What is Proteomics?

The analysis of the proteins from a biological sample at a given time.

The tools are mainly:

Separation methods such as chromatography and electrophoresis.

Biological assays such as ELISA immuno-assays.

Structural analysis techniques such as mass spectrometry.
Human genome

• 23 chromosome pairs
• More than 3 billions DNA base pairs
• 23’000 protein coding genes

but only

• 5’000 identified proteins
• 7’000 suspected proteins
• 11’000 missing proteins ??
Post-translational modifications

- **Phosphorylation**: serine and threonine mediated by serine/threonine kinases, also tyrosine mediated by tyrosine kinases. Associated to cell signaling.

- **Ubiqination**

- **Methylation, Acetylation, glycosylation, Oxidation, Nirtrosylation**
Proteomics

- A major challenge to Analytical Chemistry!
### Proteomics and Diagnostics

<table>
<thead>
<tr>
<th>Analysis of a whole protein content</th>
<th>Analysis of a specific protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Selectivity</td>
<td>Selectivity by affinity</td>
</tr>
<tr>
<td>Universal detection (MS)</td>
<td>Specific detection</td>
</tr>
<tr>
<td>No amplification</td>
<td>Amplification (ELISA)</td>
</tr>
<tr>
<td>LOD: 10 nM</td>
<td>LOD: 10 fM</td>
</tr>
</tbody>
</table>

LOD: Limit of Detection

Tuesday, October 12, 2010
Two major approaches of Proteomics

2-D gel separation
- Isoelectric focusing
- Size separation by SDS PAGE
- Spot transfer, e.g. Blotting
- Analysis, e.g. Mass spectrometry

Shot gun approach
- Digest proteins
- Separate polypeptides
  - Ion chromatography, HPLC, ICAT,…
- Analyse peptides by MS

Bio-informatics to identify known proteins
2D gel mapping

pl fractionated & SDS denaturated proteins

pH

Isoformes

Colorectal epithelial cell
Challenges for automatic software-based analysis include:

- Incompletely separated (overlapping) spots (less-defined and/or separated)
- Weak spots / noise (e.g., "ghost spots")
- Running differences between gels (e.g., protein migrates to different positions on different gels)
- Unmatched/undetected spots, leading to missing values
- Mismatched spots
- Errors in quantification (several distinct spots may be erroneously detected as a single spot by the software and/or parts of a spot may be excluded from quantification)
- Differences in software algorithms and therefore analysis tendencies

Generated picking lists can be used for the automated in-gel digestion of protein spots, and subsequent identification of the proteins by mass spectrometry.
Plan

• Electrophoresis & Proteomics
  • Isoelectric focusing
    • SDS PAGE
    • Capillary electrophoresis
    • Off gel electrophoresis
  • Electrospray Ionization
  • Matrix Assisted Laser Desorption Ionization
  • Scanning Electrochemical Microscopy
Ion mobility

Viscous friction

\[ f = -\zeta v \]

Electrochemical potential gradient

\[ F = -\frac{1}{N_A} \text{grad} \tilde{\mu} \]

Newton's law

\[ f + F = ma \]
Steady state flux

At the steady-state

\[ \nu = -\frac{1}{N_A \zeta} \nabla \tilde{\mu} \]

Flux in solution

\[ J_i = c_i \nu = -\frac{c_i}{N_A \zeta} \nabla \tilde{\mu}_i = -c_i \tilde{\mu}_i \nabla \tilde{\mu}_i \]

\( \tilde{\mu}_i \)  Electrochemical mobility (>0)
Viscous friction

Navier-Stokes equation in laminar flow

\[ \zeta = 6\pi \eta r \]
Phenomenological Equation

Electrochemical Potential

\[ \tilde{\mu}_i = \mu_i^\circ + RT \ln c_i + \bar{V}_i p + z_i F \phi \]

Flux

\[ J_i = - c_i \tilde{u}_i \text{grad} \mu_i - z_i F c_i \tilde{u}_i \text{grad} \phi - c_i \tilde{u}_i \bar{V}_i \text{grad} p \]
Isoelectric focusing

Ampholytes separation:
- in carrier ampholytes
- on a pH gradient gel
Carrier ampholyte

- Developed in 1961 by H. Svensson
- Strong buffering power at the pI
- Low conductivity at the pI
- Low molecular weight

```
CH₂—N—(CH₂)ₓ — N—(CH₂)ₓ
   \              \                      \(R = H \text{ or } (CH₂)ₓ\text{-COOH}, X=2 \text{ or } 3\)
   \(CH₂)ₓ          \(CH₂)ₓ\)
   \NR₂            \COOH
```
Invitrogen Carrier Ampholyte

ZOOM® Carrier Ampholytes are mixtures of amphoteric species containing sulfonate and carboxylic groups at one end, and free primary and secondary amino groups at the other. As the degree of substitution and chain length vary, many molecular components constitute a ZOOM® Carrier Ampholyte. The average molecular weight distribution is 400-700 Daltons.

A 2% ZOOM® Carrier Ampholyte solution must have the following specifications:

<table>
<thead>
<tr>
<th>Ampholytes</th>
<th>pH</th>
<th>pH Gradient</th>
<th>Conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZOOM® Carrier Ampholytes 3-10</td>
<td>6.9 ± 0.4</td>
<td>3.3 ± 0.5-10.0 ± 0.6</td>
<td>400-890 µS/cm</td>
</tr>
<tr>
<td>ZOOM® Carrier Ampholytes 4-7</td>
<td>5.79 ± 0.4</td>
<td>4.0 ± 0.3-7.0 ± 0.4</td>
<td>150-550 µS/cm</td>
</tr>
<tr>
<td>ZOOM® Carrier Ampholytes 6-9</td>
<td>7.65 ± 0.4</td>
<td>6.0 ± 0.4-9.0 ± 0.4</td>
<td>200-650 µS/cm</td>
</tr>
<tr>
<td>ZOOM® Carrier Ampholytes 9-11</td>
<td>10.4 ± 0.4</td>
<td>8.9 ± 0.4–10.7 ± 0.4</td>
<td>200-700 µS/cm</td>
</tr>
<tr>
<td>ZOOM® Carrier Ampholytes 4-6</td>
<td>5.2 ± 0.3</td>
<td>4.0 ± 0.3-6.1 ± 0.3</td>
<td>150-400 µS/cm</td>
</tr>
<tr>
<td>ZOOM® Carrier Ampholytes 5-7</td>
<td>6.4 ± 0.3</td>
<td>4.8 ± 0.3-7.3 ± 0.3</td>
<td>150-390 µS/cm</td>
</tr>
<tr>
<td>ZOOM® Carrier Ampholytes 6-8</td>
<td>7.6 ± 0.4</td>
<td>6.2 ± 0.4-8.2 ± 0.3</td>
<td>150-550 µS/cm</td>
</tr>
</tbody>
</table>
Immobilised pH Gradient (IPG) gel

Gradient mixing of ampholytes in a polyacrylamide gel

Electrophoresis in practice, R. Westermeier, Wiley.
Bromocresol titration of an IPG strip

Absorbance vs. Wavelength / nm

pH=3: yellow
pH=10: dark blue

1: pH 4.4
2: pH 4.7
3: pH 5.2
4: pH 5.4
5: pH 5.5
6: pH 5.7
7: pH 5.9
8: pH 6.0
9: pH 6.7

pH$_{exp}$ vs. pH$_{th}$

Tuesday, October 12, 2010
Amino acids with hydrophobic side groups

- Valine (val)
- Leucine (leu)
- Isoleucine (ile)
- Methionine (met)
- Phenylalanine (phe)

Amino acids with hydrophilic side groups

- Asparagine (asn)
- Glutamic acid (glu)
- Glutamine (gln)
- Histidine (his)
- Lysine (lys)
- Arginine (arg)

Amino acids that are in between

- Glycine (gly)
- Alanine (ala)
- Serine (ser)
- Threonine (thr)
- Tyrosine (tyr)
- Tryptophan (trp)

