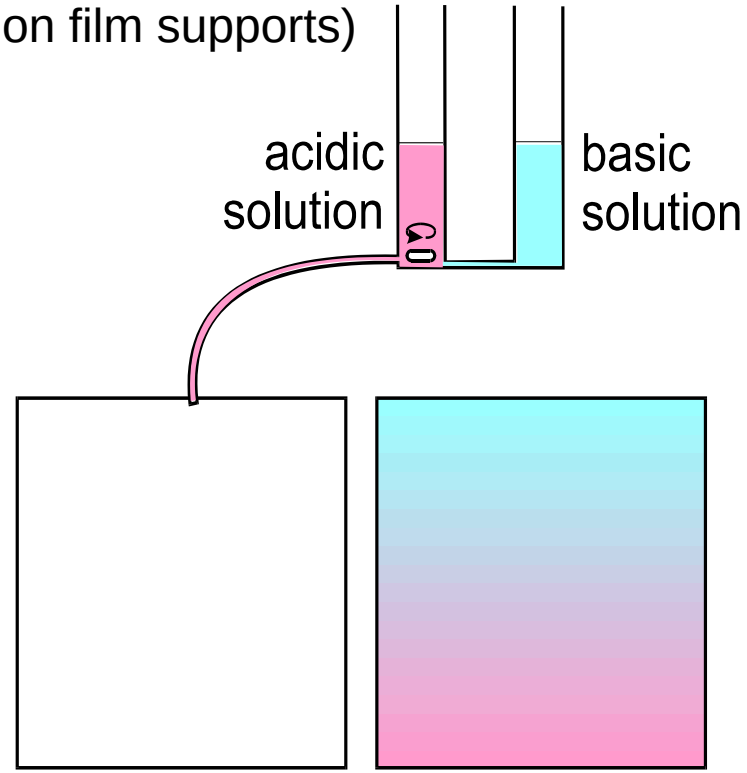
Acrylamide derivatives: Immobiline®

CH_2=CH-CO-NH-R,

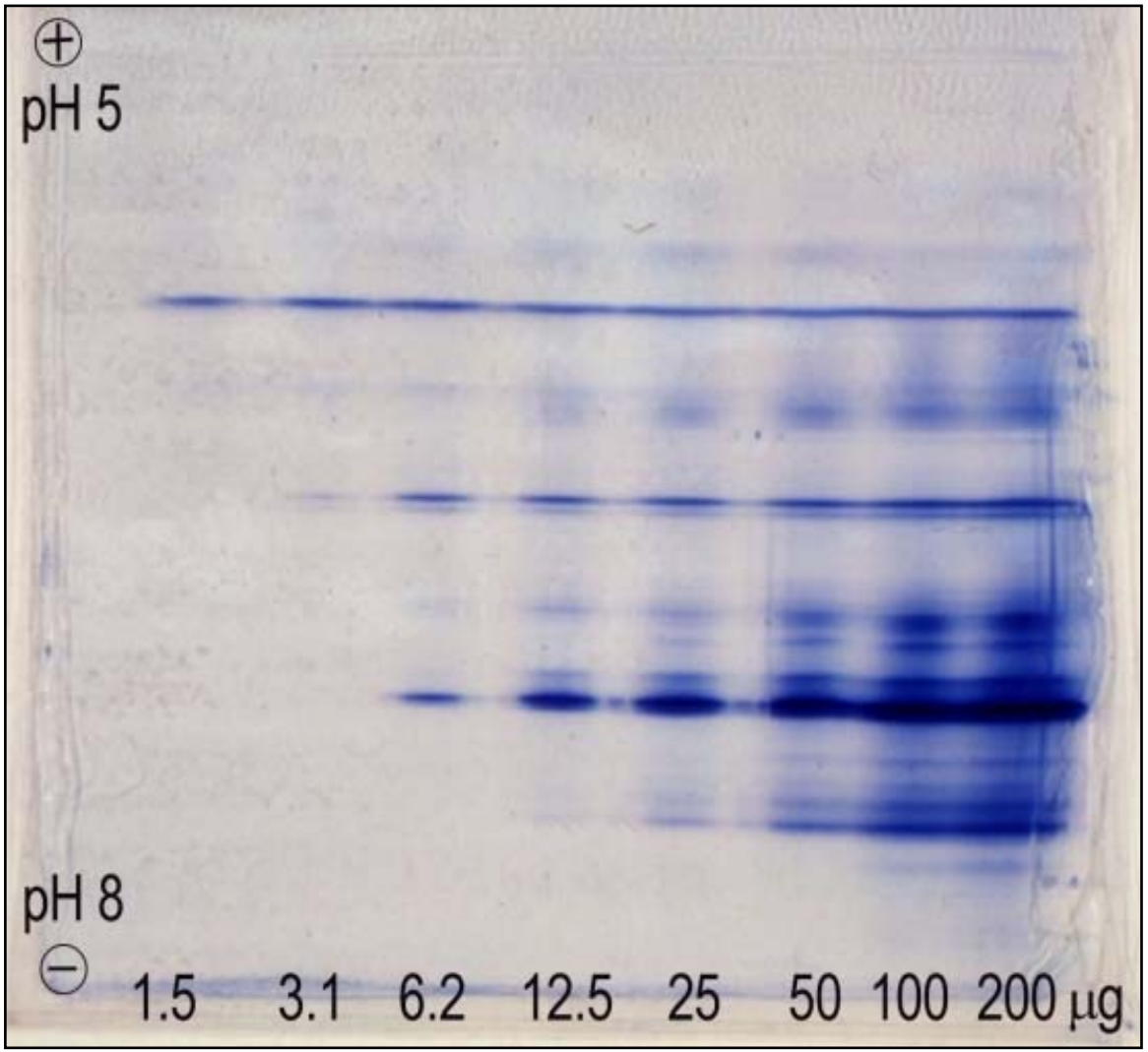
R contains a carboxylic

or a tertiary amino group

Immobiline Gels
(0.5 mm gel layers
on film supports)



