The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

**2039-5**

# Conference on 2nd Drug Development for the Third World: From Computational Molecular Biology to Experimental Approaches

***1 - 5 June 2009***

## Modes of Drug Action I

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# Many Modes of Drug Action

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Trieste
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**The modes of**
**"Drug Action"**
**in this lecture**
**includes also several examples of**
**"Inhibitor Action"**
**because of the important concepts involved.**

**But there is a tremendous difference between an Inhibitor and a Drug.**

**Drugs** are really very special molecules.
Their effects on humans are far more beneficial than damaging, even though there is nothing absolute and much is both
**drug-type & drug-dose dependent**
as well as often
**person dependent**

**Oral drugs** tend to be:
- quite small
- uncharged

## Many modes of drug action

**on many different drug targets:**

- **Proteins**
  - Active Site Blockers
  - Cofactor Site Blockers
  - Receptor Binding Site Binders
  - Conformational Change Preventers
  - Protein-Protein Interaction Preventers
  - Protein Assembly Inhibitors
  - Protein Assembly Stabilizers
  - Protein-Protein Glues
  - Protein-DNA Recognition Blockers
- **DNA**
  - Intercalators
  - Minor Groove Binders
  - Crosslinkers
  - Antisense oligonucleotides
- **Protein-DNA Complexes**
  - Topoisomerase Poisons
- **Protein-RNA Assemblies**
  - Ribosome Tunnel blockers
- **Membranes**
  - Pore Formation
- **Substrates**
  - Specific Substrate Binding
- **Small Molecules**
  - Small molecule assembly inhibition

# 1. Drugs acting on Proteins

Proteins perform an incredible number of different functions in living cells, many of which are crucial for the organism.

Essential proteins from pathogens or cancer cells are promising drug targets for infectious diseases and cancers.

Many normal human proteins are also drug targets for treatment of human diseases or afflictions.

Abnormal, defective proteins responsible for a wide spectrum of *genetic* diseases are in principle also good drug targets. This is, however, very difficult since:

(i) often closely related proteins need to be avoided;

(ii) the number of patients suffering from one and the same point mutation is often very small.

## How many drug binding sites are there in an organism?

It is of interest to see how many proteins are (estimated to be) encoded by various genomes:

| | |
|---|---|
| • Hepatitis C Virus | ~ 10 |
| • HIV | ~ 12 |
| • *Haemophilus influenza* | ~ 1,743 |
| • *Mycobacterium tuberculosis* | ~ 4,000 |
| • Yeast | ~ 5,885 |
| • *Plasmodium falciparum* | ~ 5,500 |
| • C. *elegans* | ~ 19,000 |
| • Humans | ~ 25,000 |

How many, and which, of these proteins *can be exploited* in the battle against infectious agents or cancer cells remains to a large extent still to be determined.

How many of these *have to be avoided* in human cells is also still a question with an incomplete answer.



## 1.1. Active Site Inhibitors

**Important Issues**

- **Is the target active site unique to a pathogen, or are there similar proteins in pathogen and host?**
- **If the target is unique to a pathogen, or humans, quite small molecules mimicking the transition state of the catalyzed reaction might work with great affinity *and* selectivity.**
- **If the target in a pathogen has a homologous counterpart in the human host, then small molecules blocking the true active site of the pathogen protein are likely to hit the active site of the corresponding human enzyme as well.**
- **Hence, larger molecules are preferred in such cases since these confer greater specificity because they can utilize pockets and charges unique to the pathogen protein.**
- **However, then non-essential regions of the pathogen protein are used for obtaining selectivity and resistance is possibly soon around the corner.**

# 1.1. Active Site Inhibitors (ctd.)

Examples

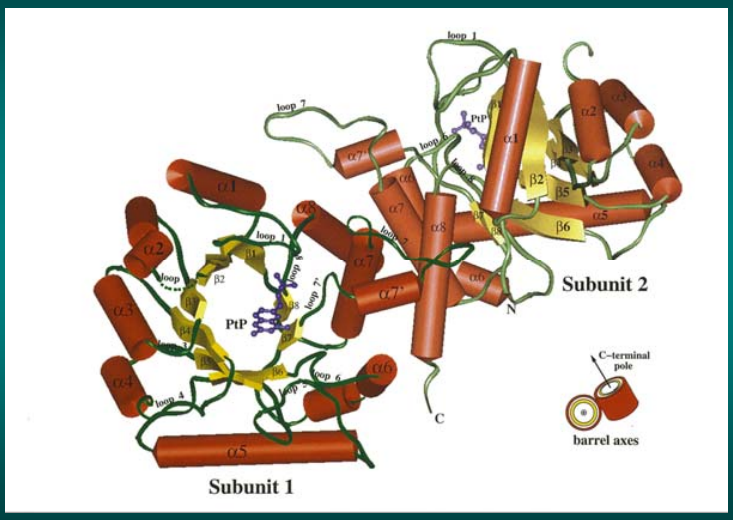
Famous groups of Active site inhibitors are:

- **HIV protease inhibitors**
- **Influenza Virus Neuraminidase inhibitors**
- **HIV ReverseTranscriptase Inhibitors**
- **Sulfa drugs: targeting dihydropteroate synthase (DHPS) in bacteria**

Other famous examples are:

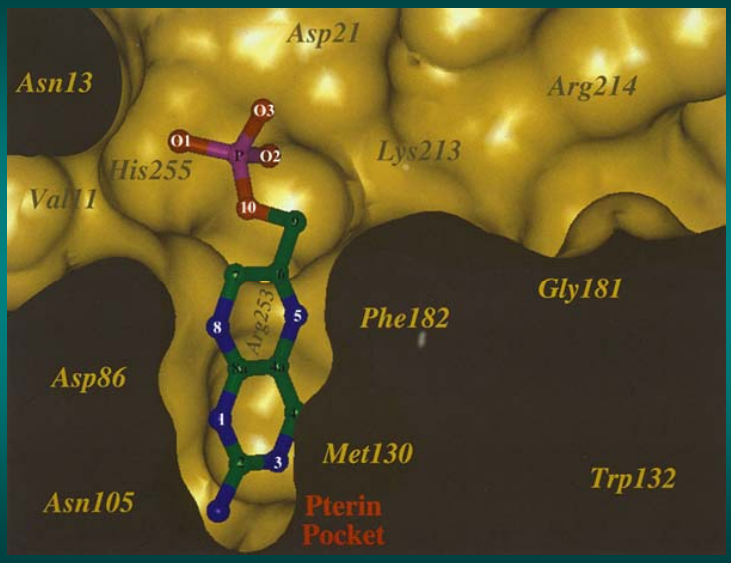
- **Penicillins: targeting bacterial cell wall synthesis enzymes**
- **Aspirin: targeting cyclo-oxygenase**
- **Pyrimethamine: targeting dihydrofolate reductase (DHFR) in the malaria parasite**
- **Trimethoprim: targeting dihydrofolate reductase (DHFR) in bacteria**
- **Methotrexate: targeting dihydrofolate reductase (DHFR) in humans**

***M. tuberculosis* DHPS:Substrate-analog Complex**




Baca et al , *J. Mol. Biol.* **302**, 1193-1212 (2000).

*M. tuberculosis* DHPS:Substrate-analog Complex




Baca et al , *J. Mol. Biol.* **302**, 1193-1212 (2000).

1. Drugs acting on Proteins

## 1.2. Cofactor Site Blockers

**Many "diffusing" cofactors like NAD, ATP, GTP, CoA are critical for living cells and are used by a large number of proteins in humans and pathogens. This immediately raises the question of whether or not the binding pockets for these cofactors are good drug targets.**

**Positive aspects of such cofactor binding areas are:**

- **They extend usually quite far away from the "true" active site (where bond breaking and making takes place). Hence these cofactor binding sites tend to differ more than active sites among proteins and hence they offer likely opportunities for *selectivity* between species (but see below!);**
- **They tend to be rather large molecules containing ring systems and hence bind to rather large and somewhat hydrophobic pockets. Hence these sites offer opportunities for high *affinity*.**

**At the same time, on the negative side:**

- **Many identical cofactors are used by many different proteins in humans and hence selectivity among human proteins might be difficult to obtain.**

# An example of

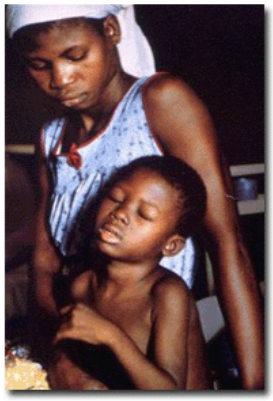
# Structure-based Design of

# Selective Inhibitors

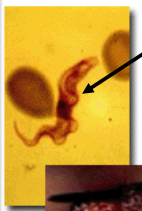
**Target:**
**the enzyme GAPDH**
**from**
**the sleeping sickness parasite,**
**a "Trypanosomatid"**

## Sleeping Sickness

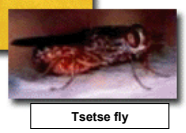
a.k.a "African Trypanosomiasis"



Lumbar puncture
for diagnosis of parasites in CNS



Blood stream
form of
parasite



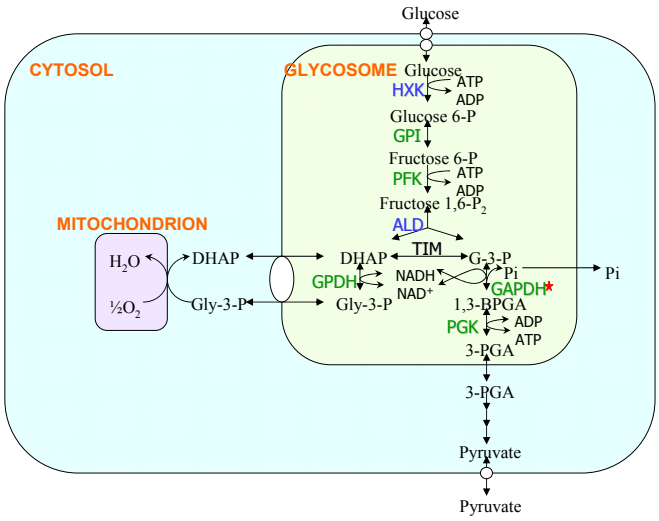
Tsetse fly

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.

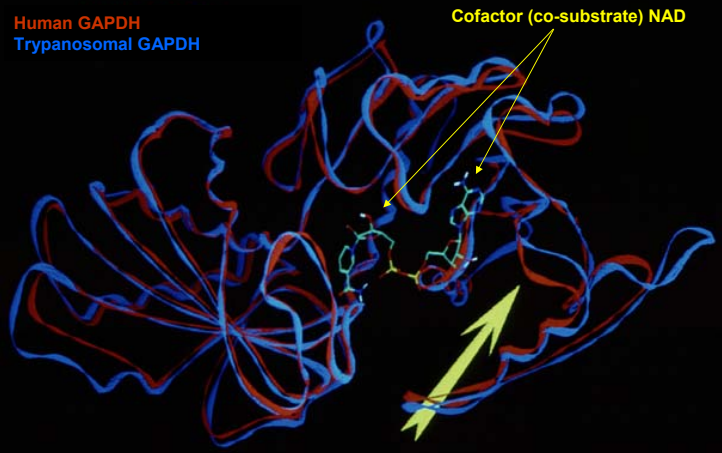
With thanks to Wes Van Voorhis

# 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)

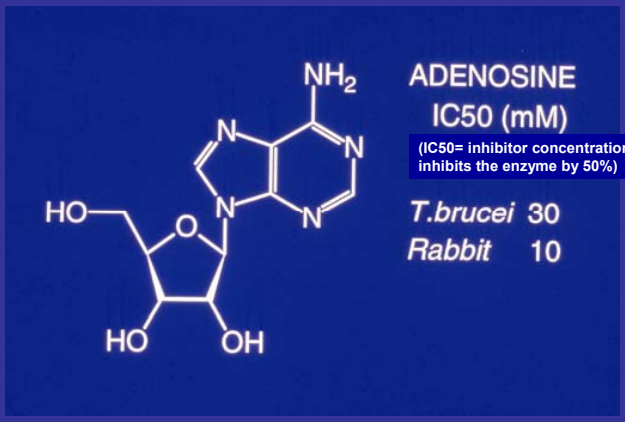



# Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the Sleeping Sickness Parasite and the human host




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
IC50 (mM)

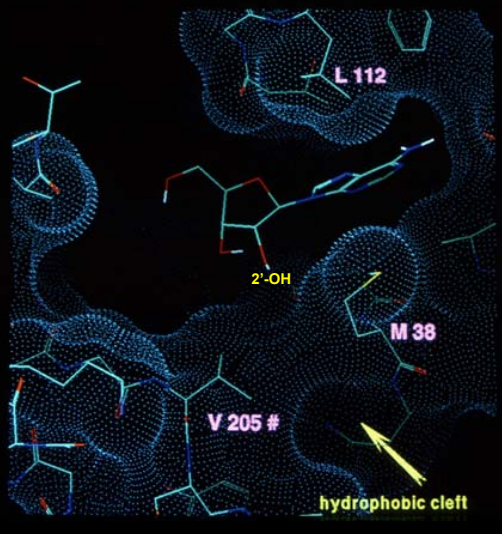
(IC50= inhibitor concentration which inhibits the enzyme by 50%)

*T.brucei* 30
*Rabbit* 10

- 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 slighty *worse* than the mammalian enzyme.

## Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

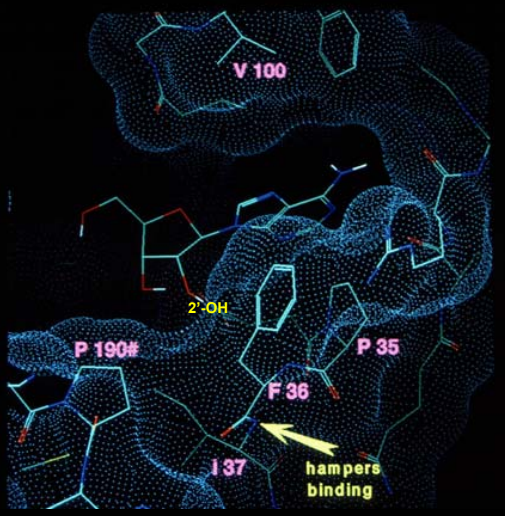
Sleeping sickness parasite GAPDH : Hydrophobic Groove near 2'OH of Adenosine




Fred Vellieux
Christophe Verlinde
Fred Opperdoes
Paul Michels

## Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

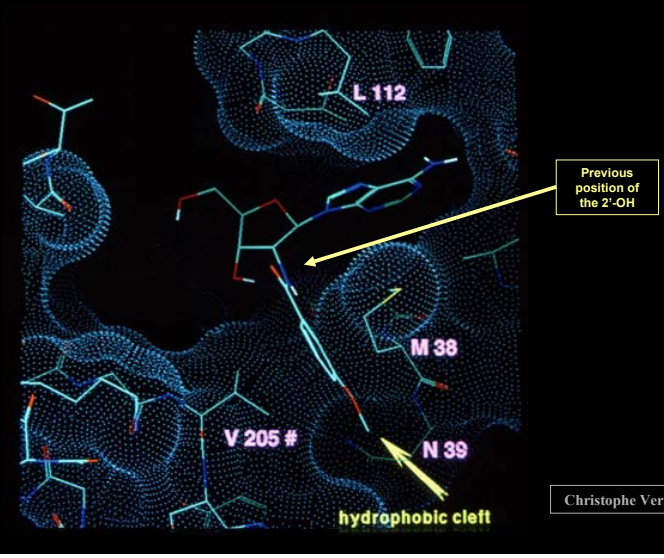
Human GAPDH : NO groove near 2'OH of Adenosine




Randy Read
Christophe Verlinde
Herman Watson

## Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

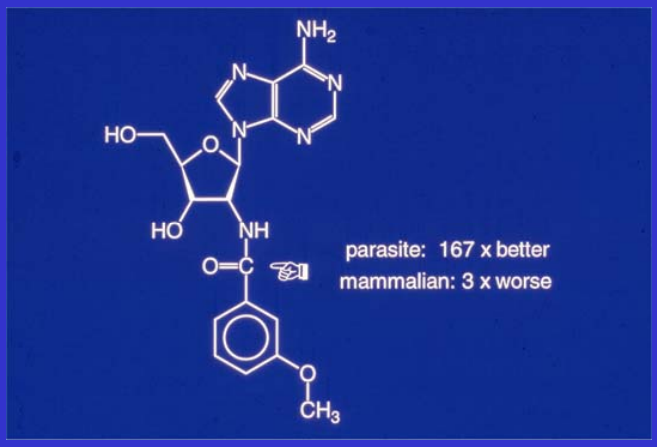
Sleeping Sickness parasite GAPDH : Substituent Modeled in Hydrophobic Groove near 2'OH of Adenosine



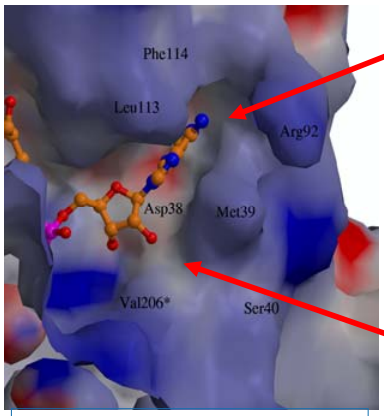

Christophe Verlinde

## Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

### Selectivity of Structure-based Designed Inhibitors




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




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

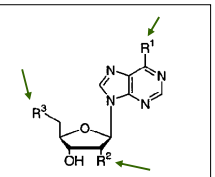
Hydrophobic Groove

**Structure leads to "Targeted Combinatorial Chemistry" to fill the grooves optimally**

Hydrophobic Groove

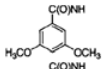
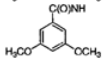
•Note: *Leishmania mexicana* GAPDH is ~77% sequence identical to *Trypanosoma brucei* GAPDH and all residues in the region of interest are identical in these two pathogenic "Trypanosomatids". So these two enzymes are used interchangeably.

## 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.

### A small and focused combinatorial library

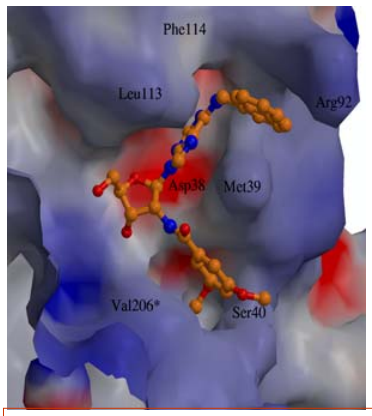
| Compound | $R^1$ | $R^2$ | $R^3$ | $IC_{50}$ (μM) |
|---|---|---|---|---|
| 35 | $NH_2$ | OH |  | 250 |
| 36 | $NH_2$ | OH |  | 250 |
| 41 |  | OH |  | inactive |
| 42 |  | OH |  | inactive |
| 48 | $NH_2$ |  |  | 100 |
| 49 | $NH_2$ |  |  | 60 |

[a] Inactive = inactive at 50 μM.

($IC_{50}$= inhibitor concentration which inhibits the enzyme by 50%)

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.**

Stephen Suresh Antonysami

1. Drugs acting on Proteins

## 1.2. Cofactor Site Blockers (Ctd.)

**Kinase inhibitors are currently a popular way to arrive at inhibitors.**

**The anti-cancer drug Gleevec binds to the ATP binding pocket of the aberrant Bcr-Abl kinase. This the gene product of the so-called Philadelphia chromosome, formed by a reciprocal translocation between chromosome 9 and 22. The resultant hybrid kinase is part Bcr-kinase and part Abl-kinase.**

**The drug is, however, only important for a quite rare cancer: chronic myelogenous leukemia (CML).**

Druker and Lyndon, J. Clinical Investgation 105:3-7 (2000)
See also: Noble Science 303: 1800 (2004) for a general discussion of structure based protein kinase inhibitor design.

1. Drugs acting on Proteins

## 1.3. Receptor Site Binders

**Cell surface receptors**

Many proteins, circulating in human beings, secreted by pathogens, or located on the surface of cells from host and pathogen, are "receptor binding molecules". These are designed to recognize with very high specificity other molecules called "receptors" (which are proteins or saccharides or small molecules). Such receptor-ligand interactions do not involve enzymatic reactions, and offer therefore no opportunity for transition state analogues, but still are often very interesting drug targets.

Several receptor binding proteins from human pathogens recognize oligosaccharide receptors at the cell surface. One such protein is cholera toxin which recognizes the glycolipid ganglioside GM1 at the surface of epithelial cells of the host's digestive tract. Interfering with this toxin:receptor interaction is an attractive avenue towards developing anti-cholera compounds.

Viruses have to enter cells in a highly specific and well-orchestrated way. Compounds interfering with viral protein-human receptor interactions are very interesting from a drug development point of view. Such interactions are not always very extensive, as seen for instance in the case of HIV gp120 interacting with the primary receptor CD4 on the surface of human T cells. The interacting surfaces are about 750-800 $Å^2$ on each of these proteins while some cavities occur at this interface as well. A compound called "BMS488043" is a small molecule which prevents binding of HIV gp120 to CD4 on the human target cell. It is a promising oral antiviral agent

Cholera Toxin and receptor binding inhibitors: (Fan et al., IJMM 294, 217-223 (2004).
Gp120-CD4: Kwong *et al.*, *Nature* 393, 648-659 (1998) & Castagna et al , Drugs 65:879-904(2005)

1. Drugs acting on Proteins

## 1.3. Receptor Site Binders

**CNS receptors**

• There is an entirely different class of receptors - those occurring in the synapse and other parts of the central nervous system. Well-known examples are Dopamine-, Histamine-, Serotonin-, Arginine-receptors. Many existing drugs function as agonists and antagonists of these receptors.

• Very famous are the so-called **"G-coupled receptors"** (GPCRs). Very recently the first three-dimensional structure have been reported for one of these receptors. (Human beta-2 adrenergic receptor (Cherezov et al, Science 318:1258-1265 (2007); Rasmussen et al, Nature 450: 383–387 (2007); Rosenbaum et al, Science 318: 1253-1254 (2007)) This will probably allow for many SBDD studies on GPCR's in the coming days, years, and decades.

**Human proteins interacting with human cell surface receptors**

Many circulating proteins interact with cell surface receptors. Examples are human growth hormone, insulin, etc. In certain cases, like growth defects or diabetes, one might wish to either interfere with such interactions, by e.g. developing compounds which mimic the circulating proteins.

1. Drugs acting on Proteins

## 1.4. Conformational Change Affectors

**Many proteins are designed such that they undergo significant conformational changes which are crucial for their functioning in living organisms. Some of these conformational changes are quite small, such as the motion of a 10-residue loop during catalysis by triosephosphate isomerase. Other conformational changes are almost unbelievably large such as, for instance, those occurring in Elongation Factor Tu (EFTu).**

**Obviously, preventing these sophisticated flexible proteins from undergoing their proper conformational changes is a way to block the action of these proteins and treat certain diseases.**

## 1. Drugs acting on Proteins

### 1.4.1. "Conformational Change Preventers (CCPs) "

**Preventing Hexameric gp41 helix bundle formation**

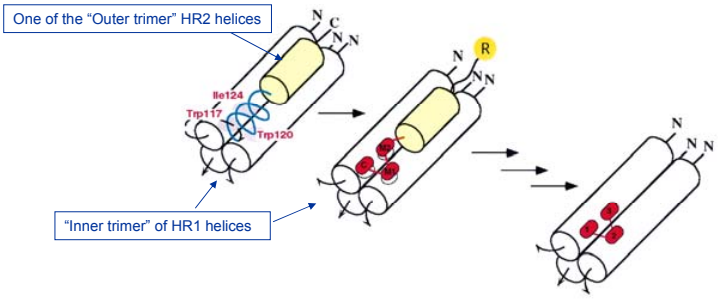
A typical example of the development of **"late CCPs"** are two reports on compounds which inhibit the final stage of a **large conformational change to be undergone by gp41** when fusing together the membranes of HIV and the T cell receptor. Here the target area is a hydrophobic pocket of an "inner" HR1 helix trimer which, at the end of the membrane fusion event, has to be occupied by hydrophobic groups from an "outer" HR2 helix packing against this inner trimer of helices. Small molecules which bind to this pocket and are able to prevent the outer helix from finding its binding site, prevent membrane fusion and thereby thwart the attempts of the HIV virus to enter the T cell.

