Gel Electrophoresis

What is Electrophoresis?

- The migration of charged molecules in solution in response to an electric field
- Proteins, at a pH other than their pI, carry a net charge
- Rate is proportional to:
  - Field strength
  - Ionic strength
  - Viscosity
  - Net charge
  - Temperature
  - Size, Shape
The Double-Layer Theory

- Ionic solutes surround the charged particle
- Counter-ions concentrate at particle surface and dissipate with distance
- Ion layer dampens particle’s electric potential
- Changes in ionic strength, dielectric constant, and temperature affect layer thickness

The Electronic Double-Layer
The Double-Layer Theory

- Ionic solutes surround the charged particle
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- Ion layer dampens particle’s electric potential
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\[ \kappa \sim (\mu / DT)^{1/2} \quad \Psi \sim \Psi_0 e^{-\kappa} \quad u \sim \Psi D/\eta \]

“Zone” Electrophoresis

- vs. Moving Boundary Electrophoresis
- Sample applied over small area
- Complete resolution of individual components
- The “zone” is now usually omitted
Electrophoretic Media

- Ideal support media should simply trap fluids, having limited interaction with sample
- Practical considerations include:
  - Transparency
  - Mechanical strength
  - Cost
  - Toxicity/Ease of use
- Common Media:
  - Filter paper, cellulose acetate
  - Starch, agar, polyacrylamide gels

Electrophoretic Media

- All media exhibit some sample interference
  - Adsorption
  - Ion exchange
  - Chemical reaction
  - Molecular sieving
  - Migration path distortion
  - Perturbation of ionic environment
  - Electro-osmotic buffer flow

- Which media do I choose?
Evolution of Gel Electrophoresis

- "Ionophoresis in Silica Jelly" 1946
  - First significant analysis of peptide using gels

- Agar gel protein separation attempted in 1907 by Field and Teague

- Agar gel separation of inorganic ions by Kendall et al., 1923-1926
  - Tube gels 5 cm dia., up to 20 meters long!
  - Electrophoresed at 100-500V for days...

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Evolution of Gel Electrophoresis

- Pectin Gel – Grabar, et al. 1956

- Starch Gel – Poulik & Smithies 1958

- Acrylamide – Raymond & Weintraub 1959

- Carboxymethyl Cellulose – Huehns & Jakubovic 1960
Polyacrylamide Gels

- Highly pure chemicals readily available
- Chemically inert
- Stable over pH, Temp
- Transparent
- Molecular sieving
- Reproducible

Native PAGE

- Most proteins have a pI = 4-7
- Most native gels run at pH = 8.0 – 9.5
  - (Most) proteins have net negative charge
  - Migrate towards anode (‘bottom’ in most gels)
- Closer pH is to the pI:
  - Increases running time
  - Increases separation
- Separation based on both size and charge
- Often used to characterize complexes
Denaturing PAGE

- Urea was first common denaturant
  - Requires high concentration (8M)
  - Does not affect intrinsic protein charge
  - Protein separated by size and charge

- Sodium Dodecyl Sulphate (SDS)
  - Better denaturant than urea
  - Quantitatively coats protein at 1.4 : 1 ratio
    - Normalizes charge density
    - Protein separated only by *size*, not charge

First SDS-PAGE Ever Run

- Jacob V. Maizel, Jr.
  - (a) July 7, 1963
    Adenovirus particles on a 5% acrylamide gel
  - (b) March 1965
    Adenovirus particles + β-mercaptoethanol on a 5% acrylamide gel
(Dis)Continuous Buffer Systems

- Continuous Buffer Electrophoresis
  - Same buffer ions & pH in sample, gel, buffer
  - Can have different concentrations
  - No stacking gel

- Discontinuous Buffer Electrophoresis
  - No constraints on buffer ion composition/pH
  - Utilizes a large pore stacking gel
  - Can handle larger sample volumes

