Water-based boundary lubrication with biomolecule additives on diamond-like carbon and stainless steel surfaces

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Abstract

Friction and wear incur high economic costs globally. It has been estimated that approximately 30% of energy is used to overcome friction. Developing new solutions, such as coatings, surface texturing and lubricants, to reduce friction in the boundary lubrication regime can have great importance to global energy savings in the future.

In this thesis, water-based lubricants with hydrophobin protein (HFBI, HFBII and FpHYD5) and quince mucilage additives were used to lubricate engineering materials such as diamond-like carbon (DLC) coatings, stainless steels and plastics. It was found that hydrophobins can form monolayers on stainless steel, diamond-like carbon (a-C:H) and PDMS surfaces. On stainless steel surfaces, HFBI and FpHYD5 layers contain 40-64% water. Increasing the water content in hydrophobin film reduced friction in hydrophobin-lubricated stainless steel vs stainless steel contacts. The same effect was seen in quince mucilage-lubricated UHMWPE vs stainless steel contact.

The lowest friction coefficients (COF) were measured in FpHYD5 hydrophobin-lubricated contacts where COF as low as 0.03 was measured. Quince mucilage-lubricated UHMWPE vs stainless steel reduced the friction coefficient to as low as 0.02. Of all the tests, the lowest friction coefficients (close to 0.01) were measured with HFBI and FpHYD5 hydrophobins in PDMS vs PDMS contacts.

Based on the results, it can be suggested that the requirements for water-based lubrication with biomolecule additives in industrial applications are
• A mild temperature range, T= 4 - 95°C
• Low contact pressures, 0.1-5 MPa
• Hydrophobic surfaces, contact angle of water >90°
• Stable conditions (pH, ionic strength)

In the future, water-based lubricants could be used in, among others, the food and beverage industry, the textile industry and biomedical applications.

Tiivistelmä

Kitka ja kuluminen aiheuttavat vuosittain merkittäviä taloudellisia menetyksiä. On arvioitu, että noin 30 % maailman energiankulutuksesta kuluu kitkahäviöihin. Uusien teknologisten ratkaisujen, kuten pinnoitteiden, pinnan teksturoinnin sekä voiteluaineiden, avulla voidaan kitkaa alentaa erityisesti rajavoitelualueella.

Tässä väitöskirjassa on tutkittu erilaisten hydrofobiini-proteiinien sekä kvitten hedelmästä liuotetun liman käytettävyyttä lisäaineena terästen, timantinkaltaisten pinnoitteiden (DLC) sekä muovien vesivoitelussa. Tutkimuksessa havaittiin, että hydrofobiinit kykenevät muodostamaan ruostumattoman teräksen, DLC:n sekä PDMS-muovin pinnoille yhden molekyylin paksuisia kerroksia. Teräksen pinnalle muodostuneessa proteiinikalvossa 40-64 % massasta oli vettä. Veden määrällä hydrofobiinien muodostamassa voitelukalvossa oli positiivinen vaikutus kitkan ja kulumisen alenemiseen teräs vs. teräs-kontaktissa. Myös kvitten limalla voidelluissa polyeteeni vs. teräs - kokeissa havaittiin, että molekyyleihin sitoutuneella vedellä on kitkaa alentava vaikutus.

Alhaisimmat kitkakertoimet (0,03) teräs vs. teräs kontaktissa mitattiin FpHYD5-nimisellä hydrofobiinilla. Alhainen kitka johtui teräskuulan pinnalle syntyneestä proteiinikalvosta. Kvitten limalla pystyttiin saavuttamaan alhaisimmillaan 0,02 kitkakerroin polyeteeni vs. teräs kontaktissa. Kaikista alhaisimmat kitkakertoimet saavutettiin PDMS vs. PDMS - kontaktissa, missä HFBI ja FpHYD5 hydrofobiinit alensivat kitkan lähelle 0,01:tä.

Tulosten perusteella voidaan todeta, että vesipohjaisilla voiteluaineilla on potentiaalia rajavoiteluun teollisissa sovelluksissa esimerkiksi elintarvike-, tekstiili- ja terveysteknologia-aloilla, jos lämpötilat, kontaktipaineet, materiaaliominaisuudet sekä olosuhteet ovat sopivat.

Preface

The research work for this PhD thesis was performed between 2010 and 2015 at VTT Technical Research Centre of Finland Ltd. Part of the experiments were carried out at Suzhou Institute of Nano-Tech and Nano-Bionics (SINANO), Aalto University, Technical University of Denmark (DTU), Saarbrücken University and University of Turku. I would like to thank all the personnel at these institutes for their help in conducting the experiments. In addition, I would like to thank the Finnish companies Picosun, Picodeon and Bionavis for their participation in the 'Nanolubrication by functional coatings and biomolecules' project. The research work was conducted in four research projects supported financially by VTT, the Academy of Finland and Tekes – the Finnish Funding Agency for Innovation.

First of all, I would like to thank Aino Helle and Erja Turunen who hired me at VTT and have always encouraged my work. At the beginning of my research career, Aino gave me some good advice and made a few comments that have helped me through these five years with the thesis. I would also like to thank my bosses Maria Oksa and Satu Tuurna at VTT who have always supported and encouraged me. When I arrived at VTT in 2010, I did not know anything about biomimetics or bio-lubrication. In the beginning, Tiina Ahlroos and Päivi Laaksonen were especially helpful, introducing me to the basics of biomimetics and lubrication.

A big part of the work was carried out in a tribology laboratory at VTT where a real tribology expert, Simo Varjus, taught me how to use different devices and perform the tests carefully to guarantee the reliability of the results. I must admit that sometimes assembling the disc with an error of less than 0.002 mm was frustrating, but at least I can now trust my results.

I would like to acknowledge Prof. Jari Koskinen from Aalto University, Prof. Kenneth Holmberg from VTT and Prof. Feng Li from SINANO. They all have different research backgrounds and they shared their different perspectives on the results.

I would also like to thank my co-authors for their support and advice while writing the thesis and conducting the experimental work. In addition, I would like to thank all my colleagues, especially at MK8 and KT3. Jarkko Metsäjoki must be mentioned. He

List of publications

This thesis is based on the following original publications, which are referred to in the text as I-VI (Appendix A). The publications are reproduced with kind permission from the publishers.

I   Ahlroos T, Hakala TJ, Helle A, Linder MB, Holmberg K, Mahlberg R, Laaksonen P, Varjus S. Biomimetic approach to water lubrication with biomolecular additives. Proceedings of the Institution of Mechanical Engineers, Part J: *Journal of Engineering Tribology*, 2011; published online: 1-10.

II   Hakala TJ, Laaksonen P, Saikko V, Ahlroos T, Helle A, Mahlberg R, Hähl H, Jacobs K, Kuosmanen P, Linder MB, Holmberg K. Adhesion and tribological properties of hydrophobin proteins in aqueous lubrication on stainless steel surfaces. RSC Advances, 2012;2:9867-9872.

III   Hakala TJ, Saikko V, Arola S, Ahlroos T, Helle A, Kuosmanen P, Holmberg K, Linder MB, Laaksonen P. Structural characterization and tribological evaluation of quince seed mucilage. *Tribology International*, 2014;77:24-31.

IV   Hakala TJ, Laaksonen P, Helle A, Linder MB, Holmberg K. Effect of operational conditions and environment on lubricity of hydrophobins in water based lubrication systems. *Tribology,* 2014:8(4):241-247.

V   Hakala TJ, Metsäjoki J, Granqvist N, Milani R, Szilvay GR, Elomaa O, Deng M, Zhang J, Li F. Adsorption and lubricating properties of HFBII hydrophobins and diblock copolymer poly(methyl methacrylate-b-sodium acrylate) additives in water-lubricated copper vs. a-C:H contacts. *Tribology International*, 2015;90:60-66.

VI   Shi Z, Hakala TJ, Metsäjoki J, Szilvay GR, Li F. Lubrication of aluminium versus diamond-like carbon contacts with hydrophobin proteins. Accepted to be published in Surface Engineering.

Author's contributions to the publications

I   Timo J Hakala wrote the introduction, participated in planning the contact angle measurements and analysed the results in the discussion. Tiina Ahlroos was responsible for the tribological experiments and submission. Riitta Mahlberg performed contact angle measurements.

II   Timo J Hakala planned the experiments, performed the tribological experiments, analysed the results and was responsible for writing the manuscript. Päivi Laaksonen carried out QCM and ellipsometer experiments. Riitta Mahlberg performed FTIR experiments. Vesa Saikko carried out CTPOD experiments

III   Timo J Hakala planned and performed the tribological experiments, which were carried out with a pin-on-disc tribometer, and analysed the results. Päivi Laaksonen was responsible for writing the manuscript. Vesa Saikko carried out CTPOD experiments

IV   Timo J Hakala planned and performed the experiments, analysed the results and was responsible for writing the manuscript. Päivi Laaksonen guided in performing the ellipsometer experiments. Suvi Arola helped in mucilage characterization.

V   Timo J Hakala planned and performed the experiments, analysed the results and was responsible for writing the manuscript. Niko Granqvist performed the SPR experiments together with Timo J. Hakala. Roberto Milani produced the polymer lubricant for experiments. Geza R. Szilvay instructed about the HFBII proteins. Mengmeng Deng helped to operate ICP-CVD coating deposition device. Simo Varjus performed part of the tribological experiments.

VI   Timo J Hakala planned the tribology experiments, analysed the tribology results and wrote the tribology part of the manuscript. Zhen Shi deposited a-C:H coating together with Timo J. Hakala, arranged the coating characterization and was responsible for writing the manuscript. Geza R. Szilvay

Contents

Appendices

     Publications I-VI

List of symbols

a-C:H   Hydrogenated amorphous carbon type of DLC coating

AFM   Atomic Force Microscopy

COF   Coefficient of Friction or Friction Coefficient

Da   Dalton, atomic mass unit, which is usually used to describe the size of biomacromolecules

DLC   Diamond-like carbon coating

IEP   Isoelectric point

MDa   Megadalton, 1000000 Da

OWLS   Optical Waveguide Lightmode Spectroscopy

PDMS   Polydimethylsiloxane

QCM   Quartz Crystal Microbalance

RP-HPLC   Reversed Phase High Performance Liquid Chromatography

SEM   Scanning Electron Microscopy

SFB   Surface Force Balance

SPR   Surface Plasmon Resonance

UHMWPE   Ultra-High Molecular Weight Polyethylene

1. Introduction

Environmental issues such as severe pollution (Fig. 1) and global warming are the result of increased use of fossil fuels and energy consumption. There are two ways to reduce pollution: 1) to find alternative, cleaner methods to produce energy and 2) to reduce the amount of energy needed. It has been suggested that about 15-35% of the world's energy is used to overcome friction (Holmberg, 2012, 2013, 2014). Thus, new ways to reduce friction and wear play an important role in decreasing the overall energy consumption. Friction and wear can be reduced by new low-friction, low-wear coatings, surface treatments and new lubrication solutions (Cai et al., 2013). One new possible lubrication solution includes a biomimetic approach to lubrication (Holmberg et al., 2012), which means learning from low-friction lubrication systems in nature and transferring these mechanisms to engineering lubrication systems.

Figure 1. Pollution is a severe problem, especially in China. Picture taken in Suzhou Industrial Park in 2014.

Lubrication can be improved by developing new solutions, such as coatings, surface texturing and lubricants (Fig. 2) that can reduce friction, especially in the boundary lubrication regime. Traditionally, oil has been the most used lubricant, but the fact that the world is running out of natural resources, such as oil, is a motivator to find alternative lubricants. In addition, there are several application areas in which oil cannot be used as a lubricant due to its harmful additives and the remnants it leaves on the final products. The use of oil also brings with it other problems such as waste treatment and harmful leakages.
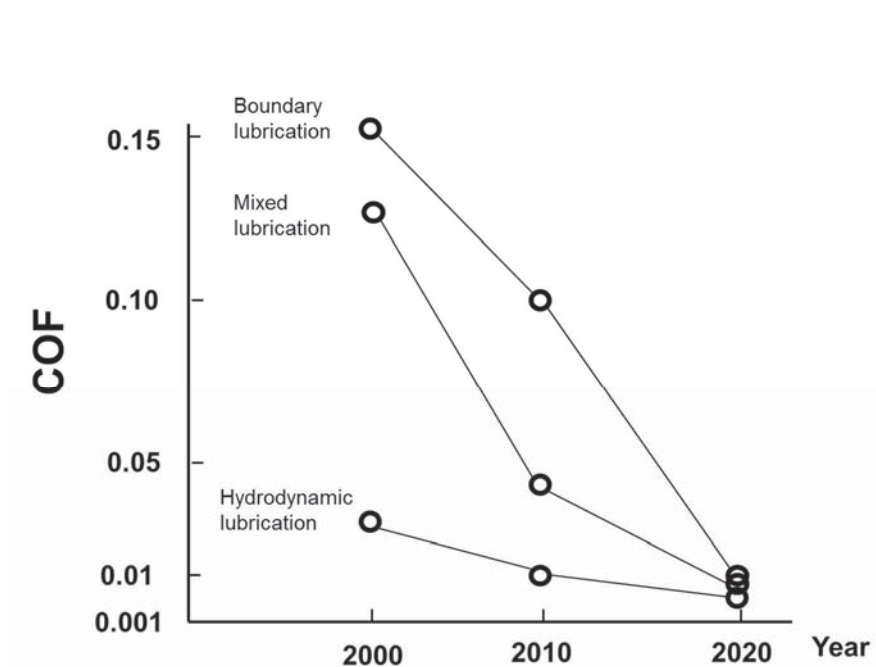
Figure 2. The development of lubricants has reduced friction in different lubrication regimes, and this trend will continue in the near future (modified from Holmberg, 2012).

