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Biological Sensing and DNA Templated Electronics Using Conjugated Polymers

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Cover: Stretched DNA decorated with tPOMT on a PDMS modified glass slide. The length of one DNA molecule is approximately 25 μm .

During the course of the research underlying this thesis, Per Björk was enrolled in Forum Scientium, a multidisciplinary doctoral programme at Linköping University, Sweden.

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Abstract

Conjugated polymers have been found useful in a wide range of applications such as solar cells, sensor elements and printed electronics, due to their optical and electronic properties. Functionalization with charged side chains has enabled water solubility, resulting in an enhanced interaction with biomolecules. This thesis focus on the emerging research fields, where these conjugated polyelectrolytes (CPEs) are combined with biomolecules for biological sensing and DNA nanowire assembling.

CPEs have shown large potential in biomolecular detection where the optical read out is due to the geometrical alternation in the backbone and aggregation state. This thesis focused on transferring the biomolecular detection to a surface of CPEs. The characterization of the CPE layer show that a hydrogel can be formed, and how the layer can undergo geometrical changes upon external stimulus such as pH change. A selective sensor surface can be created by imprinting ssDNA or an antibody in the CPE layer. The discrimination for complementary DNA hybridization and specific antibody interaction can be monitored by surface plasmon resonance or quartz crystal microbalance. We have also taken the step out from the controlled test tube experiments to the complex environment of the cell showing the potential for staining of compartments and structures in live and fixed cell. Depending on the conditions and CPE used, cell nuclei, acidic vesicles and cytoskeleton structure can be visualized. Furthermore, the live staining shows no sign of toxic effect on cultured fibroblasts.

CPEs can also be a valuable element when assembling electronics in the true nano regime. I have used DNA as building template due to its attractive size features, with a width of around 2 nm and a length scale in the μm regime, and the inbuilt base-pairing recognition

elements. This thesis shows how DNA can be decorated with CPEs and stretched on surfaces into a model for aligned semiconducting nanowire geometries. Not only making the template structures is of importance, but also how to place them on the correct surface position, i.e. on electrodes. Strategies for positioning DNA nanowires using transfer printing and surface energy patterning methods have therefore been developed in the thesis. The stretched DNA decorated with CPE also offers a way to further study the molecular binding interaction between the two molecules. Single molecular spectroscopy in combination with polarization has given information of the variation of the CPE binding along a DNA chain.

Populärvetenskaplig Sammanfattning

Kunskapen om hur naturen och människans biologi fungerar ökar ständigt. För att kunna utnyttja denna kunskap och använda den i biokemiska eller medicinska sammanhang krävs att vi har möjlighet att mäta vad som sker. Här spelar diverse sensorer en viktig roll. Likt en termometer som kan mäta en förändring i den omgivande temperaturen kan en sensor inom den biologiska världen mäta exempelvis en förändring hos en viss biomolekyl eller om den interagerar med en annan biomolekyl. I första delen av arbetet som beskrivs i denna avhandling har en speciell typ av polymer använts för att detektera biomolekylers interaktion. Dessa kallas konjugerade polymerer och har en upprepad struktur i sin kemiska formel som är alternerade dubbel- och enkelbindningar mellan kolatomer. Denna alternering ger dem deras speciella optiska och elektriska egenskaper. Deras färg och elektriska ledningsförmåga kan t.ex. ändras beroende på om polymererna är tätt tillsammans eller långt ifrån varandra. Även hur den enskilda molekylens form ändras förändrar deras egenskaper.

Genom att bilda ett komplex av en biomolekyl och en konjugerad polymer har man gjort basen för en sensor. När biomolekylen förändras eller binder till något kommer det att ske en geometriförändring som smittar av sig på polymeren som därmed också förändrar sig. Förändringen i polymeren kan avläsas som färgförändringen av det ljus som den absorberar eller skickar ut när den belyses jämfört med innan interaktionen. Jag har i denna avhandling beskrivit hur man kan göra en tunn film av en konjugerad polymer för att använda den till specifik DNA detektion och även antikroppars interaktion. DNA innehåller vår genetiska information och antikroppar är viktig del i vårt naturliga immunsystem. Det går även att låta polymererna binda in till olika strukturer i odlade celler och beroende på vad den binder till kommer den att få olika färg. Det går därmed

att visualisera t.ex. cellkärnan, sura vesiklar och strukturelement i celler vilket sedan kan användas vid t.ex. studier av cellers funktion.

Även utvecklingen mot små elektriska och optiska komponenter drivs på i en ständigt ökande takt och fysiska begränsningar i de traditionella tillverkningsmetoderna börjar göra sig mer och mer påminda. Genom att finna inspiration i naturen och kombinera det med samma konjugerade polymerer som för detektion ovan kan nya tillverkningsätt börja tas fram. Andra delen av avhandlingen handlar om forskningen i just detta gränsland. I naturen finns det många självbyggande strukturer och av dessa har jag har valt att använda DNA. DNA har genom sin sekvens inbyggda positioneringsanvisningar som kan användas för att designa strukturer och bredden på DNA är endast runt 2 nanometer, vilket är en halv miljon gånger mindre än en millimeter. Längden kan samtidigt vara många mikrometer vilket ger DNA ett längd/breddförhållande som är extremt högt. De konjugerade polymererna kan under vissa förutsättningar nå en ledningsförmåga som dåliga metaller och genom att dekorera en DNA sträng med dem och sträcka ut komplexet på en yta har man fått en modell för en ledande sladd i nanometer skala. I avhandlingen beskrivs också metoder för hur dessa nanotrådar kan placeras ut på vissa positioner på ytan genom ytmönstring och kontakttryckning.

List of Publications

Paper I

Björk P, Persson NK, Nilsson KPR, Åsberg P, Inganäs O, *Dynamics of complex formation between biological and luminescent conjugated polyelectrolytes - a surface plasmon resonance study*, Biosensors and Bioelectronics, vol 20, 9, 1764-1771, 2005

My contribution: Major part of experimental and writing. Ellipsometry part together with NK Persson.

Paper II

Åsberg P, Björk P, Höök F, Inganäs O, *Hydrogels from a water-soluble zwitterionic polythiophene: Dynamics under pH change and biomolecular interactions observed using quartz crystal microbalance with dissipation monitoring*, Langmuir, vol 21, 16, 7292-7298, 2005

My contribution: All QCM-D experiments together with P Åsberg and minor part of the writing.

Paper III

Björk P, Nilsson KPR, Lenner L, Kågedal B, Persson B, Inganäs O, Jonasson J, *Conjugated polythiophene probes target lysosome-related acidic vacuoles in cultured primary cells*, Molecular and Cellular Probes, vol 21, 5-6, 329-337, 2007

My contribution: Major part of the staining experiments together with KPR Nilsson and half of the writing.

Paper IV

Björk P, Holmström S, Inganäs O, *Soft lithographic printing of patterns of stretched DNA and DNA/electronic polymer wires by surface-energy modification and transfer*, Small, vol 2, 8-9, 1068-1074, 2006

My contribution: All experiments and writing. Prestudy by S Holmström.

Paper V

Björk P, Herland A, Scheblykin IG, Inganäs O, *Single molecular imaging and spectroscopy of conjugated polyelectrolytes decorated on stretched aligned DNA*, Nano Letters, vol 5, 10, 1948-1953, 2005

My contribution: All experiments. SMS part together with IG Scheblykin. Major part of the writing.

Paper VI

Björk P, Thomson D, Mirzov O, Wigenius J, Scheblykin IG, Inganäs O, *Structural studies of a well defined conjugated polyelectrolyte and its interaction with DNA*, In manuscript

My contribution: All experiments. SMS and polarization together with IG Scheblykin, D Thomson and O Mirzov. DLS and Abs together with J Wigenius. Half of the writing.

Publications not Included in Thesis

Herland A, Björk P, Nilsson KPR, Olsson J, Åsberg P, Konradsson P, Hammarström P, Inganäs O, *Electroactive luminescent self-assembled bio-organic nanowires: Integration of semiconducting oligoelectrolytes within amyloidogenic proteins*, Advanced Materials, vol 17, 12, 1466-1471, 2005

Herland A, Björk P, Hania PR, Scheblykin IG, Inganäs O, *Alignment of a conjugated polymer onto amyloid-like protein fibrils*, Small, vol 3, 2, 318-325, 2007

Patent Application

K. Peter R. Nilsson, Anna Herland, Per Hammarström, Per Björk and Olle Inganäs, *Methods using self-assembly/aggregation of biomolecules for the construction of electronic devices based on conjugated polymers*. PCT/SE/2005/001021

Best Poster Awards

Per Björk, Anna Herland, Peter Nilsson and Olle Inganäs, *Assembling Luminescent Conjugated Polymers with Biomacromolecules - Towards Materials and Devices*. EMRS, Strasbourg, May 2004

Per Björk, Anna Herland, Ivan Scheblykin, Sven Holmström, Peter Nilsson, Jon Jonasson, Bertil Kågedal and Olle Inganäs, *Conjugated polyelectrolytes – tools for biomolecular electronics and cell staining*. ICSM, Dublin, July 2006

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1 General Introduction

Biotechnology has in the last decades emerged as a discipline. One of the driving forces behind the fast development is the accelerating use of biosensors. “Analytical devices, which combine biospecific recognition systems with physical or electrochemical signaling” is one definition for biosensors [1]. Biosensors cover a large area of applications and it is the selectivity and defined kinetics for the biospecific reactions that form the base for biosensors [2]. DNA sensors, or gene chips, is the subject of many research groups attention [3-11]. Screening and genetic diagnostics for pharmaceutical, medical and forensic purpose, and genetic modifications for the food and plant industry are some of the more common applications for gene chips [12, 13]. Detection of pesticides [2], cells and tissue properties [2] and enzymes [1] are examples of non-DNA applications for biosensors.

Many of the products on the market today are expensive and require an advanced handling procedure, which often includes a labeling step. Affymetrix is an example of one company, dominating the high density gene chip market, which supplies highly sophisticated, but also expensive products for DNA detection [14]. A cheap, more flexible and label free system with easy handling would be of great competitive strength. The use of conjugated polymers (CP) is one direction that shows large potential for this in biological sensing. The conjugated backbone of the CP with alternating single and double bonds forms the bases for their electrical and photophysical properties. Geometrical alternation in the backbone visualized as chromic or electrical change, can be used as the sensor functionality. CPs also have the advantage that a collective response can amplify the read out compare to small molecule based sensors [15]. The CPs can be made water soluble by adding charged side chains to the polymers. Theses conjugated polyelectrolytes (CPEs) can form interaction with biomolecules and thereby open new

routes for designing biological sensors. In the first part of this thesis (paper I-III) we have studied how the CPEs function as sensor layers to be used for detection of DNA hybridization and antibody interaction. We have also taken the step out from the controlled test tube experiments to the complex environment of the cell to see what information can be extracted from animal cells, live as well as fixed.

Another area of increasing interest is biotemplated electronics. This field is still in its early research phase and the inspiration for biotemplated electronics is most often found in nature. There are so many fantastic and sophisticated assemblies evolved during millions and millions years of organism evolution and some of them can offer building blocks in the true nano size regime. The DNA molecules, carrying the genetic information, are maybe the most obvious template. DNA has an extreme aspect ratio with nm width and several μm in length. The assembling can be controlled by the inbuilt recognition elements for precise localization via base pairing of the nucleotide sequence and stretched nanowires, crossings, networks, defined multilayer structures and also to some extent moving DNA machines can be formed [16-19]. Other templates that have been used are amyloid like fibrils, viruses and bacteriophages, self assembled peptides and actin filaments [20]. System flexibility is generally gained when stability is lost for the biotemplates, why you carefully have to select the best template for the actual situation and requirements.

