Yale/NIDA Neuroproteomics Center

Biophysics Core

Ewa Folta-Stogniew, Ph.D., M.S.

Biophysics Core

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

Expands the proteomic analyses beyond the identification of proteins' networks

Allows quantitative characterization of interactions between candidates identified through mass spectrometry approaches

Biophysics Core

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)
- CD-Spectrophotometer
- Stopped-Flow Spectrofluorometer
- Surface Plasmon Resonance (SPR) Sensor [BiaCore Biosensor; T100]
- Composition Gradient Static Light Scattering (CGSLS)
- Asymmetric flow Field-Flow Fractionation (AFFF)

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

Common questions:

- how tight is the binding ? (light scattering: static LS and dynamic LS)
- how many of each molecule are in the complex ? (ITC, SPR, LS, stopped-flow)
- how fast does the complex form? (SPR and stopped-flow)
- is the binding event enthalpy or entropy-driven? (ITC)

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Application of label free technologies to find small molecule capable of blocking signaling via PrP-C (cellular prion protein) and mGluR5 (metabatropic glutamate receptor 5) that leads to disruption of neuronal function

NIDA Investigator: Steven Strittmatter

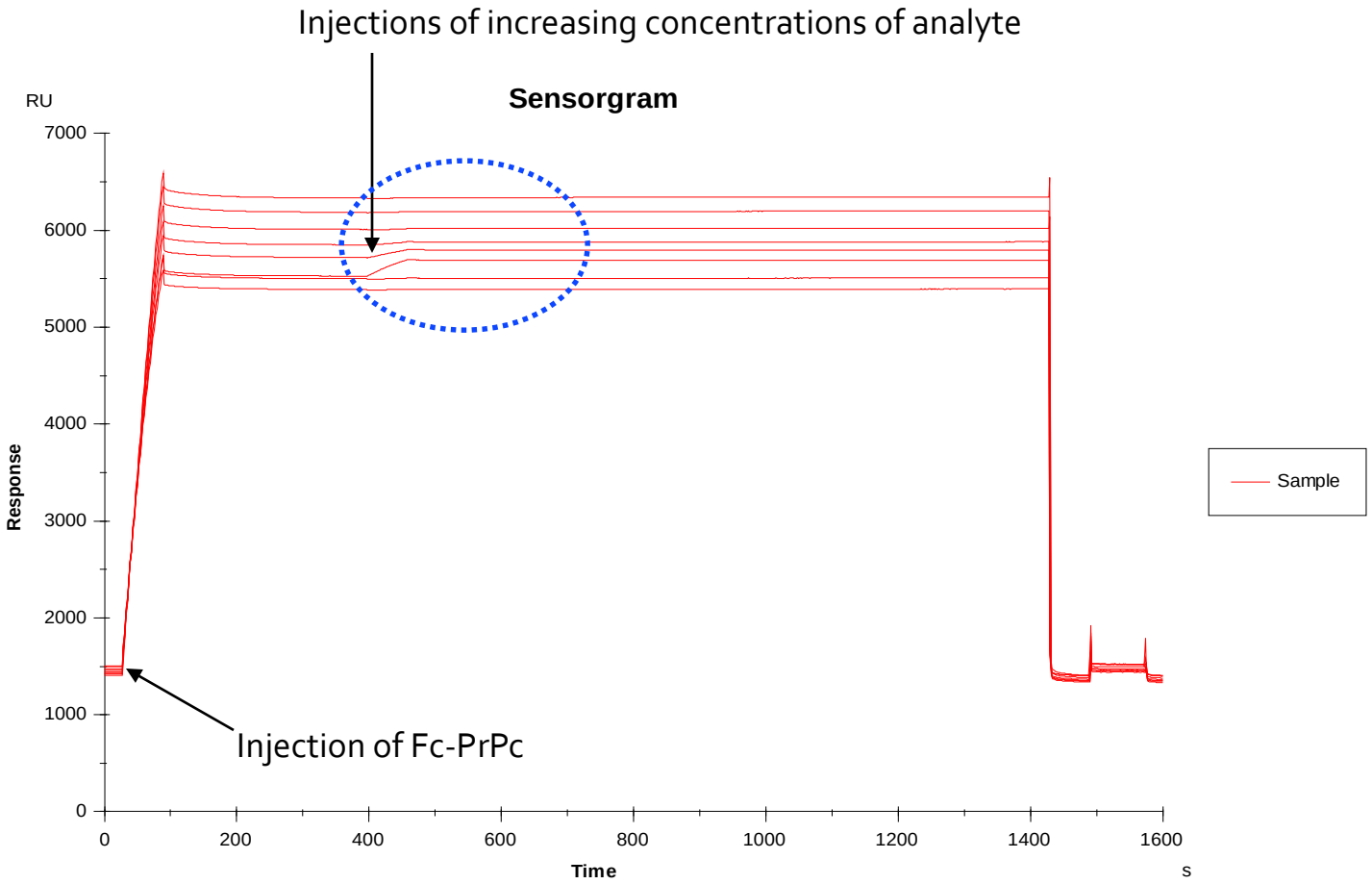
Technologies employed: **SPR**

SPR experiment is a surface based binding assay

requires immobilization of one of the interacting partners

Capture of Fc-PrPc construct on protein A surface

no purification, capture directly from cell lysate, easy regeneration

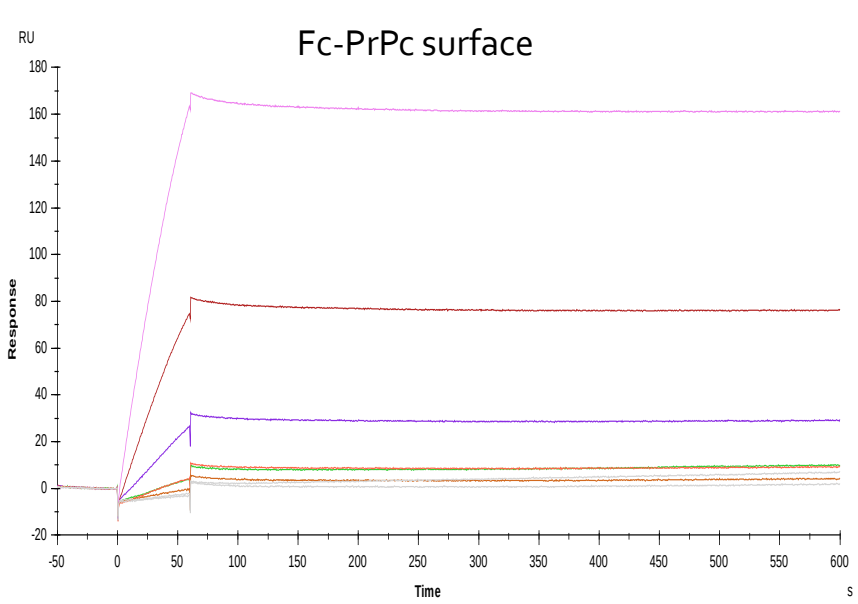


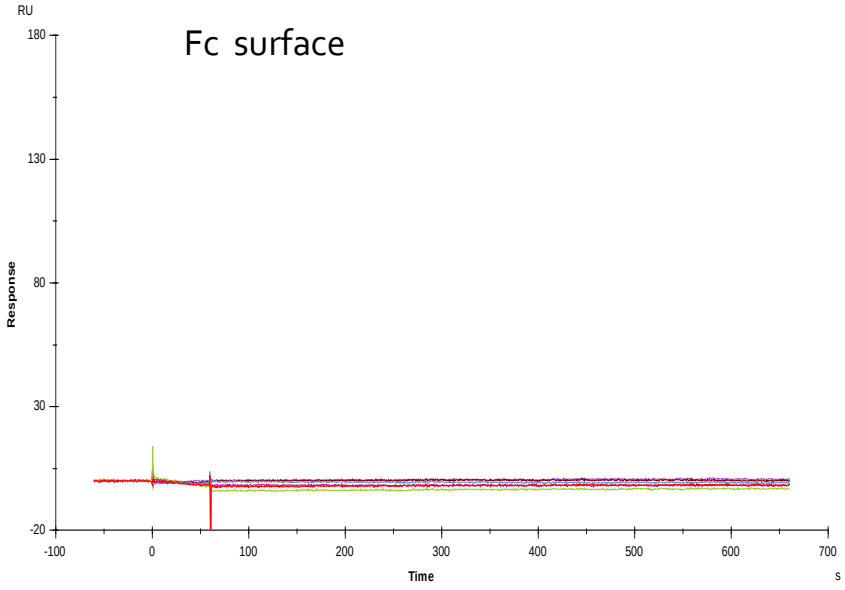
Capture of Fc-PrPc construct on protein A surface

no purification, capture directly from cell lysate, easy regeneration

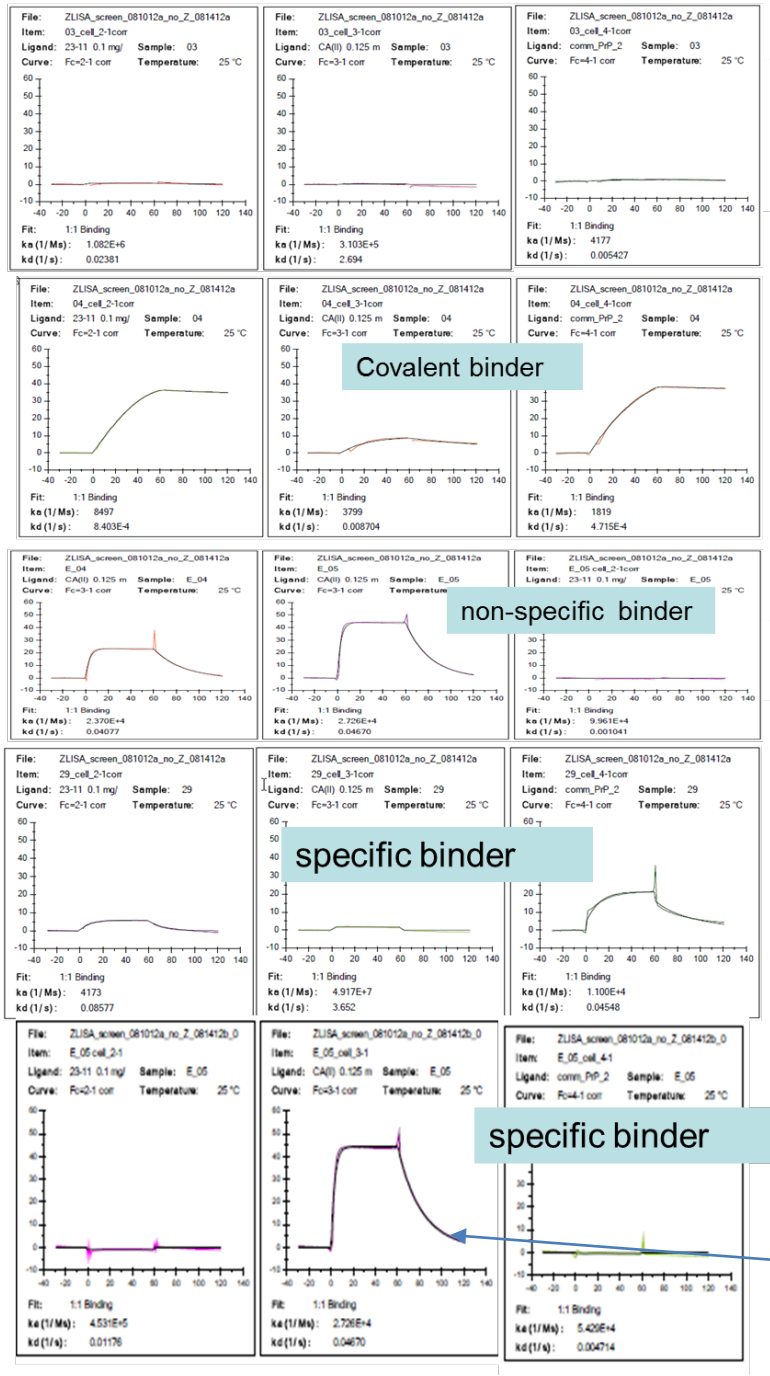
We observed a dose-dependent and specific response (no binding to reference cell or protein A surface, no binding to human Fc alone) indicative of specific complex formation between PrPc and Aβo

Aβo injections

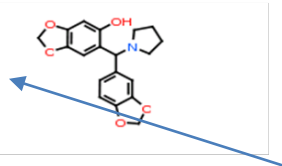




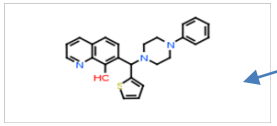
PrpP23-111 Carbonic anhydrase Full length PrP



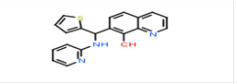
D727-0685



G856-2519



4896-2436



D402-0204

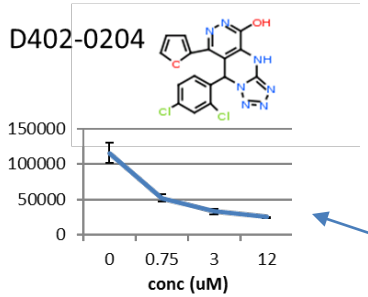
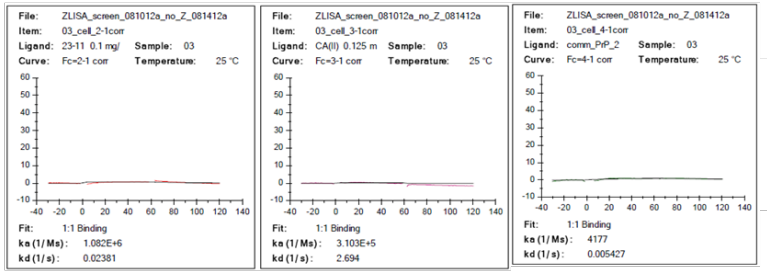


Table 2. Summary of amplitudes observed during initial 10 uM screen.