Water is a fascinating option for a lubricant due to its low viscosity, good thermal conductivity and environmental friendliness. However, water is corrosive for common engineering materials, e.g. steel, and it has poor boundary lubrication properties. The temperature range for water lubrication is also limited compared with the use of oil because of evaporation. Nowadays, water-based lubricants are mainly used in low temperature applications that operate in a hydrodynamic lubrication regime, such as water-lubricated bearings.

Additives can be used to improve wetting, extreme pressure, and the corrosion and lubricating properties of water (Mortier et al., 1997). Improvements in boundary lubrication have attracted great interest from scientist in the past few years. Different additives, such as graphite (Chen et al., 2011), nanodiamonds (Elomaa et al., 2103; Kato et al., 2009), copper-containing nanoparticles (Zhang et al., 2012), ionic liquids (Phillips et al., 2004; Omotowa et al., 2004), amines, glycols (Tomala et al., 2010), polymers (Chawla et al., 2009), surfactants (Briscoe et al., 2006) and biomolecules (Coles et al., 2010; Lee et al., 2005), have been studied. According to patents, new water-based lubricants are proposed for use to lubricate different applications, such as railway switches, conveyors in the beverage industry, textile-processing machines and machining optical glass (N.A., 1980, Ruhr et al., 2004).

1.1   Lubrication regimes

Lubrication can be divided into three regimes: 1) boundary lubrication, 2) mixed lubrication and 3) hydrodynamic lubrication. In the boundary lubrication regime, contacting surface asperities carry the load and only lubricating layers with a thickness of some nanometres can reduce wear and friction. The boundary lubrication (Fig. 3) regime is achieved when the sliding velocity and lubricant viscosity are low or the surface roughness and normal force are high. Water has poor boundary and mixed lubrication properties, but due to its low viscosity it is a fairly good lubricant in the hydrodynamic lubrication regime. Unlike oils, water does not form solid-like layers under high contact pressures and thus it does not help prevent impact wear.
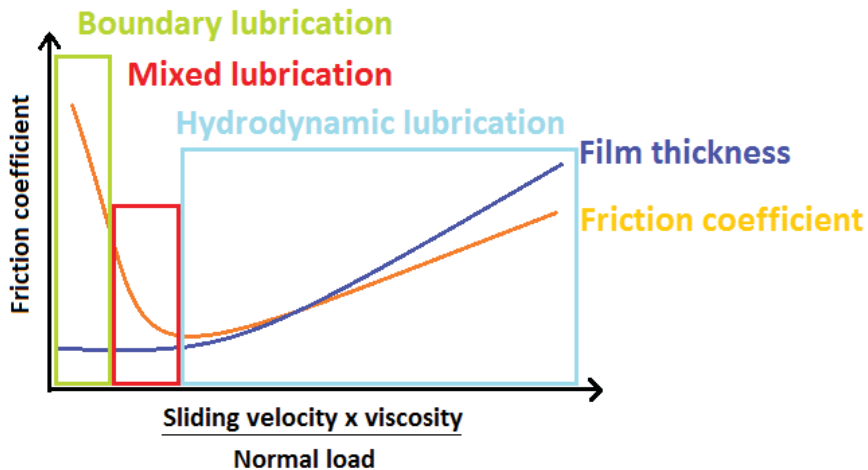
Figure 3. Stribeck's curve and thickness of lubricating film vs lubrication regimes in traditional oil lubrication

Boundary lubrication can be divided further into four regimes (Stachowiak and Batchelor, 2005):

1. Low temperature and low load
2. Low temperature and high load
3. High temperature and medium load
4. High temperature and high load

In the first two boundary lubrication regimes, the lubricating films are formed by surface-adsorbed molecules, called the adsorption lubrication regime (Stachowiak and Batchelor, 2005). These molecules adhere to the surfaces via physical or chemical bonds. The molecules that adhere via physical bonds are usually more easily removed from the surface due to shear and increased temperature. Thus, lubrication with these kinds of additives is limited to low contact pressures and has not gained much interest from scientist before (Fig. 4). In the third and fourth regimes, the lubricity is provided by lubricant additives that can react with the surface. These additives often contain sulphur, chlorine or phosphorus.
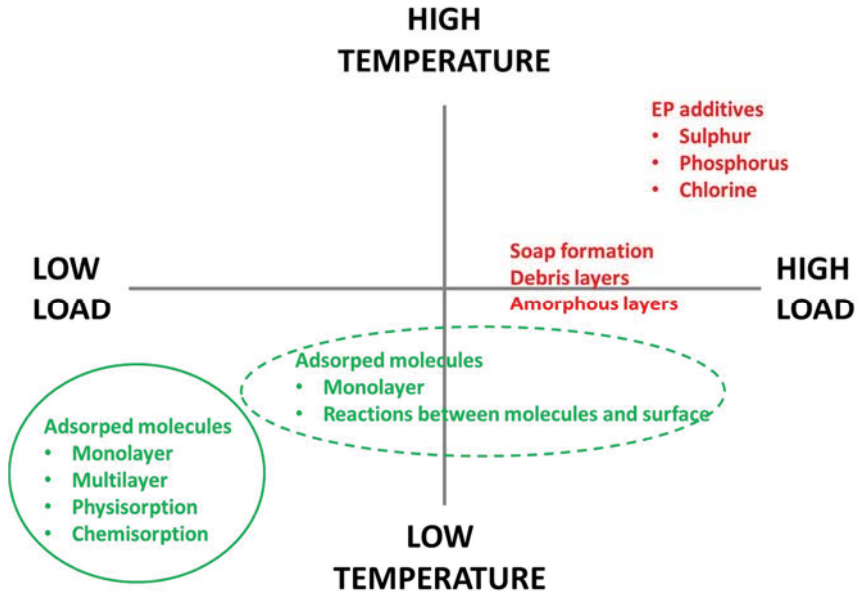
Figure 4. Lubrication mechanisms at different temperatures and under different normal loads (based on information from Stachowiak and Batchelor)

1.2 Boundary lubrication in nature

The interest in biomimetic lubrications comes from nature, where several examples of self-healing, low-friction lubrication systems can be found, such as mammal joints, lungs and eyes for instance (Neville et al., 2007; Dedinaite, 2012). The lubrication systems are complex including multilayer structures and fluid where lubrication is a result of several different molecules, including charged macromolecules and phospholipids. These highly stabilized lubrication systems with super-low friction coefficients below 0.01 can be measured in the boundary lubrication regime (Klein, 2012; IMechE; Dedinaite, 2012). Compared with oil-lubricated contacts, the friction coefficients are approximately 0.1 in the boundary lubrication regime.

The differences compared with man-made lubrication systems are that the lubricated materials are softer than industrially used materials, e.g. steel, the contact pressures and sliding speeds are relatively low, and the lubrication is water based.

1.3 Lubricating biomolecules

In nature, water-based lubrication systems include biomolecules, such as proteins, carbohydrates and phospholipids, and can remain low friction and low wear for close to 100 years, as seen in, e.g., mammal joints (Dedinaite, 2012). The adhesion forces of biomolecules to solid surfaces are high compared with traditional surfactant molecules, which can widen the adsorption lubrication regime to higher contact pressures. In addition, the biomolecules can have superior low friction properties compared with traditional surfactant molecules (Klein, 2012). At high temperatures, biomolecules can denature, which limits their use to low temperatures.

1.3.1 Proteins

Proteins are biopolymers consisting of amino acid units bonded by amide or peptide bonds. The properties of the molecule depend on the existing amino acids as well as primary, secondary, tertiary and quaternary protein structures (Hart et al., 2003; Nelson et al., 2008).
Lubricating glycoproteins in nature, such as mucin, aggrecan and lubricin, are relatively big: their size is usually a few MDas. The layers they form on solid surfaces can be sterically and electrically repulsive. Due to the big hydrophilic brush-like structures, they are able to bind large amounts of water (Lee et al., 2015; Zappone et al., 2007; Yakubov et al., 2009). While the hydrophilic part of the molecules binds water, the hydrophobic part can provide good adhesion to solid surfaces. Strong adhesion to surfaces prevents the molecules from shearing away easily from a surface under mechanical contact. Strong adhesion also helps molecules to be re-adsorbed onto the surface after being sheared away (Coles et al., 2010).

The structures of glycoproteins have inspired scientists to develop a similar type of polymer molecules. Unlike glycoproteins, the polymer molecules can be attached to the substrate via covalent bonding, which improves their load-carrying properties. With chemically attached polyzwitterionic brushes, friction coefficients as low as 0.0004 at contact pressure 7.5 MPa have been achieved (Chen et al., 2011).

1.3.1.1 Hydrophobins

Hydrophobins are small amphiphilic proteins produced by filamentous fungi that consist of about 100 amino acids. Hydrophobins are the most surface-active proteins known so far. Fungi use hydrophobins to modify their surface properties and control the interactions with their environment. For example, hydrophobins reduce the surface tension at the air-water interface to make it easier to breach the water-

air interface during their growth. On other hand, the hydrophobins can also make the surface of fungus more hydrophobic in an air atmosphere (Wösten et al., 1999; Sunde et al., 2008). Hydrophobins are divided into Classes I and II (Linder, 2004). Class II hydrophobins are suitable for lubricant additives in aqueous lubrication because they are water soluble. Class II has only been found in Ascomycetes, while Class I hydrophobins have also been found in Basidiomycetes (Linder et al., 2005).

The structure of Class II hydrophobins, such as HFBI and HFBII, consists of an aliphatic hydrophobic part and a hydrophilic part (Fig. 5). Inside the protein, there are four disulphide bridges that make the protein structure very stable against environmental changes. To act as a lubricant, additives in industrial applications, hydrophobins, have three important properties: 1) self-assembly and strong adhesion to solid surfaces, 2) high water binding ability and 3) stable structure that can withstand high temperatures (up to 90°C) without breaking (Askolin et al., 2006; Hakanpää et al., 2004).
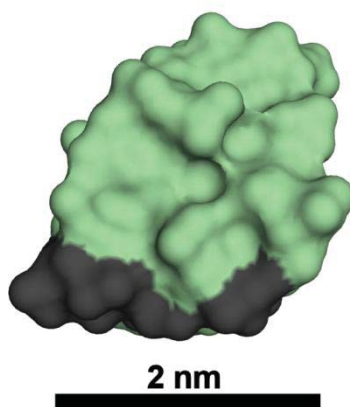
Figure 5. Structure of an HFBII hydrophobin protein. The green colour indicates the hydrophilic-exposed surface and the dark grey colour at the bottom indicates the hydrophobic patch. The diameter of the hydrophobic patch is approximately 2.2 nm. (Structure from Protein Data Bank entry 1R2M; Hakanpää et al., 2004)

The film-forming properties of hydrophobins are well known. They can form monolayer films on both air-water and water-solid interfaces without changes in their structures (Linder et al., 2005). Due to their amphiphilic structure, they can make hydrophobic surfaces more hydrophilic and hydrophilic surfaces more hydrophobic. The cationic and anionic properties also affect the adsorption and alignment of the hydrophobins on solid-water interfaces (Grunér et al., 2012). In solutions,

Class II hydrophobins form dimers and at high concentrations (10 mg/ml) they can form tetramers (Linder et al., 2005).

Earlier studies have shown that Sc3 hydrophobins are able to reduce the contact angle of water and friction in dry conditions measured on polymer surfaces in nanoscale by AFM (Misra et al., 2006). More recent studies have shown that HFBI and FpHYD5 hydrophobins are able to reduce friction significantly in water-based lubricated PMDS/PMDS contact (Lee et al., 2015). In both systems, the friction coefficients dropped to 0.01-0.02. Higher friction coefficients (0.11-1.9) were measured between two HFBI layers by surface force balance (SFB). The friction was dependent on the environment and substrate hydrophobicity, which affects the alignment of the molecules (Goldian et al., 2013).

More than 70 different hydrophobins are found in nature (Linder et al., 2005). In this study, three of these have been studied, namely HFBI, HFBII and FpHYD5. HFBI and HFBII are Class II hydrophobins, which have different amino acid sequences, and their role in the biological function is different in nature, e.g. HFBII is found in spores while HFBI is found in the mycelium of vegetative cultures (Linder et al., 2005). FpHYD5 is a hydrophobin that has a similar structure to HFBI and HFBII except that it has a glycan group (mass 1695 Da) attached to its hydrophilic side (Sarlin et al., 2012).

The isoelectric points of hydrophobins are important because the environment affects the electric charges of the proteins, and the interaction between the proteins and the surface. Thus, the pH can affect the hydrophobin adsorption and film structure on solid surfaces.

1.3.2   Carbohydrates

Carbohydrates are molecules with combined hydroxyl groups and carbonyl groups. Carbohydrates can be divided into monosaccharides, oligosaccharides and polysaccharides depending on their structure. Monosaccharides are the smallest carbohydrate compounds, which means that they cannot be hydrolysed into smaller units. Oligosaccharides consist of at least two monosaccharides, and polysaccharides can contain thousands of monosaccharide units. Cellulose is one example of a polysaccharide (Hart et al., 2003; Nelson et al., 2008).

