Exploring the Biodiversity in the Traditional Medicine: an *in silico* Target Identification Approach

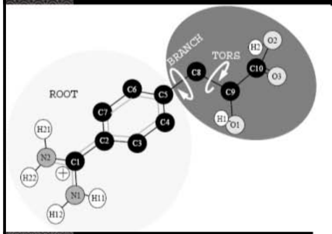
Jung-Hsin Lin
Division of Mechanics, Research Center for Applied Sciences &
Institute of Biomedical Sciences, Academia Sinica
School of Pharmacy, National Taiwan University

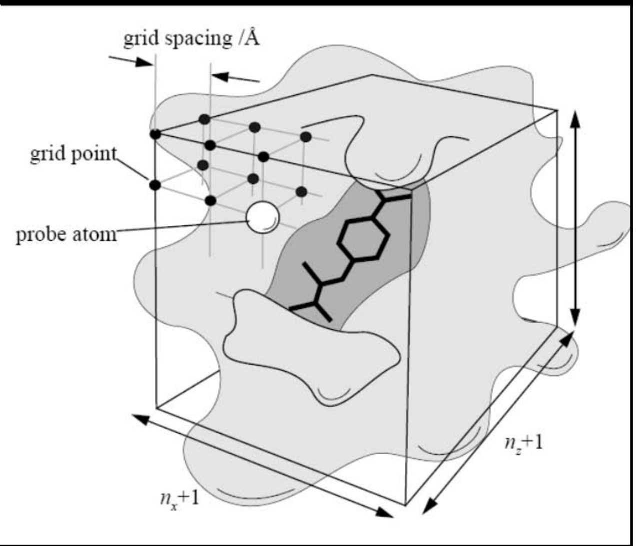
Biodiversity Cyberinfrastructure (BioCI) Southeast Asia Institute Program, Dec. 3, 2011

Outline

- The molecular docking methodology
- Virtual screening of natural products
- Identification of protein targets of natural products
- Multiple action natural products and beyond

The Flexible Docking Problem





AutoDock Scoring Function

- A free energy-based empirical approach

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{sol}$$

$$\Delta G_{vdw} = W_{vdw} \times \sum_{i,j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right)$$

$$\Delta G_{hbond} = W_{hbond} \times \sum_{i,j} E(t) \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} - E_{hbond} \right)$$

$$\Delta G_{elec} = W_{elec} \times \sum_{i,j} \frac{q_i q_j}{\varepsilon(r_{ij}) r_{ij}}$$

$$\Delta G_{tor} = W_{tor} \times N_{tor}$$

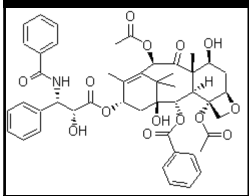
$$\Delta G_{sol} = W_{sol} \times \sum_{i,j} \left(S_i V_j + S_j V_i \right) e^{-r_{ij}^2 / 2\sigma^2}$$

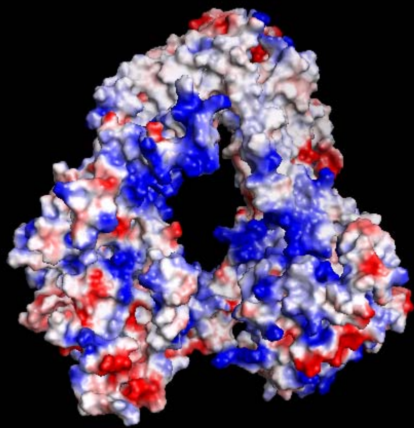
$$\Delta G_{obs} = RT \ln K_D$$

W_{vdw}	0.1485
W_{hbond}	0.0656
W_{elec}	0.1146
W_{tor}	0.3113
W_{sol}	0.1711

J. Comput. Chem. **19:** 1639-1662 (1998)

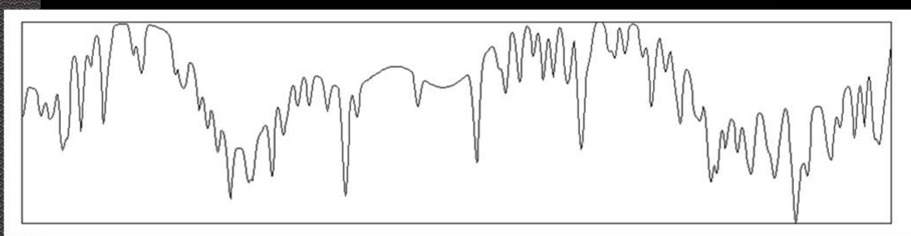
Prediction of binding pose could be challenging: the case of P-glycoprotein with Paclitaxel (Taxol)





Characteristics of Biological Complex Problems

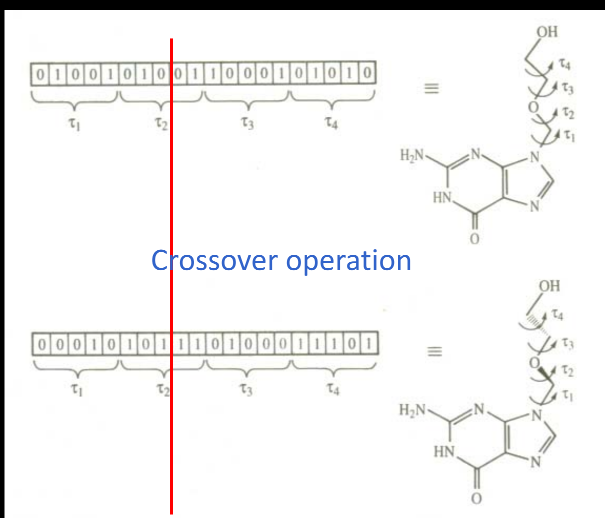
- The potential energy function is extremely rugged.
- The potential energy surface is usually highly asymmetric.
- The true global minimum is often surrounded by many deceptive local minima.
- The biological complex problems are mostly in the space of high dimensionality.



Genetic Algorithm

1. [Start] Generate random population of *n* chromosomes (suitable solutions for the problem)
2. [Fitness] Evaluate the fitness *f(x)* of each chromosome *x* in the population
3. [New population] Create a new population by repeating following steps until the new population is complete
   a. [Selection] Select two parent chromosomes from a population according to their fitness (the better fitness, the bigger chance to be selected)
   b. [Crossover] With a crossover probability cross over the parents to form new offspring (children). If no crossover was performed, offspring is the exact copy of parents.
   c. [Mutation] With a mutation probability mutate new offspring at each locus (position in chromosome).
   d. [Accepting] Place new offspring in the new population
4. [Replace] Use new generated population for a further run of the algorithm
5. [Test] If the end condition is satisfied, **stop**, and return the best solution in current population
6. [Loop] Go to step **2**

"Chromosomes" for Flexible-Ligand Docking



Leach, 2001.

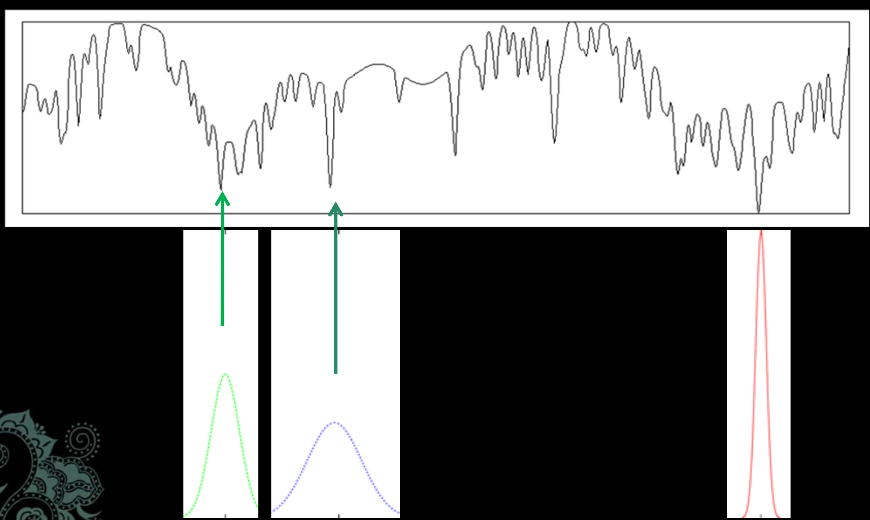
The Evolutionary Gaussians Algorithm

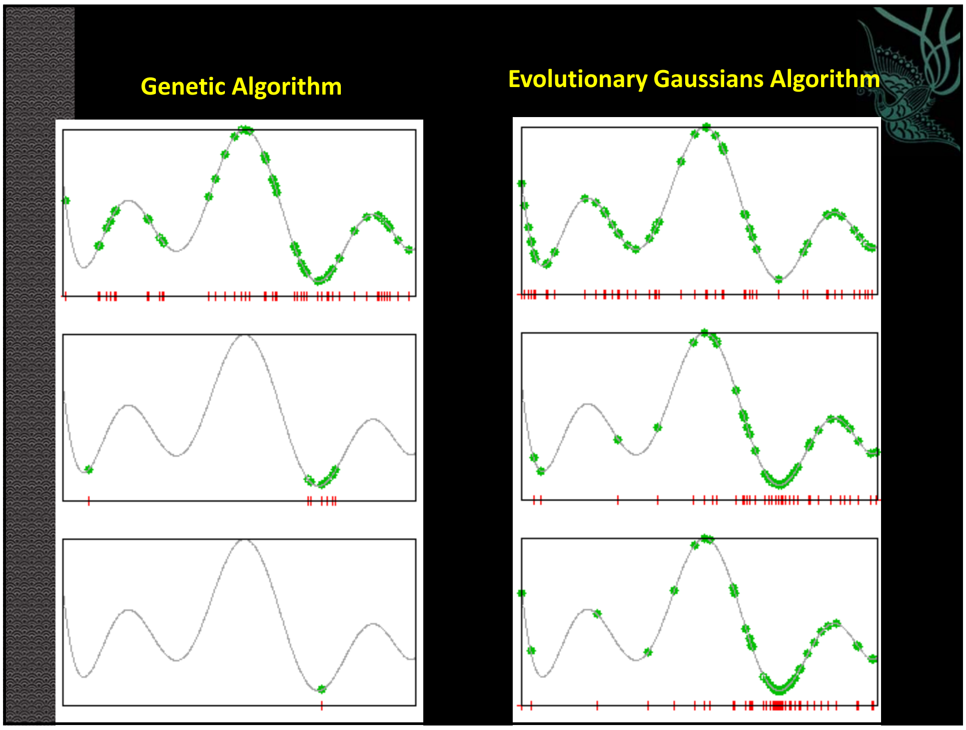
- n individuals, denoted by $\boldsymbol{s_1}, \boldsymbol{s_2}, \ldots, \boldsymbol{s_n}$, are generated. Each $\boldsymbol{s_i}$ is a vector corresponding to a point in the domain of the objective function f. In order to achieve a scale-free representation, each component of $\boldsymbol{s_i}$ is linearly mapped to the numerical range of [0,1].
- The individuals in each generation of population are then sorted in the ascending order based on the values of the energy function on evaluated on these individuals. Let $\boldsymbol{t_1}, \boldsymbol{t_2}, \ldots \boldsymbol{t_n}$ denote the ordered individuals and we have $f(\boldsymbol{t_1}) < f(\boldsymbol{t_2}) < f(\boldsymbol{t_n})$.
- n Gaussian distributions, denoted by $G_1, G_2, \ldots G_n$, are generated before the new generation of population is created. The center of each Gaussian distribution is selected randomly and independently from $\boldsymbol{t_1}, \boldsymbol{t_2}, \ldots \boldsymbol{t_n}$, where the probability is not uniform but instead follows a discrete diminishing distribution, $n : n\text{-}1 : \ldots : 1$.

