ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

LANGMUIR BLODGETT ASSEMBLY OF PEPTIDE FUNCTIONALIZED NANOPARTICLES ONTO SILICATEBASED SURFACES AND THEIR CHARACTERIZATION

M.Sc. THESIS

Nur MUSTAFAOĞLU

Department of Nano Science and Nano Engineering

Nano Science and Nano Engineering Programme

Thesis Advisor: Prof. Dr. Mustafa ÜRGEN

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

PEPTİT İLE FONKSİYONLANDIRILMIŞ NANOPARÇACIKLARIN LANGMUİR BLODGETT YÖNTEMİ İLE SİLİKA TABANLI YÜZEY ÜZERİNE KAPLANMASI VE KARAKTERİZASYONU

YÜKSEK LİSANS TEZİ

Nur MUSTAFAOĞLU
(513101015)

Nano Bilim ve Nano Mühendislik Anabilim Dalı

Nano Bilim ve Nano Mühendislik Programı

Tez Danışmanı: Prof. Dr. Mustafa ÜRGEN

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Nur Mustafaoğlu, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID **513101015**, successfully defended the **thesis** entitled "**LANGMUIR BLODGETT ASSEMBLY OF PEPTIDE FUNCTIONALIZED NANOPARTICLES ONTO SILICATEBASED SURFACES AND THEIR CHARACTERIZATION**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Mustafa ÜRGEN** Istanbul Technical University	.....
Jury Members :	**Prof.Dr. Mustafa ÜRGEN** Istanbul Technical University	.....
	Assoc. Prof. Dr. H. Özgür ÖZER Istanbul Technical University	.....
	Assis. Prof.Dr. Fatma Neşe KÖK Istanbul Technical University	.....

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To my family,

FOREWORD

In our age, most of the high impact scientific results are achieved through interdisciplinary studies. Scientist needs to collaborate with colleagues from different disciplines to exhibit efficient results and to analyze complex systems in nature. Nevertheless, in these studies basic science knowledge is inevitable. After graduate from Molecular Biology and Genetic and Physics Engineering Departments, it was indispensable for me to conduct my future work on nano science and technology. In this regard, I would like to thank firstly to father of this new era Richard Feynman and all my teachers.

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ABBREVIATIONS

AFM **:** Atomic Force Microscopy

AuBP **:** Gold Binding Peptide

AuBP4-QBP2: Bifunctional peptide which ability to bind both gold and quartz

AuNP **:** Gold nanoparticle

DNA **:** Deoxyribonucleic Acid

dH_2O **:** Distiled water

EDL **:** Electrical Double Layer

EtOH **:** Ethanol

FEG-SEM **:** Field Emission Guns – Scanning Electron Microscopy

FT-IR **:** Fourier Transform Infrared Spectroscopy

GEPI **:** Genetically Engineered Peptides for Inorganics

LB **:** Langmuir Blodgett film

NP **:** Nanoparticle

QBP **:**Quartz Binding Peptide

SAM **:** Self-Assembled Monolayer

SEM **:** Scanning Electron Microscopy

SPR **:** Surface Plasmon Resonance

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LANGMUIR BLODGETT ASSEMBLY OF PEPTIDE FUNCTIONALIZED NANOPARTICLES ONTO SILICATEBASED SURFACES AND THEIR CHARACTERIZATION

SUMMARY

Dimension of systems in vival organisms allows us to benefit from the outstanding property of nano-scale materials especially in medical, biological and small-scale engineering applications. In nature, there are many examples of inorganic – organic hybrid composite structures such as bone, teeth, shells, sponge spicules, silk, magnetotactic bacteria. However, mankind has not been as successful as mother nature in the production of these structures by simple, environmentally friendly, and hierarchical manner. Biomimetics is the science and technology of mimicking nature both for understanding the production methodology and characteristics of these extraordinary materials. By a better understanding of the nature's way of production of these hybrid nano structured materials, several revolutionary achievements are expected to occur in areas such as contraction of the original cell detection systems, signal and imaging systems, bio-electronic and photonic systems, controlled release systems and biosensors. The implementation of molecular recognition and self-assembly systems is vital for understanding stated materials.

In nature, proteins and peptides have an important role in the formation of biological hard tissues. The growth of these biological materials is arisen at room temperature and at neutral pH values. These production processes are directed and controlled by proteins and peptides with molecular recognition and self-assembly methods. Therefore, it is important finding these specific peptides sequences in order to design new materials by mimicking the nature.

Selection of peptides can be connected to inorganic substances are carried out using phage or cell display systems via combinatorial biology. After the selection of peptides, it is possible to re-designs them using computer-based methods, and peptides can be produced not only bygenetic cloning but also synthesized by Fmoc method. In the literature, there are studies on surface binding properties of peptide functionalized nanoparticles produced via self-assembly and the PDMS soft lithography methods. Another alternative for controlled deposition of organic materials onto solid surfaces with controlled thicknesses as monolayer / multilayer is the Langmuir Blodgett method. There are several studies in the literature that are utilizing LB technique to assembly metal nanoparticles onto solid supports; however, in these studies complex and harsh chemicals are used to modify the surface structure and gold nanoparticles in order to obtain stable monolayers. These chemical processes are not biologically friendly and limits the applications of sensing in the biomedical area.

The originality and the aim of this study are to benefit from the selective binding properties of GEPI for binding nanoparticles to inorganic surfaces (instead of using detrimental chemicals used for this purpose) by LB method. For this purpose silicate based substrates and gold nanoparticles are selected as a model system. We used multi-functional inorganic binding peptide to both for functionalizing gold nanoparticles and the silicate based substrate. These multi-functional inorganic binding peptides were a combination of two different genetically engineered inorganic binding peptides (GEPI) joined a flexible linker.

Peptide sequence that was selected from combinatorial peptide phage or cell surface display libraries is synthesized via Fmoc method using CSBio 336s peptide synthesizer by Mustafa Güngörmüs in Washington University. In this regard, AuBP4-QBP2, which has an ability to bind to both gold and quartz, was used in this study. The sequence of the AuBP4-QBP2 was RAVRRRSVRREVGGGPWLPPSLPPWPP.

For establishing a monolayer of AuBP4-QBP2 on water surface which is required for mono layer coating on the silicate solid surface using LB method different solvents are used; ultrapure water, ethanol and 1:1 ethanol:ultrapure water. Ethanol allowed standing of the peptide layer on water surface thanks to its volatility properties. Peptide solutions were mixed with the gold colloids in different proportions to create peptide functionalized gold nanoparticles. The amount of nanoparticles required for the production of LB films was determined by spectroscopic analysis of the mixtures. For this purpose, UV/Vis spectrophotometer, spectrofluorimeter and FTIR were used.

Solutions comprising of only peptides, only the gold nanoparticles, peptides and gold nanoparticles, mixture of peptide-functionalized gold nanoparticles were transferred on the silica-based surfaces using the LB technique. Surface pressure/Area isotherm graphics were obtained. Transfer ratio and isotherm graphs provided information about the thin film. Coated surfaces were investigated by using AFM and FEG-SEM.

Results indicate that the peptides were specifically bind to gold nanoparticles and caused to aggregation of them. The solvent of the peptides significantly influenced the aggregation of the gold nanoparticles and also the LB film formation of peptides onto substrate. According to the AFM and FEG-SEM results, the structure of the LB films consisting of peptides as bottom layer and gold nanoparticles as upper layer were influenced by the solvent used in preparation of peptide solution, the size of gold nanoparticles.

PEPTİT İLE FONKSİYONLANDIRILMIŞ NANOPARÇACIKLARIN LANGMUİR BLODGETT YÖNTEMİ İLE SİLİKA TABANLI YÜZEY ÜZERİNE KAPLANMASI VE KARAKTERİZASYONU

ÖZET

Doğada gözlemlenen birçok yapı hem organik hem de inorganik malzemelerden oluşmuştur. Bu malzemeler makro ve mikro boyutlarda olabiliceği gibi büyük çoğunluğu nano boyutlardadır ve üretimleri nano boyutlarda gerçekleşir. Günümüzde doğanın bu müthiş üretim mekanizmanı taklit ederek yeni malzemeler tasarlamaya ve üretmeye dayalı araştırmalar hız kazanmıştır. Bu hızlı ilerlemede nano bilim ve nano teknolojinin de katkıları büyüktür. Nano bilim, 1-100 nm arasındaki malzeme ve sistemleri inceleyen bilim dalıdır. Doğada bulunan birçok yapı ve doğada gerçekleşen birçok olay nano boyutlarda olduğu için bu bilim alanındaki gelişmeler biyoteknoloji alanındaki gelişmeleri doğrudan etkilemektedir. Nano bilim ve biyoteknoloji alanlanlarının kesiştiği yeni bilim alanına nanobiyoteknoloji adı verilmektedir.

Doğanın organik ve inorganik yapıları büyük bir uyum içerisinde sergilediği birçok örnek sayılabilir. İnsan vücudu bunlardan en önemlisidir. Bunların yanısıra doğada organik ve inorganik malzemelerin düzenli bir hibrit yapı oluşturduğu örnekler de mevcuttur. Diş minesi, deniz kabukları, ipek ve manyetik bakteriler bunlara örnek gösterilebilir. Bu yapıların üretiminde doğa hiçbir kimyasal malzeme, yüksek sıcaklık ve pH değerlerine gereksinim duymaz. Bu hibrit yapılı malzemelerin üretiminde moleküler tanıma ve kendiliğinden montaj (self-assembly) mekanizmaları rol oynar. Moleküler tanıma, iki ya da daha fazla molekülün geometrik ve fizikokimyasal özelliklerine dayanarak birleşmesi olarak tanımlanabilir. Bu mekanizma, sıvı bir ortamda birleşen moleküller arasında Van der Waals, elektrostatik etkileşimler ve hidrojen bağları gibi özel bir etkileşim gerçekleşmesi ile olur. Kendiliğinden montaj yönteminde ise dışarıdan herhangi bir müdahaleye gerek kalmadan, moleküllerin kendi kendilerine düzenlenmelerine dayanır. Kendiğinden montaj yöntemi doğanın çok fonksiyonlu malzemeler ve sistemler oluşturmakta sıklıkla uyguladığı bir yöntemdir. Bakterin koloni oluşturmasından, galaksilerin oluşmasına kadar kendiliğinden montaj yönteminin etkin olduğu görülür.

Doğanın kullandığı kendiğinden montaj ve moleküler tanıma gibi yöntemlerin protein ve peptitlerin yardımı ile gerçekleşmektedir. Proteinlerin sert dokuların oluşmasında iki önemli görevleri vardır. Bunlardan ilki, mineral kristallerine bağlanarak bunların büyümelerini sağlamak; ikincisi ise, minerallerin bağlanmaları için iskele görevi görmektir. Organik – inorganik hibrit yapıların görevinde rol oynayan bu peptit ve proteinler birçok bilim adamının ilgisini çekmektedir. İnorganik yapıları kendiliğinden ve özel olarak tanıyan peptitlerin ilk eldesi, özel organizmalardan bu peptitlerin moleküler biyoloji yöntemleri ile çıkarılması ile gerçekleştirilmiştir. Ancak bu yöntemin oldukça zahmetli, zaman alıcı ve elde edilebilecek peptitlerin doğada olanlar ile sınırlı olması gibi çeşitli dezavantajları

vardır. Bu sebeple bilim adamları ilgilendilendikleri malzemeye özel olarak bağlabilecek peptitlerin seçmek için faj ve hücre gösterim sistemlerini geliştirmişlerdir. Bu alandaki ilginin artması ile de faj ve hücre gösterim kütüphaneleri oluşturulmuştur. Peptitlerin seçiminden sonra bilgisayara dayalı yöntemlerle yeniden dizaynları mümkün olabilmekte, ve peptitler genetik klonlama ile üretilebildikleri gibi, çoğunlukla Fmoc yöntemi ile sentezlenmektedirler. 7-14 amino asit büyüklüğünde sentezlenebilen bu kısa peptitler belirlenen inorganik yüzeylere fizikokimyasal kovalent olmayan bağlarla özel olarak bağlabilirler. Bu peptitler, genetik olarak modifiye edilmiş inorganiğe bağlanabilen peptitler (Genetically Engineered Inorganic Binding Peptides (GEPI)) olarak isimlendirilir.

GEPI'ler doğadan esinlenerek yeni malzeme tasarlama ve üretme alanında yapılan çalışmalara hız kazandırmış ve yeni bir bakış açısı katmıştır. Son on yılda, metaller (Au, Ag, Pt ve Pb), oksitler (ZnO, Al_2O_3 ve SiO_2), yarıiletkenler (GaN, Cu_2O, TiO_2 ve ITO) gibi birçok farklı inorganik malzemeye ve bunların yanısıra grafit, kalsit, mika ve hidroksiapatit gibi fonksiyonel malzemelere bağlanabilen GEPI'ler sentezlenmiştir. GEPI'lerin malzemeye özel olmaları onları diğer peptit ve proteinlerden ayrı kılmakta ve çok geniş uygulama alanlarında kullanılabilmelerini sağlamaktadır. Farklı uygulamalarda kullanılabilirlikleri arttırmak için GEPI'lerin yüzeye bağlanma mekanizmaları araştırılmaktadır. Literatürde bu amaçla yapılan hem deneysel hemde moleküler dinamik yöntemlerle birçok çalışma mevcuttur. GEPI'ler inorganik yüzeyleri fonksiyonlandırmak, hibrit yapılar üretmek ve nanoparçacıklar sentezlemek için moleküler bağlayıcılar olarak kullanılmaktadırlar. Son yapılan çalışmalarda, iki farklı yüzeye özel olarak bağlanabilen ikili fonksiyonlandırılmış GEPI'ler üretilmiştir. Bu ikili fonksiyona sahip peptitler, altın nanoparçacıkların tek bir aşama ile üretiminde ajan olarak ve düzenli hibrit yapıların elde edilmesinde moleküler bağlayıcı olarak kullanılmaktadır. Birden çok yüzeye tanımlı olarak bağlanabilen peptitler oluşturulan hibrit yapıların stabilitelerinin ve fonksiyonlandırılabilirliklerinin artmasında önemli rol oynamaktadır.

Nano boyutlarda yüzeylerin modifiye edilmeleri ve fonksiyonlandırılmaları nano-biyoteknoloji ve nanobilim alanlarında büyük öneme sahiptir. Fonksiyonlandırılmış yüzeyler mikro- ve nano-elektronik, kimya, biyokimya ve biyomedikal endüstrilerinde yeni aletlerin üretiminde kullanılmaktadır.Yüzeylerin fonksiyonlandırılmasında kimyasal ve biyokimyasal moleküller kullanılmaktadır. Tiyol ve silan grupları yüzey fonksiyonladırılması amacıyla en yaygın olarak kullanılan moleküllerdir. Yüzeylerde oluşturulan kimyasal gruplar yüzeylere üzerlerine bağlanan hücre, protein ve DNA gibi moleküllere, sıcaklık, pH ve hidrofobiklik/hidrofiliklik gibi çevresel faktörlere karşı sinyal ve seçicilik özellikleri kazandırırlar. Günümüzde, yüzeylerin modifiye edilmelerinde kendiliğinden monolayer oluşturma (SAM) yöntemi ve litografik metotlar yaygın olarak kullanılmaktadırlar. Ancak bu yöntemler yüksek fiyatları, kompleks yapıları, çevresel faktörlerden etkilenmeleri ve özel ekipmanlar gerektirmeleri gibi çeşitli dezavantajlara sahiptirler. Bunların yanı sıra bu yöntemlerde kullanılan kimyasalların kompleks ve zararlı olmaları biyolojik çaışmalar için uygun değildir ve biyomedikal uygulama alanlarını kısıtlar niteliktedir.

Literatürde yapılan çalışmalarda peptit bağlı nanopartiküllerin yüzeye bağlanmaları kendiliğinden montaj ile olabilmekte, bunun yanı sıra PDMS gibi soft litografi yöntemleri de kullanılarak istenilen array ortamına yerleştirilebilmektedir.

Langmuir Blodgett yöntemini kullanılarak organik bileşikler istenilen kalınlıklarda tek veya çok katmanlı olarak katı yüzeylere kaplanabilir. LB yöntemiyle metal nanoparçacıkların da katı yüzeylere kaplanması ile ilgili birçok çalışma mevcuttur.LB metodunda küvet içerisinde doldurulmuş olan sıvı bir madde, genellikle su, moleküllerin hava/su arafazında monolayer oluşturulması için kullanılır. Bu nedenle, katı yüzeye çekilmesi istenen moleküller uçucu bir çözücü içerisinde çözülürler. Su üzerine damlatılan solusyondan uçucu çözücü buharlaşırken içerisinde bulunan moleküllerinde suyun üst kısmına doğru çıkmalarına yardımcı olur. Su üzerinde oluşan tek molekül katmanının düzenlenmesi bariyerler yardımı ile gerçekleştirilir. Hava/su arafazında oluşturulan tek molekül katmanının katı bir yüzeye alınması, bu katı yüzeyin belli bir sabit hızla suya daldırılıp çıkarılması ile gerçekleştirilir. LB filmin yapısı faklı parametrelere bağlıdır, bunların en önemlileri moleküllerin yapısı, yüzeyin hidrofilik/hidrofobik olması, pH ve sıcaklıktır. LB tekniği, zorlu ortam şartları ve ekstra zararlı kimyasallar gerektirmemesi nediyle biyolojik olarak uyumlu yapıların üretilmesi için oldukça uygundur.

Bu çalışma kapsamında, altın nanoparçacıkların fonksiyonlanmaları için inorganik yüzeylere bağlanabilen multi-fonksiyonel peptitler kullanılmıştır. Bu multi-fonksiyonel peptitler iki adet genetik olarak modifiye edilmiş inorganiğe bağlanabilen peptitlerin (GEPI) esnek bir bağlayıcı ile birleştirilmesinden oluşmaktadır. Peptit sekansı kombinatriyal peptit faj yöntemi veya hücre görüntüleme kütüphaneleri kullanılarak seçilip Fmoc yöntemi ile CSBio 336s peptit sentezleyici cihaz kullanılarak sentezlenmiştir. Bu çalışmada hem altına hem de kuartz'a bağlanma özelliği olan AuBP4-QBP2 peptiti kullanılmıştır. Bu peptidin sekansı RAVRRRSVRREVGGGPWLPPSLPPWPP şeklindedir.

AuBP4-QBP2'nin LB yöntemiyle katı yüzeye kaplanabilmesi için su üzerinde monolayer oluşturması gerekmedir. Bu amaçla su, ethanol ve 1:1 ethanol:su çözeltilerinde çözülmüştür. Ethanol uçucu özelliği nedeniyle peptidin suyun üzerinde durmasını sağlayacaktır. Altın nanopartiküllerin peptit ile fonksiyonlandırılmasını sağlamak için oluşturulan peptit solusyonları ile altın kolloidler farklı oranlarda karıştırılmıştır. Bu karışımların spektroskopik analizleri yapılarak, LB film tekniği ile katıyüzeye kaplanması için en uygun oranları içeren solusyonun tayini yapılmıştır. Bu amaçla UV/Vis spektrofotometre ve FT-IR yöntemleri kullanılmıştır.

LB film tekniği kullanılarak sadece peptitlerin, sadece altın nanopartiküllerin, peptit ve altın nanopartiküllerin sırayla ve peptit fonksiyonlandırılmış altın naopartiküllerin kuartz yüzey üzerine transferleri gerçekleştirilmiştir. Bu esnada elde edilen isotherm eğrileri ve transfer oranları grafikleri katı yüzey üzerinde elde edilen monolayer film hakkında bilgiler vermektedir. Kaplanmış yüzeylerin mikroskobik incelemeleri AFM ve FEG-SEM yöntemleri ile yapılmıştır.

Elde edilen sonuçlar göstermiştir ki, ikili fonksiyona sahip olan peptitler altın nanoparçacıklara bağlanmaktadır ve onları aglomere olmalarına neden olmaktadır. Peptitlerin çözücüleri altın nanoparçacıkların birleşerek çökelmelerinde ve peptitlerin LB film oluşturmasında önemli etkene sahiptir. AFM ve FEG-SEM sonuçlarında, LB film yöntemi ile oluşturulan alt katmanı peptit, üst katmanı altın nanoparçacık olmak üzere oluşturulan iki katmanlı yapıların peptit solusyonundan ve altın nanoparçacıkların boyutlarından etkilendikleri görülmüştür.

1. INTRODUCTION

Surface modification and functionalization in nanoscale is an important phenomenon in nano-bio technology and science. Chemical and biochemical groups can be used as functional groups for this purpose. Such functionalized surfaces are widely used in fabrication of new devices in micro- and nano-electronics(Y. H. Wang, Mirkin, & Park, 2009), chemical, biochemical and biomedical industry(Ratner & Bryant, 2004). These surface chemical groups give specificity to the surface in order to select or signal some specific molecule adhesion such as cells, proteins and DNA depending on environmental conditions such as hydrophobicity/hybrophilicity, pH and temperature. Besides that, it is possible to adapt these surfaces for designing and fabrication of micro and nano scale devices for as electronics, photonics and sensing applications.

Currently, lithographic methods including photo-(Gates et al., 2005), e-beam(Watt, Bettiol, Van Kan, Teo, & Breese, 2005), nano-sphere(Haynes & Van Duyne, 2001), scanning probe (Liu, Xu, & Qian, 2000) and soft-lithography(Xia & Whitesides, 1998) are the most common approaches that are used for fabrication of functionalized surfaces. However they have several drawbacks such as complexity, high cost, requiring well-controlled environment and sophisticated equipment(Gates, et al., 2005). Self-Assembling Monolayer (SAM) method (Harrington, Rodgers, & Rev, 1985)is also widely used in order to produce well-assembled structures. However, SAM has some additional limitations about controllability of system and biologically unfriendly conditions used for modifying the solid surfaces.

Conventionally, functional surfaces can be created utilizing many different chemical molecules. Thiols are the leading molecules that are widely used for surface functionalization. They are used as a molecular linker for immobilizing of molecules to the surface of interest. Thiols have several advantages for designing functional surfaces such as availability, easy synthesis procedures. Carboxyl, amine or a benzene group can be used to functionalize thiol groups utilizing chemical reactions(Galperin & Koonin, 1997; Goren, Galley, & Lennox, 2006; Sainsbury et

al., 2007; Walt, 2002). Besides their advantages, thiols have several disadvantages especially in multi-material systems. They are not good enough to immobilize a desired surface, specifically. Thiol and silane based molecules form SAM on substrate through a covalent bonding(Bain & Whitesides, 1988; Love, Estroff, Kriebel, Nuzzo, & Whitesides, 2005). Although they can form SAMs on various kind of metallic surfaces, this binding process is not specific, therefore this does not give an advantage for multi-material systems as selectivity.

The requirement of new kind of molecular linkers that have specificity and material selectivity properties is gained importance in parallel with developments in the area of nano and micro-technologies. Specific recognition capability of molecules is necessary for designing selectivity systems in micro and nano scale. In other words, molecular linkers that have an ability to distinguish different surfaces are required for systems composed of multi-materials (Sarikaya, Tamerler, Schwartz, & Baneyx, 2004). Although material scientist try to manipulate nano-scale objects on solid surfaces to functionalize them with various methods as mentioned above, they have not achieved the process selectivity of binding specific to different substrates. As pointed out by many leading scientists the engineering ability of nature can be the answer to this problem. Nature has ability to fabricate micro- and nano- scale objects that can recognize certain materials. The main tools that used in nature for this purpose are molecular recognition, self-assembly and precise immobilization(Tamerler & Sarikaya, 2007; Zhang, 2003). These are the molecular machines of nature.

