Examining the Binding Mechanism and Public Health Implications of Alcohol Consumption and Poisoning

Team DRINK

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ABSTRACT

Alcohol is a commonly abused drug, especially in the United States, which contributes to a death rate of over 2,000 people a year from alcohol poisoning. In particular on college campuses, social binge drinking is prevalent, which can lead to alcohol poisoning, defined as excessive alcohol diffusion into the brain. In the case of alcohol poisoning, ethanol molecules diffuse through the blood brain barrier (BBB) and bind to extrasynaptic gamma aminobutyric acid type A (GABA-AR) receptors which causes neuronal inhibition, nausea, breathing difficulties, and potential death. However, specific binding mechanisms remain unknown. To analyze the biological impacts of alcohol poisoning, we created computational models of the lipid bilayer with an inserted GABA protein and the corresponding ethanol concentration of 0.3 g/dL using molecular dynamics. To examine the social impacts of alcohol consumption and college students' drinking habits, we conducted a survey of undergraduate students at the University of Maryland. After examining the relationship between family history, parental knowledge of alcohol consumption, and drinking frequency and quantity, we elucidated relationships between these variables. By understanding both the biological and social implications of alcohol consumption, future researchers may be able to develop campaigns to prevent deaths from overconsumption of alcohol.

LITERATURE REVIEW

Alcohol is one of the most commonly abused drugs among individuals in a variety of age groups. As of 2018, more than 14 million adults over the age of 18 had suffered from an alcohol abuse disorder at some point in their life (NIAAA, 2019). Additionally, between the years 2000 and 2016, the age-standardized rate of deaths from alcohol related causes rose significantly in the US from an 4.0 per 100,000 deaths in 2000 to 12.0 per 100,000 deaths in 2016, which may be

explained by a breadth of research reflecting trends of higher incidences of alcohol-use disorders and high-risk drinking (Spillane et al., 2020). This mortality from alcohol-related incidents has increased across a broad range of demographics, especially minoritized groups and younger populations. The total annual burden of alcohol use disorder amounts to almost $250 billion in the United States and more than 3 million deaths globally, making research in this area vital to solving this enormous public health crisis (Abrahao et al., 2017).

College students and young adults are at the highest risk of irresponsible drinking habits. Whether one has experienced excessive drunkenness oneself, has had to help an intoxicated peer, or has been a bystander to someone else suffering from intoxication, irresponsible drinking is not a rare situation for college students to find themselves in or around. In a previous study on motives for drinking, it was determined that two motives for heavy drinking are to deal with personal stress and peer pressure in social settings (Abbey et al., 1993). However, these are not the only two reasons why people drink, as many people drink because of the reward pathway that alcohol consumption leads to. According to the National Library of Medicine, “Drinking patterns have changed over time, with the frequency of binge drinking (consuming four/five or more drinks [on a single occasion] for women/men) remaining high (30% to 40%). Young adults in the college age range are developmentally and socially at higher risk for drinking at binge levels” (Krieger et al., 2018). Drinking has become so heavily ingrained in today’s culture that underage drinkers are now at the highest risk for excessive drinking. The National Institute on Alcohol Abuse and Alcoholism states that issues like changes in brain development, unintentional injuries, and even alcohol poisoning are common due to excessive drinking, which is very concerning especially in young populations (Krieger, 2019).

Excessive alcohol consumption is dangerous in a variety of ways, not the least of which is its impact on human inhibition. Excessive drinking results in suppression of the frontal lobe's activity, specifically in decision making. When experiencing effects of intoxication, people are more likely to forget their own limits and become more susceptible to peer pressure. When the frontal lobe is suppressed by ethanol, intoxicated people fail to understand the risks of drunk driving, which contributes to dangerous car crashes. This is reflected strongly in drunk driving statistics in the United States. According to the National Highway Traffic Safety Administration (NHTSA) in 2020, 11,654 people died in drunk driving crashes (*Drunk Driving | NHTSA*, n.d.). Additionally, excessive alcohol consumption over a short period of time, usually just a few hours, can cause the severe health condition known as alcohol poisoning. Alcohol poisoning is characterized by a blood alcohol content of 0.25-0.399% and results in symptoms such as irregular breathing, unresponsiveness, a comatose state, vomiting, lowered body temperature, and more (Alcohol Rehab Guide). Moreover, according to the Alcohol Rehab Guide, untreated alcohol poisoning can result in subsequent life threatening health issues such as hypothermia, hypoglycemia, brain damage, irregular breathing and heartbeat, or in severe cases, death.

Despite the dangers of excessive alcohol consumption, alcohol poisoning is not considered a prevalent problem in America. However, American drinking culture does contribute to alcohol poisoning, which could become a more pressing issue if trends of increasing alcohol use and abuse continue as they are. Drinking culture in America is characterized by social binge drinking (drinking large amounts of alcohol in a short amount of time in social settings such as parties), which can cause alcohol poisoning. This binge drinking mentality is heavily influenced by the types of advertisements that alcohol companies produce to entice their intended audiences to purchase their products. This advertising typically depicts the feelings of youth and heavily

associates drinking alcohol with having fun. This creates a social bias where people feel as though they need to have alcohol at social events, which encourages them to drink. According to the Harvard School of Public Health, extreme forms of binge drinking are increasing on college campuses, while typical binge drinking levels have maintained a steady rate (Wechsler, 2000). In their study of binge drinking, they found that the hubs for binge drinking on college campuses are fraternities, sororities, and athletics departments. Binge drinking within these large groups can easily lead to alcohol poisoning, especially since some college students are under the legal drinking age of 21, and it is possible that these underage students will not seek medical attention out of fear of legal or institutional repercussions.

Alcohol consumption plays a role in other aspects of people's lives beyond just legal and institutional consequences. According to one 2010 study, positive correlations were found for both men and women between having a family history of alcohol abuse and alcohol consumption expectancies, the number of drinks consumed per week, and negative alcohol-related consequences (LaBrie et al., 2010). This indicates that family history can contribute to higher levels of dangerous alcohol-related behaviors. Finally, another factor that influences drinking behaviors is parental messaging around alcohol. A zero-tolerance approach is more protective against college drinking and negative alcohol-related consequences (Abar et al., 2012). Based on this research, we decided to examine the influences of family history and parental knowledge of alcohol consumption on alcohol consumption behaviors in our sample of UMD undergraduate students along with the biological mechanisms by which alcohol intoxication occurs.

CHAPTER 1: COMPUTATIONAL

Introduction

Neuronal Lipid Membrane

Neuronal membranes contain phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), cholesterol, oxysterols and sphingolipids. PI is not commonly found in most human cells, but is concentrated in neural cells. PI is essential for synapse function, as PI plays a role in generating "metabolites" that allow synaptic vesicles to be directed/primed for the presynaptic membrane (Lim and Wenk, 2009). Essentially, PI is an important factor in the synaptic vesicle cycle. PS is primarily found in the inner leaflet of neural membranes and its high concentration is attributed to the inner leaflet's negative charge (as PS is a negatively charged lipid). PS lipids bind to some proteins at their C2 domain, including many important signaling regulator proteins, and this mechanism is done via the surface charge protein (Yeung et al., 2008). Specific to neuronal cells, PS may also act as a storage buffer for fatty acids (Guo et al., 2007). Similarly to PS, PE is primarily found in the inner leaflet of neuronal membranes. PE and PC can both form ether linkages, which form plasmenyl ethanolamine (pPE) that may play a role in membrane fusion and storage of arachidonic acid. PC is highly abundant in lipid membranes, making up about a third of membrane lipids, and is proposed to have a role in membrane structure. Additionally, PC has a role in maintaining membrane fluidity. Specifically, a ratio of PC to PE needs to be maintained to ensure that the membrane does not become too leaky (Lim and Wenk, 2009).

When ethanol is introduced into cell systems, membrane fluidity increases (Da Silveira et al., 2003). This may be due to ethanol interactions with PE and PC lipids or lipid synthesis, throwing off the ratio of PE to PC in the membrane.

GABA and GABA Receptors

Gamma-aminobutyric acid (GABA) is a neurotransmitter that acts as the primary inhibitor in the brain and a major neurotransmitter in the spinal cord (Jewett and Sharma, 2020). After it is synthesized in the cytoplasm of the presynaptic neuron, it is transported in vesicles which are docked in the plasma membrane of cells. After an action potential is reached, the vesicles fuse with the plasma membrane and GABA is released into the synaptic cleft, free to bind with its receptors on the postsynaptic neuron. It can either be degraded extracellularly in the presynaptic neuron or returned back to the glia, where it is degraded by the GABA transaminase enzyme. GABA acts as one of the most important homeostatic controllers of the body, as it prompts the inhibition of many bodily controls. High levels of GABA produce more relaxation and sedation to the body (Hampe et. al., 2017). GABA functions as an inhibitory neurotransmitter because it increases chloride conductance and causes hyperpolarization in the postsynaptic membrane, increasing the amount of stimulus needed for an action potential to occur. GABA receptors have been found to have three distinct classes, A (GABA-AR), B (GABA-BR) and C (GABA-CR) (Lobo and Harris, 2008). GABA-AR and GABA-CR are ligand-gated while GABA-BR is G protein coupled. GABA primarily signals through GABA-AR and GABA-BR. Ethanol has been found to predominantly affect GABA-AR. GABA-ARs are found mostly in postsynaptic membranes and are shown to have subunits which bind differently with various substances (Davies, 2003). GABA receptors, however, are not exclusive. Several modulatory agents, including benzodiazepines and anesthetics, have been found to be able to bind to these receptors. Benzodiazepines are used as positive allosteric modulators, meaning they increase the activity of GABA receptors without directly imitating GABA neurotransmitters. While ethanol has never been found to be a positive allosteric modulator, like

benzodiazepines are, it is known that ethanol has GABA-mimetic effects (Olsen and Liang, 2017).

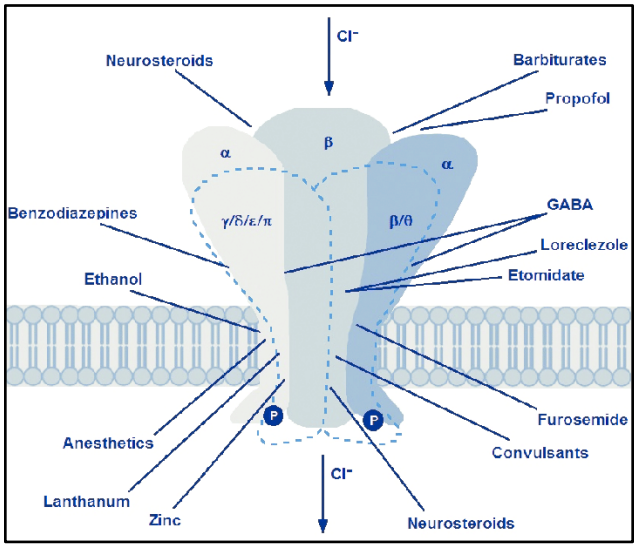

Fig. 1 Schematic representation of GABA-A receptor complex and its subunits (Samardzic & Strac, 2016)

GABA-AR (Figure 1) are pentameric proteins. GABA-AR has at least 2 alpha (α) subunits, and 1 beta (β) subunit. The fourth can vary between a β and theta (θ) subunit and the fifth between gamma (γ), delta (δ), epsilon (ε), and pi (π) subunit. There are 6 different classifications of α subunits, 3 β subunits, 3 γ subunits, and 1 form for the other subunits. These subunits are isoforms and determine the receptor's agonist affinity, chance of opening, conductance, and other properties. Depending on the characteristics of each subunit, GABA-AR affinity with ethanol will be varied. The majority of GABA-A receptors contain 2 α subunits, 2 β subunits and 1 γ or ρ subunit. The most common GABA-AR in mammalian tissue is α1-β2-γ2 (2 α1, 2 β2, 1 γ2), which has been extensively studied in relation to ethanol due to the receptors prevalence in the CNS (Davies, 2003). However, other combinations of subunits have begun to be experimented on to test their responses to ethanol, such as α1β3γ2 (Lobo and Harris, 2008).

