Prediction of the Passive Membrane Permeability of Molecules

2022.08.06 Literature Seminar
M1 Hiromu Kakizawa

Contents

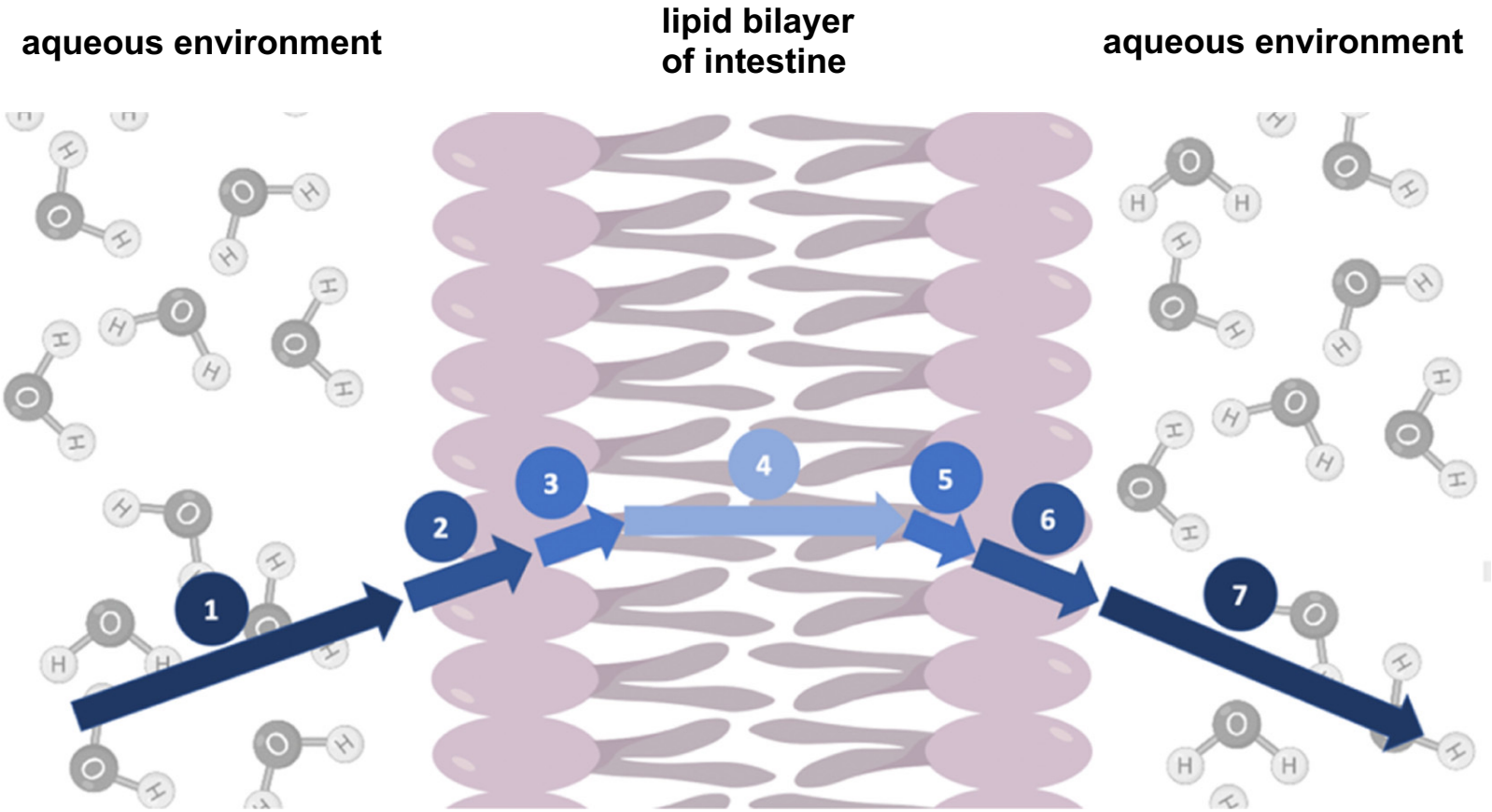
1. Introduction

2. Conformational and structural determinants for cell permeability
   (*Nat. Chem. Biol.* **2016**, *12*, 1065)

3. Integration of calculated 3D properties of molecules into permeability prediction
   (main paper, *J. Med. Chem.* **2022**)

Contents

Requirements for Oral Drugs



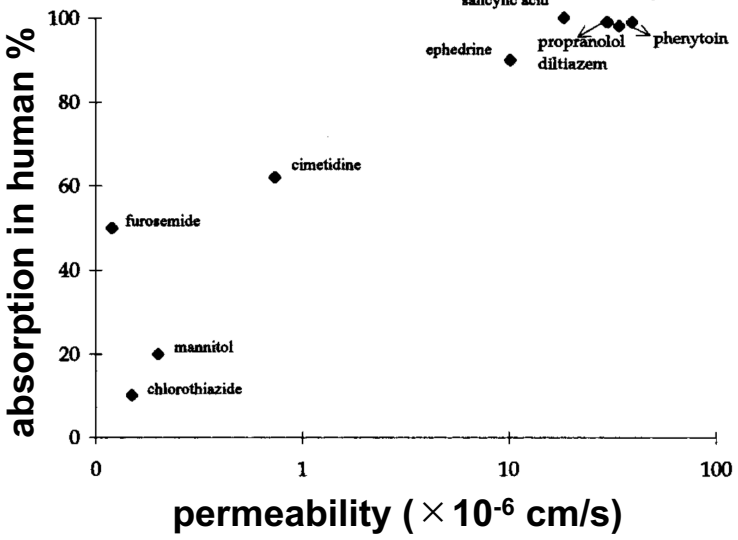
Necessities for molecules to be successfully absorbed at intestine:

- **sufficient solubility in aqueous environment**
- **good lipophilicity to enter into and pass through the membrane (= membrane permeability)**

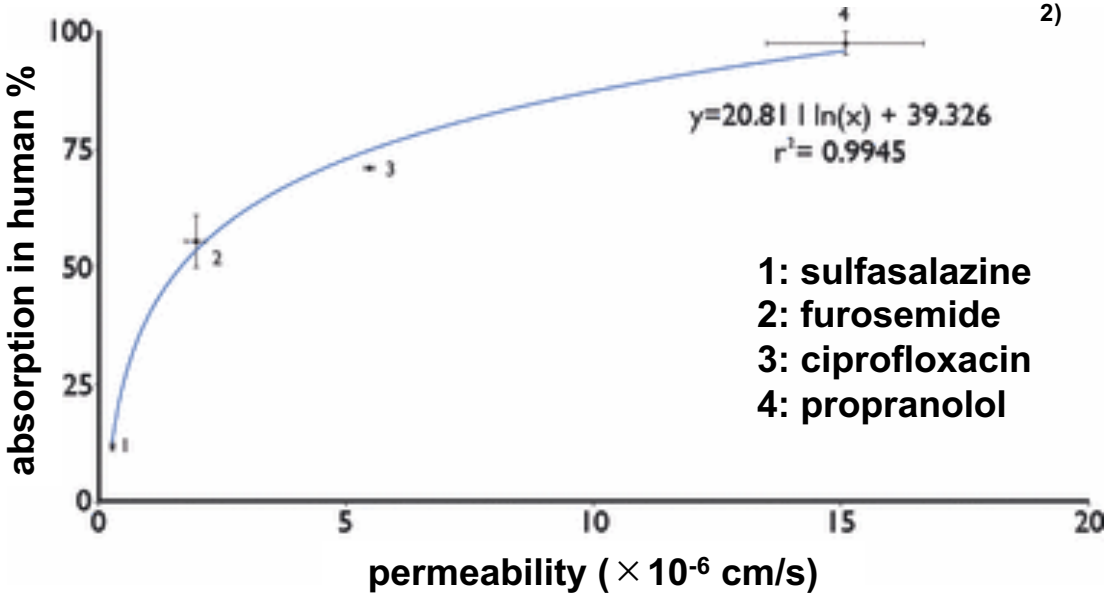
Permeability Affecting Absorption in Human

The extent to which the oral drug is absorbed in humans highly depends on its cell permeability.
(the rate of drug absorbed in human out of the dose is plotted against permeability of the drugs)

1)



2)



Drugs with higher cell permeability show efficient absorption in humans (= can possess good bioavailability).

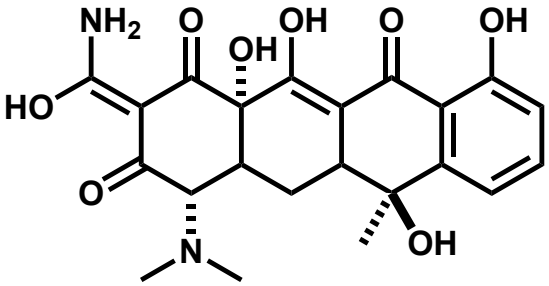
1) Pade, V.; Stavchansky, S. *J. Pharm. Sci.* **1998**, *87*, 1604. 2) Fortuna, A.; Alves, G.; Falcão, A.; Soares-da-Silva, P. *Epilepsia.* **2012**, *53*, 529.

