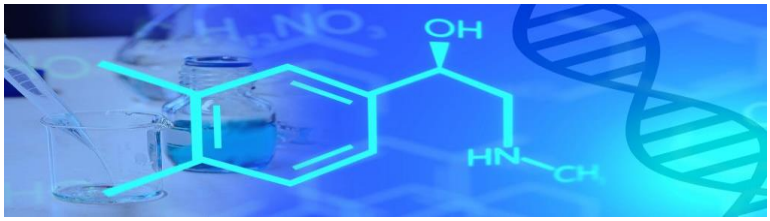
INTRODUCTORY COURSE "MD-SIMULATION RESEARCH

(FROM ATOMIC FRAGMENTS TO MOLECULAR COMPOUND)



Nada Atef Kamal Mohamed

Bachelor's degree of Science, Biochemistry and Chemistry department, Faculty of Science, Fayoum University, 2014.

Diploma in Medical Chemistry, Chemistry department, Faculty of Science, Fayoum University, 2016.

Master Grant, 2016, Academy of Scientific Research and Technology (ASRT),

, Scientists Next Generation Grant (SNG cycle 5).

Pre-master of Biotechnology and Life Sciences, department of Biotechnology, Faculty of Postgraduate Studies for Advanced Science, Beni-Suef University ,2017.

Supervised by

Prof. Dr. Kholmirzo Kholmurodov

Professor, Sci. Dr. Phys.-Math., FLNP JINR.

Dubna State University, Dubna, Moscow region, Russian.

24 May - 02 July, 2021.

Egypt

Contents

INTRODUCTION

1. Background

Molecular dynamics is a way of solving equations of motion numerically (hence you need a computer).Equations of motion are coupled differential equations and hence cannot be solved analytically. The trajectories of atoms and molecules are determined by numerically solving Newton's equations of motion for a system of interacting particles, where forces between the particles and their potential energies are often calculated using interatomic potentials or molecular mechanics force fields. The method is applied mostly in chemical physics, materials science, and biophysics**.[1, 2]** Molecular dynamics simulations are powerful tools for studying the physical basis of biological macromolecule structure and function. The traditional understanding of proteins as relatively inflexible structures has given way to a dynamic model in which internal movements and resultant conformational changes are critical to their function. This article provides a brief history of biomolecular simulations and its early applications. It then discusses several recent research that demonstrate the value of such simulations, before concluding with a discussion of their ever-increasing potential to contribute to biology.**[3]** Another essential feature of simulations is that, while the potentials used in simulations are approximate, they are entirely within the control of the user, allowing the user to investigate the significance of certain contributions in defining a given attribute by eliminating or changing them. This is best illustrated graphically by the use of 'computer alchemy' to transmute the potential from one system to another during a simulation in the computation of free energy differences**.[4]**

Basic equations, potentials and simulation techniques.

1.1Quantum mechanical MD: is basically the molecular orbital calculation and offers the most detailed description of a molecule's chemical behavior. The advantage of this method is that it breaks the many-electron Schrodinger equation into many simpler one-electron equations,and also Each one electron equation is solved to yield a single-electron wave function, called an orbital, and energy called orbital energy.

Quantum mechanics is based on Schrödinger equation

$$H\Psi = E\Psi = (U + K)\,\Psi$$

E = energy of the system relative to one in which all atomic particles are separated to infinite distances

H = Hamiltonian for the system.

It is an "operator", a mathematical construct that operates on the molecular orbital, Ψ, to determine the energy.

U = potential energy K = kinetic energy

Ψ = wave function describes the electron distribution around the

Molecule.

Schrödinger equation – linear, partial differential equation that describes how the quantum state of some physical system changes with time analog to Newton's 2nd Law. The "solutions" are functions which describe wave-like motion for a group of particles.[5]

$$E\Psi(\mathbf{r}) = \frac{-\hbar^2}{2m}\nabla^2\Psi(\mathbf{r}) + V(\mathbf{r})\Psi(\mathbf{r})$$

wavefunction :

probability (density) amplitude describing the quantum state of a particle (electrons, nuclei) and how it behaves as a function of space and time.

Semi-empirical models - simplest of the quantum chemical schemes use Hartree-Fock method, but make approximations and obtain some parameters from use empirical data (like pre-calculated orbitals). (PM3, MNDO, AM1, RM1, PM3, and PM6).

The Hamiltonian operator H is, in general.

$$\hat{H} = -\sum_{i}^{\text{particles}} \frac{\nabla_i^2}{2m_i} + \sum_{i<j}^{\text{particles}} \sum \frac{q_i q_j}{r_{ij}}$$

$$\nabla_i^2 = \frac{\partial^2}{\partial x_i^2} + \frac{\partial^2}{\partial y_i^2} + \frac{\partial^2}{\partial z_i^2}$$

Where Vi2 is the Laplacian operator acting on particle i. Particles are both electrons and nuclei. The symbols mi and qi are the mass and charge of particle I, and rij is the distance between particles

The first term gives the kinetic energy of the particle within a wave formulation.

The second term is the energy due to the Coulombic attraction or repulsion of particles.

$$\hat{H} = -\sum_{i}^{\text{electrons}} \frac{\nabla_i^2}{2} - \sum_{i}^{\text{nuclei}} \sum_{j}^{\text{electrons}} \frac{Z_i}{r_{ij}} + \sum_{i<j}^{\text{electrons}} \sum \frac{1}{r_{ij}}$$

Kinetic energy

Attraction of Electrons to nuclei

Repulsion Betweenelectrons

In currently available software, the Hamiltonian above is nearly never

used. The problem can be simplified by separating the nuclear and electron motions.

