AI in Drug Discovery and Development

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Welcome to the course "AI in Drug Discovery and Development." In today's session, we will talk about molecular dynamics simulations. So, by the end of this lecture, you will be able to understand the fundamentals and principles of molecular dynamics simulation, explore force fields, non-bonded interactions, and thermodynamic ensembles. As well as learning AI-driven advancements in MD for improved accuracy and efficiency, and discovering emerging trends and applications of molecular dynamic simulation in drug discovery and development. So, let us dive into what molecular dynamic simulation is. So, you can think about anything in this world that is still.

So, everything in this world is in motion; actually, everything is moving, and it has been rightly said by Professor Richard Feynman, who was a great physicist and teacher. So, it is said that everything that living things do can be understood in terms of the jiggling and wiggling of atoms. So, if we can understand the motions of these molecules and how they are, you know, interacting with each other and how they are moving. So, we can understand a lot about not only the biology but all those physical phenomena as well, and that is the purpose of, you know, using molecular dynamics simulation.

So, it is a computational method used to study the physical movements of atoms and molecules over time. So, it means that we see what happens to the molecules over time, actually. For example, at time t, what are the configurations or confirmations of those molecules, and then at time t+1, what are the confirmations of those molecules that we see? So, it is based on the principle of Newtonian mechanics, where we treat the atoms as classical particles and their trajectories are determined by using Newton's equations of motion. And regarding the dynamics, we see the displacement from the average structure. For example, we can see how the local side chain motion acts as conformational gates in oxygen transport, like myoglobin.

Or we can see how we can understand the functions of enzymes, like how they do the catalysis. Or we can understand how the ion channels are working, where the ions are being transported from one side of the cell membrane to the other side. And then we can understand the thermodynamics as well, where we can, you know, use the equilibrium behavior. We can determine various thermodynamic properties, such as the energy of ligand binding or the energy of solvation. So, all those thermodynamic properties can be

calculated using molecular dynamic simulation as well.

So, let us say that in this picture, what you see here is a small molecule bound to a ligand that is in solution, and those small dots you see are the ions, actually. But this single snapshot or this single picture is just showing you one moment of its, you know, motion; actually, in reality, it is happening like this. Where both the protein and ligands are mobile, they are moving, and the ligand comes and binds to the binding pocket and then goes away, so binding and unbinding are happening. And also, the protein is moving through its side chains as well as the backbone. So, the purpose of molecular dynamics simulation is to model this kind of motion of the molecules, where we can understand their dynamics and how they move over time.

So what we do is use, as I said, Newtonian mechanics to calculate the net force and acceleration experienced by each atom. Where each atom I is treated as a point with mass m and fixed charge qi, for example, we have here these.

$$\vec{F}_i = m_i a_i = -\frac{\mathrm{d}V}{\mathrm{d}r_i}$$

For example, these five atoms, which are in a system, all have their unique position and velocity. And all of these atoms are, you know, exerting force on the other atoms as well. For example, here you can see in this picture that atom 1 is exerting force on atom 2, atom 5, and atom 3, and then atom 5 is exerting force on, you know, atom 1 and other atoms as well.

So, if we can, we use Newton's second law of motion, F is equal to ma, where we know how much force they are exerting on each other. And, because we already know the mass of those atoms, we can calculate the acceleration. And, if we can calculate the acceleration, then we can calculate their position after a fixed time frame. So, for example, after 2 femtoseconds or after 2 nanoseconds, what will be the position of those atoms? We can calculate that by using Newtonian mechanics or Newton's second law of motion. So, we do that to calculate the position at time Tn plus Tn, Tn plus 1 actually, and the forces.

So, as I said, we already know the mass, and if we can determine the force, we derive it from the interatomic potential functions. And these are just the analytical approximations, which are called, as you know, force fields. So, what do we do with the molecular dynamic simulation, knowing that the macroscopic properties are often determined by the molecular-level behavior? So, for example, you can see here that intermolecular potentials can be used for the microscopic properties. Molecule-level behavior can be used to determine the phases, such as the conversion of solid to liquid or liquid to gas. So, that can

be understood if we can understand the molecular level behavior by using molecular dynamic simulation.

