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GRADUATE SCHOOL OF SCIENCE

MASTER THESIS

Determination of the Interaction Between Amino Acids and Gold Nanoparticles: Molecular Simulation Study

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THESIS APPROVAL

This master thesis study under the name of "**Determination of the interaction between Amino Acids and Gold Nanoparticles: Molecular Simulation Study**" which is prepared and presented by Mehri Niazi, is approved as Master Thesis in Nanoscience and Nanotechnology Department at Ondokuz Mayıs University by committee members listed below on .../ ../2019.

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ETHICAL STATEMENT

I declare that all the information given in this dissertation is true and absolute which is prepared in conformity with the regulations for Ondokuz Mayıs University Graduate School of Science and thesis writing rules, all the information were referred and kept on the right side of the laws according to scientific ethic during the stage of production of information.

Mehri Niazi
June 2019

ABSTRACT

Master's Thesis

Determination of the interaction between Amino Acids and Gold Nanoparticles: Molecular Simulation Study

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Ondokuz Mayıs University

Graduate School of Science

Department of Nanoscience and Nanotechnology

Supervisor: Asst. Prof. İbrahim İNANÇ

For a long time, it has been understood that the nanotechnology could contribute to great achievements in different fields and its role in medicine will be increased significantly as time goes by. A lot of studies have been performed to improve nanomedical applications in diffferent diseases. They could be applied to prevent, to diagnose and to treat. Among all nanoparticles, gold derivatives are popular due to their interesting physical and chemical properties. In this study, we evaluated interactions between bulding blocks of proteins, Amino acids with gold nanoparticles by simulation study. Material studio software in Monte Carlo method was used for this study. The structure of Amino acids and gold nanorods were designed by RASPA software. Results demonstrated different patterns of interactions for polar, non-polar, basic and acidic amino acids. Energy levels and binding affinity as well as stability after binding were assessed. *Ser* in polar amino acids had the highest loading, while *gly* as a non-polar amino acid had the lowest laoding rate. Binding energy studies revealed that the lowest energy levels resulted in highest affinity between amino acids and gold nanorods. We did the same experiments for two other shapes of gold nanoparticles including Au nanosheet and Au spherical nanoparticles. For polar amino acids, shape of nanoparticles did not result in specific changes. As previous one, in this group for both new structures, *Ser* has shown the highest loading capacity and *Tyr* has shown the fastest attachment and separation on the surface among polar ones. These results could be applied in drug design systems for various dieases especially cancer drug delievery.

Keywords: Amino Acids, Gold nanorods, Interaction, Molecular Simulation, Monte Carlo, Nanomedicine

ÖZET

Yüksek Lisans Tezi

Amino Asitler ve Altın Nanopartiküller Arasındaki Etkileşimin Belirlenmesi: Moleküler Simülasyon Çalışması

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Nanoteknolojinin farklı alanlarda büyük başarılara imza atacağı ve özelllikle tıp alanındaki rolünün gün geçtikçe daha hayati hale geleceği uzun zaman önce anlaşmıştır. Farklı hastalıklarda nanomedikal uygulamalarını geliştirmek için çok sayıda çalışma yapılmıştır. Önlemek, teşhis ve tedavi etmek için uygulanabilirler. Tüm nanopartiküller arasında, altın fiziki ve kimyasal özellikleri nedeniyle popülerdir. Bu tez çalışmasında, simülasyon yardımı ile proteinlerin yapıtaşları, amino asitler ile altın nanopartiküller arasındaki etkileşimleri hesapladık. Bu çalışma için Monte Carlo yönteminde RASPA yazılımı kullanılmıştır. Amino asitler ve altın nanoçubukların yazılım tarafından tasarlanmıştır. Sonuçlar, polar, apolar, bazik ve asidik amino asitler için farklı etkileşimler desenleri göstermiştir. Enerji seviyeleri ve bağlanma meyili ve bağlanma sonrası stabilite değerlendirildi. Polar amino asitlerdeki *ser* en yüksek yüke sahipken, apolar bir amino asit olarak *Gly* en düşük yükleme sahiptir. Bağlanma enerjisi çalışmaları, en düşük enerji seviyelerinin amino asitler ve altın nanoçubukları arasında görülen en yüksek afinite ile sonuçlandığını ortaya koymuştur. Aynı deneyleri Au nano tabaka ve Au küresel nano parçacıklar da yaptık. Polar amino acitler için nanopartiküllerin şekli özel değişikliklerle sonuçlanmadı. Önceden olduğu gibi, her iki yeni yapı için bu grupta, *Ser* en yüksek yükleme kapasitesini ve *Tyr* kutup yüzeyleri arasındaki yüzeyde en hızlı bağlantı ve ayrılığı göstermiştir. Bu sonuçlar, özellikle kanser ilacının vucuda dağıtımı başta olmak üzere çeşitli hastalıklar için üretilecek ilaçların sistemlerinde uygulanabilir.

Anahtar kelimeler: Amino Asitler, Altın Nanoçubuk, Etkileşim, Moleküler Simülasyon, Monte Carlo, Nanomedikal

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This thesis is dedicated to humanity and love.

Thank you.

Author
Mehri NIAZI

1. CONTENTS

ABBREVIATIONS

AA	Amino Acids
Ala	Alanine
Arg	Arginine
Asn	Aspargine
Asp	Aspartic Acid
Cys	Cysteine
Gln	Glutamine
GNPs	Gold Nanoparticles
His	Histidine
Ile	Isoleucine
Lys	Lysine
Leu	Leucine
Met	Methonine
Phe	Phenylaanine
Pro	Prolin
Ser	Seine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

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TABLE LIST

1. INTRODUTION

1.1 Nanotechnology

Nanotechnology and nanoscience illustrates field of research and applications at the nano level. Nano is a Greek word meaning "dwarf" and a nanomaterial represents dimensions of 1 billionth in meter or $10^{-9}m$ (1, 2). In this regard, objects and materials which have one dimension in range of 1 to 100 nm (3), are called nanomaterials (4). It was in 1995, winner of Nobel Prize, Richard Feynman mentioned "There is a plenty room at the bottom" (5) and stated dimension of nm (6). He defined nanotechnology as "the processing, separation, consolidation and deformation of materials by one atom or molecule."(7). A lot of properties have been introduced for nanotechnology including mechanical, optical, electrical and chemical behaviors (8). Therefore, it has resulted in so many achievements in different fields including industry, medicine, communication, information and agriculture. The most significant advancement happened in field of medicine and pharmaceutics and it is developing very fast. Nanomedicine is the mixture of science and technology which provides diagnosis, treatment and prevention of diseases using nanomaterials (9). Some examples include fluorescent biological labels, drug and gene delivery, bio detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumor destruction via heating (hyperthermia), separation and purification of biological molecules and cells, and MRI contrast enhancement (10-16).

1.2. Cancer and its Therapies

Cancer (uncontrolled growth of abnormal cells) is still one of the most challenging healthcare issues in the world. It is known as the first and the second culminating cause of death in developed and developing countries, respectively (17). In this regard, there has been a growing concern over applying diagnostics and therapeutic agents to reduce mortality caused by cancer (18-21). In addition, their attempt also considers devising new methods to prevent, early diagnosis and treatment of cancer. Main cancer treatments include surgery, chemotherapy or radiotherapy (22, 23). All are efficient in their own place, however they could face some limitations. For example, surgery is limited to primary and accessible tumors (24). Chemotherapy is an indispensable part

of cancer treatment (18, 22). Nanomedicine seems to be very helpful and effective to approach to cancer treatment. One of the main considerations in cancer treatment is selective killing of tumor cells which is partially possible by applying nanomedical solutions (25).

1.3. Nanomedicine and Cancer

Application of nanotechnology in medicine and cancer is generally based on early detection and selective targeting. As mentioned above, the most challenging idea in cancer related treatments is to decrease side effects of drugs which is in close relation with targeted drug delivery. So far, vast variety of nanocarriers have been developed to administer pharmaceutics specifically to tumor sites (26). There are some criteria to design an appropriate nanomedicine to target cancer cells including reasonable safety and efficacy, stability, ease of administration, acceptable size, shape, surface activity, surface area and pharmacokinetic properties to accumulate in cancerous cells (27, 28). Some of most recently used nanoparticles in cancer drug delivery are protein-drug conjugated nanoparticles, liposomal nanopaticles, polymeric nanoparticles, dendrimers and hydrogels as well as inorganic and metal nanoparticles (29). It has been approved that among all nanoparticles, gold nanoparticles (*GNPs*) attracted many attentions in field of cancer and this is especially originated from remarkable properties of them (30).

1.3.1 Gold Nanoparticles

Gold is one of the oldest known metals that have been discovered (31). Au [I] and Au [III] are known as common oxidation states of gold, but GNPs exist in non-oxidated form of Au [0]. The history of GNPs refers to 16th century when Paracelsus applied colloidal gold for treatment of mental disorders caused by syphilis. In spite of ancient history of gold, application of GNPs in medicine was founded in 19th century (31, 32). Then it was in 20th century when characterization studies for GNPs were performed by TEM and AFM microscopes and this boiled down to improvements in GNPs properties and coating research (33). So far, a lot of studies have been designed to apply GNPs into cancer research field. They could be applicable as drug delivery

agents, tumor sensors and eradicators of cancer cells by photothermal therapy method (34).

There are several properties including physical and chemical ones which make GNPs as a unique mean in cancer therapy as following: flexible size and shape range, large surface area rather than volume ratio, light scattering and absorbance ability and easily functionalized with different biomolecules that particularly leads them to be single in kind (35-37). Albeit, there are some inevitable issues about GNPs application in cancer field especially in clinical trials. First issue could be mentioned as biocompatibility. It is approved that more and less GNPs they are almost biocompatible agents due to their inert nature, but on the other hand, there is an issue of cytotoxicity. Cytotoxicity is almost dependent on size, shape, chemical compounds and surface properties of GNPs and should be studied more in detail in further research (30). In the meanwhile, there are controversial opinions over cytotoxicity. Some studies demonstrated that GNPs lack the potency inducing acute and intensive toxicity (38). Others illustrated cytotoxicity is more in comparison with previous studies and it is closely related to size and shape (8, 39).

