Synthesis of different size amino acid capped nanoparticles varying the external conditions

M.Sc. Thesis

By

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DISCIPLINE OF CHEMISTRY

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Synthesis of different size amino acid capped nanoparticles varying the external conditions

A THESIS

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Synthesis of different size amino acid capped nanoparticles varying the external conditions** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DISCIPLINE OF CHEMISTRY, Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the time period from July 2019 to June 2020 Dr. Anjan Chakraborty, Associate Professor, IIT 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.

M. Ateeque
29.06.2020

Signature of the student with date
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This is to certify that the above statement made by the candidate is correct to the best of my/our knowledge.

Dr. Anjan Chakraborty

Mohammad Ateeque successfully given his/her M.Sc. Oral Examination held on **25 JUNE 2020**.

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Date:

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Mohammad Ateeque

M.Sc. 2nd year

Dedicated to My

Family

Abstract

Herein we systematically investigated the effect of different temperatures, pH, and pressure (hydrothermal method) on the size of the aromatic amino acid-functionalized gold nanoparticles where amino acids act as a reducing agent as well as capping agent. We found that temperature plays a key role in the size tunability during the nanoparticle synthesis. Tryptophan and phenylalanine functionalized nanoparticles have the excellent ability to tune the size of the nanoparticle whereas the size of the tyrosine functionalized nanoparticle is independent of temperature. Further, the formation of gold nanocluster was also observed during the hydrothermal method. Surprisingly, we found that except phenylalanine tryptophan and tyrosine can form the gold nanoclusters with excellent luminescent properties.

TABLE OF CONTENTS

LIST OF FIGURES 12

NOMENCLATURE 13

ACRONYMS 14

Chapter 1: Introduction 15-17

Chapter 2: Experimental section 18

Chapter 3: Results and Discussion 19-23

Chapter : Instrumentation 24

Chapter 5: Conclusion 25

REFERENCES 26-27

LIST OF FIGURES

Figure 1: a) Normalized UV-Vis spectra of Au-Trp at different temperatures. Transmission electron microscopy images of Au-Trp at b) 50 °C and C) 80 °C. Scale bar 20 nm.

Figure 2: a) Normalized UV-Vis spectra of Au-Phe at different temperature and b) transmission electron microscopy images of Au-Phe at 80 °C. Scale bar 50 nm.

Figure 3: a) Normalized UV-Vis spectra of Au-Tyr at different temperature and b) transmission electron microscopy images of Au-Tyr at 80 °C. Scale bar 50 nm.

Figure 4: a) Excitation dependent emission and b) excitation dependent normalized emission of tryptophan functionalized gold nanoclusters.

Figure 5: Excitation dependent emission of tyrosine functionalized gold nanoclusters.

Figure 6: Atomic force microscopy (AFM) images of a) Au-Trp NCs and b) Au-Tyr NCs. Scale bar 100 nm.

Figure 7: luminescence stability of the Au-Trp Nano-clusters against variable pH conditions.

NOMENCLATURE

nm	Nanometer
mM	Milli molar
mL	Milliliter
µL	Microliter

ACRONYMS

AuNPs	Gold Nanoparticles
Phe	Phenylalanine
Tyr	Tyrosine
Trp	Tryptophan
$HAuCl_4$	Tetrachloroauric(III) acid
NaOH	Sodium Hydroxide
TEM	Transmission Electron Microscopy
NPs	nanoparticles
NCs	nanoclusters