The high OH-group content of carbohydrate molecules makes them water soluble and highly hydrated (Stokes, et al., 2011). Their lubrication properties are dependent on the shape of the molecule, which affects lubricant rheology, the ability to entrain the contact zone and the adsorption properties (Garrec and Norton, 2012b). Surface hydrophilicity has been observed to improve spreadability of some carbohydrates on surfaces, though sometimes their adsorption is expected to occur via hydrophobic interactions (Zinoviadou et al., 2008; Stokes et al., 2011).

Some carbohydrates can retain their structure and properties in a wide range of environmental condition such as a temperature range of 25-70°C and pH values of 2-9, but bacterial growth is a problem and thus industrial use in lubrication will be limited.

1.3.2.1   Quince mucilage

Quince mucilage is extracted from the seeds of the quince fruit. When dry seeds are immersed in water, the mucilage is self-formed at room temperature. The quince mucilage contains water-soluble cellulose and other carbohydrates (Vignon and Gey). The cellulose consists of cellulose nanofibrils and hemicelluloses such as glucoronoxylans. The mucilage has several beneficial properties such as a large amount of swelling and a slippery appearance, probably due to the hydration of the carbohydrate structures, which has been observed to enhance lubrication in water-based systems and lower friction to a level of 0.005 measured between the mucilage surface and glass (Li J. et al. 2012). As the bacteria use carbohydrates as a nutrient, the problem with the quince mucilage is the bacterial growth, which contaminates the lubricant relatively quickly if the bacterial growth is not restricted by chemicals.

1.3.3   Phospholipids

Phospholipids are similar to fats and oils with regard to their molecular structure. The phospholipid molecules consist of a non-polar tail and polar headgroup. Phospholipids prefer to arrange membrane structures, which have an important role in, for example, biological systems.
The amphiphilic molecular structure of phospholipids makes them able to adsorb on different surfaces and form multilayer structures (Hills, 2012; Trunfio-Sfarghiu et al., 2008; Goldberg et al., 2011). Although phospholipids are not easily water soluble, they have been used in water-based lubrication systems as a lubricant additive. Their solubility in water requires higher temperatures (~ over 40 °C) or carrier molecules, such as lubricin, which improve their solubility in water (Hills, 2002). The lubrication properties of phospholipids are highly dependent on pH and ionic strength (Dekkiche et. al., 2010). Phospholipids are one of the few biomolecules that have been tested to lubricate engineering materials and still been observed to provide low friction under high contact pressures (Goldberg et al. 2011; Hills, 1995).

1.3.4   Buffer solutions

As biomolecules are sensitive to changes in the environment and their properties depend highly on pH and ionic strength, buffer solutions are used. Buffer solutions

are used to stabilize the pH and ionic strength to a chosen level. pH affects the electric charges in molecules, and ionic strength can affect the interaction between molecules as well as the water-binding ability and structure of the lubricating layer (Lee et al., 2005). Buffer solutions consist of small molecules and ions and can have a certain ability to lubricate in themselves. For example, anions can form hydrated structures and increase the local viscosity between sliding surfaces and thus lubricate the contact more effectively than water (Garrec and Norton, 2012a).

1.4   Boundary lubrication mechanisms

Biomolecules can form different kinds of structures on solid surfaces and lubricate the contacts via different mechanisms in the boundary lubrication regime.

1.4.1   Hydration lubrication

Most of the glycoproteins and polymers that form monolayers on surfaces are expected to lubricate solid surfaces via hydration lubrication. In hydration lubrication, the sliding occurs between the hydrophilic parts of the two molecule layers that are adhered to both sliding surfaces (Fig. 6), and low friction is related to the bound water and its fluidity (Klein, 2004).

Lubricating molecules prevent the water from escaping from the contact zone. Under high-contact pressures, the water molecules move between the liquid and the surface-attached molecules. This phenomenon provides low friction in lubrication under higher contact pressures. Due to the nature of water, i.e. that the viscosity is not pressure dependent, its lubrication properties differ significantly from those of oil. The lubricating macromolecules usually contain large carbohydrate structures and thus they have the ability to bind high amounts of water (Roba et al., 2009; Yang et al., 2006; Stokes et al., 2011; Li J. et al.; 2012).

Other important properties of lubricating macromolecules are their strong adhesion to surfaces, repulsion between molecule layers, and lateral forces in the layer and surface. All these properties affect the stability and structure of the lubricating film (Stachowiak and Batchelor, 2005; Klein, 2006). The hydration lubrication mechanism can be disturbed by the bridging of molecule layers adsorbed on both sliding surfaces. The bridging occurs most easily with neutral molecules. The charged molecules have stronger repulsion to each other, which prevents the bridging effectively (Klein, 2003).
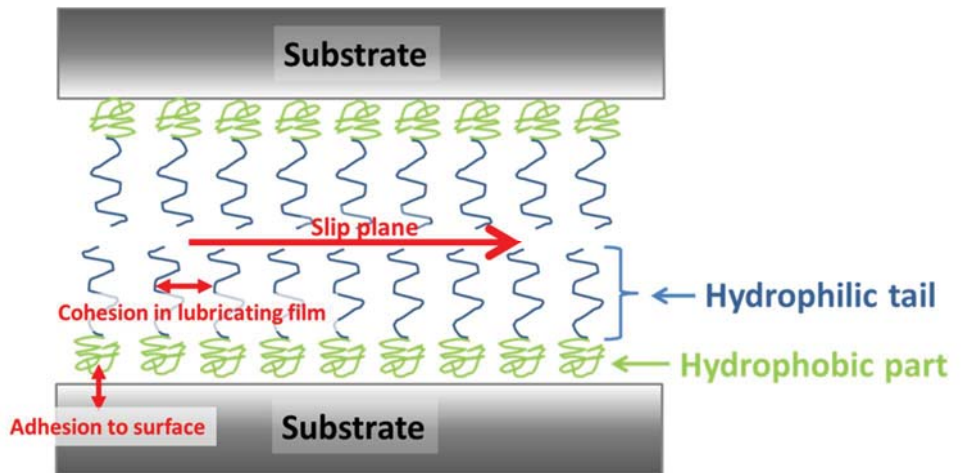
Figure 6. Schematic picture of hydration lubrication where the slip plane is located between adsorbed layers. Strong adhesion to the surface and cohesion inside the lubricating film increase the load-carrying properties of the lubricant film.

In some cases, the hydration lubrication can occur between the lubricating molecules and the substrate. This has been shown with polar surfactants where the sliding occurred between the polar headgroups and substrate via hydration lubrication (Briscoe et al., 2006).

1.4.2   Other boundary lubrication mechanisms

In addition to hydration lubrication, there are different mechanisms to explain low friction achieved by biomolecule additives in the boundary lubrication regime. These mechanisms are viscous boundary lubrication, slip plane between adsorbed layers, slip effect on the surface and graphitization.

In viscous boundary lubrication, friction is dependent on the molecule concentration in the lubricant. In this mechanism, biomolecules form multilayer films between the sliding surfaces, and increasing the concentration increases the film thickness (Fig. 7). An increase in film thickness reduces the local shear rate and shear stress and thus reduces friction in the sliding contact (Yakubov et al., 2009). An increase in local viscosity may also have hydrodynamic effects between two sliding surfaces.
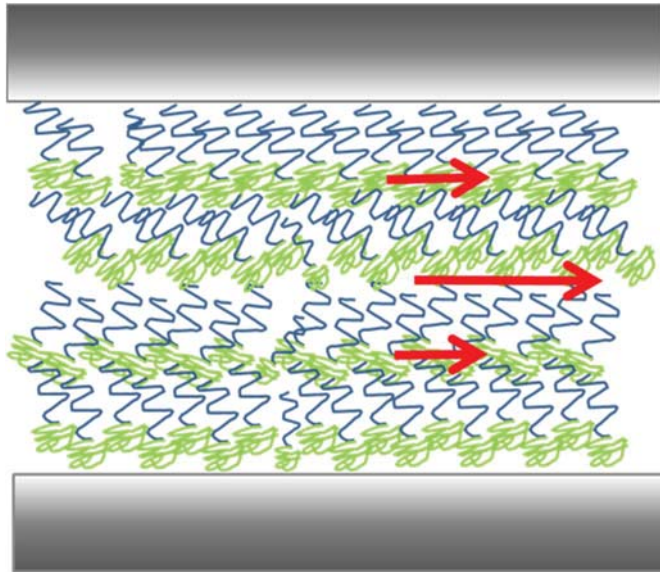
Figure 7. Water-based lubrication improved with biomolecule additives. Biomolecules are attached to the surfaces and bind water between two sliding surfaces.

Biomolecules mainly consist of carbon, nitrogen, oxygen and hydrogen. At high temperatures and pressures, biomolecules can degrade and form graphite on the sliding surfaces (Liao et al., 2011). This phenomenon depends on the surface materials, because the reaction to form graphite requires a catalytic material, e.g. a transition metal such as cobalt. In tribological contact, fresh surfaces are formed that can act as a catalyst for reactions. Graphite is a solid lubricant and known to lubricate well in humid conditions.

1.5   The effect of the environment

Environmental factors, such as ionic strength, pH and temperature, can affect the adsorption and film structure of lubricating films formed of biomolecules on solid surfaces (Lee et al., 2005; Madsen et al., 2014). The film structure affects, for example, the repulsion forces between opposing molecule layers, the cohesive forces in lubricating film, the adhesion forces and the amount of hydration.
It has been found that low friction with brush-like molecules is achieved when hydrophilic brushes protrude away from the solid surface, which happens, e.g., in water (Pettersson et al., 2008). The film may compress due to changes in pH and ionic strength, which can increase friction. There are also examples where the ionic strength affects the film structure but does not affect the lubricating ability of the biomolecule layer, i.e. the friction coefficient, but the wear of the layer at higher normal loads (Macakova, 2011). The wear may be increased due to reduced co-

hesive forces in the film, changes in adhesion forces or an increased number of entanglements in the contact.

It has been found that the larger the area of one molecule on a solid surface, the lower the internal cohesion inside the lubricating film. This has an effect on the adhesion forces between the film and the substrate (Heuberger, et al., 2005; Lee et al. 2015). Stronger adhesion between the molecule and solid surface increases the load-carrying capacity by preventing the shearing away of lubricating film.

High temperatures or shear forces can cause denaturation of the biomolecules. This affects their lubricity because of the formation of compact and well-adhered protein layers onto the sliding surfaces, which have a lower level of hydration (Heuberger et al., 2005; Fang et al., 2007). Unfolded molecules can have a lower water-binding ability than molecules in a natural state.

1.6   Water-based lubricant vs oil

Oil is still the most used lubricant in many applications. It has relatively good boundary lubrication properties and several other benefits compared with water-based lubricants. Oil is not as sensitive to aging and temperatures as water-based lubricants. However, the friction coefficients in the boundary lubrication regime can be magnitudes lower in water- and biomolecule-lubricated contacts than in oil-lubricated contacts (Stachowiak and Batchelor, 2005; Lee et al., 2005; Harvey et al., 2011).

A general comparison between oil and water-based lubricants in the boundary lubrication regime is difficult to make because the lubrication performance is highly dependent on the lubricated materials. For example, oil lubricates hydrophilic vs hydrophilic contacts well but water lubricates hydrophilic vs hydrophobic contact better (Borruto et al., 1998). Water with lubricant additives is found to lubricate hydrophilic-hydrophobic contacts most effectively (Pawlak et al., 2011)**.** Thus, surface wettability has a strong effect on lubrication by water-based lubricants. When lubricating metallic materials under high contact pressures, the oil additives containing reactive groups of sulphur, phosphorus and chlorine can react chemically with the surface and form very thin easily shearing layers that prevent excess wear (Stachowiak and Batchelor, 2005). Most biomolecules are physically adsorbed and cannot form this type of chemically reacted layers. Thus, their ability to lubricate is limited to low-contact pressures.

1.7 Aim of the work

The aim of this thesis is to study the potential of biomolecules for use as lubricant additives in water-based lubrication for materials used in engineering applications in the future (Fig. 8). It requires understanding of the connection between film formation and lubrication performance as well as determining the conditions where low friction and wear can be obtained. The performance of biomolecules in lubrication has been studied with artificial materials before but not with engineering materials such as stainless steel and diamond-like carbon coatings.
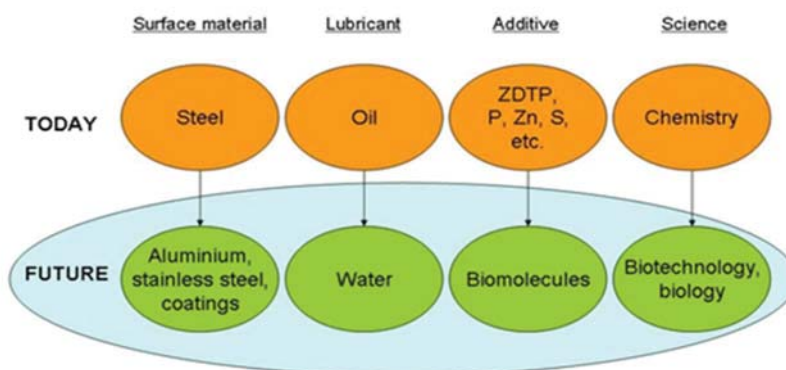
Figure 8. Possible changes in lubrication systems toward environmentally friendly lubrication in the future may include utilizing biotechnology and water (Publication I).

