AN INVERSE KINEMATICS SYSTEM FOR F_1ATPASE NANO-MOTOR MANIPULATION

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F_1ATPase is a protein that fully folds with adenosine triphosphate (ATP) hydrolytic activity. Its three-dimensional (3-D) conformations play a crucial role as high-efficiency ATP-driven motors at nano-level. Computer-aided design technology allows us to modify F_1ATPase and manipulate its rotation before experimental testing. Over the last decade, several computer-aided systems have been developed to simulate protein dynamics; for instance, *Dezymer*, *Procheck*, and *Metal-search*. However, mathematical models have rarely been implemented for engineering purposes. Our ultimate goal is to generate a system that will predict conformational transition of rotary protein motors, including their mechanical properties, with low computational cost. The inverse kinematics model for a binary manipulator with many actuators, a robotics algorithm, has the potential to be used in developing a prototype system. Our computer-aided system will include the following steps: 1) ATP binding-site searching 2) sidechain/backbone substitution 3) steric-crash detection, and 4) calculation of global minimum energy. For site searching, geometric deformation of F_1ATPase needs to be determined. The method for resolving our problem is using forward kinematics algorithms to determine coordinates of the active sites on F_1ATPase. In this paper, we propose to investigate an algorithm for searching the ATP binding sites, in order to obtain the best solutions corresponding to an inverse kinematics system for sidechain/backbone substitution. We expect that our computer-aided system will increase the efficiency of mechanochemical motor controlling.

INTRODUCTION

Rotary protein motors are biomolecular machines in an organism which facilitate homeostasis in cells. Two well-characterized biomolecular motors are bacterial flagella motor (BFM) and ATP synthase (also called F_oF_1ATPase). We need to control and program these rotary engines to serve nanomedical applications such as pharmaceutical transportation and nanorobotics machinery. Rotary protein motors demonstrate that they have the potential to act as a part of bionano-mobile robot.

ISBN: 978-81-904262-8-2(RPS)

ATP synthase or F_oF_1ATPase is composed of two rotary subunits: F_o and F_1 motors (Figure 1a) [1]. F_o- and F_1ATPase connect to each other serially. These cellular motors generate mechanical forces during enzymatic reactions (intermolecular binding at active sites). The F_o motor is driven by proton-motive force while the F_1 component is rotated by nucleotide (ATP or GTP) hydrolysis. Conformational changes of F_1ATPase, during nucleotide breaking, cause the γ subunit to spin in a counterclockwise direction. In contrast, F_oATPase rotates clockwise when protons pass through ion-binding sites. The F_o motor generates large torque which subsequently drives the F_1 motor in the direction of ATP synthesis.

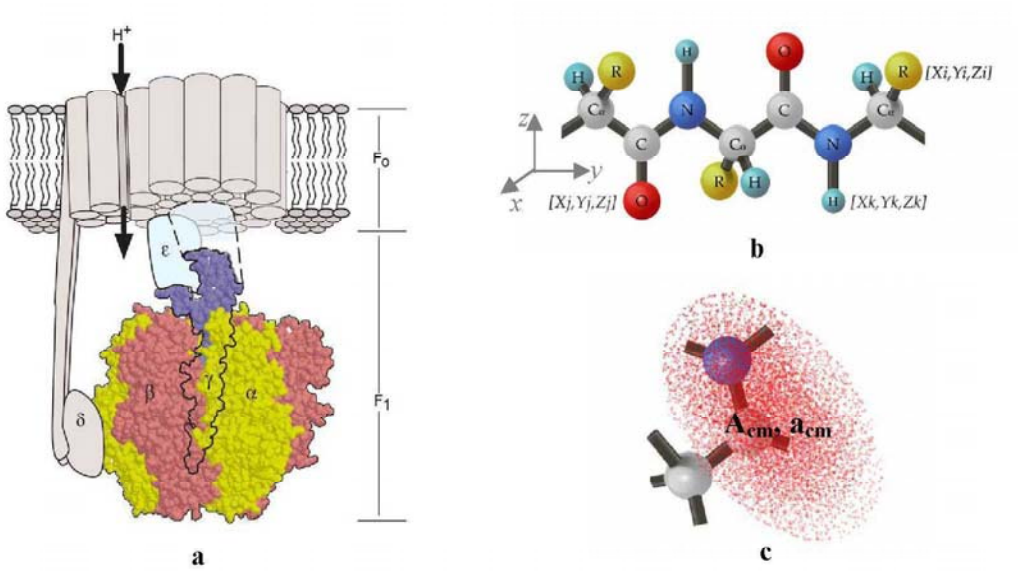

Figure 1. **a)** Organization of ATP synthase (adapted from [1]): a symmetric $\alpha 3\beta 3$ hexamer of F_1ATPase composed of three ATP binding sites, **b)** coordinate assignment of each amino acid residue on F_1ATPase in order to determine the ATP binding-site and **c)** the concept of the mean of the workspace density function of each molecule along the F_1ATPase backbone

As intact F_oATPase crystal is not available in the Protein Data Bank (PDB), the organization of F_o motor could be neglected. Generally, there are two hypothetic models to describe the intermolecular binding energy governs the rotation of F_1ATPase; the former is the *Power Stroke* model and the latter is the *Brownian Ratchet* theory [2]. Both laws explain the same mechanism of ATP hydrolysis in F_1ATPase but differ in their fundamental terminology. The *Power Stroke* theory defines ATP hydrolysis as the following reaction [3, 4]:

$$Enz + ATP \underset{\Delta G \sim 14 k_B T}{\overset{\text{Binding}}{\rightleftharpoons}} Enz \cdot ATP \underset{\Delta G \sim 0 k_B T}{\overset{\text{Hydrolysis}}{\rightleftharpoons}} Enz(ADP + Pi) \underset{\Delta G \sim 10 k_B T}{\overset{\text{Pi Release}}{\rightleftharpoons}} Enz \cdot ADP + Pi \underset{\Delta G \sim 0 k_B T}{\overset{\text{ADP Release}}{\rightleftharpoons}} Enz + ADP.$$

The total free energy of ATP hydrolysis is approximately 24 $k_B T$ where k_B is Boltzmann's constant and T is the absolute temperature. We hypothesize that if the rate of ATP binding and/or phosphate released is/are altered, the mechanics of F_1ATPase rotation may also change.