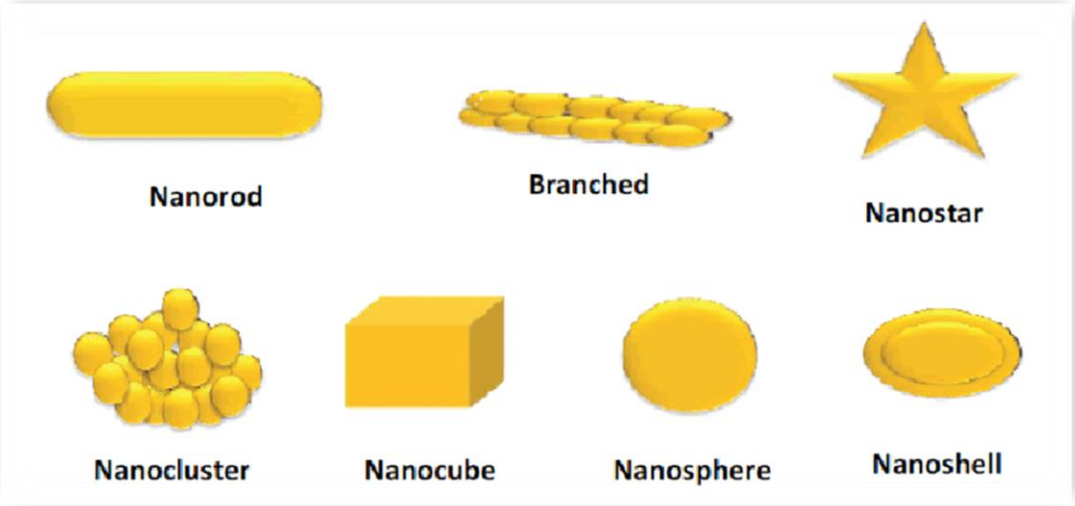

Figure1.1. Different Shapes of GNPs (Imran A, 2017)

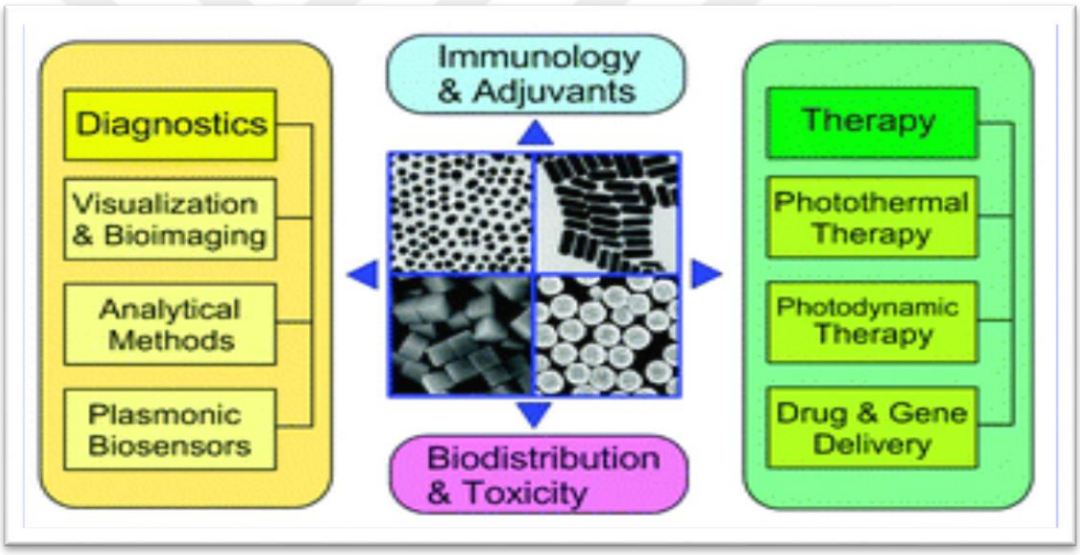

Figure1.2. GNPs Applications in Biomedicine (Khlebtsoy N, 2012)

1.3.2. Main Application of Gold Nanoparticles in Cancer

1.3.2.1. Capable sensors for tumor detection

AuNPs could be suitable candidates to label because of their capability to interact with visible light. This occurs based on accumulation of AuNPs in targeted site and scattering light to give an image of region by means of different tools such as optical microscopy, dark field microscopy, photothermal imaging, and photoacoustic imaging (40, 41).

1.3.2.2. Drug carriers

Recently, there has been growing interest in modifying pharmacokinetic properties of available drugs to reduce specific side effects in addition to increasing bioavailability in targeted cells. AuNPs have been vastly used as drug carriers owing to high loading capacity of agents, easy surface modification, release control system by external and internal stimulus as well as passive targeting ability which is called enhanced permeability and retention (EPR) effect (35, 42).

1.3.2.3. Photothermal therapy

This is the point where central application of GNPs comes into discussion. One of the unique abilities of AuNPs is to absorb photons efficiently and convert them to heat which basically destroys cancer cells. Hyperthermia is more hazardous to cancer cells rather than normal cells because of abnormal vascular structure in tumor cells. Therefore, heat generated by GNPs leads to biomolecule destruction and cellular membrane of tumor cells is disrupted, consequently (43). Hyperthermia is generally applied by other treatments like chemotherapy and radiotherapy. However, the lack of heating of deep tumor tissues is still challenging (44, 45).

1.3.2.4. Future perspectives

Research on GNPs has shown their potential ability to be applied in cancer treatment and imaging. So far, studies focused on synthesis and characterization of AuNPs. Above mentioned applications including hyperthermia and drug delivery are in second

phase and first phase of clinical trials, respectively. In spite of many improvements in different areas, toxicity remains as crucial challenge in application of AuNPs (46, 47).

Apart from what mentioned above, when nanoparticles enter cells, a coating is formed around them by proteins which are present in physiological environment and resulting protein-nanoparticle complex (48). It plays an important role in distribution of nanoparticles in biological fluid. In this regard, studying the relationship between nanoparticles and constitutive components of proteins that are amino acids is fundamental part of research for GNPs as well (49).

1.3.3. Amino Acids

Amino acids (AA) are known as basic building compartments of peptides and proteins. In different studies, almost 300 types of amino acids are explained, however only 20 of them participate in proteins structure. Some of them exist in nature, but others are added to genetic code (50). There are two main groups in AA including amino group and carboxylic group and the main difference between amino acids is in their functional group (51). AA are classified into four main groups including polar, non-polar, acidic and basic. For instance, amino acids with aromatic sides and hydrocarbon alkyl are non-polar ones such as Glycine (*Gly*), Valine (*Val*), Leucine (*Leu*), Alanine (*Ala*), Isoleucine (*Ile*), Proline (*Pro*), Methionine (*Met*) and Tryptophan (*Trp*). Side chains in AA with amides, acids and alcohols give them polar features. Tyrosine (*Tyr*), Serine (*Ser*), Asparagine (*Asn*), Threonine (*Thr*), Glutamine (*Gln*), and Cysteine (*Cys*) are classified as polar amino acids. Meanwhile, Aspartic Acid (*Asp*) and Glutamic Acid (*Glu*) are considered acidic amino acids and Lysine (*Lys*), Arginine (*Arg*), and Histidine (*His*) are basic.

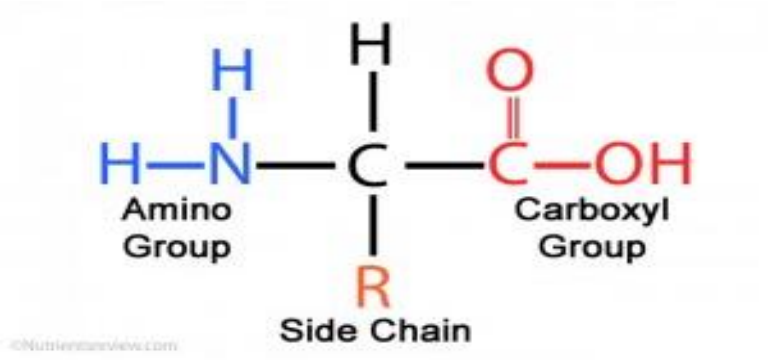

Figure 1.3. Chemical Structure of Amino Acid http://www.nutrientsreview.com/proteins/amino-acids (wikibooks)

1.4. Molecular Simulation

By growing importance of studying nanomaterials and biomolecules interface, there have been a lot of performed research in the field of bionanosciense. There are exponential number of products as therapeutics to apply containing nanoparticles (52), while the main challenge is applying nanoparticles to the interface of biomolecules. Generally, proteins and peptides are basically prone to be absorbed on the surface of NPs and cause inevitable side effects like modifying function and structure of *NPs* (53-56). Moreover, as GNPs have been used alot in medicine and biology and their conjugation affinity to biomolecules especially proteins (57) is a debatable part of nanobiosciences. Therefore, not only properties should be evaluated, but also interface evaluation is an indespensible necessity to contribute useful and effective applications of AuNPs in the medicine (58, 59). This is where molecular simulation could be very helpful and valuable to visualize conjugation and affinity between biomolecules and *NPs* as it is difficult to find and obtain actual data for such systems by performing experiments (48, 60, 61).

1.5. Molecular Dynamics Simulation

The history refers to late 70s (62, 63). The molecular dynamics (*MD*) method was first introduced by Alder and Wainwright in the late 1950's (Alder and Wainwright, 1957,1959) to study the interactions of hard spheres. Many important insights concerning the behavior of simple liquids emerged from their studies. Its main feature is to investigate interactions and movements between molecules and atoms. The development of advanced high-performance materials has gained importance with the

speed of computation and advances in new computational algorithms. MD is a method to simulate materials on an atomic scale. MD is a deterministic technique to simulate the motion of atoms. The simulation is divided into a series of time stages, usually in femtosecond order. For each step, all forces between all atoms are calculated and then combined to obtain new positions and speeds. This process is repeated until the end of the simulation. In time steps the material properties can be calculated from positions, speeds and forces. In fact, it generally facilitates examination of materials under investigation or the search for materials that are not available, and the development of more accurate and realistic interaction wwhich ensures the reliability and feasibility of the simulations.

The concept of MD simulations can be applied at different scales. From chemical intuition and classical point of view, it is quite simple to use the model of the whole atom, where each atom is represented by a particle with a given mass. For computational efficiency, intermolecular interaction potentials were modelled with simple and approximate functions such as Lennard-Jones potential for van der Waals interaction, point loaded Coulomb potential for electrostatic interaction, and harmonic potential for chemical bonds (64). MD simulation is a very suitable method for understanding the behavior of nanometer-scale defects due to the fact that it has the ability to track the position, velocity and acceleration of atoms, or the atomic trajectory of atoms.

1.6. RASPA Program

RASPA is newly devised program to simulate adsorption and diffusion of molecules. The RASPA code was written as a collaboration among Northwestern University (USA), the University of Amsterdam (The Netherlands) and the University Pablo de Olavide (Spain). Two main computational approaches to tackle these systems are (i) quantum mechanical calculations and (ii) force field-based simulations. Force field-based approaches include Monte Carlo (MC) simulations, molecular dynamics (MD) simulations and energy minimizations. We introduce here a code, RASPA that focuses on MC, MD and minimization of systems described by classical force fields. In RASPA, a single point of an isotherm can be obtained within hours for a simple system and in days for more complicated systems. Here, simulations are independent and are

run as batches of serial simulations that differ in temperature, pressure, etc. RASPA used three generic ‘types’ or ‘groups’ for the particles: (1) ‘framework atoms’, (2) ‘adsorbates’ and (3) ‘cations’.The advantage is that the different components of the total energy are available and the interactions can be examined (65).

