Protein adsorption: Data mining and analysis

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Abstract

Protein adsorption on solid surfaces is a ubiquitous process central to a vast array of applications, ranging from medical interests, such as biomaterials, drug delivery and release, and devices for diagnostics and high throughput screening, to the more classical ones, such as air conditioning installations and clothing. Consequently, a very large body of research has focused on the study of protein adsorption at the liquid-solid interface in the last few decades. These protein adsorption data collected from the literature were analyzed using nonlinear and piecewise linear regression. Interestingly, a consistently better fit is obtained if the data is divided based on the detection methods and also in two separate sub-sets representing protein adsorption on hydrophilic and hydrophobic surfaces, respectively. Protein properties such as hydrophobicity, hydrophilicity, surface area etc. were calculated for each protein with the software called protein surface properties calculator. In addition, protein surface area, number of protein layers adsorbed and its thickness were also estimated. Piecewise linear regression with breakpoint applied to the protein adsorption data for the quartz crystal microbalance hydrophobic data set gave a Langmuir isotherm fit and it suggests that the input variables to protein adsorption, i.e., protein concentration in solution; protein descriptors derived from primary structure (protein area, protein hydrophobicity and hydrophilicity, isoelectric point); surface descriptors (surface tension); and fluid environment descriptors (pH, ionic strength), correlate well with the output variable – the protein concentration on the surface with the intersection corresponding to the number of protein layer of ~1.2. This can be approximated as a protein monolayer and can be considered as a critical point below which protein adsorbs as a monolayer on the surface and above which protein adsorption will continue to happen on the protein monolayer underneath instead of the surface.

Résumé

L’adsorption de protéines sur des surfaces solides est un processus omniprésent au cœur d’une vaste gamme d’applications: par exemple, dans le domaine médical, les biomatériaux, les systèmes de délivrance de médicaments, les dispositifs de diagnostic et le criblage à haut débit, ou dans les domaines moins spécialisés, par exemple, la climatisation et la conception de vêtements. Par conséquent, au cours des dernières décennies, beaucoup l'études sur l'adsorption de protéines à l'interface liquide-solide ont été effectuées. Ces données d’adsorption de protéines recueillies dans la littérature ont été analysées par régression non linéaire et linéaire par morceaux. Remarquablement, un meilleur ajustement de courbe est systématiquement obtenu si les données sont triées par les méthodes de détection et également par deux sous-catégories différentes représentant respectivement l’adsorption de protéines sur des surfaces hydrophiles et hydrophobes. Les propriétés des protéines, telles que l'hydrophobie, l'hydrophilie, la surface spécifique, etc., ont été calculées pour chaque protéine à l'aide du logiciel appelé calculateur de propriétés de surface des protéines. De plus, la superficie de la protéine, le nombre de couches de protéine adsorbées et son épaisseur ont également été estimés. La régression linéaire par morceaux avec point de rupture appliqué aux données d'adsorption de protéines pour l'ensemble de données hydrophobes de microbalance à cristal de quartz donné un ajustement isotherme de Langmuir. Cela suggère que les variables d'entrée pour l'adsorption de protéines, c'est-à-dire la concentration de protéines en solution, les descripteurs de protéines dérivés de la structure primaire (surface protéique, hydrophobicité et hydrophilie des protéines, point isoélectrique), les descripteurs de surface (tension superficielle), et les descripteurs de l’environnement des fluides (pH, force ionique) sont bien corrélés avec la variable de sortie - la concentration de protéines à la surface avec l’intersection correspondant au nombre de couches de protéines de ~ 1,2. Ceci peut être considéré comme une monocouche de protéine et comme un point critique en dessous duquel la protéine

s'adsorbe sous forme de monocouche à la surface et au-dessus duquel l'adsorption de protéine continuera à se produire sur la monocouche de protéine au lieu de la surface.

Chapter 1 – Project description

1.1 Motivation

Proteins are large and complex biomolecules formed during the translation process and plays many crucial roles in the body. These include regulatory, structural and functional roles. For instance, proteins such as, cargo proteins (myosin, dynein, kinesin, etc.) are involved in intercellular transport, transcription factors (TFIIA, TFIIB, etc) are proteins involved in the transcription process to form mRNA, enzymes (lipase, amylase, trypsin, polymerase, etc.) are the class of proteins that are involved in biochemical reactions within the body, antibodies (IgG, IgM, etc.) are the class of proteins that are involved in maintaining immunity and are the defense mechanism of the body.

Proteins have a tendency to attach/adhere to almost any surface with either specific or non-specific interaction and this process is commonly known as “protein adsorption”. Protein adsorption can be a practical asset as well as a problem. For instance, the first step that is initiated after a biomaterial is implanted in the body is the adsorption of proteins on to the implanted surface. The proteins involved are mostly serum proteins present in the blood and these proteins are the point of contact where the host cells interact with the biomedical implant. This is the classical foreign body reaction and it determines the success of the biomedical implant. On the contrary, for tissue engineering applications, protein adsorption is important for the attachment and spreading of cells and in the synthesis of organs.

Protein adsorption, hence is critical to a large number of biomedical and industrial applications, including, but not limited to, biomedical implants, biomaterials, microarrays, lab-on-a-chip, surgical instruments, catheters, air conditioning, etc.

Many parameters influence protein adsorption at solid-liquid interface such as, bulk protein concentration in solution, diffusion, affinity of proteins to the surface. Protein's inherent properties such as its charge, hydrophobicity and hydrophilicity also contributes to the adsorption phenomena and these properties are dependent upon the amino acid residues of proteins. Charge of the protein is also influenced by the solution pH. Surface properties plays a critical role in the adsorption process as well and it involves parameters such as, surface charge, surface energy, and morphology.

This thesis entails data collection for protein adsorption from the literature published in the last 10 years. These literature were reviewed based on the protein adsorption parameters such as, concentration of proteins in solution and on surface, pH, ionic strength, surface contact angle etc. Literature reporting protein adsorption on nanoparticles were not studied. Adsorption data was also included from our lab's protein adsorption database reported previously (The database reports adsorption data from the literature since 1980s).[1] The thesis also involves analysis of the protein adsorption parameters using nonlinear least square regression and piecewise linear regression on the current data and the past data. These analysis are performed to check how well the reported data fits the Langmuir adsorption isotherm curve.

1.2 Project Goals and Specific Aims

The goal of this project is to update the protein adsorption database and to study factors affecting protein adsorption.

The specific aims of this research work are the following

1. Update protein adsorption database from the literature.

2. Calculate protein parameters such as, charge, hydrophobicity and hydrophilicity using protein surface properties (PSPC) calculator for all the reported proteins.

3. Perform non-linear regression analysis on protein parameters and other external parameters (pH, temperature, contact angle etc.) using Langmuir isotherm equation to check if there is a good correlation among different parameters.

1.3 Contribution of Authors

Data mining was performed by Prasad Shetty and Maru Arias. Calculation of protein parameters on PSPC was done by Giulia Ippoliti and Prasad Shetty. Regression analysis was performed by Prasad Shetty.

1.4 Thesis Composition

This is a manuscript-based thesis and Chapter 1 outlines the motivation for the current project; Chapter 2 provides an introduction to the current research project; Chapter 3 contains the draft manuscript with experimental details, results, and discussion; Chapter 4 provides a conclusion of the work.

Chapter 2 - Introduction

2.1 Proteins

2.1.1 Protein properties

Proteins are the most abundant macromolecules and play several crucial roles in the body. These roles are quite diverse and include structural, functional and regulatory roles.[2] Proteins formed during the translation stage are made up of amino acids which are encoded in the gene. There are 20 different amino acids that can be combined to make different proteins and these dictate protein's unique function and its structure. Based on the amino acid sequence, a protein may be big or small (Titin, most commonly found in human muscles is the largest protein with ~27,000 amino acids and with a molecular weight of $3*10^6$ Da and TAL protein is the smallest reported protein found in *Drosophila melanogaster* with 11 amino acids).[3-4] Amino acid sequence also dictate if a protein or a part of the protein is either hydrophilic or hydrophobic, which also influences protein's structure and its folding.[5] The hydrophobicity and hydrophilicity of a protein play an important role among other factors in the adsorption of protein to a surface. [6-7]

2.1.2 Protein adsorption

Protein adsorbs to a surface or an analyte by means of different interactions such as, ionic interactions, hydrogen bonding, van der Waal, hydrophobic interactions etc.[8-10] These interactions depends on the protein residues as well as residues/charge on the surface/analyte among other factors affecting the protein adsorption. Protein adsorption can be of practical value and a problem as described by V. Hlady et. al.[11] When a biomaterial is implanted inside a body, within a minute albumin proteins from the blood gets adsorbed on the implant surface, making protein adsorption

the first process that happens after an implant, which is problematic. This is followed by neutrophil and macrophage attachment and subsequent collagenous encapsulation, which is a classic foreign body reaction as shown in figure 1.[12-13] This raises the issue of biocompatibility.

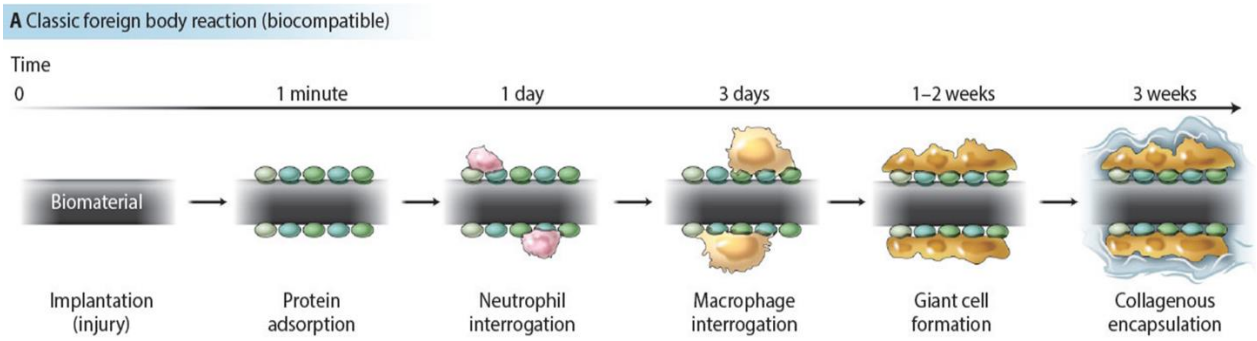

Figure 1: Classic foreign body reaction. Proteins adsorbing to the biomaterial after its implant.[13]

Protein adsorption, hence is critical to a large number of biomedical and industrial applications, including, but not limited to, biomaterials, biomedical implants, microarrays, drug delivery, surgical instruments, etc.

For biomaterials, protein adsorption is important for understanding its performance and is the first process that occurs after a biomaterial implantation in the body. Protein adsorption is much less desirable for biomaterial-based or biomedical implants since it can elicit host immune response.[13] On the other hand, it is important in tissue engineering applications since it influences cell activation, adhesion and wound healing.[14-16]

2.2 Deposition of proteins on the surface

Prior to the work on protein adsorption database, the Wikipedia page on protein adsorption was updated (https://en.wikipedia.org/wiki/Protein_adsorption) with the supervision of Professor Dan V. Nicolau, which makes the subject more accessible to the general audience and to the scientific community. Different sections were added/edited in Wikipedia, specifically, 'Experimental approaches for studying protein adsorption', 'Biomolecular adsorption database', 'Forces and interactions influencing protein adsorption'.

This was followed by my work on data analysis on protein microarrays.[17-18] Clancy et al reports how the protein uniformity and hence the signal is affected on different surfaces, among other factors and on the type of microarray printer: microcontact (μCP), inkjet and pin printing. Protein depositions were studied on a range of substrates such as, 3-Glycidoxypropyl-dimethoxymethyl silane (GPS), Trichloro(octyl) silane (OTS), and 3-(Aminopropyl)-triethoxy silane (APTES) and Trichloro(1H,1H,2H,2H-perfluorooctyl) silane (PFS). Fluorophore tagged BSA (Cy5) and IgG (Alexa Fluor 647) were used in this study and the protein patterns from different microarray printers were analyzed on different substrates based on 7 parameters such as, spot size, eccentricity, coffee-ring ratio, mean fluorescence intensities, smoothness and contrast of the fluorescence intensity profile.[18] Figure 2 describes a radar chart where all these parameters were integrated for the specific surfaces and proteins printed by each of the three methods. The values were normalized to 1 and a higher value indicate better performance. Although using fluorescence technique does not provide a direct estimation of the amount of deposited proteins such as, in QCM and ellipsometry, fluorescence however, gives a visual deposition of proteins on the surface with information about spot size, eccentricity, spot uniformity etc.

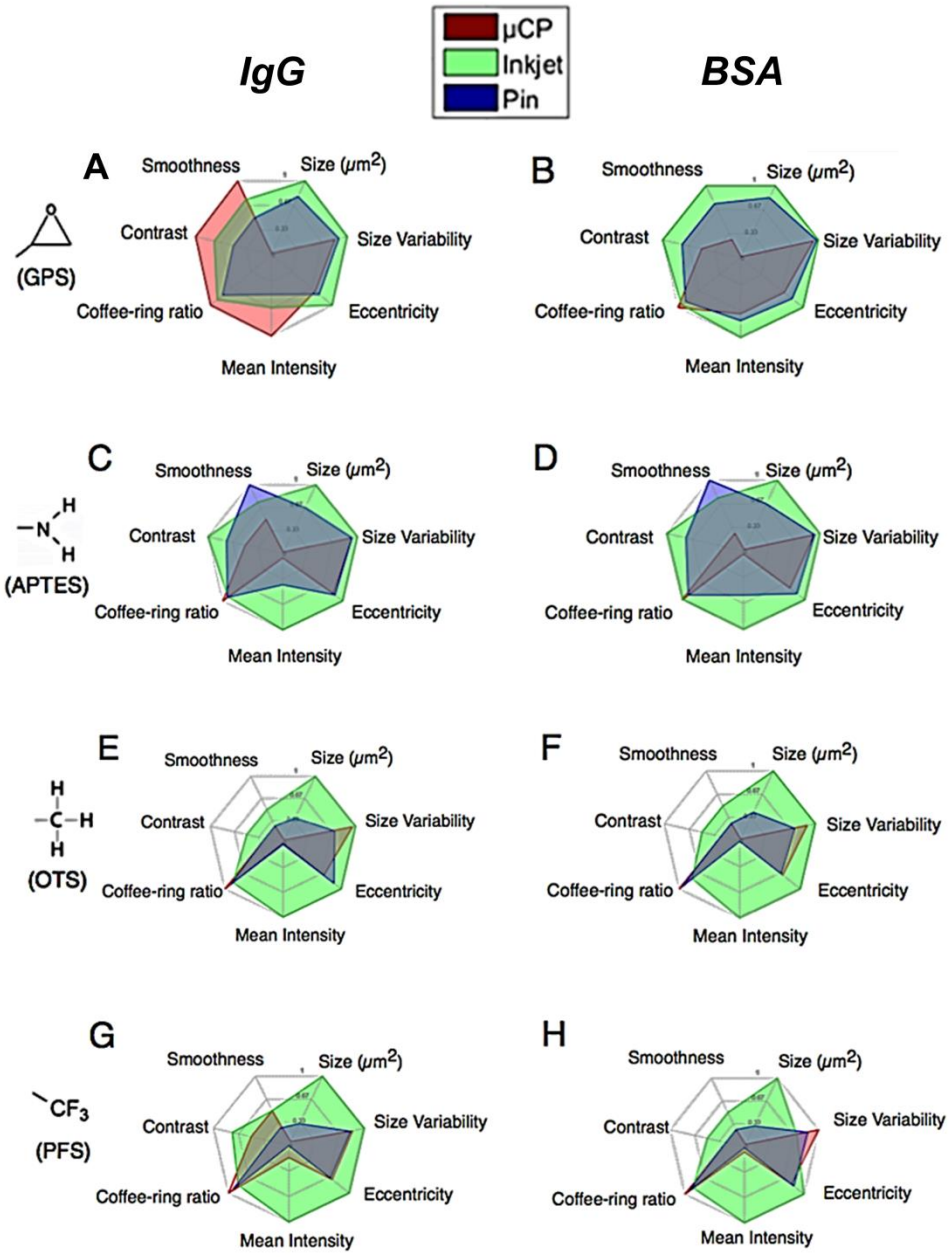

Figure 2: Representation of deposition technique performance on different surfaces based on the parameters investigated. Radar charts showing how the 3 methods (µCP (red), inkjet (green), and pin (blue) printing) compare in the 7 parameters investigated in this work when printing IgG (left) and BSA (right) on GPS- (A,B), APTES- (C,D), OTS- (E,F), and PFS-functionalized (G,H) glass slides. For each parameter, except size, a larger area covered represents a better performance of the method for this parameter.[17]

