Origin of Blue-Fluorescence of Tyrosine and Tryptophan Functionalized Silver Nanoparticle Solution and the Effect of Lipid Coating on Nanoparticle Stability

M.Sc. Thesis

by

SACHIN DEBNATH

DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY INDORE
MAY, 2023

Origin of Blue-Fluorescence of Tyrosine and Tryptophan Functionalized Silver Nanoparticle Solution and the Effect of Lipid Coating on Nanoparticle Stability

A THESIS

Submitted in partial fulfillment of the
requirements for the award of the degree
of
Master of Science

by
SACHIN DEBNATH
Roll No. 2103131021

DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY INDORE
May, 2023

INDIAN INSTITUTE OF TECHNOLOGY INDORE

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Origin of Blue-Fluorescence of Tyrosine and Tryptophan Functionalized Silver Nanoparticle Solution and the Effect of Lipid Coating on Nanoparticle Stability**, is an authentic record of my own work carried out during the time period from July 2021 to June 2023 under the supervision of Prof. Anjan Chakraborty, Professor, Department of Chemistry, Indian Institute of Technology Indore.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

Sachin Debnath. 15-05-2023
Signature of the student with date
(Sachin Debnath)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

A Chakraborty
15/05/2023
Signature of the Supervisor of M.Sc. thesis with date
(Prof. Anjan Chakraborty)

Sachin Debnath has successfully given his M.Sc. Oral Examination held on **17th of May, 2023**

A Chakraborty
Signature of Supervisor of M.Sc. thesis
Prof. Anjan Chakraborty
Date: 15/05/2023

S. Selvakumar (Dr. Selvakumar)
Convener, DPGC (Acting DPGC)

Date: 22.05.2023

22.05.23
Signature of PSPC Member
Dr. Tridib Kumar Sarma
Date:

Signature of PSPC Member
Prof. Tushar Kanti Mukherjee
Date: 23|5|23

Acknowledgments

With great pleasure, I want to express my deepest gratitude to my thesis supervisor **Prof. Anjan Chakraborty** (Professor, Department of Chemistry, Indian Institute of Technology Indore), for providing me an opportunity to undertake my research project under his research group. His constant guidance, timely support, and motivation have been constructive to complete this M.Sc. project. His enthusiasm and dedication have always inspired me.

My sincere thanks must also go to the PSPC members of my thesis: **Prof. Tushar Kanti Mukherjee** and **Dr. Tridib Kumar Sarma**, for their valuable suggestions and kind support.

I am grateful to Prof. Suhas S. Joshi, Director, Indian Institute of Technology Indore, for encouraging and providing the required facilities at the institute.

I would like to acknowledge the Indian Institute of Technology Indore for providing laboratory and computational facilities during my research project.

I am very grateful to Head, Department of Chemistry, Indian Institute of Technology Indore to constantly motivate me to research and providing us a nice environment. I would also like to thank Prof. Rajneesh Misra, Prof. Suman Mukhopadhyay, Prof. Apurba K. Das, Prof. Sampak Samanta, Prof. Biswarup Pathak, Dr. Tushar Kanti Mukherjee, Dr. Shaikh M. Mobin, Dr. Satya S. Bulusu, Dr. Chelvam Venkatesh, Dr. Amrendra Kumar Singh, Dr. Abhinav Raghuvanshi, Dr. Dipak Kumar Roy, Dr. Selvakumar Sermadurai, and Dr. Umesh A. Kshirsagar for their guidance and help throughout my M.Sc. course. I have benefited significantly from the Chemistry office staff's indeed professional support: Mr. Ghanashyam A. Bhavsar, Mr. Parthiban P. K., Mr. Manish Kushwaha, Ms. Vinita Kothari, and Mr. Rameshwar Dauhare.

I owe a special thanks to my project mentors **Mr. Avijit Maity** & **Mr. Debanjan Bagchi** for helping and guiding me throughout the project. I am also very fortunate to have such senior lab members. I would also like to thank them for the help in every aspect, friendly discussions, lunch breaks, endless treats, and good times outside the lab.

I would personally like to extend my admiration to all my batchmates during my complete M.Sc. journey.

Most importantly, I sincerely thank my parents (Mr. Chandra Sekhar Debnath and Mrs. Chinu Rani Debnath), Sister Rajlaxmi and my family members for their unconditional trust and support.

SACHIN DEBNATH

Dedication

This thesis is dedicated to my parents.

Abstract

The formation of lipid corona and lipid-induced aggregates on nanoparticles surface is an emerging field in nanoscience. Till now, several properties of Au NPs (surface charge, size, and surface ligand) and lipids (surface charge and phase state) have been discussed in literature. There is no such report in literature where silver nanoparticles can also form lipid corona and lipid-induced aggregates. In our work, we aim to investigate the formation of lipid corona and lipid induced aggregates on tyrosine and tryptophan functionalized silver nanoparticles and to analyze how the coating of lipid around the nanoparticle surface can boost its stability in the harsh environment of biological fluid. Additionally, our objective is to study the origin of nanoparticles. Among these numerous studies, Our results showed that tyrosine functionalized silver nanoparticles (Ag-Tyr NPs) are stable (possibly form lipid corona) in presence of charge lipid vesicles (Positive) and neutral (zwitterionic) lipid vesicle of high concentration. whereas at low concentration, it undergoes aggregation. Interestingly, at low lysozyme concentration, tryptophan functionalized silver nanoparticles (Ag-Trp NPs) are stable in presence of zwitterionic lipid vesicles but form aggregates in presence of lipid vesicles having positive charge. The Ag-Trp and Ag-Tyr NPs do not interact with negatively charged lipid vesicles suggesting the negative surface charge of nanoparticles. It has been also found stability of nanoparticle increases after coating of Lipid around its Surface i.e., lipid coating prevents the nanoparticle aggregation in different environmental Condition. Followed by this there are found to be quenching of fluorescence by nanoparticles where acceptor Ag NP accept the transferred energy from the fluorescent donor components in the solution.

Table of Contents

List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 3

List of Tables

CHAPTER 1

CHAPTER 2

CHAPTER 3

Nomenclature

° C	Degree Celsius
mL	Mililiter
mM	Milimolar
µL	Microliter
nm	Nanometer
nM	Nanomolar

Acronyms

AA	Amino Acid
AA-Ag NPs	Amino Acid functionalized Silver Nanoparticles
DMPC	1,2-Dimyristoyl-sn-Glycerol-3-Phosphocholine
DMPG	1,2-Dimyristoyl-sn-Glycerol-3- Phosphoglyceral sodium salt
DMTAP	2,3-di (tetradecanoyloxy) propyl-trimethylazanium
GUV	Giant unilamellar Vesicle
LUV	Large unilamellar Vesicle
MUV	Multi Lamellar Vesicle
NC	Nanocluster
MUV	Phenylalanine Nanocluster
phe	Phenylalanine
Ag NPs	Silver Nanoparticles
$AgNO_3$	Silver Nitrate
SUV	Small Unilamellar Vesicle
SPR	Surface Plasmon Resonance
Trp	Tryptophan
Ag-Trp NPs	Tryptophan Functionalized Silver Nanoparticles
Tyr	Tyrosine
Ag-Tyr NP	Tyrosine Functionalized Silver Nanoparticles

Chapter 1

Introduction

1.1 Nanoparticles

Nanoparticles are smaller particles with sizes ranging from 1 nm to 100 nm[1]. It holds a significant role in the modern realm of nanotechnology due to its unique physical and chemical properties[2]. It has excellent antibacterial activity as it is smaller in size than the pathogens[3]. Because of their unique properties at the nanoscale level, nanoparticles have sparked a lot of interest in biological applications[4]. Their compact size and high surface area-to-volume ratio make them adaptable and appropriate for various biomedical and biotechnological applications. Some significant areas in biology where nanoparticles have applications are in Drug Delivery, Imaging and Diagnostics, Cancer Therapy, Biosensors, Tissue Engineering, Tissue Engineering, Gene Delivery, etc[5–7]. In recent years, researchers have focused on the Nanoparticle fluorescence property to examine the self-assembly mechanism of amyloid aggregation to understand better disease-related structures in the presence or absence of nanoparticles[8].

Also, Nanoparticle has been widely used in electronic system. The term "nanoelectronics" refers to the use of nanotechnology in electrical components. It has a wide range of uses in fields like computing and the production of electronic goods like iPod Nanos' Flash memory chips and mouse, keyboard, and cell phone castings with antimicrobial and antibacterial coatings[9,10].

According to their size, shape, and material characteristics, nanoparticles can be categorized into a wide range of different categories. Dendrimers, liposomes, and polymeric nanoparticles are included in the organic category of certain classifications, whereas fullerenes, quantum dots, and gold nanoparticles are included in the inorganic category[11,12]. Other categories

categories nanoparticles depending on their carbon, ceramic, semiconducting, or polymeric composition. In addition, nanoparticles can be categorized as soft (like liposomes, vesicles, and nanodroplets) or hard (like titania (titanium dioxide), silica (silica dioxide), and fullerenes). The manner that nanoparticles are categorized often depends on their use, such as in therapy or diagnostics as opposed to basic research, or it could be connected to how they were made[13].

1.2 Synthesis of Nanoparticles:

Metal is the primary component of nanoparticles with a stabilizing agent acting as a ligand on their surface[14]. there are numerous processes for the synthesis of nanoparticles. Chemically, metal salt can be used to synthesis nanoparticles by combining it with a reducing agent and a stabilising agent, with the reducing agent converting the metal ion present in the metal salt into pure metal, creating the metal nanoparticle core that the stabilizing agent then stabilizes[15,16]. also, naturally plant, algae, fungi, yeast etc are found to be useful in the formation of Nanoparticles Because it imparts various types of color due to absorption in the visible range, it can be characterized by UV-Vis Spectroscopy[17,18]. Additionally, characterization methods like TEM (Transmission Electron Microscopy), SEM (scanning electron microscope), AFM (Atomic Force Microscope), etc. are used[19].

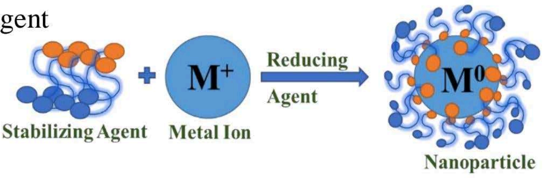

Figure 1: Schemetic Diagram of Nanoparticle Formation

1.3 Silver Nanoparticles:

Now, among different metal nanoparticles, Research Taking more interest on Silver Nanoparticles because of its low toxicity than others[20]. For the preparation of Silver Nanoparticles, the most useful substance is citric acid or trisodium citrate followed by sodium borohydrate[21]. But because of low toxicity and better caping agent, amino acid are used frequently[22].

1.4 Amino Acid Functionalized Silver Nanoparticles:

Aromatic Amino Acids contains $-NH_2$ and $-COOH^-$ group. They act as stabilizing as well as reducing agent. Thus, three aromatic amino acids—tryptophan, tyrosine, and phenylalanine—were used in the production of silver nanoparticles[23]. NaOH is also used in our work to increase the reducing power of amino acids. Both the concentration of Amino Acids and NaOH had an impact on the size of the Ag-NPs[24]. Temperature is another important factor in the creation of nanoparticles. It has been noted that at room temperature and in heated environments, the kinetics for the creation of nanoparticles change.

1.5 Fluorescence Property

The size and form of nanoparticles during synthesis have an impact on their optical properties and molecular structure[25–27]. Smaller nanoparticles typically have a fluorescent characteristic, setting them apart from larger ones[28]. The topic of tiny nanoparticles and bulk bodies is highly contradictory[29]. which makes the subject a fantastic and fascinating area of study. the smaller nanoparticle has been denoted by a term Nanocluster[30,31]. Some reports suggested that nanocluster is the initial forming step and has been formed during the formation of nanoparticle in its growing stage[32]. There is a lot of report regarding the formation of nanocluster during the nanoparticle synthesis characterized by studying the fluorescence nature of the supernatant part of nanoparticle solution[3]

Additionally, the amino acid residues (Tyr & Trp) found in the supernatant part are fluorescent by nature. Trp is said to include the Indole group, which makes it far more sensitive to fluorescence and the main source of absorption and emission[33–35]. Furthermore, some reports claim that amino acids undergo clusterization in the presence of metal ions, which are fluorescent by nature[36].

These substances give the Nanoparticle solution's fluorescence properties. In this work, we intend to investigate the fluorescence behavior of Nanoparticle Solution.

