Making sense of adsorption

Attempting to explain the adsorption of histatin 5 with models, metaphors, and machines

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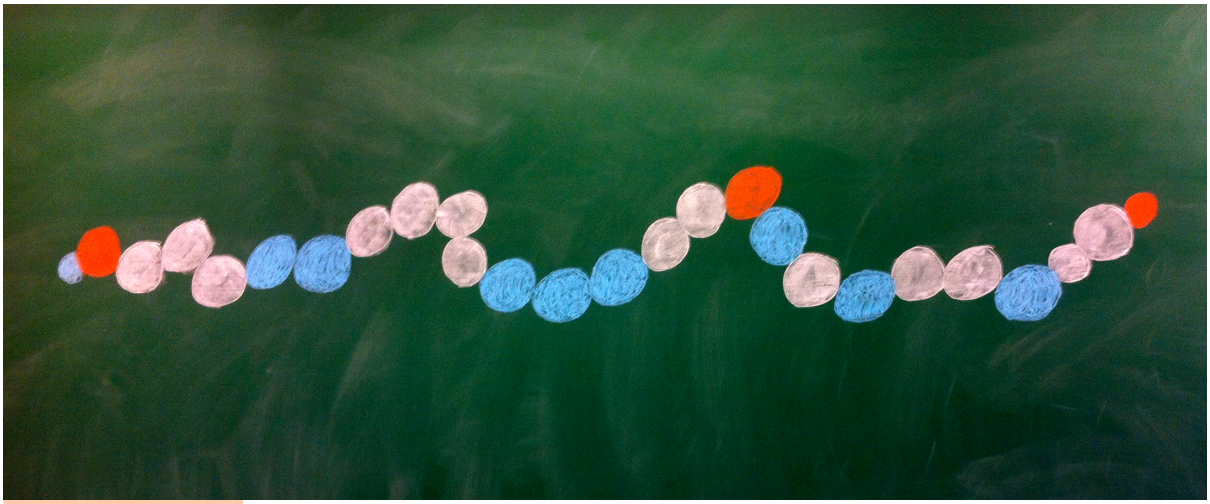

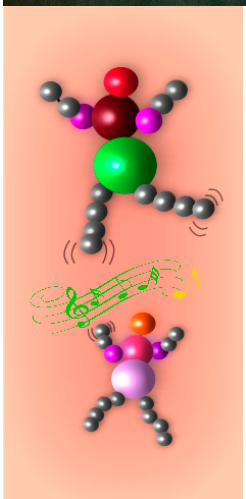

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THEORETICAL CHEMISTRY | FACULTY OF SCIENCE | LUND UNIVERSITY
KRISTIN HYLTEGREN

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Kristin Hyltegren

Lund University

LICENTIATE THESIS
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Abstract
This thesis summarises two studies in which the main purpose was to find out *how* and *why* the amount of the protein histatin 5 that adsorbs to negatively charged surfaces changes with pH and ionic strength. Histatin 5 is an intrinsically disordered saliva protein, and in the oral environment it adsorbs to tooth enamel. The approach here is to simplify the biological system by using an aqueous buffer solution instead of saliva, and a silica surface to represent the tooth enamel. Ellipsometry measurements were performed in order to answer the question of *how* the adsorbed amount changes with pH and ionic strength. Coarse-grained Monte Carlo simulations were used to investigate the molecular mechanisms behind the adsorption of histatin 5 and try to elucidate *why* the experiments give certain trends. The obtained results could have implications for the understanding of the role of histatin 5 in the oral environment, as well as for fundamental understanding of the adsorption of flexible proteins or polyelectrolytes.

The main take-home messages are the following: (i) the adsorbed amount of histatin 5 changes with ionic strength – but the trends are different depending on the pH of the solution, (ii) the change in surface charge with pH and ionic strength can strongly affect the adsorbed amount, (iii) the electrostatic interactions between charged groups are not enough to account for the experimentally observed adsorption of histatin 5 to silica surfaces, and (iv) the coarse-grained model used in these studies cannot explain the experimentally observed pH-dependence of the adsorbed amount as a function of ionic strength. However, the simulations cast light on the balance between the electrostatic *attraction* between the protein and the surface and the electrostatic *repulsion* between adsorbed proteins, the deficiencies of the Langmuir isotherm, and the implications of protein charge regulation for adsorption.

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Front cover: A model, a metaphor, and a machine.
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Dedicated to the principal supervisors of my three theses in Chemistry.

Till huvudhandledarna för mina tre examensarbeten i kemi.

*Till **Magnus Gustafsson** som med glädje tog sig an mig som student när jag under mitt andra år på kemiprogrammet på Göteborgs universitet skulle göra ett litet projekt inom ramen för kursen "kemi avancerat". Det hade varit svårt att skriva denna avhandling utan kunskap om fysik och programmering och jag har dig att tacka för att mitt intresse för båda dessa ämnen vaknade. Jag minns med värme våra timslånga diskussioner om pedagogik, livet och den akademiska banan. Det betydde mycket att du lyssnade på mig och tog alla mina förslag och frågor på allvar. Tack också för stödet när jag mådde som sämst.*

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*Till **Roland Kjellander** som, med sin unika entusiasm, bjöd med oss studenter in i molekylernas värld och uppmanade oss att själva leva oss in i vad vi skulle uppleva om vi vore molekyler. Utan dig hade jag kanske levt i villfarelsen att statistisk termodynamik bara är tungt och tråkigt. Med inspiration från bland annat dig blev skrivandet av denna licentiatavhandling till en ren fröjd. Tack också för de intensiva diskussionerna på ditt kontor i början av mitt **masterarbete**. Det är vid sådana tillfällen som vetenskap är som bäst!*

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Stort tack också för att du har hängt med i och accepterat turerna fram och tillbaka kring huruvida det blir en fortsättning på den här avhandlingen eller inte. Jag hoppas du kan känna en viss stolthet över vad vi åstadkommit såhär långt.

Abstract

This thesis summarises two studies in which the main purpose was to find out how and why the amount of the protein histatin 5 that adsorbs to negatively charged surfaces changes with pH and ionic strength. Histatin 5 is an intrinsically disordered saliva protein, and in the oral environment it adsorbs to tooth enamel. The approach here is to simplify the biological system by using an aqueous buffer solution instead of saliva, and a silica surface to represent the tooth enamel. Ellipsometry measurements were performed in order to answer the question of *how* the adsorbed amount changes with pH and ionic strength. Coarse-grained Monte Carlo simulations were used to investigate the molecular mechanisms behind the adsorption of histatin 5 and try to elucidate *why* the experiments give certain trends. The obtained results could have implications for the understanding of the role of histatin 5 in the oral environment, as well as for fundamental understanding of the adsorption of flexible proteins or polyelectrolytes.

The main take-home messages are the following: (i) the adsorbed amount of histatin 5 changes with ionic strength – but the trends are different depending on the pH of the solution, (ii) the change in surface charge with pH and ionic strength can strongly affect the adsorbed amount, (iii) the electrostatic interactions between charged groups are not enough to account for the experimentally observed adsorption of histatin 5 to silica surfaces, and (iv) the coarse-grained model used in these studies cannot explain the experimentally observed pH-dependence of the adsorbed amount as a function of ionic strength. However, the simulations cast light on the balance between the electrostatic *attraction* between the protein and the surface and the electrostatic *repulsion* between adsorbed proteins, the deficiencies of the Langmuir isotherm, and the implications of protein charge regulation for adsorption.

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Preface

> My mother made me a scientist without ever intending it. Every other Jewish mother in Brooklyn would ask her child after school: 'So? Did you learn anything today?' But not my mother. She always asked me a different question. 'Izzy,' she would say, 'did you ask a good question today?' That difference – asking good questions – made me become a scientist!
>
> *Isidor I. Rabi, Nobel laureate in physics (discoverer of NMR!), when asked by Arthur Sackler why he, unlike the other immigrant kids in his neighbourhood, became a scientist*

As a child, I never dreamt of becoming a scientist. I wanted to be an actress, a singer, a farmer or a bus driver (because bus drivers are needed!). But most of all I wanted to be an author. So how did this thesis come to be? What was it that made me choose the path towards a PhD in Theoretical Chemistry?

When I was approximately 13 years old I started to be interested in science. I was fascinated by how everything from fabric to air to humans are built up by atoms and I wanted to understand the nature of atoms and molecules and their interactions. My impression was that while physics could *describe* the world, chemistry could do something much more powerful, namely *explain* the world.

As a 14–15-year-old, I started to have doubts about my choice to go for a career as an author. I still *wanted* to become an author but the probability of earning money by writing fiction seemed dauntingly low. Also, I wanted to learn more about science. Then the thought struck me: "I can become a scientist and write books about science!" Ever since then, that is what I have been striving for. I never cared much about *doing* science, I just wanted to *learn* a lot about science and write books about it. I thought that with a PhD in a science discipline I would earn the credibility and the knowledge I needed to get there.

Unfortunately, it turned out that a PhD in Chemistry is an awful lot about

doing science. Doing science is nothing like learning about science from others! There are no explanations there ready for you to read and there is no teacher there ready to explain your research to you in case your thoughts get stuck. According to my experiences, doing science is like swimming in an ocean of confusion and doubt, not knowing whether you will ever find an island where you can experience solid ground under your feet again. Many times I have seriously doubted whether I would be able to stand there on my big day and *defend* my thesis. I just wanted to throw everything I had done into the garbage bin, where I imagined it would fit in perfectly well.*

I decided to quit my PhD studies and get the licentiate degree instead. I surprised myself with the sudden decision but within a couple of days the date for the licentiate seminar was set and I started to prepare for thesis writing and a spectacular 1/2 PhD party. To be honest, I was mostly planning the party.

However, all of a sudden I was not afraid of defending my thesis anymore. I believe that good or innovative research is more about asking interesting questions than about defending what you did in the past or trying to be the one who knows the most. I decided to hide nothing. I would show my doubts and conceptual difficulties openly.

The thought of quitting gave me a wonderful sense of freedom. Now I didn't need to care about what people would think about me. I didn't even need to care about what the opponent or the examiner would think about my thesis. If this thesis would not earn me a degree, that would no longer matter much. What would matter was whether *I* would like the thesis or not. Whether *I* would be proud of it. Therefore, I started writing the thesis that I would have liked to read but, most importantly, the thesis that I would enjoy writing. And since it's my work, it probably has both my strengths and my flaws woven into it.

You will notice that I have used a book with the ambitious title *Making sense of life*, written by science historian and philosopher Evelyn Fox Keller, as one of my main sources of inspiration. At the time I bought it I was taking a master's course called "Historical and philosophical perspectives on science". During this course we read important texts by historians of science, among them Keller. I took her texts immediately to heart with a feeling that I had found an academic soulmate.

I decided to buy her book *Making sense of life* and I started reading it. However, it is quite heavy reading and since I had to focus on more urgent things it ended up collecting dust in my bookcase. When I had decided to quit my PhD studies and just started writing this licentiate thesis, I returned to Keller's book once again to pick some nice quotes from it.

I was caught already by the subtitle. It reads "Explaining biological development with models, metaphors, and machines". It was a coincidence too good to ignore: Coarse-grained *models*, daily life *metaphors*, and *machines*, such as the

*Similar feelings are commonly experienced by PhD students during a phase of the PhD studies that has been called "The valley of shit". See https://thesiswhisperer.com/2012/05/08/the-valley-of-shit/

ellipsometer or supercomputers, were the core tools behind the thesis I imagined I was going to write.

I flipped through to the conclusion chapter and saw that – not only had she used the same tools as I had done – she had arrived at similar conclusions too! Although much more wisely and elegantly expressed than my dim, negative thoughts centered on failure. Now, I realised that I had been wrong. My inability to find the answers I sought was neither a personal failure nor a failure of science. It was a simply a fact of life. Finally, my research started to make sense.

Kristin Hyltegren, Lund, August and September 2016

Populärvetenskaplig sammanfattning

I denna sammanfattning kommer jag att börja med att försöka ge en bild av mina forskningsområden i stort (teoretisk kemi och statistisk mekanik). Frågorna jag har försökt besvara är vilken typ av frågeställningar vi sysslar med och vilka metoder vi använder. Därefter kommer jag att beskriva mitt eget projekt: vad det innebär att forska på adsorption av histatin 5, varför vi har valt att göra det och vad som skulle kunna bli möjliga tillämpningar av forskningen.

Vad sysslar vi med för frågor inom teoretisk kemi?

Inom området teoretisk kemi används datorer och beräkningar för att besvara kemiska frågeställningar. Eftersom en sådan frågeställning ofta formuleras på ett sätt som är svårt att förstå för en utomstående så tänkte jag istället försöka förklara vad det kan handla om genom att ta ett exempel och använda mig av liknelser från vår vardag (och fest). I det här fallet så tar vår frågeställning avstamp i ett enkelt experiment som är lätt att göra hemma:

Ta en behållare som går att försluta. Häll sedan i vatten och en skvätt matolja. Oljan kommer då att lägga sig ovanpå vattnet. Om du skakar behållaren våldsamt kommer du att kunna bilda en så kallad dispersion. I dispersionen finns små droppar av olja som simmar runt i vattnet. Denna blandning är dock inte stabil. Om du låter den stå i några minuter så kommer den återigen att bilda två lager – oljan hamnar överst och vattnet underst.

En fråga som vi kan ställa utifrån denna observation är: Vad är det som gör att olja och vatten inte vill blandas? Det här är en typ av frågeställning som en teoretisk kemist skulle kunna ta sig an.

För att ha möjlighet att hitta ett svar på frågan måste vi ge oss in i molekylernas värld. Vi måste förstå vilka *krafter* som verkar mellan molekylerna – med andra ord varför vattenmolekyler dras till andra vattenmolekyler men ogillar oljemolekyler. Min forskning handlar till stor del om just så kallade intermolekylära krafter, så därför ska jag försöka förklara vad det handlar om.

Att mingla som en molekyl

Det som gör att vattenmolekyler bildar en vätska vid rumstemperatur istället för att fara runt i gasform är att det finns intermolekylära krafter som håller samman molekylerna. Krafterna är dock inte tillräckligt starka för att vattnet ska vara i fast form.

För att använda en liknelse: Låt oss säga att människan är en molekyl. Vi består av olika kroppsdelar (i molekylernas värld motsvaras de av atomer), till exempel två händer, två fötter, ett huvud med mera (i molekylernas värld kan det vara till exempel två syreatomer, två väteatomer och en kolatom). Om några människor håller varandra i händerna känner de av en stark mellanmänsklig kraft som håller samman dem och tvingar dem att förflytta sig som en enhet (ett fast objekt). Denna kraft kan liknas vid starka intermolekylära krafter mellan molekyler.

Kraften gör dock inte att människorna som ingår i enheten (aggregatet) smälter samman – de är fortfarande väl avgränsade personer (molekyler). Intermolekylära krafter är svaga i jämförelse med kemiska bindningar, som är vad som håller samman atomerna i en molekyl. Detta ser vi även i analogin med människorna: det krävs mycket små krafter för att dra isär två människor som håller varandra i handen (två molekyler som interagerar) jämfört med den kraft som skulle krävas för att dra loss en hand (atom) från resten av den människokropp (molekyl) som den sitter fast på.

Svagare mellanmänskliga krafter (motsvarande lite svagare intermolekylära krafter av den typ som finns i en vätska) kan observeras på en fest där människor minglar runt. Krafterna är attraktiva, vilket får människorna att samlas i en stor grupp, men varje enskild person kan röra sig fritt inom sällskapet och interagera med olika människor.

Ensamvargar som går på fest försvinner ofta iväg från gruppen. De upplever en väldigt svag attraktion till andra människor, vilket i molekylernas värld skulle motsvara att de befinner sig i gasform.[†]

För att närma oss fallet med vatten och olja kan vi ta analogin med festen ett steg vidare. Låt oss säga att det finns två slags människor på festen som gillar folk som ingår i samma grupp men inte folk som ingår i den andra gruppen. Vi kan till exempel tänka oss att vi har ett gäng MFF:are och ett gäng AIK:are. Om vi tillför energi i form av en käck lek kan vi kanske tvinga människorna från de två olika lägren att beblanda sig en stund, men efteråt delar de återigen spontant upp sig i två olika grupper.

[†]Notera att även de mer sociala personerna på festen till slut övergår i gasform. Det kan till exempel gå till så att temperaturen höjs på dansgolvet tills kokpunkten nås och folk går upp i rök (det vill säga beger sig hemåt). En annan, kanske vanligare, mekanism är att människor försvinner från dansgolvet på grund av att trycket är för lågt.

Att studera molekyler i verkligheten

Om vi kunde intervjua en molekyl skulle den säkert invända att det finns många väsentliga skillnader mellan min liknelse ovan och hur det verkligen fungerar i molekylernas värld. Även om liknelser kan vara ovärderliga då det gäller att få en känsla för ett fenomen så kan vi sällan dra slutsatser om verkligheten baserat på dessa liknelser. Vi kan tyvärr alltså inte lära oss särskilt mycket om molekyler genom att gå på fest (om vi undantar kunskapen om effekten av vissa kemiska substanser som är vanligt förekommande på just fester...). Inte heller lär vi oss något om molekylers bevekelsegrunder genom att intervjua en MFF:are om varför hen inte gillar AIK:are.

Att intervjua själva molekylerna inser nog alla är en fullständigt urflippad idé, så intervjuer som metod får vi lämna åt kollegorna på andra institutioner. Faktum är att vi knappt ens kan titta på molekylerna. De är alldeles för små! Vad vi däremot kan göra är att använda datorer för att simulera hur de förflyttar sig.

Genom simuleringar kan man på ett kontrollerat sätt uppleva eller studera ett modellsystem eller en imitation av verkligheten. Ett exempel är flygsimulatorer, där man kan pröva på vissa aspekter av att vara pilot i en kontrollerad miljö. En fördel med att använda datorsimuleringar för att studera processer är att vi i datorn har möjlighet att slå av och på olika krafter/funktioner och se vad som händer – något som vanligen är helt omöjligt att göra i experiment utanför datorn.

Vad är statistisk mekanik?

Jag arbetar inom fältet statistisk mekanik där man tittar just på hur molekyler rör sig, vilka molekyler som dras till varandra, om molekylerna bildar stabila aggregat med mera. Vi tittar alltså inte på kemiska reaktioner som många andra kemister.

Statistisk mekanik handlar, precis som namnet antyder, mycket om mekanik och därför ligger det här forskningsområdet i gränslandet mellan kemi och fysik. Så var kommer statistiken in då? Är jag måhända expert på t-test eller anova-tabeller, som man kan lära sig om i en statistikkurs? Nej, långtifrån. Den statistik vi använder oss av handlar om att molekyler kan fördela sig på olika sätt och ha olika egenskaper men fortfarande ge upphov till samma makroskopiska egenskaper (till exempel tryck) och att vi alltid måste simulera ett tag och sedan ta medelvärden för att få fram de egenskaper som vi är intresserade av, till exempel var molekylerna helst befinner sig eller vilket tryck systemet har.

Datorsimuleringar, modellering och experiment

Alla system vi tittar på med hjälp av datorsimuleringar är förenklade jämfört med verkligheten. Precis som att en enkel leksaksbil bestående av en träkloss på fyra hjul inte på långa vägar kan räknas som en riktig bil är våra digitala

molekyler något helt annat än verklighetens molekyler. De kan beskriva och förklara vissa fenomen, men går fullständigt bet när det gäller andra. För att illustrera med en liknelse: Om vi låter vår leksaksbil rulla ner för en backe så kommer dess rörelse någorlunda korrekt att beskriva hur en verklig bil skulle accelerera i en nedförsbacke om vi tryckte ner kopplingspedalen och lät den rulla. Vi kommer dock aldrig att med hjälp av denna leksaksbil kunna förstå hur en riktig bil accelererar med hjälp av sin motor. Om vi är ute efter att våra resultat ska vara tillämpbara på den verkliga världen gäller det alltså att välja en bra modell och att *enbart använda modellen för sådant som den klarar av att beskriva*.

För att se om de modeller vi använder kan beskriva och förklara verkligheten behövs också jämförelser med experiment. Problemet är att det är svårt att hitta system som är direkt jämförbara. I simuleringar måste man nöja sig med ett mindre antal molekyler – här har jag använt mellan en och fyrtio molekyler av ett protein som finns i saliv och heter histatin 5 – medan jag i ett verkligt experiment har cirka 50 000 000 000 000 000 (femtio miljoner miljarder) proteinmolekyler. Förutom det har jag cirka 200 000 000 000 000 000 000 000 (tvåhundratusen miljarders miljarder) vattenmolekyler, kanske en del föroreningar, en yta vars egenskaper beror på hur jag har tvättat den och vilket pH jag har i min lösning, en buffert som behövs för att hålla pH konstant men förhoppningsvis inte påverkar mätningen och så vidare... Det är här någonstans som det börjar bli komplicerat. Och då är vi fortfarande kvar i ett relativt idealt system som endast med hjälp av vår goda vilja kan liknas vid systemet där histatin 5 vanligen befinner sig – nämligen munhålan.

En svårighet vid jämförelse mellan teori och experiment är att veta om resultaten skiljer sig åt för att teorin inte klarar av att beskriva verkligheten eller om de skiljer sig på grund av att vi inte har det vi tror att vi har i vårt experimentella system. Det är också värt att komma ihåg att många experimentella tekniker bygger på att modeller används för att översätta den data man får ut från experimentet till något som är begripligt och jämförbart med annan data. Så är det till exempel med den experimentella teknik som jag har använt och som kallas ellipsometri. Det är inte omöjligt att teoretiskt framtagen data ibland beskriver verkligheten bättre än vad ”översatta” data från experiment gör.

Var kommer mitt projekt in i vetenskapsbilden?

Vid det här laget har du nog förstått att mitt projekt är grundforskning – alltså mer inriktat på förståelse och observation än på något särskilt användningsområde.

Till skillnad från Neil Armstrong, som deklarerade att han med ett litet steg för en människa tog ett stort steg för mänskligheten, skulle jag vilja påstå att denna licentiatavhandling är ett stort steg för en människa men ett mikroskopiskt steg för mänskligheten. Huruvida min forskning kommer att leda till någon tillämpning eller någon upptäckt av betydelse för fortsatt forskning är ännu ovisst. Men precis som Neil Armstrong inte hade kunnat ta sitt berömda lilla

steg utan en massa andra små steg så kan det mycket väl hända att detta lilla projekt blir en viktig del i något större.