One of the other most prevalent forms of GABA-AR in the CNS is α1β3γ2, containing 2 α1 subunits, 2 β3subunits and 1 γ2 subunit (Mortensen et. al., 2012). Despite its abundance in the BBB, there is no literature pertaining to ethanol response, making it a perfect candidate for further study.

Inhibition of High Affinity Ethanol Binding Sites

Ethanol has been noted as having several mechanisms within the brain that modulate brain chemistry to produce the state of "being drunk." However, the most notable danger in this case is the interactions of ethanol with neurons containing GABA receptors. GABA is primarily responsible for producing the "rest and relaxation" state as the major inhibitor via the parasympathetic nervous system. As GABAergic activity increases, the activity of other neurons that happen to fall within the GABA signaling pathway to fire is reduced. Ethanol acts as an agonist, increasing the chances of firing for GABAergic neurons (Valenzuela, 1997), so it produces a state of relaxation, which can have dangerous results. Through this GABA pathway, alcohol functions as a major depressant within the nervous system. Our concern with this function of alcohol is that, at very high concentrations of ethanol, alcohol depresses processes integral to life to the point of death. In approximation, blood alcohol concentrations (BAC) above 300 mg/dL (BAC = 0.3%) of alcohol can result in severely slowed respiration, hypotension, hypothermia, and coma. Concentrations above 400 mg/dL (BAC = 0.4%) can be fatal (McIntosh & Chick, 2004).

This portion of the project aims to develop an in silico model of a lipid bilayer membrane in the brain to study ethanol-GABA and ethanol-lipid effects. We will perform molecular dynamics simulations to examine two research aims: 1) how inserting ethanol impacts bilayer properties and 2) how inserting ethanol influences protein-ethanol interactions. For the first

research aim, we theorize that ethanol will increase DPPC and DPPE aggregation. Past research used nuclear magnetic resonance (NMR) to examine the interaction of ethanol with phospholipid bilayers. Ethanol was found to strongly interact with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) bilayers (Barry & Gawrisch, 1994). More specifically, ethanol binding altered the orientation of lipid headgroups resulting in lipid disordering. This disordering behavior corresponded with an increase in the number of lipids per area ratio. For the second research aim, we theorize ethanol will interact with serine and threonine more frequently than other residues via hydrogen bonding (Kruse et al., 2003).

Methods

Bilayer Creation

The neuronal lipid ratios that were utilized were adapted from Ingolfsson et al., (2017). The cholesterol concentration was reduced from 50% (Ingoldsson et al, 2017) to 25% of the total lipid number according to work from Dietschy & Turley (2004). We did not use the original 50% cholesterol ratio from Ingolffsson et al., (2017) due to concerns that it may adversely impact the membrane fluidity. The full lipid composition and their ratio in the upper/lower leaflets for each simulation are listed in Tables 1 & 2. The bilayer was primarily composed of dipalmitoylphosphocholine (DPPC), dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidylinositol (DPPI), dipalmitoylphosphatidylserine (DPPS), dipalmitoylphosphate (DPPA), and cholesterol (CHOL).

Table 1.1 Composition of bilayer, no ethanol.

Lipid	Outer Layer (#)	Inner Layer (#)
DPPC	268	72
DPPE	240	228
DPPI	0	24

DPPS	0	228
DPPA	0	60
CHOL	288	144

Table 1.2 Composition of bilayer with protein, no ethanol.

Lipid	Outer Layer (#)	Inner Layer (#)
DPPC	276	72
DPPE	240	228
DPPI	0	24
DPPS	0	228
DPPA	0	60
CHOL	288	144

Protein Insertion

Protein 6I53, a cryo-EM structure of the human synaptic alpha1-beta3-gamma2 GABAA receptor in a lipid nanodisc, was retrieved from the Protein Data Bank (PDB) and used as the starting protein structure. We removed the attached megabody38 complex using PyMOL to isolate the protein receptor.

Ethanol Concentration

We used a concentration of 0.3 BAC (blood alcohol concentration, mg/dL) to model the condition of as our point of alcohol poisoning and extreme intoxication (McIntosh & Chick, 2004) which was obtained by calculating and determining the number of ethanol molecules based off of the number of water molecules in each simulation, shown in Tables 3 & 4.

Table 2.1 Composition of bilayer with ethanol.

Lipid	Outer Layer (#)	Inner Layer (#)
DPPC	268	72
DPPE	240	228

DPPI	0	24
DPPS	0	228
DPPA	0	60
CHOL	288	144
ETHANOL (E)	8	9

Table 2.2 Composition of bilayer with protein and ethanol.

Lipid	Outer Layer (#)	Inner Layer (#)
DPPC	276	72
DPPE	240	228
DPPI	0	24
DPPS	0	228
DPPA	0	60
CHOL	288	144
ETHANOL (E)	24	25

Simulation Parameters

Four lipid bilayer systems (bilayer, bilayer with ethanol, bilayer with protein, bilayer with protein and ethanol) were first built with the CHARMM-GUI Martini Marker Bilayer Builder. They were simulated with coarse-grained molecule dynamics using Gromacs 2019 package (Lindahl et al., 2018). Martini's coarse-grained force field version 2.2 was used for amino acids while version 2.0 was used for lipids and non-polarizable water (Arnarez et al., 2015; de Jong 2012 et al., 2012). Both bilayer and bilayer with ethanol systems were solvated in a 18.39 x 18.39 x 10.06 nm^3 cubic box with 14890 water molecules and neutralized with 372 sodium ions. Both bilayer with protein and bilayer with protein and ethanol systems were solvated in a 20.13 x 20.13 x 17.33 nm^3 cubic box with 42449 water molecules and neutralized with 352 sodium ions. The system was minimized using the steepest descent method, followed by isochoric-isothermal (NVT) and isobaric-isothermal (NPT) runs. There were two minimization runs and four

equilibration runs. Pressure for NPT was set to 1 bar. All subsequent production runs were run for 3 μs, at 303.15 K, pressure of 1 bar, and a timestep of 0.02 ps with the exception of bilayer with protein, which was run for 3.003 3 μs. The last 3 μs of the bilayer with protein run were used for analysis. The systems' compressibilities were 3 x 10^{-4} bar^{-1} for all simulations. The cutoff for short range Coulomb and Vander Waals interactions was 1.0 nm. Pressure and temperature was controlled using Parrinello-Rahman barostat and V-rescale thermostat.

Analysis

The lipid aggregation plots were generated using a Python script which counted the number of contacts between coarse-grained beads that if they were within a 7 angstrom distance of each other. Root Mean Square Deviation (RMSD) and partial density plots were generated using the gmx_rms and gmx_density tools in Gromacs 2019. All images were visualized using Visual Molecular Dynamics (VMD) 2022 (Humphrey et al., 1996). Frequency polygon plots were generated using Google Sheets.

Results

Research Aim One: Examining Ethanol-Lipid Interactions and Lipid-Lipid Aggregation

Frequency polygon plots were generated to determine the number of ethanol-lipid contacts for the bilayer with ethanol model and the bilayer-protein with ethanol model. Analysis was split based on leaflets due to the difference in bilayer composition (See Tables 1.1-2.1). In the upper leaflet, ethanol was observed to have the highest number of contacts with DPPC and DPPE. Ethanol was also observed to have a higher number of contacts with DPPC, more frequently, in the presence of the GABAA protein versus no protein.

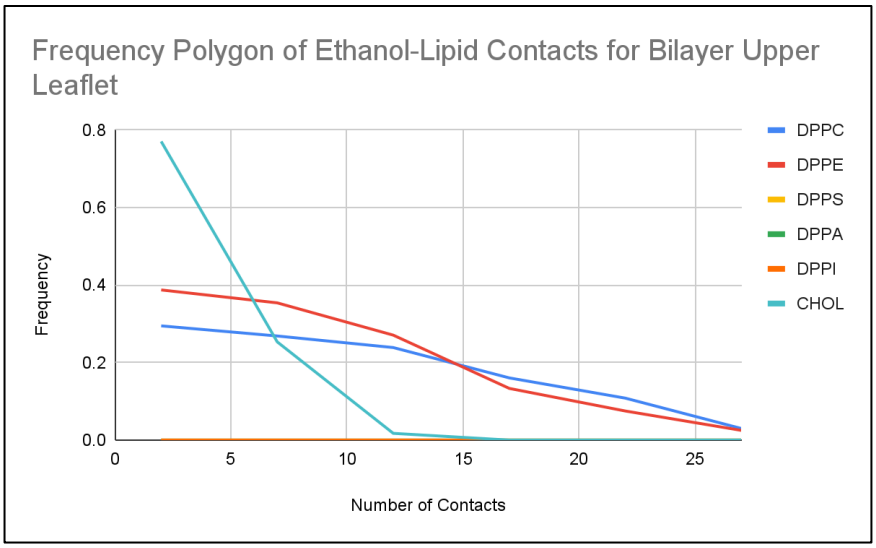

Fig. 2.1 Frequency polygon plot of ethanol-lipid contacts in upper leaflet of bilayer model.

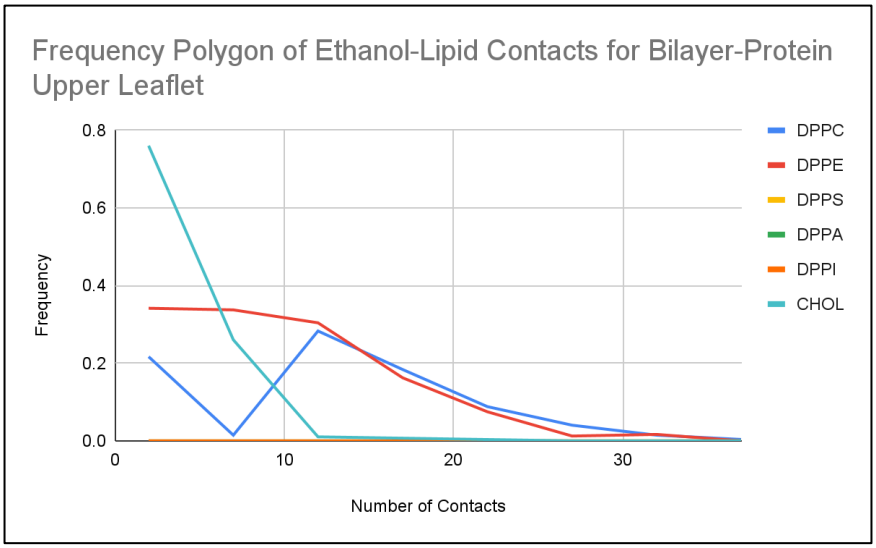

Fig. 2.2 Frequency polygon plot of ethanol-lipid contacts in upper leaflet of bilayer with protein model.

In the lower leaflet, ethanol was observed to have the highest number of contacts with DPPE and DPPS. Ethanol was also observed to have a higher number of contacts with DPPE and DPPS, more frequently, in the presence of the GABAA protein versus no protein.

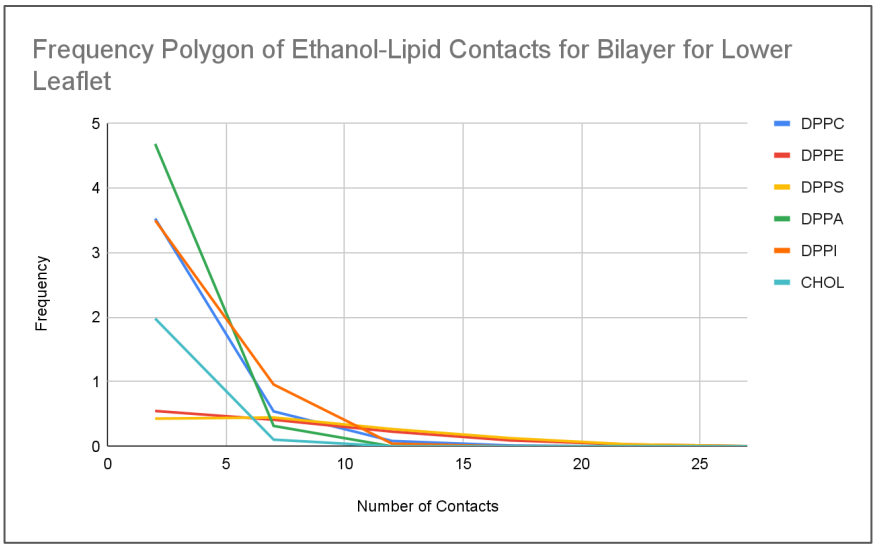

Fig. 3.1 Frequency polygon plot of ethanol-lipid contacts in lower leaflet of bilayer model.

