ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

MOLECULAR SIMULATION OF FIBRINOGEN ADSORPTION ONTO POLYURETHANE SURFACES

M.Sc. THESIS

Kamal PORHONAR BONAB

Department of Chemical Engineering

Chemical Engineering Programme

SEPTEMBER 2013

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

MOLECULAR SIMULATION OF FIBRINOGEN ADSORPTION ONTO POLYURETHANE SURFACES

M.Sc. THESIS

Kamal PORHONAR BONAB

Department of Chemical Engineering

Chemical Engineering Programme

Thesis Advisor: Assoc.Prof.Dr.M. Göktuğ AHUNBAY

FEBRUARY 2014

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

POLİÜRETAN YÜZEYLERE FİBRİNOJEN ADSORPSİYONUNUN MOLEKÜLER SİMÜLASYONU

YÜKSEK LİSANS TEZİ

Kamal PORHONAR BONAB
(506101038)

Kimya Mühendisliği Anabilim Dalı

Kimya Mühendisliği Programı

Tez Danışmanı: Doç.Dr. Göktuğ AHUNBAY

ŞUBAT 2014

Kamal porhonar bonab, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID **506101038**, successfully defended the **thesis** entitled "**MOLECULAR SIMULATION OF FIBRINOGEN ADSORPTION ONTO POLYURETHANE SURFACES**", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Assoc.Prof.Dr. M. Göktuğ AHUNBAY** İstanbul Technical University	....
Jury Members :	**Assist.Prof.Dr. Özge KÜRKÇÜOĞLU** İstanbul Technical University	....
	Assoc.Prof. Dr. Mehmet SAYAR Koç University	....

Date of Submission : 04 June 2013
Date of Defense : 09 September 2013

FOREWORD

I want to record my sincere thanks to all contributors whose cooperation and assistance helped me through my M.Sc. study reported herein. First of all, thanks to my supervisor Assoc. Prof. Dr. M. Göktuğ Ahunbay for inspiring me and for providing such an intresting project for me to work on. Thank you for your direction and your help in the development for my mind. Thank you for teaching me. I am honored to have worked with you and I have learned more from you than you know.

I want to record my special thanks to Barış Demir who always helped and encouraged me. I also wish to thank Ramin Ekhtiari Salmas for his great helps and sympathy.

I would also thank to the Department of Chemical Engineering at the Istanbul Technical University for giving me the opportunity to come here and for providing an exciting and dynamic enviroment for me to mature as a scientist.

Finally, I want to convey my thanks to my family for their support and encourage and understanding.

June 2013 Kamal PORHONAR BONAB

TABLE OF CONTENTS

Page

FOREWORD ... **vii**
TABLE OF CONTENTS ... **vii**
ABBREVIATIONS ... **x**
LIST OF TABLES ... **xiii**
LIST OF FIGURES ... **xiv**
SUMMARY ... **xvii**
ÖZET ... **xix**
1. INTRODUCTION ... **1**
2. POLYMERIC BIOMATERIALS AND PROTEIN INTERACTIONS ... **5**
  2.1 Biomaterials ... 5
  2.2 Polyurethanes ... 6
  2.3 Proteins ... 8
  2.4 Modeling of Protein-Surface Interactions ... 13
  2.5 Key Factors for the Molecular Simulation of Peptide/Protein- Surface interaction ... 14
  2.6 Solvation Effects ... 15
    2.6.1 Simulation with explicit solvent. ... 16
    2.6.2 Simulation with implicit solvent. ... 17
  2.7 Protein-Surface Interaction ... 19
  2.8 Protein Orientation ... 20
  2.9 Surface Interaction ... 21
3. THE BIOMATERIAL INTERFACE ... **22**
  3.1 Protein Adsorption ... 22
  3.2 Protein Adsorption onto Polyurethane ... 23
  3.3 Fibrinogen Adsorption ... 24
4. MOLECULAR SIMULATION ... **27**
  4.1 Molecular Mechanics ... 28
  4.2 Molecular Dynamics ... 30
  4.3 Forcefield Parameters ... 33
  4.4 Molecular Simulation of Ensembles ... 34
5. SIMULATION METHODS ... **37**
  5.1 System ... 37
  5.2 Modeling Protein−Surface Interactions ... 39
  5.3 Protein-Surface Interaction Determination ... 39
  5.4 Initial Adsorption Stage in the Dielectric Medium ... 42
  5.5 Calculation of Changes in Phi (Φ) and Psi (ψ) Torsion Angles ... 44
6. ANALYSIS OF ADSORPTION ENERGETICS AND ORIENTATION ... **47**
  6.1 Adsorption in the Implicit Water Molecules ... 47
  6.2 Adsorption in the Explicit Water Molecules ... 54

7. CONCLUSION…………………………………………...………………. 65
REFERENCES………………………………………………………………. 67
CURRICULUM VITAE……………………………………………………….. 71

ABBREVIATIONS

AMBER : Assisted Model Building with Energy Refinement
CED : Cohesiv Energy Density
CHARMM : Chemistry at HARvard Macromolecular Mechanics
COMPASS : Condensed-phase Optimized Molecular Potentials for Atomistic Simulation Studies
CO : Castor Oil
COM : Center of Mass
CVFF : Consistent-Valence Force field
ESFF : Extensible Systematic Force Field
HSA : Human Serum Albumin
HDI : Hexamethylene diisocyanate
IgG : Gamma-globulin
GROMOS : Groningen molecular simulation
kDa : kiloDalton
MM : Molecular Mechanic
MD : Molecular Dynamic
MC : Monte Carlo
NPT : Isothermal - Isobaric Ensemble
NVT : Canonic Ensemble
NVE : Microcanonical Ensemble
PEG : Polyethylene Glycol
PCFF : Polymer-Consistent Force Field
PB : Poisson–Boltzmann
PDB : Protein Data Bank
PU1 : Polyethylene Glycol + Hexamethylene diisocyanate Copolymer
PU2 : Castor Oil + Hexamethylene diisocyanate Copolymer
PVA : Polyvinil alcohol
RMSD : Root-Mean-Square Deviation
UFF : Universal Force Field

LIST OF TABLES

Page

Table 2.1 : Amino acid classifications...…………………….………………..... 10
Table 2.2 : Blood protein properties from considering protein primary structure.....11
Table 2.3 : Strenghs of bonds controlling tertiary protein structure. ……….……..12
Table 5.1 : Fibrinogen C-terminus, amino acid number and polarity. …………......41
Table 6.1 : Number of amino acids within 3Å, 5Å and 7Å at 6 different…...……... Orientation. ..……….…………………….………..……………..…50
Table 6.2 : Graphics slope values of interaction energy in relation to the number…. of amino acids and atomic. ..……..………..………….……………... 53
Table 6.3 : Graphics slope values of strain energy in relation to the number of……. amino acids and atomic…….………………….……………...……......54
Table 6.4 : Number of amino acids within 3Å, 5Å and 7Å at 6 orientation……..… 58
Table 6.5 : N amino acids within an adsorption layer of 3Å, 5Å, 7Å in implicit…… and explicit system…….……………………………...……………... 58

LIST OF FIGURES

Page

Figure 2.1 : Polyurethane structure and urethane group…..………………….……….. 7
Figure 2.2 : Amino acid group..…….…………………………….….………….… 8
Figure 2.3 : Amino acid residues..………………………………………….……. 9
Figure 2.4 : Molecular simulation studies on adsorption. ……………………..…... 13
Figure 2.5 : An all-atom antibody molecule with (a) end-on or (b) head-on orientation on a charged surfaces..……..…………………...……….. 21
Figure 4.1 : Atom bond, angles..……………………………………………….. 29
Figure 4.2 : A simple flow diagram of standard MD algorithm...………………… 31
Figure 5.1 : Polyurethane monomers used in this study...………………………… 38
Figure 5.2 : Bulk polymer matrices (a) PEG-HDI, (b) CO-HDI..……………....... 38
Figure 5.3 : Structure of human gamma fibrinogen 30 KD carboxyl terminal fragment..…………………….……………………………….…… 40
Figure 5.4 : Residues sequence of gamma fibrinogen 30 KD carboxyl…………….. terminal fragment..……………………………………..……..... 40
Figure 5.5 : Fibrinogen module inscribed in a rectangular prism...………………… 43
Figure 6.1 : The 6 initialtrial geometries of fibrinogen placed close to the PU1 polyurethane surface. …………………………..……..………… 48
Figure 6.2 : The 6 initialtrial geometries of fibrinogen placed close to the PU1 polyurethane surface. ……………………………………………..… 48
Figure 6.3 : Interaction energies with respect to protein amino acids within an adsorption layer of 3Å, 5Å and 7Å. ..………………..…...…....… 51
Figure 6.4 : Interaction energies with respect to protein amino acids atoms within an adsorption layer of 3Å, 5Å and 7Å. …..…………………….…... 52
Figure 6.5 : Strain energies with respect to protein amino acids within an adsorption layer of 3Å, 5Å and 7Å……………………………….… 52
Figure 6.6 : Strain energies with respect to protein amino acids within an adsorption layer of 3Å, 5Å and 7Å. ..………….……………………... 53
Figure 6.7 : Interaction energy in relation to the number of amino acid and the atomic graphic. …….……………………………………..……..... 54
Figure 6.8 : Strain energy in relation to the number of amino acid and the atomic graphics…………………………………………..……..………… 54
Figure 6.9 : Configurational changes of fibrinogen upon adsorption on the surfaces……………………………………………………..…..... 55
Figure 6.10 : Center of mass of protein movement……………………..……... 55
Figure 6.11 : RMSD values of γFg in PU1 and PU2 surfaces.................................. 57
Figure 6.12 : Protein amino acids psi (ψ) bond angles average and absolute changes after adsorption……………………………………….... 59
Figure 6.13 : Protein amino acids phi (Φ) bond angles average and absolute changes after adsorption…………………………………………….. 59

Figure 6.14 : Phi (Φ) and psi (ψ) Ramachandran plots bond angles before and after adsorption……………………………………………..……....... 61
Figure 6.15 : Phi (Φ) and psi (ψ) Ramachandran plots bond angles before and after adsorption……………..……………………….…..…..…....... 62

MOLECULAR SIMULATION OF FIBRINOGEN ADSORPTION ONTO POLYURETHANE SURFACES

SUMMARY

Many medical devices and biomaterials, come into contact with the internal surfaces of the body (such as surgical tools and implants). The interaction between a biomaterial and living tissue occurs in a narrow interface zone. Therefore, biocompatibility of these devices will largely be determined by the material surface properties. These include: surface roughness, wettability, chemical composition and electrical charge. In addition, the presence of leachables (low molecular weight fragments, additives, contaminants) is of great importance.

In this work, we used poly(ethylene glycol) PU1 and castor oil copolymers of hexamethylene diisocyanate-based polyurethanes (PEG-HDI and CO-HDI, respectively) PU2 as biomaterials and we focused on the adsorption characteristics of polyurethanes, which constitute a very appealing class of polymers because of their high biocompatibility and excellent physical and mechanical properties. Then, we compared their mechanisms for fibrinogen adsorption using molecular mechanics and molecular dynamics simulations. These versatile polymers have been widely used in wound dressings as well as in cardiovascular and breast implants.

The crystal structure of a 30 kDa C-terminus γ-chain fragment of fibrinogen was obtained from the Protein Data Bank (PDB ID# 1FID), and was placed near polymer surfaces in six different orientations.

Simulations firstly were carried out in an effective dielectric medium, and PU1 polymer surfaces were kept rigid and PU2 surface was kept flexible. Orientations with minimum interaction energies were selected and carried out in molecular dynamic (MD) simulation. For each orientation, energy minimizations were carried out to analyze changes in the interaction energy between the surface and polymer due to adsorption, and the number of amino acids within a distance of 3,5 and 7Å from the surface was determined. Next, a linear model was fitted to the interaction energy as a function of the number of amino acids. The slope in the model yielded the absolute interaction energies between the protein and the surface, was used as a basis for the comparison of the surface biocompatibility, larger slope values indicating larger interaction strength. Beside these linear models, COM (center of mass) plots and RMSD (Root Mean Square Deviation) plots for showing distance movement as a function of time were prepared and finally Ramachandran diagrams for observing secondary structure changes were prepared.

Results from linear models, COM and RMSD plots and Ramachandran diagrams cleared that fibrinogen was adsorbed more strongly on PU2 surface in explicit water compared to PU1 surface and also PU1 shows weaker adsorption in explicit water system compared to implicit water system. By these results in hand we see that PU2 surface are very better than PU1 surface for fibrinogen adsorption.

POLİÜRETAN YÜZEYLERE FİBRİNOJEN ADSORPSİYONUNUN MOLEKÜLER SİMÜLASYONU

ÖZET

Biyomalzemeler insan vücudundaki canlı dokuların işlevini yerine getirmek ya da desteklemek amacıyla kullanılan malzemeler olup, modern tıp uygulamalarında canlıların yaşamlarını sürdürmelerini sağlamak, yaşam koşullarını iyileştirmek ve tedavi sürelerini kısaltmak amacıyla kullanılmaktadırlar. Son yıllarda, biyomalzeme bilimi büyük ilerleme kaydetmiştir, ancak biyolojik sistemlerle etkileştiğinde uyumluluğu yüksek olan yeni malzemelerin geliştirilmesi için çalışmalar sürmektedir. Biyouyumluluk, bir biyomalzemenin en önemli özelliğidir. Biyouyumlu malzeme vücutla uyumlu malzeme, yani kendisini çevreleyen dokuların normal değişimlerine engel olmayan ve dokuda istenmeyen tepkiler (iltihaplanma, pıhtı oluşumu) meydana getirmeyen malzemelerdir. Ancak halen mükemmel biyouyumluluğa sahip malzemenin sentezlenmesi gerçekleşmemiştir ve bu amaçla yapılan çalışmalar sürmektedir. Polimerler değiĢik Ģekil ve biçimlerde hazırlanabilmelerinden dolayı biyomalzeme olarak geniĢ bir kullanım alanına sahiptirler. Polimer malzemelerin kullanıldığı bazı alanlar; yapay kalp, damar ve kalp kapakçıları, göğüs implantları, kontakt lensler, ortopedik malzemeler, diyaliz üniteleri Ģeklinde özetlenebilir. Ancak yüksek avantajları yanı sıra polimerler yine de mükemmel biyouyumlu malzemeler değillerdir. Bazı uygulamalarda örneğin ortopedide mekanik dayanımlarının zayıf olması, sıvıları yapılarına alarak ĢiĢebilmeleri veya zehirli ürünler yayabilmeleri polimerlerin olumsuz özellikleri arasında yer almaktadır. Ayrıca bahsedilen tüm bu inorganik ve organik malzemelerin hibrit olarak kullanılması ile kontrol edilebilir hızlarda bozunabilen, kontrol edilebilir yüzey özelliklerine sahip kompozit malzemeler de hazırlanabilir. Ġnorganik ve organik maddelerin miktarı değiĢtirilerek malzeme özellikleri istenilen ölçüde kontrol edilebilir. Poliüretanlar üstün fiziksel, mekanik özellikleri ve yüksek biyouyumlulukları sebebiyle biyomedikal uygulamalarda geniş kullanım alanına sahip olan polimerlerdir. Tekrar birimleri, bir diol grubu ve bir diizosiyanat grubundan oluşmaktadır. Poliüretanlar ilk olarak 1960 yılında biyomalzeme alanında kullanılmaya başlanmıştır. Yüksek dayanım özellikleri ile özellikle kardiyovasküler uygulamalarda en çok tercih edilen malzemelerdir. Kardiyovasküler uygulamaların yanı sıra poliüretanlar günümüzde yara örtüsü üretiminde de kullanılmaktadırlar. Biyomalzemeler canlı vücuduna yerleştirildiklerinde biyolojik çevre ile ara yüzey oluştururlar. Vücuda yerleştirilen malzemenin yüzeyinde ilk olarak protein adsorpsiyonu meydana gelir ve ardından hücre ile birleşme başlar. Bu nedenle proteinlerin polimer yüzeyler üzerine adsorpsiyonu biyomedikal uygulamalarda büyük öneme sahiptir. Son yıllarda bu konu üzerinde birçok deneysel çalışma yapılmıştır ancak protein yapıları büyük yapılar olduğundan ve sürekli hareket halinde olduklarından deneysel olarak protein-yüzey etkileşimlerinin incelenmesi oldukça karmaşıktır. Bu konuda birçok çalışma yapıldığı halde halen protein adsorpsiyonunun uygun şekilde kontrol edilebilmesi için ara yüzeyde gerçekleşen protein-yüzey etkileşimleri tam olarak açıklanamamıştır. Deneysel metotlar ara yüzeyde gerçekleşen dinamik süreçleri ve konformasyon değişimlerini açıklamada yetersiz kalmaktadır bu nedenle bu konuda yeni yöntemlerin geliştirilmesi gerekmektedir. Son yıllarda biyomalzemelerin yüzey

özellikleri ve yüzeylere protein adsorpsiyonu moleküler simülasyon yöntemleri ile araştırılmaktadır. Moleküler simülasyon yöntemlerinin protein-yüzey ilişkilerinde kullanılması diğer uygulamalara kıyasla daha yeni olup her geçen gün gelişme göstermektedir. Moleküler simülasyon yöntemleri protein adsorpsiyonuna atomik boyuttan bakabilmeyi sağlar. Böylelikle moleküler simülasyon yöntemleri kullanılarak önceden elde edilen deneysel verilerin atomik boyutta elde edilen veriler ile birleştirilerek proteinlerin yüzey üzerine adsorpsiyonu hakkında yeni bir bakış açısı kazanılması beklenmektedir. Moleküler simülasyon yöntemleri kullanılarak yapılan protein adsorpsiyonu çalışmaları genel olarak incelenirse; malzeme ve protein yapılarının modellenmesinin ardından; Protein-Yüzey etkileşimleri, Proteinlerin farklı konumları, Protein konformasyonlarında meydana gelen değişimleri incelenir.

Poliüretanlar döşeme, tekstil, kâğıt, ambalajlama, arabaların gösterge panellerinde ayakkabı tabanlarında, izolasyonda, halılarda, ilaç sektörü gibi birçok değişik alanda da kullanılmaktadırlar. Üstün fiziksel özellikleri ve yüksek kan uyumluluğu sayesinde poliüretanlar biyomalzeme olarak da kullanılmaktadırlar ve en çok gelecek vadeden polimer biyomalzemeler olarak görülmektedirler. Daha önceden de bahsedildiği gibi bir malzemenin biyomalzeme olarak kullanılması için sadece fiziksel özeliklerinin yeterli olması yetmez. Birçok değişik faktörün bir arada tutulması ile uygun malzeme seçilir. Poliüretanların mükemmel fiziksel özelliklere sahip olmalarının yanı sıra kan uyumlulukları da oldukça yüksektir.

Moleküler simülasyon yöntemleri deneysel çalışmaları desteklemek ya da deneysel çalışma yapmadan elde edilecek sonuçları önceden tahmin edebilmek amacıyla hesaplamalı yöntemleri kullanır. Moleküler simülasyon yöntemlerinin ilk basamağı moleküler modellemedir. Moleküler modellemede moleküllerin davranışlarının gerçek sistemlere benzer şekilde modellenmesi için teorik ve hesaplamalı kimyayı bir arada kullanır. Moleküler simülasyon ise oluşturulan bu moleküler modeller üzerine kurulmuş sayısal deneydir. Moleküler simülasyon yöntemleri küçük kimyasal sistemlerden geniş biyolojik moleküllere ve malzeme topluluklarına kadar değişen bir aralıkta kullanılmakta ve inorganik, biyolojik ve polimerik sistemlerin yapı, dinamik ve termodinamik özelliklerinin araştırılmasında kullanılmaktadır. Basit hesaplamalar el ile yapılabilmektedir, fakat büyük ölçüdeki sistemlere moleküler simülasyon yöntemlerinin uygulanması bilgisayar kullanımını gerektirmektedir. Moleküler simülasyon yöntemleri temel olarak iki kategoriye ayrılabilirler. Bunlardan ilki kuantum mekaniği yöntemleri olup diğeri ise klasik mekanik yöntemlerdir. Kuantum mekaniği yöntemlerinde elektronlar temel alınır ve Schrödinger denklemleri kullanılarak moleküllerin özellikleri ve davranıÇları incelenir. Klasik mekanik yöntemlerde ise yalnız atomlar veya atom grupları temel alınır ve deneysel kuvvet alanları kullanılarak molekülün davranıÇları ve özellikleri incelenir. Kuantum mekaniği kullanan yöntemler daha kesin yöntemlerdir ve incelenen sitemle ilgili deneysel veri gerektirmezler ancak daha çok hesaplamaya dayanırlar ve küçük miktarda atom gurupları için kullanılırlar. Protein-yüzey iliÇkisi incelenen çalıÇmalarda çok fazla atomla çalıÇıldığından kuantum mekaniği kullanıÇlı değildir. Diğer tarafta klasik mekanik yöntemler kuantum yöntemlerine göre daha az hesaplama gerektirir ve binlerce atom içeren büyük sistemlerin bile simülasyonu yapılabilir. Bu nedenle protein-yüzey iliÇkisi çalıÇmalarında daha çok klasik mekanik yöntemler tercih edilirler.

Bu çalışmada farklı monomerlerden oluşan iki farklı poliüretan malzemelerin ara yüzey biyouyumlulukları Moleküler Mekanik (MM) ve Moleküler Dinamik (MD) yöntemleri kullanılarak incelenmiştir. Çalışmanın hedefi, literatürdeki deneysel verilerle simülasyon sonuçları arasında bir bağıntı kurarak, ilerde yapılacak deneysel çalışmalarda deney sayısını azaltmak ve üretilecek malzemelerin özellikleri hakkında öngörüde bulunmaktır. Yapılan çalışmada biri kristal diğeri amorf olan iki farklı poliüretan yüzey kullanılmıştır. Bu çerçevede proteinlerin farklı poliüretan yüzeylere adsorplanması karşılaştırılarak, bu yüzeylerin biyouyumlulukları değerlendirilmiştir. Böylece malzeme yüzeyi ile protein bağlanması arasındaki ilişki değerlendirilmiş ve sentezlenmesi planlanan malzemelerin bir ön elemeden geçirilmesi amaçlanmıştır.