$$G_i(\mathbf{x}) = \left(\frac{1}{\sqrt{2\pi} \cdot \sigma_i}\right) \exp\left(-\frac{(\mathbf{x} - \mathbf{t}_k)^2}{2\sigma_i^2}\right)$$

$$\sigma_i^2 = \alpha + \frac{(\beta - \alpha)(k-1)}{n-1}$$

Nucleic Acids Research 33: W233-W238 (2005)





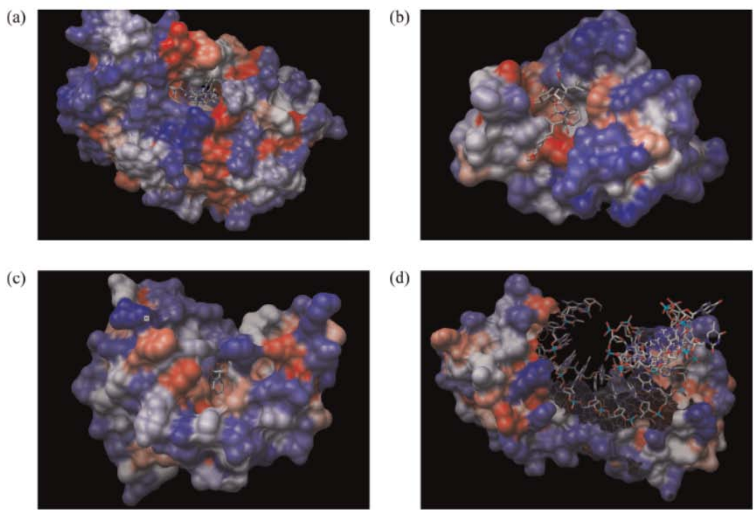


Figure 2. Molecular graphics display of the four benchmark cases: (**a**) HIV-II protease complexed with its inhibitor L-735,524 (PDB ID: 1HSH); (**b**) FKBP-FK506, an immunophilin-immunosuppressant complex (PDB ID: 1FKF); (**c**) Complex formed between phospholipase A2 and aspirin (PDB ID: 1OXR); and (**d**) TATA-box binding protein (YTBP) complexed with DNA containing a TATA-box (PDB ID: 1YTB).

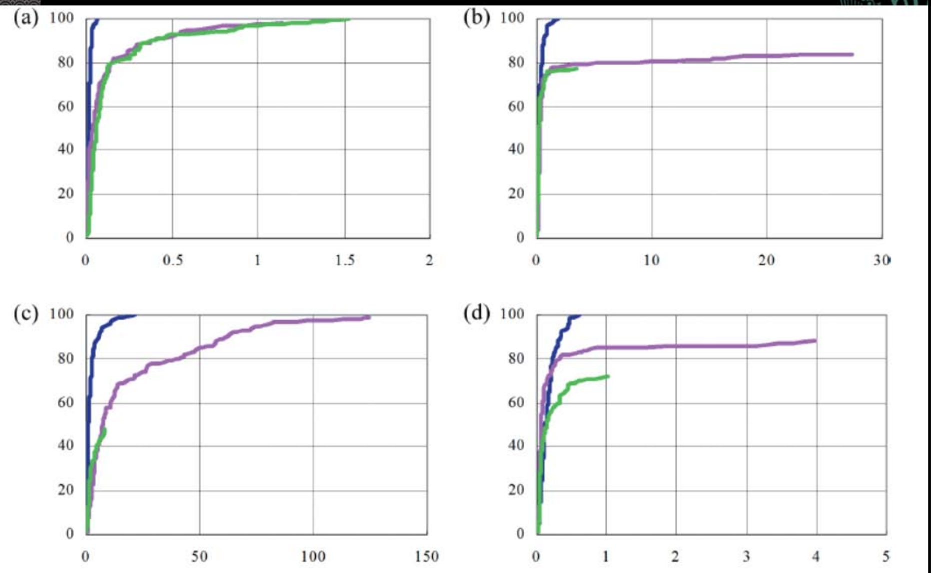


Figure 3. Number of runs to reach convergence versus the number of energy evaluations consumed (in units of 10^7): blue, MEDock results; magenta, LGA results (with parameters tuned); green, LGA results (with default parameters). **(a)** HIV-II protease complexed with its inhibitor L-735,524 (PDB ID: 1HSH); **(b)** FKBP-FK506, an immunophilin-immunosuppressant complex (PDB ID: 1FKF); **(c)** complex formed between phospholipase A2 and aspirin (PDB ID: 1OXR); and **(d)** TATA-box binding protein (YTBP) complexed with DNA containing a TATA-box (PDB ID: 1YTB).

Functional form of AutoDock4 scoring function

$$\begin{aligned}
\Delta G_{bind} = {} & W_{vdw} \times \sum_{i,j}\left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) \\
& + W_{H-bond} \times \sum_{i,j} E(t)\left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}\right) \\
& + W_{estat} \times \sum_{i,j} \frac{q_i q_j}{\varepsilon(r_{ij}) r_{ij}} \\
& + W_{desol} \times \sum_{i,j} \left(S_i V_j + S_j V_i\right) e^{\left(-r_{ij}^2/2\sigma^2\right)} \\
& + W_{tor} \times N_{tors}
\end{aligned},$$

The Gasteiger charge model was used.

The desolvation energy is accounted for by calculating the surrounding volume of an atom (V_i), weighted by the atomic solvation parameter (S_i) and an exponential term with a distance weighting factor σ (0.35Å in AutoDock4).

$$S_i = (ASP_k + QASP \times |q_i|), \quad k = \mathrm{C,A,N,O,S,H}$$

Huey *et al.*, *J. of Comput. Chem.* **28**: 1145-1152 (2007)

Least square (LS) regression

Gauss, 1800

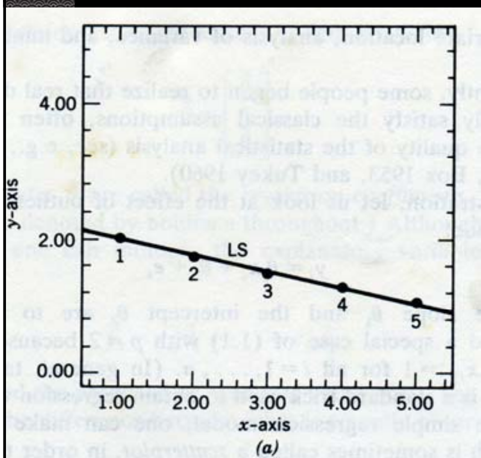
$$y_i = w_1 x_{i1} + w_2 x_{i2} + \cdots + w_p x_{ip} + e_i$$

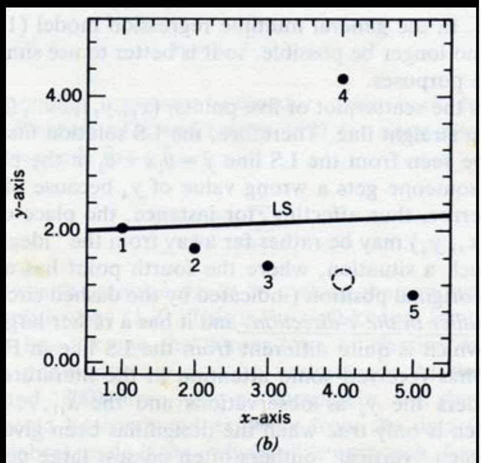
$i = 1, \ldots, n$ Sample size

$$r_i = y_i - \hat{y}_i \qquad \mathbf{w} = \left(w_1, w_2, \cdots, w_p\right)$$

$$\underset{\mathbf{w}}{Minimize} \sum_{i=1}^{n} r_i^2$$

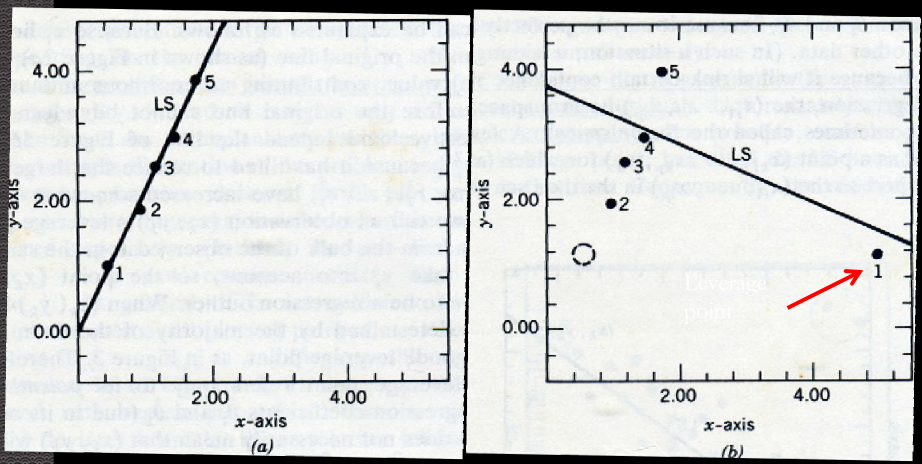
The problem of least square (LS) regression





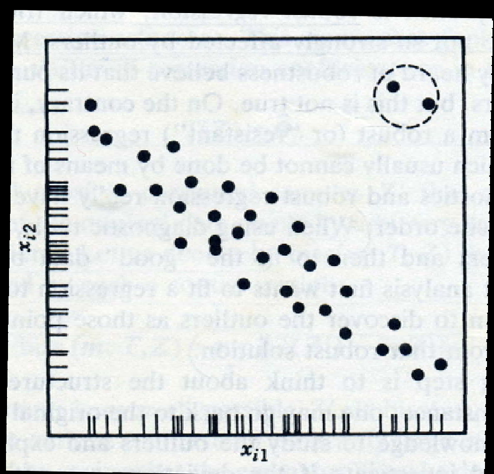
Outlier in the y-direction

The problem of least square (LS) regression



Outlier in the x-direction

Leverage points in two dimension

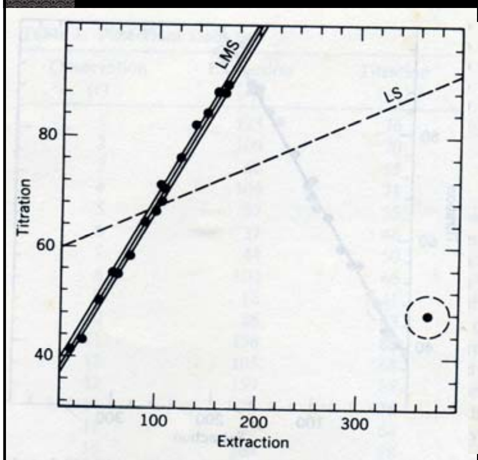


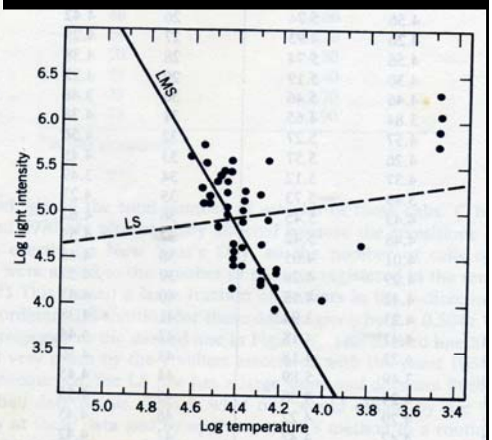
Leverage points are not easy to detect by checking the ranges of variables

Regression diagnostics versus robust regression

- Regression outliers pose a serious threat to standard least square analysis.
- Regression diagnostics: Use some quantity to pinpoint the influential points, remove the outliers, and then LS.
- Robust regression: Devise estimators not so strongly affected by outliers. Fit to the majority of data.

