Enhancing Molecular Docking Efficiency for Computer-Aided Drug Design via Systems Theorem

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ABSTRACT

This paper has developed a new computational approach to simulating the evolution of molecular docking and protein folding in drug discovery via Lyapunov equation. The approach is based on the use of minimum energy to prove the stable in protein – ligand interaction. Computational docking of drug candidates to bio-molecular targets has become a standard method in drug lead identification and optimization. One important challenge emerges that proteins are not static structures. There are motions at all scales that require a flexible representation for the docking target, as well as the ligand. Authors have carried out studies on expected movements of side chains in protein structures, and have developed Lyapunov computational approaches to provide focused stability of protein folding to drug docking calculations. Protein structure is that given the uncountable number of possible conformations for a protein, how authors can determine the lowest energy structure. In this paper, the authors have demonstration some examples in protein folding kinetics and drug docking computations, and this work succeeds in citing Lyapunov equation and molecular dynamics to support this theme. Finally, molecular docking and protein folding kinetics'will be discussed.

Keywords: System theorem, Molecular docking, Lyapunov, Minimum energy, Protein folding Kinetics.

I. INTRODUCTION

The process of bringing a new drug to the market is very complex. According to a 1997 U.S. government report, it takes 12 years and 500 million dollars for the average new drug to go from the research laboratory to patient use. More than half of this time is required for clinical and pre¬clinical in vivo testing. Today the time-to-market for a new drug is considerably shorter than in the past thanks to the application of computer modelling techniques to the design process. High performance computer techniques allow an effective pre-screening of the compounds before final synthesis and testing, the most costly phases, both in time and money. To reap the benefits of computer modelling, substantial resources for computing power and technical expertise are required. While large companies can afford it, this is not the case for small and medium sized drug developing enterprises with a limited budget.[1]

To carry out the simulation of biologically active molecules, we can use the molecular kinetics to simulate drug docking in receptor (protein or antibody). In molecular docking we attempt to predict the structures of the intermolecular complex formed between two or more molecules. The docking problem involves many degrees of freedom. There are six degrees of translational and rotational freedom of one molecule relative to the other, as well as the conformational degrees of freedom of each molecule. Various algorithms have been developed to tackle the docking problem. These algorithms can be characterized according to the number of degrees of freedom that they ignore, as well as to the algorithm adopted in order to obtain the structure of the intermolecular complex. The most common are Simulated Annealing, Molecular Dynamics, Monte Carlo, geometrical approaches or a combination of such techniques.

All of the methods and approaches mentioned above face the same problem: the existence of what might be called "false-positive" results among the set of solutions, i.e., the native-like structures is higher energy than some nonnative structures. Shoichet and Kuntz have tested the reliability for differentiating the native conformation from nonnative ones with different energy evaluation methods such as buried surface area, free energy of solvation, mechanical constraints, packing, electrostatic complementarity, and energy minimization using standard mechanical force fields [2]. They found that only the energy-minimization technique was able to discriminate, in most cases, the native from nonnative conformations. Electrostatic complementarity using a DELPHI type of calculation showed significant improvement. Guida et al. used the energy-minimization technique coupled with a MC method to calculate the bound structure of four inhibitors of thermolysin. They found that, in each case, the crystallographically observed conformations were among the low-energy conformers discovered, with three of them having the lowest energy. Caflisch et al. also applied the local energy-minimization technique, but within a different scheme, to the HIV type 1 aspartic acid protein's complex. Their results also did not lead to any false-positive results; their lowest-energy conformation was the closest to the

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x-ray structure.

Lyapunov developed a general theory of dynamic stability applicable to both linear and nonlinear systems. The authors attempt to use Lyapunov in drug docking for ligand –proteins interaction stability. When a global minimum energy is happened, the ligand is the key for new drug compound; the authors seek to the global minimum energy states via Lyapunov Equation.[3]

II. DRUG DOCKING

In the 1970s people began molecular dynamics simulation. In 1982 Kuntz et al. published the DOCK algorithm. It was the first docking program to approach the problem from a non-simulation approach. It instead used a clique-search approach. This is done by matching features in the ligand to features in the receptor. Another approach in rigid docking is geometric hashing. Geometric hashing has its origins in computer vision. These rigid models worked well for some examples and not so well in other examples. In reality molecules are not rigid and are often quite flexible.[4]

