Application of Light Scattering for Analysis of Protein-Protein Interaction and Aggregation

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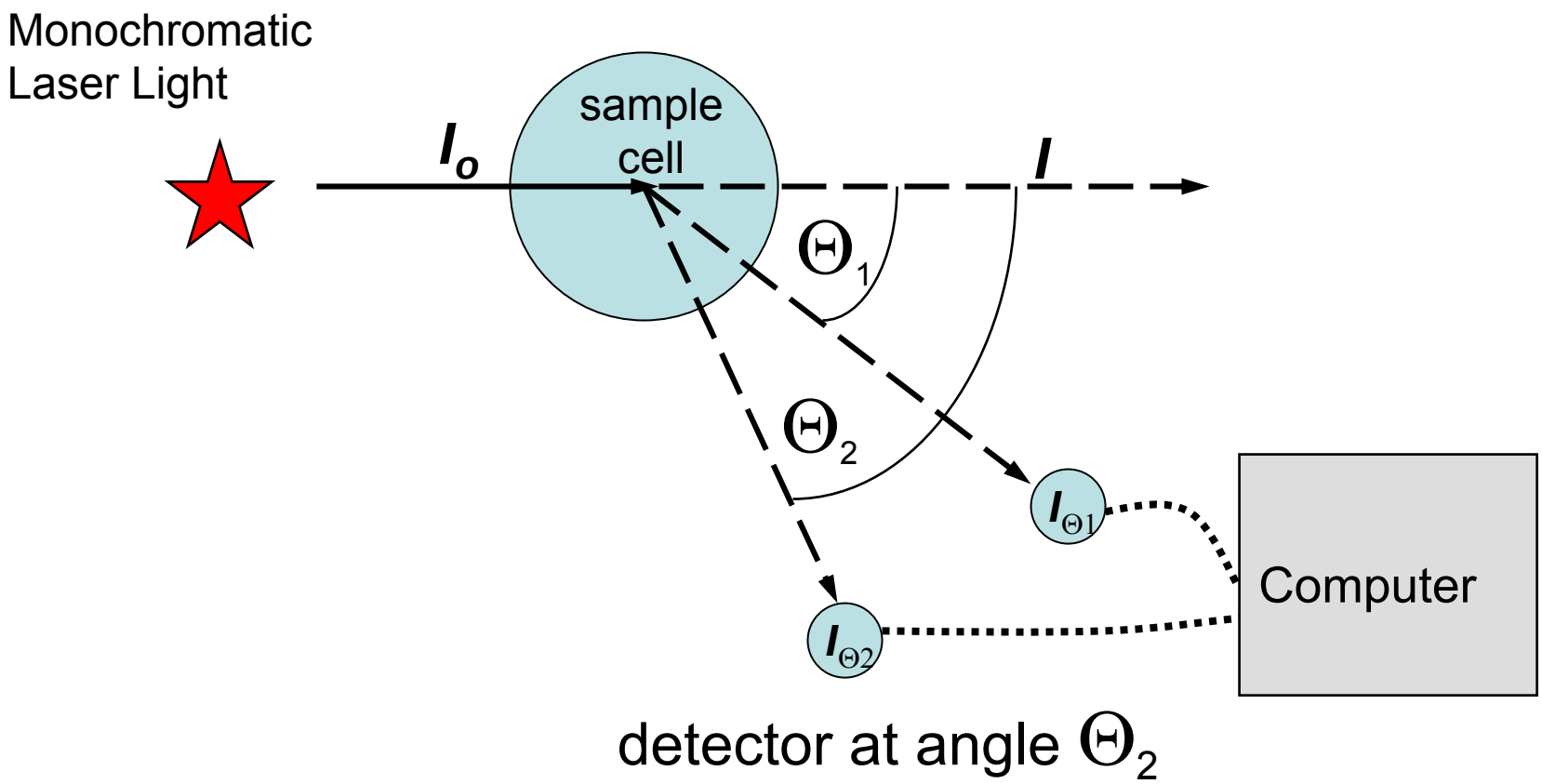
- Light Scattering Technologies
  - Static and dynamic light scattering
  - Parameters derived from SLS and DLS measurements
- Flow Mode Light Scattering Applications
  - Molar mass distributions and differences in populations
  - Determination of an oligomeric state of modified proteins from SEC-LS/UV/RI measurement
  - Determination of dimerization constant from SEC-LS measurements
- Capabilities and limitation of LS measurements

Light Scattering Experiments



Light Scattering Experiments

- **Static (classical)**

  time-averaged intensity of scattered light

- **Dynamic (quasielastic)**

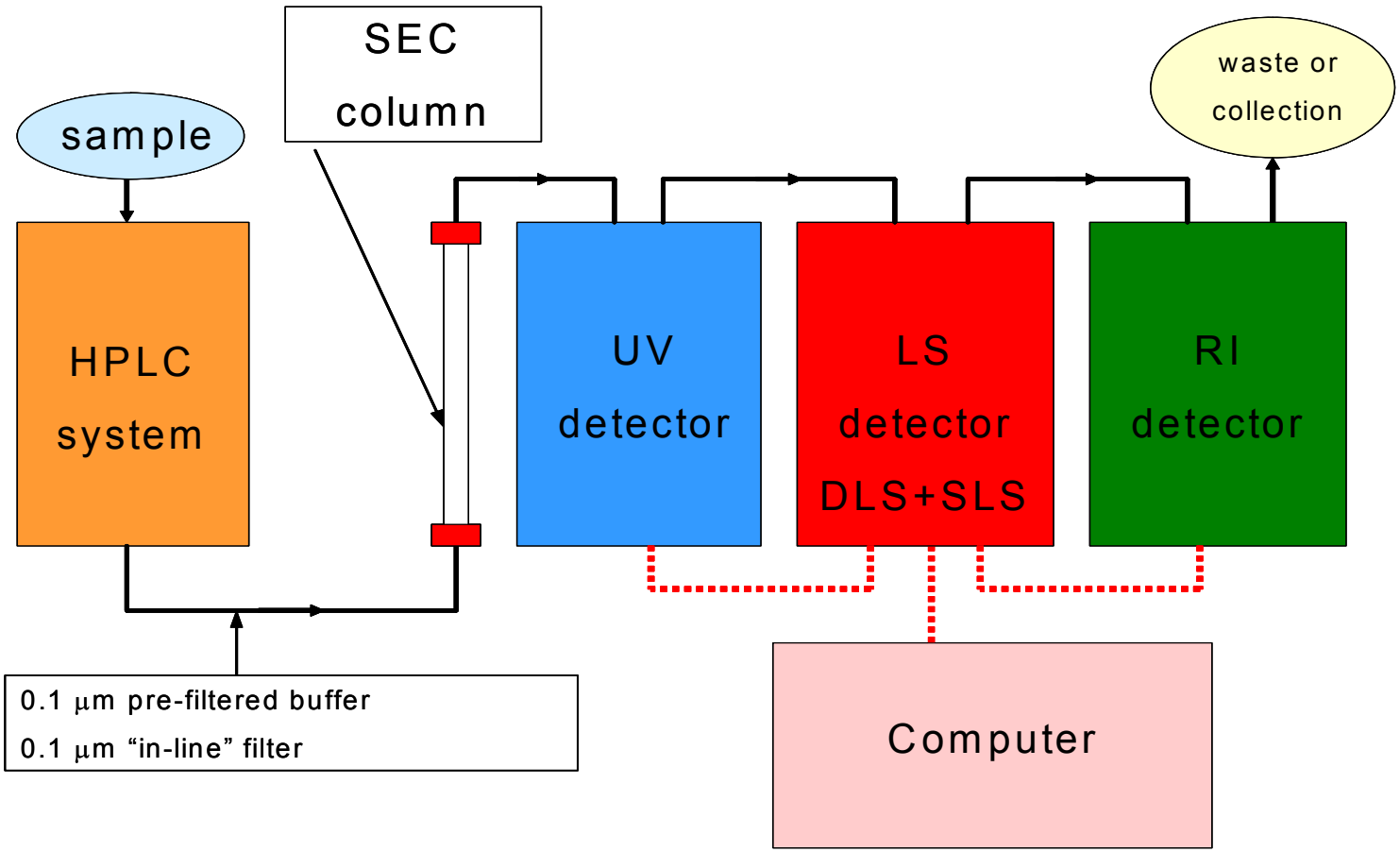
  fluctuation of intensity of scattered light with time

Measurements:

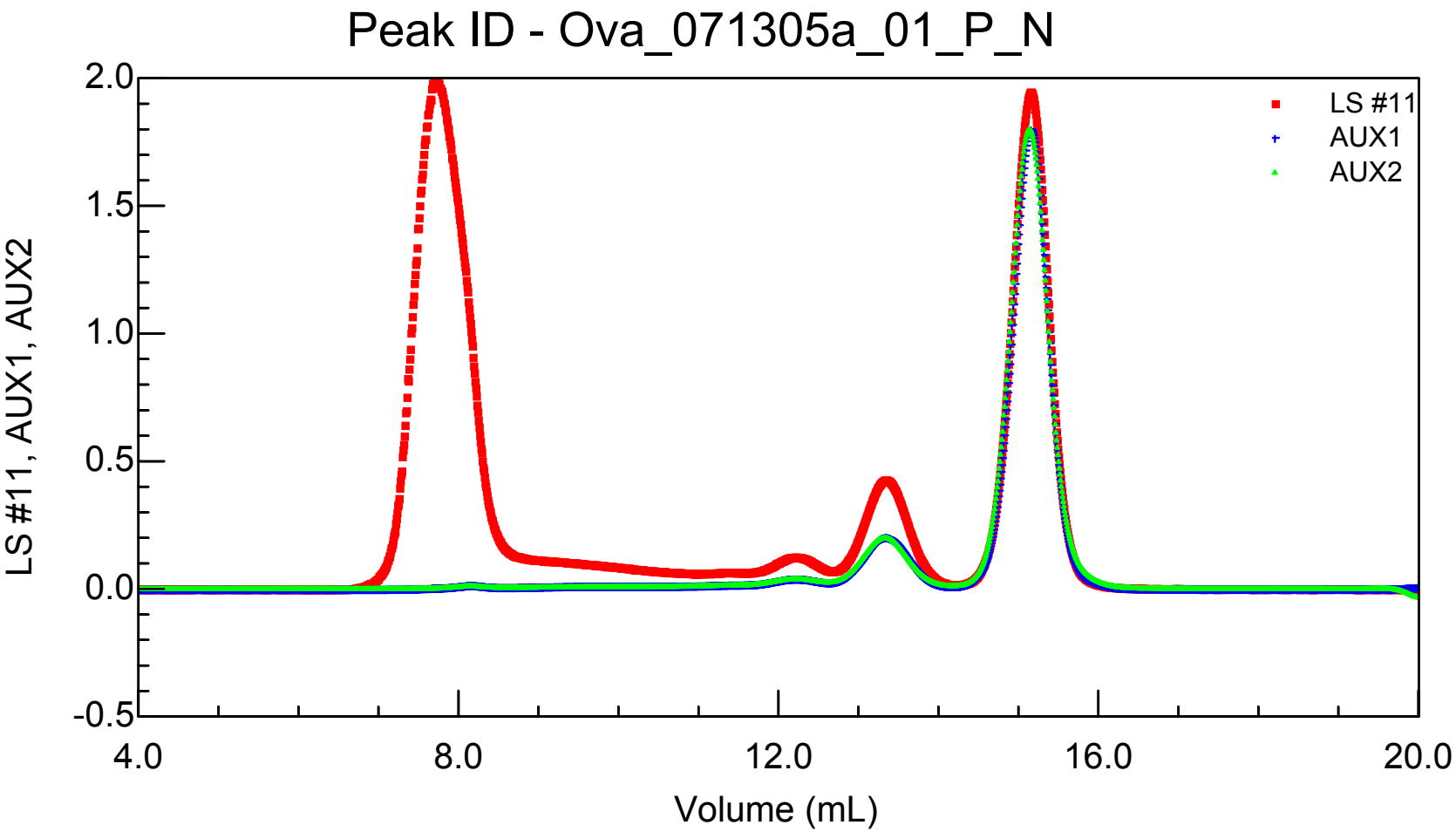
- batch mode
- "in-line" mode combined with a fractionation step,

  i.e. chromatography, mainly Size Exclusion Chromatography, Flow Field Fractionation

Typical SEC-MALLS system



Three Detector monitoring



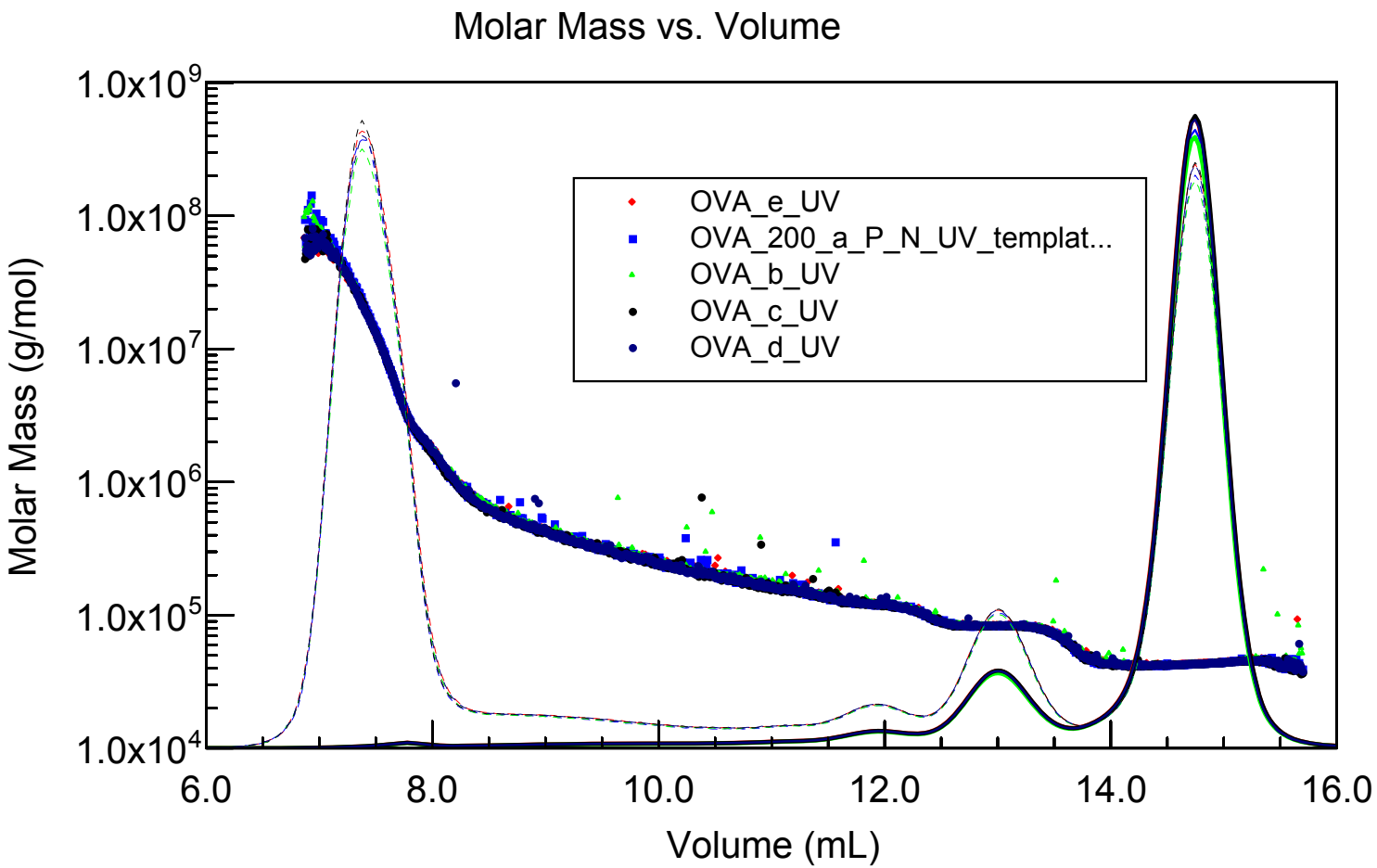
UV at 280 nm

RI

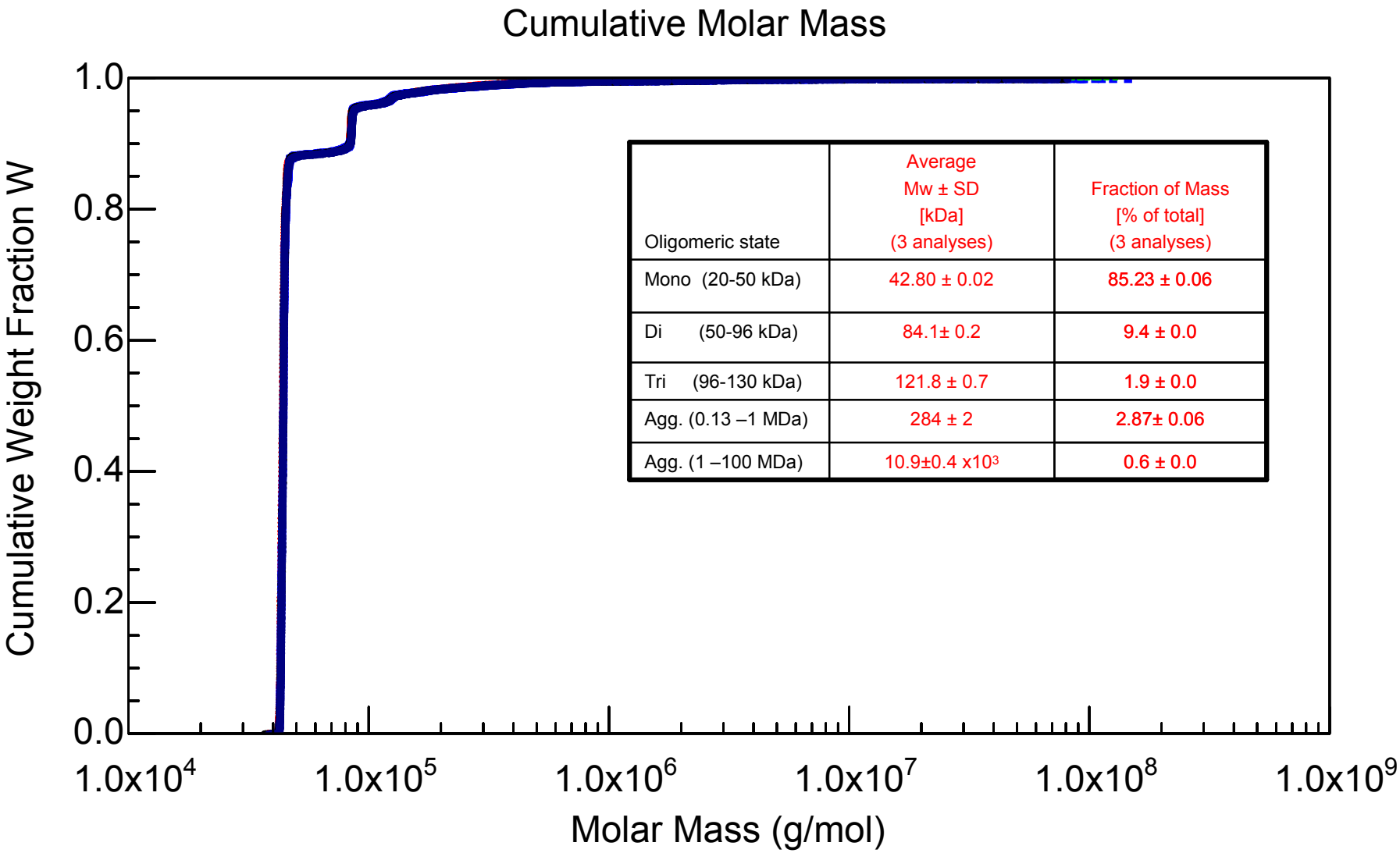
LS at 90°

Molar mass distribution for multiple analyses

Ovalbumin 43 kDa automated template processing of five data sets

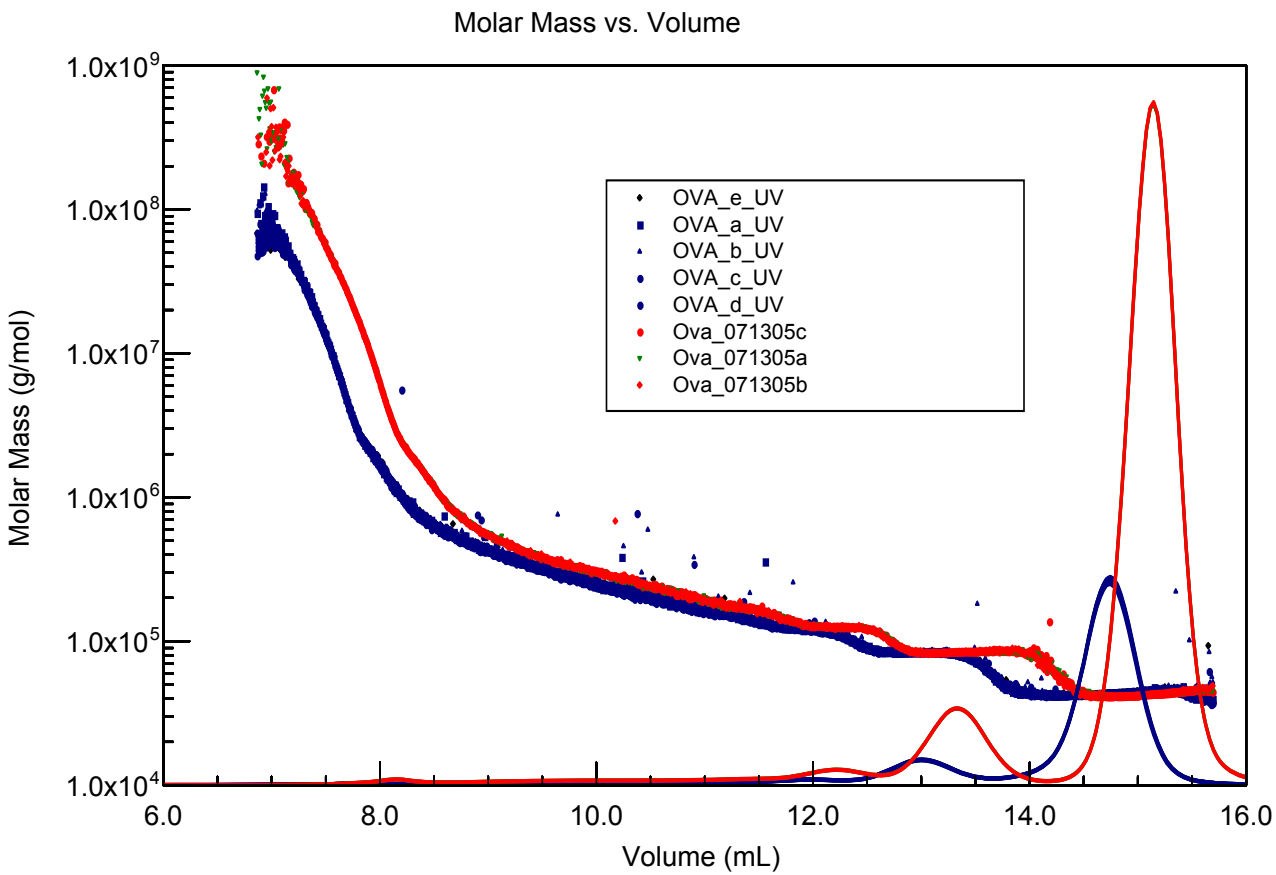


Determination of Weight Fractions



Oligomeric state	Average Mw ± SD [kDa] (3 analyses)	Fraction of Mass [% of total] (3 analyses)
Mono (20-50 kDa)	42.80 ± 0.02	85.23 ± 0.06
Di (50-96 kDa)	84.1± 0.2	9.4 ± 0.0
Tri (96-130 kDa)	121.8 ± 0.7	1.9 ± 0.0
Agg. (0.13 –1 MDa)	284 ± 2	2.87± 0.06
Agg. (1 –100 MDa)	10.9±0.4 x10^3	0.6 ± 0.0

