Structure formation at solid/liquid interfaces

Understanding self-assembly and environmental challenges

SHIRIN NOUHI
Abstract


The work described in the present dissertation has explored the structure of particles and molecules at solid/liquid interfaces, aiming to understand the physics of self-organizing systems and use this knowledge to address some environmental issues. Surface-sensitive neutron scattering techniques, such as reflectometry and grazing incidence small angle scattering, have been used as the primary tool to investigate structures in proximity to an interface. Some of the challenges in the interpretation of neutron scattering data are discussed, and new methods for analyzing the signal have been proposed.

It was shown that charged stabilized colloidal particles can self-assemble and form large areas (20 cm$^2$) of crystalline structures, close to a smooth solid surface extending to depths of several micrometers, while orienting themselves into smaller crystallites in the bulk of the suspension. The adsorption of proteins from the seeds of different species of Moringa trees on alumina, silica and polystyrene surfaces was studied, as a means for using proteins from different sources and with different properties, for the water clarification step in the purification process. The seed proteins also showed to enable locking the structure of colloidal particles at the solid/liquid interface, acting as a molecular glue.

Perfluorinated surfactants (PFASs), widely used in industrial, pharmaceutical and food packing products, have been identified as emerging pollutants, raising a global concern for the environment and wildlife. The present study has shown how PFASs molecules of different fluorocarbon chain length and with different functional groups, create defects in model membranes by partitioning and removing phospholipids from the bilayer, making the bilayer thin and less dense.

The effect of interface roughness was studied on the lamellar structure of a non-ionic surfactant. Concentrated solutions of the surfactant have been shown to form well-ordered and well-aligned structures at a smooth interface, which could be modified further by simply heating the sample. However it was found that even small roughness, of the same order as the bilayer thickness, can distort the structure to a depth of several micrometers from the interface. Heating the sample could improve the alignment but not as much as that formed at a smooth surface.

Keywords: Solid/liquid interface, neutron scattering, colloidal particles, self-assembly, perfluoroalkyl substance, Moringa seed proteins, adsorption, lamellar disorder, thermal fluctuations.

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To my wonderful father
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Comparative study of flocculation and adsorption behavior of *Moringa peregrina* and *Moringa oleifera* seed proteins
Shirin Nouhi, Habauka M. Kwaambwa, Philipp Gutfreund and Adrian R. Rennie, *Submitted to the Journal of Environmental Engineering Science*

II Grazing incidence small angle neutron scattering from structures below an Interface

III Sticking particles to solid surfaces using *Moringa oleifera* proteins as a glue

IV Interactions of perfluoroalkyl substances with a phospholipid bilayer studied by neutron reflectometry

V Distortion of surfactant lamellar phases induced by surface roughness
Shirin Nouhi, Alexandros Koutsoubas, Vassilios Kapaklis and Adrian Rennie, *Submitted to Physical Review E*

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Comments on my participation

I. Planning and performing experiments, sample preparation, data analysis and responsible for writing the majority of the manuscript.

II. Planning and performing experiments, sample preparation (apart from synthesizing the particles*), data analysis and responsible for writing the majority of the manuscript.

III. Planning and performing experiments (apart from QCM-D measurements† and some of the AFM measurements‡), sample preparation, data analysis and responsible for writing the majority of the manuscript.

IV. Planning and performing experiments, sample preparation, data analysis and responsible for writing the majority of the manuscript.

V. Planning and performing experiments, sample preparation, data analysis and responsible for writing the majority of the manuscript.

Parts of this dissertation have been presented in my licentiate, some figures and equations have been reproduced.

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1. Soft matter and interfaces

Some of the materials we use on a daily basis are difficult to classify into the conventional categories of solid, liquid and gas, for example, toothpaste, cosmetics, yogurt, paints, lubricants, detergents, etc. This class of materials is called soft matter. Soft matter includes a large variety of materials, but all of them show some common features/behavior. Soft matter deforms easily under stress, thermal fluctuations and magnetic or electric forces, and shows a large, slow and non-linear response compared to a liquid or solid. Commonly the response time of fluid is in the order of nanoseconds whereas for a colloidal or polymer solution this can be a billion times slower [1, 2].

Soft matter consists of components which are larger than an atom but smaller than micrometers, such as polymers, colloids, surfactants, and liquid crystals, etc. These components are normally dispersed in another medium (solid, liquid or gas) and can interact with each other or their medium to form different structures with unique properties and a wide variety of applications. However, when these materials meet an interface, the symmetry of the forces between their components breaks and they can show completely different behavior and form a different structure. For the application of many of these systems, their interactions with interfaces is of great importance. A common goal in industry and materials science is to tune the interaction of these components to obtain the desired structures with required properties. To achieve this goal a fundamental understanding of the interactions and the interface effects is necessary. For example, to improve the quality of paints, the interaction and adsorption of dispersed particles in the paint with the solid wall is essential and can be studied for further improvements [3].

The present work describes the physics of the interactions between the components of soft matter at solid/liquid interfaces. Particular colloidal, amphiphiles and protein dispersion have been chosen in order to understand the basic physics of their interactions, explore the structure, and to address some relevant environmental issues. In all systems, the components were dispersed in water, and the structure has been studied in equilibrium. Solid/liquid interfaces are known as buried interfaces, studying these structures at molecular level, requires tools that are specifically sensitive to the interface and allow measurements at nanometer and ångström length scales. The primary technique used in this dissertation is neutron scattering. Neutrons have low absorption compared to X-rays and can penetrate deep into some substrates that allows experiments to probe samples on the side of the substrate immersed in liquid. This offers a great opportunity to explore the nanostructures at solid/liquid, so-called buried interfaces.
1.1 Colloidal particles

Colloids include phases of insoluble particles, gas or liquid droplets dispersed in a different material (medium). Depending on the phase of the medium or dispersed phase, different terms are used for the colloidal systems [1].

- if the medium is gas: "aerosol"
- if the medium is liquid: "sol" or "dispersion" (for a solid dispersed phase), "foam" (for a gas dispersed phase) or "emulsion" (for a liquid dispersed phase)
- if the medium is solid: "solid dispersion" (for a solid dispersed phase), "solid emulsion" (for a liquid dispersed phase) or "solid foam" (for a gas dispersed phase)

The current work has been studying sol/dispersion type of colloids; hence the term colloidal systems will be used accordingly from this point on.

The molecules (or micromolecules) of the dispersed phase can have different morphologies, they can be spherical, rod-like or plate-like and is generally between 1 ångström up to 1 micrometer; too small to sediment or float due to gravitational forces, but small enough to be affected by temperature and exhibit Brownian motion [4].

Colloidal particles dispersed in a medium undergo constant Brownian motion and since they are also attracted to each other by van der Waals forces, they may approach and collide which results in their aggregation and sedimentation or creaming. Such a system is no longer stabilized. The common mechanisms of stabilizing a colloidal dispersion are: steric stabilization [5], charge stabilization [6] or a combination of both [7].

In steric stabilization, particles are coated with flexible polymers which usually are self-avoiding. The forces between polymers overcome the van der Waals (attractive) forces between particles and do not allow particles to approach each other. The strength of repulsion between particles in steric stabilization is adjusted by the length of chains, grafting density or the solubility of the polymers in the solvent, which can be altered by, for example, temperature.

In the charge stabilization process, particles have similar surface charge and the stabilization is achieved through the balance between electrostatic repulsion (Coulomb force) and van der Waals forces. The van der Waals force between particles separated by a distance $r$, scales as $r^{-6}$ for intermediate and with $r^{-7}$ for large distances, due to retardation effects.

For a charged particle dispersed in an aqueous solution, electrostatic potential decreases exponentially as $\exp(-\kappa d)$ away from the surface of particles, where $d$ is the distance from the surface of the particle, and the decay length, $\kappa^{-1}$, can be calculated as:

$$\kappa^{-1} = \left(\frac{k_BT\epsilon_0\epsilon}{2q^2NAI}\right)^{1/2}$$  \hspace{1cm} (1.1)
where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, $\varepsilon_0$ and $\varepsilon$ are the permittivity of vacuum and the dielectric constant of dispersion respectively, $q$ is the elementary charge, $N_A$ is the Avogadro’s number, and $I$ is the ionic strength of the dispersion.

When charged colloidal particles are dispersed in a liquid, an electrical double layer forms on their surface. The electrical double layer, as shown in Figure 1.1, consists of two parts: Stern layer, oppositely charged counter ions which chemically adsorb to the surface of the particles, and the diffuse layer which consists of free counter ions surrounding the particles to screen the first layer. The outer edge of the electrical double layer is called the slipping plane.

In a charge-stabilized system, when the electrical double layer of particles overlap they repel each other and prevent the aggregation.

The interaction between charged particles was described by Derjaguin-Landau-Verwey-Overbeek (DLVO) over 70 years ago [8, 9]. where $V_a$ is the attractive energy potential (arising from long-range van der Waals or Hamaker force) and $V_r$ is the repulsive potential (originated from similar charge repulsion and short-range force arising from Born repulsion).

According to this theory, the potential energy of the interaction between particles is defined as:

$$ V = V_a + V_r \quad (1.2) $$

The resulting force, as shown in Figure 1.1, leads to the primary and secondary minima and an energy barrier. The primary or deep minimum occurs where particles come very close to each other and go through irreversible coagulation. The secondary minimum or so-called metastable condition is where the attractive forces are dominant, but the interaction energy of the particles is not enough to overcome the energy barrier and particles remain at a certain distance from each other.

Self-assembly is defined as a process by which individual components of a system arrange themselves into an ordered structure [10]. Self-assembly is a unique and important feature of colloidal systems. Charged stabilized colloidal suspensions can assemble themselves into a variety of structures from a colloidal fluid to colloidal crystal and amorphous glassy phases. Particles diffuse in the suspension via Brownian motion; at low concentrations, they may show short-range positional ordering but no regular structure over a larger volume. This phase is called a "colloidal fluid" where the concentration of particles in an aqueous solution is typically up to 1 or 0.5 % in volume (this can vary depending on the concentration of counter ions in the solution etc.). At higher concentrations, long-range interactions cause particles to order themselves and form close packed structures, called a "colloidal crystal". At intermediate concentrations, part of the sample can order, meaning that colloidal crystal and colloidal fluid phase can coexist. At very high concentrations, heterogeneous and homogeneous crystallization can occur depending on the available nucleating sites or the concentration of nucleating
agents. Finally, above a certain concentration (known as glass transition concentration), amorphous glassy phase structures form [11, 12].

Colloidal systems can provide an excellent model system for understanding many physical problems such as phase transitions [13], kinetics of crystallization [14, 15] and the physics of nucleation and growth of atoms in a crystals [16].

**Colloidal particles at solid/liquid interfaces**

When a colloidal dispersion comes in contact with solid surfaces, hard boundaries imposed by the interface can induce ordering or self-assembly of particles and assist the formation of crystalline structure even at lower concentrations than expected.

In recent years, there has been a great interest in self-assembly of colloidal particles at solid/liquid interfaces as they can provide a low-cost and simple method to produce useful nanostructures, for example, making templates for photonic devices [17]. However, achieving the desired structure can require some level of fine control over the interactions governing the ordering [18] which individually or simultaneously alter the final structure. Parameters such as particles concentration [19] and surface charge [20], solvent property (free counter ions) temperature, pH [21] and chemical and physical properties
of the surface/interface can influence the ordering of colloidal particles both in bulk and at interface. Sometimes self-assembly of the particles can be manipulated using external forces such as shear and flow [23], optical [24], magnetic [25] and electric forces [26].

Once the interfacial structure is formed, an essential but challenging step is to preserve it during the drying process. When the liquid film evaporates capillary forces created by the menisci that form around the particles can deform the structure at the surface. These attractive forces can pull the particles together to form densely packed regions while leaving some areas devoid of particles [27].

1.2 Amphiphiles

Amphiphiles (Greek origin meaning: loving both) include molecules with a segment that is water soluble (hydrophilic) and a segment which tends to be water-insoluble or oil soluble (hydrophobic or oleophilic). A balance between these two parts of amphiphiles defines the solubility of molecules. Amphiphiles occur in a wide range of industrial products such as biological systems, detergents, pharmaceutical, food, and cosmetic formulations, etc. A wide range of molecules that have hydrophobic and hydrophilic regions can be characterized as amphiphiles, for example, surfactants, lipids or proteins.