However, these templates have all one property in common; that none of them have the intrinsic possibility for electronic conduction. They need to be functionalized in order to assemble functional electronic devices. Conjugated polymers, and especially the conjugated polyelectrolytes, are candidates that possess many of the requirements for successful functionalization. CPEs can be both water soluble and biocompatible and at the same time have semiconducting properties. The second part of this thesis shows how we can use the knowledge from the CPE biosensor area, described in the first part of the thesis, and transfer them into the biotemplated electronics area for assembling of CPE decorated DNA nanowires (paper IV-VI). Not only making the template structures is important, but also how to place them on the correct surface position, i.e. on electrodes. Strategies for positioning DNA nanowires using soft lithography methods have therefore been developed in the thesis (paper IV).

The biological reporting functionality and the nanowire decoration assembling have many common scientific questions. Not only have the biomolecular detections using

CPEs opened the way for DNA wire decoration, but evaluation of the stretched and decorated DNA nanowires have given new information of the binding properties of CPE to biomolecules. The later can be used in the future development to improve the performance of CPE biosensors.

2 Conjugated Polymers

Polymers are molecules built up by repeating monomer units. During the synthesis of polymers, monomers are linked together by covalent bonds to form a long-chained macromolecule. If only one monomer is used, the polymer is called a homopolymer and if the polymer consists of two or more monomers it is called a copolymer.

In the 1970s, Chiang et al. discovered that the polymer polyacetylene could reach a conductivity close to that of a metal if the polymer was doped [21]. The discovery started a growing interest for a class of polymers, named conjugated polymers, with many special properties. A conjugated polymer is an organic molecule where the backbone of the polymer is built up by carbon atoms covalently connected via alternating single and double σ -bonds [22]. This alternation, called π -conjugation, gives highly delocalised electrons (π -electrons) that can move quite freely along the polymer chain. The distance an electron can move along the chain is called the conjugation length. Due to these π -electrons, conjugated polymers have unique one-dimensional electronic and optical properties. When conjugated polymers are in their undoped state, they are medium to wide band gap semiconductors making them quite poor conductors. However, the conductivity can be tuned by doping and high conductivity can be obtained. Changes in absorption and photo-luminescence related to the doping and conformational state can also often be observed [23, 24]. The basic structures for some commonly used conjugated polymers are shown in figure 2.1.

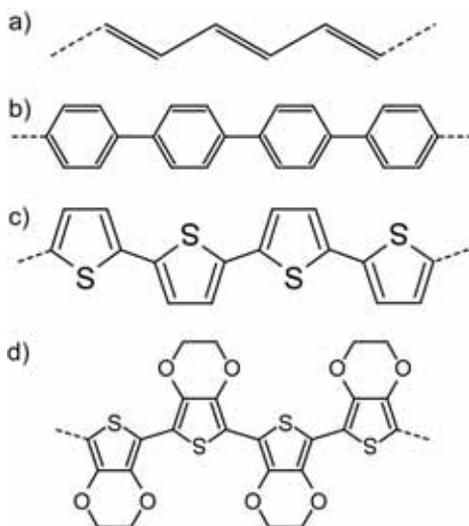


Figure 2.1: Basic chemical structures of some commonly used conjugated polymers. a) polyacetylene, b) polyparaphenylene, c) polythiophene, d) PEDOT.

2.1 Orbital Structure

Carbon, the main building block of most conjugated polymers, has six electrons of which four are valence electrons. In conjugated polymers, three of these valence electrons, two 2p and one 2s, form three sp^2 hybridized orbitals. Two of the sp^2 orbitals are responsible for σ -bonds (Fig. 2.2b) in the polymer backbone and the third sp^2 forms a σ -bond with the side chains of the polymer. The remaining unhybridized p_z orbital points orthogonally to the σ -bond orbital plane, and can overlap with the p_z orbital of neighboring carbon atoms to form a π -bonds (Fig. 2.2c). This π -conjugation, resulting into delocalization of electrons along the polymer backbone, are dominating when it comes to optical and electronic properties for conjugated polymers. The π -band forms the valence band with the highest occupied molecular orbital, HOMO, while the π^* -band forms the conduction band with the lowest occupied molecular orbital, LUMO (Fig. 2.2d). The difference in energy between these bands is termed the band gap energy, E_g .

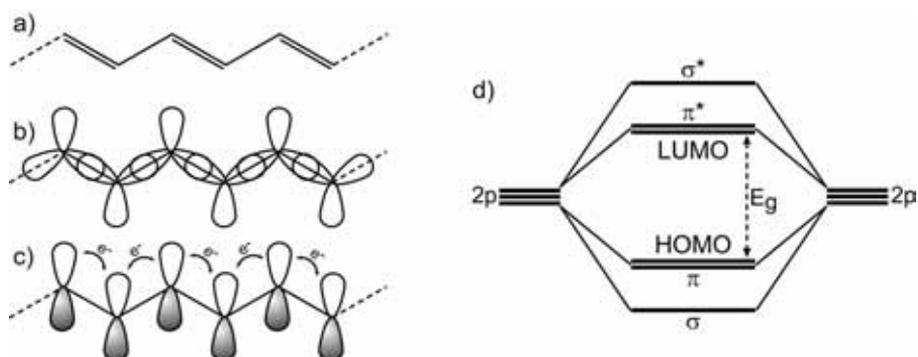


Figure 2.2: a) Conjugated segment of a backbone (polyacetylene). b) sp^2 orbitals forming σ -bonds in the polymer backbone. c) p_z orbitals forming π -bonds in an orthogonal direction to the σ -bond plane. d) Sketch of the HOMO-LUMO levels.

2.2 Photophysical Properties

Conjugated polymers absorb a photon when the photon energy matches the band gap for the polymer [25]. Due to molecular vibration of the C-C bonds in the conjugated system, conjugated polymers have discrete levels in both the ground (π -band) and excited state (π^* -band). When a photon is absorbed, an electron is excited from the singlet ground state S_{00} to any of the vibrational levels in the excited S_{1m} states (Fig. 2.3).

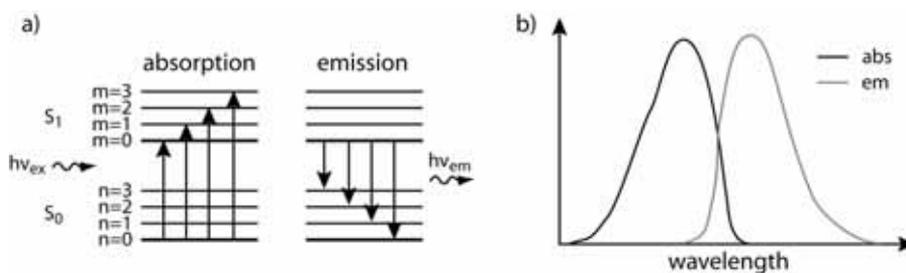


Figure 2.3: The principles for absorption and emission for a conjugated molecule. a) The transitions between ground and excited state for absorption and emission. b) Example spectra for absorption (abs) and emission (em).

The energy acquired from the absorption in conjugated polymer decays via radiative and non-radiative pathways [26]. Radiative decay in conjugated polymers leads to a photoluminescence (PL) process, hence emission of photons (Fig. 2.3). Compared to the absorbance energy, vibrational relaxation to the lowest excited states S_{10} before it returns to one of its ground state S_{0n} , gives longer wavelength of the emitted photon. Aggregation of polymers can alter the excitation and decay mechanism further by introducing intermolecular processes and lowering the transition photon energy by a shift of the energy levels and the dipole moments [27].

Conjugated polymers generally have, due to polydispersity of the material, difference in the backbone conformation and aggregation phenomena, a broader adsorption and photoluminescence spectra compared to small more stiff molecular dyes. As will be shown in the next section, different stimulus that affects the conjugated polymer geometry on single chain or on collective multimolecular level, can shift the optical as well as the electrical properties of CPs.

2.3 Conjugated Polyelectrolytes

A conjugated polymer backbone has very poor solubility in most solvents. By introducing side chains, the solubility can be increased and thereby their processability and compatibility to different solvents. For interaction with biological material solubility in polar solvents is crucial and water soluble CP can be achieved by synthesizing polymers with charged side chains. The introduced charges make the CP to a polyelectrolyte, and therefore we use the term conjugated polyelectrolytes (CPEs) for this class of conjugated polymers. Some examples of reported water soluble CPEs are polythiophene [26, 28-30], polyphenylene vinylene [31], polyphenylene ethynylene [32] and polyaniline [33]. In this thesis, CPEs based on polythiophenes have been used (Fig. 2.4). The names of the CPEs are mainly trivial names, with some exceptions i.e. that the letter t in the beginning indicates a trimer version.

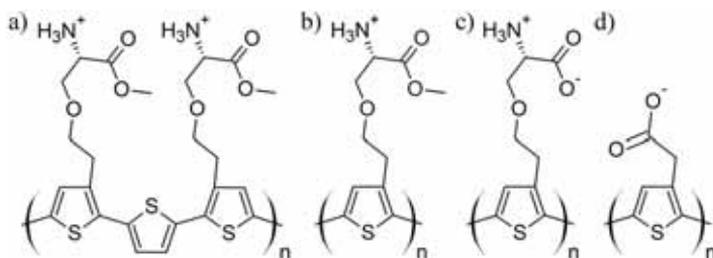


Figure 2.4: The chemical structure of the repeating units of the conjugated polyelectrolytes used in this thesis.

The CPEs used are polymerized from either monomer (POWT, POMT, PTAA) or trimer units (tPOMT). There are some differences between these polymers. The first is that the polymerizing process gives a more polydisperse material for monomer based polymers compared to trimer based polymers. POMT range from 11-26 monomers in size [34], while its trimer version tPOMT consists of ca 80% 12 repeating and 20% 9 repeating thiophene rings in its backbone. Another difference is that tPOMT gives a regioregular polymer while POWT, POMT and PTAA are regiorregular with random distribution of the side chain position. The more controlled length and structure opens for more defined photophysical properties of tPOMT.

Many polythiophenes are sensitive to external stimuli, giving chromic transition as well as changes in the electrical properties [35]. For optical transitions, the effects from different stimuli have been termed solvatochromism (solvents) [36], thermochromism (heat) [36-39], photochromism (light) [40], ionochromism (chemicals) [41] and biochromism (biomolecules such as proteins) [42, 43]. The later phenomena can be used for biosensor functionality. The induced changes are due to geometrical alternations on a single polymer chain level or a collective response of the aggregation status for multiple inter-molecular polymer interactions. Intra-molecular conformational changes in the conjugated backbone give rise to change in the conjugation length [44]. It is often steric interactions, which are responsible for the resulting twisting associated with a spectral blue shift or planarization associated with a spectral red shift [35]. Red shift and reduction in the photoluminescence intensity can also be obtained through inter-chain processes due to aggregation of polymer chains [27]. The exact reason for spectral and

intensity shifts is in many cases not yet fully understood. Geometrical changes on non-conjugative polymers has been thoroughly reviewed supporting the above main theory, even though they have less rigid backbone structure compared to conjugative polymers [45]. A special case of twisting is when a helical geometry is induced, evident by i.e. absorption difference of right handed and left handed circular polarized light in the 300 - 700 nm range of circular dichroism (CD) measurements [27]. Whether the helical geometry is an intra-chain phenomena [46, 47] or a result of helical pi-stacking of several conjugated polymer molecules [27, 48] is still an open question (Fig. 2.5).