Interesting papers (Eckert, et al. *Cell* 99, 103-115 (1999) and Ferrer, et al. *Nat. Struct. Biol.* 6, 953-960 (1999)) describe Structure-Guided Combinatorial Chemistry and Biochemistry approaches. These papers show the power of structural information for the early stages of the lead discovery process.

## 1. Drugs acting on Proteins

### 1.4.1. "Conformational Change Preventers (CCPs) " (ctd.)

**Preventing Hexameric gp41 helix bundle formation**




A structure-based combinatorial approach to identify inhibitors of gp41-mediated viral entry. A fragment of the gp41 outer helix peptide is attached to the bead (R) and used to target a three-position non-peptide library into a hydrophobic cavity (purple ovals) on the surface of the inner coiled coil (middle). C, M1 and M2 (red ovals) represent the three library positions — cap, monomer 1 and monomer 2, respectively. The cavity is occupied in the original gp41 structure by residues Trp 117, Trp 120, and Ile 124 (left). Ultimately, the nonnatural elements in the three library positions will be optimized to produce an inhibitor without the native peptide sequence (right).

Ferrer et al.. *Nat. Struct. Biol.* **1999**, *6*, 953-960.

## 1. Drugs acting on Proteins

### 1.4.1. "Conformational Change Preventers (CCPs) " (ctd.)

**HIV gp41 Inhibitor screening**

**STRATEGY:**

**From HR2 Peptide**

**to**

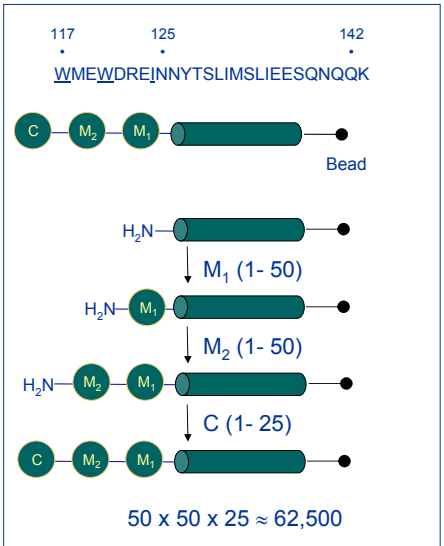
**Peptide-plus-synthetic**

**to**

**Synthetic Ligands**

**by**

**Combinatorial Chemistry**




Ferrer et al.. *Nat. Struct. Biol.* **1999**, *6*, 953-960.



## 1. Drugs acting on Proteins

### 1.4.1. "Conformational Change Preventers (CCPs) " (ctd.)

**Preventing Hexameric gp41 helix bundle formation**

**A conceptually simpler approach was very successful:** Wild et al PNAS 91:9770-9774(1994) explored peptides as preventers of the conformational change in gp41, occurring after g9120 has bound it its receptor CD4.

This led to a **peptide that mimics the HR2 helix of gp41**, and thought to act by binding into the grooves of the HR1 trimeric helical bundle of gp41. The peptide corresponds to gp160 residues 643-678.

This peptide, with an N-terminal acetyl group and a C-terminal amide is the new drug **Enfuvirtide**, previously known as DP178, then as T-20.

It is no surprise that the drug of about 36 amino acids and a molecular weight of about 4000, is not an oral drug. Enfuvirtide needs to be administered subcutaneously by injection.

Discovery: Wild etal PNAS 91:9770-9774(1994)
Description: Greenberg et al Rev. Med. Virol. 14: 321-337 (2004)

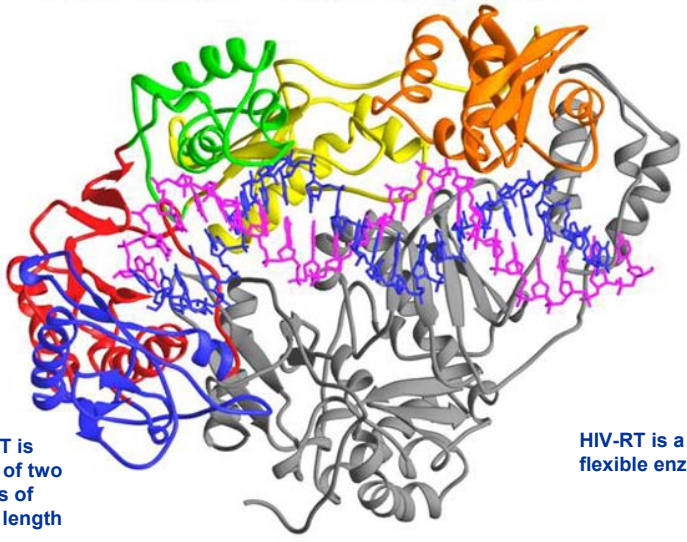
1. Drugs acting on Proteins

1.4. Conformational Change Preventers

# HIV Reverse Transcriptase

## Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI's)

HIV-1 Reverse Transcriptase in Complex with RNA/DNA



HIV-RT is consists of two chains of different length

HIV-RT is a very flexible enzyme

From: http://home.ncifcrf.gov/hivdrp/rt/PPT_f1A.html – 20 Feb 2006

1. Drugs acting on Proteins

1.4. Conformational Change Preventers

## Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI's)

**NNRTIs were discovered by drug screening programs.**

**Subsequent crystal structures showed that:**

1. **Nonnucleoside inhibitors NNRTIs bind in a hydrophobic pocket of HIV-1 RT near the polymerase active site.**
2. **In the available RT structures, there is no NNRTI binding pocket if there is not a bound NNRTI.**
3. **NNRTI binding distorts the enzyme. Both the nucleic acid and the incoming dNTP can still be bound; however, an NNRTI-inhibited enzyme cannot carry out the chemical step of the polymerization reaction.**
4. **NNRTI's are chemically very diverse – yet bind in the same pocket/region!**

1. Drugs acting on Proteins

1.4. Conformational Change Preventers

## Allosteric site binders

**Many proteins, often enzymes but e,g. also hemoglobin, are regulated by compounds which bind at "allosteric sites" which are often far removed from active sites.**

**Binding of allosteric effectors at allosteric sites causes conformational changes which activate or inactivate the enzyme's activity.**

**Hence targeting allosteric sites and thereby preventing conformational changes, most likely by locking the protein in one state, leads to de-regulation of the enzyme's activity.**

**This is another approach to designing compounds which interfere with the proper functioning of a key protein in a target cell.**

## 1.5. Protein Assembly Inhibitors

**Many proteins function as part of one, or several, multi-protein complexes. Hence, compounds which would interfere with the formation of multi-protein complexes are in many cases potential drugs since the formation of a stable multi-protein assembly is often essential for the proper functioning of components of that assembly.**

**There is often a major problem, however. Many protein-protein interfaces of multi-protein complexes are extensive** (Jones & Thornton. *Proc. Natl. Acad. Sci. USA* 93, 13-20 (1996)) **and it is often hard to come up with small compounds which disrupt the affinity of one protein for another protein in such well-defined multi-protein complexes.**

**However, not *all* protein interfaces are extensive. An interesting case is ribonucleotide reductase (RNR) - which shows the feasibility of Protein Assembly Inhibitors.**

1. Drugs acting on Proteins

## 1.5. Protein Assembly Inhibitors

Interference with protein-protein interactions Is not often seen in actual cases and in structure-based drug design projects is often looked at quite skeptically. There are good reasons for doing so. After all, **protein-protein interfaces resolved by structural biologists look quite formidable because of :**
**(i) their large interface;**
**(ii) often large affinity of the two protein partners;**
**(iii) the lack of crevices in the protein-protein interface.**

However, one might argue that only multimeric assemblies with extensive interfaces and a quite favorable free energy of association have been studied so far. These are, of course, way easier to investigate than weak interactions. So one might argue that in the future there will be increasing opportunities for interfering with protein-protein interactions

Wells and McClendon (Nature 450:1001-1009 (2007)) reviewed the state of the art in this field. The number of success stories is still quite small. But in some instances certain small molecules have been surprisingly successful in preventing two protein molecules to associate.

## 1. Drugs acting on Proteins

### 1.5 Protein Assembly Inhibitors (ctd)

## Ribonucleotide Reductase (RNR)

In 1986, two reports appeared describing the serendipitous(!) discovery of peptides which prevented the assembly of the R1 and R2 components of Herpes Simplex Virus **RNR** (Dutia, et al. *Nature* **321**, 439-441 (1986); Cohen, et al. *Nature* **321**, 441-443 (1986)). Since RNR's are crucial for many, if not all, organisms, and since this $R_1R_2$ interface is likely to differ among organisms, this discovery has been followed up - with very interesting results.

For instance, Liuzzi *et al.* (*Nature* **372**, 695-698 (1994)) reported the peptidomimetic BILD 1263 which is 200,000 times more potent in preventing HSV RNR assembly than the starting pentapeptide AVVNDL.

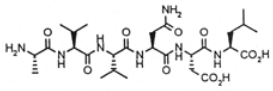
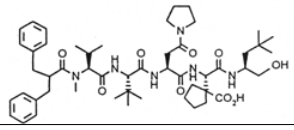
Moss *et al.* (*J. Med. Chem.* **39**, 4173-4180 (1996)) described BILD 1357, a significantly more potent antiviral compound than BILD 1263. Another peptidomimetic, BILD 1633SE, was reported to be active against acyclovir-resistant Herpes Simplex Virus (HSV) infections (Duan, et al. *Antimicrob. Agents Chemother.* **42**, 1629-1635 (1998)).

Hence, in favorable cases development of protein assembly inhibitors is possible.

## 1. Drugs acting on Proteins

### 1.5 Protein Assembly Inhibitors (ctd)

## Ribonucleotide Reductase (RNR)

| Compound | Structure | IC50 (nM) |
|---|---|---|
| AVVNDL |  | 58,000 ± 14,000 |
| BILD 1263 |  | 0.30 ± 0.08 |

Structure and potency of **HSV RNR subunit-association inhibitors**.

Compounds were tested in a competitive binding assay as described previously. $IC_{50}$ (inhibitor concentration to give 50% inhibition) values represent the mean ±s.d. of 3 determinations. None of the compounds inhibited human RNR up to 400 μM.

A potent peptidomimetic inhibitor of HSV ribonucleotide reductase with antiviral activity in vivo.
Liuzzi, M., Deziel, R., Moss, N., Beaulieu, P., Bonneau, A. M., Bousquet, C., Chafouleas, J. G., Garneau, M., Jaramillo, J., Krogsrud, R. L. *et al.* (1994). *Nature* **372**, 695-698.

## 1. Drugs acting on Proteins

### 1.5 Protein Assembly Inhibitors (ctd)

## Tumor Necrosis Factor-α (TNFα )

A small molecule inhibitor of Tumor Necrosis Factor-α has been described with a remarkable mode of action.

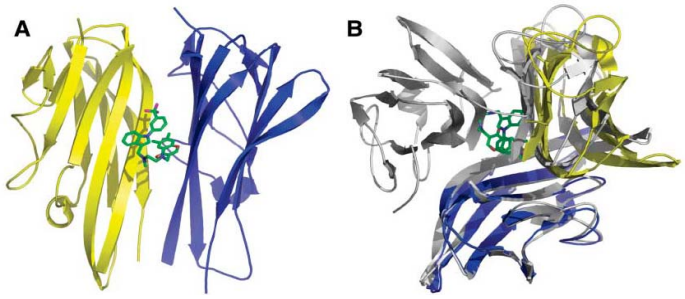
It binds to the intact biologically TNFα *trimer* and accelerates subunit dissociation to rapidly inactivate the cytokine. It appears even to form a new TNFα *dimer* (!) with the compound lodged between two subunits.