Bu çalışmada, poliüretanların protein ile ilişkilerini incelemek için kristal polietilen glikol+hekzametilen diizosiyanat (PU1) ve amorf hint yağı+hekzametilen diizosiyanat (PU2) poliüretanları kullanılmıştır. Daha sonra C-terminus gama chain Fibrinogen (1FID) proteini polimerler üzerine altı farklı konumda yerleştirilmiştir. Simülasyonlarda suyun etkisini aktarabilmek için dielektrik kaysayısı 78'e sabitlenmiştir ayrıca PU1 polimer atomları hareketsiz ve PU2 polimer atomlari ise hareketli tutularak simülasyonlar gerçekleştirilmiştir. Her bir konum için enerji minimizasyonu yapılmıştır. Polimer-protein etkileşim enerjisinin en düşük olduğu konumlar seçilmiş ve sulu ortamda moleküler dinamik simülasyonlari gerçekleştirilmiştir. Ardından polimer yüzeyinden 3, 5 ve 7Å mesafede bulunan amino asitlerin yüzey ile teması sonucu ortaya çıkan etkileşim enerjileri incelenmiş ve 7Å mesafede bulunan amino asitlerin yüzeye adsorplanmış kabul edilmesinin en doğru yaklaşım olduğu gözlenmiştir. Daha sonra, amino asit sayısına karşılık etkileşim enerjisi grafiklerinin eğimlerinden mutlak etkileşim enerjisi hesaplanmıştır. Eğim değeri fazla olan yüzey protein ile daha güçlü etkileşim göstermektedir. Böylece yüzeylerin biyouyumluluğu konusunda nano ölçekte bilgi sahibi olunmuştur.

Ağırlık Merkezi Graflari zamana karşılık ve Ortalama Karekök Sapma Graflari proteinin polimer yüzeyinden mesafesi hesaplanmiştir ve ramaçandran diagramları MD simülasyonu yapılan konumlar için hazırlanmıştır. Ayrıca adsorpsiyon çalışmalarında amino asitlerin yüzeye olan ilgilerini gözlemlemek için ψ ve Φ bağ açılarındaki değişim incelenmiştir. Amino asit gruplarının, yüzeyle etkileşimlerinin incelenmesi, yüzeylerin farklı proteinler için özel tasarlanarak, kontrollü protein adsorpsiyon yöntemlerinin geliştirmesinin sağlayacaktır.

Elde edilen sonuçlara göre saklı (implicit) çözücü ortamda daha fazla hidrofilik olan PU1 yüzeyi fibrinogen ile daha güçlü etkileşim göstermektedir. Bu farklılık, kristal PU1‟in düzgün bir yüzeye sahip olmasına ve böylece protein ile daha iyi ilişki kurabilmesine dayandırılabilir.

Çözücünün Açık (expilicit) olarak gösterildiği simülasyon sonuçlarına göre, Lineer modeller, Ağırlık Merkezi Graflari (COM) zamana karşılık ve Ortalama Karekök Sapma Graflari (RMSD) zamana karşılık ve Ramachandran şemaları, fibrinojenin PU1 yüzeyinde PU2 yüzeyine göre daha güçlü adsorbe olması ve ayrıca PU1 açık su sisteminde saklı su sistemine göre zayıf adsorpsiyon gösteriyor. Eldeki sonuçlar gore, fibrinojen adsorpsiyonu için PU2 yüzeyi, PU1 yüzeyinden daha çok iyi olduğunu görünmektedir.

1. INTRODUCTION

Biomaterials are natural and synthetic materials used to mimic functions of living cells and to support the damaged organs. Biocompatibility is the most important property of a biomaterial. Because of their high biocompatibility, polyurethanes are among the most appealing class of biocompatible polymers. Polyurethanes are especially used in cardiovascular field, breast implants and wound dressing production [1]. The interaction between a biomaterial and living tissue occurs in a narrow interface zone. Therefore, biocompatibility will largely be determined by the material surface properties. These include : surface roughness, wettability, chemical composition and electrical charge. In addition the presence of leachables (low molecular weight fragments, additives, contaminants) is of great importance. Polyurethanes are a very appealing class of polymers for use in the biomedical field. This is because of their relatively good biocompatibility, their excellent physical and mechanical properties, and the ease with which their properties can be tailored to the end use. Polyurethanes were introduced as biomaterials during the 1960s. At present, the use of segmented polyurethane elastomers in clinical devices is well established [2].

Then development of biomaterials is not a new area of science, having existed for around half a century. The study of biomaterials is called biomaterial science. It is a provocative field of science, having experienced steady and strong growth over its history, with many companies investing large amounts of money in the development of new products. Biomaterial scince encompasses elements of medicine, biology, chemistry, tissue engineering and materials science.

Biomaterials can have a benign function, such as being used for a heart valve, or may be bioactive; used for a more interactive purpose such as hydroxylapatite coated implants. Biomaterials are also used every day in dental applications, surgery and drug delivery [5].

At present the use of segmented polyurethane elastomers in clinical devices is well established [2]. Polyurethanes, having extensive structure/property diversity, are one of the most bio- and blood-compatible materials known today. These materials played a major role in the development of many medical devices ranging from catheters to total artificial heart. Properties such as durability, elasticity, elastomer-like character, fatigue resistance, compliance, and acceptance or tolerance in the body during the healing, became often associated with polyurethanes. Furthermore, propensity for bulk and surface modification via hydrophilic/hydrophobic balance or by attachments of biologically active species such as anticoagulants or biorecognizable groups are possible via chemical groups typical for polyurethane structure. These modifications are designed to mediate and enhance the acceptance and healing of the device or implant. Many innovative processing technologies are used to fabricate functional devices, feeling and often behaving like natural tissue [6].

Since materials interact with environment through their interfaces, both the kind and the strength of such interactions are largely dependent on the surface properties of the materials. While a material is in contact with a biological environment, the surface chemistry and topography of the material are important parameters that may influence protein adsorption, cell interaction, and ultimately the host response. However, materials that are polymeric, ceramic, or metallic with totally different surface properties may induce similar responses *in vivo*, and this has been attributed to non-specific protein adsorption on the surface. Protein adsorption on the material surface is believed to be the initial event when a material comes into contact with a biological environment. The adsorbed protein layer will influence the subsequent biological reactions including platelet adhesion and activation. Therefore, understanding the interaction between proteins and material surfaces is critical, and control of protein–surface interactions continues to be an important factor for consideration in the design of biocompatible surfaces [7].

Thus, understanding the mechanism and energetics of protein solid surface interactions is critical to efficiently design novel biomaterials. From a theoretical viewpoint, the surface peculiarities and the hierarchical structure of proteins cannot be handled analytically or through coarse-grained simulations, except for very broad and general features, but require a fully atomistic description. Moreover, owing to the large system size typically involving tens of thousands of atoms, quantum methods cannot be used. Hence, in recent years, an atomistic picture of protein adsorption on specific surfaces has been obtained through different computational tools, such as molecular dynamics (MD), molecular mechanics (MM), and Monte Carlo methods.. Because proteins are biological macromolecules formed by tens of residues, at least, their rearrangements, for instance close to a surface, involve motion at different length scales, ranging from the local ones within a single residue with very short time scales, to the collective ones involving long adjacent strands at much longer time scales, whereas macromolecular connectivity gives rise to cooperative processes. Such events pose a noticeable challenge to current simulations. The information obtained using these computational methods can be very useful to tailor optimized surfaces to control adsorption of target proteins, e.g., by means of grafting with specific functional groups [3,10].

In this work adsorption characteristics of polyurethanes, which constitute a very appealing class of polymers because of their high biocompatibility and excellent physical and mechanical properties were focused. These versatile polymers have been widely used in wound dressings as well as in cardiovascular and breast implants. Swelling of the poly(ethylene glycol) (PEG)-based polyurethanes is an important issue in biomedical applications.

In order to better understand the alterations in the adsorption characteristics due to the incorporation of castor oil into PEG-based polyurethanes, we studied adsorption of the crystal structure of a 30 kDa C-terminus γ-chain fragment of fibrinogen (γFg) on two model polyurethane systems: (1) crystalline PEG-hexamethylene diisocyanate (PEG-HDI) and (2) amorphous castor oil-hexamethylene diisocyanate (CO-HDI) polymers, which constitute two limiting compositions. We used atomistic models of these two polymers, calculated their mechanical properties driven by adsorption energetics, and compared these results with the available experimental data. Finally, we performed molecular mechanics calculations and molecular dynamics simulations to compare the affinity of these polyurethanes to the fibrinogen protein in the presence of implicit and explicit water.

2. POLYMERIC BIOMATERIALS AND PROTEINS INTERACTION

2.1 Biomaterials

Biomaterial is defined as “material that is compatible with living tissue — material that can be safely implanted into the human body and left there without causing an adverse reaction”. This definition can be extended to include material that is compatible with living cells [8].

A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems. Biomaterials may be used singularly to replace or augment a specific tissue, or in combination to perform a more complex function, in organ replacement. Biomedical materials include metals, ceramics, pyrolytic carbon materials, composites and polymers. Of these groups, polymers represent the largest class. There are three fundamental properties that a biomaterial should possess; mechanical strength, a functional characteristic, and biocompatibility. The functional characteristic is required so that the material has the specific property to perform the required task. Mechanical strength is required to retain an adequate level of performance [9].

Biocompatibility is one of the most important characteristics of a biomedical polymer material whose surface is required to interact with a biological system. Such interactions between polymer surfaces and organisms have been the focus of many studies. Since proteins are viewed as the primary and the most important player in mediating polymer–organism interactions, the status of the proteins on a material surface is believed to determine the ultimate biocompatibility of a given polymer. In order to achieve specific responses between polymer surfaces and the adjacent cells and to reduce non-specific interactions, the principles for designing biocompatible polymer materials are brought forth, such as passivating the polymer surfaces to minimize non-specific protein interaction, or decorating polymer surfaces with biomolecules to induce specific protein adsorption and cell responses [10, 11].

A large number of polymers have been used in biomedical applications. Developments in polymer science opened up the variety of materials that were available. Polymers that are used in medical applications include naturally occurring materials such as natural rubber and cellulose. Synthetic biomaterials include silicone rubber (SR), polyvinylchloride (PVC), nylon, polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET) and of course the polyurethanes. Polyurethanes account for very little of the total amount of polymers used for medical and pharmaceutical applications. This does not reflect the diversity of their applications and their relative success as a biomaterial. Polyurethane elastomers combine excellent mechanical properties with good blood compatibility, which has favored their use and development as biomaterials, particularly as components of implanted devices [9].

2.2 Polyurethanes

Polyurethanes are a family of heterogeneous polymers; they contain the urethane linkage, analogous to the carbamate group in organic chemistry, within the polymer chains. Urethane groups usually do not constitute the majority of the functional groups within a polyurethane. It is the ability to incorporate other functional groups into the polymer network that contributes to the range of properties exhibited by polyurethane materials. Consequently, the properties of polyurethanes range from rigid hard thermosetting materials to those of much softer elastomers.

Generally, thermoplastic polyurethanes, which comprise the most important group for implantable devices, have very high tensile strength, toughness, abrasion resistance, and resistance to degradation, in addition to biocompatibility that has sustained their use as biomaterials [9].

By composition, the polyurethanes are either thermoplastic or thermosets. The thermoplastic PU are block copolymers composed of hard segment (A) and soft segment (B) blocks are arranged in $(AB)_n$ structure. The hard segment (HS) block is composed of diisocyanate and the chain extender, usually low molecular weight diol or diamine. The soft segment (SS) is usually a polyol, either hydroxyl or amine terminated polyester, polyether, polycarbonate, and in special cases, polyolefin or hydrocarbon as shown in Figure 2.1.

Swelling of the poly(ethylene glycol) (PEG)-based polyurethanes is an important issue in biomedical applications and is needed to be controlled by addition of cross-linkers. Alternatively, castor oil (CO), which is attractive due its safety and effectiveness in biomedical applications, can be used as the polyol component for decreasing swelling degree of PEG-based polyurethanes due to its high content of ricinoleic acid, which has a hydroxyl functional group on the 12th carbon. Since the amount of castor oil in the polyurethane determines strongly polymer properties, the performance of the castor oil and PEG-based polyurethane films in protein adsorption has been studied in great detail [13].

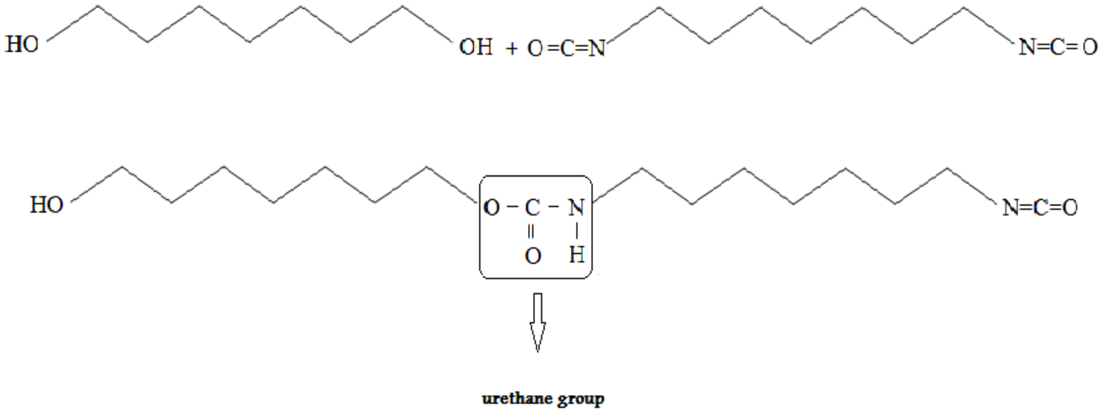


Figure 2.1 : Polyurethane structure and urethane group [12].

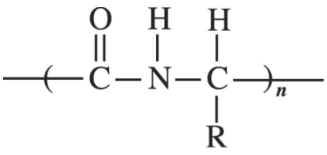
Having extensive structure/property diversity, polyurethanes are one of the most bio- and blood-compatible materials. They had a major role in the development of many medical devices ranging from catheters to total artificial heart. Properties such as durability, elasticity, elastomer-like character, fatigue resistance, compliance, and acceptance or tolerance in the body during the healing, became often associated with polyurethanes. Furthermore, propensity for bulk and surface modification via hydrophilic/hydrophobic balance or by attachments of biologically active species such as anticoagulants or biorecognizable groups are possible via chemical groups typical for polyurethane structure. These modifications are designed to mediate and enhance the acceptance and healing of the device or implant. Many innovative processing technologies are used to fabricate functional devices, often react as well as natural tissue.

The hydrolytically unstable polyester polyurethanes were replaced by more resistance but oxidation- sensitive polyether polyols based polyurethanes and their clones containing silicone and other modifying polymeric intermediates. Chronic *in vivo* instability, however, observed on prolonged implantation, became a major roadblock for many applications [13].

Presently, utilization of more oxidation resistant polycarbonate polyols as soft segments, in combination with antioxidants such as vitamin E, offer materials which can endure in the body for several years. The applications cover cardiovascular devices, artificial organs, tissue replacement and augmentation, performance enhancing coatings and many others [6].

2.3 Proteins

Protein, in fact, mean ‘‘of first importance’’ in Greek, this is for a very good reason because initial protein interactions with material surfaces control subsequent cell functions essential for tissue engineering/implant applications, antibody interactions for immunoassays and/or biodetection devices, and bacteria interactions involved in problems such as marine fouling and infection. Clearly, understanding protein size and structure will aid investigators in either maximizing or minimizing protein interactions with surfaces to improve these technologies. Proteins are polyamides formed by a step reaction polymerization between the amino and carboxyl groups of amino acids: where R- is a characteristic side group and n is the number of repeating units. Depending on the side group, the molecular structure of the protein will drastically change [14].



$$-\left(\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle H}{|}}{N}-\underset{\underset{\displaystyle R}{|}}{\overset{\overset{\displaystyle H}{|}}{C}}\right)_n-$$

Figure 2.2 : Amino Acid group.

To date, the fundamental nanometer size of proteins has been largely ignored and not appreciated when considering approaches to control initial protein interactions with surfaces. Thus it is first important to describe protein structure to discuss how nanotechnology can be utilized to influence protein interactions. There are four important levels of protein structure to consider: primary, secondary, tertiary, and quaternary; these will be discussed in the sections that follow.

- Primary Structure: The primary structure of a protein is its linear sequence of amino acids. Each amino acid is linked to another through peptide bonds Fig. 2.2. Since there are 20 amino acids found in humans (11 of which are synthesized in the human body: Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, Pro, Ser, and Tyr; and 9 of which are synthesized elsewhere but are essential to human diet: His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val), many sequences are possible to create numerous proteins with diverse properties.

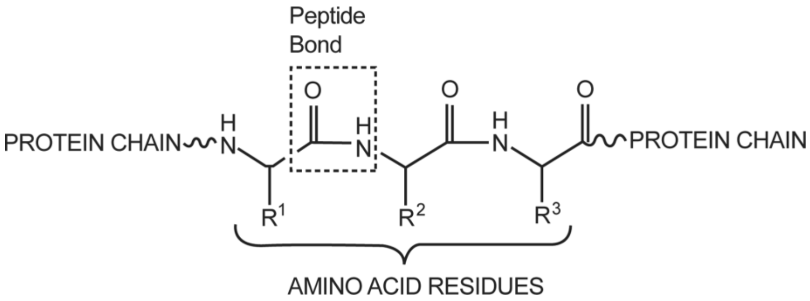


Figure 2.3 : Amino acid residues

Moreover, these 20 amino acids are themselves diverse (Table 2.1) and may be classified according to a number of categories: such as either aliphatic amino acids, amino acids with hydroxyl-containing or sulfur-containing side chains, aromatic amino acids, basic amino acids, cyclic amino acids, or acidic amino acids and their amides. Other amino acid classifications that are often utilized when considering initial protein interactions with material surfaces are either non-polar, polar, or ionized (**Table 2.1**).

Some amino acids have side chains (or residues) that carry no charge at any pH yet exhibit strong polar character Ionizable side chains vary from fairly acidic ones (such as Asp and Glu, which are fully negative at the physiological pH of 7.4) to more basic amino acids (such as the imidazole group in His, which has a positive charge at a pH of 7.4) and the still more basic amino acids that carry full charges at a pH of 7.4 (specifically, Lys and Arg).

Yet another group of amino acids has no acid, base, or polar character in their residues as shown by their generally much lower solubility in water (such as Ala, Val, Leu, Ile, Met, Trp, and Pro). For these reasons, it should not be surprising why proteins exist with a wide range of properties, as shown in **Table 2.2**, and this is just from considering protein primary structure.

Table 2.1 : amino acid classifications [15].

Amino Acid lowcring (three-letter and one-letter abbreviations) of the surface tension of water	**Charge**	**Hydrophobicity (kcal/mol)***	**erg/cm^2/mol/l**
Isoleucine (Ile or I)	Neutral	0.73	-15.2
Phenylalanine (Phe or F)	Neutral	0.61	-17.3
Valine (Val or V)	Neutral	0.54	-3.74
Leucine (Leu or L)	Neutral	0.53	-21.9
Tryptophan (Trp or W)	Neutral	0.37	-9.6
Methionine (Met or M)	Neutral	0.26	-3.01
Alanine (Ala or A)	Neutral	0.25	0.96
Glycine (Gly or G)	Neutral	0.16	1.12
Cysteine (Cys or C)	0 to -1	0.04	0.69
Tyrosine (Tyr or Y)	0 to -1	0.02	-15.1
Proline (Pro or P)	Neutral	-0.07	-0.49
Threonine (Thr or T)	Neutral	-0.18	0.59
Serine (Ser or S)	Neutral	-0.26	0.76
Histidine (His or H)	0 to 1	-0.40	1.03
Glutamic acid (Glu or E)	0 to -1	-0.62	0.86
Asparagine (Asn or N)	Neutral	-0.64	1.17
Glutamine (Gln or Q)	Neutral	-0.69	1.21
Aspartic acid (Asp or D)	0 to -1	-0.72	0.96
Lysine (Lys or K)	0 to 1	-1.1	0.92
Arginine (Arg or R)	0 to 1	-1.8	1.03

*Transfer from hydrophobic to more hydrophilic phase; increased positive value means more hydrophobic.

- Secondary Structure: The secondary structure of proteins consists of regions of ordered structures in the protein chain. Two main secondary structures of proteins are very common: the α-helix and the β-pleated sheet. The degree of secondary structures in proteins varies to a large amount. Both the α-helix and β-pleated sheet secondary protein structures are controlled by hydrogen bonding mechanisms. Hydrogen bonding mechanisms are electrostatic attractions between oxygen of one chemical group and hydrogen of another chemical group. The α-helix results from the coiling of the protein chain such that the peptide bonds making up the backbone are able to form hydrogen bonds with each other. These hydrogen bonds are directed along the axis of the helix, and thus amino acid residues (portions of the amino acid not participating in the peptide bond) extend at right angles from the helix with minimal steric hindrance that further stabilizes the α-helix. The β-pleated sheet is a layering of protein chains in which each layer is held together by hydrogen bonds between the peptide links. The amino acid residues are situated at right angles to the sheets with, again, minimal steric hindrance that further stabilizes the β-pleated sheet.

Table 2.2 : Diverse properties of proteins [15].

Protein activity	Function	location	Size kDa	Shape (Å)	Stability	Surface
Albumin	Carrier	Blood	65	42 x 141	Denatures at 60^0C	Low on polyethylene
Fibrinogen	Clotting	Blood	340	460 x 60	Denatures at 56^0C	High on polyethylene (trinodular string)
IgG	Antibody	Blood	165	T-shaped		Low on polyethylene
Lysozyme	Bacterial Iysis	Tear;	14,6	45 x 30	ΔG_n = -14 kcal/mol	High on negatively hen egg (globular) charged surfaces
Hemoglobin	Oxygen carrier	Red blood	65	55	Normal form	Very high on Cell (spherical) polyethylene
Hemoglobin S	Oxygen carrier	Sickle red	65	55	Less than hemoglobin	Much higher blood cells (spherical) air – water activity than hemoglobin
Myoglobin	Oxygen carrier	Muscle	16,7	45 x 35 x 25	ΔG_n = -12 kcal/mol	(spherical)
Collagen	Matrix factor	Tissue	285	3000 x 15	Melts at 39^0C	(triple helical rod)
Bacteriorhodopsin	Membrane protein		26	30 - 40 long		High at cell membrane
Tryptophan	Enzyme		27		ΔG_n = -8.8 kcal/mol	High air- water synthase denatures at 55^0C
Tryptophan	Enzyme		27		ΔG_n = -16.8 kcal/mol	Much less active syntha at air – water variant α subunit interface than wild type

- Tertiary Structure: The tertiary structure of proteins is the overall three dimensional shape of the protein. Protein tertiary structure can either possess little order for many structural proteins, or be extremely complicated for other proteins such as enzymes that fold up on themselves to form more complex structures. Clearly, the tertiary structure of proteins is a consequence of primary structure as it depends on spontaneous interactions between different amino acids and, under aqueous conditions, spontaneous interactions between amino acids and water as will be discussed. Because of the properties of the amino acids and the order in which amino acids are bonded (i.e., protein primary structure), select electrostatic interactions will be created for predictable tertiary structures.