Hydrophilic

Lipophilic
# Properties of natural amino acids

<table>
<thead>
<tr>
<th>Aminoacid</th>
<th>M</th>
<th>pK COOH</th>
<th>pK NH3</th>
<th>pK side</th>
<th>pl</th>
<th>Hydropathy</th>
<th>Occurrence%</th>
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<td><strong>Nonpolar</strong></td>
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<td>Leu</td>
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<td>I</td>
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<td>M</td>
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<td>Glutamine</td>
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<td>5.7</td>
<td>–3.5</td>
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<td><strong>Polar charged</strong></td>
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<tr>
<td>Glutamic acid</td>
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<td>9.7</td>
<td>4.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Amino acid charge

Acid terminal

\[ c_{A^-} + c_{AH} = 1 \]
\[ K_{a1} = \frac{c_{A^-} - c_{H^+}}{c_{AH}} \]

\[ c_{A^-} = \frac{K_{a1} \cdot 10^{pH}}{1 + K_{a1} \cdot 10^{pH}} \]
\[ c_{AH} = \frac{1}{1 + K_{a1} \cdot 10^{pH}} \]

Base terminal

\[ c_B + c_{BH^+} = 1 \]
\[ K_{a2} = \frac{c_B c_{H^+}}{c_{BH^+}} \]

\[ c_{BH^+} = \frac{1}{1 + K_{a2} \cdot 10^{pH}} \]
\[ c_B = \frac{K_{a2} \cdot 10^{pH}}{1 + K_{a2} \cdot 10^{pH}} \]
Amino acid

Cation
\[ c_{AH-BH^+} = c_{AH}c_{BH^+} = \left( \frac{1}{1 + K_{a1}\cdot10^{pH}} \right)\left( \frac{1}{1 + K_{a2}\cdot10^{pH}} \right) \]

Anion
\[ c_{A^-B} = c_{A^-}c_{B} = \left( \frac{K_{a1}\cdot10^{pH}}{1 + K_{a1}\cdot10^{pH}} \right)\left( \frac{K_{a2}\cdot10^{pH}}{1 + K_{a2}\cdot10^{pH}} \right) \]

Neutral
\[ c_{AH-B} = c_{AH}c_{B} = \left( \frac{1}{1 + K_{a1}\cdot10^{pH}} \right)\left( \frac{K_{a2}\cdot10^{pH}}{1 + K_{a2}\cdot10^{pH}} \right) \]

Zwitterionic
\[ c_{A^-BH^+} = c_{A^-}c_{BH^+} = \left( \frac{K_{a1}\cdot10^{pH}}{1 + K_{a1}\cdot10^{pH}} \right)\left( \frac{1}{1 + K_{a2}\cdot10^{pH}} \right) \]
Zwitterionic ampholyte: $A^-B, A^-B^+, AB^+$

$$K_2 = \frac{c_Bc_{H^+}}{c_{BH^+}}$$

$$K_2 = 10^{-6}$$

$$K_1 = \frac{c_A-c_{H^+}}{c_{AH}}$$

$$K_1 = 10^{-4}$$
Amino acid

pK=2.5  pK=9.5

\[ z(\text{pH}) = F\left( c_{\text{AH}} c_{\text{BH}^+} - c_{\text{A}^-} c_{\text{B}} \right) = F\left( c_{\text{BH}^+} - c_{\text{A}^-} \right) \]
Isoelectric focusing

Diffusion-migration flux

\[ J_i = -c_i \tilde{u}_i \ \text{grad} \tilde{\mu}_i = -c_i \tilde{u}_i \ \text{grad} \mu_i - c_i \tilde{u}_i z_i F \ \text{grad} \phi \]

Equation of conservation

\[ \frac{\partial c_i}{\partial t} = -\text{div} J_i = 0 \]

\[- \frac{\partial}{\partial x} \left[ - \frac{c_i(x) \tilde{u}_i RT}{c_i(x)} \frac{\partial c_i(x)}{\partial x} - c_i(x) \tilde{u}_i z_i(x) F \frac{\partial \phi}{\partial x} \right] = 0 \]

\[ RT \ \frac{\partial c_i(x)}{\partial x} = c_i(x) z_i(x) FE \]
Resolution

Linear approximation of the charge at the pl

\[ z_i = -p_i \ x \]

\[ RT \ \frac{\partial c_i}{\partial x} = -c_i p_i F E \ x \]

Gaussian distribution

\[ c_i = c_i^{\text{max}} \ \exp \left( \frac{-p_i F E x^2}{2RT} \right) \]

Standard deviation

\[ \sigma = \frac{RT}{p_i F E} \]

\[ p_i = -\left( \frac{dz_i}{dpH} \right) \left( \frac{dpH}{dx} \right) \]
Concentration

Linear pH gradient gel

\[
\frac{dc}{c} = z(pH) \frac{FE}{RT} \, dpH = \left[ c_{BH^+} - c_{A^-} \right] \frac{FE}{RT} \, dpH
\]

\[
= \left[ \left( \frac{1}{1 + K_{a2} \cdot 10^{pH}} \right) - \left( \frac{K_{a1} \cdot 10^{pH}}{1 + K_{a1} \cdot 10^{pH}} \right) \right] \frac{FE}{RT} \, dpH
\]

Concentration profile

\[
c = c_{\text{max}} \exp \left[ \frac{FE}{RT} \right] \left[ \text{pH} - \log \left( \frac{(1 + K_{a1} \cdot 10^{pH})(1 + K_{a2} \cdot 10^{pH})}{\left( \frac{1}{\sqrt{K_{a1}}} + \frac{1}{\sqrt{K_{a2}}} \right)^2} \right) \right]
\]
Amino acid

\[ pK_1 = 2.5, \ pK_2 = 9.5 \]

\[ \frac{FE}{RT} = 1, 10, 1000 \]
Isoelectric focusing of a zwitterionic ampholyte

\[ A^{-}B, \, A^{-}B^{+}, \, AB^{+} \]
Peptide charge

Negative peptides

N-term = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-2.5}}

asp = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-3.9}}

glu = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-4.2}}

cys = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-8.3}}

ty = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{-\text{pH}} \cdot 10^{-10.1}}

Positive peptides

N-term = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-9.0}}

his = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-6.0}}

lys = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-10.0}}

arg = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-\text{pKa}}} = \frac{1}{1 + 10^{\text{pH}} \cdot 10^{-12.5}}
Peptide charge

\[ z(pH) = N\text{-term} + n_{\text{his}} \text{his} + n_{\text{lys}} \text{lys} + n_{\text{arg}} \text{arg} \]

\[- C\text{-term} - n_{\text{asp}} \text{asp} - n_{\text{glu}} \text{glu} - n_{\text{cys}} \text{cys} - n_{\text{tyr}} \text{tyr} \]

\[ n_{\text{his}} = 1 \]
\[ n_{\text{lys}} = 1 \]
\[ n_{\text{arg}} = 1 \]
\[ n_{\text{asp}} = 1 \]
\[ n_{\text{glu}} = 1 \]
\[ n_{\text{cys}} = 1 \]
\[ n_{\text{tyr}} = 1 \]
Peptide focusing