Differences in population based on molar mass distribution



Ovalbumin (5 runs)

Mw = 108 ± 17 kDa

Polydispersity Mw/Mn

2.3 ± 0.4

Ovalbumin (3 runs)

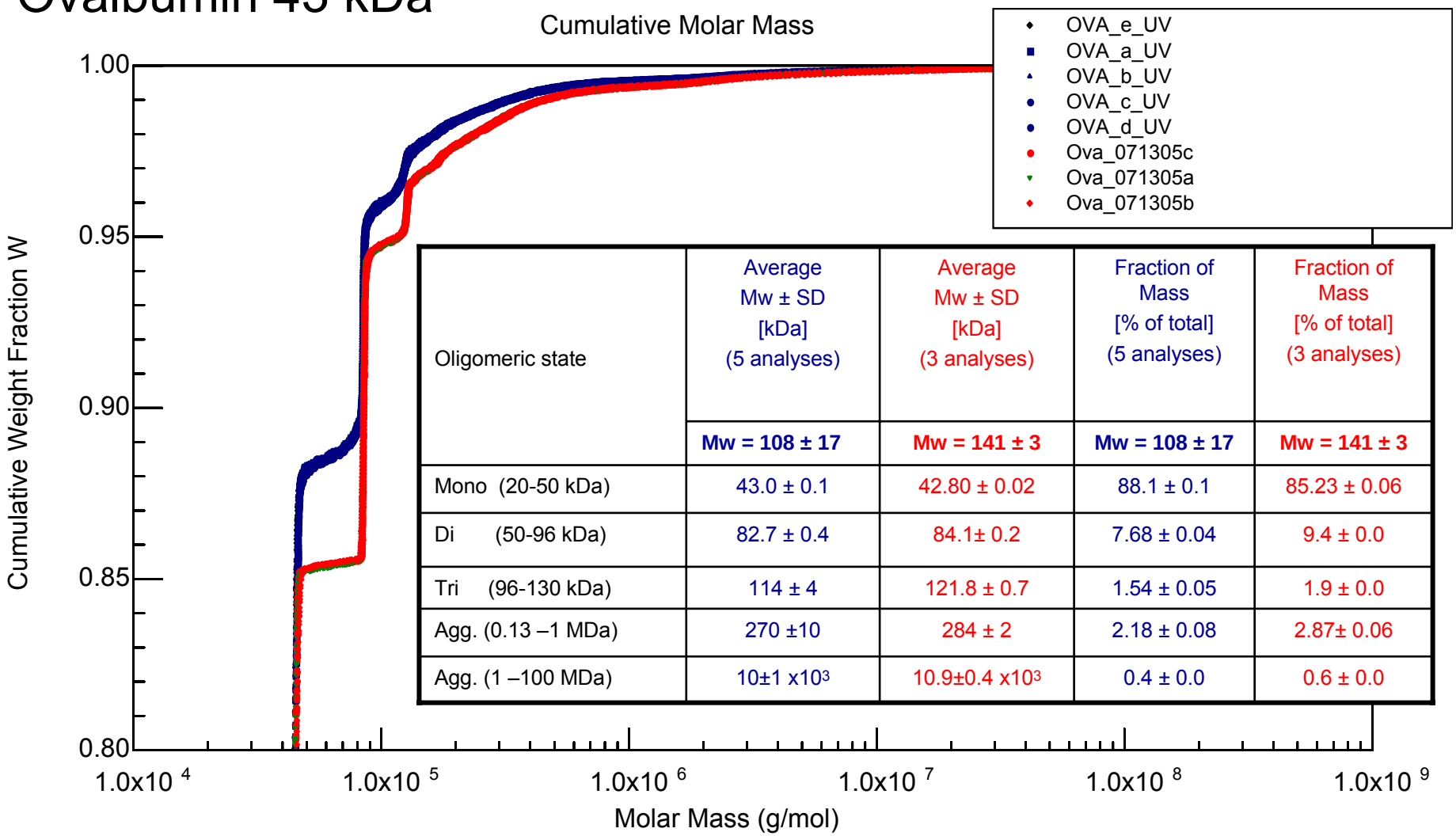
Mw = 141 ± 3 kDa

Polydispersity Mw/Mn

2.92 ± 0.06

Differences in population based on molar mass distribution

Ovalbumin 43 kDa



Oligomeric state	Average Mw ± SD [kDa] (5 analyses)	Average Mw ± SD [kDa] (3 analyses)	Fraction of Mass [% of total] (5 analyses)	Fraction of Mass [% of total] (3 analyses)
	Mw = 108 ± 17	**Mw = 141 ± 3**	**Mw = 108 ± 17**	**Mw = 141 ± 3**
Mono (20-50 kDa)	43.0 ± 0.1	42.80 ± 0.02	88.1 ± 0.1	85.23 ± 0.06
Di (50-96 kDa)	82.7 ± 0.4	84.1± 0.2	7.68 ± 0.04	9.4 ± 0.0
Tri (96-130 kDa)	114 ± 4	121.8 ± 0.7	1.54 ± 0.05	1.9 ± 0.0
Agg. (0.13 –1 MDa)	270 ±10	284 ± 2	2.18 ± 0.08	2.87± 0.06
Agg. (1 –100 MDa)	10±1 x10^3	10.9±0.4 x10^3	0.4 ± 0.0	0.6 ± 0.0

Determination of the oligomeric state of modified proteins from SEC-LS/UV/RI analysis

1. Glycosylated proteins
2. Proteins conjugated with polyethylene glycol
3. Membrane protein present as a complex with lipids and detergents

Input:

- Polypeptide sequence
- *Chemical nature of the modifier*

Results:

- Oligomeric state of the polypeptide
- Extend of modification (grams of modifier /gram of polypeptide)

"three detector method"

Yutaro Hayashi, Hideo Matsui and Toshio Takagi (1989) Methods Enzymol,172:514-28
Jie Wen, Tsutomu Arakawa and John S. Philo (1996) Anal Biochem, 240:155-66
Ewa Folta-Stogniew (2006) Methods in Molecular Biology: New and Emerging Proteomics Techniques, pp. 97–112

Three Detector Method

$$MW_p = \frac{k*(LS)(UV)}{\varepsilon(RI)^2}$$

MW_p	Molecular Weight (polypeptide)
ε	extinction coefficient
LS	light scattering intensity
UV	absorbance (ε)
RI	refractive index change
k	*calibration constant*

Yutaro Hayashi, Hideo Matsui and Toshio Takagi (1989) Methods Enzymol,172:514-28

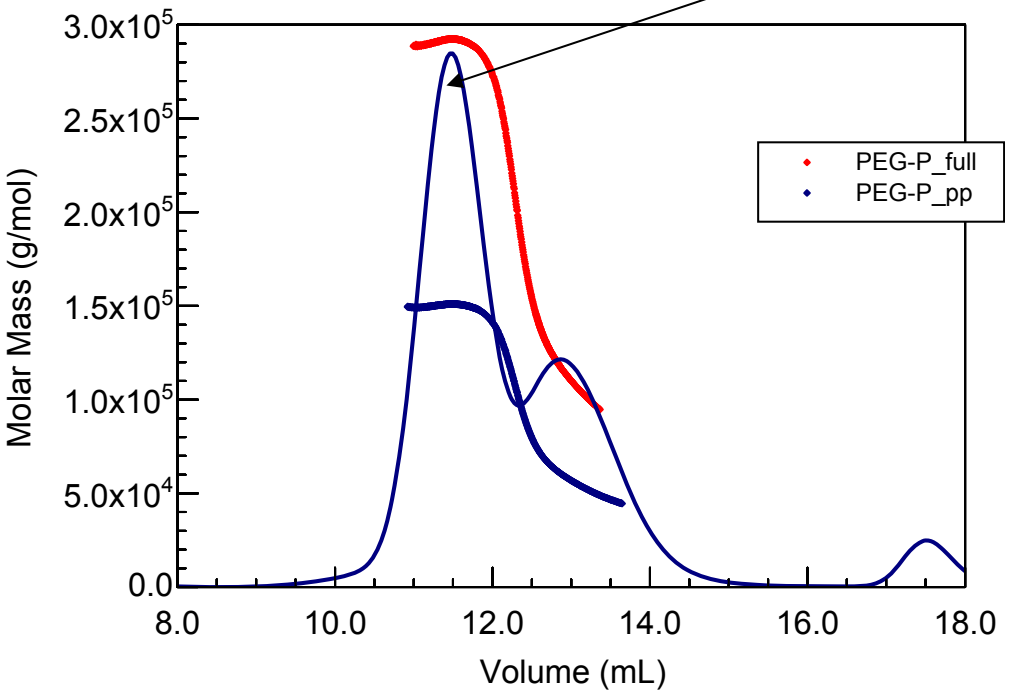
Jie Wen, Tsutomu Arakawa and John S. Philo (1996) Anal Biochem, 240:155-66

Ewa Folta-Stogniew (2006) Methods in Molecular Biology: New and Emerging Proteomics Techniques, pp. 97–112

Modified proteins: PEG-ylated and Glycoproteins

PEG-ylated protein: 75 kDa

36 kDa polypeptide + 39 kDa PEG



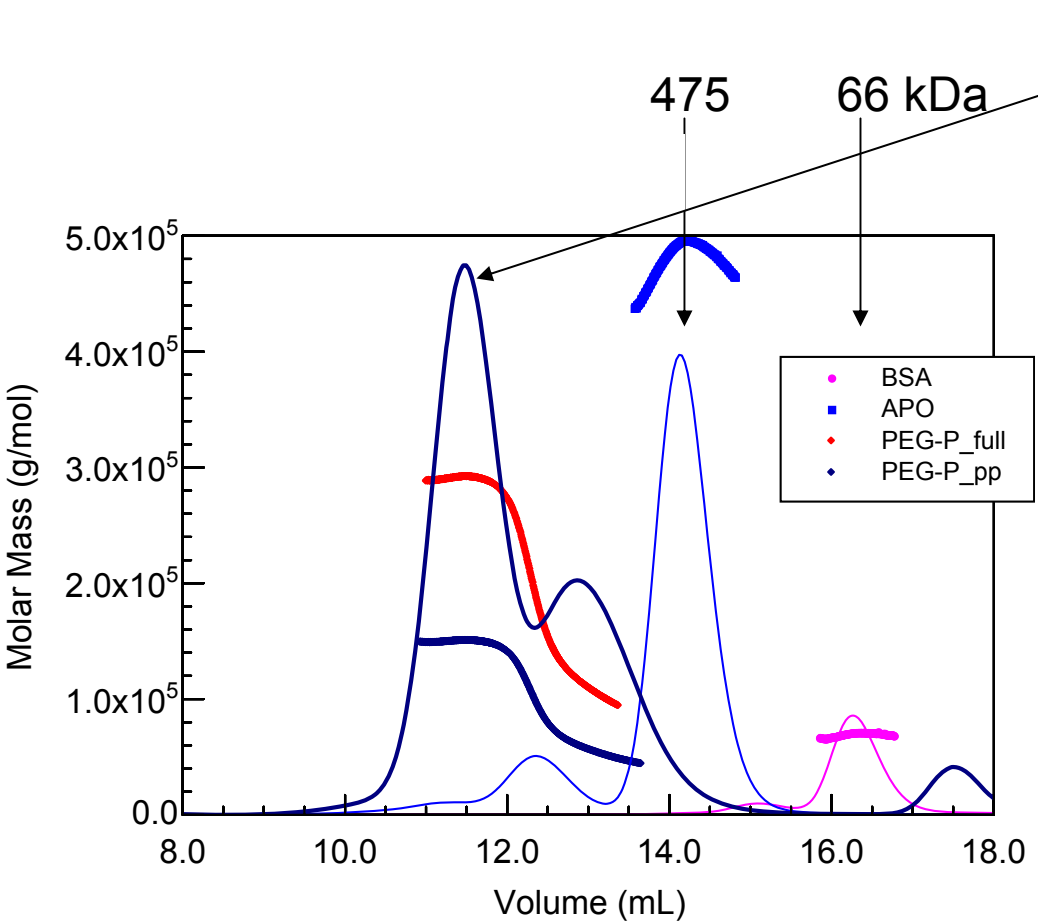
Polypeptide: 146 kDa
(tetramer: 144 kDa)

Full protein: 291 kDa

(tetramer: 300 kDa)

PEG-ylated protein: 75 kDa

36 kDa polypeptide + 39 kDa PEG

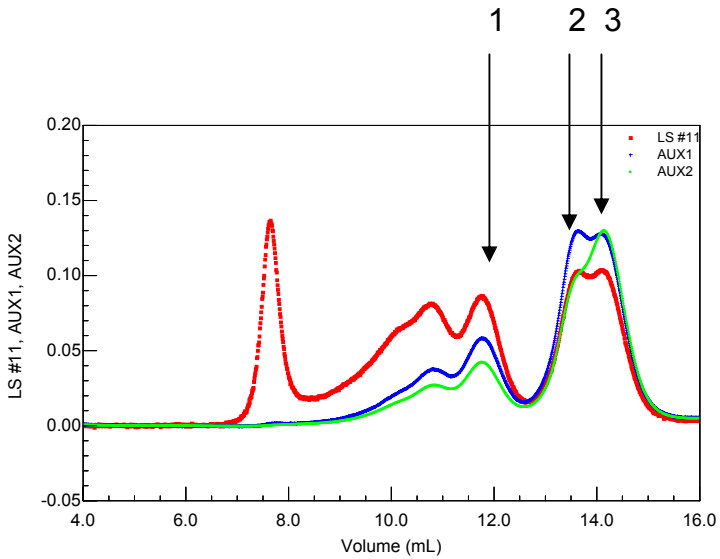


Polypeptide: 146 kDa
(tetramer: 144 kDa)

Full protein: 291 kDa

(tetramer: 300 kDa)

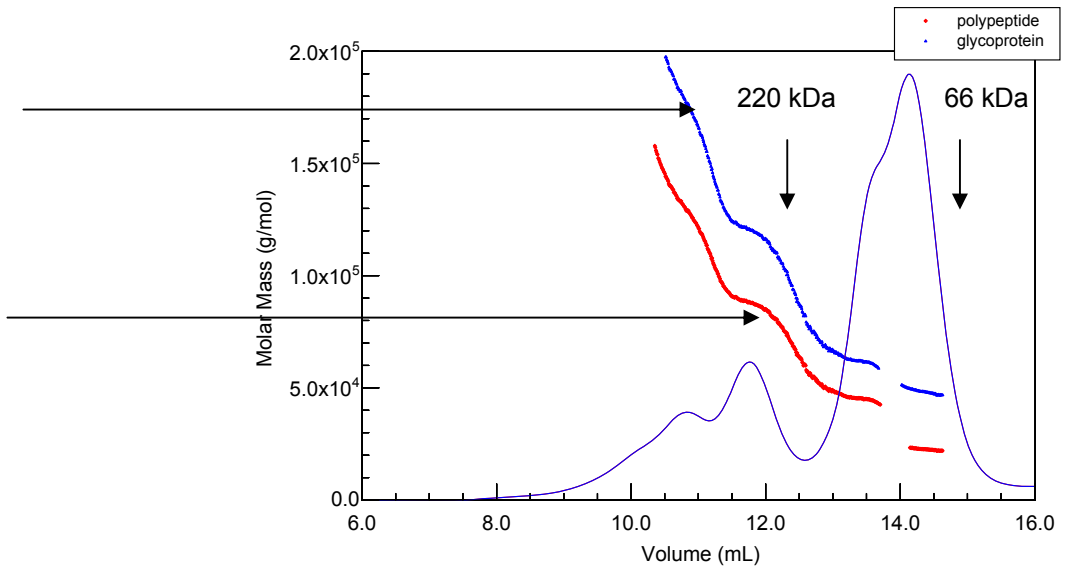
Glycoprotein 44.1 kDa polypeptide; unknown level of glycosylation



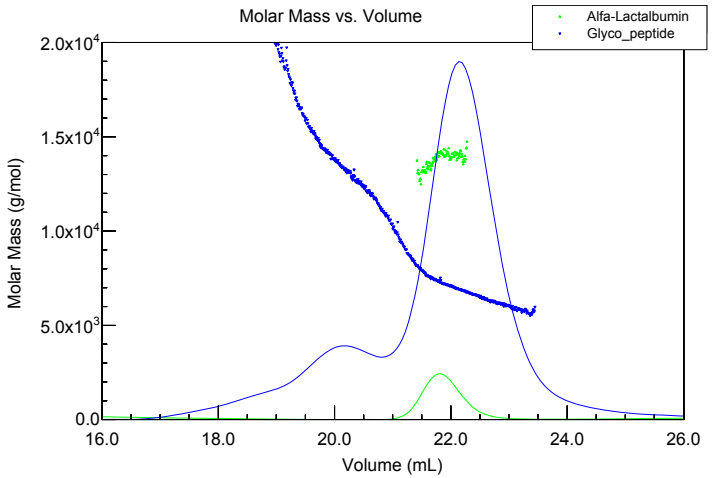
peak	UV/RI	MM_{pp} (kDa)	Grams of sugar/gram of polypeptide	Full Glycoprotein (kDa)
1	0.54	90	0.4	122
2	0.52	45	0.4	63
3	0.37	23	1.1	48

Full glycoprotein

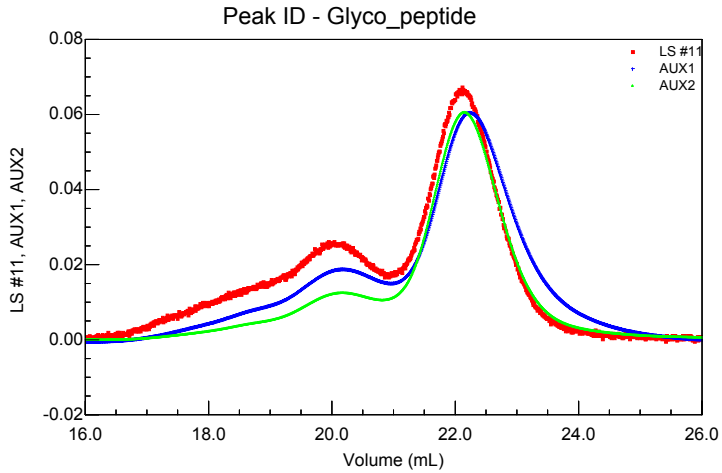
Polypeptide

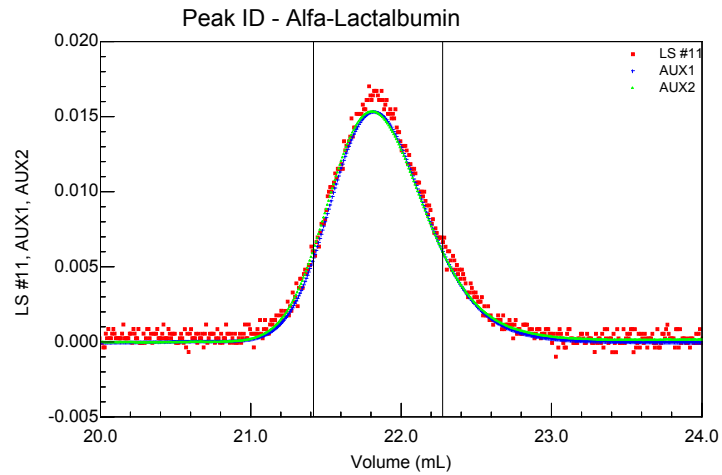


Glycosylated peptide: 9.13 kDa; 350 μg



Alfa-lactalbumin 14 kDa; 20 μg





Alfa-lactalbumin 14 kDa; 20 μg

Determination of the oligomeric state of a complex of glycosylated protein+peptide

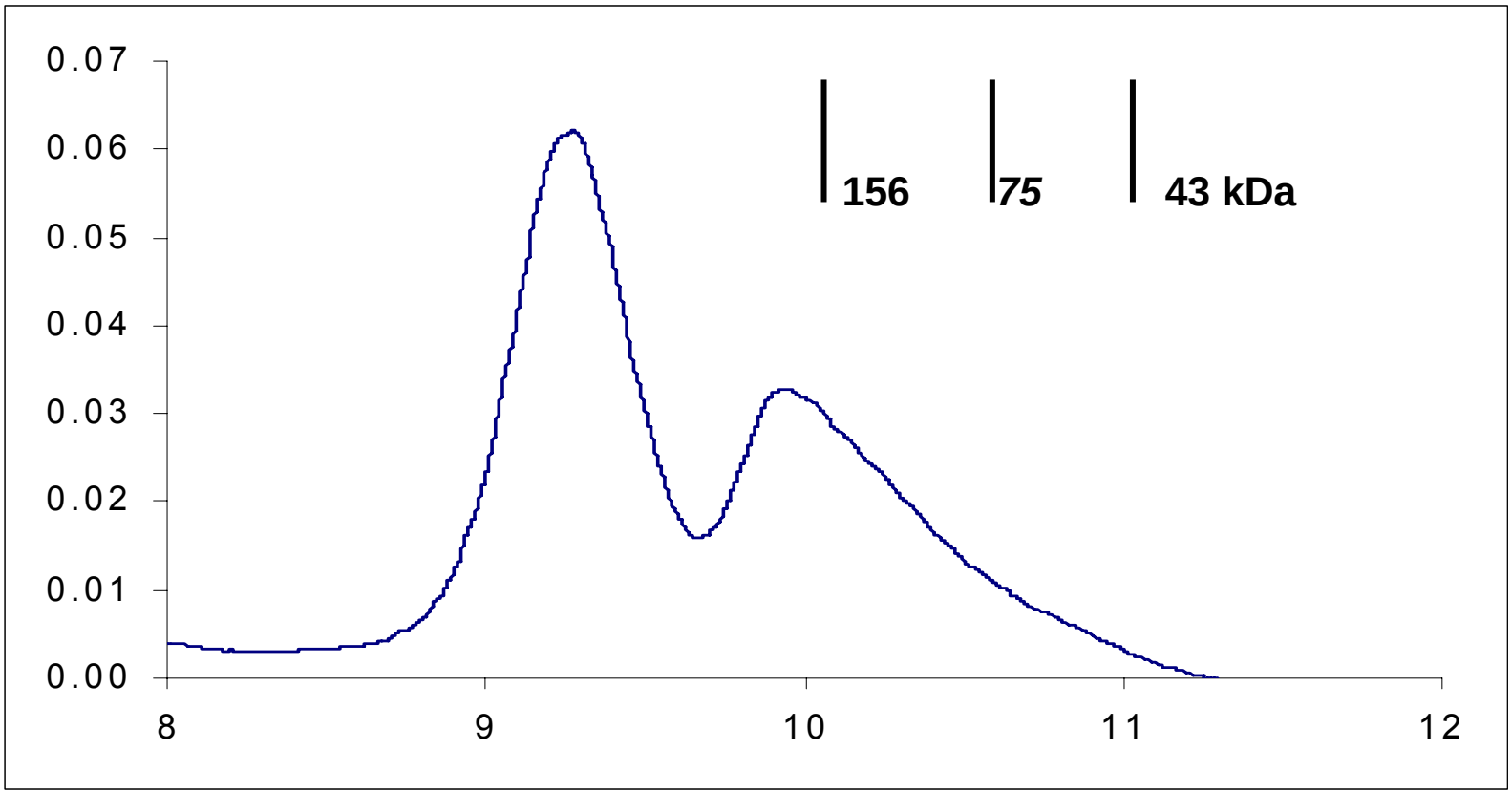
protein 58 kDa extracellular ANP-binding domain (ECD) of cell-surface receptor 16% of mass is sugar