1.7. Metropolis Monte Carlo Method (MC)

In MC, a molecular system is controlled by a few parameters inculding volume and temperature. Generally and experimentally, there are a few parameters which could describe molecular system including temperature and volume. A chain of configurations is generated to assemble sample (66). The process is made of two-stages. Firstly, a trial chain of configuration is generated with the probability of transition, and consequently proposed configuration is formed.

1.8. Adsorption Theory

Simulation of of pure or mixed sorbates is performed by Sorption. Adsorption reduces the imbalance of attractive forces which exists at the surface. Three ways are possible to simulate adsorption equilibrium consisting of fixed loading (canonical ensemble), fixed pressure (grand canonical ensemble), and Henry constant (uniform ensemble). Furthermore, a series of fixed pressure (67) simulations are performed in a specific fugacity range by adsorption isotherm and provides the amount of sorbates at given temperature and pressure. On the other hand, fixed loading module in which temperature is graually decreased over the series of simulation. Namely, it facilitates determination of binding sites and energies of fixed number of sorbates. Henry constant calculates loading limitation when pressure moves forward zero. This theory supports two kinds of Monte Carlo Methods. First is the Metropolis MC method (Metropolis et al., 1953) in which bias does not exists in trials. In addition, the structure of sorbate is considered to be rigid and there is an incorporation of rigid orientations. The second one is the configurational bias MC method in which there is a bias toward low energies and it includes torsional degrees of freedom. Almost all of physical and chemical processes happen at various interfaces. In this regard, adsorption is considered as one of the fundamental interface reactions. This process occurs

spontaneously. Adsorption generally increases balance of attractive energy at the surface and hence free energy is formed.

1.8.1. Adsorption Isotherm Simulation

This property enables researches to perform experiments and simulations in fixed pressure at fixed temperature in a single step. Starting and ending fugacities are available to perform simulation in a specified number of steps. The theory is that the logarithm of start fugacity for components increases linearly to logarithm of end fugacity. To put it simply, there is an exponential increase in fugacity and the result is sum of total fugacity which is used for analysis. Equilibrium generally demonstrates a state in which the amount of adsorption of molecules onto the surface is exactly counte balanced by the rate of desorption of molecules. For instance, when the rate of adsorption equals the rate of desorption, dynamic equilibrium occurs. Rate of adsorption is influenced mainly by pressure and presence of available adsorption sites.

1.8.2. Henry's Concept

Henry Concept (H) is calculated as Henry's law. Equilibrium is formed of with components reservior and loading of that component where N is proportional to the fugacity of the component, f.

$$N=Hf \qquad Eq\ (1.1)$$

In calculations, loading is the number of molecules.Therefore, inverse units of the fugacity is carried by the H and the units of loading per cell per kPa^{-1}. In this simulations, configurations which overlap are excluded from energy fields and they are not counted in reports of the MC steps.

2. LITERATURE REVIEW

It is approved that collecting real experimental data for visualization of interactions between GNPs and Amino acids or their conjugates have been challenging for years (58). So, simulation is always a valuable contribution to science and provides accurate access to structural properties as well as any kind of bonding (68). A lot of papers and scientific works followed this field. For example, a study was designed to find out adsorption of *Cys* AA on surface of Au by periodic density functional calculations in 2003. Results demonstrated that Au sites are favorite for *Cys* and a sort of chemical binding occurred because of thiol group on Au and its strong affinity with *Cys* (69).

It was 2005, Podstawka et al. performed an experiment by Surface Enhanced Raman Spectroscopy to see deposition of various AA such as *Met*, *Gly*, and *Cys* on surface of colloidal gold surface. Spectral analysis predicted AA orientations and competitive interaction of AA functional groups on the gold surface (70). Preferred conformation of amino acids on metal surface of Au was studied by Au force field described as a method in which Au atoms are constant. It is concluded that almost all of AA had interaction with Au surface, however some of them had partial interactions and affinity (68).

Peptides were also studied in simulation studies to understand factors involved in interaction affinity of them with Au surface and it was illustrated that the interaction is related to AA sequence (58). Another study showed adsorption behavior of *His* AA and *His*-containing peptides on Au surface conducted by MD simulation and COMPASS force field. This study presented the idea that adsorption on Au surface occurred due to the presence of imine nitrogen in *His (71)*. Molecular dynamics of AA adsorption on *GNPs* was performed for all 20 AA by OPLS AA force field. In this system, Au atoms were dynamic. Stability, flexibility, molecular structure of adsorbed amino acids were evaluated in this study.

All evaluated data revealed that none of 20 AA are equal. *Asp* is adsorbed faster than others. Moreover, there were more *Arg* and *Thr* on gold rather than other AA in the same time (72). A study illustrated binding-related mechanisms, which govern interaction preferences between 20 AA and 5 different types of GNPs. Among twenty, twelve of them preferred to bind to the 4 nm GNP and seven of them tended to GNP of 2 nm GNP (73).

Another study was conducted to study interaction between fullerene-like gold nanocage and selected amino acids. Most studies approved applicability of DFT calculations method in studying interactions between molecular systems and nanostructures. In this study, adsorption energy was calculated between AA and gold nanocages. Results of this study demonstrated that AA

form stable binding with gold nanocages via their potential active sites. Interaction between Au and *Gly* is exothermic and stable. The interaction happens because of amino N-active site. It was also indicated that tripeptide of *Gly* had stronger bond to Au rather than *Gly* due to the presence of carbonyl and carboxyl O atoms active sites (74).

Other AA including *Cys, His, Phe* and *Tyr* with sulfur, imidazole and aromatic groups were also evaluated for interaction studies with Au nanocages. They strongly bound to Au and further studies provided results that peptides and proteins with AA which contain hydroxyl oxygen, amino nitrogen and carbonyl oxygen active sites can form stable bindings with Au via its potential active sites (74).

3. OBJECTIVES

The main objective is to determine binding affinity between amino acids and gold nanoparticles in addition to the involved mechanisms by molecular simulation. To conduct this experiment, GNPs designed will be designed and 20 different AA will also be designed. Then, binding affinity of AA to different shapes of Au will be assessed according to involved energy. We will try to understand mechanism which is involved in binding affinity to Au surface. We tend to know whether difference in AA classification could affect binding affinity of AA to Au surface. Are structure and side chains affect binding mechanism to GNPs? Can size and shape of nanostructures affect binding affinity of AA. Generally, the main objective is to determine binding preferences of AA for different types of Au derivatives in variety of shapes and size. Au derivatives includes Au nanorods, Au sheets and Au 1nm nanoparticles.

4. MATERIALS AND METHOD

4.1.Design and Modelling Chemical Structure of Amino Acids

Chemical structures of AA were checked from Protein Data Bank (PDB). All 20 AA were designed manually by Material Studio Software 7.0. In addition, Au nanorods were designed in four corners of cubic box by the same software. Besides, Au sheet and 1 nm spherical Au nanoparticle were also designed to compare shape and size effect on AA binding. RASPA program was used for data analysis. According to figure 4.1, different AA are listed according to their functional groups. Figure 4.2 illustrated designed AA in four different groups including polar, non-polar, acidic and basic ones.

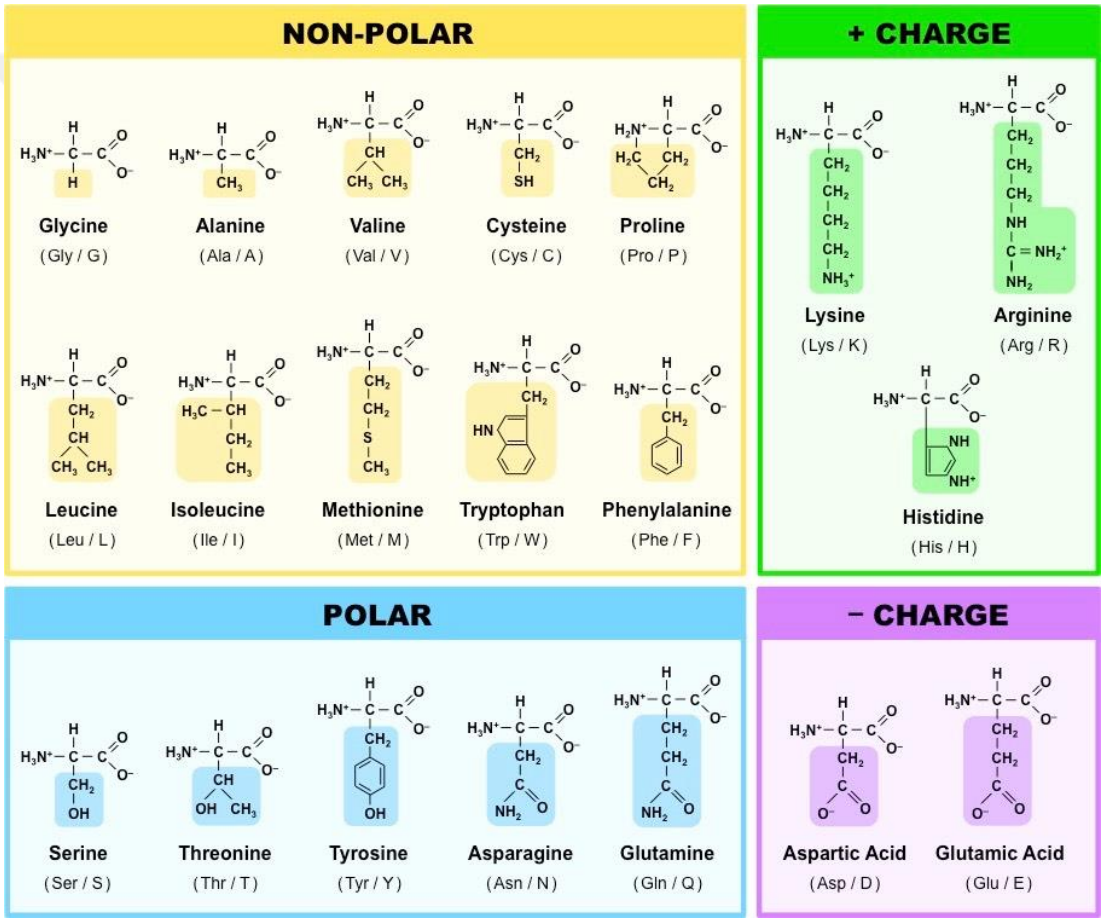

Figure. 4.1. Classification of Amino Acids (www.onlinesciencenotes.com)

4.2. Adsorption Calculations

In this study, Metropolis Algorithm was used to study affinity between AA and Gold nanorod. Binding energy of AA were obtained and compared in fixed temperature and pressure.

