Advanced Thermodynamics and Molecular Simulations

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Lecture - 50
MD Simulations – Case Studies III

Hello all of you, so in the last lecture we have been discussing some case studies of MD simulations taken from some of my own research. So, today I will present some more case studies. So, this particular problem that I am discussing borrows similarity to the previous problem when I was looking at complexation of polyelectrolytes but in this case we are looking at single polyelectrolyte species where you have simply aggregation of polymer chains of the same charge.

So, we are looking at the poly-acrylic acid same as in the previous example, but we are not looking at the other cationic polymer that we had in there and we are interested in looking at the phase behavior of this solution that means when will the polymer chains aggregate, when will they dissolve in solution and how will that change with the pH of the solution?

So, the ultimate motivation for doing this is to use this as pH responsive drug carriers and this is why we are doing this as some kind of a study leading up to that but first we study the phase behavior of single polymer chain species and see how do they change with the pH.

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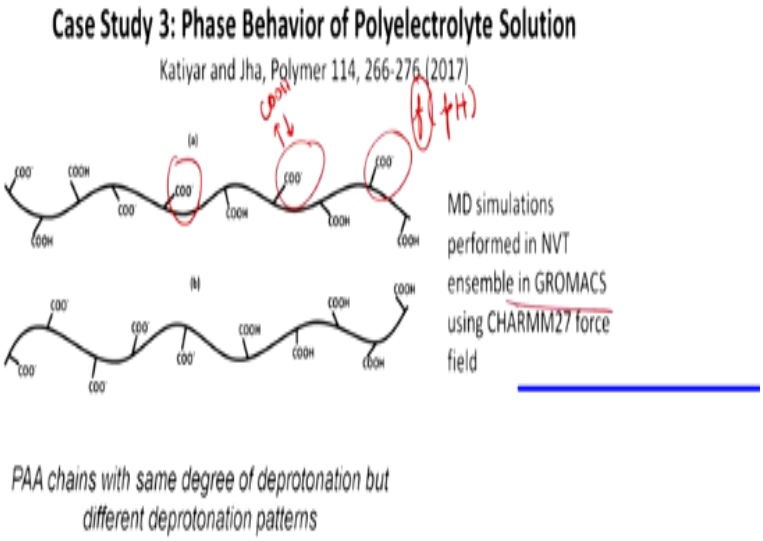

So, if you think about this particular problem I have been telling you that the number of the COO minus groups on the polymer chain that represents the charge on the polymer chain is basically a function of the pH and in fact this particular transition from COOH to COO minus is always happening that is the dynamic in nature but we always work with a constant f in molecular dynamics because, we cannot account for the protonation deprotonation processes in fact there are some methods called constant pH molecular dynamics that does that, but they are not really standard MD that we are using or is coded inside the software's.

So, with this particular idea we want to see how will the aggregation or the phase behavior will change as the pattern of the deprotonation changing, although we are not doing protonation deprotonation process. We can still change the pattern of deprotonation. So, let us say for example if you have 20 COOH groups they can all be COO minus or 50% of them can be COO minus or 20% can be COO minus depending on the pH.

Now let us say if 20% are COO minus this 20% can be located anywhere in the chain but then we can imagine different kinds of patterns of deprotonation, for example if they are randomly located versus if they are located only at one end, one of the reasons why we want to do that is because when I put them on one end, that represents the conditions present in many block copolymers because you have blocks which are charged.

So, although I am doing a homo-polymer chain with the same repeat unit, that particular thing when I am putting a block of negative charge kind of mimics the behavior of block copolymer chains but since we do not know where the deprotonation will take place, both of them are pretty much valid patterns to for deprotonation and since we are not doing it dynamically we are anyway missing out on that particular process of deprotonation and protonation.

So, therefore both remains approximate unless we have some mechanism to compare that, do experiments we can validate that in those cases but in general it remains approximate with the assumption that we have made. So, we are doing MD simulations in GROMACS again, all the simulations are in NVT in sample for this example and the same is true for the last case study that we discussed. We have been using the CHARMM27 force field using swiss param software, that is the same thing that we do also here and then the first thing that we do is we do a single chain simulation. So, it turns out that the polymer chains can have different tacticity.