We can understand the structure as well as, you know, the folding of proteins or peptides into 3D structures that can be understood. And then we can also see the domain motion, actually, like how the transport of ions or the binding and unbinding of the substrates or the product from the enzyme binding site is happening. So, what we actually do is make a model because now we are simulating it. So, we are making a model, and our objective shall be to create a model that completely replicates the actual system. So, we make a model, and then we try to, you know, take help from the real system, and then we can calculate the experimental properties as well.

And then when we make a model, we do the theoretical predictions and get the simulation results. and then we compare the simulation results with the experimental results to gain confidence in our modeling of the system. So, why do we need MD simulations? So, the first thing is that we cannot see atoms; although with the help of, you know, some microscopy techniques like cryo-electron microscopy, we can see the atoms. However, we cannot see the motion of the atoms; actually, we cannot see the dynamics of how they move with time. With that, we cannot see, and generally, what we know about the biomolecules is based on the models that represent them.

We cannot determine all the structures by using biophysical techniques, such as X-ray crystallography, which we cannot use because of some problems with crystallization. Many of the proteins, especially those membrane proteins, do not crystallize very easily. We cannot use NMR spectroscopy for determining the structures of some proteins because of oversolubility. Or we cannot even use cryo-EM for some of them because of the low contrast. And as we cannot measure the dynamics of the system at an atomic level, that is why we need MD simulation.

So that we can make a realistic model of the system, simulate it, and try to understand what happens to that system over time, we can further calculate those microscopic properties. So, one of the examples is, for example, here we have water. So, if I wanted to model this water in a molecular dynamics simulation. So, it will look like this, actually, where we have all those water molecules simulated in a small simulation box. So, let us understand some of the governing principles of MD simulations.

So, the first thing is the force field parameters, and then we will understand how to treat the non-bonded interactions. We will understand the boundary conditions, integrating the time steps, treatment of temperature and pressure, environment, solvation effects, science, etcetera, starting configuration, and the choice of degree of freedom. So, let us see what

you know about force fields and the interaction. So, as I said, if I want to simulate these water molecules in this flask. So, what will happen is I have to make a system that closely replicates reality, okay.

So, I will make a small box, for example, and then I will put all those water molecules in it. So, now as we know that mass is what we already know. So, we actually need to calculate the force. So, the force field comes into the picture, which is a collection of equations and associated constants designed to reproduce molecular geometry and selected properties of the tested structures. So, a single atom will be affected by the potential energy functions of every atom in the system.

So, what this water system means is that we will have one water molecule that will be affected by the bonded neighbors. And then the non-bonded atoms will come either from the same molecule or from different molecules. So, the total potential energy function will be equal to the bonded contribution and the non-bonded contribution.

$$V(R) = E_{bonded} + E_{non-bonded}$$

So, let us see what those bonded and non-bonded contributions are. So, the non-bonded contributions are two potential functions that we need to be concerned about between the non-bonded atoms: the first one is the van der Waals potential, and the other one is the electrostatic potential.

So, when we talk about the Enon-bonded. So, we came to know that we can explain it using the van der Waals and electrostatic potential.

$$E_{non-bonded} = E_{van-der-Waals} + E_{electrostatic}$$

And the van der Waals potential says that the atoms that have no electrostatic charge will still tend to attract each other at short distances as long as they do not get too close. There are if we have two atoms that are coming close to each other. So, until unless they intermediate between their van der Waal radii.

So, they will attract, and then if they are touching their van der Waals radii, they will start repelling each other. So, this is what the van der Waals potential says, and then that repulsion is coming from, you know, the overlapping electron clouds. So, then they will start repelling each other with astounding force. So, Lennard-Jones potential is one of the

simplest models that approximate the interaction between a pair of neutral atoms or molecules. You can see here if these two atoms are coming close to each other.

So, if they are at a distance, then there is no, you know, energy associated with them, but if they start coming close to each other, then there will be attraction as soon as they come close to the Van der Waals radii. So, they will start to repel each other. So, this function is the determined potential energy function, which is determined by using this equation.

$$E_{electrostatic} = \sum_{\substack{nonbonded \\ pairs}} \frac{q_i q_k}{Dr_{ik}} \qquad F = \frac{q_1 q_2}{4\pi\varepsilon_0 r^2}$$

Coulomb's Law

So, this is what contributes to the van der Waals interaction, and then another non-bonded interaction is electrostatic. So, this can be explained by using Coulomb's law, which says that the force of attraction is inversely proportional to the square of the distance.