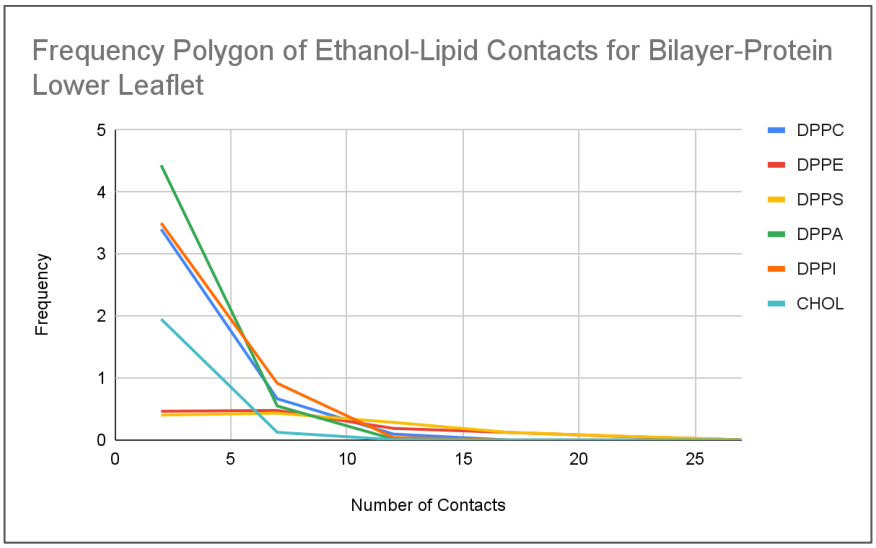

Fig. 3.2 Frequency polygon plot of ethanol-lipid contacts in lower leaflet of bilayer with protein model.

The subteam visualized the bilayer with ethanol simulation and bilayer-protein with ethanol simulation in VMD to see if specific lipids were aggregating in the leaflets or around the protein respectively. For the bilayer with ethanol simulation, there was no clear lipid aggregation in the upper or lower leaflets observable to the human eye. For the bilayer-protein with ethanol

simulation, in the lower leaflet, DPPE and DPPS appeared to aggregate around the GABAA protein by the 3 μs timestamp.

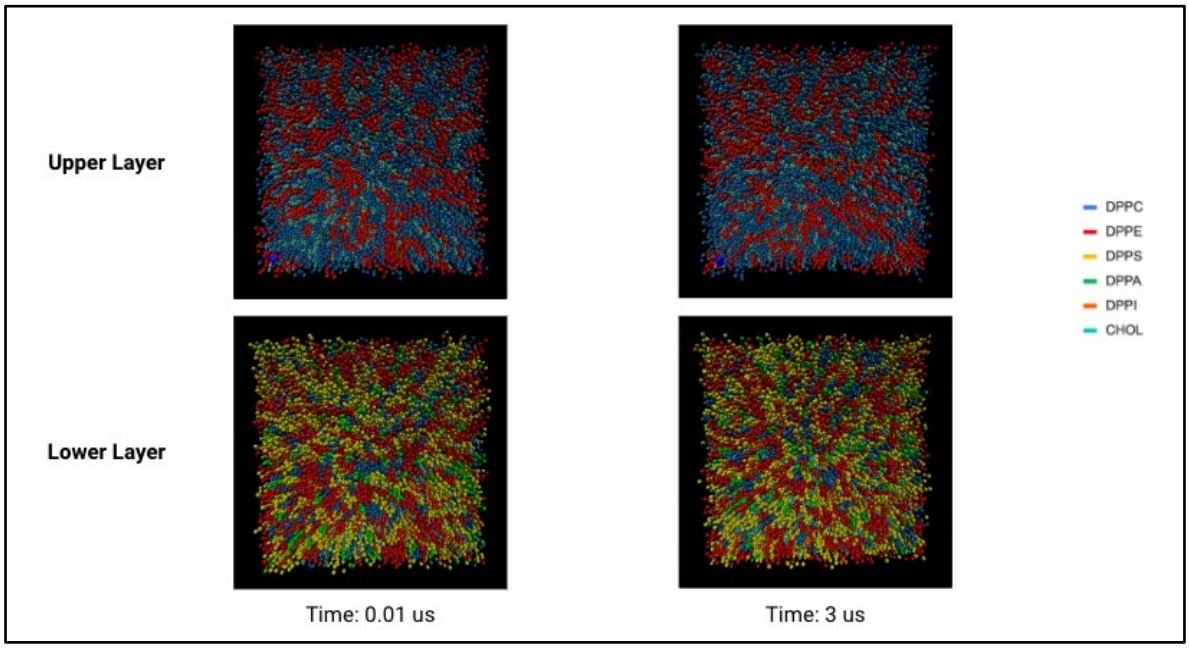

Fig. 4.1 Bilayer with ethanol simulation split by leaflet at 0.01 μs and 3 μs.

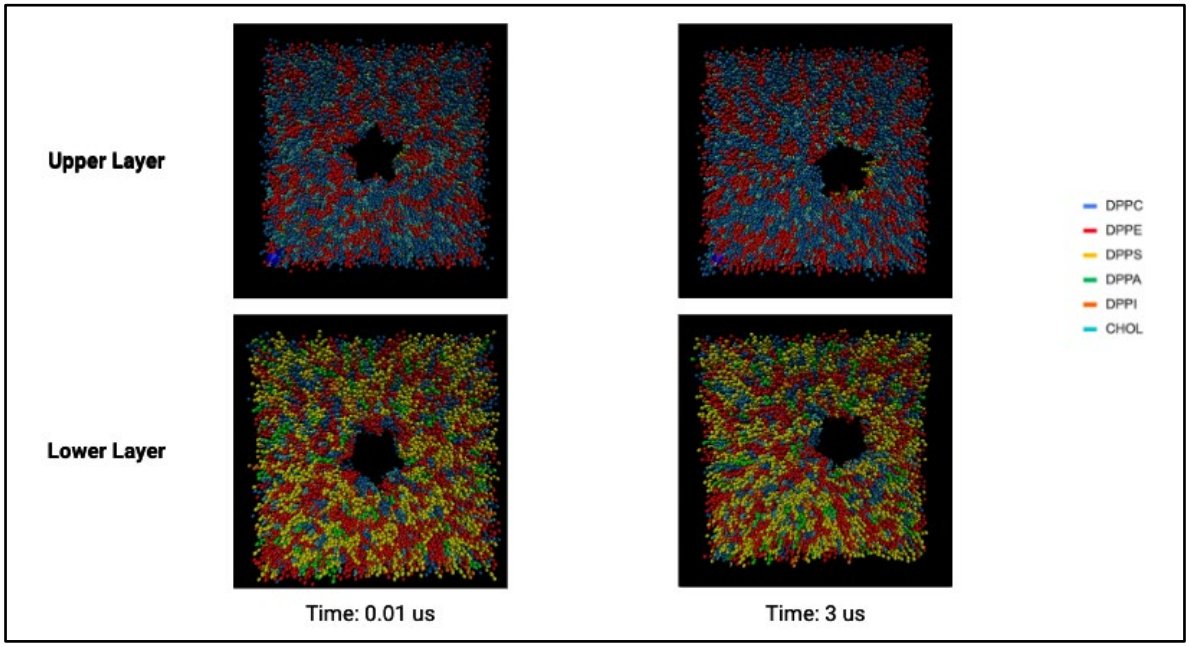

Fig. 4.2 Bilayer-protein with ethanol simulation split by leaflet at 0.01 μs and 3 μs.

Taking into consideration the higher interactions between ethanol and DPPE/DPPS compared to other lipids as well as changes in lipid behavior seen visually, lipid aggregation plots were generated for DPPE and DPPS for all four simulations. In Figs. 5.1 and. 5.2, there are clear changes in lipid aggregation when comparing simulations with and without ethanol. In Fig. 5.1, DPPE aggregation plateaued over time for the bilayer with protein simulation, but sharply increased over time for the bilayer with protein and ethanol simulation. In Fig. 5.2, DPPS aggregation increased over time for the bilayer simulation, but sharply decreased for the bilayer with ethanol simulation. A similar downward trend in DPPS aggregation was observed for the bilayer with protein and ethanol simulation.

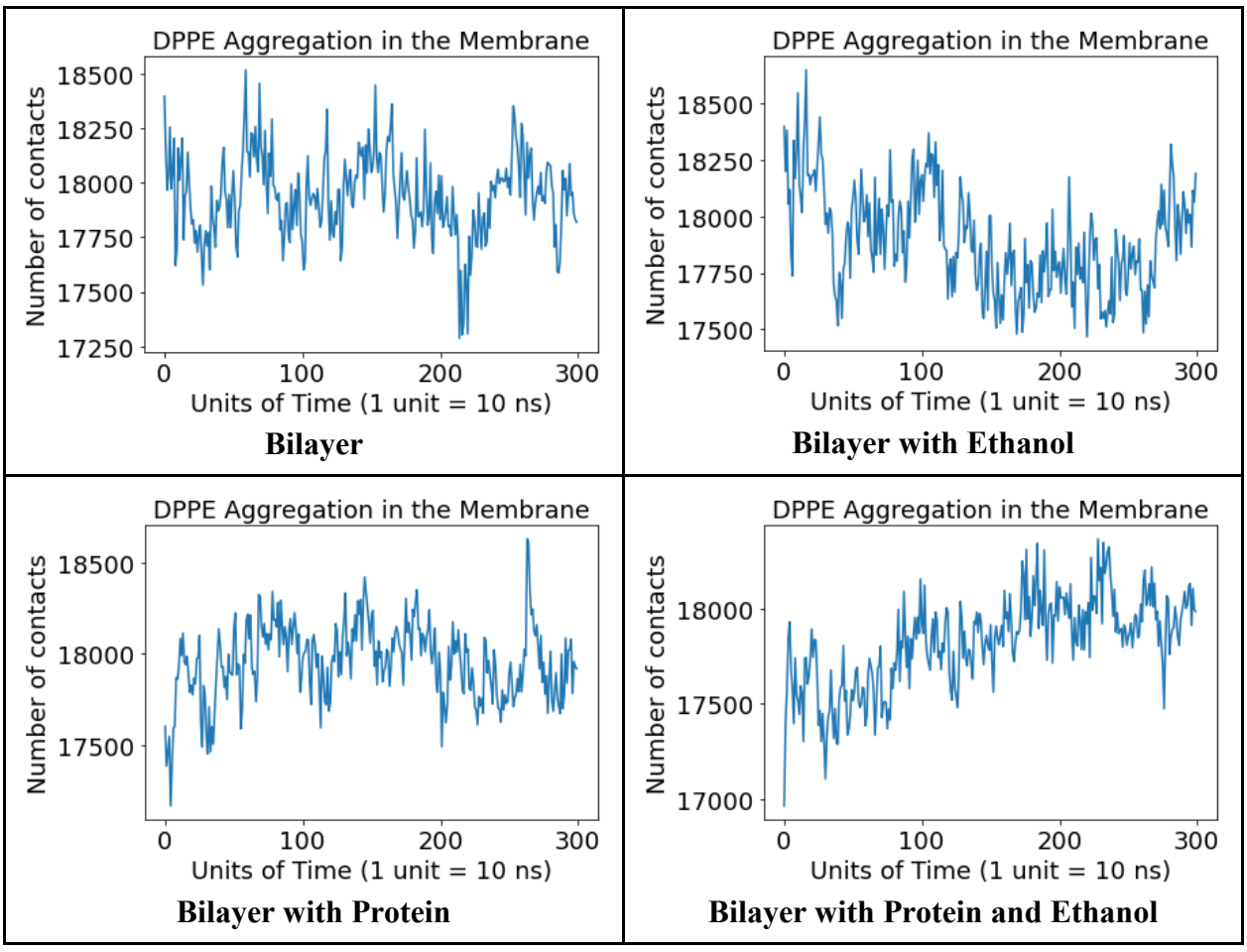

Fig. 5.1 DPPE aggregation in the bilayer, bilayer with ethanol, bilayer with protein, and bilayer with protein and ethanol simulations.