$dn/dc_t = 0.179$ g/mL

48 kDa polypeptide portion

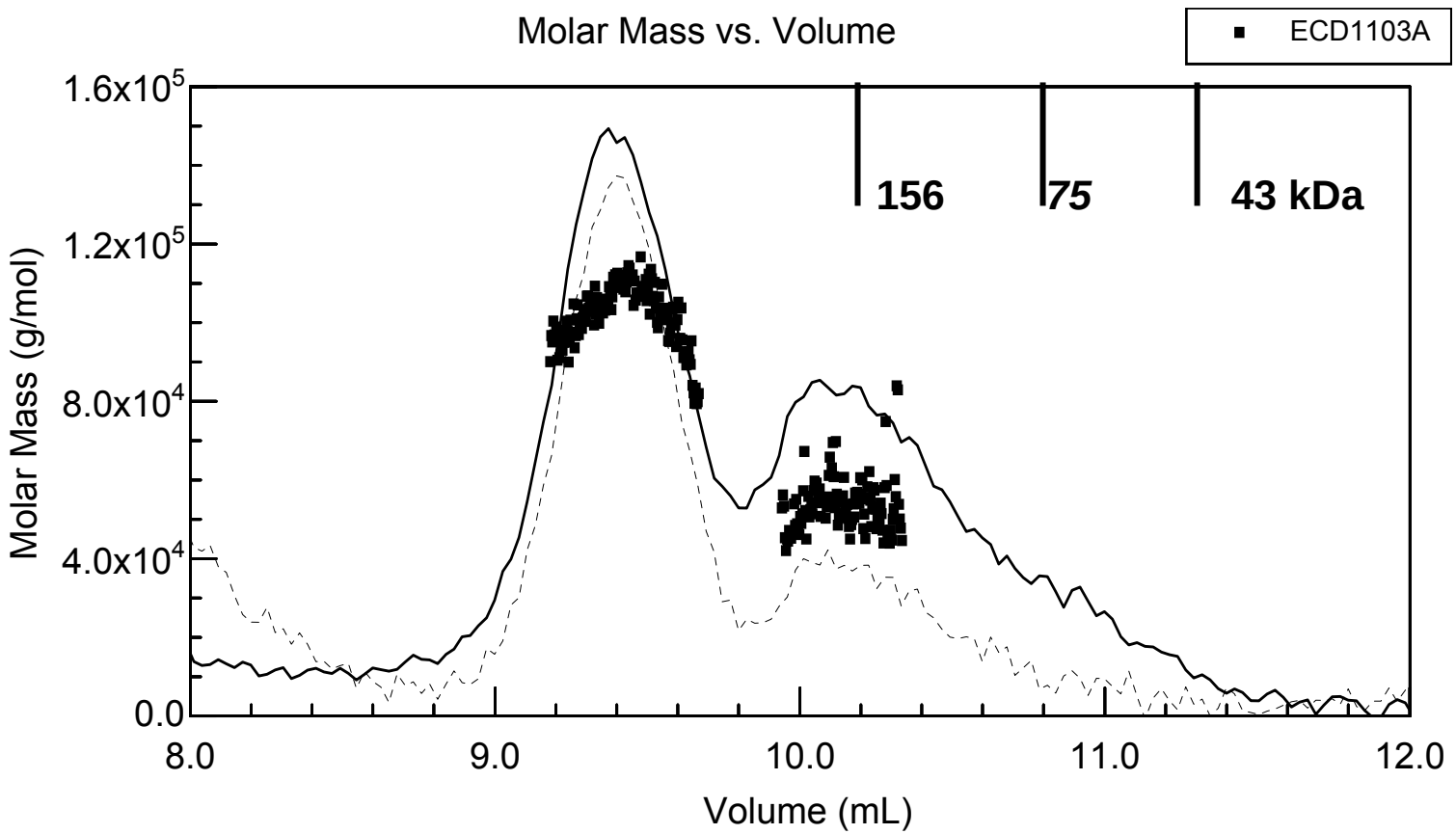
ligand 2.7 kDa atrial natriuretic peptide (ANP)

Injected sample complex (ECD : ANP) 2:1



ECD $_{dimer}$ = 2 x 58 = 116 kDa (polypeptide 96 kDa) ANP = 2.7 kDa

Injected sample complex (ECD : ANP) 2:1



ECD $_{dimer}$ = 2 x 58 = 116 kDa (polypeptide 96 kDa) ANP = 2.7 kDa

ECD-ANP complex; ~3 μg

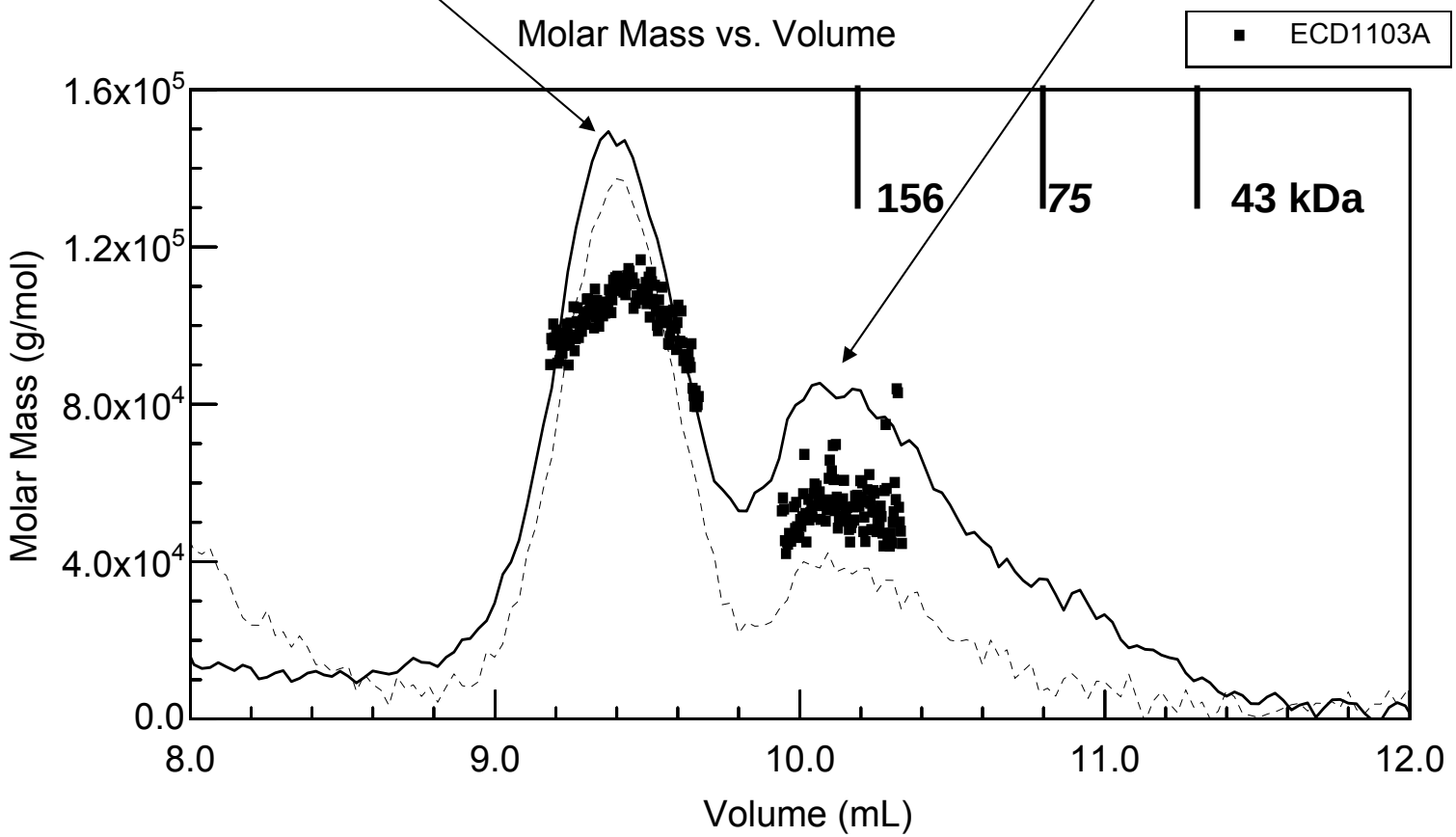
$MW_{glycoprotein}$ = 103 ± 10 kDa

$MW_{polypeptide}$ = 96 ± 7 kDa

ECD; ~2 μg

$MW_{glycoprotein}$ = 54 ± 6 kDa

$MW_{polypeptide}$ = 44 ± 5 kDa



ECD_{dimer} = 2 x 58 = 116 kDa (polypeptide 96 kDa)

ANP = 2.7 kDa

Hydrophobic proteins

Determination of the oligomeric state of detergent solubilized proteins:

polypeptide+lipids+detergent complexes of unknown detergent+lipids content

Proteins: 47 kDa porin **LamB** **trimer = 141 kDa**

33 kDa hemolysin **α-HL** **heptamer = 231 kDa**

detergent

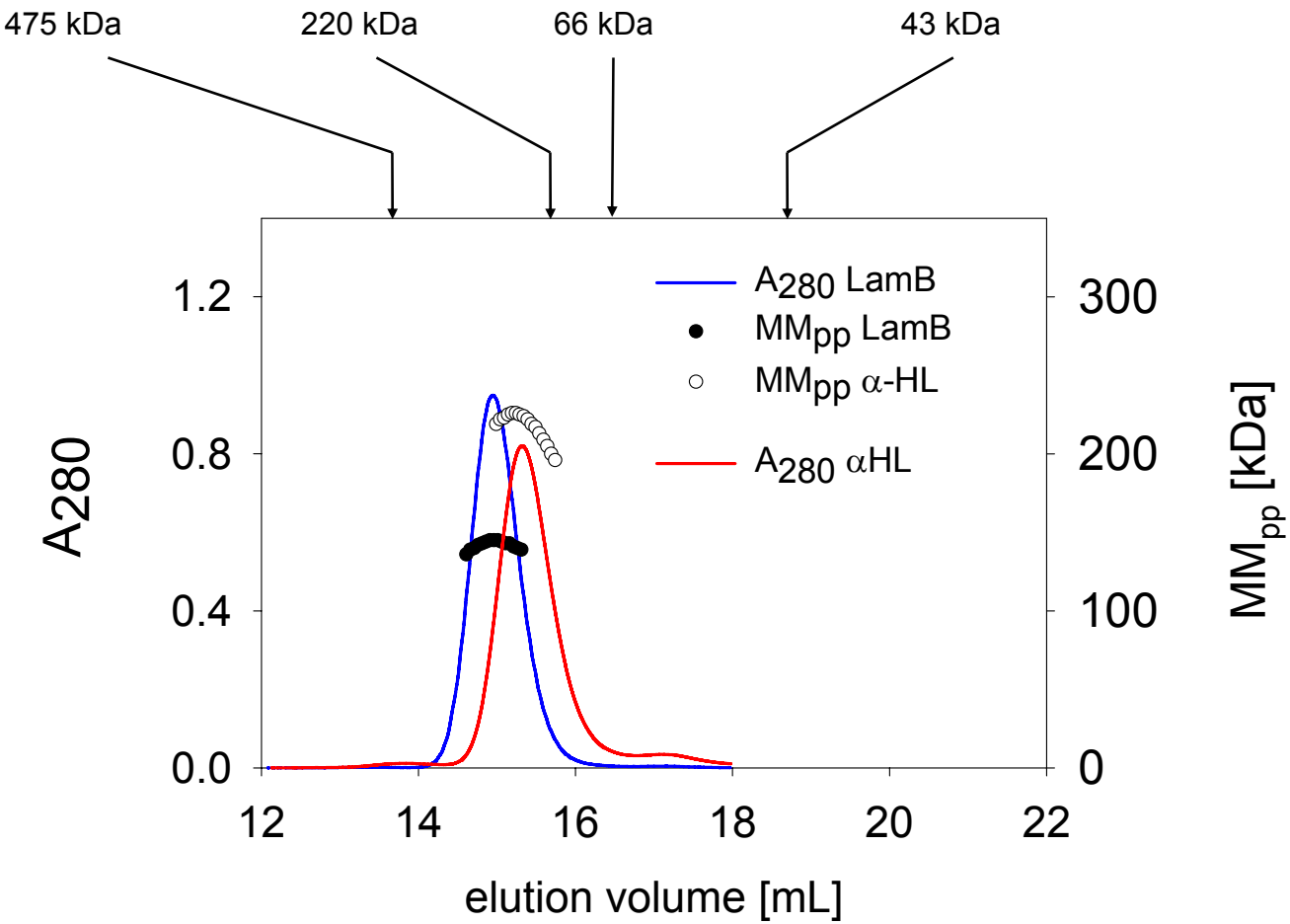
dodecyl maltoside (C12M) MW = 511 g/mol

0.5g/L i.e. 0.05%

CMC = 0.008% micelle size 50-70 kDa

Proteins: 47 kDa porin **LamB** **trimer = 141±3 kDa** (141 kDa)

33 kDa hemolysin **α-HL** **heptamer = 215±20 kDa** (231 kDa)



Three Detector Method

Yutaro Hayashi, Hideo Matsui and Toshio Takagi

Methods Enzymol 1989;172:514-28

allows determination of mass of detergent/lipids bound to a polypeptide

$$\left(\frac{dn}{dc}\right)_{app} = k_2 A \frac{(RI)}{(UV)}$$

$$\left(\frac{dn}{dc}\right)_{app} = \left(\frac{dn}{dc}\right)_{pp} + \delta \left(\frac{dn}{dc}\right)_{d+l} = K' \frac{(RI)}{\varepsilon(UV)}$$

δ is mass of detergent and/or lipids per 1 gram of polypeptide

Assumption : detergent does not produce any signal in UV

$MW_{complex}$ = 285 kDa

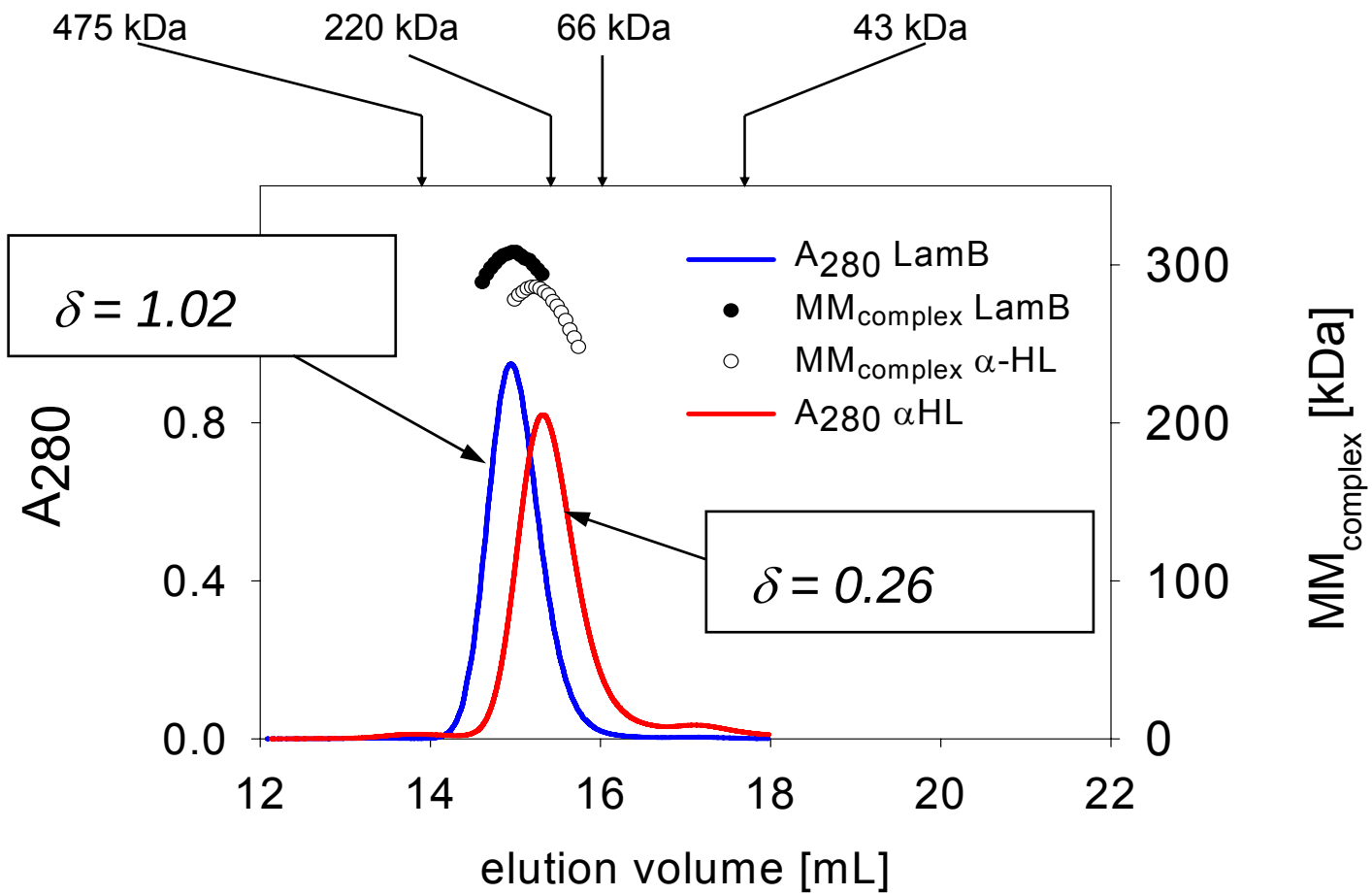
$MW_{polypeptide}$ = 141 kDa

δ = 1.02 lipids per 1 gram of polypeptide

$MW_{complex}$ = 271 kDa

$MW_{polypeptide}$ = 215 kDa

δ = 0.26 lipids per 1 gram of polypeptide



Yernool D, Boudker O., Folta-Stogniew E., and Gouaux E. (2003) Trimeric subunit stoichiometry of the glutamate transporters from Bacillus caldotenax and Bacillus stearothermophilus. *Biochemistry* **42**: 12981-12988

Determination of dimerization constant from SEC-LS measurements

SecA protein

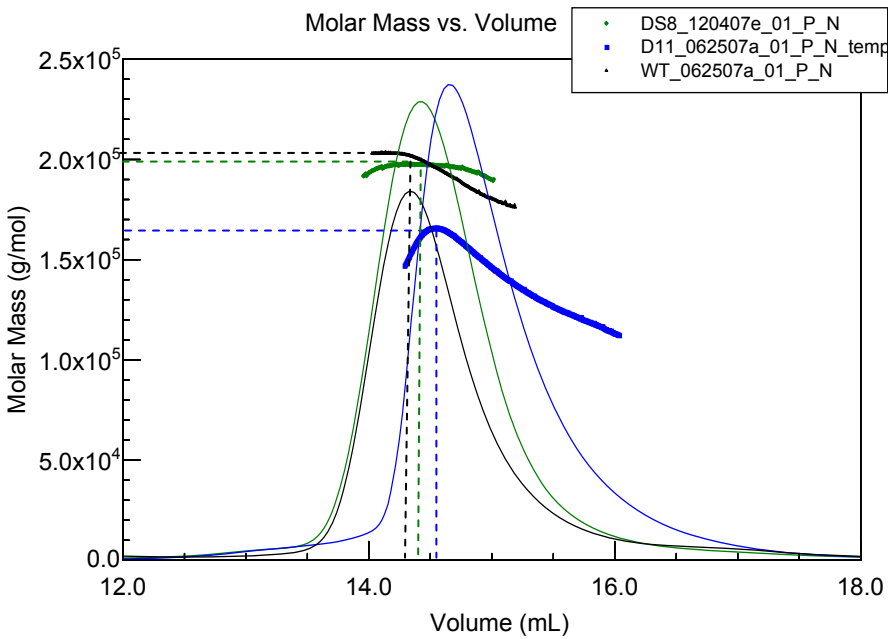
WT	monomer = 102 kDa
DS8 deletion mutant	monomer = 101 kDa
D11 deletion mutant	monomer = 100 kDa

SecA protein

WT	102 kDa
DS8 deletion mutant	101 kDa
D11 deletion mutant	100 kDa

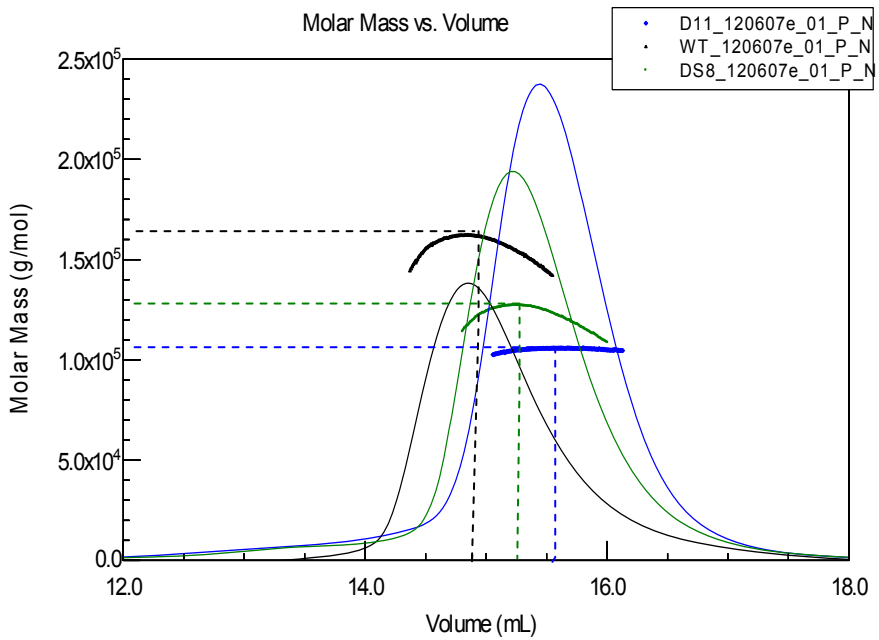
Low salt buffer:

10 mM Tris pH 7.5, 5 mM Mg2+, 100 mM KCl



High salt buffer:

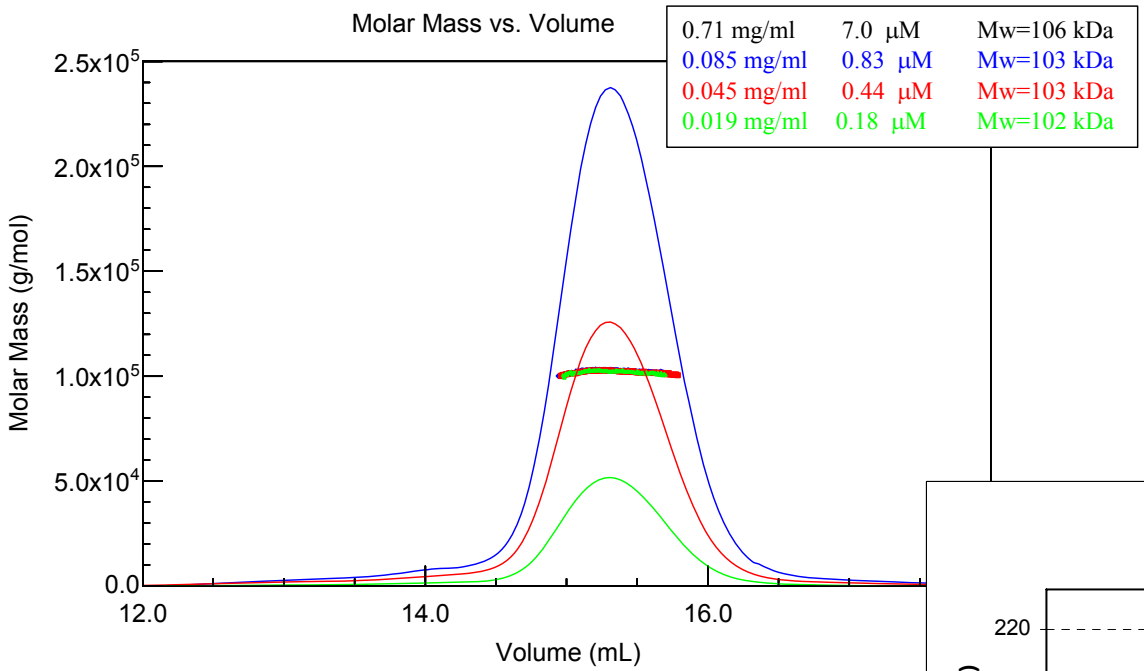
10 mM Tris pH 7.5, 5 mM Mg2+, 300 mM KCl

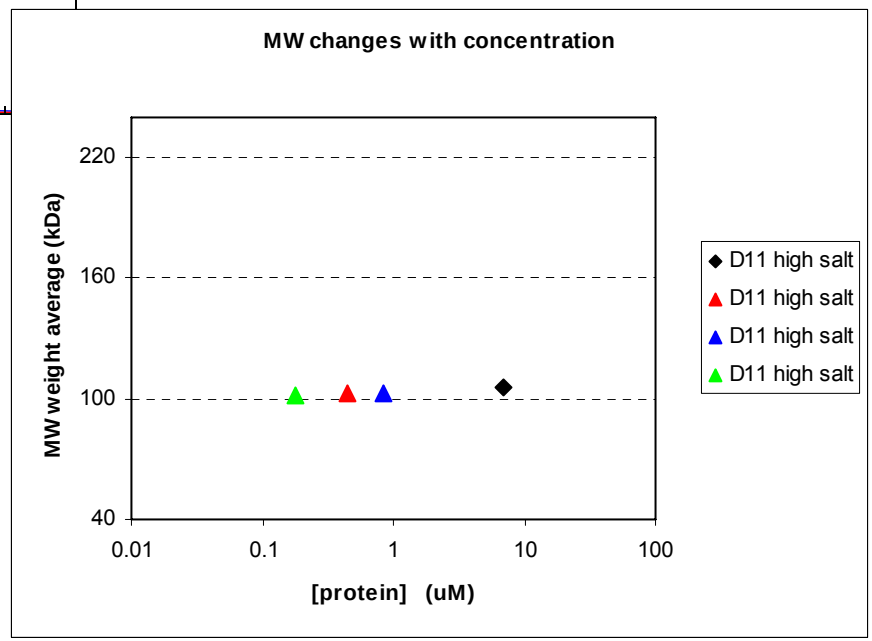


D11 deletion mutant mono= **101 kDa**

High salt buffer:

10 mM Tris pH 7.5, 5 mM Mg2+, 300 mM KCl,

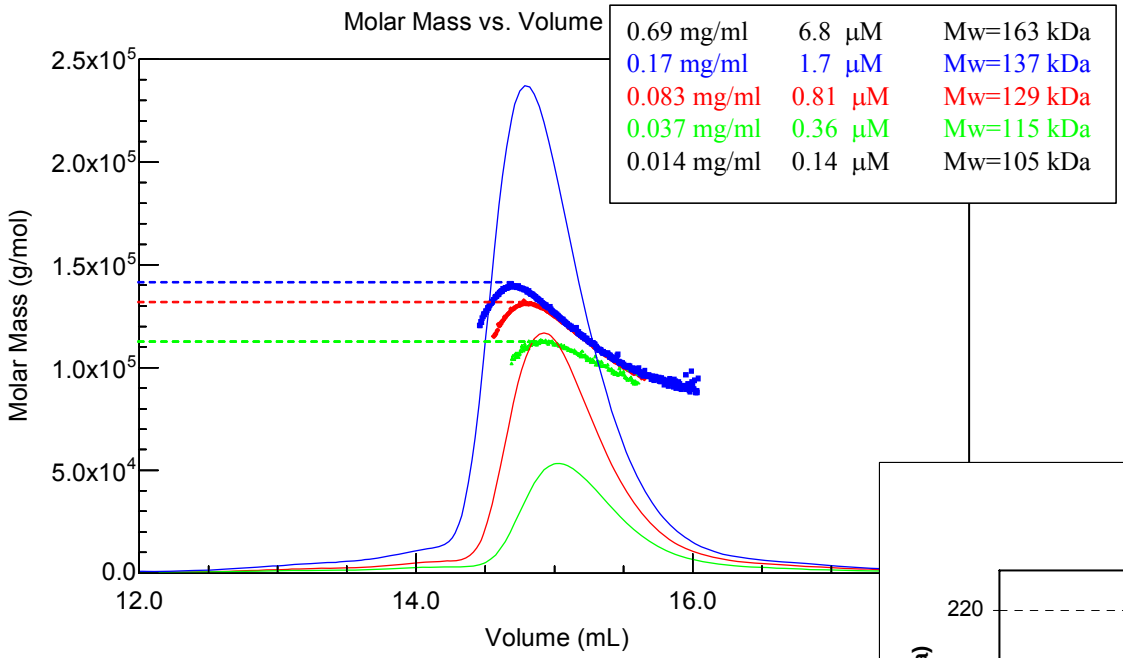


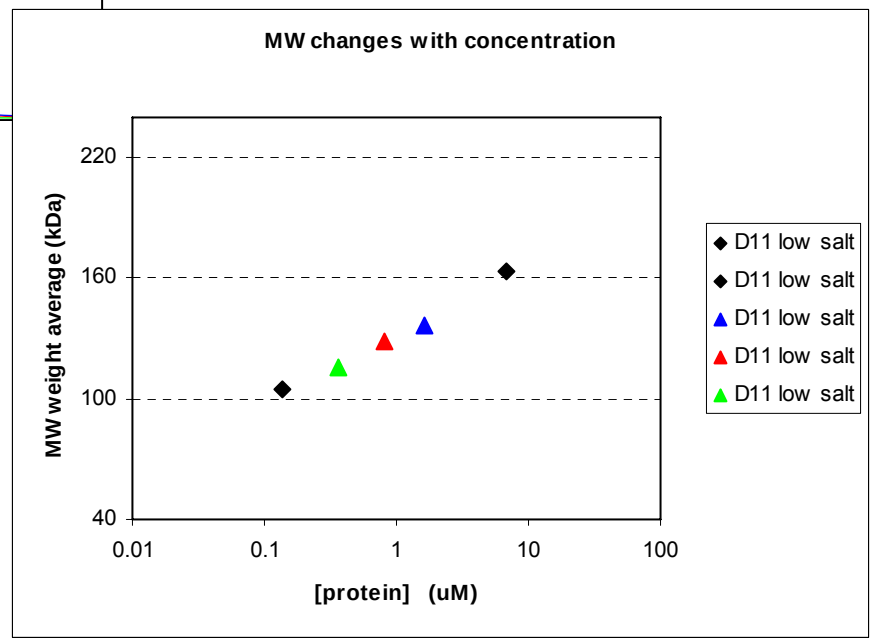


D11 deletion mutant mono= **101 kDa**

Low salt buffer:

10 mM Tris pH 7.5, 5 mM Mg2+, 100 mM KCl,





$$M_w = f_m M_m + f_d M_d = M_m(2 - f_m)$$

$$2M = D$$

$$K_a = \frac{[D]}{[M]^2} = \frac{(1-f_m)}{2(f_m)^2 c_t}$$

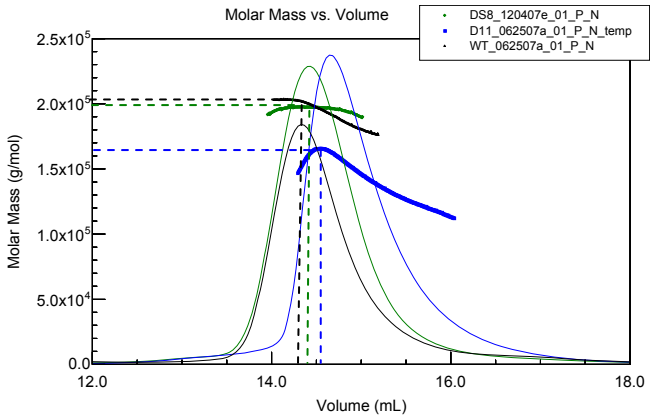
$$f_m = \frac{-1+\sqrt{1+8K_a c_t}}{4K_a c_t}$$

WT monomer = 102 kDa

DS8 deletion mutant monomer = 101 kDa

D11 deletion mutant monomer = 100 kDa

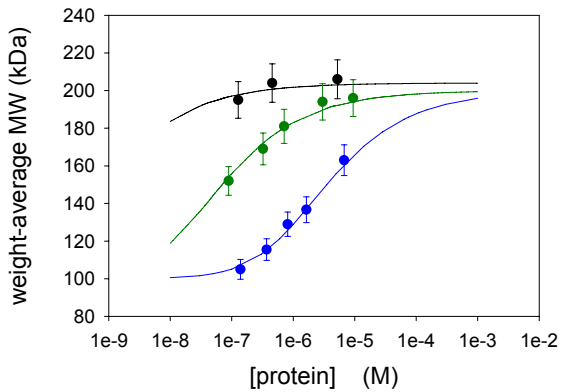
Low salt buffer: 100 mM KCl



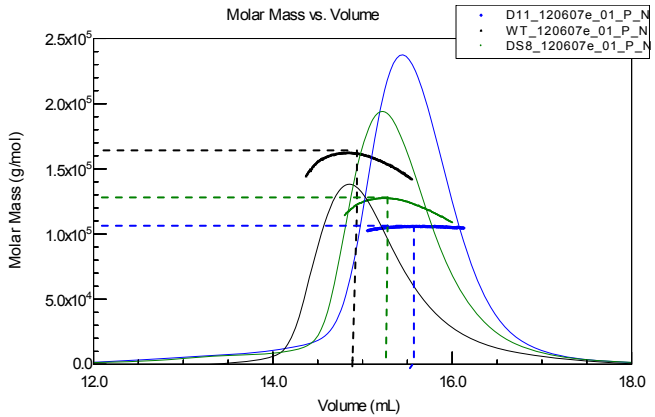
WT Kd= <1e-9

DS8 Kd=7±1e-8 M

D11 Kd=3.5±0.2e-6 M



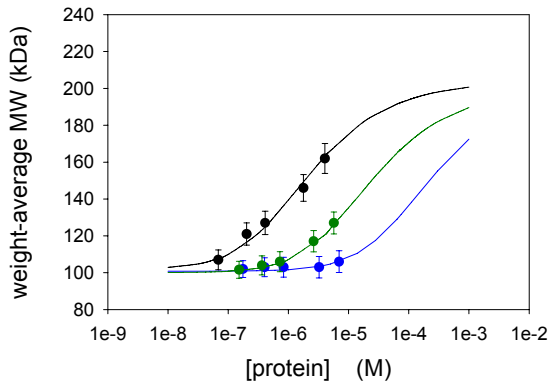
High salt buffer: 300 mM KCl



WT Kd= 2.2±0.2e-6 M

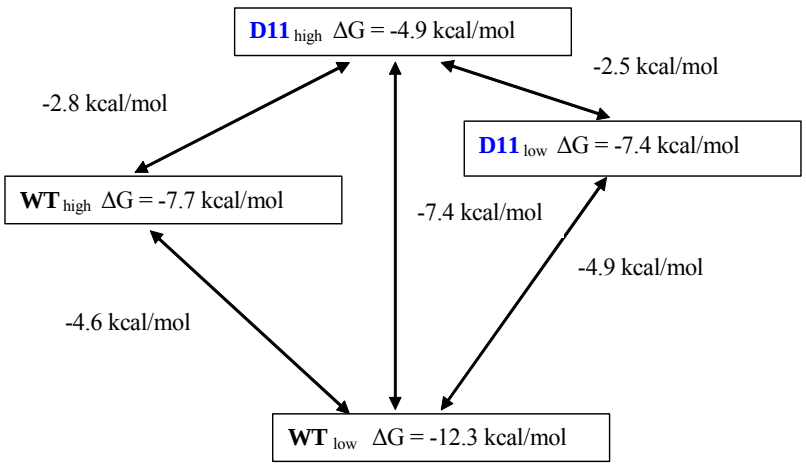
DS8 Kd= 2.41±0.05e-5 M

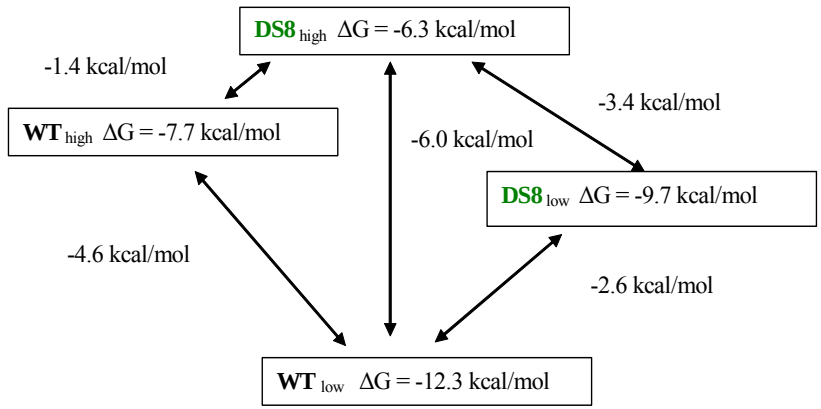
D11 Kd> 2.4e-4 M



Thermodynamic linkage for SecA dimerization

Protein	Low Salt 100 mM KCl		High Salt 300 mM KCl	
	Kd [M]	ΔG dimer (kcal/mol)	Kd [M]	ΔG dimer (kcal/mol)
WT	$<1\times10^{-9}$	-12.3	$2.2\pm0.2\times10^{-6}$	-7.7
DS8	$7\pm1\times10^{-8}$	-9.7	$2.41\pm0.05\times10^{-5}$	-6.3
D11	$3.5\pm0.2\times10^{-6}$	-7.4	$>2.4\times10^{-4}$	-4.9





Capabilities

Static LS

- fast and accurate determination of molar masses (weight average)
  - glycosylated protein, conjugated with PEG, protein-lipids-detergent complexes, protein-nucleic acid complexes
- accuracy of ± 5% in molar mass determination
- easy to implement, fully automated (data collection and data analysis)
- highly reproducible (no operator's bias)
- SEC/MALS excellent in detecting and quantifying population with various oligomeric state in protein
- excellent approach for determination of oligomeric state of modified proteins and peptides
- can be used to determine association constant (concentration gradient measurements)

Limitations

Static LS

- measures *weight average* molar mass – needs fractionation to resolve different oligomeric states
- possible losses of sample during filtration and fractionation
- limitation on solvent choices (related to a fractionation step)
- SEC/MALS dilution during experiment

Ken Williams
Director of W.M. Keck Biotechnology Resource Laboratory at Yale
University School of Medicine

NIH

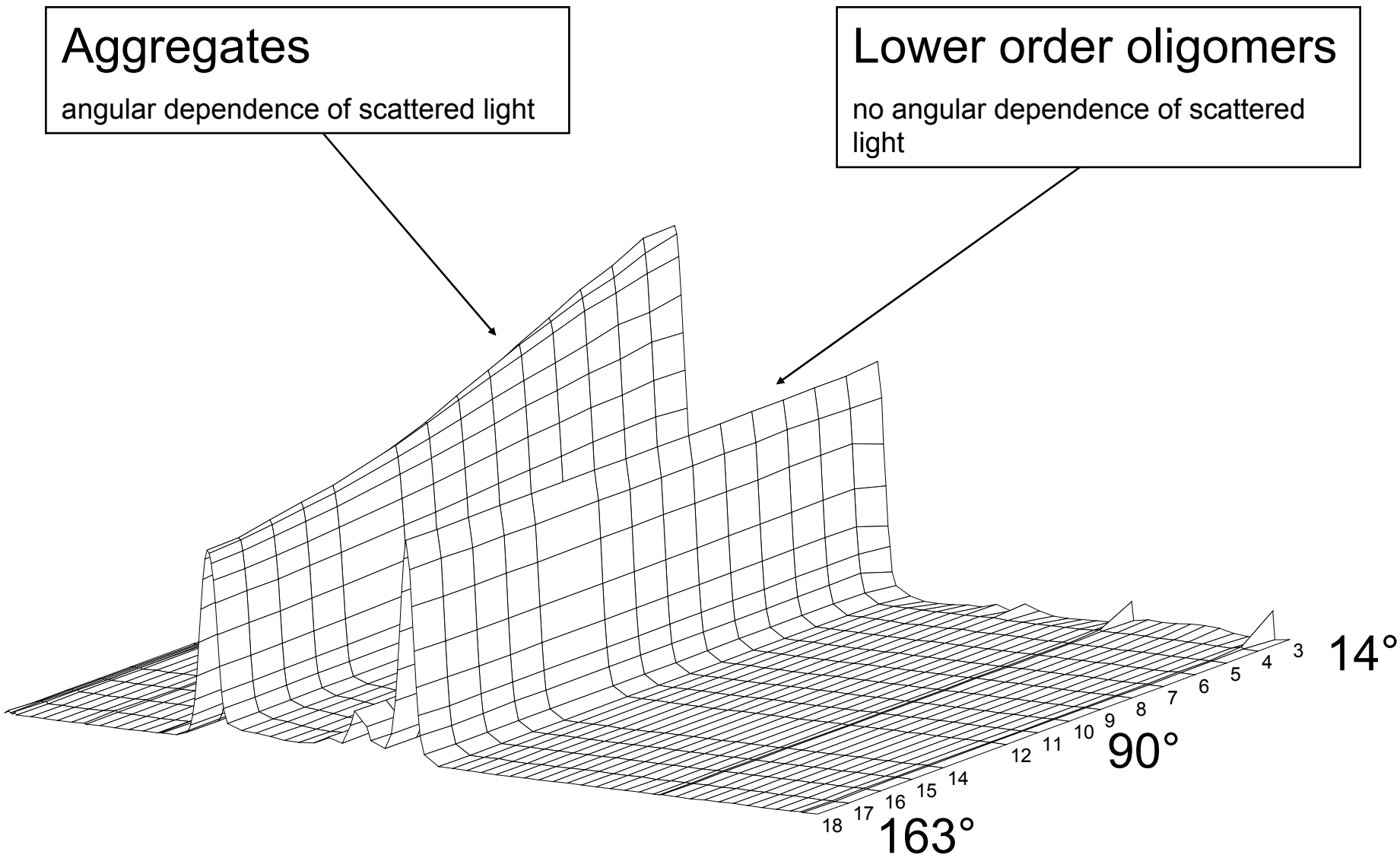
Users of SEC/LS Service

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

Ewa.Folta-Stogniew@yale.edu

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

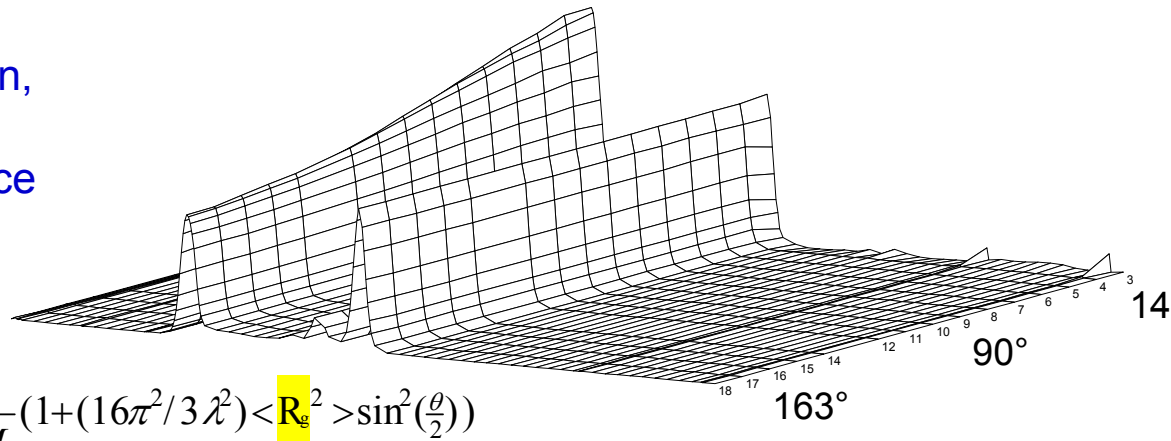
Ovalbumin 43 kDa



Morphology of aggregates from angular dependence of LS signal;

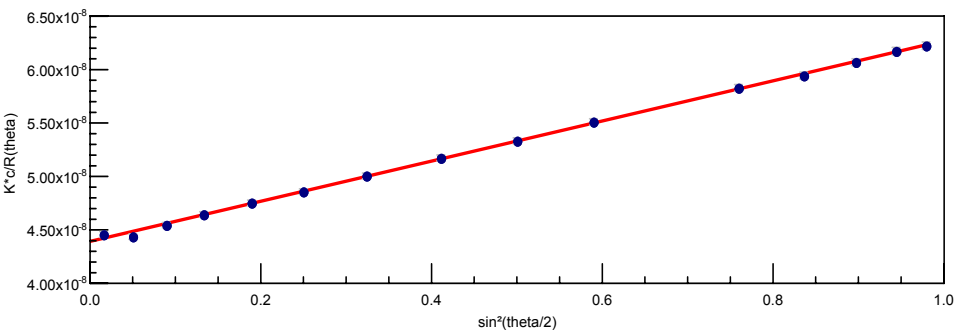
size determination- Rg

Determination of radius of gyration, R_g, (root mean square radius, R.M.S.,) from angular dependence of scattered light



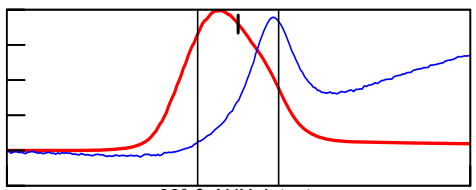
Zimm Plot

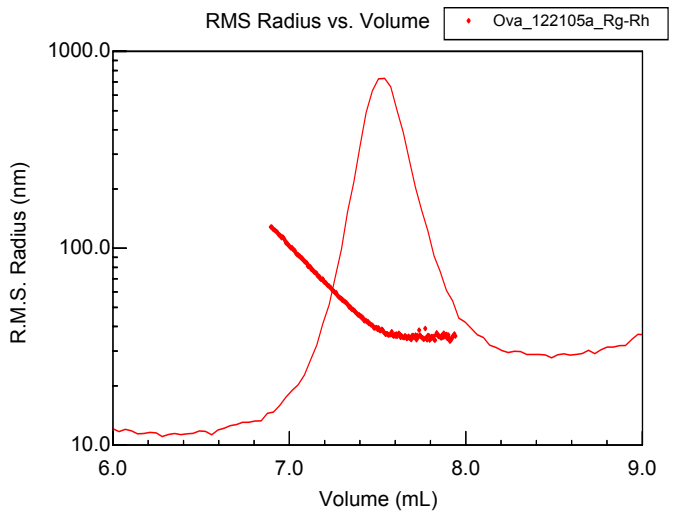
$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w}\left(1+\left(16\pi^2/3\lambda^2\right)\langle R_g^2\rangle \sin^2\left(\frac{\theta}{2}\right)\right)$$



