Biomimetic Model Membranes to Study Protein-membrane Interactions and their Role in Alzheimer's Disease

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BIOMIMETIC MODEL MEMBRANES TO STUDY PROTEIN-MEMBRANE INTERACTIONS AND THEIR ROLE IN ALZHEIMER’S DISEASE

by

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An undergraduate honors thesis submitted in partial fulfillment of the requirements for the degree of

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Thesis Adviser

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BIOMIMETIC MODEL MEMBRANES TO STUDY PROTEIN-MEMBRANE INTERACTIONS AND THEIR ROLE IN ALZHEIMER’S DISEASE

(JUNE 2015)

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ABSTRACT

Lipid-coated AuNPs are prepared as cellular membrane mimics to study the protein-membrane interactions that play a role in neurodegeneration. PC and PC/Chol (1:1) lipid-coated AuNPs are used to monitor Aβ oligomer and monomer cellular membrane interactions. We used fluorescence anisotropy and TAMRA conjugated Aβ1-42 (TAMRA-Aβ1-42) to determine Aβ binding to lipid-coated AuNPs. An increase in the fluorescence anisotropy (r) of TAMRA-Aβ1-42 in the presence of the lipid-coated AuNPs indicate that the Aβ monomers bind to the membrane surface. Based on the change in r the binding affinity of Aβ to the AuNPs at pH 6.5 is higher than at pH 8 and is greater for Au-SO-PC-HT compared Au-SO-PC-Chol-HT. Dynamic light scattering (DLS) confirmed that Aβ1-42 monomers as well as oligomers bind to the lipid bilayers of the AuNPs as is evident by an increase in the hydrodynamic diameter (HD) of the lipid-coated AuNPs. When incubated with Aβ monomers, DLS show the greatest increase in the HD of Au-SO-PC-HT verses the Au-SO-PC-Chol-HT. When incubated with Aβ oligomers, a greater increase in HD is observed at pH 6.5 compared to 8 with DLS, demonstrating that Aβ binding is pH dependent. To investigate if Aβ binding to lipid-coated AuNPs cause significant disruption in membrane integrity cyanide etching studies are performed. UV-vis showed no shift in the surface plasmon resonance or change in optical density of lipid-coated AuNPs for either membrane type at pH 6.5 or 8.0 demonstrating that Aβ binding to the AuNP membranes does not lead to significant membrane disruption.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTERS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>6</td>
</tr>
<tr>
<td>II. EXPERIMENTAL SECTION</td>
<td>10</td>
</tr>
<tr>
<td>III. PREPARATION OF LIPID-COATED AUNPS AND BETA-AMYLOID BINDING STUDIES</td>
<td>13</td>
</tr>
<tr>
<td>Lipid encapsulation of AuNPs</td>
<td>13</td>
</tr>
<tr>
<td>Cyanide stability studies of lipid-coated AuNPs</td>
<td>15</td>
</tr>
<tr>
<td>The effect of pH and membrane cholesterol on Aβ (monomer) interactions with hybrid lipid-coated AuNPs</td>
<td>17</td>
</tr>
<tr>
<td>The effect of pH and membrane cholesterol on Aβ (oligomer) interactions with hybrid lipid-coated AuNPs</td>
<td>23</td>
</tr>
<tr>
<td>Evaluating if Aβ monomers or oligomers disrupt membrane integrity</td>
<td>26</td>
</tr>
<tr>
<td>IV. CONCLUDING REMARKS</td>
<td>28</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>31</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

I.1. Cartoon of surface of lipid-coated AuNP. ...............................................................8

III.1. A) Representative UV-vis spectra for cyanide studies of Au-SO-PC-Chol-HT,
   B) Cartoon of PC-Chol AuNP. ..............................................................................16

III.2. Representative fluorescence anisotropy time based plot of TAMRA-Aβ1-42 in
   10 mM PBS at pH 8.0 with Au-SO-PC-HT.............................................................19

III.3. Bar chart of change in fluorescence anisotropy of TAMRA-Aβ1-42 monomers after
   incubation with Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles
   .........................................................................................................................20

III.4. Representative dynamic light scattering distribution of average hydrodynamic
   diameter of Au-SO-PC-HT in 10 mM PBS at pH 8.0 with 2 µM Aβ1-42 monomers
   .........................................................................................................................22

III.5. Bar chart of average hydrodynamic diameter of Au-SO-PC-HT and Au-SO-PC-
   Chol-HT nanoparticles incubated with Aβ1-42 monomers.......................................23

III.6. Bar chart of average hydrodynamic diameter of Au-SO-PC-HT and Au-SO-PC-
   Chol-HT nanoparticles incubated with Aβ1-42 oligomers ........................................26

III.7. Representative UV-vis spectra of Au-SO-PC-HT nanoparticles in 10 mM PBS pH
   8.0, with 2 µM Aβ oligomers, and 1 hour incubation with 3 mM cyanide
   .............................................................................................................................27
CHAPTER 1

INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia. It affects approximately 5.4 million Americans and over 36 million people worldwide.\textsuperscript{1,2} The number of people with AD is projected to double every 20 years, and it is estimated that 115 million will be afflicted by 2050. In 2010, the worldwide costs of dementia are 604 billion dollars, which amounted to more than 1% of the global gross domestic product.\textsuperscript{2} Furthermore, there is currently no effective treatment to delay, halt, or reverse the symptoms of Alzheimer’s. This makes AD one of the most common illnesses in the world for which there is no treatment or cure. Therefore, there is a strong motivation to learn more about the etiology of AD for the purposes of developing drug therapies to slow, halt, or even reverse its effects.

