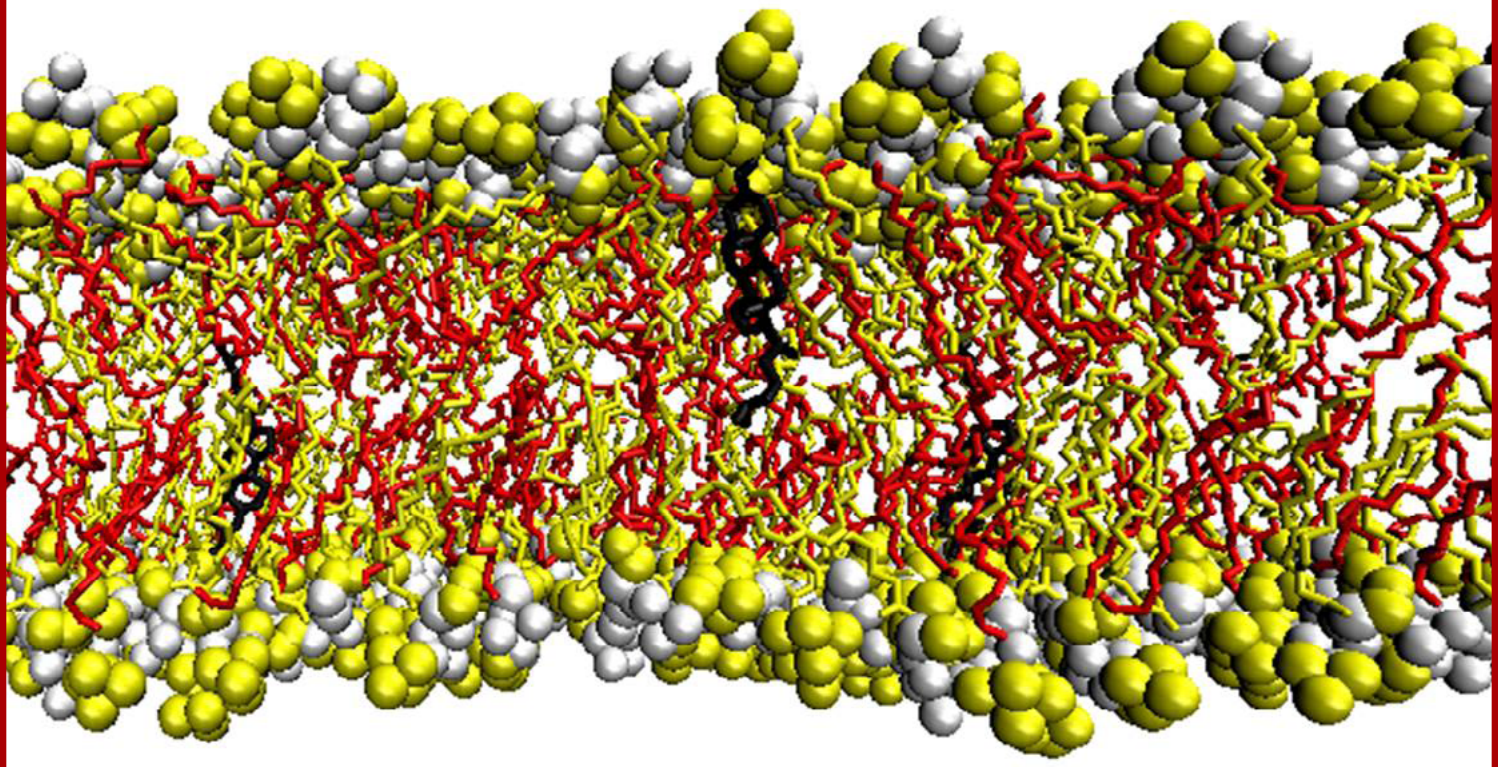

THE CATALYST

University of Maryland's Undergraduate Bioengineering Research Journal
College Park, MD

Issue No. 2 - Winter 2015

Want to get published in the next issue?

THE CATALYST

The Catalyst is UMD's undergraduate bioengineering research journal. We are looking to publish a variety of related undergraduate research with our third issue coming this Summer 2015! If you are an undergraduate student working on publishable research related to biomedical engineering and biotechnology, you are qualified to participate. Contact us via email or submit your research abstract through the Google Doc link provided below.

No research experience?

You can still take part in the journal's News Updates sections, which showcases topics such as recent advances in the field. Email us if you are intersted in contributing.

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Oppurtunities in Research

Curiosity. We're born with it. It's what motivates us to make that great transition from crawling to walking – allowing us to explore farther and faster. It's the bane of our parents' existence through elementary school with our incessant "but why?" questioning. It leads to anticipation as we collect those first precious data points for our middle school science fair project. It is why we, the faculty, the graduate students, and the undergraduate students, are all here at the University of Maryland; we are curious about the world around us, and we want to understand it and make it better. Undergraduate research is a career-building opportunity and for many the first major step in a lifetime of following one's curiosity.

The opportunities to participate in undergraduate research are abound. Our own Fischell Department of Bioengineering faculty and affiliated faculty have established incredibly productive and ground-breaking research programs. Working in a lab on campus has the benefits not only of convenience and proximity but also allows students, over the course of multiple semesters, to take ownership of a larger, potentially more impactful project. Of course, working in a lab away from campus affords its own benefits: expanding a student's network and introducing them to new and different projects, problems, and perspectives. Colleges across the country offer summer Research Experiences for Undergraduates (REU), often in the hopes of identifying and recruiting promising young scientists and engineers to their graduate program. National labs/institutes and companies in the private sector offer undergraduate summer internships that expose the participants to government and industrial research, respectively. They, too, offer these programs to seek out potential future employees and colleagues. If a student wants to experience life beyond the U.S., there are opportunities to collaborate with researchers at institutes and universities all over the world.

Students, now is the time! Never again will you have so many opportunities to try out different research areas, meet different people, and explore different locations. Take advantage of this time to discover what career path will best satiate your curiosity.

Provided by Dr. Angela Jones, Lecturer, Fischell Department of Bioengineering, University of Maryland, College Park

Letter From the Editor

Dear Reader,

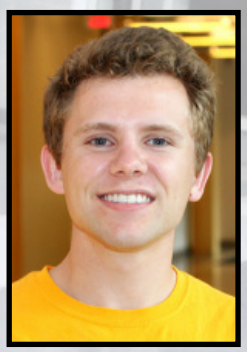
Welcome to the second issue of *The Catalyst*: University of Maryland's Undergraduate Research Journal for Bioengineering and Biotechnology. After the success of the first issue, the editorial board's goal throughout the fall 2014 semester has been to bring students an improved version of *The Catalyst* with more research, applicable advice and guidance from professionals in the field, and more student perspective. Furthermore, the second issue provides updates regarding the first-ever University of Maryland iGEM team's gold medal success in Boston and new sections showcasing international travel and research conducted by BioE students here in the Fischell Department of Bioengineering.