Differential equation

\[
\frac{dc}{c} = z(pH) \frac{FE}{RT} \, dpH
\]

\[
= \left[ -c_{C-\text{ter}} - n_{\text{asp}} c^{-}_{\text{Asp}} - n_{\text{glu}} c^{-}_{\text{Glu}} + n_{\text{his}} c^{+}_{\text{His}} - n_{\text{cys}} c^{-}_{\text{Cys}} \right. \\
\left. -n_{\text{tyr}} c^{-}_{\text{Tyr}} + n_{\text{lys}} c^{+}_{\text{Lys}} + n_{\text{arg}} c^{+}_{\text{Arg}} + c^{+}_{\text{N-ter}} \right] \frac{FE}{RT} \, dpH
\]

\[
\begin{align*}
n_{\text{his}} &= 1 \\
n_{\text{lys}} &= 1 \\
n_{\text{arg}} &= 1 \\
n_{\text{asp}} &= 1 \\
n_{\text{glu}} &= 1 \\
n_{\text{cys}} &= 1 \\
n_{\text{tyr}} &= 1 \\
\end{align*}
\]

\[FE/RT = 1, 10, 1000\]
Distribution at the pI

C-ter < 0
asp < 0
glu < 0
arg > 0
lys > 0
tyr = 0
N-ter > 0

his > 0
cys = 0

his = 0

cys < 0

his > 0, cys < 0

his = 0

cys = 0
Before focusing

After focusing
Exercice

Calculate the pI of: GAVLM, LLHCR
Draw the pH dependence of the charge
# Properties of natural amino acids

<table>
<thead>
<tr>
<th>Aminoacid</th>
<th>M</th>
<th>pK COOH</th>
<th>pK NH₃</th>
<th>pK side</th>
<th>pI</th>
<th>Hydropathy</th>
<th>Occurrence%</th>
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<td>Glycine</td>
<td>Gly</td>
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<td>Ala</td>
<td>A</td>
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<td>9.7</td>
<td>6.0</td>
<td>1.8</td>
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<td>Phe</td>
<td>F</td>
<td>1.8</td>
<td>9.1</td>
<td>5.5</td>
<td>2.8</td>
<td>3.5</td>
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<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
<td>2.2</td>
<td>9.1</td>
<td>10.1</td>
<td>−1.3</td>
<td>3.5</td>
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<td>W</td>
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<td>9.4</td>
<td>5.9</td>
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<td>Ser</td>
<td>S</td>
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<td>9.2</td>
<td>5.7</td>
<td>−0.8</td>
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<tr>
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<td>Thr</td>
<td>T</td>
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<td>10.4</td>
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<td>−0.7</td>
<td>6.0</td>
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<td>C</td>
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<td>8.3</td>
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<td>−3.5</td>
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<td>Lysine</td>
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<td>10.0</td>
<td>9.7</td>
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</tr>
<tr>
<td>Hystidine</td>
<td>Hys</td>
<td>H</td>
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<td>9.2</td>
<td>6.0</td>
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<td>2.1</td>
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<td>9.0</td>
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<td>4.7</td>
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<td>Asp</td>
<td>D</td>
<td>2.1</td>
<td>9.8</td>
<td>3.9</td>
<td>2.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Glu</td>
<td>E</td>
<td>2.2</td>
<td>9.7</td>
<td>4.2</td>
<td>3.2</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Exercice

GAVLM:
\[ pK_{N\text{-ter}}(G) = 9.0, \quad pK_{C\text{-ter}}(M) = 2.3, \quad \text{pI} = 5.65 \]

LLHCR:

Between 8.3 and 9.6, we have 2 positive and 2 negative charges, the \( \text{pI} = 8.95 \)
Protein titration on an IPG
Haemoglobin titration

Accelerated 100 times

from Pharmacia Biotech
Protein pl distribution

- S Cerevisiae downloaded from SwissProt (6229 proteins)
Peptide pI & Phosphorylation

proteome of *Saccharomyces Cerevisiae*

Normalized cumulative distribution of isoelectric points of non-phosphorylated peptides (thick black line), and singly to 20 times phosphorylated peptides (thin lines). No miscleavage was allowed. pH bins used for peptide counts are 0.1 pH unit wide.
Mini IEF gel

Scanned pictures of IEF electrophoresis on A) 1 cm² and B) 16 cm² gels with a HP scanner with a resolution of 1200 pixels. Only one channel of the whole gel is shown.
**pH gradient on 1 cm**

Figure 1. pH gradient achieved in A) 1 cm$^2$ and B) 16 cm$^2$ gels with 5% of ampholytes for a pH gradient between 3 to 10. The prefocusing and focusing conditions are shown in the table 2.
Plan

- Electrophoresis & Proteomics
  - Isoelectric focusing
- SDS PAGE
  - Capillary electrophoresis
  - Off gel electrophoresis
- Electrospray Ionization
- Matrix Assisted Laser Desorption Ionization
- Scanning Electrochemical Microscopy
In SDS polyacrylamide gel electrophoresis (SDS-PAGE) separations, migration is determined not by intrinsic electric charge of polypeptides but by molecular weight. Sodium dodecylsulphate (SDS) is an anionic detergent that denatures proteins by wrapping the hydrophobic tail around the polypeptide backbone. For almost all proteins, SDS binds at a ratio of approximately 1.4 g SDS per gram of protein, thus conferring a net negative charge to the polypeptide in proportion to its length.

The SDS also disrupts hydrogen bonds, blocks hydrophobic interactions, and substantially unfolds the protein molecules, minimizing differences in molecular form by eliminating the tertiary and secondary structures. The proteins can be totally unfolded when a reducing agent such as dithiothreitol (DTT) is employed. DTT cleaves any disulphide bonds between cysteine residues. The SDS-denatured and reduced polypeptides are flexible rods with uniform negative charge per unit length. Thus, because molecular weight is essentially a linear function of peptide chain length, in sieving gels the proteins separate by molecular weight.

There are two types of buffer systems used in protein gel electrophoresis: continuous and discontinuous.
SDS micelle
SDS denaturation

The detergent sodium dodecyl sulfate (SDS) is used to solubilize proteins for SDS polyacrylamide-gel electrophoresis.

Protein with two subunits, A and B, joined by a disulfide bridge.

Single subunit protein.

Heat with SDS and mercaptoethanol.

Negatively charged SDS molecules.

Polyacrylamide-gel electrophoresis.

SDS polyacrylamide-gel electrophoresis (SDS-PAGE) Individually polypeptide chains form a complex with negatively charged molecules of sodium dodecyl sulfate (SDS) and therefore migrate as a negatively charged SDS-protein complex through a slab of porous polyacrylamide gel. The apparatus used for this electrophoresis technique is shown above (left). A reducing agent (mercaptoethanol) is usually added to break any –S–S– linkages in or between proteins. Under these conditions, proteins migrate at a rate that reflects their molecular weight.

Slab of polyacrylamide gel.
**SDS-PAGE**

27 cm Total Length Capillary
1 = Orange G
2 = α-lactalbumin (14.2 KDa)
3 = carbonic anhydrase (29 KDa)
4 = ovalbumin (45 KDa)
5 = bovine serum albumin (66 KDa)
6 = phosphorylase B (97.4 KDa)
7 = β-galactosidase (116 KDa)
8 = myosin (205 KDa)

Standard test mix separation on an eCAP-SDS 14-200 capillary

y = -3.377x + 6.833, R-squared: .995
SDS-PAGE

Figure 45
SDS-PAGE separation of protein standards

Conditions: Bis-crosslinked polyacrylamide (7.5 % T, 5 % C), 100 mM trisborate, 0.1 % SDS, 8 M urea, pH 7.3, E = 300 V/cm, i = 12 µA, l = 15 cm, id = 75 µm, λ = 280 nm, polyacrylamide coated capillary

Figure 44
PCR analysis of single and double stranded DNA

Conditions: Uncrosslinked polyacrylamide (9 % T, 0 % C), 100 mM Tris-borate, pH 8.3, E = 300 V/cm, i = 9 µA, l = 20 cm, L = 40 cm, id = 75 µm, polyacrylamide coated capillary
Influence of the molecular weight

http://www.sfu.ca/bisc/bisc-429/electrophoresis.html

http://www.sfu.ca/bisc/bisc-429/electrophoresis.html
Continuous elution electrophoresis. After samples are loaded onto the upper surface of the gel, molecules are electrophoresed through the cylindrical gel matrix where they separate into ring shaped bands. Individual bands migrate off the bottom of the gel where they pass into a thin elution frit contained in the elution chamber. A dialysis membrane underneath traps proteins in the elution frit. Elution buffer flows into the elution chamber around the perimeter of the elution frit. As individual bands exit the gel, they are drawn to the center of the frit and out through the collection tube to a peristaltic pump. The pump drives the separated molecules to a fraction collector. As molecules are purified they are collected in discrete liquid fractions and are available for assay and characterization.
Motion in a viscous medium

\[ f_{\text{friction}} = -\zeta v \]

Steady state

\[ v = \frac{f}{\zeta} = \tilde{u}f \]

Sphere in a viscous medium

\[ \zeta = 6\pi \eta r \]

Diffusion coefficient

\[ D = kT\tilde{u} = \frac{kT}{\zeta} \]

Electrophoreoretic mobility

\[ u = zF\tilde{u} = \frac{zFkT}{\zeta} \]
Zone electrophoresis

\[ u_i = z_i F \tilde{u}_i = \frac{z_i F}{N_A \zeta_i} \]

Separation as a function of the charge over friction coefficient ratio
### Table 6.1: Constante de friction $\zeta$ en fonction de la masse moléculaire pour différentes géométries.

