Prediction of Dissociation Constant in Protein-Ligand Interaction

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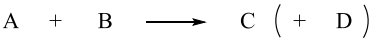
In protein-ligand interactions, such as antigen-antibody interactions and hormone-receptor interactions, a correlation between the equilibrium dissociation constant K_D and the reduced mass of the protein and ligand was found. The correlation of dissociation constants as pK_D ($-\log K_D$) between literature values and predicted values was confirmed in high coefficient of determination R^2 over 0.98.

Keywords: Dissociation Constant |Protein-Ligand Interaction |Correlation |Reduced Mass |Prediction

The author has reported the correlation between reduced mass and yield in many organic chemical reactions, such as C-C, C-N, C-O coupling reactions, cycloaddition reactions, Bingel reaction of fullerene, and general reactions in the synthesis of natural products and has shown that the relationship expressed in the following equations 1a-e for the reaction scheme 1 is maintained even in enzymatic reactions including formation of ES complex.[1-5] Therefore, the author



Scheme 1. Reaction of molecule A with B to produce C (and D)

$$y = -0.186\frac{M'_{AB}}{n_A n_B n_I} + 100 \quad (1a)$$

$$M'_{AB} = \frac{M'_A M'_B}{M'_A + M'_B} \quad (1b)$$

$$M'_{A(B)} = M_{A(B)} - 14.03aR_{A(B)} \quad (1c)$$

$$a = 0.00177M_{A(B)} \quad (M_{A(B)} \le 768) \quad (1d)$$

$$a = 1.36 \quad (M_{A(B)} > 768) \quad (1e)$$

y : yield of molecule C
M'_{AB} : adjusted reduced mass of molecular A and B
$M'_{A(B)}$: adjusted molecular weight of molecular A(B)
$M_{A(B)}$: molecular weight of molecular A(B)
$n_{A(B)}$: number of reaction sites A(B)
n_I : intermolecular reaction = 1,
intramolecular or enzymatic reaction = 2
$R_{A(B)}$: number of rotatable bonds of molecular A(B)
a : coefficient for adjustment by molecular weight

was interested in the relationship between reduced mass and protein-ligand interactions, such as antigen-antibody reactions and hormone-receptor interactions. For these protein-ligand interactions, it was considered appropriate to replace the yield, which is a measure of the correlation with reduced mass used in the analysis of organic reactions reported previously, with the equilibrium dissociation constant K_D. The K_D, which represents binding affinity, is very important in studying protein-ligand interactions, and methods for measuring, evaluating, and predicting K_D are considered important.[6] In this report, we would like to show a simple K_D prediction method using reduced mass, which has not been published before.

As for antigen-antibody reactions, the author analyzed the reactions between antigens, that cause malignant tumors, allergies, and autoimmune diseases, and their antibody drugs, as well as the reactions between proteins on the outer membrane of SARS-CoV-2 virus that causes the new coronavirus infection (COVID-19), and receptors on the cell membranes of infected cells. For drug-receptor interactions, the reactions with receptors for insulin, insulin-like growth factor, calcitonin, calcitriol, and thyroid hormone were analyzed. In an equilibrium reaction shown in scheme 2, the dissociation constant K_D is expressed by the equation 2a. Since the yield y is expressed in the equation 2b, if the concentrations of A and B are equal and the initial concentration of molecule A is $[A]_0$ M, the equation relating K_D and y can be obtained and is shown in the equation 2d. The

Scheme 2. Equilibrium reaction: AB ⇄ A + B

$$K_D = \frac{[A][B]}{[AB]} \quad (2a)$$

$$y = 100\frac{[AB]}{[A]_0} = 100\frac{[A]_0 - [A]}{[A]_0} \quad (2b)$$

$$[A] = [B] \quad (2c)$$

$$K_D = [A]_0\frac{(100-y)^2}{100y} \quad (2d)$$

K_D was calculated by substituting y, which was obtained using the equation 1a, into the equation 2d. The number of rotatable bonds (NORB) of a protein was determined by first dividing the molecular weight by the average mass of amino acid

residues ($\frac{M_{A(B)}}{118.8}$), multiplying it by the average number of rotatable bonds of amino acids (2.90), and then subtracting the number of amide bonds ($\frac{M_{A(B)}}{118.8}$) from it as follows:[7]

$$R_{A(B)} = 2.90\frac{M_{A(B)}}{118.8} - \frac{M_{A(B)}}{118.8} = 0.01599M_{A(B)}.$$