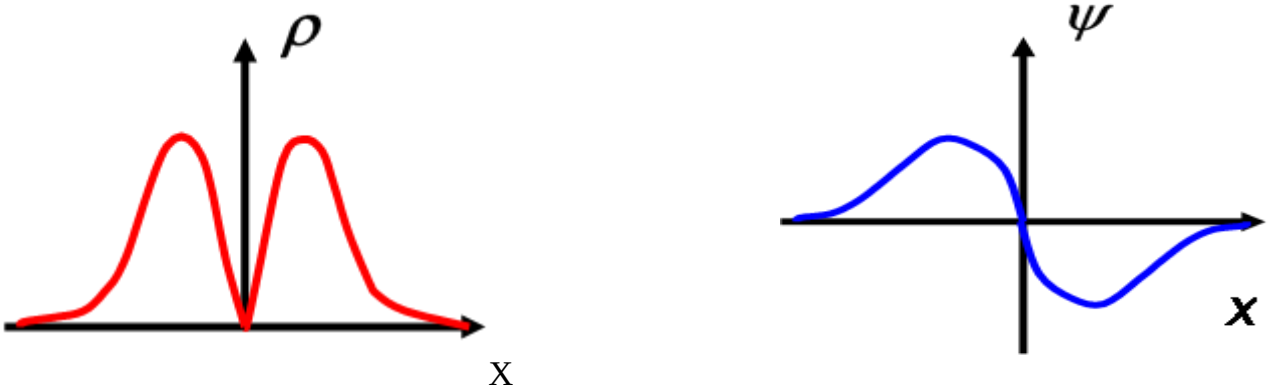
Thus, each electronic structure calculation is performed for a fixed nuclear configuration, and therefore the positions of all atoms must be specified in an input file.

1.2 Density functional theory (DFT):

The development of density functional theory has considerably improved the use of computational chemistry as a tool in the design and development of organic corrosion inhibitors (DFT). Historically, corrosion scientists have found novel corrosion inhibitor molecules by slowly altering the structures of existing inhibitors or by testing hundreds of compounds in the laboratory; however, these experimental methods are frequently prohibitively expensive and time-consuming. Thus, continuous hardware and software advancements have paved the way for the effective use of theoretical chemistry in corrosion inhibition research at a lower cost. Based on electronic/molecular characteristics and reactivity indices, DFT has enabled corrosion scientists to precisely estimate the inhibitory efficacies of organic corrosion inhibitors.**[6]**

The electron density is the square of wave function and integrated over electron coordinates. The complexity of a wave function increases as the number of electrons grows up, but the electron density still depends only on 3 coordinates.

With this theory, the properties of a many-electron system can be determined by using functional, i.e. functions of another function, which in this case is the spatially dependent electron density.



There are difficulties in using density functional theory to properly describe intermolecular interactions, especially van der Waals forces (dispersion); charge transfer excitations; transition states, global potential energy surfaces and some other strongly correlated systems.**[7]**

DFT was originated with a theorem by Hoenburg and Kohn. The original H-K theorems held only for non-degenerate ground states in the absence of a magnetic field.

The first H-K theorem demonstrates that the ground state properties of a many-electron system are uniquely determined by an electron density that depends on only 3 spatial coordinates.

It lays the groundwork for reducing the many-body problem of N electrons with 3N spatial coordinates to only 3 spatial coordinates, through the use of functional of the electron density.

This theorem can be extended to the time-dependent domain to develop time-dependent density functional theory (TDDFT), which can be used to describe excited states.

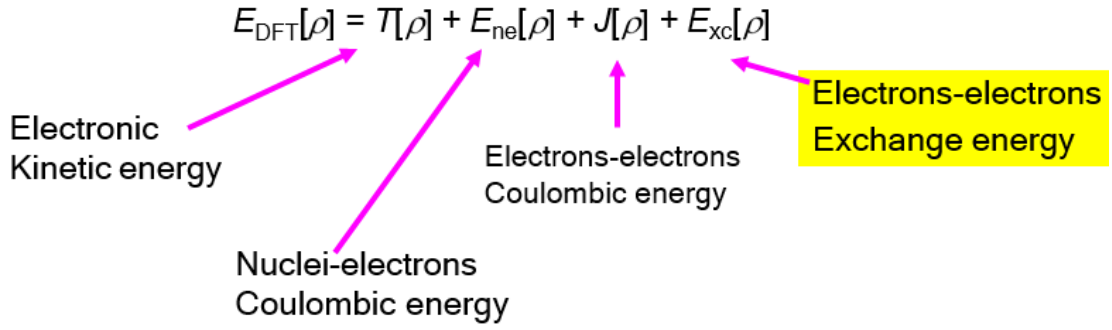
The second H-K theorem defines an energy functional for the system and proves that the correct ground state electron density minimizes this energy functional.**[8]**

1.3 kohn-Sham theory

Within the framework of Kohn-Sham DFT, the intractable many-body problem of interacting electrons in a static external potential is reduced to a tractable problem of non-interacting electrons moving in an effective potential.

The effective potential includes the external potential and the effects of the Coulomb interactions between the electrons**.[9]**

e.g. the exchange and correlation interactions.



- ❖ Modeling the latter two interactions becomes the difficulty within KS DFT.
- ❖ In this formulation, the electron density is expressed as a linear combination of basis functions similar in mathematical form to HF orbitals.
- ❖ Adeterminant is then formed from these functions, called Kohn±Sham orbitals
- ❖ It is the electron density from this determinant of orbitals that is used to compute the energy.

2. Molecular mechanics. (MM)

Molecular mechanics is a mathematical formalism which attempts to reproduce molecular geometries, energies and other features by adjusting bond lengths, bond angles and torsion angles to equilibrium values that are dependent on the hybridization of an atom and its bonding scheme. The Process of finding the minimum of an empirical potential energy function and produce a molecule of idealized geometry.**[10]**

• Molecular mechanics breaks down pair wise interaction into

√ Bonded interaction (internal coordination)

- Atoms that are connected via one to three bonds

√ Non bonded interaction.