Self-assembly and molecular recognition are the most important fabrication strategies of nature. In self-assembly organization system as its name indicates, molecules assemble spontaneously and form a regular structure without requirement any human intervention(Whitesides & Grzybowski, 2002). In nature, self-assembly organization can be observed in diverse range of systems from bacterial colonies to galaxies. Besides that, most of the biological processes require molecular recognition. Protein folding, nucleic acid hybridization, enzyme-substrate, cofactor or inhibitor interactions, signaling pathways of receptor molecules are examples of all processes that benefit from molecular recognition strategies(Babine & Bender, 1997; Whitesides & Lipomi, 2009; Zhang, 2003). Molecular recognition can be defined as: desired combination of two or more molecules based on complementary

geometrical and physicochemical properties. Molecular recognition occurs in aqueous media, a place that supports weak intermolecular forces like Van der Waals, electrostatic interactions and hydrogen bonds, specific interactions arises between connecting regions of related molecules.

In nature, there are many examples of the self-organization and molecular recognition mechanisms that take place between organic – inorganic interactions and organic – organic interactions. Bone, teeth, shells, sponge spicules, silk, magnetotactic bacteria are some of these examples that have their own molecular control to organize their molecules(Sarikaya, 1999). Proteins and peptides are the most important control elements for growth and shape of these hybrid structures(Sarikaya, Tamerler, Jen, Schulten, & Baneyx, 2003; Tamerler et al., 2010).The proteins can act in two ways to control the growth of hard tissues. One is controlling the growth of mineral crystals by binding them and the second is serving as scaffolds for minerals to deposit (Gungormus et al., 2008; Yuca et al., 2011). The most of the reactions controlled by peptides does not require any harsh conditions such as high temperature and high pressure. In nature, hard tissues and hybrid structures are synthesized at room temperature and natural pH values.

The control mechanism of nature for inorganic tissue synthesis inspired the scientists for a long time. The advent of technology and characterization tools in nano scale in last few decades made a great impact in understanding processes and methods used by the nature. Now, it is possible to analyze natural processes at molecular level. The studies at nano dimensions give a new perspective to the scientist for building structures beginning from single molecule as processed in nature. The combination of biomimetics and nano science opened interesting possibilities for synthesis and control of new structures by using a combination of molecular biology and material science knowledge.

The first idea of synthesizing materials using proteins by mimicking nature was put forward by Sarikaya (Sarikaya & Aksay, 1995). This requires synthesis and selection of biomolecules with desired inorganic binding capability. At the beginning, inorganic binding proteins (IBPs) were obtained by extracting them from biological hard tissues. Nevertheless, this process is limited with materials found in nature and it is also inconvenient and time-consuming. After that, the first cell surface library were generated by Stanley Brown in order to screen the binding affinity of the iron

oxide(S. Brown, 1992). He also identified the first synthetic inorganic binding peptides for gold and chromium(S. Brown, 1997). Sarikaya group has added a new perspective to this area and created new generation of biomimetic linker molecules and using this inorganic binding peptides as template molecules(Sarikaya, et al., 2003). Moreover, they did the first studies for understanding molecular binding mechanisms of genetically engineered peptides to inorganic materials(Braun, Sarikaya, & Schulten, 2002; Donatan, Sarikaya, Tamerler, & Urgen, 2012; Hnilova et al., 2008). The peptides used as molecular linkers are selected utilizing cell surface display and phage display methods(Donatan et al., 2009) via biocombinatorial biology. These peptides with short amino acid sequences (7-14 amino acids) have capability to bind specifically with the inorganic materials through physicochemical non-covalent bonds, called as "Genetically Engineered Inorganic Binding Peptides" (GEPI) (Tamerler & Sarikaya, 2009).

In last decade, a number of different GEPI were synthesis that can bind to diverse range of inorganics: metals as Au, Ag, Pt and Pb, oxides as ZnO, Al_2O_3 and SiO_2, semiconductors as GaN, Cu_2O, TiO_2, ITO and besides that other functional materials such as graphite, calcite, mica and hydroxyapatite. The GEPIs have prodigious potential for various applications in diverse range of areas from biomedicine, nano-biotechnology to nano-electronics and photonics. This broad range of application area is caused from the advantage of GEPIs' material specific properties that is the hallmark of the GEPIs besides other proteins and peptides(Sarikaya, et al., 2003; Tamerler, et al., 2010). In order to use in different applications, many researches to characterize GEPIs and to understand their binding mechanism were carried out (Hnilova, et al., 2008; Seker, Zengin, Tamerler, Sarikaya, & Demir, 2011; Tamerler, Oren, Duman, Venkatasubramanian, & Sarikaya, 2006). GEPIs are already used as molecular linkers to functionalize the inorganic surfaces, to create hybrid structures and also to synthesis nano-particles. Besides that, the studies with GEPIs that has affinity to bind quantum dots open the area of usage in optical visualization systems(Seker, Ozel, & Demir, 2011). Recently, new kinds of GEPIs are designed that has affinity to bind two distinct materials specifically. These bi-functional peptides are used as molecular agents to synthesis of gold nanoparticles in single fabrication method(Hnilova, Khatayevich, et al., 2012) and as molecular linkers to

build hierarchical hybrid structures(Davies & Aggeli, 2011; Hnilova et al., 2011; Hnilova, So, et al., 2012).

Langmuir Blodgett (LB)(Akpo, Weber, & Reiche, 2006; Green et al., 2000; Oliveira Jr, 1992; Rajagopal & Schneider, 2004) is a well-known method to create mono and multilayer organic thin films on solid surfaces. This method can also be used for thin films consisting of inorganic nano-particles(Siffalovic et al., 2012). In this method, a liquid substance, mostly water, is used to form a monolayer at air/water interface. In this regard, molecules that are desired to be transferred onto a solid surface is mixed with a volatile solvent. By the evaporation of this solvent, molecules move up to the air/water interface and produce a monolayer of molecules or nanoparticles. It is possible to control the formation of this layer with barriers. While compressing the barriers, a condensed monolayer formation of the molecules can be obtained on water surface. This monolayer is transferred to the substrate as a thin film. The transfer method is based on the lowering or rising of the substrate, which shows strong dependence on the hydrophobicity/hydrophilicity of the substrate. Additionally, monolayered molecules can also be transferred to solid surfaces vertically using Langmuir-Scheaffer (LS) technique(Roberts, 1990). The structure of the LB films depends on parameters such as molecular structure, hydrophobicity/hydrophilicity, pH and temperature.LB technique is a very suitable method to create biologically friendly structures since for their production harsh-conditions and additional chemicals are not required.

In this thesis, we aimed to demonstrate a bio-enabled fabrication method based on Langmuir Blodgett (LB) technique for creating gold nanoparticle patterns on solid surfaces. Schematics of the aimed structure are presented in the Figure 1.1 and Figure 1.2.Assembly of gold nanoparticles onto a solid support as a film is required for several applications such as electrochemical sensor chips and bio-electronic devices. For this purpose, the bi-functional GEPIs are used as molecular linker between gold nanoparticles and solid surface. The bi-functional peptides named as AuBP4-QBP2 possess the ability to bind gold and silicate materials selectively. The sequence of the AuBP4-QBP2 is RAVRRRSVRREVGGGPWLPPSLPPWPP. The hydrophobicity of this peptide is suitable to form a monolayer on the water surface.

The desired LB thin film composed of bi-functional peptide and gold nanoparticles is produced by transferring the bi-functional peptide on to silica-based substrate with

LB technique and Au nanoparticle are attached to the peptide by incubating in Au nanoparticle containing solutions.

For optimization of LB film transfer conditions of peptides on glass surfaces different solvents are used and the effect of the solvents on LB film structure were compared via surface pressure/area isotherms.

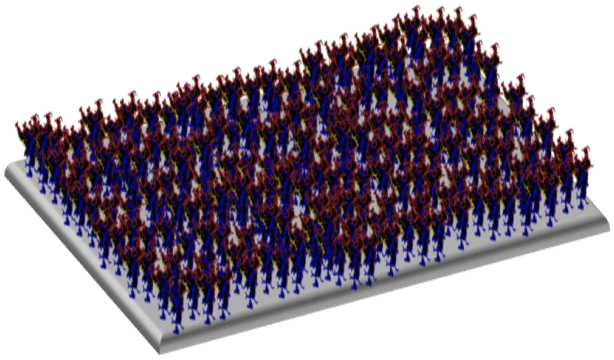

Figure 1.1: Schematic view of AuBP4-QBP2 monolayer LB film on solid surface.

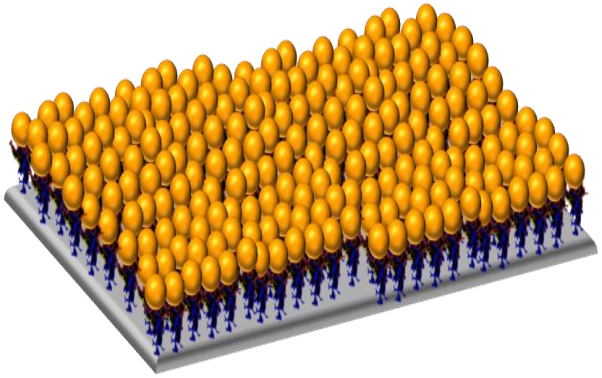

Figure 1.2: Schematic view of two layer LB film constructed AuBP4-QBP2 and gold nanoparticles.

The isotherms also generated for gold nanoparticles and peptide functionalized gold nanoparticles. Additionally, optical and structural properties of the peptide functionalized gold nanoparticles were investigated using UV/Vis and FT-IR spectrophotometer. The structure of the LB films was characterized by UV/Vis spectroscopy, FTIR, FEG-SEM and AFM.

2. BACKGROUND INFORMATION

2.1 Biomimetic and Bio-inspiration

The most beautiful examples of harmony of organic and inorganic structure can be seen in nature. Their mechanical, physical and chemical properties deeply inspired the scientists. Over the last 10 – 15 years, nanotechnology accelerated the studies on mimicking the nature because tools for the investigation of their structure and production techniques became available. This new research area is named as biomimetics and can be defined as "the design of new biological devices with the hierarchical combination of organic and inorganic materials". The aim of this area is to develop new approaches and technologies using the features of biological organisms in nature and their interactions with inorganic materials in nano scale (Figure 2.1). These techniques can be used for cancer diagnostic and treatment(Haley & Frenkel, 2008), disease treatment (Azarmi, Roa, & Löbenberg, 2008), imaging techniques (Z. L. Wang et al., 2011), and also detection of single cells.

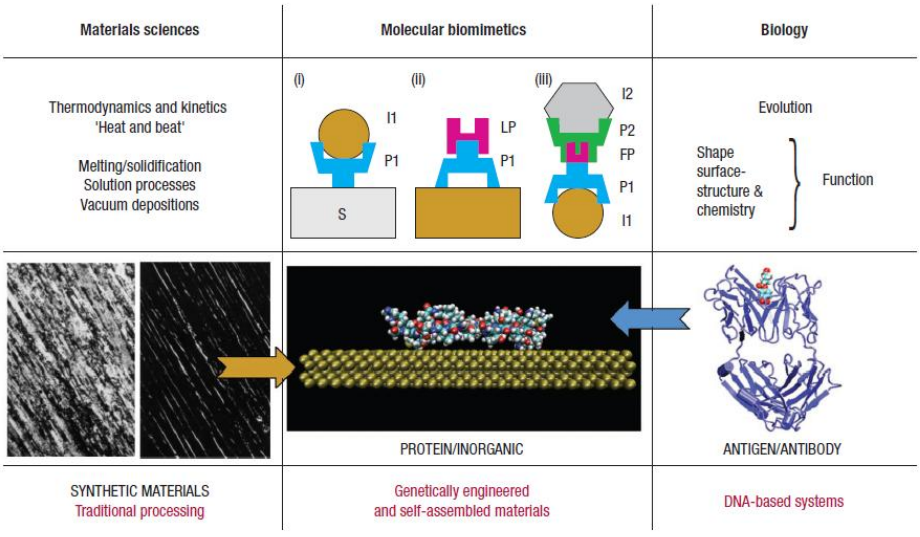

Figure 2.1: Molecular Biomimetic (Sarikaya, et al., 2003)

The nanostructures in the biological organisms give novel ideas to scientists and engineers for designing new products with better functionality, efficiency and

flexibility. The energy conversion efficiency of routinely used machines are low they caused to pollution, and having a limited lifetime. On the other hand, in nature, biological structures in our body or in other organisms are perfectly nonstop working, without any detrimental effect on nature. Therefore understanding the properties and production methods of these biological structures are of prime importance for new engineering applications.

Nanobiotechnological studies are interdisciplinary and requires collaboration among researchers from; material science, biology, physics, chemistry and engineering. The rapid increase in knowledge of function of the biological and organic materials opens up new horizons to researchers. Improvements on the microscopic and characterization techniques of biological materials at the atomic level give rise to the increasing knowledge of biological structures, manufacturing methods of nanobiological devices. Furthermore, the results achieved through nanobiotechnology can extend to practical applications of this scientific area in different industry sectors. The health industry is one of the main benefitor of these results. Industries such as food, electronic, mechanical and chemical are the other leading industries that can benefit from nanobiotechnological research results.

2.1.1 Self-assembly systems

Genetic materials (DNA and RNA), peptides and proteins are in nano dimensions. A different scientific and technological approach is required for understanding their function and processing routes due to their unusual physical, chemical and mechanical properties. They are living structures with a capacity of self – assembling.

The structure of the DNA is appropriate for sequential design, which makes it a very suitable material for the nanobioengineering applications such as nano mechanical devices.

One of the most important components of the biological organisms is proteins, which are the main structural constituent of all types of living organisms. They have several functions in the organism such as transportation, modification, structure and catalyzing chemical reactions. Proteins can be combined with nanoparticles efficiently for nano-devices applications (Astier, Bayley, & Howorka, 2005; Moll et al., 2002; Ringler & Schulz, 2003). With their self – assembly, repair and also

replication ability they are expected to be one of the structural elements of the man-made structures.

Lipids are the self – assembled structures in the living organisms. They are mostly found in the membrane of the cells and they have both hydrophobic and hydrophilic parts. Similar to DNA and protein, biological membranes are also excellent examples for structuring and organization in the molecular level. The self – association properties of biological macromolecules can be used to design biomimetic membranes, which can be constituted in vitro. Biomimetic membranes have various application areas in nanobiotechnology. For instance; encapsulation and controlled release systems for drug delivery, gene therapy and composite structures. These are also used in detection applications, nanobiosensors, chip technology and nano carriers (Boisseau, Houdy, & Lahmani, 2009).

2.1.2 Hierarchical self-assembly processes in nature

In nature, organic and inorganic compounds are in perfect compatibility. The rigidity, durability, toughness, conductance and stability properties of the inorganic materials give specific qualities and concessions to the organic materials. There are various examples of these in nature, especially in the organisms, which live in the extreme environments (Tamerler & Sarikaya, 2007)(Figure 2.2).

One of the examples of organisms which are combination of inorganic and inorganic materials is magnetotactic bacteria (*Aquaspirillum magnetotacticum*) (Figure 2.2-A) (Tamerler & Sarikaya, 2007, 2009). These bacteria include ordered single crystallized octahedral-shaped magnetite (Fe_3O_4) particles which helps them to align themselves according to the earth's magnetic field. This property of these bacteria can be adapted to medicine to engineering and even mining. Another example is Rosella (*Rosella recavitzea*) which is a kind of sponge, which has mutualism living style with algae. It has star shape cells at the tip that has perfect silica structure to collect the light. Then the light is transferred through the silica based biological optical fibers to the interior of the sponge (Figure 2.2-B)(Tamerler & Sarikaya, 2007, 2009). Furthermore, mother of pearl, the interior part of is shell is called nacre, is a segmented laminated composite of calcium carbonate. This perfect ordered „brick and mortar' structure gives highest toughness and fracture strength of all known ceramic based structures (Figure 2.2-C) (Tamerler & Sarikaya, 2007, 2009).

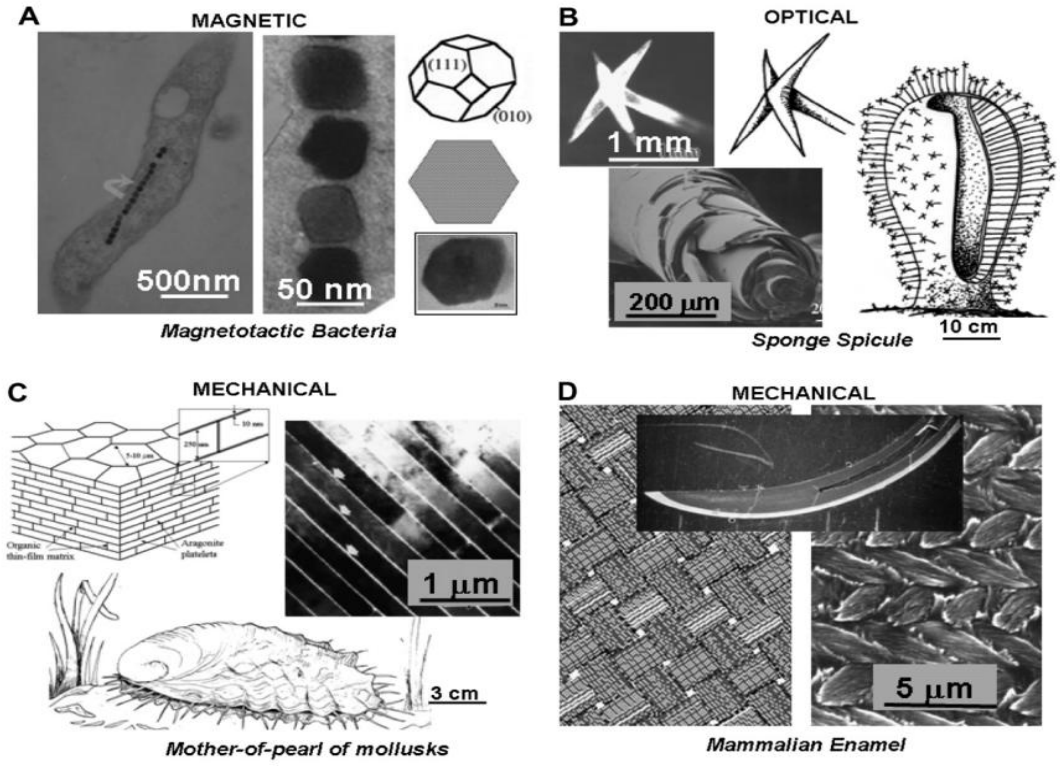

Figure 2.2: Examples of organisms living in extreme environments A) Magnetotactic Bacteria B) Sponge Spicule C) Mother of pearl of mollusks D) Mammalian (Tamerler & Sarikaya, 2007)

As a last example, tooth is one of the hardest materials in the mammalian; this hardest property is given by hydroxyapatite material in the enamel of the mammalian tooth. This hierarchically ordered structure of the tooth absorbs the energy in the chewing and cutting processes in the mouth, hence the other chin structures can be protect from mechanical stress (Figure 2.2-D) (Sarikaya, et al., 2003; Sarikaya, et al., 2004; Tamerler & Sarikaya, 2007).

2.2 Genetically Engineered Peptides for Inorganics

Although, proteins have a significant role for molecular biomimetic application with their central role in assembly of biological materials, their synthetic production is very hard because of their complex structure and conformation. To design a protein based hybrid structure, peptides that can specifically bind to solids, must be selected from the nature. This selection can be in two ways. One of them is a complex and time-consuming process including isolation, purification, and sequence and structure analysis of proteins (Arakaki, Webb, & Matsunaga, 2003; Mock et al., 2008; Shimizu, Cha, Stucky, & Morse, 1998). The other one is based on existing proteins

that are known to bind inorganic surfaces (Martin Jézéquel, Hildebrand, & Brzezinski, 2000) (which are selected from organisms which already seen in nature (Figure 2.2)). Peptides have shorter amino acid sequences, are the alternative to design protein – inorganic hybrid structure. Their designs and synthesis is easier than the whole protein. By using genetic engineering techniques such as molecular design of recombinant proteins and site directed mutagenesis, surface specific peptides can be synthesized.

2.2.1 Selection and characterization of GEPIs

The peptides that have the capability to bind solid surfaces that are selected through affinity-based biopanning protocols; phage display (Kehoe & Kay, 2005) and cell-surface display (S. Brown, 1992) techniques. A schematic picture shows the principle of these techniques are represented in Figure 2.3. The basic principle of these techniques relies on the interaction of the peptides from peptide libraries with the specific substrate (binding stage). With different washing steps, weak and non-binding peptides are removed from the surface. Washing step are repeated to isolate for the specifically high binding peptides to the interested surface.

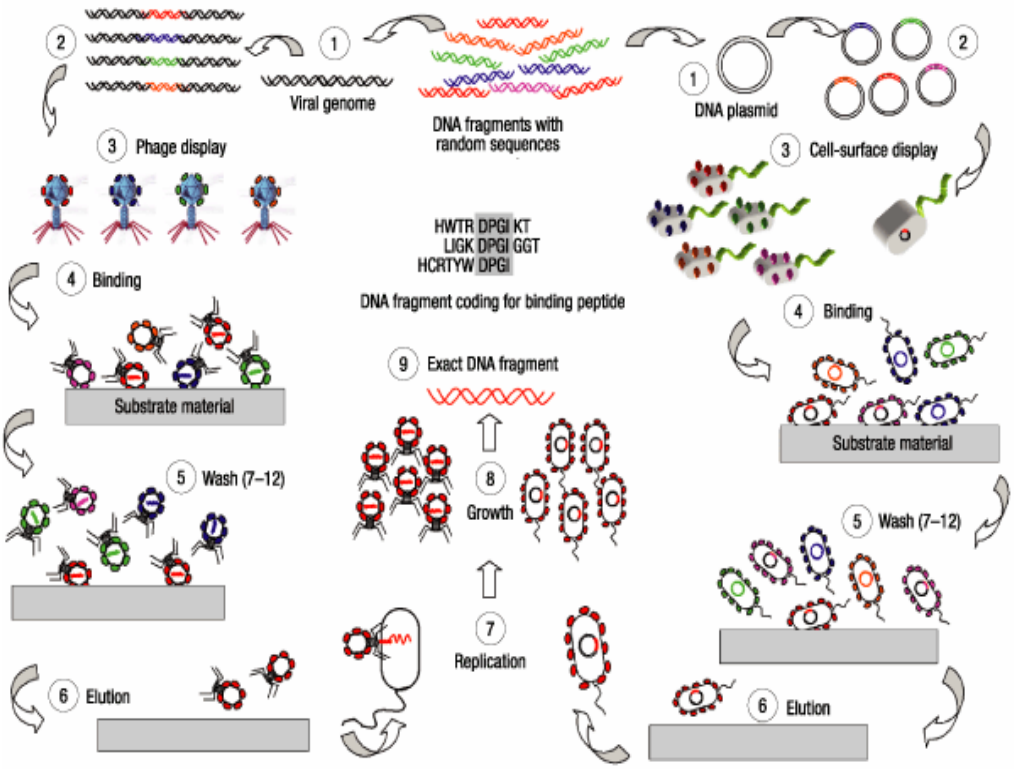

Figure 2.3: General protocols for selection of the GEPIs using phage display and cell surface display technologies (Sarikaya, et al., 2003)

Specific binding peptides have been selected wide range of variety of metals and materials including gold (Au) (S. Brown, 1997; Slocik, Stone, & Naik, 2005), platinum (Pt) (Sarikaya, et al., 2004) and lead (Pd), silver (Ag) (Naik et al., 2004), iron (Fe) and titanium (Ti) (Donatan, et al., 2012), oxides such as Al_2O_3, SiO_2(Kacar et al., 2009; Oren et al., 2010), ZnO (Thai et al., 2004) and semiconductors such as Cu_2O(Thai, et al., 2004), GaAs (Whaley, English, Hu, Barbara, & Belcher, 2000), TiO_2(Chen, Su, Neoh, & Choe, 2006; Dickerson et al., 2008) CdS and ZnS (S. W. Lee, Mao, Flynn, & Belcher, 2002)and minerals; such as mica (Donatan, et al., 2009), hydroxyapatite (Gungormus, et al., 2008), calcite (DeOliveira & Laursen, 1997) and sapphire (Krauland, Peelle, Wittrup, & Belcher, 2007), and biocompatible substrates such as silica, titania and alumina by utilizing the above stated techniques. Although there has been considerable effort in the fields physics, chemistry and material science to understand how proteins bind to solid surface with high affinity, it is not clearly answered yet. There are strong indications showing that the recognition mechanism of the peptide to the solid surface relies on both chemical and physical properties of the substrate (H. Lee, Rho, & Messersmith, 2009). Hydrogen bindings, polarity of the surface or peptide, charge effects are the several of the chemical effects and conformation, size and morphology of the peptide and surface are the several of physical effects that play significant roles on recognition mechanisms.

