A New Computer Method for Drug Target Search and Application to Probing the Molecular Mechanism of Chinese Natural Products

Y. Z. Chen and C. Y. Ung

Department of Computational Science
National University of Singapore

E-mail: yzchen@cz3.nus.edu.sg
**Outline**

- **Drug targets and new drug discovery**
  - Therapeutic targets, side effect, toxicity, pharmacogenetics.
  - Mechanism of bioactive natural products.

- **Method**
  - Ligand-protein inverse docking.

- **Application: Molecular Mechanism of Chinese natural products**
  - Protein targets of selected CNPs.
Drugs targets and new drug discovery

Therapeutic Targets

A new target for aspirin

Dr. A. O'Neill

Two aspirin and some are in the "aspirin family" of drugs. Aspirin is a non-steroidal anti-inflammatory drug (NSAID) that is used to relieve pain, reduce fever, and reduce inflammation. Its mechanism of action is thought to involve the inhibition of cyclooxygenase (COX), an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins, which are mediators of inflammation.

Inhibitors of COX-2 are known to be more effective in treating chronic inflammatory conditions than aspirin, which inhibits both COX-1 and COX-2. However, inhibitores of COX-2 have been associated with an increased risk of gastrointestinal bleeding and ulceration.

Recent studies have suggested that a new target for aspirin may be the inhibition of protein kinase C (PKC). PKC is a family of serine/threonine kinases that are involved in a variety of cellular processes, including signal transduction, cell proliferation, and apoptosis. Inhibition of PKC has been shown to have anti-inflammatory effects.

Although the exact mechanism by which aspirin inhibits PKC is not fully understood, it is believed that aspirin may inhibit PKC by interfering with the activation of its upstream regulators, such as diacylglycerol kinase (DGK) and phosphatidylinositol-3-kinase (PI3K).

The discovery of a new target for aspirin has the potential to improve the therapeutic profile of this widely used drug, offering a new avenue for the development of more selective and effective non-steroidal anti-inflammatory drugs.
Drug targets and new drug discovery

Side effect, toxicity, pharmacogenetics

Mitochondrial Targets of Drug Toxicity

K. B. Wallace and A. A. Starkov
Department of Biochemistry and Molecular Biology, University of Minnesota School of Medicine, Duluth, Minnesota 55812; e-mail: kwallace@d.umn.edu
astarkov@d.umn.edu

Key Words oxidative phosphorylation, uncouplers, bioenergetics, permeability transition, redox cycling

Abstract Mitochondria have long been recognized as the generators of energy for the cell. Like any other power source, however, mitochondria are highly vulnerable to inhibition or uncoupling of the energy harnessing process and run a high risk for catastrophic damage to the cell. The exquisite structural and functional characteristics of mitochondria provide a number of primary targets for xenobiotic-induced bioenergetic failure. They also provide opportunities for selective delivery of drugs to the mitochondrion. In light of the large number of natural, commercial, pharmaceutical, and environmental chemicals that manifest their toxicity by interfering with mitochondrial bioenergetics, it is important to understand the underlying mechanisms. The significance is further underscored by the recent identification of bioenergetic control points for cell replication and differentiation and the realization that mitochondria play a determinant role in cell signaling and apoptotic modes of cell death.

Molecular Mechanisms of Genetic Polymorphisms of Drug Metabolism

Urs A. Meyer
Biozentrum of the University of Basel, CH-4056 Basel, Switzerland

Ulrich M. Zanger
Dr. Margarete Fischer-Bosch-Institute for Clinical Pharmacology, D-70376 Stuttgart, Germany

KEY WORDS: Genetic polymorphism, CYP2D6, CYP2C19, N-acetyltransferases, drug metabolism, pharmacogenetics

Abstract One of the major causes of interindividual variation of drug effects is genetic variation of drug metabolism. Genetic polymorphisms of drug-metabolizing enzymes give rise to distinct subgroups in the population that differ in their ability to perform certain drug biotransformation reactions. Polymorphisms are generated by mutations in the genes for these enzymes, which cause decreased, increased, or absent enzyme expression or activity by multiple molecular mechanisms. Moreover, the variant alleles exist in the population at relatively high frequency. Genetic polymorphisms have been described for most drug-metabolizing enzymes. The molecular mechanisms of three polymorphisms are reviewed here.
Detection of side effect and toxicity in early stages of drug discovery

- Most drug candidates fail to reach market
- Side effect and toxicity is an important reason.
- Large part of money ($350 million per drug) and time (6-12 years for a drug) has been wasted on failed drugs.

