# Light Scattering Coupled to Refractive Index and UV Absorption Measurements in Studies of Membrane Proteins Homo- and Hetero Associations

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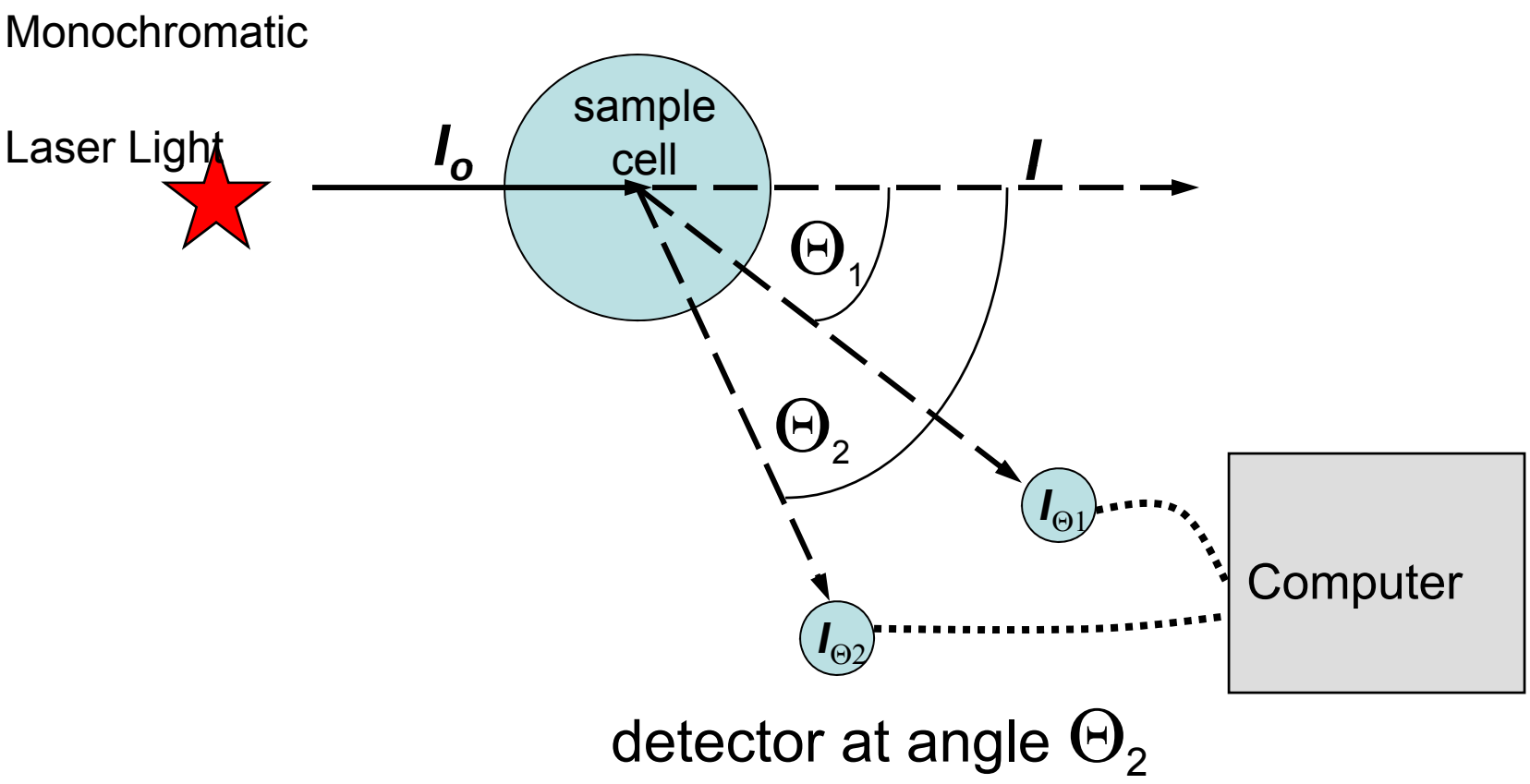
*Yale School of Medicine*

- Light Scattering Technologies
  - Static and dynamic light scattering
  - Parameters derived from SLS and DLS measurements
- Flow Mode Light Scattering Applications in Studies of Membrane Proteins
  - Determination of an oligomeric state of membrane proteins from SEC-LS/UV/RI measurement
  - Determination of complex formation and stoichiometry from SEC-LS measurements
- Capabilities and limitation of SEC-LS/RI/UV measurements

# Light Scattering Experiments



# Light Scattering Experiments

- **Static (classical)**

  time-averaged intensity of scattered light

- **Dynamic (quasielastic)**

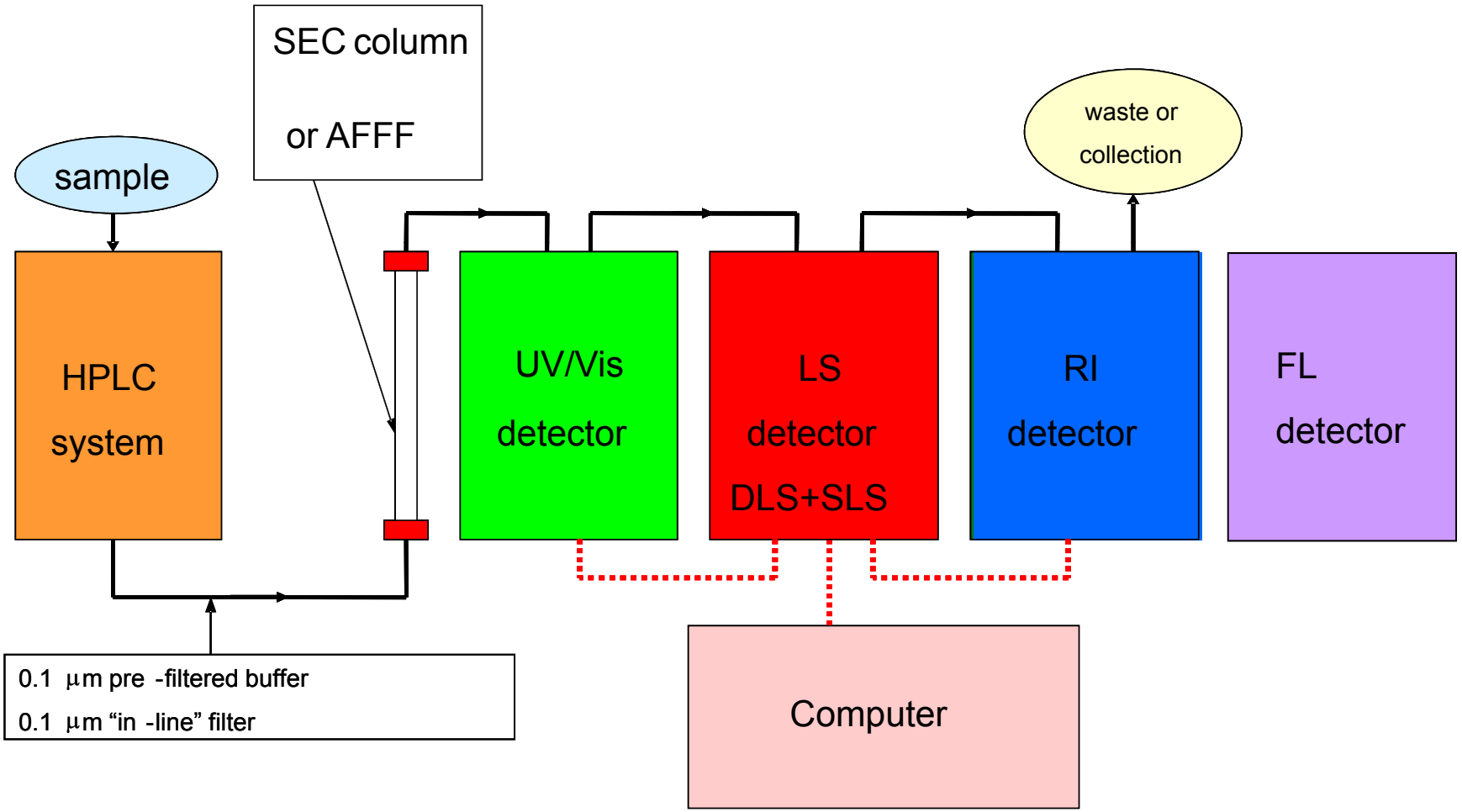
  fluctuation of intensity of scattered light with time