There are four main interactions among residues of amino acids that contribute to the tertiary structure of proteins, each with different strengths: covalent, ionic, hydrogen and van der Waals bonds. Of these interactions, covalent bonds are the strongest, as indicated in **Table 2.3**.

Table 2.3 : Strengths of bonds controlling tertiary protein structure [15].

Type of bond	**Strength (kJ/mol)**
Covalent (S – S)	250
Ionic	20
Hydrogen	7 – 40
Van der Waals	1.9

- Quaternary Structure: Only proteins that possess numerous subunits have quaternary structure. How these subunits interact will determine the quaternary structure of that protein. An example of a well-studied quaternary protein structure is hemoglobin, which is composed of four protein subunits as described by secondary structure. In any case, interactions between amino acid residues on the exterior of the protein’s tertiary structure will control quaternary structure. Thus ionic bonding mechanisms, which control the placement of hydrophilic amino acids on the exterior of protein tertiary structure in an aqueous environment, now play another important role in determining quaternary structure. Similarly, hydrophobic interactions have an essential role in determining quaternary structure. For example, it is clearly not possible for all hydrophobic amino acids to be placed on the interior of proteins; some may be present on the exterior. Thus, a small hydrophobic area between adjacent subunits in the quaternary structure of proteins may be formed through hydrophobic interactions because these groups would have a preferred interaction of facing each other, rather than being exposed to the aqueous environment. This is another example of how important hydrophobic interactions are in controlling protein structure. Although the structure of proteins has been well studied for a number of years, only in the past several decades have investigators begun to elucidate mechanisms of protein interactions with solid substrates.

Knowledge of protein structure has certainly aided these researchers in understanding properties of surfaces that mediate protein interactions. ‘‘Protein Interactions with Surfaces’’ will describe some of these findings [15].

2.4 Modeling of Protein –Surface Interactions

To date, molecular simulation has been successfully used to examine adsorption processes at different levels, including adsorbent modeling and visualization, adsorption of small molecules, and protein adsorption on solid surfaces, as summarized in **Figure 2.4**.

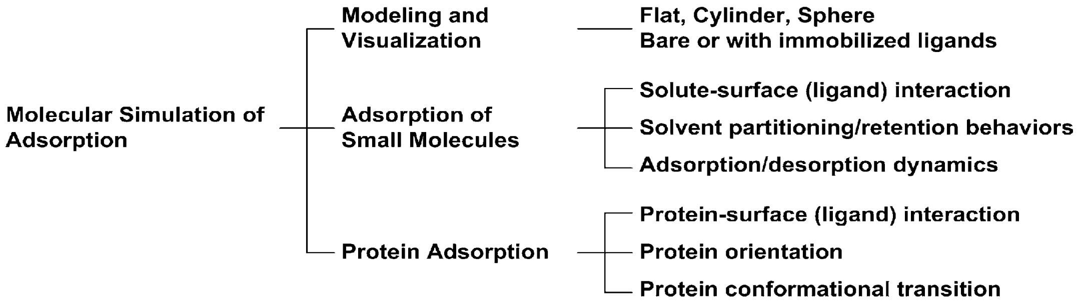

Molecular Simulation of Adsorption
Modeling and Visualization
Adsorption of Small Molecules
Protein Adsorption
Flat, Cylinder, Sphere
Bare or with immobilized ligands
Solute-surface (ligand) interaction
Solvent partitioning/retention behaviors
Adsorption/desorption dynamics
Protein-surface (ligand) interaction
Protein orientation
Protein conformational transition

Figure 2.4 : Molecular simulation studies on adsorption [16].

Modeling of adsorbents is the first step for the molecular simulation of adsorption and protein chromatography. According to the actual surface shape, adsorbent is usually modeled as a flat, cylinder or sphere surface (implicit or explicit surface). All-atom models are usually used due to its high precision and abundant chemical information. However, coarse-grained models have also been used to enlarge the simulation system and save computational cost. With these models, ligand conformation and surface morphology are visualized and examined through MC (Monte Carlo) or MD (Molecular Dynamic) simulations.

Utilizing the models mentioned above, the adsorption of small molecules on solid surface can be investigated by MC or MD simulations to achieve molecular details. The researches usually focus on the solute–surface interaction, solvent retention behaviors, and adsorption/desorption dynamics. In this study, adsorption of fibrinogen protein on polyurethane surface is investigated. Molecular interaction between solute and surface is the driving force for adsorption.

In comparison with the adsorption of small molecules, protein adsorption is more complex due to its large volume, heterogeneous surface, and structural flexibility. Therefore, diverse studies of protein adsorption by molecular simulations have been reported, including protein–surface interaction, protein orientation on surfaces, and the conformational transition on surfaces. Protein–surface interaction is the driving force for protein adsorption. Various molecular simulations have been performed to examine the interactions, which are sometimes represented by adsorption free energy [16].

There is some forces that promote the adsorption of proteins at surfaces and there is still much discussion as to the relative importance of these. A number of interactions contribute to the adsorption of proteins at surfaces. The major contributing factors are believed to be entropic in origin. Of these the principal factor promoting adsorption is believed to be the displacement of water from the interfacial region by amino acid residues. Another contributing factor is the entropy change associated with changes in the secondary and tertiary structure of the protein on adsorption, which may promote or oppose adsorption depending on the nature of the protein molecule. The contribution of amino acid chain entropy to protein adsorption is an area that is still under discussion. Have put forward the argument that unfolding of protein secondary structure gives an increase in chain entropy that is sufficient to compensate for both the loss of chain conformational entropy and the loss of enthalpy due to breakage of bonds holding together the secondary structure. Both experiments and simulations have demonstrated that unfolding of secondary structure does occur in an adsorbed protein on the evidence that ordered structures in adsorbed proteins can increase at hydrophobic surfaces. A further complication is that Monte Carlo simulations of hydrophobic copolymers have also shown that even in the absence of specific secondary structure unfolding, the entropy change for adsorption can be favorable. This suggests that some conformations adopted by adsorbed proteins may be more flexible than the native state. At fluid interfaces the simulated entropy change for globular molecules can be even bigger. Suggesting that interface fluidity allows the protein to adopt a greater range of adsorbed conformations. To date, the precise role of protein structural change in driving surface adsorption remains unresolved [20].

2.5 Key Factors for the Molecular Simulation of Peptide/Protein-Surface Interactions

Of the three basic classes of molecular simulation methods, the one most directly applicable for the simulation of peptide-surface or protein-surface interactions is the class of methods that uses an all-atom empirical force field. If properly parameterized and applied, these methods can accurately represent the atomic-level behavior for a system containing a sufficiently large numbers of atoms to represent an adsorbent surface, peptides or a small protein, and the water and ions of the solvent. The key phrase in the preceding sentence is “if properly parameterized and applied”. There are three main issues that must be appropriately addressed in order to perform a useful molecular simulation of peptide-surface interactions. The first is force field parameterization. The force field equation determines how atoms interact with one another during a simulation, and the accuracy of a simulation depends directly on the suitability of the set of force field parameters that are used to represent the types of atom-atom interactions involved. The second key issue pertains to how solvation effects are accounted for. If solvent molecules are explicitly included in the molecular system being evaluated, then this relates directly to the previous issue, namely, force field parameterization to accurately represent the molecular-level behavior of water and ions in solution. Alternatively, if the solvent is represented using some type of mean-field approximation, as with implicit solvation methods, then the accuracy of this approximation must be considered. The third key issue is related to system sampling and what is called sampling ergodicity. In practical terms, sampling ergodicity refers to the need to sample a sufficient number of configurational states of a system in order to calculate a representative ensemble-average property of the system, such as the average potential energy or change in free energy for a given process. This becomes problematic when different states of the system are separated by relatively high energy barriers, which tend to trap the system in localized areas, thus preventing other important states from being sampled. When this occurs, it results in errors in the calculated properties of the system [24].

2.6 Solvation Effects

During the process of peptide/protein adsorption, the water molecules and salt ions in solution do not just provide an inert medium that the reactions take place in; but rather, they are active components of the system. As such, it is essential that solvation effects be accurately represented in any molecular simulation of peptide-surface or protein-surface interactions. A simulation composed of only a peptide and a surface, without the presence of solvent molecules or the representation of solvation effects, represents molecular behavior under vacuum conditions, which has little to do with processes that occur in aqueous solution. The most direct and accurate way of including solvation effects in an all-atom empirical force field simulation, whether it employ an MM (Molecular Mechanic), MC (Monte Carlo) or MD (Molecular Dynamic) method, is to include the molecules of the solvent explicitly using a water model that was specifically designed to be used with the selected force field along with the appropriate concentration of salt ions. Numerous special water models have been developed for use with these simulations, such as SPC, TIP3P, TIP4P, TIP4P/EW, TIP5P, and polarizable water. The benefit of the use of explicit solvation in a simulation of peptide adsorption is that the water molecules are then able to specifically interact with the functional groups of both the amino acid residues of the peptide and the adsorbent surface, with these interactions being in direct competition with the interactions between the water molecules themselves and the amino acid functional groups with those of the adsorbent surface. When used with a properly SPC/E, tuned force field, this not only enables adsorption processes to be accurately represented but also enables the effects of adsorption processes on the surrounding water structure to be evaluated and characterized, thus providing insights into the types of atomic-level interactions that influence adsorption behavior [22].

2.6.1 Simulations with explicit solvent

Explicit inclusion of water molecules provides, as realistically as possible, the kinetic and thermodynamic properties of the protein surface interaction process. Simulations with explicit water are carried out in a periodic box scheme; the box is usually rectangular, but other shapes are also possible. A less common treatment is to perform simulations in a thin layer of water around a protein molecule restrained with a weak harmonic potential. There are currently a number of water models used in MD simulations. These models were parameterized assuming that a cut-off is applied to nonbonded interactions, but they are often used with Ewald summation to treat long-range electrostatics.

All these models treat water as a rigid molecule. Although bond stretching and bond-angle bending, or polarization effects and many-body interactions, have been introduced into water models, they involve a large increase of computational expense, which has limited their use as widely as the SPC or TIP models. The water models are usually parameterized at a single temperature (~298 K) and therefore do not correctly capture the temperature dependence of properties such as the solvent density or diffusion coefficients.

The presence of water molecules in the system dramatically increases the number of degrees of freedom (typically by more than 1000 degrees of freedom). Because of this limitation, along with the small values of the time step in integrating the equations of motion (of the order of femtoseconds), explicit-solvent all-atom MD algorithms can simulate events in the range of 10^{-9} s to 10^{-8} s for typical proteins and 10^{-6} s for very small proteins. These timescales are at least one order of magnitude smaller than the folding times of proteins [23].

One of the primary problems with the use of explicit water molecules in a simulation is that such a large number of water molecules must be used in order to appropriately represent a peptide in aqueous solution that the water itself often represents more than 90% of the atoms in the system. Accordingly, over 90% of the computational time is spent simulating the behavior of the bulk water as opposed to the peptide-water-surface interactions, which are of primary interest. As an approach to circumvent this problem, many different types of implicit aqueous solvation methods have been developed [22].

2.6.2 Implicit-solvent methods

The use of continuum representations of the solvent greatly decreases the number of degrees of freedom in the system and, consequently, the sampling time. The rigorous implicit treatment of solvent in MD involves

(*a*) Designing an effective potential function that describes the change of the free energy of the system on the change of the conformation of the solute molecule and

(*b*) Direct effect of the solvent on the dynamics of the solute molecule through collisions, which results in the appearance of net friction and random forces.

The most common treatment of electrostatic interactions between the solute and solvent makes use of the generalized Born surface area (GBSA) model, which includes an approximation to the solution of the Poisson-Boltzmann equation for a system comprising the solute molecule immersed in a dielectric with counter-ions and also takes into account the loss of free energy owing to the formation of a cavity in the solvent. Simpler models have also been developed in which the free energy of solvation is expressed in terms of solvent-accessible surface areas of solute atoms or solvent-excluded volumes owing to the contributions from pairs of atoms, but they are used in other applications than MD. The GBSA model can lead to discontinuous forces because of its explicit use of molecular surface area. Use of the GBSA model eliminates the need for the lengthy equilibration of water necessary in explicit water simulations. However, this model does not reproduce the all-atom free-energy landscape of folding and can overestimate the stability of the native state. With the addition of the Berendsen thermostat or other thermostats, canonical simulations can be carried out with implicit-solvent models; such a treatment corresponds to a low-viscosity limit and has been applied with success to all-atom *ab initio* folding simulations of proteins by canonical MD. However, ignoring solute-solvent friction makes the folding times, calculated with implicit-solvent MD simulations, the lower bounds of the true experimental folding times of proteins [23].

These methods all attempt to represent the effects of the aqueous solution by the incorporation of some type of mean-field approximation that is directly integrated into the force field equation as opposed to explicitly representing individual atoms of the solvent. This greatly reduces the number of degrees of freedom of the molecular system that is being simulated, thus reducing the computational requirements for the simulation. While this benefit comes at a cost of decreased accuracy, it does enable system size and time scale to be greatly extended for a given computational system and time frame available for the simulation. There are basically two important components of solvation that must be represented for a reasonably accurate implicit solvation method:

1. The electrostatic shielding provided by the water molecules and ions in solution, which represents solvation effects around polar and charged functional groups, and

2. Hydrophobic effects, which represent hydration effects around nonpolar groups.

Several different approaches to represent solvation effects implicitly have been developed and used for protein folding simulations. Many of these, however, are only appropriate for the specific applications that they were developed for, and even then may provide a poor representation of solvation effects. Unfortunately, this has led to substantial misuse of implicit solvation effects for the simulation of peptide/protein-surface interactions, with the generation of completely erroneous results because of the improper representation of the system [25].

2.7 Protein - Surface Interactions

Protein–surface interaction is the driving force for protein adsorption. Various molecular simulations have been performed to examine the interactions, which are sometimes represented by adsorption free energy. The effects of protein orientation, surface properties, and protein–surface distance were examined. MC simulations were also used to examine the interaction, including the enantioselective adsorption on nanostructured surfaces and slit pores, and the interaction of a coarse-grained model polymer chain on a planar surface. The simulations also indicated that the protein–surface interaction was dependent on the surface hydration and properties. With these results, it is expected that the parameters involved in protein–surface interactions can be improved to accurately simulate protein adsorption on biomaterial surfaces and to develop the force field. This issue is mainly related to the accurate description of the nonbonded interactions, which is always approximately described by Lennard–Jones and Coulomb potential energies with empirical parameters. Improved parameters based on exact adsorption energy from quantum computational chemistry or experiments will be helpful to exactly explore the adsorption phenomena. Moreover, the information on the effect of surface properties would help to improve the design and modification of adsorption surfaces. However, it should be noted that the protein–surface interaction is very complicated due to the flexibility and heterogeneous surface of protein. There is no general procedure proposed for the calculation of protein–surface interaction. The results from different models cannot be quantitatively compared to each other. Therefore, more accurate method should be developed. At present, however, the calculation may not be doable for macromolecules such as proteins. So, suitable and meaningful simplifications are still necessary [16].

The affinity of a protein molecule for a surface depends on several factors that include both the nature of the adsorbent surface and the surface of the protein that first contacts the surface. Several simulation studies have demonstrated the importance of the orientation of protein (or protein-like) molecules on their adsorption at surfaces. Raffaini and Ganazzoli [24] have used MD to investigate the adsorption of peptide subdomains from human serum albumin and fibronectin on a graphite surface. Panos et al, using a similar model, have also noted that the importance of orientation of fibronectin adsorption onto polyurethane surface that depend on the side on which the molecule initially adsorbs [3]. In both cases, the initial energy minimization for conformations at a surface gave differing values depending on the orientation of the subdomain [25].

2.8 Protein Orientation

Protein orientation has significant effect on protein–surface interaction and thus on the adsorption behaviors, because the protein surface is usually heterogeneous with hydrophobic, hydrophilic and charged regions. For example, when the hydrophobic region on protein surface approaches the hydrophobic ligands, the protein is attracted. However, if the hydrophilic region on protein surface approaches the hydrophobic ligands, the protein will be repulsed. Using molecular simulations, the protein orientation on surfaces, for instance, head-on and end-on, at varied chromatographic conditions can be monitored Fig 2.5 shows an example of protein orientation on solid surface, where the head-on and end-on orientations have been drawn. Jiang and coworkers examined the adsorption and orientation of proteins on charged surfaces [17, 18] and SAMs [19] using MC simulation with both colloidal and all-atom models [20]. Ravichandran et al. investigated lysozyme adsorption at a positively charged planar solid surface using Brownian dynamics simulations through allatom model and simplified uniformly charged sphere model [21]. Latour and coworkers examined the effect of surface properties on adsorption using MD simulations on an all-atom model of fibrinogen fragment over SAMs (Self Assembeld Monolyers) [4]. These studies showed that the orientation of adsorbed proteins was nonuniform, depending on the protein and adsorbent surface properties. The proteins underwent substantial rotational and translational motions over the surface before stabilizing in various preferred orientations, and the final ordering of adsorbed proteins might strongly depend on the relative location of the hydrophilic and hydrophobic patches on their surfaces. These results indicated the importance of protein orientation and surface properties on protein adsorption. However, the quantitative description is difficult due to the abundant possible orientation of protein. The exact description of protein orientation should be developed to explore the energy map as a function of protein orientation, similar as the protein energy pit model [17, 18, 19, 20, 20, 21, 4].

2.9 Surface Interaction

Virtually all implant surfaces that contact soft or hard tissues become rapidly coated with a 1- to 10-nm thick protein layer, most often from blood plasma. Such layers have different compositions, depending on the underlying chemistry and topography, and they activate different homeostatic systems. Proteins or their surface-bound proteolytic degradation fragments also offer binding sites to cells, leading to the surface–cell communication necessary for the expression of cell morphology, proliferation, differentiation, and function. Implants inserted into tissues for extended periods of time may be isolated from surrounding tissues by a 50- to 250-μm thick fibrous encapsulation. Poor revascularization is then observed 50 to 100 μm away from the surface [26].

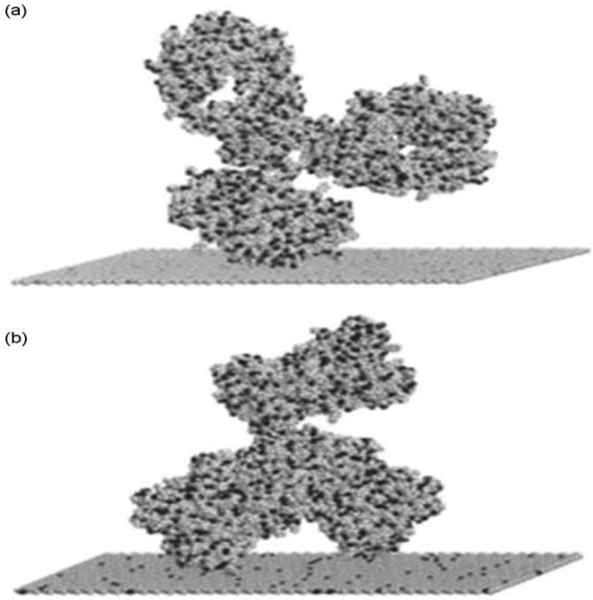


Figure 2.5: An all-atom antibody molecule with (a) end-on or (b) head-on orientation on charged surfaces [16].

3. THE BIOMATERIAL INTERFACE

The outermost atomic layer of a general biomaterial interface at the moment of insertion is a combination of inorganic or organic oxides and hydroxides with low chemical reactivity and low solubility at physiological conditions. Different surfaces possess different basic water chemistries as represented by their elemental compositions and functionalities, such as –OH, CH, PO4 , -NH , -COOH, and -SiOH groups. This chemistry also gives rise to different free surface energies, water-retaining capacities, surface mobilities, etc. — properties that may or may not be important for blood and tissue responses [26].

3.1 Protein Adsorption

Blood consists of approximately 45% formed elements and 55% plasma. The formed elements include red and white blood cells, and platelets. Of the plasma, 93% is water. The remaining 7% is made up of ions, sugars, hormones, enzymes, amino acids and hundreds of proteins. Plasma protein adsorption to biomaterial surfaces is frequently considered to be the initial biological interaction with the host environment. Proteins are high molecular weight solutes that can be considered as copolymers of amino acids. Proteins are heterogeneous molecules and contain regions of differing polarity, charge and hydrophilicity. Thus, proteins exhibit amphoteric and amphiphilic properties. The precise structure of protein is not only determined by its specific sequence of amino acids, but by the secondary and tertiary structure that determines the conformation of the molecule and the distribution and orientation of the side groups. A protein may contain anywhere from fifty to over ten thousand residues with a specific chain sequence of amino acids.

The major driving force for the folding of a polypeptide in solution is dehydration of hydrophobic amino acid side chains tend to be at the periphery of the molecule. The net result is a spontaneously folding protein with a hydrophobic core and a complex irregular exterior surface formed by the polar or hydrophilic side chains. Proteins are generally prefer an aqueous environment. However, when a protein solution is contacted with another phases, there is a tendency for the protein molecules to accumulate at the interface. At a solid interface, the complex structure of proteins give rise to a number of interactions with the surface, involving hydrophobic, electrostatic and polar forces. The extent of these interactions is not only depended on the number of the surface and nature of the protein , but also on the number and concentration of proteins in the contacting solution, the temperature of the system, the length of time of contact and rheological conditions. Thermodynamic driving forces may cause protein molecules to condense onto the surface. Adsorption to the surface may invoke conformational changes in the protein to denature.

Adsorption is not a statistic event as adsorbed proteins can undergo conformational changes with time, and exchange with other molecules in the contacting solution. It is generally accepted that the adsorption of plasma proteins onto an artificial surface is the first event to occur when blood contacts a biomaterial, usually within a few seconds, preceded only by the adsorption of water and inorganic ions. Blood platelets and other formed elements arrive at the surface shortly thereafter, and at the time, interact with a protein layer on the other of a few hundred Angstroms. It is also accepted that the adsorbed protein layer influences the nature of subsequent events, as other blood components such as blood cells must interact with this protein layer. Adhesive proteins may act as bridging molecules for cellular adhesion. Thus, studies of protein adsorption onto biomaterial surfaces is not only relevant to gaining an understanding of blood-material interactions, but also is an important step in the design of improved materials for clinical applications [9].

3.2 Protein Adsorption onto Polyurethanes

The investigation of protein adsorption to artificial surfaces has been significant in biocompatibility research. A large part of this research has been focused on glass, silicone rubber, and hydrogel surfaces. Protein adsorption studies on polyurethane surfaces have been relatively limited. Some studies have been carried out exclusively on polyurethanes in order to gain a better understanding of the effect of polyurethane surface chemistry or surface architecture on protein adsorption. Other studies have incorporated a polyurethane as one of many surfaces evaluated.

