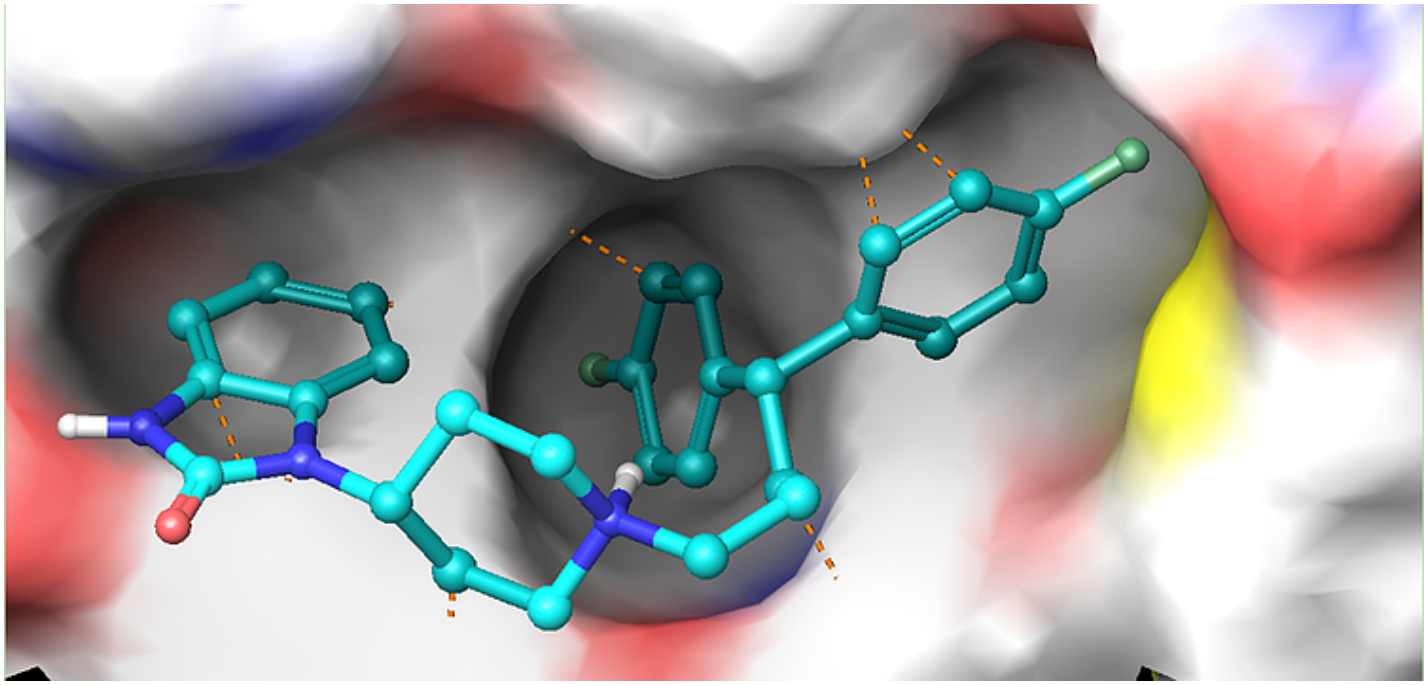
In Silico Drug Design and Development



Joseph Badillo

MacMillan Group Meeting

March 1, 2016

In Silico Drug Design and Development

Outline

General outline

1) Some vocabluary

2) Intro to docking and scoring functions

3) Examples of drug lead discovery using *in silico* methods

a) Mycobacterium tuberculosis

b) Type-II diabeties

c) Cancer

4) Computaional method used to find modifiers for siRNA

In Silico Drug Design and Development

CADD

- **Computer aided drug design (CADD):** is the use of computing power to streamline the drug discovery and development process**.**

***In silico* vocabulary:**

Virtual screening: computational technique used to evaluate vast libraries chemical structures as potential new drug compounds (docking a library of compounds).

Docking: predicts the orientation (or pose) in which a ligand will bind to a host protein.

Scoring: a fast approximate mathematical method used to predict the strength of non-covalent interactions (binding affinity) between two molecules after they have been docked.
The scoring function is one of the most important components in structure-based drug design.

Bioinformatics: Most important is structural information about potential biological targets

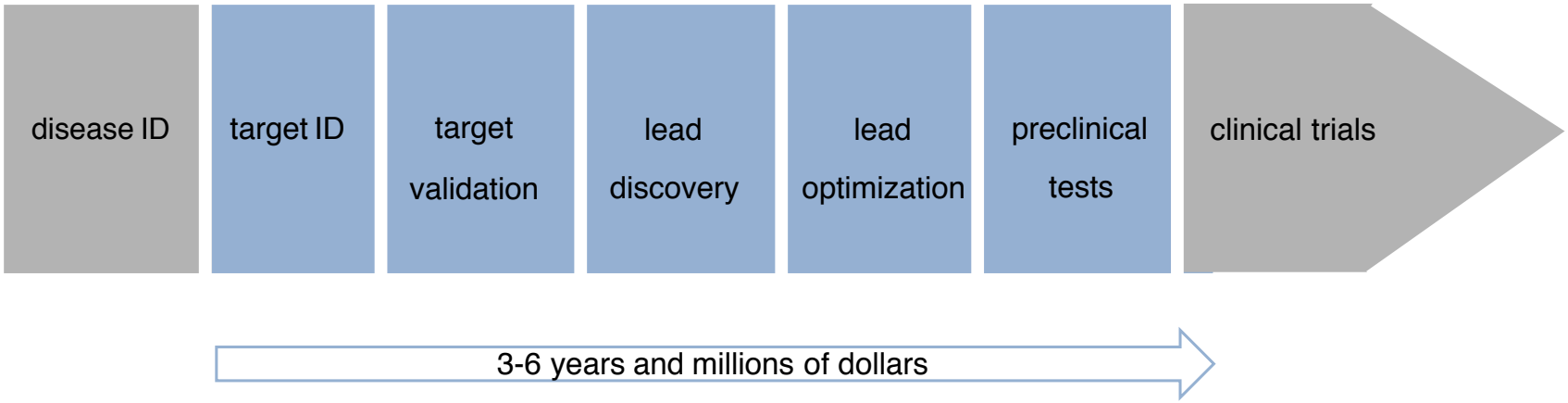
Chemical informatics: Design of *in silico* filters to eliminate compounds with undesirable properties.
Prediction of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) based on criteia such as polar surface area (PSA),calculted log P, and the number of H-bond donors and accepters.
How "druglike" are a set of compounds.

Kapetanovic, I. M. *Chem.-Biol. Interact.* **2008**, *171*, 165.

In Silico Drug Design and Development

tradtional vs CADD

Tradtional drug development:



Computer-aided drug design (CADD) applications in various stages of drug development:

Target ID: Bioinformatics, reverse docking, protein structure prediction

Target validation: Target drugability, tool compound design

Lead discovery: Library design, docking/scoring, virtual screening

Lead optimization: Quantitative structure-activity relationship, structure-based optimization

Preclinical tests: In silico ADMET (absorption, distribution, metabolism, excretion, toxicity), physiologically-based pharmacokinetic (PBPK) simulations

In Silico Drug Design and Development

Docking

- **Molecular docking:** is a tool used in structural molecular biology and computer-assisted drug design. The goal of molecular docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure.

The question:

"Given the structure of a protein and that of a potential ligand, can the two form a favorable complex? What are the bases for binding and specificity?" -Brian Shoichet (UCSF)

Three main challenges associated with docking:

Molecular flexibility: There are many states to consider in docking flexible molecules (both target and ligand).

Accurate scoring: Evaluating fit for the docking molecules from a database, and ranking them accordingly.

Specificity: Understanding important interactions.

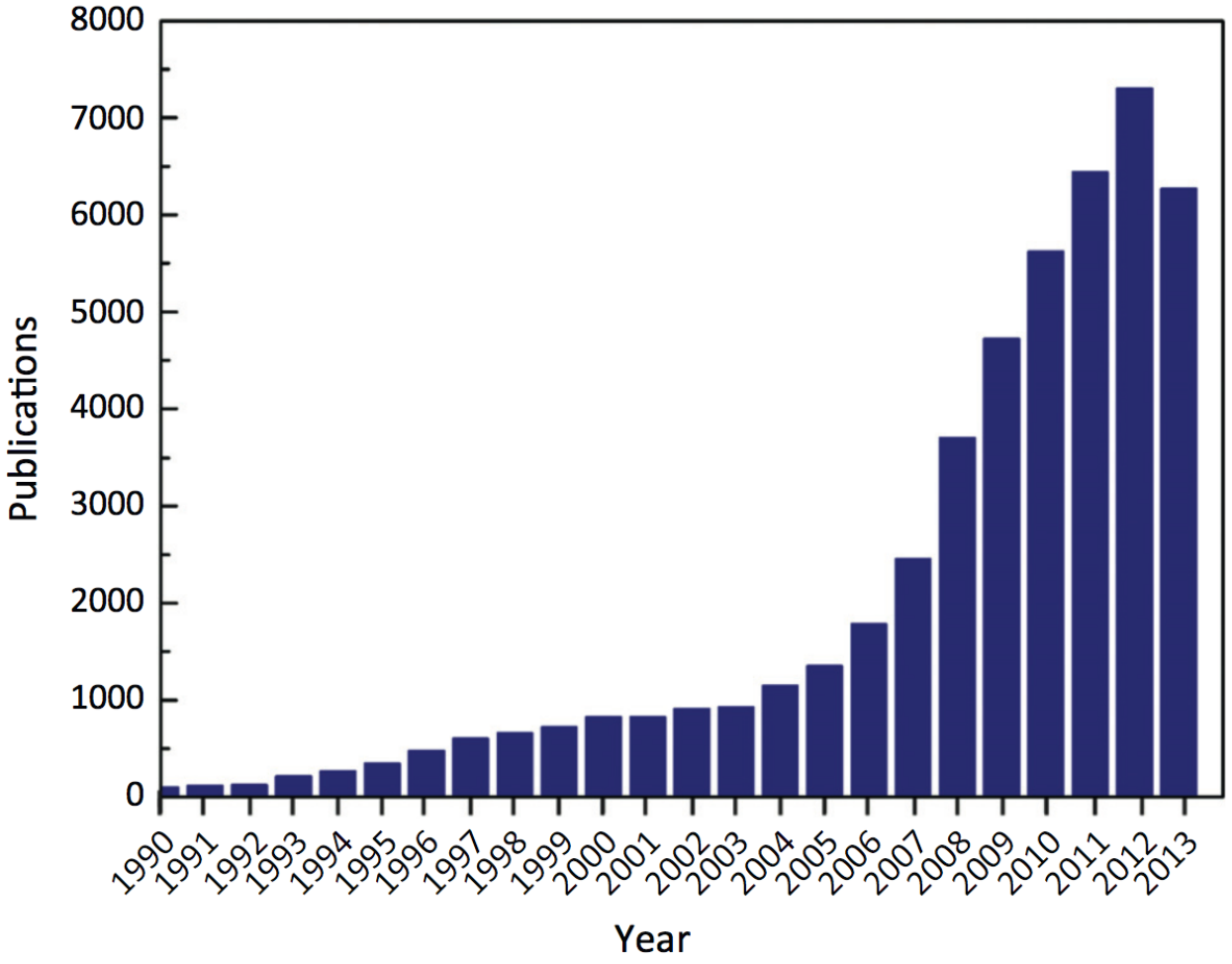
Su, A. I.; Lorber, D. M.; Weston, G. S.; Baase, W. A.; Matthews, B. W.; Shoichet, B. K. *Proteins: Struct., Funct., Genet.* **2001**, *42*, 279

http://www.bkslab.org/docking.php

In Silico Drug Design and Development

Docking

- **Increase in the number of docking of papers from 1990 to 2013 (PMC-NCBI database):**



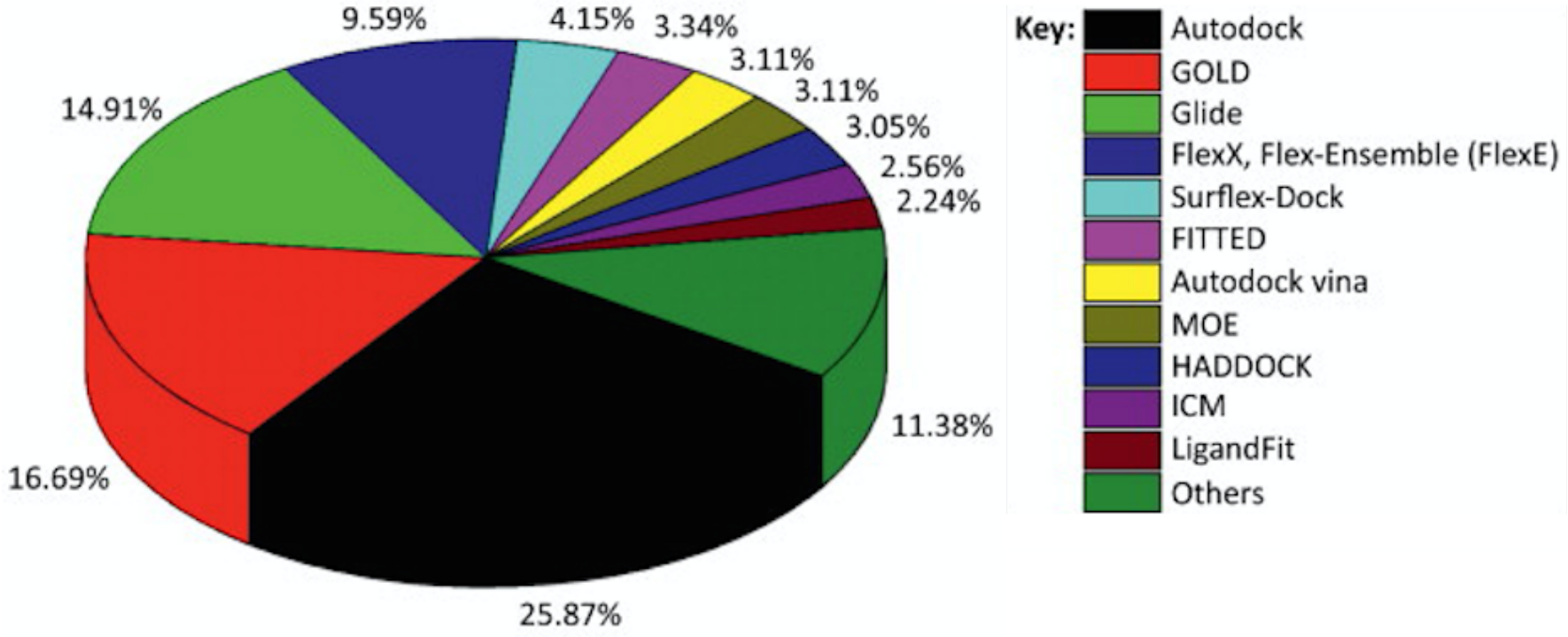
Keywords: 'dock' or 'docking'

Chen, Y.-C. *Trends Pharmacol. Sci.* **2015**, *36*, 78.