## Measurements:

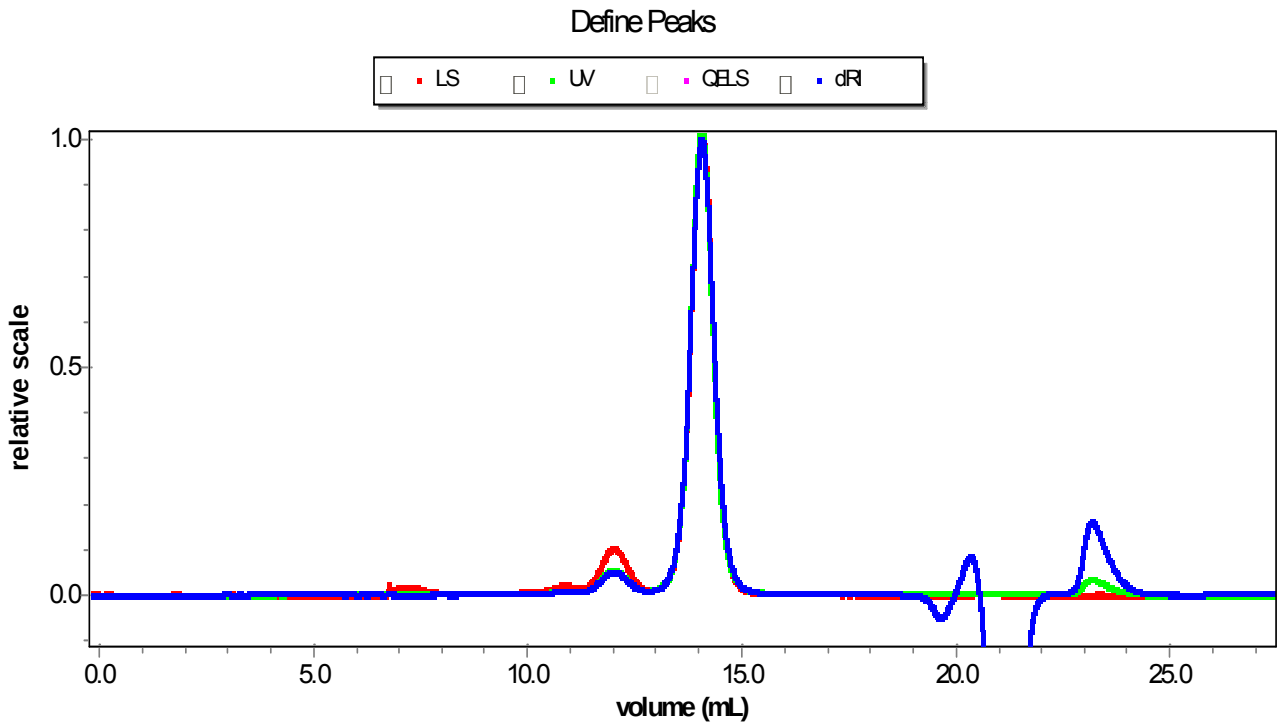
- batch mode
- "in-line" mode combined with a fractionation step,

