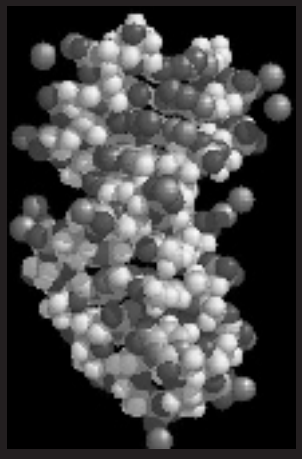
Then and Now...

MOLECULAR SIMULATIONS IN MODERN SCIENCE

By Eva Nong



Biomolecule Models: Then and Now: Before the dynamic protein model of the 1970's, biological molecules were predominantly represented as rigid constructs, similar to the original steel model of Watson and Crick's DNA double helix. Scientists now understand biological molecules as a collection of dynamic structures, filled with thousands of vibrating atoms (above).

credits: "Super Models in the Laboratory." *Nature* 400 (1999): 485.

It's the first week of school and you stroll into your biology class laboratory, ready to start your experiment. However, you walk past the familiar latex gloves and geeky goggles to pick up a wireless mouse. An hour later, you harvest your results not from the dark blue smudges on a gel but from the computer screen, where a newly synthesized DNA helix gracefully rotates to your mouse clicks. Welcome to the wonderful world of molecular dynamics (MD) simulations, a new branch of experimental science and an increasingly important tool for the study of biological macromolecules.

With rapidly advancing computing power, MD simulations are becoming more sophisticated, with complex arrays of reactant molecules and force fields. MD not only provides a viable alternative to conducting chemical reactions but also resolves problems that cannot be addressed in a conventional lab setting (1). Thirty years since its start, MD has already made great strides and is revolutionizing the way we do research in science.

What is MD simulation?

MD is a computer-generated mimic of chemical reactions with proteins and DNA as the players. It does so by using physical principles to calculate the random motion of biomolecules at an atomic level. Cells are bathed in an aqueous environment, in which all atoms have free energies that give rise to random vibrating motion (2). While the movement of each atom is microscopic compared to the size of a DNA molecule, the combination of hundreds and thousands of atoms wiggling at the same time is instrumental in how DNA acts as a whole. An MD reconstruction takes into account this constant vibration of atoms in solution. Therefore, by perceiving virtual DNA molecules as composed of dynamic units, MD provides a platform for realistic simulation of reactions and has been experimentally proven to be highly accurate (1).

The Advantages of Molecular Simulations

Because MD applies physical principles to calculate reactions, it generates quantitative measurements that are otherwise unobtainable in a laboratory setting. It is very difficult for real-life chemical reactions to resolve quantitative data on a molecular level. For example, during a polymerase chain reaction (PCR) reaction, it is impossible to see how the polymerase and DNA interact inside the chamber, let alone record the exact reaction rate for one round of replication. With MD, however, obtaining such information would be child's play. MD simulates macromolecules based on the motion and position of each atom, calculating the changes to these parameters as a function of time. This process yields quantitative data, such as reaction rates, relative stabilities of the reactants and transient intermediates generated during the course of replication (1).

The power of MD does not stop here. It also passes the magic wand to its users, giving them complete control to design experiments at will (1). Normally, setting up a new experiment could mean months or even years of work (5). For example, if you want to compare the reaction rates of two different polymerases, you would have to prepare proteins and set up two separate experiments. Each procedure consumes a lot of time and reagents, in addition to increasing the likelihood of human error (1). However, MD users can preserve the original settings and change only the necessary components. For example, one can load the original reaction file, substitute substrates, change computer experimental parameters, and start the experiment immediately. With MD, comparison projects can now be done simply by changing inputs of the computer program.

MD offers an easy alternative to certain conventional laboratory experiments that are incredibly expensive and difficult to set up. For example, a recent focus of many researchers has been the "optical tweezers" experiment: researchers use lasers to pull on one end of molecules, such as DNA, with the other end attached to a bead (3, 5). But because lasers are expensive and entail precise installation of multiple auxiliary structures, researchers may opt to use MD to predict the results instead (5). MD can create a virtual laser from force fields that come with the software package and thereby simulate the optical tweezers experiment without actually doing it (1). This saves both lab expenses and many headaches.