In Silico Drug Design and Development

Docking

- **All docking publications from 1990 to 2013:**

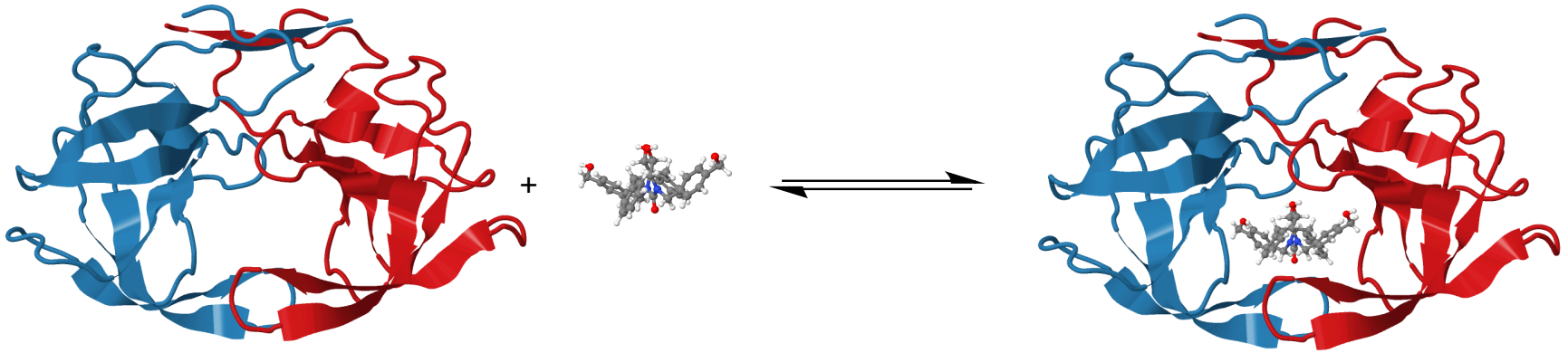


Chen, Y.-C. *Trends Pharmacol. Sci.* **2015**, *36*, 78.

In Silico Drug Design and Development

Predicting binding affinity

- **Free energy of binding (ΔG) is related to binding affinity (K_i):**



[E] [I] [EI]

$$\Delta G = -RTLnK_A \quad \Longrightarrow \quad K_A = K_i^{-1} = \frac{[EI]}{[E][I]}$$

Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. *Nat. Rev. Drug Discovery* **2004**, *3*, 935.

In Silico Drug Design and Development

Scoring functions

- **Three common types of scoring funtions:**

Force field

affinities are estimated by summing the strength of intermolecular interactions between all atoms using a force field

Empirical

based on counting the number of various types of interactions between two binding partners

Knowledge-based

based on statistical observations of intermolecular close contacts in large 3D databases (aka statistical potentials)

Huang, S.-Y.; Grinter, S. Z.; Zou, X. *Phys. Chem. Chem. Phys.* **2010**, *12*, 12899.

In Silico Drug Design and Development

Scoring functions

- **Common scoring functions:**

type	scoring function
Force field-based	DOCK, DOCK3.5 (PBSA), DOCK/GBSA(SDOCK), AutoDock, GOLD, SYBYL/D-Score, SYBYL/G-Score
Emperical	FlexX, Glide, ICM, LUDI, PLP, ChemScore, X-Score, Surflex, SYBYL/F-Score, LigScore, MedusaScore, AIScore, SFCscore
Knowledge-based	ITScore, PMF, DrugScore, DFIRE, SMoG, BLEEP, MScore, GOLD/ASP, KScore

Huang, S.-Y.; Grinter, S. Z.; Zou, X. *Phys. Chem. Chem. Phys.* **2010**, *12*, 12899.

In Silico Drug Design and Development

Docking

- **Success rates of 16 scoring functions for a test set of 100 diverse protein-ligand complexes, using the criterion of rmsd ≤ 2 Å**

Scoring function	Type of scoring	Success rate (%)
ITScore/SE	K	91
DrugScore	K	87
ITScore	K	82
Cerius2/PLP	E	76
SYBYL/F-Score	E	74
Cerius2/LigScore	E	74
DrugScore	K	72
Cerius2/LUDI	E	67
X-Score	E	66
AutoDock	F	62
DFIRE	K	58
DOCK/FF	F	58
Cerius2/PMF	K	52
SYBYL/G-Score	F	42
SYBYL/ChemScore	E	35
SYBYL/D-Score	F	26

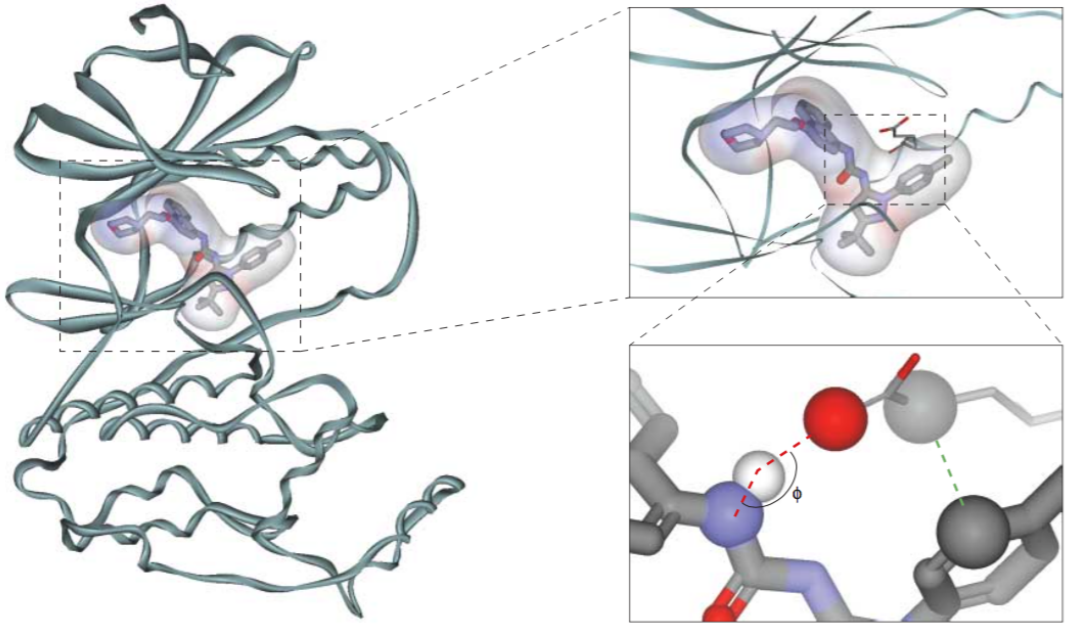
K = knowledge-based, E = empirical, and F = force field

Huang, S.-Y.; Grinter, S. Z.; Zou, X. *Phys. Chem. Chem. Phys.* **2010**, *12*, 12899.

In Silico Drug Design and Development

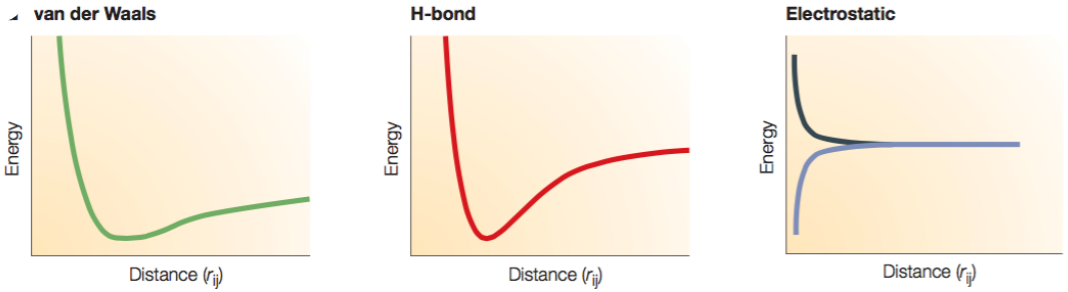
Scoring functions

- **Common force field scoring functions:** **Structure of p38 mitogen-activated protein kinase with bound inhibitor BIRB796 (PDB code: 1KV2).**



⇐ electrostatic potential

⇐ interaction between Glu71 and BIRB796



- - - van der Waals

- - - H-bond (angle dependent)

2 like chagres

2 opposite charges

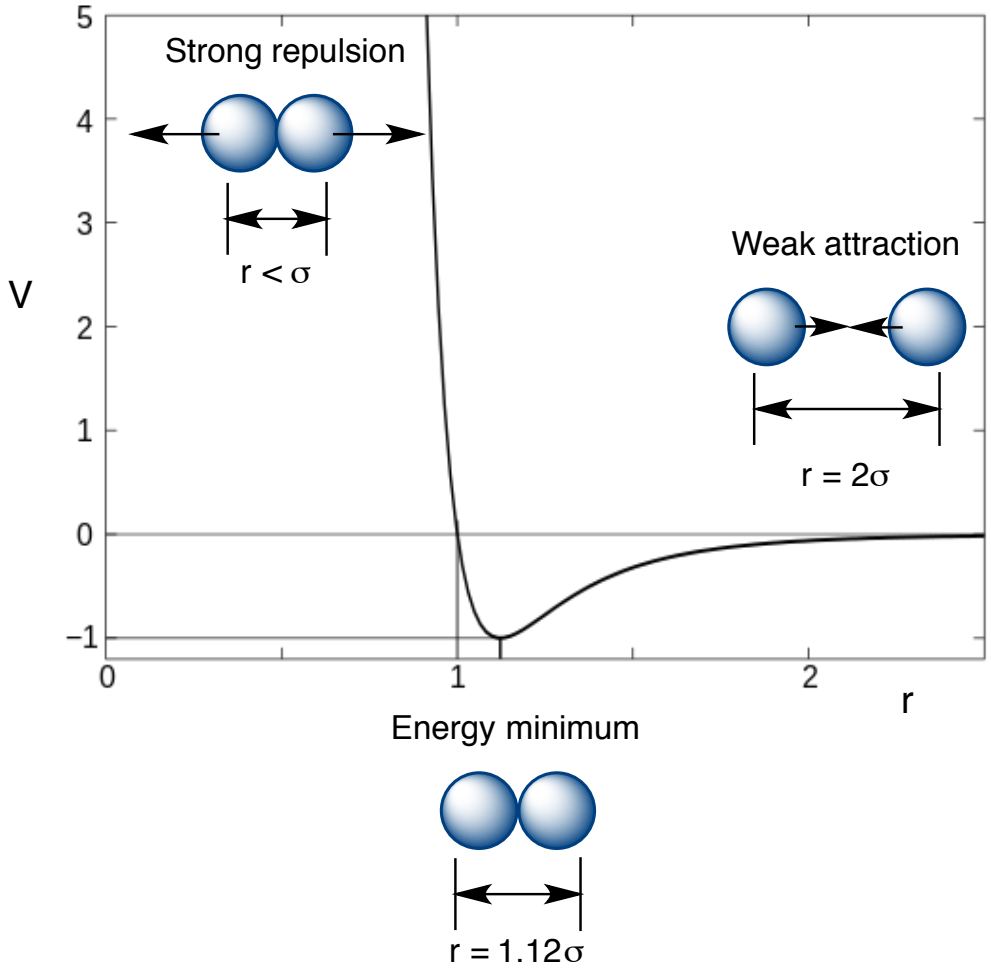
(PDB code: 1KV2)

Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. *Nat. Rev. Drug Discovery* **2004**, *3*, 935

In Silico Drug Design and Development

Scoring functions

- **Lennard-Jones potential:**



$$V = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$$

ε = well depth

r = distance between particles

σ = finite distance at which the inter-partical potential is zero

In Silico Drug Design and Development

calculating free energy

- **In simple force-field potentials the individual terms are summed to give $\Delta G_{binding}$:**

$$\Delta G_{binding} = \Delta G_{vdw} + \Delta G_{H-Bond} + \Delta G_{electrostatic} + \Delta G_{tortional} + \Delta G_{solvation}$$

Three main force-field scoring function limitaions:

Implicit solvation models are often inadequate and based on a continuous medium instead of "explicit" solvent molecules.

There are is universal set of weighting coefficients for different energy terms for diverse protein–ligand complexes.

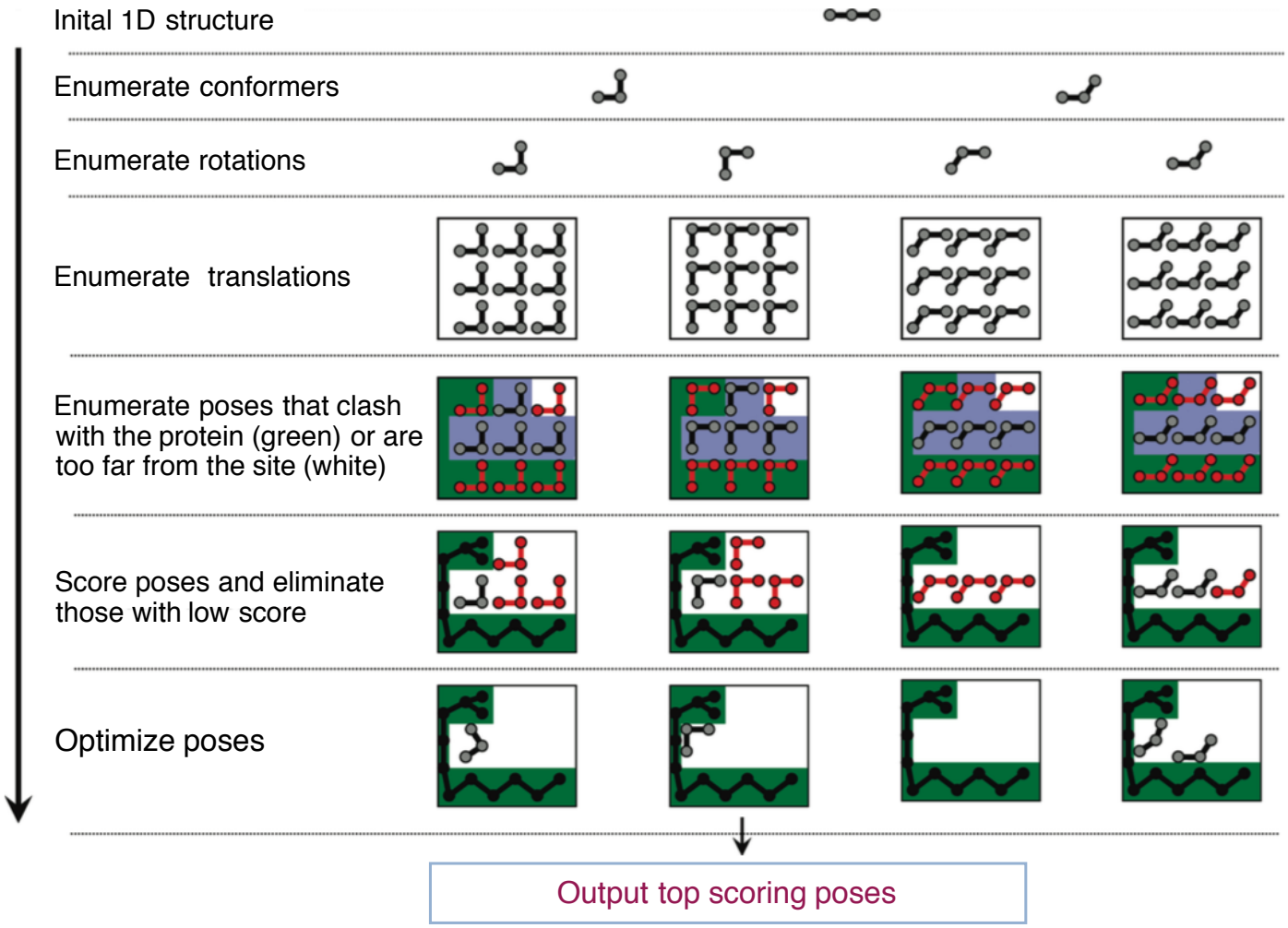
Inaccurate treatment of entropic effects may easily render useless the accuracy of electrostatic calculations

Huang, S.-Y.; Grinter, S. Z.; Zou, X. *Phys. Chem. Chem. Phys.* **2010**, *12*, 1289

In Silico Drug Design and Development

Docking

Docking ensembles using OpenEye FREAD: Finding a good pose.