He et al," Small molecule Inhibition of TNF-α", Science, 310: 1022-1025 (2005).

## 1. Drugs acting on Proteins

### 1.5 Protein Assembly Inhibitors (ctd)

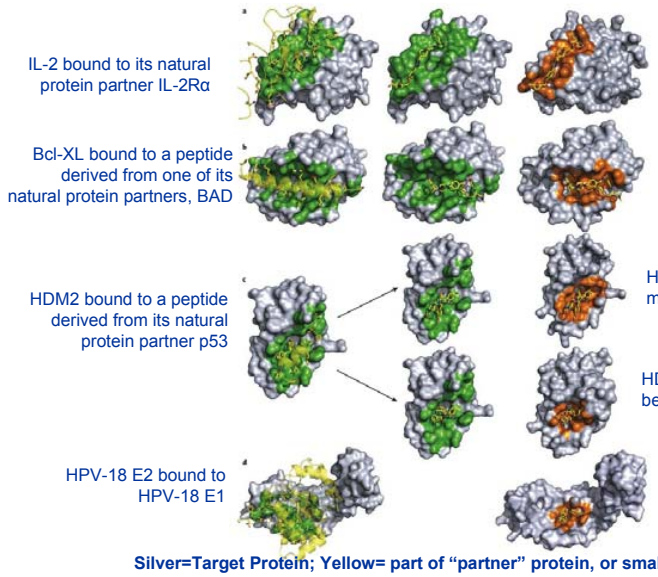
## Tumor Necrosis Factor-α (TNFα )




**(A) X-ray crystallography structure of the TNF-a dimer–compound complex. (B) Shift in subunit orientation within the TNF-a dimer–compound complex. Superposition of the TNF-a trimer structure (gray) with the TNF-a dimer–compound complex structure (yellow-blue) shows a slight widening in the angle between the subunits at the compound binding site.**

He et al," Small molecule Inhibition of TNF-α", Science, 310: 1022-1025 (2005).

## 1.5 Protein Assembly Inhibitors (ctd)




**Silver=Target Protein; Yellow= part of "partner" protein, or small molecule;**
**Green= contact surface partner protein; Orange= contact surface small molecule**

Wells and McClendon Nature 450:1001-1009 (2007)

### 1. Drugs acting on Proteins

# 1.6. Protein Assembly Stabilizers

## Picorna Virus Capsid Stabilizers

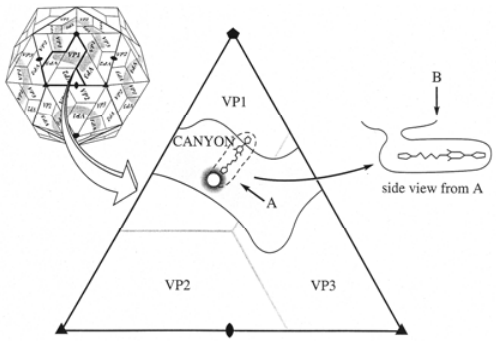
For several multi-protein assemblies it is crucial that they ***dis*assemble** at exactly the proper moment. One class of proteins in this category consists of the protein capsid of many viruses. Of great interest are a series of compounds which bind near the receptor-binding region of the picorna viruses. Several of these viruses, especially rhinovirus, the causative agent of the common cold, have been structurally well characterized with respect to the binding site of Disassembly Inhibitors.

Several of these are called "WIN compounds" and their most unusual binding site in a pocket underneath the "canyon" floor of rhinovirus has been studied in detail (Zhao, et al. *Structure* 4, 1205-1220 (1996)). These compounds stabilize the virus capsid, thereby prevent uncoating of the RNA, thereby interrupting the life cycle of the virus.

## 1. Drugs acting on Proteins

### 1.6. Protein Assembly Stabilizers

**Picorna Virus Capsid Stabilizers**



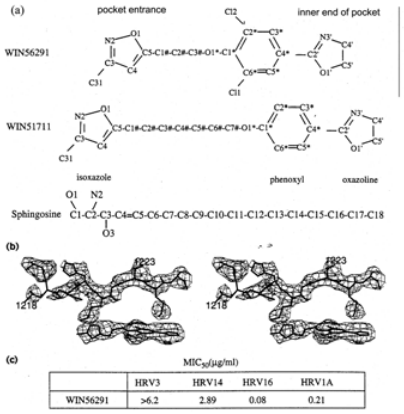

Diagrammatic view of picornavirus with enlargement of one icosahedral asymmetric unit showing the outline of the canyon and the entrance to the antiviral-binding pocket. The protomeric assembly unit (which differs from the geometric definition of the asymmetric unit) is shown in heavy outline on the icosahedron.

Zhao, R., Pevear, D. C., Kremer, M. J., Giranda, V. L., Kofron, J. A., Kuhn, R. J. & Rossmann, M. G. (1996). Human rhinovirus 3 at 3.0 Å resolution. *Structure* **4**, 1205-1220.


## 1. Drugs acting on Proteins

### 1.6. Protein Assembly Stabilizers

**Picorna Virus Capsid Stabilizers**


| | HRV3 | HRV14 | HRV16 | HRV1A |
|---|---|---|---|---|
| WIN56291 | >6.2 | 2.89 | 0.08 | 0.21 |

**Capsid-binding antiviral compounds.**

**(a) Structures of WIN56291, WIN51711 and a sphingosine molecule.**

**(b) Electron density for the HRV3-WIN56291 complex showing the flexible GH loop and the WIN compound conformation.**

**(c) $MIC_{50}$ values for WIN56291.**


Zhao, R., Pevear, D. C., Kremer, M. J., Giranda, V. L., Kofron, J. A., Kuhn, R. J. & Rossmann, M. G. (1996). Human rhinovirus 3 at 3.0 Å resolution. *Structure* **4**, 1205-1220.

1. Drugs acting on Proteins

1.6. Protein Assembly Stabilizers

## Tubulin Stabilizers

Another multi-protein system is tubulin, the major component of microfilaments, which are made and unmade continuously in cells. Tubulin is one of the sites of action of **Taxol (tamoxafen),** an anti-cancer drug. This compound binds to specific pockets of tubulin and stabilizes the tubulin polymer.

(Nogales, et al. *Nature* 391, 199-203 (1998);
Downing & Nogales. *Curr. Opin. Struct. Biol.* 8, 785-791 (1998)).

1. Drugs acting on Proteins

# 1.7. Protein-Protein Glues

These differ from Protein Disassembly Inhibitors in that these "glues" bring proteins together which should not interact with each other under normal circumstances.

Examples of Protein-Protein Glues are:

(1) Cyclosporin A which promotes the association of cyclophilin with calcineurin;
(2) FK506 which promotes the association of FKBP12 with calcineurin; and,
(3) Rapamycin which promotes the association of FKBP12 and "Target of Rapamycin" (TOR).

- Cyclophilin and FKBP12 are peptidyl-prolyl isomerases;
- Calcineurin is a two-component phosphatase, made of an A and a B subunit;
- TOR, and family members, are protein kinases.

The end result of the action of Cyclosporin and FK506 is that calcineurin, a protein phosphatase, can no longer function properly By itself, cyclosporin inhibits the peptidyl prolyl isomerase activity of cyclophilin, just like FK506 inhibits the prolyl isomerase activity of FKBP12. Yet, it is the formation of the ternary complexes with calcineurin, thereby blocking the action of the latter enzyme, which leads to a decrease of IL-2 production in T cells, and to immunosuppression.

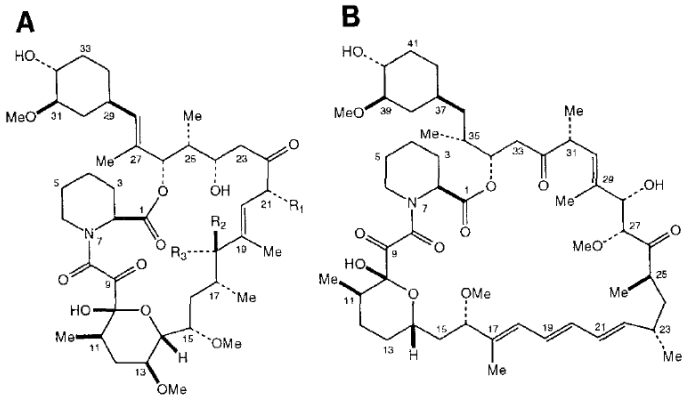
These compounds, cyclic peptides, produced by microorganisms, are a defense mechanism against competing fungi. Remarkably, these targets are so well conserved in humans that these compounds can be used as immunosuppressants.

Hemenway & Heitman. Cell Biochem. Biophys. 30, 115-151 (1999);Arndt, Mucribiol. 145:1989-2000 (1999); Wang&Heitman, Genome Bioloy 6:226 (2005);
(Kissinger, et al. *Nature* **378**, 641-644 (1995); Griffith, et al. *Cell* **82**, 507-522 (1995)).

## 1. Drugs acting on Proteins

### 1.7. Protein-Protein Glues (ctd.)

**Two immunosuppressants which act as protein-protein glues**




FK-506 | Rapamycin

From: Griffith Cell 82:507-522 (1995)

## 1. Drugs acting on Proteins

### 1.7. Protein-Protein Glues (ctd.)

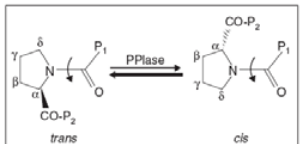
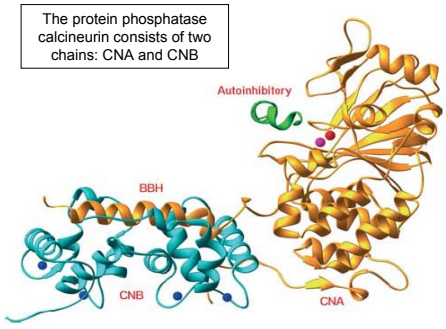



Figure 1
A schematic illustration of the *trans* and *cis* isomers of the peptide bond between proline (on the left of each structure shown) and another amino acid ($P_1$, on the right). The interconversion between the two forms is catalyzed by cyclophilins and other peptidyl-prolyl isomerases (PPIases) [7]. The carbon atoms of the proline are indicated by Greek letters; $P_2$ indicates a third amino acid on the other side of the proline. The peptide bond has some double-bond character and is planar.

The immunosuppressants inhibit the peptidyl prolyl isomerases cyclophilin or FKBP12, but this is **NOT** their key property in immune modulation.

From: Ping Wang and Joseph Heitman – *Genome Biology* 2005, 6:226

The protein phosphatase calcineurin consists of two chains: CNA and CNB



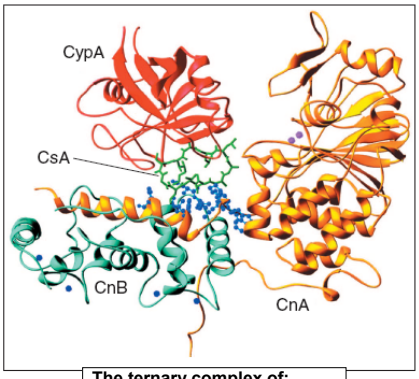

**A ribbon diagram of the unliganded calcineurin**. Golden = CNA, cyan =CNB, green = autoinhibitory loop. The red, pink, and blue spheres represent $Zn^{2+}$ , $Fe^{3+}$ and $Ca^{2+}$, respectively. (PDB entry 1AUI.)

Ke, H. & Huai, Q. Structures of calcineurin and its complexes with immunophilins-immunosuppressants. *Biochem Biophys Res Commun* **311**, 1095-102 (2003).