1.5.1 Energy Quenching: According to numerous studies the surface of certain nanoparticles has been found to contain ligands that are fluorescent. As was already mentioned, the fluid of nanoparticles solution contains fluorescent amino residue or amino acid clusters. Furthermore, During the synthesis of nanoparticles, fluorescent nanoclusters have reportedly been found to develop. Thus, there is a possibility of the energy quenching of fluorescent amino acid residue or nanocluster by the nanoparticle may thus lead to energy transfer between them. Where the fluorescent components in the solution serves as a donor while the nanoparticle acts as an acceptor. These type study has already been reported. however there have been no reports of silver nanoparticle-related events of this nature.

1.6 Lipid Bilayers (Liposomes):

A lipid-based liposome is a tiny artificial vesicle. Liposomes are formed of lipid bilayers and contain the two essential components hydrophobic tail and hydrophilic head, lying in the size range of µm to nm. In the aqueous medium, it takes on a spherical structure[37]. Liposomes are utilized as chemical carriers in medicine delivery systems since they are naturally non-toxic. In the aqueous medium, it takes on a spherical structure. Liposomes are utilized as chemical carriers in medicine delivery systems since they are naturally non-toxic.

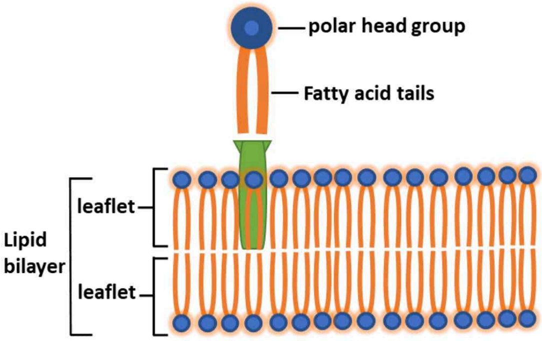

Figure 2: Phospholipid Bilayer

(I) SUV (Small Unilamellar Vesicles) (II) MLV (Multi-Lamellar-Vesicles) (III) GUV (Giant-Unilamellar Vesicles) (IV) LUV (Large- Unilamellar Vesicles).

The preparation of liposomes can be done in a number of ways. Some of the most popular techniques are the (I) reverse phase evaporation method, (II) the solvent injection method, and (III) the thin-film hydration method[37–40]. In this work, several lipids (DMPC, DMTAP, and DMPG) have been employed to produce liposomes utilizing the solvent injection method.

1.7 Studying the Nano-Bio Interaction of Nanoparticles with Lipid Bilayers:

There are many studies regarding the interaction of NP with various lipid bilayer in the literature. The head part of the lipid has the most important significance in the nano-bio interaction[41,42]. We previously reported the interaction of different lipid vesicle of different charge with Au NPs and optimized that high concentration of lipid leads to give promising stable lipid corona[5]. But at low concentration lipid-induced aggregation was observed whether it is positively charged or neutral lipid[43,44]. In this work our interest is to investigate the same i.e., the interaction of Ag-AA NPs with the lipids of different charge remaining the same stabilizing capping ligands (AA).

1.8 Effect of Lipid Coating on the Stability of Nanoparticles:

Lipid nanoparticles have made significant strides in research, and because of their excellent biocompatibility and high efficacy, they are now considered to be excellent carriers for many components and play a significant part in drug delivery system[45–47]. The significance of lipid nanoparticles stems mostly from the lipid coating's improvement of nanoparticle stability[48–50]. Although bare nanoparticles are typically stable, their stability is often diminished in an aqueous suspension and results in induced toxicity[51]. Additionally, the environment had an impact on the stability of the nanoparticles by neutralizing the surface charge by lessening the electrostatic attraction between the nanoparticles, leading to surface oxidation, the dissolution of silver ions, or nanoparticle aggregation[52]. By improving biocompatibility and protecting the nanoparticle from harsh environmental conditions of a specified pH, different ions concentration and temperature coating of lipid surrounding the nanoparticles provides outstanding stability[45,53]. In this work we intend to study the effect of lipid coating around nanoparticles surface on its stability by using the different pH conditions, different NaCl concentrations, and through freeze-Thaw Cycle.

1.8.1 Freeze-Thaw Cycle: Freeze-Thaw Cycle is a straightforward method to assess the stability of nanoparticles[54]. In this procedure, the nanoparticles are cycled between being kept at extremely low temperatures for freezing and returning to normal temperature for thawing. In this instance, nanoparticles are essentially excluded from the arrangement of water molecules and crystallization of ice during the freezing process, boosting both the local particle concentration and ionic strength in the liquid that has not yet frozen[55]. We examine the stability using this process by measuring the amount of cycle nanoparticle can stop from entering aggregation.

1.8.2 Varying pH level & Salt Concentration: This is a fairly popular technique where the salt and H+ concentrations are varied to investigate the effects on Nanoparticle stability. The concentration of H^+ and ions in the solution has a general tendency to neutralize the surface charge of the nanoparticles, which causes them to aggregate[41,56]. However, coating the nanoparticles can prevent these phenomena to a greater extent. All of this study can be optimized by varying the concentration of both the Salt and H^+ Concentration i.e., pH.

1.9 Objectives:

Our goal in this work is to optimize the production of nanoparticles by varying the Concentration of both NaOH & amino acids and then the origin of fluorescence in the resulting nanoparticles. We'll also research how different charged lipid liposomes interact with nanoparticles, and we'll also work to investigate how the lipid coating on the particles can affect their stability. This thesis's work is divided into three further chapters.

Chapter 2: It will provide a summary of all experimental techniques and works in.

Chapter 3: It will include all of the results that have been compiled and a brief explanation of each.

Chapter 4: The third chapter will serve as the overall conclusion of our research.

Chapter 2

Experimental Section

2.1 Chemicals and Reagents:

We purchased Three Amino Acids Phenylalanine (Phe), Tryptophan (Trp), Tyrosine (Tyr), Silver Nitrate salt ($AgNO_3$), and HEPES (4-(2-hydroxyetyl)-1-piperazineethanesulfonic acid from Sigma-Aldrich. The phospholipids i.e., DMTAP *(*2,3-di(tetradecanoyloxy)propyl-trimethylazanium chloride), DMPG (1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol sodium salt*),* and DMPC (1,2-Dimyristoyl-sn-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids. NaOH (Sodium Hydroxide) and Milli-Q water were purchased from Merck. All these chemicals were used received without further purification. All the glassware were kept for overnight in aqua regia (HCl/HNO3- 3:1) and after that cleaned it properly before the experiments.

2.2 Synthesis of Amino Acid functionalized Silver Nanoparticles:

With the use of three distinct AA and NaOH, we synthesized the Ag-NPs in situ, by altering the AA and NaOH concentrations. We primarily optimize the size variation of Ag-NPs functionalized with AA. To synthesize colloidal and stable AA functionalized Ag NPs, we use two distinct AA concentrations of 1 mM and 0.5 mM as well as altering the concentration of NaOH from 2 mM to 10 mM.

To make the Ag NPs, we first combined 8.75 ml of Milli-Q water with 250 µL of 0.75 mM aqueous silver nitrate solution. The 9ml solution underwent continuous stirring for 30 minutes at 80°C. The necessary quantity of AA solution at a particular concentration was then added to it, followed by the addition of NaOH at a particular concentration. The entire solution was then left at 80°C for 2 hours while being constantly stirred. As colloidal Ag NPs develop, the color of the solution changes from colorless to yellow.

2.3 Preparation of Supernatant Solution:

We had poured about 2 ml units of nanoparticle solution into a few glass vials, put them in a centrifuge (REMI RM 12C BL), and let it spin for about 20 minutes at 800 rpm. Following that, two distinct layers were created, with the lower layer being the naked nanoparticle and the upper layer being the supernatant. From each 2 mL nanoparticle solution, we extract roughly 1.6 mL of supernatant solution. Further research was done on the supernatant solution's fluorescence characteristic.

2.4 Kinetic Study of the Silver Nanoparticles:

We used the same synthesis process to make the nanoparticles in order to evaluate the kinetics of their formation. However, at that point, both the absorbance and emission data had been optimized within a certain time interval. Initially, A vial was taken, properly cleaned, and dried using evaporation. A 125 mL solution of 30 mM AgNO3 was prepared to 4.5 mL and set aside for continuous stirring at 720 rpm at room temperature. After around 30 minutes, which were used to homogenize the solution, 500 mL of 10 mM tyrosine solution was added, preferentially followed by 50 mL of 1 M NaOH solution. The sample was obtained immediately and after 1 minute of addition to analyze its emission and absorption spectra. The data was collected between the times of 5 min, 10 min, 20 min, 30 min, 40 min, 40 min, 50 min, 60 min, 80 min, 100 min, 120 min, 150 min, and 12 hours.

In a hot environment, the kinetics are currently too fast. Therefore, it is somewhat unpredictable to analyze the Absorption and Emission phenomenon. In order to better understand the kinetics involved in the production of nanoparticles, we repeated the same experiments using a comparable reaction mixture at room temperature in addition to the heated setting. Throughout the whole experimental phase, simultaneous recordings of the emission and absorption phenomena were made.

2.5 Preparation of Lipid Vesicles:

In a HEPES (pH-7.22) medium, we prepared three distinct surface-charged liposomes at a concentration of 0.8 mM. The round bottom flask was first filled with 3 ml of the HEPES solution, which was then heated at 80 °C for roughly one hour. The required amount of lipid was then included. For the lipid dissolution, we utilize 1% ethanol. After one hour, we added the lipid sample to the HEPES solution and let the cap stay open for ten to fifteen minutes to let the ethanol evaporate. After that, the heating was turned off, and it was left stirring vigorously for 4-5 hours. Since the DMTAP and DMPG are charged lipids, we mix neutral DMPC and charged DMTAP/DMPG in a 7:3 ratio to ignore any form of repulsion between the charged lipids.

2.6 Lipid Vesicle-Nanoparticle Mixture Preparation:

In order to study the interaction between lipid vesicles and NP, we mixed these three different surface-charged liposomes with a set concentration of Ag-Tyr and Ag-. By using fixed concentrations of Ag NPs and various concentrations of lipid, we were able to change the numerical ratio of Ag NPs to liposomes per ml (1:1, 1:2, 1:5, 1:10, 1:20, 1:40, 1:80, and 1:160). For incubation, the entire Ag NPs solution containing various lipids in varying concentration ratios was left overnight.

2.7 Instrumentation:

Fluoromax-4p spectrofluorometer from Horiba Jobin Yvon (model: FM-100) was used to record all the fluorescence spectra, using a quartz cuvette of 1010 mm2, while UV-Vis Spectrophotometer (Cary 100 Bio) was utilized to analyze all the absorption spectra. The software OriginPro 8.1 was used to analyze the absorption spectra.

Chapter 3

Results and Discussion

3.1 Optimization and Characterization of Different Amino Acid Functionalized Ag NPs:

At first, we aim to know the optimum reaction condition to synthesize aromatic amino acid functionalized silver nanoparticles (Ag-AA NPs) by the use of various aromatic amino acids, including (I) Tyrosine (Tyr), (II) Tryptophan (Trp), and (III) Phenylalanine (Phe). Here, amino acids act both as reducing agents & stabilizing agent. The Structure of these three aromatic amino acids (AA) is given below

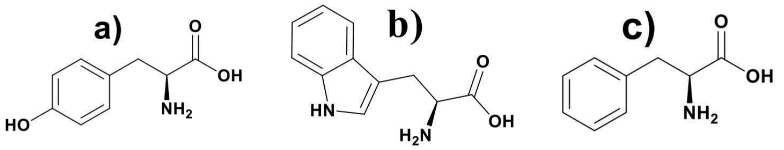

Figure 3: Molecular Structure of a) Tyrosine b) Tryptophan c) Phenylalanine.

Tyr (mM)	NaOH (mM)	SPR Peak (nm)
1	3	423
1	5	418
1	10	407
0.5	10	412
0.5	5	415
0.5	3	419
0.5	2.5	430
0.5	2	431

Tyr (mM)	NaOH (mM)	SPR Peak (nm)
1	5	411
1	3	418
1	10	407
1	2.5	422
1	2	427
0.5	5	409
0.5	3	407
0.5	2.5	410
0.5	2	413

Table 1: Optimization Table for the Synthesis of Tyrosine Functionalized (Ag- Tyr NPs) and Tryptophan Functionalized (Ag-Trp NPs) Silver Nanoparticles.

As we discussed earlier that we varied the reaction condition by changing the concentration of NaOH (2 mM to 10 mM) and AA (1 mM & 0.5 mM) at fixed concentration of $AgNO_3$ and optimize the formation of AA functionalized Ag NPs. The same thing has been followed for the synthesis of all three AA functionalized Ag NPs.

3.1.1 Tyrosine (Tyr) Functionalized Ag-NP: It was found that the smallest sized Tyr functionalized Ag NPs of 20-25 nm was observed by the optimize condition of 1 mM Tyr and 10 mM NaOH, which gives the SPR Peak at about ~407 nm in UV-Visible Spectra.

