Light Scattering

STATIC Light Scattering

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

Measures avg. intensity of scattered light for

Absolute Molecular Weight (*r_g Radius of Gyration*)

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 (**R_h** Size)

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

and http://info.med.yale.edu/wmkeck/biophysics

Define "*Raleigh Ratio*" as $R_\theta = (i_\theta / I_\theta)(r^2 / (1 + \cos^2\theta))$**, and thus**

$$R_\theta = [2p^2 n_o^2 (dn/dC)^2 / l^4 N^o]\, CM \text{ , or}$$

$$R_\theta = KCM.$$

Note: Different experimental methods yield different types of experimentally arrived at "Molecular Weights." Light scattering yields a **"weight average"** molar mass– solutions must be scrupulously clean since dust will contribute to average as very large molecules.

		$\Sigma C_i M_i^y / \Sigma C_i M_i^{y-1}$		
Number Average M_n	$\Sigma N_i M_i / \Sigma N_i$	$\Sigma C_i / \Sigma(C_i / M_i)$	$y = 0$	Osmotic Press / F.Pt.
Weight Average M_w	$\Sigma N_i M_i^2 / \Sigma N_i M_i$	$\Sigma C_i M_i / \Sigma C_i$	$y = 1$	Light Scatt. / Sed. Eq.
"Z" Average M_Z	$\Sigma N_i M_i^3 / \Sigma N_i M_i^2$	$\Sigma C_i M_i^2 / \Sigma C_i M_i$	$y = 2$	Sedimentation Equil.

(Mw/Mn) Polydispersity

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Examples:

Sample 1 - 98% major component M = 30,000 + 2% dimer M ~ 60,000

Mn = [98(30,000) + 2(60,000)] / 98 + 2 = (2,940,000 + 120,000) / 100 = 30,600

Mw = [98(30,000)^2 + 2(60,000)^2] / (98(30,000) + 2(60,000) =

(88,200,000,000 + 7,200,000,000) / (2,940,000 + 120,000) =

95,400,000,000 / 3,060,000 = 31,176

(Mw/Mn) Polydispersity = 31,176 / 30,600 = 1.02

Sample 2 - 98% major component M = 30,000 + 2% aggregate M ~ 600,000

Mn = [98(30,000) + 2(600,000)] / 98 + 2 = (2,940,000 + 1,200,000) / 100 = 41,400

Mw = [98(30,000)^2 + 2(600,000)^2] / (98(30,000) + 2(600,000) =

(88,200,000,000 + 720,000,000,000) / (2,940,000 + 1,200,000) =

808,200,000,000 / 4,140,000 = 195,217

(Mw/Mn) Polydispersity = 195,217 / 41,400 = 4.72

How it can be used to characterize a protein sample?

Static LS can easily detect aggregates

Light Scattering Signal $R(\Theta) \sim Mw^*c$

because of their big Mw, aggregates scatter strongly

Angular variation of the scattered light is related to the size of the molecule

the light scattering signal from aggregates will show angular dependence, while LS signal produces by lower order oligomers like monomers, dimers et c. will not

Ken Williams
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Protein K: octamer 8 x 16.3 kDa = 130 kDa