2.2.2 Current applications of GEPIs

The GEPIs have prodigious potential for various applications in diverse range of areas from biomedicine, nano-biotechnology to nano-electronics and photonics. GEPIs differentiate from other proteins with their specific recognition property (Sarikaya, et al., 2003; Tamerler, et al., 2010) that is the main reason for the popularity in broad range of application areas.

Specific inorganic substrate recognizing ability of GEPIs makes them a very suitable linker, assembler and bridger in self and directed assembly of nano-dimensional objects and molecules. Therefore, GEPIs can be used for functionalization of surfaces. For this purpose, a number of studies were carried out to understand the binding mechanisms of GEPIs (Hnilova, et al., 2008; Seker, Ozel, et al., 2011; Tamerler, Oren, et al., 2006). Up to now specific binders for wide range and variety

of materials has been selected and genetically engineered for improving their specific binding properties (Oren et al., 2007; Zin et al., 2009).

GEPIs are also used in the process of targeted assembly of the nanostructures on patterned complex solid surfaces. In this case, the surface or nanoparticles is functionalized with GEPIs and then nanoparticles are attached to the surface as SAMs benefiting from the specific binding property of the peptide to the nanoparticle material such as quantum dots (Seker, Ozel, et al., 2011) and gold nanoparticles (Tamerler et al., 2006).

Currently, new kinds of GEPIs are designed that has affinity to bind two or more distinct materials specifically. These multi-functional peptides are used as molecular agents to synthesis of gold nanoparticles in single fabrication method (Hnilova, Khatayevich, et al., 2012) and as molecular linkers to build hierarchical hybrid structures (Davies & Aggeli, 2011; Hnilova, et al., 2011; Hnilova, So, et al., 2012).

2.2.3 Multi-functional GEPIs

Multi-functional peptides have the ability to bind to more than one inorganic substrate. They are synthesized with the same method as for GEPIs. They are combination of two GEPIs with a flexible linker mostly including GGG sequence, due to glycine is the smallest amino acid. Multi-functional peptides can be designed in order to bridge desired materials.

There are experimental and modeling researches about designing multi-functional peptides including silicate and gold binding sequences with GGG linker (Hnilova, Khatayevich, et al., 2012)(Figure 2.4) and gold binding peptide and maltose-binding protein with SGGG or PGPGPG linkers (Hnilova, et al., 2011). These sequences can be combined in two different combinations to form bi-functional peptide: QBP – AuBP or AuBP – QBP. The structure of the peptide is changed by sequence of it due to electrostatic forces and interactions. On this regard, binding performance of QBP – AuBP sequence is significantly better than the reverse combination (Hnilova, Khatayevich, et al., 2012). The sequence of the AuBP-QBP is WAGAKRLVLRREGGGPPPWLPYMPPWS and QBP-AuBP is PPPWLPYMPPWSGGGWAGAKRLVLRRE, their structures are given in Figure 2.4.

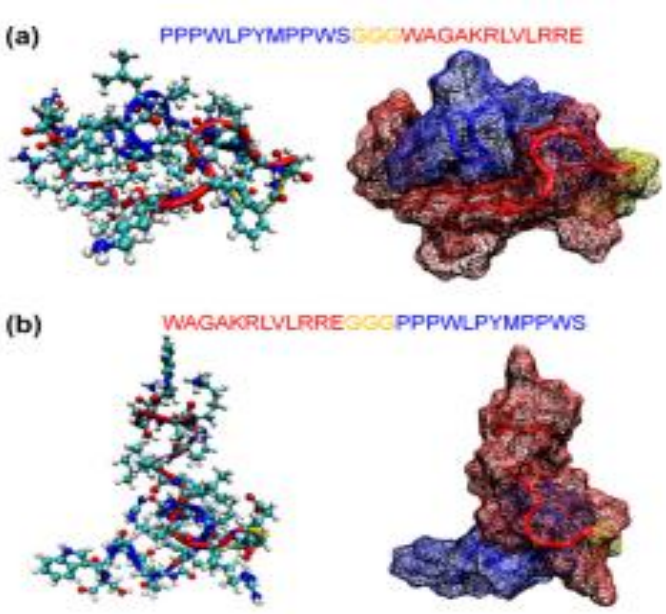

Figure 2.4: Predicted structures of multi-functional peptide combinations. Molecular conformations of a) QBP-AuBP and b) AuBP-QBP (Hnilova, Khatayevich, et al., 2012).

2.3 Hierarchical Fabrication of Multi-functional GEPIs and Nanoparticles at Solid Surfaces

A number of methods are used to fabricate organic-inorganic nanostructures on solid surfaces. Although these methods are, quite various they can be categorized in two subtitle as top-down and bottom up nanofabrication. Lithographic techniques including optical, e-beam, nano-imprint, soft-, scanning probe and block copolymer are the mostly used top-down approaches that enable multi-directional patterning.

Besides that, bottom-up approaches such as atomic layer deposition, sol-gel nanofabrication and molecular self-assembly, vapor-phase deposition are the most common methods to produce such hybrid structures. The combination of lithographic techniques and molecular self-assembly is also quite effective method to produce organic-inorganic hybrid films. However, they have several drawbacks such as complexity, high cost, requirement well-controlled environment and sophisticated equipment. In this regard, scientists are looking for and trying to develop new simpler techniques in accordance to the requirements of the desired hybrid structures.

Langmuir-Blodgett film coating technique is another method that is used for producing both organic and/or inorganic structures. This technique is a quite controllable method to produce monolayer/multilayer thin films of the molecules.

The first condition to produce a well structure thin film is to obtain a monolayer at air/water surface. It is relatively easier to achieve this condition for organic molecules due to their amphiphilic features. Additional functional groups must be added to inorganic nanoparticles in order to obtain Langmuir Blodgett films of them. These functionalizations are mostly done chemically, but these processes are not biologically friendly and not appropriate for biomedical applications. Design of bio-enabled inorganic thin films is quite important for applications in biomedical area. A novel method that using GEPIs to functionalize gold nanoparticles in order to produce hybrid Langmuir Blodgett films is demonstrated in this study.

2.3.1 Langmuir Blodgett film technique

Water surfaces, water-air interfaces and monolayer films are started studying from 1770s. The idea of monolayer films on water surface was began with Benjamin Franklin who realized that an oil drop wave from a teaspoon spread in a large area, however he did not realize that this oil film was monolayer. Over a century later, in 1879, Rayleigh was interested in water surfaces and he quantified the observations of Franklin. He calculated the thickness of the oil film on water was about 1.6 nm by taking into account of volume and covered area of oil droplet. First experiment in this field had been performed by Agnes Pockels in her kitchen sink in 1882. She showed that the monolayer films on water-air interface can be controlled with barriers. Pockels send a letter to Franklin about her studies, which are directly related with Franklin's. Franklin kindly send her results to Nature to be published in 1891 on behalf of Pockels (Pockels, 1891). Langmuir is known as the father of this field due to his progressive contributions to surface science. Langmuir and his assistant student Katharine Blodgett was awarded by Nobel Prize in 1932.

LB method is used in the construction of biosensors, of p-n junctions, non-linear optical devices, optical fiber coatings, energy transfer systems, for the production of electronic devices (Hubert Motschmann & Möhwald, 2001; Paul et al., 2003) and in polymer industry. Especially, the Langmuir Blodgett film techniques are still keeping the importance for molecular electronic systems because of active role of organic materials in processing of information, transformation and also storage (Hubert Motschmann & Möhwald, 2001).

2.3.1.1 Self-assembled monolayers at air-water interfaces

Coating of organic materials on to solid surfaces with controlled thicknesses as monolayer / multilayer is possible with Langmuir Blodgett (LB) method (Akpo, et al., 2006; Oliveira Jr, 1992; Rajagopal & Schneider, 2004).

A plot of surface pressure as a function of the area of water surface is the single and the most important indicator of the monolayer formation of molecules. This are mostly known as a surface pressure/area isotherm, and often abbreviated to "isotherm". This plot is obtained at constant temperature and can be measured on a point-to-point basis. Most common approach is to obtain the pseudo-equilibrium conditions while compressing the film at a constant rate and continuously monitoring the surface pressure. Monolayer formation on water surface is described schematically in Figure 2.5 and Figure 2.6.

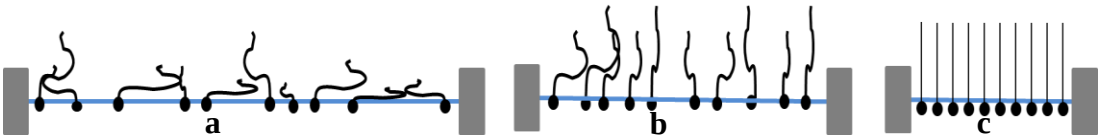

Figure 2.5: Monolayer of molecules on water surfaces: a) expanded b) partly compressed c) close packed.

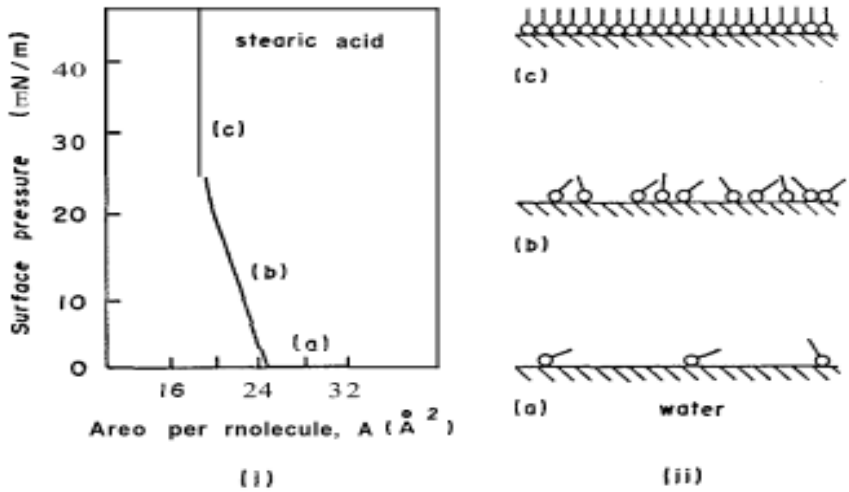

Figure 2.6: Surface pressure /area isotherm (left) and orientation of the surfactants in different phases(Oliveira Jr, 1992).

Reproducibility of the isotherms depends on the materials being investigated; it can be controlled by achieving repeated compression, and expansions of the barriers while recording surface pressure.

The isotherms exhibit several “distinct” regions that are characteristic for the molecules. Generally, four distinct regions are apparent on examining the isotherms. In the first region, surface pressure stays constant with decreasing molecular area (Figure 2.6). This suggests that nanoparticle-molecule domains are widely dispersed on the sub-phase in a gas-like manner. In the second region, a nonlinear increase is seen in the surface pressure, which means that the domains begin to interact with each other. In the third region is a steep linear increase in surface pressure associated with the formation of a close-packed film. Finally, in the fourth region, the rate of change of surface pressure suddenly decreases which indicate the collapse of the monolayer.

In this method, cleanness of the slides is very important, thus in general, the studies are carried out in a clean room. The quality of LB film depends on the pH, temperature, ionic content and speed of immersion, hydrophilic and hydrophobic properties of the substrate.

2.3.1.2 Transfer of monolayers to solid surfaces

The films formed by LB method are named as Y – type, X – type and Z – type according to the symmetry of the layers (Oliveira Jr, 1992) (**Figure 2.7**). The majority of the materials which can form as a film have amphipathic or amphiphilic nature (Akpo, et al., 2006). The film occurs as a stable monolayer at the top of the surface because both hydrophilic and hydrophobic groups co-exist on liquid surface. (Rajagopal & Schneider, 2004).

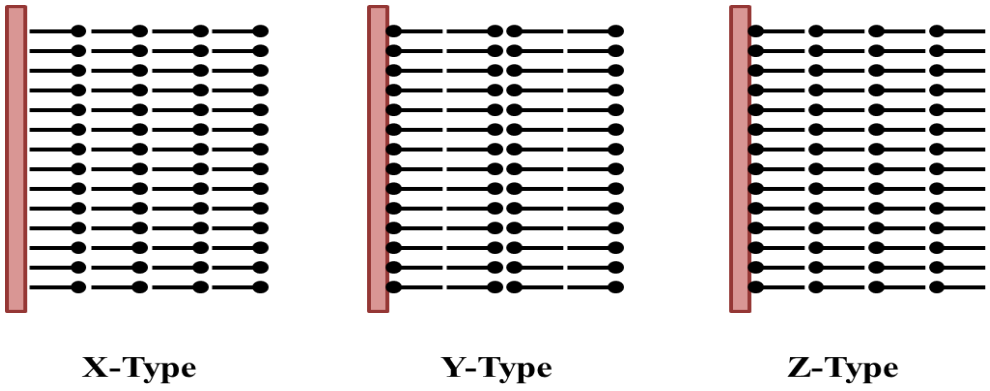

Figure 2.7: Different deposition types of LB films.

Transfer ratio value is a good indicator to control the monolayer deposited on a substrate. It is calculated by dividing the area of the barriers that have moved inwards (to maintain the surface pressure) with the area of the substrate.

$$Transfer\ ratio = \frac{Trough\ area\ reduced\ by\ bariers}{Substrate\ area\ deposited} \quad \textbf{(2.1)}$$

If hydrophilic substrates are used, the substrate must be lowered into the water before the molecules are spread on the surface. Thereby, prior attachment of some of the molecules during the lowering of the substrate can be prevented.

The deposition of the first layer is relatively easy; placing second and other layers on top of it is more difficult. The substrate must be allowed to properly dry before the next dipping. Dipping speed, surface pressure, temperature, pH, substrate properties (hydrophilic/hydrophobic), drying time and salt in subphase are important variables that must be adjusted to improve the results.

2.3.1.3 Surface pressure measurements

The examination of parameters such as surface pressure, surface potential is important for characterization of films, which are produced by LB applications. The surface pressure is defined as the reduction of surface tension of liquid in the presence of monolayer of organic matter. The graphics of pressure vs area are the most valuable data that helps us to investigate the formation of the monolayer and allows us to make qualitative analysis. Surface pressure can be determined by several methods such as Langmuir balance and Wilhelmy plates(Roberts, 1990).

In the Wilhelmy method; surface tension force of a reference plate, which is partially immersed in the subphase, is determined and compared to the similar absolute measurement on a clean surface. The sensitivity of this technique is about $5x10^{-3}$ mNm^{-1}(Munger & Leblanc, 1980). The calculations are made according to the dimensions and angle of immersed part of the Wilhelmy plate which is shown in the Figure 2.8.

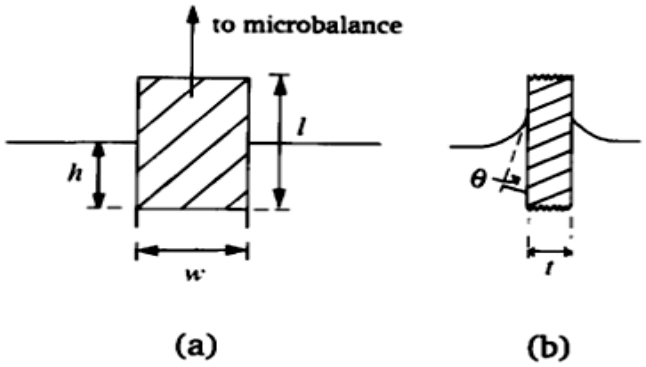

Figure 2.8: Wilhelmy Plate: (a) Front view (b) side view (Petty, 1996).

Net downward force F can be determined as;

$$F = \rho_p glwt + 2\gamma\ t + w\ cos\theta - \rho_L gtwh \tag{2.2}$$

where ρ is density of material, ρL is density of liquid, γ is the surface tension of the liquid, θ is the contact angle on the solid plate and g is the gravitational constant. In practical procedure, the Wilhelmy plate is wetted completely by the liquid and change in "F" for a stationary plate or change in "h" for a constant applied force is measured. Therefore, the change in surface tension is given by;

$$\Delta\gamma = \frac{\Delta F}{2\ t + w} \tag{2.3}$$

If we assume that the plate is thin enough (t << w);

$$\Delta\gamma = \frac{\Delta F}{2w} \tag{2.4}$$

The surface pressure (Π) can be determined as reduction of the pure liquid surface tension by the film and thus it can be formulated as;

$$\Pi = -\Delta\gamma \tag{2.5}$$

Surface potential techniques are the other methods that are used for analyzing of monolayer formed by LB method. In this method, the potential difference between the clean liquid surface and a monolayer-coated surface is measured. The measurements such as surface pressure and surface potential can provide information about the behavior of the self-assembled molecules.

2.3.1.4 LB films of gold nanoparticles

Langmuir Blodgett films of gold nanoparticles have been achieved by functionalizing of them with different chemical agents. Gold particles have a high tendency for interacting with thiol groups, that's why, in most of the studies, gold nanoparticles are functionalized with thiol groups using different chemicals such as dodecanethiol (Bourgoin, Kergueris, Lefèvre, & Palacin, 1998), 1,4-benzenedimethanethiol (Sbrana, Parodi, Ricci, & Di Zitti, 2002), octadecylamine (Zhou, Liu, Zhang, Jiang, & Li, 2005) in order to obtain well-established Langmuir-Blodgett film formation.

For the characterization of Langmuir Blodgett films of thiol capped gold nanoparticles, TEM, STM has been used. Strong covalent binding of thiol groups allow to obtain high resolution 2-D images of gold structures even after a long time (Sbrana, et al., 2002). In practice, it is hard to obtain gold monolayer film images by the scanning probe techniques, since the tip of cantilever in contact mode destroys the film. A way of overcoming this problem is to use non-contact modes or stabilizing of the film by treating with 2,5′′'-bis(acetylthio)-5,2′,5′,2′′ -terthienyl (T3) (Bourgoin, et al., 1998).

2.3.1.5 LB films of peptides

Langmuir Blodgett is a quite efficient technique to perform thin films of organic molecules. A number of studies were conducted on organic molecules that can form a monolayer at air/water interface. The structure of the thin films depends on the construction of the organic molecules. Amphiphilic, hydrophobic, hydrophilic or aromatic molecules can produce X, Y or Z type thin films (Figure 2.7).

LB films of biological molecules including lipids, peptides, proteins, and DNA were produced for different purposes. Two types of lipid molecules are present, cholesterol and phospholids, and both of them are amphiphilic. Therefore, LB film technique is quite appropriate for performing lipid bi-layer structure by mimicking the nature in order to understand dynamics of the cell membrane and the interaction of the membrane with proteins and other molecules. Fluorescently labeled phospholipid(Teissie & Tsong, 1981; Thompson, Brian, & McConnell, 1984)can be used to investigate the degree of penetration of a protein such as cytochrome c (K. S. Lee, Won, Noh, & Shim, 2010)into a lipid monolayer which is of great help in

understanding electron transfer processes such as respiration and photosynthesis. Moreover, LB film technique is one of the major methods used in photosynthesis research(Kim, Lee, Lee, & Park, 2012). There are numerous studies of chlorophyll(Jones, Tredgold, & O'Mullane, 1980; Silvia & Porter, 1974), porphyrins (Jones, Tredgold, & Hodge, 1983) and electron donor-acceptor systems(Flanagan, 1983) on mimicking membranes produced by LB films. In this regard, physical properties of the biological molecules that can be used in LB studies(Tredgold, 1977) are available in the literature.

Biosensor applications (Ramanathan, Ram, Malhotra, & Murthy, 1995) of LB films provides a widespread interest area since many scientists believe that the incorporation of biological molecules such as proteins, enzymes (Sriyudthsak, Yamagishi, & Moriizumi, 1988) and DNA (Karymov et al., 1992; Sukhorukov, Montrel, Petrov, Shabarchina, & Sukhorukov, 1996) into LB films will lead to novel integrated solid state devices. The formation of thin films with LB technique provides several advantages such as ability to produce the films in controllable thicknesses, ease of functionalization for signaling and detection, and most importantly possibility to form hybrid structures consisting of monolayers. For this purpose, LB films of biological molecules such as peptides (Rapaport, 2006) which have possibility to use in a sensor, detection or biomedical device was investigated in detail both experimentally and modeling. Especially for experimental and modeling comparisons, peptides are used since proteins, enzymes and DNA are more complex for analysis. Although the characterization of LB films of peptide with AFM is relatively hard and requires special tips, they are very helpful for understanding the assembly and comparison with modeling results (Powers, Yang, Lieber, & Kelly, 2002). The pattern of the peptides on solid surface depends on both the structure of the peptide; single stranded antiparallel β-sheets (Whitehouse et al., 2005), single stranded parallel β-sheets (Sneer, Weygand, Kjaer, Tirrell, & Rapaport, 2004), double stranded β-sheets (Powers, et al., 2002), multi-stranded β-sheets (C. L. Brown, Aksay, Saville, & Hecht, 2002), ordered amphiphilic helicals (Niwa, Yokoi, Kinoshita, & Zhang, 2005), and the length of the peptides (Rapaport, 2006) (Figure 2.9).

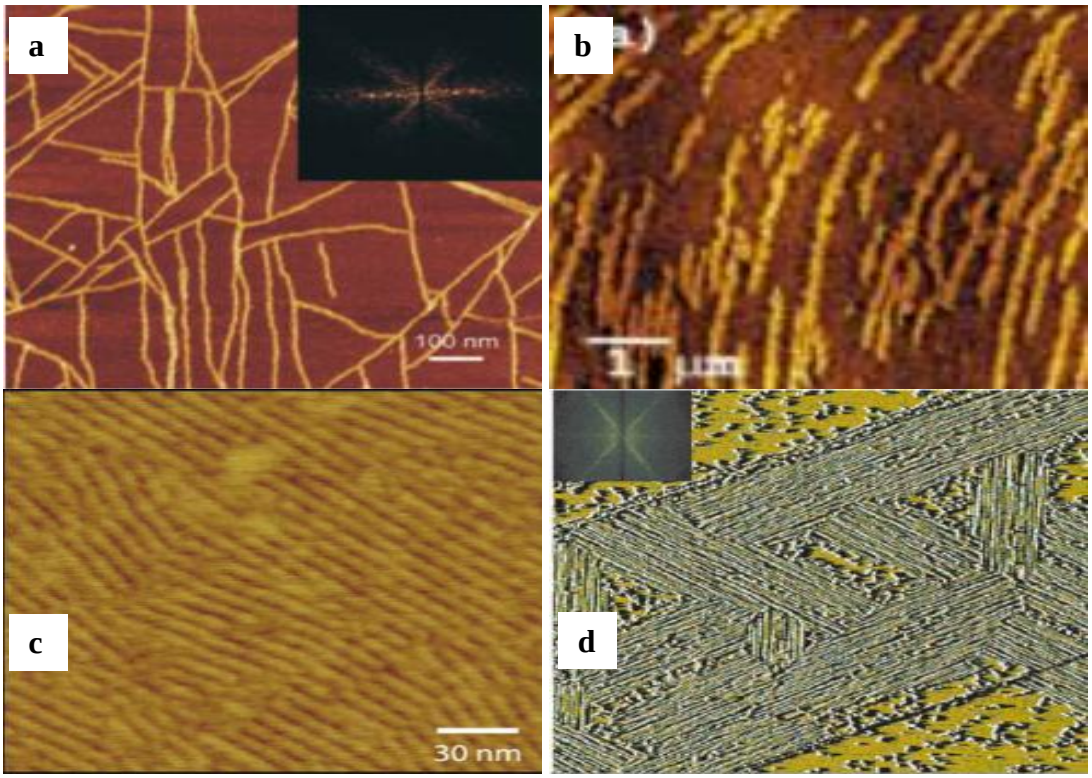

Figure 2.9: a) AFM image of LB film of single stranded antiparallel peptide β-sheets on mica substrate (Peptide sequence: CH_3CO–Gln–Gln–Arg–Phe–Gln–Trp–Gln–Phe–Glu–Gln–Gln–$CONH_2$ (P11-2)) b) AFM image of LB film of single stranded parallel β-sheets on mica surface (Peptide sequence: CH_3CO–Pro–Cys–Phe–Ser–Phe–Glu–Phe–Lys–Pro–NH_2). c) AFM image of LB film of double stranded β-sheets on mica substrate (Peptide sequence: NH_2-Glu-Nle-Glu-Nle-Glu-Nle-Glu-Val-Gly—Dpro-Val-Glu-Nle-Glu-Nle-Glu-Cys(DMBDY)-Glu-H) d) AFM image of LB film of multi-stranded β-sheets on highly ordered pyrolytic graphite (HOPG) (Peptide sequence:MDYEIKFHGDGDNFDLNLDDSGGDLQLQIRGPGGRVQVHIHSSSG KVDFHVHNDGGDVEVKMH). This figure is adapted from references a) (Whitehouse, et al., 2005) b) (Sneer, et al., 2004) c) (Powers, et al., 2002) d) (C. L. Brown, et al., 2002).

