Introduction to Drug Discovery: Application to Viral Targets

Fall 2004, AMS-691 Section 2
Topics in Applied Mathematics

Introduction to Computational Structural Biology and Drug Design

Meeting 02, 09/01/04, Topics 1 and 2

Robert C. Rizzo

Food and Drug Administration (~ 100 years)

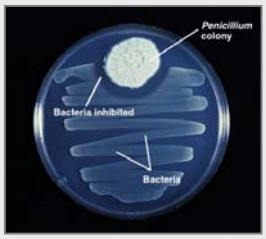
- 1862 Abraham Lincoln appointed chemist Charles M. Wetherill to head the chemical division in the newly created Dept. of Agriculture.
- 1938 Following the death of 107 persons (children) who took a poisonous "Elixir of Sulfanilamide", Congress strengthened public health protection by passing the Federal Food, Drug and Cosmetic Act
- 1962 Dr. Frances Kelsey, an FDA officer, refused to allow the marketing of thalidomide, a sleeping pill that lacked sufficient evidence of safety. Responding to thousand of birth defects that the drug was causing in Europe and South America, Congress passed the Kefauver-Harris Amendments requiring evidence of drug effectiveness as well as greater safety.

The Birth of Modern Chemotherapy

- Treatment with chemicals is chemotherapy. Quinine was one of the first known chemical used to treat a disease (malaria).
- 1796 Edward Jenner inoculated a person with cowpox virus. The person was then protected from smallpox.
- 1861 Beginning with Louis Pasteur's work, discoveries included the relationship between microbes and disease, and antimicrobial drugs
- 1910 Paul Ehrlich discovered salvarsan, an arsenic derivative, was effective against syphilis.

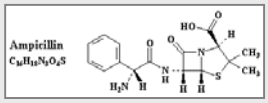
The Birth of Modern Chemotherapy

- 1928 Alexander Fleming discovered the first antibiotic.
- He observed that *Penicillium* fungus made an antibiotic, penicillin, that killed S. *aureus*.
- 1939 Florey, Chain, and Associates began work on isolating and synthesizing large amounts of *Penicillin*.
- 1944 Used in WWII to treat infections.



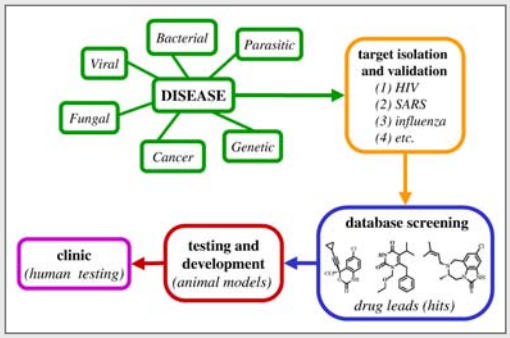
Penicillin Binds to a Specific Bacterial Protein

- Penicillin is a member of a class of compounds called β-lactams.

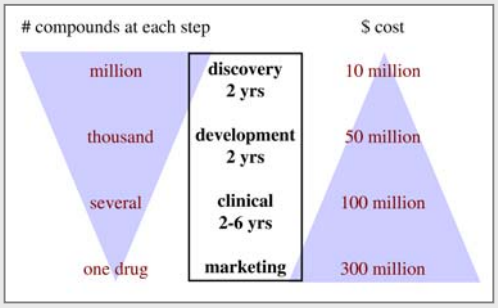


- These compounds interfere with bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs) which are located in bacterial cell walls
- Inhibition of PBPs leads to inhibition of peptidoglycan synthesis needed to build the bacterial cell wall.
- The bacterial cell dies.

Modern Drug Development



Drug Design Pyramid*



*From I. D. Kuntz (UCSF)

Drug Discovery Disciplines

- Medicine
- Physiology/pathology
- Pharmacology
- Molecular/cellular biology
- Automation/robotics
- Medicinal, analytical,and combinatorial chemistry
- Bioinformatics, structural and computational chemistries

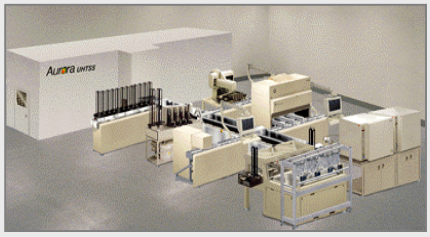
Some Corporate Decisions

- What is the market for this potential therapeutic?
- Is the indication a chronic disease or a potentially fatal disease
- What is the competition?
- Can it be 'Fast Tracked' (accelerated approval process with FDA) ?
- How many people contracted the disease in the U.S.?
- Can it be manufactured at a reasonable cost?