Dobroiu et al reports on using fluorescence interference contrast (FLIC) enabled structures to improve the performance of microarray by modulating the fluorescence. By changing the width

and height of microarray pillars, amplification of fluorescence and signal to noise ratio was achieved. The data analysis also showed uniformity in fluorescence signal from the microarray spots.[17]

2.3 Factors affecting protein adsorption

2.3.1 Protein properties affecting adsorption

Molecular weight

Protein adsorption is influenced by the rate of diffusion and the rate of diffusion depends on the protein size/weight. Smaller proteins tend to diffuse more and get adsorbed to the surface faster than larger proteins. On the other hand, higher molecular weight protein will have more amino acid sequence and hence will have more binding domains for interacting with the surfaces, thus favoring adsorption.[12, 19]

Isoelectric point

The isoelectric point (pI) is the pH at which a molecule carries no net charge. The pH of the buffer determines the ionic state of the protein. At a pH higher than the protein pI, a protein will carry net negative charge and at a lower pH than the pI, the protein will carry a positive charge. Protein adsorption is higher at the isoelectric point because it minimizes protein – protein repulsion and results in a higher packing density on a surface. [12, 20]

Protein folding and stability

Protein's structure might be critical for its adsorption to a surface because the availability of certain binding domains depends on the protein conformation. Proteins are usually made of primary,

secondary, tertiary and quaternary structure and adsorption on a surface can result in a partial or significant conformational change wherein the amino acid residues interact with the surface resulting in the deviation of the native state of the protein as shown in figure 3.[21] This unfolding of the protein may also result in the decrease or loss of bioactivity.[22] This is especially true for hydrophobic surfaces where the protein can unfold to expose its hydrophobic core and results in denaturation.[23] Proteins that have less thermodynamic stability and less crosslinks, commonly referred as "soft proteins" tend to adsorb more easily than the proteins with higher thermodynamic stability also known as "hard proteins". Unfolding of these soft proteins are easily achieved due to lower thermodynamic stability.[12]

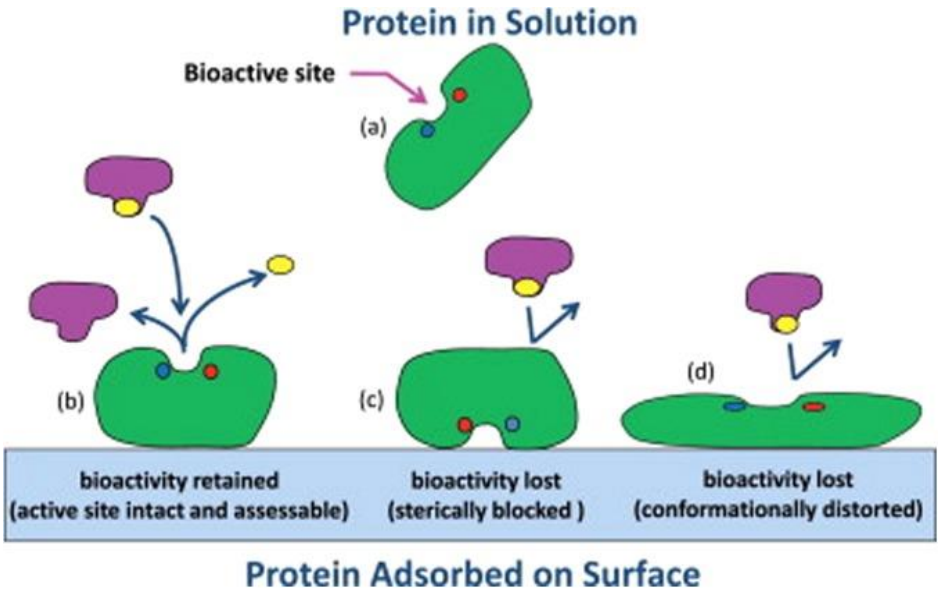

Figure 3: Schematic of the activity of protein upon adsorption.[21]

Protein surface

The amino acid sequence of proteins dictate its hydrophobicity, hydrophilicity and surface charge and these properties affect protein – protein interaction and protein – surface interactions. These properties also play a critical role in protein folding. Hydrophobicity and hydrophilicity has been

reported to be measured based on two methods: 1) using amino acid residues (amino acid level) and 2) using atoms of amino acid (atomic level). Protein charge on the other hand is measured at the atomic level.[24] The distribution of charges and hydrophobic and hydrophilic moieties as shown in figure 4, will provide a better understanding or prediction of the adsorption of protein to a surface.

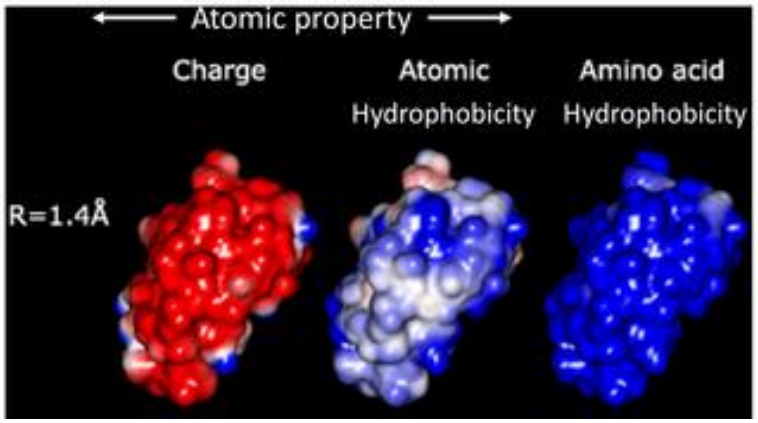

Figure 4: Comparison of the molecular surface of ribonuclease. Atom-based properties, i.e., charges (left column; red=negative, blue=positive), atomic hydrophobicity (middle column; red=hydrophobic and blue=hydrophilic region); and amino acid-based hydrophobicity (right column) are studied using a probe of 1.4Å radius on the surface of ribonuclease (PDB ID: 1AFU).[24]

2.3.2 External parameters affecting protein adsorption

pH

The pH of a buffer determines the electrostatic state of proteins in that buffer. When the pH of the solution equals the isoelectric point (IP) of the protein, then the protein carries no net charge. This favors proper packing of proteins on the surface by minimizing protein – protein repulsion. But at a higher pH when pH > IP, proteins are negatively charged and at a lower pH with pH < IP, proteins are positively charged.[12, 20]

Temperature

Temperature can alter protein structure and folding and hence can affect its adsorption on a surface. Temperature hence influences the equilibrium state and kinetics of protein adsorption. Increase in the temperature increases the rate of diffusion and hence favors protein adsorption.[20] The driving force for protein adsorption is entropy driven and since there is a conformational change in protein during adsorption, this process might be associated with conformational entropy gain. The other mechanisms for entropy change on adsorption might involve release of water molecules from protein and the surface and the release or binding of ions.[20, 25] However, increase in temperature can also result in protein denaturation. This exposes the hydrophobic core of the protein favoring adsorption process. Like adsorption, desorption also happens at a higher temperature, but depends on the type of protein, buffer conditions and also the surface. Desorption of proteins is seen in a buffer solution whereas adsorption is seen in protein solution as shown in figure 5. Hard proteins such as lysozyme and fibronectin stay mobile at the interface whereas soft proteins like BSA denature at the interface increasing the contact area with the surface preventing desorption.[26]

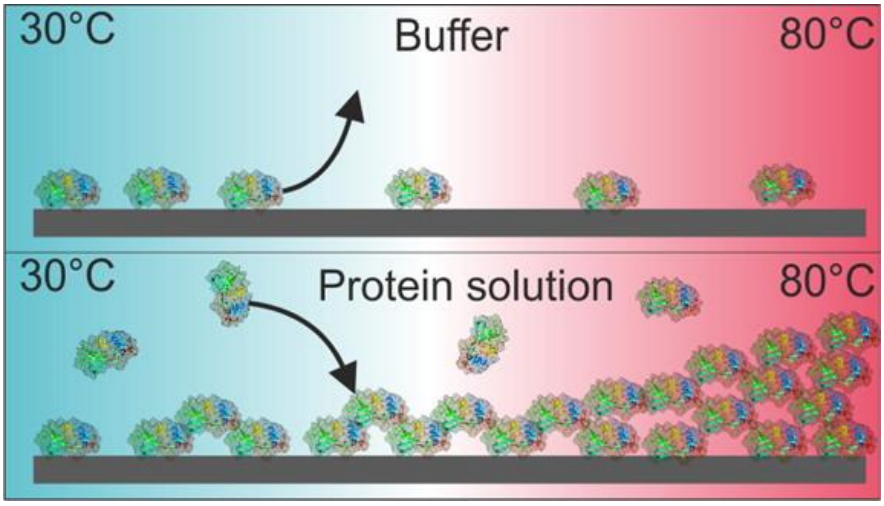

Figure 5: Effect of temperature on protein adsorption. In the buffer solution, the adsorbed proteins tend to desorb as the temperature increases (upper panel). In the protein solution, proteins tend to aggregated and adsorb rather than desorb as the temperature rises (lower panel).[26]

Ionic strength

Ionic strength refers to the concentration of ions dissolved in a solution. Ionic strength determines the Debye length which is a measure of charge carrier's net electrostatic effect in a solution. The higher the ionic strength the shorter are the electrostatic interactions between charged molecules. This means that the adsorption of charged proteins to the oppositely charged surface gets inhibited whereas the adsorption to like charged surface gets amplified.[20] With the increase in ionic strength, lateral diffusivity of proteins decreases. This also increases the surface pH and net protein charge at the surface.[27-28] In addition, a very high concentrations of ions can cause proteins to precipitate commonly known as 'salting out'. [29]

2.3.3 Surface properties affecting adsorption

Surface morphology

Surface size can be categorized into macro/micro sized and nano sized. The size of the surface influences the amount of adsorbed proteins. Nano surfaces have higher surface area and hence more proteins adsorption can be expected. However, protein adsorption on nanoparticles is complex and not well studied because it depends on different factors such as nano particle size, type and shape of nanoparticle, protein type, surrounding medium and other factors.[30] Protein's conformation change upon adsorption is also dependent on the size of the nanoparticles.[31] When nanoparticles are injected into the system, blood serum proteins get adsorbed onto nanoparticles forming a protein layer known as 'protein corona'. Protein corona can be categorized into 'hard corona' where proteins are tightly bound and 'soft corona' with loosely bound proteins as shown in figure 6.[32] This affects the efficiency and bioavailability of nanoparticles, resulting in rapid clearance from the blood and lower target specificity.[30, 33-34]

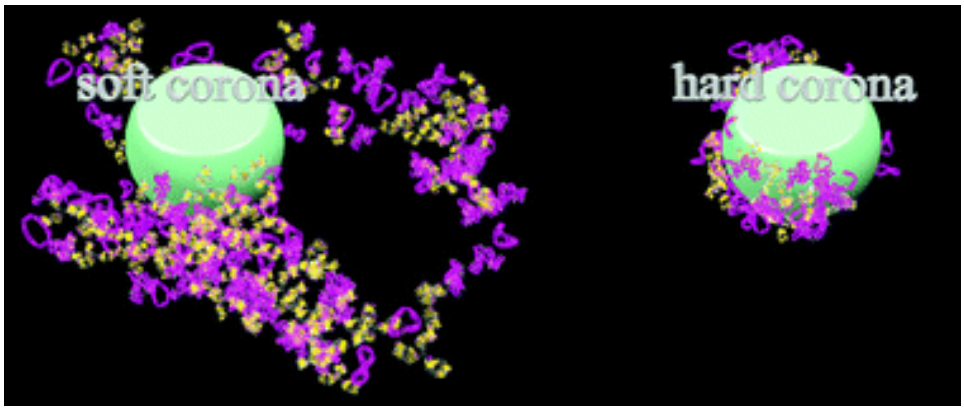

Figure 6: Different types of protein corona: Soft corona, where proteins loosely adsorb on nanoparticles; and hard corona, where proteins tightly bind to nanoparticles.[32]

Surface chemistry

Surface chemistry dictates whether a surface will be hydrophilic or hydrophobic, the charge it carries and the type of interaction with the adsorbing protein. Table 1 summarizes the nature of surface based on different functional groups. Protein adsorption is favorable on hydrophobic surface because of the increase in entropy caused by the replacement of water molecules by the proteins. On the other hand, on hydrophilic surfaces, water molecules form hydrogen bonds with the surface and hence the replacement of water molecules by proteins is minimized, thus less

Functional group	Properties	Effect on proteins and cells
$-CH_3$	Neutral; hydrophobic	Has high affinity/binding with fibrinogen; binds strongly with IgG; promotes increased leukocyte adhesion and phagocyte migration
–OH	Neutral; hydrophilic	Has decreased affinity for plasma proteins; induces exposure of cell adhesive domains on fibronectin; increases differentiation of osteoblasts
$-NH_2$	Positive; hydrophilic	Has high affinity for fibronectin; promotes increased myoblast proliferation; promotes differentiation of osteoblasts; promotes increased endothelial cell proliferation
–COOH	Negative; hydrophilic	Has increased affinity for fibronectin and albumin

Note: These are generalized observations and may vary depending on experimental conditions and the presence of other proteins in solution.

Table 1: Different surface functional groups and their hydrophobic/hydrophilic nature affecting protein adsorption .[12]

protein adsorption and conformational change.[12]Charged surfaces tend to adsorb more protein of oppositely charged, but like charged proteins also get adsorbed because the amino acid residues tend to have positive and negative charges and hence protein will have positive and negative charged domains irrespective of their net charge.

2.4 Calculating protein surface properties

Protein surface properties calculator (PSPC) is a proprietary software developed by Dr. Dan V. Nicolau and his research group for the calculation of protein properties such as hydrophobicity, hydrophilicity and the charge at the amino acid and atomic level. The hydrophicity and hydrophilicity is measured using two hydrophobicity scales; the hydrophobicity of an amino acid is measured based on the enthalpy for its transfer (i) through a lipid membrane (DGwif); and (ii) from water to octanol (DGoct).[24]The molecular surfaces of the selected proteins is probed with a virtual rolling probing ball with a set radius scanning the atoms on the surface of the protein. Figure 7 represents the PSPC software.

