# Application of Light Scattering

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*Yale University*

# Biophysics Resource of Keck Laboratory: Yale School of Medicine

**Mission:** quantitative characterization of interactions between biomolecules using in solution biophysical methods

## Common questions:

- how tight is the binding ? ( binding affinity: $K_d$, $K_a$)
- how many of each molecule are in the complex (stoichiometry)
- how fast does the complex form? (kinetics)
- is the binding event enthalpy or entropy-driven? (thermodynamics)

## List of technologies:

- Size Exclusion Chromatography coupled with Light Scattering (SEC/LS)
- Dynamic Light Scattering (DLS)
- Isothermal MicroCalorimeter (ITC)
- Spectrofluorometer
- Stopped-Flow Spectrofluorometer
- Surface Plasmon Resonance (SPR) Sensor [BiaCore Biosensor; T100]
- Composition Gradient Static Light Scattering (CGSLS)
- Asymmetric flow Field-Flow Fractionation (AFFF)

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

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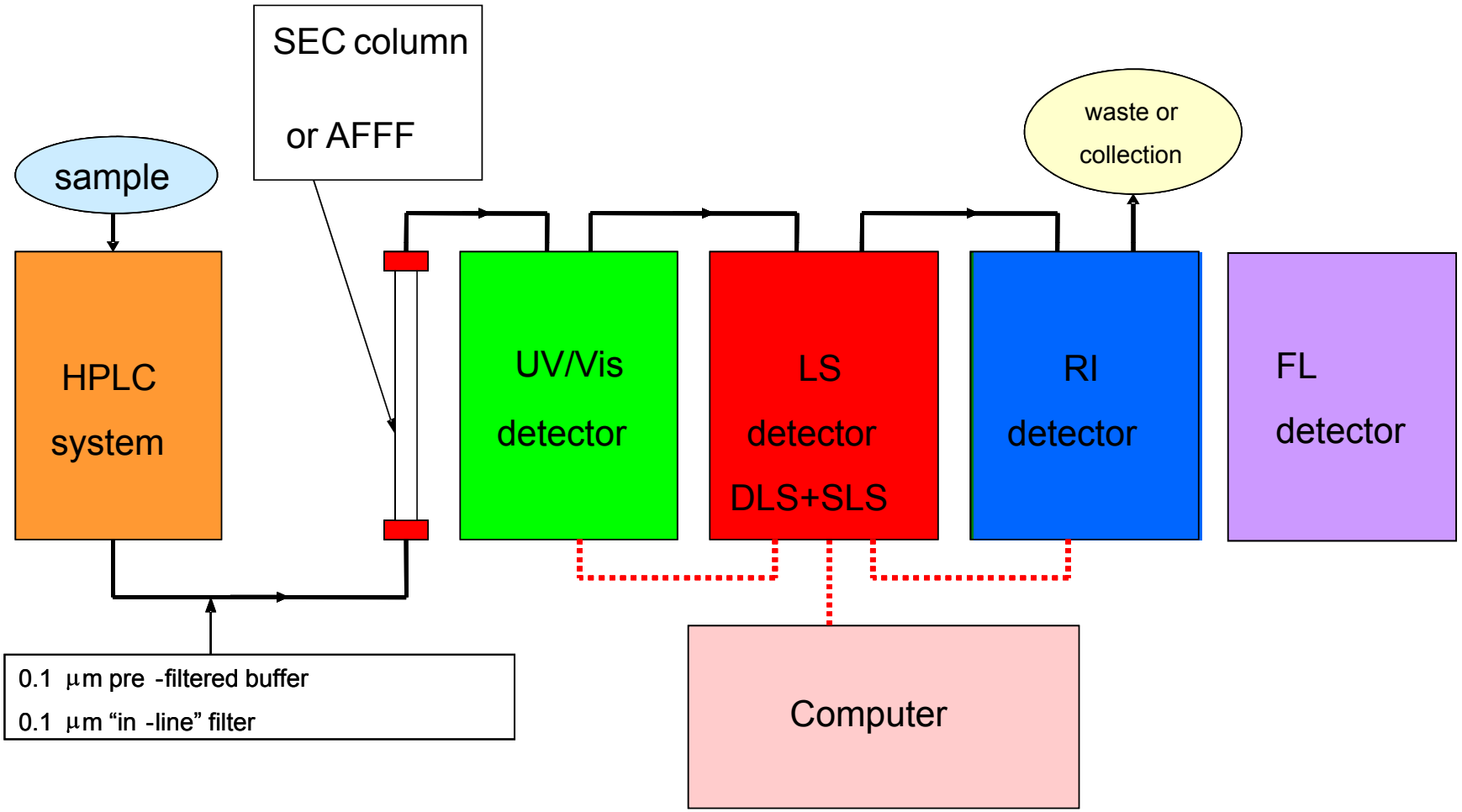
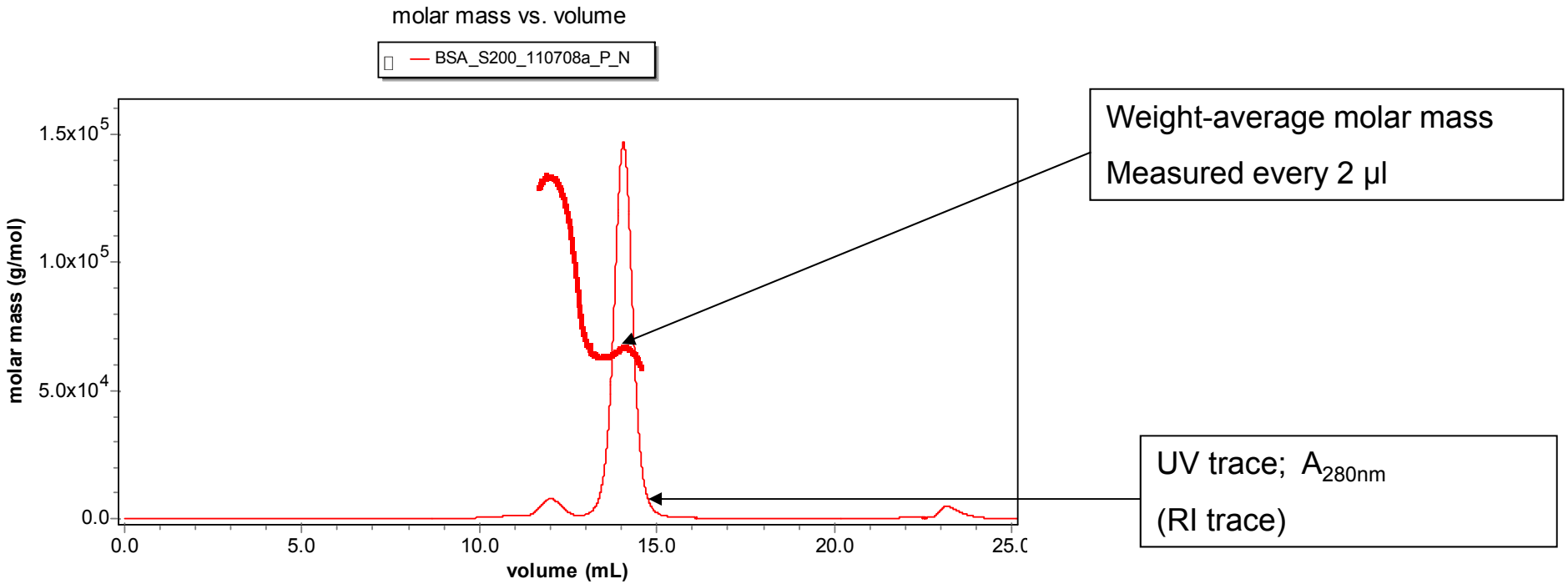
# Typical SEC(AFFF); MALLS system



SEC/LS results: Molar Mass Distribution Plot

BSA

Monomer: 66 kDa



# Is that ALL?

SEC/LS results: Molar Mass Distribution Plot

BSA

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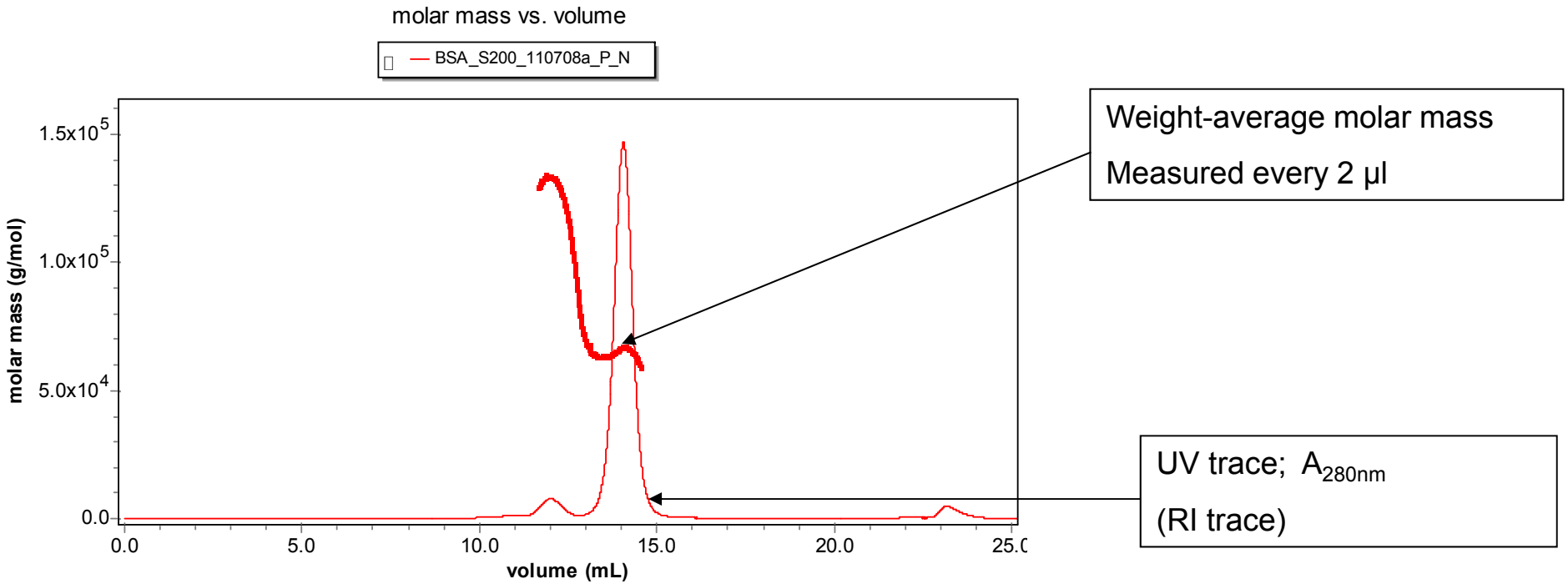
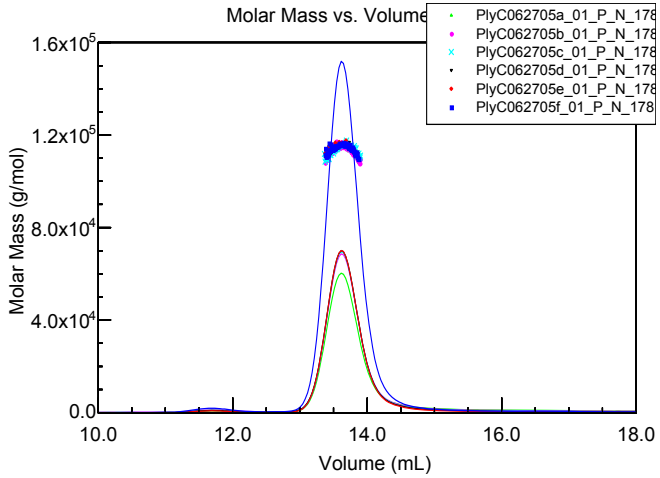