One of the prominent features found in AD brain is the presence of senile plaques (SP) and neurofibrillary tangles (NFT), which contain aggregates of the peptide beta-amyloid (Aβ).\textsuperscript{2} One of the dominant beliefs for the past 15 years on what causes AD has been the “amyloid cascade hypothesis”, which originally suggested that a build up of Aβ in the brain causes fibril formation that lead to the formation of SPs and NFTs.\textsuperscript{1,3} The appearance of these SPs and NFTs then lead to a series of changes in the brain that cause neurodegeneration, and eventually death. However, more recent evidence has shown that SPs and NFTs may actually be the products of neurodegeneration instead of its cause.\textsuperscript{3} Furthermore, some studies have indicated Aβ oligomers as the main culprit as opposed to fibrils.\textsuperscript{1,4,5} These oligomers have been shown to cause cellular membrane disruption and form channels, causing cellular [Ca\textsuperscript{2+}]}
dysregulation, neurotoxicity, and death.\(^1\)\(^6\) However, this is still unproven, and even though the pathology of AD is well understood its exact cause is still uncertain. Furthermore, even though Aβ is hypothesized to be the central cause of AD it is widely accepted that it relies on a complex series of events with multiple agents for this neurodegeneration to occur. These other factors involve, but are not limited to, hyperphosphorylated tau, metal ions, membrane cholesterol content, and reactive oxygen species.\(^1\)\(^6\) These factors compounded with multiple pathological agents make it difficult to narrow down the cause and effect of AD etiology. Therefore, future research in AD would benefit from methods that have the ability to simplify the cellular environment. This would allow for the isolation and clarification of the individual steps occurring in the overall AD pathology. More specifically, interactions of Aβ with neuronal membranes and how these interactions influence membrane disruption and instability that lead to neuronal cell death.

Cellular membranes in biological systems are complex and comprised of heterogeneous mixtures of lipids, proteins, and carbohydrates.\(^7\) Due to this complexity, one method that has been used to isolate AD etiology is the use of membrane mimics such as liposomes and planar lipid bilayers.\(^6\)\(^8\) These membranes can consist of one or two types of lipids, which is a far simpler environment than biological cellular membranes. This allows for more control of membrane composition and structural features that might influence protein-membrane interactions, which play a role in neuronal cell death. However, there are shortcomings to using liposomes and planar lipid bilayers as membrane mimics. Liposomes tend to be unstable and rearrange quickly in solution, and planar lipid bilayers lack the dynamics of cellular membrane curvature.\(^7\) As an answer to both of these problems hybrid bilayer membrane mimics have been used. This type of model membrane is first pioneered
by Anne Plant on planar surfaces, and consists of a lipid bilayer tethered to a metal surface with an alkanethiol. More recently Mackiewicz et al. anchored lipid bilayers to gold nanoparticles with alkanethiols, which gives it far more stability than liposomes but the curvature of a cell membrane. This curvature can be tuned by changing the size of the nanoparticle, which can be used to investigate the effect of membrane curvature on protein-membrane interactions, similar to clathrin and caveolin proteins found biologically. The gold core of lipid-coated AuNPs can be used as optical agents, and the surface plasmon resonance (SPR) of AuNPs can be used to monitor their interactions in solution. Furthermore, their high surface to volume ratio exposes a large area of lipids on their surface, which can be functionalized to aid in the study of protein-membrane interactions (Figure I.1). For instance, Mackiewicz et al. showed that hybrid membrane mimics can detect and bind to proteins such as C-reactive protein (CRP), a cardiovascular biomarker. Both UV-vis and dynamic light scattering measurements showed that CRP binds reversibly in a calcium-dependent manner to lipid-coated nanoparticles, which leads to membrane rearrangement causing the nanoparticles to cluster. This is significant because it provided a method for visualizing how low density lipoprotein aggregates, which is a key step in atherosclerosis.
In this study hybrid lipid-coated AuNPs are used to investigate the interactions of Aβ with phosphatidylcholine (PC) and cholesterol-rich membranes. Changes in the SPR and absorbance of the solutions are monitored in the presence of Aβ to discover if lipid-coated AuNPs aggregated in the presence of the peptide. We also use a well-known etchant of AuNPs, cyanide, to determine if the Aβ binding causes membrane disruption or pore formation. Fluorescence anisotropy using TAMRA-labeled Aβ1-42 is conducted to investigate if Aβ binds to the lipid-coated AuNPs. Furthermore, the change in the average HD of the lipid-coated AuNPs is also monitor to confirm Aβ binds to lipid-coated AuNPs. Here we demonstrate that these hybrid membrane mimics can be useful platforms for intricate features of membranes that influence Aβ-membrane binding interactions and instability that play a role in Alzheimer’s disease.
CHAPTER 2

EXPERIMENTAL SECTION

Materials and General Methods

Reagents and Buffer Solutions

Aqueous solutions of 10 nm gold citrate-capped nanoparticles are from BBI solutions. The hybrid bilayer lipids consisted of 95% L-α-phosphatidylcholine (PC) and cholesterol (Chol) from Matheson Coleman & Bell. A 10 mM PBS at pH 7.0 is used to reconstitute lipid films. 1 M PBS at pH 8.0 and 1 M HEPES at pH 6.5 are used to buffer the AuNP solutions before binding studies. Solutions of 95% 1-hexanethiol (HT) and hexafluoroisopropanol (HFIP) are purchased from Sigma Aldrich. Beta-amyloid, Aβ_{1-42} and TAMRA-Aβ_{1-42} is purchased from Anaspec as lyophilized powders and stored at -20°C in HFIP. The Aβ_{1-42} is modified with a TAMRA dye at the N-terminal end of aspartic amino acid residue of the Aβ_{1-42} sequence Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH.