Adsorption av histatin 5 – vad är det och varför forskar vi på det?

Liksom titeln på min avhandling avslöjar handlar min forskning om adsorption av histatin 5. Adsorption är när en molekyl fastnar på en yta. Det är alltså inte samma sak som absorption, som handlar om att något sugs upp av något annat – till exempel vatten som absorberas av en disktrasa. Adsorption är snarare molekylernas motsvarighet till att klista fast ett klistermärke (motsvarande en molekyl) på en yta.

Jag har studerat bland annat fria energier för adsorption. Det kan sägas motsvara hur starkt ”klistret” är som fäster molekylen på ytan. ”Klistret” kan vara till exempel attraktion mellan positiva och negativa laddningar, vilket är vad jag främst har studerat. Det visade sig dock under arbetets gång att denna attraktion inte var nog för att förklara vad vi ser i experiment, så jag fick inkludera ytterligare en attraktion i mina simuleringar. Den representerar andra typer av krafter som finns mellan molekylerna i lösning (proteinerna) och atomerna som ytan består av.

Anledningarna till att vi forskar på just adsorption av proteinet histatin 5 är flera. Denna pusselbit är en del av ett större projekt där vi försöker förstå egenskaperna hos den familj av proteiner som histatin 5 tillhör (så kallade oordnade proteiner). Genom att använda histatin 5 som modellprotein och jämföra simuleringar med experiment vill vi dels testa och utveckla vår modell för oordnade proteiner och dels nå en mer grundläggande förståelse för hur histatin 5 beter sig i olika miljöer. Miljöerna har vi varierat genom att ändra pH och salthalt hos lösningen där proteinet och ytan befinner sig.

Varför är det då intressant att veta hur histatin 5 och dess adsorption till ytor påverkas av att vi har proteinet och ytan i olika typer av lösningar? Jo, histatin 5 är ett viktigt protein för salivens funktion. Det dödar en sorts svamp som heter *Candida albicans* och finns naturligt i våra munnar. Dessutom adsorberar histatin 5 till tandemaljen och kan, tillsammans med andra adsorberade proteiner, skydda den mot att skadas av de syraattacker som tänderna utsätts för när vi äter. Det faktum att miljön är föränderlig i våra munnar gör att det är intressant att studera hur histatin beter sig i lösningar med olika pH och salthalt.

Möjliga tillämpningar

Möjliga områden där våra resultat skulle kunna tillämpas i framtiden är till exempel utveckling av bättre saliversättningsmedel för personer som av någon anledning inte producerar egen saliv (till exempel efter strålbehandling mot cancer i huvud eller hals). Ett intressant spår är att billiga mjölkproteiner

skulle kunna tillsättas i saliversättningsmedel för att ge liknande funktion som salivproteiner.

En kemisk förening av histatin 5 och en molekyl som heter spermidin‡ har visat sig ha potential att bli ett läkemedel mot svamp i munnen, vilket gör det intressant att förstå histatin 5:s egenskaper i förhållande till den nya molekylen.

Intresserad av att läsa vidare?

I resten av denna bok kommer jag att mer detaljerat gå in på vad jag har gjort, vad jag har observerat och vilka metoder jag har använt. Här och var har jag lagt in rutor där jag på ett lättsammare sätt beskriver olika fenomen med hjälp av liknelser. Välkommen med på upptäcktsfärd i mikrokosmos!

‡Namnet kommer av att spermidinmolekylen först upptäcktes i sperma.

List of Publications

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

I **Adsorption of the intrinsically disordered saliva protein histatin 5 to silica surfaces. A Monte Carlo simulation and ellipsometry study**
K. Hyltegren, T. Nylander, M. Lund, M. Skepö.
Journal of Colloid and Interface Science, 467, **2016**, pp. 280–290.

II **Adsorption of polyelectrolyte-like proteins to silica surfaces and the impact of pH on the response to ionic strength. A Monte Carlo simulation study.**
K. Hyltegren, M. Skepö.
Manuscript submitted to Journal of Colloid and Interface Science.

Contribution Report

I **Adsorption of the intrinsically disordered saliva protein histatin 5 to silica surfaces. A Monte Carlo simulation and ellipsometry study**
I performed the experiments and the simulations. I analysed the data and wrote the manuscript with support from the co-authors.

II **Adsorption of polyelectrolyte-like proteins to silica surfaces and the impact of pH on the response to ionic strength. A Monte Carlo simulation study.**
I took part in designing the study. I performed the simulations, analysed the data, and wrote the manuscript with support from M. Skepö.

Part I

Introduction and Context

Chapter 1

Introduction

> "As scientists in the modern era we generally proceed under the assumption that phenomena, if they are natural, are ipso facto explicable—obliged, as it were, to make sense to us. [...] The prevailing assumption over the last two hundred years has been that only divine intervention would be capable of releasing the world from its obligation to make sense to us"
>
> *Evelyn Fox Keller,* Making sense of life *(2002), p. 295*

The title of this thesis – *Making sense of adsorption: Attempting to explain the adsorption of histatin 5 with models, metaphors, and machines* – is a paraphrase of the wonderfully ambitious title *Making sense of life: explaining biological development with models, metaphors, and machines*, which Evelyn Fox Keller (professor emerita of history and philosophy of science at MIT and a trained physicist) used for her book published in 2002 [1]. However, I decided to tone down the boldness of the original title a bit by adding the word "attempting". Also, this word correctly indicates that this is work in progress.

1.1 Why do we attempt to make sense of adsorption?

Adsorption is the adhesion of molecules to a surface. It is a very common phenomenon occurring for example when something is painted. As we shall see, another example is the adhesion of saliva proteins, such as histatin 5, to the tooth surfaces in the mouth.

The adsorption of proteins to solid surfaces has been described as a common but very complicated phenomenon [2] and it has important implications for medical procedures (for example, proteins adsorb to implants that are introduced into the body), and for the food industry (adsorption can lead to fouling – the accumulation of unwanted material on a surface). The complexity, the commonness, and the relevance make the adsorption of proteins a perfect topic for scientific inquiry.

Ideally, if we manage to make perfect sense of adsorption using a theoretical/computational approach, we could make a software that predicts how many molecules that would adsorb under given circumstances. Then we would not have to make time-consuming and expensive experiments.

1.2 Models, metaphors, and machines as tools for making sense

It should come as no surprise that models and machines are essential tools for making sense of physical/chemical phenomena. The models that we have used for computer simulations and experiments are described in chapters 7 and 8, respectively. The machines that we have used are mainly supercomputers (introduced in chapter 7) and an instrument called an ellipsometer (chapter 8).

However, the inclusion of metaphors among the tools might require some more explanation.

According to the Cambridge English dictionary, a metaphor is "an expression, often found in literature, that describes a person or object by referring to something that is considered to have similar characteristics to that person or object" [3]. Within this thesis, I will use the word metaphor as a synonym to the word analogy, even though the term metaphor is mostly used for short expressions such as "the ozone hole", where "hole" is a metaphor for the lack of ozone rather than an actual description of the phenomenon.

Metaphors are often used as educational tools but history also has a couple of examples where metaphors/analogies have helped scientists make important discoveries. For example, it is said that Johannes Kepler understood the motions of the planets by means of an analogy with a working clock [4]. Some people even go so far as to say that thinking about and understanding science without metaphors and analogies is impossible [5].

Analogies have been called a two-edged sword due to the fact that, even though they are often helpful, they can also be dangerous since they all break down at some point. Harrison and Treagust warns agains using only one analogy for a wide range of phenomena since that often means that you take the analogy too far. Instead, they argue that several different analogies should be used in teaching, even though some students might be disturbed by the fact that they don't get a single explanation [6].

In this thesis I have decided to use metaphors from our daily lives to cast

light on some of the phenomena that I am describing. The metaphors can be found in boxes with the heading "Explaining x with metaphors".

1.3 How do we make sense of something?

What are the criteria that need to be fulfilled in order for us to make sense of something? What counts as an explanation? I believe that such questions have no simple answers – it depends on the field of study and the preferences of the person that tries to make sense of a phenomenon. However, even without a clear definition of what counts as an explanation, our feelings can tell us when something has made sense. Therefore, I will use the simple definition that something has made sense when the person trying to make sense of it thinks "Aha".

One might argue that this definition is a bad one since it accepts also fundamental misconceptions as valid tools for making sense of something. However, I would argue that misconceptions can be essential tools when we try to make sense of reality. Some misconceptions have worked remarkably well so far.

Science has changed and developed throughout the centuries and chances are that many of the widely accepted assumptions of today will be disproved before the next century begins. However, that doesn't mean that the explanations built on these assumptions don't make sense now. It also doesn't mean that the theory we have is completely wrong – maybe we just haven't understood its limitations yet. For example, Newtonian mechanics is perfectly valid for most systems that we encounter in our daily lives, but when we look at very small systems we need quantum mechanics and at high speeds we need Einstein's theory of relativity.

However, my view that misconceptions can sometimes be acceptable tools for making sense of something doesn't mean that I find it appropriate to use them in case we *know* that they are misconceptions. For example, when teaching science or presenting it to a wider audience I think that care should be taken to provide the students/audience with the best explanatory tools that we can.

Within physics and chemistry, an accepted explanation of a phenomenon is often a reductionist one – one that breaks a problem into parts and then describes how the parts together give rise to the phenomenon, with the help of laws or principles and logical reasoning. In the physics textbook *Matter and interactions I: Modern mechanics* this view is nicely expressed in the beginning of the first chapter:

"The main goal of this textbook is to have you engage in a process central to science: the attempt to explain in detail a broad range of phenomena using a small set of powerful fundamental principles." [7, p. 1]

The idea behind this project was exactly the same. I proceeded under the assumption that if we properly combined simple models based on fundamental laws with experiments, the adsorption of histatin 5 would in the end be obliged to make sense.

Chapter 2

Context and relevance

> "The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter – for the future. His duty is to lay the foundation for those who are to come, and point the way. He lives and labors and hopes."
>
> *Nikola Tesla, Radio power will revolutionize the world,* Modern Mechanix & Invention *(1934)*

This work focuses on a rather specific problem of adsorption: The adsorption of the saliva protein histatin 5 to a silica surface. The purpose of this chapter is to describe the relevance of the research project and put it into context.

2.1 The context of the research project

2.1.1 Disordered proteins

The PhD project presented in this licentiate thesis is part of a larger project where we are modelling proteins that are intrinsically disordered (the concept of intrinsically disordered proteins will be introduced in section 3.3). We are also trying to understand their physicochemical properties in solution and at interfaces. The idea is that by appropriately modelling these proteins, we can gain a deeper understanding of how they behave in the biological environments where they are naturally present. The topic of intrinsically disordered proteins has attracted a lot of interest during the past 15 years since it was relatively

recently discovered that disordered proteins are responsible for many important biological functions [8–12].

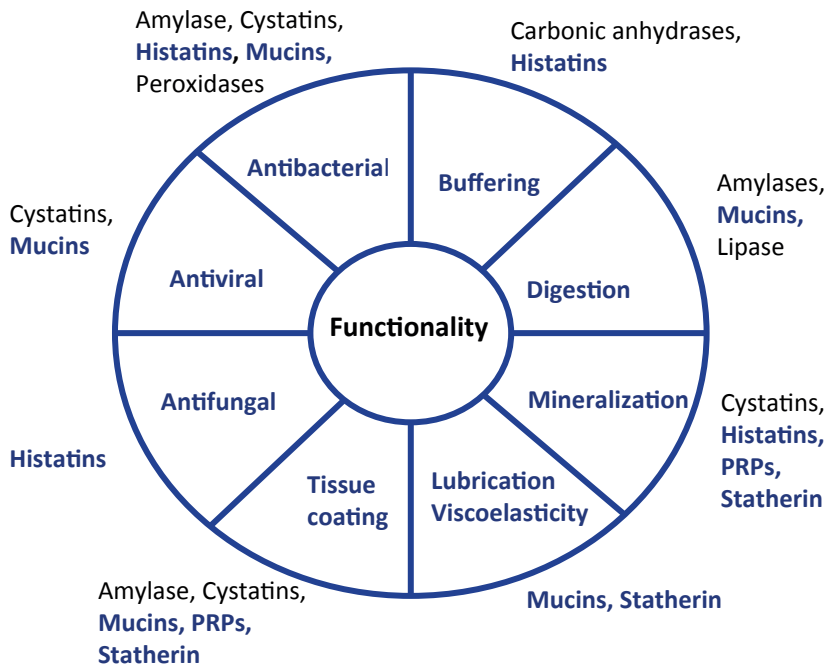


Figure 2.1: The functions of saliva and the proteins responsible for each function, adapted from Levine [13]. The intrinsically disordered proteins are marked in blue. (PRPs = proline-rich proteins)

2.1.2 Saliva

Saliva consists of 99.5 % water. However, saliva also contains proteins – some of which are intrinsically disordered (proline-rich proteins, statherin, histatins, mucins [14]). Even though proteins constitute such a small fraction of saliva (approximately 0.2 % [34]), they are important for almost all of its functions, see Fig. 2.1, and thus for our oral health. If we can understand the saliva proteins and their functions better, it may become easier to develop better saliva replacement products and new peptide-based drugs, for example against fungal infections.

2.2 The relevance of the research project

2.2.1 The biological relevance: Histatin 5 – a potential drug

Histatin 5 kills a fungus called *Candida albicans* and this has received much attention in the literature [15–21]. Interestingly, histatin 5 is still effective against *Candida albicans* when adsorbed to hard surfaces present in the mouth [22]. It has been suggested that developing antimicrobial peptides that selectively adsorb to tooth enamel could be a way to increase their therapeutic activity by making it easier for them to escape degradation by enzymes in the mouth [23]. The rationale behind this idea is the observation that histatin 1 is able to resist degradation in this way, and the same is expected for histatin 5 [24]. Thus, a fundamental understanding of what governs the adsorption of histatin 5 could aid in the development of new peptide-based antifungal drugs.

2.2.2 The relevance for model evaluation and development: Histatin 5 as a model protein and the novelty of our approach

Within the framework of the larger project concerning intrinsically disordered proteins, we are evaluating and improving a coarse-grained model for such proteins, *i.e.* a rather crude model that does not take all the atoms of the molecule into account. In order to evaluate the generality of the model, it needs to be applied to different types of intrinsically disordered proteins, and comparisons with experiments need to be made.

Histatin 5 is a short, cationic protein that can be produced synthetically. The availability makes it a good choice of protein for performing experiments. From a physicochemical point of view, histatin 5 is an interesting choice of model protein due to its unusual capability of changing its net charge depending on the external electric field (this concept of protein charge regulation is described in section 5.1.2). While flexible proteins/polyelectrolytes have been modelled by many others over the years, the inclusion of charge regulation in the model is somewhat unique to our approach.

2.3 The studied systems

The systems that we have studied are simplified versions of the oral environment where histatin 5 is naturally present – meaning that they can be seen as models of the real system. In the experimental model system, saliva is exchanged for histatin 5 dissolved in an aqueous buffer, the tooth enamel is exchanged for a silica surface, and the rest of the mouth environment is simply ignored. The simulated systems contain one or several histatin 5 molecules which are coarse-grained. The surface is represented as a completely flat interface with a smeared charge. Water and salt are not explicitly included.

Are these reasonable model systems compared to the biological one? In most respects, probably not. But hopefully the mechanisms governing the adsorption

of histatin 5 to the solid surfaces in the mouth are the same as those in our model system.

2.4 Outline for the thesis

Before we proceed to the results of the research we must understand the system itself and the tools that we are using. Part II of this thesis describes histatin 5 (the adsorbate) and the surface to which it adsorbs (the adsorbent). In Part III, some fundamental theory is provided. In Part IV, the methods and models are described, and in Part V the main results of the papers are presented, followed by a discussion and an outlook.

Part II

Adsorbate and Adsorbent

Chapter 3

Histatin 5 – an intrinsically disordered saliva protein

> "The suggestion that the native state of many proteins is intrinsically disordered (or, as originally termed, unstructured) is now integral to our general view of protein structure and function. A little more than 10 years ago, however, such challenge to the almost dogmatic 'structure–function paradigm' was pure heresy due to the overwhelming evidence that structure determines function. A decade of steady progress turned skepticism around[...]. I show that the evidence for the generality and importance of this phenomenon [of disordered proteins] is now so insurmountable that it demands the inclusion of 'unstructural' biology into mainstream biology and biochemistry textbooks."
>
> *Peter Tompa, Intrinsically disordered proteins: a 10-year recap,* Trends in biochemical sciences *37, no. 12 (2012)*

The goal of this chapter is to explain the concepts in the chapter title and then provide some additional information about histatin 5 and saliva.

3.1 Proteins and polypeptides

A protein is a large molecule made from amino acids which are bound to each other through a peptide bond, see Fig. 3.1. Since the amino acids lose a few of their original atoms (a water molecule) upon forming a peptide bond, the

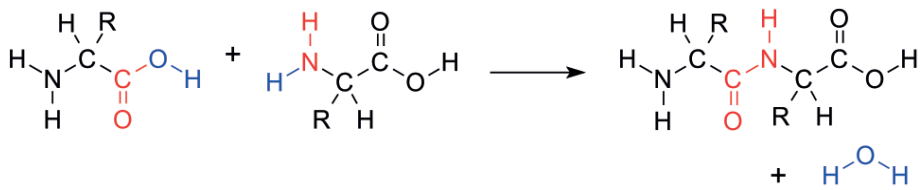


Figure 3.1: The formation of a peptide bond between two amino acids. R denotes the side chains of the amino acids and the other atoms form the *backbone* of the peptide. (Public domain, https://commons.wikimedia.org/wiki/File:AminoacidCondensation.svg)

residual parts that make up the proteins are called amino acid *residues*. A molecule where several amino acid residues are connected by peptide bonds is called a polypeptide.

Proteins are a type of polypeptides. The building blocks are 20 different amino acids, see Fig. 3.2. The word protein is generally reserved only for longer polypeptides. There is no absolute requirement for how long a polypeptide has to be to be called a protein, but most proteins contain between 50 and 2000 residues [25]. Interestingly, histatin 5 is referred to as a protein in the literature even though it is only 24 amino acid residues long [15]. In earlier literature, it was named HRP-5 (histidine-rich polypeptide 5) [26, 27] but it seems that as its antifungal function was discovered, it started to be referred to as a protein [15].

3.2 Protein structure and function

The structure of a protein is generally described as divided into four different layers: primary, secondary, tertiary and quaternary structure. The definitions are given below.

3.2.1 Primary structure

The primary structure of a protein is defined as the sequence of amino acid residues from the N- to the C-terminal. The N-terminal is the end of the protein that contains the amino group (NH_2) and the C-terminal contains the carboxylic acid group (COOH) of the backbone, see Fig. 3.1.

3.2.2 Secondary structure

The secondary structure are common structural elements of proteins, for example α-helices and β-sheets. Such regular patterns are made possible by specific hydrogen-bonding patterns of the protein backbone (see Fig. 3.1 for a definition of the backbone). The oxygen atoms of the C=O-groups bind to the hydrogens of

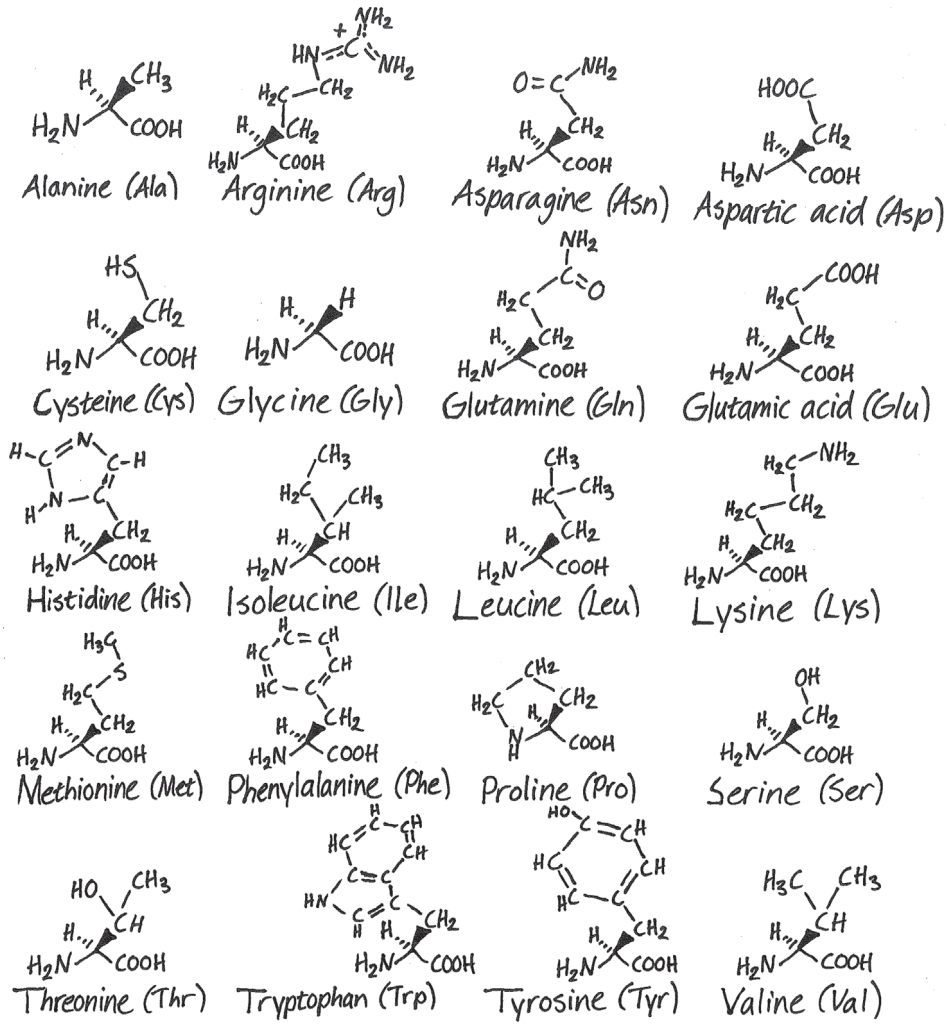


Figure 3.2: The structures of the 20 different amino acids that are the building blocks for proteins.

the N-H-groups. Several of these bonds in a row connect two different parts of the backbone with each other and a secondary structure is formed.

3.2.3 Tertiary structure

The tertiary structure is the three-dimensional structure of the protein as a whole. Hydrophobic interactions are the main driving force for the process where a protein folds into its tertiary structure [28]. However, covalent bonds (sulfur bridges), electrostatic interactions (such as salt bridges) and hydrogen bonds are also important. Most of these interactions are described in more detail in chapter 5.