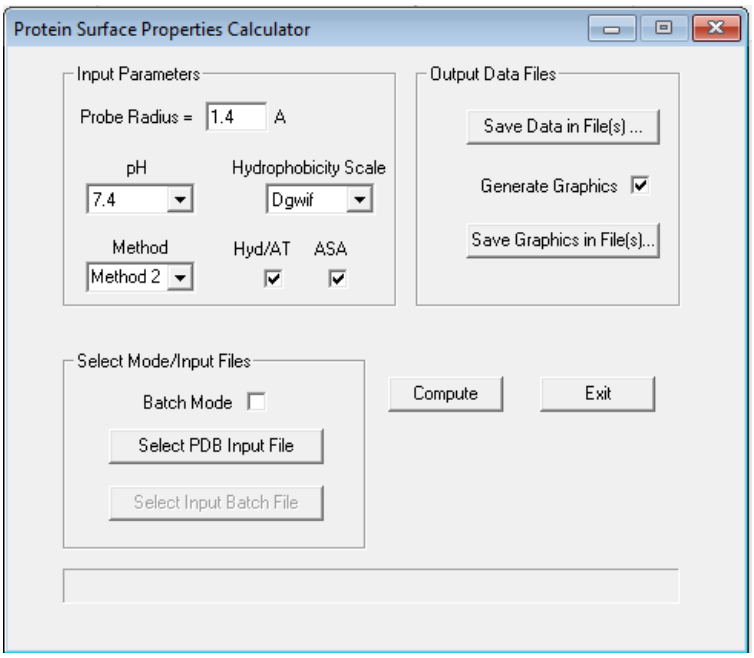

Figure 7: Protein surface properties calculator. PSPC for the calculation of hydrophobicity, hydrophilicity and charge of proteins.[24]

2.5 Methods for measuring protein adsorption

Understanding protein adsorption is critical for several biomedical and industrial application. The choice of a measurement technique depends on the type of study and may involve studying adsorption kinetics, the amount of adsorbed protein, the activity and the structure of the adsorbed proteins.[12, 35-36] Many label free approaches such as, ellipsometry, UV spectrometry, surface plasmon resonance (SPR), optical waveguide lightmode spectroscopy (OWLS), etc. have been used to study adsorption kinetics and some have also been also used to measure the thickness of the adsorbed protein layer.[20] Spectroscopy techniques such as, X-ray photoelectron spectroscopy (XPS) has been employed to study the composition of adsorbed protein layers.[12] Labelled techniques such as, radiolabelling, lowry assay, bicinchoninic acid (BCA) assay have also been employed.[35, 37-39] Radiolabelling technique, which uses radio isotopes for labelling proteins was one of the widely used technique for measuring adsorption and has been used since 1980. Lowry

and BCA assay measures protein adsorption based on absorption spectra. Fluorescence measurements of adsorption can be performed using fluorescein isothiocyanate (FITC) labels and microscopy techniques.[18, 35] Techniques such as total internal reflection fluorescence (TIRF) and Förster resonance energy transfer (FRET) have been used for measuring protein adsorption. TIRF has been used to study protein adsorption kinetics and FRET has been used for studying protein folding/unfolding.[20] On the other hand, to study the structure of adsorbed proteins and its conformational changes upon adsorption, infrared spectroscopy (IR), attenuated total internal reflectance-infrared spectroscopy (ATR-IR) and circular dichroism (CD) spectroscopy has been used.[20] Atomic force microscopy (AFM) has also been used to study protein adsorption by imaging of the adsorbed protein and can provide information, such as the height of the protein. AFM combined with scanning tunneling microscope (STM) has also been used to characterize single adsorbed protein molecule with improved lateral information. Quartz crystal microbalance (QCM) has been used widely for measuring the adsorbed protein mass; however, it does not measure the dry protein mass because it incorporates the mass of water in the protein layer. Recent advances also include optics-based label free new tools for measuring protein adsorption such as, neutron reflectometer, interferometric reflectance imaging sensor (IRIS), formerly known as spectral reflectance imaging biosensor (SRIB) and whispering gallery mode (WGM).[40-42]

2.6 Adsorption isotherm

Besides the different measurement techniques for measuring or quantifying protein adsorption, developing an adsorption isotherm is one of the simplest methods that can be used for studying protein adsorption.[43] Among these isotherms, Langmuir isotherm is the simplest and one of the widely used adsorption isotherm method. Freundlich isotherm and Brunauer-Emmett-Teller (BET)

are other adsorption isotherm methods.[44] Adsorption isotherms are constructed by plotting surface concentration of proteins at different solution concentration of proteins as shown in figure 8 thus it gives us an understanding of how proteins and surfaces interact. Each adsorption models have their own characteristic shapes. Langmuir model assumes that the adsorption forms as a 'monolayer' on the homogenous surface. Freundlich model describes adsorption on heterogenous

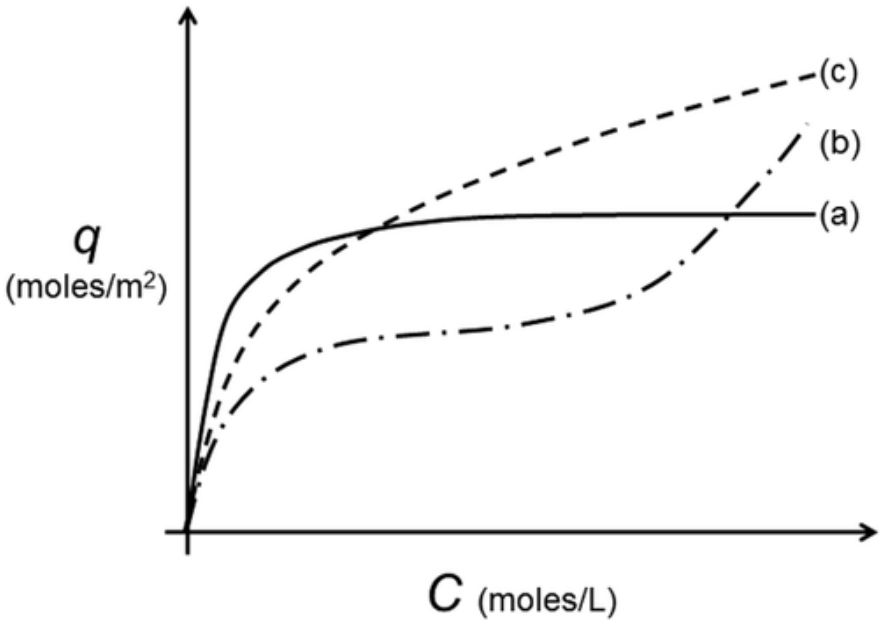

Figure 8: Shape of different adsorption isotherms: Plot of surface concentration (q) vs solution concentration (C) with (a) Langmuir isotherm (—), (b) BET isotherm (— · —), (c) Freundlich isotherm (- - -).[44]

surfaces, whereas BET model describes multi-layer protein adsorption on different sites on a surface, which is usually the case.[44]

Chapter 3 - Data mining and analysis for protein adsorption

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(Manuscript in preparation)

3.1 Abstract

Protein adsorption on solid surfaces is a ubiquitous process central to a vast array of applications, ranging from medical interests, such as biomaterials, drug delivery and release, and devices for diagnostics and high throughput screening, to the more classical ones, such as air conditioning installations and clothing. Consequently, a very large body of research has focused on the study of protein adsorption at the liquid-solid interface in the last seven decades. The protein adsorption data collected from the literature were analyzed using nonlinear and piecewise linear regression. Interestingly, a consistently better fit is obtained if the data is divided based on the detection methods and also in two separate sub-sets representing protein adsorption on hydrophilic and hydrophobic surfaces, respectively. In addition, protein surface area, number of protein layers adsorbed and its thickness has been estimated. Piecewise linear regression with breakpoint applied to the protein adsorption hydrophobic data for the quartz crystal microbalance gave a Langmuir isotherm fit and it suggests that the input variables to protein adsorption, i.e., protein concentration in solution; protein descriptors derived from primary structure (protein area, protein hydrophobicity and hydrophilicity, isoelectric point); surface descriptors (surface tension); and fluid environment descriptors (pH, ionic strength), correlate well with the output variable – the protein concentration on the surface with the intersection corresponding to the number of protein

layer of ~1.2. This can be approximated as a protein monolayer and can be considered as a critical point above which protein adsorption will continue to happen on the protein monolayer below instead of the surface. While the database is of general interest, the prediction of the thickness and the number of protein-covered layers are of particular relevance to the design of microfluidics devices.

3.2. Introduction

Protein adsorption is critical to a large number of biomedical and industrial applications, including, but not limited to, biomaterials, biomedical implants, microarrays, drug delivery, surgical instruments, etc. For biomaterials, protein adsorption is important for understanding its performance and is the first process that occurs after a biomaterial implantation in the body.[12] Protein adsorption is much less desirable for biomaterial-based or biomedical implants since it can elicit host immune response.[45-47] On the other hand, it is important in tissue engineering applications since it influences cell activation, adhesion and wound healing.[48-49] For protein microarrays, one needs to find the optimum balance between higher protein concentration on surfaces, which leads to an increase in overall sensitivity; and protein denaturation, which leads to sensitivity decrease.

One of the parameters that influences protein adsorption at solid-liquid interface is the bulk protein concentration in solution. Adsorption on the surface increases with a higher concentration of single protein.[12] Diffusion also plays a significant role; with higher diffusing proteins (smaller proteins) adsorbing on the surface faster than heavy proteins.[12, 19, 50] Another important factor is the affinity of proteins to the surface, with proteins having high affinity to a surface tend to form stronger bonds and hence stronger adsorption. However, proteins adsorbed on the surface tend to either

desorb or replaced by other proteins, a process known as Vroman effect.[19, 50] Protein's inherent properties such as its charge, hydrophobicity and hydrophilicity also affect the adsorption phenomena. These properties depends upon the amino acid residues of proteins and larger proteins with high molecular weight have more residues for binding to the surface. Proteins with a positive charge on their surface tend to bind to the negatively charged surface and vice versa. Charge of the protein is also influenced by the solution pH. At the isoelectric point of the protein, there is no net charge and that seems to favour the adsorption process because it minimizes electrostatic repulsion. [31, 51-52]

Surface properties play a critical role in the adsorption process and it involves parameters such as, surface charge, surface energy, scale and topography.[20] These properties can be modified to suit protein adsorption applications. Commonly used surfaces include polymers, silicon wafer (modified and unmodified), oxides (including unmodified silica), phospholipids, metals/non metals (such as gold, germanium, alloys, carbon etc.), modified silica, self-assembled monolayers (SAMs), glass, quartz and mica. Surfaces can be tuned by chemical treatments thereby changing their properties and the most commonly used ones are the silanes. Silanes modify the hydroxyl groups present on the surfaces of glass, quartz, metal oxides, silicon etc. with alkoxysilane groups rendering the surface hydrophobic.[20] Similarly, SAMs are formed by the assembly/adsorption of amphiphilic molecules such as, alkanethiols on a substrates such as gold, silicon, etc.[52]

One of the recent advances in this field involves studying protein adsorption on nanomaterials. Protein adsorption also depends on the type of material scale i.e. adsorption on a macro/micro sized surface is different than on nano sized surface and the process is not very well understood.[30] Nanoparticles have larger surface area and hence could result in increased protein adsorption. Proteins adsorbing on the nanoparticles forms a layer around it, called protein corona. For

therapeutic nanoparticles, protein corona might be undesirable because it results in nanotoxicity, rapid clearance from the blood and hence lower target specificity.[32-34] Unlike other regular macro/micro materials, nanomaterials might better mimic the physiological makeup since cells and tissues have patterns comprising of biomolecules in the nano scale and moreover, protein's size is in the nanometre range (3-15 nm for most blood proteins).[12] This might favor subsequent protein adsorption followed by cell attachment and other process.

Understanding protein adsorption involves devices/tools for measuring and studying adsorption and may involve studying adsorption kinetics, the amount of adsorbed protein, the activity and the structure of the adsorbed proteins.[12] Many label free approaches such as, ellipsometry, UV spectrometry, surface plasmon resonance (SPR), optical waveguide lightmode spectroscopy (OWLS), etc. have been used to study adsorption kinetics and some have also been used to measure the thickness of the adsorbed protein layer.[20] Labelled techniques such as, radiolabelling, lowry assay, bicinchoninic acid (BCA) assay have also been employed.[35, 37-39] On the other hand, to study the structure of adsorbed proteins and its conformational changes upon adsorption, infrared spectroscopy (IR), attenuated total internal reflectance-infrared spectroscopy (ATR-IR) and circular dichroism (CD) spectroscopy has been used.[20] Atomic force microscopy (AFM) has also been used to study protein adsorption by imaging of the adsorbed protein and can provide information, such as the height of the protein. AFM combined with scanning tunneling microscope (STM) has also been used to characterize single adsorbed protein molecule with improved lateral information. Quartz crystal microbalance (QCM) has been used widely for measuring the adsorbed protein mass; however, it does not measure the dry protein mass because it incorporates the mass of water in the protein layer. Recent advances also include optics-based label free new tools for measuring protein adsorption such as, neutron reflectometer, interferometric reflectance imaging

sensor (IRIS), formerly known as spectral reflectance imaging biosensor (SRIB) and whispering gallery mode (WGM).[40-42]

To this end, we describe a database, which aggregates published data regarding protein adsorption. Regression analysis was performed on different parameters that influence protein adsorption. These parameters include buffer pH and ionic strength, surface contact angle, isoelectric point, hydrophobicity and hydrophilicity of proteins. We were able to construct a Langmuir fit with piecewise linear regression and a predictive tool was used to estimate the protein layer thickness and the number of adsorbed protein layer. The database can be used for the selection of materials, operation conditions and for designing microfluidics or microarray devices.

3.3. Methods

3.3.1. Data collection for the database

The database comprises of only literature data that reports quantitatively the protein, surface and fluid parameters. Currently, the database does not include data from any nanoparticles or nano-surfaces. The database comprises of data from different experimental studies with different methods used to study protein adsorption. The primary data has been collected from the open literature (see Appendix I and II) using the major literature search engines (e.g., PubMed, Scopus, Wiley, Springer, Science Direct, etc.). This initial search was followed by the detailed analysis of the published data for extracting adsorption parameters for the database. The database consists of 964 records of protein adsorption experiments.

3.3.2. Protein adsorption variables reported in the database

The database reports on several parameters that affects protein adsorption process, i.e., related to the protein, surface and fluid environment, as well as the different measurement techniques employed. Some of the protein parameters such as charge, hydrophobicity and hydrophilicity are not reported in the literature, but calculated using protein surface properties calculator (PSPC).[24]

Protein variables. Presently the database comprises data regarding the adsorption of 28 representative proteins, namely: albumin (HSA and BSA) 33.2%; immunoglobulin G 13.8%; lysozyme 13.3%; fibrinogen 11.2%; alpha-lactalbumin 7.4%; myoglobin 3.1%; fibronectin 2.3%; ribonuclease A 1.9%; alpha-chymotrypsin 1.8%; insulin 1.7%; beta-casein 1.7%; cutinase 1.3 %; Glucose oxidase 0.8%; human growth hormone 0.6%; immunoglobulin M 0.6%; alpha-2-macroglobulin 0.6%; alpha-s1-casein 0.6%; beta-lactoglobulin 0.6%; cholesterol esterase 0.5%; collagen 0.5%; alpha-amylase 0.4%; Cry1Ac 1.1%; cytochrome c 0.1%; Lactoferrin 0.4%; prothrombin 0.2%; protein A 0.1% and hemoglobin 0.1%.

PDB ids for the proteins used in the adsorption studies are listed in the database. Based on these PDB ids, charge, hydrophobicity and hydrophilicity of these proteins that are not reported in the literature are measured using PSPC software.[24] Amino acid sequence in the form of FASTA format[53] is used to measure proteins isoelectric point (http://isoelectric.org/).

Surface variables. The database consists of 9 types of surfaces on which protein adsorption has been studied: polymers 38.53%; silicon wafer (modified and unmodified) 18.8%; oxides (including unmodified silica) 13.64%; phospholipids 6.92%; metals/non metals 6.92%; modified silica 6.61%; self-assembled monolayers (SAMs) 5.89%; glass 2.48%; quartz 0.21%. The central surface parameter is the surface hydrophobicity or hydrophilicity and they are measured using

either contact angle or surface energy/tension. Contact angle data is more predominantly reported in the literature rather than the surface tension. Surface tension is actually calculated based on the surface contact angle using a MATLAB script. Average contact angles are reported in the database when both the advancing and receding contact angles are mentioned in the literature.[54]When the original literature does not report the surface contact angle, its value is reported from other literatures, either by the same author or research group; or an average value reported elsewhere for the same surface.

Fluid media variables. Currently, there are 17 different buffer solutions with distinct concentrations and composition represented in the database. The buffer parameters includes pH and ionic strength. It also includes the temperature of the buffer during adsorption studies. For the experiments reported at room temperature, the value was assumed 22 ºC.

Protein concentration on the surface and in solution. Protein concentrations on the surface is measured as the amount of protein adsorbed per area of the surface (mg/m^2). It is measured either using protein adsorption isotherms, such as Langmuir, Freundlich etc.[44] or using different measurement techniques, such as radiolabelling, ellipsometry, UV-based absorption etc. [20] The concentration of proteins in the buffer media (mg/ml) is quite important since higher solution concentration increase protein adsorption on surfaces among other factors affecting adsorption.

Protein adsorption measurement techniques. 16 different measurement methods have been listed in the database that were used for the quantification of the amount of protein adsorbed on surfaces, with the proportion as follows: UV absorption 28.42%; ellipsometry 21.89%; radio-labelling 21.47%; quartz micro balance (QCM) 14.21%; Lowry method 2.28%; sedimentation field-flow fractionation (SdFFF) 1.97%; total internal reflection fluorescence (TIRF) 1.97%; bicinchoninic acid protein assay (BCA) 1.66%; Neutron reflectivity 1.04%; spectral reflectance imaging

biosensor (SRIB) 0.93%; surface plasmon resonance (SPR) 0.93%; whispering gallery mode (WGM) 0.83%; X-ray reflectomrtey 0.83%; attenuated total reflectance-fourier transform infrared (ATR-FTIR) 0.83%; high performance liquid chromatography (HPLC) 0.62% and fluorescence spectroscopy 0.10%.

3.3.3. Statistical analysis

Before performing statistical analysis, data validation was performed, especially the range and constraint check; some data were excluded that stands out from the database trends, e.g., very high surface concentration (>50 mg/m^2), very high protein concentration in solution (> 5 mg/ml), and temperature outside the room temperature range (>19^{o}C and < 26^{o}C). Furthermore, the regression has been applied separately to data representing adsorption on hydrophilic (contact angle lower than 45^o; 292 cases) and hydrophobic surfaces (321 cases).

