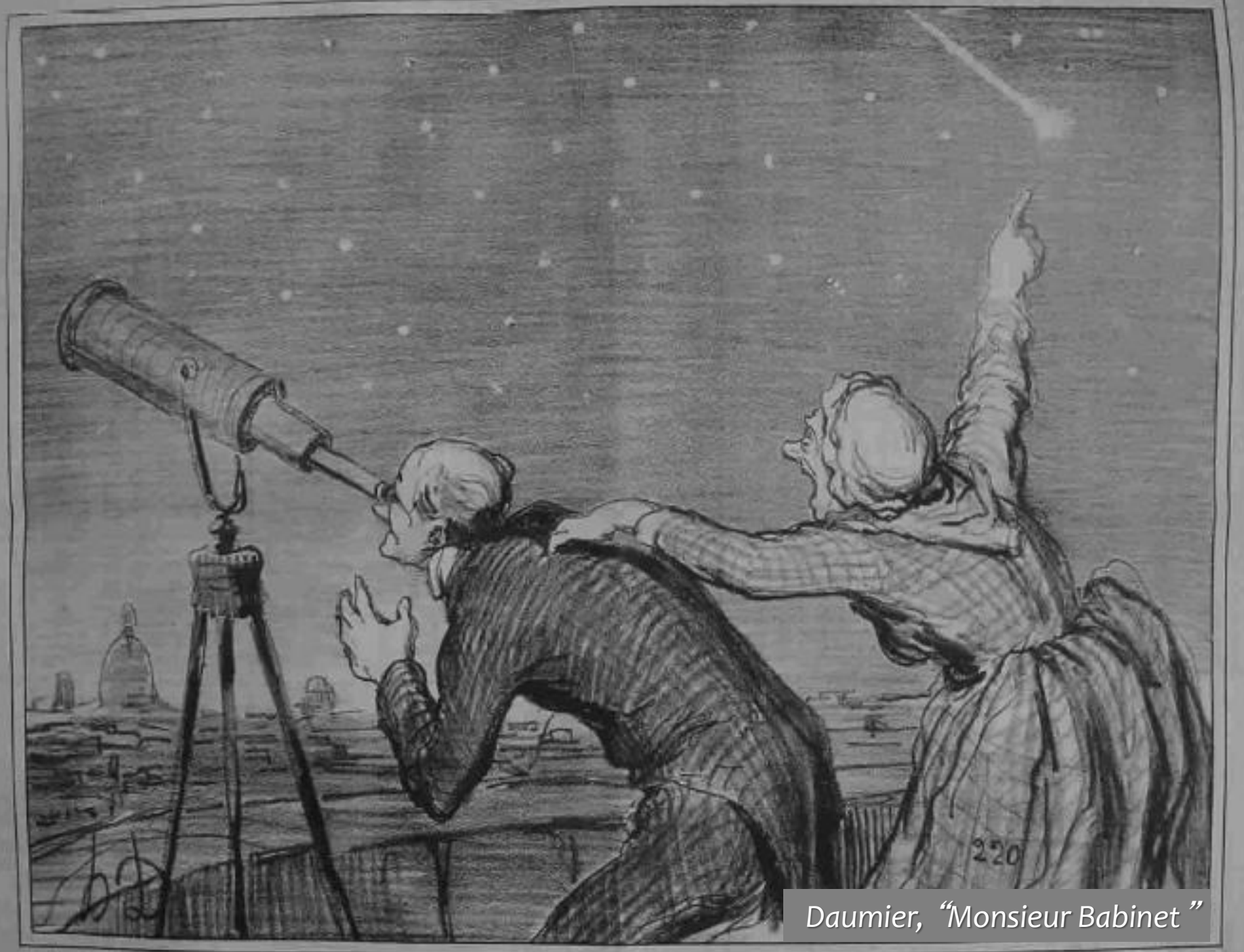
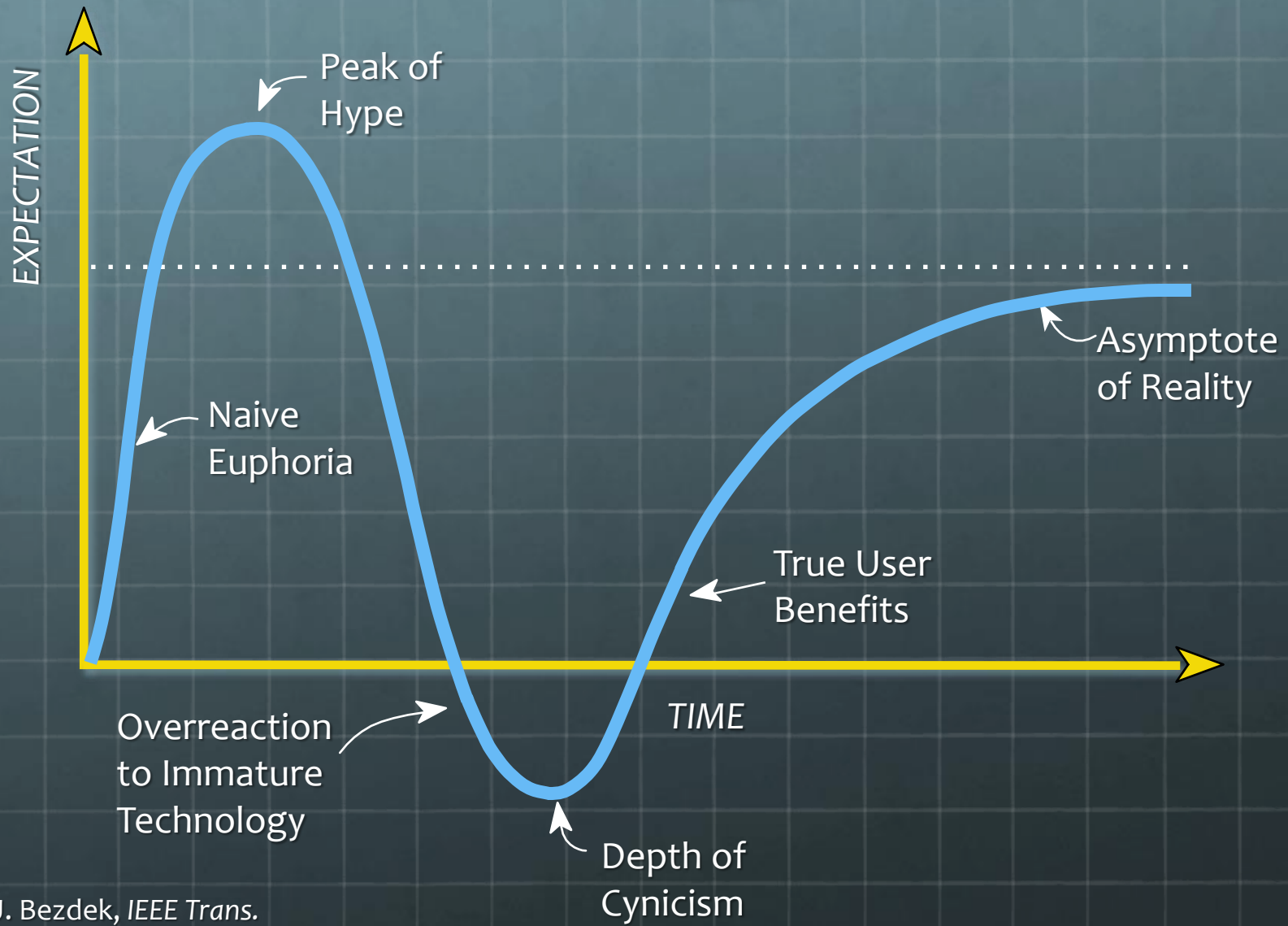
We don't know a millionth of a percent about anything.

-- Thomas Alva Edison

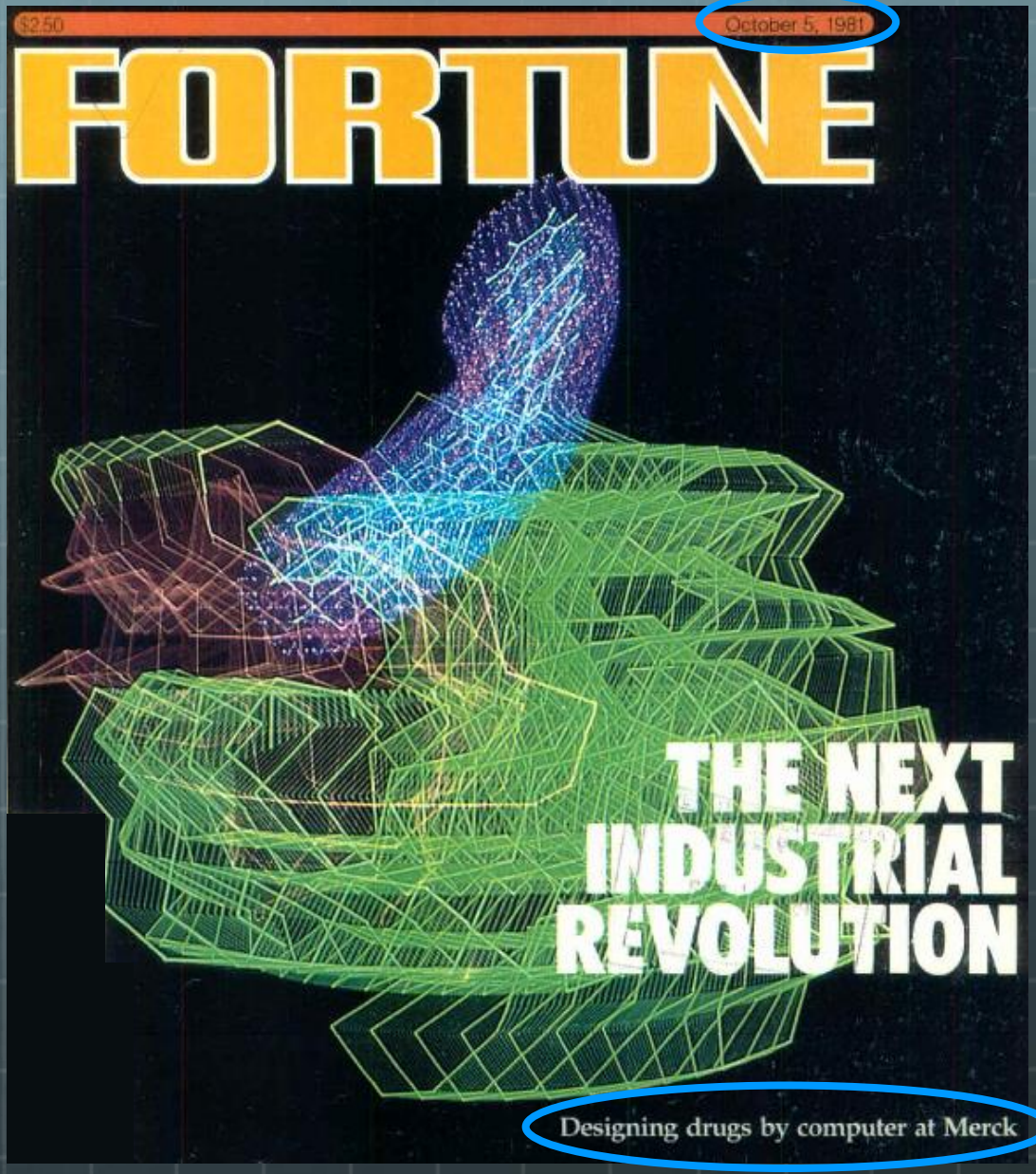


Daumier, "Monsieur Babinet"

Technology Cycle



J. Bezdek, *IEEE Trans. Fuzzy Sys.*, 1, 1-5 (1993)

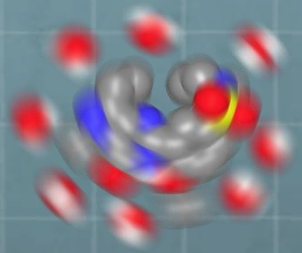


Part 1:
SBDD Primer

Targets Which Have Yielded Clinical Candidates With the Help of Structure-Based Drug Design

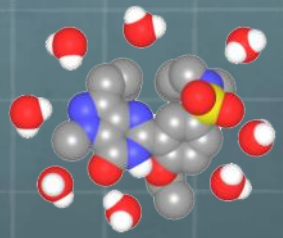
Therapeutic Area	Targets
Cardiovascular	ACE, Renin, Thrombin, Factor VII, Factor Xa
Glaucoma	Carbonic anhydrase
Inflam / immun	Human neutrophil elastase, P38, IMPDH, ICE, COX2, MMP-X, JAK3
Cancer	Purine nucleoside phosphorylase, Thymidylate synthase, VEGF kinase (KDR), Aurora-2, CDK2, EGF kinase (erbB), Glycinamide ribonucleotide formyl-transferase, HSP90, BTK, ……
Antivirals	HIV protease, Influenza sialidase (neuraminidase), HCV protease, HCV polymerase, rhinovirus 3C protease, rhinovirus coat proteins
Sepsis	Caspases (broad), secretory PLA2
Diabetes	PPAR-gamma, DPP-IV, Aldose reductase
Osteoporosis	Cathepsin K
Various CNS	GSK3 kinase, Acetylcholinesterase, BACE

Thermodynamic Decomposition of Ligand/Protein Binding



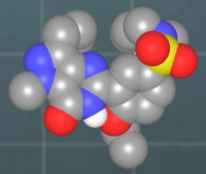
Solvated Ligand

ΔG(1)