In the previous report on the correlation between reduced mass and yield in enzymatic reactions, it was suggested that n_I in the equation 1 a represents affinity. Therefore, n_I was expressed as the equation 3 using the number of binding sites or ligands (n_A, n_B), and the coefficient c was adjusted to optimal value in each concentration $[A]_0$ so that the regression coefficient of the relationship between the literature and predicted value of pK_D (pK_D = $-\log K_D$) calculated by using the rewritten equation 1a' and the equation 2d, in the graph would be closer to 1.

$$n_I = cn_An_B \qquad (3)$$

$$\therefore \quad y = -\frac{0.186M'_{AB}}{c(n_An_B)^2} + 100 \qquad (1a')$$

Table 1 summarizes protein-ligand combinations analyzed (sample size = 64), molecular weight, number of rotatable bonds, reduced mass adjusted with NORB, n_A, n_B, n (= $c(n_An_B)^2$), and the literature values of dissociation constant and their predictions in the case of $[A]_0 = 1\times10^{-6}$ M.[8-42] In the table, the literature values of K_D were listed without considering the differences in measurement methods and conditions such as temperature, pH, and salt concentration. Literature versus predicted dissociation constants pK_D in the cases of protein concentration of 1 M, 1×10^{-2} M, 1×10^{-4} M, and 1×10^{-6} M are shown in Figure 1.

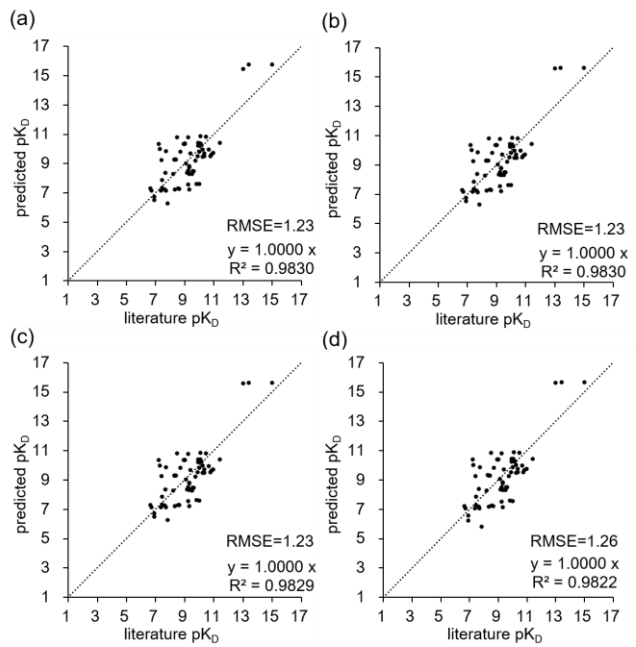


Figure 1. Literature versus predicted pK_D plot of protein-ligand interaction. (a) $[A]_0$ = 1 M, (b) $[A]_0$ = 1×10^{-2} M, (c) $[A]_0$ = 1×10^{-4} M, (d) $[A]_0$ = 1×10^{-6} M.

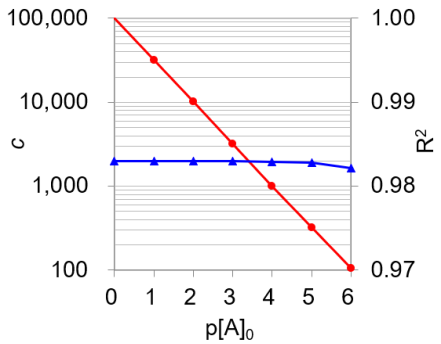


Figure 2. c value (●) for each $[A]_0$ and coefficient of determination R^2 (▲) in the correlation between literature and predicted pK_D.

As shown in Figure 2, the c value refers to the collision frequency, and the relationship with $[A]_0$ is expressed by the equation:

$$c = 99882[A]_0^{0.4973} \qquad (R^2 = 1.00) \qquad (4).$$

Coefficient of determination R^2 in the correlation between the literature and predicted pK_D is over 0.98 in a wide range of $[A]_0$. Although this method does not consider the structure or free energy at all, a higher coefficient of determination was obtained than the prediction method by machine learning using X-ray crystal resolution.[6(b,c)] Furthermore, this K_D prediction method can also be used to estimate the number of binding sites by changing n_A and n_B to see if it agrees with the predicted value, if the exact measurement of K_D is known.