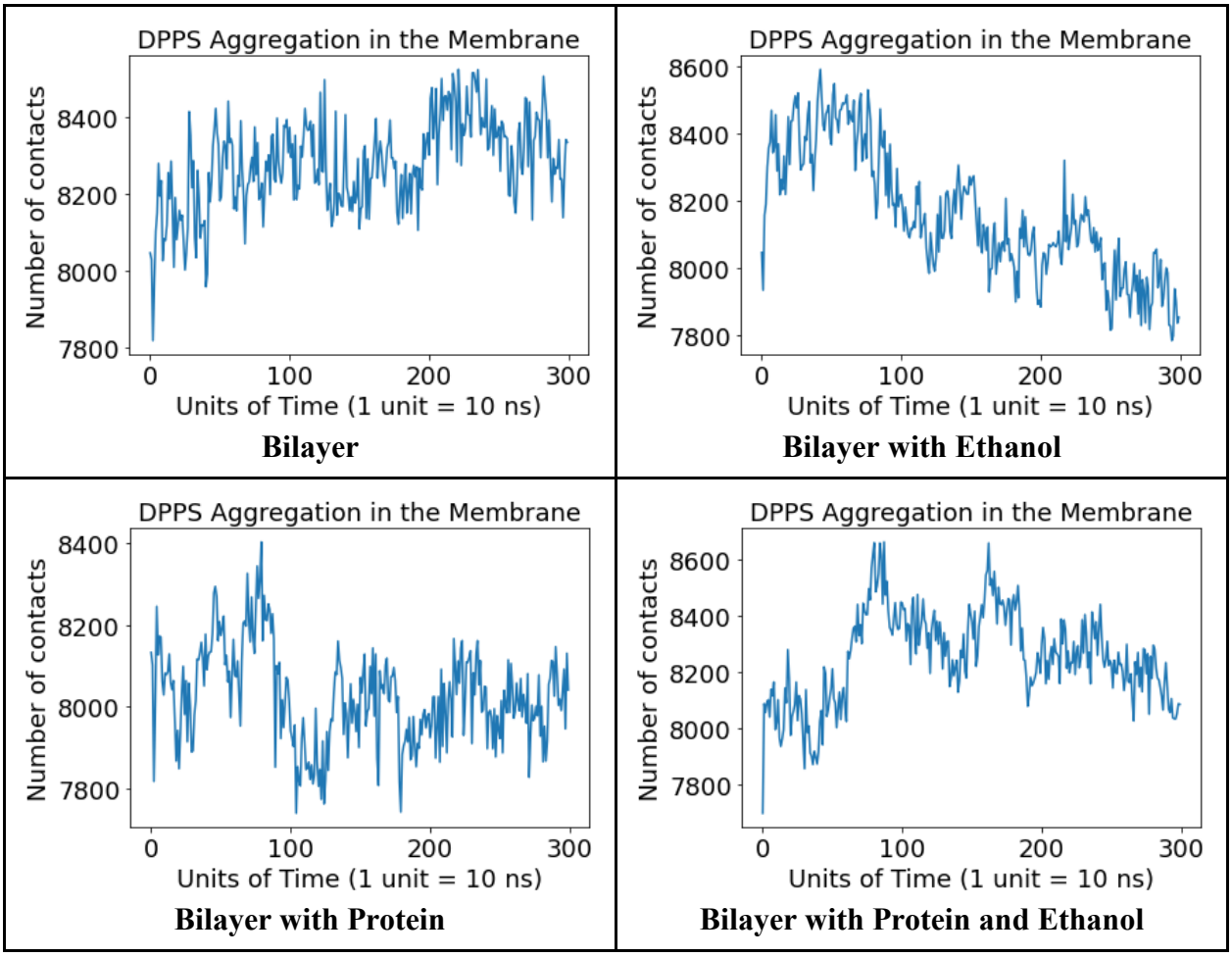

Fig. 5.2 DPPS aggregation in the bilayer, bilayer with ethanol, bilayer with protein, and bilayer with protein and ethanol simulations.

Frequency polygon plots were generated to determine the number of protein-lipid contacts for the bilayer-protein model and the bilayer-protein with ethanol model. While no significant changes were observed in the upper leaflet, there was a clear change in the number of protein-lipid contacts for DPPE and DPPS in the lower leaflet. As seen in Fig. 6, the number of protein-DPPE contacts increased with the addition of ethanol. Meanwhile, the number of protein-DPPS contacts slightly decreased with the addition of ethanol.

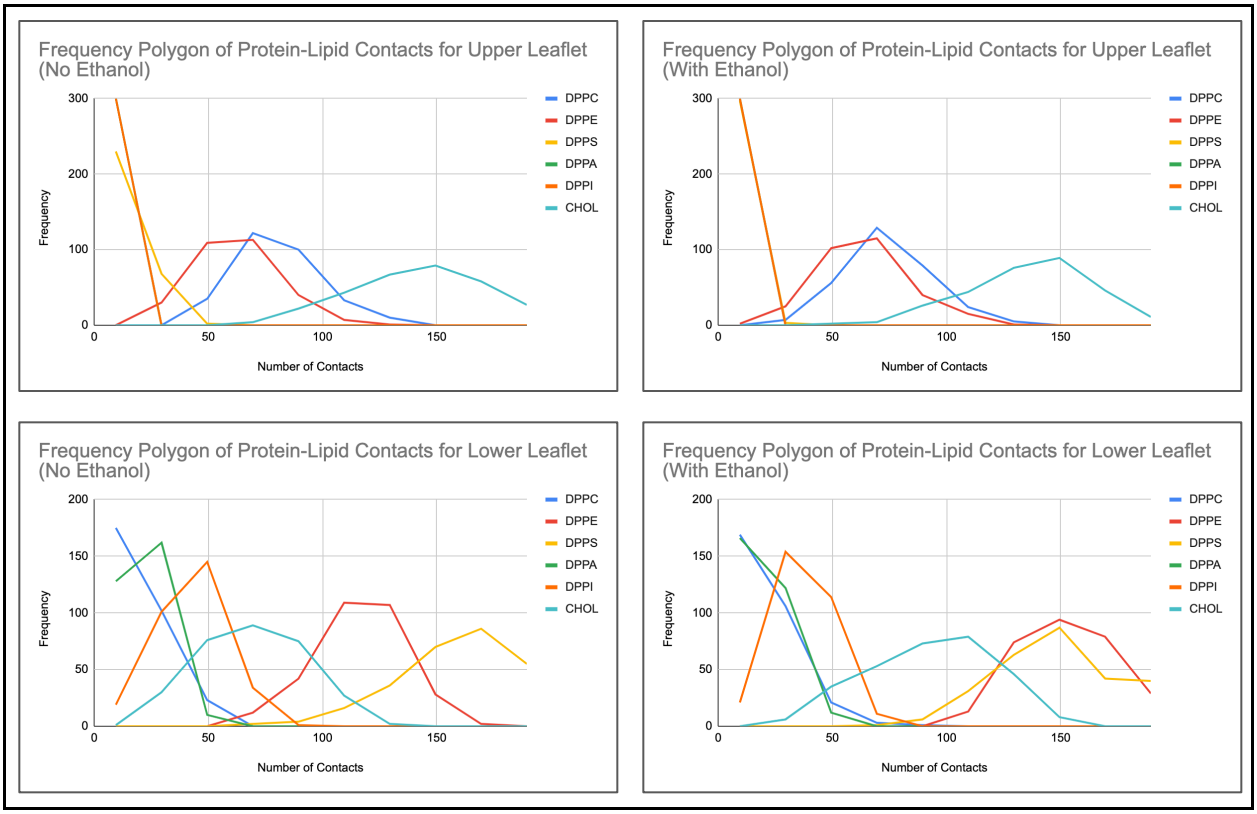

Fig. 6 Frequency polygon plots of protein-lipid contacts split by leaflet for the bilayer with protein model (no ethanol) and bilayer with protein and ethanol model.

Research Aim Two: Examining Ethanol-Protein Interactions

The bilayer-protein with ethanol simulation was visualized in VMD. As seen in Fig. 7, the GABAA protein's backbone and all ethanol beads were isolated from other elements in the system. By running the simulation, subteam members were able to select for specific amino acids in the backbone which appeared to interact with the ethanol beads. The subteam identified a possible binding pocket in the protein consisting of serine, alanine, arginine, tryptophan, and phenylalanine residues.

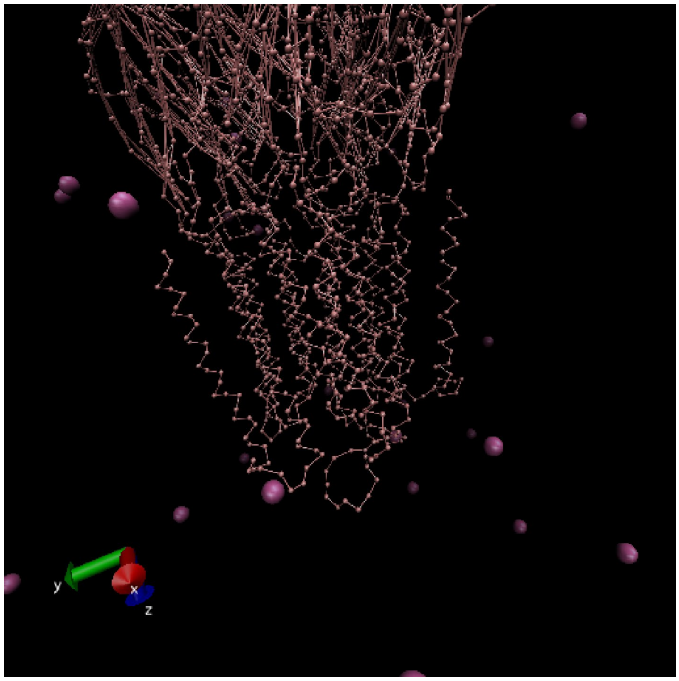

Fig. 7 Bilayer with protein and ethanol simulation at 0.01 μs. Selections made for the GABAA protein backbone and ethanol beads (purple).

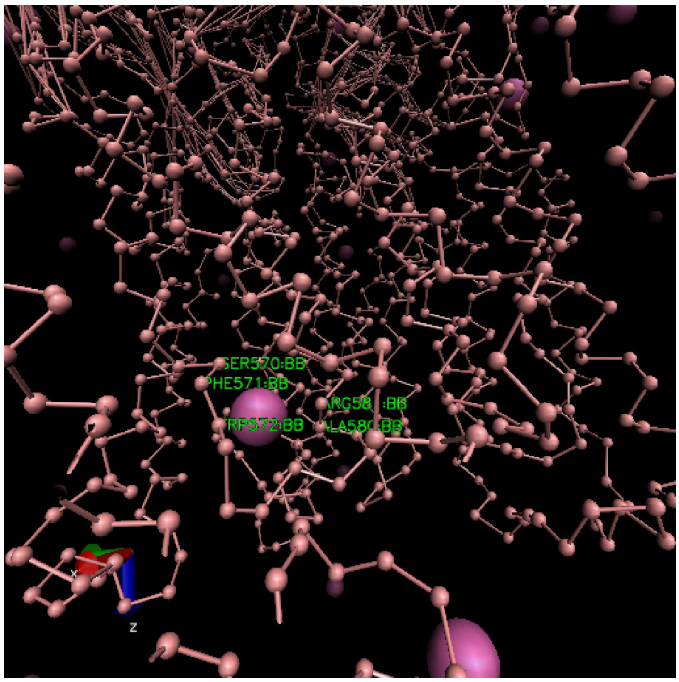

Fig. 8 Bilayer with protein and ethanol simulation at 3 μs. Selections made for serine, alanine, arginine, tryptophan, and phenylalanine residues.

A frequency polygon plot was generated to determine the number of ethanol-amino acid contacts for the previously listed residues. Ethanol was found to have the highest number of contacts with serine, tryptophan, threonine, and phenylalanine compared to other residues.

