Application of Light Scattering in a Core Facility Setting

Ewa Folta-Stogniew
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/

Biophysics Resource of Keck Laboratory: Yale School of Medicine

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

Common questions:

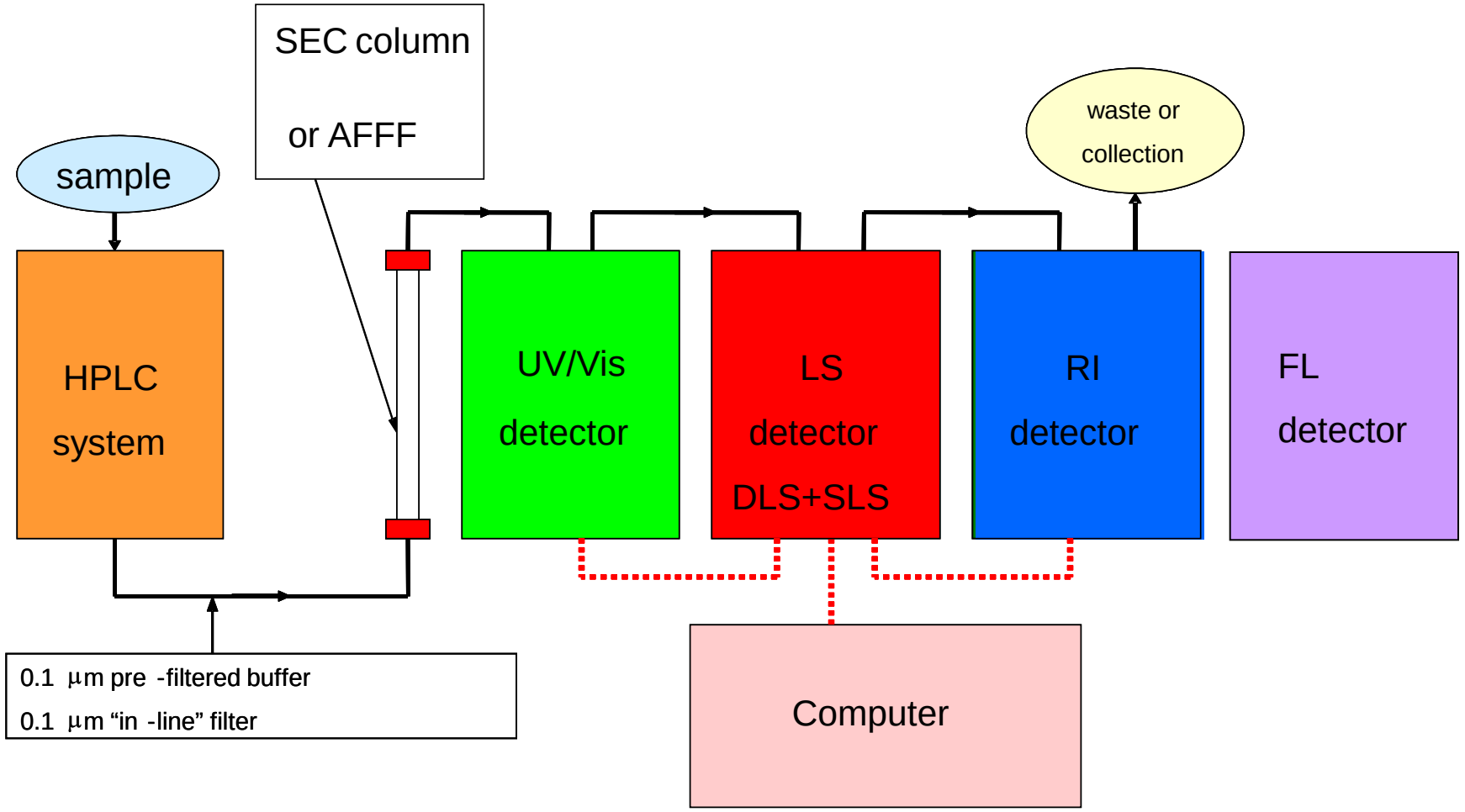
- how tight is the binding ? (binding affinity: K_d, K_a)
- how many of each molecule are in the complex (stoichiometry)
- how fast does the complex form? (kinetics)
- is the binding event enthalpy or entropy-driven? (thermodynamics)

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/

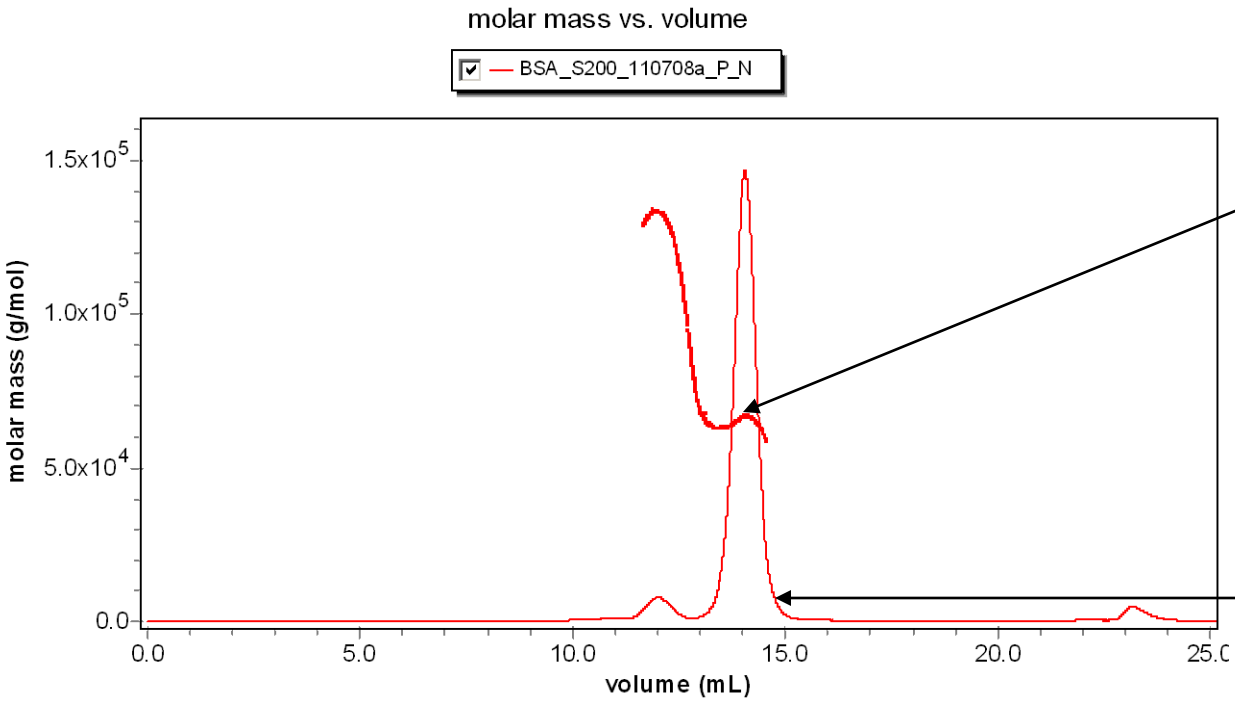
Typical SEC(AFFF); MALLS system



SEC/LS results: Molar Mass Distribution Plot

BSA

Monomer: 66 kDa



Weight-average molar mass

Measured every 2 µl

UV trace; A_{280nm}

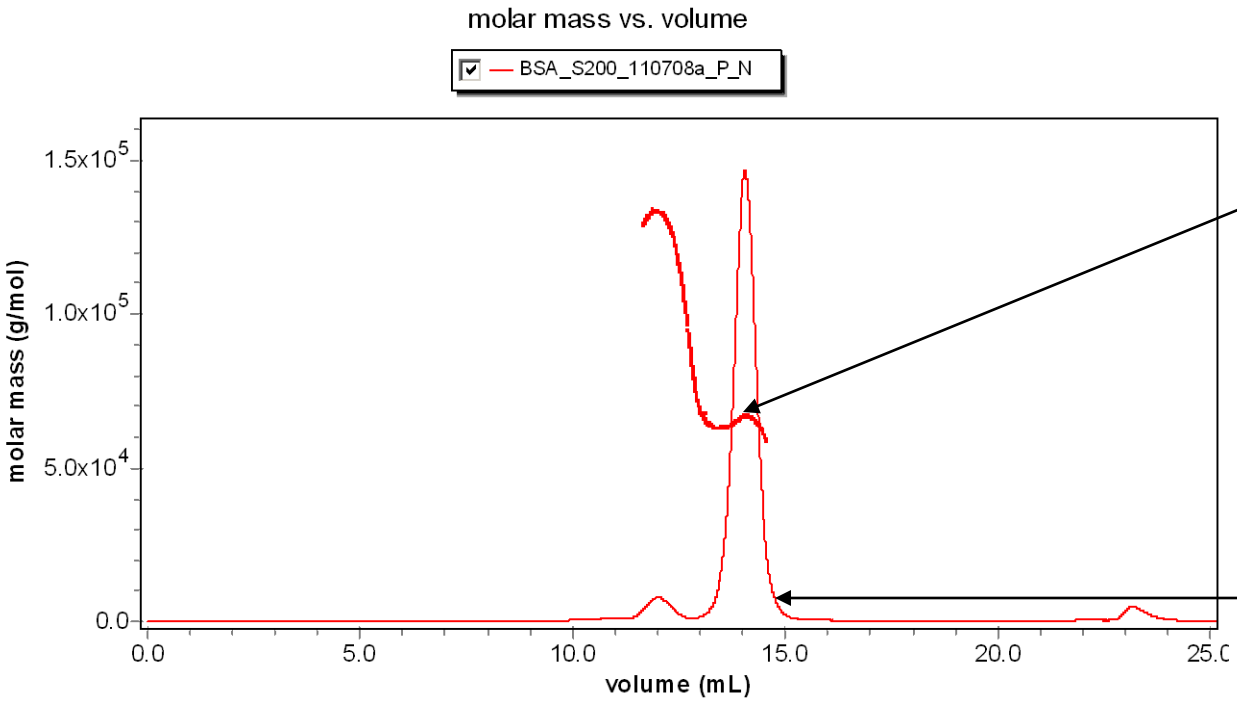
(RI trace)

Is that ALL?

SEC/LS results: Molar Mass Distribution Plot

BSA

Monomer: 66 kDa



Weight-average molar mass

Measured every 2 µl

UV trace; A_{280nm}

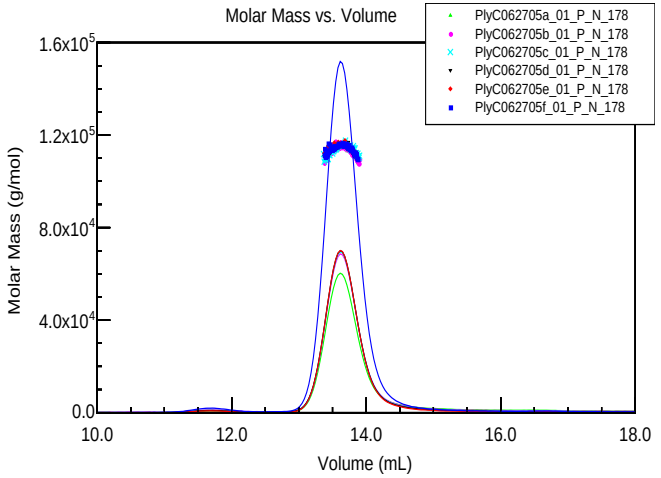
(RI trace)

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

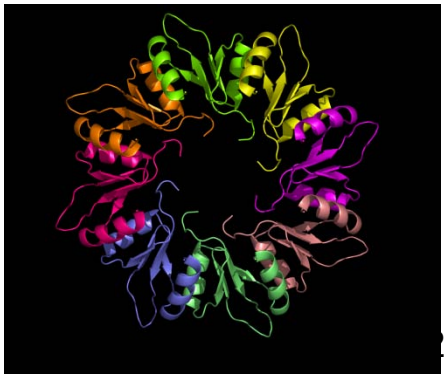
SEC/LS accuracy ~3 % , i.e. ~ 3kDa for PlyC

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

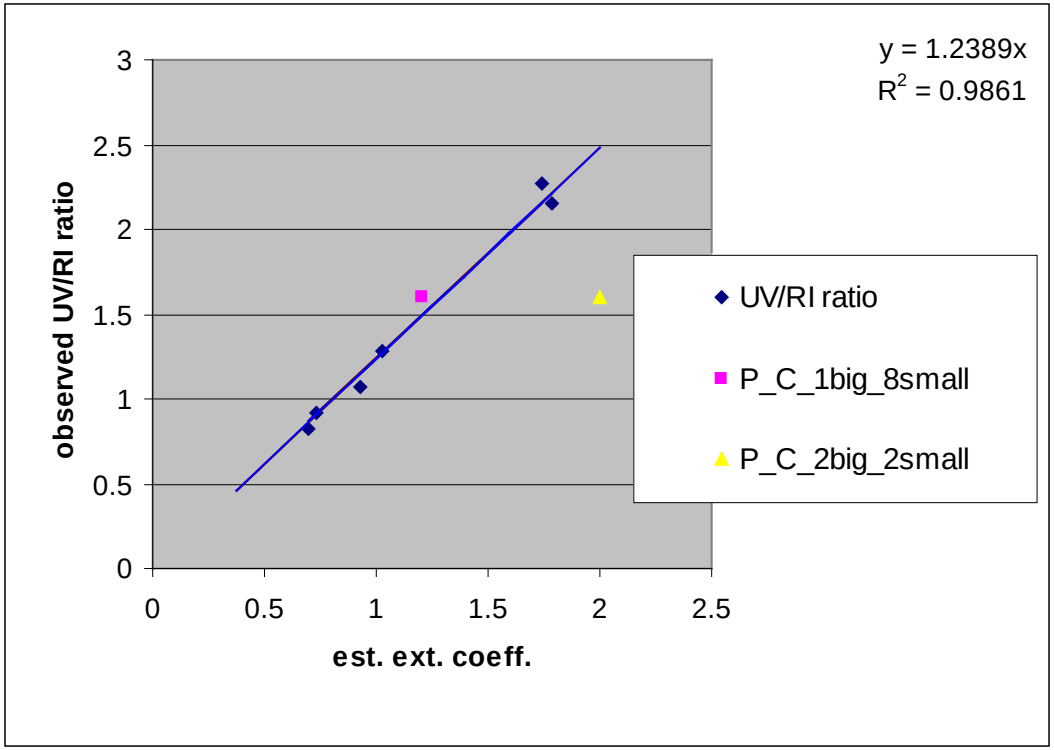


Octameric PlyCB. The eight PlyCB subunits arranged in a ring as observed in the crystal structure of PlyC.

