Structure-Based Ligand Design

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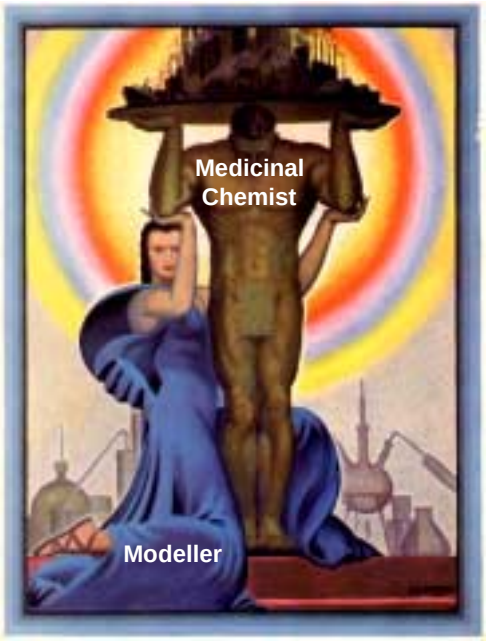


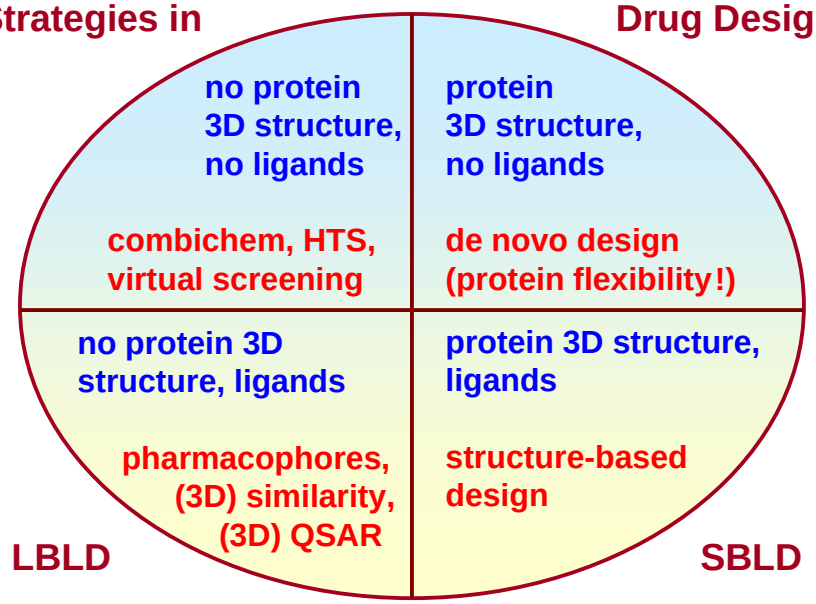
A. Cressy Morrison

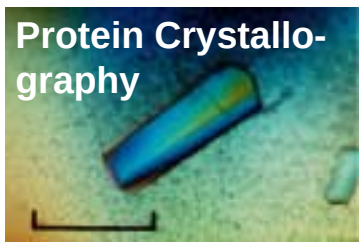
Man in a Chemical World
The Service of Chemical Industry

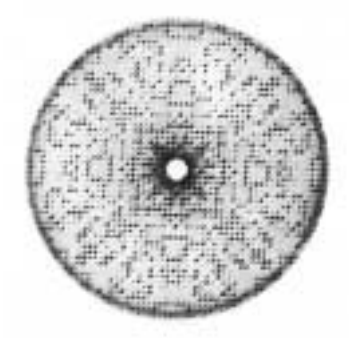
Ch. Scribner's Sons, NY, 1937

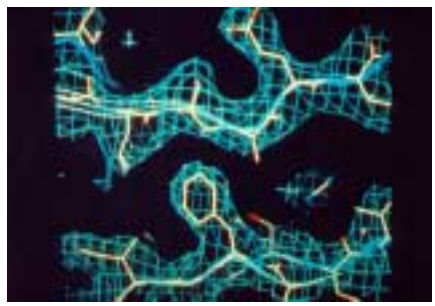
„Chemical Industry, Upheld by Pure Science, Sustains the Production of Man's Necessities"