1.2.1 Surfactants

Many surfactants are water-soluble amphiphiles where the hydrophobic part is usually a chain composed of carbon and hydrogen, and hydrophilic part consists of an ionic or polar head group. One of the standard classifications of surfactants is based on the charge of their head group, and according to that, they are characterized into three main groups of ionic, non-ionic and zwitterionic surfactant. Ionic surfactants carry a charge on their functional group in an aqueous solution. The charge on the head group of ionic surfactants can be negative (anionic surfactants) or positive (cationic surfactants). Sodium dodecyl sulphate (SDS) is an example of a synthetic anionic surfactant which is commonly used in detergents and personal care products such as toothpaste. Hexadecyltrimethylammonium bromide (CTAB) is an example of cationic surfactants with antiseptic properties against bacteria and fungi and is used in some industrial detergents or cleaning solutions. Nonionic surfactants do not carry a particular charge on their head group. Brij series surfactants (e.g. polyethylene glycol dodecyl ether) are examples of nonionic surfactants which are commonly used in detergents. Zwitterionic surfactants carry both negative and positive charge on their head
group, they are mild surfactants which are used to prepare health care products for sensitive skins, for example, dodecyl betaine or dodecyl alanine.

Similar to colloidal particles, surfactants interact with each other, the medium or the interfaces to decrease the free energy. When surfactants are dissolved in a solvent like water, the hydrophobic chain causes an unfavorable distortion and increases the free energy of the system. At water/air interface, surfactants tend to adsorb at the interface as a monolayer (called Gibbs monolayer) and expose their hydrophobic tail to the air to lower the free energy. The adsorption of surfactant to water/air interface decreases the tension* of water. Figure 1.2 a is a schematic illustration of changes in the surface tension of water when plotted against surfactant’s concentration. The surface tension curve usually falls into a lower limit after which further increase in the concentration of surfactants does not change the surface tension further. This concentration is the critical micelle concentration (cmc) of surfactants. At the cmc, surfactants begin to form spherical/ellipsoidal structures, celled micelles, in which the chain of surfactants go to the middle and form the core, and their functional group stays on the shell (see figure 1.2). The size of micelles defines the aggregation number of surfactants. Aggregation number of surfactants, in particular, nonionic surfactants, is highly influenced by the temperature.

Changes in the temperature can also affect the cmc and solubility of the surfactants in water. Figure 1.2b shows changes in solubility and the cmc for an ethylene oxide surfactants as a function of temperature and surfactant concentration. The solubility increases with the temperature, but when the temperature reaches a specific value, there is a sharp increase in the solubility above which it exceeds the cmc values. This temperature is called the Krafft temperature. For the nonionic surfactants, the solubility decreases with the temperature. At a certain temperature, surfactants tend to come out of the solution as large aggregates and make the solution cloudy. This point is known as cloud point and the temperature where it occurs depends on the hydrophobic/hydrophilic regions of the surfactants.

Surfactants can have different phases in bulk depending on the temperature and their concentration in the solution. Below the Krafft temperature surfactants remain in crystalline form at all concentrations. Above the Krafft temperature, at low concentrations, molecules are freely dispersed in water and lower the surface tension of water by adsorbing at the interface. As the concentration increases, it becomes favorable for the tail region of surfactants to gather together and form micelles. However, when the concentration increases further the micelles can grow larger and eventually can change shape and form cylinders, cubic or hexagonal structures. At even higher concentrations molecules may form multilayer or vesicles. Figure 1.3 is a

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*Surface tension is the measure of the energy required for increasing the surface of a liquid by a unit of area, it is typically shown in energy per unit area or force per unit length.
Figure 1.2. a) Surface tension changes with the concentration of surfactants b) Schematic of temperature dependence of cmc and solubility of anionic surfactant, sodium dodecyl sulphonate (redrawn from reference [28], chapter 4, page 79).

Typical phase diagram of surfactants in water. Depending on the shape, size and the chemistry of the chain or functional group, surfactants may or may not experience all the phases. For example, surfactants with two tails or small functional group usually, prefer to form cylinder instead of micelles or form curved vesicles instead of lamellar sheets, etc.

**Surfactants at solid/liquid interfaces**

Similar to the colloidal particles, when surfactants in an aqueous solution are introduced to an interface or when external forces are applied, their structures might be affected in various ways, and a new structure with completely new properties may form. For example even below the cmc, where surfactant molecules are expected to be freely dissolved in the solvent, they can form ordered structures at an interface. Understanding the effect of interfaces on surfactant structure is very important in many industrial and biological applications, for example, the efficiency of detergents, mineral flotation, corrosion inhibition, oil recovery, etc. The present dissertation explores the effect of solid/liquid interfaces.

Surfactants tend to adsorb from an aqueous solution onto a solid surface. The adsorption of surfactants at solid/liquid interfaces can occur through different mechanisms [29]:

- ion exchange: where the ions adsorbed from the solution onto the solid substrate are replaced with the surfactant ions
- ion pairing: where the surfactant ions pair up with the ions adsorbed from the solution onto the solid surface
- hydrophobic bonding: where the hydrophobic interaction between the surfactant and the solution drives the surfactants towards the surface or another adsorbed layer of surfactant
- permanent dipole interactions: when the surfactant contains electron-rich nuclei and the solid substrate strongly positive sites
- dispersion forces: where the adsorption is driven by London-van der Waals force between the surfactant and solid surface

The preferred mechanism depends on the type of the surfactant and the adsorbing solid surface. For example, some surfactants adsorb by exposing their tail to the surface, to protect their hydrophobic tail from water and form a monolayer on the surface. However some surfactants can adsorb exposing their head group to the surface and since exposing their tail to the aqueous solution is not favorable for them, another surfactant layer adsorbs onto this layer covering the tails and forming a bilayer on the surface. Depending on the concentration of the surfactants, further layers can adsorb to the interfacial layer and form lamellar structures aligned with the solid surface. In some cases when surfactants carry a similar charge as the solid surface, the adsorption may be opposed; in contrast, the opposite charge can give rise to the adsorption, etc.

Adsorption of surfactants (or generally molecules) at solid/liquid interfaces is usually quantified by the surface excess, $\Gamma$, which is defined as the amount of surfactants adsorbed over a unit area of the surface. The kinetics of adsorption of surfactants to solid/liquid interfaces can be described using alternative model isotherms [30]. The adsorption isotherm of a surfactant onto a specific surface is defined as the adsorbed amount at different equilibrium bulk concentration of the solution at a certain temperature. One of the simple and commonly used isotherm models is the Langmuir adsorption isotherm [31]. According to the Langmuir adsorption isotherm, surface excess of material on the surface, $\Gamma_A$ is given by:

$$\Gamma_A = \Gamma_m \frac{KC}{1 + KC}$$
where $\Gamma_m$ is the maximum surface excess value (plateau value in the adsorption isotherm), $C$ is the concentration of the surfactant in the solution and $K$ is a constant. According to Langmuir adsorption, $\Gamma_A$ is maximum when $C \to \infty$.

1.2.2 Lipids

Lipids are a class of organic amphiphiles which are insoluble in water, but are soluble in non-polar solvents such as chloroform, hydrocarbons or alcohols. Lipids are the building block of cell membranes which act as a source of energy and signaling in biological systems. Lipids rarely exist in an organism as 'free' molecules but rather combined with proteins or carbohydrates as lipoproteins or lipopolysaccharides.

In biophysics and biochemistry lipids are classified into three major groups of simple, compound or derived lipids [32]. Simple lipids are fatty acid esters of different alcohols which carry no other substance, for example triglycerides (fats) and triacylglycerols (oils). Compound lipids are fatty acid esters of different alcohols carrying additional chemical group such as phosphates, nitrogenous bases, carbohydrates and proteins. Phospholipids, glycolipids, and lipoproteins are examples of compound lipids. Derived lipids are obtained from the hydrolysis of simple or compound lipids, for example, steroids and terpenes.

The present dissertation, has used some phospholipids as simple model systems to mimic the behavior of biological membranes. Generally, phospholipids consist of two hydrophobic fatty acid ester tails and a hydrophilic head containing a phosphate group. Phosphatidylcholine (e.g. 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMPC) is an example of a phospholipid with two saturated C$_{14}$ alkyl chains attached to the head group, and is one of the major components found in cell membranes.

**Lipids at solid/liquid interfaces**

As for the synthesis surfactants, the amphiphilic nature of phospholipid makes them assemble into structures that minimize the interaction of the hydrophobic
tails with surrounding water and maximize the exposure of the hydrophilic end groups towards the water. When lipids are mixed in water, they often form multilamellar vesicles with several bilayers. A few of the lipids spontaneously, and some others after mixing well (or often sonicated) can break into smaller unilamellar vesicles. Vesicles are closed spherical shells with water both on the inside and the outside (see Figure 1.4). When the dispersion of vesicles comes close to a suitable solid surface, the vesicles fuse onto the surface and form bilayers. Bilayers are best formed from unilamellar or single-wall vesicles, this is the motivation for sonicating lipid dispersion to break the multilamellar vesicle into smaller vesicles. Phospholipid bilayers are widely used as a simple proxy to understand the behavior of more complex structures such as cell membranes.

1.2.3 Proteins

Proteins are a group of biological macromolecules which can show amphiphilic behavior. The primary structure of proteins is made of a sequence of amino acids that are linked together by peptide bonds. Amino acids are small organic molecules, consist of a central carbon atom linked to an amino (-NH$_2$) and a carboxyl group (-COOH), a hydrogen atom and a variable group (known as R-group). R-group of amino acids can consist of only an atom (for example H) or of a group of atoms bond to the central carbon atom. Amino acids vary in size and other physiochemical properties, for example, some are polar, some apolar and some charged. The amino acids are normally one of the 20 standard groups of [33]:

- polar amino acids: serine, cysteine, threonine, asparagine, glutamine, tyrosine, tryptophan.
- non-polar amino acids: glycine, alanine, proline, valine, isoleucine, leucine, phenylalanine, methionine.
- charged amino acids: aspartic acid, glutamic acid, lysine, arginine, histidine

The specific sequence by which amino acids are attached to each other determines the folding and hence three-dimensional structure of proteins. For example, oppositely charged amino acids approach each other and create a bridge, or non-polar amino acids cluster in the interior of the proteins (typically in the solution) and originate the folding of proteins.

Proteins form a variety of three-dimensional structures to serve their different functions. For example in biological systems proteins can provide structural framework, strength and mechanical support for various organs, like skin, blood vessels or bones; they can act as catalysts for the biochemical reactions of metabolism and assist the replication of DNA; or they can serve as an antibody by recognizing and repelling attacking pathogens; etc. [33].
Proteins at solid/liquid interfaces

Proteins can adsorb strongly to solid surfaces [34, 35]. This adsorption is desirable in some cases for example in the use of proteins as a stabilizer of colloidal suspensions for cosmetics and pharmaceutical products [36] or protein extraction using chromatographic columns [37]; however in some other cases can be undesirable, for example, in the functionality of biosensors [38] or drug delivery from polymeric microcarriers [39]. Understanding different factors governing the interactions, adsorption, and the structure of proteins at solid/liquid interfaces are of relevance in both natural and technological processes.

1.3 Outline of this dissertation

In the next chapters, the tools to access structural information at nm and ångström length scales at buried interfaces (primarily neutron scattering) will be discussed, followed by a description of the interpretation and understanding of the data. The dissertation will then introduce the materials and describe the motivation for the choice of different systems studied. Finally, the results of this dissertation will be presented as following:

The interactions of single component dispersions and the structures that can be formed at solid/liquid interfaces will be described in two different length scales of soft matter: for proteins which are a few nm big molecules and can form layers up to several nm thick (article I); and for colloidal particles which are several hundreds of nm in size, and can form structures that can extend several micrometers from the interface (article II). The understanding of the adsorption and structure of small molecules will be used for tuning and preserving the structure of large particles at an interface (article III). The dissertation will continue with a description of a two components systems (lipid and surfactants) to explore the interaction of surfactant molecules with a model system biological interface (article IV). When describing interfacial structures, the property of the interface itself plays a key role. As such towards the end, the dissertation will explain how and to what extent physical properties of the surface, such as roughness can affect the self-assembly of soft matter components at an interface (article V).