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

# Typical SEC(AFFF); MALLS system



## Three Detector monitoring




RI

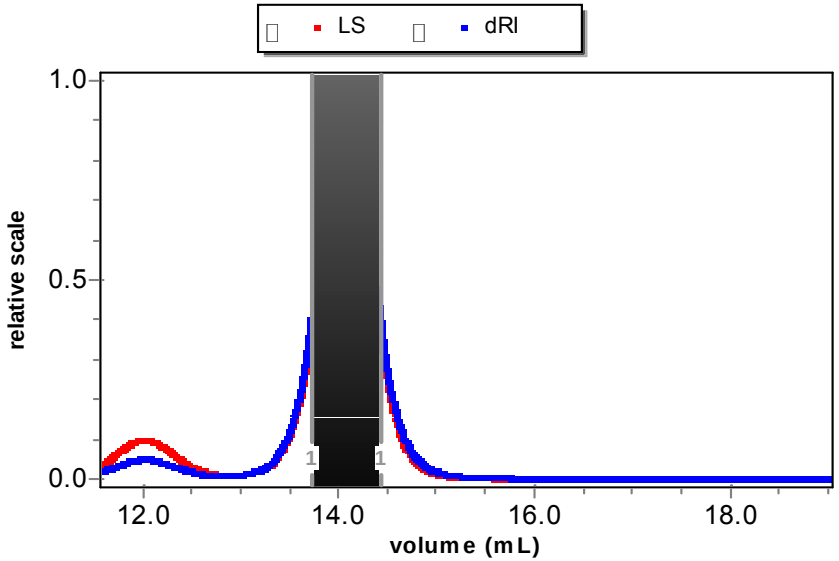
UV at 280 nm

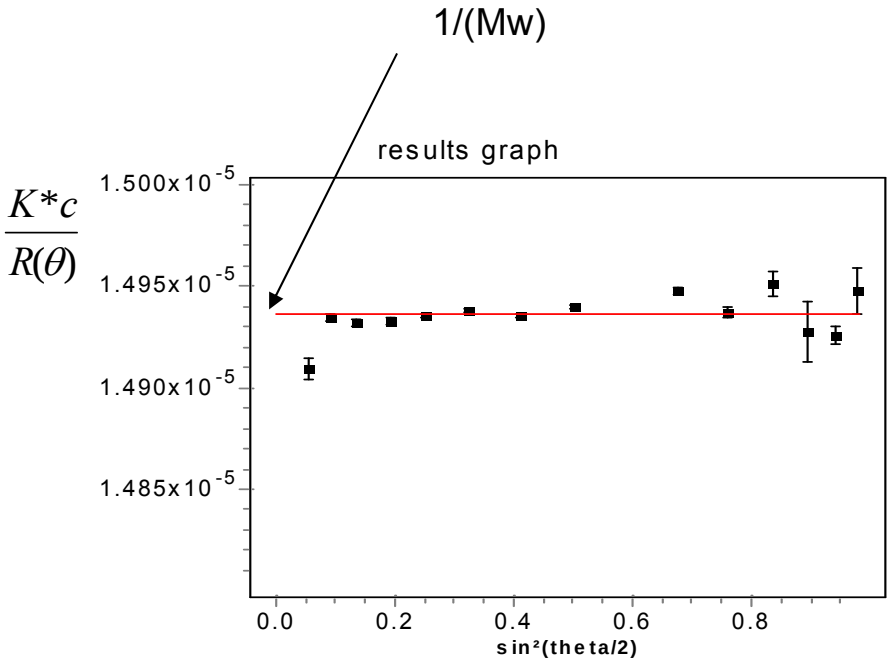
LS at 90°

## Rayleigh-Debye-Zimm formalism

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

| | |
|---|---|
| R(Θ) | Rayleigh ratio (excess scattered light) |
| c | sample concentration (g/ml) |
| Mw | weight-average molecular weight (molar mass) |
| A2 | second virial coefficient (ml-mol/g2) |
| P(Θ) | form factor (angular dependence) |
| K | optical constant $[4\pi^2 n^2 (dn/dc)^2 / (\lambda_o^4 N_A)]$ |

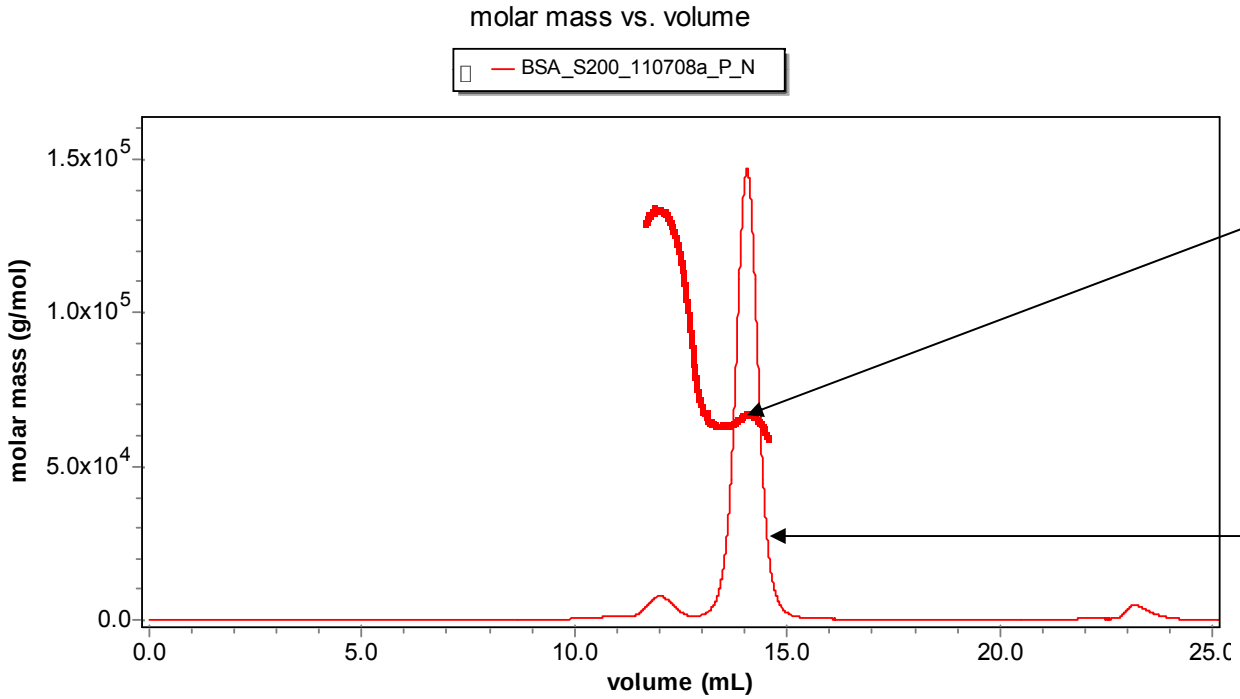


SEC/LS results: Molar Mass Distribution Plot

BSA

Monomer: 66 kDa




Weight-average molar mass

Measured every 2 µl

UV trace; $A_{280nm}$

(RI trace)

## Protein

47 kDa (monomer)  well characterized porin

detergent

dodecyl maltoside (C12M) MW = 511 g/mol

0.5g/L  i.e. 0.05%

CMC = 0.008%  micelle size 50-70 kDa

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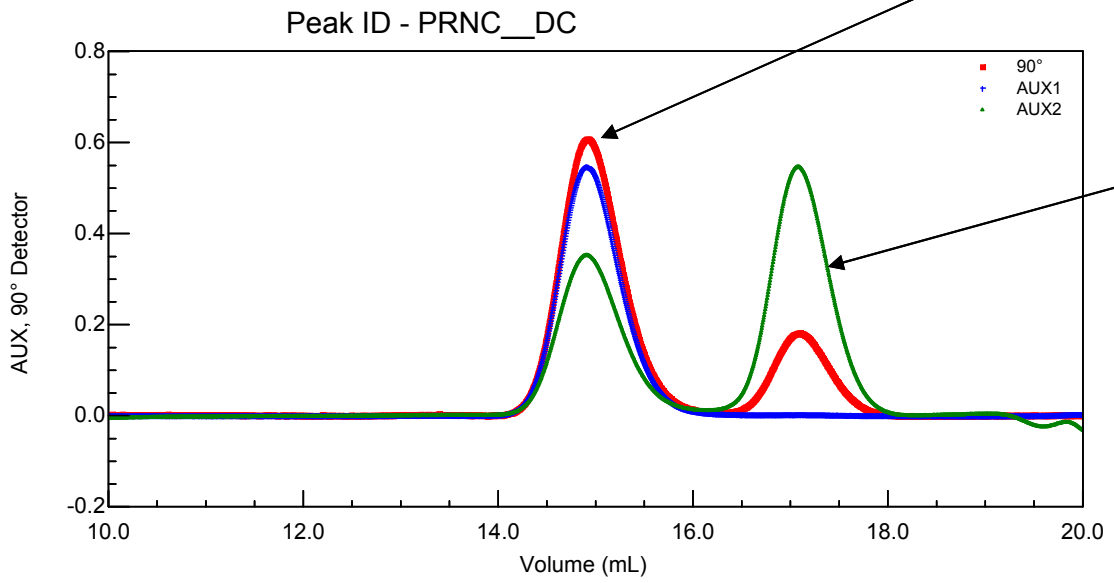
Protein+lipid+detergent

empty micelle




Protein+det.+lipids — LS @ 90 degree

Protein+det.+lipids — RI

Protein — UV @ 280 nm

# 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

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

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

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

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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