Strategies in Drug Design

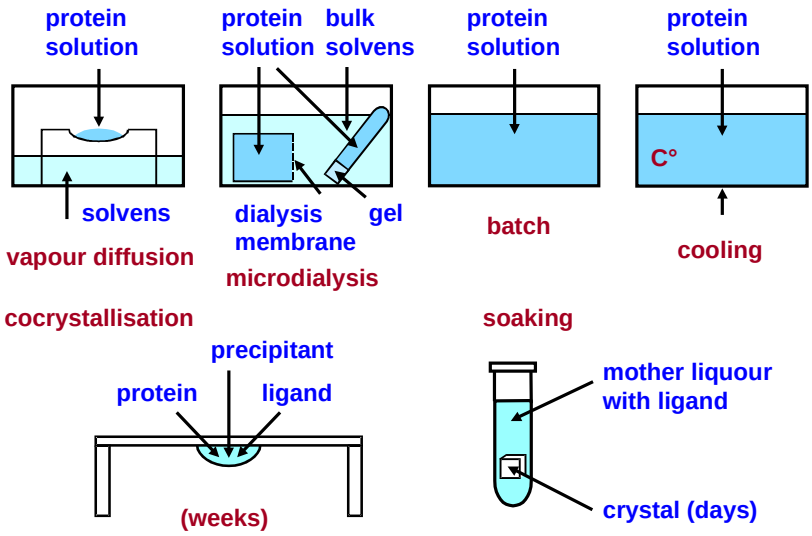




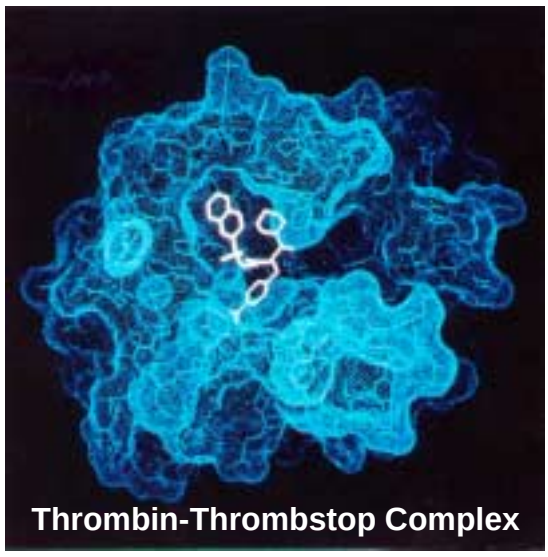


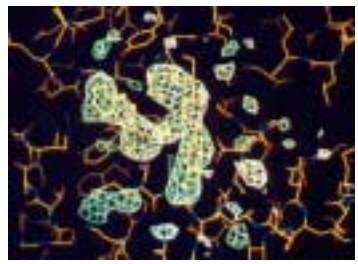


Crystallisation Techniques

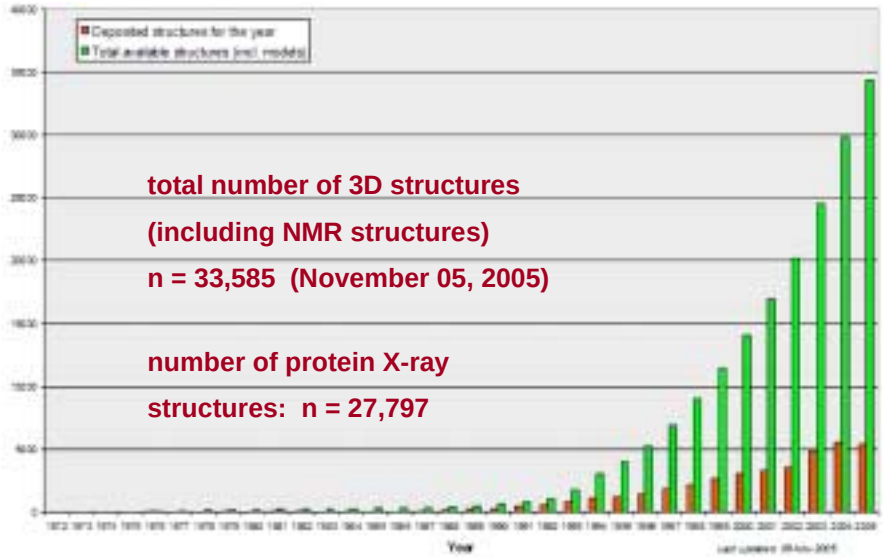


Thrombin and Thrombin Inhibitor Complexes



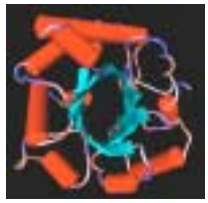


Number of Protein 3D Structures in the PDB



Protein Domain Superfold Families

α/β doubly wound
2FOX - flavodoxin



TIM barrel
7TIM

α/β sandwich
1APS - hydrolase

greek key - Ig
2RHE - Bence
Jones

α up-down
256B -
cytochrome

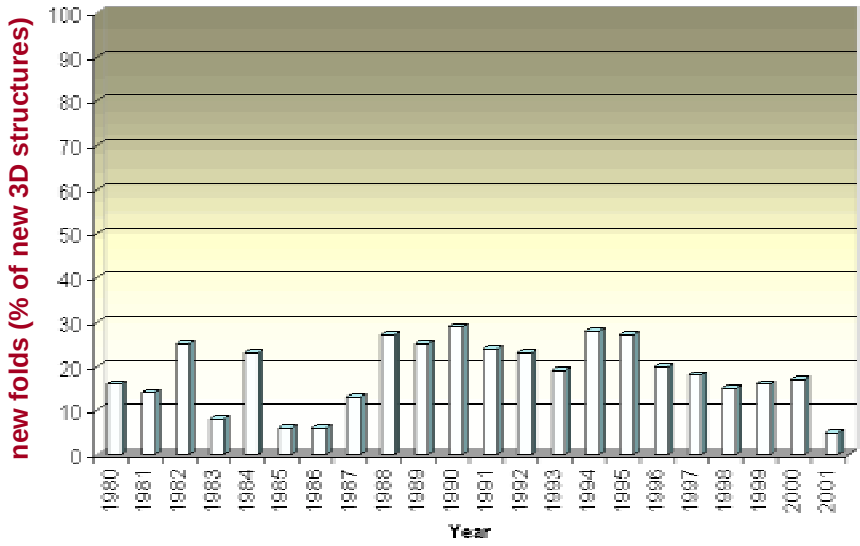
globin
1THB - hemoglobin

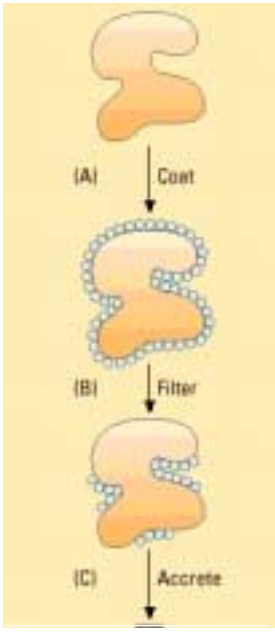
jelly roll 2STV - virus

trefoil
1I1B - IL-1β

αβ roll

Proportion of New Protein Folds / Year



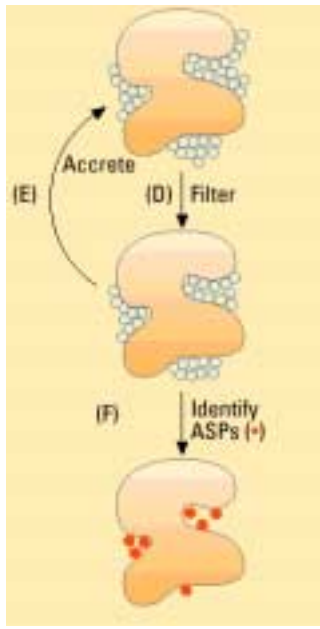


Binding Site Recognition

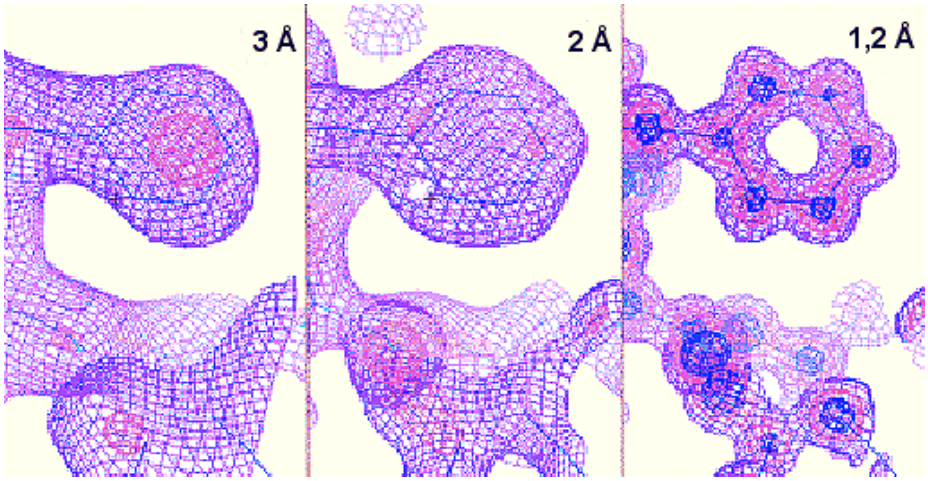
a) coat the surface with water
b) remove surface-exposed water
c) accrete new water molecules
d) filter
e) repeat process
f) identify „active site points“

(PASS algorithm, G. P. Brady Jr., JCICS 14, 383-401 (2002))

R. C. Willis, Mod. Drug Discov., Sept. 2002, 28-34.



The Problem of Protein 3D Structure Resolution

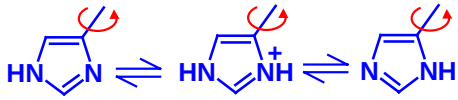


Problems of PDB Protein 3D Structures

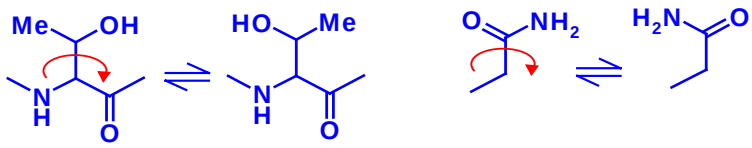
Binding site geometry may be different in free protein and complex

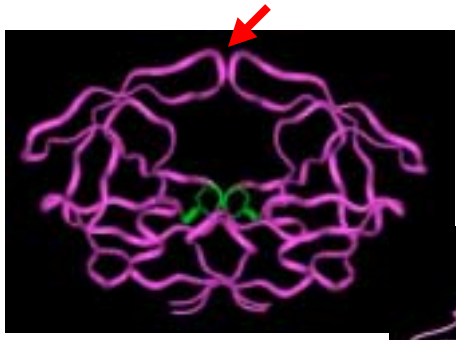
Lacking hydrogen atoms, orientation of hydroxyl groups
can be added automatically

Protonation of acidic and basic side chains
Amino acid pK_a values: pK_a shifts?
His: protonation equilibrium

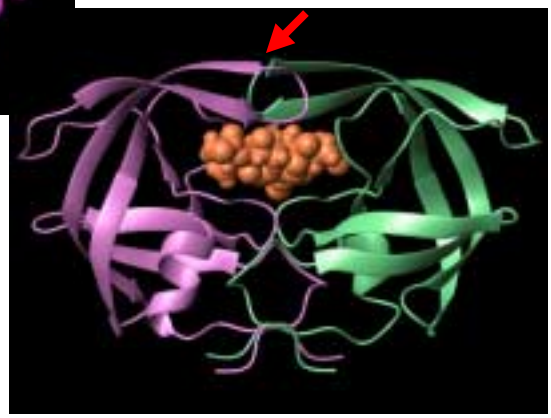


No differentiation between C, O and N
rotamer equilibria of his, thr, asn, gln



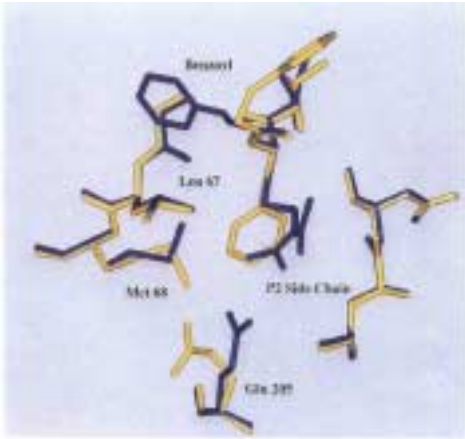


HIV Protease, without a ligand

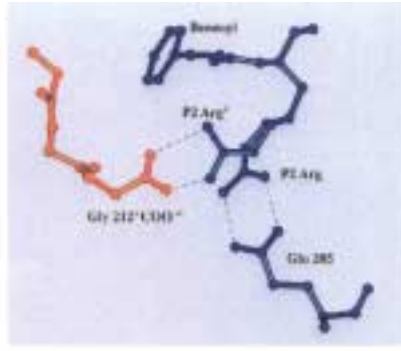


HIV Protease, with a ligand

Flexibility of the Binding Site of Cruzain



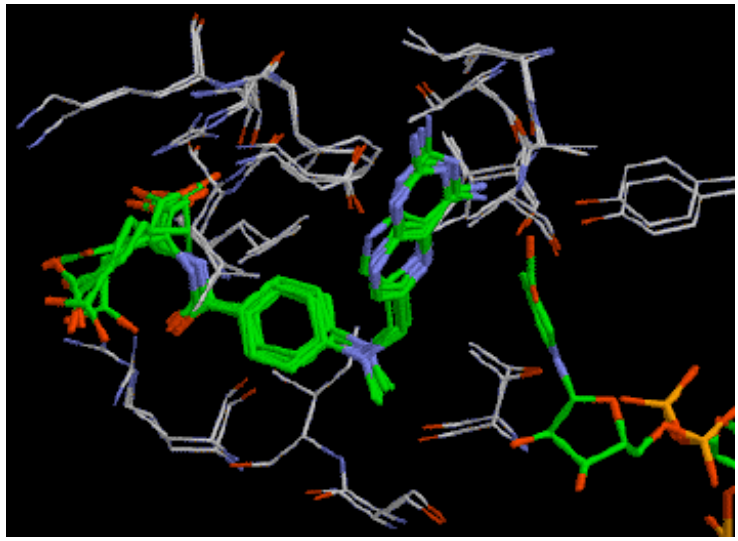
a) Glu 205 flexibility, induced by Phe (gray) and Arg (blue) inhibitors



