Structure-Based Drug Design

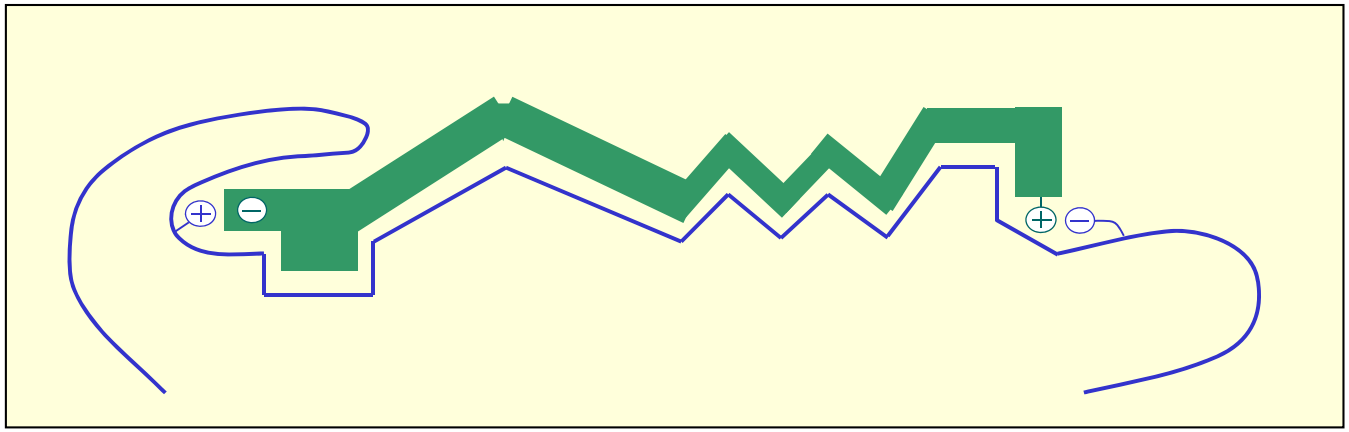
A few general principles and case studies

BC530

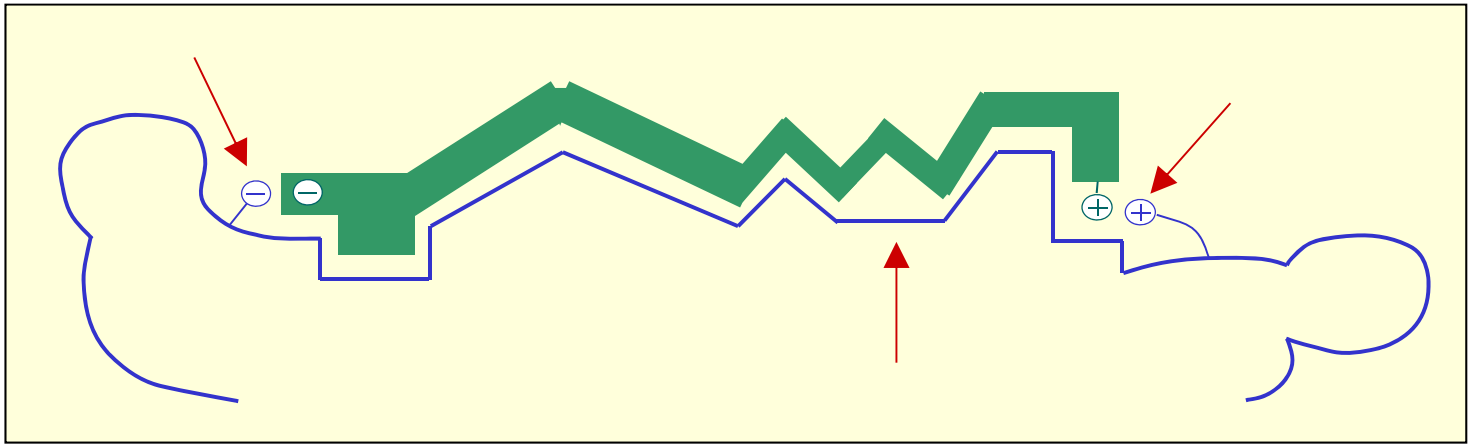
Fall Quarter 2015

Wim G. J. Hol
http://www.bmsc.washington.edu/WimHol/

Simplified View of Structure-based Drug Design



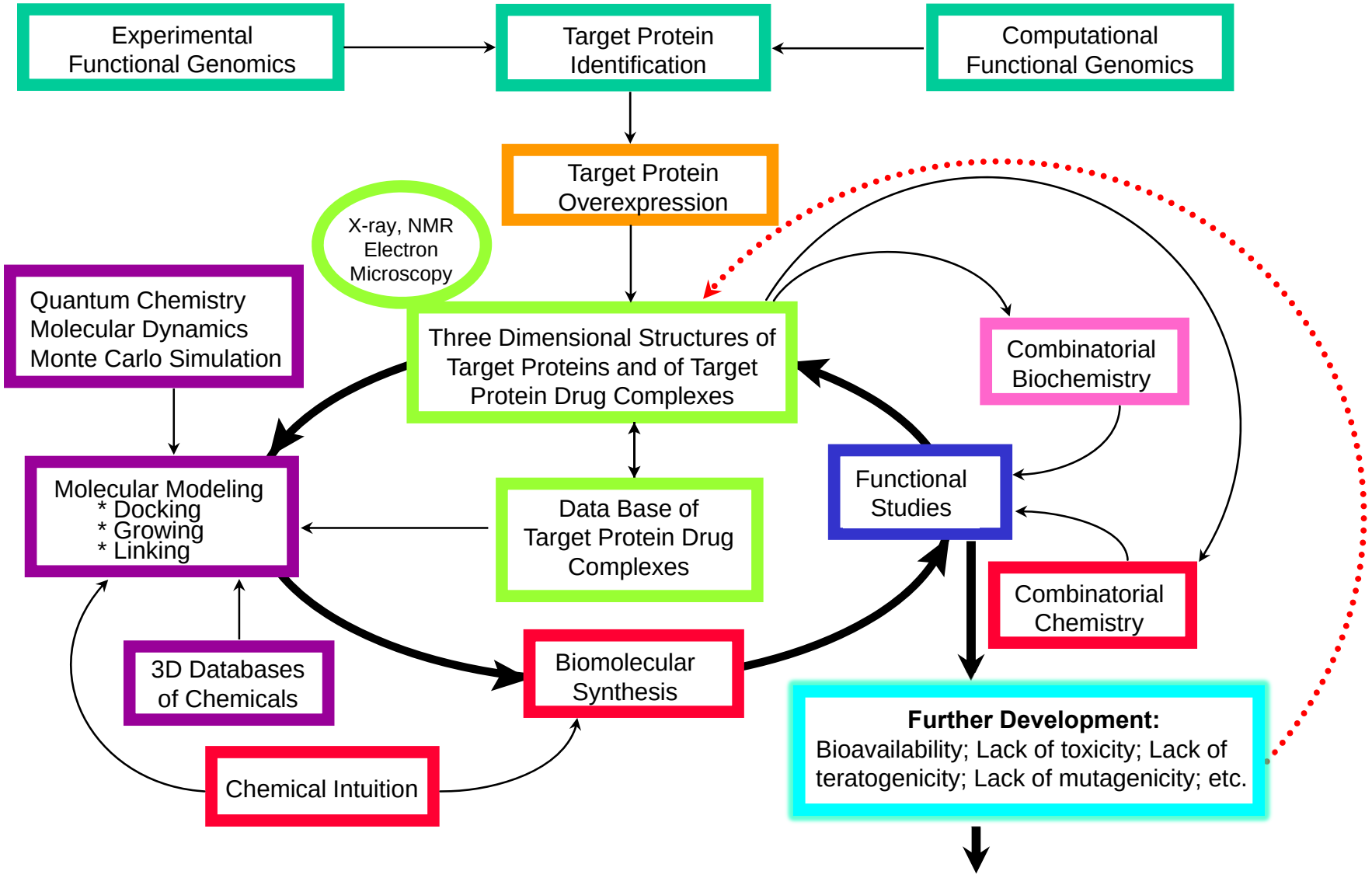
High Affinity for Drug Target



Low Affinity for Homologues of Drug Target

Selective Inhibition is often, but not always (!), CRUCIAL

STRUCTURE-GUIDED DRUG DESIGN



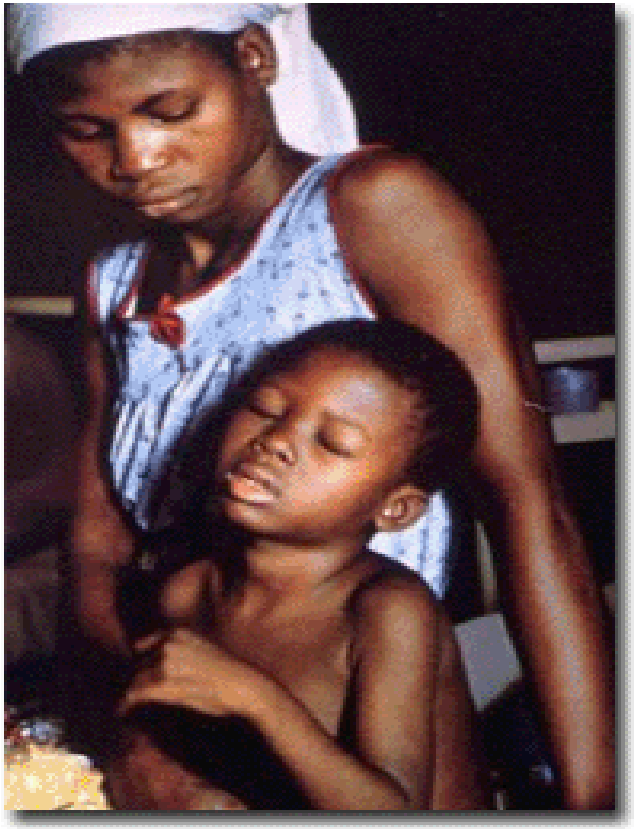
Drug Design

A case study

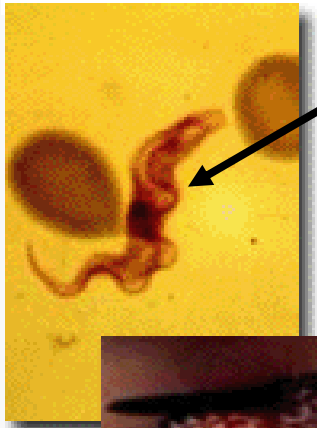
Structure-Based Inhibitor Design of the enzyme GAPDH from the sleeping sickness parasite, a “Trypanosomatid”

Sleeping Sickness

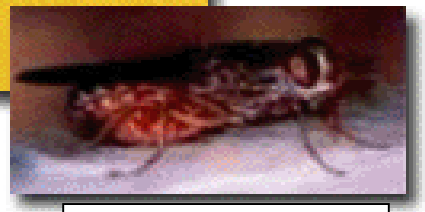
a.k.a “African Trypanosomiasis”



Lumbar puncture
for diagnosis of parasites in CNS



Blood stream
form of parasite



Tsetse fly

Sleeping sickness is caused by a unicellular eukarytote: *Trypanosoma brucei* – a “Trypanosomatid”

Other pathogenic trypanosomatids are whole set of *Leishmania* species.

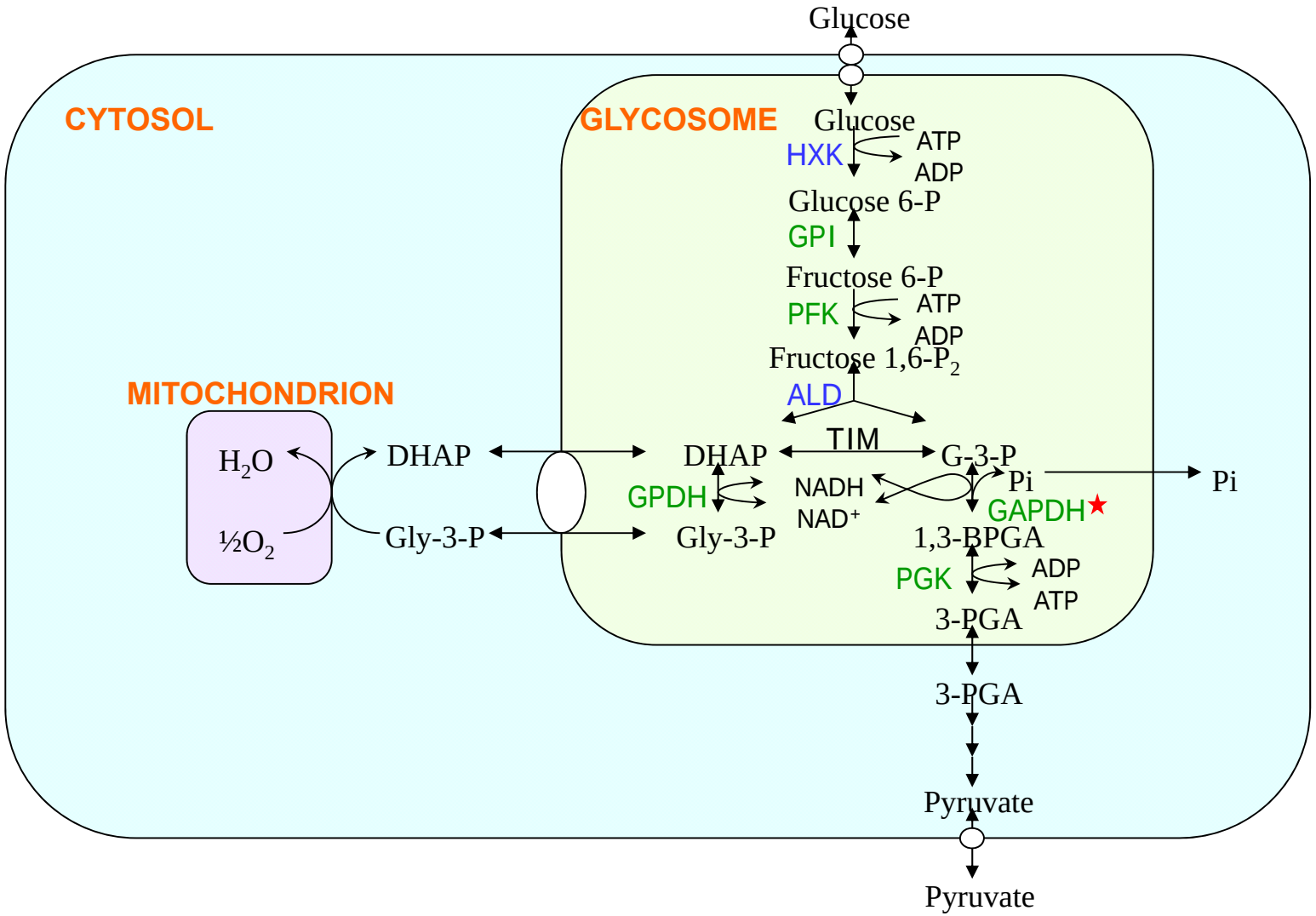
These cause a spectrum of different tropical diseases, called “leishmaniasis”.

Many enzymes in *Trypanosoma brucei* and *Leishmania* species are very similar in amino acid seqeunce.

With **thanks to Wes Van Voorhis**

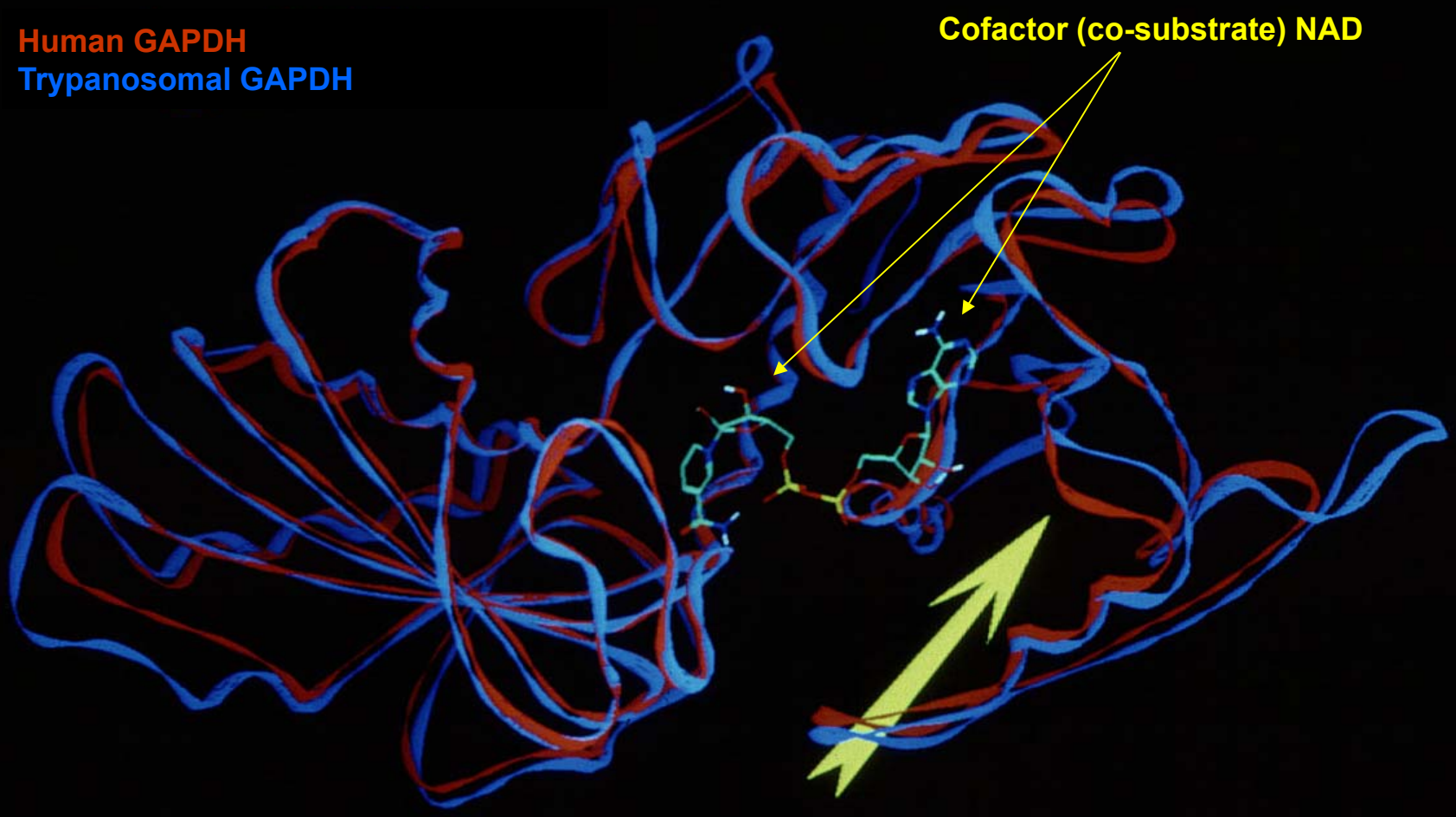
Glycolytic enzymes are critical for the blood stream form of *Trypanosoma brucei*

(ONLY in this group of parasites most of the glycolytic enzymes are sequestered in a unique organelle: the glycosome)



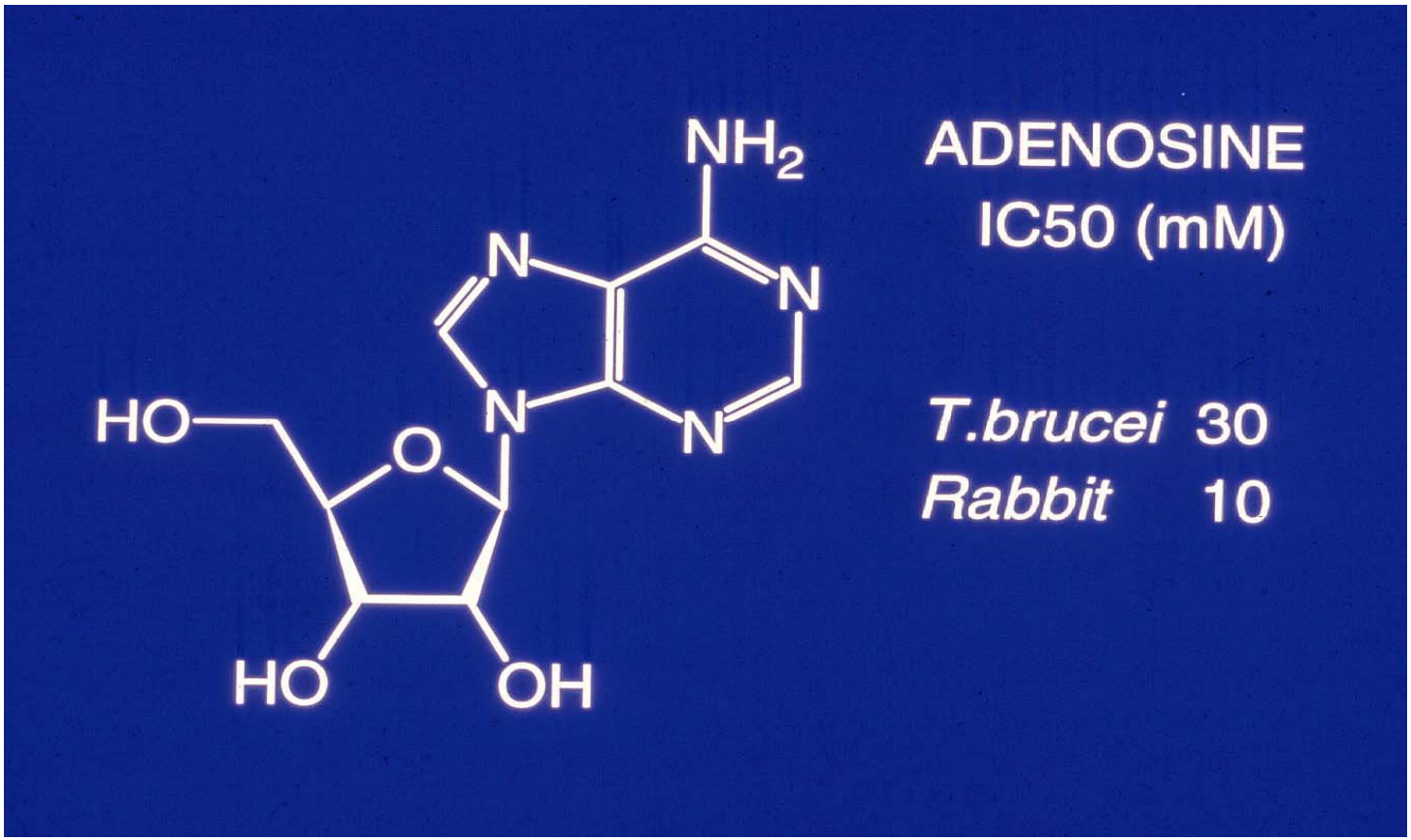
With thanks to Paul Michels and Fred Opperdoes

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the Sleeping Sickness Parasite and the human host



Note the difference in conformation near the ribose of the NAD cofactor in the homologous proteins of host and parasite.