Robust regression





Cross validations

Combination	LOO-CV		MCCV	
	S_{PRESS}	q^2	S_{PRESS}	q^2
AutoDock4^{RGG}	1.732	0.675	1.782	0.657
AutoDock4^{RAP}	1.707	0.684	1.749	0.670
AutoDock4^{RRP}	1.711	0.683	1.755	0.668

All RMSE values and S_{PRESS} are in kcal/mol.

Assessment with external complexes

Performance of the robust AutoDock4 scoring functions and two other recent scoring functions tested with the PDBbind data sets

scoring function	N_{train}	N_{test}	R_p	R_s	SD	ME
AutoDock4^{RGG}	147	1427	0.604	0.615	1.61	1.26
AutoDock4^{RAP}	147	1427 v2009	0.606	0.617	1.60	1.25
AutoDock4^{RRP}	147	1427	0.595	0.610	1.62	1.26
original AutoDock4^{GG}	187	1427	0.562	0.594	1.66	1.31
sfc_290m	290	919	0.492	0.555		
sfc_229m	229	919 v2005	0.501	0.558		
sfc_frag	130	919	0.525	0.576		
PDSE-SVM	278	977 v2005	0.517	0.535	1.84	1.42

R_p: Pearson's correlation coefficient; R_s: Spearman's correlation coefficient
SD (standard error) and ME (mean error) are presented in the *pKd* unit. The binding free energy in kcal/mol at 298 K was converted to the *pKd* unit by dividing with the factor of -1.36.

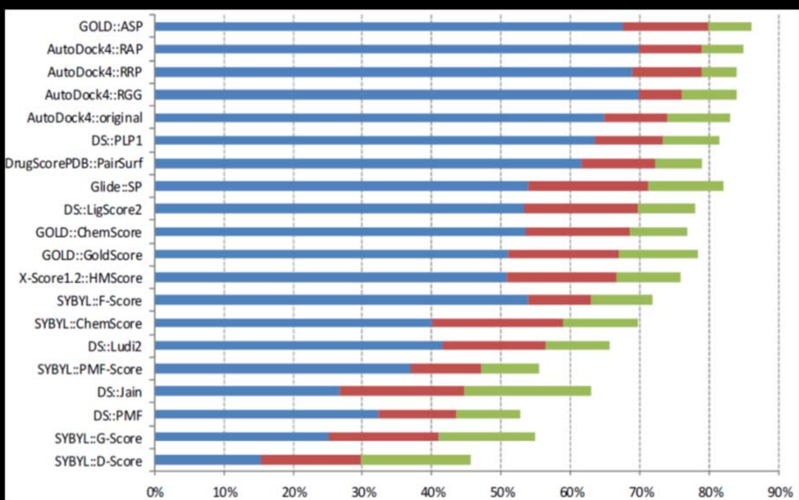
Sotriffer *et al.*, *Proteins* **73:** 395-419 (2008).
Das *et al.*, *J. of Chemi. Inf.Model.* **50:** 298-308 (2010).

Binding pose prediction with Wang *et al.* 2003 decoys

scoring function	success rate (%) for different rmsd criteria ≦1Å	≦1.5Å	≦2Å	≦2.5Å	≦3Å
DrugScore[CSD]	83	85	87		
AutoDock4[RAP]	83	85	87	87	87
AutoDock4[RGG]	80	82	86	86	86
AutoDock4[RRP]	79	81	84	85	85
original AutoDock4[GG]	74	76	79	79	79
Cerius2/PLP	63	69	76	79	80
SYBYL/F-Score	56	66	74	77	77
Cerius2/LigScore	64	68	74	75	76
DrugScore	63	68	72	74	74
Cerius2/LUDI	43	55	67	67	67
X-Score	37	54	66	72	74
AutoDock3	34	52	62	68	72
Cerius2/PMF	40	46	52	54	57
SYBYL/G-Score	24	32	42	49	56
SYBYL/ChemScore	12	26	35	37	40
SYBYL/D-Score	8	16	26	30	41

[a] Except for the results of the AutoDock4 scoring functions, the results of DrugScore[CSD] and other scoring functions were taken from Velec *et al.* and Wang *et al.*, respectively.
[b] Scoring functions are sorted by the number of cases under 2Å.

Binding pose prediction with Cheng *et al.* 2009 decoys



Class-dependence of robust scoring functions

scoring function	success rate (%; rmsd ≦2Å) overall (100)	hydrophilic (44)	mixed (32)	hydrophobic (24)
AutoDock4[RAP]	87	89	91	79
AutoDock4[RGG]	86	86	91	79
AutoDock4[RRP]	84	84	91	75
original AutoDock4[GG]	79	77	81	79
Cerius2/PLP	76	77	78	71
SYBYL/F-Score	74	75	75	71
Cerius2/LigScore	74	77	75	67
DrugScore[PDB]	72	73	81	58
Cerius2/LUDI	67	75	66	54
X-Score	66	82	59	46
AutoDock3	62	73	53	54
Cerius2/PMF	52	68	44	33
SYBYL/G-Score	42	55	34	29
SYBYL/ChemScore	35	32	34	42
SYBYL/D-Score	26	23	28	29

[a] Data were adopted from Wang *et al.*[23] except for AutoDock4 scoring functions.

[b] Scoring functions are sorted according to the overall success rates.

JOURNAL OF CHEMICAL INFORMATION AND MODELING

ARTICLE

pubs.acs.org/jcim

Robust Scoring Functions for Protein–Ligand Interactions with Quantum Chemical Charge Models

Jui-Chih Wang,[†] Jung-Hsin Lin,[*,§,‡,∥] Chung-Ming Chen,[†] Alex L. Perryman,[⊥] and Arthur J. Olson[⊥]

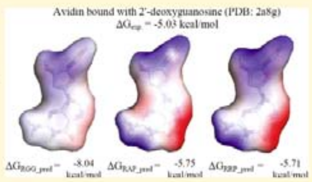
[†]Institute of Biomedical Engineering and [‡]School of Pharmacy, National Taiwan University, Taipei, Taiwan

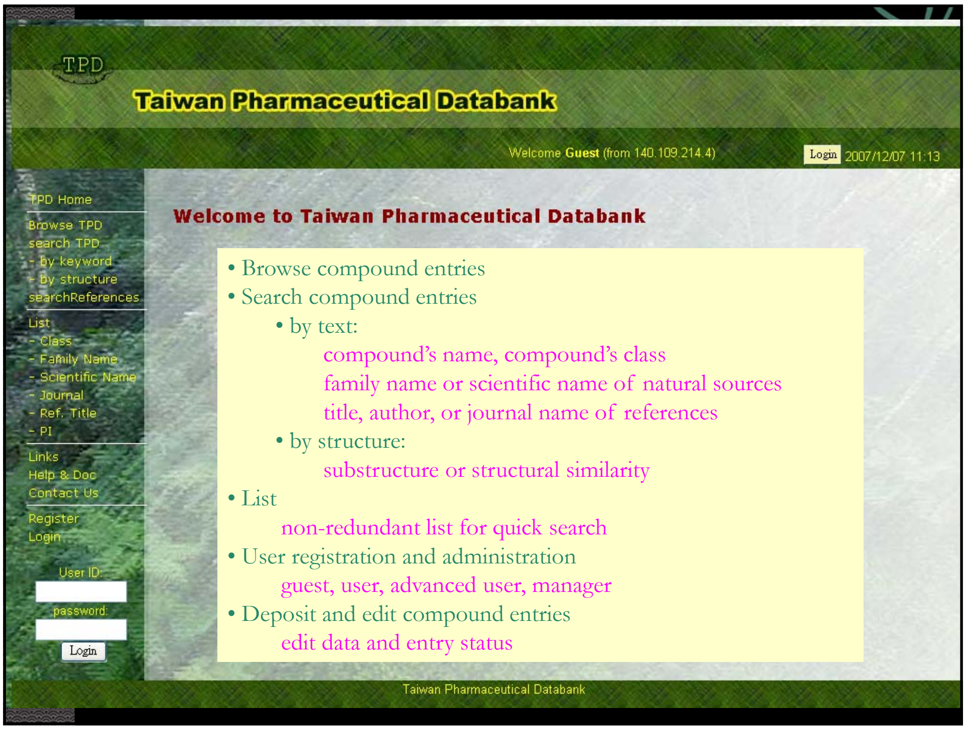
[§]Division of Mechanics, Research Center for Applied Sciences and [∥]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

[⊥]Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, United States

Supporting Information

ABSTRACT: Ordinary least-squares (OLS) regression has been used widely for constructing the scoring functions for protein–ligand interactions. However, OLS is very sensitive to the existence of outliers, and models constructed using it are easily affected by the outliers or even the choice of the data set. On the other hand, determination of atomic charges is regarded as of central importance, because the electrostatic interaction is known to be a key contributing factor for biomolecular association. In the development of the AutoDock4 scoring function, only OLS was conducted, and the simple Gasteiger method was adopted. It is therefore of considerable interest to see whether more rigorous charge models could improve the statistical performance of the AutoDock4 scoring function. In this study, we have employed two well-established quantum chemical approaches, namely the restrained electrostatic potential (RESP) and the Austin-model 1-bond charge correction (AM1-BCC) methods, to obtain atomic partial charges, and we have compared how different charge models affect the performance of AutoDock4 scoring functions. In combination with robust regression analysis and outlier exclusion, our new protein–ligand free energy regression model with AM1-BCC charges for ligands and Amber99SB charges for proteins achieve lowest root-mean-squared error of 1.637 kcal/mol for the training set of 147 complexes and 2.176 kcal/mol for the external test set of 1427 complexes. The assessment for binding pose prediction with the 100 external decoy sets indicates very high success rate of 87% with the criteria of predicted root-mean-squared deviation of less than 2 Å. The success rates and statistical performance of our robust scoring functions are only weakly class-dependent (hydrophobic, hydrophilic, or mixed).





