Review

Revisiting the Hansch–Fujita approach and development of a fundamental QSAR

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This review paper is based on the lecture presented in Kyoto on August 25th, 2012, by one of the authors (HC) in the Memorial Symposium for the 50th Anniversary of the Hansch–Fujita Approach; however, several parts have been added. The current paper contains (I) a formulation of an extended one of the well-known Hansch–Fujita QSAR analysis, but a new promising one (called linear expression by representative energy terms (LERE)-QSAR), which can consider ligand–protein interactions explicitly by using modern molecular calculations on a whole ligand–protein complex, and (II) applications of the LERE-QSAR analysis to several cases. © Pesticide Science Society of Japan

Keywords: *ab initio* molecular orbital calculation, Hansch–Fujita analysis, ligand–protein interaction, linear expression by representative energy terms (LERE), quantitative structure–activity relationship (QSAR), solvation energy calculation.

Introduction

The advent of QSAR began with the encounter of Professors Corwin Hansch and Toshio Fujita in June 1961, as described by themselves.^{1,2)} More than half a century has passed since their discovery of a general approach to the formulation of QSAR. Since its conception, this approach has provided a new perspective for chemical–biological interactions as well as a number of successes in drug discovery. Now it is time to develop a new and promising approach based on their QSAR with the aid of modern, powerful molecular calculations on a whole ligand–protein complex structure while staying true to the original ideas of Hansch and Fujita as much as possible. Their seminal ideas are succinctly and clearly expressed in their early publications.^{3–5)} With respect to their key concepts, which focused on (1) linear free-energy principle (LFEP)⁶⁾ and additivity/decomposition of free-energy change and (2) LFEP parameters represented by Hammett σ , we now will discuss two topics relating to (1) and (2) by comparing results and implications obtained using the original and our QSAR approaches.

From Hansch–Fujita Type of QSAR to a Novel One Using Molecular Calculations on Ligand–Protein Complexes

1. Hansch–Fujita type of QSAR

A few remarks concerning the Hansch–Fujita equation may be

appropriate. A general form of the Hansch–Fujita equation is expressed by Eq. (1):

$$\log(1/C) = a\sigma + b\log P + \text{const} \quad (1)$$

where C is a concentration having a standard response in a standard time. σ and $\log P$ are the Hammett constant and partition coefficient (to be more precise, logarithm of the partition coefficient P between 1-octanol and water), respectively. When C can be assumed to correspond to the equilibrium constant, $\log(1/C)$ is proportional to the free-energy change ($\log(1/C) = -\Delta G/(2.303RT)$) of the overall process from administration of a drug to interaction with a target receptor. Hence, Eq. (1) states that the overall free-energy change for a congeneric series of ligands is expressible as the sum of representative energy terms, each of which follows LFEP.

2. Reassessment of parameters used in traditional QSAR, using molecular calculations

2.1. Hammett σ

The Hammett σ constant has long been known as one of the most important LFEP parameters that correlate with biological activity. It is a conventionally used electronic parameter in studies of enzymatic QSAR. However, it is not necessarily obvious why σ represents variations in the free-energy change associated with the complex formation between a congeneric series of ligands and their target protein. We comprehensively reevaluate experimentally derived parameter σ , confirming that it represents intermolecular interaction energy terms, by applying a molecular orbital (MO) calculation (Hartree–Fock (HF)/6-31G(d,p)) to a simple ligand–protein complex model, complexes of a series of *para* and *meta* mono-substituted acetophenones

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with *N'*-methylacetamide.⁷⁾ Figure 1 shows that the total binding energy ($\Delta E_{\text{bind}}^{\text{HF}}$) is nicely linear with σ . Variations of energy components obtained with the energy decomposition analysis developed by Kitaura and Morokuma⁸⁾ are also plotted in Fig. 1, revealing that all the energy components are linear with σ and that the electrostatic component (*ES*) governs $\Delta E_{\text{bind}}^{\text{HF}}$. Similarly, the hydration (ΔG_{sol}) and dispersion (E_{disp}) energy changes accompanying the complex formation are nicely linear with σ ($r=0.967$, $\text{slope}=-0.855$ (conductor-like polarizable continuum model (CPCM)⁹⁾/HF/6-31G(d,p)) and $r=0.973$, $\text{slope}=1.19$ (MP2¹⁰⁾/6-31G(d,p)), respectively). It is noteworthy that ΔG_{sol} shows a nearly perfect correlation with $\Delta E_{\text{bind}}^{\text{HF}}$ ($r=0.995$, $\text{slope}=-0.693$). These results suggest that the intermolecular energy terms obtained through MO calculations for full complex structures of a congeneric series of ligands with a protein basically follow LFEP, as represented by Hammett σ , and can bridge the gap between the traditional Hansch–Fujita and modern QSAR approaches.