The Catalyst remains open for the submission of abstracts from any University of Maryland student conducting research related to bioengineering and biotechnology throughout the year. This issue features novel research in computational modeling of cholesterol's interactions within a lipid bilayer. The editorial board is also looking for photos and videos from student research. Photos and videos will be placed in upcoming issues as highlights. Credit to the student and lab will be given. For example, see the background of the Table of Contents. The background is an image of fibroblast cells taken by teaching assistant, Kelly Snead, during the Biology for Engineers Laboratory, BIOE121.

The Catalyst was fortunate this past fall 2014 semester to co-host the "In Silico Medicine Forum Series: Using Big Data for Age-Related Diseases" seminar at Johns Hopkins Eastern led by the Emerging Technology Center. In addition to supporting student research on campus, the editorial board would like to provide students opportunities at future seminars and conferences.

We are also pleased to welcome and announce our new adviser, Dr. Angela Jones, lecturer in the Fischell Department of Bioengineering. We recommend current BioE students to see the Opportunities in Research section written by Dr. Jones for inspiration and guidance in your studies and research.

The editorial board of The Catalyst has many people to thank for the second issue's publication. We would like to thank the A. James Clark School of Engineering and Fischell Department of Bioengineering for supporting undergraduate research and providing space on the Department's website for the issue. We would also like to thank freshman students interviewed; by sharing your experiences you will help guide other freshman in their studies and give upperclassmen a time to reflect back on their first year. Thank you to the authors who went through journal review process of submission, editing, and review. Most importantly, I would personally like to thank the editorial board of The Catalyst; we continue to grow each semester – thank you for your energy, innovative ideas, and passionate focus on other students.



Thank you and enjoy reading,

Kevin Pineault

News Updates

Beckton Dickinson Lab Tour

By Kenneth Ke, Guest Contributor

BD represents a lot more to me than to the casual observer. They are more than just "that place that makes all of our test tubes". BD is the epitome of big scale industry, which is one of the multitude of career opportunities that exist to a bioengineering major. BD (Becton, Dickinson and Company) is a Fortune 500 company that specializes in producing medical supplies, devices, laboratory equipment and diagnostic products that are used in everyday life, from agar plates to rapid flu tests. These facts can be pulled straight from their company website. What was offered to me, as a member of the Biomedical Engineering Society, was a behind the scenes look at how an engineer exists as the cog in a gear of BD.

Walking into the first factory was an absolute assault on the senses all the way through. Before we could enter we had to don slightly comical hair (and beard) nets along with safety goggles. Once inside the machinery of the factory we saw as every gear moved in perfect harmony in order to turn raw plastic into differential agar medias. The smells, the sounds, the incredible precision brought out a boyish sense of wonder that really brought me back to why I wanted to be an engineer in the first place. I could envision myself outside of the classroom and optimizing and redesigning this well oiled assembly line. This tour was more than eye opening, it was a sneak peek into the answer of that question that all undergraduates fear: what do you want to do when you graduate? At least I have one answer for myself.

Startup Shell Starting Up

By Lily Sookal, Guest Contributor, Events Director of Startup Shell

When you ask bioengineering students at UMD what the main career paths are in bioengineering they'll give you three tried-and-true answers: medical school, graduate school, or industry. By industry, they usually mean starting as an Engineer I at some company and eventually moving up the company through technical or managerial achievements. Surprisingly, few will give another answer: entrepreneurship.

My dream, as an engineer, is to create a medical device that will improve the health of millions of people, whether it be through rapid diagnosis of a disease or a therapy to treat one. I want to be the person to think of an innovative idea and have the creativity and flexibility to make it a reality, to make my stamp on the world. For this reason, I chose to apply to be part of UMD's student-run entrepreneurial community called Startup Shell. Startup Shell is part think-tank, part startup incubator and an incredible resource for those students passionate about starting their own company. With over 100 members and 20 ventures represented, we are becoming one of the strongest student-led entrepreneurial forces in the nation and plan to expand further. If you believe you have an idea that could become the next big thing in the biotech industry, I highly encourage you to think about applying to this collaborative community of entrepreneurs.

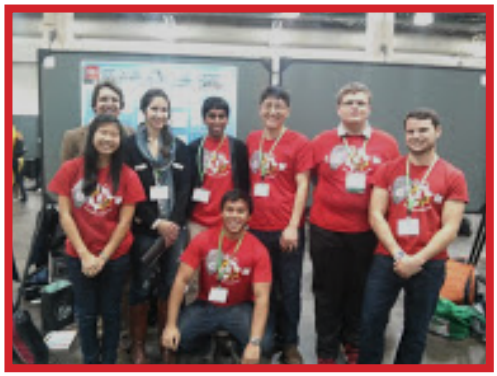
iGem Update: After the Big Win

By Nathan Barber, Staff Contributor, Member of iGem

This was the University of Maryland's inaugural iGEM team. For our first endeavor, we set out to develop a biosensor for the oyster pathogen Perkinsus Marinus, which is found in the Chesapeake Bay as well as around the world. Over the past year, what started as an initial thought turned into a full scale project. We had a very dedicated team of over 10 undergraduate students working tirelessly throughout the summer on fundraising, research, human practices, and beyond. We were able to generate four genetic constructs, design and create two new chimera proteins for our design, and conduct an extensive human practices campaign by surveying different communities affected by P. marinus, all of which can be seen on our team website (2014.igem.org/Team:UMaryland).

Through the generous support of our sponsors, we were able to send 7 students, 2 faculty mentors, and one graduate advisor to the 2014 iGEM International Jamboree (Oct 30th – Nov 3rd). This was an amazing trip filled with wonderful presentations, insights into how iGEM was run, and many educational opportunities. During the course of the jamboree, we presented our project to hundreds of fellow iGEM'ers during two 3 hour poster sessions and a 20 minute full-length presentation. We were delighted to talk with a marine biologist from California as well as an oyster expert from France, along with other experts in the field of synthetic biology, demonstrating that the scope of our project extended further than we could have ever imagined. The international aspect of this competition was truly amazing and was everything we had hoped for. All of this work culminated in us receiving a gold medal at the competition. We were very honored to receive this in only our first year.

While we are extremely proud of all of accomplishments this year, we will not rest on our laurels. We will continue to expand our team in order to reach even greater heights next year. Ultimately our hope is to use synthetic biology to continue to positively impact our community. Please feel free to contact us if you have any further questions on what's been going on, and what our next steps are! Please direct all emails to our mentor and coordinator, Dr. Boots Quimby (bquimby@umd.edu).