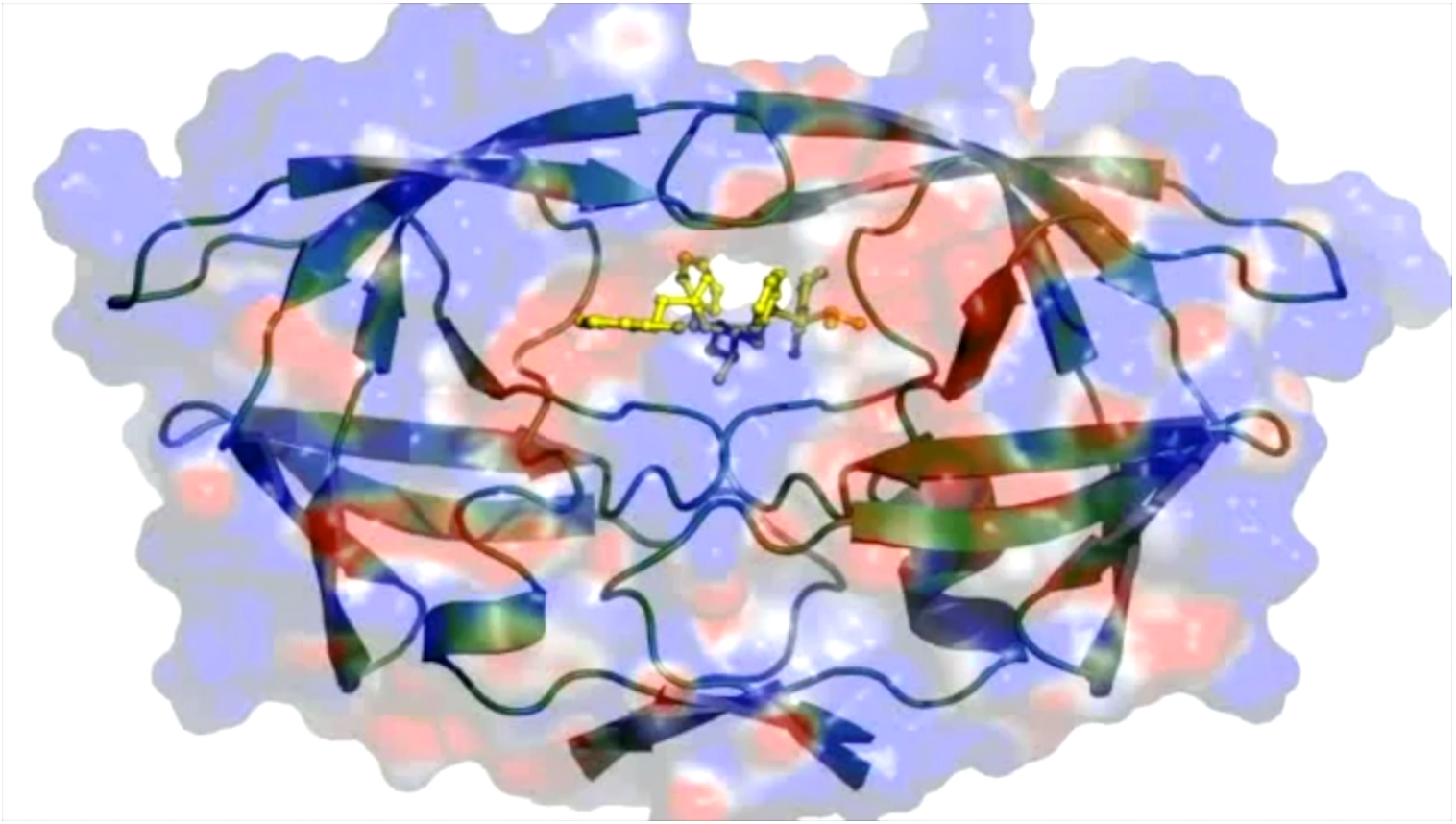
Typically evaluates 10^5 to 10^9 confromations for each molecule in seconds

McGann, M. *J. Chem. Inf. Model.* **2011**, *51*, 578.

In Silico Drug Design and Development

Docking

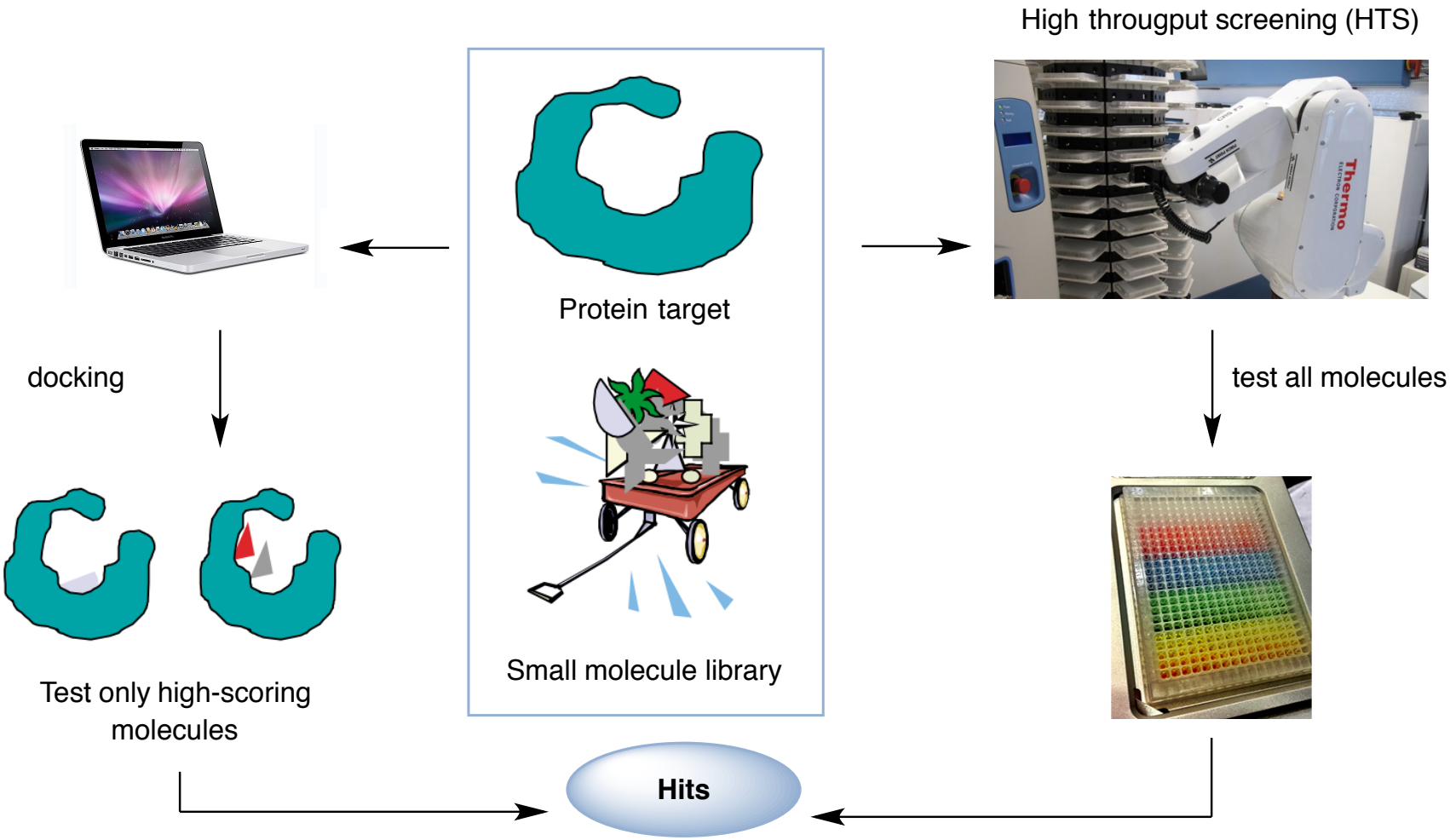
- **Flexx mechanism for drug fragment docking into HIV protease:**



In Silico Drug Design and Development

Docking

- **Docking vs.HTS for lead discovery**

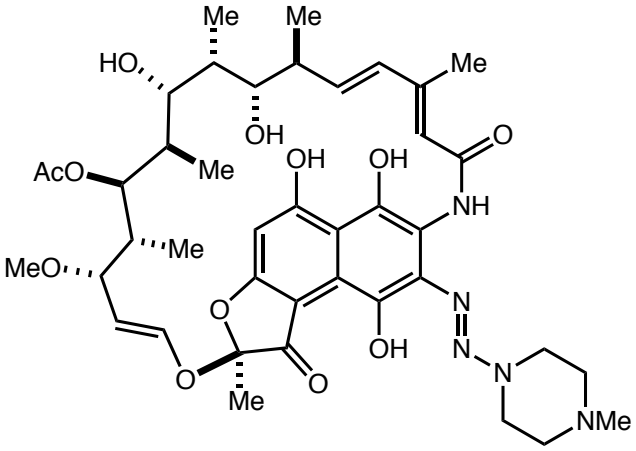


In Silico Drug Design and Development

Inhibitors of DHPR via in silico screening

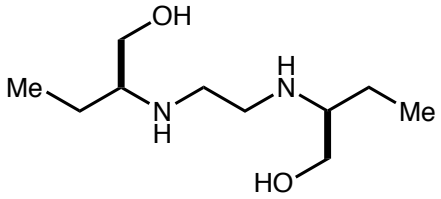
- ***Mycobacterium tuberculosis* (MTB):** a leading cuase of death in developing countries, especially for people with compromised immune systems as a consequence of HIV infection.

Current treatments:



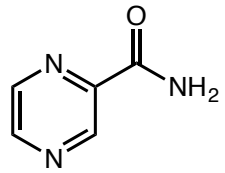
rifampicin

inhibits nucleic acid synthesis



ethambutol

distrupts cell wall bio-synthesis



pyrazinamide

(pro-drug for pyrazinonic acid)

inhibits translation

Resistance to these therapys have emerged so new new enzyme tragets are needed

In Silico Drug Design and Development

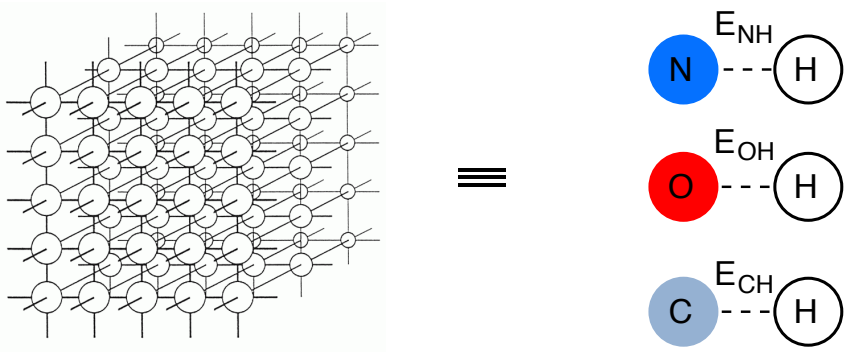
Inhibitors of DHPR via in silico screening

- ***Screening of compounds against DHPR from the Merck chemical collection by two approaches:***

DHPR (dihydrodipicolinate reductase): has been found to play an essential role in bacterial cell wall synthesis and is a potential therapeutic target for MTB.

Virtual sceening approach: Docking using FLOG (flexible ligands oriented on a grid) algorithm

FLOG: at each point (on a grid) the potential energy is calculated and stored for each atom type.



Traditional screening approach: Screen thousands of compounds *in vitro*

DHPR catalyzed hydride transfer was monitored by following NADPH oxidation to NAD^+ (via absorbance or fluorometric anaiysis)

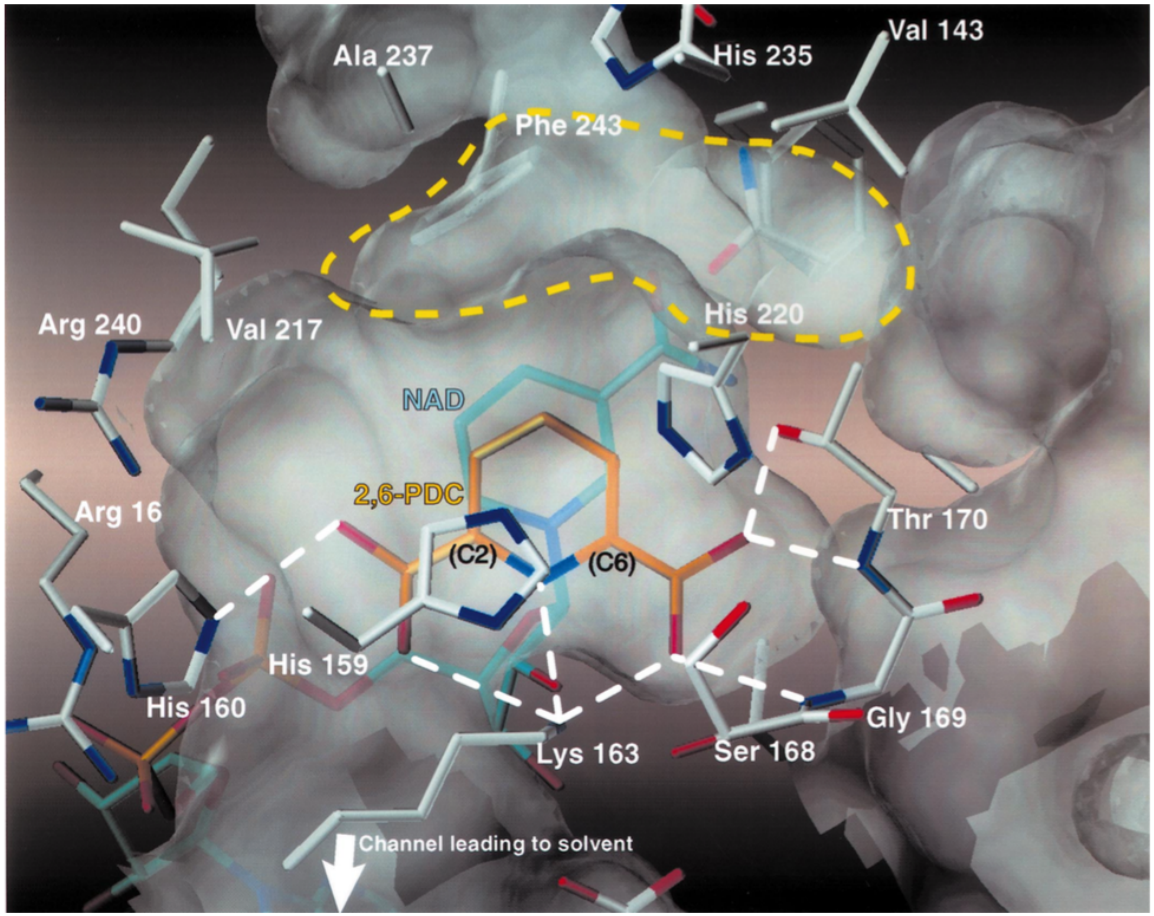
Paiva, A. M.; Vanderwall, D. E.; Blanchard, J. S.; Kozarich, J. W.; Williamson, J. M.; Kelly, T. M. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **2001**, *1545*, 67.

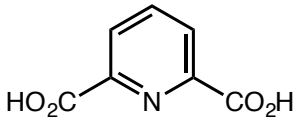
Miller, M. D.; Kearsley, S. K.; Underwood, D. J.; Sheridan, R. P. *J. Comput.-Aided Mol. Des.* **1994**, *8*, 153.