The scientific hypothesis is that biomolecule additives can form lubricating layers on engineering materials and reduce friction in the boundary lubrication regime compared to traditional lubricants such as mineral oil with extreme pressure (EP) additives.

By understanding the molecule structure and properties and their relation to lubrication performance, new lubricant additives with tailored properties can be developed in the future. In addition, new lubrication systems could be designed by taking account of the requirements for operational conditions, environment and lubricated materials.

2. Experimental

2.1 Materials

2.1.1 Lubricants

The hydrophobins (HFBI, HFBII and FpHYD5) and quince mucilage lubricants were all produced at VTT Technical Research Centre of Finland. The lubricants and their most important properties are listed in Table 1.

Table 1. Lubricants and their main properties related to lubrication performance

Lubricant	Molecule mass (g/mol)	Isoelectric point	Properties
HFBI	7540	5.7*	Strong adhesion to solid surfaces
HFBII	7200***	6.7**	Strong adhesion to solid surfaces, lower cost to produce than HFBI
FpHYD5	9210	4-5	Higher water-binding ability compared with HFBI
Quince muci-lage	-	-	High water-binding ability

*Wang et al. 2010, **Kisko et al., 2008, ***Staminirova et al., 2013

Hydrophobins were produced using recombinant strains of Trichoderma reesei and purified by two-phase extraction and reversed phase high-performance liquid chromatography (RP-HPLC) as described previously (Linder et al., 2004; Sarlin et al., 2012). The proteins were dissolved in different buffer solutions and water for the experiments.

Quince seeds (Cydoniaoblonga) were separated from fresh fruit, dried in an ambient temperature and stored in dry conditions before use. Mucilage was extracted from the quince seeds by immersing the seeds in fresh water. The seeds were weighed and kept in a 40 mg/ml concentration in deionized water overnight. The solution was decanted and the extraction repeated once more. The extract contained some solid impurities originating from the seeds, which were removed by gentle centrifugation and filtration through three layers of cotton gauze. The mucilage was stored at 4°C. The dry mass of the mucilage, 0.55 m%, was determined by lyophilization. The mucilage could also be lyophilized and re-dispersed in deionized water to gain a higher concentration. The mucilage was washed with ethanol as described earlier (Vignon and Gey, 1998). Concentrated mucilage was dispersed in three parts ethanol and centrifuged. The excess ethanol was removed and the mucilage dialyzed and freeze-dried. Part of the mucilage was freeze-dried without dialysis and used in the lubrication studies (Publication III).

The buffer solutions used in the experiments are listed in Table 2 (Publication IV).

Table 2. Buffer solutions used in the adsorption and tribology experiments

pH	Ionic Strength	Buffer solution
pH 3	50 mM	50 mM citric acid – sodium citrate
pH 5	50 mM	50 mM sodium acetate
pH 7	50 mM	50 mM tris(hydroxymethyl)aminomethane – HCl
pH 9	50 mM	50 mM glycine-sodium hydroxide
pH 5	500 mM	500 mM sodium acetate

2.1.2   Materials for tribological experiments

Tribological experiments were carried out with pins and discs manufactured from different materials (Fig. 9). The materials and their properties are listed in Table 3.
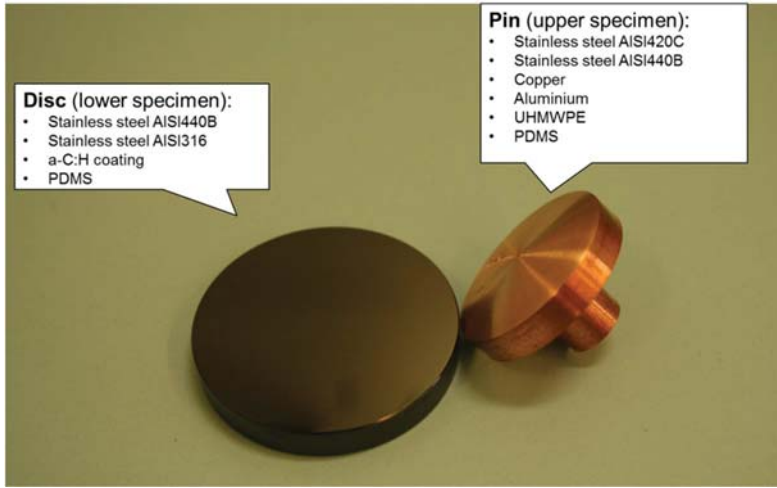
Figure 9. Discs and pins for tribotests

Table 3. Material properties of pins and discs

Pin/disc material	Surface roughness (R_a)	Hardness
AISI440B	0.05 µm or lower	567 HV1
AISI420C		927 ± 22 HV1
a-C:H	below 0.05 µm	20.3 GPa (nanohardness)
AISI316	0.01 µm	200 HV1
Aluminium	0.05 µm or lower	82.1 HV1
Copper	0.44 ± 0.03 µm	96.0 ± 1.1 HV1,
UHMWPE	Not measured	Not measured
PDMS	Not measured	Not measured

Stainless steel spheres (AISI420C) with a diameter of 10 mm were purchased from a commercial source. The spherical-shaped pins with a radius of 50 mm were made of AISI440B, copper and aluminium. PDMS pins were spherical shaped. The ultra-high molecule-weight polyethylene pins (UHMWPE) were used in the POD and CTPOD experiments. The UHMWPE pins had flat contact surface areas of 12.6 mm^2 (POD) and 63.6 mm^2 (CTPOD). An unused surface of the pin was used in each experiment.

Stainless steel (AISI440B) discs were purchased from a commercial source and then polished in-house at VTT. The diameter of the discs was 40 mm and the surface roughness R_a value was 0.05 µm or better. The same disc was used in

several tests and before each tests it was cleaned by ultrasonic washing in petro-
leum ether and in ethanol, for 5 minutes each.

The a-C:H coatings were deposited on stainless steel discs by ICP-CVD. The
coatings had a total thickness of approximately 1 μm. The coating thickness in-
cluded a SiN$_x$ adhesion layer with a thickness of 300 nm.

2.2 Adsorption measurements

To study the adsorption and film-forming ability of hydrophobin on stainless steel
and other surfaces, the experiments were carried out using the following methods:
a quartz crystal microbalance with dissipation monitoring (QCM-D; Publication II),
an ellipsometer (Publications II and IV), surface plasmon resonance (SPR; Publi-
cation V) and optical waveguide lightmode spectroscopy (OWLS; Hakala and Lee,
2011). The ellipsometer, SPR and OWLS measure the dry mass of adsorbed
molecules on solid surfaces while QCM-D also takes account of the mass of
bound water

Adsorption measurements by QCM-D (Publication II)
A quartz crystal microbalance with dissipation monitoring (QCM-D) was used for
simultaneous measurement of frequency and dissipation to follow the binding of
proteins to stainless steel sensors (SS2343; Biolin Scientific Sweden). First, the
sensor chips were cleaned in a standard UV/ozone chamber for 10 min and then
exposed to a hot $H_2O/NH_3/H_2O_2$ mixture (1 : 1 : 5) for 10 min, followed by rinsing
with Milli-Q water. The protein adsorption measurements were carried out by in-
jecting 1 ml of protein solutions into either Milli-Q water (Millipore, US) or 50 mM
sodium acetate (Sigma-Aldrich, US) using a flow of ~ 0.1 ml/min to the chamber
and following the frequency and dissipation responses.

Adsorption measurements in liquid by ellipsometry (Publication II)
Ellipsometric measurements were carried out using a multiwavelength ellipsome-
ter operated at a single wavelength of 532 nm. The device was set up in a PCSA
(polarizer-compensator-sample-analyser) configuration with an angle of incidence
of 65° to the surface normal. A stainless steel QCM-D sensor was placed in a
home-made liquid cell that was filled with the corresponding liquid. After equilibra-
tion of the surface, the protein solution was injected into the cell by hand with a
syringe. Before performing the experiments, the sensor was cleaned in a similar
manner to the QCM-D experiments. The ellipsometric angles D and Y were rec-
orded continuously over time via the nulling ellipsometry principle in two zones
(Tompkins and Irene, 2005).

Adsorption measurements of dry films by ellipsometry (Publication IV)
The adsorbed amount of protein was estimated by measuring the effective film thickness using a multiwavelength ellipsometer operated at a single wavelength of 532 nm. A polarizer-compensator-sample-analyser configuration was employed. Data were collected between the angle of incidence from 45 to 83° reflected from a polished stainless steel surface that had been immersed in the liquid of interest for 60 min, rinsed and dried. The experiments were carried out at 40-45% relative humidity and a temperature of 20.4°C. The film thickness results were obtained by fitting the measured data to an optical box model consisting of stainless steel and the protein film. The complex refractive index for the stainless steel was measured from a clean surface, and a constant refractive index of 1.460 was assumed for the protein film (Arwin, 1998). The absolute amount of adsorbed protein for which the effective thickness was calculated was determined with de Feijter's formula (de Feijter et al., 1978).

Surface Plasmon Resonance (SPR; Publication V) is a surface-bound optical phenomenon that can be used to study interactions and optical layer properties, such as the refractive index (RI) and thickness (d) of materials. While commonly applied in biomolecular screening (Rich and Myszka, 2010), new methods such as the now utilized Multi-Parametric surface plasmon resonance (MP-SPR) allow us to characterize interactions between biomolecules and surfactants with different coatings (Granqvist et al., 2013; Orelma et al., 2011).
The MP-SPR instrument together with gold and copper-coated sensors was used in the measurements. The a-C:H coating was deposited on the gold sensor as described in Section 2.1. The measurements were performed at 20°C using a flow rate of 30 µL/min. The HFBII interaction between a-C:H and Cu surfaces was studied in pure water (Milli-Q) and in an acetate buffer (50 mM sodium acetate, pH 5.0). Each interaction experiment consisted of a series of HFBII or polymer injections with concentrations of 3.9, 15.6 and 62.5 µg/mL respectively. An exception to this was a polymer interaction with a Cu surface, which was performed with a single injection of 250 µg/mL. The amount of HFBII was calculated from the SPR signal level after all the injections, and the initial slope of the first HFBII sample injection was used to calculate the relative interaction rate. The a-C:H coating thickness was determined using SPR Navi LayerSolver v.1.0.2.2.4.

Adsorption experiments by Optical waveguide lightmode spectroscopy (OWLS)
OWLS was used to study the adsorption of the proteins on polydimethylsiloxane (PDMS) surfaces. At the inlet of OWLS, hydrophobin sample solution was injected into the device and allowed to mix with the flow of buffer solution (50 mM sodium acetate buffer, pH 5), finally reaching the target surfaces. Adsorption of the proteins was determined as the change in refraction index in the vicinity of the surfaces (Hakala and Lee, 2011).

2.3   Tribological experiments

Tribological experiments were carried out with a pin-on-disc (POD) tribometer designed and manufactured at VTT Technical Research Centre of Finland (Fig. 10; Publications I-V). Ultra-high molecular weight polyethylene (UHMWPE) vs stainless steel experiments were carried out by POD (Publication III) and Circular translation pin-on-disc (CTPOD; Publications II-III; Saikko, 2006). In these experiments, a flat-on-flat type of contact was applied. Polydimethylsiloxane (PDMS) vs PDMS reference tests were carried out with a pin-on-disk tribometer (CSM Instruments, software version 4.4 M, Switzerland). In these tests, 5 N normal load and sliding velocity 50 mm/s were applied (Hakala and Lee, 2011).

In POD tests, different test parameters (normal load, sliding speed, sliding distance) were applied (Fig. 11). Most of the experiments were carried out with 0.05 m/s sliding speed and normal load of 2 N or 10 N. The volume of the lubricants used was 0.6 ml in each experiment. Although here, bath lubrication was not used and the lubricant covered the entire wear track during the experiment (Fig. 12). All the tests were carried out at room temperature (22 ± 1°C) and evaporation was neglected in the tests. The duration was 40 minutes, unless stated otherwise.

The friction force was measured constantly during the tests. After the tests, the wear volume of the ball or pin was measured from the dimensions of the wear track and both sliding surfaces (ball and disc) were examined by optical microscopy. In interesting cases, the wear tracks were studied by scanning electron microscopy (SEM) and a 2D profilometer. It was not possible to determine the wear volume from the disc due to the duration of the experiments (usually 40 minutes), which did not cause a deep wear track on the disc even if lubricated with a relatively poor lubricant such as 50 mM sodium acetate (Fig. 13). Thus, the wear volume of the lubricated contacts was calculated by measuring the dimensions of the wear tracks of the pins that were sliding against the discs. The wear track dimensions have been used to calculate the apparent contact pressures.

