Computer Simulations of Biomolecular Machines and Switches

Jana Chocholoušová and Jaroslav Vacek
Institute of Organic Chemistry and Biochemistry
Prague, Czech Republic

1. Introduction

Cell life is supported by many biomolecular motors, actuators, pumps, active gates and other machines. They are responsible for harvesting energy, maintaining homeostasis, transport of nutrients, storage and transfer of genetic information, molecular recognition etc. Much is yet to be learnt about their structure and effective function in the midst of Brownian environment. Deep understanding of biomachine action will have tremendous impact on broad areas of our everyday life with applications ranging from medicine to nanotechnology and molecular electronic. Such an understanding is not expected to emerge without detailed knowledge of their 3-D structure and dynamics.

We are using molecular and Langevin dynamics to theoretically investigate the structure and dynamic operation of molecular machinery and we are trying to gain some more insight into their structure-function relationship.

Our research is focused on molecular modeling and simulations of artificial molecular devices, such as rotors and motor, which may be attached to a grid or not. Such devices may be driven by the gas flow or liquid. Also we have investigated light driven motors. Biological motors and other devices are of interested because they can serve as a model for the artificial motors or find use in biomimetics etc. The F1-ATPase is one of the most profound biological motors, it is responsible for energy management. Other devices, such as aptamers or riboswitches, can work as natural sensors or transporters for specific targets.

2. Methodology

Molecular Dynamics (MD) describes intra- and intermolecular interactions either by "force fields" (classical MD) or by quantum chemical model ("quantum dynamics"). Newton's equations of motion are being solved and trajectory is being produced as the simulation proceeds.

Quantum Chemical Calculations are used for precise calculation of smaller molecules or components and their properties, such as vibration spectra, pKa, rotation barriers etc.

Graphics and analysis. To visualize the results we mostly use VMD, moil-view or pymol. Other analysis is usually done by ptraj, charmm or tink analysis tools.

3. Systems: Molecular Machines

A molecular machine is a single macromolecule or macromolecular complex that performs a specific function for a living system [Schneider, 1991] Examples of molecular machines operation: (i) for a DNA it means base pairing and information storage (ii) for RNA aptamers it is recognition of the target (iii) for a riboswitch it is recognition of target connected with a conformational change and a biosynthesis regulation as result. (iv) for the ATPase it is the ATP hydrolysis and motion

In many molecular machines, decrease in thermal motion has been observed as a consequence of machine action, in other cases energy can be taken from ATP hydrolysis.

4. Examples of systems studied in our lab

4.1 ATPase.[1] Molecular motors are mechano-chemical biological nanomachines that fulfill a variety of central functions in cellular metabolism. They can generate force and torque by converting chemical energy, mainly adenosine triphosphate (ATP) into mechanical work. F_0F_1-ATP synthase is a prototype rotary molecular machine and has evolved into a paradigm for rotational energy conversion in biology. Theoretical modeling can produce important insight into the mode of action of nanomachines. Therefore, experimental observations of single nanodevice actions should be complemented with computer simulations. We investigate dynamics and function of the ATPase to gain more knowledge about its key elements, action and energy management. This should finally lead to an integrative view of the various conformational alterations and their interplay and interdependencies in a rotary biological motor. So far we have done the normal mode analysis and some simulations of smaller parts of the ATPase. **This research is performed in collaboration with Dirk Bald and the Amsterdam group.**

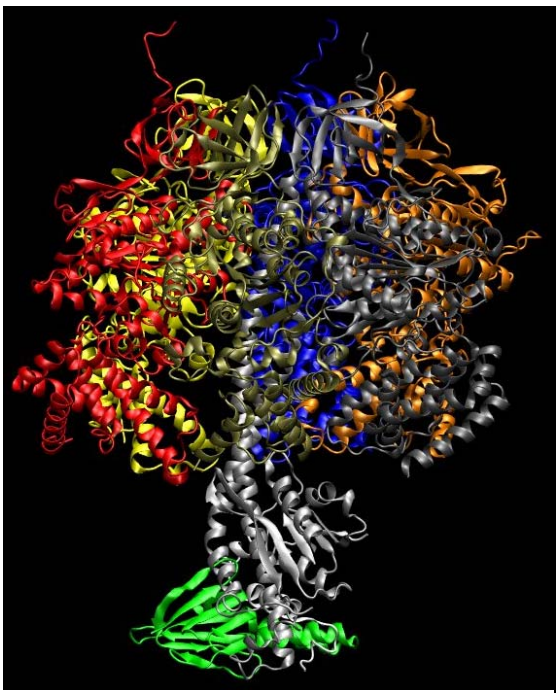

Figure 1. F1 subunit of the ATPase (pdb id 1E79)

4.2 SAM riboswitch.[2,3,4] Riboswitch is a part of mRNA that can directly bind a small target molecule. It works as a natural sensor controlling gene's activity by binding a specific ligand. Such an mRNA is thus involved in regulating its own activity. A riboswitch consists of an aptamer part and an expression platform. The aptamer changes its conformation in response to binding the target. Expression platform, which is the mechanism for gene expression regulation, is also affected by the structural rearrangement. Examples of known riboswitches are S-adenosylmethionine (SAM) riboswitches (Figure 1), which bind SAM and regulate methionine biosynthesis and transport, T-box, TPP, FMN, Cobalamin, purine and glycine riboswitches. Although there is a known crystal structure of a SAM riboswitch with its ligand, so far experimentalists are struggling with determination of the free form structure.