Mw = 137 kDa

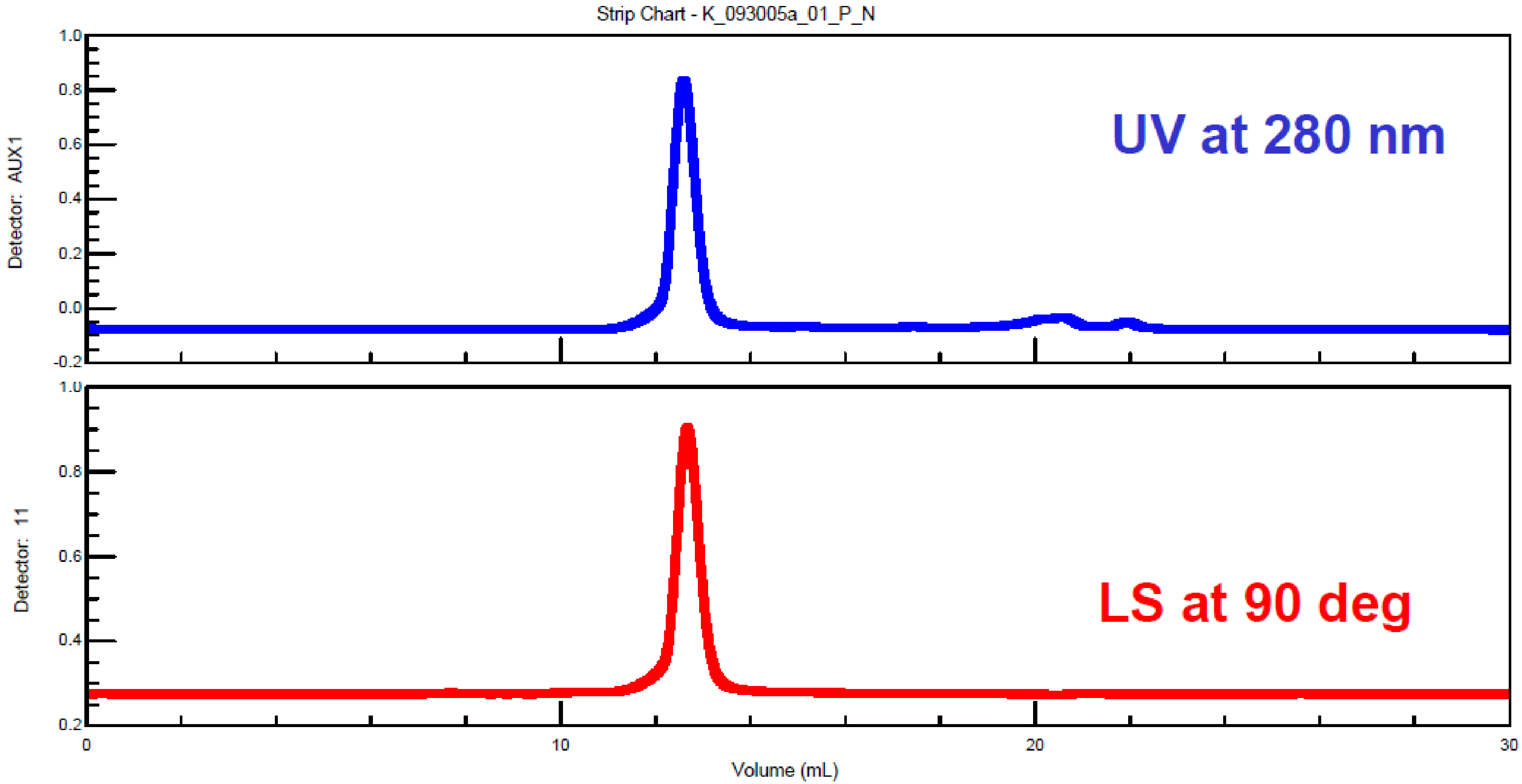
Polydispersity Mw/Mn 1.01

Concentration at apex = 0.09 mg/mL

98.9 % at 133 kDa

1.1 % at 192 kDa

0.0 % 0.5-100 MDa



Protein K: octamer 8 x 16.3 kDa=130 kDa

Mw = 141 kDa

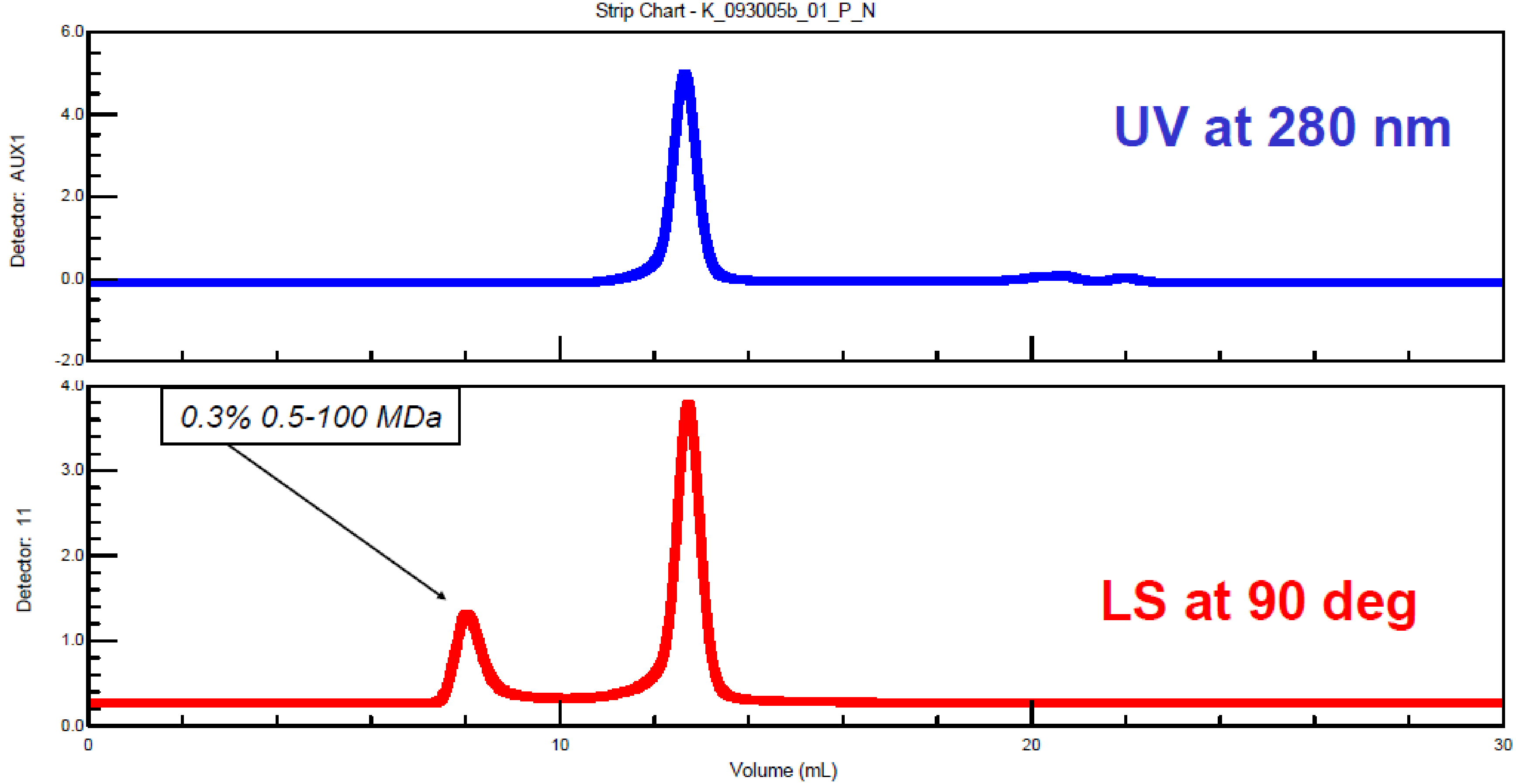
Polydispersity Mw/Mn 1.05

Concentration at apex = 0.5 mg/mL

95.8 % at 133 kDa

3.9 % at 217 kDa

0.3% 0.5-100 MDa



Feature detected in a batch mode LS measurements for sample containing aggregates

- ***Static (classical)***

Aggregates present:

- elevated weight average Molar Mass (M_w weight average)
- angular dependence in scattered light

- ***Dynamic (quasielastic)***

Aggregates present:

- autocorrelation function cannot be described by single exponential (cumulant fit)

Missing information: how much and what size?