Seventh Annual Fischell Festival a Success

By Dimitri Tito, Staff Contributor

"The greatest achievement that engineering can make is to improve the quality of life for millions of people. Our gift will help young engineers develop their ideas to improve healthcare for human beings throughout the world."
Robert E. Fischell, M.S. '53, Physics

Bioengineers play a significant role in designing innovative technologies that enrich the qualities of human health care, and enhance novel systems to satisfy unmet needs. The seventh year of the Fischell Festival was marked by unprecedented attendance. More than one hundred University of Maryland Bioengineering faculties, students, Alumni, and members of biotech industry attended the event to support the effort of research scientists in the medical field to design progressive cures of disease, vaccines, drug delivery methods, and biomedical devices. From the Kay Boardrooms, Dr. Stephen Redd, Centers for Disease Control and Prevention Director, addressed improving responses to public health threats including Influenza and Ebola. From the detection of Novel swine influenza to the 2009 pandemic, lessons learned from Influenza responses are being applied to control and prevent Ebola outbreak. Dr. Don Milton, Director of Maryland Institute for Applied Environmental Health presented his research about Influenza Pandemic Preparedness. He evaluated several modes of influenza transmission. From the Fischell Department of Bioengineering, two faculties and ten graduate students presented their research at the festival. Dr. Ian White introduced his sample preparation for point-of care infectious disease diagnostics in both improving patient care and preventing the spread of bacteria infections. Dr. Christopher Jewell presented his research on engineering synthetic vaccines to promote immunity and to combat autoimmune diseases. Graduate students Matt Dowling, Eric Hoppmann, Wei Yu, Sean Virgile, Alek Nacev, Darryl Sampey, Tony Melchiorri, Rasa Ghaffarian, Stefanie Cohen, and Shawn Greenspan also presented their research to the Bioengineering community. At 5 pm, Dr. Rita Colwell, Former NSF Director and Distinguished University professor concluded the festival with a Whiting-Turner lecture on infectious diseases in a world of climate change.

On the Cover

By Adam Berger, Design Chair

This issue's front cover image was generated by the author of this issues research section, Anastasiya Belyaeva. The graphic was modeled in VMD and represents a phospholipid bilayer embedded with cholesterol. Of course, in honor of the University of Maryland, the colors are represented as the school colors. See Anastasiya's paper on page 12 to read more about computer modeling in bioengineering.

Interview with Freshman BIOE

Haroula (Rou) Tzamaras '18

Rou is a freshman BIOE major that enjoys EWB and running in her spare time. She is a part of university honors and the Flexus program.

What made you chose to do BioE? What specifically within bioengineering piques your interest?
"I originally wanted to do neuroscience. In high school, my engineering teacher told me that doing bioengineering would open up many more doors even within neuroscience. I started to gain a real liking for neuroengineering. I am also really interested in anything having to do with bioengineering and the environment. "

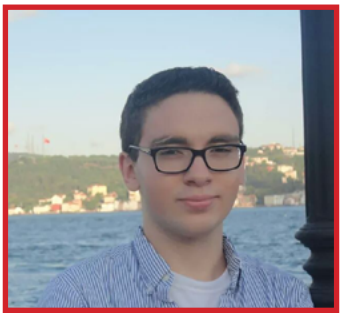
What has been your favorite class thus far?
"My favorite class so far has been BIOE121 with Dr. Jones. I like the fact that we get to do hands on experiments that are enjoyable. They also take what we learn in class and allow us to apply it."

What class do you look forward to the most these next few years?
"I look forward to the more specialized BioE classes. I look forward to learning the detailsnot just the basics."

Looking into the future, where do you see yourself in ten years?
"In the future, I honestly have no idea where I will be. There are so many options here and I am keeping my options open! I can get interested in anything pretty easily."

Metecan (Mete) Erdi '18

Mete is a freshman bioengineering major. He is first generation American and speaks Turkish fluently. He has participated in many robotics competitions and is an Eagle Scout.



What made you chose to do BioE? What specifically within bioengineering piques your interest?
"I chose BIOE because I like working with kids. As a teen, I always wanted to be a babysitter. But as a male, it is harder to get jobs in that sphere. Thus, I thought that the next best thing would to be a pediatrician. I knew that I really liked engineering because I like using my analytical and critical thinking skills. I knew that I wanted to be a doctor, but wanted to apply my academic knowledge beyond that which biology teaches. I really enjoy biomechanics type of stuff such as prosthetics because I enjoy working with my hands and being at the cusp of mechanical engineering in biomedical applications".

What has been your favorite class thus far?
"So far I have really enjoyed ENES100 because I have the opportunity to lead the group, as a group leader, and I also enjoy the group work and talking with other freshman of various backgrounds and majors. I also really enjoy the programming and electronics."

Looking into the future, where do you see yourself in ten years?
"In terms of career I see myself as a pediatrician leading a practice due to my love of working with others and my love for leadership. I love helping people out whether it be physically or mentally. In the future years, I hope that this dream becomes a reality. I don't think that I want to specialize in anything within pediatrics. If I do not see a solution to the issue immediately, I think that BIOE will help me learn other ways to approach the problem that I may not have thought of without a background in BioE."

Bioengineering Abroad

By Nariman Ziaee, Staff Contributor; Interviewing Dan Hogan

Last Spring, Bioengineering student Dan Hogan, was awarded the Freeman Asia Global Engineering Scholarship to fund his exchange at Hong Kong Polytechnic University (HKPU). As part of the Global E3 program, every course Dan enrolled in at HKPU transferred back to the University of Maryland and kept him on track with his degree. Dan took Bioimaging, Bioinstrumentation, Biotransport, Microfabrication of Biomedical Devices, and Global Biomedical Device Regulation.

Dan says, "The coursework in Hong Kong was challenging, but I felt a lot less pressure since I knew the classes would be transferring back to Maryland as Pass/Fail. It was also very interesting for me to work with teams of Chinese students on a bioinstrumentation project designing a prototype device that allowed paraplegic patients to surf the web. From the sensor and microcontroller side, there was little difference between this project and the control system from my hovercraft in ENES 100."

In addition to his studies, Dan took part in research work with the HKPU Rehabilitation Engineering Department in an ongoing stroke rehabilitation clinical trial. This exciting project coupled a wearable robot, functional electrical stimulation, electromyography readings, and a video game interface to guide stroke patients as they worked to restore lost upper limb mobility. In an aging population increasingly sensitive to showing weakness in public, this project seeks to develop a low cost at-home rehabilitation device. For Dan, the opportunity to work with patients at the interface of technology and health-care, was a dream come true.

"The stakes for finishing a project are a lot higher when you know you have a patient coming in the next day to use the device. I was able to practice a lot of programming, signal processing, the mechanics of electrical and direct forces applied to the limb by the

Figure 1. In May 2014, Dan had the opportunity to showcase his stroke rehabilitation research work at the Hong Kong Polytechnic University in an exhibit at the International Medical Device Fair in Hong Kong.

robot, and work directly with three patients throughout their recovery. It's often easy to lose sight of why we learn the things we do as engineering students: fluids, mechanics, math, electronics---what's the point? But, when you see someone lift their hand in a way they thought they'd lost forever, and you know your work played a part, it all comes together and there is really nothing more rewarding." says Dan.