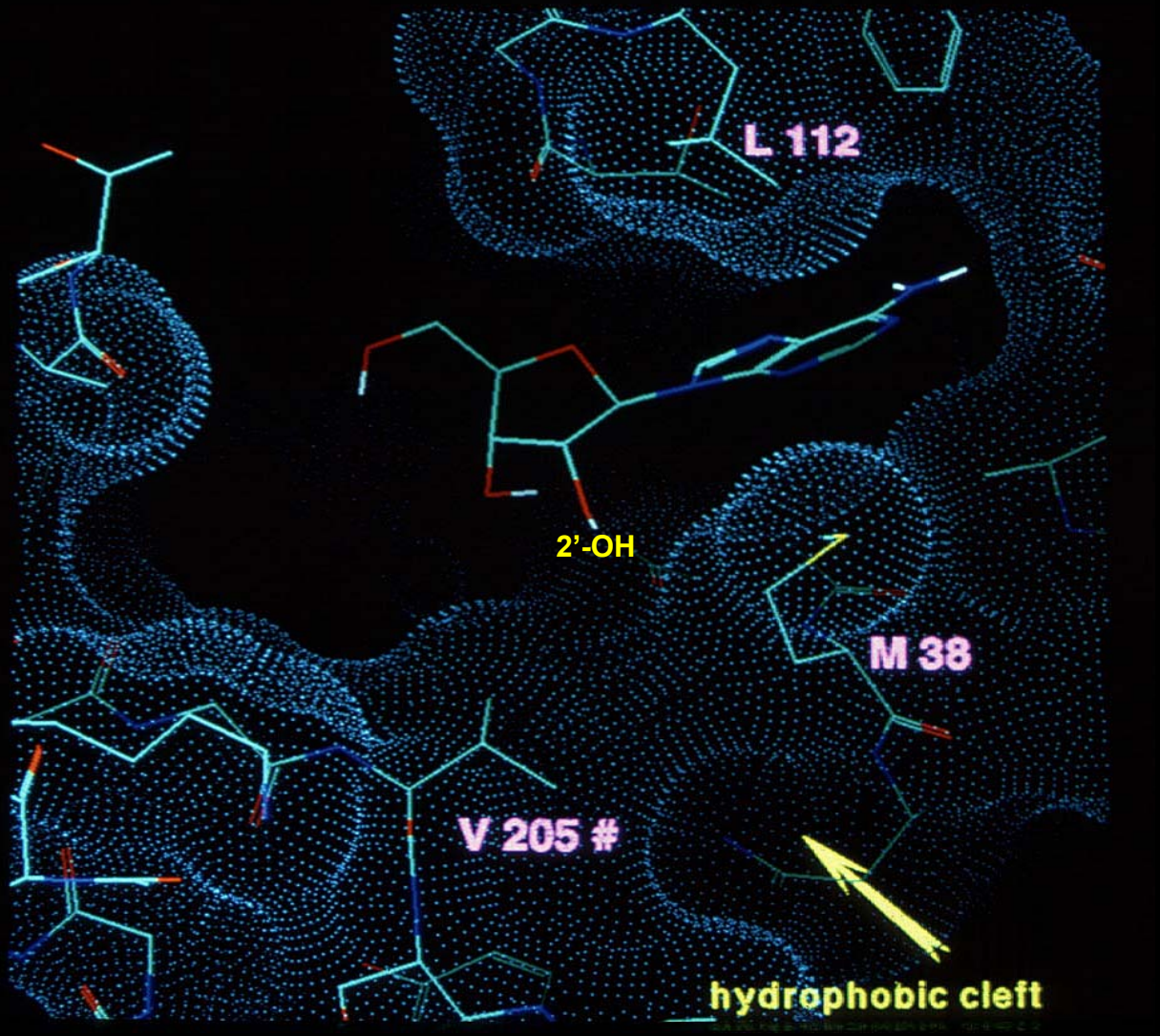
Adenosine – the starting point



- Adenosine is part of the cofactor (co-substrate) NAD of the enzyme GAPDH
- It is by itself a poor inhibitor of mammalian and *T. brucei* parasite GAPDH
- Moreover, it inhibits the sleeping sickness parasite enzyme slightly *worse* than the mammalian enzyme.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

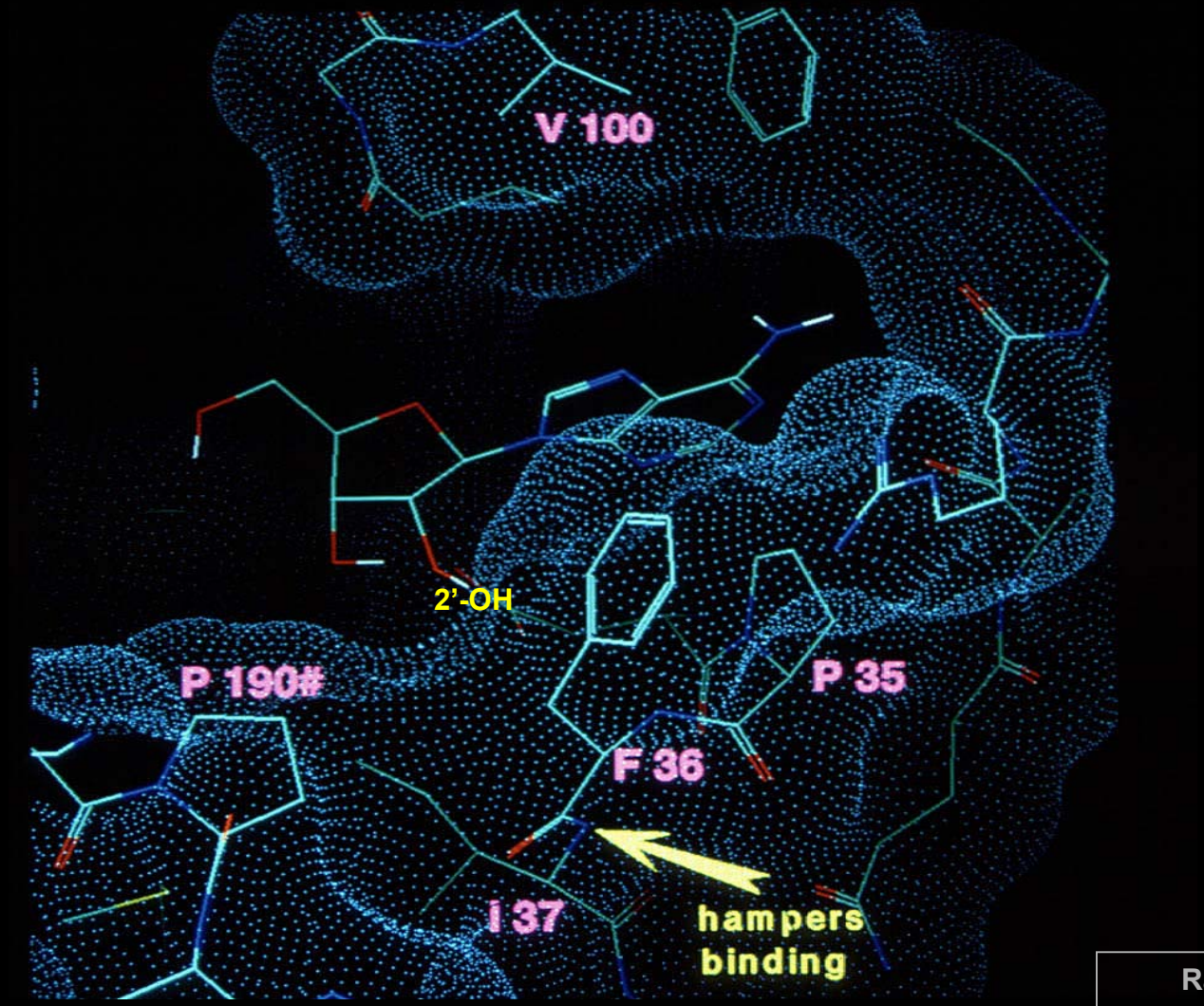
Sleeping sickness parasite GAPDH : Hydrophobic Groove near 2'OH of Adenosine



Fred Vellieux
Christophe Verlinde

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Human GAPDH : NO groove near 2'OH of Adenosine

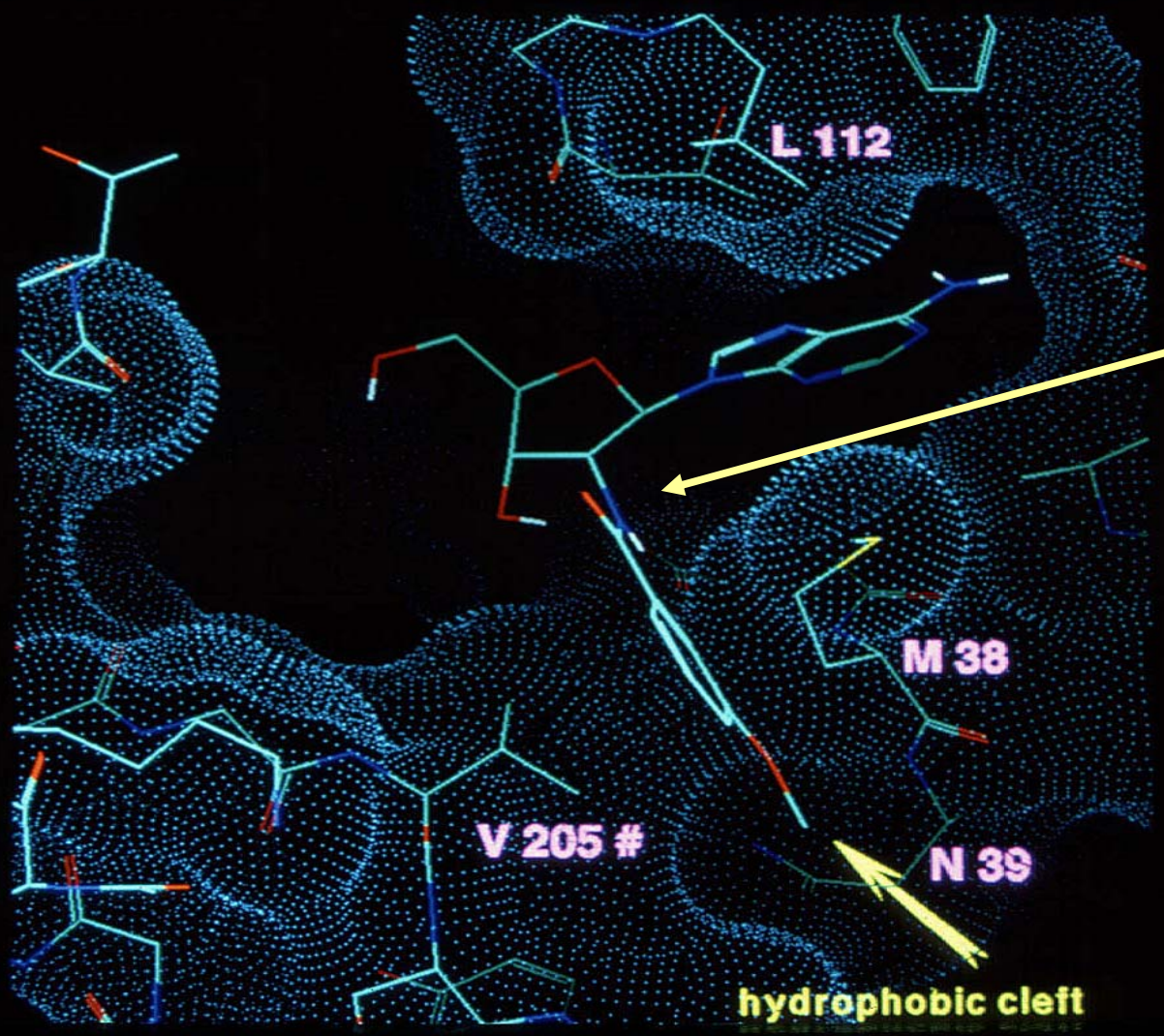


Randy Read

Christophe Verlinde

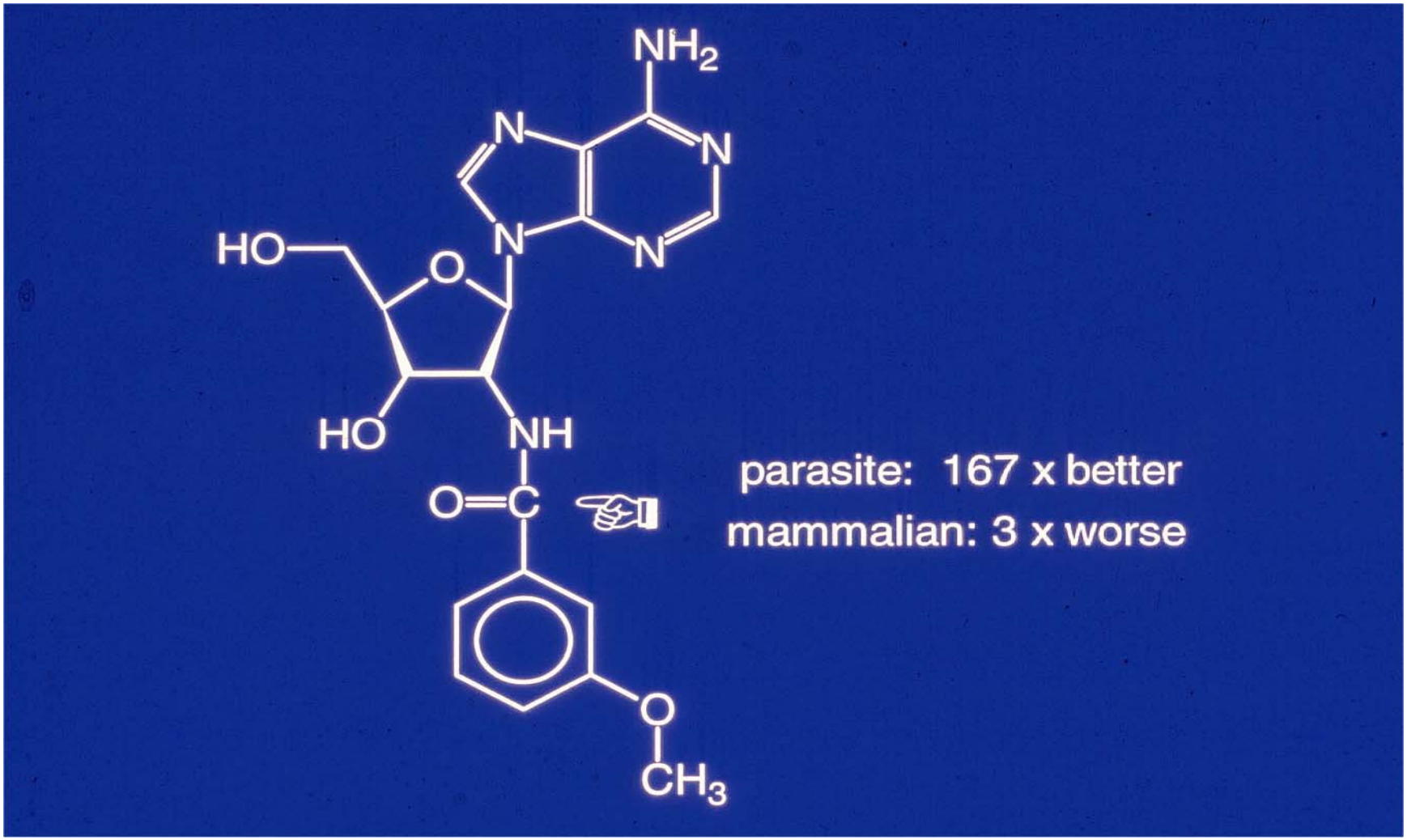
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Sleeping Sickness parasite GAPDH : Substituent Modeled in Hydrophobic Groove near 2'OH of Adenosine



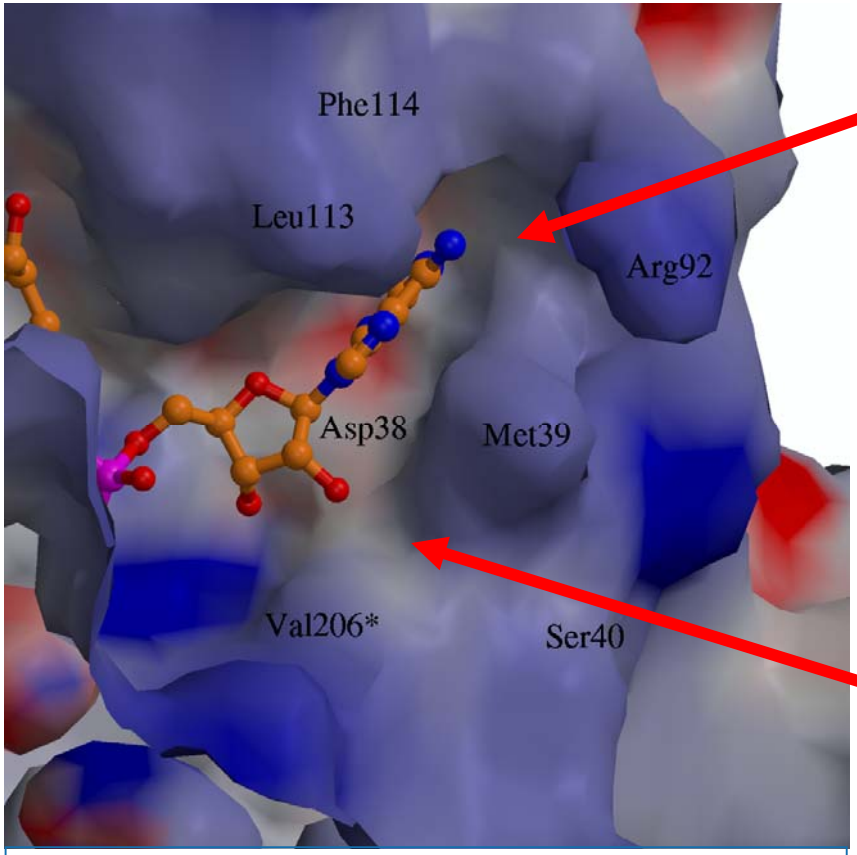
Christophe Verlinde

Selectivity of Structure-based Designed GAPDH Inhibitors



Selectivity changes of 2'-OH substituted compound *versus* adenosine

Exploring additional hydrophobic grooves near the adenosine binding pocket of *Leishmania mexicana* GAPDH



Surface of *L. mexicana GAPDH with NAD bound.**

Hydrophobic Groove

Fill for affinity enhancement

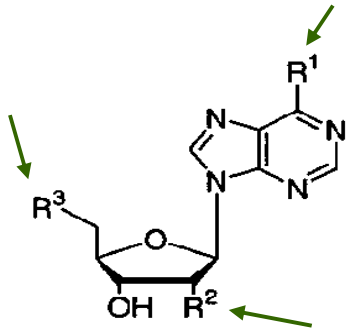
Structure leads to "Focused Combinatorial Chemistry" to fill the grooves optimally

Hydrophobic Groove

Fill for affinity and selectivity improvement

•Note: *Leishmania mexicana* GAPDH is ~77% sequence identical to *Trypanosoma brucei* GAPDH and all residues in the region of interest are identical in these two pathogenic "Trypanosomatids". So these two enzymes are used interchangeably.

Inhibition of *L. mexicana* GAPDH by Adenosine Derivatives

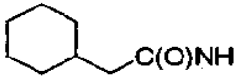
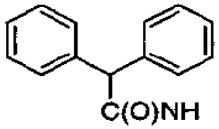
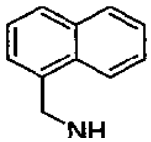
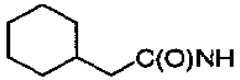
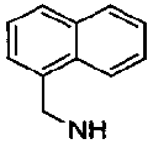
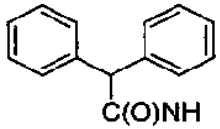
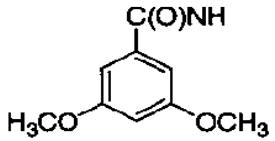
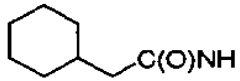
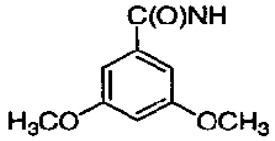
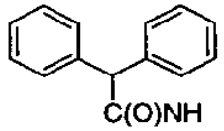


35, 36, 41, 42, 48, and 49
These numbers are just "short-hand names" for variant molecules with different substituents.

Principle:

Make a diverse set of inhibitors by different substituents at three positions R^1, R^2 and R^3 of a so-called "scaffold molecule" (shown above).

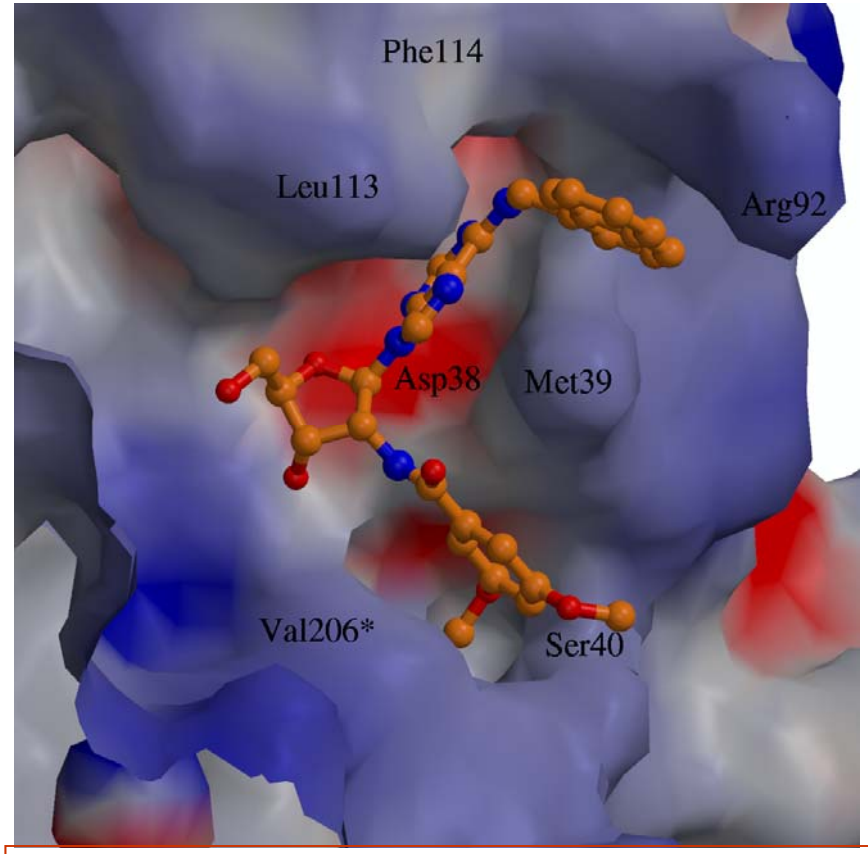
Using a "focused combinatorial library"

Compound	R^1	R^2	R^3	IC_{50} (μM)
35	NH_2	OH		250
36	NH_2	OH		250
41		OH		inactive
42		OH		inactive
48	NH_2			100
49	NH_2			60

[a] Inactive = inactive at 50 μM.

Michael Gelb and coworkers, Wes Van Voorhis, Fred Buckner

Inhibition of *L. mexicana* GAPDH by Adenosine Derivatives



Crystal structure of L. mexicana GAPDH with "NMDBA"

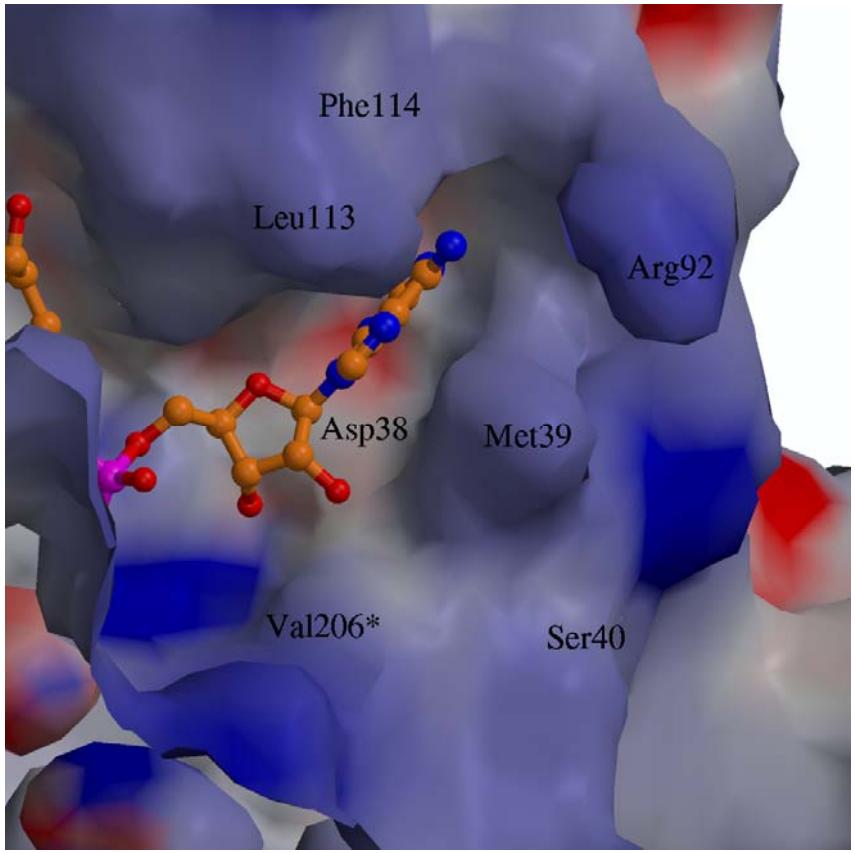
Clearly visible is the selectivity cleft between Met39 and Val206* (from the neighboring monomer), with the dimethoxybenzamido group of NMDBA inserted into it.

The surface has been color coded according to the electrostatic potential. Red represents negative potential and blue positive potential.