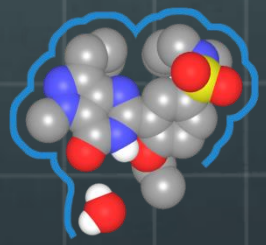
Solvated Ligand in Bioactive Conformation

ΔG(3)



Desolvated Ligand

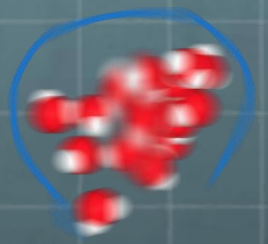
ΔG(5)



Protein / Ligand /Water Complex

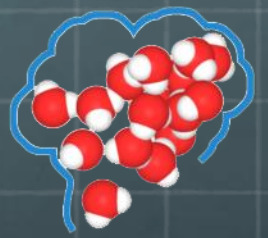
Solvated Apo Protein

ΔG(2)



Solvated Protein in Ligand-Induced Conformation

ΔG(4)



Ligand-Induced Desolvated Protein Binding Site

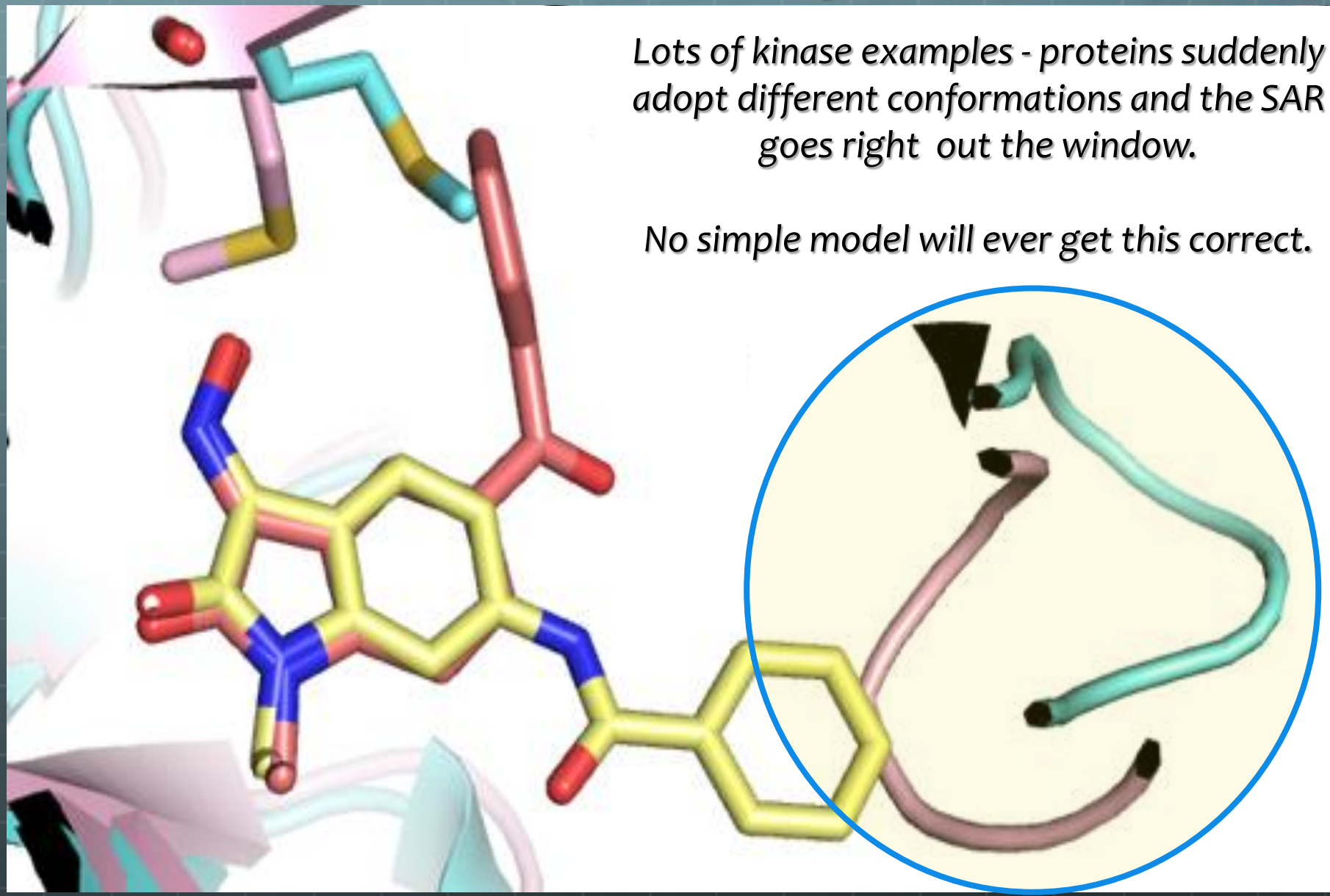
$$\Delta G_{bind} = \sum_{i=1}^{5} \Delta G(i)$$

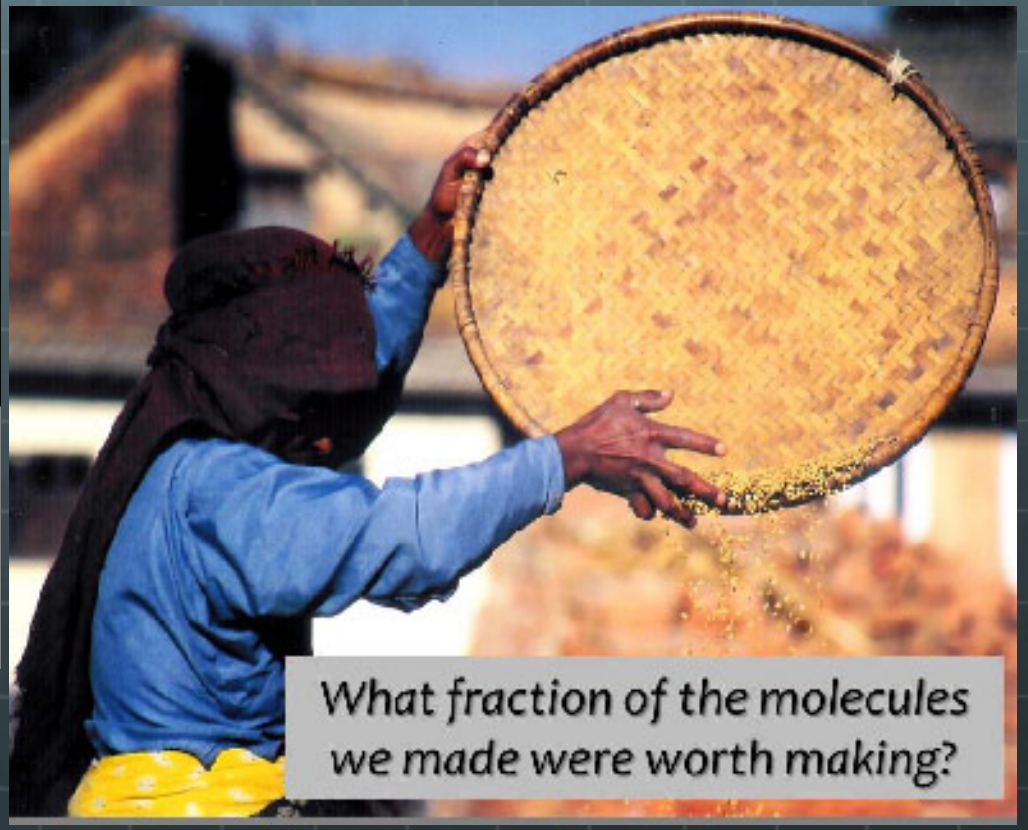
Source: Schrödinger

Proteins are Dynamic

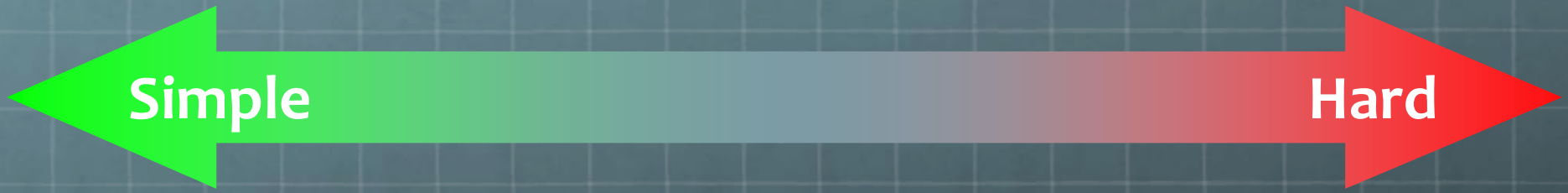
Lots of kinase examples - proteins suddenly adopt different conformations and the SAR goes right out the window.

No simple model will ever get this correct.





SBDD Toolkit



Simple filters | Empirical data / rules | Classification; QSAR | Structural mapping | Pharmacophores; 3D-QSAR | Structure; Dynamics | First principles (QM; Stat mech)

Binding … Selectivity … Oral Absorption … BBB … Protein binding … Solubility … Vss … Metabolism … hERG … CYP inhibition … CYP reactivity … Dose … CYP induction … Toxicity … Production of reactive metabolites … Tissue Distribution … Cell permeability … Transporter inhibition … Active transport … Gut stability … Oral half-life … IV half-life …

Part 2:
IL-1β Converting Enzyme (ICE; Caspase-1)

Observations from Vertex: What Factors Lead to Successful Structure-Based Drug Design?

- Structures available early in each project
  - Willingness and ability to produce protein
- Real-time structures (rapid feedback)
- Experts at interpreting / applying structure
  - Diverse backgrounds, savvy, practical
- Strong links between chem, modeling, x-ray
  - Broad exploration of chemotypes
- Realism about value & limitations of SBDD
  - Don't oversell the technology - use appropriately
- Focus on *drug* design goals
  - Willing to trade *good binding* for *good properties*

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Required heroic biochemical efforts but saved a year+

X-ray structures of >10 of distinct scaffolds

4 modelers with diverse backgrounds, plus savvy crystallographers

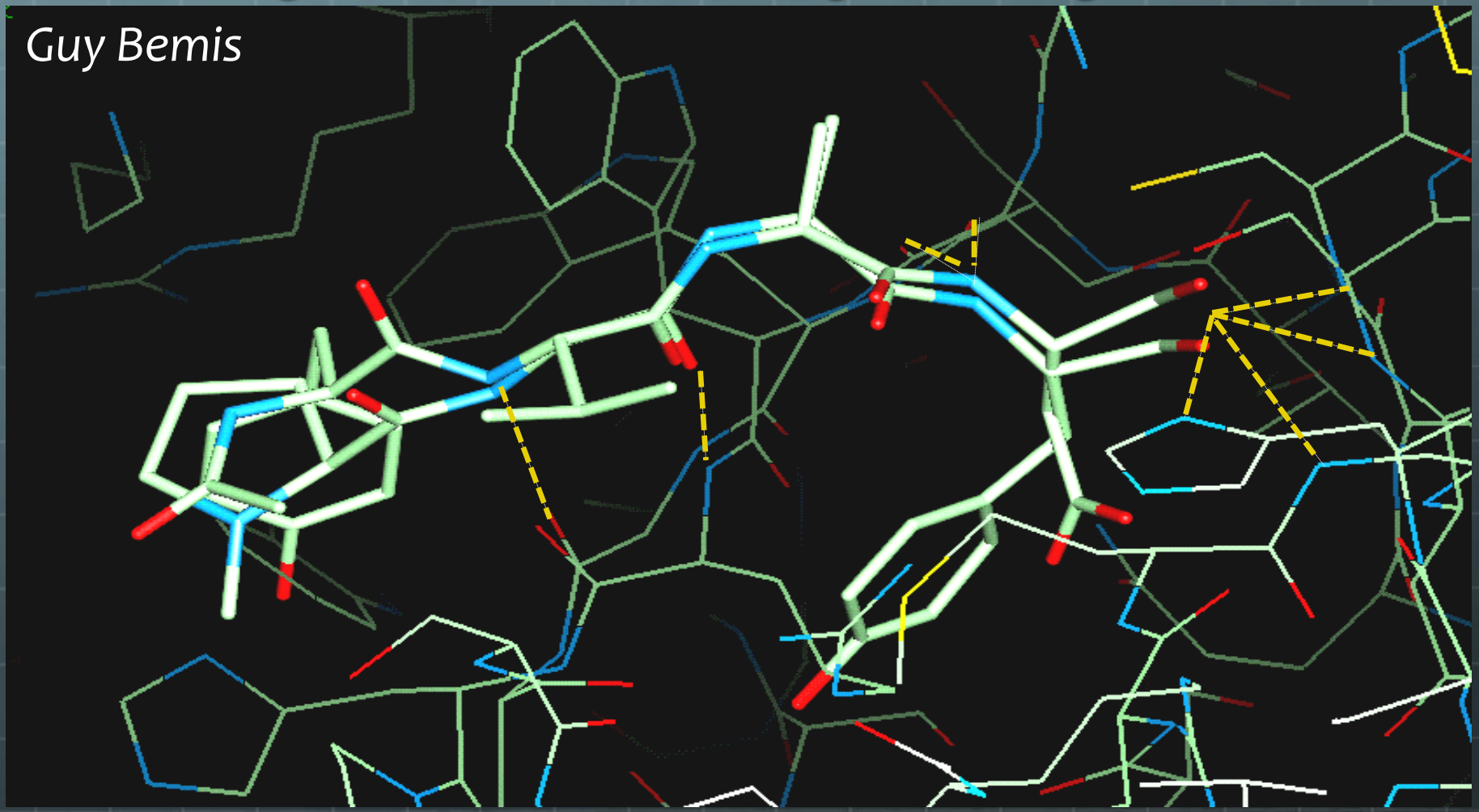
Broad exploration of chemotypes; aggressive use of structural info.