Tacticity here refers to the fact that ultimately on a polymer chain, every carbon has say two hydrogens attached to it and I can replace one of the hydrogens with another functional group like COOH or COO minus, now as I do that I can do that to any of the hydrogen atom but if I do it to these two atoms they essentially give rise to slightly different structures and this is what is referred to tacticity, it depends on where my functional groups is present on the chain. And actually does not depend on one repeat unit as such it depends on how they are present on every repeat unit. If they happen to be on the same side of the polymer chain or the polymer backbone it is called Isotactic. If they are kind of alternating one below one above it is called syndiotactic. If for example it is randomly up and down this can be atactic.

So, all these polymers will show different conformations because the function groups are present on different sides of the polymer chain and clearly I am talking about in a three dimensional space, so now the conformation will also depend on the fraction of charge or the pH, so we are looking at the effect of tacticity and we are looking at the effect of pH.

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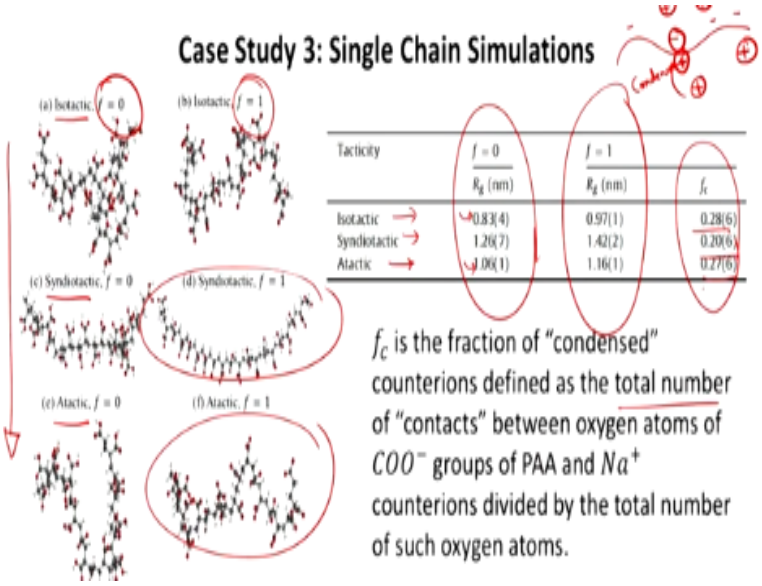

So, I am showing you here the effect of tacticity, so I am looking at Isotactic, Syndiotactic and Atactic chain all of the same number of repeat units just varying in tacticity and I am doing it for f equal to 0 case, when it is all neutral and f equal to 1 case when it is fully ionized that means all the COOH groups are deprotonated, they are present in the COO minus form.

So, clearly when it is deprotonated the chain has to be elongated because you will have repulsion between the COO minus groups on the chain. When it is all protonated that is in the form of COOH then there is no such repulsion. So, the chain can pretty much fold because there is no repulsion between the groups on the chain and this is precisely what we see here, the chains are more elongated when we are looking at f equal to 1 case then compared to f equal to 0 case. There is also effect of tacticity, so different tacticity gives me different conformations and actually different size of the polymer chain.

Now I have been telling you that if I want to characterize the size of a polymer chain I can use the radius of gyration. So, therefore we compute radius of gyration for both f cases and also for the 3 tacticity and it tells me that clearly R_g for f equal to 1 case, for all these three cases are larger than compared to f equal to 0 case and there is also some pattern in tacticity that is syndiotactic chains happen to be having highest R_g that is most elongated followed by the atactic chains and followed by the isotactic chains and this has been confirmed by previous studies as well.

In addition to that we also compute a quantity called the fraction of condensed ions and what it means is that, when you have a polymer chain clearly if the polymer chain has a negative charge to make the system neutral we have to have some counter ions present in the system which are mobile, but then these counter ions which are mobile experiences strong attraction with the negative charge present on the chain and therefore what may happen is some of these counter ions will tend to come very close to the polymer chain and compensate its negative charge, and this phenomena is referred as condensation and it turns out as the polymer chains become more and more charged, the condensation increases.

In fact if you have a multivalent ion you will have even larger condensation, because it offers more coulomb attraction with the counter ions. So, therefore we are measuring the fraction of condensed counter ions, which in this case is defined by simply a contact criteria. So, we count the total number of contacts between the oxygen atoms in the COO minus group and sodium counter ions in this particular case and we normalize this by the total number of such oxygen atoms and this gives me a fraction between 0 to1.

