Successes and Failures in Structure-Based Drug Design

by Ren Wei
overview

• Choice of drug target
• Determination of the accurate structural information
• Identification of the Target Site
• Drug Design Methods
• Protein and Ligand Flexibility
• Some successful examples
• Reference
Structure-Based Drug Design: The first success

• The first success: peptide-based HIV protease inhibitor; (Roberts, N., 1990)
The process of structure-based drug design: an overview

- The Iterative Process of Structure-Based Drug Design
Choice of drug target: modulate function of human protein

- closely linked to human disease and binds a small molecule in order to carry out a function;
- usually has a well-defined binding pocket;
- other designed small molecules can compete, at a required level of potency, with the natural small molecule in order to modulate the function of the target;
Choice of drug target: modulate function of human protein

- Drug target are usually *Protein*;
- Drug design against *RNA* targets with well-defined secondary structure, like the bacterial ribosome and portions of the HIV genome, has also been effective;
Choice of drug target: modulate function of human protein

<table>
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<th>Main drug products of the current market</th>
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<td>small molecule drugs against G protein coupled receptors (GPCRs)</td>
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<td>at least 25%</td>
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Choice of drug target: total inhibition against pathogenic organisms

Criteria:
• Antimicrobial drug targets should be essential, have a unique function in the pathogen, be present only in the pathogen, and be able to be inhibited by a small molecule.
• The target should be essential, in that it is part of a crucial cycle in the cell, and its elimination should lead to the pathogen’s death.
• The target should be unique: no other pathway should be able to supplement the function of the target and overcome the presence of the inhibitor.
• Finally, the target molecule should be able to be inhibited by binding a small molecule.
Determination of the accurate structural information: Techniques

- X-ray crystallography
- nuclear magnetic resonance spectroscopy (NMR)
- computational methods (modeling)
Determination of the accurate structural information: X-ray Crystallography

- purify protein to 99% purity
- grow small crystals from the pure protein
- measure X-ray diffraction data from the crystal
- solve the "phase problem"
- build model in the electron density map
Crystallography in Drug Design

Crystal + inhibitor soak $\rightarrow$ difference map
Determination of the accurate structural information: X-ray Crystallography

**Advantage:**
- high resolution image of all atoms in your protein
- no size limitations (up to 998kDa, and even virus particles!)
- very easy to study complexes
- ordered water molecules are visible in the experimental data and are often useful in drug lead design

**Disadvantage:**
- you NEED a crystal
- protein is not in solution but packed in a crystal
Determination of the accurate structural information: NMR

**Advantage:**
- protein in its natural environment
- information about dynamics/folding

**Disadvantage:**
- size limitation (about 30 kD)
- structure not completely defined
- complicated experiments require exotic nuclei
Determination of the accurate structural information: homology modeling

Quality of a model...

• Evaluated by a confidence factor per residue (SWISS-MODEL);
• Other factors for judgment of an experimental data like stereo chemical soundness and residue in most favored region of Ramachandran plot as well;
Identification of the Target Site

- The ligand binding site can be the active site, as in an enzyme, an assembly site with another macromolecule, or a communication site necessary in mechanism of the molecule. Additionally, RNA secondary structural can provide excellent target sites since they are species specific, bind ligands, and can be specific for a disease state.
- Target sites for protein-protein interactions can be difficult to locate since these surfaces are often flat, large, and hydrophobic.
- Co-crystallization studies can be invaluable for the determination of a good target site.
Drug Design Methods

• Experiment: high-throughput screening with combinatorial chemistry;

• Computer-aided methods:
  1. inspection
  2. virtual screening
  3. de novo generation
Drug Design Methods: Questions to decide the methods

(1) Are molecules available which can be modified to be inhibitors?
(2) Is there a means for synthesizing novel molecules?
(3) what is the degree of accuracy required at a particular stage of the design process versus the time needed for the calculation?
Drug Design Methods: molecules to be modified...