IEF of carbonic anhydrase in an IPG gel

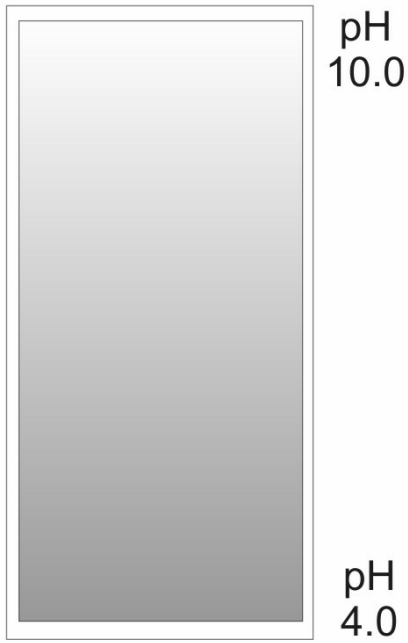


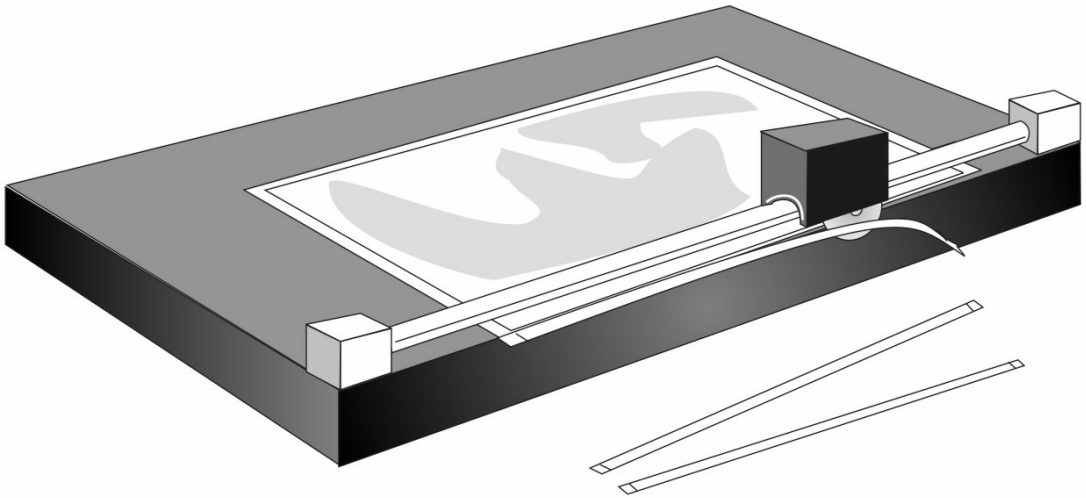
0.5 mm gel

pH 5 - 8

Preparation of an IPG Strip

dried gel with
immobilized pH gradient





Features of Immobilized pH Gradients

Few acrylamide derivatives

No gradient drift

Tailor-made gradients

Very narrow gradients possible: e.g. pH 4.2-4.4

Stable basic pH gradients

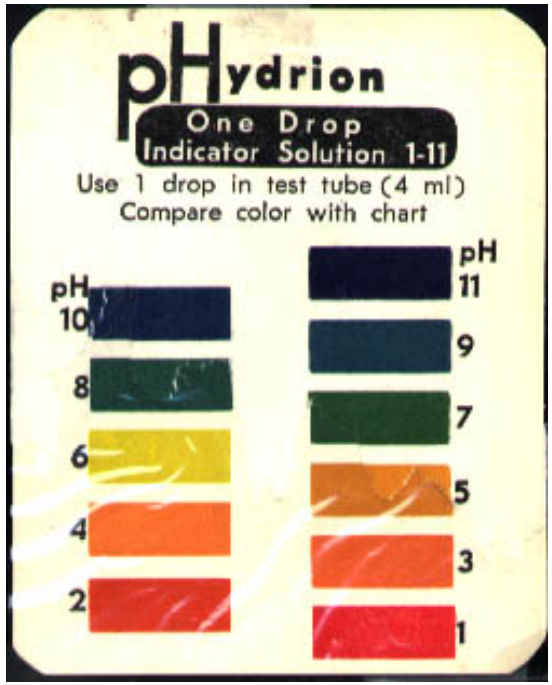
Gradient profile is stable

True equilibrium method

High loading capacity

IEF in narrow strips

IPG strip pH gradient 3 - 10 L

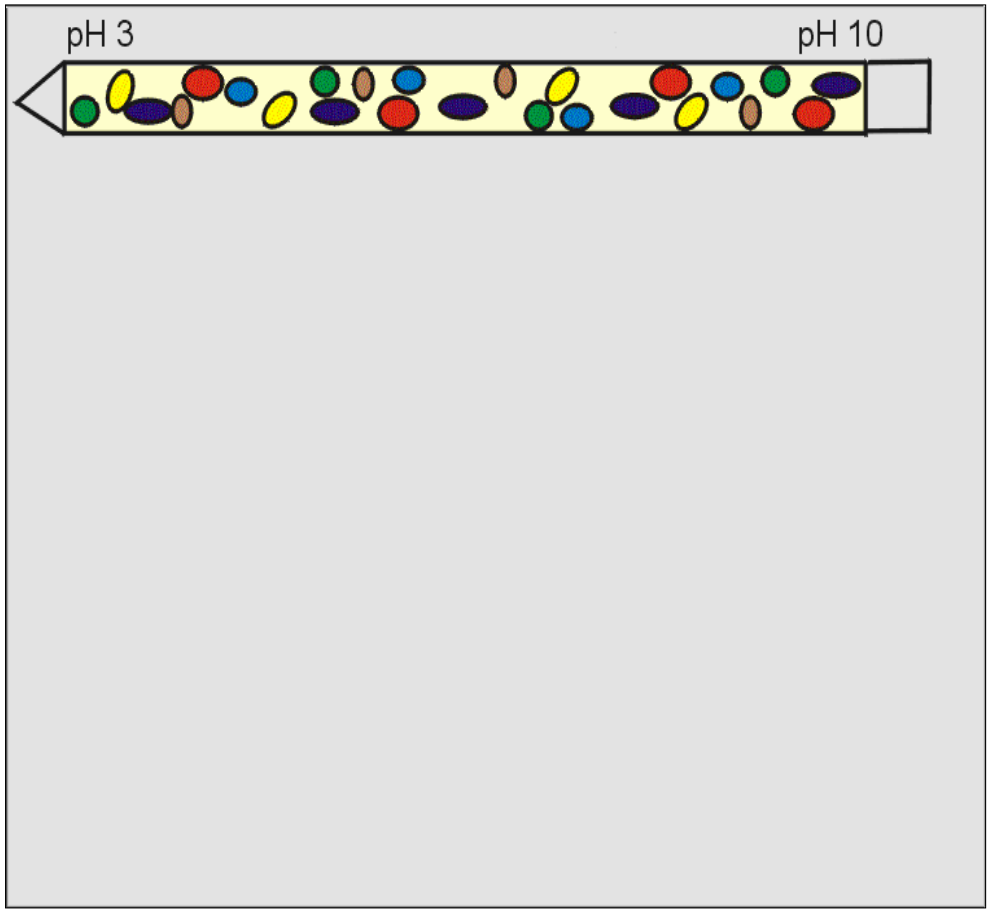


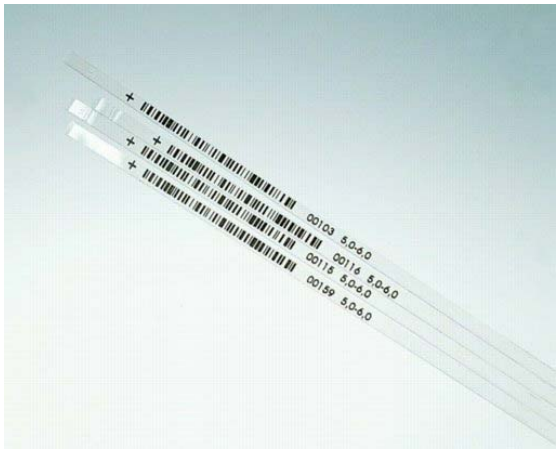
Principle of 2-D electrophoresis with IPG

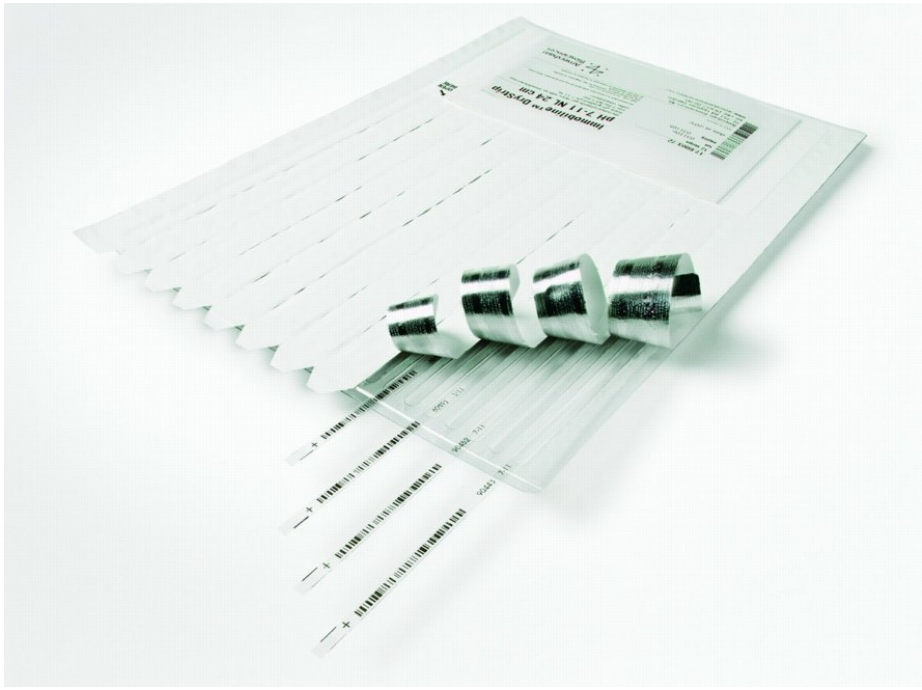
1. First dimension:
   denaturing isoelectric focusing separation according to the isoelectric point

2. Second dimension:
   SDS electrophoresis separation according to the molecular weight

2-D electrophoresis resolves a few thousand protein spots.



