Supplemental Material:

A model for specific and nonspecific binding of ligand to multi-protein complexes by native mass spectrometry

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Section a

a.1 masses of α-amanitin/Pol II complexes

	# of alpha-amanitin	0	1	2	3	4	5	6	7	8
	Theoretical mass	469682	470601	471520	472439	473358	474277	475196	476115	477034
1uM Man	Measured mass	470100	471080							
5uM Man	Measured mass	470220	471100							
10uM Man	Measured mass	470120	471100	472140	473160					
20uM Man	Measured mass	470320	471080	472080	473060	474060	475020	475920		
50uM Man	Measured mass	470001	471020	471920	472880	473800	474780	475720	476640	477600

Table Sa1. Theoretical and measured masses for α-amanitin/Pol II 10mer complexes.

	# of alpha-amanitin	0	1	2	3	4	5	6	7	8
	Theoretical mass	514154	515073	515992	516911	517830	518749	519668	520587	521506
1uM Man	Measured mass	514780	515880							
5uM Man	Measured mass	514780	515760							
10uM Man	Measured mass	514820	515780	516760	517799					
20uM Man	Measured mass	514279	515700	516680	517660	518620	519580	520720		
50uM Man	Measured mass	514140	515560	516540	517460	518380	519360	520300	521300	522239

Table Sa2. Theoretical and measured masses for α-amanitin/Pol II 12mer complexes.

The measured masses were obtained by deconvolution of charge states. To avoid overlapping peaks the following charge states were used: 10mer: 50-53; 12mer 47-50.