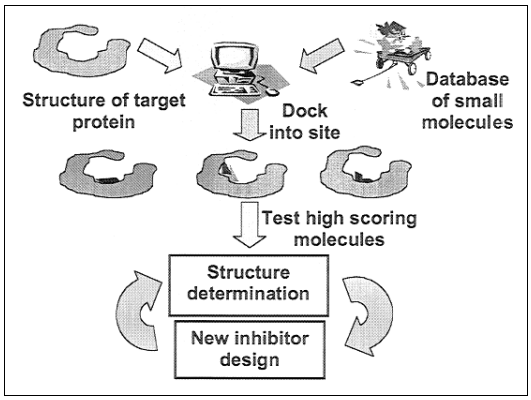


Figure.1. Drug docking flowcharts [4]

The ligand was the first part to become flexible. The ligand is usually a small molecule with few degrees of freedom. The first approach was to find all or most of the conformations the flexible ligand would take on and feed those conformations into a rigid docking program. The Flexibase/FLOG docking programs were based on this conformation ensemble approach. Another popular approach is fragmentation. In this case the ligand is broken up in some way and then placed piece by piece into the receptor. There are two major subsets of this approach. One is "place and join" where all of the fragments are placed independently and then the program tries to connect them together. The other is an incremental approach, where the fragments are placed one by one according to the orientation of the previous pieces. FlexX is one of the more popular incremental approach programs. A third approach in flexible ligand docking is genetic algorithms and evolutionary programming. This approach mimics biological evolution, where the individuals are different configurations of the molecules. A fitness function determines whether or not different configurations are used in the next generation of configurations. One of the first programs developed using this approach was GOLD based on the ideas of Jones et al. There are various other techniques for flexible docking. A program that we used was AutoDock, which uses simulated annealing to find the docked conformation. Later a genetic algorithm approach was added to AutoDock. There is other less used approaches and approaches that combine the previously mentioned to round out the work done on flexible-ligand docking.

Many good results have come from introducing ligand flexibility. However, the problem becomes much more difficult when trying to add in protein flexibility. A ligand is usually a small molecule with from 10 to 100 atoms. However, a protein has thousands of atoms, and thus has many more degrees of freedom. This makes the problem difficult, and solving this problem is what we've been working on. Our approach uses various search and dimensionality reduction techniques to solve the problem.

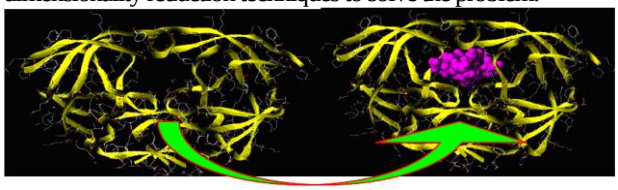


Figure 2: Drug binds to a protein by lock and key mechanism [5]

III. The Minimization energy function

An energy function takes in the conformation of a molecule and outputs a number representing the energy. The actual number is not important, what is important the relation between different conformations and their energies. The lower the energy the better the conformation is. If you have a good energy function then the minimum should be where the molecule is naturally. In our case we want the minimum to be the docked conformation of the ligand and the receptor. When we first started building our program we were using the CHARMM energy function to calculate the energy of the molecules. Normally CHARMM is used to study large macromolecules such as proteins. It turned out that CHARMM is not a good energy function to use in docking. The minimum was not the docked conformation, and the results from using CHARMM were nowhere close to the docked conformation. We decided to use the AutoDock energy, or scoring function. We choose it because AutoDock is open source and so we would be able to see exactly what it was doing. The AutoDock energy function has fewer terms than the CHARMM energy function and focuses on the aspects most related to docking. After implementing the AutoDock force field correctly we got much better results.

The authors had developed conjugate gradient minimizer for the CHARMM force field. It used the analytical derivate of the CHARMM function and was fairly fast. Minimizing is used in investigating the flexibility of a protein using principal components. When we change conformations of molecules we do not want to perturb the bonds or bond angles very much, because in nature they do not change much either. The principal components are in

Cartesian space, and so when we travel along them distortions in the structure of the molecule can occur. Minimization helps reduce these distortions. With respect to searching, minimizing at key times during the search might help find the docked conformation quicker. This summer we discovered a package called OPT++ developed by Sandia Labs. It was fast and flexible and so we used it instead of the home grown minimizer.[6]