2.2 Partition coefficient log *P*

The partition coefficient log P_{sol} ($P_{\text{sol}}=C_{\text{sol}}/C_{\text{water}}$) can be expressed with the Gibbs free-energy, enthalpy, and entropy differences.

$$\begin{aligned}\log P_{\text{sol}} &= -\Delta G_{\text{sol}} / (2.303RT) \\ &= -\Delta H_{\text{sol}} / (2.303RT) + \Delta S_{\text{sol}} / (2.303R)\end{aligned}\quad (2)$$

Starting from the basic relation Eq. (2), we formulated a semiempirical correlation Eq. (3) for log P_{sol} (sol=chloroform

(CL) and 1-octanol (oct))^{11–13)}:

$$\log P_{\text{sol}} = a\Delta E_{\text{sol}} + b\text{ASA} + cI_{\text{HAc}} + dI_{\text{sol}} + \text{const} \quad (3)$$

where ΔE_{sol} (calculated with CPCM/HF/6-31+G(d)//HF/3-21G*) is the solvation energy difference between aqueous and organic solvent phases, and ASA is the accessible surface area of solute molecules. I_{HAc} and I_{sol} are indicator variables. I_{HAc} takes unity for hydrogen-bonding acceptors but otherwise zero, and I_{sol} takes unity for log P_{CL} and zero for log P_{oct} . Although I_{HAc} and I_{sol} were introduced empirically, physicochemical meanings of these indicator variables were discussed adequately in the original paper.¹²⁾ Equation (3) can give an excellent prediction of log P_{sol} for non-hydrogen bonders and hydrogen-bonding acceptors ($r=0.972$, $s=0.283$ for training compounds ($n=208$) and $r=0.973$, $s=0.296$ for external test ones ($n=51$)). When deriving Eq. (3) from Eq. (2), we assumed that (1) ΔE_{sol} on the right-hand side of Eq. (3) corresponds to ΔH_{sol} in Eq. (2) (note that $\Delta H \approx \Delta E$ in solution) and that (2) ASA, I_{HAc} , and I_{sol} effectively express ΔS_{sol} . In fact, the coefficient of ΔE_{sol} in Eq. (3) takes a value ($a=-0.776$) close to the expected one ($-1/(2.303RT)=-0.734$ at 298 K). However, for hydrogen-bonding donors, that is approximately half of the expected value (~ 0.34). This result probably suggests the deficiency in continuum solvation models (*i.e.*, assumption of uniform distribution) used in PCM,⁹⁾ generalized Born (GB),¹⁴⁾ and Poisson–Boltzmann (PB)¹⁴⁾ types of calculations. Figure 2 shows observed and calculated log P_{CL} values (chloroform/water) of simple molecules, together with calculated ones with reference interaction site model (RISM),^{15,16)} which explicitly considers the (non-uniform) distribution of solvent molecules. The standard error of log P_{CL} calculated with RISM (0.670) is, however, considerably larger than that with CPCM (0.296).

At present, it is difficult to reproduce log P_{sol} within chemical accuracy (~ 0.5 kcal/mol) only by means of theoretical calculations such as the *ab initio* self-consistent reaction field (SCRf) type of MOs and RISM. It should be very important to make the status of the current solvation models higher in predicting the solvation energy by extending and elaborating the current models.

3. Formulation of the LERE-QSAR equation

We previously proposed a novel QSAR procedure called linear expression by representative energy terms (LERE)-QSAR^{17–21)} involving molecular calculations such as an *ab initio* fragment molecular orbital (FMO) one.²²⁾ The idea of LERE-QSAR follows that of the Hansch–Fujita type of QSAR. The first assumption made in formulating the LERE-QSAR equation is that the free-energy terms comprising the overall free-energy change associated with complex formation are all additive.

$$\Delta G_{\text{obs}} = \Delta G_{\text{bind}} + \Delta G_{\text{sol}} + \Delta G_{\text{others}} \quad (4)$$

ΔG_{obs} on the left-hand side is the overall free-energy change calculated from the observed inhibitory potency ($\Delta G_{\text{obs}}=RT\ln C$, where C is the same in Eq. (1)). ΔG_{bind} and ΔG_{sol} (typically taken

Fig. 1. Plot of Hammett σ and energy components in complex formation of *N'*-methylacetamide with 41 *para* and *meta* mono-substituted acetophenones (shown in the graph). $\Delta E_{\text{bind}}^{\text{HF}}$ represents the total binding energy. $\Delta E_{\text{bind}}^{\text{HF}}$ is further decomposed⁸⁾ into *ES* (electrostatic), *EX* (exchange), *CT* (charge transfer), *PL* (polarization), and *MIX* (others) components ($\Delta E_{\text{bind}}^{\text{HF}}=\text{ES}+\text{EX}+\text{CT}+\text{PL}+\text{MIX}$).

