3D-SAR Analysis of EDs Based on Target Receptor Structure

Nobuo Tomioka, Akiko Itai

Institute of Medicial Molecular Design, Inc.

At the beginning of my talk, I would like to thank organizing committee and Dr. Kanno for giving me a chance to talk in this Symposium.

Our company, IMMD, was established in 1995 for research and application of computer-assisted drug design. Today, I'm going to talk about our computational technology first, and then about application of the technology to endocrine disrupters study.

This slide shows a typical flow of drug design and development. A protein having a role in a certain biological activity is selected as a target, and compounds that can modulate the protein's activity are selected from compound library by high-throughput screening (HTS). These compounds are called "leads". Lead compounds are further optimized for their activity, and a few good compounds are selected as drug candidates and subjected to the clinical trials.

In typical cases, these steps require many bench experiments and long time. Our mission is to provide technology that can accelerate these steps by using computers. Since computers are made of silicons, such technology is called "*in silico*" technology.

First, I will talk about main component of our *in-silico* technology, the "docking simulation".

A drug molecule gives rise to its activity by binding to a specific protein in the body. It forms a stable complex with the target protein as shown here. In this picture, a backbone of the protein structure is shown as a wire. A drug molecule binds to a well-formed pocket in the protein. This structure of protein-ligand complex was elucidated by protein crystallography.

The purpose of the docking simulation is to predict such structure of the complex without the crystallographic experiments.

Docking simulation is not a trivial problem for solving. We must consider enormous degrees of freedom including ligand rotation and translation relative to the protein, and ligand conformations due to the bond rotations. A conformation of the ligand when it binds to the protein is called "active conformation". The active conformation of a ligand is not the same as the stable conformation of the isolated ligand.

For solving the docking problem, we have developed an automatic docking method, called "ADAM".

ADAM performs systematic and efficient search of relative orientation and conformation of the ligand, based on matching of hydrogen-bond patterns, with stepwise optimizations of the docking model. Docking models are evaluated by energy scores. The advantage of the ADAM method is its high accuracy and speed.

In an ideal docking simulation, the structure of top-ranked docking model should coincide with the structure found in crystal.

This slide shows results of ADAM docking for two proteins. Structure of ligand observed in crystal is shown in green, and predicted structure of the docking model is shown in yellow. In spite of flexible conformation of ligands shown here, ADAM can predict the conformation and position of the ligands correctly. Root-mean-square deviations of the ligand atoms from the experimental positions are quite small.

Accuracy of the ADAM docking has been tested in many protein-ligand systems. In most of these cases, we could predict very accurately the positions and conformations of the bound ligands.

With an accurate and fast automatic docking method, we can use it for the purpose of *in silico* screening.

This slide shows a typical flow of our *in silico* screening using the ADAM method.

A three-dimensional database of commercially-available or virtual compounds are prepared, and each compound is docked to the target protein by using the ADAM method. Compounds that could be docked successfully, are scored based on the structures of the most stable complexes. Based on the scores and other criteria, a small number of compounds are selected as hits.

In typical lead-discovery projects, we select about 100 compounds at this stage, and purchase or synthesize the compounds and apply them to experimental assays to test their activity. In the best case, about 40 % of the selected compounds are active and submitted to the optimization steps.

This slide shows results of our *in silico* screening in actual drug design projects. “Success ratio” of active compounds versus selected hits, varies from several percents to 40 percents, depending on the difficulties and assumptions in the target proteins.

This slide summarizes advantage of *in silico* screening compared to high-throughput screening.

First, we can handle out-of-stock compounds or virtual compounds. Compound samples are necessary only after the screening. Since a small number of compounds are selected, we can use elaborate assays that are unsuitable for HTS. Since structures of protein-ligand complexes are obtained by docking, we can get hints for structure modification in order to improve activity of the hit compounds. Due to the rapid increase of solved crystal structures of various proteins, number of applicable targets of *in silico* screening is increasing rapidly as well.