Testing multiple compounds for each scaffold; "bracketing

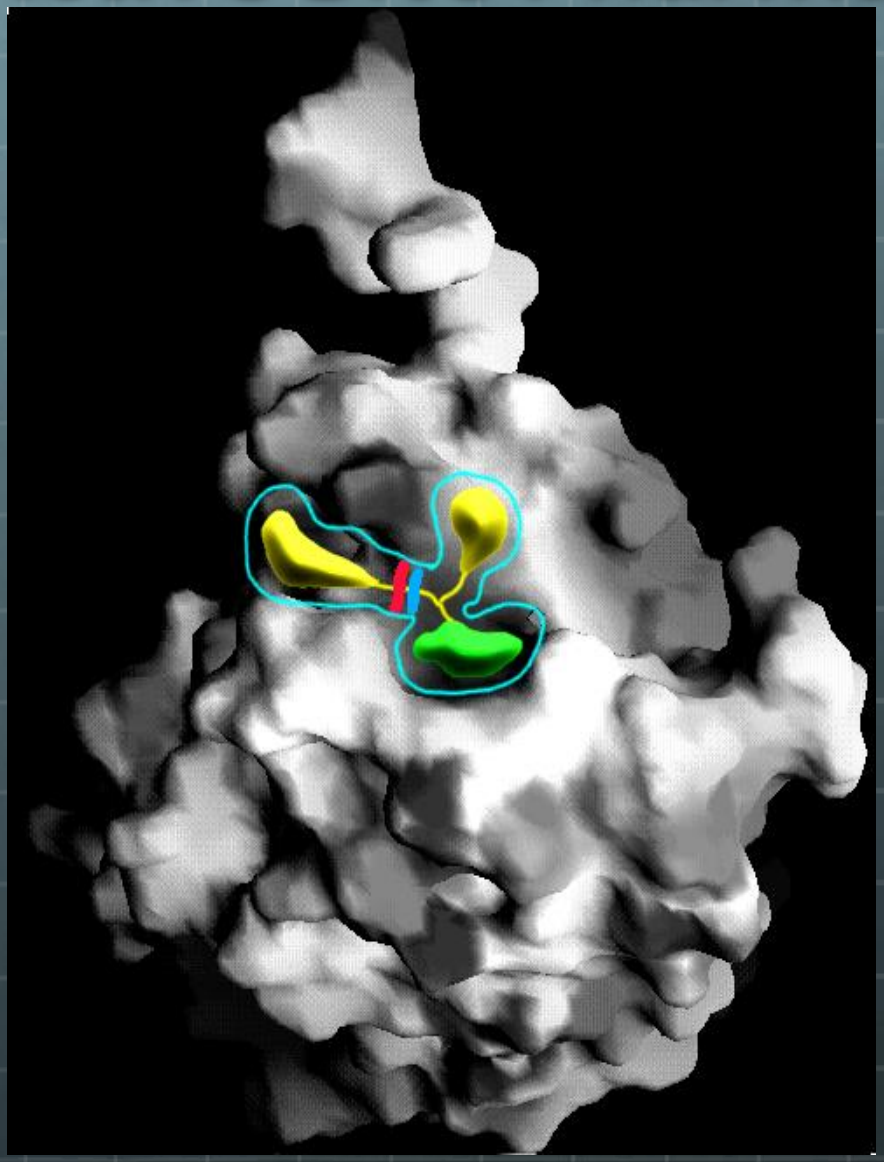
Chemistry centered on drug-like cmpds; early focus on PK, whole-blood cell efficacy

The ICE - Chymotrypsin Connection

Different global folds - similar ligand recognition motifs



The ICE Active Site Pharmacophore



ICE: Good or Bad Outcome?

- Dev. candidate series designed within 5 wks of xray
- First compound synthesized was 20 nM
- Sixth compound: decent oral rat clearance and t-1/2
- Development candidate 2 years after that
- Efficacious in 280 patient Phase 2A RA study

ICE: Good or Bad Outcome?

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- Sixth compound: decent oral rat clearance and t-1/2
- Development candidate 2 years after that
- Efficacious in 280 patient Phase 2A RA study
- **But:**
  - **Molecules were high-MW acids with poor permeability, poor WB cell activity, low human half-life, and high dose**
  - **Pralnacasan showed fibrosis in dogs after 9 months at very high dose**
  - **Aventis dropped program during Phase 2B RA trial**

ICE: Lessons

- Fantastic SBDD effort → blistering speed
- Creative insight re: chymotypsin fold led to breakthrough
- Deep understanding of relevant history led to dev candidate scaffold
- Deep understanding of protein-ligand recognition motifs led to broad patent claims
- But in the end, the molecule was sub-standard and our understanding of the disease biology was inadequate.
- So: a SBDD ***failure***.

Part 3: Covalency

Covalent Drugs: More Common Than You'd Think

Table 2. Targets, Indications, and Mechanism of Action of Covalently Interacting Small Molecules

mechanism	target	indication	name of drug or representative drug[a]	reacting functionality	reversibility	dose (mg)[b]
acylation	serine-type D-Ala-D-Ala carboxypeptidase	bacterial infection	amoxicillin[c]	β-lactam	irreversible	100—500
	triacylglycerol lipase	obesity	orlistat	lactone	reversible	360
	acetylcholinesterase	Alzheimer's	rivastigmine	carbamate	reversible	6—12
	β-lactamase	bacterial infection	clavulanate[c]	β-lactam	irreversible	500
	prostaglandin endoperoxidase synthase	pain	aspirin	ester	reversible	1000
	vitamin K epoxide reductase (warfarin-sensitive)	anticoagulant	warfarin	coumarin		2—10
	enol—acyl carrier protein reductase	bacterial infection (tuberculosis)	*isoniazid*	hydrazide[d]	irreversible	300
	aldehyde dehydrogenase	alcoholism	*disulfiram*	disulfide	irreversible	500[e]
alkylation	UDP-*N*-acetylglucosamine-1-carboxyvinyltransferase	bacterial infection	fosfomycin	epoxide		3000
	alanine racemase	bacterial infection (tuberculosis)	D-cycloserine	amine[d]		>250
	GABA-AT[f]	epilepsy	vigabatrin	amine[d]	irreversible	3000[e]
metal/metalloid binding	aromatase	breast cancer	*exemstane*[c]	methyl	irreversible	25
	proteasome	multiple myeloma	bortezomib	boronic acid	reversible	3
disulfide bond formation	H^+/K^+ ATPase	gastresophageal reflux disease	*omeprazole*[c]	sulfenamide	irreversible	20
	P2Y12 purinoceptor antagonist	platelette aggregation inhibitor	*clopidogrel*	thiol	irreversible	75
(seleno-enzyme)	thyroxine 5′-deiodinase (type 1)	hyperthyroidism	propylthiouracil	thiourea		450
hemiketal formation	serine protease hepatitis C virus NS3[g]	viral infection	VX-950 (**1q**)	ketoamide	reversible	n/a
Michael addition	ribonucleoside diphosphate reductase	cancer	*gemcitabine*[c]	vinyl ketone		≥150—000[h]
	thymidylate synthase	cancer	floxuridine[c]	unsaturated amide	reversible	0.1—0.6 (mg/kg)/d
	ErbB1/2[g]	cancer (NSCLC)	HKI-272 (**1t**)	unsaturated amide	irreversible	n/a
	5-α-reductase	benign prostatic hyperplasia	finasteride[c]	unsaturated amide[d]	reversible	5
	MAO-B	Parkinson's disease[i]	*selegiline*[c]	aceylenic imine[d]	irreversible	1
Pinner reaction	DPP IV[g]	diabetes	vildagliptin	nitrile	reversible	100
	cathepsin K[g]	osteoporosis	odanacatib	nitrile	reversible	10—50[j]

[a] Prodrugs are indicated in italics. [b] As determined from the FDA label or other medical references. [c] Because of the large number of drugs developed for these targets, one representative drug is indicated in the table. [d] Indicates functionality covalently modified by the cofactor. [e] Estimated dose. [f] Approved in Canada, U.K., and Mexico. [g] Under clinical investigation. [h] Dose = 1000 mg/m^2 weekly. The average body surface area of a person is approximately 1.5—2 mm^2. [i] Several irreversible MAO inhibitors are on the market for the treatment of depression. [j] Weekly dose used in the clinical trial "MK0822 (Odanacatib) Late Phase II Dose-Finding Study" described at www.clinicaltrials.gov.

Source: Potashman, Michele H., and Mark E. Duggan. "Covalent Modifiers: An Orthogonal Approach to Drug Design." *Journal of Medicinal Chemistry* 52, no. 5 (2009): 1231-46.

Aspirin MOA Finally Revealed

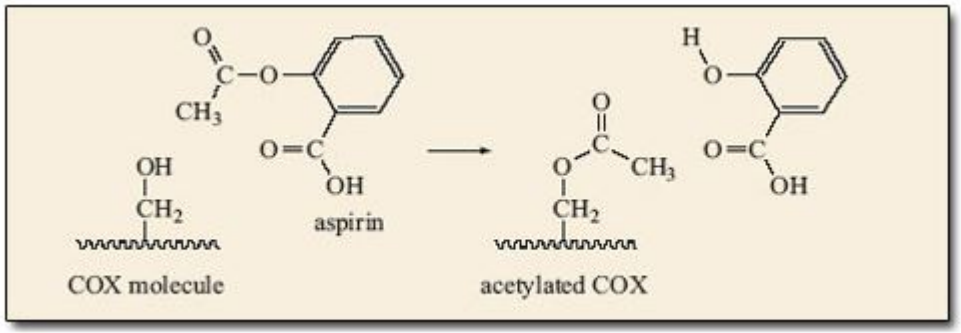
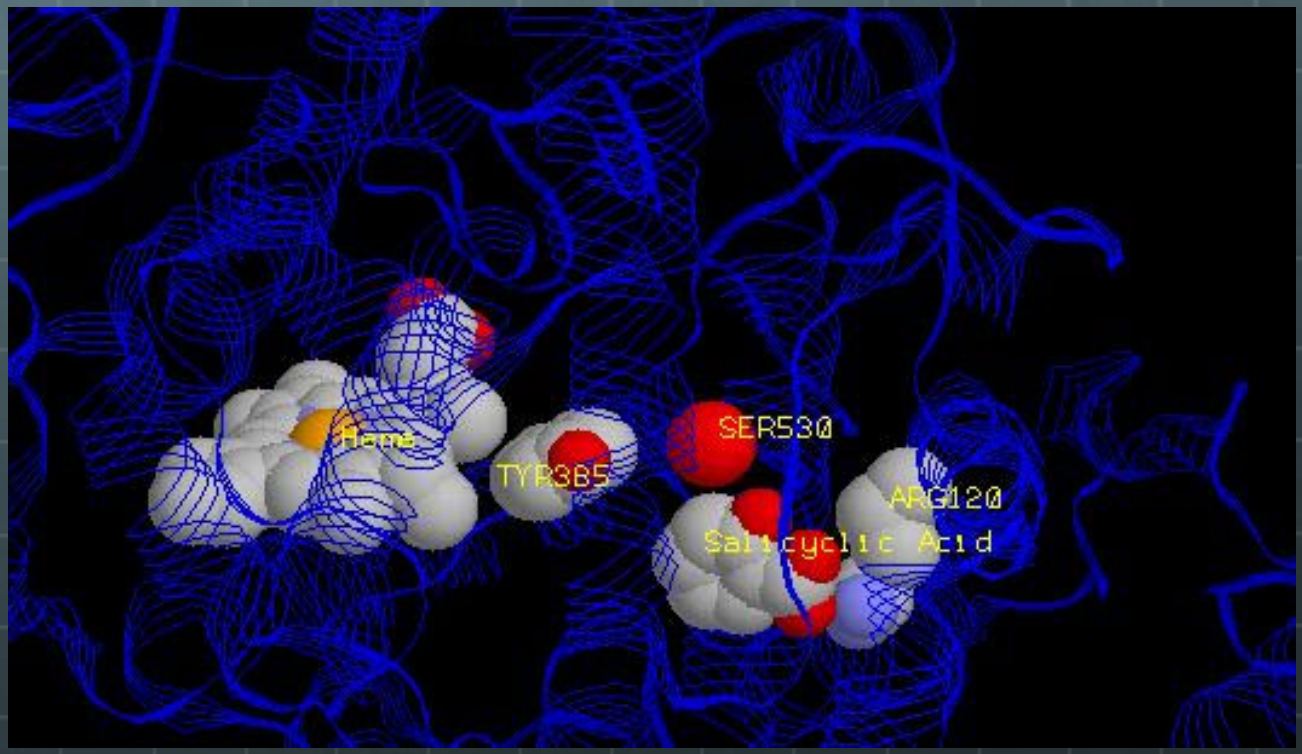
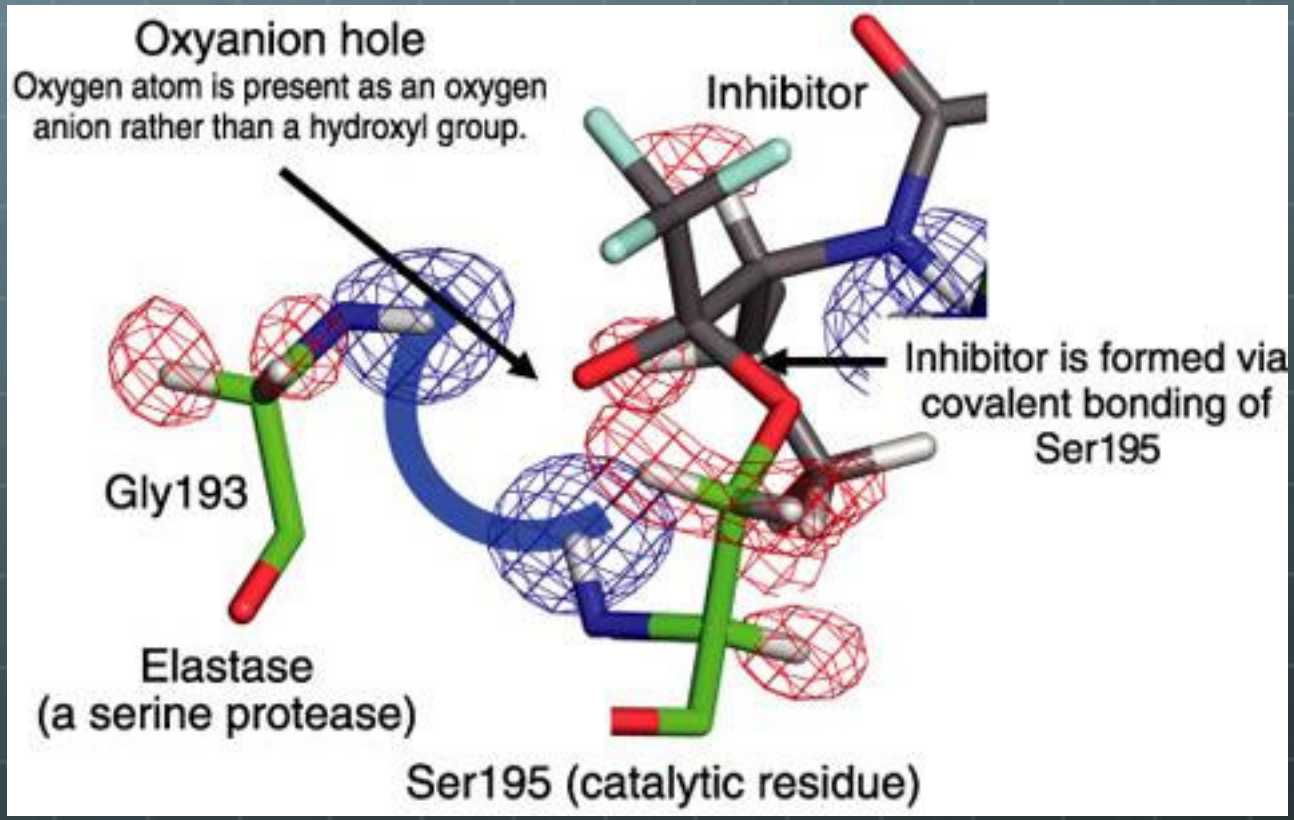