## 1. Drugs acting on Proteins

# 1.7. Protein-Protein Glues

**Figure 2**
The structure of the ternary complex between the drug cyclosporin A (CsA), human cyclophilin A (CypA) and human calcineurin [37]. The CsA-CypA binary complex lies at the base of the helical arm of the catalytic subunit of calcineurin (CnA) that binds the regulatory subunit calcineurin (CnB); it nestles in a hydrophobic groove in intimate contac with both subunits, at a region unique to calcineurin and not found in other phosphatases, and this intimate contact gives the interaction high specificity. Reproduced with permission from [37].




**The ternary complex of:**
**Calcineurin : CnA and CnB**
**Cyclosporin A : CsA**
**Cyclophilin A : CypA**

Figure from: Ping Wang and Joseph Heitman *Genome Biology* 2005, 6:226
Structure from: HUai et al PNAS 99: 12037-12041 (2002)

## 1. Drugs acting on Proteins

### 1.7. Protein-Protein Glues

**FK506: An immunosuppressant which acts as a protein-protein glue**

Another example of a glue is FK506 forming the calcineurin:FKBP12:FK506 complex.

The eventual effect of the glue FK506 on calcineurin is a surprise.

In the calcineurin:FKBP12:FK506 complex (hereafter called the "ternary complex") the active site of the phosphatase calcineurin is **neither** blocked by the protein FKBP12 **nor** by the macrolide FK506. Neither has the active site of the phosphatase been deformed upon ternary complex formation. Actually, the activity of calcineurin in the ternary complex towards certain small molecules is *enhanced* compared with uncomplexed calcineurin! But the real substrates of calcineurin are proteins, such as NF-AT.

**Upon binding of FKBP12:FK506 (as well as of cyclophilin:cyclosporin A) to calcineurin, the protein substrates can no longer reach the active site of the phosphate,** hence these proteins remain phosphorylated, with dire consequences for the signaling pathways in the T cell.

Griffith Cell 82,507-522, (1995)
Kissinger, et al. *Nature* **378**, 641-644 (1995)
Huai Q, Kim HY, Liu Y, Zhao Y, Mondragon A, Liu JO, Ke H *Proc Natl Acad Sci USA* 2002, **99:**12037-12042
Jin L, Harrison SC: *Proc Natl Acad Sci USA* 2002, **99:**13522-13526.

## 1.8 Protein-DNA Recognition Blockers

No "pure" example of this mode of action of a drug has been discovered so far by the writer of this slide. The paucity of examples of small molecules interfering with protein DNA interactions has probably several reasons.

First, these interactions often involve very large interfaces and hence it is not easy for a small molecule to compete with very tight interactions (however, not all protein-DNA interactions need always to be very tight).

Second, protein-DNA interfaces tend to bring a large number of charged and polar groups together (although the complex of the TATA box binding protein (TBP) with DNA appears to be an exception). Small molecules which are able to bind to such charged and polar interfaces, might themselves have to be highly polar and, therefore, have difficulty in passing cellular membranes.

A paper aiming for blocking such a DNA-protein interaction (See "A ß-sheet peptide inhibitor of E47 dimerization and DNA binding" (Ghosh, I. & Chmielewski, J. *Chem. Biol.* 5, 439-445 (1998)), arrives indeed at molecules which inhibit DNA binding by E47. However, it turns out to be a peptidic dimerization-preventer of E47 which is homodimeric.

1. Drugs acting on Proteins

## 1.8 Protein-DNA Recognition Blockers (ctd.)

Since many DNA-regulating proteins are dimers, interference with the dimerization process is in principle an interesting way to interfere with DNA-binding (Ghosh, I. & Chmielewski, J. *Chem. Biol.* 5, 439-445 (1998)) . However, the dimer interfaces are often quite large (more accurately: the affinities of the subunits for each other is often quite large), and to obtain small molecules with have enough affinity to interfere with this dimerization process might be a challenge.

A favorable factor is that some of the DNA-binding regulators might only be present in small quantities in the cell, and therefore the law of mass action can perhaps in some cases work in favor of a compound which is able to enter a cell at quite high concentrations.

# 2. Drugs Acting on DNA

**Compounds binding to DNA and then**
**(i) altering the structure of DNA, or**
**(ii) interfering with access to the minor or major grooves of DNA**
**is a great way of causing troubles to cell given the fact that DNA is so crucial and that so many proteins interact with DNA.**

**And indeed, there are many compounds of great medical importance which have DNA as target. There are:**

**2.1 Intercalators**
**Many exist. They alter the structure of DNA dramatically**

**2.2 Minor Groove Binders**
**Several exist. Selectivity is being increased by several approaches such as pursued by the Dervan group. Access by proteins to the minor groove is prevented.**

**2.3 Covalent Complexes**
**Cisplatin is an anti-cancer agent which covalently binds to adjacent guanines in a single strand of duplex DNA, or may act by cross-linking different duplexes.**

**2.4 Antisense nucleotides**
**Being tried experimentally. Are highly charged. Modifications of phosphate groups increase uptake.**

## 2. Drugs acting on DNA

### 2.1. Intercalators

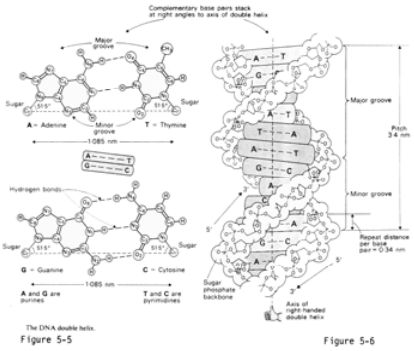



Figure 5-5 Figure 5-6

**Canonical Structure of B-DNA with stacked adjacent base-pairs .**

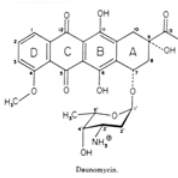
When looking at the structure of DNA (above) is not obvious at all where hydrophobic pockets would reside which can by used by Ligands for high-affinity binding. It appears, however, that a remarkable conformational change of the DNA duplex creates a marvelous hydrophobic binding pocket for the so-called "intercalators".

Figure probably from Rees and Sternberg – or from R.E. Dickerson's Sc. American article.

## 2. Drugs acting on DNA

### 2.1. Intercalators

**DNA Intercalators** occupy the place of a base pair! The phosphate ribose backbone of DNA accommodates this extra planar molecule quite readily!



Daunomycin.

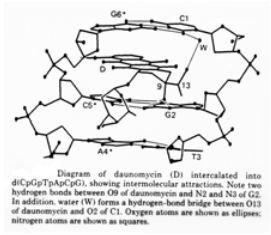


Diagram of daunomycin (D) intercalated into d(CpGpTpApCpG), showing intermolecular attractions. Note two hydrogen bonds between O9 of daunomycin and N2 and N3 of G2. In addition, water (W) forms a hydrogen-bond bridge between O13 of daunomycin and O2 of C1. Oxygen atoms are shown as ellipses; nitrogen atoms are shown as squares.

A dramatic conformational change creates a terrific hydrophobic binding site for planar molecules.

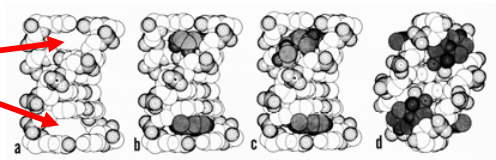


FIG. 2. Space-filling drawings of the daunomycin–d(CpGpTpApCpG) complex. In *a*–*c*, the molecular 2-fold axis is horizontal and in the plane of the paper. (*a*) Right-handed helical hexanucleotide duplex by itself. (*b*) Hexanucleotide and aglycone without the acetyl group on C9. Note the way O9 projects down over G2 from the aglycone at the top. (*c*) Hexanucleotide and complete daunomycin. The amino sugar extends into the minor groove. (*d*) The complex as viewed into the minor groove down the molecular 2-fold axis perpendicular to the paper. The amino sugars and ring A fill most of the minor groove. The C4 methoxy group can be seen extending through into the major groove. Heavy stippling, daunomycin atoms; open circles, carbon; dotted circles, nitrogen; solid circles, oxygen; radial-spiked circles, phosphorus. Hydrogen atoms are not shown.

Note the very significant conformational change the duplex DNA undergoes upon binding the intercalator daunomycin. **It is obvious that the degree of specificity is quite limited since very few sequence-specific functional groups of the DNA are contacted.**

Quigley, G.J. et al. Molecular structure of an anticancer drug-DNA complex: daunomycin plus d(CpGpTpApCpG). *Proc Natl Acad Sci U S A* **77**, 7204-8 (1980).

## 2. Drugs acting on DNA

### 2.1. Intercalators

**Linked Intercalators:**

We saw earlier that intercalators are "creating" their own binding sites in DNA by replacing base-pairs. However, selectivity, that is selectivity for a specific DNA base *sequence*, is a problem for such intercalators.

There are however, interesting variations on this intercalator theme possible. Such as creating a bivalent intercalator where the linker could in principle be optimized for selectivity. Here the problem of too large a molecular weight quickly arises, however.

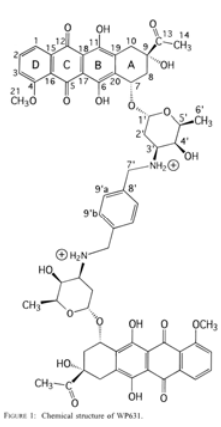
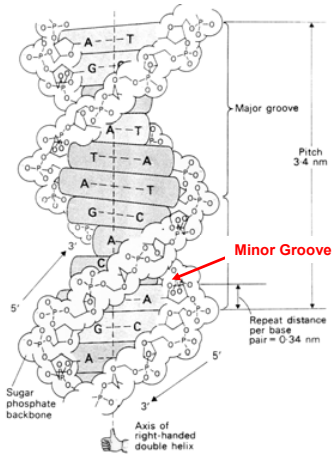


FIGURE 1: Chemical structure of WP631.

Hu, G.G. et al. Structure of a DNA-bisdaunomycin complex. *Biochemistry* **36**, 5940-6 (1997).

## 2. Drugs acting on DNA

### 2.2. Minor Groove Binders




A surface of DNA where sequence-specific contacts can in principle be made quite easily is the minor groove. Hence, minor groove binders (MGBs) have received considerable attention over the last few decades.

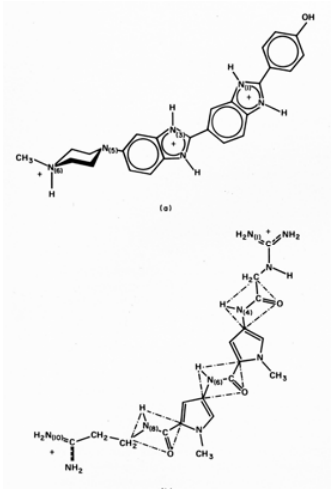
Actually in the always ongoing battle between species, the minor groove was already the target of certain natural occurring MGBs such as netropsin.

The mode of binding of this compound and of several synthetic compounds proved to be very interesting.

And *two* binding modes appeared to occur in this class of Ligands.

## 2. Drugs acting on DNA

### 2.2. Minor Groove Binders



Pjura, P.E., Grzeskowiak, K. & Dickerson, R.E. Binding of Hoechst 33258 to the minor groove of B-DNA. *J Mol Biol* **197**, 257-71 (1987).