Serendipity and Drug Discovery

- Often molecules are discovered/developed for one indication and then turn out to be useful for others
  - Tamoxifen (birth control and cancer)
  - Viagra (hypertension and erectile dysfunction)
  - Salvarsan (Sleeping sickness and syphilis)
  - Interferon-α (hairy cell leukemia and Hepatitis C)
- Compounds can bind to multiple protein targets (side-effects)

Traditional Drug Discovery: High-Throughput Screening (HTS)

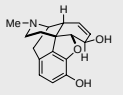


Screening perhaps millions of compounds in a library to see if any show activity against a certain disease (196, 3184, 1500 well plates)

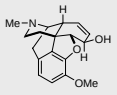
Traditional Drug Discovery: High-Throughput Screening (HTS)

- HTS Pros
  - no knowledge needed (but need a good assay)
  - anything might turn up
  - easy to scale up (robotics)
  - the major paradigm for drug discovery (~ 30% of research dollars)
- HTS Cons
  - hard to use prior knowledge
  - many false positives
  - expensive to keep "production line" running (overhead)
  - data are yes/no rather than quantitative
  - inventory problems (libraries may contain many errors)
  - garbage in/garbage out

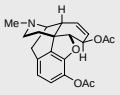
Lead Refinement: Quantitative Structure Activity Relationships (QSAR) for Opiates



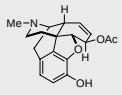
morphine
relative potency = 1
addictive



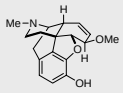
codeine
relative potency = 0.2
addictive



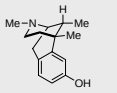
heroin
relative potency = 2
addictive



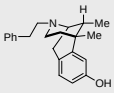
6-acetylmorphine
relative potency = 4
addictive



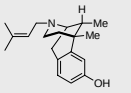
heterocodeine
relative potency = 5
addictive



metazocine
relative potency = 1
addictive



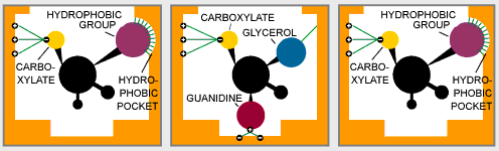
phenazocene
relative potency = 4
NON-addictive



pentazocine
relative potency = 0.33
NON-addictive

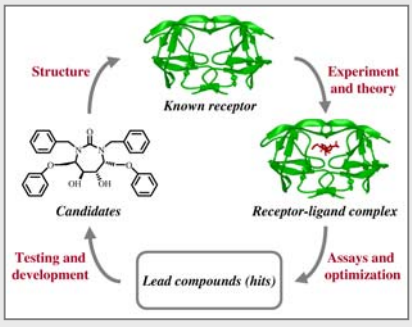
Anti-Influenza Drugs: Binding-site Schematic

Sialic acid | **Zanamivir (inhaled)** | **Oseltamivir (oral)**



Structure and function (potency) intimately related to activity

Structure-based Design and Discovery*



*Irwin D. Kuntz *Science* **1992**, *257*, 1078-1082

Computational Structure-based Drug Design

- Structural: (homology modeling, docking)
- Lead discovery and optimization: (database screening via computers, SAR: structure activity relationships)
- Pharmacological: (ADME: absorption, distribution, metabolism, and excretion)

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- Molecular recognition: (affinity, resistance, selectivity)

Computational methods yield *energetic* and *structural* information about the system being modeled that can be used to understand experimental systems

Advances in Computing Power

"If the automobile industry had made as much progress in the past fifty years, a car today would cost a hundredth of a cent and go faster than the speed of light."