2.3.2 Characterization techniques of LB films

The thin films obtained by LB technique can be characterized by several methods. The thickness of the LB film can be measured with methods such as ellipsometer, SPR (surface plasmon resonance) (Seker, Zengin, et al., 2011), XRD (X-ray diffraction), neutron diffraction and reflection and electron diffraction. The Raman scattering, FTIR (Fourier Transform Infrared), SEM (Scanning Electron Microscope), TEM (Transmission Electron Microscope), AFM (Atomic Force Microscope) and optical microscope can be used to view the layout of the molecules on the surface of the film and investigate the structure of them (Oliveira Jr, 1992). A table (Table 2.1) containing overview of the more relevant techniques for characterizing Langmuir Blodgett Film structure and organization is given

below(Girard-Egrot, Godoy, & Blum, 2005). In addition to these, a number of variable analyzing methods depending on the film's features can be used for characterization of them.

Table 2.1: Overview of the more relevant techniques for characterizing Langmuir–Blodgett film structure and organization (Girard-Egrot, et al., 2005).

Techniques	Information deduced
Ellipsometry	Thickness determination (accuracy of 2Å) and refractive index
Neutron diffraction and reflection	Structural information, overall thickness of LB film, average thickness of a molecular layer
X-ray diffraction	Multilayer thickness, monolayer thicknesses, interplanar periodicity, electron profile density normal to the substrate plane, crystalline structure of LB films (positional order of hydrocarbon chain).
Grazing incidence – X-ray diffraction (GIXD)	In-plane lattice structure of molecular assembly and hydrocarbon chain tilt
Electron diffraction • Transmission electron diffraction (TED) • Reflection high energy electron diffraction (RHEED) • Low energy electron diffraction (LEED)	• In-plane intermolecular spacing, crystalline structure and defects. • In-plane structure information, tilt angle and tilt direction of the aliphatic chain. • Inter-chain spacing measurement (intermolecular distances).
Infrared spectroscopy • Polarized IR spectroscopy, infrared reflection adsorption spectroscopy (IRRAS), attenuated total reflection–Fourier transform infrared (ATR– FTIR)	• Hydrocarbon chain conformation, degree of ionization of the head groups, H-bonding, chemical and structural changes. • Hydrocarbon chain packing, hydrocarbon chain tilt angle measurement, molecular orientation.
Surface-enhanced Raman spectroscopy (SERS)	Conformation of alkyl chains and head groups, biomolecule interactions with LB films.
X-ray photoelectron spectroscopy (XPS) or electron spectroscopy for chemical analysis (ESCA)	Quantitative surface chemical composition analysis.
Optical microscopies • Nomarski differential interface contrast microscopy • Fluorescence near-field scanning optical microscopy (FL-NSOM)	• Visualization of defects (birefringent crystallites), steps in the optical thickness of the film having different number of monolayers (accuracy of the 30 Å with a lateral resolution of 1 μm). • Molecule orientation, lipid domain morphology, grain boundaries, micro collapsed region.
Electron microscopies • Scanning electron microscopy (SEM) • Transmission electron microscopy (TEM) (with shadowing, replication, silver decoration and charge decoration)	Surface morphology, lipid domain structure, patterns, pinholes and defects (in-homogeneous crystalline domains, micro collapse…) (resolution of 50 nm).
Scanning tunneling microscopy (STM) Atomic force microscopy (AFM)	Surface topography, lipid domain morphology, visualization of defects (grains, pinholes, lateral heterogeneity, disclinations…)

Characterization of LB film starts from preparation of monolayer in air-water interface through the computer-aided systems. These measurements are mainly used

to control and characterize the monolayer formation on the water before transferring on solid substrate. Surface pressure/area isotherms give information about the limiting area of the molecules, which forms only one monolayer on air-water interface. Besides that, it is possible to record the data of barrier positions, surface pressure, transformation layers, temperature and pH while both forming monolayer and transferring of it on to substrate. Thus, this controllability of the system gives several advantages to examine and compare the results of the monolayer formations.

After obtaining the thin film, the morphological characteristics of the films can be determined microscopically with Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM). Fourier Transformation of Infrared spectroscopy (FTIR) or Raman Spectroscopy can be used for investigation of specific interactions/bonds between the solid surface and peptides and peptides and nanoparticles.

3. MATERIALS and METHODS

3.1 Materials

In this study, multifunctional peptide (AuBP4 – QBP2) suspended in ultrapure water (Merck (Lot: Z260633 207)) and Ethanol (Merck (Lot: K42351083 121)) was used for functionalizing gold nanoparticles in order to transfer easily silicate substrates via Langmuir Blodgett Method.

3.1.1 Multi-functional peptides: AuBP4 – QBP2

The peptide, which is used in this study, was synthesis by Fmoc method using an automated solid-phase peptide synthesizer (CS336X, CSBio Inc., Menlo Park, Ca). After synthesis, the peptide was purified by C-18 reverse phase liquid chromatography (RPLC) to a level >95%.

Bi-functional peptides have two different sequences with a GGG linker one can bind silicate (QBP) (Hnilova, Khatayevich, et al., 2012) and the other one can bind to gold (AuBP) (Hnilova, Khatayevich, et al., 2012). The peptide sequence of the AuBP4-QBP2 peptide is RAVRRRSVRREVGGGPWLPPSLPPWPP. The pdb form of this sequence has been obtained using PEPstr (Kaur, Garg, & Raghava, 2007). Schematic view of this pdb file converted by using PyMOL is given Figure 3.1.

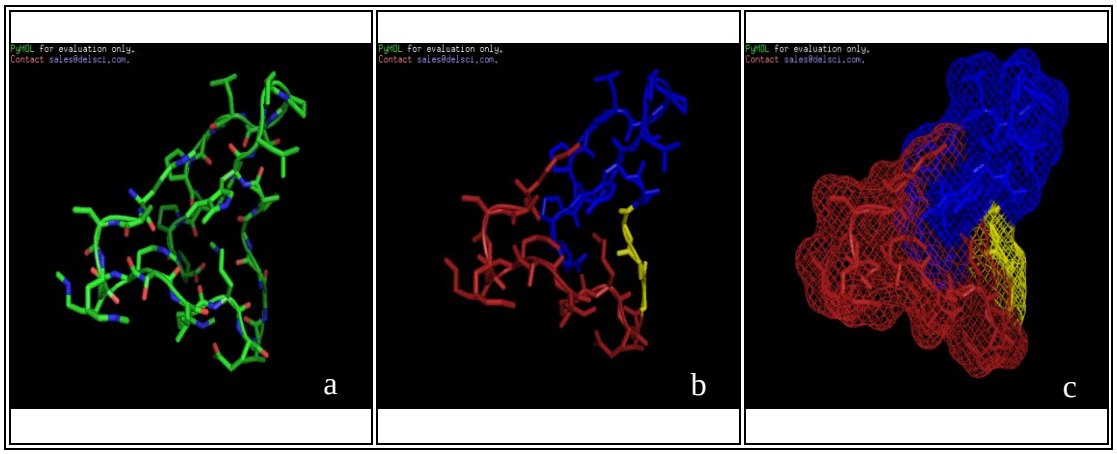

Figure 3.1: The schematic view of the AuBP4-QBP2 a) cartoon view b) cartoon view: red indicates AuBP4, yellow indicates ggg linker and blue indicates QBP2 c) mash view: red indicates AuBP4, yellow indicates ggg linker and blue indicates QBP2.

Hydrophobicity analysis of this peptide is important for determining its suitability of it for the formation of monolayer at air/water interface. The hydrophilic and hydrophobic residues of the peptide are seen in Figure 3.2. 56% of this peptide is hydrophobic which makes it suitable for the formation a monolayer on water surface (Figure 3.3). The net charge of the peptide is 5 at pH 7 and the iso-electric point is pH 12.7. The calculated molecular weight of peptide is 3076.61 g/mol and extinction coefficient is 11380 $M^{-1}cm^{-1}$ (calculated online Innovagen - Peptide property calculator (Innovagen, 2011)).

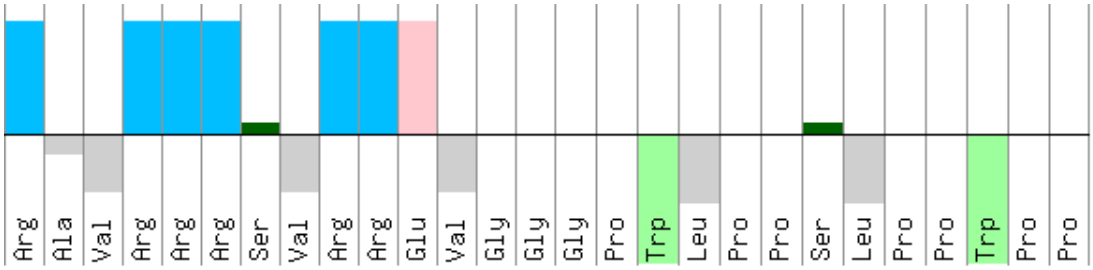

Figure 3.2: Hydrophilic (top) and Hydrophobic (bottom) residues of AuBP4-QBP2. Color codes: blue: basic, pink: acidic, dark green: polar, green: aromatic, grey: aliphatic. The scheme has been obtained using online Innovagen - Peptide property calculator (Innovagen, 2011).

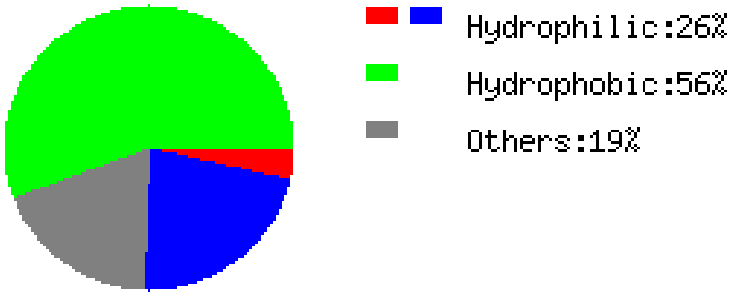

Figure 3.3: Pie chart of the peptide according to its hydrophobicity analysis. This graph has been obtained using online Gemsec – Peptide property calculator(GenScript-The Biology CRO, 2011).

3.1.2 Gold nanoparticles

2 nm and 7 nm gold colloid nanoparticles were used in this study. “Accurate Spherical Gold Nanoparticle” (7nm) was purchased from Nanopartz (Source Lot: SPA296D) with a concentration of 0.05 mg/ml. Its absorption wavelength is 522 nm and red in color.

2 nm Gold Colloid nanoparticles was purchased from BBI international (Product code: EM.GC2) which is suspended in H_2O and do not include any preservatives. Its optical density is more than 0.015 at 400 nm. Gold chloride concentration is 0.001%. The color of 2 nm gold colloid is pale yellow.

3.1.3 Silicate based surfaces

As substrates optical grade glass was used (CORNING® (2947-75x25). Their dimensions are 75x25 mm. Their thickness is between 0.96 - 1.06 mm. They have plain surfaces. Light transmittance of these glasses is higher than 95%.

3.2 Instruments and Methods

3.2.1 Langmuir Blodgett system

KSV Minimicro Langmuir Blodgett system is used in the experiments (Figure 3.4). This system is suitable for transferring small volumes of molecules. Trough is made by Teflon, which is super hydrophobic, has 240 cm^2 surface area and 85 ml subphase volume. The barriers are hydrophilic and produced from Delrin®. Surface pressure was measured by using platinum-iridium Wilhemny plate.

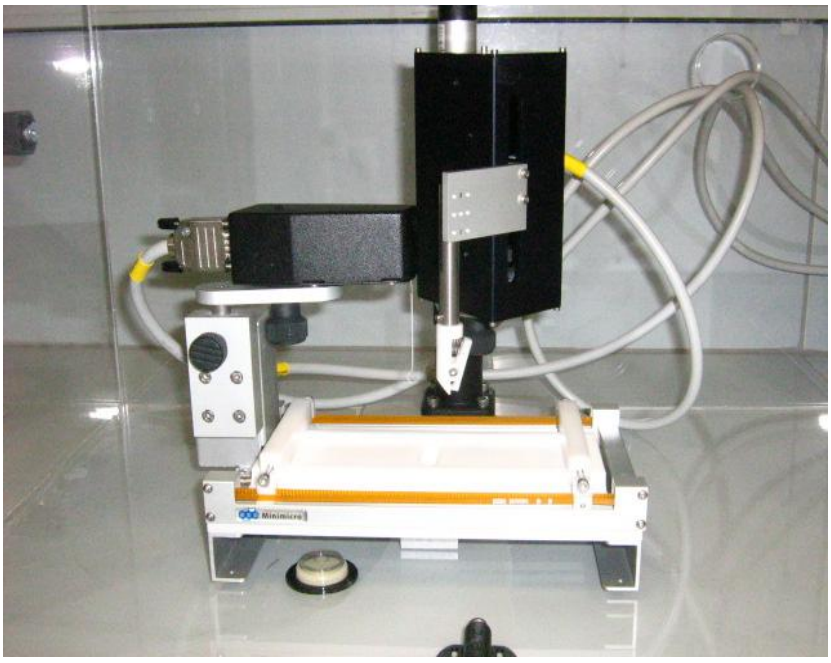

Figure 3.4: KSV Minimicro Langmuir Blodgett system.

3.2.2 Characterization systems

3.2.2.1 Optical characterization

UV/Vis spectrophotometer (Bio-Rad Benchmark Plus microplate spectrophotometer) was used to measure absorption spectra of the gold nanoparticles and peptide functionalized gold nanoparticles. FTIR measurements were conducted by Spectrum 100 series FI-IR Spectrometer (Perkin Elmer).

3.2.2.2 Microscopic characterization

Atomic Force Microscopy (NanoMagnetics Instruments) was used for surface characterization of the Langmuir Blodgett films. The thin films of the peptides, gold and peptide functionalized gold surfaces were imaged by using silicon tips.

Field Emission Scanning Electron Microscope (FEG-SEM) (JEOL JSM-7000F) was also used for imaging of LB films. A thin film of platinum was covered on to LB films in order to increasing conductivity of the surface. The SEI and BSE images of the surface were taken in the 10.000 and 50.000 magnifications using 5 kV acceleration voltages.

3.2.3 Preparation of Langmuir Blodgett films of peptide functionalized gold nanoparticles

Multi-functional peptides that have capability to bind both gold and silicates were dissolved in three different solvents: ultrapure water, 1:1 ethanol: ultrapure water and ethanol. Dissolving of peptides in a volatile solvent is important to form a monolayer at air/water interface. These peptide solutions were mixed with gold colloid solutions in different proportions. First, these suspensions were examined spectroscopically in order to understand interaction of gold and peptides in water or ethanol.

The trough was filled with ultrapure water and any surface contaminant was removed using a pipette attached to a suction pump. The Wilhemny plate was cleaned by ethanol and distilled water, respectively, and heated until it turns to red for several seconds. The solid surface was lowered into the water since we want to transfer only one layer on it while it lifts upwards. The solutions containing the molecules that we want to form as monolayer were spread on the ultrapure water. Conventional isotherms and compression cycles were measured with waiting 10 minutes at room

temperature after spreading with a compression rate of 10 cm^2 min^{-1}.Surface pressure – Area isotherms indicates that a monolayer was present on the air/water interface. The solid substrate was pulled up with a rate of 10mm/min by keeping the surface pressure was constant.

4. RESULTS and DISCUSSION

In this thesis, we studied LB film formation of AuBP4-QBP2, which is bi-functional peptide that has ability to bind to both silicate and gold. LB film formation of gold nanoparticles is also investigated in order to fabricate organic-inorganic hierarchical hybrid structures. The optical and structural characterizations of the peptide functionalized gold nanoparticle containing solutions were performed by using UV/Vis spectroscopy and FT-IR. LB films were also characterized by UV/Vis spectroscopy, FTIR, AFM and SEM.

4.1 Spectroscopic Investigations of Gold Nanoparticle and Peptide Functionalized Gold Nanoparticle Solutions

Optical properties of the gold nanoparticles, peptides and peptide functionalized gold nanoparticles solutions were investigated. Color changes, stabilities and behavior of gold nanoparticles, peptide molecules and their combinations were characterized. Absorption spectra of the solutions in different concentrations were measured.

4.1.1 Gold colloid nanoparticles solutions

Optical properties of the gold colloid nanoparticles show different characteristics according to their dimensions. The color of 5 -20 nm Au nanoparticles containing solutions are red or in red scales. Below the 5 nm they are transparent or tune yellowish. The commercial gold colloid nanoparticles used in this study are red in 7 nm (Nanopartz) and tune yellowish in 2 nm (BBI). The absorption spectra of the gold colloid nanoparticles are measured in the range of 400 nm – 800 nm wavelengths. Gold colloid nanoparticles with 7 nm size have an absorption peak at 522 nm (Figure 4.1-a), whereas 2 nm gold colloid nanoparticles have no absorption peak in visible region (Figure 4.1-b).

Intensity of absorption peak decreases with the decrease in the gold concentration (Figure 4.2-a).

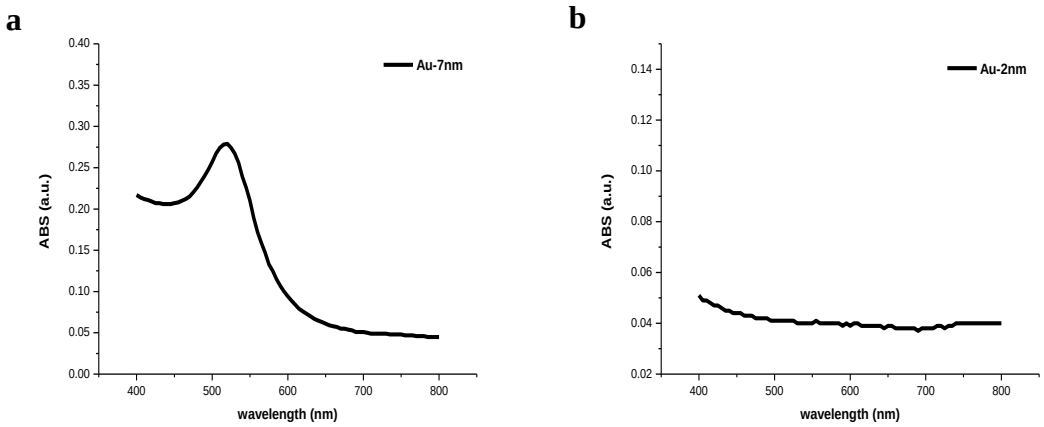

Figure 4.1: Absorption spectrum of gold colloid nanoparticles a) 7 nm b) 2 nm.

As shown in the Figure 4.2, the absorption intensity changes linearly in accordance to the concentration. A linear equation easily fit to this line and the concentration of the gold suspensions can be determined according to this equation.

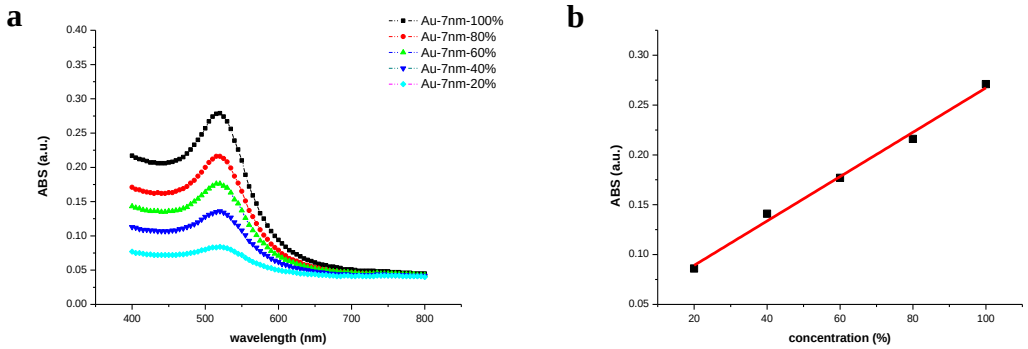

Figure 4.2: a) Absorption spectra of gold colloid nanoparticles (7nm) in different concentrations b) Concentration vs absorption intensity graph of gold colloid nanoparticles (7 nm). Linear fitting equation: y=0.223x+0.0447, R^2=0.992.

4.1.2 Peptide containing solutions

Multifunctional peptides synthesized with Fmoc method are in solid form. They are dissolved in three different solutions in order to understand their functionality and stability in various aqueous media. These solutions are prepared using ultrapure water, ethanol and 50% ethanol and 50% ultrapure water. Peptides solutions in these three liquid solutions are transparent in the visible region and thus, they have no absorption peak in the region of 400 nm – 800 nm. The absorption spectra of the peptide solutions are shown in Figure 4.3.

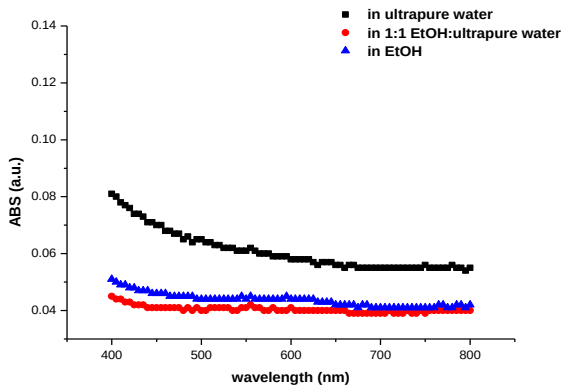

Figure 4.3: Absorption spectra of peptides (100 μM) in different solutions.

Since peptide does not give any optical absorption in the Vis range, it was not possible to get any qualitative or quantitative data from these measurements.

4.1.3 Peptide functionalized gold nanoparticles

Multi-functional peptides that have capability to bind both gold and silicates (optical glass) were combined with gold nanoparticles prior to LB transfer onto glass substrate. In this regard, the binding behavior and optical properties of peptide – gold colloid nanoparticle solution is expected to give information with respect to the stability and suitability of this solution for LB process. Peptides were dissolved in different solutions as ultrapure water, 1:1 EtOH:ultrapure water and EtOH in different concentrations 100μM, 10μM, 1μM in each solutions. These peptide solutions were combined with different amounts of 7nm gold colloid solutions, 80%, 60%, 40%, and 20% Absorption spectra of these solutions were measured and compared to find appropriate solution and the amount of gold colloid to suspend. Since it was not possible to obtain any absorbance for 2 nm gold particles these solutions are not used in this set of experiments.