Figure 2. Dan travelled to Cambodia during his exchange and had the opportunity to observe ongoing malaria research activities along that country's northern border with Thailand. He also made time to visit the famous temples at Angkor Wat.

During his time abroad, Dan was also certified by the Hong Kong Medical Device Regulatory body and the Asia Regulatory Professionals Association in global medical device regulation. This certification is increasingly important to employers looking to bring products to market in Asia and the European Union.

Dan also travelled throughout Southeast Asia during his time in Hong Kong. In addition to leisure trips to Vietnam, the Philippines, Thailand, Singapore, and Malaysia; Dan participated in a research exchange with Malaria research physicians along Cambodia's northern border with Thailand.

Dan told us, "It's easy to lose sight of how good we have things in Western society. We often take for granted our access to clean water, healthcare, and information. In Cambodia, a country that lost virtually all of its intellectual capital under Pol Pot and the Khmer Rouge, they are working to build up this infrastructure from scratch. Smart engineers have designed microfluidic diagnostics and money is pouring in from the Gate's Foundation and World Malaria Fund, but only time and investment in people will produce a lasting impact in this region. There's a real need for problem solving engineers to innovate in this space."

Dan has returned to the University of Maryland and is currently finishing his final year in the Fischell Department of Bioengineering. He is looking forward to graduating in May and matriculating to medical school next fall. His advice to students is simple, "Increase your distance. Break your routine, change your perspective, and make yourself a competitive applicant and interesting person in the process. There's much more to education than the courses we take."

The Education Abroad Office and the International & Leadership Program Coordinators at the Clark School of Engineering are dedicated to assisting students and helping them to take advantage of experiences offered overseas. Contact Ramsey Jabaji at rjabaji@mail.umd.edu or explore the Global E3 program for yourself at http://www.iie.org/programs/globale3.

Anastasiya Belyaeva

Anastasiya Belyaeva is a senior bioengineering student with a minor in statistics. She is currently involved in computational bioengineering research in the Biomolecular Modeling Laboratory under the guidance of Dr. Silvina Matysiak. Her project aims to determine the mechanism driving amyloid aggregation in Alzheimer's disease using computer simulations. This summer she participated in the Amgen Scholars program at University of Washington, also using computer simulations to understand the behavior of lipid bilayers. Anastasiya is involved in the Math Success program at University of Maryland, where she weekly teaches students mathematics. Combining her passion for mathematics, computer science, and bioengineering, she plans to pursue a Ph.D. in computational bioengineering upon graduation.

ABSTRACT

Cholesterol plays an important role in the organization of phospholipid bilayers. One of the most remarkable effects of cholesterol on lipid bilayers is the condensing effect: the area per molecule decreases with increasing cholesterol concentration. This occurs due to ordering of phospholipid carbon chains in the presence of cholesterol, which enables the transition of lipids from liquid-disordered to liquid-ordered state. Although the condensing effect of cholesterol has been observed both computationally and experimentally, the exact molecular mechanism remains unknown. In this study, we use coarse-grained molecular dynamics simulations to examine the effect of different cholesterol concentrations on dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. We observe that the area per molecule decreases with increasing cholesterol concentration, confirming the condensing effect of cholesterol. As the area per molecule decreases, the average order of phospholipid carbon chains increases. Thus, cholesterol straightens and orders the lipid carbon chains. After measuring the order of lipids as a function of distance from a single cholesterol, we determine that phospholipid order is enhanced over a distance of approximately 1.5 nm, which spans at least two cholesterol solvation shells. This finding indicates that cholesterol affects not only the phospholipids that are its immediate neighbors but also the phospholipids that are further away. Understanding how cholesterol orders phospholipids will enable us to explain the driving mechanism behind the liquid-disordered to liquid-ordered state transition as well as the origin of the condensing effect.

Keywords: molecular dynamics, membrane, cholesterol, simulation

Effect of Cholesterol on Lipid Bilayer Phase Behavior by Molecular Dynamics Simulations

Anastasiya Belyaeva[1], Shushan He[2], and Lutz Maibaum, Ph.D.[2]
[1]Fischell Department of Bioengineering, University of Maryland, College Park, MD; [2]Department of Chemistry, University of Washington, Seattle, WA

1. Introduction

Membranes are complex systems, consisting of proteins, a variety of phospholipids, and cholesterol. Cholesterol is known to play an important role in controlling the properties of the membrane by stiffening the lipid bilayer and regulating protein function [1]. Multicomponent systems of phospholipids and cholesterol have been extensively explored by both experimental and computational studies. However, essential questions remain about the molecular basis of cholesterol's impact on membrane properties. To understand the basic interactions between phospholipids and cholesterol in the lipid bilayer, this study focuses on a binary mixture of phospholipid and cholesterol.

One of the most remarkable effects of cholesterol on lipid bilayers is the condensing effect: area per molecule decreases upon addition of cholesterol more than expected by ideal mixing [2]. In the case of ideal mixing, the total bilayer area is estimated based on the area a single cholesterol and single phospholipid occupy. However, the total bilayer area is observed to be smaller than simply the sum of the areas of cholesterol and phospholipid molecules, forming the basis of the condensing effect. Thus, the condensing effect involves a more complex reorganization of the lipid bilayer, causing the observed nonideal behavior and the dramatic decrease in area per molecule [3]. Many experimental and computational studies confirmed the condensing effect and the corresponding increase in lipid bilayer thickness and lipid order [2, 5, 9]. However, as of this article, the exact molecular basis of the condensing effect remains unknown.

The effect of cholesterol on lipid bilayers has been described in terms of lipid phase behavior. Few phase diagrams have been proposed for binary mixtures of lipids with high melting temperature and cholesterol (Figure 1). Three phases have been reported in these mixtures: solid-ordered (S_o), liquid-disordered (L_α), and liquid-ordered (L_o). The solid-ordered or gel phase occurs at low cholesterol concentrations and low temperatures. This phase is characterized by ordered lipid chains that exhibit slow lateral diffusion. The liquid-disordered phase consists of disordered lipid chains with fast lateral diffusion and is observed at low cholesterol concentrations but high temperatures. As the cholesterol concentration increases, the liquid-ordered phase emerges, which is described by ordered lipid chains with fast lateral diffusion. Although a variety of both computational and experimental techniques have been used to elucidate the phase behavior of lipid bilayers, the transitions from one phase to another and the presence of coexistence regions remain a controversial topic due to differences in time and distance scales used by different methods [4-6]. The phase diagram in Figure 1a, suggests a distinct L_α - L_o coexistence region at higher temperatures, which is separated by a triple point from the S_o - L_o phase. Interpretation of data based on methods such as "differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) [13], electron spin resonance (ESR) [14], fluorescence resonance energy transfer (FRET) [15], fluorescence recovery after photobleaching (FRAP) [16], fluorescence anisotropy, and freeze fracture electron microscopy [17]" provide evidence for this phase diagram, according to a review by Veatch and Keller [4]. The phase diagram presented in Figure 1b proposes a gradual transition from the L_α to L_o phase without a separate high temperature coexistence region or a triple point. Evidence for this diagram is provided by "DSC [18], NMR [19], and FRAP [20]" experiments [4]. In the context of cell membranes, it is important to determine the precise phase behavior of lipid bilayers to better model membrane rafts that have important biological function in the cell [4, 6].