Peak, Slice : 1, 944
Volume : 7.867 mL
Fit degree : 1
Conc. : (1.915 ± 0.020)e-6 g/mL
Mw : (2.277 ± 0.024)e+7 g/mol

Radius: 46.8±0.2 nm





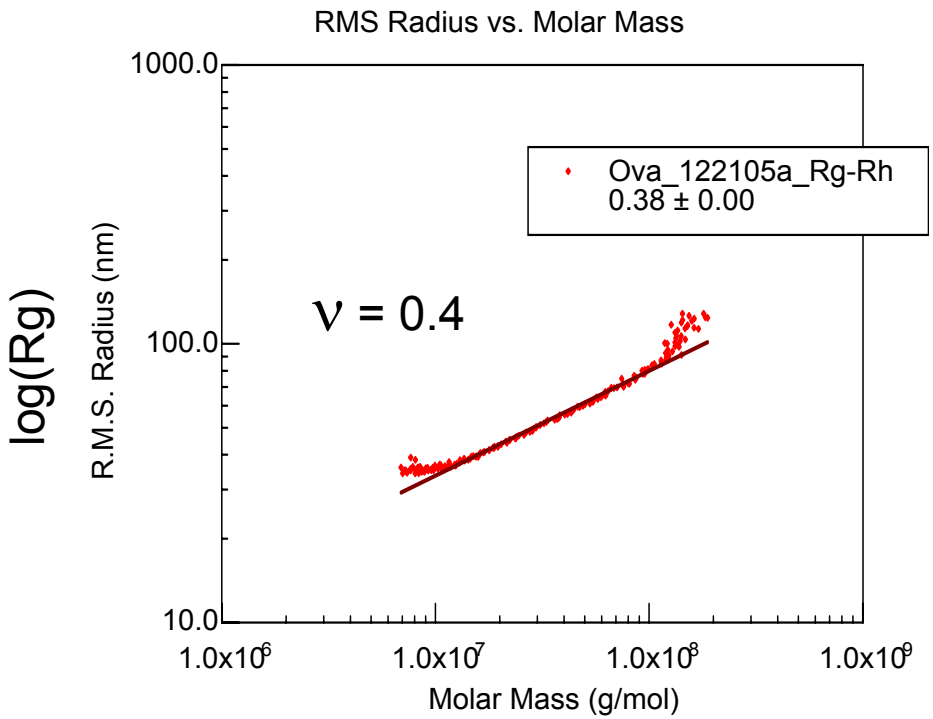
Inferring conformational information from the relationship between molecular size (Rg) and molecular weight (Molar Mass)

$$R_g \sim M^{\nu}$$

$\log(R_g)$ *versus* log(MM)

Slope = ν

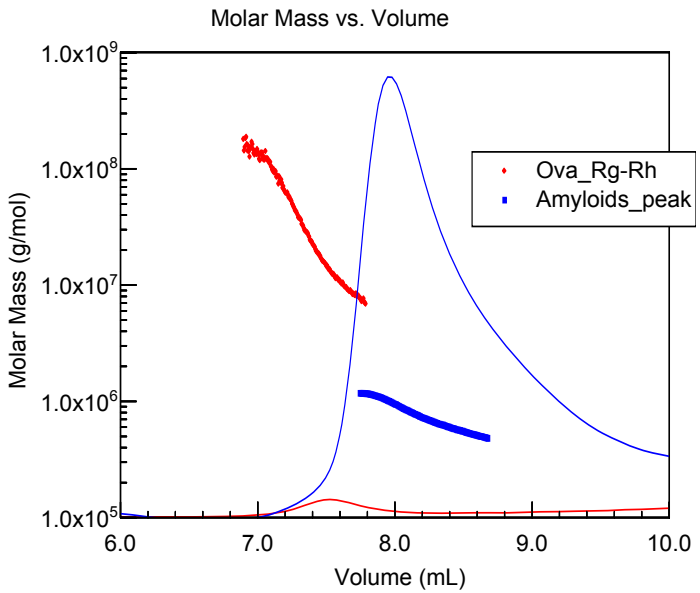
For	ν
Sphere	0.33
Coil	0.5
Rod	1

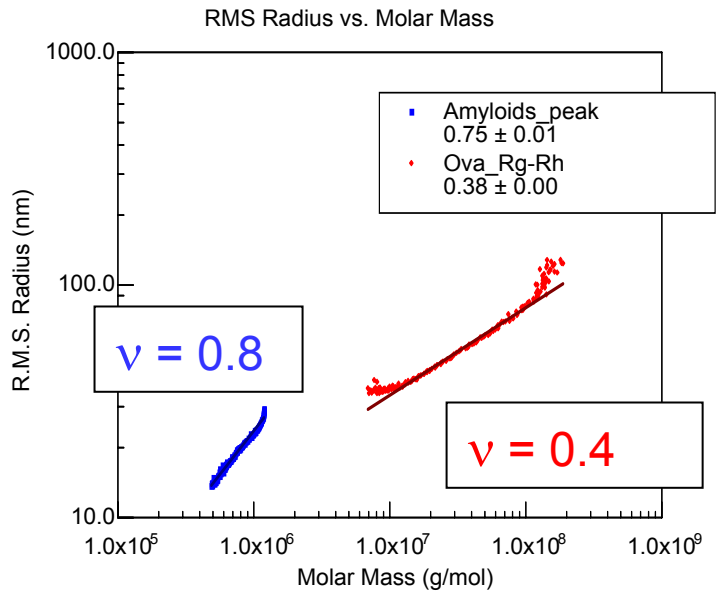


Rollings, J.E. (1992) in *"Laser Light Scattering in Biochemistry"*, Eds. S.E. Harding, D. B. Sattelle and V. A. Bloomfield; p. 275-293

Shape analysis: **log(Rg) *versus* log(MM)**

Aggregates of Ovalbumin *vs.* "amyloid-type" fibers





For	ν
Sphere	0.33
Coil	0.5
Rod	1

Ova_aggr ν = 0.4 Sphere/Coil

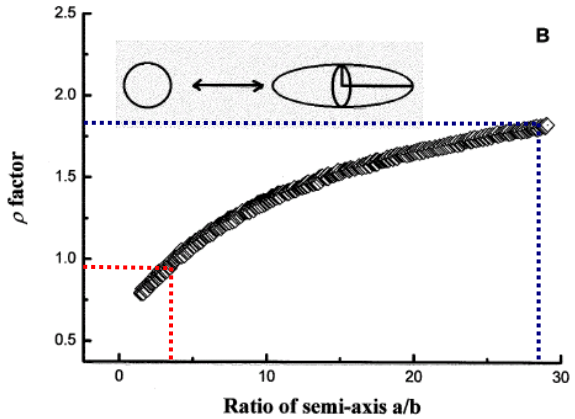
Amyloids ν = 0.8 Coil/Rod

Shape analysis: **shape factor $\rho = R_g/R_h$**

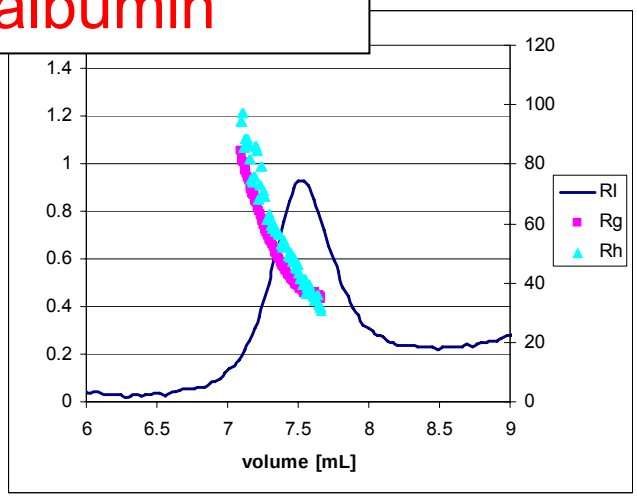
Aggregates of Ovalbumin *vs.* amyloid fibers

Shape factor: $\rho = R_g/R_h$

Combination of MALS (R_g) and DLS (R_h)

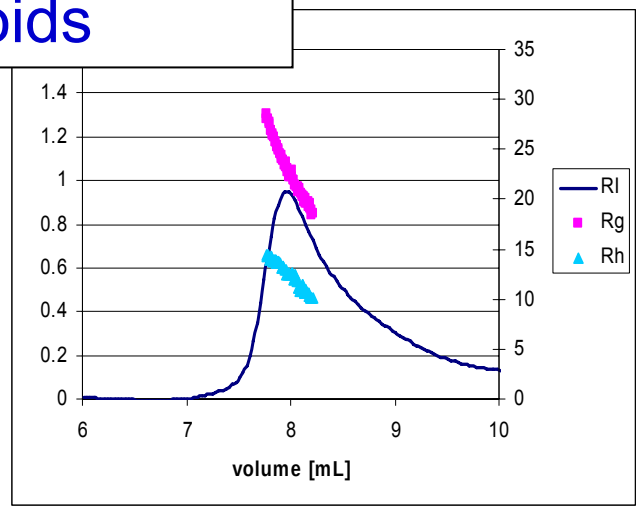


Ovalbumin



$R_g/R_h = 0.91$

Amyloids



$R_g/R_h = 1.84$

[B] Rizos, A. K., Spandidos, D. A., and Krambovitis, E., (2003) *Int. J. Med.,* 12, 559-563

Shape analysis: **shape factor $\rho = R_g/R_h$**

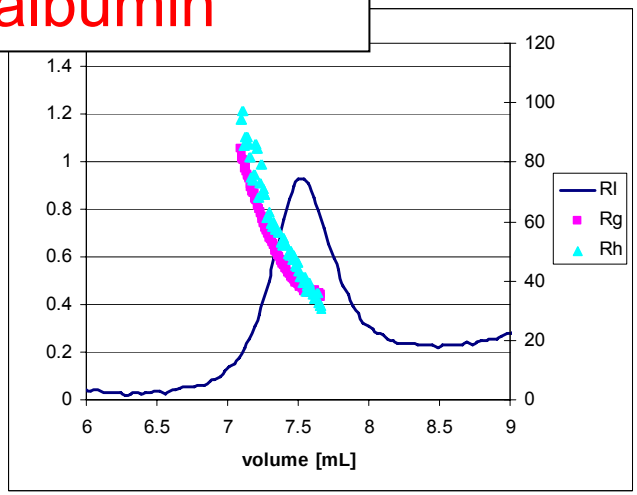
Aggregates of Ovalbumin *vs.* amyloid fibers

Shape factor: $\rho = R_g/R_h$

Combination of MALS (R_g) and DLS (R_h)

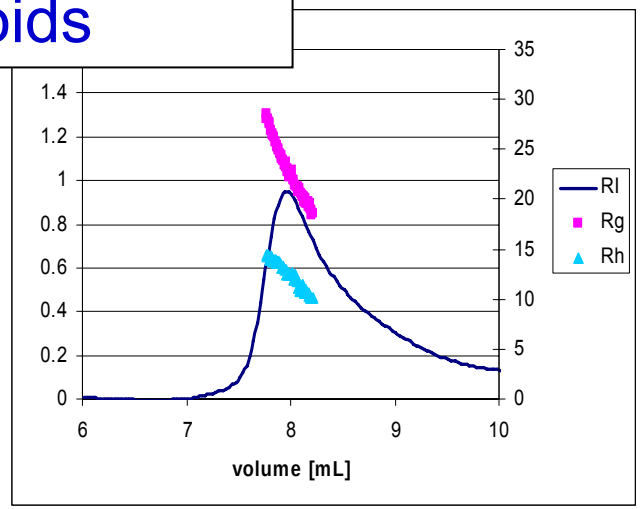
For	$\rho = R_g/R_h$
Sphere	0.774
Coil	0.816
Rod	1.732

Ovalbumin



$R_g/R_h = 0.91$ Coil

Amyloids



$R_g/R_h = 1.84$ Rod

[B] Rizos, A. K., Spandidos, D. A., and Krambovitis, E., (2003) *Int. J. Med.,* 12, 559-563

Shape analysis:

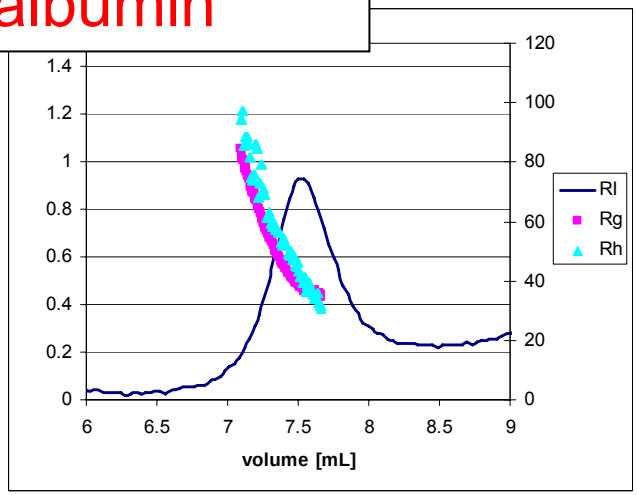
$R_g \sim M^{\nu}$

$\rho = R_g/R_h$

For	ν
Sphere	0.33
Coil	0.5
Rod	1

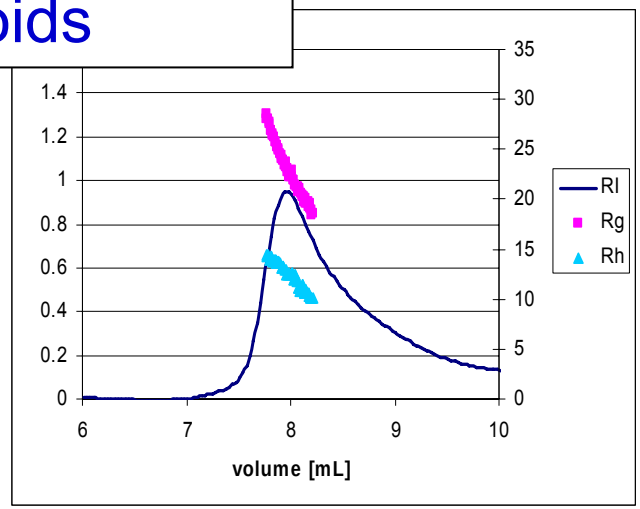
For	$\rho = R_g/R_h$
Sphere	0.774
Coil	0.816
Rod	1.732

Ovalbumin



$R_g/R_h = 0.91$ Coil

Amyloids



$R_g/R_h = 1.84$ Rod

Ova_aggr $\nu = 0.4$ Sphere/Coil

Amyloids $\nu = 0.8$ Coil/Rod

Shape analysis:

shape factor $\rho = R_g/R_h$

$\rho = R_g/R_h = 1.84$ Rod

$\log(R_g)$ *versus* log(MM) Slope = ν

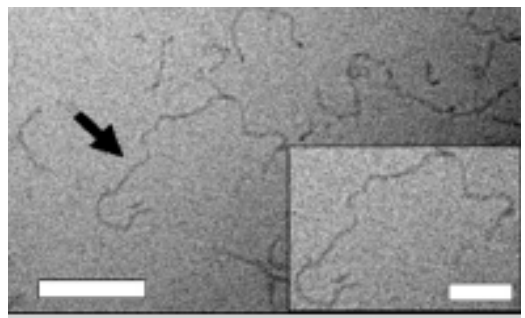
Amyloids $\nu = 0.8$ Coil/Rod

TABLE 1 Summary of scaling exponents and average ρ-ratio values [a]

	γ_c	η_c	Avg ρ–ratio	γ_m (ν)	η_m
aCgn (α-chymotrypsinogen A)	–0.3 ± 0.1	–0.27 ± 0.07	1.65 ± 0.1	0.74 ± 0.16	0.64 ± 0.12
bG-CSF (bovine granulocyte-colony stimulating factor)	–1.13 ± 0.34	–1.25 ± 0.34	1.76 ± 0.13	0.74 ± 0.15	0.8 ± 0.4

[a] Weiss W F, IV, Hodgdon T. K., Kaler E. W., Lenhoff A. M., and Roberts C. J. (2007) Nonnative Protein Polymers: Structure, Morphology, and Relation to Nucleation and Growth. *Biophysical Journal* **93**: 4392-4403

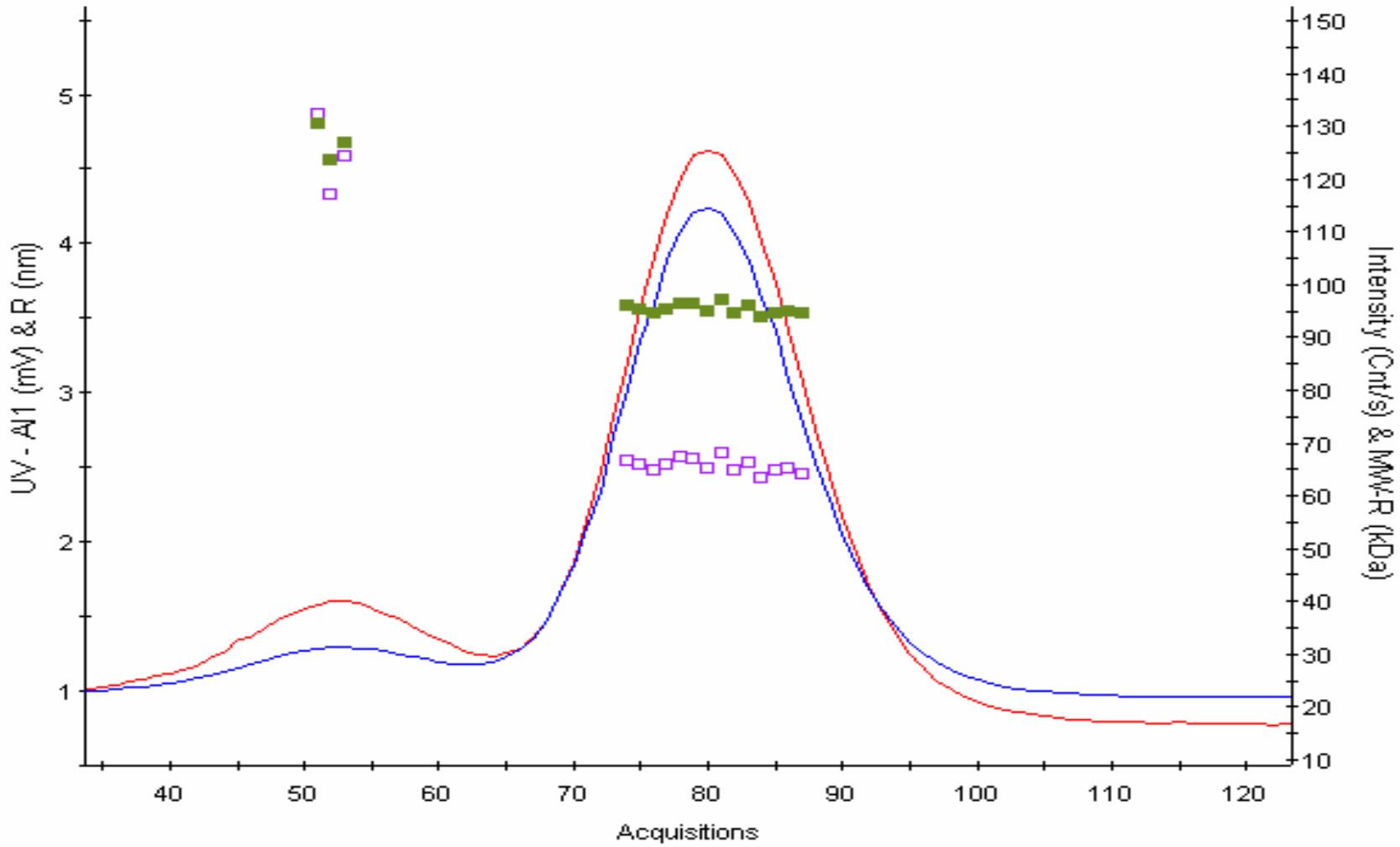
Cryo-TEM micrograph of aCgn samples (c_0 = 1 mg/mL) at m = 0.05



Weiss W F, IV, Hodgdon T. K., Kaler E. W., Lenhoff A. M., and Roberts C. J. (2007) Nonnative Protein Polymers: Structure, Morphology, and Relation to Nucleation and Growth. *Biophysical Journal* **93**: 4392-4403