The streptococcal C1 bacteriophage lysin, PlyC,

Holoenzyme is a multimeric protein:

50.3 kDa, “catalytic” subunit  Ext. coeff. $A^{0.1\%}_{280}$ = 2.2

8.0 kDa, “binding” subunit  Ext. coeff. $A^{0.1\%}_{280}$ = 0.3




SEC/LS MW= 114.0±0.4 kDa

PlyC 1 big+8 small predicted MW = 114.3 kDa

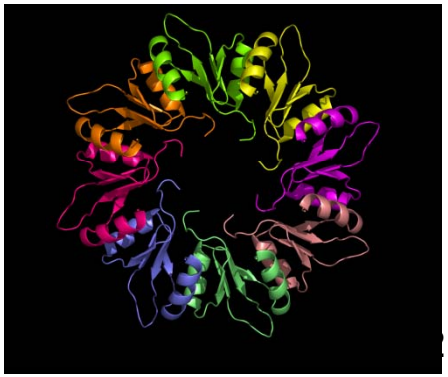
SEC/LS accuracy ~3 % , i.e. ~ 3kDa for PlyC

PlyC 1 big+8 small MW = 114.3 kDa  Ext. coeff. $A^{0.1\%}_{280}$ = 1.2

PlyC_bis 2 big+2 small MW = 116.6 kDa  Ext. coeff. $A^{0.1\%}_{280}$ = 2.0

Nelson D, Schuch R., Chahales P., Zhu S., and Fischetti V. A. (2006) PlyC: A multimeric bacteriophage lysin. *Proceedings of the National Academy of Sciences* **103**: 10765-10770

"on-line" determination of extinction coefficient [a] from UV/RI ratio

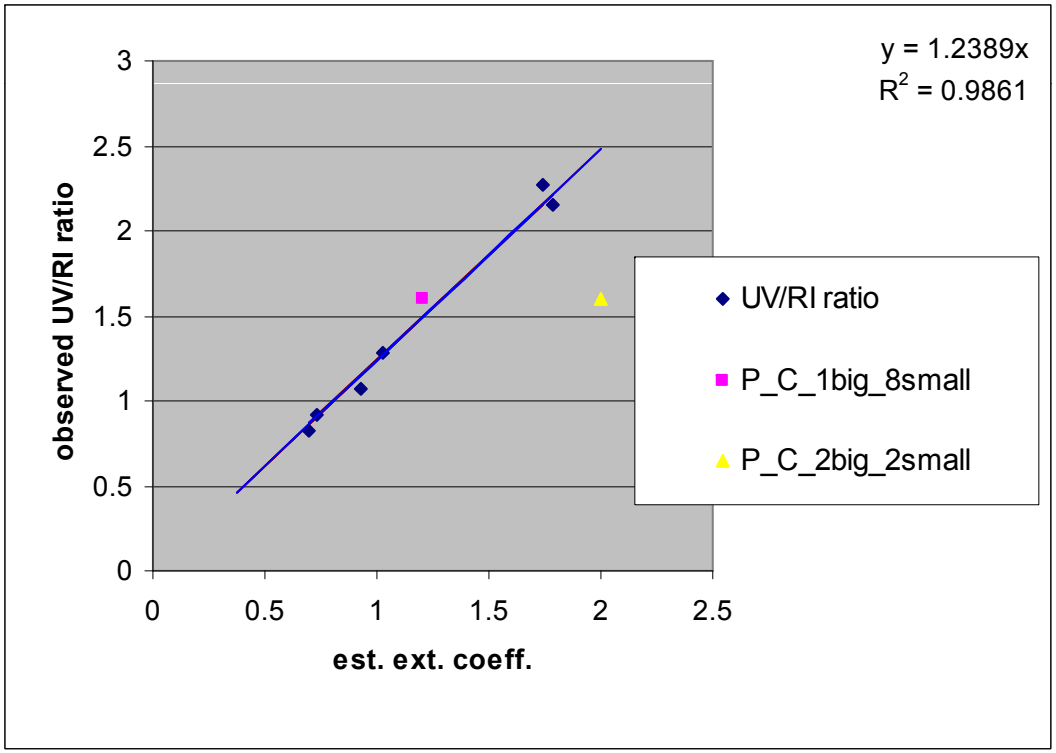


**Octameric PlyCB.** The eight PlyCB subunits arranged in a ring as observed in the crystal structure of PlyC.

**Evaluated models:**

1 big+8 small MW= PlyC model (1+8)

2 big+2 small MW= PlyC_bis model (2+2)




| Protein | Ext. coeff. Est. | UV/RI ratio | | residual ^2 |
|---|---|---|---|---|
| | | observed | computed | |
| Apo | 1.026 | 1.279 | 1.271 | 0.000 |
| BAM | 1.788 | 2.147 | 2.215 | 0.005 |
| BSA | 0.700 | 0.821 | 0.867 | 0.002 |
| CA | 1.737 | 2.273 | 2.152 | 0.015 |
| OVA | 0.730 | 0.919 | 0.904 | 0.000 |
| Ti | 0.928 | 1.070 | 1.150 | 0.006 |
| **PlyC (1+8)** | **1.204** | **1.600** | **1.491** | **0.012** |
| **PlyC_bis (2+2)** | **2.000** | **1.600** | **2.478** | **0.770** |


[a] Philo J S, Aoki K. H., Arakawa T., Narhi L. O., and Wen J. (1996) Dimerization of the Extracellular Domain of the Erythropoietin (EPO) Receptor by EPO: One High-Affinity and One Low-Affinity Interaction. *Biochemistry* **35**: 1681-1691

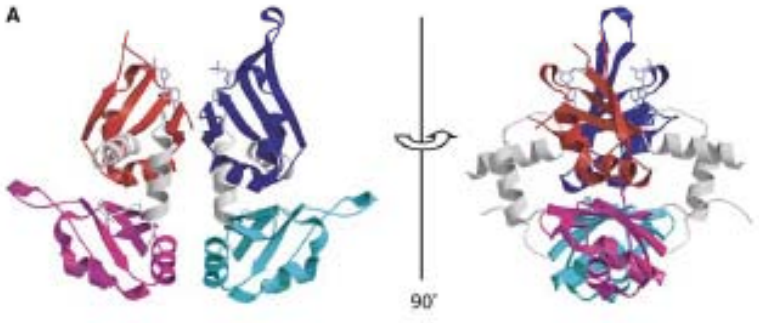
Nelson D, Schuch R., Chahales P., Zhu S., and Fischetti V. A. (2006) PlyC: A multimeric bacteriophage lysin. Proceedings of the National Academy of Sciences 103; 10765-10770

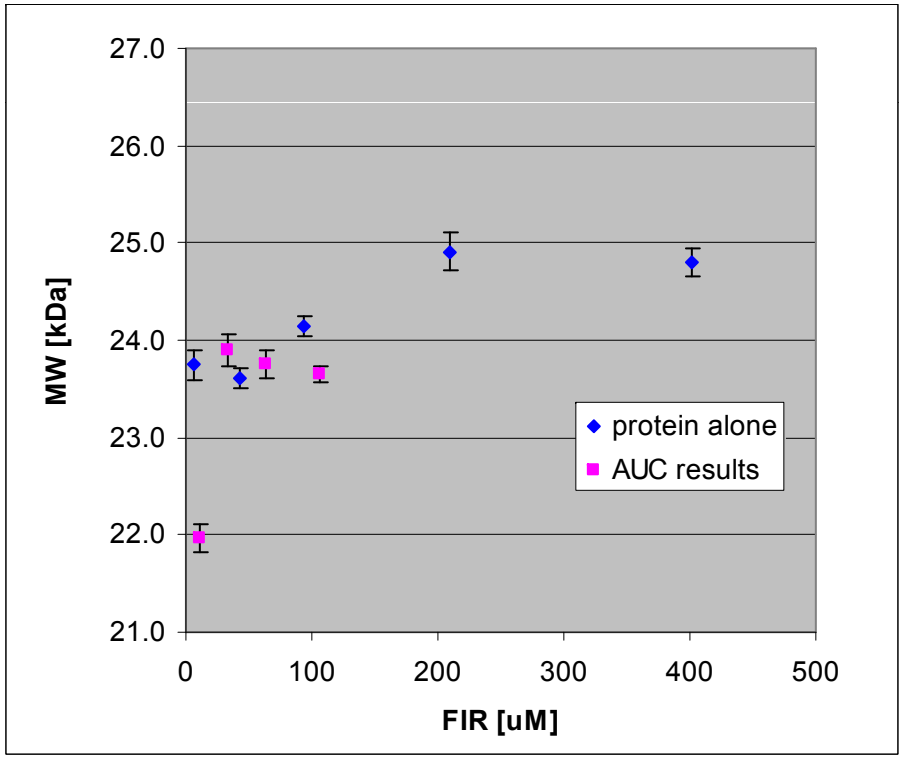
# Dimerization of FIR

FIR: human *c-myc* FarUpStream Element (FUSE) Binding Protein (FBP) Interacting Repressor (FIR)

FIR protein fragment: first two RRM domains

FIR: 23.4  kDa monomer; seen as a dimer in the X-ray structure



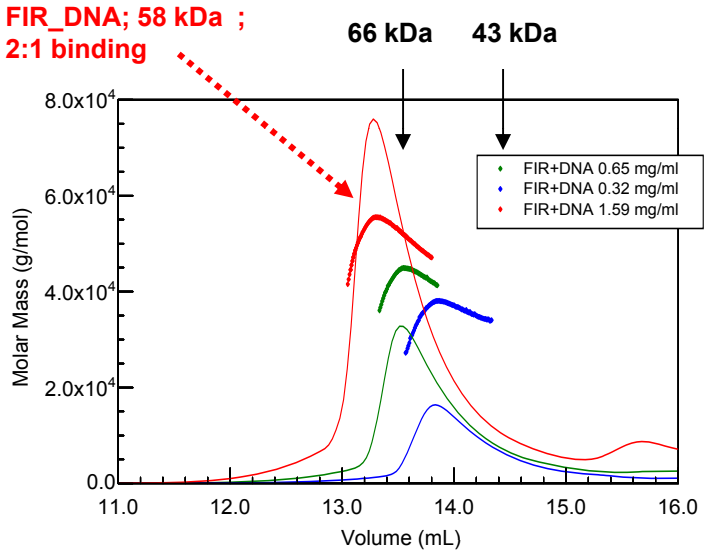

Crichlow G V, Zhou H., Hsiao H. H., Frederick K. B., Debrosse M., Yang Y., Folta-Stogniew E. J., Chung H. J., Fan C., De la Cruz E. M., Levens D., Lolis E., and Braddock D. (2008) Dimerization of FIR upon FUSE DNA binding suggests a mechanism of c-myc inhibition. *EMBO J* **27**: 277-289