Chapter 1

Introduction

In recent years, various fluorescent probes such as fluorescent carbon dots, semiconductor quantum dots, photo-luminescent silicon nanoparticles, metallic nanoparticles (NPs) and nanoclusters (NCs) have gained considerable attention due to their potential applications in the field of catalysis, biosensing, bio-labelling, in vivo and in vitro bioimaging, cancer therapy etc.[1-9]. Ideally, fluorescent NPs or NCs should have excellent biocompatibility, good stability and high targeting selectivity [10]. Moreover, the ability of these fluorescent NPs or NCs to accommodate various target biomolecules and drugs allow their use not only in gaining information about the structures and functioning of subcellular organelles but also in the targeted imaging and various cancer therapies. The development of various NCs with well-defined surface chemistry, well-controlled shape and size distributions and unique optical and electronic properties is the basis for all such biological applications.[11] The conventional semiconductor quantum dots (QDs) are larger in dimensions (usually larger than 3 nm) and contain toxic heavy metals. The ultrafine size, strong fluorescence, favourable biocompatibility and excellent photostability of gold NCs make them highly promising alternatives for biosensing and bioimaging applications.[12-14] Gold NCs have low toxicity and due to their unique optical and electronic properties and easy surface functionalization, they are very useful in the area of catalysis, sensing and other biomedical applications.[15,16] Gold NCs exhibit molecule-like properties which include discrete electronic states and size-dependent fluorescence because they consist

of a few to hundreds of atoms and typically possess sizes comparable to the Fermi wavelength of the conduction electrons.[17] The gold NCs which contains only a few Au atoms show unique physical and chemical properties such as well-defined molecular structure, discrete electronic transitions and characteristic size-tunable photoluminescence properties [18]. The fluorescence of a gold NC could be tuned by changing the particle size. Some other structural parameters such as valence state of gold, the surface-bonded ligand or the crystallinity of the NCs also have significant influences on the fluorescence features. A lot of attention has been given to the applications of gold size using green ligands and bright fluorescence is still a challenge. The biomolecules with the well-defined chemical composition are generally used as templates to conveniently synthesize the Au NCs which are biocompatible and are with well-defined morphologies [19-21]. Previously, albumin and other protein stabilized synthesis of gold NCs have been reported in which, the structure of the NCs depends on the molar ratio of the precursor, $AuCl_4^-$ and the protein [22]. The amino acids area particularly attractive class of reducing agents because of their nearly universal presence in biological and environmental systems. Despite the many excellent articles/reviews the mechanisms of the formation of amino acid-based Au NCs/NPs as well as the origin of the luminescence properties are not clearly understood.

The applications of gold NPs greatly depend on the size of NPs as well as on the nature of the capping agent. Thus a synthesis procedure must have the flexibility to tune the nanoparticle size by keeping the capping agent common. Zhoa et. al. reported the citrate capped gold NPs in which the tunable particle

size.[23] They demonstrate the effect of chloride ions on the size of the gold nanoparticles was investigated by varying the experimental parameters including pH and the ratio of reducing agent ($NaBH_4$/Citrate) to the gold precursors. Other than citrate capped gold nanoparticles the tenability of the size of the nanoparticles is rare. Therefore, we used aromatic amino acids as a reducing agent as well as a capping agent to control the particle size by maintaining the pH of the experiment, temperature condition and also concentration of the aromatic amino acids. Three different aromatic amino acids (tyrosine, tryptophan and phenylalanine) are used to prepare the gold nanoparticles and gold nanoclusters. Initially, the effect of temperature on gold nanoparticle synthesis by using these amino acids was investigated. Then, the effect of pH and the hydrothermal method was further investigated by using the same precursors.

Chapter 2

EXPERIMENTAL SECTION

Materials:

Chloroauric acid ($HAuCl_4.3H_2O$), phenylalanine, tyrosine, tryptophan were obtained from Sigma-Aldrich. The hydrogen chloride solution (HCl, 37%) and Sodium Hydroxide (NaOH) were obtained from Merck. Milli-Q water was used throughout the synthesis procedure. All the chemicals were commercially available and used as received without any further purification.