Liposome Preparation

Lipid solutions of PC and Chol are prepared from stock solutions dissolved in CHCl₃: PC (50 µL 21.6 mM PC), PC/Chol 1:1 (25 µL 21.6 mM PC: 25 µL 21.6 mM Chol). Thin films are formed from drying the above CHCl₃ lipid solutions in 20 mL glass vials by solvent evaporation under N₂. The lipid films are further desiccated under vacuum for an additional
12 h prior to re-suspension in 2 mL of 10 mM PBS at pH 7.0 followed by 90 min of sonication.

**Nanoparticle preparation Hybrid Lipid-coated AuNPs**

Sodium oleate (SO) (2.2 µL of 9.3 mM solution) is added to 10 nm gold citrate nanoparticles (1 ml, 0.8 OD at $\lambda_{\text{max}} = 519$ nm in H$_2$O) and stirred for 20 min. From the above prepared liposome solutions, 42 µL is added to the nanoparticle SO solution and incubated for 40 min. This comes to (22.7 nmol of PC) for PC only membrane or (11.3 nmol PC and 11.3 nmol Chol) for PC/Chol 1:1 membrane. Hexanethiol (1 µL of a 10 mM solution in ethanol) is then added to the mixture and stirred for an additional 30 min.

**Aβ Oligomer and Monomer Preparation Sample Preparation for Aβ Binding Studies**

Aβ oligomers are prepared by adding Aβ$_{1-42}$ (10 µL 222 µM) in HFIP solvent to 90 µL in 10 mM HEPES for pH 6.5 or 10 mM PBS. The Aβ$_{1-42}$ peptide solutions are incubated at 4 °C for 24 hours to allow for oligomerization. Aβ monomers are prepared by adding Aβ$_{1-42}$ (10 µL 222 µM) in HFIP solvent directly to buffered hybrid lipid-coated AuNP solutions.

**UV-vis Spectroscopy and Dynamic Light Scattering (DLS) Studies**

Au-SO-PC-HT and Au-SO-PC-Chol-HTs (1:1) are buffered with 10 µL of 1 M HEPES at pH 6.5 or PBS at pH 8.0. Aβ monomers or oligomers are then introduced to the lipid coated AuNP solutions to yield final concentrations of 2 µM for monomers or 2 µM for oligomers. The UV-vis spectra or DLS is taken before and 15 min after the introduction of Aβ. To probe the effect of Aβ on membrane integrity, samples are exposed to cyanide and
UV-Vis spectra is taken. Cyanide (10 µL 307 mM in H₂O) is added to the samples and UV-Vis spectra taken after 1 h of incubation. The final concentration of cyanide in the sample is 3 mM. UV-vis spectra is taken with an Ocean Optics USB4000 UV-vis-NIR spectrophotometer using a 1 cm quartz cuvette. Dynamic Light Scattering measurements are recorded with Horiba LB-550 particle size analyzer with a quartz cuvette.

**Fluorescence Anisotropy**

TAMRA-Αβ₁₋₄₂ (47 nM monomer) is added to 1000 µL 10 mM PBS at pH 8.0 or 10 mM HEPES at pH 6.5. The anisotropy of the TAMRA-Αβ₁₋₄₂ is monitored for 10 min before the lipid-coated AuNPs (400 µL of 0.8 O.D.) are added to the solution. The sample is incubated for 10 min before the anisotropy is recorded for an additional 10 min. Fluorescence measurements are conducted on a PTI spectrophotometer using Felix32 software. Measurements are taken using a quartz cuvette at an excitation of 544 nm, an emission of 580 nm, and a 12 nm bandpass on both monochromators. The steady-state anisotropy (r) is calculated from equation 1:

\[
 r = \frac{I_{VV} - G I_{VH}}{I_{VV} + 2 G I_{VH}}
\]

where \( I_{VV} \) and \( I_{VH} \) are the fluorescence intensities measured, the subscripts indicate the orientation of the excitation and emission polarizers, and \( G = \frac{I_{HV}}{I_{HH}} \) is the wavelength dependent sensitivity of the instrument.⁶ Reported Δr values are the average of three independent samples each averaged over 10 min.
CHAPTER 3
PREPARATION OF LIPID-COATED AUNPS AND BETA-AMYLOID BINDING STUDIES

Lipid-Encapsulation of AuNPs

Previously, a method for the preparation of hybrid lipid-coated AuNPs with phospholipid vesicles was developed by Mackiewicz et al.\textsuperscript{9} Since then, Yang and Murphy have prepared hybrids of varying types of lipid membranes, and characterized their arrangement on the nanoparticle surface with TEM imaging.\textsuperscript{17} These techniques involve coating AuNPs with pre-formed lipid vesicles of a uniform size and stabilization of the membrane on the surface with a long chain hydrophobic thiol.\textsuperscript{10,17} Using a similar procedure we first determined the minimal amount of lipids to coat a 10 nm AuNP colloid. This strategy reduces the amount of free liposomes available in solution that could complicate binding studies and ensures enough lipids are present to cover each colloid. In this synthesis the same technique for producing these phospholipid-covered AuNPs is used to prepare cholesterol-rich membranes. Enough lipids are added to the colloid solution to form a bilayer over the AuNP surface based off these calculations.