Drug Discov Today 1997; 2:72

Drug targets and new drug discovery

Drugs from Natural Products

From natural products to therapeutic drugs

TIPS, May 1999, 20:190

- Screening of bioactive compounds
- Molecular mechanism
- Further development
Drug targets and new drug discovery

Drugs from Traditional Medicine

- Great potential, widely used.
- Need standardization, quantitative analysis and validation.

- Molecular mechanism
- Safety profile
- Further development

Pharmacology & Therapeutics 2000, 86:191-198

Associate editor: P.K. Chiang

Traditional Chinese medicine: an approach to scientific proof and clinical validation

Robert Yuan*, Yuan Linb

*Department of Cell Biology and Molecular Genetics, University of Maryland, 1109 Microbiology Building, College Park, MD 20742, USA
bMaroo Polo Technologies, Bethesda, MD 20817, USA

Abstract

The classical Chinese pharmacopoeia describes a large number of herbal formulations that are used for the treatment of a wide variety of diseases. This therapeutic approach is ignored by many and considered to be an alternative to conventional medicine by others. The scientific proof and clinical validation of these herbal formulations require a rigorous approach that includes chemical standardization, biological assays, animal models, and clinical trials. Such Western methodologies need to take into consideration the complex mixture of chemicals and how they are to be used in humans. This review examines relevant studies on the use of traditional Chinese medicines for the treatment of such diseases as bronchial asthma, atopic dermatitis, and irritable bowel syndrome. An interdisciplinary approach to traditional Chinese medicine may provide a platform for the discovery of novel therapeutics composed of multiple chemical compounds. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Chinese herbal formulations; Chemical standardization; Biological assays; Bronchial asthma; Atopic dermatitis; Irritable bowel syndrome

Abbreviations: AA, adjuvant arthritis; AIA, antigen-induced arthritis; DGNIT, Daengui-Nann-Tong-Tang; IBS, irritable bowel syndrome; LT, leukotrienes; TCM, traditional Chinese medicine.
Drug targets and new drug discovery

Drugs from Traditional Chinese Medicine

- Mixture of multiple herbs etc.
- Maintaining and restoring balance.
- Mutual accentuation, mutual enhancement, mutual counteraction, mutual suppression, mutual antagonism, mutual incompatibility

- Multiple targets: therapeutic effect, symptom treatment, toxicity modulation, drug delivery, harmonization

![Diagram showing the relationship between TCM recipes, herbs, active compounds, receptors, and various effects such as main therapeutic effects, toxicity, side effects, and 2nd therapeutic effects.](image)
Drug targets and new drug discovery

Understanding of Molecular Mechanism of Traditional Medicine

The need for a low cost and fast-seed method:
- Cost and Difficulty in testing large number of protein targets.
- Limited resources of herbal extracts, purified bioactive compounds etc.

Computer approach as a helping tool?
- Lower cost and more powerful computer.
- Advances in structural and functional genomics.
- Software development.
Existing Methods:
Given a Protein, Find Putative Binding Ligands From a Chemical Database

New Method:
Given a Ligand, Find Putative Protein Targets From a Protein Database

Compound Database
Compound 1
...
Compound n

Protein
Successfully Docked Compounds as Putative Ligands

Protein Database
Protein 1
...
Protein n

Ligand
Successfully Docked Proteins as Putative Targets

Science 1992;257: 1078
Feasibility

Proteins
- Protein numbers: >12,000 3D structures in PDB.
- Protein diversity: 17% in PDB with unique sequence.
- Development of structural genomics: 10,000 unique proteins within 5 years.


Method
- Ligand-protein docking docking algorithms capable of finding binding conformations.

*Proteins.* 1999; 36:1

Cost and efficiency
- Increasing performance (docking of 100,000 compounds in days).
- Decreasing cost (Linux PC, Multi-processor machine)
How to Model Ligand-Protein Binding?
Learn from the Mechanism of Ligand-Protein Binding

Before Binding
Cavity A accessible

After Binding
Cavity A blocked
Ligand Binding Site

Drug binding site in a cavity of protein
Drug binds to a protein by lock and key mechanism
Modeling Procedure

Ligand–protein docking:
Step 1: Creation of spheres to fit a cavity
Modeling Procedure

Ligand–protein docking:
Step 2: Place a ligand to match the positions of spheres
Modeling Procedure

Ligand-protein docking:
Step 3: Check chemical complementarity.

Conformation change
Binding affinity
How to Check Chemical Complementarity?