Figure 10. Pin-on-disc designed and manufactured at VTT
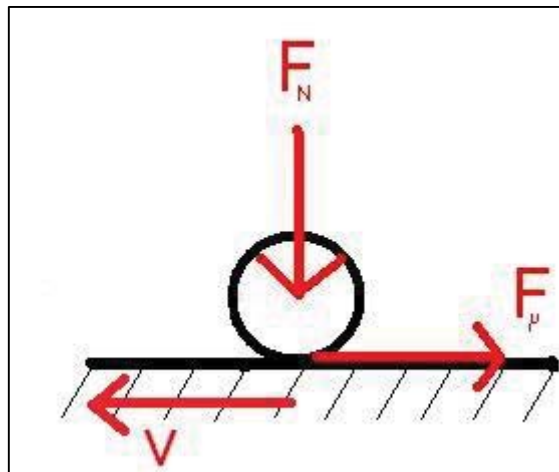
Figure 11. Ball on a flat-type contact. After initial contact of the surfaces, the contact type changes to flat on flat and the contact pressure decreases as a function of wear
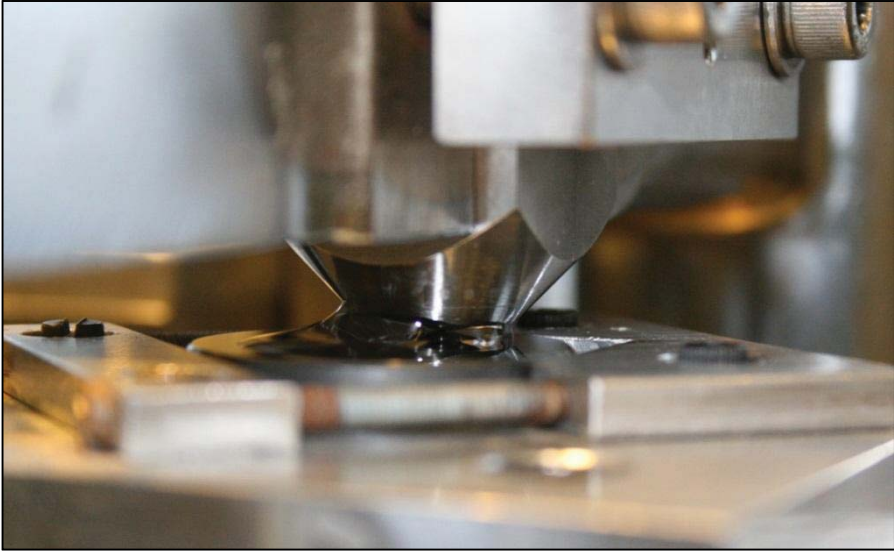
3

Figure 12. Contact of stainless steel ball and stainless steel disc lubricated with 50 mM sodium acetate buffer, pH 5
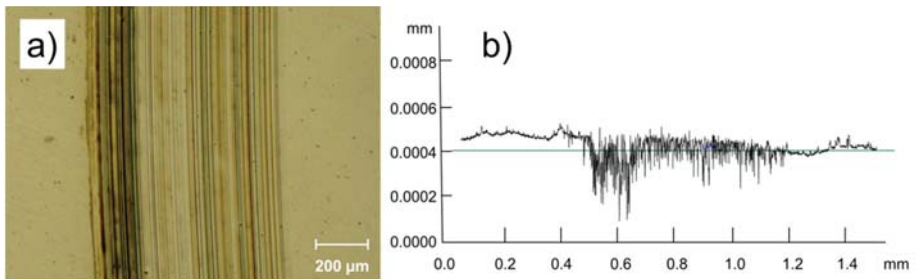
Figure 13. a) Optical microscopy image and b) 2D profile of wear track on stainless steel disc after pin-on-disc experiment lubricated with 50 mM sodium acetate buffer (pH 5). The test parameters were normal load 10 N, sliding velocity 0.1 m/s and duration 40 min. The amount of wear was hard to determine quantitatively.

2.4   Characterization

For the contact angle measurements an Attension Theta optical tensiometer (from Biolin Scientific) was used at 22.5°C and 50% relative humidity (RH). The sessile drop method was used to measure the contact angles of water on surfaces. The Young-Laplace equation was used for fitting the drop profiles. The size of

the water droplet was 5 µl and the contact angle of the water was determined as an average from 5 parallel experiments. For the contact angle, the measurements concentration of HFBII solutions was 0.1 mg/ml. The HFBII layer was formed by an addition of a 0.5 ml solution on the a-C:H surface, and after 30 minutes of adsorption, the excess solution was washed away with 1.0 ml of Milli-Q water. After washing, the a-C:H surface was dried with air (Publication V).

Scanning electron microscopy (SEM) + energy dispersive spectroscopy (EDS) (Zeiss Ultraplus + Thermo Fisher Scientific Ultradry EDS detector) was used to characterize wear tracks on copper and aluminium pins as well as a-C:H coating (Publication V).

Raman spectroscopy (Horiba Jobin-Yvon Labram HR Raman, 488 nm) was used to characterize oxide films on a copper pin (Publication V). The Raman spectrum of tribofilm on a stainless steel surface was obtained with a spectrometer equipped with a microscope with 615 and 640 objectives. The laser was an Innova 300C FreDTM frequency doubled Ar^+ ion laser, which was operated at a 244 nm wavelength. A charge-coupled device camera was used for detecting the scattered light. The system was controlled and the data were processed with Grams32 software.

3. Results and discussion

3.1 Adsorption and film forming

In measurements carried out with a Quartz Crystal Microbalance (QCM) and ellipsometer it was found that both HFBI and FpHYD5 hydrophobins can form monolayers on stainless steel surfaces. On stainless steel surfaces, the maximum adsorbed mass was achieved with a 0.05 mg/ml concentration. The higher concentrations do not increase the amount of adsorbed molecules on the surface, which can be explained by the monolayer formation (Publication II). Similar results were observed in the experiments carried out on a-C:H and PDMS surfaces measured by SPR and OWLS, respectively (Publication V; Hakala and Lee, 2011). The adsorbed masses shown in Fig. 15 represent the maximum amounts of adsorbed molecules after additional injections did not increase the amount of adsorbed mass. Thus the error rates are small.

By comparing the dry masses of adsorbed molecules (Fig. 15) it can be seen that there is no great difference between the adsorbed amounts of hydrophobins on different surfaces. It is interesting that higher amounts of hydrophobins are adsorbed on the surfaces in water than in buffer solution. Water has a higher pH than acetate buffer (pH 5), which affects the surface charges of the hydrophobin proteins. Changes in surface charges may affect lateral interactions between hydrophobins and their interactions with the surface. In water, the net electric charges of hydrophobins are closer to 0 or negative because of the isoelectric points of hydrophobins, which are between 4 and 6.7 (Kisko, 2008; Table 1).
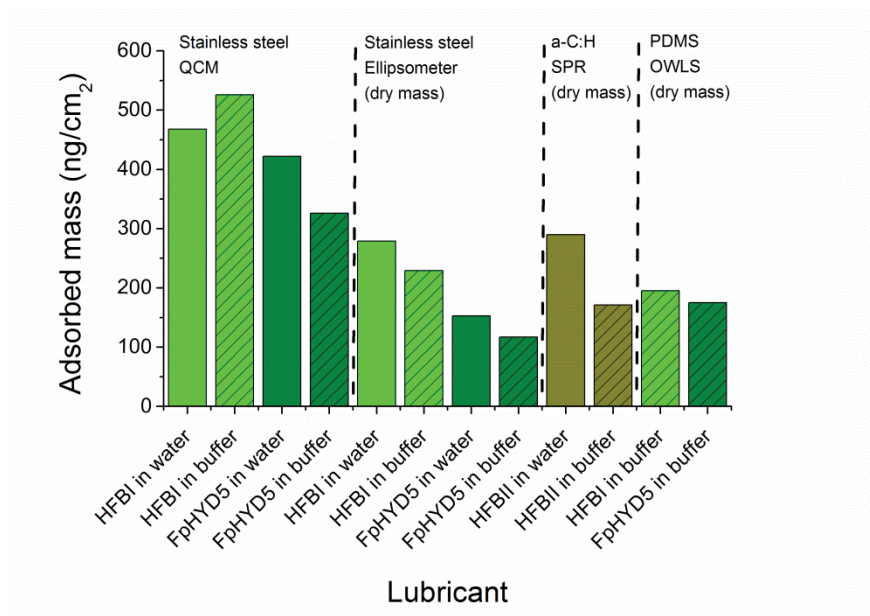
Figure 15. Adsorbed mass of hydrophobins on different surfaces. Ellipsometer, SPR and OWLS measure the dry mass of hydrophobins adsorbed on the surface while QCM takes bound water into account (Publications II and V; Hakala and Lee, 2011).

The effect of pH and ionic strength on the adsorption of FpHYD5 hydrophobins on stainless steel surfaces was studied with an ellipsometer (Publication IV). Increasing the ionic strength clearly decreased the amount of bound protein, whereas an increase in pH from 5 to 9 more than doubled the thickness of the bound protein layer (Fig. 16). The pH affects the net electric charge of the molecule and solid surface. Stainless steel surfaces consist mainly of iron oxides and chromium oxides. The isoelectric points (IEP) for these oxides are 5.2-8.6 and 7.0, respectively (Parks, 1965). For FpHYD5, the isoelectric point is about 4-5. The structure of the glycan group of FpHYD5 molecules is not known exactly and, thus, the exact IEP cannot be calculated. According to the adsorption results, it can be seen that the adhesion is increased significantly when the electric charges are both negative. At pH 5, the adsorbed mass of FpHYD5 on the stainless steel surface was significantly lower.
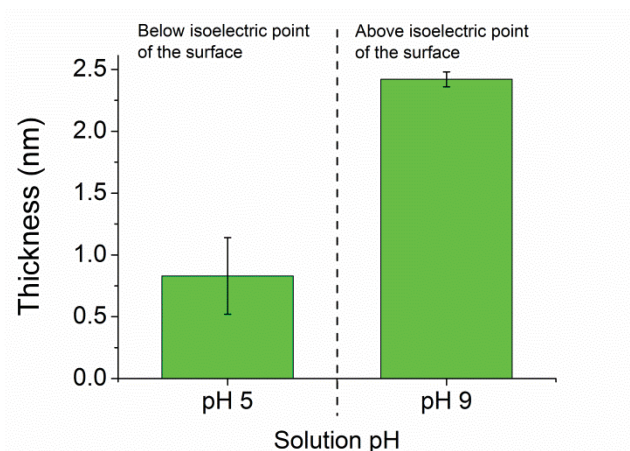
Figure 16. The thickness of FpHYD5 adsorbed on a stainless steel surface at pH 5 and pH 9 (Publication IV).

On the a-C:H surface, the HFBII hydrophobins reduced the contact angle from 75° to 60° (Publication V). On the stainless steel surface, HFBI hydrophobins did not affect the contact angle significantly, which was ~60° for a clean surface except with the highest concentration of 5 mg/ml (Publication I). The water contact angles on FpHYD5 layers formed on stainless steel surfaces at pH 5 and pH 9 were 59 and 40, respectively. It must be taken into account that the contact angles were measured on dried hydrophobin layers that may have had a different structure to the monolayer formed on a solution/solid interface. The results may also have been affected by the surface roughness.

The diameters of HFBI and HFBII hydrophobins are about 2-3 nm. The FpHYD5 molecule has a similar size except that it has a glycan group attached to its hydrophilic part. The size of the hydrophobic patch of hydrophobin proteins is about 4 nm^2. The molecular masses of hydrophobins are HFBII 7200 g/mol, HFBI 7540 g/mol and FpHYD5 9210 g/mol. The average area that the molecules occupy on different surfaces can be calculated from the adsorbed masses with the knowledge that hydrophobins form monolayer structures both on stainless steel and a-C:H surfaces. The average values for area per molecule on different surfaces are presented in Table 4.

Table 4. Area per molecule measured on different surfaces

Molecule	Surface	Area per molecule (nm^2)
HFBI[Publication II]	Stainless steel	4.6
FpHYD5[Publication II]	Stainless steel	14.7
HFBII[Publication V]	a-C:H	4.1
HFBI[Hakala and Lee, 2011]	PDMS	6.4
FpHYD5[Hakala and Lee, 2011]	PDMS	8.7

When comparing the area per molecule on stainless steel and a-C:H surfaces with the area on PDMS surfaces, the density of hydrophobins is higher on stainless steel and a-C:H surfaces. The only exception is the FpHYD5 molecule, which has a higher density on a PDMS surface, which is supported by the results published by Lee et al, 2015. This may originate from different alignments of the molecules on stainless steel surfaces compared with PDMS surfaces where hydrophobins are expected to adhere with their hydrophobic patch.