SPR cycle #	compound #	Binding level values 2-1corr with 3*STDVE subtracted	3-1 corr	4-1 corr
0	blank	-4.9	-1.6	-2.8
1	DHPA	7.0	-0.8	5.2
2	D727-0684	-3.6	-0.8	0.6
3	D727-0685	-3.4	-0.6	-0.1
4	G856-2519	30.7	8.2	34.8
5	J033-0157	36.9	4.5	2.6
6	J033-0178	3.0	-0.7	0.9
7	C200-5997	5.8	0.6	4.8
8	C301-7797	0.7	0.0	0.6
9	C429-0410	5.4	4.5	5.1
10	D420-4969	2.1	1.5	10.3
11	4896-2436	12.0	8.5	17.8
12	6405-0005	-0.3	-0.9	1.0
	blank	-3.8	-1.8	-2.5
13	C239-0780	-3.2	0.0	2.1
14	D727-0683	-3.6	-0.9	-1.4
16	E218-0164	0.6	0.8	5.3
17	E218-0320	-1.6	1.6	0.5
18	E218-0324	8.0	7.9	3.5
19	E218-0327	9.3	6.6	6.3
	solvent correction			
20	E218-0665	0.3	0.3	1.9
21	E234-0004	-0.8	2.0	2.3
22	E234-0056	11.0	9.5	7.6
23	F296-0458	-3.0	-0.5	1.5
24	F685-0437	-1.1	-0.5	4.1
25	F685-0578	3.3	-0.5	6.3
	blank	-6.5	-1.9	-2.3
26	F685-1196	-2.0	-0.6	2.1
27	F685-1228	-5.0	-1.7	-0.9
28	F685-1588	-3.0	-1.2	0.5
29	D402-0204	1.6	0.8	20.4
30	8562-00038	-4.7	-1.0	-1.6
31	C561-2995	-1.7	0.9	1.6
32	C761-0116	-3.0	-0.8	0.1
33	D402-0188	-1.5	-0.3	2.0
34	G628-0193	-4.9	-1.0	-1.1
E_1	ACT	-5.1	28.2	-1.7
E_2	AMBS	-4.9	18.2	-1.6
E_3	SULF	-4.8	19.8	-1.5
E_4	4SA	-4.5	22.1	-1.6
	solvent correction			
E_5	FUR	-5.1	42.9	-1.6
Z	z	457.9	6.6	428.5

PrpP23-111 Carbonic anhydrase Full length PrP



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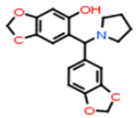
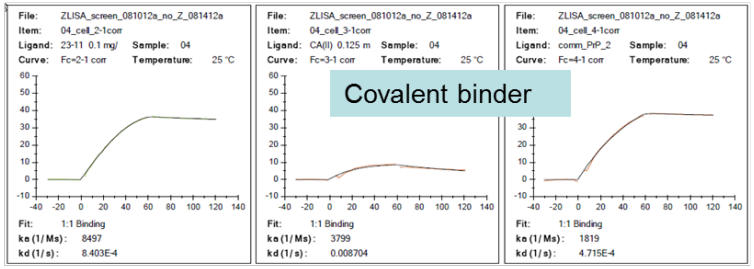
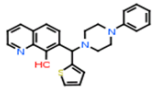
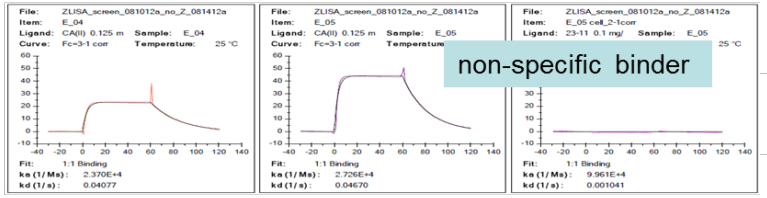




Covalent binder

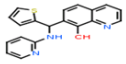
G856-2519

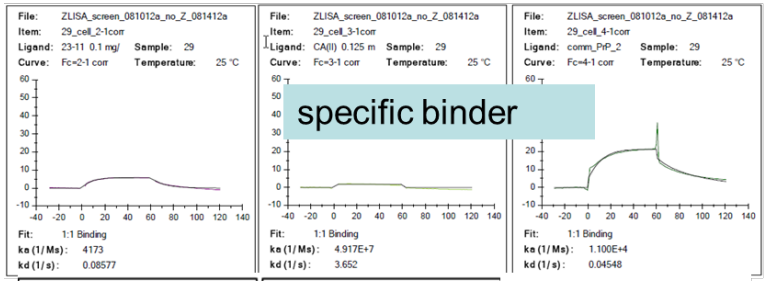




non-specific binder

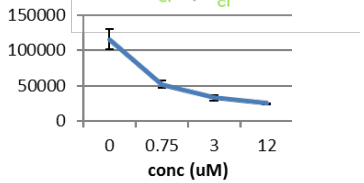
4896-2436

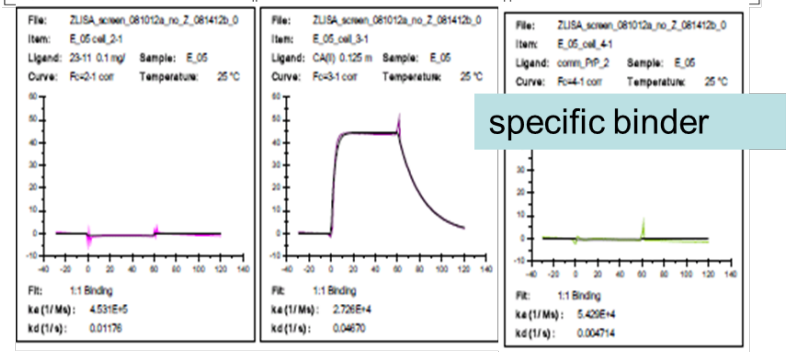




specific binder

D402-0204





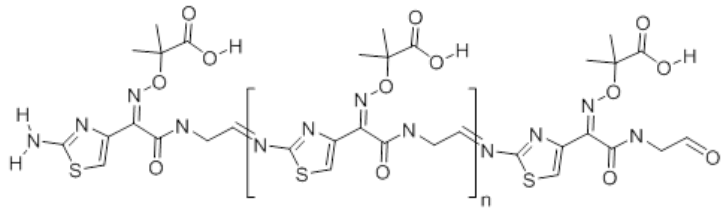
specific binder

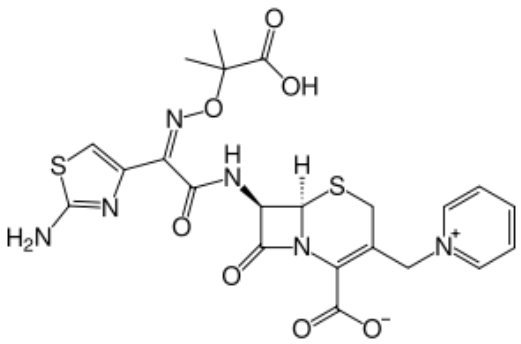
For T100 Biacore biosensor all data analysis for secondary screens was done manually

NIDA funds were used to upgrade the instrument to T200 capabilities in April 2019, which adds automation to data processing for results of secondary screens

Characterization of interaction between PrP protein and drug candidates

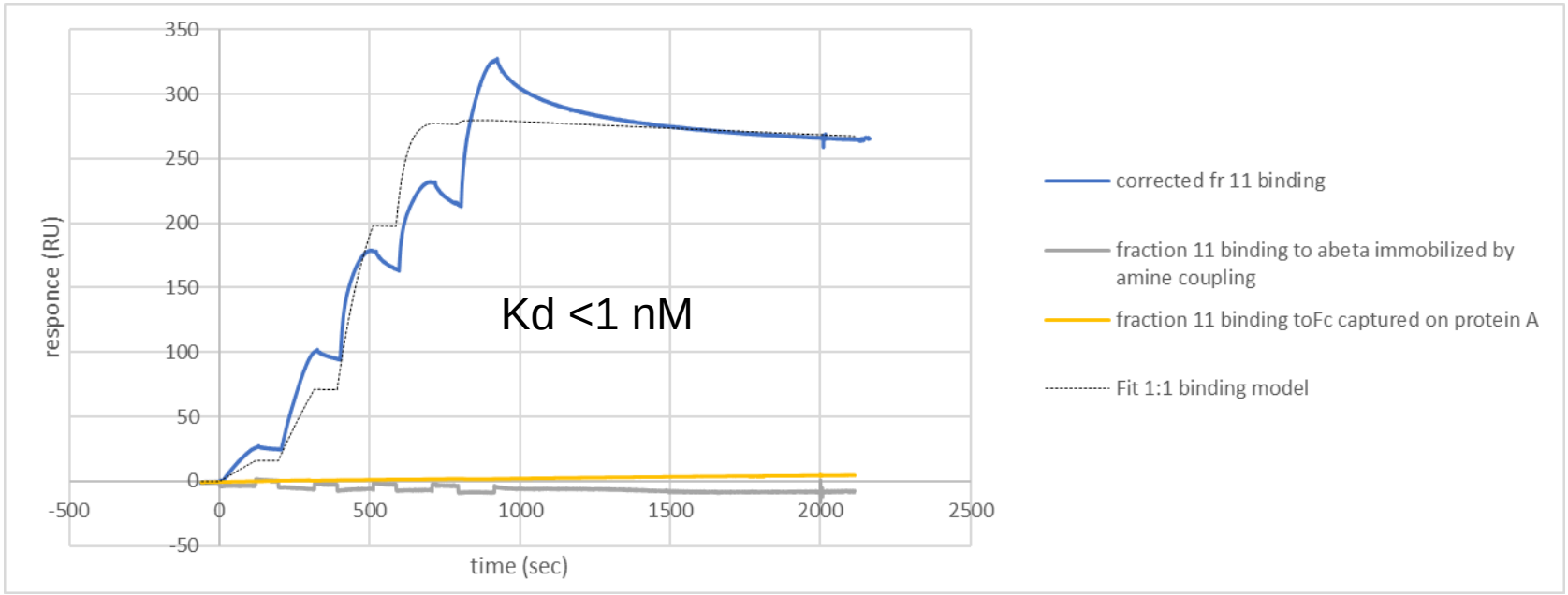
Z is a polymer that forms from a degradation product of ceftazidime.





Ceftazidime MW = 546

		amine-coupled		capture
	cell 1	blank		
	cell 2	Protein A	4667.5	Fc-PrP
	cell3	Protein A	4686.5	human Fc
	cell4	abeta		



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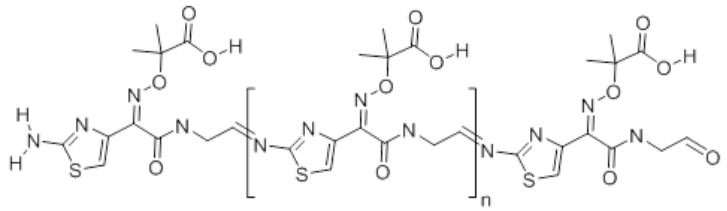

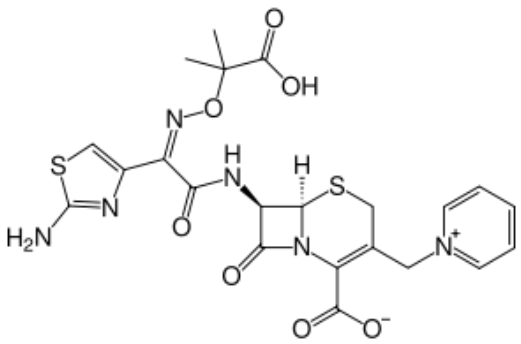

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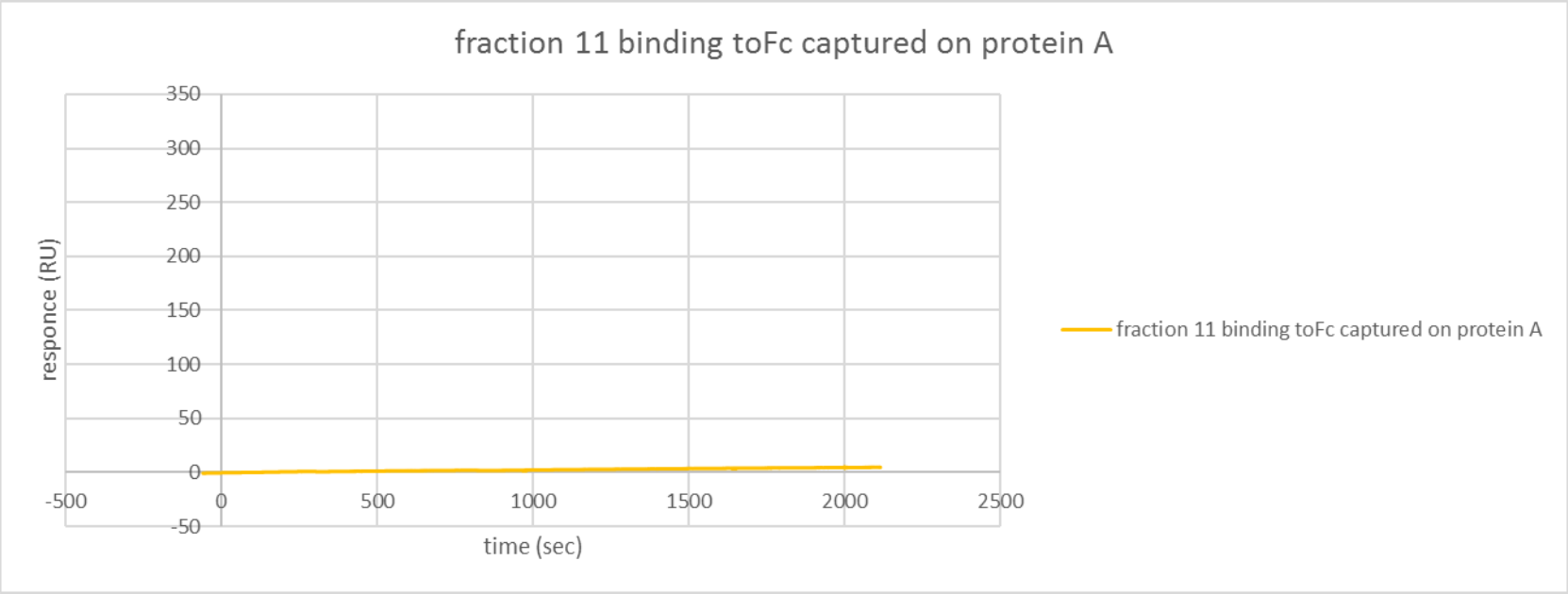