-Electrostatic and Van der Waals component

❖ The general form of the force field equation is;

$$E_P(X) = E_{bonded} + E_{nonbonded}$$

2.1 Bonded interactions

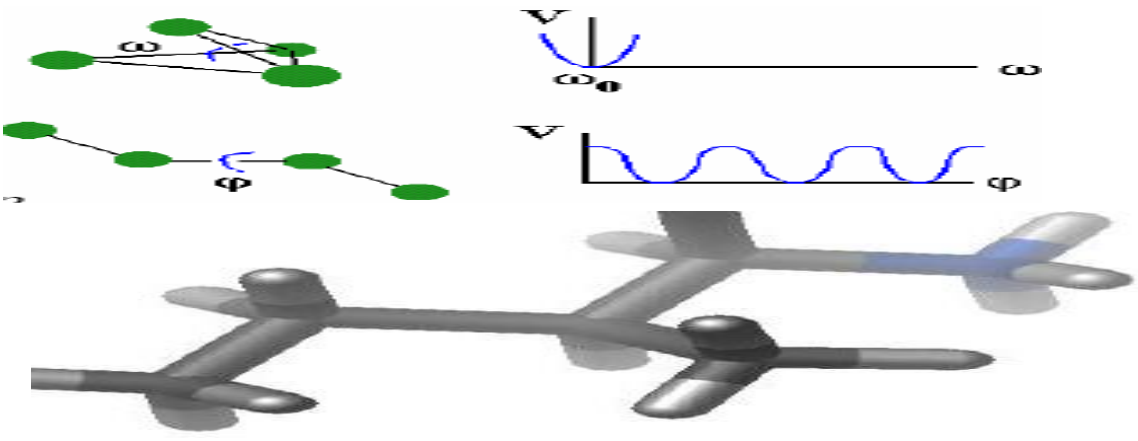
Used to better approximate the interaction of the adjacent atoms.Calculations in the molecular mechanics is similar to the Newtonians law of classical mechanics and it will calculate geometry as a function of steric energy.

<u>Hooke's law is applied here</u>

f = kx

f = force on the spring needed to stretch an ideal spring is proportional to its elongation x ,and where k is the force constant or spring constant of the spring.

$$E_{bonded} = E_{bond} + E_{angle} + E_{dihedral}$$



- Bond term

$$E_{bond} = \tfrac{1}{2}\, k_b\, (b - b_o)^2$$

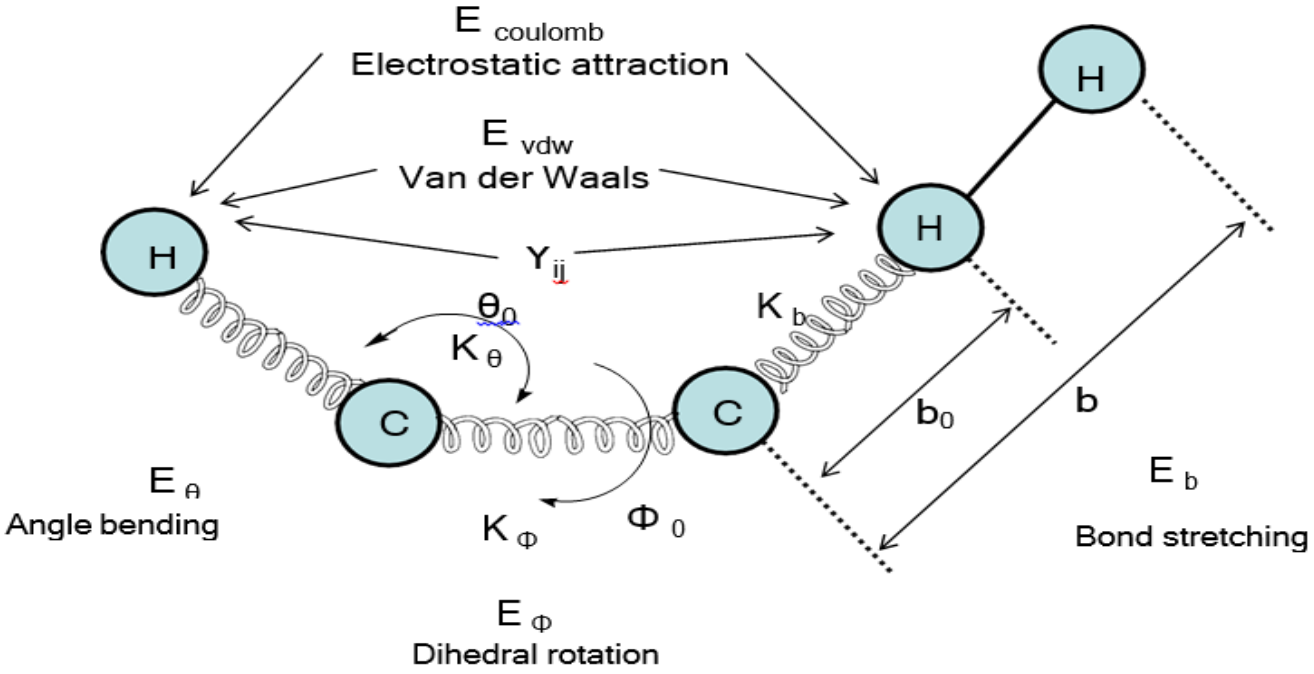
- Angle term

$$E_{Angle} = \tfrac{1}{2}\, k_\theta\, (\theta - \theta_0)$$

- Energy of the dihedral angles

$$E_{dihedral} = \tfrac{1}{2}\, k_\Phi (1 - \cos(n\Phi + \delta))$$

Graphical representation of the bonded and non bonded interaction and the corresponding energy terms.



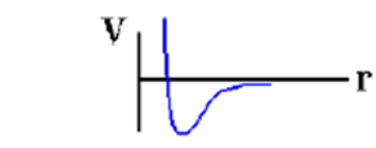
2.2 Non bonded interactions

The nonbonded interaction terms usually include electrostatic interactions and van der waals interaction , which are expressed as coloumbic interaction as well as Lennard-Jones type potentials, respectively,and nearly applied to all pairs of atoms.

• All of them are a function of the distance between atom pairs.