What we find is that there is quite significant condensation in all these cases that means that we have a significant amount of the counter ions which are in the vicinity of the polymer chain, because of the coulomb attractions you may very ask that, why not all of the counter ions come on the chain and the reason is because they have entropy, so entropy favors the ions to be in the solution on the other hand the coulomb interactions want them to come together.

So, if the entropy is dominant then the chains want to be farther from the polymer chain they want to be dissolved they want to be mobile everywhere. If on the other hand coulomb attraction dominates they have to be condensed on the polymer chain. So, clearly we can also vary that by varying the temperature along with the valency of the polymers.

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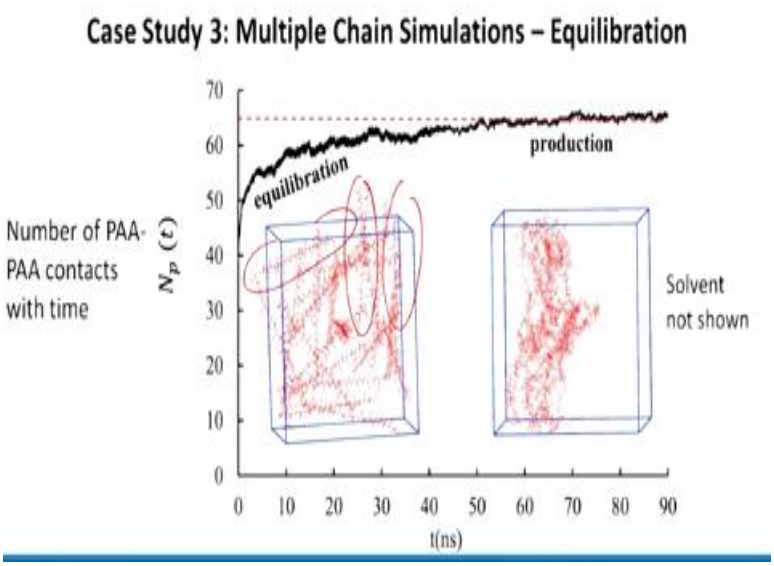

So, the next thing that we do is we extend that to multiple chains because ultimately we are interested in the aggregation behavior of the polymer chains. So, to do that we first have to build the polymer chains and then place them into a box and clearly we have to fill that with solvent again I am not showing you solvent and then we start running the MD simulation after doing necessary minimization or preprocessing that we have to do. We start doing the MD simulation and then at some point we have to say that now we have reached the equilibrium of the simulation, what will be that particular point? How will I define equilibration in this particular system?

And the answer is, we can look at the number of the PAA-PAA contacts with time as the polymer chains start coming together the number of contacts increases and eventually they start converging around some value and it is those values where we should, we can stop the simulation because the polymers have now aggregated. Again I have been telling you that we can never be sure that if I run the simulation for 10 times more time will I get the same result or not? And it really depends on how much computation that we can do how much computing power do we have. We can of course try to run for some cases and see that it is the same equilibrium state but in the end it really boils down to the fact that how much computing power we can afford, that dictates the presence of the equilibrium.

So, what we see here for example is that the number of contacts appears to plateau near a particular value after 99 nanosecond and therefore we stop here. We have done some runs for longer time to see that whether we get some other equilibrium and we do not find that, but that does mean that we can rest assured that it will always be here. It may happen that over 100 times more time it can come to some new equilibrium state the MD is simply not able to explore that state in whatever times that we have simulated.

This is particularly important when you have systems that tend to crystallize, crystallization itself is a slow process typically in the time scales we can do MD simulations, it is not easy to see crystallization behavior. It can only be seen for very simple molecules for complex molecules and MD will never show crystal formation and in those cases we have to be happy with whatever we can do in the time scales that we can simulate the same implies here, so we have used the criteria of the number of contacts with time. And therefore we think that this is an equilibrium state, but we can never be sure until one has done long enough simulation.