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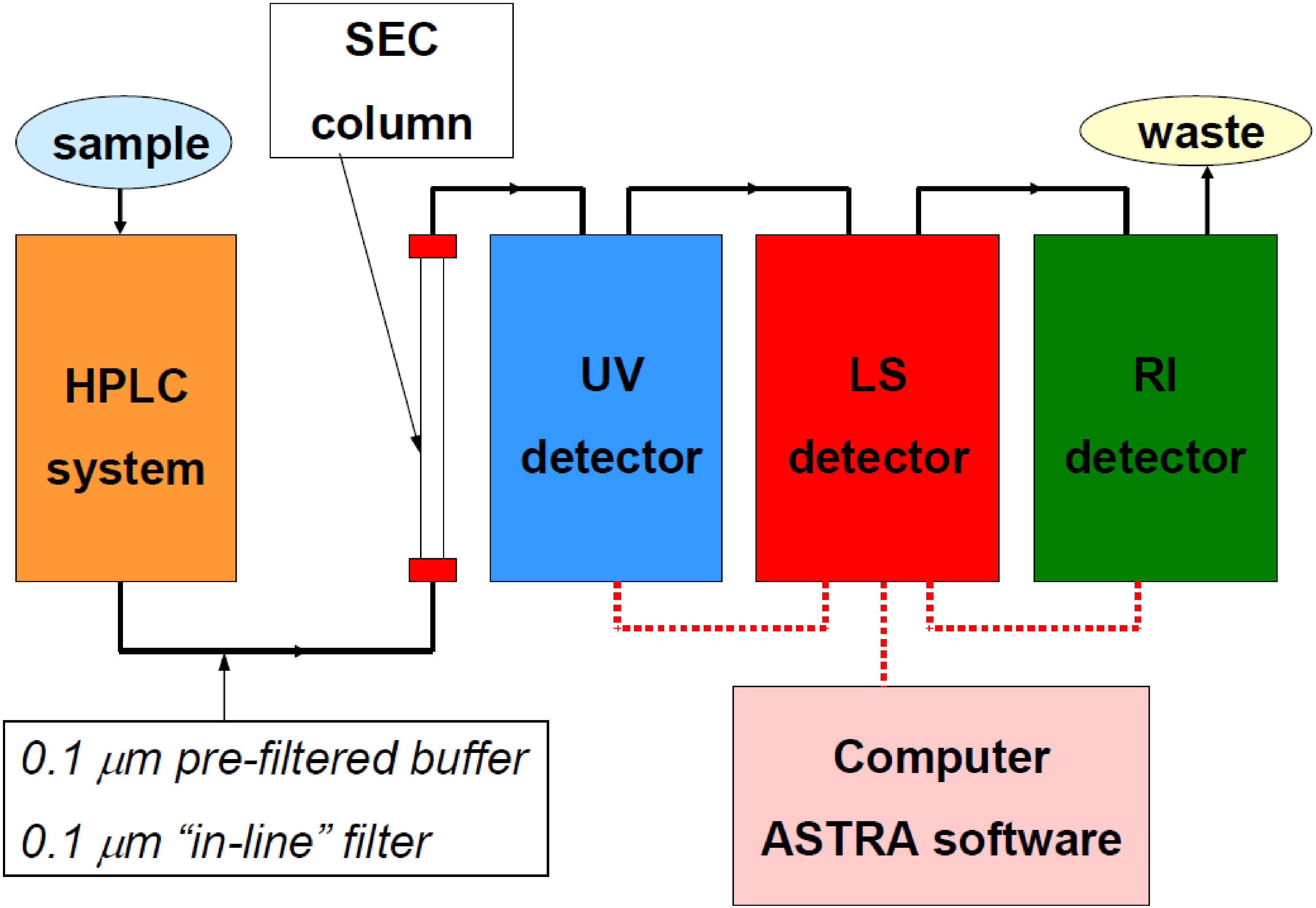
Missing information: how much and what size?

- **Fractionate sample**
- **Combine LS measurement with SEC (size excludion chromotography)**