**Two Minor Groove Binders:**

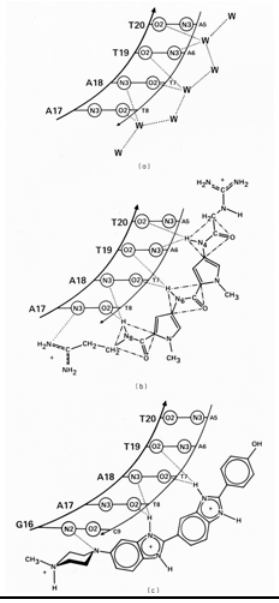
- **Netropsin**
- **Hoechst 33528**

**Note their crescent shape ("banana shape"), coupled with hydrogen bonding donors along their concave edge. They also contain some planar aromatic ring systems.**

**Both also have positive charges.**

**These are typical characteristics of Minor Groove Binders.**

## 2.2 Minor Groove Binders bound to DNA




**Schematics of the minor groove in free B-DNA of sequence C-G-C-G-A-A-T-T-C-G-C-G, and in its complexes with 2 drug molecules. Sugar-phosphate backbones are the curved sides of a ladder with rungs for base-pairs. PyrimidineO-2 and purine N-3 (N-2 for guanines) are circled on the rungs.**

**Letters W indicate water oxygens. Dotted lines mark hydrogen bonds, although some of these are long by conventional standards.**

**(a) Spine of hydration (indicated by "W"'s) in the drug-free B-DNA helix.**

**(b) Complex with netropsin in which amide NH groups replace the 1st shell waters of the spine.**

**(c) Complex with Hoechst 33258, with a residual continuation of the spine of hydration beyond the drug binding site.**

Pjura, P.E., Grzeskowiak, K. & Dickerson, R.E. Binding of Hoechst 33258 to the minor groove of B-DNA. *J Mol Biol* **197**, 257-71 (1987).

## 2.2 Minor Groove Binders and DNA

**While for quite some time it was thought that the netropsins and homologs bound as a monomer in the minor groove, it appeared that several also bound as side-by-side dimers in this groove!**

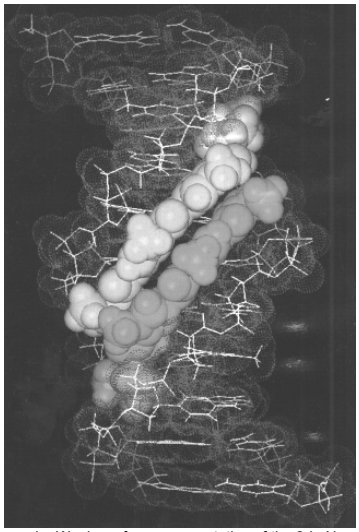
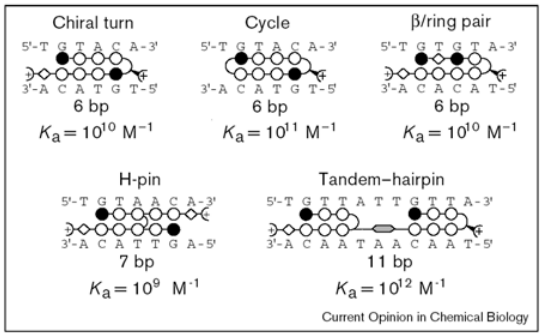


Fig. 4. van der Waals surface representation of the 2-ImN-d(GCATCACTCGG).d(CCGAGTCATGC) complex obtained by energy refinement using semiquantitative distance constraints derived from NOESY.

Mrksich, M. et al. Antiparallel side-by-side dimeric motif for sequence-specific recognition in the minor groove of DNA by the designed peptide 1-methylimidazole-2-carboxamide netropsin. *Proc Natl Acad Sci U S A* **89**, 7586-90 (1992).

## 2.2 Minor Groove Binders to DNA

The discovery of the side-by-side binding mode of certain MGB-s led to the design of linked, or kinked, MGB variants by the Dervan group.




MGB design by the Dervan group:

Novel polyamide-DNA biding motifs with equilibrium constants ($K_a$) shown.

Chiral turn: amino-substitution at the α-position of the γ turn residue leading to enhanced binding affinity;

Cycle: cyclic poylamides show higher affinity with respect to the hairpin structure;

β/ring pair: the β/ring pair relaxes the ligand curvature. In some cases, the binding affinity of the β/ring pair–polyamides is significantly higher than that of the ring/ring analog;

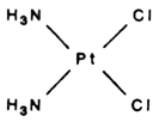
H-pin: compared to their non-linked analogs, H-pins exhibit higher binding affinity.

Tandem-hairpin: tandem polyamides recognize large DNA sequences with excellent binding affinity and specificity.

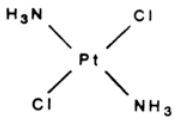
Dervan, P.B. & Burli, R.W. Sequence-specific DNA recognition by polyamides. *Curr Opin Chem Biol* **3**, 688-93 (1999).

## 2. Drugs acting on DNA

### 2.2. Covalent crosslinkers




*cis*-DDP
(cisplatin)

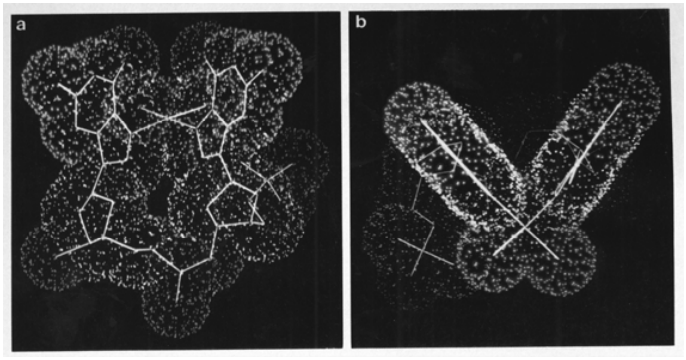



*trans*-DDP

Cis-Diamino dichloro platina ( *cis*-DDP or “cisplatin”) is a frequently used anti-cancer drug, which reacts with guanine bases of DNA. It causes cross links which are fatal for cells. Interestingly, the isomer *trans*-DDP (see above) is much less active.

## 2. Drugs acting on DNA

### 2.2. Covalent crosslinkers




Two views of the van der Waals spheres of
Cis-Platina crosslinking two adjacent guanidine bases.

Sherman, S. E., Gibson, D., Wang, A. H.-J. & Lippard, S. J. (1985). X-ray structure of the major adduct of the anticancer drug cisplatin with DNA: *cis* -[Pt(NH3)2{d(pGpG)}].

*Science* **230**, 412-417.

## 3. Drugs Acting on Protein-DNA Complexes (Turning Proteins into "Poisons" )

**Many of the compounds acting on proteins have to take a very large percentage of the target protein out of commission in order to have a significant effect (although this percentage has not often been truly quantitated is this writer's guess).**

**There are more clever ways to act as drug, however. For instance, when the effect of a drug on a relatively small percentage of the Target biomacromolecules in the cell can be converted into a devastating cascade of events then this looks like an effective way to interrupt the machinery of a targeted cell.**

3. Drugs acting on Protein-DNA complexes

## Turning Proteins into Poisons (Ctd)

The **"topoisomerase poisons"** fall into this category. Topoisomerases are ubiquitous enzymes that solve topological problems generated by key nuclear processes such as DNA replication, transcription, recombination, repair, chromatin assembly, and chromosome segregation.

**There are two types of topoisomerases:**

**Type I** enzymes are monomeric and transiently break one strand of duplex DNA, allowing for single-step changes in the linking number of circular DNAs (the number of times one strand of DNA crosses the other).

**Type II** enzymes are dimeric and break both strands of a duplex to generate a gate through which another region of dsDNA can be passed, resulting in linking number changes in steps of two.

Type I and type II enzymes are fundamentally different in both mechanism and cellular function. The medical importance of these enzymes is underscored by the fact that they are the specific targets of many promising anti-cancer and antimicrobial drugs.

3. Drugs acting on Protein-DNA complexes

## Turning Proteins into Poisons (Ctd)

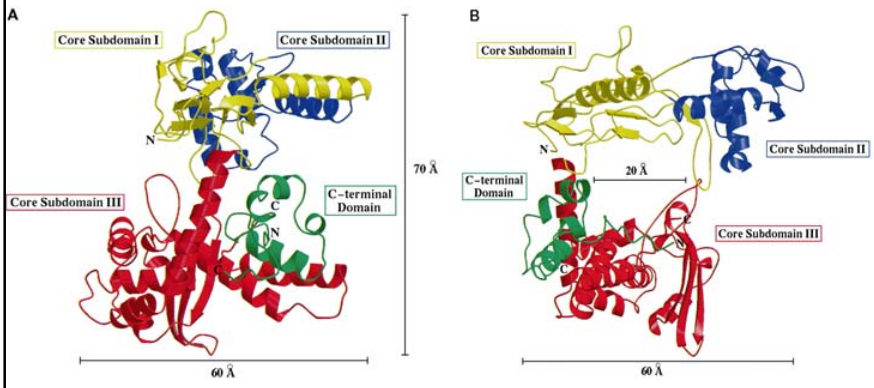
Both eukaryotic type I and type II topoisomerases are the target of certain drugs called **"topo poisons". These compounds utilize the fact that the topoisomerases form a transient covalent link between one, or two, tyrosine residues with one, or two, strands of DNA. Topo poisons are compounds which prolong the life time of this transient covalent complex creating thereby a major road block on the DNA highway.** RNA polymerases, for instance, do not appreciate meeting such a large (one to several hundred kDa) protein obstacle covalently attached on what should be a splendid road to travel on. The precise downstream effects of topo poisons are not fully unraveled but they are certainly very unhealthy for the cell.

The figures on the next pages illustrate the structure and function of human topoisomerase I, and show how the **anti-cancer drug camptothecin binds to the topo I-DNA complex.**

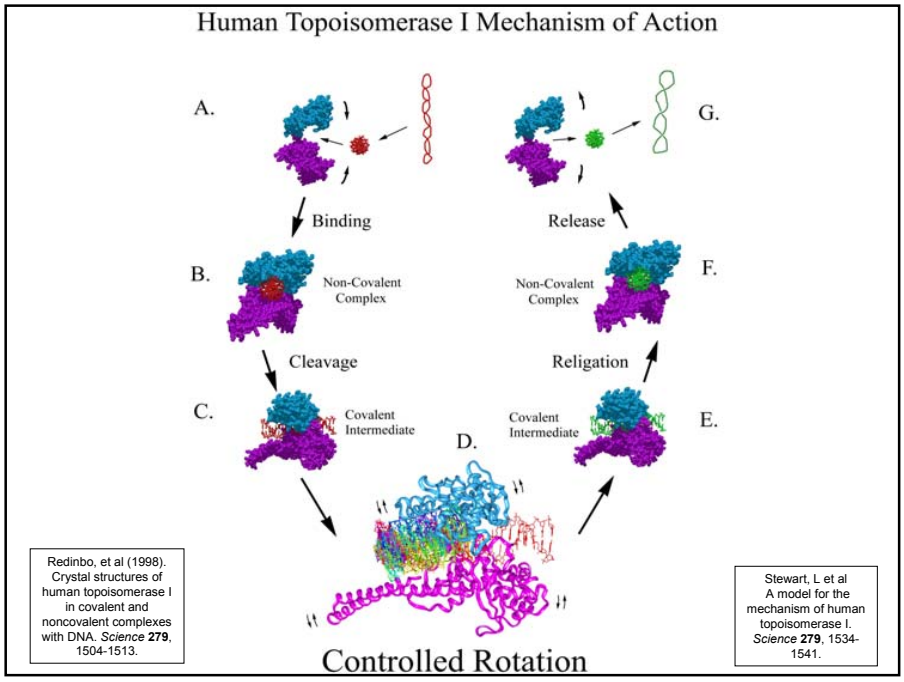
(Redinbo, et al. *Science* 279, 1504-1513 (1998); Stewart, et al. *Science* 279, 1534-1541 (1998);
Redinbo, et al. *Curr. Opin. Struct. Biol.* 9, 29-36 (1999)
Staker et al & Stewart,L. PNAS 99, 15837-15392 (2002).

# Turning Proteins into Poisons (Ctd)

## The structure of Human Topoisomerase IB




Redinbo, M. R., et al *Science* **279**, 1504-1513. (1998).
Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA.




Redinbo, et al (1998). Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science* **279**, 1504-1513.