In Silico Drug Design and Development

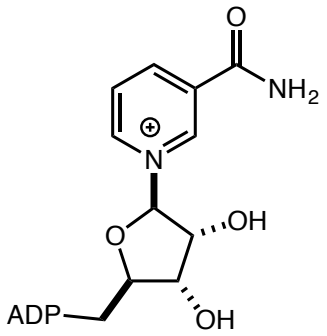
Inhibitors of DHPR via in silico screening

- ***Active site of dihydrodipicolinate reductase (DHPR):***





2,6-PDC (orange)



NAD^+ (cyan)

DHPR (carbons white)

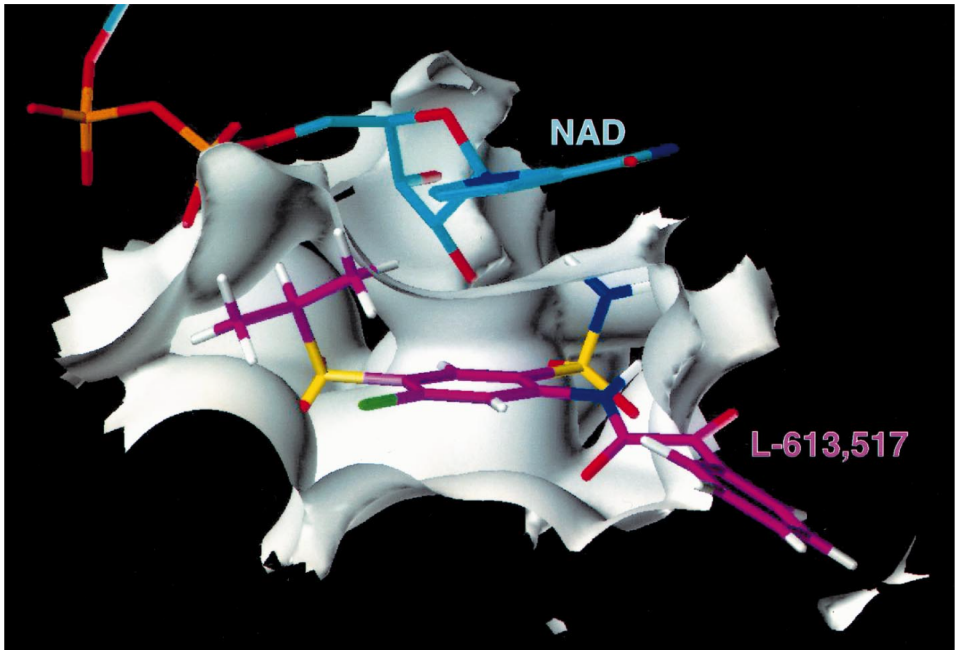
X-ray stucture of *E.coli* DHPR with 2,6-PDC bound in active site stacked with NAD^+ cofactor

Paiva, A. M.; Vanderwall, D. E.; Blanchard, J. S.; Kozarich, J. W.; Williamson, J. M.; Kelly, T. M. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **2001**, *1545*, 67

In Silico Drug Design and Development

Inhibitors of DHPR via in silico screening

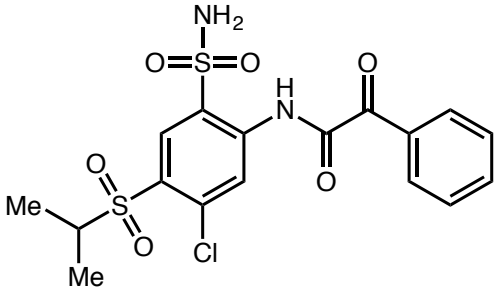
- ***View of L-613,517 docked into the DHPR active site:***



1.6 X 10^6 compounds docked

500 scoring compounds chozen for *in vitro* assay

Lead compound:



L-613,517 (magenta)

IC_{50} = 7.2 μM (*E. coli*)

IC_{50} = 7.2 μM (*M. tuberculosis*)

The over all hit rate (IC_{50} values < 100 μM) for the virtually screened compounds was 6%

Paiva, A. M.; Vanderwall, D. E.; Blanchard, J. S.; Kozarich, J. W.; Williamson, J. M.; Kelly, T. M. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **2001**, *1545*, 67.

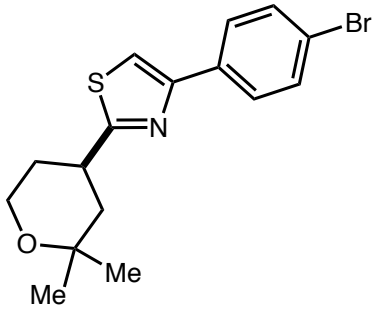
In Silico Drug Design and Development

Inhibitors of DHPR via in silico screening

Compounds identified through traditional HTS:

Thousands of compounds screened from the Merck Chemical Repository

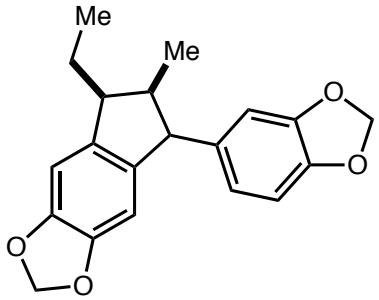
Lead compounds identified:



L-298,878

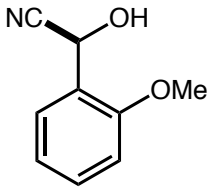
IC_{50} = 54 μM (*E. coli*)

IC_{50} = 35 μM (*M. tuberculosis*)



L-245,060

IC_{50} = 20 μM (*E. coli*)



L-273,552

IC_{50} = 93 μM (*E. coli*)

The over all hit rate (IC_{50} values < 100 μM) for the Merck HTS compounds was ≤2%

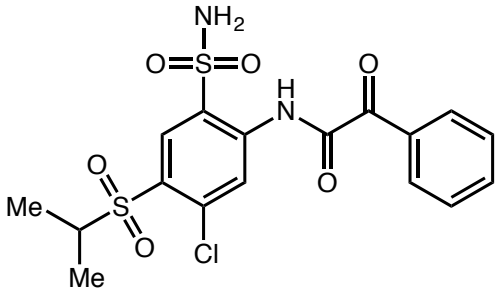
Paiva, A. M.; Vanderwall, D. E.; Blanchard, J. S.; Kozarich, J. W.; Williamson, J. M.; Kelly, T. M. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **2001**, *1545*, 67.

In Silico Drug Design and Development

Inhibitors of DHPR via in silico screening

- **Virtual screening vs. traditional high throughput screening:**

Lead identified using VS:



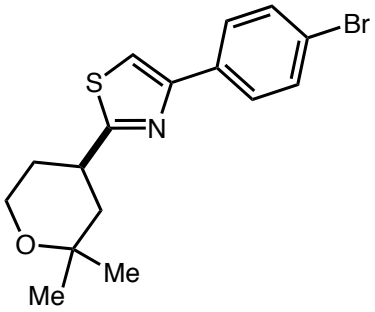
L-613,517

IC_{50} = 7.2 μM (*E. coli*)

IC_{50} = 7.2 μM (*M. tuberculosis*)

over all hit rate was 6%

Lead identified using HTS:



L-298,878

IC_{50} = 54 μM (*E. coli*)

IC_{50} = 35 μM (*M. tuberculosis*)

over all hit rate was ≤2%

Paiva, A. M.; Vanderwall, D. E.; Blanchard, J. S.; Kozarich, J. W.; Williamson, J. M.; Kelly, T. M. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **2001**, *1545*, 67.

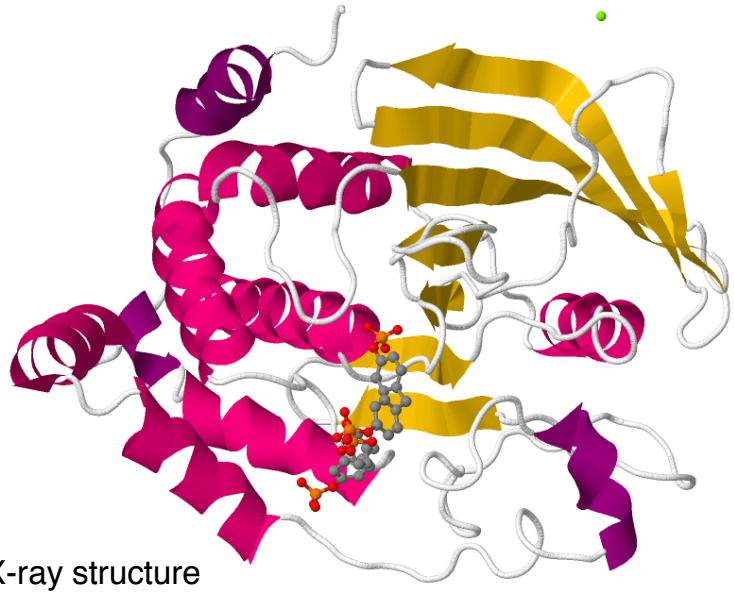
In Silico Drug Design and Development

Inhibitors of protein tyrosine phosphatase-1B

- ***Type-II diabetes*:** metabolic disorder characterized by high blood sugar due to inulin resistance or lack of inulin.

  Long-term effects lead to heart disease, stroke, kidney failure, and nerve damage.

Protein tyrosine phosphate-1B (PTP1B): overproduction has been implicated in the onset of type-II diabetes, shown to deactivate the insulin receptor by hydrolyzing phosphotyrosines, and is therefore a potential drug target.

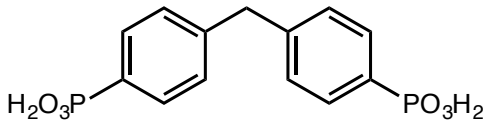


X-ray structure

1.90 Å

PTP1B complexed with two BPPM molecules

Size: 38 kD, 321 residues



BPPM

Puius, Y. A.; Zhao, Y.; Sullivan, M.; Lawrence, D. S.; Almo, S. C.; Zhang, Z.-Y. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 13420.

Elchebly, M.et al.. *Science* **1999**, *283*, 1544.

In Silico Drug Design and Development

Inhibitors of protein tyrosine phosphatase-1B

Molecular docking and HTS for the discovery of novel PTP1B inhibitors:

HTS: a 400,000 "corporate" compound library was screened against PTP1B.
543 compounds inhibited the enzyme at 300 μM.
85 had IC_{50} values ranging from 1-100 μM.
Hit rate of 0.021%.

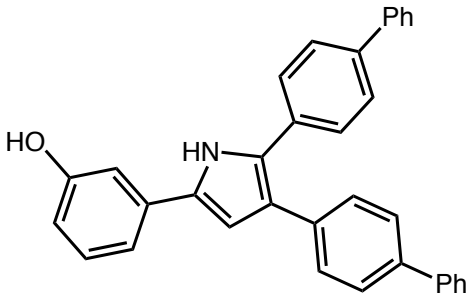
Moleculr docking: 250,000 commercial compounds from the ACD, Biospecs, and Maybridge databases were evaluated in DOCK 3.5.
An average of 350 conformations per compound (~90 x 10^6 conformers).
1000 compounds were considered for further evaluation (889 commercially available).
365 compounds (178 "spanners" and 187 "nonspanners") were tested *in vitro.*
127 compounds had $IC_{50} < 100$ μM
21 hits with $IC_{50} < 10$ μM
Hit rate of 34.6%.

Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.

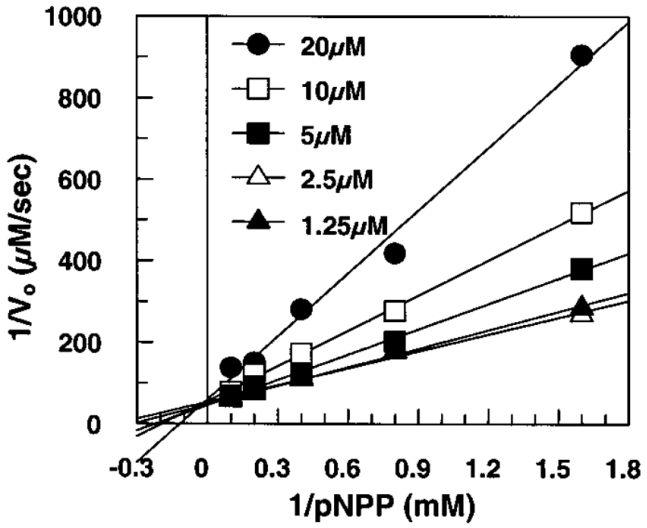
In Silico Drug Design and Development

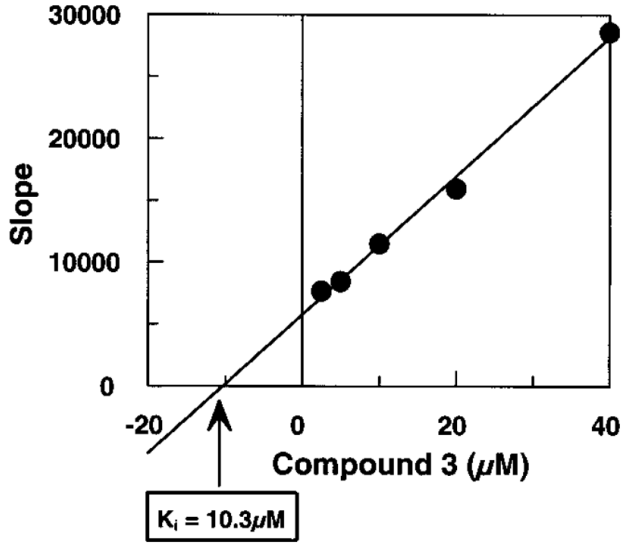
Inhibitors of protein tyrosine phosphatase-1B

■ **Kinetic analysis of docking hits:** 4 out of the 127 hits behaved as simple competitive inhibitors.



Compound 3
Docking rank: 39
Docking score: -39.6 kcal/mol
IC_{50} = 8.6 μM (vs. PTP1B)





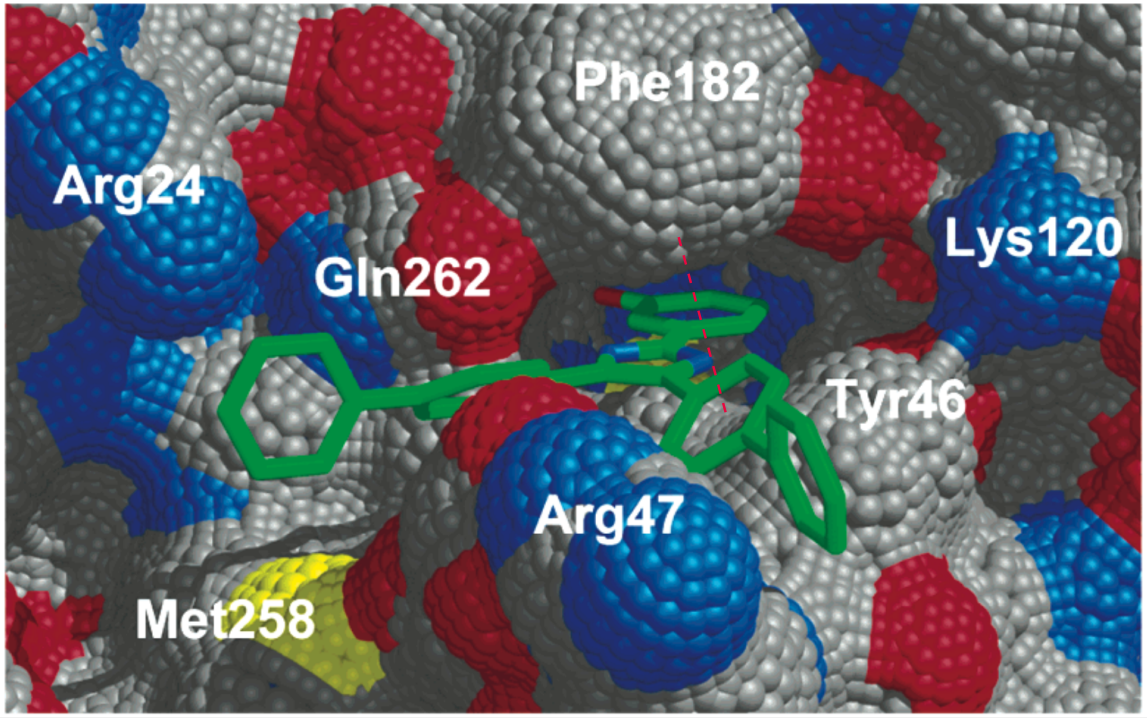
Lineweaver-Burk analysis of competitive inhibitor compound 3 (pNPP = *p*-nitrophenyl phosphate).

Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.

In Silico Drug Design and Development

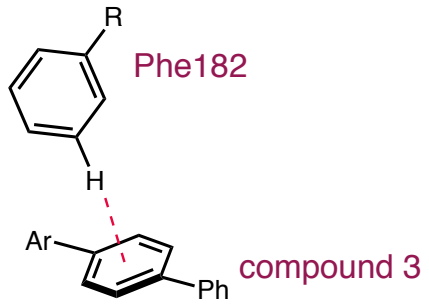
Inhibitors of protein tyrosine phosphatase-1B

- **The molecular surface of PTP1B docked with compound 3 (K_i = 10.3 μM):**



Extensive shape complementarity

Aryl ring sandwiched in between Phe183 and Tyr46 (stacked with Tyr46)



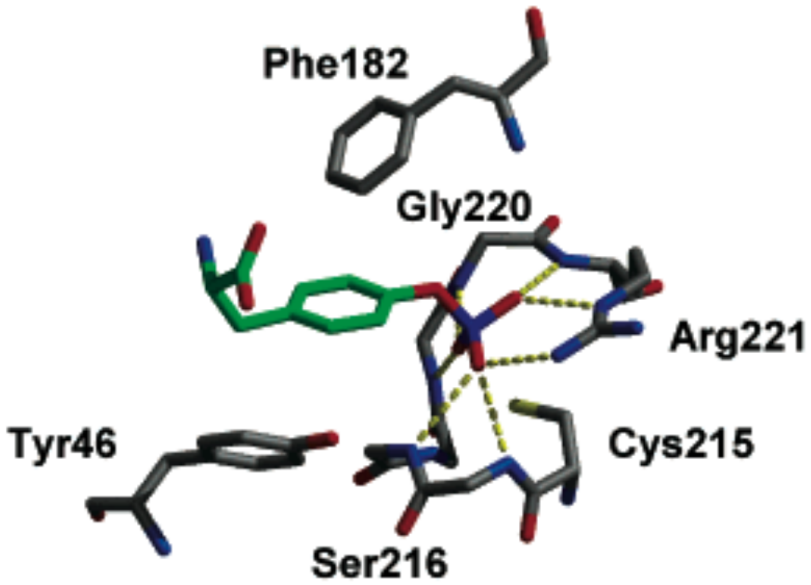
Herringbone interaction
(C–H - - π–interaction)

Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.

In Silico Drug Design and Development

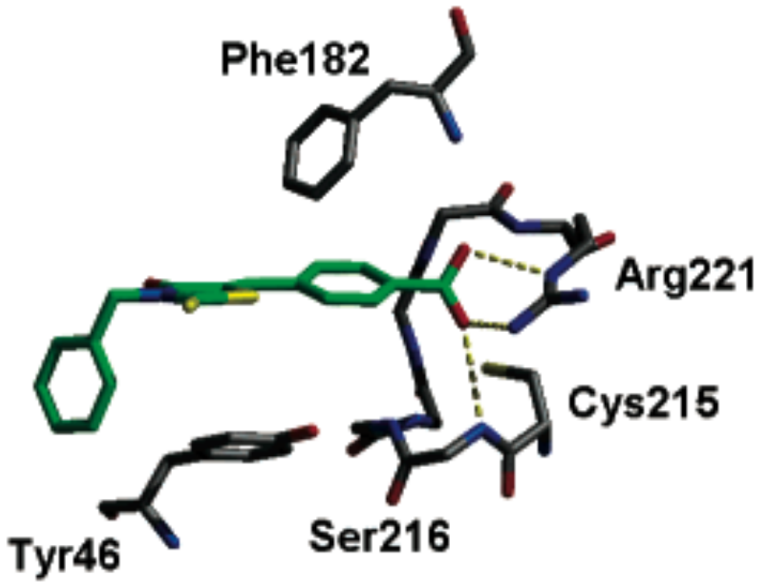
Inhibitors of protein tyrosine phosphatase-1B

Comparision of the docked ligands to phosphotyrosine:

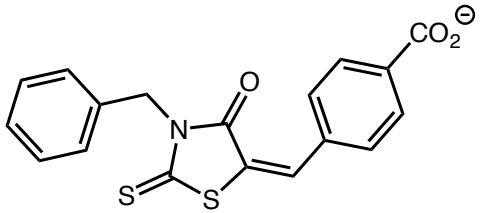


X-ray structure of the PTP1B catalytic site bound to phosphotyrosine.

Charged compounds featuring carboxylic or salicylic acid groups hydrogen bond with the phosphate recognition residues: Ser216, Gly220, and Arg221.



Compound 8 docking orientation



IC_{50} = 21.6 μM (vs. PTP1B)

Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.

In Silico Drug Design and Development

Inhibitors of protein tyrosine phosphatase-1B

- **Chemical informatics:** Compare and contrast the "druglikeness" of the docked vs. HTS hit list.

Lipinski's rule of five (RO5):

1) molecular weight ≤ 500
2) calculated lopP ≤ 5
3) ≤ 5 hydrogen bond donors
4) ≤ 10 hydrogen bond acceptors

list	# of compounds	passing 3/4	passing 4/4	average RO5 score
HTS hits	81	49	19	2.73
high-scoring docking molecules	889	773	577	3.47
docking molecules selected for testing	365	332	248	3.57
bioactive docking hits	127	116	73	3.49

Bioactive hits from docking are more drug like!

Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.

In Silico Drug Design and Development

Inhibitors of protein tyrosine phosphatase-1B

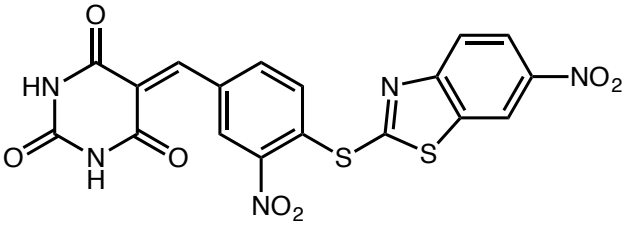
Important things to consider:

1) Although there is a 1700-fold enrichment in the docking hit rate vs. HTS.

   These libraries contain fundamentally different structures. A apples to apples comparison is needed.

2) Are the docking molecules binding at the active site as predicted?

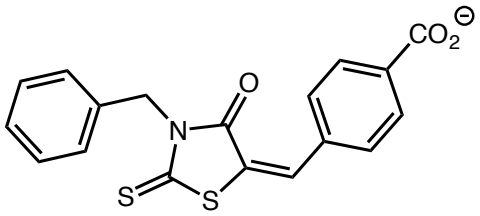
3) Are the scoring functions accurate?



compound 1

docking score -33.4 kcal/mol

IC_{50} = 4.1 μM (vs. PTP1B)



compound 8

docking score -42.0 kcal/mol

IC_{50} = 21.6 μM (vs. PTP1B)

These inaccuracies may be attributed to failure to consider enzyme desolvation and conformational changes.

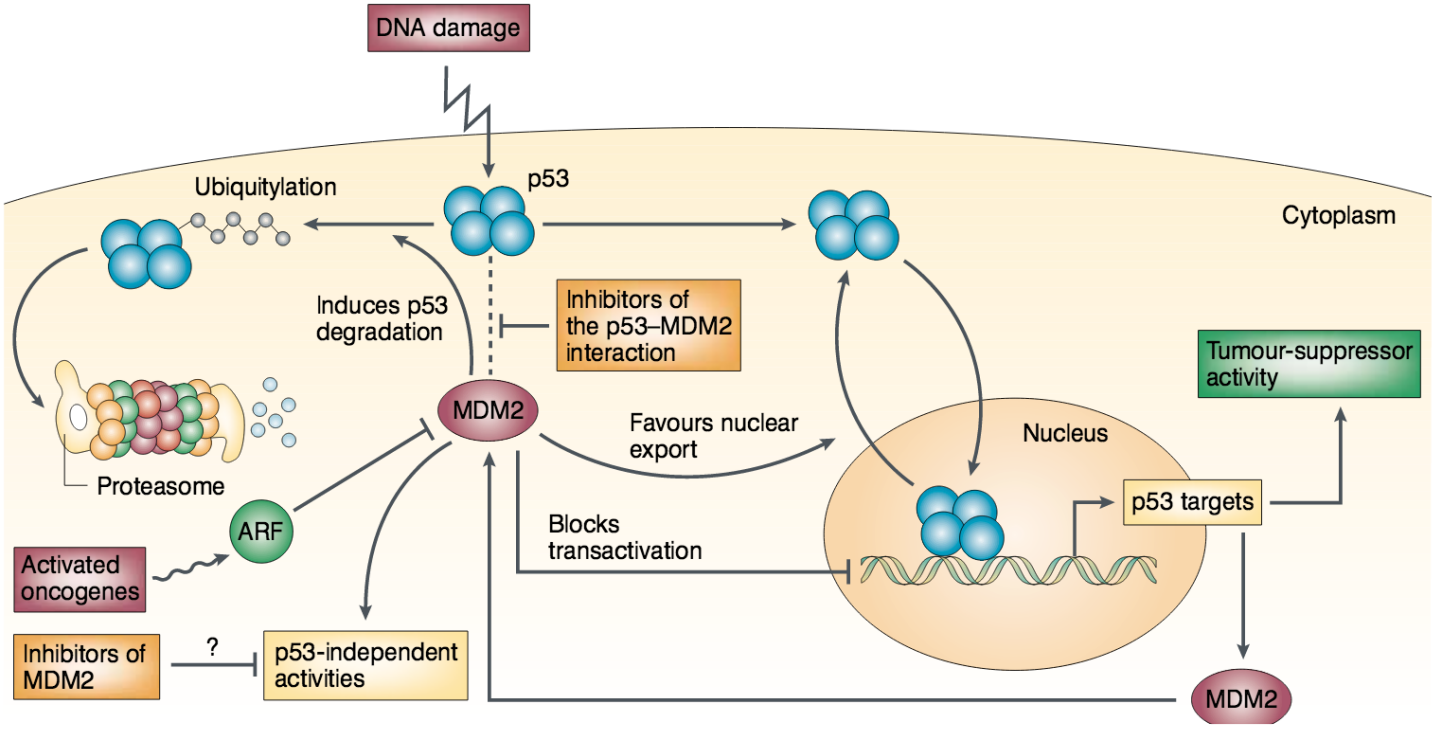
Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.

In Silico Drug Design and Development

p53-MDM2 inhibitor

New potential cancer therapy:

The tumor suppressor p53 is mutated in 50% of human cancers. In the remaining 50% it is rendered inactive due to interactions with the murine double minute 2 (MDM2) protein.



Inhibiting the p53-MDM2 interaction presents a novel strategy for the development of therapeutics against a variety of cancers.

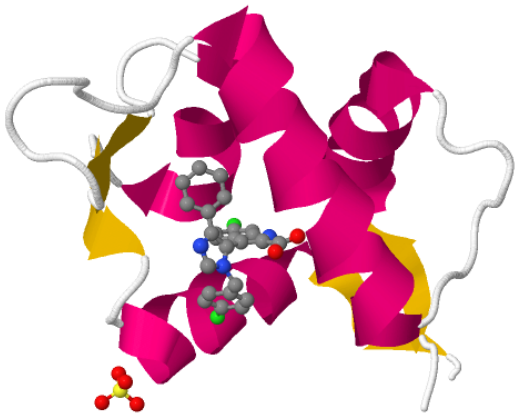
Chene, P. *Nat. Rev. Cancer* **2003**, *3*, 102.

In Silico Drug Design and Development

p53-MDM2 inhibitor

- ***X-ray structures of MDM2 bound to 3 diffrent inhibitors:***

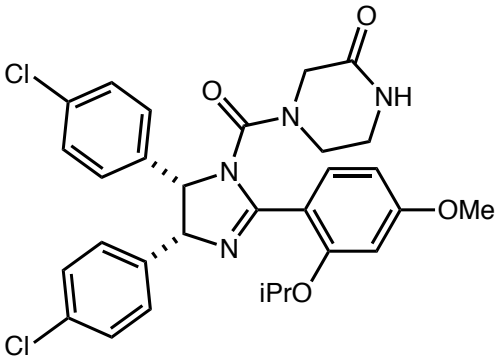
  Each ligand type induces different MDM2 confromers used in docking analysis

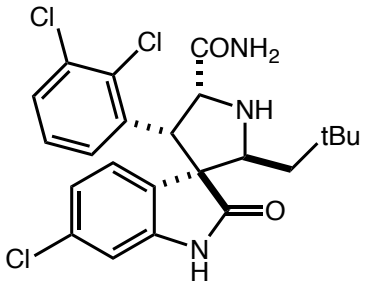


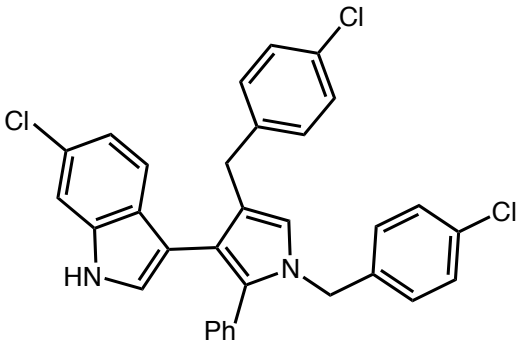
MDM2/nutlin

MDM2/MI63

MDM2/K23





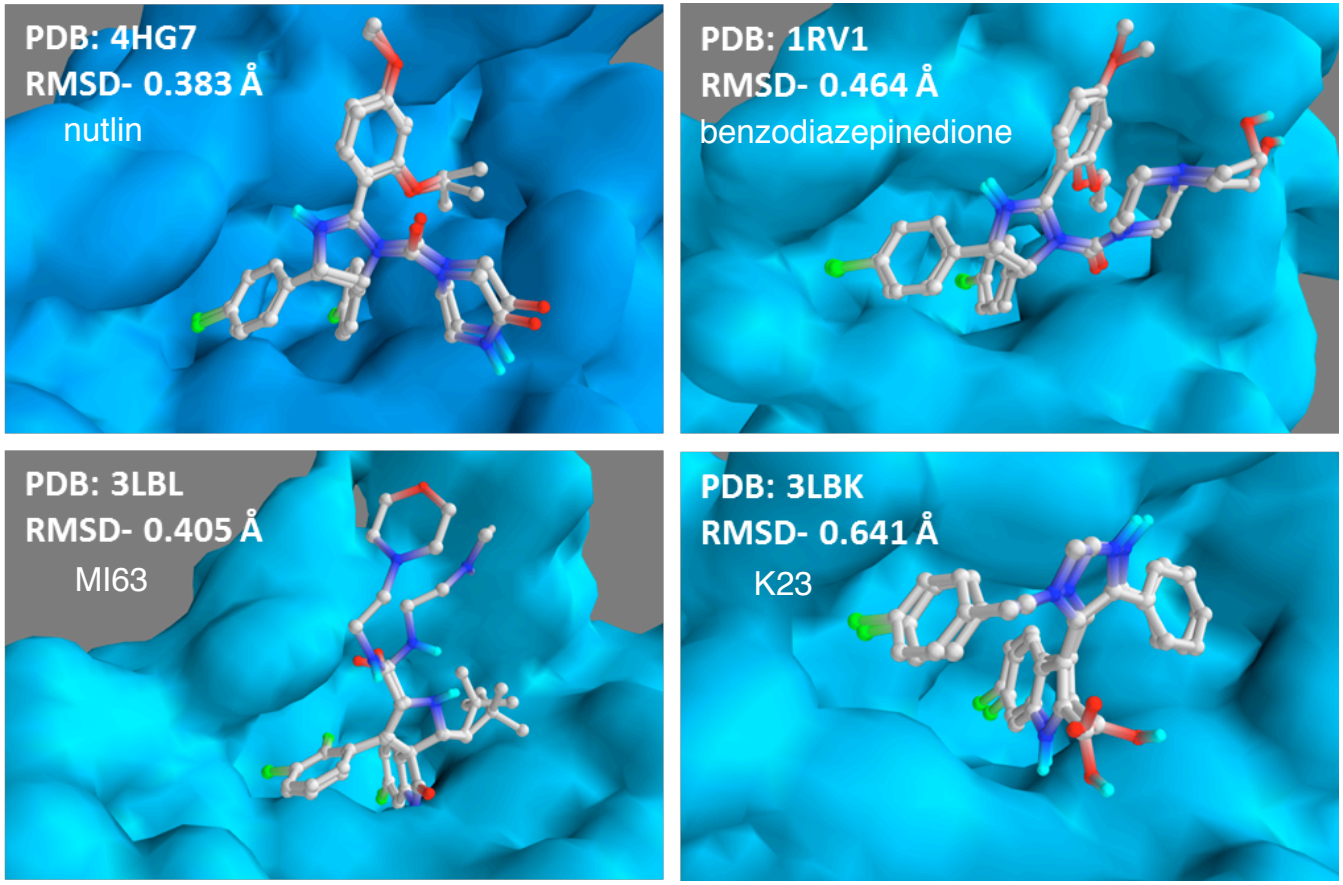


Patil, S. P.; Pacitti, M. F.; Gilroy, K. S.; Ruggiero, J. C.; Griffin, J. D.; Butera, J. J.; Notarfrancesco, J. M.; Tran, S.; Stoddart, J. W. *J. Comput.-Aided Mol. Des.* **2015**, *29*, 15