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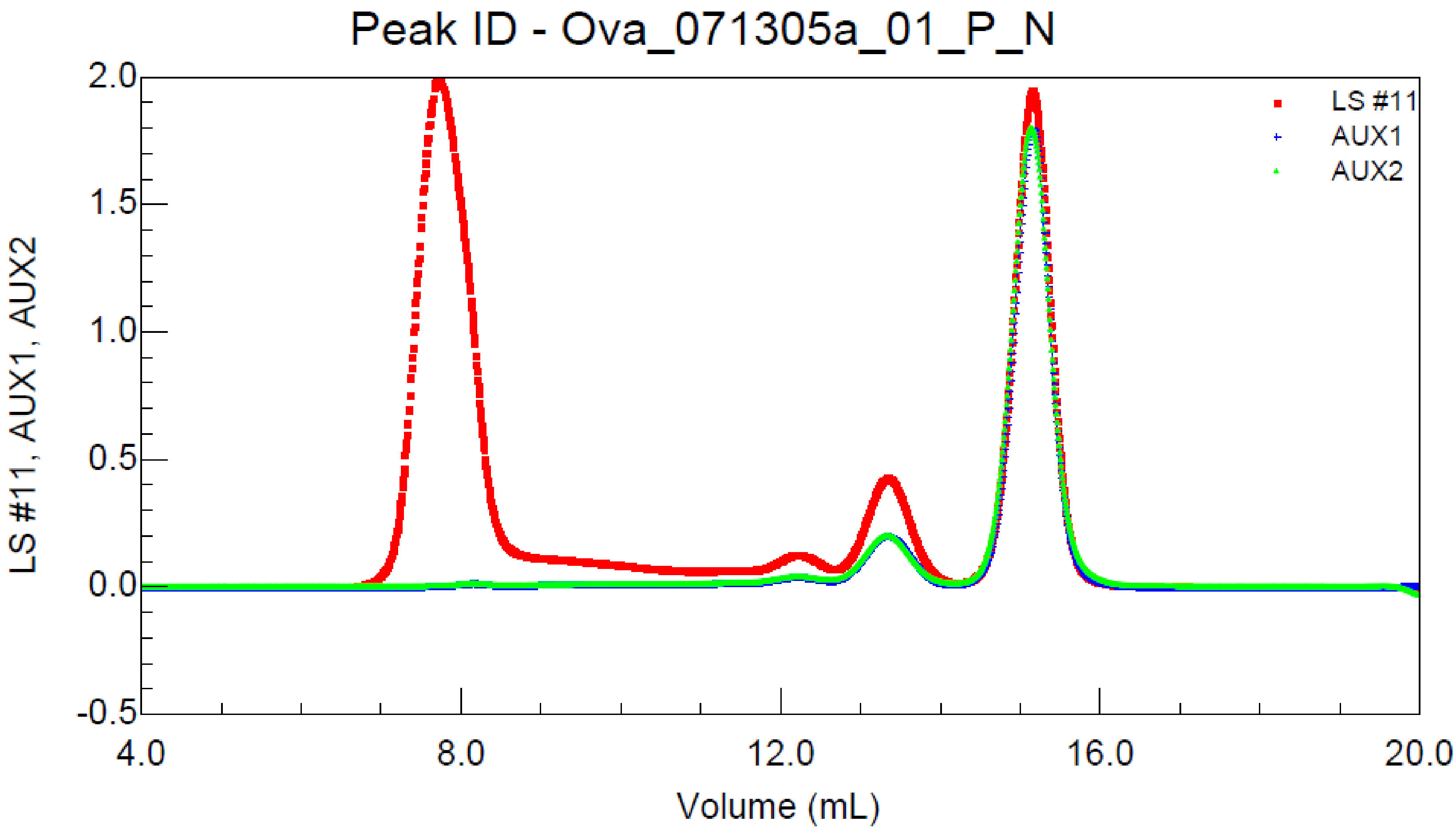
Three Detector monitoring