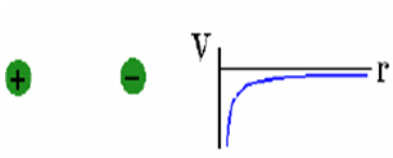
E Nonbonded = E van der waals + E electrostatic

$$E_{\text{van der waals}} = \sum_{atoms\_i,k} \left(\frac{A_{ik}}{r_{ik}^{12}} - \frac{C_{ik}}{r_{ik}^{6}} \right)$$



Lennard Jones potential

$$E_{\text{electrostatic}} = \sum_{atoms\_i,k} \frac{q_i q_k}{D r_{ik}}$$

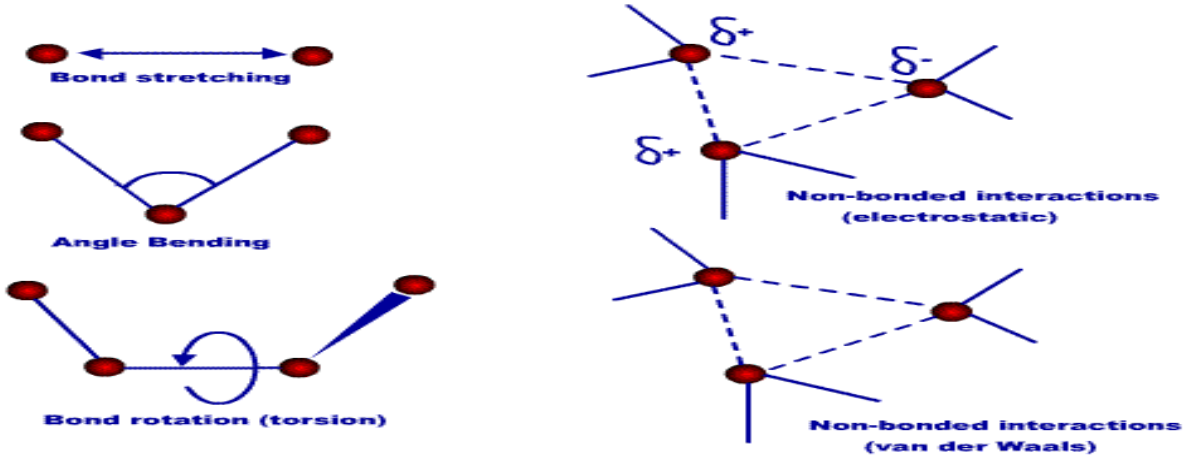


Coulomb's Law

3. Force field parameters for molecular dynamics simulations.

For a given time step, classical molecular dynamics (MD) simulations integrate Newton's equations of motion over a molecule. On biological (s) timescales, this technique has been utilized to examine condensed phase biomolecular systems such as proteins, nucleic acids, carbohydrates, and lipids. The accuracy of the underlying parameters, generally referred to as a force field, is critical to the success of classical MD simulations. Such equations describes the various aspects of the equation like stretching, bending, torsions, electronic interactions van der Waals forces and hydrogen bonding.**[11]** Some force fields simplify the complexity of the

calculations by omitting most of the hydrogen atoms. The parameters describing the each backbone atom are then modified to describe the behavior of the atoms with the attached hydrogens. Thus the calculations uses a CH_2 group rather than a *Sp3* carbon bonded to two hydrogens. These are called united atom force field or intrinsic hydrogen methods.**[12]**



This figure is a schematic representation of the four key contributions to a molecular mechanics force field: bond stretching, angle bending, torsional terms and non-bonded interactions.

3.1 Some popular force fields are:

[AMBER, CHARMM, DL POLY]

A- AMBER

The name of both a Assisted model building with energy refinementis force field and a molecular mechanics program It was parameterized specifically for the protein and nucleic acids It uses only five bonding and nonbonding terms and no any cross term. This equation calculates energy as the sum of a harmonic potential for bonds and angles, a truncated Fourier series for dihedrals, and Lennard-Jones and pairwise electrostatic potential function for nonbonded forces, with the prime on the nonbonded term sum indicating that the calculation is only performed for atoms in different molecules or separated by at least three bonds.Partial derivatives of this equation with respect to atom position in the x, y, and z directions provide the forces from which to propagate Newton's equations of motion.

B- CHARMM

Chemistry at Harvard macromolecular mechanics is the name of both a force field and program incorporating the force field.It was originally devised for the proteins and nucleic acids. But now it is applied to the range of the bimolecules , molecular dynamics, solvation , crystal packing , vibrational analysis and QM/MM studies.

It uses the five valance terms and one of them is an electrostatic term.

C- DL- POLY

DL_POLY is a general-purpose molecular dynamics simulation package, which was developed by Daresbury Laboratory in the mid-1990s for the molecular simulation community in the United Kingdom. The package now has a world-wide user base and applications in many areas of molecular simulation. In this article we briefly review the history and design of the package and highlight some recent applications in the areas of; liquids and solutions; spectroscopy; ionic solids; molecular crystals; polymers; glasses; membranes; proteins; solid and liquid interfaces; catalysis; liquid crystals; intercalation and clathrates; and novel systems.

4. Molecular mechanics energy minimization.

Energy minimization is an important step in molecular modeling of proteins. Molecular mechanics energy minimization means to finds stable, low energy conformations by changing the geometry of a structure or identifying a point in the configuration space at which the net force on atom vanishes .In other words, it is to find the coordinates where the first derivative of the potential energy function equals zero. Such a conformation represents one of the many different conformations that a molecule might assume at a temperature of 0 k0.The potential energy function is evaluated by a certain algorithm or minimizer that moves the atoms in the molecule to a nearest local minimum.**[13]**Geometry optimization is an iterative procedure of computing the energy of a structure and then making incremental increase changes to reduce the energy.

4.1Minimization involves two steps

A- An equation describes the energy of the system as a function of its coordinates must be defined and evaluated for a given conformation.

B- The conformation is adjusted to lower the value of the potential function.

4.2 Internal coordinates

In internal coordinates presentation, the potential energy surface looks like a valley surrounded by high mountains.

• The high peaks corresponds to stretching and bending terms and close Vander Waals contacts while the bottom of the valley represents the torsional degree of freedom.

• Using the internal coordinates there is a clear separation of variables into the hard ones (those whose small changes produces large changes in the function values) and soft ones (those whose changes do not affect the function value substantially).

• During the function optimization in the internal coordinates, the minimizer first minimizes the hard variables and in the subsequent iterations cleans up the details by optimizing the soft variables.

• While in the Cartesian spaces all variables are of the same type.

