<table>
<thead>
<tr>
<th>Datum/Zeit</th>
<th>Veranstaltungsort</th>
<th>Thema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo, 08.02.2010</td>
<td>Hörsaal Institut für Glaschemie Fraunhoferstrasse 6</td>
<td><em>Albert Einstein and the Viscosity of Macromolecules</em></td>
</tr>
<tr>
<td>10.00-11.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo, 08.02.2010</td>
<td>Hörsaal Haus 1,IAAC, August-Bebel-Str. 2</td>
<td><em>Light Scattering and SEC-MALLs</em></td>
</tr>
<tr>
<td>12.15-13.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di, 09.02.2010</td>
<td>Institut für Materialwissenschaft und Werkstofftechnologie, HS 124 Löbdergraben 32</td>
<td><em>Dynamic Light Scattering</em></td>
</tr>
<tr>
<td>12.15-13.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mi, 10.02.2010</td>
<td>Hörsaal 3, Carl-Zeiss-Str. 3</td>
<td><em>Analytical Ultracentrifugation I</em></td>
</tr>
<tr>
<td>16.15-17.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do, 11.02.2010</td>
<td>Döbereiner Hörsaal</td>
<td><em>Analytical Ultracentrifugation II: Interactions</em></td>
</tr>
<tr>
<td>14.15-15.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lecture 2:
Light Scattering & SEC MALLs

Part 1: Light scattering basic theory (overhead transparencies)
Part 2: Instrumentation, SEC-MALLs and “Triple” Detection
2 Main Types

1) Classical or "Static" Light Scattering
   - Measure scattered intensity $i_\theta$ as a function of angle $\theta$ $\rightarrow M_w$ (weight average molecular weight) $R_g$ (radius of gyration)

2) Dynamic or "Quasi-Elastic" Light Scattering
   - Measure rapid fluctuations of $i_\theta$ as a function of time $t$ $\rightarrow D$ (diffusion coefficient)
CLASSICAL LIGHT SCATTERING

... for characterisation of biomolecular molecular weights and conformations (via

"radius of gyration," $R_g$ )
The blue colour of the sky and the polarisation of skylight ... constitute, in the opinion of our most eminent authorities, the two great standing enigmas of meteorology. Indeed it was the interest manifested in them by Sir John Herschel in a letter of singular speculative power that caused me to enter upon the consideration of these questions so soon.

J. Tyndall, 1969
Historical

17th C
SNELL
NEWTON
HUYGENS
FERMAT

NATURE OF LIGHT;
GEOMETRIC OPTICS

19th C
YOUNG
FRESNEL

DIFFRACTION/INTERFERENCE

1865: MAXWELL - ELECTROMAGNETIC THEORY

1869: TYNDALL - LIGHT SCATTERING;
"THE 2 GREAT STANDING ENIGMAS"
JOHN WILLIAM STRUTT (LORD RAYLEIGH)

In 1874, aged 28, photographed by himself with a wet collodion plate.
1881: Rayleigh - Single Particle Theory \{ Small Scatterers \}

1908: MIE
1909: Debye \} General Theory

1914: Rayleigh
1915: Debye \} Approximate Theory
1925: Gans

1908: Smoluchowski \} Thermodynamic Theory of Solution Scattering
1911: Einstein \} 

1947-50: Debye
Zimm \} Scattering by Solutions of Macromolecules
Classification of Light Scattering

(by particle type) \[ d \]

(1) Rayleigh \[ d \leq \frac{\lambda}{20} \] \[ M \leq 40000 \]
- Lysozyme, Myoglobin etc.

(2) Rayleigh - Gans - Debye (RGD) \[ d \sim \lambda/20 \Rightarrow \lambda \]
\[ \frac{n_p}{n_o} - 1 \leq 0.1 \]
\[
\left\{ \right. \]
- OF MOST INTEREST!

(3) Mie \[ d \geq \lambda \]
- LARGE VIRUSES, BACTERIA etc.
RAYLEIGH - GANS - DEBYE (RGD) SCATTERING.