Synthesis of amino acid-functionalized gold nanoparticles:

All glassware was thoroughly cleaned overnight with freshly prepared 3:1 HCl/HNO_3 (*aqua regia*) and rinsed thoroughly with Mili-Q water before use. Aromatic amino acids functionalized gold nanoparticles were synthesized by previously reported in situ green synthesis.[1] Briefly, 0.25 mL chloroauric acid solution (0.75 mM) was added in 3.75 mL of Mili-Q water and the solution was gently stirred at preheated oil bath. After a few minutes, 1mL of freshly prepared 5 mM aromatic amino acid solutions (1 mM concentration in solution) were added into the previous solution followed by addition of freshly prepared 50 µL of 1 M NaOH (10 mM concentration in solution) under vigorous stirring for 1-2 hours. The formation of AuNPs was confirmed by observing the ruby red solution from a colourless solution.

Synthesis of amino acid-functionalized gold nanoclusters:

The gold nanoclusters were prepared by taking Chloroauric acid (30mM, 166 µL) and Milli Q (9mL) into autoclave then added the amino acid (5mM, 1mL) into the same autoclave and immediately added hydrogen chloride solution (1M) in the solution to maintain pH approximate equal to ~2.0. The autoclave kept into the oven for 24h at 120°C. The colour of the solution turned from colourless to dim yellow and obtained gold nanocluster given blue colour emission under UV light.

Chapter 3

RESULTS AND DISCUSSION

The temperature of the reaction solution can play an essential role in the nucleation process as well as the size of the nanoparticles. The reaction conditions were varied by temperature (25, 50, and 80 °C) however the $HAuCl_4$, tryptophan, and NaOH concentrations were kept constant. Tryptophan functionalized gold nanoparticles were synthesized by using the previously described method. We found that the influence of temperature on the UV−Vis spectra of the Au NPs was studied. The normalized UV-Vis spectra of the Au NPs at different temperature were shown in **Fig 1a**. The UV-Vis spectra reveal that with increasing temperature Au NPs show a narrow SPR band indicating, decrease in the size of the nanoparticles with increasing reaction temperature. **Fig. 1b** and **c** represent the transmission electron microscopy (TEM) images of the Au Trp at 50 °C and 80 °C respectively. TEM images reveal that at 50 °C the size of the Au-Trp NPs was ~ 15-20 nm whereas at 80 °C the sizes of the NPs were in the range of 6-10 nm.

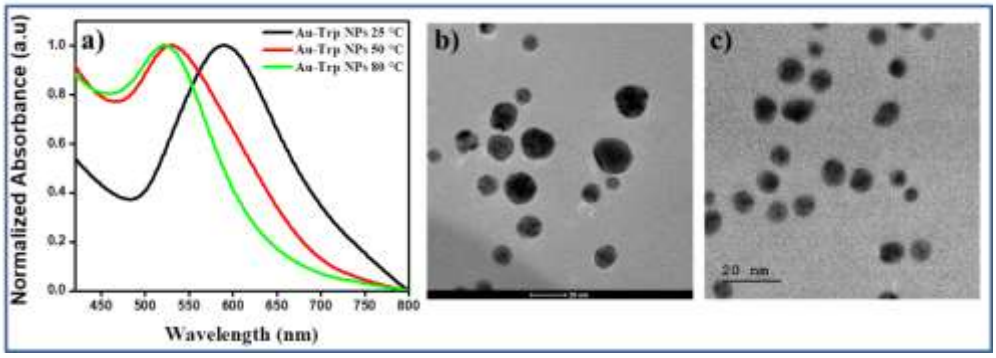

Figure 1: a) Normalized UV-Vis spectra of Au-Trp at different temperatures. Transmission electron microscopy images of Au-Trp at b) 50 °C and C) 80 °C. Scale bar 20 nm.

Similarly, for the Phenylalanine functionalized gold nanoparticle, Temperature has an important role in modulating the size of the AuNPs. **Fig. 2a** represents the UV-Visible spectra of the AuNPs at different reaction temperatures ($HAuCl_4$, phenylalanine, and NaOH concentrations were kept constant). **Fig. 2b** represents the TEM image of the AuNPs at 80 °C. At first, we synthesized

the AuNPs at room temperature which forms black colour solution indicating the formation of a large size of the AuNPs. The UV-Visible spectra of the AuNPs show that the SPR peak is at ~ 560 nm which is also a good agreement of the colour and size of the AuNPs. Then, we increase the temperature of the solution to the reaction mixture. With the increase in the temperature, the SPR peak shifts to the left in the UV-Visible spectra which indicates the formation of small size AuNPs. At temperature 80 °C, The TEM image reveals that the size of the AuNPs was 10-15 nm which is also in good agreement with the SPR peak of the AuNPs. All of these data indicate that the temperature plays a major role in the nucleation process of the AuNPs and the nucleation process governs the size of the AuNPs.