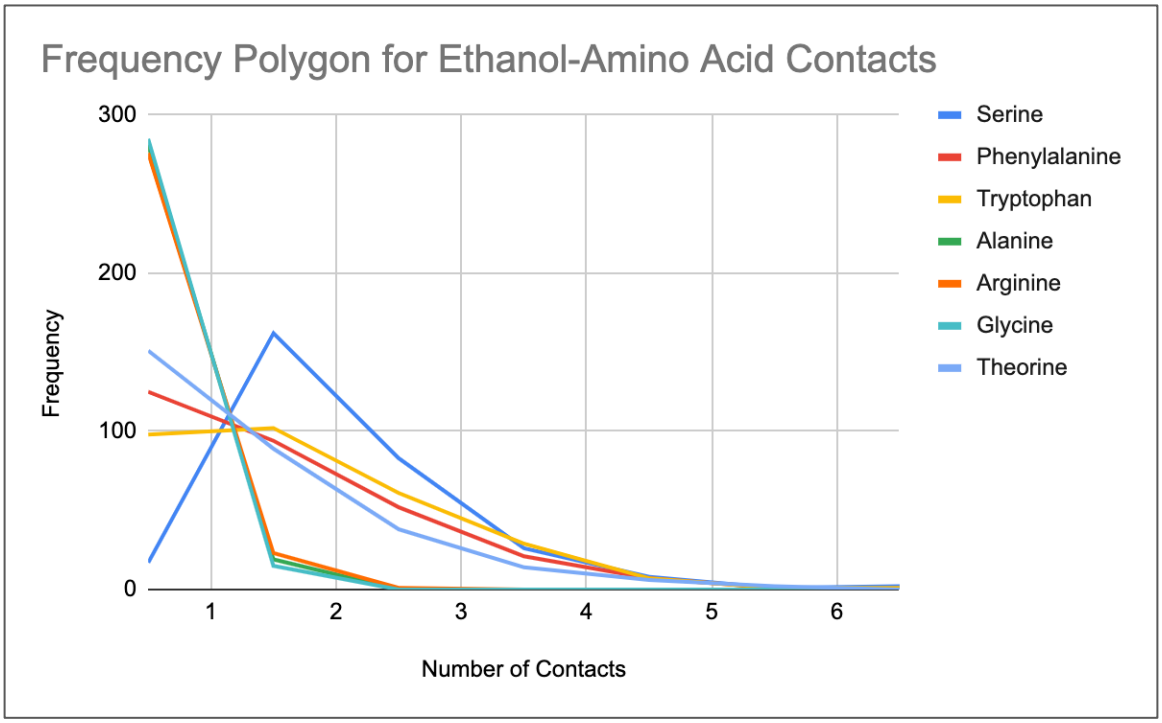

Fig. 9 Frequency polygon plot of ethanol-amino acid contacts for the bilayer-protein with ethanol model.

Discussion

Prior research suggests that ethanol exerts effects on both membrane lipids and proteins, which can in turn alter GABA receptor function (Peoples et al., 1996). Overall, DPPE and DPPS were two lipids observed in our simulation to change in the presence of ethanol. Our results show a decrease in DPPS aggregation when ethanol is introduced into the bilayer, but an increase in DPPS aggregation when ethanol is introduced to the protein embedded in the bilayer. The same trend is seen on DPPE. This suggests that the presence of GABA-AR embedded in the protein may promote lipid contacts, likely due to lipid's roles in neurotransmission.

Neurotransmitters are known to be more attracted to membranes that contain anionic lipids, such as DPPS, rather than neutral membranes, even including GABA. GABA is hydrophilic, which would typically not adhere to the lipid membrane, but is attracted to membranes containing DPPS regardless (Postila et. al., 2019). It's possible that DPPS aggregates near GABA to promote GABA binding to GABA-AR. However, ethanol is easily transferred through the blood brain barrier since it is lipophilic, which is congruent with our results that show aggregation decreasing when ethanol is introduced into the system with only the bilayer. A possible explanation for ethanol increasing DPPS and DPPE aggregation in the protein-bilayer simulation is that the aggregation response is signaled by the GABA-AR protein rather than the molecules themselves. If ethanol typically downregulates DPPS and DPPE aggregation, then it should downregulate aggregation with the protein inserted. However, we observed upregulation of DPPE and DPPS aggregation when ethanol was introduced into the protein-bilayer, suggesting the receptor itself may have a role in lipid aggregation.

Among common targets of ethanol, such as ligand-gated ion channels (e.g., GABA-AR), we can observe a common theme in the amino acids that ethanol prefers. Many of ethanol's known binding sites to proteins contain pockets with multiple α helices that often contain serine and threonine residues (Harris et al., 2008). As seen in Figure 8, the ethanol bound in the pocket of our protein has high contacts with both serine and threonine, as well as tryptophan and phenylalanine. It's possible that the ethanol was attracted to the serine and threonine in the binding pocket and the increased contacts with tryptophan and phenylalanine is due to their proximity to the serine and tryptophan residues.

While the exact downstream effects on GABA-AR binding and function from ethanol-lipid interactions is unknown, proposed mechanisms of ethanol action include increasing the

disordering of lipids in the membrane which may impact ion transport and neurotransmitter release (Strong and Wood, 1984), decreasing the temperature which lipids membrane transition phases which would decrease ion channel function, and disruption of hydrogen bonds between proteins and the lipid-head groups (Peoples et al., 1996). Prior spectroscopy work on ligand-gated ion channels, namely nicotinic acetylcholine receptors which share similar structural features as GABA-ARs, show that changes in lipid environment can lead to desensitization—when receptor responses decrease—conformational change in the protein (Baenziger et al., 2000). Ethanol binding itself can also lead to GABA-AR desensitization, but this effect may also vary based on the subunit combination (Dopico & Lovinger, 2009).

Limitations

There are a few major limitations to our research that should be addressed in future studies. Our system models the surrounding fluid as almost entirely water, which is not accurate to the actual molecular makeup of the space around the neuronal membrane. Since our system does not mimic the exact conditions of the brain during alcohol intoxication, it is possible that GABA-AR's response to ethanol could be impacted by factors in the bloodstream or extracellular fluid. As mentioned in the introduction, GABA-AR is a chlorine ion channel. Our simulation did not include any chlorine ions, so future studies should include chlorine to determine how/if ethanol interacts with the chlorine influx causing neuronal excitation. Our simulation did not include multiple runs, meaning that observations from our results may not be reproducible since we were not able to gather data over repeated simulations and compare the ethanol response. The protein that we used within the protein data bank was in complex with Megabody38, which may have caused conformational changes not represented in GABA-AR naturally embedded in the BBB. Due to these conformational changes, ethanol response

(specifically potential protein conformational changes caused by ethanol interactions) may be different from how GABA-AR typically responds in the BBB. It is also possible that GABA-AR responds differently at different concentrations of ethanol. Our model only observed dynamics at a BAC of 0.3%, which is noted at the concentration of ethanol in the blood where symptoms associated with alcohol poisoning begin to occur. At 0.4% (lethal levels of ethanol), GABA-AR may interact differently with ethanol as physiological changes occur within the body.

In the future, it would be beneficial for our experiment to be repeated with multiple runs using the same parameters to ensure that the response to ethanol observed is significant and measure the error. Additionally, the conditions of the surrounding area of the BBB should be mimicked to determine if ethanol response is impacted by external factors in the human body. As mentioned before, GABA-AR may respond differently at varying concentrations of ethanol, so simulations should be done at concentrations of ethanol greater and less than 0.3% BAC. If these findings are significant, determining a way to prevent ethanol response or block ethanol binding to GABA-AR receptors could be done to potentially discover a way to treat/prevent alcohol poisoning. Our model also only contains sodium ions and lacks chloride ions, which are the primary ions that pass through the GABA-AR ion channel. Thus, it is difficult to examine properties of the channel such as agonist effects.

Future Directions and Industry Impact

Currently, the binding of ethanol and the role of other compounds such as neurosteroids within GABA receptors are not well-understood, remaining an area of interest for scientists interested in alcohol-use disorders (Olsen et al., 2017). While there is a large body of research examining the role in increased ethanol concentration in GABAergic neurotransmission, many questions remain such as which GABA receptor subunits are sensitive to differing concentrations

of ethanol. Additionally, current medications for alcohol-use disorders can contain serious side effects which impact patient health. Understanding receptor function and binding mechanisms will not only allow for the discovery and development of more efficient drugs, but also help link specific behavioral actions resulting from alcohol to receptor action (Lobo & Harris, 2008). We hope that our model will serve as a prototype for future, more complex models of ethanol diffusion and binding in the brain.

CHAPTER 2: PUBLIC HEALTH

Introduction

The study's main objective was to comprehend the patterns of alcohol intake among UMD students. Instead of drawing conclusions about particular variable connections, the objective was to learn more about the study's participants: the students. In order to investigate potential causes for why some people drink more than others among UMD students, we selected to distribute a survey pertaining to alcohol consumption patterns across campus. As alcohol is such a commonly abused drug on college campuses, the goal was to understand drinking on our campus since this is a community very close to us. Based on prior evidence, we hypothesized that there would be relationships between variables including family history and parental knowledge of alcohol consumption, and these along with other factors may influence students' drinking behaviors.

Methods

Survey Creation and Distribution

To collect data on the population of undergraduate University of Maryland students, the public health subteam began by creating a Qualtrics survey consisting of three parts. Survey 1 (Appendix A) was a consent survey including an age acknowledgement that the participant was over 18 years old. Survey 2 (Appendix A) contained 34 questions pertaining to alcohol consumption and behavior questions and self-reported demographic information. For most questions, participants were given an N/A or preferred not to answer option to give them the option to avoid sharing information they were uncomfortable with sharing. Survey 3 (Appendix A) served to obtain contact information from participants so they could be compensated. In order to protect respondents anonymity, the three surveys were disconnected from each other. In

Qualtrics, IP address tracking was turned off and participants had to click to proceed to an entirely new survey once they completed the one prior to it. For example, if participants were not 18 years old as confirmed on Survey 1, they could not proceed to Survey 2 to answer the questions about alcohol consumption behaviors. The results from each survey cannot be connected to the personal identifying information that was collected in surveys 1 and 3. In order to compensate participants, an incentivized raffle was conducted, and a random number generator was used to select 40 participants to receive a $25 Terrapin Express credit (UMD specific debit account).

Once the survey was created based on questions previous studies had asked and based on what we wanted to know about UMD students' behavior, we went through an Institutional Review Board (IRB) proposal and approval process to ensure that the study being conducted with human subjects was being conducted correctly. The IRB process began in September 2021 and the study was approved in February 2022. Once IRB approval was obtained, the survey was distributed to the wider campus.

Three main methods were used to distribute the survey: word-of-mouth, social media, and physical flyers placed around campus. Each member of the full team was instructed to send the survey link in any campus group chats they were part of, including those for clubs and classes. The survey was also advertised on the Gemstone listserv and the College of Mathematics and Natural Sciences (CMNS) listserv. Because team members were involved in diverse activities, this increased the diversity of responses. A digital flyer (Appendix B) for the survey was posted on the Team DRINK Instagram page on February 18, 2022. Finally, physical advertising posters were placed on campus in various places, including educational buildings, the student union, apartments, and in various bathrooms around campus. Because the team was

composed entirely of women, the physical flyers were only able to be put up in women's bathrooms, which affected the demographic split of the respondents, skewing the team to have approximately 1.5 times as many women respondents as men.

Data Analysis

All data was stored in UMD Box for security purposes. To work with the data, we first removed all respondents who answered "other" to the year in college question so our data was representative only of undergraduates at UMD. Our total sample size was 189. To code drinking frequency consistently, all data was converted to times per month and if that produced a range, the higher value of the range was taken (ex. 2-3 times per week = 8-12 times per month = 12 for new freq.). For respondents who reported drinking on special occasions, their drinking frequency was recorded as 0 times per month. For the number of drinks consumed in a drinking period, the highest number of the range reported in the free response was used. For gender analysis, one person self-identified as a transgender woman so they were coded as woman. Four responses were coded as other so they were excluded from the analyses based on gender. There were four responses that only answered the gender question that were excluded from all data analysis.