The amount of lipids necessary to fully encapsulate the AuNPs is derived by utilizing beer’s law and equation 2 to convert the absorbance of the stock solution into concentration. This is based on previous studies using similar sized gold nanospheres.\textsuperscript{9,18}

\[
\ln \epsilon = k \ln D + a \quad \text{(2)}
\]
where $D$ is the diameter of the nanoparticles to be covered, $k$ and $a$ are constants 3.32 and 10.8 and $\varepsilon$ is the extinction coefficient.$^{9,18}$ The number of entities in a mL of solution is then derived by multiplying the concentration of gold nanoparticles, $C$ by Avogadro’s number, $N_A$.\textsuperscript{9}

$$N = N_A C \quad (3)$$

When compared to PC, the hydroxyl head group of cholesterol is significantly smaller.$^{19}$ With this in mind, the rough estimate made for the amount of lipids needed to completely cover the surface of the AuNPs are based off the larger PC head group size of 69.4 Å$^2$.\textsuperscript{20} Using these parameters it is estimated that a single AuNP of 10 nm diameter can be completely covered by a monolayer of roughly 453 molecules of lipids. An additional 887 lipids is needed to cover this monolayer, which would produce a bilayer on the nanoparticle surface with an outer diameter of 14 nm. This comes to a total of 10.5 nmol of lipids needed to produce a lipid bilayer on 1 mL 10 nm AuNPs at 0.8 OD. However, a 1 nm water cushion has been anticipated to form between the nanoparticle surface and lipid bilayer, as shown with lipid-coated glass beads in previous studies.\textsuperscript{21} Furthermore, there is the possibility of a loss of lipids during their transfer to the nanoparticle surface. In addition, the nanoparticles are an average of 10 nm but there could be slightly smaller or larger nanoparticles. All of these factors as well as the smaller cholesterol head group size can lead to the above calculations being an underestimate. To compensate for these possibilities, 22.7 nmol of lipids are used per 1 mL of AuNP solution at 0.8 O.D.

It has been found, that hydrophobic alkanethiols can be used to anchor lipid bilayers to a gold surface in order to form robust hybrid bilayer nanoparticles (HBM$s$).\textsuperscript{9,10,22} Similarly we employed hexanethiol (HT) to anchor the bilayer on the AuNP surface. When there are scarce amounts of lipids present in solution the addition of hydrophobic thiols can cause
nanoparticle aggregation. This is visually evident by a change in AuNP solution color from red to purple, and can be seen instrumentally by a red-shift in the SPR band into the near-infrared region, and a decrease in O.D. of the AuNPs. This aggregation can be avoided by adding sodium oleate (SO) to the AuNP solution prior to the addition of lipids. AuNP are incubated with SO prior to addition of lipids and hexanethiol. As discussed previously, the SO nonpolar end is attracted to the AuNP surface while its polar head is facing away from the nanoparticle surface. These polar head groups can interact with water molecules keeping the AuNPs water-soluble.\textsuperscript{9,10} SO does not form a covalent bond with the AuNP but instead serves as a hydrophobic binding partner to the hydrophobic thiol preventing aggregation. After a 20 min incubation with SO, the AuNPs are then incubated with the lipids for an additional 40 min. This is followed by the addition of hexanethiol for a final 30 min incubation.

\textbf{Cyanide stability studies of lipid-coated AuNPs}

Cyanide stability studies are performed to determine if the AuNP surface is completely covered with the cholesterol-rich membranes (Figure III.1A). Cyanide oxidizes Au\textsuperscript{0} to Au\textsuperscript{1} and is a well-known etchant of gold.\textsuperscript{10,23} Therefore, if the lipid-coated AuNPs are not etched this is an indication that the lipid bilayer is fully covering the nanoparticle surface. It has been demonstrated by Mackiewicz \textit{et al.} that AuNPs become resistant to cyanide upon the addition of long chain hydrophobic alkyl thiols and demonstrates that AuNPs are covered by membranes evenly.\textsuperscript{9,10} As further evidence that lipids are coating the surface of these hybrid AuNPs, Yang and Murphy prepared lipid-coated AuNPs with varying types of lipids and have shown that they are on the surface using TEM.\textsuperscript{17} Cyanide (3 mM) is added to Au-SOA-PC-Chol-HT nanoparticles (a > 7 fold excess compared to gold atoms in 1 mL at 0.8 OD)
and the change in the SPR and O.D. are monitored for 30 min. The UV-vis spectra revealed no change in the SPR or O.D. for the Au-SOA-PC-Chol-HT (Figure III.1A) indicating that the cyanide ions are unable to penetrate through the lipid bilayer to etch the gold surface. Furthermore, this indicates that the lipid bilayer is evenly distributed, fully intact, and ion-impermeable on the AuNP surface. In previous studies by Mackiewicz et al. lipid-coated AuNPs without the thiol anchor are unstable to cyanide etch over the same amount of time. This same cyanide stability study is conducted in this experiment for Au-SO-PC-HT as well. Both types of lipid-coated AuNPs used in this study showed no significant change in SPR or absorbance at 30 min. This suggests they have cyanide stable lipid bilayers, and this stability is due to the anchoring of the bilayer by alkanethiols, as depicted in Figure III.1B.

![Figure III.1](image.png)

**Figure III.1.** A) UV-vis absorption spectra of i) Au-SO-PC-Chol-HT nanoparticles ii) Au-SOA-PC-Chol-HT nanoparticles in the presence of 3 mM cyanide for 30 min B) Cartoon of Au-SO-PC-Chol-HT nanoparticles.