3.2   Tribology

3.2.1   Friction and wear

The hydrophobins reduced the friction in stainless steel vs stainless steel compared with water in the experiments performed with 2 N normal load and a sliding velocity of 0.05 m/s. In some experiments, the friction decreased to below the level of oil-lubricated contacts, though the wear volume was higher in the hydrophobin-lubricated contacts (Fig. 17). The lowest friction coefficient was 0.09, achieved with FpHYD5 hydrophobins in 50 mM sodium acetate buffer at pH 5. This friction coefficient was 24% lower than for the pure mineral oil-lubricated contact. Addition of 2% EP additives (yellow points in Fig. 17) reduced friction by 0.01 in mineral oil lubricated contacts compared to pure mineral oil. The lowest wear volume for a stainless steel ball was also measured in an FpHYD5-lubricated contact.
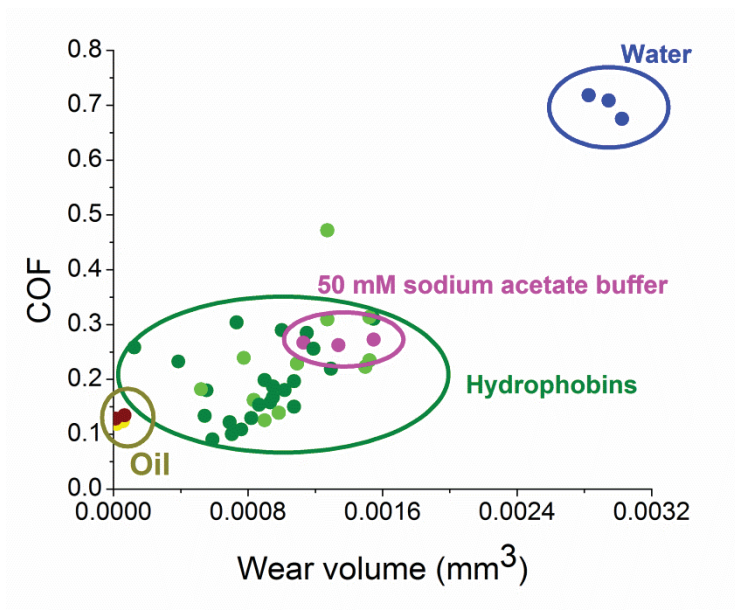
Figure 17. Friction coefficients (COF) vs wear volumes in stainless steel vs stainless steel experiments lubricated with oil, hydrophobins, buffer and water

Hydrophobins were also able to lubricate copper vs a-C:H and PDMS vs PDMS contacts better than water or acetate buffer. Quince mucilage was not an effective lubricant in stainless steel vs stainless steel contact but reduced friction significantly in UHMWPE vs stainless steel contact where hydrophobins had no significant effect on friction (Fig. 18).
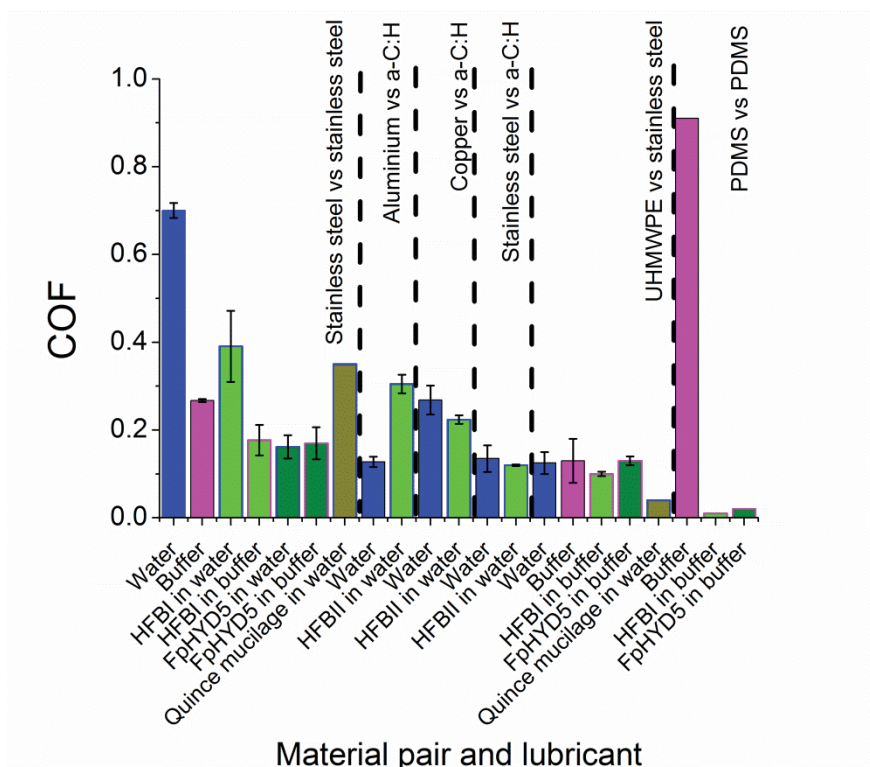
Figure 18. Lubrication of different material pairs with water, 50 mM sodium acetate buffer, hydrophobin proteins and quince mucilage (Publications I-VI; Hakala and Lee, 2011)

Water-lubricated stainless steel vs stainless steel contacts had friction coefficients of approximately 0.6-0.7. In hydrophobin-lubricated contacts, the friction coefficient decreased to a level of 0.1-0.2. Sodium acetate buffer (50 mM) solution was able to reduce the friction coefficient to 0.25-0.35. In hydrophobin-lubricated experiments, the friction coefficient was not dependent on normal load (2-100 N), but an increase in sliding velocity to 0.10 m/s caused an occasional decrease in friction coefficient in FpHYD5-lubricated experiments (Publications II and IV).

Quince mucilage was not able to lubricate stainless steel vs stainless steel contact. The reason for the poor lubricating ability of quince mucilage may be related to the repulsion between the negatively charged quince mucilage and stainless steel surfaces, which led to the removal of the lubricant from the contact zone (Publication III). However, while hydrophobins were not able to reduce the friction in UHMWPE vs stainless steel contact compared with water or sodium acetate buffer solution, the quince mucilage performed as an excellent lubricant reducing the friction down to 0.02-0.07 (Publication III). This shows that the material pair as

well as contact geometry can have a significant effect on lubrication performance with biomolecules.

The hydrophobin concentration did not have a big effect on lubrication after exceeding a concentration of 1.0 mg/ml. In smaller concentrations, there was a slight increase in friction and wear, which may be explained by breakage or partial removal of the lubricating film during the POD experiment (Publication II). The adsorption speed of hydrophobins on a stainless steel surface depends on the concentration. At low concentrations, the sheared molecules are not replaced by new ones as rapidly. The increase in quince mucilage concentration, however, reduced the friction in UHMWPE vs stainless steel contact. This indicated that while hydrophobins do lubricate by a monolayer, the quince mucilage lubricates better when it forms thicker multilayer structures between sliding surfaces (Publications II and III).

The lubrication of contacts with a-C:H-coated surfaces with hydrophobins was challenging. When the HFBII hydrophobins were added to water, the friction coefficient and wear increased in aluminium vs a-C:H contacts compared with water-lubricated experiments (Publication VI). In copper vs a-C:H contacts, the hydrophobins reduced friction by 13-30% compared with water but increased wear (Publication V). In stainless steel vs a-C:H contacts, hydrophobins did not have a significant effect on friction and wear.

The PDMS vs PDMS contact was the only one for which the hydrophobins were able to reduce friction to a level of 0.01-0.02. This contact has significantly lower contact pressures (0.36 MPa) and both surfaces are hydrophobic. In addition, in this contact, no significant wear of the surfaces occur. Lee et al., 2015, have published results on the performance of HFBI and FpHYD molecules in PDMS vs PDMS contact showing that friction was significantly lower compared with pure buffer solution in the boundary lubrication regime.

Determining the effect of hydrophobins and quince mucilage on wear in different contacts was challenging. In many contacts, the wear was below the determination limit, which depends on the profilometer. The effect on wear is presented in Table 5. In stainless steel vs stainless steel contacts, hydrophobins and quince mucilage reduced the wear compared with water. In experiments in which a-C:H coating was used, the wear of the counter materials (aluminium and copper) was increased when hydrophobins were added to the water.

Table 5. The effect of lubricants on wear compared with water or buffer solution in different contacts. (- = no experiments carried out)

Lubricant	Hydrophobins	Quince mucilage
Contact		
Stainless steel vs stainless steel	Reduced	Reduced
UHMWPE vs stainless steel	Not detected	Not detected
Aluminium vs a-C:H	Increased	-
Copper vs a-C:H	Increased	-
Stainless steel vs a-C:H	Not detected	-
PDMS vs PDMS	Not detected	-

3.2.2 The effect of adhered molecules on friction and wear

As seen in Section 3.1, although the hydrophobins form monolayers on different solid surfaces, the amount of adhered hydrophobin can affect the friction and wear. Based on the tribological experiments with stainless steel counter bodies (Publications I, II, IV), the increased dry mass of hydrophobins actually increases the friction and wear (Fig. 19). This indicates that the hydrophobin proteins are not lubricating in themselves. As Heuberger et al. and Lee et al. (2005) have shown, the increased number of adsorbed molecules increases cohesion between lubricant molecules and decreases the adhesion force between molecules and substrate and thus cause increased friction. This can explain the increased friction and wear in hydrophobin-lubricated contacts. However, a certain number of molecules are needed to adhere to surfaces because in pure water and buffer solution, lubricated contacts had higher wear compared with contacts lubricated with hydrophobin proteins.
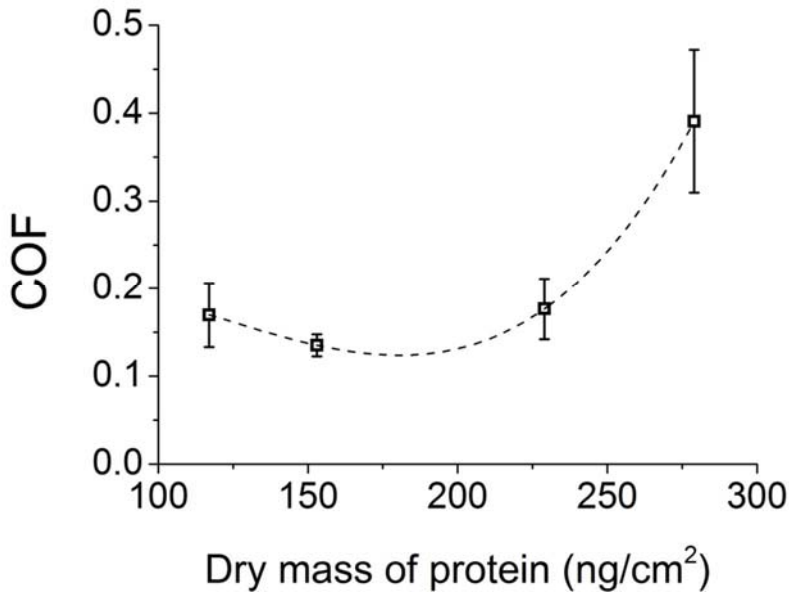
Figure 19. Friction coefficient of the dry mass of hydrophobin proteins adhered to a stainless steel surface (Publication II)

As seen before, the dry mass of FpHYD5 hydrophobins on stainless steel surfaces increased with increasing pH. The friction and wear of FpHYD5-lubricated contacts were measured at different pH levels from pH 3 to pH 9 using a constant load of 10 N and constant sliding velocity of 0.05 m/s (Fig. 20). The buffer solutions were studied as a reference system. At lower pH values, the friction and wear of the stainless steel vs stainless steel contact remained constant, but increasing the pH to 7 caused a small increase in both friction and wear. When the pH was increased to 9, the friction coefficient and wear of the buffer solution-lubricated contacts decreased slightly, whereas the FpHYD5 hydrophobins caused an increase in both friction and wear (Publication IV). The isoelectric point of FpHYD5 proteins is between 4 and 5 and chromium oxide has an isoelectric point at 7 (Parks, 1965). The increase in friction in FpHYD5-lubricated contacts occurs when both surface and molecule are negatively charged and the adsorbed mass of hydrophobins is also high (pH 9). It may be that at pH 9, hydrophobins form a very dense molecule layer on stainless steel surfaces, thus limiting the amount of water bound to the layer. This could explain the higher friction measured at pH 9.
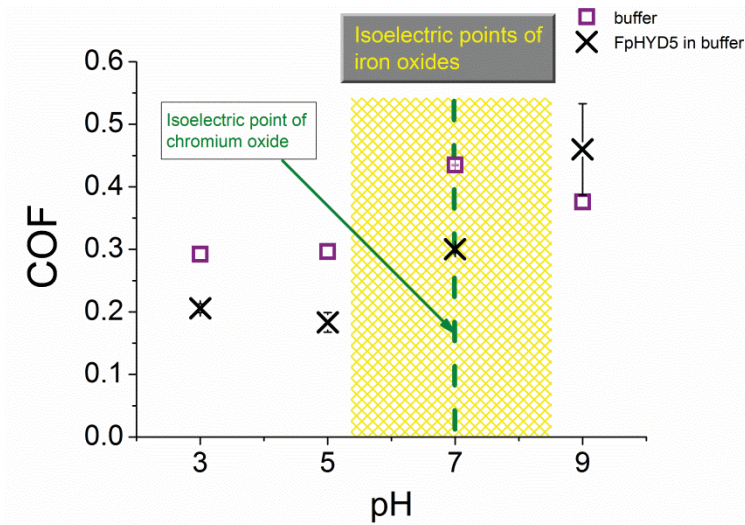
Figure 20. Friction coefficient vs solution pH in FpHYD5-lubricated stainless steel vs stainless steel contacts. The friction below the isoelectric points of chromium oxide and iron oxides is lower than the friction-measured pH above the isoelectric points.