This method was applied to the prediction of dissociation constant of an insect pheromone and the receptor. The dissociation constants for the pheromone (+)-disparlure of the gypsy moth *Lymantria dispar* and the pheromone binding proteins PBP2 and DNS-PB2 are 2.9×10^{-6} M and 2.6×10^{-6} M, respectively.[43] To make the predictions from equation 1a' and 2d agree with these literature values, c had to be lowered to 0.43 (predicted values of 2.8×10^{-6} M and 2.9×10^{-6}), which is much smaller than the value (106) calculated as $[A]_0 = 1\times10^{-6}$ M using the equation 4. According to the literature, this interaction takes place in two steps at two different sites of the receptor, which may be one of the reasons.

Table 1. Analysis of protein-ligand interaction in the case of $[A]_0 = 1\times10^{-6}$ M.

A	B	M_A[a]	ref.[b]	M_B[c]	ref.[d]	R_A[e]	R_B[f]	M'_{AB}[g]	n_A[h]	n_B[i]	n[j]	K_D (M) literature[k]	K_D (M) predicted	ref.[l]
Melanotransferrin (mTF)	anti mTF scFv	80215	44	28000	8	1283	448	14421	1	1	106	2.04×10^{-8}	8.7×10^{-8}	8
mTF	anti mTF IgG	80215		150000	8	1283	2399	36316	2	2	1688	4.82×10^{-10}	1.7×10^{-9}	8
CD20, B-lymphocyte antigen	murine anti-CD20 mAb 2B8	35000	9	150000	51	560	2399	19718	2	2	1688	3.88×10^{-9}	4.8×10^{-10}	9
CD20	rituximab	35000		144187	46	560	2306	19569	2	2	1688	5.45×10^{-9}	4.8×10^{-10}	9
CD20	ofatumumab	35000		146060	46	560	2336	19618	2	2	1688	4.76×10^{-9}	4.8×10^{-10}	9
human epidermal growth factor receptor 2 (HER2)	trastuzumab	137910	44	145532	47	2206	2328	49201	2	4	6752	4×10^{-10}	1.9×10^{-10}	10
HER2	pertuzumab	137910		148000	48	2206	2367	49603	2	2	1688	3×10^{-10}	3.2×10^{-9}	10
Vascular endothelial growth factor (VEGF)-A165	aflibercept	45000	44	96897	47	720	1550	21352	2	2	1688	1.9×10^{-10}	5.7×10^{-10}	12
VEGF-A165	bevacizumab	27042		149197	47	432	2386	15907	2	2	1688	5.8×10^{-11}	3.1×10^{-10}	12
VEGF-A165	ranibizumab	27042		48379	47	432	774	12053	2	2	1688	4.6×10^{-11}	1.8×10^{-10}	12
epidermal growth factor receptor (EGFR)	cetuximab	134277	44	152000	49	2148	2431	49538	2	2	1688	2.5×10^{-10}	3.2×10^{-9}	13
EGFR	imgatuzumab (GA201)	134277		145027	47	2148	2319	48446	2	2	1688	6×10^{-10}	3.0×10^{-9}	11
hen egg white lysozyme (HEL)	hen egg white lysozyme antibody (HyHEL-10)	14305	14	150000	14	229	2399	9074	2	2	1688	2.2×10^{-11}	1.0×10^{-11}	14
cluster of differentiation 4 (CD4)	ibalizumab	51111	44	150000	50	817	2399	26488	2	2	1688	8×10^{-10}	8.8×10^{-10}	11
surface antigen CD319 (SLAMF7)	elotuzumab	37421	44	145452	46	598	2326	20681	2	2	1688	4.04×10^{-8}	5.3×10^{-10}	15