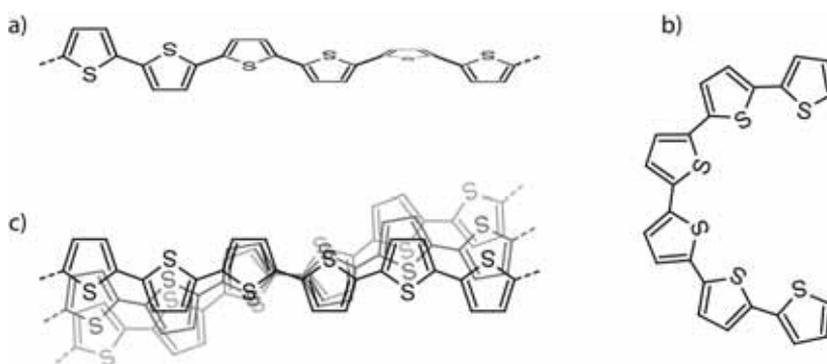


Figure 2.5: Different possibilities for helical organization of polythiophene backbones. a) Helical transoid. Intra-chain rotation of the thiophene ring angles orthogonally to the backbone direction. b) Helical cisoid. Intra-chain twisting of the backbone into a circular geometry. c) Helical packing. Inter-chain stacking of several polymer chains into a helical spiral.

Some initial studies have been done to evaluate the geometrical status of the CPEs used in our group. Dynamic light scattering (DSL), fluorescence correlation spectroscopy (FCS) and ultracentrifuge measurements points towards that they forms clusters of various sizes in water-based solutions. DLS and FCS gives an estimation that clusters of more than 500 polymer chains (3400 g/mol) or 1 μm in diameter is present when POWT is solved in double distilled water. However, the DLS measurements also show traces that there might be particles with a radius of a few nm in the solution, indicating that very small clusters or individual chains also can be present at the same time. The ultracentrifuge measurements on two polymers called tPOWT and tPTAA (trimer

versions of POWT and POMT) in acidic respectively basic solutions have given a cluster size of circa 10 polymer chains [49].

3 Conjugated Polyelectrolytes as Biological Reporters

The increasing understanding of our genome expression and the regulation mechanisms of organisms through biomolecular interaction and signaling has led to a continuing increase in the development of biological sensors. A biosensor is a device where a physical transducer translates response of a biomolecule from one or a few analyte stimulus into a readable out signal [50, 51]. Optical, electronic, acoustical, mechanical and calorimetric are all examples of different principles for transducers used in biosensing. Clinical diagnostics, pharmaceutical research, environmental and food applications are among the wide range of areas where biological sensing day by day increases its importance.

Lately, the vast amount of information enclosed in the fully sequenced genomes of several organisms including human has given an extensive development of different array techniques for high throughput DNA analysis [52]. DNA chips are now used on regular basis, but in further understanding of the expression of the genome on cell and organism level, protein sensors play a crucial part and the research in this area is therefore expanding [53]. The constructions of protein chips are however less straight forward, with a number of delicate problems such as detection of protein interactions, stability issues regarding the preservation of protein conformation and biological activity after anchoring to a surface, and also how to retain high selectivity [54].

However, you should bear in mind that these fancy and high tech array detection mechanisms only have a small part of the overall market for biosensor. The dominant biosensor is still the disposable glucose sensor kits that stand for around 85 % of the total

market [55]. Inspired by this knowledge, a cheap, versatile and label free system with easy handling would be of great value. The use of conjugated polymers (CPs) compatible with aqueous environment is one candidate that shows potential for this in biological sensing. Among the benefits of using CPs are that they, compared to small molecule based sensors, have the advantage that minor perturbations can be amplified by a collective response (Fig. 3.1) [15, 56]. They also usually have the possibility for cheap production methods.

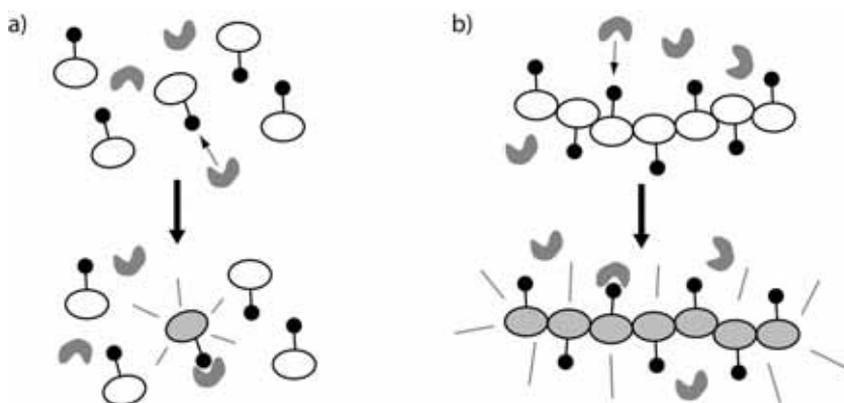


Figure 3.1: Detection principles. a) Biosensor using a single fluorophore reporter as reporter for detection of an interaction. b) Biosensor using the collective response of the conjugated polymer for detection of an interaction.

3.1 Solution Detection of Biomolecules Using CPEs

Probing interactions and changes in biological system and molecules has been done using CPs since the early 90's [57]. The principles for detection can rely on chromic changes of the CP, detected i.e. by intensity or spectral shifts of the polymer in absorption or photo luminescence. It may also include fluorescence resonance energy transfer (FRET) of the CP to/from a small molecular chromophore (detected by lighting of the CP or the chromophore) or use the energy transfer phenomena to quench the CP fluorescence by transferring the energy to a quencher molecule (detection by turning off the CP fluorescence) (Fig. 3.2). Combination of these methods can also be a powerful tool to improve the detection limits and results.

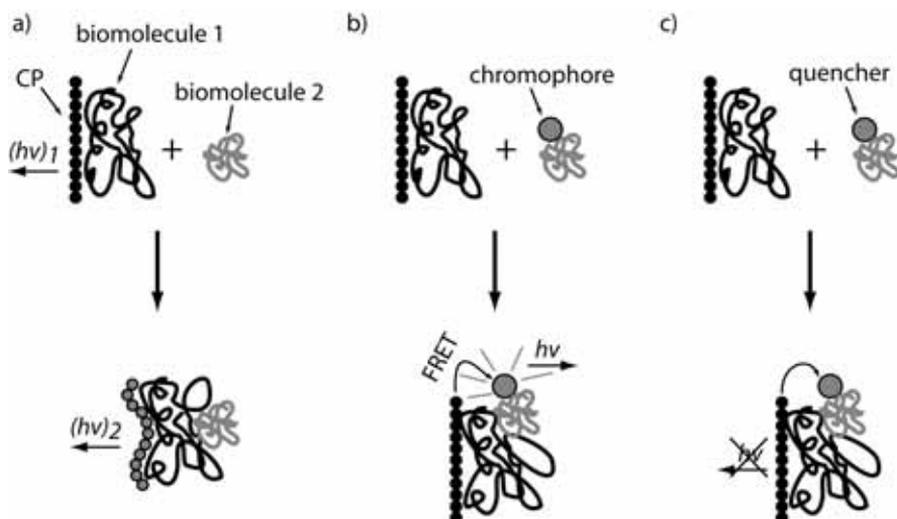


Figure 3.2: Examples of three different principles for optical detection of biological targets using conjugated polymers. a) Conformational geometry change of the CP leading to a chromic alternation upon binding of a biomolecule. b) Triggering of energy transfer (FRET) between the CP and a small molecule chromophore upon binding of the biomolecule. c) Quenching of the fluorescence from the polymer upon binding of a biomolecule.

Chromic change methods, shown as colorimetric or intensity alteration, are based on the conformational changes in the geometry of the conjugated polymer backbone or in their state of aggregation (Fig. 3.2a). A CP may be associated with a biomolecule via covalent bonds or via weaker interactions such as electrostatic and hydrophobic forces. When a change occurs in the biomolecule, for instance by interaction of a receptor with its ligand, the CP will also be affected with a detectable spectral or electronic change as a result. The CP can be more or less twisted by the change in the biomolecule, they can also be separated from each other or aggregation can be induced.

DNA detection using the conjugated polyelectrolyte POWT is a good example of this method (Fig. 3.3) [58]. The CPE/DNA binding is based on non-covalent interactions. Addition of ssDNA to a POWT solution is believed to force the polymer to a more planar

structure and also to induce aggregation. Electrostatic interaction between the negative phosphate backbone of the DNA strand and the positive amino groups of the polymer is one component of the binding. The DNA bases also have the possibility to form hydrogen bonds to the amino- and carboxyl groups of the polymer. Formation of hydrogen bonds between one DNA chain and several polymer chains, and also between different polymer chains, may contribute to the resulting aggregation. Stacking of exposed thiophene rings might also be a factor in the aggregation process. The result can be seen in fluorescence as a red shift and a decrease in intensity.

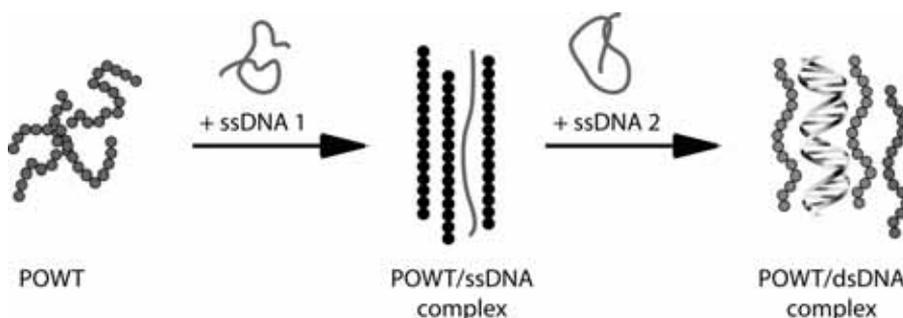


Figure 3.3: DNA detection using POWT. The twisted POWT chains bind to the ssDNA 1 probe and forms a complex where POWT is aggregated and more planar (red shift and decrease in intensity of the photoluminescence). When the complementary ssDNA 2 is added, POWT again adopts a more twisted conformation and possibly some separation of the polymer occurs (blue shift and increase in photoluminescence).

Upon addition of a complementary DNA strand, hybridization will occur and a separation effect of polymer chains can be seen. Disruption of the hydrogen bond when the DNA bases binds to its matching base instead of a nearby polymer chains is suggested to be responsible for the separation. The electrostatic interaction is probably less affected, but the double helix may drive the polymers to a more non-planar structure. The result is a blue shift and an increase in intensity of the emission. This is the main system I have used in this thesis to evaluate the biomolecular sensor possibilities of POWT layers. The chromic change principle has also been used by various groups for solution detection of conformational changes in proteins [59-62], synthetic peptides [34], E-Coli, lectines and influenza virus [63], lectines [64] and biotin-avidin interaction [65].

FRET is a dipole-dipole coupling process in which energy is transferred from an excited donor chromophore to an acceptor chromophore, in this case from/to a CP [66]. The FRET can be characterized by some calculations [67]. The emission of the donor in the absence (F_0) or presence (F) of the acceptor is used for calculating the transfer efficiency (E) by

$$E = 1 - (F/F_0) \quad (\text{Eq 3.1})$$

The overlap integral (J) correlating the donor emission with the acceptor absorption is given by

$$J = \int F_D(\lambda)\epsilon(\lambda)\lambda^4 d\lambda \quad (\text{Eq 3.2})$$

where F_D is the peak-normalized fluorescence spectrum of the donor, λ is the wavelength and $\epsilon(\lambda)$ is the molar extinction coefficient of the acceptor. The Förster distance (R_0), at which the transfer efficiency is 50%, can be calculated to get a value of the distance between the donor and acceptor according to

$$R_0^6 = 8.785 \times 10^{-5} k^2 Q_D J / n^4 \quad (\text{Eq 3.3})$$

Where k is the orientation factor between donor and acceptor dipoles, Q_D is the quantum yield and n is the refractive index of medium. The distance (R) between the chromophores can then be obtained by

$$R = R_0 (1/E - 1)^{1/6} \quad (\text{Eq 3.4})$$

Detection using FRET (or lighting) techniques (Fig. 3.2b) has been demonstrated on DNA hybridization [68, 69], RNA-protein interaction [70], binding of streptavidin conjugated with a fluorophore to biotinylated PPE polymer [71], protease enzyme activity [72] and kinase-phosphatase interactions [73]. The group of Mario Leclerc has taken the concept of DNA detection by FRET technique to an impressive zeptomole level.