By switching to the AutoDock function we no longer have an analytical derivative. We would have to use a numerical derivative, which is much slower. Fortunately the AutoDock function is much simpler than CHARMM and so it was not as bad as we had feared. However, the results from the minimization were awful because the bond and bond angles were not maintained. This is because the AutoDock function only contains terms for non-bonded interactions, so the bonds between molecules were being ignored in the minimization. The two solutions were to add fake bond and bond angle terms to the AutoDock function in order for the minimization to take then into account, or to do minimization in dihedral space. Adding the fake terms worked pretty well, but there were still slight changes in the bond and bond angles. The better solution is to minimize in dihedral space. Unfortunately, something about the dihedral spaces makes OPT++ break. So we do not have a minimizer at this point that can minimize in dihedral space.

IV. MOLECULAR MECHANICS AND DYNAMICS (MM AND MD)

The mechanical molecular model was developed out of a need to describe molecular structures and properties in as practical a manner as possible. The range of applicability of molecular mechanics (MM) includes:[7]

(a) Molecules containing thousands of atoms

(b) Organics, oligonucleotides, peptides, and saccharides

(c) Vacuum, implicit, or explicit solvent environments.

(d) Ground state only

(e) Thermodynamic and kinetic properties

The object of MM is to predict the energy associated with a given conformation of a molecule. However, MM energies have no meaning as absolute quantities. Only differences in energy between two or more conformations have meaning. A simple MM energy equation is given.by:

$$Energy(E) = E_{stretch} + E_{bending} + E_{torsion} + E_{non\text{-}bonded\ \ interactions} \quad (1)$$

These equations together with the parameters required to describe the behavior of different kinds of atoms and bonds, is called a force-field. Many different kinds of force-fields have been developed over the years. Some include additional energy terms that describe other kinds of deformations. Some force-fields account for coupling between bending and stretching in adjacent bonds in order to improve the accuracy of the mechanical model. All of the potential energy functions are illustrated in the graph below:[8]

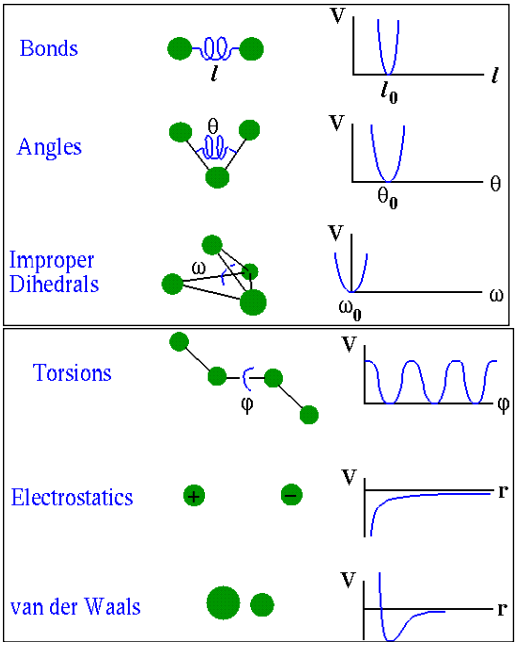


Figure 3: Potential energy functions

V. Thermodynamics of folding

Various approaches are used to correlate experimental findings with theoretical models for protein folding is stability. The authors investigated the effect of local minima in the rates of protein folding reactions. Using Kramers theory of diffusion limited barrier crossing that these intermediated can accelerate folding under certain conditions, due to a entropic effect. The physical biochemist would probably discuss protein stability primarily in terms of the thermodynamic stability of a protein that unfolds and refolds rapidly, reversibly, cooperatively, and with a simple, two-state mechanism: [9]

$$F(olded) \underset{k_u}{\longleftrightarrow} U(nfolded) \quad (2)$$

Where the K_u is the equilibrium constant for unfolding. (Negative free energy of folding comes from a balance of opposing large forces)

The easiest proteins to study folding and stability are those that exhibit this sort of rapid reversibility. Both experimental design and also theoretical treatment of data are simplified by reversible systems. Thus, it is no surprise that most of the literature reports about stability discuss this type of reversible system. The bulk of this dissertation will also focus on thermodynamic stability. In these cases, the stability of the protein is simply the difference in Gibbs free energy ΔG, between the folded and the unfolded states. The only factors affecting stability are the relative free energies of the folded (G_f) and the unfolded (G_u) states.