The effect of pH and Membrane Cholesterol on Aβ (Monomer) Interactions with Hybrid Lipid-coated AuNPs
Aβ has been found to bind and form aggregates on lipid membrane models as well as membranes in biological systems.\textsuperscript{24,25} It has been shown that Aβ binds to phospholipid membranes with high affinity and should therefore bind to the phosphatidylcholine membranes of the AuNP membrane mimics.\textsuperscript{26} Furthermore, the effect that membrane cholesterol content has on Aβ binding to membranes is a controversial subject.\textsuperscript{6,27,28} It is still unclear as to whether cholesterol is an agent for Aβ disruption of cellular membranes, a protectant, or neither. Therefore, two types of lipid-coated AuNPs have been prepared for these studies. One membrane type is completely composed of PC and the other membrane is composed of PC-Chol 1:1 ratio. It has also been shown that Aβ takes on different structural conformations at different pH, and it appears that Aβ may cause greater membrane disruption at more acidic pH.\textsuperscript{29,30} With this in mind, Aβ binding studies are performed in 10 mM PBS pH 8.0 and compared to 10 mM HEPES pH 6.5 buffers.

To determine if Aβ\textsubscript{1-42} monomers bind to the surface of the lipid-coated AuNPs, we developed a fluorescence anisotropy assay. A TAMRA dye is covalently linked to the N-terminal aspartic acid residue of the Aβ peptide (purchased from Anaspec) and serves as a reporter of Aβ binding to hybrid lipid-coated AuNPs. The increase in the fluorescence anisotropy of TAMRA-Aβ\textsubscript{1-42} is expected upon its binding to lipid-coated AuNPs. This is due to the increase in the hydrodynamic radius (HR) upon Aβ binding to the AuNP surface (Scheme 1).
A representative fluorescence anisotropy plot of the TAMRA-\(\alpha\)-\(\beta\)1-42 in the absence and presence of Au-SO-PC-HT is shown in Figure III.2. An increase in the anisotropy (\(\Delta r = 0.1\)) is observed at 10 min after addition of Au-SO-PC-HT to the TAMRA-\(\alpha\)-\(\beta\)1-42, which suggest the hydrodynamic radius is increased and the molecular rotation is slowed, indicating binding to the AuNP surface (Figure III.2, (ii)). The beginning of the experiment (time = 0) begins with 47 nM TAMRA-\(\alpha\)-\(\beta\)1-42 in 10 mM PBS buffer pH 8.0, which has an intrinsic anisotropy of 0.12 in 10 mM PBS buffer at pH 8. The anisotropy of monomeric TAMRA-\(\alpha\)-\(\beta\)1-42 alone does not increase over 30 min in the absence of lipid-coated AuNPs. Therefore, the observed \(\Delta\) in \(r\) is due to TAMRA-\(\alpha\)-\(\beta\)1-42 binding to the lipid bilayer of the AuNPs. Furthermore, the \(\Delta\) in \(r\) is indicative of the binding efficiency (\% of TAMRA-\(\alpha\)-\(\beta\)1-42 binding to the lipid-coated AuNPs) and the relative size of the resulting \(\alpha\)-\(\beta\)-lipid-coated AuNP conjugate. This could be because the stronger the affinity \(\alpha\)-\(\beta\) has for the lipid membrane, the more monomers that will bind to its surface, and the greater the hydrodynamic radius of the TAMRA fluorophore on its surface.
Figure III.2. Representative fluorescence anisotropy of i) 47 nM TAMRA-Aβ1-42 in 10 mM PBS buffer pH 8.0 after addition of ii) 400 µL Au-SO-PC-HT nanoparticles O.D. 0.8 at 15-30 min.

Similar trials are conducted with TAMRA-Aβ1-42 monomers for Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5. Three trials are conducted for each lipid-coated AuNP membrane type as well as at each pH and the average ∆ in r (Δravg) of these samples is calculated (Figure III.3). The ranking of trials by membrane type and pH from largest average increase in r to least is: Au-SO-PC-HT at pH 6.5 (0.324 ± 0.014) > Au-SO-PC-Chol-HT at pH 6.5 (0.278 ± 0.008) > Au-SO-PC-HT at pH 8.0 (0.115 ± 0.008) > Au-SO-PC-Chol-HT at pH 8.0 (0.094 ± 0.017). This suggests that Aβ1-42 monomers have a stronger binding affinity for lipid-coated AuNPs at the more acidic pH of 6.5. The larger ∆ in r could be from a greater amount of TAMRA-Aβ1-42 monomers binding to the surface, forming AuNP-Aβ species with a larger hydrodynamic radius. At pH 6.5, Aβ1-42 has a greater hydrophobic surface verses 8.0, which could cause it to adopt a more folded structure.29,30 This folded structure might aggregate with itself more rapidly in solution and bind to the lipid-coated AuNPs with higher affinity. This increase in binding would form a larger hydrodynamic radius and lead to a slower molecular rotation of the TAMRA fluorophore. The anisotropy results also revealed a slightly greater ∆ in r for the
Au-SO-PC-HT nanoparticles verses the Au-SO-PC-Chol-HT. This could be indicative of Aβ₁-₄₂ monomers having a greater binding affinity for membranes without cholesterol verses cholesterol-rich ones. Previous studies with cholesterol membrane mimics have also shown Aβ₁-₄₂ to spontaneously insert itself into lipid bilayers with high cholesterol content.²⁷,²⁸ This insertion is occurs through the hydrophobic C-terminal end of the peptide. At lower pH the hydrophobic regions of Aβ₁-₄₂ could be even more buried in its folded structure and therefore less accessible. This could lead to a higher affinity of Aβ₁-₄₂ for the Au-SO-PC-HT AuNPs instead of the cholesterol-rich ones. This greater affinity would lead to more Aβ₁-₄₂ binding to the PC membrane surface, which would increase its hydrodynamic radius and therefore decrease the molecular rotation of the TAMRA-Aβ₁-₄₂ bound to the lipid-coated AuNPs surface.