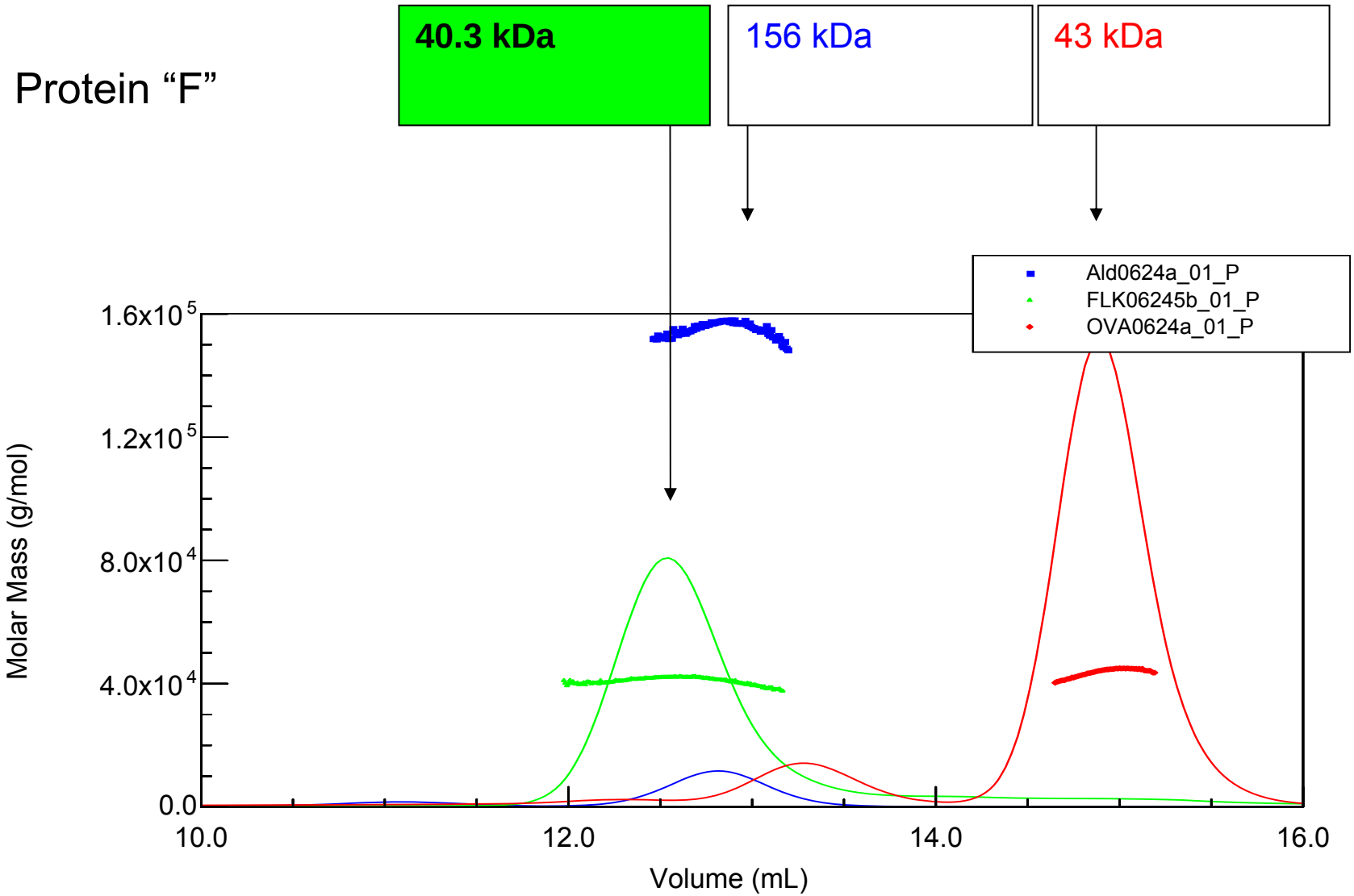
Molar Mass Distribution Plot

BSA 66 kDa



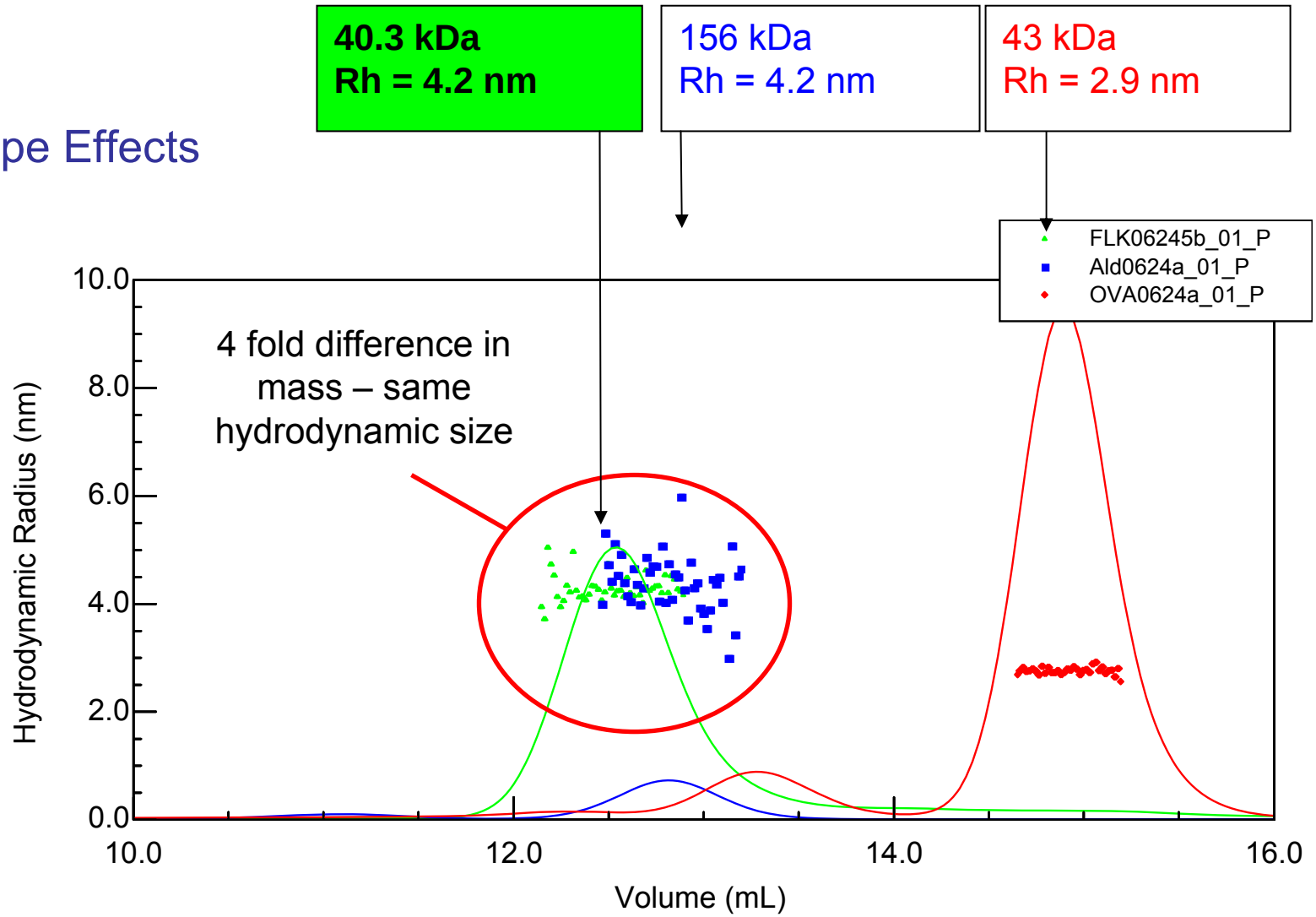
Shape information from light scattering measurements

Protein “F”



Protein “F” frictional ratio R_h/R_s = 1.85 non-spherical shape

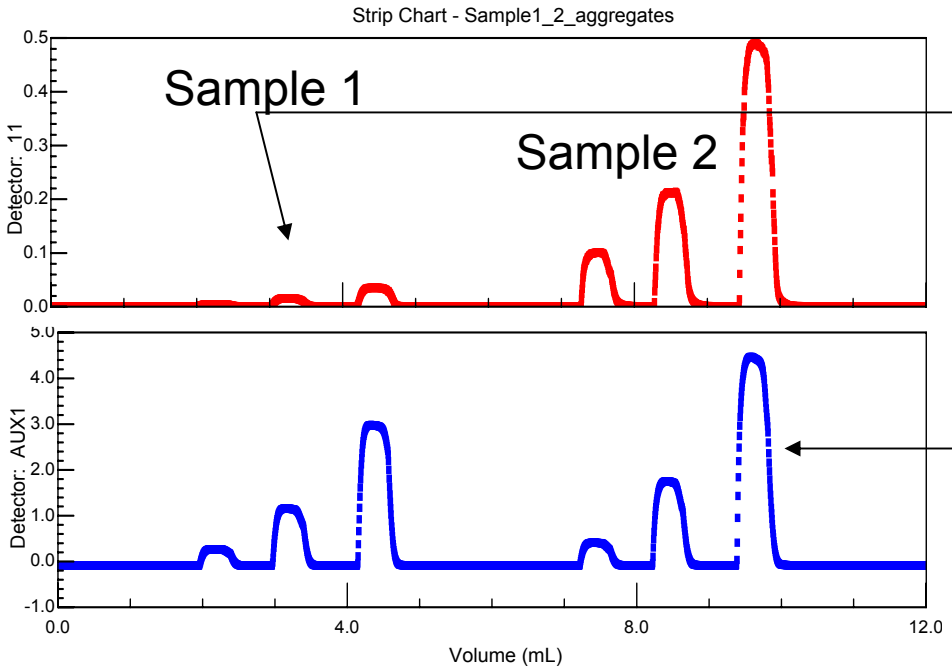
Shape Effects

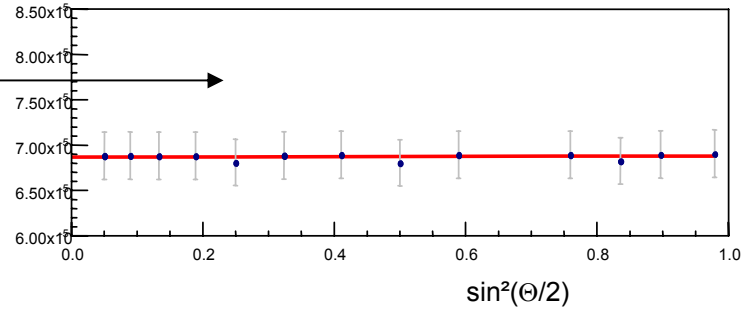


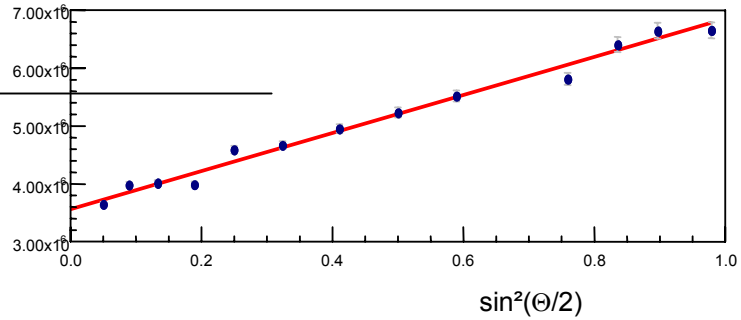
- Batch Mode Light Scattering Applications
  - Detection of aggregates in DLS and SLS measurement

Batch Mode Static MALLS experiment

Monomer 14 kDa







Sample	Weight Average MM, Mw ± SD* [kDa]	RMS [nm]
1	15 ± 1	0
2	126 ± 8	56 ± 10

Angular dependence of scattered light clearly indicates presence of aggregates

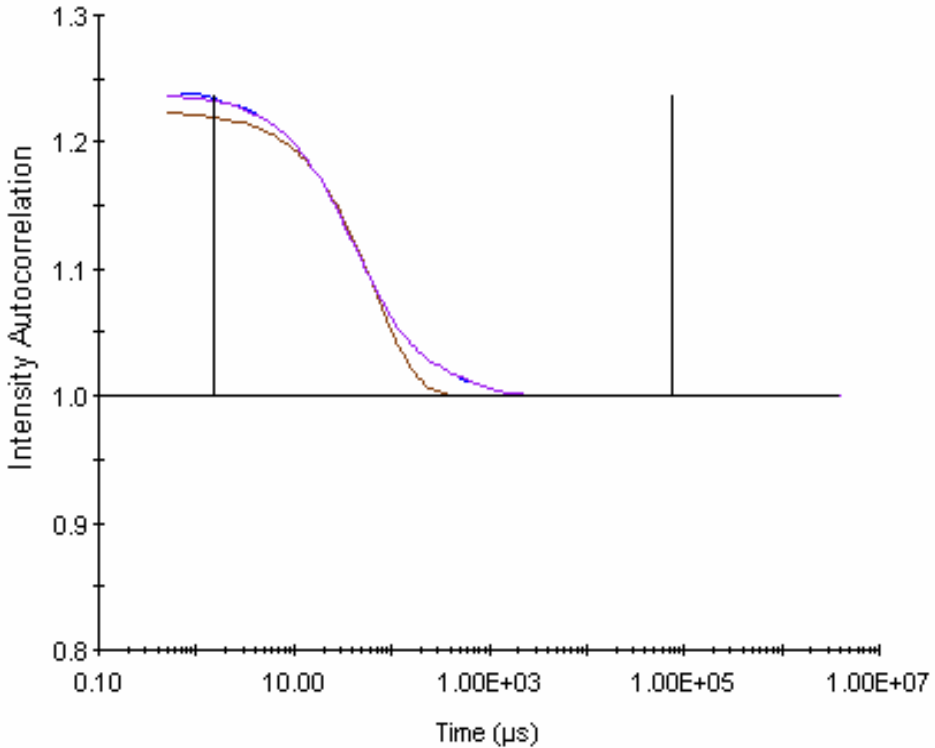
Determination of hydrodynamic radius, R_h, from a Dynamic LS experiment

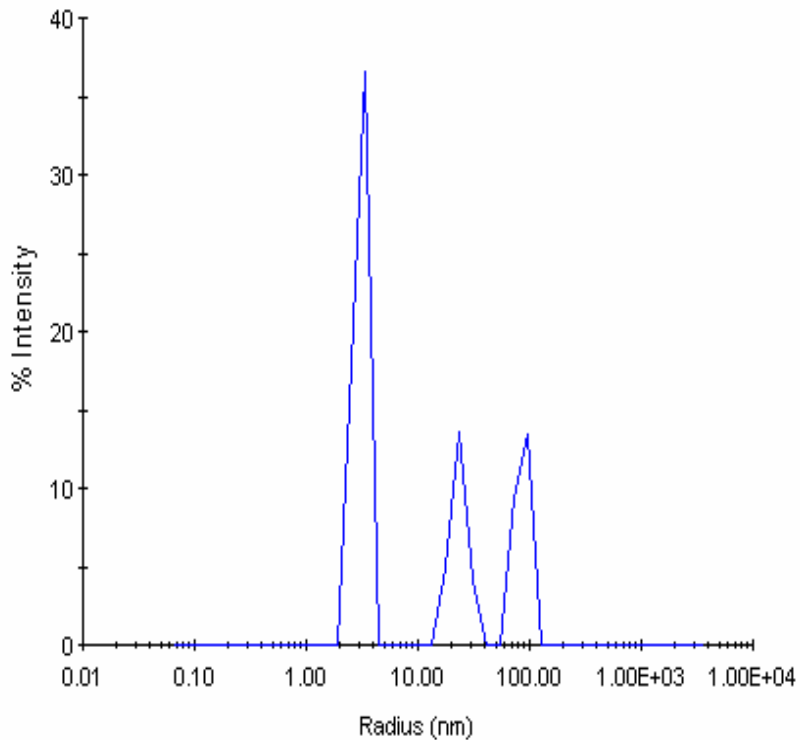
Ovalbumin; monomer: 43 kDa; Rh=3.0 nm

Rh = 8±7 nm from Cumulant Fit (Polydispersity 93%)

Regularization Fit:

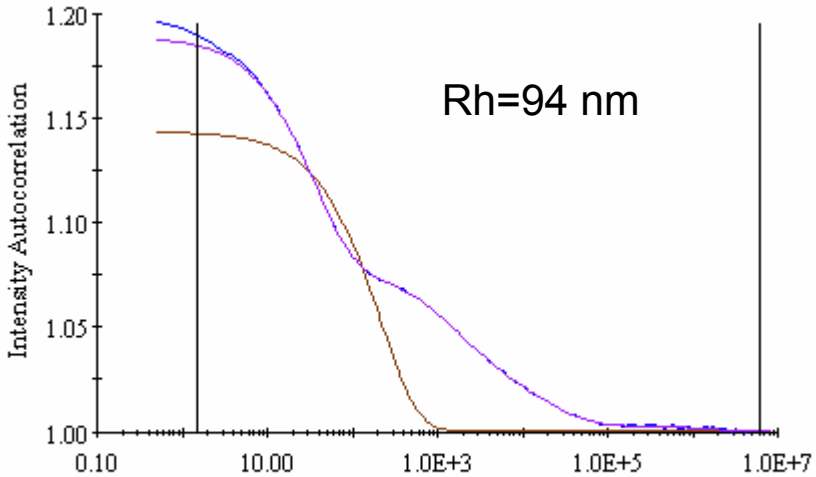
Peak	Rh (nm)	Polydispersity (%)	MW (R) kDa	% Intensity	% Mass
1	3.1	12.8	46	54	99.9
2	24	17.8	>1MDa	23	0.1
3	86	13.4	>1MDa	23	<0.1

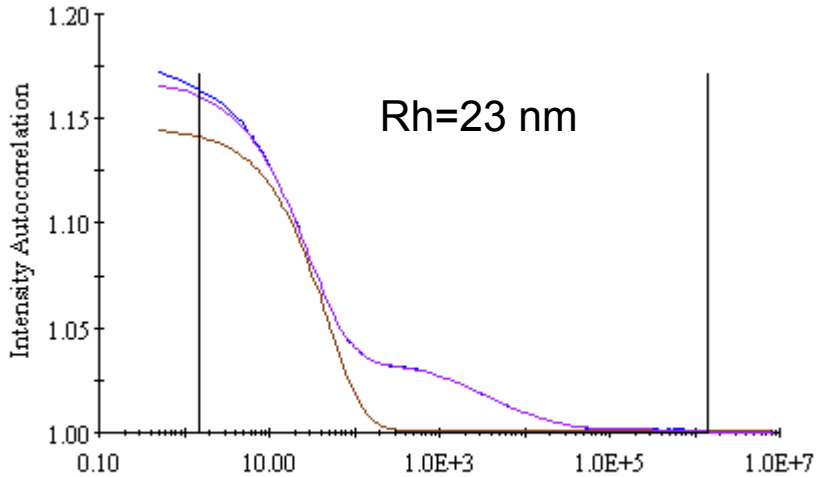


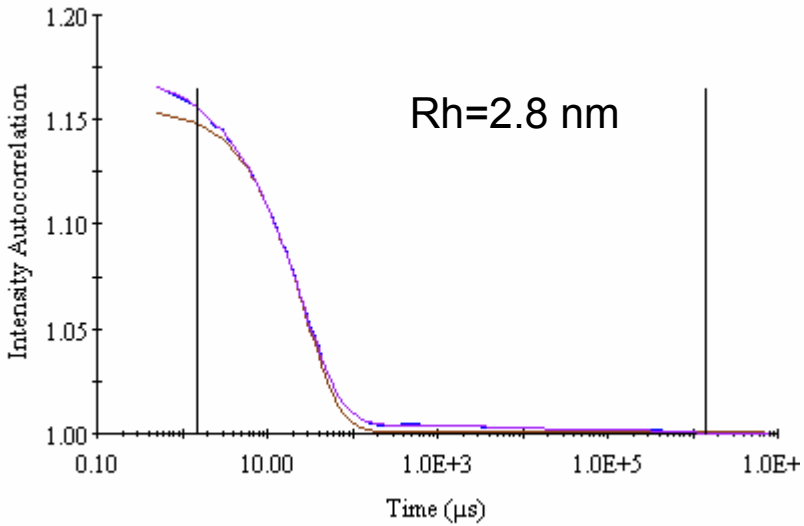


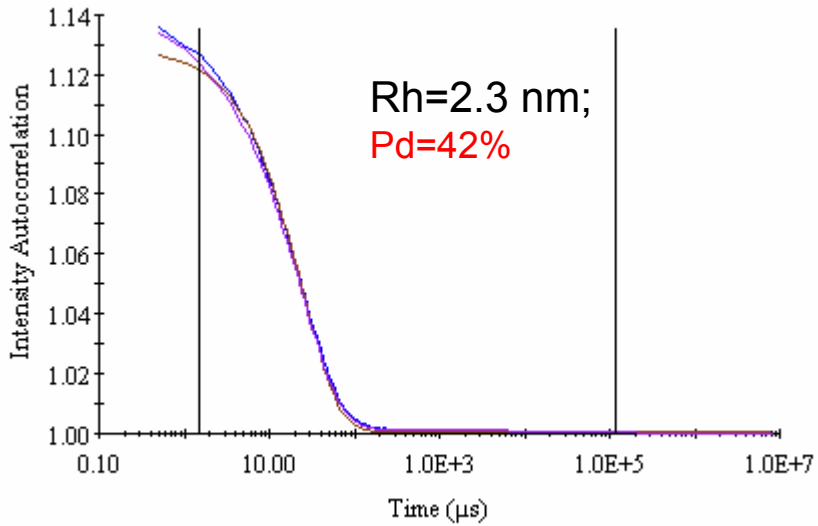
Dissociation of aggregates upon dilution; time course

Protein H 23 kDa; Rh=2.3 nm









Ovalbumin 43 kDa

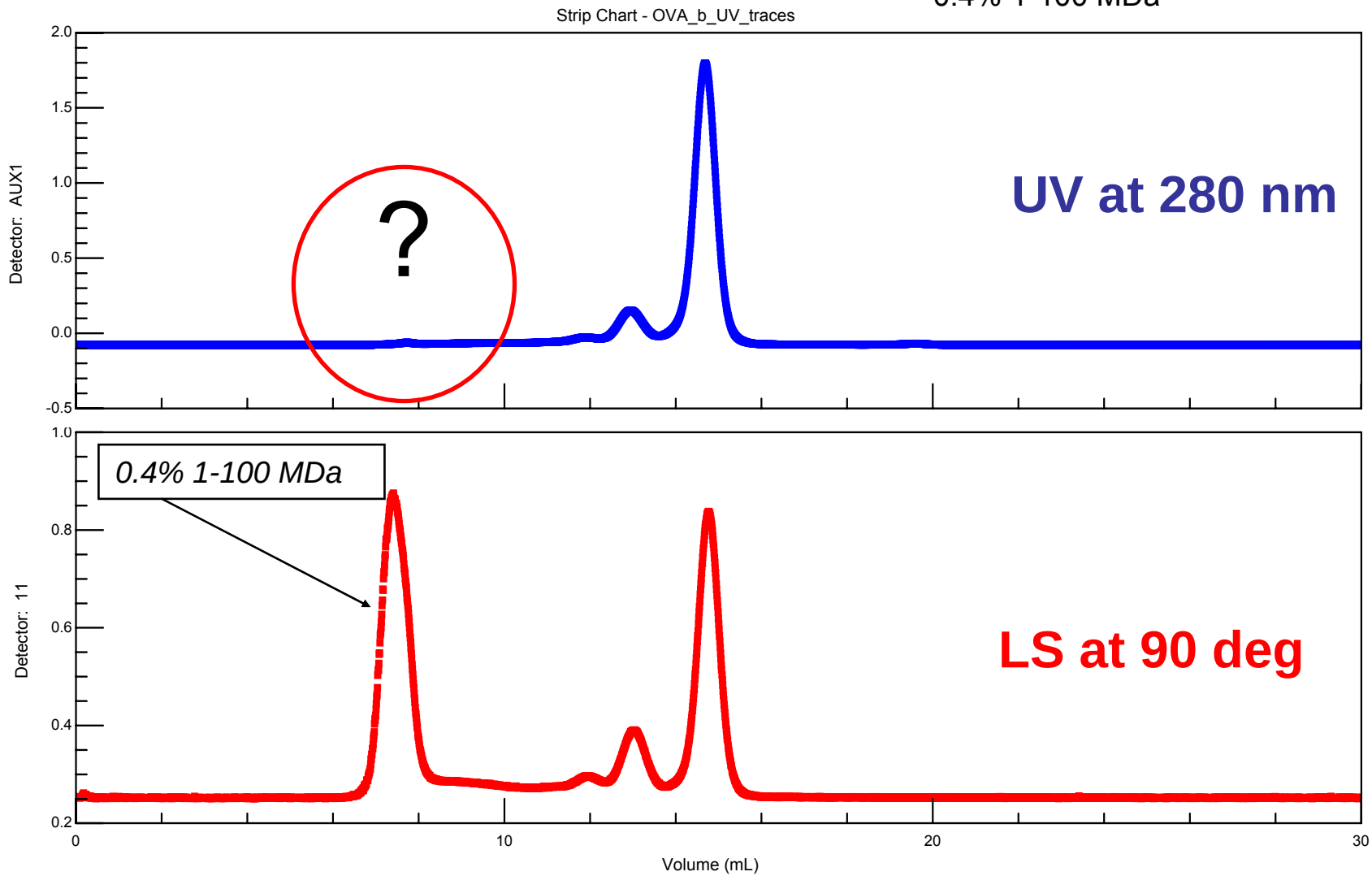
88% monomer

8% dimer

1.5% trimer

3% aggregates < 1MDa

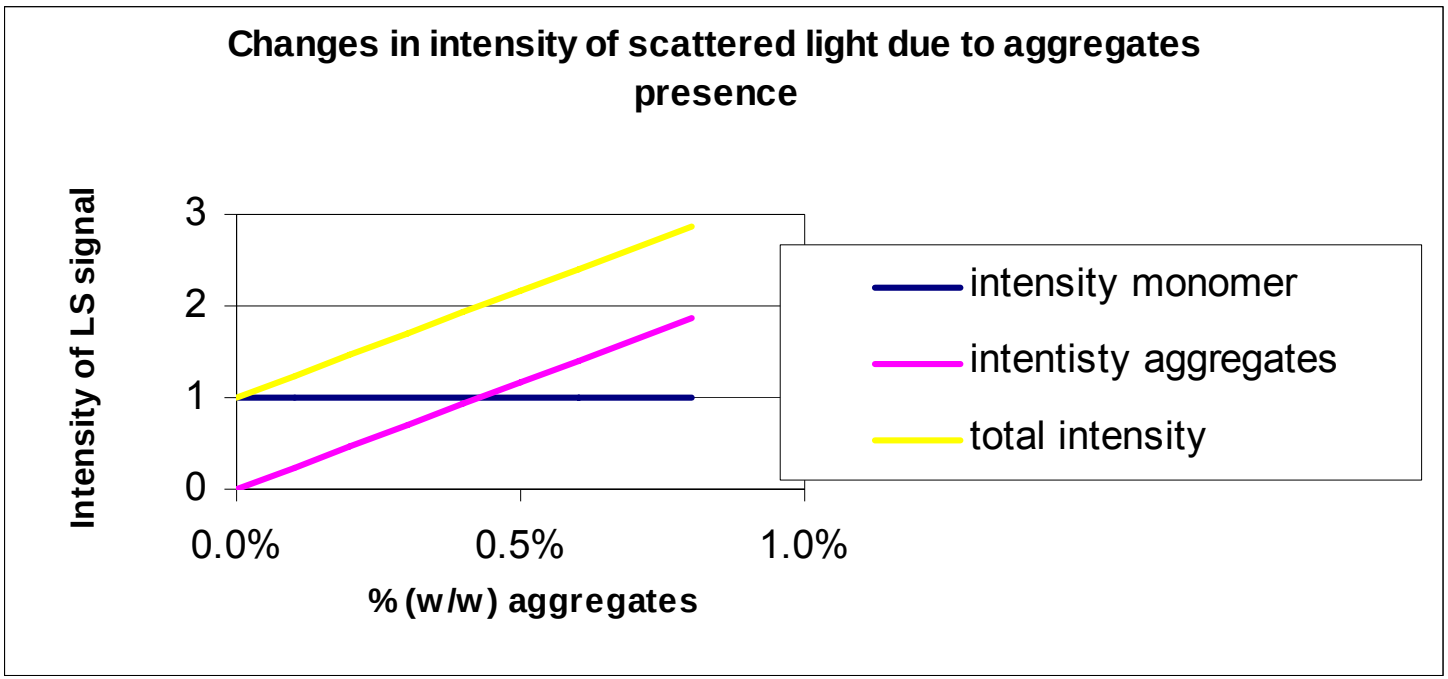
0.4% 1-100 MDa



Intensity of scattered light ~ M_w*c

due to their high Mw aggregates scatter very strongly

A monomeric protein 43 kDa and aggregates 10 MDa at 2 mg/mL:



Why Light Scattering?