TPD: Taiwan Pharmaceutical Databank

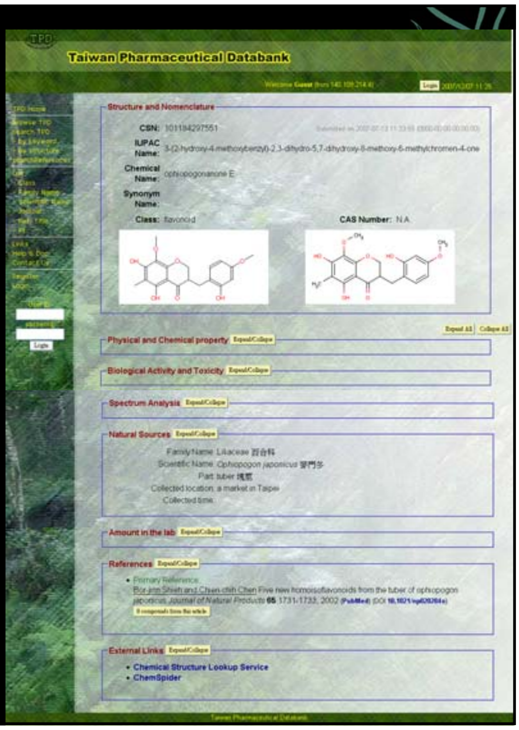
http://tpd.mc.ntu.edu.tw/

- Web-based Database
  - LAMP: Linux OS, Apache web server, MySQL relational database, PHP
    - Dynamic HTML, JAVA scripts
  - JAVA applets are used for structure drawing and presentation
    - JME and Marvin from ChemAxon
- User interface in Chinese and/or in English
- Currently TPD contains entries for
  - 1600+ compounds which are classified into 130 classes
  - 200+ natural sources which are from 84 families
  - 340+ references which are published in 37 journals

TPD: Taiwan Pharmaceutical Databank

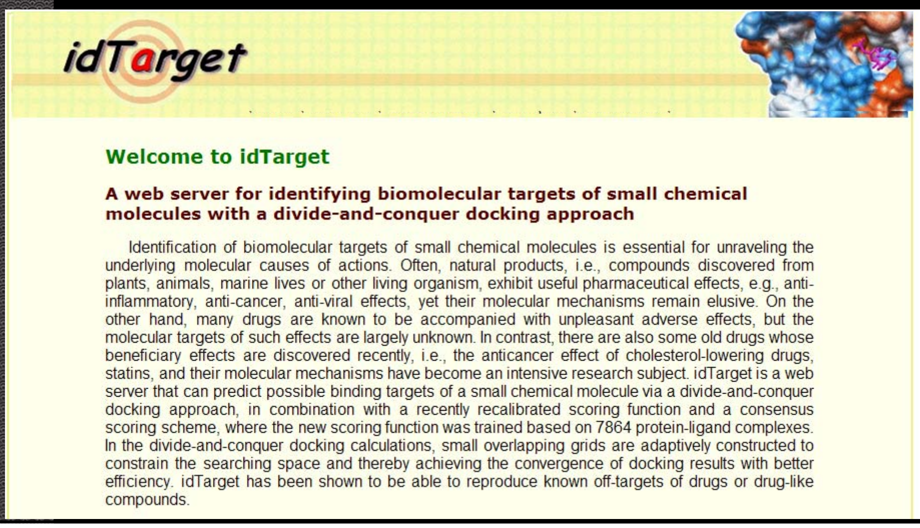
The information for each compound entry may include:

- Structure and Nomenclature
  - Structure (still image and interactive presentation), compound's class chemical name, IUPAC name, synonym, CAS Registry Number
- Physical and Chemical Properties
  - molecular formula, molecular weight, melting point, solubility, store condition
- Biological Activity and Toxicity
  - text and/or image
- Spectrum Analysis
  - text and/or image
- Natural Sources
  - family name, scientific name, part used, collected time and location
- Amount in the lab
- References
  - reference information and links to PubMed and/or DOI if available
- External links
  - Chemical Structure Lookup Service, ChemSpider

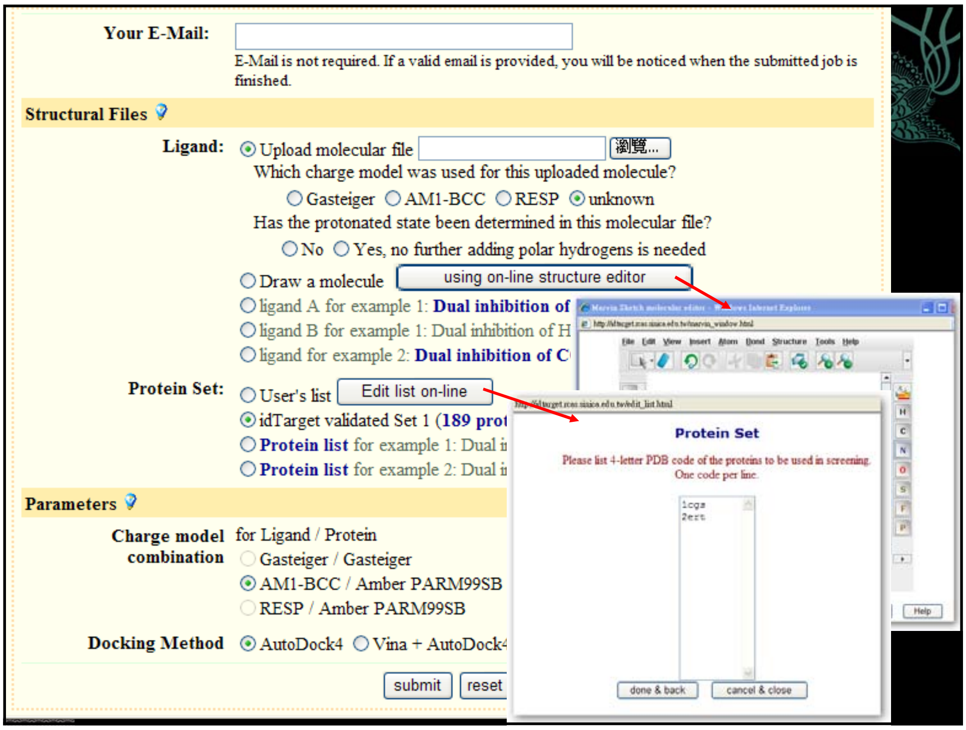


idTarget: A web server for identifying biomolecular targets of small chemical molecules with a divide-and-conquer docking approach

http://idtarget.rcas.sinica.edu.tw/




PDB
PROTEIN DATA BANK
PDB-101
An Information Portal to Biological Macromolecular Structures
Search
Biological Macromolecular Resource
Full Description
Featured Molecules
Structural View of Biology
Molecule of the Month
PDB Pioneers
Full Article
Protein Structure Initiative Featured System
New Structures
New Features
wwPDB News


Your E-Mail:
E-Mail is not required. If a valid email is provided, you will be noticed when the submitted job is finished.
Structural Files
Ligand: Upload molecular file 瀏覽...
Which charge model was used for this uploaded molecule?
Gasteiger AM1-BCC RESP unknown
Has the protonated state been determined in this molecular file?
No Yes, no further adding polar hydrogens is needed
Draw a molecule using on-line structure editor
ligand A for example 1: Dual inhibition of
ligand B for example 1: Dual inhibition of H
ligand for example 2: Dual inhibition of C
Protein Set: User's list Edit list on-line
idTarget validated Set 1 (189 prot
Protein list for example 1: Dual i
Protein list for example 2: Dual i
Parameters
Charge model combination for Ligand / Protein
Gasteiger / Gasteiger
AM1-BCC / Amber PARM99SB
RESP / Amber PARM99SB
Docking Method AutoDock4 Vina + AutoDock4
submit reset
Protein Set
Please list 4-letter PDB code of the proteins to be used in screening.
One code per line.
done & back cancel & close
Help

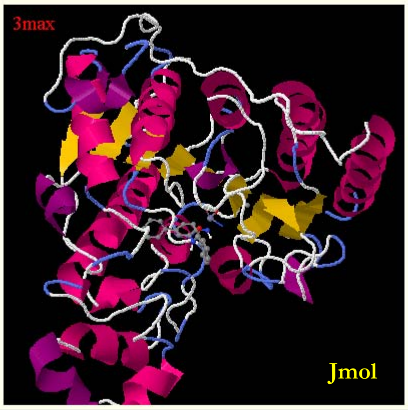
Example: Dual inhibition of HMG-CoA and HDAC

Off-target screening for 3max inhibitor

idTarget

| Home | Services | Documentaion | Examples | Contact us |

Result of 1293705965mj



This idTarget job is finished. 10 proteins were screened. You may download the result.

Rank by Energy

#	PDB ID	Energy (kcal/mol)	XScore	ligand pose	PDB Link
1	3max	-15.01	7.70	Download	PDB
2	1hw9	-10.08	6.59	Download	PDB
3	1ajv	-8.37	6.94	Download	PDB
4	1cim	-8.21	6.34	Download	PDB
5	1tng	-8.00	6.20	Download	PDB
6	1hhi	-7.86	6.44	Download	PDB
7	1mcb	-7.75	7.19	Download	PDB
8	1epo	-7.13	6.52	Download	PDB
9	4hmg	-6.65	6.07	Download	PDB
10	1aee	-4.68	5.52	Download	PDB

Statins: the Aspirin of the 21st century? - Windows Internet Explorer

http://www.cbc.ca/health/story/2008/11/10/f-statins.html

檔案(F) 編輯(E) 檢視(V) 我的最愛(A) 工具(T) 說明(H)

cbc.ca | News | Sports | Entertainment | Radio | TV | My Region | Search cbc.ca | Radio-Canada.ca

Member Centre: Login | Sign Up
Toronto | Change City
22.6°c Partly Cloudy
Tomorrow: 27°c Sunny

CBCnews.ca Health

Home | World | Canada | Health | Arts & Entertainment | Technology & Science | Money | Consumer Life | Diversions | Weather | Your Voice

Story Tools: EMAIL | PRINT | Text Size: S M L XL | REPORT TYPO | SEND YOUR FEEDBACK | SHARE

Statins: the Aspirin of the 21st century?

Last Updated: Monday, November 10, 2008 | 3:23 PM ET Comments 17 Recommend 37
CBC News

They're the best-selling family of drugs of all time, with annual worldwide sales estimated at more than $20 billion. Every year, Canadian doctors write more than 12 million prescriptions for statins, making them the most-prescribed drugs in the country. They're in a class of drugs that has proven very effective at lowering cholesterol levels and reducing the risk of heart attacks.

The possible effectiveness of statins is so great that surprised researchers reported in November 2008 they have stopped a four-year study two years early in order to present their findings as soon as possible on the drugs' benefits to patients.

The study, which followed nearly 18,000 patients from 27 different countries, found the strongest evidence yet that people with high levels of a particular protein are at increased

Related

Protein important marker of heart disease: researchers

IN DEPTH: Statins and the Adverse Drug Reaction Database

Health Headlines

Top Headlines | Recommended | Commented

- Canada's heart attack death rates declined rapidly after 1994 16 | 11
- 6-year-old's death believed to be linked to swine flu 30 | 20
- Contaminated onions suspected in North Bay's E. coli outbreak 14 | 18
- Staying social may keep seniors' motor skills sharp 1 | 0
- Dog, cat bites could spread MRSA 2 | 5

STATINS AND CANCER PREVENTION

*Marie-France Demierre**#, Peter D. R. Higgins‡#, Stephen B. Gruber§, Ernest Hawk|| and Scott M. Lippman¶*

Abstract | Randomized controlled trials for preventing cardiovascular disease indicated that statins had provocative and unexpected benefits for reducing colorectal cancer and melanoma. These findings have led to the intensive study of statins in cancer prevention, including recent, large population-based studies showing statin-associated reductions in overall, colorectal and prostate cancer. Understanding the complex cellular effects (for example, on angiogenesis and inflammation) and the underlying molecular mechanisms of statins (for example, 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase-dependent processes that involve geranylgeranylation of Rho proteins, and HMG-CoA-independent processes that involve lymphocyte-function-associated antigen 1) will advance the development of molecularly targeted agents for preventing cancer. This understanding might also help the development of drugs for other ageing-related diseases with interrelated molecular pathways.

Demierre *et al., Nature Rev. Cancer* **5:** 930-942 (2005)

The Risk of Cancer in Users of Statins

Matthijs R. Graaf, Annette B. Beiderbeck, Antoine C.G. Egberts, Dick J. Richel, and Henk-Jan Guchelaar

A B S T R A C T

Purpose
Several preclinical studies suggested a role for 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) in the treatment of cancer. The objective of this study was to compare the risk of incident cancer between users of statins and users of other cardiovascular medication.

