VIROLOGY

Research, Development and Applications of Antiviral Agents 1

EPIDEMIOLOGY OF VIRAL DISEASES

Prevention and control

Vaccines:
the proven best defense
against viruses

Vaccines have been very successful in preventing some viral diseases; however, they provide modest to no therapeutic effect in individuals that are already infected

Prevention and control

Consequently, the second arm of antiviral defense has been the development and use of molecules capable of preventing an infection or stopping it once started.

Antiviral drugs:
small molecules that block virus replication

EPIDEMIOLOGY OF VIRAL DISEASES

Prevention and control

Antiviral drugs:
small molecules that block virus replication

However, despite 50 years of research, our arsenal of antiviral drugs remains dangerously small.

Only less than 50 antiviral drugs are available on the market

Most against HIV, HBV, HCV, Herpesviruses (persistent infections)

THE CONUNDRUM OF ANTIVIRAL CHEMOTHERAPY:
So Much Knowledge, So Few Antivirals

• Safety is the overriding concern in anviral drug discovery! Compounds interfering with virus growth often have adverse severe effects on the host:

- Side effects are common (unacceptable)
- Every step in viral replicative cycle engages host functions

• No broad-spectrum of antiviral agents are currently available

THE CONUNDRUM OF ANTIVIRAL CHEMOTHERAPY:
So Much Knowledge, So Few Antivirals

- **Another serious problem for antiviral discovery:**
  - Many acute infections are of short duration and by the time the indiduals feel ill the virus is no longer replicating and indeed may have already been cleared from the host. Thus, too late to impact clinical disease.
  - Antivirals drugs for these viruses must be given early in infection or prophylactically to populations at risk.
  - Safety issues: giving drugs to healthy people not wise

- The lack of rapid diagnostic tests has hampered development of antiviral drugs

- Many medically important viruses are difficult or impossible to grow in laboratory (eg. HBV, HPV), too dangerous (Ebola).

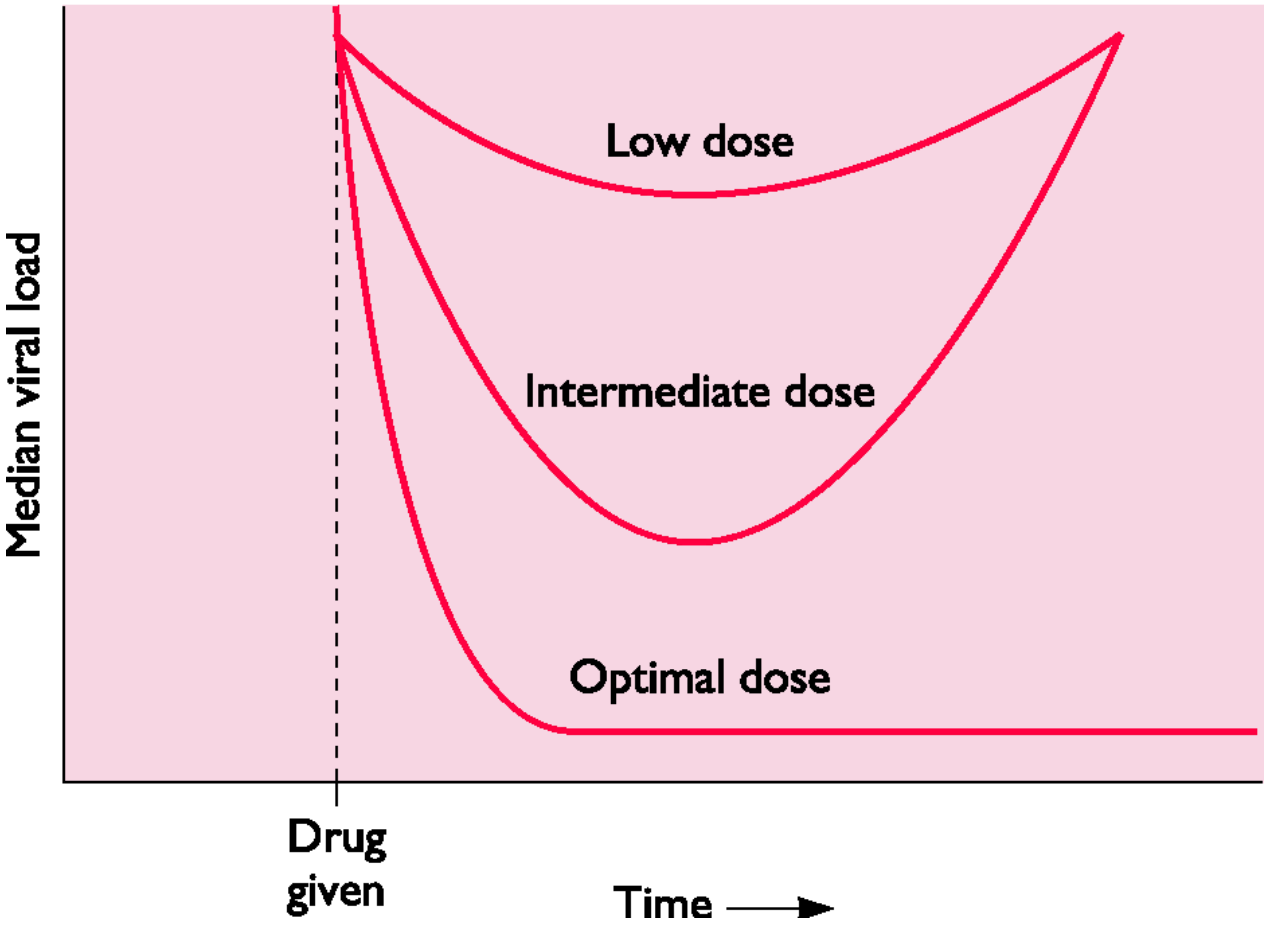
THE CONUNDRUM OF ANTIVIRAL CHEMOTHERAPY:
So Much Knowledge, So Few Antivirals

An unappreciated reason may be the most important

- Antiviral compounds must be virtually 100% efficient in blocking virus growth.
- A compound must block virus replication completely!
- So, it must be extremely potent!!!
- Partially inhibition is not acceptable for an antiviral: even a modest viral replication in the presence of a drug provides the opportunity that drug–resistant mutants arise.
- Makes drug discovery expensive.

The problem of viral resistances

REPLICATION=MUTATION



If the administered drug dose does not completely suppresses viral replication, mutant viruses will be selected

ANTIVIRAL RESEARCH HISTORY

- The first modest search for antiviral drugs occurred in 1950s.
  - *Chemists looked at derivatives of the sulfonamide antibiotics.*
  - *Synthesis of thiosemicarbazones actve against poxviruses.*
  - *Smallpox was still a major threat after WWII.*
- 1960s and 1970s: “blind screening” programs to find chemicals with antiviral activity spurred on by successes in the treatment of bacterial infections with antibiotics.
- No attempt to focus discovery on a virus or a virus-specific mechanism
- Random chemicals and natural product mixtures tested for ability to block replication of a variety of viruses in cell culture systems.
- Considerable effort, very little success: one exception!
- Symmetrel (Amantadine) approved late 1960s for Influenza A virus infections. Mechanism of action discovered only in 1990s.

Antiviral discovery today

Modern antiviral discovery

The advent of modern molecular virology, recombinant DNA technology and sophisticated chemistry make possible the TARGETED DISCOVERY

- Essential viral genes cloned, expressed in genetically tractable organisms, purified, and analyzed in atomic detail.
- Life cycles of most viruses known, targets for intervention can be generalized.
- Modern technology allows inhibitors to be found even for viruses that cannot be propagated in cell culture

Blind screening  Is no longer attractive

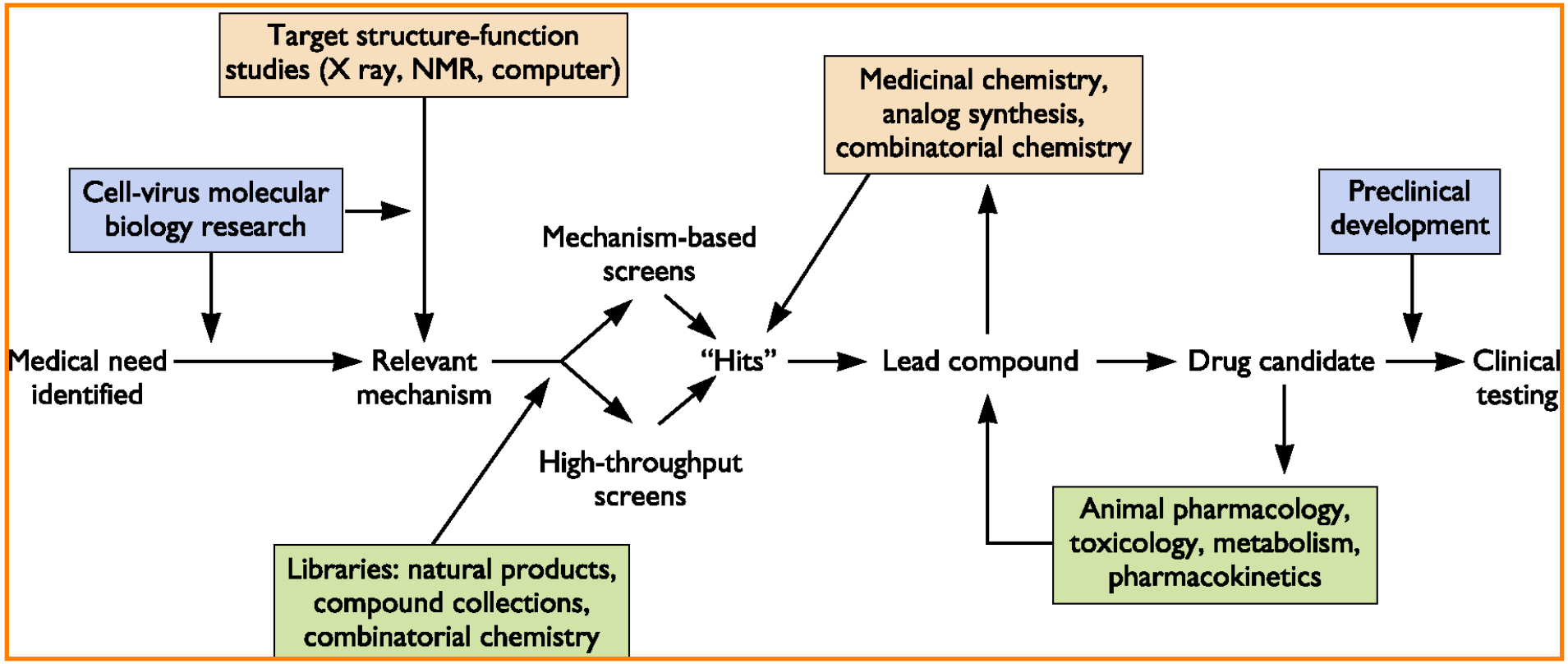
Antiviral discovery today

Blind screening  Is no longer attractive

Modern antiviral discovery

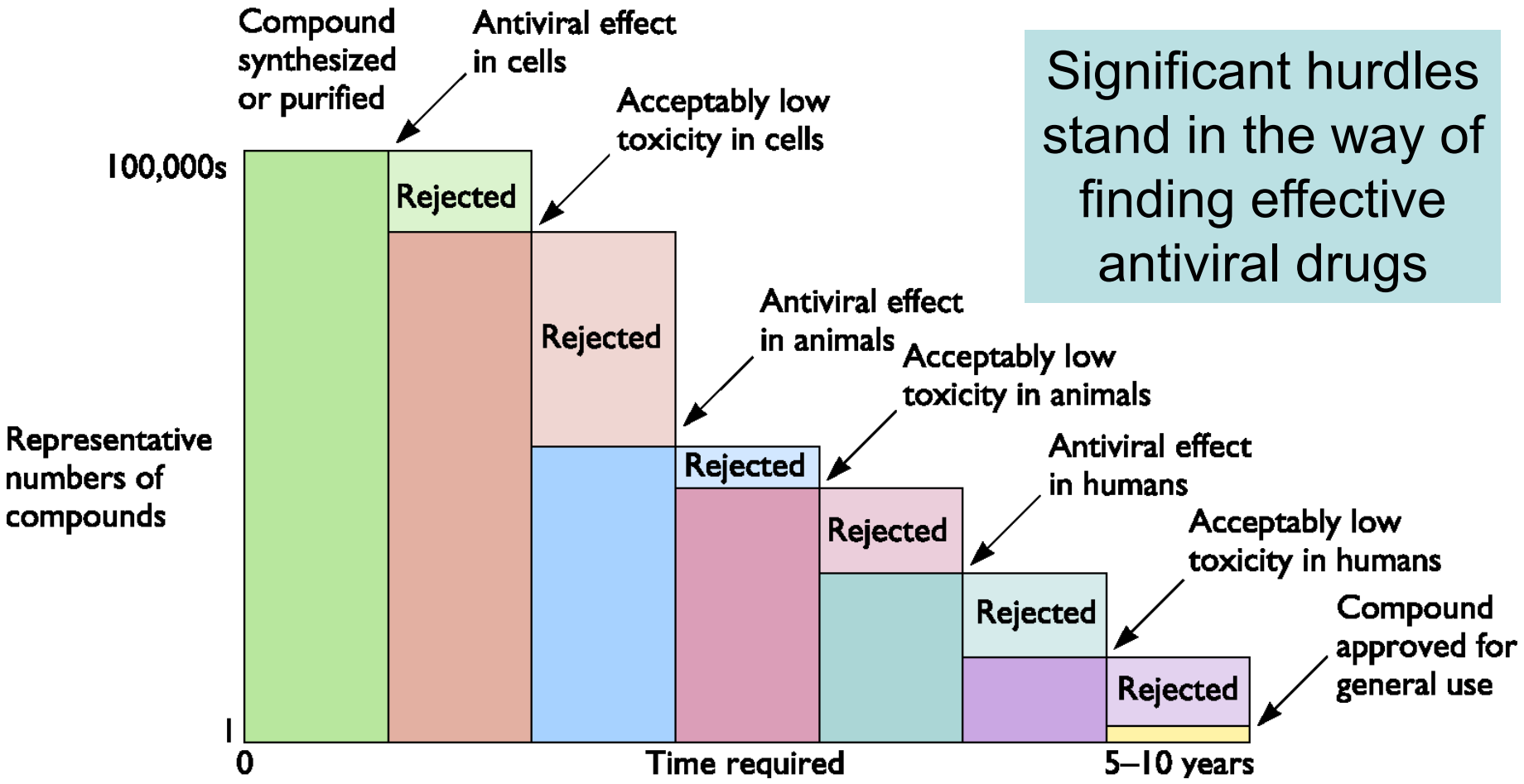
- Mechanism-based screens
- Cell-based assays
- Combinatorial chemistry
- High-throughput screens
- Computational Approaches:
  - Structure-assisted drug design
  - Genome sequencing, proteomic analyses
  - *In silico* discovery via Virtual Screening

Search and development of antiviral drugs



The path of modern antiviral drug discovery

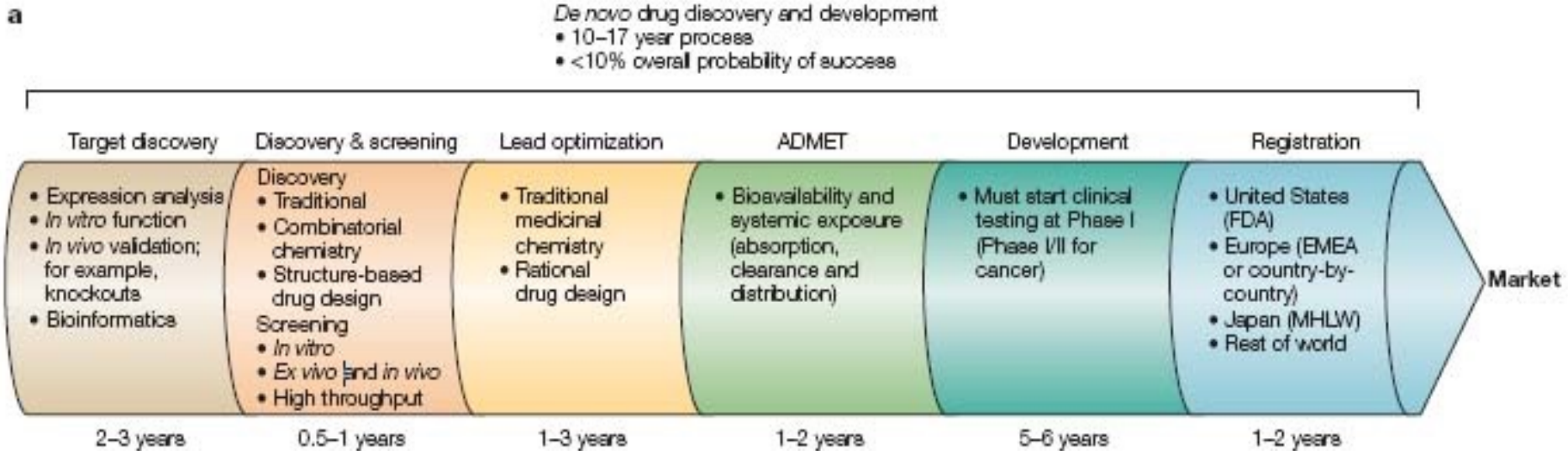
R&D of antiviral discovery: antiviral drugs are expensive to discovery, develop, and bring to market



Significant hurdles stand in the way of finding effective antiviral drugs

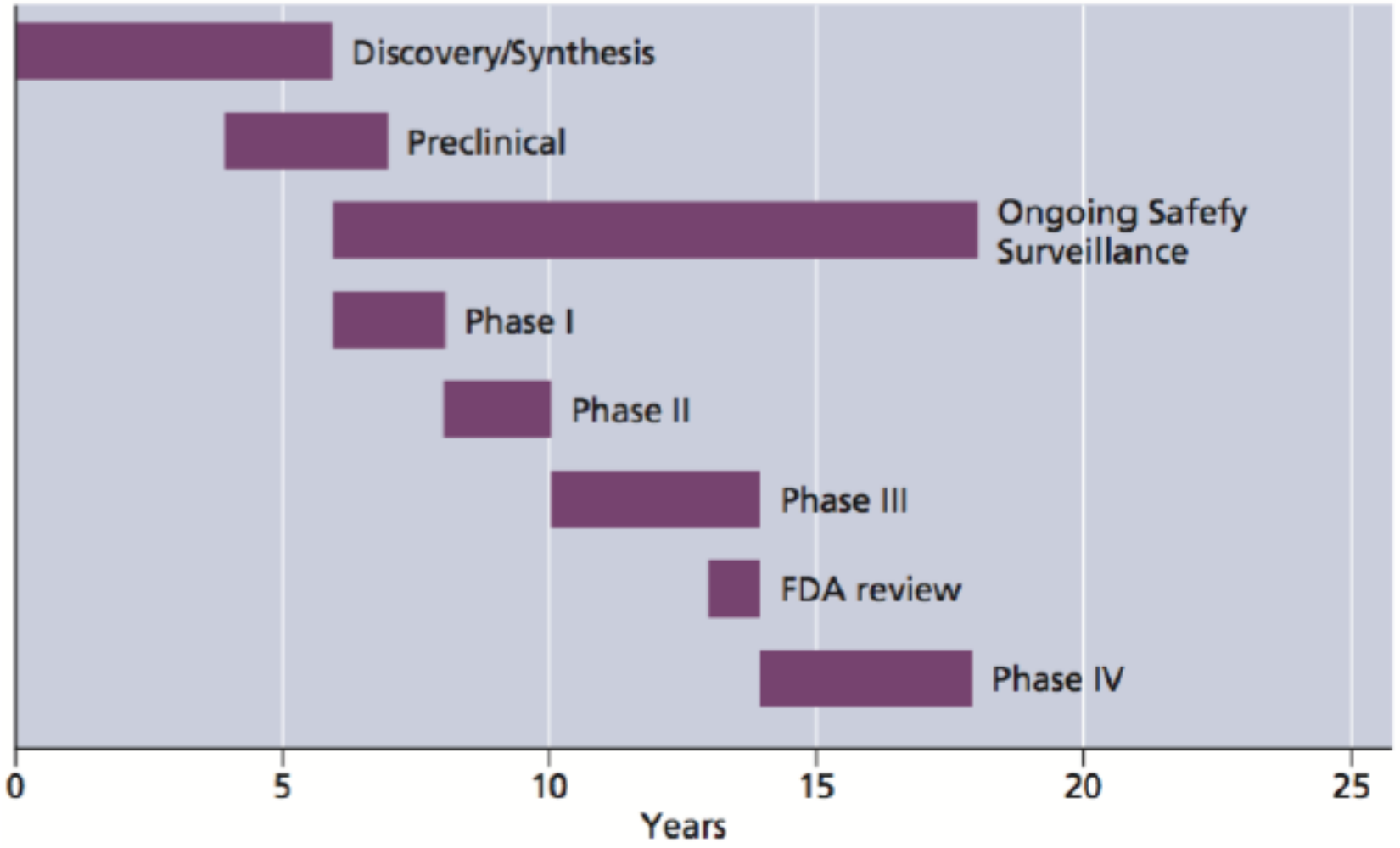
Staircase of drug discovery: it can takes 5 to 10 years after the initial lead is found to get a drug to the market and it may cost 100M$ to 500M$