4.2.1. Adsorption Isotherm Calculations

The simulation was performed at fixed pressure where the temperature was 300 K. To establish an equilibrium in order to equalize number of AA as adsorbate with Au nanorod molecules, Au nanosheet and 1 nm spherical nanoparticle as adsorbent, adsorption isotherm were performed in metropolis algorithm. In addition, citrate affinity was also evaluated the difference of sheet crystal adjustment with three different crystals including Au [100], Au [111] and Au [211]. Citrate was used due to the fact that it is a commonly used material which is used to synthesize Au nanomaterials.

4.2.2. Adsorption Energy Calculations at Fixed Pressure

For further data, we preferred to perform fixed pressure adsorption, but this time we calculated energy in group of AA and compared them together.

4.2.3. Henry's Constant Calculation

To calculate Henry constant, adsorption isotherm is used and the slope of adsorption isotherm is considered as Henry constant. In this study, we performed Henry's constant simulation which is a method in adsorption for all AA to assess average loading at lower pressures. This is done once for individual AA and then we compared it with group of AA.

5. RESULTS

5.1. Design of Amino Acids and Various Shapes of Au

All amino acids were designed and modelled in Material Studio 7.0. Figure 5.1(a, b, c and d) shows obtained AA that we modelled. The building elements are illustrated beside each one and AA are classified in four main groups including non-polar which are called hydrophobic AA and they do not have charge on the 'R' group (Alanine, Leucine, Isoleucine, Valine, Methionine, Phenyl alanine, Tryptophan and Proline). Polar AA which do not have charge on the 'R' group, but they carry polar groups like hydroxyl, sulfhydryl and amide groups in them (Glycine, Serine, Threonine, Cysteine, Glutamine, Asparagine and Tyrosine). Basic ones which are polar amino acids with positive 'R' group (Lysine, Arginine and Histidine), and acidic ones which are polar amino acids with negative 'R' group (Aspartic acid and Glutamic acid). Au nonorods, Au sheets and 1 nm spherical nanoparticles were also designed by this software which are illustrated in Figure 5.2 (A, B and C).

Besides, side chains and backbones of AA were modelled as their real structures to examine the roles of AA backbone and side chains in binding preferences. We did not change N terminals and C terminals of AA and we left them uncapped in their natural form. Au nanorods, Au sheets and Au spherical nanoparticles were 100 Å, 30 Å and 50 Å, respectively.