The larger and more positive ΔG_u, the more stable is the protein to denaturizing.

$$\Delta G_u = G_u - G_f \quad (3)$$

The Gibbs free energy, G, is made up the two terms enthalpy (H) and entropy (S), related by the equation:

$$G = H - TS \quad (4)$$

Where T is the temperature in Kelvin.

The folding free energy difference ΔG_u , is typically small, of the order of 5- 15 kcal/mol for a globular protein (compared to e.g. ~30 - 100 kcal/mol for a covalent bond). On the other hand, the biotechnologist is more concerned with the practical utility of the definition: Is the protein stable enough to function under harsh conditions of temperature or solvent? It may also lie either simply in reversibility or, for irreversibly or slowly unfolding proteins, in kinetic stability. If a protein unfolds reversibly it may be fully unfolded and inactive at high temperatures, but once it cools to room temperature, it will refold and fully recover activity. From a functional standpoint this may be all that is required for it to be classified as thermostable. However, from a thermodynamic standpoint (and in terms of this dissertation) it is classified as non-thermostable. In the case of irreversible or slowly unfolding proteins, it is kinetic stability or the rate of unfolding that is important. A protein that is kinetically stable will unfold more slowly than a kinetically unstable protein. In a kinetically stable protein, a large free energy barrier to unfolding is required and the factors affecting stability are the relative free energies of the folded (Gf) and the transition state (Gts) for the first committed step on the unfolding pathway. Irreversible loss of protein folded structure is represented by:[10]

$$F \underset{k_u}{\longleftrightarrow} U \xrightarrow[k_i]{} I(nactive)$$

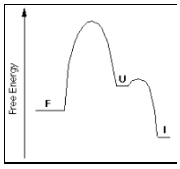


Figure 4: Potential folding free energy

The ki is the rate constant for some irreversible inactivation process. The free energy profile for a rapidly inactivating protein is shown below. Note that once the unfolded form is reached, the energy barrier to inactivation is lower than that to refolding.

As an aside, this observation (in reverse: unfolded-to-folded) has some consequences for the theory that a folded protein reflects the global energy minimum for the structure. This is not true if the activation energy required reaching that global minimum is so high that it cannot be reached. In such a case, the structure will be to all intents and purposes are trapped in a local minimum, and that locally minimized structure will be the folded state for that protein.[11]

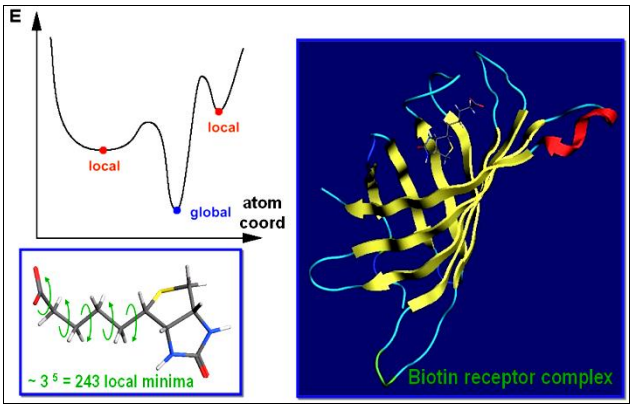


Figure 5: Potential folding free energy in stability conformation

This case is illustrated by subtilisin, which requires a pro-sequence as a catalyst for folding; the pro-sequence is not required in the final structure, but lowers the activation energy of folding is more than 27 kcal/mol. In the absence of this pro-sequence, the low energy final folded structure would not be attained.

For a two state transition the equilibrium constant can be determined from the average fraction unfolded in the transition region (α):

$$K_{eq} = \frac{[N]}{[U]} = \frac{1-\alpha}{\alpha} \quad (5)$$

The free energy of the folded state relative to the unfolded state can be derived for each set of conditions:

$$\Delta G_{fold} = G_n - G_U = -RT \ln K_{eq} \quad (6)$$

Determine the Stability of Docking System by Lyapunov Direct Method [9][15]

In a non-equilibrium linear region, entropy S generates P according to thermodynamics second law:

$$P \geq 0, \text{ and } \frac{dP}{dt} \leq 0 \quad (7)$$

Where P denotes the thermodynamics probability, entropy S and P satisfies the Boltzman relation equation:

$$S = K_B \ln P \quad (8)$$

Where KB represents the Boltzman equation constant value. Therefore, entropy S generates P according to the Lyapunov function in linear region. According to the Lyapunov stability theorem, the non-equilibrium constant state is stability in the linear area. In the nonlinear region of non-equilibrium state a Lyapunov function, $\delta^2 S$ also exists, producing $\delta^2 S \leq 0$ and $\frac{d}{dt}(\delta^2 S)$ non-constant sign. From the Lyapunov stability theorem, the nonlinear region's non-equilibrium state may be stability or instability since $\frac{d}{dt}(\delta^2 S)$ can be positive, negative or zero.