“Rule of Five” And Its Outliers

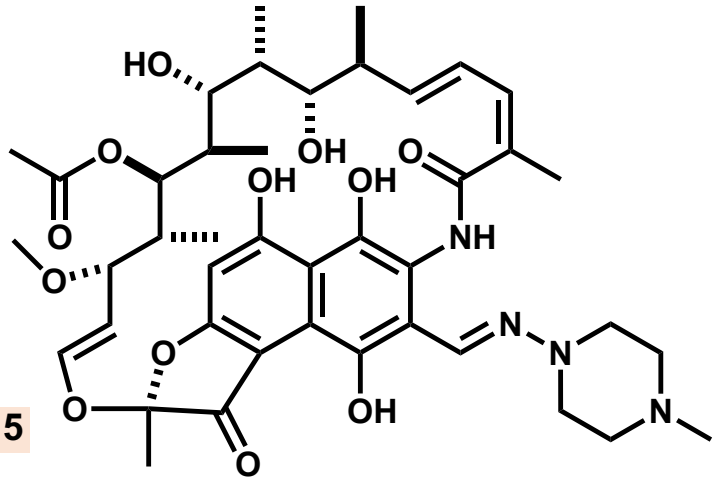
In 1996, Lipinski et al. found the tendency that poor permeation is more likely when[1]:

- **number of hydrogen bond donors (HBD, -NH or -OH) > 5**
- **molecular weight (MW) > 500**
- **ClogP (calculated lipophilicity) > 5, or other parameter MLogP > 4.15**
- **number of hydrogen bond acceptor (HBA, N and O atoms) > 10**

However, there are many drugs which break the rule but can permeate membrane[2].



tetracycline
MW = 444, HBD = 6,
ClogP = –0.90, HBA = 9

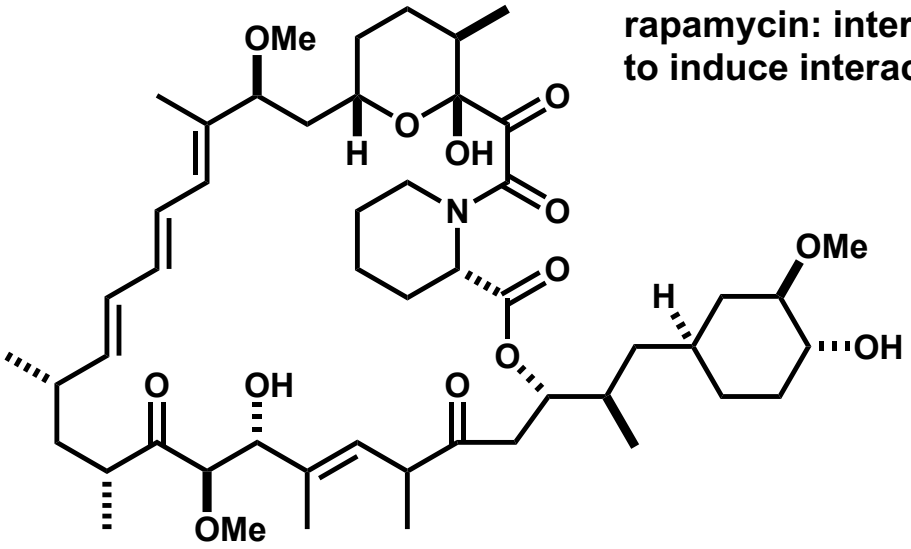


Rifampicin
MW = 823, HBD = 6,
ClogP = 3.7, HBA = 15

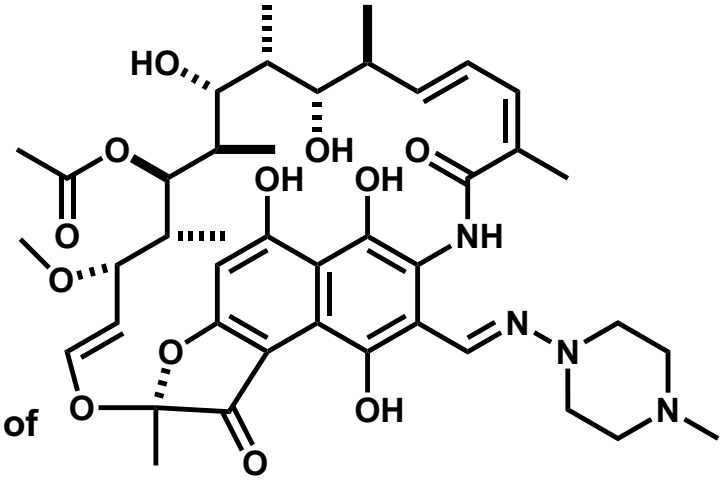
1) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3. 2) Krämer, S. D.; Aschmann, H. E.; Hatibovic, M.; Hermann, K. F.; Neuhaus, C. S.; Brunner, C.; Belli, S. *Adv. Drug Delivery Rev.* **2016**, *101*, 62.

Macrocycle in a Drug Discovery

Macrocyclic compounds, usually with high MW and many HBAs and HBDs, are currently in use as drugs and some of them can address targets challenging for small molecules[1)].



rapamycin: interaction with protein FKBP12 to induce interaction with FRAP



rifampicin: inhibition of RNA polymerase

More general method to predict the permeability of macrocycles, many of which are "beyond rule of five" molecules, is strongly desired.

1) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discovery* **2008**, *7*, 608.

Contents

Prof. Jan Kihlberg

1988 Ph.D at Lund Institute of technology (organic chemistry)

1996 Professor at Umeå University

2003 Director of medicinal chemistry at AstraZeneca

2009 Director competitive intelligence and business foresight analysis at AstraZeneca R&D molndal

2013 Professor at Uppsala University

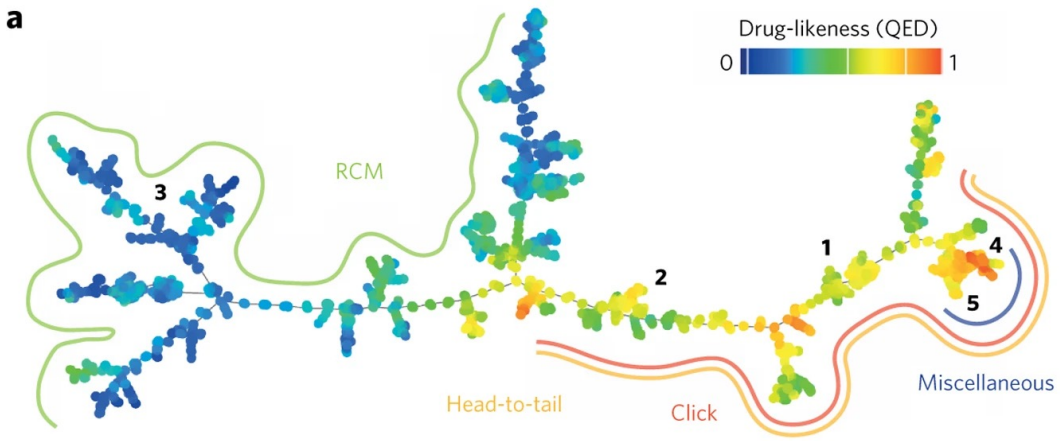
Research Interests:

- Drug design beyond the "rule of 5"

- Investigation for immunological mechanism of rheumatoid arthritis

- Chemical probes to elucidate the pathway for chronic kidney disease

Compound Selection for Study



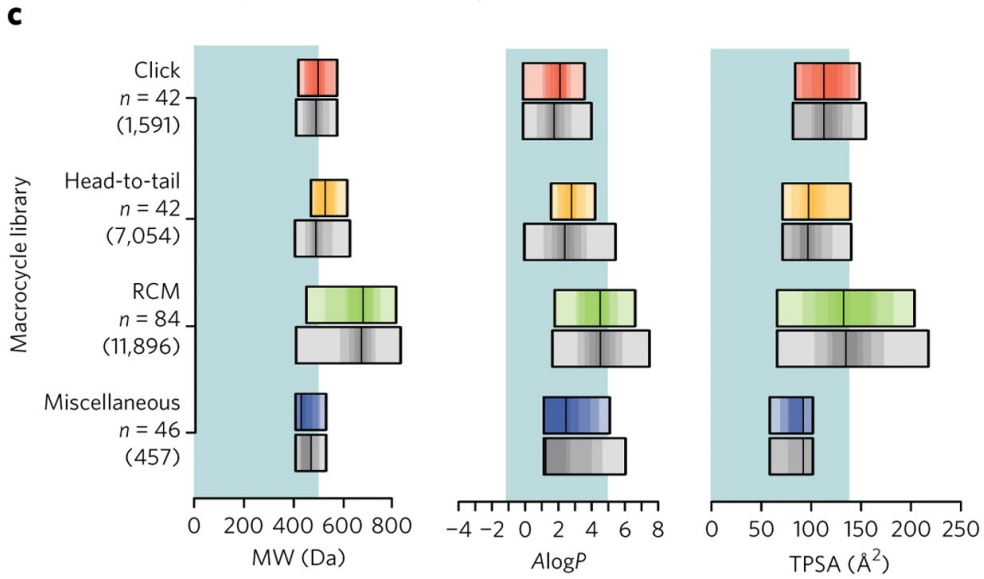
214 macrocycles were selected from Broad Institute's screening collection based on similarity network.