<table>
<thead>
<tr>
<th>Geometry</th>
<th>$\zeta \sim M^{n}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphère solide</td>
<td>$\zeta \sim M^{1/3}$</td>
</tr>
<tr>
<td>Chaîne polymère non structurée</td>
<td>$\zeta \sim M^{0.5}$</td>
</tr>
<tr>
<td>Cylindre solide</td>
<td>$\zeta \sim M^{0.8}$</td>
</tr>
<tr>
<td>Disque solide</td>
<td>$\zeta \sim M^{2/3}$</td>
</tr>
<tr>
<td>ADN</td>
<td>$\zeta \sim M$</td>
</tr>
</tbody>
</table>

**Zimm’s equation**

$$D(M) = \frac{kT}{6\pi \eta R_H(M)}$$

$R_H(M)$ : Hydrodynamic radius
Figure 8. Velocity vs. the electric field intensity ($E < 3 \text{ V/cm}$) for various dsDNA sizes.

Migration velocity independent of the size
**Diffusion-convection**

**Diffusion-migration**

\[
\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial y^2} - v_y \frac{\partial c_i}{\partial y}
\]

**Change of variable**

\[Y = y - v_y t\]

\[
\frac{\partial c_i(Y,t)}{\partial t} = D_i \frac{\partial^2 c_i(Y,t)}{\partial Y^2}
\]

\[c_i(Y,t) = \frac{c_o}{2\sqrt{\pi Dt}} \exp \left[ -\frac{Y^2}{4Dt} \right] = \frac{c_o}{2\sqrt{\pi Dt}} \exp \left[ -\frac{(y-vt)^2}{4Dt} \right]\]
Zimm’s Equation

Diffusion coefficient measured from peak widening

With & without electric field

Figure 11. Diffusion coefficient vs. dsDNA lengths (in base pairs). The slope (~0.57) indicates a Zimm’s scaling for all molecular lengths.

A. Nkodo, J. Garnier, B. Tinland, Hongji Ren, C. Desruisseaux, L. McCormick, G. Drouin, Gary W. Slater
Diffusion coefficient of DNA molecules during free solution electrophoresis
Electrophoresis 2001, 22, 2424–2432
Figure 10. Translational diffusion coefficient $D$ for various DNA sizes vs. field intensity $E$.

The Nersnt-Einstein equation is not verified

\[ \frac{D}{u} \neq \frac{kT}{Q} \]

A. Nkodo, J. Garnier, B. Tinland, Hongji Ren, C. Desruisseaux, L. McCormick, G. Drouin, Gary W. Slater

Diffusion coefficient of DNA molecules during free solution electrophoresis

Electrophoresis 2001, 22, 2424–2432

Tuesday, October 12, 2010
Long-chain molecules cause a solution to become viscous (A) because they interfere with one another as the solution flows. As their concentration increases, the molecules become entangled, yielding a viscoelastic behavior that partakes of both solid and liquid traits (B). If the intertwined molecules bond with one another, the result is a crosslinked gel (C).

Adapted with permission from “Intelligent Gels,” Osada, Y., and Ross-Murphy, S. B., Scientific American 268, 82 (1993). Copyright Scientific American,
Motion in a polymer

\[ v = v_0 e^{-K_R P^\%} \]

- \( v_0 \) \text{ Free solution velocity}
- \( K_R \) \text{ Retardation coefficient}
- \( P^\% \) \text{ Polymer concentration}
- \( e^{-K_R P^\%} \) \text{ Molecular sieving effect}
Ferguson plot of seven standard proteins

\[ v = v_0 e^{-KR P^\%} \]
Molecular sieving

In gel electrophoresis, molecular sieving can be described by the Ogston theory (Ogston, 1958): the average pore size of the matrix is in the same range as that of the hydrodynamic radius of the migrating solute.

In this case the logarithm of the velocity of the migrating solute is proportional to the size of the solute:

\[ v \approx \exp(-n) \]

This theory also assumes that the migrating particles behave as unperturbed spherical objects.
Polymer Gel

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Concentration</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crosslinked polymers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyacrylamide/bis-acrylamide</td>
<td>2– 6 % T, 3 – 6 % C</td>
<td>Oligonucleotides, DNA sequencing,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Native and SDS-bound proteins</td>
</tr>
<tr>
<td><strong>Linear polymers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyacrylamide</td>
<td>&lt; 0.1– 6 %</td>
<td>Restriction fragments</td>
</tr>
<tr>
<td>Hydroxylalkyl cellulose, polyvinyl alcohol, dextran</td>
<td>6 –15 %</td>
<td>Oligonucleotides, DNA sequencing, proteins</td>
</tr>
<tr>
<td>Agarose</td>
<td>0.05 –1.2 %</td>
<td>Restriction fragments, Proteins</td>
</tr>
</tbody>
</table>
Polyacrylamide

\[
\text{Acrylamide}
\]

\[
\text{CH}_2 = \text{CH} \quad \text{CH}_2 = \text{CH} \\
\ | \quad | \\
\ \text{C} = \text{O} \quad \text{C} = \text{O} \\
\ | \quad | \\
\ \text{NH}_2 \quad \text{NH}_2 \\
\]

\[
\text{N,N'}-\text{methylene bisacrylamide}
\]

\[
\text{CH}_2 - \text{CH} - [\text{CH}_2 - \text{CH} - \text{CH}_2 - \text{CH}]_n \text{CH}_2 - \text{CH} - [\text{CH}_2 - \text{CH} - \text{CH}_2 - \text{CH}]_n \text{CH}_2 - \\
\ | \quad | \quad | \quad | \\
\ \text{CO} \quad \text{CO} \quad \text{CO} \\
\ | \quad | \quad | \\
\ \text{NH}_2 \quad \text{NH}_2 \quad \text{NH}_2 \\
\ | \quad | \\
\ \text{CH}_2 \quad \text{CH}_2 \\
\ | \quad | \\
\ \text{NH} \quad \text{NH} \\
\ | \quad | \\
\ \text{CO} \quad \text{CO} \\
\ | \\
\ \text{CO} \\
\ | \\
\ \text{NH}_2 \quad \text{NH}_2 \\
\]

Polyacrylamide gel
Agarose

SEM photo of a 1% LE Agarose gel at 22kX magnification.

http://www.bioscience-beads.com/stdspec.htm

Tuesday, October 12, 2010
Interaction DNA-gel

DNA, can still migrate through a polymer network which has a pore size that is significantly smaller than the size of the solute. This phenomenon can be explained by the reptation model.

A schematic representation of the entanglement coupling interaction of DNA with the polymer chains of the sieving matrix.

DNA separation

Schematic diagram of a solute migrating through a polymer network by the Ogston mechanism. The DNA percolates through the mesh as if it were a rigid particle.

Schematic diagram of a solute migrating by the reptation mechanism. In this case, the DNA is forced to squeeze through the tubes formed by the polymer network.