tumor necrosis factor (TNFα)	golimumab	25644	44	146943	46	410	2350	15171	2	2	1688	1.8×10^{-11}	2.8×10^{-10}	16
TNFα	etanercept	25644		51167	46	410	818	11870	2	2	1688	1.1×10^{-11}	1.7×10^{-10}	16
TNFα	infliximab	25644		165000	56	410	2639	15422	2	2	1688	4.4×10^{-11}	2.9×10^{-10}	16
TNFα	adalimumab	25644		150000	56	410	2399	15217	2	2	1688	1.3×10^{-10}	2.9×10^{-10}	16
complement component 5 (C5)	eculizumab	188305	44	148000	46	3012	2367	57580	2	2	1688	6.9×10^{-10}	4.3×10^{-9}	17
C5	ravulizumab	188305		144937	46	3012	2318	56907	2	2	1688	2.2×10^{-8}	4.2×10^{-9}	17
SARS-CoV-2 S	angiotensin-converting enzyme 2 (ACE2)	141178	44	92463	44	2258	1479	38821	1	1	106	1.47×10^{-8}	1.5×10^{-6}	18
SARS-CoV-2 S1	ACE2	78300	57	92463		1252	1479	29459	1	1	106	1.2×10^{-7}	5.6×10^{-7}	19
SARS-CoV-2 S RBD	ACE2	51500	57	92463		824	1479	22983	1	1	106	1.2×10^{-7}	2.8×10^{-7}	19
SARS-CoV-2 RBD	ACE2	23400	52	92463		374	1479	12975	1	1	106	4.7×10^{-9}	6.8×10^{-8}	20
SARS-Cov-2-RBD-His tag	ACE2	26000	53	92463		416	1479	14101	1	1	106	4.42×10^{-8}	8.2×10^{-8}	20
SARS-Cov-RBD-His tag	ACE2	26500	54	92463		424	1479	14311	1	1	106	1.85×10^{-7}	8.5×10^{-8}	20
SARS-CoV-2 RBD	humanized sdAbs 1E2	23400	55	15000	21	374	240	6351	1	1	106	3.552×10^{-8}	1.4×10^{-8}	21
PIP HOE 140, a radioligand	bradykinin B2 receptor	1381	45	44461	44	28	711	824	1	1	106	1.5×10^{-11}	2.1×10^{-10}	22
WAY-100635, an antagonist	5-HT(1A) receptor	423	45	46107	44	7	737	345	1	1	106	8.7×10^{-11}	3.7×10^{-11}	23
[^{3}H]CP96345, a tritium-labeled antagonist	NK1 receptor	413	45	46251	44	7	740	337	1	1	106	1.2×10^{-10}	3.6×10^{-11}	24
Olmesartan, an antagonist	Human angiotensin II AT1 receptor	559	45	41061	44	11	657	400	1	1	106	9.1×10^{-11}	5.0×10^{-11}	25
Telmisartan, an antagonist	Human angiotensin II AT1 receptor	515	45	41061		7	657	419	1	1	106	1.2×10^{-10}	5.5×10^{-11}	25
insulin	insulin receptor (IR)	5808	45	156333	44	93	2500	3891	1	1	106	6.41×10^{-9}	5.1×10^{-9}	26
Insulin-like growth factor 1 (IGF-1)	insulin-like growth factor 1 receptor (IGF-1R)	21841	45	154793	44	349	2476	13299	1	1	106	5×10^{-10}	7.2×10^{-8}	27
Insulin-like growth factor 2 (IGF-2)	IGF-1R	20140	44	154793		322	2476	12383	1	1	106	2.3×10^{-9}	6.1×10^{-8}	28
insulin	insulin receptor isoform A (IR-A)	5808		125000	58	93	1999	3856	1	1	106	3.2×10^{-10}	5.0×10^{-9}	28
IGF-1	IR-A	21841		125000		349	1999	12919	1	1	106	2.37×10^{-8}	6.7×10^{-8}	28
IGF-2	IR-A	20140		125000		322	1999	12052	1	1	106	2.9×10^{-9}	5.7×10^{-8}	28