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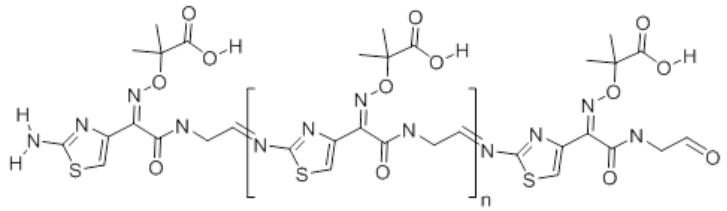

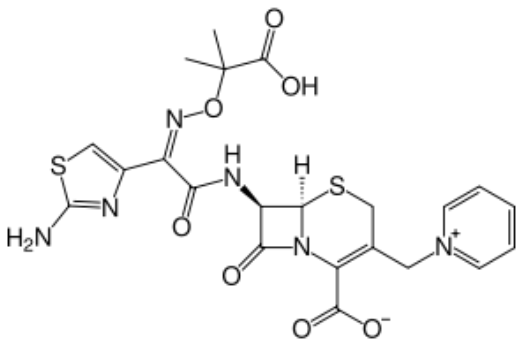

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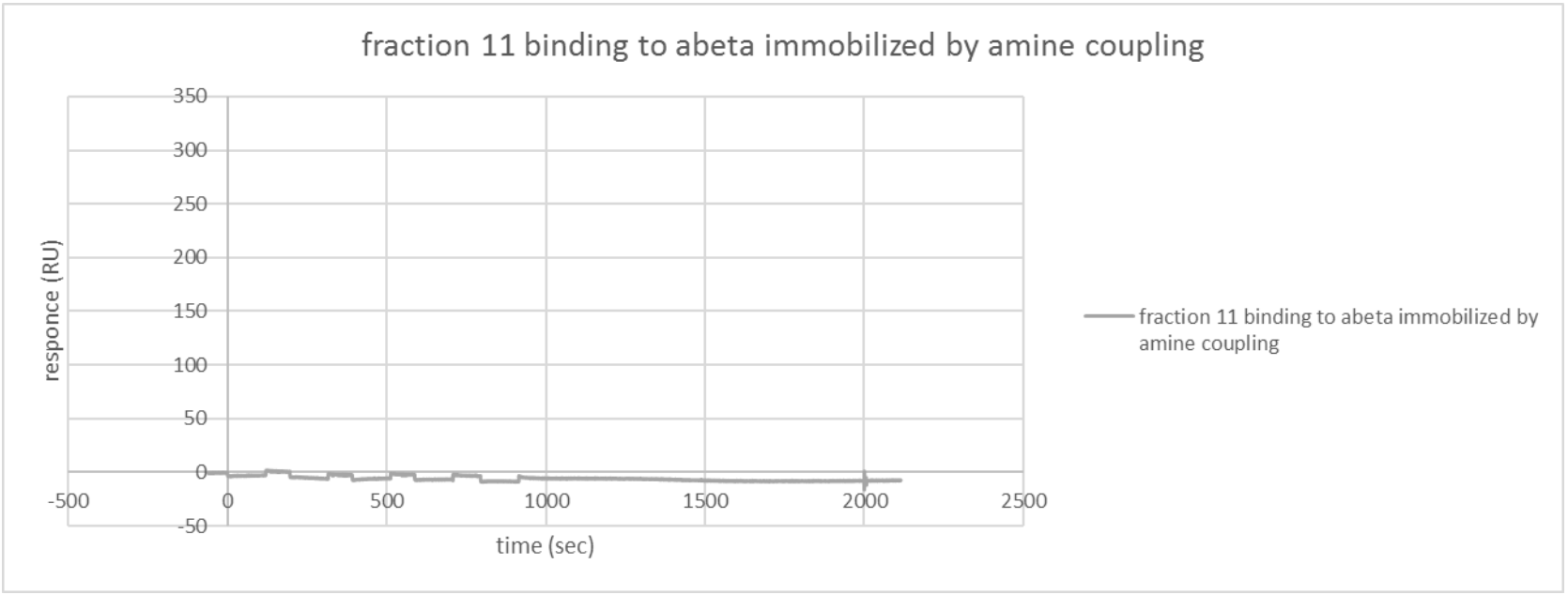

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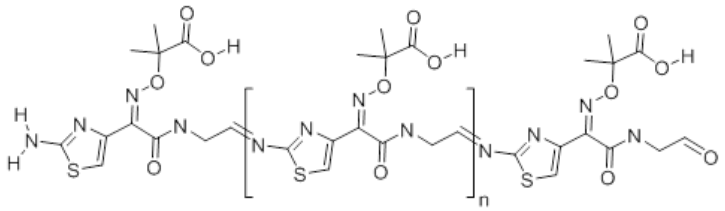
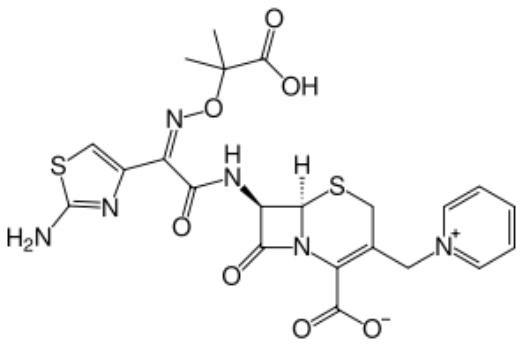
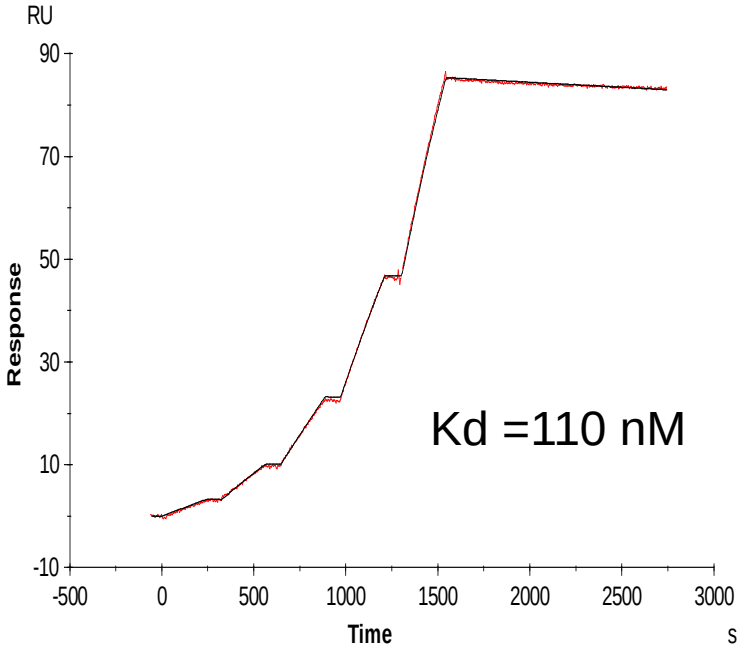


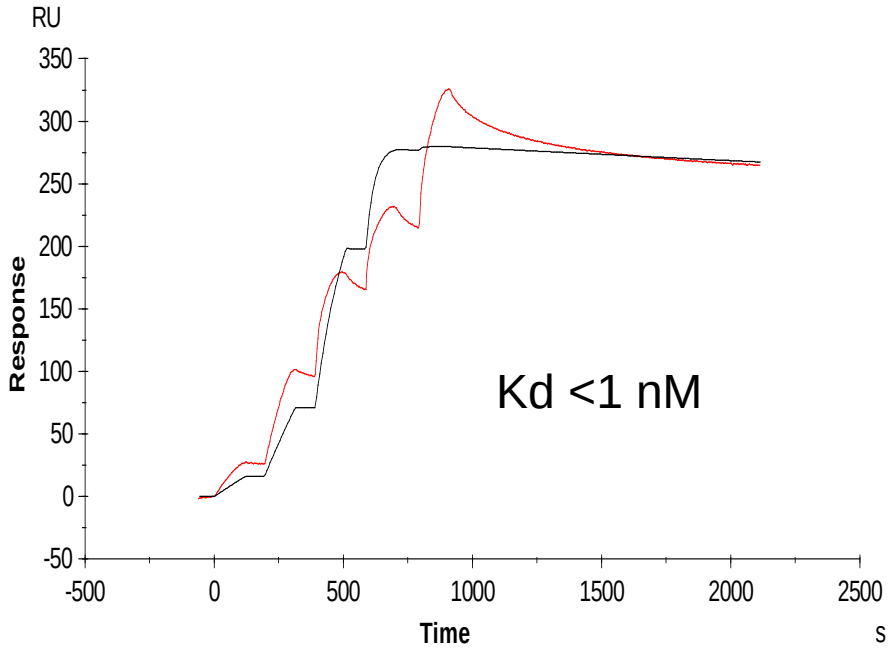


Ceftazidime MW = 546

Aβo binding to immobilized PrPc
5.5 uM (3 fold)



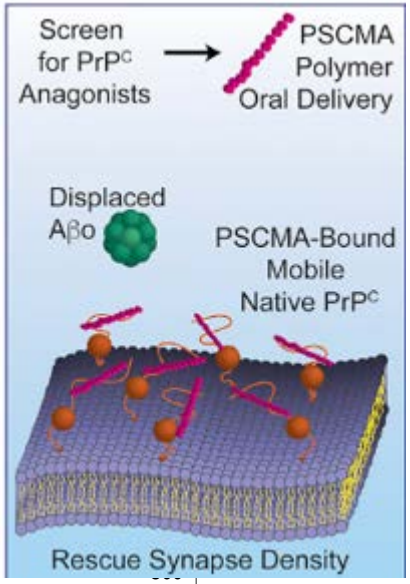
150 nM(3 fold)



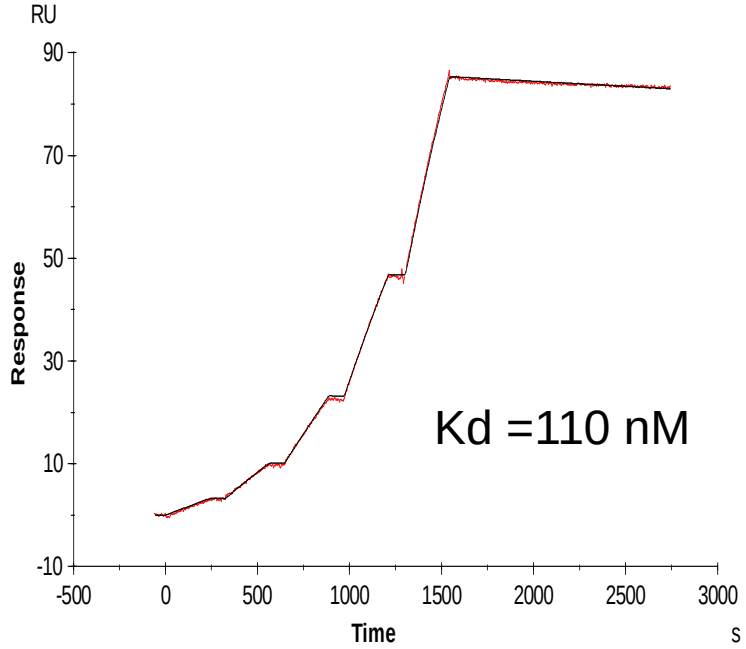
Gunther, E. C., Smith, L. M., Kostylev, M. A., Cox, T. O., Kaufman, A. C., Lee, S., Folta-Stogniew, E., Maynard, G. D., Um, J. W., Stagi, M., Heiss, J. K., Stoner, A., Noble, G. P., Takahashi, H., Haas, L. T., Schneekloth, J. S., Merkel, J., Teran, C., Naderi, Z. K., Supattapone, S., and Strittmatter, S. M. (2019) Rescue of Transgenic Alzheimer's Pathophysiology by Polymeric Cellular Prion Protein Antagonists. Cell reports 26, 1368