We believe, that molecular modeling will bring new opportunities to explore structure, dynamics and function of the SAM riboswitch and provide useful information to experiments.

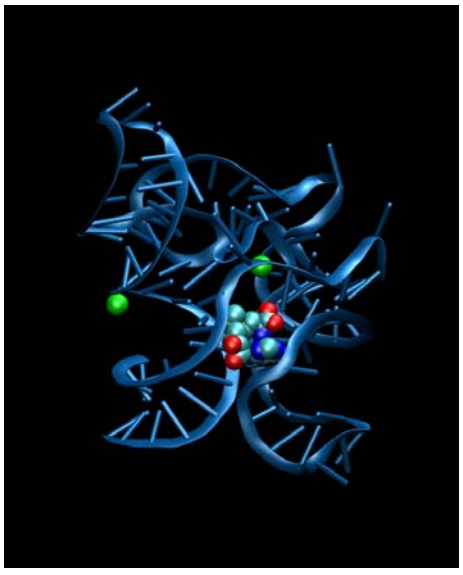

Figure 2. SAM riboswitch in the bound state (pdb id 2CIS). Ligand (SAM): van der Waals representation , Mg^{2+} ions green balls), RNA (blue).

The existence of riboswitches has only recently been found. Now that riboswitches are a known mechanism of genetic control, it is reasonable to speculate that more riboswitches will be found.

4.3 TRP aptamer.[5,6,7] Aptamers are oligonucleotide sequences with affinity for specific targets. They can be discovered experimentally using SELEX (Systematic Evolution of Ligands by Exponential Enrichment).[4] It is thus now possible to isolate oligonucleotide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. This class of molecules will rival antibodies in both therapeutic and diagnostic applications. The demand for diagnostic assays to assist in the management of existing and emerging diseases is increasing, and aptamers could potentially fulfill molecular recognition needs in those assays. Newer experimental results show that this motif is required but not sufficient to retain the functionality of the aptamer, however. Our preliminary calculations revealed that a nearby feature (red in Figure 3 right) may be required to maintain full functionality. In this example, molecular modeling is able to provide extremely valuable information, help design new experiments and bring more understanding of the function.

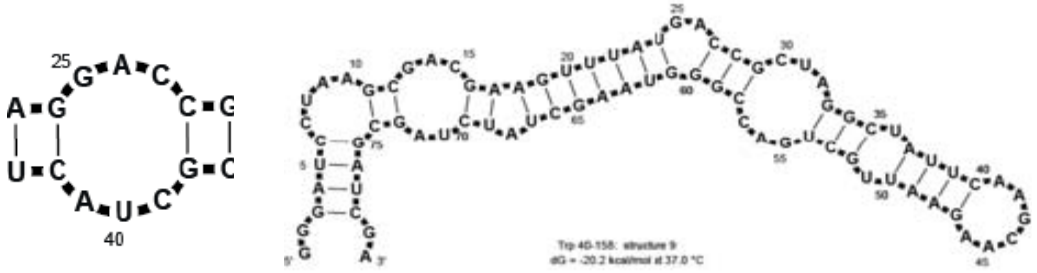

Figure 3. Tryptophane binding site. Consensus sequences and aptamer example.

4.4 Fluorescent proteins (GFP, CFP, YFP)[8] have a unique can-line shape formed by 11-strand β-barrel and a single α-helical strand carrying a chromophore running through the center. The barrel provides a microenvironment which allows for the formation of the chromophore and protects it from quenching. A significant advantage of fluorescent proteins such as GFP over small fluorescent molecules is that they are usually much less harmful when illuminated in living cells. This has redefined fluorescence microscopy and enabled observation of many biological processes such as protein folding, protein transport or RNA dynamics.

We prepared models of two barrels connected by amino acid linkers of a various length. The goal is to estimate the chromophore - chromophore distance for different linkers. **This research is performed in collaboration with Klaus Kemnitz and Werner Zustratter and the Berlin/Magdeburg groups.**

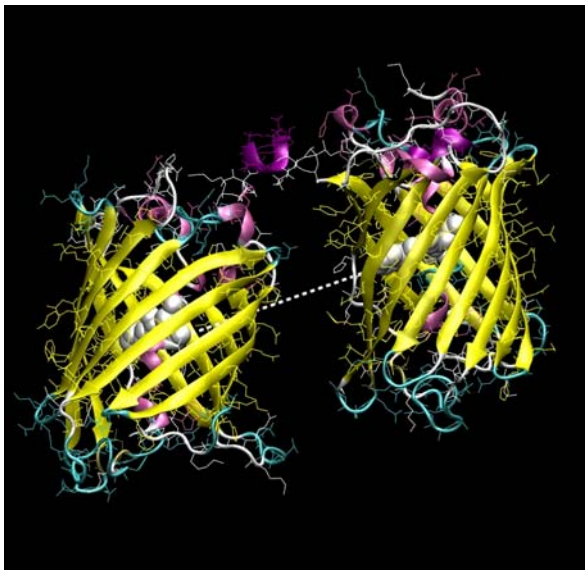

Figure 4. CFP and YFP barrels connected via an 8 amino acid linker.

5. Conclusions

We have shown that molecular modeling and theoretical chemistry methods are useful tools for exploring 3-D structure, dynamics and function of various biomolecular devices. Based on the theoretical results we have helped with setting up new experiments and with finding answers or ideas related to structural problems.

6. Acknowledgement

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7. References

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