So, we calculate the electrostatic potential by using this equation, and we know that opposite charges attract each other while like charges repel each other. So, coming to the combination of those Lennard-Jones and electrostatic potentials. So, we now know that the non-bonded potential will be equal to the van der Waals contribution from the van der Waals interactions and from the electrostatic interaction. Okay, and the other part is the bonded interactions. There are three types of bonded interactions.

So, these bonded interactions will be between the atoms that are connected through a bond, actually. So, you can see here that this oxygen atom is connected to this hydrogen atom. Now we have this OH bond. So, what kinds of energies can be associated with this? So, there will be stretching along the bond. So, these two atoms will, you know, move away from each other.

So, this bond will get stretched out. So, this energy associated with the stretching along the bond will contribute to the bonded interactions. So, Estretching and then Ebending. So, this is, you know, symmetric stretching, where both of these hydrogen atoms are moving away from this oxygen atom at the same time. However, this is asymmetric stretching where one of the hydrogen atoms is going away while another hydrogen atom is coming close to the oxygen atom.

And then another energy contribution is the E bending, where these hydrogen atoms will come close to each other or move away from each other. and the energy associated with

this one will be again contributing to the bonded interactions. And then the third one is the dihedral, where it is, you know, bending around a bond, actually. So, as you can see, for example, here we have another atom, and then rotation around the bond will lead to the dihedral contribution. So, together they all three contribute to the bonded interactions.

$$E_{bonded} = E_{stretch} + E_{bend} + E_{dihedral}$$

So, now combining all of these energy features or energy contributions that represent the different types of interactions between atoms of the protein molecule. So, these were like van der Waals energy, electrostatic energy, hydrogen bond energy, bond energy, bond angle energy, and dihedral angle energy, okay. So, the E total is bonded plus non-bonded, and then E bonded was, you know, E stretching, bending, and dihedral, and then E non-bonded was the van der Waals and electrostatic. So, that is how we determine the force that is used to calculate the acceleration. Now, why do we need MD simulations, or if we look at what we can do with MD simulations, if we compare them with, you know, physiological phenomena? For example, this is a time scale of some of the fundamental atomic or molecular scale motions where you can see that the times start from maybe femtoseconds to minutes, actually.

So, what we see here is that the absorption of light happens in less than femtoseconds, the electron transfer happens within femtoseconds, and the proton transfer happens within picoseconds. Side chain flips of, you know, molecules, at least the proteins, happen in nanoseconds, and then we have, you know, the solute permeation, like the transport of ions or, you know, other molecules. So, that happens in microseconds, and also in the microsecond time scale we have the secondary structure formation, channel gating, and domain motion; all these happen at the microsecond level. And, the active transport and action potential happen in the range of milliseconds, while protein translation occurs in the range of seconds. So, if we look at these physiological scale motions, or you can say the time taken by these processes.

So, what we can do is use, for example, molecular dynamics simulation to study the phenomena that occur from femtoseconds to milliseconds. Now, even we can go up to, you know, seconds as well with the advancements in, you know, all those GPUs and computational infrastructure; we can go up to even seconds as well. And if we use coarse-grain simulation or other advanced methods, we can go up to, you know, even tens of seconds as well. So, if we compare it with the other methods like IR and Raman spectroscopy. So, they can understand the phenomena, they can be used to understand the phenomenon that occurs between picoseconds to, you know, seconds.

And then we have NMR, the same as IR and Raman; we can use FRET, which can be used for understanding the phenomena that happen from microseconds to seconds. And we have the AFM, atomic force microscopy, or optical tweezers, which can be used to understand the phenomenon from milliseconds to seconds. And we have electrophysiology as well, which is usually used to understand the, you know, transport of ions or molecules across the membranes. So, that can be used to study the phenomena that are occurring from microseconds to seconds. So, now, if we wanted to understand, for example, secondary structure formation in any protein, channel gating, or domain motion.

So, we need to understand that we need to run the system for at least, you know, in this range of time. So, if our objective is to understand the motion of, you know, secondary structure or the domain motion, and if we are running our simulation for up to nanoseconds only, then that simulation will not be able to give us, you know, the information that we wanted to have. So, now we have seen that we got the force from the force field, we already have the mass, and now we can calculate the acceleration of those atoms in a system, and that is the purpose of, you know, molecular dynamic simulation. So, now we need to choose a simulation time step. For example, how fast I need to calculate those time points and to calculate all those properties at that time point.