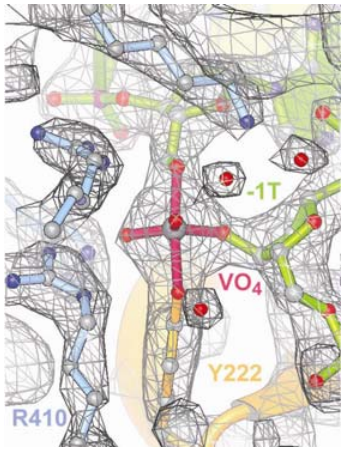
Stewart, L et al A model for the mechanism of human topoisomerase I. *Science* **279**, 1534-1541.

# Turning Proteins into Poisons (Ctd)

## Human Topoisomerase IB

**During the reaction cycle Topo I forms a transient covalent complex between a tyrosine and a 5'OH of DNA.**

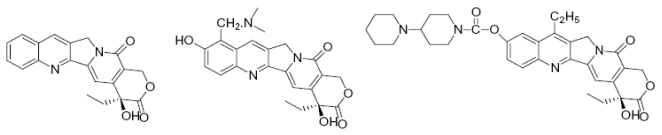
**The transition state is captured (right) by using vanadate to mimick the pentavalent transient phosphoryl group**




Davies, D.R., Mushtaq, A., Interthal, H., Champoux, J.J. & Hol, W.G.J. The structure of the transition state of the heterodimeric topoisomerase I of *Leishmania donovani* as a vanadate complex with nicked DNA. *J. Mol. Biol.* **357**, 1202-1210 (2006).

# Turning Proteins into Poisons (Ctd)

## Human Topoisomerase IB



1, CPT 2, Topotecan 3, Irinotecan

Figure 1. CPT and its analogues.

**Camptothecin (CPT), Topotecan and Irinotecan:**

**Three "Topo IB Poisons",**

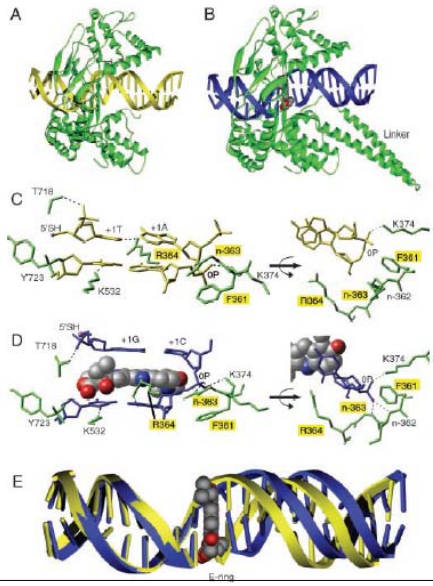
**i.e.**

**Three compounds which stabilize the covalent Topo-DNA complex.**

From: Srivastava, V., Negi, A.S., Kumar, J.K., Gupta, M.M. & Khanuja, S.P. Plant-based anticancer molecules: a chemical and biological profile of some important leads. *Bioorg Med Chem* **13**, 5892-908 (2005).

## CAMPTOTHECIN-TOPO-DNA complex

CPT nestles itself like an intercalator in between two base pairs; the result is that the 5'-OH of the cleaved strand can now no longer reach the 3'-phosphate and replace the covalent bond with Topo's catalytic tyrosine. The ~ 100 kDa enzyme is now a roadblock on the DNA highway next to a single strand break!!




Topo I-DNA complexes

Active Site without Camptothecin (CPT)

Active Site with Camptothecin (CPT) bound

Camptothecin intercalates near active site

The mechanism of topoisomerase I poisoning by a camptothecin analog
Staker et al & Stewart,L. PNAS 99, 15837-15392 (2002)

## CAMPTOTHECIN-TOPO-DNA complex

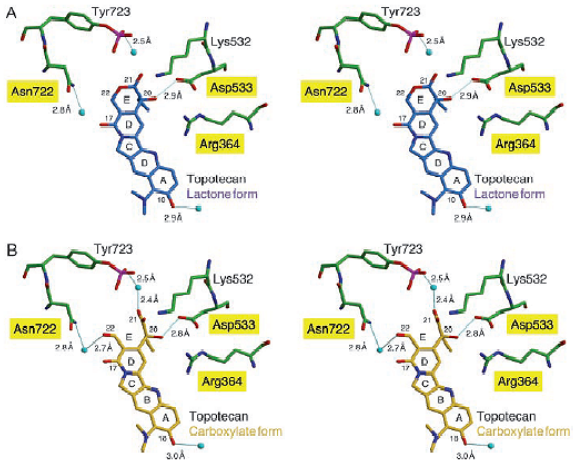



Fig. 3. Stereoview of Topotecan binding. The Topotecan interactions with protein side chains for the lactone (*A*, blue) and carboxylate (*B*, gold) forms of the drug are depicted. Hydrogen bonds (predicted by contact distance and geometry of the refined atomic positions) are shown as solid blue lines, and the calculated interatomic distances are indicated. Labels for residues that result in resistance to CPT when mutated are highlighted in yellow. The oxygen atoms of water molecules are depicted as light blue spheres. Protein side chains are green, and noncarbon atoms are colored red for oxygen, blue for nitrogen, and magenta for phosphorus. The DNA and protein side chains that do not interact with Topotecan have been omitted from the figure.

"The mechanism of topoisomerase I poisoning by a camptothecin analog"
Staker et al & Stewart,L. PNAS 99, 15837-15392 (2002)

## Turning Proteins into Poisons (Ctd)

**Also, the bacterial type II topoisomerases are the target of several drugs. The most successful topo II inhibitors are quinolones such as nalidixic acid, oxolinic acid and, in particular, ciprofloxacin These appear to be excellent antibacterial agents.**

**These compounds are stabilizing the enzyme-DNA covalent complex which, when such a complex occurs at a position ahead of replication forks, leads to quick arrest of DNA replication, irreversible DNA damage and cell death**

**In other words they are typical DNA poisons, very smart drugs. In addition, ciprofloxacin is likely to act on two related type II bacterial topoisomerases, which are called (to make life easy) "gyrase" and "topoisomerase IV".**

**Acting on *two* targets is very clever from the point of view of drug resistance.**

Shen & Chu. *Curr. Pharma. Design* 2, 195-208 (1996)

Maxwell. *Trends Microbiol.* 5, 102-109 (1997); Drlica & Zhao. *Microbiol. Mol. Biol. Rev.* 61, 377-392 (1997)

## 4. Drugs Acting on RNA

**RNA carries out numerous critical functions in living organisms, hence blocking crucial RNA molecules is a perfect way to prevent, e.g., pathogens to multiply rapidly. With more and more functions for RNA being discovered, including the so-called micro-RNA's, interfering with proper folding, processing or action of these RNAs is in principle a good strategy.**

See also : "Targeting RNA with Small-Molecule Drugs: Therapeutic Promise and Chemical Challenges." Gallego,J and Varani, G., Acc Chem Res 34, 836-843 (2001).

# 5. Drugs Acting on RNA-Protein Complexes ("Ribosome Tunnel Blockers")

**The ribosome is the protein-synthesizing machinery with ribosomal RNA catalyzing the peptide forming reaction, tRNAs bringing in the amino acids and proteins regulating many steps, and providing energy, mainly by GTP synthesis.**

**The ribosome is the target of many compounds developed in the course of evolution during the battle for survival. Many organisms produce compounds which block ribosome action – in very different manners.**

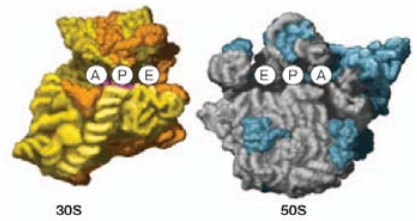
**The large, or "50S", subunit of the ribosome contains a "tunnel" through which the growing polypeptide chain travels to reach the cytoplasm. This tunnel is the target of several antibiotics.**

**In an excellent recent review** (Poehlsgaard, J. & Douthwaite, S. The bacterial ribosome as a target for antibiotics. Nat Rev Microbiol 3, 870-81 (2005)), **the mode of action of antibiotics is discussed on the basis of a wealth of crystallographic information.**

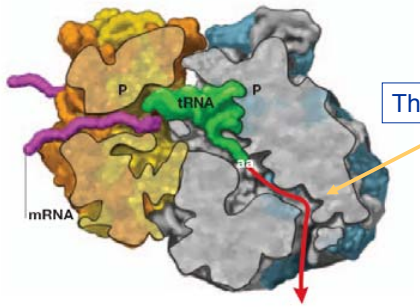
## 5. Ribosome Tunnel Blockers (ctd.)

30S Subunit ("Small Subunit")

50S Subunit ("Large Subunit")




Cross Section




The "Channel"

Poehlsgaard, J. & Douthwaite, S. The bacterial ribosome as a target for antibiotics. *Nat Rev Microbiol* **3**, 870-81 (2005).

## 5. Ribosome Tunnel Blockers (ctd.)

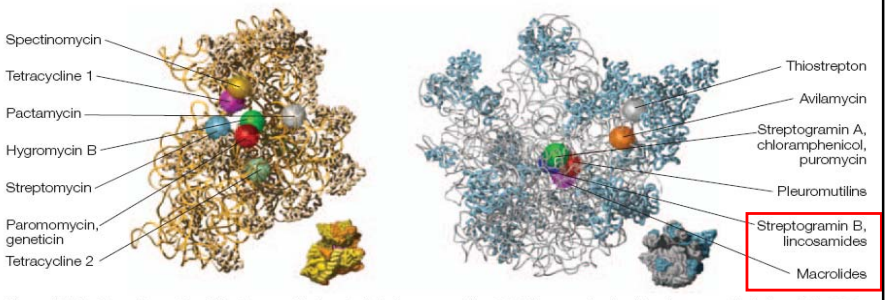



Figure 1 | **Binding sites of antibiotics on the bacterial ribosome.** The 30S ribosomal subunit is shown on the left and the 50S ribosomal subunit is shown on the right. The antibiotic-binding sites were initially determined by biochemical and genetic techniques; subsequently, many sites were revealed in greater detail by X-ray crystallography. At the overlapping sites, antibiotic binding is usually mutually exclusive (for example, for macrolide, lincosamide and streptogramin B compounds), however, streptogramin A and B compounds bind synergistically at adjacent sites. Subunit models are based on the *Thermus thermophilus* 70S ribosome structure[29]. In this figure, for clarity, part of the r-protein L9 has been omitted. Ribosomal RNAs are shown in yellow and grey and r-proteins in bronze and blue.

**The binding sites of macrolide, lincosamide and Streptogramin B(MLSB) antibitocs are located in the 50 subunit tunnel – and are boxed in red.**

Poehlsgaard, J. & Douthwaite, S. The bacterial ribosome as a target for antibiotics. *Nat Rev Microbiol* **3**, 870-81 (2005).

## 5. Ribosome Tunnel Blockers (ctd.)

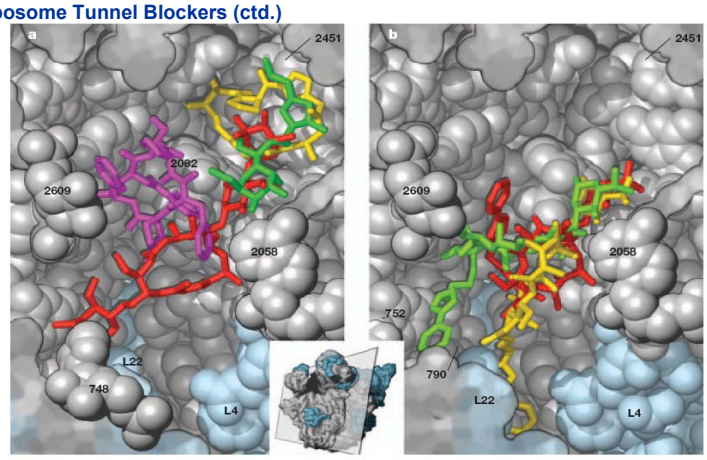



Figure 4 | **Binding sites of macrolide, lincosamide, streptogramin B ($MLS_B$) antibiotics within the 50S subunit tunnel.** **a** | Subunit cross-section showing the upper portion of the tunnel from the peptidyl-transferase centre (at A2451) to the narrow bend at r-protein L22. The subunit structure is drawn from the coordinates of Tu *et al.*[42]; r-proteins are in blue and rRNA is in grey. The relative positions of the binding sites for the macrolide tylosin (red)[28], the streptogramin A compound dalfopristin (yellow), the streptogramin B compound quinupristin (magenta) and the lincosamide clindamycin (green)[42] are shown. Key nucleotides involved in drug contact and resistance are indicated; some of the nucleotides shown (748, 2058 and 2609) are above the plane of the cross section. Methylation or mutation at nucleotide A2058 disrupts contact and confers resistance to all $MLS_B$ compounds, whereas methylation at G748 affects only tylosin[86]. **b** | The same tunnel section showing the conformations of telithromycin seen in *Haloarcula* 2058A-subunits (red)[42] and in *Deinococcus* subunits (yellow)[102]. The structure in green is a model of telithromycin binding derived from chemical protection on *Escherichia coli* ribosomes[118].