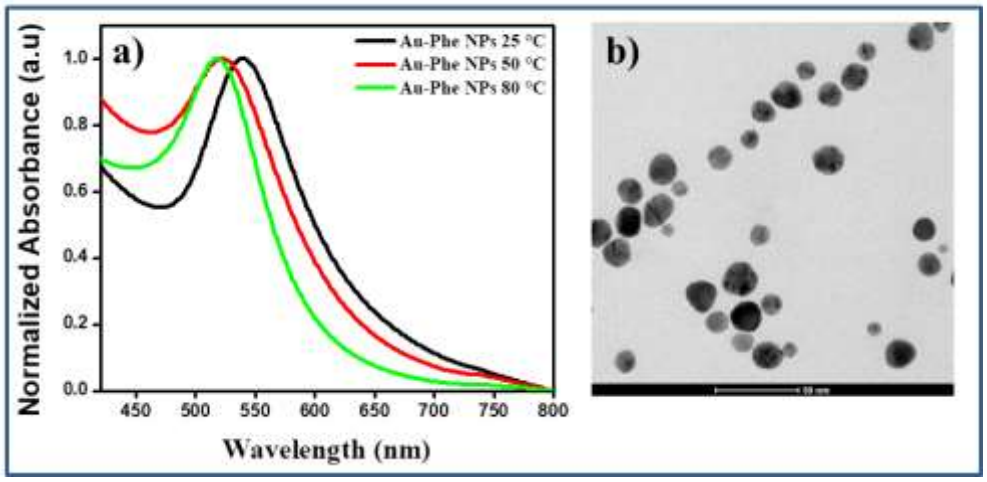

Figure 2: a) Normalized UV-Vis spectra of Au-Phe at different temperature and b) transmission electron microscopy images of Au-Phe at 80 °C. Scale bar 50 nm.

Interestingly, for the tyrosine functionalized gold nanoparticles, the temperature does not have a significant effect in the size of the AuNPs. **Fig. 3a** represents the UV-Visible spectra of the AuNPs at different reaction temperatures ($HAuCl_4$, tyrosine, and NaOH concentrations were kept constant). The TEM image of the AuNPs at 80 °C is shown in **fig. 3b**. The SPR peak of the AuNPs is at ~ 520nm in all temperatures which indicate the formation of the almost same size of AuNPs irrespective of the temperatures. This outcome may be due to the fact that intermolecular hydrogen bonding

between tyrosine molecules play a crucial role in the nucleation process of the AuNPs.