Because what we are doing is solving Newton's second law of motion at each time point. So, for example, we started at time t0 and then we will calculate the acceleration of those atoms at times t1, t2, t3, and t4. So, what should be the gap between these t2, t3, t4, or what is known as the simulation time steps? So, what should that be? So, let us discuss it. For example, we are using rigid molecules. So, usually we keep a time step of 5 femtoseconds, and the idea is that we try to understand the translation and rotation.

If we are using the flexible molecules along with the rigid bonds. So, we keep the time step at 2 femtoseconds, where the idea is to understand the translation, rotation, and torsions. If we keep the flexible molecules with the flexible bonds, we have to keep the time frames at 1 or 0.5 femtoseconds, where we try to study the translation, rotation, torsions, as well as vibrations. So why is it important to choose a suitable time step? Because if we are using a very small-time step.

For example, this is your atom, and then this one atom is moving in this direction, and you have another atom that is moving in this direction. So, if I calculate this, I keep the time step very small. So, to move from this position to this position, I have to calculate several time steps; I have to calculate all those properties and all those potential functions. For multiple times, and then that will lead to a lot of computational cost, actually, and on the other hand, if I do it very fast. So, what will happen is, for example, if I keep a very large

time step.

So, the molecule and the atom will move in this direction, even before the collision. So, the predicted velocity, predicted acceleration, and predicted position of these atoms will cross each other. Or they will collide with each other if I am using, you know, a large time step, and that will lead to instability and, you know, failure of your system. So, the appropriate time, using an appropriate time step, will look like this, where you calculate the positions of these atoms. As soon as they approach each other, they start repelling each other because now the van der Waals energy is coming into play, along with the electrostatic repulsion.

So they will start repelling each other and then they will move away; this will tell us the real trajectory of these atoms, and this is what we wanted to understand, actually. So, this is why choosing the right time step is very important for running any molecular dynamic simulation. So, now we have chosen the time step. The next step is that we usually use the constraint algorithms because we are, you know, simulating a system.

So, we do not have any control over you know the system. So, we have to make sure that all those atoms are stable and that they are bound to each other. So, we usually use a bond length constraint for the equation of motion, and then SATTLE is a very fast analytical solution for small molecules, and it is widely used to constrain bonds in water molecules. And we have another algorithm called SHAKE, which is an iterative algorithm that resets all bonds to the constrained values sequentially until the desired tolerance is achieved. And then we have the LINKS algorithm, which is a linear constraint solver that employs a power series expansion to determine how to move the atoms so that all constraints are satisfied. Because we are moving all those, you know, atoms away from calculating their positions after each time step.

So, we need to take care of the bonds because we should not let them break the bond and then become free. So, for constraining the bond lengths, we use all these different kinds of algorithms. Okay, another thing is the thermostats because on a molecular level, temperature manifests itself as a number of particles having a certain average kinetic energy. So, we need to control the temperature as well because we usually run the simulation at a constant temperature. So, we need to use some temperature control algorithms like the Berendsen thermostat, which fails to produce kinetic energy distributions that represent a correct thermodynamic ensemble.

So, there are other thermostats as well, like Nose-Hoover, which produce the correct thermodynamic ensemble but can take a long time to converge. So, even though the Berendsen thermostats fail to produce the correct thermodynamic ensemble, they can be

useful for your system relaxation as they are robust and converge fast. Likewise, we have to control the pressure as well. So, the role of the pressure control algorithm is to keep the pressure in the simulation system constant or to apply external stress to the simulated system. So, we use weak coupling methods like the Berendsen pressure bath coupling, or we can use stochastic methods like stochastic cell rescaling.

There are some extended methods as well as extended system methods like the Perinello-Rahman barostat, Nosé-Hoover barostat, and MT-TK, which is the Martina Tuckerman-Tobias Klein barostat. And the idea is to ensure stability of a system; volume must be adjusted very slowly with a small increment at each simulation step. Okay, once we know how to control the temperature, how to control the pressure, and then the most important thing is the water models. Because most of the simulations, at least the biological simulations, we are doing in the water, actually. So, the goal of biomolecular simulation is the accurate and predictive computer simulation of the physical properties of biological molecules in the requested environment.