The various systems in this study are chosen in order to understand the physics of soft matter and relate that to address some relevant environmental issues. For example article I has chosen seed proteins to understand their adsorption to different surfaces, as a mean to exploring new natural coagulant agent in water purification purposes. Article IV has chosen an emerging class of surfactants (fully- or partly-fluorinated surfactants) to contribute to a better understanding of their biological toxicity and environmental hazard.
2. Methods

The primary technique used in the present work in order to explore the structure and collect compositional information from the interfacial layer at the wet interface was neutron scattering. Neutrons are not easily accessible; they require the use of large facilities. For characterization of the surfaces and materials prior to and as a complement to the neutron data, in-house techniques such as X-ray reflectometry, atomic force microscopy, quartz crystal microbalance, light spectroscopy, and zeta potential and particle size analysis have been used. In this chapter, each method will be described briefly mentioning its application in the present study.

2.1 Neutron scattering

2.1.1 Background

Neutrons are spin 1/2 sub-atomic particles with no charge. They have a mass 1839 times larger than electrons ($1.67 \times 10^{-27}$ kg, similar to that of protons), the magnetic moment of -1.9130427 $\mu_n$ and a mean lifetime of 15 minutes (885.9 s), for a free neutron [40]. Neutrons are produced in two conventional ways: nuclear fission of uranium isotope 235 at a reactor, or by bombarding a heavy element with energetic particles (e.g. accelerated protons) at a spallation source. Fission reactors produce a continuous flux of neutrons whereas the spallation source can generate a pulsed beam. Sometimes, the quality of a neutron source is described by the flux it can produce which is defined as neutrons per square centimeter per second ($cm^{-2} s^{-1}$) [41]. The energy of neutrons released from nuclear sources is in the range of MeV, while for a typical neutron experiment meV neutrons are required. This energy shift is normally achieved through the collision of neutrons with the atoms of a so-called moderator. The moderated neutrons are then guided towards the instrument and illuminated on the sample.

Neutrons have several unique properties which makes them a powerful tool to probe nanostructures in particular at buried interfaces:

- their wavelength is usually between 0.1 nm and 1 nm which is comparable to that of inter-atomic distances in materials
- they do not damage the sample: non-destructive
- they interact with the nucleus and as nuclear forces are very short range (a few femtometers) neutrons can penetrate deep into some materials with negligible interactions: probing buried interfaces
Figure 2.1. Different scattering process for neutrons with $\mathbf{k}$ as the wave vector of neutrons and $\mathbf{Q}$ as the momentum transfer. a) Elastic scattering: the magnitude of scattered wave remains constant but the direction changes, and inelastic scattering where both magnitude and direction change. b) Coherent scattering: where the scattered waves from nuclei are in phase and incoherent scattering: where scattered waves are out of phase.

- their interactions with the nucleus enable them to distinguish between different isotopes of the same elements: isotope labeling
- they carry magnetic moment: a powerful probe for magnetic structures

It is worth mentioning that despite the benefits that neutrons offer, the use of neutrons can be limited due to the low flux, long measurement time (compared to X-rays for example), weak scattering from some materials and also the need to access large experimental facilities.

In scattering experiments, neutrons are normally viewed as waves with vector $\mathbf{k}$, which has the magnitude of $2\pi/\lambda$; where $\lambda$ is the wavelength of neutrons. Neutron scattering can be elastic or inelastic. In the case of elastic scattering, the magnitude of the wave vector for the scattered neutrons remains the same as incoming neutrons while its direction changes, whereas in an inelastic scattering both magnitude and direction change. Figure 2.1 shows a schematic of the wave vector of neutrons during each scattering process. Neutrons can also be scattered coherently or incoherently. Coherent scattering occurs when the scattered waves from all the nuclei have definite relative phases and can interfere with each other, thus provide structural information. Incoherent scattering occurs when the scattered waves have random phases so they all scatter in different directions, and therefore do not provide structural information.
The effective area presented by a nucleus to neutrons is defined as the "cross section" which is normally given in barns \((1 \text{ barn} = 10^{-28} \text{ m}^2)\). Cross section can be defined as \(4\pi b^2\) \cite{42}, where \(b\) is the scattering length of the specific nuclei for neutrons. The scattering length is an indication of the interactions between nucleus and neutron which varies randomly across the periodic table and is different for different isotopes of an element. Note that the scattering length is a complex number, where the imaginary component becomes important for nuclei with a high absorption coefficient (such as boron and cadmium); otherwise, it is treated as a real quantity. The scattering length of neutrons for different elements can be found in the literature, for example, in the compilation by Sears \cite{43} or online calculators \cite{44}.

Materials consist of a collection of different nuclei. The characteristic of a material which describes its interactions with the neutrons is called the "scattering length density" (SLD). Scattering length density is defined as:

\[
\rho = \frac{\sum_{i=0}^{n} b_i}{V}
\]  

(2.1)

where \(b_i\) is the scattering length of the element \(i\); and the sum is taken over the volume \(V\) containing \(n\) atoms.

Like other radiation waves, neutrons undergo reflection and refraction at the interfaces between different media. Interface for neutrons is defined as the plane where materials with different scattering length density meet. The refractive index of neutrons for each material is defined in terms of the scattering length density as:

\[
n = 1 - \frac{\lambda^2 \rho}{2\pi}
\]

(2.2)

where \(\rho\) is the scattering length density of the material.

The critical angle where the total reflection occurs is given by Snell’s law as:

\[
\cos \theta_c = \frac{n_2}{n_1}
\]

(2.3)

which can be converted to the critical momentum transfer, \(Q_c\):

\[
Q_c = \sqrt{16\pi(\rho_2 - \rho_1)}
\]

(2.4)

where \(\rho_2 - \rho_1\) is the difference between the scattering length density of the material 2 (below the interface) and material 1 (above the interface).

As described above, different isotopes have different scattering lengths, and hence a different scattering length density for neutrons. This offers a great advantage in using neutron scattering to study the structure of complex soft matter systems. The scattering length density of H\(_2\)O is \(-0.56 \times 10^{-6} \text{ Å}^{-2}\) while that of D\(_2\)O is \(6.35 \times 10^{-6} \text{ Å}^{-2}\). Such a big range allowed using H\(_2\)O, D\(_2\)O, or a specific mixture of them, to match the scattering length density of some components and enhanced the scattering signal from other parts.
(so-called contrast variation). Figure 2.2 is a simple illustration of contrast variation, where each color represents a different scattering length density value.

2.1.2 Neutron reflectometry

**Specular reflection**

Neutron reflectometry is a technique used to study near-surface structures. In this method, neutrons are reflected from the interface onto a detector. At specular condition ($\theta_i = \theta_f$, where $\theta_i$ is the angle of the incoming beam and $\theta_f$ is the angle of outgoing beam), the $Q$ vector is perpendicular to the interface, hence structural information such as thickness, roughness, and composition of the layers parallel to the interface can be obtained. Reflectivity is a measure of the ratio between the intensity of the reflected beam and the incident beam at the specular condition. Periodic structures repeating with equal distance give rise to the measured intensity at specific angles leading to intense Bragg peaks. The position of Bragg peaks in scattering angle is correlated with the repeat distance of the diffracting layers $d$ via Braggs’ law [45]:

$$2d \sin(\theta_i) = m\lambda$$  \hspace{1cm} (2.5)

where $m$ is a positive integer.

The scattering angle depends on the wavelength of neutron beams which varies depending on the instrument. Hence neutron scattering data are normally presented as a function of momentum transfer, $Q$ (shown in Figure 2.1) which as shown in Figure 2.1 is:

$$Q = k_f - k_i$$  \hspace{1cm} (2.6)

with the magnitude of:

$$Q = \frac{4\pi \sin(\theta)}{\lambda}$$  \hspace{1cm} (2.7)

where $\theta$ is half of the scattering angle which in the case of specular reflection is the same as the incident angle of the beam.
Figure 2.3. Interference of reflected beam at the specular condition. When the reflected wave from parallel interfaces are in-phase, constructive interference happens which gives rise to the amplitude of the scattered wave, and if they are in opposite phase, destructive interference happens, and no reflection is observed. There are conditions which fall in between these two, and the amplitude of reflected beam becomes smaller than the constructive interference but not zero.

Neutron reflectometry instruments operate in two different modes: monochromatic or time-of-flight (TOF). In the monochromatic mode, neutrons with a single wavelength are illuminated onto the sample, and to access different Q, the incident and scattering angle are varied. This configuration is also known as θ-2θ scan. While in the time-of-flight mode, the incident and scattering angle are both fixed, but a spectrum of neutrons with the different wavelength is illuminated onto the sample. In this configuration, neutrons with a longer wavelength (lower energy) provide information about the smaller Q and neutrons with a shorter wavelength, about the larger Q region.

In TOF mode, neutrons are sent in a pulsed white beam (a distribution of different wavelengths) to the sample. The time that it takes for a neutron to hit the detector determines the wavelength. On some of the spallation sources such as ISIS in the UK, neutrons are already produced as pulses, while at a reactor-base facilities, usually a chopper is used to measure in TOF mode, for example, on the D17 instrument [46, 47].

Off-specular scattering and grazing incidence small angle scattering

Specular reflection measures the scattering at θ_i = θ_f. In this condition the momentum transfer is normal to the surface (as shown in Figure 2.3) thus only the structural information perpendicular to the interface can be obtained. For θ_i \neq θ_f, the momentum transfer is no longer perpendicular to the surface, so the scattering will include signals which come from possible correlations in other directions. For example, if the interface is rough or
domains with different density are formed on the surface, there will be lateral correlations depending on the size of domains. By introducing a small in-plane component to the scattering wave vector, it is possible to probe these correlations. There are different ways to measure off-specular scattering, a traditional way is to perform a so-called rocking scan. In this type of scan, the detector is kept in a fixed position, and the angle of the sample varies. Under these conditions, scattered neutrons at a constant angle ($\theta_i + \theta_f = \text{constant}$) provide lateral structural information. This method is normally used when a point detector is available on the instrument. More detailed explanation of various ways to measure off-specular data and the features seen in off-specular data is discussed in reference [48]. Another typical way of recording off-specular scattering is to use a position sensitive detector where all the neutrons including specular and off-specular are captured. Figure 2.4 is a schematic showing scattering collected on the two-dimensional position sensitive detector.

Off-specular scattering is limited to probing rather large correlation lengths, typically $> 50$ nm [49]. To access smaller length scales, a further modification of the method is required to enhance the resolution. For this purpose, Grazing-incidence small-angle scattering (GiSANS) is used. GiSANS allows measurement of roughness, sizes, shapes and lateral correlations with a length scale from 3 to 100 nm [50, 51, 52]. In this technique, all the neutrons scattered from the sample are collected onto a two-dimensional position sensitive detector. The neutrons on each pixel then will have a contribution of all three components of the $Q$ ($Q_x$, $Q_y$, $Q_z$). Typically in this approach, the beam is highly collimated as a point to increase the resolution in different directions, and illuminates the sample at small angles, close to the critical angle of the interface. By carefully choosing the interface and incidence angle one can control the depth of the penetrating beam into the sample and determine the in-plane structure of the sample at the certain depth. The penetration depth, in a GiSANS experiment, is defined as a depth where the intensity of the wave falls to $1/e$ of the initial intensity and is given by [53]:

$$z_{1/e} = \frac{\sqrt{2} \lambda}{4\pi \sqrt{\left(\theta_i^2 - \theta_c^2\right)^2 + \left(\frac{A}{2\pi} \mu\right)^2 - \left(\theta_i^2 - \theta_c^2\right)}}$$  \hspace{1cm} (2.8)

At small angles and below the critical edge the penetration depth is determined by the interface contrast and evanescent wave [54]. At high angles generally, the penetration is limited by the attenuation coefficient ($\mu$). For X-rays, $\mu$ is dominated by the absorption whereas for neutrons, the absorption is usually low, but the scattering (mostly incoherent scattering) causes attenuation of the beam that gives an effect analogous to absorption in reducing the coherent scattering signal.

A further development required to increase the resolution for a GiSANS is to move the detector further away from the sample so that the scattered
neutrons from small objects could be resolved. This is a motivation for using SANS (small angle neutron scattering) instruments for GiSANS experiments, for example, NG3 at NIST [55] or D22 at ILL [56]. However, depending on the system and geometry of the instrument, some reflectometers are also used for GiSANS experiments such as MARIA at MLZ [57].