b) Interactions of an Arg inhibitor with Glu 205 and a C-terminal Gly 212‘

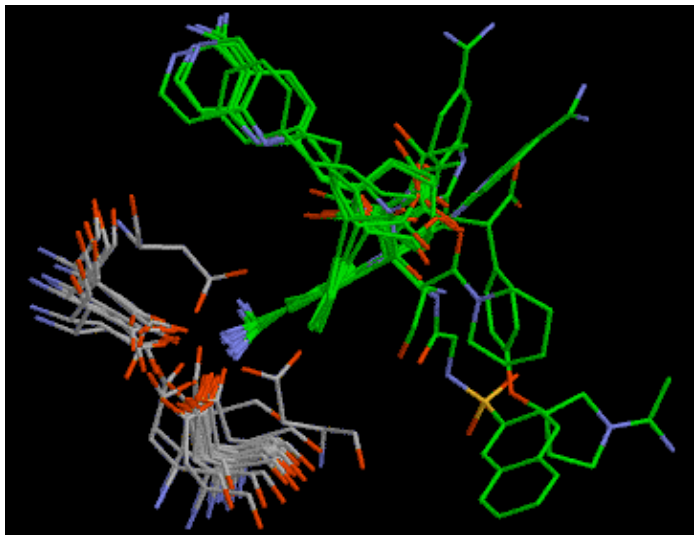
S. A. Gillmor et al., Protein Sci. 6, 1603-1611 (1997)

RELIBASE - Comparison of MTX Binding Sites

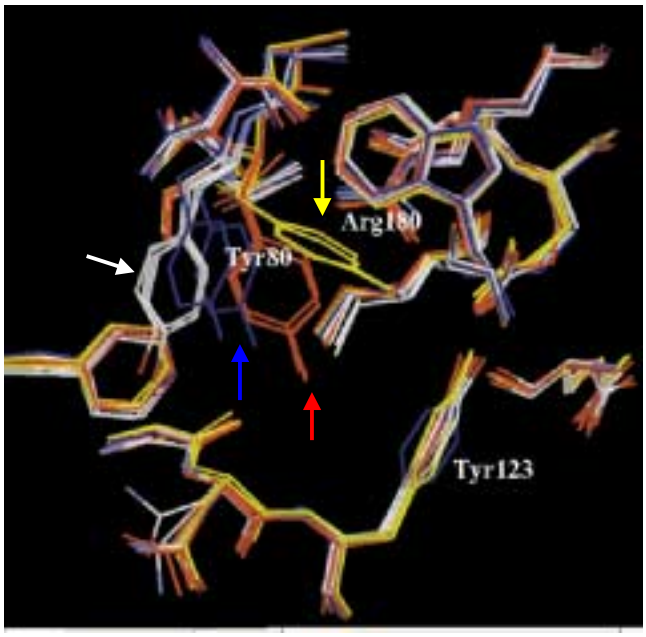


www.ccdc.cam.ac.uk/prods/relibase/index.html

RELIBASE - Benzamidine / COO^- Interactions



www.ccdc.cam.ac.uk/prods/relibase/index.html



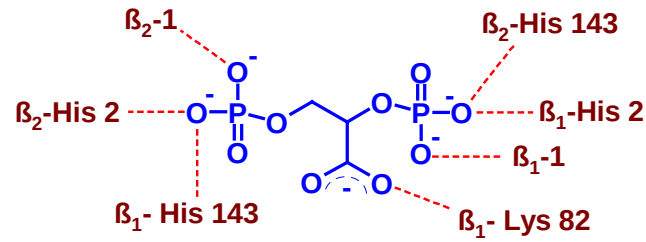
RELIBASE

Binding Site Flexibility

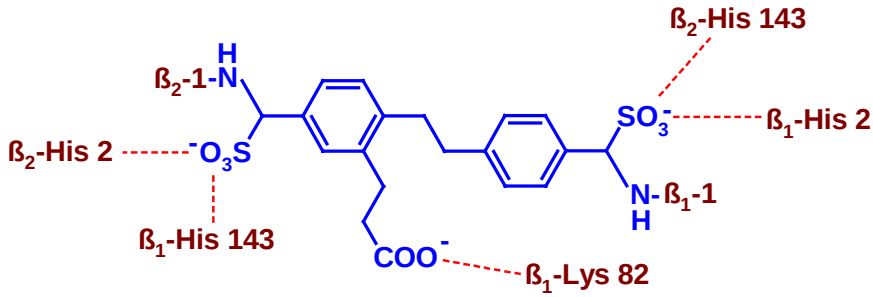
white: e.g. 1BR6
blue: 1IFS and 1APG
red: ligand-free structures 2All, 1RTC and 1IFT
yellow: R180H mutants 1OBS and 1OBT

J. Günther et al., J. Mol. Biol. 326, 621-636 (2003)

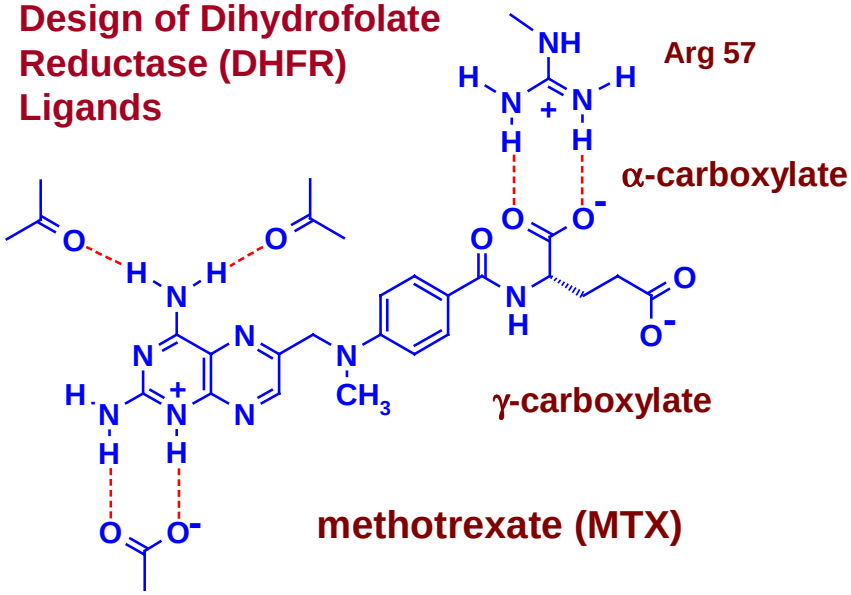
Structure-based Design of Protein Ligands



2,3-DPG reduces the oxygen affinity of hemoglobin

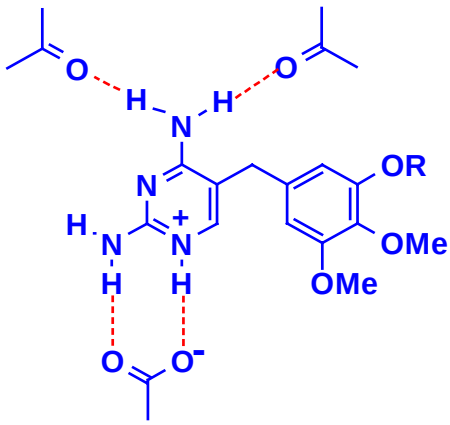


Design of Dihydrofolate Reductase (DHFR) Ligands



methotrexate (MTX)

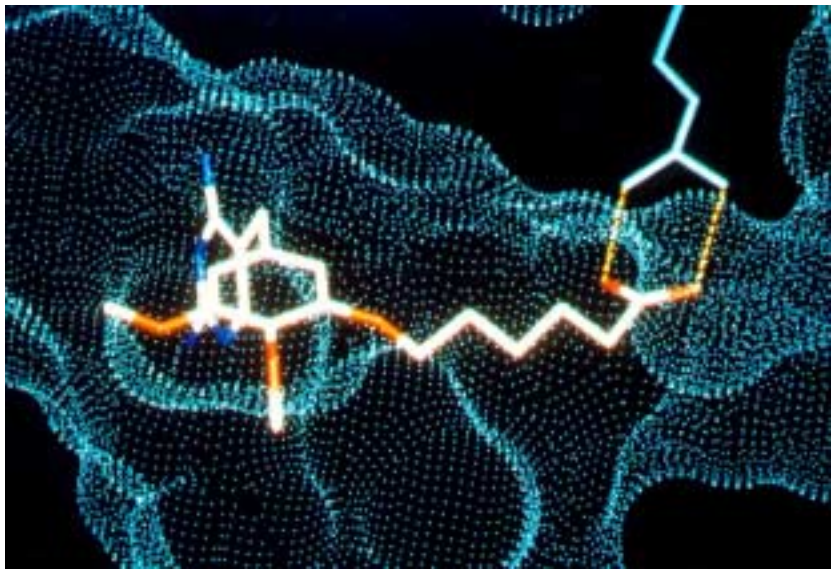
Design of Dihydrofolate Reductase (DHFR) Ligands