Fig. 2. Comparison of observed and calculated $\log P_{CL}$ values (chloroform/water). Black, gray and dotted bars represent observed $\log P_{CL}$ and calculated ones with CPCM¹²⁾ and RISM,^{15,16)} respectively.

Fig. 3. Entropy-enthalpy compensation. (a) Human carbonic anhydrase II with a series of substituted benzenesulfonamides²⁴⁾ and (b) concanavalin A, pea lectin, and lentil lectin with monosaccharides.²⁵⁾

as dominant free-energy terms) are the intrinsic binding interaction free-energy of an inhibitor with a protein and the solvation free-energy change associated with complex formation, respectively. ΔG_{others} , the sum of free-energy terms other than representative free-energy terms ΔG_{bind} and ΔG_{sol} , is assumed to be linear with that of representative free-energy terms (LERE approximation). ΔG_{others} consists of, for instance, the structural deformation (adaptation/induced fitting) energy associated with complex formation

$$\Delta G_{\text{others}} = \beta(\Delta G_{\text{bind}} + \Delta G_{\text{sol}}) + \text{const}_1 \quad (5)$$

where ΔG_{others} is expected to work as a penalty term ($\beta < 0$ and/or $\text{const}_1 > 0$). The last assumption is the entropy-enthalpy compensation,²³⁾ which is expected to effectively hold for the binding of a series of ligands with a protein:

$$T\Delta S = \alpha\Delta H + \text{const}_2 \quad (6)$$

where $\alpha > 0$.

ΔG_{sol} is replaced by the dominant polar (electrostatic)

contribution $\Delta G_{\text{sol}}^{\text{pol}}$ because the non-polar contribution is often negligible, and most of $\Delta G_{\text{sol}}^{\text{pol}}$ is considered to come from the enthalpic contribution.¹⁹⁾ Combining the above three equations yields the following expression (LERE-QSAR equation):

$$\Delta G_{\text{obs}} = \gamma(\Delta E_{\text{bind}} + \Delta G_{\text{sol}}^{\text{pol}}) + \text{const}_3 \quad (7)$$

The coefficient γ on the right-hand side of the above equation is a constant determined by α and β ($\gamma = (1 - \alpha)(1 + \beta)$). Representative energy terms other than ΔE_{bind} and $\Delta G_{\text{sol}}^{\text{pol}}$, such as the dissociation of a ligand (ΔG_{diss}) and deformation free-energy changes, are added to Eq. (7) if needed. ΔE_{bind} is computable using *ab initio* MO calculations such as FMO²²⁾ and ONIOM,²⁶⁾ and $\Delta G_{\text{sol}}^{\text{pol}}$ is computable with continuum solvation models such as GB,¹⁴⁾ PB,¹⁴⁾ PCM,⁹⁾ and RISM.²⁷⁾ Table 1 concisely lists an estimation of representative energy terms and the entropic energy used in the LERE procedure. A general procedure for the LERE-QSAR analysis consists of two steps: (1) determine a complex structure, usually by carrying out classical molecular mechanics and molecular dynamics calculations repeatedly, and (2) calculate

Table 1. Estimation of representative energy terms and the entropic energy

Energy	Symbol	Method (program)	Ref.
Binding energy	ΔE_{bind}	Molecular Mechanics (AMBER)	28
		Fraction with Caps (MFCC)	29
		FMO (ABINIT-MP and GAMESS)	22
		QM/MM (ONIOM)	26
Solvation energy	ΔG_{sol}	GB/PB (AMBER)	14
		PCM (Gaussian and GAMESS)	9
		Hybrid (PCM/PB(GB))	30
		RISM	27
Entropic energy	$-T\Delta S$	Normal mode calculation	31, 32
		Quasi-harmonic approximation	33
		Entropy–enthalpy compensation	23

ΔX ($X = E_{\text{bind}}$ and $G_{\text{sol}}^{\text{pol}}$) as $\Delta X = X_{\text{complex}} - X_{\text{protein}} - X_{\text{ligand}}$ (super-molecular approximation).

Although several free-energy calculation methods³⁴⁾ have been proposed such as the thermodynamics integration, free-energy perturbation, Bennett's acceptance ratio, linear interaction energy (LIE),³⁵⁾ and solvated interaction energy (SIE),³⁶⁾ the purpose of our research is to develop an LFEP-based QSAR that can predict the overall binding free-energy change within chemical accuracy, which is unattainable by approaches other than LERE-QSAR, at least for now.