Poehlsgaard, J. & Douthwaite, S. The bacterial ribosome as a target for antibiotics. *Nat Rev Microbiol* **3**, 870-81 (2005).

# 6. Drugs acting on Membranes

For Structure Based Drug Design, the "structure" of the membrane bilayer is not an easy, well-defined starting point. Yet, for the sake of compleness, and for inspiration, it is good to know that nature has made quite a large number of molecules used in interspecies warfare, and which are acting on membranes. Sometimes well-defined structures can be obtained of such membrane-function disrupters in action. Such structures might serve as starting points for a next generation of designed compounds.

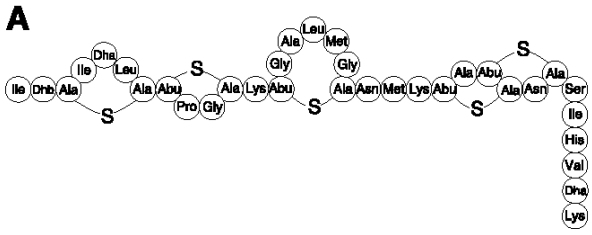
Clearly the issue of specificity is an intriguing one since all membrane bilayers look so very similar - and hence one would expect membrane damaging compounds to be very toxic for every membrane. Yet, in nature compounds acting on specific membranes are very common - even though their mode of action is often ill understood. The specificity issue for one of these pore-forming peptide antibiotics, nisin Z, has been unraveled by Breukink et al., Science 286, 2361-2364 (1999)).

It appears that this 34-residue containing peptide uses Lipid II, a precursor in cell wall synthesis, as an anchor. Actually, the D-Ala-D-Ala peptide of this same Lipid II is the target of the antibiotic vancomycin and, indeed, in the presence of vancomycin, nisin Z is less active in forming pores in Lipid II-containing membranes. So nisin Z apparently contains a "specificity determining region" and a "pore forming region". The precise three-dimensional structure of nisin Z bound to Lipid II and embedded in the membrane still remains to be unraveled.

(Side remark: In spite of its use as a food preservative for almost 50 years, no resistance to nisin has been reported yet!)



## Nisin Z




(A) Primary structure of nisin Z. Dha, dehydroalanine; Dhb, dehydrobutyrine; Ala-S-Ala, lanthionine; Abu-S-Ala, β-methyllanthionine; S, the sulfur atom of the thioether bond.

Breukink, E., Wiedemann, I., van Kraaij, C., Kuipers, O. P., Sahl, H.-G. & de Kruijff, B. (1999). Use of the cell wall precursor Lipid II by a pore-forming peptide antibiotic. *Science* **286**, 2361-364.

## 6. Drugs acting on membranes (Ctd.)

**Since Vancomycin binds to the D-Ala-D-Ala terminal end of the substrate and blocks Nisin Z action, it is highly likely that Nisin Z also recognizes D-Ala-D-Ala.**

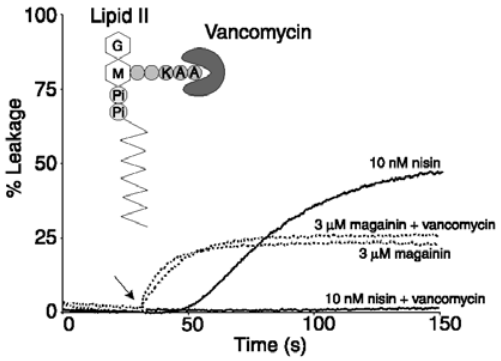



**Fig. 2.** Vancomycin blocks the activity of nisin against *M. flavus* cells. The activity of the peptides was monitored as described for Fig. 1. When present, vancomycin was added 2 min before the addition of nisin or magainin. Washing the cells after treatment with vancomycin gave similar results. The arrow marks the time point of peptide addition. Inset: Schematic structure of Lipid II with vancomycin bound to the COOH-terminal Lys-D-Ala-D-Ala sequence. The structure consists of a membrane-incorporated undecaprenyl moiety to which the amino sugar MurNAc is attached via a pyrophosphate. To the MurNAc (M) a pentapeptide is attached, of which the composition may slightly differ within different bacterial genera. The final subunit of Lipid II is GlcNAc (G). Pi, phosphate.

Breukink, E., Wiedemann, I., van Kraaij, C., Kuipers, O. P., Sahl, H.-G. & de Kruijff, B. (1999). Use of the cell wall precursor Lipid II by a pore-forming peptide antibiotic. *Science* **286**, 2361-364.

# 7. Drugs Acting on Substrates

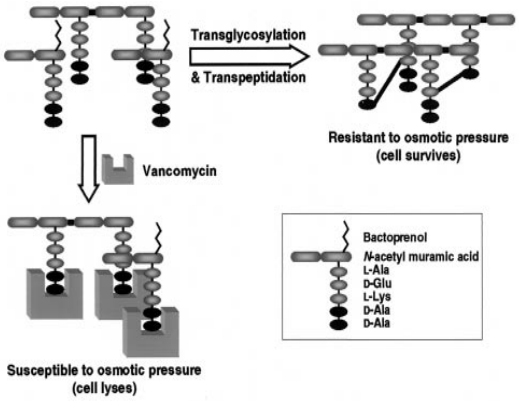
Inhibitors can also act on substrates since preventing proteins or ribozymes or RNA molecules to reach their substrate is in principle an as valid approach as preventing the substrate from reaching the protein or ribozyme. However, the substrates are often flexible and usually have no pocket which can be the binding site of “substrate binders”.

Therefore the number of Drugs acting on Substrates found so far in nature is very small. A famous exception is **Vancomycin** – to be explained on the next slide.

Vancomycin is important as a “Drug of last resort” in cases of multi-drug resistant bacteria not responding to treatment with conventional antibiotics. However, Vancomycin itself appears to be subject to a most unexpected resistance mechanism as we will see at the end of the course.

7. Drugs acting on substrates (Ctd.)

## Mode of Action of Vancomycin




**Vancomycin binds the D-Ala-D-Ala moiety of the growing peptidoglycan and sterically occludes the transglycosylation and transpeptidation steps of cell-wall assembly. The resulting incomplete cell wall yields cells susceptible to lysis through osmotic shock.**

Lessard, I. A. D. & Walsh, C. T. (1999). VanX, a bacterial D-alanyl-D-alanine dipeptidase: Resistance, immunity, or survival function? *Proc. Natl. Acad. Sci. USA* **96**, 11028-11032.

7. Drugs acting on substrates (Ctd.)

## Mode of Action of Vancomycin

The surprising binding mode of vancomycin binding to D-Ala-D-Ala peptidoglycan termini.

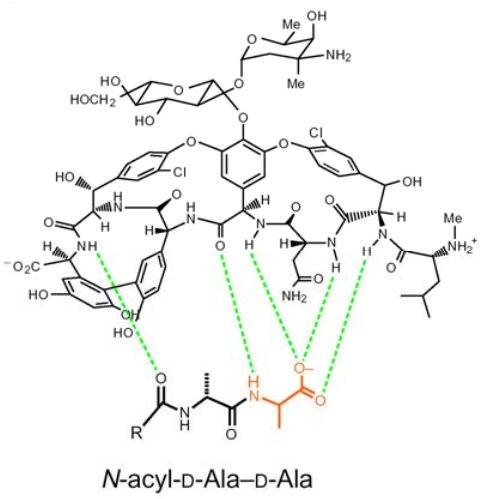
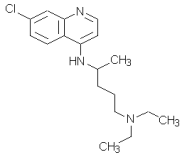



Figure 2a from: Healy, V. L., Lessard, I. A. D., Roper, D. I., Knox, J. R. & Walsh, C. T. (2000). Vancomycin resistance in enterococci: reprogramming of the D-Ala-D-Ala ligases in bacterial peptidoglycan biosynthesis. *Chem. Biol.* **7**, R109-R119.

## 8. Drugs Preventing Small Molecules Aggregation

# Chloroquine



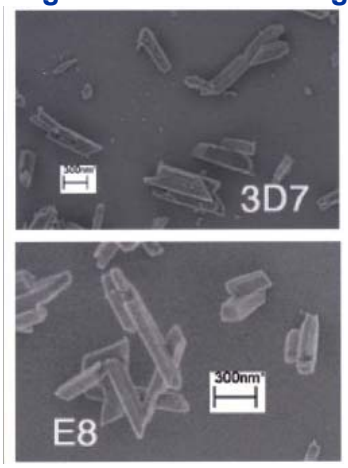

**The malaria parasite infects the red blood cell of the human host and it uses hemoglobin as its sole source of energy. During hemoglobin digestion the heme group comes free and forms a threat to the parasite since the iron ion generates radicals which damage the cell. The formation of a complex of many heme groups into an assembly called "hemozoin", which is no longer able to generate radicals, is thought to be the way in which the parasite is protected from the dangerous effects of heme.**

**Chloroquine has been for decades the drug of choice in the treatment of malaria. Its mode of action is not yet absolutely established but the most widely accepted way of killing parasites is most remarkable.**

**First it "hyperconcentrates" to millimolar levels in the "Digestive vacuole" of the parasite. The major theory about the mode of action of Chloroquine is that the compound interferes with hemozoin formation! This is a truly astonishing mode of action – entirely different from all modes of drug action observed elsewhere.**

**A good account of the history and uncertainties surrounding the mode of action of chloroquine can be found in: Sullivan, Int J Parasitology 32:1645-1653 (2002).**

## 8. Drugs Preventing Small Molecules Aggregation (Ctd.)



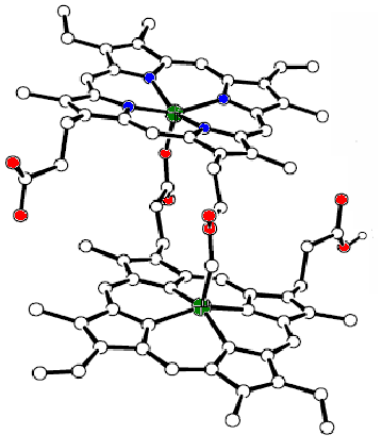

Chloroquine is thought to prevent hemozoin formation

**Field Emission in-lens Scanning Electron Microscopy (FEISEM) view of Hemozoin** – the remnants of hemoglobin after *P.falciparum* has devoured the protein, i.e. the globin part of hemoglobin. The packing of heme groups in the hemozoin aggregates is thought to render the heme innocuous, i.e. the Fe-ion does not generate dangerous radicals any more.

Akompong et al. J Biol chem 277: 28923- 28933 (2002)

## 8. Drugs Preventing Small Molecules Aggregation (Ctd.)

Chloroquine is thought to prevent hemozoin formation by preventing stacking of heme groups



The advantage of interfering with hemozoin formation is that the target is "immutable" which provides advantages from a drug resistance perspective

**The structure of malaria pigment beta-haematin**.

Pagola et al Nature. (2000) 404:307-310