- LS measurements are non-invasive and non-destructive
  - small sample volumes
  - great dynamic range for sizing: hydrodynamic radii ~ 2nm to 500 nm
  - great dynamic range for Mw determination: < 1kDa to >10 MDa
  - wide range of concentrations (non-ideality can be addressed through the determination of second virial coefficient)
  - perfectly suited for determination of oligomeric state of modified proteins without prior knowledge of extend of modification (glycosylated proteins, proteins modified by polyethylene glycol, or membrane proteins present as complexes with lipids and detergents)
- LS measurements are perfect tools for detection and characterization of aggregates
  - Scattering Intensity, $R(\Theta) \sim Mw*c$

    because of their big Mw, aggregates scatter strongly even when present at low concentrations; easily detectable
  - Angular variation of the scattered light is related to the size and shape of the molecule

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

Various uses of Light Scattering for assessing protein aggregates

Experiment	Detects Aggregates	Information about population (distribution)	Challenge in use	Sample dilution	Speed
DLS	Yes	No	Low	No	Fast
Micro-batch MALS	Yes	No	High	No	Medium
SEC/MALLS/DLS	Yes	Yes	Medium	Yes	Medium

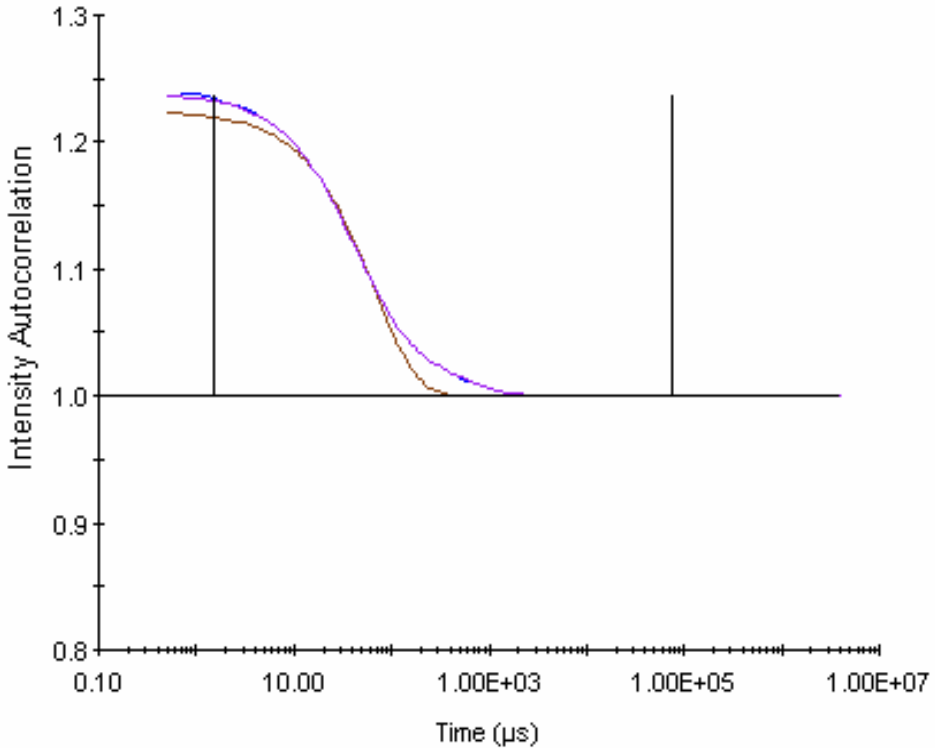
Determination of hydrodynamic radius, R_h, from a Dynamic LS experiment

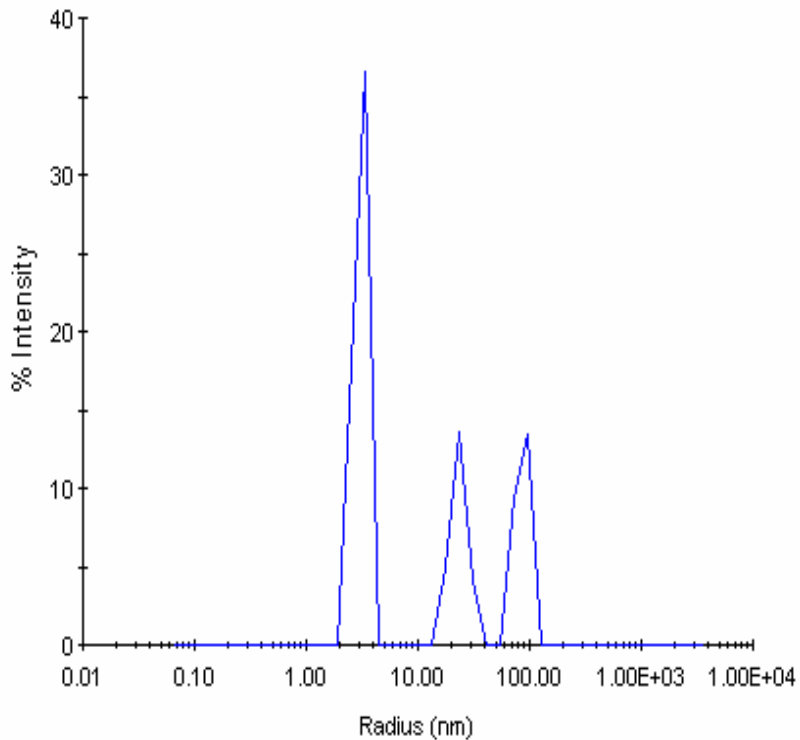
Ovalbumin; monomer: 43 kDa; Rh=3.0 nm

Rh = 8±7 nm from Cumulant Fit (Polydispersity 93%)

Regularization Fit:

Peak	Rh (nm)	Polydispersity (%)	MW (R) kDa	% Intensity	% Mass
1	3.1	12.8	46	54	99.9
2	24	17.8	>1MDa	23	0.1
3	86	13.4	>1MDa	23	<0.1





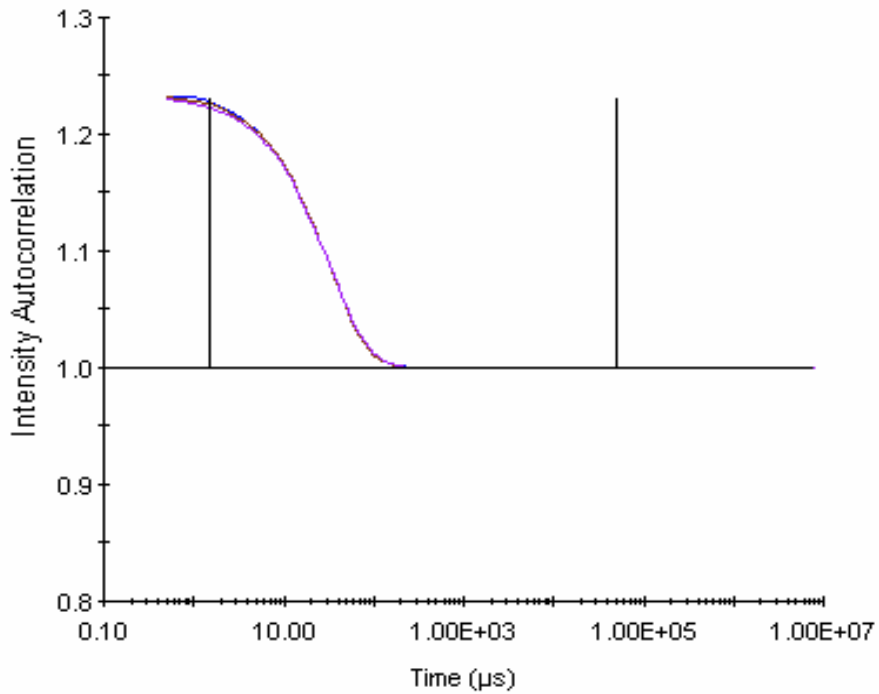
Results from a batch mode Dynamic LS experiment:

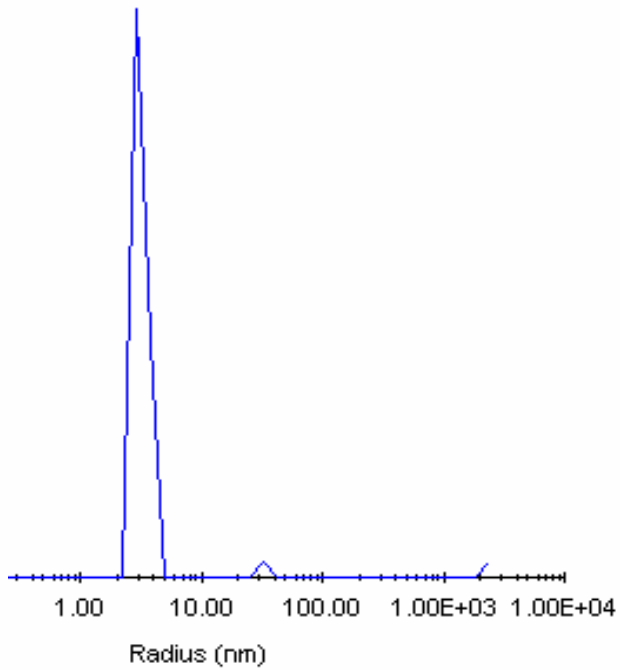
Ovalbumin 43 kDa; Rh=3.0 nm

Rh = 3.2±0.6 nm from Cumulant Fit (Polydispersity 19%)

Regularization Fit:

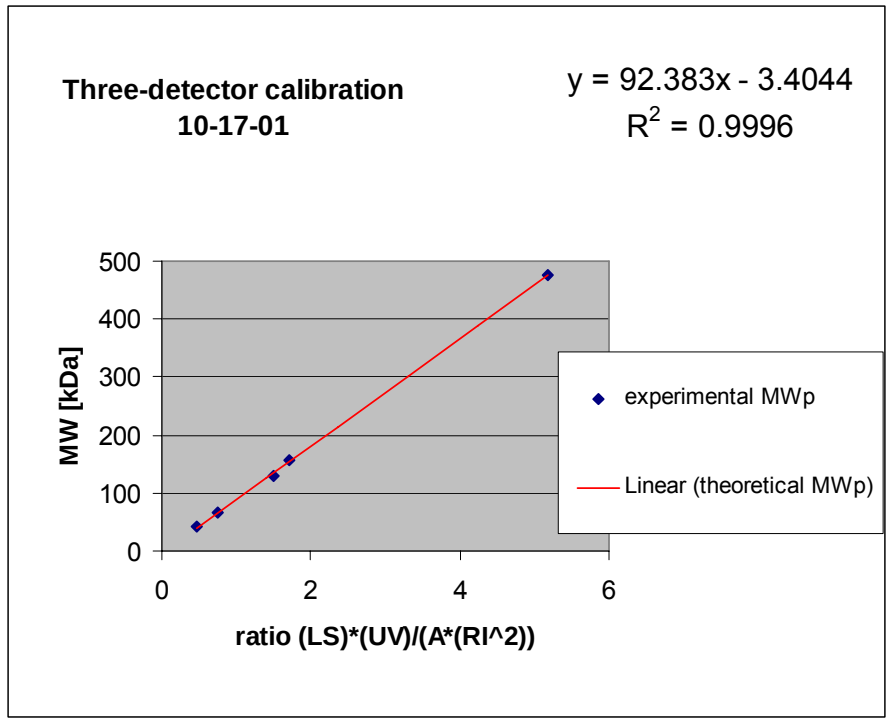
Peak	Rh (nm)	Polydispersity (%)	MW (R) kDa	% Intensity	% Mass
1	3.1	12.9	46	96	100
2	32	0	>1MDa	2	0
3	2423	0	>1MDa	2	0





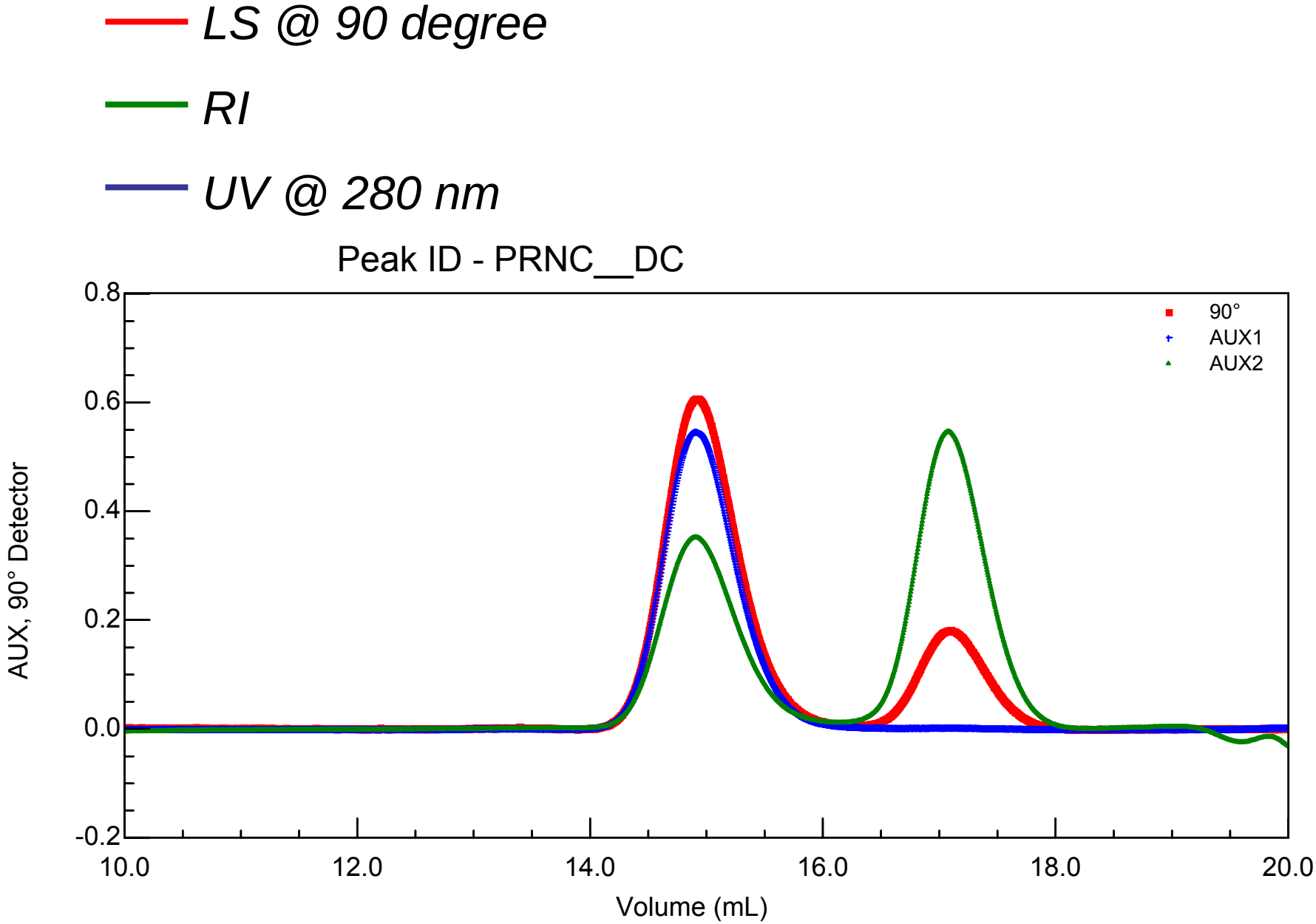
$$MW_p = \frac{k*(LS)(UV)}{\varepsilon(RI)^2}$$

Protein	MW (kDa)
Ova	43
BSA(1)	66
BSA(2)	132
Ald	156
Apo-Fer	475



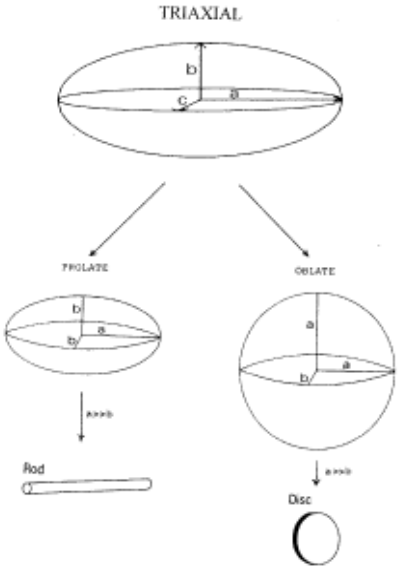
Flow Mode Light Scattering Applications

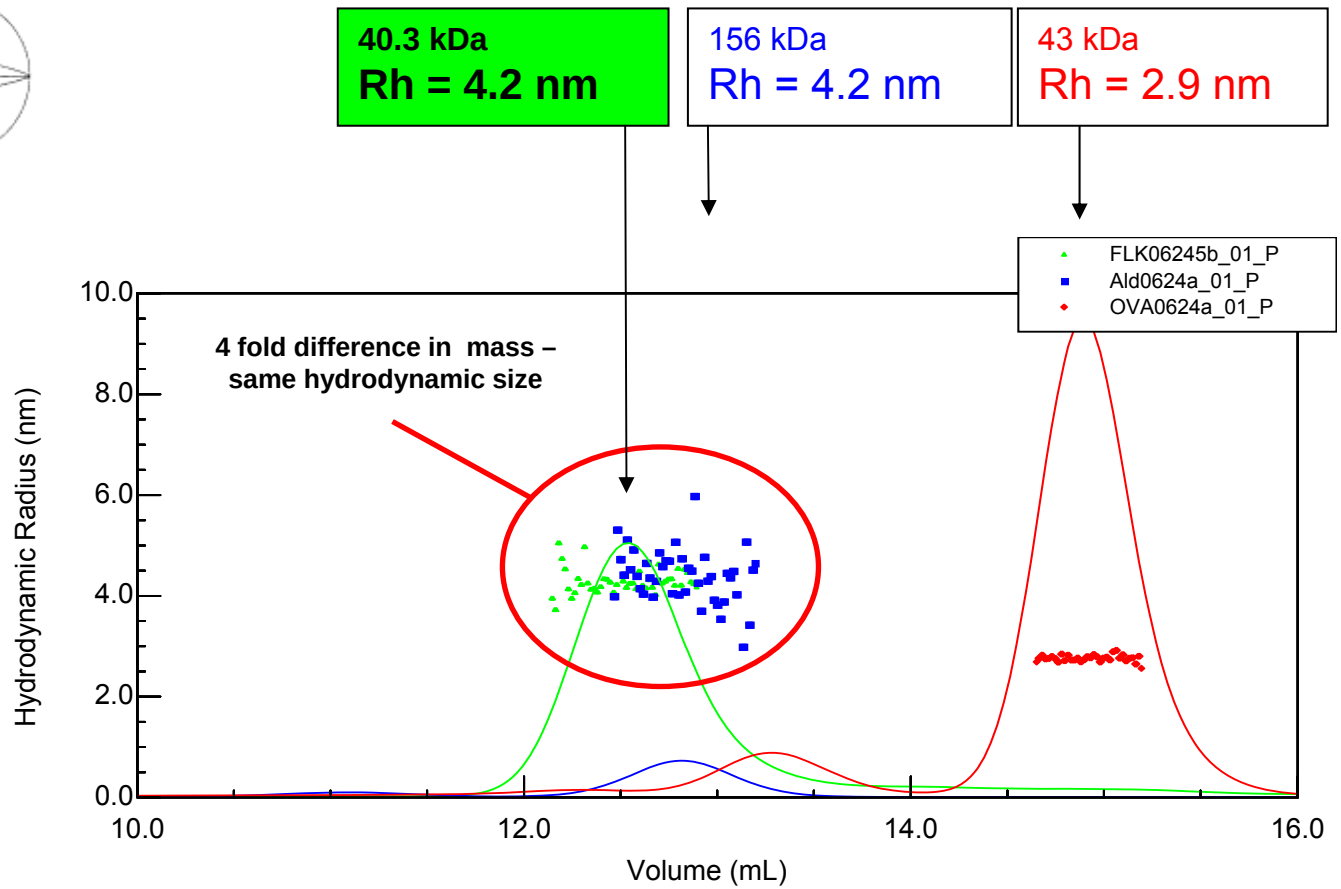
- Molar mass distributions and differences in populations
- Determination of an oligomeric state of modified proteins and oligos from SEC-LS/UV/RI measurement
- Determination of dimerization constant from SEC-LS measurements


LS @ 90 degree
RI
UV @ 280 nm
Peak ID - PRNC__DC
90°
AUX1
AUX2
AUX, 90° Detector
0.8
0.6
0.4
0.2
0.0
-0.2
10.0
12.0
14.0
16.0
18.0
20.0
Volume (mL)

Protein “F” frictional ratio $P = R_h/R_s = 1.85$ non-spherical shape

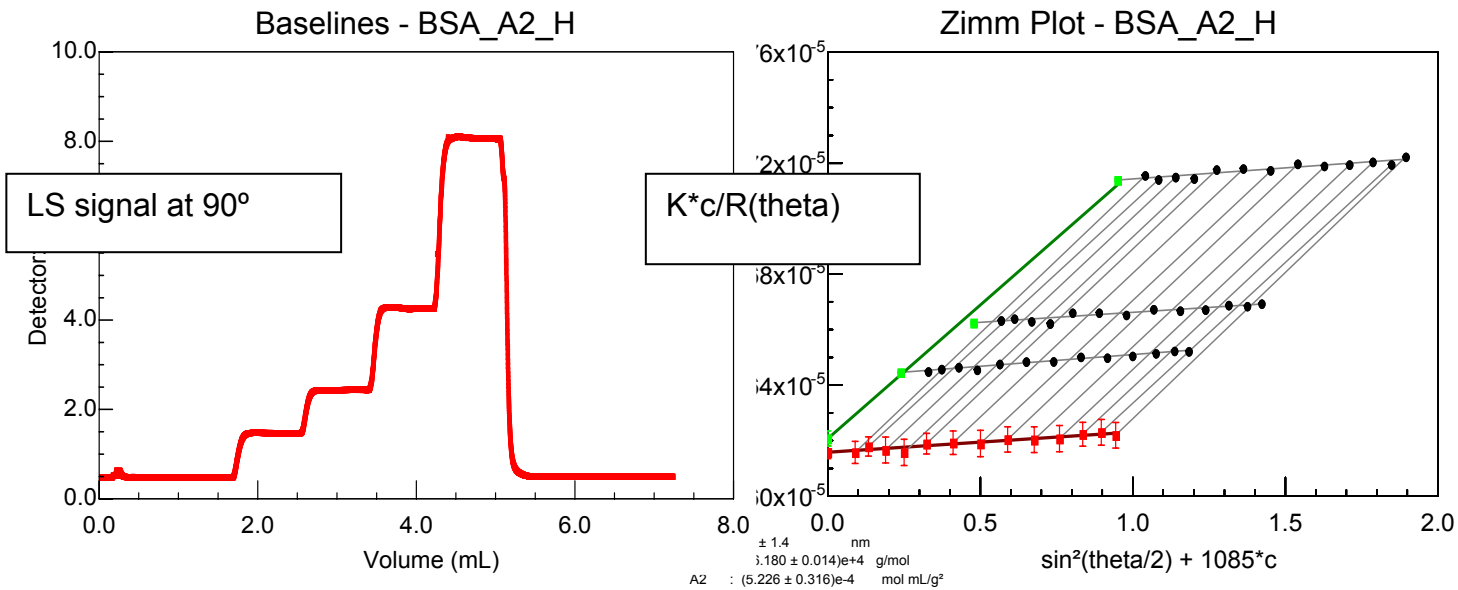
Axial ratio a/b (prolate) = 16.6 (oblate) = 22.9





Harding S E, Longman E., Carrasco B., Ortega A., and de la Torre J. G. (2004) Studying antibody conformations by ultracentrifugation and hydrodynamic modeling. *Methods Mol Biol* **248**: 93-113

Determination of Mw and second virial coefficient from Zimm plot analysis of light scattering data.