In Figure 4., absorption spectra of peptide functionalized gold nanoparticles are shown. Peptide solutions in different solvents were mixed with gold colloid nanoparticles in different proportions. Gold colloid nanoparticles have an absorption peak at 522 nm (Figure 4.1-a). When added to the Au colloid solutions, peptides cause an immediate color changes from red to dark red/purple(Figure 4.4) and the absorption peak shift to the higher wavelengths (Figure 4.), signifying aggregation of nanoparticles.

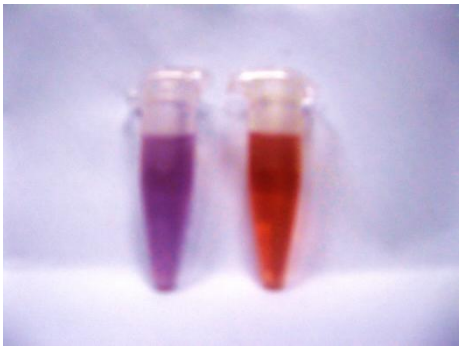

Figure 4.4: Color change with the addition of peptide in gold colloid nanoparticles (50% AuBP4-QBP2 in 1:1 EtOH:ultrapure water and 50% gold colloid nanoparticles were mixed).

Two possible mechanisms can be proposed for explaining this aggregation of nanoparticles by the addition of peptides. The first one is the binding of peptides to more than one particle at a time, leading to an effect called "bridging" flocculation (Myers, 1999). And the other possibility is the electrostatic repulsion in the electrical double layer (EDL) of the particles is decrease of by the addition of positively charged peptides to negatively charged gold nanoparticle containing solutions allowing colliding particles to aggregate (Lévy et al., 2004; Myers, 1999). The concentration of the peptide and the gold nanoparticle is also an important parameter to determine the cause of the aggregation of gold colloids (Tullman, Finney, Lin, & Bishnoi, 2007). If the aggregation is caused by bridging flocculation, it should decrease with the increasing peptide concentration, because gold nanoparticle surface will be saturated with the individual peptide. On the other hand, if the aggregation is seen in peptide functionalized gold nanoparticles due to electrostatic effects, the degree of particle aggregation should increase with peptide concentration.

A series of experiments in three different solutions (pure water, ethanol 50% + pure water 50 %, and pure ethanol) containing different amounts of Au nanoparticles and peptides are conducted to reveal the nanoparticle-peptide binding mechanism (Figure 4.). In all experiments, it is observed that aggregation increased with the increase of peptide concentration indicating that the decrease of electrostatic repulsion in the electrical double layer (EDL) of the particles is the responsible mechanism for the aggregation.

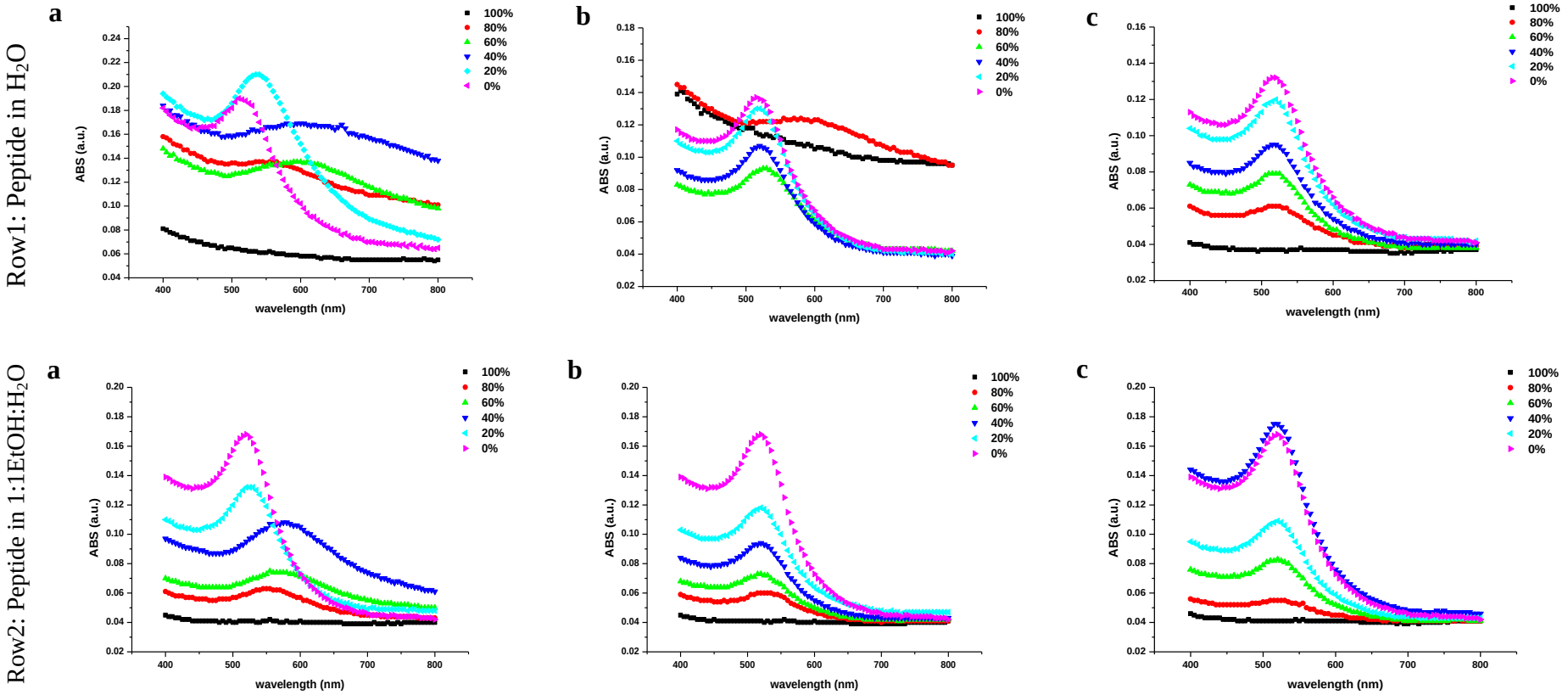

Figure 4.5: Absorption spectra of the solutions including different amount of peptide and gold nanoparticles in different concentrations of peptides. Line 1: Peptide in ultrapure water in a) 100µM b)10 µM c)1 µM concentrations suspended with different amount of gold nanoparticles (7nm). Line 2: Peptide in 1:1 EtOH: ultrapure water in a) 100µM b)10 µM c)1 µM concentrations suspended with different amount of gold nanoparticles (7nm). Line 3: Peptide in EtOH in a) 100µM b)10 µM c)1 µM concentrations suspended with different amount of gold nanoparticles (7nm). Line 4: Comparison of three kind of solutions including different amount of peptides (100 µM) a) 80% b) 60% c) 40% d) 20% and gold (7nm) a) 20% b) 40% c) 60% d) 80%.

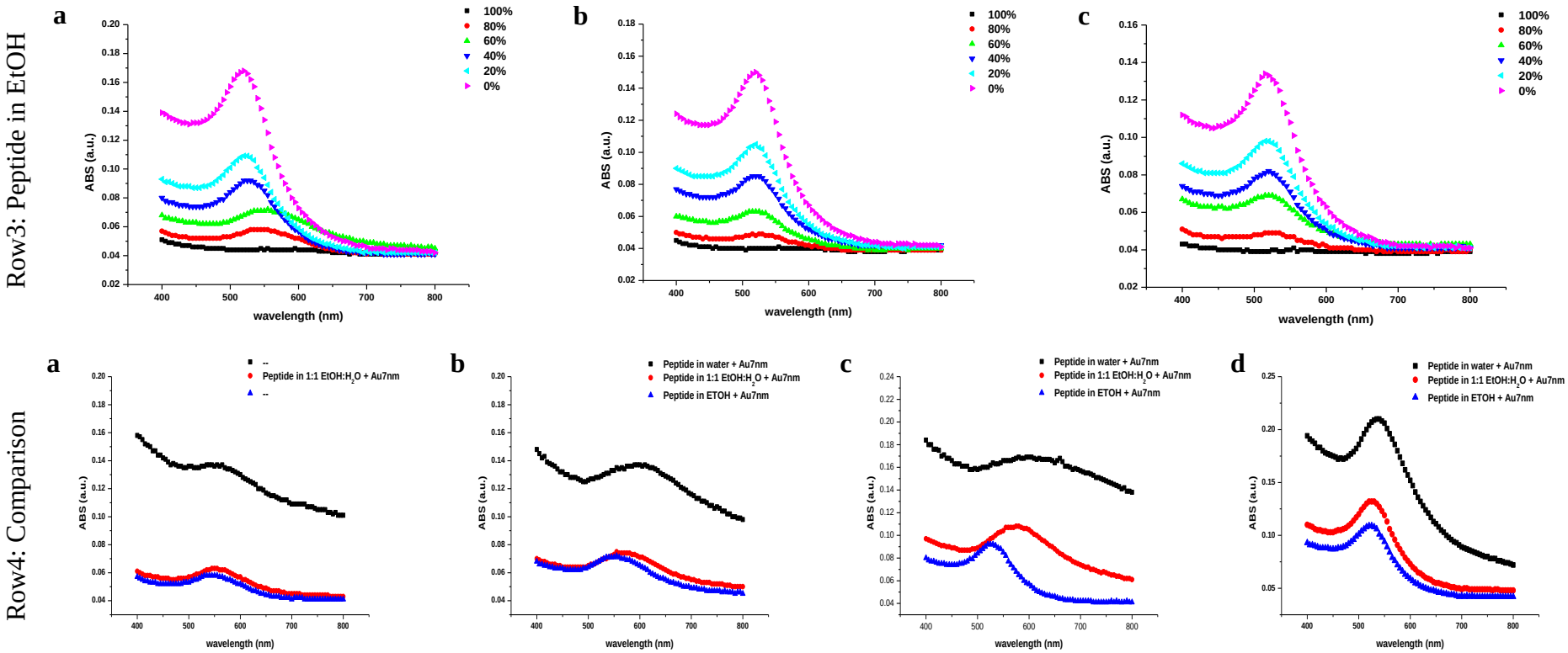

Figure 4.5 (continued): Absorption spectra of the solutions including different amount of peptide and gold nanoparticles in different concentrations of peptides. Line 1: Peptide in ultrapure water in a) 100µM b)10 µM c)1 µM concentrations suspended with different amount of gold nanoparticles (7nm). Line 2: Peptide in 1:1 EtOH: ultrapure water in a) 100µM b)10 µM c)1 µM concentrations suspended with different amount of gold nanoparticles (7nm). Line 3: Peptide in EtOH in a) 100µM b)10 µM c)1 µM concentrations suspended with different amount of gold nanoparticles (7nm). Line 4: Comparison of three kind of solutions including different amount of peptides (100 µM) a) 80% b) 60% c) 40% d) 20% and gold (7nm) a) 20% b) 40% c) 60% d) 80%.

In pure water solutions with 100 μM peptide concentration (Figure 4.-Row1-a), aggregation was observed at all Au nanoparticle concentrations. However, more the aggregation was observed in higher amount of peptide containing solutions. In pure water solutions with 10 μM peptide concentration (Figure 4.-Row1-b), aggregation started to be observed at 80% and 60% peptide concentrations. With 1 μM peptide concentrations (Figure 4.-Row1-c), no aggregation was observed irrespective of the gold nanoparticle concentration.

In 1:1 EtOH:ultrapure water solutions with 100 μM peptide concentration (Figure 4.-Row2-a), aggregation results were similar with the pure water solutions with 100 μM peptide concentrations. However, the amount of aggregation was less than the pure water solutions. The amount of aggregation was decreased with decreasing molar concentrations of peptide, 10 μM (Figure 4.-Row2-b) and 1 μM (Figure 4.-Row2-c). Similar results were observed for ethanol solutions with 100 μM peptide concentration.

In all solution with 1 μM peptide concentration, no aggregation was observed irrespective of the Au nanoparticles content (Figure 4.-column c). In solutions with 10 μM peptide concentration (Figure 4.-column b) aggregation started to be observed at 60 %, and 80 % peptide concentrations in pure water and ethanol containing solutions respectively. In pure water with 100 μM peptide concentration, aggregation was observed at all Au nanoparticle concentrations. However, in 50% ethanol and pure ethanol solutions aggregation started to be observed at 60% and 40 % Au-nanoparticle concentrations respectively (Figure 4.-column a).

The aggregation behavior comparison of gold colloid solutions with the addition of peptides in different solvents (100 μM) are given in Figure 4.5-Row4. According to these graphs, the amount of aggregation of gold nanoparticles was less in ethanol solutions. This is a positive result for suitability of the peptides in such a volatile solution including ethanol for formation of LB films. However, it can be noted that ethanol can cause degradation of peptides (Neuman Jr & Gerig, 2008).

On the other hand, much higher aggregation of gold nanoparticles was observed in pure water solutions. These results indicated that the peptides binds to the gold nanoparticles specifically, however this peptide solution is not suitable for formation of LB film because of the aggregation tendency. In the view of results, the 1:1

EtOH:ultrapure water solution is selected for formation of the LB films. The aggregation amount was enough to see the attraction of the peptides to the gold nanoparticles. Besides, the ethanol in the solution helps the monolayer formation of the peptides on air/water surface for LB film.

Time dependent aggregation behavior is investigated in more detail reaction with measuring the time dependency of aggregation in 1:1 EtOH:ultrapure water mixed with gold colloid nanoparticles (7nm) and bi-functional peptides in proportion of 50% (Figure 4.6). The absorption spectra of the solution were measured every minute for a duration of 51 minutes. The color of the solutions changes from red to dark red/purple, blue and then transparent. The aggregation process occurs during almost an hour, than as determined from the exponential fit equation absorption peak is saturated at 701 nm. In this wavelength, the solution is transparent. Maximum absorption wavelengths vs time graph was plotted. The data perfectly fit to exponential function, y = 701.25 – 116.709*exp(-0.05*x) R^2=0.98 (Figure 4.6). This results shows that gold nanoparticles were agglomerated with peptide in an exponential manner.

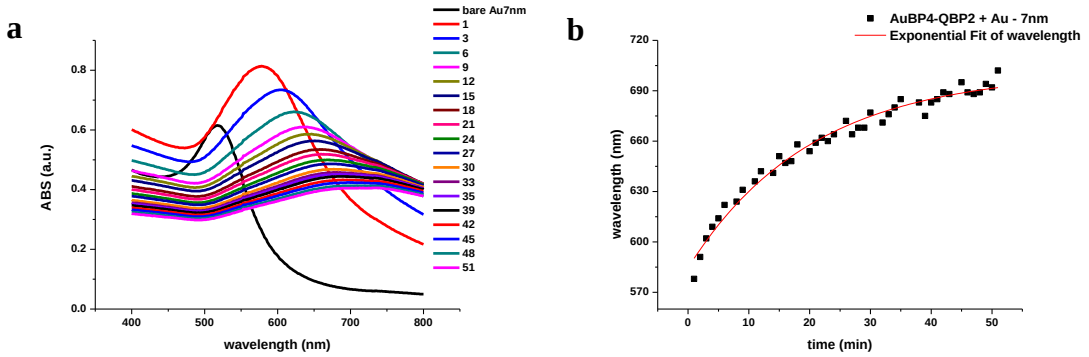

Figure 4.6: Red shift of the peptide functionalized gold nanoparticles (7nm) (%50 peptide in 1:1 EtOH:ultrapure solution and %50 Au-7nm). a) Absorption spectra, b) shift of the peaks.

4.2 FT-IR Analysis of Peptide and Peptide Functionalized Gold Nanoparticle Solutions

Fourier Transform Infrared spectroscopy is another spectroscopic technique to examine the organic structures. It gives the fingerprints of the molecules. The bi-functional peptides soluted in various solutions, peptide functionalized gold colloid

nanoparticle solutions were investigated by FTIR in order to find the relationship between bi-functional peptide and gold nanoparticles.

4.2.1 FT-IR analysis of peptide solutions

Peptide solutions prepared by using different solvents have been investigated by FT-IR. FT-IR spectrum of ethanol and water has been taken from FDM library (Fiveash Data Management Inc. (FDM), 2012).

Peptides dissolved in pure ethanol gave similar ethanol spectrum except one peak at 1641 cm^{-1}. This peak clearly indicates the presence of amide bonds in the spectra. Amide I and amide II peaks determine the peptide structure. Amide I peak is in the range of 1600 cm^{-1} and 1700 cm^{-1} and amide II peaks are in the range of 1350 cm^{-1} and 1500 cm^{-1} in FTIR absorption spectra (Gallagher, 1997; Haris & Severcan, 1999).

In solutions containing water the situation becomes more complex because the transmission FTIR spectrum of a water soluble protein overlaps with water spectrum(Haris & Severcan, 1999). The overlapping water spectrum can be digitally subtracted from the spectrum of the protein and broad infrared band can be analyzed using second-derivative and deconvolution procedures (Byler & Susi, 1986; Haris & Chapman, 1992; Jackson & Mantsch, 1995). Therefore, well-resolved and high-qualified FTIR spectrum of the peptides diluted in water can be obtained.

The similarity of the FT-IR spectra of peptide dissolved in ultrapure water similarity water is clearly observable in Figure 4.7and Figure 4.8.

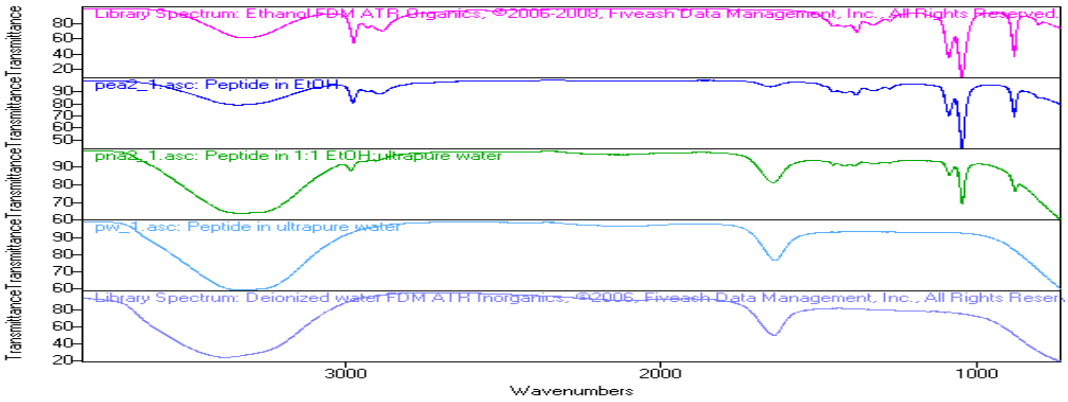

Figure 4.7: FTIR spectrum of the ethanol (pink), peptide in ethanol (dark blue), peptide in 1:1 ethanol:ultrapure water (green), peptide in ultrapure water (light blue), deionized water (blue).

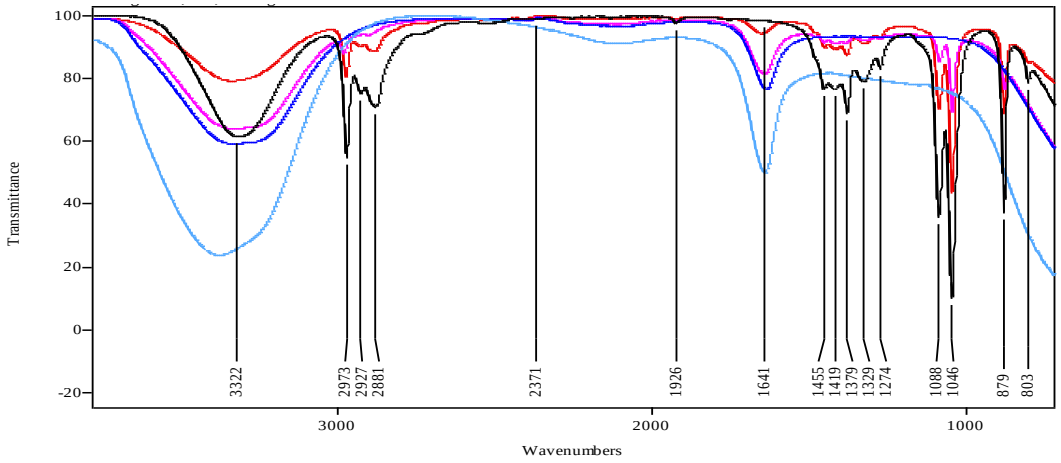

Figure 4.8: FTIR spectrum of the ethanol (black), peptide in ethanol (red), peptide in 1:1 ethanol:ultrapure water (pink), peptide in ultrapure water (blue), deionized water (light blue).

In water containing solutions, we could not determine the peptide peaks since it overlapped with water spectrum. However, the results obtained in pure ethanol solution strongly indicate the presence of amide bonds in the solutions.

4.2.2 FT-IR analysis of peptide functionalized gold nanoparticle solutions

For investigation of the interaction of gold nanoparticles with peptides FT-IR spectra of 2 nm gold nanoparticle and peptide containing solutions are obtained in various solvents by varying the both gold nanoparticle and peptide concentrations.

In the FT-IR spectra in which only pure water is used as solvent no difference in the amide bands at 1641 cm^{-1} is observed with the increase of peptide concentration (Figure 4.9).

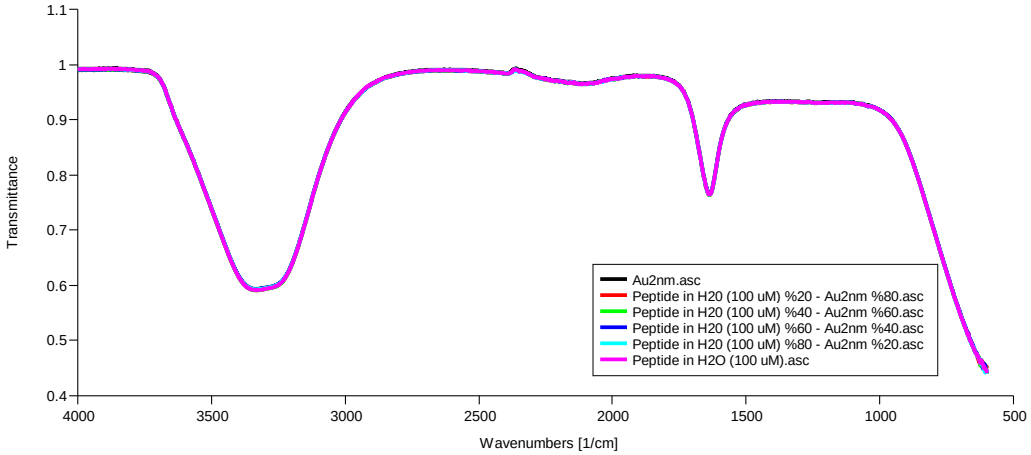

Figure 4.9: FTIR spectra of the mixture of the peptide in ultrapure water and gold colloid nanoparticles (2nm) in different proportions.

This can be attributed to the aggregations observed in water-based solvents (Section 4.1.3). It seems that aggregation of peptide functionalized nanoparticles proceeds through amide bonds since no increase in the intensity is observed by the increase of peptide concentration.

In the FT-IR spectra in which 1:1 EtOH:ultrapure water mixtures is used as solvent an increase in the amide bands at 1641 cm^{-1} is observed with the increase of peptide concentration (Figure 4.10). Moreover, a slight shift in the amide peak positions is also observed.