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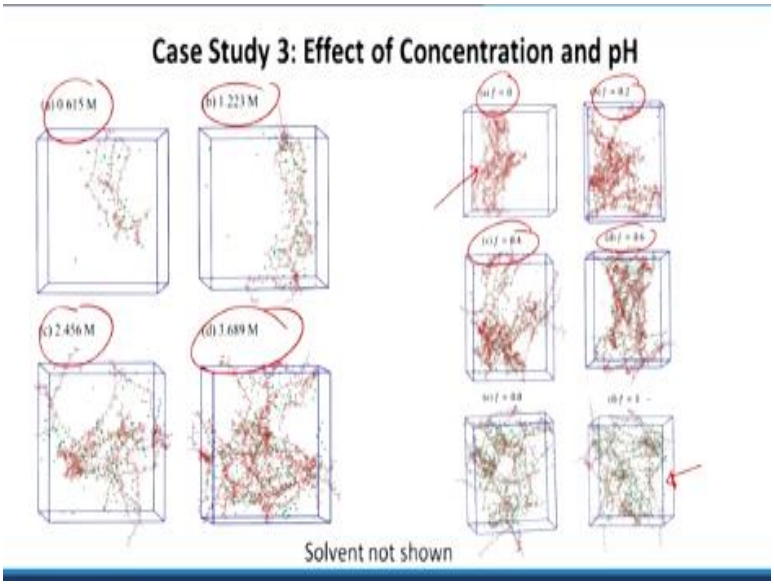

So, next we can look at the effect of concentration and the pH. So, in this case I am simulating for different concentrations of the repeat units and the results are quite natural. The system appears to be more dense as I go to higher and higher concentrations. But then we further analyze this data to look at how the hydrogen bonding and all those things depend on the concentration.

Similarly we can do the effect of pH by simulating at different values of f and what you can see here is when I am at f equal to 0 the polymer chains are neutral and we can see and aggregate as I start increasing f the aggregation starts to be lesser and lesser, and in the case when we do f equal to 1 you pretty much see no aggregate that means the polymer chains like to be dissolved in the solution and this is expected because at f equal to 1 you have electrostatic repulsion between the polymer chains and therefore the tendency of aggregation is not present but at f equal to 0 there is no such repulsion so they come together because the polymer is hydrophobic.

We can analyze the hydrogen bonding in more detail and an interesting physics that we found in this particular case, again that is not very relevant for this course as such but just to tell you what all information can MD can provide you we found that not only there is hydrogen bonding between COOH and COOH, there is also a significant hydrogen bonding between COO minus groups and COOH.

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Case Study 3: Hydrogen Bonding

f	0.615 M		1.223 M	
	COO– and *COOH*	*COOH* and *COOH*	*COO*– and *COOH*	*COOH* and *COOH*
0	–	0.05(3)	–	0.19(1)
0.2	0.13(1)	0.01(1)	0.18(1)	0.05(1)
0.4	0.18(2)	0.03(1)	0.21(1)	0.02(0)
0.6	0.17(1)	<0.01	0.20(1)	<0.01
0.8	0.12(1)	<0.01	0.07(1)	<0.01

f	2.456 M		3.689 M	
	COO– and *COOH*	*COOH* and *COOH*	*COO* and *COOH*	*COOH* and *COOH*
0	–	0.25(2)	–	0.22(1)
0.2	0.23(1)	0.04(1)	0.24(1)	0.05(1)
0.4	0.22(1)	0.02(1)	0.25(1)	<0.01
0.6	0.19(0)	<0.01	0.21(1)	<0.01
0.8	0.08(0)	<0.01	0.11(0)	<0.01

So, when we go from COOH to COO minus or as I increase f, the hydrogen bonding does not decrease in a linear fashion because when see 50% of them are COO minus and 50% are COOH, you can still have hydrogen bonding between the COO minus and COOH apart from the COOH COOH hydrogen bonding. And therefore the hydrogen bonding does not quite decrease as I increase the pH in fact it turns out that it goes to some kind of a maximum before it start to fall

down because eventually when we have all COO minus then there is no hydrogen bonding but as long as we have some COOH present in the system you have hydrogen bonding between COOH COOH and COOH at COO minus and there is an interesting pattern to be seen here as I increase concentration for example and how these hydrogen bonds change how the aggregation change and so on again the details are in the paper but I want to demonstrate what information can MD provide for simulations of these kind of systems.