a.2 Processing of α-amanitin/Pol II binding with two specific sites

The binding model is

$$Kap_1 = Ks1 + Kn_1 = Ks1 + \beta$$

$$Kap_2 = Ks2 + Kn_2 = Ks2 + \beta/2$$

$$Kap_j = Kn_j = \frac{\beta}{j^\gamma}, \; j=3,\ldots,N$$

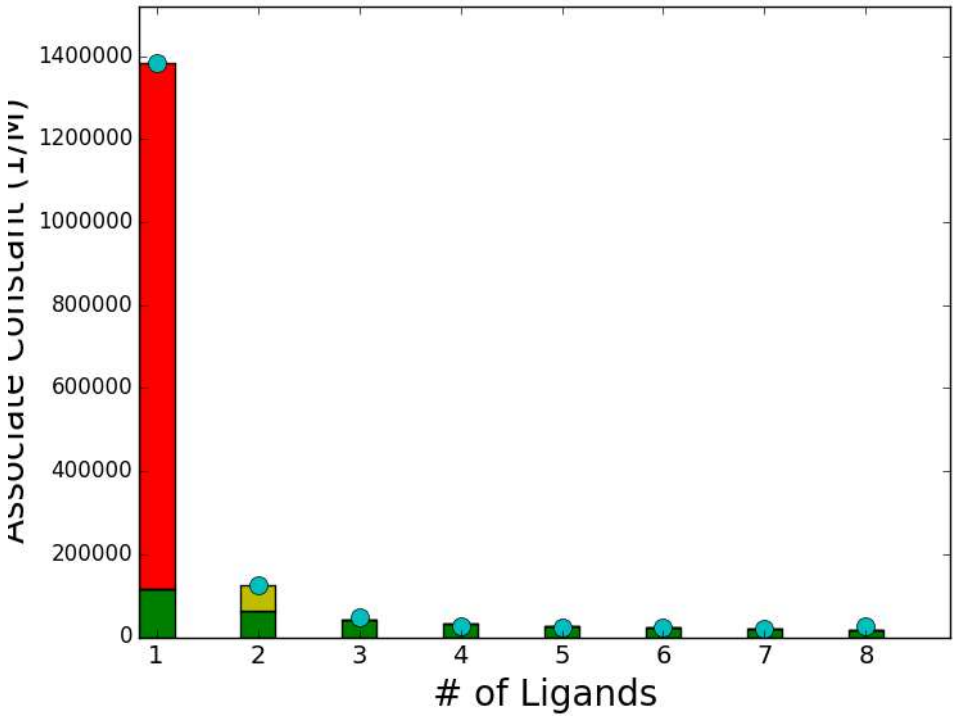

Figure Sb1. Deconvolution of specific binding from nonspecific exponential association constants. Blue circles: apparent association constants; Green bars: contributions of nonspecific binding; Red bar: the first specific binding component (Ks1); Yellow bar: the second specific binding component (Ks2).

Ks1= 1267405.0111 (0.79 μM)

Ks2= 63398.9060156 (15.8 μM)

beta= 115004.378632 (8.7 μM)

gamma= -0.895174261954

a.3 Deconvolution with an exponential function for nonspecific binding

The functional form is

$$Kn_j = \beta e^{-\gamma j}$$

The deconvolution results are Ks = 1.2×10^6M, β= 4.0×10^5M and γ=0.62

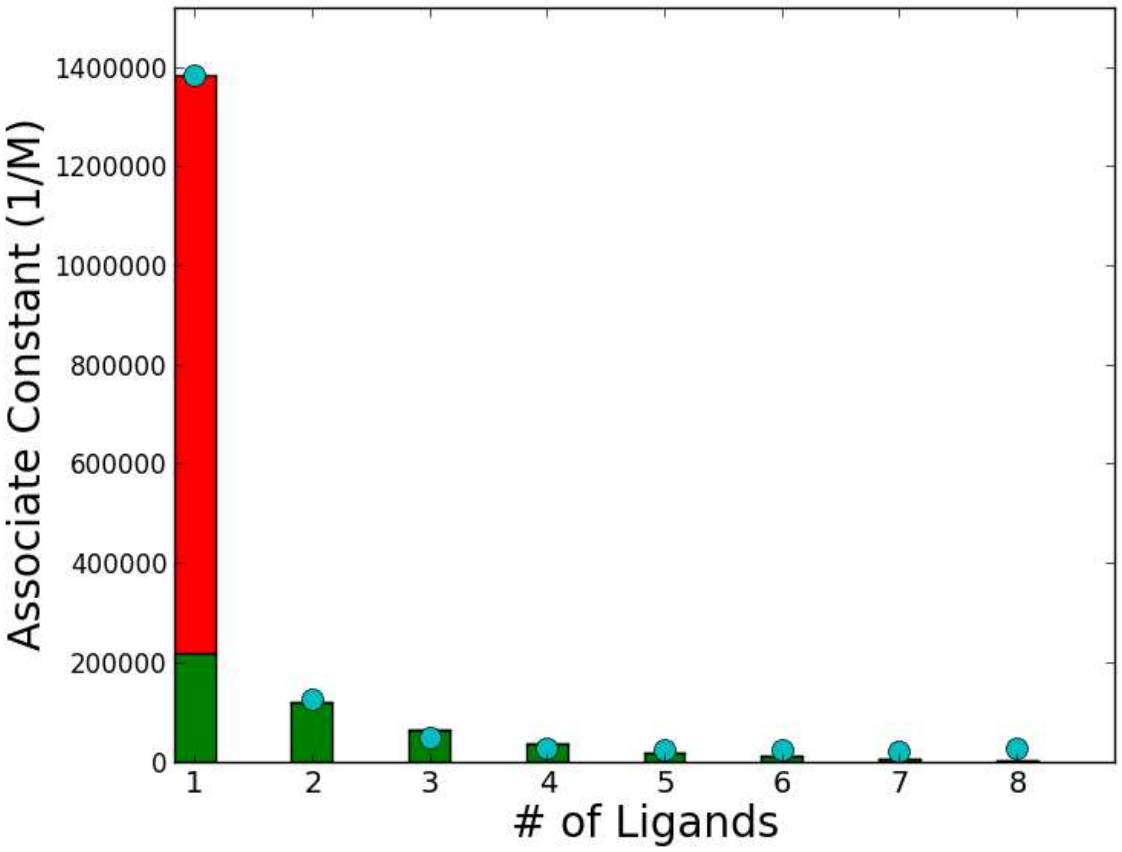

Figure Sd1. Deconvolution of specific binding from nonspecific exponential association constants. Blue circles: apparent association constants; Green bars: contributions of nonspecific binding; Red bar: specific binding component.