Protein adsorption studies on polyurethanes have focused primarily on the adsorption of albumin, fibrinogen and γ-globulins. Since albumin "passivates" while fibrinogen or γ-globulins "activates" surfaces, some investigators have studied competitive adsorption from mixed solutions, and have used albumin/fibrinogen or albumin/ γ-globulins adsorption ratio as indicators of blood compatibility [27].

3.3 Fibrinogen Adsorption

Adsorption of fibrinogen onto biomaterial surfaces is of interest, owing to the protein's role in mediating cellular responses, and as a coagulation protein. It has been postulated that it is not necessarily the amount of the protein that it is adsorbed onto a given surface that is important, but also the conformation of the adsorbed protein. The precise conformation of a protein is partly determined by the length of time that the protein has resided on a surface.

Conflicting results exist concerning the effect of hard segment and soft segment domains on protein adsorption, which has extended to the study of fibrinogen adsorption. Several workers have reported that fibrinogen interacts with the hard segment, and that the hard segment is a determinant of the thrombogenicity of polyurethane materials. It also has been observed that as the hard segment concentration at the surface increases, the surface becomes less attractive to proteins (9, 12). Fibrinogen is the third primary plasma component. In contrast to albumin and IgG, however, fibrinogen appears to play a major role in the inflammatory response. It immediately adsorbs to implanted biomaterials and experiments suggest that it undergoes denaturation following adsorption. This transformation following adsorption onto a surface leads to the fibrinogen adhering more strongly to the material. The degree of fibrinogen denaturation on a material surface correlates strongly with the degree of acute inflammatory response. Additionally, implantation into mice which lack circulating fibrinogen shows a lack of an inflammatory response to the implanted material unless the material has been pre-coated with fibrinogen or plasma. Thus, fibrinogen is a necessary component of inflammatory cell recruitment to implanted biomaterials [28].

Fibrinogen plays a central role in the mechanism of coagulation and thrombosis and is partially involved in the development of postintervention restenosis. Because of therapeutic implications, it is convenient for the vascular interventionalist to revisit its structure, function, and relationships within the vascular environment. This review focuses on the molecular structure, mechanisms of adsorption, and fibrinogen interaction with artificial surfaces [29].

4. MOLECULAR SIMULATIONS

Molecular simulation is a research tool with sufficiently small scales in both time and space, so it can offer clear microscopic information in a direct manner. It has been widely used to understand protein conformational transition at molecular level, and has the potential to complement both experimental and theoretical studies. At present, it has become particularly popular with the increase of computational power and speed of computers. In molecular simulation, both the adsorbate and surface can be visualized using coarse-grained (simplified) models or all atom (atomistic) models, depending on the description precision required and the computational power provided [16].

The complexity of the thermodynamic interactions makes adsorption a unique phenomenon to study. Thus, understanding the mechanism and energetics of protein solid surface interactions is critical to efficiently design novel biomaterials. Atomistic-level computational simulations can provide valuable insight into the details of interactions of individual amino acids with a surface. For this purpose, different computational tools could be applied to understand the mechanism of protein adsorption, such as molecular dynamics (MD), molecular mechanics (MM), and Monte Carlo (MC) methods. These methods can provide crucial atomistic level details to understand and manipulate surface-protein interactions, which would be highly challenging to obtain via experimental methods [3].

MC simulation generates a trajectory in phase space based on the use of random numbers and probability statistics, while MD simulation computes the equilibrium and transport properties of a classical many-body system based on statistical mechanics (controlled by the laws of Newtonian dynamics). MC simulation is usually used for conformational sampling and calculation of thermodynamic properties, while MD simulation is suitable to provide the time-dependent behavior of a molecular system. Moreover, it should be noted that the set of moves in MC simulation is crucial for the results. However, both MC and MD simulations can be used to provide detailed information on the fluctuations and conformational changes of biomacromolecules [16].

The information obtained using these computational methods can be very useful to tailor optimized surfaces to control adsorption of target proteins, e.g., by means of grafting with specific functional groups [3].

4.1 Molecular Mechanics

Molecular mechanics (force field) calculation is the most commonly used type of calculation in computational medicinal chemistry, and a large number of different force fields have been developed over the years. The results of a molecular mechanics (MM) calculation are highly dependent on the functional forms of the potential energy functions of the force field and of the quality of their parameterization. Thus in order to obtain reliable computational results, it is crucial that the merits and limitations of the various available force fields are taken into account. The main drawback of MM is that the method and the quality of the calculations are extremely dependent on empirical parameters. Such parameters are generally determined by experimental studies or high-level *ab initio* calculations, and the parameterization is often based on a small number of model systems.

The fundamental unit of most force fields is the atom type, determining what parameters to apply for all interactions involving the same constituent atom types. The various interaction types include bond lengths, angles, distances, etc. (see Fig 4. 2). Each bond in a structure will contribute a stretch term to the total energy. Bonds are normally described as harmonic bonds, and like springs and are characterized by a preferred length. The resistance to change from the optimum value is then defined by a ‘‘force constant,’’ and each bond type is thus described by at least two parameters and the energies calculated by Hooke’s law (Eq. 1). Here the reference bond length is l_o.

$$E\mathrm{s}=\mathrm{ks}(\mathrm{l}-\mathrm{l}_0)^2 \qquad \textbf{(4.1)}$$

The simplest approach to obtaining optimized bond angles close to the reference value θ (**Fig. 4.2**) is to introduce a quadratic energy penalty, the harmonic approximation,similar to the representation of bond energies (Eq. 4), although some methods use nonbonded interactions to model angle forces.

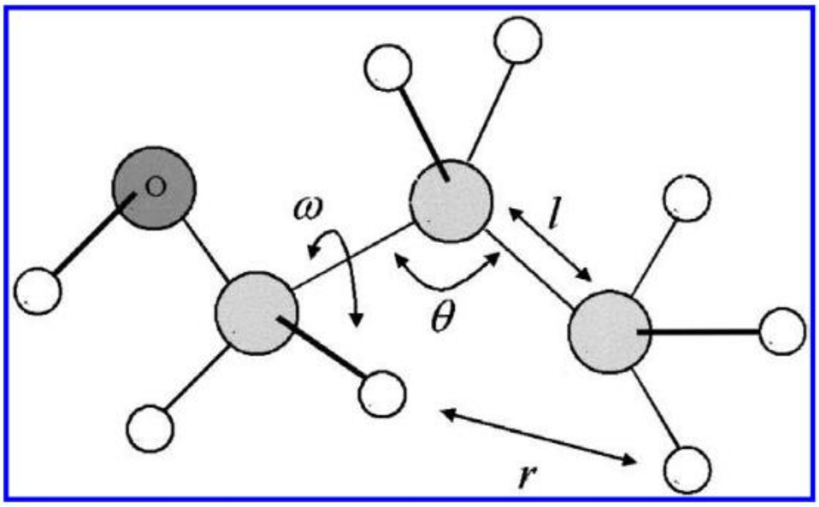


Figure 4.1 : Atom bond, angles [23].

$$E_b=k_b(\theta-\theta_0)^2 \quad \textbf{(4.2)}$$

Some other important parameters are:

Nonbonded interactions: Interactions between atoms that are not transmitted through bonds are referred to as nonbonded interactions.

Electrostatic interactions: Calculation of electrostatic interaction energies can be done simply by using Coulomb's law, (Eq. 3), providing charges q centered on each nucleus.

$$E_{el}=\frac{q_{i-}\ q_j}{Er} \quad \textbf{(4.3)}$$

Van der Waals interactions: Short-range repulsions and London dispersion attractions are balanced by a shallow energy minimum at the van der Waals distance (Eq. 4), describing the Lennard–Jones' potential, used by most force fields. Here the parameters A and B are calculated based on atomic radii and the minimum found at the sum of the two radii.

$$E_{vdw}=\frac{A}{r^{12}}-\frac{B}{r^6} \quad \textbf{(4.4)}$$

Most force fields use the Lennard–Jones functional form or close derivatives (9-6 or 14-7 functional forms as opposed to the standard 12-6 form), shown in Eq. 5.

$$E_{vdw} = Ae^{-\propto r} - \frac{B}{r^6} \quad \textbf{(4.5)}$$

Hydrogen bonding: The simplest way to handle hydrogen bonding is to rely on the other nonbonded potentials to reproduce hydrogen bonds. Some methods include specific pair parameters.

Torsional angles: Four consecutive atoms define the torsional bond (Fig.4.2) [29].

4.2 Molecular Dynamics

Molecular dynamics (MD) is a powerful technique for computing the equilibrium and dynamical properties of classical many-body systems. Over the last fifteen years, owing to the rapid development of computers, polymeric systems have been the subject of intense study with MD simulations. At the heart of this technique is the solution of the classical equations of motion, which are integrated numerically to give information on the positions and velocities of atoms in the system.

A simple flow diagram of a standard MD algorithm is shown in Fig. 4.3 and includes the following steps:

1. First, a model configuration representing a molecular-level snapshot of the corresponding physical system is chosen or constructed, and is initialized (initial positions, velocities of each particle within the system).

2. Then the total force acting on each particle within the system is computed. For polymer systems such a force has two components: intermolecular (from atoms belonging to different polymer chains) and intramolecular (from atoms belonging to the same chain).

3. The integration of the equations of motion follows with an appropriate method. The most popular of these will be described in detail in the next section.

4. Actual measurements are performed (positions, velocities, energies, etc., are stored) after the system has reached equilibration, periodically every N steps.

5. After completion of the central loop (N steps), averages of the mea- k sured quantities and of the desired properties are calculated and printed [30].

As stated above, at the heart of an MD simulation is the solution of the classical equations of motion. Let us consider a system consisting of N interacting molecules described by a potential energy function V.

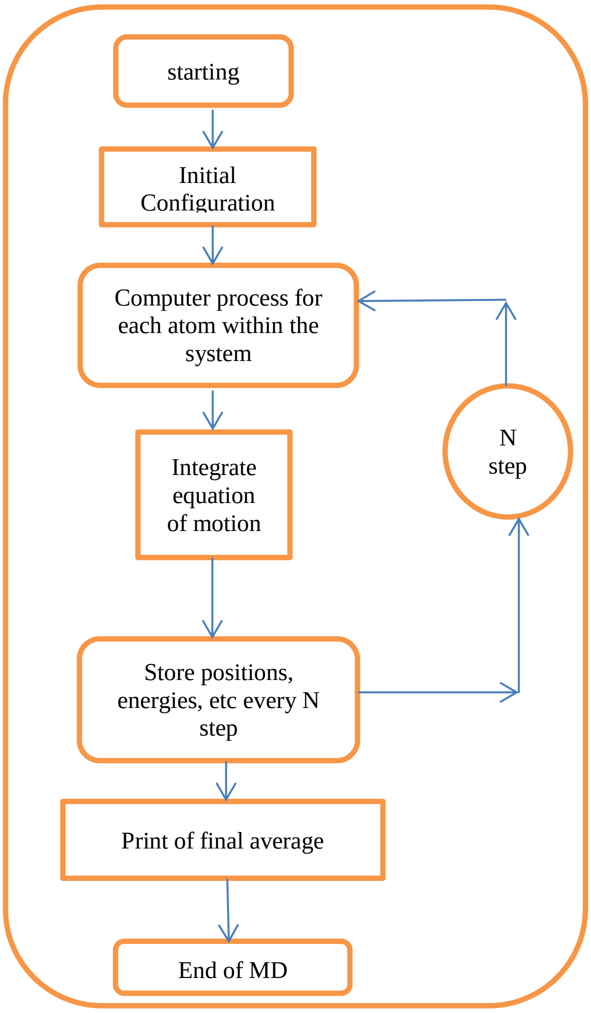

starting
Initial Configuration
Computer process for each atom within the system
Integrate equation of motion
Store positions, energies, etc every N step
Print of final average
End of MD
N step

Figure 4.2 : A simple flow diagram of standard MD algorithm

Let us also denote as q_k And $\dot{\mathrm{q}}_{\mathrm{k}}$ the generalized coordinates describing the molecular configuration and their time derivatives, respectively. The classical equations of motion for this system can be formulated in various ways. In the Lagrangian formulation, the trajectory q(t)(=q1(t), q2(t),…,qk(t),…) satisfies the following set of differential equations:

$$\frac{\partial \mathrm{L}}{\partial \mathrm{q_k}} = \frac{\mathrm{d}}{\mathrm{dt}}\left(\frac{\partial \mathrm{L}}{\partial \dot{\mathrm{q}}_{\mathrm{k}}}\right) \qquad \textbf{(4.6)}$$

where L is the Lagrangian of the system. This is defined in terms of the kinetic energy, K, and potential energy, V, as L= L(**q**, **q**˙, t) ≡ K-V. The generalized momenta p k conjugate to the generalized coordinates q k are defined as:

$$p_k = \frac{\partial L}{\partial \dot{q}_k} \qquad \textbf{(4.7)}$$

Alternatively, one can adopt the Hamiltonian formalism, which is cast in terms of the generalized coordinates and momenta. These obey Hamilton’s equations:

$$\dot{q}_k = \frac{\partial H}{\partial p_k}, \quad \textbf{(4.8)}$$

$$\dot{p}_k = -\frac{\partial H}{\partial q_k} \quad \textbf{(4.9)}$$

where H is the Hamiltonian of the system, defined through the equation:

$$H(p,q) = \sum_k \dot{q}_k p_k - L \quad \textbf{(4.10)}$$

If the potential V is independent of velocities and time, then H becomes equal to the total energy of the system:

$$H(p, q)=K(p)+V(q) \quad \textbf{(4.11)}$$

In Cartesian coordinates, Hamilton’s equations of motion read:

$$\dot{r}_i \equiv v_i = \frac{P_i}{m_i}, \quad \textbf{(4.12)}$$

$$\dot{p}_i = -\nabla_{r_i} \quad \textbf{(4.13)}$$

$$V \equiv -\frac{\partial V}{\partial r_i} = F_i \quad \textbf{(4.14)}$$

Hence

$$m_i \ddot{r}_i \equiv m_i \dot{q}_i = F_i \quad \textbf{(4.15)}$$

where Fi is the force acting on atom i. Solving the equations of motion then involves the integration of the 3N second-order differential equations (Eq.6) (Newton’s equations). The classical equations of motion possess some interesting properties, the most important one being the conservation law. If we assume that K and V do not depend explicitly on time, then it is straightforward to verify that $H^{\cdot}$=dH/dt zero, i.e., the Hamiltonian is a constant of the motion. In actual calculations this conservation law is satisfied if there exist no explicitly time- or velocity dependent forces acting on the system. A second important property is that Hamilton’s equations of motion are reversible in time. This means that, if we change the signs of all the velocities, we will cause the molecules to retrace their trajectories backwards. The computer-generated trajectories should also possess this property.

There are many different methods for solving ordinary differential equations of the form of (Eq. 6). The two most popular families of algorithms used in MD simulations for the solution of classical equations of motion is: the higher-order methods and the Verlet algorithms [30].

4.3 Force-Field Parameters

In order to set up a molecular mechanics model it is necessary to identify mathematical expressions that are able to define the molecular structures and give the corresponding strain energies. It is also necessary to find parameter values for these expressions so that the model can reproduce or predict the molecular structures and properties. These two parts of molecular mechanics package have a direct influence on the optimized structure. As the potential energy functions and the force-field parameters are interrelated, the parameters should not, in general, be transferred from one force field to another.

In theory, a properly developed force field should be able to reproduce structures, strain energies, and vibrations with similar accuracies, as the three properties are interrelated. However, structures are dependent on the nuclear coordinates (position of the energy minima), relative strain energies depend on the steepness of the overall potential energy surface (second derivative). Thus, force fields used successfully for structural predictions might not be satisfactory for conformational analyses or the prediction of vibrational spectra, and vice versa. The only way to overcome this problem is to include the appropriate type of date in the parameterization process. [31].

Traditionally, the potential forces are calculatting using empirical all- atom potential functions, such as CHARMM (Chemistry at Harvard Molecular Mechanics), AMBER (assisted model building with energy refinement), GROMOS (Groningen molecular simulation), and CVFF (consistent valence force field), which include the solvent either explicitly or as a continuum (implicit solvent treatment). The functional forms of the force fields are a trade-off between accuracy in representing forces acting on atoms and low computational cost or ease of parameterization. Thus, one usually considers only interactions between point charges in the computation of the electrostatic-interaction energy, thereby neglecting higher moments of electron-charge density and polarization effects. Harmonic functions for bond stretching and bond-angle bending are used instead of more refined ones with anharmonicity included. However, this approximation can result in unreasonably large distortions of the bond angles. The inherent inaccuracies also result in an incompatibility of properties obtained by using different force fields. Although the per-residue errors inherent in force-field inaccuracies amount to a fraction of a kilocalorie per mole, they translate into tens of kilocalories per mole for the entire protein. Therefore, optimization of the whole force field, as for simplified models, needs to be performed for all-atom force fields [23, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41].

4.4 Molecular Simulation of Ensembles

Molecular simulations are almost invariably conducted in the context of an ensemble. The choice of the ensemble determines which thermodynamic properties can be evaluated and it also governs the overall simulation algorithm. Monte Carlo and molecular dynamic algorithms for a specified ensemble are very different, reflecting fundamental differences in the two simulation methods.

The original Monte Carlo method sampled the canonical (NVT) ensemble. Metropolis et al. (1953) introduced the concept of importance sampling to construct a Markov chain [43]. The Mont Carlo method can be extended relatively easily to other ensembles because importance sampling can be used any Markov chain process. In general, the Monte Carlo method can be extended to other ensembles by sampling the variable that is conjugate to the new fixed parameter. For example, McDonald (1972) reported Monte Carlo simulations in the isobaric – isothermal (NPT) ensemble by sampling volume (the conjugate of pressure) in addition to molecular coordinates. In the grand canonical ensemble, the chemical potential is fixed and the total number of molecules N is the conjugate variable that is sampled (Valleau and Cohen, 1980). In the past, it has been possible to perform Monte Carlo simulations in the microcanonical ensemble [43].

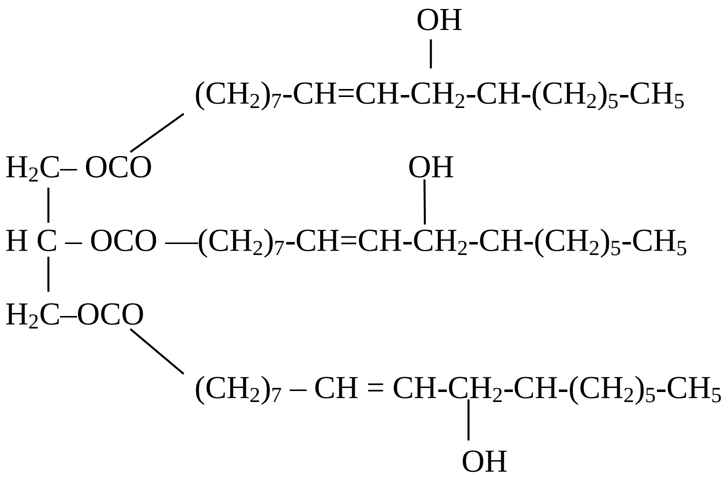
Newton’s equations of motion lead naturally to the microcanonical (NVE) ensemble because energy is conserved. Therefore, in contrast to Monte Carlo simulations, the microcanonical ensemble is the natural choice for molecular dynamics. Extending molecular dynamics to other ensembles requires some artificial tampering with the equations of motion. A canonical (NVT) Ensemble can be obtained by either using velocity scaling or explicitly adapting the equations of motion. Anderson (1980) formulated an isobaric-isoenthalpic (NPH) and isobaric-isothermal (NPT) molecular dynamic algorithms by introducing additional degrees of freedom which are coupled to the particle coordinates [44].

5. SIMULATION METHODS

5.1 System

In this work, adsorption characteristics of polyurethanes, which constitute a very appealing class of polymers because of their high biocompatibility and excellent physical and mechanical properties were focused. These versatile polymers have been widely used in wound dressings as well as in cardiovascular and breast implants. Swelling of the poly(ethylene glycol) (PEG)-based polyurethanes is an important issue in biomedical applications and is needed to be controlled by addition of cross-linkers. Alternatively, castor oil (CO), which is attractive due its safety and effectiveness in biomedical applications, can be used as the polyol component for decreasing swelling degree of PEG-based polyurethanes due to its high content of ricinoleic acid, which has a hydroxyl functional group on the 12th carbon. Since the amount of castor oil in the polyurethane determines strongly polymer properties, the performance of the castor oil and PEG-based polyurethane films in protein adsorption has been studied in great detail.

In order to better understand the alterations in the adsorption characteristics due to the incorporation of castor oil into PEG-based polyurethanes, adsorption of fibrinogen (γFg) studied on two model polyurethane systems: (1) crystalline poly(ethylene glycol)−hexamethylene diisocyanate (PEG-HDI) and (2) amorphous castor oil−hexamethylene diisocyanate (CO-HDI) polymers, which constitute two limiting compositions (Figure 5.2). In this study the atomistic models of these two polymers were used. Finally, we performed molecular mechanics calculations and molecular dynamics simulations to compare the affinity of these polyurethanes to the fibrinogen protein in the presence of implicit and explicit water.



(a)

$H-(O-CH_2-CH_2)_{68}-OH$ **(b)** $O = C = N - (CH_2)_6-N = C = O$ **(c)**

Figure 5.1 : Polyurethane monomers used in this study: (a) castor oil (CO), (b) poly(ethylene glycol) (PEG), and (c) hexamethylene diisocyanate (HDI) to create PEG-HDI and CO-HDI polyurethane copolymers [3].

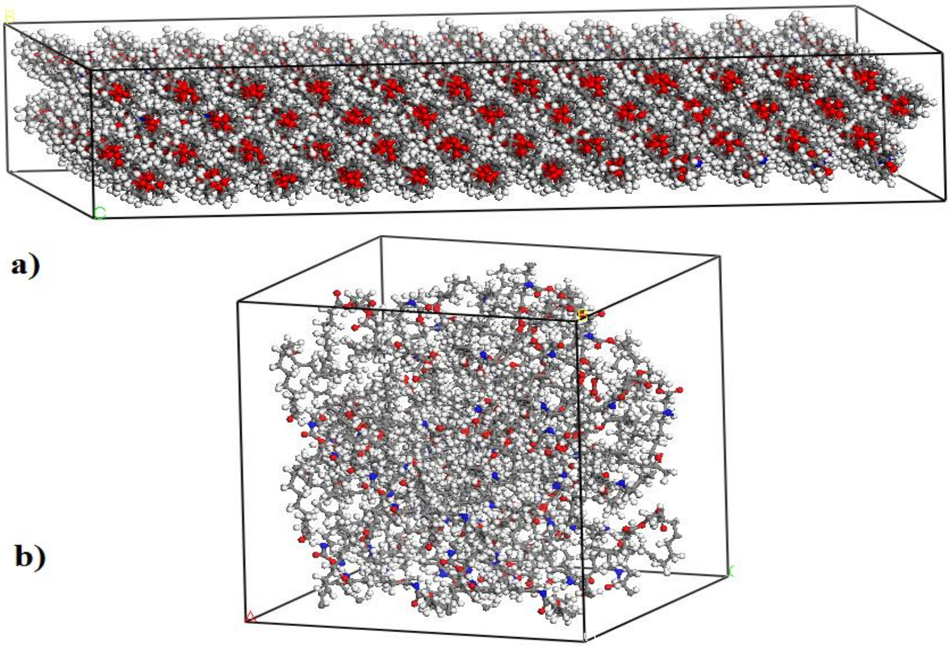


Figure 5.2 : Bulk polymer matrices (a) PEG-HDI, (b) CO-HDI. The atoms are colored in the following manner: gray for carbon atoms, red for oxygen atoms, blue for nitrogen atoms, and white for hydrogen atoms. The box around the molecules is shown to provide information about the matrix shapes used in the simulations [3].