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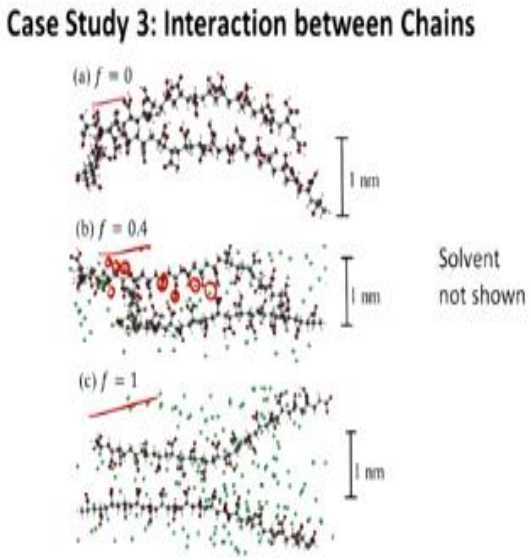

We can also look at the interaction between the chains in more detail, you can look at the polymer chains locally. Let us say if I am seeing in the VMD for example, I can zoom in and see what is happening near a polymer chain. For example I will be talking about the condensation phenomena where the counter ions come and sit on the polymer chain we can indeed see that happening by zooming into a polymer chain and see that there are for example here, counter ions which are pretty close to the polymer chain. And these are the guys which I am saying are condensed on the polymer backbone and we can see how this will change as I change conditions.

In this case I am showing for different values of f and clearly you can see as f is increasing the repulsion between the polymer segments become large that give rise to increase in the separation between the polymer chains and this is what gives rise to an aggregate getting dissolved as I increase f.

So, when I say aggregate getting dissolved, I am doing different simulations but it carries the idea of what will happen if f will change or what will happen when pH will change in the system.

We can also look at the aggregation in somewhat different perspective, we can look at the water content of these aggregates and this can be computed by a quantity known as the solvent accessible surface area essentially what you do is you make a probe particle, that is not really part of the system its external to the system.

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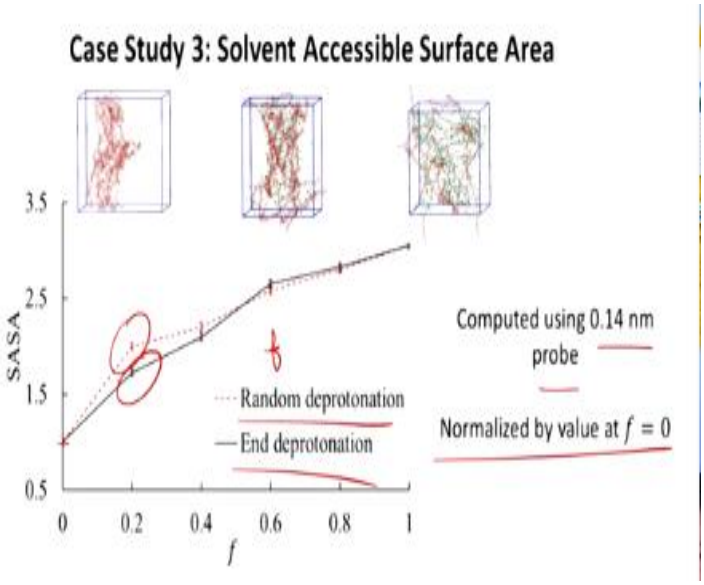

So, you move a probe particle inside the simulation trajectory whatever you have obtained already and see whether there is a room for the probe to move. If there is a room for the probe to move that means the solvent may also come in there because solvent size is also close to 0.14 nanometers. If on the other hand there is no room that means solvent cannot come inside there, if solvent cannot come inside there then that means the aggregate is very compact and in that case we can say the solvent cannot assess the polymer aggregate and in that case the aggregation is more.

So, the amount of the probes I can place in the system tells me how much the system is accessible to the solvent and clearly this is done over trajectories from which solvent has been removed, because if solvents are in there then clearly this makes no sense because if water is already present then where I am doing the probe.

So, ultimately we remove all the water and we then start moving the probe to see how much area is accessible to the water molecule and that gives me an indication of how compact is the aggregate and how much is the water content of the aggregate and in this case we are normalizing this particular value of solvent accessible surface area that we call SASA with the value we obtain at f equal to 0.

So, it starts from 1 and I find that the SASA increases with f which is natural, because the aggregate dissolves as f increases. So, the water content of aggregate or accessibility of water in the aggregate increases as f increases. But what we also saw is that for a given value of f if I compare two different patterns in one case we randomly place COO minus group. And in other case we place all the COO minus groups in the end they gives you different amount of the water content for certain values of f and that tells me that my aggregation itself will be affected not only by the f alone, but also by how I am deprotonating the polymer chain and that pretty much tells me the effect of the pattern of deprotonation or the pattern of charging of the polymer chain which is indeed a significant role.