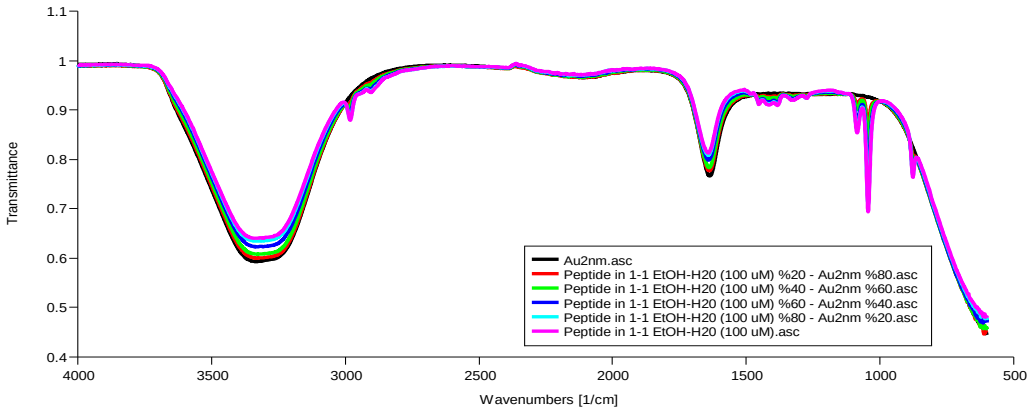

Figure 4.10: FTIR spectra of the mixture of the peptide in 1:1 EtOH:ultrapure water and gold colloid nanoparticles (2nm) in different proportions.

In the FT-IR spectra in which only ethanol is used as solvent a more distinct increase in the amide bands at 1641 cm^{-1} is observed with the increase of peptide concentration (Figure 4.11). Furthermore, a distinct shift in the amide peak positions is also observed.

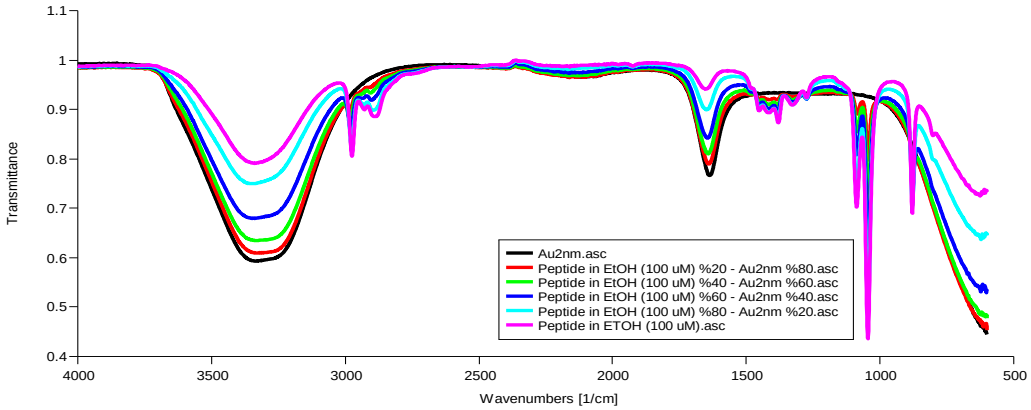

Figure 4.11: FTIR spectra of the mixture of the peptide in ethanol and gold colloid nanoparticles (2nm) in different proportions.

These results indicate the positive role of ethanol in decreasing the tendency of aggregation of peptide functionalized gold particles. Moreover, shifts in the amide band positions can be attributed to the conformation changes of peptides while attaching to the nanoparticles.

The solution containing gold nanoparticle and peptide in ethanol in different proportion gives the spectra similar with ethanol except the peaks at the region of 1500 cm^{-1}-1700cm^{-1} and 2000cm^{-1}-2200cm^{-1}. The intensity of these peaks decreases with the decreasing the amount of peptide in the solution (Figure 4.11). The peak around 3320 cm^{-1} is responsible for C-H bonds and it is observed both in water and ethanol spectra. The peak is quite broad and it is shifted from 3311 cm^{-1} to 3335 cm^{-1} gradually from bare gold nanoparticle solution to peptide in ethanol solution. Gold nanoparticles does not give any FTIR peaks, thus the spectrum from this solution represents the water peaks. The shift at the region 3320 cm^{-1} can be attributed to the differences between the characteristic peaks of water and ethanol when used as the solvent of peptide. Similar situation is observed at the peak around 1645 cm^{-1}, however, in this case the peaks shift from 1637 cm^{-1} to 1653 cm^{-1} from pure gold nanoparticle solution to peptide solution. Besides that, the structure of the peptide can be determined using the information of this peak. The deconvolation of this peak gives more information about secondary structure of peptides. The peaks at 1641 cm^{-1} represents the uncoordinated structure, whereas peaks at 1653 cm^{-1} corresponds the helix structure of the peptide (Byler & Susi, 1986; Gallagher, 1997).

As a result, from FTIR spectra, the peptide structure in different solutions has different characteristics. Peptides in ultrapure water solutions had almost the same spectra with water and the amide peak was at 1637 cm^{-1}. Therefore, the peptide has low components in its structures. On the other hand, the peak positions of amide I are shifted in the peptide solutions including ethanol. This can be interpreted as structural change of peptides in the solutions containing ethanol.

4.3 Langmuir Blodgett Films

Langmuir Blodgett films of the molecules is performed in two-steps. The first step is formation a monolayer on water surface and this can be confirmed by isotherm graphs. The second step is transfer of the monolayer on a solid substrate, can be visualized by transfer ratio and transfer graphs.

4.3.1 Isotherm process for formation monolayer on air/water interface

For this purpose, the characteristic isotherms of peptide in different solution, gold colloid nanoparticles and peptide functionalized gold nanoparticles were obtained.

4.3.1.1 Isotherms of peptide solutions

The isotherm graphs of peptides obtained for all three solvent (Figure 4.12). The solutions were prepared at the same concentration, 0.30954 mg/ ml (100 µM), for all solvents. To form a monolayer on air/water interface, the molecules dissolved in a volatile solvent to help them to move up of molecules while evaporating. For this reason, peptides in water did not show a good isotherm graphs (Figure 4.12-a) whereas peptide in a solvent including ethanol showed quite good isotherms (Figure 4.12-a and b).

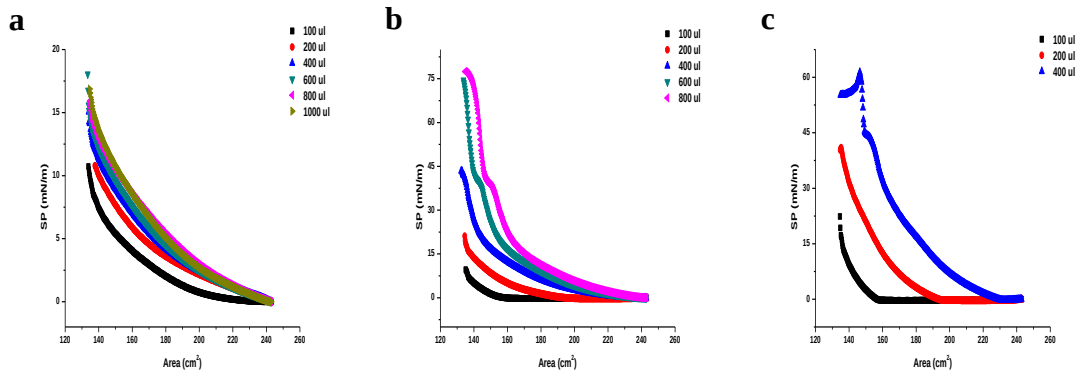

Figure 4.12: Surface Pressure / Area isotherms of 100 µM peptides in **a)** ultrapure water, **b)** 1:1 EtOH:ultrapure water, **c)** EtOH solutions.

Peptides dissolved in 1:1 EtOH:ultrapure water, the presence of ethanol as volatile solvent helps to form a monolayer on water surface. After spreading peptides on water (sub-phase) from solutions containing ethanol, at least 10 minutes time is allowed to evaporate ethanol before LB film production.

Isotherms of peptide solutions in all solvents does not include any constant surface pressure region which indicates that molecules are not widely dispersed, and they are already interacted each other. The amount of peptide spreading on the water surface is also an important parameter for increasing the surface pressure to higher levels. A linear speed increase starts after 15 mN/m for water solvent and after 40 mN/m for both 1:1 EtOH:ultrapure water and EtOH solvents, which also indicated the formation of a close-packed film.

In water based solutions it was not possible to form a monolayer even in solutions containing up to 1000 µl peptides, highly probably due to aggregations in pure water solution. However, in ethanol containing solution the collapse occurs at 75 mN/m and 60 mN/m for 1:1 EtOH:ultrapure water solution and ethanol solution, respectively indicating the formation of monolayer on subphase.

As seen in the Figure 4.12 the isotherm graphs of the peptides in different solvents, by the presence of ethanol in the solution, it became possible to form monolayers even with lower amount of peptides.

The limiting molecular areas for peptide molecules in different solvents obtained by extrapolation of the surface pressure/Area isotherm to the x axis, are 28.863Å^{2}molecule^{-1} for water solvent, 31.904 Å^{2}molecule^{-1} for 1:1 EtOH:ultrapure water solvent, 64.980 Å^{2}molecule^{-1} for ethanol solvent (Figure 4.13).

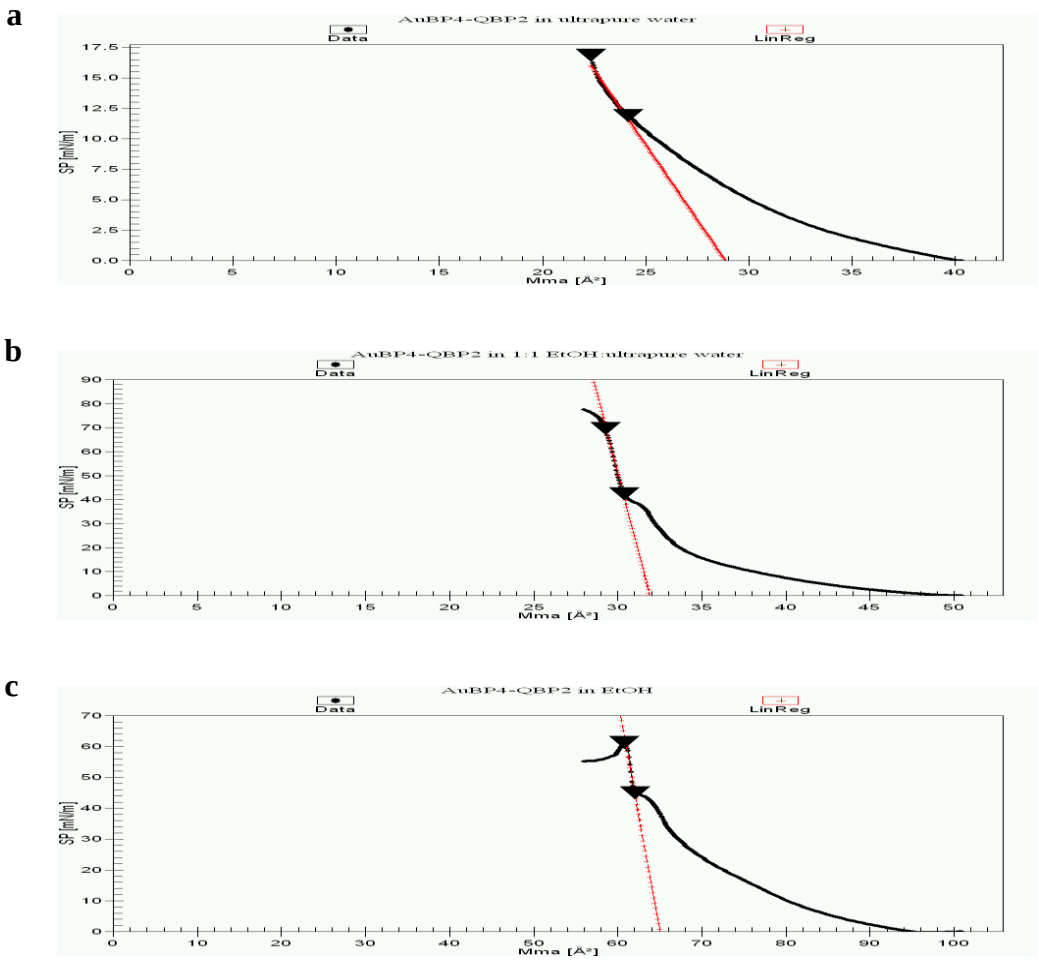

Figure 4.13: Surface Pressure / Molecular Area isotherm of AuBP4-QBP2 peptide in **a)** ultrapure water (1000 µl), **b)** 1:1 EtOH:ultrapure water (800 µl), **c)** EtOH (400µl).

4.3.1.2 Isotherms of gold nanoparticle solutions

The gold colloid does not spread on the water surface, they tend to sink immediately, and they do not show a good isotherm when pure water is used as solvent. On the other hand, gold colloid nanoparticles containing ethanol show a typical isotherm graph. The isotherm graphs are shown in Figure 4.14. These results indicate that when gold nanoparticles are suspended in a solution containing ethanol it is possible to obtain LB films of them.

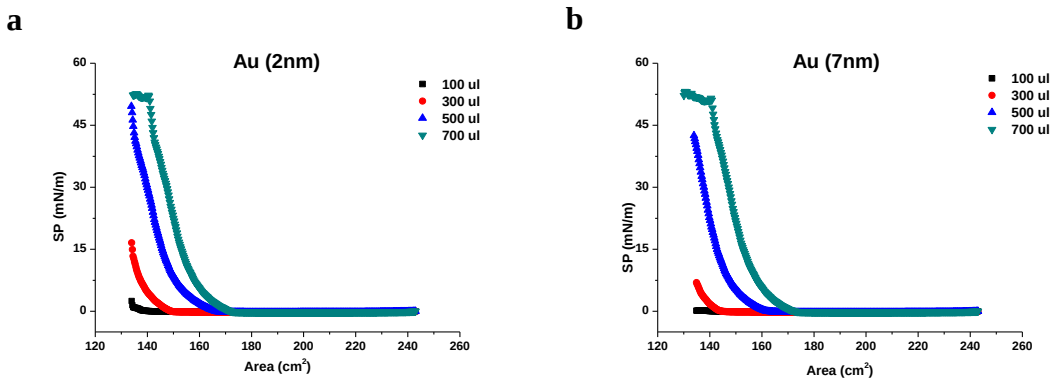

Figure 4.14: Surface Pressure / Area isotherms of gold colloid nanoparticles **a)** 2 nm, **b)** 7 nm.

The limiting molecular areas obtained by extrapolation of the surface pressure/Area isotherm to the x axis, are 28.180 $Å^2$molecule^{-1} for AuNP – 2nm (Figure 4.15) and 28.360 $Å^2$molecule^{-1} for AuNP – 7nm (Figure 4.16).

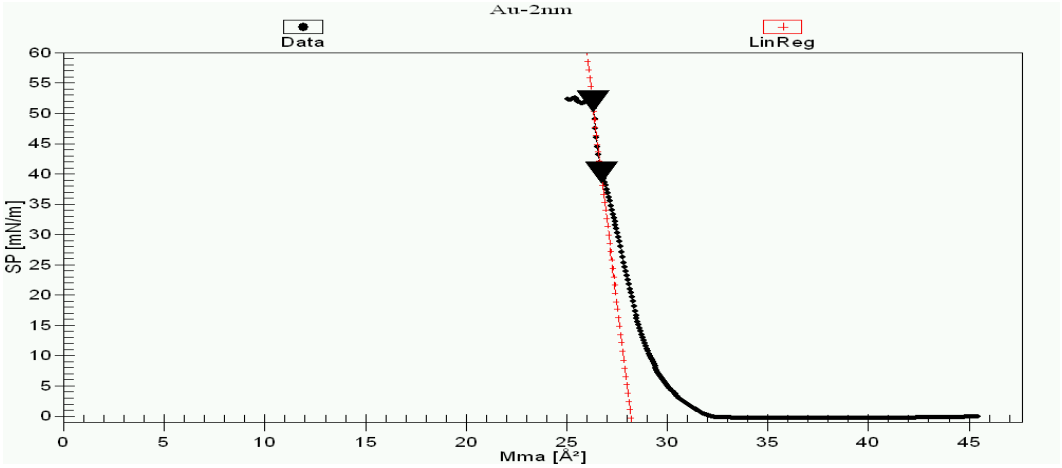

Figure 4.15: Surface Pressure / Molecular Area isotherm of 2 nm gold colloid nanoparticles mixed with EtOH in 1:1 proportion.

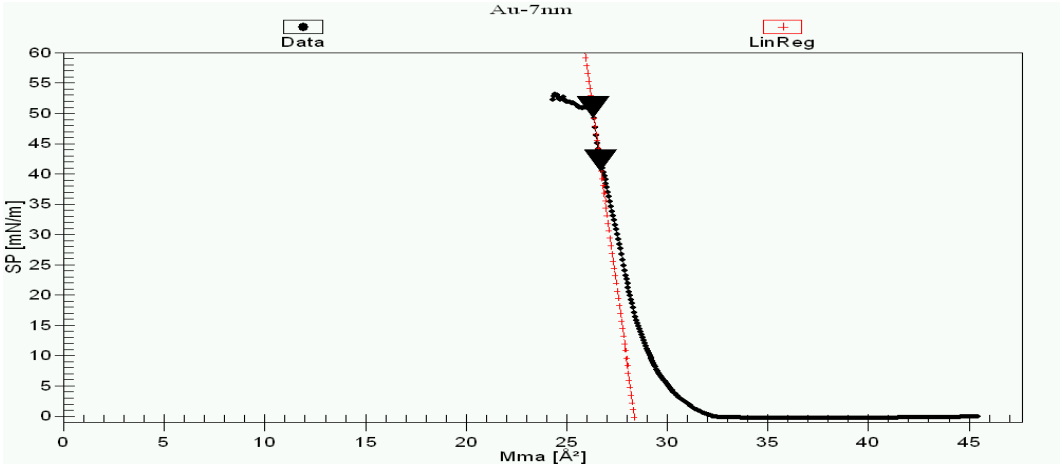

Figure 4.16: Surface Pressure / Molecular Area isotherm of 7 nm gold colloid nanoparticles mixed with EtOH in 1:1 proportion.

4.3.1.3 Isotherms of peptide functionalized gold nanoparticles

Experiments on LB film formation by using peptide functionalized surface are conducted in 1:1 ethanol: water mixtures using 50 vol % mixtures of peptide and gold nanoparticles. The isotherms of peptide functionalized gold colloid nanoparticles are shown in Figure 4.17. The volume of solution appropriate for LB film production is selected by compressing different amount of solutions on the sub-phase. It becomes possible to obtain monolayer films suitable for LB film deposition only when 1300 µl (for 2 nm Au particles) and 1400 µl (for 7 nm Au particles) of solution is used. These volumes are almost two times higher than the ones used for unfunctionalized Au nanoparticles. It is believed that aggregation and precipitation of functionalized nanoparticles cause the requirement of higher volumes. Thus in cases where LB film deposition is required for peptide functionalized gold nanoparticles higher volumes of solutions should be used.

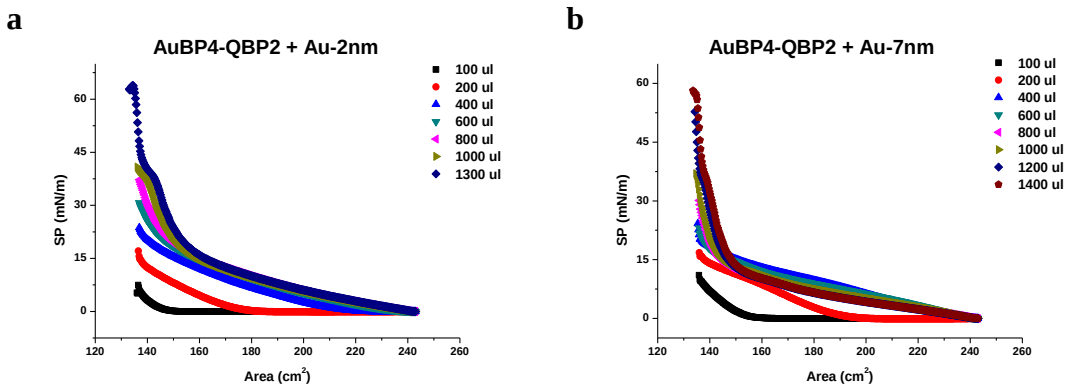

Figure 4.17: Isotherms of peptide functionalized gold nanoparticles. 50% peptide in 1:1 EtOH:ultrapure water and 50% gold colloid nanoparticles a) 2 nm b) 7 nm

The limiting molecular areas obtained by extrapolation of the surface pressure/Area isotherm to the x axis, are 33.647 $Å^2$molecule^{-1} for peptide functionalized gold NP – 2nm and 30.737 $Å^2$molecule^{-1} for AuNP – 7nm (Figure 4.18).

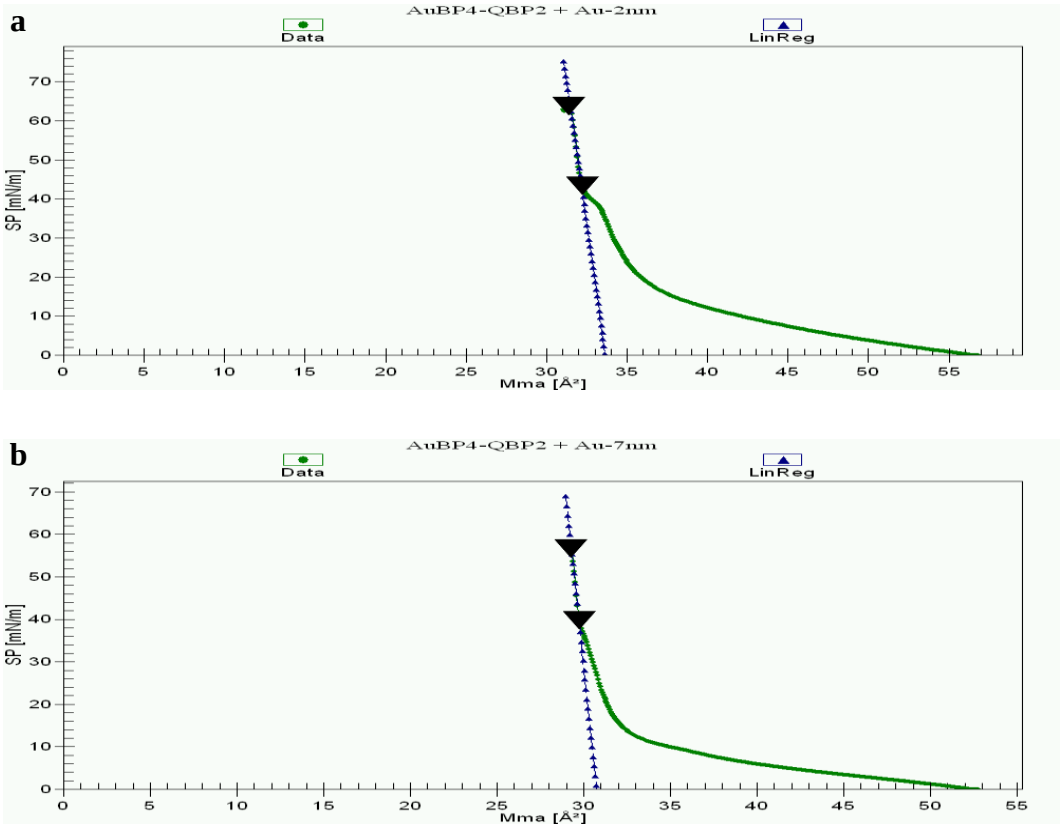

Figure 4.18: Surface Pressure / Molecular Area isotherm of peptide functionalized gold nanoparticles. 50% peptide in 1:1 EtOH:ultrapure water and 50% gold colloid nanoparticles a) 2 nm b) 7 nm

4.3.2 Dipping process optimization for the production of LB films

Dipping process is performed after formation of the monolayer on water surface. In our study, transfer of only one layer on solid surface is aimed; therefore, the substrate is submerged in sub-phase before spreading molecules on water. After compressing barriers up to a constant surface pressure and formation a monolayer, for the regular arrangement of the layer of molecules, it was waited for 10 minutes before pulling out of the substrate. The substrates are pulled out of the solution with a constant velocity, 5 mm/min without allowing the film to collapse. During the deposition process, the surface pressure fluctuates.