Methods
Data were used from the PHARMO database, containing drug dispensing records from community pharmacies and linked hospital discharge records for residents of eight Dutch cities. The study base included all patients with one or more prescriptions for cardiovascular drugs in the period between January 1, 1985 and December 31, 1998. Cases were identified as patients in the study base with a diagnosis of incident cancer and matched with four to six controls on sex, year of birth, geographic region, duration of follow-up, and index date. The analysis was adjusted for diabetes mellitus; prior hospitalizations; comorbidity; and use of diuretics, angiotensin-converting enzyme inhibitors, calcium-channel blockers, nonsteroidal anti-inflammatory drugs, sex hormones, and other lipid-lowering drug therapies.

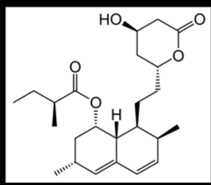
Results
In the study base, 3,129 patients were identified and matched to 16,976 controls. Statin use was associated with a risk reduction of cancer of 20% (adjusted odds ratio [OR], 0.80; 95% CI, 0.66 to 0.96). Our data suggest that statins are protective when used longer than 4 years (adjusted OR, 0.64; 95% CI, 0.44 to 0.93) or when more than 1,350 defined daily doses are taken (adjusted OR, 0.60; 95% CI, 0.40 to 0.91).

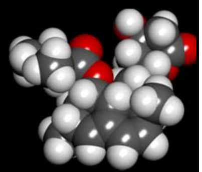
Conclusion
This observational study suggests that statins may have a protective effect against cancer.

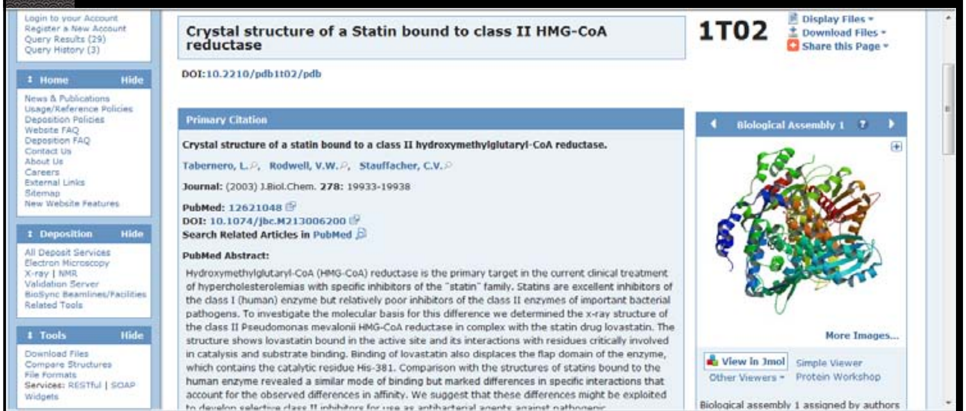
Graaf *et al.,* J. *Clin. Onco*. **22:** 2388-2394 (2004)

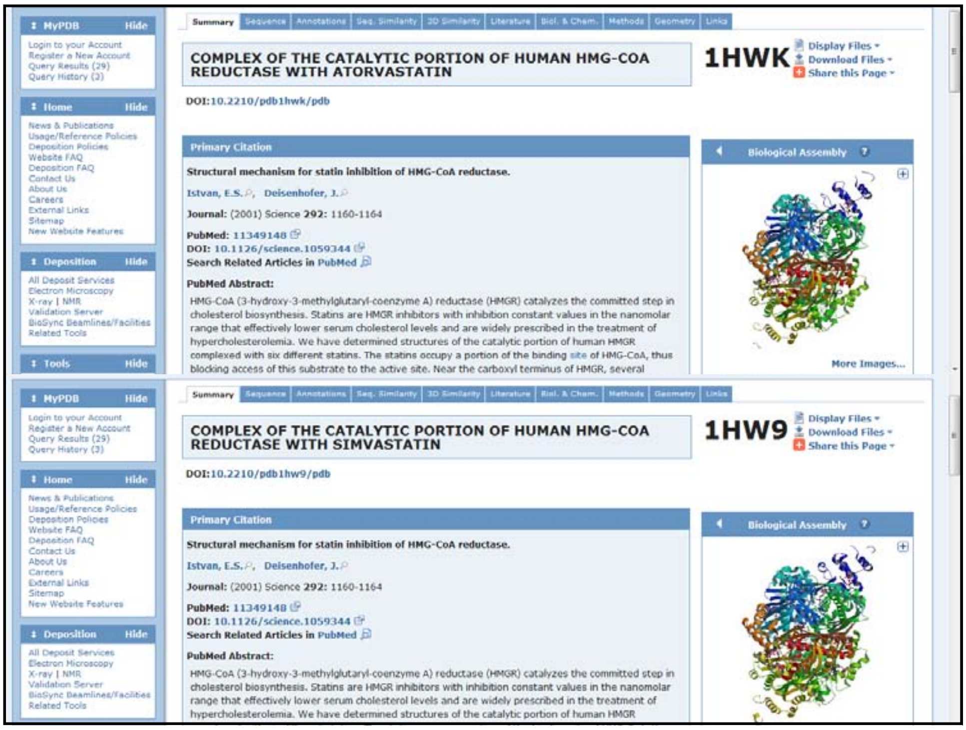
Red Yeast Rice (紅麴米、紅曲米)

- Red yeast rice is a bright reddish purple fermented rice, which acquire its color from being cultivated with the mold *Monascus purpureus*.
- In addition to its culinary use, red yeast rice is also used in traditional Chinese herbology and traditional Chinese medicine.
- Its use has been documented as far back as the Tang Dynasty in 800 AD. It is taken internally to invigorate the body, aid in digestion, and revitalize the blood. A more complete description is in the traditional Chinese pharmacopeia, Ben Cao Gang Mu, from the Ming Dynasty (1378-1644).
- In the late 1970s, researchers in the United States and Japan were isolating lovastatin from *Aspergillus* and monacolins from *Monascus*, respectively. Chemical analysis showed that lovastatin and monacolin K are identical.

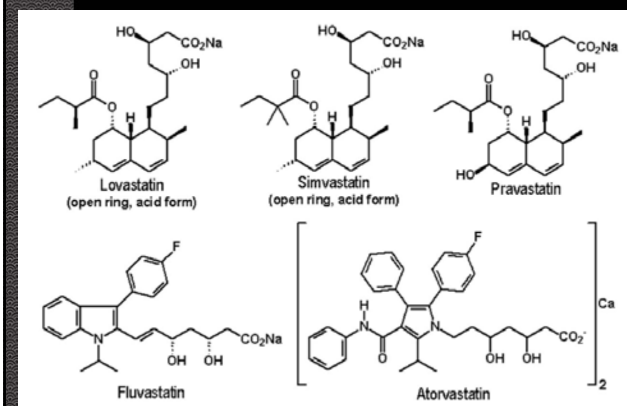








Can statins inhibit HDAC?

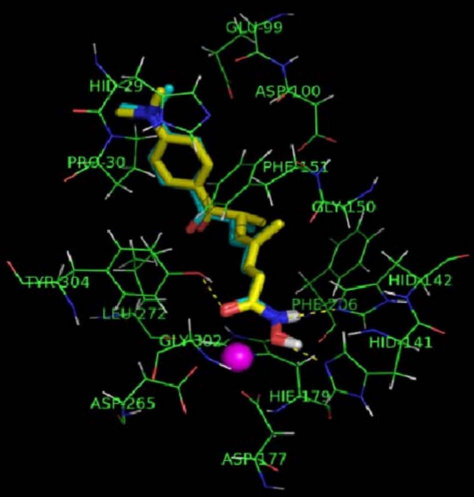


Professor Ching-Chow Chen,
Department of Pharmacology,
College of Medicine,
National Taiwan University

Validation of Docking Parameters

Structure: HDLP

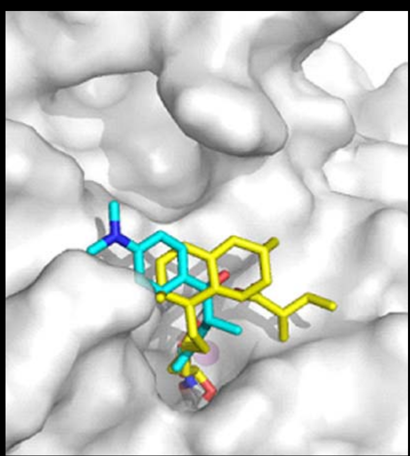
Nature **401:** 188-193 (1999)



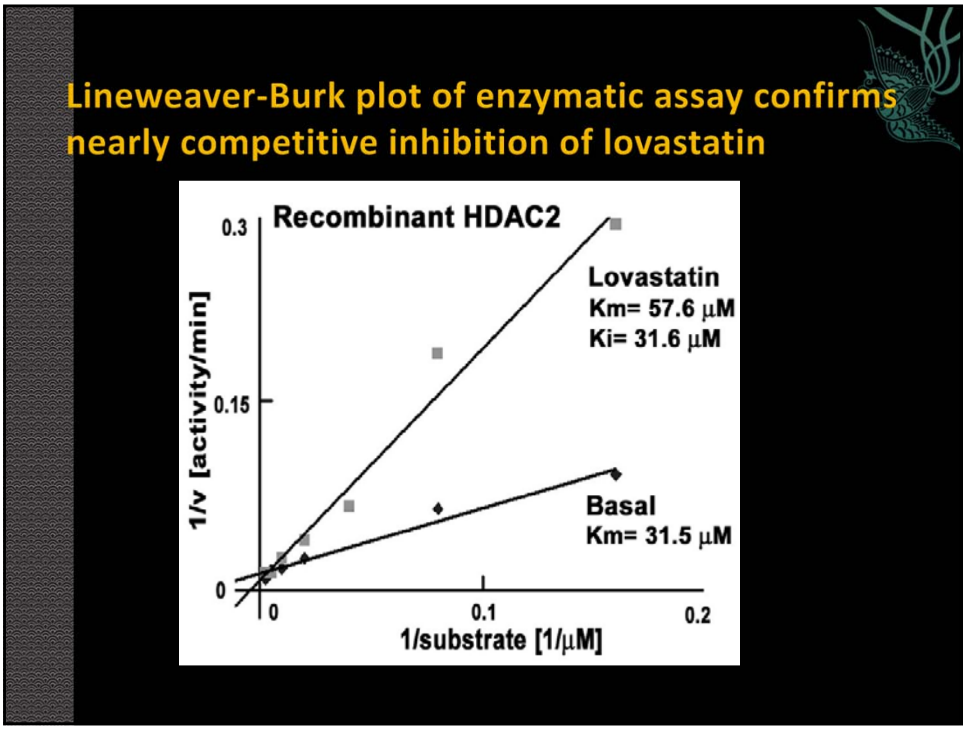
CPK: Experimental pose
Yellow: Docked pose

Lin *et al. Cancer Res.* **68:** 2375-2383 (2008)