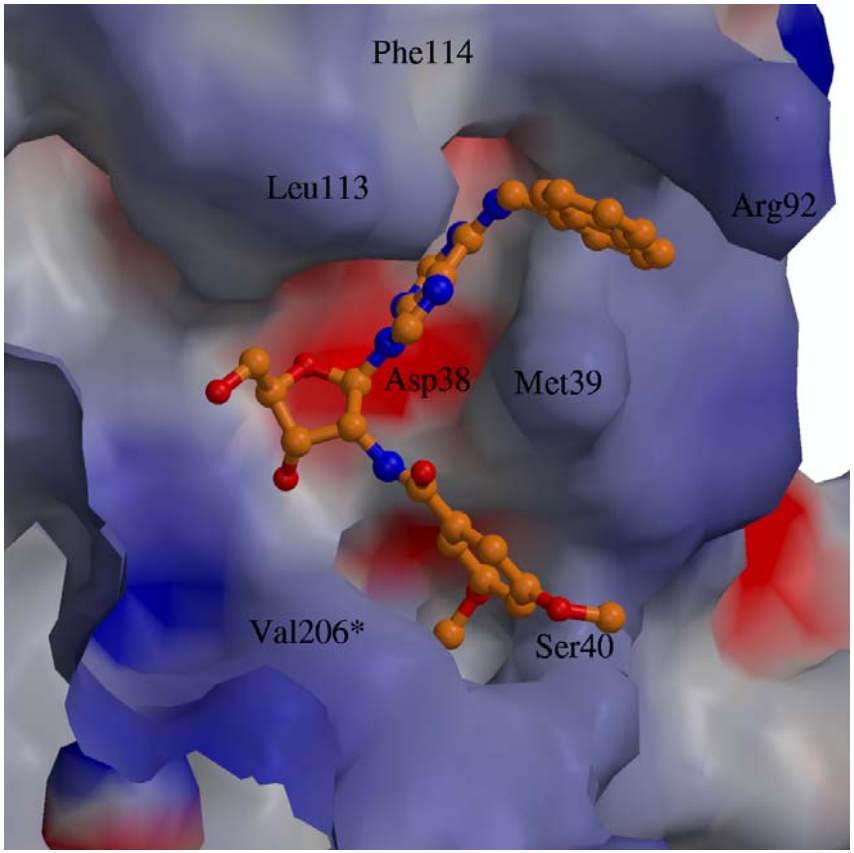
"NMDBA": A new inhibitor with 10^5-fold (!) affinity gain compared to the initial inhibitor adenosine

Suresh et al. J Mol Biol 309:423-435 (2001)

Flexibility in the structure of *L. mexicana* GAPDH



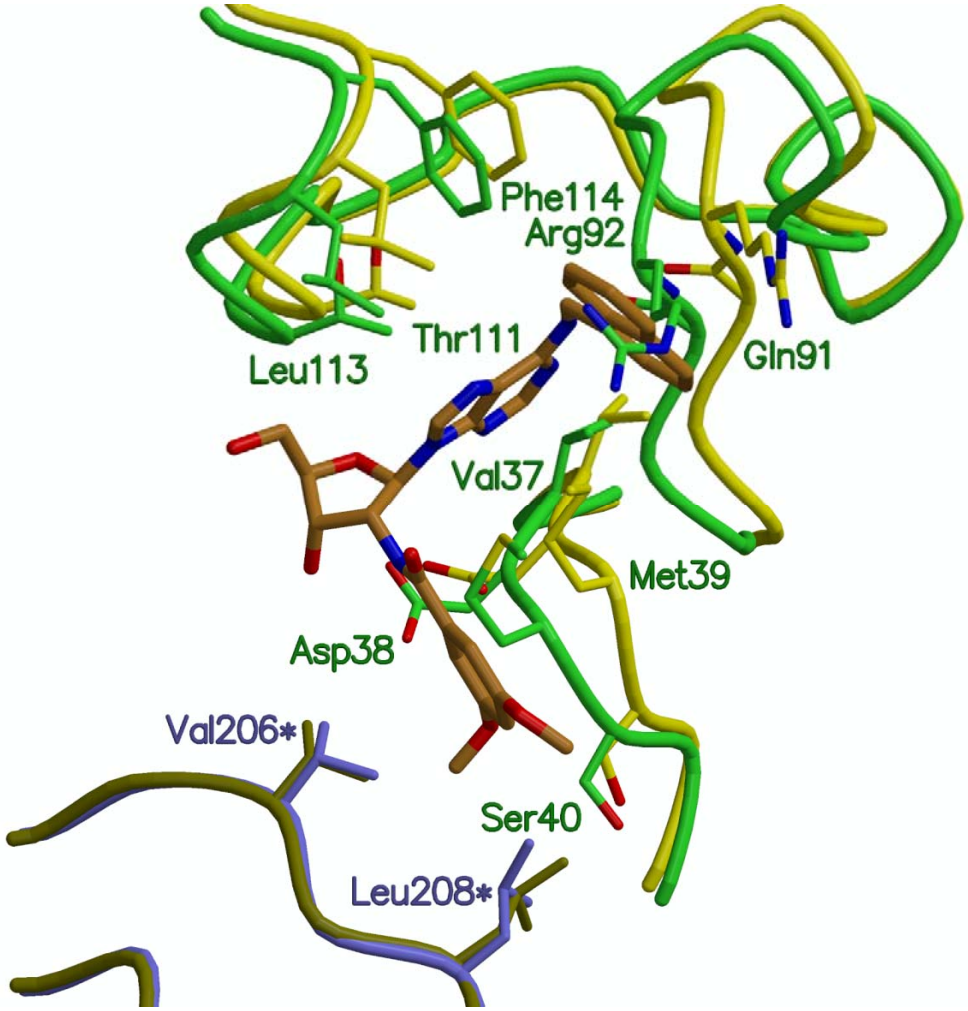
LmGAPDH + NAD



LmGAPDH + NMDBA

Suresh et al. J Mol Biol 309:423-435 (2001)

Flexibility in the structure of *L. mexicana* GAPDH



GAPDH in complex with NAD: green and violet
GAPDH in complex with TNDBA: yellow and gold
Only TNDBA shown

The figure illustrates the displacements of the protein atoms at the inhibitor binding site. In particular, the movement of Met39 effects expansion of the selectivity cleft, and this motion propagates to the other atoms involved in inhibitor binding.

Adaptation of the protein to a ligand is a very common, yet stilll an often surprising, event.

Suresh et al. J Mol Biol 309:423-435 (2001)

Influenza Virus Neuraminidase Inhibitors

A classic example of Structure-Based Drug Design (SBDD) on the basis of a

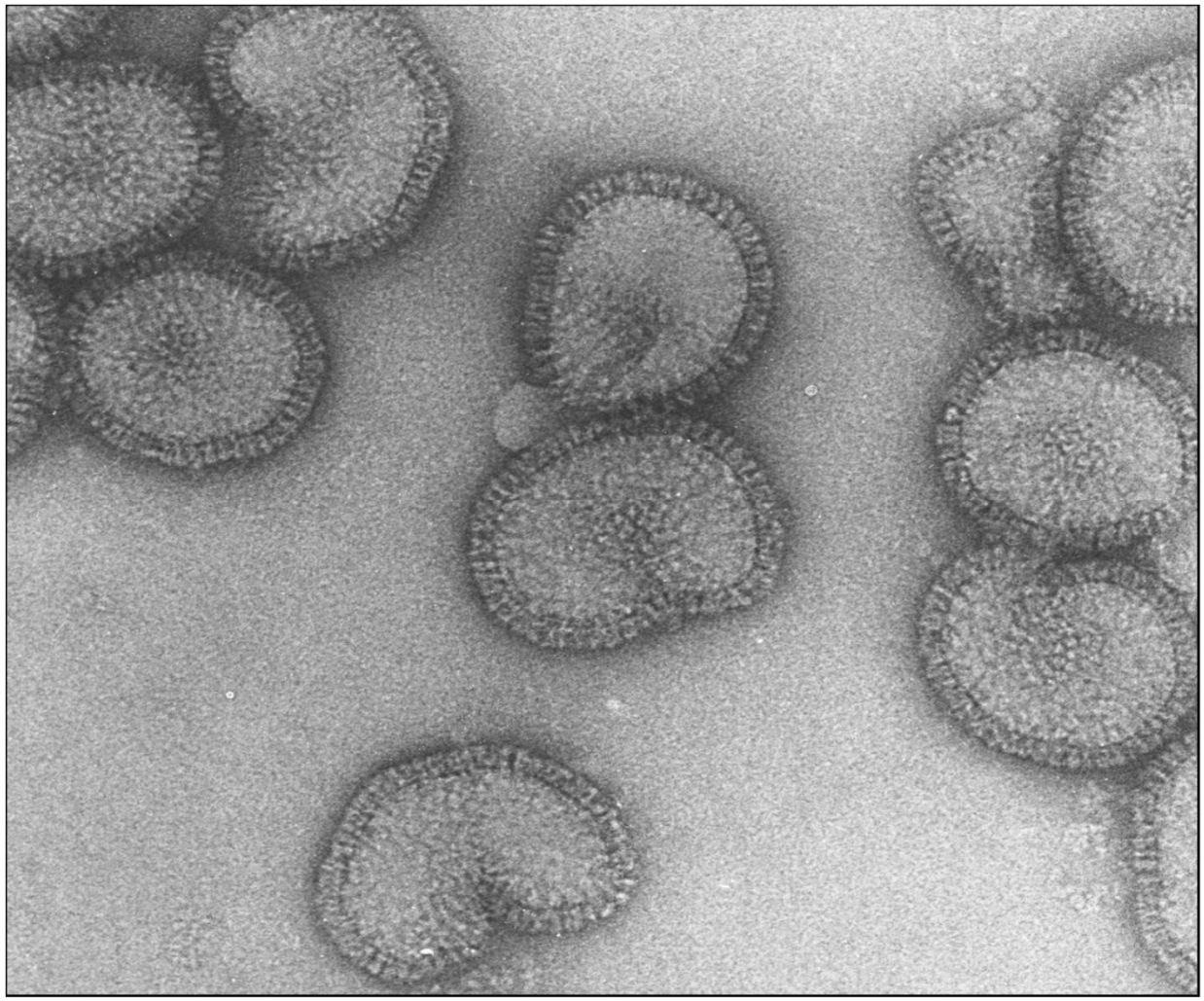
Enzyme-Transition State Analog Complex

&

affinity gain by increasing electrostatic interactions

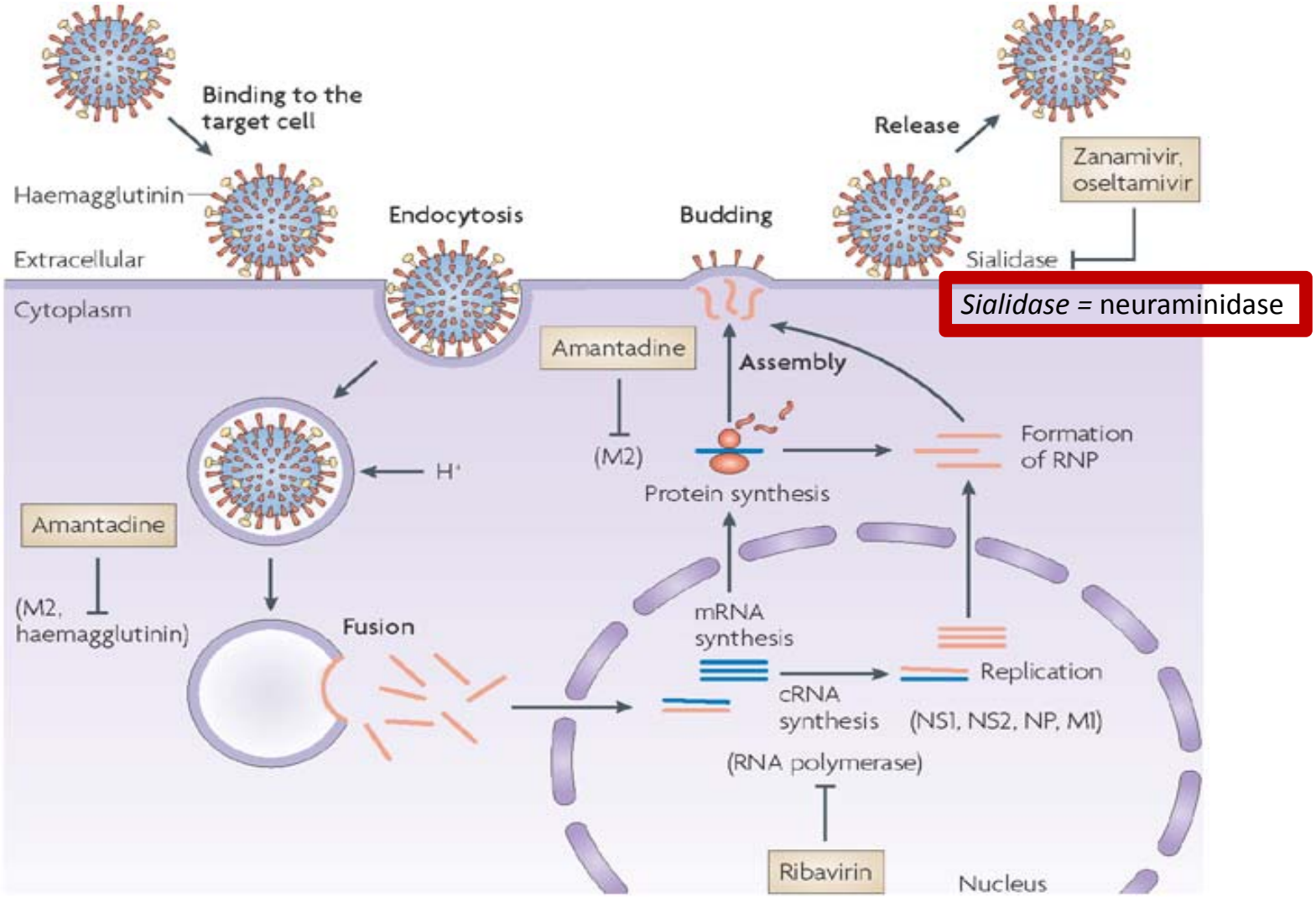
Von Itzstein et al, Nature 363:419 (1993) ; Moscona, Inhibitors for Influenza, N. Engl. J. Med. 353, 1363-1373 (2005)

INFLUENZA VIRUS



Influenza Virus has two main surface proteins: haemagglutinin (H) and neuraminidase (N).

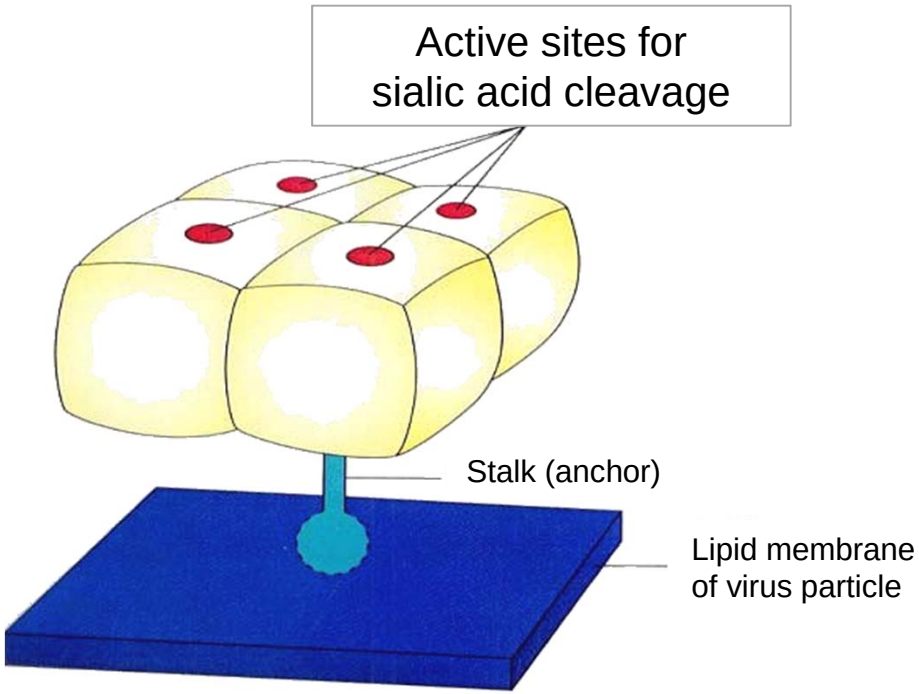
Influenza Virus



Sialidase = neuraminidase

Figure from: von Itzstein, M. (2007). *Nature Reviews Drug Discovery* **6**, 967-974

Influenza Virus Neuraminidase.

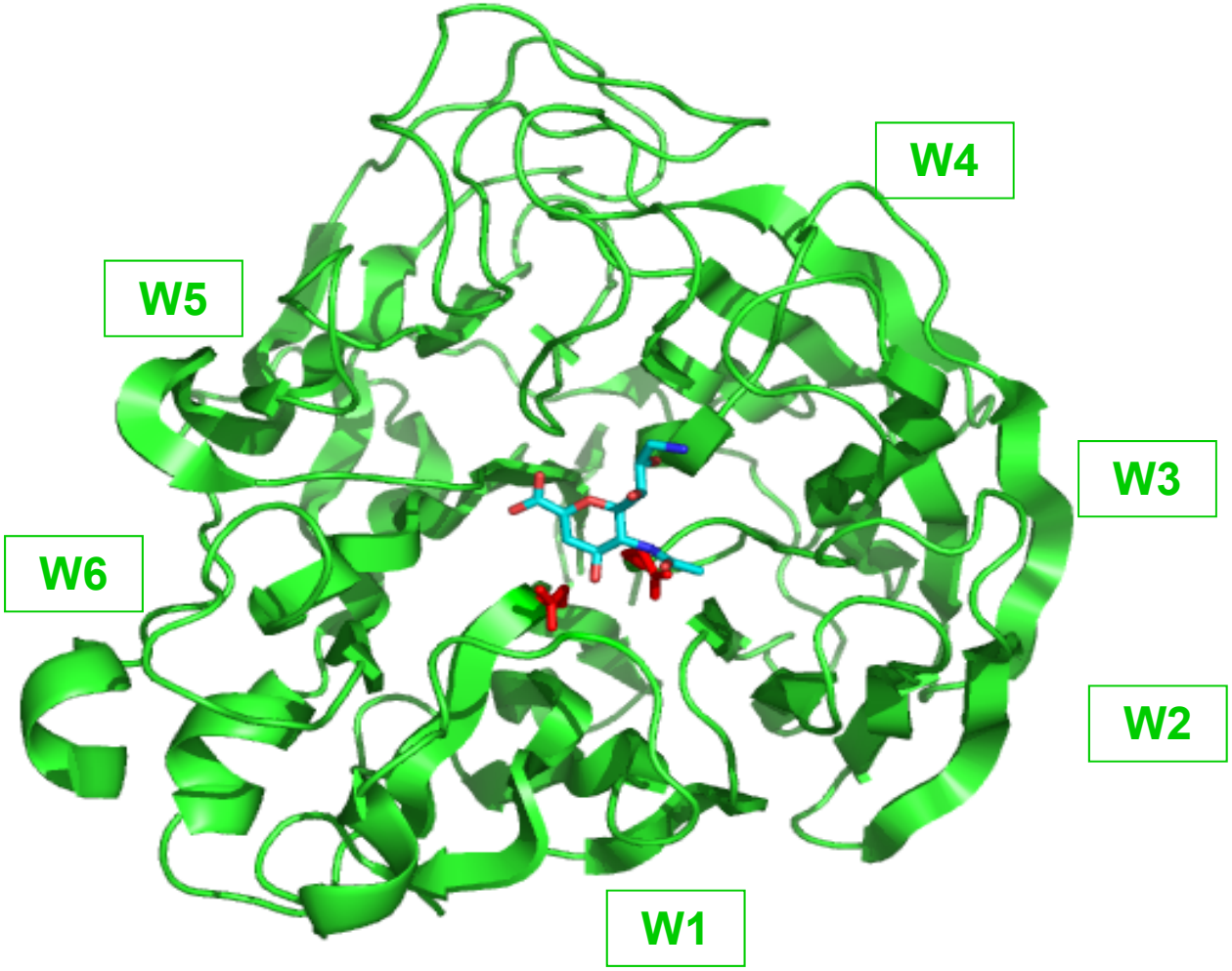


Schematic representation of the neuraminidase tetramer on the surface of the influenza A virus: the “NA spike”

The enzyme neuraminidase plays a key role in the release of new viruses from the host cell surface.

Inhibition of neuraminidase activity appeared to be a way to decrease the severity of a flu infection.

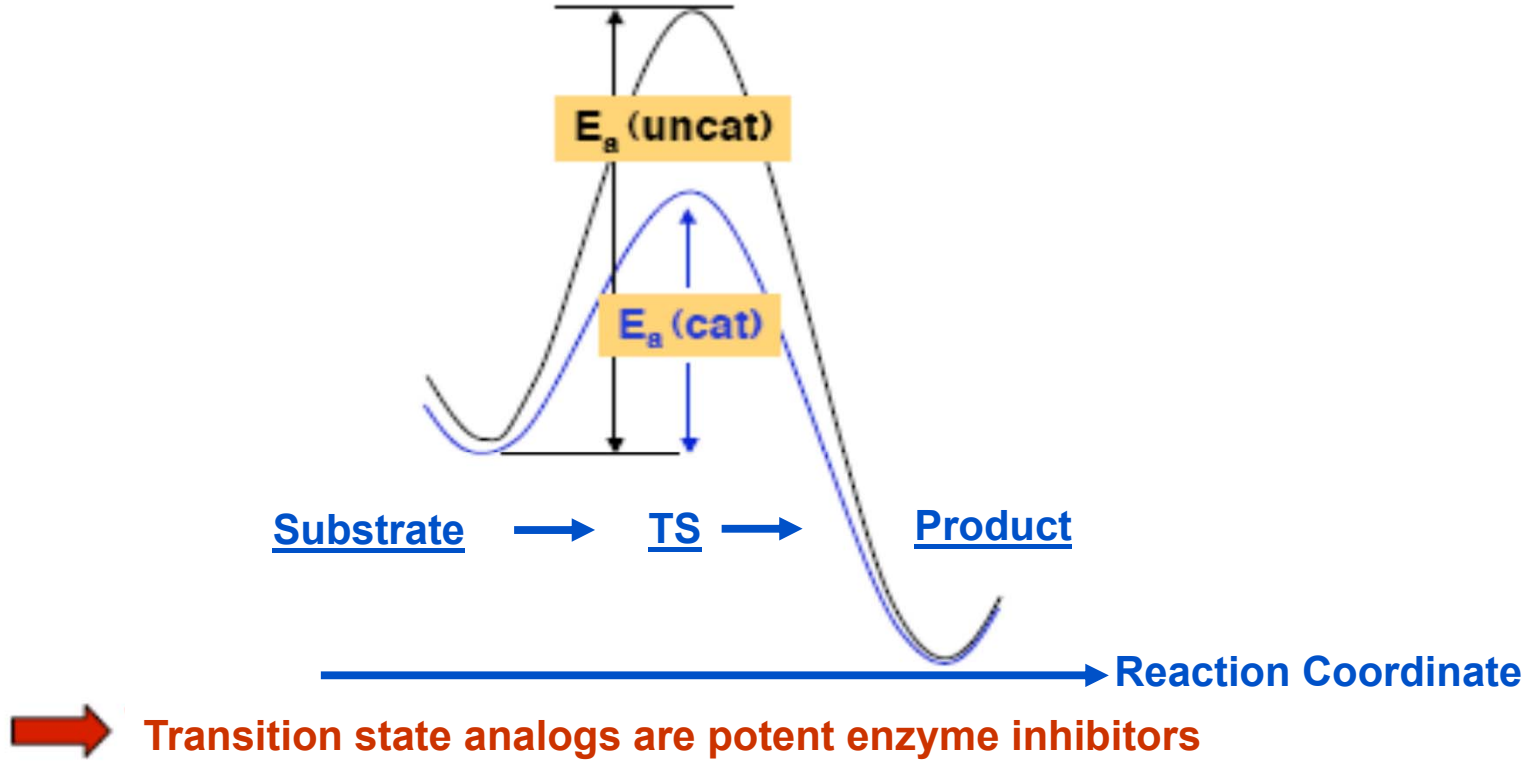
The Three-dimensional Structure of a single Subunit of Influenza Virus Neuraminidase



Wn = n-th 4-β-stranded "propeller"

SMITH et al, PROTEIN SCI. 10: 689 (2001) – PDB-code 1F8D.

Enzyme often catalyze reactions by preferential binding of the transition state *vs* the ground state

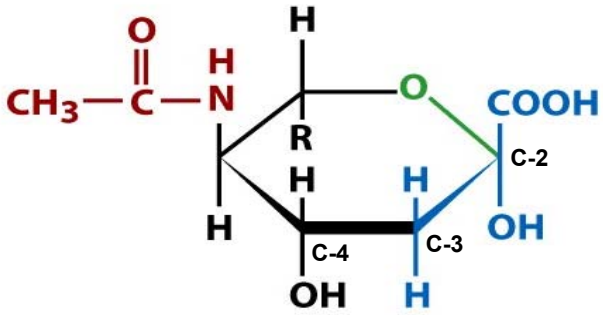


Transition state analogs are potent enzyme inhibitors

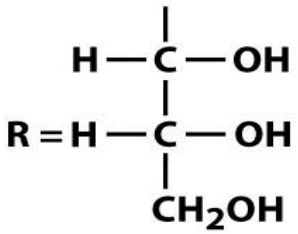
TS = Transition State

Modified from Carolyn R. Bertozzi - website: http://grtc.ucsd.edu/lecture42.pdf

The substrate of neuraminidase



***N*-Acetylneuraminic acid**
(pyranose form)

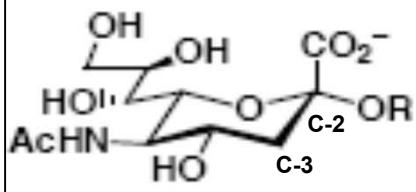


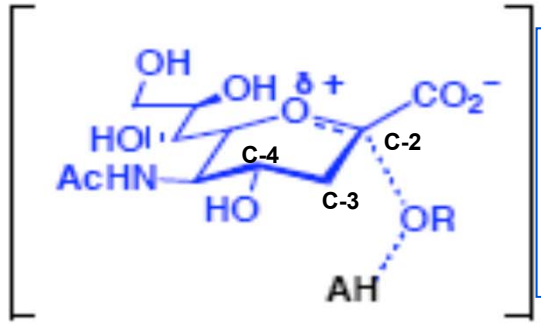
Sialic Acid ≡ N-acetylneuraminic Acid

(A complex sugar, attached to quite a few human cell surface proteins)

The Reaction catalyzed by neuraminidase

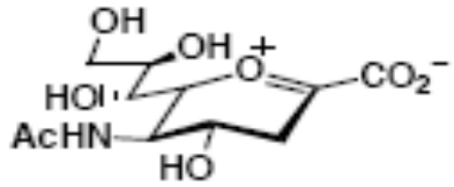
Substrate
(Sialic Acid bound to a protein via the 2-OH)

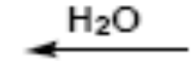


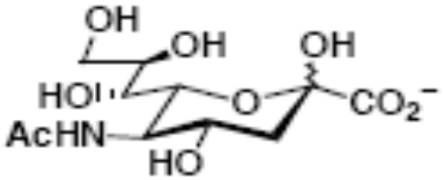


Transition State:

With the ring-O, the C-2, the carboxyl carbon and the C-3 approximately in one plane.