They have achieved this by using fluorescence signal amplification, also called superlighting, where the hybridization of one ssDNA strand affects the complex conformation of several ssDNA/CP at the same time, resulting in a more efficient energy transfer from the CPs to the chromophore and thereby amplifying the hybridization signal [69, 74]. Instead of using FRET from CPs as a pure lighting mechanism for small chromophores, it is also possible to use FRET for turning off the CP fluorescence. Quenching of a polymer system has been proven to be more sensitive compared to quenching of small chromophores [75, 76]. Variants of the kinase-phosphatase system detected via FRET has not only been used as turn-on systems, but also for quencher based turn-off detection system for energy transfer from the CP to a quencher molecule and thereby loss in photoluminescence intensity [73]. Other detection systems relying on excitation quenching have been shown for DNA [77, 78], protease enzyme activity [72, 79] and saccharide [80]. The quenching can be quantified by the Stern-Volmer equation [81]:

$$K_{sv} = \left\{ \left(\frac{\Phi^0}{\Phi} \right) - 1 \right\} / [Q] \quad (\text{Eq 3.5})$$

Where K_{sv} is the Stern-Volmer constant, Q is the quencher concentration, Φ^0 and Φ are photoluminescence quantum efficiencies in the absence and presence of the quencher, respectively. However, Whitten has shown that the quenching is dependent of the polymer concentration and that K_{sv} therefore is not always constant for CP quenching as assumed in the Stern-Volmer equation [82].

3.2 Surface Detection of Biomolecules

To scale up a measurement and to reduce the sample amount, chip based methods are often to prefer. The Affymetrix DNA chip with up to 1.8 million genetic markers, manufactured by photolithography methods, is maybe the most well known example of a commercial product within the high throughput field [14]. DNA chips are now well established as a tool in research, and the success has inspired the development of the more complex array based protein detection chips. DNA chips give the genomic expressions levels in a cell, while protein chips have more direct information about the function and regulation of this expression in the cell [54, 83]. The gene expression is also

often only the first step followed by post-translation alternation such as phosphorylation, glycosilation and various biomolecular interactions [83]. Therefore many scientists claim that protein chip can be of more relevance in understanding the cellular functions or status of an organism. The Affymetrix DNA chip and many other array biomolecular detection chips use fluorescent probing with a small molecular chromophore as detection mechanism. An alternative solution might be to use conjugated polymers for constructing chip based detection systems. Many of the principles described for CP detection in solution can be transferred to surface based methods. The CP can also function as a conformational sensitive matrix for detection with i.e. fluorescence and electrochemistry or as an affinity matrix for detection with i.e. surface plasmon resonance.

The first shown biosensor of a conjugated polymer in 1993 from Charych et al. is actually surface based [57]. A bilayered film structure was formed with a sensing toplayer of polydiacetylene (PDA) functionalized with a sialic acid analog as side chains (Fig. 3.4a). The sialic acid analog functions as a receptor for the influenza virus hemagglutamin and when the virus binds to the sensing layer, a blue shift of the CP absorption can be seen. The changed conjugation length giving the chromic transition was due to increasing fatty side chain disorder of the CP in the bilayer assembly. The technique was later further developed to detection in vesicular and membrane structures [84-86]. Similar approach for glucose detection has been demonstrated. Here, hexokinase enzyme immobilized on a lipid polydiacetylene layer undergoes a conformational change when glucose binds to it. The induced stress in the PDA layer produces a detectable color change [87]. Alteration of the electronic properties of nucleobase-functionalized polythiophenes films can also be used for the recognition processes when a complementary base is added in the electrolyte solution [3]. The binding of uracil was followed both in the electroactive properties and in the absorbance. Detection of complementary DNA hybridization using CPs on surfaces has also been demonstrated. Le Floch et al. have used a ferrocene-functionalized cationic polythiophene to amplify the electrochemical signal of complementary DNA binding to PNA probes bound to a gold surface [88]. Oligonucleotide-functionalized polypyrrole films are another possibility for electrochemical detection of DNA hybridization [8]. A significant modification of the voltammogram is observed upon addition of a complementary oligonucleotide to the electrolytic solution, which can be quantitatively

determined by amperometric methods. The electrochemical detection limit without any signal processing is about 10^{-11} mol.

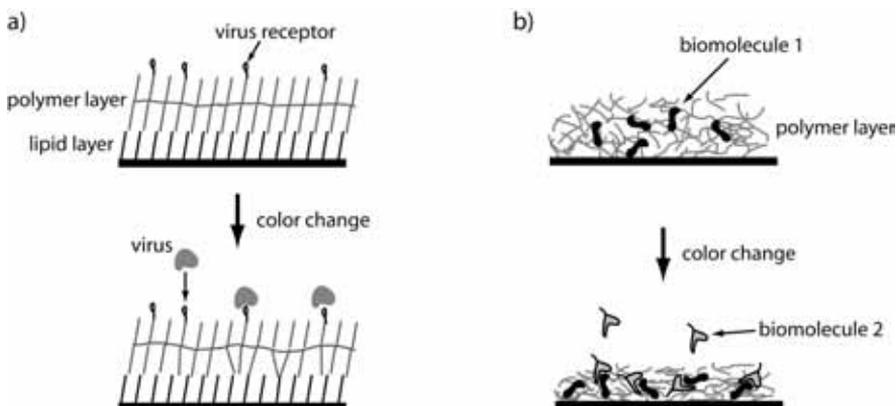


Figure 3.4: a) Influenza virus hemagglutinin detection using polydiacetylene functionalized with a sialic acid analog. Schematic illustration adopted from [57]. b) Detection of biomolecules using POWT layer. The binding signal can be i.e. fluorescence if the interaction induces conformational changes in the POWT layer or i.e. SPR if the POWT layer just functions as an affinity matrix.

In this thesis, POWT layers have been used to probe biomolecular interaction by surface plasmon resonance (SPR) [89] and quartz crystal microbalance with dissipation (QCM-D) [90]. Apart from the biomolecular interaction information, the methods also give an insight in the layer functionality. It may for instance be possible to distinguish if geometrical changes are present or if the layer only functions as an affinity matrix (Fig. 3.4b).

3.2.1 Surface Plasmon Resonance (SPR)

A surface plasmon is a p-polarized electromagnetic wave, bound to the surface, which propagates along the interface between a dielectric material (e.g. water) and a metal (e.g. gold) [91]. Surface plasmons can be described as oscillations of electrons at the surface of the metal. It is however the evanescent field associated with the surface plasmon, which functions as the sensitive part in the sensor. Due to the exponential decay of this

electromagnetic wave, sensors based on the SPR phenomenon are very surface sensitive. Sensors based on the surface plasmon phenomena have in recent time (since 1983) also extended their applications to include biosensing [92]. SPR biosensors can, often in real-time, be used to determine a number of factors regarding the interaction between a biomolecule and a surface. Some of these are: concentration, association and dissociation constants, affinity studies and determination of interaction specificity. Another interesting observation, possible to investigate utilizing SPR, is detection of conformational changes in biomolecules [93].

Biacore AB (Uppsala, Sweden) has developed a commercial biosensing system based on the surface plasmon phenomena, which utilizes the Kretschmann configuration. The sensor chip is a glass slide coated with a thin gold layer ($\sim 500 \text{ \AA}$). When molecules are close to the surface a change in the reflective index will be induced and a SPR response can be detected and expressed in resonance unit (RU). The RU response can roughly be converted to a surface mass concentration. The conversion value is dependent on the properties of the sensor surface and the molecule responsible for the change in concentration. For most proteins, one RU corresponds to about 1 pg/mm^2 . To make the chip surface active for biomolecular interactions, matrixes or biomolecules are attached to the gold surface. Biacore supplies a number of different chips, of which a chip with a covalently attached dextran matrix is the most commonly used. They also supply bare gold chips where the purchasers, on their own, can attach different surface chemistry of interest.

3.2.2 Quartz Crystal Microbalance with Dissipation (QCM-D)

An alternative method to study surface events is the quartz crystal microbalance with dissipation (QCM-D). In QCM, a quartz crystal is put into resonance by applying a RF voltage and the resonance frequencies (f), will vary with the total mass, including surface bond molecules and water. A crystal has several resonance frequencies that can be used to gain better evaluation and the fundamental resonance frequency for the crystal alone is termed f_0 . When a mass is attaching to the surface the resonance frequency will be affected and this change can be translated into a mass change. It is also possible to get knowledge of the viscosity of the bound surface mass with the addition of damping, the

energy dissipation (D). This is done by measuring the damping after turning of the RF voltage. Fast damping corresponds to a rigid mass and slow damping indicates a more viscoelastic mass (hydrogel like). Modeling of f and D gives the thickness and viscosity of the adsorbed mass.

For a rigid mass with a ΔD close to zero the Sauerbrey relation is a valid model [94]. The Sauerbrey mass m_s can be calculated by:

$$\Delta m_s = \frac{C}{n} \Delta f \quad (\text{Eq 3.6})$$

Where C is the mass sensitivity constant (for instance $17.7 \text{ ng}\cdot\text{cm}^{-3}\cdot\text{Hz}^{-1}$ at $f_0=5 \text{ MHz}$) and n is the overtone number. For a non-rigid mass where Δm is not directly proportional to Δf a more complex modeling has to be done (Fig. 3.5). A Voigt-based viscoelastic description can be applied [95]. For a detailed description and use of the model see references [95-98]. The modeling combine Δf with the ΔD at several harmonics to include the more hydrogel like structure of the mass and the effective mass, layer thickness and viscosity can be calculated.

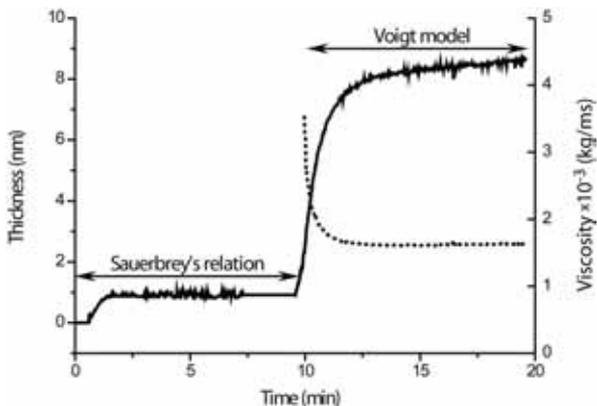


Figure 3.5: Modeling the data from a QCM-D measurement. The first part is a rigid film and the Sauerbrey relation is valid, while the second part is after swelling to a hydrogel and the Voigt model has to be applied and values for the viscosity can be obtained.

The used QCM-D setup in this thesis was from Q-Sense (Göteborg, Sweden). The quartz crystal with an interfacing layer of gold is connected to a flow system for injection of solution. QCM has the advantage that it is not affected by refractive index changes in the solution, but it has poorer possibilities for kinetic measurements. The resolution of QCM, in ng/cm^2 range, is also slightly lower compared to the SPR sensitivity.