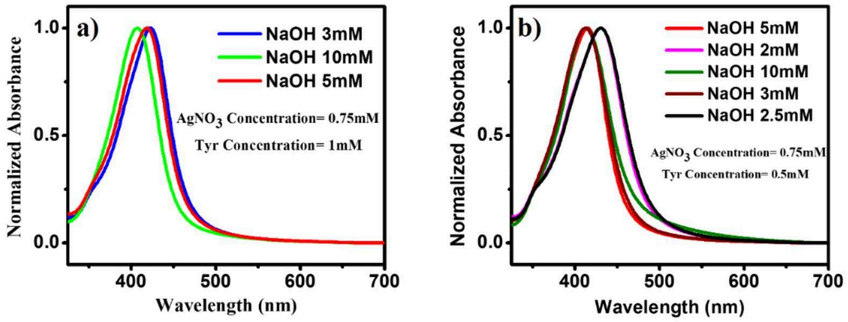

Figure 4: Normalized UV-Visible Absorption Spectrum of Ag-Tyr NPs at Various Concentration of NaOH and Fixed Concentration of Tyr (a) 1 mM and (b) 0.5 mM and Fixed Concentration of $AgNO_3$ (0.75 mM).

3.1.2 Tryptophan (Trp) Functionalized Ag NPs: Among these concentration conditions, Trp of 1 mM and NaOH of 10 mM is optimum concentration to synthesize the Trp functionalized Ag NPs having the size range of about 20-25 nm showing the SPR Peak at ~407 nm in UV-Visible Spectra.

3.1.3 Phenylalanine (Phe) Functionalized Ag NPs: We found that there was no formation of Phe functionalized Ag NPs with any of the optimized condition (Phe - 1mM and0.5mM, NaOH - 2mM to 10 mM). No characteristic SPR Peak was observe in UV-Visible Spectra.

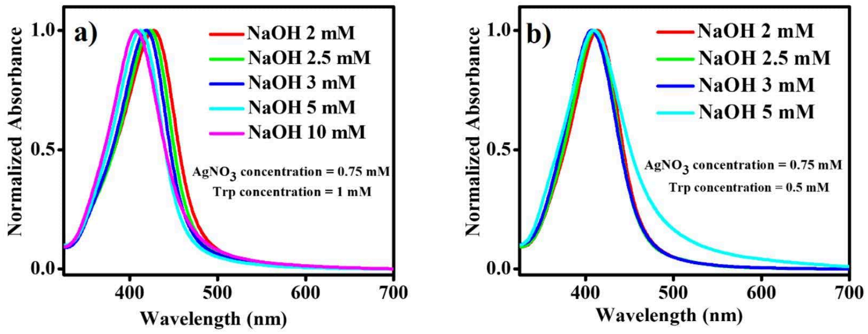

Figure 5: Normalized UV-Visible Absorption Spectrum of Ag-Trp NPs at Various Concentration of NaOH and Fixed Concentration of Trp (a) 1 mM and (b) 0.5 mM and Fixed Concentration of $AgNO_3$ (0.75 mM).

3.2 Studying the Fluorescence Property and Kinetics of the Formation of Tyr & Trp Functionalized Nanoparticles:

3.2.1 Kinetics of Nanoparticle Formation:

To understand the kinetics of nanoparticle production We simultaneously captured the absorption and emission data during a specified time period. Using our usual method of synthesis, we first began to gather data on absorption and emission during the production of nanoparticles under heated conditions. Following are the heated absorption and emission spectra for Trp and Tyr functionalized nanoparticles.

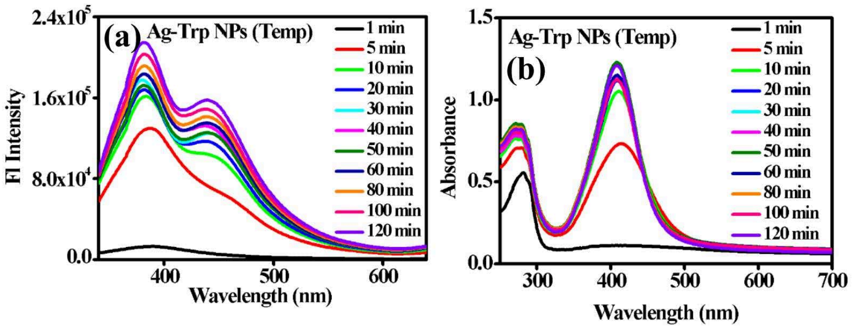

Figure 6: (a) Emission Spectra and (b) UV-Visible Absorption Spectrum of Ag-Trp NPs at Heated Condition During the Formation.

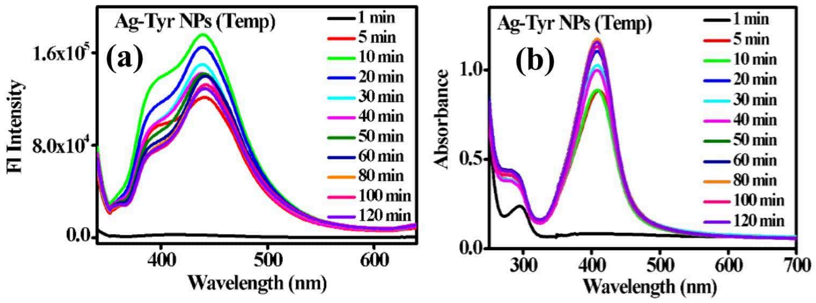

Figure 7: (a) Emission Spectra and (b) UV-Visible Absorption Spectrum of Ag-Tyr NPs at Heated Condition During the Formation.

It was found that the fluorescence emission spectrum has two peaks, one at between 380-400 nm and the other between 400 and 500 nm.The first peak is due to the Amino Acids (Tyr & Trp). Additionally, the absorption is very slow at first but suddenly increases to a greater level. It is therefore quite quick to study the kinetics. also Int. 440/Int. 380 ratio with time indicates that the initial reaction is too quick.

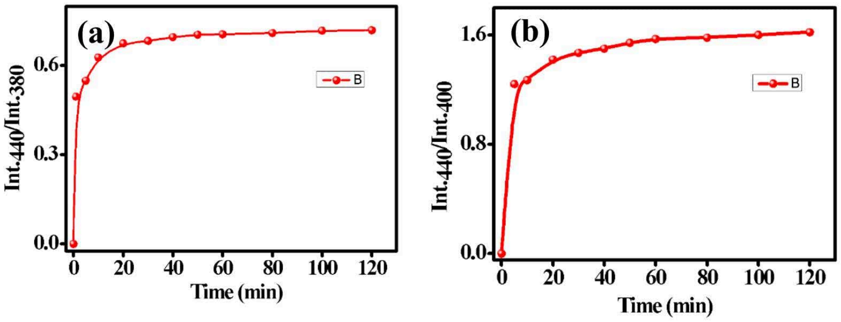

Figure 8: Plot of Int.440/Int.380 with Time for (a) Ag-Trp NP and (b) Ag-Tyr NP During Synthesis at Heated Condition.

The reaction was therefore monitored at Room temperature while using the same reaction mixture in order to better understand the kinetics. The absorption and emission spectra of both Tyr and Trp functional nanoparticle during the formation are shown bellow-

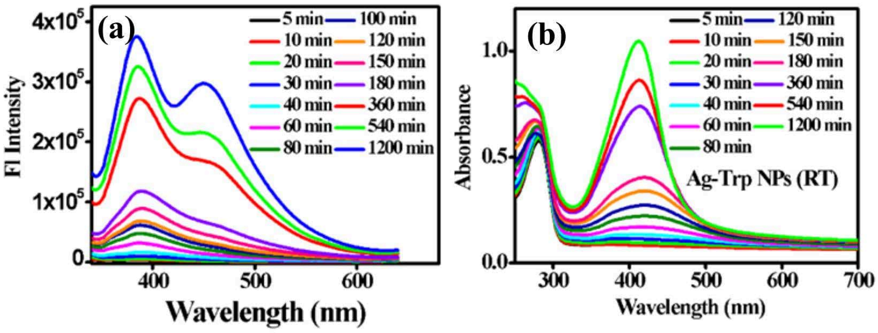

Figure 9: (a) Emission Spectra and (b) UV-Visible Absorption Spectrum of Ag-Trp NPs at Room Temperature During the Formation.

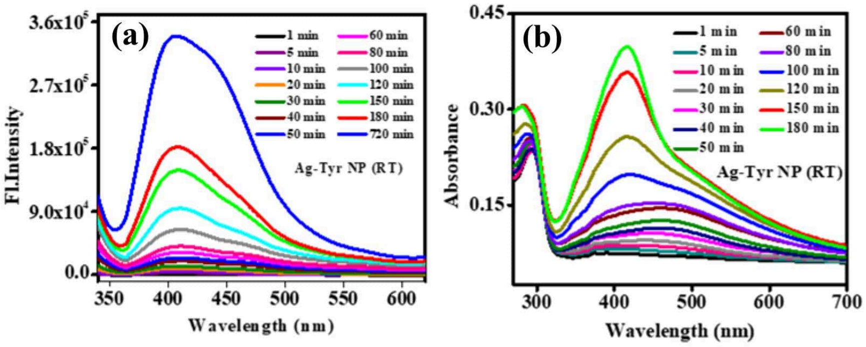

Figure 10: (a) Emission Spectra and (b) UV-Visible Absorption Spectrum of Ag-Tyr NPs at Room Temperature During the Formation.

It is observed that the first peak i.e., the peak due to the amino acid (already Reported) initially started to increase then fall down with time after a particular period. For a better understanding of the first emission, we performed the same reactions but this time without Ag salt. And this time it is found that there is an emission peak at about 350 nm which indicates the emission of free amino acid.

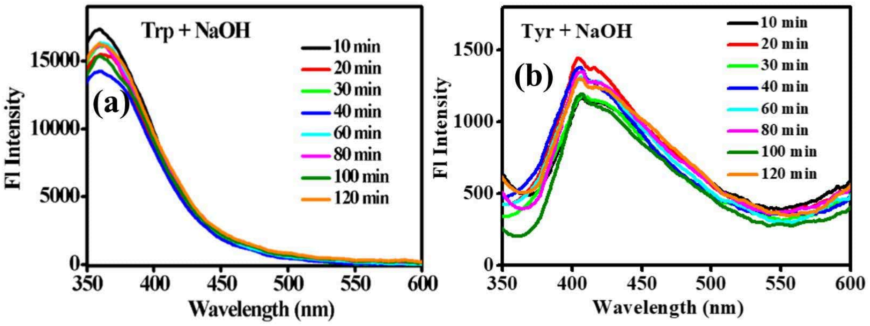

Figure 11: Emission Spectra of free (a) Tryptophan and (b) Tyrosine During Synthesis Without Ag-Salt.

This peak at about 350 nm indicate emission due to the free amino acids. Which confirms that in presence of Ag^+ ion the amino acids (Trp & Tyr) undergo clusterization result into shifting of peak between 350- 400 nm.

There are followed by a secondary peak in the 400–450 nm range. According to many studies, it may be due to the quenching by Ag Nanoparticle, where emission spectra of different donors overlap with characteristics surface plasmon resonance of Ag nanoparticle i.e., there may be a possibility of Energy Transfer between the acceptor Ag NP and the donors (amino acid cluster/nanocluster) present in the System. The actual Reason is still unclear.

3.2.2 Study of the Supernatant Portion and Investigation of Ag NP Quenching:

First separated the supernatant portion. The solution's supernatant portion of both the Nanoparticles was optimized by exciting at various wavelengths between 300 and 360 nm, and the matching emission peak was seen between 300 and 400 nm. It was found that the fluorescence property is excitation independent in nature for both the case.

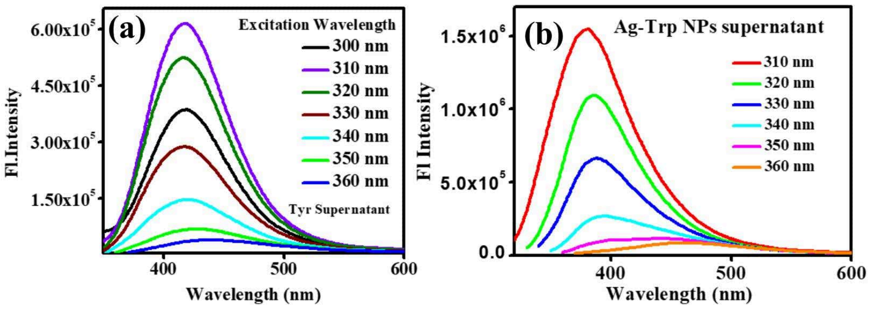

Figure 12: Excitation Independent Emission Spectra of (a) Tyrosine Supernatant and (b) Tryptophan Supernatant Portion of the Solution.