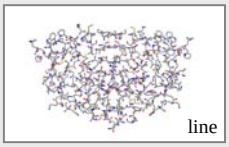
- Ray Kurzweil, *The Age of Spiritual Machines*

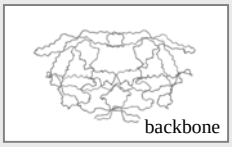
Many Biological Systems (drugs and receptors) can be Modeled on a Computer Using the Techniques of Molecular Mechanics

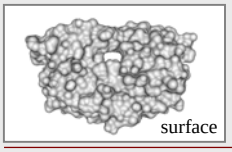
- Each atom is represented as a sphere in 3-D space
  - atom type (C, N, H, O, S, etc)
  - radius (size)
  - partial charge (electron density)
- Molecular Mechanics calculations yield detailed atomic level *structures* and *energies*
- Energy = bonds + angles + charge-charge + steric fit

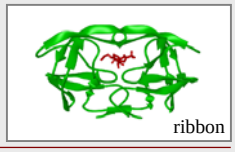
In general, lower (more favorable) energies are desirable

Pictorial representations of a receptor: Each depiction yields unique information that can be used to help understand how a drug interacts with its target


line

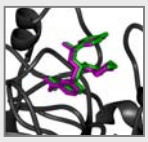

backbone


surface

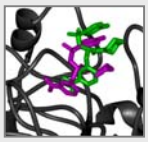

ribbon

Calculations Yield Detailed *Structures* At the Atomic Level

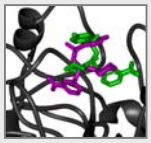
Three computational results are shown which depict how a drug (green) might interact with a receptor (gray). The experimentally observed position is also shown (magenta).


0.56 Å rmsd

correct


3.34 Å rmsd

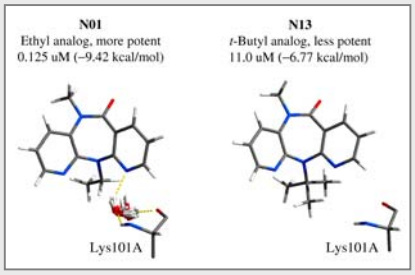
close


6.29 Å rmsd

incorrect

smaller root mean square deviation (rmsd) = better match

Calculations Yield *Energies*



For the nevirapine analogs shown above (anti-HIV compounds), the *structures* available from computer calculations are helpful in understanding why the compound on the left has greater activity (*energy*)

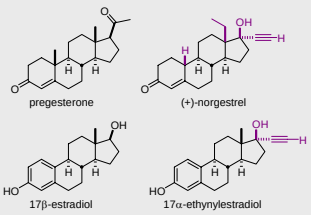
Rational Drug Design

- Computational structural biology (computational chemistry)
  - Molecular modeling
  - Property prediction
  - Statistical modeling, QSAR, etc
- Experimental structural data (3D)
  - X-ray diffraction
  - Nuclear Magnetic Resonance
- Data mining, information clustering, diversity analysis, descriptors

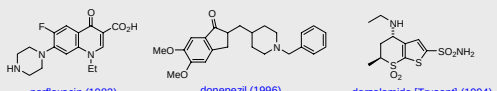
Rational Drug Design Leads May Also be Based on Natural Enzyme Substrates, Chemical Messengers…

NATURAL LEAD SYNTHETIC DRUG

Oral Conctraceptives



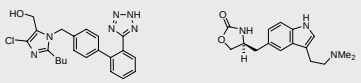
Rational Drug Design Successes



norfloxacin (1983)
antibiotic
first of the 6-fluoroquinolones
QSAR studies

donepezil (1996)
Alzheimer's treatment
acetylcholinesterase inhibitor
shape analysis and docking studies

dorzolamide [Trusopt] (1994)
glaucoma treatment
carbonic anhydrase inhibitor
SBLD and *ab initio* calcs



losartan [Cozaar] (1995)
angiotensin II antagonist
anti-hypertensive
Modeling Angiotensin II octapeptide

zolmatriptan [Zomig] 1995
5-HT_{1D} agonist
migraine treatment
Molecular modeling

Many Disease Targets (Viruses, Parasite, Bacteria)