In order to avoid the ambiguity and subtleties that accompany the interpretation of experimental data, we use a computational model to probe the lipid bilayer

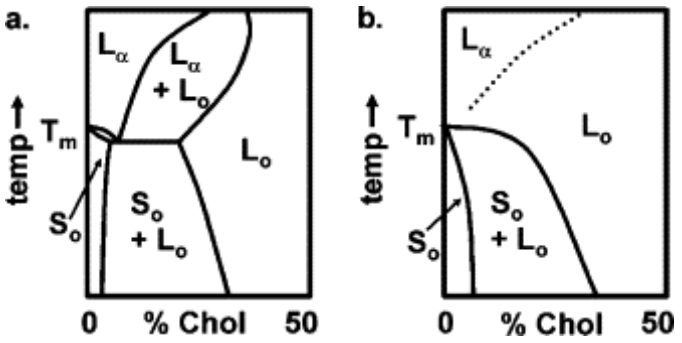


Figure 1. Two different phase diagrams for binary mixtures of lipids and cholesterol (taken from ref. 4). (a) Liquid-disordered (L_α) and liquid-ordered (L_o) are distinct thermodynamic phases, separated by a coexistence region (L_α + L_o). S_o refers to solid-ordered phase in both diagrams. This phase diagram is based on experimental methods such as "DSC, NMR [13], ESR [14], FRET [15], FRAP [16], fluorescence anisotropy, and freeze fracture electron microscopy [17]". (b) In contrast, the transition from L_α phase to L_o phase is gradual, as denoted by the dotted line and the lack of a coexistence region. This diagram is supported by evidence based on "DSC, NMR, and FRAP", according to a review by Veatch and Keller [4].

phase behavior. Since phase transitions can take place slowly, we use a coarse-grained MARTINI model, which groups atoms together for faster computation, as opposed to its slower atomistic counterpart [7-8]. This study focuses on determining the molecular mechanism behind cholesterol's control of membrane properties. We aim to identify the molecular basis of the condensing effect of cholesterol and describe the phase behavior of lipid bilayers as cholesterol concentration is increased.

2. Methods

2.1. Computational details

Molecular dynamics simulations of lipid bilayers composed of 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol were performed at increasing cholesterol concentrations. The initial structures were obtained from the MARTINI website [8]. Systems with different cholesterol concentrations were constructed by randomly replacing DPPC lipids with cholesterol and replicating the bilayer until the system consists of 1152 lipids. A total of 21 simulations were performed, with cholesterol concentrations ranging from 0% to 55.5%. The lipid bilayer was composed of 1152 lipids (DPPC or cholesterol) in 9216 solvent molecules (8 coarse-grained solvent molecules per lipid) based on previous computational studies that resulted in proper hydration of the bilayer and achievement of accurate bilayer properties, such as surface tension and area per lipid, which have been validated experimentally [8, 21]. The approximate dimensions of the lipid bilayer were 19.1×19.1×7.2 nm. The simulations were performed for 1 μs, and the first 200 ns were excluded from analysis to account for the equilibration of the lipid bilayer.

All simulations were performed using the GROMACS software package [10] with the coarse-grained MARTINI force field. The coarse-graining is done by mapping on average four atoms into one group, resulting in four main types of beads (polar, nonpolar, apolar, and charged). Instead of modeling every atom (as would be the case in atomistic simulations), DPPC is modeled using one positively-charged choline bead (Q_0), one negatively-charged phosphate bead (Q_a), two glycerol-ester beads (N_a), and eight apolar alkyl beads (C_1), shown in Figure 2a. The cholesterol molecule is modeled similarly, however it has an additional ring bead type. The coarse-grained structure of cholesterol is illustrated in Figure 2b. Cholesterol is modeled with

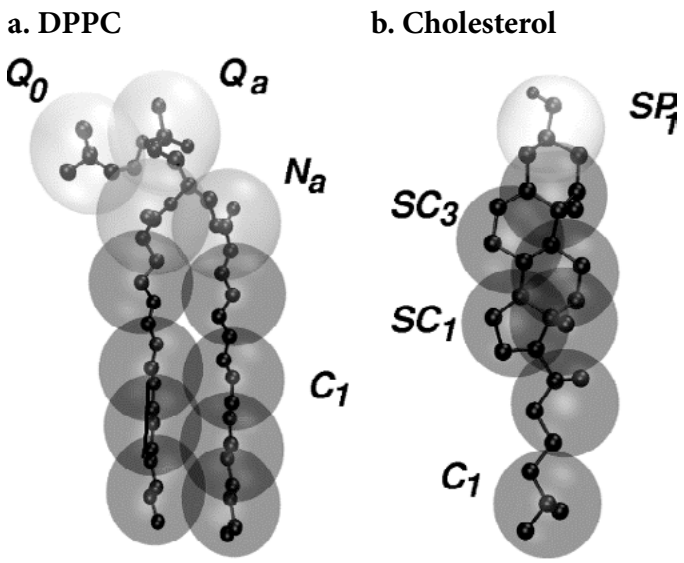


Figure 2. Grouping of DPPC and cholesterol atoms in the MARTINI coarse-grained model (figure adapted from the MARTINI website and ref. 8.) (a) The structure of DPPC and corresponding coarse-grained MARTINI beads shown as transparent spheres. Q_0 is a positively-charged bead corresponding to the choline group, Q_a is a negatively-charged bead for phosphate group. N_a is a bead for the glycerol-ester group and C_1 is an apolar bead used to represent the saturated lipid tails. Coarse-grained DPPC consists of one Q_0, one Q_a, two N_a, and four C_1 beads. (b) The structure of cholesterol is modeled with seven sterol beads (one SP_1, one SC_3, five SC_1) and one apolar bead (C_1). SP_1 is a polar ring bead, SC_1 and SC_3 are apolar ring beads of different hydrophobicities.

one polar ring bead (SP_1), one apolar ring bead (SC_3), five apolar ring beads with increased hydrophobicity (SC_1), and one apolar alkyl bead (C_1).

Parameterization of the interactions between these coarse-grained beads is based on the partitioning free energy between water and organic phases [8]. The MARTINI force field has been extensively tested and verified for accuracy [7-8]. Nonbonded interactions are defined based on the Lennard-Jones potential and are cut off at 1.2 nm and a shift starting at 0.9 nm [8]. Charged particles interact through a Coulomb potential and bonded particles are modeled using harmonic potentials of bond lengths and angles.