Figure 16: The acetyl group in aspirin reacts with an alcohol group inside the COX cavity.

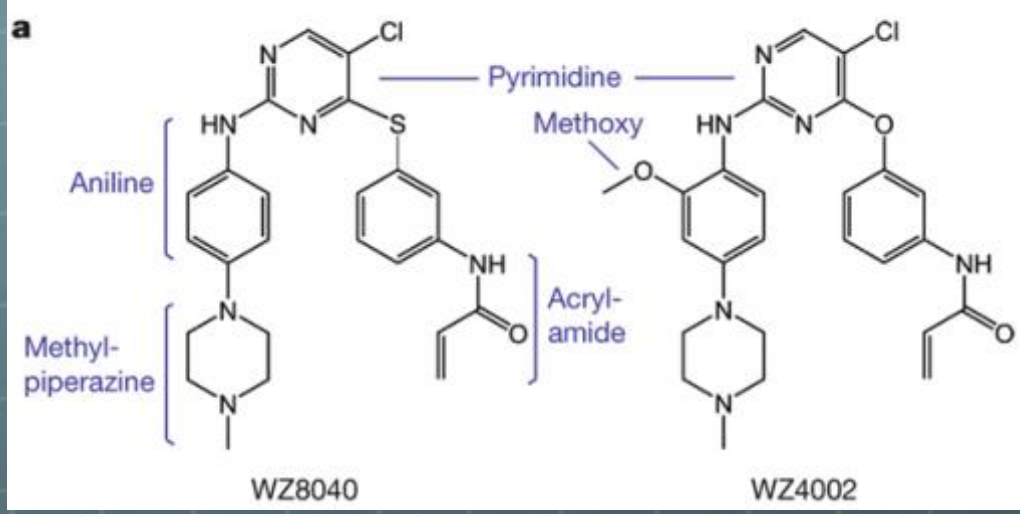


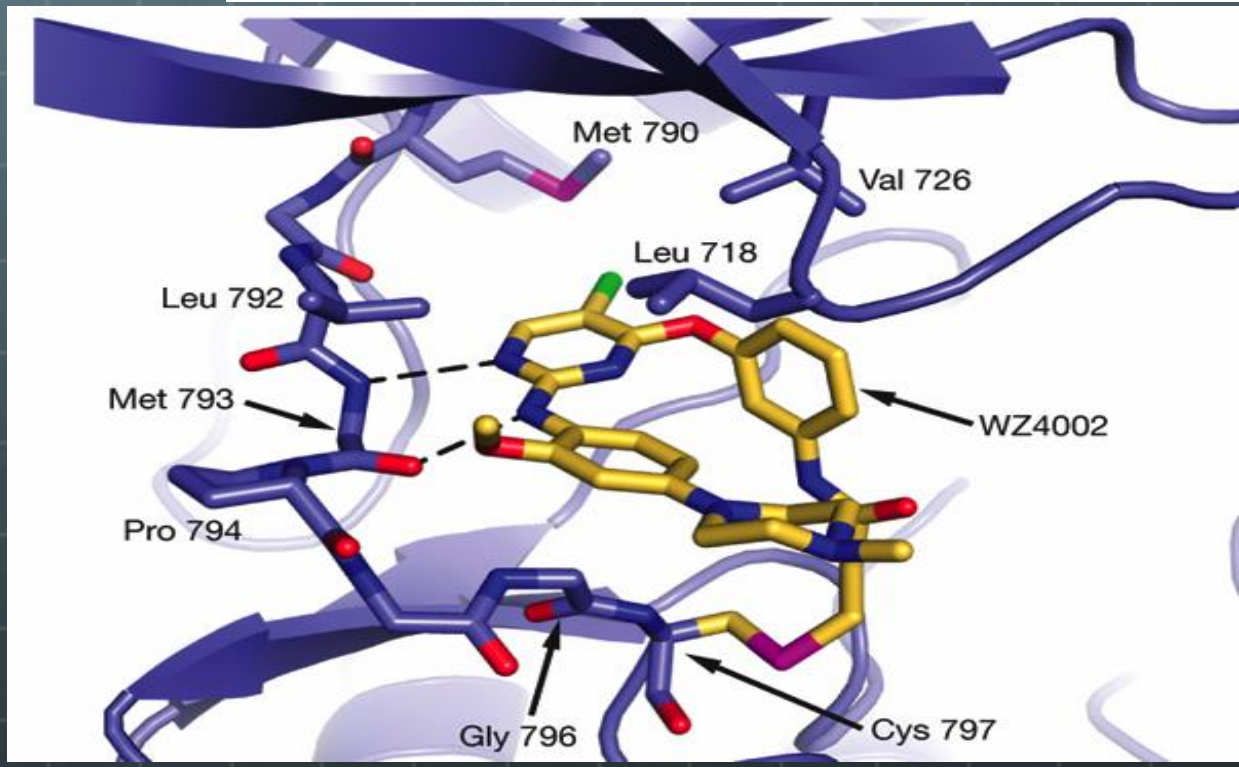
Covalent Serine Protease Inhibitors



Irreversibles Don't Have to Use Catalytic Residues

- Epidermal growth factor receptor (EGFR) kinase inhibitors
- Acrylamide moiety reacts with conserved cysteine
- Discovered by screening against mutants resistant to other EGFR inhibitors

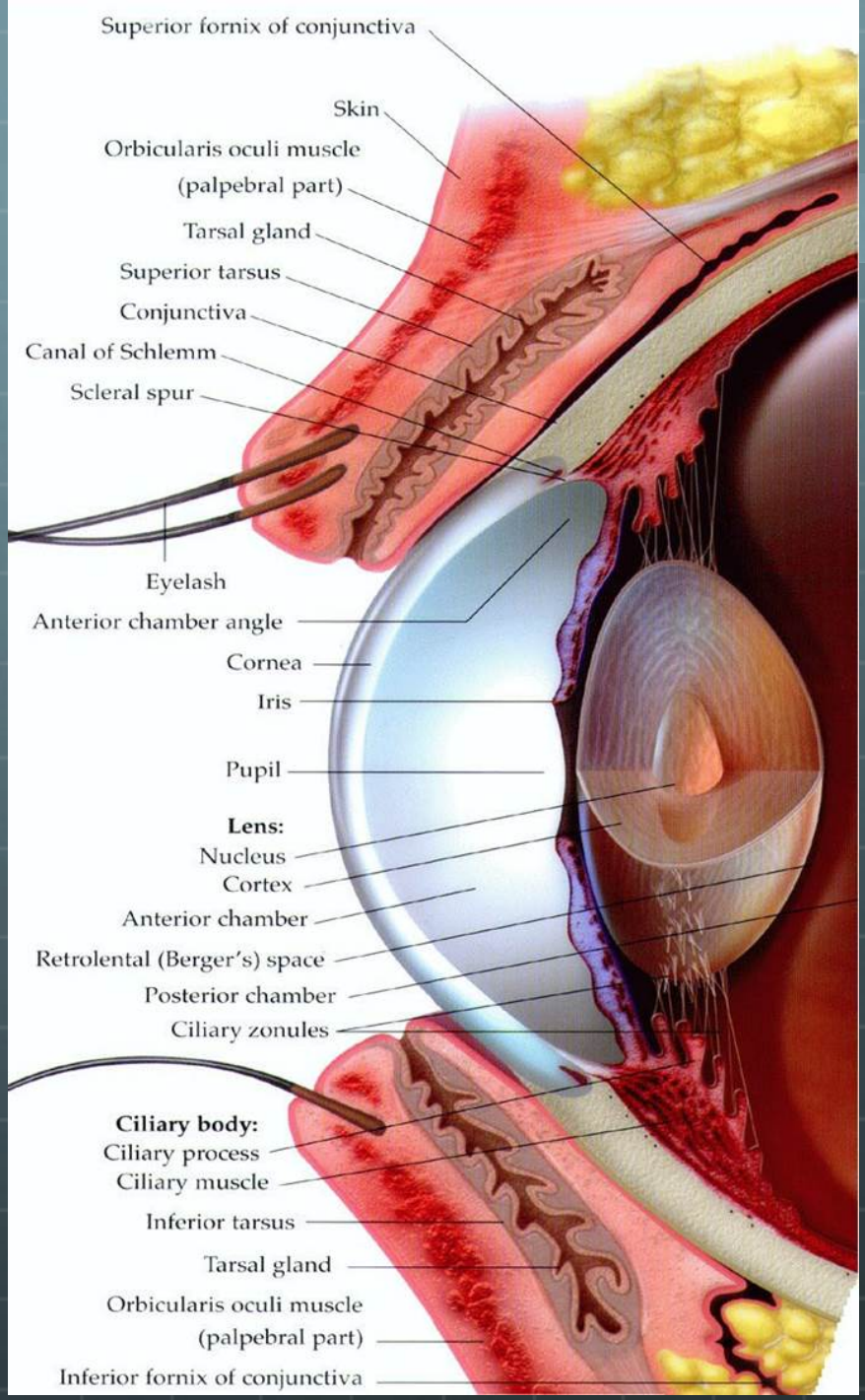


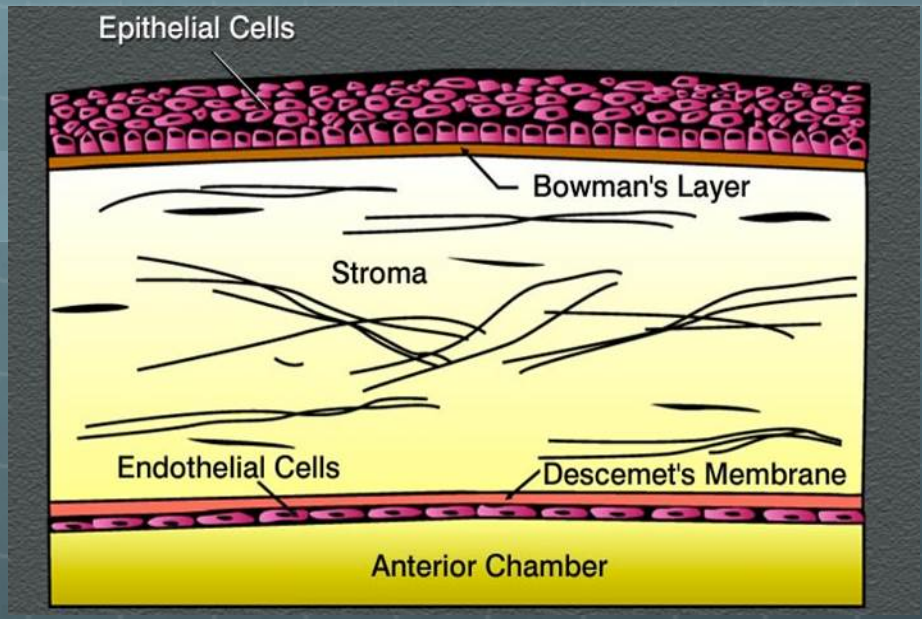


Source: Zhou, Wenjun, Dalia Ercan, et al. "Novel Mutant-Selective EGFR Kinase Inhibitors Against EGFR T790M." *Nature* 462, no. 7276 (2009): 1070-4.