The stability of a protein is determined by large number of small positive and negative interaction energies .It makes a positive contribution to the change in free energy, if DG > O then, protein folding would not be favorable, folding must be a thermodynamically favored process (DG < 0)

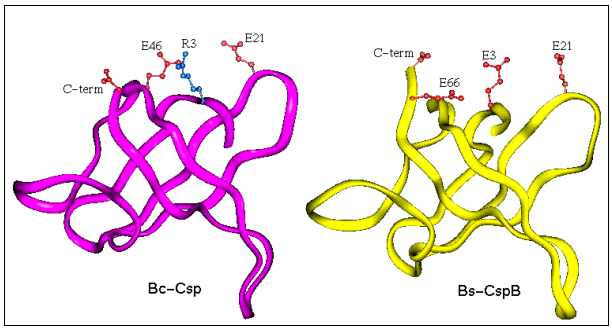


Figure 6 the major stabilizing forces of protein structures are hydrophobic and electrostatic.

While there is consensus on the hydrophobic contributions, the roles of electrostatic interactions in protein stability have been uncertain. The authors have made significant progress in modeling electrostatic effects.[12]

Ligand-protein docking: Modeling Procedure
Step 1: Creation of spheres to fit a cavity.
Step 2: Place a ligand to match the positions of spheres
Step 3: Check chemical complementarity.

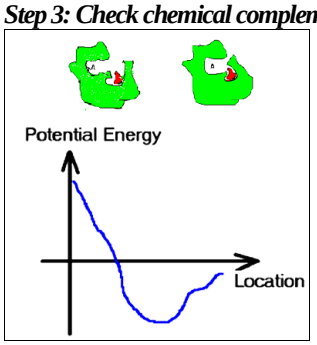


Figure 7: Ligand-protein docking: Modeling Procedure

A Lyapunov function is some kind of mathematical quantity that is maximized by a particular dynamical system as it changes according to whatever rules it works by. A general methodology in the stability analysis of equilibrium of a nonlinear dynamical system is to find a suitable Lyapunov function. This is in general a very difficult task, but for nonlinear molecular dynamic systems there often is a natural candidate Lyapunov function, namely the energy function.[13]

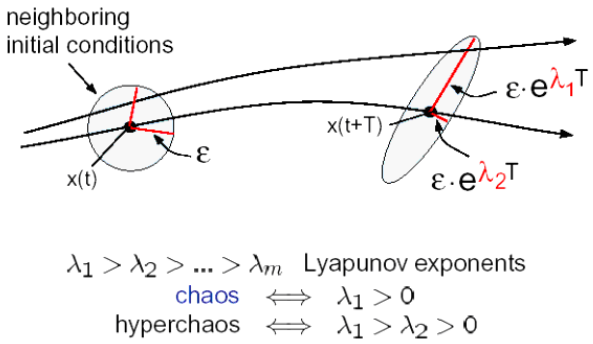


Figure 8: Energy Lyapunov Functions for Molecular Dynamic System

Lyapunov developed a general theory of dynamic stability applicable to both linear and nonlinear systems. The following sections are referencing process. According to Hess's law of heat summation, the change in free energy between two states will be the same, no matter what the path. So we can calculate the free energy of binding in solvent by the following equation:

$$G_{\text{binding,solution}} = G_{\text{binding,vacuo}} + G_{\text{solvation(EI)}} - G_{\text{solvation(E+I)}} \quad (9)$$

Since we can calculate $G_{binding,vacuo}$ from our docking simulation, and can estimate the free energy change upon solvation for the separate molecules E and I, and for the complex, EI, $G_{solvation(EI)}$ and $G_{solvation(E+I)}$ respectively, then it is also possible to calculate the free energy change upon binding of the inhibitor to the enzyme in solution, $G_{binding,solv}$. Thus, we can estimate the inhibition constant, K_i, for the inhibitor, I.