All the energy minimization calculations in this study were performed using the Materials Studio 5.0 simulation package, and the secondary structure visualizations were obtained using Discovery Studio 3.0 software (Accelrys Inc., San Diego, CA) and all molecular dynamic simulations were performed using the LAMMPS simulation program [45].

5.2 Modeling Protein−Surface Interactions

In order to compare the affinity of the model surfaces to the protein, two step procedure was employed: First, energy minimization in dielectiric medium (implicit solvent) was performed in order to determine the optimal orientations for adsorption on each surface. Then, full atomistic MD simulations with explicit water were carried out using the structures determined in the minimization step on each of the crystalline and amorphous polyurethane surfaces to investigate the effect of protein−water competition on the adsorption behavior. This combination of energy minimization in implicit solvent and MD simulations in explicit water is useful in learning about different aspects of the protein−surface interactions and had been previously used in various studies [3].

5.3 Protein-surface interaction determination

The crystal structure of a 30 kDa C-terminus γ-chain fragment of fibrinogen (γFg) was obtained from the Protein Data Bank (PDB ID# 1FID) and further processed in preparation for our simulations, which involved the removal of noncovalently bonded heterogeneous atoms (e.g., oxygen atoms from bound water molecules) and the addition of hydrogens to complete valence requirements of the crystal structure’s heavy atoms. In addition, because this is a protein fragment, the downloaded structure had an incomplete terminal residue (leu 402), which was also removed.

This protein model is particularly relevant for these studies because it contains only two disulfide cross-links between adjacent cysteine residues. Thus, its tertiary structure is predominantly stabilized by secondary bonding interactions, which the functional groups of an adsorbent surface will compete with to potentially induce protein unfolding and spreading on the surface, with possible exposure of the P1-P2 epitopes [4].

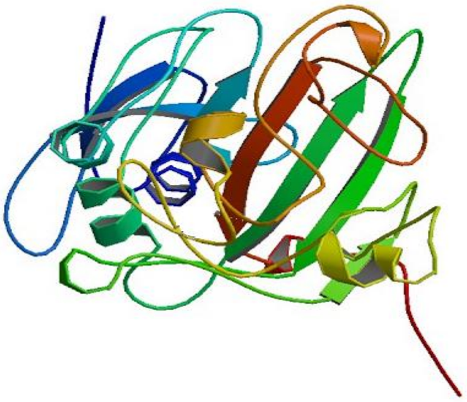


Figure 5.3 : Structure of human gamma fibrinogen 30 KD carboxyl terminal fragment [24].

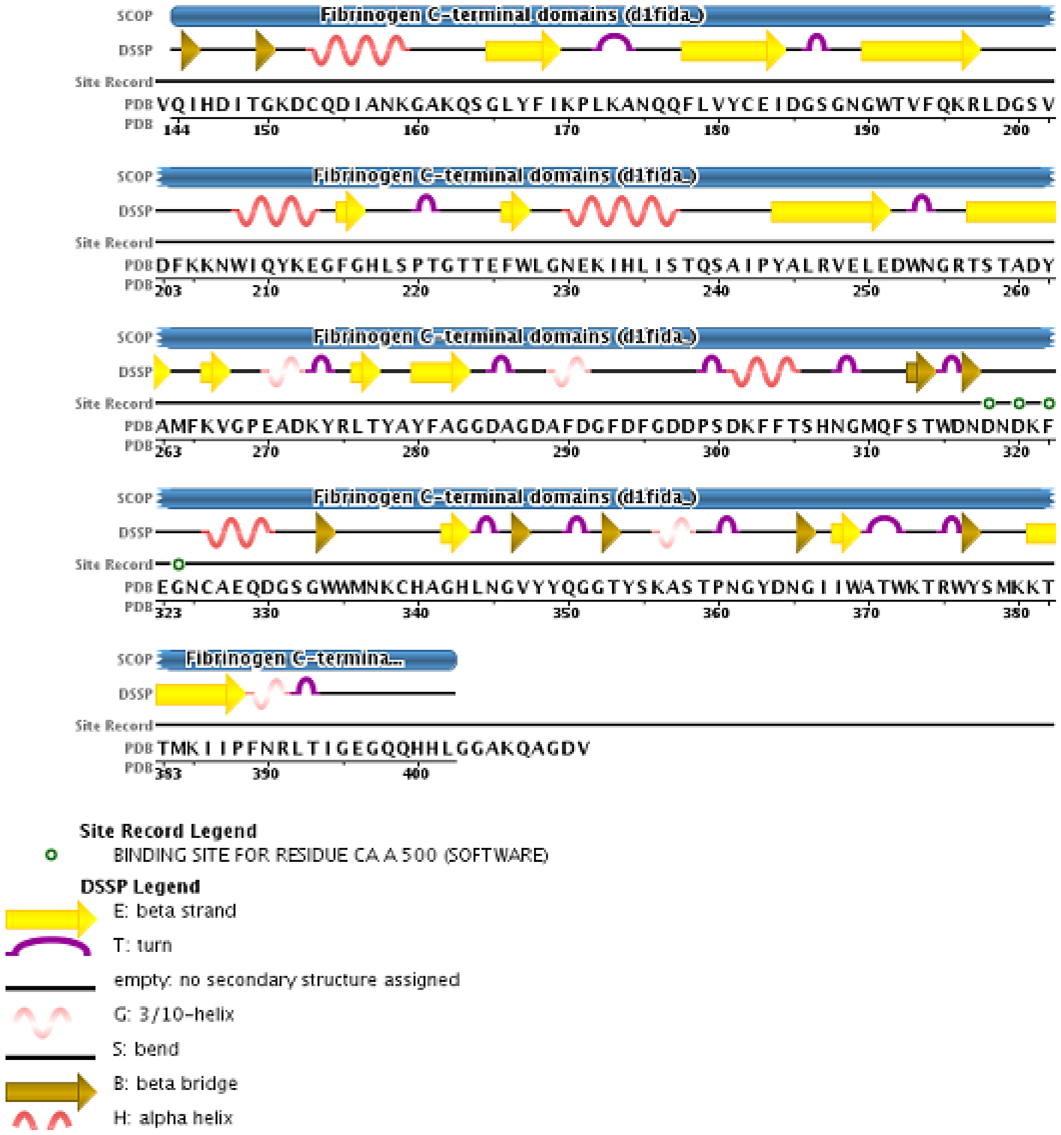

SCOP
DSSP
Site Record
PDB
Fibrinogen C-terminal domains (d1fida_)
VQIHDITGKDCQDIANKGAKQSGLYFIKPLKANQQFLVYCEIDGSGNGWTVFQKRLDGSV
144 150 160 170 180 190 200
DFKKNWIQYKEGFGHLSPTGTTEFWLGNEKIHLISTQSAIPYALRVELEDWNGRTSTADY
203 210 220 230 240 250 260
AMFKVGPEADKYRLTYAYFAGGDAGDAFDGFDFGDDPSDKFFTSHNGMQFSTWDNDNDKF
263 270 280 290 300 310 320
EGNCAEQDGSGWWMNKCHAGHLNGVYYQGGTYSKASTPNGYDNGIIWATWKTRWYSMKKT
323 330 340 350 360 370 380
Fibrinogen C-termina...
TMKIIPFNRLTIGEGQQHHLGGAKQAGDV
383 390 400
Site Record Legend
BINDING SITE FOR RESIDUE CA A 500 (SOFTWARE)
DSSP Legend
E: beta strand
T: turn
empty: no secondary structure assigned
G: 3/10-helix
S: bend
B: beta bridge
H: alpha helix

Figure 5.4 : Residues sequence of gamma fibrinogen 30 KD carboxyl terminal fragment [31].

Table 5.1 : Fibrinogen C-terminus, Amino Acid number and polarity chart [9].

No	Amino Acid	3-letter	Side chain polarity	Number of Amino Acid
1	Alanin	Ala	Non polar	18
2	Arginine	Arg	Basic polar	6
3	Asparagine	Asn	Polar	15
4	Aspartic acid	Asp	Acidic polar	22
5	Cystein	Cys	Non polar	4
6	Glutamic Acid	Glu	Acidic polar	10
7	Glutamine	Gln	Polar	14
8	Glycine	Gly	Non polar	33
9	Histidine	His	Basic polar	8
10	Isoleucine	Ile	Non polar	14
11	Leucine	Leu	Non polar	13
12	Lysine	Lys	Basic polar	21
13	Methionine	Met	Non polar	5
14	Phenylalanin	Phe	Non polar	16
15	Proline	Pro	Non polar	7
16	Serine	Ser	Polar	14
17	Threonine	Thr	Polar	18
18	Tryptophan	Trp	Non polar	10
19	Tyrosine	Tyr	Polar	13
20	Valine	val	Non polar	8

For studying multiple interactions among the protein, water molecules, and surfaces, the choice of force field was based on the accurate representation of protein−water and protein− surface interactions, which were of the primary concern since the surface of polymers in this study were kept rigid. Each force field has a unique background and assumption, and consequently presents different computational results. This study investigated the effects of consistent valence force field (CVFF) [3], which was originally parametrized for peptide and protein structures and also applicable to polymers in aqueous environments. The CVFF force field was used previously in similar studies [3, 24, 46] and was shown to reproduce the protein dynamics satisfactorily.

In order to compare the affinity of the model surfaces to the protein, the adsorption energetics of the surfaces were compared by optimizing the geometry of the protein on each surface through energy minimization in the dielectric medium with $\varepsilon = 78$. This allowed direct evaluation of the surface− protein interactions without the interference of the water molecules. For this purpose the geometry of the isolated fibrinogen domain was energy-minimized first, and then it was placed away from each of the model surfaces in six different orientations to remove the bias of approach angle. These orientations corresponded to the sides of a hypothetical rectangular box containing the protein domain as illustrated in Figure 5.5. For each starting orientation, energy minimization was carried out, while the polymer structure was kept rigid, to analyze changes in the interaction energy between the surface and polymer due to adsorption [3].

In the last part of this work, the orientations yielding minimum-energy configurations were chosen for the PEG-HDI and CO-HDI surfaces, and molecular dynamics were carried by replacing the implicit solvent by explicit water molecules in order to reveal the effect of water-protein-surface interactions. The protein was soaked with around 10,000 SPC water molecules at a density of 1 g/cm3. The starting configuration was selected after the energy minimization, and simulations were run up to 15 ns with a time step of 1 fs at 300 K until the system reached equilibrium.

5.4 Initial Adsorption Stage in the Dielectric Medium

At first, Geometry of the isolated fibrinogen module in the dielectric medium was optimized. The minimized energy, to be used later to calculate the interaction energy with the polyurethane surface, amounts to 2.4 MCal/mol. There are relatively small differences between the optimized and the experimental geometries of the backbone but different orientations of the side groups due to the lack of hydration and a slightly larger number of intramolecular H bonds. Afterward, Geometry of the fibrinogen module close to the polyurethane surface, keeping fixed throughout the carbon planes was optimized. Since the module may be roughly inscribed in a rectangular, we considered six different starting orientations, corresponding to each face of the protein lying on the surface.

Upon direct energy minimizations, all six orientations showed a significant initial surface adsorption, usually accompanied by some rearrangements of the interacting strands, though not as large as in the albumin fragments. We anticipate that all the corresponding energy minima turned out to be local ones, as it will be shown later through the MD runs and subsequent optimizations. The results of the initial optimizations are summarized.

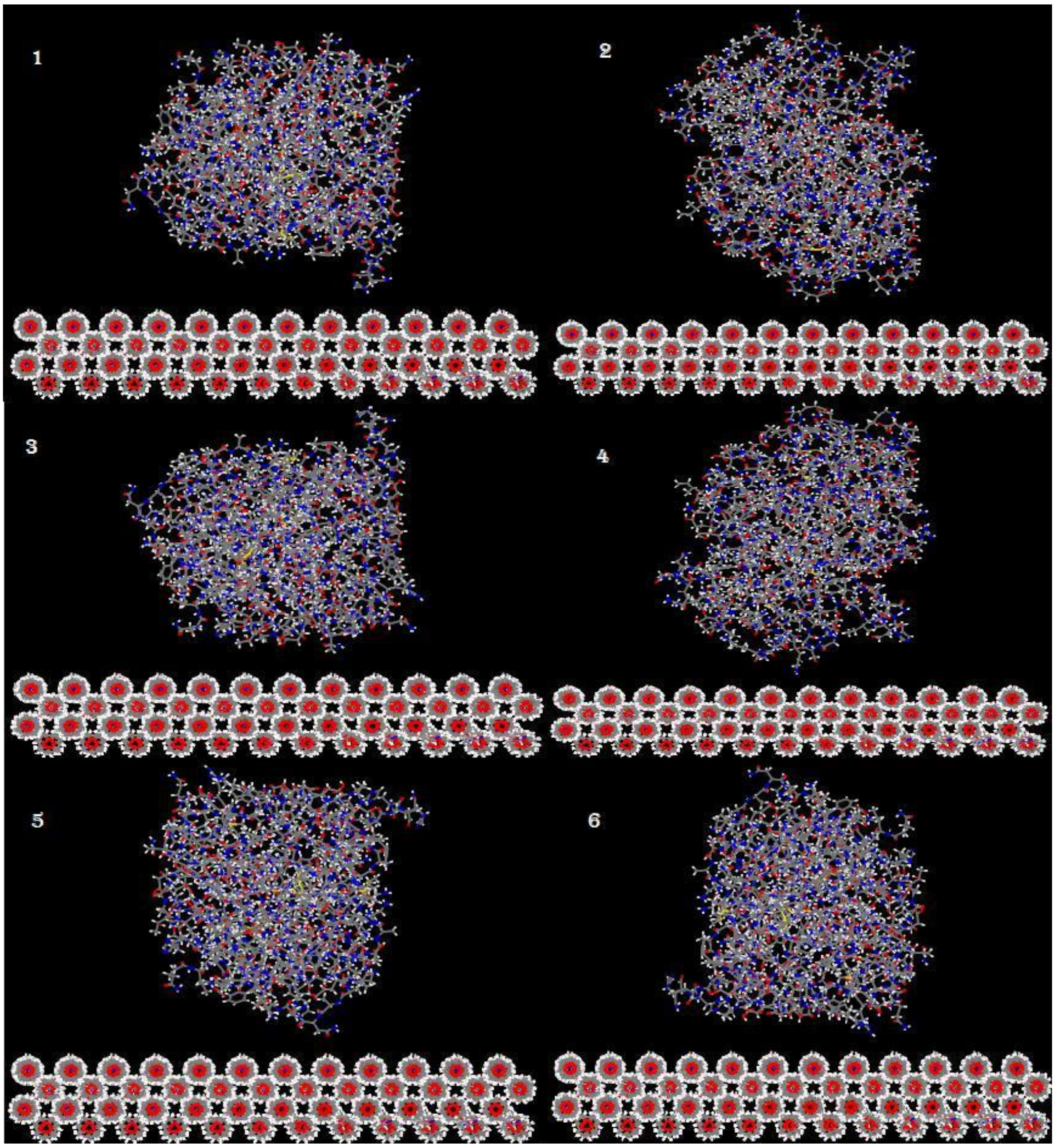


Figure 5.5 : Fibrinogen module inscribed in 6 different orientation on PU1.

Some intramolecular rearrangements are evident in the optimized geometry of the most stable initial adsorption state shown in the Figure 5.5. Such rearrangements consist of the loss of the β-sheets at the right-hand side and at the center of the molecule, although the corresponding strands do remain roughly parallel to one another and to the surface. On the other hand, very little intramolecular changes, if any, are found upon adsorption in the less stable state so that the geometry of the fibrinogen module and its secondary structure are essentially unchanged.

In addition to the relative energy of the initial adsorbed state (Erel), the interaction energy, defined as Eint =(Efree + Eplanes) - Etot, where Efree is the energy of the free, isolated molecule in the optimized geometry. According to this definition, Eint>0 is the energy required to detach the adsorbed molecule from the surface and bring it back to the free state. Also, since the planes were kept fixed in the simulations, we have *E*planes = 0, although of course all the carbon atoms do correctly interact with the protein atoms. We also define *E*strain = *E*frozen - *E*free, where *E*frozen is the energy of the fibrinogen module in the frozen geometry it adopts upon adsorption.

After optimization in material studio systems with minimum energies were comprised of 10,000 SPC water molecules and were performed using the LAMMPS program. Systems equilibrated for 100 ps at a temperature of 300 K and constant pressure of 1 atm with the protein backbone atoms restrained in order to prevent adsorption before equilibration. Following the equilibration classical and accelerated MD simulations were carried out for 15 ns with a time step of 1.0 fs enabled by the SHAKE algorithm to ensure rigid hydrogen atoms [47].

5.5 Calculation of Changes in Phi (Φ) and Psi (ψ) torsion angles

The amino acids phi (Φ) and psi (ψ) torsion angles are important to observe the secondary structures changes that occur after adsorption of proteins.

Torsion angles are among the most important local structural parameters that control protein folding - essentially, if we would have a way to predict the Ramachandran angles for a particular protein, we would be able to predict its 3D structure. The reason is that these angles provide the flexibility required for folding of the polypeptide backbone, Thus, rotation of the main chain (backbone) of a protein can be described as the rotation of the peptide bond planes relative to each other. For this purpose, fibrinogen C-terminus γ-chain Φ and ψ changes in torsion angles are investigated after adsorption.

For each orientation, all of the amino acids Φ and ψ torsion angles is calculated as in Equality 5.1 and 5.2 after the protein is adsorbed on polymer surfaces.

$$\Delta\Phi=\Phi_{End}-\Phi_{start} \qquad \textbf{(5.1)}$$

$$\Delta\psi=\psi_{End}-\psi_{strat} \qquad \textbf{(5.2)}$$

Then, from Equation 5.3 and 5.4 average changes for each amino acid Φ and ψ torsion angles is calculated in the all the six different orientation, regardless of location.

$$<\Delta\Phi>_{orientation} = \sum_{n=1}^{6} \Delta\Phi i/6 \quad \textbf{(5.3)}$$

$$<\Delta\psi>_{orientation} = \sum_{n=1}^{6} \Delta\psi i/6 \quad \textbf{(5.4)}$$

To calculate the absolute changes in Φ and ψ torsion angles, calculations reiterated in Equation 5.5 and 5.6 as follows.

$$|\Delta\Phi| = |\Phi_{End}| - |\Phi_{start}| \quad \textbf{(5.5)}$$

$$|\Delta\ \psi| = |\psi_{End}| - |\psi_{start}| \quad \textbf{(5.6)}$$

Then from Equation 5.7 and 5.8 average absolute bond angles were calculated for six different orientation and for each amino acid in the protein chain, the absolute changes in Φ and ψ are calculated again, regardless of location.

$$<|\Delta\Phi|>_{orientation} = \sum_{n=1}^{6} |\Delta\Phi i|/6 \quad \textbf{(5.7)}$$

$$<|\Delta\ \psi|>_{orientation} = \sum_{n=1}^{6} |\Delta\psi i|/6 \quad \textbf{(5.8)}$$

According to the data obtained from each of the different orientation amino acids ψ and Φ angles changes and absolute changes graphs prepared.

After calculation of all of the amino acids Φ and ψ changes and absolute changes by using Equation 5.7, and 5.8, average of the same type of amino acids found in protein were calculated by using this calculation for each amino acid Φ and ψ angles(AA), the graphs for 20 amino acid have been prepared (n: number of the individual amino acid protein)

$$<Amino\ acid\ AA\ (\Delta\Phi)> = \sum_{n=1}^{6} Amino\ acid\ AA\ (\Delta\Phi)/n \quad \textbf{(5.9)}$$

$$<Amino\ acid\ \ AA\ (\Delta\psi)> = \sum_{n=1}^{6} Amino\ acid\ AA\ (\Delta\psi)/n \quad \textbf{(5.10)}$$

By using amino acid Φ and ψ angles that are calculated before, in order to better observe of changes in secondary structure of adsorbed protein, Ramachandran plots were prepared and by using DSSP program [33], the adsorption of protein before and after the changes in the structure of β-Sheets were investigated [3,49].

6. ANALYSIS OF ADSORPTION ENERGETICS AND ORIENTATION

6.1 Adsorption in the Implicit Water Molecules

For each orientation, the interaction energy between the protein and the polymer surface (Eint) was calculated after the energy minimization:

$$E_{\mathrm{int}} = (E_{\mathrm{prot}} + E_{\mathrm{poly}}) - E_{\mathrm{tot}} \qquad \textbf{(6.1)}$$

where Eprot is the intramolecular energy of the fibrinogen before adsorption, Etot is the total energy of the protein−polymer system after adsorption, and Epoly is the intramolecular energy of the polymer surface (Epoly = 0, as the polymer structure was kept rigid). In this work PU2 surface was not kept rigid and it calculated by material studio and equals 2.1Mcal/mol. Interaction energies as a function of protein atoms and amino acids being in contact with the polymer surface within arbitrary adsorption layers of thicknesses (δ) of 3, 5, and 7 Å were plotted. A linear model was fitted to the interaction energy as a function of the number of amino acids. The slope in the model, corresponding to the absolute interaction energies between the protein and the surface, was used as a basis for the comparison of the surface affinity to the protein, larger slope values indicating larger interaction strength. The calculation procedure was repeated for the strain energy, which is defined as:

$$E\mathrm{strain} = E_{\mathrm{frozen}} - E_{\mathrm{free}} \qquad \textbf{(6.2)}$$

where Efrozen is the intramolecular energy of the protein after adsorption. The strain energy is indicative of broken H-bonds and thus breaking of β-sheets in the protein.