Part 4:
Four SBDD Drugs

Glaucoma





The epithelium - Covers the surface of the cornea, is about 5-6 cell layers thick.

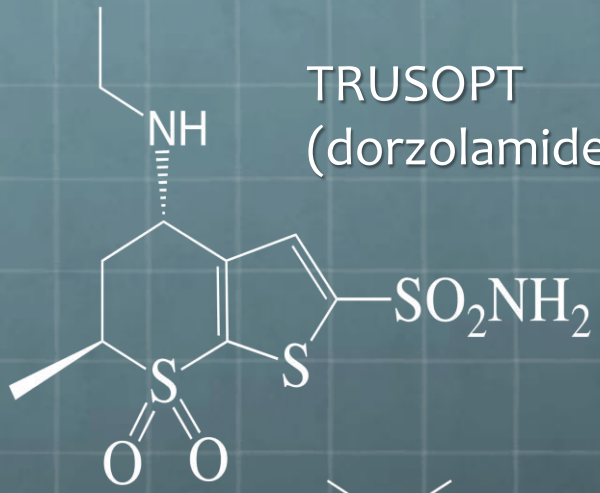
Bowman´s membrane - Very difficult to penetrate.

The stroma - The thickest layer, composed of tiny collagen fibrils that run parallel to each other, this precision formation gives the cornea its clarity, strength, elasticity, and form.

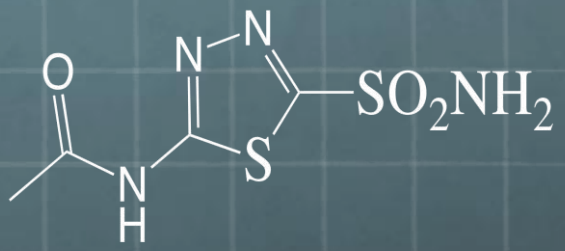
Descemet's membrane - A thin but strong sheet of tissue that acts as protection against infection and injuries. It is composed of collagen fibers (different from those of the stroma).

The endothelium - Essential in keeping the cornea clear. It pumps this excess fluid out of the stroma, which has the danger of swelling with water.

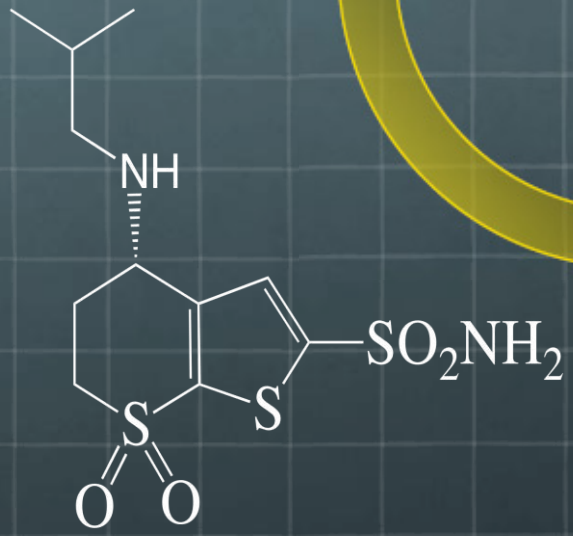
First Crystallography-Based Drug Design Example (Merck)

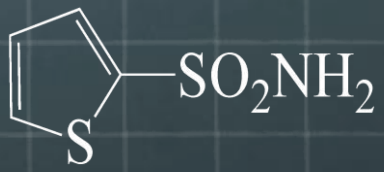


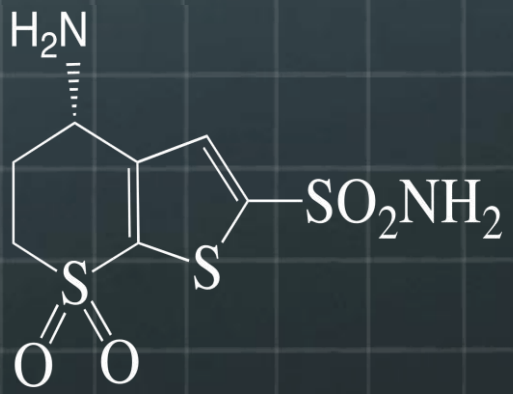
TRUSOPT
(dorzolamide HCl)



DIAMOX (acetazolamide)



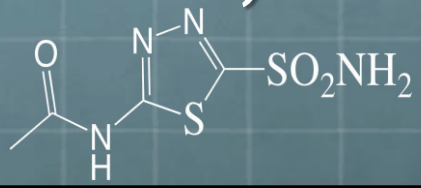




J. Med. Chem. 30, 591-599 (1987)
J. Med. Chem. 32, 2510-2522 (1989)
J. Med. Chem. 37, 1035-1054 (1994)

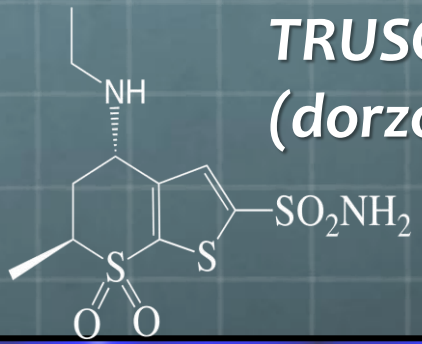
A Struggle of Biblical Proportions?

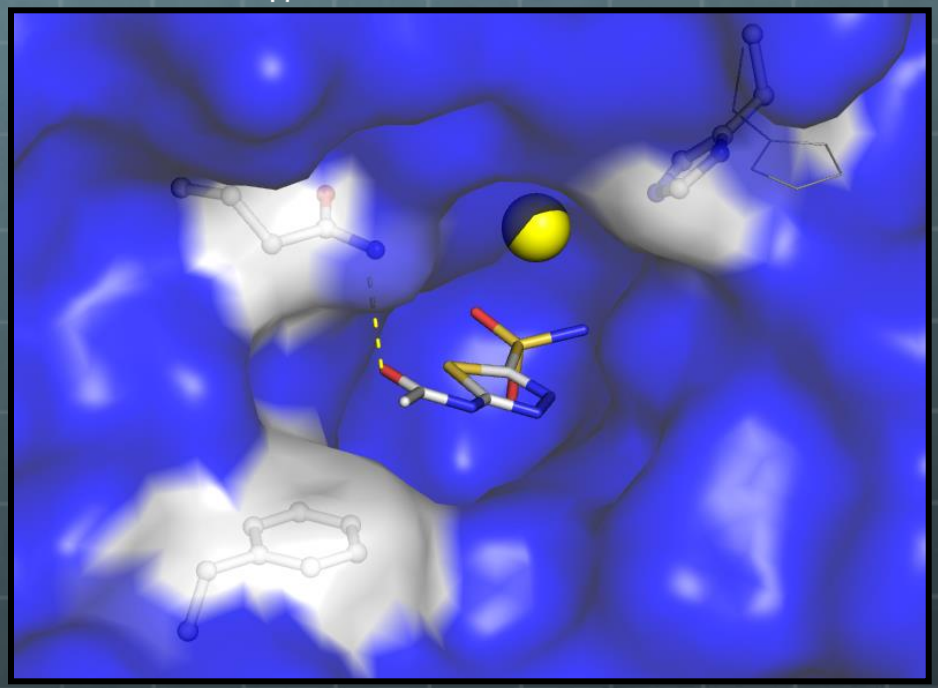
DIAMOX
(acetazolamide)

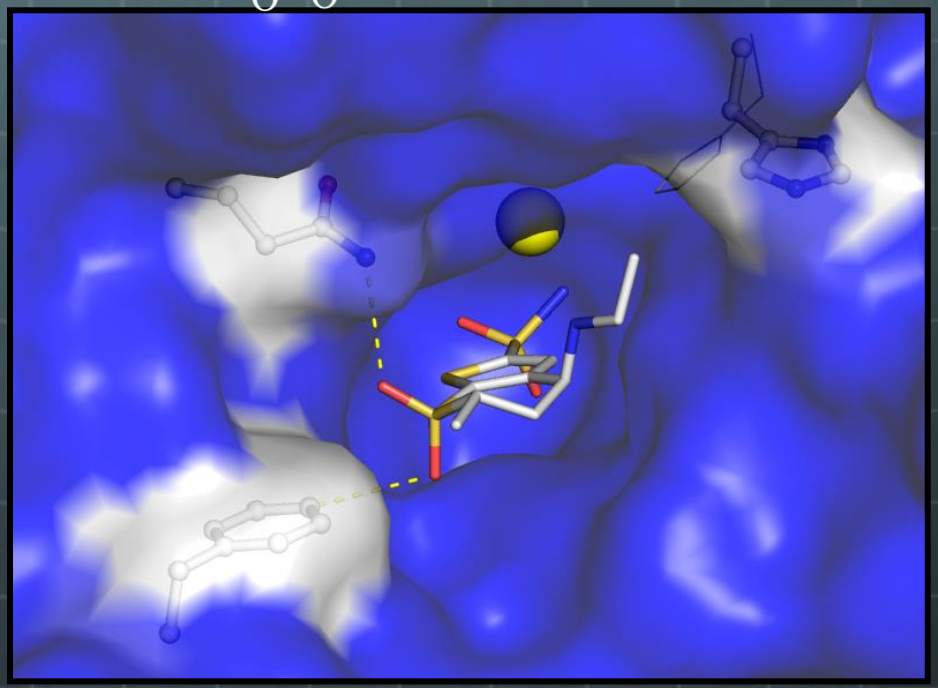


40 YEARS

TRUSOPT
(dorzolamide HCl)







1YDA, Nair et al
Biochemistry 34, 3981-3989 (1995)

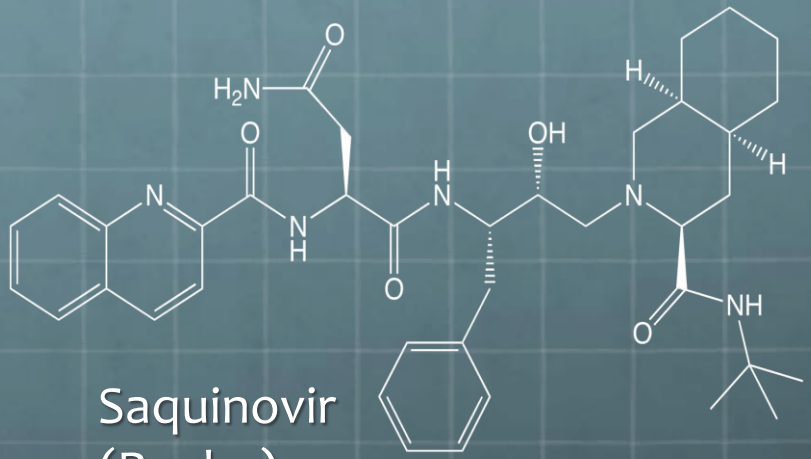
1CIL, Smith et al
Protein Sci. 3, 118-125 (1994)

Carbonic Anhydrase: Lessons

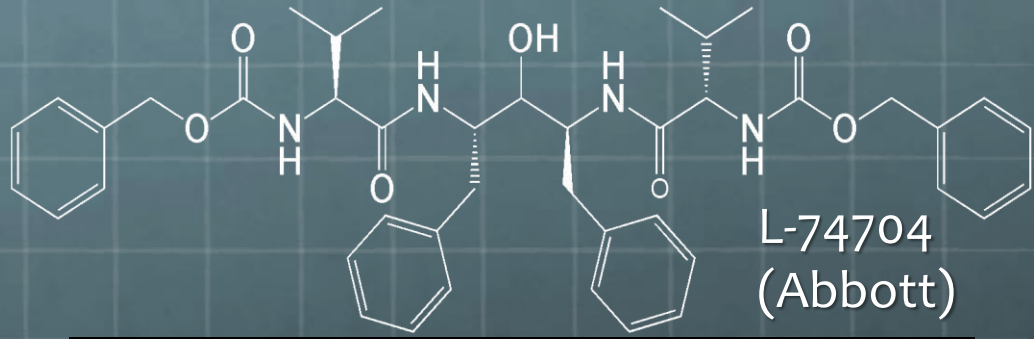
- When working on validated targets, “stay the course”
- SBDD can be used to optimize physical / biological properties
- Conformational analysis is critical