A non-linear least squares regression analysis was performed using the software package Statistica (from TIBCO Software Inc.). We used estimation algorithms: Levenberg-Marquardt and Gauss-Newton. The former gave a better correlation than the latter. The maximum number of iterations was set to 2000 and the convergence criterion was set to 10^{-6} (the optimization stops when the changes in the parameters from iteration to iteration are no more than the convergence criterion). Langmuir isotherm equation (equation 1) was used to model the adsorption data.

$$\frac{1}{V_1} = \frac{1}{(K_1.V_2)} + \frac{K_2}{K_1} \qquad (1)$$

$$K_1 = a_3.V_3 + a_4.V_4 + a_5.V_5 + a_6.V_6 + \ldots\ldots + a_n.V_n$$

$$K_2 = b_3.V_3 + b_4.V_4 + b_5.V_5 + b_6.V_6 + \ldots\ldots + b_n.V_n$$

Where V_1 is the surface concentration of the adsorbed protein; V_2 is the solution concentration of proteins; V3, V4, V5, V6, V7, V8, V9 are the parameters that influences protein adsorption such as surface tension, pH, ionic strength of buffer, molecular weight, isoelectric point, protein positive charge, negative charge, protein hydrophobicity and hydrophilicity etc., a and b are constants.

Initial non-linear least square analysis was implemented in Statistica with 613 cases. Later, these data points were segregated into hydrophilic (up to 45^o) and hydrophobic surfaces (> 45^o) and separate regression analysis was performed for each. These cases were later segregated based on widely used protein adsorption measurement techniques: QCM (90 cases), ellipsometry (74 cases), UV (184 cases) and radiolabelling (172 cases).

Piecewise linear regression with breakpoint was also applied on the database representing QCM (90 cases), ellipsometry (74 cases), which was further segregated into hydrophobic and hydrophilic surfaces. Breakpoint was set automatically by Statistica. Different estimation algorithms were used: quasi-Newton, Hooke–Jeeves, Simplex, Rosenbrock, but quasi-Newton gave a very good correlation. Breakpoint also indicates the point of surface concentration above which the protein layer is no longer a monolayer and the resulting protein adsorption does not happen on the surface but the protein layer below it. Accordingly, breakpoint can be used to estimate the approximate thickness of the protein layer.

3.4. Results and discussion

3.4.1. Distribution of the protein adsorption descriptors in the database

Proteins. The distribution of protein parameters represented in the database such as, the molecular weight and the isoelectric points are shown in Figure 9a and Figure 9b. Although the proteins represented in the database can be categorized into small, medium and large-sized, the large majority of data (approximately 69%) are cases for proteins with small molecular weights (between 6 and 100 kDa). This is due to the over representation of albumin and lysozyme in the database. A small percentage of cases comprises of proteins with high molecular weights, (e.g., 14.4% for immunoglobulin). The distribution of isoelectric points of the proteins is also due to the excessive representation of lysozyme (IP = 8.34), albumin (IP = 5.47), fibrinogen (IP = 6.15) and IgG (IP = 6.57).

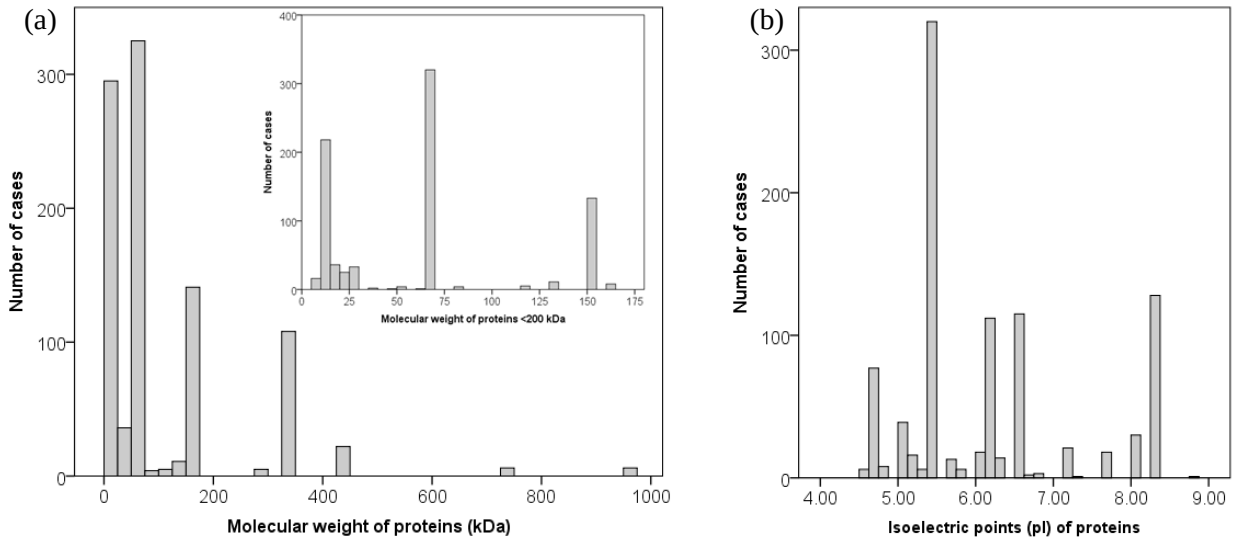

Figure 9: Distribution of the protein properties in the database: (a) molecular weights. The inset provides the distribution of weights <200 kDa, (b) isoelectric points.

The molecular weight and isolectric point of proteins, if not reported in the literature have been estimated from the amino acid composition of each protein based on their respective PDB ids form RCSB protein data bank (https://www.rcsb.org/).

Adsorption surfaces. Figure 10 shows the distribution of the contact angle data of the surfaces represented in the database. Two distinct cluster is evident from the figure: hydrophilic surfaces with contact angles between 0 to 45 degree; and hydrophobic surfaces with contact angles between 70 and 120 degree, and some data points connecting the two clusters.

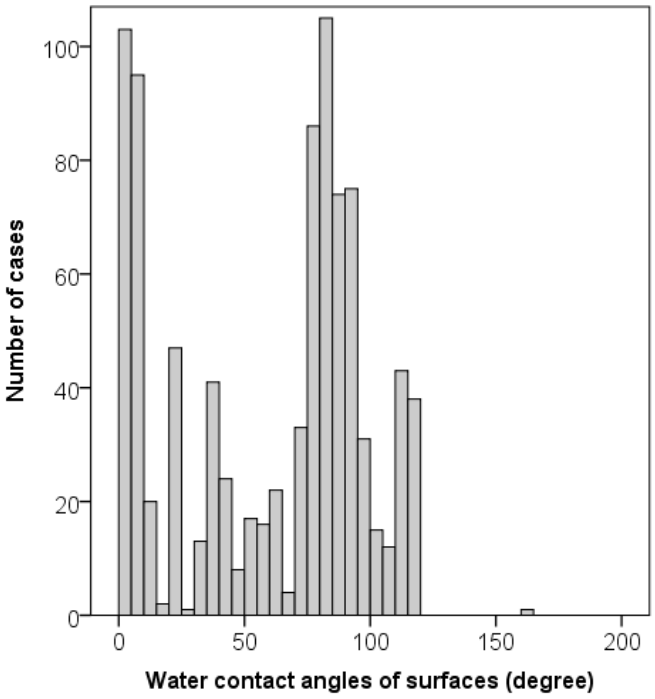

Figure 10: Distribution of the contact angle of the surfaces in the database. Hydrophilic (0 to 45 degree) and hydrophobic (70 and 120 degree) clusters can be seen.

Fluid media. Figure 11a provides the distribution of buffer pH in the database. In majority of the reported cases, experiments (964 cases) were performed in the neutral pH region (pH = 7.0 –7.4) and the total pH range in the database ranges from 2.75 to 11. Difference between the pH of the

buffer and the isoelectric point was also plotted as shown in figure 11b with a mean value of 1.01. There is an increase in the adsorption of protein on the surface when the buffer pH matches protein's respective isoelectric point. Ideally, pH-pI should be around 0 for increased adsorption. Figure 11c provides the distribution of the ionic strength of the buffer and indicates the experimental preference for buffers with low concentration of ions, or an ionic strength around

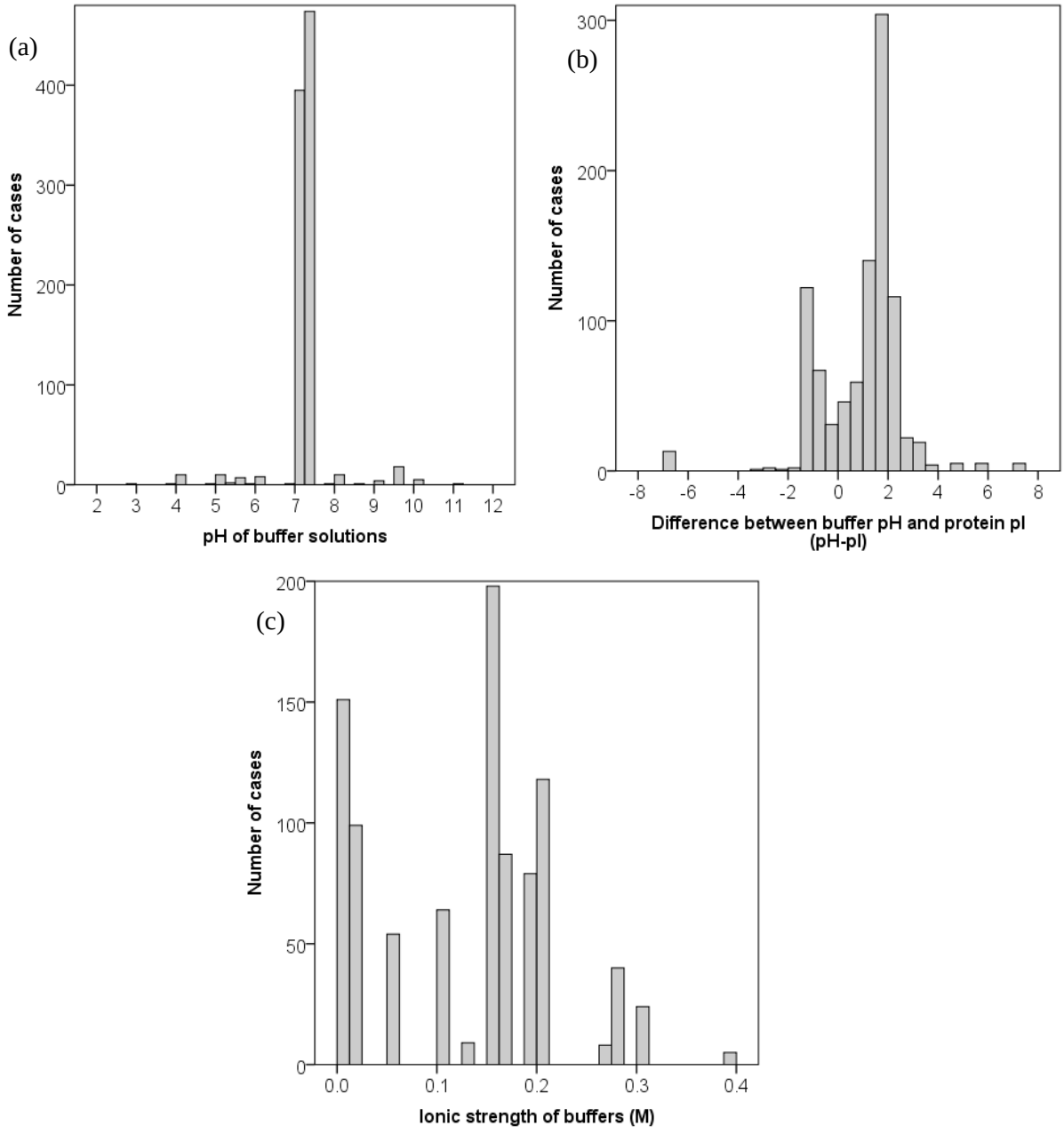

Figure 11: Distribution of the fluid media parameters in the database: (a) pH of buffers, (b) pH and isoelectric points difference, (c) ionic strength of buffers.

0.17. This corresponds to 1X phosphate buffer saline as per the database. The overall ionic strength range in the database spans from 0.001 to 0.41.

Protein concentrations in solution and on surface. Figure 12a and 12b provides the distribution of protein concentration in solution and on the adsorbing. Most of the data in the database indicate that the protein adsorption experiments were conducted at low concentrations in solution (90.56% up to 2 mg/ml), and the resulting lower concentration on the surface (76.66% up to 5 mg/m^2). However, it can be seen that the overall range of protein concentrations is quite broad, both in solution and on the surface spans.

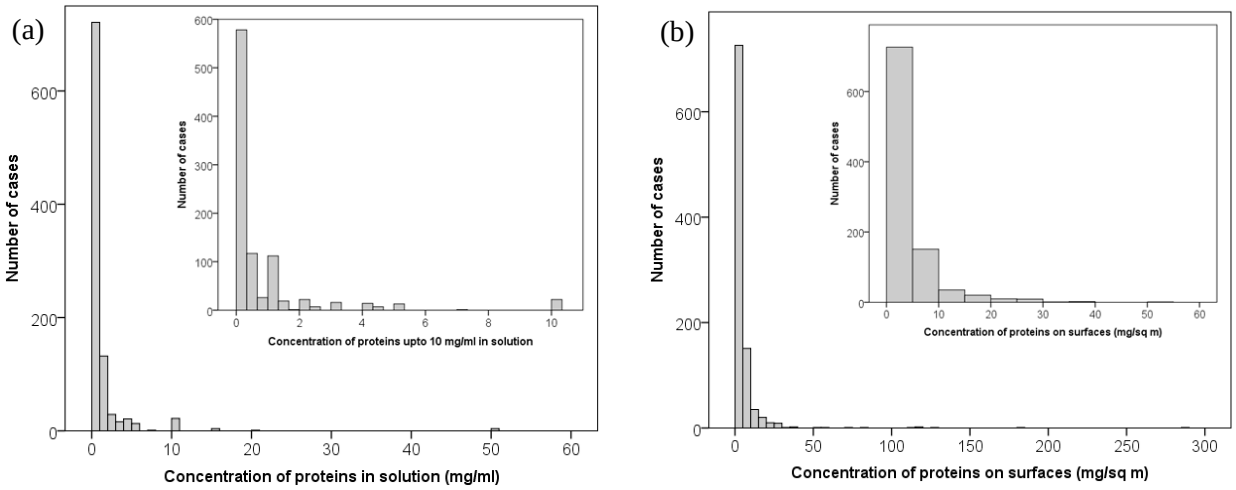

Figure 12: Distribution of protein concentrations in the database: (a) in solution. The inset provides concentration up to 10 mg/ml in solution, (b) on the surface. The inset provides concentration up to 60 mg/m^2.

Measurement methods. The distribution of different measurement technique described in the database and reported since 1980 has been shown in figure 13. Radiolabelling method was quite predominantly used since 1980, followed by UV and TIRF in 1985-1990. Ellipsometer was quite widely used since 1990s and QCM usage can be seen since 2000 based on the database.

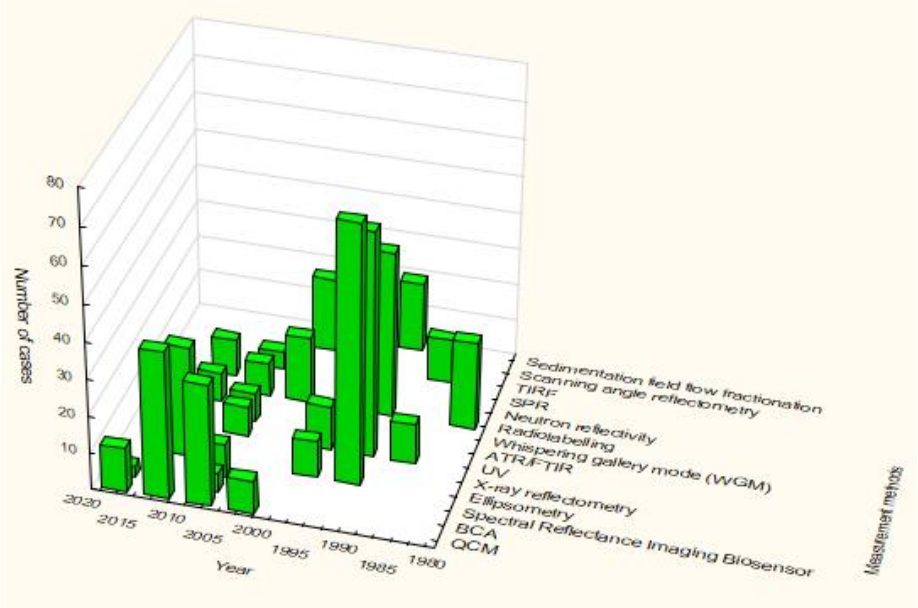

Figure 13: Distribution of protein adsorption measurement techniques reported since 1980.