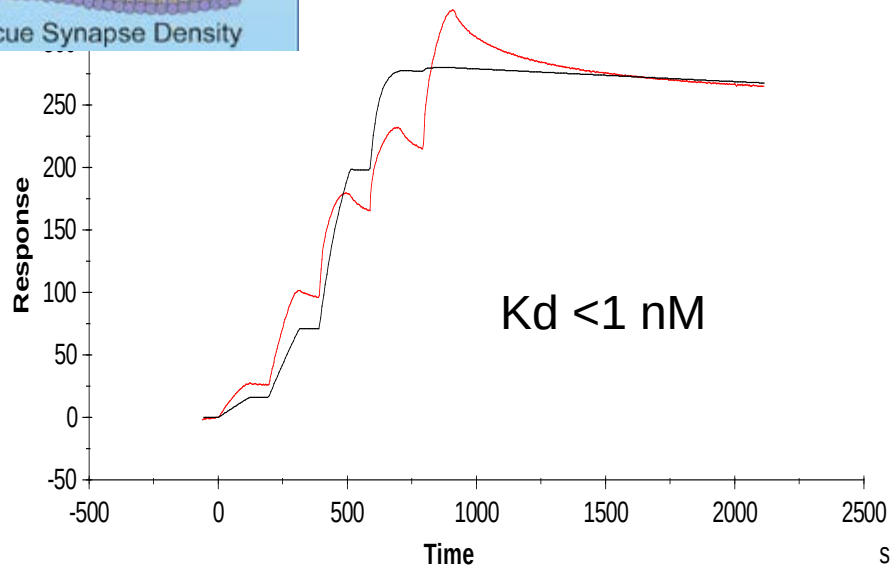
SPR assisted in screening for PrPc antagonists and selected Z as the best binder of PrPc

Z is a capable of displacing Aβo from PrPC and rescuing synapse densities



Aβo binding to immobilized PrPc





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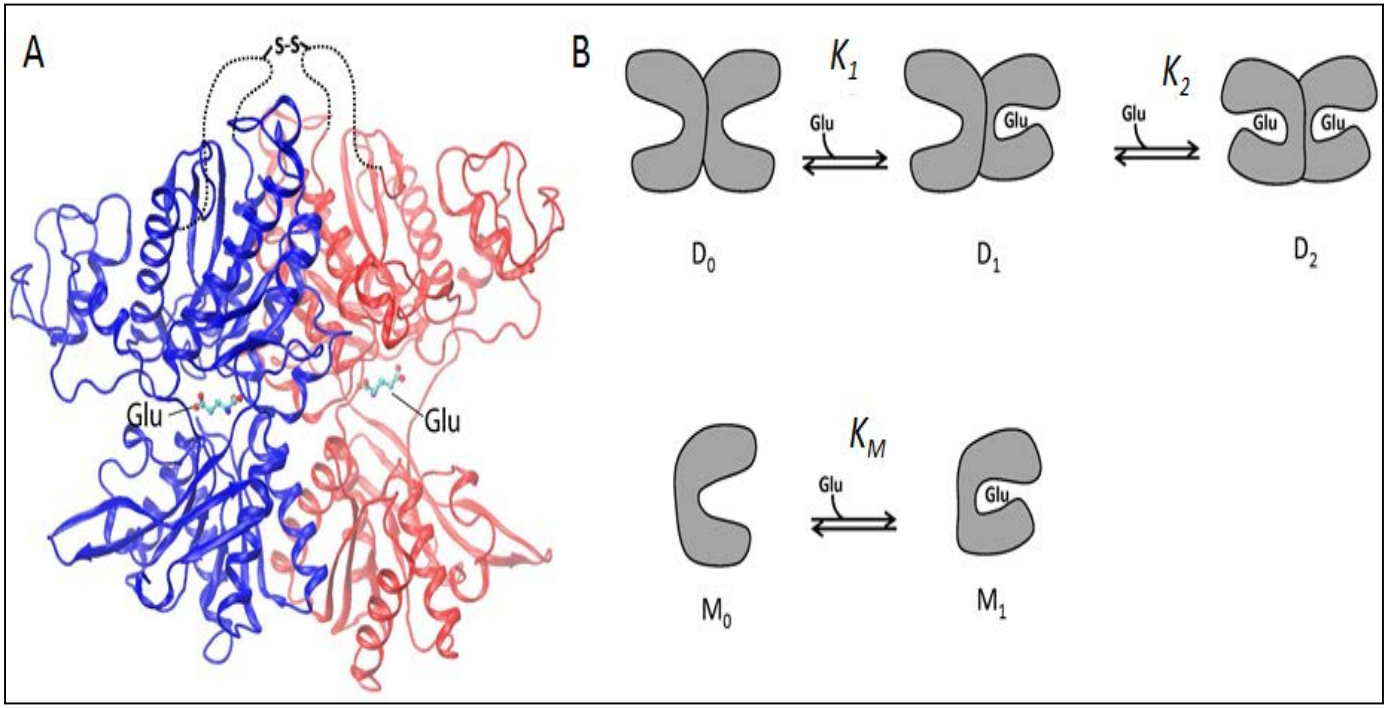
Application of light scattering to measure dimerization constant of ligand-binding domain (LBD) of Glutamate Receptor 1

NIDA Investigator: Elsa Yan (pilot project recipient)

Technologies employed: **SEC/MALS**

Activation of mGluR1 LBD in the dimeric and monomeric form upon binding of Glutamate



Serebryany E, Folta-Stogniew E, Liu J, Yan EC. (2016) Homodimerization enhances both sensitivity and dynamic range of the ligand-binding domain of type 1 metabotropic glutamate receptor. FEBS Lett. 590: 4308-4317

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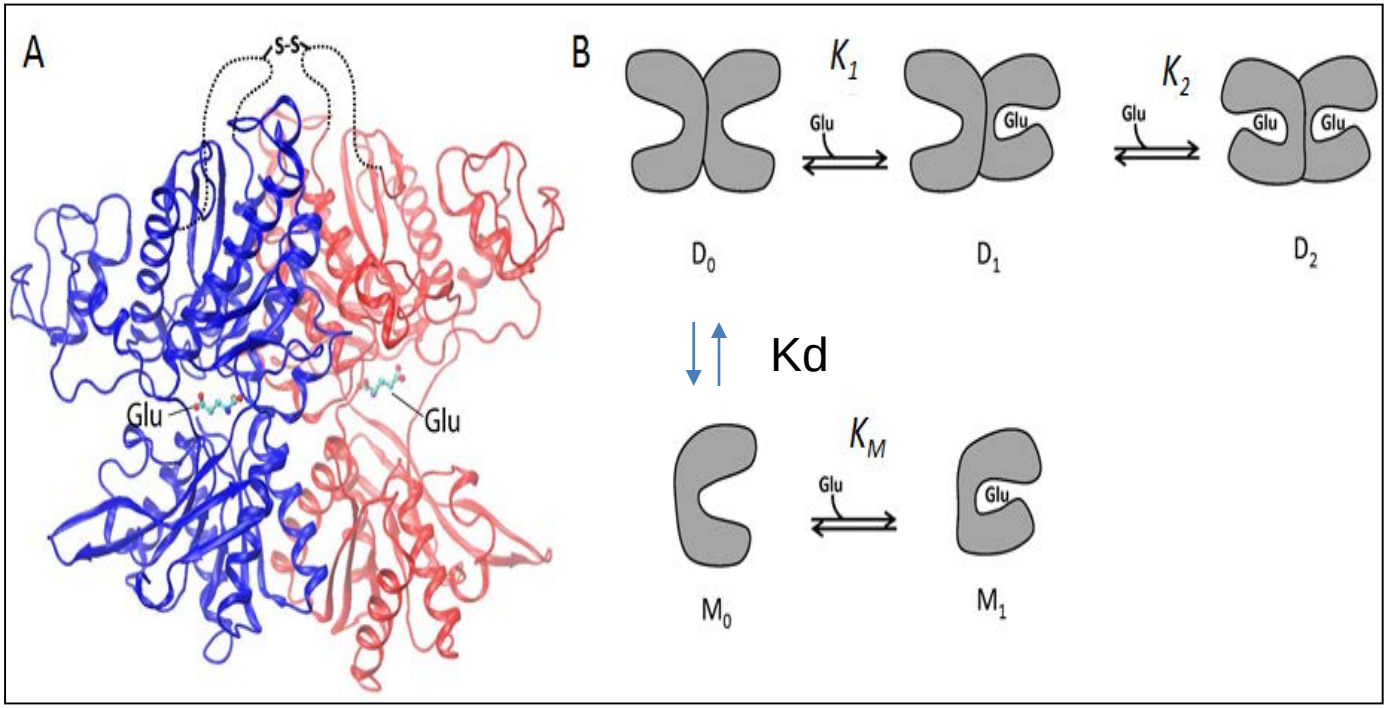


Determination of dimerization constant from SEC-MALS measurements

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

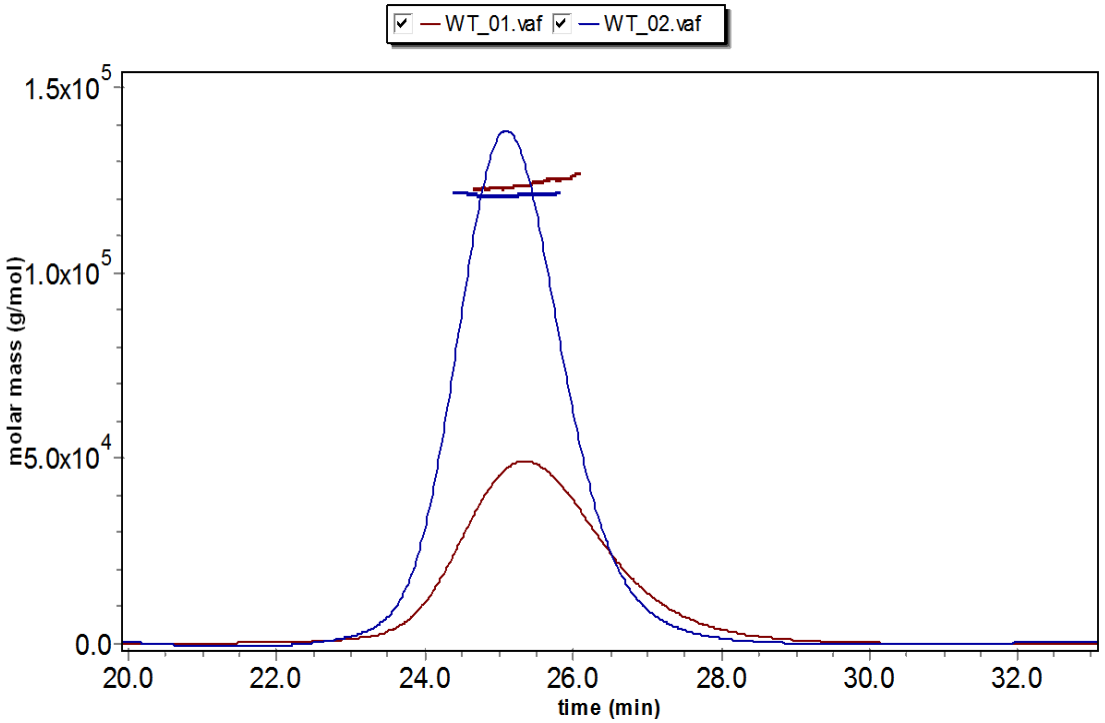
mGluR LBD is a homodimer with a glutamate binding pocket in each subunit

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 = 60 kDa *dimeric in solution*

Mutant C140S monomer = 60 kDa *destabilized dimer?*



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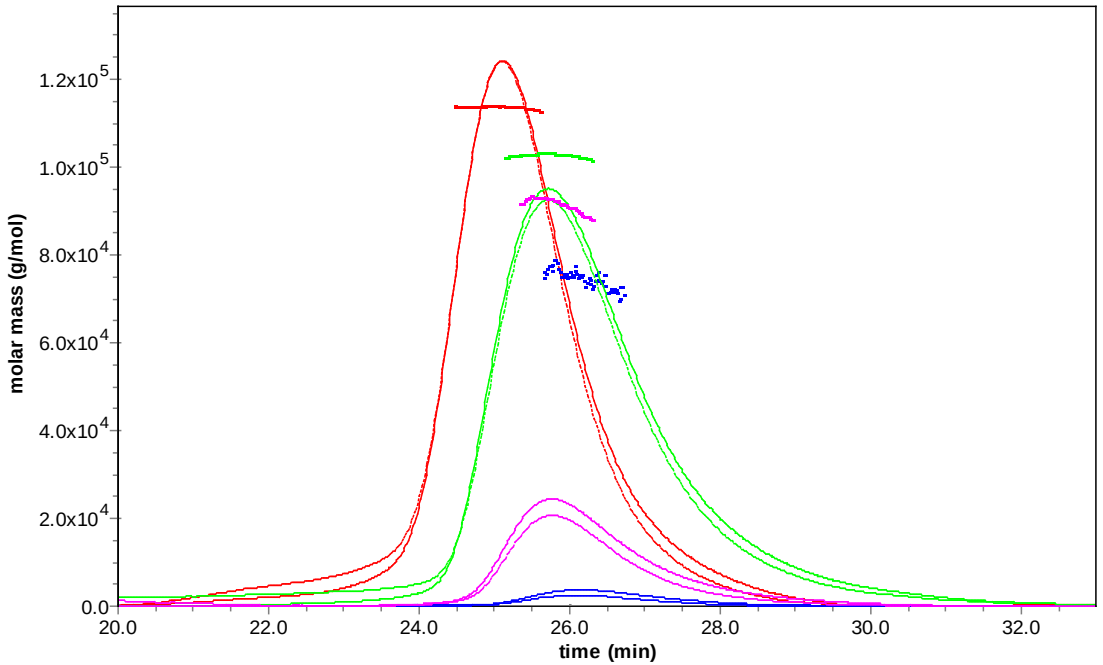

Serebryany E, Folta-Stogniew E, Liu J, Yan EC. (2016) Homodimerization enhances both sensitivity and dynamic range of the ligand-binding domain of type 1 metabotropic glutamate receptor. FEBS Lett. 590: 4308-4317