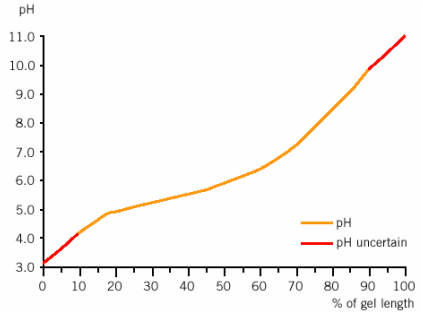




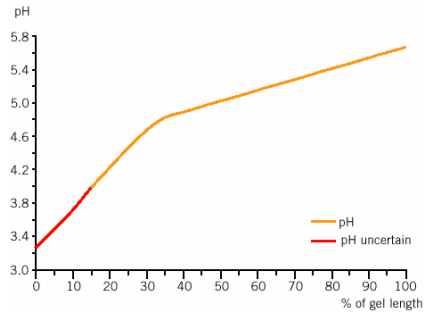
Immobiline DryStrips

New Immobiline DryStrips

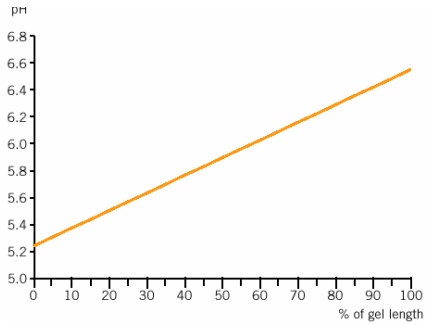
pH 3-11



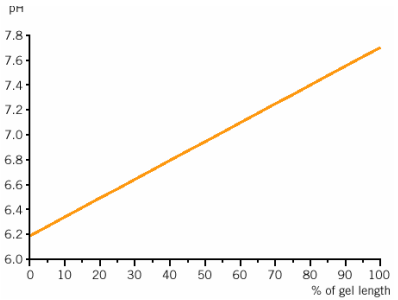
pH 3-5.6 NL



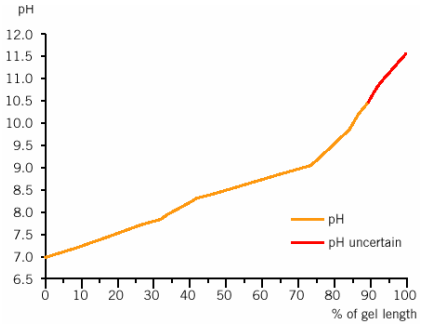
pH 5.3-6.5



pH 6.2-7.5



pH 7-11 NL



<u>Lengths:</u>

7, 11, 13,18,and 24 cm

New IPG buffers

Wide pH gradient: 3 – 11 NL

Mouse liver extract

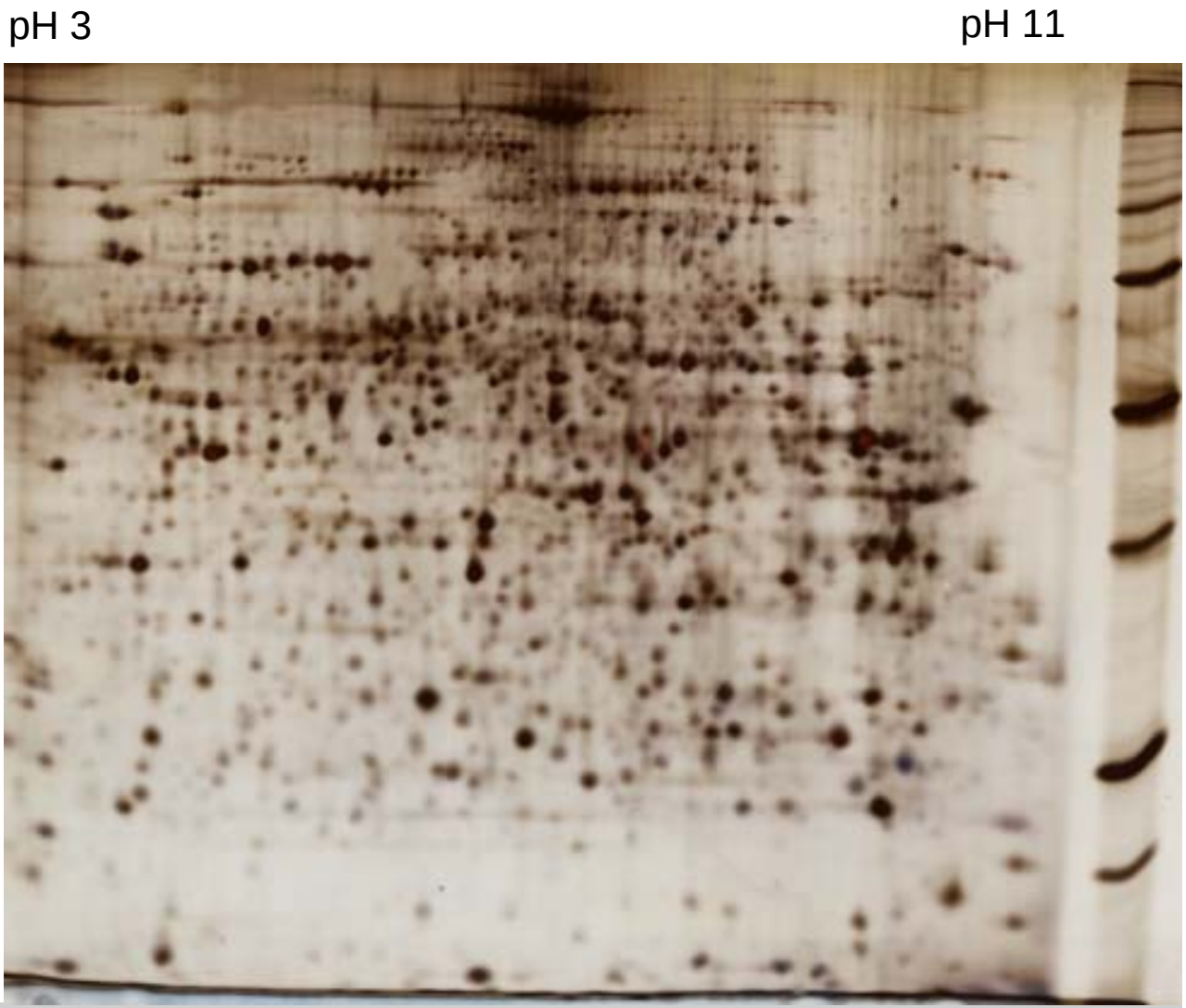
IPG 24 cm, pH 3-11

From A. Görg

Proteomics Department

Technische Universität

Munich



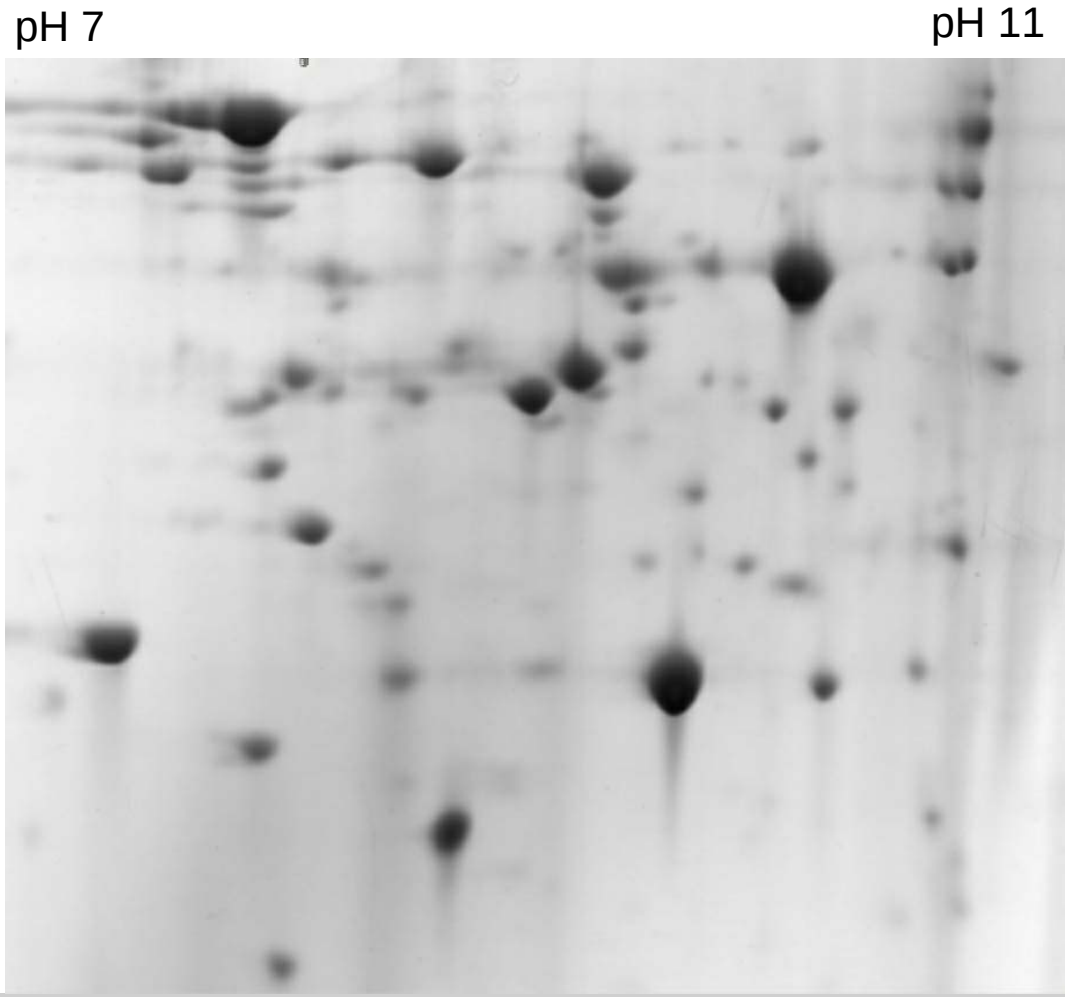
Basic gels: pH 7-11 NL

New proprietory Immobiline reagent:

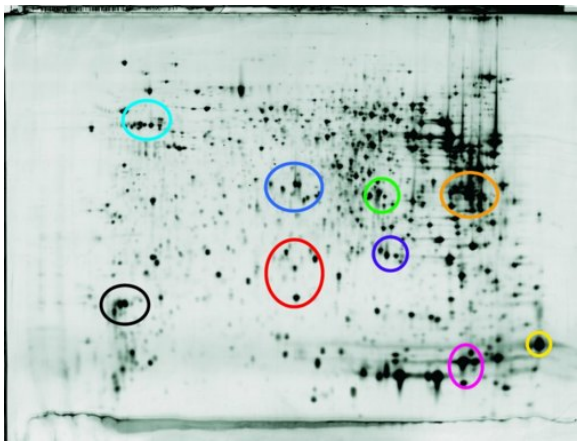
Increased buffering capacity

Stabilizes protein patterns

Mouse liver extracts



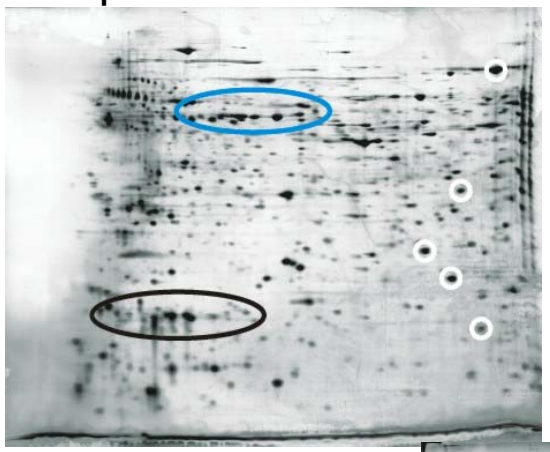
New IPG strips with overlapping pIs

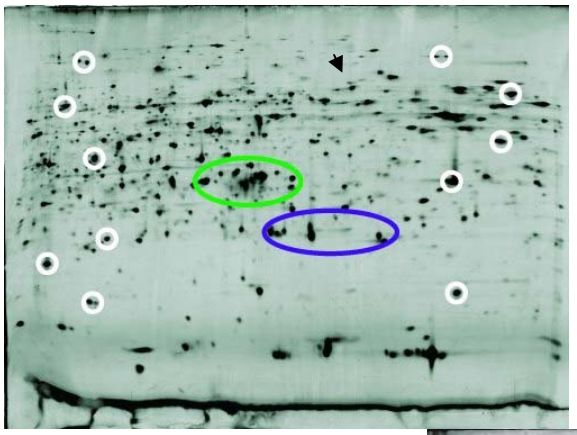


100 µg mouseliver extract +
7.5 µg alkylated lysozyme

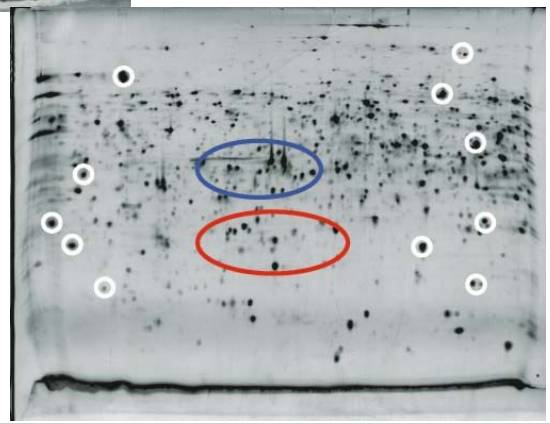
pH 3-11

pH 3.0-5.6



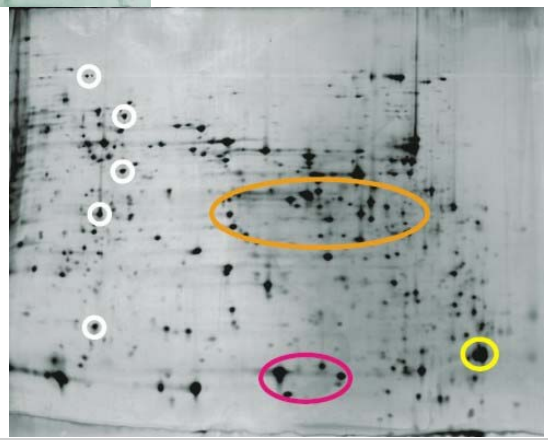


pH 6.2-7.5

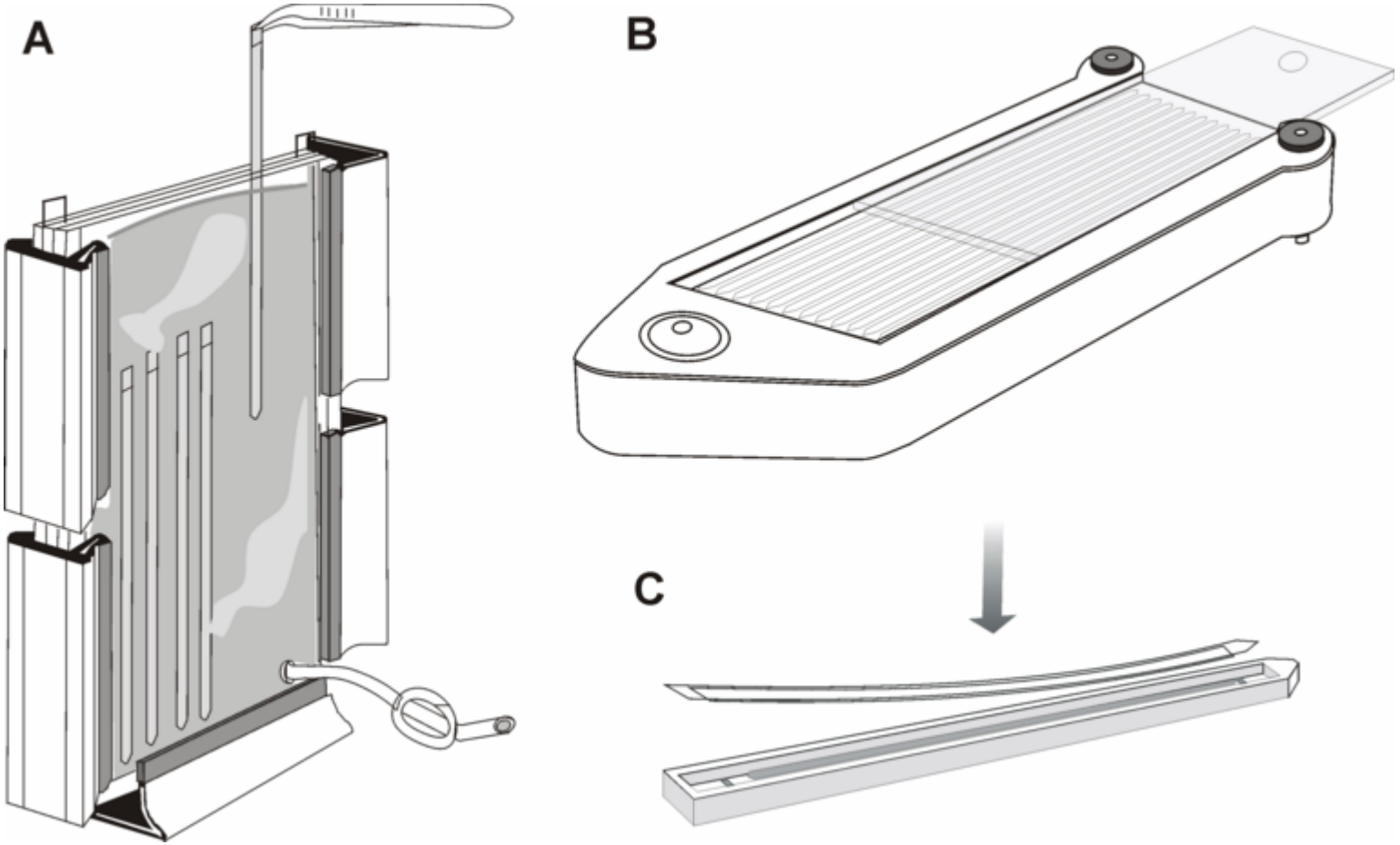


pH 5.3-6.5

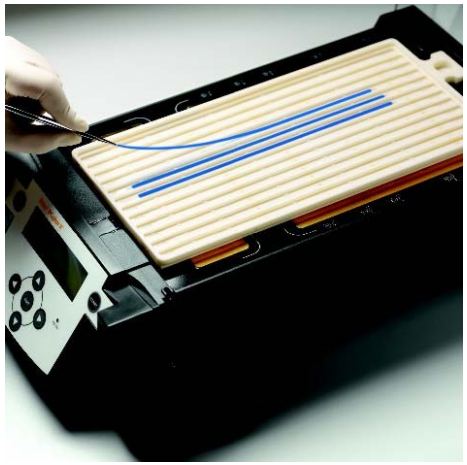
pH 7.0-11

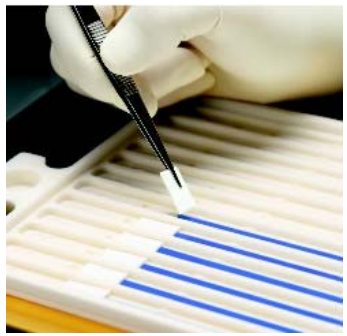


Rehydration of IPG strips



Ettan IPGphor Manifold





Preparative run on IPGphor with Manifold

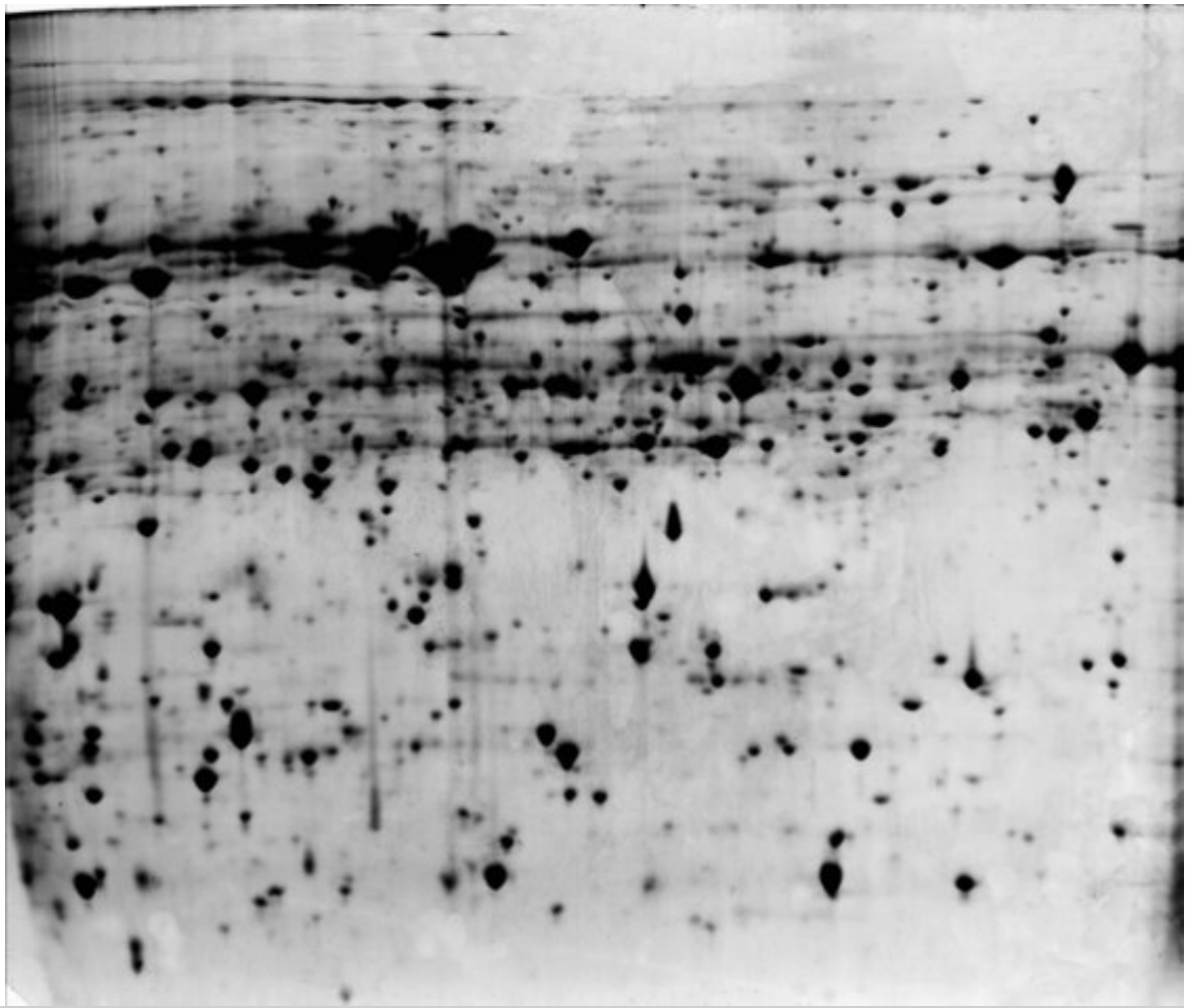
24 cm IPG DryStrip
pH 5.0-6.0

Destreak RehydrationSolution

2 mg E. coli proteins applied in the rehydration solution.

