ABSTRACT SUMMARY
Over several years our research group has been carrying out a research program involving the use of molecular dynamics simulation with all atom resolution to gain insight into the structure and function of drug delivery liposomes (DDL). Originally we were focused on PEGylation, but recently we have shifted our focus to the formulation of the DDL itself. This presentation covers several pieces of work including our study of the effect of cholesterol in the membrane [1,2], liposomes composed of novel synthetic lipids [3], The effect of replacing cholesterol with cholesterol hemisuccinate in the DDL membrane and liposomes designed to target the liver.

INTRODUCTION
Of all the forms of nanovector so far developed, the most established is the drug delivery liposome (DDL). The DDL is a versatile carrier, capable of carrying hydrophobic drugs in the membrane, or hydrophilic drugs in the internal cavity. The structure and activity in the body of the liposome can be engineered through alterations to the liposome formulation. Cosurfactants, like cholesterol, can be added to the membrane to alter its properties. Phospholipids with polymers like poly(ethylene glycol) PEG or targeting ligands can be added to achieve a protective “stealth” sheath and/or target the DDL to a specific tissue where the drug is to be released.

While drug delivery liposomes present a wide range of mechanisms that can be employed to fine tune their structure and function, the extent to which the precise effect of these alterations to the liposome can be investigated experimentally is extremely limited. There is no means to accurately observe the changes to the DDL on an atomic resolution scale. All-atom molecular dynamics simulation (AA-MD), however, provides just such a window on the liposome membrane. This has been used extensively to study the properties of biological phospholipid membranes and we have applied this methodology to the study of DDL membranes. One can, with all atom resolution, simulate a section of phospholipid membrane up to 15X15 nm in size for up to a microsecond in time. The insight gained can be directly applied to the development of DDL based drug delivery mechanisms.

In this presentation we will discuss four recent pieces of work where we have applied AA-MD: 1) the effect of cholesterol in the membrane 2) liposomes composed of novel synthetic lipids 3) liposomes with cholesterol hemisuccinate for pH dependent triggered release and 4) liposomes designed to target liver cancer.

EXPERIMENTAL METHODS
For all simulated systems a section of membrane with periodic boundary conditions was modeled in a solvent with salt concentration modeling the bloodstream environment (140 mM NaCl). The OPLS-AA parameter set was used to simulate all membranes with all atom resolution. All simulations and analysis were carried out using the GROMACS open source simulation package.

RESULTS AND DISCUSSION
We simulated liposome membranes with cholesterol with and without PEGylation. Cholesterol is known to have the effect of compacting the membrane, however preventing the formation of a gel structure. In previous work we studied the behavior of PEGylated lipid membrane [4,5] in both the liquid crystalline and gel states, and we were thus able
to study the effect on the membrane structure, specifically the PEG conformation, resulting from the addition of PEG to the membrane. We simulated membranes with cholesterol both with and without PEGylation. While our simulation of the membrane with cholesterol but no PEGylation was designed as a control, we stumbled on an interesting result of general biological significance: cholesterol in the membrane inhibits the binding of salt cations to the membrane surface. Regarding the effect on the PEG behavior, we found that, like the case of the liquid crystalline membrane, the PEG enters into the membrane core, however, in a structured rather than unstructured fashion, winding along the $\beta$ face of the cholesterol. Additionally the PEG interferes with the role cholesterol usually plays in structuring and compacting the membrane.

We studied the membrane of a novel DDL composed of synthetic lipids composed of phospholipids that have had the positions of the phosphate and choline groups interchanged. Our results show that this alteration radically changes the interaction with bloodstream ions and water ordering in the vicinity of the membrane in a fashion that promotes fusion with cell membranes, thus drug intake.

Replacing cholesterol with cholesterol hemisuccinate in DDLs has been proposed as a mechanism for pH triggered drug release. Our simulations of these systems support this hypothesis and show considerable qualitative change in the membrane structure with change in pH.

Liposomes to target liver cancer have been proposed, with a set of different possible lipids to functionalize the targeting receptor into. Our simulations demonstrated that the most effective means of raising the position of the receptor within the membrane, thus increasing its exposure, was to conjugate it to a triplet of three conjugated lipids. We were thus able to provide direction to the experimental formulation effort.

**CONCLUSION**

Our ongoing research program using computational molecular modeling with all atom resolution to study the effect of formulation changes on the structure, thus activity in the bloodstream of DDLs, continues to provide insight relevant to the development of these drug nanocarriers. The four separate pieces of work discussed here, The effect of cholesterol in the DDL membrane, synthetic lipid liposomes, liposomes with cholesterol hemisuccinate and liver targeting liposomes, demonstrate that this methodology is capable of providing practical insight capable of guiding the development of nanovectors.

**REFERENCES**


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