**Experimental set-up for neutron scattering**

*Sample holder:* The sample holder used for neutron scattering experiments have been described in details previously [58, 59, 60]. Briefly, the sample holder was a solid/liquid reflection cell with a 2 mm polytetrafluoroethylene (PTFE) gasket that contains the liquid sample. The sample was sealed between two crystals or one crystal and a polycarbonate backplate, depending on the experiment. The crystals were held together with aluminum frames that had channels for circulating water to control the temperature of the cell. The actual temperature of the cell was measured using a resistance thermometer (Pt-100). The cells are made so that according to the geometry of the instrument they could be mounted either vertically or horizontally. Figure 2.5 shows a picture of a reflection cell on the translation stage during a neutron reflectometry measurement.
2.2 Complementary techniques

2.2.1 X-ray reflectometry

Neutrons interact with the nuclei whereas X-rays interact with the electron cloud around the atoms. Since the electron cloud is several orders of magnitude larger, X-rays cannot penetrate as deep into materials. Apart from the differences in the type of interactions between X-ray and neutrons with the material, they share the same principles. X-ray instruments are more readily available as the use of them does not necessarily require accelerators or reactors. In the present dissertation, in-laboratory X-ray instruments have been used for characterization of solid surfaces and check the roughness prior to neutron experiments.

2.2.2 Atomic force microscopy

Atomic force microscopy (AFM) is a technique which can record real-space images on an atomic scale. In principle, AFM uses a physical tip which is located within a few ångström from the surface of the sample. The tip moves over the surface, and as a result of inter-atomic forces between the probe and sample, it is deflected from its initial position. The deflection of the tip is then sensed with a detector system which normally consists of a laser and a photodiode that can detect the vertical position of the probe with a high accuracy [61]. AFM can provide lateral resolution comparable to the diameter of the tip that is usually about a few nm.

AFM operates in two primary modes, contact and tapping mode. In contact mode, the tip is dragged along the sample. This mode is not recommended
for soft materials since the tip can pick up parts of the sample and this results in distortion in imaging and destruction of the sample. Often tapping mode is used to scan soft material. In this mode, the cantilever oscillates with a frequency close to its resonance frequency and lightly taps the surface while moving along the sample. The amplitude and phase of oscillations changes with shape and roughness or softness in different regions of the sample. These changes can be translated into a topographic image of the sample. Atomic force microscopy is a surface sensitive and local probe technique which is not suitable to study in-depth or buried structures or to obtain average structural information over a large area. The AFM instrument used for the work presented in this dissertation operates on dry surface only and was used mostly to determine the distribution and size of the colloidal particles adsorbed to a solid surface after drying.

2.2.3 Quartz crystal microbalance with dissipation

A quartz crystal microbalance with dissipation (QCM-D) is a physical and highly sensitive device used to study adsorption/desorption of material at a solid/liquid interface. In this technique a thin quartz disc is sandwiched between a pair of electrodes; applying an alternating current can excite the quartz to oscillate at its resonance frequency. Adding or removing a small amount of material (even in the order of nanograms) to/from the quartz changes the frequency of oscillation. Figure 2.6 shows how the frequency varies when a rigid layer adsorbs at the quartz surface. QCM-D data are normally shown as changes in the frequency, \( \Delta f \) and energy dissipation, \( \Delta D \). Using the Sauerbrey relation [62] changes in the frequency can be translated into the amount of the mass bound to the surface:

\[
\Delta f = -C \Delta m
\]

where \( C \) is a constant depending on the sensitivity factor of the used quartz. Measuring the dissipation provides extra information about conformational changes and the rigidity (viscoelasticity) of the layer. In this dissertation, QCM-D has been used to study the adsorption of proteins and particles at the solid/liquid interface.

![Figure 2.6. Changes of frequency as firm layer is adsorbed onto the crystal, steps represent injection of sample with increasing concentration (\( C_2 > C_1 \))]
2.2.4 Ultraviolet spectroscopy

Ultraviolet (UV) spectroscopy is an absorption spectroscopy technique commonly used for the qualitative analysis of concentration and identification of chemicals. In this dissertation, transmission of the visible light has been used to determine the clarity of colloidal particle dispersions. According to the Beer-Lambert law [63], when a monochromatic beam passes through a solution, the decreasing rate of the intensity of radiation depends on the thickness of the sample, the concentration of absorbers in the solution and the intensity of incident radiation. Beer-Lambert Law describes the absorbance of a sample as:

\[ A = -\log\left(\frac{I}{I_0}\right) = \varepsilon \cdot c \cdot l \]  

(2.10)

where \( \varepsilon \) is the absorptivity, \( l \) is the path length of the beam, \( c \) is the concentration of the sample, and \( \frac{I}{I_0} \) is the transmission. In this work, UV spectroscopy was used to determine the attenuation of samples that arises from scattering and could thus could be used to assess the extent of aggregation of colloidal particles.

2.2.5 Zeta potential

Zeta potential of particles or molecules is determined by applying an electric field to the dispersion and measuring the velocity of molecules or particles migrating towards the electrodes. The electrophoretic mobility of the particles is linearly related to their zeta-potential. In this dissertation, zeta potential measurements were performed to determine the surface charge of particles and proteins dispersed in water.
3. Choice of materials and sample preparation

3.1 *Moringa* seed proteins

Understanding the interaction of proteins with various interfaces is of great importance in many biological and industrial processes. In this work *Moringa* seed proteins were chosen to explore the physics of protein interactions at solid/liquid interfaces. *Moringa* trees (also known as miracle or drumstick trees) have thirteen known species which grow in various regions of the world [64]. They grow fast and relatively easy, even in regions with little rainfall, and provide a rich source of nutrition, oil, and proteins [65]. Typically the seeds consist of 40-60% protein [66], providing an easily grown and rich source to extract large quantities of proteins.

![Figure 3.1](image)

*Figure 3.1.* a and b) *Moringa* seeds used in this study with their origin marked on the map: MOI: *Moringa oleifera* and MP: *Moringa peregrina* from Iran, and MOB: *Moringa oleifera* from Botswana. The maps have been taken from Google maps (attributions: mapa GISrael, ORION, ME (Iran); AfriGIS (Pty) Ltd, Google (Botswana)). c) A photograph of *Moringa oleifera* tree with the seed pads marked on it.

*Moringa* seed proteins have unique properties, which have made them an interesting material to explore further; for example, protein extracted from the
seeds of *Moringa oleifera* trees is known as one of the most effective natural flocculating agents for impurities dispersed in waste and groundwater [67, 68]. In this work seeds from *Moringa oleifera* trees grown in Iran and Botswana and *Moringa peregrina* trees grown in Iran were chosen. Figure 3.1a and b shows the origin of the seeds and a photographs of the each seed with an example of seed pods.

### 3.1.1 Protein extraction

The procedure to extract the proteins was followed as described by [69]. The seeds were de-shelled and crushed with a kitchen blender or a pestle in a mortar. The crushed seeds were mixed with petroleum ether, 40-60 °C (each 100 g in about 500 mL petroleum ether), in a fume hood using a magnetic stirrer and then was filtered. In the filtration step, the oil is expected to dissolve in petroleum ether and come out of the filter paper, while the rest of the material including proteins to be left on the paper. The material that was left on the filter paper was dried in air overnight. The dried powder was mixed with deionized water (each 100 g in about 400 mL of water) and stirred for about an hour and then filtered two to three times until a clear solution was obtained. In this step, proteins are expected to dissolve in water; however, the solution may include other components such as water-soluble polysaccharide, etc. To precipitate the proteins from the solution, ammonium sulfate was added to the clear solution to saturation. At this point, ammonium sulfate dissolves in water (∼74 g in each 100 mL) and forces the proteins to come out of the solution. Proteins that come out of the solution in the form of a sticky paste, were separated and dissolved in water and filtrated to remove the large particles, then were dialyzed at least 15 times (each time in about 5 L of deionized water) to remove the ammonium sulfate. Dialysis tubing used for this procedure was cellulose membrane tubing purchased from Sigma-Aldrich (D9527-100FT) with a molecular weight cut-off of 14 kDa. To purify the proteins further, the dialyzed solution was added to carboxymethyl cellulose packed column, to which the proteins are expected to bind. When all the solution passed through the column, the elution of proteins was performed adding the same volume of 1 mol L\(^{-1}\) NaCl solution. The eluted protein solution was dialyzed as described above until the salt was removed and the conductivity of the dialyzing water became the same as the deionized water. Finally, the protein powder was obtained via freeze drying.

The protein extract was characterized using zeta potential measurement and amino acid compositional analysis. All the species were found to have a positive potential of about +20 mV. The amino acid composition analysis is presented in *Article I* and a comparative discussion between different species can be found in the discussions section.
3.2 Latex particles

Self-assembled structure of colloidal particles at solid/liquid interfaces has the potential to be used as lithographic masks to pattern magnetic and photonic devices [70]. However for this method to be applicable, understanding the physics of the interaction between colloidal particles at solid/liquid interfaces is essential. For this purpose, charged-stabilized spherical polystyrene latex particles (PS) were chosen as a simple colloidal model. Particles were synthesized and characterized previously [60] using scanning electron microscopy, atomic force microscopy, light scattering, zeta potential measurements, small angle neutron scattering, etc. Particles of two sizes were used during this work, PS3 latex with the average diameter of 140 nm and polydispersity < 2 % and PS11 latex with an average diameter of 72 nm and polydispersity < 5 %. Zeta potential measurements showed that particles carry a negative surface charge (-30 mV for PS3 and -35 mV for PS11). Figure 3.2 shows an AFM image from PS3 particles dried on a silicon crystal (a) and a photograph of the particles in a glass vial showing iridescent color, an indication of crystalline structure with spacing in the order of the visible light wavelength (b).

![AFM image and photograph of particles](image)

*Figure 3.2. a) AFM image of PS3 particles dried on a silicon substrate. b) PS3 particles dispersed in water in a glass vial.*

3.3 Phospholipids

Phospholipids are the building blocks of cell membranes and are commonly used for fundamental studies to understand the behavior of cell membranes. In this study, two types of phospholipids were chosen: 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). DMPC is a fully saturated lipid which makes it form a relatively rigid bilayer whereas DOPC is an unsaturated lipid which forms a more flexible bilayer. The gel-to-fluid transition temperature for DMPC bilayers is reported to be 24 °C and for DOPC -17 °C [71, 72].
3.3.1 Preparation of lipid bilayers

Bilayers of DMPC and DOPC were prepared with a similar procedure: The lipids were first dissolved in chloroform (0.03 mg/mL) in a glass vial. The chloroform then evaporated with nitrogen gas in a fume hood until a thin film was formed on the walls of vial. Deionized water was added to the dried lipids to make vesicles with the concentration of 1 mg/mL. The solution was sonicated using a tip sonicator until a relatively clear solution was obtained (see Figure 3.4). The sonication time varied between 20-40 minutes depending on the power of the available instrument. In this process, the aggregates of lipids break into small vesicles so the solution becomes more transparent for the visible light.

Vesicle dispersion was injected into the reflection cell at 55 °C to allow the vesicles to fuse onto the surface and form a bilayer. The temperature of the cells was then decreased to 35 °C and the surface was rinsed with water by injecting more than ten times of the reflection cell volume. Bilayers were then characterized in-situ using neutron reflectivity measurements in three contrasts (H₂O, D₂O and a mixture to match the scattering length density of the substrate) so that an unambiguous model fit to the thickness of different regions of bilayer could be obtained.

![Figure 3.4. DMPC lipids in water, before (a) and after (b) sonication for about 30 minutes.](image)
3.4 Surfactants

3.4.1 Nonionic surfactant

Non-ionic surfactants are good detergents, wetting agents and emulsifiers which are used in many industrial, pharmaceutical and food products. In this work, non-ionic surfactant Brij L4 (polyethylene glycol dodecyl ether, \( \text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{CH}_2)_4\text{OH} \)) was chosen. According to the phase diagram available in literature [73], Brij L4 is expected to form a lamellar structure at concentrations above approximately 30 wt. % at room temperature. The Brij sample was purchased from Sigma-Aldrich (mass density of 0.95 g/mL at 25 °C) and was mixed with \( \text{D}_2\text{O} \) and diluted to the desired concentrations, for neutron scattering experiment.

3.4.2 Fluorinated surfactants

"Fluorinated" is a general term used for surfactants where at least one of the hydrogen atoms in their tail region is replaced by fluorine. Fluorinated surfactants are strong dirt and water repellents which have made them an attractive substance in many industrial applications such as textile, carpet, clothing and leather treatment, ski waxes, fire fighting foams, food packing products, etc. [74]. Fluorocarbons are also used in pharmaceutical applications for treating respiratory failure or controlled release drug delivery [75]. Perfluorinated alkylated substances (PFASs) are a subgroup of fluorinated surfactants in which all the C-H are replaced with C-F [76]. The main two classes of perfluorinated surfactants are perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs). PFASs are commonly referred as long- or short-chain compounds, the term long-chain for perfluoroalkyl sulfonates is used when perfluorocarbon chain includes more than 5 carbons and for perfluoroalkyl carboxylates when it includes more than 6 carbons.