HIV

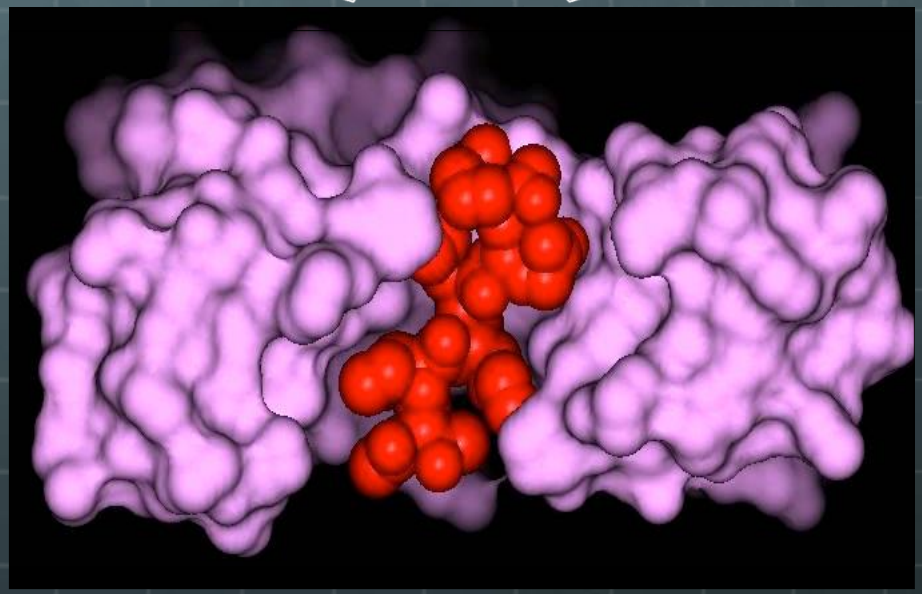
HIV Protease: Prototypes, Circa 1992



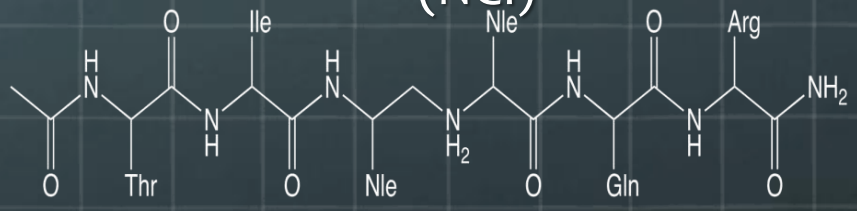
Saquinovir
(Roche)



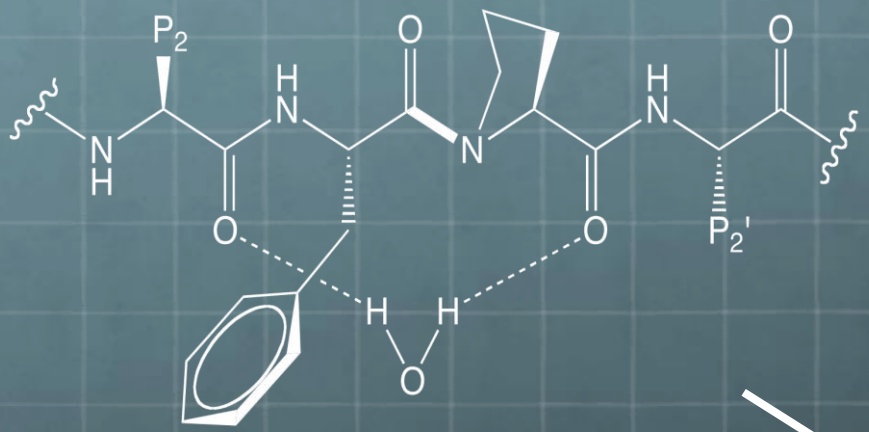
L-74704
(Abbott)



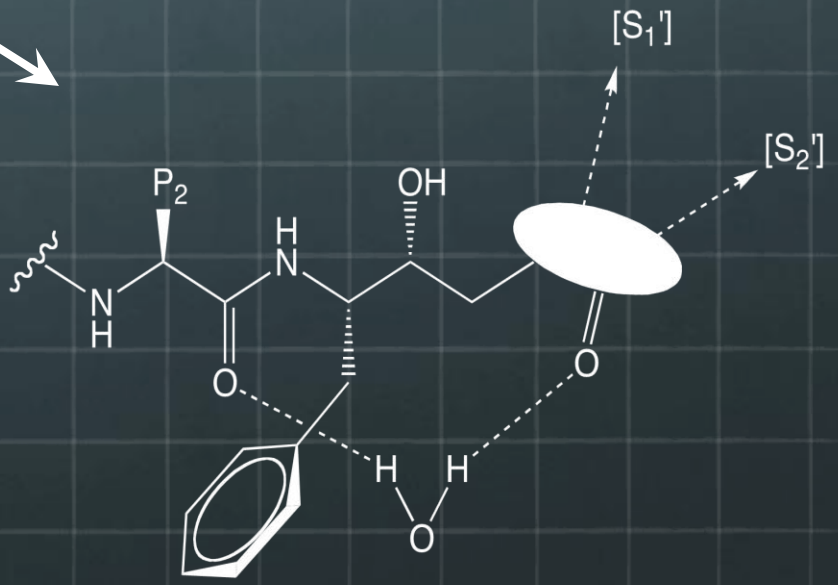
MVT-101
(NCI)



Novel Scaffolding → Simpler Molecules?

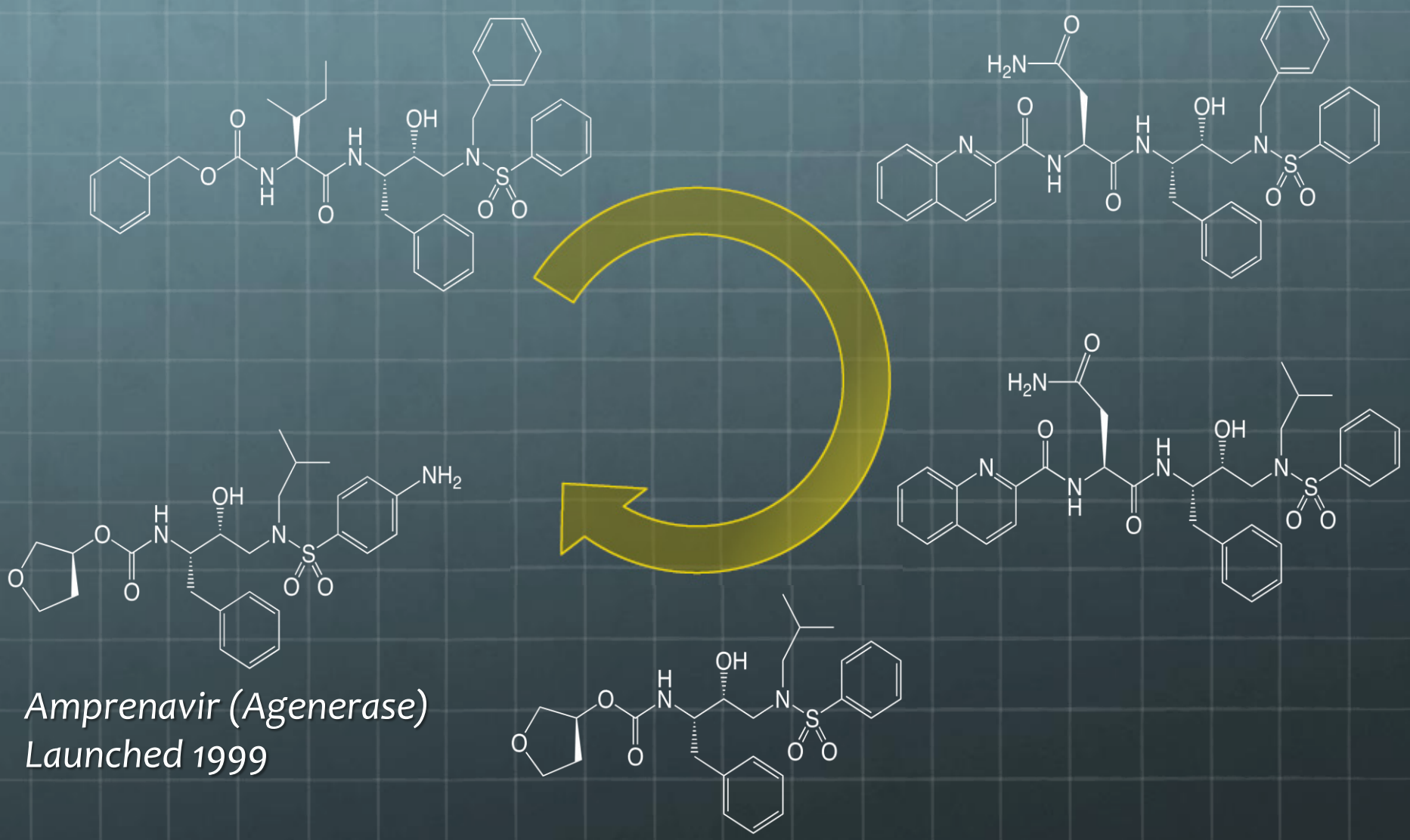


- Preserve the interactions with catalytic Asps
- Maintain the hydrogen bonds to the flap water
- Design a scaffold which can reach S_1' and S_2'
- Design a scaffold with minimal binding strain



Roger Tung, Govinda Rao

5 Chemists, 12 Months, 204 Compounds

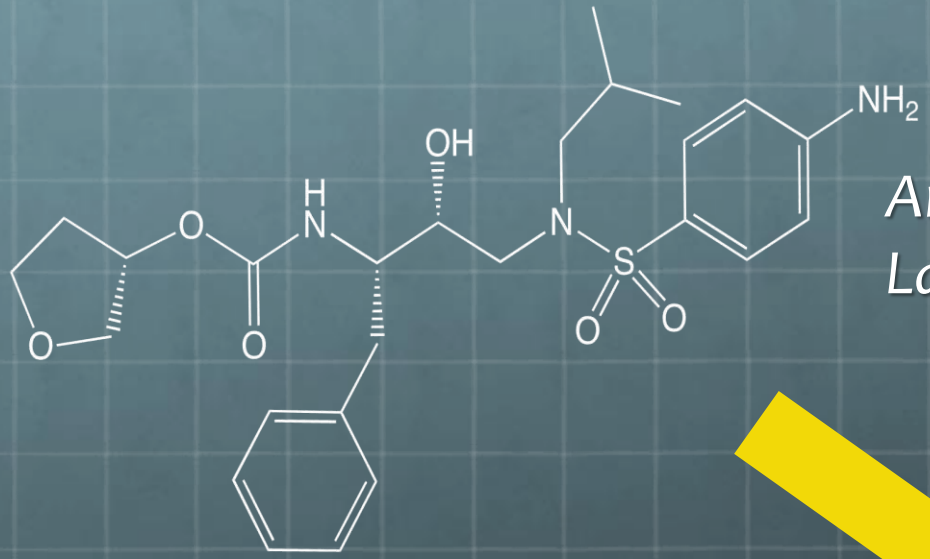


Amprenavir (Agenerase)
Launched 1999

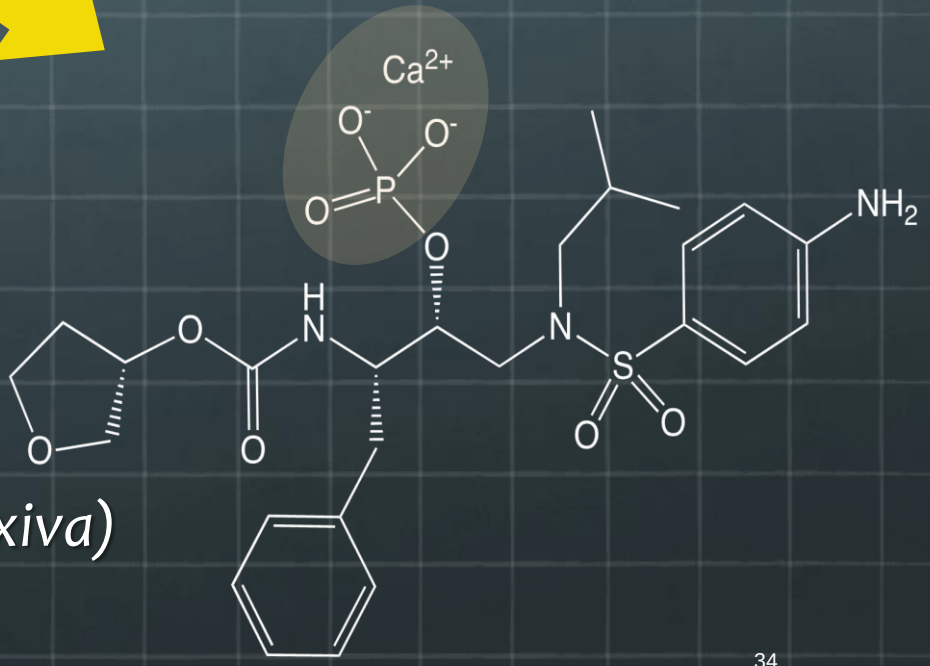
JACS 1995, 117, 1181-1182
Protease Inhibitors in AIDS Therapy, Flexner & Ogden, ed. pp 101-118

D'Oh !