- For biomolecules of $M = 40,000 \rightarrow 20 \times 10^6$

Measure a parameter 'RAYLEIGH RATIO'

$$R_0 = \frac{i \omega}{I_0} \left[ \frac{C^2}{1 + \cos^2 \theta} \right]$$

dir. of particle from detector

Light scattering by a solution of macromolecules can be summarized by the equation:

$$\frac{KC}{R_0} \sim \left\{ 1 + \frac{16\pi^2 R_g^2 \sin^2 \theta}{3 \lambda^2} \right\} \left( \frac{1}{M} + 2BC \right)$$

$k$ is a collection of constants: $\frac{2\pi^2 n_0^2 (dn/dC)^2}{N_A \lambda^4}$

$B$: 2nd virial coeff.; $C$: concentration

$R_g$: "radius of gyration".

If $B$ is known, or $C$ is small enough $(20C \approx 0)$

A plot of $\frac{KC}{R_0}$ vs $\sin^2 \frac{\theta}{2} \rightarrow$ Mol. wt $+ R_g$. 

\[ w23 \]
ZIMM PLOT: ALGINATE POLYSACCHARIDE (M ~ 200,000)

\[
\frac{K_C}{R_0} |_{c=0} = \frac{1}{M} \left\{ 1 + \frac{16\pi^2 R_g^2}{3} \frac{\sin^2 \theta}{\lambda^2} \right\}
\]

\[
3\left( \frac{1}{n^2} - \frac{1}{n_0^2} \right) = \frac{1}{M} \left\{ 1 + \frac{16\pi^2 R_g^2}{3} \frac{\sin^2 \theta}{\lambda^2} \right\}
\]
Besides classical light scattering, $R_g$ can also be obtained from solution x-ray scattering or neutron scattering. Why might these alternative techniques be more suitable for smaller biomolecules ($M \leq 40000$)?
**Radius of Gyration** $R_g$

- Root mean square distance of mass elements in a particle from centre of mass

**Sphere** ($\text{radius } R$):
$$R_g = \sqrt{\frac{3}{5}} \cdot R$$

**Rod** ($\text{length } L$):
$$R_g = \frac{L}{\sqrt{12}}$$

**Ellipsoid** ($\text{semi-axes } a, b, c$):
$$R_g = \sqrt{\frac{a^2 + b^2 + c^2}{5}}$$

- (nb. prolate $c = b$
  oblate $c = a$)

**Random coil** ($\text{mean square end-to-end distance } R^2$):
$$R_g = \sqrt[\frac{1}{2}]{\langle R^2 \rangle} / \sqrt{6}$$

**Bead models** $R_g = \text{some complicated function}$!
### Typical $R_g$ values

<table>
<thead>
<tr>
<th>Material</th>
<th>$M$</th>
<th>$R_g$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>14,100</td>
<td>1.52</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>70,000</td>
<td>2.98</td>
</tr>
<tr>
<td>Turnip yellow mosaic virus</td>
<td>$5 \times 10^6$</td>
<td>30.0</td>
</tr>
<tr>
<td>Myosin</td>
<td>493,000</td>
<td>46.8</td>
</tr>
<tr>
<td>DNA sample</td>
<td>$4 \times 10^6$</td>
<td>117.0</td>
</tr>
</tbody>
</table>
Molecular Weight: Light scattering

“MALLs” detector
with 120mW laser operating at 658 nm
Molecular Weight: Light scattering
Molecular Weight: Zimm plot

\( 10^6 \frac{(Kc)}{R_\theta} \)

\( \frac{1}{M_w} \)

\( R_g \) from slope

\( \sin^2(\theta/2) + kc \) (k=100)
Molecular Weight: Light scattering
Molecular Weight: Light scattering
Molecular Weight: SEC–MALLS

- MALLs detector
- SEC - columns
- Concentration detector
Molecular Weight: SEC–MALLS

Fogg FJJ et al, Biochemical Journal. 1996
Molecular Weight: SEC–MALLS

3

Combined Differential Light Scattering with Various Liquid Chromatography Separation Techniques

By Philip J. Wyatt

WYATT TECHNOLOGY CORPORATION, SANTA BARBARA, CALIFORNIA 93130-3003, U.S.A.