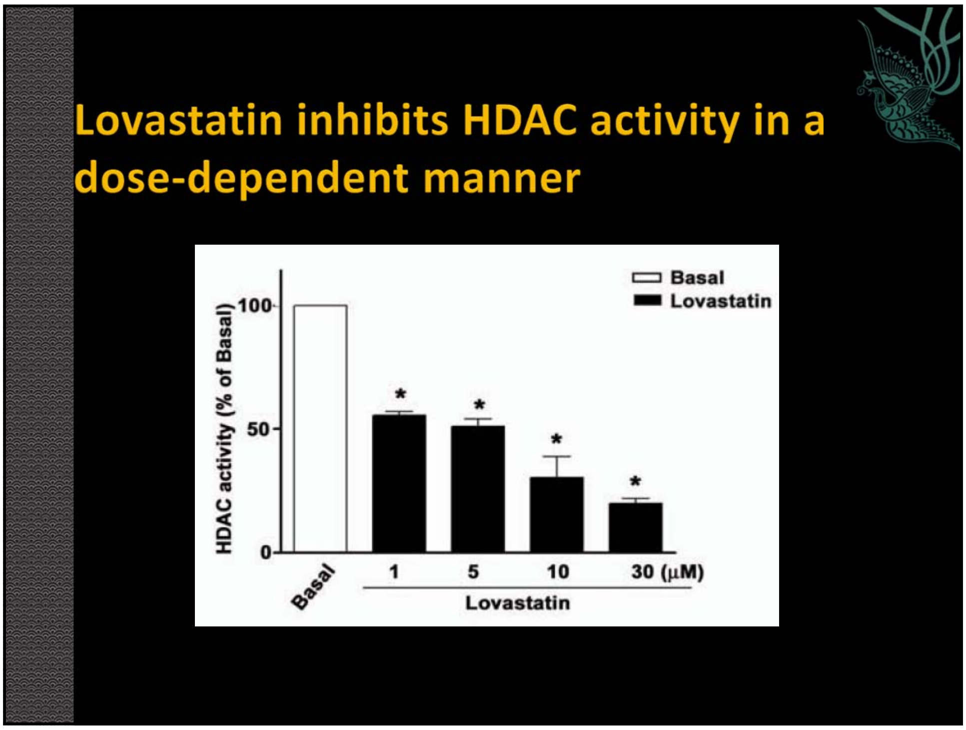
Lovastatin and TSA both can bind competitively at the catalytic site



Cyan: TSA
Yellow: Lovastatin

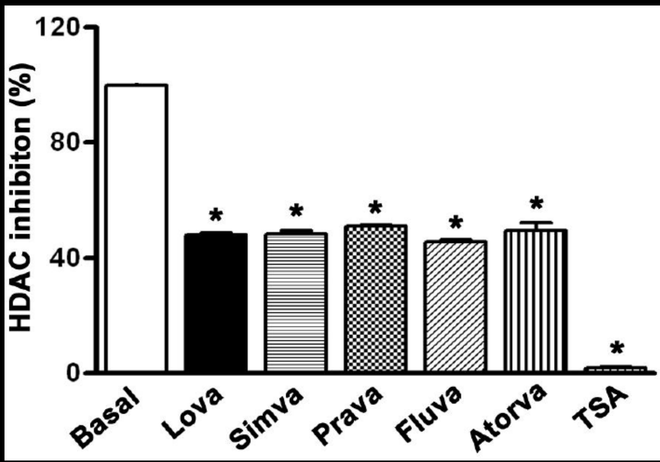


Lineweaver-Burk plot of enzymatic assay confirms nearly competitive inhibition of lovastatin
Recombinant HDAC2
0.3
0.15
0
1/v [activity/min]
Lovastatin
Km= 57.6 μM
Ki= 31.6 μM
Basal
Km= 31.5 μM
0
0.1
0.2
1/substrate [1/μM]

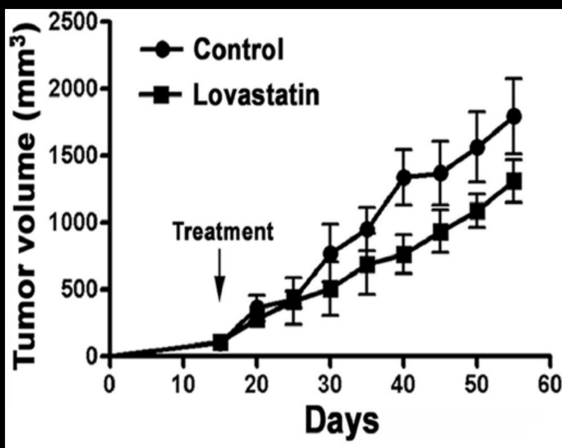


Lovastatin inhibits HDAC activity in a dose-dependent manner
Basal
Lovastatin
HDAC activity (% of Basal)
100
50
0
*
Basal
1
5
10
30 (μM)
Lovastatin

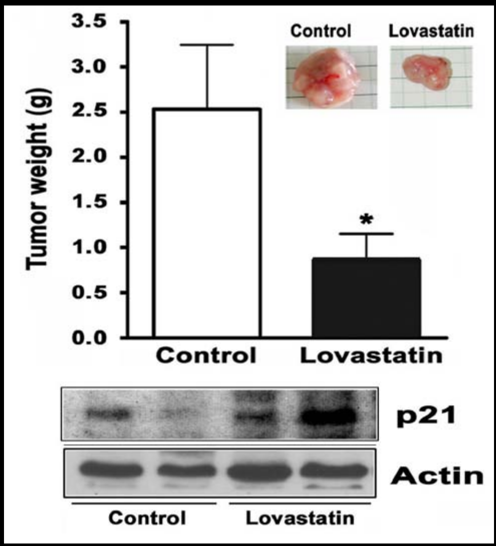
Nuclear HDAC activity in A549 cells were reduced by statins



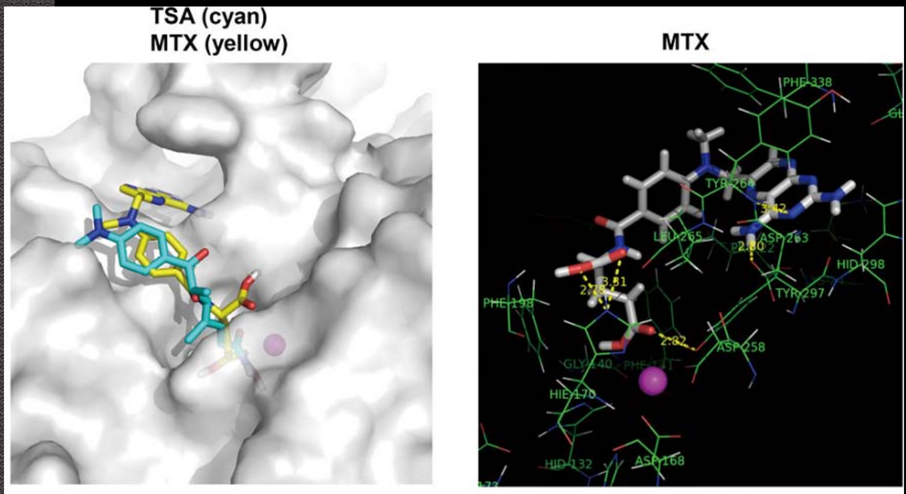
Statins reduced tumor growth rate in the xenograft nude mice



Lovastatin reduced the tumor size and weight after 45 days

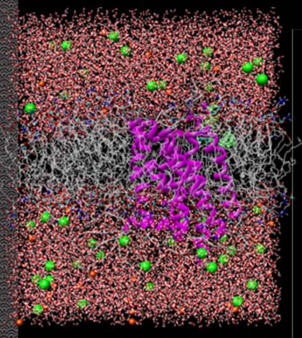


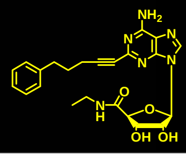
The antifolate drug methotrexate is also an HDAC inhibitor

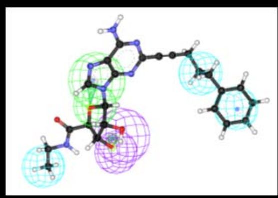


Biochem. Biophys. Res. Comm. **391** 1396-1399 (2010)

Designing new therapeutic agents for treating Huntington's disease

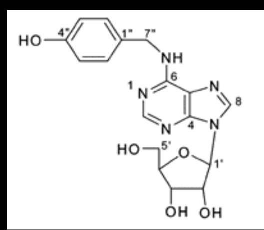






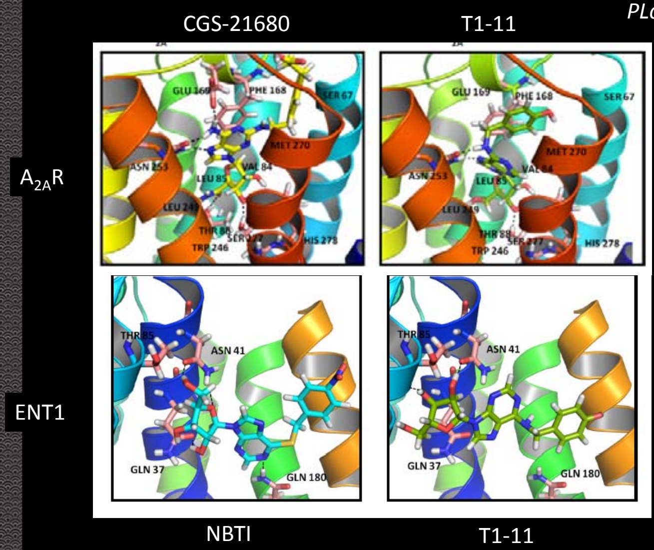
Adenosine A_{2A} Receptor and Its Agonists

- There are four adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3)
- Among them, the A_{2A}R has attracted much attention as a potential drug target for *Huntington's disease* and other *neurodegenerative diseases*.
- Some A_{2A} receptor agonists:
  - **CGS21680** is a potent and selective inhibitor which attenuates HD symptoms in a transgenic mouse model, but it has severe side effects.
  - **T1-11** is recently isolated from a Chinese medicine *Gastrodia elata* (天麻)

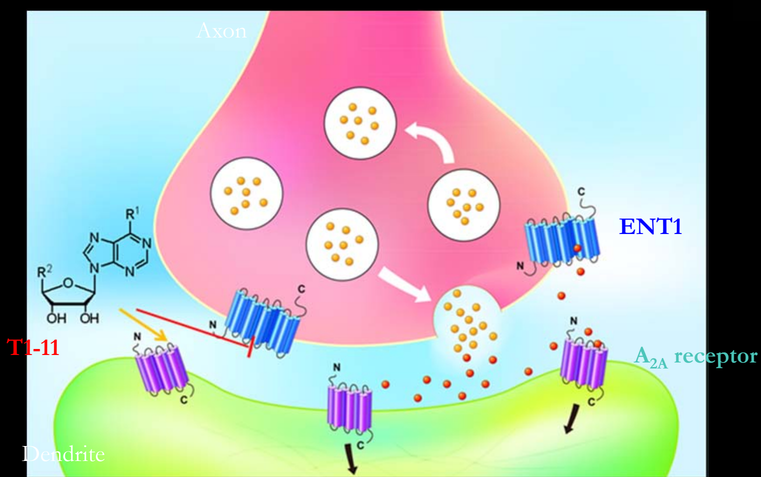


T1-11 specifically targets adenosine A_{2A} receptor and ENT1 transporter

PLoS ONE **6**: e20934(2011)



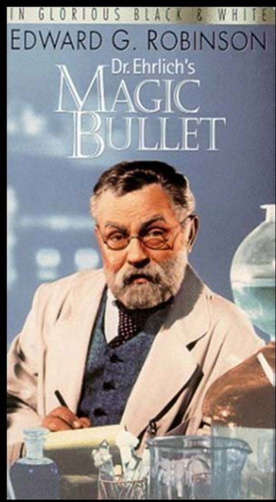
Goal



To explore the chemical space around T1-11 and rationally design compounds that bind both human adenosine A_{2A} receptor and hENT1.