PFASs are very stable and do not degrade readily [77], and therefore tend to accumulate and contaminate the environment, wildlife, and the human body. Several studies have reported an increasing concentration of PFASs, found in groundwater [78], house-hold dust [79] and even blood serum of human in the past decades. For example in 2012 in Uppsala, Sweden, the level of PFASs found in blood samples of pregnant women living near Arlanda airport, showed an increase over the time. The reason for that was found to be the contamination of groundwater since many firefighting practices took place in that area [80]. Lately there has been an increasing concern regarding the toxicity of these compounds, in particular for the long-chain compounds. PFOS (long-chain sulfonated PFAS) has been banned as a persistent organic pollutant in several countries, and the use of many others are under debate [81]. Despite various regulations for the use of these compounds, there
are considerable uncertainties as to how these compounds can damage the biological systems.

Earlier reports have established that fluorinated surfactants similar to the hydrocarbon surfactants need to reach specific chain length before they can affect the membrane fluidity [82]. In recent years, shorter chain (C₄ and C₅) or partly-fluorinated substances have been introduced to the market as alternative safer replacements [83, 84]. However there is not a clear description regarding this choice of type.

<table>
<thead>
<tr>
<th>Perfluoroalkyl carboxylates</th>
<th>Perfluoroalkyl sulfonates</th>
<th>Effect of functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorohexanoic acid (PFHxA, C₅)</td>
<td>Perfluorobutanesulfonic acid (PFBS, C₄)</td>
<td>Perfluorononanoic acid (PFNA, C₈)</td>
</tr>
<tr>
<td>Perfluorononanoic acid (PFNA, C₈)</td>
<td>Perfluorohexane sulfonate (PFHxS, C₆)</td>
<td>Perfluorooctanesulfonic acid (PFOS, C₈)</td>
</tr>
<tr>
<td></td>
<td>Perfluoroctanesulfonic acid (PFOS, C₈)</td>
<td>Perfluorooctanesulfonamide (FOSA, C₈)</td>
</tr>
</tbody>
</table>

**Table 3.1.** PFASs used in this study: PFASs in each column were compared together, substances presented in the first two columns were used to compare the effect of chain length; those in the third column were used to understand the effect of functional group.

PFASs in this study were chosen to allow a systematic comparison between the interaction of molecules with different effect chain length and functional group. Table 3.1 shows a list of perfluorinated substances with the abbreviations, which is commonly used in the literature, as they have been compared in this work. To compare the effect of chain length on the interactions of PFASs, perfluoroalkyl carboxylates with two chain lengths (C₅ and C₈) were compared with each other, and three perfluoroalkyl sulfonates with 4, 6 and 8 carbons in their chain group, to each other. To explore the effect of functional group, PFNA (perfluoroalkyl carboxylates with 8 carbons in its chain group), PFOS (perfluoroalkyl sulfonates with 8 carbon in its chain group) and FOSA (perfluorooctanesulfonamide with 8 carbons in its chain group) were chosen, as examples of PFASs with the same chain but different functional group.

All the substances were purchased from Sigma-Aldrich with a reported purity of > 97-98%. PFASs are acidic and can alter the pH of the medium significantly. In order to avoid confusing the structural changes due to the pH variation with that of molecules interactions, the experiments using PFASs were performed in a buffer solution. The solubility limit for some of PFASs has been reported to vary slightly depending on the
type of buffer [85]. HEPES is one of the buffers in which PFASs show higher solubility compared to others, it has been used frequently to study the behavior of biological membranes, and we have seen that it does not affect the structure of bilayer. PFASs were diluted in 20 mmol L$^{-1}$ HEPES ((2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid) D$_2$O and H$_2$O buffers to the desired concentrations for the neutron scattering experiments.

3.5 Solid surfaces

In this work, silicon crystals cut on (111) face, sapphire crystals (0001) face, and quartz crystals were used. Silicon and quartz crystals were purchased from Crystran and sapphire crystals from PI-KEM. The quality of the crystals was checked by testing one of each batch using X-ray reflectometry after the purchase. The crystals used for neutron scattering experiments were measured individually in different contrasts (H$_2$O, D$_2$O and scattering length density matched to that of the crystal) after cleaning prior to exposing them to the samples.

The cleaning procedure of surfaces can affect the surface chemistry and roughness of the surfaces and hence influence the structures formed at the interface [86]. All the surfaces in this study were cleaned with the same procedure: Surfaces were immersed in diluted laboratory detergents, if available Neutracon otherwise Decon-90, with approximately 1:50 ratio of detergent in water for a few minutes. Neutracon is a mild and less destructive detergent compared to Decon-90. Decon-90 is strongly basic and may etch crystal in high concentration and long exposure time. After rinsing with pure water, the polished sides of the crystals were placed faced-up in a glass petri dish covered with concentrated sulfuric acid (H$_2$SO$_4$). Approximately the same volume of deionized water was then added to the acid on the surface. After about 5 minutes, surfaces were rinsed with water for at least one minute. Cleaning with sulfuric acid and water was repeated up to three times until the surfaces became highly hydrophilic. In some cases (the latest batch of purchased silicon crystals) when no improvements were seen with acid cleaning, the surfaces were cleaned using UV-Ozone cleaner for maximum 5 minutes followed with extensive rinsing with pure water.
4. Results and discussion

This chapter presents an exploration of the interactions of molecules and particles dispersed in water, at solid/liquid interfaces, in an attempt to understand their overall, equilibrium structure. In the first sections of this chapter, the structural information at two different length scales are presented. In particular, *Moringa* seed proteins and polystyrene colloidal particles are used, leading to the third section where the understanding from protein adsorption to manipulate the interactions of particles with the interface is tackled. In the fourth section, the results obtained from studying the interactions of mixed molecules (lipid and surfactants) is described and finally the effect of surface roughness on interfacial structures of surfactants, as a well-ordered and well-aligned lamellar system, is shown.

This effort aims at obtaining the physicochemical factors that affect the interactions of simple model systems of molecules and particles. Investigating the basic physics of simple model systems can provide an understanding of more challenging and complex systems. The results presented in this dissertation link these understandings to some environmental issues and challenges in material physics and nanotechnology. The experimental work presented in this dissertation is based mainly on modeling neutron reflectometry and grazing incidence small angle scattering. This poses some challenges regarding data reduction, modeling the scattering data, and understanding various components that contribute to the signal. Some of these challenges that were identified in this process will be discussed in this chapter.

4.1 Proteins at solid/liquid interfaces

Finding a readily available, low-toxicity and low-cost natural material as a flocculating agent for water purification purposes, is a major challenge. *Moringa* trees have attracted global attention for their nutritional, medical and in particular water treatment applications [87, 65]. Protein from the seeds of *Moringa oleifera* has been identified as one of the most effective flocculents that can assist sedimentation/creaming of impurity particles dispersed in water [67, 88]. Besides mineral particles, algae in drinking water present multiple problems, not only due to the bad taste but also concerning obstruction of filters, the growth of biofilm, dangerous toxins, etc. [89]. Recent practical tests have shown that the proteins of *Moringa oleifera* trees are effective as a bio-coagulant for microalgae removal [90].
There are thirteen varieties of *Moringa trees* [64], and among them *Moringa oleifera* is the most studied one. Different species can be native to, or grow more readily in different regions; using species that can grow locally can reduce the cost significantly. *Moringa peregrina* is one of the species which grows in different regions of the world, where many of them have very limited access to clean water. As a part of this dissertation, the interactions of *Moringa peregrina* seed proteins have been studied with model system minerals and colloidal particles. Figure 4.1 shows the dispersion of PS3 latex particles in the absence of (a) and in the presence of *Moringa peregrina* seed proteins (b). Sedimentation of particles due to the flocculation with proteins is evident. Quantitative analysis using UV-Visible spectroscopy (Figure 4 in *paper I*), showed that the efficiency of *Moringa peregrina* seed proteins to flocculate PS3 particles is similar to that of *Moringa oleifera* seed proteins.

The proposed mechanism behind the flocculation of particles has been the adsorption of proteins to the surface of particles that together with their tendency to aggregate (i.e., proteins adsorb to each other) can gather the particles into big flocs which can sediment or cream, depending on the density difference [68].

To further explore the behavior of *Moringa peregrina* seed proteins at the molecular level, a comparative neutron reflectometry study was performed with the proteins from the seeds of *Moringa oleifera*. In this experiment, a reflection cell was assembled with a silica surface on one side and alumina on the other, so that the adsorption of the same protein solution can be simultaneously studied on two surfaces with different surface charge [91].

The reflectivity data were modeled using fitting programs by A. R. Rennie available on the web [92]. For the adsorption of seed proteins, *cprof* program was used, which allows modeling layers with a particular scattering length density on the solid surface and an additional layer with decaying protein density moving away from the interface. Modeling reflectivity data showed that *Moringa peregrina* seed proteins, similar to the *Moringa oleifera* proteins, adsorb to both silica and alumina, with a plateau in the adsorbed amount at concentrations close to 0.1 wt.%. These results show that proteins extracted from seeds of *Moringa peregrina* can be used in a similar way as the

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*Figure 4.1.* a) PS3 particles dispersed in water, b) PS3 particle dispersion mixed with *Moringa* proteins.
previously suggested *Moringa oleifera*. There are however differences in the amount and the structure of the adsorbed layer. Figure 4.2 shows the amount of *Moringa peregrina* seed proteins adsorbed at each surfaces (a), and *Moringa oleifera* (b). Note that the adsorption of *Moringa oleifera* on alumina was measured only at the highest concentration because of limited measurement time. Previous studies [69] have reported the values at lower concentrations. The results of this study showed that proteins extracted from seeds of *Moringa peregrina* trees adsorb more onto alumina whereas those of *Moringa oleifera* more onto silica. This can be beneficial information for improving the efficiency of water clarification, as it will allow choosing the more effective species depending on the kind of impurity.

Besides the difference in the adsorbed amount, the structures of the adsorbed layers of proteins were different. *Moringa peregrina* proteins formed a dense layer, with the protein fraction close to 70% near the alumina surface, while a layer with protein fraction about 50% was formed near the silica surface. The results showed that these differences could not be due to differences in surface charge, as they both have an overall positive charge in the same range (approximately +20 mV). The differences in the amino acid composition of the proteins in this study, were found to be small. It has been found that even small differences in the amino acid composition can result in significant differences in adsorption behavior of seed proteins [93]. There is also a possibility that different adsorption behavior of proteins is due to different sequences. Previous studies on *Moringa oleifera* seed proteins has shown that proteins with similar aminoacid composition but different sequence can show a very different adsorption at slid surfaces [93].

### 4.2 Colloidal particles at solid/liquid interfaces

The ordering of charge-stabilized, monodisperse polystyrene latex particles close to solid walls has been observed previously [94]. Quartz crystal microbalance with dissipation (QCM-D) has shown that the structure forms close to but not directly at the interface [Hellsing et al. manuscript 2018]. It was found that the separation of particles from the solid surface is in the order of the particles spacing.

Figure 4.3a shows a schematic of particles ordering close to the interface. The structural information perpendicular to the interface can be studied by neutron reflectometry and in-plane ordering with GiSANS. Figure 4.3b shows two-dimensional scattering pattern recorded in a GiSANS experiment from 8 wt.% PS3 particles at silica/water interface. The scattering shows several orders of Bragg reflection appearing intensely on the detector. The sum of the intensity in the \( Q_x \) direction with different orders Bragg peaks marked on it, is shown in figure 4.3c),
Figure 4.2. The adsorbed amount of seed proteins from *Moringa peregrina* (a) compared to that of *Moringa oleifera* (b) at water/silica and water/alumina interfaces. As shown in the schematic, *Moringa oleifera* seed proteins show higher adsorption at the silica surface whereas *Moringa peregrina* seed proteins show higher adsorption at alumina surface.

Several orders of Bragg peaks, equally spaced from each other is an indication of ordering and uniform alignment of particles over an area of about 20 cm$^2$ (the illuminated area of the interface). The spacing of the peaks along $Q_x$ direction shows a lattice parameter of approximately 400 nm, which is similar to that of the bulk for this concentration and size of particles, when forming a close-packed (e.g., face centered cubic) structure. Figure 4.5 shows GiSANS data from PS3 particles, when the incident beam illuminates the sample at angles close to the critical angle of the interface.