Why a Stacking Gel Stacks

- Tris-glycine, pH=8.3 in reservoir buffer
- Tris-HCl, pH=6.8 in low % stacking gel
- Tris-HCl, pH=8.8 in high % resolving gel
- Both glycine and Cl⁻ migrate to anode
- The pI of glycine is ~6
  - Glycine in buffer is more dissociated
  - Glycine in stacking gel is less dissociated
  - Glycine in resolving gel is more dissociated
Stacking: Pre-Run

Stacking: Running, #1A

No ions = Big Voltage Gradient
Stacking: Running, #1B

Stack pH=6.8

Resolving pH=8.8

Stacking: Running, #2

Stack pH=6.8

Resolving pH=8.8
Stacking: Running, #3

Stack pH=6.8

Resolving pH=8.8

Anode (+)

Stacked: Resolving

Stack pH=6.8

Resolving pH=8.8

Anode (+)
Isoelectric Focusing (IEF)

- Electrophoretic technique in which amphoteric compounds are fractionated according to their isoelectric points along a continuous pH gradient
- Sample must be a good ampholyte
  - Sharp titration curve (i.e. not short peptides)
- Support media exhibits limited sieving
- Protein complexes must be very stable
  - Remain in tact at pH = pI
  - The complex $K_d < 1 \mu M$

Advantages:
- IEF is an equilibrium technique
- pIs can be measured to ~0.001 pH units

Disadvantages:
- Limited effectiveness with pI<3.5 & pI>10
- Large complexes restrained by support media

Biggest challenge: pH gradient
- Synthetic Carrier Ampholytes (SCA)
- Immobilized pH Gradients (IPG)
Synthetic Carrier Ampholytes

- A complex mixture of soluble amphoteric compounds with closely spaced pls, spanning a given pH range

- Oligoamino-oligocarboxylic acids
  - pl range 3-10, gradient resolution to ~0.02 pH
  - Average mol mass ~750 mw (600-900)
  - Good buffering capacity

Problems With SCA-IEF

- Basic SCA form complexes with hydrophobic proteins
- Acidic SCA can chelate metal ions
- Low ionic strength can cause protein ppts
- Uneven buffering & conductivity
- Cathodic drift (Electro-osmosis)
  - pH gradient is pulled towards cathode
  - Basic proteins are lost (NEPHGE)
  - pH gradient towards anode plateaus
**Immovilized pH Gradients**

- A pH gradient that is copolymerized with the support matrix via the inclusion of modified monomers

- Immobilines:
  - Based on Acrylamide: \( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}_2 \)
  - Append side group: \( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}–\text{R} \)
  - \( \text{R}= \) Non-amphoteric weak acid or base

- Pour gels as gradients of low to high \( pK_a \)

- Gradient resolution to ~0.001 pH units

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**Acidic Immobilines**

<table>
<thead>
<tr>
<th>pK&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Formula</th>
<th>Name</th>
<th>( M_r )</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}–\text{C}–\text{CH}_3 )</td>
<td>2-Acrylamido-2-methylpropanesulfonic acid</td>
<td>207</td>
<td>( b )</td>
</tr>
<tr>
<td>3.1</td>
<td>( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}–\text{CH}–\text{COOH} )</td>
<td>2-Acrylamidoglycolic acid</td>
<td>145</td>
<td>( c )</td>
</tr>
<tr>
<td>3.6</td>
<td>( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}–\text{CH}_3–\text{COOH} )</td>
<td>N-Acrylsarcosine</td>
<td>129</td>
<td>( d )</td>
</tr>
<tr>
<td>4.4</td>
<td>( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}–(\text{CH}_3)_2–\text{COOH} )</td>
<td>3-Acrylamidopropanoic acid</td>
<td>143</td>
<td>( d )</td>
</tr>
<tr>
<td>4.6</td>
<td>( \text{CH}_2=\text{CH}–\text{CO}–\text{NH}–(\text{CH}_3)_2–\text{COOH} )</td>
<td>4-Acrylamidobutyric acid</td>
<td>157</td>
<td>( d )</td>
</tr>
</tbody>
</table>

* The pK values for the three Immobilines and for 2-acrylamidoglycolic acid are given at 25°, for AMPS (pK 1.0) the temperature of pK measurement is not reported.