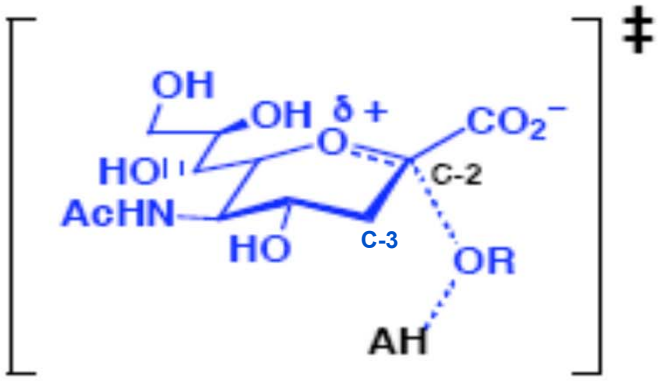
Product

(Free Sialic Acid)

Sialic acid = N-ACETYL-NEURAMINIC ACID

Modified from Carolyn R. Bertozzi - website: http://grtc.ucsd.edu/lecture42.pdf

Design of Transition State Analog neuraminidase inhibitors



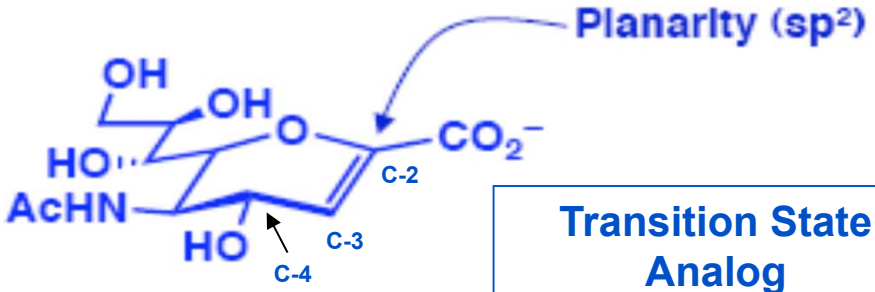
Transition State

Features:
- Planarity at C-2
- Buildup of + charge

DANA

$K_I = 10^{-6}$ M

Also inhibits human neuraminidase



Transition State Analog

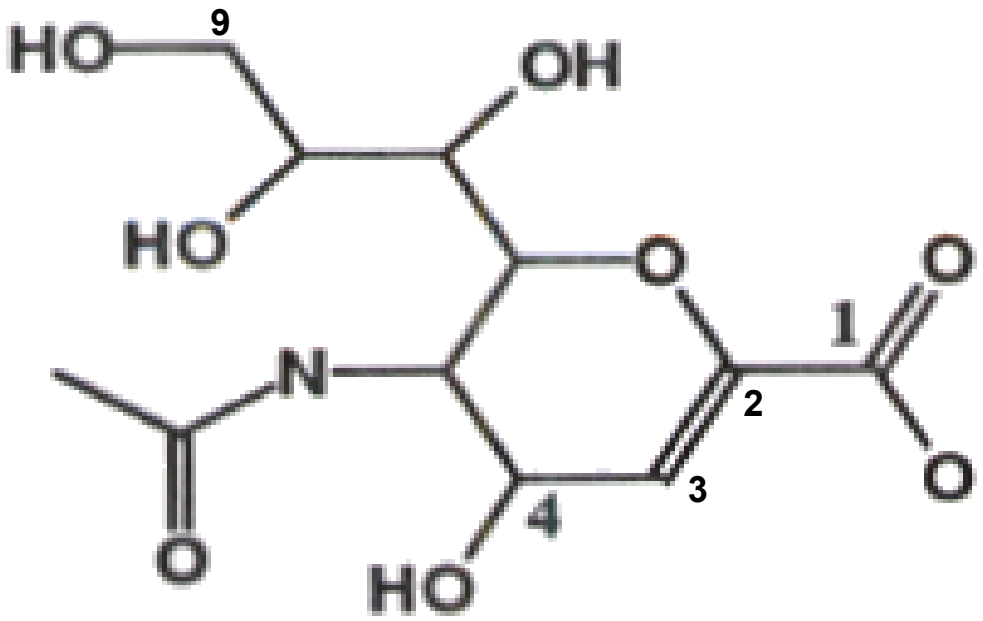
DANA ≡

2-DEOXY-2,3-DEHYDRO-N-ACETYL-NEURAMINIC ACID

Adapted from Carolyn R. Bertozzi - website: http://grtc.ucsd.edu/lecture42.pdf

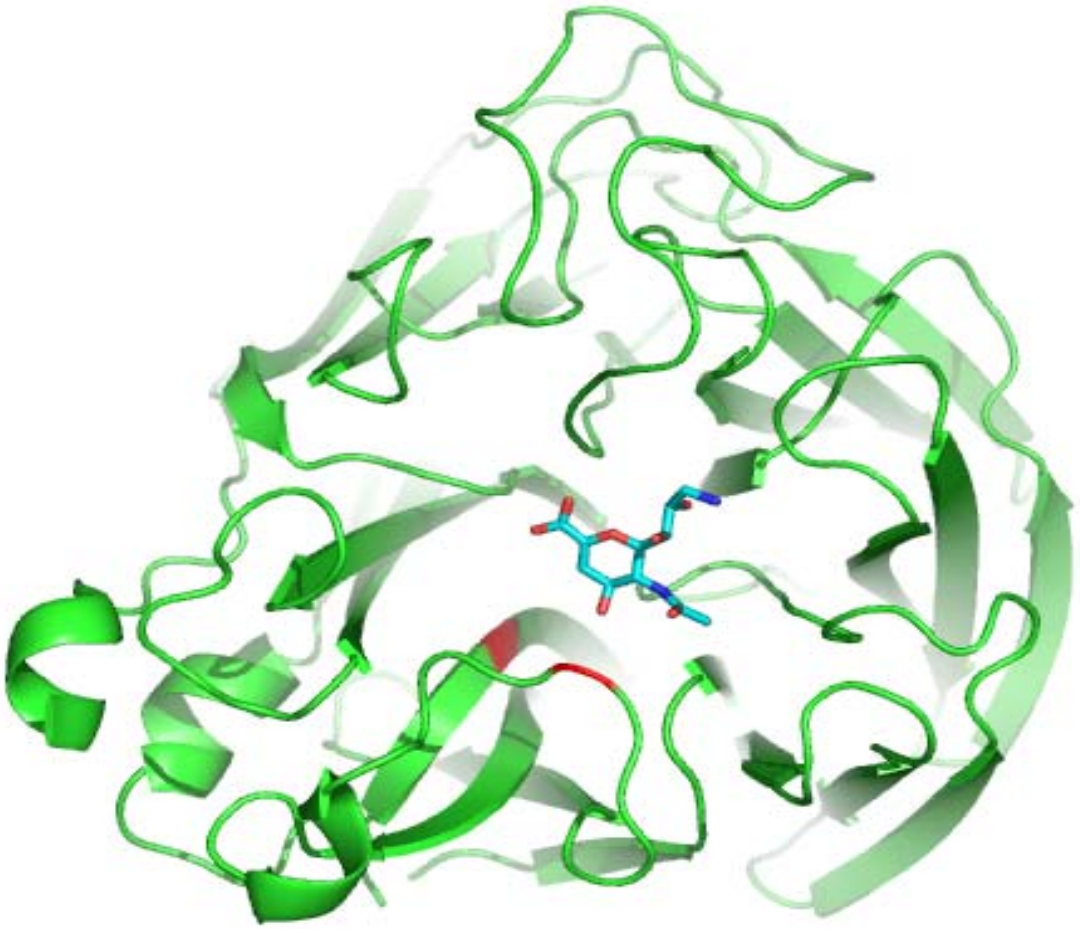
The Starting Point

The Transition State Analog (TSA) DANA



DANA

Influenza Virus Neuraminidase in complex with 9-amino-DANA

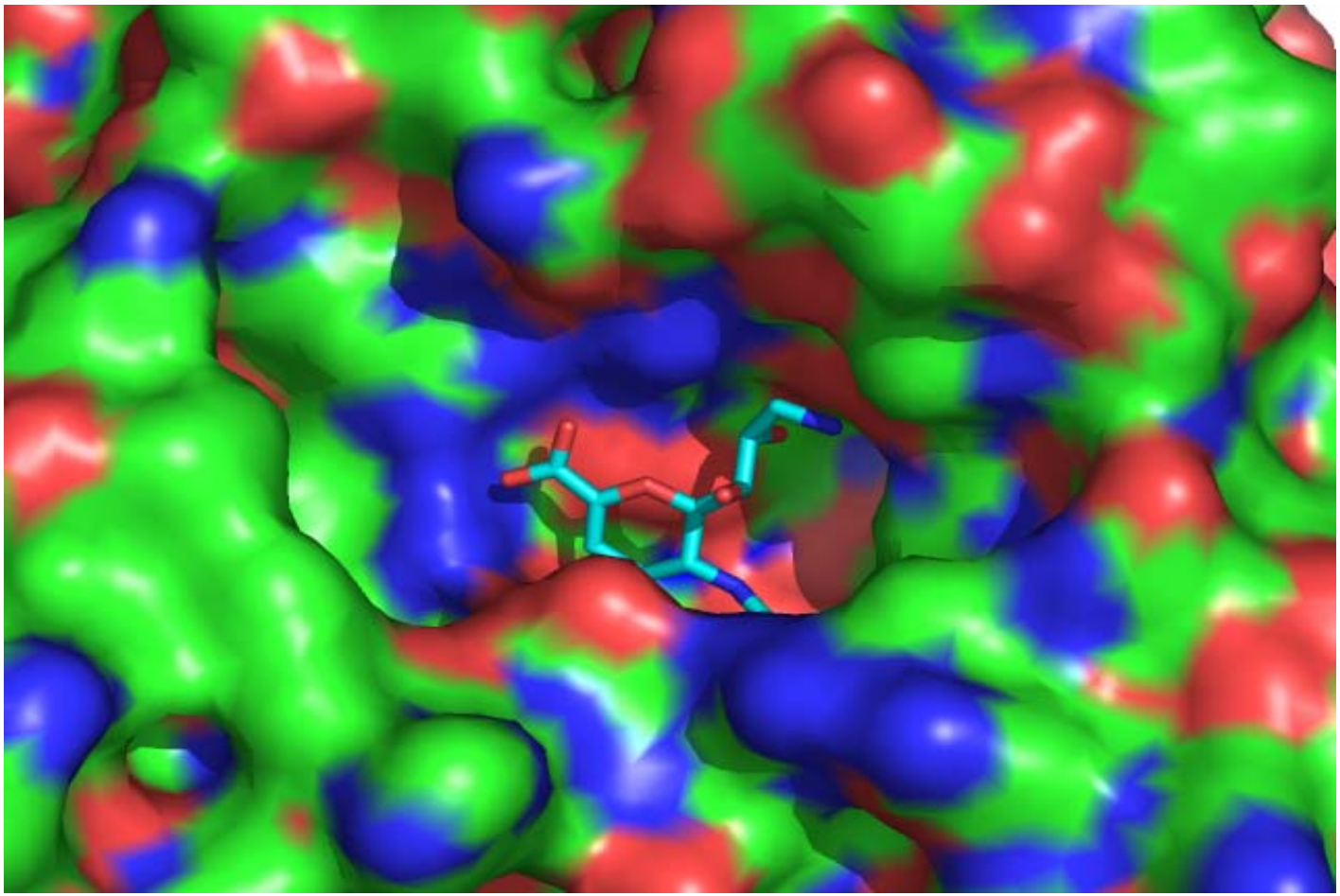


9-amino-DANA =

9-AMINO-2-DEOXY-2,3-DEHYDRO-N-ACETYL-NEURAMINIC ACID

(The 9-amino group is irrelevant for the drug development story)

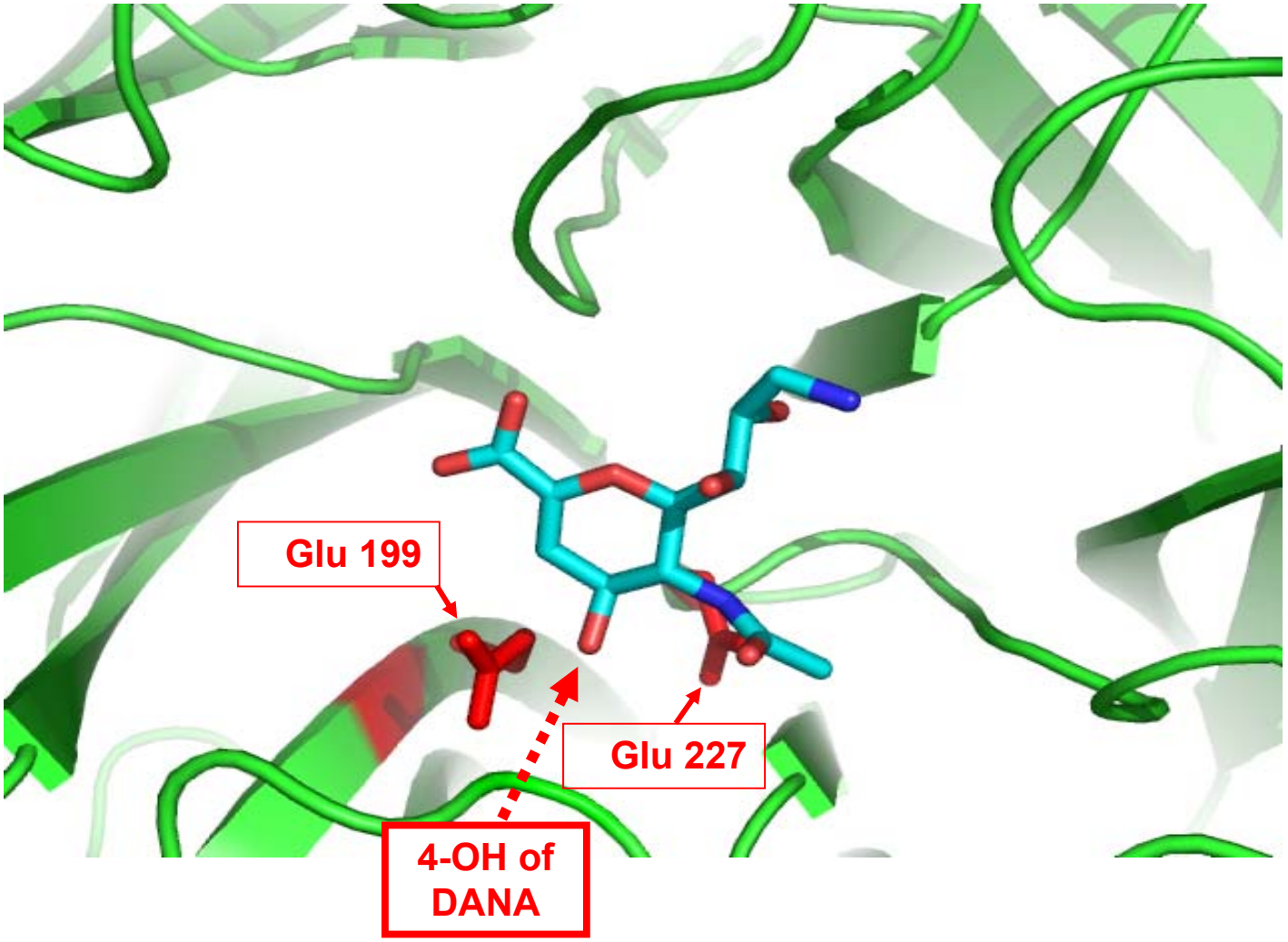
Influenza Virus Neuraminidase in complex with 9-amino-DANA



9-amino-DANA sits clearly in a pocket.

This is the active site of neuraminidase

Influenza Virus Neuraminidase in complex with 9-amino-DANA

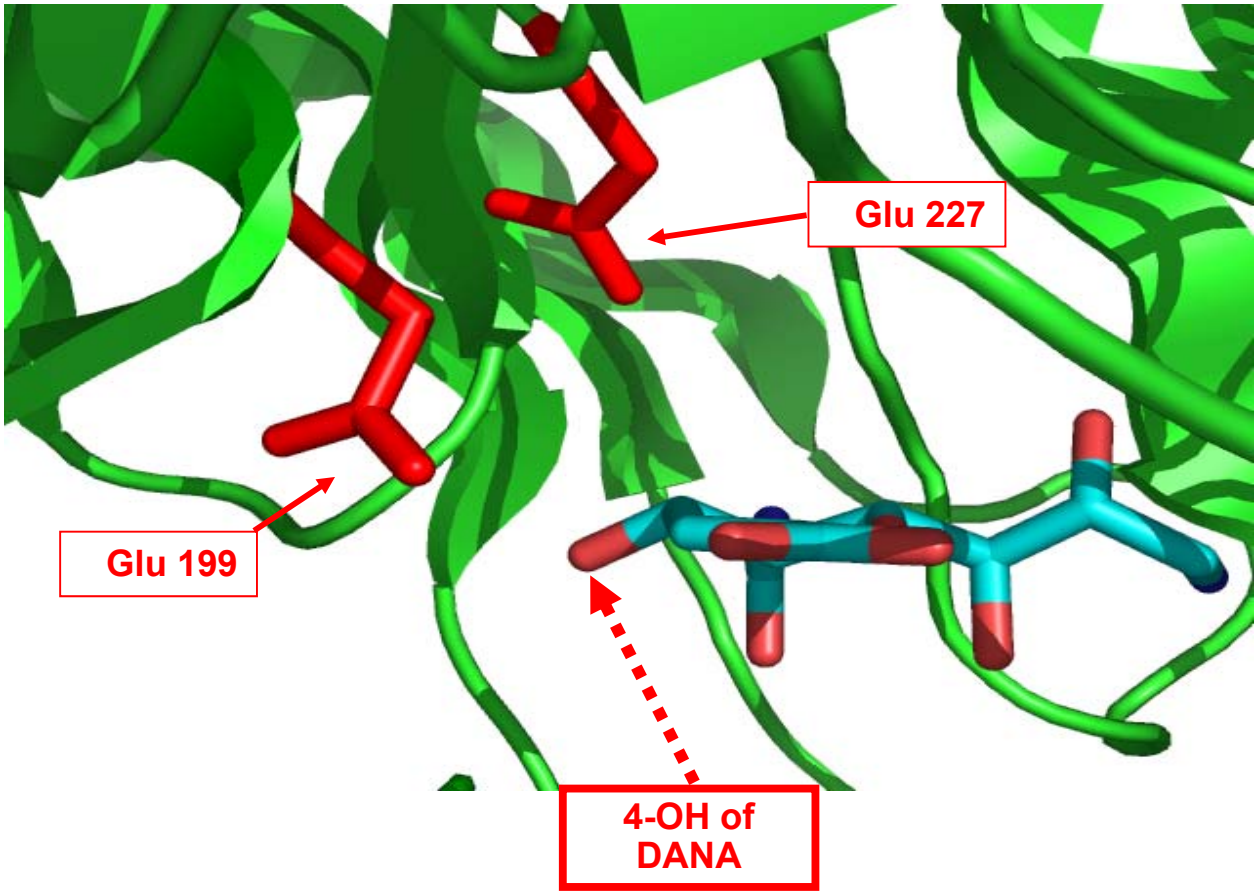


View of two key Neuraminidase residues near the 4-OH of 9-amino-DANA

Influenza Virus Neuraminidase in complex with 9-amino-DANA

Close-up and 90 degrees rotated

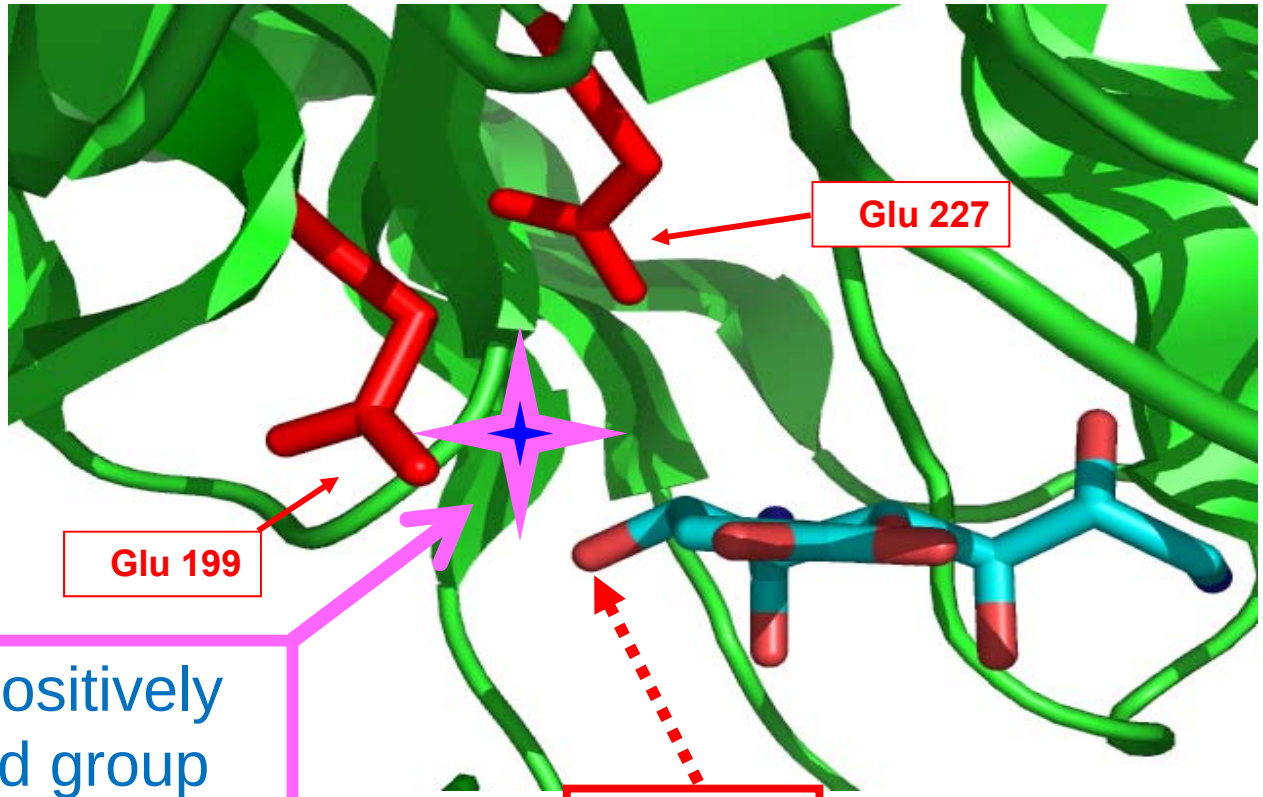
Influenza Virus Neuraminidase in complex with 9-amino-DANA



Two negatively charged carboxylates are quite close to the 4-OH

Bivalent Neuraminidase Inhibitors

Influenza Virus Neuraminidase in complex with 9-amino-DANA

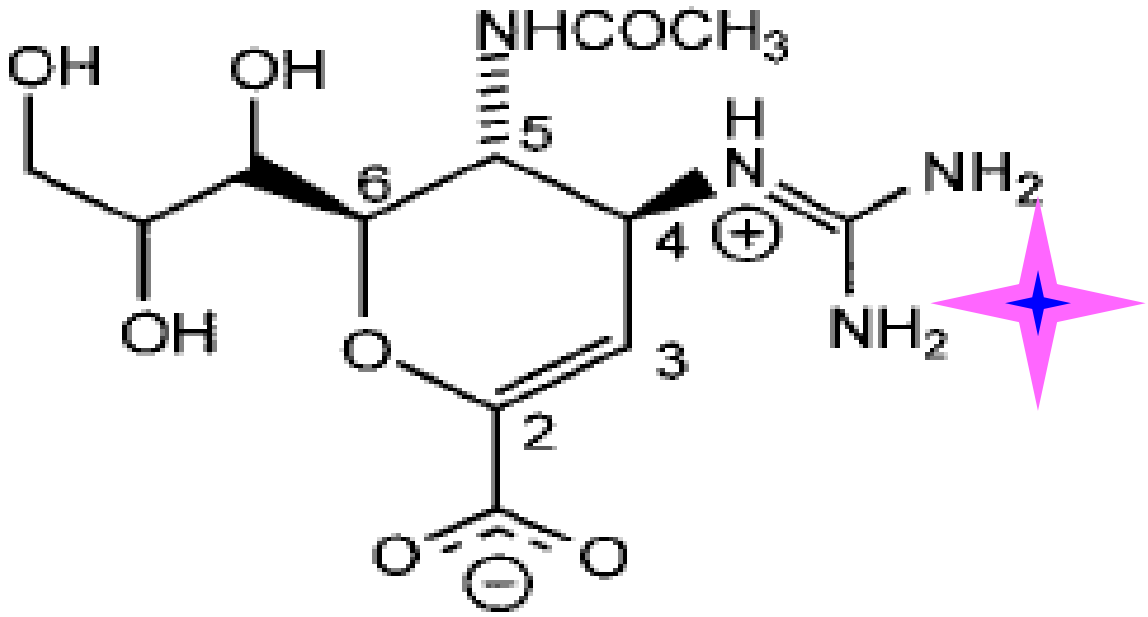


Place positively charged group **THERE**

Quite obvious what to do to gain affinity – TWO compounds made

Compound made: 4-guanidino-DANA

A guanidino substituent at the 4-position instead of a hydroxyl



Does it indeed live up to the expectations?
I.e. of being a better inhibitor than DANA?