3.2.4 Quaternary structure

Quaternary structure is the three-dimensional structure of two or more proteins forming a larger functional unit. An example of such a unit is hemoglobin, which consists of four protein subunits in most vertebrates.

3.2.5 The connection between structure and function

Many proteins are like molecular machines performing different tasks in the body, for example catalysing chemical reactions (these proteins are called enzymes), transporting molecules and replicating DNA. Some proteins are also essential for providing structure for various tissues etc. For example, collagen – the most common protein in mammals – is an extracellular protein that is an important constituent of skin, bone, teeth, cartilage, and tendon [25].

For a long time, it was assumed that proteins needed a well-defined 3D-structure in order to be functional. For example, an enzyme is often thought to be like a lock, which can only perform its function (open) when the right substrate (key) enters the active site (keyhole). Proteins that are important for structure also depend on having a specific structure themselves.

However, there are many proteins that do not adopt a well-defined 3D-structure under physiological conditions, and they are still functional – a discovery that ended the so-called "structure–function paradigm" within protein science [8–12].

3.3 Intrinsically disordered proteins

Intrinsically disordered proteins are proteins that do not adopt a well-defined secondary or tertiary structure under physiological conditions (pH $\approx$ 7, ionic strength $\approx$ 150 mM). Instead, they behave essentially as random coils in solution. A random coil is like a chain – the different parts are bonded to each other but apart from that condition, the chain/molecule can adopt essentially any conformation. The likelihood that a part of the chain has a specific conformation is unaffected by the conformation of the rest of the chain.

Approximately 30 % of the proteins present in eukaryotes have disordered regions with a length of ≥ 50 residues [9]. Counting also shorter proteins, the number would increase since short proteins are less likely to fold into well-defined tertiary structures.

Examples of intrinsically disordered proteins are the milk protein β-casein (209 residues) [29] and the saliva protein histatin 5 (24 residues) [16, 30]. Both of them have important functions – β-casein transports calcium to the suckling infant [31] and histatin 5 has antimicrobial effects [15, 32].

Explaining protein disorder with metaphors

The H.C. Andersen of proteins

In the protein world, those individuals that lack a stable structure under physiological conditions are called intrinsically disordered. Perhaps this is similar to how we categorise a certain type of humans who are not mentally stable in our society as having bipolar disorder.

One might think that people with a disorder are of little use for society – instead of contributing they are costing resources since they might need different types of treatments. Similarly, it was assumed that proteins needed to have an ordered structure to be able to perform anything useful inside our bodies.

However, it turned out that intrinsically disordered proteins are responsible for many important functions. Similarly, people with mental disorders can contribute greatly to society! People with creative professions, such as artists or scientists, are more often diagnosed with bipolar disorder than the general population [33]. Since creativity/flexibility was probably important for solving critical problems that humans had in the past, natural selection criteria might have favoured traits connected to mental disorder.

So how can disordered people function? Do they need to be in a specific environment where they are stable or can they function even without stability? This question has an equivalence in the protein world: Is it so that an intrinsically disordered protein must acquire a structure by a change in environment to be able to function, or can it perform its task also in its disordered state? Quite possibly, the answer depends on which person/protein we are considering.

3.4 The primary structure of histatin 5

The amino acid sequence of histatin 5 is Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr [15]. Notably, one third (7 out of 24) of the amino acid residues are histidines.

Histidine is an unusual amino acid since it is uncharged above pH 6 and positively charged below pH 6. The other amino acids that can be positively charged (lysine and arginine) are so below pH 11 and 12, respectively. The amino acids that can be negatively charged (aspartic and glutamic acid) are so above a pH of 4. This means that histidine is the only amino acid that can change its charge at physiological pH (pH $\approx$ 7). This so-called "charge regulation" mechanism of histatin 5 will be described in more detail in chapter 5.

3.5 Saliva proteins

3.5.1 The composition and protein content of saliva

Saliva consists of approximately 99.5 % water. The rest can be divided into inorganic constituents – for example sodium, potassium, chloride, and iodide – and organic constituents such as proteins, carbohydrates, and amino acids [34]. Proteins make up between 1.0 and 6.4 g/l of saliva. There are conflicting reports about the concentration of histatin 5 in human saliva – according to Castagnola *et al.* the mean concentration is 47 µg/ml [35] and according to Campese *et al.* it is 8 µg/ml [36].

Even though proteins such as histatin 5 constitute only a tiny fraction of saliva, they are of large importance for our oral health, as described in section 2.1.2.

3.5.2 How the acquired enamel pellicle protects your teeth

Proteins adsorb to the tooth enamel, creating a layer that protects the teeth against acid degradation and affects the attachment of bacteria to the tooth surface [37]. This layer is called the acquired enamel pellicle.

Histatin 5 selectively adsorbs to hydroxyapatite (the main constituent of the tooth enamel) [38], making it a so-called pellicle precursor. It has been shown that intact histatin 5 is present in the *in vivo* acquired enamel pellicle to some degree and that it can protect the enamel from acid injury [39]. However, histatin 5 has another function which is even more important.

3.6 The antifungal effect of histatin 5

3.6.1 *Candida albicans* and oral candidiasis

Histatin 5 kills the fungus *Candida albicans* [15], which is commonly present in the mouths of humans.* A healthy person who carries *Candida albicans* in the mouth generally experiences no problems. However, if the fungus grows too much it gives an infection called oral candidiasis (or oral thrush). It shows up as white patches in some parts of the mouth, often on the tongue, see Fig. 3.3.

*More exact estimates of the prevalence of *Candida albicans* in human mouths have varied greatly and depend on the method of measurement [40].

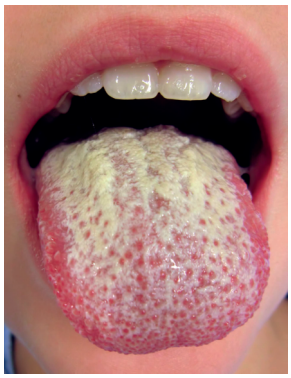


Figure 3.3: Human tongue infected by oral candidiasis (thrush). Photo by James Heilman, MD, distributed under a CC BY-SA 3.0 license. (https://commons.wikimedia.org/wiki/File:Human_tongue_infected_with_oral_candidiasis.jpg)

The infection can cause discomfort or bad taste. In extreme cases it can spread through the bloodstream or the upper gastrointestinal tract and even become fatal [41].

People with HIV, people with very dry mouth, smokers and people with diabetes are examples of people who are extra susceptible to oral candidiasis [41].

3.6.2 Histatin 5 as a drug candidate against oral candidiasis

It has been suggested that histatin 5 could work as a drug against oral candidiasis [42]. There are already antifungal drugs that work but as the fungi become resistant, new drugs are needed. The problem with histatin 5 is that it is quickly degraded in the oral environment, which means that it is impractical and uneconomical to use the protein as it is. An interesting possibility is to connect an active fragment of histatin 5 with a molecule called spermidine. This histatin–spermidine-conjugate has shown promising results as an anticandidal drug in mice [42].

Chapter 4

Silica, tooth enamel and hydroxyapatite – scratching the surface

> "It is remarkable that silica, the major component of the earth's solid surface, has never become a separate branch of study or instruction. Science students graduate with little or no knowledge of its properties or chemistry."
>
> *Ralph K. Iler,* The Chemistry of Silica *(1979), Preface, p. vii*

In this chapter, I am "scratching the surface" of the complex surface chemistry of silica and tooth enamel/hydroxyapatite – the types of surfaces that are most important for my work.

Silica is a convenient model surface to use for ellipsometry measurements, which is why I have used it for the experiments in this thesis. However, tooth enamel, which consists mainly of hydroxyapatite, is the surface that is relevant in the biological system where histatin 5 is present naturally.

4.1 Silica

Silica is another name for silicon dioxide (SiO_2). It is by far the most common material of the earth's crust. It is most commonly found in the form of the mineral quartz.

4.1.1 The silica surface and its silanol groups

A silica surface that is exposed to air at ordinary temperatures reacts with water so that the surface becomes covered with silanol groups (SiOH) [43]. Immersed in an aqueous solution, these silanol groups can lose an H-atom, yielding charged SiO^--groups. This is the main mechanism by which silica surfaces acquire a negative charge. In an aqueous solution, the extent to which this happens is determined by the pH and the ionic strength of the solution.

4.1.2 The surface charge of silica

The surface charge densities of silica particles at different pH and salt concentrations have been measured by Bolt [44] and by Samoshina *et al.* [45]. The values are given in Table 4.1.

Table 4.1: Surface charge densities of silica particles at different pH-values and concentrations of monovalent salt (c_s) in $\mu C/cm^2$. The surface charge densities determined by Samoshina *et al.* have been measured from a graph and the numbers in this table are therefore approximate.

pH	c_s = 10 mM		c_s = 100 mM	
	Bolt [44]	Samoshina *et al.* [45]	Bolt [44]	Samoshina *et al.* [45]
6	−0.9	−0.25	−1.5	−0.50
7	−1.8	−1.00	−3.2	−2.00
8	−3.8	−2.75	−6.2	−5.75
9	−6.9	−7.00	−11.6	−12.75

Even though the numerical values differ, the trends are the same. The surface charge of silica increases approximately by a factor of two when the salt concentration is increased from 10 mM to 100 mM. We have used the surface charge densities determined by Samoshina *et al.* [45] for the simulations included in this thesis.

The negative charge of the hydroxylated silica surface keeps increasing with pH until about pH 10.7. The maximum charge is limited by the density of silanol groups on the surface, which is 4–5 per nm^2 [43]. However, above a pH of 8–9, silica starts to dissolve, forming silicate ions ($HSiO_3^-$). Complete ionisation of the surface can be achieved just below the pH where silica dissolves completely, in the presence of a concentrated salt solution.

4.2 Tooth enamel and hydroxyapatite

4.2.1 Tooth enamel

The tooth enamel surface consists of a mineral component – impure hydroxyapatite – and organic materials forming a matrix. The organic matrix holds the mineral crystals together and regulates their formation [34]. When a newly

formed tooth has appeared in the mouth, the tissue-forming cells are worn off, and the fate of the enamel is no longer determined by cellular processes but by the interactions with the fluids in the mouth [34].

Hydroxyapatite occupies approximately 86 % of the volume of enamel and makes up 96–97 % of its weight [46].

4.2.2 Hydroxyapatite

The chemical formula of hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$. In addition to being the main component of tooth enamel, it also constitutes up to 50 % of human bone by volume.

The surface of hydroxyapatite is dominated by calcium, phosphate and hydroxyl groups, as indicated by its chemical formula. In aqueous solutions at pH 7, the surface is likely to be dominated by HPO_4^{2-} and $H_2PO_4^-$.

4.2.3 The surface charge of enamel and hydroxyapatite

The surface charge densities of enamel and hydroxyapatite vary significantly depending on the pH of the solution, the solid-to-liquid ratio, the pretreatment of the surface and, in case of hydroxyapatite, its purity [47].

According to Arends [47], enamel surfaces and hydroxyapatite surfaces of various purities are positively charged in doubly distilled water at high solid-to-liquid ratio (similar to the conditions in the mouth). However, after pretreatment with saliva from the submandibular glands, the surfaces became negatively charged, indicating that the acquired pellicle would make the enamel surfaces negatively charged *in vivo*.

At low solid-to-liquid ratios, Arends found that the enamel and hydroxyapatite surfaces were negatively charged above pH $\approx 5.5 - 6$ [47]. However, not as negatively charged as a silica surface would be under similar conditions.

Part III

Fundamental Theory

Chapter 5

Interactions in the molecular world

> "I have lived much of my life among molecules. They are good company. I tell my students to try to know molecules, so well that when they have some question involving molecules, they can ask themselves, What would I do if I were that molecule?"
>
> *George Wald, Nobel laureate in Physiology or Medicine, speech at the Nobel Banquet in Stockholm, 10 December, 1967*

In order to understand how and why proteins form structures, interact with each other and adsorb to surfaces (the theme of this work), we must understand what kinds of interactions that are present between molecules and atoms in general. In this chapter, I will describe the different interactions that are important in the world that molecules experience.

Most of the information in this chapter is taken from Israelachvili [48] and for more in-depth information, I refer the reader to this book.

5.1 Coulomb interactions

The electrostatic interaction between two charged atoms (ions) is described by Coulomb's law:

$$F = \frac{Q_1 Q_2}{4\pi\varepsilon_0 \varepsilon r^2} \tag{5.1}$$

where F is the electrostatic force between the atoms, Q_1 and Q_2 are the charges of atoms 1 and 2, ε_0 is the vacuum permittivity, ε is the relative permittivity of the medium surrounding the atoms, and r is the distance between the atoms.

The potential energy between two charges is given by

$$V = \frac{Q_1 Q_2}{4\pi\varepsilon_0\varepsilon r} \tag{5.2}$$

which means that the distance dependence is $1/r$.

5.1.1 Screened Coulomb interactions

In a solution containing ions, the ionic atmosphere causes the Coulomb potential to decay more rapidly with distance than Eq. (5.2) implies. This *electrostatic screening* effect is taken into account by the screened Coulomb potential:

$$V = \frac{Q_1 Q_2}{4\pi\varepsilon_0\varepsilon r} \mathrm{e}^{-\kappa r} \tag{5.3}$$

where κ^{-1} is the *Debye length*.

$$\frac{1}{\kappa} = \sqrt{\frac{\varepsilon_0 \varepsilon k_\mathrm{B} T}{2 N_\mathrm{A} e^2 I}} \tag{5.4}$$

where k_B is the Boltzmann constant, T is the temperature, N_A is Avogadro's constant, e is the elementary charge, and I is the *ionic strength* defined by

$$I = \frac{1}{2} \sum_{i=1}^{n} z_i^2 c_i \tag{5.5}$$

where n is the number of different ionic species, z_i is the charge number of an ion of type i and c_i is the concentration of ions of type i.

5.1.2 Charge regulation and protein charge capacitance

The charge of a molecule that contains acidic or basic groups can change in the presence of an electric field. This phenomenon is called *charge regulation* since the molecule regulates its charge depending on the environment. In case it is close to a positively charged object, it is more favourable for it to become negatively charged and, conversely, if it is close to a negatively charged object it is favourable for it to become positively charged.

At a certain pH (the pK_a or pK_b) an isolated (non-interacting) acid or base has an equal probability of being charged or neutral. Around this pH it is particularly easy to perturb the degree of ionisation because there is a large natural fluctuation, which can be expressed as a high *charge capacitance* [49].

The pK_a of the amino acid histidine is approximately 6, which is close to physiological pH. This means that histatin 5 has an unusually high charge

capacitance compared to other proteins under physiological conditions. Thus, charge regulation can sometimes contribute significantly to the driving forces behind the adsorption of histatin 5 [50].

5.2 Interactions involving permanent dipoles

Most molecules have no net charge, however many molecules are polar. This means that the equal number of positive and negative charges are not evenly distributed, but the molecule has an excess negative charge at one end and a positive excess at the other end. Polar molecules/objects can be characterised by a vector quantity called the dipole moment. For the case of two point charges separated by a distance l the dipole moment is:

$$\boldsymbol{\mu} = q\boldsymbol{l}, \tag{5.6}$$

where q is the absolute value of the charges ($+q$ and $-q$), and $\boldsymbol{l}$ is the distance vector from the negative charge to the positive charge.

A more general expression for the dipole moment is:

$$\boldsymbol{\mu}(\boldsymbol{r}) = \int_V \rho(\boldsymbol{r}_0)\,(\boldsymbol{r}_0 - \boldsymbol{r})\,\mathrm{d}^3\boldsymbol{r}_0, \tag{5.7}$$

where $\rho(\boldsymbol{r}_0)$ is the charge density at $\boldsymbol{r}_0$, and $\mathrm{d}^3\boldsymbol{r}_0$ denotes a volume element in the volume V.

5.2.1 Ion–dipole interactions

When a polar molecule interacts with an ion and $r \gg l$ (the distance from the ion to the centre of the dipole is much greater than the distance between the charges of the dipole), the potential energy becomes:

$$V = -\frac{Qql\cos\theta}{4\pi\varepsilon_0\varepsilon r^2} = -\frac{Q\mu\cos\theta}{4\pi\varepsilon_0\varepsilon r^2} \tag{5.8}$$

where Q is the charge of the ion, l is the distance between the two charges of the dipole and θ is the angle between $\boldsymbol{r}$ (the vector from the ion to the centre of the dipole) and $\boldsymbol{l}$. Note that the distance dependence is $1/r^2$.

In case the factor determining the magnitude of the angular dependence in Eq. (5.8) $\left(\frac{Q\mu}{4\pi\varepsilon_0\varepsilon r^2}\right)$ falls below $k_\mathrm{B}T$, the dipole can rotate more or less freely. However, since the attractive interactions are more favourable, the angularly averaged potential will not be zero. By averaging in a way that takes into account that some orientations are more favourable than others (more specifically, by using Boltzmann averaging) one gets the following expression for the interactions between ions and rotating dipoles [48]:

$$V \approx -\frac{Q^2\mu^2}{6\left(4\pi\varepsilon_0\varepsilon\right)^2 k_\mathrm{B}Tr^4} \text{ for } k_\mathrm{B}T > \frac{Q\mu}{4\pi\varepsilon_0\varepsilon r^2} \tag{5.9}$$

Note that this potential decays more rapidly with distance than the one described by Eq. (5.8).

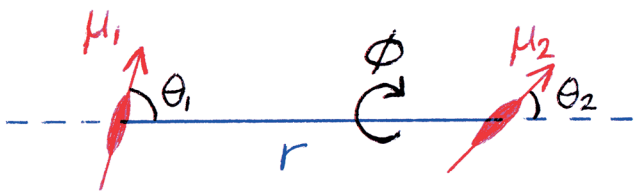


Figure 5.1: The angles θ_1, θ_2, and ϕ between two stationary dipoles separated by a distance r.

5.2.2 Dipole–dipole interactions

The potential energy between two stationary dipoles is given by:

$$V = -\frac{\mu_1\mu_2}{4\pi\varepsilon_0\varepsilon r^3}\left(2\cos\theta_1\cos\theta_2 - \sin\theta_1\sin\theta_2\cos\phi\right), \tag{5.10}$$

where ϕ is the azimuthal angle, see Fig. 5.1. However, in solution the dipoles rotate. Thus, the angle-averaged potential found by using the Boltzmann weight is more relevant for this type of system [48]:

$$V \approx -\frac{\mu_1^2\mu_2^2}{3\left(4\pi\varepsilon_0\varepsilon\right)^2 k_\mathrm{B}Tr^6} \text{ for } k_\mathrm{B}T > \frac{\mu_1\mu_2}{4\pi\varepsilon_0\varepsilon r^3} \tag{5.11}$$

Note that the distance dependence is now $1/r^6$ instead of $1/r^3$ as in Eq. (5.10).

The Boltzmann-averaged interaction described by Eq. (5.11) is called the *Keesom* interaction and it is one of three $1/r^6$-dependent interactions which are included in the so-called *van der Waals* interactions.

Hydrogen bonding

The hydrogen bond is mainly an unusually strong dipole–dipole interaction which can orient neighbouring molecules in both liquids and gases [48]. A hydrogen bond can form between an electronegative atom with a free electron

pair, such as O, N or F, and a hydrogen atom bound to such an electronegative atom. The reasons for the strength of this type of dipole–dipole interactions are the small size of the H-atom, allowing for smaller separations, and the high electronegativity of O-, N- and F-atoms leading to a relatively large positive charge on the H-atom.

5.2.3 Dipole–induced dipole interactions

A polar molecule can induce a dipole in a nonpolar molecule since its electric field makes it more favourable for the electrons of the nonpolar molecule to reside on a specific side of the molecule. This process is called *polarisation* and gives rise to another $1/r^6$-dependent potential which is the second component belonging to the van der Waals interactions. It is often called the *Debye interaction*.*

5.3 London dispersion forces

London dispersion forces are interactions occurring between all molecules – both polar and nonpolar. Dispersion forces are of quantum mechanical origin [48], but they can be understood without diving deeply into quantum mechanics.

A nonpolar molecule has zero dipole moment averaged over time. However, at a specific moment in time, it has a dipole moment due to the specific positions of the electrons around the nuclei. This dipole generates an electric field, which polarises nearby atoms. Therefore, there is an instantaneous attractive force between nearby molecules – the dispersion attraction. Dispersion forces are stronger between larger molecules.

The London dispersion interaction energy has a distance dependence of $1/r^6$ and it is the third component belonging to the van der Waals interactions.

5.4 Excluded volume/Pauli repulsion

According to the Pauli exclusion principle, two electrons cannot occupy the same space/state. This means that when two atoms come close to each other they will feel a Pauli repulsion, which is of quantum mechanical origin. This gives rise to excluded volume – a volume that is impossible for an atom or molecule to enter since it is already occupied.

*Note that ions can also induce dipoles, but a description of this type of interactions has been omitted since the mechanism is the same as for the Debye interaction.

5.5 Pauli repulsion + van der Waals attraction ≈ the Lennard-Jones potential

A common way of describing Pauli repulsion and van der Waals attraction between two particles mathematically is by using the Lennard-Jones potential:

$$V = 4\varepsilon_{\mathrm{LJ}} \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^{6} \right], \tag{5.12}$$

where $\varepsilon_{\mathrm{LJ}}$ is the depth of the potential well between the interacting particles, σ is the distance where the potential is zero (equal to the sum of the Lennard-Jones radii of the two interacting particles), and r is the distance between the particles.

As we saw before, the van der Waals interaction potential has a distance dependence of $1/r^6$. The repulsion should be steep but the specific choice of a $1/r^{12}$-dependence is only motivated by computational convenience $\left(1/r^{12} = (1/r^6)^2\right)$.

5.6 Hydrophobic interactions

Water is a solvent with unusual properties, partly due to its strong tendency to hydrogen bond. This means that it is unfavourable to dissolve nonpolar molecules, which are unable to form hydrogen bonds, in water. The low solubility of nonpolar molecules/fragments in water gives rise to a "hydrophobic attraction" between such molecules/fragments in water.

5.7 Adsorption and the forces between macromolecules and charged surfaces

The forces between surfaces and molecules are similar to the ones described above – after all, a surface also consists of atoms, molecules, and/or ions. However, there are some interactions that become more important when we look at molecules in relation to surfaces.

