Light Scattering for Analysis of Oligomerization and Protein Interactions

New York Structural Biology Group

4th Winter Meeting

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New York Academy of Sciences
7 World Trade Center
Manhattan, NY

Ewa Folta-Stogniew

Biophysics Resource; Keck Laboratory
Yale School of Medicine

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/

Biophysics Resource of Keck Laboratory: Yale School of Medicine

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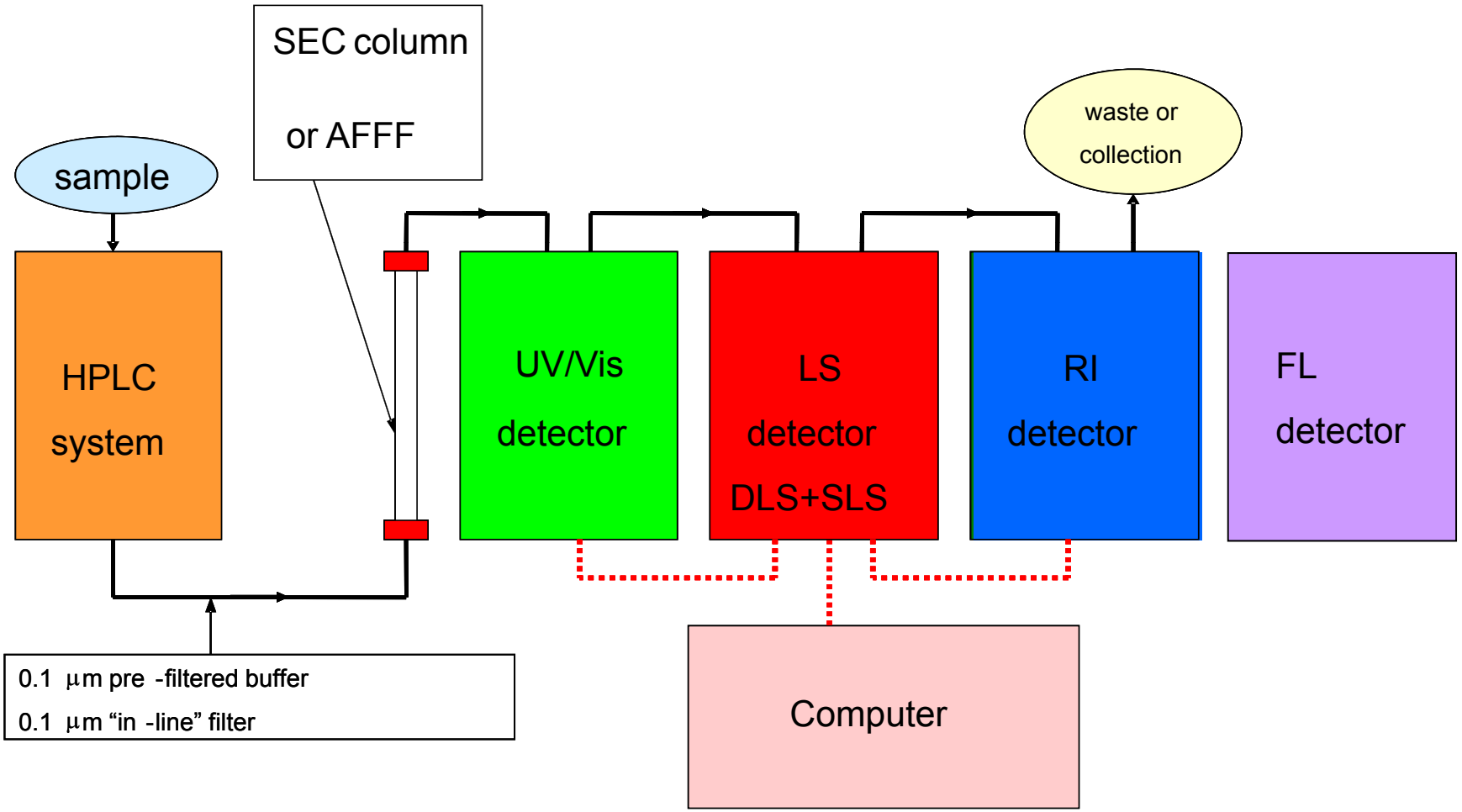
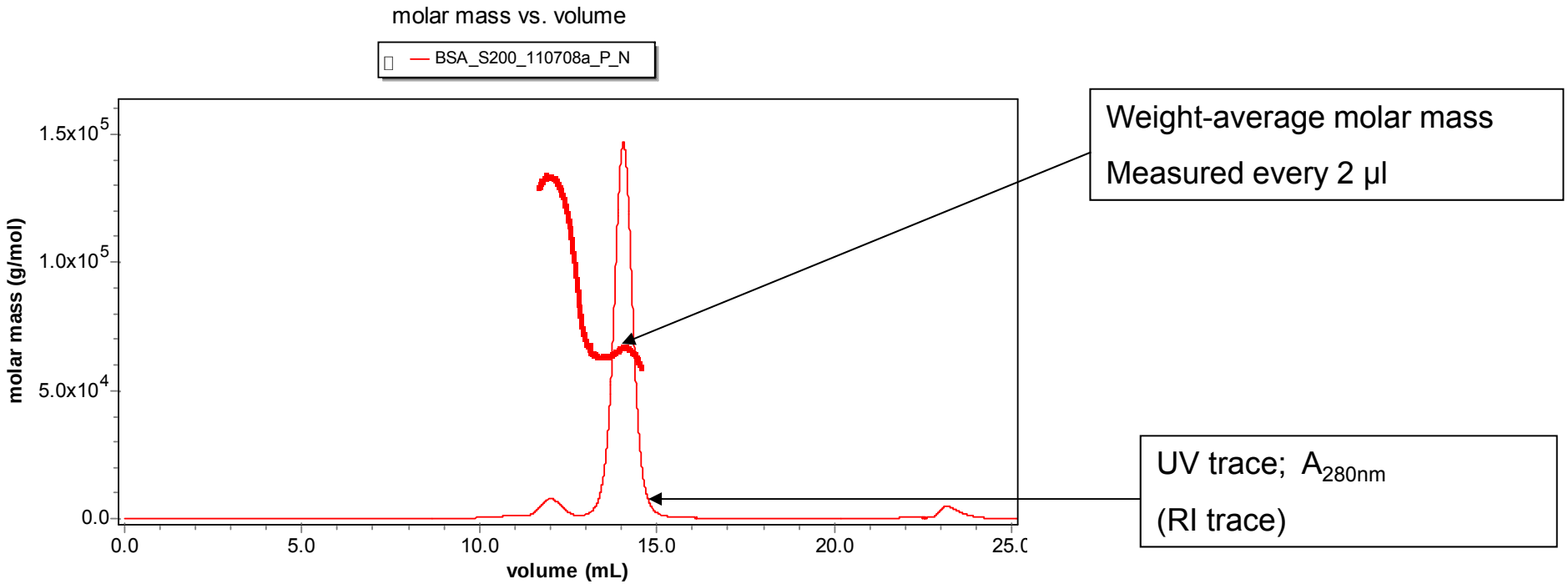
Typical SEC(AFFF); MALLS system