In Silico Drug Design and Development

p53-MDM2 inhibitor

- **The binding modes of MDM2 crystal ligands predicted by the AutoDock Vina*:***



Excellent agreement with X-ray pose

Patil, S. P.; Pacitti, M. F.; Gilroy, K. S.; Ruggiero, J. C.; Griffin, J. D.; Butera, J. J.; Notarfrancesco, J. M.; Tran, S.; Stoddart, J. W. *J. Comput.-Aided Mol. Des.* **2015**, *29*, 15

In Silico Drug Design and Development

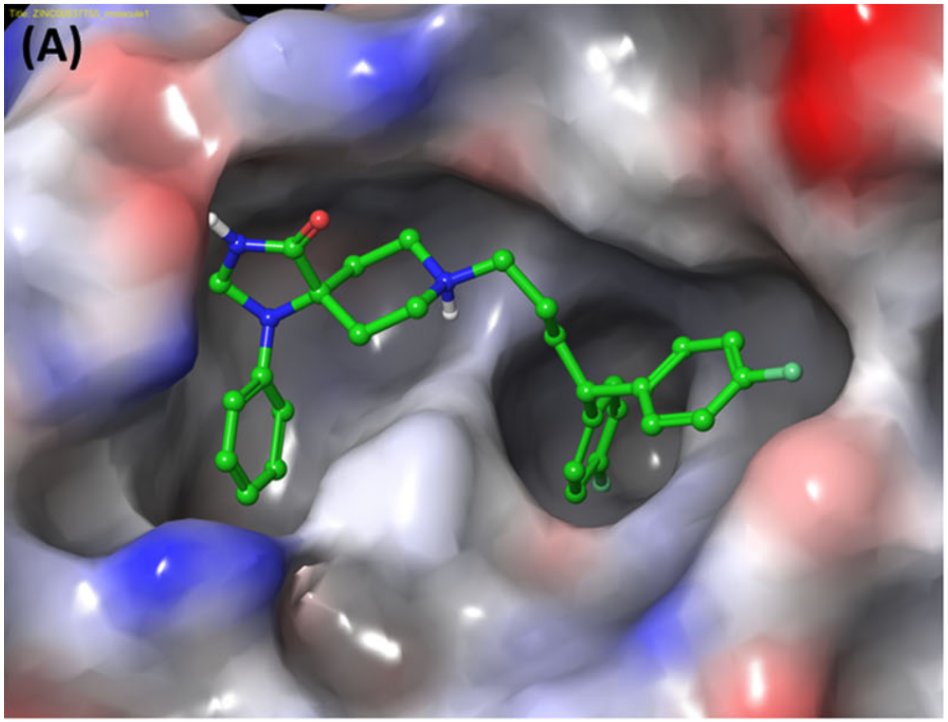
p53-MDM2 inhibitor

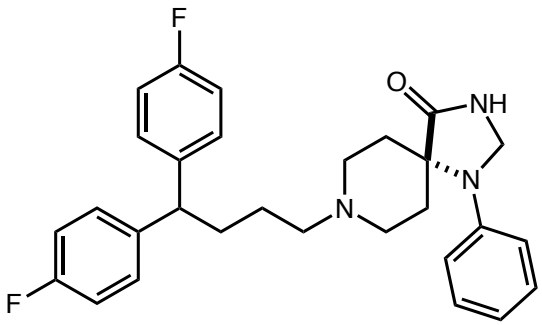
- ***Repurposing drugs through virtual screening:***

7,800 approved drugs from the ZINC database were selected for virtual screening against MDM2

6 hit compounds were identified.

ZINCC00537755 (fluspiriline) had the highest binding energy





Fluspirilene

Discovered in 1963 at Janssen Pharmaceutica

schizophrenia

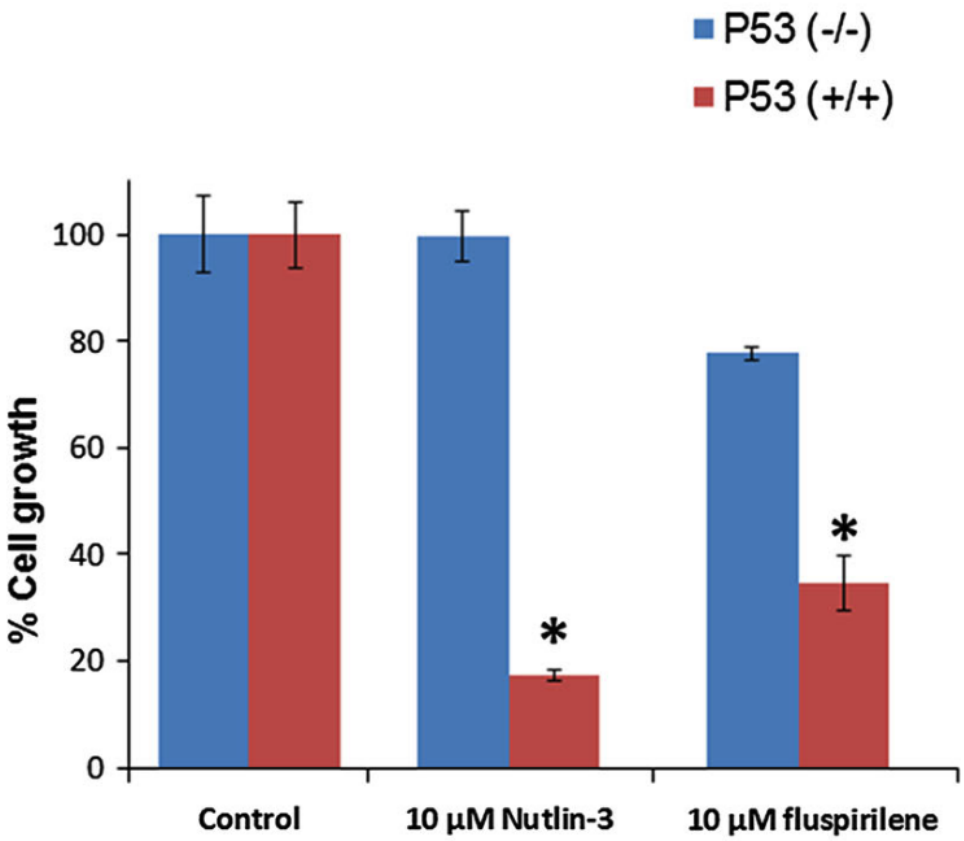
molecule	binding free energy
fluspiriline	-122 kcal/mol
MI63	-126 kcal/mol

Patil, S. P.; Pacitti, M. F.; Gilroy, K. S.; Ruggiero, J. C.; Griffin, J. D.; Butera, J. J.; Notarfrancesco, J. M.; Tran, S.; Stoddart, J. W. *J. Comput.-Aided Mol. Des.* **2015**, *29*, 15

In Silico Drug Design and Development

p53-MDM2 inhibitor

- **Fluspirilene showed comparible inhibiton to known inhibitor nutlin:**



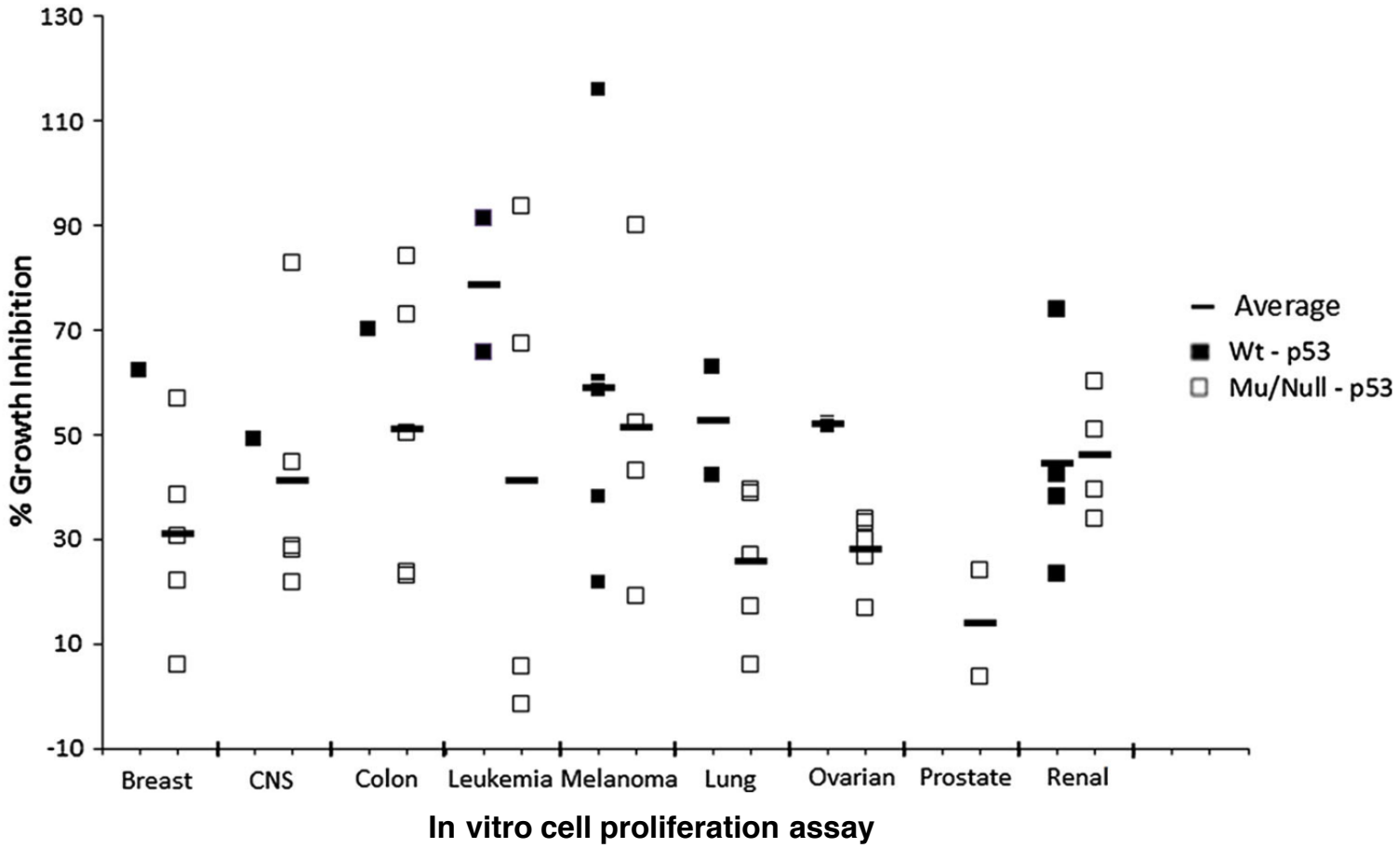
In vitro cell proliferation assay

Patil, S. P.; Pacitti, M. F.; Gilroy, K. S.; Ruggiero, J. C.; Griffin, J. D.; Butera, J. J.; Notarfrancesco, J. M.; Tran, S.; Stoddart, J. W. *J. Comput.-Aided Mol. Des.* **2015**, *29*, 15

In Silico Drug Design and Development

p53-MDM2 inhibitor

- **Fluspirilene (10 μM) showed a broad-spectrum in the NCI60 human tumor cell lines:**

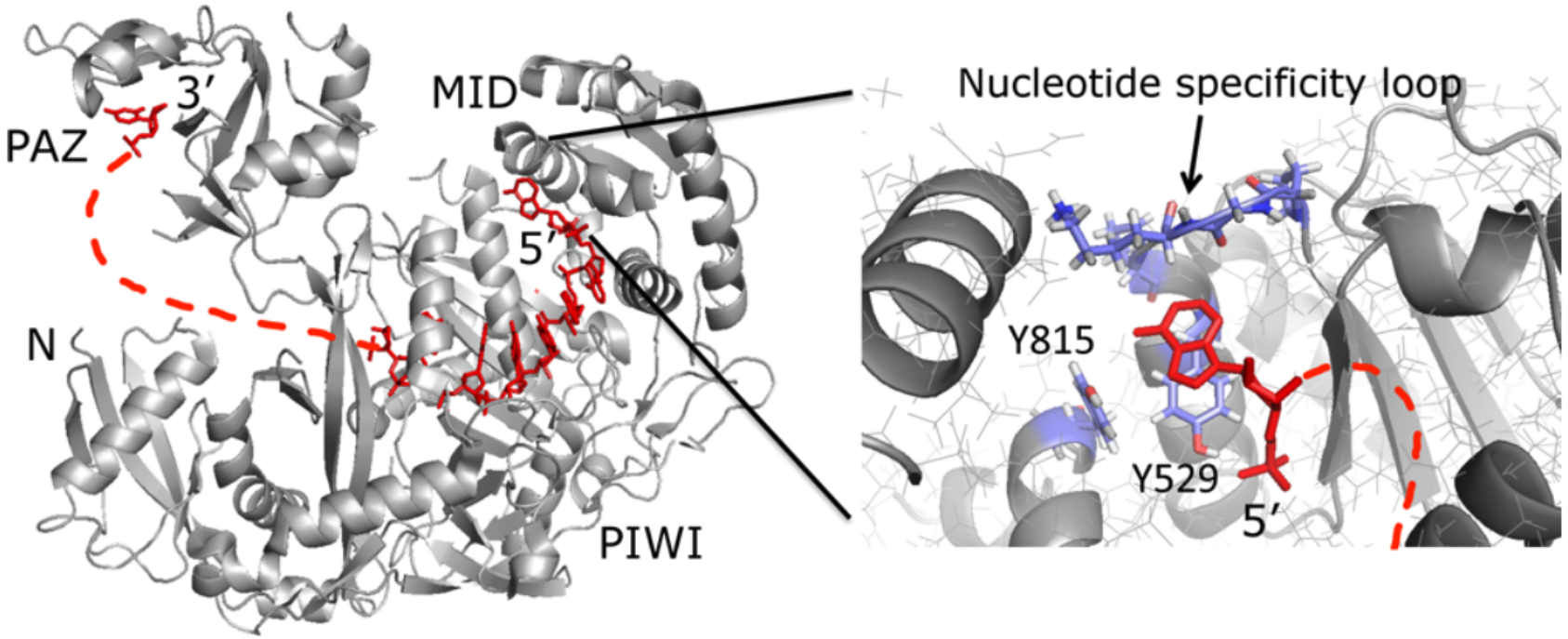


Patil, S. P.; Pacitti, M. F.; Gilroy, K. S.; Ruggiero, J. C.; Griffin, J. D.; Butera, J. J.; Notarfrancesco, J. M.; Tran, S.; Stoddart, J. W. *J. Comput.-Aided Mol. Des.* **2015**, *29*, 15

