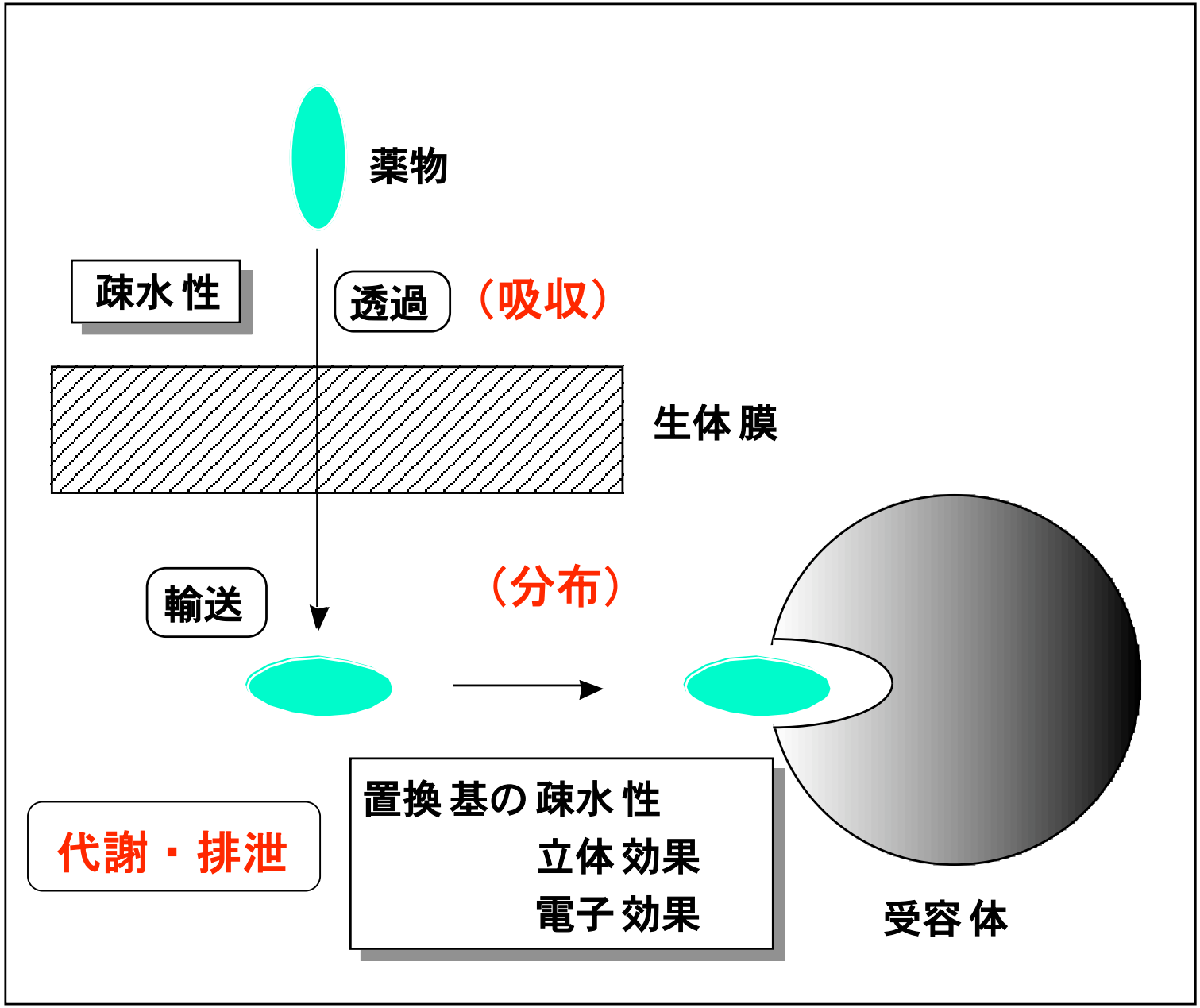
薬学講義 2007年1月16日

定量的構造活性相関

Quantitative Structure-Activity Relationships : QSAR

京都大学大学院
農学研究科
赤松美紀

薬物の輸送・透過・受容体との相互作用



ADME/Tox

医農薬の動態

Absorption	吸収
Distribution	分布
Metabolism	代謝
Excretion	排泄
Toxicity	毒性

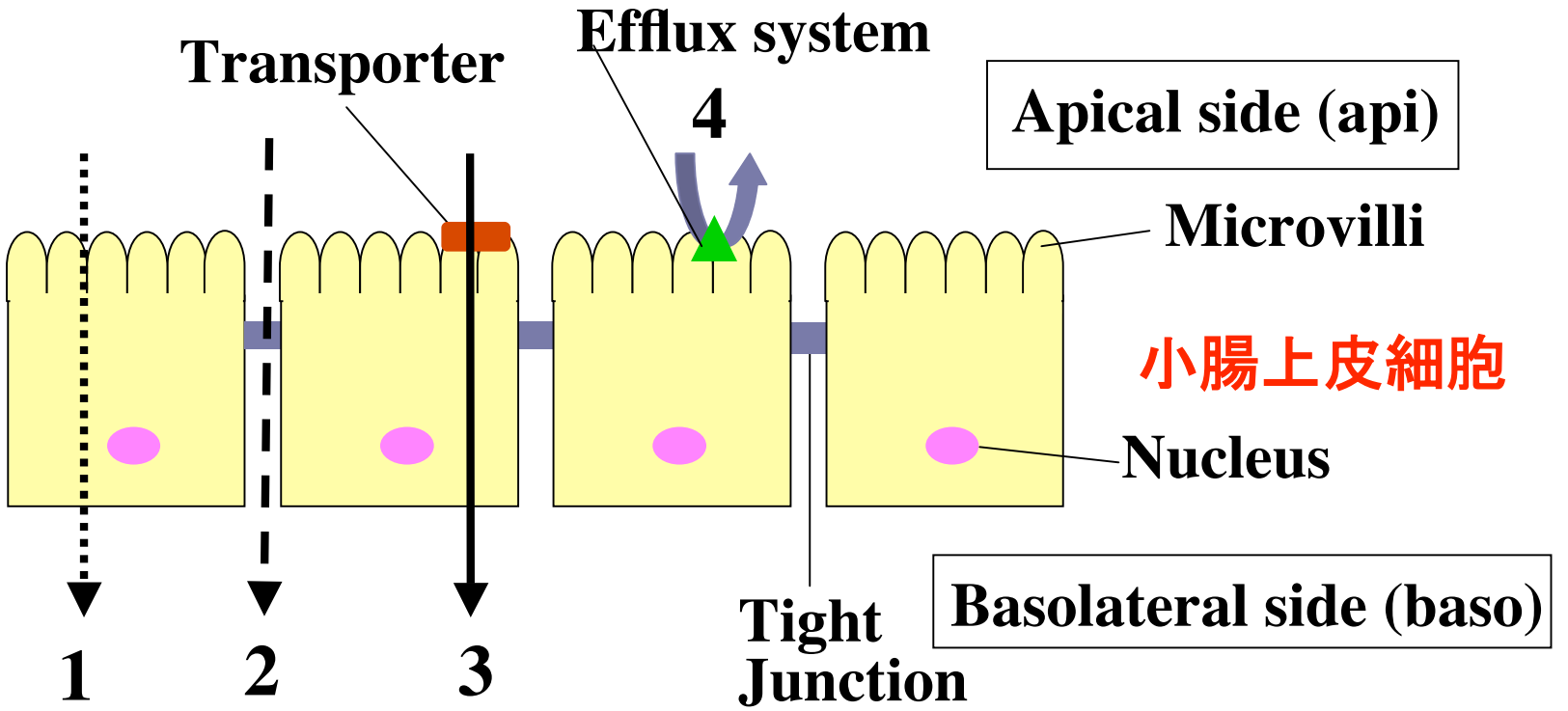
ADME/Tox

医農薬の動態

Absorption	吸収
Distribution	分布
Metabolism	代謝
Excretion	排泄
Toxicity	毒性

医農薬の構造と細胞透過性との関係

小腸における吸収