SEC/LS results: Molar Mass Distribution Plot

BSA

Monomer: 66 kDa

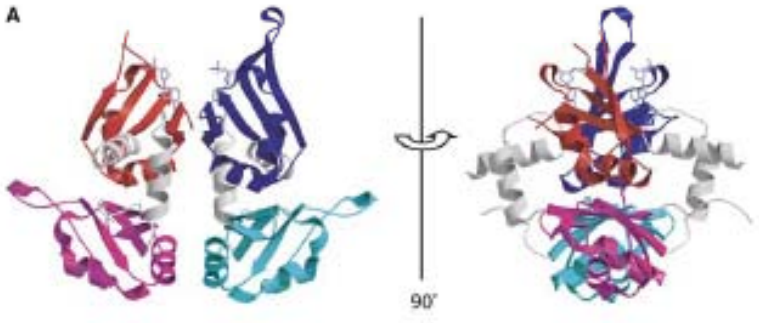


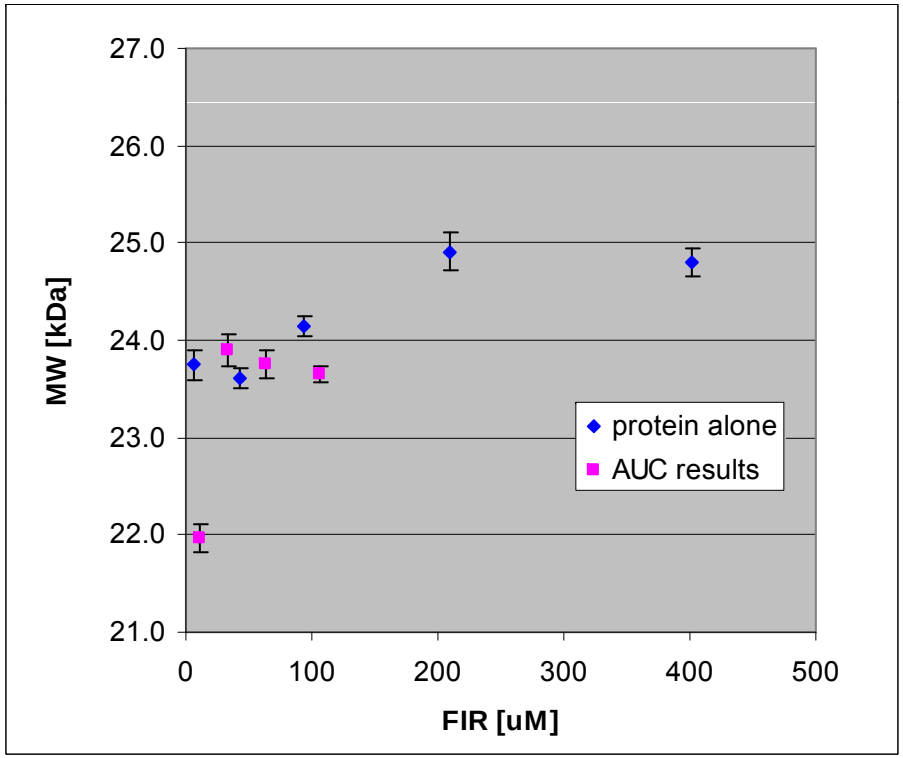
Dimerization of FIR

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





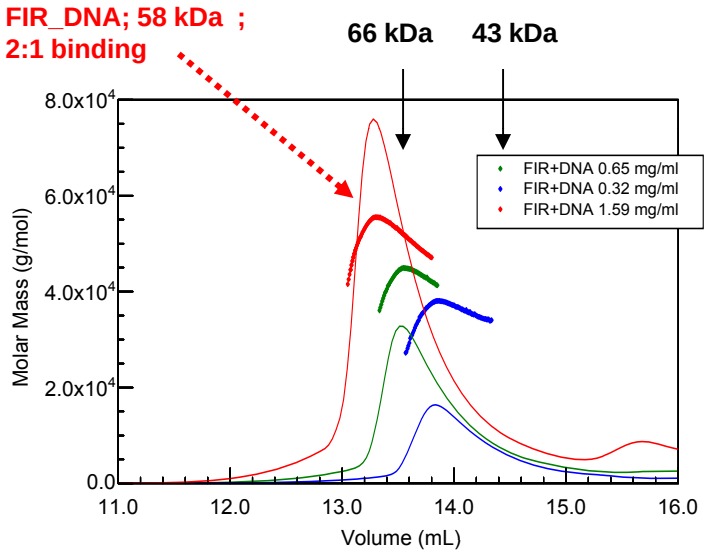
Crichlow G V, Zhou H., Hsiao H. H., Frederick K. B., Debrosse M., Yang Y., Folta-Stogniew E. J., Chung H. J., Fan C., De la Cruz E. M., Levens D., Lolis E., and Braddock D. (2008) Dimerization of FIR upon FUSE DNA binding suggests a mechanism of c-myc inhibition. *EMBO J* **27**: 277-289

Dimerization of FIR depends on DNA binding event

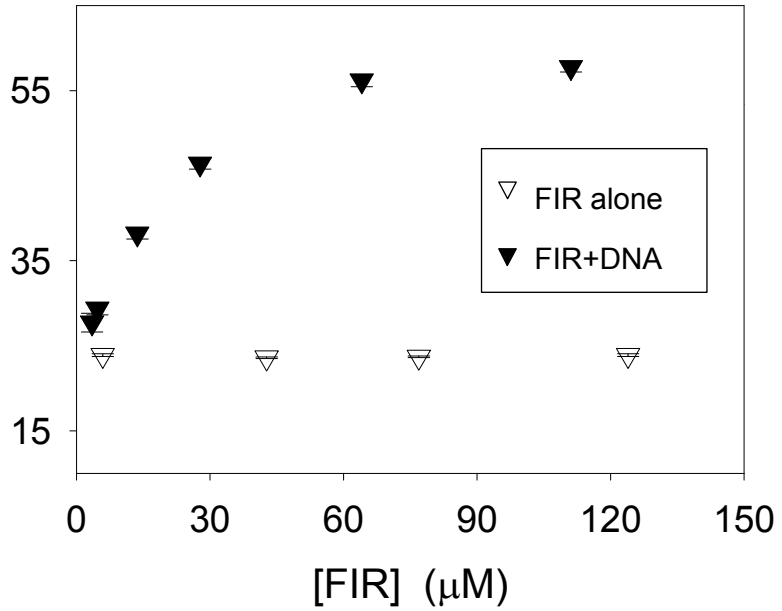
FIR protein: 23 kDa monomer

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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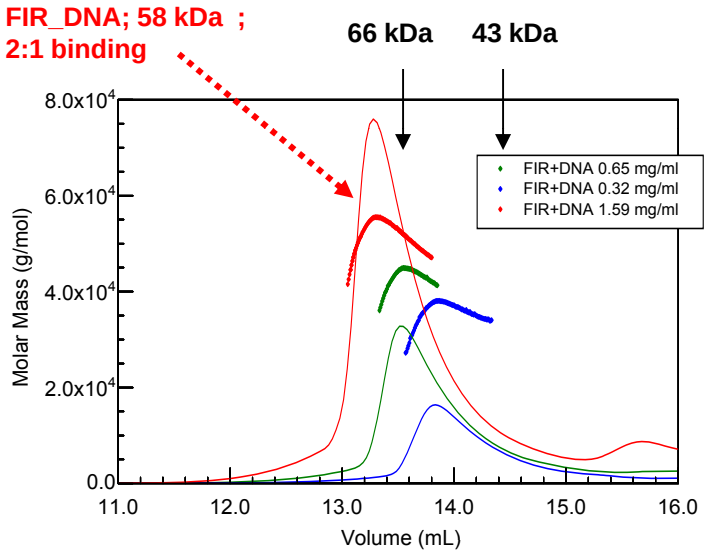

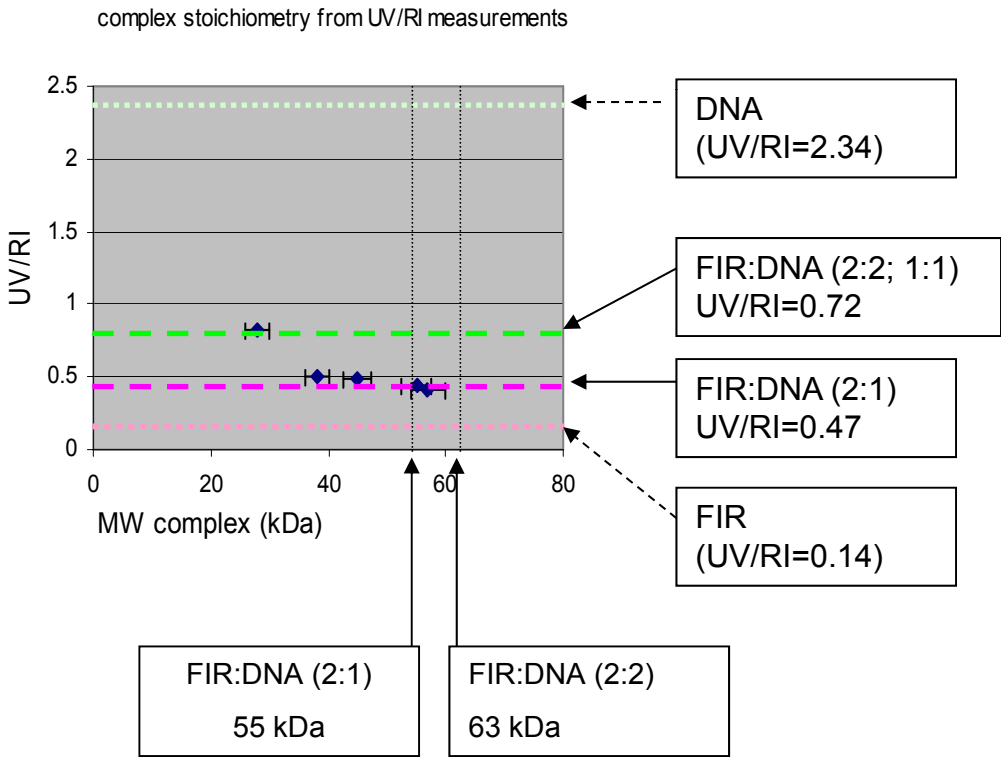

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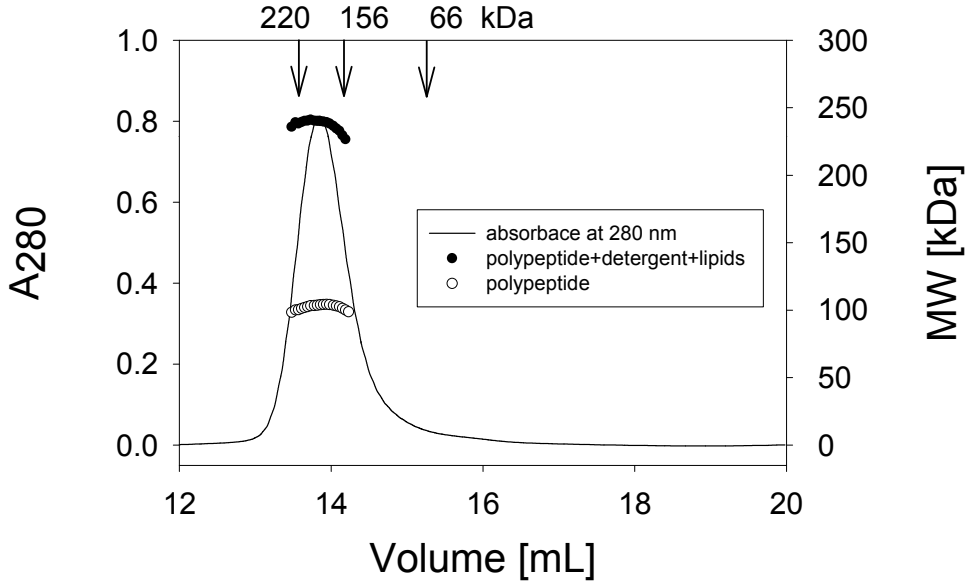
Multiple oligomeric states for reconstituted KtrAB K^+ Transporter