Section b

b.1 Extraction of appearent associate constants (Kas).

Intensity data from the supplemental material of (Shimon, L.; Sharon, M.; Horovitz, A., A method for removing effects of nonspecific binding on the distribution of binding stoichiometries: application to mass spectroscopy data. Biophys J 2010, 99 (5), 1645-9.) is (a) from charge satate 19 and (b) normalized within a spectrum (at the certain concentration). The signals of the charge state 19 consist of majority of the all signal intensities. Intensities from all charge states can be aggregated together. The results are essentially the same. The input data for the data model is shown in the following table

# of ADP	0	1	2	3	4	5
5.0E-06	0.783609	0.216391198	0	0	0	0
1.0E-05	0.538314	0.366569845	0.095116	0	0	0
1.5E-05	0.500266	0.409456555	0.090277	0	0	0
2.0E-05	0.36327	0.45620863	0.161786	0.018736	0	0
3.0E-05	0.209488	0.428831067	0.27487	0.070328	0.016483	0
4.0E-05	0.146669	0.329642607	0.301703	0.156927	0.065058	0
5.0E-05	0.040476	0.238603971	0.380873	0.223052	0.087106	0.029889
1.0E-04	0.014055	0.16539788	0.36554	0.275353	0.134499	0.045155

Table Sb1. Refromated raw data from supplmental material of Shimon et al.

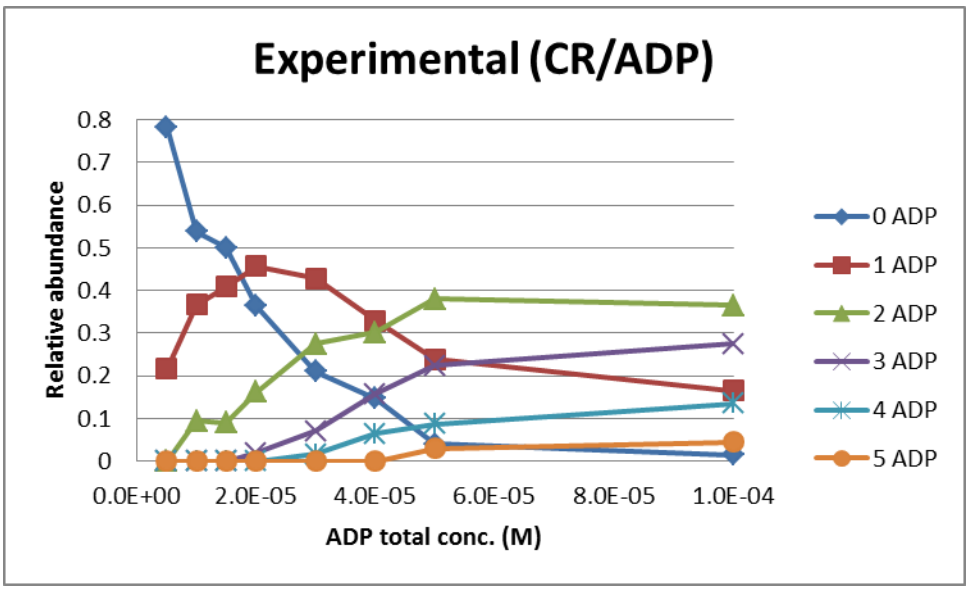

Figure Sb1. Data of Table Sb1

The first level of processing for appearant association constants gives the following results