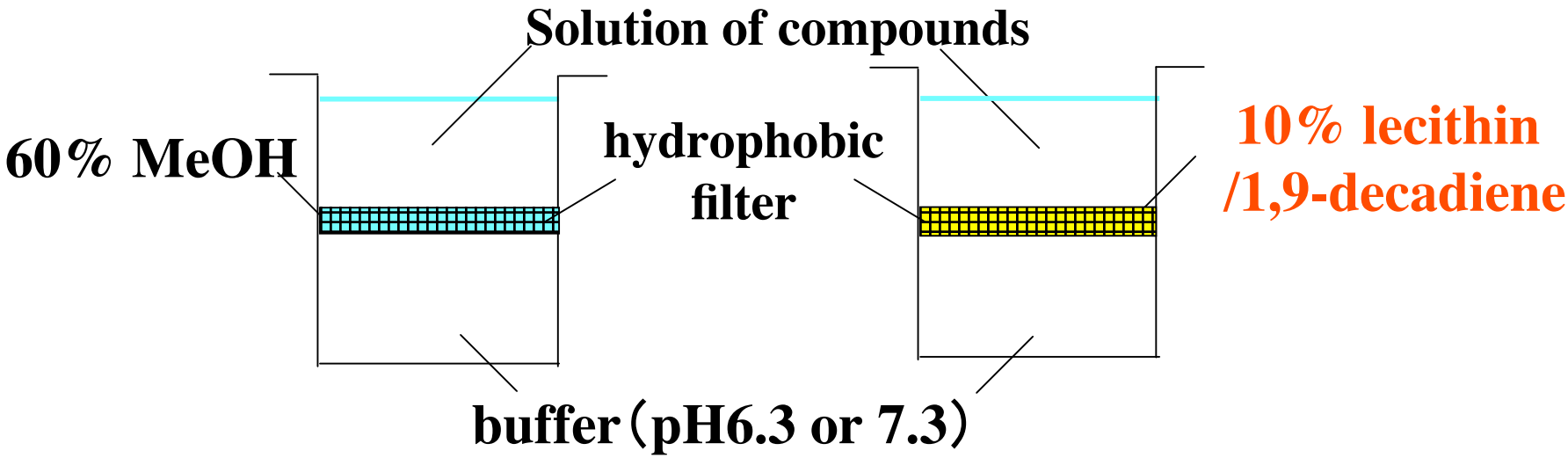


1. Passive transcellular route
2. Passive paracellular route
3. Active carrier-mediated route
4. Efflux systems (P-glycoprotein etc.)

→ 人工脂質膜透過性 (PAMPA)

Caco-2 細胞透過性

Method of PAMPA



PAMPA: Parallel Artificial Membrane Permeation Assay

Descriptors for Classical QSAR Analyses

$\log P_{oct}$:

1-オクタノール/水系における分配係数の対数

$|pK_a - pH|$:

化合物のpK_a と実測した pH 7.3の差の絶対値

非解離型/解離型の存在比に関係

SA_{HA}, SA_{HD}:

水素結合受容性基および供与性基のvan der Waals 表面積($Å^2$) x 1/100

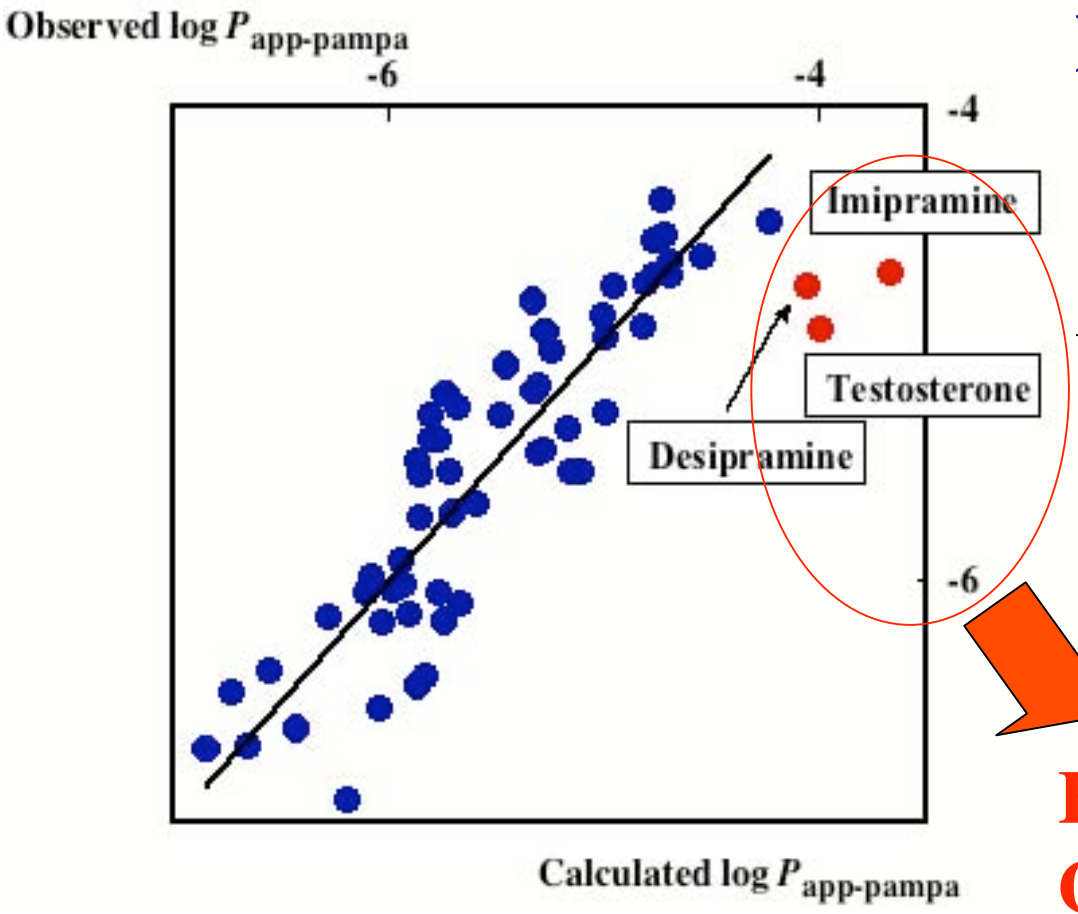
Test Compounds

Peptide related compounds: 22 compounds
Commercial drugs :38 compounds (shown as below)

Compound	log P_{oct}	Compound	log P_{oct}	Compound	log P_{oct}
norfloxacin	-1.03	coumarin	1.39	phenytoin	2.26
caffeine	-0.07	clonidine	1.43	diltiazem	2.80
hydrochlorothiazide	-0.07	hydrocortisone	1.61	alprenolol	2.89
theophylline	-0.02	prednisolone	1.62	propranolol	2.98
pirenzepine	0.10	acebutolol	1.71	labetalol	3.09
antipyrine	0.23	pindolol	1.75	ketoprofen	3.12
ranitidine	0.27	metoprolol	1.88	testosterone	3.32
acetaminophen	0.51	corticosterone	1.94	naproxen	3.34
nadolol	0.71	piroxicam	1.98	ibuprofen	3.50
practolol	0.79	dexamethasone	2.01	verapamil	3.79
trimethoprim	0.91	furosemide	2.03	imipramine	4.44
aminopyrine	1.00	oxprenolol	2.10	desipramine	4.54
chloramphenicol	1.14	salicylic acid	2.26		

Total 60 compounds → **QSAR analysis 57 compounds**

PAMPA Permeability of Structurally Diverse Compounds



Compound	log P_{oct}
desipramine	4.54
imipramine	4.44
testosterone	3.32

Excluded from the QSAR equation

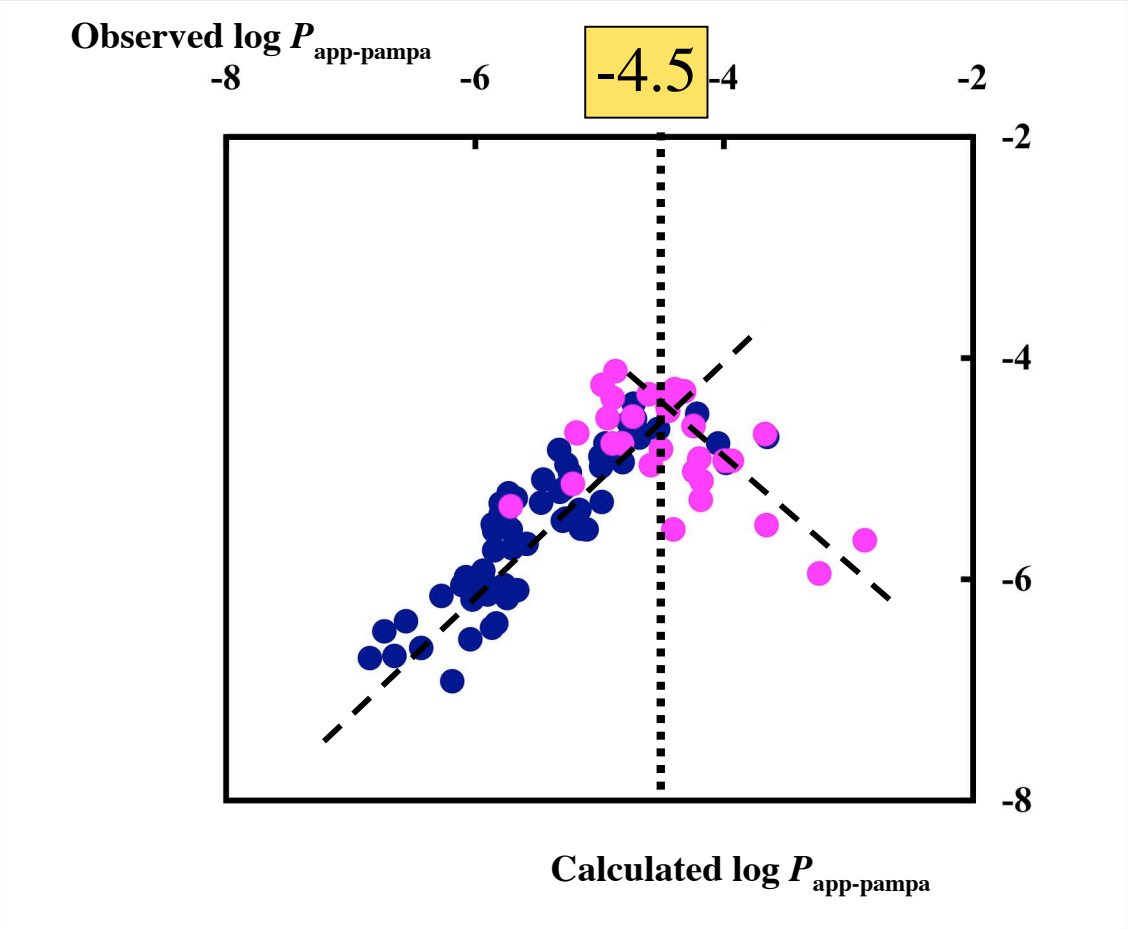
$$\log P_{app\text{-}pampa} = 0.43\ (\pm 0.09)\ \log P_{oct} - 0.25\ (\pm 0.08)\ |pK_a - pH| - 1.07\ (\pm 0.49)\ SA_{HA} - 1.00\ (\pm 0.42)\ SA_{HD} - 4.98\ (\pm 0.31)$$

$$n = 57 \quad s = 0.33 \quad r^2 = 0.76 \quad q^2 = 0.74$$

Fujikawa, M. et al., Bioorg. Med. Chem., 13, 4721 (2005) (QSAR of PAMPA permeability)