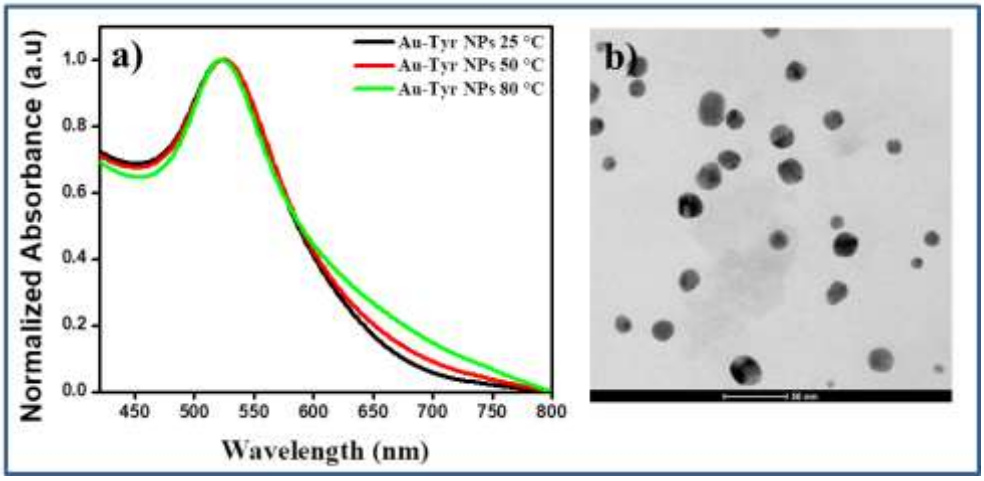

Figure 3: a) Normalized UV-Vis spectra of Au-Tyr at different temperature and b) transmission electron microscopy images of Au-Tyr at 80 °C. Scale bar 50 nm.

The hydrothermal method was used for the preparation of the blue-emitting AuNCs by using $HAuCl_4$, aromatic amino acids in acidic conditions. **Fig. 4(a-b)** represents the excitation dependent emission and excitation dependent normalized emission spectra of the tryptophan functionalized AuNCs. The aqueous solution of Au NCs exhibited high blue fluorescence under UV light (365 nm) irradiation. The maximum emission peak of Au NCs was observed at 455 nm, and the maximum absorption wavelength of the excitation spectrum was 360 nm.

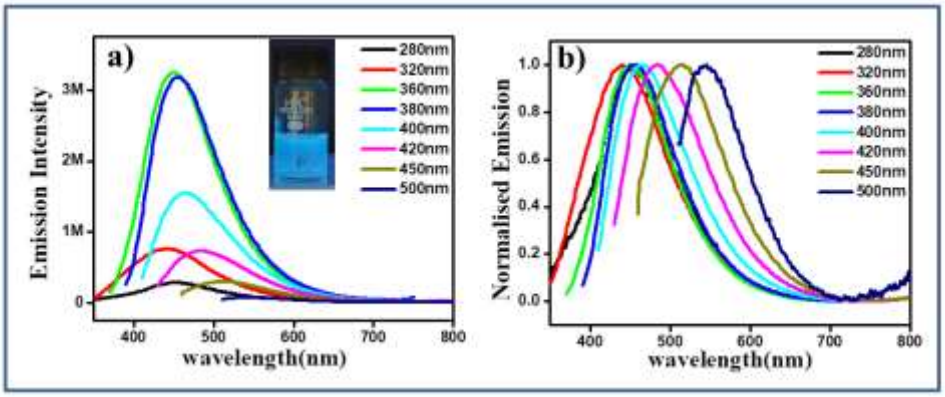

Figure 4: a) Excitation dependent emission and b) excitation dependent normalized emission of tryptophan functionalized gold nanoclusters.

Similarly, the aqueous solution of tyrosine functionalized AuNCs also exhibits high blue fluorescence under UV light (365 nm) irradiation. **Fig. 5** represents the excitation dependent emission spectra of the tyrosine functionalized AuNCs. The maximum emission peak of Au NCs was observed at 458 nm, and the maximum absorption wavelength of the excitation spectrum was 360 nm.