# Dimerization of FIR depends on DNA binding event

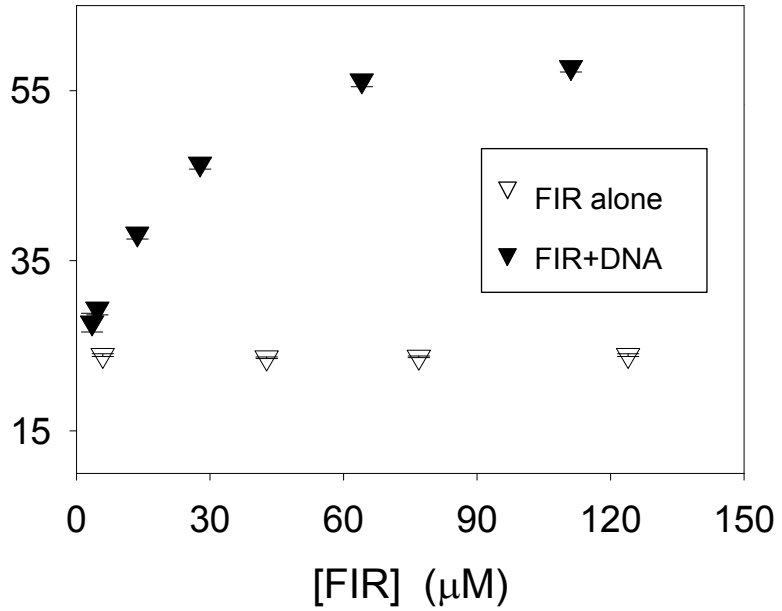
FIR protein: 23 kDa monomer

ssDNA fragment upstream of the P1 promoter, known as FUSE; 8 kDa

**FIR+DNA complex**; task: determine stoichiometry of the FIR+DNA complex in solution




| FIR-DNA complexes | MW (kDa) |
|---|---|
| FIR+DNA (2:1) complex | 54.7 |
| FIR+DNA (2:2) complex | 62.8 |
| Observed MW | 57.7 |




Concentration dependent measurements reveal that in solution the dimerization is driven by DNA binding

Crichlow, G. V., Zhou, H., Hsiao, H-h., Frederick, K. B.,, Debrosse, M., Yuande Yang, Y., Folta-Stogniew, E. J., Chung, H-J., Chengpeng Fan, C., De La Cruz, E., Levens, D., **Lolis, E.,** and **Braddock, D.** (2008) "Dimerization of FIR upon FUSE binding suggests a mechanism of c-myc inhibition", *EMBO J* ***27****: 277-289*

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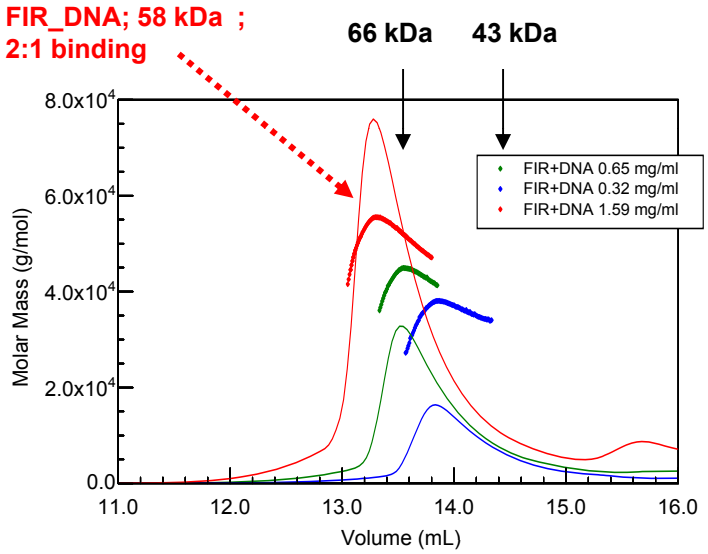


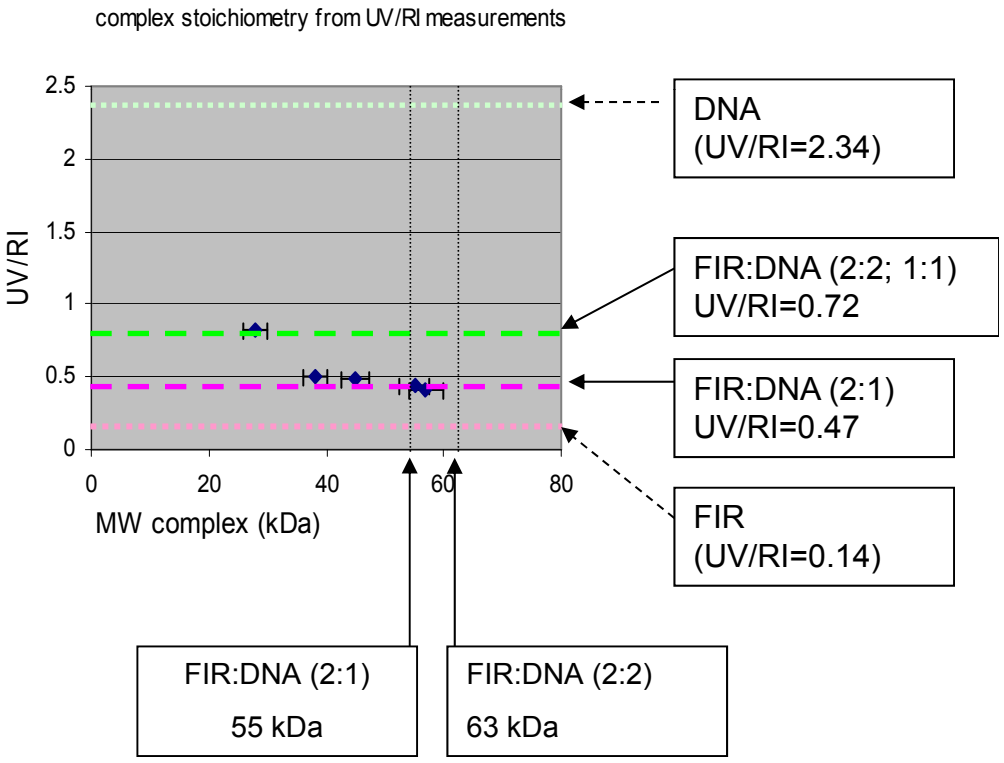


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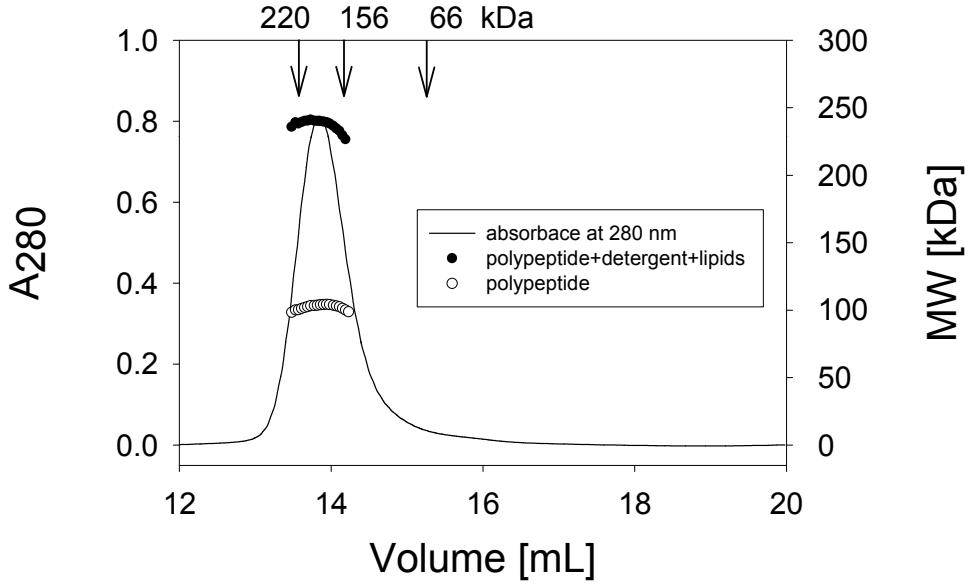
# Multiple oligomeric states for reconstituted KtrAB $K^+$ Transporter

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

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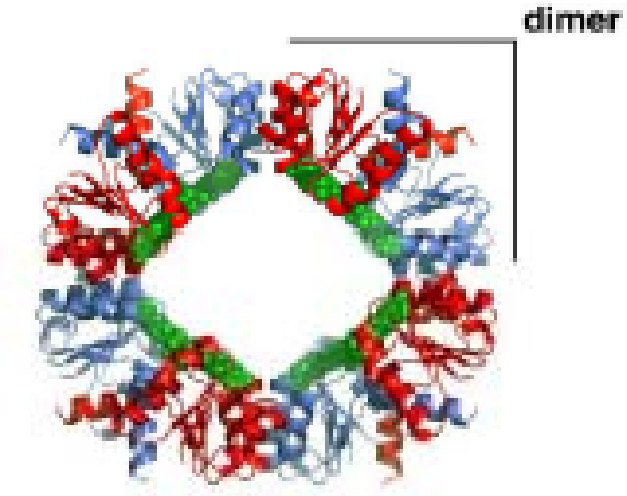
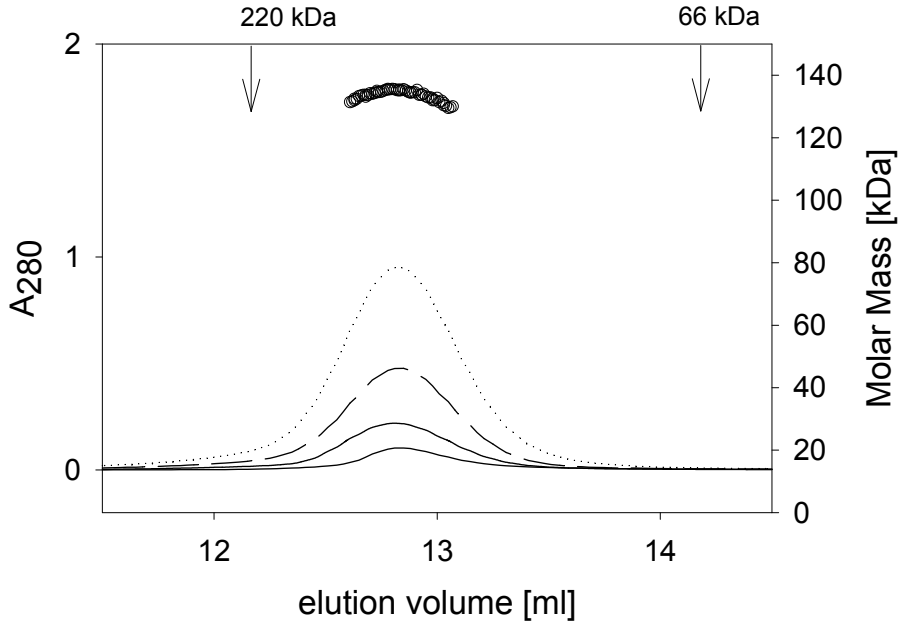
complex of KtrB membrane protein and KtrA RCK domain (regulating and conductance of $K^+$)

KtrA RCK domain : basic assembly dimer, higher order oligomers: tetramer or octamer

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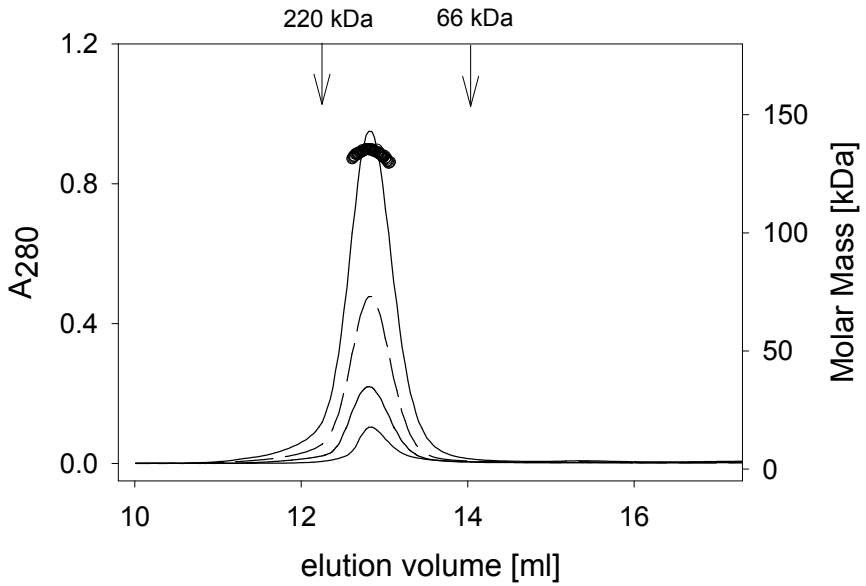
Effects of detergent on oligomeric state of KtrA RCK domain