3.4.2. Estimation of the thickness and number of adsorbed protein layer

The estimation of the thickness of the protein layer adsorbed on surfaces and the number of adsorbed layers, described in the previous work, is implemented in the database as an applet that allows the user to estimate the protein layer thickness as a function of protein radius and surface area.[1] While the vast majority of the data in the database represents protein layer thicknesses up to one monolayer, the prediction can estimate higher values. The values estimated for protein layer thickness are minimum values, as we assumed the closest packing of proteins and ignored the inherent uptake of water in the protein layer.

3.4.3. Regression analysis

The regression analysis using non-linear least square did not result in good statistical fit for the dataset, i.e. a correlation coefficient, R^2 of 39.31%, 30.32% and 8.8% for the whole data, the

hydrophilic and the hydrophobic data. The dataset with 613 cases were segregated based on the protein adsorption measurement technique, namely radiolabelling, QCM and ellipsometry to identify if we can find a better correlation with the measurement techniques. Currently, QCM and ellipsometry are the most widely used measurement technique for protein adsorption with 22.97% and 18.61% of the data respectively. Radiolabelling was the most widely used technique prior to 2010 and accounts for 19% of the dataset.

Regression analysis on radiolabelling data did not yield good statistical fits with an R^2 of 41.72% and 39.49% for the whole radiolabelling data and hydrophilic data. However, an R^2 of 86.66% was achieved for hydrophobic data. QCM data gave a very good statistical fits with an R^2 of 98.73%, 85.39% and 99.87% for the whole QCM data, hydrophobic data and hydrophilic data respectively. Ellipsometry gave a good statistical fit with an R^2 of 82.36% and 84.36% for the whole ellipsometry data and hydrophobic data respectively. However, despite of having good correlation (*supplementary section*), we were not able to fit a Langmuir curve, perhaps the reason could be due to using too many parameters with few data sets (~10 parameters for 60 data points for QCM hydrophobic) and that could result in noise and hence a high R^2 value.

Method	All surfaces			Hydrophobic surfaces			Hydrophilic surfaces		
	Protein types	No. of cases	R^2	Protein types	No. of cases	R^2	Protein types	No. of cases	R^2
QCM	6	90	98.73	5	60	85.39	5	30	99.87
Ellipsometry	5	74	82.36	5	67	84.36	4	7	NA
Radiolabelling	7	172	41.72	6	96	86.66	5	76	39.49
UV	6	184	46.56	5	38	93.58	6	146	57.22

Table 2: Non-linear regression analysis of protein adsorption data based on different measurement methods

Piecewise linear regression on QCM hydrophobic data gave a good R^2 value of 88.02% (breakpoint of 2.458 mg/m^2). Radiolabelling data had an R^2 value of 95.08% (breakpoint of 6 mg/m^2) for both whole radiolabelling and hydrophilic data. The hydrophobic data of radiolabelling had an R^2 value of 96.16% (breakpoint of 6 mg/m^2). Ellipsometry had an R^2 of 93.37% (breakpoint of 2.9 mg/m^2) and 94.3% (breakpoint of 2.9 mg/m^2) for the whole data and hydrophobic data. UV based measurement had an R^2 of 98.88% (breakpoint of 1.7 mg/m^2), 90.32% (breakpoint of 2 mg/m^2) and 99.74% (breakpoint of 2.74 mg/m^2) for the whole UV data, hydrophilic data and hydrophobic data respectively. Breakpoint values were varied and the resulting R^2 values were plotted against the breakpoint (*supplementary section*). The piecewise regression for QCM hydrophobic method provided coefficients for different variables (*supplementary section*). These coefficients were used to construct two equations and these equations were plotted for the surface concentration vs solution concentration as shown in figure 14a to get a Langmuir distribution with the intersection at (2, 5.47). A separate plot (figure 14b) of number of protein layers vs surface concentration gave us two clusters: one comprised only of lysozyme (on the left) and the other cluster comprised of other proteins (on the right). At a surface concentration of 5.4 mg/m^2 and below one can expect a monolayer. A separate cluster for lysozyme could be due to a high isoelectric point compared to the buffer pH. Piecewise regression for other measurement techniques did not yield a Langmuir distribution.

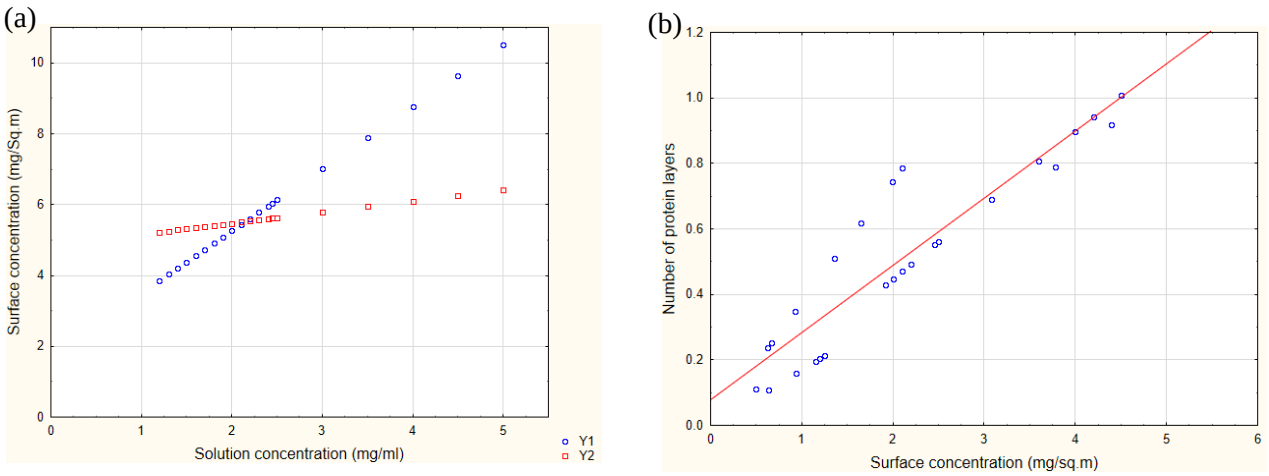

Figure 14: (a) Piecewise linear regression of QCM hydrophobic method with a Langmuir distribution with the intersection at (2, 5.47), (b) No. of protein layers vs surface concentration plot. Surface concentration of 5.47 mg/m^2 correspond to the protein layer of ~1.2

3.5 Conclusions

Protein adsorption at solid-liquid interfaces is important to many applications. Protein adsorption data reported in the literature since 1980s has been compiled into a database. Database was arranged based on the different parameters influencing protein adsorption: buffer pH and ionic strength, surface contact angle, isoelectric point, hydrophobicity and hydrophilicity of proteins. The distribution of these parameters in the database gives an overview of the trend and the scale of its distribution; for instance, contact angle distribution produced a hydrophilic and hydrophilic cluster; in case of buffer pH, pH of 7.4 was predominantly used. Despite having a good correlation (R^2>85%) for QCM method, nonlinear regression did not produce a Langmuir fit. On the other hand, piecewise linear regression produced a Langmuir fit for QCM hydrophobic method, albeit not with ellipsometer, UV and radiolabelling, The plot of surface concentration vs solution concentration generated by piecewise, corresponds to the surface concentration of 5.47 mg/m^2, the concentration above which adsorption does not happen as a monolayer, but multilayer.

Furthermore, the data present in the database used a predictive tool that can estimate the thickness of the adsorbed protein layer, and the number of adsorbed protein layers and was used for plotting number of layers vs. surface concentration for QCM with hydrophobic data and the concentration of 5.47 corresponds to ~1.2 layers of adsorbed protein. The database hence can be used for the selection of materials, operation conditions and for designing microfluidics or microarray devices.

3.6 Acknowledgements

The research reported here was sponsored by grants from Natural Sciences and Engineering Research Council (NSERC) of Canada.

3.7 Supplementary information

Piecewise linear regression results for QCM hydrophobic

Model is: Piecewise linear regression with breakpoint (BAD regression M1_AA_Dgwif 2018 for statistica) Dependent variable: surf_conc Loss: Least squares Final loss:10.498676850 R= .93819 Variance explained: 88.020%									
Const.B0	**solution conc**	**Surface tension, Ysl (calculated) (mJ/m^2)**	**Ionic strength**	**pH-IP**	**Protein total area**	**Hydrophobicity**	**Hydrophilicity**	**Hydrophobicity /hydrophobic area**	
16.86694	1.754476	0.068644	-5.08785	-0.327917	0.000055	-0.041085	-0.019716	1733.140	

Const.B0	solution conc	Surface tension, Ysl (calculated) (mJ/m^2)	Ionic strength	pH-IP	Protein total area	Hydrophobicity	Hydrophilicity	Hydrophobicity /hydrophobic area	Breakpt.
7.287768	0.312958	-0.027103	31.02625	-1.45199	-0.000236	0.017158	0.011136	0.065555	2.458409

For the first equation:

	Average	Coeff*Average
Bo		16.86694
YsI	26.29933	1.8053
IS	0.131477	-0.66894
pH-IP	1.134773	-0.37211
Protein area	24634.27	1.363563
Hypho	-14.6262	0.600917
Hyphi	170.8352	-3.36822
Hypho/Hypho area	-0.00835	-14.4788
Total		1.74865

Y1 = 1.7544*X + 1.74865

For the second equation

	Average	Coeff*Average
Bo		7.287768
YsI	26.29933	-0.7128
IS	0.131477	4.079246
pH-IP	1.134773	-1.64767
Protein area	24634.27	-5.8092
Hypho	-14.6262	-0.25096
Hyphi	170.8352	1.902362
Hypho/Hypho area	-0.00835	-0.00055
Total		4.848193

Y2 = 0.3129*X + 4.848193

Piecewise linear regression: Plot of R^2 vs breakpoints for different measurement techniques. Ellipsometer and UV plots does not have hydrophilic results because of insufficient data.

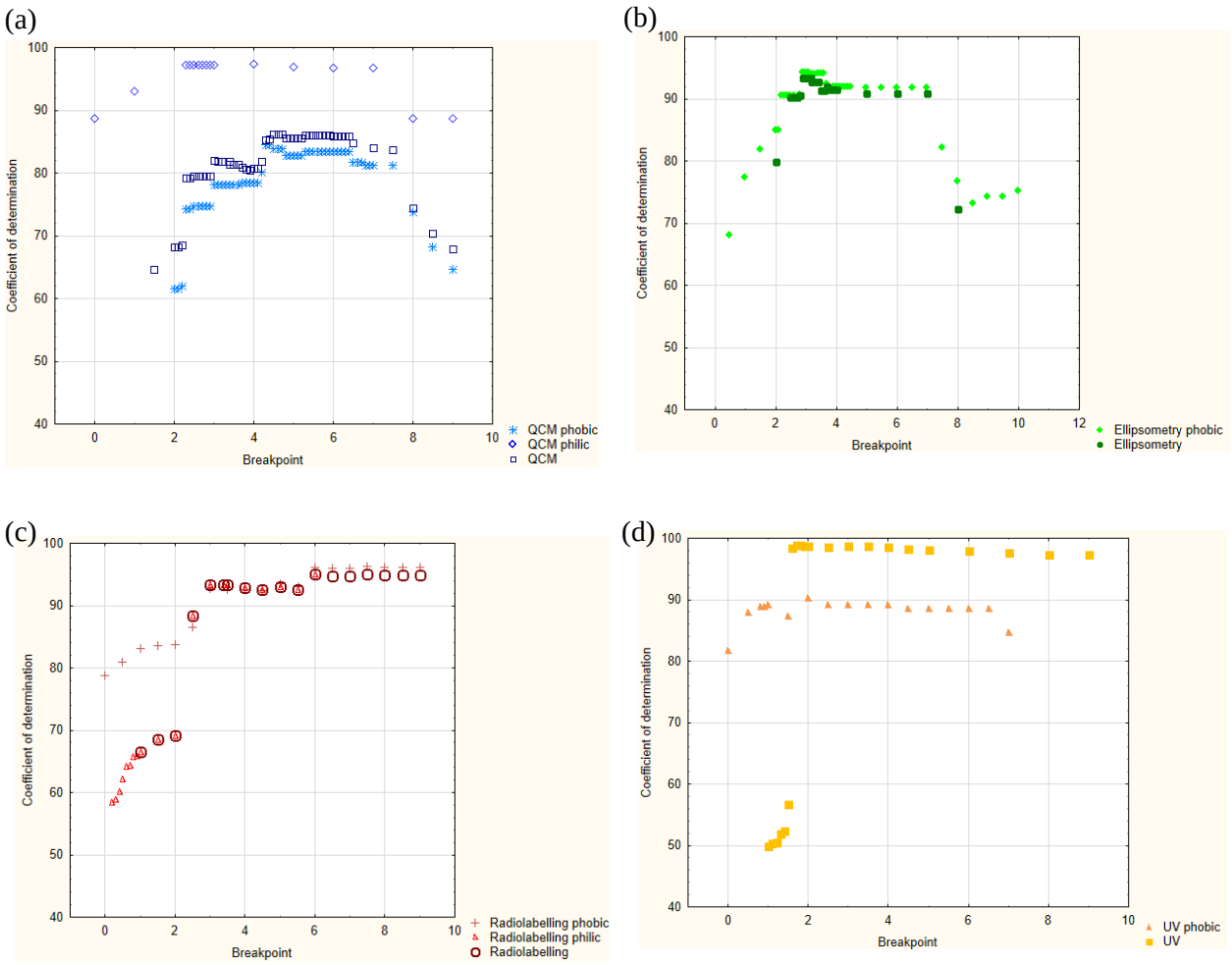

Figure S1: Coefficient of determination at different breakpoints for piecewise linear regression: (a) QCM, (b) Ellipsometry, (c) Radiolabeling, (d) UV

Chapter 4 – Conclusion and future scope

4.1 Conclusion

Protein adsorption is crucial to a vast array of applications, ranging from medical interests, such as biomaterials, drug delivery and release, and devices for diagnostics and other industrial applications. This thesis detailed data collection of the protein adsorption data from the literature published since the last decade and the prior data, which was collected from the literature since 1980s to form a database and its analysis. Prior to the work on protein adsorption database, the Wikipedia page on "protein adsorption" was updated, this was followed by data analysis of protein microarray data.[17-18]

Data collection of protein adsorption data involved critical examination of the published work to get protein, fluid and surface parameters influencing protein adsorption. Protein parameters include protein's PDB id, protein's molecular weight and isoelectric point. Properties such as hydrophobicity, hydrophilicity and charge were calculated using PSPC software. Fluid parameters comprises of buffer pH, ionic strength and buffer temperature. Surface parameters consists of the type of surface and its contact angle. This thesis does not include data or its analysis for nano-surfaces since protein adsorption is quite different on nanoparticles/nano-surfaces and is one of the limitations of this study.

Initial data analysis involved studying distribution of different parameters reported since 1980s. This was followed by performing non-linear analysis using Langmuir isotherm equation. Analysis was performed on the database which was segregated into different methods used to study protein adsorption: QCM, ellipsometry, UV and radiolabeling and was further divided into hydrophobic and hydrophilic surfaces. Non-linear analysis gave a very good correlation for QCM (>85%)

compared to other techniques, however, failed to construct Langmuir isotherm curve. The high correlation could be due to the noise generated by using too many parameters (~10) with limited data points (~60). Piecewise-linear regression on the other hand gave a Langmuir fit for QCM hydrophobic dataset. The intersection corresponds to the surface concentration of 5.47 mg/m^2, the concentration above which adsorption does not happen as a monolayer, but multilayer. Furthermore, a predictive tool was used to estimate the thickness of the adsorbed protein layer and the number of adsorbed protein layers. Number of layers vs. surface concentration for QCM with hydrophobic data was plotted and the concentration of 5.47 mg/sq.m corresponds to ~1.2 layers of adsorbed protein. The database hence can be used for the selection of materials, operation conditions and for designing microfluidics or microarray devices.