3.2.3 POWT Layer Properties

The properties of conjugated polyelectrolyte layers are in themselves interesting to study. Is it possible to get a hydrogel structure of a CPE layer? Can this layer interact with its surrounding and are the geometry changes on intra-chain and inter-chain level upon interaction still present? The later is a key feature for using the geometrical changes in CPE films, visible in i.e. fluorescence, as sensors. Combining the techniques of SPR, QCM-D, ellipsometry, fluorescence and absorption gives a good insight in the behavior of the CPE films from both an optical and a physical point of view. To render quality films with defined thicknesses, spin coating or physisorption to a surface can be used. Ellipsometric measurements give an approximate thickness of around 8 nm for dry films spun from 5 mg/ml water solutions on gold surfaces.

Illustrated by the fluorescence microscope images in figure 3.6, the optical measurements show how the color of the POWT layer is changed in buffers with different pH. At high pH, the films red shifts due to collapsed film structure and the polymer chains come closer to each other [24, 90, 99]. Lowering the pH gives a film structure with less aggregated and more twisted polymer chains with a blue shift in color. At pH below pI (~5,9), POWT starts to detach from the surface due to chain separation effects and very little fluorescence is seen from the remaining thin POWT layer.

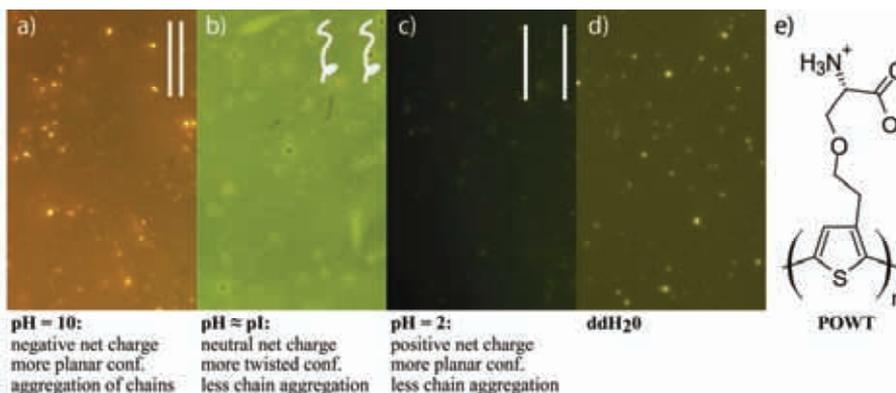


Figure 3.6: Fluorescence images of POWT layers affected by different buffers. a) Carbonate buffer pH 10. b) Acetate buffer pH 5. c) Phosphate buffer pH 2. Most of the CPEs have detached from the surface due to separation effect. d) ddH₂O. e) The chemical structure of POWT.

POWT from water solution can easily be adsorbed to a gold surface on a QCM-D crystal [90]. The measurements on such surfaces in QCM-D confirm the optical results and that the layer can undergo changes from a quite rigid film at high pH to a hydrogel like layer with high water content, for pH around the polymers pI. The structural change can be followed in the QCM-D as an increased layer thickness and a lower viscosity upon switching the buffer (Fig. 3.7). The thickness of the layer can increase as much as up to 100% for a pH 10 to pH 4 transition and the related viscosity change can be up to 50%. The changes are also reversible. The conclusions from the different characterization methods are that all basic properties necessary for interaction studies using chromic change as detection mechanism is fulfilled for the POWT layer.

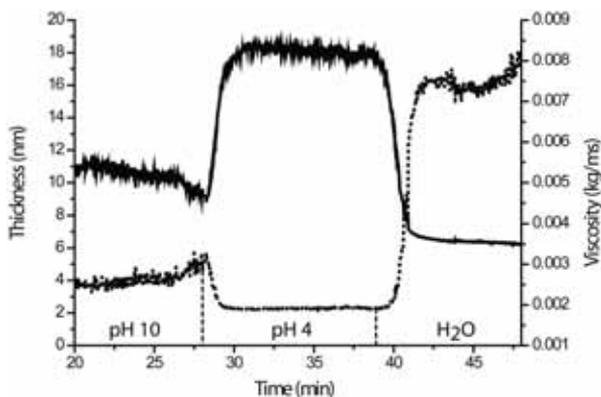


Figure 3.7: QCM-D measurement of the pH dependence of a POWT film adsorbed to a gold surface. The graph shows the calculated thickness (line) and viscosity (dots). pH change is marked with vertical dashed lines.

3.2.4 POWT Layer Dynamics in DNA Detection

Detection using chromic transitions of POWT for DNA hybridization has been demonstrated in solution and also on a surface [58]. The surface detection was done by coating a POWT layer with ssDNA followed by flowing a complementary respectively non-complementary ssDNA over the surface using micro channels. A color shift could be seen for the complementary strand in fluorescence microscopy, indicating a geometrical change of the polymers POWT layer upon hybridization. To further study this process we used SPR and QCM-D. The general evaluation process is illustrated in figure 3.8 with a SPR DNA measurement. From the SPR measurements a distinct response can be seen for the complementary ssDNA strand, while there is only a low response from loosely associated non-complementary ssDNA that is washed away by the buffer [89]. QCM-D gives the same result, however with slightly less distinct responses [90]. The QCM-D also shows that the first DNA addition initially gives decrease in thickness and an increase in viscosity, which is then changed into an increase in thickness and a decrease in viscosity. This signals a significant structural change in the POWT layer upon DNA binding.

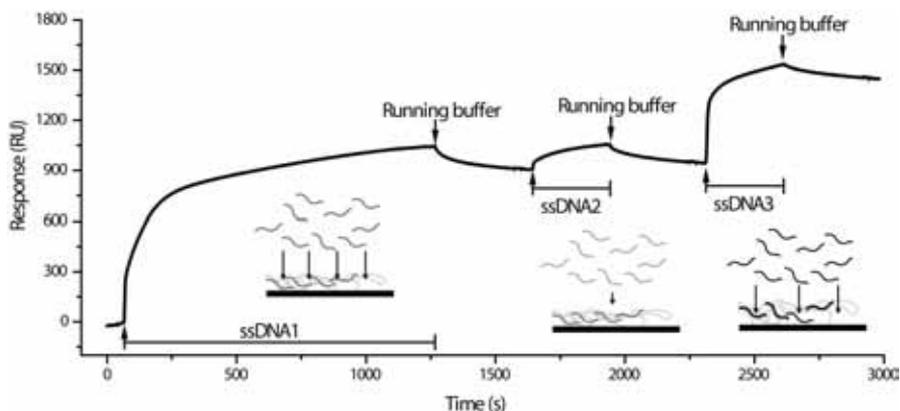
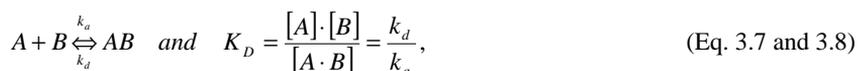


Figure 3.8: Sensorgram showing the injection sequence of 20 bp long ssDNA and illustrations of the possible ssDNA binding in the POWT layer. The layer is first equilibrated with the probing ssDNA, followed by injection of non-complementary and complementary ssDNA.

The binding strength of molecules can be evaluated using kinetic analysis of SPR sensorgrams (Fig. 3.9). In its simplest form, with a 1:1 Langmuir binding event with no change in conformation, the dissociation constant can be calculated using the equation $K_D = k_d/k_a$, where k_a is the rate constant for the association phase (injection of analyte) and k_d is the rate constant for the dissociation phase (wash out after injection of analyte) (Fig. 3.9). The rate constants are related to the reaction according to:



where A for instance is a biomolecule and B its binding partner. For a correct interpretation of the SPR curve, it must be adjusted from effects that do not derive from the ligand/analyte interaction. Different refractive index between the injected buffer containing the analyte and the running buffer, unspecific binding and baseline drift are example of such major unwanted contributions. Therefore, a control where no analyte is present is usually done and subtracted from the response curve. For best results, different analyte concentration should be evaluated. Of great importance is of course to evaluate if

the matching model has biological relevance, since several different models can fit equally well [100]. It is therefore vital to have good knowledge of the systems and its biological characteristics.

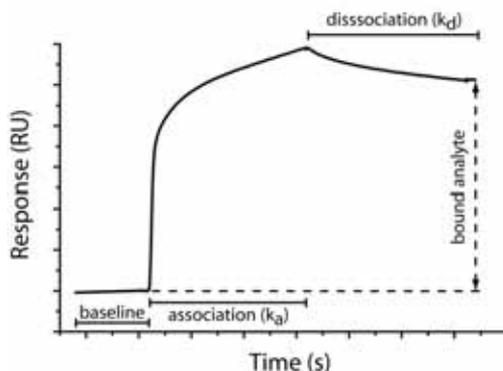


Figure 3.9: Sensorgram showing the association and dissociation phases from which information for binding rate constants of the analyte can be extracted.

When analyzing the binding of analytes where a conformational/structural change is induced upon binding, 1:1 Langmuir binding is not enough to describe the whole process. A second step taking the refractive index change induced by the conformational/structural change upon binding has to be added. The dissociation constant can then be calculated using $K_D = (k_{d1}/k_{a1}) \times (k_{d2}/k_{a2})$, where k_{d1} and k_{a1} are the rate constants for the 1:1 Langmuir binding step and k_{d2} and k_{a2} are the rate constants for the conformational change step. This model matches the known theory for the DNA/POWT interaction (see chapter 3.1), where the binding of ssDNA induces a change in the polymer conformation or/and aggregation state. Using the model, a dissociation constant for the ssDNA binding is determined to $(6.3 \pm 3.4) \times 10^{-8}$ M [89]. The order of magnitude for K_D has later been confirmed by solution FRET experiments [101]. Some typical K_D values can be seen in figure 3.10.

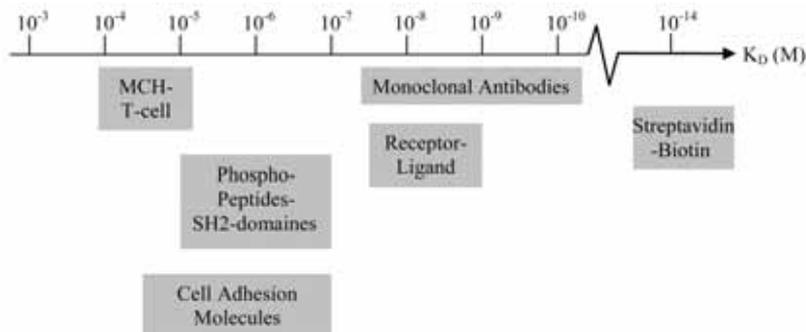


Figure 3.10: K_D values for some common molecular interactions.

3.2.5 POWT Layer Dynamics in Antibody/Antigen Interaction

Antibodies, a natural part of our immune system, are often used as recognition elements in research development and diagnostics. There are different classes of antibodies, but their basic structure is quite similar with a Y-shaped geometry [102]. As illustrated by immunoglobulin G (IgG) in figure 3.11, they consist of two parts. The two F_{ab} arms with the recognition elements toward the antigens in the top parts and the F_c stem. The major

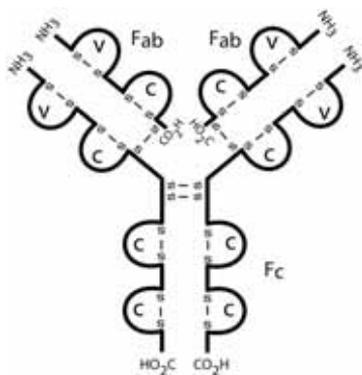


Figure 3.11: Schematic illustration of an IgG antibody. F_c and F_{ab} are the stem respectively the recognition element parts. V and C stands for variable respectively constant parts of the antibody.

part of the antibodies has a more or less constant sequence and it is mainly the top part of the F_{ab} that differs considerably between different antibodies. It is these two highly variable F_{ab} regions that enable the antibodies to bind to virtually everything from small molecules to large macromolecules. The F_c part is more hydrophobic compared to the F_{ab} arms.