R&D of antiviral discovery: antiviral drugs are expensive to discovery, develop, and bring to market



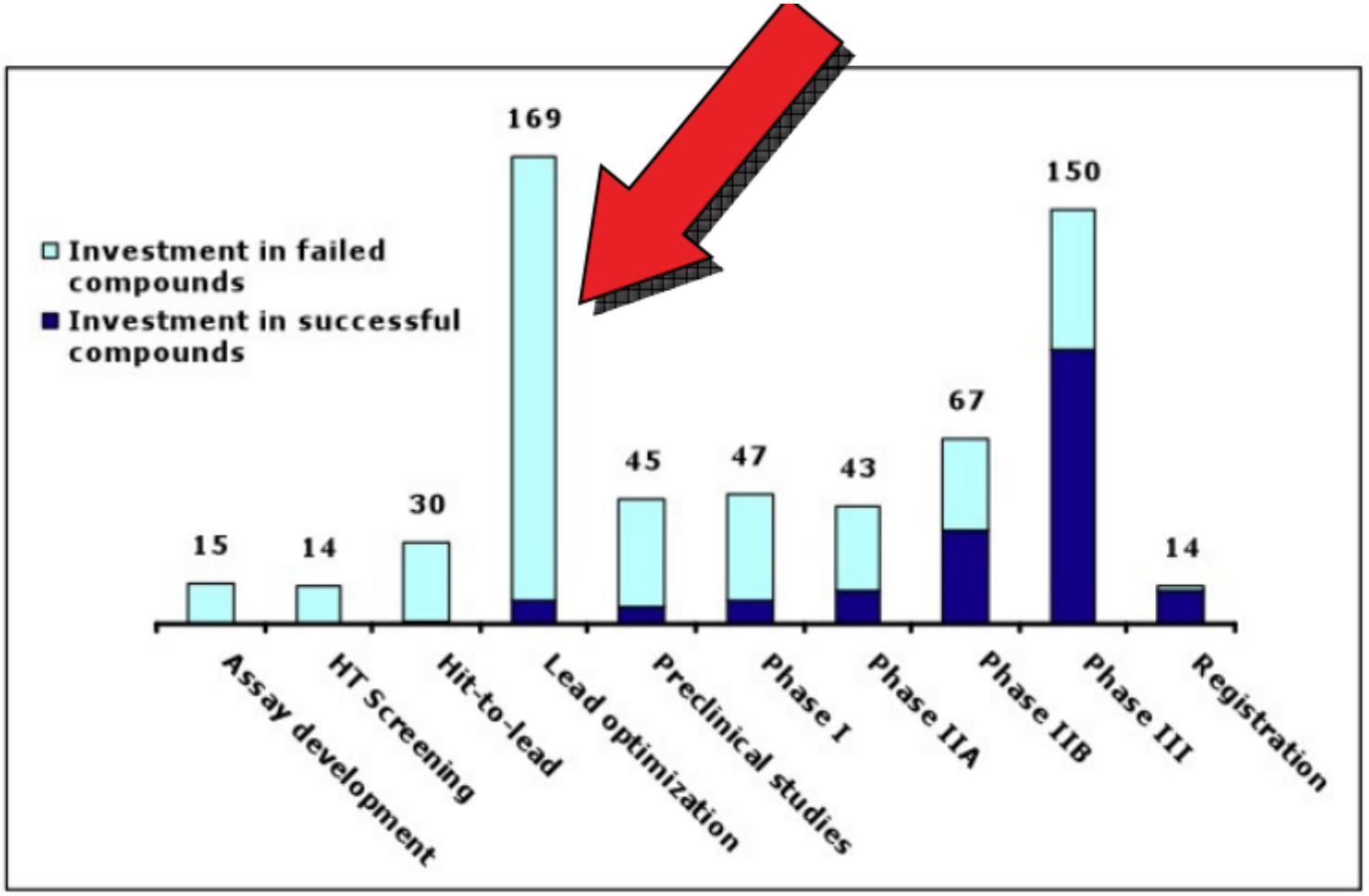
- Long process, extimated time from 10 to 17 yrs
- Low overall probability of success: <10%;
- Cost-expensive process:
  high ratio failed compounds/successfull compounds.

R&D of antiviral discovery:preclinical and clinical testing



From drug discovery to the hospital

R&D of antiviral discovery: costs and risks



Investment per phase of drug discovery and development for one successful drug (USD millions)

G.M. Milne, Jr., Annual Reports in Medicinal Chemistry, 2003, 38, 383-396

Search and development of antiviral drugs

Table 19.8 Key points for drug hunters seeking commercially viable antiviral drugs

Virology issues

Must obtain proof of principle as soon as possible.

- What is the molecular mechanism? Does the drug work in tissue culture and in animal models as predicted?

Compound should block viral spread early to limit cytopathology and host cytokine/inflammatory response.

- Drug must block replication completely and must not make an infection "persistent" by slow replication and spread.

Resistance to the antiviral drug must be manageable.

- Resistance mutations arise when any virus is permitted to repli-

Search and development of antiviral drugs

Business issues

Compound should be safe with no side effects.

Compound should be inexpensive to manufacture.

Compound should be easy to formulate and deliver.

- A pill to be swallowed is much preferred over injection.

Compound must satisfy an unmet medical need.

- That is, it must be better than any competitive drug or, better yet, have no competition.

Ultimately, a profit should be possible.

- The market should be large enough to allow a profit to be made.

Search and development of antiviral drugs

Blind screening  Is no longer attractive

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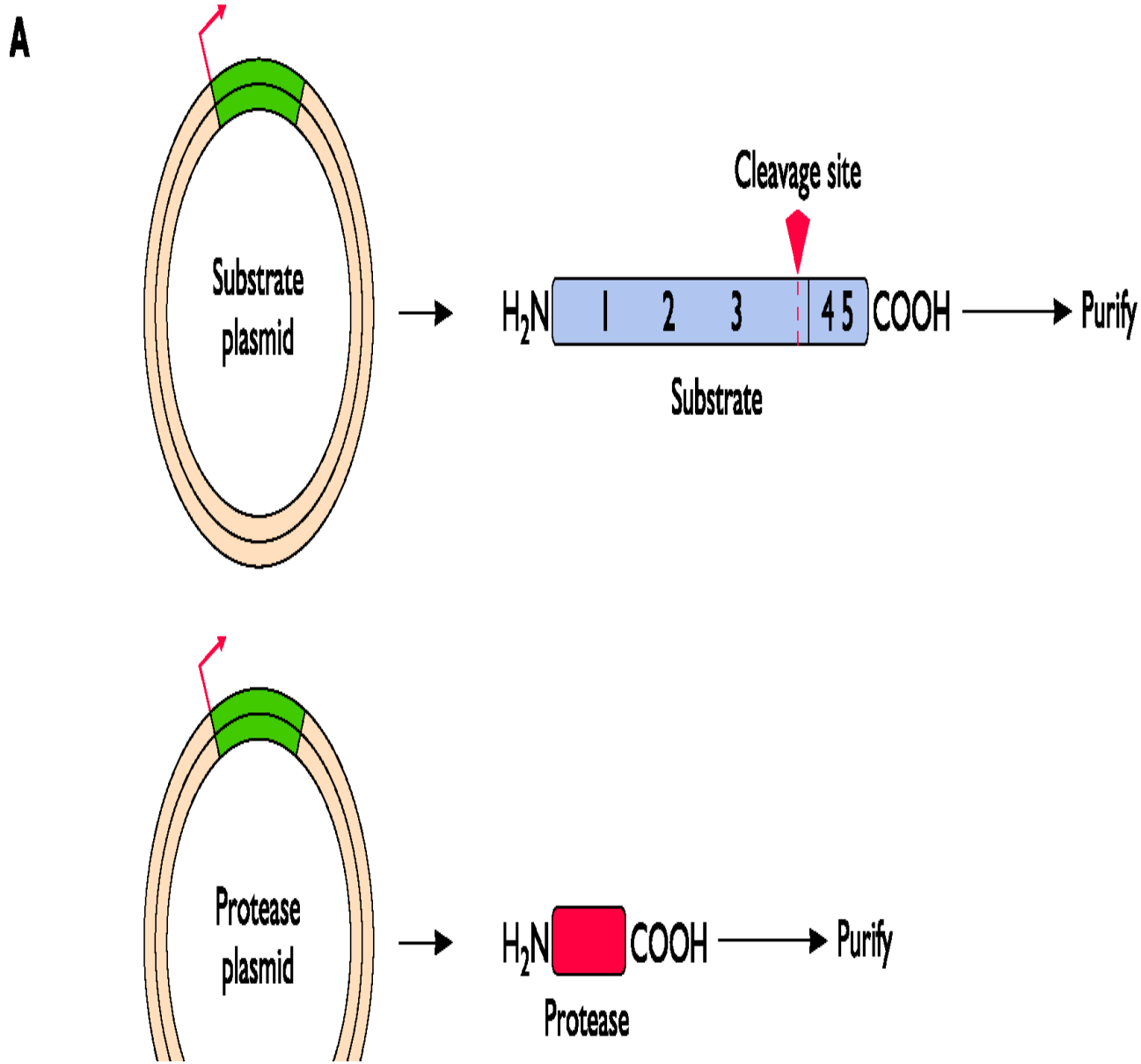
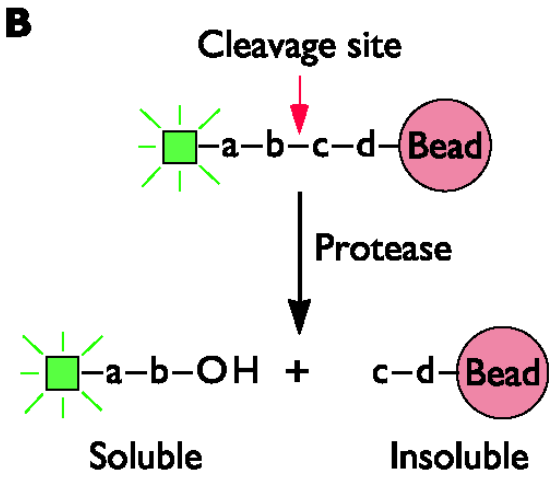
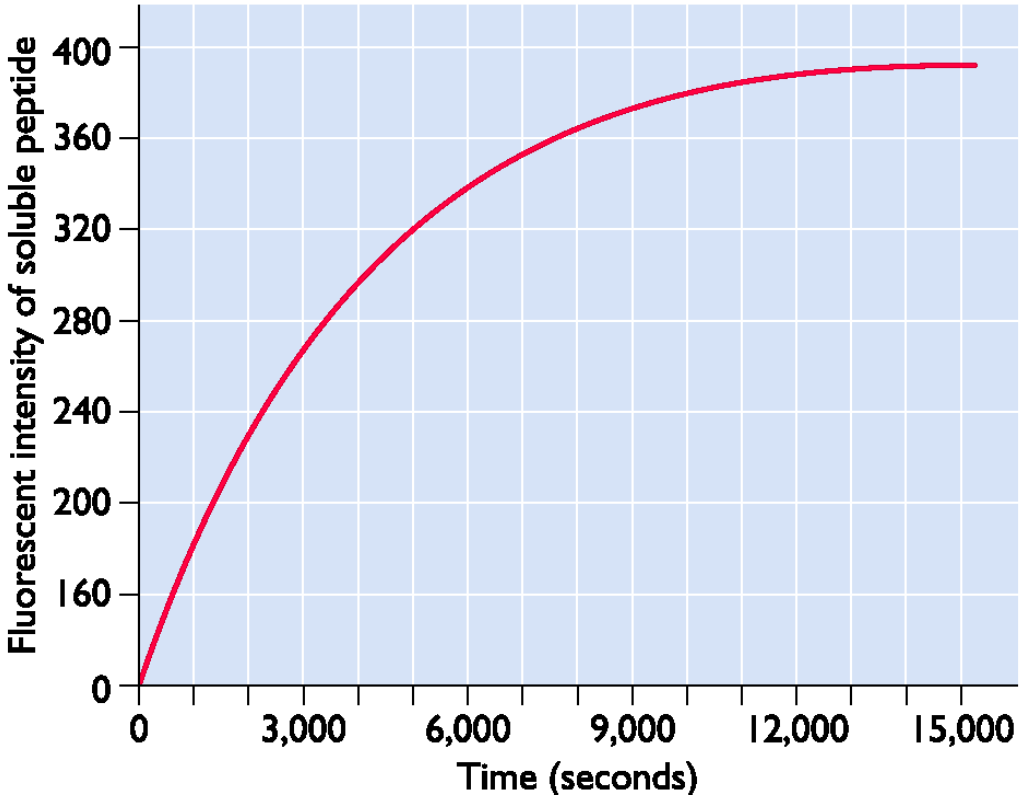
Mechanism-based assay to select inhibitors of a viral protease



Mechanism-based assay to select inhibitors of a viral protease



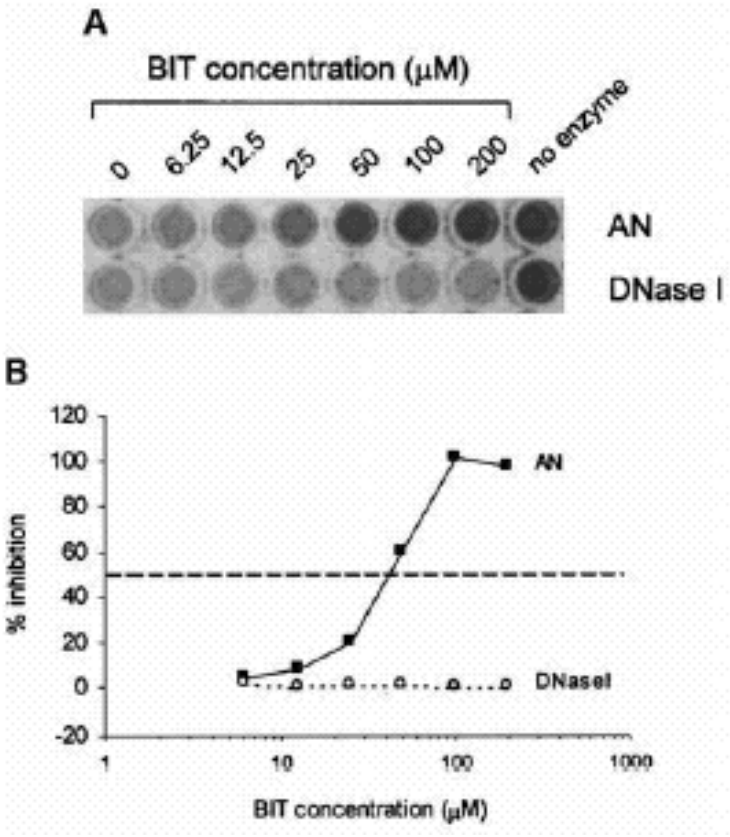


An example of a mechanism-based assay to select inhibitors of a viral nuclease

Analytical Biochemistry 293, 239–245 (2001)
doi:10.1006/abio.2001.5144, available online at http://www.idealibrary.com on IDEAL®

A Colorimetric Assay for High-Throughput Screening of Inhibitors of Herpes Simplex Virus Type 1 Alkaline Nuclease

Joel C. Bronstein and Peter C. Weber[1]
Infectious Diseases Section, Pfizer Global Research and Development, Ann Arbor, Michigan 48105



Nuclease assays. The DNA–methyl green degradation assay utilized a DNA substrate complexed with the intercalating dye methyl green and was obtained commercially (Sigma Chemical Co.). Degradation of the DNA substrate resulted in release of the dye and a corresponding loss of solution color. Assays were carried out as 150-μl reactions in 96-well plates and included 75 μl water, 15 μl 10× assay buffer (0.5 M Tris–HCl, pH 7.5, 30 mM $MgSO_4$), 5 μl compound stock prepared in methanol, 30 μl AN (2 μg) or bovine pancreatic DNaseI (0.15 units) diluted in DE buffer just prior to use, and 25 μl DNA–methyl green substrate (25 μg from a 1 mg/ml stock). Typically all of the components of the reaction were added with the exception of the substrate, and the plate was mixed and incubated at 37°C for 5 min. The DNA–methyl green substrate was then added to start the reaction, and the plate was then mixed again and incubated at 37°C for 3 h. The reaction was terminated by the addition of 50 μL sodium citrate (0.2 M, pH 7.5), and the plate was then mixed, sealed, and stored in the dark at room temperature for at least 12 h to allow color equilibration before reading on a spectrophotometer to determine A_{650}. AN inhibitor screens typically included the

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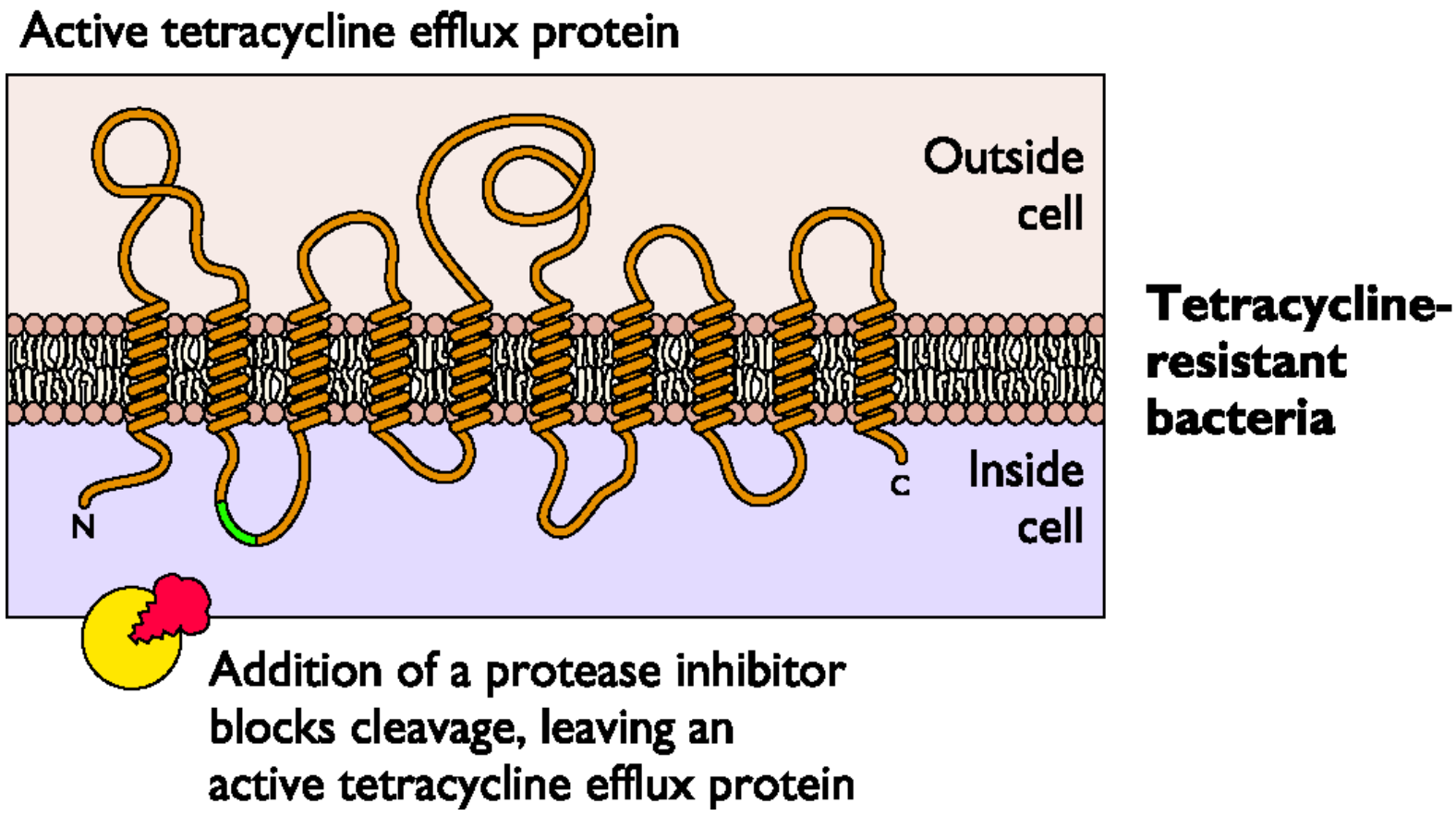
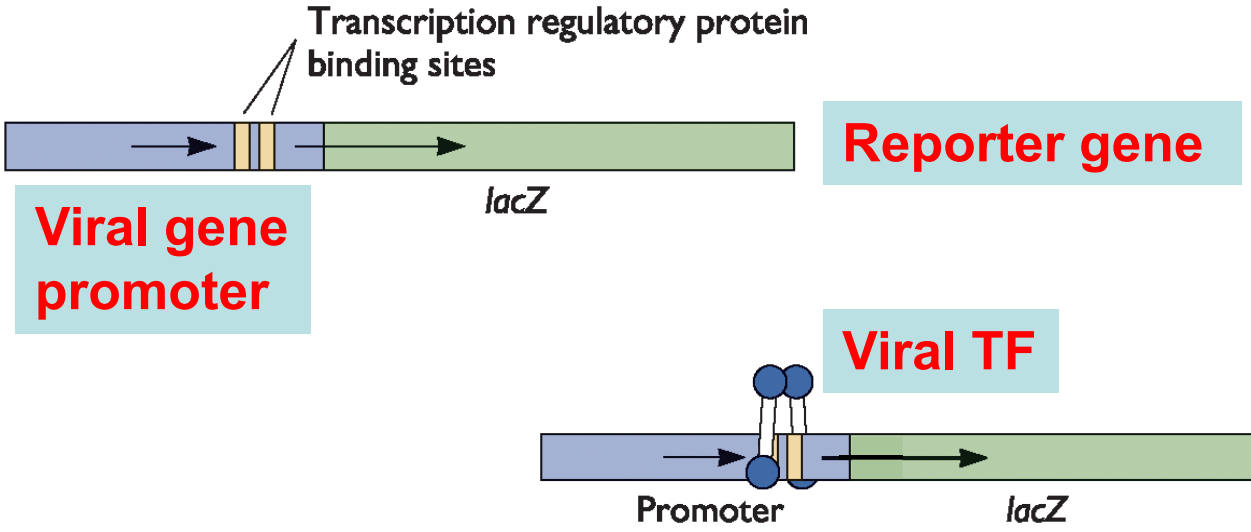
A cell-based assay to select inhibitors of HIV protease