Added Test Compounds

Compound	log P_{oct}	Compound	log P_{oct}	Compound	log P_{oct}
Chemicals		**Chemicals**		**Agrochemicals**	
2,4-dichlorophenol	3.06	4-chloroaniline	1.88	atrazine	2.61
2,4-dimethylphenol	2.30	4-cyanophenol	1.60	benthiocarb	3.42
2,5-dichloronitorbenzene	3.03	4-nitroaniline	1.39	biphenyl	4.01
2-chloroaniline	1.90	aniline	0.90	BPMC	2.78
2-chlorophenol	2.15	diethyl phtalate	2.47	diazinon	3.30
2-methylphenol	1.95	dimethyl phtalate	1.56	DMTP	2.50
2-nitoraniline	1.85	diphenylamine	3.50	IBP	3.21
2-nitorphenol	1.79	hydroquinone	0.59	imidacloprid	0.59
3-chloroaniline	1.88	phenol	1.47	MEP	3.30
3-nitroaniline	1.37	pyrene	4.88	salithion	2.67
3-nitrophenol	2.00	diphenylamine-2-carboxylic acid	4.36	tebufenozide	4.25
				methoxyfenozide	3.70
				halofenozide	3.22
				chromafenozide	2.70
				RH-5849	2.45



PAMPA Permeability Including Hydrophobic Compounds

- Previous compound set
- Added compounds set

Predicted log $P_{app\text{-}pampa} \leq$ -4.5: Increase with apparent hydrophobicity

Predicted log $P_{app\text{-}pampa}$ > -4.5: Decrease with apparent hydrophobicity

Apparent hydrophobicity: $\log P_{app} = \log P_{oct} - \alpha |pK_a - pH|$

QSAR Equations for PAMPA Permeability

Predicted log $P_{app\text{-}pampa} \leq -4.5$

$$\log P_{app\text{-}pampa} = 0.41\ (\pm 0.09)\ \log P_{oct} - 0.28\ (\pm 0.07)\ |pK_a - pH| - 1.19\ (\pm 0.43)\ SA_{HA} - 1.09\ (\pm 0.40)\ SA_{HD} - 4.79\ (\pm 0.28)$$

$n = 74 \quad s = 0.35 \quad r^2 = 0.77$

Predicted log $P_{app\text{-}pampa} > -4.5$

$$\log P_{app\text{-}pampa} = -0.40\ (\pm 0.20)\ \log P_{oct} + 0.28\ (\pm 0.15)\ |pK_a - pH| - 3.69\ (\pm 0.62)$$

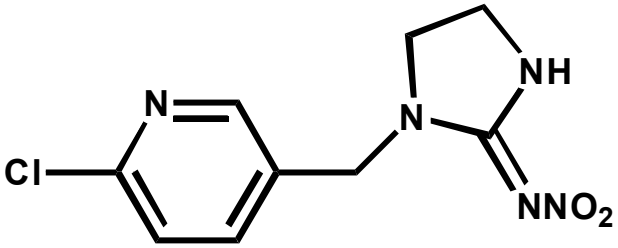
$n = 23 \quad s = 0.32 \quad r^2 = 0.50$

All compounds
(Bilinear equation for $\log P_{app} = \log P_{oct} - 0.68\ |pK_a - pH|$

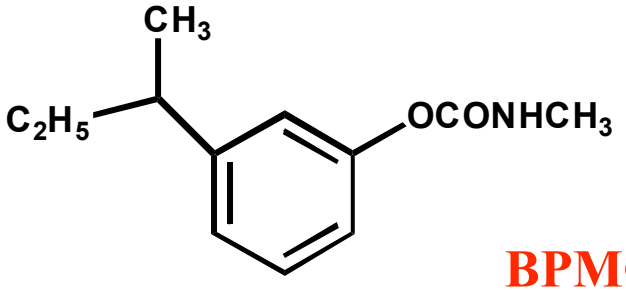
$$\log P_{app\text{-}pampa} = 0.53\ (\pm 0.10)\ \log P_{app} - 1.18\ (\pm 0.25)\ \log(\beta 10^{\log P_{app}} + 1) - 0.74\ (\pm 0.35)\ SA_{HA} - 1.14\ (\pm 0.40)\ SA_{HD} - 4.99\ (\pm 0.24)$$

$n = 97 \quad s = 0.36 \quad r^2 = 0.72 \quad q^2 = 0.68 \quad \log P_{app}\ (opt) = 2.08 \quad \log \beta = -2.17$