3.2.2.1 Study the Quenching of Ag Nanoparticle:

Firstly, we prepare the naked nanoparticle, centrifuge it at 8000 rpm and after that separating the supernatant portion. secondly, we centrifuged the raw nanoparticle for the same time period of 20 min but this time the rotation was 20000 rpm, after that we take this supernatant portion as our useful material. Followed by these, after optimizing the emission spectra of the fluorescent supernatant solution, the addition of the nanoparticle component at various concentrations began. The emission phenomenon was recorded in every step.

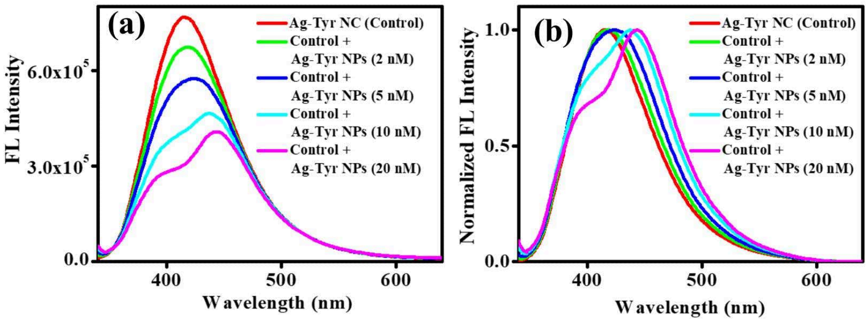

Figure 13: (a) Emission Spectra & (b) Normalized Emission Spectra of Tyr NC & Tyr NP Fluorescence Quenching.

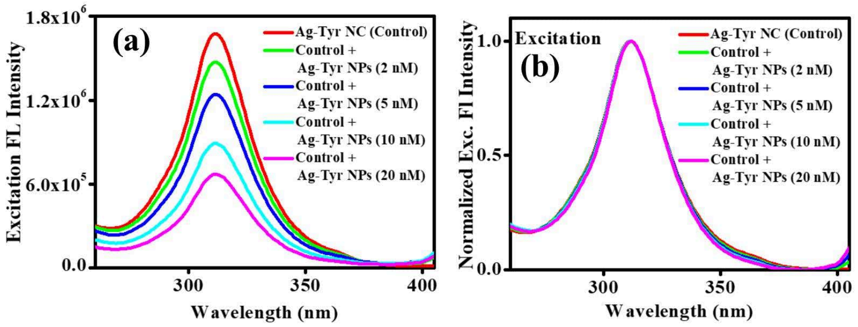

Figure 14: (a) Excitation Spectra & (b) Normalized Excitation Spectra of Tyrosine NC & Tyrosine Nanoparticle Fluorescence Quenching.

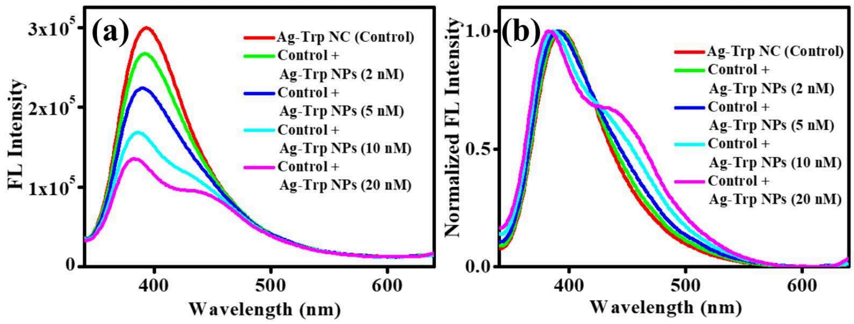

Figure 15: (a) Emission Spectra & (b) Normalized Emission Spectra of Trp NC & Trp NP Fluorescence Quenching.

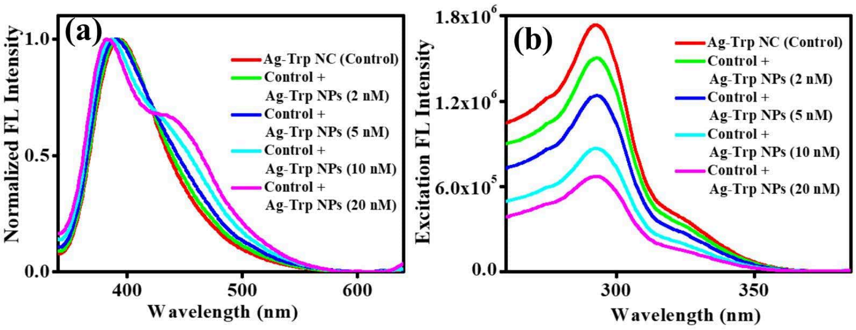

Figure 16: (a) Excitation Spectra & (b) Normalized Excitation Spectra of Trp NC & Trp NP Fluorescence Quenching.

It was noted that when the concentration of nanoparticles in the supernatant solution increased, the characteristic emission peak of the supernatant portion started to decline and a secondary peak formed in the 400–450 nm region. With a rise in the concentration of nanoparticles, the second peak becomes stronger and more dominating. This study shows unequivocally that an energy transfer between the fluorescent supernatant component and the nanoparticle is happening, where the supernatant sample acting as a donor and the nanoparticle acting as an acceptor. The schematic diagram of energy transfer is shown bellow-

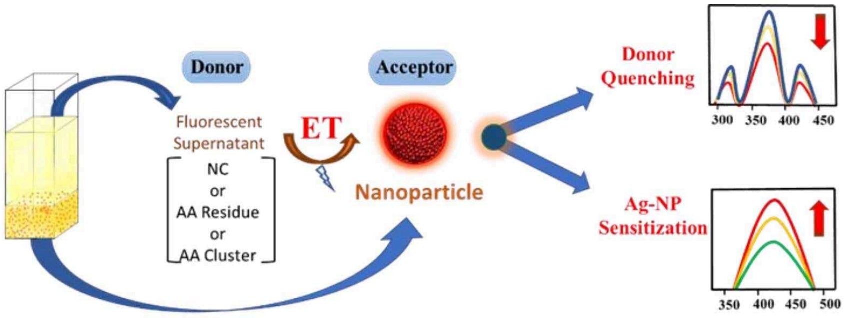

Figure 17: Schematic Diagram of Energy transfer

Followed by these to understand the origin of fluorescence in Nanoparticle i.e., which part of the nanoparticle accepting energy, we further investigated following the same quenching method. But this time the process was carried out by the use of citrate functionalized nanoparticle.

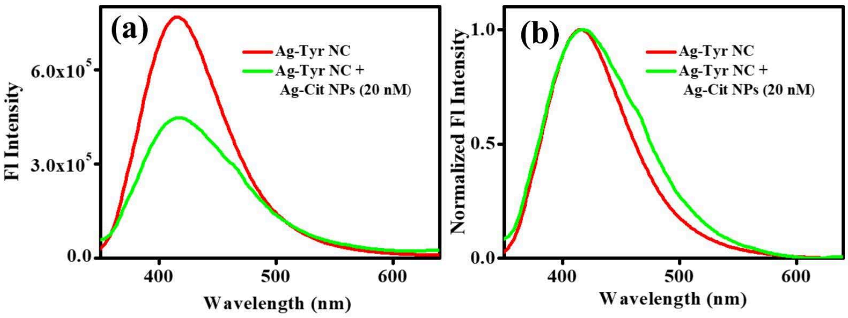

Figure 18: (a) Emission Spectra & (b) Normalized Emission Spectra of Tyr NC & Cit NP Fluorescence Quenching.

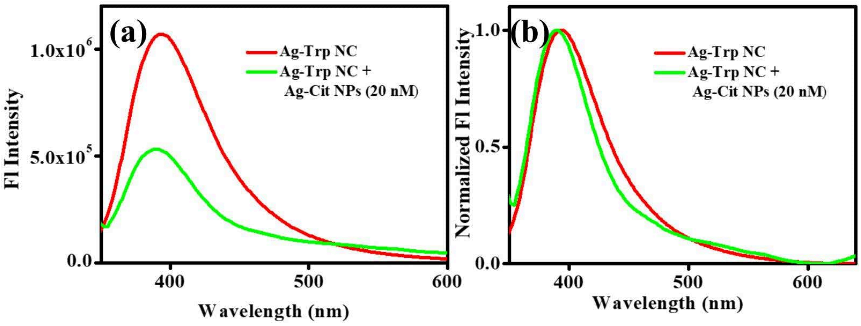

Figure 19: (a) Emission Spectra & (b) Normalized Emission Spectra of Trp NC & Cit NP Fluorescence Quenching.

It was found that this time there was no any secondary peak which gives us a possibility that the AA functionalized Nanoparticle contains some AA cluster on the surface of the Nanoparticle which are Fluorescent in nature and this is the one who accepting all the energy.

3.3 Studying the Interaction of Different Lipid Vesicles with Ag-NPs:

We investigated the interaction of three lipid vesicles having different surface charged namely DMPC (zwitterionic), DMTAP (positive) and DMPG (negative) With Tyr and Trp Functionalized Ag NPs. We used 7:3 ratio of DMPC-DMTAP and DMPC/DMPG as the DMTAP and DMPG are charged in nature, whereas the DMPC is neutral. We started with 0.8 mM 500 μL of lipid with 500μL 20 nM Ag NPs of Tyr and Trp both. This concentration indicates the 1:1 ratio of Liposome to Nanoparticle numbers per ml. Next, we varied the lipid concentration at fixed ag NPs concentration to get the desired ratio from ratio 1:1 to 1:160. The lipid-Ag NPs mixture was incubated overnight to reach the equilibrium. The UV-Vis absorption spectroscopy was used to establish the interaction.

3.3.1 Ag NPs Interaction with Negatively Charged Lipid Vesicles (DMPC: DMPG):

The UV-Visible spectra showed that the Ag-Tyr and Ag-Trp NPs did not interact with negatively charged lipid vesicles at any concentration as the SPR peak was not shifted. The results suggested that the Ag NPs are negatively charged.

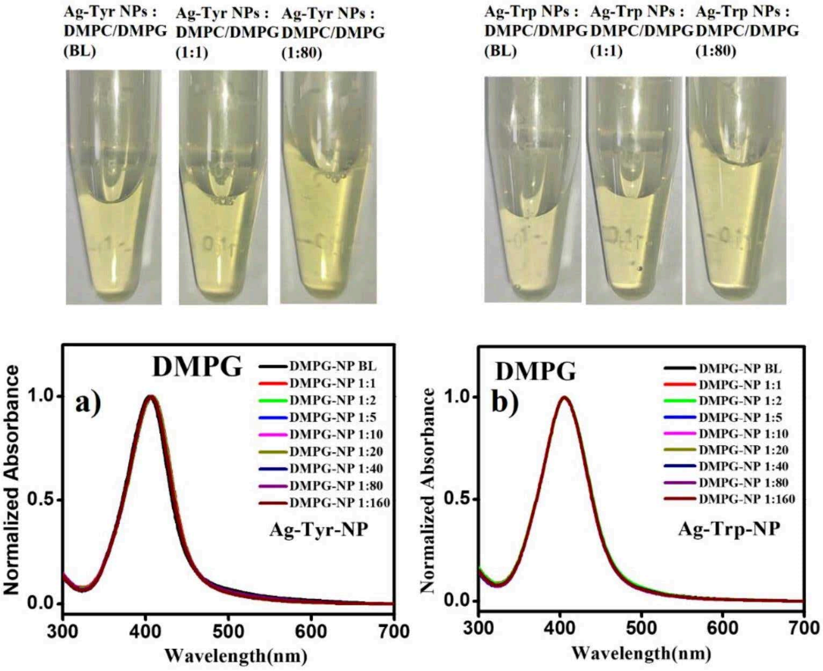

Figure 20: Normalized UV-Visible Spectra of the (a) Ag-Tyr NPs and (b) Ag-Trp NPs in Presence of Different Concentration of DMPC/DMPG (Negative Charged) Lipid Vesicles.

3.3.2 Ag NPs Interaction with Lipid Vesicles Having Positive Charge (DMPC: DMTAP):

The UV-Visible spectroscopy showed a SPR peak shift of about ~12nm for Ag-Tyr NPs and ~ 8nm for Ag-Trp-NPs at high lipid concentrations of positively charged lipid vesicles. With the decrease in the lipid concentration, both the Ag-Tyr NPs and Ag-Trp NPs underwent aggregation upto the number ratio 1:160. This result suggests that when the number of nanoparticles are 160 times the number of lipid vesicles having positive charge (1), the Ag-Tyr and Ag-Trp NPs form lipid-induced aggregates.