Von Itzstein et al, Nature 363:419 (1993)

Inhibitory Properties of modified 4-guanidino-DANA

Based on the structure of the TSA DANA in complex with influenza virus neuraminidase, the compound 4-guanidino-DANA was designed and synthesized.

The K_i-values (in M) were as follows:

	Flu Neura	Human Neura
DANA	1×10^{-6}	1.2×10^{-5}
4-guanidino-DANA	2×10^{-10}	1×10^{-3}

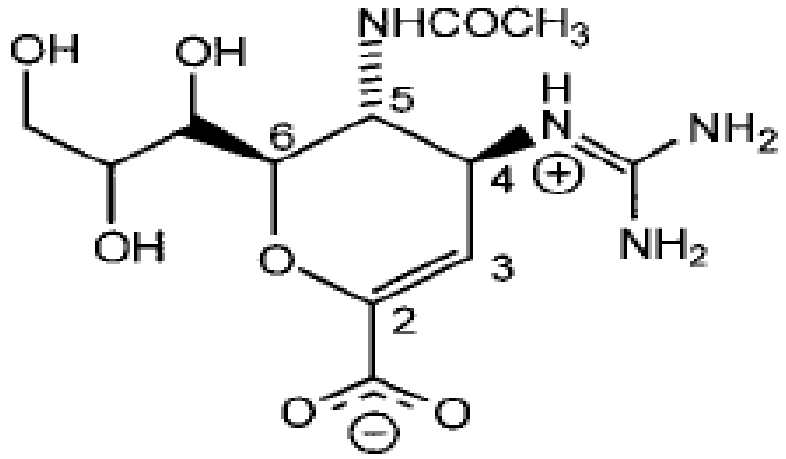
By changing one single functional group:

- **The affinity for the target flu enzyme was enhanced by a factor of ~10,000.**
- **The affinity for the human homologous enzyme was decreased by a factor of ~100.**
- **The selectivity was improved by a factor of ~1,000,000 !**

Von Itzstein et al, Nature 363:419 (1993)

Properties of 4-guanidino-DANA

Zanamivir (Relenza)



Zanamivir

This compound is obviously very hydrophilic:
One guanidinium group & One carboxylate & Three hydroxyls & One NH-C=O group!

Therefore this medicine is NOT active when given orally.

However, influenza virus enters host lung cells.
Hence, the compound can be administered with an inhalator.

Physical Chemical Requirements of (most) Oral Drugs

The (four) Lipinski "Rules of Five"

"From the 50,427 compounds in the WDI (World Drug Index) File ....2245 were selected which are likely to have superior physico-chemical properties.

Poor absorption or permeation are more likely when:

- **The MWT is over 500**
- **There are more than 5 H-bond donors**
- **There are more than 10 H-bond acceptors**
- **The Log P is over 5**

... orally active therapeutic classes outside the 'rule of 5' are:
antibiotics, antifungals, vitamins and cardiac glycosides.

….We suggest that these few therapeutic classes contain orally active drugs that violate the 'rule of 5' because members of these classes have structural features that allow the drugs to act as substrates for naturally occurring transporters."

Lipinski et al., Advanced Drug Delivery Reviews 46: 3–26 (2001)

Medicines have to fulfil many requirements

Drugs are VERY precious compounds

For *orally available* medicines a fine balance is required between :

(i) Sufficient capacity to cross membranes,
so it can be taken up from the digestive tract;
(ii) Sufficient water solubility,
so it can reach the site of action in sufficient concentrations.

Some other requirements of an ideal medicine are:
(iii) Not being converted to an inactive substance by human enzymes;
(iv) Not being cleared rapidly from the blood;
(v) No teratogenicity;
(vi) No mutagenicity;
(vii) No toxicity;
(viii) And more…

Hence, it is not really a surprise that it is a major challenge to make a new safe, effective, orally available, affordable medicine.

The power of multivalency

Multivalent Inhibitors of Cholera Toxin (CT)

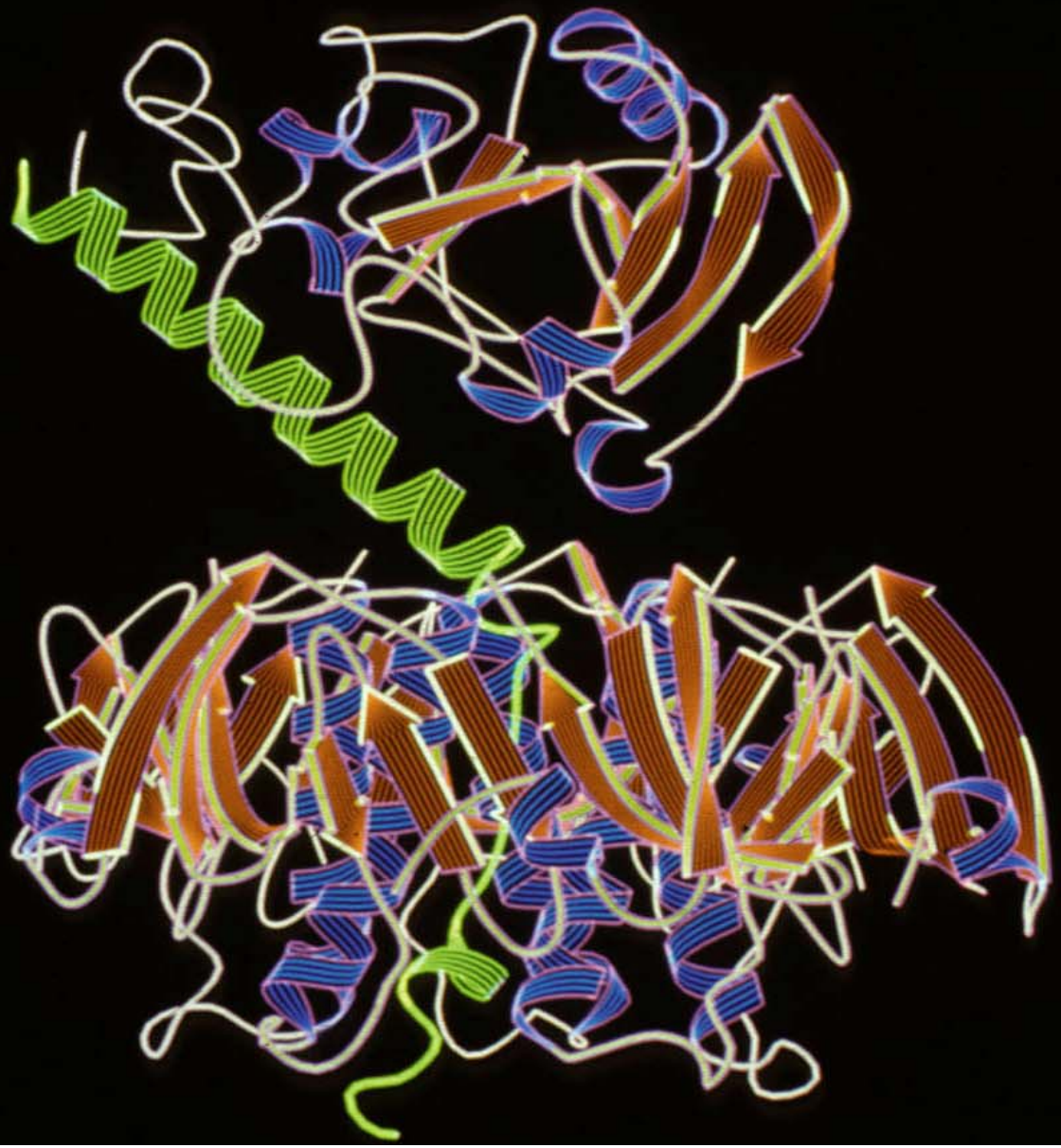
A toxin produced by *Vibrio cholerae.*

CT is a close relative of Heat-Labile Enterotoxin (LT) produced by enterotoxigenic *E. coli,* the cause of much of children's and traveler's diarrhea

Minke,. The role of waters in flexible docking strategies for carbohydrate derivatives: heat-labile enterotoxin, a multivalent test case. *J. Med. Chem.* 42, 1778-1788 (1999).

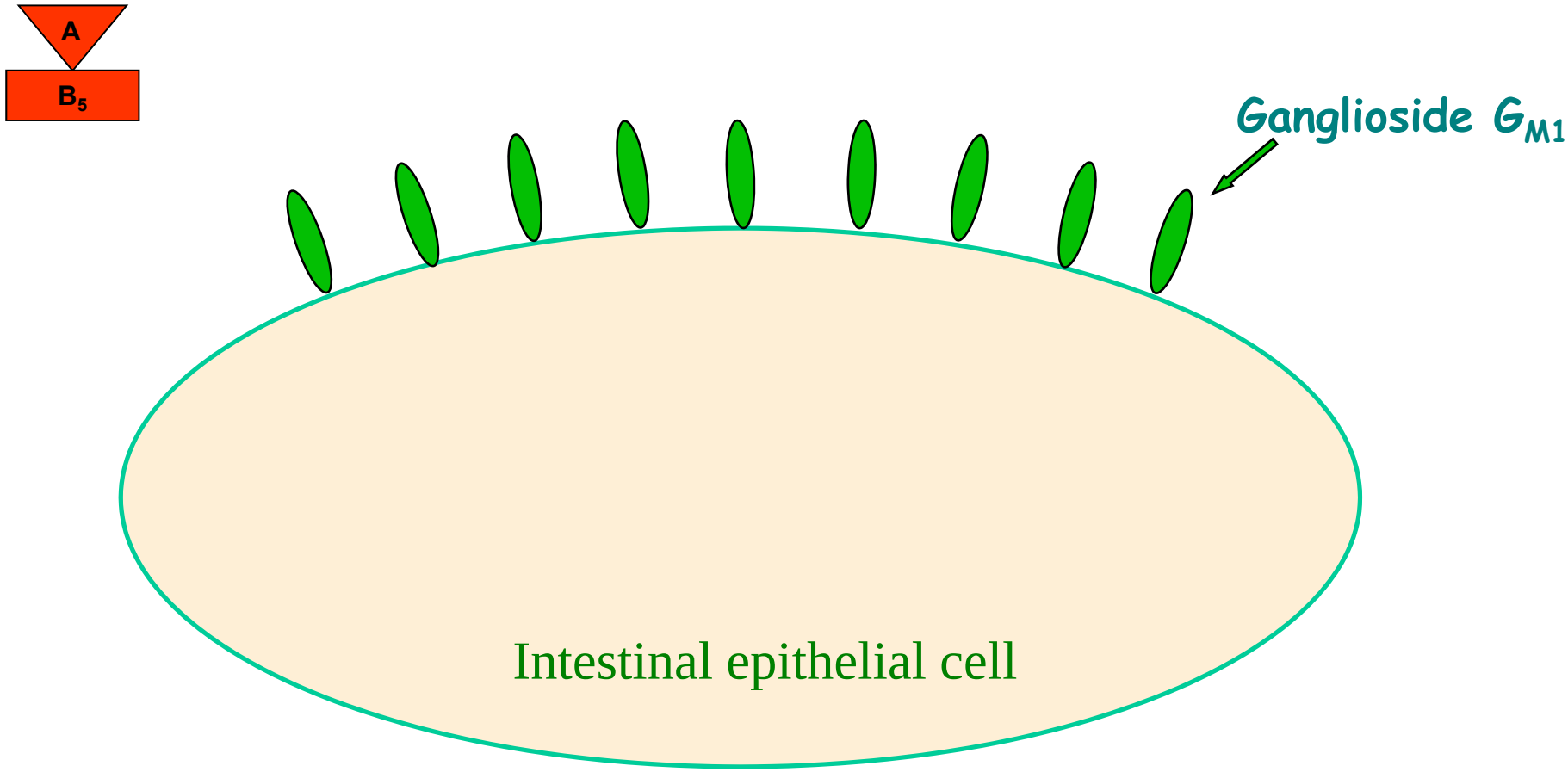
Fan, A 10^5 gain in affinity for pentavalent ligands of *E. coli* heat-labile enterotoxin by modular structure-based design. *J. Am. Chem. Soc.* 122, 2663 (2000)

Heat-labile Enterotoxin (LT), a very close relative of Cholera toxin (CT)



Titia Sixma

CT and LT Receptor Binding

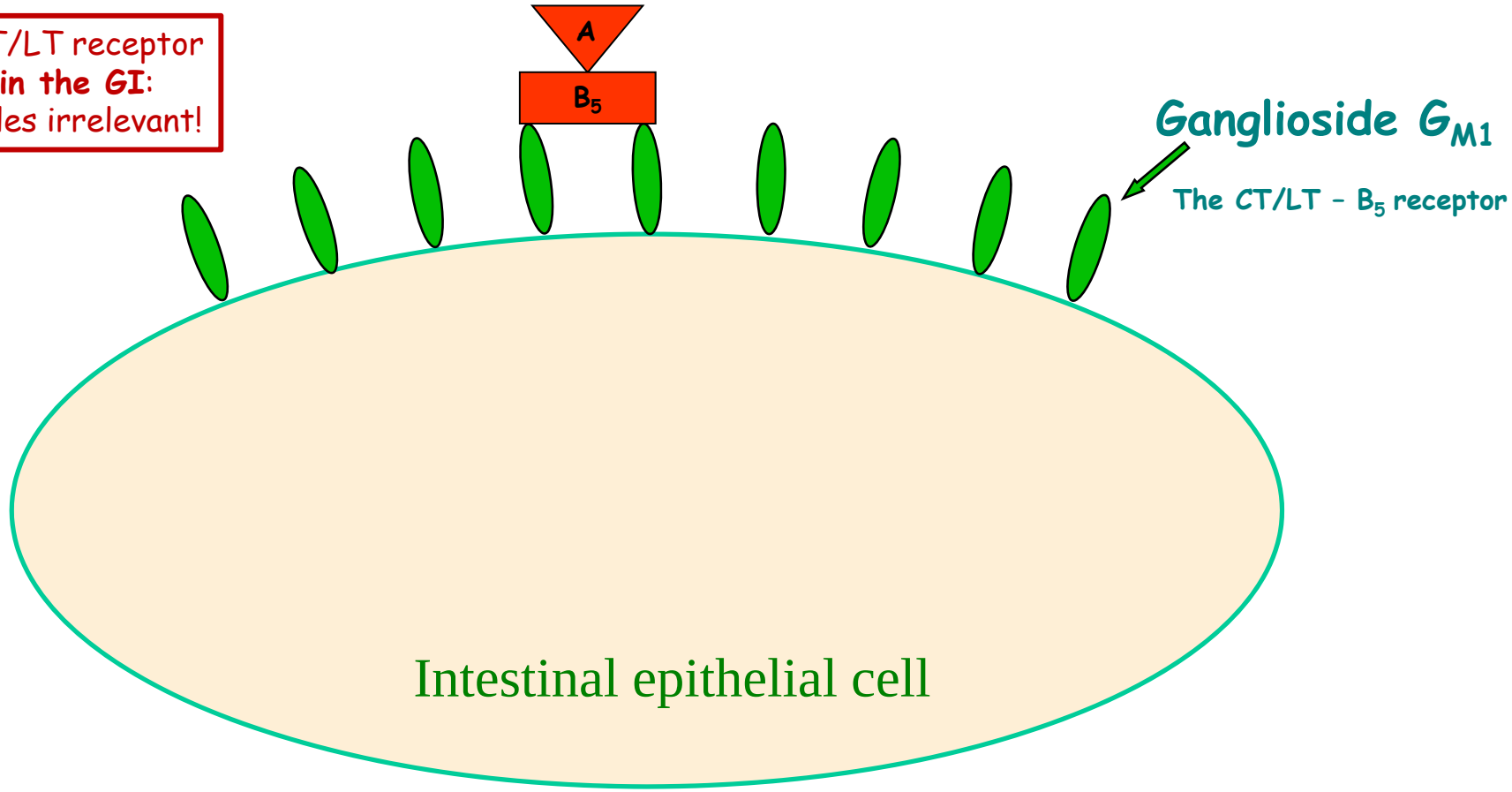


CT : Cholera

LT : Traveller's & Children's diarrhea

CT and LT Receptor Binding

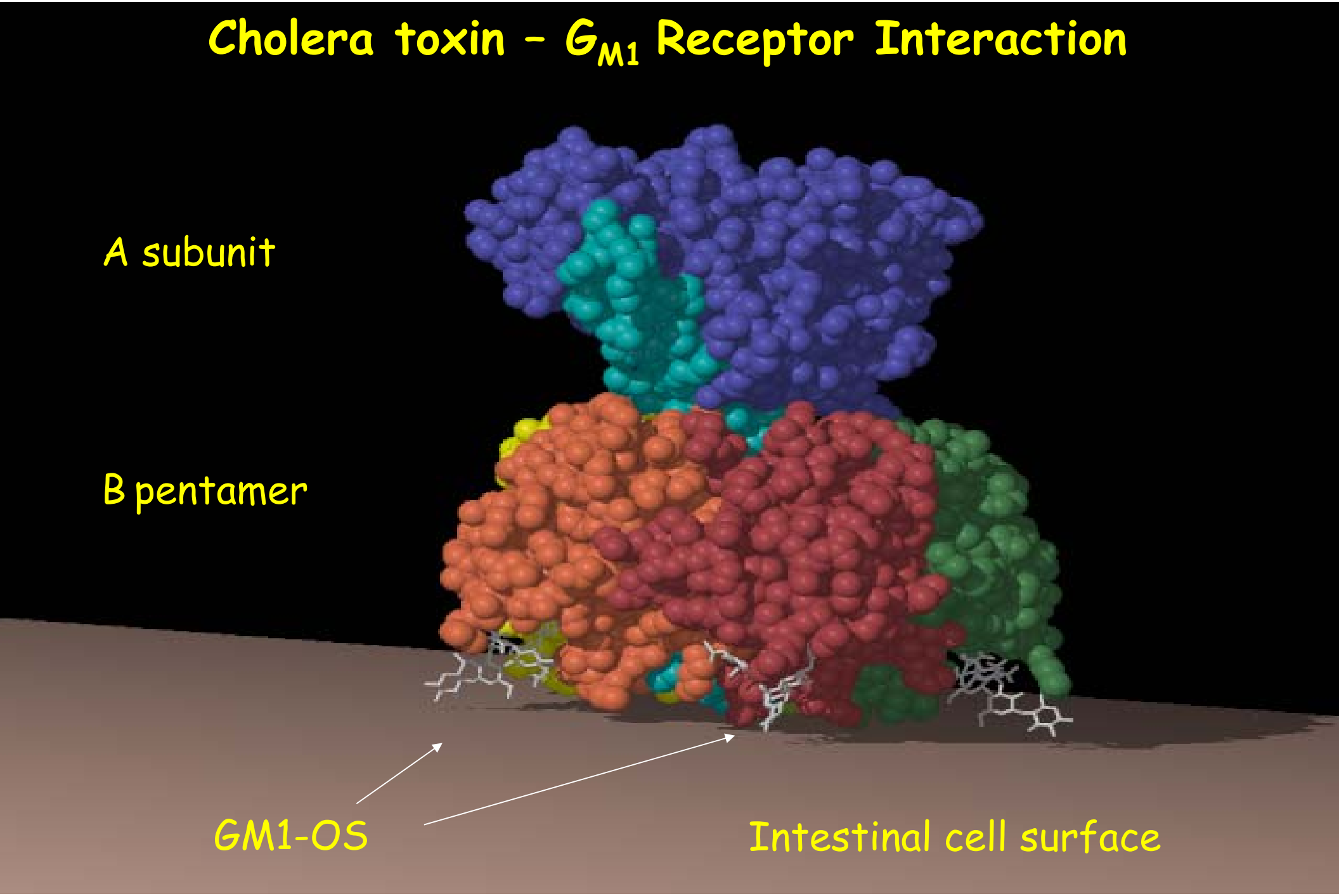
For blocking CT/LT receptor recognition **in the GI**:
The Lipinski rules irrelevant!