R	K_i [nM]
CH_3	1.3
CH_2COOH	2.6
$(CH_2)_2COOH$	0.37
$(CH_2)_3COOH$	0.035
$(CH_2)_4COOH$	0.066
$(CH_2)_5COOH$	0.024
$(CH_2)_6COOH$	0.050

trimethoprim, R = CH_3

Design of DHFR Ligands



General Strategies for the Rational Design of Protease Inhibitors

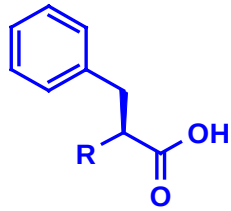
a) Exchange of the scissile bond in the substrate („substrate-like“aspartyl protease inhibitors)

b) Introduction of a group that interacts with a metal ion („carboxy-terminal“ metalloprotease inhibitors)

c) Introduction of a group that covalently interacts with the catalytic amino acid („amino-terminal“ serine and cysteine protease inhibitors)

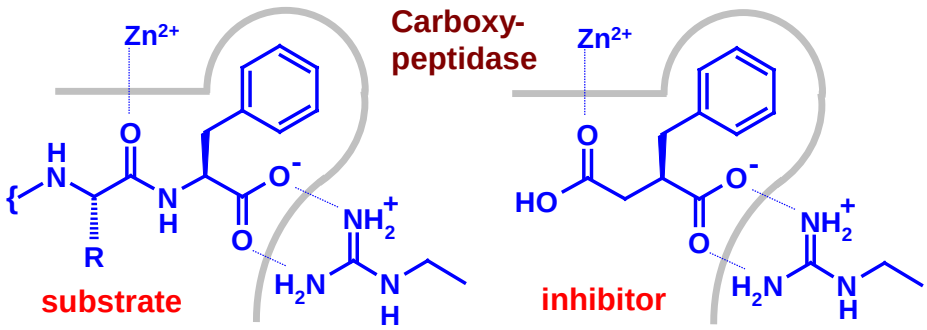
Strong non-covalent interactions with the binding site pockets (especially hydrophobic interactions)

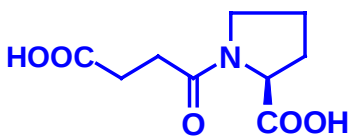
Relative Potency of Zn^{++}-Complexing Groups

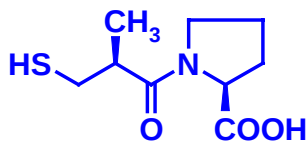
	K_i [nM]
R = H	6200
CH_2COOH	450
$CH_2S(=NH)_2CH_3$	250
$OP(=O)(OH)_2$	140
CH_2SH	11

Hugo Kubinyi, www.kubinyi.de

Structure-Based Design of Captopril



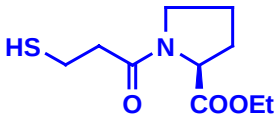


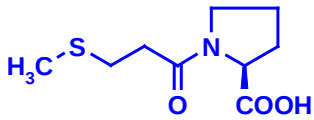


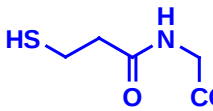
IC_{50} = 330 µM

Captopril IC_{50} = 23 nM

Captopril Analogs

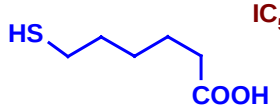






IC_{50} = 17 µM

IC_{50} = 4300 µM



IC_{50} = 2.8 µM

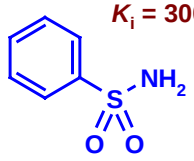
IC_{50} = 1100 µM

Free thiol and carboxylate groups are required for binding. Esterification of the carboxylate group reduces the binding affinity by nearly two orders of magnitude. S-methylation leads to a 20,000-fold reduction in binding affinities.
Also the central carbonyl group seems to be essential for high binding affinity (lower right analog).

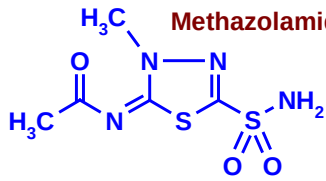
Hugo Kubinyi, www.kubinyi.de

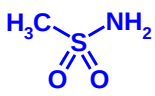
Structure-Based Design of Dorzolamide

K_i = 300 nM

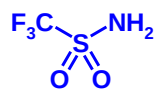


Methazolamide

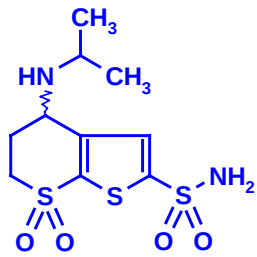




K_i = 100 µM

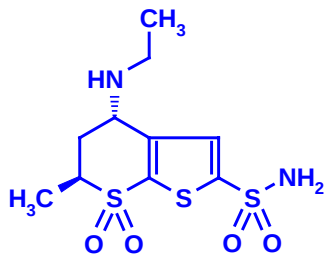


K_i = 2.0 nM



MK 927

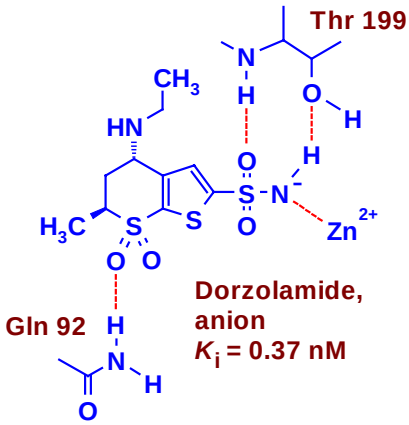
K_i = 0.7 nM

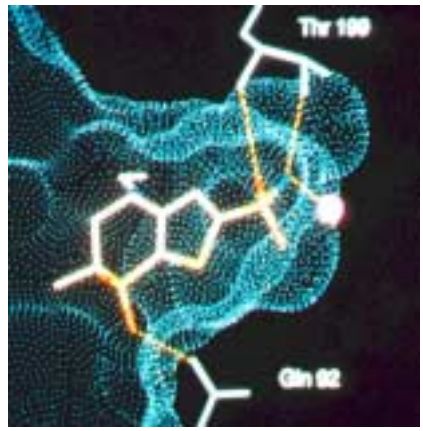


Dorzolamide

K_i = 0.37 nM

Binding Mode of Carbonic Anhydrase Inhibitors



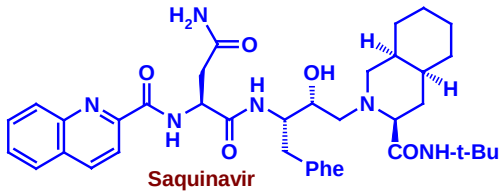


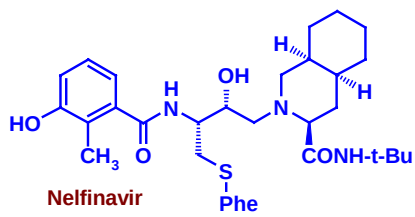
$CH_3SO_2NH_2$, K_i = 100 µM, pK_a = 10.5