In the previous slides, I have talked about our *in silico* technology very briefly.

In the rest part, I will talk about the application of our technlogy to the estrogen receptor for studying structure-activity relationships of endocrine disrupters.

Estrogen receptor is a typical target protein for many EDs.

Estrogen receptor consists of N-terminal modulating domain and DNA-binding domain, and a C-terminal ligand-binding domain. Crystal structures of the ligand-binding domain have been solved with different compounds as ligands.

This picture shows a crystal structure of the ligand-binding domain complexed with DES. A pocket here, which is buried within the protein, is the binding site of DES. EDs that modulate estrogen receptor are considered to bind to this pocket.

This slide shows a computational procedure for analyzing structure-activity relationships of known ED-compounds.

Chemical structures of ED-compounds are converted to a three-dimentional structure and are docked to the binding site in estrogen receptor with program ADAM. The structures of protein-ligand complexes are further optimized by an energy minimization program, and scores for binding free energy are evaluated by program GenB. Finally, these scores are compared with the experimental relative binding affinity.

Binding affinity of ligands are governed by the relative binding free energy - “delta-G-bind”.

In the program GenB, “delta-G-bind” of a protein-ligand complex is estimated by considering conformational term, hydration term, and interaction term, by using these equations.

The program GenB has been parametrized by using protein-ligand complexes of several protein families, in order to reproduce experimental binding affinity. For these ligand sets, experimental binding affinity can be predicted with an root-mean-square error of about one order of magnitude.

This slide summarizes the crystal structures used in the present study.

Estrogen receptor changes its conformation in some of its parts, depending on the types of bound ligands. In order to account for these conformational changes, we used multiple crystal structures

complexed with different ligands including agonist and antagonist. We also considered conformational change of some amino acid residue near the binding site.

For each protein structure, we docked 110 known ED-compounds by the program ADAM.

We then scored each compound by the program GenB, and selected best scores for each compound by using the regression analyses. In this trial, 82 compounds could be scored among the 110.

This slide shows a comparison between the scores from the docking simulation and observed relative binding affinity for the 82 compounds. Since the docking score represents the relative binding free energy, more negative score corresponds to the stronger binding affinity. logRBA of 2 corresponds to the binding affinity of beta-estradiol.

The correlation between the predicted and observed binding affinity was fairly good, in spite of the diversity of the compounds included in this analysis. We still have some over-estimating and under-estimating cases, and we are now revising the procedures and parameters of the simulation, in order to reduce such errors.

As another example, we performed a preliminary test of *in silico* screening.

In this test, we put 50 known ED-compounds in a larger pool of arbitrary compounds taken from Available Chemicals Directory and each compound was docked to the estrogen receptor. Hit compounds were scored as in the previous analysis, and ranked with the scores.

Since most of the compounds in Available Chemicals Directory are non-EDs, known ED-compounds should be ranked at the top of the hits, in an ideal situation.

This slide shows the ranking of the known EDs among 24,000 arbitrary compounds. Lower end of the plot corresponds to the highest rank.

Among the 50 EDs tested, 42 were ranked within top 1000, and many of them within top 100. This result implies the applicability of our *in silico* technology for seaching novel ED candidates from a compound database. In this trial, several compounds failed to be ranked highly or could not be docked to the target protein structure. We will still need to revise our computational methods, in order to reduce these “false negative” cases.

Finally, I would like to thank these people of IMMD for the present work, and for the people at NIHS and CERI for providing us experimental data and suggestions.

Thank you.

Q&A

Kavlock: Thank you. Questions from the audience?

Q: I have two questions. First of all, does ADAM allow you to introduce flexibility into the backbone of the protein?

Tomioka: No. That is why I used several crystal structures for ER.

Q: And the second question. Of the compounds that you tested, does ADAM allow you to accommodate compounds that have halogens like chlorine or bromine? Can you model those into the pocket?

Tomioka: I beg your pardon.