5. Molecular dynamics and Simulations.

Actually time evaluation of the molecular system and the information generated from simulation methods can be used to fully characterize the thermodynamic state of the system. Here the molecular system is studied as the series of the snapshots taken at the close time intervals. (Femtoseconds usually).

Based on the potential energy function we can find components F_i of the force F acting on atom as

$$F_i = - dV / dx_i$$

This force in an acceleration according to Newton's equation of motion.

$$F = m a$$

By knowing the acceleration we can calculate the velocity of an atom in the next time step. From atom position , velocities and acceleration at any moment in time, we can calculate atom positions and velocities at the next time step.And so integrating these infiniteimal steps yields the trajectories of the system for any desired time range.

Molecular dynamics for larger molecules or systems in which solvent molecules are explicitly taken into account is a computationally intensive task even for supercomputers.

For such a conditions we have two approximations.

- Periodic boundary conditions.
- Stochastic boundary conditions.

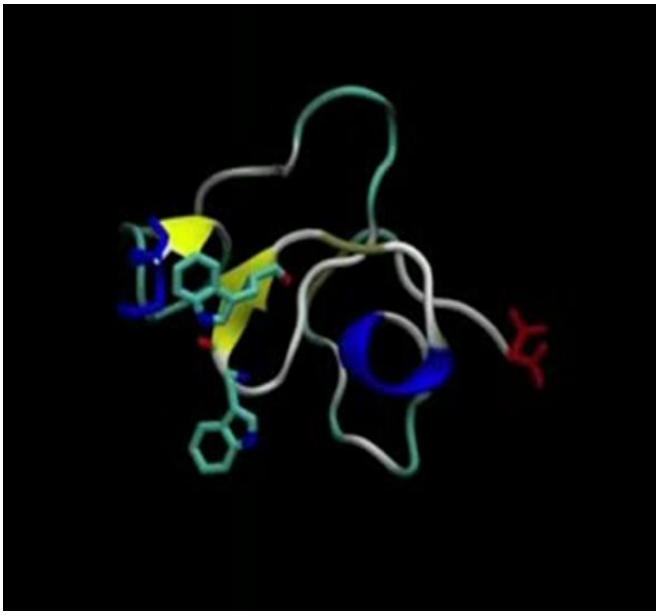
5.1 Molecular dynamics on proteins

Although normally represented as static structures, proteins are in fact dynamic.Most experimental properties, for example, measure a time average or an ensemble average over the range of possible configurations the molecule can adopt.One way to investigate the range of accessible configurations is to simulate the motions or dynamics of a molecule numerically. This can be done

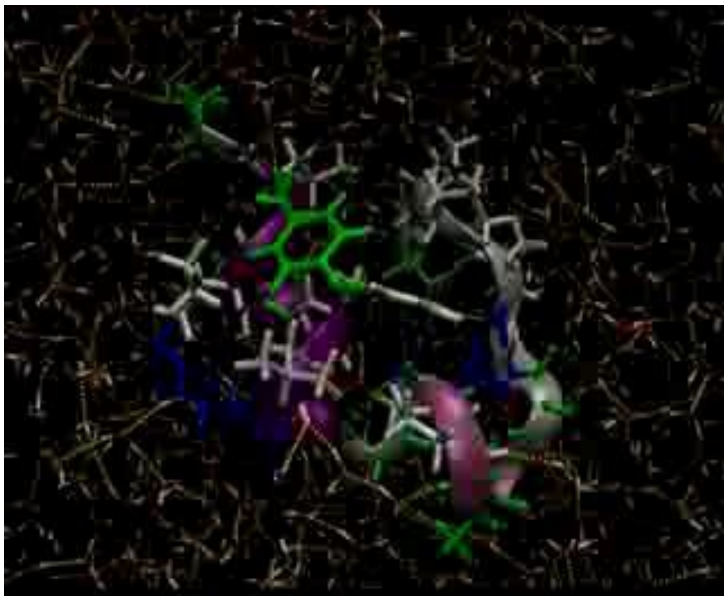
by computing a trajectory, a series of molecular configurations as a function of time, by the simultaneous integration of Newton's equations of motion.

So exactly the Molecular Dynamics is the process of giving the movements to proteins internally which is produced by increasing the temperature of the system and cooling them rapidly in a very short time scale.During these conditions the steric interactions or the imperfect bonds between the amino acid residues and the peptides are removed or modified.It generates the most stable and the energy minimized conformations of the protein.While doing so it computes many different frames or trajectories of the same protein.

MD of protein in vacuum



MD of protein in Water



5.2 Molecular Dynamics of SUMO proteins

Small Ubiquitin-like Modifier or SUMO proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function.The function performed by SUMO proteins is known as SUMOylation.post-trnalational modification involved in various cellular processes such as transcriptional regulation, apoptosis, protein stability etc.Similar to ubiquitin and SUMOylation is directed by an enzymatic cascade analogous to that involved in ubiquitination. In contrast to ubiquitin, SUMO is not used to tag proteins for degradation.**[14]**

A-Structure schematic of human SUMO protein.

NMR structure of SUMO: the backbone of the protein is represented as a ribbon, highlighting secondary structure; N- terminus in blue, C-terminus in red.

5.3 Function of SUMO

> SUMO modification of proteins has many functions. Among the most frequent and best studied are protein stability, nuclear- cytosolic transport, and transcriptional regulation.

> Typically, only a small fraction of a given protein is SUMOylated and this modification is rapidly reversed by the action of deSUMOylating enzymes.

> The SUMO-1 modification of RanGAP1 (the first identified SUMO substrate) leads to its trafficking from cytosol to nuclear pore complex.The SUMO modification of protein leads to its movement from the centrosome to the nucleus .

6. Giving Dynamics to the protein

Step 1 : generation of structures.

Step 2: performing molecular dynamics on each of the topologies.

Step 3: Recording the potential energy changes in protein during Dynamics.

Step 4: Clustering of the best minimized structures.

Programs/software's used:

1-Cyana

2-NAMD/VMD

3-VEGA ZZ

4-GROMACS

(Hands-on exercise)

6.1 MD test modeling.

Through this course, I attempted to create a small protein simulation Practical application of lopinavir and its efficacy against model newlyEmerged Coronavirus Atomistic Insights into the Inhibitory Mechanisms through COVID-19 Main Protease (Mpro).