[a] molecular weight of molecule A, [b,d] reference for molecular weight of molecule A(B) [c] molecular weight of molecule B, [e] number of rotatable bonds of molecule A, [f] number of rotatable bonds of molecule B, [g] reduced mass adjusted with NORB (R_A, R_B), [h] number of bonding sites or number of ligands (molecule A), [i] number of bonding sites or number of ligands (molecule B), [h,i] In the case of antigen/monoclonal antibody interaction, n_A and n_B were both set to 2 unless otherwise stated in the literature, [j] $n = c(n_A n_B)^2$, $c = 105.5$, [k] If there was a range of literature values, the value with the higher affinity (smaller K_D value) was listed. [l] reference for K_D.

Table 1. continued

A	B	M_A[a]	ref.[b]	M_B[c]	ref.[d]	R_A[e]	R_B[f]	M'_{AB}[g]	n_A[h]	n_B[i]	n[j]	K_D (M) literature[k]	K_D (M) predicted	ref.[l]
insulin	insulin receptor isoform B (IR-B)	5808		90000	58	93	1439	3791	1	1	106	5×10^{-10}	4.8×10^{-9}	28
IGF-1	IR-B	21841		90000		349	1439	12212	1	1	106	2.24×10^{-7}	5.9×10^{-8}	28
IGF-2	IR-B	20140		90000		322	1439	11435	1	1	106	3.55×10^{-8}	5.1×10^{-8}	28
IGF-1	Insulin-like growth factor-binding protein 1 (IGFBP-1)	21841		27904	44	349	446	8513	1	1	106	1×10^{-10}	2.7×10^{-8}	29
IGF-1	insulin-like growth factor-binding protein 3 (IGFBP-3)	21841		31674	44	349	507	8982	1	1	106	6×10^{-10}	3.0×10^{-8}	30
calcitonin (chicken), a peptide hormone	chicken calcitonin receptor	3372	45	32423	59	54	519	2122	1	1	106	5×10^{-10}	1.5×10^{-9}	31
calcitonin	calcitonin receptors in rat osteoclasts	15103	44	60292	44	242	964	8392	1	1	106	1.6×10^{-10}	2.6×10^{-8}	32
calcitriol, the active form of vitamin D	endoplasmic reticulum resident protein 57 (ERp57)	417	45	56782	44	5	908	362	1	1	106	1×10^{-9}	4.1×10^{-11}	33
calcitriol	vitamin D receptor (VDR)	417		48289	44	5	772	361	1	1	106	1.2×10^{-9}	4.1×10^{-11}	34
calcitriol	caveolae-enriched membrane fraction (CMF)	417		23000	60	5	368	357	1	1	106	1×10^{-9}	4.0×10^{-11}	34
calcitriol	plasma vitamin D binding protein (DBP)	417		58000	61	5	928	362	1	1	106	6×10^{-8}	4.1×10^{-11}	34
AMG580, a PET tracer	phosphodiesterase 10A (PED10A)	473	35	88412	44	6	1414	400	1	1	106	7.19×10^{-11}	5.0×10^{-11}	35
propranolol, a beta blocker	β-1-adrenergic receptor	259	45	51323	44	7	821	213	1	1	106	3.3×10^{-9}	1.4×10^{-11}	36
propranolol	β-2-adrenergic receptor	259	45	46459	44	6	743	219	1	1	106	6.0×10^{-10}	1.5×10^{-11}	36
[^{3}H]-dihydroalprenolol (DHA), a trithium-labeled blocker	β-2-adrenergic receptor	251	45	46459		8	743	200	1	1	106	8.35×10^{-11}	1.2×10^{-11}	37
[^{125}I]-iodocyanopindolol (CYP), a radiolabeled antagonist	β-2-adrenergic receptor	399	45	46459		6	743	336	1	1	106	4.1×10^{-12}	3.5×10^{-11}	37
triiodothyronine (T3), a thyroid hormone	thyroid hormone receptor α1 (THRα1)	651	45	54816	44	5	877	562	1	1	106	6×10^{-11}	9.9×10^{-11}	38
thyroxine (T4), a thyroid hormone	THRα1	777	45	54816		5	877	669	1	1	106	2×10^{-9}	1.4×10^{-10}	38
T4	thyroxinebinding globulin (TBG)	777		46325	44	5	741	667	1	1	106	1×10^{-10}	1.4×10^{-10}	39
T3	transthyretin(TTR)	651		15887	44	5	254	542	1	1	106	5.326×10^{-8}	9.2×10^{-11}	40
T4	TTR	777		15887		5	254	642	1	1	106	1.973×10^{-8}	1.3×10^{-10}	40
biotin, vitamin B7	avidin, a biotin-binding protein	244	45	54700	62	5	875	213	4	4	27008	1×10^{-15}	2.1×10^{-16}	41
thiobiotin	avidin	260	45	54700		5	875	227	4	4	27008	1×10^{-13}	2.4×10^{-16}	41
iminobiotin	avidin	243	45	54700		5	875	212	1	1	106	3.5×10^{-11}	1.4×10^{-11}	41
biotin	streptavidin	244		53361	63	5	853	213	4	4	27008	4×10^{-14}	2.1×10^{-16}	42

[a] molecular weight of molecule A, [b,d] reference for molecular weight of molecule A(B) [c] molecular weight of molecule B, [e] number of rotatable bonds of molecule A, [f] number of rotatable bonds of molecule B, [g] reduced mass adjusted with NORB (R_A, R_B), [h] number of bonding sites or number of ligands (molecule A), [i] number of bonding sites or number of ligands (molecule B), [h,i] In the case of antigen/monoclonal antibody interaction, n_A and n_B were both set to 2 unless otherwise stated in the literature, [j] $n = c(n_A n_B)^2$, $c = 105.5$, [k] If there was a range of literature values, the value with the higher affinity (smaller K_D value) was listed. [l] reference for K_D.

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