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

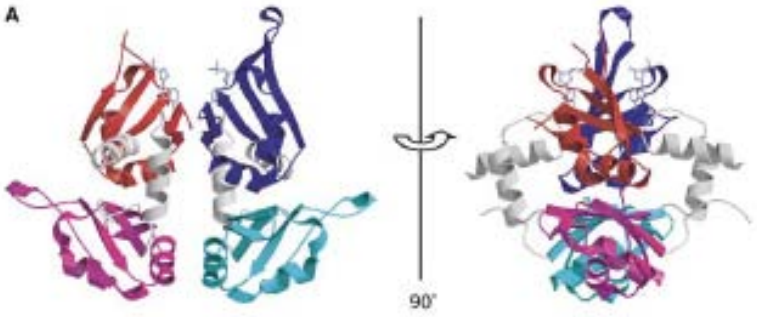
Nelson D, Schuch R., Chahales P., Zhu S., and Fischetti V. A. (2006) PlyC: A multimeric bacteriophage lysin. Proceedings of the National Academy of Sciences 103; 10765-10770

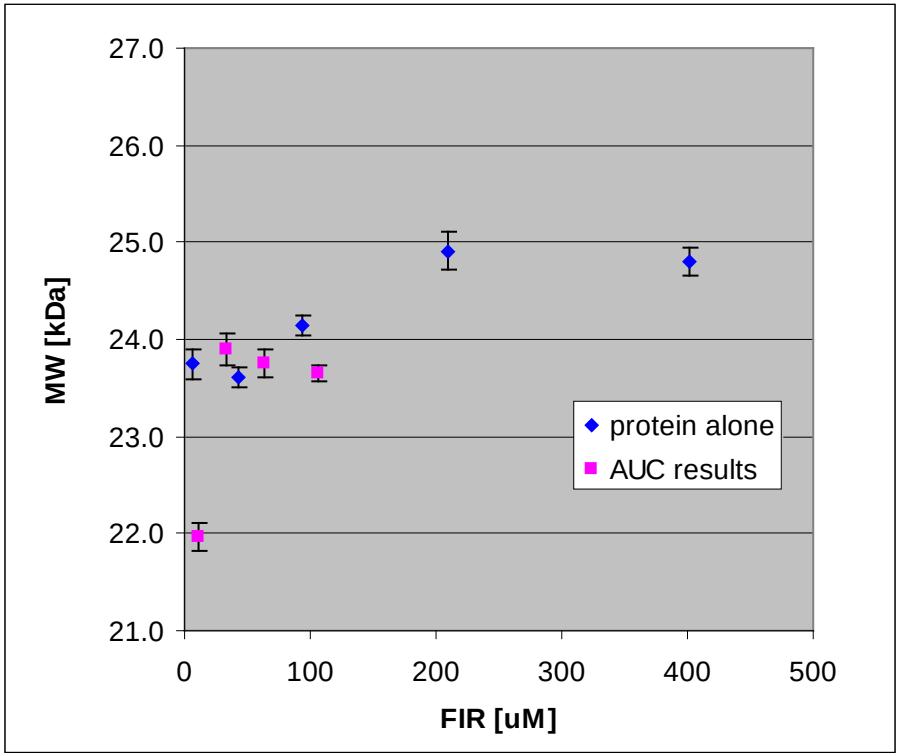
Dimerization of FIR

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





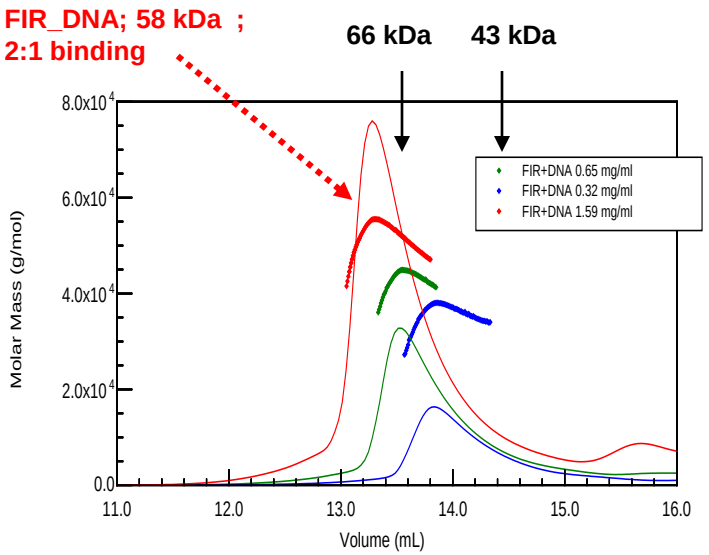
Crichlow G V, Zhou H., Hsiao H. H., Frederick K. B., Debrosse M., Yang Y., Folta-Stogniew E. J., Chung H. J., Fan C., De la Cruz E. M., Levens D., Lolis E., and Braddock D. (2008) Dimerization of FIR upon FUSE DNA binding suggests a mechanism of c-myc inhibition. *EMBO J* **27**: 277-289

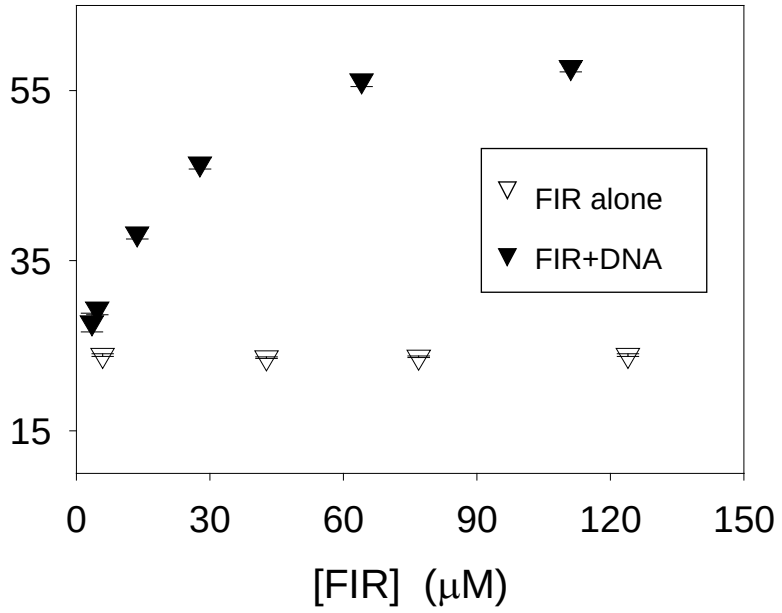
Dimerization of FIR depends on DNA binding event

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

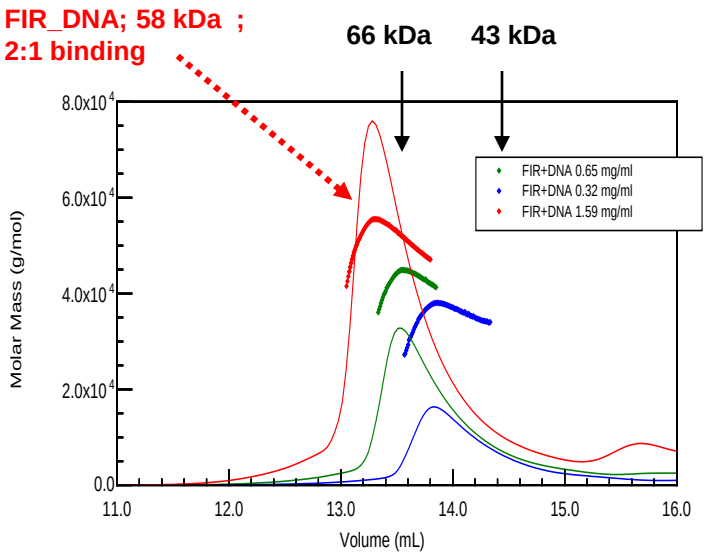
Crichlow, G. V., Zhou, H., Hsiao, H-h., Frederick, K. B.,, Debrosse, M., Yuande Yang, Y., Folta-Stogniew, E. J., Chung, H-J., Chengpeng Fan, C., De La Cruz, E., Levens, D., **Lolis, E.,** and **Braddock, D.** (2008) "Dimerization of FIR upon FUSE binding suggests a mechanism of c-myc inhibition", *EMBO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

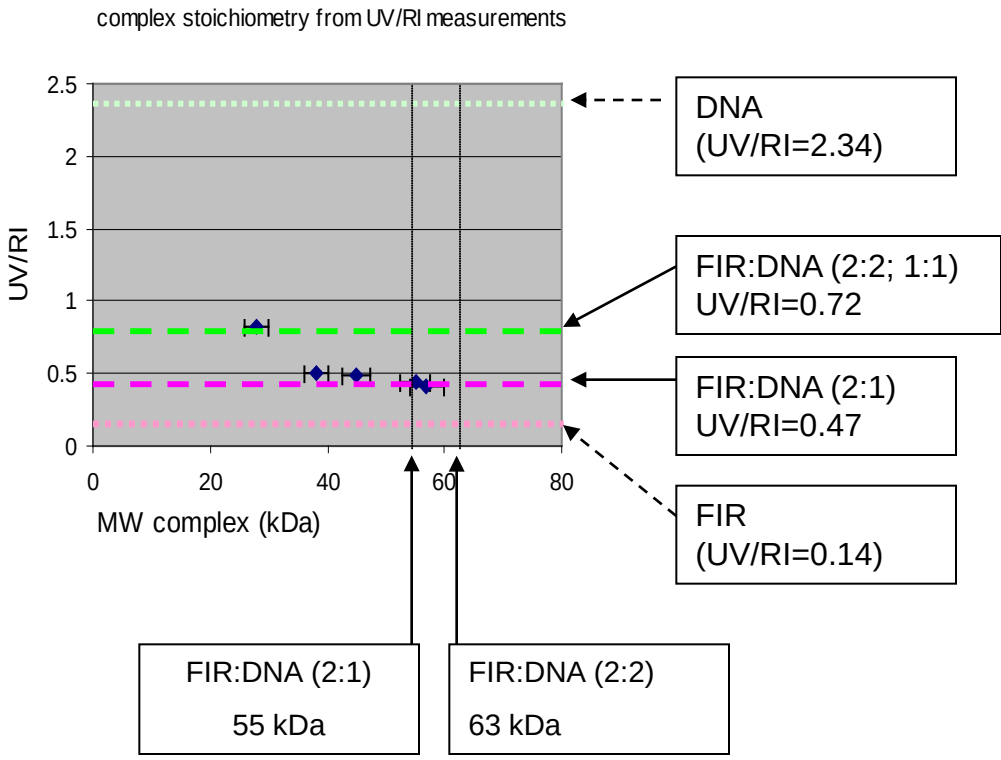
Dimerization of FIR depends on DNA binding event

FIR protein: 23 kDa monomer; seen as a dimer in X-ray structure

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

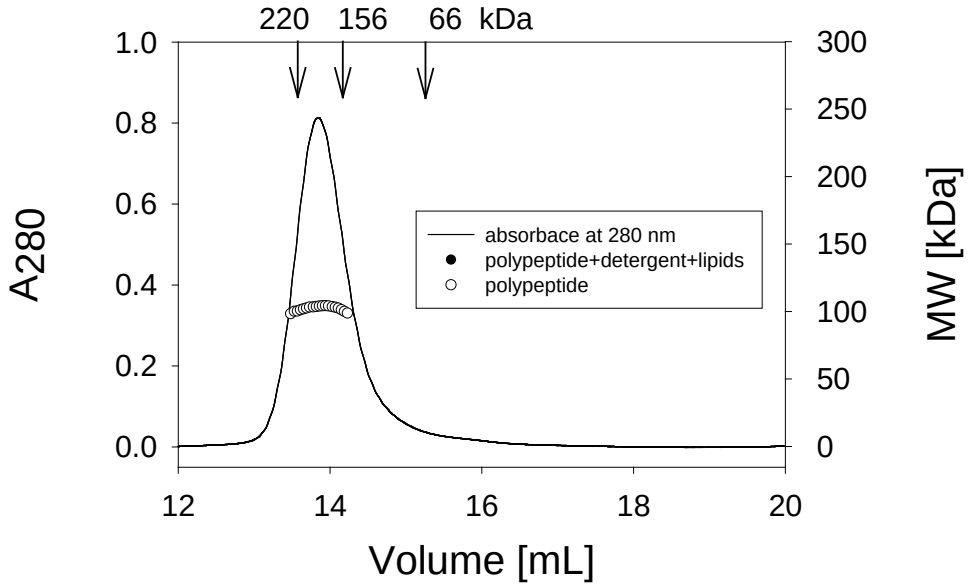
Crichlow, G. V., Zhou, H., Hsiao, H-h., Frederick, K. B.,, Debrosse, M., Yuande Yang, Y., Folta-Stogniew, E. J., Chung, H-J., Chengpeng Fan, C., De La Cruz, E., Levens, D., **Lolis, E.,** and **Braddock, D.** (2008) "Dimerization of FIR upon FUSE binding suggests a mechanism of c-myc inhibition", *EMBO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

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

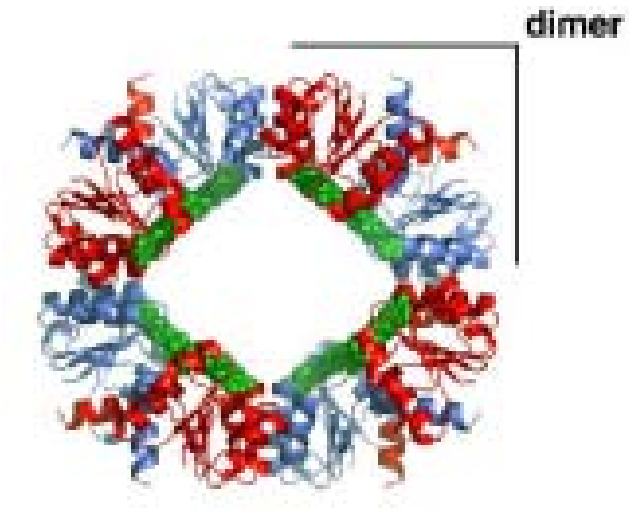
Albright R A, Ibar J. L. V., Kim C. U., Gruner S. M., and Morais-Cabral J. H. (2006) The RCK Domain of the KtrAB K+ Transporter: Multiple Conformations of an Octameric Ring. *Cell* **126**: 1147-1159