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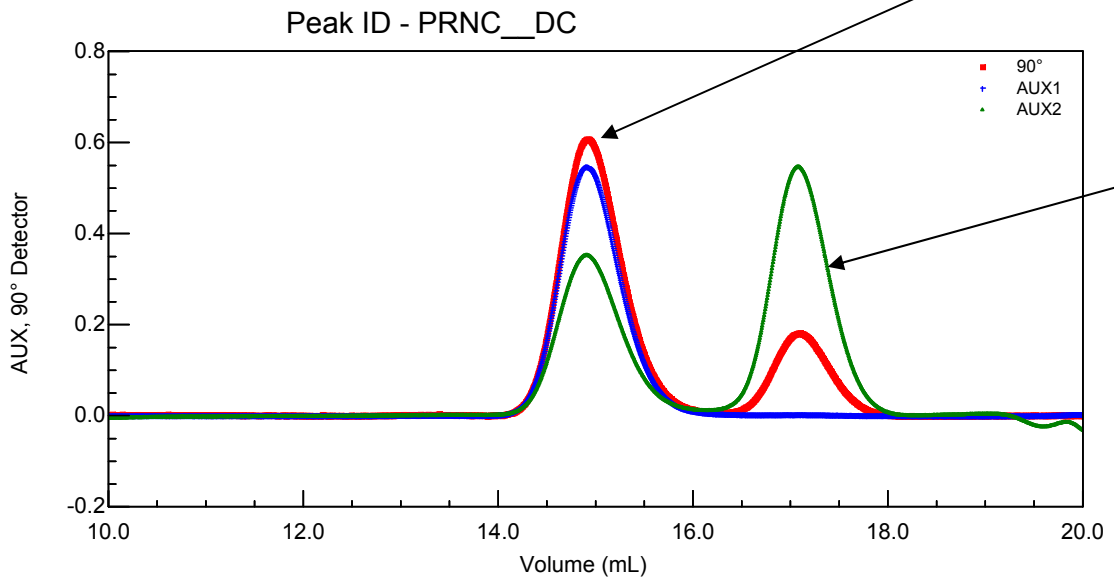
Protein+lipid+detergent