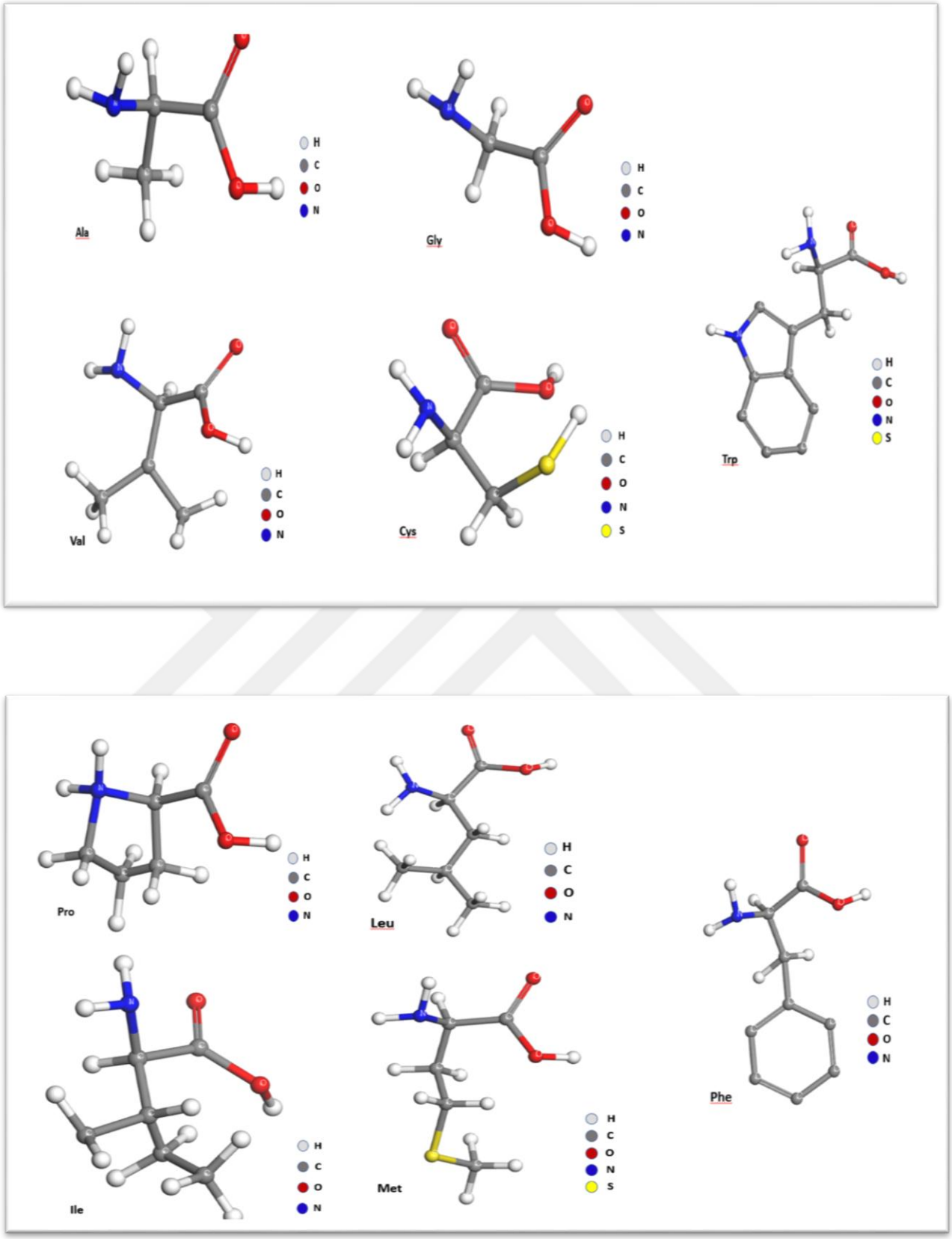

Figure 5.1(a). Designed non-polar AA

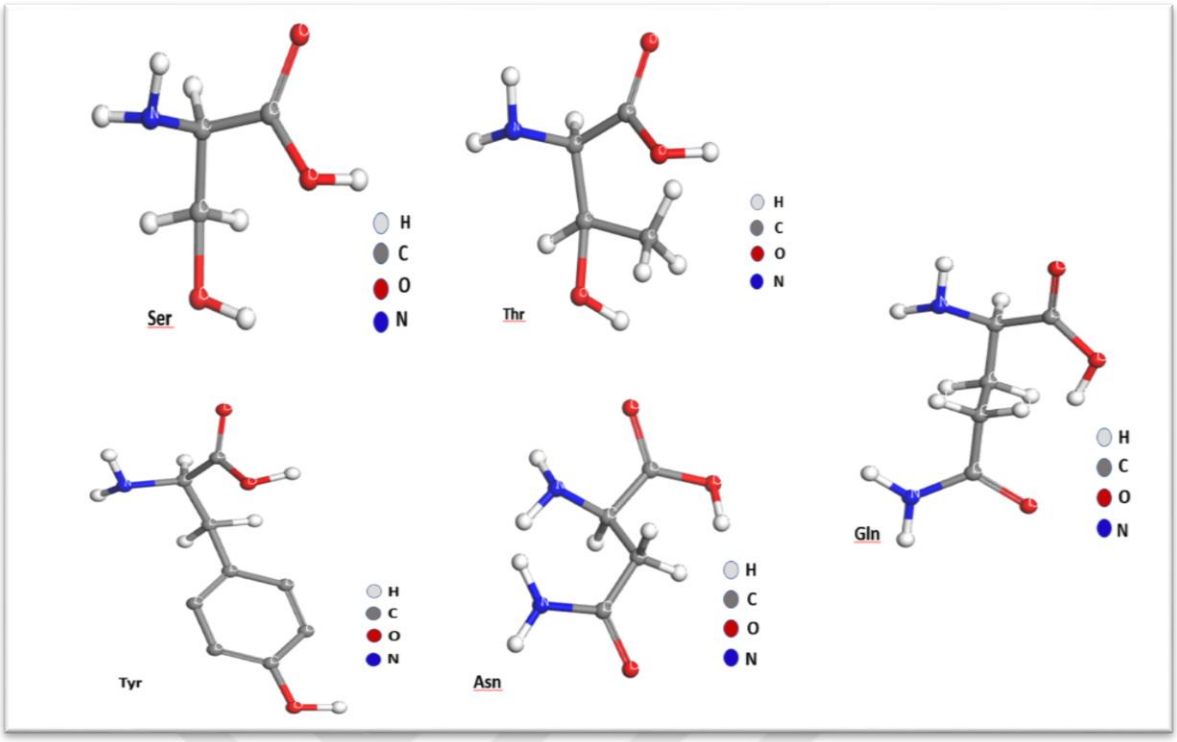

Figure 5.1(b). Designed polar AA

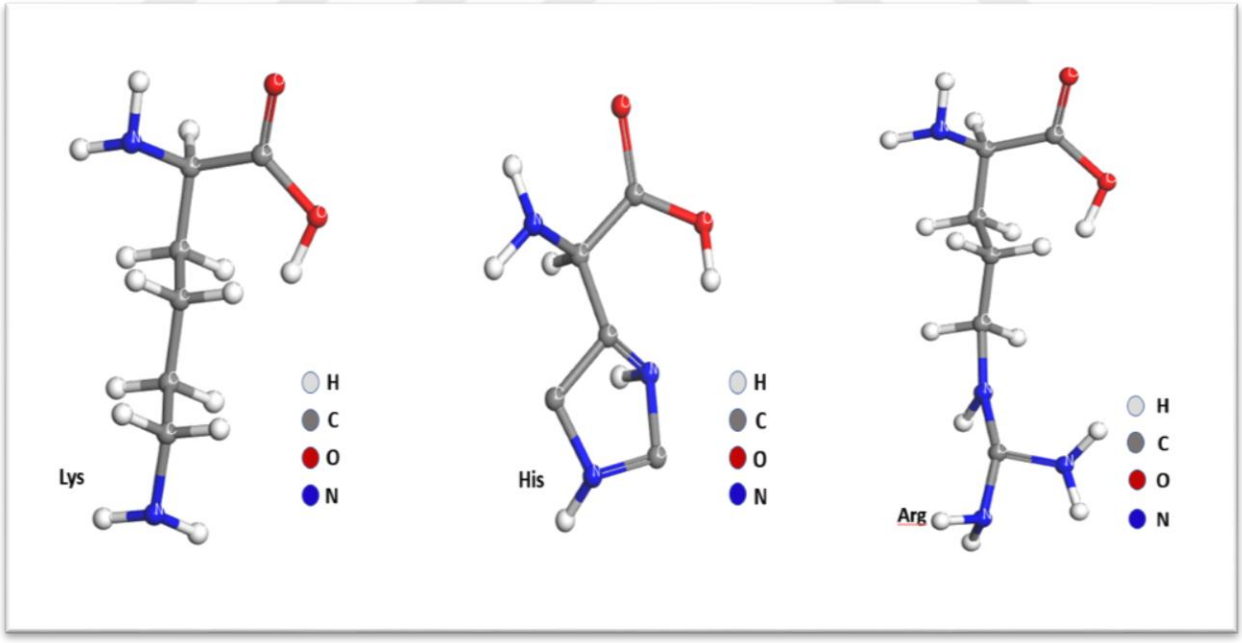

Figure 5.1(c). Designed basic AA

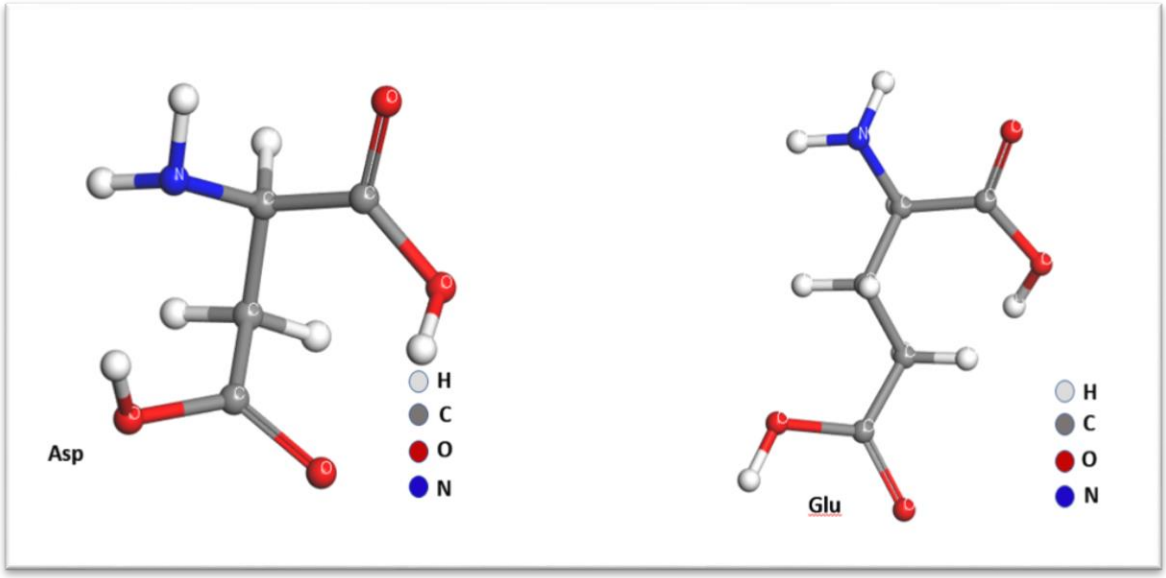

Figure 5.1(d). Designed acidic AA

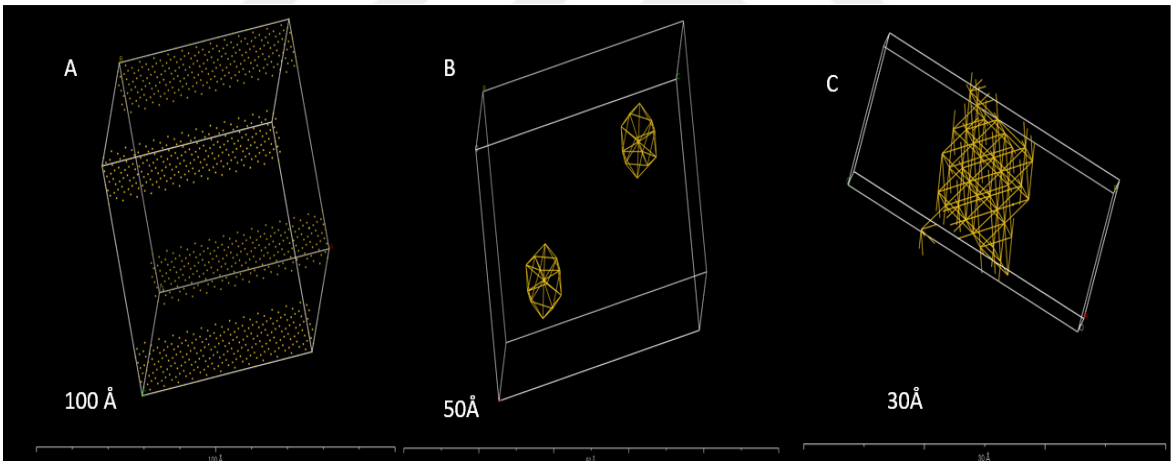

Figure 5.2. (A) Au Cell nanorod, (B) Au nanoparticle (B), (C) Au Sheet

5.2. Adsorption Isotherm and Stability of Amino Acids on Adsorbed Surface

In adsorption isotherm, the graphs are generated by loading per cell in vertical axis on fugacity (kPa), which is roughly shows pressure on horizontal axis. This graph Figure. Figure 5.3 illustrated stability of adsorption on surface of Au nanorods for polar, non-polar, basic and acidic AA.

Results in polar AA showed that *Tyr* has the lowest capacity and the slowest rate in attachment and separation process from surface. It occurred when the loading per cell is almost 200 AA per cell. *Cys* and *Thr* showed similar behavior and there was a sharp increase in adsorption rate for both until it is reached to plateau in almost 350 AA per cell. *Asn* and *Ser* also behaved similarly, however *Asn* adsorbed and separated sooner than *Ser*.

On the other hand, for non-polar AA, the story was a bit complex. It is proved that *Gly* has shown strange pattern in which loading was less than expectation in addition to having a steady increase till 1000 kPa of total fugacity. While *Ala* loading has dramatically risen up and then it showed a slight change but did not reach plateau. *Trp, Met, Pro, Val, Ile* and *Phe* were almost same in loading and adsorption behavior. Among all non-polar amino acids, *Leu* adsorbed in high loading levels and it seemed to be more stable on surface of Au nanorods.

For non-polar AA, *Leu* and *Ala* had a significant rise. The lowest rate was for *Gly*. While other AA had shown same behavior. In basic AA, *His* absorbed sooner than *Arg* and *Lys* and it was also less stable on surface of Au nanorods. *Lys* had the highest loading per cell. Acidic AA were same and they had high loading rate, but both *Asp* and *Glu* had separated fast in almost zero fugacity. *Glu* and *Asp* have illustrated same pattern, however loading for *Asp* is a bit more than *Glu*.

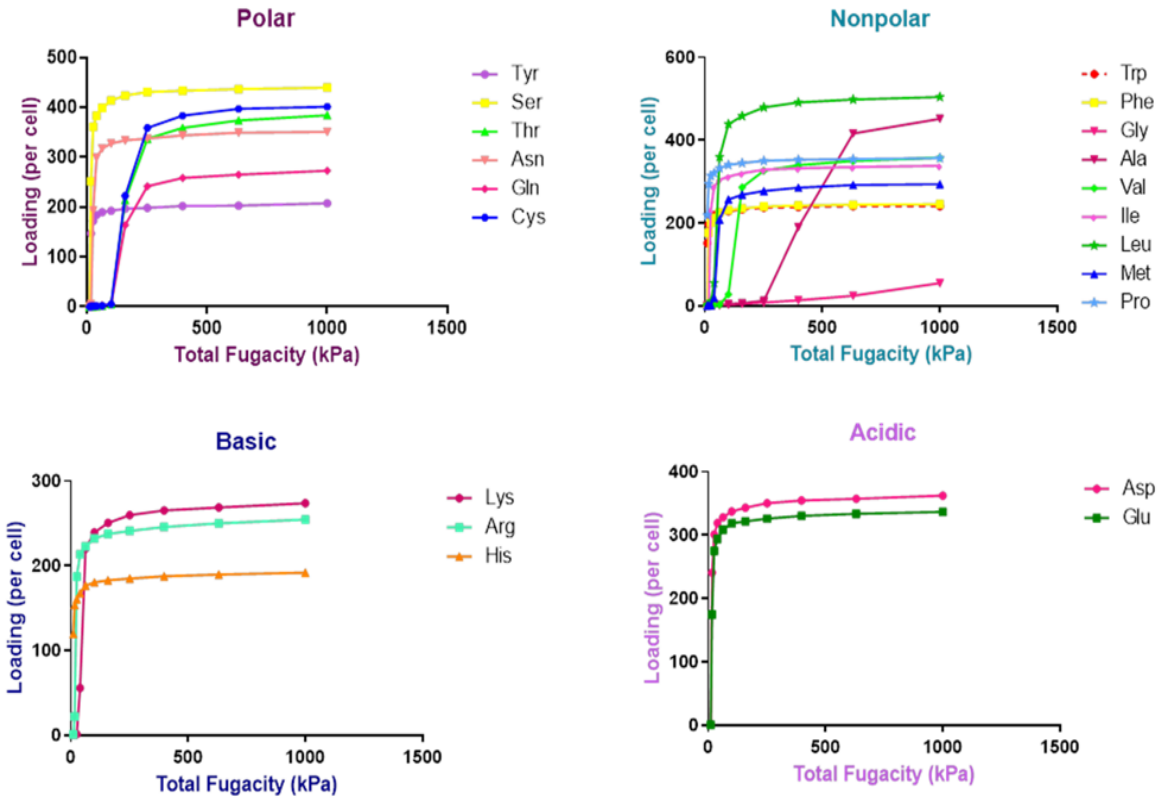

Figure 5.3. Adsorption isotherm on surface of Au Nanorods for polar, non -polar, basic and acidic AA

To see the effect of shape on binding affinity of AA on different structures derived from Au, AA were selected based on results of experiments of Au nanorods. From each group of AA acids, the ones which had significant patterns were chosen for further simulation on 1 nm spherical gold and gold sheets. Results illustrated in Figure 5.4 that for polar AA, shape of nanoparticles did not result in specific changes. In other word, in this study shape did not affect loading amount for polar AA. As previous one, in this group for both new structures, *Ser* has shown the highest loading capacity and *Tyr* has shown the fastest attachment and separation on the surface among polar ones.

On the other hand, the pattern for non-polar AA is a bit different. For Au sheet, some fluctuations were seen and a stable pattern was not approved. Besides, for 1nm spherical Au, *Gly* has had the highest absorption even more than *Leu*. So, we can conclude that shape is a factor which is responsible for binding affinity of AA on surface of Au nanostructures. Figure 5.4 shows changes for polar and non-polar, basic and acidic AA more clearly for gold sheets.