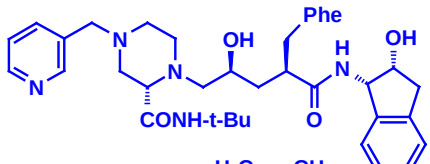
$CF_3SO_2NH_2$, K_i = 2 nM, pK_a = 5.8

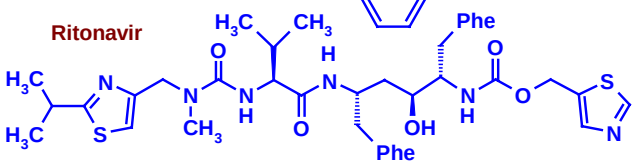
Hugo Kubinyi, www.kubinyi.de

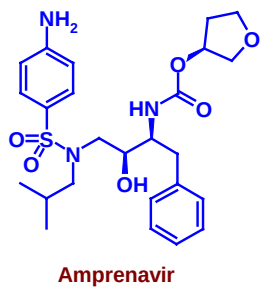
Design of HIV Protease Inhibitors



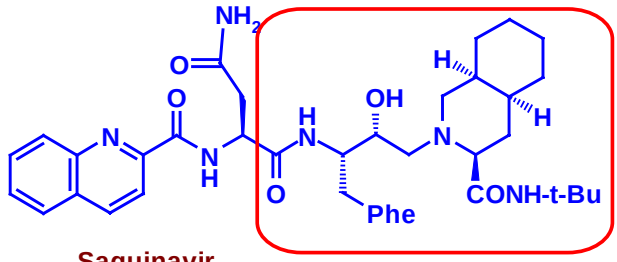




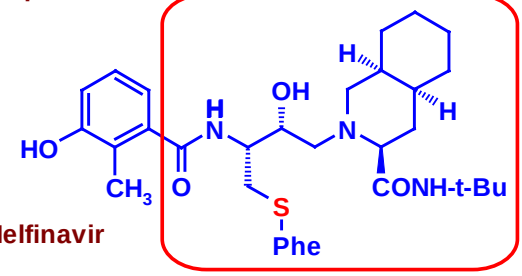




Design of HIV Protease Inhibitors



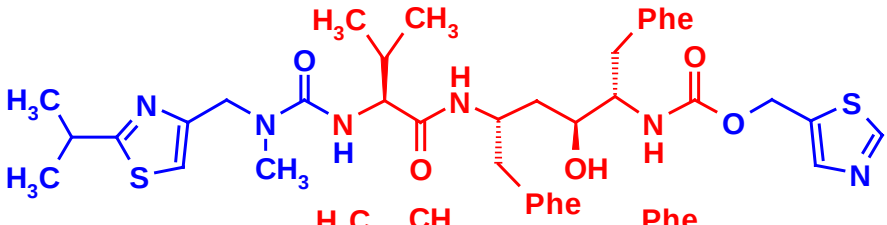
Saquinavir



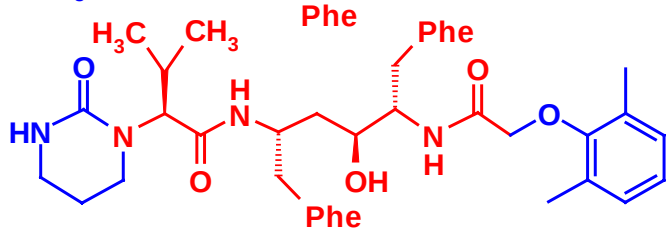
Nelfinavir

Hugo Kubinyi, www.kubinyi.de

HIV Protease Inhibitors Against Resistant Strains



Ritonavir

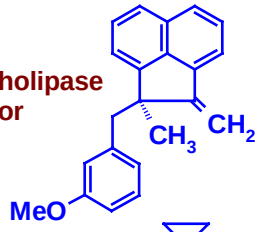


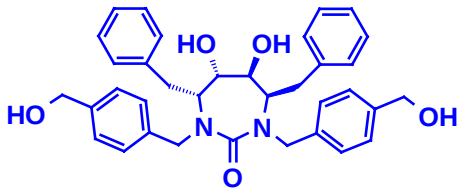
Lopinavir

therapeutically applied in combination: lopinavir is active against ritonavir-resistant strains; its pharmacokinetics is improved by the CYP 3A4 inhibitor ritonavir.

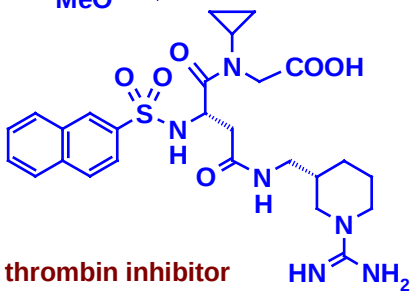
Success Stories and Failures of Structure-based Design

phospholipase inhibitor

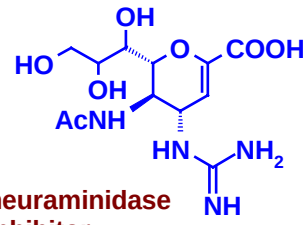




HIV protease inhibitor



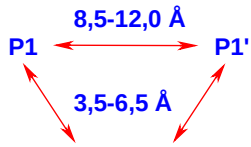
thrombin inhibitor



neuraminidase inhibitor

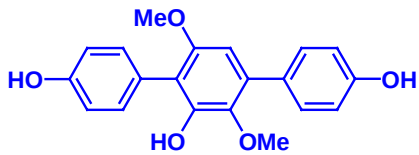
Rational Design of HIV-Protease Inhibitors

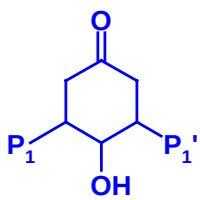
pharmacophore hypothesis

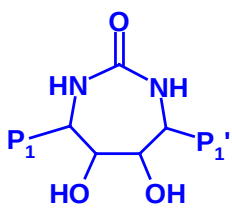


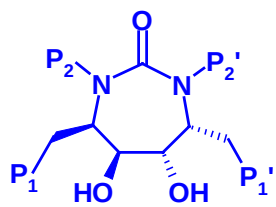
H-bond donor / acceptor

hit from 3D search

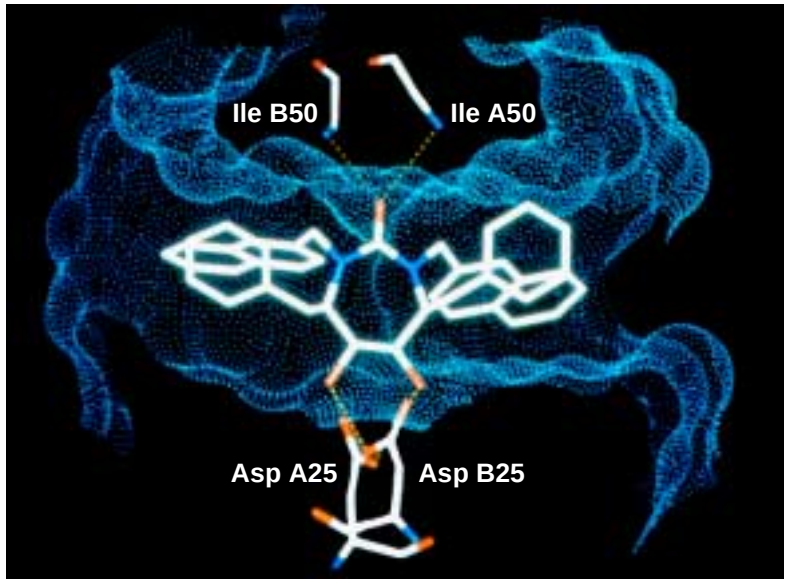




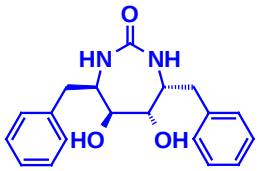




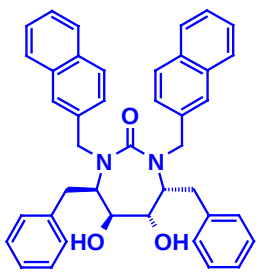
Binding Mode of the DuPont HIV-Protease Inhibitors



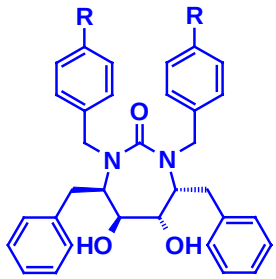
DuPont HIV-Protease Inhibitors



K_i = 4500 nM

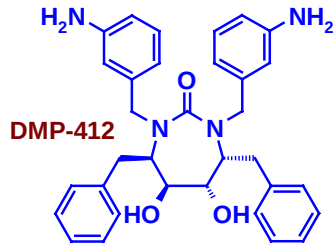


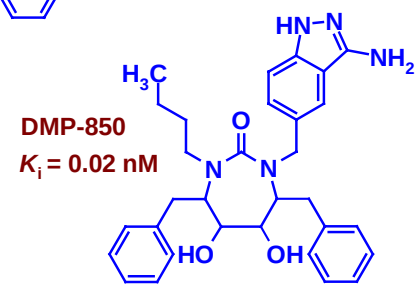
K_i = 0.3 nM



DMP-323
R = -CH_2OH
K_i = 0.3 nM

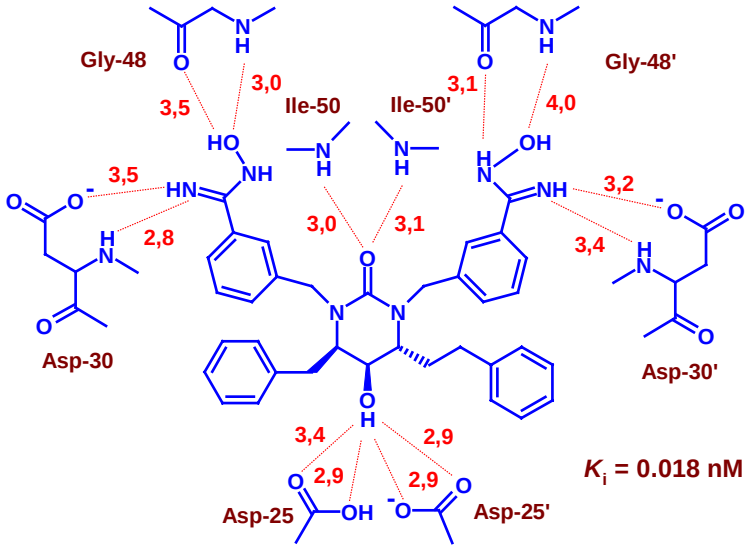
DMP-450
R = -NH_2
K_i = 0.3 nM





DMP-850
K_i = 0.02 nM

HIV-Protease Inhibitor *vs*. Resistant Strains



K_i = 0.018 nM

General Recommendations for Ligand Design

Complementary hydrophobic surfaces contribute to affinity but lipophilicity increase reduces solubility.