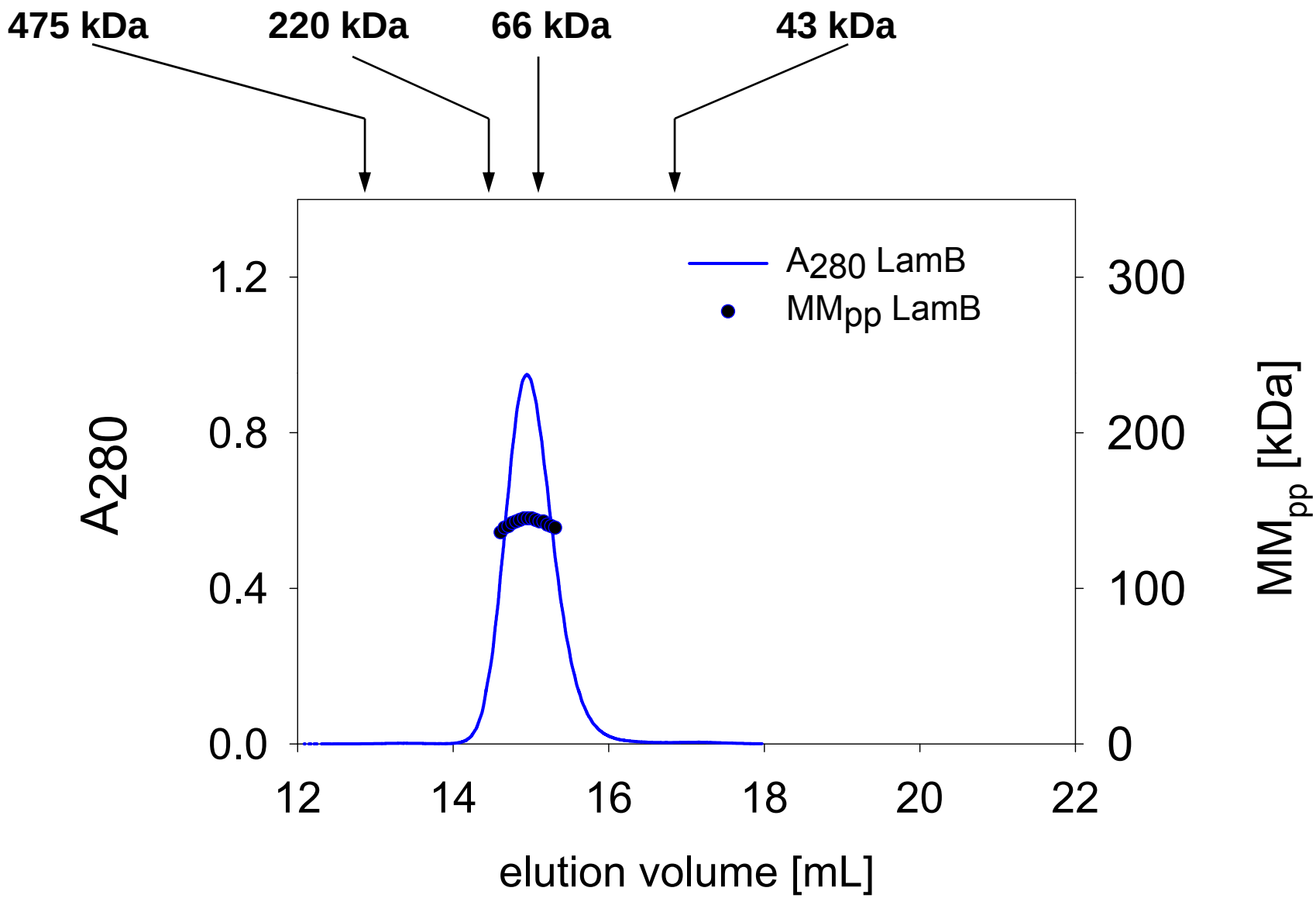
empty micelle




Protein+det.+lipids — LS @ 90 degree

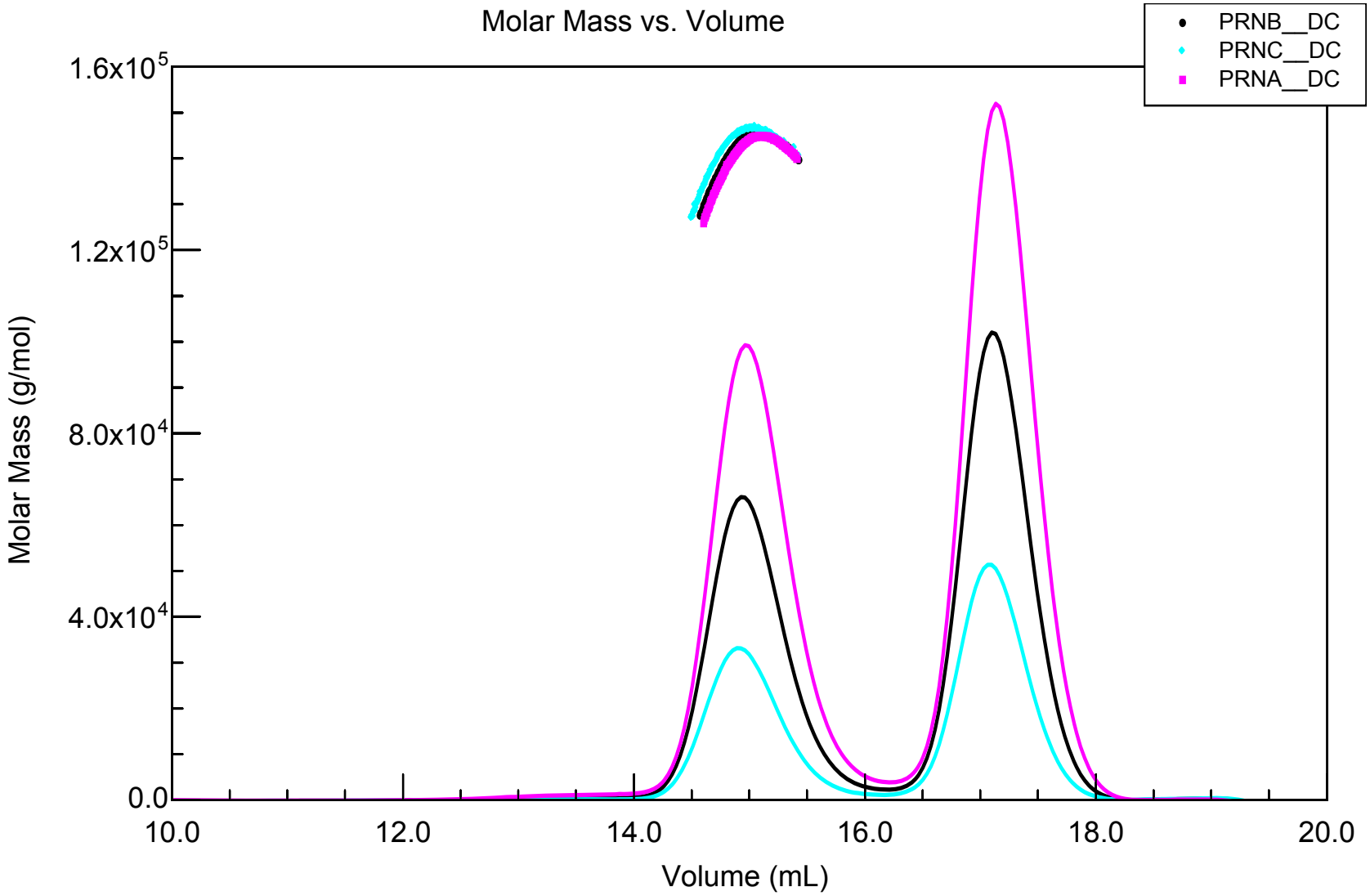
Protein+det.+lipids — RI

Protein — UV @ 280 nm


475 kDa
220 kDa
66 kDa
43 kDa
A280 LamB
MMpp LamB
A280
MMpp [kDa]
elution volume [mL]
0.0
0.4
0.8
1.2
0
100
200
300
12
14
16
18
20
22