Small angle neutron scattering (SANS) data measured by rotating the cell 90 degrees (Figure 4.4) showed ring shape scattering (powder like distribution of particles) when 2 mm beam was illuminated onto the sample. However, when the beam is further collimated (to 1 mm for example), Bragg scattering spots started appearing which can give an estimate of the grain size of a crystallite. A comparison between the results of SANS and GiSANS suggests that particles self-assemble themselves into well-ordered structures aligned with the solid surface to a depth of few micrometer (depth probed by GiSANS) over a large area, while smaller crystallites are randomly distributed deeper into the bulk (millimeter length scale probed by SANS).

Figure 4.5 shows two-dimensional detector images in Q space measured at several incident angles close to the critical angle. Figure 1, *paper II* shows the experimental setup for this experiment. The y axis is the direction of the
Figure 4.3. Monodisperse latex particles ordered close to a solid surface (a),
two-dimensional GiSANS scattering (a) providing in-plane correlations of the
particles, and sum of the signal vertically highlighting features of scattering which
provides information in $Q_x$ direction (c).

beam and the structural information in x and z direction are projected onto
the detector. Note that the horizontal axis contains components of $Q_y$. The
Bragg reflections from the ordered particles appear at incident angles smaller
than the critical angle. The particles are 140 nm in diameter and according
to QCM-D data, they form structures with a separation of a few hundred nm
from the solid wall, implying that a penetration depth of several hundred nm is
required to probe the structure of particles. However at incident angles below
the critical angle, the evanescent wave is expected to penetrate only a few nm
into the sample. This raises the question, what depth is the GiSANS signal
coming from? The penetration depth of the beam in a GISANS experiment is
known to be given by equation 2.8 where the penetration depth at each angle
is defined for a single wavelength and a defined incident angle. However, in
reality, neutron instruments do not provide a single wavelength, but rather a
distribution, depending on the resolution of the instrument.

The penetration depth for a single wavelength, indicted with $z_1/e(\theta_i, \lambda)^*$,
is shown versus the penetration depth which is smeared due to wavelength
distribution, indicated with $z_1/e$, in Figure 4.6. In this case the resolution
function for the D22 instrument (triangular distribution with $\Delta\lambda/\lambda = 9.7\%$ )

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*The notation has been modified compared to that used in Paper II.
Figure 4.4. Small angle neutron scattering data measured with different beam size on the sample.

is taken for 1.4 nm wavelength. In addition to the wavelength, for a particular incident angle, an angular distribution around the set value occurs, depending on the collimation of the beam. This is an important challenge especially for the case of neutrons due to the broader wavelength distribution compared to X-rays. This issue has been discussed [95] but rarely addressed in the literature prior to the work of this dissertation. Recently there has been new calculation models proposed which account for the resolution effect [96]. However to our knowledge, nearly no study has been showing the effect directly on experimental data.

In paper II we propose a model to calculate changes of measured intensity in GiSANS experiment as a function of incident angle, accounting for both wavelength and angular resolution of the instrument. In this approach, the intensity is considered to be a function of the contrast in the scattering length density of the structure for neutrons, and the intensity profile of the evanescent wave.

The model calculates the scattering length density of the sample by slicing the structure into planes parallel to the solid surface and estimating the fraction of each plane occupied by particles. Figure 4.7 is an illustration of parameters used to calculate the fraction of particles in each plane parallel to the surface. The chord $c$ of the spherical particles at a distance $\zeta$ from their center is calculated using:

$$R^2 = \left(\frac{c}{2}\right)^2 + \zeta^2$$  \hspace{1cm} (4.1)
where $R$ is the radius of particles and $\zeta$ is the distance of the chord from the center of the particle. The cross-sectional area is then given by:

$$\pi \left( \frac{c}{2} \right)^2 = \pi (R^2 - \zeta^2)$$

(4.2)

The fractional occupancy by material in a sphere at a distance $\zeta$ from the centre is:

$$f_s = \pi (R^2 - \zeta^2) / \pi R^2 = (1 - \zeta^2 / R^2)$$

(4.3)

Knowing the fraction of material in each slice of particles, the scattering length density at depth $z$ can be calculated as:

$$\rho_z = f_s \rho_{PS} + (1 - f_s) \rho_{water}$$

(4.4)

in which, $\rho_{PS}$ is the scattering length density of polystyrene and $\rho_{water}$ is that of water (which can change to any other materials). Figure 4 of paper II shows the scattering length density profile calculated for dispersion of particles with radius $R$, lattice parameter $a$, and separation of the structure from the solid surface of $z_g$, in D$_2$O. The contrast in the scattering

Figure 4.5. GiSANS signal measured for 8 wt. % PS3 latex in D$_2$O at silicon/water interface measured with $\lambda = 1.4$ nm close to the critical angle ($\theta_c = 0.9^\circ$).
length density is the difference between the scattering length density profile of the sample and that of the solvent. The amplitude of the intensity of the evanescent wave decreases exponentially as it penetrates deeper into the sample:

$$A(\lambda, \theta) = A_0(\lambda, \theta) \exp(-z_{1/e}/z)$$  \hspace{1cm} (4.5)$$

where $A_0(\lambda, \theta)$ is the initial intensity, $z_{1/e}$ is the penetration depth of the evanescent wave and $z$ is the depth into the sample.

The model creates a distribution of angles and wavelengths around the set values depending on the resolution function of the instrument. There is a probability associated with each wavelength or incident angle; the sum of all the probabilities is equal to 1. An example probability function created by the model is shown in Figure S6, supplementary information of paper II. The model calculates the penetration depth for all the possible angle and wavelength within the distribution and from that calculates the intensity of the evanescent wave at various depth.

Finally, the expected GiSANS intensities at each depth are calculated as a convolution of the contrast in scattering length density and the intensity profile of the evanescent wave which is weighted by the probability function.

$$I_s(\theta_i) = A(\lambda, \theta)^2 \Delta \rho(z)$$  \hspace{1cm} (4.6)$$

The intensities calculated using this approach provide information about the relative changes of GiSANS intensity as a function of incident angle of the beam but in no absolute values. Figure 4.8 shows the evolution of GiSANS intensities calculated for 8 wt. % PS3 particles in D$_2$O where the structure

![Figure 4.6. Penetration depth profile in a GiSANS experiment with and without accounting for the wavelength distribution of the instrument shown as $z_{1/e}$ and $z_{1/e}(\theta_i, \lambda)$, respectively.](image-url)
Figure 4.8. Changes of the measured intensity in a GiSANS experiment modeled for 8 wt. % PS3 particles in D$_2$O (lattice parameter of approximately 400 nm) forming a close-packed structure with different separations, $z_g$, from the interface. Data points represent the sum of scattering at first order Bragg peak, measured on the D22 instrument.

is formed: at the interface, with 100 and 140 nm separation from the solid surface. The plot shows the model calculation based on the resolution function of D22 instrument. The model has been tested against the experimental data by taking the intensity measured the first order Bragg peak at each incident angle. Figure 4.8 shows that the calculations follow the experimental data and can model the smearing of the intensity close to the critical angle well. The deviation of the curve representing different separations of the particles from a solid interface occurs at angles where several orders of magnitude higher flux is required to measure. Currently, available neutron sources cannot provide such a high flux, and even if the upcoming sources such as ESS could offer such a high flux, it is possible that the signal would be veiled by the dominant strong background intensity. This suggests that the GiSANS technique cannot be useful to probe the separation of particles from the interface, but a powerful tool to determine the spacing of particles from each other and defining the lattice parameter. The result of this dissertation showed that GiSANS is a powerful technique for studying in-plane correlation and even large structures (particles in the order of hundred nm) at buried interfaces. When the evanescent wave is expected to penetrate only a few nm from the interface, there will be a transmitted wave to a depth of several micrometers due to the spread in wavelengths and angles of incidence, which can allow studying ordered structure extending micrometers into bulk. This highlights the importance of the instrument resolution on understanding the measured signal. One of the critical challenges in a GiSANS experiment is the limitation of suitable programs available to quantitatively analyze the data.
in particular when it comes to the structures at solid/liquid interfaces. The proposed approach provides an analysis tool for experimental data to a certain extent, but more importantly offers a simple tool for calculations prior to experiments in order to develop the sample, choose the appropriate instrument or modify instrumental settings. The method is not limited to the structure of a particular system or instrument; it can be used to estimate the expected intensities for any known/expected structure measured on any instrument with a known resolution function. For instance, the model has also been tested on PS11 particles measured on NG3, SANS instrument with a lower wavelength resolution (see figure S3 supplementary document paper II).

4.3 Tuning the interactions of particles with the interface using proteins

It has been suggested that the self-assembly of colloidal particles offers a low-cost and readily available technique, for patterning useful nanostructures for photonic and electronic devices [70]. Polystyrene latex particles have been shown to self-assemble themselves into highly crystalline structures close to the solid/liquid interface. Self-assembly properties of such systems have made them an interesting and potentially applicable system. However finding a method to lock the particles in place and retain the structure has been a challenge; particles tend to move due to capillary forces, and the structure tends to crack in this process.

So far we have shown that Moringa seed proteins can bind to a variety of surface (i.e., polystyrene, silica, and alumina) and also to themselves. This suggests that they can influence the interactions between the particles and solid surface by binding to both. QCM measurements from the dispersion of latex particle on bare quartz crystal showed an increase in the frequency of the oscillating quartz crystal with added particle dispersion. When the dispersion of particles was injected onto a pre-adsorbed Moringa seed protein layer, the frequency decreased. As expected from the Sauerbrey equation, the decrease of the oscillating frequency is an indication for the adsorption of firmly interacting components at the interface. AFM images of silicon substrates showed sticking of particles to the surface with adsorbed protein layer, even after rinsing the surface with water. AFM was used to acquire real space images of the surface from dried samples, complemented by neutron reflectometry experiments, to obtain structural information of stuck particles at the wet interfaces. Figure 4.9 shows neutron reflectivity data from the 0.5 wt. % PS3 particles dispersed in D$_2$O at a quartz surface before and after rinsing with water, when no protein layer was adsorbed to the interface (top panel) and when particles were injected into the cell after the adsorption of protein layer (bottom panel).
Figure 4.9. Schematic of the structure of particles near solid surface in the presence and absence of *Moringa* seed proteins before and after rinsing with water. Reflectivity data and model fits of each schematic are shown on the right panel.

In the absence of a protein layer, particles were rinsed away from the surface, and the reflectivity data could be modeled with the same parameters used for modeling the bare surface. In the presence of the protein layer however, fringes appear which are an indication of an adsorbed layer on the surface that was not removed with extensive rinsing. Rinsing with water only made the features in reflectivity more visible, as it could remove particles and flocks from the bulk of the cell and decrease the diffuse scattering which can broaden the peaks.

The method was tested for smaller particles (PS11), model fits to the reflectivity data showed adsorption of particle layer with coverage of 17 % for PS3 and 13 % for PS11, from the dispersions with the particle concentration of 0.5 wt. % in water.

It is worth mentioning that one of the disadvantages for using protein seeds in water purification (described in the first section of this chapter) purposes is that seeds can release water-soluble proteins and organic matter in the processed water. There have been suggestions that sand pre-coated with *Moringa* seed proteins after rinsing with clean water, can be used as antimicrobial filters [97]. The result of the present work can provide complementary information regarding the surface excess of proteins and particles in order to adjust the required amount depending on the turbidity of water.

In summary, the results showed that seed proteins could act as a molecular glue to lock particles in place. This can be valuable information for various
applications: for instance, to obtained patterned nanostructures by simply preadsorbing protein layers on parts of a solid surface solid, or separating desired components of a complex system.

4.4 Surfactants and lipids at solid/liquid interfaces

Perfluoroalkyl substances are emerging pollutants posing challenges for human health and the environment. In the past decades, there have been increasing concerns regarding their biological and environmental toxicity. There are two conceptually different hypotheses proposed to explain bioaccumulation behavior of perfluorinated substances (PFASs): one hypothesis is based on the partitioning into phospholipids, as they have a moderate affinity for PFASs, and the other is based on the binding of PFASs to proteins [98]. The present dissertation has focused on exploring the first hypothesis. Several studies have reported that PFASs accumulate highly in lipid-rich tissues of the brain (e. g. brain stem) [99, 100]. It has been suggested that PFASs can partition into the membranes and change the fluidity of the bilayer [101].