Case Studies of LERE-QSAR Applications

1. Comparative QSAR analysis of zinc containing enzyme inhibitors^{18,20,21)}

Substituted benzenesulfonamides (BSAs) and biphenylsulfonamides (BSs), shown in Figs. 4(a) and (b), inhibit bovine carbonic anhydrase II (CA) and matrix metalloproteinase-9 (MMP), respectively.

During their complex formation, the anionized sulfonamide (SO_2NH^-) in BSAs and carboxylate (CO_2^-) in BSs coordinate to the zinc atom (Zn^{2+}) in the active sites of CA and MMP, respectively. Hansch³⁷⁾ and Verma³⁸⁾ reported QSAR Eqs. (8a) and (8b) for inhibition of CA by BSAs and that of MMP by BSs, respectively:

$$\log(1/K_i) = 0.946\sigma + 0.177\pi + 5.52 \quad (8a)$$

$$n = 16, \quad r = 0.956, \quad s = 0.193$$

$$\log(1/IC_{50}) = -1.14\sigma + 1.24MR + 4.38 \quad (8b)$$

$$n = 9, \quad r = 0.944, \quad s = 0.195$$

where π in Eq. (8a) is a hydrophobic constant of a substituent (X in Fig. 4(a)), which is defined as $\log P$ (substituted benzene) – $\log P$ (benzene). MR in Eq. (8b) represents the dispersion interaction of a substituent (X in Fig. 4(b)). It should be noted that signs of coefficient of σ are opposite in Eqs. (8a) and (8b); thus, we focused on the reasons the opposite direction of substituent electronic effect is observed in complex formations CA–BSA and MMP–BS. We performed the LERE-QSAR

Fig. 4. Structure of (a) BSAs (*para*- and *meta*-substituted benzenesulfonamides) and (b) BSs (*para*-substituted biphenylsulfonamides).

analysis using the three representative energy terms: ΔE_{bind} (ONIOM (HF-D³⁹⁾/6-31G:Amber), electronic embedding scheme), $\Delta G_{\text{sol}}^{\text{pol}}$ (GB), and ΔG_{diss} (integral equation formalism PCM (IEFPCM)⁴⁰⁾/B3LYP⁴¹⁾/6-31+G(d,p) and IEFPCM/HF/6-31+G(d) for CA–BSAs^{18,20)} and MMP–BSs,²¹⁾ respectively) for complexes CA–BSA and MMP–BS and obtained Eqs. (9a) and (9b) with s (standard deviation) < 0.3 kcal/mol:

$$\Delta G_{\text{obs}} = \gamma(\Delta E_{\text{bind}} + \Delta G_{\text{sol}}^{\text{pol}}) + 0.337\Delta G_{\text{diss}} + 2.14 \quad (9a)$$

$$n = 16, \quad r = 0.952, \quad s = 0.268 \text{ kcal/mol}, \quad \gamma = 0.205$$

$$\Delta G_{\text{obs}} = \gamma(\Delta E_{\text{bind}} + \Delta G_{\text{sol}}^{\text{pol}}) - 1.56\Delta G_{\text{diss}} + 29.5 \quad (9b)$$

$$n = 9, \quad r = 0.957, \quad s = 0.234 \text{ kcal/mol}, \quad \gamma = 0.231$$

It is noted that the coefficients (γ) in Eqs. (9a) and (9b) are nearly the same value. Figure 5(a) shows that the relative contribution from $\Delta G_{\text{sol}}^{\text{pol}}$ and ΔG_{diss} overwhelms that from ΔE_{bind} and that the former governs the overall free-energy change ΔG_{obs} . Conversely, Fig. 5(b) shows that the latter governs ΔG_{obs} . There is an anticorrelation between ΔE_{bind} and $\Delta G_{\text{sol}}^{\text{pol}}$ in Eqs. (9a) and (9b) ($r = -0.922$ and -0.862 , respectively (the negative sign of r indicates a negative slope hereafter)), as frequently observed. As demonstrated in the previous section “Hammett σ ,” ΔE_{bind} and $\Delta G_{\text{sol}}^{\text{pol}}$ are expected to show positive and negative correlations with Hammett σ , respectively.

As can be seen in Figs. 6(a) and (b), variations of $\Delta G_{\text{sol}}^{\text{pol}}$ in CA–BSAs and ΔE_{bind} in MMP–BSs are mostly from the zinc atom.

The opposite signs of the coefficients of σ in Eqs. (8a) and (8b) are now clearly explainable from differences in representative energy terms between complex formations CA–BSA and MMP–BS. This instructive case study demonstrates that LERE-QSAR has a considerable advantage over the traditional QSAR, although there is no logical conflict between the two.