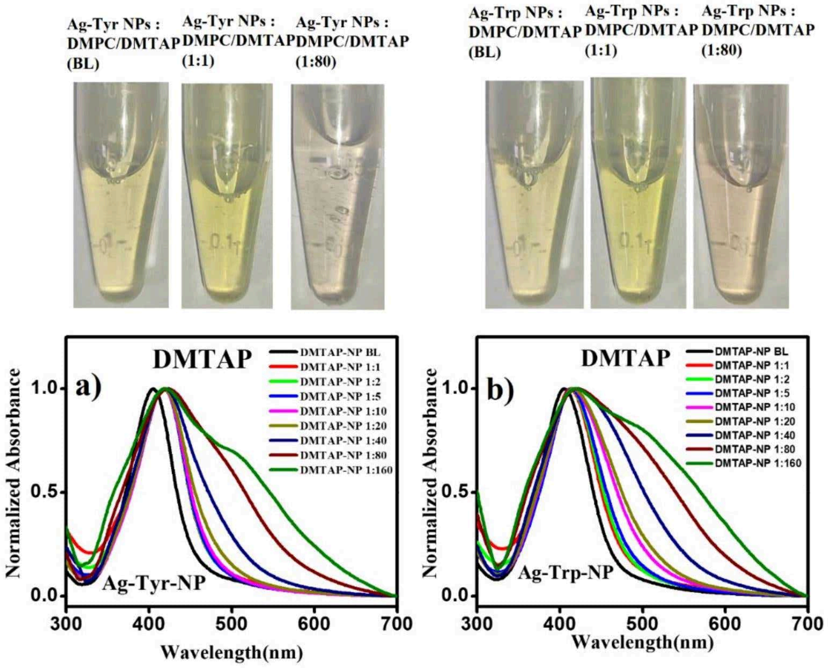

Figure 21: Photograph and Normalized UV-Vis Absorption Spectrum of the (a) Ag-Tyr NPs and (b) Ag-Trp NPs in Presence of Different Concentration of DMPC/DMTAP (Positively Charged) Lipid Vesicles.

3.3.3 Ag NPs Interaction with Neutral (Zwitterionic) Lipid Vesicles (DMPC):

In presence of zwitterionic lipid vesicles (DMPC) at high concentration, both the Ag-Tyr and Ag-Trp NPs were stable. But at low concentration of zwitterionic lipid vesicles (DMPC), the Ag-Tyr NPs underwent aggregation, but the Ag-Trp NPs did not. The UV-Visible spectra showed a spectral shift of SPR peak ~ 8nm for Ag-Tyr NPs whereas the SPR peak was slightly broadened when the lipid concentration [Ag NPs: DMPC (1:1)] is high. At low concentration of lipid [Ag-Tyr NPs: DMPC (80:1)], the SPR peak was shifted at longer wavelength along with a new broadened peak. But for Ag-Trp NPs [Ag-Trp NPs: DMPC (80:1)], the SPR peak of ag-Trp NPs was not at all shifted.

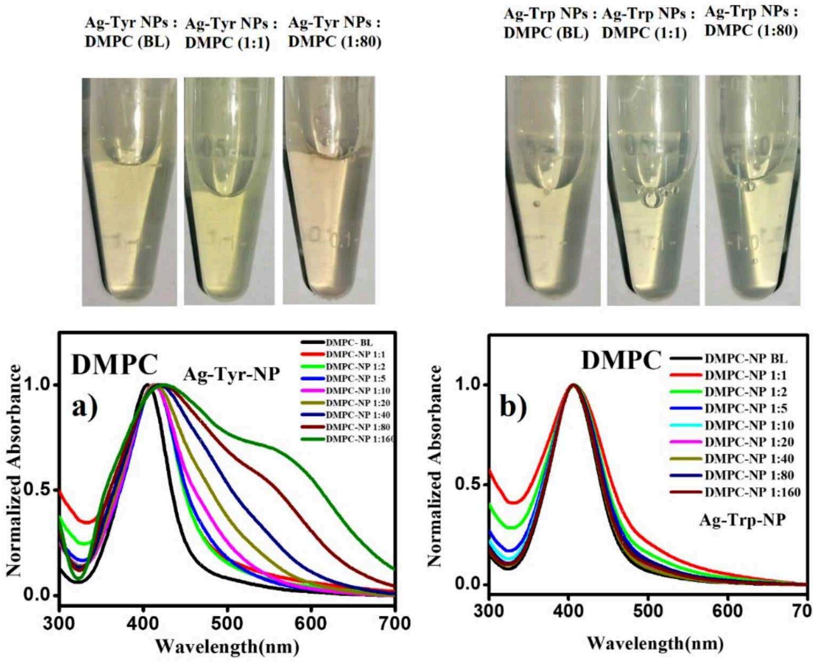

Figure 22: Photograph and Normalized UV-Vis Absorption Spectrum of the (a) Ag-Tyr NPs and (b) Ag-Trp NPs in Presence of Different Concentration of DMPC (Zwitterionic) Lipid Vesicles.

3.3.4 Ag NPs Interaction with Neutral (Zwitterionic) Lipid Vesicles (DPPC):

When the amount of lipid is relatively low (Ag-Trp NPs: DPPC (80:1)), the UV-Vis absorption spectrum shows a broader peak, indicating that the nanoparticles are aggregating. However, Stable interaction were found when the amount of lipid is significantly high (Ag-Trp NPs: DPPC 1:1, 1:2, or 1:5), a small spectral shift of about 6-7 nm in the SPR peak was observed. While Tyr functionalized nanoparticles showed several diverse outcomes, including stable nanoparticles with a spectrum shift of roughly

6-7 nm at high lipid concentration (Ag-Tyr NPs: DPPC 1:1, 1:2). However, there is no change in the SPR peak, indicating that there was no interaction when low lipid concentration (Ag-Tyr NPs: DMPC (80:1)) were utilized.

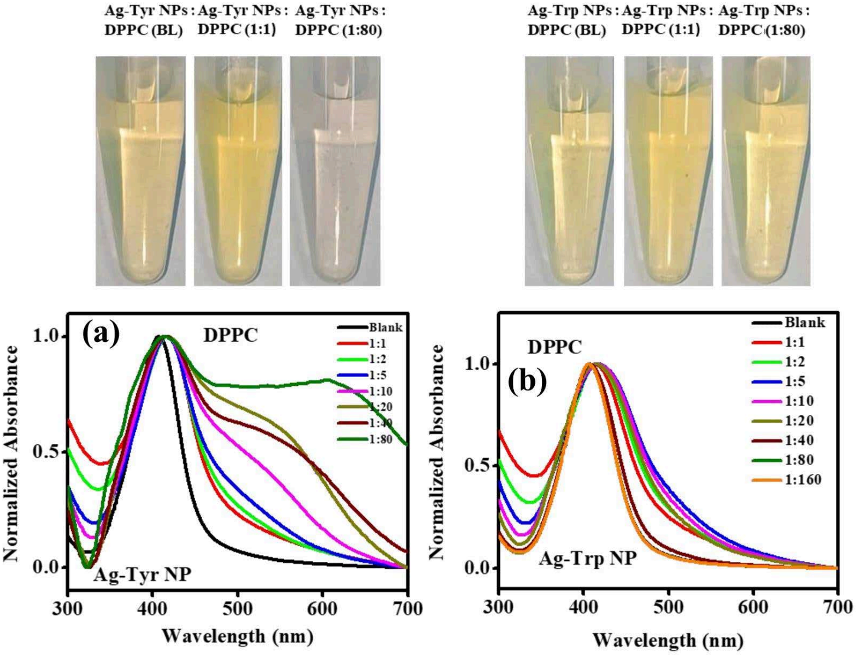

Figure 23: Photograph and Normalized UV-Vis Absorption Spectrum of the (a) Ag-Tyr NPs and (b) Ag-Trp NPs in Presence of Different Concentration of DPPC (Zwitterionic) Lipid Vesicles.

3.3.5 Ag NPs Interaction with Neutral (zwitterionic) Lipid Vesicles (DOPC):

High lipid concentration (Ag-Tyr NPs: DOPC 1:1, 1:2 & 1:5) gives the stable interaction of lipid (DOPC) and Tyr nanoparticle. we found a SPR peak shift of about 6 nm in UV-Vis spectra in this case. Followed by the Aggregation formation at lower lipid concentration of DOPC (Ag-Tyr NPs: DOPC 80:1). But a fantastic phenomenon was observed in case of Trp functionalized nanoparticle. Compare with all the other Zwitterionic lipid, DOPC shows a considerable interaction of SPR peak shifting about 3-4 nm. But similarly the interaction become diminished when the lipid concentration is low (Ag-Trp NPs: DOPC 80:1).

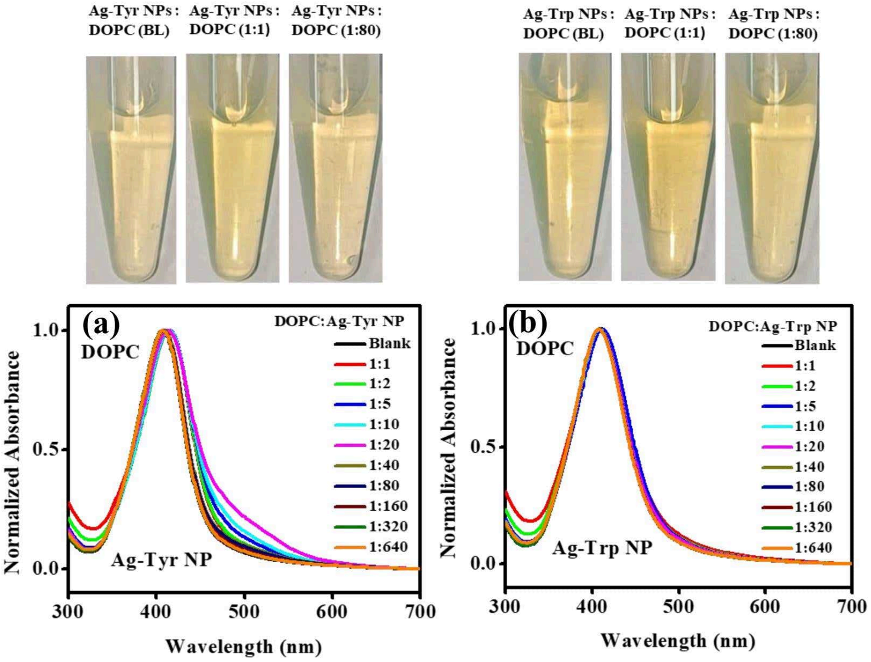

Figure 24: Photograph and Normalized UV-Vis Absorption Spectrum of the (a) Ag-Tyr NPs and (b) Ag-Trp NPs in Presence of Different Concentration of DOPC (Zwitterionic) Lipid Vesicles.

	Ag-Tyr NP:Lipid		**Ag-Trp NP:Lipid**	
	1:1	**80:1**	**1:1**	**80:1**
DMPG	No Interaction	No Interaction	No Interaction	No Interaction
DMTAP	**Stable**	Lipid-Induced Aggregation	**Stable**	Lipid-Induced Aggregation
DPPC	**Stable**	Lipid-Induced Aggregation	**Stable**	**Stable**
DMPC	**Stable**	Lipid-Induced Aggregation	**Stable**	Lipid-Induced Aggregation
DOPC	**Stable**	No Interaction	**Stable**	**Stable**

Table 2: Interaction of Different Lipid with Tyr & Trp Functionalized Silver Nanoparticle at Higher (1:1) and Lower (80:1) Lipid Concentration.

3.4 Studying the Effect of Lipid Coating on the Stability of Ag NP:

At specific pH levels and in the presence of varied ion concentrations, stability of nanoparticle decreases. Additionally, the temperature has an impact. However, compared to naked nanoparticles, lipid coated nanoparticles are far more stable because the lipid coating increases biocompatibility and protects them from the hostile environment of biological fluid. We combine 500 μL of 20 nM nanoparticles with 0.8 mM 500 μL liposomes of various lipids which indicates a 1:1 ratio of nanoparticle to liposome as an aim of examine the impact of lipid coating on the stability of nanoparticles. The ratio was then adjusted up to 1: 160. We next added various quantities of NaCl to study the stability at various concentration level of ions. Finally, we performed a freeze-thaw cycle while keeping the solution at -20. and allowed it to freeze then kept it at Room temperature until it thawed. It was found that the lipid coating offers the nanoparticles an exceptional degree of durability. The interaction was examined using UV-Vis absorption spectroscopy.