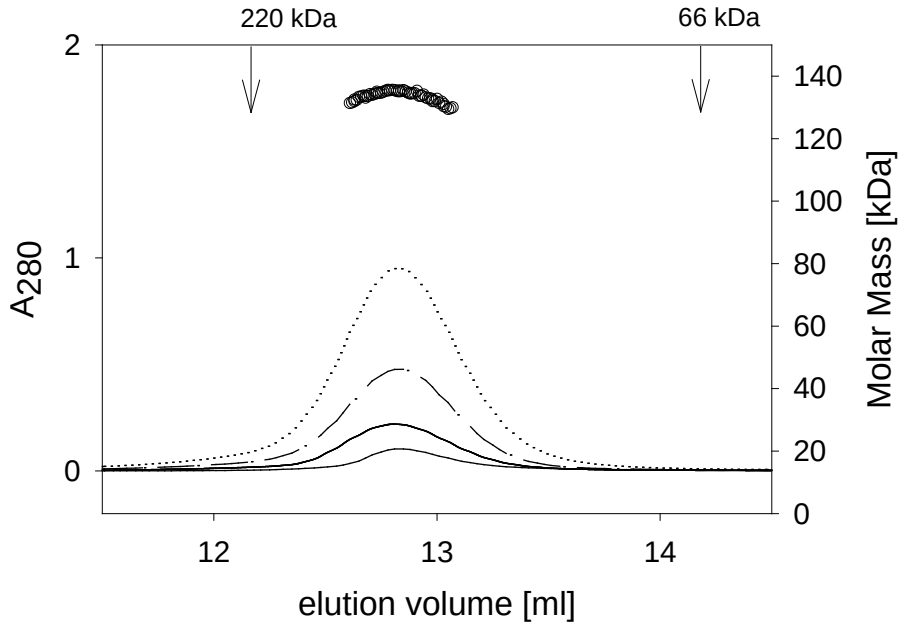
Multiple oligomeric states for reconstituted KtrAB K^+ Transporter

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





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

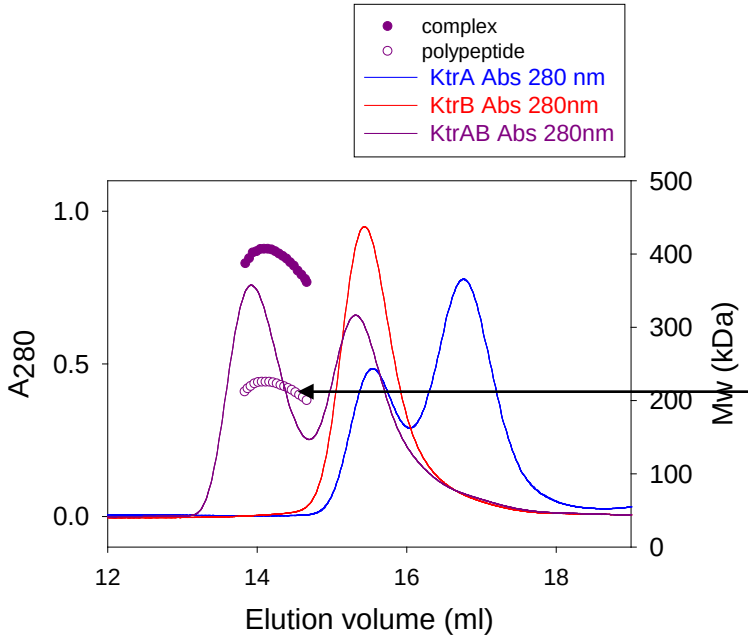
Multiple oligomeric states for reconstituted KtrAB K^+ Transporter

KtrAB ion transporter

complex : 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

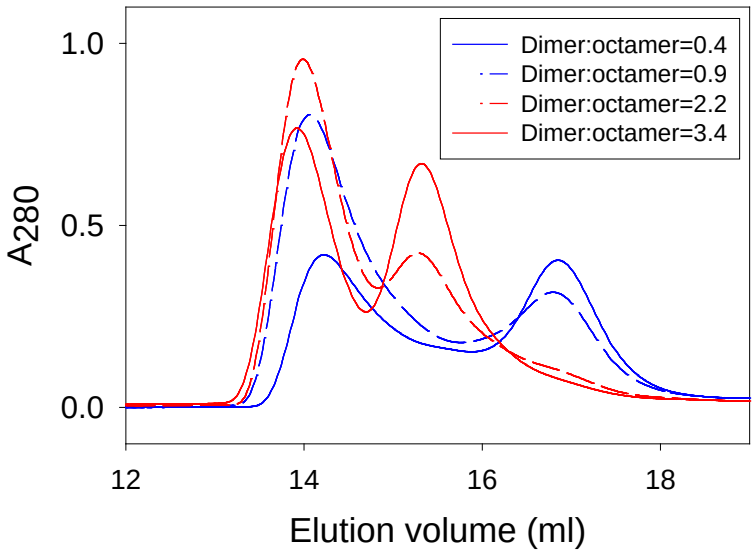
Buffer: 25 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM NADH, 1 mM DDM

Multiple oligomeric states for reconstituted KtrAB K^+ Transporter

KtrAB ion transporter

(8:2) model polypeptide = 228 kDa

(8:4) model polypeptide = 325 kDa



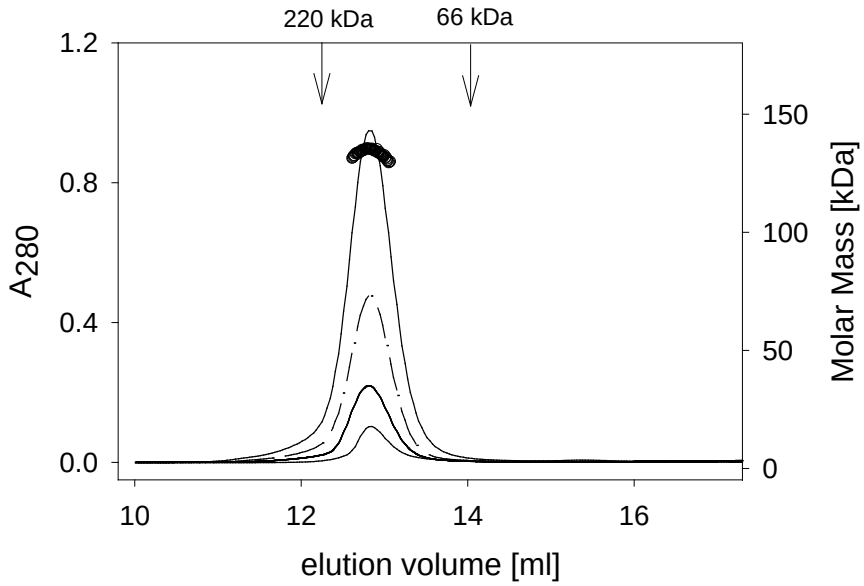
dimer:octamer KtrB:KtrA	complex	Elution volume (ml)	Total mass of complex (kDa)	Poly-peptide (kDa)	lipids (kDa)
0.4	8:2	14.23	486	228	256
0.9	8:2	14.05	521	240	281
2.2	8:4	13.99	552	302	261
3.7	8:4	13.91	560	299	251

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*

Effects of detergent on oligomeric state of KtrA RCK domain

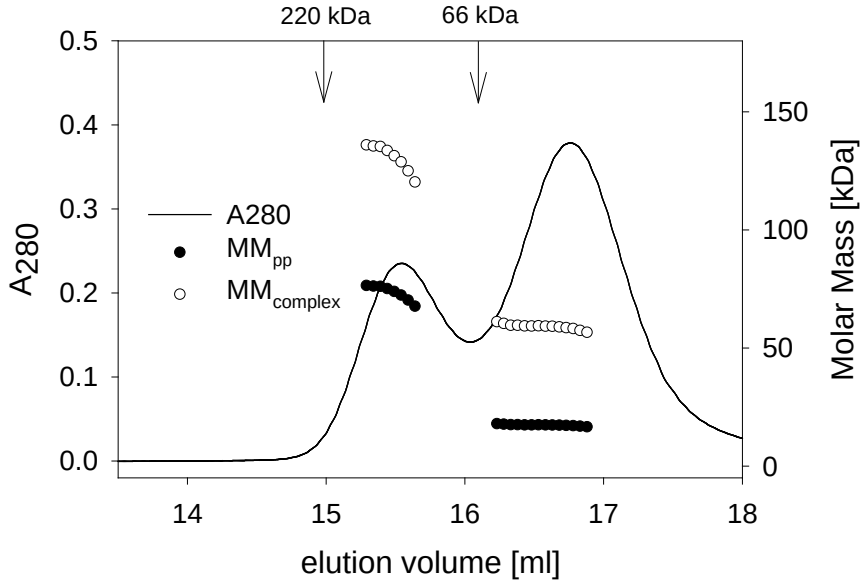
KtrA RCK domain no detergent

(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

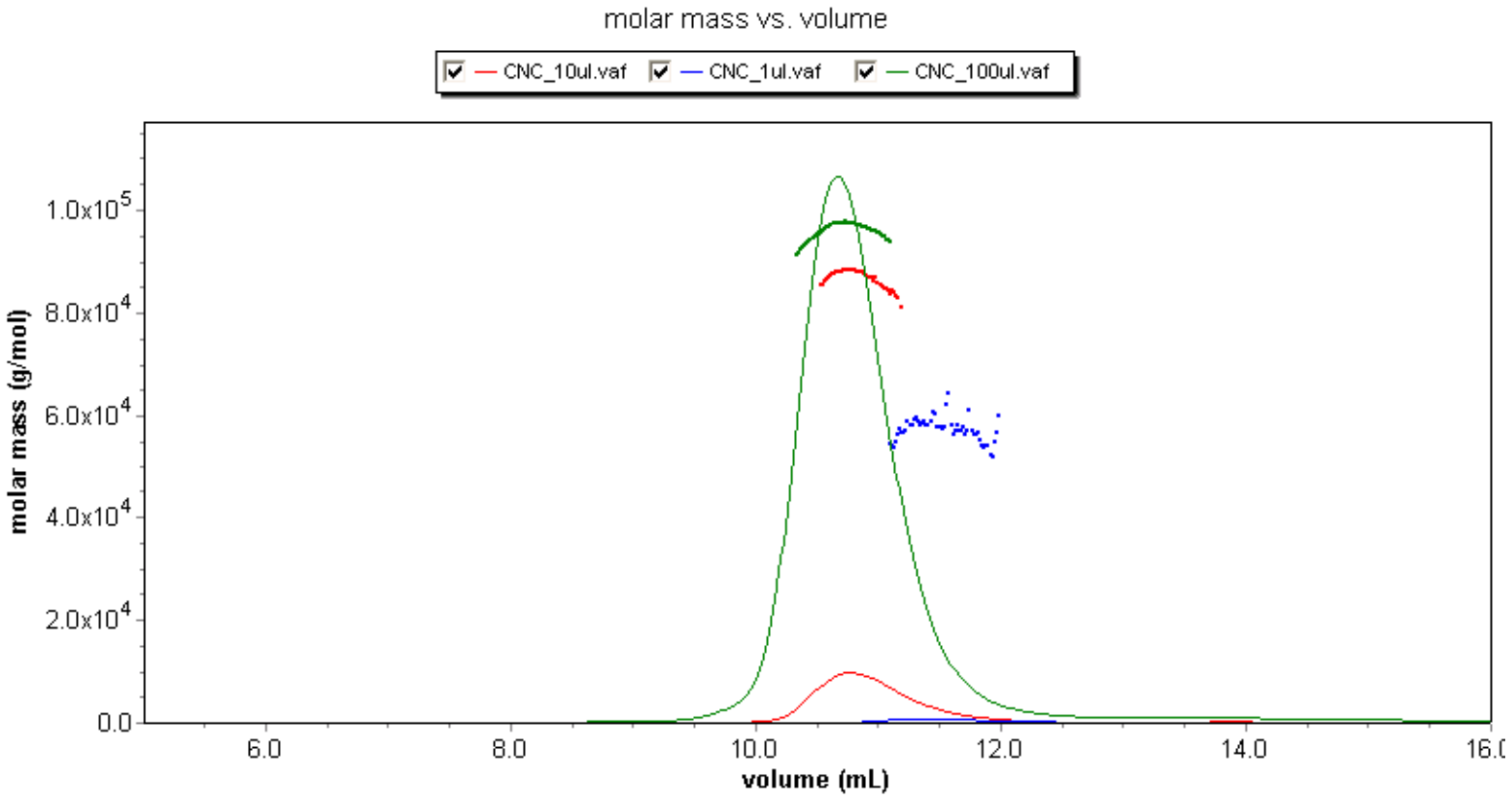
Results:

- Determination of dimerization constant

Determination of dimerization constant from SEC-LS measurements

Nucleobindin 1 (NUCB1) is a widely expressed multidomain calcium-binding protein whose precise physiological and biochemical functions are not well understood;

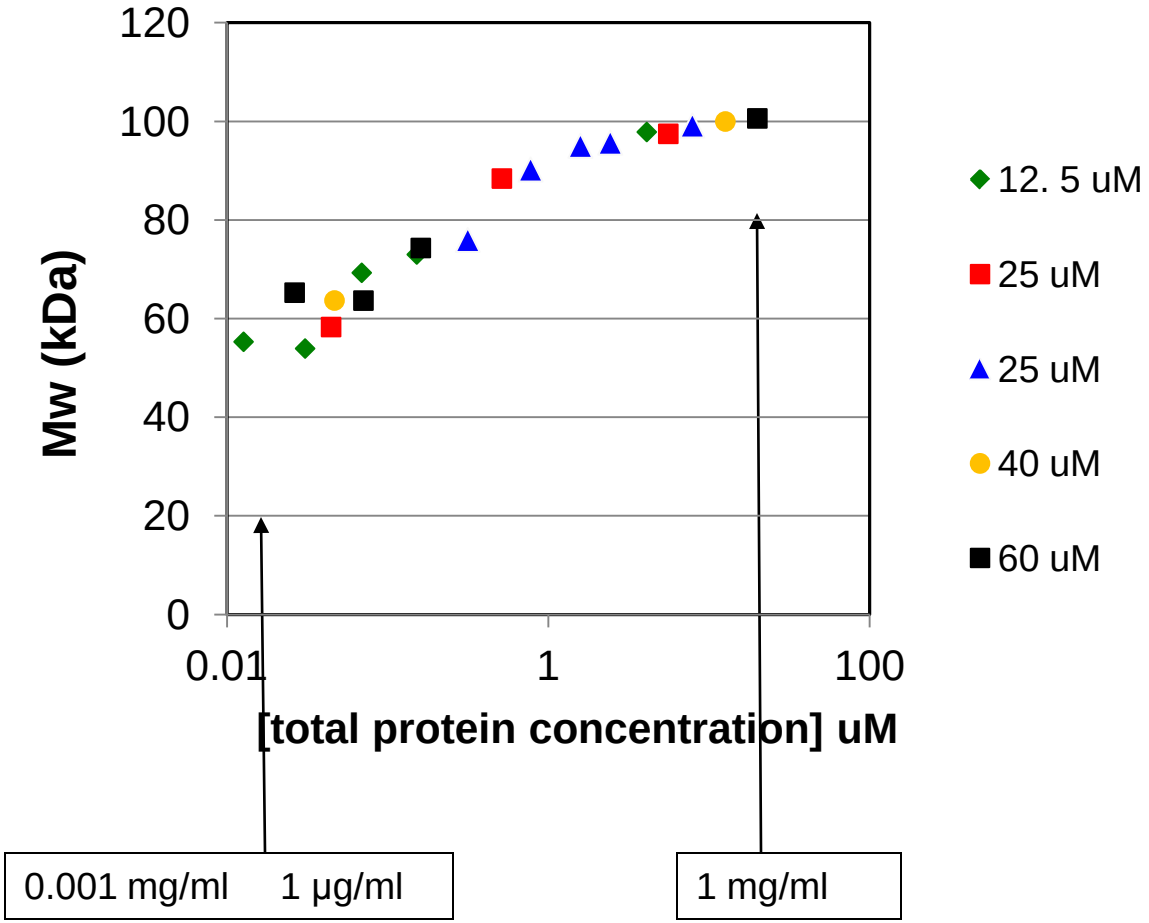
soluble form of NUCB1 (*sNUCB1) ;* Monomer 51 kDa