VI. VAN DER WAALS POTENTIAL ENERGY

The pairwise potential energy, $V(r)$, between two non-bonded atoms can be expressed as a function of internuclear separation, r, as follows,

$$V(r) = \frac{Ae^{-\varepsilon r}}{r} - \frac{C_6}{r^6} \quad (10)$$

Graphically, if reqm is the *equilibrium internuclear separation*, and e is the *well depth* at reqm, then:

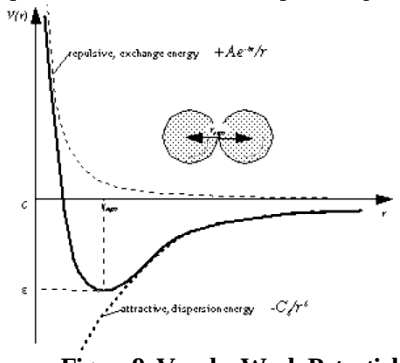


Figure 9: Van der Waals Potential Energy

The exponential, repulsive, exchange energy is often approximated thus,

$$\frac{A}{r}e^{-\varepsilon r} \approx \frac{C_n}{r^n} \quad (11)$$

Hence pairwise-atomic interaction energies can be approximated using the following general equation,

$$V(r) \approx \frac{C_n}{r^n} - \frac{C_m}{r^m} = C_n r^n - C_m r^m \quad (12)$$

where m and n are integers, and C n and C m are constants whose values depend on the depth of the energy well and the equilibrium separation of the two atoms' nuclei. [14] Score equation is taking account for VDW interaction,

hydrogen bonding, hydrophobic interaction, deformation entropy loss and metal-bonding upon protein-ligand binding process.

$$pK_d = K_{VDW} + K_{H-bond} + K_{Hydrophobic} + K_{Rotor} + K_{metal} + const. \quad (13)$$

VII. EXPERIMENT 1: LYAPUNOV STABILITY FUNCTIONS

The following illustrate the relation between minimum energy and molecular modeling from Lyapunov Equation. The authors devised a general method for generating reaction coordinates and to characterize the stability basins of the energy landscape for systems of interest in biomolecular simulation. The method, based on stability theory, decomposes the molecular system into subsystems and constructs a suitable vector Lyapunov function whose components are individual stability functions for each subsystem.

First, consider n differential equations of molecular motion with n particles.

$$\begin{aligned} \frac{dX_1}{dt} &= f_1(X_1, X_2 \ldots X_n) \\ \frac{dX_2}{dt} &= f_2(X_1, X_2 \ldots X_n) \\ &\vdots \\ \frac{dX_n}{dt} &= f_n(X_1, X_2 \ldots X_n) \end{aligned} \quad (14)$$

The system's constant solution $(X_{1s}, X_{2s} \ldots\ldots X_{ns})$ becomes the reference state.

Therefore, the general solution of the (14) linearized equation is as follows:

$$\begin{aligned} x_1 &= c_1 \mathrm{A}_1 e^{\lambda_1 t} + c_2 \mathrm{A}_2 e^{\lambda_2 t} + \cdots + c_n \mathrm{A}_n e^{\lambda_n t} \\ x_2 &= c_1 \mathrm{B}_1 e^{\lambda_1 t} + c_2 \mathrm{B}_2 e^{\lambda_2 t} + \cdots + c_n \mathrm{B}_n e^{\lambda_n t} \\ &\vdots \\ x_n &= c_1 \mathrm{N}_1 e^{\lambda_1 t} + c_2 \mathrm{N}_2 e^{\lambda_2 t} + \cdots + c_n \mathrm{N}_n e^{\lambda_n t} \end{aligned} \quad (15)$$

In Equations (15) above, c1 , c2 …cn are discerned by the initial condition. According to these n solutions, the stability constant state solution can be summarized using the Lyapunov function as follows:

1. If λ_1 , λ_2 ...λ_n are less than zero in the real number part, then $\lim_{t\to\infty} |x_i| = 0$ is **asymptotically stable** for linearized equation (14) with two particles. Since the small molecular perturbation motion x_i falls with increasing time *t*, the constant state (X_{1s}, X_{2s}) of nonlinear equation (15) is also **asymptotically stable.**
2. If either λ_1 or λ_2 ...λ_n exceed zero in the real number part, then $\lim_{t\to\infty} |x_i| = \infty$ is **unstable** for linearized equation (14) with two particles. Because the small molecular perturbation motion x_i rises exponentially with increasing time *t*, the constant state $(X_{1s}, X_{2s}, \cdots, X_{ns})$ of the (15) nonlinear equation is also **unstable.**
3. If λ_1 or λ_2 ...λ_n are below zero and at least one eigenvalue equals zero in the real number part, then $\lim_{t\to\infty} |x_i| = 0$ is **stable**, but is not **asymptotically stable** for linearized equation (14) with two particles. The equation is **marginally stable** at location (X_{1s}, X_{2s}). In this condition, the small molecular perturbation motion may alter the solution for the nonlinear equation. Therefore, when x_i is only stable and not **asymptotically stable** from the linearized equation (15), the solution for the nonlinear equation state of solution (14) cannot be determined.

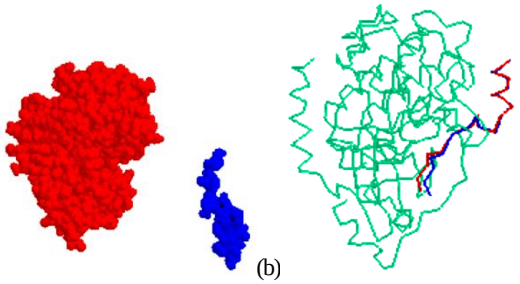
Except in the boundary situation, the eigenvalues of the linearized system completely demonstrate the stability characteristics of an equilibrium point of a nonlinear system. If boundary eigenvalues exist they must be analyzed separately. The linearized stability theorem is employed to identify the stability of the solution in a nonlinear system. This technique is termed "linearized stability analysis".

VIII.Experiment 2: (1)Protein-protein docking of 1ydr and (2) Protein-drug docking of la30

The program receives as input a pair of PDB files, as a "receptor" and a "ligand" molecule. It finds transformations of the "ligand" that will achieve the best shape complementarity between the two molecules. The rigid docking program returns a set of hypotheses sorted by the value of this shape complementarity between the receptor and the ligand. The shape complementarity score favors molecular surface interactions and penalizes "small" penetrations of the ligand into the receptor.

1. Protein-protein docking of 1ydr (transformed chain I on chain E)

This is the structure Of Camp-Dependent Protein Kinase (chain E) and its peptide inhibitor (chain I).



(a) (b)

Figure 10 :(a) Input to docking program (receptor in red and ligand in blue) (b) Solution (red) relative to ligand in complex (blue): RMSD 0.76

2. Protein-drug docking of la30:

Cyclooxygenase-2 (Prostaglandin H2 synthase from Mouse) with Flurbiprofen.

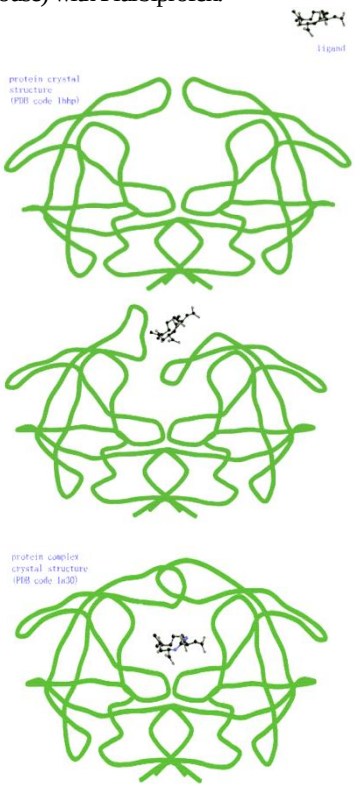


Figure 11 :(a) Input to docking program (receptor in grey and flurbiprofen drug in red) (b) Best solution relative to complex flurbiprofen: RMSD: 0.89 (c) Closer look at the solution.

IX. CONCLUSIONS AND FUTURE WORK

Drug was founded to demonstrate that high performance computing computer assisted drug design (CADD) not only dramatically improves the process of discovering new drugs, but also is an affordable tool for CADD industries.This study proposed a novel minimum energy scheme for drug docking applications. The proposed scheme preserves the important advantages inherent in a protein folding process and ligand become locked key. In final, authors cited Lyapunov Equation to prove the stability of drug docking.

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REFERENCE

[1] M. Rarey, S. Wefing & T. Lengauer, "Placement of medium sized molecular fragmets into the active site of proteins", J. Comput.-Aided Mol. Design 10, 41-54, (1996).