In Silico Drug Design and Development

siRNA

- **Argonaute protiens form complexes responsible for RNA silencing in eukaryotes :**



X-ray stucture of hAgo2 interactions with the RNA '5-guide strand

siRNAs are promising targets for potentail theraputics many o which have been considered to be "undrugable"

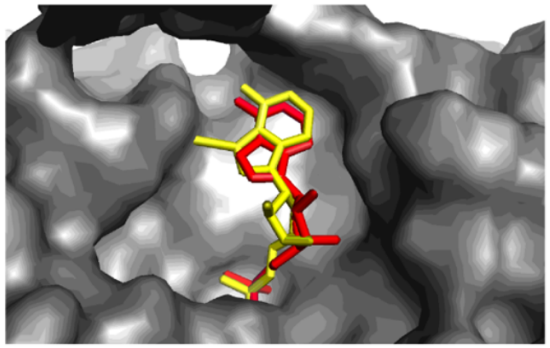
Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

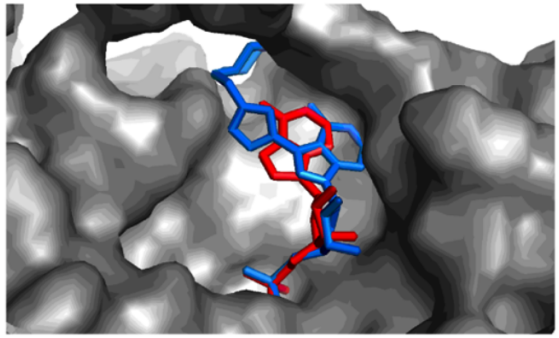
Schirle, N. T.; MacRae, I. J. *Science* **2012**, *336*, 1037.

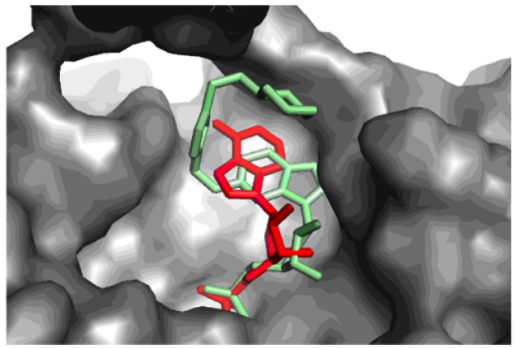
In Silico Drug Design and Development

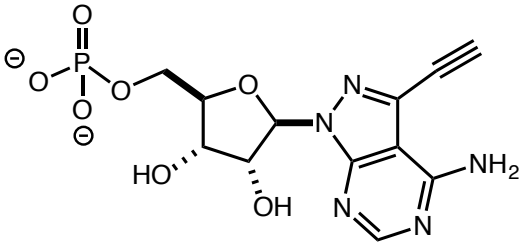
siRNA

Predicted hAgo2 binding modes for 5'-end guide strand analogs purine analogs :

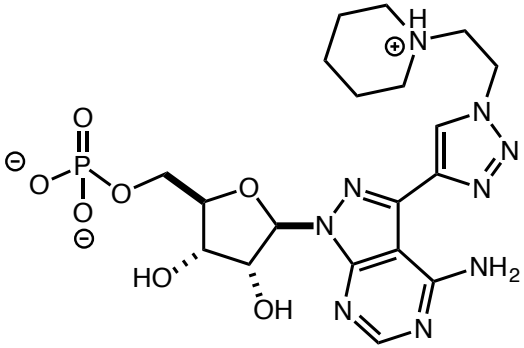




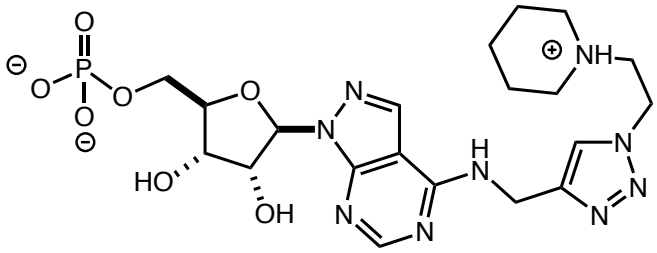




7-EAA



7-EAA triazole



2-AP triazole

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

Schirle, N. T.; MacRae, I. J. *Science* **2012**, *336*, 1037.

In Silico Drug Design and Development

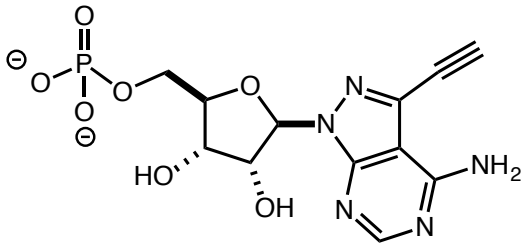
siRNA

Predicted hAgo2 binding modes for 5'-end guide strand analogs purine analogs :

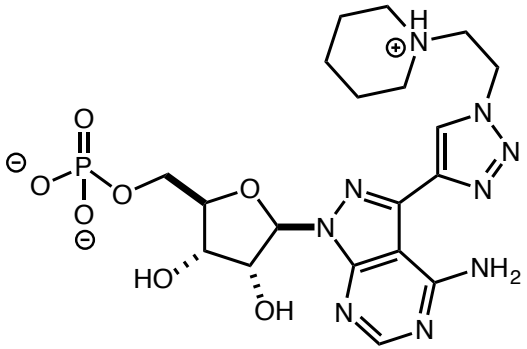
	predicted hAgo2 binding[a]	siRNA activity[b]
adenosine	1.0	++
7-EAA	8.8	+
7-EAA triazole	9.2	+
2-AP-triazole	9.5	+

[a]Lower number represents better-predicted binding

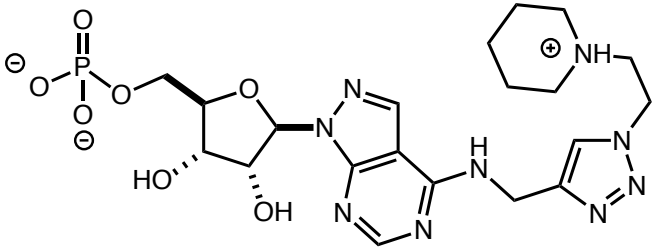
[b]+++ = <10% luciferase activity remaining after knockdown; ++ = 10-40%; + = 41-70%; ,– = >70%



7-EAA



7-EAA triazole



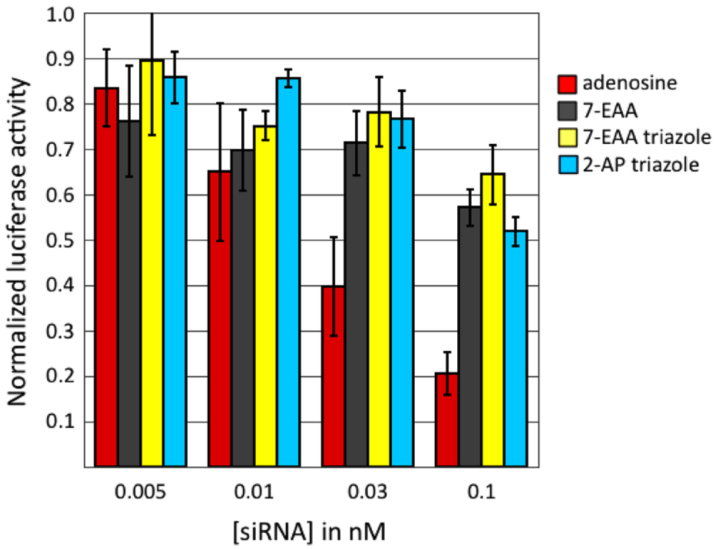
2-AP triazole

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

In Silico Drug Design and Development

siRNA

Luciferase knockdown activity in HeLa cells :



All siRNAs were prepared with a 5'-phosphorylated guide strand

5′-p-XUAGGAUUCAUAUUAGGAGAU-3′ guide

3′-ACUAUCCUAAGUAUAAUCCUC-5′ passenger

X = adenosine or nucleoside analog

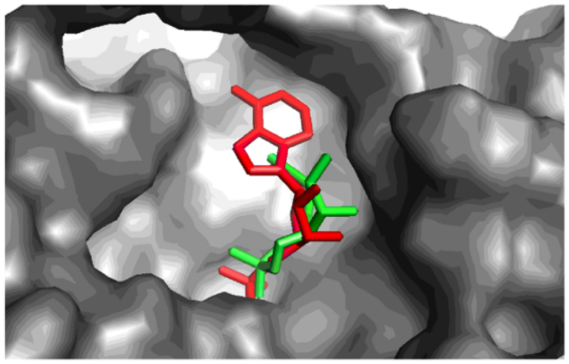
Sequence of siRNA used in this study. X indicates guide strand position 1.

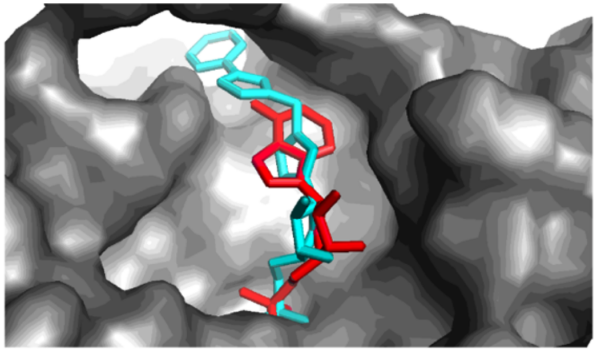
Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

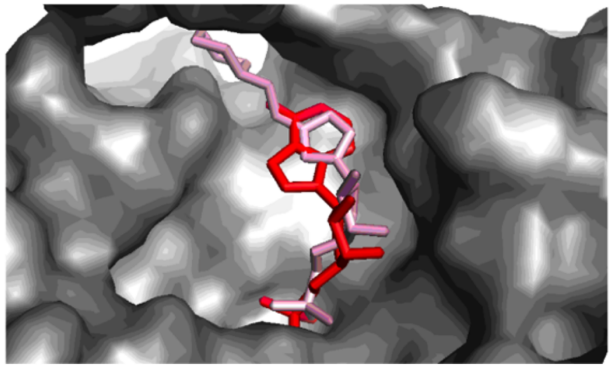
In Silico Drug Design and Development

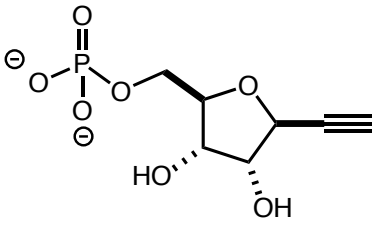
siRNA

Predicted hAgo2 binding modes for 5'-end guide strand 1-ethynyl ribose derivatives :

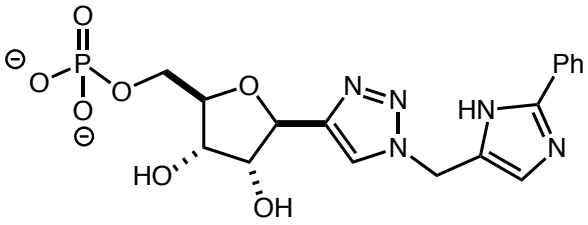




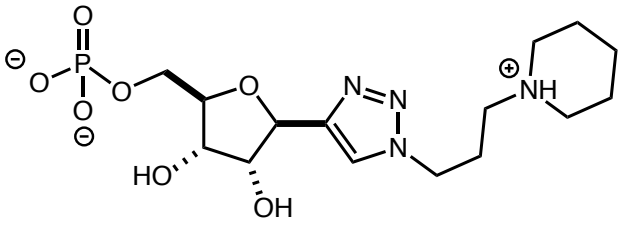




7-ER



7-ER triazole 1



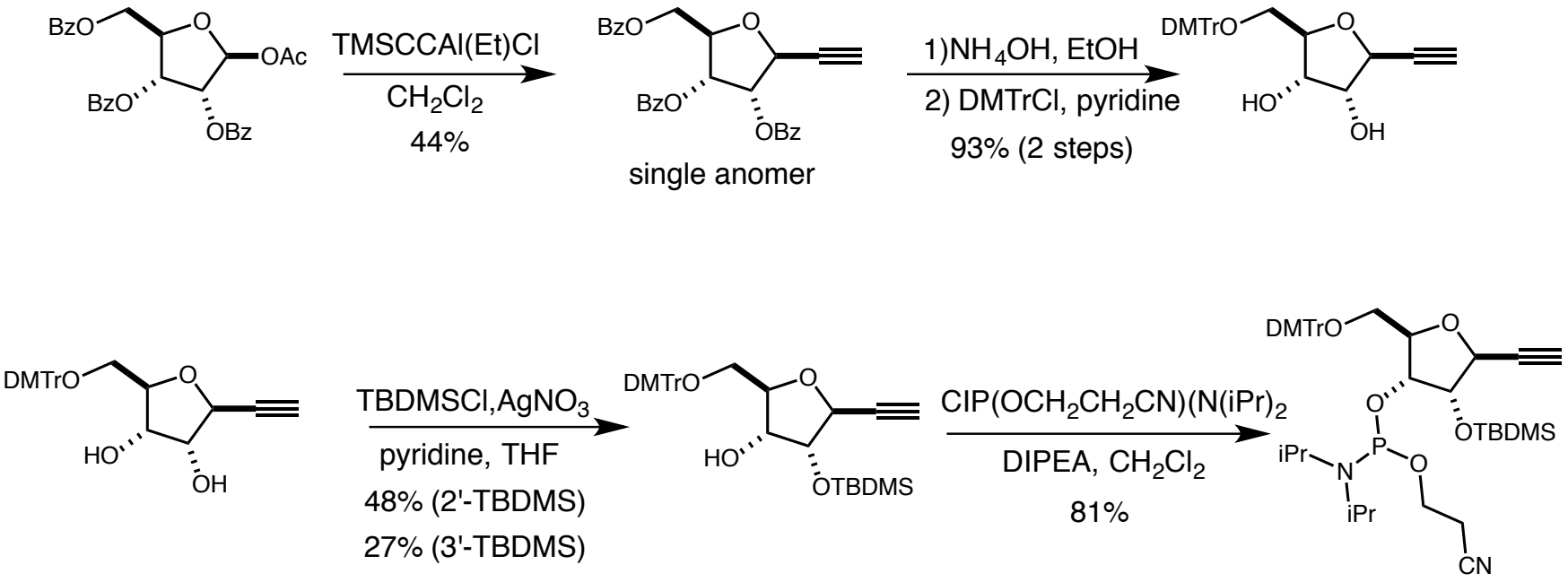
7-ER triazole 2

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.
Schirle, N. T.; MacRae, I. J. *Science* **2012**, *336*, 1037.