2. Comparative LERE-QSAR analysis of inhibitory activity of sialic acid analogues on two neuraminidases^{17,19)}

Neuraminidases (NAs) are ubiquitous exoglycosylhydrolases that hydrolyze the terminal sialic acids of glycoproteins and glycolipids and are widely distributed in nature, from viruses, microorganisms, and fungi to higher animals and humans.⁴²⁾ Among them, influenza virus NAs have been studied extensively as a target for influenza disease, and anti-influenza drugs have been developed. These drugs have the potential to affect the activities of endogenous human NAs.^{43,44)} Thus, it is of great importance to examine differences in the binding mechanism between the influenza virus and human NAs with a series of sialic acid analogues. We selected influenza virus neuraminidase-1 (N1-NA)

Fig. 5. Variation of energy components. (a) CA-BSAs and (b) MMP-BSs.

Fig. 6. Variance of (a) $\Delta G_{\text{sol}}^{\text{pol}}$ of CA-BSAs and (b) ΔE_{bind} of MMP-BSs, together with interaction schemes of (a) and (b). $\Delta G_{\text{sol}}^{\text{pol}}$ and ΔE_{bind} are calculated with GB and molecular mechanics (AMBER), respectively. The variance of quantities (X_i , $i=1, n$) is defined as $\Sigma(X_i - \langle X \rangle)^2$, where $\langle X \rangle = (1/n) \Sigma X_i$.

and human neuraminidase-2 (hNEU2). Amino acid residues important for recognition of sialic acids are basically conserved in N1-NA and hNEU2.¹⁹⁾

Table 2 lists sialic acid analogues, Sets I and II compounds used in the study. We constructed complexes of N1-NA and hNEU2 with Sets I and II compounds, respectively, and then performed the LERE-QSAR analysis using the three representative energy terms: ΔE_{bind} ($=\Delta E_{\text{bind}}^{\text{HF}} + E_{\text{disp}}$, FMO/MP2/6-31G),

$\Delta G_{\text{sol}}^{\text{pol}}$ (hybrid (CPCM/PB)³⁰⁾ and PB for N1-NA-Set I and hNEU2-Set II compounds, respectively), and ΔG_{diss} (CPCM/HF/6-31+G(d,p)).

The observed overall free-energy change ΔG_{obs} for complex formation of N1-NA and hNEU2 with sialic acid analogues is excellently reproducible with the LERE-QSAR Eqs. (10a) and (10b), respectively ($s < 0.4$ kcal/mol).

$$\Delta G_{\text{obs}} = \gamma(\Delta E_{\text{bind}}^{\text{HF}} + E_{\text{disp}} + \Delta G_{\text{sol}}^{\text{pol}}) + 0.0708\Delta G_{\text{diss}} + 25.4 \quad (10a)$$

$$n=8, \quad r=0.988, \quad s=0.389 \text{ kcal/mol}, \quad \gamma=0.561$$

$$\Delta G_{\text{obs}} = \gamma(\Delta E_{\text{bind}}^{\text{HF}} + E_{\text{disp}} + \Delta G_{\text{sol}}^{\text{pol}}) + 0.0976\Delta G_{\text{diss}} - 2.25 \quad (10b)$$

$$n=7, \quad r=0.985, \quad s=0.237 \text{ kcal/mol}, \quad \gamma=0.0476$$

As can be seen in Figs. 7(a) and (b), ΔG_{diss} varies depending on the types of cationic groups in fragment C (NH_3^+ and $\text{NHC}(=\text{NH}_2)\text{NH}_2$, shown in Table 2). Figure 7(a) for N1-NA-Set I compounds shows that the electrostatic contribution from two electrostatic energy terms ($\Delta E_{\text{bind}}^{\text{HF}}$ and $\Delta G_{\text{sol}}^{\text{pol}}$) in Eq. (10a) is considerably smaller than that from the dispersion energy term (E_{disp}) because of compensation between the two electrostatic energy terms ($r = -0.950$). Consequently, E_{disp} governs ΔG_{obs} in complex formation of N1-NA with Set I compounds.¹⁷⁾ Conversely, Fig. 7(b) for hNEU2-Set II compounds shows that the contribution of E_{disp} is considerably smaller than that of the electrostatic energy contribution in Eq. (10b). Hence, $\Delta E_{\text{bind}}^{\text{HF}}$ governs ΔG_{obs} in complex formation of hNEU2 with Set II compounds.¹⁹⁾