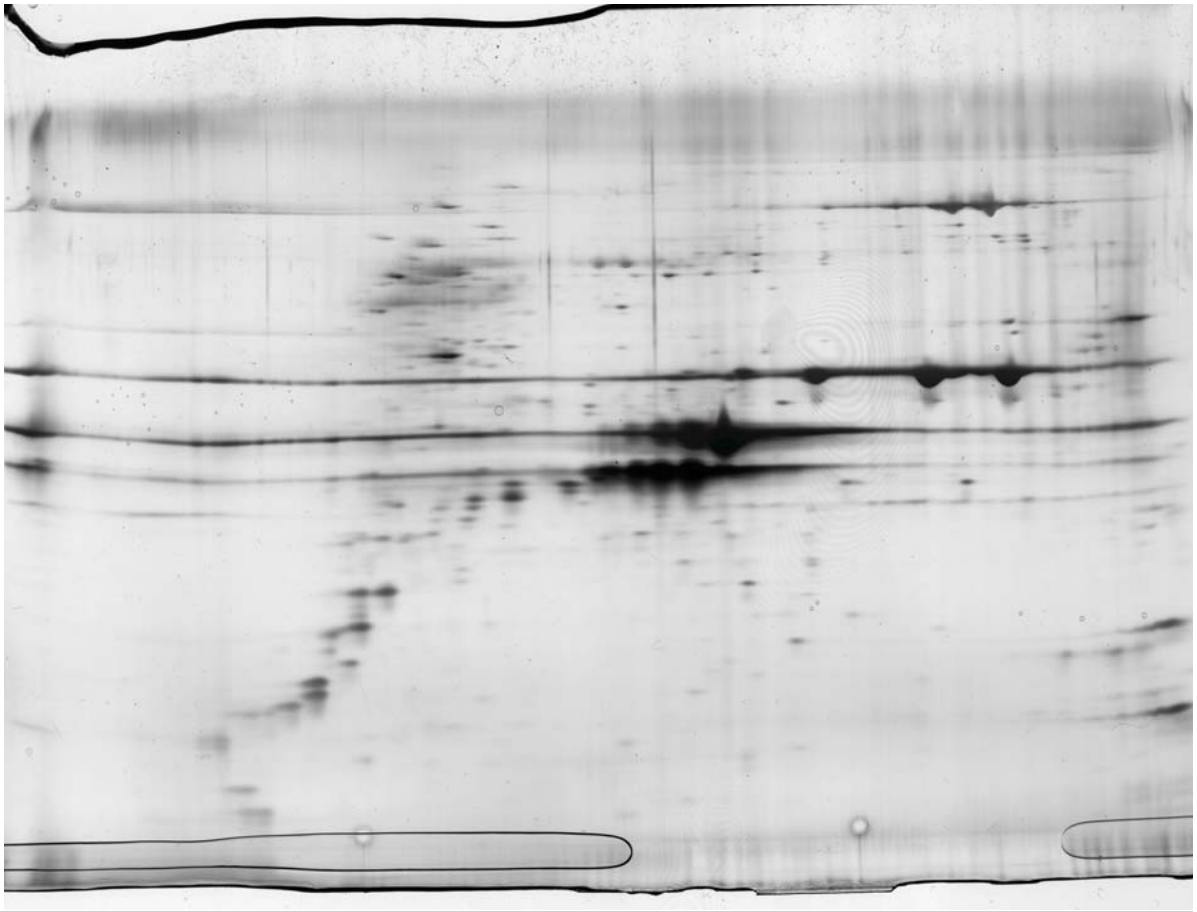
Coomassie stained.

From Ingmar Olsson, Amersham Biosciences Uppsala



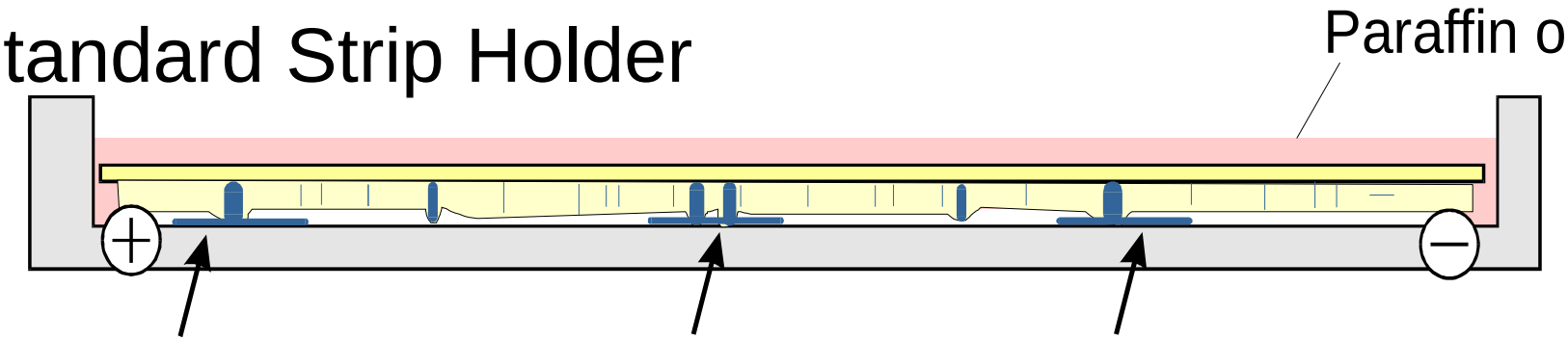
High Protein Loads

Problem: Highly Abundant Proteins

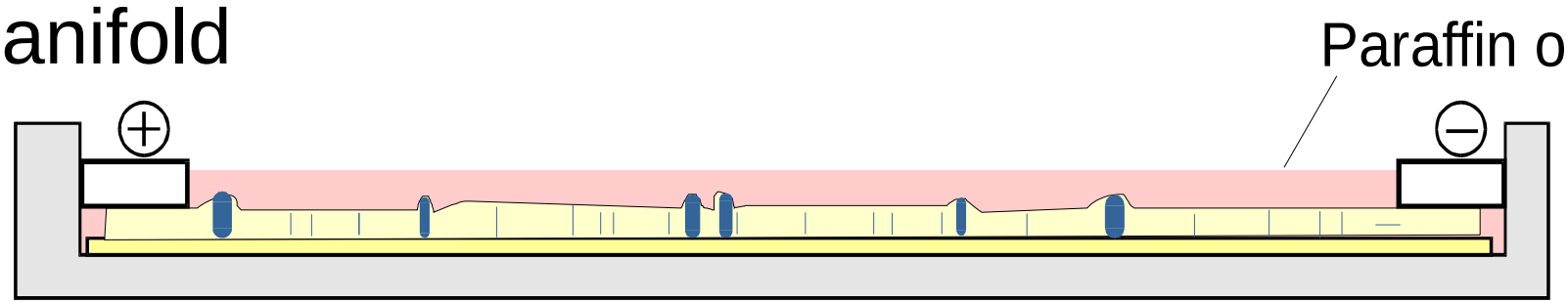


Highly Abundant Proteins

Standard Strip Holder



Manifold



Analytical vs. preparative 2-D PAGE

Analytical

Gel length:	7 to 24 cm
pH gradients:	3 – 10, 4 – 7, 6 – 11
Sample loads:	up to 500 µg protein
Separation system:	Strip holder, CLSH or Manifold
Separation conditions:	IPG gel side up or down, Vh acc. to instruction
Second dimension:	Vertical gels, 1 mm Horizontal gels, 0.5 mm
Detection:	Silver, SYPRO Ruby, DIGE

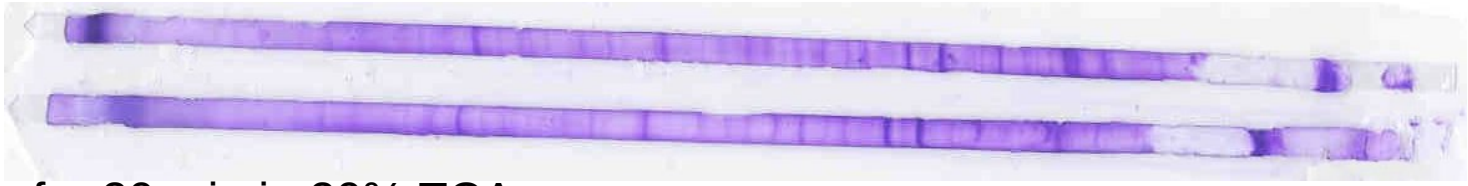
Preparative

Gel length:	18 to 24 cm
pH gradients:	mainly narrow intervals
Sample loads:	more than 500 µg protein
Separation system:	CLSH or Manifold
Separation conditions:	IPG gel side up, Vh + 10 %
Second dimension:	Vertical gels, 1 – 1.5 mm
Detection:	Coomassie, Imidazol-Zinc, DIGE

Staining of IPG Strips (cont. urea, detergent)

Acid Violet 17 Staining:

(Patestos NP *et al.* Electrophoresis. 9 (1988) 488-496)



fix for 20 min in 20% TCA,

wash for 1 min in 3% phosphoric acid,

stain for 10 min in 0.1 % Acid Violet 17 solution in 10% phosphoric acid,

destain 3 × in 3% phosphoric acid until background is clear,

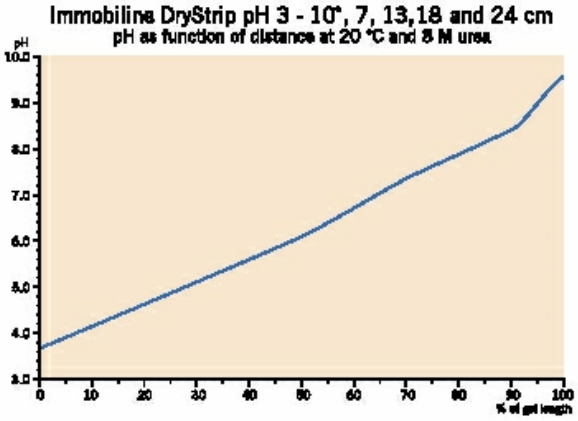
wash 3 × 1 min with H_2O_{dist},

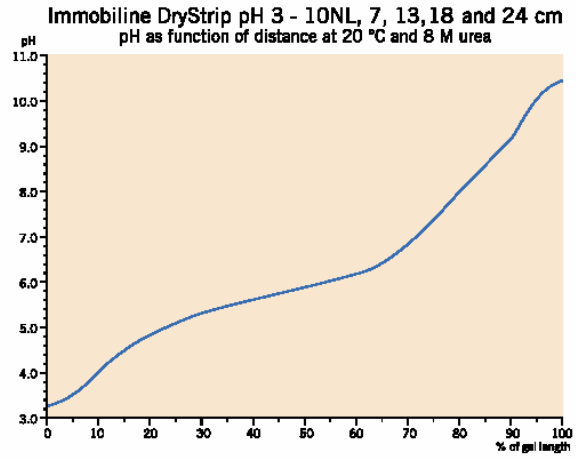
impregnate with 5 % glycerol,

air dry.

Determination of the pH gradient

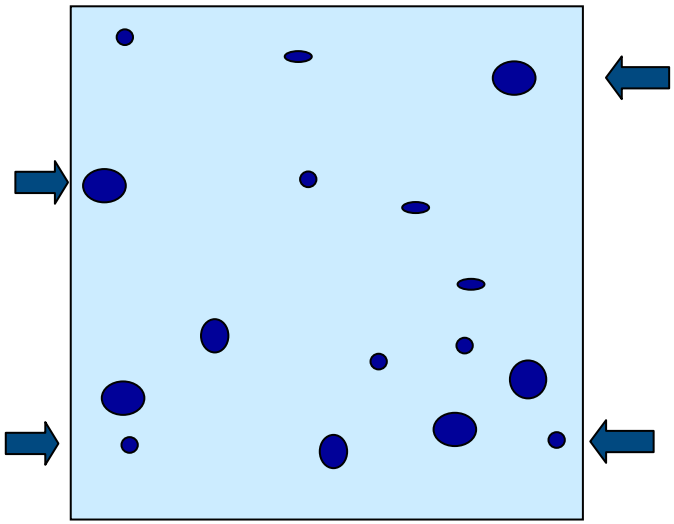
With graphs from IPG pH gradient datafiles





or

Interpolation between identified spots with known pI with image analysis software



Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez J-C, Frutiger S, Hochstrasser D. Electrophoresis 14 (1993) 1023-1031.