1. INTRODUCTION

The combination of light scattering measurements with various particle/molecular separation techniques often permits an unparalleled characterization of the separated particles. In a sense, this is but an application of the so-called "inverse scattering" problem.1,2, i.e., from measurements of the light scattering...
Viscosity detector
Pressure Imbalance Viscometer:

\[ \eta_r = 1 + (4\Delta P)(P_i - 2\Delta P) \]
Pressure Imbalance Viscometer:

$$\eta_r = 1 + \{(4\Delta P). (P_i - 2\Delta P)\}$$
Wyatt Technology Viscostar System
Viscotek System
**Viscostar - pullulans**

- **LS 90°**
  - $M_w = 396,000$ g/mol (0.1 %)
  - $[\eta] = 107.6$ ml/g (1%)
  - Injected mass = 18 μg

- **DRI**
  - $M_w = 120,000$ g/mol (0.1 %)
  - $[\eta] = 46.2$ ml/g (0.3 %)
  - Injected mass = 92 μg

- **DPV**
  - $M_w = 620$ g/mol (7 %)
  - $[\eta] = 2.8$ ml/g (0.4 %)
  - Injected mass = 423 μg

The graph shows the elution time (min) on the x-axis and the normalized detector response on the y-axis.
Chicken Fibrinogen

$M_w = 335000 \text{ g/mol (0.1 \%)}$

$[\eta] = 27.7 \text{ ml/g (0.3 \%)}$

Injected mass = 383 $\mu$g

Hydrodynamic and mass spectrometry analysis of nearly-intact human fibrinogen, chicken fibrinogen, and of a substantially monodisperse human fibrinogen fragment X

Barbara Cardinalli ², Aldo Profumo ³, Anna Aprile ³, Owlyn Byron ⁴, Gordon Morris ⁵, Stephen E. Harding ⁶, Walter F. Stafford ⁷, Mattia Rocco ⁸
Identifying Differences in Solution Conformations of Two Chimeric IgG3 Antibodies through Triple Detection SEC

Jan 1, 2006
By: Emma Longman, Stephen E. Harding, Nicola Marheineke
LOGC NORTH AMERICA
Volume 24, Issue 1

Viscosek

IgG3wt

[\eta] = 9.9 ml/g

Retractive index (mV)

Retention volume (mL)

IgG3wt (028.vdt):
- Refractive index
- Right angle light scattering
- Viscometer DP
Identifying Differences in Solution Conformations of Two Chimeric IgG3 Antibodies through Triple Detection SEC

Jan 1, 2006
By: Emma Longman, Stephen E. Harding, Nicola Marheineke
LOGC NORTH AMERICA
Volume 24, Issue 1

IgG3m15

![Graph showing retention volume vs refractive index with peaks for monomer, dimer, and aggregate.]

\[
\eta = 5.7 \text{ml/g}
\]

Table I: Results determined through triple detection of IgG3wt and IgG3m15 antibodies.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Molecular weight from literature (Da)</th>
<th>Experimental molecular weight (Da)</th>
<th>Intrinsic viscosity (ML/g)</th>
<th>Viscosity Increment</th>
<th>Hydrodynamic radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG3wt</td>
<td>160 000</td>
<td>170 300</td>
<td>9.9</td>
<td>7.5</td>
<td>6.4</td>
</tr>
<tr>
<td>IgG3m15</td>
<td>150 000</td>
<td>149 700</td>
<td>5.7</td>
<td>4.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>
A bead–shell model of the wild type IgG3

Lu et al, Biophysical Journal, 2006
Follow up bibliography:

1. On-line tutorials from: Wyatt Technology and Viscotek corporation (see their web sites)