TABLE 1. Some Infectious Agents and Diseases Recognized Since 1973*

1973	Rotavirus	Virus	Major cause of infantile diarrhea worldwide
1975	Parvovirus B19	Virus	Aplastic crisis in chronic hemolytic anemia
1976	*Cryptosporidium parvum*	Parasite	Acute and chronic diarrhea
1977	Ebola virus	Virus	Ebola hemorrhagic fever
1977	*Legionella pneumophila*	Bacteria	Legionnaires' disease
1977	Hantaan virus	Virus	Hemorrhagic fever with renal syndrome
1977	*Campylobacter jejuni*	Bacteria	Enteric pathogens distributed globally
1980	Human T-lymphotropic virus 1 (HTLV-1)	Virus	T-cell lymphoma-leukemia
1981	Toxin producing strains of *Staphylococcus aureus*	Bacteria	Toxic shock syndrome (tampon use)
1982	*Escherichia coli* O157:H7	Bacteria	Hemorrhagic colitis; hemolytic uremic syndrome
1982	HTLV-2	Virus	Hairy cell leukemia
1982	*Borrelia burgdorferi*	Bacteria	Lyme disease
1983	Human immunodeficiency virus (HIV)	Virus	Acquired immunodeficiency syndrome (AIDS)
1983	*Helicobacter pylori*	Bacteria	Peptic ulcer disease
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhea
1986	*Cyclospora cayatanensis*	Parasite	Persistent diarrhea
1988	Human herpes virus-6 (HHV-6)	Virus	Roseola subitum
1988	Hepatitis E	Virus	Enterically transmitted non-A, non-B hepatitis
1989	*Ehrlichia chaffeensis*	Bacteria	Human ehrlichiosis
1989	Hepatitis C	Virus	Parenterally transmitted non-A, non-B liver infection
1991	Sudnarlo virus	Virus	Venezuelan hemorrhagic fever
1991	*Encephalitozoon hellem*	Parasite	Conjunctivitis, disseminated disease
1991	New species of *Babesia*	Parasite	Atypical babesiosis
1992	*Vibrio cholerae* O139	Bacteria	New strain associated with epidemic cholera
1992	*Bartonella henselae*	Bacteria	Cat-scratch disease; bacillary angiomatosis
1993	Sin nombre	Virus	Adult respiratory distress syndrome
1993	*Encephalitozoon cuniculi*	Parasite	Disseminated disease
1994	Sabia virus	Virus	Brazilian hemorrhagic fever
1995	HHV-8	Virus	Associated with Kaposi's sarcoma in AIDS patients

*Adapted from Table 1 in *Global Microbial Threats in the 1990s, Report of the NSTC CIDI 1 Working Group on Emerging and Re-Emerging Infectious Diseases*, 1995

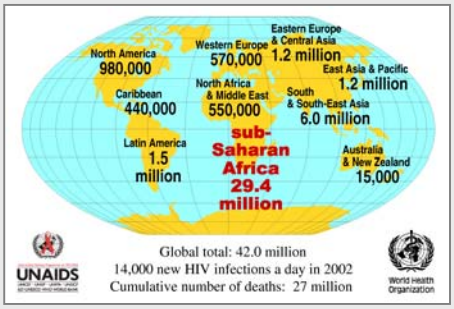
Potential Bioterrorism Targets are a NEW threat

- Small Pox
- Anthrax
- Filoviruses
- Influenza
- Botulism
- Plague
- Multi-drug resistant Tuberculosis

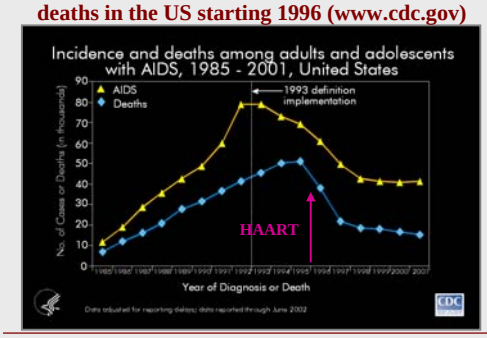
Important Viral Targets

- HIV: Human Immunodeficiency Virus
- SARS: Severe Acute Respiratory Syndrome
- Influenza
- HCV: Hepatitis C Virus

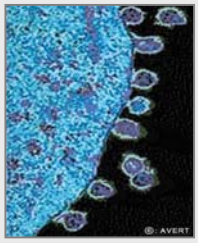
Adults and children estimated to be living with HIV/AIDS as of end 2002 (www.unaids.org)