Determination of dimerization constant from SEC-MALS measurements: Mw vs. concentration

Protein conc. (uM)	weight-average Mw (kDa)
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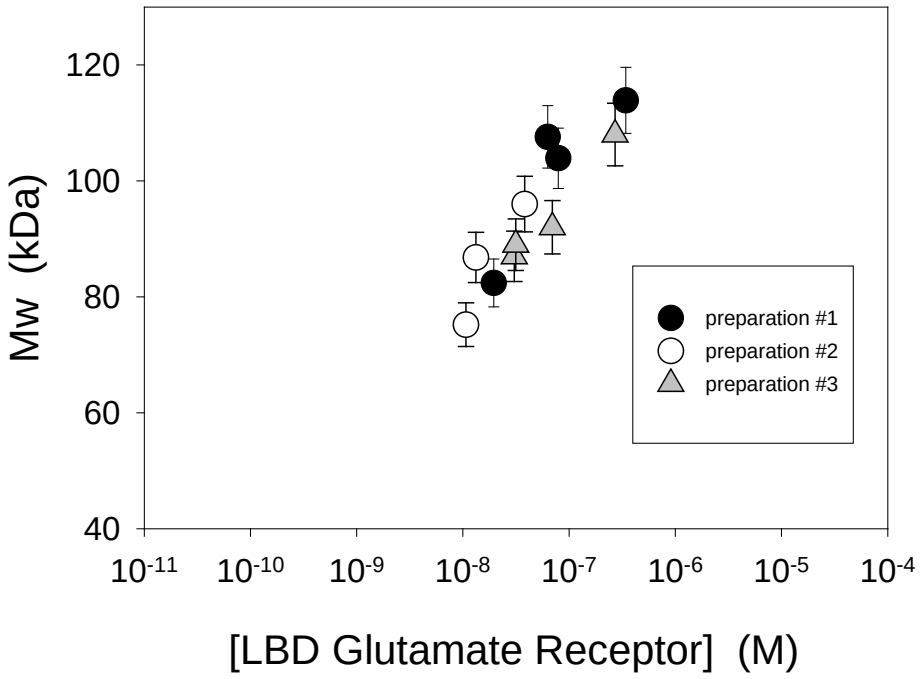

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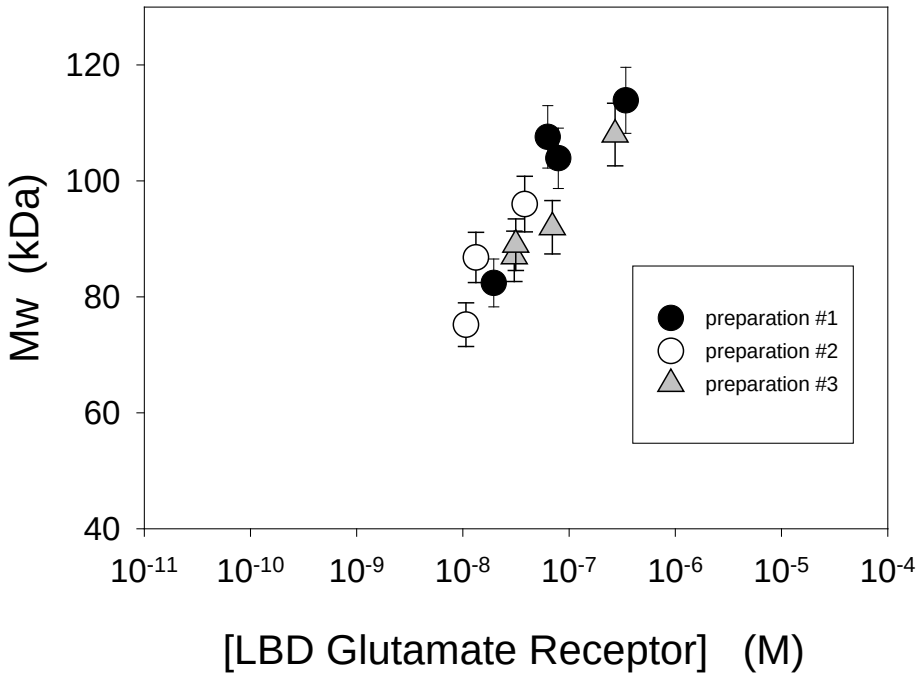

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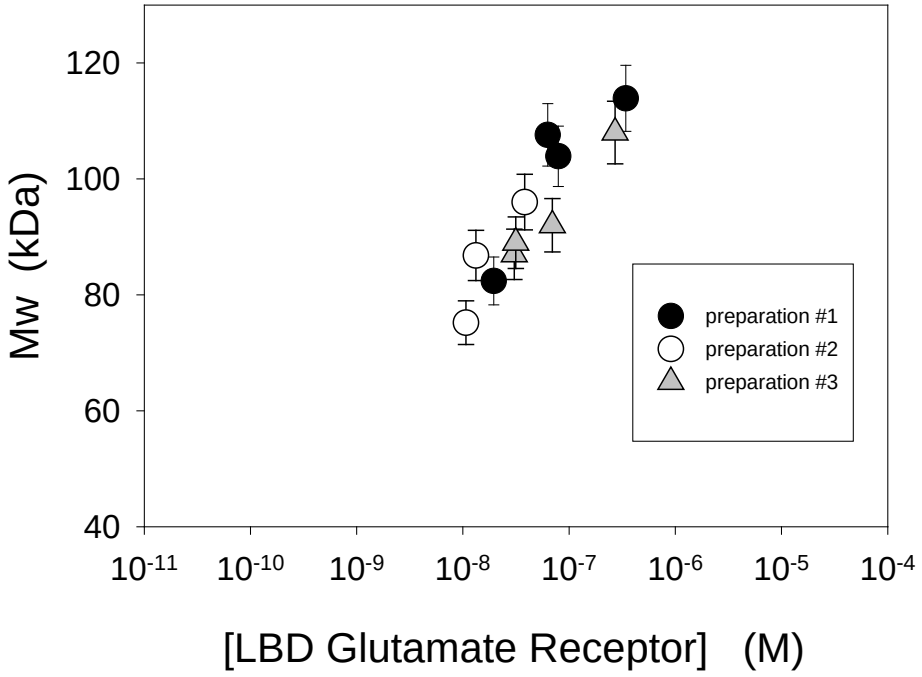

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$$2M = D$$

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

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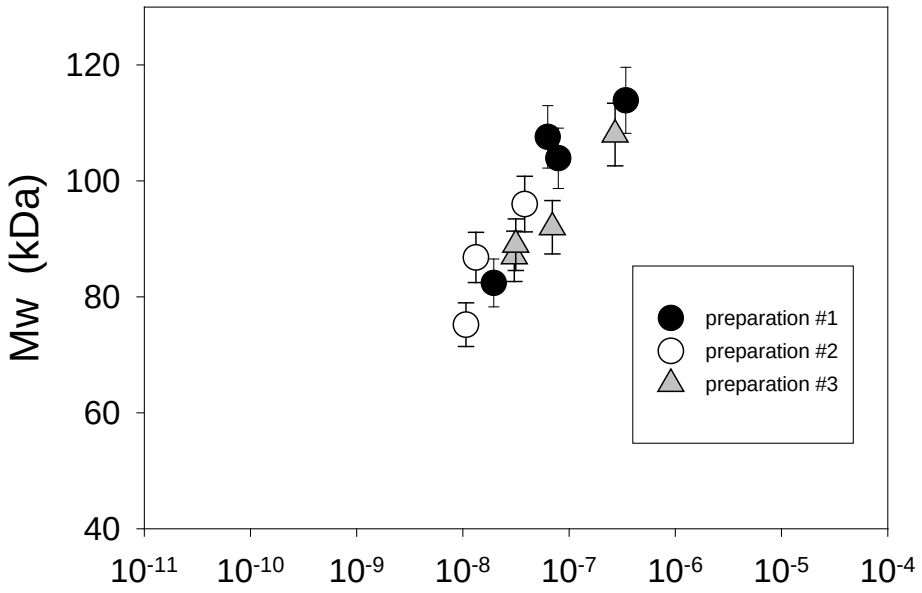

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Measure Mw

Measure total concentration

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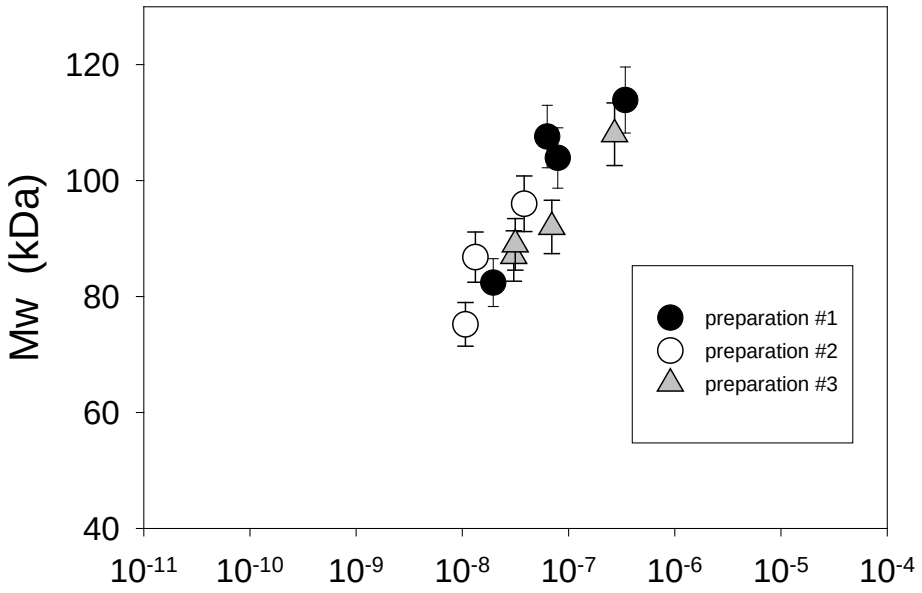

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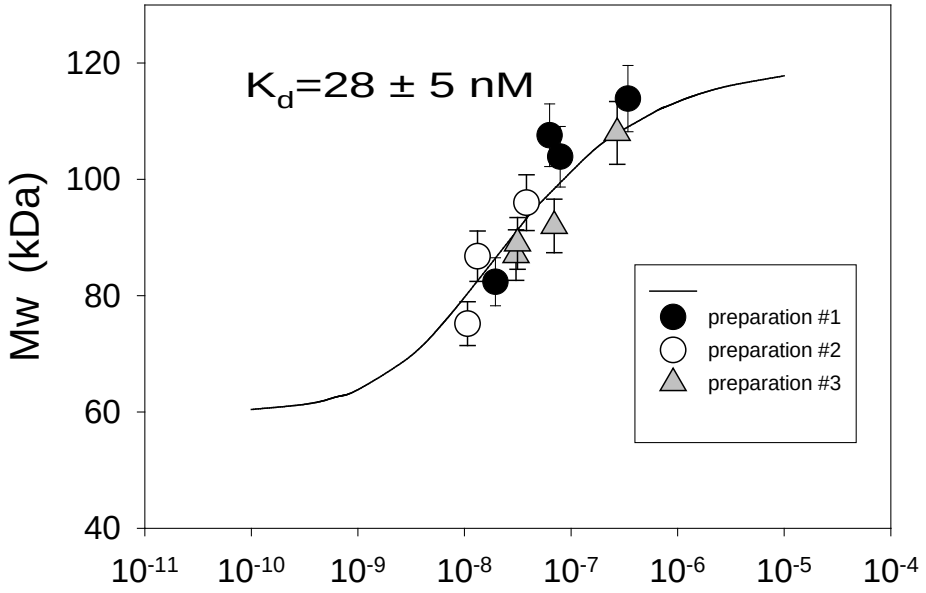

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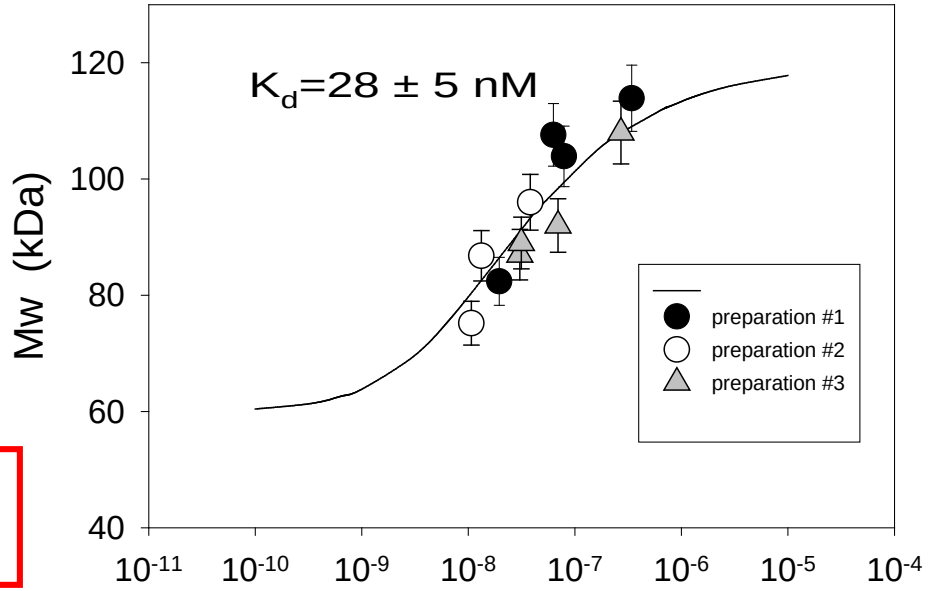

Assumes that measured Mw represents equilibrium

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[LBD Glutamate Receptor (M)