*porin  monomer = 47 kDa*

*MW = 149 ± 3  kDa  trimer*



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

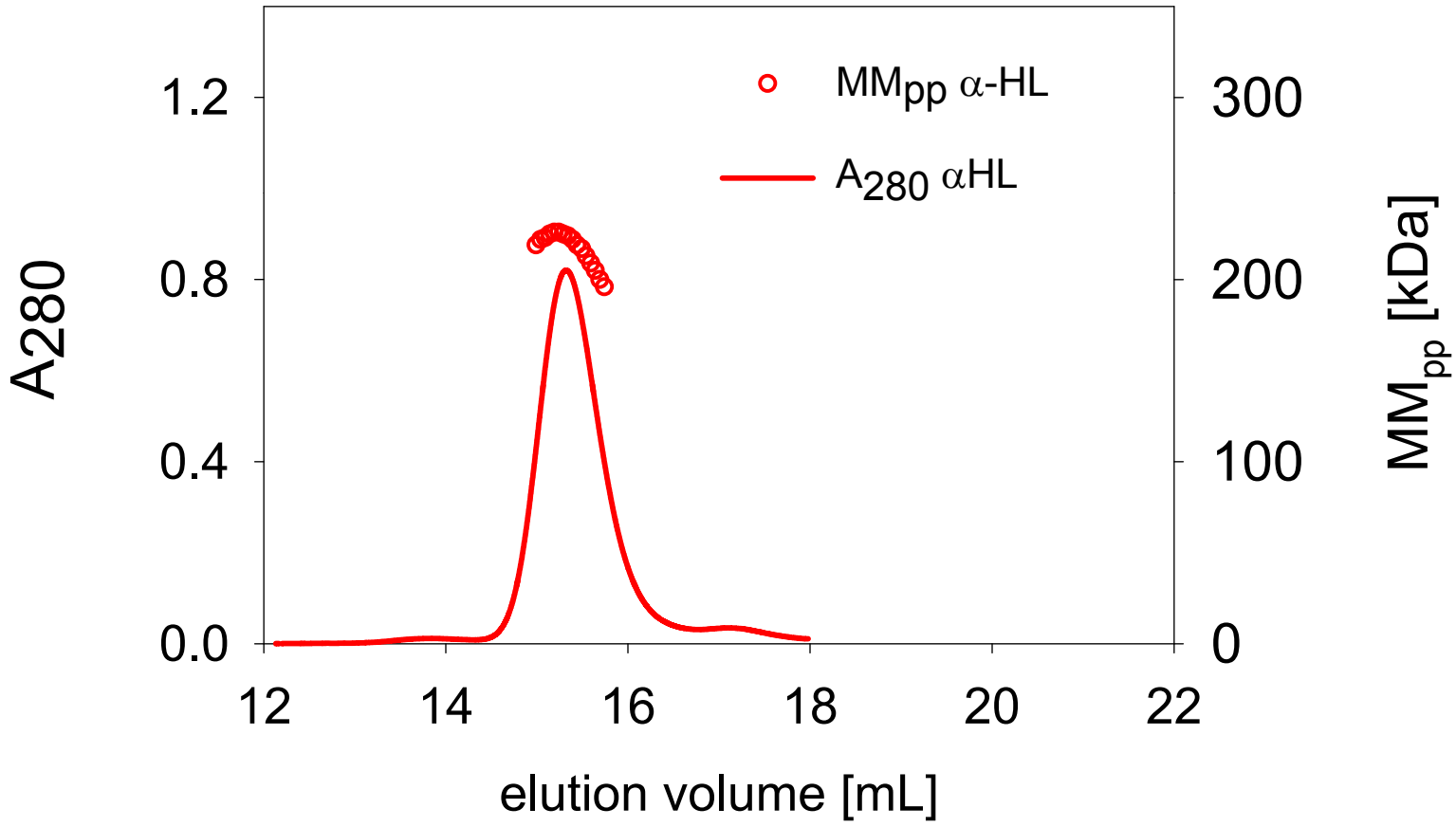
dodecyl maltoside (C12M) MW = 511 g/mol

0.5g/L i.e. 0.05%

CMC = 0.008% micelle size 50-70 kDa

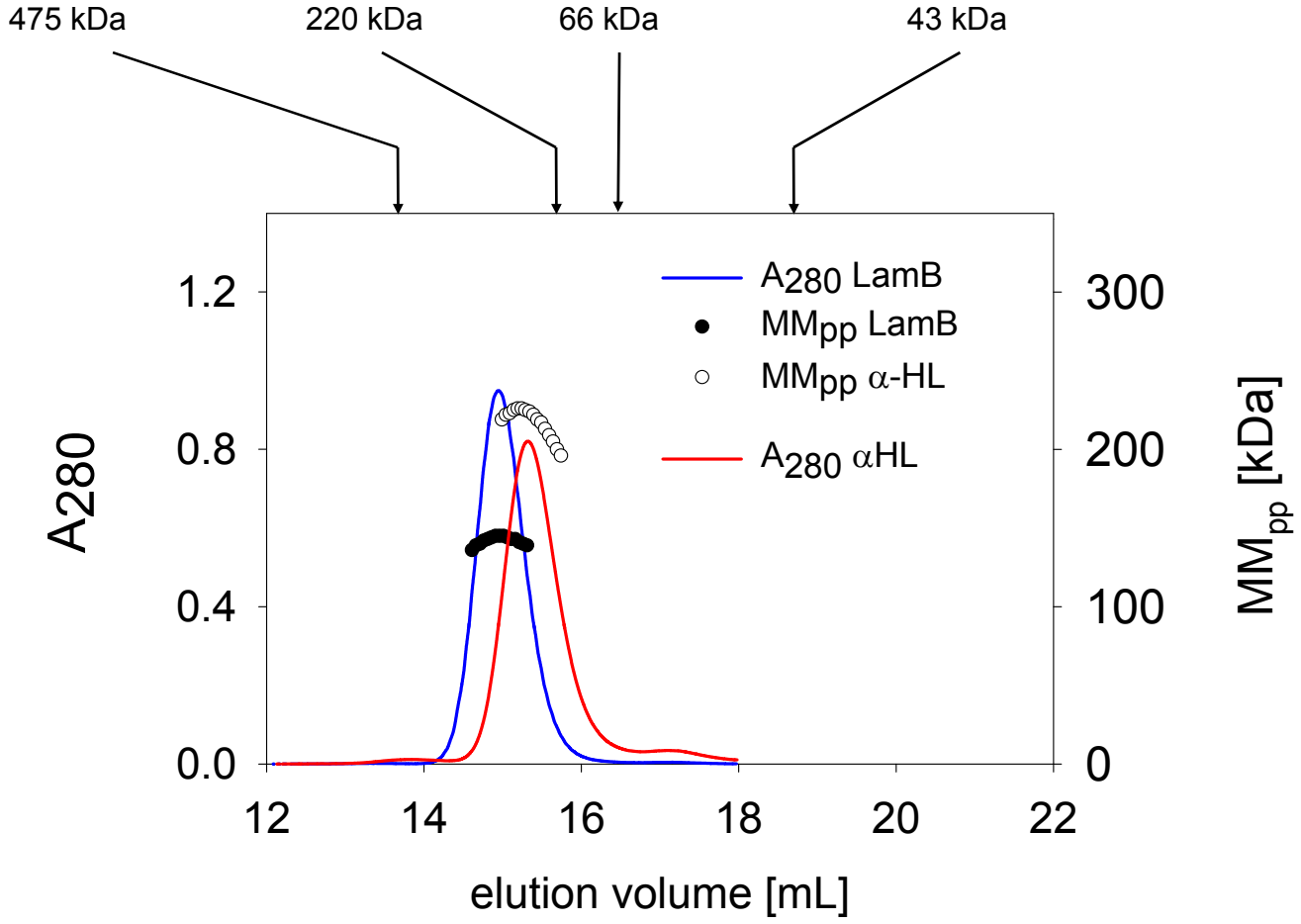
monomer = 33 kDa

MW = 225 ± 13 kDa heptamer



**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

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

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

Protein+lipid+detergent

Protein

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

$\delta$ 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

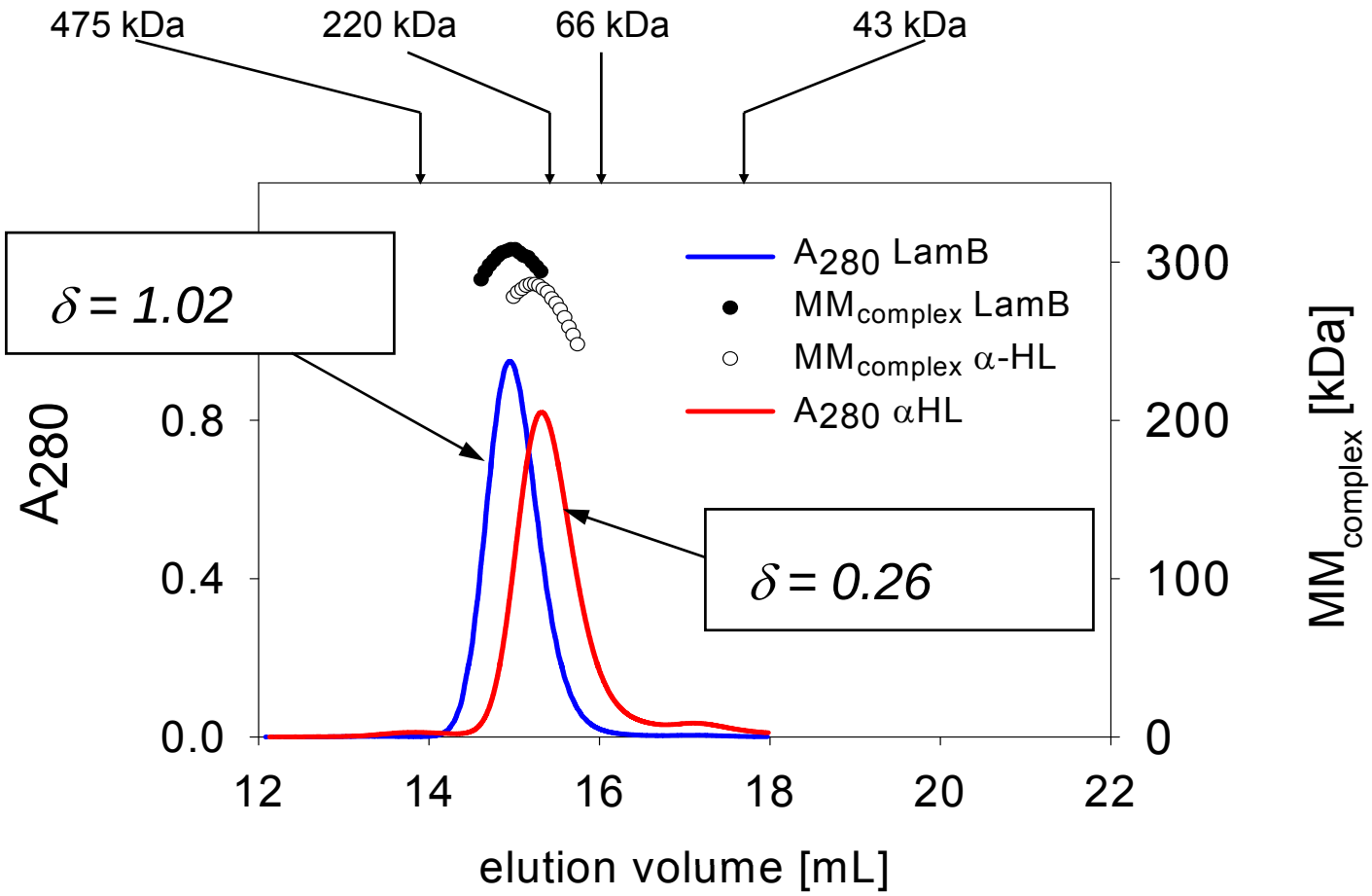
$MW_{polypeptide}$ = 141 kDa

$\delta$ = 1.02 lipids per 1 gram of polypeptide

$MW_{complex}$ = 271 kDa

$MW_{polypeptide}$ = 215 kDa

$\delta$ = 0.26 lipids per 1 gram of polypeptide



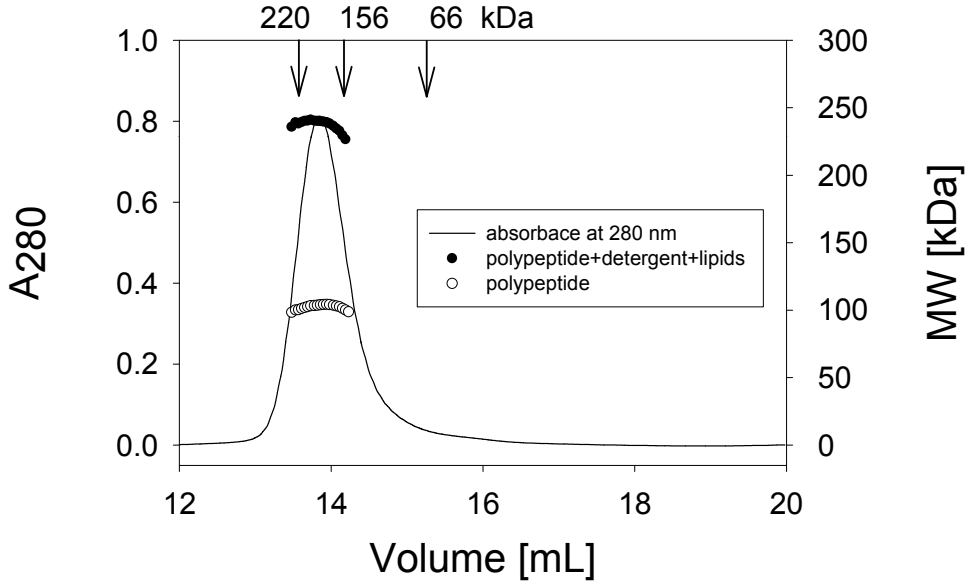