UV at 280 nm **RI** **LS at 90°**

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Ovalbumin 43 kDa

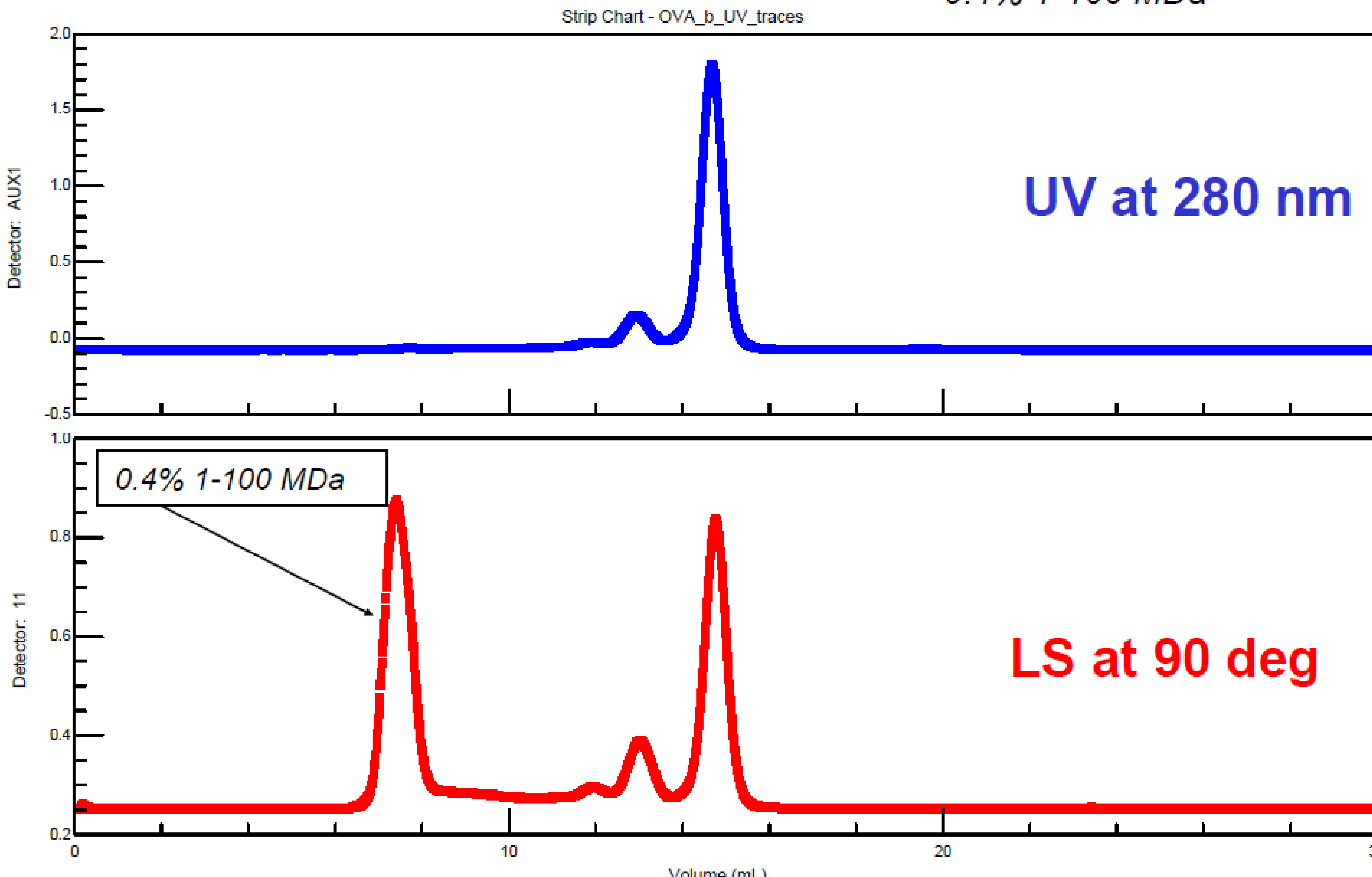
88% monomer

8% dimer

1.5% trimer

3% aggregates < 1MDa

0.4% 1-100 MDa



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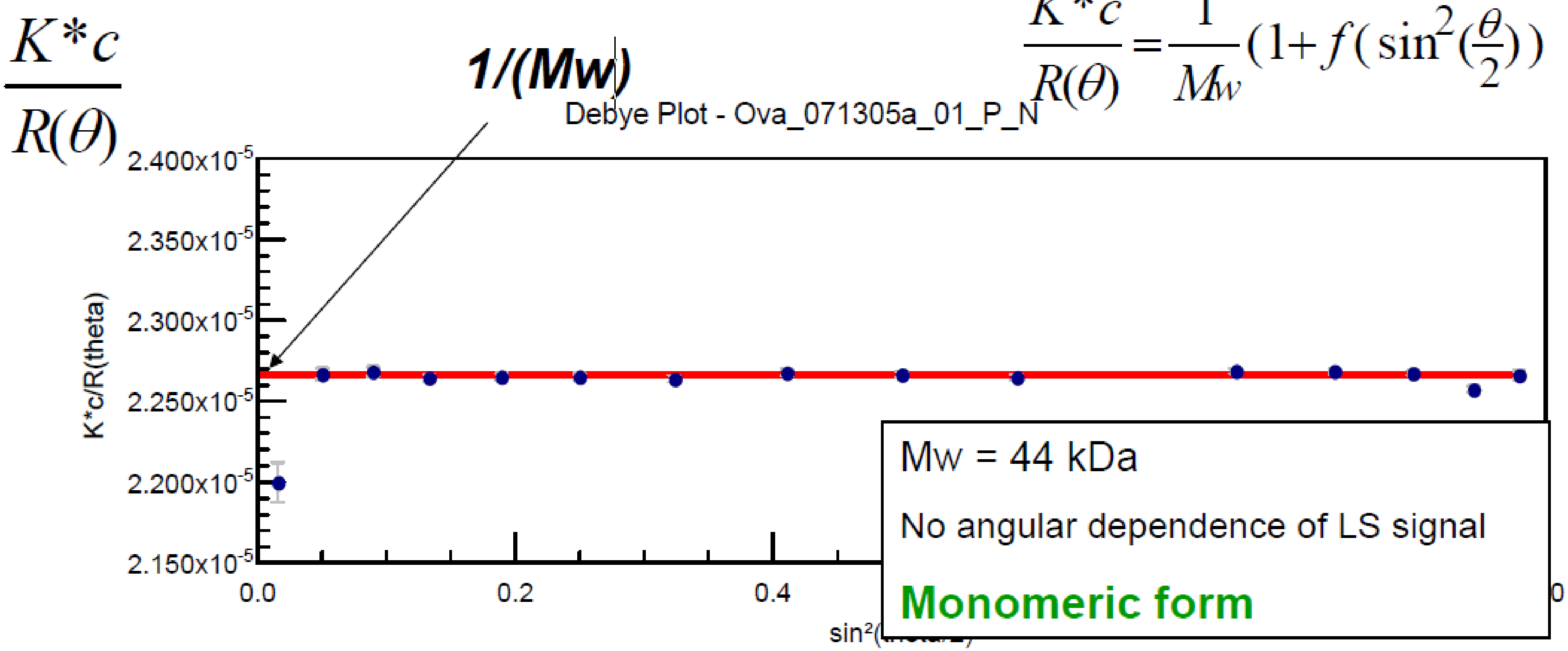
Zimm Plot Ovalbumin (43 kDa)