The intramolecular energy (Eprot) of the free-standing fibrinogen was calculated after geometry optimization by energy minimization. The resulting value of 2.34 Mcal/mol. Next, the interaction energy (Eint) and the strain (Estrain) energy of the protein−surface system were calculated for all six different protein orientations through energy minimization as described above. For each of the orientations, the number of amino acids and the number of protein atoms adsorbed on the surface were determined. The resulting interaction energies were then plotted against the number of amino acids and the number of protein atoms within the three different adsorption layers. The slopes of the linear fits to the data, which correspond to absolute interaction energies indicating the adsorption strength of the polymeric surfaces, are compared along with the strain energies. It can be seen that for the adsorption layers with thicknesses 5 and 7 Å the order of the absolute interaction energies remains unchanged.

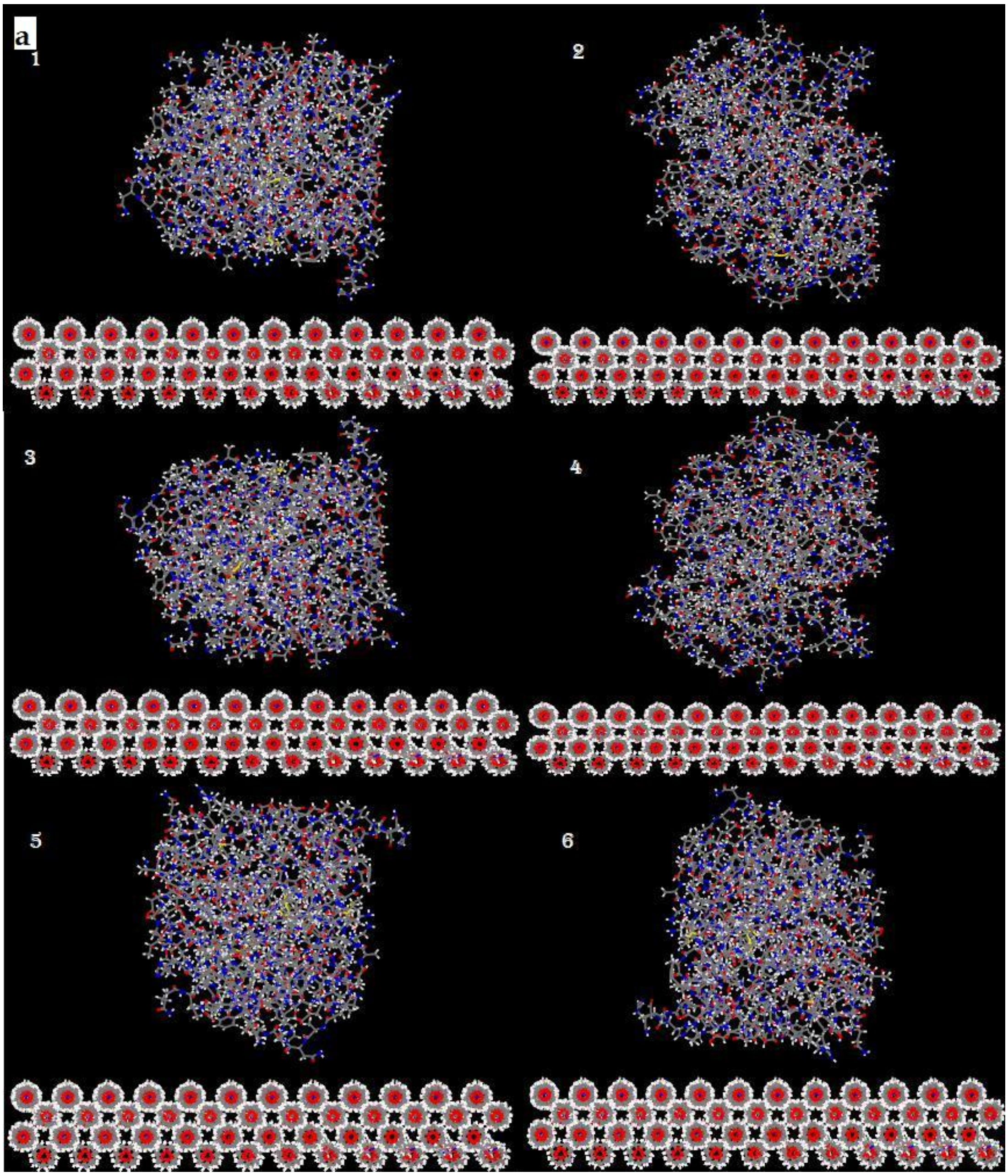


Figure 6.1 : The 6 initial geometries of fibrinogen placed close to the PU1 polyurethane surface.

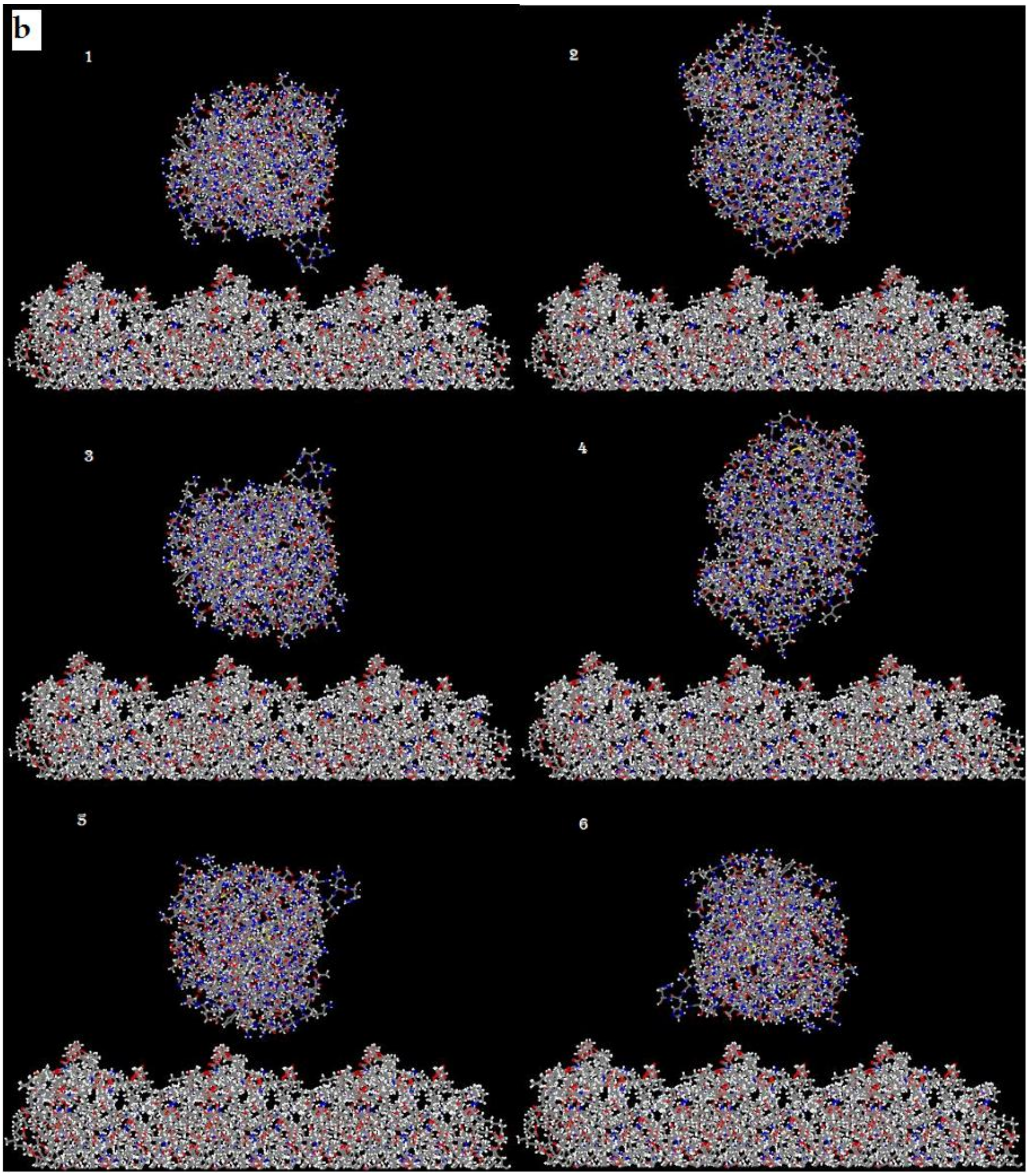


Figure 6.2: The 6 initial geometries of fibrinogen placed close to the PU2 polyurethane surface.

For each orientation, the interaction energy between the protein and the polymer surface (Eint) was calculated after the energy minimization:

$$E\text{int} = (E\text{prot} + E\text{poly}) - E\text{tot} \qquad \textbf{(6.3)}$$

where Eprot is the intramolecular energy of the fibrinogen before adsorption, Etot is the total energy of the protein-polymer system after adsorption, and Epoly is the intramolecular energy of the polymer surface (Epoly = 0, as the polymer structure was kept rigid).

Table 6.1: Number of amino acids within 3Å, 5Å and 7Å at 6 different orientation: a)PU1 , b)PU2

a)

orientation	*N-Amino acid 3Å*	*N-Amino acid 5Å*	*N-Amino acid 7Å*
PU1-1	14	24	30
PU1-2	10	15	17
PU1-3	11	19	28
PU1-4	6	7	17
PU1-5	11	14	21
PU1-6	17	22	26

b)

orientation	*N-Amino acid 3Å*	*N-Amino acid 5Å*	*N-Amino acid 7Å*
PU2-1	26	35	42
PU2-2	19	23	27
PU2-3	24	29	39
PU2-4	14	17	26
PU2-5	22	27	35
PU2-6	12	15	20

In this work PU2 surface was not kept rigid and it calculated by material studio and equals to 210.77 kcal/mol. Interaction energies as a function of protein atoms and amino acids being in contact with the polymer surface within arbitrary adsorption layers of thicknesses (δ) of 3, 5, and 7 Å were plotted. A linear model was fitted to the interaction energy as a function of the number of amino acids. The slope in the model, corresponding to the absolute interaction energies between the protein and the surface, was used as a basis for the comparison of the surface affinity to the protein, larger slope values indicating larger interaction strength. The calculation procedure was repeated for the strain energy, which is defined as:

$$E\text{strain} = E\text{frozen} - E\text{free} \qquad \textbf{(6.4)}$$

where Efrozen is the intramolecular energy of the protein after adsorption. The strain energy is indicative of broken H-bonds and thus breaking of β-sheets in the protein.

The intramolecular energy (Eprot) of the free-standing fibrinogen was calculated after geometry optimization by energy minimization.

The extent of deformation of the protein upon adsorption was analyzed through the calculation of interaction energy and strain energies for each orientation, which were then plotted against both the number of amino acids and the number of protein atoms as below:

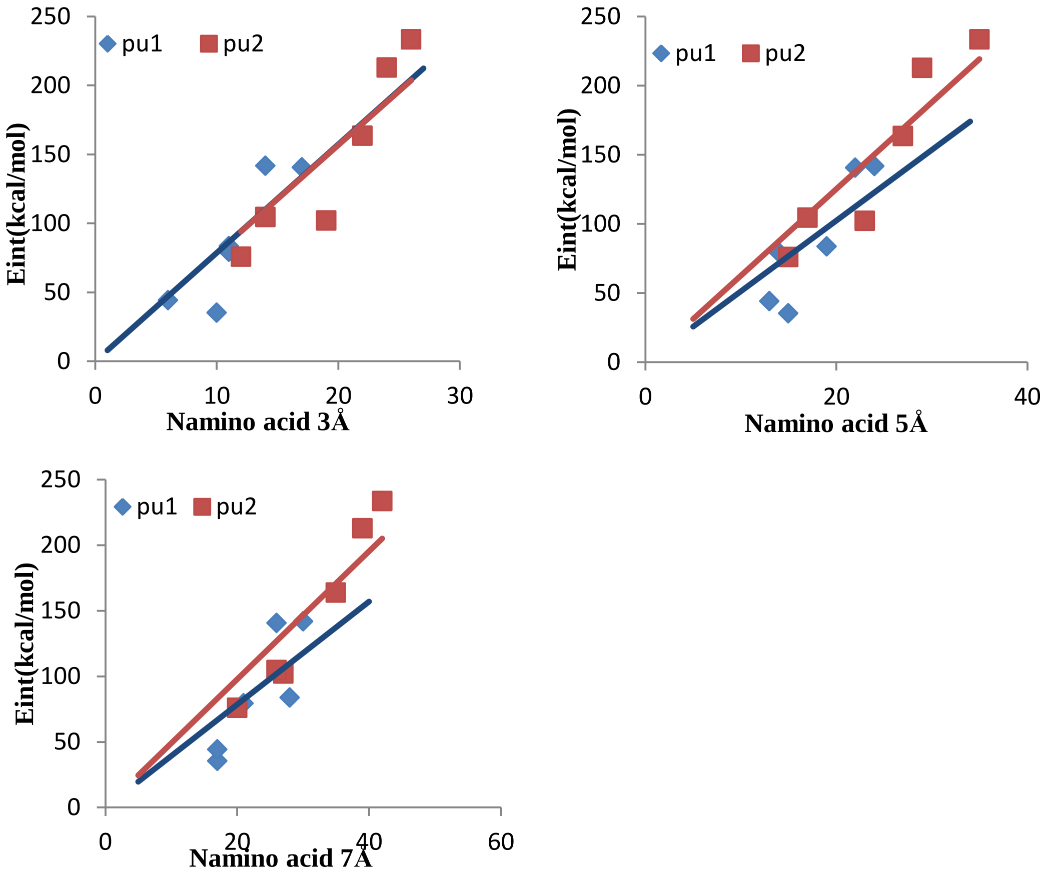
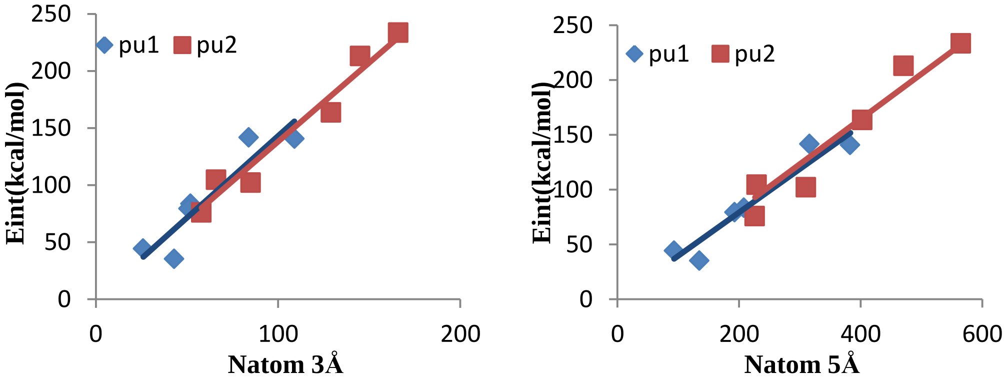


Figure 6.3: Interaction energy with respect to protein amino acids within an adsorption layer of 3Å, 5Å and 7Å for PU1 and PU2.



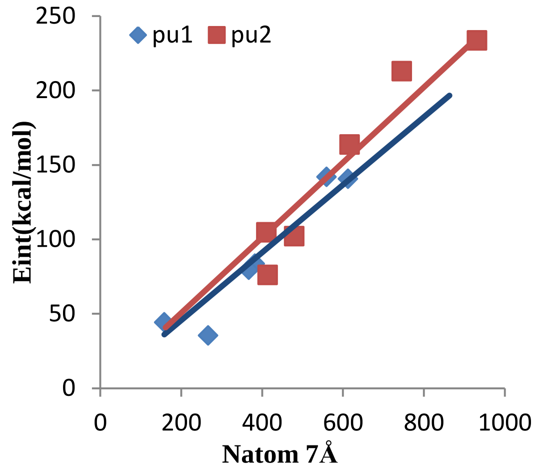


Figure 6.4: Interaction energy with respect to protein amino acid atoms within an adsorption layer of 3A, 5A and 7A for PU1 and PU2.

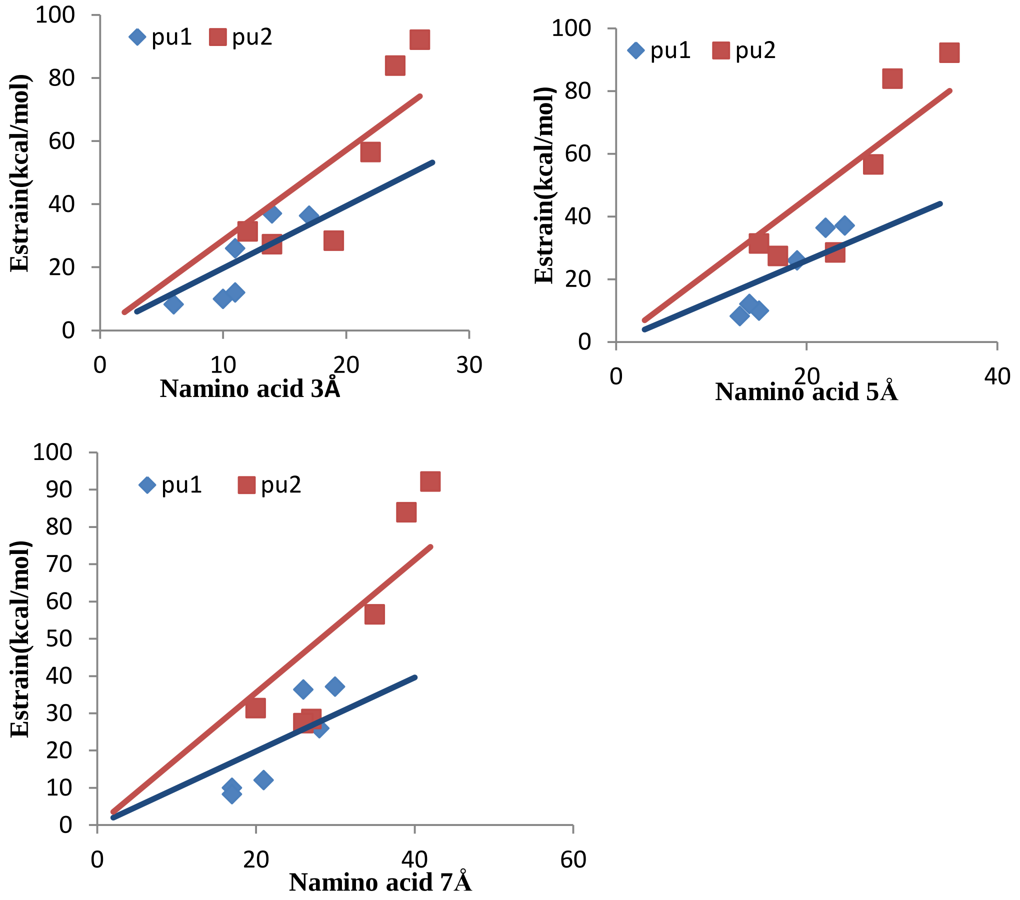


Figure 6.5: Strain energie with respect to protein amino acids within an adsorption layer of 3Å, 5Å and 7Å for PU1 and PU2 .

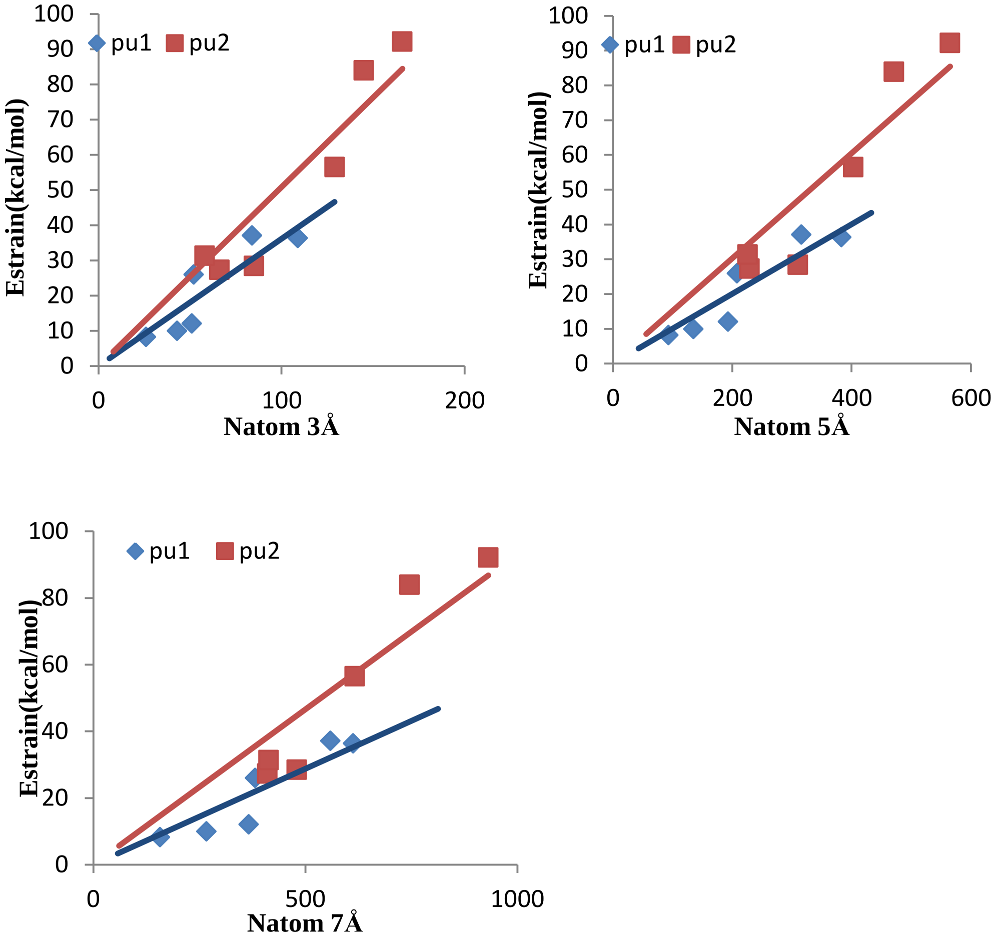


Figure 6.6: Strain energie with respect to protein amino acids atoms within an adsorption layer of 3Å, 5Å and 7Å for PU1 and PU2 .

Table 6.2: graphics slope values of strain energy in relation to the number of amino acids and atomic.