Summary

Lopinavir is an HIV-1 protease inhibitor used in to treat human immunodeficiency virus (HIV) infection. Lopinavir is currently under investigation in combination with ritonavir for the treatment of COVID-19 caused by SARS-CoV-2.

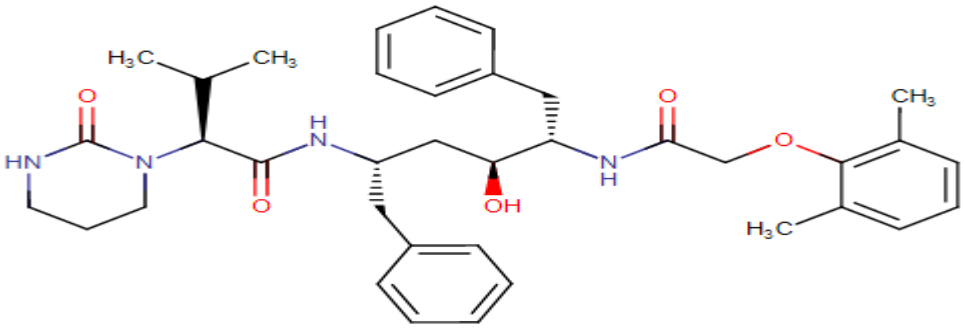
Background

Initially, the SARS-CoV-2 virus emerged from Wuhan, China and rapidly spreading across the world and urges the scientific community to develop antiviral therapeutic agents. Among several strategies, drug repurposing will help to react immediately to overcome the COVID-19 pandemic. I have chosen Lopinavir is an HIV-1 protease inhibitor, used as the inhibitors of SARS-CoV-2 main protease (Mpro) enzyme. I have a target to use inhibitors as the repurposed drugs for COVID-19, it is essential to know the molecular basis of the binding mechanism of these molecules with the SARS-CoV-2 Mpro. To understand the binding mechanism, I have performed molecular dynamics (MD) simulations. Lopinavir is an antiretroviral protease inhibitor used in combination with other antiretrovirals in the treatment of HIV-1 infection. Lopinavir is marketed and administered exclusively in combination with ritonavir - this combination, first marketed by Abbott under the brand name Kaletra in 2000, is necessary due to lopinavir's poor oral bioavailability and extensive biotransformation. Ritonavir is a potent inhibitor of the enzymes responsible for lopinavir metabolism, and its co-administration "boosts" lopinavir exposure and improves antiviral activity. Like many other protease inhibitors (e.g. saquinavir, nelfinavir), lopinavir is a peptidomimetic molecule - it contains a hydroxyethylene scaffold that mimics the peptide linkage typically targeted by the HIV-1 protease enzyme but which itself cannot be cleaved, thus preventing the activity of the HIV-1 protease**.[15]**

Type and Chemical Formula

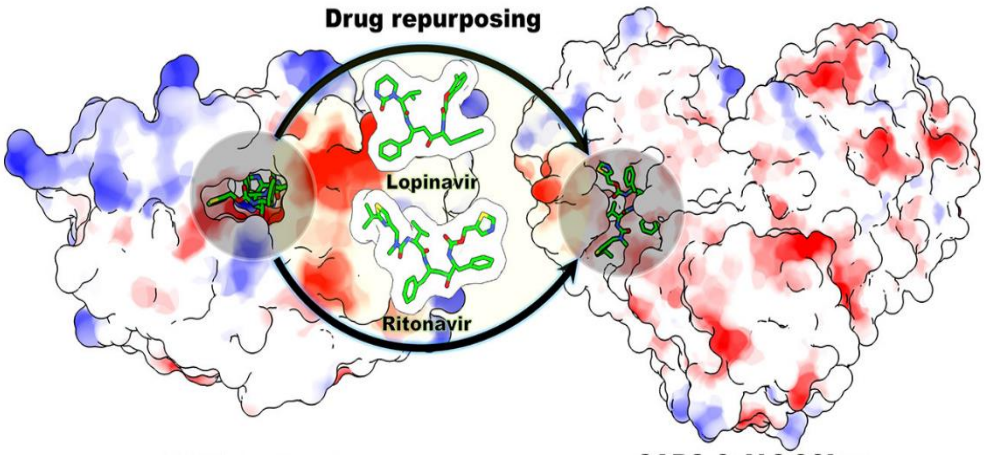
Small Molecule , C37H48N4O5

Structure of Lopinavir



Absorption

When administered alone, lopinavir has exceptionally low oral bioavailability (~25%) - for this reason, it is exclusively co-administered with ritonavir, which dramatically improves bioavailability, hinders drug metabolism, and allows for the attainment of therapeutic lopinavir concentrations. Following oral administration of lopinavir/ritonavir, maximal plasma concentrations are achieved at approximately 4.4 hours (T_{max}), and the C_{max} and AUC_{tau} are 9.8 ± 3.7 - 11.8 ± 3.7 µg/mL and 92.6 ± 36.7 - 154.1 ± 61.4 µg•h/mL, respectively.



Materials and methods

Goal: Run a short MD simulation of lopinavir and its efficacy against COVID-19 Main Protease (Mpro) using Amber Force field.