5.7.1 The electric double layer

Since there are equal amounts of positive and negative charges in nature, a charged surface must have counterions that together have an equal, but opposite, charge. When the surface is immersed in solution, the counterions are present in a layer extending out from the surface. The surface and the layer containing the counterions make up the so-called electric double layer. The double layer has been treated mathematically in several different ways. In the simulations included in this work, Gouy–Chapman theory has been used.

Gouy–Chapman theory

A charged surface is characterised by its surface charge density σ and its electrostatic potential Φ_0. Gouy–Chapman theory describes the relationship between

these two quantities and also how the potential and the distribution of ions in solution vary with the distance from the surface.

Gouy–Chapman theory is derived from a model where the charged surface is infinite in the x- and y-directions and has a smeared charge, and the ions in solution are point charges. According to Gouy–Chapman theory, the electrostatic potential decreases exponentially with distance from the surface. For a more in-depth discussion and treatment, the reader is referred for example to Evans and Wennerström [51].

5.7.2 Entropic repulsion/attraction

When a large, flexible molecule approaches a surface, its available conformations will be severely restricted by the presence of the surface. This leads to an effective repulsion of entropic origin.

However, if the molecule is oppositely charged compared to the surface it may, in addition to being electrostatically attracted to the surface, be *entropically attracted* to the surface. Let's say that we have a negatively charged surface, a flexible macromolecule with a charge of $+5$ and counterions to the surface that each have a charge of $+1$. Then it will be entropically favourable for the macromolecule to replace the counterions in the electric double layer since five counterions have more available configurations in solution than a macromolecule, which is restricted by the fact that its different parts are chemically bonded to each other. Thus, the loss in entropy is less for the adsorption of one macromolecule with a charge of $+5$ than for the adsorption of five counterions that each have a charge of $+1$.

Entropy will be discussed more in chapter 6.

Explaining intermolecular interactions with metaphors

Interactions on a crowded bus[a]

In order to get a feeling for the types of interactions that molecules experience, imagine that you are standing inside a crowded bus on a warm summer day. Many of the interactions that you experience there are similar to interactions between molecules.

The exclusion principle

According to the exclusion principle, you cannot occupy the same space as other people. Therefore, many parts of the bus will be inaccessible to you.

Van der Bus attraction

In case of sudden accelerations and breaks, the synchronisation of your movements with the neighbouring people's movements will matter. If your movements are well synchronised, this will allow for closer separations, meaning that there is a small attractive force between you. This attraction is called van der Bus attraction.

Sweatphobic interactions

In case the person beside you smells really bad from sweating too much in the heat, this leads to repulsion between you and this person. However, the people that are very sweaty will not sense the smell from other sweaty people. The sweatphobic interactions lead to segregation of sweaty people from non-sweaty people and thus give rise to an effective attraction between sweaty people.

Relatiostatic interactions

Imagine that one of your best friends enters the bus. Then you will feel a long-range attraction between you, meaning that even if you are far away from each other you will pass the other people on the bus in order to get close to each other. This is a strong relatiostatic attraction.

In case you are not as good friends, the attraction will be weaker and less long-ranged.

In case someone you dislike enters the bus, the relatiostatic interaction between the two of you will instead be repulsive.

[a]The main parts of this analogy are taken from the PhD thesis of Anıl Kurut Şabanoğlu [52].

Chapter 6

Thermodynamics and equilibrium statistical mechanics

> "With thermodynamics, one can calculate almost everything crudely; with kinetic theory, one can calculate fewer things, but more accurately; and with statistical mechanics, one can calculate almost nothing exactly."
>
> *Eugene Wigner, Nobel laureate in Physics, in Edward B. Stuart, Benjamin Gal-Or, and Alan J. Brainard (Editors)* A Critical Review of Thermodynamics *(1970), p. 205*

In this chapter, I will begin by introducing the fundamental laws of thermodynamics and statistical mechanics. Then I will introduce the concept of thermodynamic ensembles and present some of them.

6.1 What is thermodynamics and statistical mechanics?

Thermodynamics concerns the relation between heat, energy, and work. Within classical thermodynamics one can use macroscopic properties of systems, such as temperature, volume or pressure, to calculate for example enthalpy or free energy. Using classical thermodynamics, it is possible to understand how a

refrigerator works or to determine whether a chemical reaction can occur under certain conditions or not.

Equilibrium statistical mechanics (or statistical thermodynamics) is the science discipline that connects the *microscopic world* that molecules experience with the *macroscopic world* that we ourselves can experience. Another way of putting it is that statistical thermodynamics forms a bridge between classical thermodynamics and molecular physics [53]. The objective of statistical mechanics is to provide the tools to interpret equilibrium properties of macroscopic systems from a molecular perspective [54]. We want to be able to calculate the properties of a macroscopic system – such as pressure, temperature, and energy – by averaging over properties of individual molecules.

Non-equilibrium statistical mechanics will not be considered in this work.

6.2 The laws of thermodynamics

Just as mathematics has its axioms that cannot be proved, thermodynamics has its laws/postulates that have not been proved. However, they have not been disproved either and as long as they have not, it seems reasonable to assume that they hold since the mathematics built on these laws have had remarkable success when it comes to describing and predicting properties of our world.

6.2.1 The laws of classical thermodynamics

The zeroth law

The zeroth law of thermodynamics states that if two systems are each in thermal equilibrium with a third system, they are also in thermal equilibrium with each other. This means that there exists a property which is the same in all of these three systems, and this property is called temperature.

This law is a basic requirement for thermodynamics to make sense, and since it was formulated after the other three it was named the zeroth law.

The first law

The first law of thermodynamics states that the energy change in a system is a sum of the energy that passes its boundaries. This means that for an isolated system (a system that cannot exchange energy or particles with its surroundings), the energy is constant.

The first law is often expressed in the form of the energy principle: Energy cannot be destroyed or created, it can only change form.

The second law

The second law of thermodynamics states that heat cannot spontaneously flow from a colder location to a hotter location. Here, the word *spontaneously* is important. As we all know, there are inventions such as fridges, where heat is

somehow transferred from inside the fridge to its surroundings even though the inside of the fridge is cooler than the outside. However, this heat transfer is not a spontaneous flow of energy – it requires the input of electrical energy to occur. A spontaneous process happens by itself and does not require that another process occurs at the same time to provide a driving force.

The second law of thermodynamics states which kind of processes that can occur spontaneously and can also be formulated in terms of entropy, S: For all spontaneous processes that occur in an isolated system, $\Delta S \geq 0$. Thus, by calculating the total change in entropy due to a process, we can determine whether it can occur spontaneously or not. This is an extremely powerful tool *e.g.* for theoretical chemists.

The third law

The third law of thermodynamics states that as a system approaches a temperature of absolute zero (meaning 0 K), the entropy of the system approaches zero, and becomes so in the case of perfect crystalline substances [53].

6.2.2 The postulates of statistical mechanics

Postulate one: Equal *a priori* probabilities

This postulate states that, in an isolated system, all states consistent with the number of particles,* the volume and the internal energy of the system are equally probable [54].

The connection to entropy. Postulate one leads to the conclusion that a macroscopic state consistent with many different microscopic states is more probable than a macroscopic state that requires the system to alternate between a small number of microscopic states. This conclusion is intimately coupled to the concept of entropy. For an isolated system

$$S = k_{\mathrm{B}} \ln \Omega, \tag{6.1}$$

where Ω is the number of available microscopic states [53].

*Note, however, that if we have chemical reactions in the system, the number of particles will change. Perhaps it is better to say that the available states should be consistent with the *possible* numbers of particles.

Discussing the common entropy = disorder metaphor

Entropy = disorder?

In many chemistry textbooks and classrooms over the world, it is taught that entropy is essentially another word for disorder. One of the more extreme examples can be found in the textbook *Chemistry* by Olmsted and Williams [55]. The book contains a lengthy discussion of disorder and the spontaneity of various processes, where disorder is presented as a measurable quantity. According to the authors, chemists and physicists have measured changes in the organisation of matter and energy, and "They always obtain the same result, which is the **second law of thermodynamics:** ***Any spontaneous process increases the disorder of the universe.***" (p. 595, boldface and italics as in the original) [55]. This "definition" of the second law of thermodynamics is, literally, a textbook example of taking an analogy too far.

So what is the rationale behind the idea that entropy equals disorder? I will try to explain by giving an example:

Imagine a room. In the room there is a bed, a desk, a chair, a bookcase, and a book. If the room was in an ordered state the book would be in the bookcase. Let us imagine that the probabilities that the book ends up in any of the possible places in the room are equal (equal *a priori* probabilities). Is it probable that the book ends up in the bookcase?

No, it is not probable. It is more probable that it ends up in any of the other states – for example in the bed, on the floor, on the desk... The floor is a large surface corresponding to many possible positions of the book and is therefore a more probable location than the bookcase. The floor is favoured over the bookcase, meaning that the entropy of the floor state is higher! If we take all states corresponding to disorder into account, the ordered state is clearly disfavoured.

Often, an ordered state represents few possible configurations while a disordered state has many possible configurations – leading to a higher probability for disorder. Thus, disorder seems to be intimately coupled to the concept of high entropy and, by taking the analogy further, Olmsted and Williams' statement of the second law of thermodynamics follows naturally.

However, I and several others would argue that disorder is not a good translation of entropy [56–63]. It works sometimes, but it might as well guide the thoughts in the wrong direction. I am very critical of presenting the "entropy = disorder" explanation as the only tool for students to use when they attack the problem of what entropy is and what is entropically favourable or not.

Discussing the common entropy = disorder metaphor

I will go back to the books and use an analogy from Roland Kjellander (professor emeritus of physical chemistry at University of Gothenburg) to explain why [61]:

Imagine that we are packing a certain number of books in a box. If we just throw them in randomly they end up in a very disordered state. If we then shake the box, it is likely that the books have got stuck against each other in a way such that they cannot move much. Since the available configurations are few, the entropy of this disordered state is *low*. However, if we put the books in a more ordered way, side by side, we can actually increase the positional entropy of the books. The reason is that the books are packed so efficiently that they will have a lot of empty space available to move into when we shake the box. That makes more positions become available and thus the entropy for the ordered state is, in this case, higher! The same principle is behind the ordering of liquid crystals that are used in most electronic screens manufactured today.

I believe that if we want to help people to gain a molecular understanding of entropy, we should talk about the number of available configurations[a] (perhaps by talking about configurational *freedom*) rather than use a flawed description of entropy as disorder.

[a] It is, however, important to note that this way of reasoning applies not only to the positions of objects in space, but also to the distribution of energy between different energy levels.

Postulate two: Time average equals ensemble average

Within the context of statistical thermodynamics, an ensemble is a large (mental) collection of systems that have the same values of the macroscopic properties that define them (for example the number of particles, the volume, and the internal energy for the case of the microcanonical ensemble introduced in section 6.3.1). Postulate two states that a long *time average* of a mechanical property in a thermodynamic system is equal to the *ensemble average* of the same property, provided that the systems of the ensemble correctly represent the thermodynamic state and environment of the system of interest [54].

A time average is relatively easy to understand – it is simply the average value of a variable over a certain period of time. If, for example, we are driving on the motorway we could calculate the average speed that we have had during the last hour and that is then a time average of the speed. We can then repeat this for several cars to get a more general result for this particular motorway.

An ensemble average would be similar to asking a number of drivers to report their current speed at a specific moment in time, and then calculating the average. For this to be a proper ensemble average, we should not have only one motorway

but an ensemble consisting of a large number of motorways which are equal to each other.

In order for the ensemble average to equal the time average, it is important to properly sample the different parts of the road. For example, if there is a road work somewhere it will significantly lower the average speed. In case none of the drivers reporting their current speed were driving on this part of the road, our ensemble-averaged speed will differ significantly from the time-averaged speed. The problem of sampling will be discussed more in section 7.2.

6.3 Thermodynamic ensembles

Here, I will introduce some different types of thermodynamic ensembles that can be used for molecular computer simulations or theoretical discussions. For more detailed information, see for example Frenkel and Smit [64].

6.3.1 The microcanonical ensemble

In the microcanonical ensemble, the number of particles (N), the volume (V), and the internal energy (U) are constant. The microcanonical ensemble thus describes an isolated system.

6.3.2 The canonical ensemble

In the canonical ensemble, the number of particles (N), the volume (V), and the temperature (T) are constant. This is the ensemble that has been used for the simulations performed in this study.

6.3.3 The isobaric-isothermal ensemble

In case we want to describe a system with specific values for the temperature and pressure, the isobaric-isothermal ensemble is a good choice. In this ensemble, the number of particles (N), the pressure (p), and the temperature (T) are constant. The pressure can be kept constant by fluctuations in volume.

6.3.4 The grand canonical ensemble

In the grand canonical ensemble, the number of particles is allowed to fluctuate, while the chemical potential (μ), the volume (V), and the temperature (T) are constant. This ensemble describes a system of constant volume that is free to exchange energy and particles with its surroundings.

Part IV

Methods and Models

Chapter 7

Monte Carlo simulations and the coarse-grained model

"Everything should be made as simple as possible but not simpler"

A saying attributed to Albert Einstein

In this chapter, I introduce the concept of molecular Monte Carlo simulations and then I continue by describing the coarse-grained model that we have used for our simulations of the adsorption of histatin 5.

7.1 Molecular simulation

There are two main ways of conducting molecular simulations – Monte Carlo simulations and molecular dynamics simulations.

In molecular dynamics simulations, one follows the movements of molecules with time. Newtonian mechanics are used to describe the molecules. The velocities and positions of the molecules change with time depending on the forces that the molecules experience from the surroundings. The time that is simulated is usually on the order of nanoseconds but the time it takes for the computer to perform the calculations is usually more on the order of a few days.

Of course, the exact times vary greatly depending on the studied system and the level of complexity of the model.

In Monte Carlo simulations, a different technique is used. Instead of taking a time average, an ensemble average is taken, meaning that the purpose of a simulation is to generate many configurations to average over. An efficient method for doing so is the Metropolis scheme, described in section 7.2.2.

According to postulate two of statistical mechanics (section 6.2.2), also called *the ergodic hypothesis*, the results from molecular dynamics simulations and Monte Carlo simulations will be the same if the model and conditions are the same.

7.2 Moving particles – a basic trick of Monte Carlo simulations

7.2.1 The problem with naive Monte Carlo simulations

The most naive way of carrying out Monte Carlo simulations would be to generate lots of configurations where the molecules are placed randomly, and then calculate, for example, the potential energy of the system by averaging the potential energies of the different configurations. Each contribution to the average must be weighted by the Boltzmann factor for the configuration ($e^{-U/k_B T}$), since the more likely types of configurations contribute more to the average than the unlikely ones.

The problem is that, for somewhat concentrated systems, this method is extremely computationally inefficient. Over and over again, systems would be generated in which molecules overlap, and where $e^{-U/k_B T}$ is too low for the configuration to give a significant contribution to the average. Thus, another method of generating configurations is needed.

7.2.2 Importance sampling – the solution provided by Metropolis *et al.*

The Metropolis scheme [65] solves the problem. The procedure goes like this:

(i) Generate a start configuration.

(ii) Pick a molecule and try to move it to a new coordinate: $X \rightarrow X + \alpha\xi$, where α is the maximum allowed displacement and ξ is a random number between -1 and 1.

(iii) Compare the internal energy of the configuration before the trial move with that of the configuration after the trial move. If the new internal energy is lower ($\Delta U < 0$), accept the trial move. If the new internal energy is higher, accept the trial move with the probability $e^{-\Delta U/k_B T}$.

(iv) Repeat from step (ii).

The averaging is performed over all accepted configurations. An old configuration counts as an accepted one in case the trial move was rejected, meaning that some configurations will contribute several times to the average. All accepted configurations now contribute equally to the average, since the configurations are generated according to their Boltzmann weight.

By using this scheme, we will find the most important configurations meaning that we will need fewer configurations and, thus, less time to obtain a good estimate of the ensemble average. Therefore, the computational resources are used more efficiently than with the naive method.

7.2.3 Monte Carlo moves

Within the Metropolis scheme, Monte Carlo simulations involve the movement of molecules. However, many of the moves are not physical, as in molecular dynamics simulations, but generated randomly from predetermined types of possible moves. It is important to choose these moves in such a way that the generated configurations will sample the relevant parts configurational space.

Since the types of moves that need to be used are dependent on the system and the model, the Monte Carlo moves used for our simulations of histatin 5 are described in section 7.5, after the coarse-grained model has been introduced.

7.3 The Faunus framework

For the simulations in this work, an open-source library called Faunus has been used. It is a C++ framework for Metropolis Monte Carlo simulations [66].

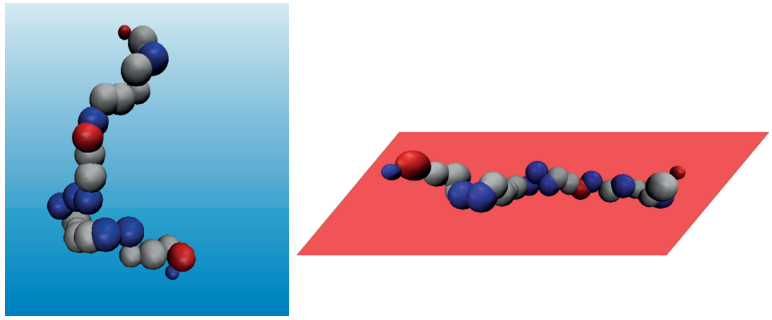
7.4 The coarse-grained model

In order to make sense of the adsorption of histatin 5, we want to model it in a simple way. Simple models are powerful explanatory tools since they are possible for us to grasp. However, if the model is too simple it gives qualitatively wrong results and may even be useless as an explanatory tool. Often, we end up somewhere in between, where some parts of the complex reality can be captured by the model and some parts not.

Here, I will introduce the coarse-grained model that I have used in my computer simulations. The same type of model has been used earlier to study the adsorption of intrinsically disordered proteins [50, 67, 68].

7.4.1 The bead model

Histatin 5 has been modelled as consisting of 26 differently sized beads on a small necklace. The beads each represent one amino acid residue, except for the ones at the ends which represent the N- and C-terminals, respectively. Figure 7.1 shows snapshots of histatin 5 from simulations using this model.



(a) (b)

Figure 7.1: Snapshots from coarse-grained simulations showing histatin 5 (a) in bulk solution and (b) adsorbed to a negatively charged surface. The blue beads are positively charged, the red ones are negatively charged and the grey ones are neutral.

The beads are connected by harmonic bonds (springs). Non-neighbouring beads experience a soft repulsion when they come close to each other (there is a Lennard-Jones potential with $\varepsilon_{\mathrm{LJ}} = 0.05\ k_{\mathrm{B}}T$ between non-neighbouring beads).

7.4.2 Charge regulation

The acidic and basic amino acid residues and the N- and C-terminals are titrating in our coarse-grained model. This means that their charges will depend on the pH, the ionic strength and the external electric field at their respective positions.

7.4.3 The surface

The surface is modelled as a completely flat surface with a smeared charge. The interactions between the charged amino acids and the surface are affected by the exponential decrease in surface potential with distance from the surface according to Gouy–Chapman theory (section 5.7.1).

In addition to the electrostatic interaction between the surface and the charged amino acid beads, we added a potential of the same form as the Lennard-Jones potential between two particles (Eq. (5.12)) between each bead and the surface:*

$$V = \varepsilon_{\mathrm{s}} \left[\left(\frac{\sigma/2}{r_{\mathrm{s}} + \sigma/2} \right)^{12} - 2 \left(\frac{\sigma/2}{r_{\mathrm{s}} + \sigma/2} \right)^{6} \right], \tag{7.1}$$

where ε_{s} is the depth of the minimum of the potential, σ is the diameter of the bead, and r_{s} is the distance between the mass centre of the bead and the surface.

*A more accurate potential between a Lennard-Jones particle and a surface is given by Eq. (3.1) in Steele [69].

The reason for including a short-range attractive interaction is that it is needed in order for our simulations to better match experiments. This potential accounts for van der Waals forces, hydrogen bonding, and possibly an overestimation of the entropy of the free histatin 5 chain in our model system.

7.4.4 Implicit salt

In most of our simulations, salt ions have not been included explicitly. Instead, the ionic strength has entered via the Debye length in the screened Coulomb potential (Eq. (5.3)).

7.4.5 Implicit water

Similarly, the water molecules are not included explicitly in the simulations. Instead, water is represented as a dielectric continuum which affects the electrostatics of the system through its relative permittivity ε. Since the most prevalent component of the experimental system is water, it might seem strange to describe water as crudely as we have done. However, since the water molecules are small compared to most amino acids, it makes little sense to include explicit water when the amino acids are described only as spheres. Interactions arising from the arrangement of water molecules are likely to be unphysical. However, it is a weakness of our model not to include explicit water since we are dealing with adsorption, where confinement of water molecules may occur. Effects coming from the local water structure are not included by the implicit solvent model.

7.4.6 The simulation box

Since it would be too time-consuming to simulate a system as large as the one used in the experiments, our digital protein molecules are placed in a small box. For single-protein simulations, I have used a cubic box with side lengths of 300 Å. In order to compensate for the fact that the box is so small, periodic boundaries are used. This means that whenever a molecule exits the box on one side, it reenters the box on the opposite side. Molecules also interact across the boundaries of the box. (One can view the simulation box as being in contact with a replicate of itself on each side where periodic boundary conditions are applied.)

Periodic boundary conditions cannot be applied in the direction corresponding to the normal to the surface since molecules cannot be allowed to pass through the surface. Therefore, hard boundaries were applied in the z-direction for the simulations where a surface was included.

Since multi-protein simulations are computationally more demanding, a smaller simulation box was used for these simulations. This box had side lengths of 150 Å in the xy-directions and 300 Å in the z-direction. 150 Å corresponds to approximately ten times the radius of gyration of histatin 5 [70] and 1.5 times the contour length.

The minimum image convention

The histatin 5 molecules interact with each other via the minimum image convention. This means that out of the infinite number of images of the same molecules that are present due to the periodic boundary conditions, a specific molecule only interacts with one image of each molecule – the one that is closest.

7.5 The Monte Carlo moves

Here, the different types of trial moves that were used are described.

Single bead translation

The movement of a single bead of one of the histatin 5 molecules.

Protein translation/rotation

Translation or rotation of a whole molecule.

Crankshaft rotation

Defines a rotation axis between two randomly chosen beads and rotates the residues between the chosen beads around the axis.

Pivot rotation

Defines a rotation axis in the same manner as for the crankshaft move and rotates the residues at one end of the protein around the axis.