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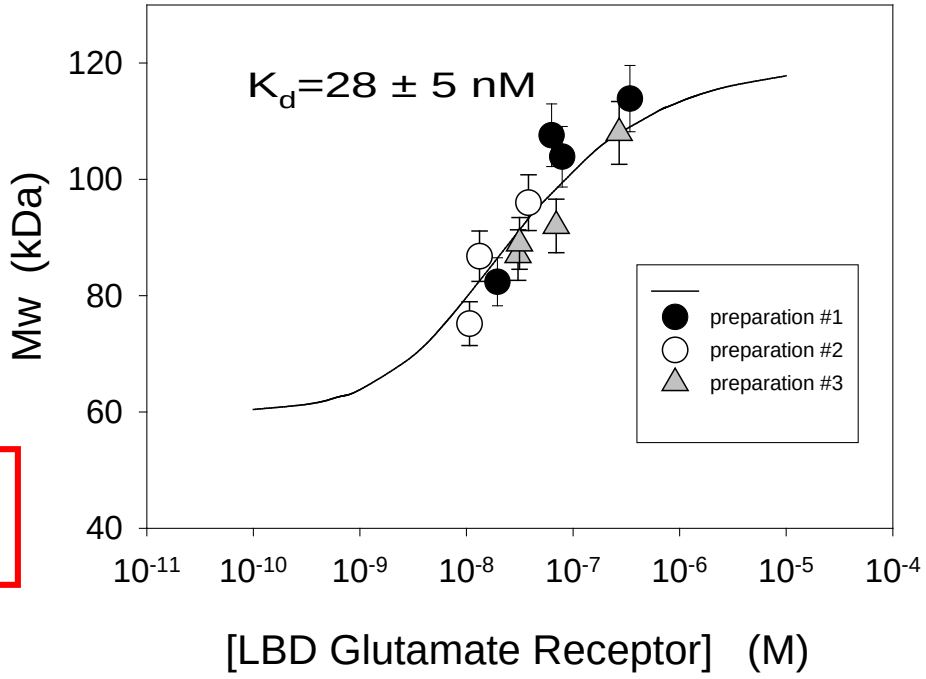

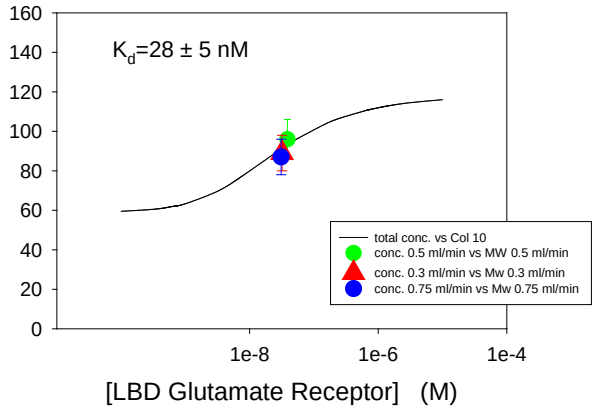

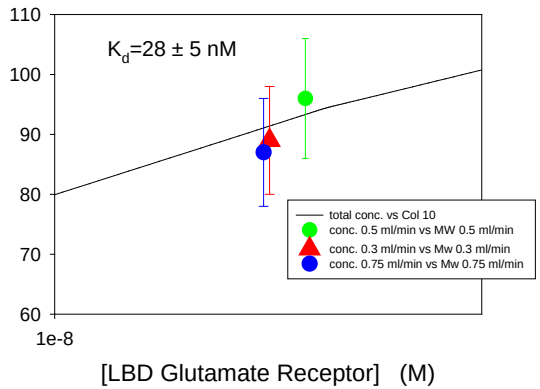

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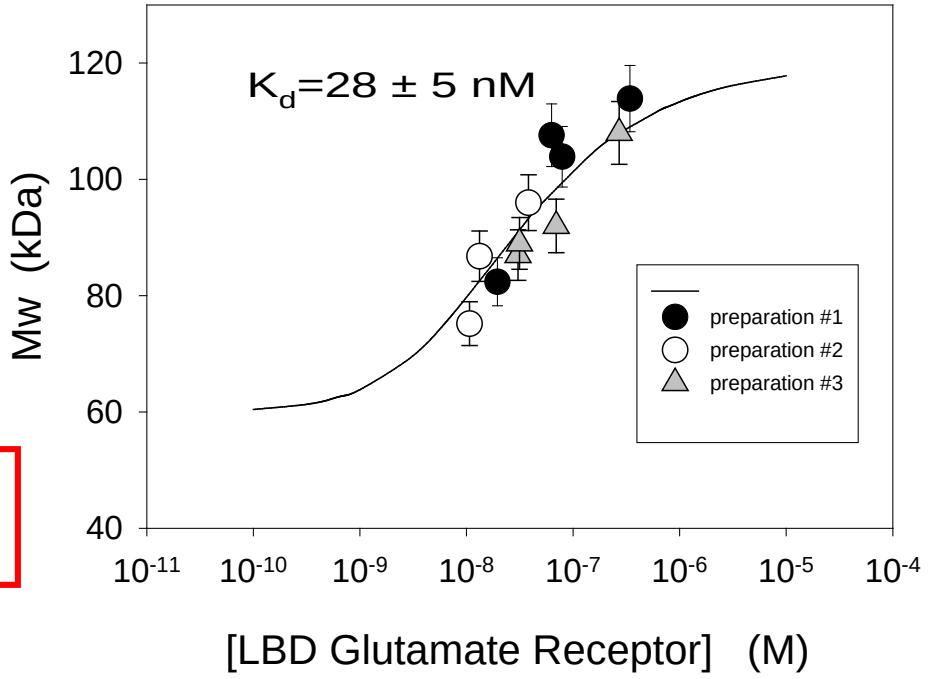

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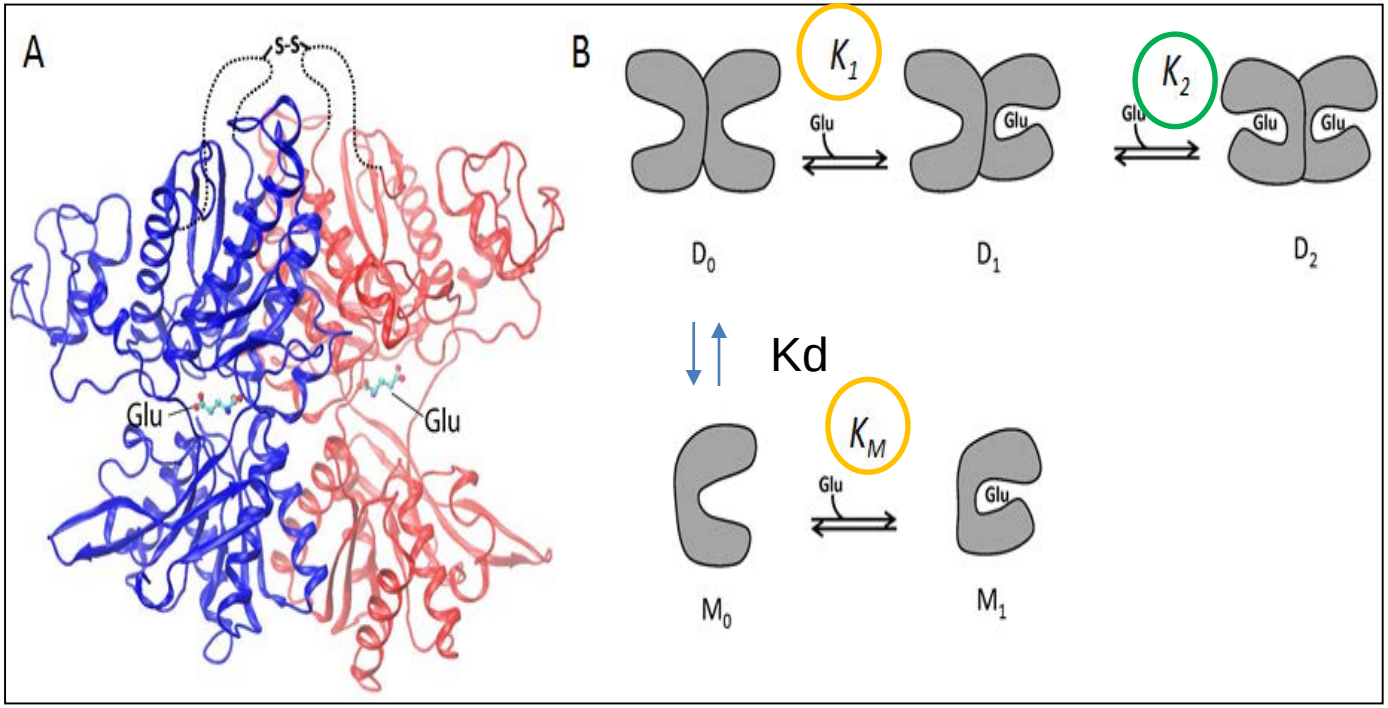
Protein sourse	# of measurements	Fitted Kd (nM)	P value
Preparation 1+2+3	11	28±5	0.0002
Preparation 1+2	7	26±7	0.0088
Preparation 1	4	18±8	0.1156
Preparation 2	3	36±8	0.0395
Preparation 3	4	22±4	0.0111

Activation of mGluR1 LBD in the dimeric and monomeric form upon binding of Glutamate



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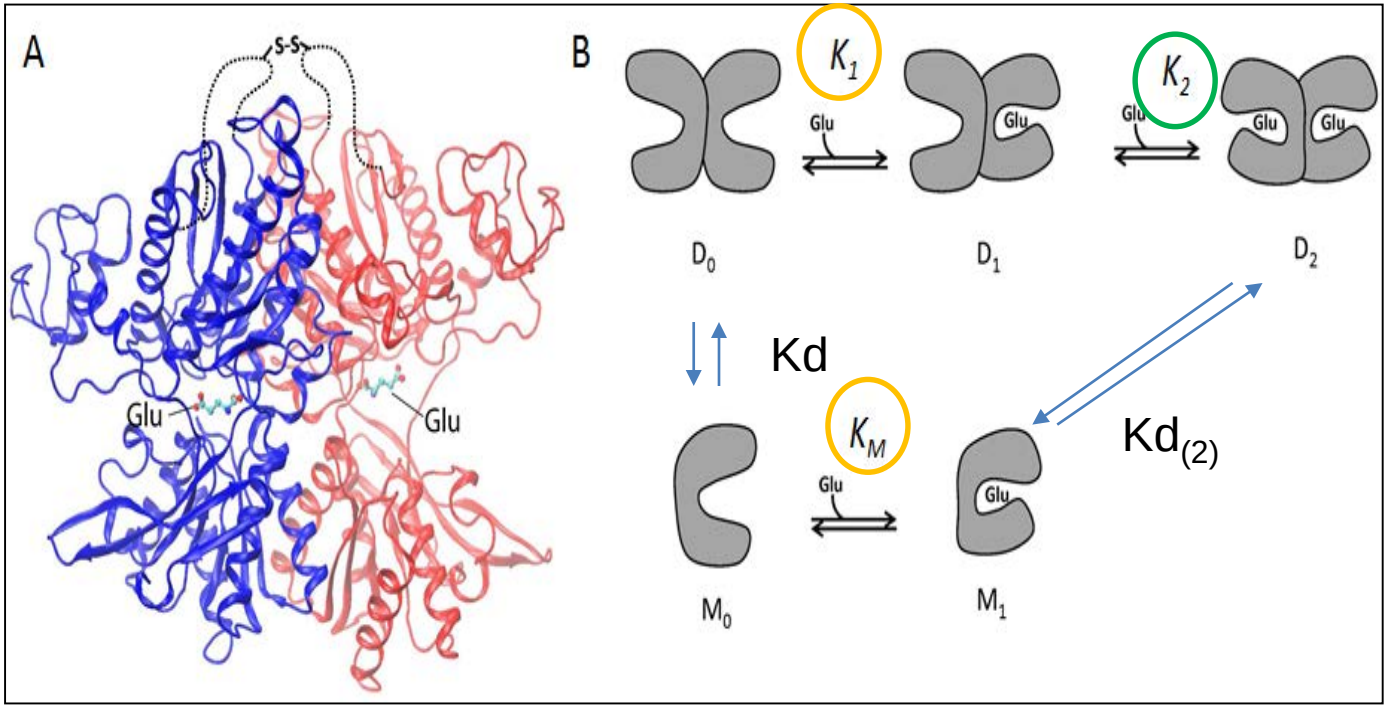