Transfer ratio, which is a good indicator of the monolayer deposition on a substrate, can be calculated as in the equation **2-1**. Total transfer ratios of LB films are given in

Table 4.1.Approach of ratio to 1 indicates that good deposition conditions are attained. A ratio less than 1, means partial coverage of the substrate. On the other hand, a ratio over 1 indicates irregularities on the LB film surface due to impurities or aggregations. In our case, transfer ratios are all over 1 which can be attributed to the impurities (since the experiments are not conducted in clean room environments) and or suspended aggregates of peptides or Au nanoparticles that are formed prior to pulling out below the floating monolayer.

Table 4.1: Total Transfer Ratio Values of LB films

	Peptide in 1:1 EtOH:ultrapure water	Peptide in EtOH	AuNP (diluted with EtOH)	Peptide functionalized AuNP
Transfer Ratio	1.590	1.386	1.493	1.388
Amount (µl)	800	400	600	250
Target pressure (mN/m)	35	35	40	10
Compression Rate (mm/min)	10	10	10	10

As the glass is lifted, the monolayer is deposited onto the substrate and the amount of molecules on the water surface decreases. Hence, the surface pressure decreases and the barriers move closer together to compensate. This accounts for the sinusoidal nature of the surface pressure curve. An example graph for the surface pressure change and transfer during deposition is given in Figure 4.19.

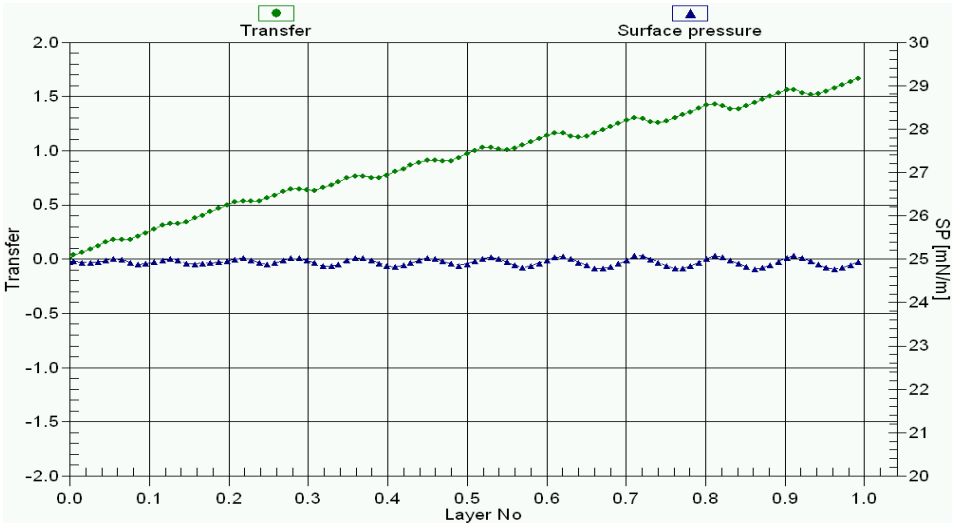

Figure 4.19: An example of transfer and surface pressure relation during deposition.

The transfer and surface pressure graphs of LB films that are imaged by AFM are given in Appendix A. The results of LB deposition experiments on optical grade glass can be summarized as follows:

1. All solutions (only Au nanoparticle, only peptide and peptide functionalized Au nanoparticles) containing ethanol can easily transferred on optical grade glass with transfer ratios over 1.
2. The volume of solutions required for monolayer LB film deposition shows changes depending on the type of particles/molecules to be suspended. The lowest volume required was 600 μl for gold nanoparticle, 800 and 400 μl for peptides and 250 μl for functionalized gold nanoparticles.

4.4 Characterization of Langmuir Blodgett Films

4.4.1 Atomic force microscope investigation of the LB films

Langmuir Blodgett films can be characterized by various methods. One of those methods is atomic force microscopy provides three-dimensional analysis of thin film surfaces. Pre-determination of solid surface roughness is also important for examining of thin films. For this purpose, AFM image of the optical grade glass is taken as a control. Then AFM images of peptide coated optical grade glass surface are investigated in order to determine the altitude and patterns of the thin films. The thin film pattern of peptide and gold nanoparticles coated on glass surface respectively is examined and compared with the LB film of the peptide functionalized gold nanoparticles. AFM image of gold nanoparticles on mica surface are given in Appendix B.

4.4.1.1 Optical grade glass

Optical grade glass is used as a solid substrate. The surface roughness of the glass is 4-5 nm in height. The line profile of glass the surface is given in Figure 4.20. Unfortunately, the surface of the glass is not smooth enough for good AFM measurements.

4.4.1.2 LB films of peptides

Langmuir Blodgett films on optical grade glass of the peptides obtained as monolayer, by using peptide containing 1:1 EtOH:ultrapure water solution.

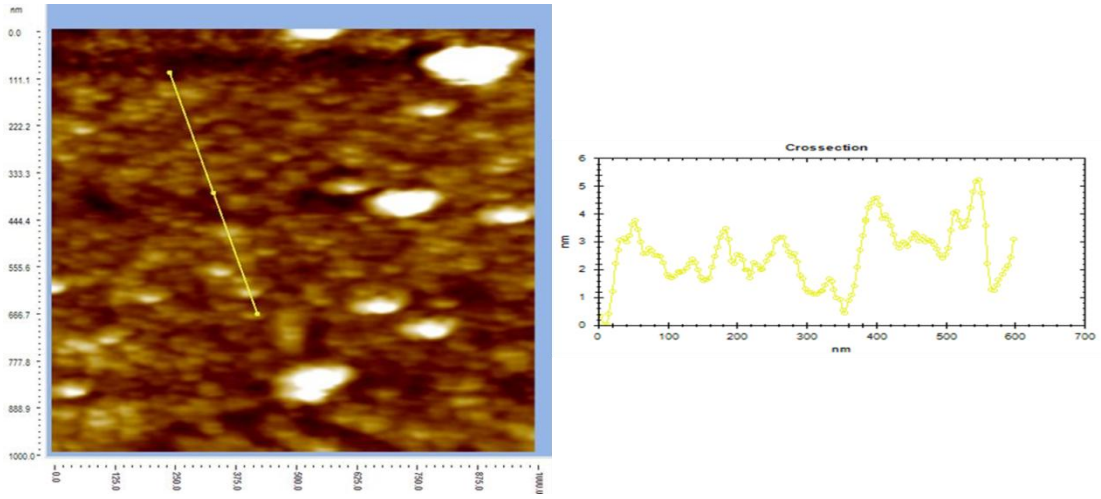

Figure 4.20: AFM image of the optical grade glass

The AFM image of peptide-covered glass is quite different from bare glass image. The line profile graph gives a height of 7-8 nm, which higher than the roughness of the bare glass (Figure 4.21).

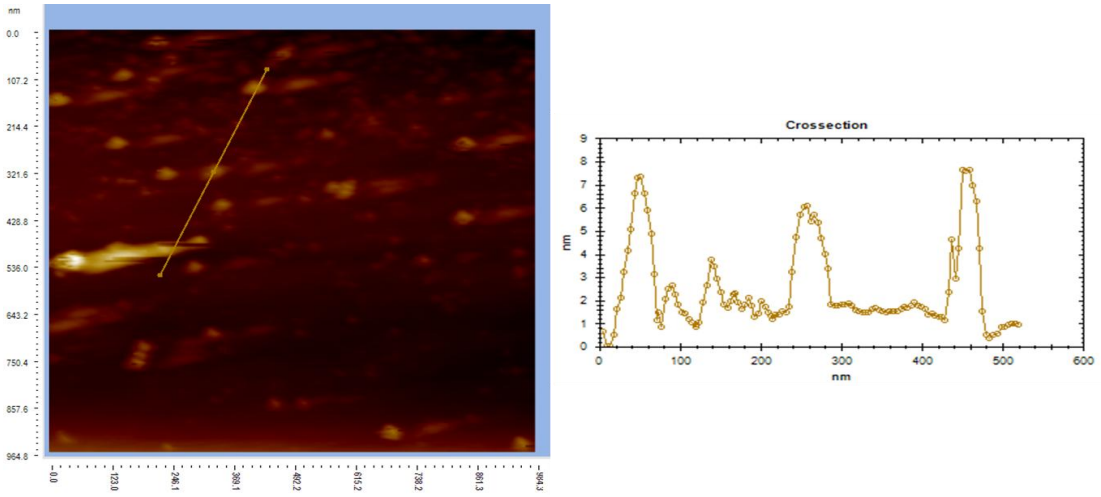

Figure 4.21: AFM image of the peptide covered optical grade glass.

4.4.1.3 LB films bilayer structures obtained by peptide and gold nanoparticle deposition following each other

After providing Langmuir Blodgett film of peptides on optical grade glass, gold nanoparticles are covered as a second layer on top of them. The final thin film, exhibit a hybrid two-layer structure, is characterized by AFM. The height of the surface structure is approximately 10-12 nm (Figure 4.22) which is higher than that of the peptide thin film.

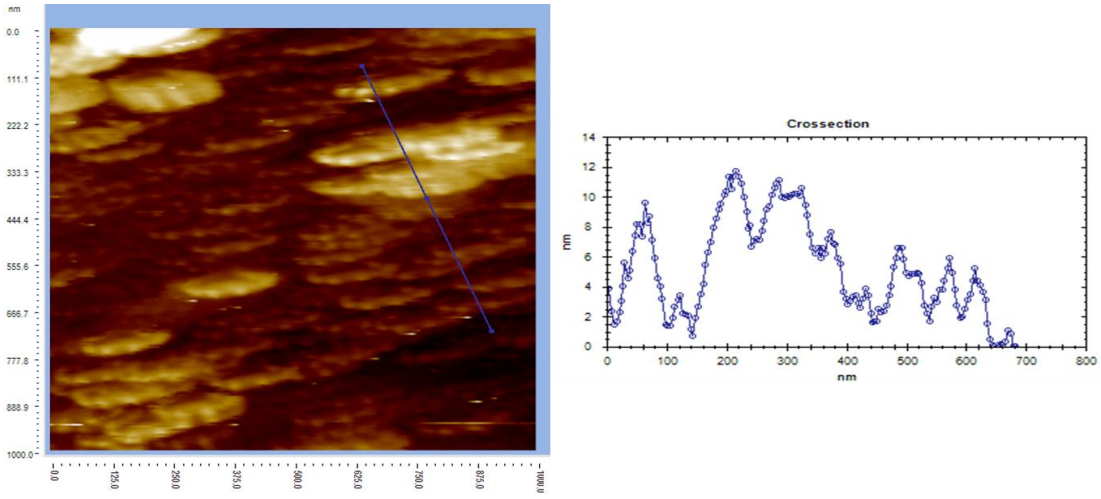

Figure 4.22: AFM image of optical grade glass covered by peptide and gold nanoparticles, respectively.

4.4.1.4 LB films obtained from peptide functionalized gold nanoparticles

7 nm gold colloid nanoparticle solution is mixed with peptides dissolved in 1:1 EtOH:ultrapure water. 250 µl of the solution is spread on the water for the formation of a monolayer and then transferred on the optical grade glass. The produced thin film is investigated by AFM (Figure 4.23).

The structure of LB film of peptide functionalized gold nanoparticles is similar with the LB film, which are produced by bi-layer transferring peptides and gold nanoparticles. However, the height disturbance of peptide functionalized gold nanoparticles is lower than the hybrid two-layer LB film.

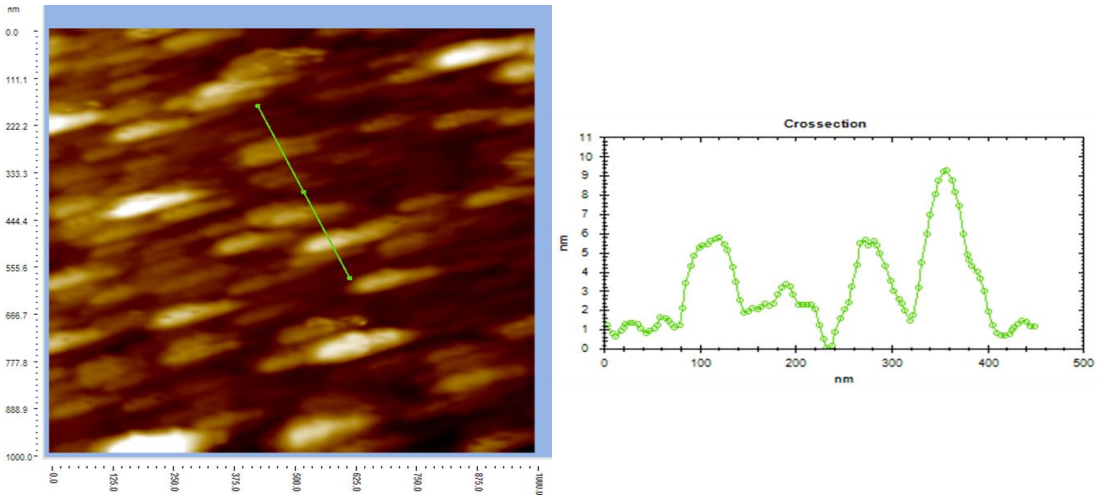

Figure 4.23: AFM image of the Langmuir Blodgett film of the peptide functionalized gold nanoparticles o optical grade glass

4.4.2 Scanning electron microscopy investigation of the LB films

SEM images of the LB films were measured after deposition of a very thin film of platinum for ensuring conductivity. The poor conductivity of the films can be attributed to two main situations. First, the gold nanoparticles on the surface do not exhibit continuity. Second, the peptide linkage to the gold nanoparticles may be reducing the conductivity. Therefore, we needed to cover a thin conductive layer on top of LB films before SEM imaging.

4.4.2.1 LB films bilayer structures obtained by peptide (in 1:1 EtOH:ultrapure water) deposition and incubation in gold nanoparticle solution

LB film of peptide (AuBP4-QBP2) is expected to assemble as a pattern on optical grade glass due to its affinity to bind on silicate surface. LB films of peptide were incubated in gold colloid nanoparticles with 2 nm and 7 nm. SEM images of the films were obtained in different magnifications: 10000 and 50000 (Figure 4.24).

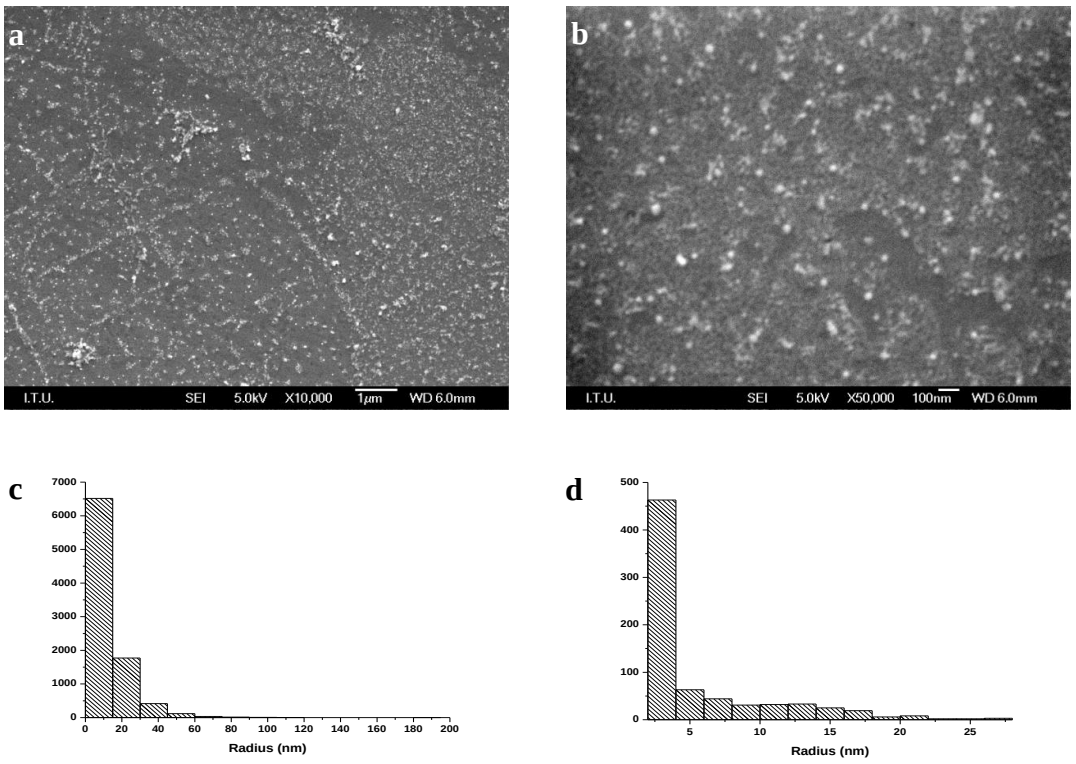

Figure 4.24: SEM images of the peptide LB films produced in 1:1 EtOH:ultrapure water and then incubated with 2 nm gold colloid solution at different magnifications a) 10000 b) 50000. Size distribution of the particles on the image: c) 10000 d) 50000.

The SEM image of the film consist of peptide in 1:1 EtOH:ultrapure water and gold nanoparticles (2nm) was shown in Figure 4.24. Although there are some agglomerations on the surface, the pattern of the film can be seen easily due to the gold nanoparticles that bind on the peptides specifically and overlay the pattern of the peptide LB film. The size distributions of the particles are given in the images (Figure 4.24-c and d). According to these analyses, the size distribution of the particles does not show a normal distribution because of the aggregation of the particles. This aggregation may be result of the peptide linkage. These results are parallel with the UV/Vis absorptions (Section 4.1.3).

The LB films consisting produced by using peptides dissolved in 1:1 EtOH:ultrapure water and then incubated with gold nanoparticles (7nm) are presented in Figure 4.25. However, the nanoparticles seem much larger than their original sizes. This agglomeration may be caused through peptide linkage between the nanoparticles that is an expected result from UV/Vis Spectroscopy. The size distributions of the particles on the surface have a homogenous distribution around 25-30 nm. This is 4 times larger than the original size of gold nanoparticles.

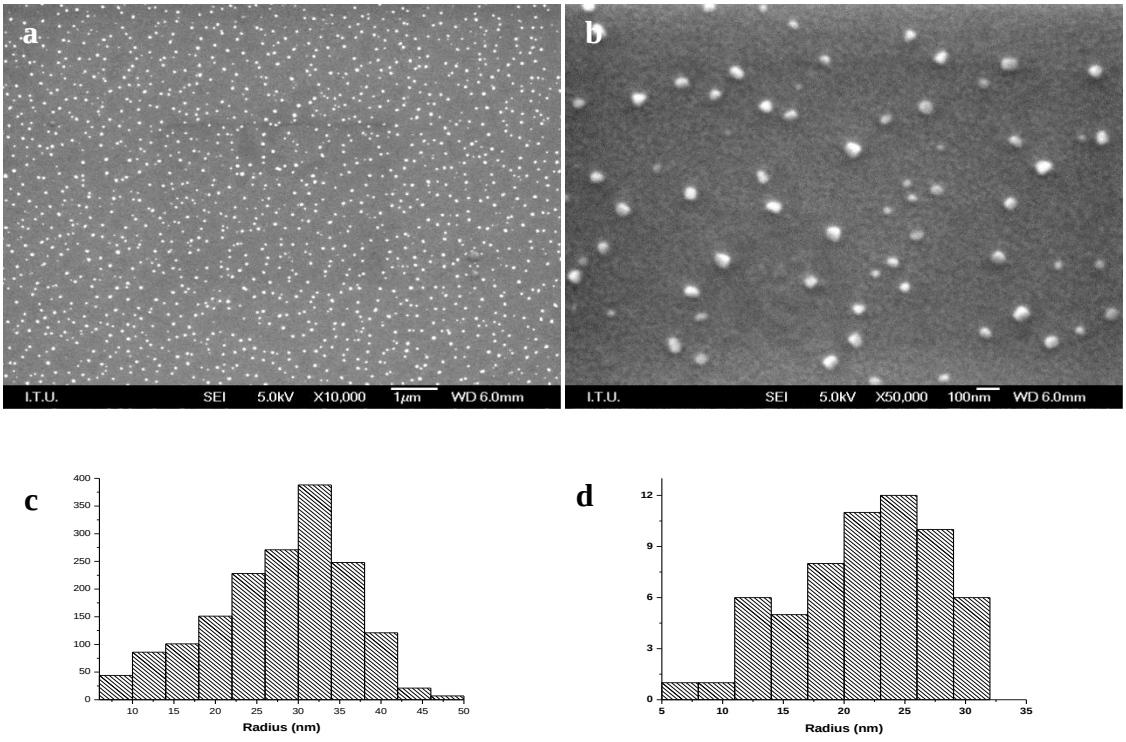

Figure 4.25: SEM images of the peptide LB films produced in 1:1 EtOH:ultrapure water and then incubated with 7 nm gold colloid solution at different magnifications a) 10000 b) 50000. Size distribution of the particles on the image: c) 10000 d) 50000.

4.4.2.2 LB films bilayer structures obtained by peptide (in ethanol) deposition and incubation in gold nanoparticle solution

In Figure 4.26, the LB films that were prepared by dissolving peptides in pure EtOH solutions and then incubated with gold nanoparticles (2nm) are shown. The pattern of the peptides can be observed at the background; however, most of the gold nanoparticles are agglomerated as bigger particles. According to results of size distributions, this agglomeration is confirmed. Although most of the particles are in smaller sizes, there are particles that are much more bigger than the others. Thus, the size distributions do not show a normal distribution. However, the distribution of small sized nanoparticles on the surface gives information about the pattern of the peptides on the surface.

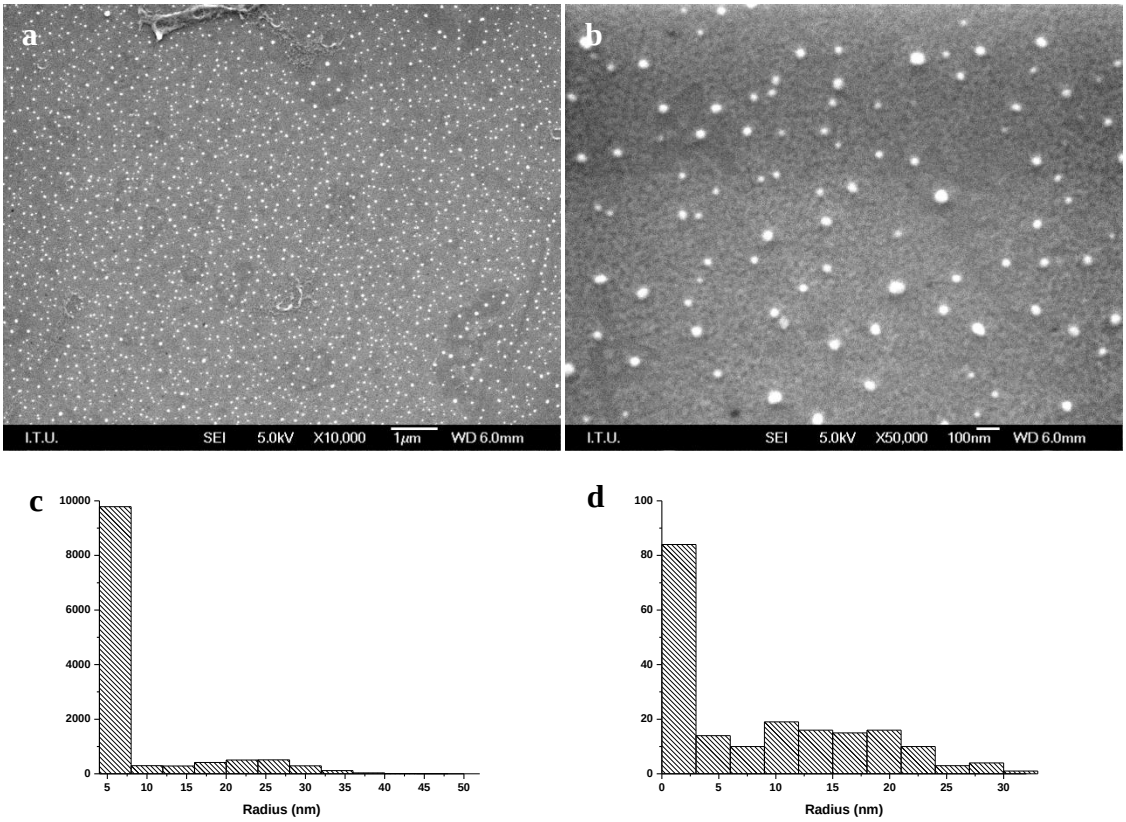

Figure 4.26: SEM images of the peptide LB films produced in pure EtOH and then incubated with 2 nm gold colloid solution at different magnifications a) 10000 b) 50000. Size distribution of the particles on the image: c) 10000 d) 50000.

