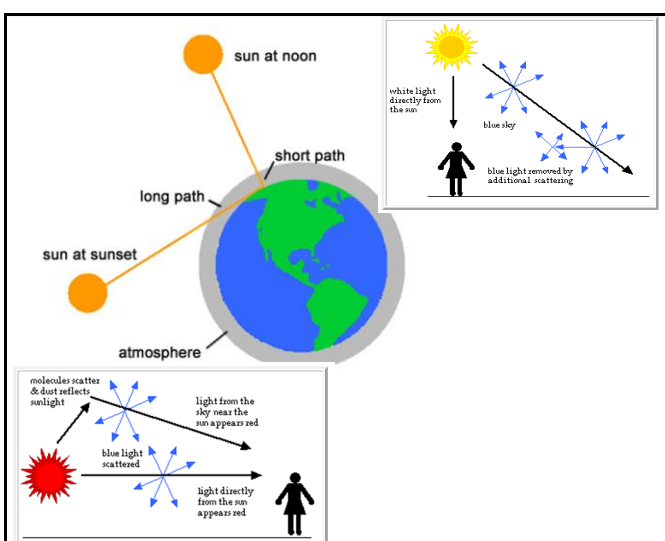
## Primary Rainbow


# Light Scattering

**Goals for this unit:**

- **Theory 101 – just the basics – Know why sky is blue, sunset burnt orange**
- **Rayleigh Scattering (Lord Rayleigh ~1871) / Rayleigh Ratio**
- **How does LS yield an "absolute" molecular weight? LS vs. RI**
- **What is polydispersity? How is it defined?**
- **Types of "Molecular Weight Averages"**
- **LS Instrument / Practical Considerations**
- **Static vs. Dynamic vs. X-ray Scattering (info from each)**
  ***(M vs. Rh vs. Internal shape)***




# Light Scattering

**STATIC** *Light Scattering*

**Also known as *Rayleigh* or *Classical* Light Scattering**

Measures avg. intensity of scattered light for

***Absolute*** Molecular Weight

**Dynamic** *Light Scattering (DLS)*

**Also known as *Quasi-elastic* Light Scattering *(QUELS)***
**or *Photon Correlation Spectroscopy (PCS)***

Measures microsecond fluctuations of single photons

Hydrodynamic Radius (Size)

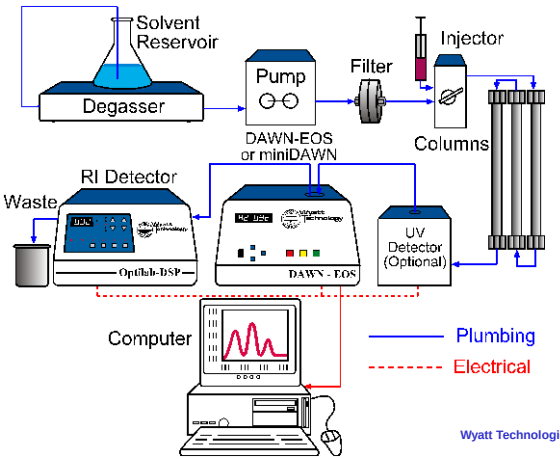
*M.L.Hackert (with figures from Precesion Detectors and Wyatt Tech.)*

## What Do We Mean By ABSOLUTE?

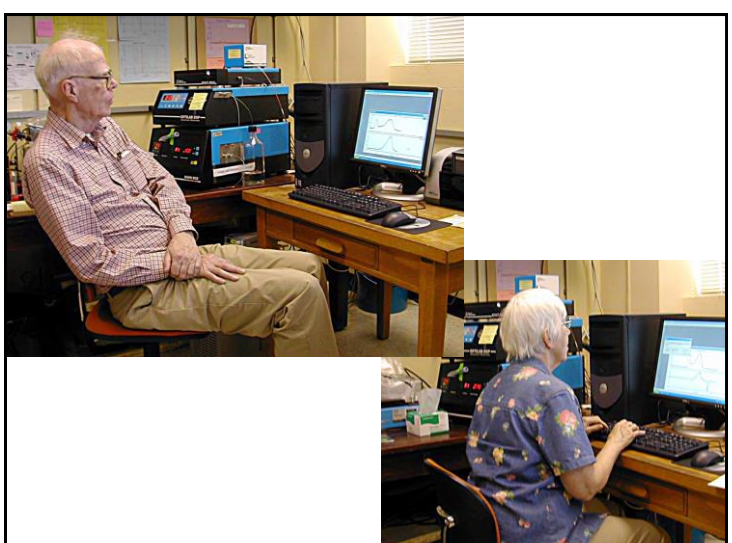
- There are **4 Absolute Methods of Measuring MW**
- 1) Membrane **Osmometry** (Number Average MW)
- 2) Light Scattering (Weight Average MW)
- 3) Sedimentation Equilibrium (Ultracentrifugation) (z-average MW)
- 4) **Mass spectroscopy**
- ***NO*** Reference to standards of mass
- ***NO*** assumptions of molecular model/conformation
- ***ALL*** parameters measured directly from 1st principles
- ***Refractive indices***
- ***geometries of cell and detector***
- ***wavelength***
- ***concentrations***
- ***detector response***
- ***temperature***
- ***dn/dc (~0.186 mL/g for average proteins)***