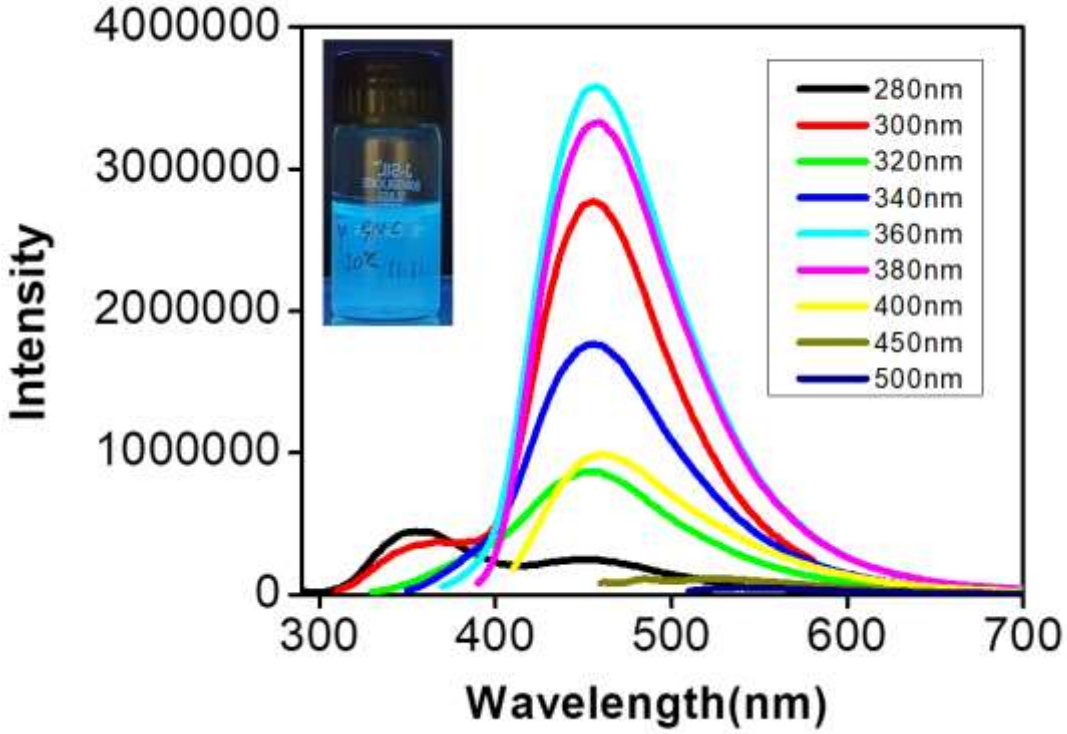

Figure 5: Excitation dependent emission of tyrosine functionalized gold nanoclusters.

This observed fluorescence property of the AuNCs is attributed to the small size of the AuNCs. So, We use Atomic Force Microscopy (AFM) to measure the size of the AuNCs. **Fig. 6 (a-b)** represents the AFM images of the tryptophan and tyrosine functionalized AuNCs respectively. The images indicate the hight of the AuNCs is in the range of 3-4 nm.

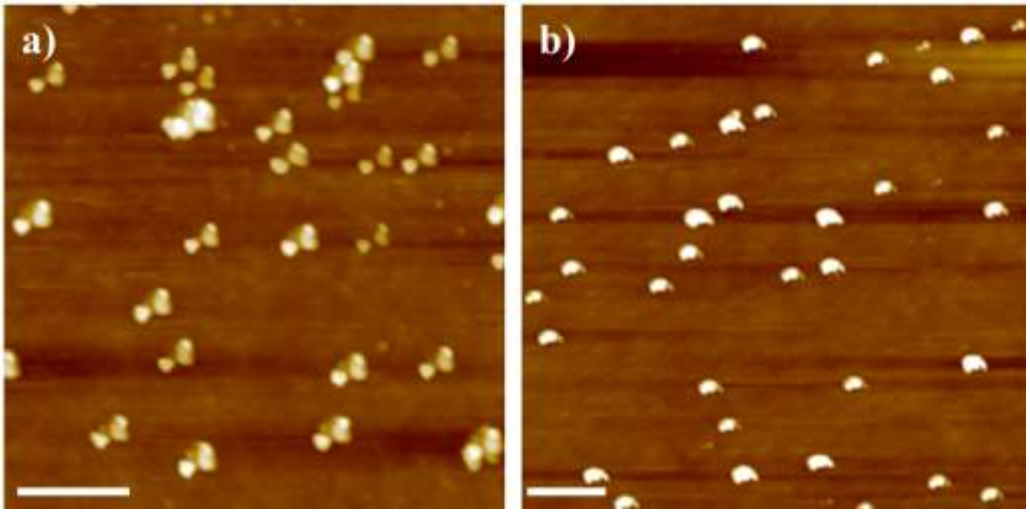

Figure 6: Atomic force microscopy (AFM) images of a) Au-Trp NCs and b) Au-Tyr NCs. Scale bar 100 nm.