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

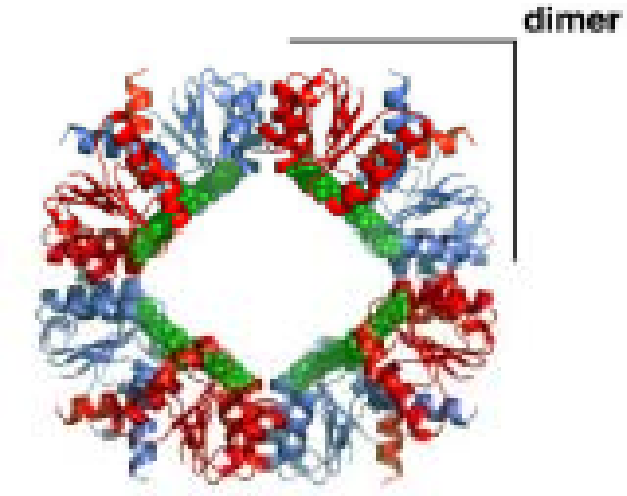
Albright R A, Ibar J. L. V., Kim C. U., Gruner S. M., and Morais-Cabral J. H. (2006) The RCK Domain of the KtrAB K+ Transporter: Multiple Conformations of an Octameric Ring. *Cell* **126**: 1147-1159

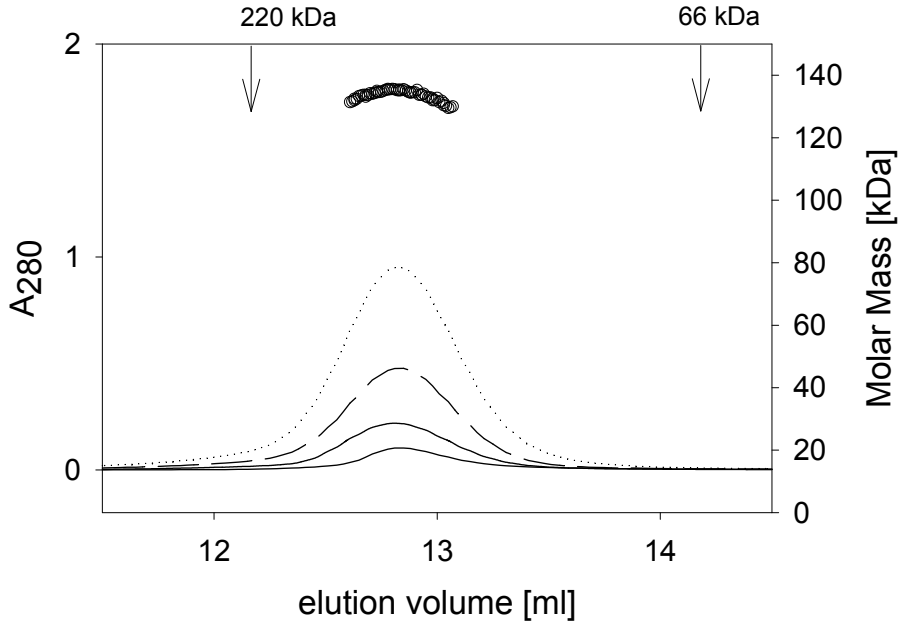
Multiple oligomeric states for reconstituted KtrAB K^+ Transporter

KtrAB ion transporter:

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

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





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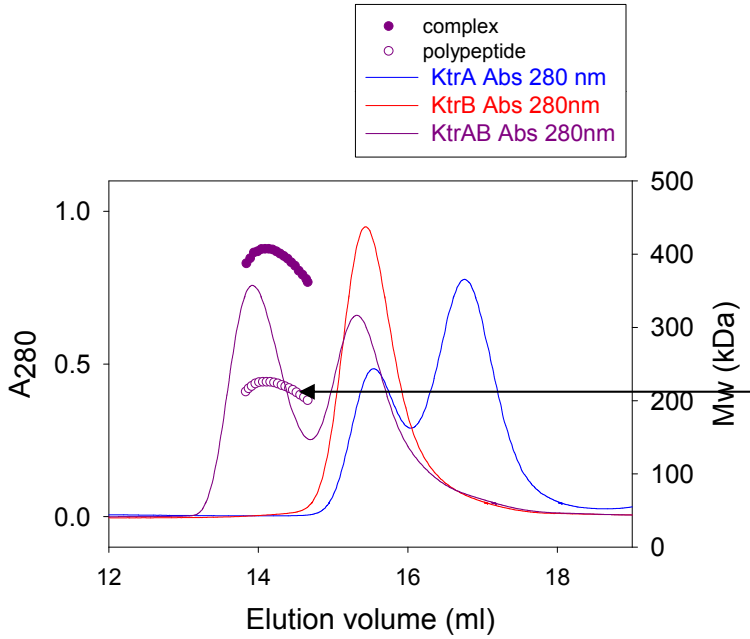
Multiple oligomeric states for reconstituted KtrAB K^+ Transporter

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

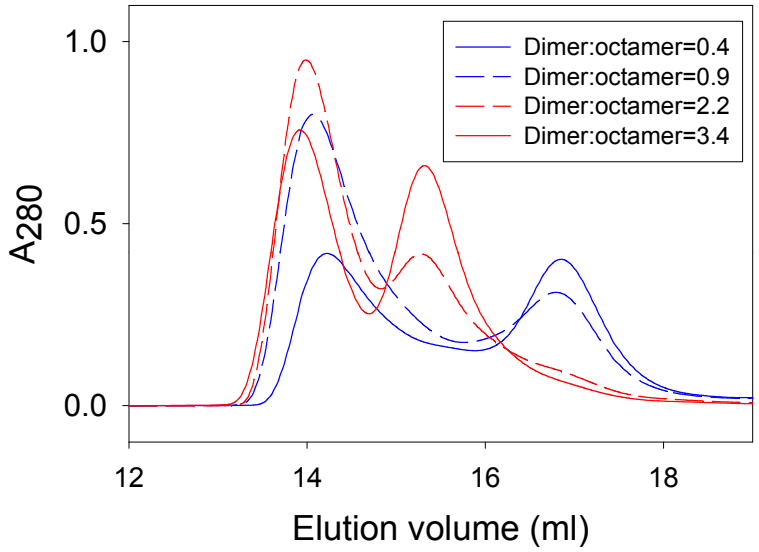
Buffer: 25 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM NADH, 1 mM DDM

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*

Determination of dimerization constant from SEC-LS measurements

SecA protein (nanomotor promotes protein translocation in eubacteria)

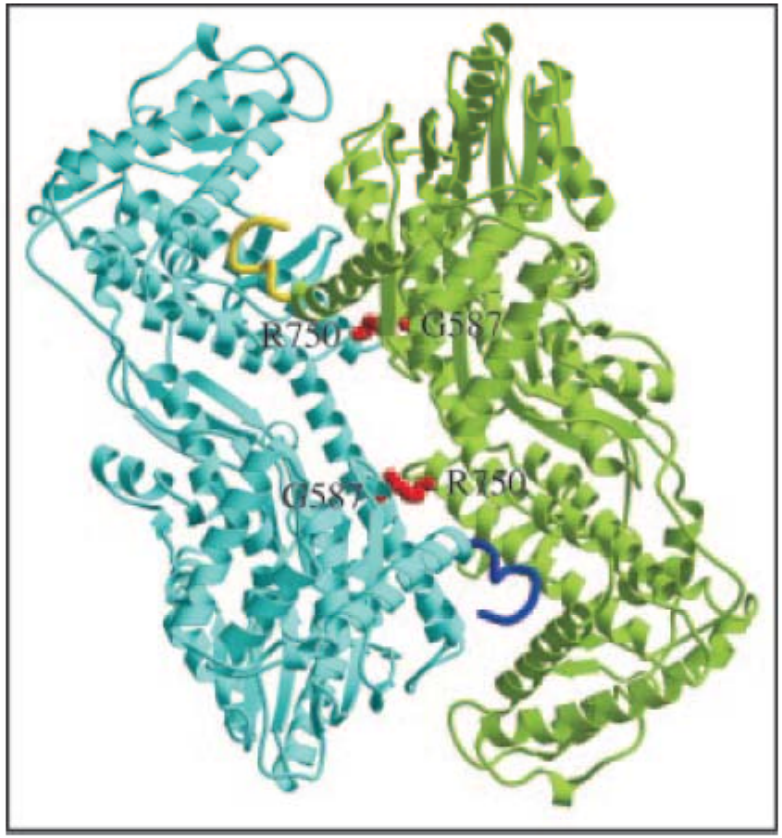
conflicting reports about whether SecA functions as a monomer or dimer