First the fitted total concentration of the Creatine kinase (CK) is 4.72μM with is 18% higher in value than 4μM reported in the paper. Second, the values of the five binding constants are shown below

ADP binding	0→1	1→2	2→3	3→4	4→5
Ka (M^{-1})	77359	28551	11322	5722	3591
Kd (M)	1.29E-05	3.50E-05	8.83E-05	1.73E-4	2.79E-4

Table Sb2. Appearent binding constants of ADP to Creatine kinase (CK)

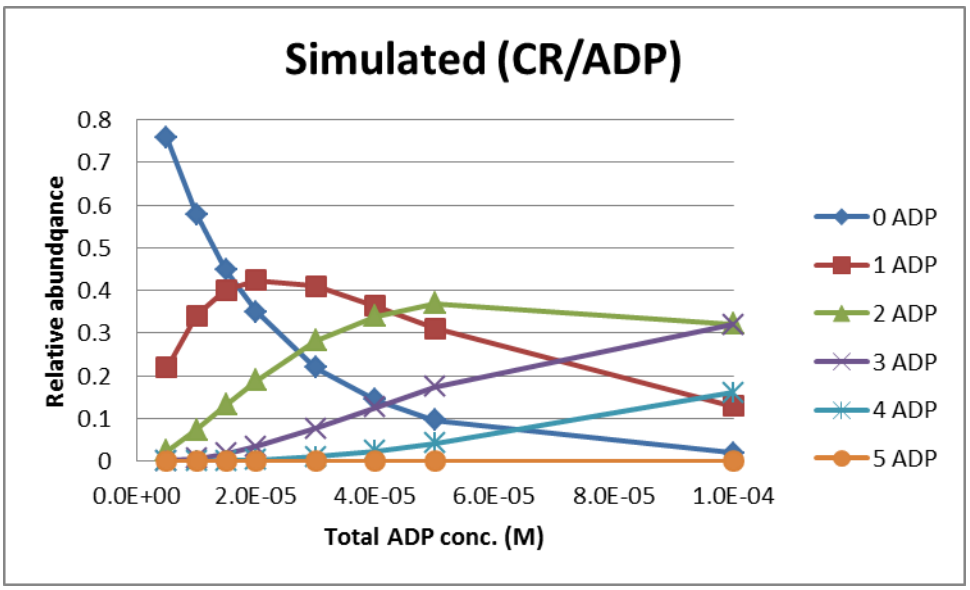

Figure Sb2. Simulated relative abundances of CK/ADP complexes using apprearent binding constants of Table Sb2 and fitted conc of CK of 4.7μM

b.2 Deconvolution of specific binding (2 independent sites) from nonspecific binding (constant)