The simulations were carried out under NPT ensemble with constant particle number, pressure (1 atm), and temperature (323 K). The pressure was applied semi-isotropically to the coarse-grained lipids and water molecules. The equations of motion were integrated using a leap-frog algorithm.

2.2. Analysis

In order to describe the order of DPPC lipids in each phospholipid-cholesterol system, an average order parameter (S) is computed, where θ is the angle between the bilayer normal and each of the acyl chain bonds, shown in Figure 3 [11]. The order parameter is averaged over all six acyl chain bonds and over all time points. This measurement indicates the degree of alignment of the acyl chains to the bilayer normal: if the acyl chains are more aligned, the bilayer is more ordered. Calculation of the order parameter provides us with a framework to describe whether lipids are in the liquid-disordered or liquid-ordered phase.

$$S = \frac{1}{2}(3\langle \cos^2 \theta \rangle - 1) \qquad (1)$$

In addition to describing the changes in the average order parameter, the probability distribution of the order parameter is computed for each concentration. For each lipid, six order parameters (corresponding to six acyl chain bonds) are calculated, as shown in eq. 1 and their probabilities are averaged, using eq. 2, where *i* is the lipid number, *j* is the acyl bond, and *x* is a variable order parameter.

$$P(S) = \frac{1}{6}\sum_{j=1}^{6} P(S_{ij}(t) = x) \qquad (2)$$

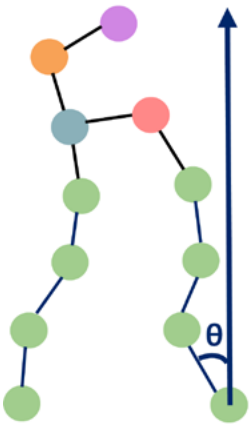


Figure 3. DPPC, represented by coarse-grained beads. Purple corresponds to the choline bead (Q_0), orange represents the phosphate bead (Q_a), blue and pink correspond to two glycerol-ester beads (N_a), and green represents acyl carbon beads (C_1). Angle θ is measured between each of the six bonds connecting acyl carbon beads (dark blue vectors connecting green beads) and the bilayer normal (in our case, bilayer normal is simply the z-axis).

DPPC lipids are then classified based on the number of neighboring cholesterols. A cholesterol is considered to be in the neighborhood of a DPPC if it is in the same leaflet and within 0.8 nm of the DPPC molecule. A cutoff of 0.8 nm is determined based on the distance of the first solvation shell in the radial distribution function between DPPC and cholesterol. The conditional order parameter distribution is calculated for DPPC lipids with 0, 1, 2, and 3 cholesterol neighbors.

In order to quantify the extent of cholesterol's ordering effect on DPPC, a normalized average order parameter (S_{norm}) is calculated with eq. 3. Average lipid order, $S(r,r+\Delta r)$, is computed using eq. 1 for lipids that are a distance *r* to $r+\Delta r$ away from a cholesterol molecule and are in the same leaflet as the cholesterol. To observe enhancement of lipid order over a system with no cholesterol, the average lipid order is normalized by the average order parameter of a system with no cholesterol ($S_{no\ chol}$).

$$S_{norm}(r) = \frac{S(r, r+\Delta r)}{S_{no\ chol}} \qquad (3)$$

The overall organization of the lipid bilayer is described by the radial distribution function, $g_{AB}(r)$, which quantifies the density of atoms of type B a distance *r* away from reference atoms A, normalized by the bulk density of B, provided by eq. 4.

$$g_{AB}(r) = \frac{\langle \rho_A(\vec{0})\rho_B(\vec{r})\rangle}{\langle \rho_A(\vec{0})\rangle\langle \rho_B(\vec{r})\rangle} \qquad (4)$$

3. Results

3.1. Condensing effect of cholesterol

The presence of the condensing effect of cholesterol was explored in our simulations. In Figure 4a, the average area per molecule versus cholesterol concentration, is shown for both the observed data as well as the predicted ideal mixing case. The ideal mixing

illustrates the expected decrease in average area per molecule due to replacement of DPPC molecules with smaller size cholesterol molecules. Based on the simulation data, the average area per molecule decreases with increasing cholesterol concentrations. More importantly, the average area per molecule decreases more than expected through ideal mixing, convincingly illustrating the condensing effect of cholesterol. As the lipids (DPPC and cholesterol) pack closer together with the condensing effect, the average order parameter of DPPC acyl chains, increases with cholesterol concentration until the saturation limit is reached (Figure 4b). This demonstrates alignment of lipid acyl chains to the bilayer normal, thus enhancing the order in the lipid bilayer.

3.2. Order parameter distribution

Next we turn to a more detailed analysis of the order parameter. We calculate the order parameter distribution for systems with no cholesterol (0%), medium concentration of cholesterol (27.8%), and high concentration of cholesterol (55.5%) that is near the saturation limit of cholesterol. Under physiological conditions, eukaryotic plasma membranes usually consist of 20-50 mol% cholesterol depending on the location of the membrane in the cell [23]. We are interested in characterizing how the order parameter changes, specifically whether all lipids become more ordered collectively or whether the percent of disordered lipid decreases. Figure 5 demonstrates a shift in the order parameter distribution as more cholesterol is present in the system. Order parameter of 1.0 indicates perfect alignment of the acyl chain with the bilayer normal, and thus a perfectly ordered bond. Order parameter of -0.5 means that the acyl chain bond is in bilayer plane, implying that the acyl chain is very disordered. As the