The SEM images in the Figure 4.27 shows the LB films of peptides formed in EtOH and then incubated with gold nanoparticles (7 nm). The pattern of gold nanoparticles on the surface is regular; however, there are some defects at the background that

indicating discontinuous in the peptide layer of the film. This situation may be caused by two reasons. First, the LB film of peptides does not deposited to the solid surface properly. Second, the EtOH may cause the deformation or degradation of the peptides. Therefore, the peptides does not setting up a LB film on the surface. The particle sizes have normal distribution, but they are two times bigger than the original size of the particles that indicates the aggregation of the gold nanoparticles with peptides.

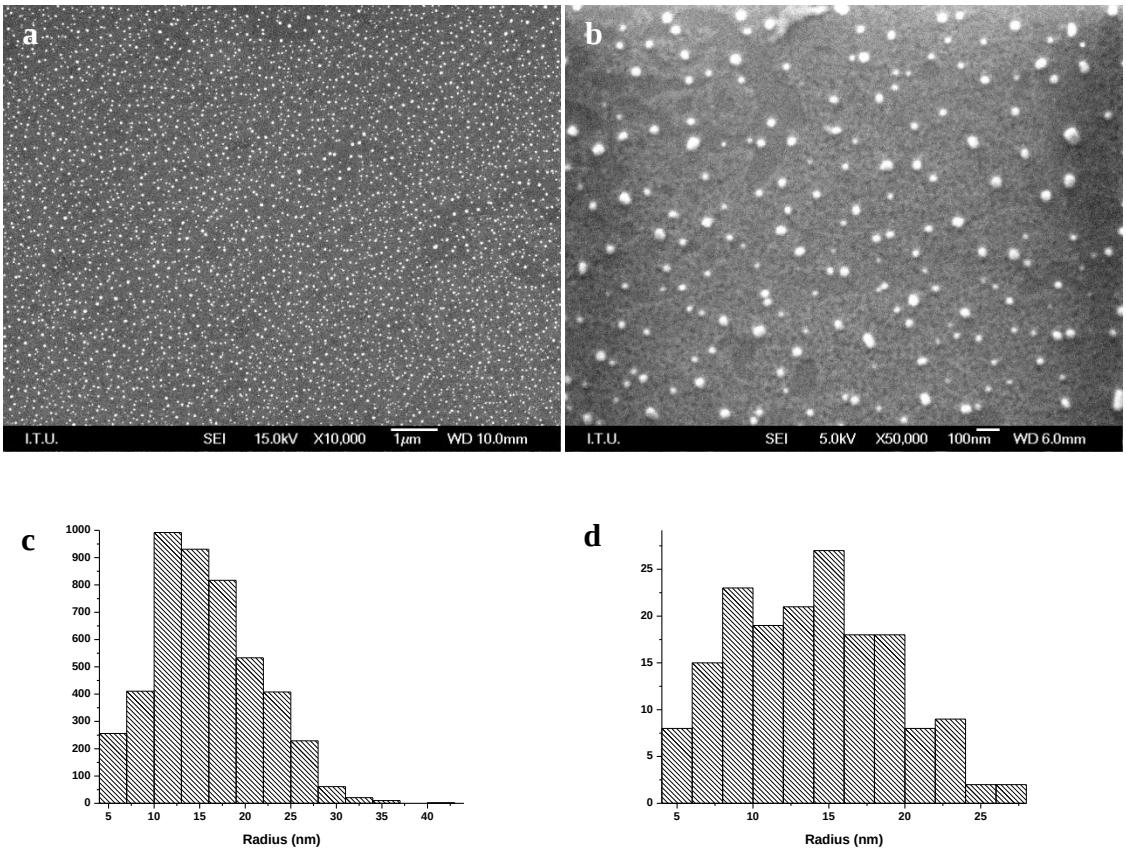

Figure 4.27: SEM images of the peptide LB films produced in pure EtOH and then incubated with 7 nm gold colloid solution at different magnifications a) 10000 b) 50000. Size distribution of the particles on the image: c) 10000 d) 50000.

The pattern of the peptides in 1:1 EtOH:ultrapure water can most easily observed with 2 nm gold NPs since smaller sized gold NPs can specifically attached to the peptides. On the other hand, with 7 nm gold NPs, although the peptide pattern can observed on background, gold nanoparticles cannot overlay the peptide pattern, as successively as 2 nm particles.

The patterns of peptides formed in pure EtOH have some defects. This can be caused from disruption of peptide structure by EtOH. As a result, binding affinity of the peptide to silicate and gold may be reduced.

5. CONCLUSIONS

In this study, we investigated the LB film formation of bi-functional peptides on silicate based surfaces and functionalization of this films with gold nanoparticles.

In the first part of the study, spectroscopic characterization of gold nanoparticle solutions, peptide solutions in different solvents and peptide functionalized gold nanoparticle solutions are conducted. 7 nm gold nanoparticles have an absorption spectra in UV/Vis range to give us information about the spectral differences with the addition of peptide. On the other hand, 2 nm gold nanoparticles do not give a response in UV/Vis range. Because of this reason, we used 7 nm gold nanoparticles in this part of the experiments. Peptide solution in pure water, 1:1 EtOH:ultrapure water and ethanol solvent do not give a absorption spectrum in UV/Vis range. The absorption peak of the bare gold nanoparticle solutions is at 522 nm, and red shift is observed with addition of peptide. The red shift of the absorption spectra indicates the aggregation of gold nanoparticles due to electrostatic repulsion effects. The aggregation of the gold nanoparticles is influenced with the solvent type of peptide solutions. More aggregation of gold nanoparticles is observed in the mixture containing peptide solutions with pure water. This indicates that peptides in pure water solutions have much more affinity to bind to gold nanoparticles. On the other hand, in peptide solutions with ethanol solvent, the aggregation amount of the gold nanoparticles is much less than other solutions. Although this is a good result for the formation of LB film easily, this lead to loss of affinity of peptides to bind gold nanoparticles. The 1:1 EtOH:ultrapure water solution shows less aggregation than water solution and higher aggregation than ethanol solution. Relying on the experiments conducted on time dependent aggregate in this solution, the peptide solution prepared with 1:1 EtOH:ultrapure water is determined to be suitable for LB film formation.

FT-IR analyses of the peptide solution are measured in order to get information about peptide structures in the solutions. Amide I and amide II peaks indicates the peptide formation, however this peaks are also seen in FT-IR spectrum of water. For this

reason, the FT-IR spectra of the solution are compared to pure water and pure ethanol spectra. The peptide solution prepared with pure ethanol gives an specific peak at 1641 cm^{-1}indicating the amide bonds which is not seen in ethanol spectrum. This peak is also seen in other peptide solutions containing water, but pure water spectra have the same characteristic peak. It is hard to distinguish the peak of water and peptide. In any case, we can ensure that the peptide are in the solutions due to the characteristic peaks of peptides are observed.

The mixtures of peptide and 2 nm gold nanoparticles are also investigated with FT-IR. The solutions containing gold nanoparticles and peptides with pure water in different amounts give exactly the same spectra. The amount of peptide or gold nanoparticles does not affect the FT-IR results. Nevertheless, the intensity of peaks decrease in other solutions while decreasing the amount of peptide solution in the mixture and peaks are shifted. This effect is observed more easily in the mixture containing peptide with pure ethanol. The amide bonds shift from 1641 cm^{-1} to 1653 cm^{-1}. The deconvolution of the amide bonds may give more detailed information about the peptide structures. While the peak at 1641 cm^{-1} represents the uncoordinated structures, the peak at 1653 cm^{-1}indicates the helix structure. Therefore, this can be interpreted as the change in peptide conformations, which may be attributed to the binding effects of gold nanoparticles or destructive effects of the solvent.

The LB film formation ability of the peptide solutions, gold nanoparticle solutions and peptide functionalized gold nanoparticle solutions are investigated with the surface pressure/area isotherms. The peptide solutions containing ethanol as a volatile agent gave good surface pressure/area isotherms including all distinct regions of a typical isotherm. After spreading the peptides on the solution of water surface, evaporation of ethanol helps the peptide molecules to move up to water surface and form a monolayer on the surface. Surface pressure/area isotherms give us information about the necessary amount of the solution to form a good monolayer. Limiting molecular areas is obtained by extrapolation of the surface pressure/area isotherm to the x-axis, which indicates the area for much closed packed form of the molecules. In order to form a monolayer on the water surface, gold nanoparticle solutions are diluted with ethanol in 50%. The isotherms graphs are obtained both 2 nm and 7 nm gold nanoparticle solutions and their isotherms are similar. The

isotherms of the mixtures including gold nanoparticles (2 nm and 7 nm) and peptide solutions with 1:1 EtOH:ultrapure water are also measured and their limited molecular areas are calculated.

The transfer of the molecules to solid surface is performed by using these informations from isotherms. Optical grade glass is used as substrate. The transfer of the molecules is performed while rising up to the substrate. The transfer ratio must be close to 1 indicating the better transfer. The transfer ratio for all solutions is measured close to 1 and greater than 1 that represent an instability in the film formation for these cases.

The LB films are characterized with AFM and FEG-SEM. All LB films of peptides for AFM images are prepared using 1:1 EtOH:ultrapure solutions. The film formation of peptides are imaged by AFM which indicates the peptide height is around 3-4 nm. After transferring the LB film of 7 nm gold nanoparticles as a second layer on to peptide covered substrate, the thickness become 8-9 nm. This increment is not as higher as expected, however it can be explained by the conformational change of peptide after binding to gold nanoparticles. In order to confirm this, the AFM images of the LB film prepared using peptide functionalized gold nanoparticle (7nm) solution are measured. Approximately the same thickness (7-8 nm) is measured for these films. The pattern is also similar to the LB films prepared using peptide and gold nanoparticles, respectively and using peptide functionalized gold nanoparticles.

FEG-SEM images gave us more information about the pattern of the films. The LB films are prepared on the optical grade glass with peptides with different solvents: 1:1 EtOH:ultrapure water and pure ethanol and then incubating in gold nanoparticle solutions (2 nm and 7 nm) and finally washing with ultrapure water. The effects of the peptide solvent and size of nanoparticles on the formation of LB film is compared using FEG-SEM images. The pattern of the peptides with 1:1 EtOH:ultrapure water can most easily observed with 2 nm gold nanoparticles due to small sized gold NPs that specifically attached to peptides. Nonetheless, with 7 nm gold NPs, the peptide pattern is not as clearly observed as 2 nm particles since these particles are larger than the peptides. The patterns of peptides in ethanol solvent have some defects. This can be caused from disruption of peptide structure by ethanol,

which can result in a decrease of the binding affinity of the peptide to quartz and gold.

As a conclusion, the binding affinity of bi-functional peptides to nanoparticles is confirmed by absorption spectra. A suitable solvent for peptides, which does not change the structure of the peptide and affinity to bind gold and silicate based materials and has properties for the best formation of the LB film, is chosen as 1:1 EtOH:ultrapure water utilizing analysis such as UV/Vis spectra, FT-IR and Surface Pressure/Area isotherms. LB film formation of the peptides, gold nanoparticles and peptide functionalized gold nanoparticles has been shown and characterized using AFM and FEG-SEM. As a result, organic – inorganic hybrid structures has been obtained with bi-functional peptide that has ability to bind gold and silicate based materials specifically utilizing LB method without using any harsh conditions and chemicals.

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APPENDICES

APPENDIX A: Surface pressure change and transfer graphs during deposition of LB film onto solid surface
APPENDIX B: AFM study of LB film of gold nanoparticles in size of 5 nm on mica surface

APPENDIX A: Surface pressure change and transfer graphs during deposition of LB film onto solid surface

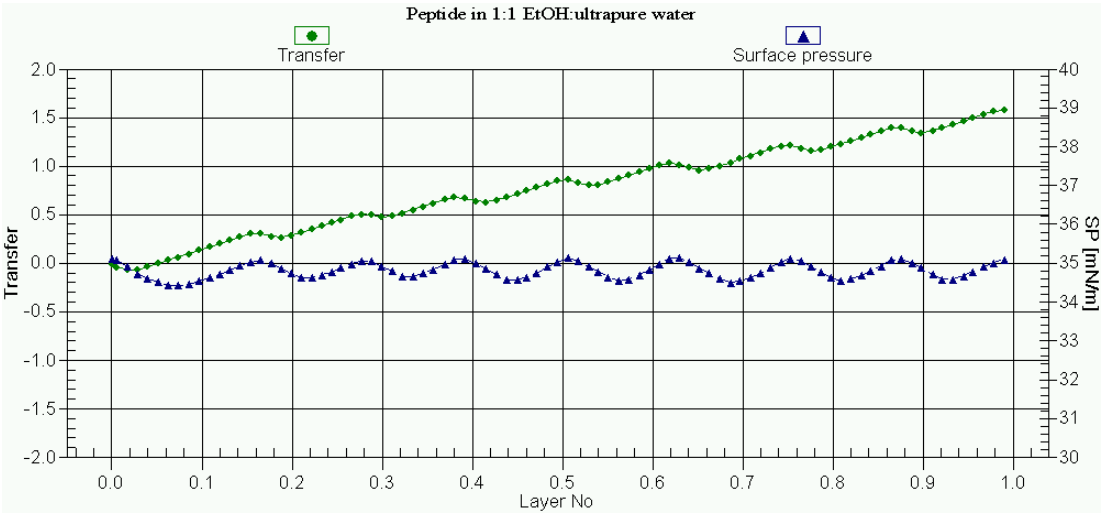

Figure A.1: Surface pressure change and transfer graph of peptide in 1:1 EtOH:ultrapure water during LB film formation.

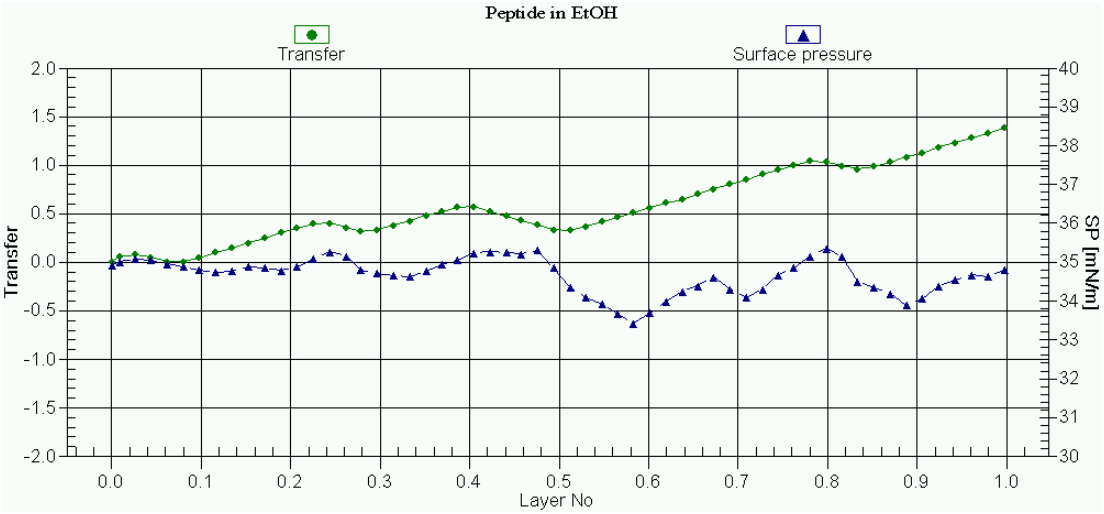

Figure A.2: Surface pressure change and transfer graph of peptide in EtOH during LB film formation.

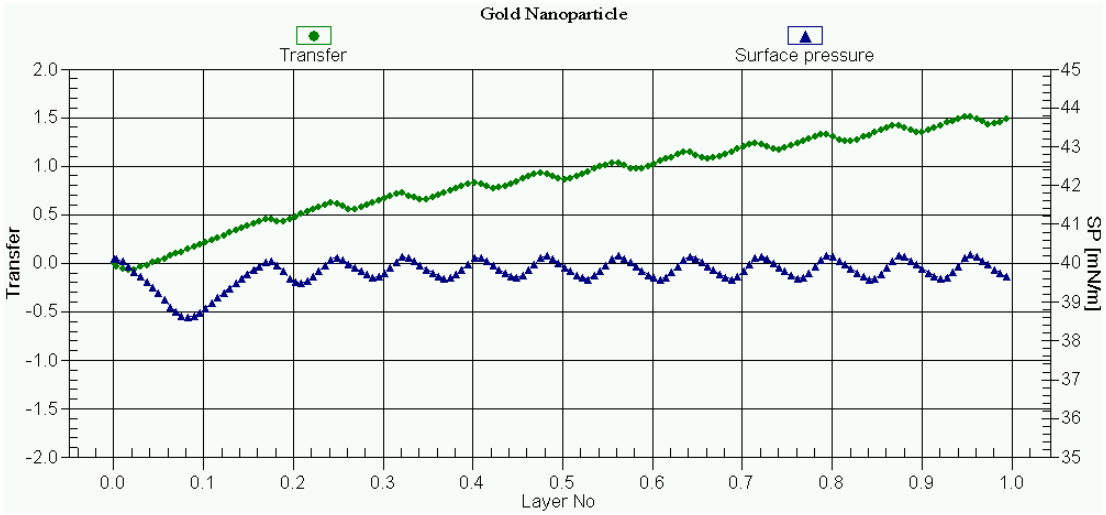

Figure A.3: Surface pressure change and transfer graph of gold nanoparticles during LB film formation.

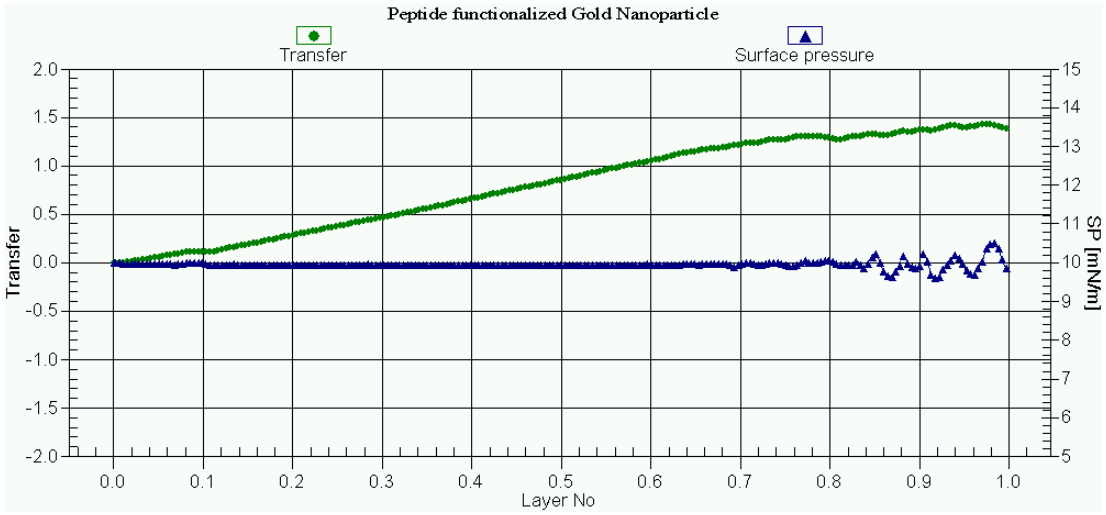

Figure A.4: Surface pressure change and transfer graph of peptide functionalized gold nanoparticles during LB film formation.

APPENDIX B: AFM study of LB film of gold nanoparticles in size of 5 nm on mica surface

Gold nanoparticles are covered on the mica surface using Langmuir Blodgett technique and characterized by AFM. However, imaging of gold nanoparticles with silicon AFM tip is quite difficult due the electrostatic effect of the particles to the tip. Mica surface is clearly flat, nanoparticles can be seen easily in Figure B.1, and their cross-section graph is given below the AFM image.

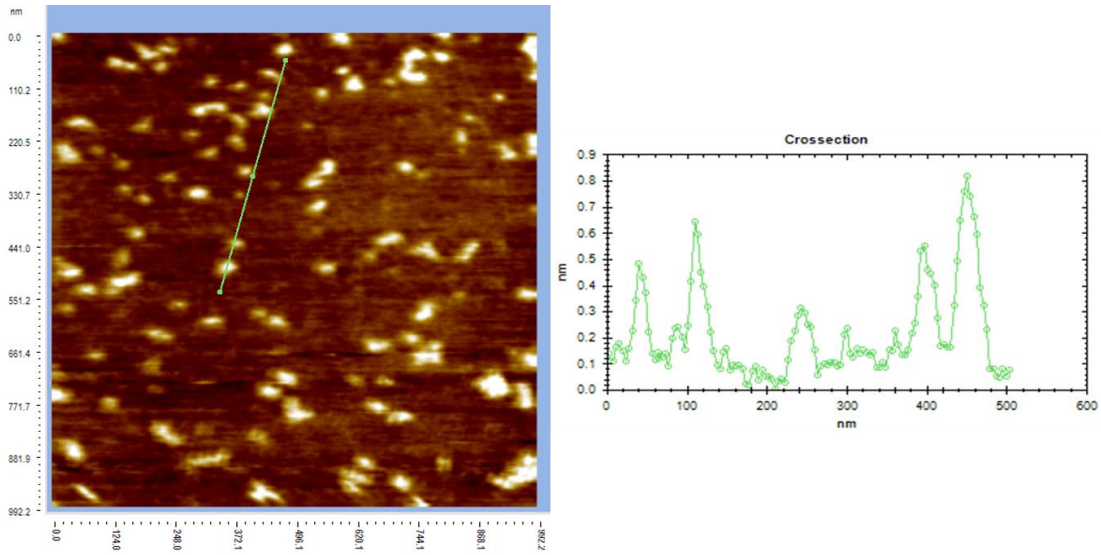

Figure B.1: AFM image of gold nanoparticles on mica surface.

CURRICULUM VITAE

Name Surname: Nur Mustafaoğlu

Place and Date of Birth: Bursa / 13.02.1987

E-Mail: nur.mustafaoglu@gmail.com

B.Sc.: ITU, Molecular Biology and Genetics, 2010

ITU, Physics Engineering, 2011

Professional Experience and Rewards:

- TEV scholarship, Turkish Educational Foundation (TEV), January 2011 – June 2012
- Prime Minister's Scholarship, Credit Residential Institution (KYK), January 2006 - June 2010

List of Publications and Patents:

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