(3.4.1) Stability of Naked Ag-Tyr NP:

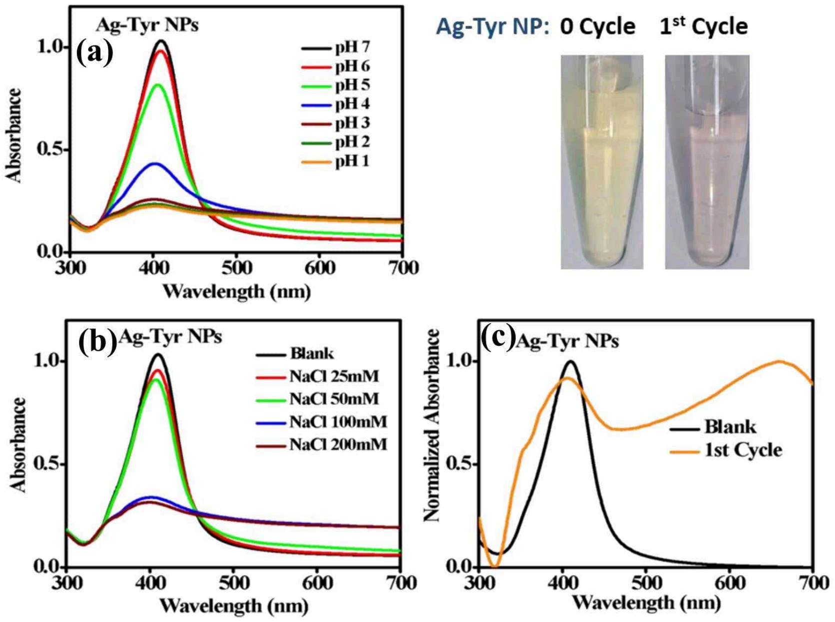

Figure 25: Absorption Spectra of Ag-Tyr NP in (a) Different pH Level, (b) Different NaCl Concentration, (c) Freeze Thaw Cycle.

It is observed that the pH of the initially synthesized nanoparticle is 7. After adjusting the pH of the solution, it was discovered that when the pH scale is below 5, nanoparticles undergo aggregation. Additionally, it can only neutralize the effects of NaCl at concentrations up to 50 mM. However, in the case of the freeze-thaw cycle, nanoparticle aggregation only occurs after the first cycle.

3.4.2 Stability of Naked Ag-Trp NP:

The Nanoparticle in the case of Trp Functionalized Ag NP becomes aggragated in a similar manner with pH scales below 5 and NaCl concentrations above 50 mM.However, it can stop the first round of FTC. After the first round, nanoparticles started to aggregate, and after round three, there was a saturated solution of aggregated nanoparticles..

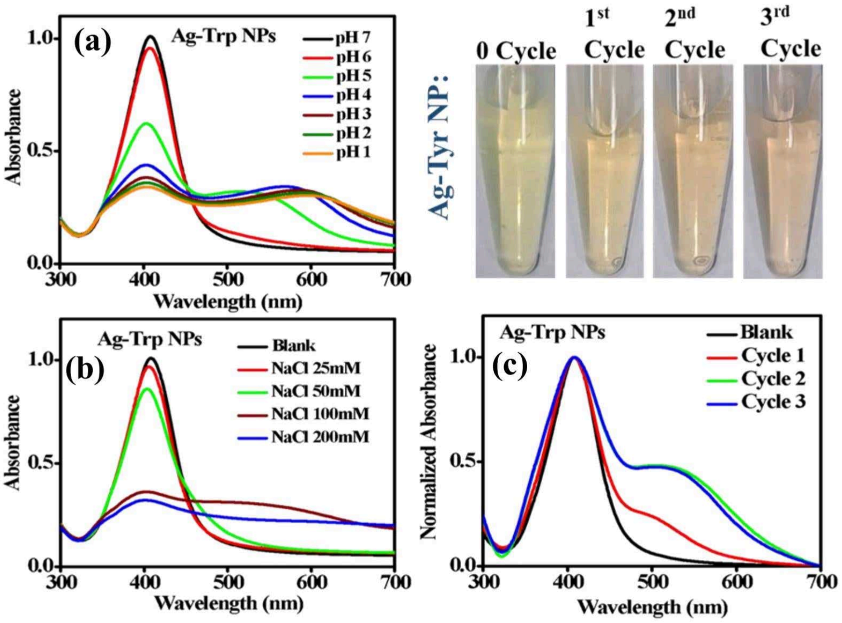

Figure 26: Absorption Spectra of Ag-Trp NP in (a) Different pH Level, (b) Different NaCl Concentration, (c) Freeze Thaw Cycle.

3.4.3 Stability of Lipid Coated Ag-Tyr NP:

For the study of stability of nanoparticle after coated with lipid around its surface, we choose those nanoparticle-lipid conjugations of each lipid (DOPC, DPPC, DMPC, and DMPC/DMTAP) where the interaction of nanoparticle and lipid is quite good among the numerous types of interaction of nanoparticle to lipid of varied ratios. We followed the same three methods of pH variation, Salt Effect, and freeze-Thaw Cycle to investigate the stability of nanoparticle-lipid conjugates. All the Characteristics Absorption spectra of the samples were taken using UV-Vis spectrophotometer.

3.4.3.1 Charged Lipid Vesicles: Firstly, two charged lipids were employed.one was DMPG and the other was DMTAP. Since DMPG, which is negatively charged, could not interact with nanoparticles, there was no production of nanoparticle-lipid conjugates, as was already discussed above. So, we test the stability of DMTAP (positively charged) lipid-nanoparticle conjugates.

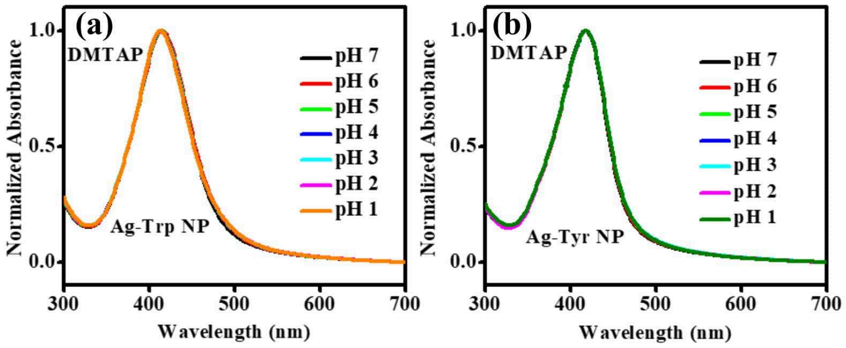

Figure 27: UV-Vis Absorption Spectrum of DMTAP (Lipid) Coated (a) Ag-Trp NPs and (b)Ag-Tyr NPs in Different pH Level.

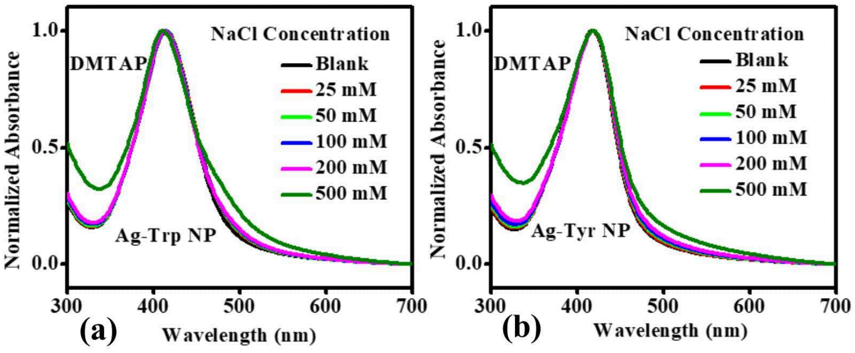

Figure 28: UV-Vis Absorption Spectrum of DMTAP (Lipid) Coated (a) Ag-Trp NPs and (b)Ag-Tyr NPs in Different NaCl Concentration.

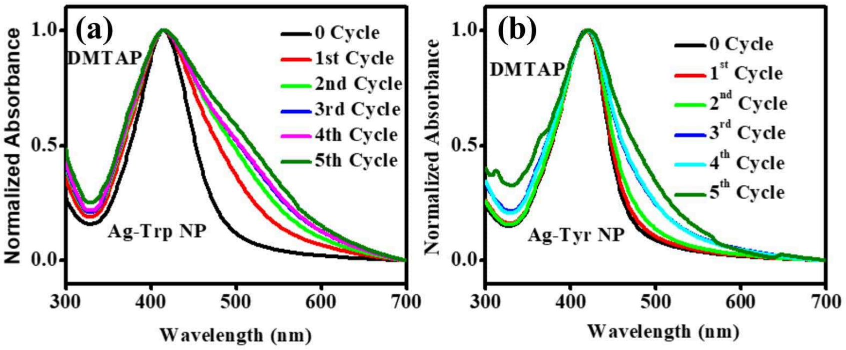

Figure 29: UV-Vis Absorption Spectrum of DMTAP (Lipid) Coated (a) Ag-Trp NPs and (b) Ag-Tyr NPs in Different Freeze Thaw Cycle.

It has been noted that when the nanoparticle is coated with lipid (DMTAP), the pH level has no impact since the SPR peak does not alter. Additionally, it can stop the aggregation process up to a concentration of 200 mM, but after that, it starts to aggregate. The graph clearly illustrates how the plot widens when the concentration of NaCl reaches 500 mM.

Following these in the freeze-thaw cycle, Trp functionalized nanoparticles can delay aggregation up to the third cycle when coated, but after that the absorption peak widened, indicating that aggregation had begun.

3.4.3.2 Neutral (Zwitterionic) Lipid Vesicle:

Three distinct neutral lipids combined with nanoparticles were used. DMPC, DOPC, and DPPC are these. Except for DPPC, none of these three showed any lipid-nanoparticle tryptophan functionalization relationship. Thus, for DMPC and DOPC, we solely use lipid-nanoparticle conjugates of tyrosine functionalized, but for DPPC, we use both tryptophan and tyrosine functionalized conjugates.

3.4.3.2.1 Lipid-Nanoparticle Conjugates (DPPC):

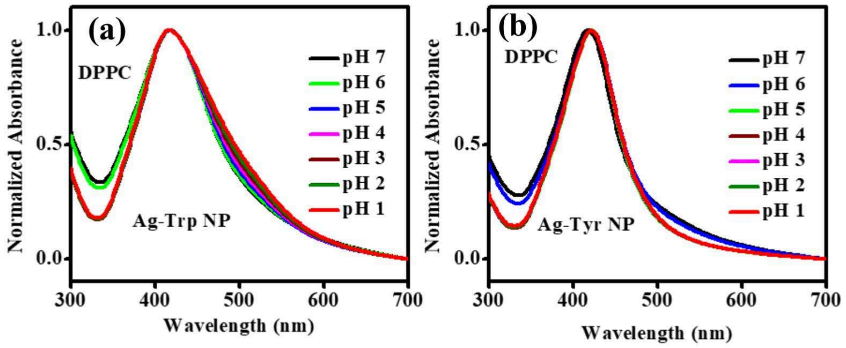

Figure 30: UV-Vis Absorption Spectrum of DPPC (Lipid) Coated (a) Ag-Trp NPs and (b) Ag-Tyr NPs in Different pH Level.

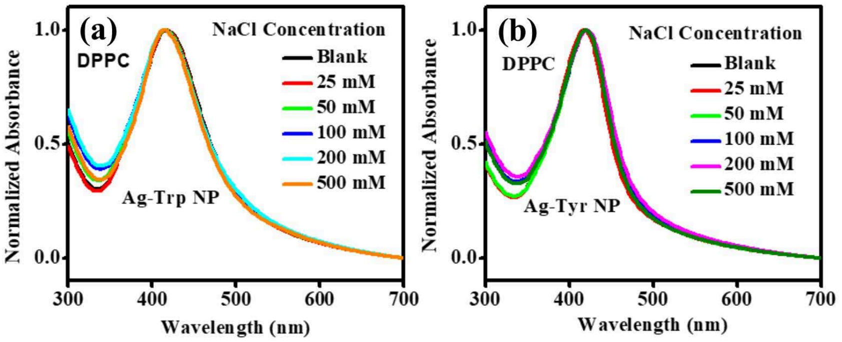

Figure 31: UV-Vis Absorption Spectrum of DPPC (Lipid) Coated (a) Ag-Trp NPs and (b) Ag-Tyr NPs in Different NaCl Concentration.