Running SPR on antibodies immobilized in POWT layers gives very interesting results on the POWT-antibody interaction [89]. Changing the injection sequence of an arbitrary IgG antibody and an IgG that is specific towards the highly conserved F_c part of IgGs shows that the binding of antibody to POWT layer has a defined direction. The F_{ab} parts interact with the POWT layer and are after immobilization hidden from the medium, while the F_c part is still accessible (Fig. 3.12). This is an important result for designing antibody biosensors with POWT as reporter molecule.

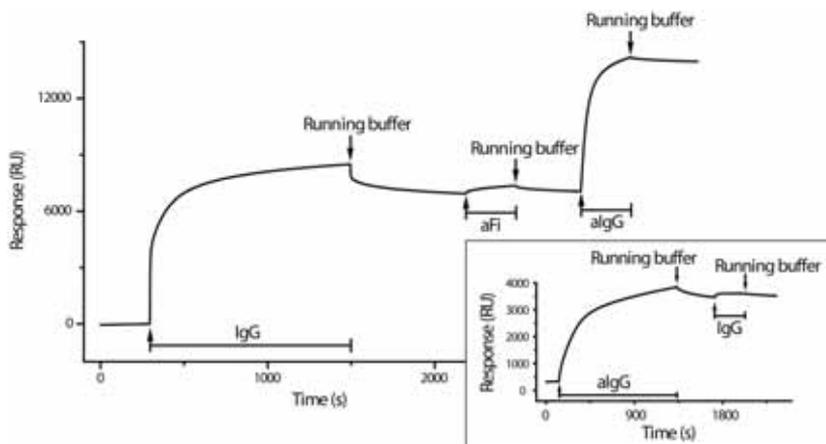


Figure 3.12: Antibody binding to POWT layer. Injection of algG gives a distinct response to the IgG immobilized in the POWT layer. However, reversing the order give no response for IgG on algG immobilized in the POWT layer (see inset).

3.3 Cell Staining

The conjugated polymers have so far mainly been used to study responses and biomolecular binding in test tubes. Lately however, with focus on histopathology, tissue

slides have been stained using the CPEs to evaluate the occurrence of amyloid deposits [103]. It has been shown that by altering the conditions for the staining, selectivity for the highly regular structure of amyloid plaques can be obtained. Probably hydrophobic interaction between the CPE backbone and the plaque is dominating, but the side chain seems to determine to what type of plaque the CPE can bind. In this thesis we have applied the CPE to cells in monolayer cultures *in vitro*. Both fixed cells and living cells have been stained. The eukaryotic cell is a most complex environment for biological sensing and, therefore, it is a challenge to extract information on what processes are going on in the living cell using conjugated polymers. In the present work, the aim with the CPE cell staining has been to investigate to which subcellular structures the CPEs might bind and under what conditions, and also to get an indication if the CPEs might have any toxic effects.

3.3.1 Structure of the Cell

The basic structure of a eukaryotic animal cell is sketched in figure 3.13 [104]. The outer boundary is defined by the cell membrane. This membrane consists of a double layer of lipids that also hosts various other molecules such as membrane proteins and cholesterol. Associated with the membrane on the outer side is the extra-cellular matrix in which the major components are structural proteins (e.g. collagen and elastin), other specialized proteins (e.g. fibrillin, fibronectin, and laminin) and proteoglycans. Proteoglycans have a protein core with attached long chains of repeating disaccharide units termed glycosaminoglycans (GAGs) forming complex and high molecular weight components. Inside the cell, incorporated in the viscous cytosol, there are many compartments, so called organelles. The cell nucleus containing the genome is usually the largest. Other organelles with specialized functions are: the ER (endoplasmic reticulum) that is responsible for synthesis and transport of lipids and proteins, the Golgi apparatus responsible for modifications, sorting and packing of macromolecules for delivery to other organelles or secretion out of the cell, peroxisomes with their oxidative enzymes, lysosomes that contain hydrolytic enzymes to digest intracellular materials, and mitochondria that provide energy to the cell via ATP synthesis. To help the cell to maintain its shape the cell relies on cytoskeleton filaments. These filaments, microtubules, actin filaments and intermediate filaments, also provide the basis for

movement. Different vesicles such as endosomes, which are transport and secretory vesicles connected to the cytoskeletal filaments provides a route for the cell to transport materials in and out of the cell, through the cell wall, to the membrane itself, and also between organelles within the cell.

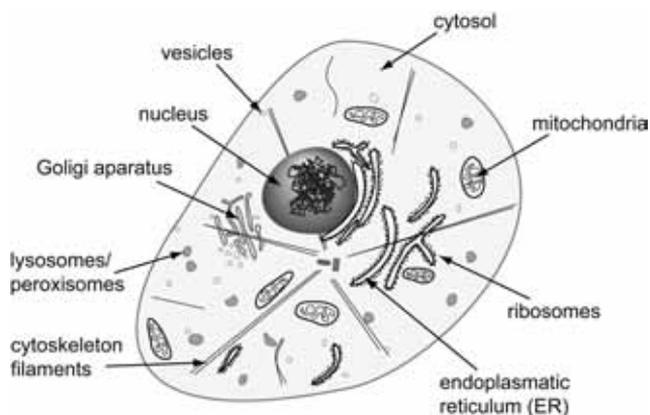


Figure 3.13: Schematic illustration of an animal cell including organelles and cytoskeletal elements.

3.3.2 CPE in Fixed Cells

Among the necessary conditions for the cells to stay in good shape are controlled temperature and pH, essential growth factors, a suitable energy source and for most cells an appropriate surface for the cell to attach to. If one of these requirements is missing, the cell may no longer be a representative model to evaluate. Therefore, when doing experiments that may influence the cells negatively, different fixation schemes are often applied to the cell before the experiment is actually performed. Depending on the fixation method, the cell morphology is preserved to a different extent and the access to intracellular structures can vary. The most preservative fixatives are paraformaldehyde and formalin, which heavily cross-link amine groups. Surfactants such as Triton X-100 can be used to open up the cell membrane for more effective diffusion of larger molecules into the cell. Ethanol and methanol, with or without acid additives, does not

cross link as much, and most of the phospholipids in the cell membrane are washed away giving a more collapsed morphology.

To stain cells grown and fixed on microscope slides, a dilute solution of the CPE in buffer was applied to the cells for 20 minutes [105]. The cells were washed in the staining buffer before and after the staining to clean and remove unspecific staining. To evaluate the staining results, fluorescence microscope was used. Our first aim was to see if CPEs could be used for in situ hybridization with DNA oligomers. This was not of any success, since chromosomes (Fig. 3.14a) and also some subcellular structures stained nicely with the CPEs, presumptively obfuscating any possible hybridization signal.

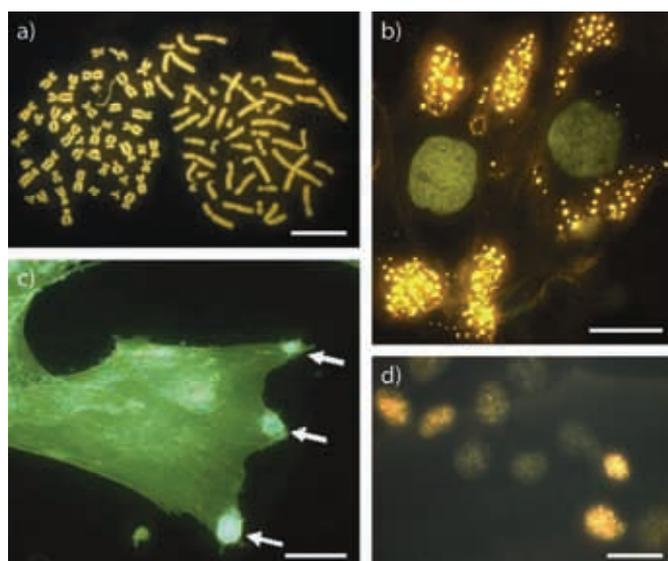


Figure 3.14: Fluorescence images of CPE stained fixed cells. a) tPOMT staining of lymphocytes in mitosis fixed with paraformaldehyde. b) tPOMT staining of EtOH:HAc fixed human fibroblasts. c) PTAA staining of paraformaldehyde fixed human fibroblast (pseudopodia marked with arrows). d) tPOMT staining of EtOH:HAc fixed prostate cancer cell lines. Scale bars: 20 μ m.

Different results of the cell staining were also obtained depending on the combination of fixation method, CPE and buffers. By using paraformaldehyde fixation in combination

with PTTA staining at neutral pH, the cell surface and pseudopodia of human fibroblasts were visualized (Fig. 3.14b). Using tPOMT in acidic buffer on acetic alcohol (EtOH:HAc) fixed human fibroblasts gave distinctive green coloring of nuclei and intense red coloring of acidic vesicles (Fig. 3.14c). The red and green color of tPOMT indicates a completely different geometry of the polymer with a planar and/or aggregated conformation in the acid vesicles and a more separated and/or twisted conformation for the chromatin staining. The binding dependence of the CPE color demonstrates the potential power of the CPE in cell staining. The cell type also affected the staining of acidic vesicles. In malignant cells (melanoma, neuroblastoma and prostate cancer cell lines), we have so far not been able to stain any acidic vesicles in the cytosol (Fig. 3.14d). In spite of that Acridine Orange, a basic dye, can stain the acidic vesicles in both cell types in live experiments [105]. The reason for this difference is still unknown and highly interesting. The staining, conditions and results are summarized in table 3.1.

Table 3.1: Summary of CPE cell staining conditions and the results. (+ + +) indicates strong fluorescence, (-) no fluorescence and (*) strong staining after permeabilization with Triton X-100.

Fixative	Acetic alcohol (EtOH:HAc)		Acid formalin alcohol		Paraformaldehyde	
	PTAA	POWT/tPOMT	PTAA	POWT/tPOMT	PTAA	POWT/tPOMT
Normal cells (Human fibroblasts and leukocytes, murine macrophages and myoblasts)						
Cell surface	-	-	+	+	++	++
Cytoplasmic vesicles	+++	+++	++	++	-	-
Nucleus and chromatin	-	+++	-	++	-	-*
Malignant cell lines (HTB-72, JKM86-4, CRL-1740, CCL-127)						
Cell surface	-	-	+	+	++	++
Cytoplasmic vesicles	-	-	-	-	-	-
Nucleus and chromatin	-	+++	-	++	-	-*

3.3.3 CPE in Live Cells

Live staining of intra-cellular components relies on a mechanism for transport over the cell membrane. It can be passive uptake by diffusion through a concentration gradient or active transport over the cell membrane mediated by for instance endocytosis or ion

pumping [104]. Macromolecules such as proteins, polynucleotides, and polysaccharides are usually taken up by endocytosis, that for the most part is accomplished by receptor-mediated activation of clathrin coated pits and vesicles. PTAA (Fig. 2.4d) with its negatively charged side chains is relatively stable at physiological pH, which makes this CPE possible to use for live staining of cells. Only a few minutes of staining were required and this indicates active uptake by endocytosis, i.e. the CPE forms complexes with some component on the cell surface, which is then redistributed from the cell surface to the interior of the cell. Once there, the complexes appeared stable since they could be observed accumulating in endosomal/lysosomal compartments (Fig. 3.15). Furthermore, no toxic effect on the cultured fibroblast cells was detected. The cells were still proliferating with intact morphology more than 48 hours after staining.