Mechanical controlling of F_1motor

According to its architecture, F_1ATPase has high mechanical efficiency with 40 pN torque generation per 120° revolution step [3, 5]. The stroke efficiency of γ shaft of F_1ATPase depends on three factors: rotational frictional drag coefficient, average rotational velocity, and free energy of ATP hydrolysis. Therefore, the mechanochemical rotation of F_1ATPase can be regulated by:

1. Controlling the admission of nucleotides to catalytic sites
2. Controlling the release of product
3. Repositioning of the catalytic residues

ROBOT KINEMATICS SYSTEM

Forward kinematics for ATP binding-site searching

Initial crystallographic data of F_1ATPase were obtained from the PDB. We can register the representative molecules in our coordinates system (Figure 1b). Bond length, bond angles, and dihedral angles are the essential variables for our 3-D computer simulation. Each parameter was treated with Z-Y-X Euler angles. We discovered that there was registration error during the simulation of a single protein backbone (N-C_α-C) (data not shown). The obtained poses could not follow molecular constraints such as overlapping of atomic spheres.

Inverse kinematics for geometric deformation of ATP binding-sites: sidechain/backbone substitution

In the case of achieved site searching, we will acquire the volume within each ATP binding site with economic calculation by means of integration of a workspace density function (WDF) [6]. The algorithm is an extension of the Ebert-Uphoff and Chirikjian algorithm, for discrete motions of multi-actuator manipulator with higher-order degrees of freedom. The algorithm was contingent on the critical assumption that all manipulator structures connect serially to each other, which is equivalent to protein backbones.

The point cloud (red dots) in Figure 1c represents the workspace of a three-atom link system. A single red dot contains a transformation element $\boldsymbol{g}$ for one discrete movement. The center-of-mass $\boldsymbol{g}$ is denoted as

$$\mathbf{g}_{\mathrm{cm}} = \begin{pmatrix} \mathbf{A}_{\mathrm{cm}} & \mathbf{a}_{\mathrm{cm}} \\ \mathbf{0}^{\mathrm{T}} & 1 \end{pmatrix},$$

where $\mathbf{A}_{\mathrm{cm}}$ is a 3-by-3 mean rotation matrix and $\mathbf{a}_{\mathrm{cm}}$ is a mean translation in 3-D space of the representative atom along the momentum axis (intermolecular bond). We can calculate the values of $\mathbf{A}_{\mathrm{cm}}$ and $\mathbf{a}_{\mathrm{cm}}$ of P-residue protein backbone by following these equations: the mean $\mathbf{M}$ of a rotational matrix $\mathbf{A}$ is given by

$$\mathbf{M} = \int_{SO(N)} \mathbf{A}\rho(\mathbf{A})\, d\mathbf{A},$$

where $\boldsymbol{\rho(\mathbf{A})}$ is probability density function of rotation matrix $\mathbf{A}$.

$$\mathbf{A}_{\mathrm{cm}}^{1^*\ldots^*P} = \mathbf{M}_{1^*\ldots^*p}\left(\mathbf{M}_{1^*\ldots^*p}^{\mathrm{T}}\mathbf{M}_{1^*\ldots^*p}\right)^{(-1/2)}$$

where

$$\mathbf{M}_{1^*\ldots^*P} = \mathbf{M}_1 \cdot \mathbf{M}_2 \cdot \mathbf{M}_3 \cdot \ldots \cdot \mathbf{M}_P,$$

and

$$\mathbf{a}_{cm}^{1*\ldots*P} = \left(\mathbf{I} + \sum_{k=1}^{P-1} \mathbf{M}^k \right) \mathbf{a}_{cm}, \; k \in \{1, \ldots, P\},$$

where **I** is the identical matrix.

The second important concept of this kinematics notation is that the center of mass $\boldsymbol{x}_{cm}$, of the whole manipulator, is obtained by summing each of $\boldsymbol{x}_{cm}$ in the manipulator workspace (point cloud) at the first module (base) while the distal part of the manipulator is untouched. After $\boldsymbol{x}_{cm}$ maximization, a computation of the possible $\boldsymbol{x}_{cm}$ of the next modules will be performed until reaching the module before the end-effector. Using this idea, we can generate possible points within the protein workspace in order to customize any amino acid residues surrounding the ATP binding sites. As a result, repositioning the boundary of ATP active sites, by sidechain/backbone substitution, possibly changes the mechanics via our model.

CONCLUSION

F_1ATPase behaves like a rotary engine and could have a strong potential for driving bionanomachines. We need to develop a predictive system, and investigate the potential of the robotic kinematics algorithm, for modelling the behavior of F_1ATPase. According to our procedures, the algorithm for searching the ATP-binding sites has been investigating and, in order to avoid registration errors of the molecular coordinate, a calibration of forward kinematic system needs to be done. We expect that the computer-aided system will support rotary protein motor modification and design. We will also investigate the effects of substituting of sidechain/backbone with amino acid rotamers. The results should increase our understanding of the virtual mechanochemistry of F_1ATPase during computer simulation.

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