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

-- Thomas Alva Edison
Technology Cycle

**EXPECTATION**

- **Pea of Hype**
- **Naive Euphoria**

- **Depth of Cynicism**
- **True User Benefits**
- **Asymptote of Reality**

**TIME**

- **Overreaction to Immature Technology**

---

Part 1: SBDD Primer
# Targets Which Have Yielded Clinical Candidates With the Help of Structure-Based Drug Design

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

\[ \Delta G_{\text{bind}} = \sum_{i=1}^{5} \Delta G(i) \]

Source: Schrödinger

© Schrödinger.com. All rights reserved. This content is excluded from our Creative Commons license. For more information, see [http://ocw.mit.edu/help/faq-fair-use/](http://ocw.mit.edu/help/faq-fair-use/).
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.
What fraction of the possible molecules have we made?
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
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

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

Guy Bemis
The ICE Active Site Pharmacophore

© Vertex Pharmaceuticals. All rights reserved. This content is excluded from our Creative Commons license. For more information, see http://ocw.mit.edu/help/faq-fair-use/.
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?

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

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
Fantastic SBDD effort → blistering speed
Creative insight re: chymotrypsin 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 | reacting functionality | reversibility | dose (mg) |
| acylation | serine-type D-Ala-D-Ala carboxypeptidase | bacterial infection | amoxicillin* | β-lactam | irreversible | 100–500 |
| | triacylglycerol lipase | obesity | orlistat | lipase | irreversible | 360 |
| | acetylcholinesterase | Alzheimer’s | rivastigmine | carbamate | reversible | 6–12 |
| | β-lactamase | bacterial infection | clavulanate* | β-lactam | reversible | 500 |
| | prostaglandin | pain | aspirin | ester | reversible | 1000 |
| | endoperoxidase synthase | vitamin K epoxide | warfarin | coumarin | 2–10 |
| | (warfarin-sensitive) | reductase | | | | |
| | enol–acyl carrier protein reductase | bacterial infection (tuberculosis) | isoniazid | hydrazide* | irreversible | 300 |
| | aldehyde dehydrogenase | alcoholism | disulfiram | disulfide | irreversible | 500* |
| alkylation | UDP-N-acetylglucosamine-1-carboxyvinyltransferase | bacterial infection | foscarnet | epoxide | | 3000 |
| | alanine racemase | | | | | |
| | | bacterial infection (tuberculosis) | d-cycloserine | amine* | >250 |
| metal/metalloid binding | GABA-AT| epilepsy | vigabatrin | amine* | irreversible | 3000* |
| | aromatase | breast cancer | exemestane* | methyl | reversible | 25 |
| | proteasome | multiple myeloma | bortezomib | boronic acid | reversible | 3 |
| | H/K+ ATPase | gastrinophagel reflux disease | omeprazole* | sulfenamide | irreversible | 20 |
| | P2Y12 purinoceptor antagonist | platelet aggregation inhibitor | clodipogrel | thiol | irreversible | 75 |
| | (seleno-enzyme) | thyroxine 5' deiodinase (type 1) | hyperthyroidism | propylthiouracil | thiourea | 450 |
| | hemiketal formation | acetylprotein | hepatitis C virus NS3| VX-950 (1g) | ketoamide | reversible | n/a |
| | | | | | | |
| Michael addition | ribonucleoside | cancer | gemcitabine* | vinyl ketone | ≥150–000* |
| | diposphatase | | | | |
| | reductase | | | | |
| | thymidylate synthase | cancer | floxuridine* | unsaturated amide | reversible | 0.1–0.6 (mg/kg)/d |
| | ErbB1/2* | cancer (NSCLC) | HKI-272 (11) | unsaturated amide | reversible | n/a |
| | 5-α-reductase | benign prostatic hyperplasia | finasteride* | amide* | reversible | 5 |
| | MAO-B | Parkinson’s disease | selegiline* | acetylcholine imine* | irreversible | 1 |
| | | | | | |
| Pinner reaction | DPP IV* | diabetes | vildagliptin | nitrile | reversible | 100 |
| | cathepsin K* | osteoporosis | odanacatib | nitrile | reversible | 10–50 |