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

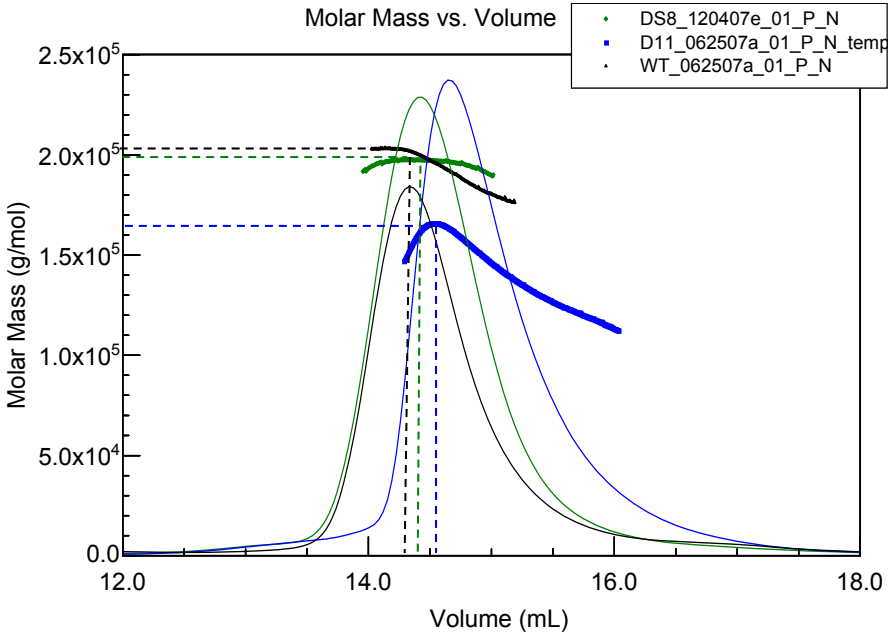
WT monomer = 102 kDa

DS8 deletion mutant monomer = 101 kDa

D11 deletion mutant monomer = 100 kDa

Low salt buffer:

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



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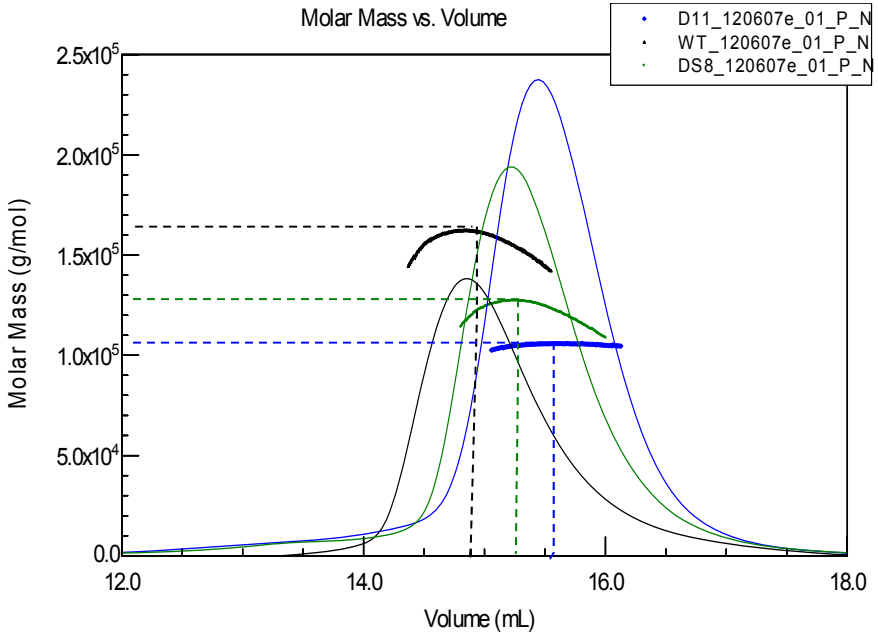
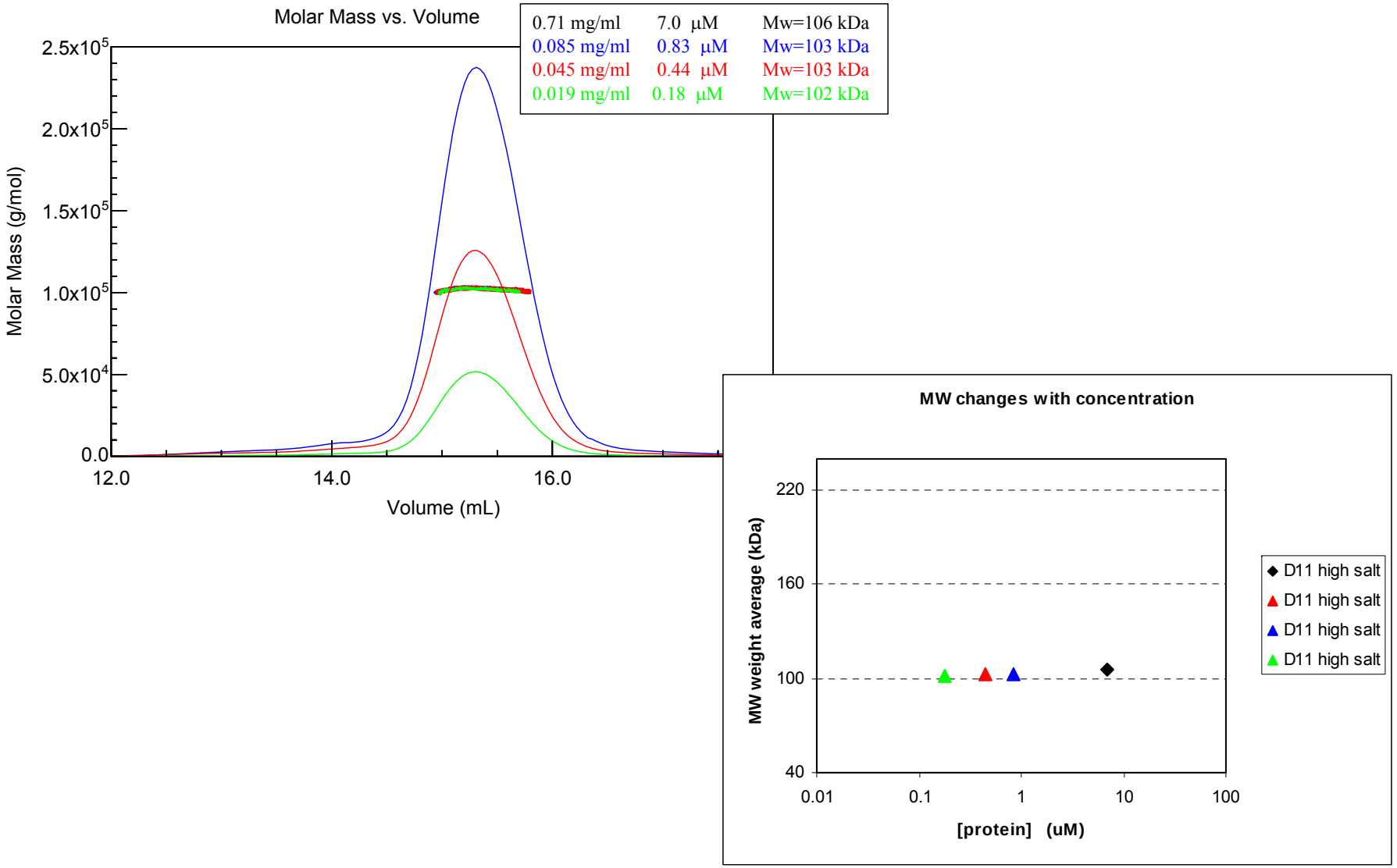


D11 deletion mutant
mono= **101 kDa**

High salt buffer:

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



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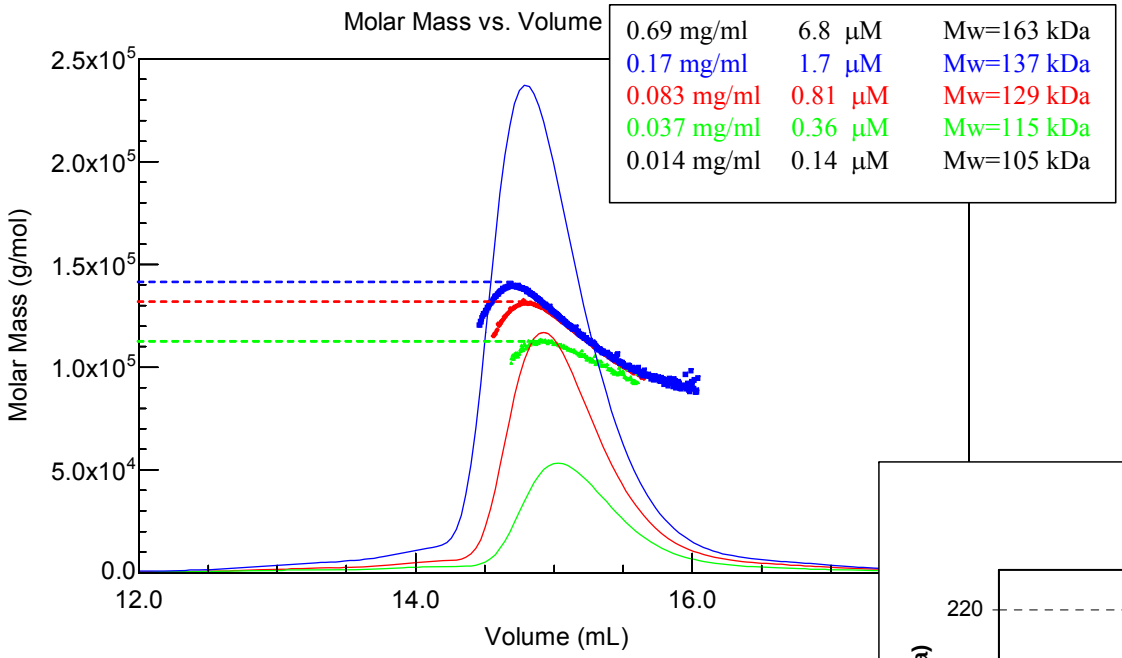
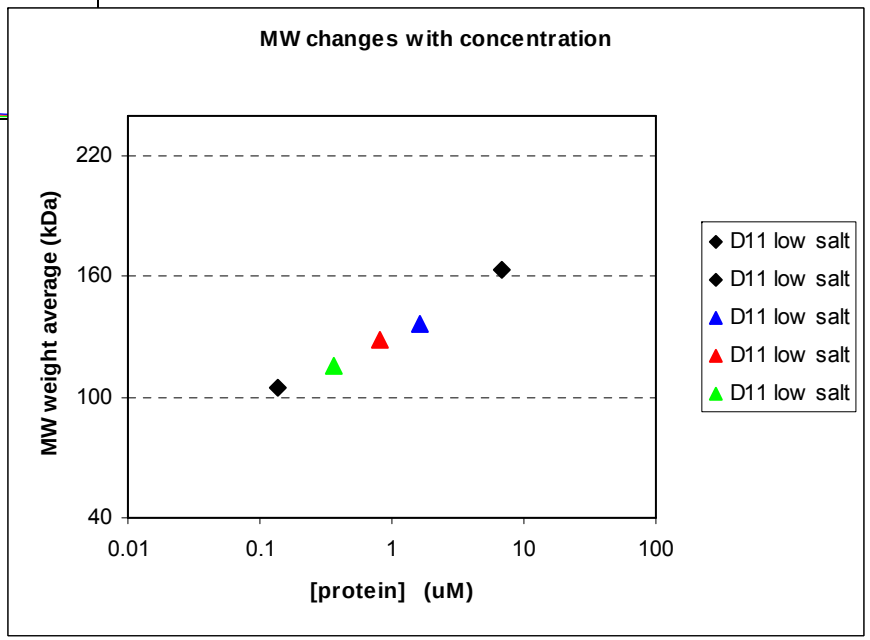