So, all the simulations we discussed in the last case study was for the case where you just have a polymer solution where you have simple polymer in water of course some counter ions in there. But now I want to actually use it in the context of polymeric drug carriers, so now I want to simulate this polymers along with drug molecules and we also want to simulate with contents of the human body.

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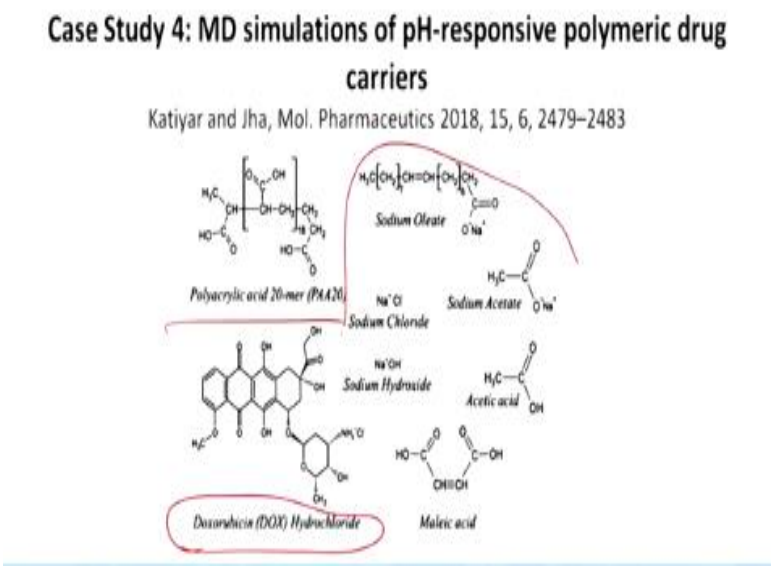

So, in this particular case study we simulate the same polymer chain that I have used in the last case study PAA, but we also simulate a drug molecule. This is a doxorubicin drug that is an anti-cancer drug and we simulate along with many other constituents that are typically present in our gastrointestinal tract or our stomach and intestine. So, until we want to see that if I make a tablet of this polymer with this particular drug and if I take that tablet in, how will the polymer and drug interact in the first place and how the polymer and drug system or the polymer itself will interact with the contents of the stomach and intestine.

This can be useful in determining whether this polymer is good enough as a carrier for this particular drug. Now if you are not comfortable with drug delivery, you only keep in mind that polymer here is working as a vehicle of the drug. If there was no polymer present the drug will not easily get inside the body, so polymer is kind of helping it get there or polymer is increasing what is known as the bioavailability that is the amount of drug that is getting inside the bloodstream is increased when I use it along with polymer, so polymer serves as a vehicle and it takes the drug there. How it does that there is a whole number of ways we can do that but for this particular problem we are only interested in how much the polymer is compatible with the drug and how much the polymer is compatible with the contents of the human body.

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Case Study 4: Systems Simulated

medium	component name	box size (nm)	number of molecules	concentration (M)	pH
Fasted State Simulated Gastric Fluid (FaSSGF)	PAA20, f = 0	10	74	0.123	1.6
	sodium chloride		21	0.035	
Fed State Simulated Gastric Fluid (FeSSGF)	PAA20, f = 0.05	10	74	0.123	5
	sodium chloride		143	0.237	
	acetic acid		10	0.017	
	sodium acetate		18	0.03	
Fed State Simulated Intestinal Fluid (FeSSIF)	PAA20, f = 0.25	10	74	0.123	5.8
	sodium chloride		74	0.123	
	maleic acid		27	0.045	
	sodium hydroxide		39	0.065	
	sodium oleate		18	0.03	
water	PAA20, f = 0.0.05, 0.25,0.6,0.85	6	16	0.123	1.6, 5, 5.8, 6.5, 7
	DOX		50	0.384	

So, with this particular idea we have simulated all this kind of systems, so we are simulating a gastric fluid which have essentially sodium chloride present in there. But then we simulate in two or three states we simulate a fed state and fasted state, so in fasted state you only have sodium chloride in fed state. In addition to sodium chloride you also have acetic acid and sodium acetate present. This is taken from the actual biological condition inside the inside the stomach and intestine. And we are looking at the components which are present in relatively high concentrations. Because components with low concentrations will be difficult to simulate in MD, because you simply will not have any molecule within the small box size that you are simulating.