Motion of an ideal chain

By isotropy

\[ \langle h^2 \rangle = \sum_i \sum_j \langle a_i \cdot a_j \rangle = \sum_i \langle a_n^2 \rangle = Na^2 \]

Distance "Head to Tail" \( R_N = a\sqrt{N} \)
**Sliding tube**

- "blob"
- Hernia
- Head

**Linear length**

\[ L = N \alpha \]
Sliding motion

Sliding rate

\[ v_g = \tilde{u}_g f \]

Sliding mobility

\[ \tilde{u}_g = \frac{1}{N \zeta_g} \]

Friction coefficient of a sliding blob

\[ \zeta_g \]

Sliding diffusion coefficient

\[ D_g = kT \tilde{u}_g = \frac{kT}{N \zeta_g} \]
Scaling law

Sliding time

\[ \tau_g = \frac{L^2}{2D_g} = \tau_1 N^3 \]

Reptation diffusion coefficient

\[ D_{\text{rep}} = \frac{R_N^2}{\tau_g} = \frac{D_1}{N^2} = \frac{kT}{\zeta_{\text{rep}}} \]

A porous matrix has a scaling law in \(1/N^2\)
Reptation with an electric field

Force acting on a chain in the presence of an electric field $E$

$$f = \sum_i q_i E \cdot \hat{t}_i = \frac{q}{a} \sum_i E \cdot a_i = \frac{qE}{a} \sum_i \hat{i} \cdot a_i = \frac{qE}{a} h_x$$

Speed of a chain in an electric field $E$

$$v_g = \frac{qE \tilde{u}_g}{a} h_x = \frac{qE}{Na \zeta_g} h_x = \frac{qE}{L \zeta_g} h_x$$
Center of mass

Position of the center of mass

\[ MR_{cm} = \sum_{i} m_i r_i \]

\[ m_i = \frac{M}{L} a_i \]

Speed of the center of mass

\[ MV_{cm} = \sum_{i} M \frac{a_i}{L} v_{gi} \]
Speed of the chain

All the elements slide at the same speed

\[ v_{gi} = v_g \hat{t}_i \]

Speed of the center of mass

\[ V_{cm} = \frac{v_g}{L} \sum_i a_i \hat{t}_i = \frac{v_g}{L} \sum_i a_i = \frac{v_g}{L} h = \tau_g^{-1} h \]
Average speed

Speed of the center of mass along the electric field

\[ V_{xcn} = \frac{v}{L} h_x \]

Average speed of the center of mass

\[ < V_{xcn} > = \frac{qE}{\zeta_g L^2} < h_x^2 > \]

Chains aligned with the field migrate faster
Weak fields

\[ <h^2> = <h_x^2 + h_y^2 + h_z^2> = \frac{1}{n} \sum_{i=1}^{n} h_{xi}^2 + h_{yi}^2 + h_{zi}^2 \]

\[ = \left( \frac{1}{n} \sum_{i=1}^{n} h_{xi}^2 \right) + \left( \frac{1}{n} \sum_{i=1}^{n} h_{yi}^2 \right) + \left( \frac{1}{n} \sum_{i=1}^{n} h_{zi}^2 \right) = <h_x^2> + <h_y^2> + <h_z^2> \]

Quasi-Isotropy

\[ <h_x^2> = \frac{<h^2>}{3} = \frac{Na^2}{3} \]

Electrophoretic mobility

\[ u_{\text{rep}} = \frac{<V_{xcm}>}{E} = \frac{qNa^2}{3\zeta_g L^2} = \frac{q}{3\zeta_g N} \]

The migration speed is inversely proportional to the length
Biased reptation

How does the head chooses the path?

\[ h_x = \sum_{i=1}^{N} a_{xi} \]

\[ h_x^2 = \sum_{i=1}^{N} \sum_{j=1}^{N} a_{xi} a_{xj} = \sum_{i=1}^{N} a_{xi}^2 + \sum_{i=1}^{N} \sum_{j \neq i}^{N-1} a_{xi} a_{xj} \]

\[ < h_x^2 > = N < a_x^2 > + N(N-1) < a_x >^2 \]
Annexe

\[ \langle h_x^2 \rangle = \frac{1}{n} \sum_{k=1}^{n} \left( \sum_{i=1}^{N} a_{xik}^2 + \sum_{i=1}^{N} \sum_{j \neq i}^{N-1} a_{xik} a_{xjk} \right) \]

\[ = \frac{1}{n} \sum_{k=1}^{n} \left( \sum_{i=1}^{N} a_{xik}^2 \right) + \frac{1}{n} \sum_{k=1}^{n} \left( \sum_{i=1}^{N} \sum_{j \neq i}^{N-1} a_{xik} a_{xjk} \right) \]

\[ = \sum_{i=1}^{N} \left( \frac{1}{n} \sum_{k=1}^{n} a_{xik}^2 \right) + \sum_{i=1}^{N} \left( \frac{1}{n} \sum_{k=1}^{n} a_{xik} \right) \sum_{j \neq i}^{N-1} \left( \frac{1}{n} \sum_{k=1}^{n} a_{xjk} \right) \]

\[ = N \langle a_x^2 \rangle + N(N-1) \langle a_x \rangle^2 \]
Influence of the electric field

Field alignment \[ a_x = a \cos \theta \]

\[ < h_x^2 > = N a^2 < \cos^2 \theta > + N(N - 1)a^2 < \cos \theta >^2 \]

Electrostatic work

\[ w_e = \int_0^a V d\lambda = \int_0^a V(x) \lambda dx = -\int_0^a E \cos \theta \lambda dx \]

\[ = -\frac{1}{2} \lambda a^2 E \cos \theta = -\frac{1}{2} qaE \cos \theta \]
Boltzmann distribution

\[ < w_e > = \frac{\int_0^\pi w_e \exp \left( -\frac{w_e}{kT} \right) \sin \theta d\theta}{\int_0^\pi \exp \left( -\frac{w_e}{kT} \right) \sin \theta d\theta} \]

Change of variables

\[ x = aqE \cos \theta / 2 \quad u = aqE / 2 \]

\[ < w_e > = -\frac{\int_u^0 \exp \left( \frac{x}{kT} \right) dx}{\int_u^0 \exp \left( \frac{x}{kT} \right) dx} = -\left[ \frac{xkT \exp \left( \frac{x}{kT} \right) - k^2 T^2 \exp \left( \frac{x}{kT} \right) }{kT \exp \left( \frac{x}{kT} \right) } \right]_u^{\infty} \]

\[ = -uc \coth \left( \frac{u}{kT} \right) + kT = -u L \left( \frac{u}{kT} \right) \]
The Langevin function is defined as:

\[ L(x) = \coth(x) - \frac{1}{x} \]

The slope at the origin is 1/3, and the saturation value is 1. The function is given by:

\[ \lim_{u \to 0} \langle w_e \rangle = \lim_{u \to 0} \left[ u L \left( \frac{u}{kT} \right) \right] = \frac{u^2}{3kT} \]

The expectation of \( \cos \theta \) is:

\[ \langle \cos \theta \rangle = \frac{\langle w_e \rangle}{-u} = L \left( \frac{u}{kT} \right) \]
\[
< w_e^2 > = \frac{\int_0^\pi w_e^2 \exp \frac{-w_e}{kT} \sin \theta d\theta}{\int_0^\pi \exp \frac{-w_e}{kT} \sin \theta d\theta}
\]

\[
< w_e^2 > = \int_{-u}^{u} x^2 \exp^{x/kT} dx \int_{-u}^{u} \exp^{x/kT} dx = \left[ x^2 kT \exp^{x/kT} \right]_{-u}^{u} - 2kT \int_{-u}^{u} x \exp^{x/kT} dx
\]

\[
= u^2 - 2kTu \coth\left(\frac{u}{kT}\right) + 2k^2 T^2 = u^2 - 2kTu L\left(\frac{u}{kT}\right)
\]

\[
\lim_{u \to 0} < w_e^2 > = \lim_{u \to 0} \left[ u^2 - 2kTu L\left(\frac{u}{kT}\right) \right] = \frac{u^2}{3}
\]

\[
< \cos^2 \theta > = \frac{< w_e^2 >}{u^2} = 1 - \frac{2kT}{u} L\left(\frac{u}{kT}\right)
\]
Finally...