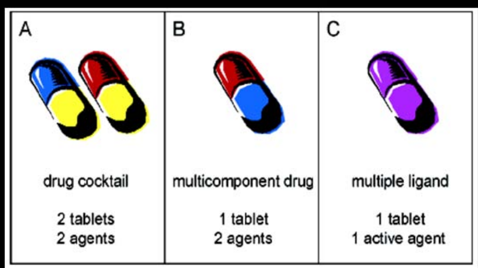
Magic Bullet vs. Magic Shotgun

- Magic Bullet (1890s):
  - To design a single chemical entity that inhibits one well-defined molecular target (one-target, one-drug)
- Complexity of human diseases:
  - modulating a multiplicity of targets could treat a disorders more efficiently
- Promiscuous drug/ transient drug
  - Extremely potent and highly selective compounds may disrupt its normal physiological function and causes side effect
- Designed Multiple Ligands (Morphy 2005)
  - based on medicinal chemistry knowledge to rationally design compounds which modulate multiple targets of relevance to a disease



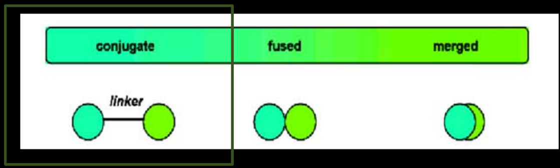
Multi-pharmacology approaches

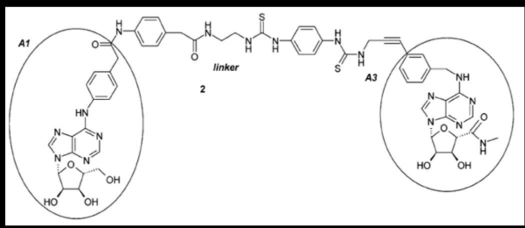
- **Drug cocktail**
  - Poor patient compliance
  - Unpredictable PK
- **Multicomponent drug**
  - Unpredictable PK
- **Multiple ligand**
  - Simple PK profile
  - Lower risk of drug-drug interaction



Journal of Medicinal Chemistry **48**, 6523-6543 (2005)

How to Design Dual-Action Compounds? (I)



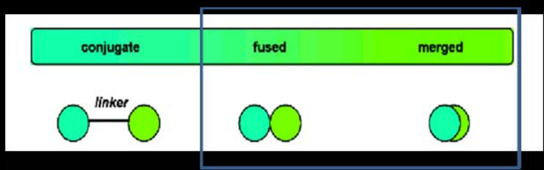


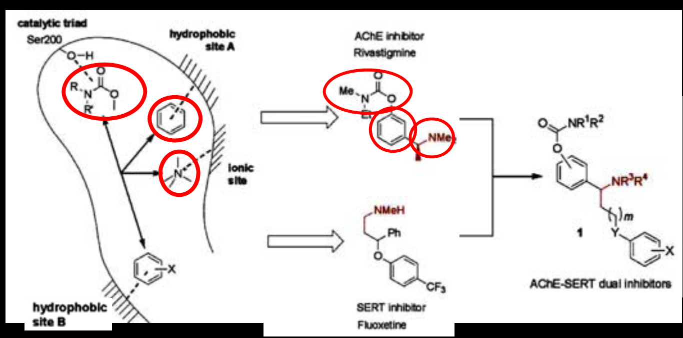
Adenosine 1 agonist

High molecular weight (>1000)
Multiple H bond donors
→ Violation of Lipinski's rules for drug-likeness

Journal of Medicinal Chemistry **48**, 6523-6543 (2005)

How to Design Dual-Action Compounds? (II)





Journal of Medicinal Chemistry **48**, 6523-6543 (2005)

Q:

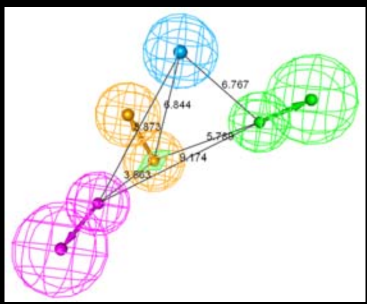
How can we rationally design dual function ligand without structural information of both targets?

Pharmacophore

- The term pharmacophore, introduced by ***Paul Ehrlich*** (1909), refers to the molecular framework that carries *(phoros)* the essential features responsible for a drug's biological activity *(pharmacon)*. *Ehrlich. Dtsch. Chem. Ges. 1909*
- **IUPAC definition (1998)**: An ensemble of steric and electronic features that is necessary to ensure the optimal supermolecular interactions with a specific biological target and to trigger (or block) its biological response.

Applications:

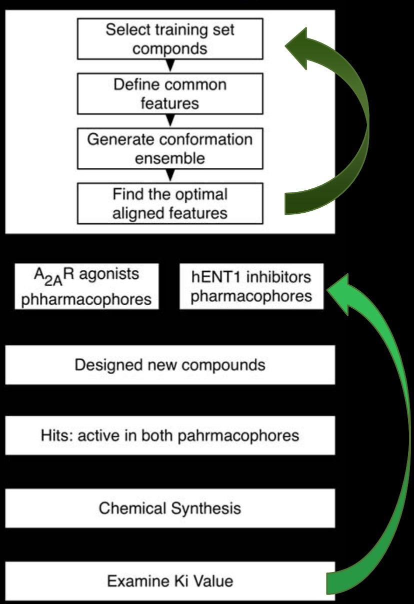
- **Virtual screening:** identify new compounds from 3D database
- **Activity prediction:** evaluate newly synthesized compound's potency (Ki, IC_{50})
- **SAR elucidation:** explain important chemical features among of a set of active compounds
- **Receptor mapping**
- **Active conformation prediction**
- **Homology modeling validation**



Process of pharmacophore construction

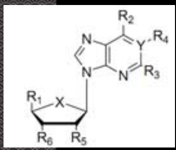
- General rules: at least 25 compounds are needed. Diversity of chemical skeletons should be considered.
- Rule out the outliers to obtain good statistics so that good predictive power can be achieved.
- The constructed pharmacophore may not be a general model, but it serves our purpose for expanding the chemical space of such dual-action compounds.

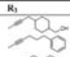
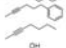
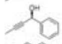
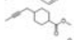
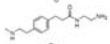
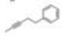
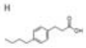
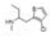
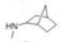
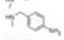
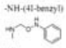
Our Strategy



- Collect 25-40 compounds form literature with K_i value which are evenly distributed among 6 order of magnitudes
- Rule out outliers
- Verify by CatScramble test

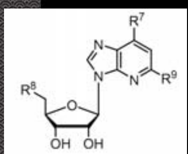
Training Set Compounds of Adenosine A_{2A} Receptor Agonists



Compound	R_1	R_2	R_3	R_4	R_5	R_6	X	Y	pK_i
A1	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	9.32
A2	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	8.92
A3	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	8.66
A4	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	8.59
A5	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	8.26
A6	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	8.24
A7	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	8.15
A8	$-CONHCH_2$-cyclopropane	$-NH_2$	H	-	OH	OH	O	N	7.92
A9	$-CONHC_2H_5$	$-NH_2$		-	OH	OH	O	N	7.72
A10	$-CONHC_2H_5$		H	H	OH	OH	O	C	7.25
A11	$-CONHC_2H_5$	$-NH_2$	$-C_2H_4Ph$	-	OH	OH	O	N	6.92
A12	$-CONHCH_3$	$-NH_2$	H	-	OH	OH	O	N	6.48
A13	$-CONHC_2H_5$	$-NH_2$	H	-	OH	OH	O	N	6.24
A14	$-CH_2OH$		Cl	-	OH	OH	O	N	5.85
A15	$-CH_2OH$		Cl	-	OH	OH	O	N	5.82
A16	$-CONHC_2H_5$		H	-	OH	OH	O	N	5.8
A17	$-C_2H_4$	$-NHOCH_3$	Cl	-	OH	OH	O	N	5.72
A18	$-CH_2OH$	$-NH_2$	H	-	OH	OH	O	N	5.54
A19	$-CONHC_2H_5$	-NH-(4I-benzyl)	Cl	-	OH	OH	O	N	5.33
A20	$-CONHC_2H_5$		Cl	-	OH	OH	O	N	5.27
A21	$-CH_2OH$	$-NH_2$	Cl	-	OH	OH	S	N	5.15
A22	$-CH_2OH$	-NH-cyclopentane	O	-	OH	OH	O	N	5.12
A23	$-CH_2OH$	-NH-cyclooctane	Cl	-	OH	OH	O	N	4.7
A24	$-CH_2OH$	$-NH_2$	O	$-CH_2C_2H_4$	OH	OH	O	N	4.4
A25	$-CH_2OH$	$-NH_2$	Cl	H	H	H	O	C	4.26

ChemMedChem **6**: 1390-1400(2011)

Training Set Compounds of hENT1 Inhibitors



Compound	R^7	R^9	R^8	pIC_{50}[a]
E1	-S-(4-NO_2-benzyl)	H	OH	9.54
E2	-NH-(4-NO_2-benzyl)	H	H	7.66
E3	-S-α,α-Dimethylbenzyl	H	OH	7.56
E4	-S-3-Me-benzyl	H	OH	7.48
E5	$-CH_2$-S-(4-I-phenyl)	H	OH	7.16
E6	$-CH_2$-S-(4-CN-phenyl)	H	OH	7.09
E7	-NH-(4-NO_2-benzyl)	H	Cl	6.97
E8	$-CH_2$-S-(4-F-phenyl)	H	OH	6.80
E9	$-CH_2$-S-(4-Br-phenyl)	H	OH	6.75
E10	$-CH_2$-S-(3-F-phenyl)	H	OH	6.70
E11	$-CH_2$-S-(3,4-Cl_2-phenyl)	H	OH	6.65
E12	$-CH_2$-S-(3-I-phenyl)	H	OH	6.61
E13	$-CH_2$-S-(4-Cl-phenyl)	H	OH	6.60
E14	$-CH_2$-S-(2-I-phenyl)	H	OH	6.55
E15	$-CH_2$-S-(3-Cl-phenyl)	H	OH	6.55
E16	$-CH_2$-S-(3-CF_3-phenyl)	H	OH	6.48
E17	$-CH_2$-S-(3-Br-phenyl)	H	OH	6.44
E18	$-CH_2$-S-(2-F-phenyl)	H	OH	6.44
E19	$-CH_2$-S-(4-MeO-phenyl)	H	OH	6.21
E20	$-CH_2$-S-(2-Br-phenyl)	H	OH	6.14
E21	-NH-(4-NH_2-benzyl)	H	OH	5.38
E22	-S-Isopropyl	NH_2	OH	5.32
E23	-S-Ethyl	NH_2	OH	5.28
E24	-S-Phenylpropyl	H	OH	5.20
E25	-S-Ethyl	H	OH	4.49

ChemMedChem **6**: 1390-1400(2011)

3D Pharmacophore Model of Adenosine A_{2A} Receptor Agonists

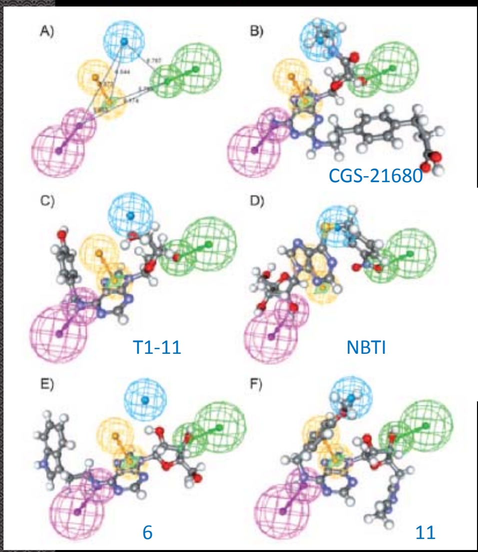


Table 3. Comparison of activities of compounds with the fitted number of features of the A_{2A}R agonist pharmacophore model.[a]

Compound	HBD	HBA	RA	HP	Fit value
CGS21680	0.166	0.125	0.201	0.436	10.6482
1 (T1-11)	0.121	0.135	0.248	×	8.68049
NBTI	0.422	0.304	×	0.901	8.62848
6	0.377	0.359	0.403	×	8.52907
11	1.181	0.445	0.434	0.544	9.59155

[a] All values are reported in Å.