Selected compounds included groups of stereo/regioisomers.

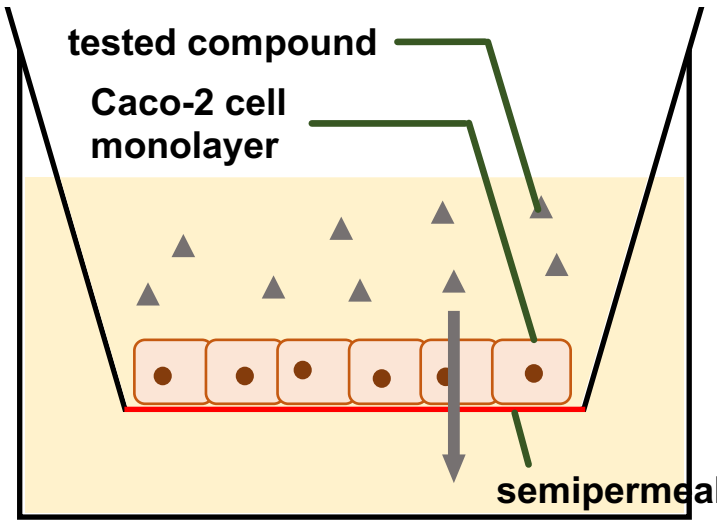
comparison of selected compounds (colored) and the screening collection (gray) in terms of molecular properties

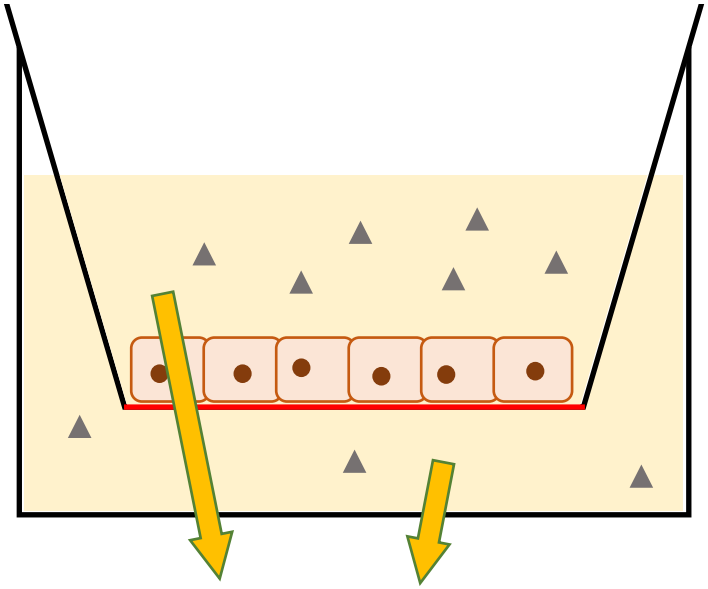
***A*log*P*: calculated lipophilicity**

TPSA: surface areas of polar regions calculated based on 2D structure

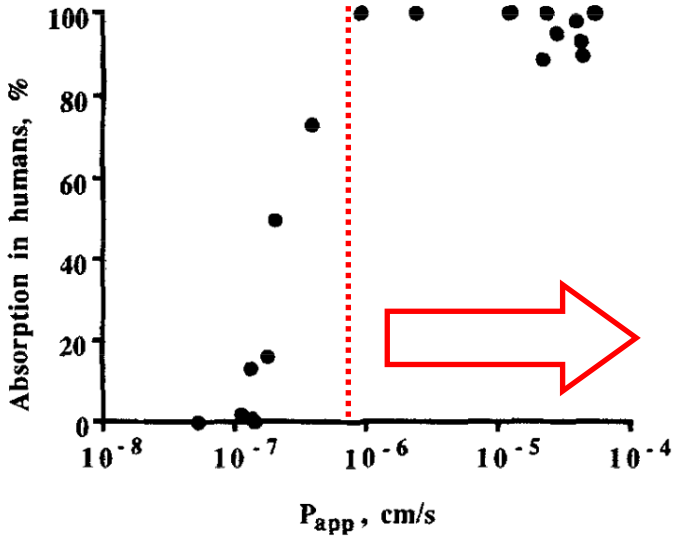


Caco-2 Cell Permeability Assay[1)]





At various time, compound concentration of each compartment is measured and permeation rate (P_{app}) is calculated.

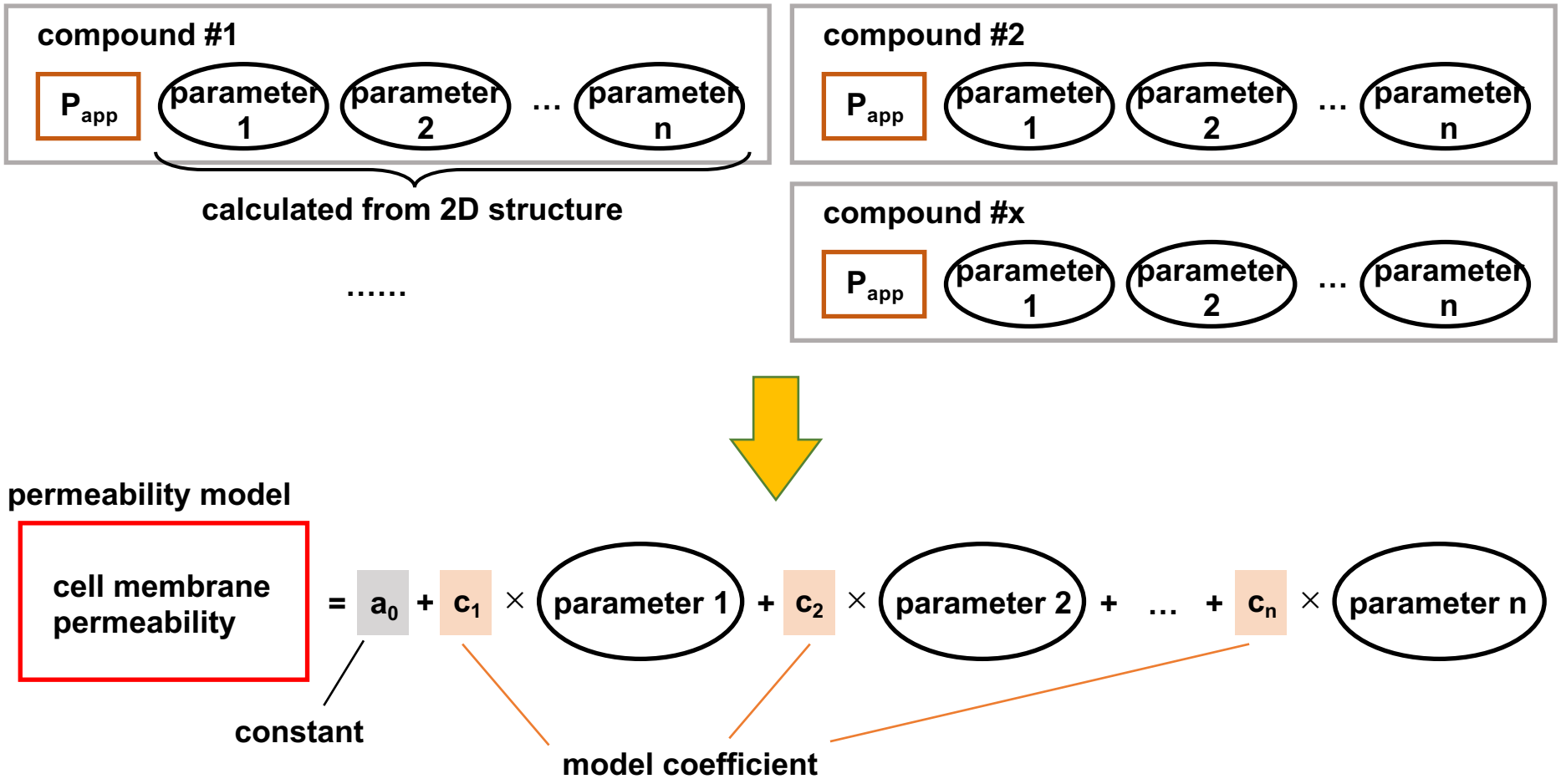


Drugs almost completely absorbed in humans are shown to have $P_{app} > 1 \times 10^{-6}$ (moderate to high cell permeability)[2)].

1) Breemen, R. B.; Li, Y. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 175. 2) Artursson, P.; Karlsson, J. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880.