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w}\left(1+f\left(\sin^2\left(\frac{\theta}{2}\right)\right)\right)$$

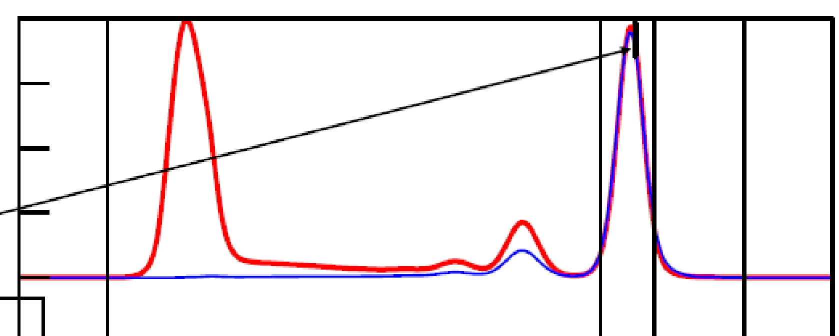


Mw = 44 kDa

No angular dependence of LS signal

Monomeric form

Peak, Slice : 2, 1826
Volume : 15.217 mL
Fit degree : 0
Conc. : (8.320 ± 0.000)e-4 g/mL
Mw : (4.413 ± 0.002)e+4 g/mol
Radius : 0.0 ± 0.0 nm