Reptation move (simulations with more than one protein molecule)

Translates the first or last bead of a protein molecule to a random position a bond distance away from the original position and then moves the neighbouring bead to the former position of the bead that was translated first. The third bead is then moved to the former position of the second bead etc.

Titration move

Protonates or deprotonates (changes the charge of) a bead.

Explaining supercomputers with metaphors

The human supercomputer

Imagine that you were living in 1922 and wanted to make a weather forecast. At that time, there were no computers to aid in such a task. At least not the kind of computers that we mean when we say the word "computer" today.

Lewis Fry Richardson was a meteorologist who was not afraid of thinking big. In 1922, he proposed a way of predicting the weather using what could be called a human supercomputer. He imagined that you could fill a theatre with "computers" (people who would compute things). Each "computer" would be responsible for a small part of the map and they would show the results of their computations to their neighbours in order for them to use them in subsequent computations.

In the middle of the room, a man would sit observing all of the computers. His function would be similar to that of a conductor of an orchestra – to make sure that everyone computed their equations at the same pace. He would shine rosy light upon a region running ahead of the others and blue light upon a region running behind. Four clerks would collect the weather forecast as it was being computed and give it to people responsible for telephoning the radio stations.

This way of thinking is indeed very close to the principles behind the supercomputers, or computer clusters, of today. Several units are connected to each other and using so called parallel computing, each of them can perform calculations at the same time. Sometimes, the different computer cores have to communicate with each other, just like Richardson's "computers" who needed to show other computers what they had found. When the computer cores have got the information necessary for continuing the computation they go on. Often, the communication between cores is the rate-limiting step in parallel computing.

Chapter 8

Experimental methods

> We have to learn again that science without contact with experiments is an enterprise which is likely to go completely astray into imaginary conjecture.
>
> *Hannes Alfvén, Nobel laureate in Physics*

In this chapter, I will introduce the experimental techniques and instruments that I have been using – mainly ellipsometry.

8.1 Purification of surface and protein

Since even a 99 % pure protein sample may not be pure enough for controlled ellipsometry if the impurity is surface active [71], care was taken to ensure the use of clean surfaces and pure protein.

8.1.1 Surface cleaning procedure

The silica surfaces were cleaned by heating them in two different solutions – firstly in an alkaline solution containing ammonia, hydrogen peroxide and water and secondly in an acidic solution containing hydrochloric acid, hydrogen peroxide and water, according to the procedure described by Landgren and Jönsson [72].

This type of cleaning procedure has been shown to effectively remove metal ions and organic contaminants [73].*

The surfaces were stored in ethanol and just before use they were cleaned in a plasma cleaner in order to remove any residual organic contaminants.

8.1.2 Purification of histatin 5 using size exclusion chromatography

The freeze-dried synthetic histatin 5 (purchased from American Peptide Company, U.S.A.) had a peptide content of 59.7 % and the purity of the peptide was 95.8 %. In order to remove excess salt and other impurities, the protein was purified using size exclusion chromatography.

Size exclusion chromatography is a way of separating molecules according to size. The principle is that a concentrated solution of the compound to be purified is injected into a buffer which runs through a column packed with a material that separates molecules according to size (and, to some extent, shape). Small molecules are held up in the column longer than large molecules. The reason is that the small molecules are able to penetrate the pores in the material and thus they experience something similar to a maze, while large molecules travel with the flow of the buffer and bypass the pores making up the labyrinth since they cannot enter due to their size. Molecules of intermediate size will be able to enter some of the pores and will thus have an intermediate retention time.

An important difference between size exclusion chromatography and other chromatographic procedures is that no chemical or physical interactions should occur between the analyte (the molecules to be separated) and the stationary phase (the column packing material) [75].

8.2 Null ellipsometry

Ellipsometry is based on the use of a special type of light – elliptically polarised light. When this type of light is reflected by a surface, the polarisation changes. The change is different depending on the characteristics of the surface and, if there is an adsorbed layer, on the thickness and refractive index of the adsorbed layer.

It is possible to find, for a given surface, an ellipticity of the incoming light that gives linearly polarised light after reflection. Letting the reflected linearly polarised light pass through a polariser, we can align the polariser in a way such that no light passes through. A light detector will then measure a null signal.† This is the principle behind null ellipsometry.

*It is worth noting that the surface charge density of the silica surface depends on the cleaning procedure – if the surface is washed in an acidic solution last it gets a less negative surface charge than if finishing with the alkaline solution [74].

†In practice you will not measure a null signal but rather a minimum in the signal strength.

8.2.1 The polarisation of light

Light is an electromagnetic wave that propagates through space. The electric and magnetic field are perpendicular to each other and to the direction of travel.

For completely unpolarised light, the electric field oscillates in all possible directions. However, if the light is linearly polarised, it oscillates only in one direction and if it is circularly or elliptically polarised, the direction of polarisation rotates. Polarisation refers to the *behaviour in time* of the field vector *observed at a fixed point in space* [76].

Linear polarisers

Linearly polarised light can be created using a linear polariser. An ideal linear polariser transmits light freely in one direction while it absorbs or deflects it completely in the perpendicular direction. The direction with zero absorption coefficient is called the transmission axis while the direction with infinite absorption or deflection coefficient is called the extinction axis [76]. Two ideal linear polariser with the transmission axes perpendicular to each other extinguish light completely.

Waveplates and elliptically polarised light

In order to get elliptically polarised light, linearly polarised light can be passed through a waveplate (also called retarder). A waveplate has a different index of refraction depending on the orientation of the electric field.

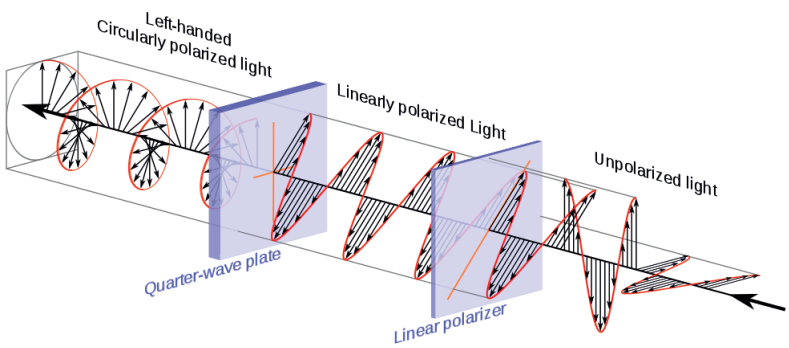


Figure 8.1: The creation of circularly polarised light. (Public domain, https://commons.wikimedia.org/wiki/File:Circular.Polarization.Circularly.Polarized.Light_Circular.Polarizer_Creating.Left.Handed.Helix.View.svg)

Polarised light can be described as composed of two perpendicular components. When linearly polarised light passes through a waveplate, one of the components are retarded relative to the other (a phase shift is introduced between

the components). This leads to elliptical or circular polarisation, since the sum of the two polarisation components will now vary in direction. The creation of circularly polarised light is depicted in Fig. 8.1.

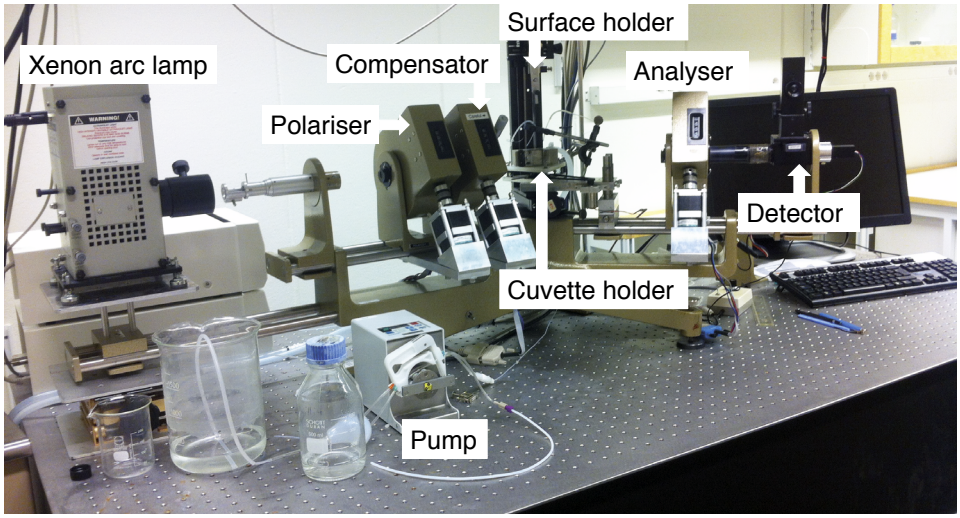


Figure 8.2: The Rudolph thin film ellipsometer used for the adsorption experiments.

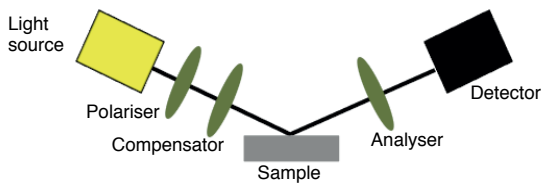


Figure 8.3: A schematic drawing of the ellipsometry setup in Fig. 8.2

8.2.2 Null ellipsometry setup

I have used a Rudolph thin film ellipsometer (Fig. 8.2) with the setup shown in Fig. 8.3. Light from a xenon arc lamp (wavelength 401.5 nm) first passes through a linear polariser. The resulting linearly polarised light passes through the compensator, which creates elliptically polarised light. The light is reflected by the sample, and after that it travels through a second polariser (the analyser). The detector detects the intensity of the light that passes through the analyser.

By adjusting the angles of the polariser and the analyser while following the change in the detected light intensity, the angles that give the weakest light signal (corresponding to linear polarisation of the light that has been reflected by the sample) are found. From the angles of the polariser, the compensator, and the analyser, the so-called ellipsometric angles Ψ and Δ can be calculated.

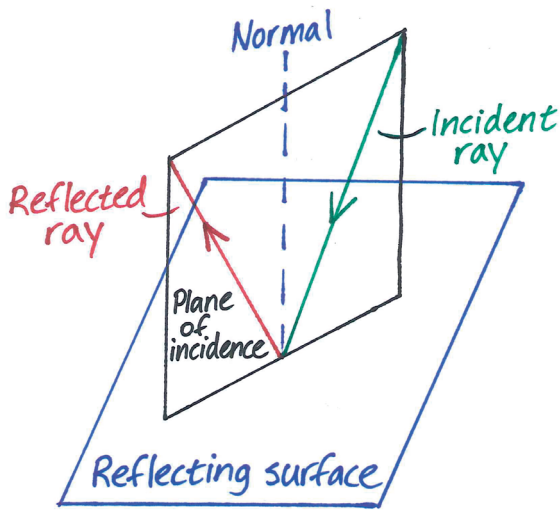


Figure 8.4: The plane of incidence is the plane that contains the incident ray and the normal to the surface at the point where the incident ray strikes the surface.

8.2.3 Models and calculations behind ellipsometry

Here, I will describe how the transformation from the ellipsometric angles to data about layer thickness, refractive index, and adsorbed amount takes place. Since our aim is to compare these experimental values to values from simulations, it is important to understand the assumptions involved in calculating the adsorbed amount.

s- and p-polarisation

As already mentioned, polarised light can be regarded as a superposition of two waves oscillating in perpendicular directions. In the case of light which is

reflected by a surface, the wave is often divided into a so-called p-component and an s-component. The p-component is parallel to the plane of incidence and the s-component is perpendicular to the plane of incidence, meaning that it is parallel to the surface. Figure 8.4 shows the location of the plane of incidence.

Upon reflection at the interface, the polarisation of light changes. This change is described by the reflection coefficient, which is the amplitude of the reflected wave divided by the amplitude of the incident wave, denoted R_p and R_s for the different components of the wave.

The ellipsometric angles (Ψ and Δ)

The ellipsometric angles, Ψ and Δ, are related to the reflection coefficients by the following relation [76]:

$$\frac{R_\mathrm{p}}{R_\mathrm{s}} = \frac{|R_\mathrm{p}|}{|R_\mathrm{s}|} \mathrm{e}^{\mathrm{i}\left(\delta_\mathrm{p} - \delta_\mathrm{s}\right)} = \tan\Psi \mathrm{e}^{\mathrm{i}\Delta}, \tag{8.1}$$

where

$$\tan\Psi = \frac{|R_\mathrm{p}|}{|R_\mathrm{s}|}, \tag{8.2}$$

and

$$\Delta = \delta_\mathrm{p} - \delta_\mathrm{s}, \tag{8.3}$$

where δ_p and δ_s are the phase shifts experienced by the p- and s-polarised light upon reflection. From the ellipsometric measurements, the values of Ψ and Δ are found.

The optical model

The experimental system was described using a model where there were four different layers separated by completely flat interfaces. The four layers are:

- The buffer (medium 0)
- The adsorbed protein film (medium f)
- The silica layer (medium 1)
- The silicon (medium 2)

The buffer, the protein film, and the silica layer were assumed to be transparent, meaning that their refractive indices are real numbers. However, the silicon is not transparent and thus its refractive index is a complex value [72].

Reflection coefficients in the optical model

The overall reflection coefficients R_p and R_s for a surface composed of several layers are functions of the reflection coefficients at each interface. In this system, there is one interface between the buffer and the film, one between the film and the silica, and one between the silica and the silicon. The reflection coefficients for the individual interfaces are denoted r_{ij}, where i and j refer to the media on each side of the interface.

$$R_x = \frac{r_{0\mathrm{f}}^x + r_{\mathrm{f}1}^x \mathrm{e}^{-\mathrm{i}2\beta_\mathrm{f}} + (r_{0\mathrm{f}}^x r_{\mathrm{f}1}^x + \mathrm{e}^{-\mathrm{i}2\beta_\mathrm{f}}) r_{12}^x \mathrm{e}^{-\mathrm{i}2\beta_1}}{1 + r_{0\mathrm{f}}^x r_{\mathrm{f}1}^x \mathrm{e}^{-\mathrm{i}2\beta_\mathrm{f}} + (r_{\mathrm{f}1}^x + r_{0\mathrm{f}}^x \mathrm{e}^{-\mathrm{i}2\beta_\mathrm{f}}) r_{12}^x \mathrm{e}^{-\mathrm{i}2\beta_1}}, \tag{8.4}$$

where x is either p or s,

$$\beta_1 = \frac{2\pi d_1 n_1 \cos\phi_1}{\lambda}, \tag{8.5}$$

$$\beta_\mathrm{f} = \frac{2\pi d_\mathrm{f} n_\mathrm{f} \cos\phi_\mathrm{f}}{\lambda}, \tag{8.6}$$

$$r_{ij}^\mathrm{p} = \frac{N_j \cos\phi_i - N_i \cos\phi_j}{N_j \cos\phi_i + N_i \cos\phi_j}, \tag{8.7}$$

and

$$r_{ij}^\mathrm{s} = \frac{N_i \cos\phi_i - N_j \cos\phi_j}{N_i \cos\phi_i + N_j \cos\phi_j}. \tag{8.8}$$

N_i and N_j are the complex refractive indices of layers i and j, ϕ_i and ϕ_j are the angles of incidence at layers i and j, and λ is the wavelength of the light [77].‡

In order to use the optical model described above to determine the properties of the adsorbed layer, we first need to know the thickness of the silica layer. It is found by measuring in two different ambient media (air and pure buffer solution), as described by Landgren and Jönsson [72].

Determination of the thickness and refractive index of the adsorbed layer

The thickness of the adsorbed film, d_f, and the refractive index of the adsorbed film, n_f, can be determined from Eq. (8.6). This is done using an iterative procedure. The value obtained for d_f is a complex value. However, the thickness of the layer is a real quantity. The iterative procedure continues until the imaginary part of d_f has been minimised. As can be seen from Eq. (8.6), this gives a strong covariance between d_f and n_f. For a fixed value of β_f, if the value of d_f is found to be small, the value of n_f becomes large instead.

When the thickness of the adsorbed layer is small, it is difficult to resolve both d_f and n_f, and unphysical values are often found. Therefore, we did not use the procedure described above for this study, but instead the refractive index of the protein layer was fixed to 1.5, which should be a reasonable value for adsorbed protein layers [78].

‡In the reference, the λ has mistakenly been replaced by a 1.

Determination of the adsorbed mass

The adsorbed amount (mass per surface area), Γ, can be obtained in different manners. This is the formula from Cuypers *et al.* [79]:[§]

$$\Gamma = \frac{3 d_\mathrm{f} f(n_\mathrm{f})}{\frac{A}{M} - v\frac{n_0^2-1}{n_0^2+2}} \left(n_\mathrm{f} - n_0\right), \tag{8.9}$$

where A is the molar refractivity of the compound, M is the molar weight, v is the specific volume and

$$f(n_\mathrm{f}) = \frac{n_\mathrm{f} + n_0}{\left(n_\mathrm{f}^2 + 2\right)\left(n_0^2 + 2\right)}. \tag{8.10}$$

This more simple expression has been devised by de Feijter *et al.* [80]:

$$\Gamma = \frac{d_\mathrm{f}\left(n_\mathrm{f} - n_0\right)}{\left(\frac{\mathrm{d}n}{\mathrm{d}c}\right)} \tag{8.11}$$

Here, the refractive index of the adsorbed film is assumed to increase linearly with the concentration and $\mathrm{d}n/\mathrm{d}c$ is the refractive index increment of the adsorbed compound.

Due to a cancellation of errors, the adsorbed amount can be determined to a much higher degree of accuracy than the thickness and refractive index of the adsorbed layer. In the de Feijter formula (8.11), the product of the layer thickness and the refractive index is taken, which means that the uncertainties introduced in the iterative procedure described above disappear in this product. In the formula by Cuypers *et al.* a similar cancellation of errors occurs.

Using Cuypers' formula to determine the adsorbed amount of histatin 5

We have used the formula by Cuypers *et al.* to determine the adsorbed amount of histatin 5 from ellipsometry measurements. The value used for the specific volume was 0.70 ml/g, as calculated from the specific volumes of the amino acids of histatin 5 [81]. M/A was similarly determined to 3.95 g/ml [82]. It should be noted that the calculated adsorbed amount depends directly on these values.

[§]In the original version of the formula, Cuypers *et al.* used a prefactor of 0.3 instead of 3. This is only a matter of units – they wanted to make a unit conversion.

Explaining the principle of ellipsometry with metaphors

Reflections in a mirror

Imagine that you have a small mirror in your hand. It reflects light very well, which makes it possible for you to see an image of yourself.

If you breathe onto the mirror, a layer of small water droplets forms. Now, you can no longer see yourself as well as you did before. This is because the reflection of light has changed and your eyes detect the difference.

This is similar to the principle behind ellipsometry – the silica surface that we measure on is like a mirror (actually the glass of a mirror consists mainly of silica). The layer of water droplets represents the layer of adsorbed proteins and your eyes are like the detector of the ellipsometer.

The main differences between ellipsometry and the analogy with the mirror and the water droplets is that in ellipsometry, we are using a special type of light which is elliptically polarised, and we are able not only to see that there is protein on the surface but also to determine the *amount* of protein.

Part V

The Research

Chapter 9

Summary and results

> "It is necessary to look at the results of observation objectively, because you, the experimenter, might like one result better than another."
>
> *Richard P. Feynman, Nobel laureate in Physics*, The Meaning of It All: Thoughts of a Citizen-Scientist *(1998), Chapter 1*

In this chapter, I will summarise the two papers included in this thesis and then present some of the results in more detail.

9.1 Summary of papers

9.1.1 Paper I

In this paper, we investigate the adsorption of the intrinsically disordered protein histatin 5 to hydrophilic silica surfaces. The silica surfaces were immersed in aqueous buffer solutions of different pH and ionic strengths. This experimental model system bears some resemblance to the oral environment where histatin 5 is present in saliva and adsorbs to tooth enamel surfaces.

The methods that were used are ellipsometry and coarse-grained Monte Carlo simulations. In the simulations, the protein was represented as a chain of spheres (representing amino acid residues) and the surface as an interface with a smeared charge. Water and salt were included implicitly and the electrostatics were described in terms of Gouy–Chapman and Debye–Hückel theories. Only a single

protein molecule was studied in the simulations, meaning that protein–protein interactions were neglected.

The main objective of the study was to gain an increased understanding of the mechanisms governing the adsorption of intrinsically disordered proteins.

The main result was that charge–charge interactions, as represented in our model, are far from enough to account for the strong adsorption of histatin 5 that was observed experimentally.

9.1.2 Paper II

This paper is a follow-up to Paper I. It is a Monte Carlo simulation study where we investigate the same model system as before, with the difference that more than one protein molecule is now included, in order to find out whether the simulated system can account for the experimentally observed trends in adsorbed amount as a function of ionic strength.

The main objective was to evaluate the previously used coarse-grained model for intrinsically disordered proteins by comparison with experiments and try to achieve an understanding of how the balance between *attractive* electrostatic interactions between the protein molecules and the surface, and *repulsive* electrostatic interactions between the adsorbed proteins, affects adsorption in this system.

The major result is that the simple coarse-grained model fails to qualitatively account for the changes in adsorbed amount as a function of ionic strength.

9.2 Results in more detail

9.2.1 We need a short-range attraction to explain the adsorbed amounts that we see experimentally (Paper I)

Even though the net charge of histatin 5 at pH 7 is approximately +5, the electrostatically driven adsorption seen in the simulations is too weak to explain the adsorbed amounts that we see in experiments. In order to induce more attraction between the protein and the surface, a short-range attraction was added in the simulations. The Langmuir isotherm was used in order to find a value of the interaction strength (ε_s in Eq. (7.1)) giving reasonable agreement between single-protein simulations and experiments.

9.2.2 The effect of salt depends on pH (Paper I)

Ellipsometry measurements have shown that the adsorbed amount is affected differently by an increase in ionic strength depending on pH, see Fig. 9.1 and Table 9.1.

Interestingly, the system at pH 6 and ionic strength 10 mM seemed much more sensitive to disturbances than the other systems, leading to a large standard deviation, as marked in the figure.