So, what we use is either the continuum models or the explicit models, and in the explicit models, we usually use TIP3P or SPCE. So, as you can see, these are the water models; like this is SPCE and this is TIP3PE. Okay, so now another important thing is, for example, if I wanted to simulate the water in this flask, I cannot take, you know, all the water molecules, which can go up to, you know, billions of water molecules. I cannot take billions of water molecules, create a system, and try to understand the properties because that would be computationally super expensive. So, what we usually do is try to take a small, you know, sample of this system, and we assume that the sample represents the overall solution, overall system, or overall population, we can say.

So, when we are taking the sample, this is not an isolated system because we have taken a small sample of it. So, these water molecules or anything else we are simulating. So, they should be allowed to cross these, you know, boundaries of the system of this cube that we have made. So, to do that, we use kind of imaginary blocks, imaginary boxes around this actual box.

So, there will be this actual box inside in the center. So, you can just imagine it as a Rubik's cube, actually, and then inside the Rubik's cube, which is 3 by 3 by 3, you have an actual sphere that contains your system, your protein, or your water molecules, or whatever you are simulating. And then surrounding it, you will have those imaginary cubes, which are an exact copy of this system, so that what happens is your molecules can cross this boundary and then re-enter from this boundary, ensuring that this system remains as real as the original system. So, we are using a different book size and shape. So, there are multiple ways to represent the system and one of the idea is that we try to reduce the volume

actually, because if we reduce the simulation volume that will lead us to reduce computational cost as well. So, we can use the hexagonal, truncated octahedron, rhombic dodecahedron, cubic, or, you know, all those kinds of box sizes and shapes we can use, okay.

So, let us discuss the thermodynamic ensembles. So, now, when we are simulating a system, we can have, you know, multiple thermodynamic ensembles. We will talk about NVE, NVT, and NPT. So, the NVE, which is where we are using a constant number of particles, n, and the volume is also constant, and the energy is also kept constant during the simulation. And then we have another canonical or constant NVT ensemble where we keep the number of particles, the volume, and the temperature constant. And then we have another ensemble, known as NPT, where we keep the number of particles, the system's pressure, and temperature constant, and then we run the simulation.

So, another important thing is the degrees of freedom; for example, the molecular degrees of freedom refer to the number of unique ways a molecule may move. If you look at this molecule, you know. So, now, this molecule has, you know, 40 atoms, okay. So, if you wanted to simulate these 40 atoms in a system. So, if I calculate the degrees of freedom for this non-linear molecule, it will be n 3 minus 6.

So, using these 40 particles or 40 atoms, we will have at least 114 degrees of freedom, and these 140 degrees of freedom mean that we have all those, you know, 140 bond lengths, bond angles, dihedrals, okay, all these properties. So, if I wanted to simulate this system, So, at each time step, I have to calculate all those 114 degrees of freedom and all those properties. So that I can get the trajectory dynamics, and you know those microscopic properties. So, that will be highly computationally intensive, but instead of using all those atomistic particles, can we coarse-grain them? So, can we, you know, just represent this whole benzene ring with a large bead or large particle? So, likewise, if I reduce it, if I coarse-grain it.

So, we can reduce it to, you know, 7 particles, okay. So, now these 7 particles will have only the 15 properties we have to calculate at each time step. So, you can see that we have almost reduced the computational power to at least 10 times less than that of the atomistic simulation. And that is the idea of using coarse-grain simulation so that we can go longer in the time frame, actually. So, if I use the same computational power to simulate this, we can go maybe up to 1 microsecond, but with this, we can go up to 1 second as well.

So, that is the power of coarse-grained simulation. So, this is a little practical thing when we start with the .gro file, which contains the structure we give all those input parameters contained in the MDP file. And this is specifically for GROMACS, which is one of the

popular open-source molecular dynamics simulation programs as well. So, we use the potential functions to describe the molecular system in the top file, which is called the topology file. We get the outputs: an ensemble of structures called configurations, an XTC file, a trajectory file, EDR containing the energy profiles, and the log files.