It should be noted that remarkable differences in the coefficient (γ) and intercept (corresponds to const_3 in Eq. (7)) exist between Eqs. (10a) and (10b). The coefficient (β) and intercept (const_1) in Eq. (5) (LERE approximation) are expressed with α , γ , const_2 , and const_3 in Eqs. (6) and (7): $(\alpha + \gamma - 1)/(1 - \alpha)$ and $(\text{const}_2 + \text{const}_3)/(1 - \alpha)$, respectively. When assuming $\alpha = 0.70$,^{25,47-49)} β takes 0.87 and -0.84 , and const_3 is $85 + 3.3 \text{ const}_2$ (relative value: 85 kcal/mol) and $-7.5 + 3.3 \text{ const}_2$ (-7.5 kcal/mol) for Eqs. (10a) and (10b), respectively. Using β and const_1

Table 2. Chemical structure and inhibitory potency of Sets I and II compounds toward N1-NA and hNEU2

(a) Set I compound					N1-NA
No.	Type	R ₁ (fragment A) ^{a)}	R ₂ (fragment C) ^{a)}	IC ₅₀ ^{b)}	ΔG _{obs} ^{c)}
1	I	OH	NH ₃ ⁺	6300	−7.38
2	I	OCH ₂ CH ₂ CH ₃	NH ₃ ⁺	180	−9.57
3	I	OCH(CH ₃)(CH ₂ CH ₃)(R)	NH ₃ ⁺	10	−11.35
4 ^{d)}	I	OCH(CH ₂ CH ₃) ₂	NH ₃ ⁺	1	−12.77
5	I	OH	NHC(=NH ₂ ⁺)NH ₂	100	−9.93
6	I	OCH ₂ CH ₂ CH ₃	NHC(=NH ₂ ⁺)NH ₂	2	−12.34
7	I	OCH(CH ₃)(CH ₂ CH ₃)(R)	NHC(=NH ₂ ⁺)NH ₂	0.5	−13.19
8	I	OCH(CH ₂ CH ₃) ₂	NHC(=NH ₂ ⁺)NH ₂	0.5	−13.19
(b) Set II compound					hNEU2
No.	Type	R ₁ (fragment A) ^{a)}	R ₂ (fragment C) ^{a)}	K _i ^{e)}	ΔG _{obs} ^{f)}
1	II	OCH ₂ CH(CH ₃) ₂	OH	0.88	−4.33
2	II	OCH ₂ CH ₂ CH ₂ (OH)	OH	0.74	−4.44
3	II	OCH ₂ CH(OH)CH ₂ (OH)	OH	1.4	−4.05
4	II	CH(OH)CH(OH)CH ₂ (OH)	OH	0.14	−5.47
5 ^{g)}	II	CH(OH)CH(OH)CH ₂ (OH)	NHC(=NH ₂ ⁺)NH ₂	0.017	−6.77
6 ^{h)}	III	CH(CH ₂ CH ₃) ₂	NHC(=NH ₂ ⁺)NH ₂	0.33	−4.94
7 ^{d)}	I	OCH(CH ₂ CH ₃) ₂	NH ₃ ⁺	5	−3.26

^{a)} Fragments A and C correspond to R₁ and R₂, respectively. Fragment B denotes the rest of structure (without fragments A and C). ^{b)} Taken from Ref. 45 (In nM). ^{c)} ΔG_{obs} = RT ln IC₅₀ (T = 310 K). ^{d)} Oseltamivir (Tamiflu). ^{e)} Taken from Ref. 46 (In mM). ^{f)} ΔG_{obs} = RT ln K_i (T = 310 K). ^{g)} Zanamivir (Relenza). ^{h)} Peramivir (Rapiacta).

Fig. 7. Variation of energy components. (a) N1-NA and (b) hNEU2.

in Eq. (5) (LERE approximation/assumption), we will now discuss the effect of ΔG_{others} on the coefficient and intercept in Eqs. (10a) and (10b). The positive β (0.87) and large const₁ (85 kcal/mol) for Eq. (10a) indicate that ΔG_{others} varies in a parallel manner with the sum of representative energy terms and yields a

large positive constant in Eq. (10a) as a penalty energy. In contrast, the negative β (−0.84) and smaller const₁ (−7.5 kcal/mol) for Eq. (10b) indicate that ΔG_{others} nearly cancels out the sum of representative energy terms because of negative correlation with the sum. As expected (β < 0 and/or const₁ > 0), ΔG_{others} is

Fig. 8. (a) Set I compound 4 (oseltamivir) accommodated in the active site of N1-NA. (b) Dispersion interaction energies of fragment A in Set I compound 4 with amino acid residues in pockets A and B of N1-NA. Pockets A and B correspond to amino acid residues surrounding fragments A and B, respectively. (c) Set II compound 5 (zanamivir) accommodated in the active site of hNEU2. (d) Electrostatic interaction energies of fragment A in Set II compound 5 with amino acid residues in pockets A and B of hNEU2.

now confirmed to work as the penalty energy both in Eqs. (10a) and (10b) but behaves differently. This is probably due to differences in the primary interaction governing the overall free-energy change, E_{disp} and $\Delta E_{\text{bind}}^{\text{HF}}$ for N1-NA-Set I and hNEU2-Set II compounds, respectively. Figures 8(a), (b) and (c), (d) show that contributions of E_{disp} (N1-NA-Set I compounds) and $\Delta E_{\text{bind}}^{\text{HF}}$ (hNEU2-Set II compounds) from amino acid residues located in close proximity to fragment A (shown in Table 2) dominantly determine the overall free-energy change ΔG_{obs} , respectively.