90° & AUX detector

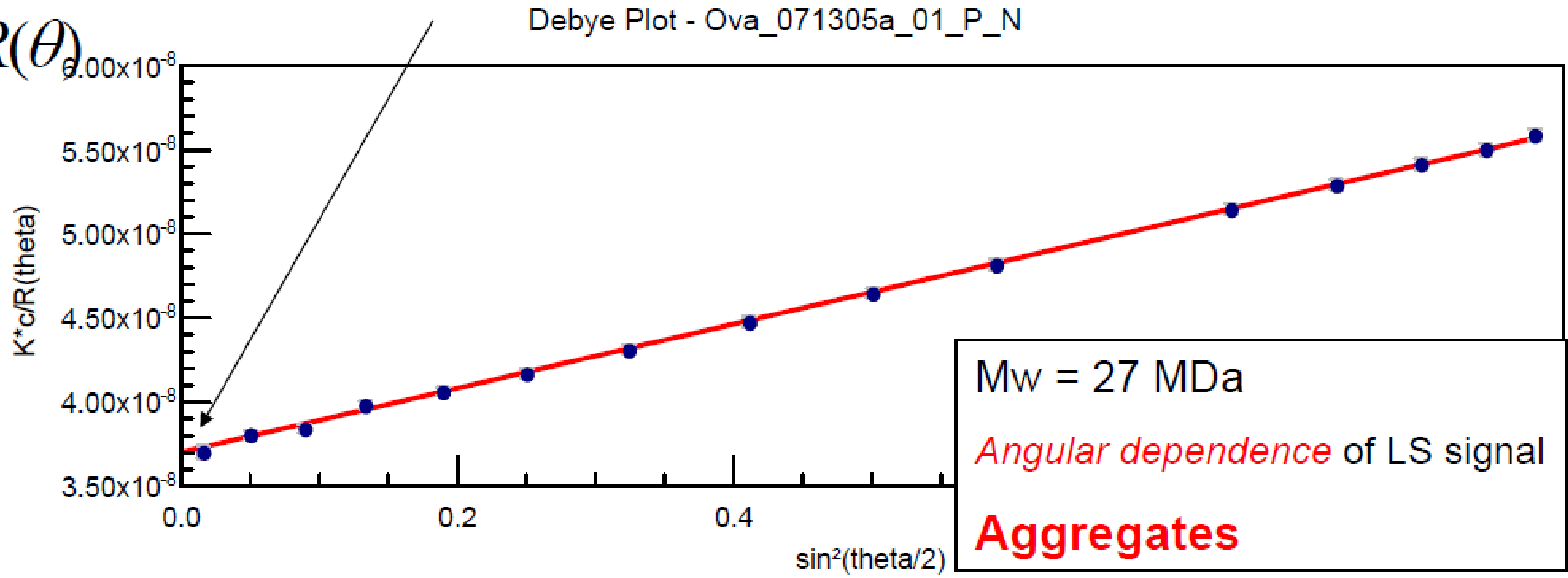
Results for last peak in elution profile

Zimm Plot Ovalbumin (43 kDa)

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w}\left(1 + f\left(\sin^2\left(\frac{\theta}{2}\right)\right)\right)$$

$\frac{K^*c}{R(\theta)}$

1/(Mw)

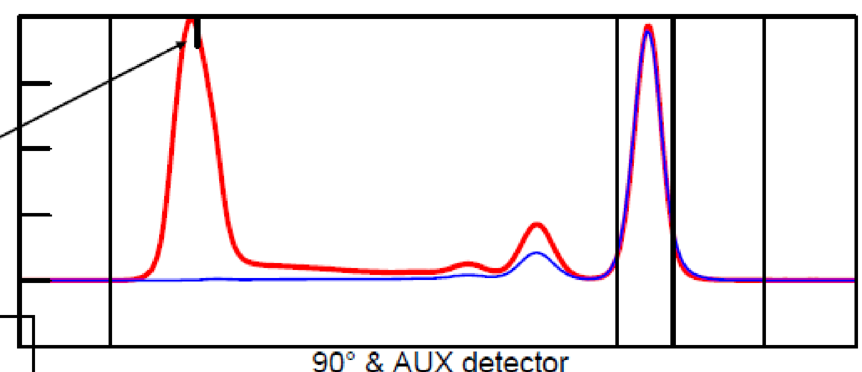


Mw = 27 MDa

Angular dependence of LS signal

Aggregates

Peak, Slice : 1, 938
Volume : 7.817 mL
Fit degree : 1
Conc. : (1.768 ± 0.021)e-6 g/mL
Mw : (2.702 ± 0.033)e+7 g/mol
Radius : 51.3 ± 0.2 nm



Results for initial peak in elution profile

http://info.med.yale.edu/wmkeck/biophysics

Light Scattering Experiments

- ***Static (classical)***

  time-averaged intensity of scattered light

- ***Dynamic (quasielastic)***

  fluctuation of intensity of scattered light with time

Parameters derived:

- *Molar Mass (weight-average) accuracy ~5%*
- ($<r_g^2>^{1/2}$) root mean square radii for ($<r_g^2>^{1/2}$)> *(λ/ 20) ~ 30 nm*

Parameters derived:

- D_T translation diffusion coefficient
- R_h <u>*hydrodynamic*</u> radius (Stokes radius)

Uncertainty of ~10% for monodisperse sample

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