In this dissertation, the interaction of a wide range of PFASs has been studied with phospholipid bilayers, to allow comparison of the effect of chain length and the functional group of PFAS molecules. Neutron reflectometry and off-specular scattering were used to model the structural changes and a molecular level.

The experiments were primarily performed with a DMPC bilayer at 35 °C to explore the effect at temperatures where the lipid bilayer is in the same phase as is in a biological membrane. The bilayer was deposited on the silicon crystal by vesicle fusion and characterized in three contrasts to obtain the thickness of different region, the amount of lipid and roughness of the layer. Reflection from the bilayer at different concentrations of PFASs was then measured in two contrasts (H2O and D2O) and the bilayer thickness, area that each lipid bilayer occupies, the number of PFASs in bilayer were modeled by simultaneous fitting of reflectivity in both contrasts at each concentration.

Figure 4.10 shows examples of reflectivity data and model fits for the bilayer without, and when exposed to 0.06 and 0.3 mmol L⁻¹ FOSA (C₈, sulfonamide head group), as an example.

Model fits to the data showed that nearly all the measured PFASs in this study, interact with the phospholipid bilayers by partitioning into the layer and displace some of the lipids to accommodate themselves. As PFASs penetrate into the bilayer, the bilayer becomes rougher and for some of the PFASs (e. g. PFHxA and PFBS) possibly patchy. The interaction of PFASs of the same head group with the bilayer was found to increase with the length of the fluorocarbon chain of the PFASs. A comparison between the penetration of PFASs which have the same chain length but different functional groups
Figure 4.10. Reflectivity data and model fits from DMPC bilayer, and the DMPC exposed to 0.06 and 0.3 mmol L$^{-1}$ FOSA in D$_2$O (blue) and H$_2$O (green). The schematic represents the interactions as the concentration of PFASs in the cell increases.

(third column in Table 3.1) showed that sulphonamide groups have the highest partitioning into the bilayer, followed by the sulfonic acids and then carboxylic acids. These results are summarised in Figure 4.11, choosing the concentrations which are in common among different compounds.

PFBS (C$_4$, with sulfonic acid in its head group) and PFHxA (C$_6$, with carboxylic acids in its head group) were the short-chain substances measured in this study. PFBS was measured up to 88 mmol L$^{-1}$ and PFHxA up to 50 mmol L$^{-1}$. Two-dimensional scattering maps from these compounds at the highest measured concentration, showed a diffuse off-specular scattering, which is possibly the Yoneda scattering [102]. Yoneda scattering is an optical effect, which occurs when the incident angle of the beam is close to the critical angle of the interface (the relative refractive index is close to unity). At this condition, the intensity of the scattering (including the diffuse scattering) is greatly enhanced, resulting in the appearance of so-called Yoneda peaks. The roughness of the surface provides a wide range of angles for the incident beam, which means there is a higher probability for parts of the beam to meet the critical condition of the interface where Yoneda scattering can be enhanced. For the samples described here, the Yoneda scattering appears due to the bilayer becoming rough or possibly patchy. Aggregation of PFASs in an aqueous solution has been reported previously [103]. There is a possibility that
Figure 4.11. Penetration of PFASs into DMPC bilayer, as volume fraction of bilayer occupied by PFASs, comparing the effect of chain length and functional group of PFASs on their interactions. Parameters used to model lipid reflectivity data from lipid bilayer are shown on the figure.

at high concentrations, PFASs aggregate within the bilayer making the bilayer appear rough or patchy. These results show that even short-chain PFASs (e.g. PFBS and PFHxA) can partition into the bilayer, deform the layer, make it rough and create defects in the bilayer.

Rinsing the bilayer with water showed to remove most of the PFASs; however the bilayer left behind was rough and less dense compared to its initial state. It has been reported that deformation or disorder of bilayers in living organisms can increase the risk of neurological diseases such as Alzheimer’s [104, 105]. The results of this dissertation suggest that even short-chain compounds which are known as safe substances [83], can interact with the bilayer and deform the structure, but at higher concentrations compared with long-chain molecules.

DMPC has the transition temperature of about 24 °C, above which the lipid bilayer is in a liquid phase and below in a gel phase. In the liquid phase, individual lipids have a relatively high degree of lateral mobility within the bilayer, whereas in the gel phase molecules are locked in place. In paper IV, the interactions of PFASs have been discussed with DMPC in the liquid phase of the bilayer. Follow-up experiments were performed to understand whether the partitioning of PFASs into the bilayer is different for the same bilayer at a
more rigid phase. For this test, PFBS at a concentration close to its solubility limit was chosen, since it had shown strong interactions with DMPC bilayer at its liquid phase. After characterizing the bilayer at 35 °C, the temperature of the cell was decreased to 15 °C and PFBS solution was injected into the cell. Neutron reflectivity from the bilayer was then measured at both 15 and 35 °C. Figure 4.12 shows reflectivity data from DMPC bilayer at 35 °C before being exposed to PFBS solution (in red) and after 88 mmol L$^{-1}$ PFBS exposed to the bilayer at 35 (a) and 15 °C (b) (in blue). Modeling the data showed that penetration of PFBS into the bilayer was significantly lower in the gel phase. When PFBS was injected at 35 °C, approximately 15-20 % of the bilayer was occupied by PFBS molecules whereas when PFBS was injected at 15 °C PFBS molecules occupied only up to 5 % of the bilayer.

To further explore how the membrane rigidity can influence penetration of PFAS molecules into the bilayer, penetration of PFBS into DMPC bilayer was compared DOPC lipid bilayer. DOPC is a phosphocholine lipid with two oleoyl chains that each contain one carbon-carbon double bond, and forms a softer bilayer compared to DMPC. After characterizing DOPC bilayer at 35 °C, 88 mmol L$^{-1}$ PFBS was injected into the cell, and similar to DMPC bilayer, DOPC bilayer was characterized in two contrasts. Figure 4.12c shows reflectivity from DOPC bilayer before and after 88 mmol L$^{-1}$ PFBS at 35 °C. Comparing Figure 4.12a and c, it is evident that changes in reflectivity for DOPC bilayer is significantly stronger. Reflectivity data could no longer be modeled using the bilayer scattering with a defined head and tail region, but rather a rough and thicker layer. Fitting the data for both DMPC and DOPC bilayer with the same model, indicated a layer with nearly two times higher PFBS in DOPC bilayer compared to the DMPC bilayer.

Figure 4.12. The effect of bilayer rigidity on the PFASs penetration, all the curves represent measurements at 35 °C: DMPC bilayer before and after injecting PFBS at 35 °C (a); DMPC bilayer before and after injecting PFBS at 15 °C, measured at 35 °C (b); and DOPC bilayer before and after injecting PFBS at 35 °C [Nouhi et al. 2018 unpublished data]
4.5 Interface roughness effect on self-assembled multilayers

So far, we have shown how some components of soft matter like particles, proteins and a mixed system of surfactants and phospholipids interact and form structures at solid/liquid interfaces. All these structures have been studied on the polished face of single crystals (typical roughness < 1 nm). In more realistic systems, however, interfaces are not as smooth. For example, healthcare products are applied to the skin which typically has micrometer length scale roughness, or ink in a simple pen functions by sticking to a paper with a roughness much greater than our polished crystals.

![Figure 4.13. Schematic representation of the lamellar structure and an example reflectivity data with the model fit from 55 vol.% Brij L4 in D₂O.](image)

The physiochemical properties of the interface profoundly influence the interaction of soft matter components. It has been shown previously that the structure and adsorption of surfactants and lipids are different against smooth and rough surfaces [106, 107]. In this section, we will discuss to what extent the roughness of the crystal can be important for the self-assembly of a highly ordered system. For this purpose, Brij L4 (C₁₂H₂₅(OCH₂CH₂)₄OH), a non-ionic surfactant was chosen at concentrations of 55 and 40 vol.% in water, where lamellar structures are formed. The structure of the Brij sample was measured against two different surfaces: a smooth polished silicon crystal, and a silicon crystal of the same batch which had been etched for 24 hours using pure Decon-90 (pH ∼ 13). Neutron reflectivity showed that the root mean square roughness for the smooth surface was < 6 Å and for the rough surface approximately 30 Å. In order to distinguish between the distortion due to the surface roughness and thermal distortion of the sample, the sample against each surface was measured at five temperatures (between 20 to 60°C).

The structure of lamellae was modeled using the byban model available on the web page electronically [92]. The model calculates reflectivity of \( N \) repeating bilayers by divining each bilayer into four layers of head, tail, head, and tail, each of which can be varied independently.

![Diagram of lamellar structure and byban model](image)
Figure 4.14. Reflectivity data and model fit for 55 vol.% Brij L4 in D$_2$O at the smooth surface after heating (red triangle) compared with the reflectivity at 20 °C to show the effect of heating the sample (a), and compared to the reflectivity from the same sample at a rough surface to indicate the effect of surface roughness (b)

and a solvent layer, and allows an extra term for thermal fluctuations of the bilayers. For the data presented in this study, the thickness of head, tail and solvent layer, area per surfactant molecule, number of repeats of the bilayer, and the root mean square fluctuation amplitude were fitted to obtain the best model for the measured data. Figure 4.13 shows reflectivity data, and the model fit from 55 vol.% Brij L4 in D$_2$O, against the smooth surface with a schematic representing the structure and fitting parameter used to model Brij lamellar phase.

Reflectivity data from the Brij sample, at the smooth surface, showed several orders of intense Bragg peaks which could be modeled with a lamellar structure extending from the interface several micrometers into the bulk. The intensity of the Bragg peaks increased with raising the temperature of the sample; fits to the data showed that increasing the temperature assists the ordering and alignment of the lamellae with the interface, while the thickness of different regions of an individual bilayer remained nearly constant at different temperatures. Heating the sample to 60 °C and cooling down to 20 °C (annealing the sample) showed a significant increase in the alignment of the bilayers.
The mean root displacement ($\xi$) of the bilayers due to thermal fluctuations were found to be similar for the sample against both surfaces (approximately 10% of the bilayer thickness) and showed an increase with rising temperature. $\xi$ for bilayer with a repeating distance $d$ can be related to Caillé parameter [108], $\eta$ by:

$$\eta = \frac{\xi^2 \pi^2}{d^2}$$  \hspace{1cm} (4.7)

and Caillé parameter for a membrane can give an estimate of bending modulus, $K$, and the compression modulus, $B$, using [109]:

$$\eta = \frac{\pi k_B T}{2d^2 \sqrt{KB}}$$  \hspace{1cm} (4.8)

where $k_B$ is Boltzmann’s constant, $T$ is the temperature, and $d$ is the repeat distance of the bilayer. Caillé parameters calculated for the lamellae were found to increase from 0.04 to 0.18 (at 20 °C and 60 °C respectively). These values are in the same range as reported for DMPC bilayer for instance [109], which suggests that Brij L4 lamellar is expected to have similar elastic moduli as a saturated phospholipid bilayer.

Figure 4.14a shows reflectivity data and model fits from 55 vol.% Brij L4 in D$_2$O after injecting the sample into the reflection cell at 20 °C and after annealing the sample, measured at 20 °C. The increase in the intensity of the peaks showed that the number of layers parallel with the interface increased about 25% after annealing.

Figure 4.14b shows the difference between reflectivity data at the smooth and rough surface after heating and cooling the sample at 20 °C. Reflectivity from the sample at the smooth surface showed sharper and more intense Bragg peaks, in particular for the higher order peaks. This is an indication of a better-aligned sample. Fitting the number of bilayers that are contributing to the observed reflectivity, showed about 70 sheets parallel with the interface at the rough surface, while 120 against the smooth surface.

Figure 4.15 shows the detector image at a fixed angle for the sample at the smooth surface on the left and at the rough surface on the right side, both when the first order Bragg peaks are at the specular reflection condition. The plots on the bottom panel show the sum over vertical pixels on the detector for the same peak at different sample temperatures. Increasing the temperature increased the intensity of Bragg peak and made it sharper. The Debye-Scherrer formula [110] allows determination the grain size of the crystallite, $D$, by:

$$D = \frac{0.9 \lambda}{\beta \cos(\theta)}$$  \hspace{1cm} (4.9)

where $\beta$ is the full width half maximum (FWHM) of the Bragg peak in radians and $\theta$ is half of the scattering angle at which the Bragg reflection occurs. Grain sizes calculated using the Debye-Scherrer relation from the width of Bragg
peak at the specular reflection condition showed similar values as the results obtained from model fits. The number of layers parallel to the surface showed an increase with temperature for the sample against both surfaces; this trend at all the temperatures was noticeably more significant for the sample against the smooth surface.