In Silico Drug Design and Development

siRNA

Synthesis of 1-ER Phosphoramidite :



Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

In Silico Drug Design and Development

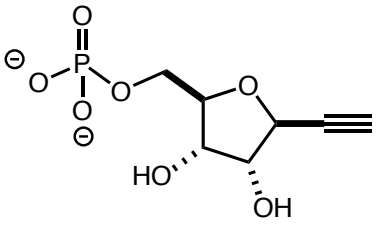
siRNA

Predicted hAgo2 binding modes for 5'-end guide strand 1-ethynyl ribose derivatives :

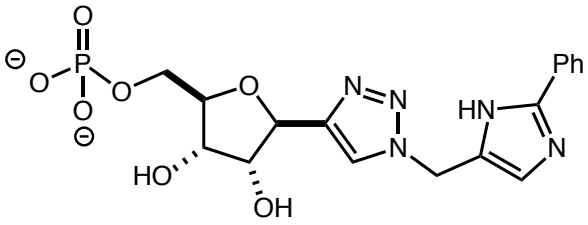
	predicted hAgo2 binding[a]	siRNA activity[b]
adenosine	1.0	++
1-ER	2.1	++
1-ER triazole I	1.2	+++
1-ER triazole II	1.3	+++

[a]Lower number represents better-predicted binding

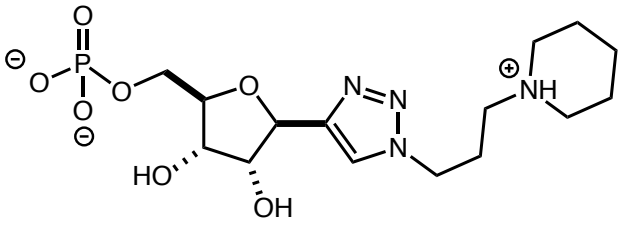
[b]+++ = <10% luciferase activity remaining after knockdown; ++ = 10-40%; + = 41-70%; ,– = >70%



7-ER



7-ER triazole 1



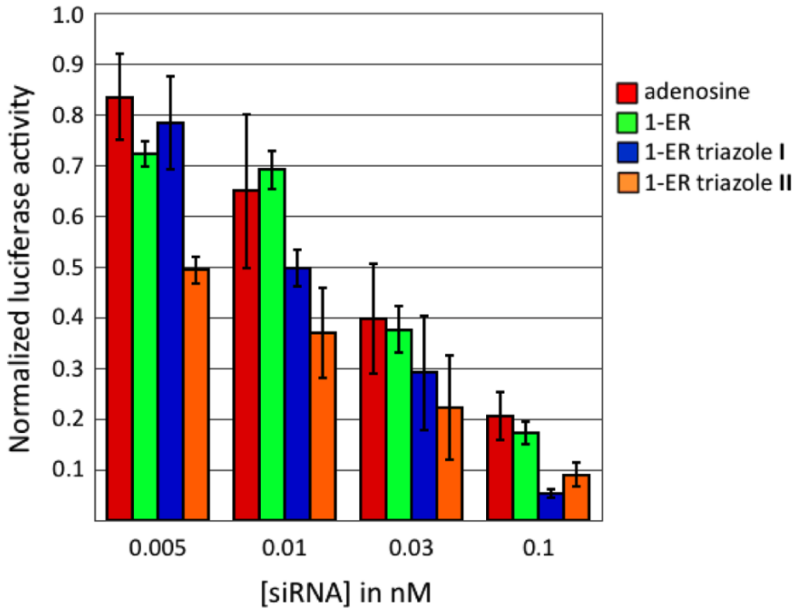
7-ER triazole 2

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

In Silico Drug Design and Development

siRNA

Luciferase knockdown activity in HeLa cells :



5′-p-XUAGGAUUCAUAUUAGGAGAU-3′ guide

3′-ACUAUCCUAAGUAUAAUCCUC-5′ passenger

X = adenosine or nucleoside analog

Sequence of siRNA used in this study. X indicates guide strand position 1.

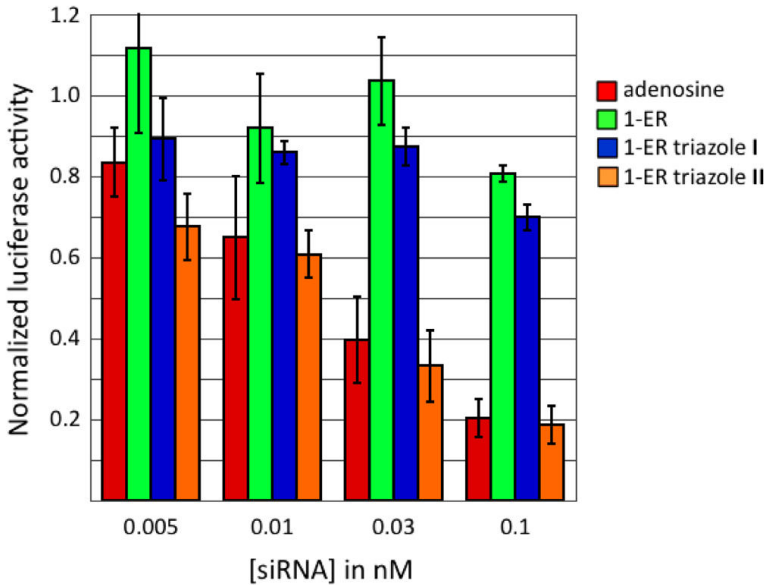
All siRNAs were prepared with a 5'-phosphorylated guide strand

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

In Silico Drug Design and Development

siRNA

Luciferase knockdown activity in HeLa cells :



5′-p-XUAGGAUUCAUAUUAGGAGAU-3′ guide

3′-ACUAUCCUAAGUAUAAUCCUC-5′ passenger

X = 5'-end ○ = ER anaolg

Sequence of siRNA used in this study. X indicates guide strand position 1.

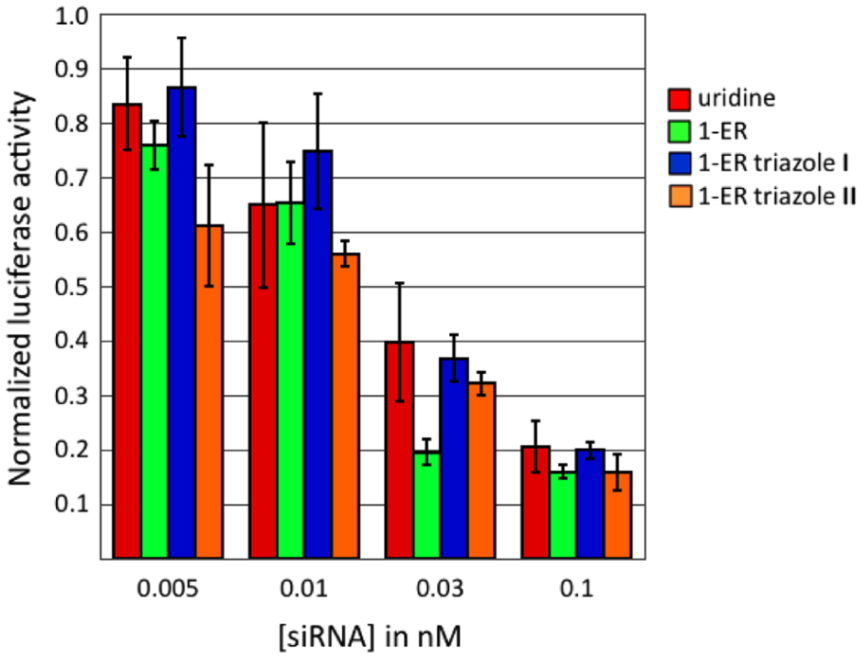
All siRNAs were prepared with a 12 position-phosphorylated guide strand

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

In Silico Drug Design and Development

siRNA

Luciferase knockdown activity in HeLa cells :



All siRNAs were prepared with a 5'-phosphorylated guide strand.
Modifications made at the 19-position of the passenger strand

5′-p-AUAGGAUUCAUAUUAGGAGAU-3′ guide
3′-ACXAUCCUAAGUAUAAUCCUC-5′ passenger

X = uridine or nucleoside analog

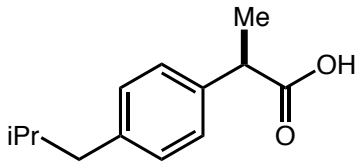
Sequence of siRNA used in this study.

Onizuka, K.; Harrison, J. G.; Ball-Jones, A. A.; Ibarra-Soza, J. M.; Zheng, Y.; Ly, D.; Lam, W.; Mac, S.; Tantillo, D. J.; Beal, P. A. *J. Am. Chem. Soc.* **2013**, *135*, 17069.

In Silico Drug Design and Development

How easy is it?

SMILES to HIts

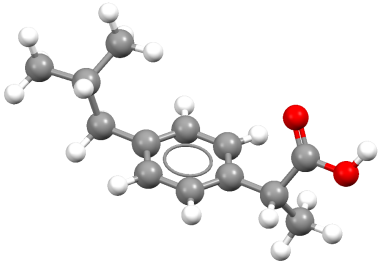


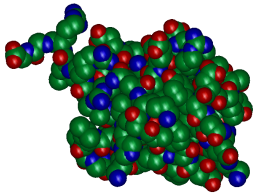
2D Sketch
Porgram

OC([C@H](C)C1=CC=C(CC(C)C)C=C1)=O

Simplified Molecular Input Line Entry Specification (SMILES)

convert to 3D stucture





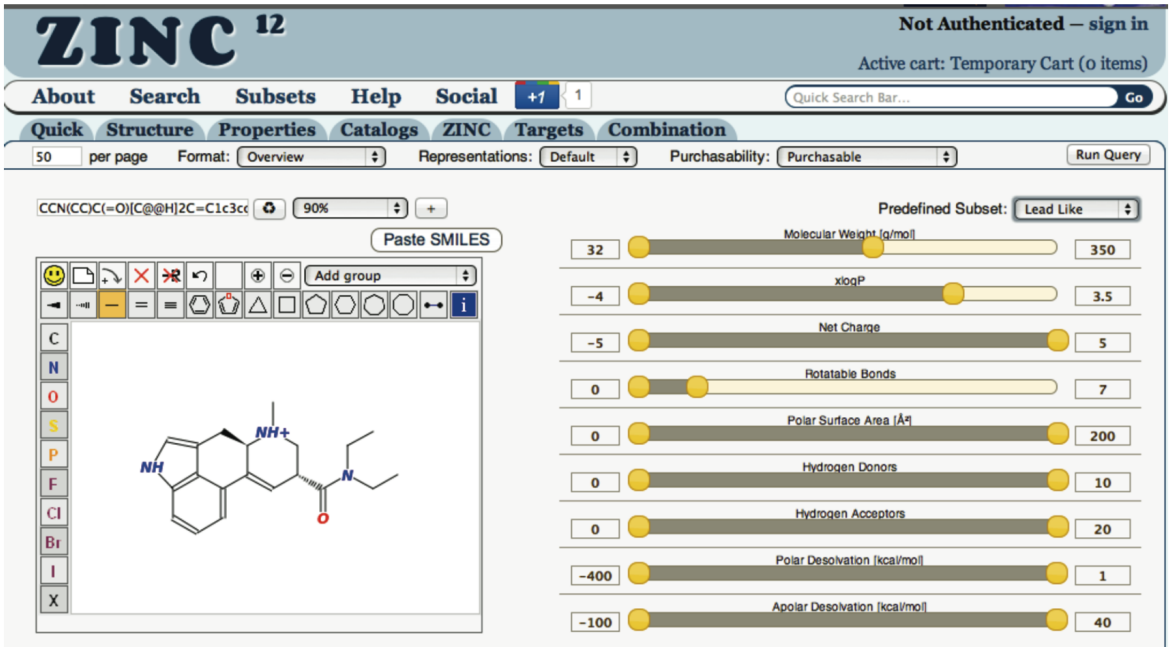
AutoDock

Enzyme substate
complex
binding score

In Silico Drug Design and Development

ZINC database

- **ZINC:** a free public resource for ligand discovery. The ZINC database contains 120 million commercially available "drug like" molecules in biologically relevant 3D representations that can be downloaded in popular ready-to-dock formats.



- Designed for investigators who are not computer specialists

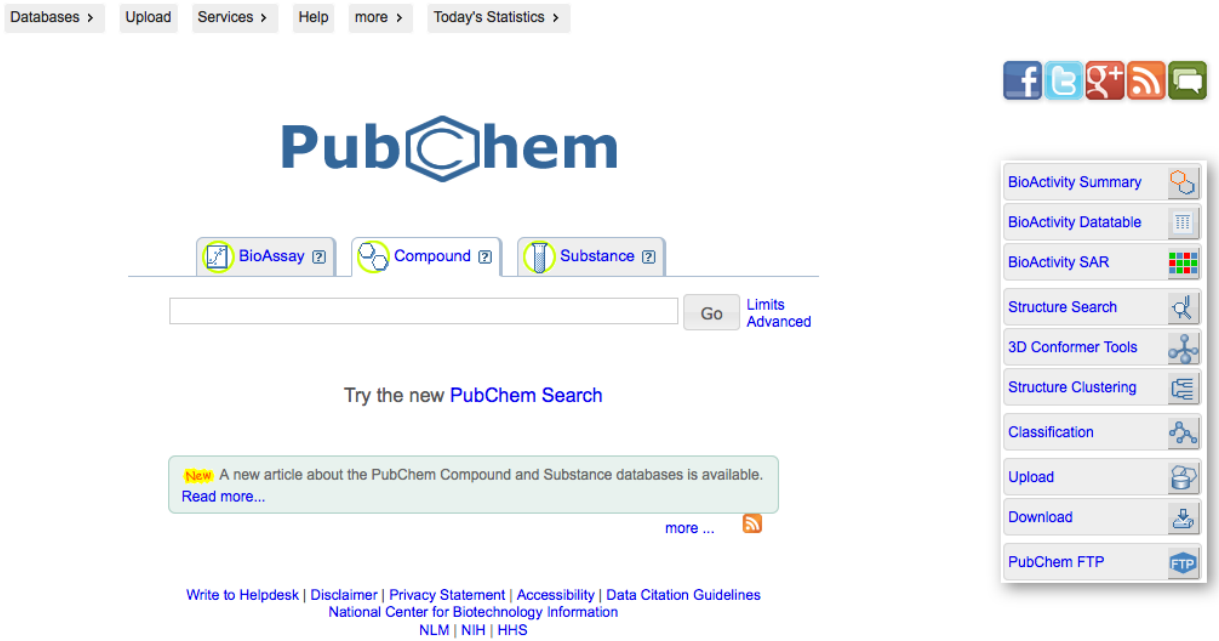
Irwin, J. J.; Sterling, T.; Mysinger, M. M.; Bolstad, E. S.; Coleman, R. G. *J. Chem. Inf. Model.* **2012**, *52*, 1757.

http://zinc.docking.org/

In Silico Drug Design and Development

PubChem database

- **PubChem:** is a database containing compounds and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI).



- Contains over 7.7 million compounds
- Over 2.1 million compounds tested
- 1.2 million bioactivity results
- Over 10 thousand protein targets

https://pubchem.ncbi.nlm.nih.gov/

In Silico Drug Design and Development

summary

- ***In silico drug design advantages and limitaions:***

Advantages:

Inexpensive
Low waste generation
Inconceivable amounts of chemical space can be investigated in seconds

Disadvantages/Challenges:

Potential problems with synthetic accessibility
Addressing receptor flexibility (reverse docking) is a major challenge
Developing new filters for removing promiscuous binders and reactive inhibitors

"Given the structure of a protein and that of a potential ligand, can the two form a favorable complex? What are the bases for binding and specificity?"

Probably...Yes!