3.2.3   The effect of water content on lubricating film

In nature, the lubricating molecules often contain carbohydrate structures that increase water-binding ability. When comparing the friction and wear results of HFBI and FpHYD5 molecules in stainless steel vs stainless steel contacts, it was observed that the increased water content in the hydrophobin film led to decreased friction and wear (Fig. 21; Publication II). Similar results were achieved with non-treated quince mucilage in UHMWPE vs stainless steel contact when the friction decreased with increased water-binding ability of quince mucilage. Although the lubricated materials are hard and the lubricating film is not stable, the importance of water in the sliding contact to reduce friction and wear seems to be significant.
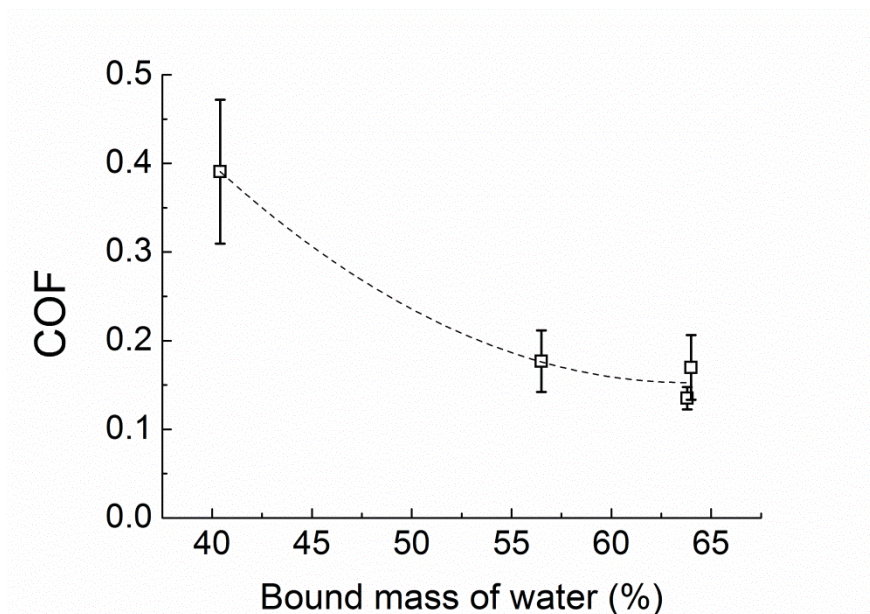
Figure 21. Friction coefficient vs water content in hydrophobin film formed on a stainless steel surface (Publication II)

3.2.4   The effect on tribofilm formation

In the contact with a-C:H-coated surfaces, the hydrophobins disturbed the lubrication by increasing wear. In water-lubricated contacts an oxide-containing tribofilm formed on aluminium and copper surfaces. The hydrophobin prevented the oxide film formation and it also affected friction and wear in the sliding contacts with a-C:H coatings. The wear of copper and aluminium increased because no oxide film was formed. In aluminium vs a-C:H, contact, friction also increased compared with the water-lubricated contacts.

In the water-lubricated contacts, Cu_2O film formed on the copper surface. In the SPR experiments, it was observed that hydrophobins were able to react with a copper surface and remove the oxide layer (Publication V).

3.2.5   Low friction with hydrophobins

Occasionally in the tribotests with FpHYD5 hydrophobins in stainless steel vs stainless steel contacts, friction started to decrease to a level below 0.05 (Fig. 22)

although the conditions were kept identical (Publication IV). Low friction in FpHYD5-lubricated contacts was always related to tribofilm formation on the worn surface on the stainless steel sphere observed by optical microscopy (Fig. 23). The tribofilm appeared as scale-like patterns formed on the sliding surface (Fig. 23 a). The film was studied by Raman spectrometry (Ref. Publication IV), which showed the presence of protein. The spectrum was deconvoluted into separate bands that were identified as amide II and III bands and a c_α-H amide bending vibration band and were comparable with those reported in the literature for proteins having b-sheet and random coil secondary structures. (Chi et al., 1998)
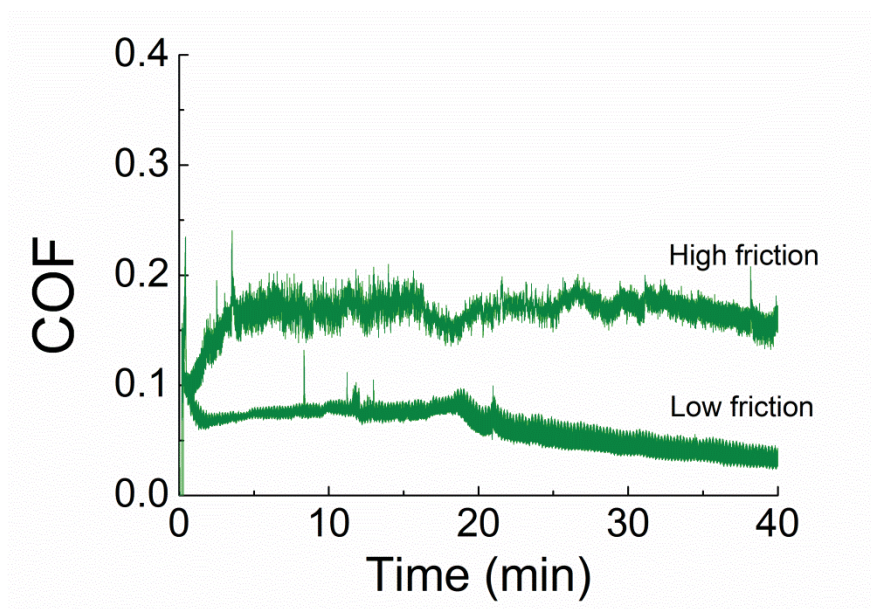
Figure 22. Two friction curves measured in a stainless steel vs stainless steel POD experiment. Although the conditions were identical, the friction was significantly lower in the second experiment. The contacts were lubricated with FpHYD5 in 50 mM acetate buffer. The test parameters were normal load 10 N, sliding velocity 0.1 m/s and duration 40 min.
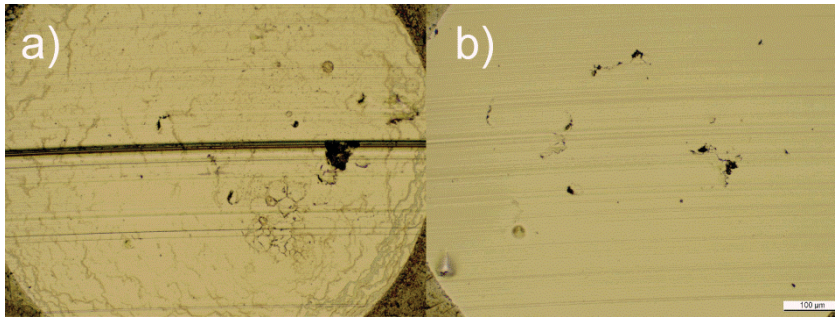
Figure 23. a) Tribofilm formed on the stainless steel surface, b) no tribofilm formed

The Formation of tribofilm, which consists of proteins, on the wear track of the pin allows a situation in which there is a protein layer on both the disc and pin surfaces. This indicates that low friction is achieved when there is a protein layer on both sliding surfaces. However, the layer on the ball/pin surface is not a monolayer of hydrophobins because a monolayer (thickness ~2-3 nanometres) cannot be detected by optical microscopy.

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4. Discussion on lubrication performance

4.1 Film formation, friction and wear performance

To provide effective boundary lubrication, the biomolecule additives need to adhere to sliding surfaces and prevent the contact between surface asperities. In biomolecule-lubricated contacts, low friction coefficients are usually achieved via hydration lubrication. Hydration lubrication requires:

1. Hydrated molecules on both sliding surfaces
2. Perfect alignment of the molecules, which allows the sliding to occur between the hydrated moieties
3. Low contact pressures

Hydrophobins are well known for their adsorption properties. The adsorption experiments showed that hydrophobins adhered well to stainless steel, a-C:H and PDMS surfaces. There is no significant difference in the amounts of hydrophobins that adhered to different surfaces. Although the surface properties did not affect the amount of adsorbed molecules, the environment in which adsorption occurs had an effect on adsorption. It was observed that the amount of adsorbed molecules was higher from water than 50 mM sodium acetate buffer solution. It may be that the ions in buffer solutions and pH affect the interactions between hydrophobins on solid surfaces. In water, hydrophobins are able to form a more closely packed monolayer compared with a buffer solution at pH 5. Strong lateral interactions are typical for hydrophobin films and have been observed earlier in an air/water interface (Szilvay et al., 2007). The adsorbed amount of FpHYD5 molecules on the stainless steel surface was lower than the amount of HFBI molecules. A probable reason for this is the rather large carbohydrate structure (1.7 kDa) in the FpHYD5 molecule, which may cause electrostatic repulsion and steric hindrance between the molecules, thus weakening the lateral interaction in the protein layer. This can also explain why the FpHYD5-lubricated contacts showed more wear than the HFBI-lubricated contacts.

Hydrophobins have relatively large hydrophilic parts for their size, which makes them able to bind high amounts of water. The water-binding ability of the lubricating hydrophobin films was dependent on both the environment and the molecule

structure. The glycosylated FpHYD5 formed layers on stainless steel with over 60% of the mass being water. An increased water-binding ability compared with the HFBI molecule is assumed to be due to the carbohydrate structures attached to the FpHYD5 molecule. Thus, a combination of an anchoring group (hydrophobin) and a water-absorbing group (carbohydrate) enabled the formation of a reasonably thick layer of water on the surface. In stainless steel vs stainless steel contacts, the FpHYD5 molecule generally lubricated more effectively than HFBI. These results indicate that the hydrophobin proteins do not lubricate in themselves very effectively but, at the same time, the bound water plays an important role in lubrication. The experiments with quince mucilage in UHMWPE vs stainless steel contact also showed the importance of water in lubrication. The addition of 1M NaCl increased friction by 36% in quince mucilage-lubricated contact, most probably due to the reduced level of hydration of the mucilage.

The alignment of hydrated molecules is an important property in hydration lubrication because low friction is achieved when the sliding occurs between the hydrated moieties. Although adsorption experiments showed that hydrophobins are able to form monolayers on different surfaces, we do not know how they are aligned. The adsorption of hydrophobins is affected by both hydrophobic interactions and electric charges. The alignment on different surfaces may be different although the amounts of adsorbed molecules would be similar. The HFBI, HFBII and FpHYD5 molecules have hydrophobic patches that are similar in size ($4 \ nm^2$) in all of the molecules. If we assume that on hydrophobic PDMS surfaces, which have contact angles of water over 90°, the FpHYD5 and HFBI hydrophobins are adhered with their hydrophobic patch. In addition, in PDMS vs PDMS contacts, both hydrophobins were able to reduce the friction coefficients close to 0.01, which is at similar level to friction coefficients in effective hydration lubrication. We do not know the alignment of the molecules on stainless steel and a-C:H surfaces, which have lower hydrophobicity compared with PDMS surfaces. It is interesting that the area per FpHYD5 molecule on a stainless steel surface was $14.7 \ nm^2$ but on a PDMS surface, the area for the same molecule was only $8.7 \ nm^2$. As the environment (pH, ionic strength) was the same in both cases, there should be no difference in the interaction between molecules. Thus, the results indicate that there are differences in the molecule alignment. The possible alignments on stainless steel and PDMS surfaces are schematically illustrated in Fig. 24. For a smaller HFBI molecule, there was no such difference in the amount of adsorbed molecules on stainless steel and PDMS surfaces, which can be explained by the globular shape of the molecule.
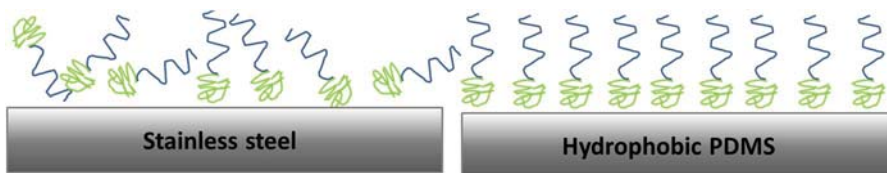
Figure 24. Adsorption and possible alignment of FpHYD5 molecules on stainless steel and hydrophobic PDMS surfaces

The lowest friction coefficients for all the experiments were measured in PDMS vs PDMS contacts lubricated with HFBI and FpHYD5 hydrophobins. The friction coefficients decreased from ~0.9 to close to 0.01 when hydrophobins were added to the buffer solution. Low friction compared with other lubrication systems may be explained by three main differences. The first factor is the hydrophobicity of PDMS surfaces. In PDMS vs PDMS contacts, the hydrophobins are probably adhered on both surfaces with their hydrophobic patches. This leads to a situation in which sliding can occur between the hydrated moieties as illustrated in Fig. 6. Secondly, in PDMS vs PDMS contacts, there are low contact pressures. While in other lubricated contacts, the contact pressures decreased down to a few MPas due to wear. Those are significantly higher than for the PDMS vs PDMS contacts for which the contact pressure is approximately 0.36 MPa. However, when the contact pressure exceeded a few MPas it had no effect on lubrication with hydrophobin molecules (Fig. 25). At higher contact pressures, the material pair has more effect on lubrication performance than contact pressure itself. Thirdly, PDMS surfaces do not wear significantly. In the contacts with the harder materials, the upper specimen in particular has wear because it is constantly in sliding contact.

Figure 25. COF vs contact pressure in different lubricated contacts.