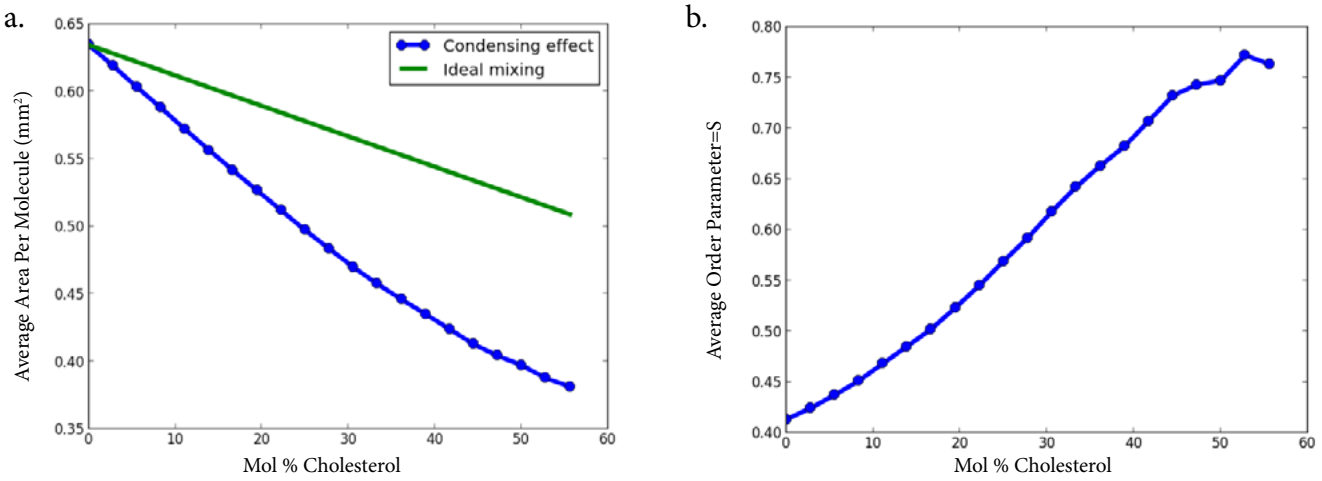


Figure 4. (a) Average area per molecule as a function of cholesterol concentration from simulation results (blue) and the predicted ideal mixing case (green), which was extrapolated as a line based on the last two area per molecule data points that approach cholesterol saturation limit. (b) Average DPPC acyl chain order parameter as a function of cholesterol concentration, calculated using eq. 1.

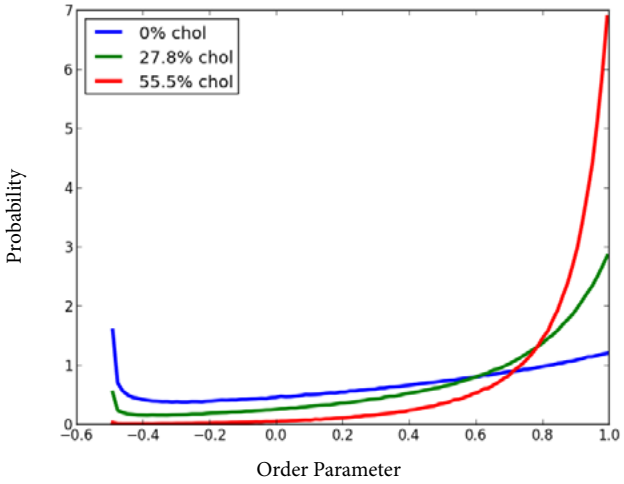


Figure 5. Order parameter distribution for low (0%), medium (27.8%), and high (55.5%) cholesterol concentrations, computed using eq. 2.

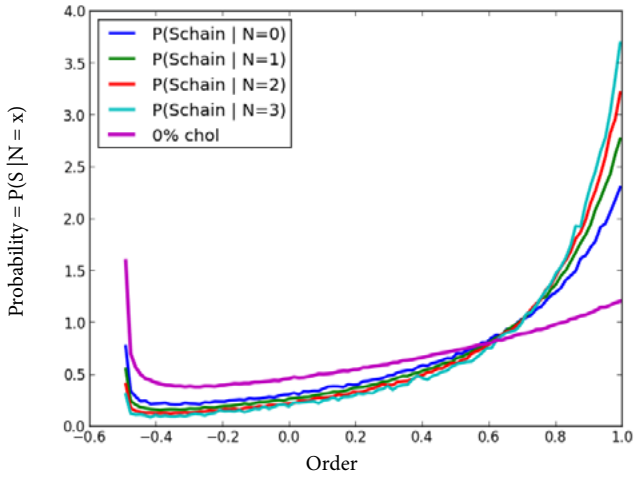


Figure 6. P(S) for 0% cholesterol system (purple) and P(S |N = x) for 27.8% cholesterol concentration where x is the number of cholesterols within 0.8 nm of DPPC.

cholesterol concentration increases from 0% to 55.5% cholesterol, the probability of observing an ordered bond increases and the probability of observing a disordered bond decreases. This type of change in the order parameter distribution suggests that the percent of disordered lipids decreases upon the addition of cholesterol to the system.

In order to analyze the effect of cholesterol on adjacent DPPC lipids, a conditional order parameter distribution was calculated. Each DPPC lipid was classified based on the number of cholesterol molecules in the immediate neighborhood of DPPC (within 0.8 nm of DPPC). Figure 6 focuses on the conditional order parameter distribution for the medium cholesterol concentration (27.8%). This figure details the shifts in the order parameter distribution as the number of neighboring cholesterols (N) increases. In addition, the figure includes the order parameter distribution for the 0% cholesterol system for comparison. Figure 6 clearly shows that as the number of cholesterol neighbors around DPPC lipids increases, the probability that the acyl chain is perfectly aligned with the bilayer normal (order parameter = 1.0) increases and the probability of observing an acyl chain bond in the bilayer plane (order parameter = -0.5) decreases. This indicates that the percent of ordered lipids increases with higher number of cholesterol neighbors. Another observation of interest is the comparison between *P(S|N=0)* and the 0% cholesterol system. Both of these distributions describe lipids with no cholesterol molecules around them. However, the order parameter distribution for *P(S|N=0)* appears to have a higher fraction of ordered lipids than the 0% cholesterol system. This result shows that cholesterol orders lipids beyond its immediate neighboring lipids. This observation is also supported by fluorescence studies, which suggest that cholesterol induces lateral organization in the bilayer [22].

3.3. Cholesterol enhances lipid order for at least two solvation shells

In order to determine the range of cholesterol's ordering effect on DPPC, a system with a single cholesterol in the DPPC lipid bilayer was considered. To quantify the order of the system, the average normalized order parameter of lipids versus their distance away from the cholesterol molecule was computed (Figure 7). The order parameter was normalized against an average order of a 0% cholesterol system, thus any increase from the 1.0 baseline indicates enhanced lipid order. Based on the results presented in Figure 7, the average order of DPPC lipids is enhanced over a distance of approximately 1.5 nm from cholesterol. In order to evaluate this distance in terms of the number of solvation shells around the cholesterol molecule, the radial distribution function, *g(r)*, between cholesterol and DPPC was calculated, where *r* is the radial distance from the OH group in the cholesterol molecule to the PO_4 group in DPPC. The *g(r)* function, shown in Figure 7, describes the density variation of DPPC molecules with respect to the bulk density of DPPC molecules as a function of distance. Three distinct peaks are observed in the *g(r)* function, corresponding to the first, second, and third solvation shells. Combining the normalized order parameter data with the location of the cholesterol's solvation shells, Figure 7 shows that the order of DPPC lipids is enhanced for at least two solvation shells. This result indicates that cholesterol increases the order of the lipids that are its immediate neighbors in the first solvation shell as well as lipids that are further away in the second and third solvation shells.

3.4. Cholesterols prefer few direct cholesterol-cholesterol contacts

After the analysis of a single cholesterol system, we characterize the organization of cholesterols in the DPPC lipid bilayer at low, medium and high cholesterol concentrations (13.89%, 27.8%, and 55.5%). In Figure 8, the *g(r)* based on the distance between the OH groups of the cholesterol molecules was computed, where *g(r)* is proportional to the probability of finding two cholesterols a distance *r* away from each other.