![Bar chart](image)

**Figure III.3.** Bar chart of average Δ in r (Δr(avg)) for 3 trials of TAMRA-Aβ₁-₄₂ monomers incubated with Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5.

To confirm if Aβ₁-₄₂ monomers are binding to the surface of the lipid-coated AuNPs, we conducted dynamic light scattering (DLS). Solutions of Au-SO-PC-HT and Au-SO-PC-Chol-HT are monitored for their average hydrodynamic diameter (HD) of particles in
solution with DLS before and after the addition of Aβ_{1-42}. The binding of the Aβ_{1-42} monomers to the lipid-coated AuNPs is expected to result in an increase in the average HD of the particles in solution. This is due to the increase in the HD of the lipid-coated AuNPs when Aβ_{1-42} binds to their lipid bilayers (Scheme 2).

A representative distribution of the average HD of particles in a Au-SO-PC-HT solution before and after addition of Aβ_{1-42} monomers is shown in Figure III.4. An increase in the average HD of particles in solution is observed 15 min after the addition of Aβ_{1-42} indicating binding (Figure III.4 (ii)). The start of the experiment (Figure III.4 (i)) begins with 1 mL Au-SO-PC-HT OD 0.8 in 10 mM PBS buffer pH 8.0. The small change in the HD is consistent with a layer of Aβ on the nanoparticle surface. This small change of the HD suggests that there are no significant nanoparticle-Aβ-nanoparticle bridging interactions. DLS of Aβ_{1-42} monomers alone only show an average HD of 1 nm in size.
Further trials are conducted with Aβ₁₋₄₂ monomers for Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5. Two samples are conducted for each lipid-coated AuNP membrane type and at each pH. The average HD of the particles in solution is calculated for each set of samples 15 min after the addition of Aβ₁₋₄₂ monomers to it (Figure III.5). The ranking of trials by membrane type and pH from largest average increase in the HD to the least is: Au-SO-PC-HT at pH 6.5 had an increase in HD of 14 nm > Au-SO-PC-HT at pH 8.0 had an increase in HD by 13 nm > Au-SO-PC-Chol-HT at pH 6.5 and pH 8.0 had an equal increase in HD of 9 nm. This suggests a greater binding affinity of Aβ₁₋₄₂ monomers for Au-SO-PC-HT over Au-SO-PC-Chol-HT due to a larger increase in the average HD at both pH. This follows the similar trend of the fluorescence anisotropy trials, which also had a larger increase in the hydrodynamic radius of the Au-SO-PC-HT membranes. This could be an indicator of more Aβ₁₋₄₂ binding to the cholesterol-free membranes due to higher binding affinity. In contrast, there is no significant

Figure III.4. Representative dynamic light scattering distribution of the average HD of i) Au-SO-PC-HT in 10 mM PBS at pH 8.0 and 15 min after addition of ii) 2 μM Aβ₁₋₄₂ monomers.
difference between similar membrane types at different pH. This lack of change in the HD at different pH could be due to the Aβ₁₋₄₂ monomers being added to the lipid-coated AuNP buffer solution 15 min before conducting DLS. Where as the Aβ₁₋₄₂ monomers are incubating in the buffer solution for 15 min before the addition of AuNPs, which is followed by an additional 15 min of incubation with the AuNPs for the fluorescence anisotropy. This extra incubation time for Aβ₁₋₄₂ monomers during the fluorescence anisotropy trials could have led to more of a pH influence over their structure verses the DLS Aβ₁₋₄₂ monomer trials.

Figure III.5. Bar chart of average HD of particles in solution for 2 trials each of Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles incubated with Aβ₁₋₄₂ monomers in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5.

The effect of pH and Membrane Cholesterol on Aβ (Oligomer) Interactions with Hybrid Lipid-coated AuNPs

In recent years, it has been shown that the original “amyloid cascade hypothesis”, naming Aβ fibrils as the main culprit of AD, is unclear and has its limitations. Furthermore, it has been found that the Aβ oligomeric species are very toxic and now thought to be the main cause if not at least a major contributor to AD. Therefore, research characterizing how Aβ oligomers react with cellular membranes and how that differs from
other species of Aβ is an important area of AD research. For these experiments we prepared Aβ oligomers using the Stine protocol to produce a species stated by the literature to be of approximately 2 – 4 nm in size.\(^5\) In this study we compare and contrast the interactions of the oligomers and the monomers with our lipid-coated gold nanoparticles to learn more about their impact on the cellular environment. Previous studies have shown that not only does pH play a large role in the formation of Aβ oligomers, but that at acidic pH it may be more disruptive towards cellular membranes.\(^{29}\) Furthermore, there are previous studies \textit{in vitro} showing membranes with high cholesterol content preventing Aβ fibril formation, but causing oligomers to form instead.\(^{27,28}\) To investigate the difference in monomer and oligomer binding, here we conduct studies at two different pH and types lipid-coated AuNPs with and without cholesterol.