Determination of dimerization constant from SEC-LS measurements

Nucleobindin 1 (NUCB1) is a widely expressed multidomain calcium-binding protein whose precise physiological and biochemical functions are not well understood;

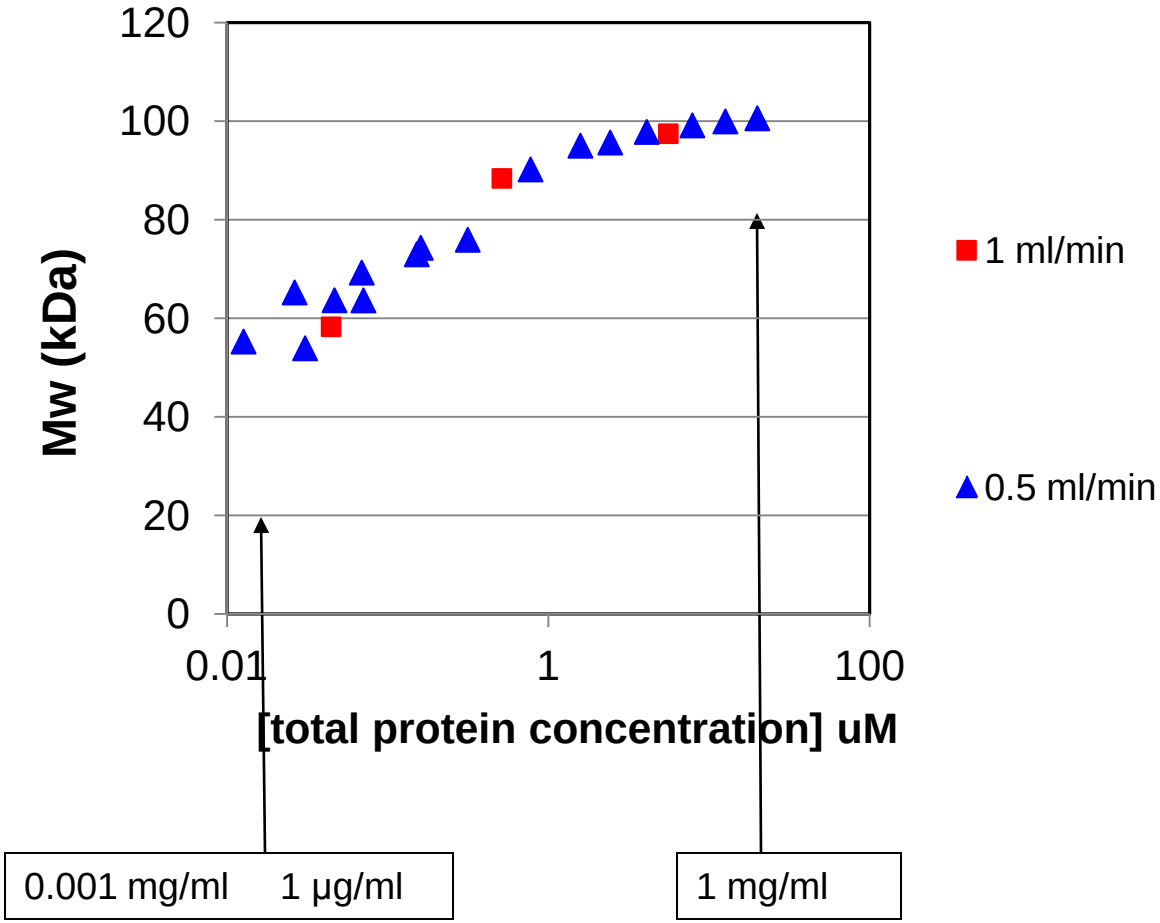
soluble form of NUCB1 (*sNUCB1) ;* Monomer 51 kDa



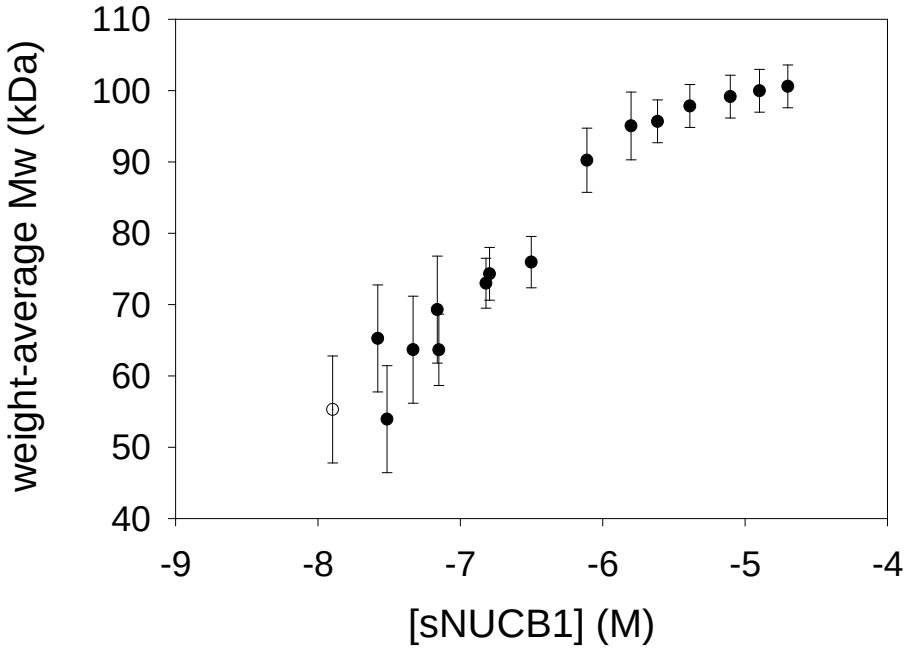
Determination of dimerization constant from SEC-LS measurements

Nucleobindin 1 (NUCB1) is a widely expressed multidomain calcium-binding protein whose precise physiological and biochemical functions are not well understood;

soluble form of NUCB1 (*sNUCB1) ;* Monomer 51 kDa



protein conc (M)	Mw (kDa)
1.27E-08	55.3
2.63E-08	65.26
3.06E-08	53.93
4.67E-08	63.67
6.89E-08	69.29
7.06E-08	63.65
1.52E-07	73
1.61E-07	74.32
4.10E-06	97.84
7.88E-06	99.16
1.26E-05	99.97
2.00E-05	100.6
1.58E-06	95.04
3.15E-07	75.95
2.43E-06	95.68
7.75E-07	90.23



$$2M = D$$

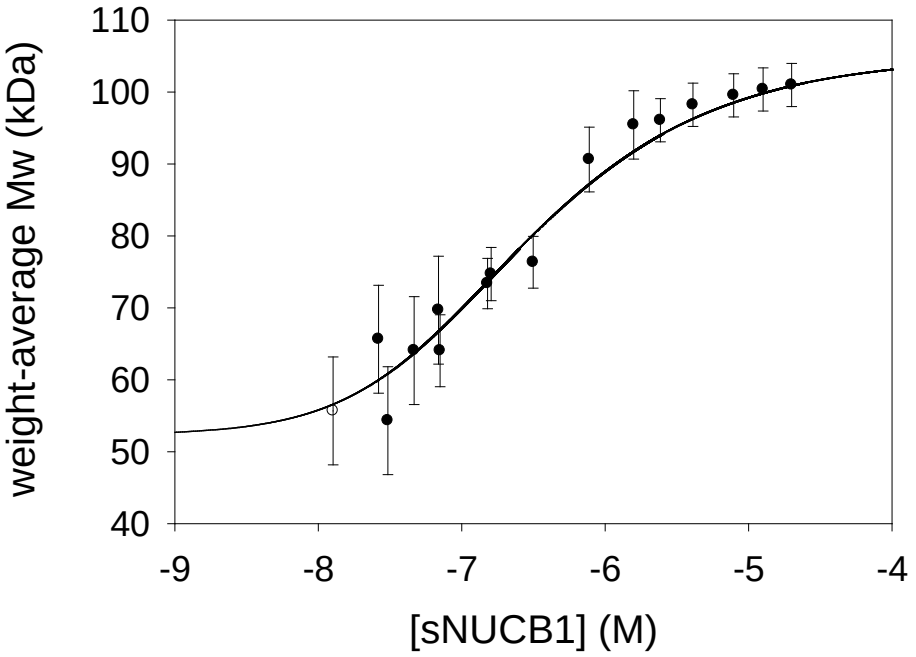
$$M_w = f_m M_m + f_d M_d = M_m(2 - f_m)$$

$$K_a = \frac{[D]}{[M]^2} = \frac{(1 - f_m)}{2(f_m)^2 c_t} \qquad f_m = \frac{-1 + \sqrt{1 + 8K_a c_t}}{4K_a c_t}$$

Kapoor N, Gupta R., Menon S. T., Folta-Stogniew E., Raleigh D. P., and Sakmar T. P. (2010) Nucleobindin 1 is a calcium-regulated guanine nucleotide dissociation inhibitor of G{alpha}i1. *J.Biol.Chem.* **285**; 31647-31660

protein conc (M)	Mw (kDa)
1.27E-08	55.3
2.63E-08	65.26
3.06E-08	53.93
4.67E-08	63.67
6.89E-08	69.29
7.06E-08	63.65
1.52E-07	73
1.61E-07	74.32
4.10E-06	97.84
7.88E-06	99.16
1.26E-05	99.97
2.00E-05	100.6
1.58E-06	95.04
3.15E-07	75.95
2.43E-06	95.68
7.75E-07	90.23

Kd=0.26μM ± 0.03 μM (12%)

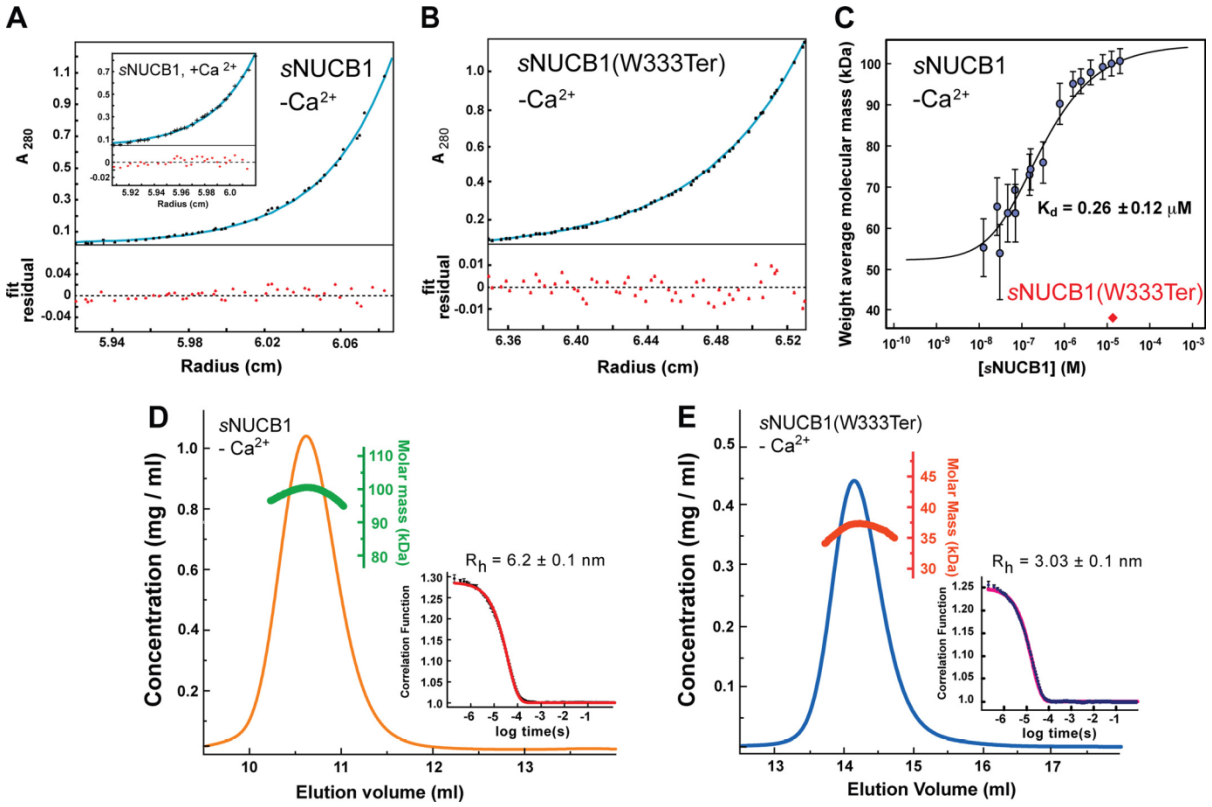


$$2M = D$$

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

$$K_a = \frac{[D]}{[M]^2} = \frac{(1 - f_m)}{2(f_m)^2 c_t} \qquad f_m = \frac{-1 + \sqrt{1 + 8K_a c_t}}{4K_a c_t}$$

Kapoor N, Gupta R., Menon S. T., Folta-Stogniew E., Raleigh D. P., and Sakmar T. P. (2010) Nucleobindin 1 is a calcium-regulated guanine nucleotide dissociation inhibitor of G{alpha}i1. *J.Biol.Chem.* **285**; 31647-31660