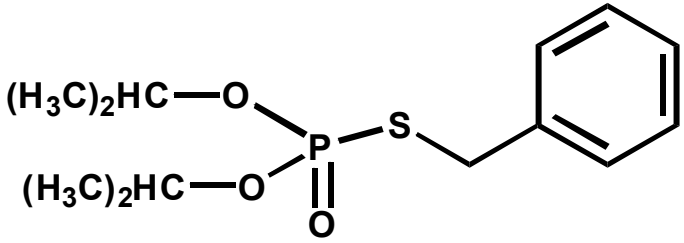
Structure of Agrochemicals



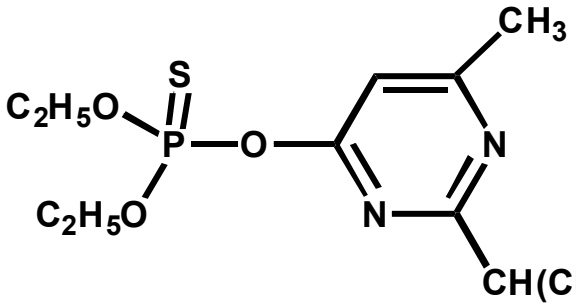
imidacloprid



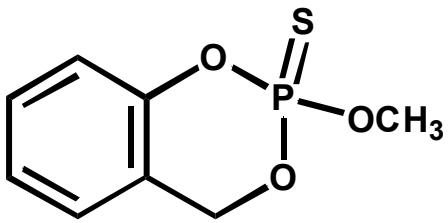
BPMC



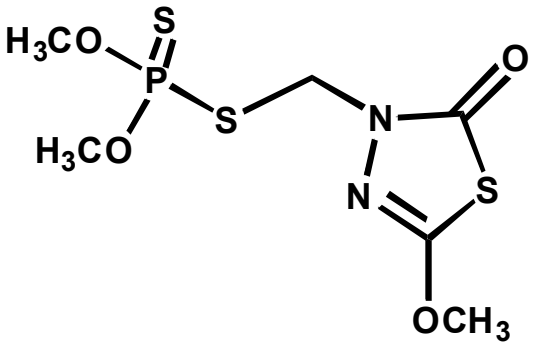
IBP



diazinon

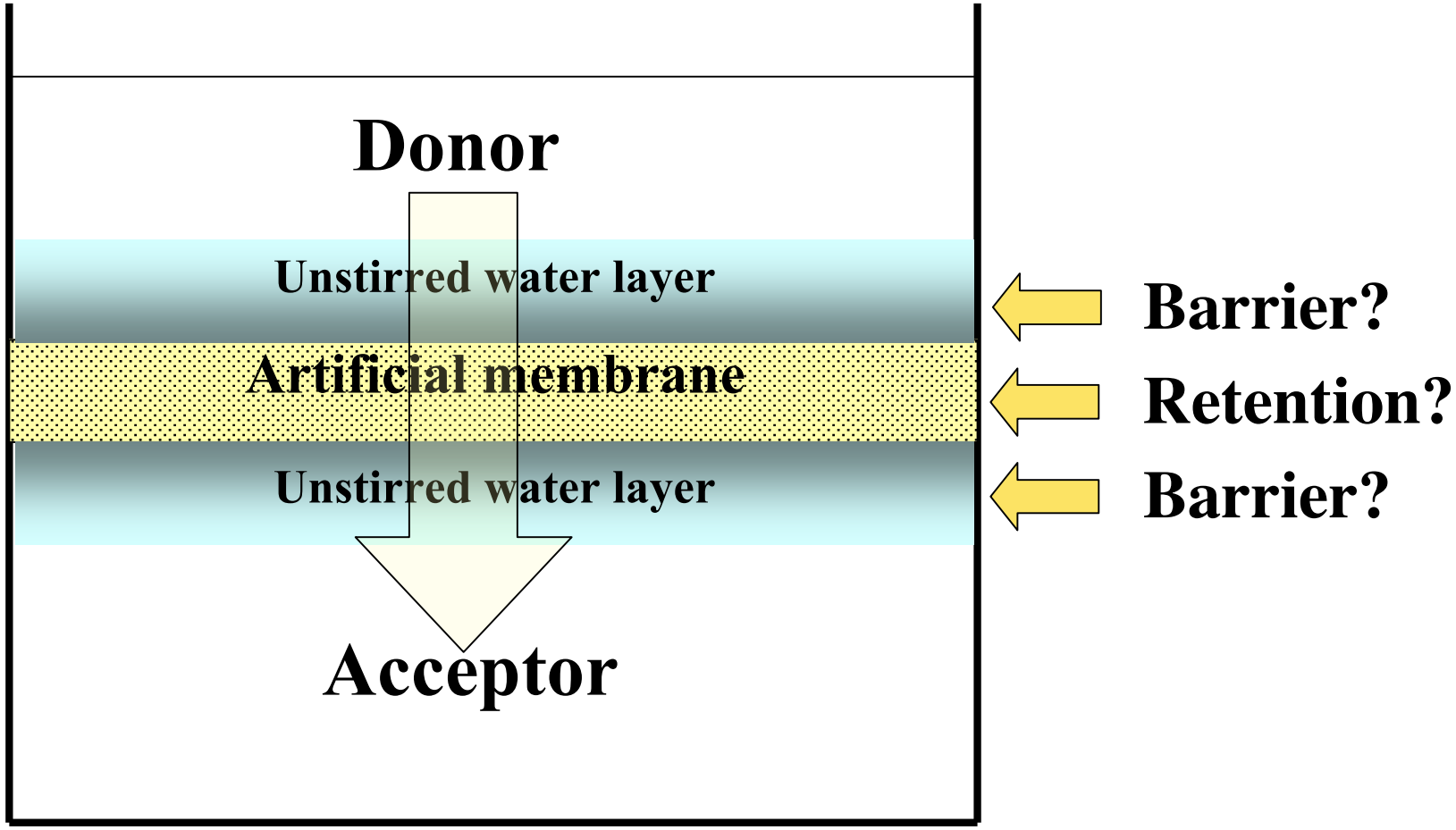


salithion



DMTP

Why Does PAMPA Permeability Decrease with Apparent Hydrophobicity for Hydrophobic Compounds?



Membrane Retention

Membrane Retention:

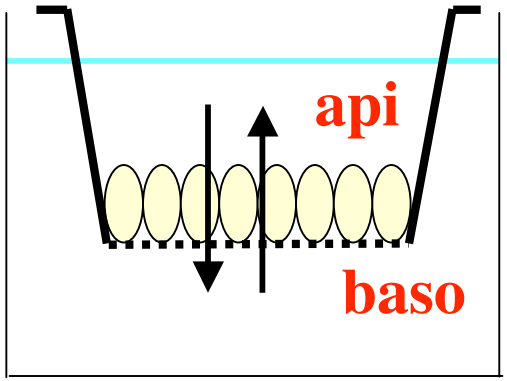
$$R\,(\%) = \frac{[\text{applied amount} - (\text{final amount in donor and acceptor compartments})\,] \times 100}{\text{applied amount}}$$

Compounds	log P_{oct}	%R	Compounds	log P_{oct}	%R	Compounds	log P_{oct}	%R
Drugs			**Chemicals**			**Agrochemicals**		
imipramine	4.44	44	2,4-dichlorophenol	3.06	42	atrazine	2.61	3
testosterone	3.32	5	2,4-dimethylphenol	2.30	20	benthiocarb	3.42	23
desipramine	4.54	2	2,5-dichloronitorbenzene	3.03	44	biphenyl	4.01	69
			2-methylphenol	1.95	3	BPMC	2.78	9
			2-nitroaniline	1.85	14	diazinon	3.30	65
			3-chloroaniline	1.88	7	DMTP	2.50	40
			aniline	0.90	1	IBP	3.21	27
			diethyl phtalate	2.47	37	imidacloprid	0.59	4
			dimethyl phtalate	1.56	13	MEP	3.30	47
			hydroquinone	0.59	2	salithion	2.67	43
			phenol	1.47	8	tebufenozide	4.25	3
			pyrene	4.88	58			

PAMPA permeability is also affected by membrane retention.