To investigate Aβ\(_{1-42}\) oligomers binding to the surface of the lipid-coated AuNPs, DLS is similarly conducted. Solutions of Au-SO-PC-HT and Au-SO-PC-Chol-HT had the change in their average hydrodynamic diameter (HD) of particles in solution monitored with DLS before and after the addition of Aβ\(_{1-42}\) oligomers (\textit{Scheme 2} and \textit{Figure III.4}). These trials are conducted in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5, and are the average of the HD over two trials for each membrane type at each pH. The ranking of trials by membrane type and pH from largest average increase in the HD to the least is: Au-SO-PC-HT at pH 6.5 had an increase in HD by 37 nm > Au-SO-PC-Chol-HT at pH 6.5 had an increase in HD by 27 nm > Au-SO-PC-Chol-HT at pH 8.0 had an increase in HD by 19 nm > Au-SO-PC-HT at pH 8.0 had an increase in HD by 12 nm. These results show that not only are Aβ\(_{1-42}\) oligomers more sensitive to pH, but their interaction with membranes without cholesterol are more influenced by it. An increase in HD with Aβ\(_{1-42}\) oligomers overall could
be due to oligomers having a stronger affinity for cellular membranes at either pH than monomers. This larger change in the HD could also be due to the larger size of the oligomers versus monomers, making their binding to the lipid-coated AuNPs more prominent. Incidentally this larger size of the oligomers could also be why there is more of a change seen in the HD between pH 6.5 and 8.0. Structural rearrangements due to pH by the bigger oligomers would show up more than with the monomers. Previous studies have shown that Aβ_{1-42} has a larger hydrophobic area at pH 6.5 and therefore can take on a more globular form.²⁷,²⁹ This form at the more acidic pH would be more prominent than the suggested more unstructured form of Aβ_{1-42} at pH 8.0.³⁰ It is also possible that Aβ_{1-42} oligomers have a stronger binding affinity for the membranes of the lipid-coated AuNPs at lower pH. The possible reason behind the stronger influence of pH on the Aβ_{1-42} oligomers in the presence of the Au-SO-PC-HT membranes could be due to Aβ_{1-42} insertion into membranes with high cholesterol content. As stated previously, it has been shown that Aβ_{1-42} tends to insert itself into membranes composed of high amounts of cholesterol.²⁷,²⁸ Furthermore, it has been shown that Aβ_{1-42} is less structurally influenced by solution pH in the hydrophobic bilayer of a membrane.²⁷ If Aβ_{1-42} is inside the bilayer of the high cholesterol lipid-coated AuNPs it would explain why it doesn’t have as large of an increase in HD at pH 6.5 as the Au-SO-PC-HT nanoparticles. The greater increase in the HD of the Au-SO-PC-Chol-HT AuNPs at pH 8.0 with the Aβ_{1-42} oligomers could also have to do with the structural arrangement of the oligomers causing them to have a stronger binding affinity for the cholesterol-rich membranes at the higher pH. Previous studies have shown that membranes with high cholesterol content are more prone to disruption, and oligomers have been shown to possibly be a more toxic form of Aβ_{1-42}.⁵,⁶,²⁸ Therefore, this larger increase in the HD of the Au-SO-
PC-Chol-HT AuNPs in the presence of Aβ₁-42 oligomers at pH 8.0 verses the Au-SO-PC-HT AuNPs could be due to a higher binding affinity of Aβ₁-42 oligomers for cholesterol-rich membranes at the higher pH. This suggests that at the higher pH cholesterol free membranes are more resistant to Aβ₁-42 oligomer binding than cholesterol-rich membranes.

![Bar chart of average HD of particles in solution for 2 trials each of Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles incubated with Aβ₁-42 oligomers in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5.]

**Figure III.6.** Bar chart of average HD of particles in solution for 2 trials each of Au-SO-PC-HT and Au-SO-PC-Chol-HT nanoparticles incubated with Aβ₁-42 oligomers in 10 mM PBS buffer pH 8.0 and 10 mM HEPES buffer pH 6.5.

**Evaluating the Effect of Aβ Monomers or Oligomers on Membrane Integrity**

UV-vis spectroscopy is used to monitor the binding interactions of Aβ₁-42 oligomers and monomers to the lipid-coated AuNPs. A 2 μM Aβ is incubated with Au-SO-PC-HT and Au-SO-PC-Chol-HT for 15 min in 10 mM PBS pH 8.0 or 10 mM HEPES pH 6.5. A representative plot of the UV-vis of a Au-SO-PC-HT solution before and after the addition of Aβ₁-42 oligomers is shown in **Figure III.7 (a-b).** The start of the experiment (**Figure III.7 (a)) begins with 1 mL Au-SO-PC-HT OD 0.8 in 10 mM PBS buffer pH 8.0. The Au-SO-PC-HT nanoparticles are then incubated with 2 μM Aβ oligomers (**Figure III.7 (b)). Since no significant red-shift in the SPR and change in O.D. of the AuNPs is observed this confirms that no nanoparticle-Aβ-nanoparticle bridging interactions are occurring in the presence of 2
μM Aβ oligomers. This is conducted for both membrane types, at each pH, for both Aβ oligomers and monomers for three trials each. For all trials no significant SPR shift or change in OD is observed.

KCN stability is used to determine if the binding of Aβ to the lipid-coated AuNP surface induces significant membrane disruption since Aβ is hypothesized to disrupt membrane stability and induce pore formation.6,8 If Aβ has compromised the lipid bilayer of the nanoparticles, then the gold core should be susceptible to KCN etch.10 Au-SOA-PC-HT is incubated with 2 μM Aβ oligomers for 15 min and followed by incubation with 3 mM KCN for 1 h. A representative UV-vis spectra of Au-SOA-PC-HT in 10 mM PBS at pH 8.0 shows no significant change in the SPR or O.D. as shown in Figure III.7 (a-c). This is conducted for both membrane types, at each pH, for both Aβ oligomers and monomers for three trials each. For all trials no significant SPR shift or change in OD is observed. These findings demonstrate that even though Aβ binds to hybrid lipid-coated AuNP surface there is no significant disruption in membrane integrity that leaves the lipid bilayer ion impermeable.

