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Supporting Material

A Method for Removing Effects of Non-Specific Binding on the Distribution of Binding Stoichiometries: Application to Mass-Spectroscopy Data

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Calculation of binding constants

Four independent experiments were carried out in order to calculate the binding constants from the plots in Fig. 3. In what follows, we provide the raw data and calculations corresponding to one out of the four experiments. The average error in the intensities is about 2% and in the ratios of intensities it is about 3%.

Table 1: Measured peak areas obtained after titration of CK with ADP. The peak areas for the free CK (I_0) and its ligand-bound states ($I_{n\geq1}$) were calculated using a deconvolution algorithm (peakfit v4, Jandel Scientific). For each concentration, peak areas for the 18^+, 19^+ and 20^+ charge states are indicated.

5 µM	I_0	I_1
18^+	5806400	1455700
19^+	14996000	4141100
20^+	8602800	2079900

10 µM	I_0	I_1	I_2
18^+	732260	504890	140380
19^+	1868100	1272100	330080
20^+	696660	442870	123290

15 µM	I_0	I_1	I_2
18^+	97151	68814	14916
19^+	977620	800160	176420
20^+	1130300	871970	207040

20 µM	I_0	I_1	I_2	I_3
18^+	305720	364980	140710	14586
19^+	1091700	1371000	486200	56304
20^+	495590	569690	215530	16623

30 µM	I_0	I_1	I_2	I_3	I_4
18^+	255600	504500	346490	85742	14510
19^+	607900	1244400	797630	204080	47832
20^+	223430	402780	302640	71914	12142

40 µM	I_0	I_1	I_2	I_3	I_4
18^+	30736	70566	76879	43399	19131
19^+	38720	87024	79648	41428	17175
20^+	3272	7125	7438	3706	1481

50 µM	I_0	I_1	I_2	I_3	I_4	I_5
18^+	55784	319750	554260	331720	136960	32527
19^+	127450	751320	1199300	702350	274280	94116
20^+	44138	220620	423010	247420	107550	28810

100 µM	I_0	I_1	I_2	I_3	I_4	I_5
18^+	10007	111980	257520	183310	82153	20927
19^+	20874	245640	542880	408940	199750	67062
20^+	4611	50672	145340	98654	45977	10672

Table 2: Calculation of the average non-specific binding constant (K_n).

30 µM	18^+	19^+	20^+
I_4/I_3=	0.17	0.23	0.17

40 µM	18^+	19^+	20^+
I_4/I_3 =	0.44	0.41	0.40

50 µM	18^+	19^+	20^+
I_4/I_3 =	0.41	0.39	0.43
I_5/I_4 =	0.24	0.34	0.27

100 µM	18^+	19^+	20^+
I_4/I_3 =	0.45	0.49	0.47
I_5/I_4 =	0.25	0.34	0.23

µM	30	40	50	100	**Average**	**STD**
Kn	0.006	0.011	0.007	0.004	0.007	0.003

Table 3: Correction of nonspecific binding effects on the distribution of binding stoichiometries (Eq. 8).

5 µM	$C_0/[E]$	$C_1/[E]$	$C_2/[E]$
18+	1.04	0.22	0
19+	1.04	0.25	0
20+	1.04	0.21	0
Average	1.04	0.23	0

10 µM	$C_0/[E]$	$C_1/[E]$	$C_2/[E]$
18+	1.07	0.67	0.15
19+	1.07	0.66	0.14
20+	1.07	0.61	0.14
Average	1.07	0.64	0.15

15 µM	$C_0/[E]$	$C_1/[E]$	$C_2/[E]$
18+	1.12	0.67	0.09
19+	1.12	0.80	0.11
20+	1.12	0.74	0.11
Average	1.12	0.74	0.10

20 µM	$C_0/[E]$	$C_1/[E]$	$C_2/[E]$
18+	1.16	1.22	0.34
19+	1.16	1.30	0.31
20+	1.16	1.17	0.32
Average	1.16	1.23	0.32

30 µM	$C_0/[E]$	$C_1/[E]$	$C_2/[E]$
18+	1.27	2.23	1.19
19+	1.27	2.32	1.11
20+	1.27	2.02	1.23
Average	1.27	2.19	1.18

40 µM	$C_0/[E]$	$C_1/[E]$	$C_2/[E]$
18+	1.39	2.80	2.57
19+	1.39	2.73	1.97
20+	1.39	2.63	2.30
Average	1.39	2.72	2.28

50 μM	C_0/[E]	C_1/[E]	C_2/[E]
18+	1.54	8.26	12.14
19+	1.54	8.52	11.24
20+	1.54	7.14	11.99
Average	1.54	7.97	11.79

100 μM	C_0/[E]	C_1/[E]	C_2/[E]
18+	2.94	30.85	49.64
19+	2.94	32.55	49.28
20+	2.94	30.27	66.08
Average	2.94	31.22	55.00

Table 4: The corrected ligand-bound concentrations (using Eq. 9 and given that C_T = 4 μM).

5 μM	C_0	C_1	C_2
18+	3.29	0.71	0
19+	3.22	0.78	0
20+	3.31	0.69	0
Average	3.28	0.72	0

10 μM	C_0	C_1	C_2
18^+	2.27	1.41	0.33
19^+	2.30	1.40	0.30
20^+	2.36	1.33	0.31
Average	2.31	1.38	0.31

15 μM	C_0	C_1	C_2
18^+	2.38	1.43	0.19
19^+	2.21	1.58	0.21
20^+	2.26	1.51	0.23
Average	2.28	1.51	0.21

20 μM	C_0	C_1	C_2
18^+	1.70	1.80	0.50
19^+	1.67	1.87	0.45
20^+	1.75	1.77	0.48
Average	1.71	1.81	0.48

30 μM	C_0	C_1	C_2
18^+	1.08	1.91	1.01
19^+	1.08	1.98	0.95
20^+	1.12	1.79	1.09
Average	1.09	1.89	1.02

40 μM	C_0	C_1	C_2
18^+	0.82	1.66	1.52
19^+	0.91	1.79	1.30
20^+	0.88	1.67	1.45
Average	0.87	1.71	1.42

50 μM	C_0	C_1	C_2
18^+	0.28	1.51	2.21
19^+	0.29	1.60	2.11
20^+	0.30	1.38	2.32
Average	0.29	1.50	2.22

100 μM	C_0	C_1	C_2
18^+	0.14	1.48	2.38
19^+	0.14	1.54	2.33
20^+	0.12	1.22	2.66
Average	0.13	1.41	2.46