Q: Compounds that have chlorines and bromines, were they in your test?

Tomioka: That is OK. We can use chlorine and bromine compounds. But our program still has some limitations. The compound must have at least one hydrogen bonding to the protein. So we are now revising the program to overcome that limitation.

Q: I'd like to ask my question in Japanese if you don't mind. I think you might have mentioned this, but regarding estrogen receptors, can the strength of estrogenic activity itself be known from the structure? Although I think that DS, E2 and bisphenol A are probably involved, but can the strength be roughly estimated from the structure?

Tomioka: Yes, it can. Here are the results. The vertical axis represents experimental relative binding affinity and the horizontal axis represents estimated relative binding affinity.

Kavlock: Any other questions?

Q: Can I ask? How fast can you handle 24,000 chemicals? How long did it take to virtually screen that?

Tomioka: The computational time required for one compound is about 10 to 20 seconds for docking, and about one minute for structural optimization, and another one minute for scoring. It is not so fast, it is about two or three minutes. In total, we used parallel Linux machines, so we can perform the whole analysis within several days, two or three days.

Q: Did you find a binding molecule, which is unusual, I mean in the classic common sense, something weird ligand, some new molecule, which was not listed as estrogen binding?

Tomioka: As regards estrogen receptor, we could find one or two non-steroidal compounds by this method. Our main purpose is to find drugs for other proteins; for other protein cases, we have still other successes.

Q: I have one question. This may be an unfair question to you, but if the U.S. EPA were to use your model right now, do I detect that it would identify about 2 or 3% of the chemicals as needing further analysis?

Tomioka: You mean…

Q: You had the 50 positives that were in there, and they were in the top 500, so that was 500 out of 24,000. So that is 2 or 3%. So is that about what would get flagged for activity?

Tomioka: You mean the compounds in these regions active? I think there are many false positives in these compounds. So if you test these 500 or 1000 compounds, probably the active vs. inactive ratio will be about 30 or 40%, and other false positive hits.

Q: I just have one question. Probably I did not understand fully, but you have a rating and with high efficiency you got a result, but out of them, 500 different…

Q: The items with a high rate are highly predictable, and extremely scaled out items also have estrogen binding ability. I don't really understand the significance of this. Would you…

Tomioka: You mean this? Scale out?

Q: Yes. It doesn't appear here. Efficiency was extremely high, but was it the program's fault that some of the items were scaled out, or was it something else?

Tomioka: No, I think it is still the problem of the evaluation method. So if you want to know which it is, I would say it close to false negative. Actually I would like it to be conducted on a higher level, but the process of the protein structure is extremely important to this method, and as Dr. Kanno said a little while ago, helix 10 to helix 12 are extremely flexible for estrogen receptors, and structure changes due to it. Thus due to the process, I think in some cases evaluation is still poor depending upon the compound. I think it might occur in such cases.

Q: Another thing concerning this, it's okay for false negative to become extremely low: we actually want it to drop without limit. If not, screening has no significance. This would mean establishing such amount in first step screening. What do you think about this?

Tomioka: Frankly speaking, I think zero would still be out of the question. As I said a little while ago, technical advances that would for example make docking possible even in case where there is no hydrogen bond may enable us to approach zero without limit.

Q: Thank you very much.

Kanno: Sorry, but could you make it short?

Q: To make it quick, I'll speak in Japanese. With this docking model, holding of the receptors differs for antagonists and agonists. In other words, is this model designed to be an active form or when C-terminals are not included, in the case of an antagonist, C-terminal is not included. I think therefore the evaluation might differ for agonists and antagonists. What do you think about this with this model?

Tomioka: I think both of these are using protein. We actually attempted docking with both proteins. When we did this, we got compounds that are contained only in protein of the antagonist form, so we are handling it so the results merge later on.

At the present only relative binding affinity can be predicted. We can't yet predict whether it will be an antagonist or an agonist.

Kanno: Thank you very much.