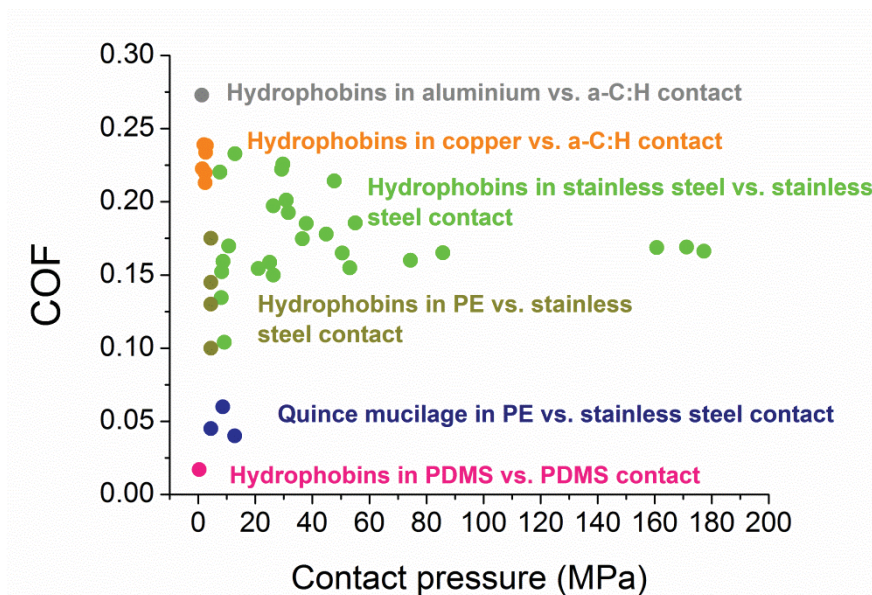As seen in Fig. 25, the friction coefficients in hydrophobin-lubricated contacts are usually between 0.1 and 0.3. Relatively high friction coefficients could be explained by higher contact pressures that may disturb low friction mechanisms by causing the entanglement of molecule layers adsorbed on both sliding surfaces (Klein, 2012; Harvey et al., 2011), removal of surface- adhered molecules (Raviv et al., 2008) and bridging where molecules are adhered on both sliding surfaces (Harvey et al. 2011; Carapeto et al., 2010). The importance of the correct alignment of molecules on surfaces in hydration lubrication was discussed earlier. In the contacts in which stainless steel, aluminium, copper and a-C:H were used, there was also wear of the materials. Due to the wear, stable lubricating films cannot be formed by adhered hydrophobins on both sliding surfaces, which prevents the existence of a hydration lubrication situation (Fig. 26 a). When surfaces are worn, the molecules are also sheared away from the contact (Fig. 26 b). The wear rates and contact pressures decrease during the experiment, and at some points there may be a situation in which the molecules are adhered on both surfaces, as seen in tribological experiments with FpHYD5. It was observed that FpHYD5 was able to form a protein layer occasionally on the worn surface of the stainless steel ball, and that led to low-friction coefficients below 0.05 in stainless steel vs stainless steel contacts. This is not the case with all materials. It is not likely that hydrophobins could form stable lubricating film on a constantly sliding pin/ball surface because the adsorption of hydrophobins on stainless steel and a-C:H surfaces takes a few minutes and hence so does the formation of a full mono-

layer. Thus, the formation of a monolayer in a kinetic situation, such as tribocontact, may be impossible. In addition, the adsorption rates of the hydrophobins depends on their concentration, which means that the recovery of the hydrophobin layer on the disc surface was faster at higher protein concentrations. This could explain why the friction remained low with higher protein concentrations while at a concentration of 0.1, friction and wear increased compared with the buffer solution. The higher protein concentration in the lubricant may also affect the amount of molecules between the sliding surfaces and the local viscosity. An increase in local viscosity can improve the lubrication by causing a hydrodynamic effect between the sliding surfaces.
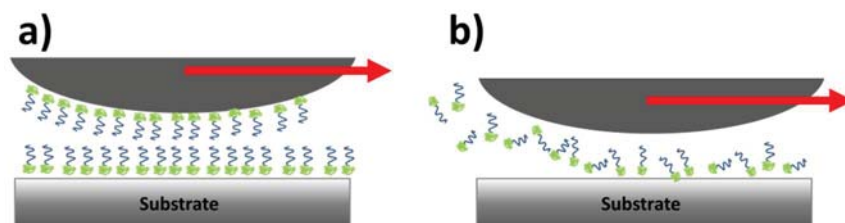
Figure 26. a) A situation in which hydration lubrication is possible in a POD experiment, b) a situation with hard materials in which wear and shear force have removed molecules from the upper surface, which is constantly in contact

In the contacts where the upper specimen was made of copper or aluminium there was also significant wear of the upper sliding surface. It was also observed that hydrophobins prevented the formation of oxides on the sliding surfaces. In SPR experiments, it was seen that HFBII hydrophobins actually removed the oxide film from the copper surface in a static situation without any mechanical contact. Thus, in these cases, the hydrophobins were not able to form lubricating layers on the upper surface, which can explain the relatively high friction coefficients of 0.2-0.3. When no oxide film was formed on the pin surface, the wear on the pin increased.

A summary of the results is presented in Table 6. It can be seen that the best results were achieved by FpHYD5 in stainless steel vs stainless steel contact, HFBI and FpHYD5 in PDMS vs PDMS contacts and quince mucilage in UHMWPE vs stainless steel contact.

Table 6. Summary of the friction and wear results for different lubricated contacts

Materials	Lubricant	Water	Buffer	Oil	HFBI	HFBII	FpHYD5	Quince mucilage
Stainless steel vs stainless steel	COF	0.6-0.8	0.25-0.35	0.1-0.15	0.15-0.25		**0.1-0.25**	0.3-0.4
	Wear	High	Average	Low	Average		Average	High
UHMWPE vs stainless steel	COF	0.1-0.15	0.08-0.18		0.1-0.18		0.1-0.18	**0.02-0.09**
Stainless steel vs a-C:H	COF	0.13-0.14				0.12		
	Wear	Low				Average		
Aluminium vs a-C:H	COF	0.12-0.14				0.28-0.32		
	Wear	Average				High		
Copper vs a-C:H	COF	0.24-0.30				0.21-0.24		
	Wear	Average				High		
PDMS vs PDMS	COF		0.8-0.9		**0.01-0.05**		**0.01-0.02**	

4.2 Potential application areas for biomolecular lubrication in the future

Based on the tribotests and literature, it can be suggested that the requirements for water-based lubrication with biomolecule additives in industrial applications are

- a mild temperature range that is suitable for water lubrication and bio-molecules, T= 4-95°C
- low contact pressures, 0.1- 5 MPa
- hydrophobic surfaces, contact angle of water >90°
- a stable environment (pH, ionic strength)

Compared with oil lubrication, water-based lubrication with biomolecules is sensitive to changes in the environment, such as temperature, pH and ionic strength. In short-term lubrication processes, such as metal working, there may be fewer limitations regarding contact pressures and temperatures, because no long stability of the molecules is needed. In these kinds of processes, for example the graphitiza-

tion mechanism, in which molecules are broken down and form graphite on sliding surfaces, can provide effective lubrication.

Possible applications for water-based lubrication improved with biomolecule additives could be existing applications in which water is already used and there is a need for lubricants that are not harmful to the environmental or humans and do not leave harmful remnants on the products. With biomolecule lubricants, friction can also be reduced compared with water-lubricated contacts as seen in the tribological experiments with quince mucilage. Aging and bacterial growth can of course cause problems in biomolecule-lubricated contacts, as carbohydrates are especially sensitive to bacterial growth. Hydrophobin proteins can survive longer without any clear worsening of performance. However, the aging of hydrophobin solutions has not yet been studied properly.

The benefit of using biomolecule-containing lubricants is their low friction in the boundary lubrication regime. This could lead to energy savings in various bearings and motors for instance. In addition, waste treatment of lubricants is much easier compared with oil lubricants because biomolecules are environmentally friendly.

Examples of some of the lubrication systems and applications with most potential in which biomolecules could be used in the future are described below.

The food and beverage industry
In certain applications in the food industry, lubrication must be arranged so that the lubricant cannot contaminate the food or beverage. There are several applications in which there is a high risk of leakage. Thus, lubricants suitable for the food and beverage industry are National Sanitary Foundation (NSF) H1 grade lubricants. The NSF H1 grade is given to lubricants that do not contaminate the food in case of contact between the food or beverage and the lubricant. The certificate also takes into account hygiene during processing and storage of the lubricant. It is important for food grade biolubricants to prevent the growth of bacteria without poisonous additives. For carbohydrate-containing lubricants, this can cause limitations on their usage. Hydrophobins can survive for a relatively long time if stored properly. The hydrophobin lubricants did not have any visual changes even after 1-2 years when stored in a refrigerator. In addition, hydrophobins can be ingested by human without health risks.

Traditional oil lubricants and their additives cannot meet the requirements for the NSF H1 grade, and synthetic lubricants have been developed for the food and beverage industry. Non-harmful bio-based lubricants could be one alternative for the food and beverage industry if they satisfy the health requirements for food grade lubricants. Many biomolecules are extracted from plants, such as the quince fruit, and are not harmful to humans (Florea and Luca, 2008).

Nowadays, food grade grease is commonly used in many applications because the risks of leakage are small compared with low viscosity oils. However, replacing grease with low viscosity lubricants would have a great effect on energy savings. Examples of applications in which bio-based lubricants could be used in the food industry are shafts and bearings, depending on the materials and conditions.

The textile industry

In the textile industry, lubricants are used in, for example, fibre processing. Lubricants need to prevent abrasive wear of the fibres. Water-based lubricants can easily be removed from the final products by washing, unlike oil-based lubricants (Li, Y et al., 2012). Textile materials, such as polyethylene and polypropylene, are hydrophobic and can be lubricated well by water-based lubricants and additives that are adsorbed to surfaces by hydrophobic forces (Song et al., 2014). The problem of hydrophobins and some other biomolecule additives in the textile industry could be high velocities of lubricated systems. Hydrophobins easily form foam, which should be avoided.

Biomedical and pharmaceutical applications

Biomolecules can be used as surgical or medical lubricants to make operations more comfortable. In some applications, the surfaces can be tailored by biomolecule additives to reduce friction and, especially, wear of the biomaterials, e.g. catheters (Zhang et al, 2013). The biomolecule layer on the surface could be used as an alternative to synthetic polymers with enhanced lubricating ability and environmental friendliness. The manufacturing of biomedical devices may need lubricants that are easily removed from the final products. Biomolecules could act as anti-stick coatings in certain production steps, e.g. printing or pressing in tablet processing and other pharmaceutical processes. For example, hydrophobins can easily be washed away with ethanol.

Conclusions

In this thesis, water-based lubricants with hydrophobin proteins (HFBI, HFBII and FpHYD5) and quince mucilage additives were used to lubricate engineering materials such as diamond-like carbon (DLC) coatings, stainless steels and plastics.

Generally, the lowest friction coefficients were measured in contacts where at least one of the surfaces was a polymeric material. In hard vs hard-type contacts, low friction coefficients and wear rates were occasionally achieved.

The main results of this thesis are as follows:
- Hydrophobins can form monolayers on stainless steel, diamond-like carbon (a-C:H) and PDMS surfaces. On stainless steel surfaces, HFBI and FpHYD5 layers contain 40-64% water (Publication II).
- An increase in water content in lubricating film reduced friction in hydrophobin-lubricated stainless steel vs stainless steel contacts (Publication II). The same effect was seen in quince mucilage-lubricated UHMWPE vs stainless steel contact (Publication III).
- FpHYD5 hydrophobins reduced the friction coefficient in stainless steel vs stainless steel contacts from about 0.7 to as low as 0.03. Low friction was related to protein film formation on the wear track of the stainless steel ball (Publication IV).
- Quince mucilage-lubricated UHMWPE vs stainless steel reduced the friction coefficient from about 0.12 to as low as 0.02 (Publication III).
- HFBI and FpHYD5 hydrophobins decreased the friction coefficient from 0.9 to close to 0.01 in PDMS vs PDMS contacts (Hakala and Lee, 2011).

Based on these results from adsorption and tribological experiments, biomolecules can form lubricating layers on engineering materials and lubricate with friction coefficients well below the friction coefficients measured with mineral oil and EP additives. This is in accordance with the scientific hypothesis of this thesis.

It can be suggested that the requirements for water-based lubrication with biomolecule additives in industrial applications are

- a mild temperature range that is suitable for water lubrication and bio-molecules, T= 4-95°C
- low contact pressures, 0.1- 5 MPa
- hydrophobic surfaces, contact angle of water >90°
- Stable conditions (pH, ionic strength)

From the work, it can be concluded that biomolecules have the potential to act as effective lubricants in the boundary lubrication regime when the conditions are mild and the environment stable.

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Friction and wear incur high economic costs globally. It has been estimated that approximately 30% of energy is used to overcome friction. Developing new solutions, such as coatings, surface texturing and lubricants, to reduce friction in the boundary lubrication regime can have great importance to global energy savings in the future.

In this thesis, water-based lubricants with hydrophobin protein (HFBI, HFBII and FpHYD5) and quince mucilage additives were used to lubricate engineering materials such as diamond-like carbon (DLC) coatings, stainless steels and plastics.It was found that hydrophobins can form monolayers on stainless steel, diamond-like carbon (a-C:H) and PDMS surfaces. Increasing the water content in hydrophobin film reduced friction in hydrophobin-lubricated stainless steel vs stainless steel contacts. The same effect was seen in quince mucilage-lubricated UHMWPE vs stainless steel contact.

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