• Inhibitors for Thymidylate Synthase Were Designed Based on Modifications of the Cofactor 5,10-Methylene Tetrahydrofolate
  Several potent inhibitors are shown: (B) CB3717, (C) OSI 1843U89, and (D) ZD1694 (Tomudex).

molecules to be modified as inhibitors

• Substrate or cofactor in the case of enzyme
• Peptides in the case of protein : protein or protein : nucleic acid interaction
Drug Design Methods: means for synthesizing novel molecules

Virtual screening: docking compounds from databases like Available Chemicals Database (ACD) can be tested using biochemical assays;

De novo generation: novel compounds can be assembled in silico, but they require also to be synthesized practically in laboratory;
Drug Design in limited time

- Protein and ligand flexibility
- Solvent effects
- ...

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structure-based drug design
Protein and Ligand Flexibility: why?

- A single structure of a protein implies an all-or-nothing folding funnel, perhaps best described with the mathematic singularity shown on the right. The folding funnels on the left and in the center demonstrate the conformational flexibility of a “standard” flexible protein and a rigid protein, respectively. Although the rigid system may be described adequately by a single structure (depending on the degree of rigidity as reflected by the narrowness of the funnel), a typical system is not.
Protein and Ligand Flexibility

- Proteins and ligands are quite flexible in solution with lots of possible conformations.
- Leads generation only from a single, rigid structure may have wrong results in silico than in in solution.
- Modeling molecular flexibility increase the computing time.
- Flexibility programming: Incorporation of multiple protein structure.
Protein and Ligand Flexibility: Determine ensembles of multiple protein structure

- NMR ensembles (s. left: 6 structures of dehydrofolate reductase);
- Multiple crystal structure;
- Computationally prediction by molecular dynamics (MD, one example is the model of HIV-1 integrase, Carlson et al.);
- Using rotamers of protein side chain;
Solvent Effects

• In one capacity, ordered water molecules seen in the structure can be incorporated into the designed ligand, effectively increasing ligand binding by increasing the entropy of the system.

• In a second capacity, ordered water molecules can be treated as bound ligands, and contacts with them can be maximized.

• In a third capacity, the effect of the solvent can be incorporated into the scoring scheme for the target:ligand interaction.
Solvent Effects: incorporated to the designed Ligand

• Nonpeptide HIV Protease Inhibitors Based on Cyclic Urea Compounds Incorporate an Oxygen Atom Where a Bound Water Molecule Was Visualized in X-Ray Structures;
Solvent Effects

• The steps of increased accuracy in modeling the solvent effect during scoring are as follows:
  (1) making the assumption that the molecules are in a vacuum, i.e., no solvent modeling;
  (2) using a fixed dielectric constant in estimating electrostatic contributions;
  (3) explicit solvation models;
  (4) modeling the Born equation. The Born equation calculates the polarization contribution to solvation when a charge is placed within a spherical solvent cavity.
Solvent Effects: Practical Approaches

- a modified Born equation to calculate solvation energies,
- an approximation to the electrostatic desolvation by modeling the first solvation shell at the binding interface,
- an implicit model which accounts for desolvation by computationally generating possible positions of water molecules in the binding.
Some successful drugs of market using structure-based drug design

<table>
<thead>
<tr>
<th>Drugs</th>
<th>against / inhibits</th>
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<tr>
<td>Amprenavir (Agenerase)</td>
<td>HIV protease</td>
</tr>
<tr>
<td>Nelfinavir (Viracept)</td>
<td>HIV protease</td>
</tr>
<tr>
<td>Zanamivir (Relenza)</td>
<td>neuraminidase</td>
</tr>
<tr>
<td>Tomudex</td>
<td>thymidylate synthase</td>
</tr>
<tr>
<td>Imitinab mesylate (Gilvec)</td>
<td>Abi tyrosine kinase</td>
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A successful example: Drug Design against AmpC $\beta$-Lactamase

(A) Ball-and-stick representation of compound 1 (red), discovered with a DOCK screen, bound to AmpC $\beta$-lactamase.

(B) Compound 1 (space filling) bound to AmpC $\beta$-lactamase (residues within 7 Å are shown with van der Waals surfaces).
Reference


Thank you for your attention!