Hydrogen bonds are important for recognition and orientation; they increase or reduce affinity.

The effect of repulsive interactions is hardly predictable.

The effect of MEP and dipole interactions is most often neglected.

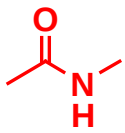
Replacement of water molecules may reduce affinity.

Small and/or flexible ligands are only weakly active; they may bind to many different targets.

binding site:	+	+	-	+	-	-	+	-	+	+	+
complex ligand	-	-	+	-	+	+	-	+	-	-	-
small ligand		-	+	-			-	+	-		

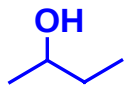
M. M. Hann et al., J. Chem. Inf. Comput. Sci. 41, 856-864 (2001)

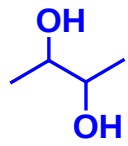
Rational Design of Protease Inhibitors: Transition State Mimics

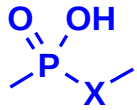


substrate

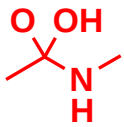
mimics





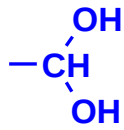


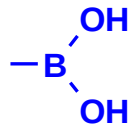
X = -CH_2-, -NH-, -O-

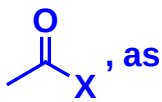


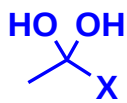
transition state

—CHO , as



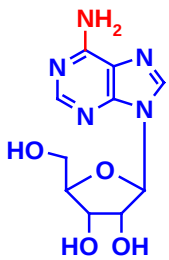






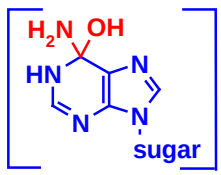
X = -CF_3, -CF_2-R, -Aryl

Rational Design: Transition State Mimics



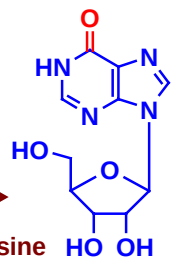
adenosine

hypothetical transition state

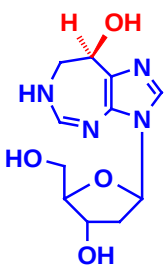


sugar

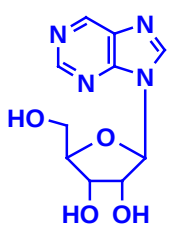
adenosine deaminase



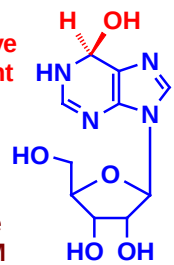
inosine



pentostatin
K_i = 2.5 pM

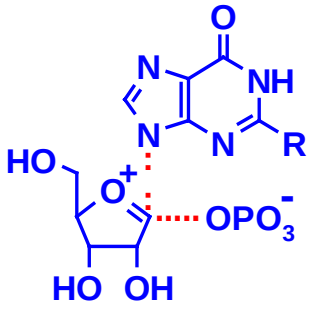


active agent

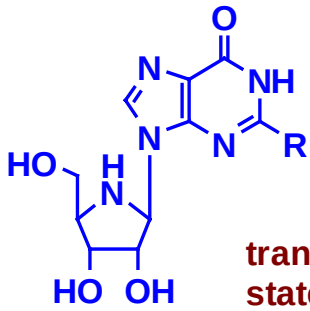


nebularine
K_i = 0.3 pM

Transition State Mimics are Picomolar Inhibitors



transition state



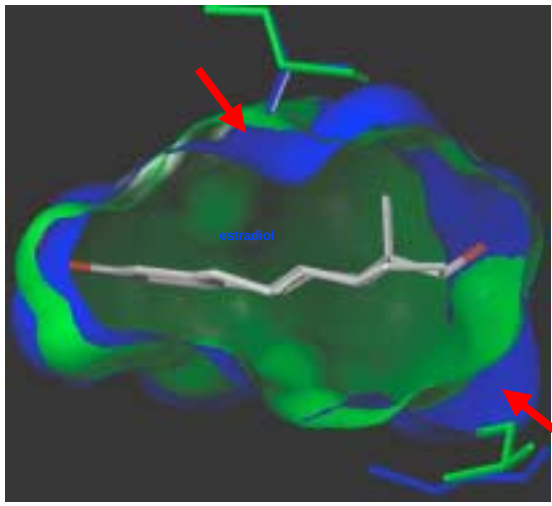
transition state mimic

R. W. Miles et al., Biochemistry 37, 8615-8621 (1998)
G. B. Evans et al., J. Med. Chem. 46, 155-160 (2003)

R = H, Immucillin-H
K_i hPNP = 72 pM

R = NH_2, Immucillin-G
K_i hPNP = 29 pM

Design of Selective ERα and ERβ Ligands



blue: hERα

green: hERß

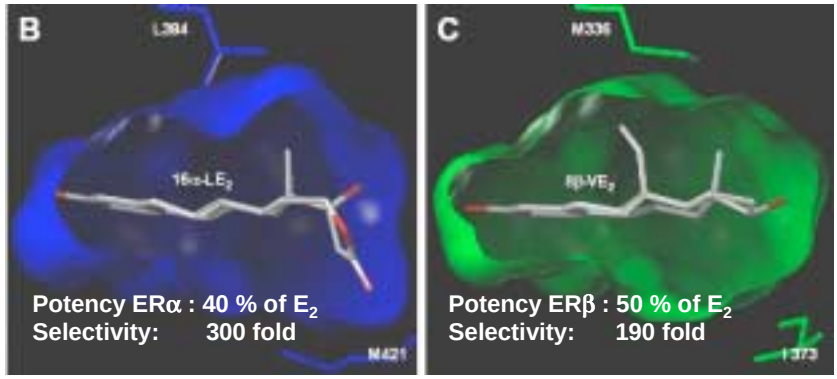
hERα → hERß

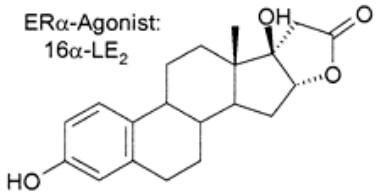
„upper“ side:
Leu384 → Met336

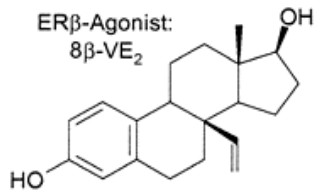
„lower“ side:
Met421 → Ile373

A. Hillisch et al., Ernst Schering Res. Found. Workshop 46, 47-62 (2004); A. Hillisch et al., Mol. Endocrinol. 18, 1599-1609 (2004)

Design of Selective ERα and ERβ Ligands



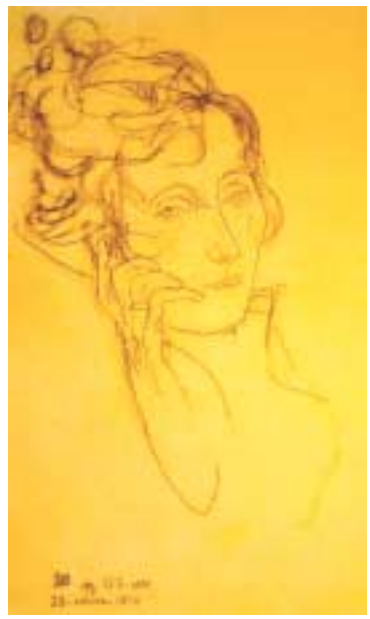




Influenza

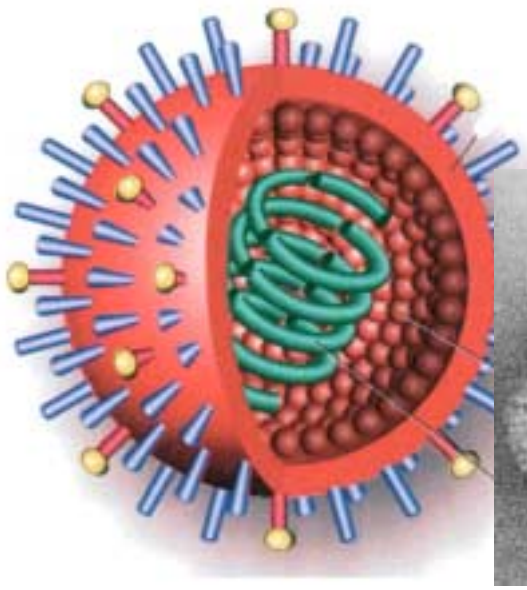
In 1918/19, the „Spanish Flu“ killed about 20-40 mio people. Especially young and very old people died from influenza. The heavy death toll of this pandemic disease has to be compared to the number of 11 mio victims of World War I.