Comparison Against NSDUH 2019 National Data

In order to perform a Chi-Square Test for Goodness of Fit to compare gender distribution of alcohol consumption against a national sample (Appendix C), responses for the questions "Do you consume alcohol?" and "What is your gender?" were used to divide respondents into four categories: Woman Consumes Alcohol, Man Consumes Alcohol, Woman Doesn't Consume Alcohol, and Man Doesn't Consume Alcohol. For the sake of this test, respondents who didn't answer either of the questions or who identified as nonbinary were discarded. The proportions of women and men who consumed alcohol were determined and used as the observed values for a

Chi-Square Test for Goodness of Fit. For the expected values, the proportions of male and female full-time college students who reported that they consumed alcohol in the 2019 NSDUH survey were obtained (*Section 6 PE Tables*, 2020, Table 6.21b). The data on the distributions of alcohol consumption in male and female students from the NSDUH survey could then be compared to the distributions of alcohol consumption in men and women from our sample. The Chi-Square Test was performed at a significance level of 0.05.

In order to perform a Chi-Square Test for Goodness of Fit to compare gender distribution of binge drinking against a national sample (Appendix C), responses for the questions "If you consume alcohol, how many drinks on average do you consume in one 'drinking period?'", "Do you consume alcohol?" and "What is your gender?" were used to divide respondents into four categories: Woman Binge Drinker, Man Binge Drinker, Woman Non-Binge Drinker, and Man Non-Binge Drinker. In accordance with the NSDUH survey standards (*2019 National Survey on Drug Use and and Health*, 2020, p. 21), respondents who were women were considered binge drinkers if they reported consuming four or more alcoholic drinks while respondents who were men were considered binge drinkers if they reported consuming five or more alcoholic drinks. Respondents that answered "no" to the question "Do you consume alcohol?" but answered the question "If you consume alcohol, how many drinks on average do you consume in one 'drinking period?'" with a non-zero number were excluded. Respondents that didn't answer either of the test questions were excluded. Again, those who identified as nonbinary were discarded. The proportions of men and women who reported binge drinking were determined by dividing the number of binge drinkers by the number of people that consumed alcohol for each gender, and these proportions were used as the observed values. For the expected values, the number of male and female full-time college students who reported that they consumed alcohol

and reported binge drinking in the 2019 NSDUH survey were obtained (*Section 6 PE Tables*, 2020, , Table 6.21a) and compared against the proportions of alcohol consumers that binge drank in each gender category in our sample. The Chi-Square Test was performed at a significance level of 0.05.

In order to perform a two-tailed z-test to compare the proportion of alcohol consumers who binge drank, the questions "Do you consume alcohol?" and "If you consume alcohol, how many drinks on average do you consume in one 'drinking period?'" were used to determine the proportion of respondents who were binge drinkers. Again, respondents who identified as women were considered binge drinkers if they reported consuming four or more alcoholic drinks while respondents who identified as men were considered binge drinkers if they reported consuming five or more alcoholic drinks. Those who didn't report a gender or who identified as some variant of gender nonbinary were given a point between the two. While summing binge drinkers, a respondent who was a woman that drank four drinks in a drinking period was added as "1", a respondent who was a man that drank four drinks was added as "0", and a nonbinary respondent that drank four drinks was added as "0.5". Responses from full-time college students from the NSDUH 2019 sample of both genders were used to determine a proportion against which we could compare our data (*Section 6 PE Tables*, 2020, Table 6.21a). A two-tailed z-test of proportions was performed at a significance level of 0.05.

Results

After controlling to only include undergraduate students, the sample size was 189 participants. The majority of respondents were women (n=136) and the minority were men (n=42). There were 7 nonrespondents for gender and 4 respondents answered "other". Of the participants, 28 were freshmen, 43 were sophomores, 77 were juniors, and 41 were seniors.

54.3% of survey respondents were under the age of 21, with the mean age being 20.3 years. At the time of the survey, 88.9% of participants reported living on or near campus, 8.47% reported not living on or near campus, and 2.65% reported that they used to live on or near campus but no longer did.

Table 3: Demographic frequencies of survey respondents.

		n	%	$\bar{x}$	St. Dev
Age (years)		175		20.3	1.244
Gender					
	Men	42	22.2		
	Women	136	72.0		
	Other	7	3.7		
	Non response	4			
Year in School					
	Freshman	28	14.8		
	Sophomore	43	22.8		
	Junior	77	63.5		
	Senior	41	21.7		
Race					
	White	91	48.2		
	Black or African American	19	10.1		
	American Indian or Alaska Native	0			
	Asian and Native Hawaiian or other Pacific Islander	64	33.9		
	Multiracial	10	5.3		
	Nonresponse	5			
Currently lives on/near campus		168	88.9		
Currently Consumes Alcohol	Yes	148	78.3		
Drinking Frequency (times per month)		189		3.3	4.4
Drinking Freq. (times per month)	Of those who consume alcohol	148		4.3	4.6
Number of Drinks Per Drinking Period		189		3.0	2.5
Drinks Per Drinking Period	Of those who consume alcohol	148		3.7	2.2
Self-reported family history of excessive alcohol consumption or alcoholism					
	Yes	62	33.0		
	No	126	67.0		
Parental Knowledge					
	Yes	123	67.2		
	No	60	32.8		

We were interested in how many times per month participants drank (drinking frequency) and the number of drinks consumed in a drinking period. For drinking frequency, 4.76% of respondents reported drinking once in the last month, 23.81% reported drinking three times within the last month, 17.46% reported drinking 4 times per month, and 14.82% reported

drinking 12 or more times per month. The average drinking frequency of the entire sample (n=189) was 3.3 ± 4.4 times per month. After controlling just for people who consume alcohol (n=148), the average drinking frequency was 4.3 ± 4.6 times per month. For the number of drinks consumed in a drinking period, 41.8% of respondents reported consuming 1-3 drinks in a drinking period, 29.1% reported drinking 4-6 drinks, and 8.47% reported drinking 7 or more drinks in a drinking period. The average number of drinks per drinking period for those who consume alcohol (n=148) was 3.7 ± 2.2. We were also interested in binge drinking behaviors, defined in this context as drinking 4 or more drinks within a 12 hour period. When asked to estimate the number of times within the last month participants drank 4 or more drinks within a 12 hour period, 52.38% never did, 35.45% did 3 times, 10.05% did 6 times, and 2.12% did 9 or more times. According to national statistics, 17% of US adults partake in binge drinking behaviors and 25% of those adults binge drink at least weekly (CDC, 2022). Another measure we were interested in was family history of excessive alcohol consumption or alcoholism. 67.0% of respondents reported not having a family history of excessive alcohol consumption or alcoholism and 33.0% self-reported having family history. Finally, we were interested in parental knowledge of alcohol consumption, and 67.2% of respondents reported that their parents know they consume alcohol.

Association Tables

Table 4: Distributions of drinking frequency and quantity for those respondents who do or don't communicate their drinking habits to their parents.

	Parental Knowledge (+)	Parental Knowledge (-)
Number of times drinking per month	n = 123 Mean: 5.84 Std Dev: 4.73	n = 59 Mean: 4.12 Std Dev: 3.04

Number of drinks per drinking period	n = 123 Mean: 3.66 Std Dev:2.25	n = 59 Mean: 3.97 Std Dev: 2.13

Table __: Distributions of drinking frequency and quantity for those respondents who do or don't have a family history of alcohol abuse or excessive alcohol consumption.

	Family History (+)	Family History (-)
Number of times drinking per month	n = 62 Mean: 5.98 Std Dev: 4.22	n = 120 Mean: 5.08 Std Dev: 4.54
Number of drinks per drinking period	n = 62 Mean: 3.67 Std Dev: 2.03	n = 120 Mean: 3.77 Std Dev: 2.32

Table __: Distributions of drinking frequency and quantity for underage respondents who do or don't communicate their drinking habits to their parents.

UNDERAGE (< 21)	Parental Knowledge (+)	Parental Knowledge (-)
Number of times drinking per month	n = 55 Mean: 4.98 Std Dev: 3.64	n = 38 Mean: 3.21 Std Dev: 0.80
Number of Drinks (cups per drinking period)	n = 55 Mean: 4.30 Std Dev: 2.60	n = 38 Mean: 4.30 Std Dev: 2.15

Table __: Distributions of drinking frequency and quantity for respondents of drinking age who do or don't communicate their drinking habits to their parents.

LEGAL AGE (21+)	Parental Knowledge (+)	Parental Knowledge (-)
Number of times drinking per month	n = 59 Mean: 6.20 Std Dev: 5.44	n = 17 Mean: 5.55 Std Dev: 4.23
Number of Drinks (cups per drinking period)	n = 59 Mean: 3.18 Std Dev:1.86	n = 17 Mean: 3.5 Std Dev: 2.02

*the number after +/- is the std dev

In these association tables, the n value is equal to the number of students who fit the criteria. For example, in the first chart, there were 123 students whose parents were aware of them drinking. In the last chart, it is evident that only 59 of those 123 students are of legal drinking age. While there were a total of 123 students who reported having parents who are aware of their drinking habits, 11 of those students did not report their age, which is why there is a gap in the n values. The mean value represents the average number of drinks/number of times a student drank per month. The standard deviation was included to emphasize the variations in responses that were received.

Comparison Against NSDUH 2019 Data

Table 5: Chi-Square Analysis Comparing Alcohol Consumption Habits by Gender Against a National Sample

	Observed (Team DRINK)	Expected (NSDUH 2019)	(O-E)^2/E
Men	85.71428571	51.4	22.90798062
Women	75	53.4	8.737078652
Total			χ = 31.64505928

The Chi-Square Test to compare alcohol consumption habits was performed with a significance level of $\alpha = 0.05$. Given that there is one degree of freedom and $\chi = 31.65$, the resultant p-value was $p < 0.001$. This indicates that there was a significant difference between the alcohol consumption habits of respondents when compared against the NSDUH 2019 National Sample. Both men and women reported that they drank alcohol more than the NSDUH proportions.

Table 6: Chi-Square Analysis Comparing Binge Drinking Habits by Gender Against a National Sample

	Observed (Team DRINK)	Expected (NSDUH 2019)	(O-E)^2/E

Men	33.33333333	67.8944475	17.59305299
Women	49.01960784	58.66177819	1.584872668
Total			χ = 19.17792565

The Chi-Square Test to compare binge drinking habits was performed with a significance level of $\alpha = 0.05$. Given that there is one degree of freedom and $\chi = 19.18$, the resultant p-value was $p < 0.001$. This indicates that there was a significant difference between the binge drinking habits of respondents when compared against the NSDUH 2019 National Sample. The proportion of both men and women that consumed alcohol and reported that they drank enough to qualify as binge drinkers was less than the NSDUH proportions.

Table 7: Two-Tailed Z-Test Comparing Binge Drinking Habits Against a National Sample

	Team DRINK	NSDUH 2019	
Proportion	0.4459459459	0.6285928518	
Sample Size	148	4,001,000	
			Z = 4.456305762

The Z-Test to compare binge drinking habits was performed with a significance level of $\alpha = 0.05$. The resulting p-value was < 0.0004. Even with the gender factor removed, fewer respondents reported binge drinking behavior than those from the NSDUH 2019 National Sample.

Discussion

The overall aim of the study was to better understand UMD undergraduates' alcohol consumption behaviors. More specific aims included finding possible associations between parental knowledge of drinking behaviors and students' alcohol consumption, family history of

excessive alcohol consumption and students' drinking behaviors, and comparing the parental knowledge effect on underage vs legal age drinkers. The goal was to learn about the students in our study and understand the relationship between different variables we surveyed about. We wanted to create a survey to explore possible reasons why some people drink more than others.

Previous studies have found that parents who spend more time communicating with their college children have fewer children who report using alcohol in college compared to students whose parents do not communicate with them as much (Abar, 2012). The same study found that parents with a zero tolerance policy for alcohol consumption have children who are much less likely to consume alcohol. While our survey did not measure the extent to which parents communicate alcohol use with their children, we did not notice a difference in the average drinks consumed per drinking period and per month with parental knowledge or the lack of.