2nd Dimension:

SDS polyacrylamide gel electrophoresis

Equilibration of IPG strips

Prior to SDS electrophoresis the strips are treated with equilibration solution:

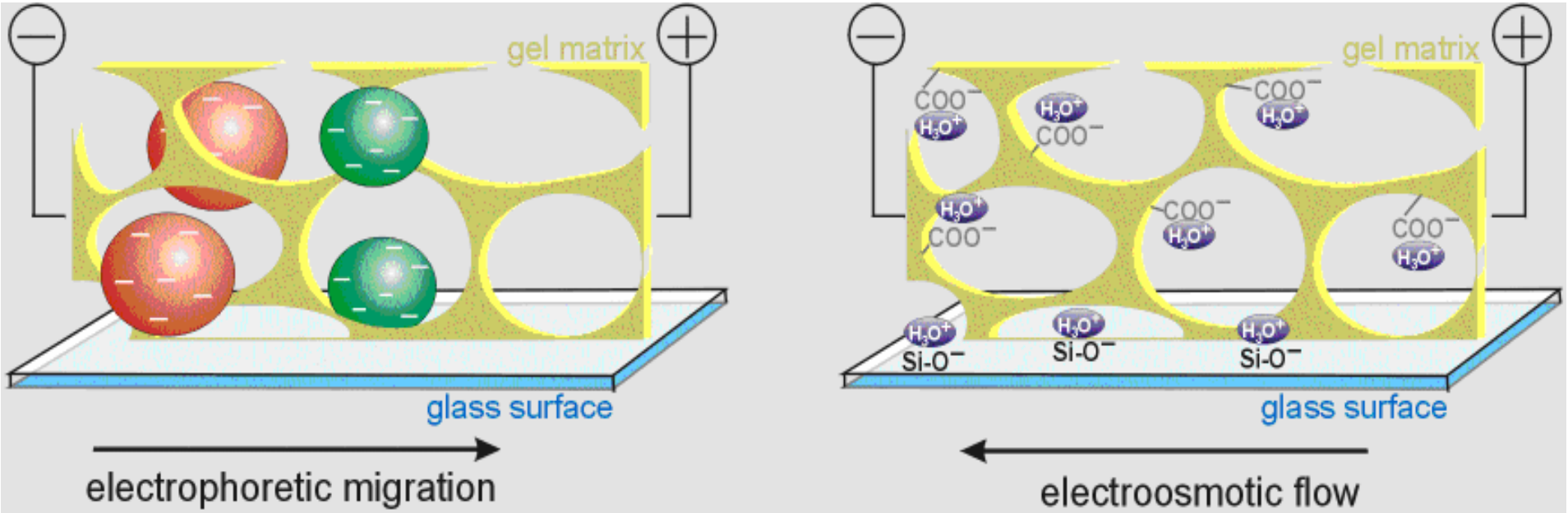
2 % SDS, 50 mMTris-HCl pH 8.8,
6 M urea, 30 % glycerol +
(1 x 15 min) 1 % DTT
(1 x 15 min) 2.5 % iodoacetamide

to cover and unfold polypeptides with SDS and DTT

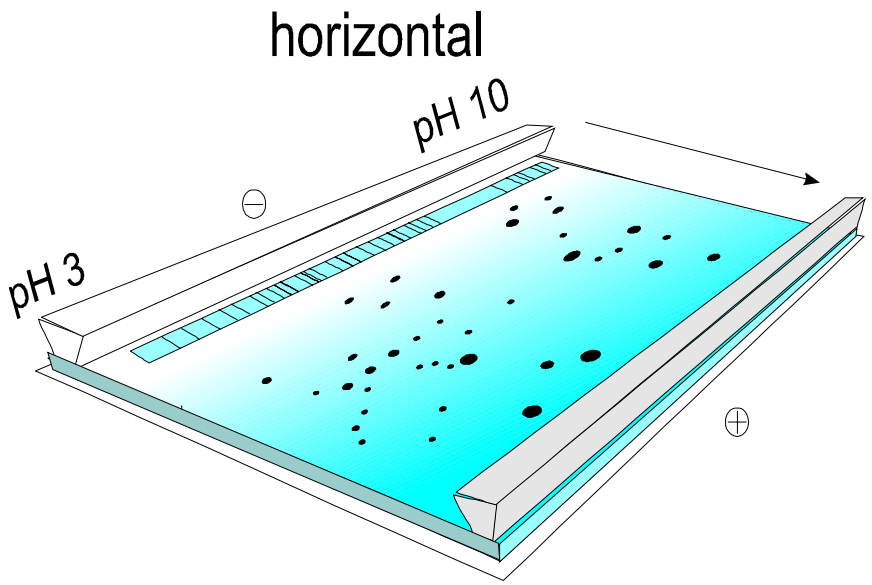
glycerol and urea for minimizing EEO effects

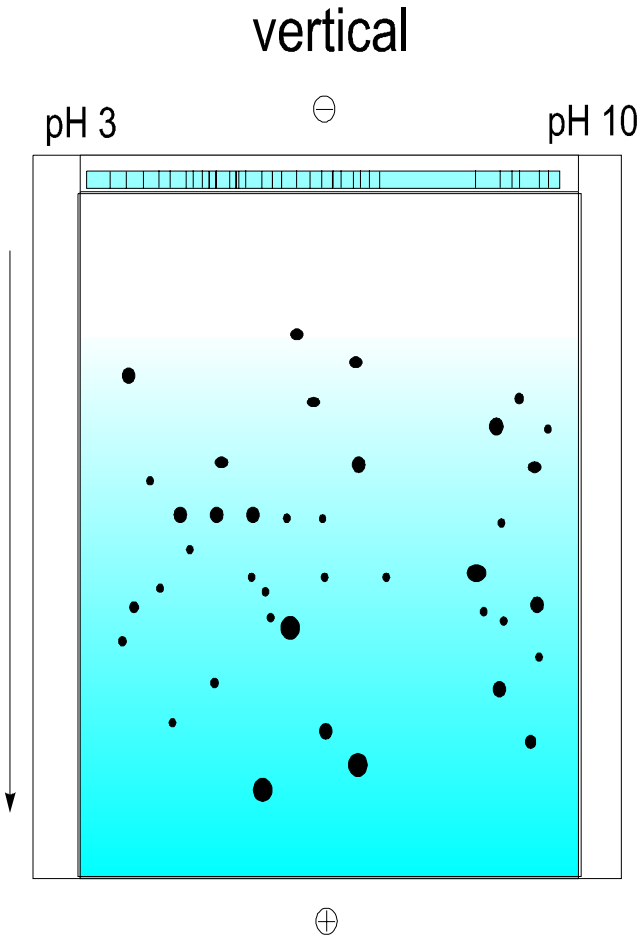
excess DTT is removed with iodoacetamide to prevent point streaking