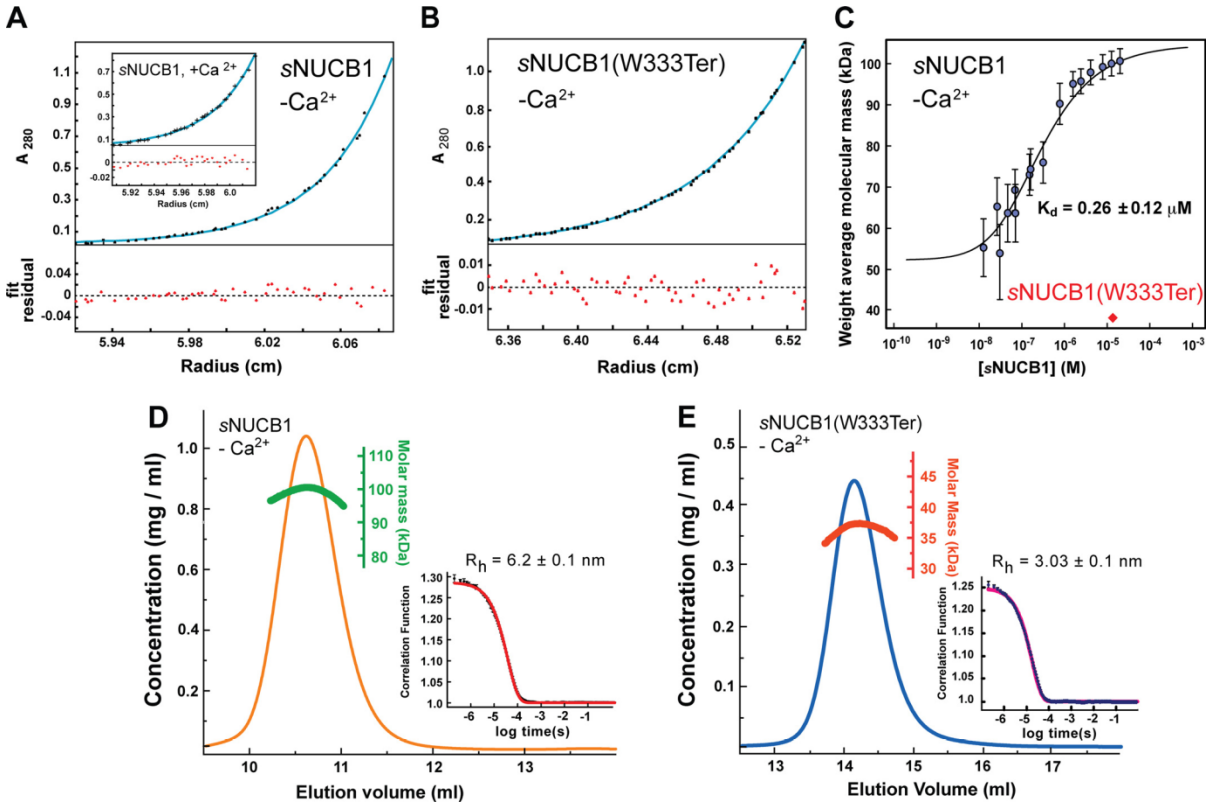


Samples		Oligomeric State in Solution			
		Molecular Weight (kDa)		Dimerization Constant	
Protein	Monomer (kDa)	AUC	SEC/MALLS	AUC	SEC/MALLS
sNUCB1-Ca2^{+}	51	98.9 ± 0.41	99 ± 1*		0.26 ± 0.03 μM
sNUCB1(W333Ter)-Ca2^{+}	37	35.2 ± 0.05	37± 2		

*Average from four SEC/MALLS analyses ± StDev

sNUCB1(W333Ter)-Ca2^{+} truncation mutant, which lacks the lucine zipper domain

Kapoor N, Gupta R., Menon S. T., Folta-Stogniew E., Raleigh D. P., and Sakmar T. P. (2010) Nucleobindin 1 is a calcium-regulated guanine nucleotide dissociation inhibitor of G{alpha}i1. *J.Biol.Chem.* **285**; 31647-31660



Samples		Oligomeric State in Solution				Shape Analysis	
		Molecular Weight (kDa)		Dimerization Constant			
Protein	Monomer (kDa)	AUC	SEC/MALLS	AUC	SEC/MALLS	Rh (DLS)	f/fo
sNUCB1-Ca2$^+$	51	98.9 ± 0.41	99 ± 1*		0.26 ± 0.03 μM	6.2 ± 0.1	2.03
sNUCB1(W333Ter)-Ca2$^+$	37	35.2 ± 0.05	37± 2			3.0 ± 0.1	1.59

*Average from four SEC/MALLS analyses ± StDev

sNUCB1(W333Ter)-Ca2$^+$ truncation mutant, which lacks the lucine zipper domain

Kapoor N, Gupta R., Menon S. T., Folta-Stogniew E., Raleigh D. P., and Sakmar T. P. (2010) Nucleobindin 1 is a calcium-regulated guanine nucleotide dissociation inhibitor of G{alpha}i1. *J.Biol.Chem.* **285**; 31647-31660

Determination of dimerization constant from SEC-LS measurements

SecA protein (nanomotor promotes protein translocation in eubacteria)

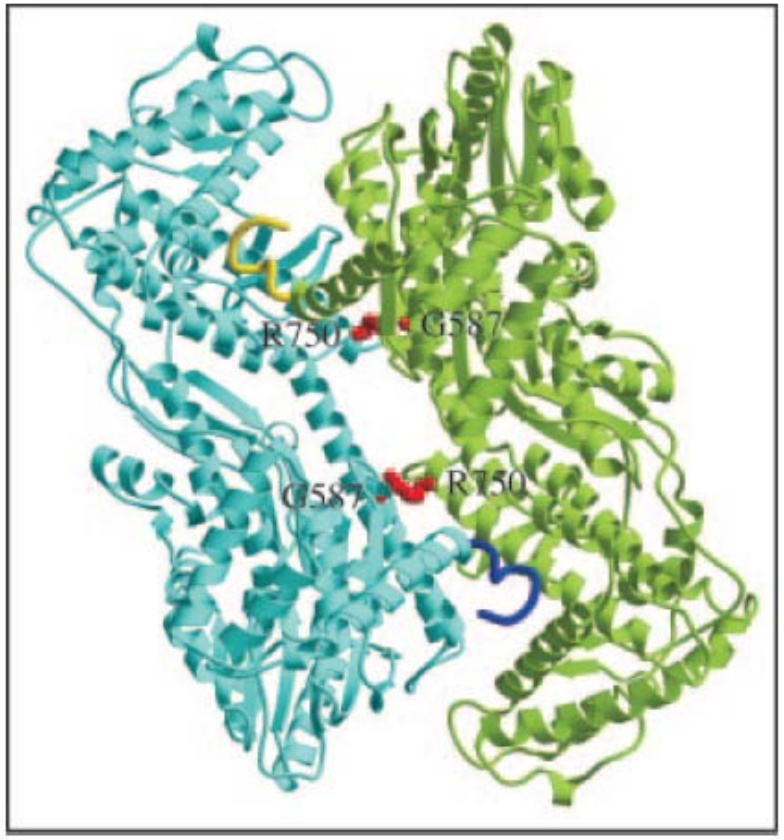
conflicting reports about whether SecA functions as a monomer or dimer

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

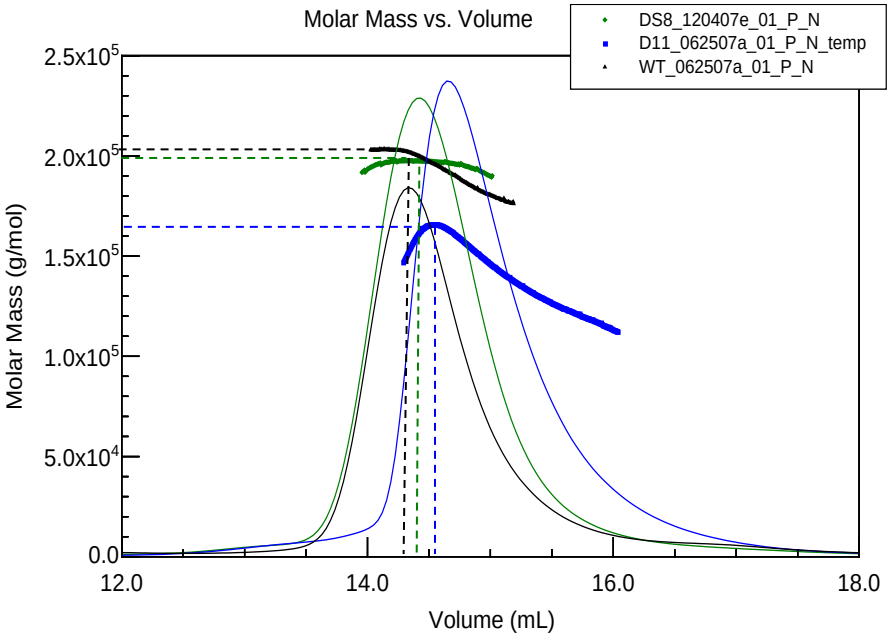
WT monomer = 102 kDa

DS8 deletion mutant monomer = 101 kDa

D11 deletion mutant monomer = 100 kDa

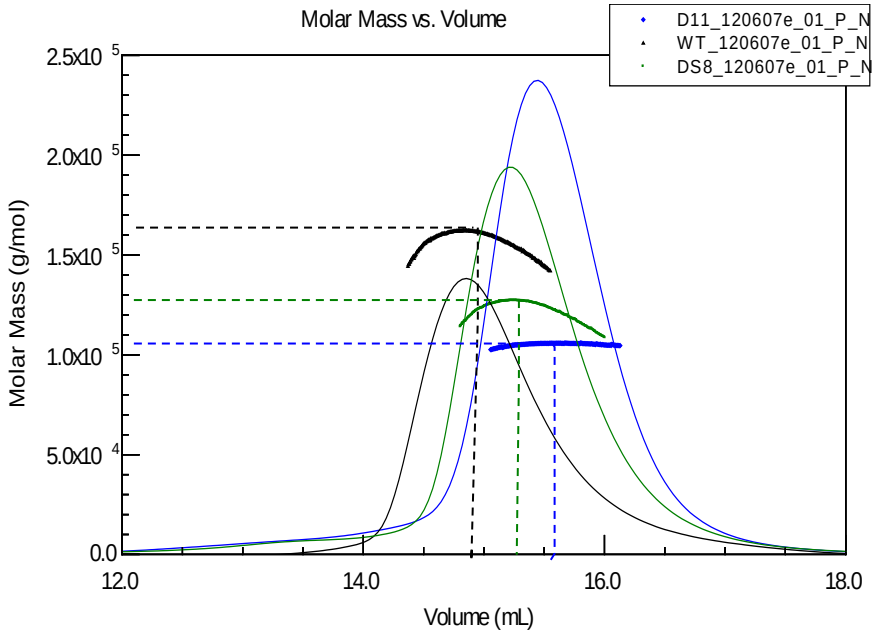
Low salt buffer:

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



High salt buffer:

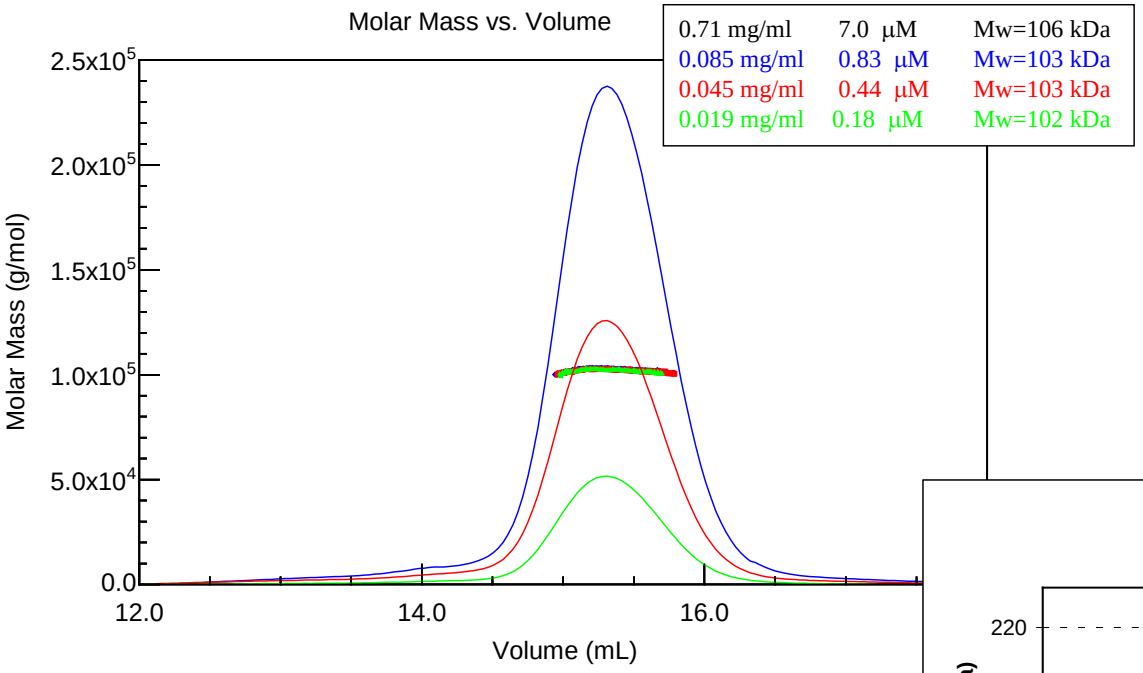
10 mM Tris pH 7.5, 5 mM Mg2+, 300 mM KCl

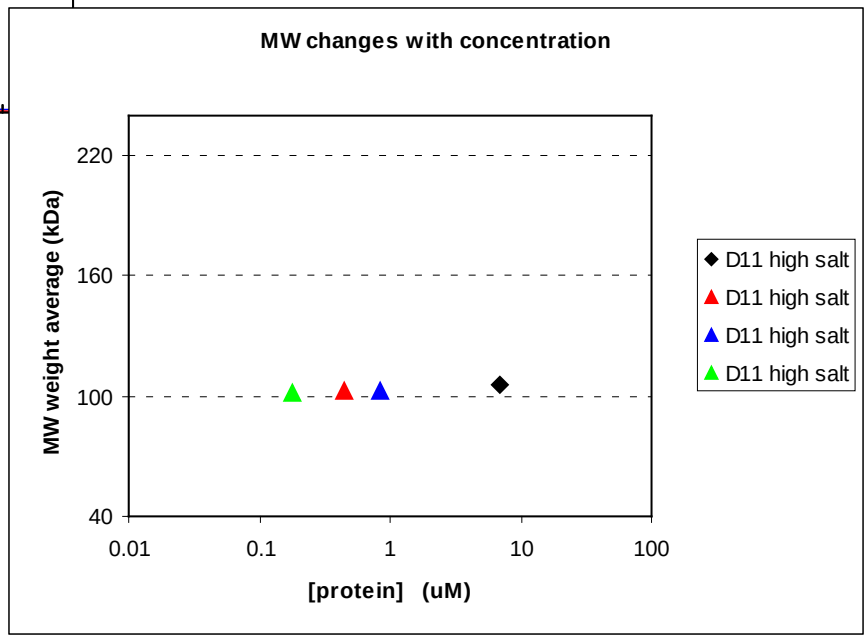


D11 deletion mutant
mono= **101 kDa**

High salt buffer:

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

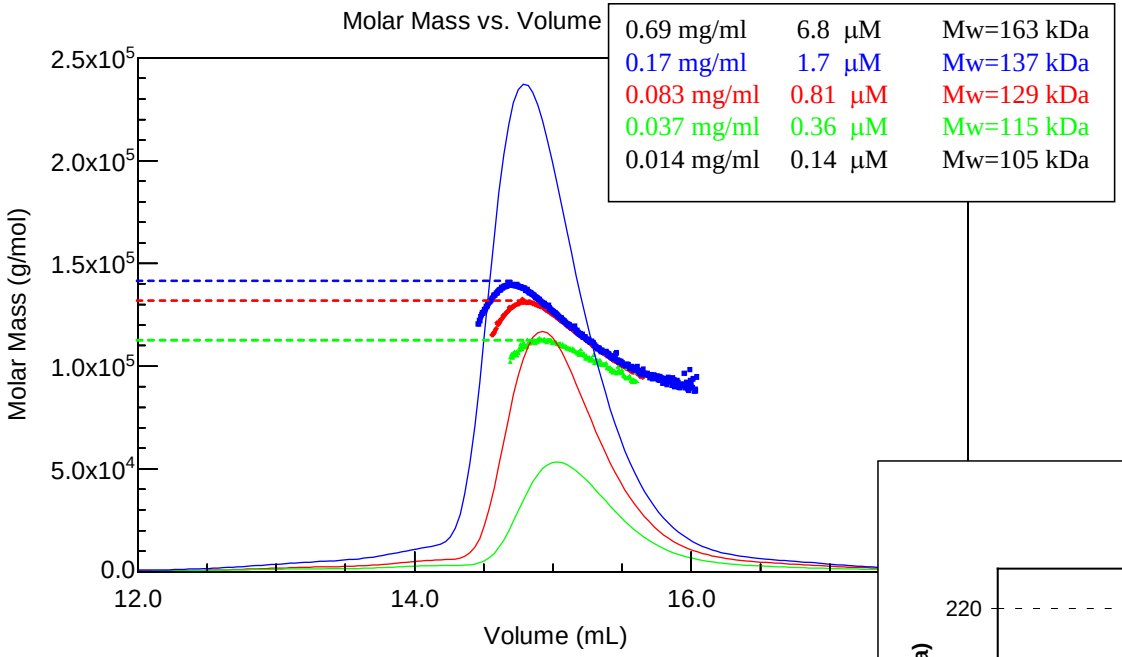


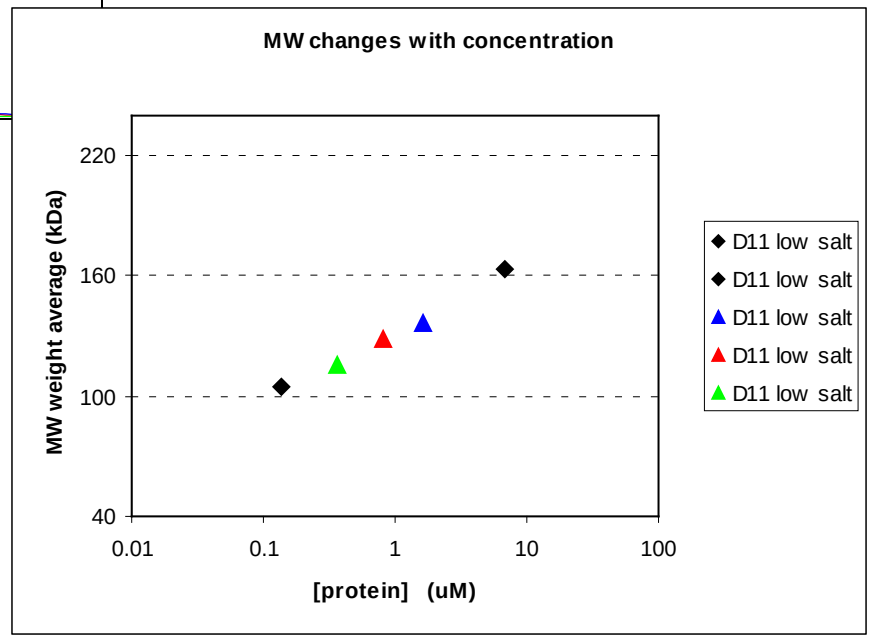


D11 deletion mutant
mono= **101 kDa**

Low salt buffer:

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

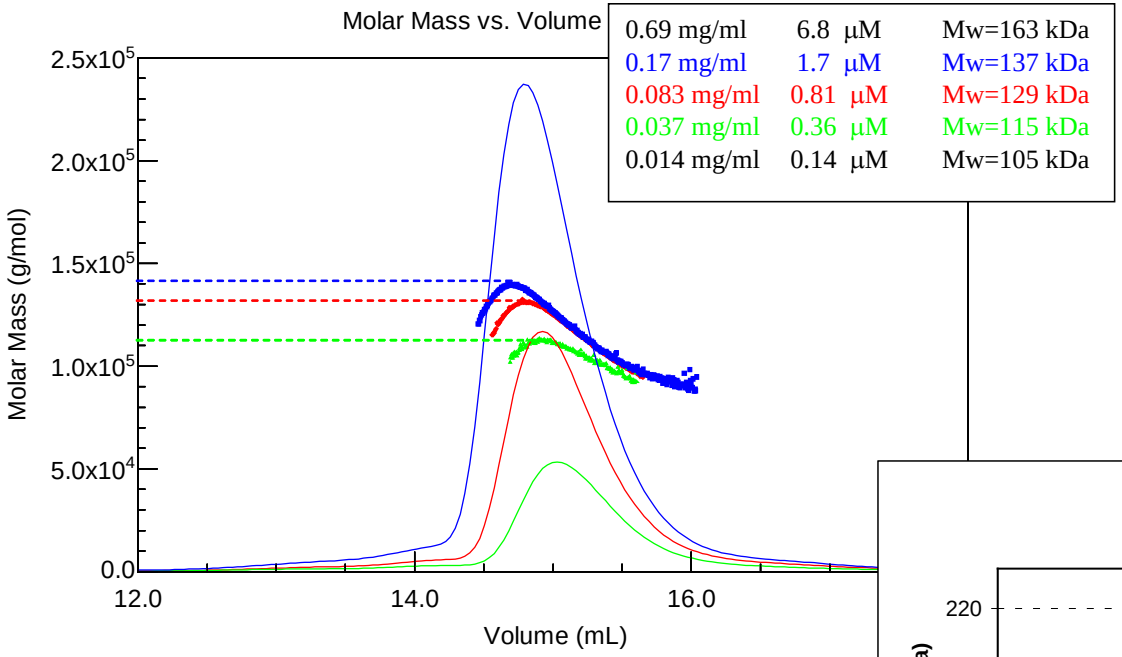


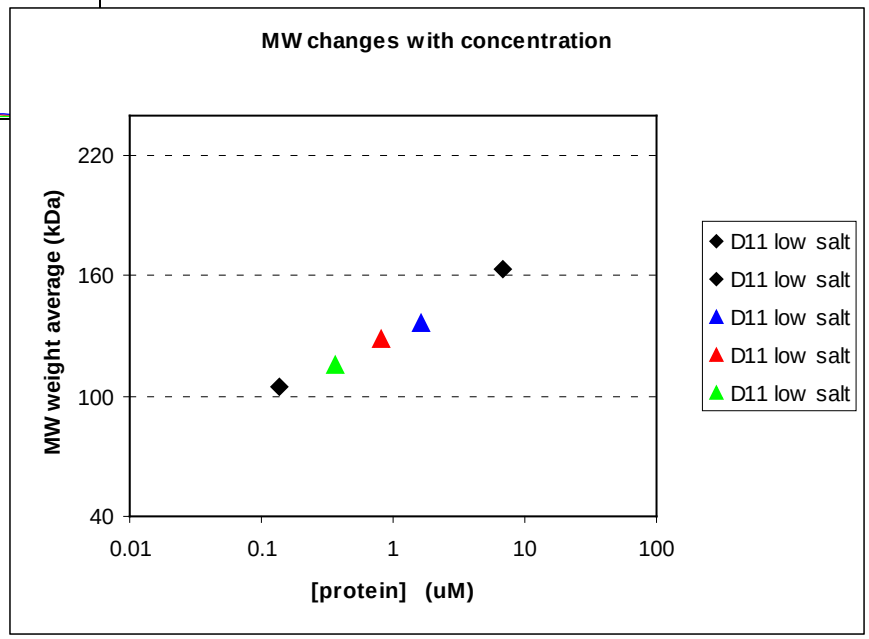


D11 deletion mutant
mono= **101 kDa**

Low salt buffer:

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





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

$$2M = D$$

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

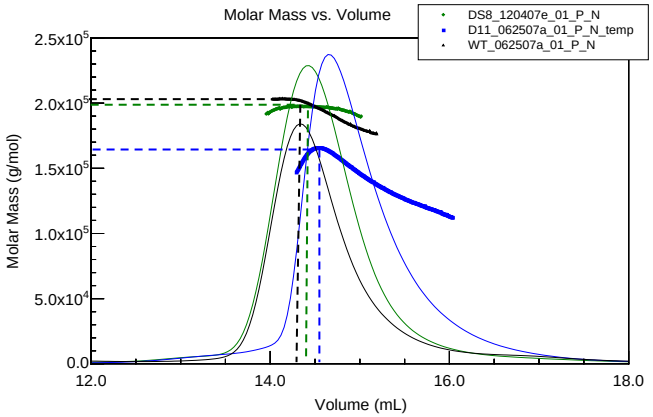
$$f_m = \frac{-1 + \sqrt{1 + 8K_a c_t}}{4K_a c_t}$$

WT monomer = 102 kDa

DS8 deletion mutant monomer = 101 kDa

D11 deletion mutant monomer = 100 kDa

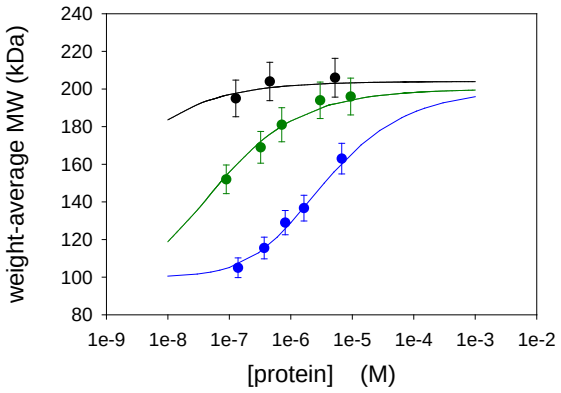
Low salt buffer: 100 mM KCl



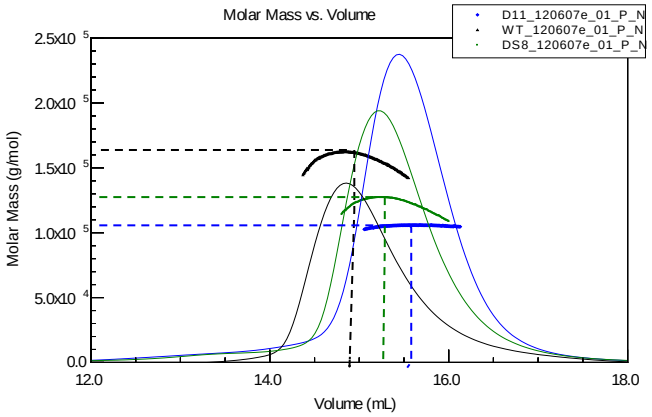
WT Kd= <1e-9

DS8 Kd=7±1e-8 M

D11 Kd=3.5±0.2e-6 M



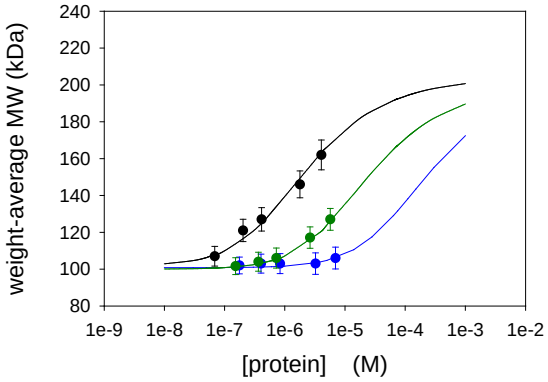
High salt buffer: 300 mM KCl



WT Kd= 2.2±0.2e-6 M

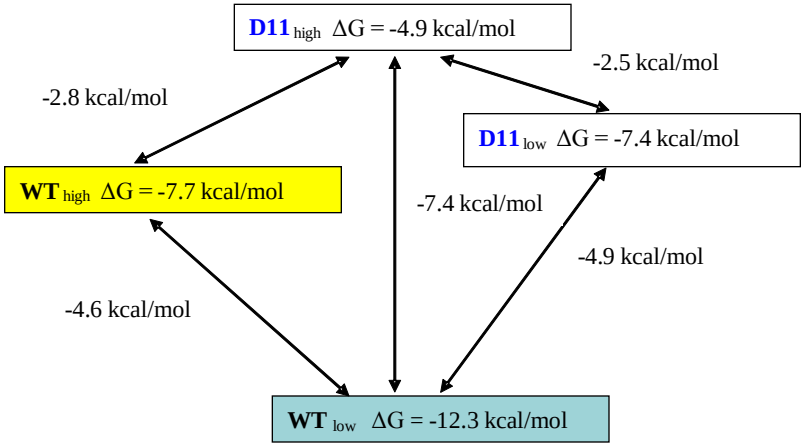
DS8 Kd= 2.41±0.05e-5 M

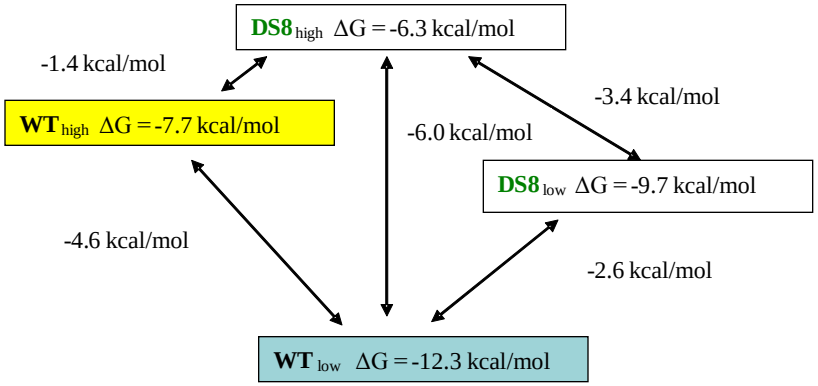
D11 Kd> 2.4e-4 M



Thermodynamic linkage for SecA dimerization 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.

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

Why no AUC data?

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.