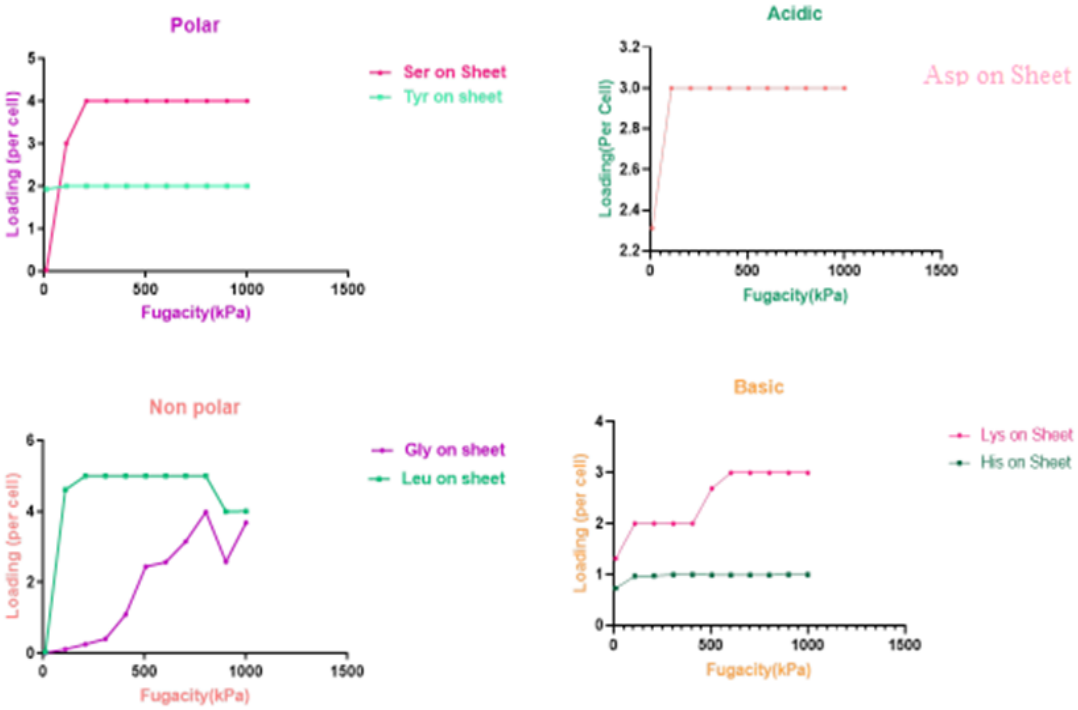

Figure 5.4. Adsorption isotherm on Surface of Au Sheets for polar, non-polar, basic and acidic AA

Figure 5.5 demonstrates the same data for Au spherical nanoparticles. For basic AA, shape did not cause any significant effects and *His* absorbed sooner that *Lys*, however, *Lys* had the highest loading per cell on sheet. Besides, *Asp* as acidic AA showed the same behavior on sheets as nanorods. 1 nm Au spherical nanoparticles had also contributed to same pattern.

Finally, results for three different crystal directions of Au were presented in Figure 5.6 to see the effect of crystal orientation on affinity of citrate as an important component of biological fluids.

As demonstrated in figure 5.6, there are different values for citrate-Au [111] which approve that citrate has no stable binding pattern on this structure. For example, till 2 citrate per cell value, the trend is upward, then it reaches to plateau and in almost 1000 kPa fugacity, it showed a decrease followed by an increase, then.

The most significant and stable pattern is seen for citrate-Au [211] which had the highest loading capacity for citrate in almost 3 citrate per cell loading. What's more, citrate on Au 100 showed stable and fixed behavior.

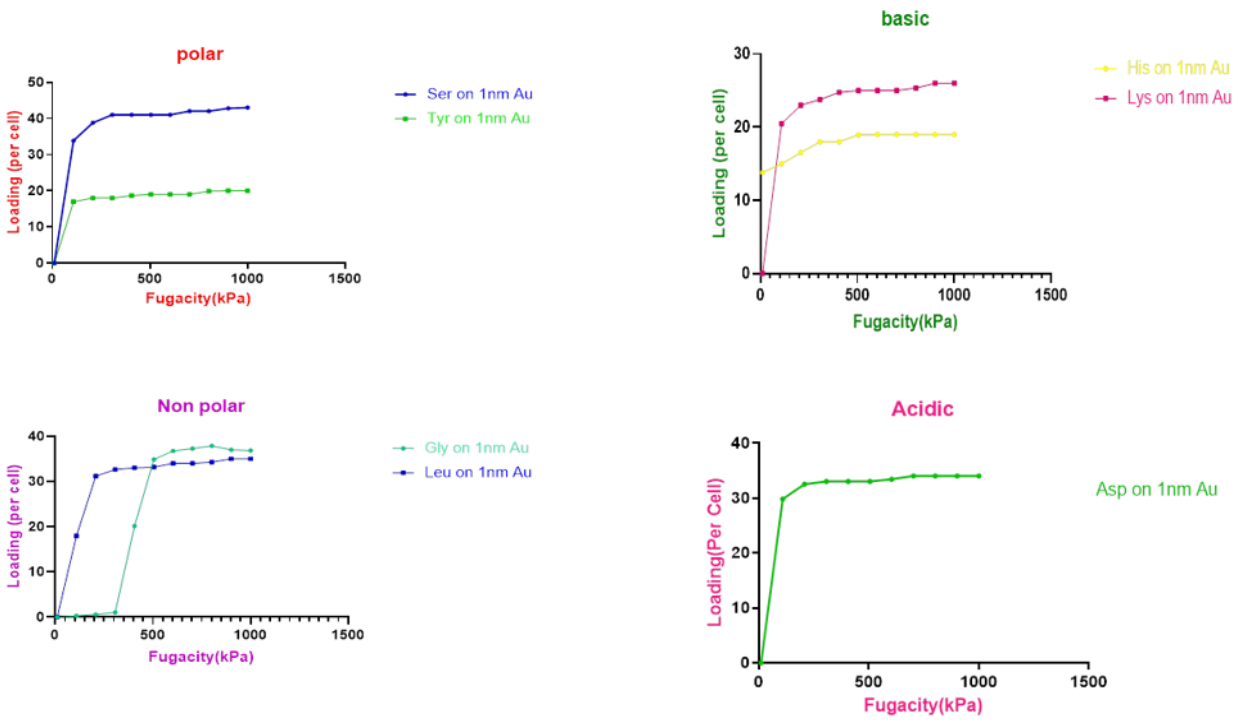

Figure 5.5.Adsorption isotherm on surface of on 1 nm Au Spherical nanoparticles for polar, non-polar, acidic and basic AA

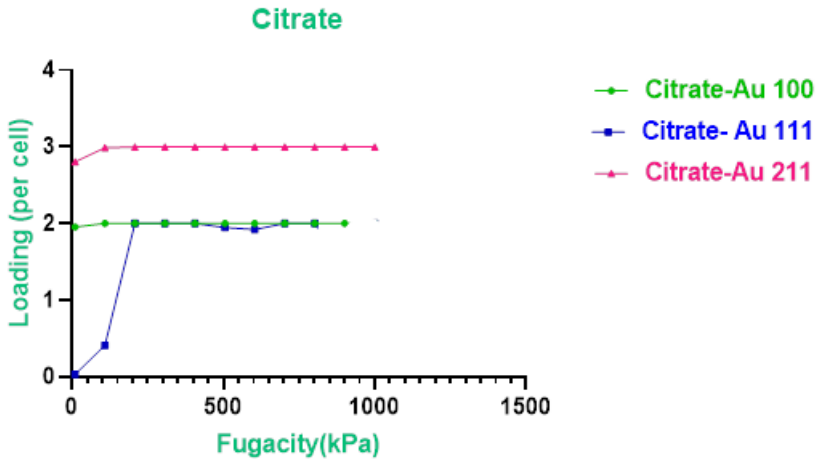

Figure 5.6. Effect of Au Crystals on Citrate affinity

5.3. Adsorption Enthalpy of Au Nanostructures

According to average Adsorption Enthalpy results that is shown in Figure 5.7, *Pro* had the highest affinity to surface with average enthalpy of 31.98 kcal/Mol, albeit *His*, *Trp*, *Tyr* and *Phe* also showed high affinity with average enthalpy of 30.67, 29.42, 20.07 and 23.14 kcal/Mol, respectively to Au nanorods. On Au sheets, *Pro* with enthalpy of 25.99, *Trp* with the enthalpy of 24.93 and *Met* with the enthalpy of 21.23 kcal/Mol had the highest affinity. For 1 nm Au nanoparticle, the pattern was almost like Au nanorods. For instance, the highest affinity was for *Pro* and *Trp* which had enthalpy of 32.75 and 32.36 kcal/Mol , respectively (Table 5.1) .

Results for selected AA on sheets have shown that *Pro* has the highest affinity among non-polar AA. In Polar ones, *Tyr* has shown the highest affinity. Shape could not affect binding energy for acidic and basic AA compared to nanorods. *Ile, Leu, Thr, Cys, Asn, Gln, Asp* and *Glu* did not have changes in average enthalpy according to shape of nanostructures (Figure 5.8). For 1 nm Au spherical nanoparticles, shape could lead to some changes. *Trp* and *Gly* have had the same pattern so far which were completely different in previous samples (Figure 5.9).