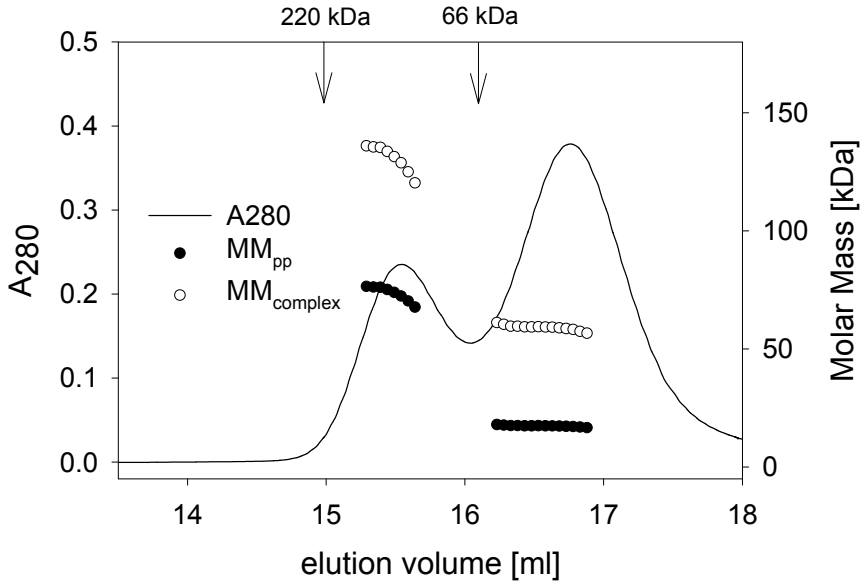
KtrA RCK domain no detergent

(octamer)




KtrA RCK domain plus detergent

(tetramer and monomer) + micelle



# Determination of dimerization constant from SEC-LS measurements

## Input:

- SEC/LS analyses at several eluting concentrations

## Results:

- Determination of dimerization constant

# Determination of dimerization constant from SEC-LS measurements

**SecA protein** (nanomotor promotes protein translocation in eubacteria)

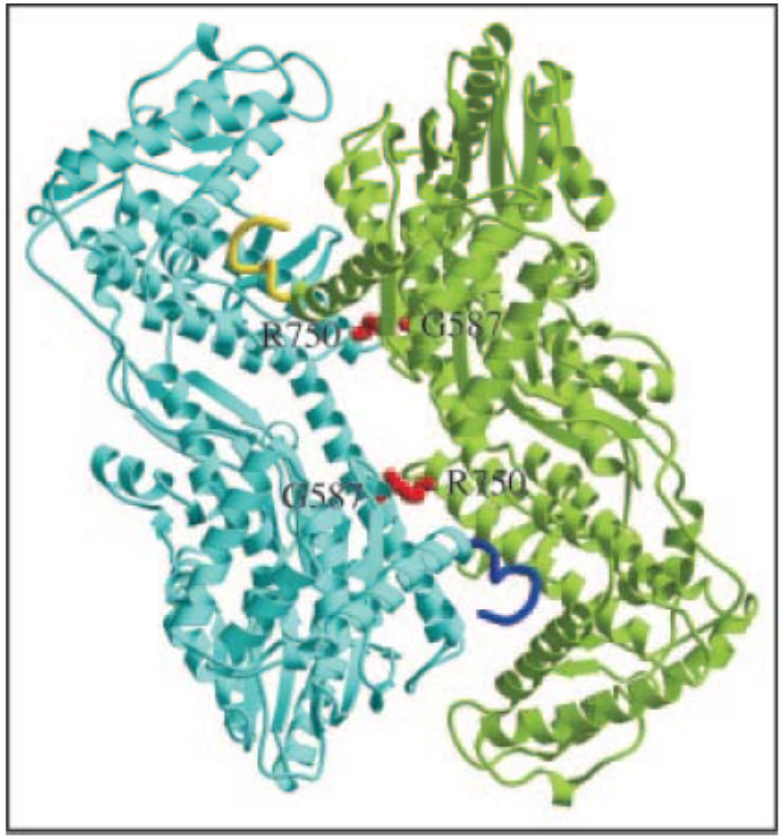
conflicting reports about whether SecA functions as a monomer or dimer

WT monomer = 102 kDa

DS8 deletion mutant monomer = 101 kDa

D11 deletion mutant monomer = 100 kDa

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|
| Met | Leu | Ile | Lys | Leu | Leu | Thr | Lys | Val | Phe | Gly |




The two subunits in the crystal structure of *B. subtilis* SecA
The first nine residues of each subunit are shown in *yellow* and *blue*[a].

[a]Picture taken from : Or, E., A. Navon, and T. Rapoport. 2002. Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane. EMBO J. 21:4470-4479

SecA protein

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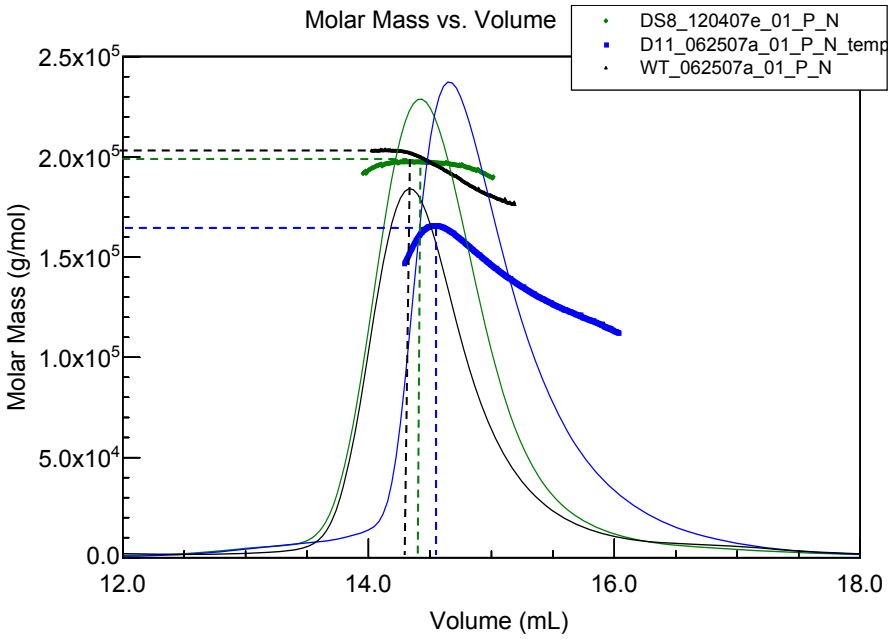
**Low salt buffer:**

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

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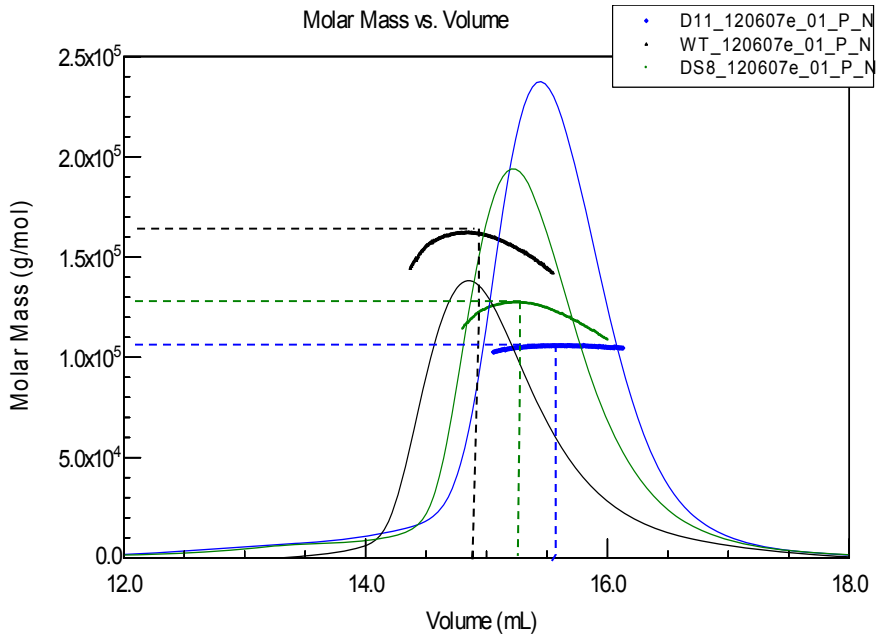
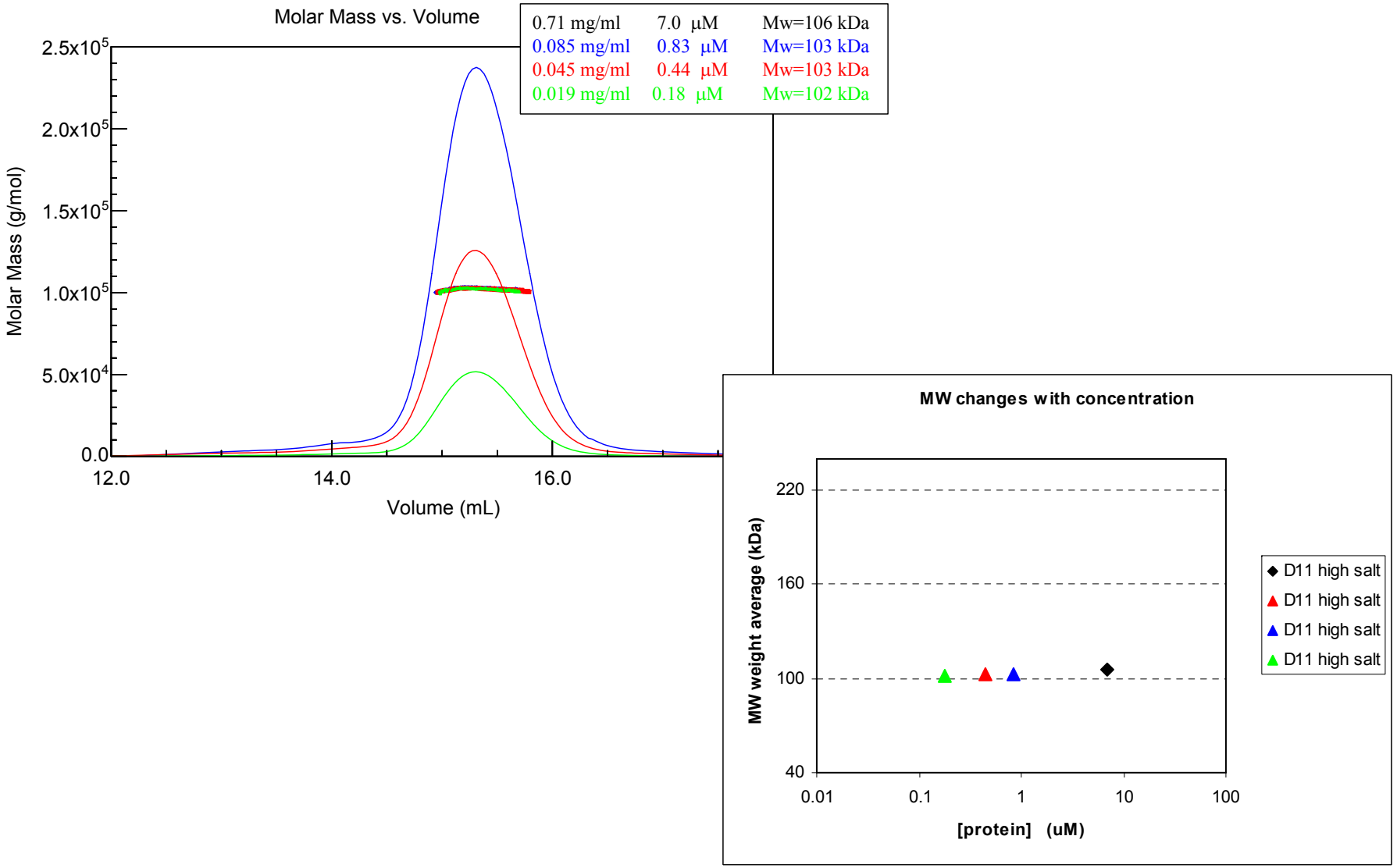