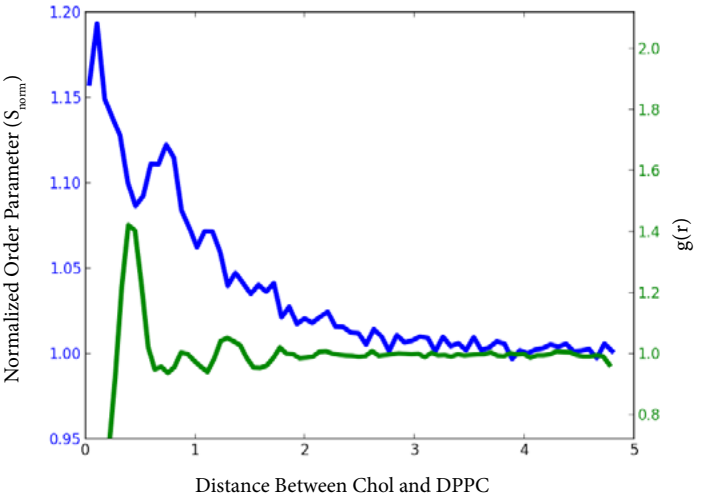


Figure 7. Order parameter of DPPC molecules *r* distance away from single cholesterol, normalized by the average order of a system with no cholesterol (blue), calculated using eq. 3 and *g(r)* between cholesterol's OH group and the PO_4 group on DPPC, calculated using eq. 4 (green).

The *g(r)* function shows two main peaks at 0.5 nm and 1.2 nm, corresponding to the first and second cholesterol solvation shells. At low and medium cholesterol concentrations (13.89% and 27.8%), the probability of finding cholesterols in the first solvation shell, adjacent to each other, is low. Instead, the probability of observing cholesterols in the second solvation shell, further away from each other, is higher. Thus at low and medium cholesterol concentrations, there are few direct cholesterol-cholesterol contacts. As the concentration of cholesterol increases (55.5%) towards the saturation limit, cholesterols are forced to reside in adjacent positions, as shown by a high peak in the first solvation shell.

4. Conclusions

In this article, we explored the molecular basis of the condensing effect of cholesterol and the phase behavior of binary mixtures of cholesterol and DPPC. Based on our simulation data, the average area per molecule decreases with increasing cholesterol concentration more than expected from ideal mixing, thus confirming the existence of the condensing effect of cholesterol on lipid bilayers. The decrease in area per molecule is paralleled by an increase in the average order parameter of DPPC acyl chains, which occurs due to reducing the percent of disordered lipids in the system. This ordering effect of cholesterol on DPPC spans at least two solvation shells, affecting lipids that are beyond cholesterol's immediate neighborhood. Based on this evidence, a cholesterol molecule is likely surrounded by ordered lipids in the bilayer, forming an ordered complex. Since cholesterols prefer few direct cholesterol-cholesterol contacts, it is likely that these ordered complexes do not aggregate together to form a liquid-ordered region. Thus, we found no evidence of a phase coexistence region between liquid-disordered (L_α) and liquid-ordered (L_o) phases. This work presents no evidence for the phase diagram in Figure 1a, a result also found by Waheed et al. [5]. However, due to computational limitations on the size and time scales of the systems, more evidence is necessary to conclusively support a gradual transition from liquid-disordered to liquid-ordered phase, shown in Figure 1b.

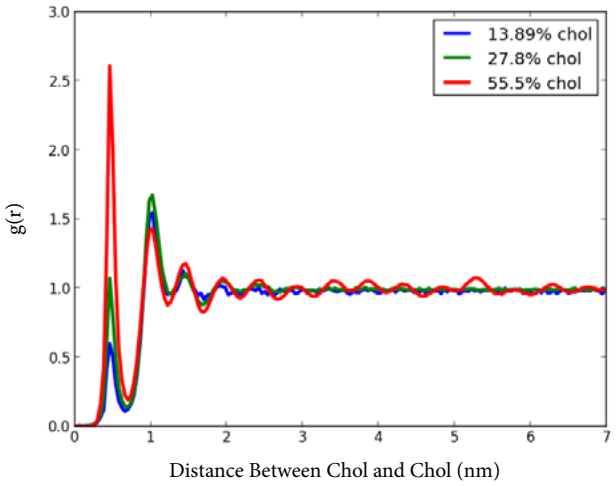


Figure 8. *g(r)* between the OH groups in cholesterols for low (13.89%), medium (27.8%) and high (55.5%) concentrations of cholesterol, calculated using eq. 4.

5. Social Implications

The study of lipid bilayer phase behavior is integral for modeling membrane rafts, which are present in cell membranes. These rafts are known to have an important role in signaling, trafficking, virus budding, endocytosis, and a variety of immune responses [12]. Understanding the formation and disintegration of membrane rafts can give insight into the mechanisms of many membrane signal-mediated diseases and thus the design of more effective therapeutics.

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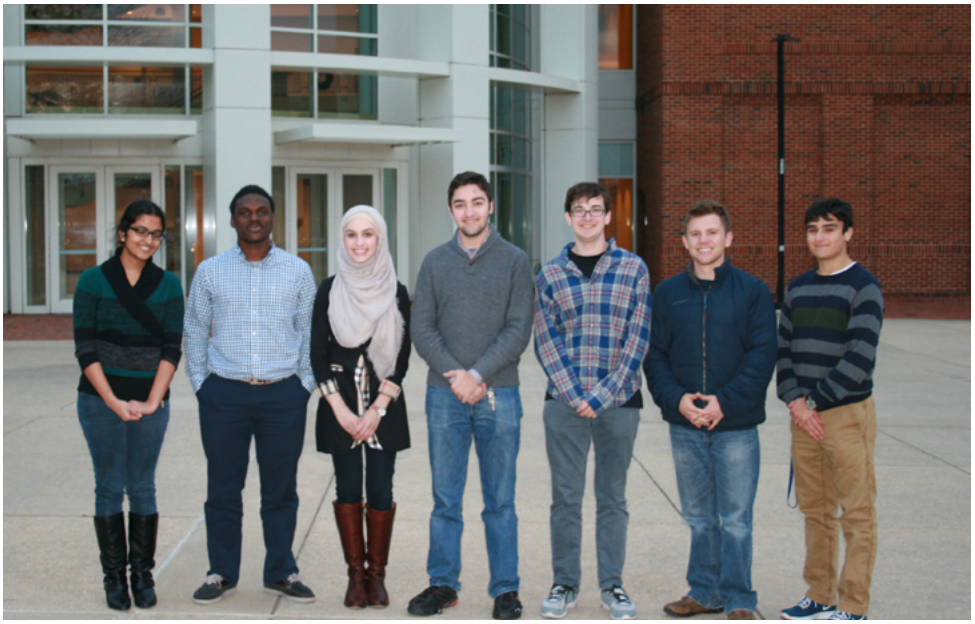

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