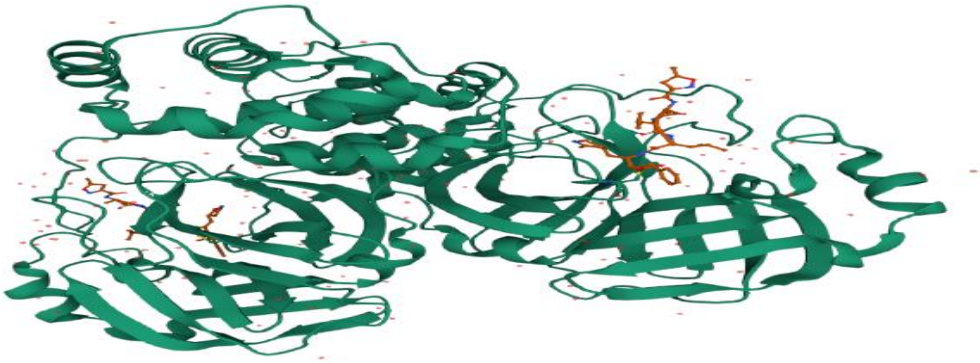
1-Examine the protein (6LU7)

2- input files from protein data bank (PDB)

3-Examine and modify a configuration file (.conf)

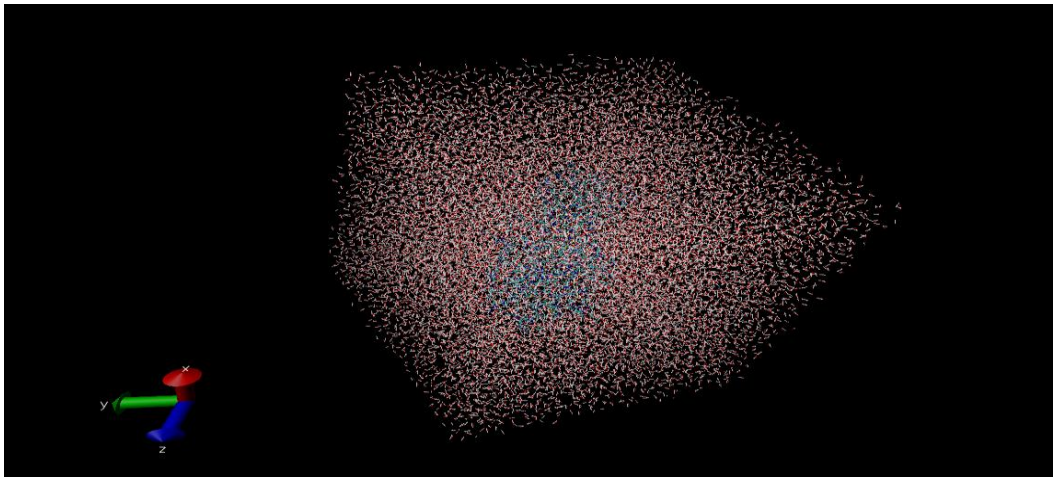
4-View trajectory (VMD)

5-Analyze run (VMD)



Ligand preparation: The 3D structure of the reference molecule, 6LU7 co-crystallized with Mpro was retrieved from the respective which was protein from Protein Data Bank. The 3D structure of each ligand (lichen online resources and compound compounds) was obtained from various databases. Polar hydrogen charges were assigned and the nonpolar hydrogens were merged by using Autodock tools.

Molecular dynamic simulation For predicting the stability of Mpro and Mpro-ligand complex, molecular dynamics simulations (MDS) were performed in a VMD package for visulization. After that ligand topologies were rejoined to the processed protein structure for building the complex system. After that, a water solvated system was built with dodecahedral periodic boundary conditions. The solutes are centered in the simulation box with a minimum distance to the box edge of 10 Å (1.0 nm)



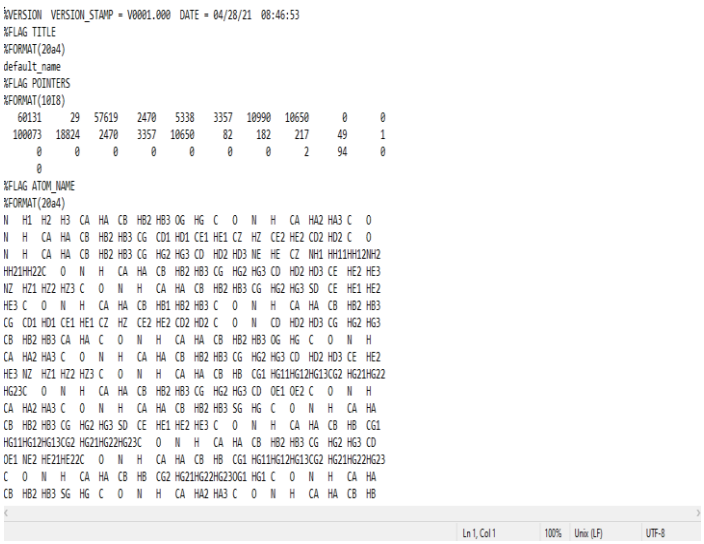
Loading AMBER rst7 and restrt files

When loading AMBER prmtop files, a new format prmtop file created with AMBER7. Select "**AMBER7 Parm**" as the type and hit **Load**.

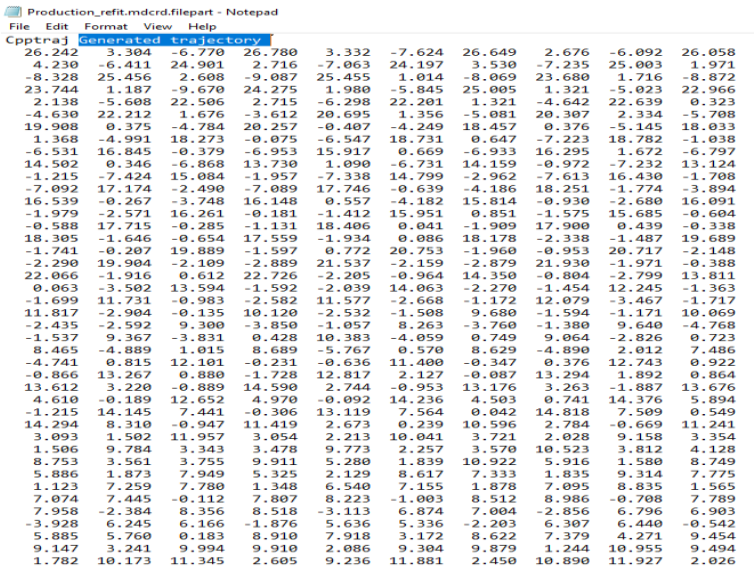
Hit **Browse** again and find the **COMPLEX.promtop**file. Select "**AMBER7 Restart**" as the type and hit **Load**. I have two molecules displayed in the "**OpenGL**" window. They will be very similar. In order to see the difference between them lets color one of them in blue.