**D11** deletion mutant
mono= **101 kDa**

**High salt buffer:**

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



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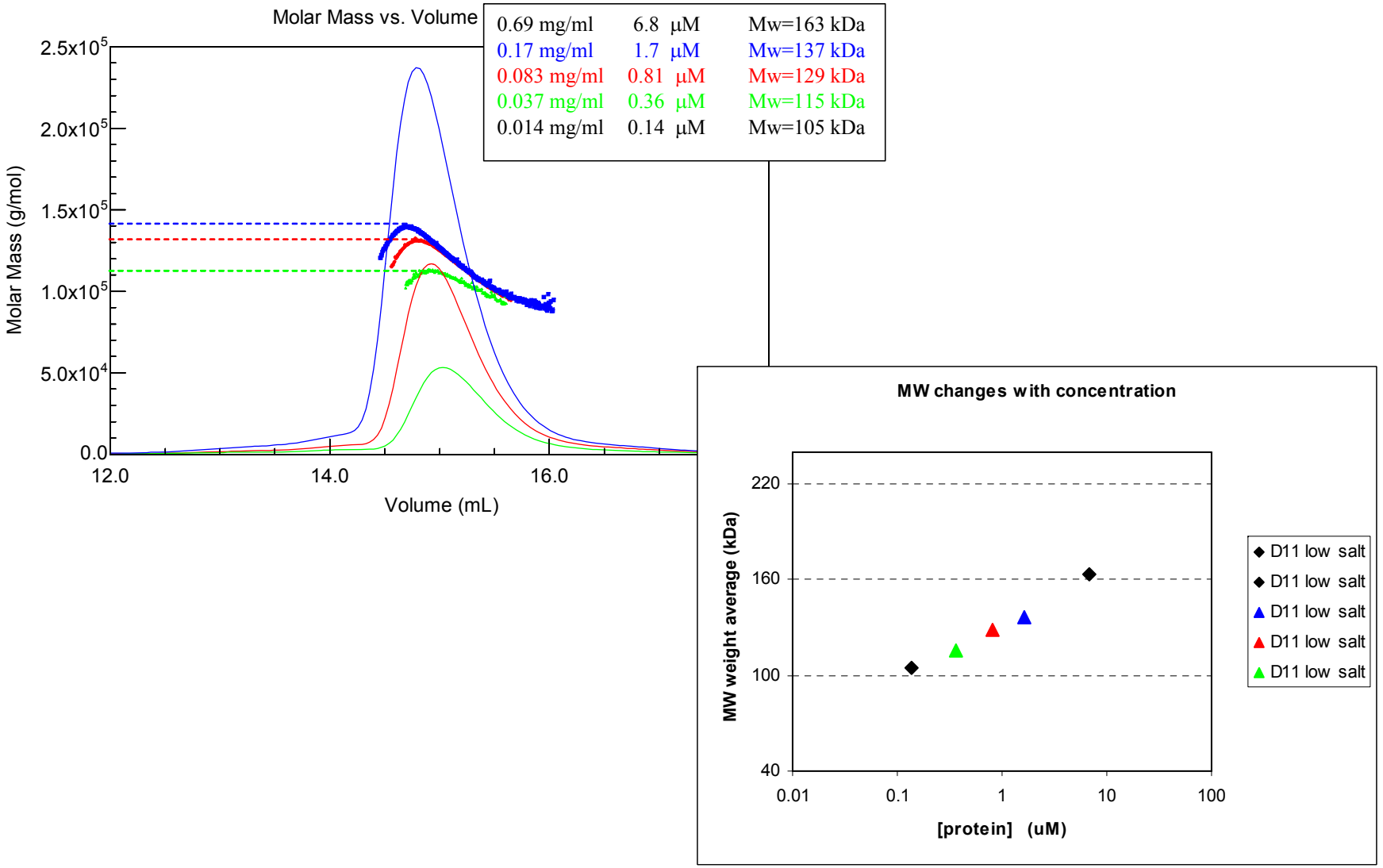

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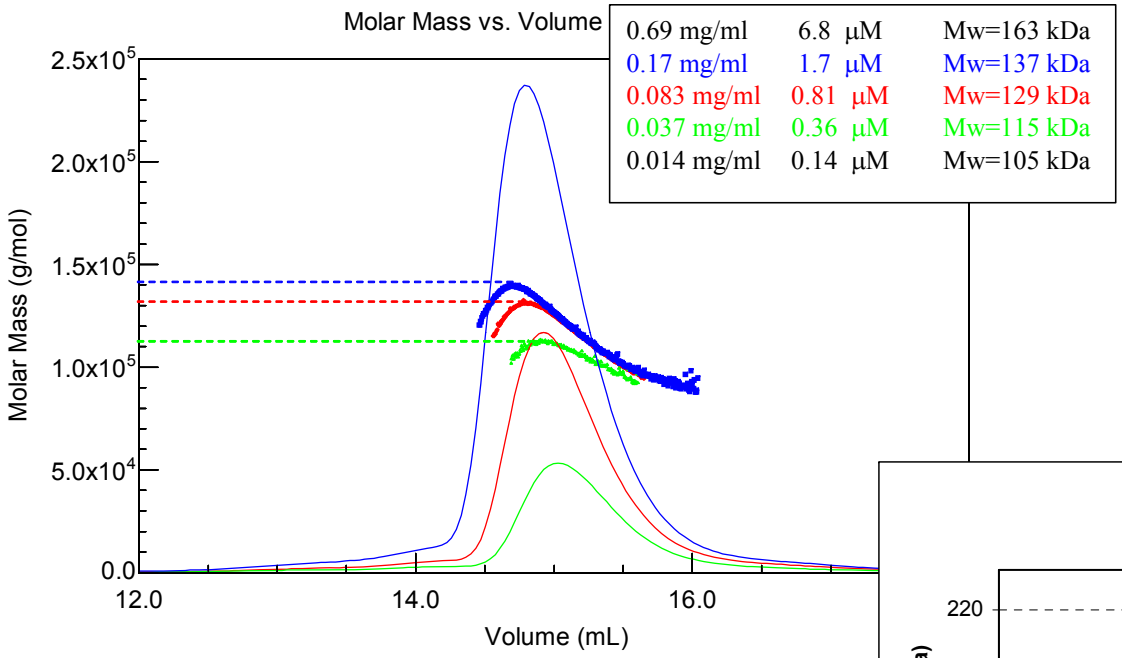


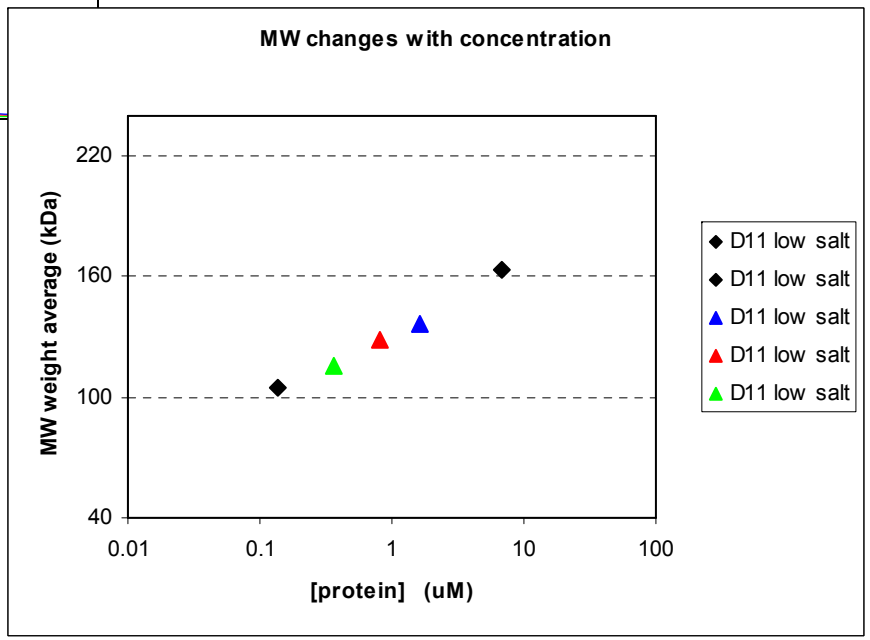


$$M_w = f_m M_m + f_d M_d = M_m(2 - f_m)$$

$$2M = D$$

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

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

WT monomer = 102 kDa

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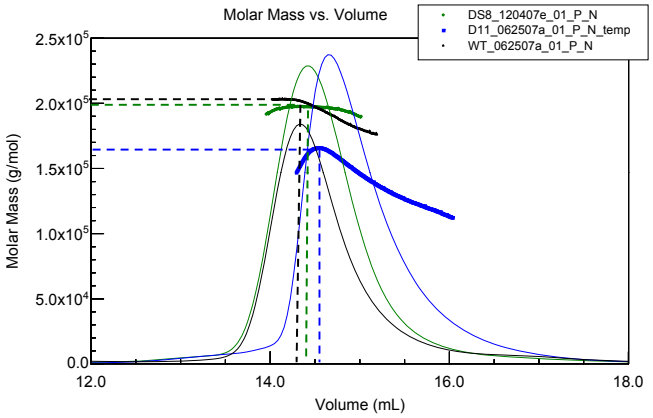
D11 deletion mutant monomer = 100 kDa