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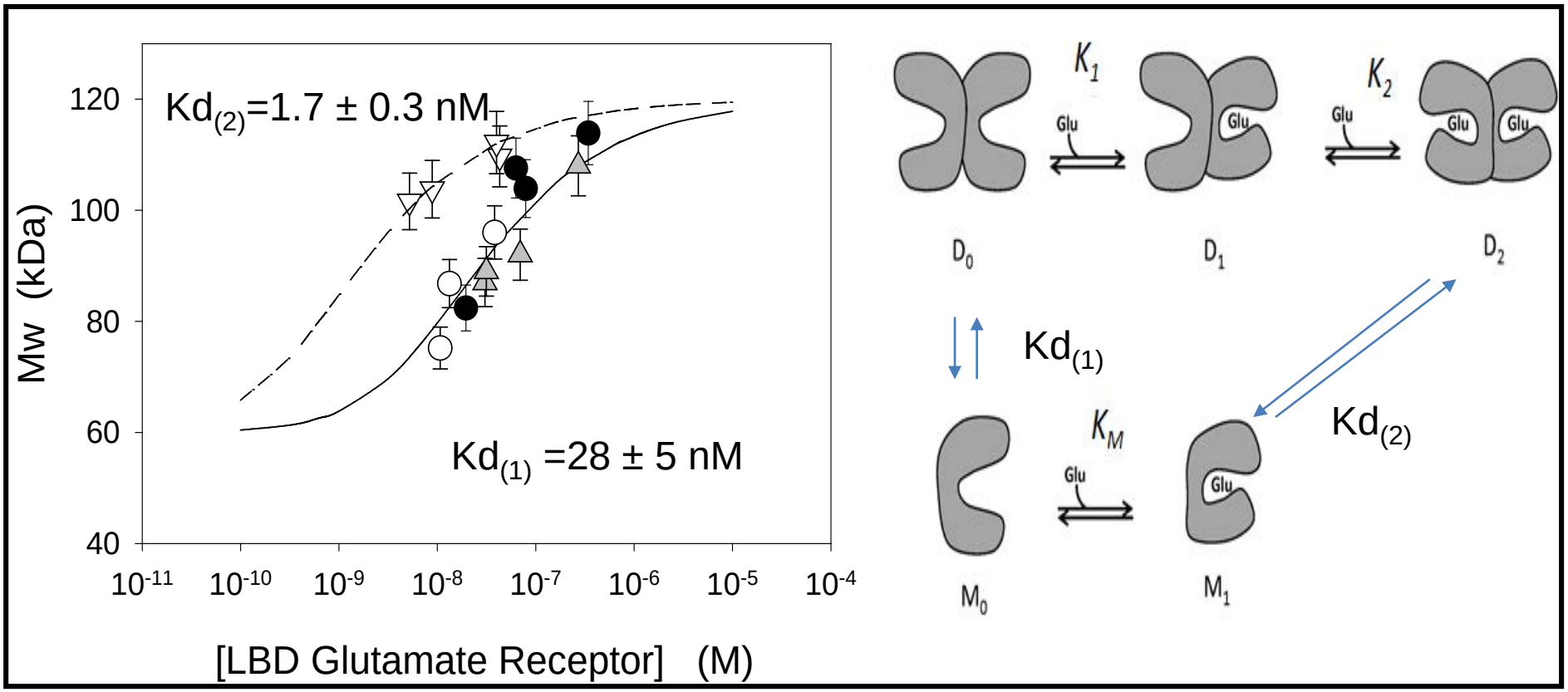

Gunther, E. C., Smith, L. M., Kostylev, M. A., Cox, T. O., Kaufman, A. C., Lee, S., Folta-Stogniew, E., Maynard, G. D., Um, J. W., Stagi, M., Heiss, J. K., Stoner, A., Noble, G. P., Takahashi, H., Haas, L. T., Schneekloth, J. S., Merkel, J., Teran, C., Naderi, Z. K., Supattapone, S., and Strittmatter, S. M. (2019) Rescue of Transgenic Alzheimer's Pathophysiology by Polymeric Cellular Prion Protein Antagonists. Cell reports 26, 1368

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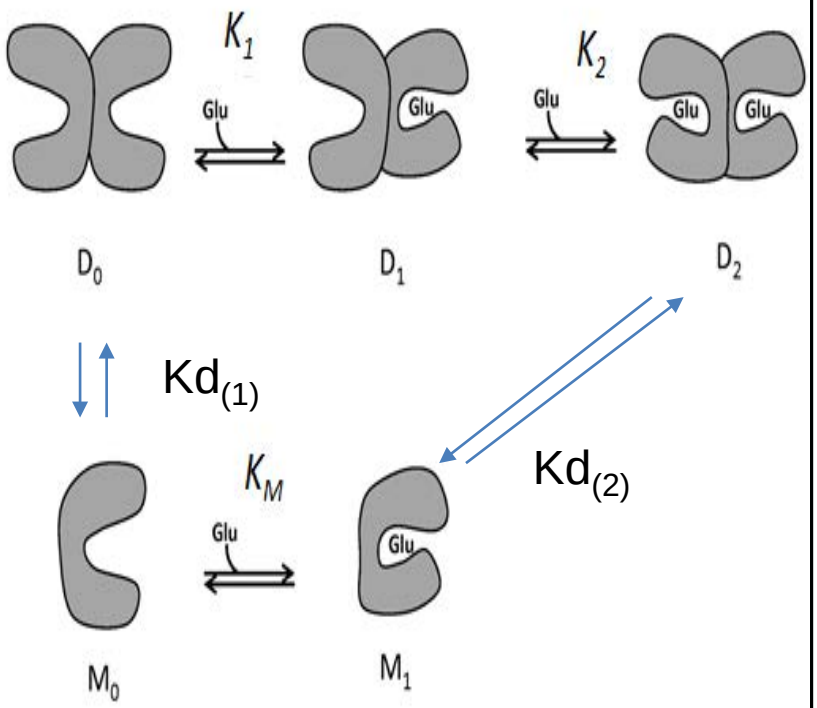
NIH

Yale/NIDA Center support

SEC/MALLS system; SIG 2007
Biacore T100; SIG 2009

Activation of mGluR1 LBD in the dimeric and monomeric form upon binding of Glutamate

K_1	0.53 ± 0.01 µM
K_2	14.5 ± 1.8 µM
K_M	24.2 ± 2.8 µM
$K_{d(1)}$	28 ± 5 nM
$K_{d(2)}$	1.7 ± 0.3 nM



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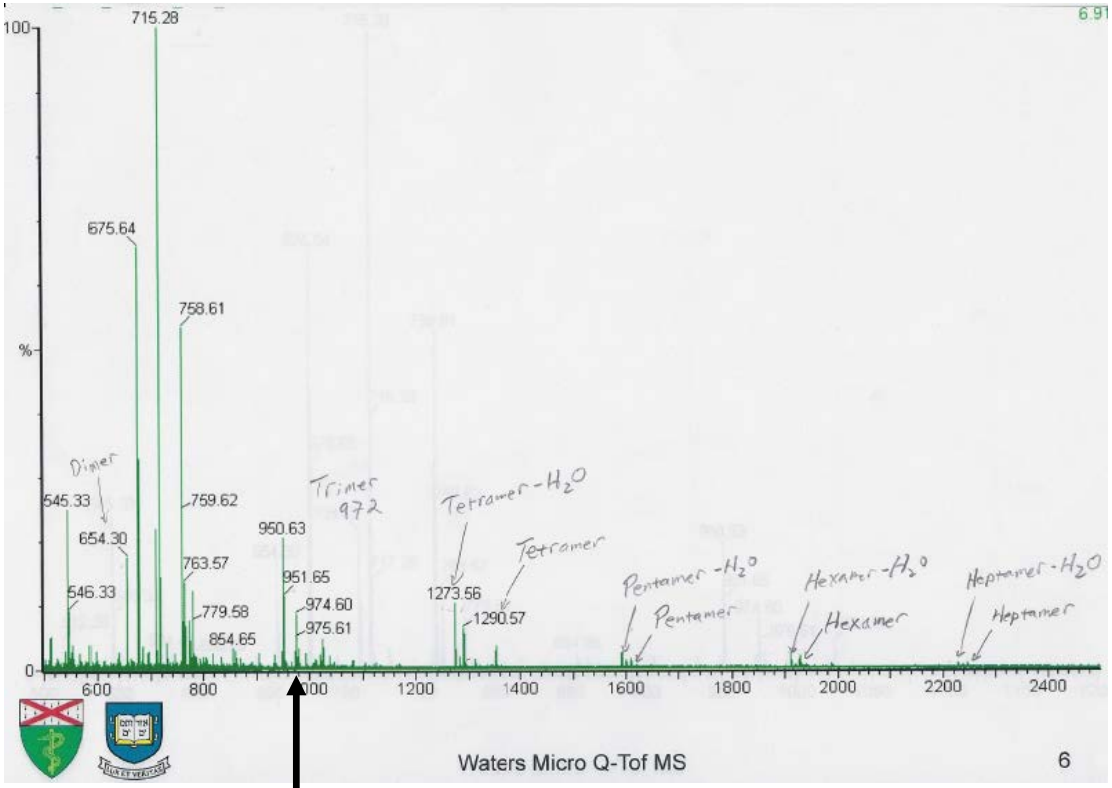
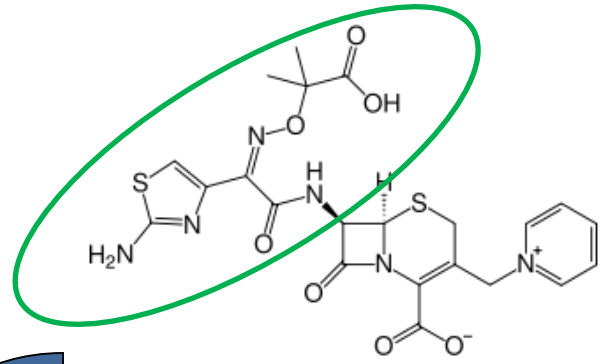
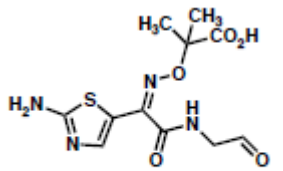
Mass spec detects polymer corresponding to sodium salt of the carboxylic acids



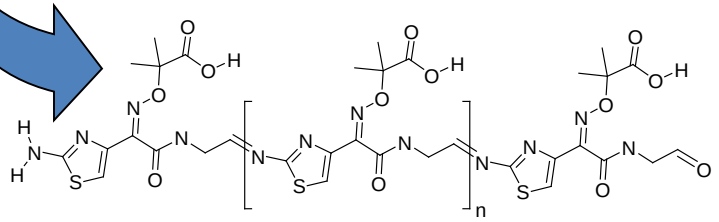


Ceftazidime MW = 546



Chemical Formula: $C_{11}H_{14}N_4O_5S$
Exact Mass: 314.07

Free monomer MW = 314



974

Trimer mass = 928 + 2 Na = 974

Application SPR for screening small molecule as drug candidates

SPR was used to performed secondary screen for direct binding of hits identified through HTS.

buffer: PBS; 5% DMSO

Cellular PRP protein was immobilized through direct amine-coupling

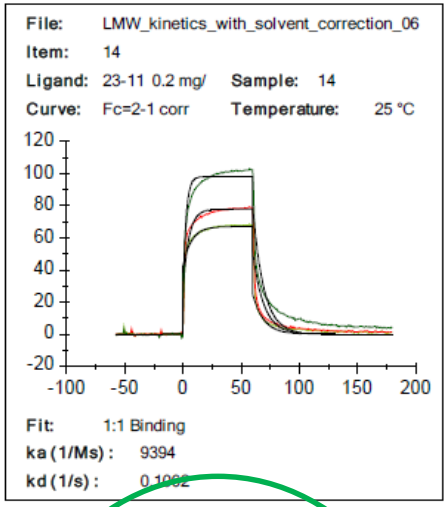
Carbonic Anhydrase was immobilized through amine-coupling and was used as a negative control and as a validation tool

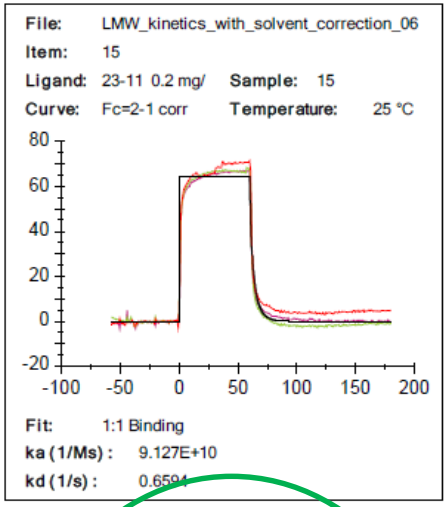
40 compounds screened at 10 uM concentration (diluted step-wise from 10 mM ; 100 % DMSO stocks)

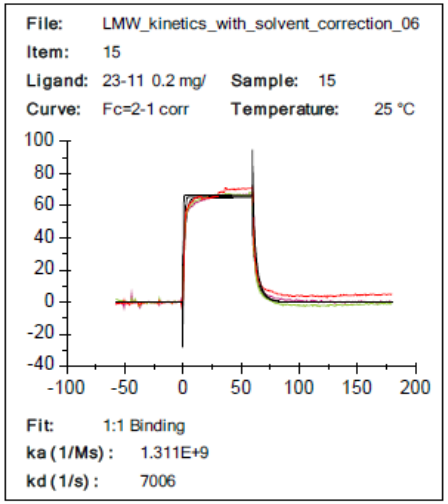
Chip: CM5 ; direct amine coupling				Response	Response
Flow cell	Procedure	Method	Ligand	Bound (RU)	Final (RU)
1	Blank	Amine			278.9
2	Time and flow	Amine	23-111 0.1 mg/ml pH 5.5	4188	2871.4
3	Time and flow	Amine	CA(II) 0.125 mg/ml pH 5.0	9276	5982.1
4	Time and flow	Amine	comm_PrP_2mg_pH_4_0_5ul+125ul	6675.3	7062.5

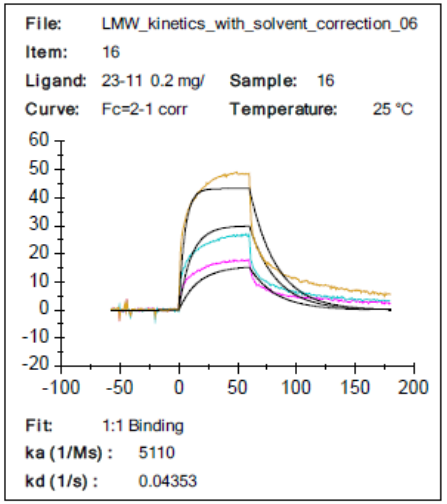
Characterization of interaction between PrP protein and drug candidates