Potential Energy Description:
Optimization and Scoring Functions in Ligand-Protein Docking

Potential Energy Description:

Conformation change

Binding strength
Energy Functions

- Chemical bonds
- Hydrogen bonding
- van der Waals interactions
- Electrostatic interactions
- Empirical solvation free energy

\[ V = V_{\text{bonds}} + \]

\[ \Sigma_{H \text{ bonds}} \left[ V_0 \left(1-e^{-a(r-r_0)}\right)^2 - V_0 \right] + \]

\[ \Sigma_{\text{non bonded}} \left[ A_{ij}/r_{ij}^{12} - B_{ij}/r_{ij}^6 + q_i q_j / \varepsilon r_{ij} \right] + \]

\[ \Sigma_{\text{atoms}} \Delta \sigma_i A_i \]
Strategy for Ligand-Protein Inverse Docking

**Ligand**

Automated Process to inversely dock a ligand to each entry in a Built-In Biomolecular Cavity Database

Successfully Docked Proteins and Nucleic Acids

Putative Targets of Ligand
- Therapeutic Targets
- Side-Effect and toxicity Targets
- Metabolism, Signalling etc

*Penicillin G binding to its target, Beta-Lactamase*

Generated by INVDOCK
Automated Protein Targets Identification Software

INVDOCK

Step 1: Vector-based docking of a ligand to a cavity
Step 2: Limited conformation optimization on the ligand and side chain of biomolecule
Step 3: Energy minimization for all atom in the binding site
Step 4: Docking evaluation by molecular mechanics energy functions and comparison with other ligands

Automated Process to inversely dock the Ligand to each entry in a Built-In Biomolecular Cavity Database (10,000 Protein and Nucleic Acid Entries)

Successfully Docked Proteins and Nucleic Acids as Putative Targets of a Ligand

Potential Applications:
Protein function, Proteomics, Ligand transport, Metabolism
Therapeutic Targets, Side-Effects, Metabolism, Toxicity
Function in Pathways
INVDOCK Cavity Models

Estrogen Receptor

Estrogen binding sites
**INVDOCK Testing Results:**

Putative Protein Targets

For an Anticancer Drug Tamoxifen

<table>
<thead>
<tr>
<th>PDB Id</th>
<th>Protein</th>
<th>Experimental Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a25</td>
<td>Protein Kinase C</td>
<td>Secondary Target</td>
</tr>
<tr>
<td>1a52</td>
<td>Estrogen Receptor</td>
<td>Drug Target</td>
</tr>
<tr>
<td>1bhs</td>
<td>17beta Hydroxysteroid dehydrogenase</td>
<td>Inhibitor</td>
</tr>
<tr>
<td>1bld</td>
<td>Basic Fibroblast Growth Factor</td>
<td>Inhibitor</td>
</tr>
<tr>
<td>1cpt</td>
<td>Cytochrome P450-TERP</td>
<td>Metabolism</td>
</tr>
<tr>
<td>1dmo</td>
<td>Calmodulin</td>
<td>Secondary Target</td>
</tr>
</tbody>
</table>

Tamoxifen is a famous anticancer drug for treatment of breast cancer. It was approved by FDA in 1998 as the 1st cancer preventive drug. 30 million people are expected to use it.
## INVDOCK Testing Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Putative Targets Identified</th>
<th>Experimentally Confirmed</th>
<th>Experimentally Implicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4H-Tamoxifen</td>
<td>17</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Aspirin</td>
<td>52</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>46</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>26</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Bioactive Chinese Natural Product</td>
<td>Plants</td>
<td>Therapeutic Effects</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Acronycine</td>
<td>Acronychia pedunculata</td>
<td>Antitumor colon 38 adenocarcinoma, L1210 leukemia cells</td>
<td></td>
</tr>
<tr>
<td>Allicin</td>
<td>Allium sativum, and Allium fistulosum.</td>
<td>Antitumor, antiinflammatory, antimicrobial, antioxidant, reduction of cholesterol and blood pressure</td>
<td></td>
</tr>
<tr>
<td>Baicalin</td>
<td>Scutellaria baicalensis and Oroxylum indicum</td>
<td>Antivirial, antitumor, antiinflammatory, diabetes.</td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>Green tea</td>
<td>Antitumor, antiinflammatory</td>
<td></td>
</tr>
<tr>
<td>Emodin</td>
<td>Rheum palmatum, Rumex dentatus, and Cassia tora</td>
<td>Antitumor, treatment of transplantation rejection and autoimmune diseases</td>
<td></td>
</tr>
</tbody>
</table>
# Application: Molecular mechanism of Chinese natural products