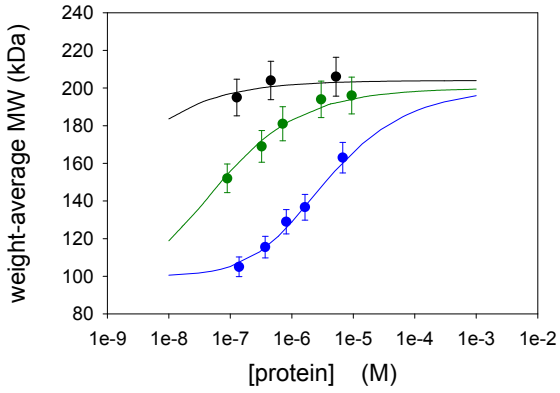
**Low salt buffer: 100 mM KCl**



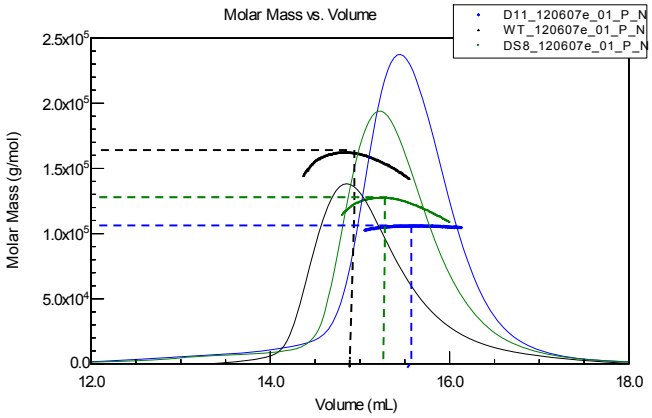

WT Kd= <1e-9

DS8 Kd=7±1e-8 M

D11 Kd=3.5±0.2e-6 M



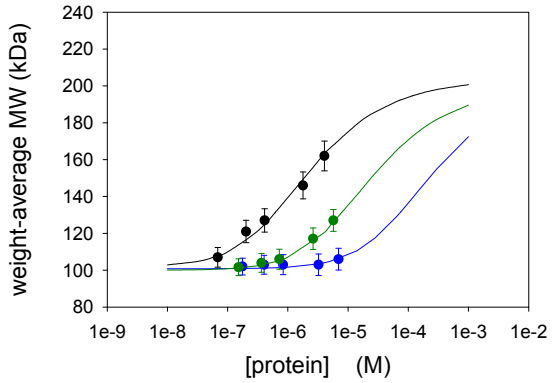

**High salt buffer: 300 mM KCl**




WT Kd= 2.2±0.2e-6 M

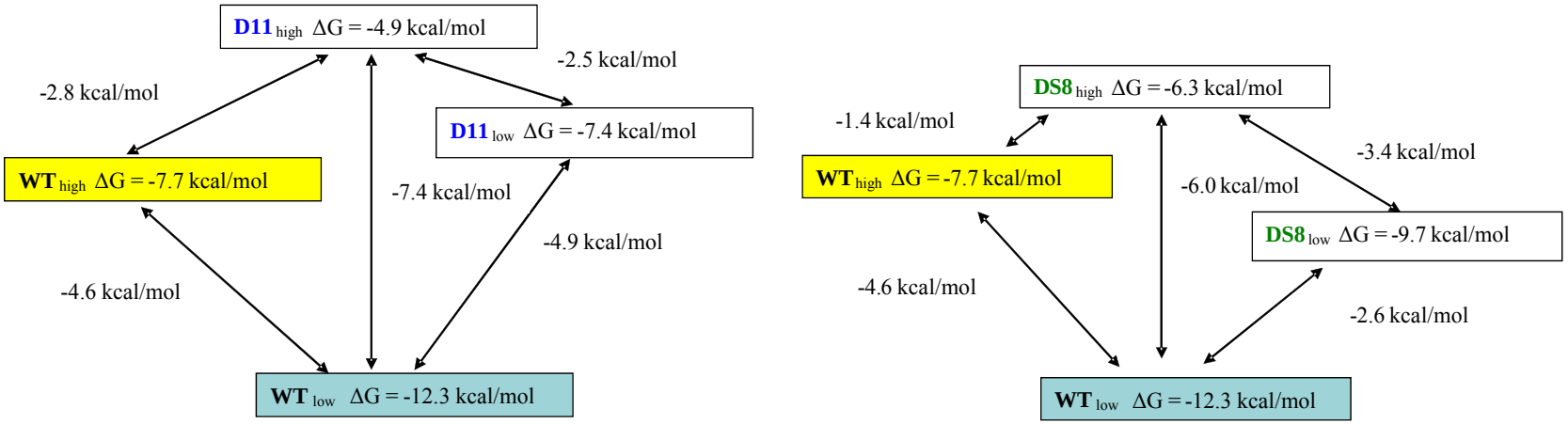
DS8 Kd= 2.41±0.05e-5 M

D11 Kd> 2.4e-4 M



# Thermodynamic linkage for SecA dimerization from SEC/MALLS

| Protein | Low salt (100 mM) | | High salt (300 mM) | |
|---|---|---|---|---|
| | $K_d$ [M] | ΔG dimer (kcal/mol) | $K_d$ [M] | ΔG dimer (kcal/mol) |
| WT | $<1x10^{-9}$ | -12.3 | $2.2\pm0.2x10^{-6}$ | -7.7 |
| DS8 | $7\pm1x10^{-8}$ | -9.7 | $2.41\pm0.05x10^{5}$ | -6.3 |
| D11 | $3.5\pm0.2x10^{-6}$ | -7.4 | $>2.4x10^{-4}$ | -4.9 |




Das S, Stivison E, Folta-Stogniew E, Oliver D. (2008) "Re-examination of the Role of the Amino-Terminus of SecA in Promoting Its Dimerization and Functional State", *J Bacteriol.* (2008) **190**;7302-7307.

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# Why no AUC data?

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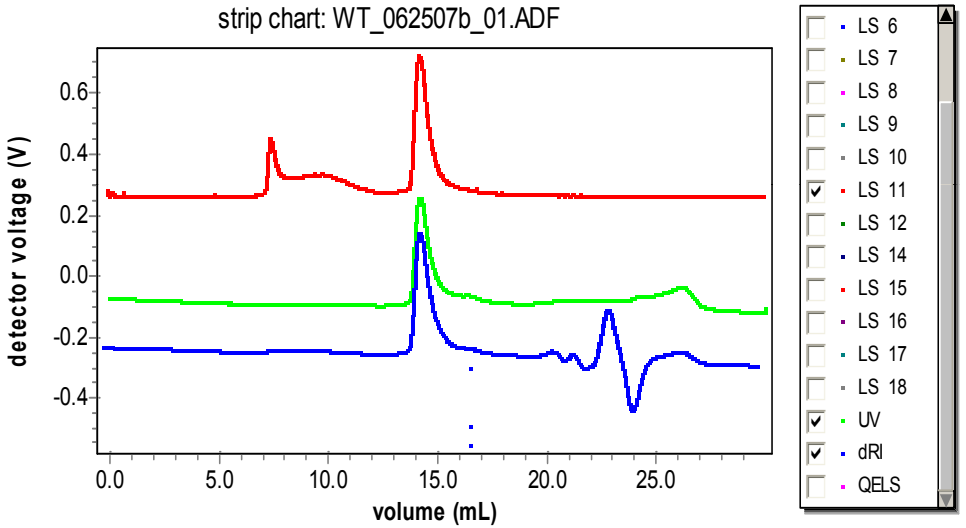
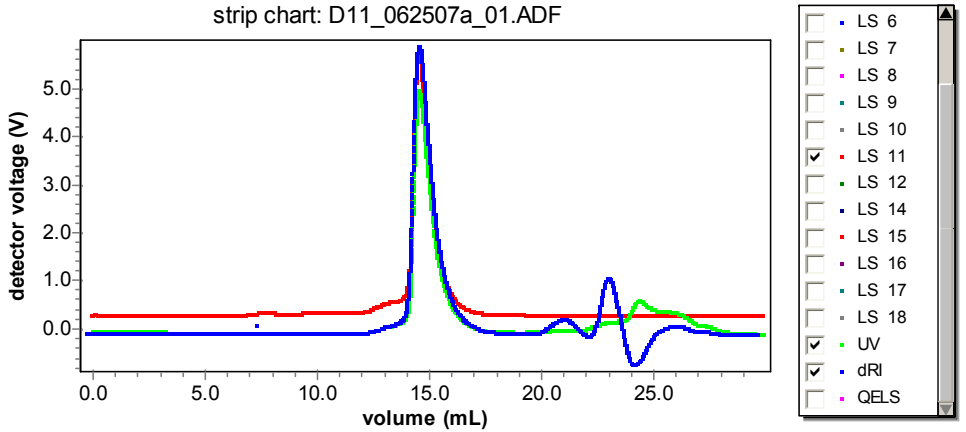
1 2 3 4 5 6 7 8 9 10 11

Met Leu Ile Lys Leu Leu Thr Lys Val Phe Gly



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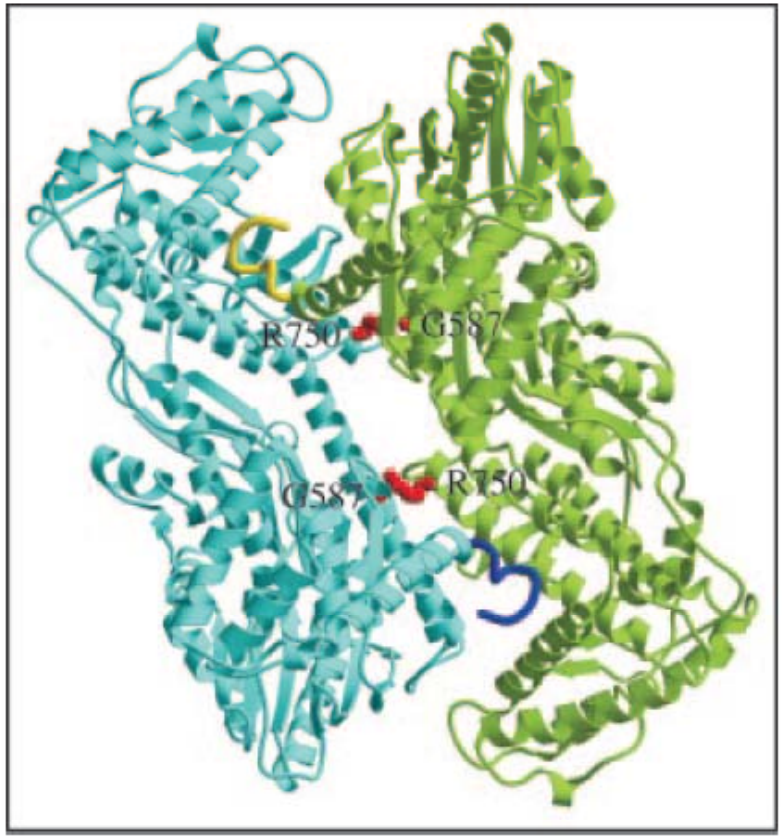
|---|---|---|---|---|---|---|---|---|---|---|
| Met | Leu | Ile | Lys | Leu | Leu | Thr | Lys | Val | Phe | Gly |




The two subunits in the crystal structure of *B. subtilis* SecA
The first nine residues of each subunit are shown in *yellow* and *blue*[a].