Yernool D, Boudker O., Folta-Stogniew E., and Gouaux E. (2003) Trimeric subunit stoichiometry of the glutamate transporters from Bacillus caldotenax and Bacillus stearothermophilus. *Biochemistry* **42**: 12981-12988

# Multiple oligomeric states for reconstituted KtrAB $K^+$ Transporter

KtrAB ion transporter:

complex of KtrB membrane protein and KtrA RCK domain (regulating and conductance of $K^+$)

**KtrB: integral membrane protein** isolated in the presence of detergent (DDM) as a polypeptide:detergent(lipid) complex




| Protein | Polypeptide [kDa] | Oligomeric state | Full complex [kDa] | Grams of detergent/lipids per gram of polypeptide |
|---|---|---|---|---|
| KtrB (monomer 49kDa) | 98 | dimer | 238 | 1.4 |

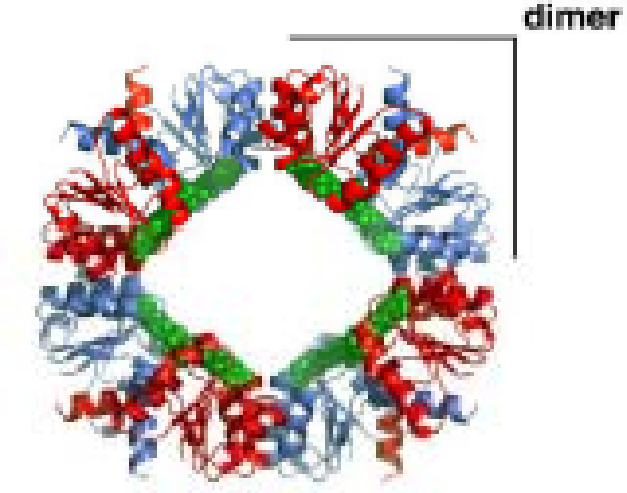
Albright R A, Ibar J. L. V., Kim C. U., Gruner S. M., and Morais-Cabral J. H. (2006) The RCK Domain of the KtrAB K+ Transporter: Multiple Conformations of an Octameric Ring. *Cell* **126**: 1147-1159

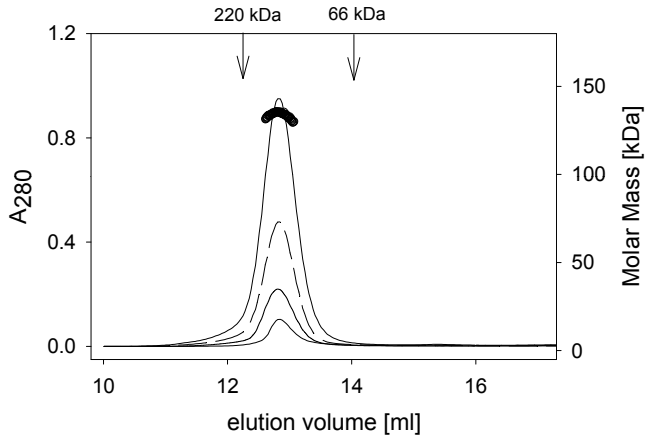
## Multiple oligomeric states for reconstituted KtrAB $K^+$ Transporter