4.2 Future works

4.2.1 Online protein adsorption database and adsorption prediction

One of the limitations of our database is that it is not an online database. Biomolecular adsorption database (BAD) is the only world-wide, protein adsorption database, freely available online, maintained by Prof. Nicolau, reporting experimental data points (http://bad.molecularsense.com/).[1] BAD also includes a protein adsorption prediction tool for predicting the amount of protein adsorption on the surface. This predictive tool is based on neural networks. However, currently, this online database is not functional due to some issues with its compatibility. Future works will involve reconstructing the database using MySQL and to incorporate the adsorption predictive tool. Since the predictive tool can estimate the amount of protein adsorption, the number of layers and the layer thickness, it could be used for different

engineering applications related to protein adsorption and can be used for designing different devices or materials for biomedical applications.

4.2.2 Combinatorial adsorption studies with microfluidics

Given the variability in the data that could be accounted for different experimental conditions, the way in which the experiment is performed, handling etc., a microfluidic platform could be used to perform large number of adsorption experiments in a single device minimizing the variability in the data. The microfluidic device could be designed to incorporate different combinations of surfaces, proteins, buffers etc. Ellipsometry could be used for measuring the adsorbed proteins on different surfaces under different conditions. Suppose one experiment within a microfluidic device requires 200x200µm footprint, which would result in an approximately one thousand experiments on $1cm^2$ microfluidic device. This would help us get large data that are reliable in one single experiment compared to the data collection from literature and minimize variability in the data.

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Appendix I: Protein adsorption database

Database with data measured at room temperature (20ºC-25ºC). Data for other temperatures were not included.

Protein	PDB	surf_conc (mg/sq. m)	sol_conc (mg/ml)	Surf_type	Contact_angle	Ysl (calculated) (mJ/m^2)	pH	IS (M)	MW (kDa)	IP	Method	Ref	Year
Fibrinogen	3GHG	16.2	0.5	ZrO2	82	24.10	7.4	0.154	340	6.15	Ellipsometry	[1]	2013
Fibrinogen	3GHG	11.4	0.5	Ta2O5	60	11.44	7.4	0.154	340	6.15	Ellipsometry	[1]	2013
Fibrinogen	3GHG	9.8	0.5	Nb2O5	72	17.98	7.4	0.154	340	6.15	Ellipsometry	[1]	2013
Fibrinogen	3GHG	8.8	0.5	TiO2	74	19.16	7.4	0.154	340	6.15	Ellipsometry	[1]	2013
Alpha-lactalbumin	1HML	1.47	0.06	polymer	82	24.10	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	1.54	0.17	polymer	82	24.10	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	1.67	0.33	polymer	82	24.10	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	1.62	0.35	polymer	82	24.10	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	1.71	0.44	polymer	82	24.10	7	0.108	14.188	4.71	UV	[2]	1995
Lysozyme	2LYZ	0.83	0.012	polymer	82	24.10	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	0.93	0.07	polymer	82	24.10	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.03	0.21	polymer	82	24.10	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.09	0.32	polymer	82	24.10	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.12	0.44	polymer	82	24.10	7	0.108	14.3	8.34	UV	[2]	1995
Alpha-lactalbumin	1HML	0.44	0.012	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	0.61	0.06	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	0.61	0.12	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	0.6	0.18	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	0.59	0.348	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995

Alpha-lactalbumin	1HML	0.59	0.504	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995
Alpha-lactalbumin	1HML	0.59	0.66	polymer	81	23.47	7	0.108	14.188	4.71	UV	[2]	1995
Lysozyme	2LYZ	0.86	0.012	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.23	0.024	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.4	0.06	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.47	0.192	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.5	0.324	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.5	0.48	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.52	0.612	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
Lysozyme	2LYZ	1.52	0.9	polymer	81	23.47	7	0.108	14.3	8.34	UV	[2]	1995
HSA	1AO6	0.3	0.005	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	0.7	0.01	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	0.8	0.025	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	1.6	0.05	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	1.6	0.075	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	2	0.1	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	2.2	0.15	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
HSA	1AO6	2.5	0.2	polymer	95.5	32.78	7.4	0.009	66.437	5.47	Radiolabelling	[3]	1995
Fibrinogen	3GHG	0.6	0.01	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	2.4	0.03	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	3.5	0.035	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	4.6	0.04	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	5.3	0.06	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	5.5	0.075	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	6	0.15	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995

Fibrinogen	3GHG	6	0.2	polymer	95.5	32.78	7.4	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	5	0.2	polymer	95.5	32.78	2.75	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	7.7	0.2	polymer	95.5	32.78	5.5	0.009	340	6.15	Radiolabelling	[3]	1995
Fibrinogen	3GHG	1.3	0.2	polymer	95.5	32.78	11	0.009	340	6.15	Radiolabelling	[3]	1995
IgG	1IGT	1.35	0.001	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	2.3	0.006	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	2.49	0.01	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	3.48	0.02	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	3.3	0.031	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	3.73	0.05	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	3.69	0.076	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	3.91	0.1	polymer	96	33.11	7	0.009	150	6.57	Radiolabelling	[4]	2001
IgG	1IGT	2.75	0.05	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	3.47	0.1	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	3.81	0.2	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.58	0.4	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.58	0.6	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.66	0.8	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.66	1	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.74	2	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.74	3	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.66	4	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.58	5	polymer	75	19.76	7	0.01	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4	5	polymer	75	19.76	4.2	0.007	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.1	5	polymer	75	19.76	5.4	0.007	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.65	5	polymer	75	19.76	6.8	0.007	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.8	5	polymer	75	19.76	7	0.007	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	4.6	5	polymer	75	19.76	7.4	0.007	150	6.57	SdF-FF	[5]	1995

IgG	1IGT	4.2	5	polymer	75	19.76	8	0.007	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	3.65	5	polymer	75	19.76	8.6	0.007	150	6.57	SdF-FF	[5]	1995
IgG	1IGT	3.8	5	polymer	75	19.76	10	0.007	150	6.57	SdF-FF	[5]	1995
Alpha-lactalbumin	1HML	0.6	0.005	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1	0.01	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.2	0.025	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.3	0.05	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.25	0.055	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.3	0.1	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.25	0.11	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.35	0.19	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.45	0.27	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.4	0.29	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.5	0.36	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.65	0.44	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Alpha-lactalbumin	1HML	1.55	0.45	polymer	82	24.10	7	0.05	14.188	4.71	UV	[6]	1995
Lysozyme	2LYZ	1	0.01	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	1.4	0.015	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	1.6	0.02	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	1.9	0.025	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	2.2	0.075	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	2.15	0.08	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	2.2	0.17	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	2.2	0.275	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	2.35	0.35	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
Lysozyme	2LYZ	2.4	0.45	polymer	82	24.10	7	0.05	14.3	8.34	UV	[6]	1995
HSA	1AO6	10	1	polymer	116	45.63	7.3	0.309	66.437	5.47	Radiolabelling	[7]	1981

HSA	1AO6	13	1.5	polymer	116	45.63	7.3	0.309	66.437	5.47	Radiolabelling	[7]	1981
HSA	1AO6	17	2.2	polymer	116	45.63	7.3	0.309	66.437	5.47	Radiolabelling	[7]	1981
HSA	1AO6	17	10	polymer	116	45.63	7.3	0.309	66.437	5.47	Radiolabelling	[7]	1981
HSA	1AO6	17	50	polymer	116	45.63	7.3	0.309	66.437	5.47	Radiolabelling	[7]	1981
HSA	1AO6	5	0.5	polymer	116	45.63	7.3	0.309	66.437	5.47	Radiolabelling	[7]	1981
Alpha-2-Macroglobulin	4U48	2.35	0.5	polymer	116	45.63	7.3	0.309	725	5.03	Radiolabelling	[7]	1981
Alpha-2-Macroglobulin	4U48	7.5	1	polymer	116	45.63	7.3	0.309	725	5.03	Radiolabelling	[7]	1981
Alpha-2-Macroglobulin	4U48	12.9	1.6	polymer	116	45.63	7.3	0.309	725	5.03	Radiolabelling	[7]	1981
Alpha-2-Macroglobulin	4U48	14.7	2.6	polymer	116	45.63	7.3	0.309	725	5.03	Radiolabelling	[7]	1981
Alpha-2-Macroglobulin	4U48	15.3	10	polymer	116	45.63	7.3	0.309	725	5.03	Radiolabelling	[7]	1981
Alpha-2-Macroglobulin	4U48	15.3	50	polymer	116	45.63	7.3	0.309	725	5.03	Radiolabelling	[7]	1981
IgG	1IGT	14	0.5	polymer	116	45.63	7.3	0.309	150	6.55	Radiolabelling	[7]	1981
IgG	1IGT	21	1	polymer	116	45.63	7.3	0.309	150	6.55	Radiolabelling	[7]	1981
IgG	1IGT	25	1.5	polymer	116	45.63	7.3	0.309	150	6.55	Radiolabelling	[7]	1981
IgG	1IGT	25	2.5	polymer	116	45.63	7.3	0.309	150	6.55	Radiolabelling	[7]	1981
IgG	1IGT	25	10	polymer	116	45.63	7.3	0.309	150	6.55	Radiolabelling	[7]	1981
IgG	1IGT	25	50	polymer	116	45.63	7.3	0.309	150	6.55	Radiolabelling	[7]	1981
Fibrinogen	3GHG	2.38	0.005	polymer	74.55	19.46	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	4.1	0.05	polymer	74.55	19.46	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	5.3	0.25	polymer	74.55	19.46	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	5.8	0.5	polymer	74.55	19.46	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	6.19	1	polymer	74.55	19.46	7.4	0.15	340	6.15	Radiolabelling	[8]	2005

Fibrinogen	3GHG	2.7	0.005	mineral	6.5	0.00	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	3.7	0.05	mineral	6.5	0.00	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	4	0.25	mineral	6.5	0.00	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	4.3	0.5	mineral	6.5	0.00	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	4.7	1	mineral	6.5	0.00	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Lysozyme	2LYZ	1.27	0.005	polymer	74.55	19.46	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	2.1	0.05	polymer	74.55	19.46	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	2.5	0.25	polymer	74.55	19.46	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	2.7	0.5	polymer	74.55	19.46	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	2.8	1	polymer	74.55	19.46	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.2	0.005	mineral	6.5	0.00	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.5	0.05	mineral	6.5	0.00	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.7	0.25	mineral	6.5	0.00	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	1	0.5	mineral	6.5	0.00	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	1.2	1	mineral	6.5	0.00	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
HSA	1AO6	8.3	0.1	Silicon doped-Diamond like Carbon 3	86.4	26.90	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
HSA	1AO6	8.2	0.1	Silicon doped-Diamond like Carbon 2	82.7	24.54	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
HSA	1AO6	7.8	0.1	Diamond like Carbon	79	22.22	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
HSA	1AO6	7.8	0.1	Silicon doped-Diamond like Carbon 1	80.1	22.90	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
HSA	1AO6	7.7	0.1	Silicon doped-Diamond like Carbon 3	86.4	26.90	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015

HSA	1AO6	7.4	0.1	Silicon doped-Diamond like Carbon 2	82.7	24.54	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
HSA	1AO6	7.2	0.1	Diamond like Carbon	79	22.22	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
HSA	1AO6	7.2	0.1	Silicon doped-Diamond like Carbon 1	80.1	22.90	7.4	0.211	66.437	5.47	Ellipsometry	[9]	2015
Lysozyme	2LYZ	0.21	0.005	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.32	0.025	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.36	0.04	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.45	0.06	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.5	0.075	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.61	0.185	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.1	0.015	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.2	0.035	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.21	0.055	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.3	0.075	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.33	0.1	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.4	0.22	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.48	0.005	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.58	0.02	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.62	0.04	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.65	0.06	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Lysozyme	2LYZ	0.75	0.175	oxide	2.5	0.00	7	0.02	14.3	8.34	UV	[10]	2001
Ribonuclease A	1A5P	0.06	0.01666	oxide	2.5	0.00	7	0.02	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.0666	0.04	oxide	2.5	0.00	7	0.02	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.08	0.06	oxide	2.5	0.00	7	0.02	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.09	0.075	oxide	2.5	0.00	7	0.02	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.1	0.1	oxide	2.5	0.00	7	0.02	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.19	0.2	oxide	2.5	0.00	7	0.02	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.05	0.02	oxide	2.5	0.00	7	0.05	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.1	0.04	oxide	2.5	0.00	7	0.05	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.18	0.06	oxide	2.5	0.00	7	0.05	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.2	0.075	oxide	2.5	0.00	7	0.05	13.7	7.72	UV	[10]	2001

Ribonuclease A	1A5P	0.28	0.1	oxide	2.5	0.00	7	0.05	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.5	0.2	oxide	2.5	0.00	7	0.05	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.2	0.005	oxide	2.5	0.00	7	0.001	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.33	0.02	oxide	2.5	0.00	7	0.001	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.38	0.035	oxide	2.5	0.00	7	0.001	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.4	0.05	oxide	2.5	0.00	7	0.001	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.42	0.075	oxide	2.5	0.00	7	0.001	13.7	7.72	UV	[10]	2001
Ribonuclease A	1A5P	0.55	0.17	oxide	2.5	0.00	7	0.001	13.7	7.72	UV	[10]	2001
Fibrinogen	3GHG	5	0.34	modified silica	91.5	30.19	7.4	0.174	340	6.15	Ellipsometry	[11]	1994
Fibrinogen	3GHG	5	1	modified silica	91.5	30.19	7.4	0.174	340	6.15	Ellipsometry	[11]	1994
Fibrinogen	3GHG	0.11	0.005	polymer	9.8	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.17	0.05	polymer	9.8	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.13	0.25	polymer	9.8	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.16	0.5	polymer	9.8	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.21	1	polymer	9.8	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.057	0.005	polymer	9.8	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.083	0.05	polymer	9.8	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.07	0.25	polymer	9.8	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.057	0.5	polymer	9.8	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.067	1	polymer	9.8	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.05	0.005	polymer	9	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.08	0.05	polymer	9	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.06	0.25	polymer	9	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.7	0.5	polymer	9	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.1	1	polymer	9	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005

Lysozyme	2LYZ	0.023	0.005	polymer	9	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.03	0.05	polymer	9	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.02	0.25	polymer	9	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.028	0.5	polymer	9	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.028	1	polymer	9	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.02	0.005	polymer	9.2	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.02	0.05	polymer	9.2	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.04	0.25	polymer	9.2	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.05	0.5	polymer	9.2	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.07	1	polymer	9.2	0.02	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.025	0.005	polymer	9.2	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.04	0.05	polymer	9.2	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.02	0.25	polymer	9.2	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.02	0.5	polymer	9.2	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.025	1	polymer	9.2	0.02	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.49	0.005	polymer	14.35	0.10	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.82	0.05	polymer	14.35	0.10	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.63	0.25	polymer	14.35	0.10	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.8	0.5	polymer	14.35	0.10	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.92	1	polymer	14.35	0.10	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.125	0.005	polymer	14.35	0.10	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.94	0.05	polymer	14.35	0.10	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005

Lysozyme	2LYZ	0.135	0.25	polymer	14.35	0.10	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.123	0.5	polymer	14.35	0.10	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.1	1	polymer	14.35	0.10	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.03	0.005	polymer	10.7	0.03	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.11	0.05	polymer	10.7	0.03	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.08	0.25	polymer	10.7	0.03	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.07	0.5	polymer	10.7	0.03	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Fibrinogen	3GHG	0.01	1	polymer	10.7	0.03	7.4	0.15	340	6.15	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.015	0.005	polymer	10.7	0.03	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.02	0.05	polymer	10.7	0.03	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.02	0.25	polymer	10.7	0.03	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.02	0.5	polymer	10.7	0.03	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Lysozyme	2LYZ	0.028	1	polymer	10.7	0.03	7.4	0.15	14.3	8.34	Radiolabelling	[8]	2005
Alpha-Chymotrypsin	2CHA	0.2	0.02	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	0.3333	0.036	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	0.5	0.05	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	0.6	0.064	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	0.9	0.1	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	1.3	0.16	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	1.8	0.22	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	2	0.46	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	2.4	0.54	oxide	2.5	0.00	7.1	0.01	25	7.16	UV	[12]	1997
Cutinase	1XZA	0.036	0.018	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997