$$M_w = f_m M_m + f_d M_d = M_m(2 - f_m)$$

$$2M = D$$

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

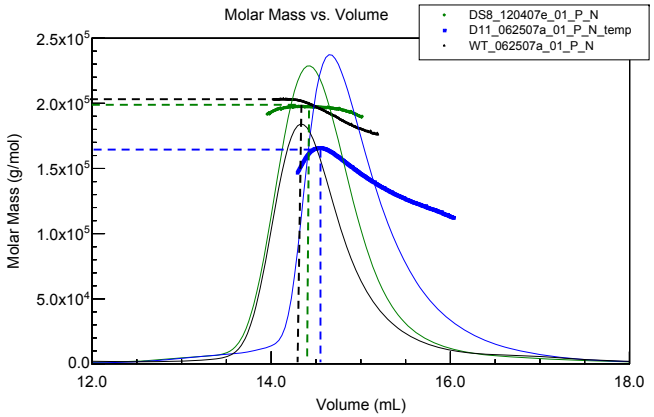
$$f_m = \frac{-1 + \sqrt{1 + 8K_a c_t}}{4K_a c_t}$$

WT monomer = 102 kDa

DS8 deletion mutant monomer = 101 kDa

D11 deletion mutant monomer = 100 kDa

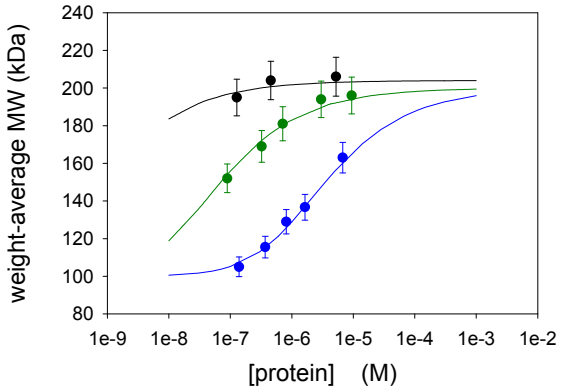
Low salt buffer: 100 mM KCl



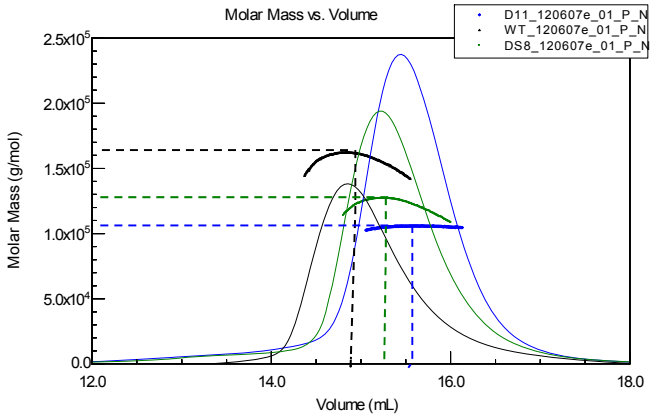
WT Kd= <1e-9

DS8 Kd=7±1e-8 M

D11 Kd=3.5±0.2e-6 M



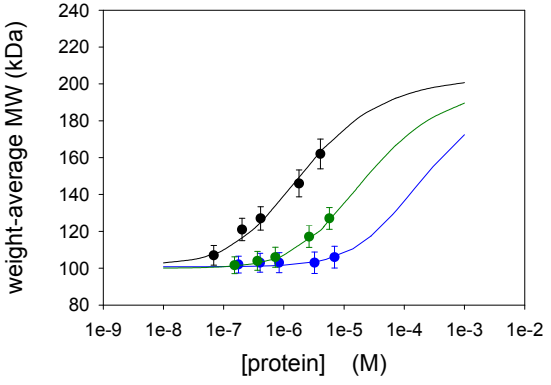
High salt buffer: 300 mM KCl



WT Kd= 2.2±0.2e-6 M

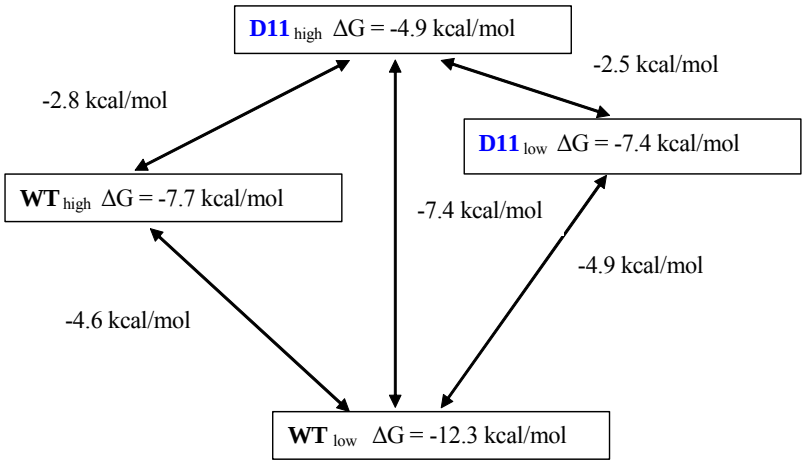
DS8 Kd= 2.41±0.05e-5 M

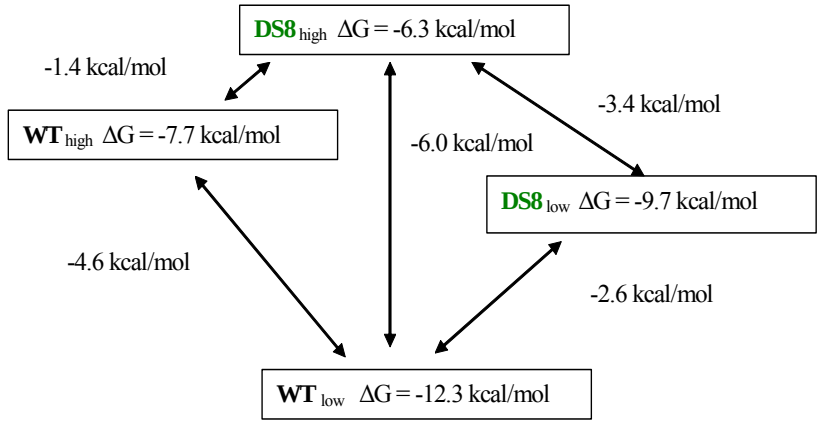
D11 Kd> 2.4e-4 M



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	$<1x10^{-9}$	-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