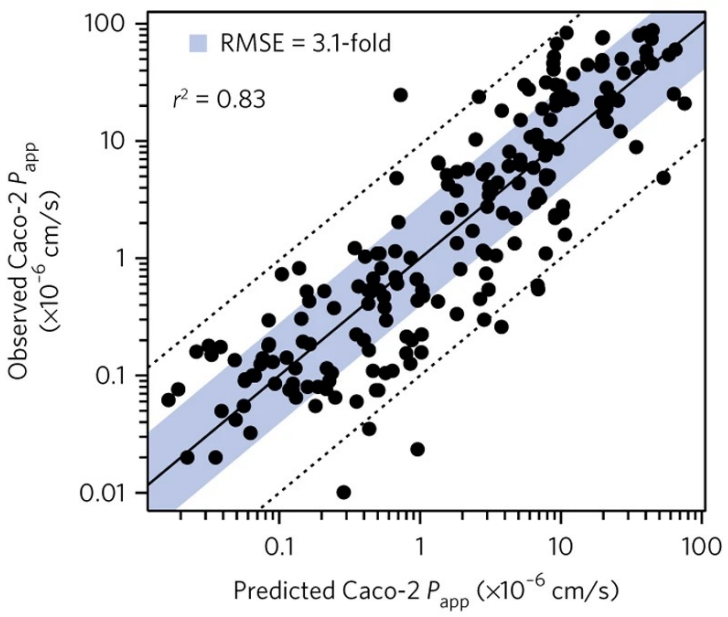
Regression Analysis

Partial least square regression (PLSR) was used, one of the methodologies of linear regression, in which the target variable is explained in a linear combination of some parameters.

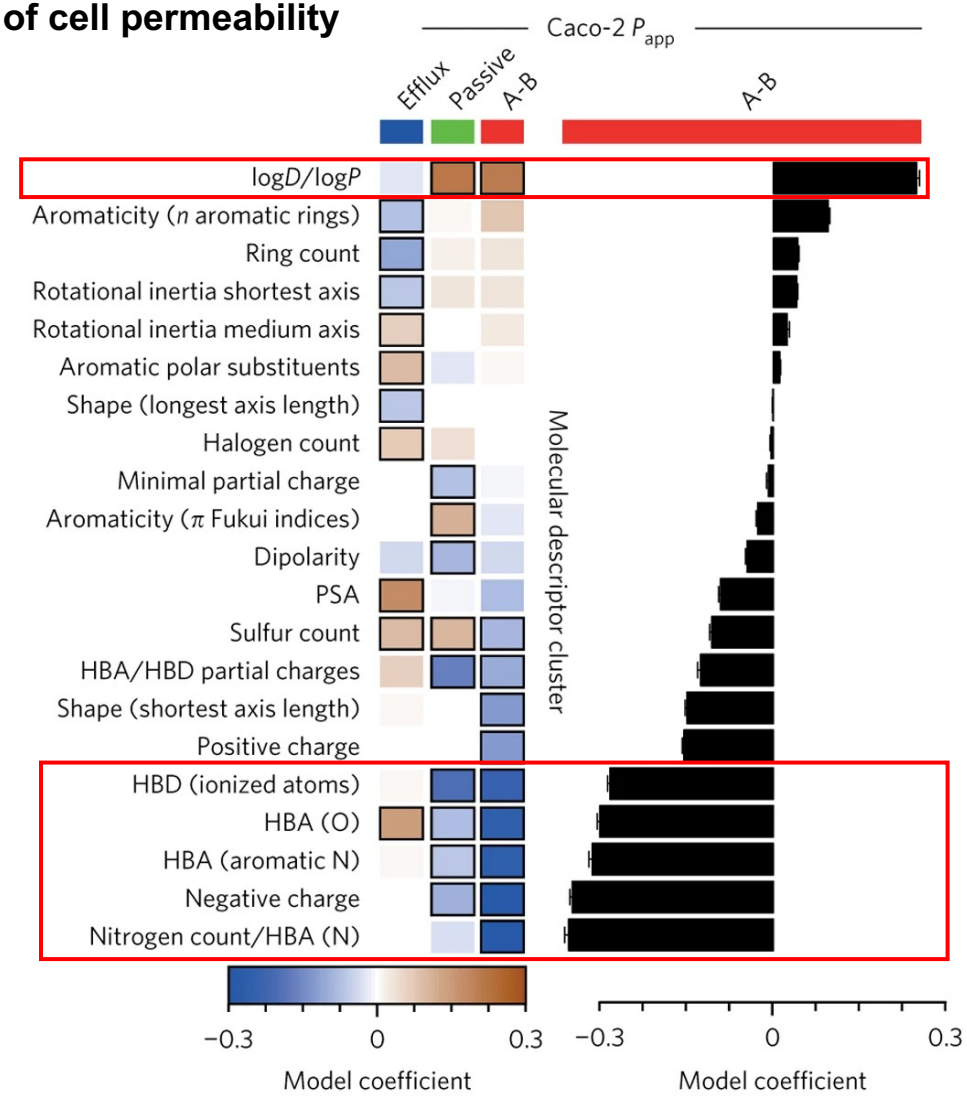


Larger model coefficient means the parameter affects the cell permeability more significantly, and can be >0 (positive effect for permeability) or <0 (negative effect for permeability).

Properties Affecting Cell Permeability



factors for prediction of cell permeability

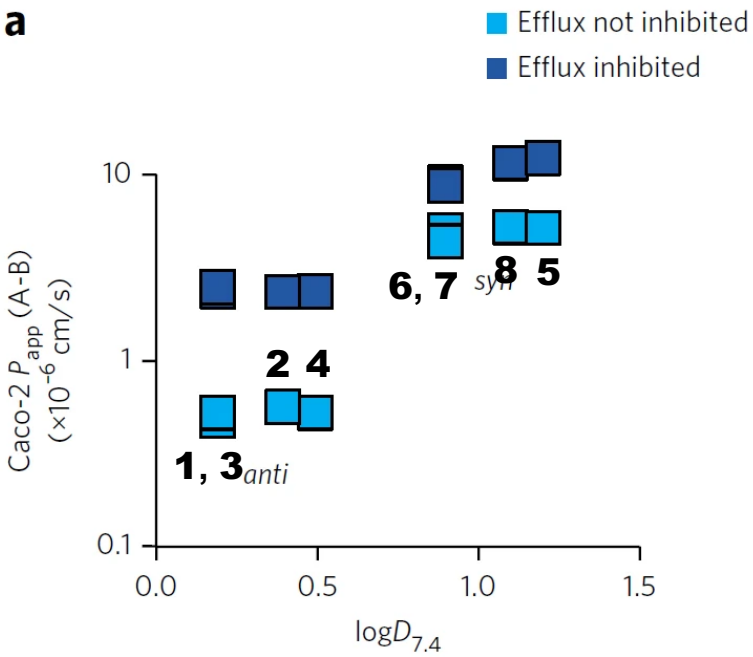


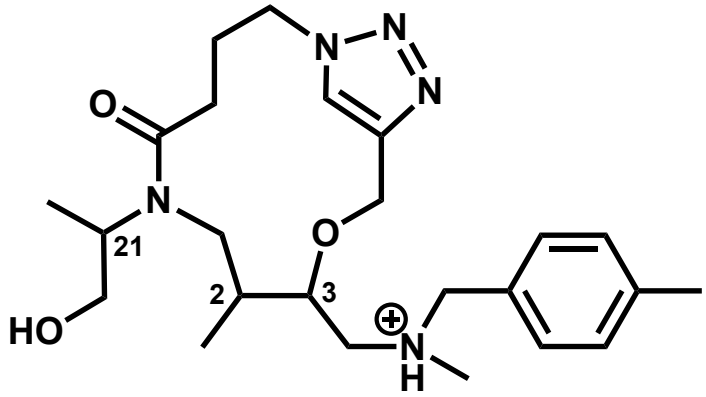
Important descriptor identified by PLSR:

- **calculated lipophilicity**

- **number of hydrogen bond donors**
- **number of hydrogen bond acceptors**
- **negative charge**

Gap of the Prediction for Diastereomers





	C2	C3	C21
1	R	R	S
2	R	R	R
3	S	S	R
4	S	S	S

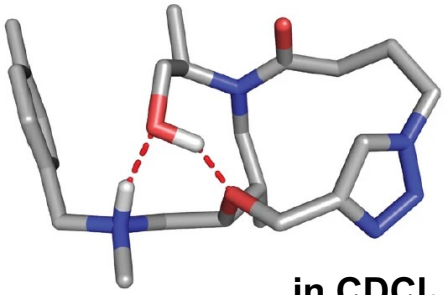
	C2	C3	C21
5	R	S	R
6	R	S	S
7	S	R	R
8	S	R	S

Although 1-8 were all calculated to have logD ≈ 2.0 and P_{app} = 2.6 × 10^{-6} to 3.4 × 10^{-6} cm/s, measured P_{app} differs between largely between *syn* and *anti* diastereomers at C2 and C3.