Cutinase	1XZA	0.1	0.035	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	0.13	0.045	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	0.14	0.056	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	0.3	0.075	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	0.39	0.09	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	0.6	0.125	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	1	0.2	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	1.4	0.36	oxide	2.5	0.00	7.1	0.01	22.367	5.7	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	1.7	0.025	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	2.2	0.05	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	2.7	0.08	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	3.8	0.13	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	4.3	0.25	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	4.5	0.4	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	4.8	0.62	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Alpha-Chymotrypsin	2CHA	5.2	0.83	polymer	116	45.63	7.1	0.01	25	7.16	UV	[12]	1997
Cutinase	1XZA	1.75	0.065	polymer	116	45.63	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	1.8	0.11	polymer	116	45.63	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	1.95	0.2	polymer	116	45.63	7.1	0.01	22.367	5.7	UV	[12]	1997
Cutinase	1XZA	2.3	0.385	polymer	116	45.63	7.1	0.01	22.367	5.7	UV	[12]	1997
BSA	3V03	0.4	0.1	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	0.6	0.25	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	0.71	0.5	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	0.8	0.9	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	0.85	1.4	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	0.9	1.75	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	0.92	2.5	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	1.05	3	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	1.1	4	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001
BSA	3V03	1.1	4.5	oxide	7	0.01	7	0.019	66.43	5.41	UV	[13]	2001

Lysozyme	2LYZ	0.07	0.11	SAM	9	0.02	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.13	0.2	SAM	9	0.02	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.22	0.35	SAM	9	0.02	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.28	0.5	SAM	9	0.02	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.39	0.75	SAM	9	0.02	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.48	1	SAM	9	0.02	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.63	0.11	SAM	108	40.75	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.93	0.2	SAM	108	40.75	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	1.36	0.35	SAM	108	40.75	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	1.65	0.5	SAM	108	40.75	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	1.99	0.75	SAM	108	40.75	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	2.1	1	SAM	108	40.75	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.17	0.11	Metal	44	4.81	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.325	0.2	Metal	44	4.81	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.54	0.35	Metal	44	4.81	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.67	0.5	Metal	44	4.81	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.89	0.75	Metal	44	4.81	7	0.169	14.3	8.34	QCM	[14]	2001
Lysozyme	2LYZ	0.996	1	Metal	44	4.81	7	0.169	14.3	8.34	QCM	[14]	2001
BSA	3V03	3	0.2	glass, inorganic polymer	0	0.00	7.4	0.128	66.43	5.41	UV	[15]	1987
BSA	3V03	4.4	0.45	glass, inorganic polymer	0	0.00	7.4	0.128	66.43	5.41	UV	[15]	1987
BSA	3V03	6.6	0.8	glass, inorganic polymer	0	0.00	7.4	0.128	66.43	5.41	UV	[15]	1987
BSA	3V03	7.1	1	glass, inorganic polymer	0	0.00	7.4	0.128	66.43	5.41	UV	[15]	1987
Fibrinogen	3GHG	2.5	0.005	polymer	112	43.21	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	4	0.05	polymer	112	43.21	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	5.5	0.25	polymer	112	43.21	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	5.9	0.5	polymer	112	43.21	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	6.2	1	polymer	112	43.21	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.1	0.005	polymer	71	17.39	7.4	0.278	340	6.15	Radiolabelling	[16]	2005

Fibrinogen	3GHG	0.2	0.05	polymer	71	17.39	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.4	0.25	polymer	71	17.39	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.5	0.5	polymer	71	17.39	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.6	1	polymer	71	17.39	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.1	0.005	polymer	76.5	20.67	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.2	0.05	polymer	76.5	20.67	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.4	0.25	polymer	76.5	20.67	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.5	0.5	polymer	76.5	20.67	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.6	1	polymer	76.5	20.67	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.1	0.005	polymer	55	9.07	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.2	0.05	polymer	55	9.07	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.4	0.25	polymer	55	9.07	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.5	0.5	polymer	55	9.07	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.6	1	polymer	55	9.07	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.2	0.005	polymer	76	20.37	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.3	0.05	polymer	76	20.37	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.4	0.25	polymer	76	20.37	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.5	0.5	polymer	76	20.37	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
Fibrinogen	3GHG	0.7	1	polymer	76	20.37	7.4	0.278	340	6.15	Radiolabelling	[16]	2005
BSA	3V03	0.46	0.13	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	0.76	0.25	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.05	0.4	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.25	0.57	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.31	0.79	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992

BSA	3V03	1.43	0.95	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.45	1.26	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.45	1.42	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.44	1.55	silica	2.5	0.00	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	0.27	0.07	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	0.53	0.16	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	0.75	0.32	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	0.88	0.45	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	0.95	0.6	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1	0.74	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.2	0.91	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1	1.05	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.3	1.2	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.3	1.37	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
BSA	3V03	1.2	1.53	hematite	21.5	0.45	7	0.19	66.43	5.41	UV	[17]	1992
Alpha-lactalbumin	1HML	0.27	0.06	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.38	0.17	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.57	0.28	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.65	0.42	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.83	0.66	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.88	0.79	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.94	1.12	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.9	1.27	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.95	1.44	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.03	0.08	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.02	0.17	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.03	0.27	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.06	0.4	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992

Alpha-lactalbumin	1HML	0.06	0.6	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.12	0.62	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.08	0.93	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.14	0.93	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.12	1.23	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.17	1.23	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.23	0.04	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.34	0.11	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.54	0.22	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.62	0.275	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.67	0.34	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.7	0.43	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.73	0.51	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.7	0.57	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.74	0.69	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.76	0.81	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.75	0.93	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.74	1.05	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.75	1.16	hematite	21.5	0.45	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.16	0.11	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.18	0.26	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.23	0.41	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.33	0.54	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992

Alpha-lactalbumin	1HML	0.47	0.65	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.51	0.88	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.56	1.17	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Alpha-lactalbumin	1HML	0.63	1.48	silica	2.5	0.00	7	0.19	14.188	4.71	UV	[17]	1992
Lysozyme	2LYZ	0.45	0.02	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	0.81	0.025	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.06	0.15	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.04	0.28	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.14	0.46	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.18	0.64	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.17	0.9	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.25	1.28	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.25	1.5	hematite	21.5	0.45	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	0.47	0.02	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	0.78	0.02	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.27	0.02	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.41	0.065	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.63	0.19	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.69	0.51	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.68	0.75	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.75	1.08	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.75	1.32	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
Lysozyme	2LYZ	1.77	1.48	silica	7	0.01	7	0.19	14.3	8.34	UV	[17]	1992
HSA	1AO6	1.2	0.01	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.37	0.02	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.5	0.03	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.82	0.05	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.67	0.06	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.9	0.08	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983

HSA	1AO6	1.78	0.1	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	2	0.14	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.9	0.22	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	2.15	0.26	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.93	0.3	Glass	110	41.99	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	0.45	0.01	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	0.75	0.02	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	0.82	0.05	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	0.9	0.08	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.28	0.1	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.3	0.14	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.45	0.196	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.28	0.24	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.4	0.26	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
HSA	1AO6	1.53	0.3	Glass	0	0.00	7.4	0.05	66.437	5.47	Radiolabelling	[18]	1983
Fibrinogen	3GHG	0	2	Polymer	31	1.60	7.4	0.174	340	6.15	SPR	[19]	2005
Fibrinogen	3GHG	0	2	Polymer	38	3.08	7.4	0.174	340	6.15	SPR	[19]	2005
BSA	3V03	0	2	Polymer	31	1.60	7.4	0.174	66.43	5.41	SPR	[19]	2005
BSA	3V03	0.06	2	Polymer	38	3.08	7.4	0.174	66.43	5.41	SPR	[19]	2005
HSA	1AO6	1.92	0.0097	Polyhydroxymethylsiloxane_Hydrophobic	90	29.22	7	0	66.437	5.47	QCM	[20]	2009
Lysozyme	2LYZ	0.67	0.005	Polyhydroxymethylsiloxane_Hydrophobic	90	29.22	7	0	14.3	8.34	QCM	[20]	2009
Lactoferrin	1B0L	3.78	0.0047	Polyhydroxymethylsiloxane_Hydrophobic	90	29.22	7	0	82.4	7.19	QCM	[20]	2009
HSA	1AO6	2.46	0.1	Polyhydroxymethyl	90	29.22	7	0	66.437	5.47	QCM	[20]	2009

				siloxane_Hydrophobic									
Lysozyme	2LYZ	7.6	0.04	Polyhydroxymethyl siloxane_Hydrophobic	90	29.22	7	0	14.3	8.34	QCM	[20]	2009
Lactoferrin	1B0L	4.4	0.0108	Polyhydroxymethyl siloxane_Hydrophobic	90	29.22	7	0	82.4	7.19	QCM	[20]	2009
HSA	1AO6	0.8	0.0097	Polyhydroxymethyl siloxane_Plasma	9.9	0.02	7	0	66.437	5.47	QCM	[20]	2009
Lysozyme	2LYZ	1.87	0.005	Polyhydroxymethyl siloxane_Plasma	9.9	0.02	7	0	14.3	8.34	QCM	[20]	2009
Lactoferrin	1B0L	4.43	0.0047	Polyhydroxymethyl siloxane_Plasma	9.9	0.02	7	0	82.4	7.19	QCM	[20]	2009
HSA	1AO6	2.23	0.1	Polyhydroxymethyl siloxane_Plasma	9.9	0.02	7	0	66.437	5.47	QCM	[20]	2009
Lysozyme	2LYZ	2.25	0.04	Polyhydroxymethyl siloxane_Plasma	9.9	0.02	7	0	14.3	8.34	QCM	[20]	2009
Lactoferrin	1B0L	7.73	0.0108	Polyhydroxymethyl siloxane_Plasma	9.9	0.02	7	0	82.4	7.19	QCM	[20]	2009
Cry1Ac	4W8J	4	0.002	Silica	36	2.59	5	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	5	0.004	Silica	36	2.59	5	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	7.43	0.01	Silica	36	2.59	5	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	8	0.02	Silica	36	2.59	5	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	5.95	0.01	Silica	36	2.59	5	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	3.88	0.01	Silica	36	2.59	6	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	3.4	0.01	Silica	36	2.59	6	0.05	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	1.1	0.01	Silica	36	2.59	7	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	0.19	0.01	Silica	36	2.59	7	0.05	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	0.33	0.01	Silica	36	2.59	8	0.01	134.138	5.01	QCM	[21]	2017
Cry1Ac	4W8J	0	0.01	Silica	36	2.59	8	0.05	134.138	5.01	QCM	[21]	2017
HSA	1AO6	3.5	1	Hexadecane thiolated gold surface	163	69.75	7.4	0.154	66.437	5.47	QCM	[22]	2017
BSA	3V03	0.025	0.063	Silicon with thermal oxide layer	36	2.59	7	0	66.43	5.41	Spectral Reflectance Imaging Biosensor [23]		2009
BSA	3V03	0.05	0.125	Silicon with thermal oxide layer	36	2.59	7	0	66.43	5.41	Spectral Reflectance Imaging		2009

											Biosensor [23]	
BSA	3V03	0.1	0.25	Silicon with thermal oxide layer	36	2.59	7	0	66.43	5.41	Spectral Reflectance Imaging Biosensor [23]	2009
BSA	3V03	0.2	0.5	Silicon with thermal oxide layer	36	2.59	7	0	66.43	5.41	Spectral Reflectance Imaging Biosensor [23]	2009
BSA	3V03	0.4	1	Silicon with thermal oxide layer	36	2.59	7	0	66.43	5.41	Spectral Reflectance Imaging Biosensor [23]	2009
IgG	1IGT	0.02	0.063	Silicon with thermal oxide layer	36	2.59	7	0	150	6.57	Spectral Reflectance Imaging Biosensor [23]	2009
IgG	1IGT	0.05	0.125	Silicon with thermal oxide layer	36	2.59	7	0	150	6.57	Spectral Reflectance Imaging Biosensor [23]	2009
IgG	1IGT	0.1	0.25	Silicon with thermal oxide layer	36	2.59	7	0	150	6.57	Spectral Reflectance Imaging Biosensor [23]	2009
IgG	1IGT	0.2	0.5	Silicon with thermal oxide layer	36	2.59	7	0	150	6.57	Spectral Reflectance Imaging Biosensor [23]	2009
BSA	3V03	7.4	10	Silica	36	2.59	5.6	0	66.43	5.41	QCM [24]	2014
BSA	3V03	7.84	10	Silica	36	2.59	5.8	0.154	66.43	5.41	QCM [24]	2014
BSA	3V03	7.65	10	Silica	36	2.59	7.4	0.154	66.43	5.41	QCM [24]	2014
BSA	3V03	8	10	Silica	36	2.59	7.4	0.154	66.43	5.41	QCM [24]	2014
BSA	3V03	7.15	10	Silica	36	2.59	7.4	0.154	66.43	5.41	QCM [24]	2014
BSA	3V03	6.48	10	Silica	36	2.59	7.4	0.154	66.43	5.41	QCM [24]	2014
BSA	3V03	7.07	10	Silica	36	2.59	8.1	0.005	66.43	5.41	QCM [24]	2014
BSA	3V03	6.07	10	Silica	36	2.59	8.1	0.154	66.43	5.41	QCM [24]	2014
Fibrinogen	3GHG	4.9	0.225	modified silica	91.5	30.19	7.4	0.174	340	6.15	Ellipsometry [11]	1994
Fibrinogen	3GHG	4.8	0.125	modified silica	91.5	30.19	7.4	0.174	340	6.15	Ellipsometry [11]	1994
BSA	3V03	1.75	1	MUOH	22.37	0.51	7	0	66.43	5.41	QCM [25]	2015

BSA	3V03	4.1	1	MUA	24.6	0.73	7	0	66.43	5.41	QCM	[25]	2015
BSA	3V03	2.5	1	DT10	96.8	33.63	7	0	66.43	5.41	QCM	[25]	2015
BSA	3V03	3.6	1	AUT	47.83	6.13	7	0	66.43	5.41	QCM	[25]	2015
BSA	3V03	3.6	1	AUT	47.83	6.13	7	0	66.43	5.41	QCM	[25]	2015
BSA	3V03	4.8	0.001	AUT	47.83	6.13	7	0	66.43	5.41	QCM	[25]	2015
BSA	3V03	2	0.0005	AUT	47.83	6.13	7	0	66.43	5.41	QCM	[25]	2015
BSA	3V03	0.5	0.0001	AUT	47.83	6.13	7	0	66.43	5.41	QCM	[25]	2015
Cytochrome c	1HRC	1.41	0.124	silicon oxide philic	6	0.00	7.4	0.154	12	8.79	X-ray reflectometry [26]		2013
Lysozyme	2LYZ	3.1	0.15	silicon oxide philic	6	0.00	7.4	0.154	14.3	8.34	X-ray reflectometry [26]		2013
Myoglobin	1MBO	1.43	0.17	silicon oxide philic	6	0.00	7.4	0.154	17	8.11	X-ray reflectometry [26]		2013
Lysozyme	2LYZ	1.36	0.15	Silicon wafer treated with OTS phobic	109.5	41.68	7.4	0.154	14.3	8.34	X-ray reflectometry [26]		2013
Myoglobin	1MBO	1.5	0.17	Silicon wafer treated with OTS phobic	109.5	41.68	7.4	0.154	17	8.11	X-ray reflectometry [26]		2013
Hemoglobin	1BUW	1.19	0.645	Silicon wafer treated with OTS phobic	109.5	41.68	7.4	0.154	64.5	7.29	X-ray reflectometry [26]		2013
BSA	3V03	1.2	0.66	Silicon wafer treated with OTS phobic	109.5	41.68	7.4	0.154	66.43	5.41	X-ray reflectometry [26]		2013
IGG	1IGT	0.76	1.5	Silicon wafer treated with OTS phobic	109.5	41.68	7.4	0.154	150	6.57	X-ray reflectometry [26]		2013
BSA	3V03	38.9	1	Polycarbonate	66.99	15.06	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	184.9	1	polyoxymethylene	75.32	19.94	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	285.2	1	polyethersulfone	79.69	22.59	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	116.1	1	polyvinylidene fluoride	85.14	26.07	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	116.2	1	polybutylene terephthalate	57.67	10.27	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	73.6	1	polysulfone	85.73	26.45	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	110.6	1	polyetherimide	84.17	25.43	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	127.2	1	polyphenylene oxide	65.9	14.51	7.4	0.154	66.43	5.41	UV	[27]	2010
BSA	3V03	10	0.1	Germanium	40	3.61	7.4	0.154	66.43	5.41	ATR/FTIR	[28]	2009