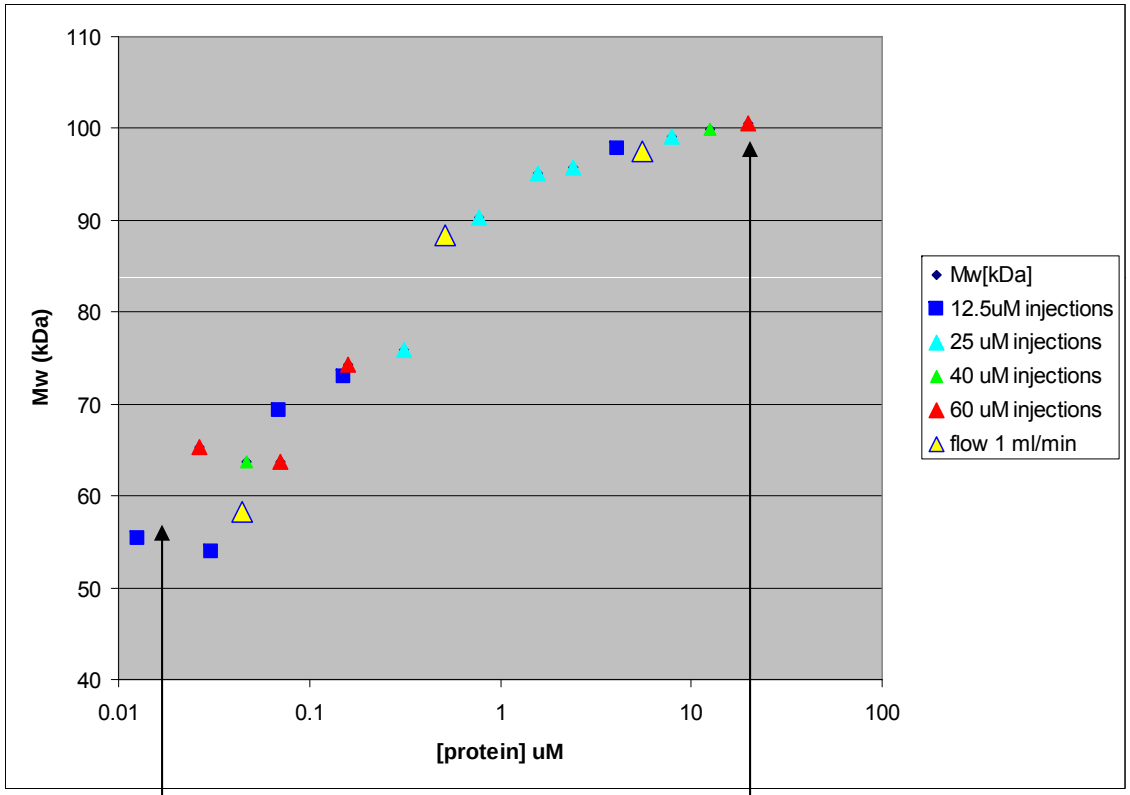
1 2 3 4 5 6 7 8 9 10 11

Met Leu Ile Lys Leu Leu Thr Lys Val Phe Gly

Das S, Stivison E., Folta-Stogniew E., and Oliver D. (2008) Reexamination of the Role of the Amino Terminus of SecA in Promoting Its Dimerization and Functional State. *The Journal of Bacteriology* **190**: 7302-7307

Monomer 52 kDa

Concentration range tested <1 µg/ml to 1 mg/ml

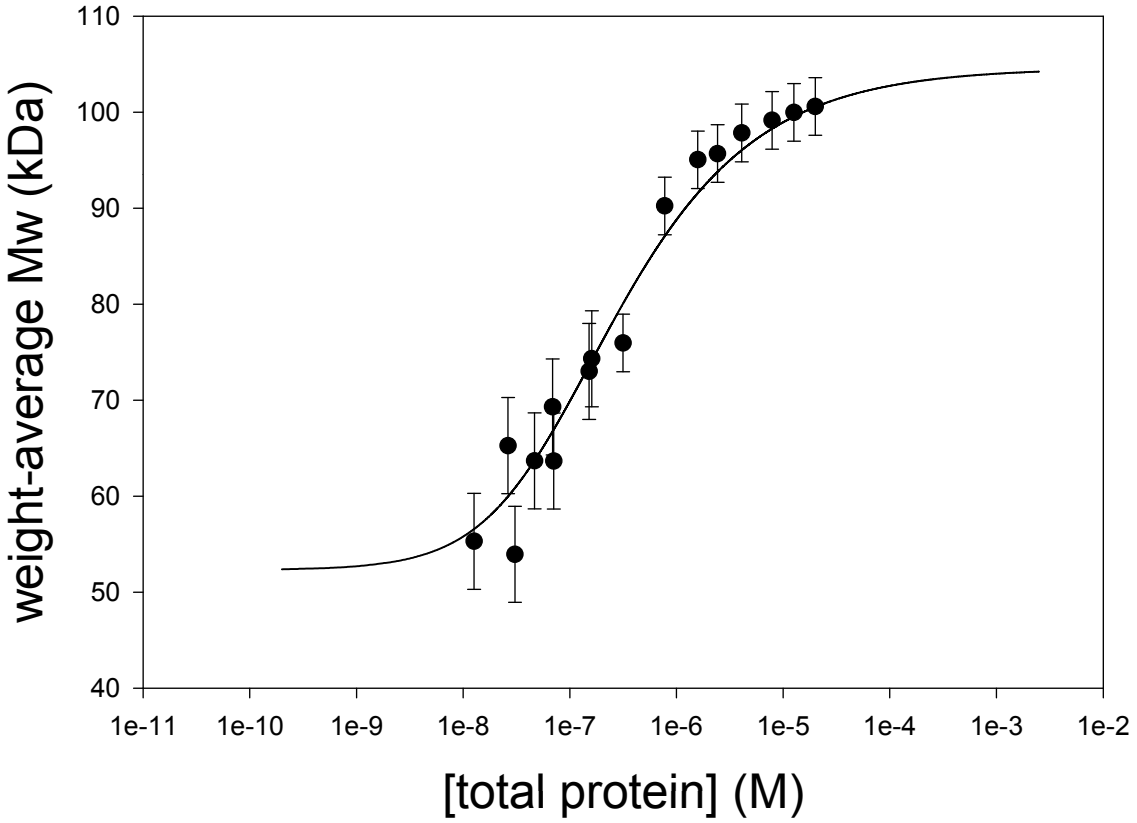


0.001 mg/ml 1 µg/ml

1 mg/ml

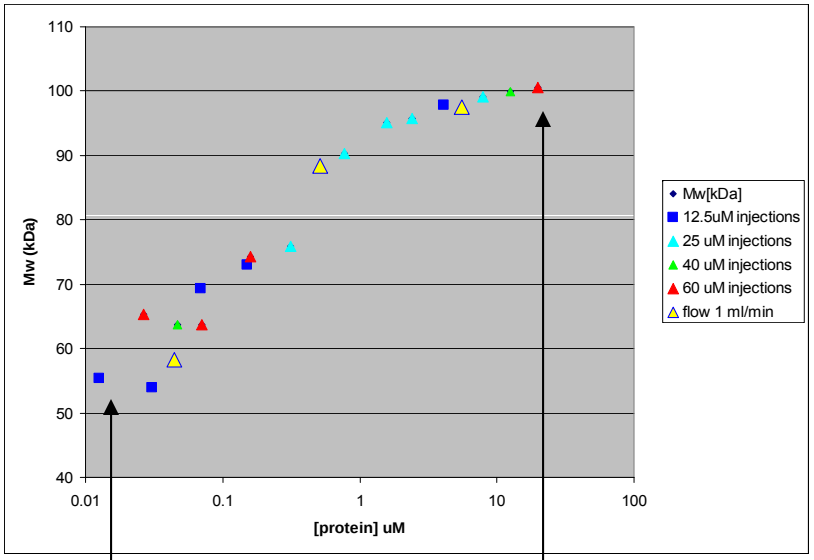
Concnetration dependance of Mw from SEC/LS measurements

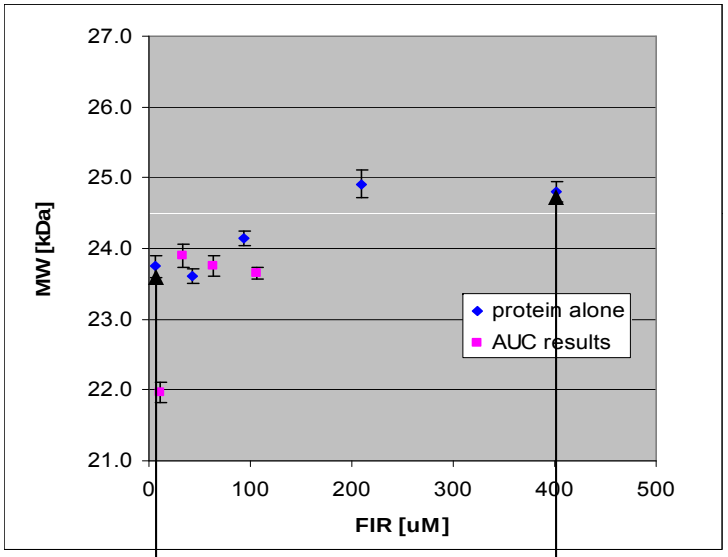
Kd=0.26μM +/- 12%



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

SEC/LS

- fast and accurate determination of molar masses (weight average) in solution
- applicable for analysis of:
  - protein-nucleic acid complexes
  - protein-lipids-detergent complexes
  - *glycosylated protein*
  - *proteins conjugated with PEG*
- can be used at wide range of protein concentrations
  from ~ 1µg/ml to >10mg/ml (correction for non-ideality)
- can be used to determine association constant (concentration gradient measurements)

Ken Williams

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

NIH

Users of SEC/LS Service

Services provided by the Biophysics Resource contributed to at least **53 publications** *(32 from Yale*)

Light Scattering Services contributed to 37 publications

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

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

Ewa.Folta-Stogniew@yale.edu

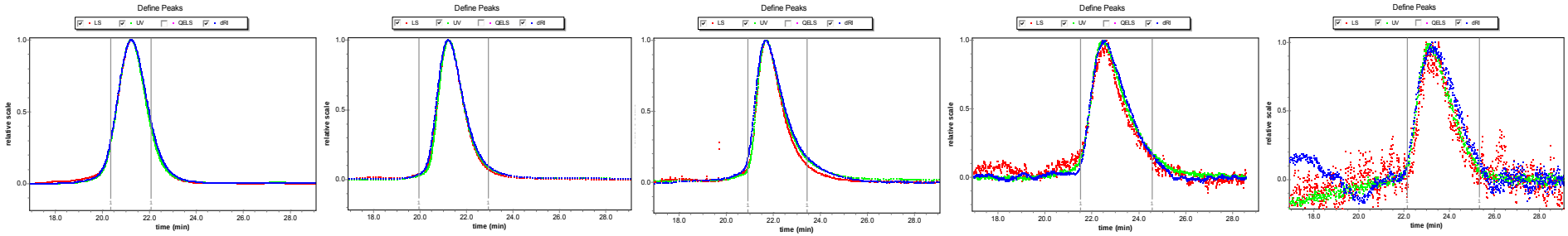
Concentration range accessible on an analytical SEC/LS system