And then, by using all this trajectory information, we can understand the thermodynamic properties, structural properties, kinetic properties, and dynamic information as well. Okay, so that was, you know, a brief introduction to what MD simulation is, how it works, and what all those factors are that affect it. So, let us see the applications that we can use it for. So, we can use it for understanding the protein structure dynamics, we can predict the conformational changes in proteins, and that helps in understanding protein folding and stability. We can understand the drug binding and interaction studies and perform the interaction studies.

We can simulate ligand-receptor interactions at an atomic level, thus helping in predicting binding affinities and hot spots that can be used in structure-based drug design. We can do the free energy calculations; we can estimate the binding free energy to rank ligands. Here we can use methods like MMPBSA, MMGBSA, or FEP, which is free energy perturbation, where we can even calculate the absolute binding free energy. We can conduct the enzyme mechanism studies where we can simulate the enzyme-substrate interactions, which helps in designing better enzyme inhibitors. We can do the membrane permeability studies where we can model the drug transport across lipid membranes and also predict the bioavailability and pharmacokinetics of the drugs.

We can do the biomolecular assemblies and aggregation studies where we can study the protein-protein and protein-lipid interactions. And investigate mechanisms behind neurodegenerative diseases, like these protein-involved diseases, proteinopathies, such as amyloidopathy or tauopathy in Alzheimer's. We can do the nucleic acid simulation, where we can analyze DNA and RNA dynamics and stability, which helps us in designing RNA-targeted therapeutics. And we can conduct the nanoparticle and drug delivery studies. So, where can we simulate the interaction of nanoparticles with biological systems, which helps in the design of nanocarriers for drug delivery as well? And these are, you know, just a very few applications of molecular dynamics simulation in drug discovery and development.

So, this is a very powerful technique that has been used a lot and it has immense potential. Okay, then let us see how AI can help us in doing MD simulations. So, we can accelerate the MD simulation by using ML-based force fields like ANI, SCHNET, or DeepMD Kit, which improve the speed and accuracy, or we can, you know, use the AI-driven core screening models, which enable the simulation of larger biomolecules. If I want you to

simulate, for example, a virus particle or a complete mammalian cell, all those things can be done by using coarse-grained simulation with AI-driven technologies. And then we can enhance the force field prediction as well, where AI can help us refine the potential energy surface, improving accuracy in molecular interactions.

Even ML models can predict interatomic forces without requiring extensive quantum mechanical simulations. So, these are called ML potentials, and they are also very popular and a hot topic nowadays. So, this can be used. And then we can use AI-driven sampling techniques like ML-based enhanced sampling, where we can use reinforcement learning and active learning, which help explore rare conformational changes efficiently. Or we can use techniques like deep language dynamics, which improve protein folding predictions.

And we can study the, you know, the protein-ligand binding as well using the AI-assisted MD models, which predict binding synergies more accurately. And we have some deep learning techniques that analyze the docking and binding site stability as well. We can use surrogate models for MD, where ML-based surrogate models replace computationally expensive simulations while retaining accuracy. So, if you know about AlphaFold ensemble. So, they have recently released a platform where you can use AlphaFold to generate ensembles of protein structures that closely replicate the MD simulation.

Although they have not reached there yet, in the future, there will definitely be DL-based methods for structure prediction. which can predict the conformational ensemble of protein structures generated by using MD simulations or that are close to the data generated by MD simulations. And it is because, in that case, it can predict even longer time-scale events that traditional MD cannot efficiently simulate. And we can do the automated analysis of MD trajectories, such as detecting important conformational states and clustering, and these ML models can classify protein folding states, ligand interactions, and transition pathways. Okay, coming to the summary, the MD simulation models atomic interactions to study biomolecular behavior and dynamics.

The key principle that we discussed is, you know, the use of force fields, non-bonded interactions, thermodynamic ensembles, and constraint algorithms for accurate simulations. So, there are advanced techniques like free energy calculations, enhanced drug binding predictions and structural refinement which can be used utilize the power of MD simulation. And then there are AI-driven approaches that improve the sampling efficiency of force fields and predictive modeling, accelerating the overall use of molecular dynamics simulations. So, in the end, I have, you know, an open question for you. So, if you could simulate molecular interactions with unlimited computational power, how would you redesign MD simulations to achieve real-time drug discovery? And I have some, you know, suggestions for further reading.

So, you can go through these papers to get more information about this topic, and with that, thank you.