## Chromatography with LS Set-up





## EM wave scattered by a molecule

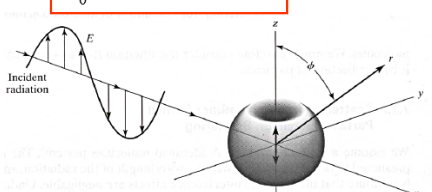
Electromagnetic wave emitted by the oscillating dipole

$$E = \frac{4\pi^2 \alpha E_0 \sin\phi}{\lambda^2 r} e^{i(\omega t - \vec{k}_s \cdot \vec{x})}$$

Scattering intensity to the incident intensity

$$\frac{I}{I_0} = \frac{16\pi^4 \alpha^2 \sin^2\phi}{\lambda^4 r^2}$$

for polarized incident light of intensity $I_0$



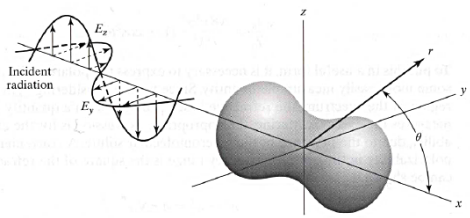
## EM wave scattered by a molecule

Scattering intensity to the incident intensity

$$\frac{I}{I_0} = \frac{8\pi^4\alpha^2(1+\cos^2\theta)}{\lambda^4 r^2}$$

for unpolarized incident light

***I ∝ 1/r² ; I ∝ 1/λ⁴ ; I depends on scattering angle***




## Scattering from molecules much smaller than λ

**molecule size << λ**

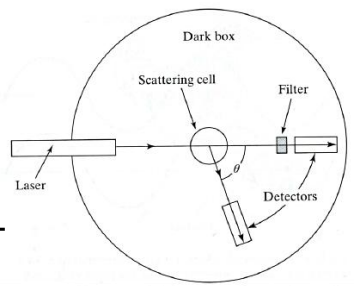
$$\frac{I(\theta)}{I_0} = \frac{2\pi^2 n_0^2}{A\lambda^4 r^2}\left(\frac{dn}{dC}\right)^2 CM(1+\cos^2\theta)$$

*Light scattering can be used to determine the molecular weight.*

Define **"Raleigh Ratio" $R_\theta$**

$$R_\theta = \frac{I(\theta)}{I_0}\frac{r^2}{(1+\cos^2\theta)}$$

$$R_\theta = KCM \quad or \quad \frac{K^*c}{R(\theta)} = \frac{1}{M}$$




## *Static* Light Scattering Detection

***Determines***

- **Absolute Molecular Weight**

  **Independent of Column Calibration**

  - The amount of **light scattered** is directly proportional to the product of the **molar mass** and the **molecular concentration**

- **Radius of Gyration ($R_g$)**

  **> 10 nm to 150 nm**

  - The **variation of scattered light** with scattering angle is proportional to the **average size** of the scattering molecules.

$$I_{LS} = C\,M_w\,(dn/dc)^2\,P_\theta\,K_\theta$$

Form Factor
Detector Constants
Specific Refractive Index Increment
Molecular Weight
Concentration of Solute
Excess Light Scattering Signal

*Rayleigh Equation*

$$\frac{K^*c}{R(\theta)} = \frac{1}{M}\left[1 + \frac{16\pi^2}{3\lambda^2}\langle r_g^2\rangle \sin^2(\theta/2) + \cdots\right] + 2A_2c$$

## RI & Light Scattering Combined for Mw

$$R_\theta = C\,M\,(dn/dc)^2\,P(\theta)\,K(\theta)$$ Rayleigh Equation

$$RI_{signal} = K_{RI}\,(dn/dc)\,C$$ RI Equation

$$\frac{R_\theta}{RI_{signal}} = \frac{K(\theta)\,M_w\,(dn/dc)\,P(\theta)}{K_{RI}}$$

$$\frac{R_\theta}{RI_{signal}} \sim M_w$$

*For a truly effective measurement of molecular weight the static light scattering detector must be combined with a well matched refractometer*