It is challenging to draw conclusions from our data because the format of our survey elicited quantitative and qualitative responses. The intent was to be able to evaluate the wide breadth of respondents' individual experiences. However, the decision to make our questions less-structured made it difficult to detect the statistical significance of correlations between different variables. Instead, we prioritized describing the trends of our data and comparing our trends against existing data.

When it comes to determining whether our data is a representative sample of the UMD population, it was invaluable to compare the demographic information from our survey respondents against the Fall 2021 demographic data acquired from the UMD Undergraduate Student Profile (Office of Institutional Research, Planning & Assessment, 2022), the same academic year that our data was collected. The undergraduate UMD population was 41.6% White, 12.6% Black or African American, 22.4% Asian, Native Hawaiian, or Pacific Islander,

10.3% Hispanic, 4.8% multiracial, 0.1% Native American or Alaskan, and the remaining 8.2% qualified as other. Meanwhile, within the respondents, 48.2% self-identified as White, 10.1% as Black or African American, 33.9% Asian, Native Hawaiian, or Pacific Islander, 5.3% as multiracial, and 2.6% didn't respond, indicating that the distribution of ethnicities within our respondents was somewhat representative of the UMD undergraduate population. The distribution of gender within the UMD undergraduate population in Fall of 2021 was 49.2% female and 50.8% male whereas our respondents had a distribution of 22.2% men, 72.0% women, 3.7% identified with neither of the binary genders, and 2.1% didn't respond. Given that our survey was heavily skewed towards women respondents, there is a disparity between the undergraduate population and the gender distribution of respondents within our sample which may be indicative of flaws in the way we searched for survey respondents. When it comes to the distribution of class standing within the UMD undergraduate population for Fall of 2021, 16.1% of students were Freshman, 20.3% Sophomores, 25.6% Juniors, and 31.5% Seniors. Within our survey respondents, 14.8% were Freshman, 22.8% Sophomores, 63.5% Juniors, and 21.7% Seniors. Again, there is a disparity between the population demographic data and the demographic data of our respondents. Our survey respondents were skewed heavily towards Juniors, which may be indicative of flaws in the methods we used to find survey participants.

As a strong point of our study, the questionnaire we created asked a variety of open-ended questions that addressed different aspects of UMD's student body drinking culture. This allowed us to gain a broader perspective of our respondents' relationship with drinking and the UMD drinking culture overall. The survey was also cost-effective, and we were able to produce and conduct it in a relatively short amount of time, which allowed us to dedicate more time to evaluating our data. Additionally, the particular questions that we chose to ask allowed us to gain

a more in depth understanding of our respondents. For example, asking about the respondents' drinking frequency in conjunction with asking about the number of drinks they consumed per drinking period allowed us to mark the difference between a frequent drinker and a binge drinker, which allowed us to develop a more holistic understanding of the respondents drinking habits. Additionally, the exploratory nature of our study allowed us to collect contemporary data about UMD students' experiences with alcohol, which provides a bit of insight into the current and local state of the dynamic and decades-long problem that is alcohol use and misuse on college campuses.

There were several limitations that impacted the reliability of our study as it was performed, including the small sample size. After the survey was closed, 194 responses had been received, and after removing non-undergraduate respondents, only 189 remained. The small sample size impeded our ability to make inferences about the UMD student body as a whole, and the results of our study aren't generalizable. It's difficult to say whether alterations to our survey could have resulted in a larger sample size; however, there were many factors that, in retrospect, may have limited our respondents. For example, there is a concern that students, especially underage students, may have been reluctant to answer a survey about alcohol use. Despite our advertising flyer and survey itself indicating that the responses would be anonymous, it's possible students could have been concerned that they might face consequences for answering the survey honestly. Additionally, the incentive to answer the survey may not have been attractive for some potential respondents. As a reward, credits on a Terrapin Express credit have limited uses at select locations around campus, which may have made this a poor incentive. Also, the rewards weren't guaranteed to every participant and were instead distributed using a lottery, which could have made participating in our survey unappealing to potential respondents as well.

Another potential concern with our respondent pool was that our survey received much more responses from individuals who drink alcohol compared to non-drinker responses. This could be related to the way we advertised our survey because we expressly indicated in our advertisements that we wanted to learn about people's drinking patterns and their awareness of issues related to alcohol (Appendix B). As a result, people who don't consume alcohol may have been less likely to respond than people that do consume alcohol, since people who don't consume alcohol may have believed that the survey wasn't relevant to them, which could have contributed to a response bias. Two more instances of potential bias became clear when we examined demographic information. First, the vast majority of our respondents were women, which may be a result of our decision to place flyers in bathrooms around campus. Since our team is made up entirely of women, the flyers naturally ended up in women's restrooms much more than they ended up in men's restrooms. Second, over half of our respondents were Juniors, which may be a result of our decision to spread links to our survey throughout class group chats and among our friends. At the time that the survey was distributed, our team was made up of Juniors, so we naturally spread the survey to Juniors more than we spread it to any other undergraduate class.

When it came time to analyze data, our questionnaire ended up being problematic. Had we found comparison data before writing the survey, we would have had a clearer direction to take with our questions so that we could maximize our understanding of specific patterns and trends in alcohol consumption. In our survey, we could have defined terms such as "one drink" as a standard drink- 12 ounces of beer, 5 ounces of wine, or 1.5 ounces of hard liquor- or "one drinking period" as a four hour period of drinking alcohol (*What Is A Standard Drink? | National*

Institute on Alcohol Abuse and Alcoholism (NIAAA), n.d.). Predefining these terms within the body of the survey could have helped us obtain more consistent data from all participants.

In the future, it may be beneficial to conduct a longitudinal study on the impact of alcohol in students' lives to round out the data from our cross-sectional study. This would provide a more comprehensive outlook of the University of Maryland's student's drinking habits. Additionally, future survey questions would consist of questions that allow for free responses, rather than only multiple-choice questions that require students to put themselves into specific categories and questions would also have better definitions of arbitrary terms such as "one drink". This is so that we have more accurate drinking frequency numbers for the students. For a deeper understanding of a parent's effect on their child's drinking habits, we may use the Abar instrument to characterize the parents and compare our results with that of Abar. We would also ask more health-related questions, as this plays a big role in the number of drinks one person can consume compared to another. Another future direction that incorporates the molecular modeling subteam would be to explore the relationship between alcohol and GABA and how that interaction is mediated by other substances such as marijuana or cocaine. This is particularly relevant to college students, as there are often substances being consumed in tandem with alcohol in collegiate settings.

Overall, our survey was a very valuable tool in learning how to conduct public health research data. While there were issues with the distribution process and with the survey itself, we were able to collect a decent sample size and analyze the sample for associations between different variables. We also learned how to go about the IRB approval process, which will be very valuable in our future endeavors. From this survey, we hope that we have contributed to

future alcohol consumption studies at UMD in order to reduce the impact of drinking on college students.

Combining the Subteams

When we first began our project in Spring 2020, we planned for a three-pronged approach to the issue of alcohol consumption and alcohol poisoning. There was to be a computational aspect, exploring the role of ethanol in the brain. From the computational models, we planned to take the models in vitro and do animal model experimentation to further elucidate the interactions of alcohol in the brain. During Fall 2020, we decided to add a public health component in order to better understand the issue of binge drinking and alcohol poisoning in our community, University of Maryland undergraduate students. Throughout the beginnings of our project, we were faced with a lot of uncertainty due to the COVID-19 pandemic. We were unsure of when we would return to campus and if we would be able to access lab space. When we first envisioned the project, the in vitro experimentation was supposed to be the bridge between computational and public health, as it would take the results from computational and apply them more directly to human-adjacent models. However, due to not being able to access a lab, we were unable to do in vitro experimentation.

The connection between the computational and public health subteams may not be as direct as it could have been if the project had been able to be completed as originally planned, however there is still a connection. By understanding the interactions of ethanol in the brain and alcohol consumption behaviors of college students, we hope to contribute to the knowledge base for future researchers that are working towards reducing alcohol-related deaths by providing the molecular and social impacts of excessive alcohol consumption.

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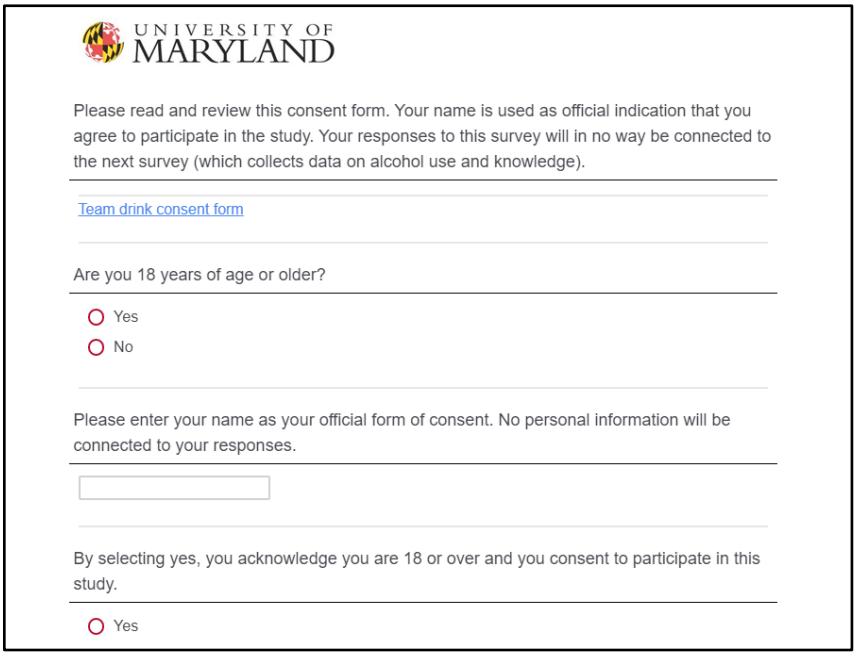
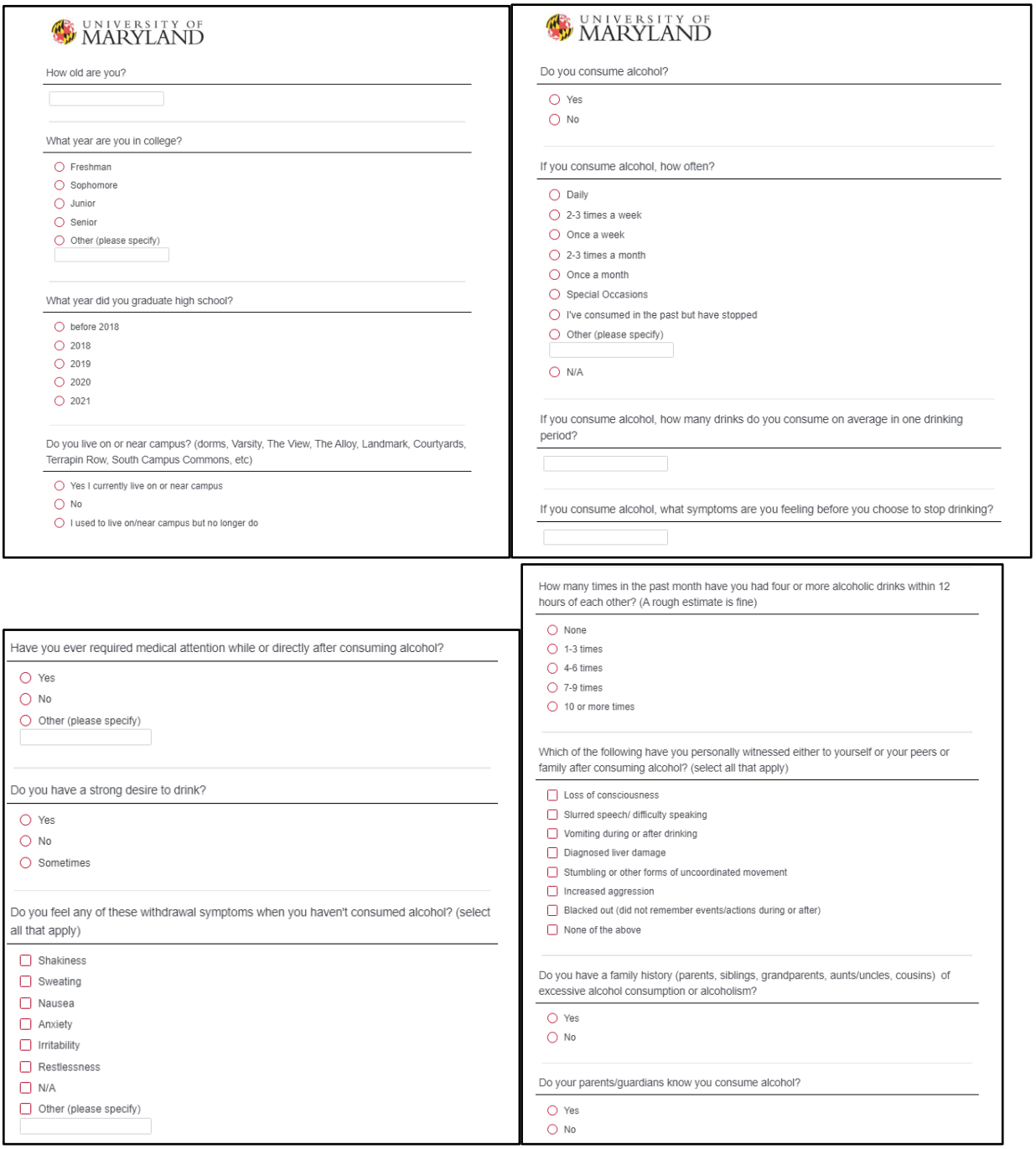
Appendix A: Qualtrics Surveys

Survey 1 (Consent Form):



Survey 2 (Alcohol Consumption and Demographic Data):



UNIVERSITY OF MARYLAND

How old are you?