Polymer	Eint(kcal/mol) Amino acid			Eint(kcal/mol) Atom		
	3Å	5Å	7Å	3Å	5Å	7Å
PU1	12.19	8.87	7.5	1.68	0.44	0.24
PU2	11.25	7.88	7.16	1.46	0.46	0.3

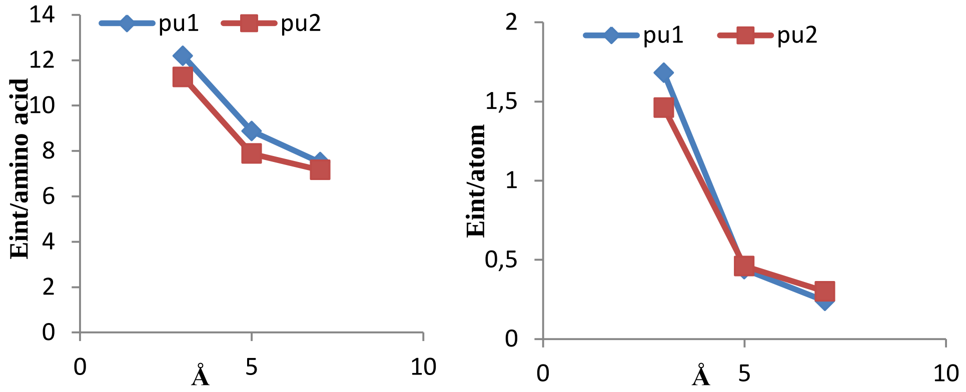


Figure 6.7: Interaction energy in relation to the number of amino acid and the atomic graphics.

Table 6.3: Graphics slope values of strain energy in relation to the number of amino acids and atomic.

polymer	Estr(kcal/mol) Amino acid			Estr(kcal/mol) Atom		
	3Å	5Å	7Å	3Å	5Å	7Å
PU1	3.61	2.62	2.2	0.5	0.13	0.07
PU2	4.34	3.04	2.76	0.56	0.18	0.12

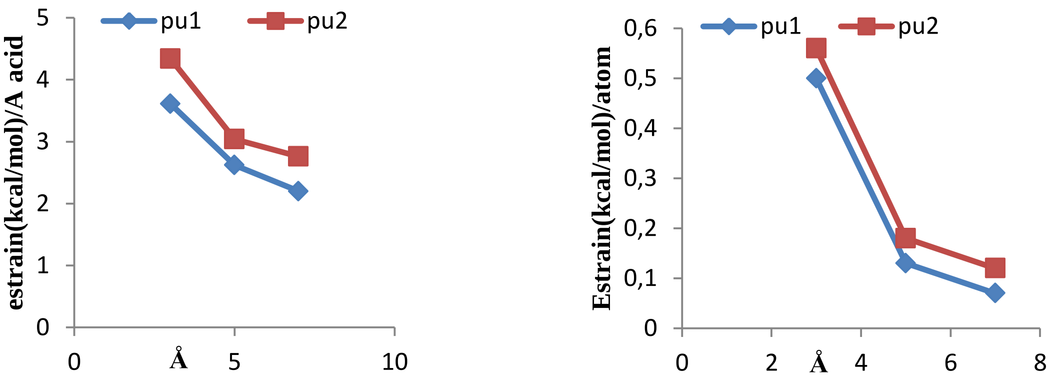


Figure 6.8: Strain energy in relation to the number of amino acid and the atomic graphics.

6.2 Adsorption in the Presence of Explicit Water Molecules

In the final step, the minimum energy yielding orientations 6 and 1 for PEG-HDI and CO-HDI, respectively, was analyzed through MD simulations by replacing the implicit solvent by explicit water molecules as described in the Simulation Method section. The 15 ns simulations were sufficient for the stabilization of the protein on the surfaces that was monitored via the evolution of the protein−surface interaction energies as well as the trajectory of the protein on the surface. The resulting configurations on the PEG-HDI and CO-HDI surfaces are given in Figure 6.9. These additional simulations took into account the interactions among the protein, water molecules, and surface, thus providing a more realistic comparison of the protein affinity for the surfaces in the presence of competitive adsorption/desorption caused by water molecules.

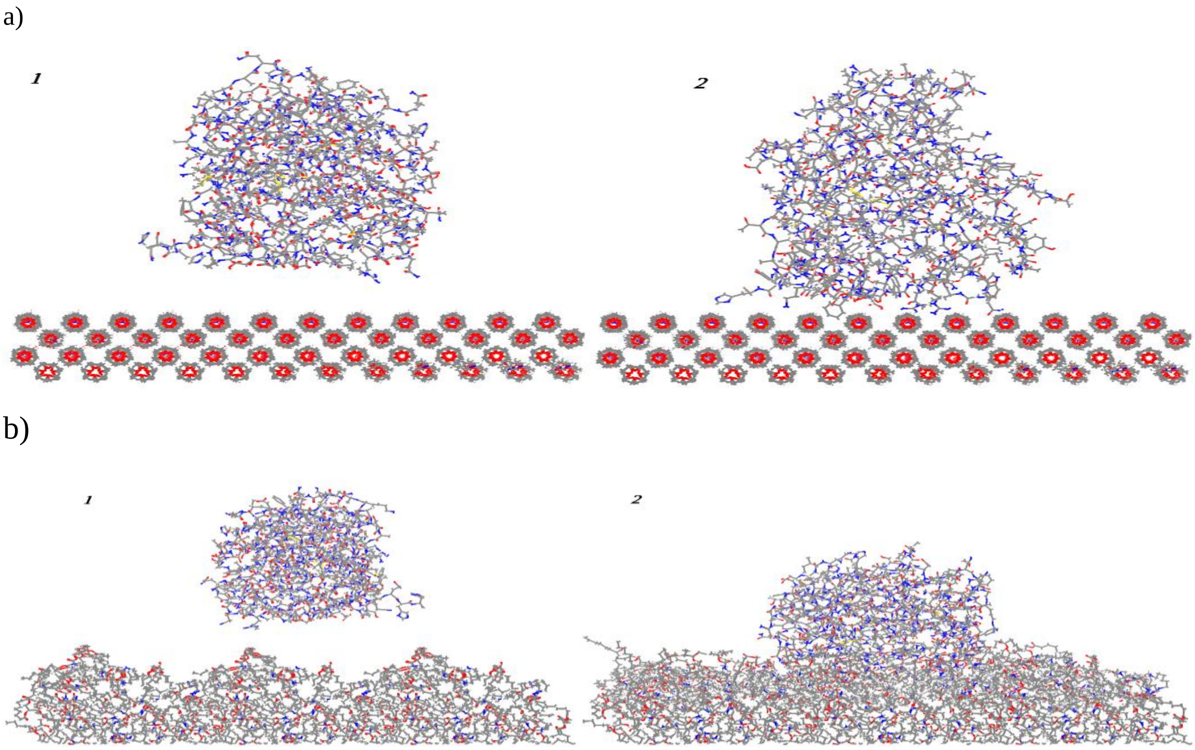


Figure 6.9: Configurational changes of fibrinogen upon adsorption on the surfaces of (a) CO-HDI, (b) PEG-HDI, in the Presence of Explicit system, (waters are invisible). 1.Before adsorption, 2.After adsorption

For a better view of the process of protein adsorption on the surface of the PU1 and PU2 in the explicit water system, distance change of center of mass of the protein (COM) was measured for each surface adsorption. Protein that quickly achive to steady state on PU surface show better adsorption, as seen in graphs PU1-6 has better approchment to surface. Graph exchange for PU1 and PU2 in 4 different orientation is as following:

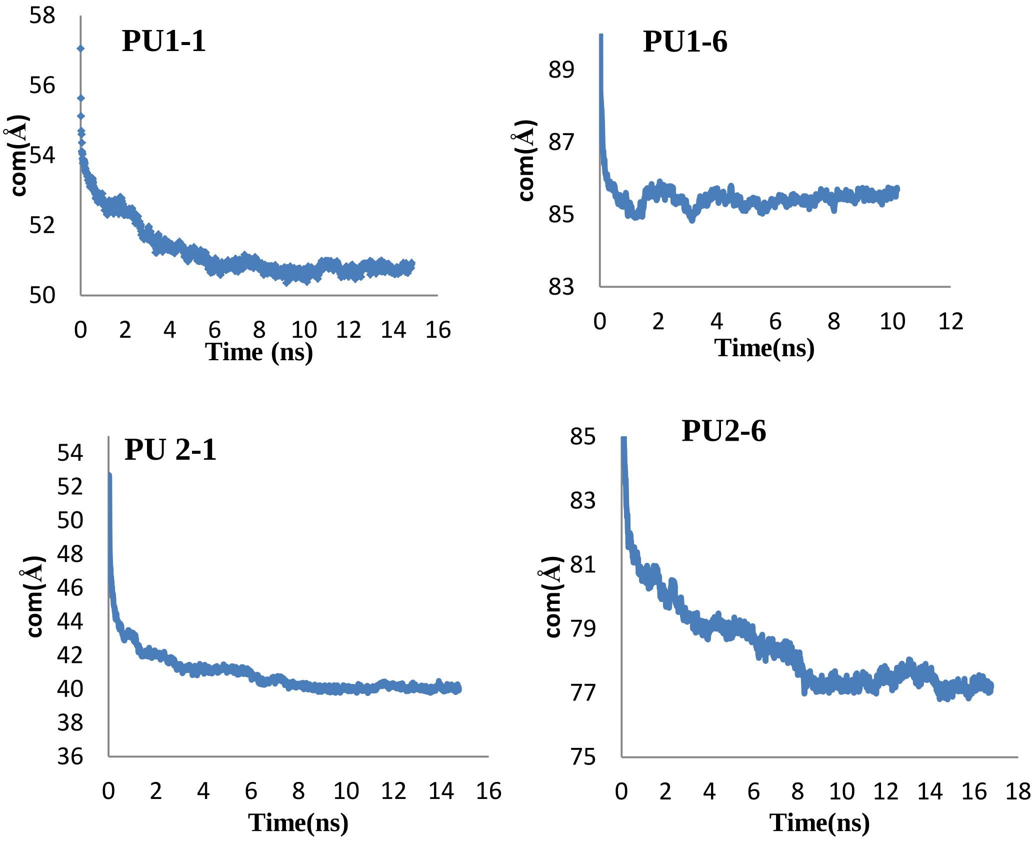


Figure 6.10: center of mass of protein movement, a) PU1-6, b) PU2-1, c) PU1-1, d)PU2-6

The trajectories were also analyzed for structural changes in the protein by looking at the root-mean-square deviation (RMSD) of the protein structure compared to the energy minimized polymer structure and the change in solvent accessible surface area (SAS) of the protein.

RMSD plots were obtained using the VMD program. This program computes the RMSD of a structure by comparing its atom coordinates with a specified reference structure. Here, the reference structure was the starting energy-minimized structure of *γ*Fg prior to beginning the MD simulations. RMSD values were calculated over the course of the simulation to characterize the change in protein structure over time.

The RMSD results are presented in Figure 6.11. The plot shows that an initial rapid change in RMSD occurred, reflecting the mobility of the residues on the protein's surface. The RMSD stabilized at about 2,5-3 Å within about 5 ns of simulation time. The overall structure of the protein did not change during the simulation. All systems with *γ*Fg over a surface exhibited similar behavior to that of the control, thus reflecting similar effects. Relatively minimal further changes in structure occurred due to specific interactions between functional groups on the protein's surface and the surface, with the RMSD increasing to about 3 Å on PU2 surfaces.

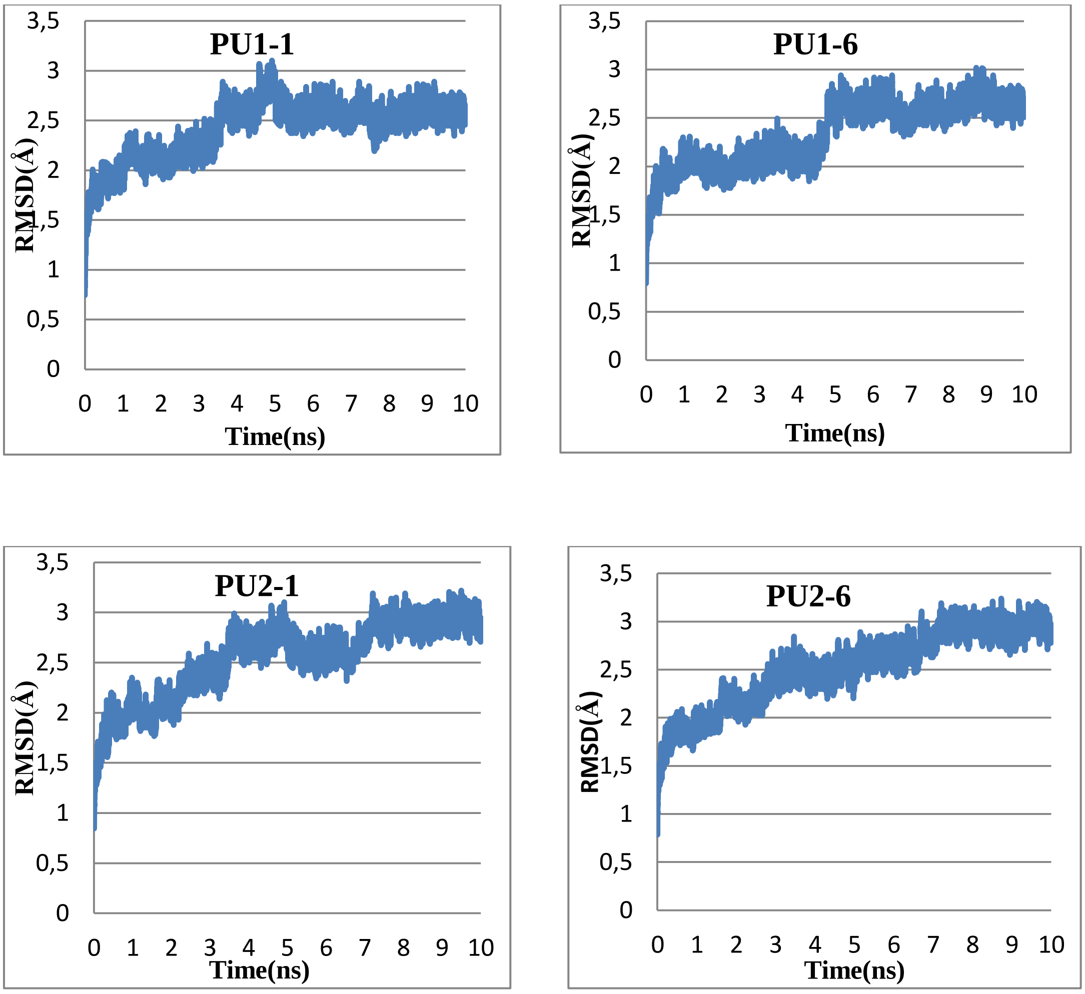


Figure 6.11: RMSD values of γFg in PU1 and PU2 surfaces.

Number of amino acids within 3Å, 5Å and 7Å for PU1 and PU2 in four different orientation in explicit system after the MD runs were calculated and then they were compared to the amino acid numbers calculated from the implicit solvent simulation of the same orientations and are amino acid numbers and are shown in Table (6.4) and Table (6.5) as following:

Table 6.4 : Number of amino acids within 3Å, 5Å and 7Å at 4 orientation after MD

polymer	orientation	Namino acid 3Å	Namino acid 5Å	Namino acid 7Å
PU1-1	1	17	25	38
PU1-6	6	15	17	22
PU2-1	1	25	31	39
PU2-6	6	23	26	31

Table 6.5 : Number of amino acids within an adsorption layer of 3Å, 5Å and 7Å in
. implicit and explicit water.

Polymer &orientation	N-3Å implicit	N-3Å explicit	N-5Å implicit	N-5Å explicit	N-7Å implicite	N-7Å explicit
PU1-1	14	17	24	25	30	32
PU1-6	17	15	22	17	26	22
PU2-1	26	25	35	31	42	39
PU2-6	12	23	15	26	20	31

The resultes show that the protein favors the hydrophobic surfaces more than the hydrophilic surface in the presence of explicit water. This difference may be explained by the competitive adsorption between the protein fragment and the water molecules on the hydrophilic surface. While 26 residues were adsorbed on the PEG-based hydrophilic surface (PU1) in the absence of explicit water molecules, the number decreased to 22 in their presence, whereas the number for adsorbed residues decreased from 42 to 39 and increase from 20 to 31 in the case of the CO-based hydrophobic surface (PU2). It is clear that competitive adsorption between the protein and water molecules disfavors protein adsorption on the hydrophilic PEG-based polyurethane due to competitive binding of water molecules, in agreement with expectations.

For each of the orientation, Φ and ψ torsion angles for average and absolute average of all the amino acids were calculated and their graphs were prepared via interaction energy and strain energy for 4-four different orientations as following:

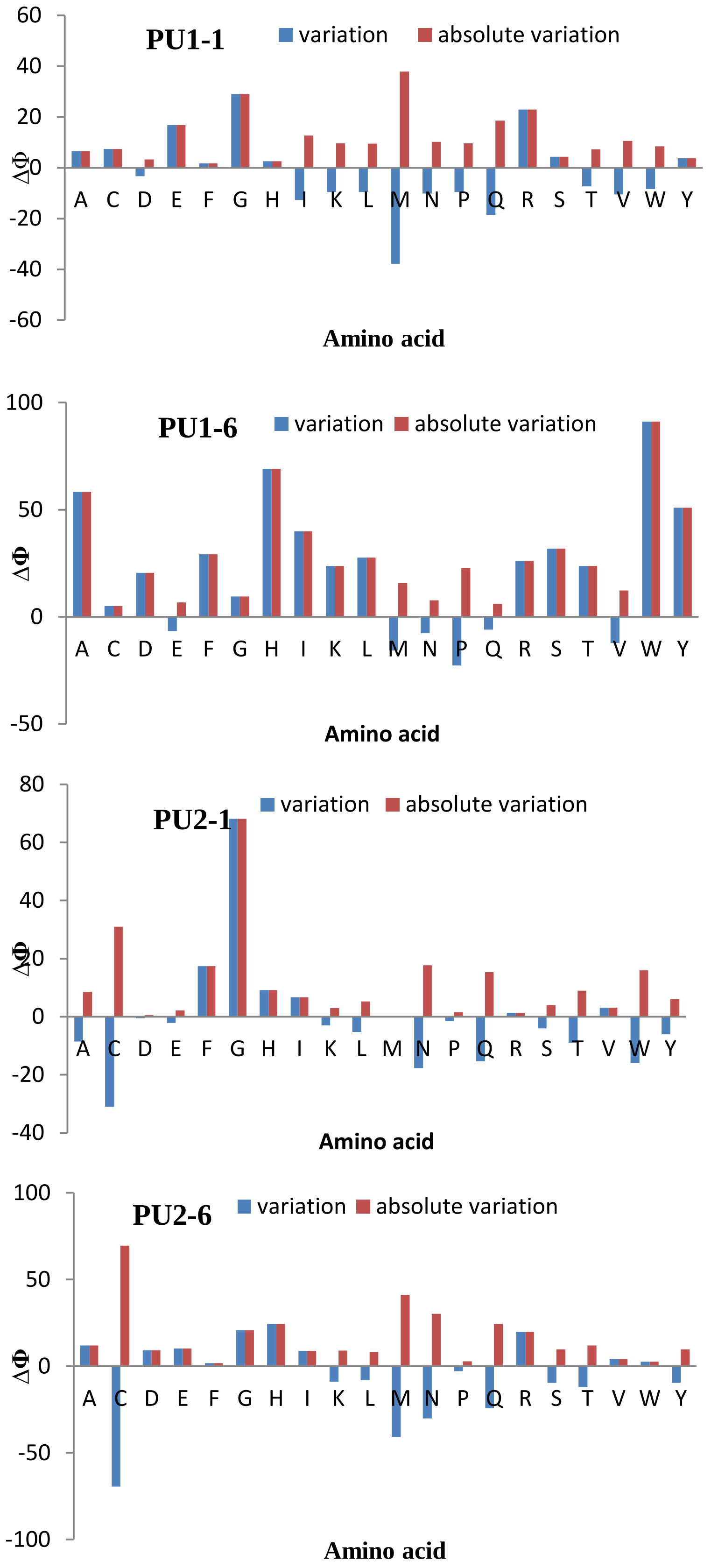


Figure 6.12: Protein amino acids psi (ψ) bond angles average and absolute changes after adsorption.

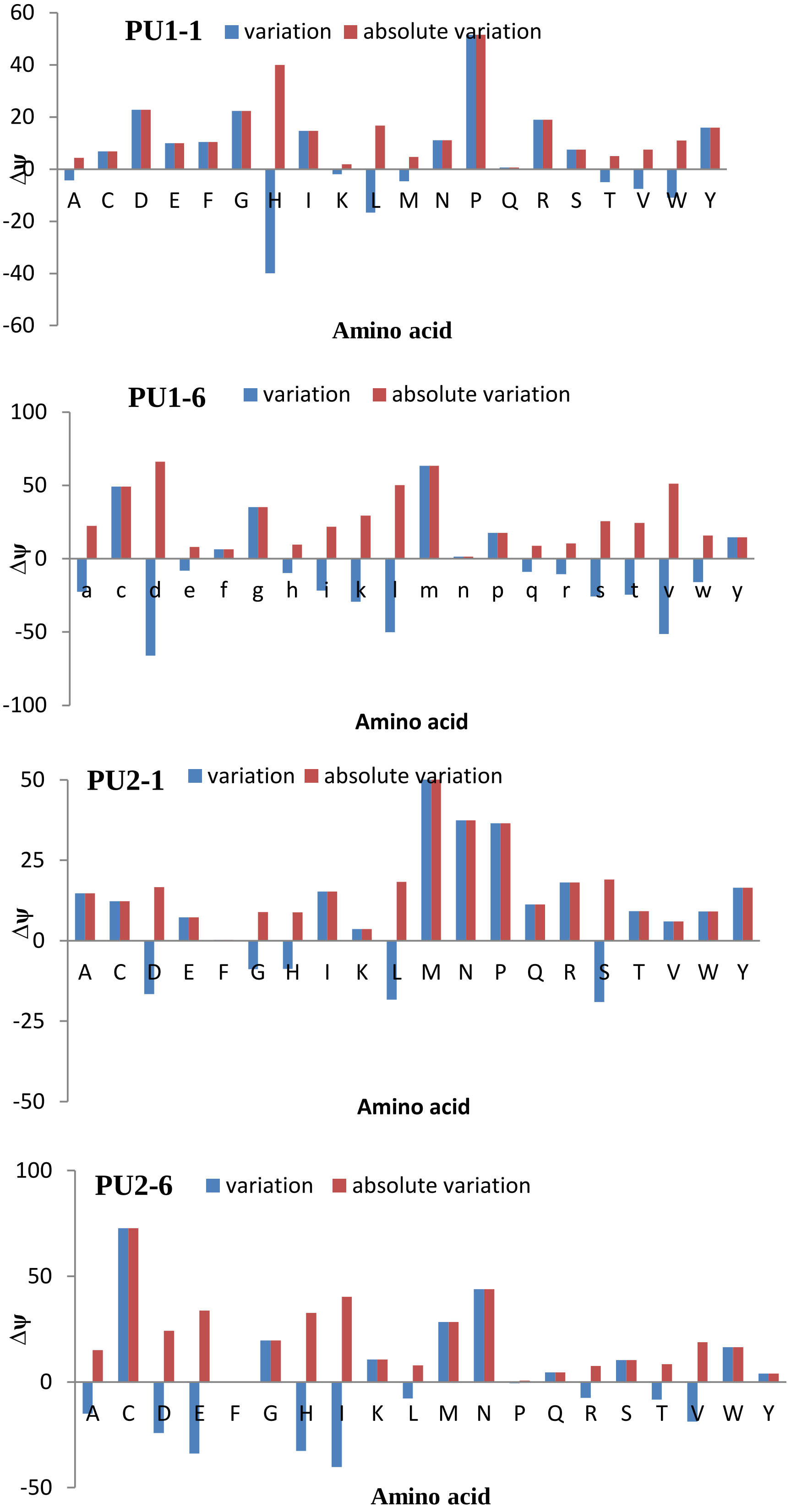


Figure 6.13: Protein amino acids phi (Φ) bond angles average and absolute changes after adsorption.