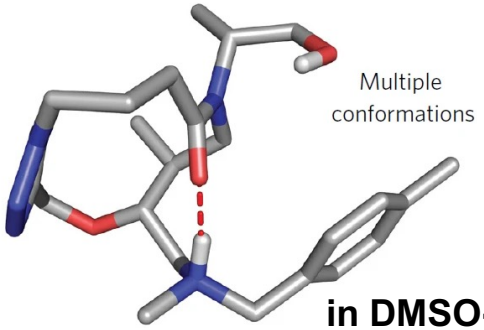
Rationale by Calculated 3D Structure

3D conformations in hydrophobic (chloroform) and hydrophilic (DMSO) environment were calculated using Molecular Operating Environment (MOE) and confirmed by NMR.

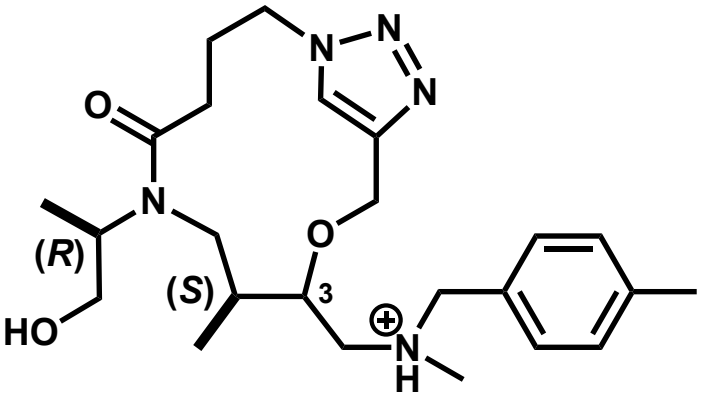
7 (*R* at C3, P_{app} = 5.48 $\times 10^{-6}$ cm/s)



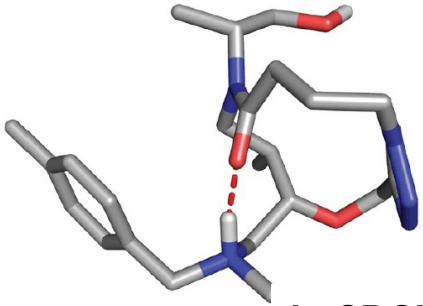
in $CDCl_3$



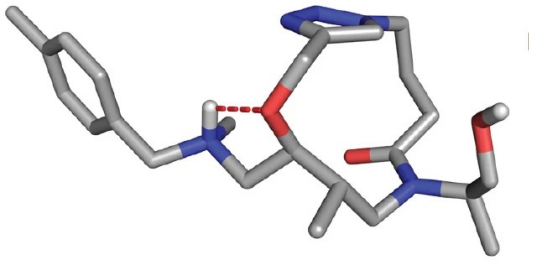
in DMSO-d_6



3 (*S* at C3, P_{app} = 0.51 $\times 10^{-6}$ cm/s)



in $CDCl_3$



in DMSO-d_6

7 could form a longer hydrogen bond network (N^+H-OH-OC) than **3** (just N^+H-OH), delocalizing positive charge at 3˚ amine under hydrophobic environment.

Models considering only 2D properties could not account for the 10-fold P_{app} difference between diastereomers.

Contents

Prof. Adrian Whitty

1991 **Ph.D at Illinoi University (Organic chemistry)**

Postdoctoral work at Brandeis University (with Prof. William P. Jencks)

1993 **Career at Biogen (including director of physical biochemistry)**

2008 **Professor at Boston University (biochemistry and chemical biology)**

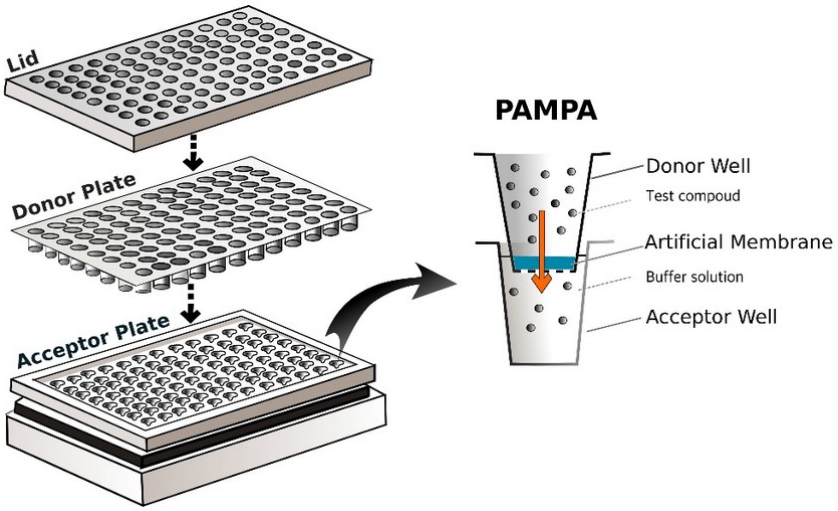
Research topics:

- inhibition of protein-protein interactions by synthetic molecules, especially macrocycles

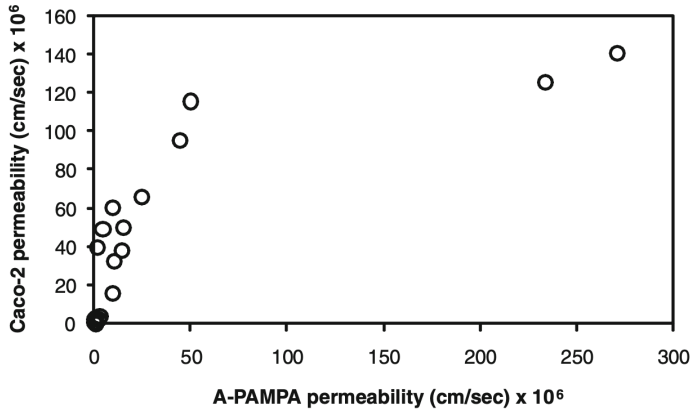
- understanding activation and signaling of growth factor receptors

Experimental Measurement of Permeability

Membrane permeability P_{eff} was measured using parallel artificial membrane permeation assay (PAMPA), which can be conducted in a very high throughput.



Each compound solution is set in "donor well". After 16 h, the amount of each compound in donor and acceptor well is measured, and then P_{eff} is calculated.



It was shown that measured permeabilities in PAMPA and Caco-2 assay are in good correlation[1].

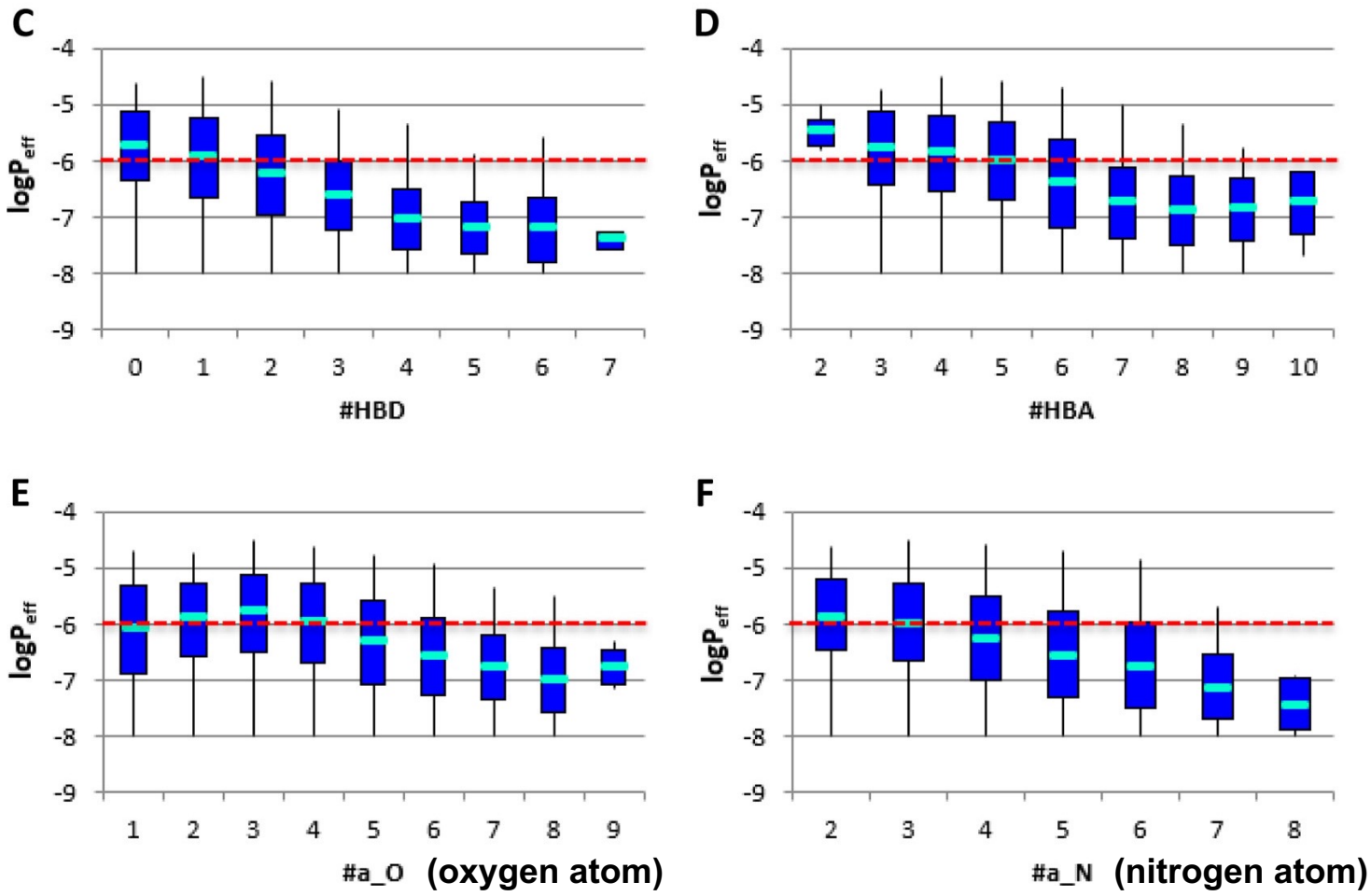
Three compounds were used as reference compounds in the research (table on the right). P_{eff} = 10^{-6} cm/s was set as the criteria for "good permeability".