Similarly we also simulate an intestinal fluid that has sodium chloride in addition we have maleic acid, sodium hydroxide and sodium oleate in addition to this we also simulate in water as in the previous study. And we also vary the fraction of charge on the polymer, the dox itself is positively charged so in some sense it is similar to the complexation of poly electrolyte example that we have done earlier except that in this case we have a drug that is positively charged that is smaller molecule in comparison to the poly cation we had in that example and looking at the composition of this different components inside the human body, I can decide how many molecules will basically go in my simulation for this particular molecule.

Clearly if I use a larger box I can accommodate more molecules and I can possibly accommodate more species present in there. For a smaller box we can only accommodate fewer molecules. And

many molecules which are present in the stomach will have such low concentration that you will have less than one molecule in the box.

So, there is no way we can capture that in a smaller box but then we are also limited by how large box we can simulate 10 nanometer is already pretty typical, we can go to 15 or 20 but that is pretty much the limit of all standard MD simulations.

So, we have tried to work with 10 nanometer, that is already pretty large system and accordingly we have chosen components which have reasonably large number of molecules in 10 nanometer, according to their concentration in the stomach and intestine.

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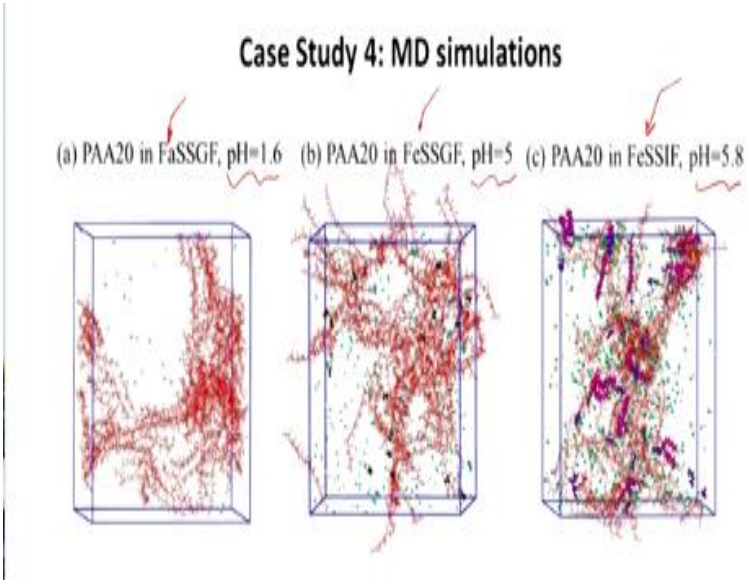

So, with this particular idea I start doing my MD simulation and now I can look at the phase behavior that is how the PAA will aggregate. In this case we are not doing drug for the moment only the polymer in the stomach fluid or intestinal fluid. This is a fasted state gastric fluid fed state gastric fluid and fed state intestinal fluid and we see how the aggregation is affected by the different fluids and of course as we do that we also change the pH and accordingly we change the fraction of charge on the PAA, and you can see beautifully how the different components of these fluids interact with the polymer and we can see from there which components have an affinity to the polymer chains which tend to repel it and we can get a whole host of information that provides a

molecular insight into how the polymer actually interacts with the components of the human body and how the aggregation is affected as we go there and aggregation, here is useful because this will determine how the tablet containing both polymer and drug will dissolve as it goes inside the stomach or intestine.

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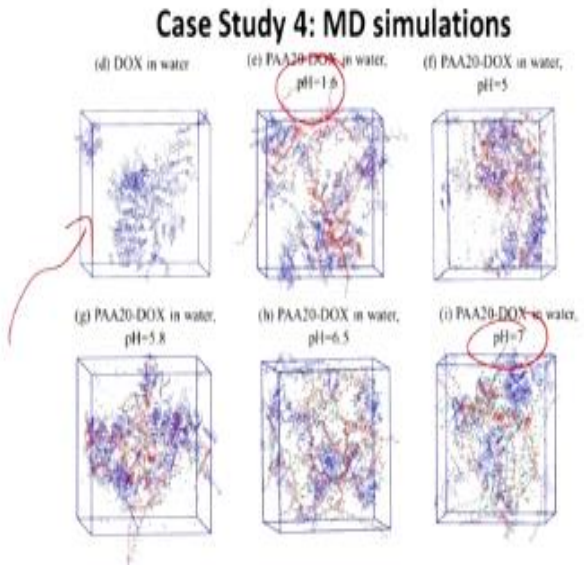

The next thing we do is we start putting in the drug as well there and in this case what we do first is I simulate one case where we have only DOX in water, that is the doxorubicin drug in water and we find that the doxorubicin tend to aggregate in water. When I add polymer, now since doxorubicin is positively charged they will tend to form complex with PAA because PAA is negatively charged and that complexation actually increases as the polymer charge increases and this is indeed what we see.