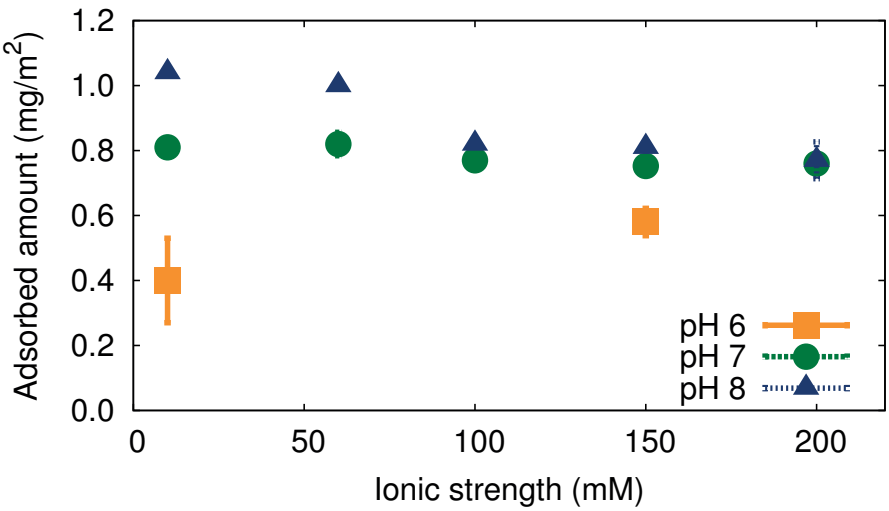


Figure 9.1: The adsorbed amount of histatin 5 to a silica surface after 60 minutes of adsorption. The error bars show the standard deviation for the cases where more than one measurement was made (for pH 7, 10 mM and 100 mM the error bars are covered by the symbols). The concentration of histatin 5 in solution was 0.05 mg/ml.

Table 9.1: The plateau adsorbed amount at different pH-values and two different ionic strengths (I). For the measurements that have been reproduced, the standard deviation is included.

pH	Adsorbed amount (mg/m^2)	
	I = 10 mM	I = 150 mM
4	0.08	0.00
5	0.48	0.44
6	0.40 ± 0.13	0.58 ± 0.042
7	0.81 ± 0.017	0.75 ± 0.005
8	1.04	0.81
9	1.23	0.94

9.2.3 Our coarse-grained model cannot reproduce the experimentally observed pH-dependence of the effect of salt (Paper II)

Simulations with many protein molecules (20–40) did not manage to reproduce the change in salt dependence observed with pH in the experiments. The value of ε_s was varied, leading to different trends – however, the pH-dependence was opposite to that of the experiments.

9.2.4 Charge regulation of histatin 5 is not important for its adsorption to a silica surface (Paper I)

It has previously been shown with simulations that charge regulation of histatin 5 can give an important contribution to its free energy of adsorption at pH 6 [50]. However, our simulations showed that the silica surface is not charged enough at pH 6 to induce a charge regulation which contributes significantly to the free energy of adsorption of a single histatin 5 molecule. A small effect can be seen at pH 8, due to the increased surface charge. However, since the charge capacitance of histatin 5 is then at a minimum, the effect is so small that we believe that it is very unlikely that charge regulation would give a significant contribution to the experimentally observed adsorbed amount.

9.2.5 Charge regulation in a multi-protein system is different from charge regulation in a single-protein system (Paper II)

The insignificance of the charge regulation of histatin 5 was further confirmed by multi-protein simulations. Here, the repulsion between the positive charges of the adsorbed histatin 5 molecules made it less favourable for each histatin 5 molecule to increase its charge upon adsorption, meaning that the effect of charge regulation became even more insignificant.

Chapter 10

Discussion

> "But by what mandate is the world obliged to make sense to us? Is such an assumption even plausible? I would say no, and on a priori grounds. One need invoke neither divine intervention nor unknown forces in order to doubt our ability to make rational sense of all that we encounter in the natural world. [...] Most of us would agree that the mind—along with its capability to make rational sense—is itself a biological phenomenon and hence a product of evolution, brought into existence by forces of natural selection. The selective advantages accruing from the ability to make sense of one's immediate and even remote environment are obvious. Yet an evolutionary process that could give rise to a mental apparatus with an *unlimited* capacity for making sense, however desirable such a capacity would seem, is difficult to imagine."
>
> *Evelyn Fox Keller,* Making sense of life *(2002), pp. 295–296*

In this chapter, I will discuss the results as summarised in the previous chapter.

10.1 Why do we need a short-range attraction? (Paper I)

Histatin 5 is similar to a positively charged polyelectrolyte. It therefore seems reasonable to assume that its adsorption to a hydrophilic and negatively charged surface is driven mainly by electrostatics. Why, then, is an additional short-

range attraction needed in order for our simulated results to match experimental results?

Hydrophobic interactions seem unlikely to be of major importance since only two of the 24 amino acid residues are hydrophobic, and since the silica surface is hydrophilic. However, they could contribute somewhat since most substances are more hydrophobic than water itself.

Hydrogen bonding could possibly occur between the protein and the surface and such interactions are not taken into account by our coarse-grained model.

It is not unusual, even for polyelectrolytes, that adsorption is driven by other interactions than electrostatic interactions between charges in case the systems are not enough strongly charged [83].

The coarse-grained model might overestimate the entropy of the free histatin 5 chain. For example, the angles between the beads are completely non-restricted. In a real protein, only certain dihedral angles of the backbone are allowed due to excluded volume effects between the side chains. An overestimation of the conformational entropy of histatin 5 in solution makes adsorption unfavourable. A short-range attraction can compensate for the overestimation.

Worth noting is also the fact that the data on the adsorbed amount that are given by ellipsometry are dependent on a model in which all interfaces are completely flat. However, errors given by this assumption have been investigated by Tiberg and Landgren and estimated to be very small [77].

Perhaps even more important is the fact that the values of the adsorbed amount calculated from ellipsometry are dependent on the parameters chosen for the Cuypers model (molar refractivity and specific volume of the protein, and the refractive index of the adsorbed layer which was fixed in this study).

10.2 How can we explain the pH-dependent salt effect? (Paper I)

Our hypothesis was that the pH-dependence could be explained by a change in the balance between electrostatic *attraction* between the positively charged amino acids and the surface, and electrostatic *repulsion* between the adsorbed histatin 5 molecules. The rationale for this hypothesis came from observations of how the protein net charge and the surface charge density change with pH. However, the simulations in Paper II could not confirm our hypothesis.

10.3 Why does our coarse-grained model fail to capture the effect of salt seen in experiments? (Paper II)

Part of the explanation could be that this is a semi-quantitative comparison (we are comparing strengths of different and opposing effects to find the net effect) and quantitative comparisons between simulations and experiments are generally difficult.

It is also not completely certain that equilibrium was established in all adsorption experiments, which would make comparison with simulations based on equilibrium statistical mechanics misleading.

There is a possibility that steric interactions or interactions involving polarisation of molecules are important. In concentrated systems, such as the protein layer adsorbed to the surface, local interactions depending on the positions of atoms could be important. These are not captured by the coarse-grained model.

If the strength of hydrophobic interactions change depending on salt content and pH, this would also not be captured by our model.

10.4 Why is charge regulation insignificant in the system where a single histatin 5 molecule adsorbs to a silica surface? (Paper I)

In order to induce charge regulation of the protein, the external electric field must be strong enough. At pH 6, the charge of the silica surface is too low to induce significant charge regulation of the histatin 5. Actually, charge regulation is more pronounced at pH 8 than at pH 6 (despite the capacitance peak of histatin 5 being approximately at pH 6), since the surface charge at pH 8 is approximately ten times higher.

10.5 How is charge regulation affected when we go from single-protein to multi-protein simulations? (Paper II)

In our system with a negatively charged surface and a positively charged adsorbing protein, the effect of charge regulation of the protein decreased when we went from simulating a single molecule to simulating many molecules. The reason is that the repulsion between the adsorbed proteins at the surface makes it less favourable for the proteins to increase their positive charge.

However, charge regulation is not always expected to decrease with the introduction of more adsorbing molecules. If the surface was instead positively charged, charge regulation of a positively charged protein would probably increase since the presence of additional positive charges in the adsorbed layer would make it more favourable for the molecules to *decrease* their charge by charge regulation.

Titration of the surface groups introduces another effect which could also lead to different conclusions regarding the charge regulation of the adsorbing molecules.

10.6 Concluding reflections: Have we made sense of the adsorption of histatin 5?

My spontaneous answer to that question would be “not really”. However, we are a fair bit closer to doing so. We have understood some of the limits of the model system that we have used so far and we have many ideas on how to continue that are outlined in the next chapter. However, one of the most important lessons that we have learned from this work is that the properties of the surface that a molecule adsorbs to are sometimes even more important than the properties of the adsorbing molecule. Perhaps histatin 5 has not been the central character of this story, after all.

A different, and for this particular problem perhaps more relevant, approach could have resulted in this thesis being titled “Making sense of adsorption: Attempting to explaining the adsorption to a hydrophilic silica surface using models, metaphors, and machines."

This is one of the reasons why I believe that the “I” in science should be made visible. We are people with our own personal driving forces and perspectives on things – not merely machines recording data. We interact with the objects or ideas that we investigate – regardless of whether we want it or not. What we believe about the world will determine what kind of answers we will accept and what kind of answers that we are able to find. As Evelyn Fox Keller puts it, “A description of a phenomenon counts as an explanation, I argue, if and only if it meets the needs of an individual or community.” [1, p. 5].

I’m not satisfied. Are you?

Chapter 11

Outlook

> "One never notices what has been done; one can only see what remains to be done"
>
> *Marie Curie, letter to her brother, 1894*

Looking out into the future of our project on the adsorption of histatin 5 to solid surfaces, this would be my to-do list:*

- Perform adsorption simulations in the grand canonical ensemble.
- Use titrating surfaces in the simulations.
- Test the hypothesis that our model overestimates the entropy of the free histatin 5 chain, for example by restricting the dihedral angles of the chain or by simulating an object with a simpler geometry.
- Test the hypothesis that the effect of charge regulation of histatin 5 is enhanced in a multi-protein system compared to a single-protein system if the surface is positively charged instead of negatively charged.
- Compare the Gouy–Chapman treatment of the surface with a model where discrete charges are placed on the surface.
- Include divalent ions in simulations and experiments.

*Note that the fact that time is limited has not been taken into account here.

- Compare Monte Carlo simulations with classical density functional theory calculations of the system.
- Perform quartz crystal microbalance (QCM-D) experiments with histatin 5 to compare to ellipsometry data.
- Perform experiments with non-titrating surfaces.
- Continue with adsorption experiments with buffers containing different amounts of urea to try to understand how urea is changing the interactions.
- Determine the titration curve for histatin 5 experimentally.
- Include surface roughness in the simulations.
- Perform adsorption experiments on other intrinsically disordered proteins and compare to data from simulations.

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Peo Emgård, thank you for all of our interesting conversations at work and at home. Even though they were sometimes distracting, I believe that your answers to some of my 120 questions contributed to my paper writing by making my working days more fun.

Annika and **Bengt Dovrinder**, thank you for warmly welcoming me into your family!

And last but not least – thank you **Carl Dovrinder** for bringing lots of music into my life, and for being more than I had dreamt of. No one can ever replace you!

Postface

> It seems that life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something, and that this thing, at whatever cost, must be attained.
>
> *Marie Curie, letter to her brother, 1894*

So, is this the end? Am I quitting my PhD studies?

The answer is no. While writing this thesis, it turned out that my choice of this path was not so wrong after all. Suddenly, I experienced the unusual benefit of being able to write and print a book while being paid with a monthly salary. And how I enjoyed it.

I might not have made sense of adsorption yet, but "By what mandate is the world obliged to make sense to us?" [1, p. 295]

Here I am with an almost complete first draft of a licentiate thesis. In a month, I will have it in print. It will be my first book about science, just like I dreamt of. The edition will be very limited, but still. My childhood goal was so close to me, and I didn't realise that until I started writing.

Now I want to write a second book – a PhD thesis. And I will do what it takes to be allowed to do that.

Kristin Hyltegren, Lund, September 2016

Paper I

Adsorption of the intrinsically disordered saliva protein histatin 5 to silica surfaces. A Monte Carlo simulation and ellipsometry study

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Adsorption of the intrinsically disordered saliva protein histatin 5 to silica surfaces. A Monte Carlo simulation and ellipsometry study

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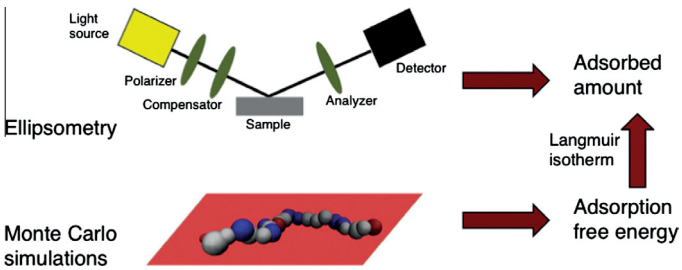
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: The adsorption of histatin 5 to hydrophilic silica surfaces is governed by electrostatic attractive forces between the positive protein and the negative surface. Hence pH and ionic strength control the adsorbed amount, which can be described by coarse-grained Monte Carlo simulations accounting for electrostatic forces and charge regulation of the protein.
Experiments: The amount of histatin 5 adsorbed to hydrophilic silica surfaces at different pH and ionic strengths was measured using null ellipsometry. The results were compared with coarse-grained Monte Carlo simulations of a single histatin 5 molecule and a surface with a fixed, smeared charge set according to experimental values for silica. The Langmuir isotherm was used to calculate the surface coverage from the simulation results. The effect of charge regulation of the protein was investigated.
Findings: Even though electrostatic attractive forces are important for the investigated system, a non-electrostatic short-ranged attraction with a strength of about 2.9 k_BT per amino acid was needed in the simulations to get surface coverages close to experimental values. The importance of electrostatics increases with increasing pH. Charge regulation of the protein affected the results from the simulations only at high surface charge and low ionic strength.

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1. Introduction

Histatin 5 is a short, basic protein containing 24 amino acid residues, of which seven are histidines (sequence: Asp-Ser-His-Al a-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-S er-His-Arg-Gly-Tyr [1]). It is present in human saliva and belongs to the family of intrinsically disordered proteins [2], as shown by CD spectroscopy [3] and NMR [4]. It has both antifungal [5] and antibacterial [6] properties, primarily against the fungus *Candida albicans*. Histatin 5 adsorbs to the cell wall/membrane of *Candida albicans* and enters and kills the cells. The mechanism by which the protein is transported into the cell may be a combination of transporter-mediated uptake across the cell wall, direct transfer across the membrane, and endocytosis [7]. Direct transfer across a liposome membrane has been observed [8]. The anticandidal activity has received much attention [1,3,7,9–12] and it has been shown that a conjugate of histatin 5 with spermidine is a potential drug against oral candidiasis [13]. Histatin 5 is also a constituent of the protective film called the enamel pellicle and offers some defence against acid-induced degradation of enamel [14].

Adsorption to negatively charged surfaces is crucial for the function of histatin 5 both as a part of the pellicle and as an antimicrobial agent. We have investigated the surface adsorption of histatin 5 to hydrophilic silica surfaces using ellipsometry and coarse-grained Monte Carlo simulations with the aim to answer three questions. The questions and the motivations behind them follow here:

Question 1: How does the adsorbed amount of histatin 5 depend on pH and ionic strength?

Motivation: In human whole saliva, the pH varies between approximately 5.7 and 7.8 [15], while the ionic strength of saliva from the parotid glands varies between 30 and 100 mM [16]. This natural variation makes it interesting to study how the adsorption of histatin 5 is affected by pH and ionic strength. Furthermore, the killing activity of histatin 5 has been shown to be dependent on these conditions. The ability of histatin 5 to kill *Candida albicans* decreases with increasing ionic strength [9,12], and while Xu et al. found no significant pH-dependence [9], Kacprzyk et al. found that histatin 5 killed *Candida albicans* more effectively at pH 7.4 than at pH 5.5 [17].

Question 2: Can we describe the adsorption of histatin 5 with a coarse-grained model with a single protein molecule?

Motivation: Coarse-grained models provide a way of investigating properties of large molecules at a reduced computational cost. The surface adsorption of histatin 5 has been studied previously using coarse-grained Monte Carlo simulations, where one coarse-grained histatin 5 molecule was adsorbed to a flat surface with a smeared charge [18]. The results were compared with atomistic simulations and density functional theory calculations. We want to extend previous work on the coarse-grained model by performing a corresponding experimental study to verify the simulation parameters and gain further insight into the adsorption mechanism by comparing experiments and simulations. By using the Langmuir adsorption isotherm to convert the adsorption free energies found from simulations into surface coverages, the results from simulations and experiments can be directly compared.

Question 3: Is charge regulation important for the adsorption of histatin 5 in a physiologically relevant system?

Motivation: The high content of histidines, which have a pK_a of ~6, makes it possible for histatin 5 to regulate its charge depending on the environment at physiological pH. The positive net charge of histatin 5 increases when the protein approaches a negatively charged surface. Earlier coarse-grained Monte Carlo simulations have shown that this mechanism increases the surface adsorption under some conditions [18]. In order to gain further knowledge on the importance of charge regulation, we use simulations to investigate whether this is the case also for the conditions studied here.

2. Materials and methods

2.1. Experimental

2.1.1. Materials

Chemicals. Synthetic histatin 5 was bought from American Peptide Company, Inc., U.S.A. (lots no V11131T1, 1312047T, and 1303040T). The peptide content was 59.7% and the purity of the peptide 95.8%. In order to remove excess salt and other impurities, the protein was purified using size exclusion chromatography, according to the procedure described in the Methods section below.

All buffer solutions were prepared using MilliQ water. Buffers containing 10 mM tris (Saveen Werner AB, Sweden, lot no. 2Z007904) were used at pH 7, 8 and 9. The pH was adjusted using 1 M hydrochloric acid. When a higher ionic strength was needed, sodium chloride (Scharlau, Spain, Prod. no. SO02271000) was added to the buffer. For the measurements at pH 6, a 10 mM bistris (Merck Millipore, U.S.A., lot no. XA27K) buffer was prepared in the same way. At pH 4 and 5, a 10 mM buffer made from acetic acid (Scharlau, Spain, batch 34335) and sodium acetate (Sigma–Aldrich, U.S.A., lot no. BCBH6230V) was used.

Substrates. Silicon wafers which were oxidized to give an approximately 300 Å thick silica (silicon dioxide) layer were purchased from Semiconductor Wafer, Inc., Taiwan. The wafers were cut into pieces and cleaned in an alkaline solution with hydrogen peroxide, followed by cleaning in an acidic solution with hydrogen peroxide, according to the procedure described by Landgren and Jönsson [20]. This procedure has been shown to effectively remove organic contaminants and metal ions [21]. The slides were then stored in ethanol (Solveco AB, Sweden). Before the measurements, the surfaces were rinsed in three steps with water, ethanol and water, dried with nitrogen and put in a plasma cleaner (model PDC-3XG, Harrick, U.S.A.) for 5 min at a pressure of approximately 0.06 mbar. The plasma cleaning was done to remove any remaining organic contaminants.

Approximate surface charge densities of silica under different conditions can be found in Table 1. Note that the surface charge varies substantially with pH and ionic strength.

2.1.2. Methods

Size exclusion chromatography. Surface chemistry experiments are very sensitive to surface active impurities, and to ensure the purity of the histatin 5, size exclusion chromatography was used. The separation range of the column (Superdex 75 10/300 GL, GE Healthcare, Sweden) was 3–70 kDa. The column was filled with a 10 mM tris buffer with 140 mM NaCl at pH 7. NaCl was included in the buffer to reduce undesired electrostatic interactions between the column material and the protein.

Table 1
Approximate surface charge densities of silica particles at different pH-values and ionic strengths (I) adjusted with KCl, taken from Samoshina et al. [19].

pH	Approximate surface charge density (μC/cm^2)	
	I = 10 mM	I = 100 mM
6	−0.25	−0.50
7	−1.00	−2.00
8	−2.75	−5.75
9	−7.00	−12.50

The freeze-dried peptide was dissolved in the running buffer at a concentration of approximately 10 mg/ml. After equilibrating the column with the buffer, 0.1 ml of the protein solution was injected. The sample corresponding to the middle of the UV absorption peak was collected; see supplementary material.

Concentration measurements. All histatin 5 concentrations were measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc., U.S.A.) at 280 nm. The extinction coefficient used was 2560 M^{-1} cm^{-1}, calculated from the tyrosine content of the peptide [22,23].

Refractive index measurements. The refractive indices of the buffers were measured using an Abbe 60 refractometer (Bellingham & Stanley Ltd., UK) at wavelengths of 579.1, 546.1 and 435.8 nm. The refractive indices at the wavelength of the ellipsometer (401.5 nm) were extrapolated from these measurements using the two-term form of the Cauchy equation.

Null ellipsometry – theory. The experimental data on the adsorption of histatin 5 have been obtained using the technique of null ellipsometry. Ellipsometry is based on the use of elliptically polarized light and the fact that when light is reflected at a surface, the polarization changes. This change is different depending on the characteristics of the surface and the thickness and refractive index of the adsorbed layer. By using an appropriate angle of the polarizer before the reflection at the surface, linearly polarized light is obtained after reflection. This linearly polarized light can be cancelled by another polarizer, the analyzer. The raw data from null ellipsometry are the polarizer and analyzer angles that can be converted into the relative phase shift, Δ, and amplitude change, Ψ. From these angles, the refractive index and the thickness of the adsorbed layer are determined. However, at low surface coverage and/or thin layers, the refractive index and the thickness are interdependent, meaning that both the thickness and refractive index cannot be resolved. Therefore, a fixed refractive index of 1.5 was used for the adsorbed layer of histatin 5. This value is reasonable for an adsorbed protein layer [24]. The effect of choosing other refractive indices is shown in the supplementary material. From the refractive index and the measured thickness of the layer, the adsorbed amount (dry mass) per surface area was calculated using the two-component model from Cuypers et al. [25]:

$$\Gamma = \frac{3 d_f f(n_f)}{\frac{A}{M} - v\frac{n_0^2-1}{n_0^2+2}}(n_f - n_0), \tag{1}$$

where d_f is the thickness of the adsorbed film, n_f is the refractive index of the film, n_0 is the refractive index of the buffer, A is the molar refractivity of the compound, M is the molar weight, v is the specific volume and

$$f(n_f) = \frac{n_f + n_0}{(n_f^2+2)(n_0^2+2)}. \tag{2}$$

Due to the interdependence between the refractive index and the layer thickness, and to cancellation of errors, the value of the adsorbed amount is less prone to errors than the refractive index and the thickness of the layer [26]. The calculated value of the adsorbed amount is also less dependent on the optical model and can therefore be used for inhomogeneous layers as verified elsewhere [25,27].