By Going to the "**Graphical Representation**" window and under "**Selected Molecule**" select the first **COMPLEX.promtop** of the two in the list. This should be initial (minimized) structure. Then under Coloring Method choose "**ColorID**" and pick 0 in the box that appear to the right of it. And see one of the molecules in the "**OpenGL**" window. I can now zoom in on this and look at the difference between the two structures.

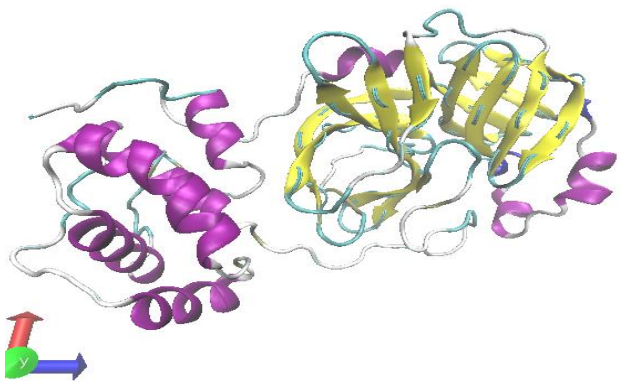
COMPLEX.promtop

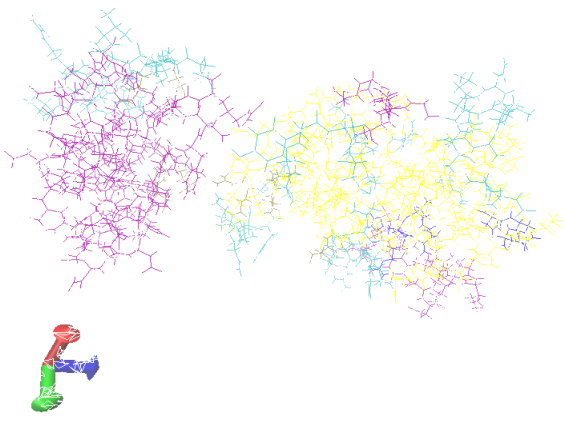


Production-refit. Generated trajectory



Graphical Representation and Visualization by VMD





7. Conclusion

Molecular dynamics and molecular mechanics are often used together to achieve the target conformer with the lowest energy configuration. The 3D shape of a molecule and Carry out a complete analysis of all possible conformations and their relative energies. Obtain a detailed electronic structure and the polarizability with taking account of molecules. The binding energy for docking a small molecule. I.e. a drug candidate, with a receptor or enzyme target. Nevertheless, molecular modeling, if used with caution, can provide very useful information to the chemist and biologist involved in medicinal research.

8. Acknowledgment

All thanks, appreciation, and respect to the great Professor Dr.Kholmirzo Kholmurodov ,For the information, he gave us during the introductory program and his permanent assistance by sending various scientific sources and his keenness to always achieve the maximum benefit during this wonderful period.

All thanks to The Joint Institute for Nuclear Research for the constant support and the various effective programs and exercises that I benefit greatly from.

9. Future Work

When I will finish discussing my master's thesis during this period in Biotechnology, Medical Sciences and Molecular Biology. I will work on looking for an opportunity to get a PhD from outside Egypt. So I am trying to find new topics to publish scientifically during the coming period so that I get a suitable opportunity. And the point that I will look for during my PhD is to work on finding a project or external scholarship on molecular dynamics, molecular modeling and simulation because this is the main goal that I will work on and find solutions through this science to model and simulate different proteins from modern diseases.

Reference

1. Hansson, T., C. Oostenbrink, and W. van Gunsteren, *Molecular dynamics simulations.* Current opinion in structural biology, 2002. **12**(2): p. 190-196.
.2.Kholmurodov, K.T., *Computer design for new drugs and materials: Molecular dynamics of nanoscale phenomena*, in *Computer Design for New Drugs and Materials: Molecular Dynamics of Nanoscale Phenomena*. 2017. p. 1-231.
3..Karplus, M. and J.A. McCammon, *Molecular dynamics simulations of biomolecules.* Nature structural biology, 2002. **9**(9): p. 646-652.
.4.Andersen, H.C., *Molecular dynamics simulations at constant pressure and/or temperature.* The Journal of chemical physics, 1980. **72**(4): p. 2384-2393.
.5.Tezcan, C. and R. Sever, *A general approach for the exact solution of the Schrödinger equation.* International Journal of Theoretical Physics, 2009. **48**(2): p. 337-350.
6..Obot, I., D. Macdonald, and Z. Gasem, *Density functional theory (DFT) as a powerful tool for designing new organic corrosion inhibitors. Part 1: an overview.* Corrosion Science, 2015. **99**: p. 1-30.
.7.Bagayoko, D., *Understanding density functional theory (DFT) and completing it in practice.* AIP Advances, 2014. **4**(12): p. 127104.
8..Fiolhais, C., F. Nogueira, and M.A. Marques, *A primer in density functional theory*. Vol. 620. 2003: Springer Science & Business Media.
9..Burke, K., *Perspective on density functional theory.* The Journal of chemical physics, 2012. **136**(15): p. 150901.
10..Rappé, A.K. and C.J. Casewit, *Molecular mechanics across chemistry*. 1997: University Science Books.
.11.Betz, R.M. and R.C. Walker, *Paramfit: Automated optimization of force field parameters for molecular dynamics simulations.* Journal of computational chemistry, 2015. **36**(2): p. 79-87.
12..Rappé, A.K., et al., *UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations.* Journal of the American chemical society, 1992. **114**(25): p. 10024-10035.
13..Zimmermann, K., *Oral: All purpose molecular mechanics simulator and energy minimizer.* Journal of computational chemistry, 1991. **12**(3): p. 310-31.9
.14.Kötter, A., H.D. Mootz, and A. Heuer, *Standard Binding Free Energy of a SIM–SUMO Complex.* Journal of chemical theory and computation, 2019. **15**(11): p. 6403-6410.
15..Khaerunnisa, S., et al., *Potential inhibitor of COVID-19 main protease (Mpro) from several medicinal plant compounds by molecular docking study.* 2020.