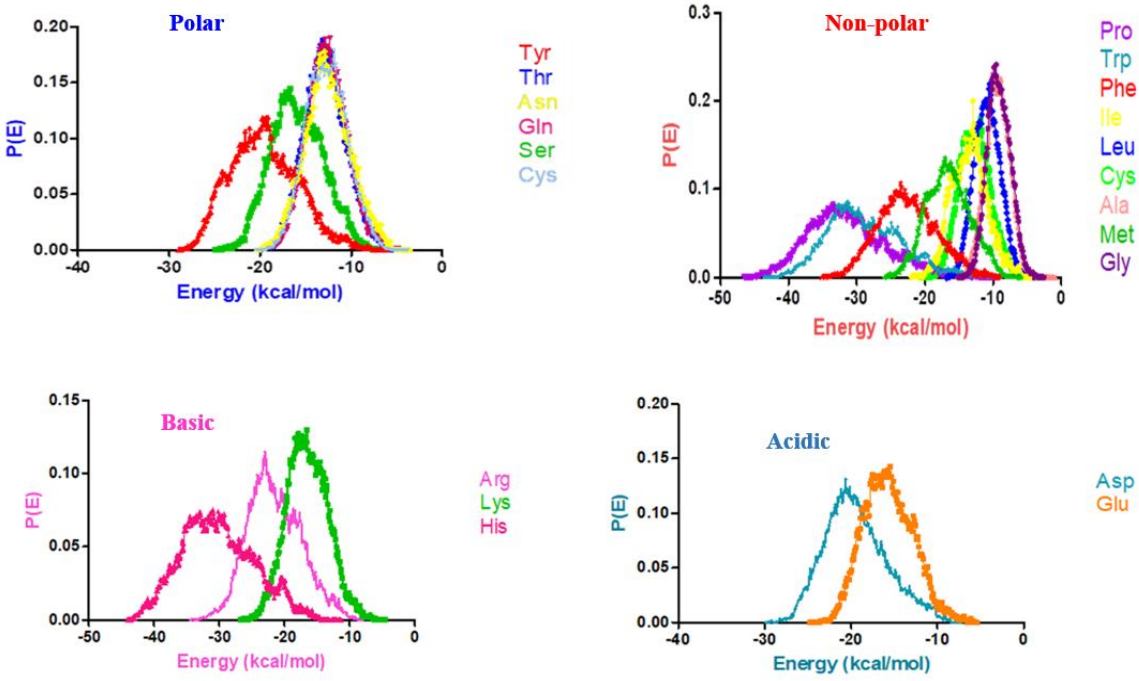

Figure 5.7.Average Adsorption Enthalpy on Au Nanorods for polar, non-polar, acidic and basic AA

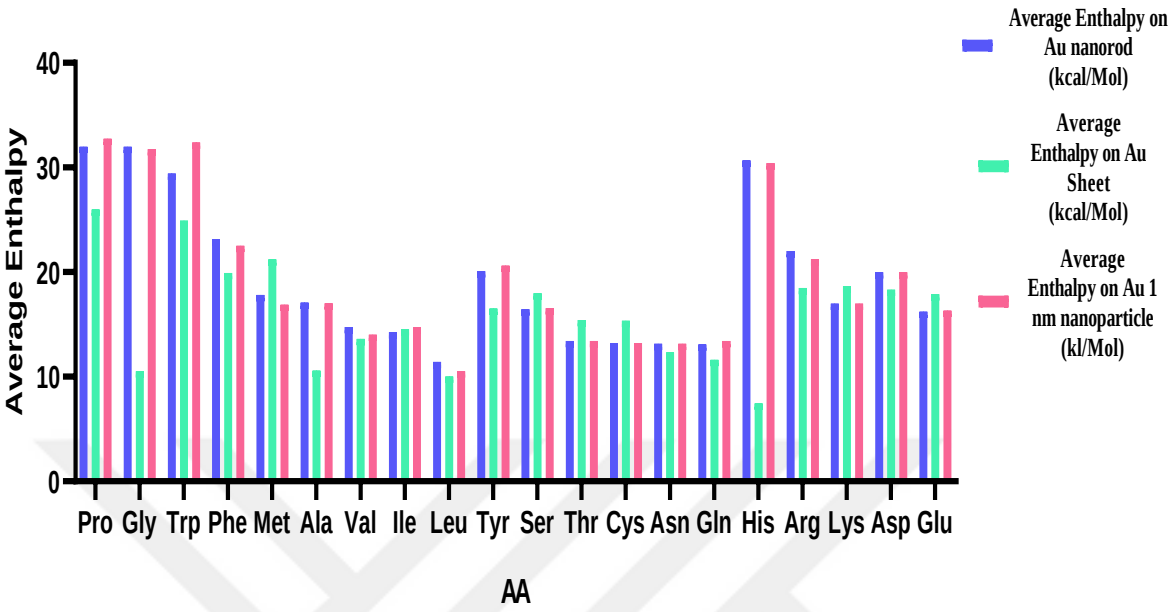

Figure 5.8. Average Enthalpy for AA on Au nanorods, Au sheet and Au 1 nm nanoparticle

Table 5.1. Average Enthalpy for All AA in Interaction with Au Nanorods, Au Sheet and Au 1 nm Nanoparticle

Type of AA	Amino Acids	Average Enthalpy on Au nanorod (kcal/Mol)	Average Enthalpy on Au Sheet (kcal/Mol)	Average Enthalpy on Au 1 nm nanoparticle (kcal/Mol)
Non Polar	*Pro*	31.98	25.99	32.75
	Gly	31.98	10.5	31.76
	Trp	29.42	24.93	32.38
	Phe	23.14	19.90	22.50
	Met	17.80	21.23	16.90
	Ala	17.08	10.58	17.50
	Val	14.76	13.60	14. 50
	Ile	14.25	14.56	14.75
	Leu	11.40	10.03	10.50
Polar	*Tyr*	20.07	16.51	20.60
	Ser	16.44	17.99	16.55
	Thr	13.41	15.41	13.40
	Cys	13.23	15.35	13.21
	Asn	13.16	12.30	13.15
	Gln	13.08	11.62	13.40
Basic	*His*	30.67	7.44	30.43
	Arg	22.01	18.49	21.22
	Lys	16.98	18.66	16.99
Acidic	*Asp*	19.98	18.31	19.99
	Glu	16.23	17.88	16.32

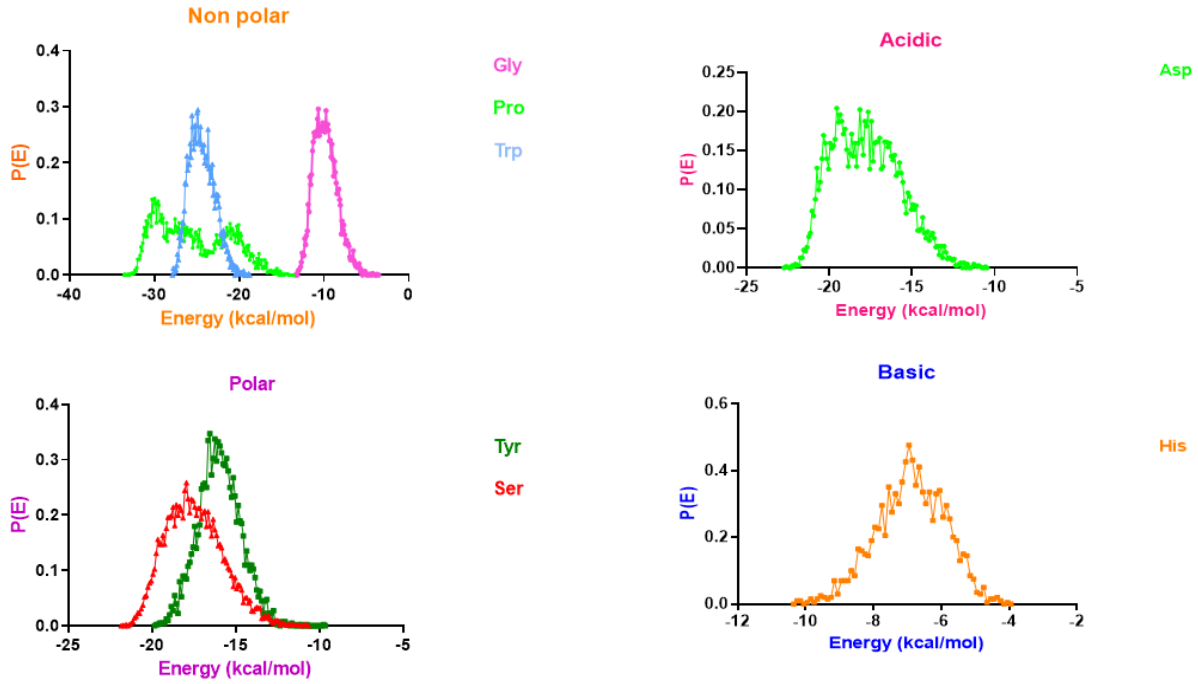

Figure 5.9. Adsorption Enthalpy values on Au Sheets for polar, non-polar, acidic and basic AA

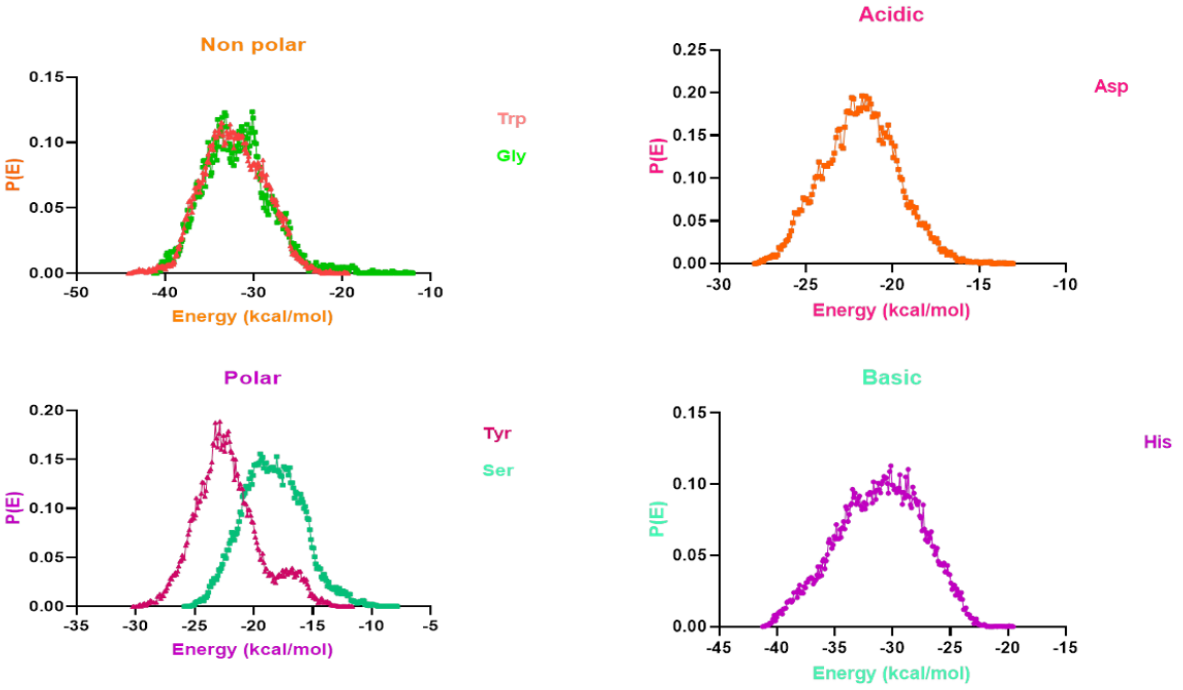

Figure 5.10. Adsorption Enthalpy values on 1 nm Au Spherical Nanoparticles for polar, non-polar, acidic and basic AA

5.4. Henry's Constant on Au Nanostructures

Results originated from this calculation are shown in figure 5.11 and 5.12, elaborated Henry Constant value which is also related to binding affinity and strength of binding of amino acids to surface of nanorods. In this calculation, it has been approved that the lower the Henry Constant is, the higher affinity is established. For *Pro* as well-known AA with the highest binding preference, the pattern was similar in Au nanorods and Au nanoparticle with Henry Constant of 0.040 and 0.006 kPa^{-1}, respectively. Henry Constant for Au sheets did not show consistent data, therefore we did not present results here. Among all AA, *Tyr* had the highest Henry Constant with the value of 0.178 kP^{-1}.