~1 µg/ml to ~10 mg/ml

UV (280nm)
RI
LS (90 degree angle)

Concentration at the apex of the eluting peak; ~ injected total mass

1 mg/ml	0.1 mg/ml	10 µg/ml	1.4 µg/ml	0.7 µg/ml



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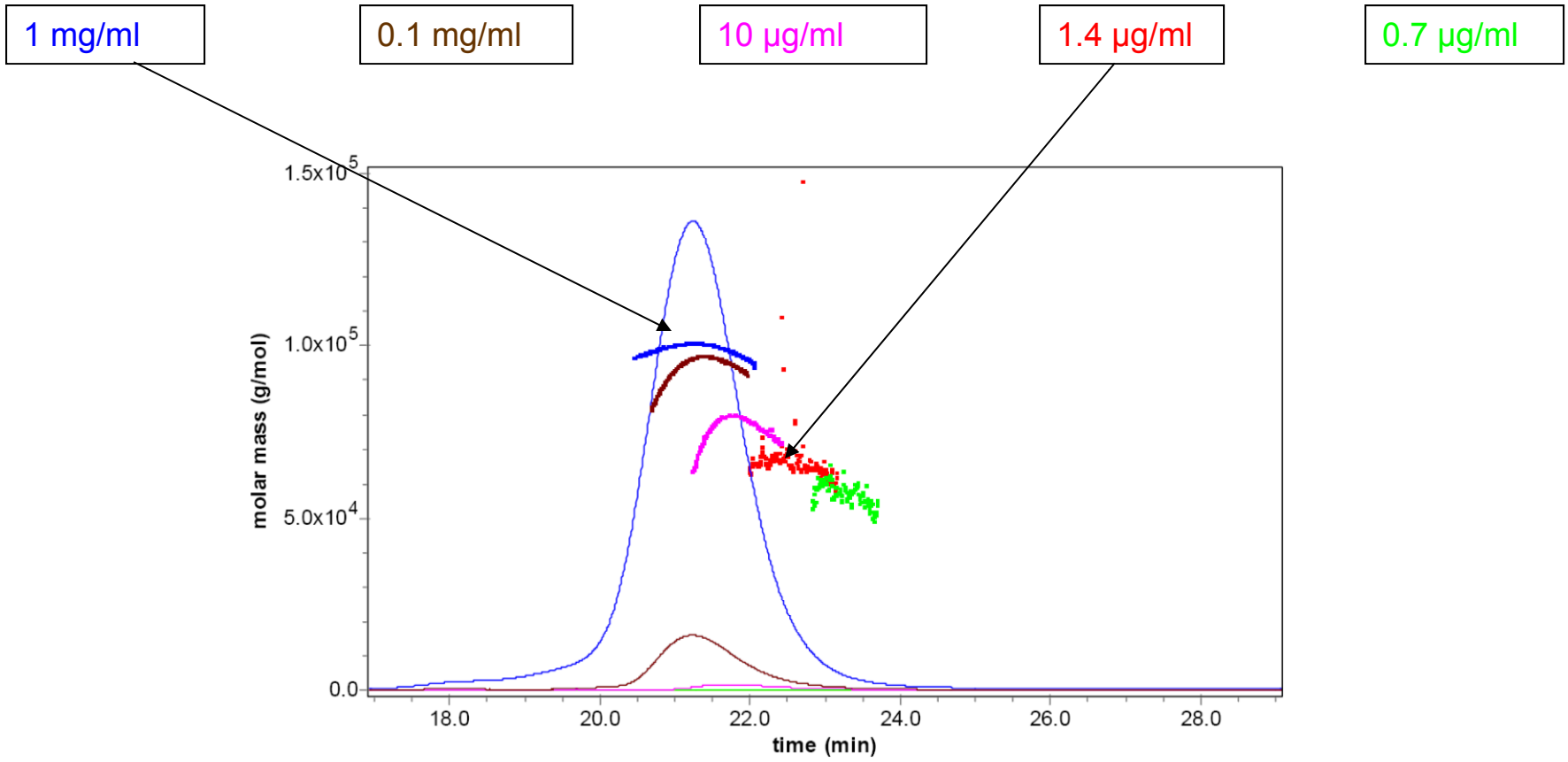
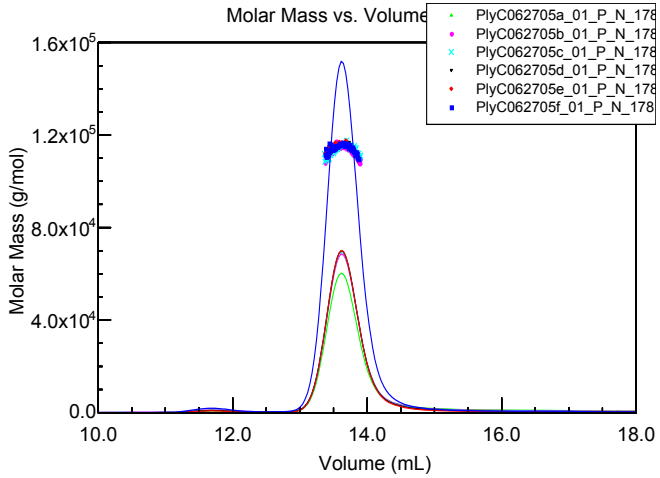


The streptococcal C1 bacteriophage lysin, PlyC,

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

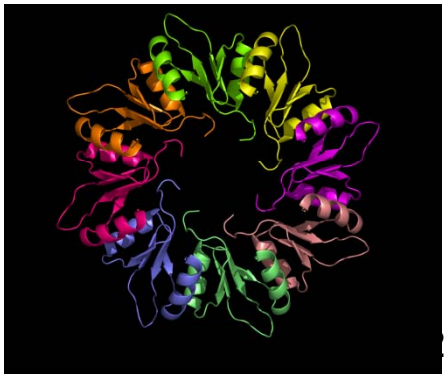
PlyC 1 big+8 small predicted MW = 114.3 kDa

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

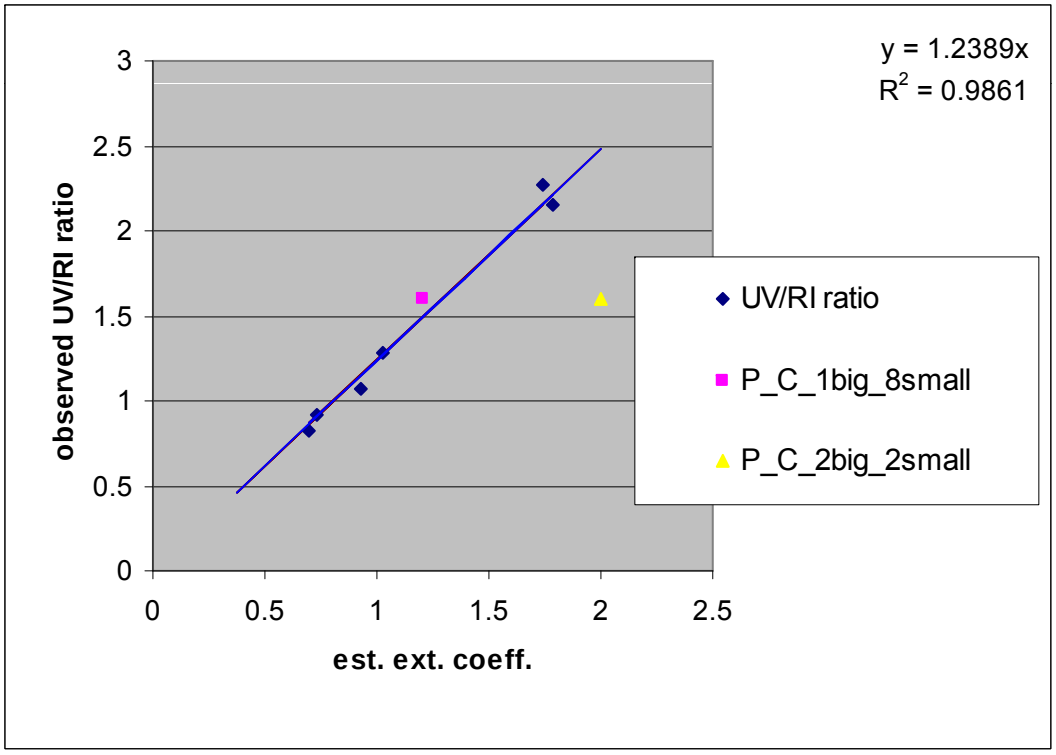


Octameric PlyCB. The eight PlyCB subunits arranged in a ring as observed in the crystal structure of PlyC.