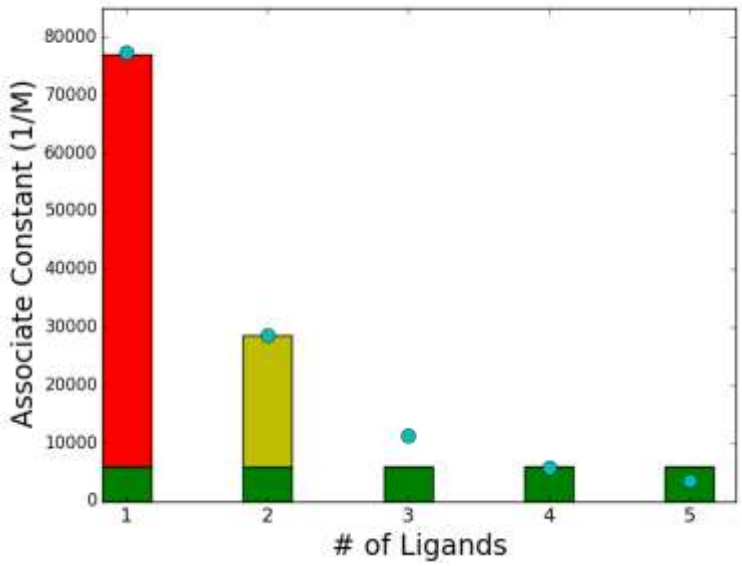

Figure Sb2. Deconvolution of specific binding from nonspecific exponential association constants. Blue circles: apparent association constants; Green bars: contributions of nonspecific (constant model) binding; Red bar: the first specific binding component (Ks1); Yellow bar: the second specific binding component (Ks2).

Ks0= 71208.3610783 M^{-1} (14.0 μM)

Ks1= 22633.4317309 M^{-1} (44.2 μM)

beta= 5917.1873316 M^{-1} (169 μM)

gamma= 0.0

b.3 Deconvolution of specific binding (2 independent sites) from nonspecific binding (inverse power law)

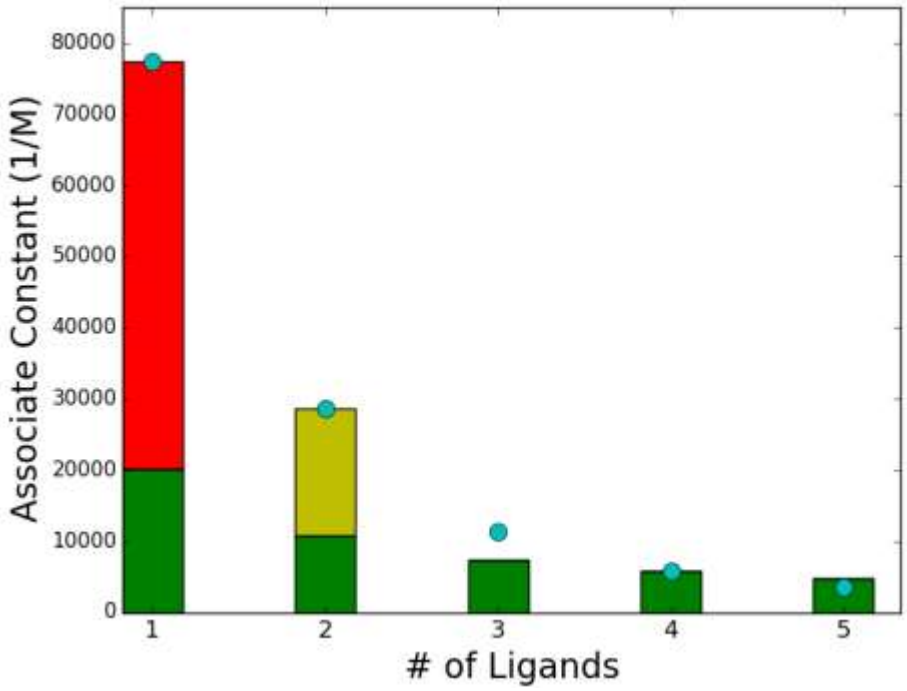

Figure Sb3. Deconvolution of specific binding from nonspecific exponential association constants. Blue circles: apparent association constants; Green bars: contributions of nonspecific (power inverse model) binding; Red bar: the first specific binding component (Ks1); Yellow bar: the second specific binding component (Ks2).

Ks1= 57246.7272953 (17.5 μM)

Ks2= 17823.01081 (56.1 μM)

beta= 20111.9720832 (49.7 μM)

gamma= 0.907

Section c

c.1 FKBP12 sequence

MHHHHHHSSGVDLGTENLYFQSNAMGVQVETISPGDGRTFPKRGQTCVVHYTGMLED
GKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGII
PPHATLVFDVELLKLE

c.2 m/z values of FKBP12/Rapamycin complexes

Species	***FKBP12***			***FKBP12+Ni***		
charge state	8+	7+	6+	8+	7+	6+
Theoretical m/z	1835.95	2098.23	2447.93	1843.29	2106.61	2457.71
measured m/z	1836.9	2099.3	2448.9	1844.1	2107.5	2458.4
Species	***FKBP12+Rapamycin***			***FKBP12+Ni+Rapamycin***		
charge state	8+	7+	6+	8+	7+	6+
Theoretical m/z	1950.22	2228.82	2600.29	1957.56	2237.21	2610.08
measured m/z	1951.2	2229.8	2601.3	1958.3	2238.0	2610.8