- **The light scattering signal is directly proportional to the Mw .**
- **The concentration source signal (e.g. RI) is indirectly proportional to the Mw.**

Precision Detectors

## *Static* LS Equation for Proteins

$$M_w = \frac{I_{LS}}{RI} K_{total}$$

1. Mw is directly proportional to the LS signal
2. Mw is indirectly proportional to the conc. source (RI)
3. The dn/dc is constant for the protein and it's aggregates (0.186 mL/g)
4. Degree of aggregation can be approximated from visual inspection

Precision Detectors

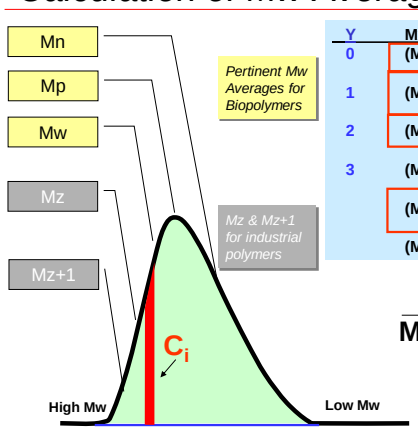
## *Accuracy of Molecular Masses of Test Proteins Determined by Light Scattering*

| Protein | Mass From Structure | Light Scattering* | Apparent Error |
|---|---|---|---|
| | [Da] | [Da] | [%] |
| Carbonic anhydrase | 29,023 | 29,800 | +2.7 |
| Alcohol dehydrogenase | 145,980 | 149,000 | +1.4 |
| β-Amylase | 224,340 | 228,000 | +1.6 |
| Apoferritin | 476,316 | 484,400 | +1.7 |
| Thyroglobulin | 669,000 | 679,000 | +1.5 |
| Ornithine decarboxylase | 990,684 | 978,000 | -1.3 |
| Octopus Hemocyanin | 3,440,000 | 3,450,000 | +0.3 |

*DAWN detector model-F, 0.19 was used as dn/dc value for all the proteins
Adapted from "Assembly of the Gigantic Hemoglobin of the Earthworm *Lumbricus terrestris* by A. Riggs *et.al.* In *J. Bio. Chem.*, Vol. 271, No. 47, pp 30007-30021, 1996.

Wyatt Technology Corporation

## *Calculation of Mw Averages*



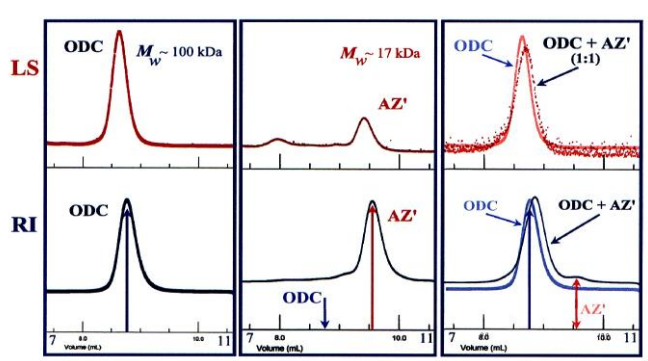

*Pertinent Mw Averages for Biopolymers*

*Mz & Mz+1 for industrial polymers*

| Y | Mx | Name |
|---|---|---|
| 0 | (Mn) | Avg. Molecular Number |
| 1 | (Mw) | Avg. Molecular Weight |
| 2 | (Mz) | Avg. Z (Velocity) |
| 3 | (Mz+1) | Avg. Z+1 |
| | (Mw/Mn) | Polydispersity |
| | (Mp) | Peak Molecular Weight |

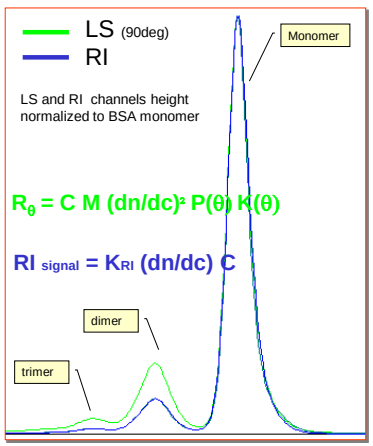
$$\bar{M}_x = \frac{\sum C_i M_i^{y}}{\sum C_i M_i^{y-1}}$$