The values used for the specific volume and M/A were 0.70 ml/g and 3.95 g/ml, respectively. These values were calculated from the amino acid composition of histatin 5 according to Cohn and Edsall [28] (specific volume) and McMeekin et al. [29] (M/A). The calculated adsorbed amount depends directly on these values.

Ellipsometry setup and procedure. The instrument used for this study was a Rudolph thin film ellipsometer (type 43603-200E, Rudolph Research Corp., U.S.A.). The measurements were performed using light at a wavelength of 401.5 nm emitted from a xenon arc lamp. The angle of incidence was 67.9°. The surface was mounted in a holder inside a trapezoid cuvette where the light passed through perpendicular to the cuvette walls. The temperature in the cuvette was maintained at 25 °C using a thermostat.

In order to determine the thickness of the oxide layer on the silicon surface, measurements were conducted both in air and in 5 ml buffer, while stirring with a magnetic stirrer. When all initial parameters had been determined, 100–300 µl of concentrated histatin 5 solution was added to the cuvette to give a final concentration of 0.05 mg/ml.

Since the purified protein was dissolved in a solution with an ionic strength of 150 mM, the buffer was exchanged before addition to the cuvette for the measurements at low ionic strength (10 and 60 mM) to make sure that extra salt from the buffer used for purification would not influence the total ionic strength in the cuvette. The exchange was made by adding the protein solution (approximately 200 µl) and the new buffer (approximately 1.5 ml) to a centrifugal concentrator (Vivaspin 2, MWCO 2 kDa, Sartorius Stedim Biotech GmbH, Germany) and then centrifuging (at ~2500g, 18 °C, in a laboratory centrifuge of model MPW-260R, MPW Med. Instruments, Poland) until a volume close to the original volume of the protein solution was reached. This procedure was then repeated a second time.

The protein was allowed to adsorb for 60 min under agitation with a magnetic stirrer. Then, buffer solution was pumped through the cuvette at a speed of ~16 ml/min. The rinsing with buffer was performed during 30 min.

2.2. Computational

Coarse-grained Monte Carlo simulations were performed using Faunus, a C++ framework for Metropolis Monte Carlo simulations [30].

2.2.1. Model

Table 2 shows all the terms contributing to the system energy Hamiltonian and the parameters of these terms are given in Table 3. All interactions were assumed to be pairwise additive. Other aspects of the model are described below.

The protein. The protein was coarse-grained using a bead model, where each amino acid and the N- and C-terminals are represented by soft spheres connected by harmonic bonds [31], see Fig. 1 for snapshots. This gives 26 beads for histatin 5. The equilibrium distance between the centres of the spheres was set to 4.0 Å,

Table 2
The terms of the system energy Hamiltonian.

Type of energy	Expression (equation number)	
Inter-bead interactions		
Debye–Hückel electrostatics	$\sum_{i=1}^{n-1}\sum_{j=i+1}^{n}\frac{q_i q_j}{4\pi\varepsilon_0\varepsilon_r r_{ij}} e^{-\kappa r_{ij}}$	(3)
Harmonic bonds	$\sum_{i=1}^{n_b} k_h (R_i - R_{eq})^2$	(4)
Lennard-Jones (non-bonded beads)	$\sum_{i=1}^{n-2}\sum_{j=i+2}^{n} 4\tilde{\varepsilon}_{nb}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]$	(5)
Titration		
Intrinsic titration energy	$\sum_{i=1}^{n_p} k_B T(\mathrm{pH} - \mathrm{p}K_{a,i}) \ln 10$, for i protonated	(6)
Protein–surface interactions		
Gouy–Chapman electrostatics	$\sum_{i=1}^{n} 2 z_i k_B T \ln\left(\frac{1+\Gamma_0 e^{-\kappa r_{s,i}}}{1-\Gamma_0 e^{-\kappa r_{s,i}}}\right)$, where $\Gamma_0 = \tanh\left[\frac{1}{4}\sinh^{-1}\left(\frac{\rho}{\sqrt{8 k_B T c_0 \varepsilon_0 \varepsilon_r}}\right)\right]$	(7)
Shifted Lennard-Jones	$\sum_{i=1}^{n} \varepsilon\left[\left(\frac{\sigma_{ij}/2}{r_{s,i}+\sigma_{ij}/2}\right)^{12} - 2\left(\frac{\sigma_{ij}/2}{r_{s,i}+\sigma_{ij}/2}\right)^{6}\right]$	(8)

Table 3
The parameters of the expressions in Table 2. All distances are measured from the centres of the beads.

Parameter = value	Description
$n = 26$	Number of beads
q_i	Charge of bead i
ε_0	Permittivity of free space
$\varepsilon_r = 78.54$	Relative permittivity
r_{ij}	Distance between beads i and j
$\kappa^{-1} = \sqrt{\frac{\varepsilon_0 \varepsilon_r k_B T}{2N_A e^2 I}}$	Debye screening length
k_B	Boltzmann's constant
$T = 298.15$ K	Temperature
N_A	Avogadro's constant
e	Elementary charge
$I = 10$ or 100 mM	Ionic strength
$n_b = n - 1$	Number of bonds
$k_b = 0.76\ k_B T/\text{Å}^2$ [31]	Harmonic bond spring constant
R_i	Bond length of bond i
$R_{eq} = 4.0$ Å	Equilibrium bond length
$\varepsilon_{nb} = 0.05\ k_B T$	Interaction strength between non-bonded beads
$\sigma_{ij} = \frac{\sigma_i + \sigma_j}{2}$	Lennard-Jones diameter
σ_i	Diameter of bead i
n_p	Number of protonated beads
$pK_{a,i}$	Acid dissociation constant of bead i
z_i	Charge number of bead i
$r_{s,i}$	Distance between bead i and the surface
ρ	Surface charge density
$c_0 = 10$ or 100 mM	Concentration of 1:1 salt
$\varepsilon = 2.9\ k_B T$	Bead–surface interaction strength

corresponding to the contour length of one amino acid [32]. The diameters of the beads were calculated from the masses of the individual amino acids under the assumption that they are spherical, using a protein density of 1.525 g/ml calculated according to Fischer et al. [33].

The amino acid beads are titrating, meaning that they will acquire different charges depending on the electrostatics of the neighbouring amino acid residues, the external potential, the pH of the solution and the intrinsic pK_a of the amino acid residue. The intrinsic pK_a values were taken from Nozaki and Tanford [34].

The buffer. Solvent and salt were modelled implicitly, entering the model as a relative permittivity and a Debye screening length κ^{-1}, respectively.

The surface. The substrate was assumed to be a smooth Gouy–Chapman surface with a smeared charge. The charge densities under the different conditions used in the simulations were taken from the experimentally determined values in Table 1. In addition to electrostatics, there was also a Lennard-Jones potential with $\varepsilon = 2.9\ k_B T$ between the amino acid beads and the surface (Eq. (8) in Table 2). The value of ε was determined by a comparison with experimental values using the Langmuir adsorption isotherm, see Section 3.3.

Each amino acid bead may approach the surface until there is zero distance between the centre of the bead and the surface. A hard wall repulsive potential prevents the bead from penetrating further into the surface. The Lennard-Jones potential between the surface and a bead is shifted so that it has its minimum at zero distance between the centre of the bead and the surface. If a normal Lennard-Jones potential is introduced, the repulsion sets in too early and the adsorption decreases instead of increasing.

2.2.2. Method

A single protein molecule was simulated using the Metropolis Monte Carlo scheme [35] in the canonical (*NVT*) ensemble. The simulation box was cubic with side lengths of 300 Å, corresponding to three times the contour length of histatin 5 and 20 times the radius of gyration. In bulk, the boundary conditions were periodic in all directions. For surface simulations, hard boundaries were applied in the z-direction.

The following Monte Carlo moves were used:

1. Protein translation
2. Protein rotation
3. Single bead translation
4. Crankshaft (picks two beads randomly and defines the vector connecting them as the rotation axis, then rotates the beads between the picked ones by 180° around the axis)
5. Pivot rotations (defines a rotation axis in the same manner as with the crankshaft move, then rotates the beads at one end of the protein 180° around the axis)
6. Titration move (protonating or deprotonating a bead)

To improve sampling of the space far from the surface, an algorithm was used to restrict the mass centre of the histatin 5 molecule to different, but overlapping, parts of the simulation box. The simulations were run during $1–50 \times 10^8$ Monte Carlo steps depending on the system.

3. Results and discussion

3.1. Histatin 5 in bulk solution

Before discussing the surface adsorption of histatin 5, some relevant properties of histatin 5 in bulk solution will be presented.

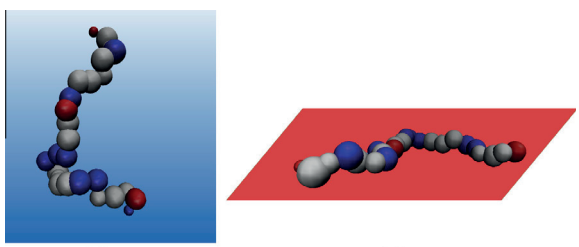

Fig. 1. Snapshots from simulations showing histatin 5 (a) in bulk solution and (b) adsorbed to a negatively charged surface. The blue beads are positively charged, the red ones are negatively charged and the grey ones are neutral. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.1.1. Monte Carlo simulations

Conformational properties. The root mean square end-to-end distance of histatin 5 was found from simulations to be 39.3 Å at pH 7 and ionic strength 100 mM. This corresponds to 38% of the contour length of the protein. The radius of gyration was determined to be 14.9 Å under the same conditions, which is close to the experimental radius of gyration of 13.3–13.8 Å determined by SAXS (beamline BM29 at ESRF, Grenoble) [36]. The radius of gyration determined by simulations was found to slightly depend on pH and for the conditions used for the adsorption studies it varies between 14.6 Å (pH 9, ionic strength 100 mM) and 17.6 Å (pH 4, ionic strength 10 mM) according to simulations. The increase with lower pH is due to the increase in net charge of the protein. In a previous adsorption study of histatin 5, the radius of gyration was estimated to be 7 Å assuming a random coil conformation in a theta solvent [37], which is apparently unrealistic.

Net charge and capacitance. The average net charge of histatin 5 found from simulations at different pH values and two different ionic strengths is shown in Table 4. The ionic strengths match available experimental conditions for the charge density of silica surfaces [19].

Electrostatic screening makes the net charge slightly higher at high ionic strength (100 mM) than at low ionic strength (10 mM). The difference in charge is largest at pH 5–6, i.e. close to the pK_a of histidine (∼6), where charge regulation of the protein is expected to have the largest effect [18]. The ability of the protein to charge regulate can be described in terms of a protein charge capacitance, $C = \langle Z^2 \rangle - \langle Z \rangle^2$, where Z is the net charge of the protein [38], which in this case is given by simulations. Fig. 2 shows that histatin 5 has a capacitance maximum at pH 5–6 and a minimum at pH 7–8.

In Fig. 3, the average charges of the different amino acid side chains and the N- and C-terminals at pH 6–8 are given. The charges are relatively evenly distributed throughout the protein, thus resembling a polyelectrolyte. Fig. 3(a) shows that at pH 6 the charges of the histidines are important for the overall protein charge, which is not the case at pH 7 and 8, see Fig. 3(b) and (c).

3.2. Surface adsorption of histatin 5

3.2.1. Ellipsometry

Effect of ionic strength. In Fig. 4 the plateau values of the total adsorbed amounts together with the irreversibly adsorbed amounts are shown for different ionic strengths at pH 7. The plateau values are recorded after 60 min of adsorption and the fraction remaining after 30 min of rinsing with buffer is considered as the irreversibly adsorbed amount. Note that the term irreversible here only concerns this aspect of reversibility.

Between ionic strengths of 10 mM and 200 mM (Debye screening lengths 30.4–6.8 Å) there is no clear effect of ionic strength on the total adsorbed amount. However, the fraction of protein that desorbs from the surface upon rinsing increases with ionic strength. This is reasonable considering the screening of the electrostatic attractive forces between histatin 5 and the surface. Thus, it is more likely that a histatin molecule will detach from the surface at a higher ionic strength. Since the electrostatic attraction is short-ranged when the ionic strength is high, it is less likely that the chain will re-attach to the surface before being removed due to the exchange of the protein solution with neat buffer.

Table 4
The average net charge of histatin 5 at different pH-values and ionic strengths (I) found from simulations.

pH	Average net charge of histatin 5	
	I = 10 mM	I = 100 mM
4	11.0	12.1
5	8.9	10.4
6	6.4	7.6
7	5.0	5.4
8	3.9	4.2
9	2.6	3.0

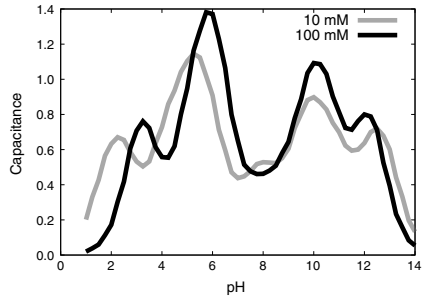

Fig. 2. The dimensionless capacitance, $C = \langle Z^2 \rangle - \langle Z \rangle^2$, of histatin 5 as a function of pH at ionic strengths 10 and 100 mM from simulations.

Interestingly, there is essentially no effect on the total adsorbed amount when increasing the ionic strength from 10 mM to 200 mM. A rough estimate (assuming spherical molecules with a radius of gyration of 13.8 Å [36] covering a surface area corresponding to the area of a circle with the same radius) gives that the adsorbed amount corresponding to a monolayer of histatin 5 is 0.84 mg/m^2. Thus, there could be an energy barrier to increasing the adsorption beyond ∼0.8 mg/m^2 since it requires more than monolayer coverage. Another possibility is that the decreased repulsion between the adsorbed histatin molecules compensates for the decrease in attraction between histatin 5 and the surface.

At an ionic strength of 500 mM, the calculated Debye screening length is 4.3 Å, meaning that any electrostatic (attractive) force between the protein and the surface is very short-ranged and not expected to be able to drive the adsorption.

Effect of pH. Table 5 shows the plateau values of the adsorbed amounts of histatin 5 at ionic strengths 10 and 150 mM for pH-values ranging from 4 to 9. The adsorbed amount increases with pH, with the possible exception of pH 6, ionic strength 10 mM, where four out of five measurements showed an adsorbed amount lower than at pH 5, ionic strength 10 mM. However, this particular system showed large fluctuations in the measured adsorbed amount leading to a high uncertainty in the reported value.

The effect of ionic strength on the adsorption depends on pH. For most pH-values, the adsorbed amount decreases with increasing ionic strength. However, at pH 5 and 7 the decrease is minor and at pH 6 there appears to be an increase. Fig. 5 shows a comparison of the dependence of the adsorbed amount on ionic strength at pH 7 and 8. Contrary to what was observed at pH 7, the adsorbed amount decreases considerably when the ionic strength increases at pH 8. Thus, at pH 8, the electrostatic attraction between the surface and the protein is strong enough for the electrostatic screening to make a difference.

3.2.2. Monte Carlo simulations

Effect of ionic strength. Fig. 6 shows the effect of changing the ionic strength from 10 mM to 100 mM on the free energy of adsorption at pH 7. Here, the screening of the electrostatic attraction between the protein and the surface that takes place at higher salt concentrations is not compensated for by the increased surface charge at higher ionic strength, i.e. the free energy minimum is

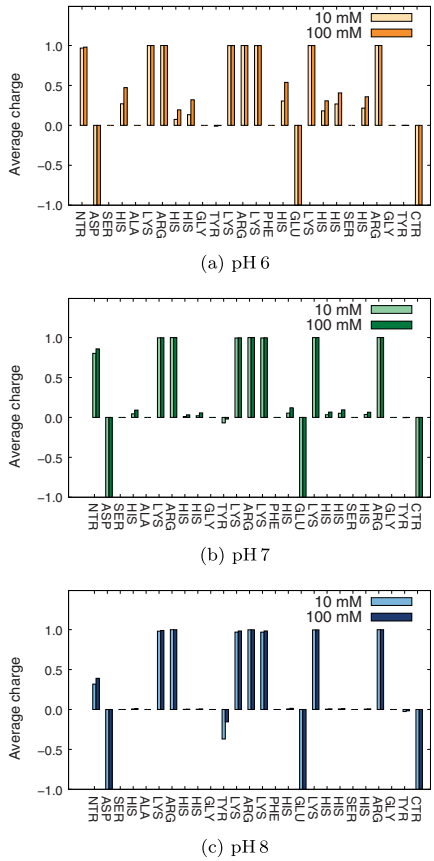

Fig. 3. Average charge for each amino acid in histatin 5 from simulations at 10 mM and 100 mM ionic strength.

lower for 10 mM than for 100 mM. At 10 mM, the free energy minimum is $-18\ k_BT$ and at 100 mM it is $-15\ k_BT$. However, this does not necessarily mean that the adsorbed amount should be lower at 100 mM than at 10 mM, since the repulsion between adsorbed proteins, that is ignored here, might have an impact. This repulsion decreases at high ionic strength and may explain the weak dependence on ionic strength in the experimentally observed adsorbed amounts.

The experimental results showed that more proteins are desorbed at high ionic strength than at low ionic strength. The fraction of protein that desorbs can be viewed as a measure of the strength of interaction with the surface, and since the coverage at pH 7 is not above monolayer coverage (0.84 mg/m^2) the proteins are all likely to interact with the surface in a similar manner. Thus, both simulations and experiments show that the attractive interaction strength is reduced at higher ionic strength.

Effect of pH. Fig. 7(a) shows the free energies of adsorption at pH 6–8 and an ionic strength of 100 mM. Here, two different processes

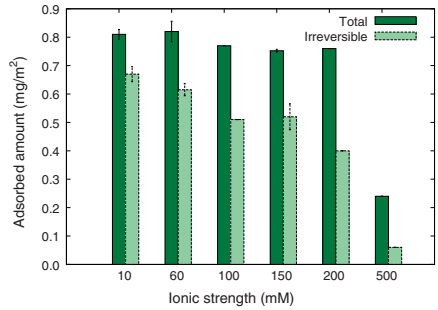

Fig. 4. Total adsorbed amount of histatin 5 recorded after 60 min and the irreversibly adsorbed amount (the amount that did not desorb after 30 min of rinsing with buffer). The buffer was 10 mM tris at pH 7, supplemented with the amount of NaCl needed to reach the desired ionic strength. The error bars show the standard deviations for the systems on which more than one measurement was made.

Table 5
The plateau adsorbed amount at different pH-values and two different ionic strengths (I). For the measurements that have been reproduced, the standard deviation is included.

pH	Adsorbed amount (mg/m^2)	
	I = 10 mM	I = 150 mM
4	0.08	0.00
5	0.48	0.44
6	0.40 ± 0.13	0.58 ± 0.042
7	0.81 ± 0.017	0.75 ± 0.005
8	1.04	0.81
9	1.23	0.94

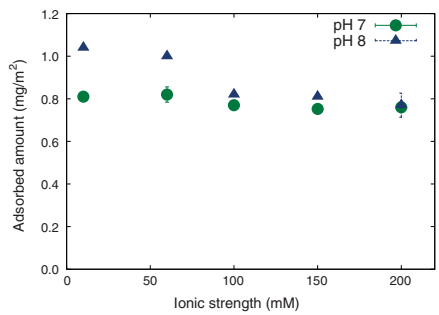

Fig. 5. The adsorbed amount recorded after 60 min as a function of ionic strength (10 mM tris + NaCl) for pH 7 and 8. The error bars show the standard deviations for the cases where more than one measurement was made (for the measurements at pH 7, ionic strength 10 mM and 150 mM, the error bars are covered by the symbols).

with opposing effects on the adsorption free energy are important. Firstly, there is the decrease of the positive protein charge with increasing pH, which decreases the attraction between the protein and the surface. Secondly, the negative surface charge increases with pH, which increases the attraction between the protein and the surface. Since the adsorption free energy minimum decreases

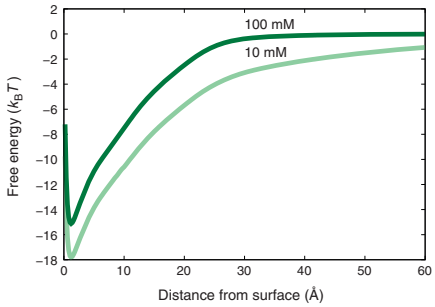

Fig. 6. Free energies of adsorption for histatin 5 at pH 7, ionic strength 10 mM and 100 mM, as a function of the distance between the surface and the protein centre-of-mass.

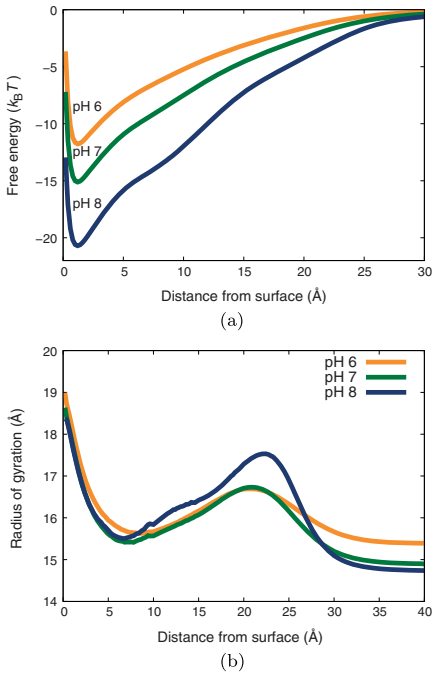

Fig. 7. (a) Free energies of adsorption, and (b) radius of gyration as a function of the mass centre distance from the surface for histatin 5 at pH 6–8, ionic strength 100 mM. Notice the different scales on the *x*-axis.

with pH, the change in surface charge is more important for the adsorption than the change in protein charge under the investigated conditions. This also agrees with the experimental results that showed an increase in absorbed amount with increasing pH, reflecting the importance of the increasing surface charge. However, it should be noted that the decreased repulsion between adsorbed proteins at higher pH could also be of importance in a system with many molecules.

Regarding the dependence of the free energy on ionic strength, all simulated pH-values from 6 to 9 gave similar results compared to the ones showed for pH 7 in Fig. 6 (results not shown). This means that the differences in behaviour between different pH-values that was observed experimentally are not captured by the model.