Evaluated models:

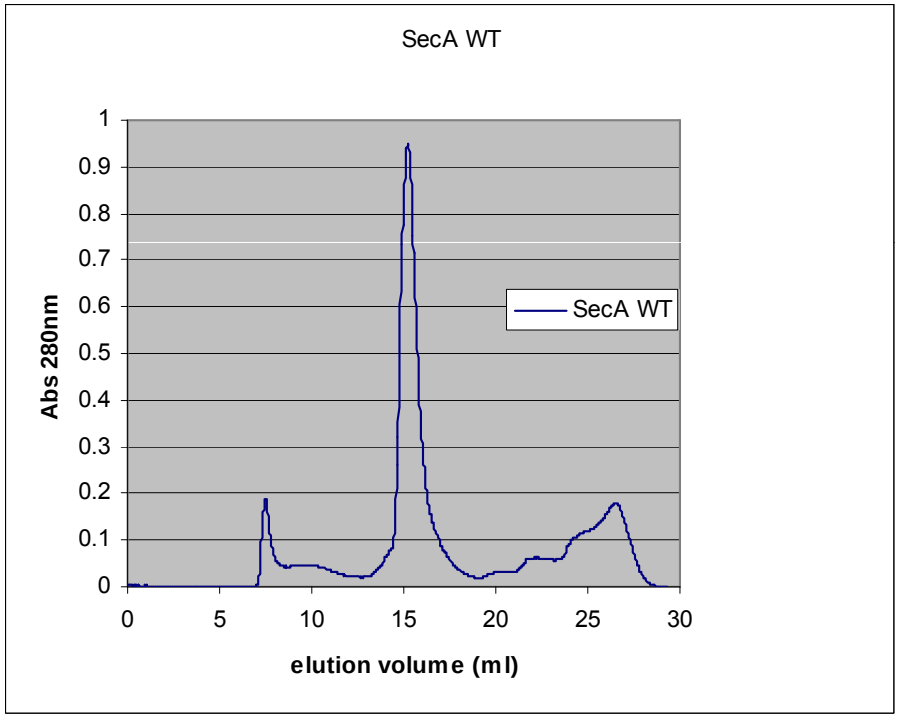
1 big+8 small MW= PlyC model (1+8)

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**

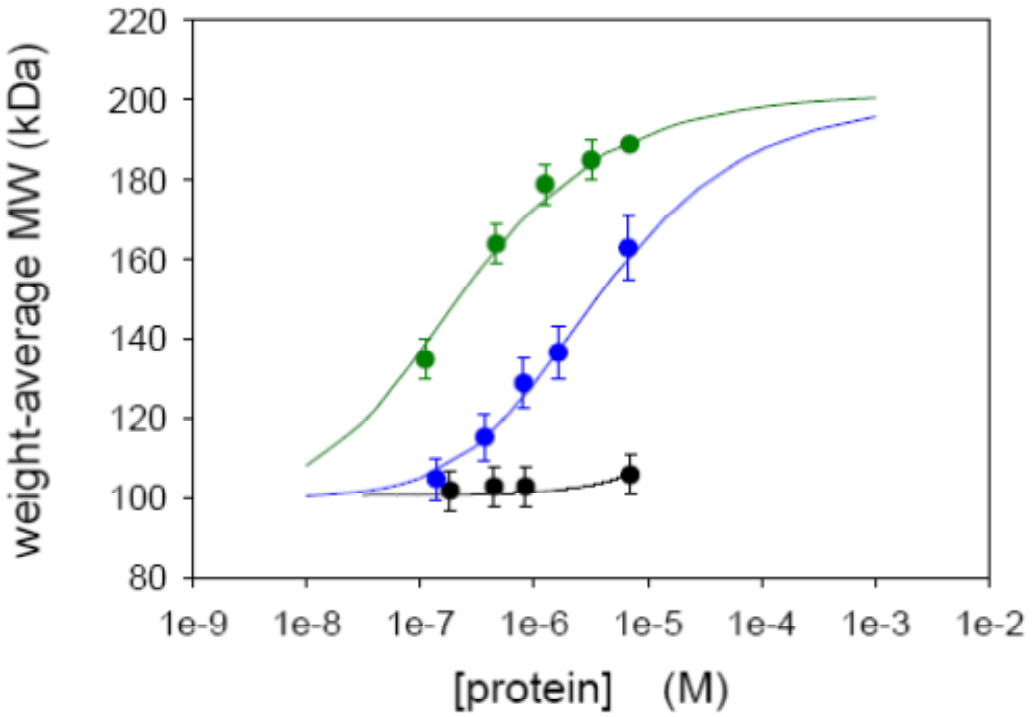
[a] Philo J S, Aoki K. H., Arakawa T., Narhi L. O., and Wen J. (1996) Dimerization of the Extracellular Domain of the Erythropoietin (EPO) Receptor by EPO: One High-Affinity and One Low-Affinity Interaction. *Biochemistry* **35**: 1681-1691


SecA WT
Abs 280nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
elution volume (ml)
SecA WT

SecAΔ11 25 mM KCl Kd=2.6×10^{-7} M

SecAΔ11 100 mM KCl Kd=3.5×10^{-6} M

SecAΔ11 300 mM KCl Kd>2.3×10^{-4}M

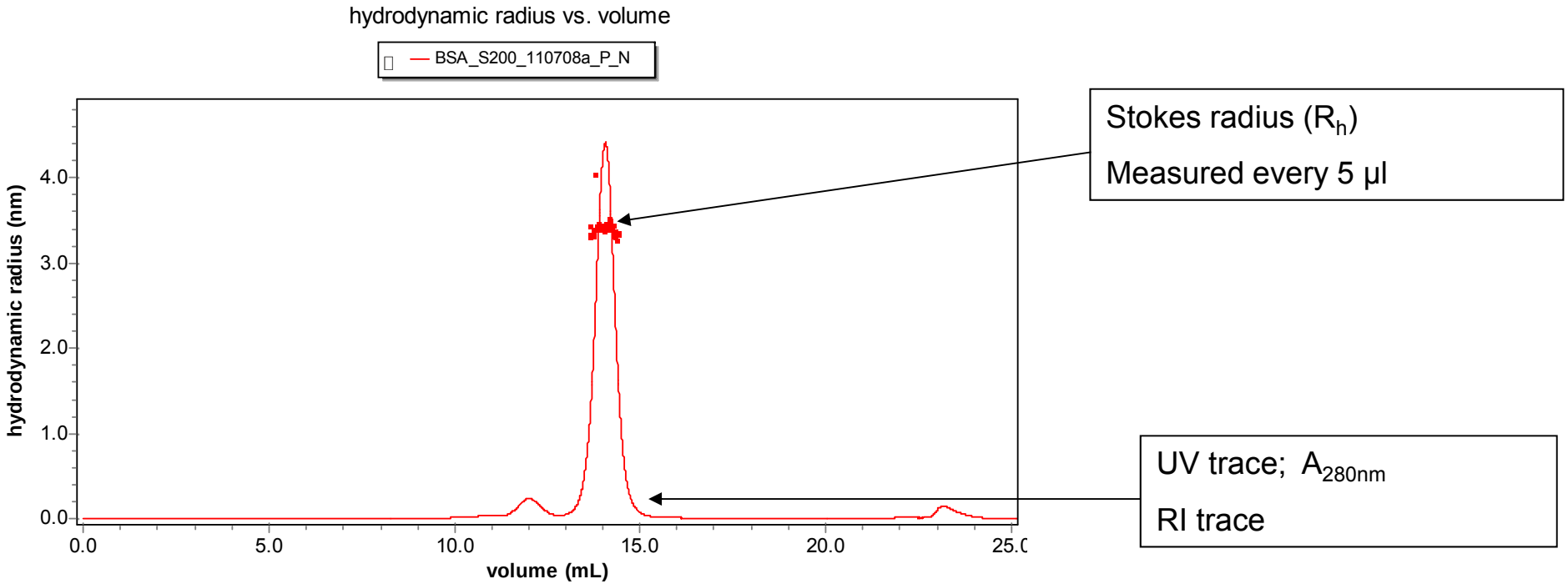


SEC/LS results: Stokes radius Distribution Plot

BSA

Monomer: 66 kDa

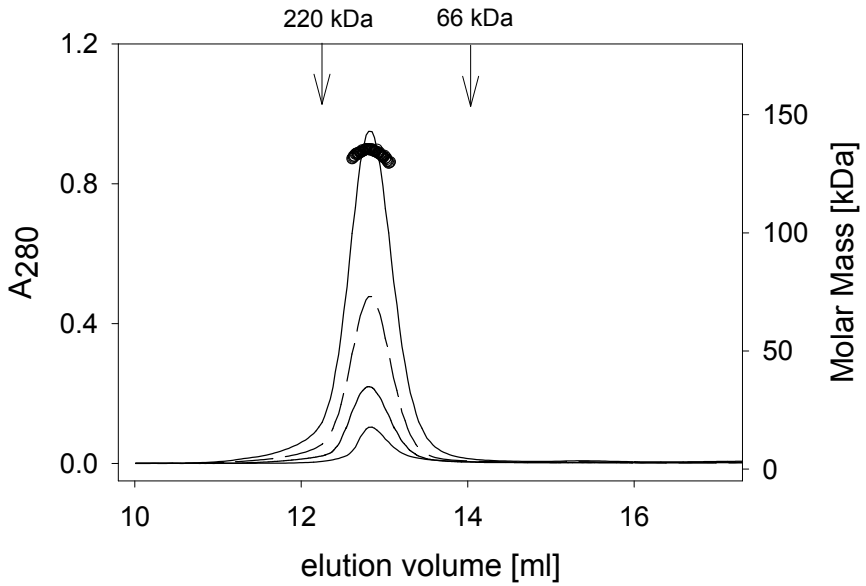
R_h = 3.4 nm



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