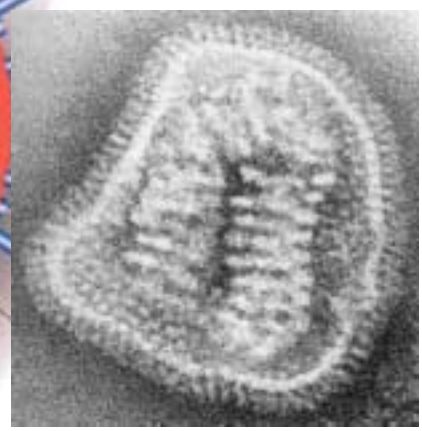
Egon Schiele prepared this drawing of his wife, one day before her death and four days before he died himself, only 28 years old.



Influenza Virus

schematic view and electron micro-scopic picture





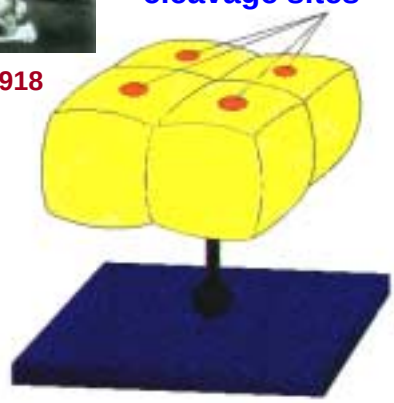
Influenza Virus

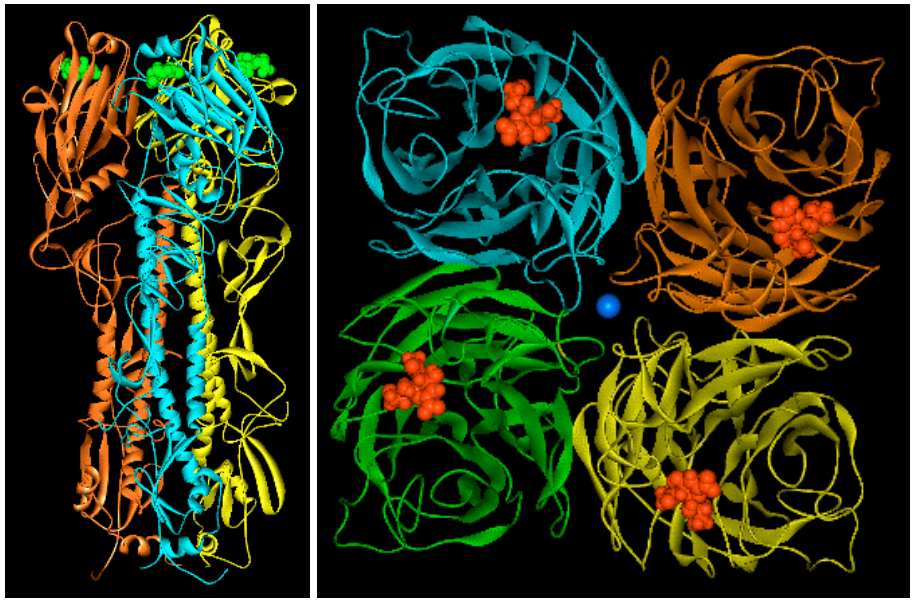
Hemagglutinine sialic acid binding sites

Kansas City, 1918

Neuraminidase sialic acid cleavage sites

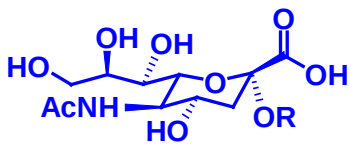
viral surface



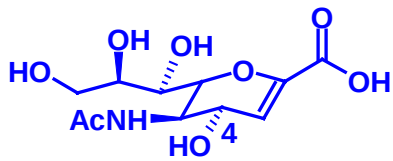


hemagglutinine + sialic acid (green) neuraminidase + DANA (red)

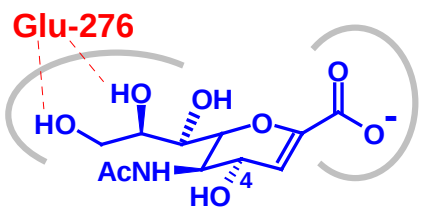
Design of Neuraminidase Inhibitors



sialic acid, R = H



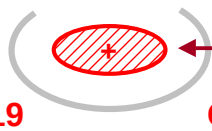
Neu5Ac2en
K_i = 1 000 nM



Arg-371

Arg-292

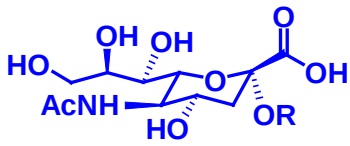
Arg-118



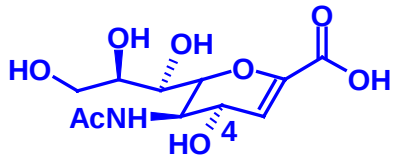
result of a GRID search with a positively charged probe

Glu-119 Glu-227

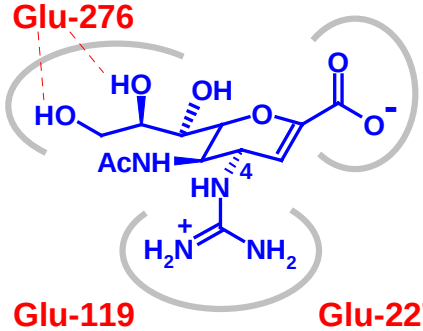
Design of Neuraminidase Inhibitors



sialic acid, R = H



Neu5Ac2en
K_i = 1 000 nM



Arg-371

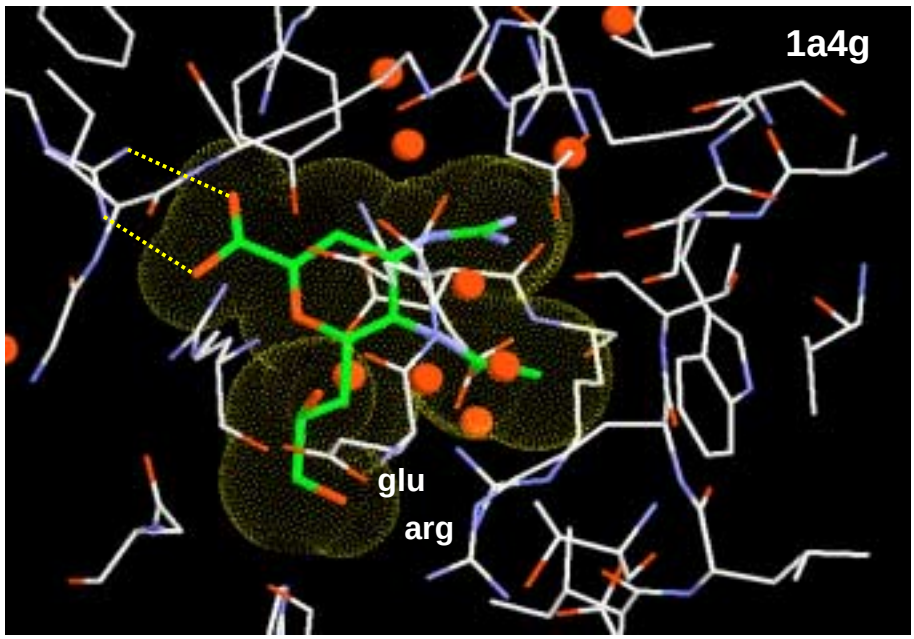
Arg-292

Arg-118

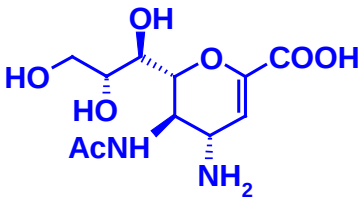
Glu-119

Glu-227

4-Guanidino-Neu5Ac2en
K_i = 0.1-0.2 nM
Zanamivir (Relenza,
Glaxo-Wellcome)