We see that the complexion increases as the fraction of charge on the PAA increases. For example for pH equal to 7 the charge on the PAA is pretty large, pretty large negative. So, in that case the complexation is maximum at lower pH the charge on the PAA is less, so there the complexation is the least and therefore the aggregation of the drug molecules decreases as I increase the pH because the drug molecules can complex with the polymer they will not aggregate among themselves.

So, this results in basically DOX molecules being far from each other, or polymer molecules sandwiched between the drug molecules or vice versa and this is useful for drug delivery context because now since the drug molecules are not aggregating they can come out as free drug, which can easily pass through the intestinal membrane. And therefore you will have high bioavailability. On the other hand if they form aggregate or some kind of crystals then these are particles of large size and once they are of large size they will find it difficult to pass through intestine into the blood stream and therefore we want doxorubicin not to form crystals but to basically form some kind of an amorphous mixture with the polymer so that once it comes out of it comes out as a free drug molecule.

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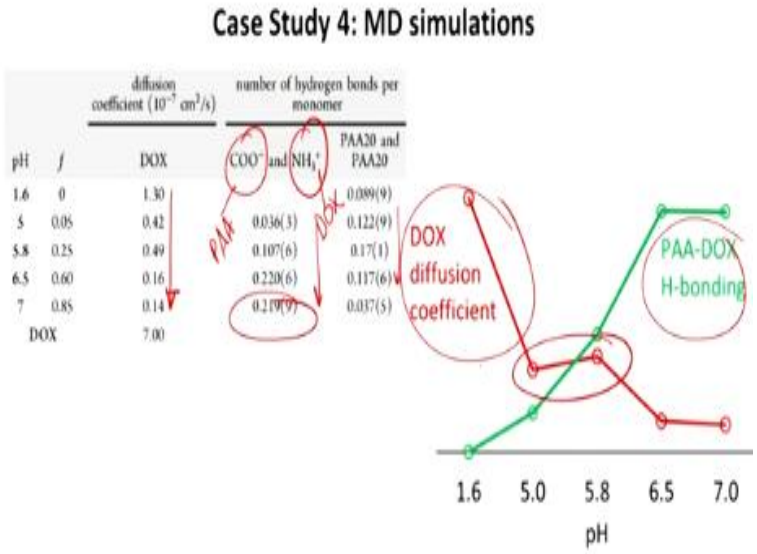

So, we can take further from there, we can look at the how the diffusion coefficient of drug changes as I change the pH so what we see here for example is that the doxorubicin diffusion decreases as I increase the pH and that happens simply because complexation with polymer increases. So, as we go to higher pH there is more complexation and since there is more complexation the doxorubicin molecule finds it difficult to come out of the polymer matrix.

On the other hand at lower pH the complexation is less, so doxorubicin finds it easier to come out of the polymer matrix we also look at the hydrogen bonding between COO minus group of the PAA and NH3 plus group that is the charge group of dox and find how the hydrogen bonding is changing as I increase the pH and clearly once we go to higher pH value there is more

complexation and that gives me larger number of hydrogen bonds because in fact the geometric criteria we are using to find the hydrogen bond pretty much does not differentiate between a hydrogen bond and ionic complexation.

And then again we find a very interesting trend of how the diffusion coefficient of DOX decreases with pH and how the hydrogen bonding increase with pH. There is some interesting trend in between and that comes from the fact that when we are at around that pH there are COO minus COOH hydrogen bonding that we have discussed in the previous case study and this is what gives rise to slightly non-monotonic behavior in a intermediate pH range.

So, with this particular example I want to conclude today's lecture. In the next lecture we will talk about some more stuff. We can do in molecular simulation such as free energy calculations and then we will discuss how can we do non-equilibrium simulations in the following week so that I want to conclude here, thank you.