Electroendosmosis



Second dimension: SDS PAGE





Second Dimension on Multiphor II

Second dimension on Multiphor II ™

pH 4

pH 7

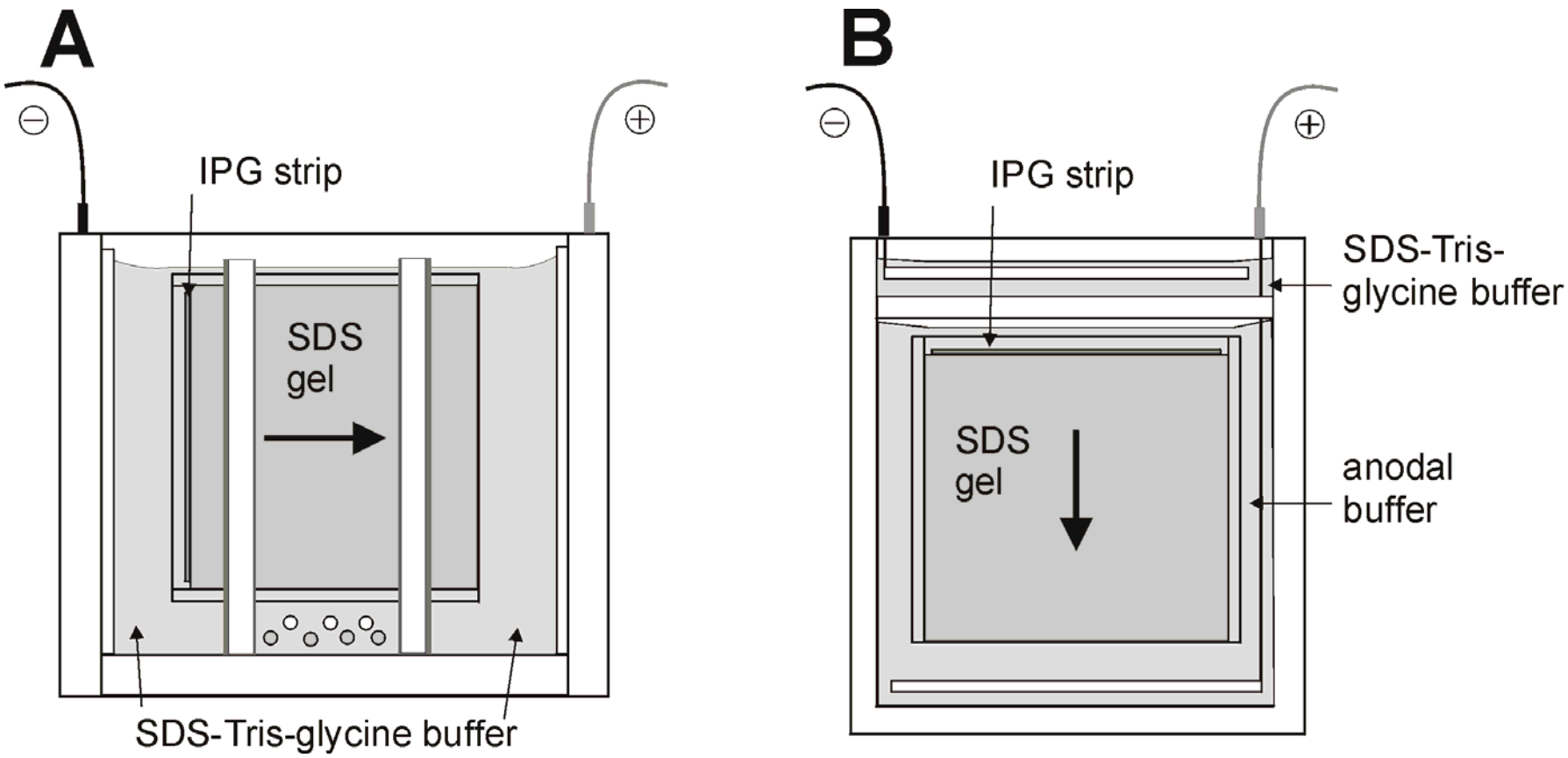
Acidic mouse liver proteins

1st dimension:
IPG 4-7 on
Multiphor II

2nd dimension:
13 % SDS gel
on
Multiphor II

Görg *et al.*
Electrophoresis 16
(1995) 1079-1086

Two ways to run the second dimension

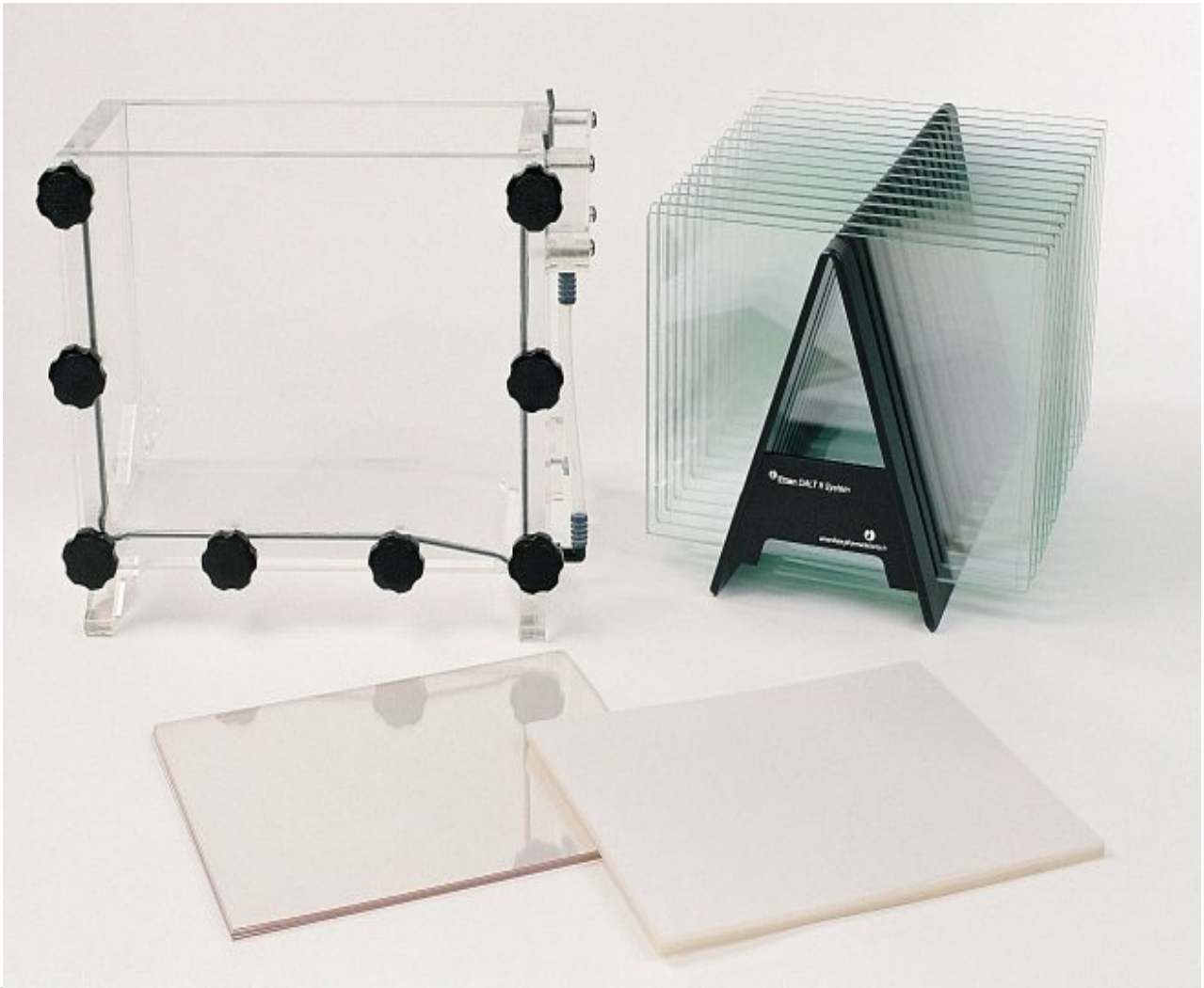


Cassettes for lab-cast Gels

Hinged cassettes with vinyl spacers

1.0 mm and 1.5 mm

Plastic sheets and filler blocks



Casting SDS gels – important points

HQ reagents: PlusOne labelled chemicals are a benchmark

TEMED not too old

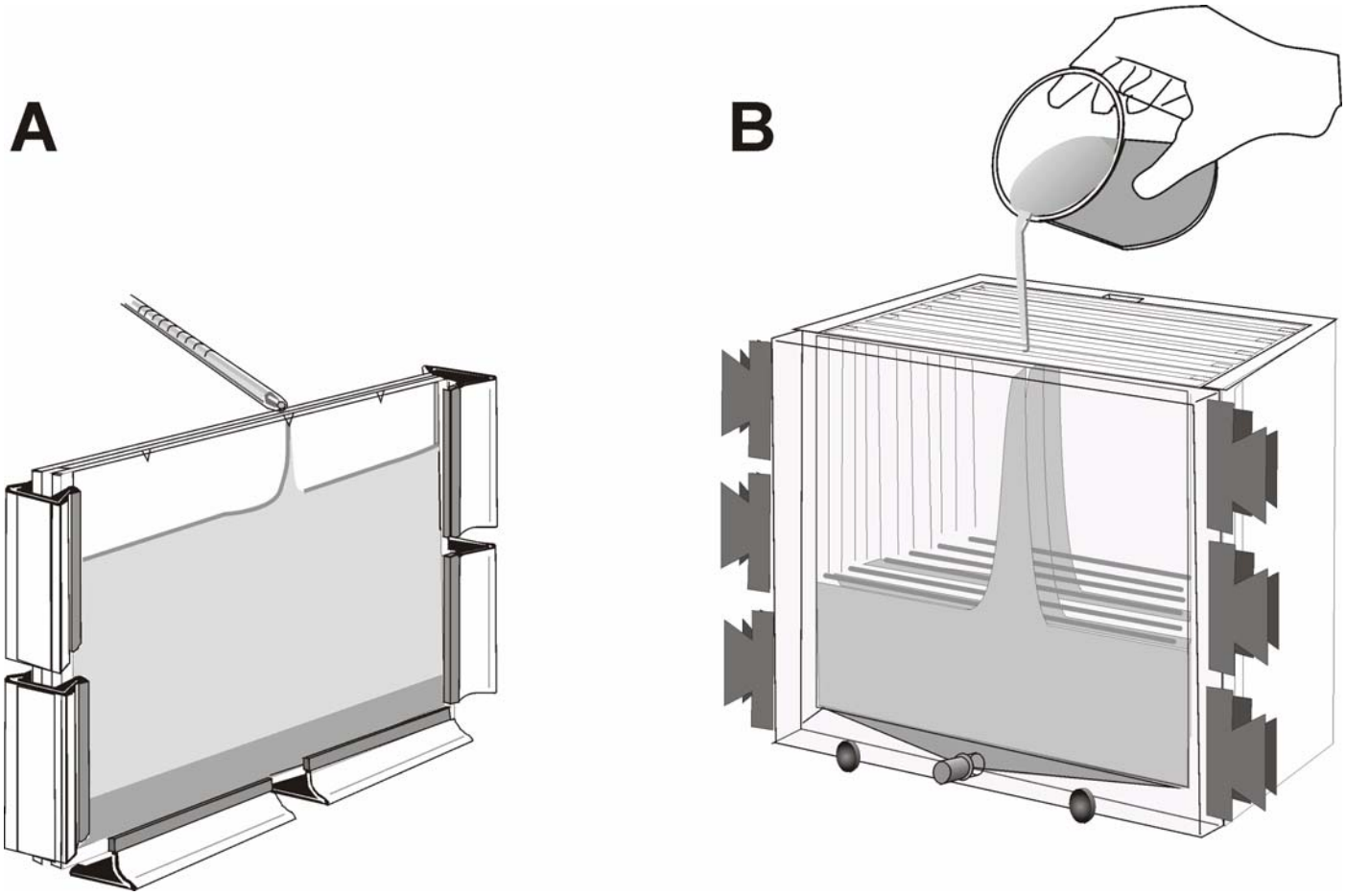
Freshly made APS

Precool monomer solution mix (containing the TEMED)