Figure III.7. Representative UV-vis spectra of a) Au-SO-PC-HT nanoparticles in 10 mM PBS pH 8.0 b) after 15 min incubation with 2 μM Aβ oligomers c) after 1 hour incubation with 3 mM cyanide.
CHAPTER 4

CONCLUDING REMARKS

This study demonstrates that hybrid lipid bilayer membrane mimics can be prepared consisting of multiple compositions of lipid membranes. Membranes consisting of PC and Chol are made and found to form robust, intact, and ion impermeable lipid bilayers around 10 nm gold nanospheres. These lipid-coated AuNPs can contribute to learning more about the etiology of Alzheimer’s disease by using them to study complex protein-membrane interactions. In this study specifically, it has been shown that these lipid-coated nanoparticles can be used to characterize how Aβ interacts with cellular membranes.

Fluorescence anisotropy and DLS show that Aβ is binding to the membranes of the lipid-coated AuNPs. Interestingly, UV-vis did not reveal aggregation of the nanoparticles due to this binding for Aβ oligomers or monomers. Furthermore, cyanide studies show that the lipid membranes of the AuNPs remain ion impermeable in the presence of Aβ. This suggests that the lipid bilayers are still intact despite being bound to Aβ. These UV-vis studies are conducted in the presence of 2 µM Aβ₁₋₄₂ oligomers and monomers, which is 4000 times greater than 0.5 nM, the concentration of Aβ found in normal cerebrospinal fluid.³⁰ Aβ₁₋₄₂ has been shown to disrupt and form channels in model membranes at concentrations of 5 µM.⁶ Therefore, these membrane mimics may be to robust to use for Aβ membrane instability tests, or higher concentrations of Aβ may be needed for future trials.

Even though the SPR band of the AuNPs do not exhibit significant changes to suggest Aβ binds to the lipid-coated AuNPs, DLS and fluorescence anisotropy revealed that pH and
membrane cholesterol content have an effect on Aβ’s interaction with lipid bilayers. Fluorescence anisotropy shows a greater increase in the hydrodynamic radius when Aβ monomers bind to lipid-coated AuNPs at pH 6.5 verse pH 8.0. It also shows a greater increase when Aβ binds to PC membranes verses cholesterol-rich membranes. DLS demonstrated similar increases in the HD in the presence of Aβ monomers for Au-SO-PC-HT AuNPs verses the cholesterol-rich ones. Previous studies show that a larger increase in the hydrodynamic radius at pH 6.5 for fluorescence anisotropy could be due to Aβ taking on a more spherical structure at a lower pH. This structural rearrangement is equated to Aβ having a larger hydrophobic region under more acidic conditions. The greater increase in the anisotropy of the cholesterol-free lipid-coated AuNPs could be equated to Aβ’s spontaneous insertion into bilayers with high cholesterol content. By becoming embedded in the lipid bilayer of the cholesterol-rich membranes, Aβ has less of an influence on increasing the profile of the membrane mimics than when it aggregates on the surface of the PC only membranes. This could also be due to a higher binding affinity of Aβ monomers for PC membranes over PC-Chol membranes. DLS shows little to no difference in the increase of the HD of the same type of lipid-coated AuNPs at different pH. This could be due to DLS not being as sensitive at showing the change in the HD as fluorescence anisotropy is at showing an increase in the hydrodynamic radius. Anisotropy is a more sensitive technique for visualizing a small Aβ peptide binding to a large AuNP surface compared to the small change in the HD expected with Aβ binds to the nanoparticle surface. The increase in hydrodynamic radius would be more drastic and also might be more influenced by the pH of the solution than the HD measured by DLS.
Dynamic light scattering is able to distinguish differences in how Aβ oligomers interacted with the membrane mimics verses monomers. In this study β oligomers binding to AuNPs showed a greater increase in the HD of the lipid-coated AuNPs. It is further revealed that this increase in the size of the HD is more strongly influenced by pH than the Aβ monomers. At pH 8.0 this increase in the HD of the membrane mimics is greatly reduced compared to pH 6.5. Furthermore, the reduction of the HD at pH 8.0 affected the membrane mimics with high cholesterol content less. Based off previous studies this reduced effect can also be linked to Aβ’s ability to spontaneously insert itself into lipid bilayers with high cholesterol content. Aβ has shown to be less structurally affected by solution pH inside a membrane than on its surface. Future studies to verify that Aβ is in fact inserting itself into the lipid bilayer should be done, as well as characterizing the cholesterol content of the membrane mimics.

To further investigate AB binding to lipid-coated AuNPs, future work proposed include TEM imaging of the lipid-coated AuNPs. Images of the cholesterol-rich membrane-coated AuNPs in the presence of Aβ monomer and oligomer species would help to further distinguish what structural influence this binding can cause to the lipid bilayer. Further images to ascertain how cholesterol is arranging itself within the bilayer, as patches or evenly distributed, would also be an important next step. Also the preparation of lipid membrane mimics of different compositions could be explored. These membrane mimics can be prepared with more complex mixtures of lipids as well as functionalized with proteins. Comparing Aβ’s interactions with these more complex models verses the simpler ones used in this study will help to further characterize Aβ’s role in cellular membrane disruption and Alzheimer’s disease.
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