<table>
<thead>
<tr>
<th>Bioactive Chinese Natural Product</th>
<th>Plants</th>
<th>Therapeutic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camptothecine</td>
<td>Canptotheca acuminata</td>
<td>Antitumor</td>
</tr>
<tr>
<td>Dicoumarin</td>
<td>Trfolium pratense, Medicago sativa, and Melilotus albus</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Genistein</td>
<td>Soy bean</td>
<td>Antitumor, Erythroleukemia cells, ventral prostate carcinomas</td>
</tr>
</tbody>
</table>

*Note: Additional entries may be required for comprehensive coverage.*
Structures

Acronycin

Baicalin

Allicin

Camptothecine
Structures

- Catechin
- Emodin
- Dicoumarin
- Genistin
<table>
<thead>
<tr>
<th>PDB</th>
<th>Protein</th>
<th>Experimental Finding</th>
<th>Target Status</th>
<th>Therapeutic Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ads</td>
<td>Aldose Reductase</td>
<td></td>
<td></td>
<td>Diabetes treatment</td>
<td></td>
</tr>
<tr>
<td>2gss</td>
<td>Glutathione S-Transferase p1-1</td>
<td>Increases intracellular glutathione</td>
<td>Implicated</td>
<td>Enhance radical scavenging activities that may useful in cancer treatment</td>
<td>Matsumoto</td>
</tr>
<tr>
<td>7ice</td>
<td>DNA Polymerase Beta</td>
<td></td>
<td></td>
<td>Anti-cancer</td>
<td></td>
</tr>
<tr>
<td>1a25</td>
<td>Protein Kinase C</td>
<td>Inhibitor</td>
<td>Confirmed</td>
<td>Induction of apoptosis in tumor.</td>
<td>Martelli Nieves-Neira</td>
</tr>
<tr>
<td>1cdk</td>
<td>CAMP-Dependent Protein Kinase</td>
<td></td>
<td></td>
<td>Anti-cancer</td>
<td></td>
</tr>
<tr>
<td>3bct</td>
<td>Beta-Catenin</td>
<td></td>
<td></td>
<td>Anti-cancer</td>
<td></td>
</tr>
<tr>
<td>1dvi</td>
<td>Calpain</td>
<td>Inhibition of calpain activities.</td>
<td>Implicated</td>
<td>Induces apoptosis in leukemic cells.</td>
<td>Eymin</td>
</tr>
<tr>
<td>1yfo</td>
<td>Receptor Protein Tyrosine Phosphatase</td>
<td>Causes elevation of PTPase in the cytosol and the nucleus which play a critical role in the induction of the differentiation of IW32 erythroleukemia cells.</td>
<td>Implicated</td>
<td>Anti-cancer</td>
<td>Wang MC</td>
</tr>
<tr>
<td>1a35</td>
<td>Topoisomerase I</td>
<td>Inhibitor</td>
<td>Confirmed</td>
<td>Anti-cancer</td>
<td>Wang MC</td>
</tr>
</tbody>
</table>
Putative and known toxicity/side effect-causing targets of Camptothecine identified from INVDOCK search of human and mammalian proteins

<table>
<thead>
<tr>
<th>PDB</th>
<th>Protein</th>
<th>Experimental Finding</th>
<th>Target Status</th>
<th>Toxicity/Side Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>2clj</td>
<td>Acetylcholinesterase</td>
<td>Reversible inhibition</td>
<td>Confirmed</td>
<td>Causes Cholinergic Toxicity</td>
<td>Dodds</td>
</tr>
<tr>
<td></td>
<td>Retinol-Binding Protein</td>
<td>Interfering with retinol transport that may cause complication in cancer and cardiovascular disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5rla</td>
<td>Arginase</td>
<td>Increase blood urea level</td>
<td>Implicated</td>
<td>Hyperammonemia that may cause nerve toxicity</td>
<td>Yu ZJ</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein</td>
<td>Experimental Finding</td>
<td>Target Status</td>
<td>Therapeutic Effect</td>
<td>Ref</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------</td>
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<td>---------------</td>
<td>-------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>2acq</td>
<td>Aldose Reductase</td>
<td></td>
<td></td>
<td>Diabetes treatment</td>
<td></td>
</tr>
<tr>
<td>1cdk</td>
<td>CAMP-Dependent · Protein Kinase</td>
<td>Inhibitor</td>
<td>Confirmed</td>
<td>Anti-cancer</td>
<td>Jinsart W</td>
</tr>
<tr>
<td>1pth</td>
<td>Prostaglandin H2 Synthase-1</td>
<td></td>
<td></td>
<td>Anti-inflammatory</td>
<td></td>
</tr>
<tr>
<td>3bct</td>
<td>Beta-Catenin</td>
<td></td>
<td></td>
<td>Anti-cancer</td>
<td></td>
</tr>
<tr>
<td>1nfk</td>
<td>Nuclear Factor Kappa-B</td>
<td>Inhibits TNF-induced NF-kappaB activation</td>
<td>Confirmed</td>
<td>Anti-inflammatory effect</td>
<td>Kumar A</td>
</tr>
<tr>
<td>1a25</td>
<td>Protein kinase C</td>
<td>Inhibitor</td>
<td>Confirmed</td>
<td>Anti-cancer</td>
<td>Jinsart W</td>
</tr>
</tbody>
</table>
Putative and known toxicity/side effect-causing targets of Emodin identified from INVDOCK search of human and mammalian proteins