Main Absorption Mechanism

Classified by $P_{app\text{-}Caco2}$ values in each direction



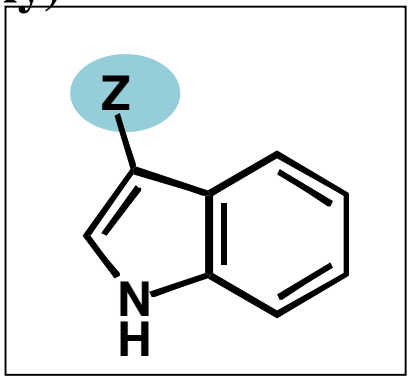
- **api → baso > baso → api**
  ⇒ influx system

Z= COOH, CH_2COOH, CH_2CH_2COOH, CH_2CONH_2

Salicylic acid

- **api → baso = baso → api**
  ⇒ passive transcellular route

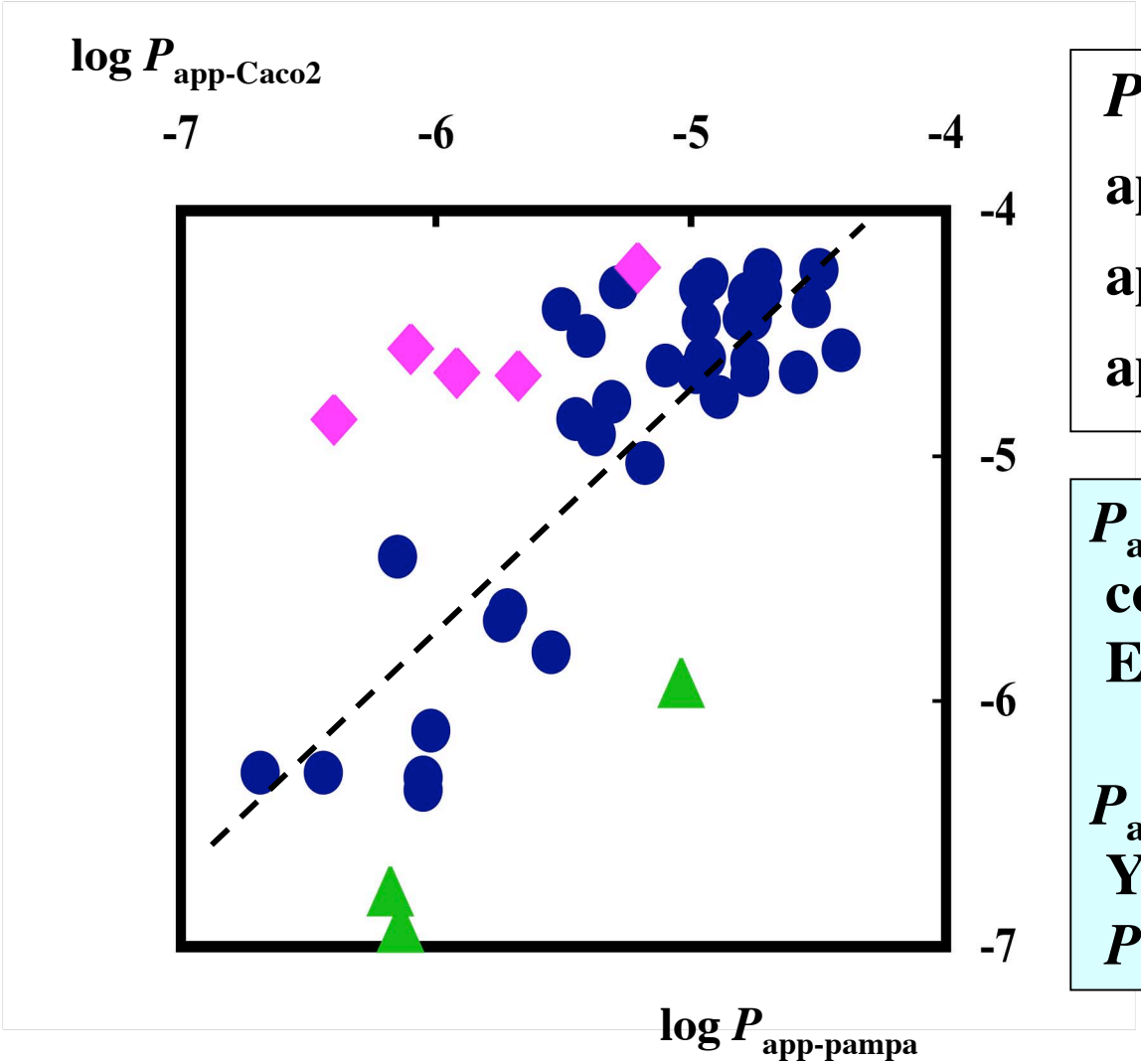
Ac-Trp-NH_2, Boc-Trp, CBZ-Trp, Fmoc-Trp, cyclo(Trp-Gly)
Z= H, CH_2OH



- **api → baso < baso → api**
  ⇒ efflux system

cyclo(Trp-Trp), Ac-Trp-Val-NH_2, Ac-D-Trp-Val-NH_2

Relationship between Caco-2 Cell and 'Unstirred' PAMPA Permeability Coefficients



$P_{app\text{-}Caco2}$:

api→baso = baso→api (●)

api→baso > baso→api (◆)

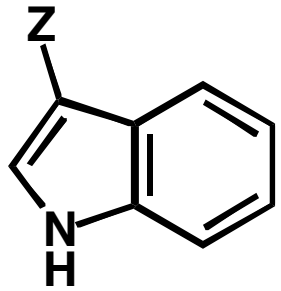
api→baso < baso→api (▲)

$P_{app\text{-}Caco2}$(peptides related compounds, agrochemicals): Experimentally measured

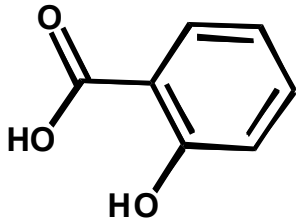
$P_{app\text{-}Caco2}$(drug): Yazdanian, M. *et al.*, *Pharm. Res.* (1998)