Fig. 7(b) shows the radius of gyration (R_g) as a function of the protein mass centre distance from the surface for pH 6, 7 and 8 at an ionic strength of 100 mM. At a distance of approximately 20 Å, the histatin molecule is affected by the potential from the surface and stretches towards it, giving rise to an increase in R_g. This effect is most pronounced at pH 8, where the surface charge is higher. Closer to the surface, R_g decreases at first but as the protein comes even closer it is forced to stretch.

Fig. 8 displays the effective free energy per amino acid residue as a function of the distance from the surface and representative snapshots. As can be seen, no part of histatin 5 is repelled by the negatively charged surface, even though there are three negatively charged residues. This is due to the Lennard-Jones potential between the beads and the surface and to the fact that the negatively charged residues are evenly distributed along the molecule, see Fig. 3. It can be seen that beads 6–7 and 12–14 are more tightly bound to the surface than the other residues at pH 7 and 8. These residues are all positively charged lysines and arginines. The protein ends are more loosely bound since they contain negative residues – the C-terminal and the aspartic acid at the N-terminal.

Effect of charge regulation. Fig. 9(a) and (b) show the variation in net charge as the protein approaches the surface at 100 and 10 mM ionic strength. The change is approximately the same for both ionic strengths. It should be noted that even though histatin 5 has a capacitance maximum at pH 6 it only changes its charge by $+0.5$ when it approaches the surface while at pH 7 and 8, where there is a minimum in the capacitance, the change is larger. It turns out that *the surface charge at pH 6 is too low to induce charge regulation of the protein in this system*. Despite the fact that the capacitance is at a minimum, charge regulation is more important at pH 7 and 8 since the surface charge is substantially higher.

Fig. 9(c) shows free energies at 100 mM ionic strength compared to a system where titration of the amino acid residues is turned off and the residues instead have average charges found from bulk simulations. Charge regulation of the protein here seems unimportant for the adsorption even though the net charge is affected.

Fig. 9(d) shows the free energies at an ionic strength of 10 mM. Here, charge regulation of the protein affects the adsorption free energy at pH 8. Thus, charge regulation may be of importance even at the capacitance minimum of histatin 5 if the surface charge is high enough and the ionic strength is low.

However, in a biologically relevant system, charge regulation of histatin 5 at pH 6 may still be important. It was shown in the study by Kurut et al. [18] that charge regulation of histatin 5 may contribute to the adsorption at pH 6, ionic strength 80–150 mM, when the charge of the surface is -5.3 μC/cm^2 (-300 Å^2/e) and there is no Lennard-Jones potential between the amino acids and the surface. This surface charge is comparable to that of a microbial membrane.

3.3. Comparison using the Langmuir isotherm

We want to directly compare the surface coverage from experiments and single-protein simulations. This can be done by using the Langmuir adsorption isotherm, which states that

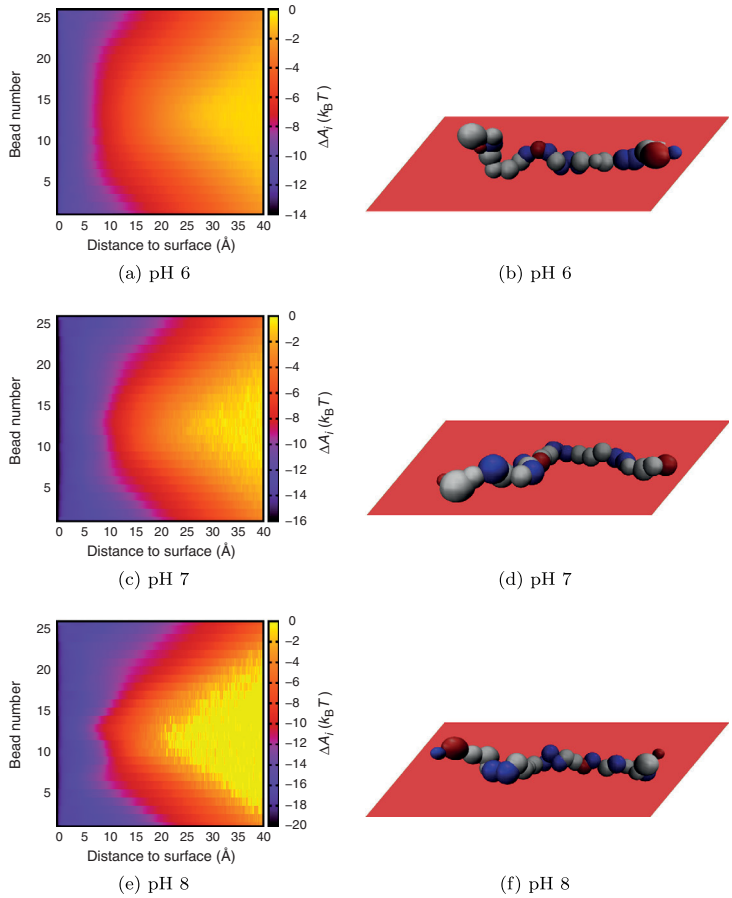

Fig. 8. (a, c, e) Free energies of adsorption per bead (ΔA_i) as a function of distance from the surface at ionic strength 100 mM. Note that the free energy scales are different! (b, d, f) Typical snapshots of adsorbed histatin 5 molecules for the different conditions.

$$\theta = \frac{KC}{1 + KC}, \tag{9}$$

where θ is the fractional coverage of the surface, K is the protein–surface binding constant and C is the concentration of the protein in solution. The constant K can be found from

$$K \approx a \int_0^\infty \left(e^{-w(r_s)/k_B T} - 1\right) dr_s, \tag{10}$$

where $w(r_s)$ is the potential of mean force (free energy) between a protein and the surface, r_s is the distance between the protein centre-of-mass and the surface, and a is the maximum coverage expressed as the surface area per molecule. Since monolayer coverage was approximated to 0.84 mg/m^2, $a \approx 600$ Å^2.

The basic conditions that must be fulfilled for the Langmuir isotherm model to be applicable are:

1. All adsorption sites are equivalent.
2. Each adsorption site can bind at most one solute molecule (maximum monolayer coverage).
3. The adsorbed molecules do not interact with each other.
4. The adsorption process is thermodynamically reversible.

For the system studied here, all of these conditions are not fulfilled, for example it appears that histatin 5 can adsorb in more than monolayer coverage (experimental coverage > 0.84 mg/m^2 in some cases). Even though all conditions are generally not fulfilled in an experimental system, the model can still serve as a tool for comparing simulations with experiments.

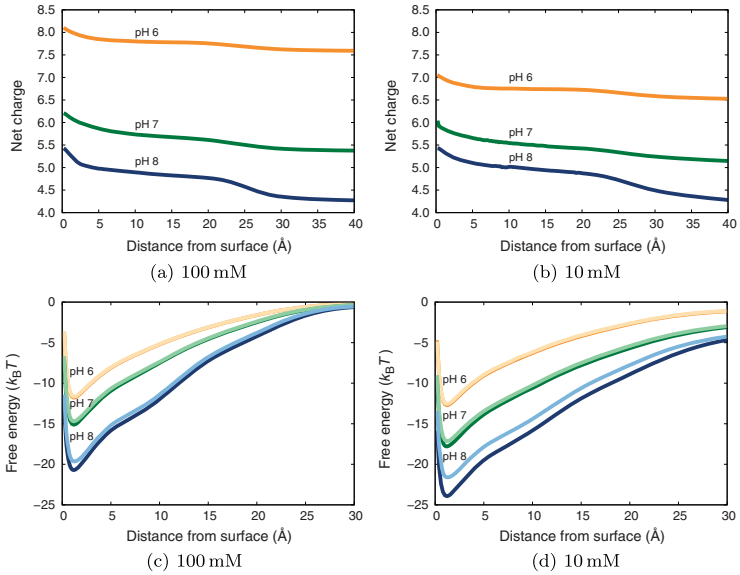

Fig. 9. (a and b) Net charge of histatin 5 as a function of the mass centre distance from the surface at pH 6–8, ionic strengths 100 and 10 mM. (c and d) Free energies of adsorption for histatin 5 at pH 6–8, ionic strengths 100 and 10 mM, modelled with (dark lines) and without (bright lines) protein charge regulation. Notice the different scales on the x-axis.

It has been shown previously for the intrinsically disordered protein β-casein that in order to observe adsorption in simulations under the same conditions as observed experimentally, a short-ranged non-electrostatic potential must be included [39]. The same observation was made here for histatin 5 at pH 6 and lower, even though the protein and the surface are oppositely charged in contrast to the case of β-casein. In the study of β-casein a Lennard-Jones potential was included in the simulations and ε was chosen as the smallest possible value that gave rise to adsorption under the studied conditions. Here, we take the comparison with experiments one step further by choosing the value of ε from the comparison using the Langmuir adsorption isotherm.

The surface coverage given by experiments and simulations with different values of ε are shown in Table 6. At pH 4 and 5, experimentally determined surface charges were not available since they are too low. The surface charge at pH 4 was set to 0 and the charge density at pH 5 was set to 1/5 of the charge density at pH 6 with the same ionic strength.

The lowest possible value of ε that still gives adsorption at pH 4, ionic strength 10 mM, is 2.0 k_BT. The value that seems most reasonable from a comparison with experiments is 2.9 k_BT, since it gives values closest to experiments for pH 5–7. However, it overestimates the adsorption at pH 4. In three cases (pH 8 and 9), the experimentally found absorbed amount was above monolayer coverage, which cannot be reproduced by the Langmuir model. Another problem that occurs regardless of the value of ε is that the behaviour when the ionic strength is increased cannot, in general, be reproduced. At pH 7, the small difference seen experimentally can be reproduced, but at lower pH the change in adsorbed amount is larger than observed experimentally and for the case of pH 6 of the wrong sign. One reason for this could be that repulsion between adsorbed proteins is important. Another possibility is that the experiments measuring the surface charges used for the simulations were made on a slightly different type of surface (silica particles), which may not correspond exactly to the ones used in the adsorption experiments. The estimated charges at pH 4 and 5 may also not be completely accurate. At lower surface coverage, the calculated surface coverage from simulations is very sensitive to small changes in surface charge and ε due to the exponential in Eq. (10). For example, an increase in ε with 0.1 k_BT may increase the calculated adsorbed amount by a factor of three.

Fig. 10 shows a correlation plot using $\varepsilon = 2.0\,k_BT$ and $\varepsilon = 2.9\,k_BT$. Since 2.9 k_BT gives the closest correspondence with experiments it was chosen as the value of ε for the simulations presented above. The main reason for the need of such a large ε is that our model of the protein overestimates the entropy of the chain by ignoring most effects of side chains and steric hindrances.

4. Conclusions

We have investigated the adsorption of histatin 5 to negatively charged surfaces using ellipsometry and coarse-grained Monte Carlo simulations of a single protein. The experimental surface was hydrophilic silica while the simulated surface was completely flat with a smeared charge corresponding to experimentally measured charge densities [19]. While previous ellipsometry measurements have been made on the effect of concentration and hydrophobicity on the adsorption to this type of surface [37], this is the first such study on the effect of pH and ionic strength. In the present study, the bulk concentration of histatin 5 was 0.05 mg/ml.

Table 6
Fractional surface coverage (θ) from experiments and simulations with different values of ε for the Lennard-Jones potential between the amino acid beads and the surface. The data from simulations were calculated using the Langmuir adsorption isotherm and the assumption of a maximum (monolayer) surface coverage of 0.84 mg/m^2.

pH, ionic strength	Experimental	$\varepsilon = 2.0\ k_BT$	$\varepsilon = 2.8\ k_BT$	$\varepsilon = 2.9\ k_BT$	$\varepsilon = 3.0\ k_BT$
4, 10 mM	0.095	1.4×10^{-4}	0.094	0.28	0.56
4, 150[a]/100[b] mM	0.00	3.9×10^{-5}	0.082	0.20	0.45
5, 10 mM	0.57	4.5×10^{-4}	0.18	0.54	0.76
5, 150[a]/100[b] mM	0.52	1.9×10^{-4}	0.095	0.28	0.53
6, 10 mM	0.48	0.0023	0.52	0.76	0.93
6, 150[a]/100[b] mM	0.69	6.0×10^{-4}	0.27	0.53	0.72
7, 10 mM	0.96	0.19	0.99	1.00	1.00
7, 100 mM	0.92	0.0091	0.85	0.97	0.99
8, 10 mM	1.2	0.98	1.00	1.00	1.00
8, 100 mM	0.98	0.55	1.00	1.00	1.00
9, 10 mM	1.5	1.00	1.00	1.00	1.00
9, 150[a]/100[b] mM	1.1	0.98	1.00	1.00	1.00

[a] Experiments.
[b] Simulations.

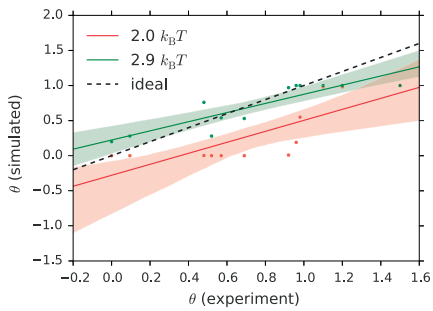

Fig. 10. The correlation between experimental and simulated data for the fractional surface coverage (θ). The coloured areas show the 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We have shown that the adsorbed amount increases with pH. In a previous simulation study of histatin 5, where the surface charge was kept constant, the adsorption was instead observed to be stronger at pH 6 than at pH 7 and 8 [18]. This difference highlights the importance of the change in surface charge depending on pH and ionic strength.

When increasing the ionic strength, our experiments show that the effect on the adsorbed amount depends on the pH of the solution. This cannot be explained by our single-protein simulations. Introducing several protein molecules is the next step to develop and evaluate our coarse-grained model.

We find that at pH 7, 0.8 mg/m^2 of histatin 5 adsorbs at ionic strengths 10–200 mM. This corresponds approximately to a monolayer (~0.84 mg/m^2). For the highest bulk concentrations used in the aforementioned earlier ellipsometry study (5–10 μg/ml) the same adsorbed amount was observed [37]. The concentration range of 5 μg/ml to 0.05 mg/ml overlaps with the concentrations of histatin 5 found in saliva [40,41].

Our hypothesis at the beginning of the study was that electrostatic interactions govern the adsorption of histatin 5. However, by using the Langmuir adsorption isotherm we find that a non-electrostatic Lennard-Jones attraction with $\varepsilon = 2.9\ k_BT$ between each amino acid and the surface is needed in the simulations in order to observe similar adsorption as in the experiments. The main reason for the need of such a high short-ranged attraction is to compensate for the overestimated entropy of the chain in the Monte Carlo simulations. The rest of the Lennard-Jones potential represents van der Waals interactions, hydrogen bonding and possibly charge regulation of the surface. Hydrophobic interactions are expected to be unimportant [37]. Work is ongoing to determine the influence of charge regulation of the surface.

Despite the histidine richness of histatin 5, charge regulation of the protein did not affect the adsorption under most conditions studied using simulations. The increase in surface charge with pH led to the counterintuitive result that charge regulation was more important at pH 8 than at pH 6 even though the protein has its highest capacity to charge regulate at pH 6.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2016.01.025.

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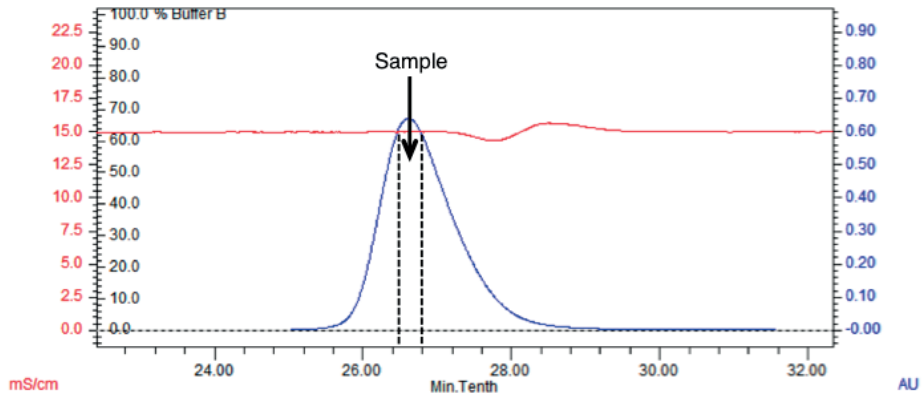

Figure 1: A chromatogram from size exclusion chromatography of histatin 5. The red curve shows the conductivity of the solution and the blue curve shows the absorbance at a wavelength of 280 nm. The sample was collected between the dotted lines as indicated in the chromatogram.

1. Purification using size exclusion chromatography

The chromatogram from one of the size exclusion chromatography runs is shown in Fig. 1. The dotted lines indicate where the sample was collected. The change in conductivity of the solution after the sample was collected indicates that counterions that were present in the freeze-dried histatin 5 were removed during the purification.

2. Reproducibility of ellipsometry measurements

The reproducibility of the ellipsometry measurements was checked by conducting four measurements on the reference system (pH 7, ionic strength 150 mM),

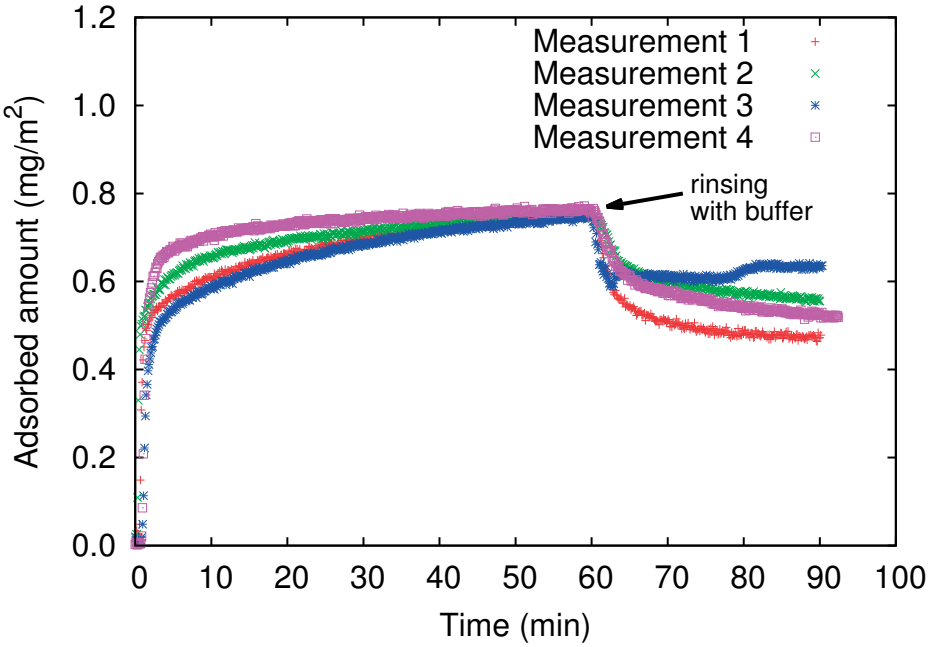

Figure 2: Results from the four ellipsometry measurements on the reference system with pH 7 and ionic strength 150 mM. Note that measurement 3 failed after the start of the rinsing with buffer.

see Fig. 2. The kinetics of the adsorption differ somewhat between the different measurements but the plateau value of the adsorbed amount after 60 minutes of adsorption is reproducible. This value is 0.75 mg/m^2 and the standard deviation is 0.005 mg/m^2. After 60 minutes of adsorption, buffer solution was pumped through the cuvette during 30 minutes. Measurement 3 failed during the rinsing. The other three measurements gave an average adsorbed amount of 0.52 mg/m^2 after rinsing. The standard deviation was 0.045 mg/m^2.

3. Effect of changing the refractive index of the adsorbed layer

Figure 3 shows how changing the value of the refractive index of the adsorbed protein layer affects the calculated adsorbed amount. As the refractive index is increased, the calculated adsorbed amount increases slightly. When the refrac-

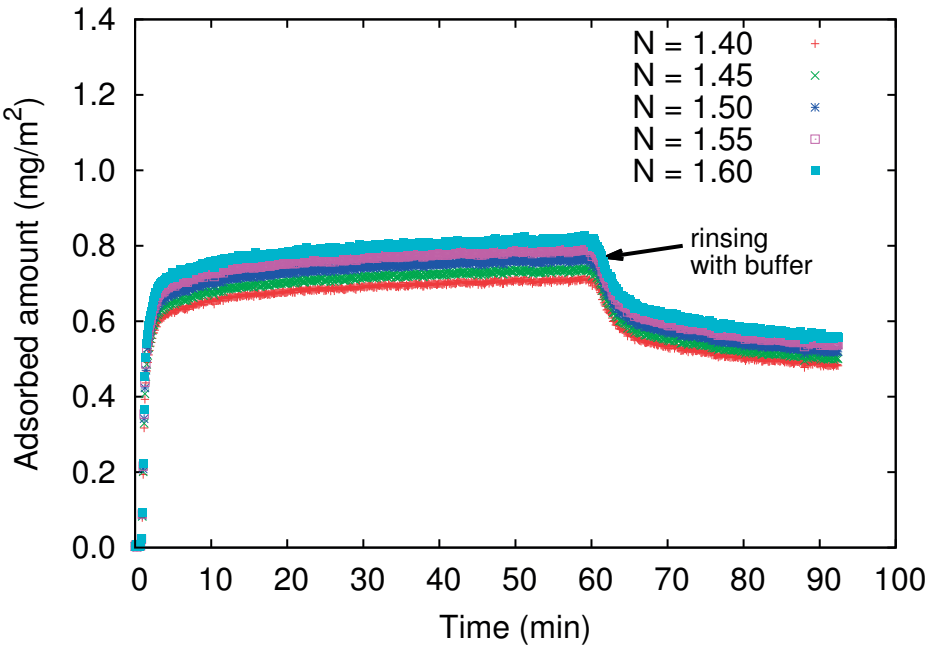

Figure 3: The adsorption isotherm from measurement 4 at pH 7, 150 mM ionic strength using different refractive indices (N) for the adsorbed protein layer.

tive index is decreased, the calculated adsorbed amount instead decreases. This is not expected to affect the comparison between different pH values and ionic strengths that is made in our study.

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Kristin Hyltegren presenting her thesis to the public in three minutes at the local Universitas 21 three minute thesis competition, September 2016. Photo: Anna Stöckl.

This licentiate thesis tells the story of an attempt to explain what governs the adsorption of the protein histatin 5 to hydrophilic silica surfaces. The tools that have been used are coarse-grained models, daily life metaphors, and machines such as the ellipsometer and supercomputers.

The thesis describes both a scientific journey and a personal one.

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