KtrAB ion transporter:

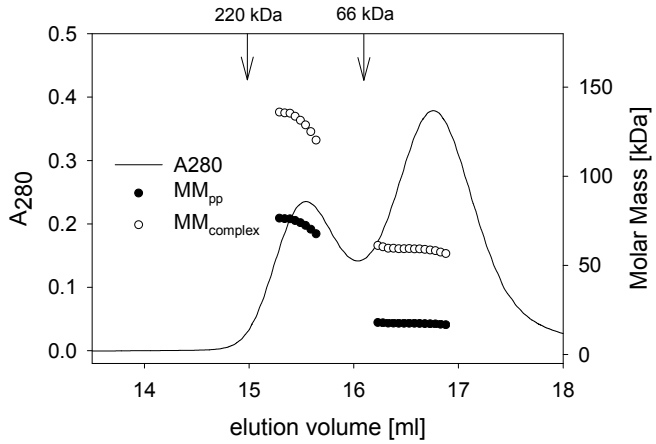
complex of KtrB membrane protein (49 kDa) and KtrA RCK domain (regulating and conductance of $K^+$)

KtrA RCK domain (16 kDa) : basic assembly dimer, higher order oligomers: tetramer or octamer




Buffer + DDM

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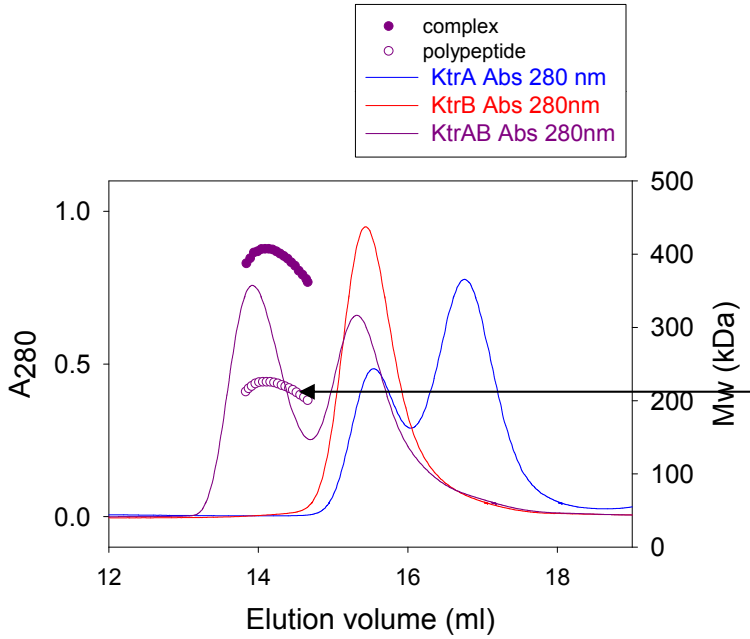
# Multiple oligomeric states for reconstituted KtrAB $K^+$ Transporter

KtrAB ion transporter

complex : KtrAB

octameric KtrA + dimeric KtrB (8:2) model polypeptide = 228 kDa

octameric KtrA + 2x dimeric KtrB (8:4) model polypeptide = 325 kDa




octameric KtrA + 2x dimeric KtrB (8:4) model
polypeptide = 325 kDa

octameric KtrA + dimeric KtrB (8:2) model
polypeptide = 228 kDa

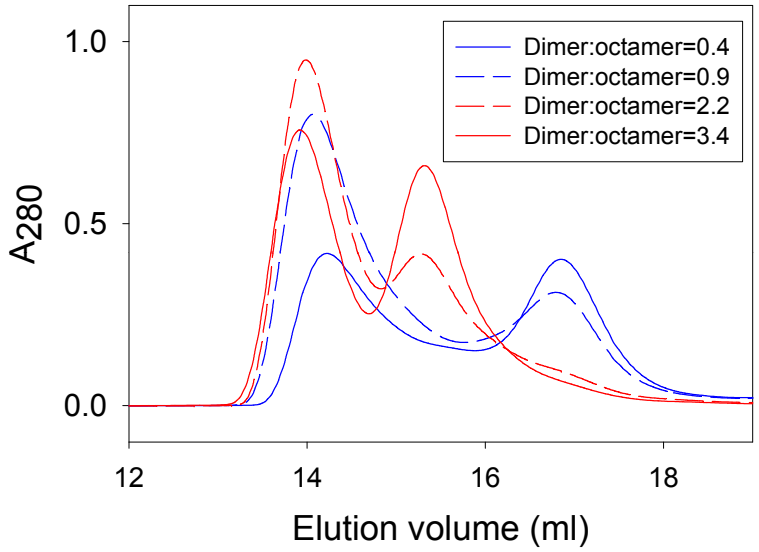
Buffer: 25 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM NADH, 1 mM DDM

# Multiple oligomeric states for reconstituted KtrAB $K^+$ Transporter

KtrAB ion transporter

(8:2) model polypeptide = 228 kDa

(8:4) model polypeptide = 325 kDa




| dimer:octamer KtrB:KtrA | complex | Elution volume (ml) | Total mass of complex (kDa) | Poly-peptide (kDa) | lipids (kDa) |
|---|---|---|---|---|---|
| 0.4 | 8:2 | 14.23 | 486 | 228 | 256 |
| 0.9 | 8:2 | 14.05 | 521 | 240 | 281 |
| 2.2 | 8:4 | 13.99 | 552 | 302 | 261 |
| 3.7 | 8:4 | 13.91 | 560 | 299 | 251 |

| dimer:octamer KtrB:KtrA | Excess KtrB dimer? | Elution volume (ml) | 8:2 model (228 kDa) | | correct model ? | 8:4 model (325 kDa) | | correct model ? |
|---|---|---|---|---|---|---|---|---|
| | | | computed MW for complex (kDa) | difference from model (kDa) | | computed MW for complex (kDa) | difference from model (kDa) | |
| 0.4 | | 14.23 | 228 | 0 | Yes | 250 | -75 | |
| 0.9 | | 14.05 | 240 | 12 | Yes | 264 | -61 | |
| 2.2 | Yes | 13.99 | 274 | 46 | | 302 | -24 | *Yes* |
| 3.7 | Yes | 13.91 | 271 | 43 | | 299 | -27 | *Yes* |

## Three detector approach

- fast and accurate determination of oligomeric state of detergent solubilized membrane proteins
- only protein sequence needed for determination of association state
- the amount of detergent and lipids associated with the polypeptide can be determined from a single SEC-LS/RI/UV analysis
- suitable for variety of detergents
- can be used at wide range of protein concentrations
  from ~ 10µg/ml to >10mg/ml (correction for non-ideality)

Ken Williams

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

# NIH

1S10 RR014776-01 “SEC/Laser Light Scattering Instrumentation”
1S10 RR023748-01 "Asymmetric flow FFF and Composition Gradient/Light Scattering System"

## Users of SEC/LS Service

Light Scattering Services contributed to> 40 publications

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

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

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