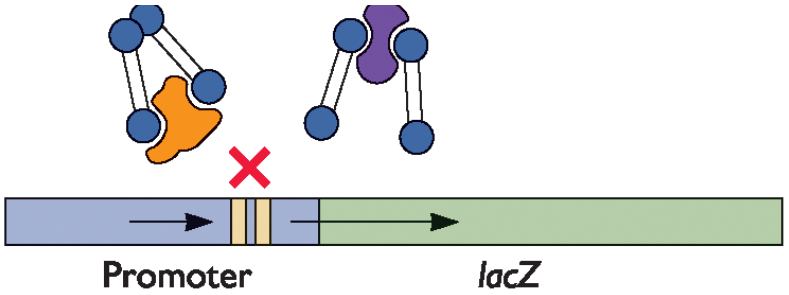
Grafsrom et al., *Adv. Exp. Med. Biol.* 312:25-40, 1992



A cell-based assay to select inhibitors of the activity of viral transcription factors



A small molecule that directly blocks DNA binding of the protein or blocks a protein-protein interaction required for the cooperative binding can be identified



ORIGINAL ARTICLE

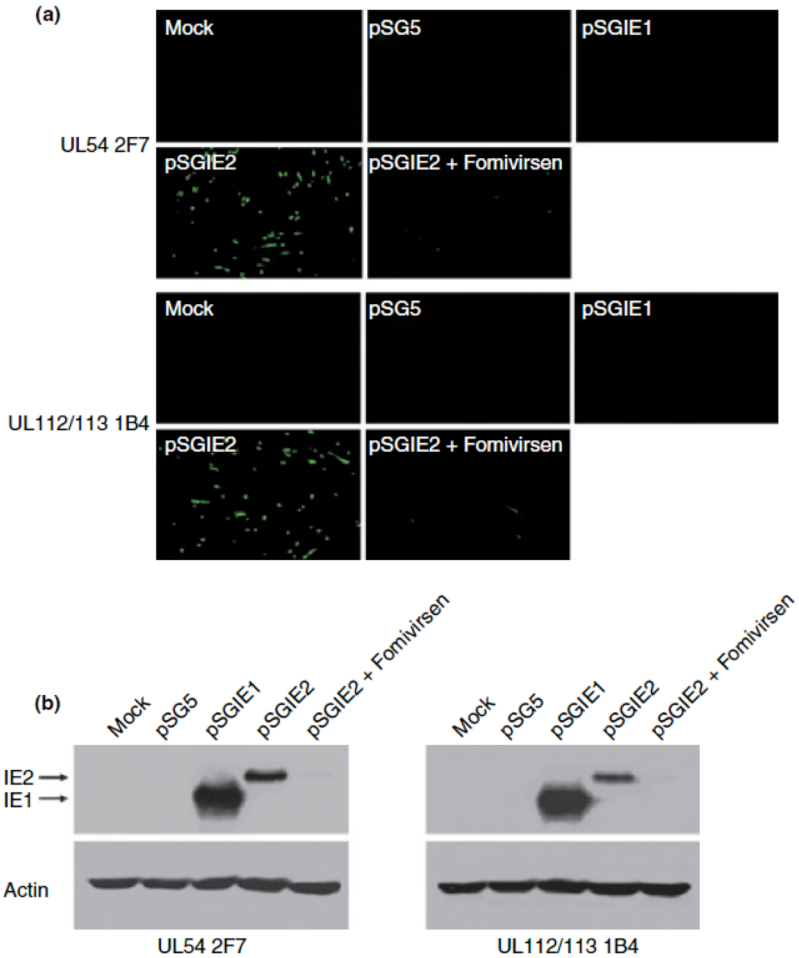
Journal of Applied Microbiology **105** (2008) 1791–1801

New cell-based indicator assays for the detection of human cytomegalovirus infection and screening of inhibitors of viral immediate-early 2 protein activity

A. Luganini[1], P. Caposio[1], M. Mondini[2], S. Landolfo[1] and G. Gribaudo[1]

1 Department of Public Health and Microbiology, University of Torino, Torino, Italy
2 Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy

HCMV IE2-dependent cell-based assay



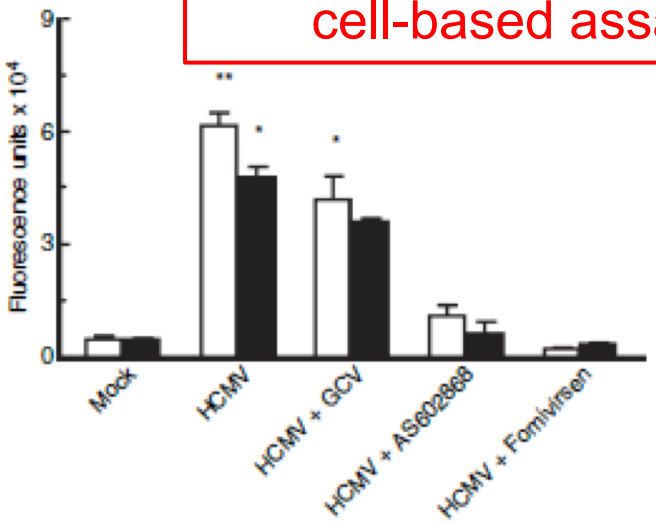
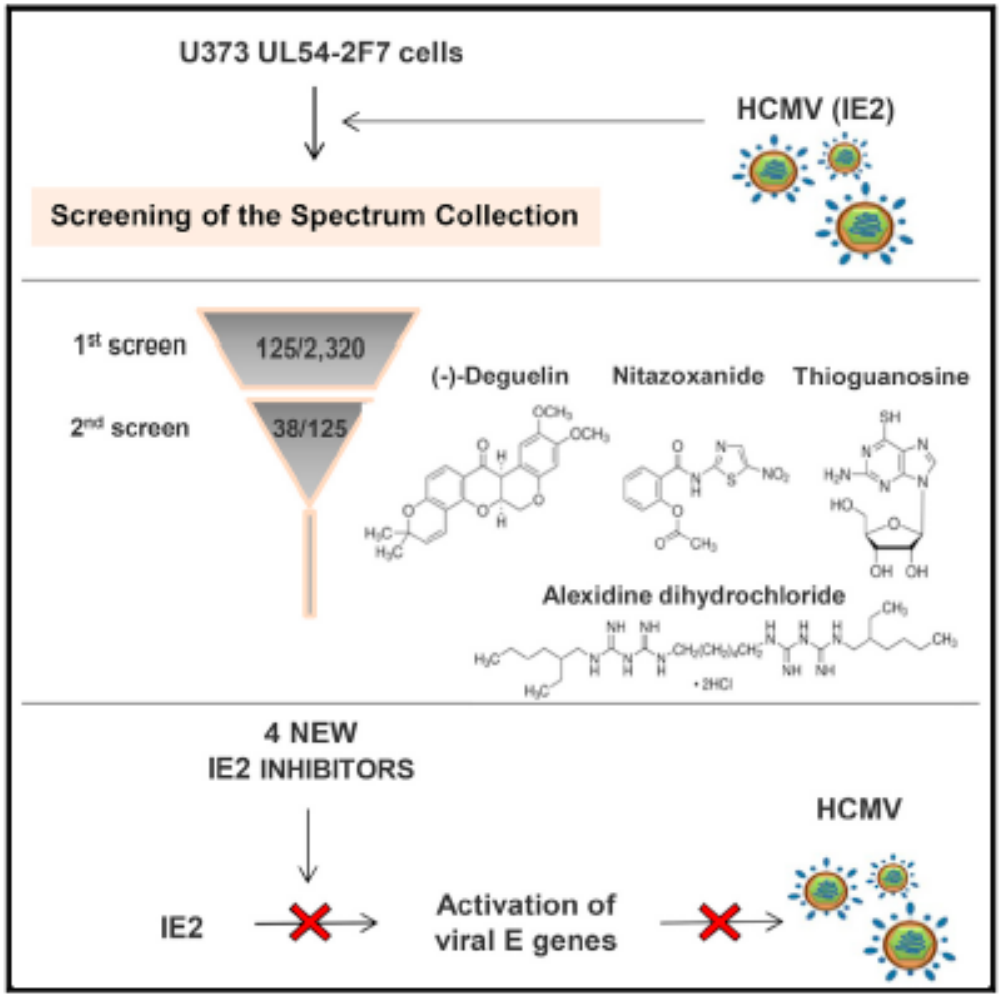


Figure 4 Effect of anti-cytomegaloviral drugs on EGFP expression in UL54 2F7 and UL112/113 1B4 cells. UL54 2F7 and UL112/113 1B4 cells were infected HCMV AD169 (moi 5) or mock-infected. Where indicated, the cells were pretreated and treated with 5 μmol l^{-1} fomivirsen (ISIS 2922) or 20 μmol l^{-1} AS602868 1 h prior to and during infection, or 100 μmol l^{-1} GCV after virus adsorption. At 72 h p.i., the cells were lysed and assayed for quantitative EGFP expression by automated fluorometry. The data shown in each column are the mean ± SD (error bars) of three independent experiments. $^{*}P < 0{\cdot}05$ compared with control, $^{**}P < 0{\cdot}01$ compared with control. (□), UL54 2F7; (■), UL112/113 1B4.

CellPress

Drug Repurposing Approach Identifies Inhibitors of the Prototypic Viral Transcription Factor IE2 that Block Human Cytomegalovirus Replication

Beatrice Mercorelli,[1,4] Anna Luganini,[2,4] Giulio Nannetti,[1] Oriana Tabarrini,[3] Giorgio Palù,[1] Giorgio Gribaudo,[2,5] and Arianna Loregian[1,5,*]
[1]Department of Molecular Medicine, University of Padua, 35121 Padua, Italy
[2]Department of Life Sciences and Systems Biology, University of Turin, 10123 Turin, Italy
[3]Department of Pharmaceutical Sciences, University of Perugia, 06123 Perugia, Italy
[4]Co-first author
[5]Co-senior author



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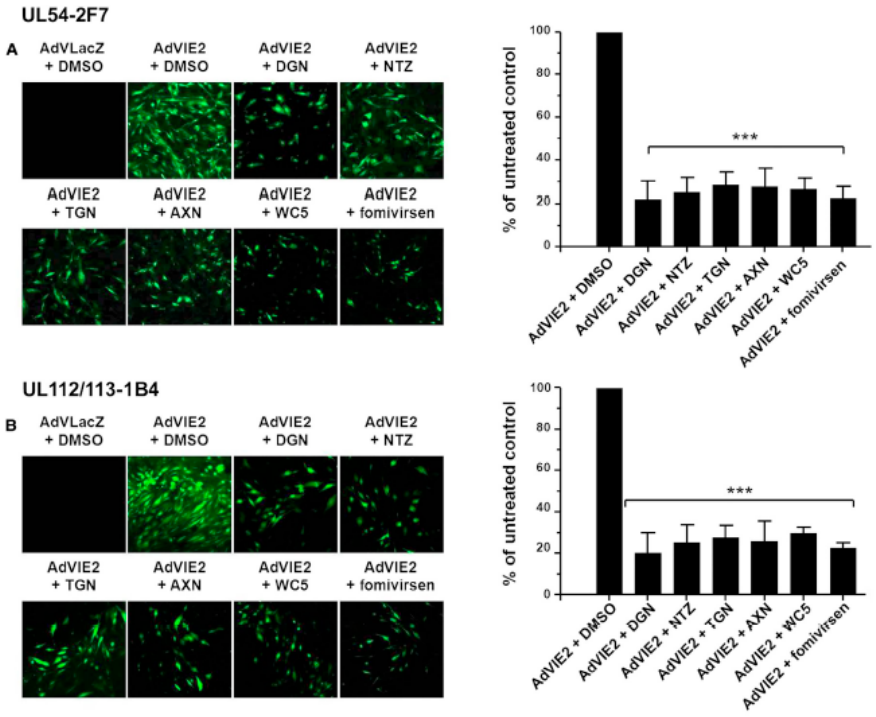
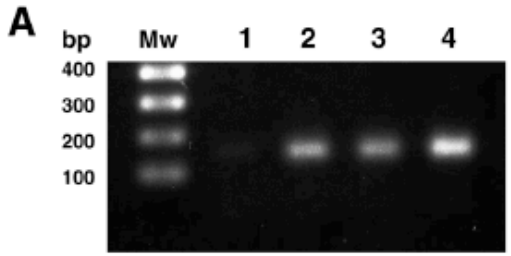
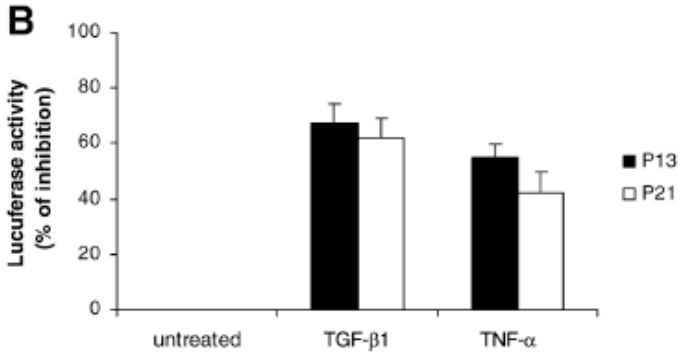


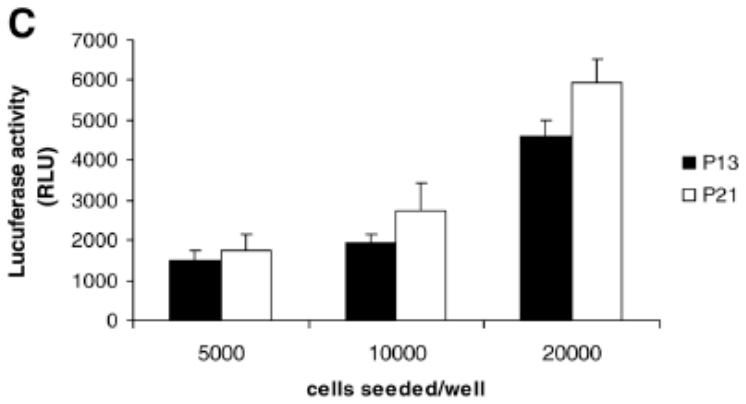
Mercorelli et al., 2016, Cell Chemical Biology 23, 1–12
March 17, 2016
http://dx.doi.org/10.1016/j.chembiol.2015.12.012

The FASEB Journal express article 10.1096/fj.05-3904fje. Published online October 27, 2005.

A cell-based high-throughput assay for screening inhibitors of human papillomavirus-16 long control region activity







Effect of cytokines on HPV-16 LCR activity

Cytokines	% of LCR inhibition (mean values ± SD)	Group[a]
Anti-inflammatory		
IL-4	56.6 ± 7.2	III
IL-10	0	I
IL-13	64.3 ± 5.8	III
TGF-β1	61.4 ± 8.4	III
TGF-β2	60.1 ± 6.5	III
TGF-β3	58.9 ± 5.9	III
Activin	32.4 ± 4.3	II
GDF-15	16.3 ± 2.1	I
Osteonectin	0.2 ± 0.03	I
Pro-inflammatory		
IL-1β	33.3 ± 4.6	II
IL-15	12.6 ± 1.8	I
IL-17	29.6 ± 3.6	I
IL-18	3 ± 0.5	I
IL-19	9 ± 1.2	I
IL-20	10.2 ± 0.8	I
IL-22	10.3 ± 1.7	I
TNF-α	53.7 ± 4.3	III
GM-CSF	12 ± 0.7	I
Growth factors		
IL-3	2.1 ± 0.09	I
IL-6	5.2 ± 0.3	I
IL-7	2.5 ± 0.5	I
IL-21	2.4 ± 0.4	I
Chemotactic factors		
IL-8	0	I
IP-10	5.2 ± 0.9	I
LEC	16.1 ± 2.3	I
MIP-1α	0	I
MIP-1β	27.3 ± 3.8	I
NAP-2	0	I
I-309	0.9 ± 0.2	I
Interferons		
IFN-α	58.2 ± 7.6	III
IFN-β	63.1 ± 4.8	III
IFN-γ	35.5 ± 4.3	II

[a]Groups are formed according to the % of LCR inhibition: I, 0–29%; II, 30–49%; III, 50–70%.