Roger Tung



Amprenavir (Agenerase)
Launched 1999



Fosamprenavir (Lexiva)
Launched 2003

HIV-Protease: Lessons

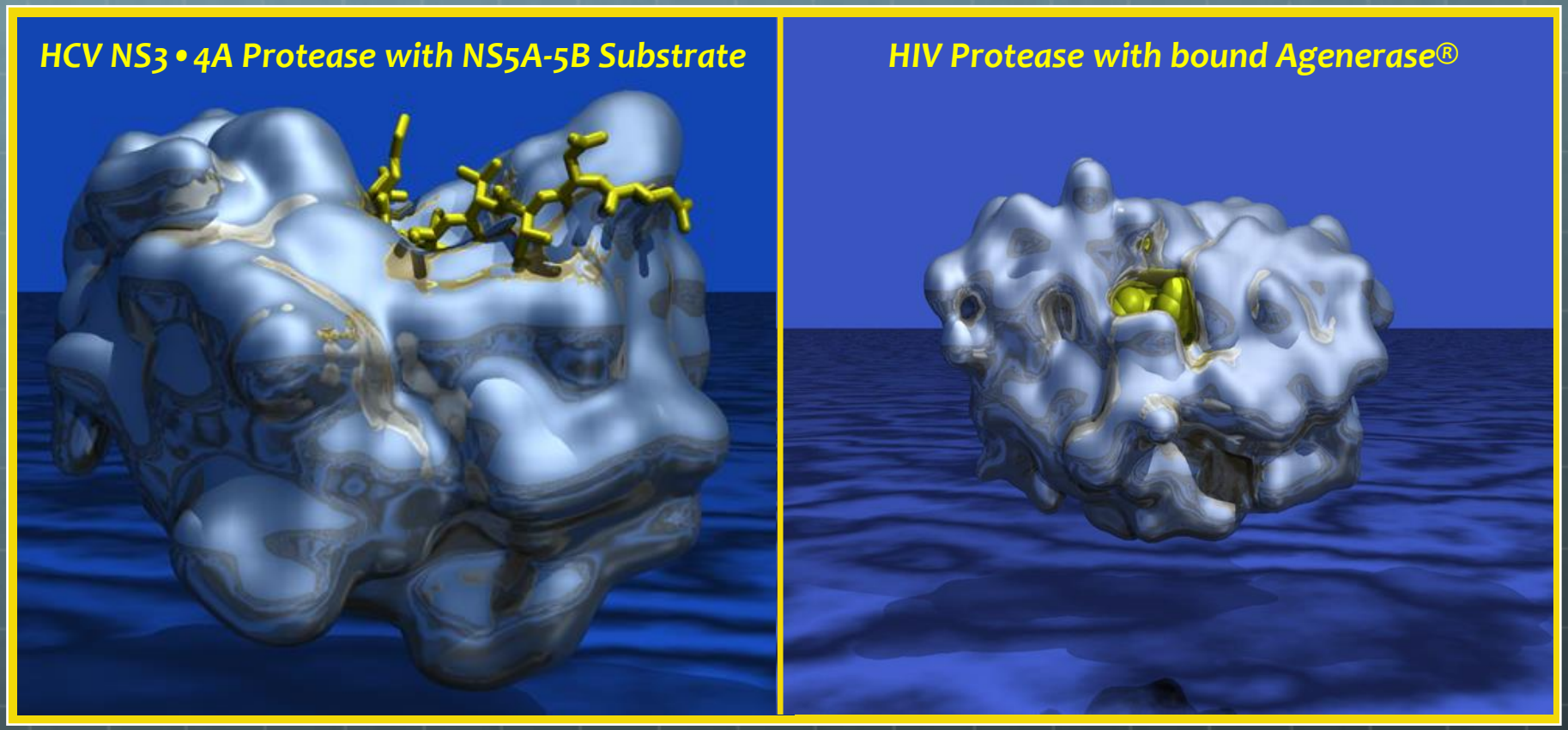
- Conformational analysis is incredibly powerful
- SBDD can help optimize physical properties
- Sometimes the marketing guys are right
- Pay attention to formulation early

HCV

Hepatitis C Infection

- Infects ~200 million people worldwide
- Progresses to cirrhosis in 20-30% of cases
- Progresses to hepatocellular carcinoma in 1-3% of cases
- Responsible for ~10,000 death / yr in US
- PEG IFN-α + Ribavirin <50% effective

HCV Protease: A Dinner Plate

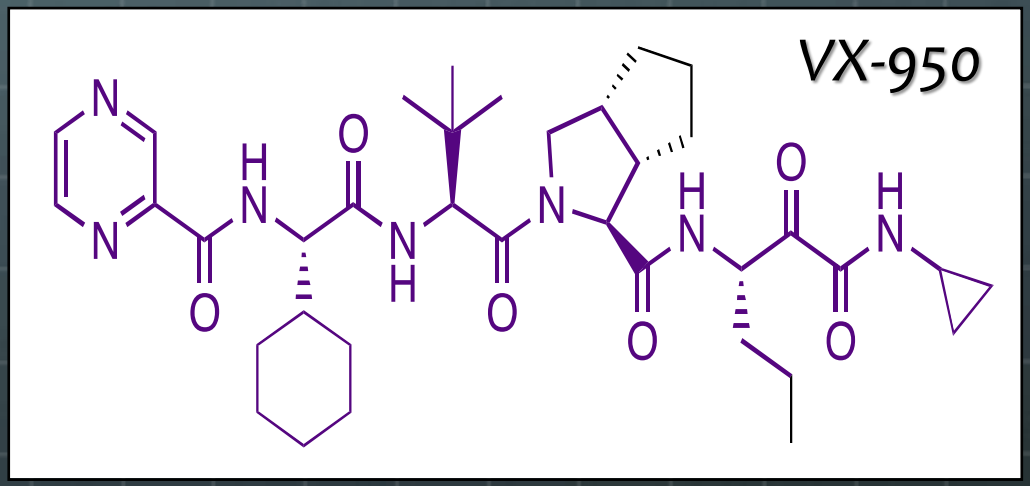


"Several loops found in other chymotrypsin family proteases are missing from HCV. These loops normally play a critical role in defining the shapes of the non-prime-side substrate-binding pockets. The absence of these loops in HCV-PR renders the binding groove ***relatively featureless****, and this constitutes a* ***challenge for drug design efforts****. It is therefore anticipated that* ***structural information*** *for enzyme-inhibitor complexes* ***may be crucial*** *for optimization of potent, drug-like inhibitors."*

Kim et al, Cell 87, 343 (1996)

HCV: Telaprevir

- Efficacy surrogate: high ratio of liver concentration to IC_{50}
  - $[C_{liver}] > 10X\ IC_{50}$
  - Fa more important than %F
- High $[C_{liver}]$ compared to other organs or tissues
  - Minimize potential for systemic toxicity
  - Some plasma exposure required to cover extra-hepatic sources of virus and for drug load monitoring in patients

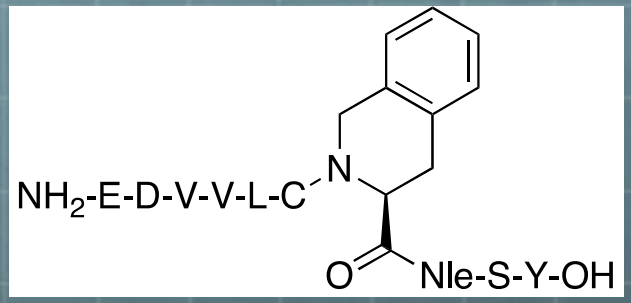


HCV Program Strategy

- Efficacy surrogate: high ratio of liver concentration to IC_{50}
  - $[C_{liver}] > 10X\ IC_{50}$
  - F_a more important than %F
- High liver concentrations are generally desirable compared to other organs or tissues
  - Minimize potential for systemic toxicity
  - Some plasma exposure required to cover extra-hepatic sources of virus and for drug load monitoring in patients

Perni, R.B. et. al. Bioorg. Med. Chem. Lett. ***2004****, 14, 1939-1942*
Y. Yip et. al. Bioorg. Med. Chem. Lett. ***2004****, 14, 251-256*
F. Victor et. al. Bioorg. Med. Chem. Lett. ***2004****, 14, 257-261*

Truncating the Decapeptide Substrate Mimic

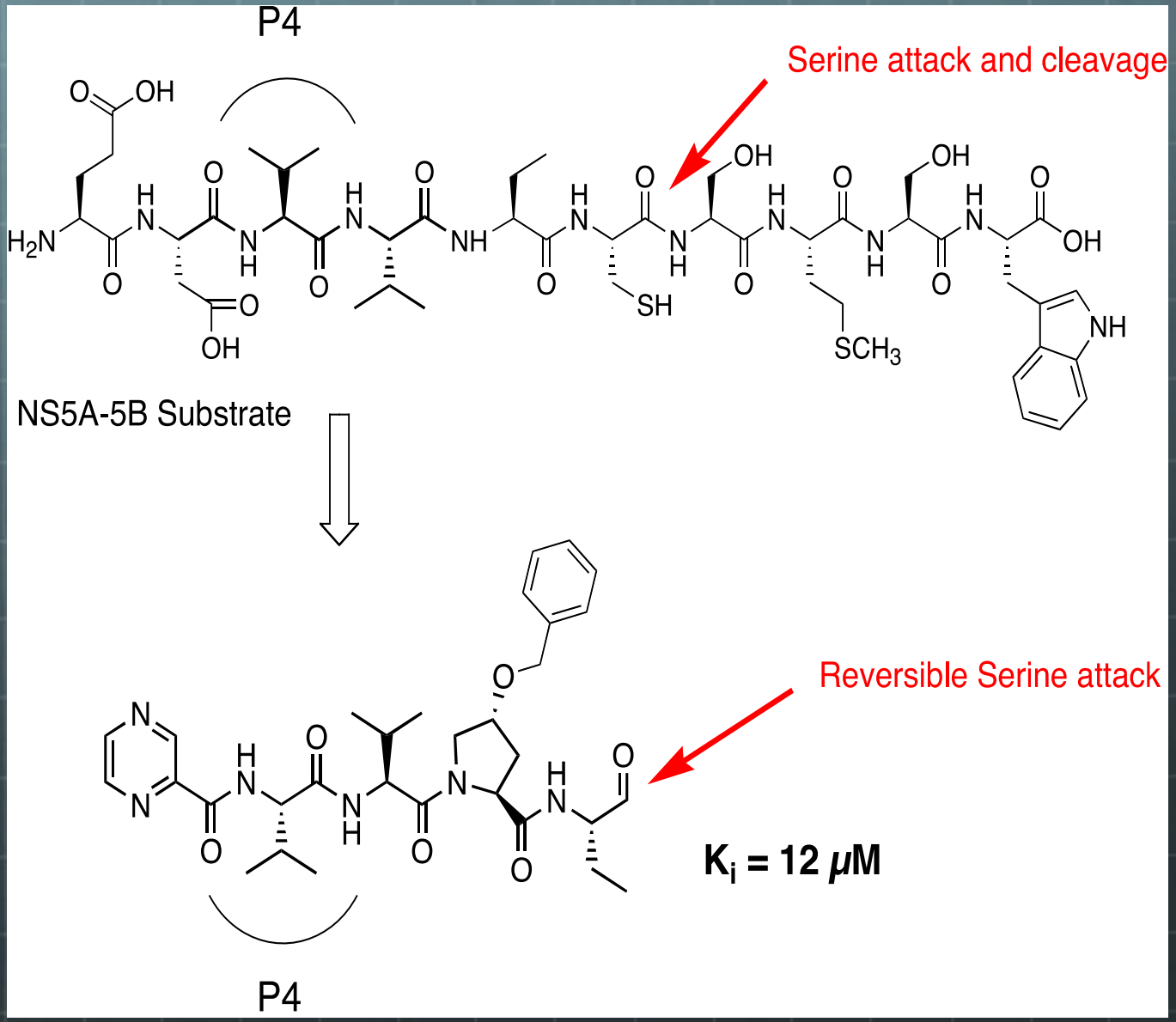


	K_i (uM)
H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	0.34
H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-OH	27
H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-OH	17
H-Glu-Asp-Val-Val-Leu-Cys-Tic-OH	14
H-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	4.4
H-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	79
H-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	500
H-Leu-Cys-Tic-Nle-Ser-Tyr-OH	2000