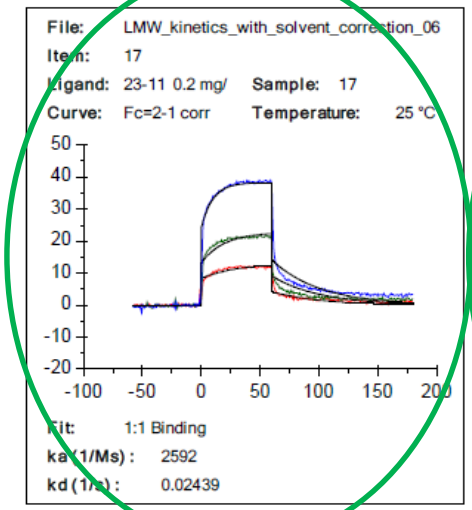
16 compounds screened at 3 concentrations 50, 16.7 and 5.56 uM

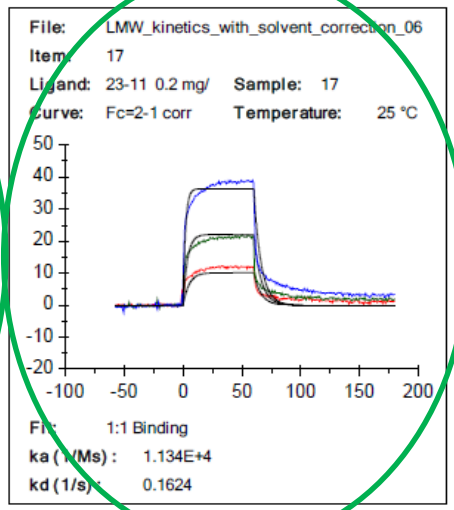


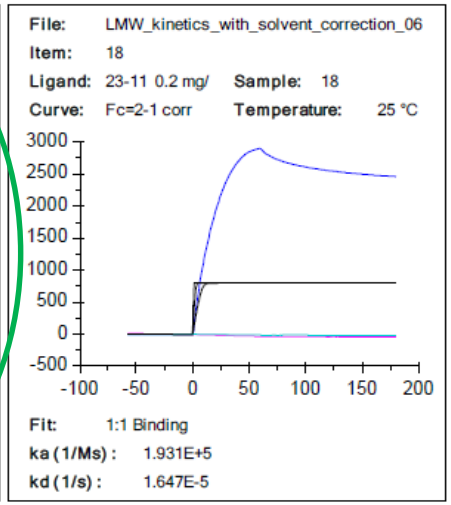


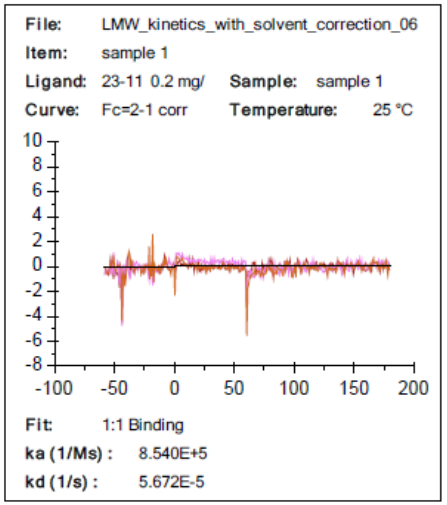






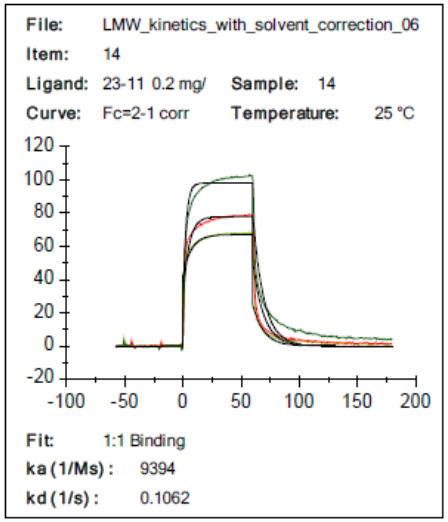


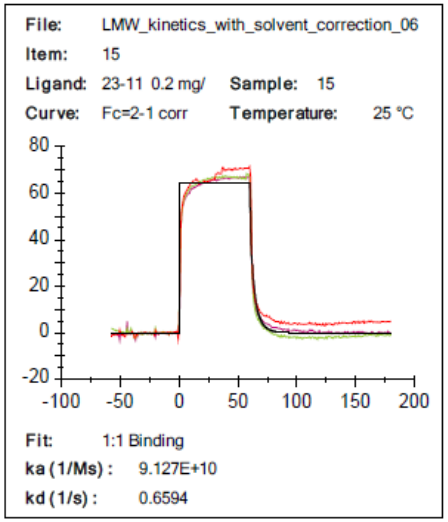


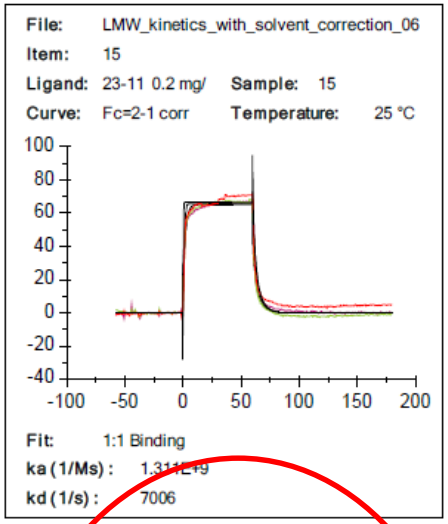


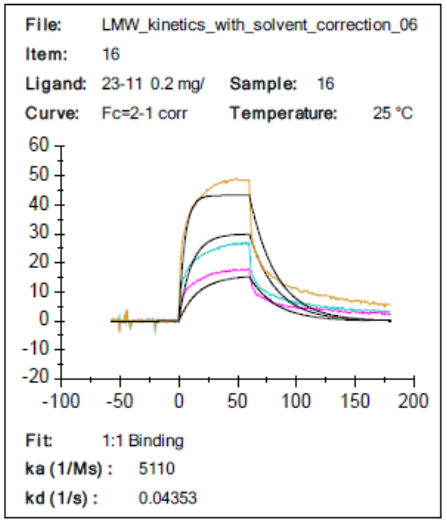
Characterization of interaction between PrP protein and drug candidates

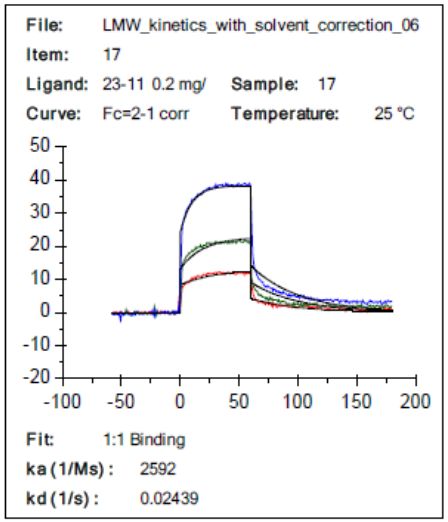
PrP protein was immobilized directly to CM5 chip via amine-coupling; binding of small molecules was monitored in PBS supplemented with 0.05% of Tween 20 detergent and 5% of DMSO.

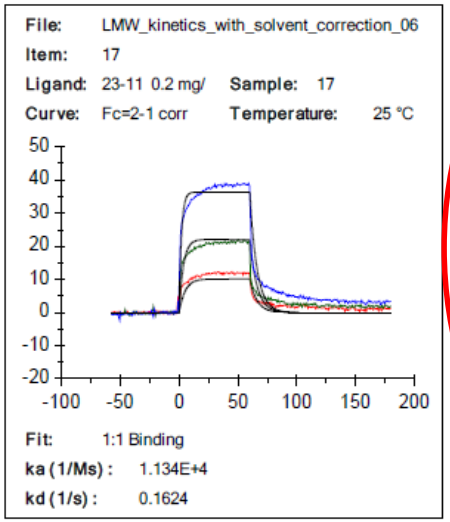


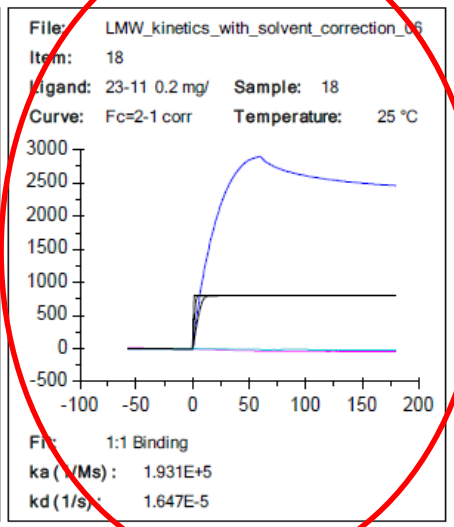


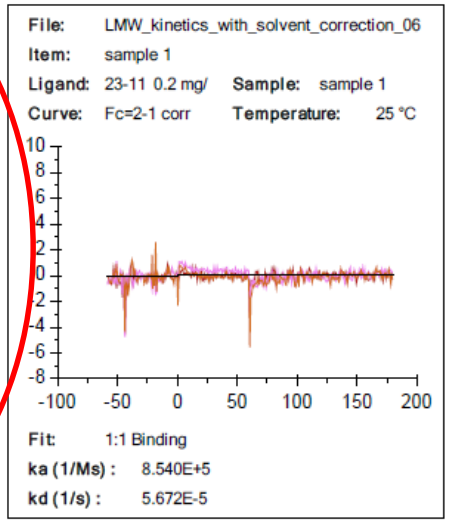












Determination of dimerization constant from SEC-MALS measurements

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

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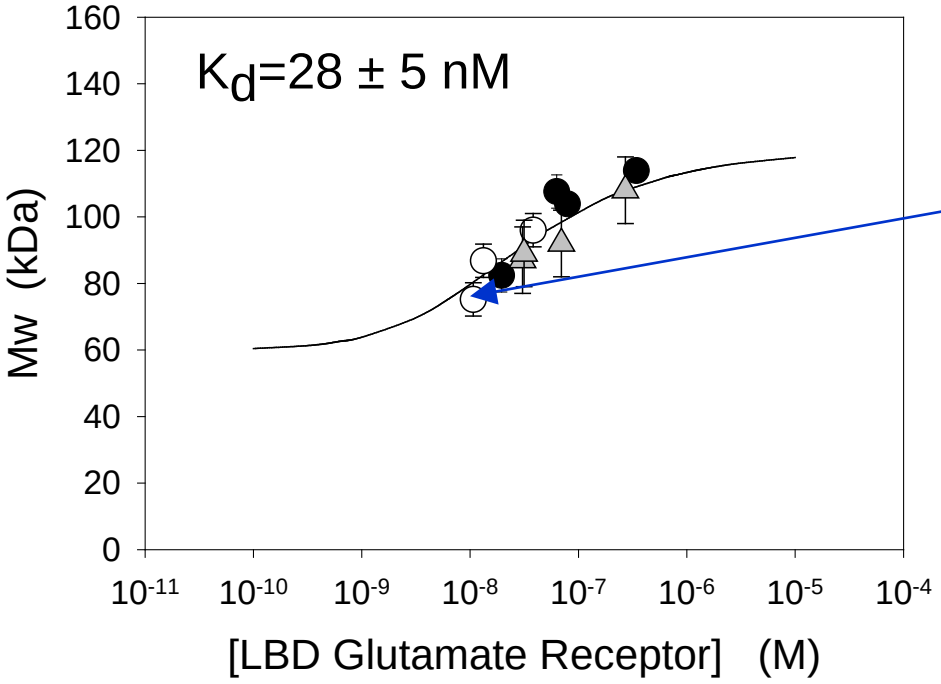
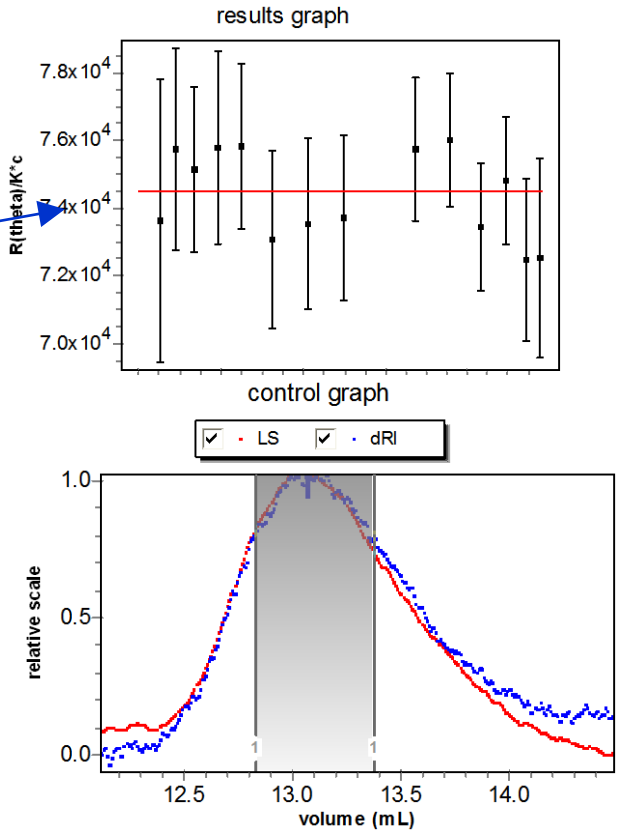
mutant monomer = 59kDa *destabilized dimer?*

Assess concentration-dependent distribution of monomer-dimer





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