\(<a href="coordinates%20with%20figures%20for%20Acidic%20Immobilines.html">Polysciences, Inc. (Warrington, PA).


\(<a href="coordinates%20with%20figures%20for%20Acidic%20Immobilines.html">Pharmacia-LKB Biotechnology (Uppsala, Sweden).
**Basic Immobilines**

<table>
<thead>
<tr>
<th>pK a</th>
<th>Formula</th>
<th>Name</th>
<th>M Wt</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>2-Morpholinoethylacrylamide</td>
<td>154</td>
<td>b</td>
</tr>
<tr>
<td>7.1</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>3-Morpholinoethylacrylamide</td>
<td>148</td>
<td>b</td>
</tr>
<tr>
<td>8.3</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Diethylaminomethylacrylamide</td>
<td>142</td>
<td>b</td>
</tr>
<tr>
<td>8.3</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Diethylaminomethylacrylamide</td>
<td>150</td>
<td>b</td>
</tr>
<tr>
<td>8.5</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Diethylaminomethylacrylamide</td>
<td>150</td>
<td>b</td>
</tr>
<tr>
<td>7.5</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Diethylaminomethylacrylamide</td>
<td>150</td>
<td>b</td>
</tr>
<tr>
<td>7.5</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Diethylaminomethylacrylamide</td>
<td>150</td>
<td>b</td>
</tr>
<tr>
<td>6.6</td>
<td>CH₃−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Diethylaminomethylacrylamide</td>
<td>150</td>
<td>b</td>
</tr>
</tbody>
</table>

*All pK a values except pK a(10.3) measured at 25°C. The value of pK a(10.3) was measured at 80°C.

1 Righetti et al, Meth Enzymol 270:235-255

**More Basic Immobilines**

<table>
<thead>
<tr>
<th>pK a</th>
<th>Formula</th>
<th>Name</th>
<th>M Wt</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6</td>
<td>CH₂−CH−CO−NH−(CH₂)₂−N</td>
<td>2-Thiopheneminocarbonylacrylamide</td>
<td>200</td>
<td>b</td>
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<tr>
<td>6.85</td>
<td>CH₂−CH−CO−NH−(CH₂)₂−N</td>
<td>1-Acetyl-4-methylpiperaizine</td>
<td>154</td>
<td>c</td>
</tr>
<tr>
<td>7.0</td>
<td>CH₂−CH−CO−NH−(CH₂)₂−N</td>
<td>2-(4-Isoxazoyl)ethylamine-2-acrylamide</td>
<td>165</td>
<td>d</td>
</tr>
<tr>
<td>7.4</td>
<td>CH₂−CH−CO−NH−(CH₂)₂−N</td>
<td>2-Thiopheneminocarbonylacrylamide</td>
<td>214</td>
<td>b</td>
</tr>
<tr>
<td>8.05</td>
<td>CH₂−CH−CO−NH−(CH₂)₂−N</td>
<td>N,N-Bis(2-hydroxyethyl)-N′-acrylamido-1,3-diaminopropane</td>
<td>200</td>
<td>c</td>
</tr>
</tbody>
</table>

*All pK a values measured at 25°C.


Righetti et al, Meth Enzymol 270:235-255
SCA/IPG-IEF

- Hydrophobic & membrane proteins can have detrimental interactions with IPGs
  - Hung up on well as entering the gel
  - Streaky bands at or near pI
- Inclusion of 3-4% SCA can resolve this
  - SCA form mixed micelles
  - Interact hydrophobically with protein
- Use as low % SCA as possible
- SCA must be pre-equilibrated in IPG