[2] P.J. Goodford, "A computational procedure for determinimg energetically favorable binding sites on biologically important macromolecules", J. Med. Chem. 28, 849-85, (1985).

[3] T. Lengauer & M. Rarey, "Computational methods for biomolecular docking", Curr. Opin. Struct. Biol. 6, 402-406, (1996).

[4] I.D. Kuntz, J.M. Blaney, S.J. Oatley, R.L. Langridge & T.E. Ferrin, "A geometric approach to macromolecule-ligand interactions", J. Mol. Biol. 161, 269-288, (1982).

[5] Wen-Tsai Sung, Hung-Yuan Chung, Yao-Chi Hsu, Kuan-Yu Chen, A Light Wind Speed Measurement System Design to Strengthen Wireless Sensor Network Functions, International Journal of Innovative Computing, Information and Control, Vol.7, No.2, pp.859-868. Feb. 2011.

[6] A.R. Leach & I.D. Kuntz, "Conformational analysis of flexible ligands in macromolecular receptor sites", J. Comp. Chem. 13, 730-748, (1992).

[7] B.K. Shoichet, D.L. Bodian & I.D. Kuntz, "Molecular docking using shape descriptors", J. Comp. Chem. 13, 380-397, (1992).

[8] T.J.A. Ewing & I.D. Kuntz, "Critical evalutation of search algorithms for automated molecular docking and database screening", J. Comp. Chem. 18, p. 1175-1189, (1997).

[9] D.A. Gschwend & I.D. Kuntz, "Orientational sampling and rigid-body minimization in molecular docking revisited: on-the-fly optimization and degeneracy removal", J. Comput.-Aided Mol. Design 10, 123-132, (1996).

[10] Duhovny D, Nussinov R, Wolfson HJ. Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics(WABI) Rome, Italy, Lecture Notes in Computer Science 2452, pp. 185-200, Springer Verlag, 2002

[11] Zhao, S., Goodsell, D.S., Olson, A.J. (2001) Proteins: Struct., Func. & Genetics, 43:271-279.

[12] Osterberg, F., Morris, G.M., Sanner, M.F., Olson, A.J., Goodsell, D.S. Proteins: Struct., Function, Ge netics 46:34-40. 2001.

[13] Wen-Tsai Sung ,Shih-Ching Ou,” Minimum Energy for Protein Folding Structure via Lyapunov Equation Molecular Dynamics” Symposium and Workshop of Bioinformatics in Taiwan 2003,Taipei,Tawan.5-7 Sept.2003.

[14] Shih-Ching Ou，Wen-Tsai Sung,” Molecular Docking for Drug Design” Symposium and Workshop of Bioinformatics in Taiwan 2003,Taipei,Taiwan. 5-7 Sept.2003.

[15] Zalili Binti Musa and Junzo Watada “A Particle Filter Approach for Multi-Camera Tracking Systems in a Large View Space”, International

Journal of Innovative Computing, Information and Control, Vol.6, No.6, pp.2827-2836. June. 2010,

[16] Vyacheslav Kalashnikov, Fernando Camacho, Ronald Askinand Nataliya Kalashnykova "Comparison of Algorithms for Solving a Bi-Level Toll Setting Problem", International Journal of Innovative Computing, Information and Control, Vol.6, No.8, pp. 3529-3550. August. 2010,

[17] Chia- Chih Tsai, Shih- Ching Ou , Chun- Yen Chung , Wen-Tsai Sung, Chin Ch Chien, and Da Yu Su " Predict protein structure and similar function via 3D Visualization tools-case study of bamboo shoot " International Proteomics Conference (IPC'03), In the Grand Hotel on May 14-17, 2004. Taipei, Taiwan.

[18] Wen-Tsai Sung, Jui-Ho Chen, Meng-Hui Wang, Yao-Chi Hsu, Remote Medical Care System Design Based on RFID and Zigbee Technology via Wireless Sensors Network, International Journal of Innovative Computing, Information and Control, Vol.6, No.11, pp.5203-5220. November. 2010.

[19] Shih- Ching Ou, Chun- Yen Chung, Wen-Tsai Sung ,Chia - Chih Tsai, Chin - Chih Chien, and Da -Yu Su, Virtual Screening and Computer-aided Drug Design in Molecular Docking via Lyapunov Function, WSEAS Trans. on BIOLOGY and BIOMEDICINE , Issue 4,Vol. 01, pp.384-390, Oct. 2004.