ChemMedChem **6**: 1390-1400(2011)

3D Pharmacophore Model of hENT1 Inhibitors

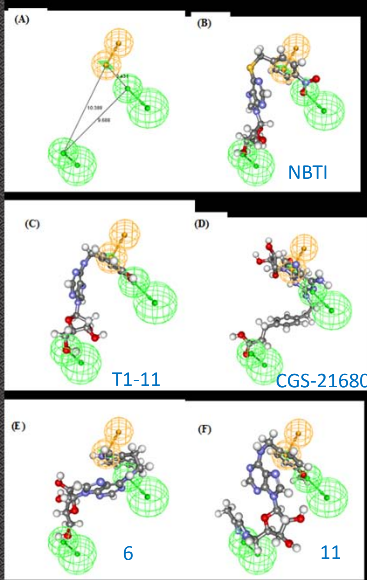


Table 4. Comparison of activities of compounds with the fitted number of features of the ENT1 inhibitor pharmacophore model.[a]

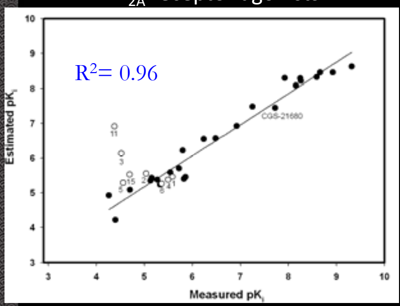
Compound	HBD	HBA	RA	Fit value
NBTI	0.421	0.647	0.793	5.92748
1	0.544	0.619	0.586	4.94133
CGS21680	0.567	1.276	0.358	5.37611
6	0.421	0.647	0.493	5.40138

[a] All values are reported in Å.

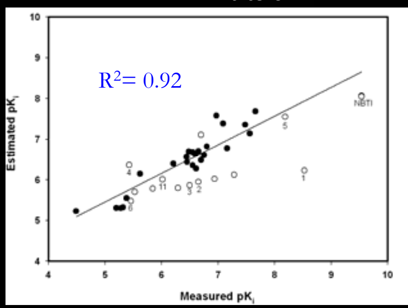
ChemMedChem **6**: 1390-1400(2011)

Statistical Assessment of Pharmacophore Models

A_{2A} receptor agonists



hENT1 inhibitors

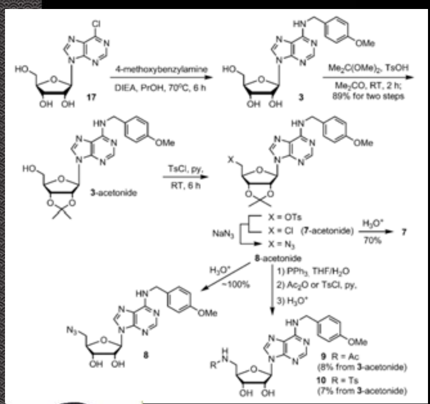


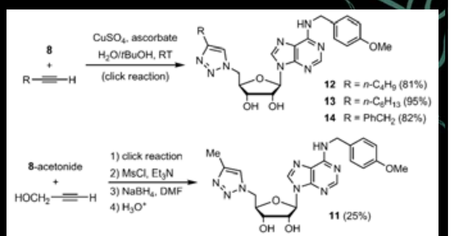
rmse: 0.658 kcal/mol

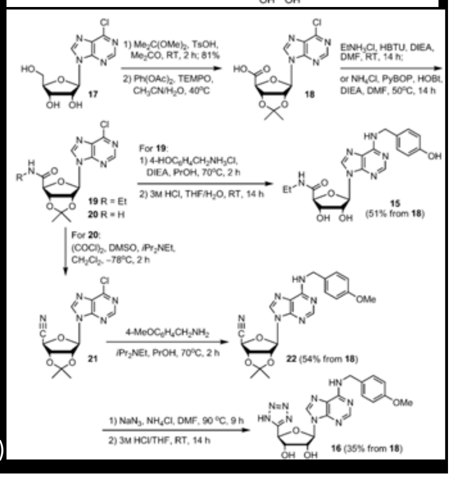
rmse: 0.85 kcal/mol

ChemMedChem **6**: 1390-1400(2011)

Chemical Synthesis







Prof. Jim-Ming Fang
Department of Chemistry
National Taiwan University

ChemMedChem **6**: 1390-1400(2011)

Biological Evaluation

Table 1. Binding activity of the N^6- and $C^{5'}$-modified adenosine derivatives with adenosine receptor and transporter.[a]

Compound	K_i [µM] $A_{2A}R$[b]	K_i [µM] ENT1[c]
CGS21680	7.77×10^{-2}	–
NBTI	>10	2.9×10^{-4}
1	2.62	5.38×10^{-1}
1-acetonide	>100	>100
2	14.4	1.44×10^{-2}
3	30.1	3.18×10^{-1}
4	3.21	3.72
5	27.7	6.51×10^{-3}
6	4.39	3.47
7	>100	2.98
8	–	5.81×10^{-1}
9	>100	1.43
10	>100	2×10^{-1}
11	41.8	9.60×10^{-1}
12	>100	5.11×10^{-1}
13	>100	5.2×10^{-2}
14	>100	1.16×10^{-1}
15	20.3	>10
16	>100	1.17

[a] Radioligand binding assays were performed by MDS Pharma Services Taiwan (Taipei, Taiwan) using standard binding protocols. [b] Human adenosine A_{2A} receptor. [c] Guinea pig equilibrium transporter 1.

Table 2. Cell viability of the N^6- and $C^{5'}$-modified adenosine derivatives.[a]

Compound	Cell viability [%]
CGS21680	88.6 ± 9.6
NBTI	29.1 ± 2.1
1	81.5 ± 1.8
2	63.7 ± 2.9
3	42.3 ± 1.8
4	83.9 ± 4.5
5	48.2 ± 1.3
6	118.8 ± 3.9
7	36.8 ± 5.3
8	29.9 ± 1.5
9	24.1 ± 4.5
10	36.4 ± 3.9
11	36.7 ± 0.6
12	28.9 ± 2.6
13	27.6 ± 0.4
14	30.6 ± 4.7
15	87.0 ± 8.4
16	30.0 ± 3.5

[a] Serum-deprived PC12 cells were treated with or without compound at 1 µM for 24 h. Cell viability was monitored by the MTT assay, and is expressed as a percentage of the MTT activity measured in the serum-containing group (100%). Serum deprivation resulted cell survival rate to 33.0 ± 1.9. Data points represent the mean ±SEM of at least three independent experiments ($n=3$–6).

ChemMedChem **6**: 1390-1400(2011)

Summary

- We have adopted a dual-pharmacophore modeling approach in the design of dual-action compounds that target the $A_{2A}R$ and hENT1, which facilitates to explore the chemical space of T1-11.
- The competitive ligand binding assays verified that the designed compounds indeed bind to both $A_{2A}R$ and ENT1 with moderate affinity.
- These compounds were shown to prevent apoptosis in serum-deprived PC12 cells, indicating their potential for treating neurodegenerative diseases. Our recent data also show that the new compounds exhibit pronounced efficacy in mouse models of neurodegenerative disease.

References

Nai-Kuei Huang,¥ Jung-Hsin Lin,¥ Jiun-Tsai Lin, Chia-I Lin, Eric Minwei Liu, Chun-Jung Lin, Wan-Ping Chen, Yuh-Chiang Shen, Hui-Mei Chen, Jhih-Bin Chen, Hsing-Lin Lai, Chieh-Wen Yang, Ming-Chang Chiang, Yu-Shuo Wu, Chen Chang, Jiang-Fan Chen, Jim-Min Fang, Yun-Lian Lin, Yijung Chern, "A new drug design targeting the adenosinergic system for Huntington's disease", *PLoS ONE* **6**: e20934(2011).

Jhih-Bin Chen, Eric Minwei Liu, Ting-Rong Chern, Chieh-Wen Yang, Chia-I Lin, Nai-Kuei Huang, Yun-Lian Lin, Yijuang Chern,Jung-Hsin Lin,* and Jim-Min Fang*, "Design and synthesis of novel dual-action compounds targeting adenosine A_{2A} receptor and adenosine transporter for neuroprotection", *ChemMedChem* **6**: 1390-1400 (2011).

Patents:

- METHOD OF MAKING AND USING AN ADENOSINE ANALOGUE, US 2008/0176816 A1 (Jul. 24, 2008)
- 結合至腺苷酸A_{2A}受體和腺苷酸轉運子以預防及治療神經退化疾病的雙功能化合物 Taiwan Patent Pending (Serial No. 99138959, filed on Nov. 12, 2010)
- DUAL FUNCTIONAL ADENOSINE ANALOGUES AND USE THEREOF IN TREATING NEURODEGENERATIVE DISEASES. Serial No. PCT/US10/56516 61/260,932 (November 12, 2010).


生活醫藥 A8 2011年9月28日 星期三 United Evening News 聯合晚報

中研院研究 中藥天麻有潛力治療失智症

未來可能研發出緩解神經退化疾病藥物 能延緩失智症到一、二十年才惡化

能活化神經 天麻鉤藤飲

10月1日開打

公費流感疫苗施打 只供高危險群 高傳播群

沒打完不會開放全民施打

施打1個月 候補對象能接種

今年度公費疫苗10月1日開打

手機上網達上限 業者竟不通知

中華電：通知有困難 未來會檢討 遠傳：智慧手機可查傳輸量

The Team

- Pharmacophore Modeling
  - Ting-Rong Chern (AS RCAS)
  - Eric Minwei Liu (NTU Pharmacy, Columbia Univ.)
- Chemical Synthesis
  - Prof. Jim-Min Fang (NTU Chemistry)
  - Jhih-Bin Chen
  - Chieh-Wen Yang
- Pharmacognosy
  - Dr. Yun-Lian Lin (NRICM)
- Biological Evaluation
  - Prof. Yi-Juang Chern (AS IBMS)
  - Nai-Kuei Huang (NRICM)
  - MDS Pharma Service Taiwan

Pharmacoinformatics Lab

Funding and support :
National Science Council
Research Center for Applied Sciences, Academia Sinica
National Center for High Performance Computing

Acknowledgements

Group members :

Wang, Jui-Chih
Lee, Po-Hsien
Chern, Ting-Rong
Shu-Hao Yeh
Chu, Pei-Ying
Gwan, Jean-Fang
Chen, Yu-Hsuan
Chang, Che-Chia
Chen, Jing-Yeh
Chen, Chun-Jui
Dhanajay Joshi
Cheng, An-Liang
Liu, Min-Wei
An-Liang Cheng
Tsai, Cheng-Che
Chou, Jing-Yu
Kuei-Ling Kuo
Hui-Hsaun Tu

Funding and support :

National Science Council,
Research Center for Applied Sciences, Academia Sinica
National Center for High Performance Computing