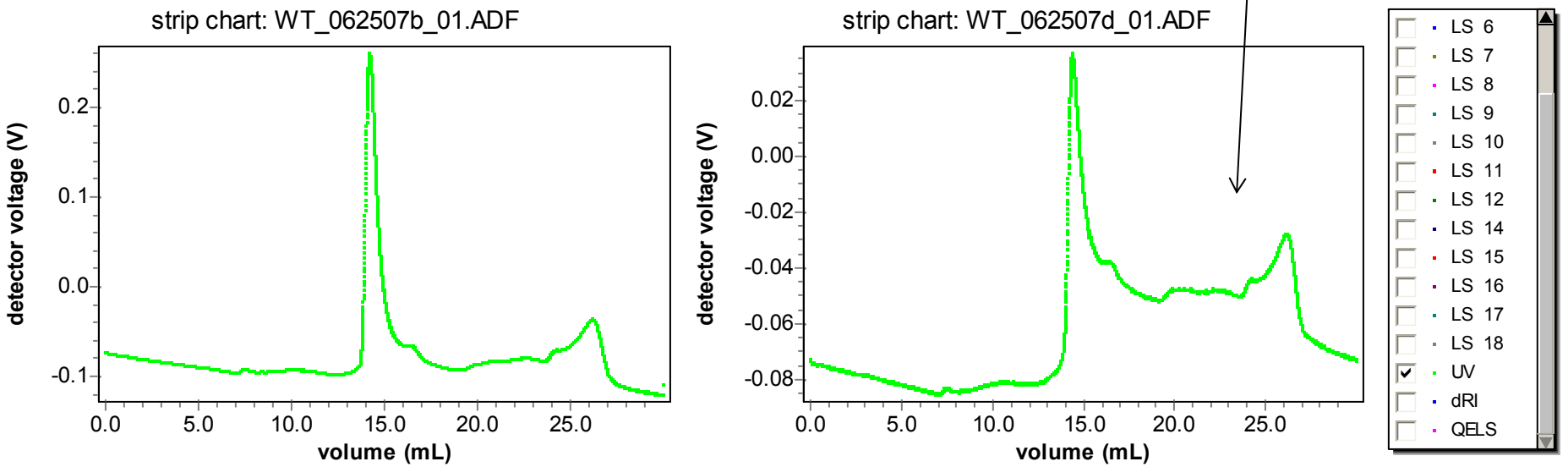
[a]Picture taken from : Or, E., A. Navon, and T. Rapoport. 2002. Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane. EMBO J. 21:4470-4479

# Determination of dimerization constant from SEC-LS measurements

**SecA protein** (nanomotor promotes protein translocation in eubacteria)

conflicting reports about whether SecA functions as a monomer or dimer

binding to quartz surface of DAD flow cell



## Determination of dimerization constant from SEC-LS measurements

**SecA protein** (nanomotor promotes protein translocation in eubacteria)

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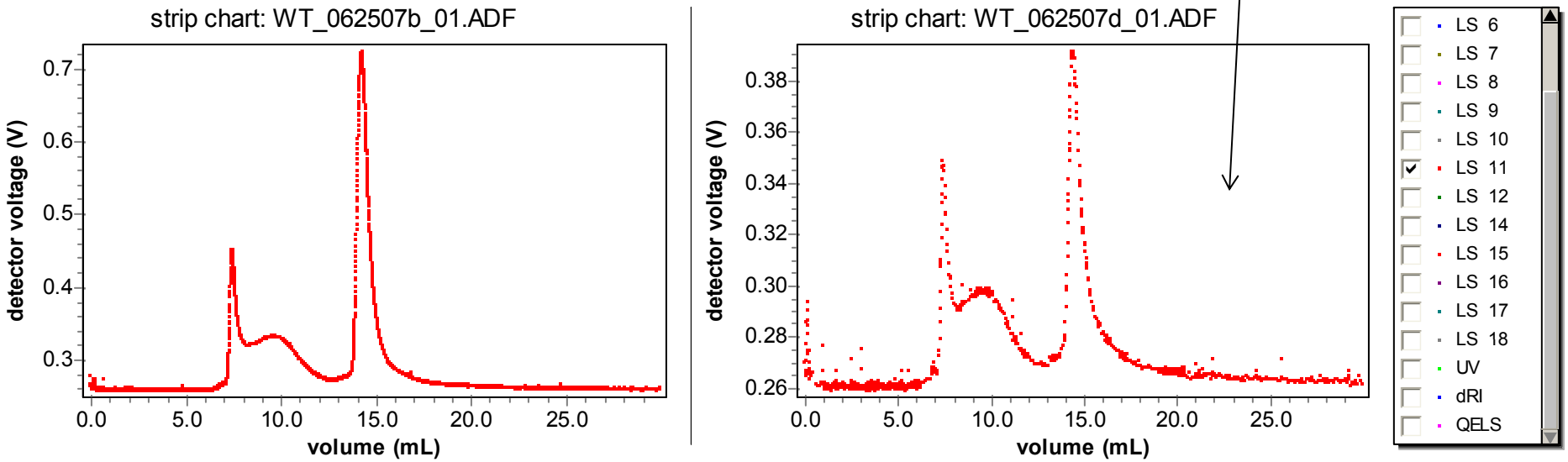
no binding to glass surface of DAWN flow cell



## Determination of dimerization constant from SEC-LS measurements

### Extracellular ligand binding domain (LBD) of the metabotropic glutamate

mGluR LBD is a homodimer with a glutamate binding pocket in each subunit

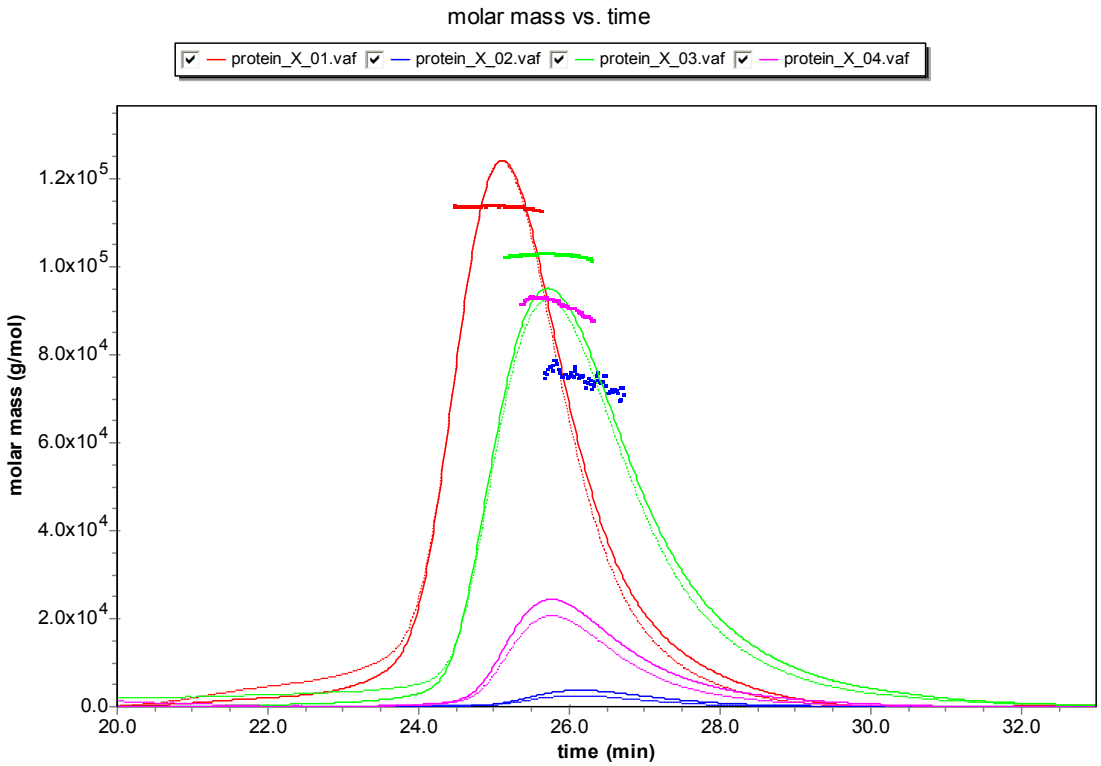
expressed in HEK293S cells; yields ~ 25 ug from a single preparation

extracellular ligand-binding domain (LBD), which acts as a detector of glutamate.

**WT** monomer = 59kDa dimeric in solution

**mutant** monomer = 59kDa destabilized dimer?

assess concentration dependent distribution of monomer-dimer



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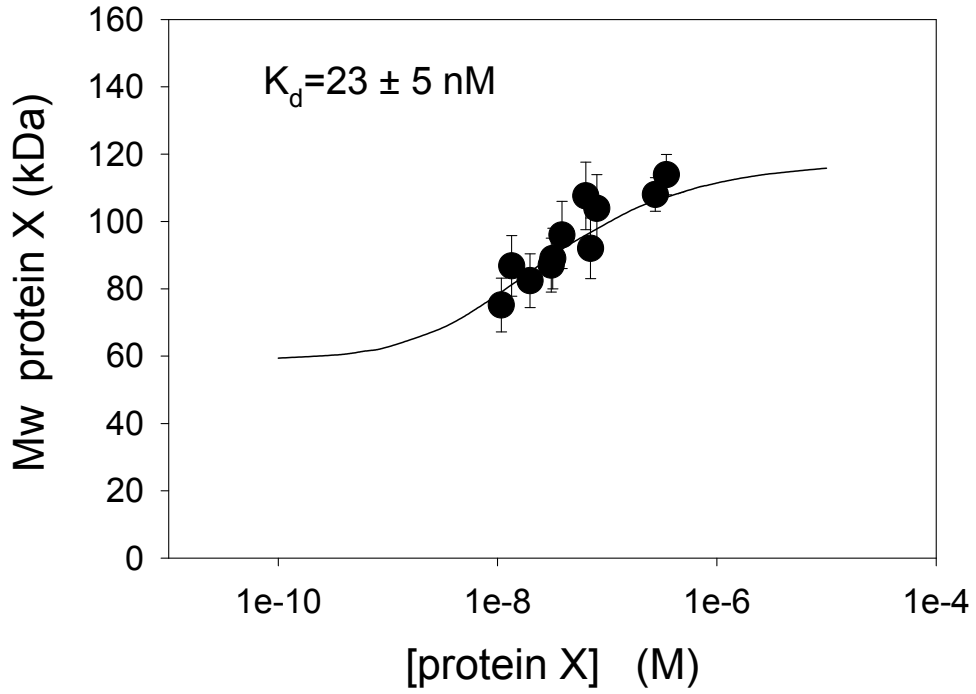

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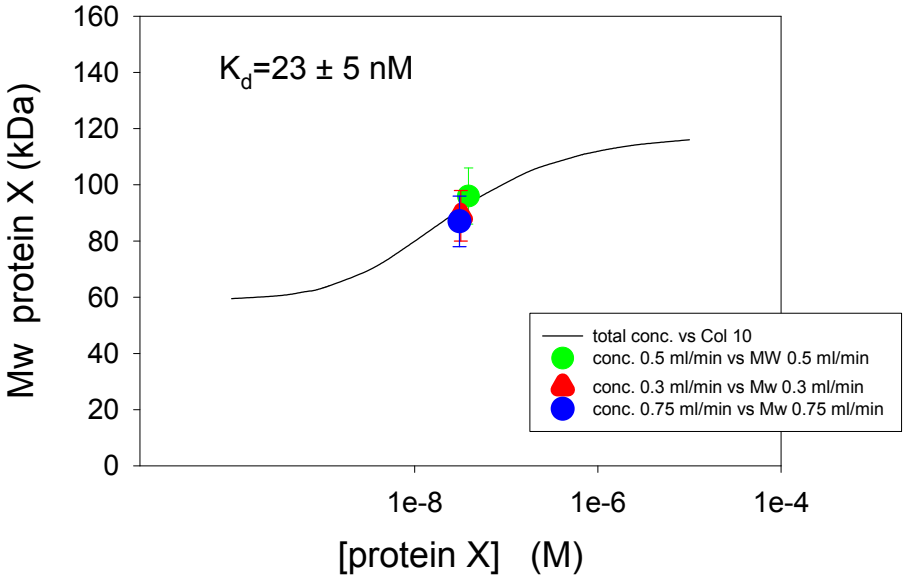