\[
\frac{\langle h_x^2 \rangle}{a^2} = N \left[ \langle \cos^2 \theta \rangle - \langle \cos \theta \rangle^2 \right] + N^2 \langle \cos \theta \rangle^2
\]

\[
= N \left[ 1 - \frac{2kT}{u} L \left( \frac{u}{kT} \right) - L^2 \left( \frac{u}{kT} \right) \right] + N^2 L^2 \left( \frac{u}{kT} \right)
\]

Series development

\[
\frac{\langle h_x^2 \rangle}{a^2} = \frac{1}{3} N + \left( \frac{N^2}{9k^2T^2} - \frac{N}{15k^2T^2} \right) u^2 + \ldots
\]
Biased reptation

\[
< V_{xcm} > = \frac{qE}{\zeta g \cdot 3} \left[ \frac{1}{N} + \frac{1}{3} \left( \frac{aqE}{2kT} \right)^2 \right]
\]

In weak fields, the speed is inversely proportional to the size
In strong fields, the speed is independent of the size
Influence of the electric field

FIG. 7. Different regimes of migration in constant-field electrophoresis: (a) Ogston sieving; (b) reptation without orientation; (c) reptation with orientation.
Influence of the size

Figure 2. “Phase diagram” of the dependence of the separation mechanism (and therefore the selectivity) on polymer concentration and on DNA size. Transition from transient entanglement coupling to sieving (I) and reptation mechanisms (III) occurs when \( c = c^* \). The entanglement threshold itself depends on the polymer molecular mass. Transition from sieving to reptation occurs when the radius of gyration of DNA is larger than the “mesh size” which in turn is dependent on the polymer concentration (see Eq. 6). Orientation (IV) occurs above a critical DNA size \( N_k^* \) and resolution is lost. This limit is shifted towards smaller sizes with increasing electric field. The exact dependence of \( N_k^* \) on the polymer concentration is still under discussion (Section 3.2). Zone II (with reduced separation) will only occur in the special case when DNA is larger than the mesh size but is too stiff to reptate (\( b_D > R_D > \zeta \)). For ssDNA, \( b_D \) is very small (\( b_D < \zeta \)) and this regime will not be observed.

*Electrophoresis* 2001, 22, 629–643
2D gel mapping

pl fractionated & SDS denaturated proteins

Electric field

Migration

M_{Wt} (kD)

Isoformes

Colorectal epithelial cell
Blotting

Passive blotting

Alternatively, electroblotting
Comparison of commonly used stains for proteins on blots. Twofold serial dilutions of protein molecular weight standards ranging from 2000 to 1 ng/band were run on six identical SDS-polyacrylamide gels and blotted to PVDF membrane.
Protein metal staining

Copper staining

- Wet membrane with methanol
- Add protein sample
- React with 0.3 M CuCl₂ (30min)
- Reduce with 0.3 M NaBH₄
- Rinse with water

Silver staining

- Wet membrane with 1% NaAc
- Sensitize with NaS₂O₃
- React with 0.1% AgNO₃ (20min)
- Reduce with 2.5% Na₂CO₃ + 1% HCHO
- Stop with 10% HAC
Silver staining

Laser-scanner picture of the sample with 5 μL 4 mg/ml BSA added PVDF membrane. The size of protein dot is about 1 mm².

SEM picture of silver clusters (with protein area of the sample).

TEM picture of silver clusters

Silver clusters are isolated nanoparticles (Ø 5~10 nm).

Plan

• Electrophoresis & Proteomics
  • Isoelectric focusing
  • SDS PAGE

• Capillary electrophoresis
  • Off gel electrophoresis

• Electrospray Ionization

• Matrix Assisted Laser Desorption Ionization

• Scanning Electrochemical Microscopy
Oxide Surface

In solution

Acidic solution

Isoelectric pH

Basic solution
Double layer

Diffuse layer
Gouy-Chapman layer

Compact layer
Stern layer

Electrode

Solvent layer

Na$^+$  Cl$^-$
Gouy-Chapman layer

Space charged region

Potential drop

Electrocapillarity
air|water interface

\[ \sigma = F \left( \Gamma_{C^+} - \Gamma_{A^-} \right) = \frac{\partial \gamma}{\partial \Delta \phi} \]
Viscosity

Let's consider a moving plane with respect to a static plane

\[ \frac{F_y}{S} = -\eta \frac{v_y}{L} = -\eta \frac{\partial v_y(x)}{\partial x} \]
**Electro-osmosis**

\[ E_y \rho(x)dx = -\left( \eta \frac{\partial v_y(x)}{\partial x} \right)_{x+dx} + \left( \eta \frac{\partial v_y(x)}{\partial x} \right)_x \]

- **Electrical driving force**
- **Friction force**

Negatively charged wall

Diffuse layer

\[ E_y \rho(x)dx = -\eta \frac{\partial^2 v_y(x)}{\partial x^2} dx \]
Zeta potential

By introducing Poisson’s equation

\[ \frac{\partial^2 \phi(x)}{\partial x^2} = -\frac{\rho}{\varepsilon_0 \varepsilon_r} \]

\[ E_y \varepsilon_0 \varepsilon_r \frac{\partial^2 \phi(x)}{\partial x^2} \ dx = \eta \frac{\partial^2 v_y(x)}{\partial x^2} \ dx \]

Boundary conditions

\[ v_y(x_c) = 0 \quad \text{for} \quad \phi(x_c) = \zeta \quad \text{at the shear plane} \]

\[ v_y(x) \to v_E \quad \text{for} \quad \phi(x) \to 0 \quad \text{in the bulk} \]
Integration

Integration between $x$ and infinity

$$\int_x^\infty E_y \varepsilon_0 \varepsilon_r \frac{\partial^2 \phi(x)}{\partial x^2} \, dx = \int_x^\infty \eta \frac{\partial^2 v_y(x)}{\partial x^2} \, dx = \left[ E_y \varepsilon_0 \varepsilon_r \frac{\partial \phi(x)}{\partial x} \right]^\infty_x = \left[ \eta \frac{\partial v_y(x)}{\partial x} \right]^\infty_x$$

soit

$$E_y \varepsilon_0 \varepsilon_r \frac{\partial \phi(x)}{\partial x} = \eta \frac{\partial v_y(x)}{\partial x}$$

Integration between the shear plan and $x$

$$\int_{x_c}^x E_y \varepsilon_0 \varepsilon_r \frac{\partial \phi(x)}{\partial x} \, dx = \int_{x_c}^x \eta \frac{\partial v_y(x)}{\partial x} \, dx = \left[ E_y \varepsilon_0 \varepsilon_r \phi(x) \right]_{x_c}^x = \left[ \eta v_y(x) \right]_{x_c}^x$$

i.e.

$$v_y(x) = \frac{E_y \varepsilon_0 \varepsilon_r}{\eta} [\phi(x) - \zeta]$$
Velocity profile

Debye approximation at the shear plane

\[ \phi(x) = \left[ \phi(0) - \phi^\infty \right] e^{-\kappa x} = \zeta e^{-\kappa x} \]

Velocity profile

\[ v_y(x) = -\frac{E_y \varepsilon_0 \varepsilon_r \zeta}{\eta} \left[ 1 - e^{-\kappa x} \right] \]

Schmoluchowski’s equation

\[ \zeta = -\frac{\eta}{\varepsilon_0 \varepsilon_r E_y} v_y \]

\[ \sigma_{\text{wall}} = -\int_0^\infty \rho(x) \, dx \approx \int_{x_c}^\infty \varepsilon_0 \varepsilon_r \left( \frac{\partial^2 \phi(x)}{\partial x^2} \right) \, dx = \varepsilon_0 \varepsilon_r \zeta \kappa \]
Electro-osmotic flow