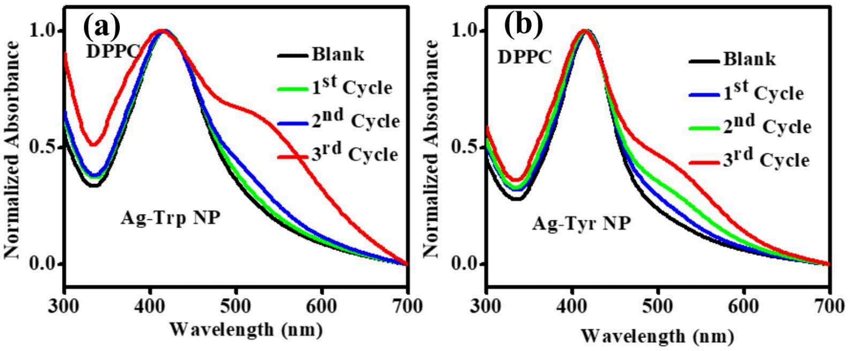

Figure 32: UV-Vis Absorption Spectrum of DPPC (Lipid) Coated (a) Ag-Trp NPs and (b) Ag-Tyr NPs in Different Freeze Thaw Cycle.

The Zwitter Ionic Lipid (DPPC) coated Silver Nanoparticle (both Tyr and Trp Functionalized) has been found to be significantly more stable than naked Nanoparticle, with no discernible effect on its stability in diverse pH levels and NaCl concentrations. The aggregation process can also be stopped up until the second cycle as there is no significant widening of absorption peak. But the absorption peak is seen to be wider than typical after 3rd Cycle, which indicates the aggregation process.

3.4.3.2.2 Lipid-Nanoparticle Conjugates (DMPC):

DMPC Lipid does not significantly interact with tryptophan-functionalized silver nanoparticles. As a result, the stability has only been examined using Ag NP that has been functionalized with Tyrosine.

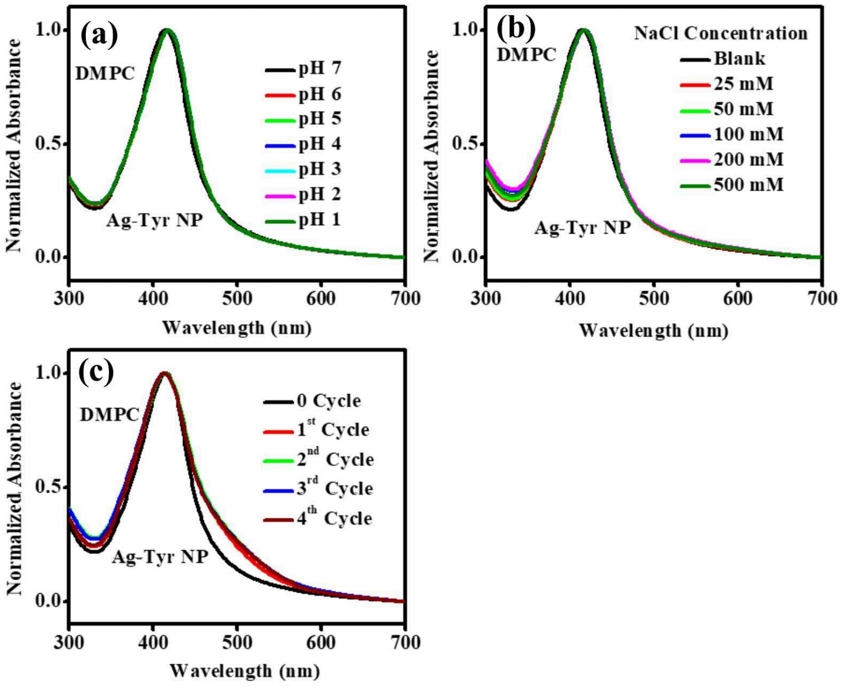

Figure 33: UV-Vis Absorption Spectrum of DMPC (Lipid) Coated Ag-Tyr NPs in D1ifferent (a) pH Level, (b) NaCl Concentration & (c) Freeze-Thaw Cycle.

Ag-Tyr NP coated with DMPC are shown to be extremely stable. As we can see, there has been no appreciable SPR peak shift and no peak broadening in the absorption spectra, which directly demonstrates the nanoparticle's stability. It can also stop the Freeze Thaw Cycle because after the first cycle, the absorption spectra barely expand, but in the following cycles, the broadening becomes saturated.

3.4.3.2.3 Lipid-Nanoparticle Conjugates (DOPC):

Similar to what we already mentioned, tryptophan-functionalized Ag NP does not significantly form a lipid-nanoparticle conjugation with DOPC lipid, thus in this study we only take into account the tyrosine-functionalized Ag NP, where the interaction between Nanoparticle and lipid (DOPC) is quite good.

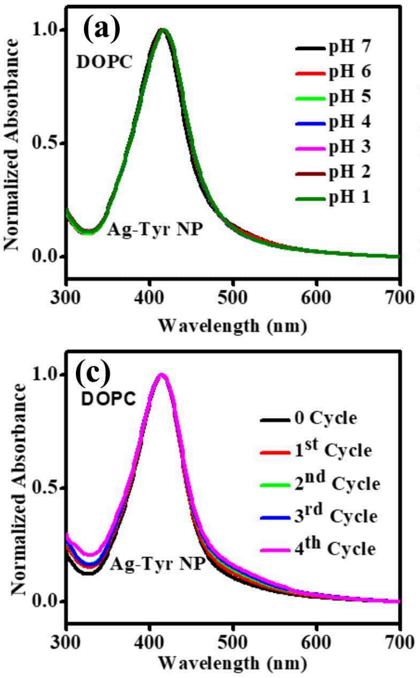

Figure 34: UV-Vis Absorption Spectrum of DOPC (Lipid) Coated Ag-Tyr NPs in Different (a) pH Level, (b) NaCl Concentration & (c) Freeze-Thaw Cycle.

Similarly, the coating of lipid (DOPC) affords the DOPC coated silver nanoparticles great durability against a variety of hostile environmental conditions. As can be seen, neither a substantial SPR peak shift nor a peak broadening are present in different pH level , NaCl Concentration & upon

Freeze Thaw Cycle, indicating that the DOPC coated nanoparticles have increased the stability of the nanoparticles.

From the above study it has been observe that lipid coatings significantly boost the durability of nanoparticles. Where the bare nanoparticles are shielded from the hostile environment by a layer of lipid, which also inhibits the nanoparticles from aggregating.

Chapter 4:

Conclusions

Herein, we have synthesized tyrosine and tryptophan functionalized silver nanoparticles and then investigate the origin of fluorescence in Nanoparticles. We also studied the interaction of these synthesized silver nanoparticles with different surface charged lipid vesicles and also analyze how the coating of Lipid around the bare nanoparticles can effect on its stability. We draw the following conclusions from our observations.

i) We have successfully optimized the reaction condition to synthesize tyrosine and tryptophan functionalized silver nanoparticles.

ii) We have investigated the fluorescence property of the Nanoparticles after synthesis the nanoparticle by our usual method.

It has been found that there is formation of Amino Acid cluster during the nanoparticle synthesis also presence of fluorescent nanocluster and free amino acid residue there is quenching of energy by Ag NP. Where Ag NP act as acceptor and the fluorescent components of the solution are donor.

iii) We have investigated the interaction of tyrosine and tryptophan functionalized silver nanoparticles with differently surface charged lipid vesicles.

iv) Tyrosine functionalized silver nanoparticles are stable with zwitterionic lipid at high concentration and with lipid vesicles having positive charge. Whereas it undergoes aggregation with lipid having positive and neutral (zwitterionic) charge at low concentration.

v) Tryptophan functionalized silver nanoparticles become aggregated with lipid having positive charged at low concentration. whereas it remains stable with neutral (zwitterionic) lipid vesicles at low concentration.

vi) Tyrosine and Tryptophan functionalized silver nanoparticles do not interact with negatively charged lipid vesicles which also suggests that the nanoparticles are negatively charged.

vii) The nanoparticles that exhibit significant interactions with various lipids have lipid molecules coating their surfaces, which boosts their durability to a greater extent by protecting them from the severe conditions found in biological fluid.

References

(1) Agnihotri, S.; Mukherji, S.; Mukherji, S. Size-Controlled Silver Nanoparticles Synthesized over the Range 5–100 Nm Using the Same Protocol and Their Antibacterial Efficacy. *RSC Adv.* **2014**, *4* (8), 3974–3983. https://doi.org/10.1039/C3RA44507K.

(2) Dong, X.-Y.; Gao, Z.-W.; Yang, K.-F.; Zhang, W.-Q.; Xu, L.-W. Nanosilver as a New Generation of Silver Catalysts in Organic Transformations for Efficient Synthesis of Fine Chemicals. *Catal. Sci. Technol.* **2015**, *5* (5), 2554–2574. https://doi.org/10.1039/C5CY00285K.

(3) Chakraborty, S.; Sagarika, P.; Rai, S.; Sahi, C.; Mukherjee, S. Tyrosine-Templated Dual-Component Silver Nanomaterials Exhibit Photoluminescence and Versatile Antimicrobial Properties through ROS Generation. *ACS Appl. Mater. Interfaces* **2021**, *13* (31), 36938–36947. https://doi.org/10.1021/acsami.1c10520.

(4) Wang, E. C.; Wang, A. Z. Nanoparticles and Their Applications in Cell and Molecular Biology. *Integrative Biology* **2014**, *6* (1), 9–26. https://doi.org/10.1039/c3ib40165k.

(5) Kanwa, N.; De, S. K.; Adhikari, C.; Chakraborty, A. Spectroscopic Study of the Interaction of Carboxyl-Modified Gold Nanoparticles with Liposomes of Different Chain Lengths and Controlled Drug Release by Layer-by-Layer Technology. *J. Phys. Chem. B* **2017**, *121* (50), 11333–11343. https://doi.org/10.1021/acs.jpcb.7b08455.

(6) Yang, X.; Yu, Y.; Gao, Z. A Highly Sensitive Plasmonic DNA Assay Based on Triangular Silver Nanoprism Etching. *ACS Nano* **2014**, *8* (5), 4902–4907. https://doi.org/10.1021/nn5008786.

(7) Qasim, M.; Udomluck, N.; Chang, J.; Park, H.; Kim, K. Antimicrobial Activity of Silver Nanoparticles Encapsulated in Poly-N-

Isopropylacrylamide-Based Polymeric Nanoparticles [Retraction]. *IJN* **2023**, *Volume 18*, 1873–1874. https://doi.org/10.2147/IJN.S415768.

(8) Cabaleiro-Lago, C.; Szczepankiewicz, O.; Linse, S. The Effect of Nanoparticles on Amyloid Aggregation Depends on the Protein Stability and Intrinsic Aggregation Rate. *Langmuir* **2012**, *28* (3), 1852–1857. https://doi.org/10.1021/la203078w.

(9) Application of Nano-Technology in Electronics. **2014**.

(10) Matsui, I. Nanoparticles for Electronic Device Applications: A Brief Review. *J. Chem. Eng. Japan / JCEJ* **2005**, *38* (8), 535–546. https://doi.org/10.1252/jcej.38.535.

(11) Joudeh, N.; Linke, D. Nanoparticle Classification, Physicochemical Properties, Characterization, and Applications: A Comprehensive Review for Biologists. *J Nanobiotechnol* **2022**, *20* (1), 262. https://doi.org/10.1186/s12951-022-01477-8.

(12) Jeevanandam, J.; Barhoum, A.; Chan, Y. S.; Dufresne, A.; Danquah, M. K. Review on Nanoparticles and Nanostructured Materials: History, Sources, Toxicity and Regulations. *Beilstein J. Nanotechnol.* **2018**, *9*, 1050–1074. https://doi.org/10.3762/bjnano.9.98.

(13) Moreno-Vega, A.-I.; Gómez-Quintero, T.; Nuñez-Anita, R.-E.; Acosta-Torres, L.-S.; Castaño, V. Polymeric and Ceramic Nanoparticles in Biomedical Applications. *Journal of Nanotechnology* **2012**, *2012*, 1–10. https://doi.org/10.1155/2012/936041.

(14) Pal, A.; Shah, S.; Devi, S. Microwave-Assisted Synthesis of Silver Nanoparticles Using Ethanol as a Reducing Agent. *Materials Chemistry and Physics* **2009**, *114* (2–3), 530–532. https://doi.org/10.1016/j.matchemphys.2008.11.056.

(15) Sharma, R. Synthesis of Amino Acid Capped Silver Nanoparticles, Characterization, and Biological Application. *Orient. J. Chem* **2020**, *36* (02), 334–338. https://doi.org/10.13005/ojc/360217.

(16) Sharma, N. K.; Vishwakarma, J.; Rai, S.; Alomar, T. S.; AlMasoud, N.; Bhattarai, A. Green Route Synthesis and Characterization Techniques of Silver Nanoparticles and Their Biological Adeptness. *ACS Omega* **2022**, *7* (31), 27004–27020. https://doi.org/10.1021/acsomega.2c01400.