"This process yields data that are impossible to observe in a test tube... an easy alternative to...conventional laboratory experiments."

A Brief History of Molecular Dynamics

Interestingly, MD models were instrumental in displacing the earlier view of DNA and proteins as relatively rigid structures. Prior to the advent of the dynamic protein model in the 1970's, biological molecules were represented as rigid fixtures similar to the steel-made double helix constructed by Watson and Crick. Sir D. L Philips once commented, "Brass models of DNA and a variety of proteins dominated the scene and much of the thinking" (1, 3).

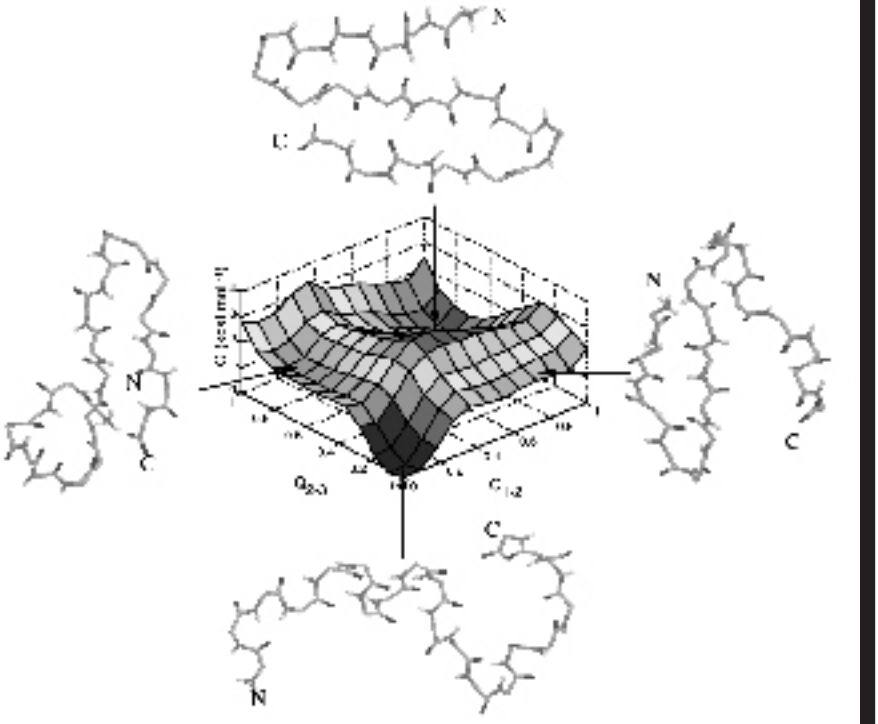
In 1975, the first MD experiment simulated a protein from the pancreas, beginning a paradigm shift towards perceiving molecules as dynamic structures (6). While the simulation itself was primitive by today's standards (it lasted only for a pico second), it demonstrated the protein as composed of thousands of vibrating atom units (1). This represented a breakthrough in the transition from brass models to dynamic models of biological molecules.

MD Today

With MD getting more and more attention from the scientific community, there has been a dramatic increase in computing power to simulate experiments. While the first MD simulation lasted only a few picoseconds, today's virtual experiments can run a thousand times as long while taking only a fraction of the original set-up time (1). Some of the commonly used MD programs, such as Chemistry at Harvard Macromolecular Mechanics (CHARMM), are developed at Harvard's Department of Chemistry and Chemical Biology by Martin Karplus. This gain in computation capacity has greatly expanded the range and complexity of experiments for MD.

In addition to the ever increasing complexity of reactant molecules, force fields and analysis packages have been incorporated into popular molecular dynamics programs, allowing users to run elaborate and complex virtual reactions. MD has evolved to include the function of an "external force," which allows users to apply tension to a region of choice (5). This way, one could simulate the results of the aforementioned "optical tweezers" experiment without having to set up laser beams and reflecting mirrors.