Plug flow

Plug flow in a capillary

Poiseuille flow under a pressure drop

\[ v_{co} = \frac{\varepsilon_0 \varepsilon_r \zeta E}{\eta} = u_{co} E = u_{co} \frac{\Delta V}{L} \]

Electro-osmotic mobility
Capillary electrophoresis

Variation of electro-osmotic mobility in a silica capillary as a function of pH

Power supply

Buffer reservoir

Sample

Capillary

Detector

Buffer reservoir

$10^4 u_{eo} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$

$pH$
Zone electrophoresis in CE

Ion transport is due to:

- Electro-osmosis (plug flow convection)
- Migration + diffusion with a background electrolyte (buffer)

\[ v = (u_{eo} + u) \frac{\Delta V}{L} \]

Electro-osmotic + Electrophoretic mobilities
Table 1. Comparison of Electrophoretic Mobilities

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Mobility* (× 10000) - Hudson (1)</th>
<th>Mobility* (× 10000) - present work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pheniramine</td>
<td>3.287</td>
<td>3.235</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>3.081</td>
<td>3.022</td>
</tr>
<tr>
<td>Brompheniramine</td>
<td>3.034</td>
<td>2.999</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>2.597</td>
<td>2.585</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>2.515</td>
<td>2.501</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>2.330</td>
<td>2.327</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>2.292</td>
<td>2.295</td>
</tr>
<tr>
<td>Methoxamine (ISTD)</td>
<td>2.072</td>
<td>2.072</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>1.985</td>
<td>1.990</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>1.941</td>
<td>1.927</td>
</tr>
<tr>
<td>Codeine</td>
<td>1.871</td>
<td>1.862</td>
</tr>
<tr>
<td>Hyroxyzine</td>
<td>1.792</td>
<td>1.777</td>
</tr>
<tr>
<td>Pentozonecitroine</td>
<td>1.703</td>
<td>1.677</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>1.666</td>
<td>1.668</td>
</tr>
<tr>
<td>Trazadone</td>
<td>1.566</td>
<td>1.558</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>1.536</td>
<td>1.528</td>
</tr>
<tr>
<td>Verapamil</td>
<td>1.391</td>
<td>1.381</td>
</tr>
<tr>
<td>Loperamide</td>
<td>1.319</td>
<td>1.310</td>
</tr>
</tbody>
</table>

*Apparent mobility of the analyte corrected for mobility of electroosmotic flow (EOF). Apparent mobility is the IL/TV where I is the capillary length to the detector (cm), L is the total capillary length (cm), t is the migration time (s) and V is the applied voltage (V). Because EOF is so low at pH 2.38, mobilities were determined relative to a reference compound, according to the method of Williams and Vigh [3].

Figure 1. TIE for drug mixture.
Micellar electrokinetic chromatography
MEKC

3.2.1 Applications of MEKC
MEKC is a dynamic mode of CE since it can be used for charged and uncharged analytes and for a wide range of substances with hydrophilic or hydrophobic characteristics. Applications include amino acids, nucleotides, vitamins, a wide range of pharmaceuticals, aromatic hydrocarbons, and explosive constituents, to name a few.

For pharmaceuticals, MEKC has been used for the determination of active drugs in tablets, creams, and injectable formulations. The separation of the active ingredients of a cold-relief preparation using SDS micelles is illustrated in figure 37. The migration order is determined by solute lipophilicity and polarity. Only cationic, and to a certain extent, neutral species become incorporated with the micelle. In this work, the effect of SDS concentration and type, pH, and organic modifier were studied. In addition, quantitative analysis of Novapon granules was performed.

**Figure 37**
MEKC separation of cold-relief medicine constituents

Conditions: 20 mM phosphate-borate, 100 mM SDS, pH 9, V = 20 kV, L = 65 cm, id = 50 μm, λ = 210 nm
Flow Cancellation

To cancel the EOF, increase the viscosity in the diffuse layer region. For example, using linear polyacrylamide.
Figure 9. Schematic diagram of the charge-reversal process at the capillary wall. (A) No surfactant added. (B) Micellar bilayer formation by hydrophobic interaction between the nonpolar chains, resulting in a reversal of the electroosmotic flow.
Schematic representation of a sequencing process ("four-color Sanger") starting from many copies of the single-stranded DNA to be sequenced, bearing a known "marker" at the beginning of the unknown sequence, a short oligonucleotide "primer" complementary to this marker is hybridized (i.e., paired) to the marker, in the presence of DNA polymerase and free nucleotides. This hybridization initiates reconstruction by the polymerase of a single strand complementary to the unknown sequence (a). Including in the nucleotide bath in which the polymerization takes place a small fraction of fluorescently labeled dideoxynucleotides (one different dye for each nucleotide type), which miss the OH group necessary for further extension of the strand, one is able to synthesize at random complementary strands with all possible arrest points (i.e., all possible lengths with an integer number of nucleotides). These newly synthesized single-stranded DNA's are then separated electrophoretically by size [see electrophoregram in (b)]: consecutive peaks correspond to DNA fragments differing by one base, and each line corresponds to one given nucleotide. Automated analysis of the data allows the determination of the sequence (symbols above the peaks). The symbol $N$ indicates ambiguous determination. In the present case, the sequence determination was faultless up to 435 bases. (Adapted from J.-L. Viovy, Rev. Mod. Phys., 72 (2000) 813-872).
DNA

- O- P-O-
- O- P-O-

- O- P-O-
- O- P-O-

C
G
A
T

C-G
A-T

Tuesday, October 12, 2010
Polymerisation

2',3'-Didésoxynucléotide
ddNTP

Fluorescent probe
Sanger’s method

Primer

Bases

Polymerase

fluo terminaison

ddGTP

ddCTP

ddTTP

ddATP
Electrophoresis of tagged strands

G

C

T

A

C-G-A-C-T
Séquencage en capillaire
Immunotyping by CE
Plan

• Electrophoresis & Proteomics
  • Isoelectric focusing
  • SDS PAGE
  • Capillary electrophoresis
  • Off gel electrophoresis
• Electrospray Ionization
• Matrix Assisted Laser Desorption Ionization
• Scanning Electrochemical Microscopy
Off-gel electrophoresis

Low pH  

pH  

High pH  

Cellular extract  
or  
Protein solutions  

**Off-gel principle**

Equal conductivity

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Peptide separation

Eglin C
pI=5.5
6.4
10.4
8.4
7.4
5.3
After 1 hour at 500V-200mA

Gel (Pharmacia pH 4→7 on 11 cm) cut & placed on Off-gel pH=5→6 over 4cm
Off-gel® electrophoresis
Iso-electric fractionation

A multi compartment cell is placed on top of a IPG gel
Each compartment is buffered by the IPG below
Solution electrophoresis in each compartment
Pre-fractionation according to pI
Off-gel on a microtiter plate

Maximum field 8.3 kV·m⁻¹
Migration during Off-gel
E-coli fractionation

Fractions

pH 4.24 – 4.43
pH 4.49 – 4.68
pH 4.73 – 4.92
pH 4.98 – 5.17
pH 5.22 – 5.42
pH 5.47 – 5.66
pH 5.71 – 5.90
pH 5.96 – 6.15

Resolution of Off-Gel

250 μg of non depleted human plasma
Pre-concentration

Distribution of $\beta$-lactoglobulin B after (A) 0, (B) 4, (C) 8 and (D) 24 h of microtiter plate off-gel electrophoresis.
The separation was performed over 10 wells with a pH gradient ranging from 4.14 to 6.8. The relative concentration represents the concentration in a given well after IEF reported to the concentration in the initial solution (100%).
Fig. 1 Instrumental setup of the MARS depletion, the Off-Gel isoelectric focusing (OGE) and the offline 2-D LC/MS system
Identification of proteins and peptides in a human serum sample

- +367 proteins
- +2000 peptides
Determining the pl of a peptide
2D-Off gel

Optional: Immunodepletion of abundant proteins

$OGE_{prot}$

pH 4

Control with 2-DE

Trypsin Digestion of fraction 6:
P pH 5.0 to 5.15

$OGE_{pept}$

pH 3

RP LC-MS/MS

Bioinformatics (OLAV)

Peptide identification acceptance/rejection after pI and retention time evaluation

Protein identification
Peptide migration

Peptide: Isoelectric pH = 5.5
Leucine enkephalin
Tyr-Gly-Gly-Phe-Leu

pK = 2.2
pK = 10.1

pH = 5
pH = 7
pH = 9

Time:
0 s
50 s
100 s
Is bioinformatics reliable?