The FWHM when the Bragg peak is away from the specular reflection condition provides an estimate of the crystallite size in the bulk of the sample. Domain sizes in the bulk of the sample against the smooth surface, corresponded to about 25 layers while for the rough surface corresponds to 17 layers. This suggests that the influence of surface roughness can extend to many layers into the bulk of the sample.

Due to the challenges in the data reduction process (discussed in paper V), reflectivity data for 40 vol.% Brij sample could not be fitted. However, changes in the intensity of different order Bragg peak with the temperature or against different surfaces were similar to the 55 vol.% sample.

One of the common parameters which gives an estimate of the alignment of the structure is the FWHM of the rocking curve around the Bragg peaks. Figure 4.16a shows the rocking curves measured around the first order Bragg

\[\text{Figure 4.15. First order Bragg peak at the specular reflection after heating the sample (top) and the vertical sum over the pixels on the detector at different temperatures (bottom).}\]
peak for 55 vol.% Brij L4, and figure 4.16b for 40 vol.% Brij L4 in D$_2$O. In both plots, the black lines indicate the rocking curves for the sample at the rough surface and the red lines for the smooth surface. Annealing of the sample (solid lines), makes the curves sharper and more intense, suggesting that it can assist the alignment of the layers with the surface. Annealing effect was similar against both surfaces. At the same temperature, rocking curves for the sample against the smooth surface were significantly sharper and more intense than those against the rough surface. This is a clear indication of distortion of the structure that is induced by the surface roughness and is seen to affect the structure up to several micrometers away from the interface.
5. Conclusions and future perspective

The present work has focused on exploring the physicochemical factors that influence the interactions and ordering of molecules and particles at a solid/liquid interface. By choosing simple model systems, relevant aspects have been studied to describe under which circumstances particular phenomena become dominant. A part of this study emphasizes understanding of the physics of self-organizing systems, and the other part applies the knowledge from simple materials to more complex systems to address relevant environmental issues. Neutron scattering was the primary technique used in this study to explore the structures below an interface at a molecular length scale. This provides a powerful tool but also can pose some challenges regarding the interpretation of the data; these methodological issues have been discussed and addressed in the present dissertation.

5.1 Environmental aspects

The use of a sustainable, environmentally friendly and low-cost alternative to conventional flocculating agents in water purification is attracting increased attention. *Moringa oleifera* seed proteins have been proposed previously as an effective flocculent for clarifying turbidity of water. A comparative study between the seed proteins of the well-studied *Moringa oleifera* and that of *Moringa peregrina* with particles and mineral model surfaces showed that the proteins extracted from seeds of both species can be used in a similar way for clarification of water. This is very valuable as *Moringa peregrina* is native to many different regions of the world and the use of species that are readily available in an area can decrease the cost and increase the efficiency significantly. Despite the similarities, structure of adsorbed layers and the amount of bound proteins were shown to be different at surfaces with different surface chemistry (i.e. alumina and silica). The choice of flocculating agents and their known different interactions with particles could be developed to optimize separation processes such as those used in the mining industry to separate different ores.

Highly fluorinated (PFASs) surfactants used in the carpet and clothing industry, food packing products, fire distinguishing foams, etc. are known to be highly toxic and bioaccumulative. As a part of this study, the interaction of these substances with phospholipid bilayers, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and
1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), as simple model membranes was investigated. It was found that PFASs damage the bilayer by inserting into the bilayer and removing some of the lipids, leaving a thinner, rough (possibly patchy) and less dense bilayer behind. This effect was shown to increase with the length of fluorocarbon chain of the PFAS molecules or with the softness of bilayers. Short-chain substances, which have been reported as safe replacement alternatives, were also found to penetrate into the bilayer and damage the layer, at higher solution concentrations. The result of this study will be a significant step forward towards finding an optimal material with the lowest toxicity and understanding the concentration limits which should not be exceeded in the environment or living organisms. The present work has been exploring the effects on a simple phospholipid bilayer, further experiments on realistic systems such as biological and plant-like membranes, or with replacement PFAS alternatives, would provide a better understanding and a more reasonable type of choice.

5.2 Physics of self-assembled structures

Self-assembled structures of charge-stabilized polystyrene colloidal particles were studied at a solid/liquid interface. Grazing incidence small angle neutron scattering (GiSANS) measurement showed that particles can arrange themselves into well-ordered crystalline arrays close to a solid wall. The lateral extent of the structure was determined by the beam illumination area, which was approximately 20 cm². The depths of structure was estimated by the penetration depth of the beam and was in the order of several micrometers. The size of 'bulk domains' was determined, by small angle neutron scattering (SANS) experiment, from the beam area and the cell thickness to be < 1 mm². The result showed that GiSANS can be successfully applied to obtain in-plane structural information. The findings in the present work highlighted the importance of instrumental resolution on the recorded signal and the interpretation of experimental data. A simple model was proposed, accounting for the resolution effect, in order to analyze the scattering data in GiSANS experiments. The model can be implemented for predicting the expected signal and can help to design more successful experiments by choosing instrument settings or type of sample.

Crystalline structures of colloidal particles at a solid/liquid interface can be used directly as templates for patterning two-dimensional nanostructures. To make such structures applicable, the major challenge is locking the particles in place and preserving the ordering during the drying process. The described in this dissertation have shown that Moringa seed proteins can act as a molecular glue with sticking probability of unity to lock the particles in place. This observation can pave the way for patterning more complex nanostructures by pre-adsorbing surfaces with the proposed molecular glue. Apart from locking
the structure, pre-adsorbing protein glue on a solid surface, can be used as a method to separate certain components from a complex dispersion, without leaving *Moringa* proteins dissolved in the solution. For example, as suggested earlier (Jerri et al. 2011) using solid surfaces with a layer of seed proteins after rinsing, helps to clarify turbidity of the waste and groundwater without leaving extra dissolved proteins or organic matter.

The present work has shown that physical properties of an interface such as surface roughness can strongly affect the self-assembly of molecules at an interface. Small roughness in the order of a bilayer thickness, can suppress the lamellar structure of a nonionic surfactant, which forms well-ordered and aligned structures at a smooth surface. The distortion due to the surface can extend to a depth of several micrometers from the solid surface. The sample of 55 vol.% polyethylene glycol dodecyl ether (Brij L4) in D$_2$O was found to be a good model system since it can self-assemble into well-ordered and aligned lamellar sheets showing intense Bragg reflection peaks. Heating the sample was shown to improve the alignment of the lamellae with the solid surface and increased the number layers to over 120. The thermal fluctuations of the sample at both surfaces were found to be similar (<10 % of bilayers thickness). Further studies are underway correlating the fluctuations due to small surface roughness to larger length scale disorders caused by addition of particles.

This work has been exploring certain factors on self-assembly in simple model systems, that typically consist of one or two components dispersed in water at equilibrium. Realistic systems include more complex structures, where different components can also interact with each other and affect the final structure. There are various other factors that may alter the structure of soft matter at solid/liquid interfaces, for example, external fields like electric field, shear or flow, changing the dispersion and condition of the sample, etc. The present work can pave the path towards a better understanding of such effects.
6. Svensk sammanfattning

Några av de material som vi använder dagligen är svåra att klassificera i de vanliga aggregationsformerna fast, flytande och gas, exempelvis tandkräm, kosmetika, yoghurt, målarfärg, smörjmedel, etc. Den här gruppen av material kallas för mjuk materia. Mjuk materia deformeras lätt när den utsätts för yttre krafter men reagerar på krafterna väldigt långsamt jämfört med fasta eller flytande material. Mjuka materia består av komponenter som är större än en atom (Ångström) men mindre än en mikrometer, exempelvis polymerer, proteiner, partiklar, etc. Dessa komponenter är vanligtvis spridda i ett annat medium (fast, flytande eller gas) och kan interagera med varandra eller med mediet för att bilda olika strukturer. När dessa material träffar på en gränssyta (gränsen mellan två medier) kan de uppvisa ett helt annorlunda beteende och bilda en annan struktur. För många av tillämpningarna av dessa system är deras interaktion med gränstor della nyckelfaktor. Till exempel, för att höja kvaliteten på målarfärg är studier av interaktionen mellan färgpartiklarna och den solida väggen av högsta vikt. I den här avhandlingen har interaktionen av partiklar och molekyler vid en gränssyta mellan ett fast och ett flytande medium utforskas. Strukturen i molekylär storleksordning har analyserats för att förstå den grundläggande fysiken, samt relaterats till aktuella miljöfrågor. Neutronspridning har varit det huvudsakliga verktyget för att studera strukturen i molekylär storlek. En konsekvens av detta är att resultaten till stor del beror på en förståelse av spridningssignalen och modelleringen av denna, vilket kan innebära vissa utmaningar. Några av dessa utmaningar diskuteras i avhandlingen.

frön kan klumpa ihop partiklarna på ett liknande sätt som Moringa oleifera frön. Morgina peregrina kan därför användas för vattenrenning i regioner där Moringa peregrina är mer lättillgängligt än oleifera. Trots likheterna skiljer sig strukturen och den adsorberade mängden proteiner på olika ytor mellan de två arterna, vilket tyder på att effektiviteten av vattenrenningen kan höjas genom att välja ett visst protein, eller en blandning av proteiner, beroende på vilken typ av orenhet som föreligger (artikel I).

Kolloida partiklar (74 samt 140 nm i diameter) dispergerade i vatten kan bilda stora regelbundna kristallstrukturer i närheten av en fast yta, medan de orienterar sig mer slumpmässigt i mindre kristaller längre bort från ytan. Med neutronspridning kan man göra mätningar som belyser en stor yta (5×5 cm²) utan att tränga djupt in i provet (sk grazing incidence small angle neutron scattering, GiSANS). Denna typen av mätning användes för att studera strukturen hos de dispergerade partiklarna närmast den fasta ytan. GiSANS är ett kraftfullt verktyg för att studera ytstrukturer, men användbarheten av GiSANS begränsas av bristen på analysprogram för att tolka data, speciellt när det kommer till hur man ska hantera instrumentets upplösning. I den här studien påvisas effekten av instrumentets upplösning på neutrondatan, och en beräkningsmetod introduceras för att modellera spridningen (artikel II).

Dispergerade kolloida partiklar som utan yttre påverkan bildar stora regelbundna kristallstrukturer, speciellt i närheten av en fast yta, har stor

Högfluorerade ämnen (PFAS) består av en fluorkolkedja och en (ofta) laddad grupp. PFAS har använts i stor omfattning sedan 1950-talet för att de är så bra på att avvisa vatten och smuts, och används i stor utsträckning i matt- och klädindustrin, som förpackningsmaterial för mat, i eldsläckarkum, etc. De senaste åren har oron ökat för deras giftighet och den mängd som ansamlas i människor, djur och natur ökat. År 2012 påvisades t.ex. i Uppsala genom regelbundna prover hos gravida kvinnor att mängden PFAS i blodet var hög och dessutom ökade. Det visade sig bero på att PFAS hade förorenat grundvattnet och läckt in i dricksvattenbrunnar. I dagsläget finns inte tillräcklig forskning om hur PFAS påverkar människors och djurs hälsa. Men de studier som finns är mycket oroande. I den här avhandlingen har interaktionerna mellan PFAS och modellcellmembran undersökts..Resultaten visar att PFAS penetrerar membranen och avlägsnar delar av dem vilket leder till att de blir både tunnare och ytan ojämnare. Det upptäcktes att den här effekten ökade med längden av fluorkolkedjan hos PFAS, och att den varierar beroende på typen av laddad grupp (artikel IV).

De ovan nämnda studierna har alla utförts på slätta, mycket högpolerade kristaller med ojämnheter mindre än 1 nm. I mer realistiska scenarier är ytorna sällan så slätta. Exempelvis kan nämns hudvårdsprodukter, som ju används på hud vilken vanligtvis har ojämnheter i storleksordningen mikrometer, eller bläcket i en vanlig penna som fastnar på papprets yta med mycket större ojämnheter än våra polerade kristaller. För att förstå effekten som ytans grovhet kan ha valdes ett system bestående av en slags molekyler kallade tensider som gärna binder till ytor. I höga koncentrationer kan de dessutom bilda, på samma sätt som de kolloidala partiklarna utan yttre påverkan, regelbundna strukturer längs med ytor. Det konstaterades att även en väldigt lätt grovhet hos ytan (från 3 nm) kan störa strukturerna både precis invid gränssyten samt flera mikrometer djupare in i provet (artikel V).
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A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)