Search and development of antiviral drugs

Blind screening  Is no longer attractive

Modern antiviral discovery

- Mechanism-based screens
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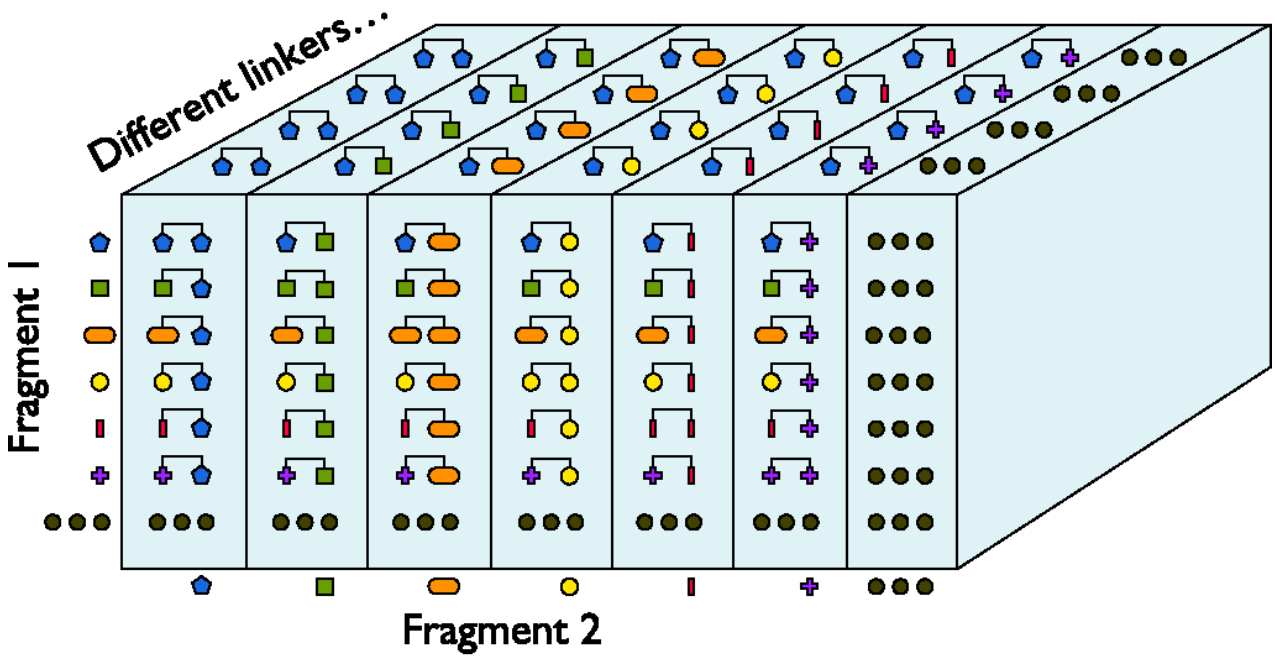
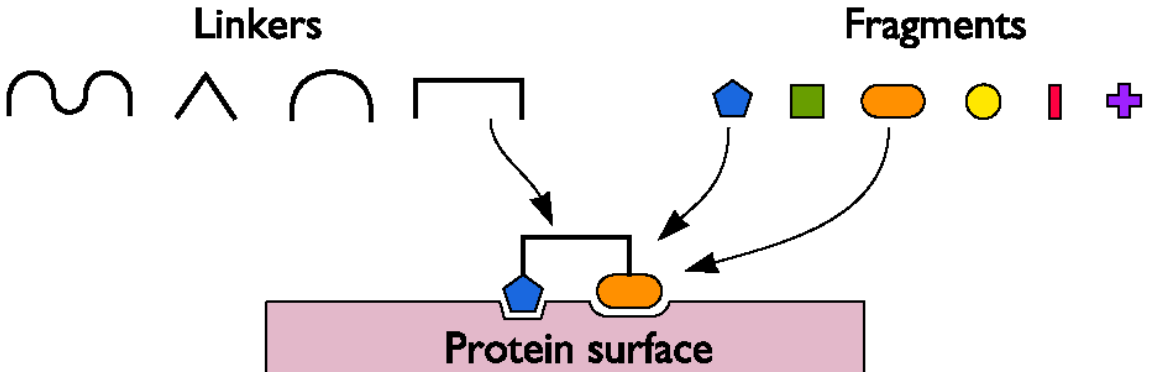
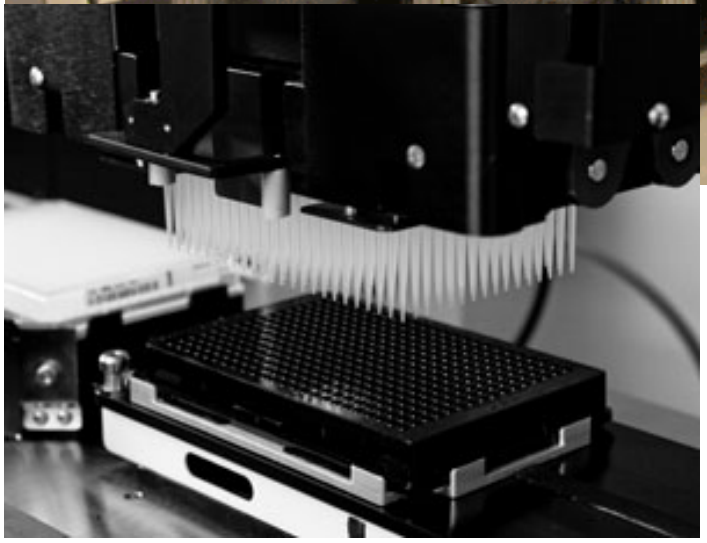
Combinatorial chemistry

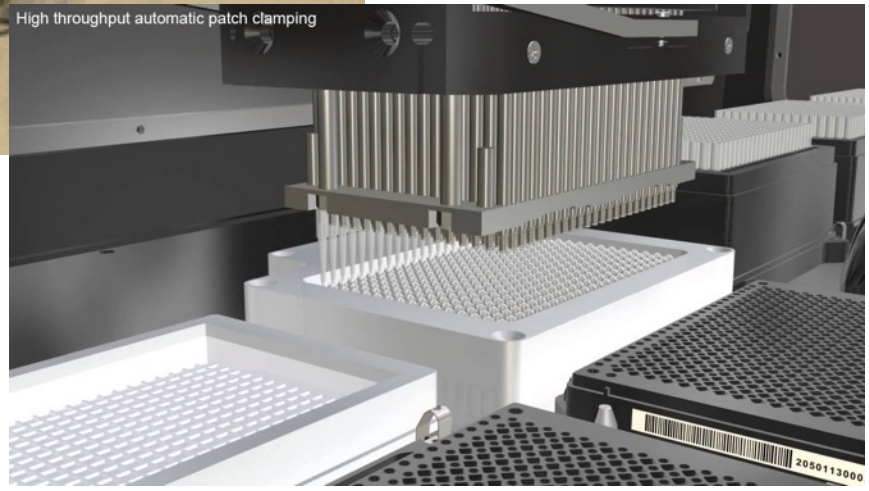


Eg. If assembled pairwise with 10 linkers, a collection of 10,000 small molecules yields a library of 10^9 new combinations.



Automated high-throughput screens





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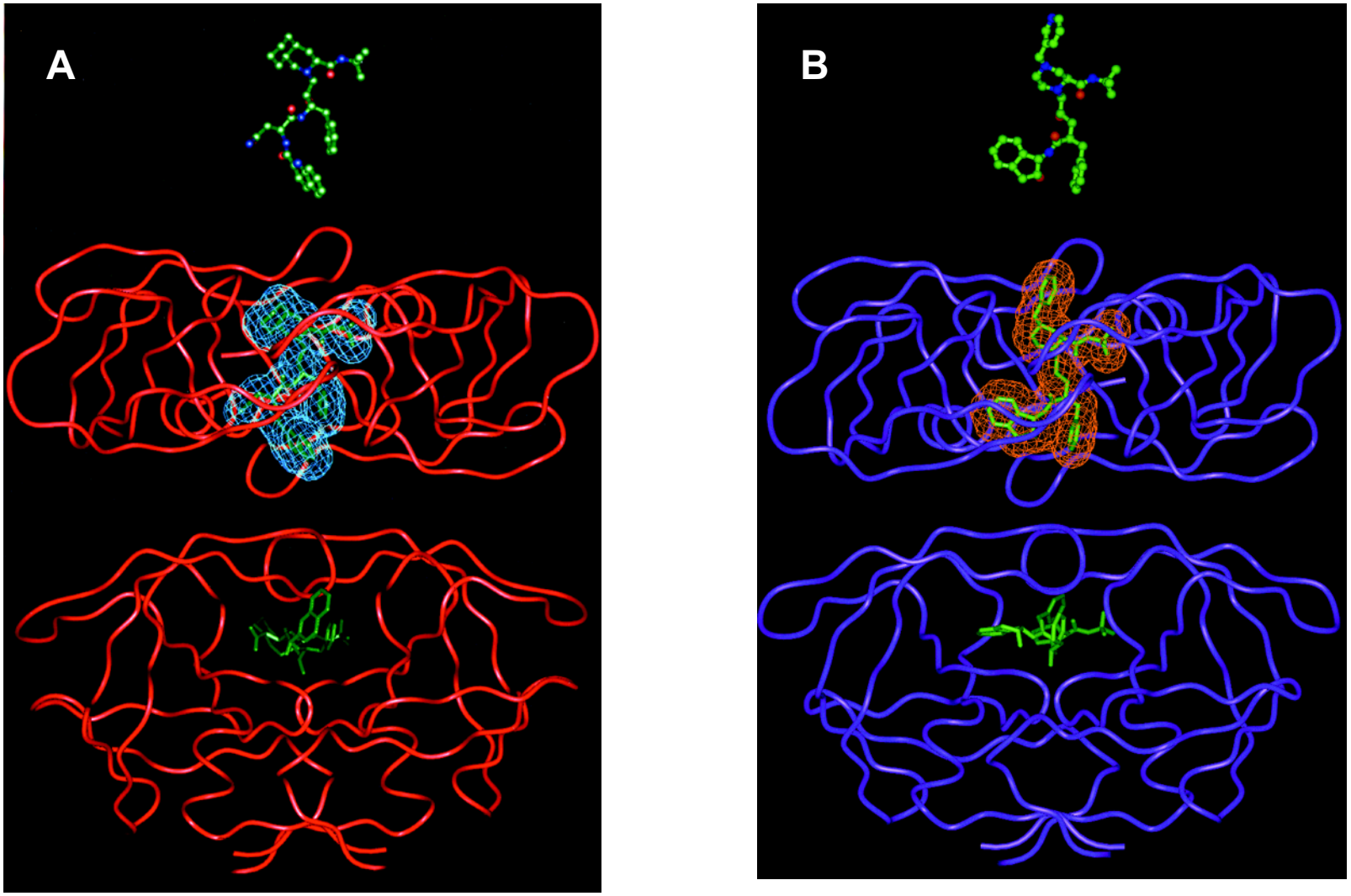
Stucture-assisted drug design as an example of computational approach to drug discovery



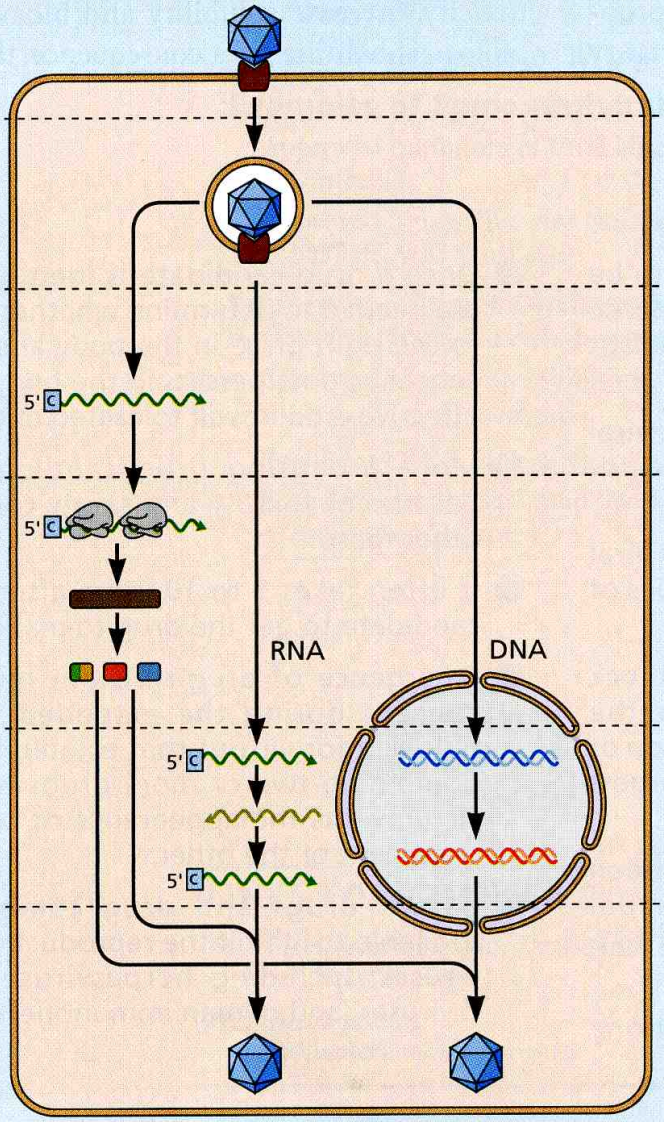
Structure of HIV protease with the inhibitors saquinavir (A) e indinavir (B)

Druggable Human Viruses

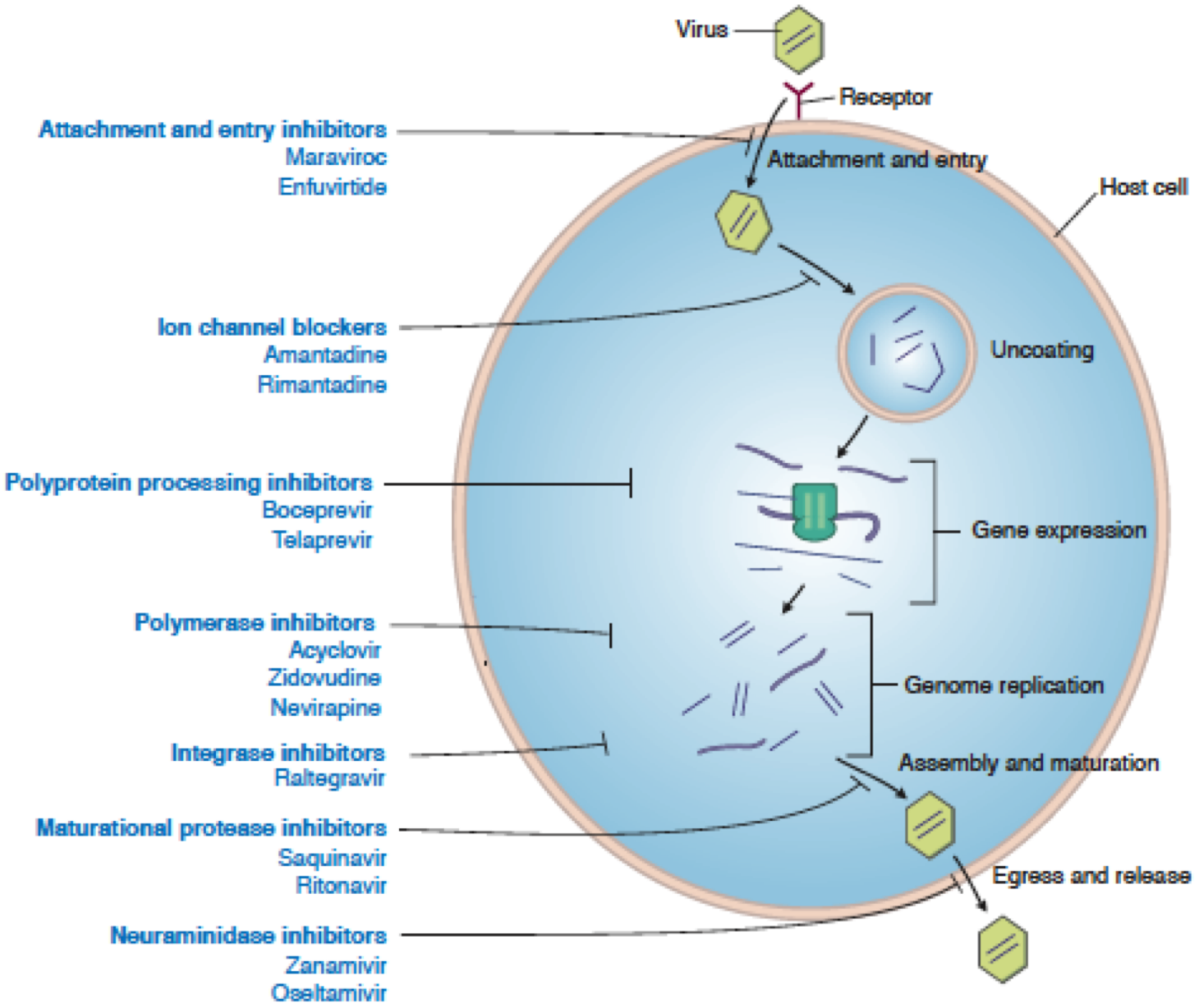
Herpes simplex virus (HSV)
Varicella-zoster virus (VZV)
Human Citomegalovirus (CMV)
Human Immunodeficiency virus (HIV)
Influenza virus
Respiratory Syncitial virus (RSV)
Hepatitis viruses A, B, C (HAV, HBV, HBC)
Human Papillomavirus (HPV)
Picornavirus

Knowledge of viral cycles identifies general targets for antiviral drug discovery

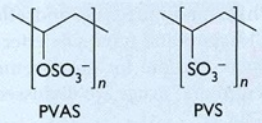
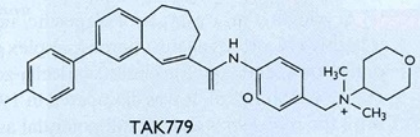
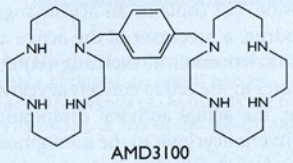
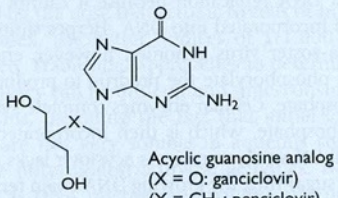
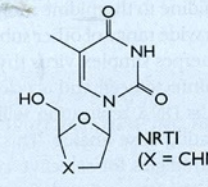
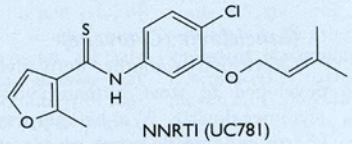
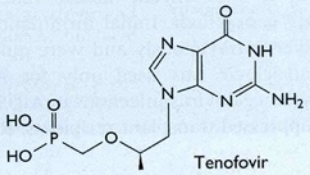
Function	Lead compound or example	Virus
Attachment	Peptide analogs of attachment protein	HIV
Penetration and uncoating	Dextran sulfate, heparin	HIV, herpes simplex virus
mRNA synthesis	Interferon	Hepatitis A, B, and C viruses; papillomavirus
	Antisense oligonucleotides	Papillomavirus, human cytomegalovirus
Protein synthesis/ Initiation	Interferon	Hepatitis A, B, and C viruses; papillomavirus
DNA/RNA replication	Nucleoside, nonnucleoside analogs	Herpesviruses, HIV, hepatitis B and C virus
Assembly	Peptidomimetics	HIV, herpes simplex virus

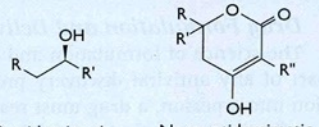
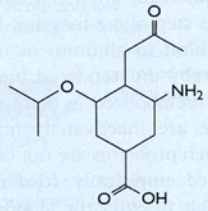
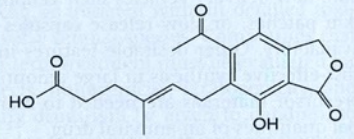
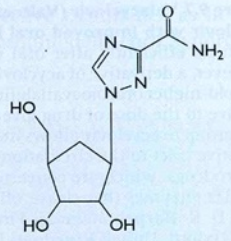
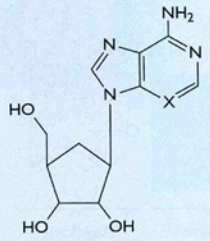


Some viral targets for antiviral drug discovery



The prototypic compounds (pharmacophores) of important classes of antiviral agents

Important classes	Structures
Viral adsorption inhibitors	 PVAS PVS
Viral-cell fusion inhibitors	 TAK779  AMD3100
Viral DNA polymerase inhibitor	 Acyclic guanosine analog (X = O: ganciclovir) (X = CH_2: penciclovir)
Reverse transcriptase inhibitors	 NRTI (X = CHN_3, AZT)  NNRTI (UC781)
Acyclic nucleoside phosphonate	 Tenofovir

Important classes	Structures
Protease inhibitors	 Peptidomimetic Nonpeptidomimetic
Neuraminidase inhibitor	 Oseltamivir
IMP dehydrogenase inhibitors	 Mycophenolic acid  Ribavirin
SAH hydrolase inhibitor	 (X = N or CH)

Examples of viral targets for antiviral drugs

- ✓**Attachment**
- ✓**Entry and uncoa[illegible]g**
- ✓**Viral gene expressio[illegible]**
- ✓**Replication of viral geno[illegible]**
- ✓**Assembly**
- ✓**Maturation and release**

Attachment

Agent	Virus
Receptor antagonists - soluble receptors - peptidomimetics - shRNAs	**HIV**
Neutralizing antibodies	**Many**
Destran sulphate, heparin, peptides	**Herpesvirus, HPV**