Then, we are interested to measure the stability of the tryptophan functionalized AuNCs against pH. We synthesized the AuNCs at pH~2. **Fig. 7** represents the stability of the AuNCs at various pH. The AuNCs are highly stable at the pH range 2-7 while the stability decreases in high basic conditions.

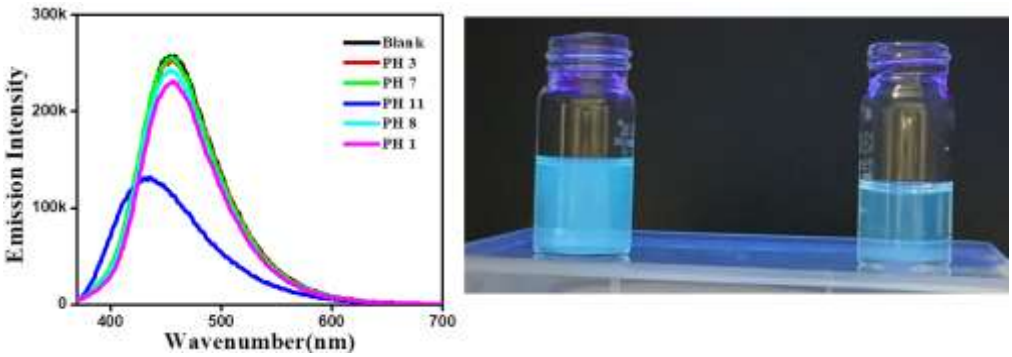

Figure 7: luminescence stability of the Au-Trp Nano-clusters against variable pH conditions.

Chapter 4

Instrumentation:

UV-Vis spectra and Steady-state fluorescence measurements: Absorption spectra of all the Au NPs were recorded using a Varian UV−vis spectrophotometer (Cary 100 Bio) in a quartz cuvette (10 × 10 mm^2). Steady-state fluorescence spectra were recorded using a Fluoromax-4p spectrofluorometer from Horiba Jobin Yvon (model: FM-100). The samples were excited at 360nm. The fluorescence spectra were corrected for the spectral sensitivity of the instrument. The emission and excitation slits were 2/2 for almost all the emission measurements. We kept temperature (T) at 25°C throughout all the measurements.

Transmission Electron microscopy: Transmission electron microscopy images were taken by using field emission gun transmission electron microscope (Model: Tecnai G2, F30) with an acceleration voltage of 300 kV. The diluted (10 times) solution of the samples were dried on a carbon-coated copper grid by slow evaporation in the air at room temperature.

Atomic force microscopy: Atomic force microscopy (AFM) images were taken by drop-casting the sample on mica substrates via tapping mode at a scan frequency of 0.65−1.0 Hz and were recorded using SmartScan software (model park NX10). The samples were immobilized on a clean cover slide by spin coating at 1000 rpm for 3 min before imaging.

Chapter 5

Conclusion

In summary, we found that temperature, pH and pressure altogether plays a key role in the synthesis of aromatic amino acid-functionalized gold nanoparticles. The formation of nanoparticles dependent on the nucleation process which further depends on the affinity and the interactions of the aromatic amino acids. We found that the size of the nanoparticles can be easily reduced from ~50 nm to ~10 nm by increasing the temperature for tryptophan and phenylalanine functionalized gold nanoparticles at the basic condition. On the other hand size of the tyrosine functionalized gold nanoparticle is independent of temperature and shows a common size around15-20 nm. The hydrothermal method along with the acidic condition brings the formation of gold nanoclusters (>5 nm) for Trptophane and tyrosine, whereas phenylalanine does not form the nanoclusters. The application of gold nanoparticles greatly depends on the size and in this context, we successfully tune the size of NPs from >5 nm to ~50 nm by using the aromatic amino acids.

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