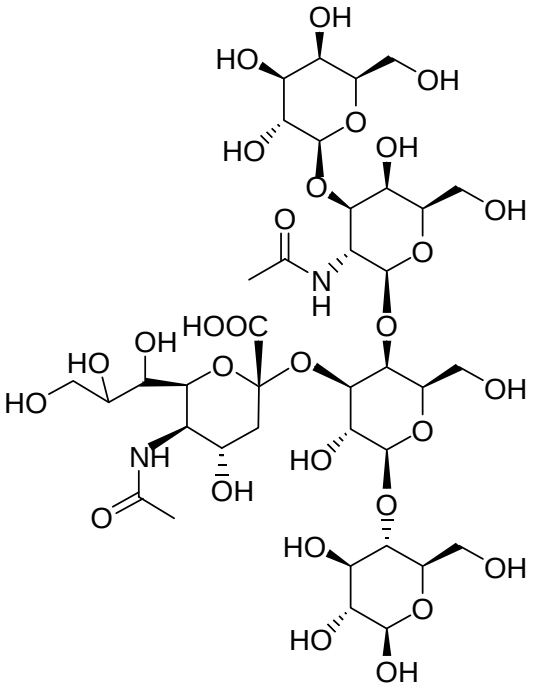
CT : Cholera

LT : Traveller's & Children's diarrhea

Cholera toxin – G_{M1} Receptor Interaction

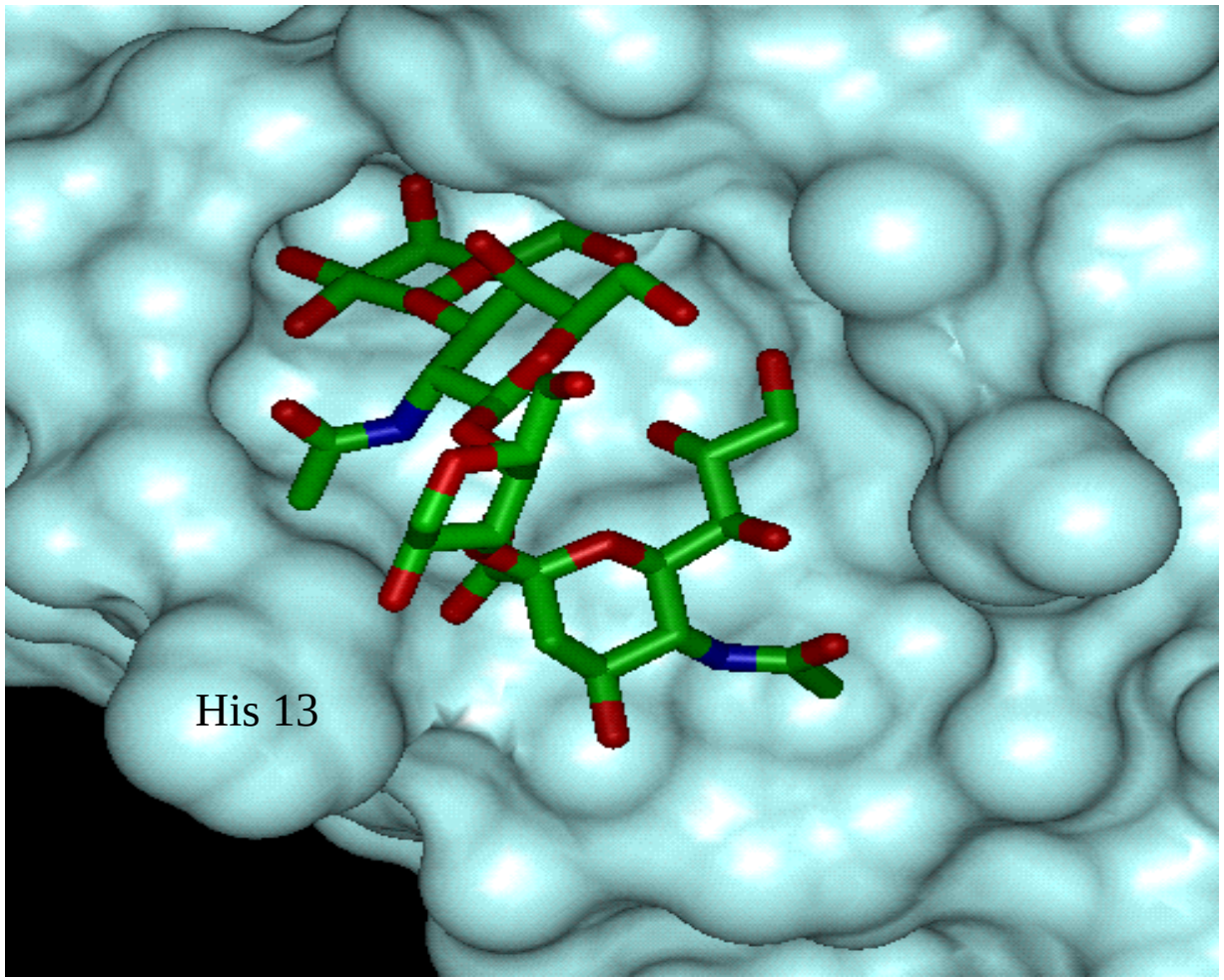


G_{M1} Pentasaccharide bound by CT



$IC_{50} = 14 \times 10^{-9}$ M

Extensive hydrophobic and H-bonding interactions

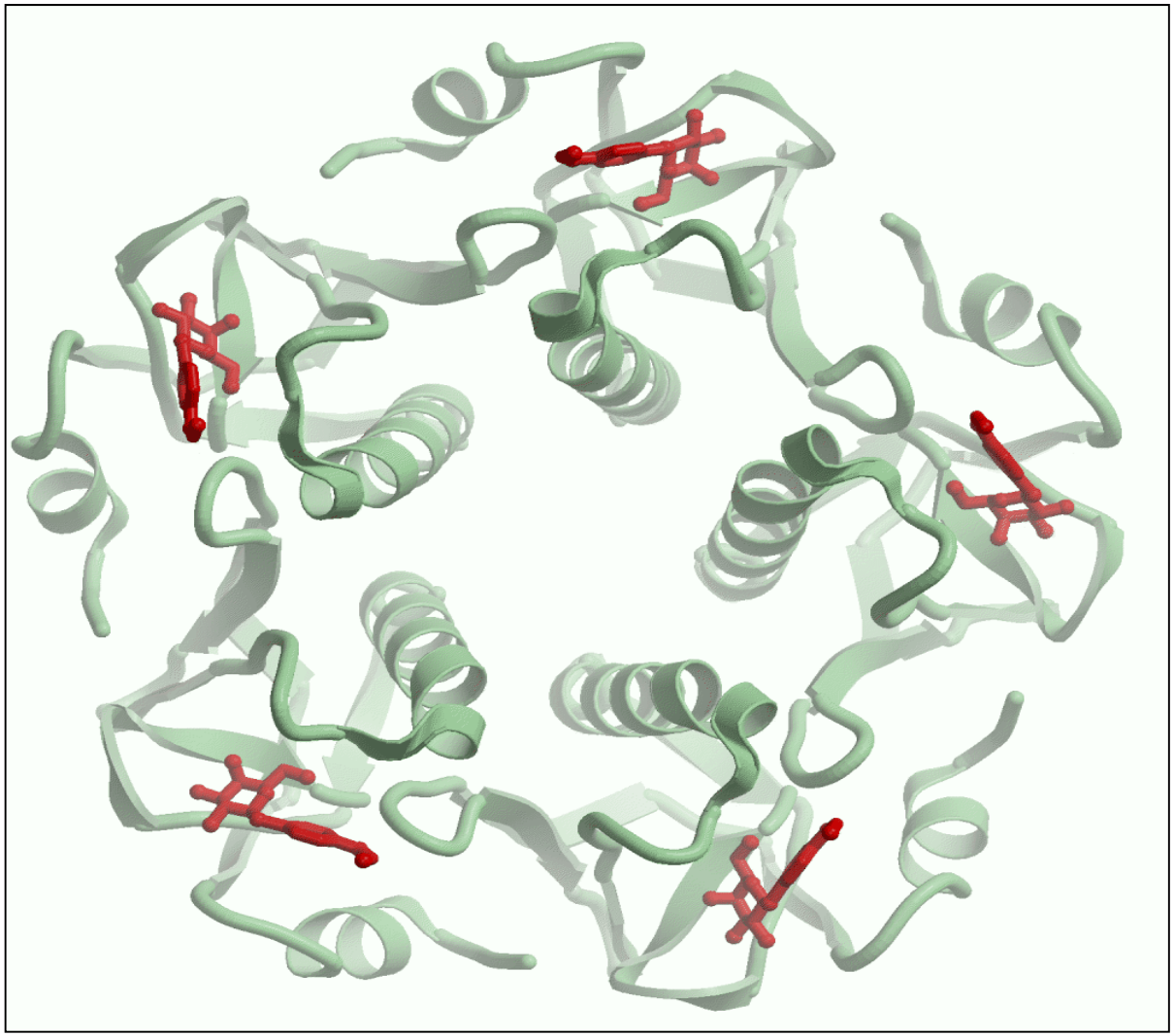


The enemy

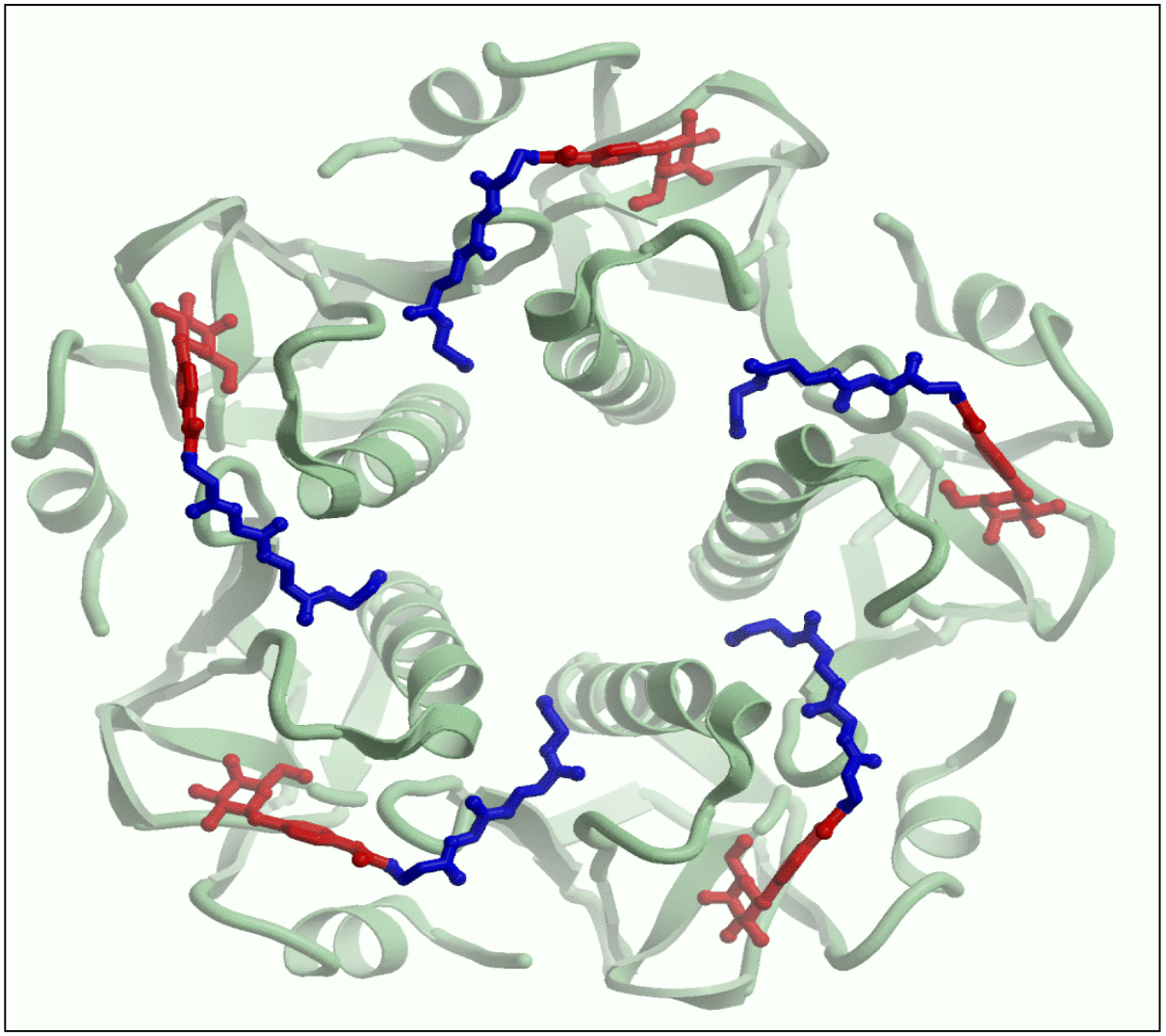
Joseph Martial

Steve Sarfaty
Ethan Merritt

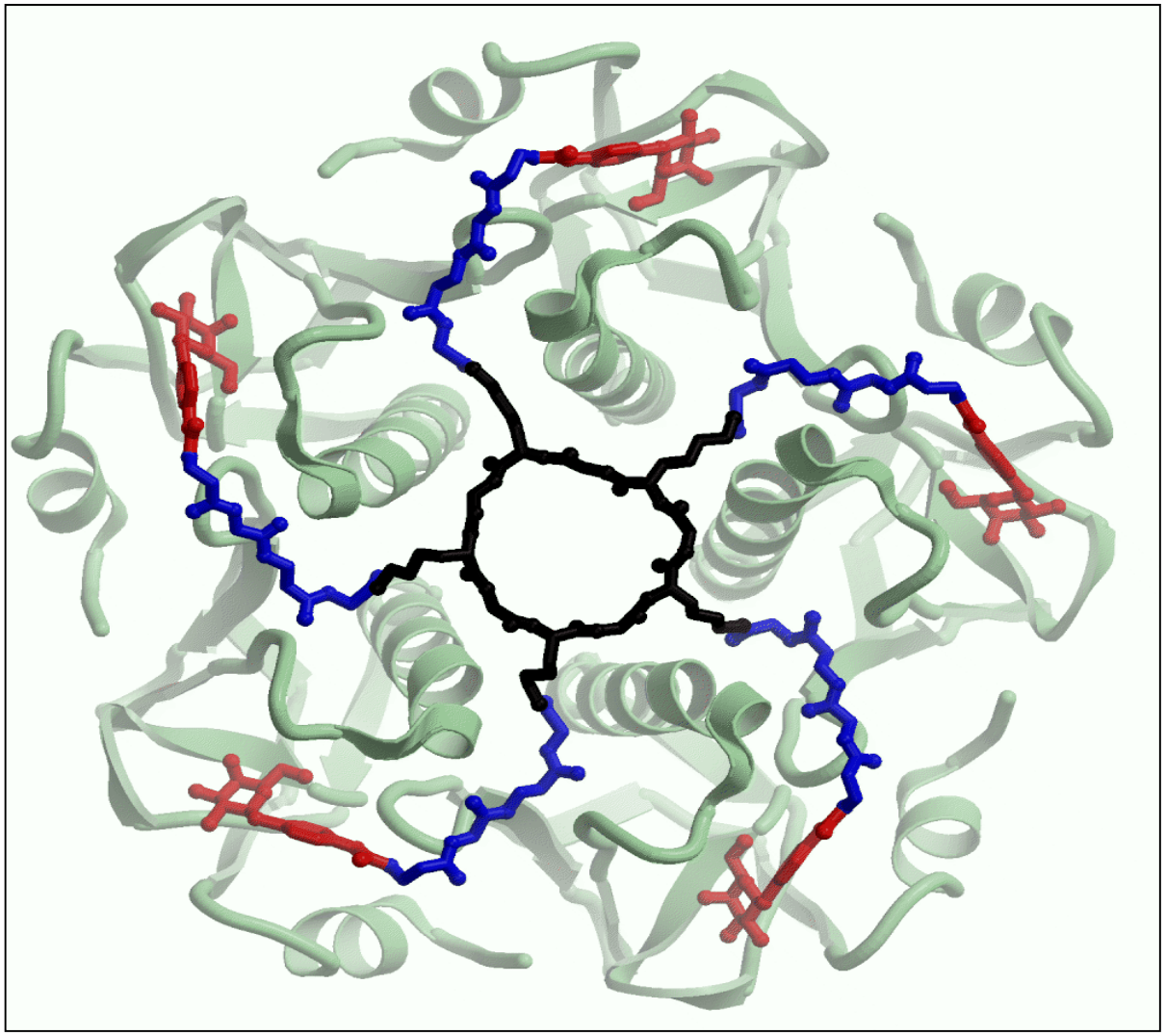
Five receptor binding sites



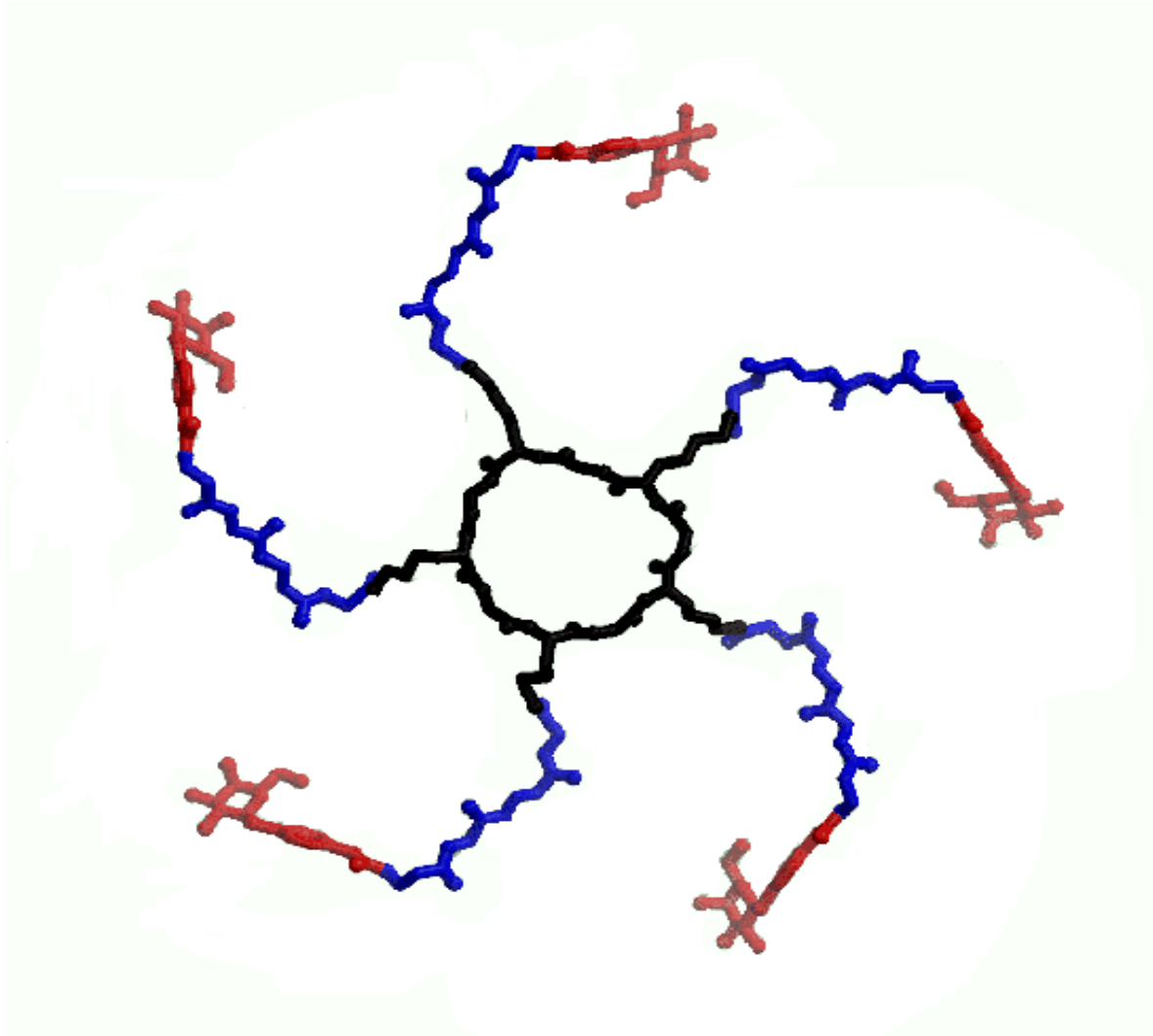
Making ligands longer



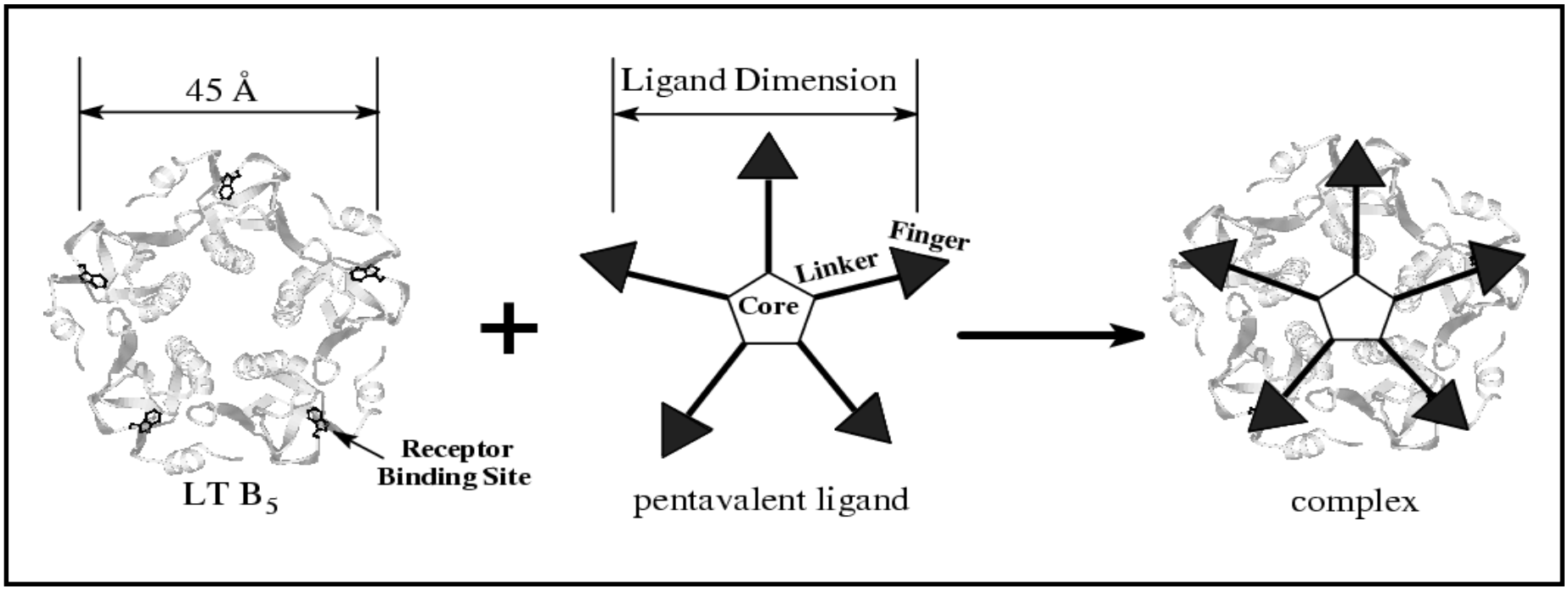
Ligand-Protein Complex



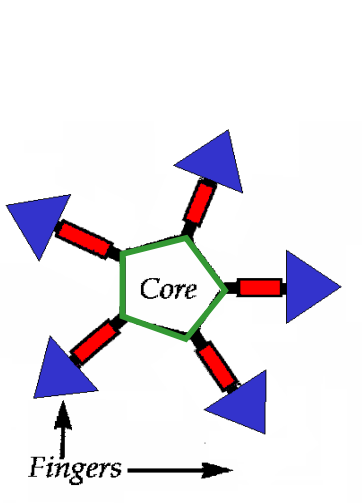
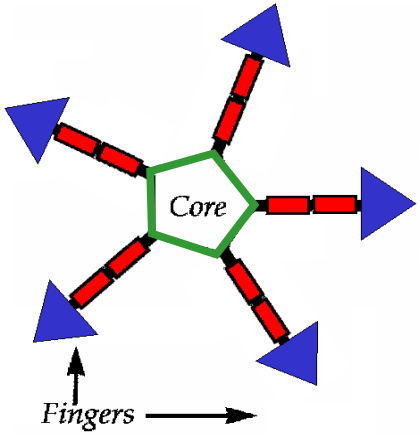
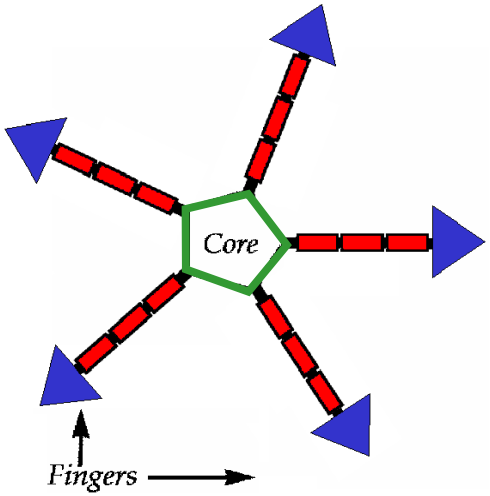
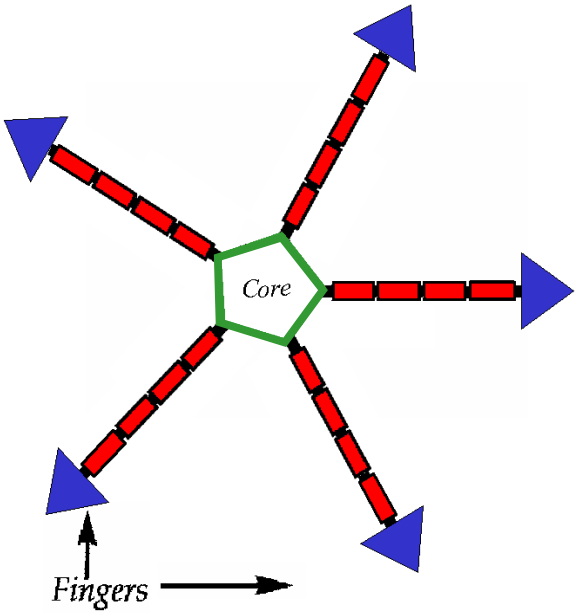
Pentavalent Ligand



THE PENTAVALENT CONCEPT

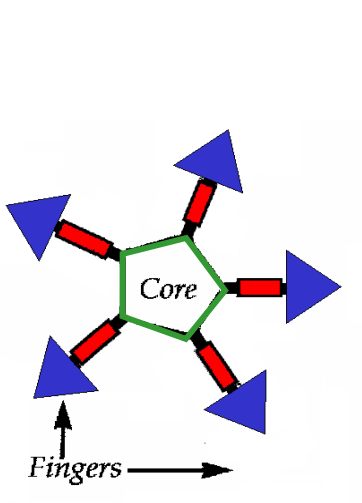
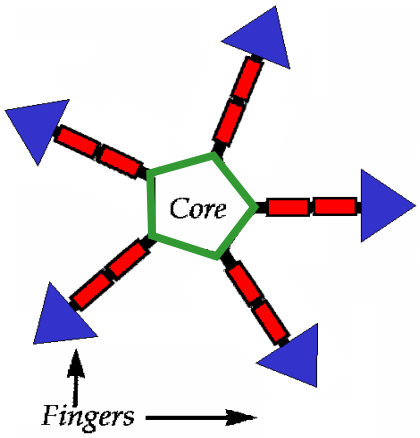
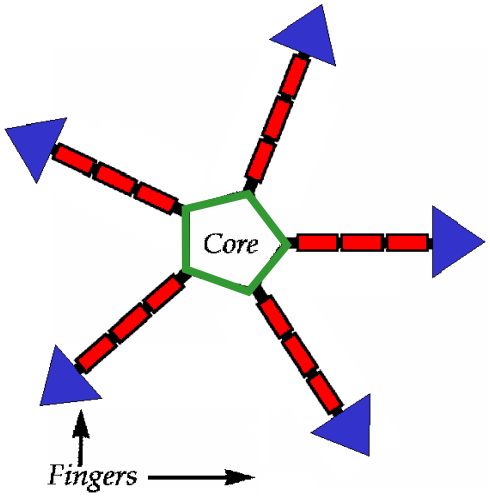
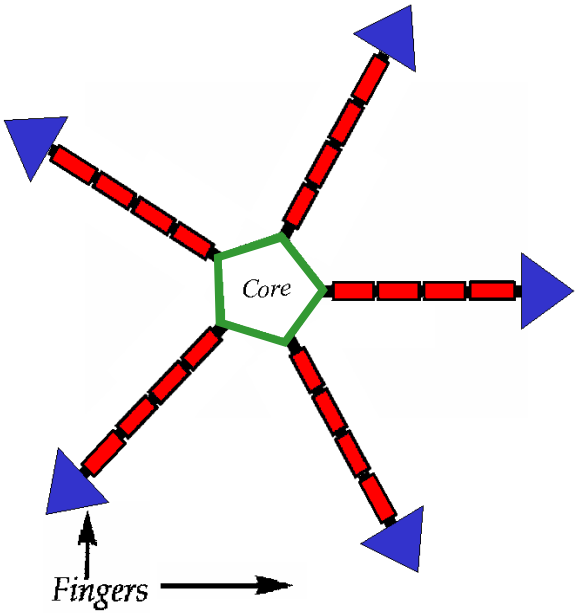


Gains in surface-receptor binding inhibition

One-Unit Linker	Two-Unit Linker	Three-Unit Linker	Four-Unit Linker
			
??? x Single Finger	???? x Single Finger	????? x Single Finger	?????? x Single Finger