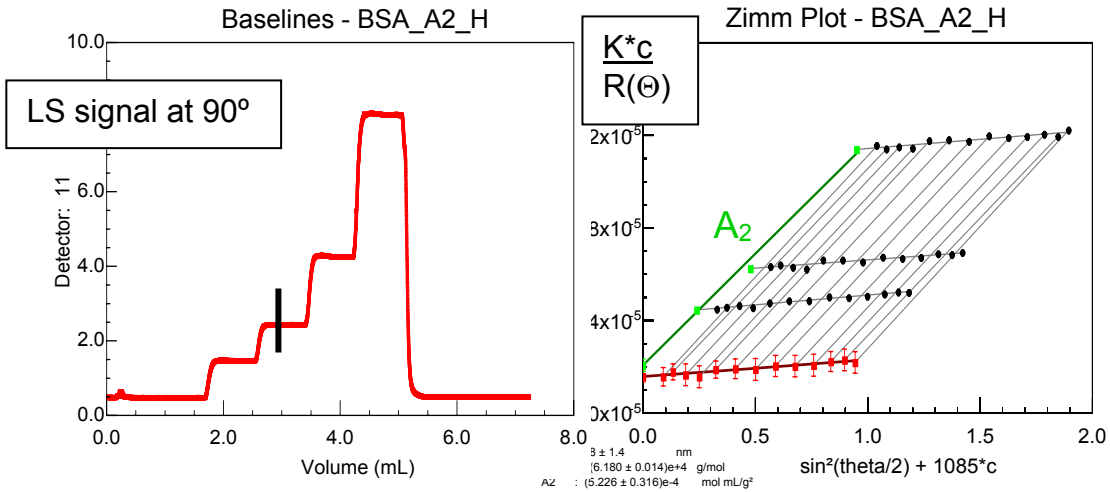
BSA solutions at concentrations: 0.22, 0.44, 0.75 and 1.1 mg/mL and the data were analyzed using Zimm formalism

Mw 65
B $(5.226 \pm 0.316)e^{-4}$ mol mL/g²

- Batch Mode Light Scattering Applications
  - Detection of aggregates in DLS and SLS measurement

Determination of Molar Mass and second virial coefficient from a batch static LS experiment

BSA 66 kDa



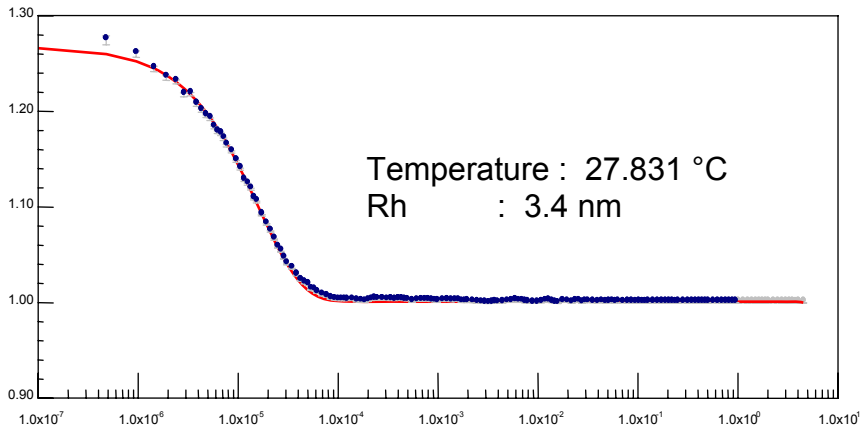
$$\frac{K^*c}{R(\theta)} = \frac{1}{MwP(\theta)} + 2A_2c$$

and Rh from DLS

Zimm plot analysis of static light scattering data

Mw = 62 kDa

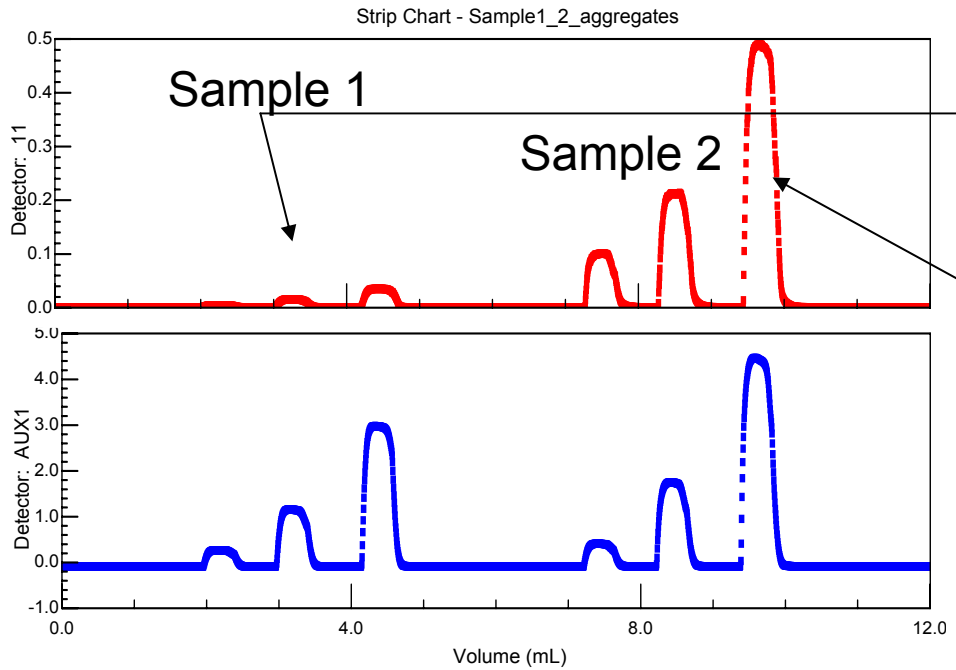
A_2 = (5.226 ± 0.316)e^{-4} mol mL/g²

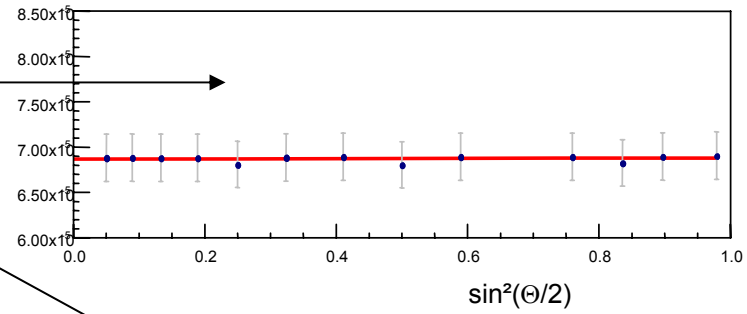


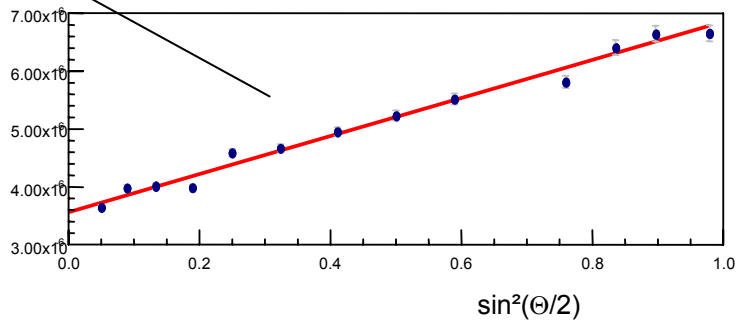
B. H. Zimm, (1948) J. Chem. Phys., 16, 1099

Batch Mode Static MALLS experiment

Monomer 14 kDa







Sample	Weight Average MM, Mw ± SD* [kDa]	RMS [nm]
1	15 ± 1	0
2	126 ± 8	56 ± 10

Angular dependence of scattered light clearly indicates presence of aggregates

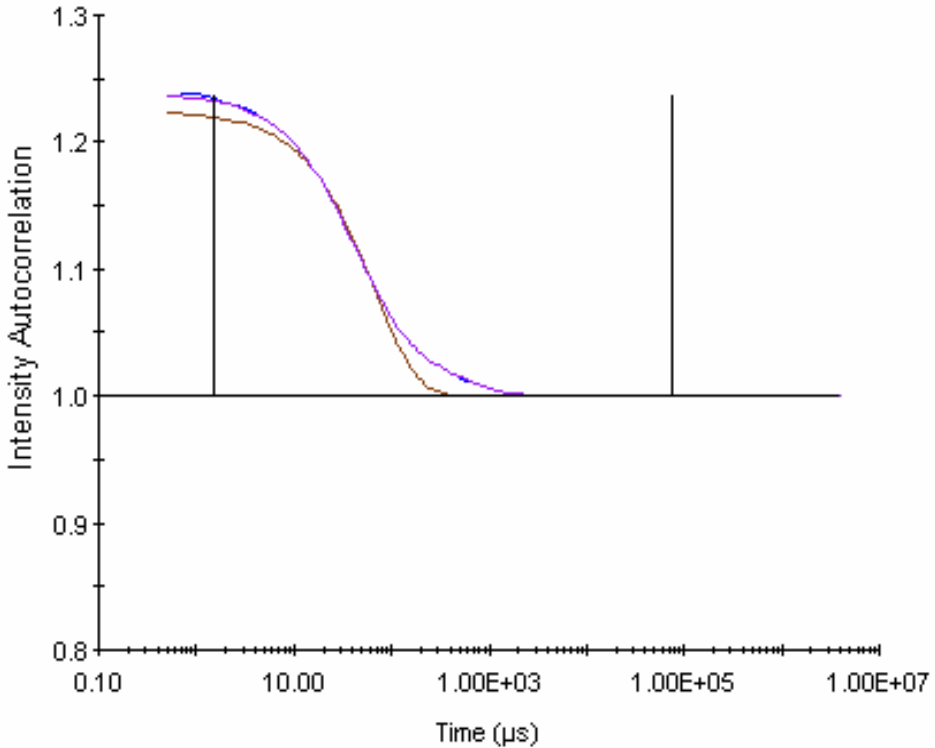
Determination of hydrodynamic radius, R_h, from a Dynamic LS experiment

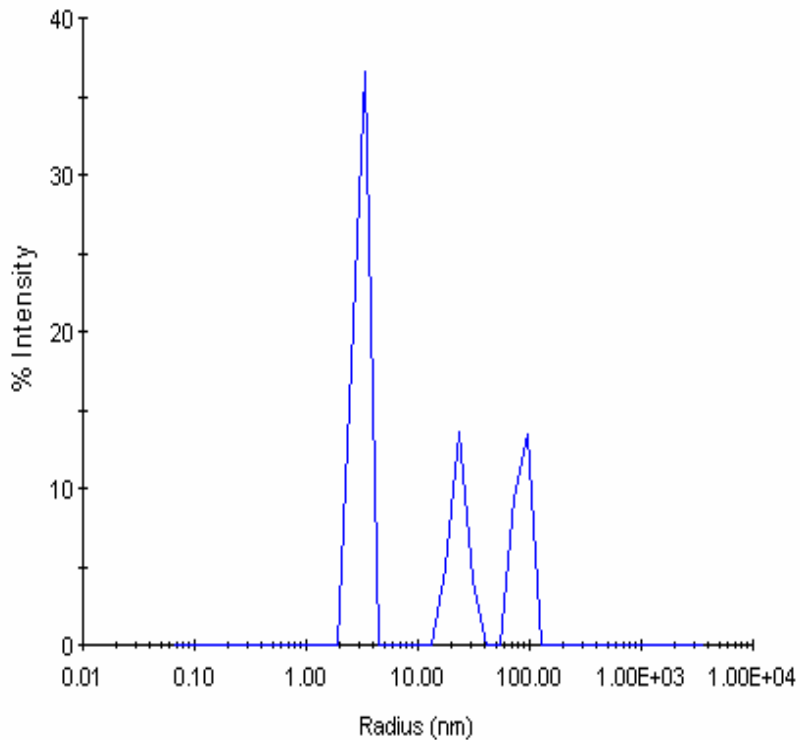
Ovalbumin; monomer: 43 kDa; Rh=3.0 nm

Rh = 8±7 nm from Cumulant Fit (Polydispersity 93%)

Regularization Fit:

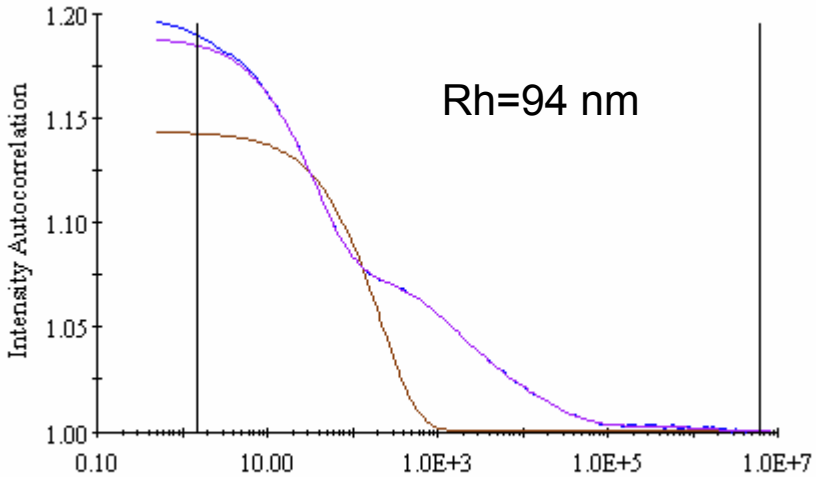
Peak	Rh (nm)	Polydispersity (%)	MW (R) kDa	% Intensity	% Mass
1	3.1	12.8	46	54	99.9
2	24	17.8	>1MDa	23	0.1
3	86	13.4	>1MDa	23	<0.1

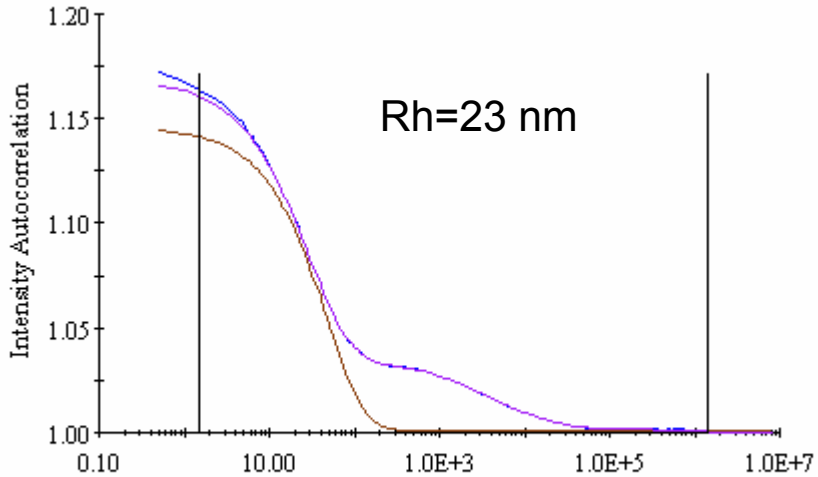


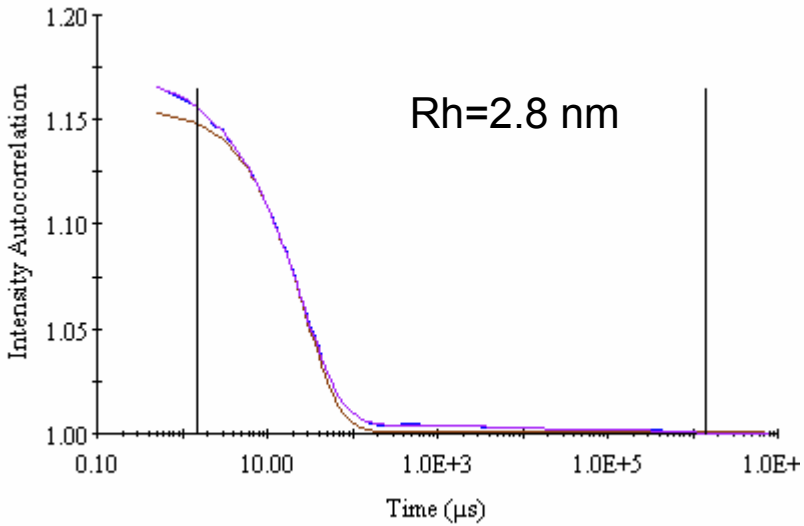


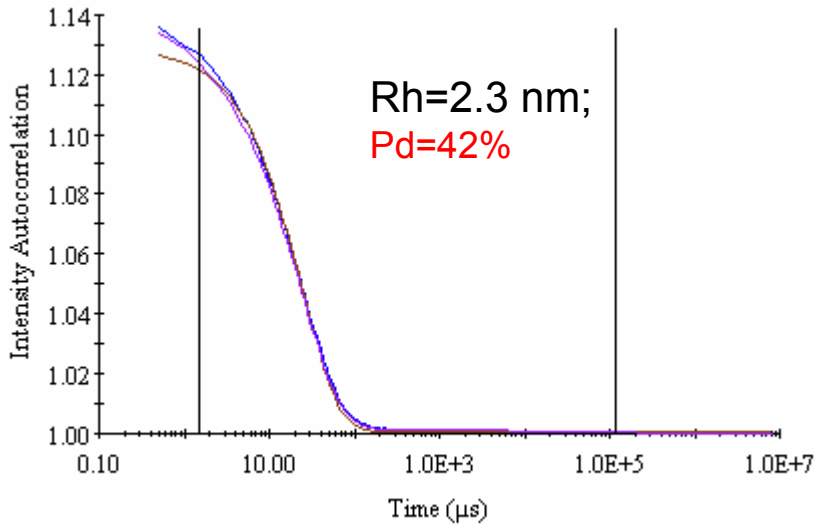
Dissociation of aggregates upon dilution; time course

Protein H 23 kDa; Rh=2.3 nm









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:

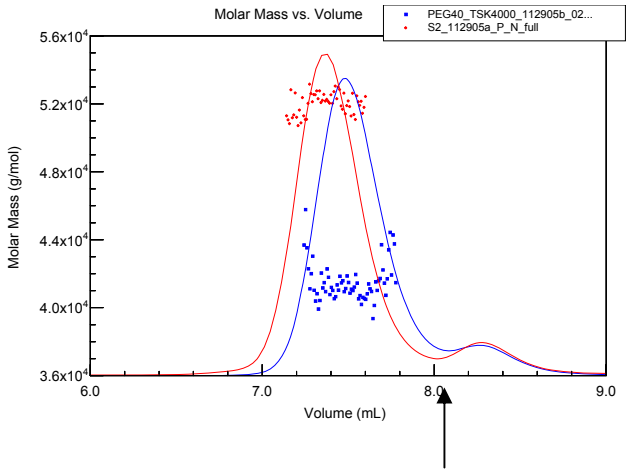
- autocorrelation function cannot be described by single exponential (cumulant fit)
- polydispersity from cumulant fit >15%

Missing information: how much and what size?

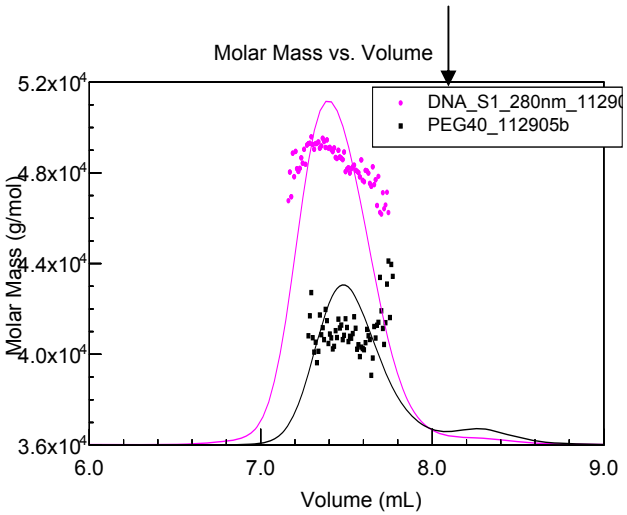
Solutions

- Sample fractionation followed by batch measurements
- **Column separation with simultaneous LS characterization**

PEG-ylated oligo



BSA 66 kDa



PEG (40K) MM = 41.0 kDa (80 μg total)

Polydispersity= 1.001

40K PEG + 12.9 kDa oligo (73 μg total)

PEG-oligo MM = 52.1 kDa

PEG (40K) MM = 41.0 kDa (40 μg total)

Polydispersity= 1.001

40K PEG + 8.3 kDa oligo (70 μg total)

PEG-oligo MM = 48.5 kDa