* Prodrugs are indicated in italics. † As determined from the FDA label or other medical references. ‡ Because of the large number of drugs developed for these targets, one representative drug is indicated in the table. ‡ Indicates functionality covalently modified by the cofactor. † Estimated dose. § Approved in Canada, U.K., and Mexico. ¶ Under clinical investigation. © Dose – 1000 mg/m² weekly. The average body surface area of a person is approximately 1.5–2 mm². Other irreversible MAO inhibitors are on the market for the treatment of depression. Weekly dose used in the clinical trial "MK0822 (Odanacatib) Late Phase II Dose-Finding Study" described at www.clinicaltrials.gov.

© American Chemical Society. All rights reserved. This content is excluded from our Creative Commons license. For more information, see [http://ocw.mit.edu/help/faq-fair-use/](http://ocw.mit.edu/help/faq-fair-use/).

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

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)

A Struggle of Biblical Proportions?

**DIAMOX**  
(acetazolamide)

**TRUSOPT**  
(dorzolamide HCl)

40 YEARS

© source unknown. All rights reserved. This content is excluded from our Creative Commons license. For more information, see [http://ocw.mit.edu/help/faq-fair-use/](http://ocw.mit.edu/help/faq-fair-use/).

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)

MVT-101 (NCI)

© source unknown. All rights reserved. This content is excluded from our Creative Commons license. For more information, see http://ocw.mit.edu/help/faq-fair-use/.
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
Amprenavir (Agenerase)  
Launched 1999 

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

Amprenavir (Agenerase)
Launched 1999

Fosamprenavir (Lexiva)
Launched 2003
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
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.”
HCV: Telaprevir

Efficacy surrogate: high ratio of liver concentration to IC$_{50}$
- \([C_{\text{liver}}] > 10 \times IC_{50}\)
- Fa more important than %F

High \([C_{\text{liver}}]\) 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

VX-950
HCV Program Strategy

Efficacy surrogate: high ratio of liver concentration to IC$_{50}$

$[C_{\text{liver}}] > 10 \times IC_{50}$

$F_a$ 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


Truncating the Decapeptide Substrate Mimic

\[
\begin{align*}
\text{NH}_2-\text{E-D-V-V-L-C}^- \\
\text{O} \quad \text{Nle-S-Y-OH}
\end{align*}
\]

\[
\begin{align*}
\text{H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH} & \quad 0.34 \\
\text{H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-OH} & \quad 27 \\
\text{H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-OH} & \quad 17 \\
\text{H-Glu-Asp-Val-Val-Leu-Cys-Tic-OH} & \quad 14 \\
\text{H-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH} & \quad 4.4 \\
\text{H-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH} & \quad 79 \\
\text{H-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH} & \quad 500 \\
\text{H-Leu-Cys-Tic-Nle-Ser-Tyr-OH} & \quad 2000
\end{align*}
\]

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

Substrate

NS5A-5B Substrate

Inhibitor

Serine attack and cleavage

Reversible Serine attack

$K_i = 12 \mu M$
Multi-Subsite Optimization

The Finish Line

\[
\begin{align*}
K_i &= 0.15 \, \mu M \\
\text{Replicon } IC_{50} &= 0.45 \, \mu M
\end{align*}
\]

\[
\begin{align*}
K_i &= 0.04 \, \mu M
\end{align*}
\]

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

\[
\begin{align*}
K_i &= 44 \, nM \text{ (15 min pre-incubation)} \\
K_i^* &= 7 \, nM \\
\text{Replicon } IC_{50} &= 0.35 \, \mu M
\end{align*}
\]
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


© source unknown. All rights reserved. This content is excluded from our Creative Commons license. For more information, see http://ocw.mit.edu/help/faq-fair-use/.
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

For information about citing these materials or our Terms of Use, visit: http://ocw.mit.edu/terms.