J.A. Landro et. al. Biochemistry ***1997****, 36, 9340-9348*

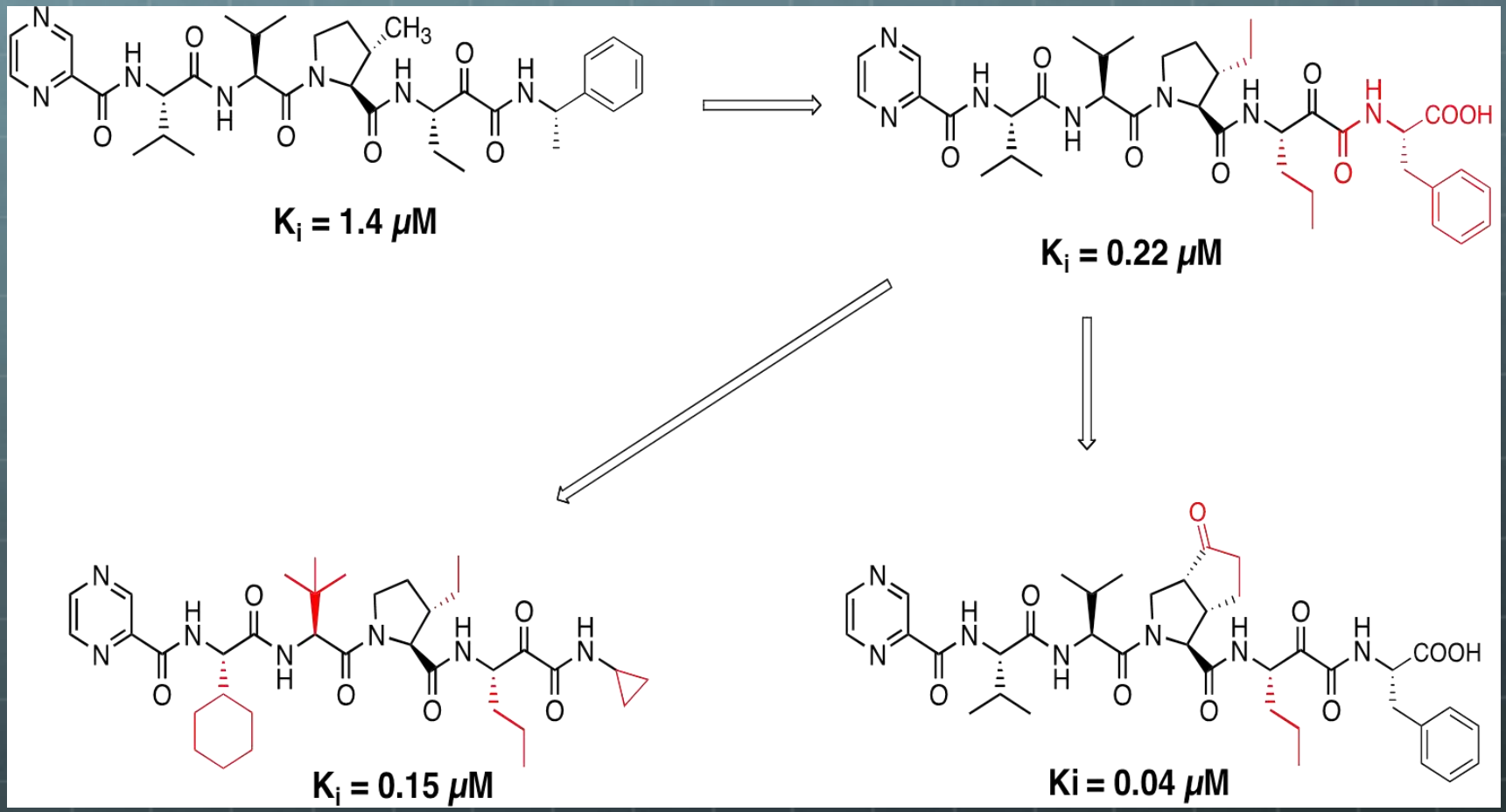
Inhibitor Evolution

Substrate



Inhibitor

Multi-Subsite Optimization

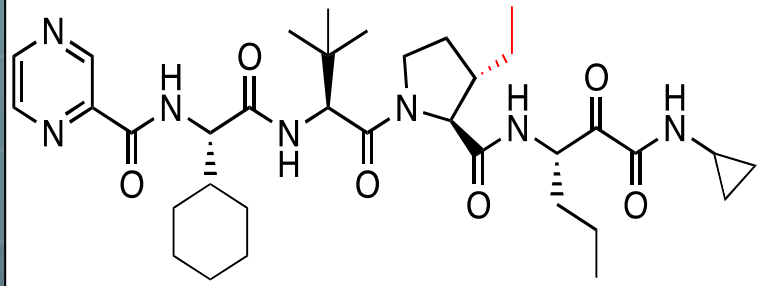


Perni, R.B. *et. al. Bioorg. Med. Chem. Lett.* **2004**, *14*, 1939-1942

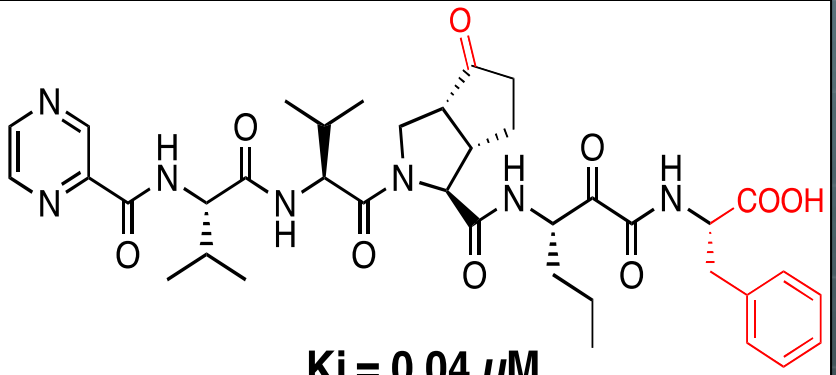
Y. Yip *et. al. Bioorg. Med. Chem. Lett.* **2004**, *14*, 251-256

F. Victor *et. al. Bioorg. Med. Chem. Lett.* **2004**, *14*, 257-261

The Finish Line

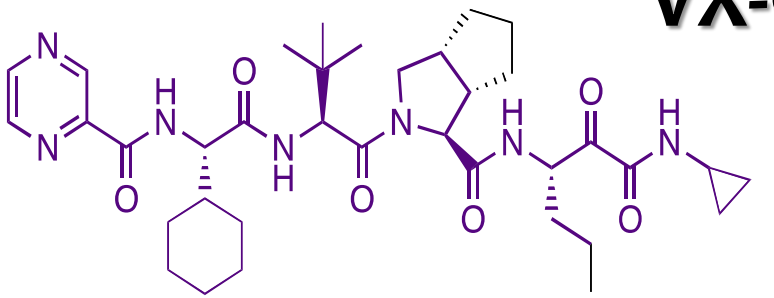


K_i = 0.15 μM
Replicon IC_{50} = 0.45 μM



Ki = 0.04 μM

VX-950



Two-step binding mode: conformational flip of both enzyme and ligand at the catalytic machinery

K_i = 44 nM (15 min pre-incubation)
K_i* = 7 nM
Replicon IC_{50} = 0.35 μM

How Do You Know You're Done?

A "good drug" --

- Serves an important need
- Has enough potency, bioavailability, and safety
- Is novel
- Can be made (formulation, synthesis, stability, …)

"Every design balances--connects--dozens of values, like a conceptual mobile, and the weights of those values, their relative utility or attractiveness, are changing constantly."

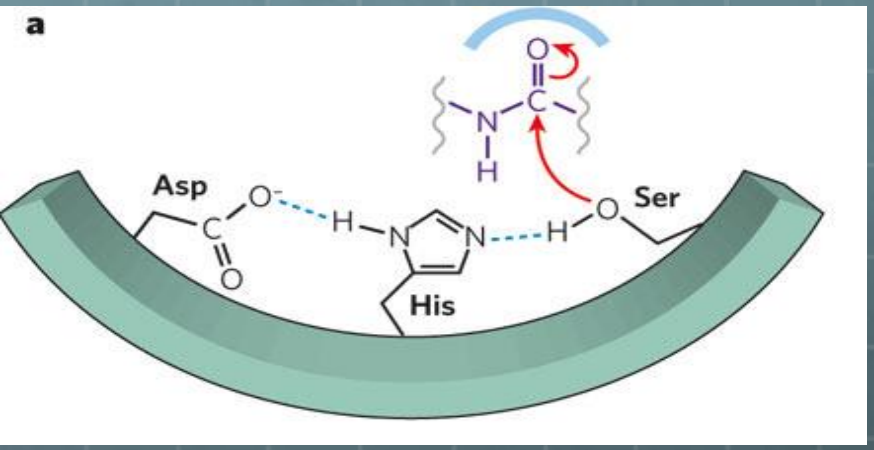
"At some point you have to shoot the engineers and ship."

"A great design attracts applications, and in doing so necessarily makes its creators look short-sighted and slightly dumb."

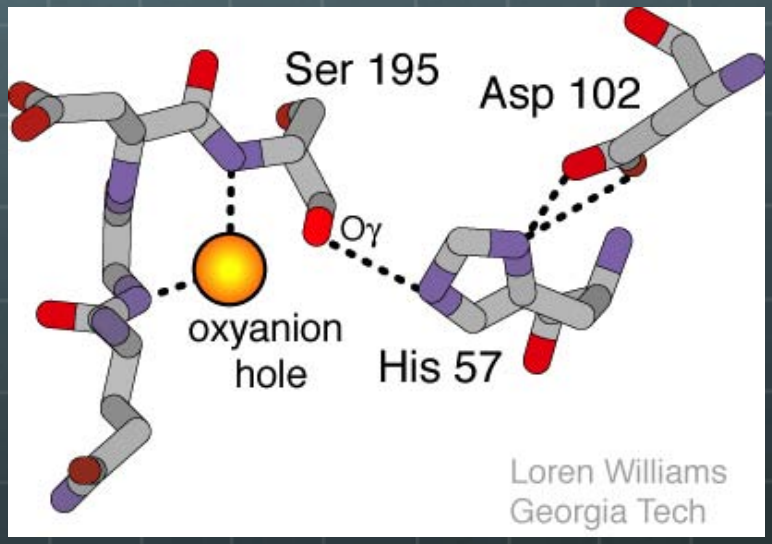
Fred Hapgood, Up the Infinite Corridor : MIT and the Technical Imagination

Telaprevir: An Insurmountable HCV-PR Inhibitor

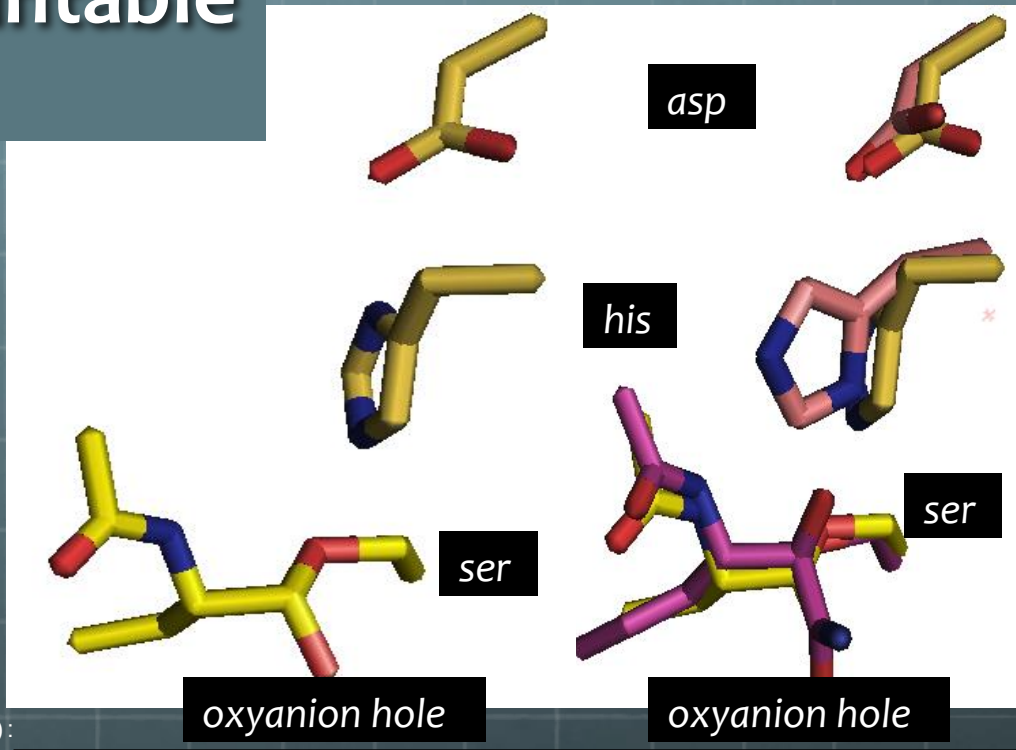
oxyanion hole

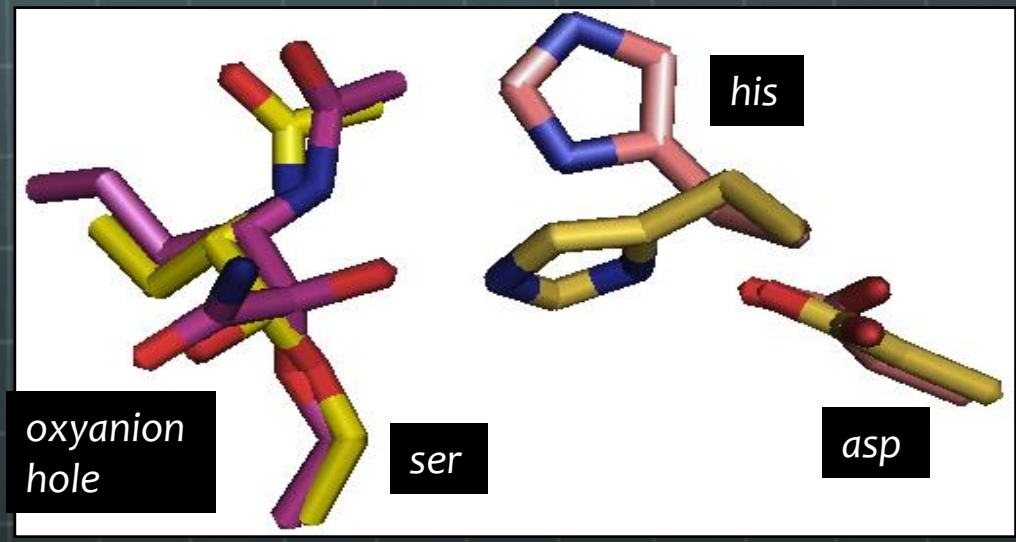


Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Erez, Elinor, Deborah Fass, et al. "How Intramembrane Proteases Bury Hydrolytic Reactions in the Membrane." *Nature* 459, no. 7245 (2009): 371-8.



Courtesy of Loren Williams. Used with permission.





HCV-Protease: Lessons

- Stick with validated targets even if hard – but be realistic about timelines
- Consider how drugs of various mechanisms can be combined
- Consider the target organ in your design
- If you're first, chances are that a better drug will come along quickly. That's OK – don't worry about looking dumb later!
- Have a vigorous 2nd generation plan

20.201 Mechanisms of Drug Actions

Fall 2013