BMC Biotechnology 2008, 8:85 doi:10.1186/1472-6750-8-85

BMC Biotechnology

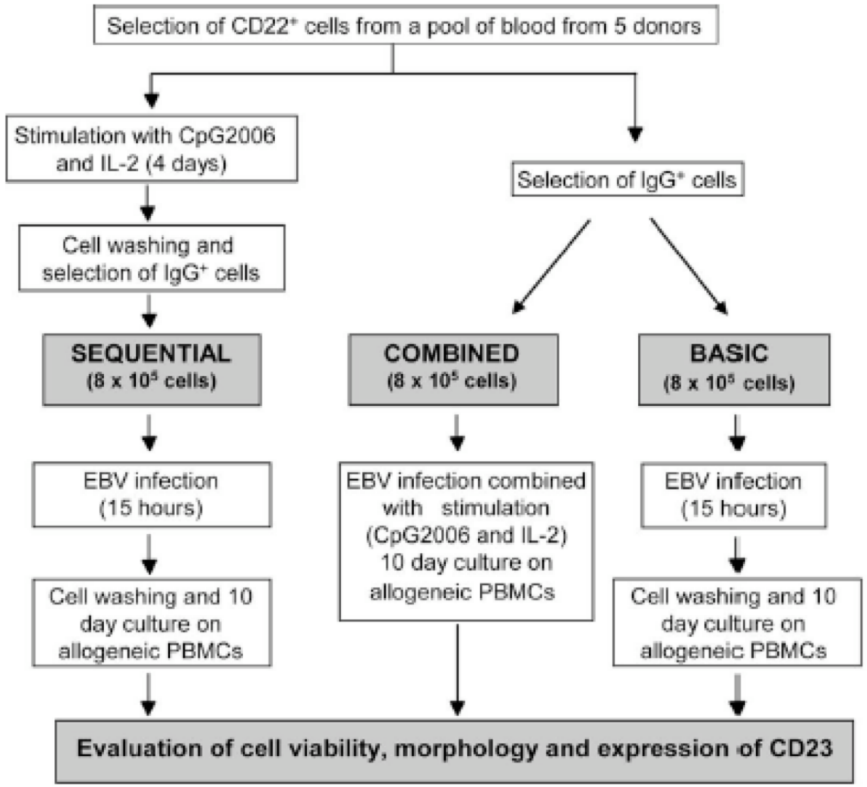
BioMed Central

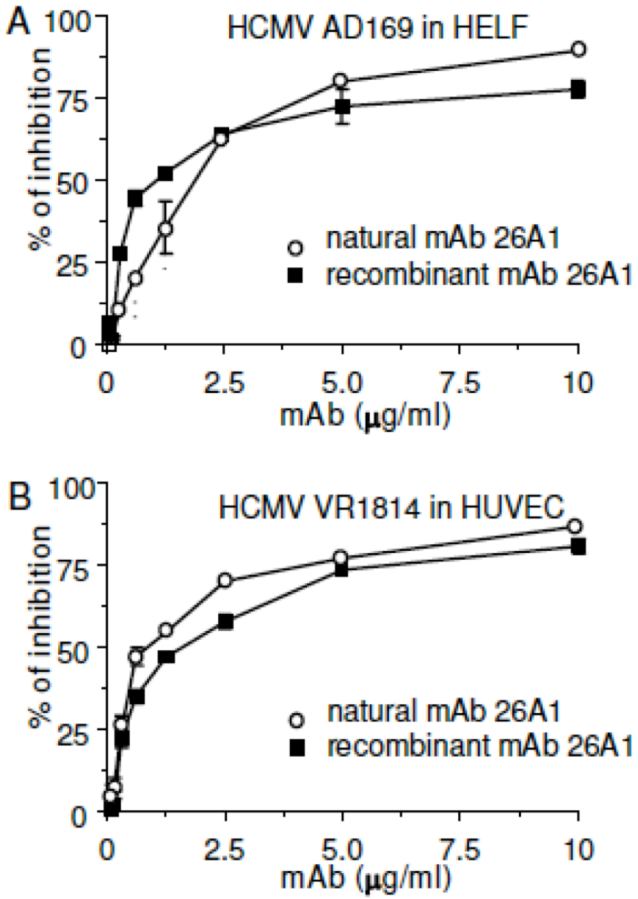
Research article

Open Access

Generation of potent neutralizing human monoclonal antibodies against cytomegalovirus infection from immune B cells

Ada Funaro*[1,2], Giorgio Gribaudo[3], Anna Luganini[3], Erika Ortolan[1,2], Nicola Lo Buono[1], Elisa Vicenzi[4], Luca Cassetta[4], Santo Landolfo[3], Richard Buick[5], Luca Falciola[6], Marianne Murphy[6], Gianni Garotta[6] and Fabio Malavasi[1,2]



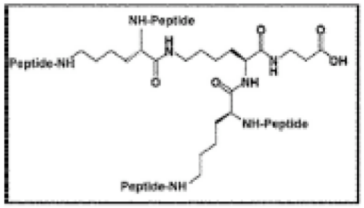


ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2011, p. 3231–3239 Vol. 55, No. 7
0066-4804/11/$12.00 doi:10.1128/AAC.00149-11

Inhibition of Herpes Simplex Virus Type 1 and Type 2 Infections by Peptide-Derivatized Dendrimers▽†

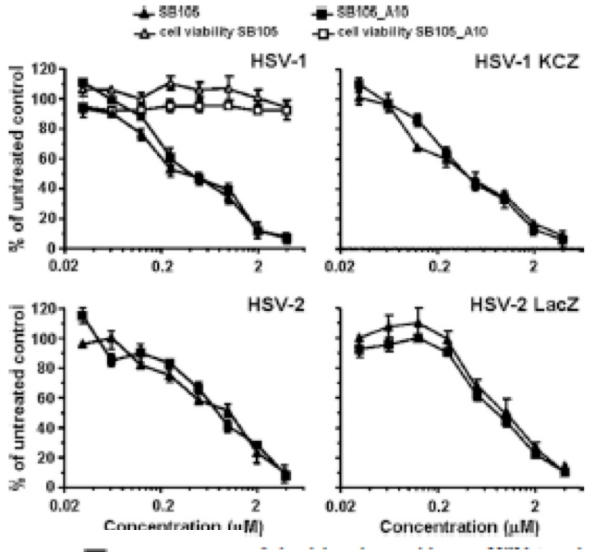
Anna Luganini,[1] Silvia Fabiole Nicoletto,[2] Lorena Pizzuto,[2] Giovanna Pirri,[2] Andrea Giuliani,[2] Santo Landolfo,[1] and Giorgio Gribaudo[1]*

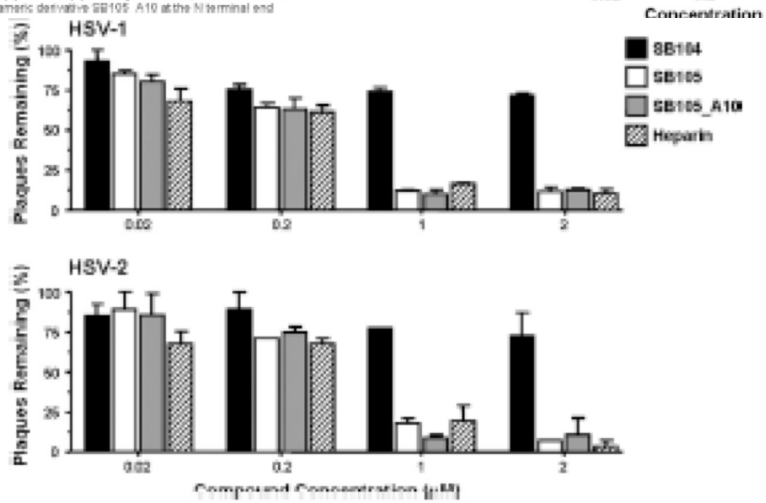
Department of Public Health and Microbiology, University of Turin, Turin,[1] and Spider Biotech S.r.l., Colleretto Giacosa, Turin,[2] Italy



Compound name	Peptide sequence
SB101[a]	QKKIRVRLSA
SB102	QKKIRVRL
SB103	QKKIRVRWSA
SB104	NKKIRVRL
SB105	ASLRVRIKKQ
SB105_LIN[b]	ASLRVRIKKQ
SB105_A10	ASLRVRIKK
SB105_A10-FAM[c]	(N$^{\alpha}$-5(6)FAM)-K-ASLRVRIKK
SB106	FKKIRVRL
SB107	QKKIRVRISA
SB108	QKKIRVRLSW
SB109	QKKIRVRFSA
SB110	QKKIRFRLSA
SB111	QKKIRIRLSA
SB112	QKKIRWRLSA
SB113	QKKFRVRLSA

a the linear peptide sequence is linked to the dimeric core Lys-β-Ala-OH
b the SB105_LIN is a 10-mer linear amido peptide, having the SB105 peptide sequence
c 5(6)-FAM is 5(6)-carboxyfluorescein, used to label the tetrameric derivative SB105_A10 at the N terminal end



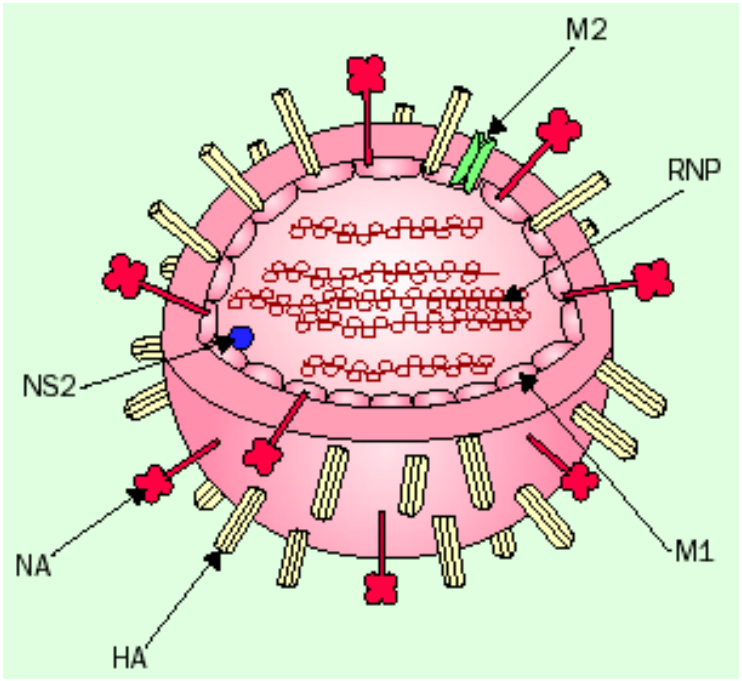


Examples of viral targets for antiviral drugs

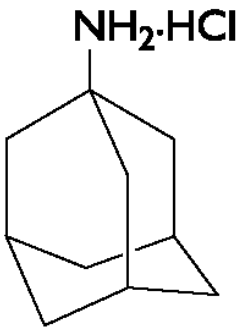
- ✓**Attachment**
- ✓**Entry and uncoating**
- ✓**Viral gene expression**
- ✓**Replication of viral genome**
- ✓**Assembly**
- ✓**Maturation and release**

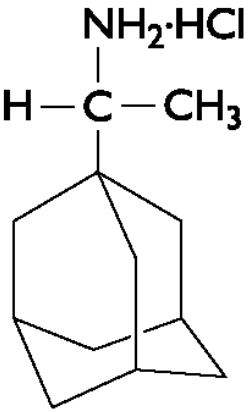
Entry and uncoating

Agent	Virus
Amantadine, rimantadine	Influenza A virus
Pleconaril	Picornavirus
Enfuvirtide	HIV



Amantadine and Rimantadine





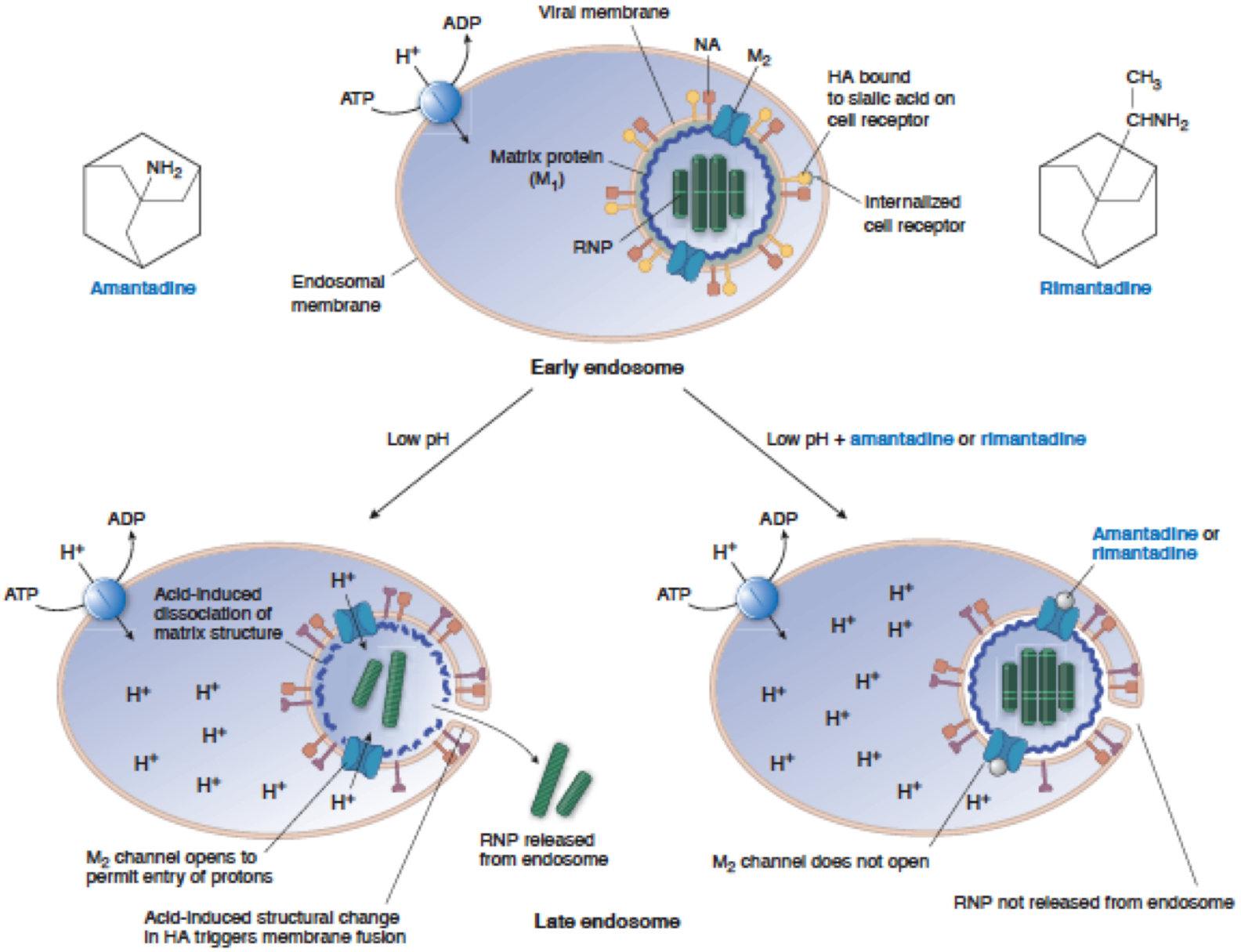
✓They are ion channel blockers that inhibit viral RNA uncoating by blocking the function of the envelope M2 protein

✓Useful in therapy and prevention of influenza A infections

✓They must be given within 24-48 hrs from symptoms onset

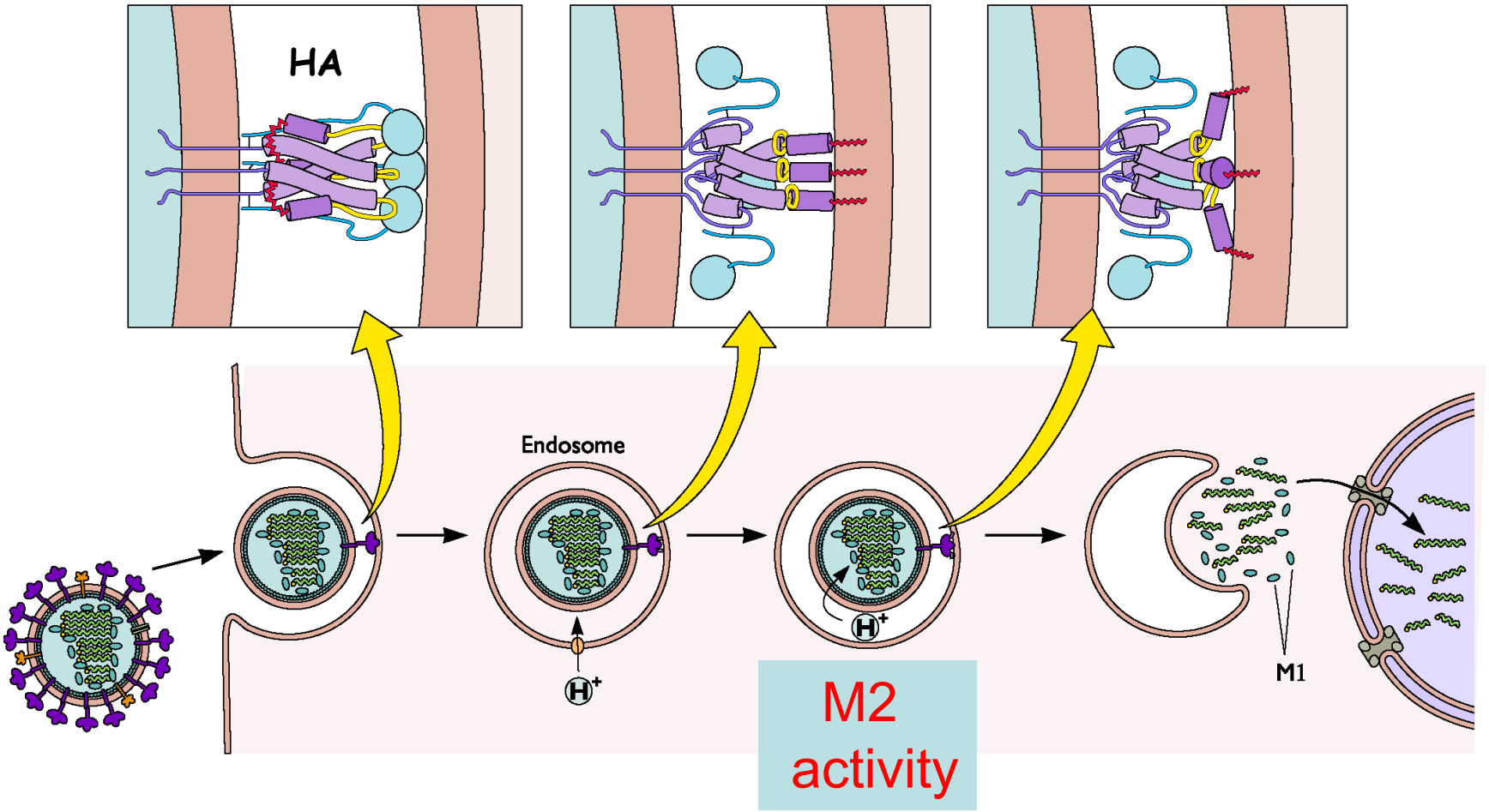
✓High frequency of resistant virus due to M2 or HA mutations

Model for uncoating Influenza A virus and effect of amantadine and rimantadine

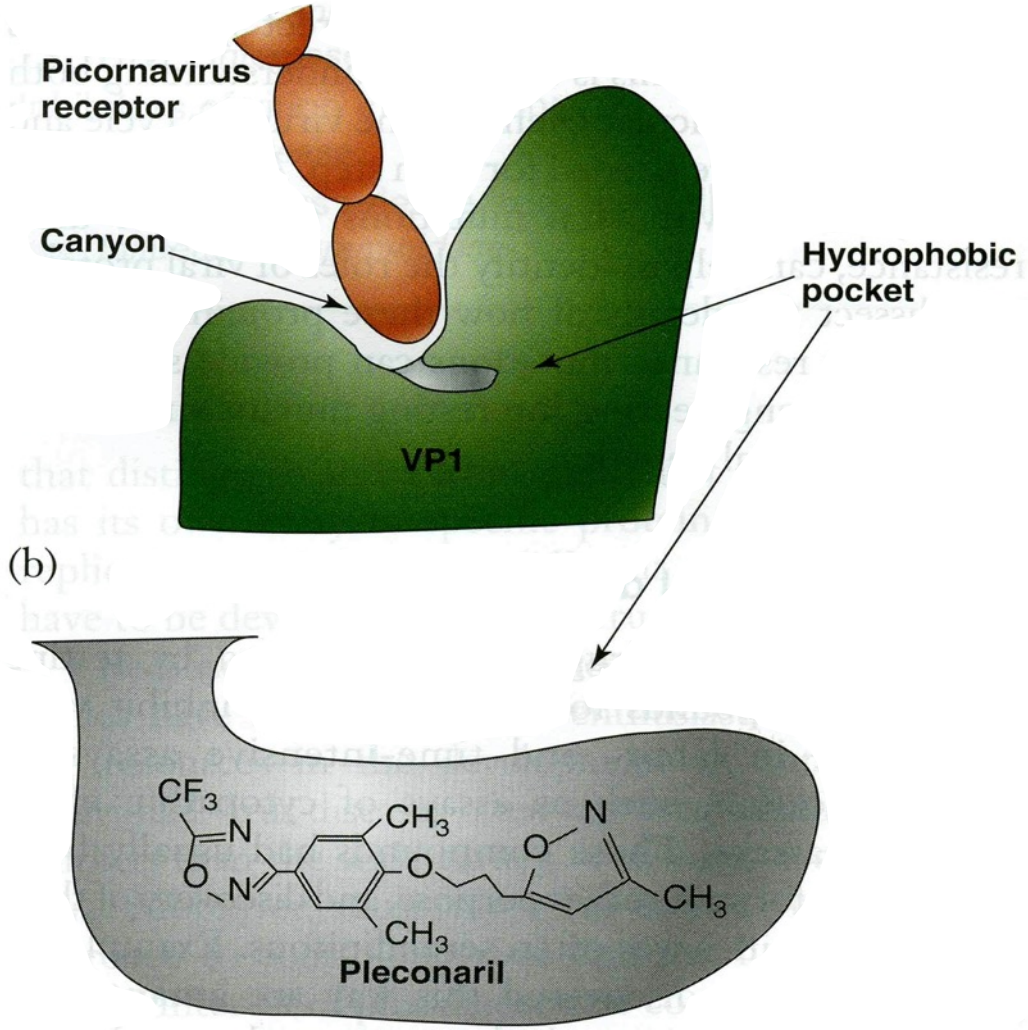


Amantadine and Rimantadine: mechanism of action

Role of M2 protein in Influenza A virus uncoating



Pleconaril : mechanism of action



Examples of viral targets for antiviral drugs

- ✓**Attachment**
- ✓**Entry and uncoating**
- ✓**Viral gene expression**
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- ✓**Maturation and release**

Viral gene expression

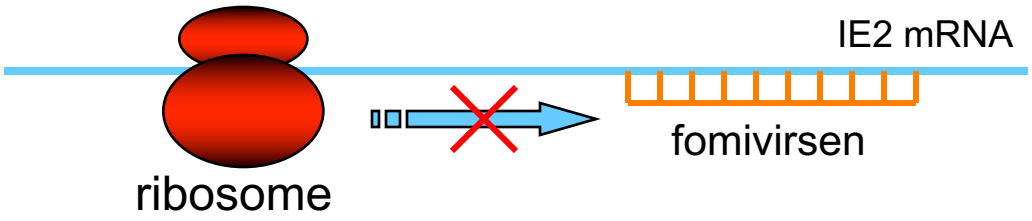
Agent	Virus
Interferons	**HAV, HBV, HCV, HPV**
Antisense oligos	**HCMV**

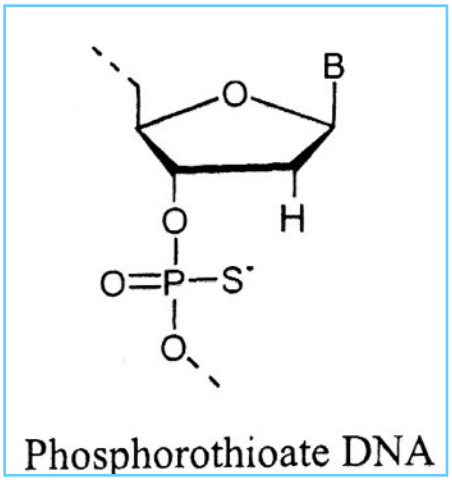
An example of an antiviral antisense oligodeoxynucleotide: Fomivirsen

- ✓It is an antisense phosphorothioate oligodeoxynucleotide complementary to a segment of HCMV IE2 mRNA

21 nt
GCG TTT GCT CTT CTT CTT GCG

- ✓It inhibits viral E and L gene expression, viral DNA synthesis, and viral replication by blocking translation of IE2 mRNA





Phosphorothioate DNA

- ✓Fomivirsen (Vitravene) was used for intravitreal treatment of HCMV retinitis in AIDS patients resistant to other anti-HCMV drugs. No longer marketed.