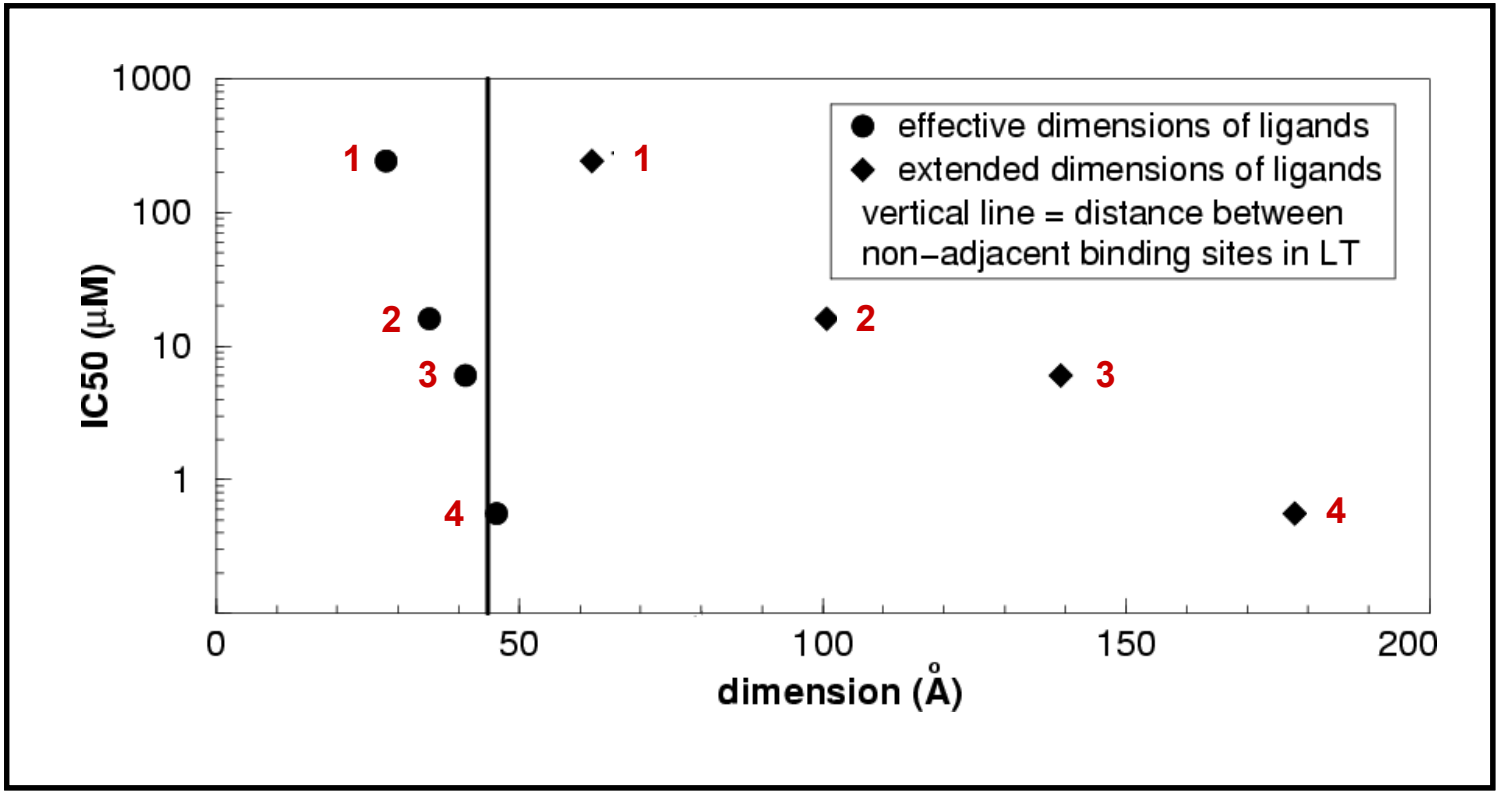
Fan et al, High affinity pentavalent ligands of Escherichia coli heat-labile enterotoxin by modular structure-based design. JACS 122: 2663-2664 (2000);
Merritt et al, Characterization and crystal structure of a high-affinity pentavalent receptor-binding inhibitor for Cholera toxin and E. coli heat-labile enterotoxin. JACS 124: 8818-8824 (2002);
Zhang et al, Solution and Crystallographic Studies of Branched Multivalent Ligands that Inhibit the Receptor-Binding Process of Cholera Toxin. JACS 124: 12991-12998 (2002).

Gains in surface-receptor binding inhibition

One-Unit Linker	Two-Unit Linker	Three-Unit Linker	Four-Unit Linker
			
240 x Single Finger	3,600 x Single Finger	10,000 x Single Finger	104,000 x Single Finger
1	2	3	4

Erkang Fan, Zhongsheng Zhang, Jason Pickens, Jiyun Liu, *et al*

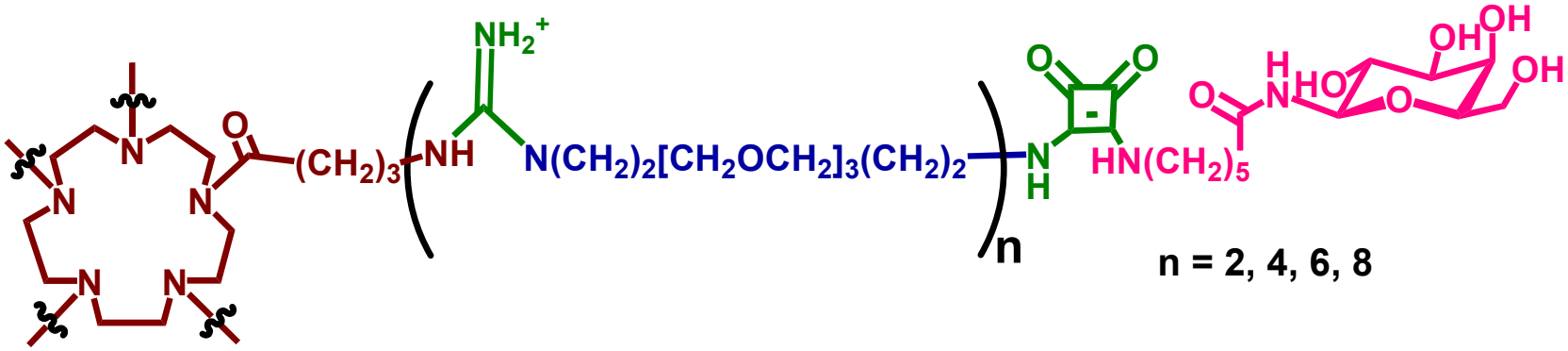
Gains in surface-receptor binding inhibition



IC_{50} *versus* EXTENDED & EFFECTIVE DIMENSIONS OF PENTAVALENT LIGANDS

Erkang Fan, Zhongsheng Zhang, Jason Pickens, Jiyun Liu, *et al*

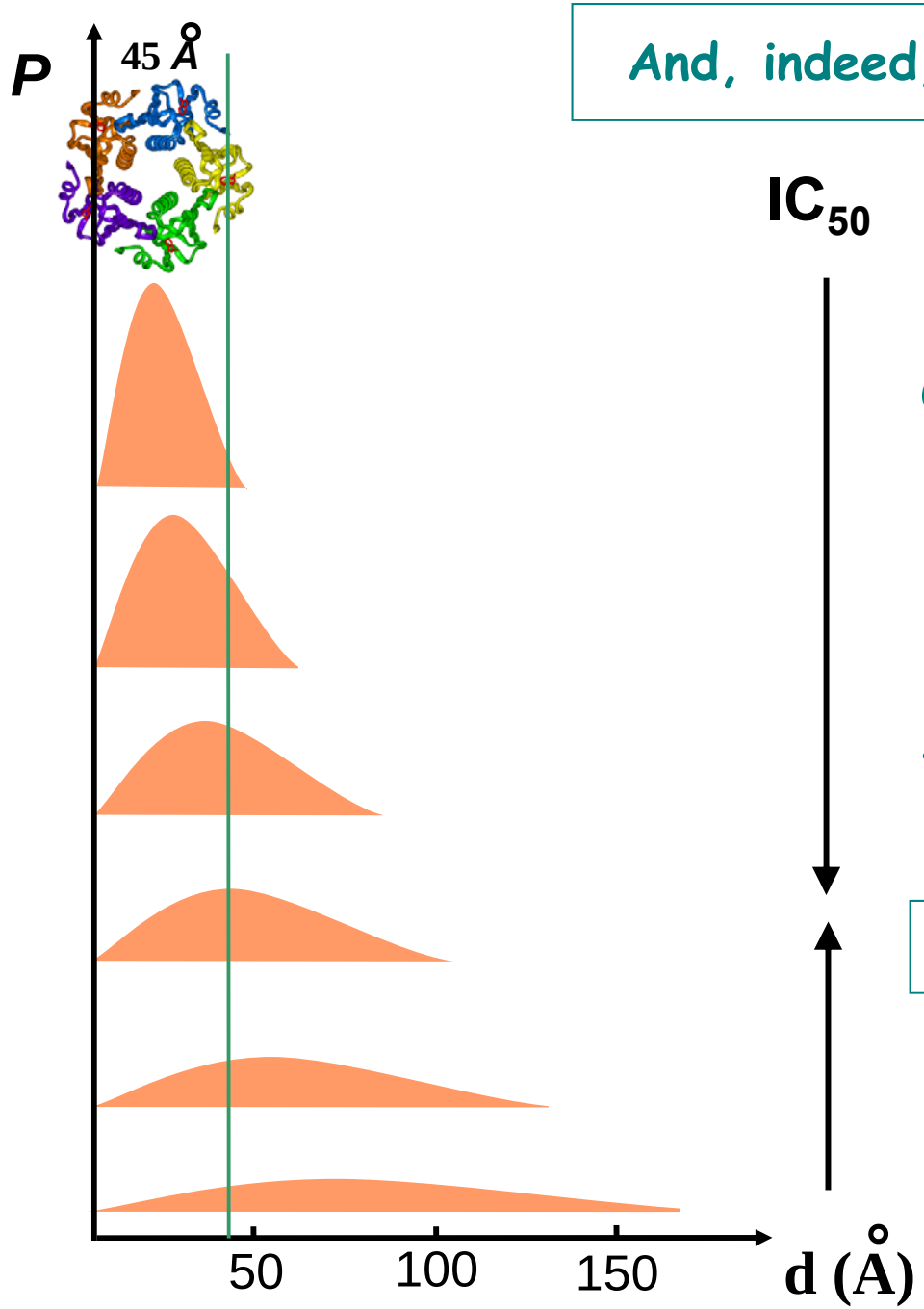
And, indeed, linker too long : *less* affinity



Linker Units	IC_{50} (μM)
n = 2	13.26 ± 0.95
n = 4	1.50 ± 0.10
n = 6	4.63 ± 0.46
n = 8	7.25 ± 0.38

(*A single galactose "finger"*: IC_{50} = ~ 100 mM =~100,000 μM)

Erkang Fan and co-workers



And, indeed, linker too long : *less* affinity

IC_{50}

One-unit linker

Two-unit linkers

Three-unit linkers

Four-unit linkers

Six-unit linkers

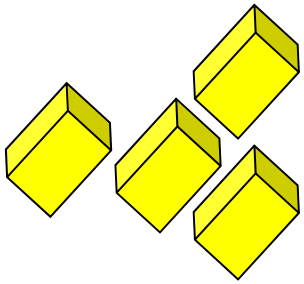
Eight-unit linkers

Fragment Cocktail Crystallography

A tool in early drug design

A useful website on Fragment Cocktail Crystallography: http://practicalfragments.blogspot.com/

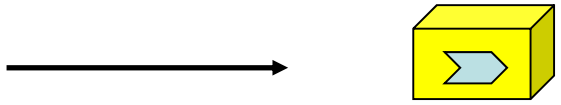
Fragment Cocktail Crystallography



Protein crystals

+

Cocktails of chemical fragments



Protein crystal with
bound chemical fragment

**Probe protein pockets by soaking crystals
in well-designed mixtures of 5-10 different chemicals,
followed by crystal structure determinations**

Fragment Cocktail Crystallography

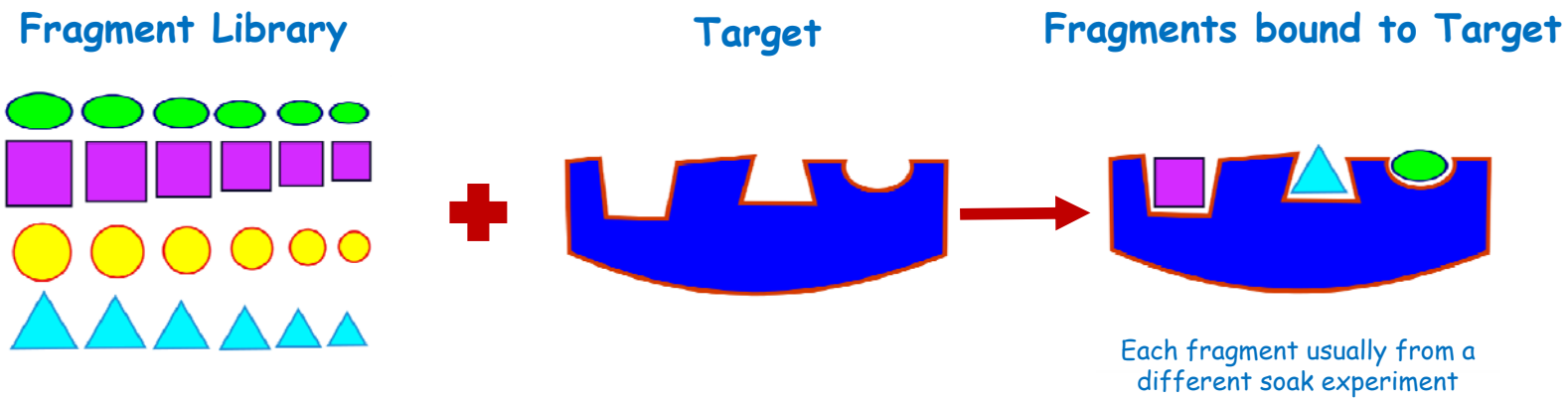
Basic idea of fragment-based drug design

- The molecules used in high-throughput screens (HTS) are quite complex.
- This complex nature of the molecules decreases the probability of finding a complementary binding site on a protein surface.
- It is easier for small molecules to find a pocket on the surface of the protein to bind to than for the typically larger molecules used in a HTS.

The probability of a snug fit of a complex molecule to a complex surface is quite small

Hann, J. Chem. Inf. Comput. Sci. 41, 856-864. (2001) ; Jhoti, Drug Discovery Today 7: 522-52 (2002)
Leach & Hann, Molecular complexity and fragment-based drug discovery, Curr Opin Chem Biol 15:489–496 (2011)
Hall, Efficient exploration of chemical space by fragment-based screening, Prog. Biophys.Mol. Biol. 116, 82–91. (2014)
Fig. after: Farmer & Reitz, Fragment-based Drug Discovery, Chpt 11 in C. Wermuth (Ed.), "The Practice of Medicinal Chemistry", 3rd Ed, Academic Press, 2008

Fragment Cocktail Crystallography



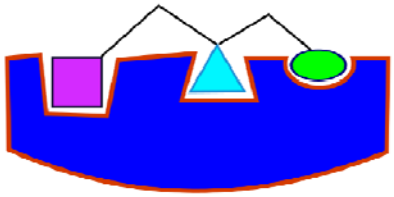
Although the probability of a fit of a simple molecule to a complex surface is quite large, the affinity of a fragment for the target is usually quite low.

Figure after:

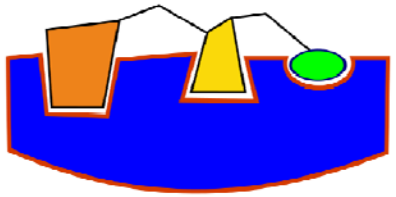
Farmer & Reitz, Fragment-based Drug Discovery, Chpt 11 in C. Wermuth (Ed.), "The Practice of Medicinal Chemistry", 3rd Ed, Academic Press, 2008

Fragment Cocktail Crystallography

Several different avenues can be explored to improve initial hits



Fragments linked to improve affinity

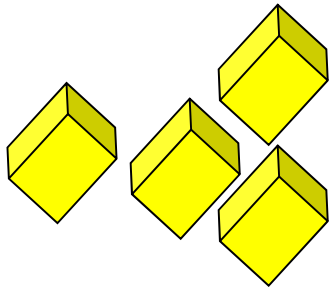


Further optimization of fit

"Growing" a single hit is perhaps even more popular than "linking" hits

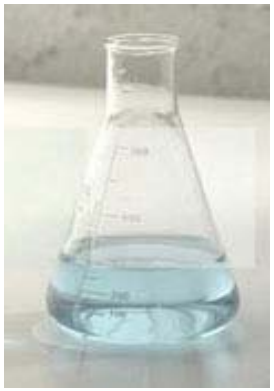
Fragment Cocktail Crystallography

Principle

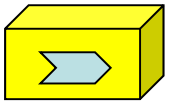


Protein crystals

+



Cocktails of chemical fragments



Protein crystal with
bound chemical fragment

Probe protein pockets by soaking crystals
in well-designed mixtures of 5-10 different chemicals,
followed by crystal structure determinations,
Followed by "growing" or "linking" the fragments to obtain higher affinity.

Fragment Cocktail Crystallography

Making the compound library

9,500 compounds

↓ *fragmentation*

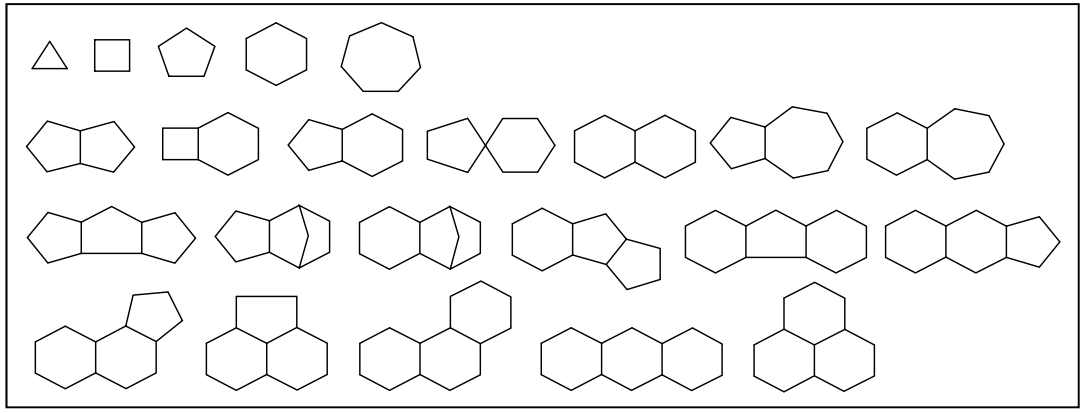
626 fragments

↓ *isolate ring systems*

23 frameworks (at connectivity level)

ACD Compound Filtering

ACD= Available Chemical Database



manual selection of compounds

→ 680 compounds

from each framework class

- ❑ eliminate mutagens, known poisons
- ❑ no highly functionalized compounds
- ❑ retain Br containing compounds

Christophe Verlinde, Erkang Fan
http://faculty.washington.edu/verlinde/

Influenza Virus

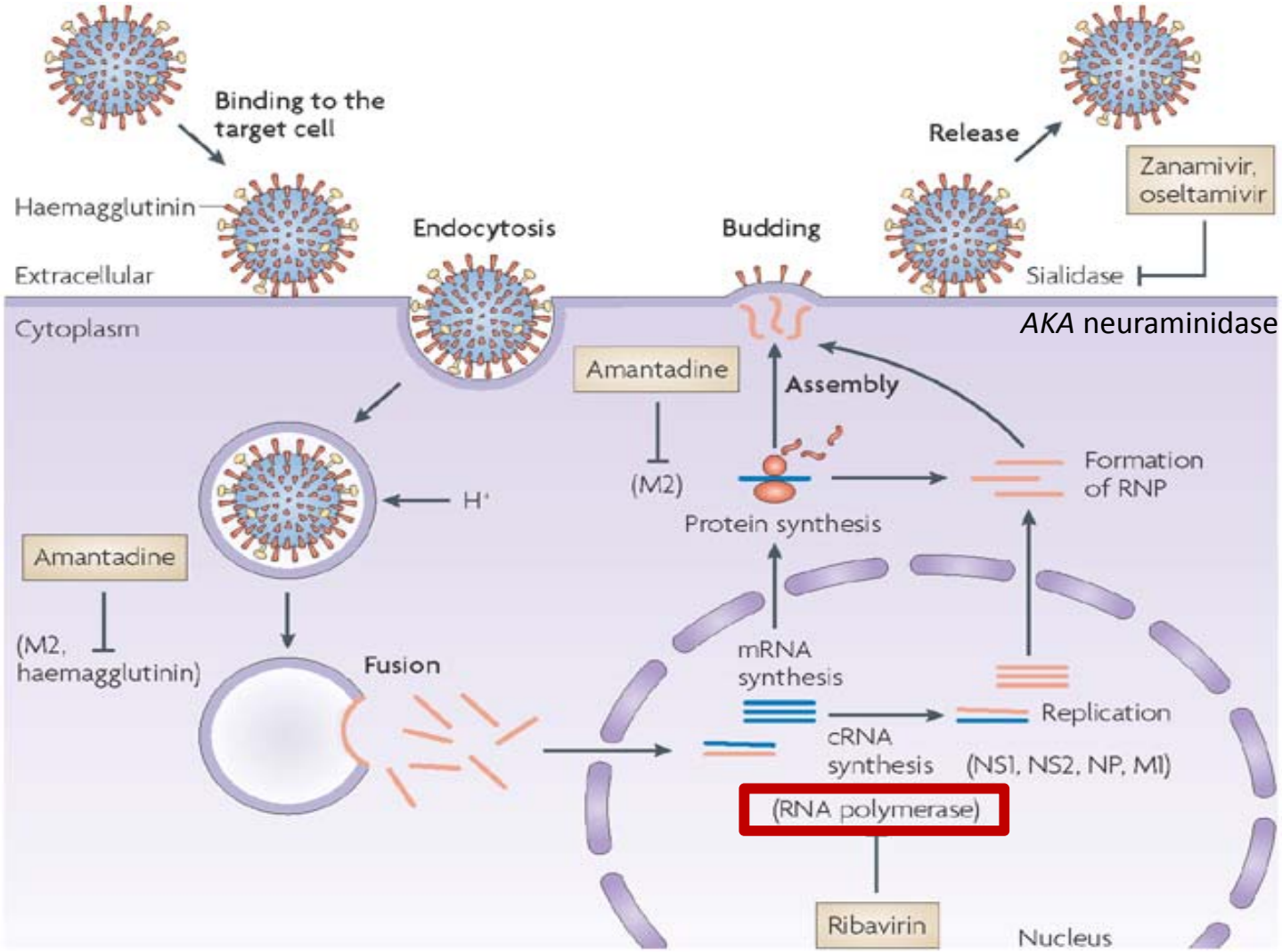


Figure from: von Itzstein, M. (2007). *Nature Reviews Drug Discovery* **6**, 967-974

Targeting Influenza Virus RNA-dependent RNA-polymerase

775 fragments in the library

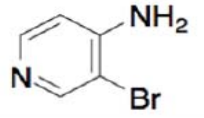
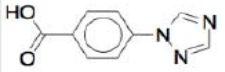
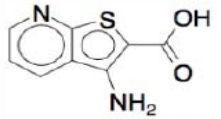
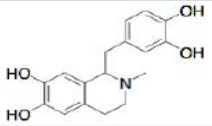
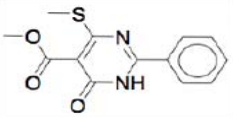
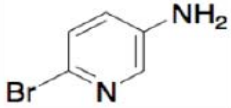
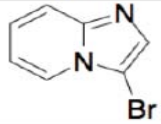
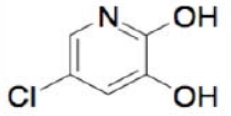
159 fragments are potentially metal chelators

8 fragments from these 159 chelators are found to be a hit in cocktail soaks, verified by individual soaks