Table Sc1. Measured m/z values of charge states of all FKBP12, FKBP12+Ni, FKBP12+Rapamycin, FKBP12+Ni+Rapamycin species.

c.3 figures for processing FKBP12/Rapamycin binding constant

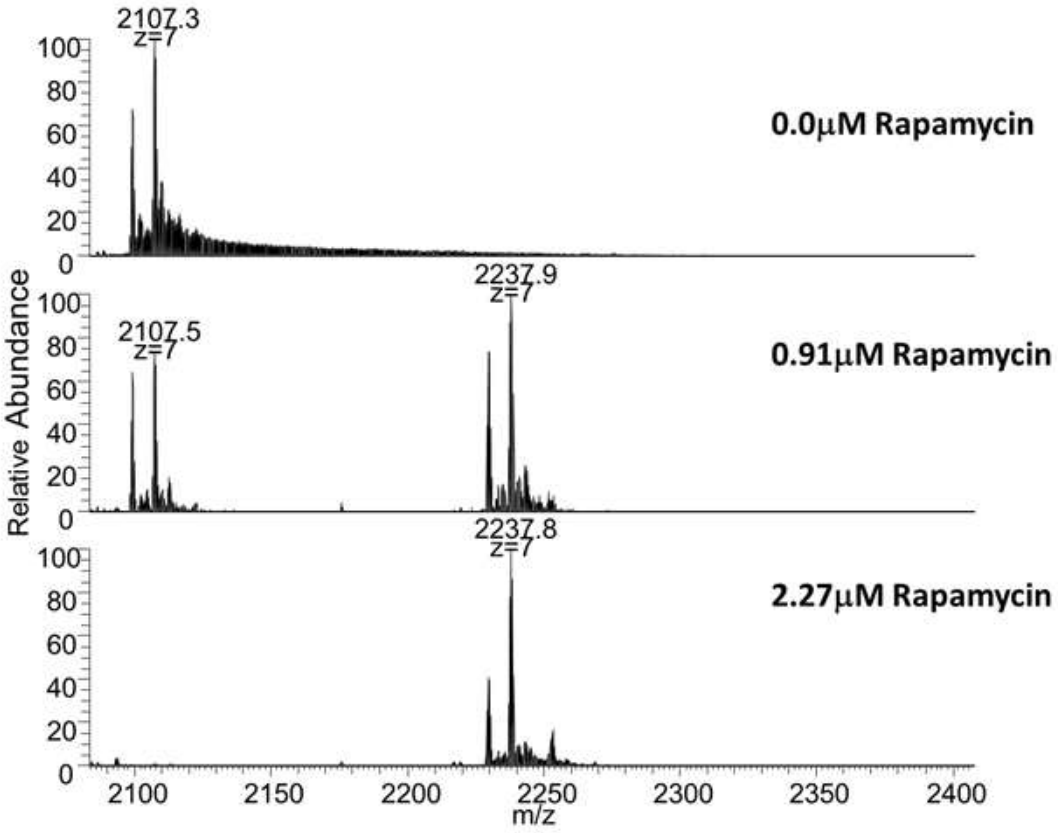

Figure Sc1. Native mass spectra of FKBP12 and Rapamycin. FKBP12 contains a His-tag and both ions with and without Ni atom were observed. Nonspecific binding products for the charge state of 7 were not observed.