Transporterと相互作用する化合物

Active transporters

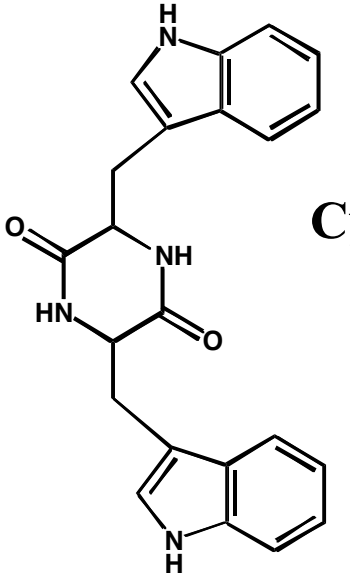


Z= COOH, CH_2COOH, CH_2CH_2COOH, CH_2CONH_2



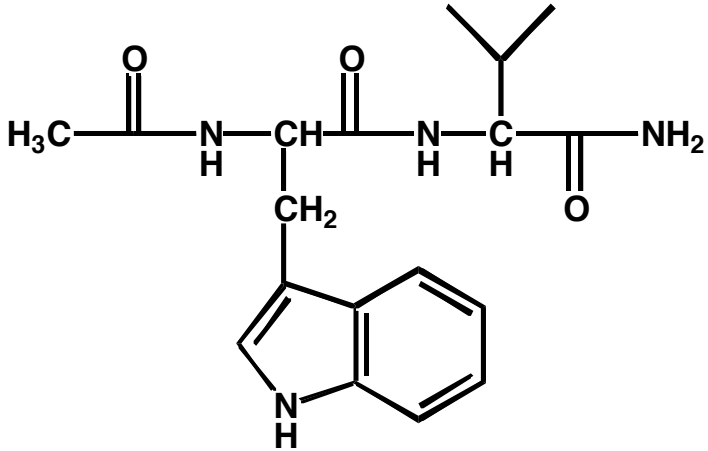
salicylic acid

Efflux systems (P-gp?)



Cyclic (Trp-Trp)

Ac-Trp-Val-NH_2, Ac-D-Trp-Val-NH_2



Transporters

能動輸送に関わるトランスポーター

アミノ酸トランスポーター：LATファミリーなど

オリゴペプチドトランスポーター：PEPT1, PEPT2

モノカルボン酸トランスポーター：MCTファミリー

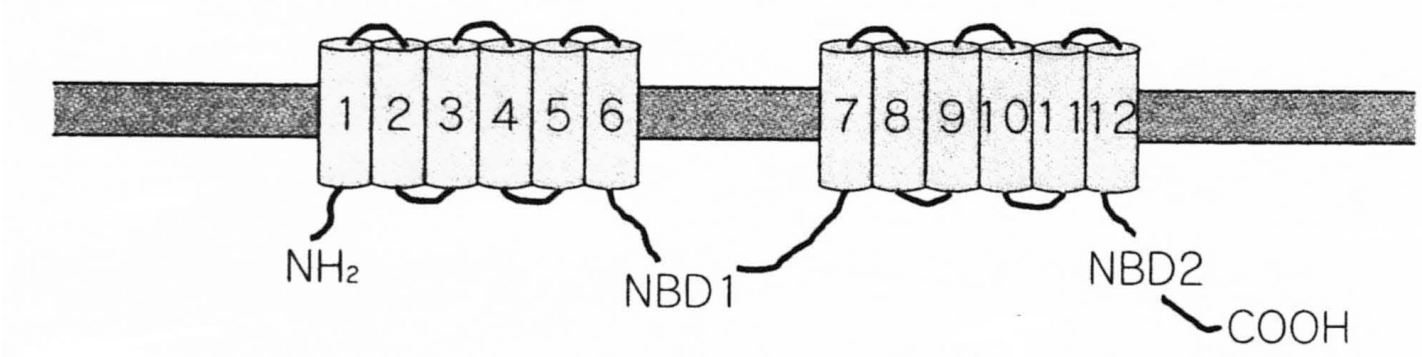
リン酸トランスポーター：NPT1

有機アニオントランスポーター：OATファミリー, OATP

有機カチオントランスポーター：OCTファミリー

P-glycoprotein (P-gp)

- ABC (ATP Binding Cassette) タンパク質の一つ
  - 12回膜貫通型タンパク質.　（Ca. 170 KDa)
  - ATP結合カセットを機能単位内に2個持つ.
- MDR (Multidrug Resistance-associated protein) ファミリーの生体異物排出ポンプ (MDR1).
  - ガン細胞の多剤耐性に関与.
- 構造に類似性のあまりない多くの基質を認識し輸送する.



ABCファミリー

MDR1

NBD: Nucleotide Binding Domain
ATP結合領域

遠藤　仁, 蛋白質　核酸　酵素, 46(5), 583-586 (2001), 図2より引用

Classical QSAR of P-gp Modulators

Wang, Lien, *et al., J. Clin. Pharm. Ther.* 28, 203 (2003)

Steroidal compounds

$$\log AR = 7.744 \log Mw + 0.137\ Clog\ P - 0.104\ H_b - 18.453$$

$n = 22 \quad s = 0.155 \quad r = 0.929$

H_b : total number of hydrogen bonds

Flavonoids

$$\log 1/k_d = 0.335\ Clog\ P - 0.007\ H_f - 0.14\ N_{lc} + 4.342$$

$n = 16 \quad s = 0.236 \quad r = 0.861$

H_f : heat of formation

N_{lc} : number of atoms of the longest chain length

Protein kinase C inhibitors

$$\log 1/IC_{50} = 4.36 \log Mw + 0.65\ E_{lumo} - 4.937$$

$n = 11 \quad s = 0.437 \quad r = 0.858$

Free-Wilson 法

- 1964年に考案
- 化合物がある構造を持っている時 1，持っていない時 0 の値をとる擬変数を用いて，活性の変化を解析する.
- Fujita-Banの修正モデル：解析対象化合物群の一つを基準化合物とし，Free-Wilson法を適用する.

Free-Wilson 法の例

Substrates of Elastase

Suc-X-Y-Ala-pNA

X,Y: amino acid

Suc: succinyl

pNA: *p*-nitroanilide

$$\log (k_{cat}/K_m) = -1.16\ \text{Gly (X)} - 0.44\ \text{Phe (X)} - 0.15\ \text{Leu (X)}$$

$$-1.05\ \text{Gly (Y)} - 0.53\ \text{Leu (Y)} - 0.67\ \text{Phe (Y)} + 0.46\ \text{Pro (Y)} + 0.35\ \text{Nle (Y)} + 0.20\ \text{Nva (Y)} + 0.24\ \text{Aba (Y)} + 3.79$$

$$n = 89 \quad s = 0.13 \quad r^2 = 0.96$$

Nomizu *et al.*, *Int. J. Peptide Protein Res.*, 42, 216 (1993)

パターン認識法

- 主成分分析 (Principal Component Analysis: PCA)
  - 元データに含まれる情報や変動を最大に保ったまま，新しい変数（主成分あるいは潜在変数）に変換し，分析する
- クラスター分析
  - 化合物間の類似度によりクラスター（群）に分割する
- SIMCA (Soft Independent Modeling of Chemical Analogy) 法