compound	measured P_{eff} ($\times 10^{-6}$ cm/s)	absorption in human %[1]
furosemide	0.024 ± 0.014	61
metoprolol	1.4 ± 0.3	95
propranolol	9.6 ± 0.5	100

1) Teksin, Z. S.; Seo, P. R.; Polli, J. E. *AAPS J.* **2010**, *12*, 238.

Prediction of Permeability

3,600 purchased macrocycles with aqueous solubility ≥ 30 μM was tested for membrane permeability.



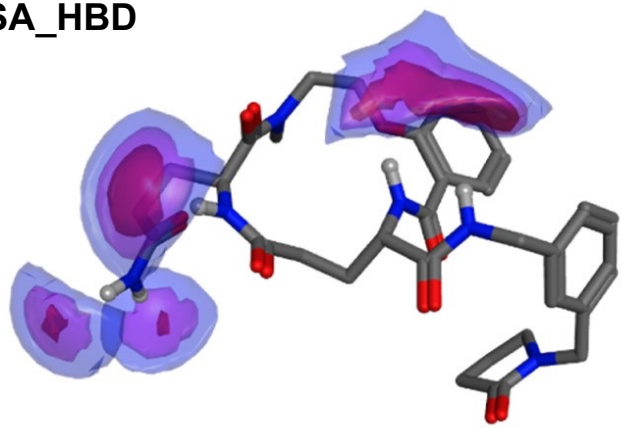
Although gradual trends were seen, no obvious cutoff value for each property was observed. Also, PLSR model using only 2D properties failed to give models better than $R^2 \sim 0.4$.

Modification of Prediction

1) Integration of molecular 3D descriptors

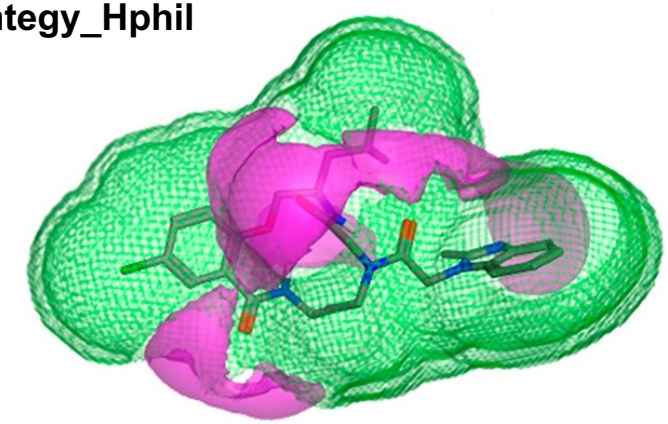
Based on the most stable 3D conformation in H_2O generated by Molecular Operating Environment (MOE), each parameter was calculated. Examples are shown below:

SA_HBD



hydrogen bond surface area

Integy_Hphil



hydrophilic integy moment:
bias of the distribution of hydrophilic surface regions (colored in magenta)

2) Division of compounds into two groups based on basicity

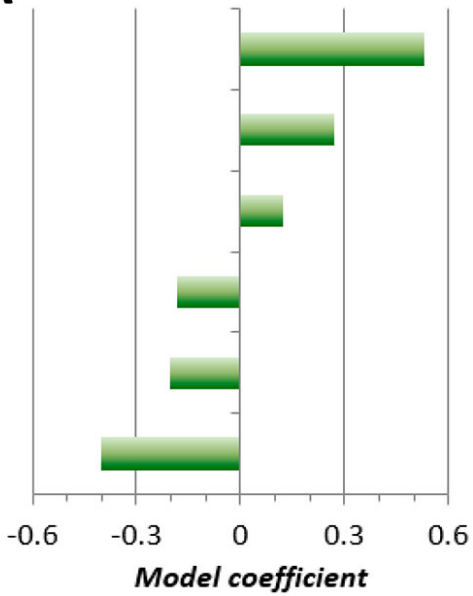
pK_a of the conjugate acid > 7.6 → **compounds existing in a cationic form at physiological pH**

< 7.6

compounds without basic centers → **compounds existing in a neutral form at physiological pH**

Permeability Model for Neutral or Weakly-Basic Compounds

A



Integy_Hphil (Hydrophilic integy moment)

ASA_H (Water accessible hydrophobic surface area)

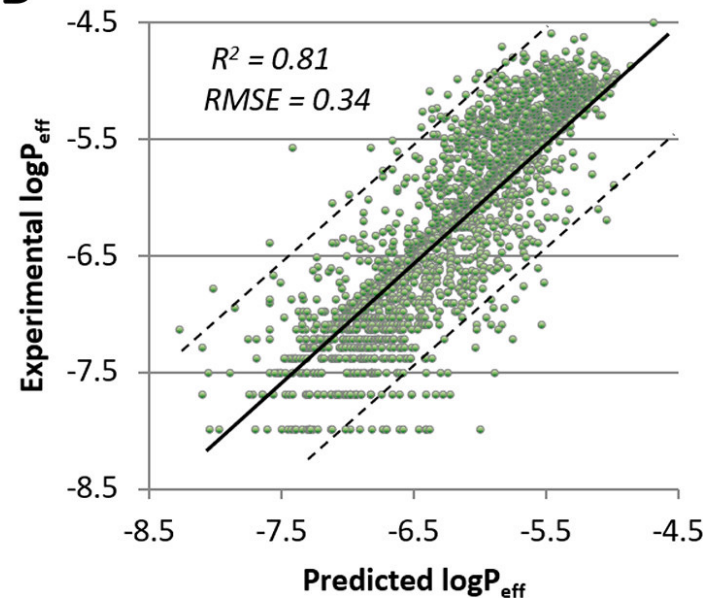
SlogP (Log of the octanol/water partition coefficient)

a_heavy (Number of heavy atoms)

SA_HBD (charge weighted, surface weighted Hydrogen bond donor area)

TPSA (Topological polar surface area)

B



In the final model, cell permeability was predicted using only six parameters.

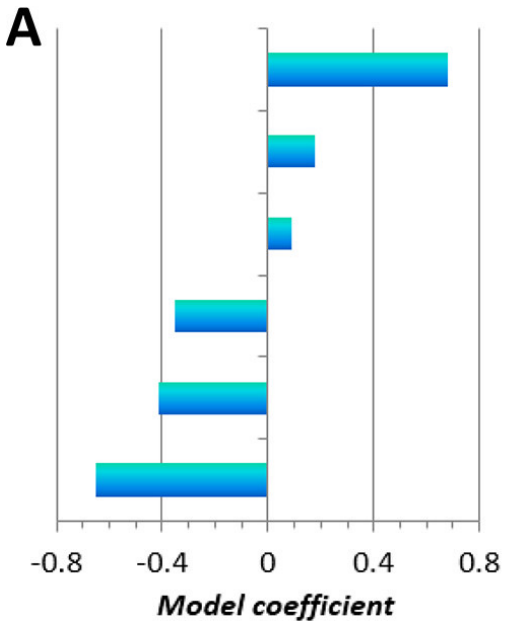
The final model included three 3D properties:

- **biased distribution of the hydrophilic regions**
- **hydrophobic surface area**
- **hydrogen bond donating area**

The remaining three 2D properties:

- **calculated lipophilicity**
- **number of heavy atoms**
- **topological polar surface area**

Permeability Model for Basic Compounds



ASA_H (Water accessible Hydrophobic surface area)