(17) Boroumand Moghaddam, A.; Namvar, F.; Moniri, M.; Md. Tahir, P.; Azizi, S.; Mohamad, R. Nanoparticles Biosynthesized by Fungi and Yeast: A Review of Their Preparation, Properties, and Medical Applications. *Molecules* **2015**, *20* (9), 16540–16565. https://doi.org/10.3390/molecules200916540.

(18) Kharissova, O. V.; Dias, H. V. R.; Kharisov, B. I.; Pérez, B. O.; Pérez, V. M. J. The Greener Synthesis of Nanoparticles. *Trends in Biotechnology* **2013**, *31* (4), 240–248. https://doi.org/10.1016/j.tibtech.2013.01.003.

(19) Mourdikoudis, S.; Pallares, R. M.; Thanh, N. T. K. Characterization Techniques for Nanoparticles: Comparison and Complementarity upon Studying Nanoparticle Properties. *Nanoscale* **2018**, *10* (27), 12871–12934. https://doi.org/10.1039/C8NR02278J.

(20) Talarska, P.; Boruczkowski, M.; Żurawski, J. Current Knowledge of Silver and Gold Nanoparticles in Laboratory Research—Application, Toxicity, Cellular Uptake. *Nanomaterials* **2021**, *11* (9), 2454. https://doi.org/10.3390/nano11092454.

(21) Almatroudi, A. Silver Nanoparticles: Synthesis, Characterisation and Biomedical Applications. *Open Life Sciences* **2020**, *15* (1), 819–839. https://doi.org/10.1515/biol-2020-0094.

(22) Santos, D. C.; Goes, J. M. R.; De Souza, V. C.; Bispo, D. F.; Otubo, L.; Andrade, G. R. S.; Camargo, Z. T.; Dos Santos, E. A. Green Synthesis of Silver Nanostructures with Amino Acid-Modified Pluronic F127 for Antibacterial Applications. *Applied Surface Science* **2020**, *505*, 144449. https://doi.org/10.1016/j.apsusc.2019.144449.

(23) De Matos, R. A.; Courrol, L. C. Biocompatible Silver Nanoparticles Prepared with Amino Acids and a Green Method. *Amino Acids* **2017**, *49* (2), 379–388. https://doi.org/10.1007/s00726-016-2371-4.

(24) Kobashigawa, J. M.; Robles, C. A.; Martínez Ricci, M. L.; Carmarán, C. C. Influence of Strong Bases on the Synthesis of Silver Nanoparticles (AgNPs) Using the Ligninolytic Fungi Trametes Trogii. *Saudi Journal of Biological Sciences* **2019**, *26* (7), 1331–1337. https://doi.org/10.1016/j.sjbs.2018.09.006.

(25) Voicescu, M.; Ionescu, S.; Calderon-Moreno, J. M.; Teodorescu, V. S.; Anastasescu, M.; Culita, D. C. Tryptophan / Dextran70 Based - Fluorescent Silver Nanoparticles: Synthesis and Physicochemical Properties. *J Fluoresc* **2019**, *29* (4), 981–992. https://doi.org/10.1007/s10895-019-02411-2.

(26) Rai, M.; Yadav, A.; Gade, A. Silver Nanoparticles as a New Generation of Antimicrobials. *Biotechnology Advances* **2009**, *27* (1), 76–83. https://doi.org/10.1016/j.biotechadv.2008.09.002.

(27) Khodashenas, B.; Ghorbani, H. R. Synthesis of Silver Nanoparticles with Different Shapes. *Arabian Journal of Chemistry* **2019**, *12* (8), 1823–1838. https://doi.org/10.1016/j.arabjc.2014.12.014.

(28) Suber, L.; Imperatori, P.; Pilloni, L.; Caschera, D.; Angelini, N.; Mezzi, A.; Kaciulis, S.; Iadecola, A.; Joseph, B.; Campi, G. Nanocluster Superstructures or Nanoparticles? The Self-Consuming Scaffold Decides.

Nanoscale **2018**, *10* (16), 7472–7483. https://doi.org/10.1039/C7NR09520A.

(29) Park, H.; Shin, D. J.; Yu, J. Categorization of Quantum Dots, Clusters, Nanoclusters, and Nanodots. *J. Chem. Educ.* **2021**, *98* (3), 703–709. https://doi.org/10.1021/acs.jchemed.0c01403.

(30) Jin, R.; Zeng, C.; Zhou, M.; Chen, Y. Atomically Precise Colloidal Metal Nanoclusters and Nanoparticles: Fundamentals and Opportunities. *Chemical reviews* **2016**, *116* (18), 10346–10413.

(31) Cui, M.; Zhao, Y.; Song, Q. Synthesis, Optical Properties and Applications of Ultra-Small Luminescent Gold Nanoclusters. *TrAC Trends in Analytical Chemistry* **2014**, *57*, 73–82. https://doi.org/10.1016/j.trac.2014.02.005.

(32) Takesue, M.; Tomura, T.; Yamada, M.; Hata, K.; Kuwamoto, S.; Yonezawa, T. Size of Elementary Clusters and Process Period in Silver Nanoparticle Formation. *J. Am. Chem. Soc.* **2011**, *133* (36), 14164–14167. https://doi.org/10.1021/ja202815y.

(33) Hui, Y.; Xue, X.; Xuesong, Z.; Yan, W. Intrinsic Fluorescence Spectra of Tryptophan, Tyrosine and Phenyloalanine.

(34) Chen, Y.; Barkley, M. D. Toward Understanding Tryptophan Fluorescence in Proteins. *Biochemistry* **1998**, *37* (28), 9976–9982. https://doi.org/10.1021/bi980274n.

(35) Pan, C.-P.; Muiño, P. L.; Barkley, M. D.; Callis, P. R. Correlation of Tryptophan Fluorescence Spectral Shifts and Lifetimes Arising Directly from Heterogeneous Environment. *J. Phys. Chem. B* **2011**, *115* (12), 3245–3253. https://doi.org/10.1021/jp111925w.

(36) Bagchi, D.; Maity, A.; De, S. K.; Chakraborty, A. Effect of Metal Ions on the Intrinsic Blue Fluorescence Property and Morphology of

Aromatic Amino Acid Self-Assembly. *J. Phys. Chem. B* **2021**, *125* (45), 12436–12445. https://doi.org/10.1021/acs.jpcb.1c07392.

(37) Maja, L.; Željko, K.; Mateja, P. Sustainable Technologies for Liposome Preparation. *The Journal of Supercritical Fluids* **2020**, *165*, 104984. https://doi.org/10.1016/j.supflu.2020.104984.

(38) Lujan, H.; Griffin, W. C.; Taube, J. H.; Sayes, C. M. Synthesis and Characterization of Nanometer-Sized Liposomes for Encapsulation and MicroRNA Transfer to Breast Cancer Cells. *IJN* **2019**, *Volume 14*, 5159–5173. https://doi.org/10.2147/IJN.S203330.

(39) Popovska, O.; Simonovska, J.; Kavrakovski, Z.; Rafajlovska, V. An Overview: Methods for Preparation and Characterization of Liposomes as Drug Delivery Systems.

(40) Akbarzadeh, A.; Rezaei-Sadabady, R.; Davaran, S.; Joo, S. W.; Zarghami, N.; Hanifehpour, Y.; Samiei, M.; Kouhi, M.; Nejati-Koshki, K. Liposome: Classification, Preparation, and Applications. *Nanoscale Res Lett* **2013**, *8* (1), 102. https://doi.org/10.1186/1556-276X-8-102.

(41) Liu, Y.; Liu, J. Zn $^{2+}$ Induced Irreversible Aggregation, Stacking, and Leakage of Choline Phosphate Liposomes. *Langmuir* **2017**, *33* (50), 14472–14479. https://doi.org/10.1021/acs.langmuir.7b03209.

(42) Reimhult, E.; Höök, F.; Kasemo, B. Intact Vesicle Adsorption and Supported Biomembrane Formation from Vesicles in Solution: Influence of Surface Chemistry, Vesicle Size, Temperature, and Osmotic Pressure. *Langmuir* **2003**, *19* (5), 1681–1691. https://doi.org/10.1021/la0263920.

(43) Maity, A.; De, S. K.; Bagchi, D.; Lee, H.; Chakraborty, A. Mechanistic Pathway of Lipid Phase-Dependent Lipid Corona Formation on Phenylalanine-Functionalized Gold Nanoparticles: A Combined Experimental and Molecular Dynamics Simulation Study. *J. Phys. Chem. B* **2022**, *126* (11), 2241–2255. https://doi.org/10.1021/acs.jpcb.2c00356.

(44) Maity, A.; De, S. K.; Chakraborty, A. Interaction of Aromatic Amino Acid-Functionalized Gold Nanoparticles with Lipid Bilayers: Insight into the Emergence of Novel Lipid Corona Formation. *J. Phys. Chem. B* **2021**, *125* (8), 2113–2123. https://doi.org/10.1021/acs.jpcb.0c10079.

(45) Tenchov, R.; Bird, R.; Curtze, A. E.; Zhou, Q. Lipid Nanoparticles─From Liposomes to MRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. *ACS Nano* **2021**, *15* (11), 16982–17015. https://doi.org/10.1021/acsnano.1c04996.

(46) Manjunath, K.; Reddy, J. S.; Venkateswarlu, V. Solid Lipid Nanoparticles as Drug Delivery Systems. *Methods Find Exp Clin Pharmacol* **2005**, *27* (2), 127. https://doi.org/10.1358/mf.2005.27.2.876286.

(47) Xu, L.; Wang, X.; Liu, Y.; Yang, G.; Falconer, R. J.; Zhao, C.-X. Lipid Nanoparticles for Drug Delivery. *Advanced NanoBiomed Research* **2022**, *2* (2), 2100109. https://doi.org/10.1002/anbr.202100109.

(48) Samimi, S.; Maghsoudnia, N.; Eftekhari, R. B.; Dorkoosh, F. Lipid-Based Nanoparticles for Drug Delivery Systems. In *Characterization and Biology of Nanomaterials for Drug Delivery*; Elsevier, 2019; pp 47–76. https://doi.org/10.1016/B978-0-12-814031-4.00003-9.

(49) Simovic, S.; Barnes, T. J.; Tan, A.; Prestidge, C. A. Assembling Nanoparticle Coatings to Improve the Drug Delivery Performance of Lipid Based Colloids. *Nanoscale* **2012**, *4* (4), 1220–1230. https://doi.org/10.1039/C1NR11273B.

(50) Luchini, A.; Vitiello, G. Understanding the Nano-Bio Interfaces: Lipid-Coatings for Inorganic Nanoparticles as Promising Strategy for Biomedical Applications. *Front. Chem.* **2019**, *7*, 343. https://doi.org/10.3389/fchem.2019.00343.

(51) Kong, B.; Seog, J. H.; Graham, L. M.; Lee, S. B. Experimental Considerations on the Cytotoxicity of Nanoparticles. *Nanomedicine* **2011**, *6* (5), 929–941. https://doi.org/10.2217/nnm.11.77.

(52) Miesen, T. J.; Engstrom, A. M.; Frost, D. C.; Ajjarapu, R.; Ajjarapu, R.; Lira, C. N.; Mackiewicz, M. R. A Hybrid Lipid Membrane Coating “Shape-Locks” Silver Nanoparticles to Prevent Surface Oxidation and Silver Ion Dissolution. *RSC Adv.* **2020**, *10* (27), 15677–15693. https://doi.org/10.1039/D0RA01727B.

(53) Ball, R.; Bajaj, P.; Whitehead, K. Achieving Long-Term Stability of Lipid Nanoparticles: Examining the Effect of PH, Temperature, and Lyophilization. *IJN* **2016**, *Volume 12*, 305–315. https://doi.org/10.2147/IJN.S123062.

(54) Farner, J. M.; De Tommaso, J.; Mantel, H.; Cheong, R. S.; Tufenkji, N. Effect of Freeze/Thaw on Aggregation and Transport of Nano-TiO_2 in Saturated Porous Media. *Environ. Sci.: Nano* **2020**, *7* (6), 1781–1793. https://doi.org/10.1039/D0EN00008F.

(55) Albert, G. C.; Roumeliotis, M.; Carson, J. J. L. The Effect of Temperature and Freeze–Thaw Processes on Gold Nanorods. *Nanotechnology* **2009**, *20* (50), 505502. https://doi.org/10.1088/0957-4484/20/50/505502.

(56) Badawy, A. M. E.; Luxton, T. P.; Silva, R. G.; Scheckel, K. G.; Suidan, M. T.; Tolaymat, T. M. Impact of Environmental Conditions (PH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions. *Environ. Sci. Technol.* **2010**, *44* (4), 1260–1266. https://doi.org/10.1021/es902240k.