1 % hit rate

IC_{50} for each hit were assayed to calculate **ligand efficiency (LE)**

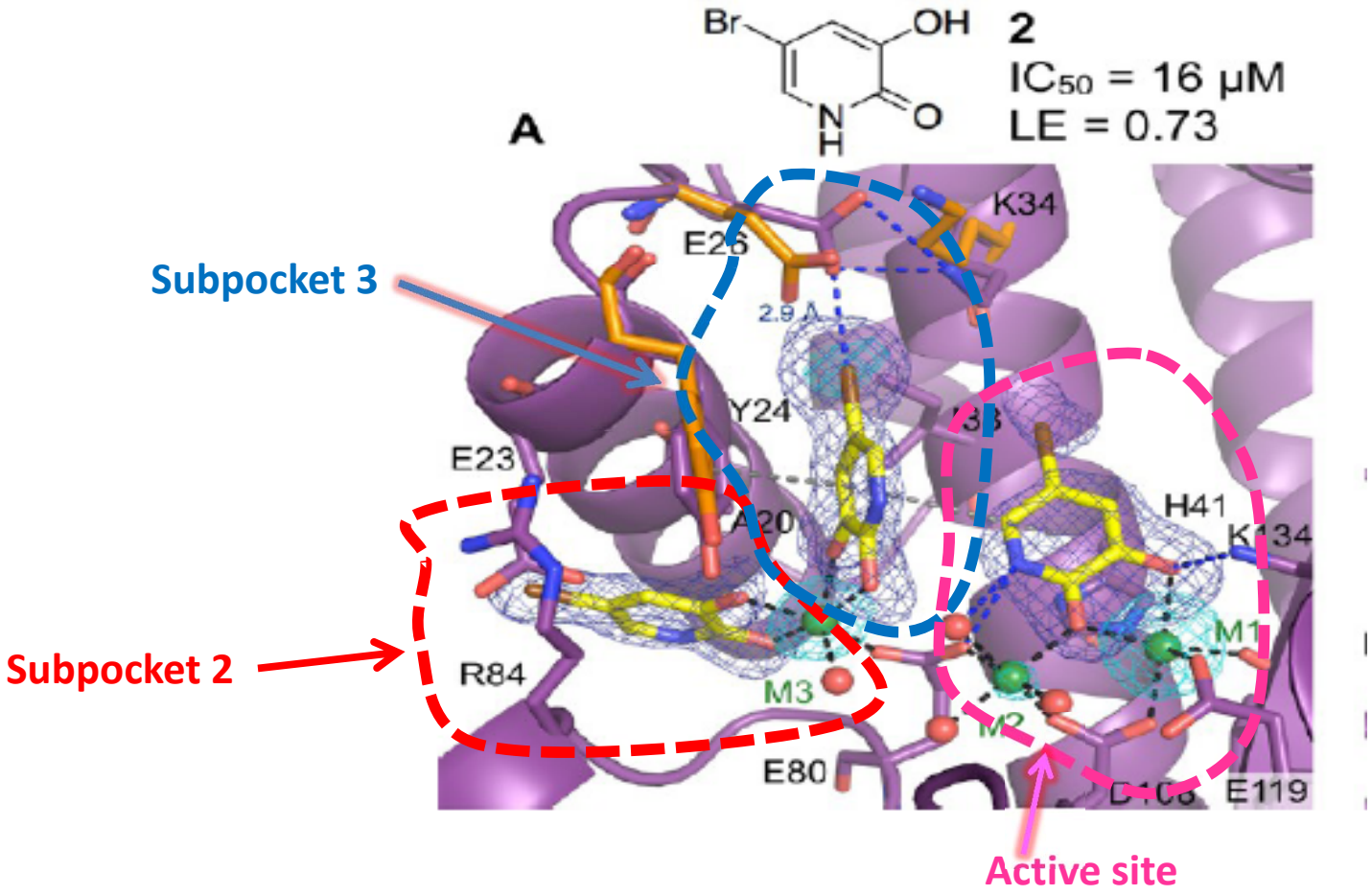
$$LE = \Delta G / N_{heavy}$$

Compound name	2D-Structure	Binding site	IC50 (μM)
4-amino-3-bromopyridine		Subpocket 2	>1000
4-(1H-1,2,4-Triazol-1-yl)benzoic acid		Subpocket 2	>1000
3-Aminothieno[2,3-b]pyridine-2-carboxylic acid		Active site	>1000
D,L-laudanosoline		Active site and subpocket 3	2
4-(1H-imidazol-1-yl)phenol		Subpockets 2 and 3	1000
methyl 4-(methylsulfanyl)-6-oxo-2-phenyl-1,6-dihydropyrimidine-5-carboxylate		Active site and subpocket 3/6	250
6-bromopyridin-3-amine		Subpocket 2	>1000
3-bromoimidazo[1,2-a]pyridine		Outside of active site cleft near Lys73	>1000
5-chloropyridine-2,3-diol		Subpockets 2, 3 and the active site	25

Bauman, Crystallographic Fragment Screening and Structure-Based Optimization Yields a New Class of Influenza Endonuclease, ACS Chemical Biology (2013)

HIT 2 & ANALOGS

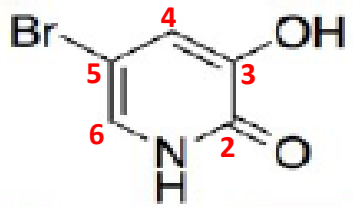
Special Length variants of N-terminal endonuclease domain required
Hit 2 is bound three times, and occupies subpockets 2, 3 and active site



Bauman, Crystallographic Fragment Screening and Structure-Based Optimization Yields a New Class of Influenza Endonuclease, ACS Chemical Biology (2013)

Hit-to-lead development

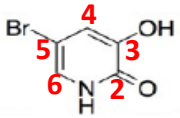
- Based on Hit 2, design modifications at 4, 5, 6 positions – synthesis – assay – crystal structure



2
IC_{50} = 16 μM
LE = 0.73

- Substitution at position 4 is compatible with binding predicted by docking, but all substitutions are deleterious experimentally
  - Maybe perturbing the electronic arrangements of chelating oxygens

Bauman, Crystallographic Fragment Screening and Structure-Based Optimization Yields a New Class of Influenza Endonuclease, ACS Chemical Biology (2013)

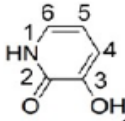


2
IC_{50} = 16 μM
LE = 0.73

Binds to subpocket 4, causing side chain rearrangements in subpocket 3

1 molecules bound (unlike 2)

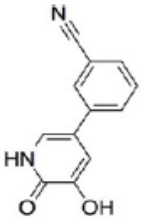
Position 5 points into subpocket 4



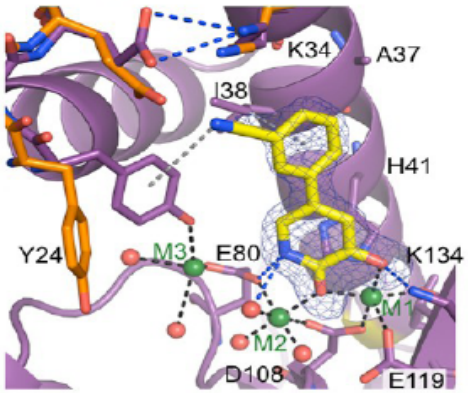
5-substitution

6-substitution

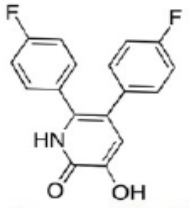
20x improvement



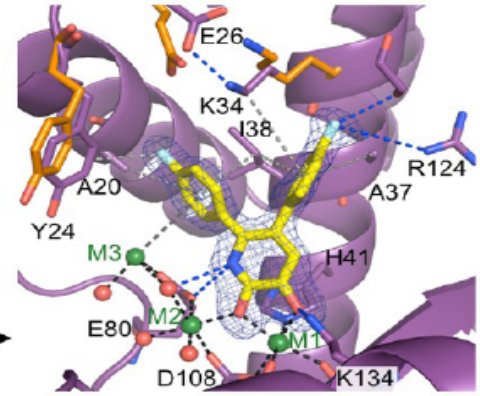
3
IC_{50} = 0.73 μM
LE = 0.54



400x improvement

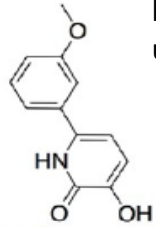


6
IC_{50} = 0.041 μM
LE = 0.50

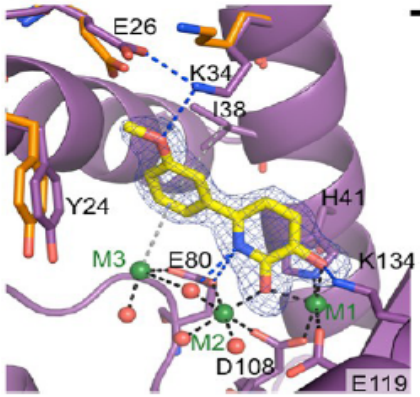


1450x improvement

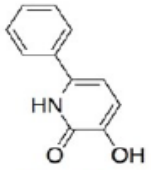
1 molecules bound (like 3 but unlike 2)



5
IC_{50} = 0.45 μM
LE = 0.54



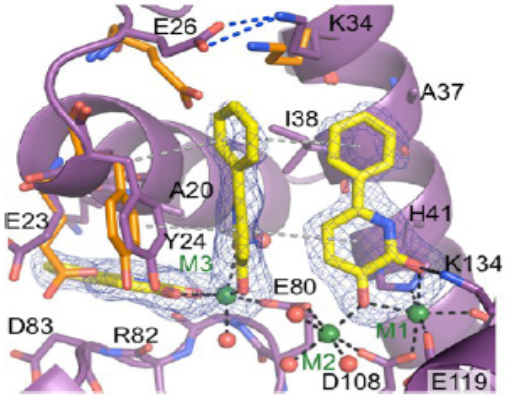
40x improvement

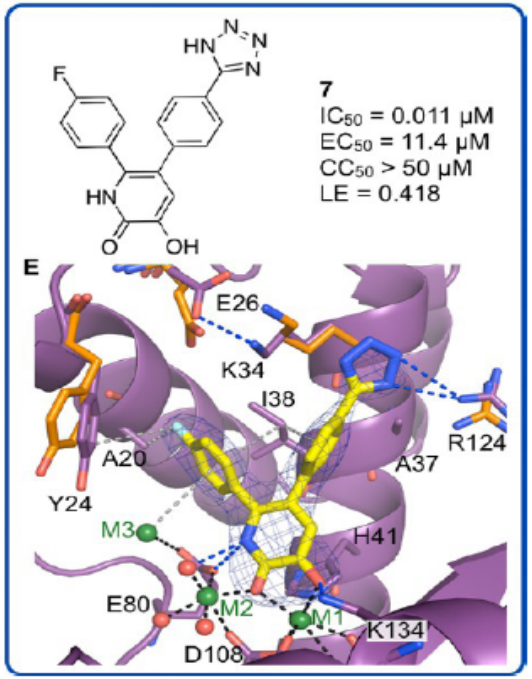


4
IC_{50} = 0.38 μM
LE = 0.63

3 molecules bound (like 2 but unlike 3)

Pyridinone ring flips 180 deg





7
IC_{50} = 0.011 μM
EC_{50} = 11.4 μM
CC_{50} > 50 μM
LE = 0.418

Protein•Protein Interaction Inhibitors

- Many proteins function as part of a *transient complex*.
- Yet, these transient interactions are critical for cell function.
- Protein interaction inhibitors interfere with these transient interactions.
- Such interactions can be relatively weak and hence are potentially an opportunity to interfere with using small molecule compounds.
- The challenge is, however, to discover “druggable pockets” in the interfaces of proteins engaged in transient interactions.
- After all, since these transient interactions are quite weak and hence pockets might be absent….

- REMARK: Please note the difference with “Protein assembly inhibitors” which interfere with the assembly of quite stable multi-protein complexes

Jones & Thornton, *Proc. Natl. Acad. Sci. USA* 93, 13-20 (1996)
Bourgeas, Atomic Analysis of Protein-Protein Interfaces with Known Inhibitors: The 2P2I Database. PLoS ONE 5(3): e9598 (2010)
A database with small molecules modulating protein-protein interactions is: TIMBAL. See: http://mordred.bioc.cam.ac.uk/timbal/
Higueruelo,. TIMBAL v2: update of a database holding small molecules modulating protein–protein interactions. Database (2013) Vol. 2013: article ID bat039

Inhibiting the interaction of HIV integrase (IN) with a human protein

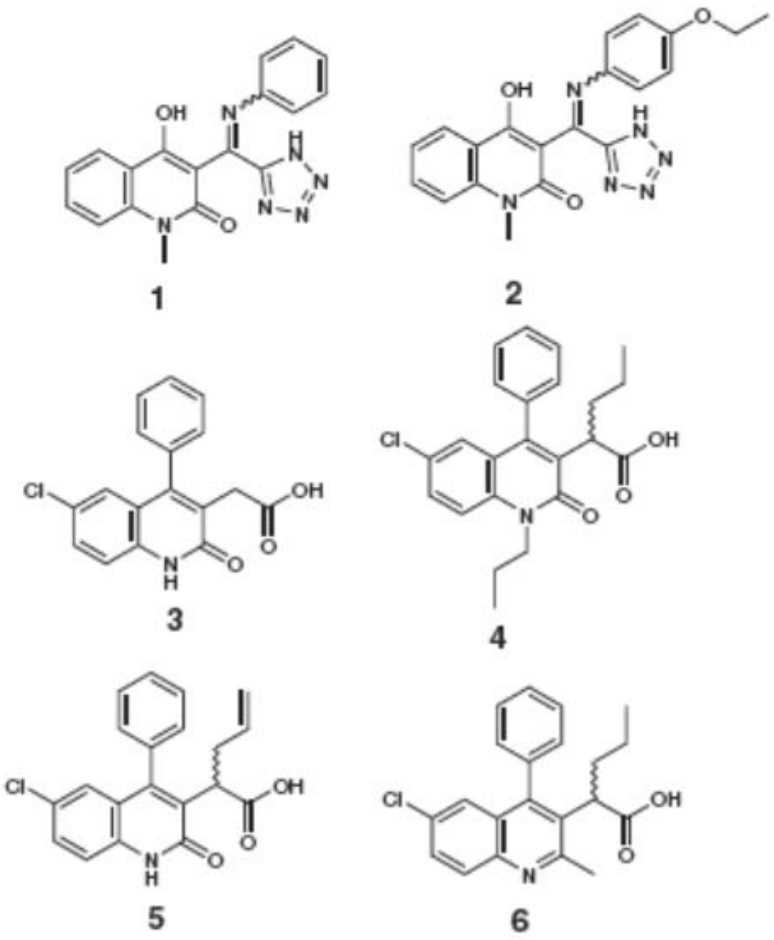
Background and procedure:

- HIV contains only about ten proteins and hence the virus needs to use many human proteins and RNA molecules to make more virus.
- One of the HIV proteins is “Integrase”, also called “HIV-IN” or “IN”.
- HIV integrase is responsible for incorporating the dsDNA made by HIV reverse transcriptase (HIV RT) into the human chromosome.
- In order to achieve this, HIV-IN needs to interact with a human protein called LEDGF/P75, hereafter called LEDGF.
- Compounds with interfere with this LEDGF•IN interaction are potential useful as anti-HIV agents.
- Virtual screening and crystal structures of domains of the LEDGF•IN complex were successfully used to arrive at compounds binding to pockets of IN needed for the interaction with LEDGF.

NOTE: LEDGF•IN means: a non-covalent complex of LEDGF and IN.

Christ, Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. Nature Chemical Biology, 6: 442-448 (2010).

Inhibiting the interaction of HIV integrase (IN) with a human protein



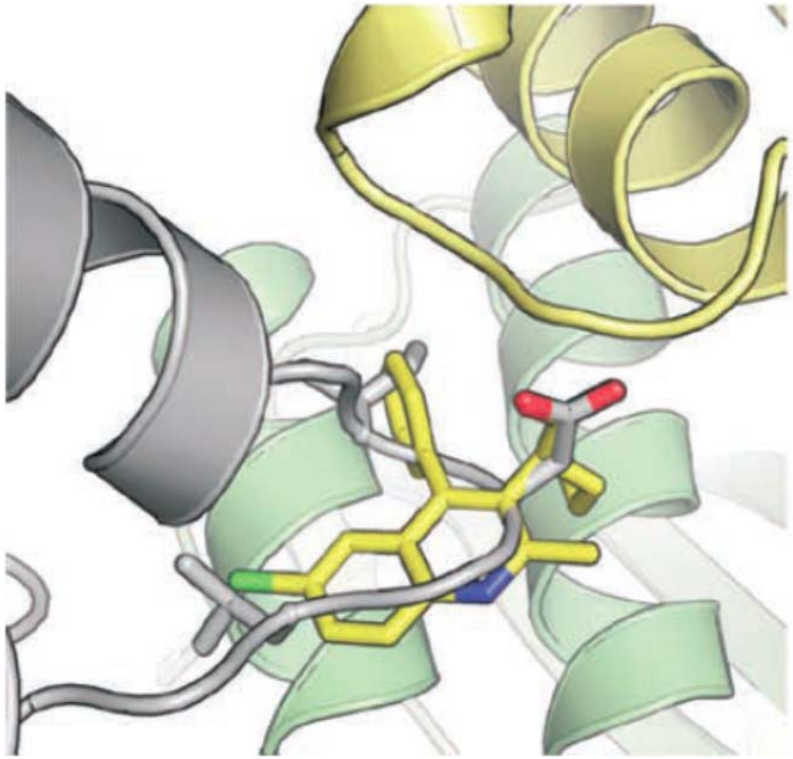
LEFT:

The major compounds considered after virtual screening of compounds binding to a pocket of the HIV integrase (IN) involved in interactions with the human protein LEDGF.

- Compound **1:** most effective in assay from 25 compounds purchased after *in silico* docking of 200,000 compounds.
- Compound **3**: After pragmatic chemical optimization
- Compound **6**: best compound obtained.
- Subsequent chemical modification resulted in yet a better compound with an IC_{50} of 0.58 µM for the IN•LEDGF interaction and an EC_{50} of 0.76 µM in an anti-HIV assay.

Christ, Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. Nature Chemical Biology, 6: 442-448 (2010).

Inhibiting the interaction of HIV integrase (IN) with a human protein



Green and yellow: HIV integrase core dimer subunits.
Yellow sticks: the soaked compound **6**.
Gray: Human LEDGF from the LEDGF•IN complex structure

Structural insight into how compound 6 binds to IN and interferes with the interaction of IN and the human protein LEDGF.

The way in which compound **6** prevents the IN•LEDGF interactions is evident from the superposition of the crystal structures of:

- the integrase binding domain of LEDGF in complex with the catalytic core of IN,

  and

- the integrase binding domain of LEDGF in complex with compound **6**.

Christ, Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. Nature Chemical Biology, 6: 442-448 (2010)
For more recent developments see: Krishnan & Engelman, Retroviral Integrase Proteins and HIV-1 DNA Integration, JBC 287: 40858–40866 (2012).

Structural Biology and Drug Development

A marvelous partnership

A Structure of a Drug Target can initiate and accelerate drug development in many important ways:

I. The Structure of the Target by itself shows immediate novel opportunities for drug design

e.g. The hexameric arrangement of helices in HIV gp41

II. A Structure of a Target with a Substrate or Co-factor or TS Analog reveals which pockets can be filled by inhibitors and suggests which types of compounds to make

e.g. HIV protease:substrate complex
Protozoan GAPDH:NAD complex
Influenza Virus Neuraminidase Inhibitors

III. Structures of the Target with Low MW-low affinity "fragments" show where fragments bind and how to modify and/or link fragments – to achieve higher affinity

e.g. "Fragment Cocktail crystallography"

IV. The structure of a compound found in a screen in complex with the Target reveals how the compound acts and how it can be modified for better affinity

e.g. NNRTI's and HIV Reverse Transcriptase
Cyclosporin in complex with Calcineurin and Cyclophilin

V. Structures of successive compounds bound to the same Target assist in understanding structure-activity relationships, binding modes and conformational changes : ITERATIVE STRUCTURE-BASED LEAD OPTIMIZATION.

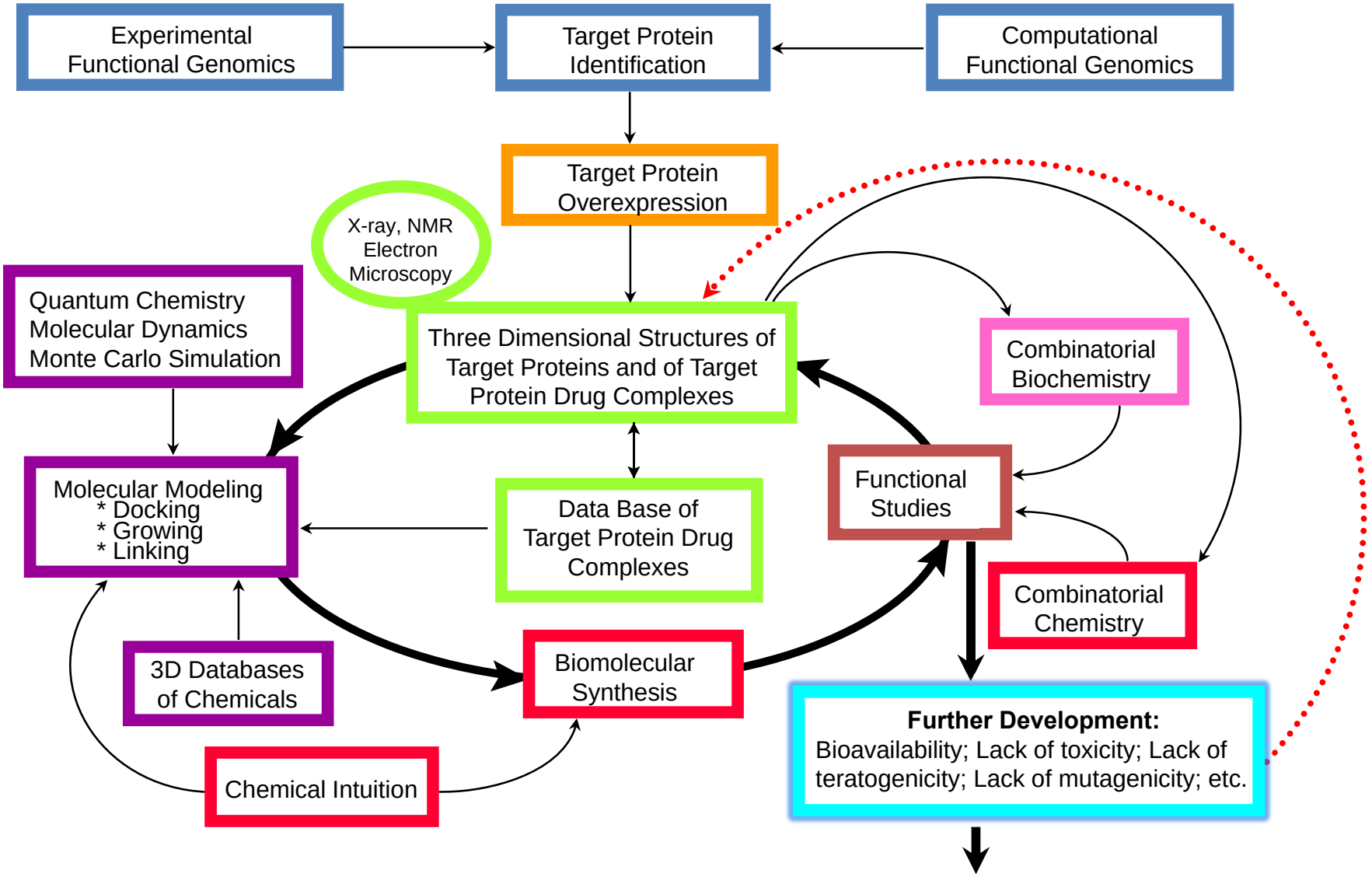
e.g. Anti-Glaucoma drug targeting carbonic anhydrase

VI. The structure of a Drug Candidate in complex with the Target can be helpful in devising strategies for modifications which MAINTAIN AFFINITY but improve e.g. drug bioavailability or decrease drug toxicity.

VII. The structure of a Drug:Target complex unravels the reasons for DRUG RESISTANCE

e.g. Gleevec and abl-src kinase

STRUCTURE-GUIDED DRUG DESIGN



Computational Approaches

An website with recent tools for Structure based drug design:

http://www.imb-jena.de/~rake/Bioinformatics_WEB/dd_tools.html

Major Journals with plenty SBDD:

J. Medicinal Chemistry

Chemistry and Biology

Nature Reviews Drug Discovery

J. Computer-Aided Molecular Design

Nature, Science, etc

REFERENCES

Inhibitor Design *Trypanosoma brucei* and *Leishmania* GAPDH

- Aronov, A. M., Verlinde, C. L. M. J., Hol, W. G. J. & Gelb, M. H. (1998). Selective tight binding inhibitors of trypanosomal glyceraldehyde-3-phosphate dehydrogenase via structure-based drug design. *J. Med. Chem.* **41**, 4790-4799.

- Aronov, A. M., Suresh, S., Buckner, F. S., van Voorhis, W. C., Verlinde, C. L. M. J., Hol, W. G. J. & Gelb, M. H. (1999). Structure-based design of sub-micromolar, biologically active inhibitors of trypanosomatid glyceraldehyde-3-phosphate dehydrogenase. *Proc. Natl. Acad. Sci. USA* **96**, 4273-4278.

- Suresh, S., Bressi, J. C., Kennedy, K. J., Verlinde, C. L. M. J., Gelb, M. H. & Hol, W. G. J. (2001). Conformational changes in *Leishmania mexicana* glyceraldehyde-3-phosphate dehydrogenase induced by designed inhibitors. J. Mol. Biol. 309, 423-435.

- Bressi, J. C., Verlinde, C. L. M. J., Aronov, A. M., Shaw, M. L., Shin, S. S., Nguyen, L. N., Suresh, S., Buckner, F. S., Van Voorhis, W. C., Kuntz, I. D., Hol, W. G. J. & Gelb, M. H. (2001). Adenosine analogues as selective inhibitors of glyceraldehyde-3-phosphate dehydrogenase of Trypanosomatidae via structure-based drug design. J. Med. Chem. 44, 2080-2093.