What year are you in college?

- Freshman
- Sophomore
- Junior
- Senior
- Other (please specify)

What year did you graduate high school?

- before 2018
- 2018
- 2019
- 2020
- 2021

Do you live on or near campus? (dorms, Varsity, The View, The Alloy, Landmark, Courtyards, Terrapin Row, South Campus Commons, etc)

- Yes I currently live on or near campus
- No
- I used to live on/near campus but no longer do

UNIVERSITY OF MARYLAND

Do you consume alcohol?

- Yes
- No

If you consume alcohol, how often?

- Daily
- 2-3 times a week
- Once a week
- 2-3 times a month
- Once a month
- Special Occasions
- I've consumed in the past but have stopped
- Other (please specify)
- N/A

If you consume alcohol, how many drinks do you consume on average in one drinking period?

If you consume alcohol, what symptoms are you feeling before you choose to stop drinking?

Have you ever required medical attention while or directly after consuming alcohol?

- Yes
- No
- Other (please specify)

Do you have a strong desire to drink?

- Yes
- No
- Sometimes

Do you feel any of these withdrawal symptoms when you haven't consumed alcohol? (select all that apply)

- Shakiness
- Sweating
- Nausea
- Anxiety
- Irritability
- Restlessness
- N/A
- Other (please specify)

How many times in the past month have you had four or more alcoholic drinks within 12 hours of each other? (A rough estimate is fine)

- None
- 1-3 times
- 4-6 times
- 7-9 times
- 10 or more times

Which of the following have you personally witnessed either to yourself or your peers or family after consuming alcohol? (select all that apply)

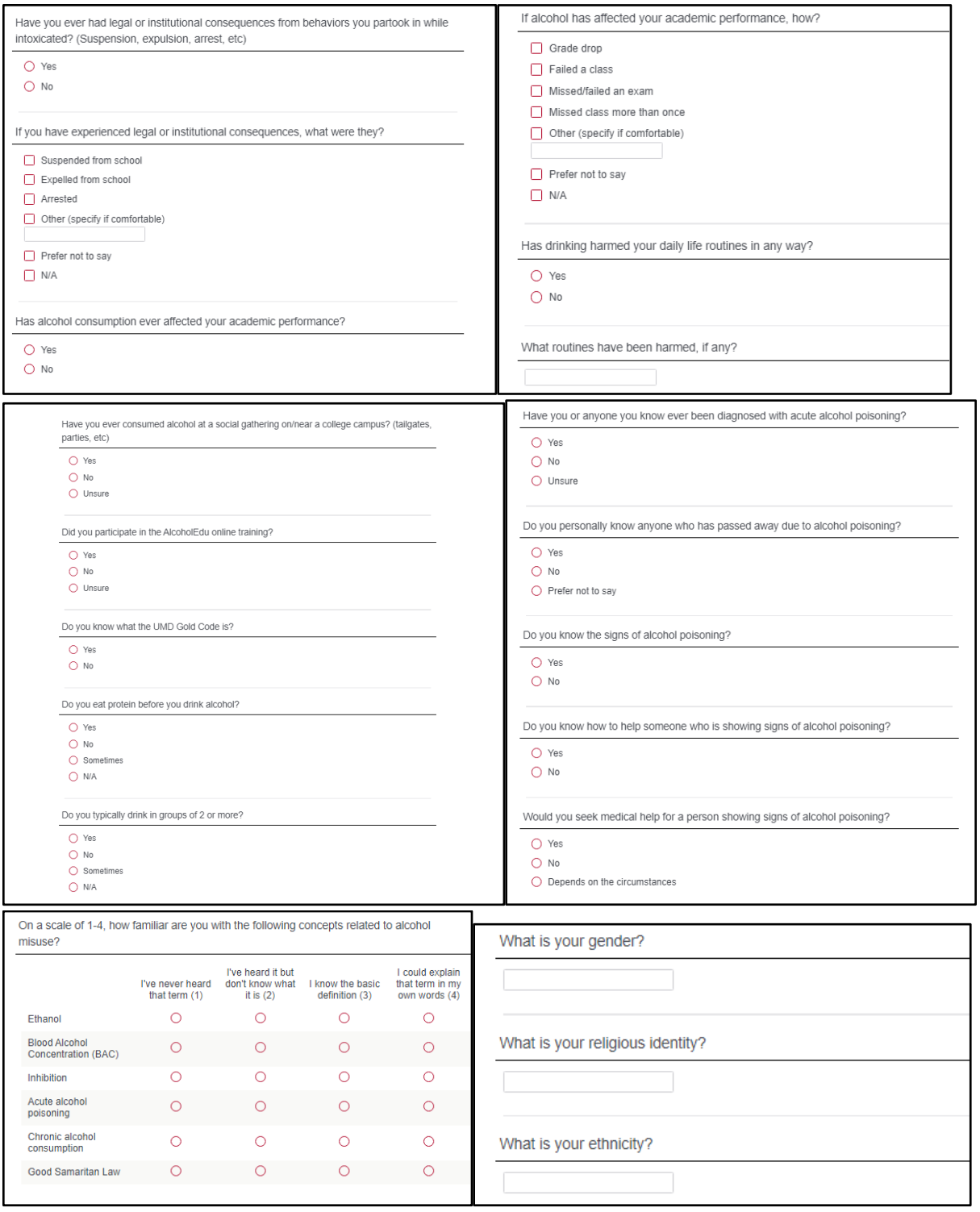
- Loss of consciousness
- Slurred speech/ difficulty speaking
- Vomiting during or after drinking
- Diagnosed liver damage
- Stumbling or other forms of uncoordinated movement
- Increased aggression
- Blacked out (did not remember events/actions during or after)
- None of the above

Do you have a family history (parents, siblings, grandparents, aunts/uncles, cousins) of excessive alcohol consumption or alcoholism?

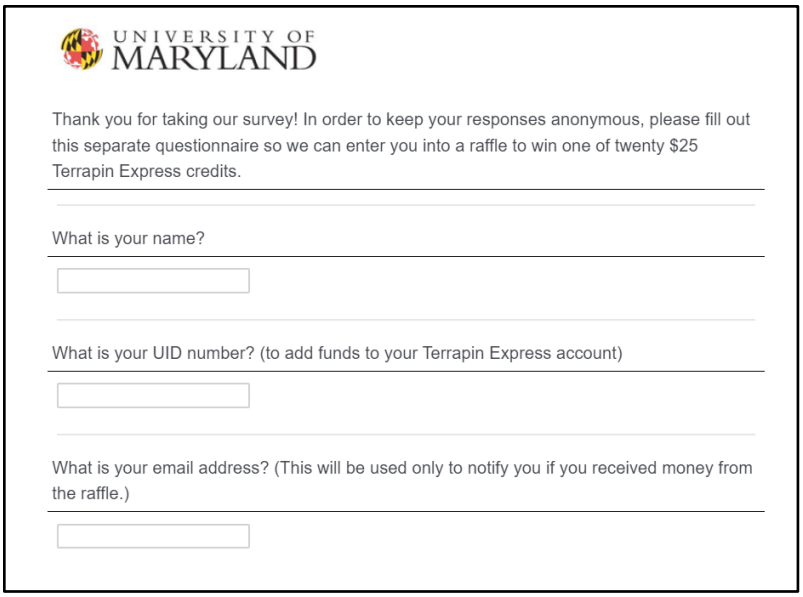
- Yes
- No

Do your parents/guardians know you consume alcohol?

- Yes
- No



Survey 3 (Personal Identifying Information):


UNIVERSITY OF MARYLAND

Thank you for taking our survey! In order to keep your responses anonymous, please fill out this separate questionnaire so we can enter you into a raffle to win one of twenty $25 Terrapin Express credits.

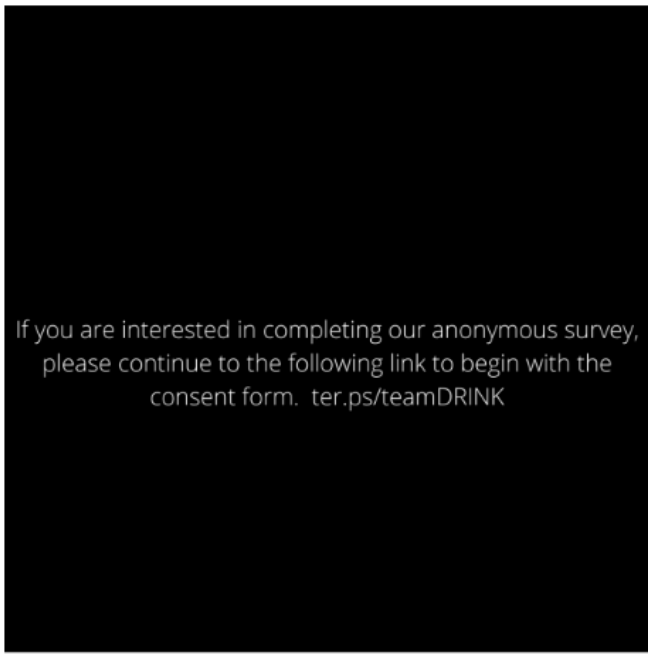
What is your name?

What is your UID number? (to add funds to your Terrapin Express account)

What is your email address? (This will be used only to notify you if you received money from the raffle.)

Appendix B: Survey Advertising Materials

Social Media Flyers:



Physical Flyers (placed around campus):

Recruiting Participants

for an anonymous survey

Hosting a survey pertaining to alcohol drinking habits and knowledge of alcohol related concerns that should take no longer than 20 minutes. Visit @umdteamdrink on Instagram for more information.

Accepting 500 participants entered into $25 Terrapin Express raffle with 40 winners

Team DRINK 2023

Appendix C: Chi Square Tables

χ^2 Test to compare the observed gender distributions of those that consume alcohol amongst survey respondents against the expected distribution obtained from the SAMHSA 2019 survey (Section 6 PE Tables, Table 6.21B, 2020).

	Observed	Expected	(O-E)	(O-E)^2	(O-E)^2/E
Male	85.71428571	51.4	34.31428571	1177.470204	22.90798062
Female	75	53.4	21.6	466.56	8.737078652
Total					31.64505928

Table __: χ^2 Test to compare the observed gender distributions of those that binge drink amongst the survey respondents that consume alcohol against the expected distribution obtained from the SAMHSA 2019 survey (Section 6 PE Tables, Table 6.21A, 2020).

	Observed	Expected	(O-E)	(O-E)^2	(O-E)^2/E
Male	33.33333333	67.8944475	-34.56111417	1194.470612	17.59305299
Female	49.01960784	58.66177819	-9.642170342	92.9714489	1.584872668
Total					19.17792565