For each amino acid, average changes in the ψ and Φ angles were analyzed. The most significant changes in the ψ average changes was seen in Asp, Met, Cys and Pro and in the Φ average changes observed in the amino acids was seen in the His, Trp, Cys and Gly.

For better observation of changes in the secondary structure of protein, by using all amino acid Φ and ψ angles, protein Ramachandran plots prepared and are shown in figure 6.13. Glycine is an amino acid that is very flexible and is in a lot of protein structure in order to observe the changes in the conformation of amino acids except glycine Ramachandran plots prepared without glycine like in Figure 6.14.

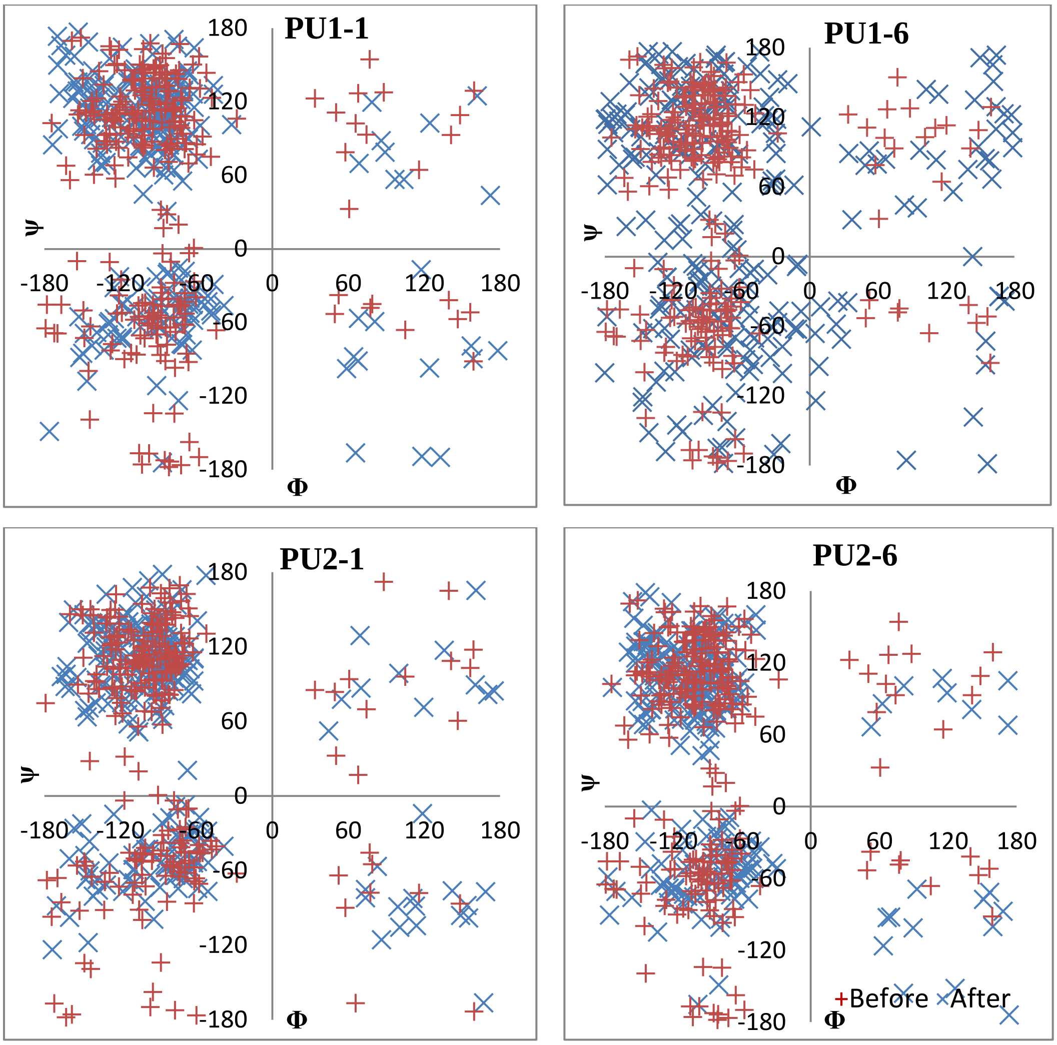


Figure 6.14: Phi (Φ) and psi (ψ) Ramachandran plots bond angles before and after adsorption.

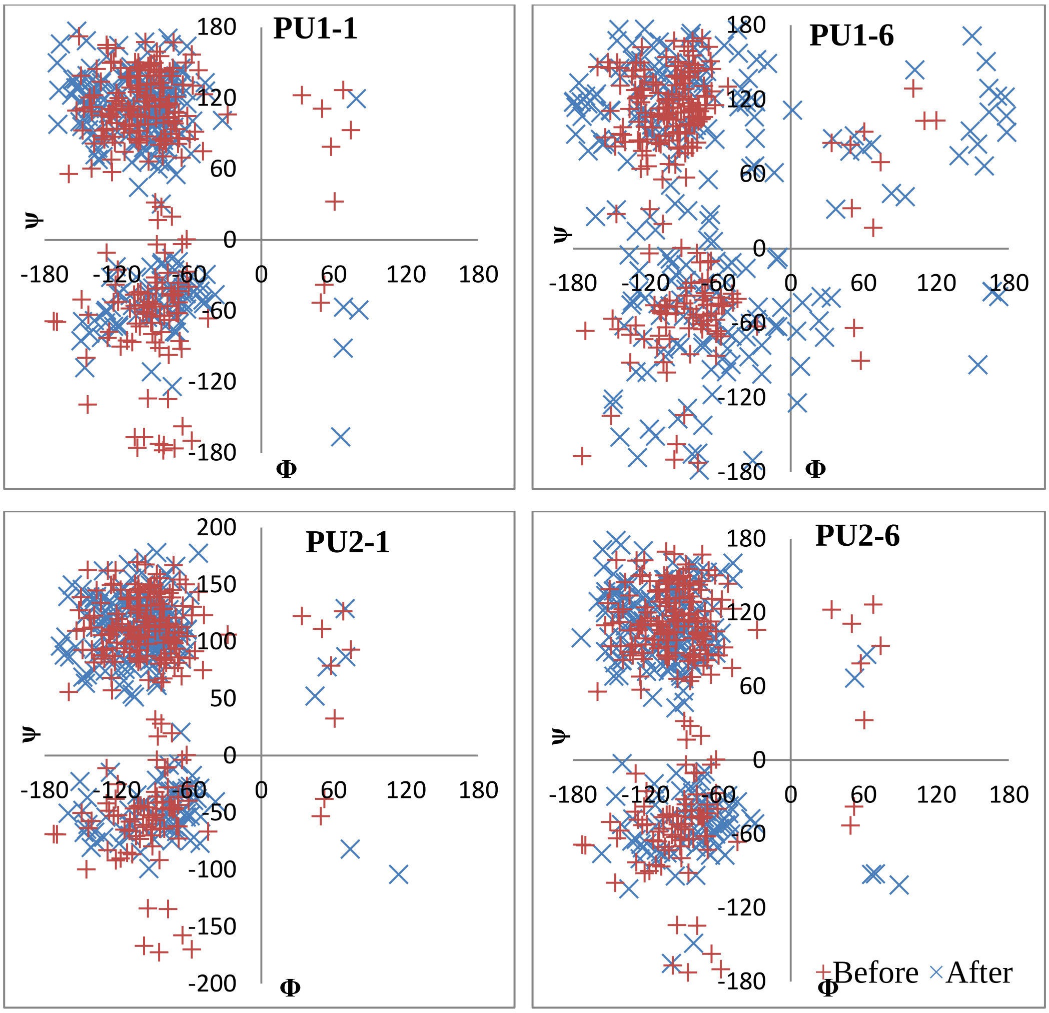


Figure 6.15: Phi (Φ) and psi (ψ) Ramachandran plots bond angles without Glysine before and after adsorption.

Ramachandran plots show some changes in the secondary structure of protein after adsorption.

There are some changes in the secondary structure of protein after adsorption. In PU1-1, large changes have occurs in amino acids like Proline, Tyrosine, Threonine, Alanine and Serine, but important changes has seen in Aspartic acid that structure of amino acid change from α-helix to β-sheets.

In PU1-6, Prolines, Serine, Alanine, Tyrosine and Phenylalanine have changes in their structures and important changes occure in alanine structure that change from β-sheets to α-helix and also in in Serine and Tyrosine structures that have changes from β-sheets region to β-allowed zone.

Important changes for PU2-1 happen for Tyrosine amino acid, that it is clear on Ramachandran plot.

There is also important changes seen in PU2-6, as largest changes, Alanine and Tyrosine had changes in β-sheet to α-helix and α-helix to β-sheet in their structures. From the above results we see that Alanine and Tyrosine show largest changes between other amino acids in the secondary structure of fibrinogen.

7. CONCLUSIONS

Adsorption of fibrinogen onto biomaterial surfaces is of interest, owing to the protein's role in mediating cellular responses, and as a coagulation protein. Polyurethane elastomers combine excellent mechanical properties with good blood compatibility, which has favored their use and development as biomaterials, particularly as components of implanted devices. To understand the adsorption behavior of fibrinogen protein onto hydrophilic and hydrophobic polyurethane surfaces, crystalline polyethylene glycol + hexamethylene diisocyanate (PU1) and amorphous castor oil + hexamethylene diisocyanate (PU2) copolymers were used for molecular simulation in this study. Fibrinogen C-terminus γ-chain (1FID) module was placed near polymer surfaces in six different orientations. Simulations were carried out in an effective dielectric medium, and polymer (PU1) surface was kept rigid but polymer (PU2) was kept flexible. For each orientation, energy minimizations were carried out to analyze changes in the interaction energy between the surface and polymer due to adsorption.

After then the number of amino acids within a distance of 3, 5 and 7Å from the surface was determined. 7Å distance from the polymer surface accepted best predictors for protein adsorption. Next, a linear model was fitted to the interaction energy as a function of the number of amino acids and number of amino acid atoms. The slope in the model yielded the absolute interaction energies between the protein and the surface, was used as a basis for the comparison of the surface biocompatibility, larger slope values indicating larger interaction strength. Simulation results showed that fibrinogen was adsorbed more strongly on PU1 surface compared to PU2 surface, although the latter is less hydrophilic.

In the final step, the most preferred orientations were used for a more realistic treatment of the adsorption process via MD simulations using explicit water molecules. the minimum energy yielding orientations 6 and 1 for PEG-HDI and CO-HDI, were analyzed through MD simulations by replacing the implicit solvent by explicit water molecules as described in the Simulation Method section. The 15 ns simulations were sufficient for the stabilization of the protein on the surfaces that was monitored via the evolution of the protein−surface interaction energies as well as the trajectory of the protein on the surface. These additional simulations took into account the interactions among the protein, water molecules, and surface, thus providing a more realistic comparison of the protein affinity for the surfaces in the presence of competitive adsorption/desorption caused by water molecules.

Results from linear models, COM and RMSD plots and Ramachandran diagrams cleared that fibrinogen was adsorbed more strongly on PU2 surface in explicit water compared to PU1 surface and PU1 shows weaker adsorption in explicit water system compared to implicit water system. These can be explained by the regular surface structure of crystalline PU1, allowing a better contact with the protein without water molecules.

These results suggest that CO-based hydrophobic surface (PU2) is very better than PEG-based hydrophilic surface (PU1) for fibrinogen adsorption. It should be noted that these results are better similler to the previous work [3] that used fibronectin protein instead of fibrinogen.

However, the results of these simulations do suggest that following initial adsorption, *γ*Fg undergoes substantial surface-induced rotational and translational motions until relatively stable low-energy adsorbed orientations are achieved. It must be emphasized, however, that the used protein segment only represents a portion of Fg and its adsorption behavior certainly can be expected to be substantially influenced by the other portions of the Fg structure, such as its α and *β* chains. As molecular simulation methods and computational power continue to improve over time, the entire Fg molecule should be able to be simulated and these effects should be able to be directly considered.

REFERENCES:

[1] **Dee, K. C., Puleo, D. A. and Bizios, R.,** 2002: An Introduction to Tissue-Biomaterial Interactions, John Wiley & Sons, Inc, New Jersey.

[2] **Corneillie, S., Lan, P. N., Schacht, E., Davies, M., Shard, A., Green, R., Denyer, S., Wassall, M., Whitfield, H. and Choong, S.,** 1997: Polyethylene glycol-containing polyurethanes for biomedical applications, Polymer International, 46, 251-259

[3] **Panos, M., Taner, Z. Sen. and Ahunbay, M. G.,** 2012: Molecular Simulation of Fibronectin Adsorption onto Polyurethane Surfaces.

[4] **Agashe, M., Raut, V., Stuart, S. J. and Latour, R. A.,** 2004: Molecular Simulation To Characterize the Adsorption Behavior of a Fibrinogen γ-Chain Fragment, Clemson, South Carolina 29634

[5] **Amogh, T., Anna P. N., Mangesh, G.,** 2010: A brief review: biomaterials and their applications

[6] **Zdrahala RJ, Zdrahala IJ.,** 1999: Biomedical applications of polyurethanes: a review of past promises, present realities, and a vibrant future, R&I Consulting International, Eden Prairie, MN 55346, USA.

[7] **Hong, C, Lin Y., Wei S., Zozg k., W, Dan L.,** 2008: Biocompatible polymer materials: Role of protein- surface intraction.

[8] **Ratner, B. D., Hoffman, A. S., Schoen F. J. and Lemons, J. E**., 1996: Biomaterials Science: An Introduction to Materials in Medicine. Academic Press, USA.

[9] **Lambda, N. M. K., Woodhouse, K. A. and Cooper, S. L**., 1998: Polyurethanes in Biomedical Applications, CRC Press, Florida.

[10] **Raffaini, G. and Ganazzoli, F.,** 2012: Molecular modelling of protein adsorption on the Surface of titanium dioxide polymorphs. Politecnico di Milano, via L. Mancinelli 7, 20131 Milano, Italy

[11] **Joon B. Park; Joseph D. Bronzino.,** 2002: Biomaterials: Principles and Applications, USA.

[12] **Richard J. Zdrahala and Ivanka J. Zdrahala.,** 1999: Biomedical Applications of Polyurethanes: A Review of Past Promises, Present Realities, and a Vibrant Future, 14: 67J Biomater Appl.

[13] **Nina M. K. L., Kimberly A. W., Stuart L. C.,** 1997: Polyurethanes in Biomedical Applications, USA.

[14] **Broglia, R. A., Serrano, L., Tiana, G.,** 2007*:* Protein Folding and Drug Design, Italy.

[15] **Thomas J. Webster**., 2004: Proteins: Structure and Interaction Patterns to Solid Surfaces, Purdue University, West Lafayette, Indiana, U.S.A.

[16] **Zhang, L. and Sun, Y.,** 2010: Molecular simulation of adsorption and its implications to protein chromatography: A review, Biochemical Engineering Journal, 48, 408–415.

[17] **Jiang, S. Y., Sheng, Y. J., Tsao, H. K., Zhou, T.,** Monte Carlo simulations of antibody adsorption and orientation on charged surfaces, J. Chem. Phys. 121 (2004) 1050–1057.

[18] **Jiang, S. Y., Sheng, Y. J., Tsao, H. K., Zhou, T.,** Orientation of a Y-shaped biomolecule adsorbed on a charged surface, Phys. Rev. E 66 (2002) 011911–11915.

[19**] Chen, S. F., Jiang, S. Y., Li, L. Y., Zheng, J.,** Molecular simulation study of water inter-actions with oligo (ethylene glycol)-terminated alkanethiol self-assembled monolayers, Langmuir 20 (2004) 8931– 8938.

[20] **Madura, J. D., Talbot, J., Ravichandran S.,** A Brownian dynamics study of the initial stages of hen egg-white lysozyme adsorption at a solid interface, J. Phys. Chem. B 105 (2001) 3610–3613.

[21] **Talbot, J., Ravichandran S.,** Mobility of adsorbed proteins: a Brownian dynamics study, Biophys. J. 78 (2000) 110–120.

[22] **Shen, J. W., Wu, T., Wang, Q., and Pan, H. H.,** 2008: Molecular simulation of protein adsorption and desorption on hydroxyapatite surfaces, Biomaterials, 29, 513–532.

[23] **Harold A. S., Mey K., and Adam Liwo.,** 2006: Protein-Folding Dynamics: Overview of Molecular Simulation Technique, Cornell University, Ithaca, New York 14853-130

[24] **Raffaini, G., and Ganazzoli, F.,** 2004: Molecular Dynamics Simulation of the Adsorption of a Fibronectin Module on a Graphite Surface, Politecnico di Milano, via L. Mancinelli 7, 20131 Milano, Italy.

[25] **Stephen R. Euson**., 2004: Computer simulation of proteins: Adsorption, gelation and self association, Edinburgh EH14 4As, United kingdom.

[26] **Kricheldorf, H. R., Nuyken, O. and Swift, G.,** 2004: Handbook of Polymer Synthesis: Second Edition, Marcel Dekker, USA.

[27] **Cristina F., Julio C. P., Eugene A. S.,** Fibrinogen: Structure, Function, and Surface Interactions From Cardiovascular Research, Department of Radiology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78229-7800

[28] **Tang L., Wu, Y., Timmons RB.,** Fibrinogen adsorption and host tissue responses to plasma functionalized surfaces. J Biomed Mater Res 1998; 42(1): 156-63.

[29] **Aggarwal, P., Hall, J. B., Mcleland, C. B., Dobrovolskaia, M. A. and Mcneil, S. E.,** 2009: Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy, Advanced Drug Delivery Reviews, 61, 428-437.

[30] **Vagelis A, H., and Vlasis G, M.,** 2004: Molecular Dynamics Simulations of Polymers, University of Patras, Patras, Greece.

[31] **Latour, R, A.,** 2008: Molecular simulation of protein-surface interactions: Benefits, problems, solutions, and future directions, Review. Biointerphases, 3, FC2/11

[32] **Brooks B. R., Bruccoleri R. E., Olafson, B. D., States, D. J., Swaminathan, S., Karplus, M.,** 1983: CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 4:187–217

[33] **Berendsen H. J. C., vander S. D., Van D. R.,** 1995: GROMACS: amessage-passing parallel molecular dynamics implementation. Comp. Phys. Commun. 91:43–56

[34] **Ewig, C. S., Thacher, T. S., Hagler, A. T.,** 1999: Derivation of class II force fields. 7. Nonbonded force field parameters for organic compounds. J. Phys. Chem. B 103:6998–7014

[35] **Van, G. W. F, Bakowies, D., Baron. R., Chandrasekhar, I., Christen, M., et al**. 2006: Biomolecular modeling: goals, problems, perspectives. Angew. Chem. Int. Ed. Engl. 43:4064–92

[36] **Mackerell A. D. J.,** 2004: Empirical force fields for biological macromolecules: overview and issues. J. Comput. Chem. 25:1584–604

[37] **Roterman, I. K., Lambert, M. H., Gibson, K.D., Scheraga, H. A.,** 1989: comparison of the CHARMM, AMBER and ECEPP potentials for peptides. II. φ-ψ maps for N-acetyl alanine N'-methyl amide: comparisons, contrasts and simple ex- perimental tests. J. Biomol. Struct. Dyn. 7:421–53

[38] **Yeh, I. C, Hummer, G.,** 2002: Peptide loop closure kinetics from microsecond molecular dynamics simulation in explicit solvent. J. Am. Chem. Soc. 124:6563– 68

[39] **Sorin, E. J., Pande, V. S.,** 2005: Exploring helix-coil transition via all-atom equilibrium ensemble simulations. Biophys. 88:2472–93

[40] **Williams, S., Causgrove, T. P., Gilmanshin, R., Fang, K. S., Callender, R. H., et al.** 1996: Fast events in protein folding: helix melting and formation in a small peptide. Biochemistry 35:691–97

[41] **Fain, B., Levitt, M.,** 2003: Funnel sculpting for in silico assembly of secondary structure elements of proteins. Proc. Natl. Acad. Sci. USA 100:10700–5

[42] **Anna, V. G., Doj, K., Joachim, K., Vladyslav, K., William, J. W.,** 2007: Prediction of fibrinogen adsorption fo biodegradable polymers: Integration of Molecular dynamics of surrogate modeling, The state University of New Jersey, USA.

[43] **Raffaini, G., and Ganazzoli, F.,** 2007: Understanding the Performance of biomaterials through molecular modeling: Crossing the bridge between their intrinsic properties and the surface Adsorption of protein. Politecnico di Milano, Italy.

[44] **Sadus, R. J.,** 2002: Molecular Simulation of Fluids Theory, Algorithms and Object-Orientation, Australia.

[45] <http://lammps.sandia.gov/> date retrieved 12.03.2013.

[46] **Raffaini, G., and Ganazzoli, F.,** 2012: Molecular modelling of protein adsorption on the surface of titanium dioxide polymorphs, Politecnico di Milano, piazza Leonardo da Vinci 32, 20133 Milano, Italy

[47] **Christian, M., Herbert M. U.,** 2013: Enhancing Protein Adsorption Simulations by Using Accelerated Molecular Dynamics, King's College, London, United Kingdom

[48] <http://swift.cmbi.ru.nl/gv/dssp/>, date retrieved 22.06.2013.

[49] <http://www.rcsb.org/pdb/home/home.do>, date retrieved 10.10.2012.

[50] **Andrew, P. K., Scott A.,** 2010: A fresh look at the Ramachandran plot and the occurrence of standard structures in Proteins, Department of Biochemistry & Biophysics, Oregon State University, Corvallis, OR 97331

CURRICULUM VITAE

Name Surname: Kamal PORHONAR BONAB

Place and Date of Birth: 16.09.1985/ IRAN

Address: ŞİŞLİ/ISTANBUL

E-Mail: kbonab@itu.edu.tr

B.Sc.: Sahand Technical University- TABRIZ/IRAN(2003-2007)