Precision Detectors




**Light Scattering (LS) and Refractive Increment (RI) Results of ODC and AZ'.** *ODC, AZ' and ODC:AZ' complex were injected onto an HPLC sizing column, separated and analyzed by LS (DAWN EOS) and RI (OptiLab DSP interfermetric refractometer). The results shown are for 7 to 11 minutes of elution volume. Frame 1 is for ODC, frame 2 for AZ', and frame 3 for the ODC:AZ' mixture at a 1:1 subunit ratio with the ODC trace from frame 1 superimposed for reference.*

## *Visualizing Aggregation State*




$$M_w = \frac{I_{LS}}{RI} K_{total}$$

$R_\theta = C\ M\ (dn/dc)^2\ P(\theta)\ K(\theta)$

$RI_{signal} = K_{RI}\ (dn/dc)\ C$

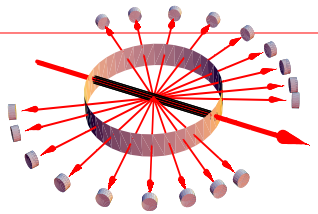
*Using visual inspection*

- Monomer (LS is equal to RI)
- Dimer (LS is 2X as large as RI)
- Trimer (LS is 3X as large as RI)

Precision Detectors

## Why Multi-Angle Detection?

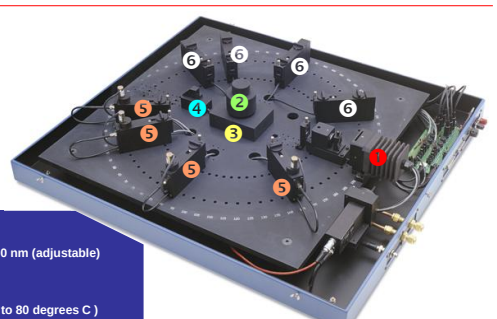
Light Scattering Intensity of Particles Shows an Angular Dependence on Size



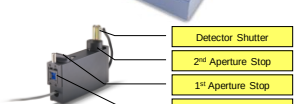
- **Low angles sensitive to large particles**
- **90 degree angle more sensitive to smaller particles**
- **High angles less sensitive to larger particles**
- **Back angles better suited for opaque matrices**

Precision Detectors

## ALS4000 Optical Platform Features



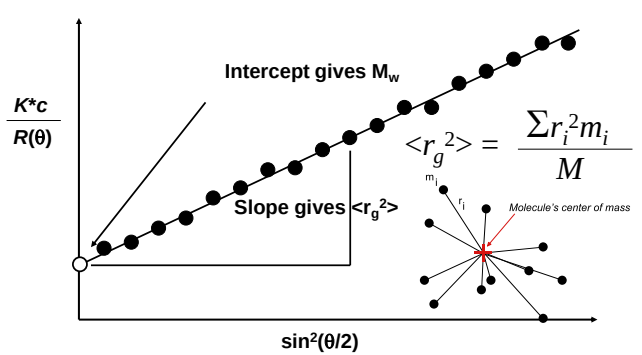
1. Diode laser: 230 mW @ 830 nm (adjustable)
2. Flow Cell (30 uL)
3. Peltier cooling/heating ( 0 to 80 degrees C )
4. Laser Beam Stop
5. DLS Detectors (Up to 4)
6. Static LS Detectors (Up to 8)




*DLS Detector*

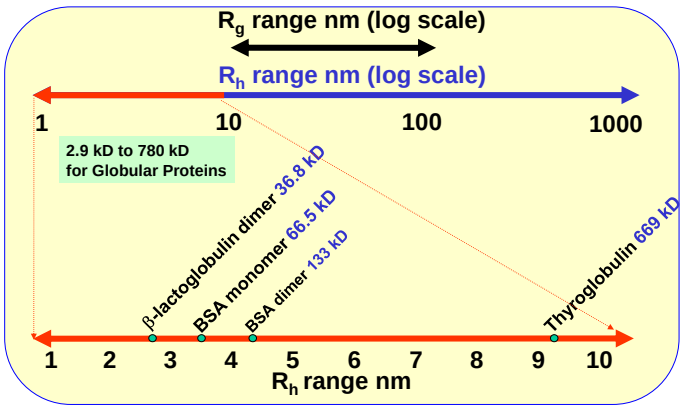
Precision Detectors

## *$M_w$ & $\langle r_g^2 \rangle$ determined by MALS*




$$\frac{K^*c}{R(\theta)} = \frac{1}{M}\left[1 + \frac{16\pi^2}{3\lambda^2}\langle r_g^2\rangle \sin^2(\theta/2) + \cdots\right] + 2A_2c$$