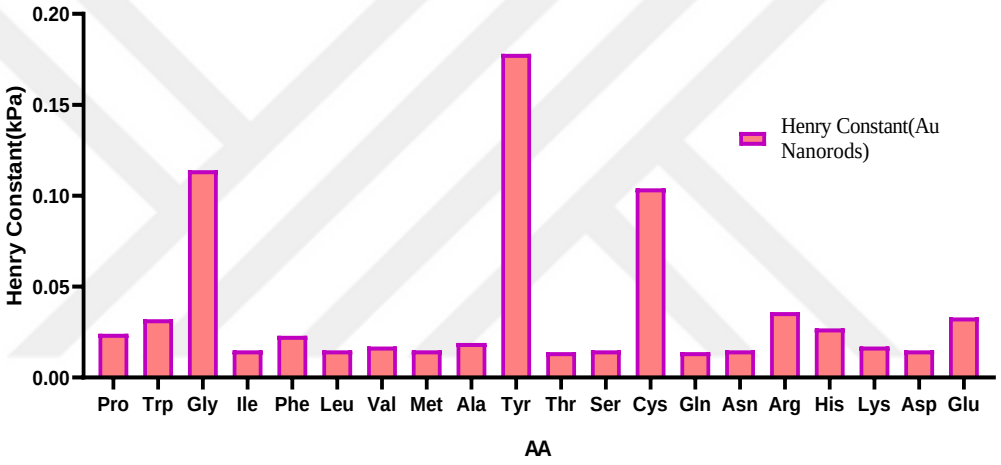

Figure 5.11.Henry Constant Diagram for Au nanorods for polar, non-polar, acidic and basic AA

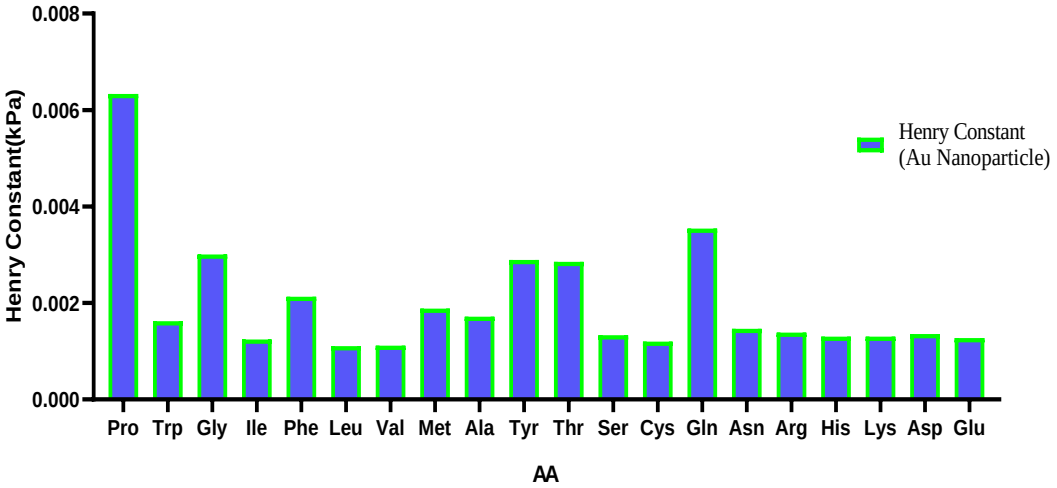

Figure 5.12. Henry Constant Diagram for Au nanoparticles for polar, non-polar, acidic and basic AA

Table 5.2. Henry Constant for All AA on Au Nanorods

Type of AA	Amino Acids	Henry Constant (kPa^{-1}) for Au Nanorods	Henry Constant (kPa^{-1}) for Au Nanoparticle
Non Polar	*Pro*	0.040	0.006335
	Trp	0.039	0.00162
	Gly	0.039	0.003004
	Ile	0.026	0.001243
	Phe	0.025	0.002131
	Leu	0.023	0.001104
	Val	0.022	0.001113
	Met	0.019	0.001884
	Ala	0.019	0.001714
Polar	*Tyr*	0.178	0.002892
	Thr	0.033	0.002851
	Ser	0.031	0.00133
	Cys	0.031	0.001199
	Gln	0.029	0.003544
	Asn	0.027	0.001467
Basic	*Arg*	0.051	0.001385
	His	0.044	0.001302
	Lys	0.037	0.001302
Acidic	*Asp*	0.044	0.001358
	Glu	0.036	0.001273

6. DISCUSSION

6.1. Determining Factors in AA Binding to Au-Derived Nanomaterials

Generally, AA demonstrated different binding behaviors and there was not a stable pattern to explain. A small change in atomic structure of AA could result in difference in adsorption of AA. This changes affinity of binding to different shapes of nanoparticles. For example, methylen is a group on side chain of AA and could modify side chain flexibility which consequently changes binding affinity to different sizes of nanoparticles.

We could determine binding affinity of AA based on their average adsorption enthalpy values. Furthermore, it is also important to understand which part of AA is responsible for binding preference including backbone, side chain or both. Studies confirm that both side chain and backbone are important in binding affinity between AA and gold nanoparticles. The mechanism behind touching surface of gold by AA is firstly occurred by backbone of AA, although there are exceptions. For example, *Arg* had its first contact by means of its sidechain on gold surface. *Arg* is the only AA with this behavior among all other 20 AA. So, it seems important AA in adsorption of peptides sequences on gold surface. In other words, we could use it to synthesize our favorite sequence of peptides for different applications (75).

It is approved that peptide sequences with aromatic side groups or sulfur atoms, including *Tyr*, *Met* and *Phe*, have strong binding energies and binding to Au surface happens due to these side chains. Aromatic ring fits the Au [111] facet of gold according to (76). *Met* has also shown strong binding and it is due to presence of sulfur element which has high binding affinity to surface of Au. There is a communality in *Tyr*, *Met* and *Phe* binding affinity in almost all simulation studies. As it is also seen in our study, *Ser* has high affinity to Au surface and this is proved in similar simulation studies as well. This could be because of natural ability of *Ser* break through first and second hydration layers and binds to gold.

A study by Joshi et al compared binding affinity between a basic AA *Lys* and an acidic AA *Asp*, with gold nanoparticles. *Asp* had shown stronger affinity in comparison with *Lys* and it is caused due to protonation of amine groups in *Asp*, while it does not happen in *Lys* (77).

Furthermore, literature focuses on the strong binding of sulfur containing amino acids to gold surfaces and several gold-binding peptides have been reported, experimental data for amino acids binding affinity to gold, that would be directly comparable with our results, are lacking. So, what is mentioned as a result in this study is computational and needs experimental data. According to peptides library, *Arg*, *Trp*, *Tyr*, and *Cys* represented in peptides with strong affinity to gold surface which is in agreement with our study in *Tyr* and *Trp* (78). Moreover, the method was also same in both studies and it was based on adhered cell counting. Our study evaluated individual AA binding affinity to different Au structures, however previous study evaluated peptides affinity to Au surface.

On the other hand, an interesting point in AA adsorption is their preference in binding to different sites of Au materials with unequal tendency. As we know, gold nanoparticles have various types of facets and edges and they are changeable based on shape and size of nanomaterails. Moreover, structure of AA and their chemical features AA (79).

Results derived from our study which is performed to understand binding affinity between AA and gold nanorods approximately are close to previous studies. We found out that there is a logical relationship between side chains present in AA and binding affinity. Our study suggested *Pro* with strong affinity and in addition, *His, Trp, Phe* and *Tyr* are in strong interaction with gold nanorods. A similar study was performed by Ganji et al, but it was between gold nanocage and AA. They mentioned *Phe*, *His*, *Cys* and *Tyr* as AA with strong affinity to gold nanocage.

Carol K. Hall et al investigated binding affinity of AA with gold nanoparticles according to gold nanoparticle size preference in three different size including 1.0, 2.0, and 4.0 nm. Results demonstrated that 12 AA (*Ala, Arg, Asn, Gln, Gly, His, Ile, Leu, Lys, Pro, Ser, and Val*) prefer to bind to the gold nanoparticle with a diameter of 4.0 nm. 7 AA (*Asp, Glu, Met, Phe, Thr, Trp, and Tyr*) prefer to bind to the nanoparticle with a diameter of 2.0 nm.We assessed our study in two different sizes, however no different result is derived. Rafii-Tabar et al studied the geometries of amino acids, when adsorbed on the nanoparticle, their flexibilities were compared with one another. The interaction of each of 20 amino acids was considered with 3 and 8 nm GNPs.

There is a significant contrast between *Pro's* binding affinity to *GNP* in our study and this study. *Pro*, *Arg* and *Asp* are not adsorbed on the surface.

7. CONCLUSION

GNPs are one of the most widely used nano products. We are able to conjugate GNPs with peptides, drugs, and other molecules to gain desirable effects. MC simulation is still proved to be a valuable tool to study the adsorption of proteins. Despite the growing applications of amino acids conjugated to gold nanoparticle, there is not much experimental work that describes the molecular level of interaction and this was one of our purposes for this study.

The demonstration of the composition of amino acids with high affinity for GNP surface reveals the fact that the 20 natural AA are not equal. It seems that chemical structure of AA, backbones, sidechains as well as characteristics of Au materials that have different size and shape could play distinct roles in binding affinity of AA to Au nanoparticles. Our study has many common results with most of studies both simulation ones as well as experimental ones.

To put it briefly, the backbone is a determining factor in binding to gold nanoparticles for *Asn, Gln, Ile, Leu,* and *Lys,* while for *Asp, Glu, Met, and Phe,* side chain is responsible for binding affinity. Finally, for a few AA, the backbone and side chain synergistically determine the binding tendency of *Thr, Trp, and Tyr*. Another important factor was different facets of gold nanoparticles which could change binding affinity of AA.

8. FURURE WORK

As applications of different GNPs are increasing, we suggest that new studies could be performed to evaluate binding affinity of AA with all types of them. On the other hand, proteins that exist in body could be simulated and this time, studies will be performed between proteins and gold nanoparticles. As AA are building blocks of peptides and proteins, we could use them to design desired sequence of peptides to have specific interaction with Au nanomaterials and use them in therapies. Besides, by applying some changes in side chains and backbones of peptides based on selected AA by molecular simulation and we can make a library of AA which are more suitable and specific to bind to Au nanomaterials.

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