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

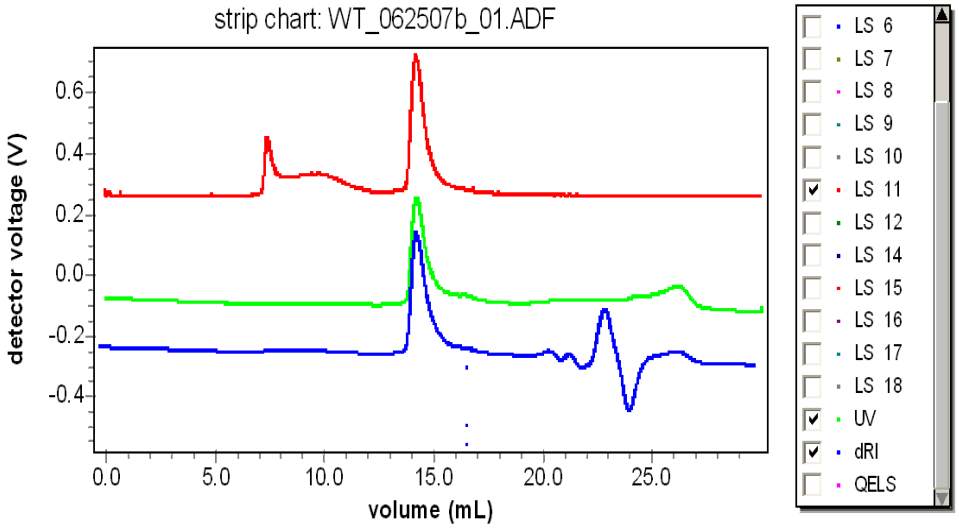
WT monomer = 102 kDa

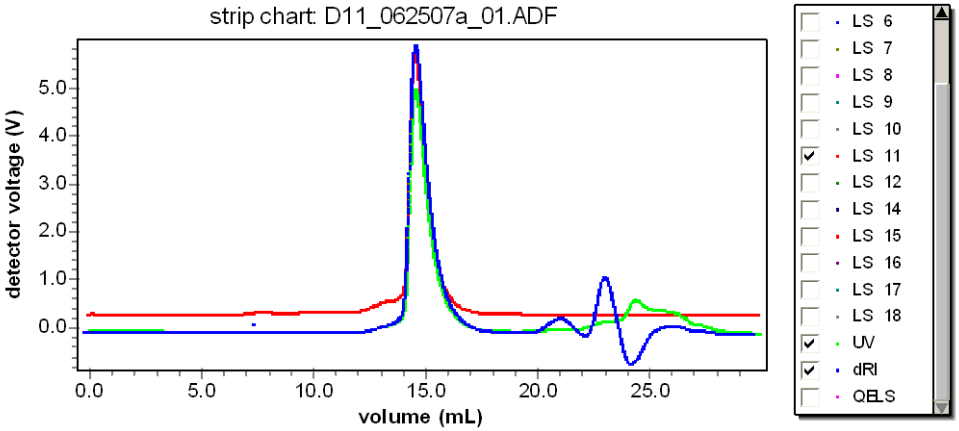
DS8 deletion mutant monomer = 101 kDa

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





Determination of dimerization constant from SEC-LS measurements

SecA protein (nanomotor promotes protein translocation in eubacteria)

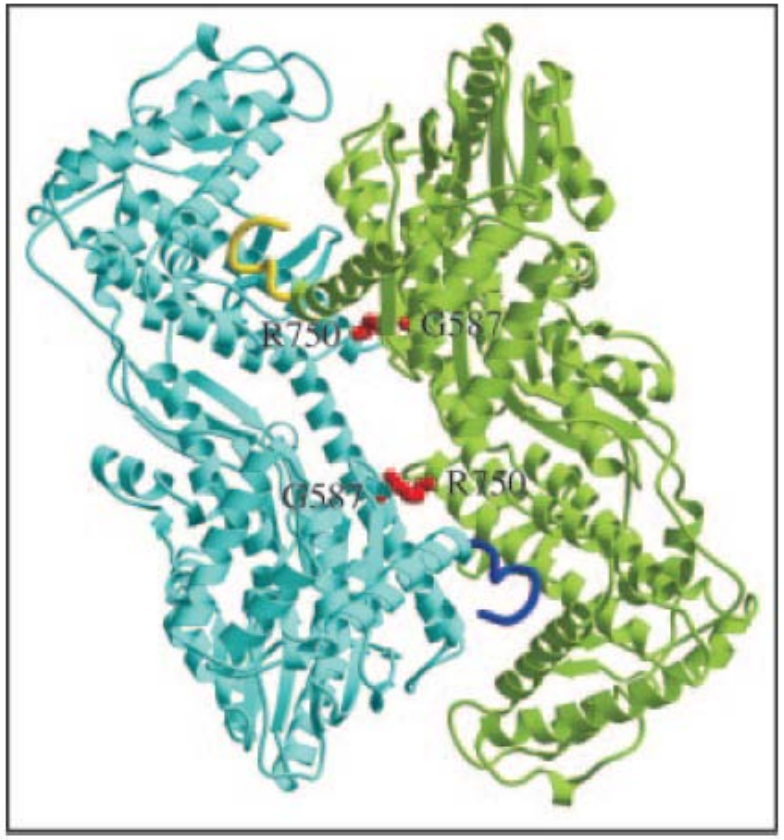
conflicting reports about whether SecA functions as a monomer or dimer

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].

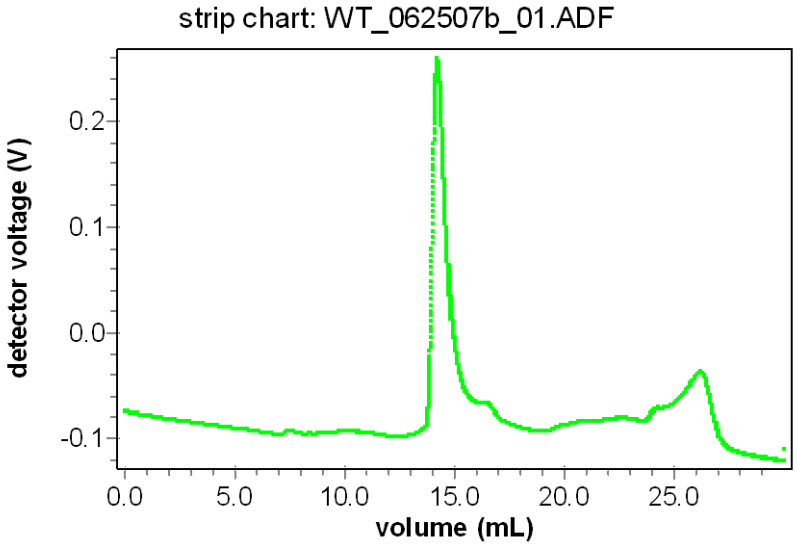
[a]Picture taken from : Or, E., A. Navon, and T. Rapoport. 2002. Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane. EMBO J. 21:4470-4479

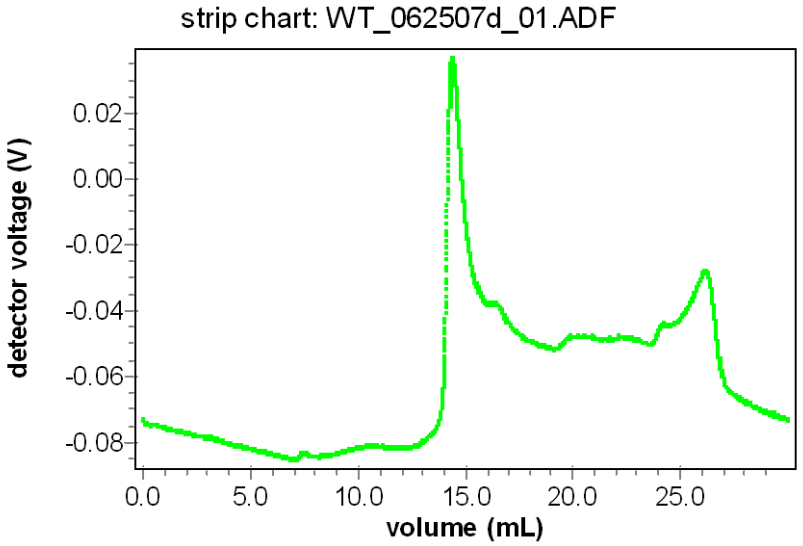
Determination of dimerization constant from SEC-LS measurements

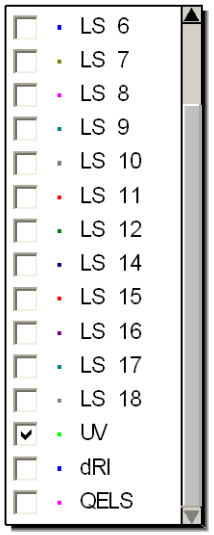
SecA protein (nanomotor promotes protein translocation in eubacteria)

conflicting reports about whether SecA functions as a monomer or dimer

WT monomer = 102 kDa





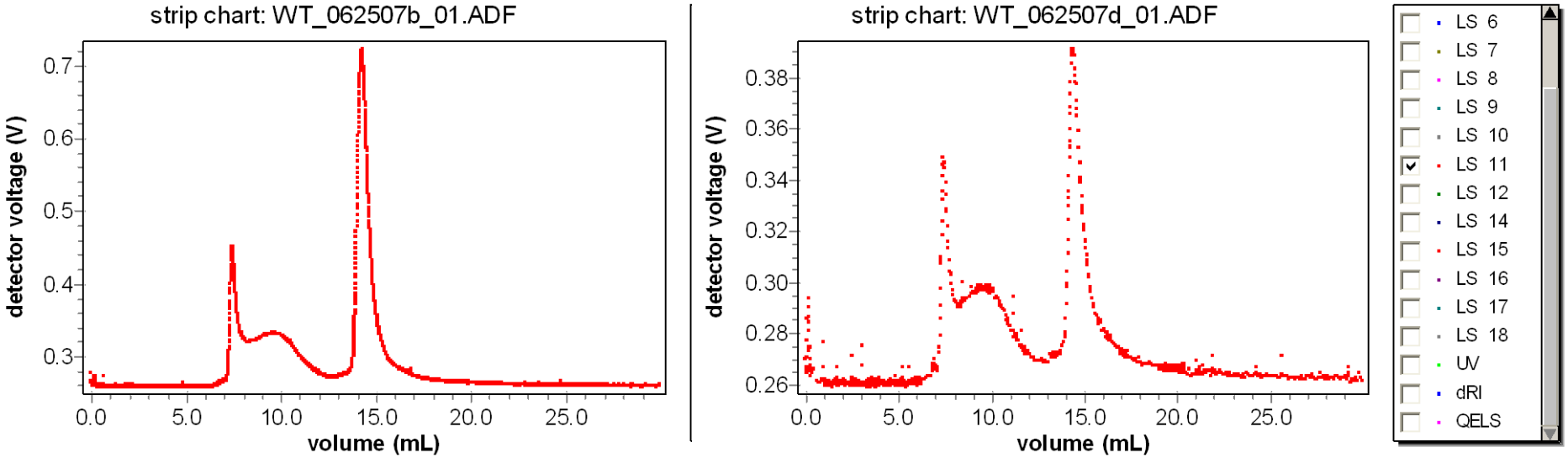


Determination of dimerization constant from SEC-LS measurements

SecA protein (nanomotor promotes protein translocation in eubacteria)

conflicting reports about whether SecA functions as a monomer or dimer

WT monomer = 102 kDa



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

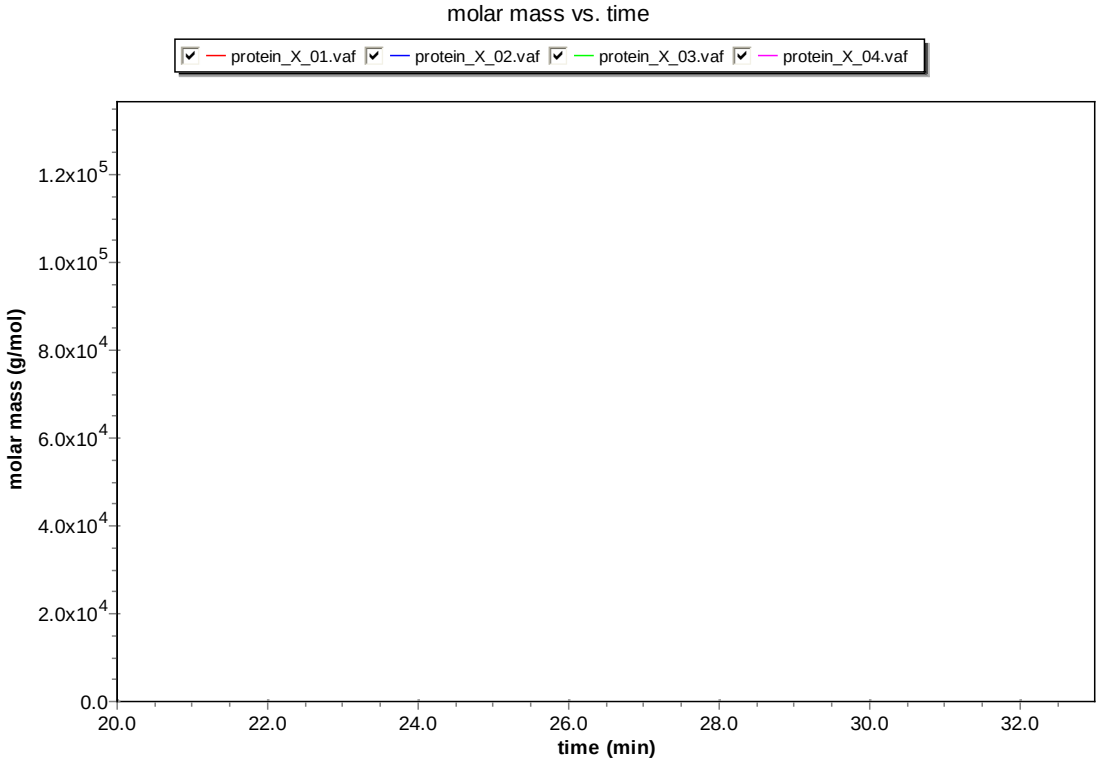
expressed in HEK293S cells; yields ~ 25 ug from a single preparation