3D-QSARの手法

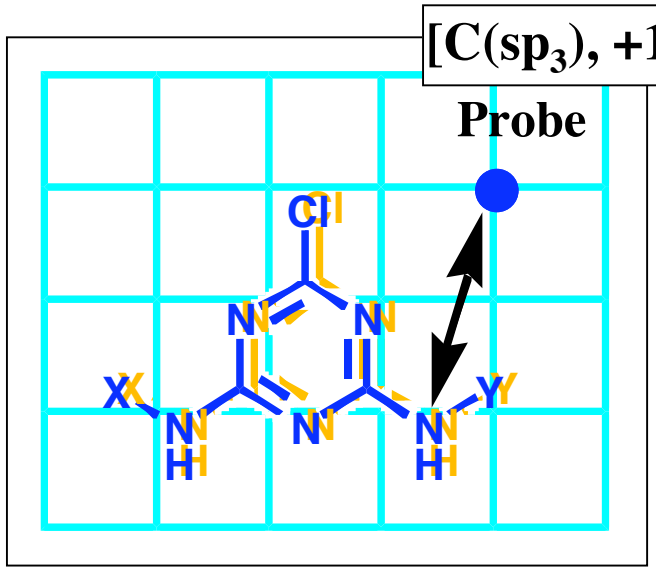
- Comparative Molecular Field Analysis (CoMFA)
- Voronoi Field analysis (VFA)
- 3D-Similarity
- CATALYST
- VolSurf

3D-QSARの手法

- Comparative Molecular Field Analysis (CoMFA)
- Voronoi Field analysis (VFA)
- 3D-Similarity
- CATALYST
- VolSurf

CoMFA

(1) 化合物のコンホメーション解析
(2) 原子電荷の計算 (MM/MO法を用いる）
(3) 化合物の重ね合わせ
(4) 立体および電子的エネルギーパラメーターの計算
(5) Partial Least Squares: PLS法による統計解析

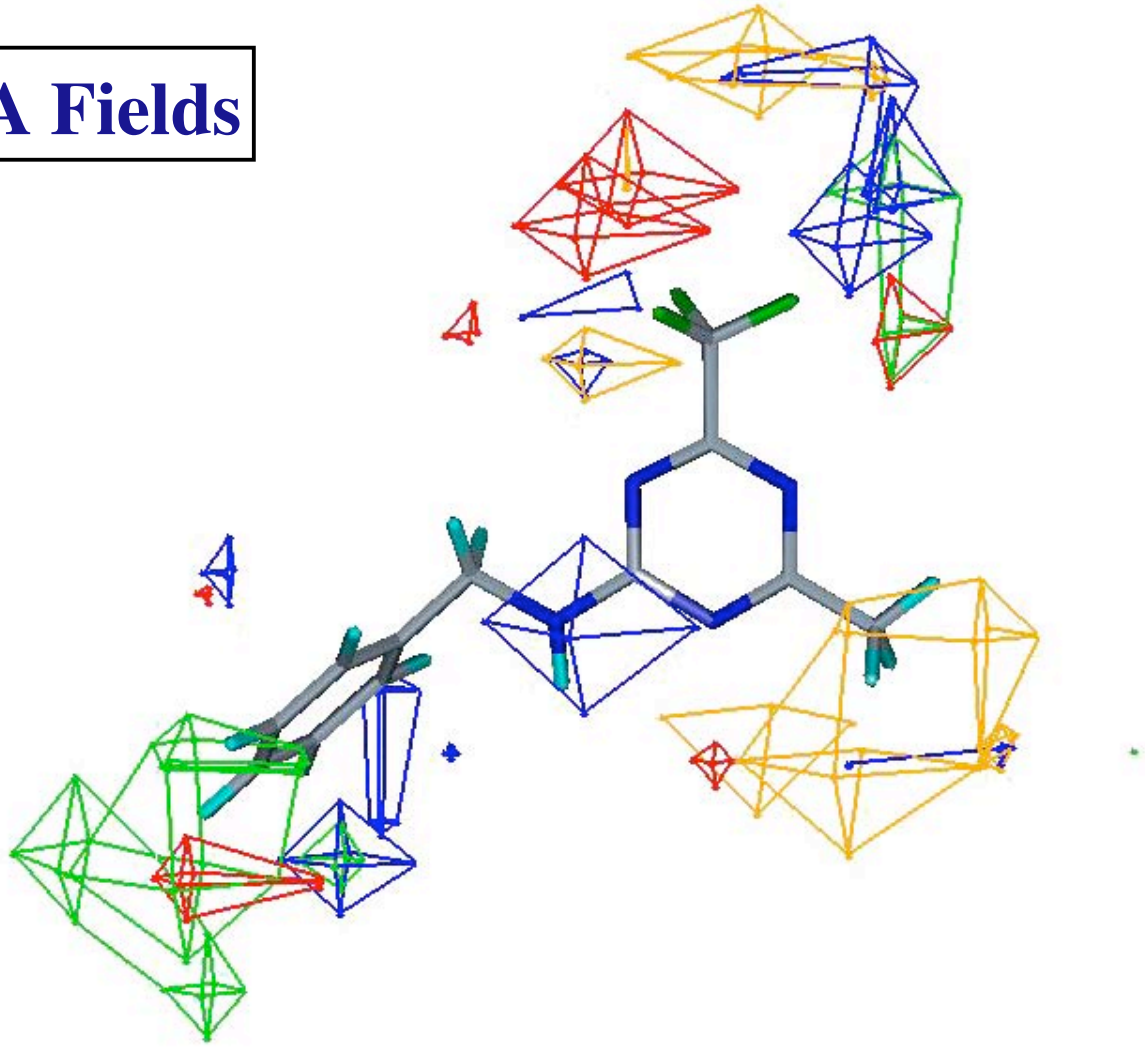


3D-Lattice

Activity =
a + [CoMFA steric and/or electrostatic terms]
+ b log *P* + c (log *P*)^2 + ...

(6) 活性にとって有利あるいは不利な
領域の視覚化

CoMFA Fields



Red: Electro-negatively favorable region

Blue: Electro-positively favorable region

Green: Sterically favorable region

Orange: Sterically unfavorable region

参考文献

17. Fujikawa, M. *et al.*, *Bioorg. Med. Chem.*, 13, 4721 (2005)
(QSAR of PAMPA permeability)

18. Nomizu, M. *et al.*, *Int. J. Peptide Protein Res.*, 42, 216 (1993)
(QSAR of elastase substrates)

19. Cramer, R. D., III *et al.*, *J. Am. Chem. Soc.*, 110, 5959 (1988)
(CoMFA)