Another salient example of MD in action is how it resolves the role of solvents in protein dynamics (9). MD is not restricted by the physical confines (i.e. temperature) that can be achieved in the lab, so it can be used to study the role of solvent quantitatively (1). It is generally known that enzymes function optimally within a particular range of temperatures (1, 2, 4). However, at temperatures below 220 K (Kelvin), or the



MD simulation allowed one group of researchers to produce the figure above by sampling thousands of conformations of a molecule, taking precise quantitative measurements for each conformation. The graph plots the free energy surface at 330K as a function of the conformation.

credits: "Molecular dynamics simulations of biomolecules." *Nature Structural Biology* 9.9 (2002): 650.

glass transition temperature (Tg), most enzymes cease to function altogether (1, 9). It was unclear whether the solvent's fluctuations, or lack thereof, were responsible for this phenomenon (1). However, this could not be determined using conventional methods because it was impossible to carry reactions at differentiating temperatures of 180K and 300 K (1). MD's virtual experiments solved this problem. Simulations were run setting an oxygen-binding protein and solutions at temperatures above and below Tg. Several simulations recorded the random motions in protein and solvent at either 300 K or 180 K (1). The results of this experiment answered the contentious question of the role of solvent in protein motion: the viscosity of the solvent was the principal factor in determining protein mobility at the temperatures tested, (180-300 K) (1). MD is thus a powerful tool that has been successful in exploring previously unexplained phenomena.

The Future of MD

The immediate goal for MD researchers is to expand its reaction substrates to include substrates at the cellular level (1). MD currently operates at the molecular level, modeling proteins and DNA on the scale of thousands of atoms. However, most biological processes operate on the cellular level as a concerted effort of many molecules. The eukaryotic DNA polymerase holoenzyme, for example, consists of several subunits as well as a regiment of auxiliary proteins such as the polymerase "clamp" and its "loader" (7). To understand the science of life, MD needs to be able to simulate large protein complexes.

The transition from proteins to cells will not be an easy one, although it is just beyond the horizon. The initial global simulation would start from a less detailed model, such as the recent example of using simplified calculations to interpret a low resolution electron microscope picture of the cowpea chlorotic mottle virus (1). While this approach merely focuses on a virus and not an actual cell, it would nonetheless imply future breakthroughs in MD.

An important application of whole-cell MD simulation would be in the field of neurobiology (8). For example, synaptic transmissions, fundamental to the workings of neurons, are ideal candidates (8). Because of MD's ability to incorporate external tension, it may simulate natural phenomena that exert force on the synapse, such as the case of mechanical stress in a contracting neuromuscular synapse and its channel openings (1, 8).

Molecular dynamics simulations have shed light on the internal motions of biological molecules since their creation in 1975 (1, 6). Today, MD plays an ever important role in previously unexplored areas. The future holds infinite possibilities for applications of this technology; perhaps, MD will be able to provide insights into the inner workings of cells and, ultimately, life itself. H

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References

1. Martin, K.,*et al*. "Molecular dynamics simulations of biomolecules." *Nature structural biology* 9.8 (2002): 646-652.
2. Brünger, A.T., *et al*. "Active site dynamics of ribonuclease." *Proc. Natl. Acad. Sci. USA* 82 (1985):8458–8462.
3. Andricioaei, I., *et al*. "Dependence of DNA polymerase replication rate on external forces: a model based on molecular dynamics simulations." *Biophysical Journal* 87 (2004): 1478-1497
4. Frauenfelder, H. *et al*. Thermal expansion of a protein. *Biochemistry* 26 (1985):254–261.
5. Goel, A., *et al*. "Tuning and switching a DNA polymerase motor with mechanical tension." *Proc. Natl. Acad. Sci. USA* 100.17 (2003):9699-704.
6. Brooks, M., *et al*. "Harmonic dynamics of proteins: normal modes and fluctuations in bovine pancreatic trypsin inhibitor." *Proc. Natl. Acad. Sci. USA* 80 (1983):6571–6575
7. Waton, J., *et al*. *Molecular Biology of the Gene* (Pearson Publishing, San Francisco; 2004).
8. Cowan, W.M., *et al*. *Synapses* (Johns Hopkins University Press, Baltimore; 2000).
9. Hagen SJ., et al. "Protein reaction kinetics in a room-temperature glass." *Science*. 18.269(5226):959-62.