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



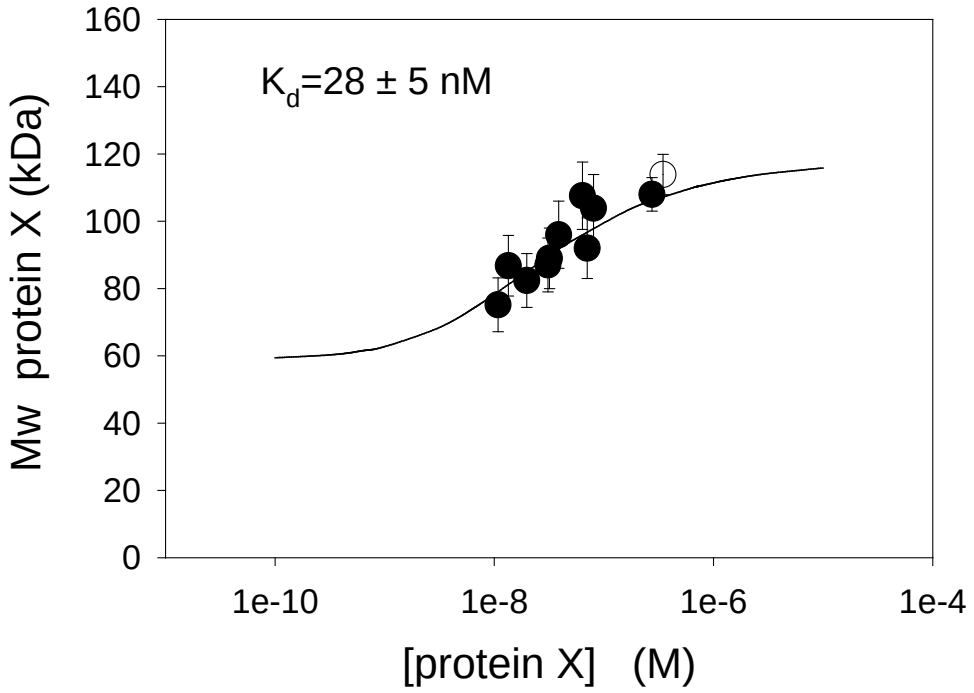
Determination of dimerization constant from SEC-LS measurements

Extracellular ligand binding domain (LBD) of the metabotropic glutamate

WT monomer = 59kDa dimeric in solution

mutant monomer = 59kDa destabilized dimer?

assess concentration dependent distribution of monomer-dimer



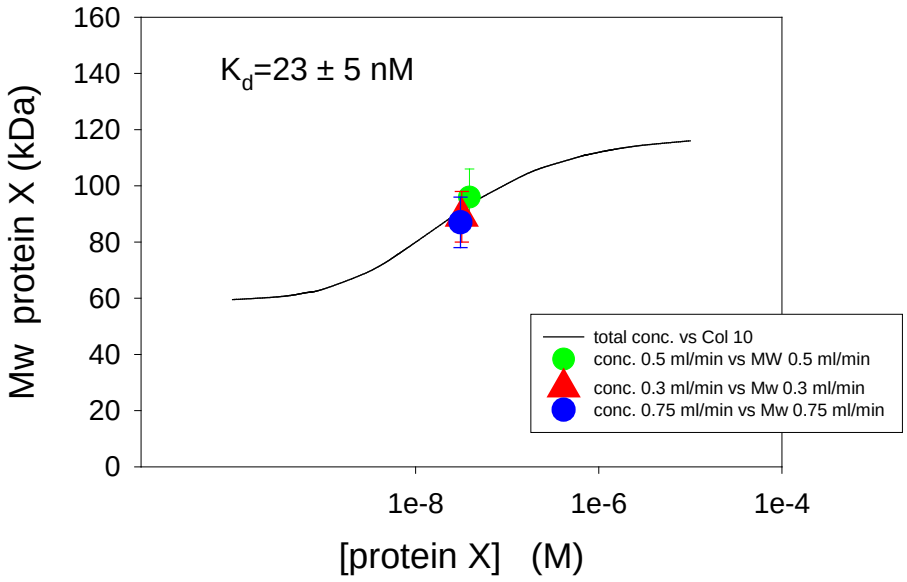
Determination of dimerization constant from SEC-LS measurements

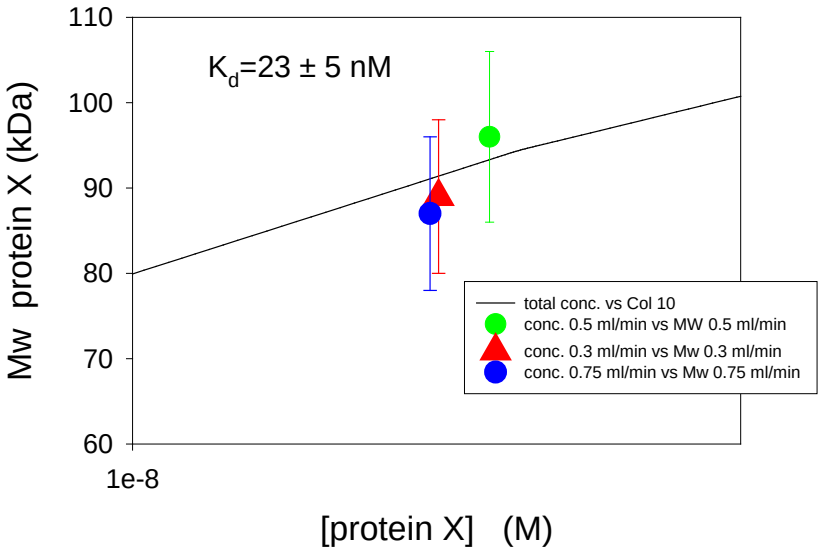
Extracellular ligand binding domain (LBD) of the metabotropic glutamate

WT monomer = 59kDa dimeric in solution

mutant monomer = 59kDa destabilized dimer?

assess concentration dependent distribution of monomer-dimer





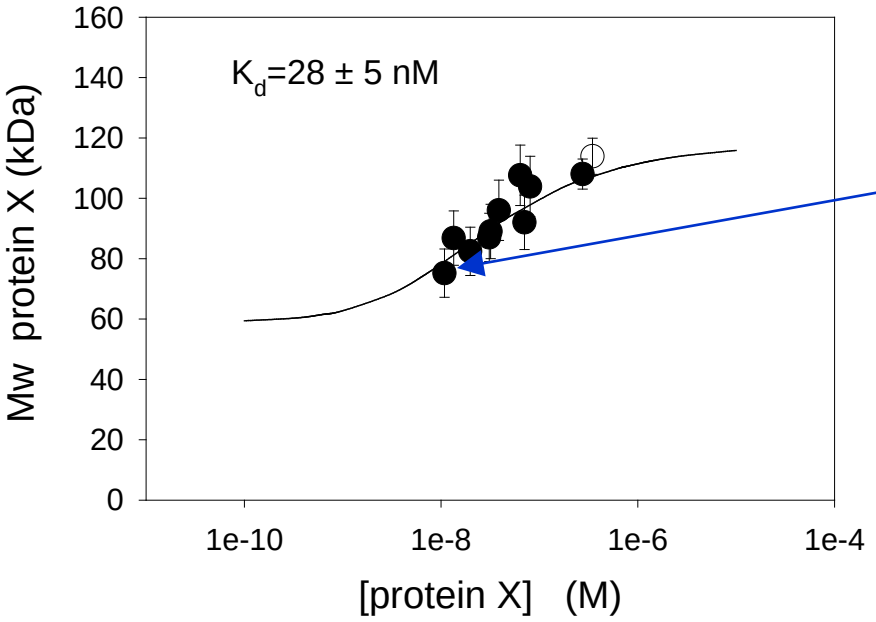
Determination of dimerization constant from SEC-LS measurements

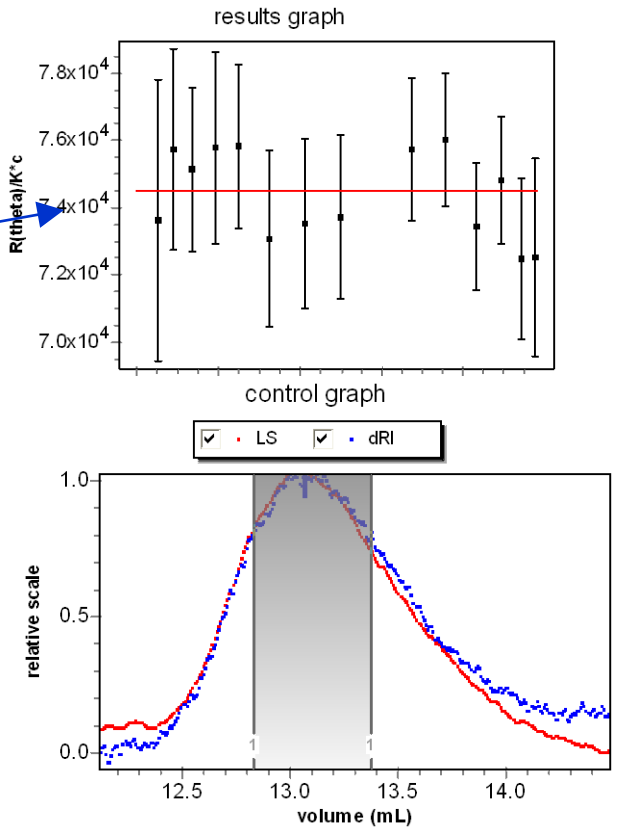
Extracellular ligand binding domain (LBD) of the metabotropic glutamate

WT monomer = 59kDa dimeric in solution

mutant monomer = 59kDa destabilized dimer?

assess concentration dependent distribution of monomer-dimer





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

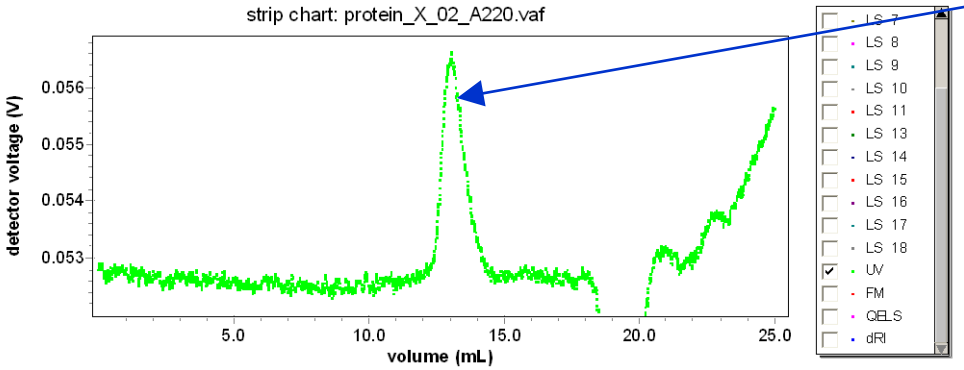
Determination of dimerization constant from SEC-LS measurements

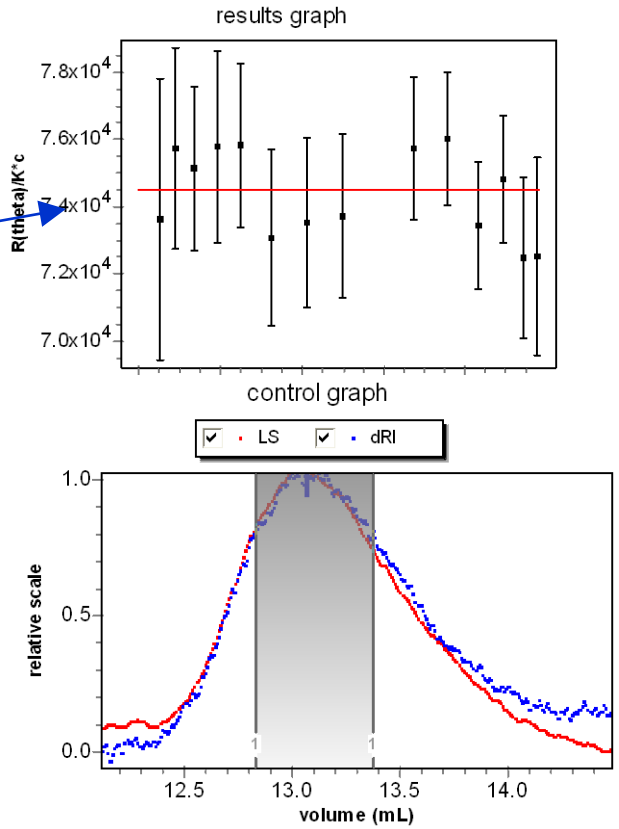
Extracellular ligand binding domain (LBD) of the metabotropic glutamate

WT monomer = 59kDa dimeric in solution

mutant monomer = 59kDa destabilized dimer?

assess concentration dependent distribution of monomer-dimer

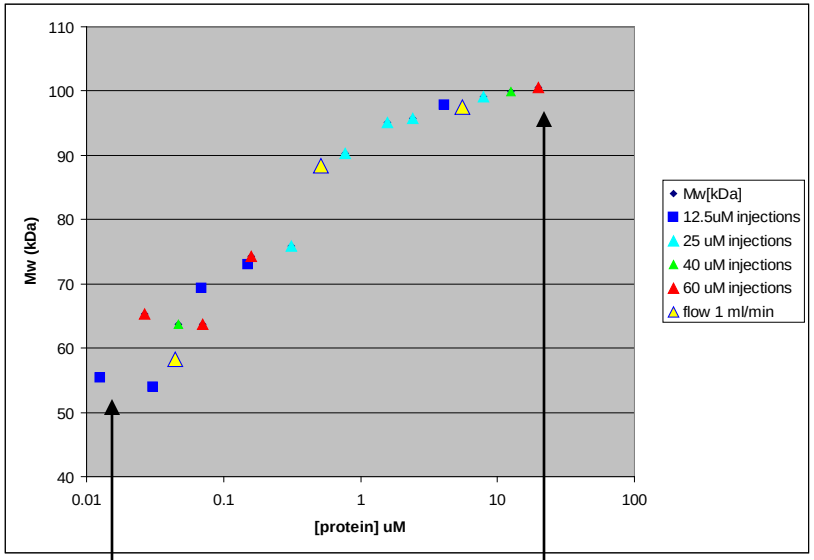


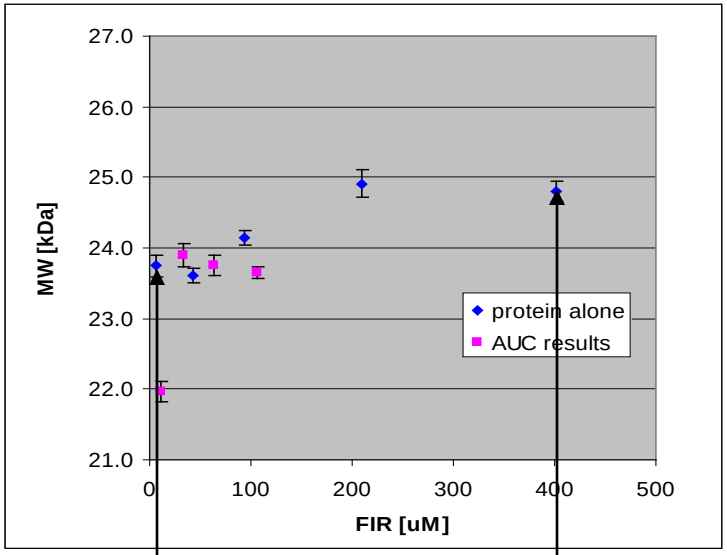


concentration= (6.426 ± 0.094) e-7 g/mL

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

Services provided by the Biophysics Resource contributed to > **60 publications**
(>30 from Yale)

Light Scattering Services contributed to > 45 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

Thank you all very much for your generosity

you have collectively contributed

$85.04 and 20 Euro pennies

for