IgG	1IGT	25	0.1	Germanium	40	3.61	7.4	0.154	150	6.57	ATR/FTIR	[28]	2009
Fibrinogen	3GHG	20	0.1	Germanium	40	3.61	7.4	0.154	340	6.15	ATR/FTIR	[28]	2009
Lysozyme	2LYZ	12.5	0.1	Germanium	40	3.61	7.4	0.154	14.3	8.34	ATR/FTIR	[28]	2009
BSA	3V03	17.5	0.1	Germanium	40	3.61	7.4	0.15	66.43	5.41	ATR/FTIR	[28]	2009
IgG	1IGT	55	0.1	Germanium	40	3.61	7.4	0.15	150	6.57	ATR/FTIR	[28]	2009
Fibrinogen	3GHG	80	0.1	Germanium	40	3.61	7.4	0.15	340	6.15	ATR/FTIR	[28]	2009
Lysozyme	2LYZ	12.5	0.1	Germanium	40	3.61	7.4	0.15	14.3	8.34	ATR/FTIR	[28]	2009
Glucose Oxidase	1CF3	0.2	0.01	Glass	0	0.00	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	0.5	0.01	DETA modiefied glass	49	6.58	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	0.85	0.01	13F modified glass	94	31.81	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	0.2	0.01	SiPEG modified glass	37	2.83	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	0.8	0.1	Glass	0	0.00	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	1.4	0.1	DETA modiefied glass	49	6.58	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	1.05	0.1	13F modified glass	94	31.81	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Glucose Oxidase	1CF3	0.2	0.1	SiPEG modified glass	37	2.83	7.4	0.154	160	4.8	Whispering gallery mode (WGM)	[29]	2015
Fibrinogen	3GHG	4.3	0.025	modified silica	91.5	30.19	7.4	0.174	340	6.15	Ellipsometry	[11]	1994
HSA	1AO6	0.099	1	poly(N-isopropylacrylamide)	58.2	10.56	7.4	0.154	66.437	5.47	Radiolabelling	[30]	2010
Fibrinogen	3GHG	0.51	1	poly(N-isopropylacrylamide)	58.2	10.56	7.4	0.154	340	6.15	Radiolabelling	[30]	2010
Lysozyme	2LYZ	0.96	1	poly(N-isopropylacrylamide)	58.2	10.56	7.4	0.154	14.3	8.34	Radiolabelling	[30]	2010
IgG	1IGT	1.5	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012

IgG	1IGT	6.45	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	2.25	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	0.75	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	6.75	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	4.5	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	3	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	2.25	0.004	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	5.25	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	12.8	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	4.5	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	2.25	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	9.75	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	6.75	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	5.25	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	0.75	0.01	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	9	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	11.6	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	8.25	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	4.12	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	10.2	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	8.25	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	7.8	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	1.95	0.02	Polystyrene	87.4	27.55	7.4	0.154	150	6.57	QCM	[31]	2012
IgG	1IGT	3.75	0.05	Silicon	77	20.98	7	0.02	150	6.57	Ellipsometry	[32]	2011
IgG	1IGT	3.7	0.02	modified silica	88	27.93	7	0.15	150	6.55	Ellipsometry	[33]	1998
Fibrinogen	3GHG	3.65	0.02	modified silica	88	27.93	7	0.15	340	6.15	Ellipsometry	[33]	1998
Fibrinogen	3GHG	3.6	0.02	modified silica	30.5	1.51	7	0.15	340	6.15	Ellipsometry	[33]	1998
IgG	1IGT	0.81	0.005	Silicon	77	20.98	7	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	1.8	0.01	Silicon	77	20.98	7	0.02	150	6.57	Neutron reflectivity [32]		2011

IgG	1IGT	2.8	0.02	Silicon	77	20.98	7	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	3.5	0.05	Silicon	77	20.98	7	0.02	150	6.57	Neutron reflectivity [32]		2011
Fibrinogen	3GHG	3.5	0.02	oxide	6.5	0.00	7	0.15	340	6.15	Ellipsometry	[33]	1998
IgG	1IGT	3.5	0.02	modified silica	30.5	1.51	7	0.15	150	6.55	Ellipsometry	[33]	1998
IgG	1IGT	3.4	0.02	modified silica	71.5	17.68	7	0.15	150	6.55	Ellipsometry	[33]	1998
IgG	1IGT	1.91	0.01	Silicon	77	20.98	3.9	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	2.6	0.01	Silicon	77	20.98	4.8	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	2.9	0.01	Silicon	77	20.98	5.3	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	2.8	0.01	Silicon	77	20.98	6.1	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	1.81	0.01	Silicon	77	20.98	7	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	1.45	0.01	Silicon	77	20.98	7.9	0.02	150	6.57	Neutron reflectivity [32]		2011
IgG	1IGT	3.2	0.425	modified silica	91.5	30.19	7.4	0.174	150	6.55	Ellipsometry	[11]	1994
IgG	1IGT	3.2	1	modified silica	91.5	30.19	7.4	0.174	150	6.55	Ellipsometry	[11]	1994
BSA	3V03	3.2	1	AUT	47.83	6.13	7	0	66.43	5.41	Ellipsometry	[25]	2015
BSA	3V03	50	4	316L Stainless steel	54	8.63	7.3	0.167	66.43	5.41	Radiolabelling	[34]	2006
BSA	3V03	29	4	CoCrMo alloy	61	11.94	7.3	0.167	66.43	5.41	Radiolabelling	[34]	2006
BSA	3V03	2.5	4	Alumina	40	3.61	7.3	0.167	66.43	5.41	Radiolabelling	[34]	2006
BSA	3V03	3.08	0.05	ultra high molecular weight polyethylene	85	26.01	7.3	0.167	66.43	5.41	QCM	[35]	2010
BSA	3V03	3.6	0.6	ultra high molecular weight	85	26.01	7.3	0.167	66.43	5.41	QCM	[35]	2010

				polyethylene									
BSA	3V03	5.86	4	ultra high molecular weight polyethylene	85	26.01	7.3	0.167	66.43	5.41	QCM	[35]	2010
BSA	3V03	6.29	10	ultra high molecular weight polyethylene	85	26.01	7.3	0.167	66.43	5.41	QCM	[35]	2010
BSA	3V03	7.12	15	ultra high molecular weight polyethylene	85	26.01	7.3	0.167	66.43	5.41	QCM	[35]	2010
BSA	3V03	7.19	20	ultra high molecular weight polyethylene	85	26.01	7.3	0.167	66.43	5.41	QCM	[35]	2010
BSA	3V03	2.5	0.05	Titanium Nitride	57.5	10.22	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	4.2	0.6	Titanium Nitride	57.5	10.22	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	5.3	4	Titanium Nitride	57.5	10.22	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	6.2	10	Titanium Nitride	57.5	10.22	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	6.7	15	Titanium Nitride	57.5	10.22	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	2.2	0.05	Titanium nniobium nitride	72.5	18.27	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	4.2	0.6	Titanium nniobium nitride	72.5	18.27	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	5	4	Titanium nniobium nitride	72.5	18.27	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	5.9	10	Titanium nniobium nitride	72.5	18.27	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	6.1	15	Titanium nniobium nitride	72.5	18.27	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	2.1	0.05	Titanium Carbonitride	70	16.81	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	4	0.6	Titanium Carbonitride	70	16.81	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	4.5	4	Titanium Carbonitride	70	16.81	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	5.5	10	Titanium Carbonitride	70	16.81	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	6.1	15	Titanium Carbonitride	70	16.81	7.3	0.167	66.43	5.41	QCM	[36]	2009
BSA	3V03	1.15	1	SAM on Gold(HS-C11CH3)	104.6	38.62	7.4	0.154	66.43	5.41	SPR	[37]	2006
BSA	3V03	0.646	1	SAM on Gold (HS-OH)	29.9	1.42	7.4	0.154	66.43	5.41	SPR	[37]	2006

BSA	3V03	1.92	1	SAM on Gold(HS-COOH)	20.8	0.40	7.4	0.154	66.43	5.41	SPR	[37]	2006
BSA	3V03	1.31	1	SAM on gold(HS-NH2)	64.4	13.70	7.4	0.154	66.43	5.41	SPR	[37]	2006
BSA	3V03	0.382	1	SAM on gold(HS-NHCO-PEG)	38.4	3.18	7.4	0.154	66.43	5.41	SPR	[37]	2006
BSA	3V03	1.57	1	SAM on gold(HS-C11CH3)	38.4	3.18	7.4	0.154	66.43	5.41	BCA	[37]	2006
BSA	3V03	0.43	1	SAM on gold(HS-OH)	38.4	3.18	7.4	0.154	66.43	5.41	BCA	[37]	2006
BSA	3V03	0.63	1	SAM on gold(HS-COOH)	38.4	3.18	7.4	0.154	66.43	5.41	BCA	[37]	2006
BSA	3V03	1.37	1	SAM on gold(HS-NH2)	38.4	3.18	7.4	0.154	66.43	5.41	BCA	[37]	2006
BSA	3V03	0.078	1	SAM on gold(HS-NHCO-PEG)	38.4	3.18	7.4	0.154	66.43	5.41	BCA	[37]	2006
IgG	1IGT	3.2	0.02	Silicon	77	20.98	7	0.02	150	6.57	Ellipsometry	[38]	2011
IgG	1IGT	3.1	0.25	modified silica	91.5	30.19	7.4	0.174	150	6.55	Ellipsometry	[11]	1994
IgG	1IGT	3	0.1	modified silica	91.5	30.19	7.4	0.174	150	6.55	Ellipsometry	[11]	1994
IgG	1IGT	2.8	0.01	Silicon	77	20.98	5	0.02	150	6.57	Ellipsometry	[38]	2011
IgG	1IGT	2.8	0.01	Silicon	77	20.98	7	0.005	150	6.57	Ellipsometry	[38]	2011
IgG	1IGT	2.7	0.01	Silicon	77	20.98	6	0.02	150	6.57	Ellipsometry	[38]	2011
BSA	3V03	2.5	1	MUA	24.6	0.73	7	0	66.43	5.41	Ellipsometry	[25]	2015
IgG	1IGT	2.3	0.05	modified silica	91.5	30.19	7.4	0.174	150	6.55	Ellipsometry	[11]	1994
HSA	1AO6	2.1	0.02	modified silica	88	27.93	7	0.15	66.437	5.47	Ellipsometry	[33]	1998
HSA	1AO6	2	0.02	modified silica	30.5	1.51	7	0.15	66.437	5.47	Ellipsometry	[33]	1998
Insulin	4INS	2	1	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	1.9	0.1	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
IgG	1IGT	1.9	0.01	Silicon	77	20.98	7	0.02	150	6.57	Ellipsometry	[32]	2011
IgG	1IGT	1.9	0.01	Silicon	77	20.98	7	0.02	150	6.57	Ellipsometry	[32]	2011

HSA	1AO6	1.8	0.02	modified silica	71.5	17.68	7	0.15	66.437	5.47	Ellipsometry	[33]	1998
HSA	1AO6	1.75	0.02	oxide	6.5	0.00	7	0.15	66.437	5.47	Ellipsometry	[33]	1998
Insulin	4INS	1.75	1	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
IgG	1IGT	1.75	0.01	Silicon	77	20.98	4	0.02	150	6.57	Ellipsometry	[32]	2011
Insulin	4INS	1.7	0.1	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	1.7	0.01	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	1.65	1	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	1.55	0.001	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	1.5	0.1	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	1.5	0.01	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
IgG	1IGT	1.5	0.01	Silicon	77	20.98	7	0.05	150	6.57	Ellipsometry	[38]	2011
Insulin	4INS	1.4	0.01	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
IgG	1IGT	1.4	0.01	Silicon	77	20.98	8	0.02	150	6.57	Ellipsometry	[38]	2011
Insulin	4INS	1.1	0.001	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
IgG	1IGT	1.1	0.01	Silicon	77	20.98	7	0.1	150	6.57	Ellipsometry	[38]	2011
Insulin	4INS	1.05	0.001	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
BSA	3V03	1	1	DT10	96.8	33.63	7	0	66.43	5.41	Ellipsometry	[25]	2015
IgG	1IGT	1	0.005	Silicon	77	20.98	7	0.02	150	6.57	Ellipsometry	[32]	2011
HSA	1AO6	0.9	0.75	modified silica	91.5	30.19	7.4	0.174	66.437	5.47	Ellipsometry	[11]	1994
HSA	1AO6	0.9	1	modified silica	91.5	30.19	7.4	0.174	66.437	5.47	Ellipsometry	[11]	1994
HSA	1AO6	0.8	0.35	modified silica	91.5	30.19	7.4	0.174	66.437	5.47	Ellipsometry	[11]	1994
HSA	1AO6	0.7	0.14	modified silica	91.5	30.19	7.4	0.174	66.437	5.47	Ellipsometry	[11]	1994

Insulin	4INS	0.7	0.0004	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
IgG	1IGT	0.625	0.002	Silicon wafers	77	20.98	7	0.02	150	6.57	Ellipsometry	[32]	2011
HSA	1AO6	0.5	0.04	modified silica	91.5	30.19	7.4	0.174	66.437	5.47	Ellipsometry	[11]	1994
BSA	3V03	0.5	1	MUOH	22.37	0.51	7	0	66.43	5.41	Ellipsometry	[25]	2015
HSA	1AO6	0.5	1	Poly(2-vinylpyridine)-poly-N-isopropylacrylamide	82.5	24.41	4	0.154	66.437	5.47	Ellipsometry	[40]	2012
Insulin	4INS	0.31	0.0001	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
Insulin	4INS	0.3	0.0001	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
HSA	1AO6	0.3	1	Poly(2-vinylpyridine)-poly-N-isopropylacrylamide	82.5	24.41	7.4	0.154	66.437	5.47	Ellipsometry	[40]	2012
IgG	1IGT	0.3	0.01	Silicon	77	20.98	7	0.15	150	6.57	Ellipsometry	[38]	2011
Insulin	4INS	0.25	0.0001	modified silica	87.5	27.61	7.4	0.024	5.808	5.2	Ellipsometry	[39]	2005
HSA	1AO6	0.2	1	Poly(2-vinylpyridine)-poly-N-isopropylacrylamide	77.5	21.29	4	0.154	66.437	5.47	Ellipsometry	[40]	2012
HSA	1AO6	0	1	Poly(2-vinylpyridine)-poly-N-isopropylacrylamide	77.5	21.29	7.4	0.154	66.437	5.47	Ellipsometry	[40]	2012
IgG	1IGT	0.2	0.01	Poly(styrene)	94	31.81	9.6	0.211	150	6.12	QCM	[41]	2015
IgG	1IGT	0.64	0.03	Poly(styrene)	94	31.81	9.6	0.211	150	6.12	QCM	[41]	2015
IgG	1IGT	0.94	0.05	Poly(styrene)	94	31.81	9.6	0.211	150	6.12	QCM	[41]	2015
IgG	1IGT	1.15	0.07	Poly(styrene)	94	31.81	9.6	0.211	150	6.12	QCM	[41]	2015
IgG	1IGT	1.2	0.09	Poly(styrene)	94	31.81	9.6	0.211	150	6.12	QCM	[41]	2015
IgG	1IGT	1.25	0.11	Poly(styrene)	94	31.81	9.6	0.211	150	6.12	QCM	[41]	2015

Appendix II: References for the database

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Appendix III: Protein surface properties calculator (PSPC).

PSPC was used to calculate hydrophobicity and hydrophilicity of proteins based on their PDB IDs. The result of PSPC for lysozyme (PDB ID: 2LYZ) with a probe radius of 20 A is shown below. The calculation was done on an amino acid level and the hydrophobicity scale was Dgwif.

993	Number of atoms	
45119	Connolly surface points (10 points/A^2)	
4450.817	96.87878	Connolly surface area (A^2)
1631.369	Area with positive charge (A^2)	
13.61962	Total positive charge	
8.3485842E-03	Average positive charge	
2818.500	Area with negative charge (A^2)	
-30.90466	Total negative charge	
-1.0964934E-02	Average negative charge	

-17.28504	Total surface charge
-3.8835653E-03	Average surface charge
3593.383	Hydrophilic area (A^2)
6.5258895E-03	Hydrophilicity index
6.4322411E-04	Hydrophilicity patch
856.8068	Hydrophobic area (A^2)
-1.0339834E-02	Hydrophobicity index
-1.0227707E-03	Hydrophobicity patch
-8.859240	Total Hydrophobicity
23.45002	Total Hydrophilicity
-79.45885	Total Protein Charge
39.74700	Xmax - Xmin (A)
35.20800	Ymax - Ymin (A)
45.88500	Zmax - Zmin (A)

INPUT PARAMETERS:

PDB file name

Z:\home\giulia\Documents\Molecular Surfaces\BAD regression\2LYZ.en

Hydrophobicity per aminoacid

ASA computed for probe contact

20.00000 ProbeRadius

7.4 pH

Dgwif Hydrophobicity scale