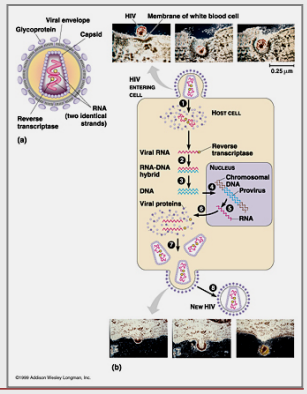


Highly Active Anti-Retroviral Therapy (HAART) attributed to dramatic declines in AIDS related deaths in the US starting 1996 (www.cdc.gov)

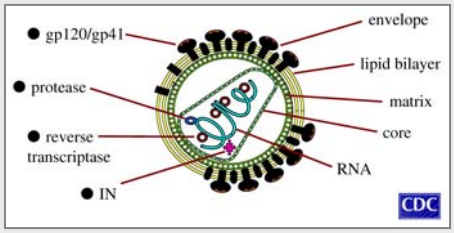


HIV Life Cycle



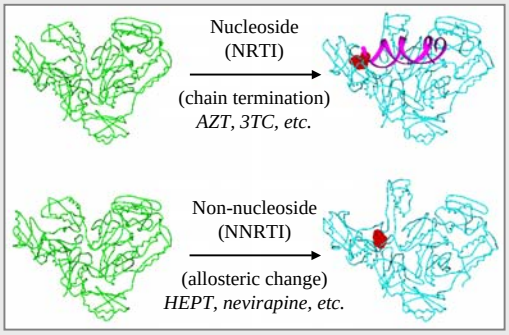


HIV viral particle and anti-viral targets

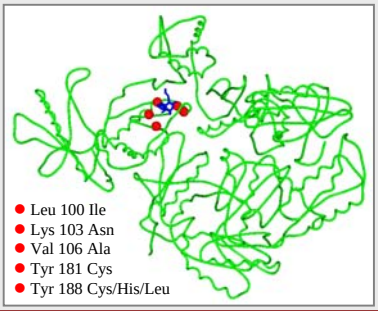


Reverse transcriptase (HIV RT) converts viral RNA to viral DNA which is then incorporated into the host cell genome

Two classes of HIV-1 RT inhibitors



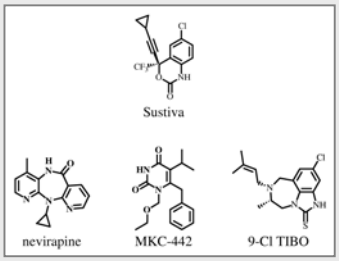
Amino acid point mutations in HIV reverse transcriptase confer drug resistance



To date, all anti-HIV drugs become less effective with continued use (resistance)

- Viral infection results in a population of HIV particles (swarm) with individual HIV proteins having small genetic differences
- The most populated amino acid sequence for a given protein is termed wild-type
- Drugs that inhibit wild-type proteins shift the population distribution
- Drug-resistant proteins with genetic mutation(s) eventually dominate the swarm (viral evolution)

Why does Sustiva have a superior fold resistance profile towards common HIV RT point mutations ?



Fold resistance (FR) = mutant activity / wild type activity

Sustiva structure-based project (example from my own work)

- Can the reason(s) for Sustiva's superior resistance profile be determined using computational structure-based design?
- Use a computational technique called DOCKING to predict how Sustiva interacts with the HIVRT protein.
- Validate that the DOCKING calculation methods and protocols are appropriate for this system (controls).
- Use the predicted *structure* of the Sustiva-HIVRT complex to compute a resistance profile (*energies*) for Sustiva and the other anti-HIVRT drugs and compare the results with experiment.

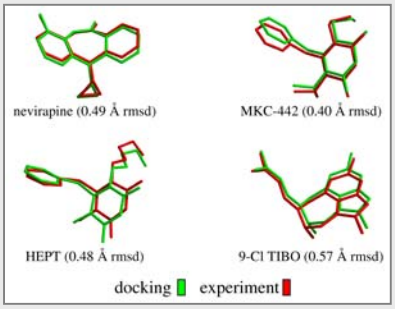
Molecular recognition via docking (How does Sustiva bind to HIV RT ?)