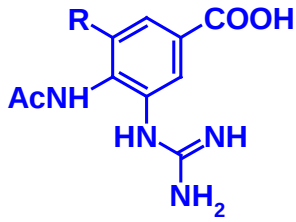


Design of Bioavailable Neuraminidase Inhibitors



4-NH_2-Neu5Ac2en

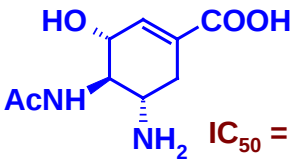
K_i = 50 nM



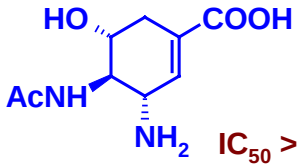
a) R = H K_i = 8 µM

b) R = $CH(OH)CH(OH)CH_2OH$

K_i > 100 µM



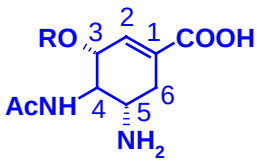
IC_{50} = 6.3 µM



IC_{50} > 200 µM

Hugo Kubinyi, www.kubinyi.de

Design of Bioavailable Neuraminidase Inhibitors



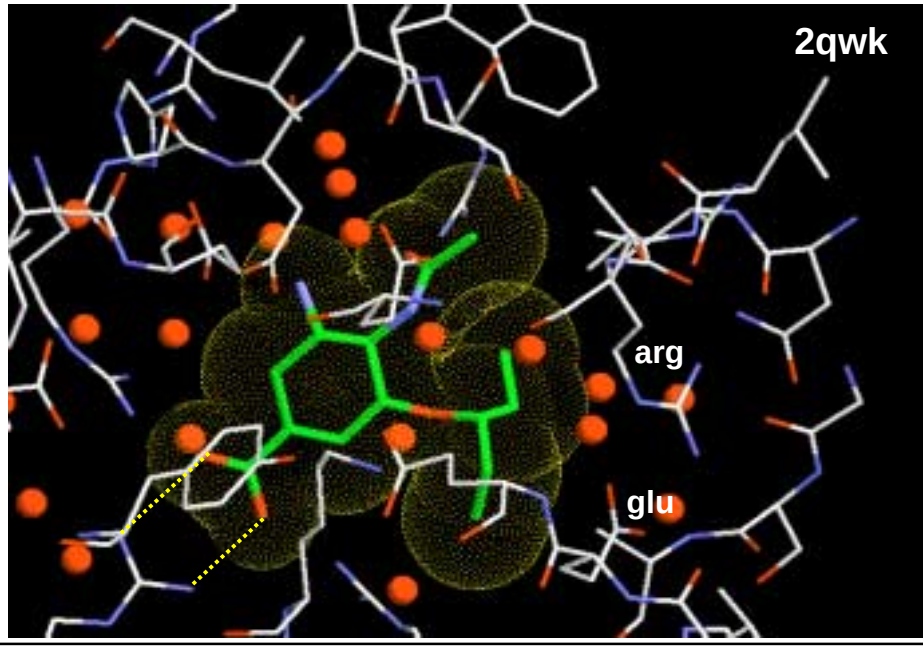
GS 4071, R = $CH(Et)_2$

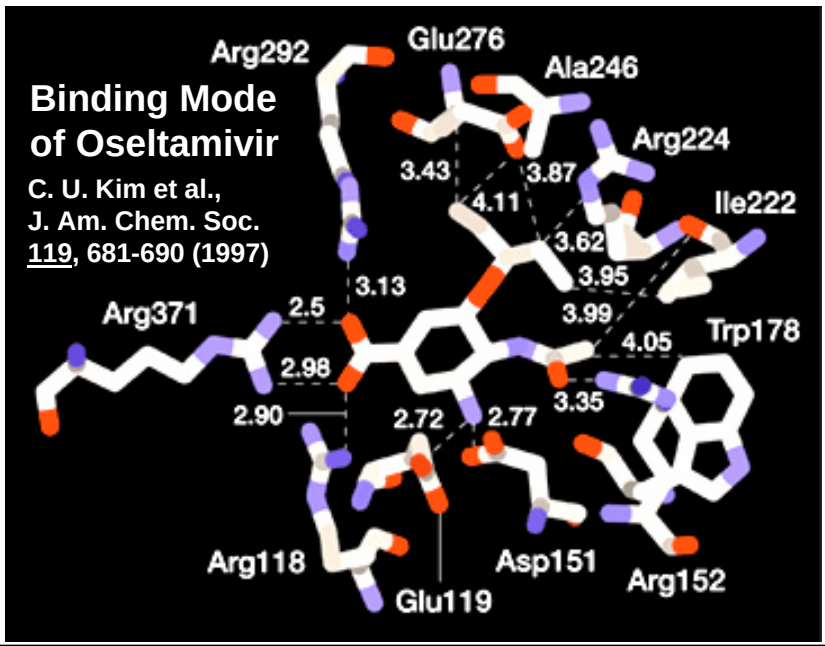
IC_{50} = 1 nM

GS 4104 (ester prodrug of GS 4071)

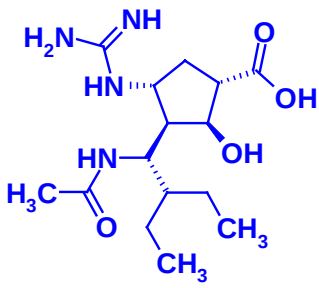
Oseltamivir (Tamiflu, Roche)

R =	IC_{50} (nM)
H	6 300
CH_3	3 700
CH_2CH_3	2 000
$CH_2CH_2CH_3$	180
$CH_2CH_2CF_3$	225
CH_2OCH_3	2 000
$CH_2CH{=}CH_2$	2 200
$CH_2CH_2CH_2CH_3$	300
$CH_2CH(CH_3)_2$	200
$CH(CH_3)CH_2CH_3$	10
$CH(CH_2CH_3)_2$	**1**
$CH(CH_2CH_2CH_3)_2$	16
Cyclopentyl	22
Cyclohexyl	60
Phenyl	530


2qwk
arg
glu

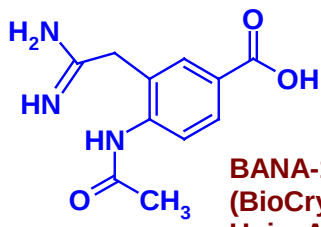

Binding Mode of Oseltamivir
C. U. Kim et al., J. Am. Chem. Soc. 119, 681-690 (1997)
Arg292
Glu276
Ala246
Arg224
Ile222
Trp178
Arg371
Arg118
Glu119
Asp151
Arg152
3.43
3.87
4.11
3.62
3.95
3.99
4.05
3.13
2.5
2.98
2.90
2.72
2.77
3.35

Other Neuraminidase Inhibitors

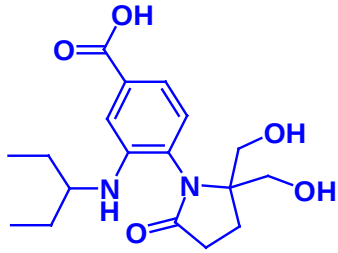


BCX-1812 =
RWJ-270201
(BioCryst, J&J)

IC_{50} = 0.1-11 nM



BANA-113
(BioCryst,
Univ. Alabama)

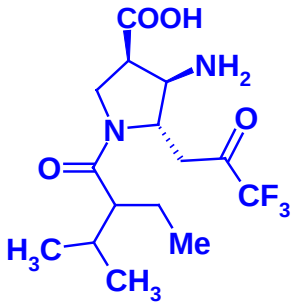


BANA-206

A. F. Abdel-Magid et al., Curr. Opin. Drug. Discov. Dev. 4, 776-791 (2001)

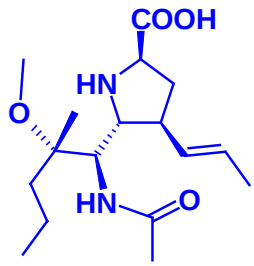
Hugo Kubinyi, www.kubinyi.de

Other Neuraminidase Inhibitors



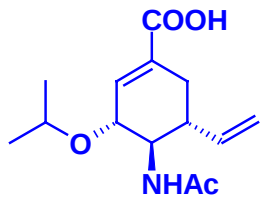
A-192 558
(Abbott Labs)

A. F. Abdel-Magid et al., Curr. Opin. Drug. Discov. Dev. 4, 776-791 (2001)



A-315 675
K_i = 0.02 - 0.31 nM
(different strains)

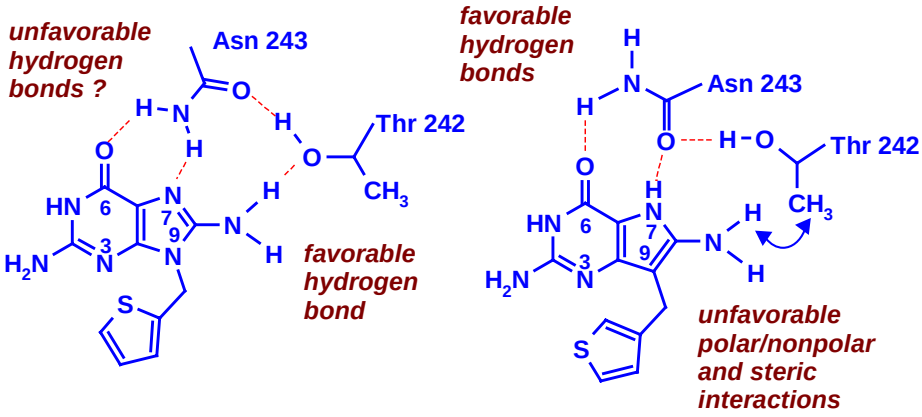
Drug Discov. Today 7, 1066 (2002)



K_i = 45 nM

S. Hanessian et al., Bioorg. Med. Chem. Lett. 12, 3425-3429 (2002)

The Relevance of Protein Crystal Structures: Conformational flexibility of PNP in the crystal



The Relevance of Protein Crystal Structures: Enzymatic activity of PNP crystals