False positive

False negative?

MS-MS analysis of the peptide fraction of pl=4

Human plasma

Tuesday, October 12, 2010
**3100 Offgel fractionator**

In the example below, immunodepleted plasma was separated into 24 fractions by OFFGEL electrophoresis (pH3-10) and into 50 fractions by SCX. Subsequent analysis by RP HPLC-Chip/MS revealed that the workflow that included OFFGEL electrophoresis identified twice as many proteins and three times as many peptides.
Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast

Lyris M. F. de Godoy et al.

Proteome-wide cellular protein concentrations of the human pathogen Leptospira interrogans

Johan Malmström et al.

**Figure 2 | Abundance levels of selected protein groups, by Gene Ontology analysis.** The number of genes, the number of identified proteins and the copies per cell for two cellular states (control and ciprofloxacin treated). Please note that hypothetical proteins are underrepresented in the copies per cell calculation as compared to gene numbers, whereas members of the protein folding and encapsulating structure group are largely overrepresented. ‘no_GO’ denotes no Gene Ontology available.
OffGel IEF Fractionator

Dépôt du brevet OffGel EPFL

Premières publications

2000

2001

2002

2006

Commercialisation mondiale par Agilent

Industrialisation

Licence à Agilent
OffGel - Capillary electrophoresis

• How orthogonal?

• Offgel is a pI separation. Ratio of acidic to basic group.

• CE is a charge/size separation. Number of positive charges per (amino acid)$^x$

• Strategy: Recharge the peptide after IEF
Figure 5. Analysis of one OGE peptide fraction (pH 4.62) by different techniques. (A) CZE in acetic acid with positively coated capillary. (B) CZE in acetic acid with neutrally coated capillary. (C) CABCE analysis at pH 3.25 in neutrally coated capillary. (D) RP-HPLC.

Figure 6. Analysis of one OGE peptide fraction (pH 5.98) by different techniques. (A) CZE in acetic acid with positively coated capillary. (B) CZE in acetic acid with neutrally coated capillary. (C) CABCE analysis at pH 3.25 in neutrally coated capillary. (D) RP-HPLC.
Fractions from OGE IEF analyzed by CABCE (neutrally coated capillary, CA narrow pH cut, pH = 3.25). Conditions: voltage 30 kV, observed current 5.5 μA, temperature 25°C, UV absorbance at 200 nm. Concentration of each tryptic digest before the OGE fractionation: 0.05 g.L⁻¹.

Fractions from OGE IEF analysed by CZE (HPC coated capillary, acetic acid 10%). Conditions: voltage 30 kV, observed current 23 μA, temperature 25°C, UV absorbance at 200 nm. Concentration of each tryptic digest before the OGE fractionation: 0.02 g.L⁻¹.
Geometric orthogonality

Figure 3. Geometric orthogonality concept. Hypothetical separation of 100 analytes in $10 \times 10$ normalized separation space. (A) Nonorthogonal system, 10% area coverage represents 0% orthogonality. (B) Hypothetical ordered system, full area coverage. (C) Random, ideally orthogonal, system, area coverage is 63% representing the 100% orthogonality.

$$O = \frac{\sum \text{bins} - \sqrt{P_{\text{max}}}}{0.63P_{\text{max}}}$$

$$N_p = P_1P_2 \frac{\sum \text{bins}}{P_{\text{max}}}$$
Offgel CE orthogonality

Practical peak capacity 700
20 pl fractions
20 cm capillary

47% orthogonality similar to SCX-RP HPLC

Based on the approach of M. Gilar et al., Anal. Chem., 2005, 77, 6426

Tuesday, October 12, 2010
In-Silico orthogonality

\[ z(N_{AA})^{-2/3} \]

Orthogonality = 44%

Sample: Bovine Serum Albumin (BSA), β-lactoglobulin (β-lac), Myoglobin (Myo) and Cytochrom. C (Cyt. C)
Electropherograms of very low-concentrated standard protein sample by FASI-CE-UV. (A) 3.2-12.8 nM; (B) 1.6-6.4 nM; (C) 0.8-3.2 nM. BGE: 83.3 mM ammonium acetate pH 4.0. Injection parameters: 3 kV, 480 sec.
Standard proteins containing Cytochrom C, Lysozyme, Bradykinin, Ribonuclease A, α-lactalbumin, Ribonuclease S, β-lactoglobulin, Lactoferine, Bovine Serum Albumin(BSA), Aldolase and Myoglobin.
Magnetism

- \( B \): magnetic induction \([T]\)
- \( H \): magnetic field \([A/m]\)
- \( M \): magnetization \([A/m]\)
- \( \mu \): permeability \([H/m]\)
- \( \mu_r \): relative permeability \([-]\)
- \( \chi \): magnetic susceptibility \([-]\)
- \( \phi \): scalar magnetic potential \([A]\)
- \( F \): magnetic force \([N]\)

\[
B = \mu_0 H
\]

\[
B = \mu_0 (H + M) = \mu_0 (1 + \chi)H = \mu_0 \mu_r H = \mu H
\]

\[
H = -\nabla \phi
\]

\[
F_{\text{mag}} = \nabla (m_{\text{bead}} \cdot B) \approx (m_{\text{bead}} \cdot \nabla)B
\]

\[
F_{\text{mag}} = \frac{V \Delta \chi}{\mu_0} (B \cdot \nabla)B
\]

\[
F_x = B_x \frac{\partial B_x}{\partial x} - B_y \frac{\partial B_x}{\partial y}
\]

\[
F_y = B_x \frac{\partial B_y}{\partial x} - B_y \frac{\partial B_y}{\partial y}
\]
Magnetic Induction B

Attraction

Repulsion

Vector representation

One magnet

Tuesday, October 12, 2010
Magnetic trapping

Magnetic forces

Tuesday, October 12, 2010
Magnetic array

Capillary junction
Magnetic tracks
Microchannel
Microchip holder

A

B

Magnetic array
Ring Magnets for CE

Repulsion

Attraction

75μm i.d.Ø
Magnetic frit for CEC

Need a CE instrument with high pressure!

C18 - 5 μm

Fe₂O₃ bead mixture 6-8μm
Capillary electrophoresis immunoassay using magnetic beads

Sample preconcentration by increasing the percolating time of the sample. Peak 1, b-LG; Peak 2, anti-b-LG antibody. Conditions: HPC-coated capillary, total/effective length 40/30 cm x 50 um id, UV absorbance at 200 nm. Injection sequence: protein A-coated MBs (0.3 uM) injection: 34.5 mbar for 3 min; anti-b-LG antibody (100 ug/mL in binding buffer) injection: 34.5 mbar for 10 min; b-LG solution (1 ug/mL in sample buffer) injection: 34.5 mbar for (A) 1 min, (B) 5 min, (C) 10 min, (D) 20 min, (E) 30 min; washing with binding buffer: 34.5 mbar for 10 min; reverse injection of separation buffer: 34.5 mbar for 5.5 min; elution and separation: applied voltage, 15 kV; MBs removal step with separation buffer: 1379 mbar for 2 min.

**Figure 1.** Schematic presentation of the procedure for online immunocapture and separation.