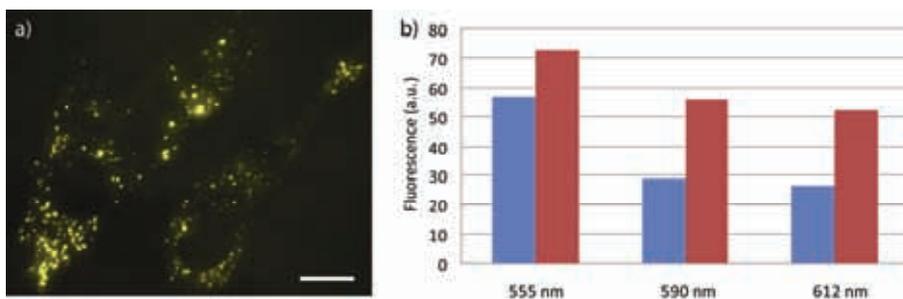


Figure 3.15: a) Live staining of human fibroblast using PTAA. The cells were put back in growth medium after the CPE staining and allowed to grow for 48 hours. The cells were then fixed prior to the fluorescence microscopy. Scale bar: 20 μm . b) The fluorescence signal of POWT at three wavelength when bound to live *Staphylococcus epidermidis* (red) and when free in PBS buffer (blue).

Initial experiments with staining of bacteria have also started. Bacteria have a rougher cell wall, and so far only the outer cell wall is stained. The aim with the staining is for instance to see if it is possible distinguish between bacteria with different types of cell wall structure. The graph in figure 3.15b shows how the POWT fluorescence is changed when binding to *Staphylococcus epidermidis*. An altered ration of the different wavelength shows that POWT red shifts when binding to the bacteria.

The CPE staining of cultured cells shows that some CPEs, like PTAA, can be applied to both fixed and live cells. By altering the conditions and type of CPE used, the staining can be directed towards different subcellular compartments and structures. The apparently low, if any, toxicity of CPEs shown in the live staining experiments, and the fact that many of these CPEs are two-photon active [106] also gives the opportunity for real time evaluation of both cultured cells in vitro and in vivo experiments, e.g. in a mouse model. Such experiments are now ongoing.

4 Biotemplated Electronics

When shrinking the size of structure to the true nano regime, researchers have looked at nature to find inspiration and model structures. In this regards, DNA is maybe the most obvious choice to use as template for construction of electronics with nanometer width. DNA offers both attractive size features with a width of around 2 nm and a length scale in the μm regime, and with built in recognition elements for precise localization via base pairing of the nucleotide sequence. The polyanion DNA molecule has an extremely high charge density with a negative charge repeated every 0.34 nm for each single strand [107]. Shielding counter ions, non-covalently associated water molecules and hydrogen bonds between complementary bases stabilize the double helix structure [108]. Hence, higher ionic strength is positive for hybridization. However, a negative effect from higher ionic strength is that mismatch hybrids are also stabilized. A decrease in ionic strength results in repelling of nearby negative charged phosphate groups and a denaturation starts, where the double helix will eventually separate.

The geometry of DNA put on surfaces can be controlled for construction of devices by a bottom-up approach where DNA functions as the template. Aligned wire geometries can be obtained by different DNA stretching techniques [109]. More advanced assembly such as networks (Fig. 4.1a) [19, 110, 111], crossings [17] and even three-dimensional structures [18] can be formed by designing ssDNA strands and let them hybridize in solution. The three-dimensional structures have been obtained by using many short DNA sequences that controls the folding of one long DNA sequence (Fig. 4.1b). The technique is called DNA origami. Such structured DNA templates can by further functionalization obtain electronic properties.

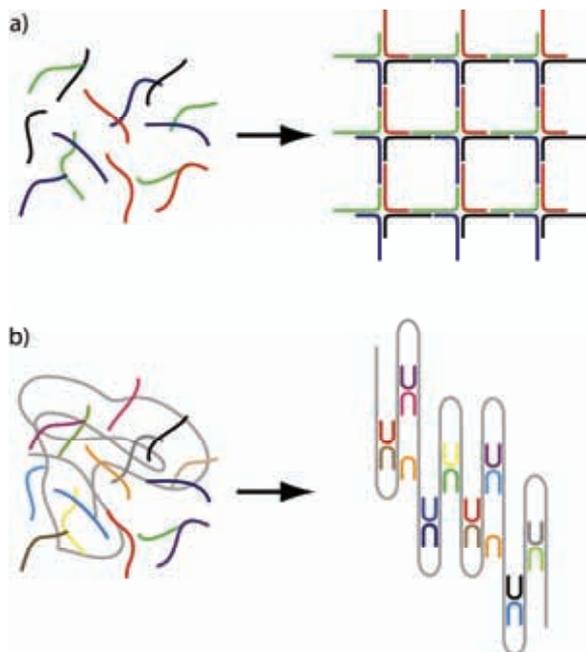


Figure 4.1: Principles for DNA assembly of structures controlled via single stranded DNA probe hybridization. a) Networks formed by repeating cross structures by assembling of four ssDNA sequences. b) The origami approach giving controlled folding of one long ssDNA sequence (grey line) by many short ssDNA sequences.

However, DNA is not the only bioinspired template used for nano electronics. Other templates that have been used are amyloid like fibrils [112, 113], self assembled peptides [114], viruses and bacteriophages [115, 116] and actin filaments [117]. All these templates have advantages and drawbacks compared to DNA. Amyloid like fibrils have for instance the advantage of being extremely stable. They can often withstand harsh environment such as high temperature and non-polar solvents. The disadvantages, on the other hand, are the uncontrollable size distribution and lack of recognition ability along the fibrils. Actin filaments have the possibility to perform motion via the use of myosin, but the actin fiber is also much more unstable compared to DNA due to its continuous polymerizing and depolymerizing process. What is gained in flexibility is often lost in stability. As for DNA, none of the above mentioned templates have the ability for electronic conduction. They all need to be functionalized in order to assembly electronic devices and metallization is the most common choice for these materials. For a more

detailed description of amyloid like fibrils functionalized with conjugated polymers, I refer to a thesis written by Anna Herland [118].

4.1 DNA Stretching

In its native state, DNA has a random coiled structure in solution, but can by different methods be stretched out to linear and also aligned geometries. The first report of stretched DNA was from Bensimon et al. 1994 [16]. They used a fluid-flow-assisted molecular combing technique, which also is the most straight forward of the so far reported methods to stretch DNA. The other methods are electrophoretic stretching [119-125] and hydrodynamic stretching [126-130]. Molecular combing has been my choice of stretching method for the work in this thesis and will therefore be treated in more detail than the other methods. The conditions for the different methods are summarized in table 4.1.

Table 4.1: Summary of the conditions and requirements for three different DNA stretching methods.

Method	Stretching force	Surface	End modification	Variables
Molecular combing [16, 131-139]	Surface tension	Hydrophobic, hydrophilic	-	Viscosity, pH, buffer, surface hydrophobicity
Electrophoretic [119-125]	Electric field	Au, Al, beads	Thiols, biotin	Field strength, frequency
Hydrodynamic [126-130]	Shear force	Au, beads, mica	Thiols, biotin	Velocity, viscosity

Both electrophoretic and hydrodynamic stretching usually requires chemical modification of the DNA for anchoring one of the ends to a surface or a bead. The modification can be thiols [125] for anchoring on metals, or biotinylation [122, 124] for anchoring on streptavidin coated surfaces. In case of anchoring to a bead, trapping by optical tweezers is normally used to retain the position for the DNA coupled bead and thereby the position of the stretched DNA [140]. Hydrodynamic stretching is due to shear force on the DNA chain generated by a flow. The DNA is aligned in the direction of the flow and the stretched length is mainly dependent on flow velocity and solution viscosity. Electrophoretic stretching relies on the polarization of the polyanionic backbone of DNA

with the counter ions. The direction of the stretching is parallel to the electric field (DC or AC). An advantage of using these methods can be that it is possible to position the DNA by directing the binding to patterned electrodes or trapped beads.

In molecular combing no modification of the DNA is required. The interaction with the surfaces is driven by either hydrophobicity on hydrophobic surfaces or electrostatic forces when using hydrophilic surfaces. The hydrophobic interaction can be due to that the DNA helix will partly be denaturated when dissolved in a slightly acidic buffer [141]. Especially the ends which will expose their hydrophobic core, which preferentially gives the desired DNA end attachment to a hydrophobic surface of i.e. polystyrene coated glass. However, stretching in TRIS pH 8 buffer has also been reported for hydrophobic surfaces indicating that partial denaturation may not be crucial or that other mechanisms are involved [136, 137]. However, λ DNA (48 502 base pair in length) is often used for DNA stretching and this phage DNA has 12 bases long single stranded sequences in both ends. These sticky ends might be enough for the hydrophobic interaction. The yield of molecular stretching has been shown to be improved by addition of small amount of a divalent ion [130]. The divalent ions added can also allow combing on negatively charged surfaces such as mica and glass slides [132].

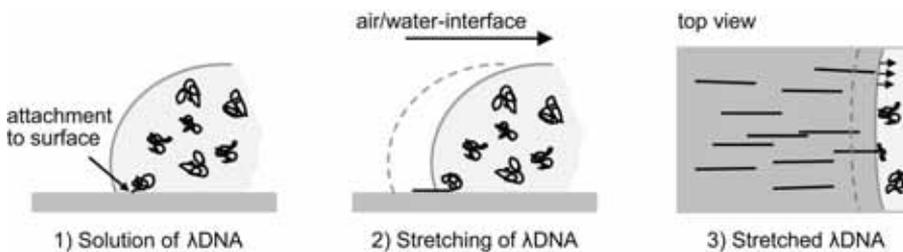


Figure 4.2: Principles of molecular combing. 1) Dispense a solution of DNA on a surface. 2) Create a withdrawing air/water meniscus. 3) DNA is stretched in the direction of the withdrawing meniscus.

The principle for the molecular combing process can be seen in figure 4.2 and an example of the result in figure 4.3. After attachment of one end, via hydrophobic or hydrophilic interaction, the surface tension will force the DNA molecule to stretch along

a withdrawing air-water interface [16]. The stretching follows the direction of the withdrawing meniscus, which has been illustrated very nicely by adjusting the meniscus shape when combing in micro channels [139]. Both ends have during the stretching process the possibility to attach to the surface. If this happens, it will result in a U-shape or a shorter stretching length with completely or partially double thickness (Fig. 4.3b). Increasing the concentration of the DNA can also affect the stretching result. By controlling the concentration, bundles instead of individual DNA molecules can be stretched [142].

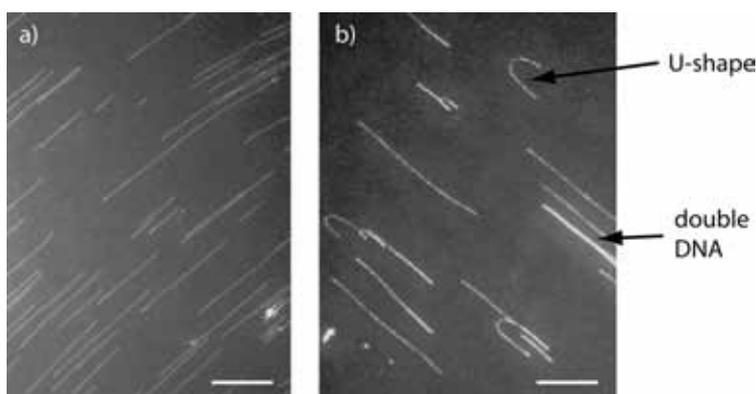


Figure 4.3: Fluorescence images of stretched DNA stained with the intercalating dye YOYO-1 on surfaces. a) Good stretching of individual DNA molecules. b) Example of U-shaped stretching and double or possibly even more multiple DNA stretching. Scale bar: a) 10 μm and b) 8 μm .