The second case study demonstrates that the LERE-QSAR equation is significantly informative concerning variations of ligand-protein interaction energy.

Concluding Remarks

We feel some concern about QSAR models in which descriptors (explaining variables) are selected “automatically” from a large pool of candidate descriptors, as sometimes found in recent publications. We believe QSAR models should be interpretable physicochemically and informative chemically and biologically, not merely statistically acceptable. It is important to make efforts for constructing a fundamental QSAR that can accurately describe the essence of chemical-biological interactions. As

mentioned at the beginning of this paper, the encounter of the two perspicacious scientists has had a strong and lasting influence on later generations.

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References

- 1) C. Hansch: *J. Comput. Aided Mol. Des.* **25**, 495–507 (2011).
- 2) T. Fujita: *J. Comput. Aided Mol. Des.* **25**, 509–517 (2011).
- 3) C. Hansch and T. Fujita: *J. Am. Chem. Soc.* **86**, 1616–1626 (1964).
- 4) T. Fujita, J. Iwasa and C. Hansch: *J. Am. Chem. Soc.* **86**, 5175–5180 (1964).
- 5) T. Fujita and T. Ban: *J. Med. Chem.* **14**, 148–152 (1971).
- 6) L. P. Hammett: “Quantitative Relationships Involving Structure and Reactivity,” in *Physical Organic Chemistry* (Chapter 11), Second ed., McGraw-Hill Kogakusha Ltd., Tokyo, pp. 347–390, 1970.
- 7) T. Yoshida, M. Shimizu, M. Harada, S. Hitaoka and H. Chuman:

- Bioorg. Med. Chem. Lett.* **22**, 124–128 (2012).
- 8) K. Kitaoura and K. Morokuma: *Int. J. Quantum Chem.* **10**, 325–340 (1976).
- 9) A. Klamt: *J. Phys. Chem.* **99**, 2224–2235 (1995).
- 10) M. Head-Gordon, J. A. Pople and M. J. Frisch: *Chem. Phys. Lett.* **153**, 503–506 (1988).
- 11) H. Chuman, A. Mori and H. Tanaka: *Anal. Sci.* **18**, 1015–1020 (2002).
- 12) H. Chuman, A. Mori, H. Tanaka, C. Yamagami and T. Fujita: *J. Pharm. Sci.* **93**, 2681–2697 (2004).
- 13) H. Chuman: *SAR QSAR Environ. Res.* **19**, 71–79 (2008).
- 14) P. A. Kollman, I. Massova, C. Reyes, B. Kuhn, S. Huo, L. Chong, M. Lee, T. Lee, Y. Duan, W. Wang, O. Donini, P. Cieplak, J. Srinivasan, D. A. Case and T. E. Cheatham 3rd: *Acc. Chem. Res.* **33**, 889–897 (2000).
- 15) K. Sato, H. Chuman and S. Ten-no: *J. Phys. Chem. B* **109**, 17290–17295 (2005).
- 16) S. Ten-no, J. Jung, H. Chuman and Y. Kawashima: *Mol. Phys.* **108**, 327–332 (2010).
- 17) S. Hitaoka, M. Harada, T. Yoshida and H. Chuman: *J. Chem. Inf. Model.* **50**, 1796–1805 (2010).
- 18) T. Yoshida, Y. Munei, S. Hitaoka and H. Chuman: *J. Chem. Inf. Model.* **50**, 850–860 (2010).
- 19) S. Hitaoka, H. Matoba, M. Harada, T. Yoshida, D. Tsuji, T. Hirokawa, K. Itoh and H. Chuman: *J. Chem. Inf. Model.* **51**, 2706–2716 (2011).
- 20) Y. Munei, K. Shimamoto, M. Harada, T. Yoshida and H. Chuman: *Bioorg. Med. Chem. Lett.* **21**, 141–144 (2011).
- 21) T. Yoshida, S. Hitaoka, A. Mashima, T. Sugimoto, H. Matoba and H. Chuman: *J. Phys. Chem. B* **116**, 10283–10289 (2012).
- 22) K. Kitaoura, E. Ikeo, T. Asada, T. Nakano and M. Uebayasi: *Chem. Phys. Lett.* **313**, 701–706 (1999).
- 23) E. Freire: *Chem. Biol. Drug Des.* **74**, 468–472 (2009).
- 24) A. D. Scott, C. Phillips, A. Alex, M. Flocco, A. Bent, A. Randall, R. O'Brien, L. Damian and L. H. Jones: *ChemMedChem* **4**, 1985–1989 (2009).
- 25) F. P. Schwarz, K. D. Puri, R. G. Bhat and A. Surolia: *J. Biol. Chem.* **268**, 7668–7677 (1993).
- 26) F. Maseras and K. Morokuma: *J. Comput. Chem.* **16**, 1170–1179 (1995).
- 27) D. Chandler and H. C. Andersen: *J. Chem. Phys.* **57**, 1930–1937 (1972).
- 28) S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta and P. Weiner: *J. Am. Chem. Soc.* **106**, 765–784 (1984).
- 29) D. W. Zhang, X. H. Chen and J. Z. H. Zhang: *J. Comput. Chem.* **24**, 1846–1852 (2003).
- 30) S. Hitaoka, H. Matoba, A. Kawano, Y. Shibata, M. Harada, S. Sakamoto, T. Yoshida and H. Chuman: *Abstr. 40th Symposium on Structure-Activity Relationships*, pp. 86–89, 2012 (in Japanese).
- 31) N. Go, T. Noguti and T. Nishikawa: *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3696–3700 (1983).
- 32) B. Brooks and M. Karplus: *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6571–6575 (1983).
- 33) J. Schlitter: *Chem. Phys. Lett.* **215**, 617–621 (1993).
- 34) J. Wereszczynski and J. A. McCammon: *Q. Rev. Biophys.* **45**, 1–25 (2012).
- 35) J. Åqvist C. Medina and J. E. Samuelsson: *Protein Eng.* **7**, 385–391 (1994).
- 36) M. Naim, S. Bhat, K. N. Rankin, S. Dennis, S. F. Chowdhury, I. Siddiqi, P. Drabik, T. Sulea, C. I. Bayly, A. Jakalian and E. O. Purisima: *J. Chem. Inf. Model.* **47**, 122–133 (2007).
- 37) C. Hansch: *Farmaco* **58**, 625–629 (2003).
- 38) R. P. Verma and C. Hansch: *Bioorg. Med. Chem.* **15**, 2223–2268 (2007).
- 39) S. J. Grimme: *J. Comput. Chem.* **27**, 1787–1799 (2006).
- 40) E. Cancès, B. Mennucci and J. Tomasi: *J. Chem. Phys.* **107**, 3032–3041 (1997).
- 41) A. D. Becke: *J. Chem. Phys.* **98**, 5648–5652 (1993).
- 42) K. E. Achyuthan and A. M. Achyuthan: *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **129**, 29–64 (2001).
- 43) K. Hata, K. Koseki, K. Yamaguchi, S. Moriya, Y. Suzuki, S. Yingsakmongkon, G. Hirai, M. Sodeoka, M. von Itzstein and T. Miyagi: *Anti-microb. Agents Chemother.* **52**, 3484–3491 (2008).
- 44) C.-Y. Li, Q. Yu, Z.-Q. Ye, Y. Sun, Q. He, X.-M. Li, W. Zhang, J. Luo, X. Gu, X. Zheng and L. Wei: *Cell Res.* **17**, 357–362 (2007).
- 45) C. U. Kim, W. Lew, M. A. Williams, H. Wu, L. Zhang, X. Chen, P. A. Escarpe, D. B. Mendel, W. G. Laver and R. C. Stevens: *J. Med. Chem.* **41**, 2451–2460 (1998).
- 46) L. M. G. Chavas, R. Kato, N. Suzuki, M. von Itzstein, M. C. Mann, R. J. Thomson, J. C. Dyason, J. McKimm-Breschkin, P. Fusi, C. Tringali, B. Venerando, G. Tettamanti, E. Monti and S. Wakatsuki: *J. Med. Chem.* **53**, 2998–3002 (2010).
- 47) D. Gupta, M. Cho, R. D. Cummings and C. F. Brewer: *Biochemistry* **35**, 15236–15243 (1996).
- 48) C. P. Swaminathan, N. Surolia and A. Surolia: *J. Am. Chem. Soc.* **120**, 5153–5159 (1998).
- 49) T. M. Gloster, P. Meloncelli, R. V. Stick, D. Zechel, A. Vasella and G. J. Davies: *J. Am. Chem. Soc.* **129**, 2345–2354 (2007).