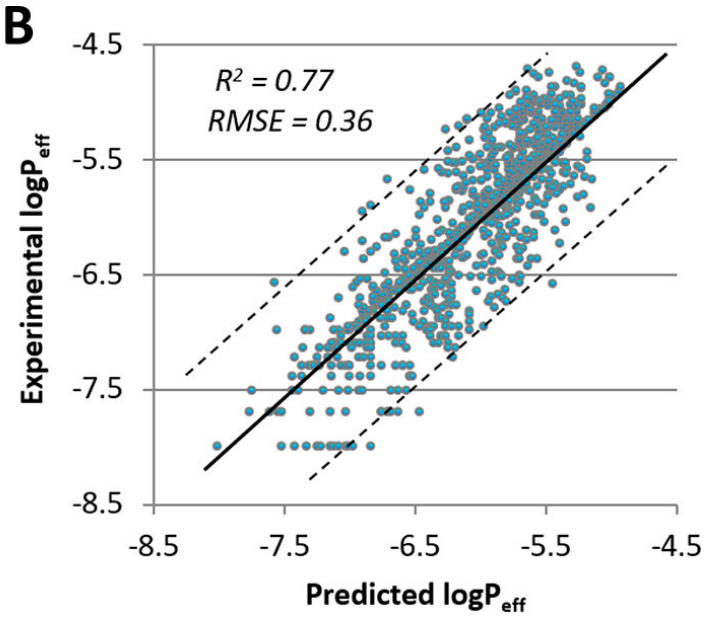
Integy_Hphob (Hydrophobic integy moment)

SlogP (octanol/water partition coefficient)

ChAx basic pKa (pKa of an basic center calculated by ChemAxon)

vsa_pol (approximation to the polar VDW surface area)

a_heavy (number of heavy atoms)



Similarly, cell permeability was predicted using six parameters.

The final model included two 3D properties:

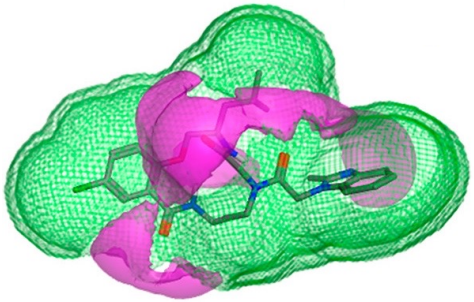
- **solvent-accessible hydrophobic area**
- **biased distribution of the hydrophobic regions**

The remaining four 2D properties:

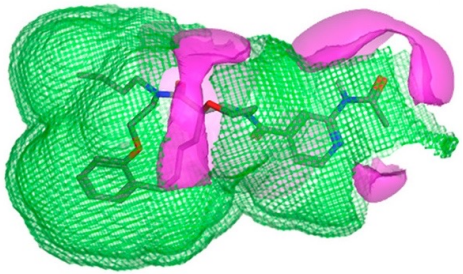
- **calculated lipophilicity**

- **pK_a of basic center**
- **number of heavy atoms**
- **surface area of polar region**

Biased Distribution of Polar Regions



A

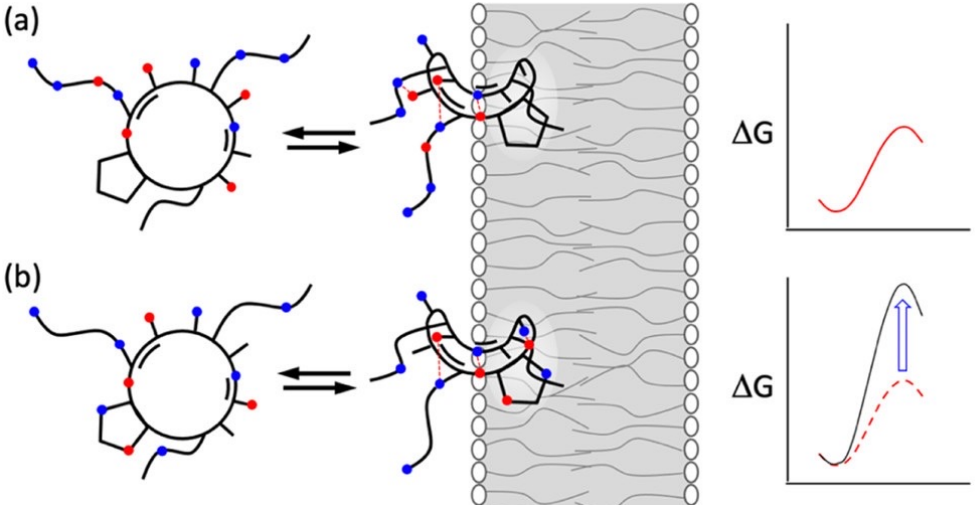


B

More biased distribution of hydrophilic area

B showed 60-fold higher P_{eff}.

	A	B
$logP_{eff}$	–7.15	–5.35
Integy_Hphil	0.63	4.45
ASA_H	645	638
clogP	4.23	3.68
a_heavy	39	38
SA_HBD	21.4	38.6
TPSA	105	101

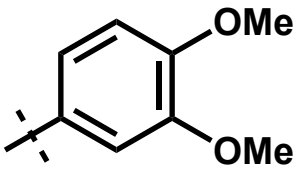
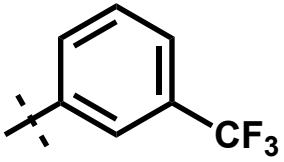
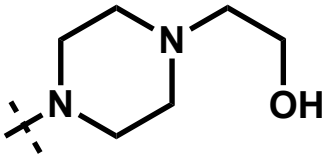
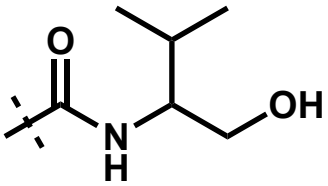
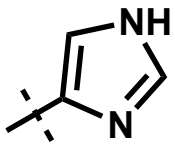
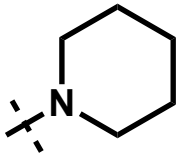
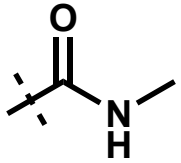
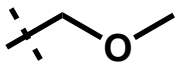


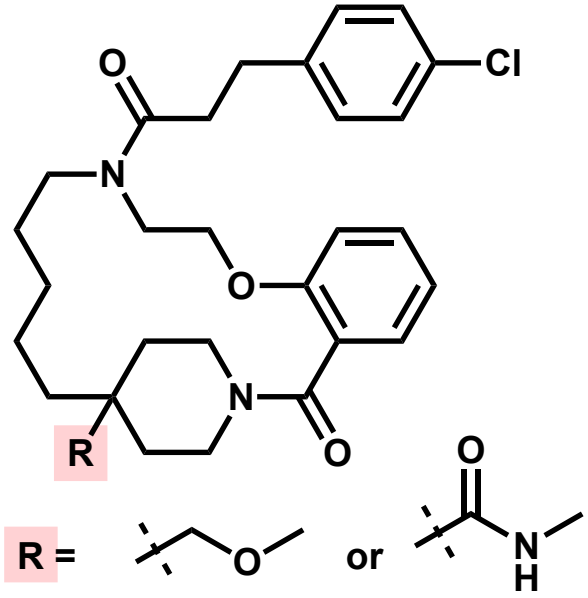
●● :relatively polar atoms

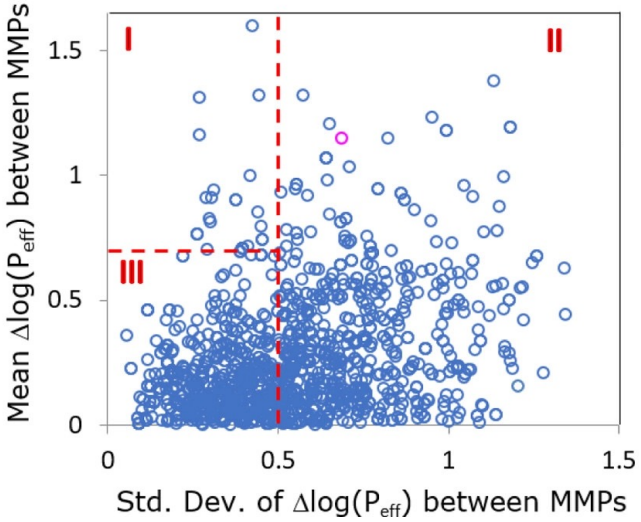
A molecule with more biased distribution of polar area can insert into membrane by its nonpolar part with lower destabilization.