- ✓Fomivirsen was the first antisense oligo approved for use in humans (FDA, 1998)

Examples of viral targets for antiviral drugs

- ✓**Attachment**
- ✓**Entry and uncoating**
- ✓**Viral gene expression**
- ✓**Replication of viral genome**
- ✓**Assembly**
- ✓**Maturation and release**

Replication of viral genome

Agent	Virus
Nucleoside analogs	Herpes, HIV, HBV, HCV
Nonnucleoside inhibitors	Herpes, HIV
Ribavirin	RSV, HCV, HEV

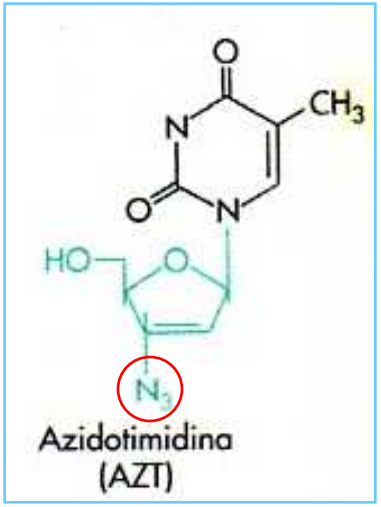
Antiviral nucleoside and nucleotide analogs

- ✓ The most widely used antiviral drugs
- ✓ They target viral DNA polymerases and viral reverse transcriptases
- ✓ They bear a base and/or a sugar modification
- ✓ Their activation to dNTPs requires phoshorylation mediated by cellular and/or viral kinases
- ✓ Viral polymerases are selectively inhibited because they bind the modified dNTP with higher affinity (100-fold) than cellular enzymes.

Nucleoside and nucleotide analogs:mechanism of action

Lack of the di un 3'-OH group of the sugar

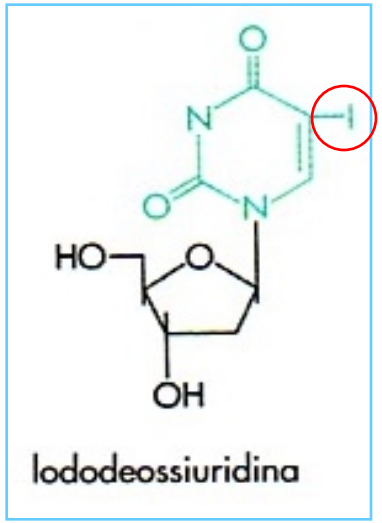
Growing DNA chain terminates and further DNA replication is blocked



Azidotimidina
(AZT)

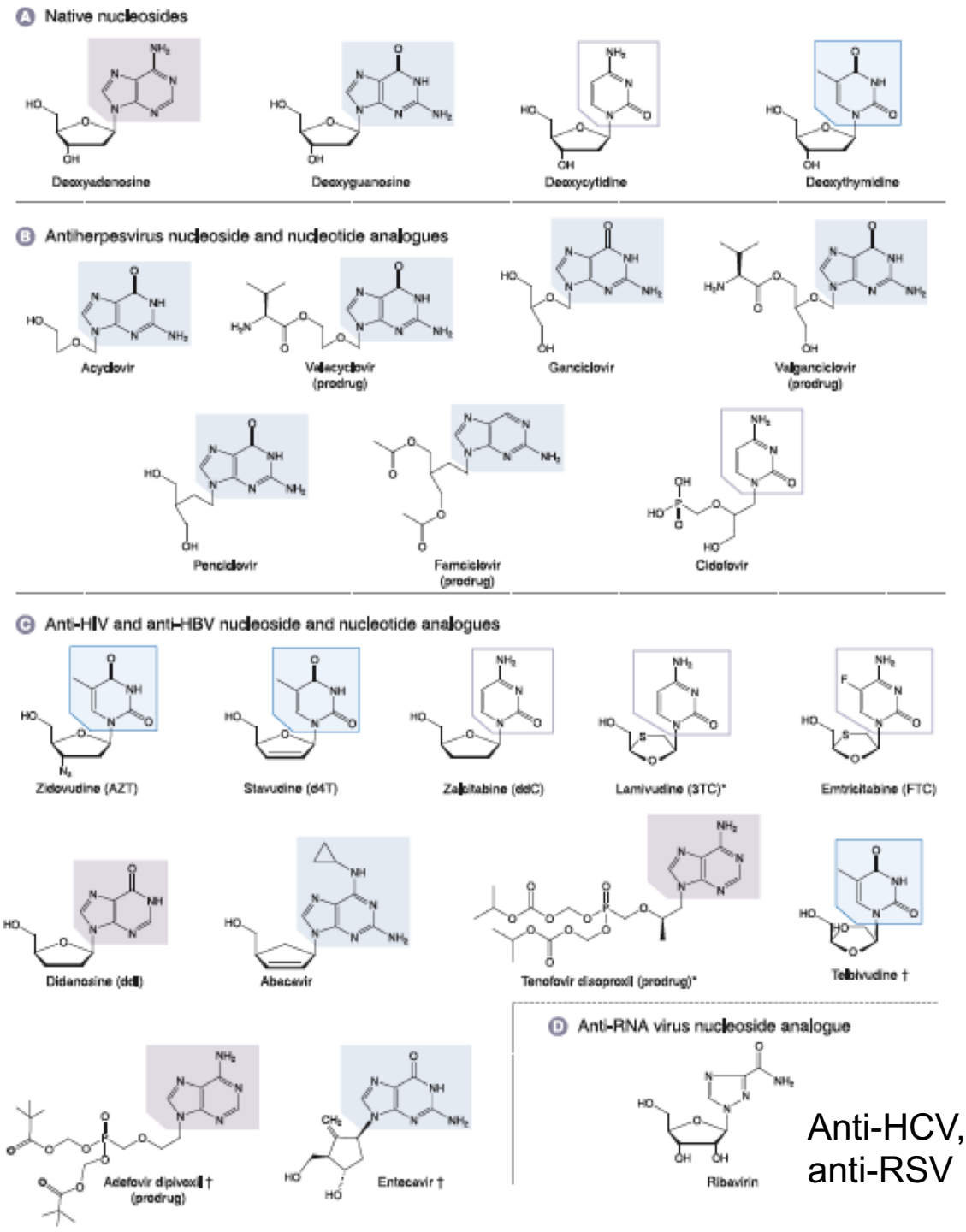
Base modification

Alteration of base pairing



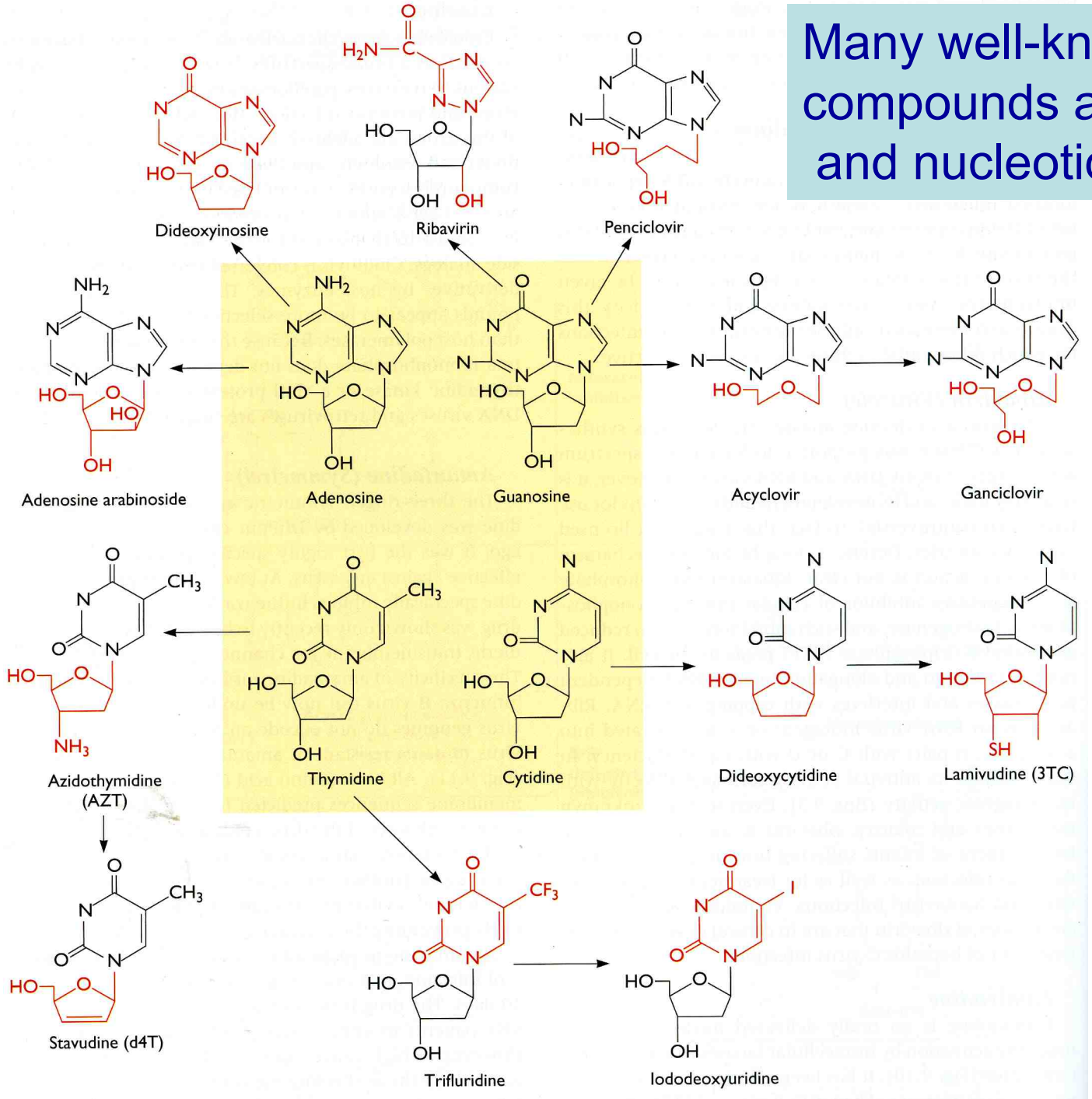
Iododeossiuridina

Structures of antiviral nucleoside analogs

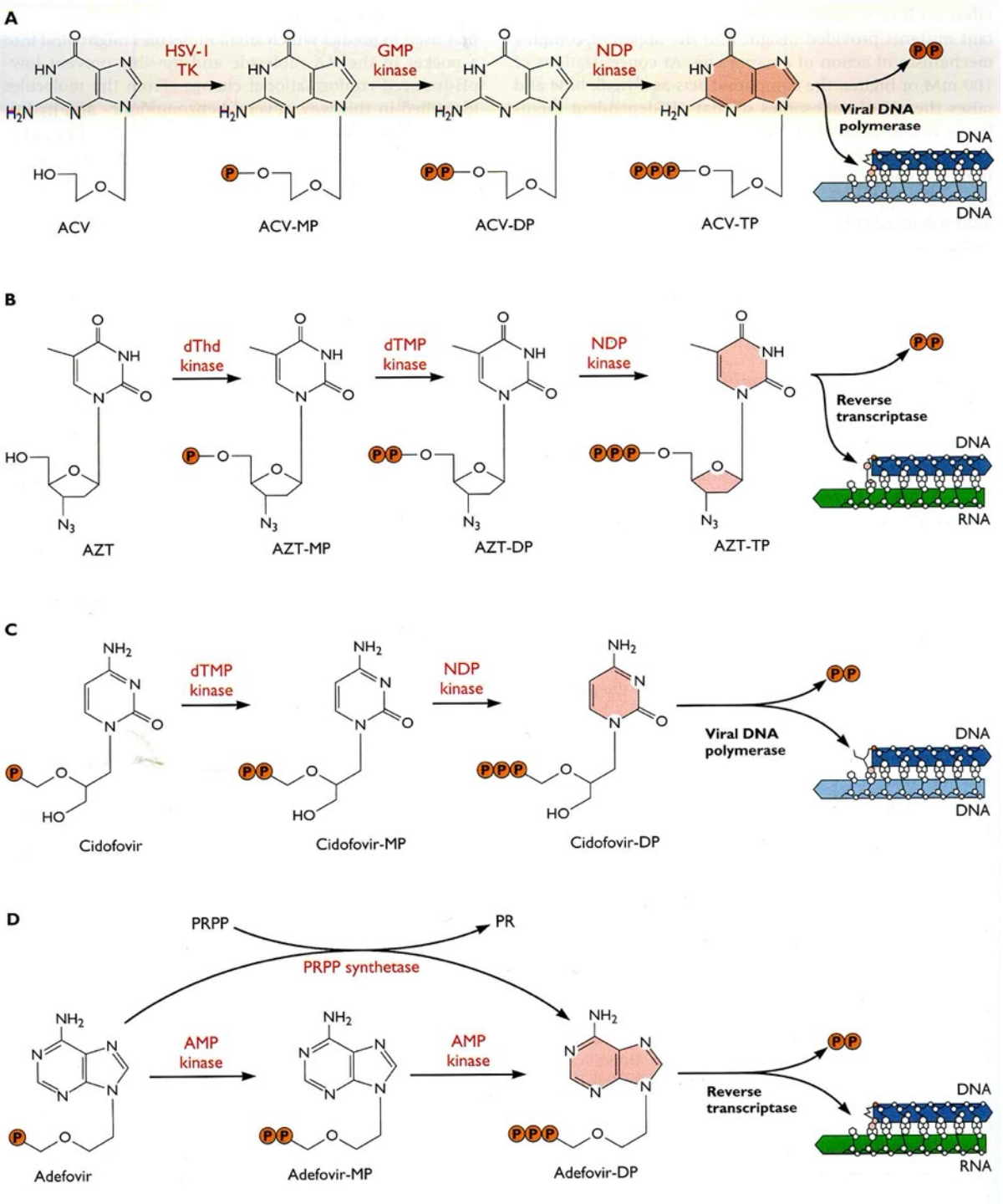


Anti-HCV, anti-RSV

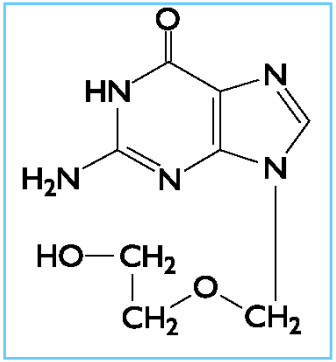
Many well-known antiviral compounds are nucleoside and nucleotide analogs



Chain termination by acyclic nucleoside analogs ACV and AZT, and by acyclic nucleotide analogs Cidofovir and Adefovir



Acyclovir (Zovirax)



✓Acyclovir is a nucleoside analog similar to guanosine, but contain an acyclic sugar group (hydroxyetossimethyl) in place of the deoxyribose. It is a **prodrug** – a precursor of the active antiviral compound

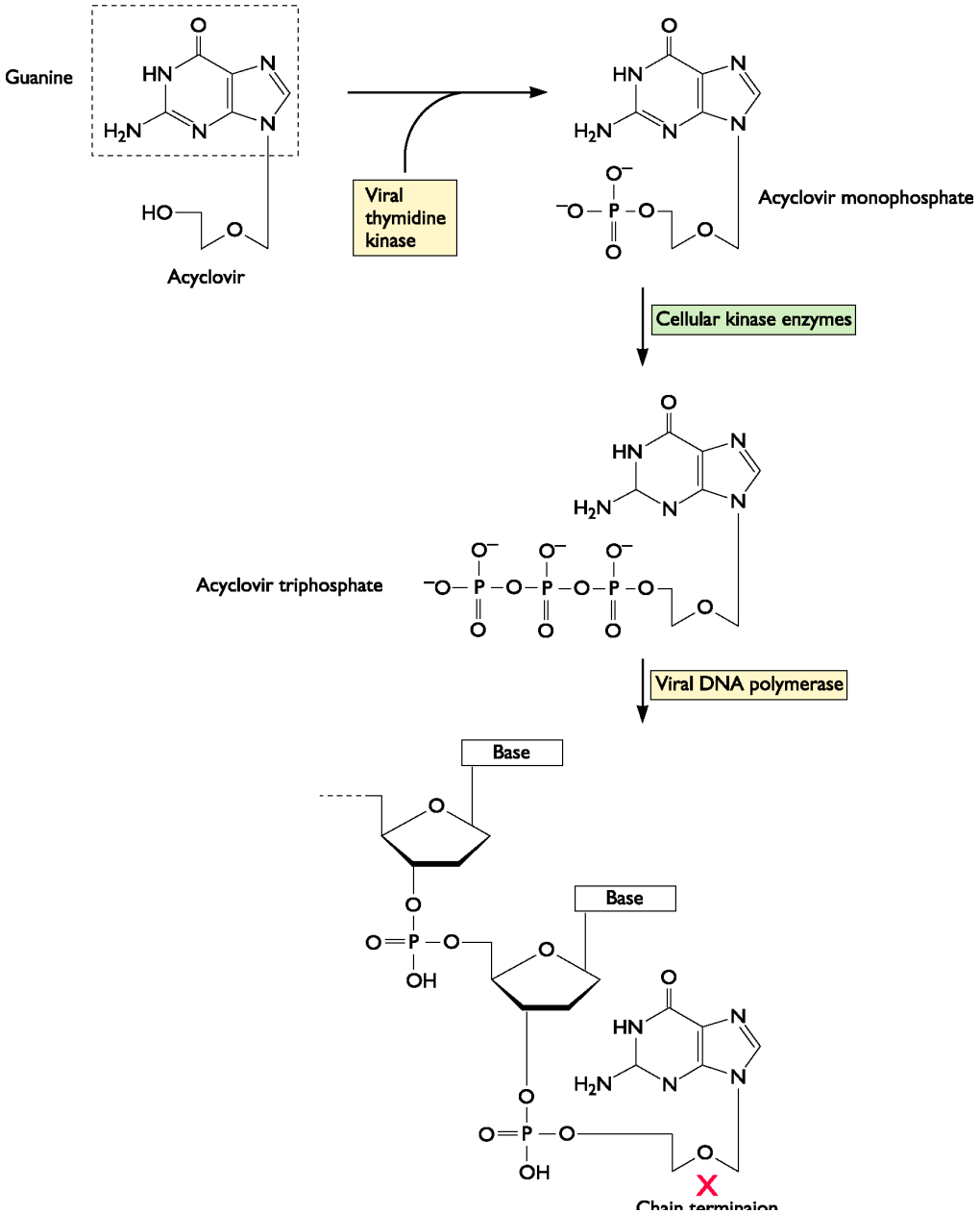
✓Acyclovir is active against HSV and VZV (to some extent)