## *Why use $R_h$ instead of $R_g$ for Biomolecules?*




Precision Detectors

## *Dynamic Light Scattering Detection*

***Determines***

- **Molecular or Particle Size**
  - As Hydrodynamic Radius (Rh)
- **Size Range**
  - 1 to 1000 nm

$$D_0 = \kappa\, T(6\pi\, \eta_0\, R_h)^{-1}$$

Diffusion Coefficient
Boltzmann Constant
Temperature
Constants
Solvent Viscosity
Hydrodynamic Radius

*Stokes-Einstein Equation*

Precision Detectors

## Hydrodynamic Radius Determination

Precision Detectors

From $g_1(\tau)$ the diffusion coefficient (D) for the scattering particles can be determined. From the diffusion coefficient, the hydrodynamic radius can be calculated.

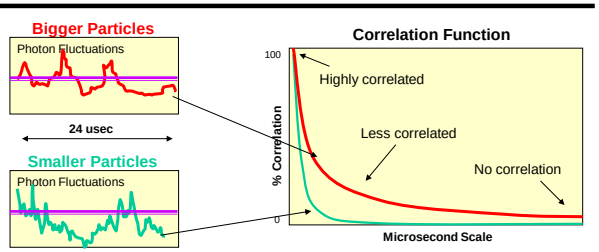
Diffusion Coefficient | Boltzmann Constant | Temperature (Kelvin) | Solvent Viscosity (Poise) | Hydrodynamic Radius (nm)

$$D_0 = \kappa\, T(6\pi\, \eta_0\, R_h)^{-1}$$

***Stokes-Einstein Equation***

***Applicable DLS Size Range***

1.5 to 1000 nm Radius

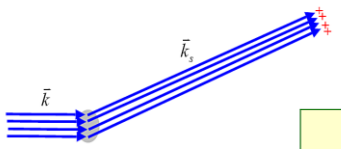



## Conclusions on Static and Dynamic LS

- Measures **Hydrodynamic Radius, Radius of Gyration, Molecular Weight. Particle Size Distribution**.
- Instrument Measures
  - **Rh size** from 1.7 nm to 1000 nm.
  - **Rg size** from 9 nm to 150 nm.
  - **Molecular Weight 200 to 20,000,000 daltons**
- Detects branching, aggregates and calculates Mw.
- **DLS**, does not require conc or dn/dc measurement for size.
- Batch Mode for non-flow system accessories.

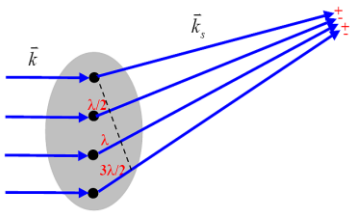
Precision Detectors

Scattering from molecules much smaller than λ




Do not need to consider interference effect.

Scattering from molecules comparable to or larger than λ




Small angle X-ray scattering (SAXS)

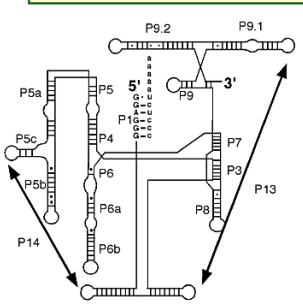
Need to consider interference effect.

## Molecular weight and size measured by Light scattering or SAXS

| Material | $M_w$ | $R_G$ (nm) |
|---|---|---|
| Ribonuclease | *12,700* | *1.48* |
| α-Lactalbumin | *13,500* | *1.45* |
| Lysozyme | *13,600* | *1.43* |
| β-Lactoglobulin | 36,000 | |
| | *36,700* | *2.17* |
| Serum albumin | 70,000 | *2.98* |
| Myosin | 493,000 | 46.8 |
| Turnip yellow mosaic virus | | *10.4* |
| Tobacco mosaic virus | $39 \times 10^6$ | 92.4 |

Values in *italic* are from low angle X-ray scattering.

## RNA folding



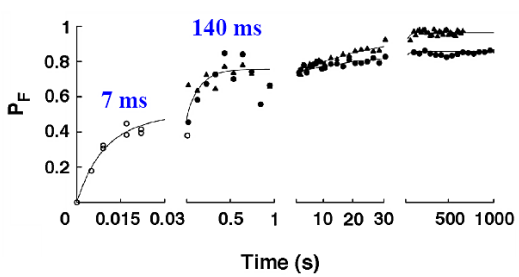

Unfolded state
Secondary structure

+ $Mg^{2+}$

Folded state with tertiary structure

SAXS has a potential to answer how fast RNA molecule compacts.

## RNA folding studied by SAXS




- Major compaction happen very fast in two phase: 7 ms and 140 ms