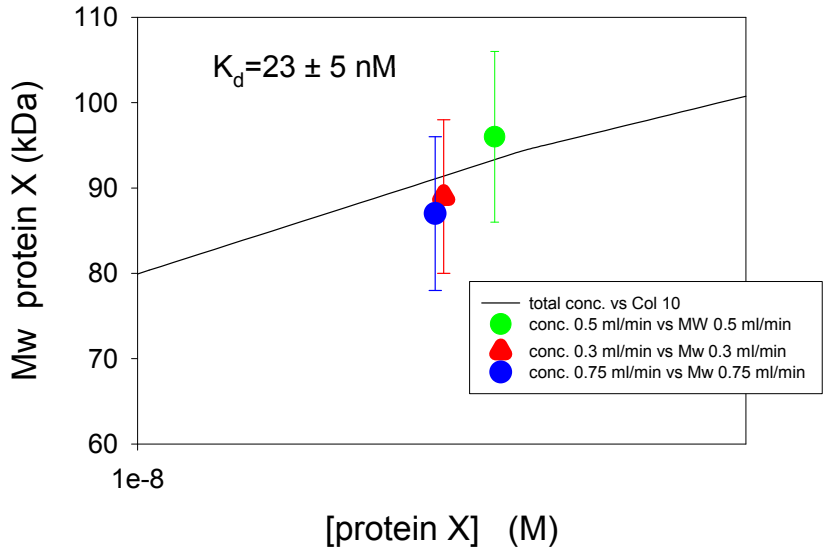

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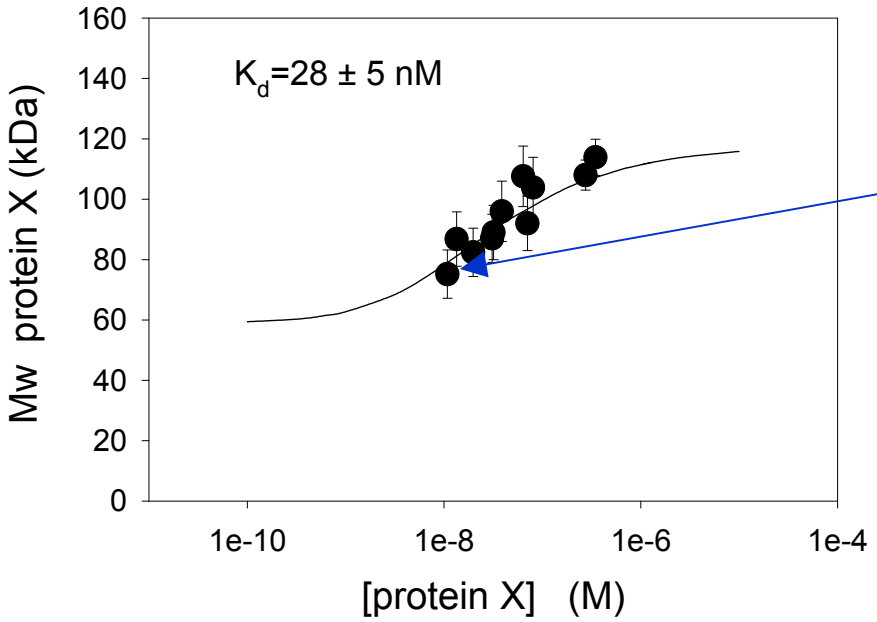


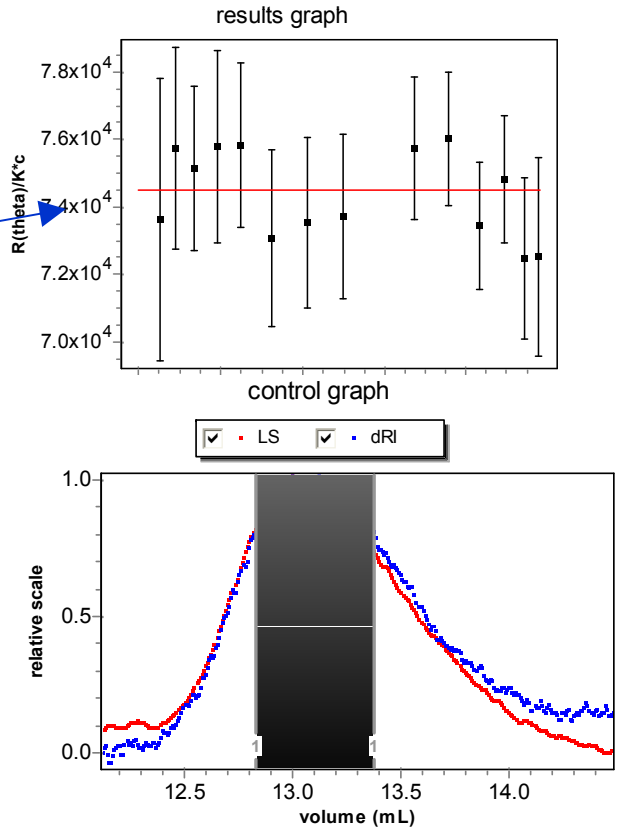


concentration= (6.426 ± 0.094) e-7 g/mL

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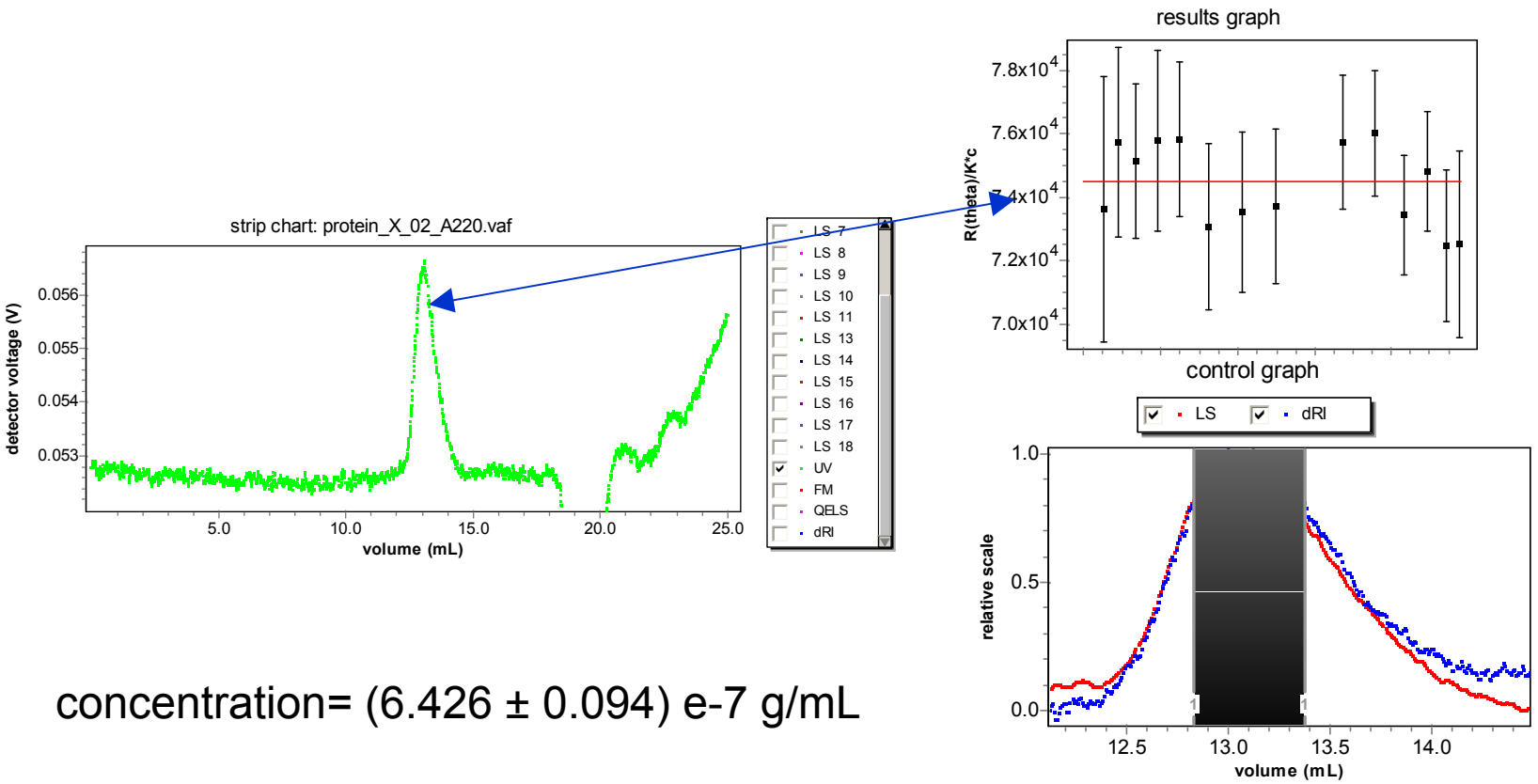
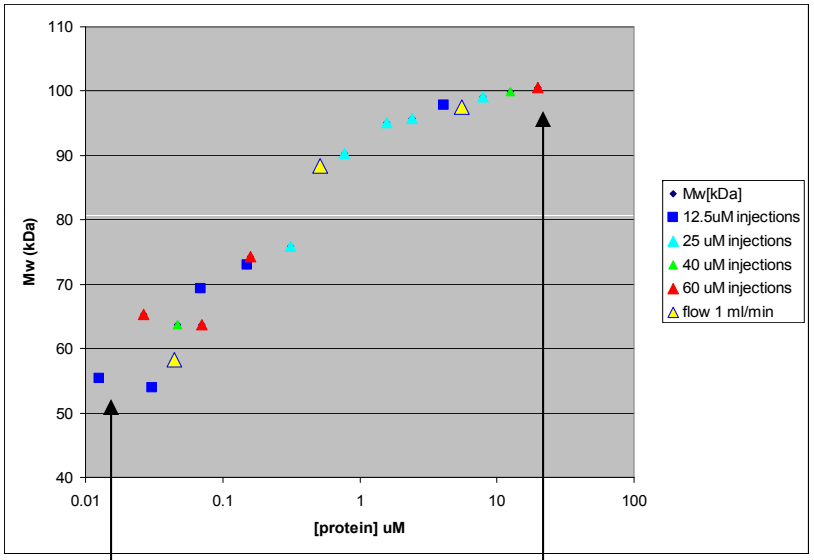
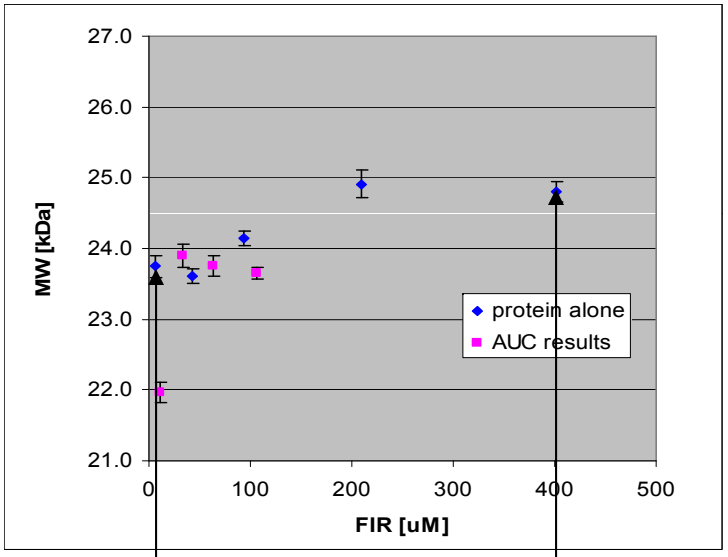



concentration= (6.426 ± 0.094) e-7 g/mL

Concentration range accessible on an analytical SEC/LS system

~1 µg/ml to ~10 mg/ml




Concentration range:

~4 orders of magnitude

0.001 mg/ml 1 µg/ml

1 mg/ml

0.1 mg/ml

9 mg/ml

# Size Exclusion Chromatography coupled with Light Scattering

- Fast and accurate determination of molar masses (weight average) in solution
- Can be used at wide range of protein concentrations from ~ 1µg/ml to >10mg/ml
  (correction for non-ideality)
- The SEC-UV/RI/LS (static and dynamic) data are very information rich and can be utilized to learn much more about the sample than “just” determination of Mw
    - Determination of stoichiometry of protein complexes:
        - protein-nucleic acid complexes
        - membrane protein in complexes with lipids and detergents
    - Provide information about shape (frictional ratio, f/fo)
    - Determination of dimerization constant

Ken Williams

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

# NIH

Users of the Biophysics Resource and SEC/LS Service

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

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