✓Useful for acute primary diseases and to prevent reactivations

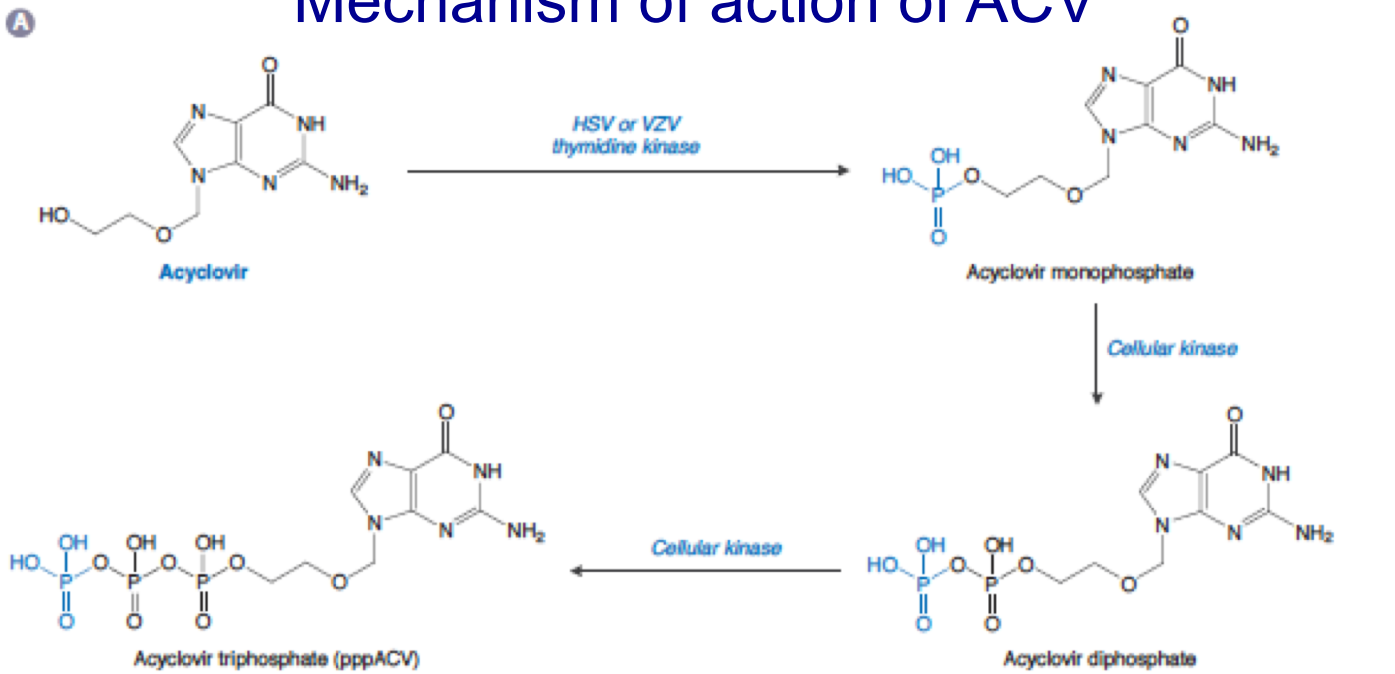
✓It does not eliminate latent infections

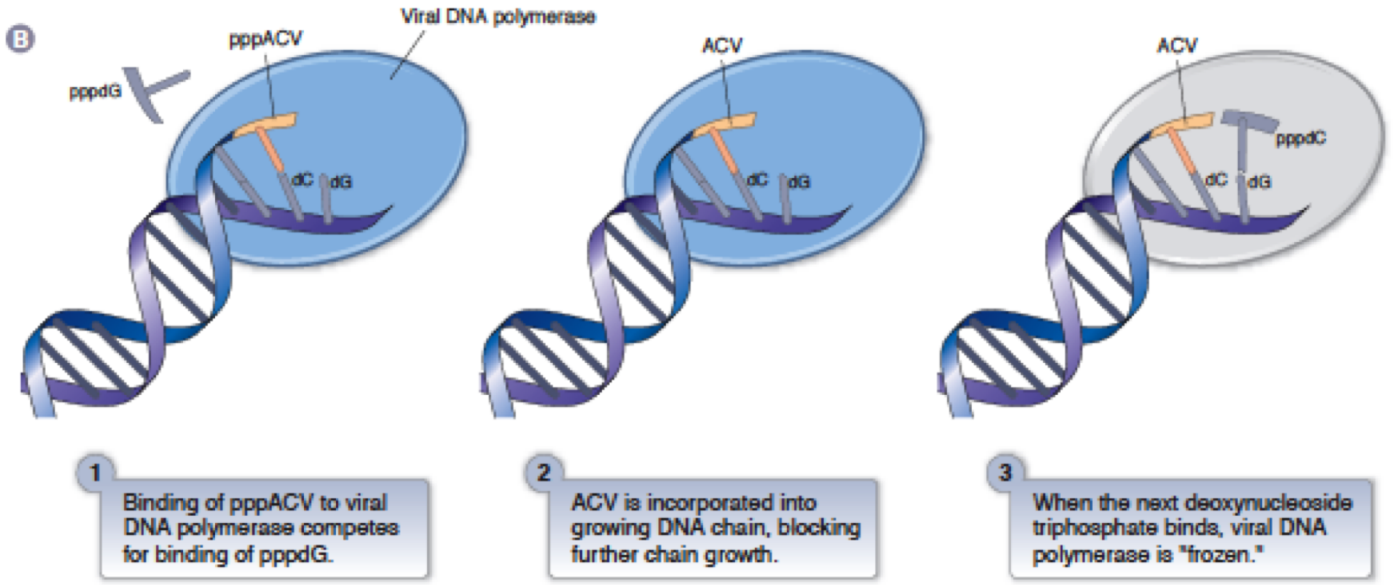
✓Resistance stems from mutations of the viral thymidine kinase (TK) and/or DNA polimerase

Acyclovir: mechanism of action



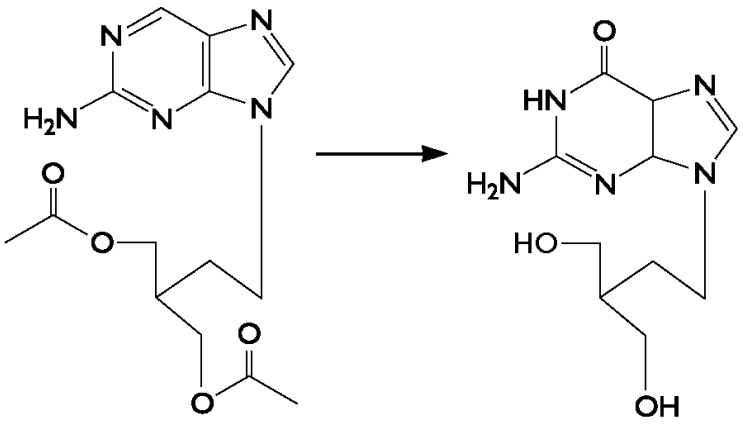
Mechanism of action of ACV





ACV derivatives: Famciclovir and Valaciclovir

Famciclovir:
9-(4-acetoxy-3-acetoxymethylbut-1-yl)guanine (Famvir)



Famciclovir

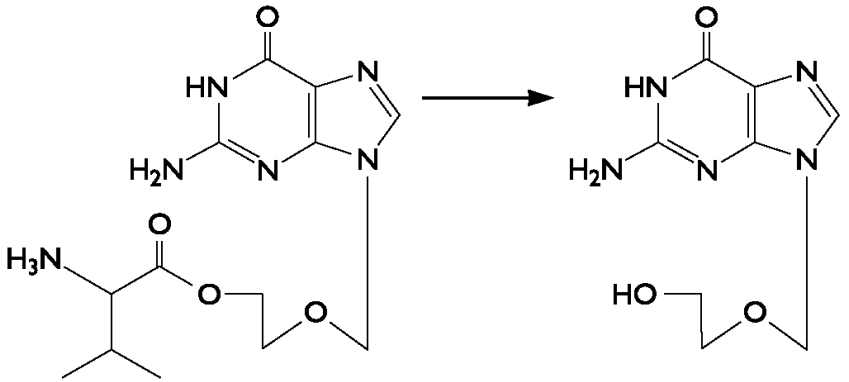
Penciclovir

✓They are **proprodrugs** that require two activation events in a row:

- cleavage of the side chain
- phoshorylation

✓Fivefold-higher oral bioavailability than ACV

Valacyclovir:
L-valyl ester of acyclovir (Valtrex)

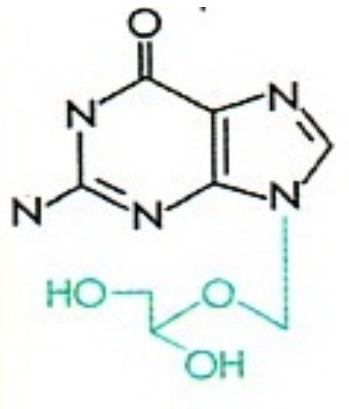


Valine Acyclovir

Acyclovir

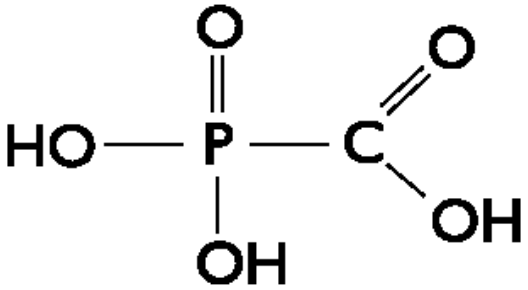
✓Active against HSV and VZV

Ganciclovir (Cytovene)



- ✓GCV is a guanosine analog with an acyclic sugar group (dihydroxyipropoximethyl) in place of deoxyribose
- ✓It is selectively active agaisnt HCMV
- ✓Useful for over HCMV diseases, prophylaxis and preemptive therapy
- ✓Intially given intravenously, quite toxic, used only for life-threatening HCMV infections
- ✓Valgancicovir, a valyl ester prodrug of GCV can be taken orally and is less toxic
- ✓Resistance stems from mutations of the viral kinase (pUL97) and/or DNA polimerase

Foscarnet (Foscavir)



✓Foscarnet it is the only nonnucleoside DNA replication inhibitor of herpesviruses. It is a pyrophosphate analog (phoshonoformic acid)

✓Foscarnet it is a noncompetitive inhibitor of the pyrophoshate-binding site of herpesvirus DNA polymerases

✓It is active against HCMV (HSV, HBV, HIV)

✓Useful for therapy of HCMV GCV-resistant strain infections

✓It accumulates in bone and causes kidney toxicity, recommended only for life-threatening infections for which other anti-HCMV drugs are no longer effective

✓Resistance stems from mutations of the viral DNA polimerase

Examples of viral targets for antiviral drugs

- ✓**Attachment**
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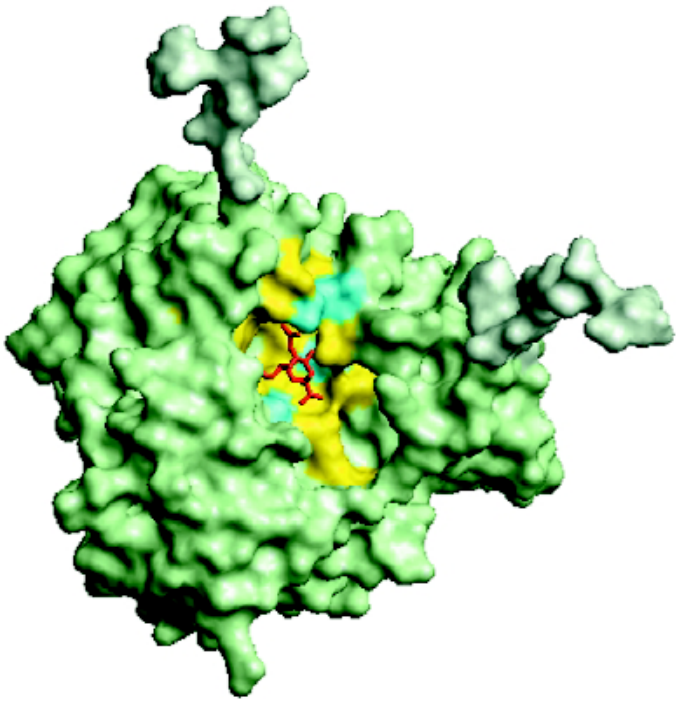
Maturation and release

Agent	Virus
Peptidomimetics, substrate analogs	**HIV**
Neuraminidase inhibitors	**Influenza**

Neuroaminidase inhibitors:
an example of structure-based design in antiviral drug discovery

Neuraminidase: structure and function



In light blue and yellow,
NA active site invariant amino acids
In red, neuraminic acid

✓NA is a tetrameric envelope glycoprotein

✓NA cuts the the a-chetosidic bond between terminal neuraminic (sialic) acid and the oligosaccharide chain

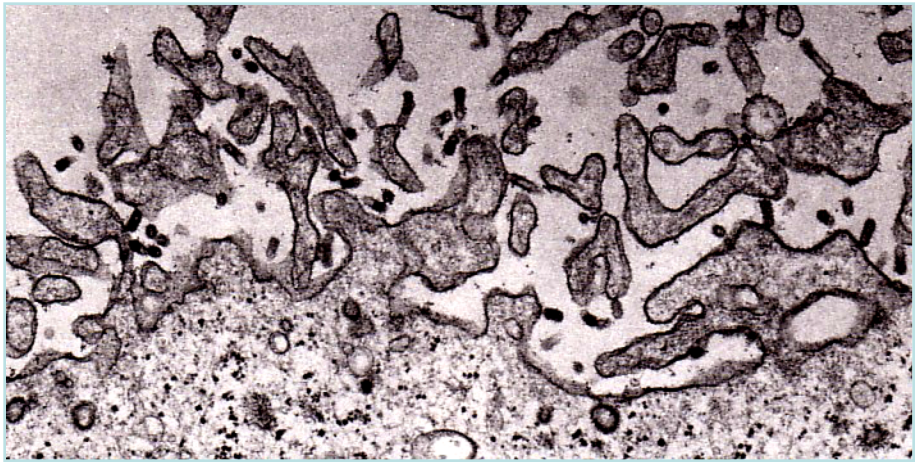
✓NA destroys HA cell surface receptors

✓NA promotes virus penetration through sialic acid-rich secretions

✓NA mediates release of viral progenies by cutting sialic acid residues of the surface of infected cells, thus breaking down non-productive re-attachment to cell receptors during exit.

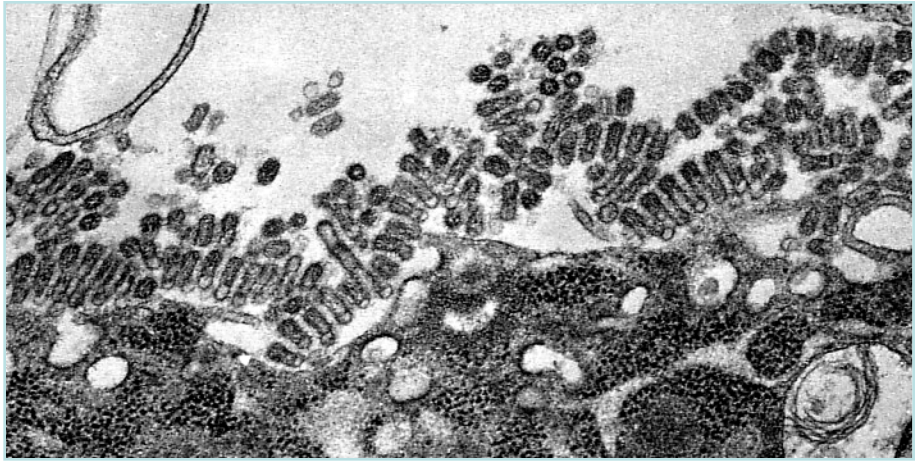
Function of Influenza virus neuraminidase

P. Palese, M. Ueda, K. Tobita, R.W. Compans. Characterization of temperature sensitive influenza virus mutants defective in neuraminidase. Virology 61:397–410 (1974).



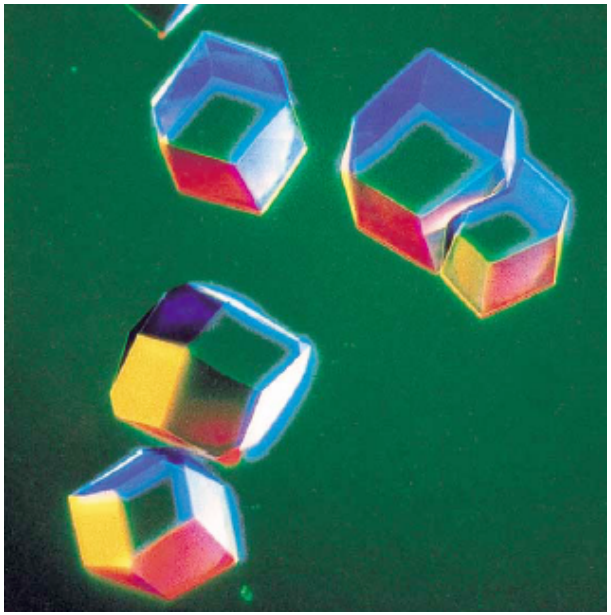
33° C
WT phenotype
Release of infectious free virions

39.5° C
NA- phenotype
Non-infectious virions aggregates



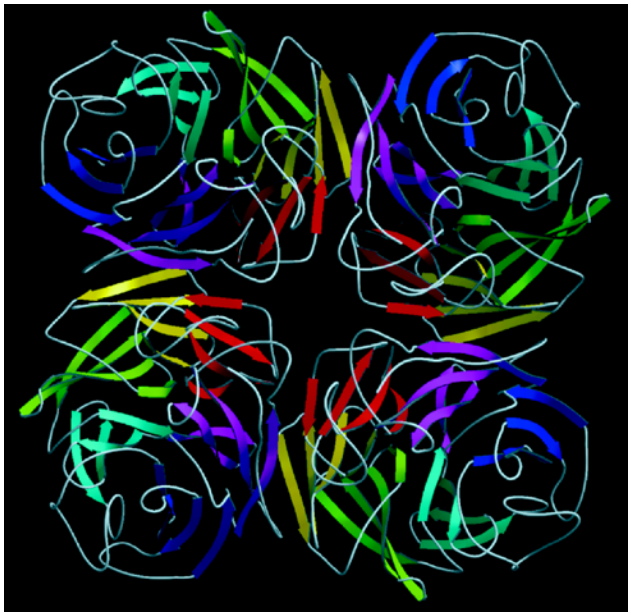
Development of neuroaminidase inhibitors