Key idea: find ligand orientations complementary to a target (solve a complicated 3-D jigsaw puzzle)

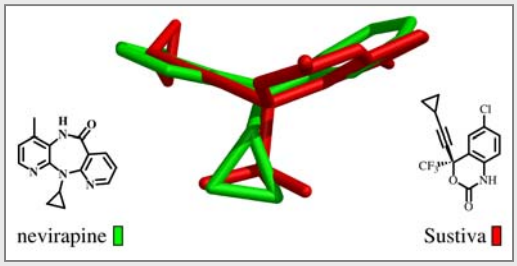
- Based on the lock and key hypothesis (Ehrlich ~ 1900)
  Lock = target (HIV RT)
  Key = ligand (Sustiva)

- First program: DOCK (I. D. Kuntz, UCSF, 1982)
  Energy score = $E_{coul} + E_{vdw}$ (electrostatic + steric energy)

Correctly docked "known" structures help provide confidence for novel predictions



The Sustiva prediction shows the butterfly binding mode observed in other NNRTI complexes



A measure of a drug's effectiveness against a mutation is given by the fold resistance

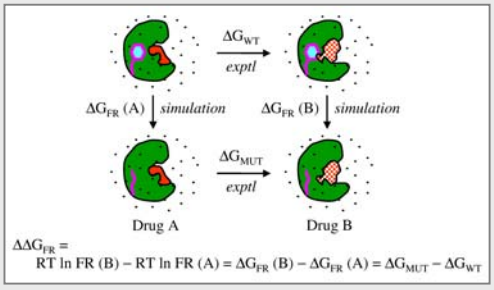
$$\text{Fold Resistance (FR)} = \frac{\text{mutant activity}}{\text{wild type activity}}$$

$$\text{Fold Resistance (FR)} = \frac{\text{Cys}\,181}{\text{Tyr}\,181}$$

$$\text{Fold Resistance (FR)} = \frac{\text{Ala}\,106}{\text{Val}\,106}$$

Common point mutations

If the predicted Sustiva-HIVRT *structure* is correct then fold resistance *energies* from computer simulations should parallel experiment



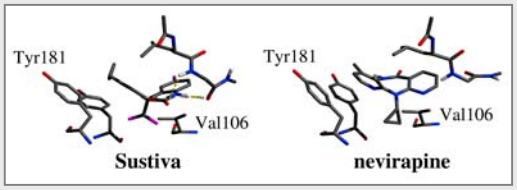
$$\Delta\Delta G_{FR} = RT \ln FR\,(B) - RT \ln FR\,(A) = \Delta G_{FR}\,(B) - \Delta G_{FR}\,(A) = \Delta G_{MUT} - \Delta G_{WT}$$

Fold resistance *energies* from computer simulations will parallel experiment[a-g] if the *structure* is correct ($\Delta G_{FR} = RT \ln (FR)$)

	Fold Resistance = Cys 181 mut / Tyr 181 wt		Fold Resistance = Ala 106 mut / Val 106 wt	
Inhibitor	**calculated**	**exptl (avg)**	**calculated**	**exptl (avg)**
Sustiva	**0.0**	**0.0**[b]	**0.0**	**0.0**[b]
nevirapine	**3.9** ± 0.29	**2.6** ± 0.36[c,d,e]	**3.3** ± 0.42	**2.5** ± 0.37[c,d,e]
MKC-442	**4.7** ± 0.29	**3.4** ± 1.51[f,g]	**0.7** ± 0.47	**2.4**[e]
9-Cl TIBO	**3.0** ± 0.33	**1.2** ± 0.95[d,e,e]	**1.3** ± 0.47	**1.1** ± 0.42[d,e,e]

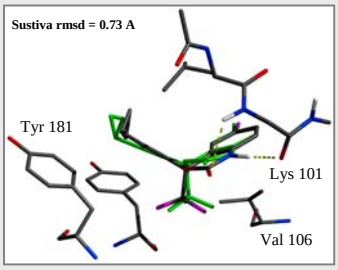
a: all values were normalized relative to those of sustiva.
b: Ki Young et al., *Antimicrob. Agents Chemother.* **1995**, *39*, 2602.
c: IC90 Levin, http://www.natap.org/reports/NR5-nnrti_update2.resis.html.
d: IC50 Byrnes et al., *Antimicrob. Agents Chemother.* **1993**, *37*, 1576.
e: EC50 Balzarini et al., *J. Virol.* **1994**, *68*, 7986.
f: EC50 Balzarini et al., *Antimicrob. Agents Chemother.* **1995**, *39*, 998.
g: EC50 Baba et al., *Antimicrob. Agents Chemother.* **1994**, *38*, 688.