Effect of Substituting Groups

Pairs of molecules with a single difference in substituting group were examined (examples shown below).

substituting group with lower P_{eff}	substituting group with higher P_{eff}	average $\Delta logP_{eff}$	StdDev of $\Delta logP_{eff}$
		1.31	0.27
		0.85	0.44
		1.38	1.13
		1.15	0.69



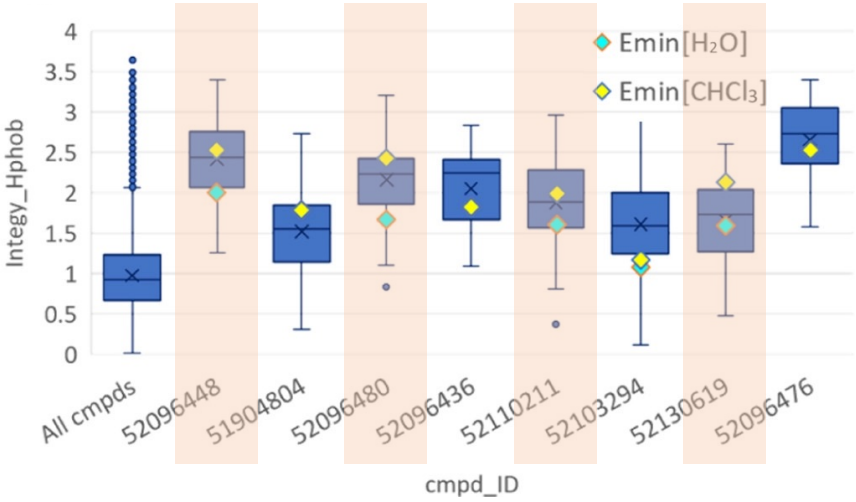


Effect of substitution on P_{eff} can be classified as following:

I. **Always large impact**
II. **Large impact depending on the other part of molecule**
III. **Small or no impact**

Limitations for the Prediction

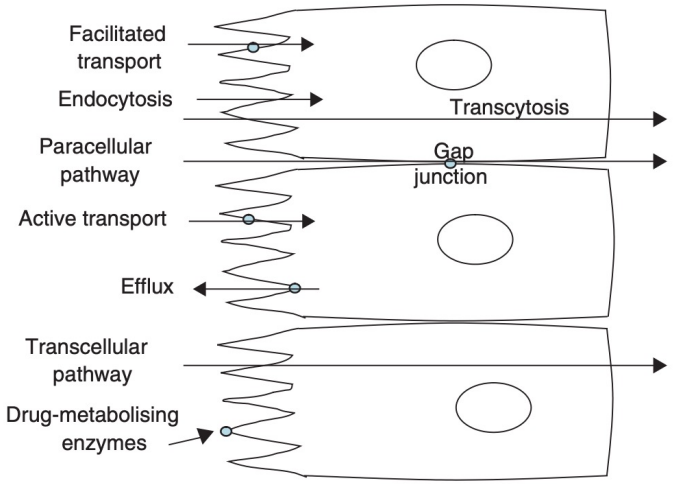
- solvent-dependent conformational change



difference of calculated 3D property in H_2O and in $CHCl_3$ for some molecules

Models using only 3D conformations calculated in H_2O may not completely predict permeability.

- neglection of other mechanisms for drug uptake

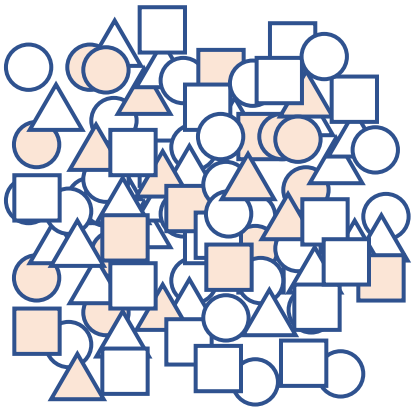


Other than passive permeation through membrane, <u>active uptake</u> by transporters or <u>efflux</u> through cell membrane can take place.
PAMPA cannot take these factors into account.

Summary and Perspective

Prediction of cell membrane permeability based on a large set of compounds were achieved using calculated 3D properties as well as the previously used 2D properties.

This prediction may be applicable for selecting more possible drug candidates for assays, saving the cost and time for preparation of physical compounds.

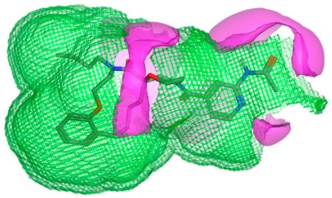


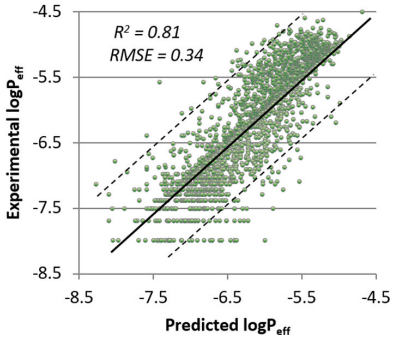
drug candidates

filtration by predicted membrane permeability

assessment of permeability with cells

rational substitution of functional group

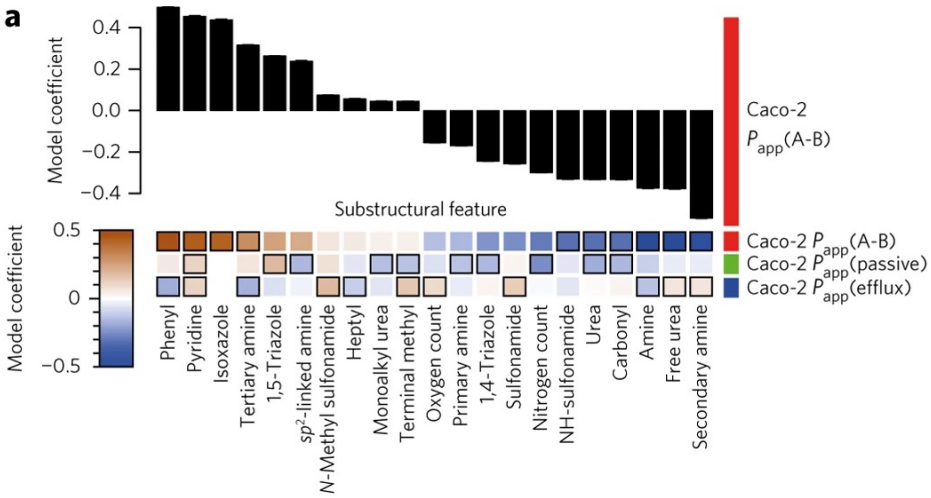




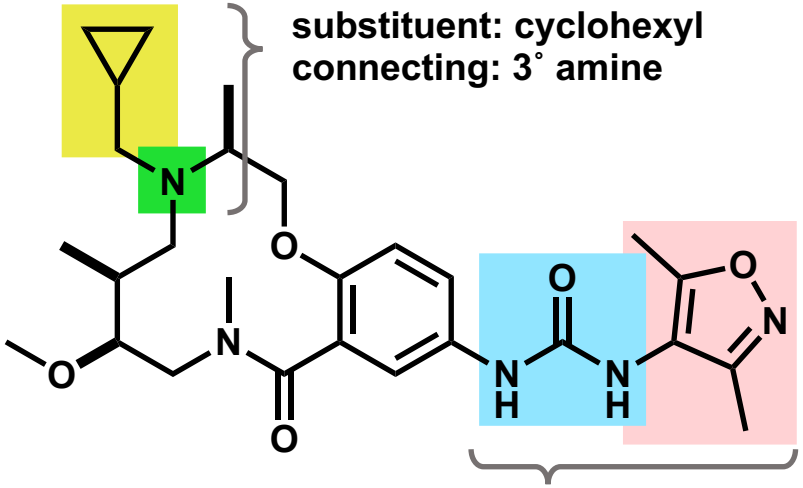
Appendix

Effect of Substitution

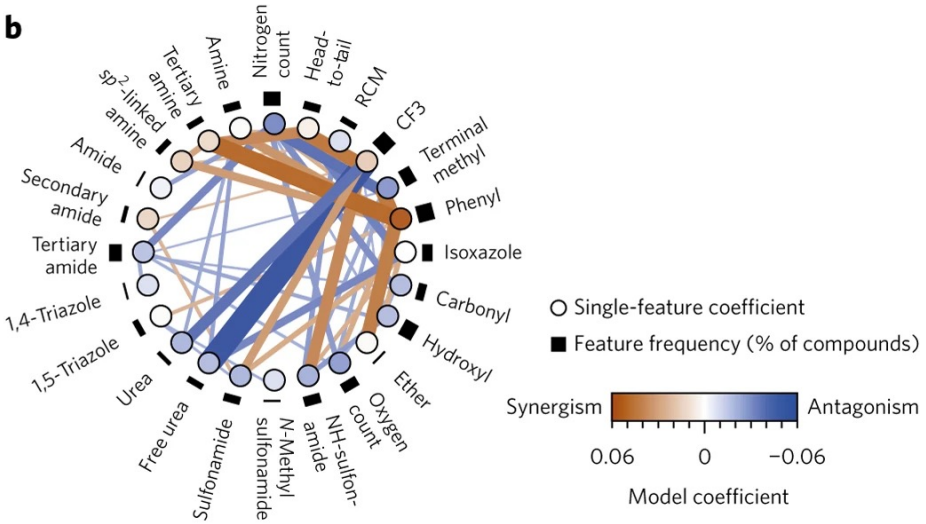
effect of each substituting group on permeability



example of "substituting groups" and its partner "connecting groups"



combinatorial effect of substituting groups and connecting groups



Combination of connecting group and substituting group, as well as substituting group alone had an effect on permeability.