1



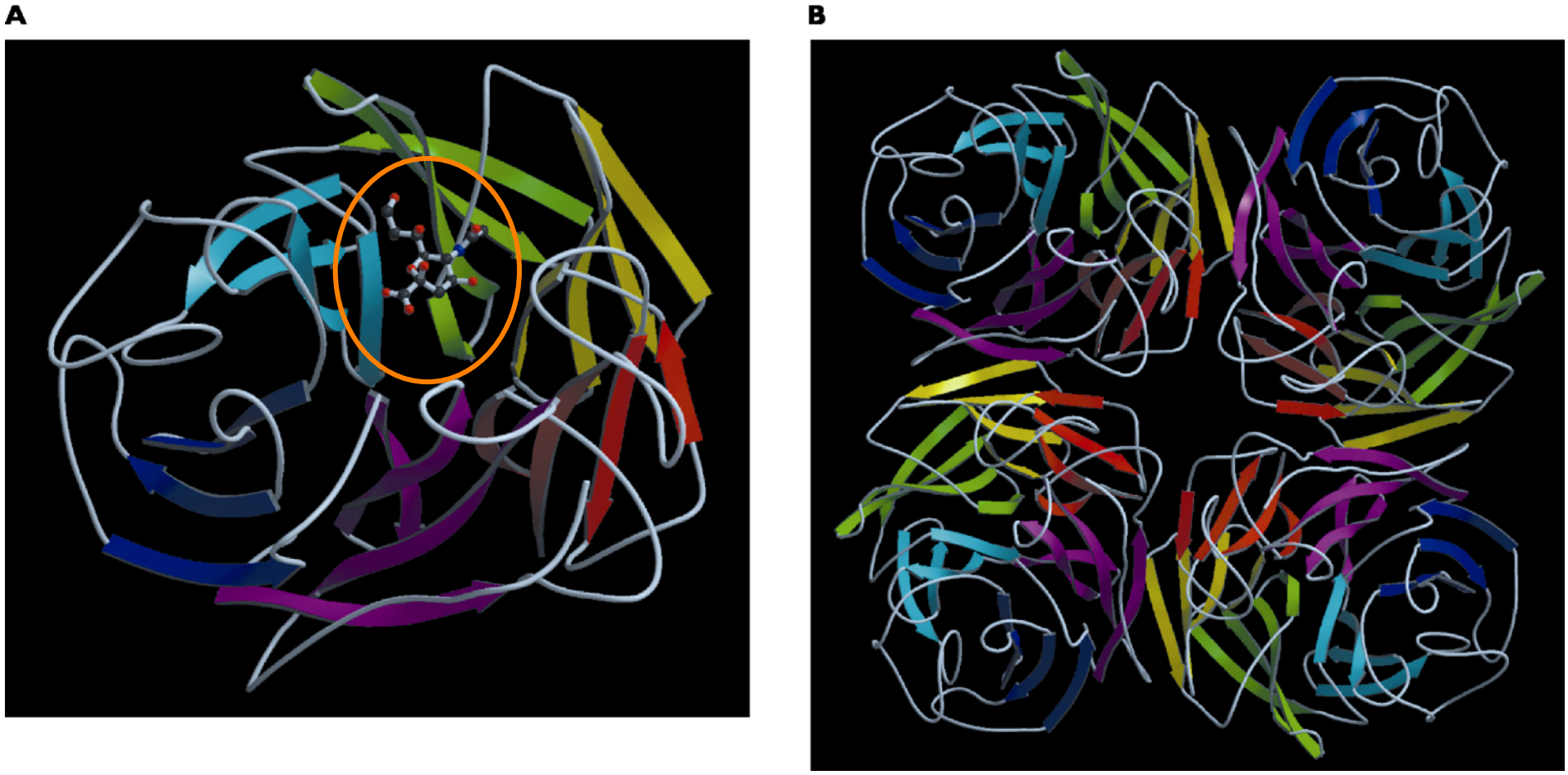
Crystallization

2



Structural analysis

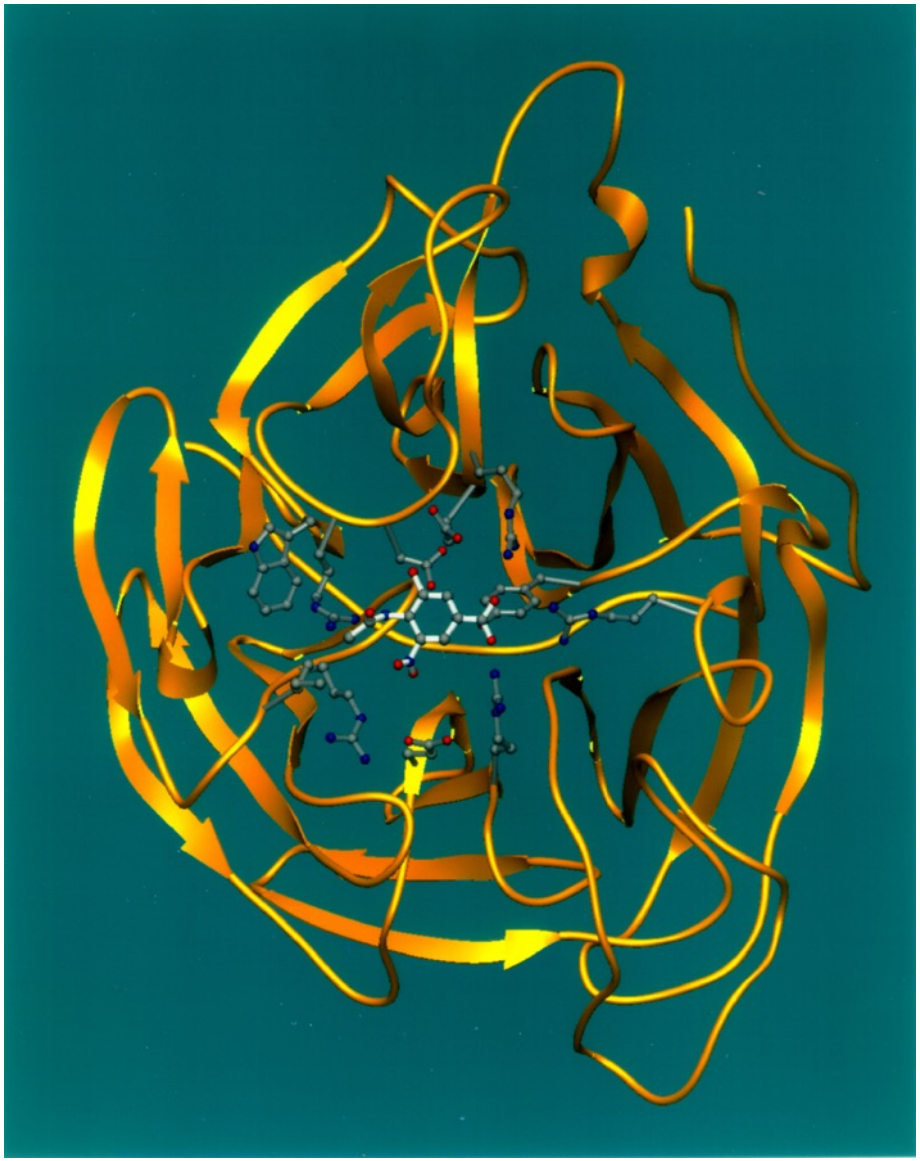
Structure of influenza A virus neuroaminidase

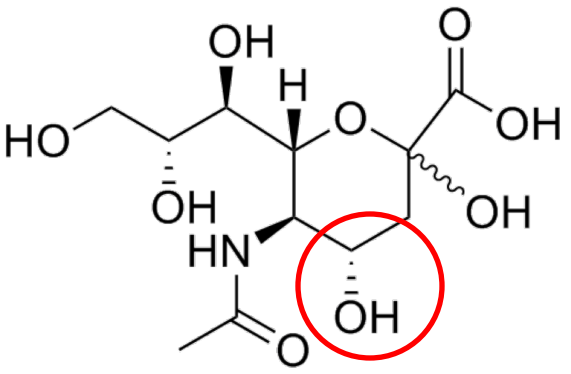


The binding site of the enzyme does not vary from strain to strain

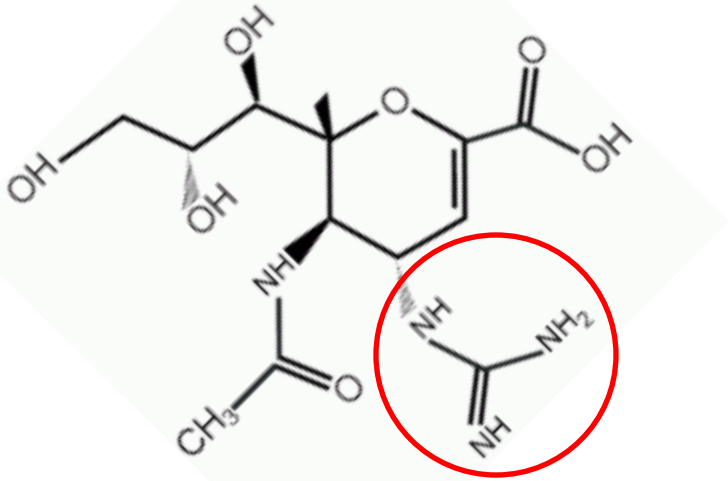
- It consists of 18 amino acid residues of which 12 are in direct contact with the bound sialic acid analogue (and presumably with sialic acid in catalytically active situations).
- Four of these 12 are positively-charged arginines, while another 4 are **negative-charged glutamic** and **aspartic acid** residues. The remainder are neutral (tyrosine, asparagine, isoleucine and tryptophan).

Structure of the influenza A neuraminidase N9 bound to an analogue of sialic acid has been determined by X-ray crystallography



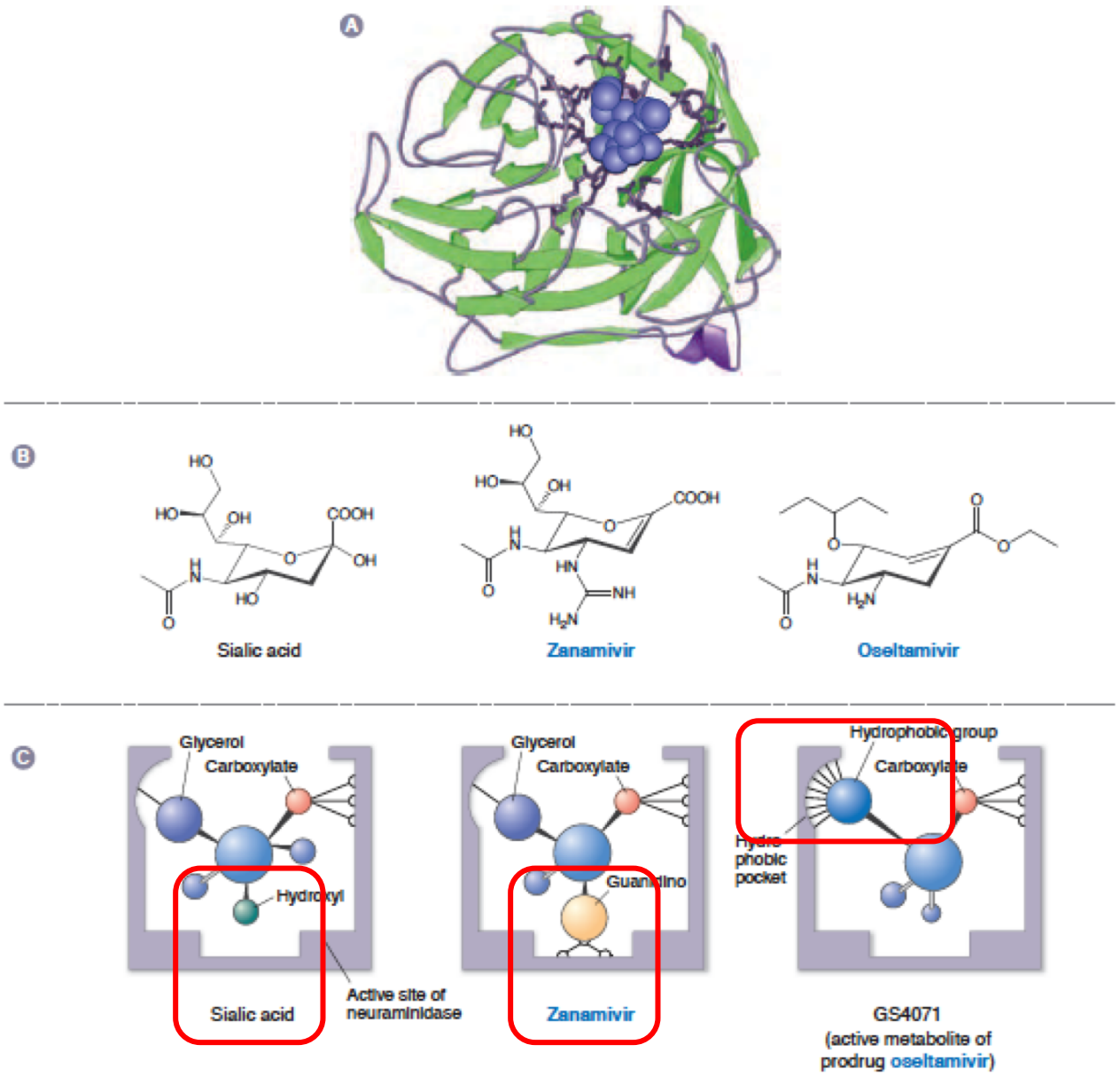


Sialic acid
(*N*-acetyl neuraminic acid)

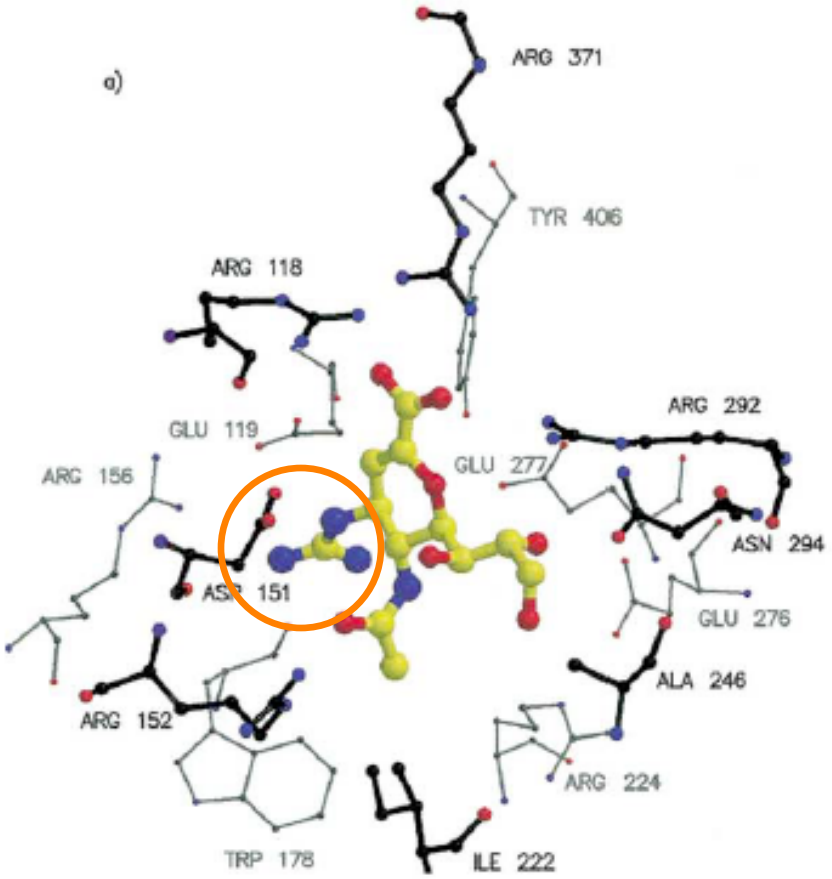


Zanamivir

Strucure-based design of inhibitors of IV A and B neuraminidase



Model of part of the active site of influenza neuraminidase bound to zanamivir

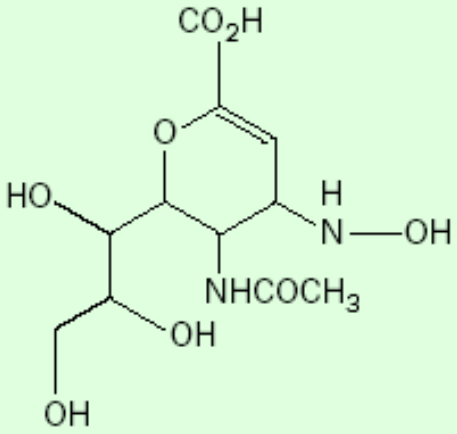


Crystallographic structure of the influenza A neuraminidase with an inhibitor (Zanamivir) bound to the active site

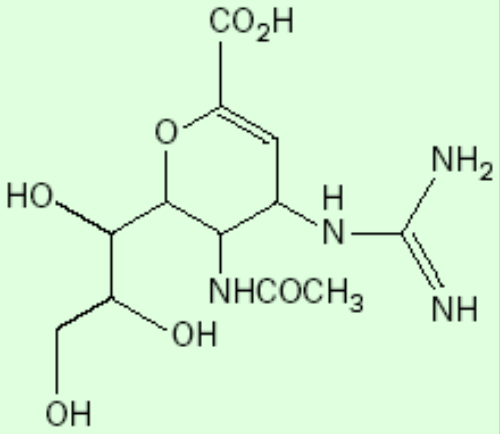
Rational drug design

Development of neuroaminidase inhibitors

Neu5Ac2en

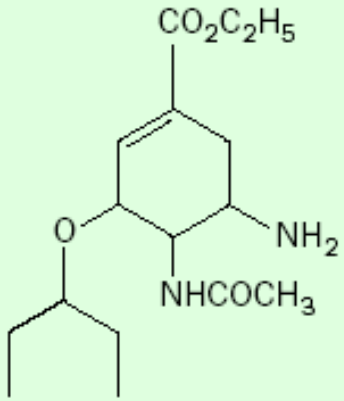


Zanamivir



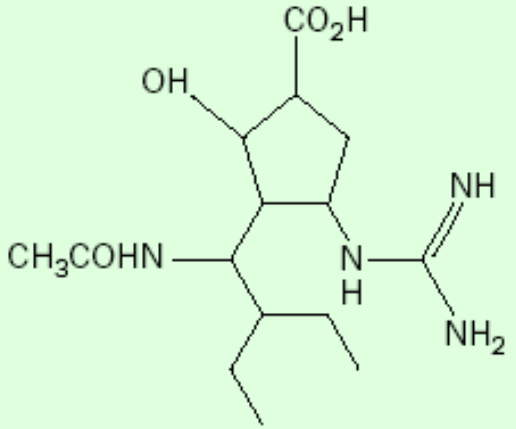
Relenza
(Biota/Glaxo)
FDA approved
Inhalation

Oseltamivir



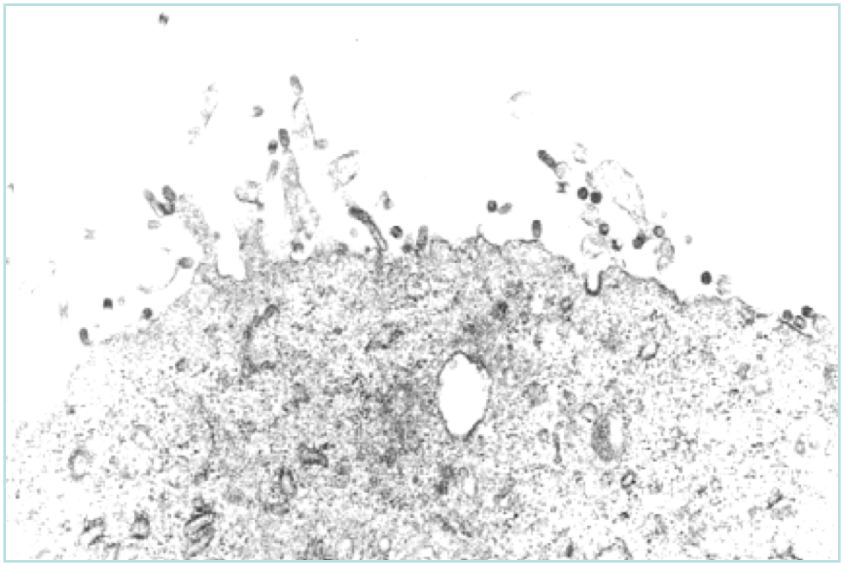
Tamiflu
(Gilead/Hoffmann
La Roche)
FDA approved
Oral

RWJ-270201

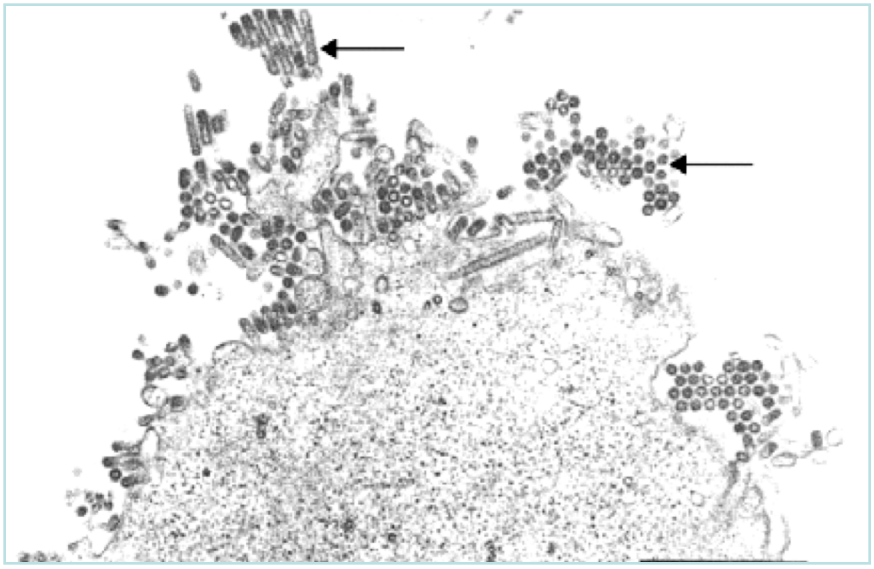


(BioCryst/
Johnson & J)
Clinical trials
Oral

Development of neuroaminidase inhibitors: *in vitro* activity



Untreated cells:
the virus is normally assembled and released from infected cells



Cells treated with a NA inhibitor:
the virus forms large non-infectious aggregates on the surface of infected cells

Zanamivir (*Relenza*) and Oseltamivir (*Tamiflu*)

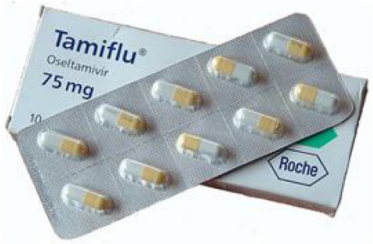
✓Neuraminic (sialic) acid analogs

✓They specifically inhibit NA of influenza and B viruses

✓Useful in therapy and prophylaxis of influenza infections. If given within 48, they will reduce disease's symptoms and duration.

Zanamivir: inhalation by Diskhaler

Oseltamivir: oral



✓Resistance stems from NA mutations