Improved resistance: Sustiva relies less on interactions with wild-type residues 181 and 106 than other NNRTIs



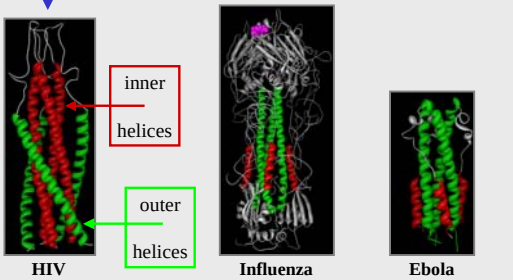
Tyr181: cyclopropyl ethynyl -smaller functional group
Val106: trifluromethyl -less hydrophobic group

A subsequently reported exptl structure (green)* fully confirmed the computational‡ prediction



*Ren, J. et al *Structure* **2000,** *8*, 1089-1094
‡Rizzo, R.C.; et al. *J. Am. Chem. Soc.*, **2000**, *122*, 12898-12900

Coiled-coil membrane fusion proteins: (a different anti-HIV target)



HIV (AIDS) **Influenza (flu)** **Ebola (hemorrhagic fever)**

A new anti-HIV compound (FUZEON) was just approved by the FDA which targets HIV membrane fusion proteins (www.trimeris.com)

Good News:

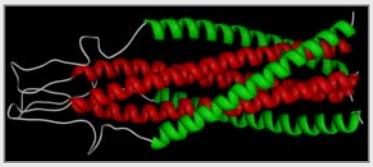
- The first new class of anti-HIV drugs in 7 yrs (first fusion inhibitor)
- Blocks HIV's ability to infect healthy immune (CD4) cells
- Reduces the amount of HIV in the blood when used with other drugs
- Slows HIV progression in patients with resistance to other drugs

Bad News:

- Must be injected (FUZEON is a large peptide)
- Expensive to produce (cost to patient about $20,000 per year)
- Resistance still occurs
- Side effects include: site injection reactions, increased pneumonia, loss of sleep , depression, decreased appetite, muscle pain, constipation, pancreas problems, allergic reactions (trouble breathing, fever, vomiting, skin rash, blood in urine, swelling of feet)

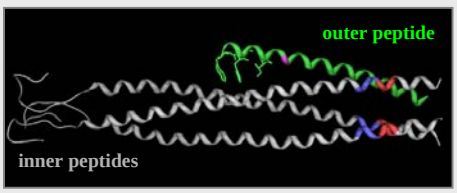
HIVgp41 model based on SIVgp41 Nuclear Magnetic Resonance (NMR) structure

Side view

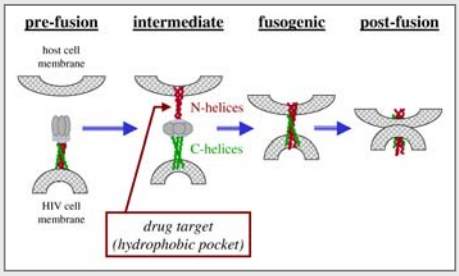


End view

FUZEON was a designed drug: A portion of the virus (an outer gp41 peptide) was taken and used against itself (inner gp41 peptides) in order to prevent infection



HIV gp41 cell membrane fusion model*



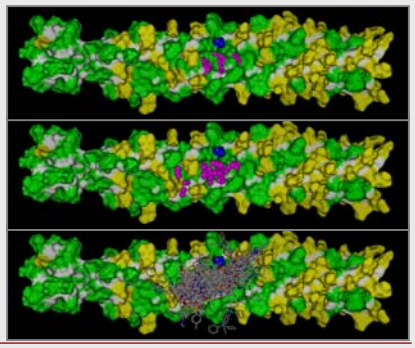
*Kim, P.; Eckert, D. M.; *PNAS* **2001,** *98*, 11187-11192

A computational drug design project

Molecular surface

A potential binding site

Computer predictions



Some Conclusions

- Drug discovery is long (~10 yrs) and expensive (~800 million $)
- Computational structural biology and "Rational Drug Design" aims to reduce the time and cost associated with getting drugs to market.
- Many disease targets (old and emerging) with no satisfactory "cure"
- Bioterrorism threats
- Humanitarian devastation: HIV, malaria
- Future (potential) pandemics : SARS, influenza (1918 ~20-40 million)
- Drug discovery is multidisciplinary (career options)