<table>
<thead>
<tr>
<th>PDB</th>
<th>Protein</th>
<th>Experimental Finding</th>
<th>Target Status</th>
<th>Toxicity/Side Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cah</td>
<td>Carbonic Anhydrase II</td>
<td></td>
<td></td>
<td>Acidity disorder in red blood cells</td>
<td></td>
</tr>
<tr>
<td>3fbp</td>
<td>Fructose-1,6-Bisphosphatase</td>
<td></td>
<td></td>
<td>Red blood cell disorder that may cause anemia</td>
<td></td>
</tr>
<tr>
<td>4cts</td>
<td>Citrate Synthase</td>
<td></td>
<td></td>
<td>Mitochondrial toxicity that may cause nerve disorder</td>
<td></td>
</tr>
<tr>
<td>4ggt</td>
<td>Glutathione Reductase</td>
<td></td>
<td></td>
<td>Genotoxicity</td>
<td></td>
</tr>
</tbody>
</table>
## Putative and known therapeutic targets of Allicin identified from INVDOCK search of human and mammalian proteins

<table>
<thead>
<tr>
<th>PDB</th>
<th>Protein</th>
<th>Experimental Finding</th>
<th>Target Status</th>
<th>Therapeutic Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1znj</td>
<td>Insulin</td>
<td>Insulin level enhancement</td>
<td>Implicated</td>
<td>Diabetes treatment</td>
<td>Mathew PT</td>
</tr>
<tr>
<td>1ah3</td>
<td>Aldose Reductase</td>
<td></td>
<td></td>
<td>Diabetes treatment</td>
<td></td>
</tr>
<tr>
<td>1cdk</td>
<td>CAMP-Dependent Protein Kinase</td>
<td></td>
<td></td>
<td>Anti-cancer</td>
<td></td>
</tr>
<tr>
<td>1pth</td>
<td>Prostaglandin H2 Synthase-1</td>
<td>Inhibitor</td>
<td>Confirmed</td>
<td>Anti-inflammatory</td>
<td>Shalinski</td>
</tr>
<tr>
<td>1rpa</td>
<td>Prostatic Acid Phosphatase</td>
<td></td>
<td></td>
<td>Anti-cancer (prostate cancer)</td>
<td></td>
</tr>
</tbody>
</table>
Putative and known potential toxicity/side effect-causing targets of Allicin identified from INVDOCK search of human and mammalian proteins

<table>
<thead>
<tr>
<th>PDB</th>
<th>Protein</th>
<th>Experimental Finding</th>
<th>Target Status</th>
<th>Toxicity/Side Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1fbt</td>
<td>Fructose-2,6-Bisphosphatase</td>
<td></td>
<td></td>
<td>Red blood cell disorder that may cause anemia</td>
<td></td>
</tr>
</tbody>
</table>
## Molecular mechanism of Chinese natural products

<table>
<thead>
<tr>
<th>Chinese Natural Product</th>
<th>Number of Identified Putative and Known Therapeutic Targets</th>
<th>Number Confirmed or Implicated Therapeutic Targets by experiment</th>
<th>Number of Identified Putative and Known Toxicity/Side effect Targets</th>
<th>Number Confirmed or Implicated Toxicity/Side Effect Targets by experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acronycine</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Allicin</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Baicalin</td>
<td>14</td>
<td>4</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Catechin</td>
<td>17</td>
<td>12</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Camptothecine</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Dicoumarin</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Emodin</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Genistin</td>
<td>22</td>
<td>7</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>
Conclusions

- Computer method useful in facilitating identification of putative therapeutic, toxicity and side effect targets.
- Short list of putative protein targets can be generated and possible mechanism can be probed.
- Application potential increases with advances in structural and functional genomics.