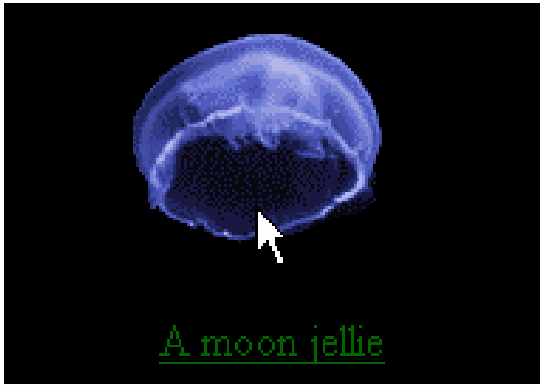
Add APS short before use

Pour solutions quickly in one go

Casting homogeneous SDS gels



Precast Ettan™ DALT gels



1 mm gels on film support

>gels cannot break

>gels cannot shrink or swell

>spot picking with data from image analysis

Special designed cassette

>to prevent uneven front

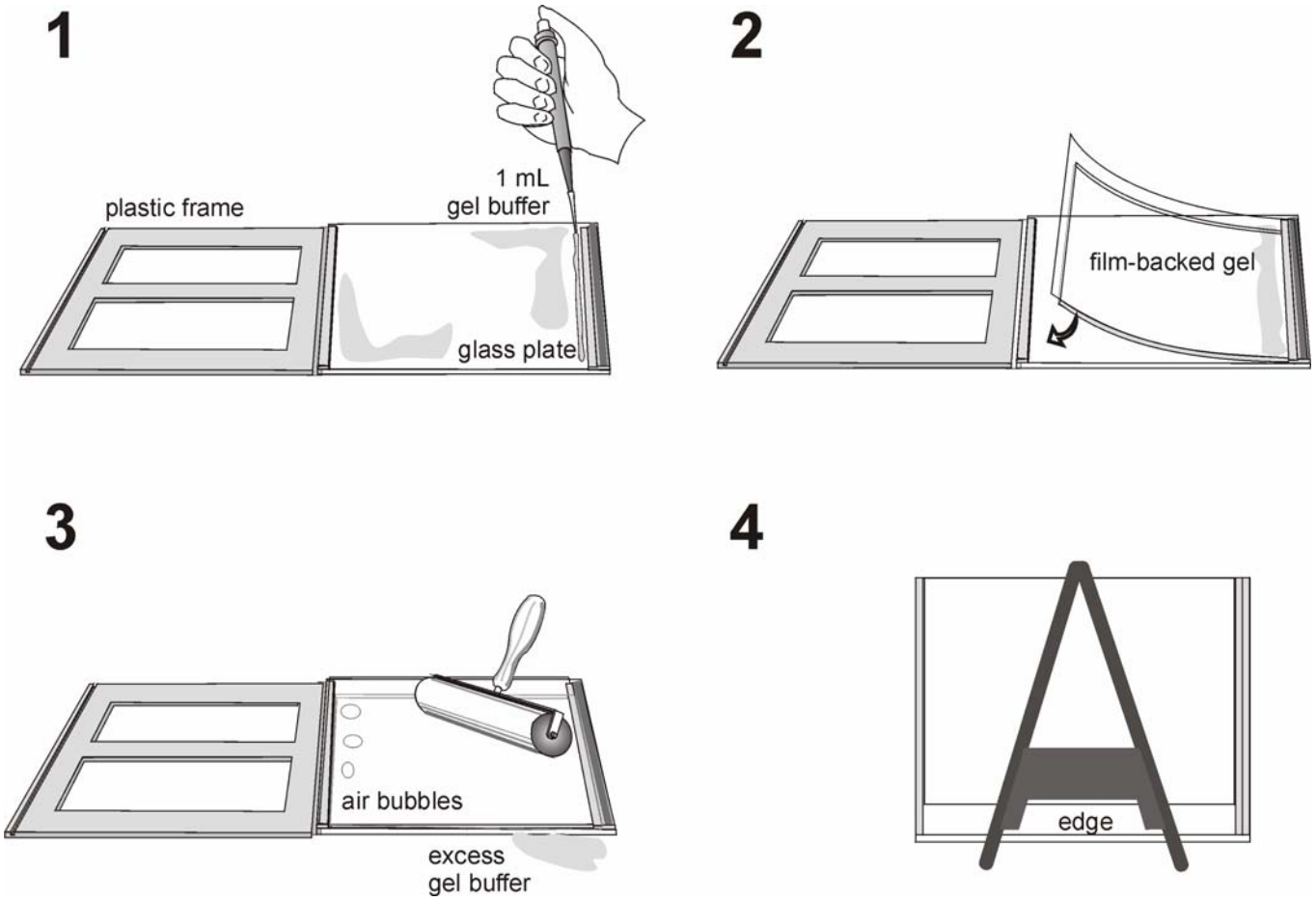
Proprietory PPA buffer system pH 7.0

>long shelflife, no matrix hydrolysis

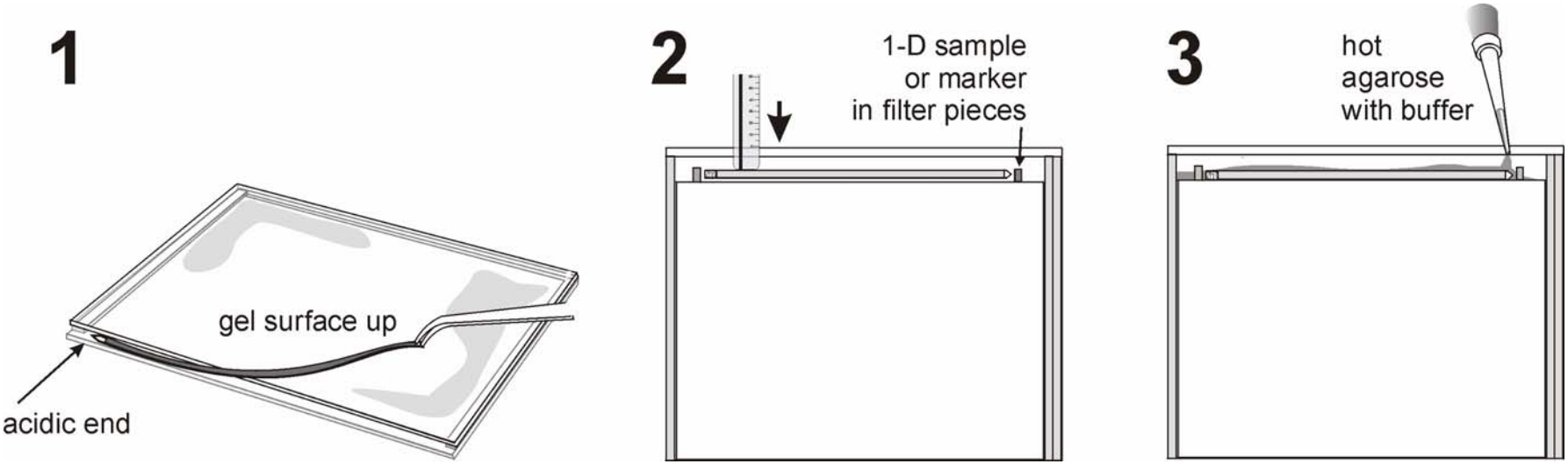
>similar M_r distribution like with Laemmli buffer (glycine in cathode)

>good and reproducible gel quality due to optimal polymerization conditions

How to set up a ready-made gel



Applying the IPG strip on the SDS gel



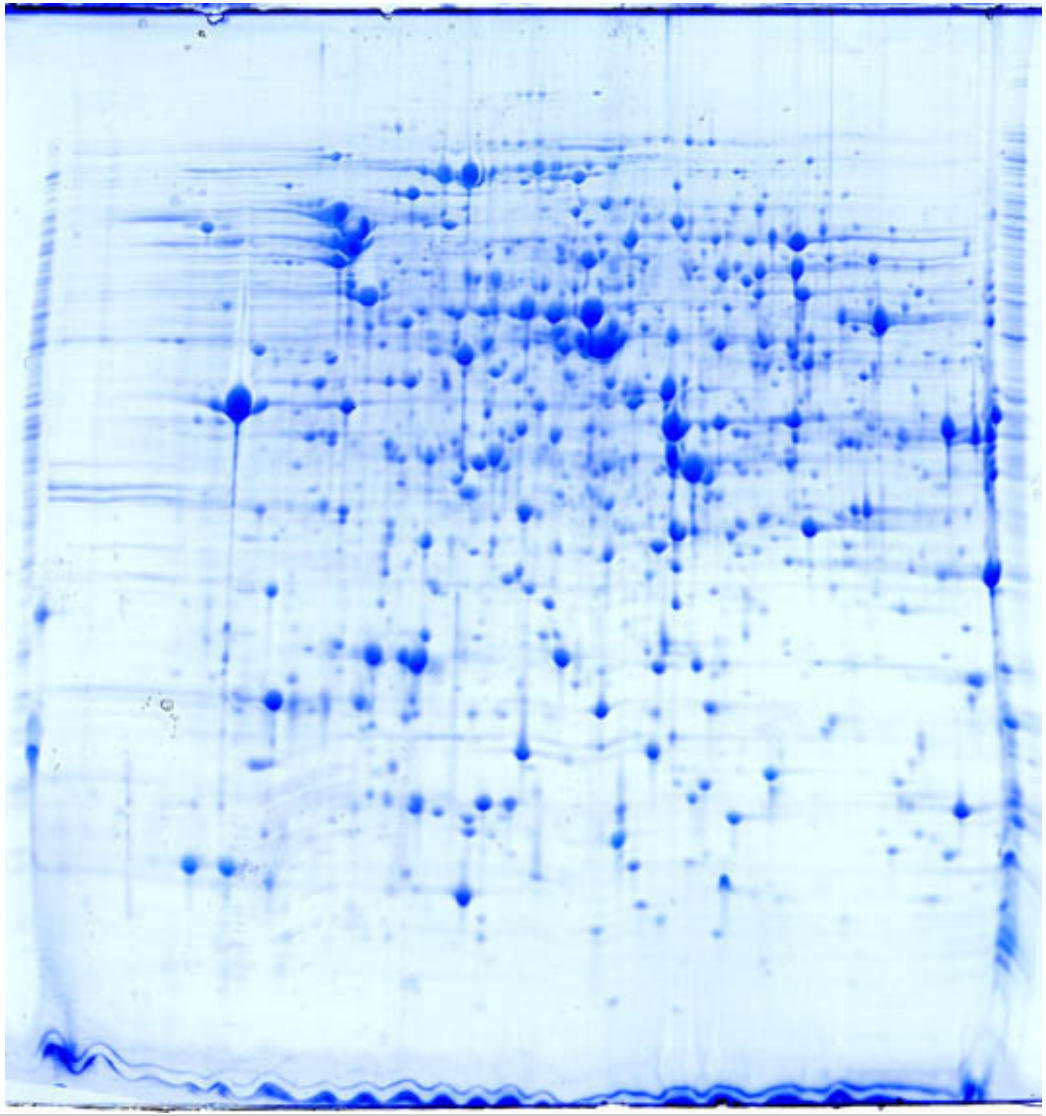
Ettan™ Dalt II Gel on film support

1 mg E. coli strain B

IPGphor
24 cm pH 4 - 7

ETTAN™ Dalt gel
12.5 % *T*

Colloidal CBB
G250 staining



Ettan™ Dalt Gel on film support

ca. 100 µg
Brassica oleracea
Floral tissue extract

IPGphor
pH 4-7
24 cm

ETTAN™ Dalt gel
12.5 % *T*