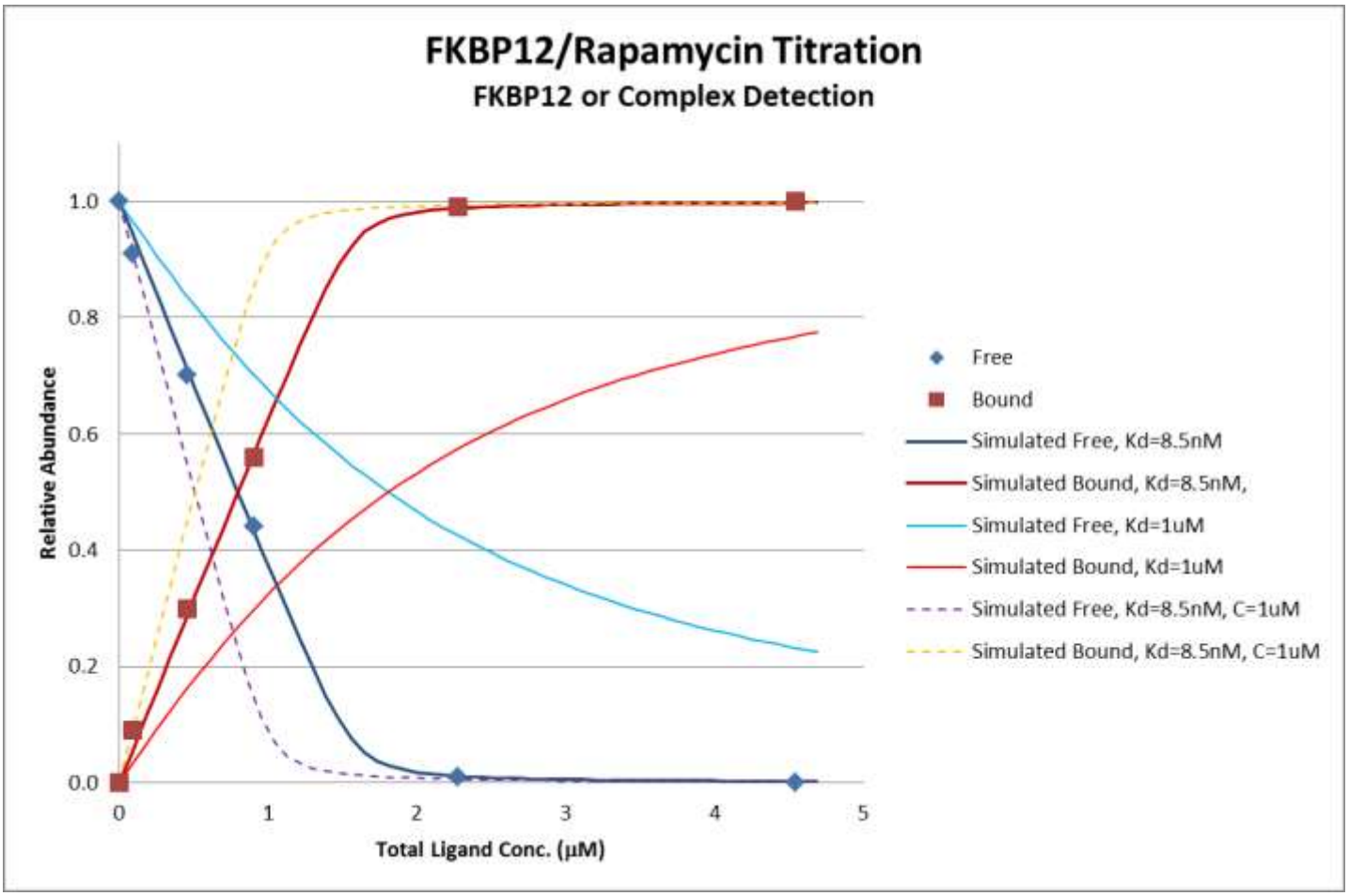

Figure Sc2. Titration curves of FKBP12 against rapamycin with FKBP12 or complex signals Intensities of FKBP12/rapamycin complexes for all charge states 6, 7, and 8 were aggregated for the relative abundances. Thick solid curves are from simulation with Kd=8.5nM and FKBP12 conc of 1.58µM (obtained by fitting the experimental data (dots)). Thin curves are from simulation with frictional Kd=1µM and FKBP12 conc of 1.58µM. Dashed lines are from Kd=8.5nM and FKBP12 conc of 1.0µM.

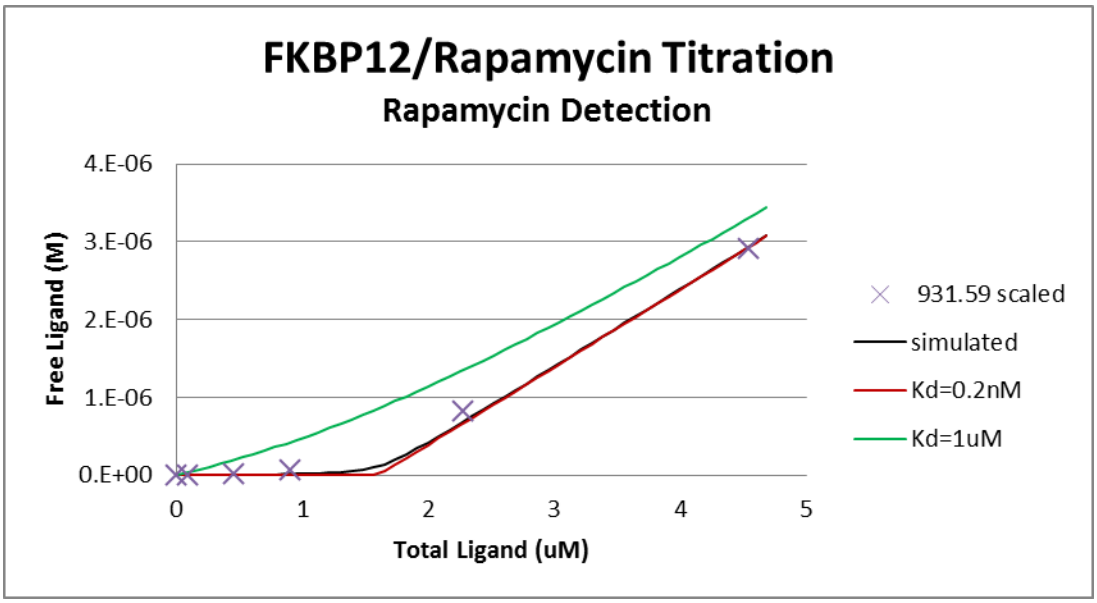

Figure Sc3. Free rapamycin (at m/z 936.6) during titration of Rapamycin against FKBP12. The verical axis has been scaled according to Rapamycin target free concentration/signal intensity ratios. The simulated curves are: Black: using Kd=8.5nM; Red: frictional Kd=0.2nM; and Green: frictional Kd of 1μM. All curves were using FKBP12 conc of 1.58μM.

Section d

d.1 Description of sample data and codes:

fsolve_withPT.py computes for apparent associate binding constants from relative intensities of native MS spectra from a series of titration samples.

fsolve_withPT.py takes two command line arguments: input file name of "MamPol2_titration_data.txt" and output file name of "Kaps_result.txt"

deconvKas_general_curveFit.py deconvolutes specific from nonspecific binding.

deconvKas_general_curveFit.py takes two command line arguments: input file name of "Kaps_result.txt" and a number of 0, 1, or 2. 0 is for the constant nonspecific binding, 1 is for the inverse power function, and 2 is for exponential function

The codes and data files were included in the CodeAndData.zip file