The receding meniscus in molecular combing can be obtained by some different methods. A droplet can be blown by a gas stream over a surface as illustrated in figure 4.2 [89, 143]. The meniscus can also be formed in micro channels [139], or just by dipping the surface into a DNA solution [144]. Blowing of a droplet is to prefer due to the easiness and low amount of DNA solution needed. If there is reason to position the DNA the use of micro channels may be preferred.

The surface tension has a strong influence on the length of the stretched DNA. An increase in the surface tension leads to an increased force on the DNA from the meniscus,

resulting in a longer length of the stretched DNA. On hydrophobic surfaces overstretching often occurs, meaning that the DNA molecule is extended beyond the contour length. For λ DNA with a contour length of ca 16,2 μm , 30-50% overstretching to 20-26 μm is not unusual when stretching on hydrophobic surfaces. The force on the DNA is then often >65 pN [134]. A reduction of the force can be achieved by lowering the surface tension. Substrate coating by a layer of 1-dodecanol on PMMA or 7-octenyltrichlorosilane is an example [145], and introduction of glycerol in the stretching medium is another way to lower the surface tension [133]. Polymethylmethacrylate (PMMA) [145], polystyrene (PS) [139, 146], hydrophobic silanes [147] and polydimethylsiloxane (PDMS) [133] are examples of hydrophobic surfaces used for DNA stretching. Examples of hydrophilic surfaces are; aminopropyltriethoxysilane (APTES) [148], glass slides [132] and MICA [130].

4.2 DNA Positioning

One dimensional stretching of DNA is often not enough when it comes to make functional electronic devices. Positioning and directing of well-aligned nanoscale building templates onto desired locations on a substrate is therefore of vital importance and this is a major challenge within the field. Surface patterning and soft lithography approaches are some methods that may overcome this problem to some extent.

4.2.1 Soft Lithography

Soft lithography is the collective name of techniques for transferring pattern by non-photolithographic methods based on an elastomeric stamp or mold [149]. Some of the included techniques are; microcontact printing (μCP), replica molding (REM), microtransfer molding (μTM), micromolding in capillaries (MIMIC) and solvent-assisted micromolding (SAMIM). The group of Whiteside has been a forerunner in the development of soft lithography, and one of the driving forces has been the cost-effectiveness and ease of use.

PDMS (polydimethylsiloxane) is often the choice of elastomeric material. PDMS is a transparent silicone material with a quite low viscosity in its uncured form. The uncured PDMS, a mixture of 10 part PDMS base and one part curing agent, is applied to a master

where it fills up the patterns. After curing, the rubber-like stamp can be peeled off and the replica with inverted pattern of the master is ready to use. The process is described in figure 4.4. The stiffness and properties of the stamp can be changed by varying the ratio or composition of the base a curing agent, adding additives, or using other materials such as hPDMS [150] and agarose gels [151].



Figure 4.4: The basic steps for PDMS stamp fabrication.

Microcontact printing is a flexible and widely used soft lithography method to transfer a pattern to a surface. A wide range of materials have been patterned using μ CP. Among them are; proteins and antibodies [152], DNA [133], alkane thiols [149], polymers [152] and conjugated polymers [153]. In μ CP, the rubber stamp with a relief pattern is incubated in an ink that contains the molecule of interest (Fig. 4.5). The ink is let to dry and the stamp is put in conformal contact with a surface. The molecule of interest is transferred to the surface during the contact and a pattern on the substrate, determined by the topographic pattern of the stamp, is formed.

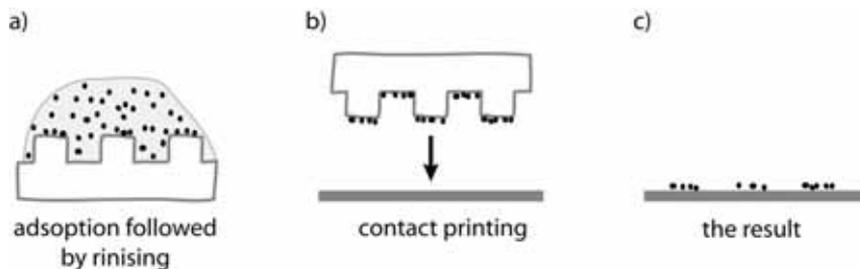


Figure 4.5: Principles for micro contact printing. a) A stamp is incubated in the solution containing the molecule of interest. The molecule is adsorbed to the stamp surface and the solution is rinsed off. b) The stamp is put in conformal contact with the substrate and the molecule of interest is during this process transferred to the substrate. c) The stamp is finally removed and a pattern has been created.

For successful printing, there usually needs to be a difference in the surface energy between the stamp and the substrate [154]. The PDMS stamp is hydrophobic in its cured and unmodified state, but can be made hydrophilic by for instance O_2 plasma treatment. The plasma treatment forms a thin layer of silicon oxides on the surface of the PDMS stamp. The surface energy of the substrate can often also easily be modified. A metal substrate can be modified by forming a self assembled monolayer of a thiol and silicon or glass can be silanized. The surface energy can be tuned by changing the end groups of the thiols and silane. Problems associated with μ CP are conformal contact over large surfaces, and also that residuals of PDMS can be transferred during the printing process of the molecule of interest [155].

4.2.2 Surface Patterning to Direct DNA

Patterning of areas to where molecular combed DNA can attach is one way of restricting the stretched DNA to defined areas. Photolithographic patterning of polystyrene lines [146], self-assembled hydrophobic thiols on gold pattern generated with standard photolithography [156], Scanning-Probe Lithographic patterning of amino terminated silanes [157] has been reported in the literature. The photolithographic step in these approaches is however both time-consuming and expensive alternative for manufacturing microstructures.

A method developed in our group is to use a bare PDMS stamp to transfer a hydrophobic pattern. As mentioned previously, contamination of PDMS residuals can be a problem in μ CP. However, the transfer of PDMS residuals can be turned into an advantage. By using the non cross linked low-molecular-weight species of the PDMS stamp as ink instead of an added ink, a thin and defined layer of PDMS residuals can be created on the surface during the contact time (Fig. 4.6) [155]. This method has been used for defining a hydrophobic pattern to where a conjugated polymer film [158, 159] and protein/polymer complexes [62] can be directed.

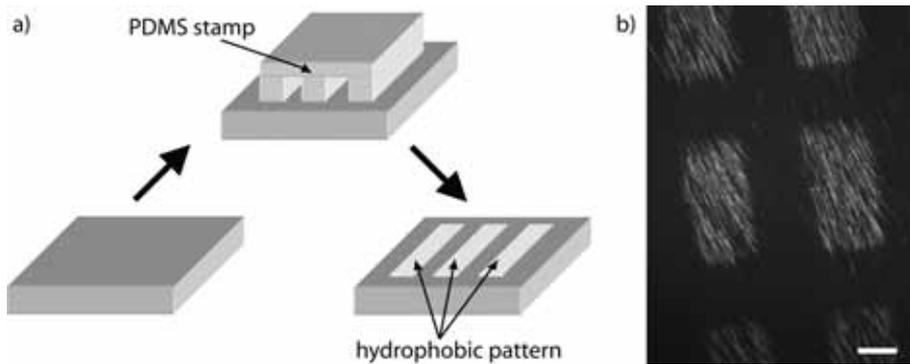


Figure 4.6: a) Surface energy patterning using PDMS stamps. b) Stretched DNA on PDMS energy modified square pattern. Scale bar: 25 μ m.

DNA can be stretched on PDMS surfaces [133, 135, 160, 161] and the surface energy patterning method by μ CP of bare PDMS can thereby offer an easy, quick and reproducible way of directing DNA to confined two-dimensional areas or between lines in a grating pattern as shown in this thesis (Fig. 4.6) [143]. The contact time for the stamp is important for achieving a good transferred PDMS pattern and often only a few minutes of contact gives the best result [unpublished results, Jens Wigenius]. Too long contact time can induce pattern instability and redistribution of the PDMS to create edge effects and discontinuous line patterns.

4.2.3 DNA Printing

An alternative to pattern the surface of the combing substrate is to transfer print the combed DNA by μ CP. The DNA is stretched using molecular combing techniques on bare [133, 135, 143, 161] or surface modified [143, 161] PDMS stamps and then printed to a substrate (Fig. 4.7a).

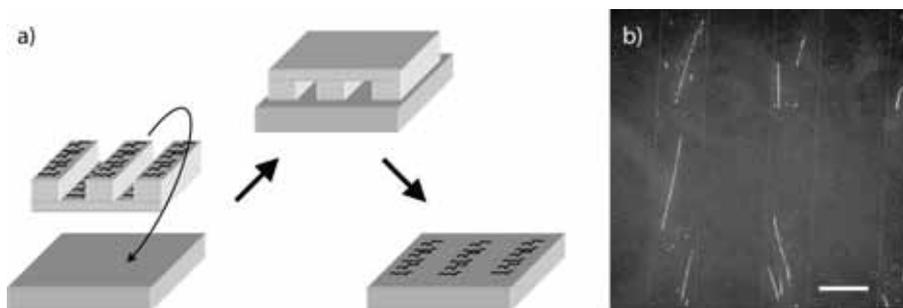


Figure 4.7: a) Schematic sketch of the DNA printing process using PDMS stamps. b) Stretched DNA transfer printed using line patterned PDMS stamp modified with PMMA [143]. Scale bar: 10 μ m.

A distribution in stretching length is often seen, but by altering of the stamp surface with a thin layer of PMMA we have achieved a more uniform stretching length that it close to the maximum overstretched length of the DNA, around 26 μ m in the case of λ DNA [143]. The stamp with the stretched DNA can then be applied to the area of interest. To even further control the positioning, structured stamp surface can be used. Guan and Lee has using this principle shown very nice results in creating highly ordered DNA arrays and crossings [133]. Circular microwells were used to force the DNA to stretch mainly from the outer border of the circle in the stretching direction. We have transfer printed stretched DNA and DNA/CPE complexes using PDMS stamps with rectangular and line pattern resulting in aligned DNA on areas with spacing in between (Fig. 4.7b). The μ CP of DNA results in positioned stretched molecules, but still aligned in only one dimension. However, double transfer printing can be used to achieve crossings [133, 135]. As always with μ CP, complete transfer over large areas can be a problem [135] as well as breakage of DNA chains during the printing process [143].

4.3 DNA Wire Functionalization

Apart from controlling the position, DNA needs to be functionalized with electronic materials in order to assemble functional electronic devices, as intrinsic conduction in DNA does not give the basis for electronics. Various functionalization methods have been suggested. Metal ions have been complexed with DNA chains and reduced to metal particles [132, 162] and sometimes also with further metallization to improve the conducting performance. [17, 163, 164]. The drawback is the thickening of the wire, and a final result around 50 nm is not unusual. Another possibility is to decorate DNA with semiconducting [165] or surface-functionalized metal nanoparticles [137, 166]. Alignment and positioning of DNA nanowires physically sputtered with the, at low temperature, superconducting $\text{Me}_{19}\text{Ge}_{79}$, have also been demonstrated [167]. Immobilization of aniline monomers to a DNA network stretched between two gold electrodes followed by polymerization to polyaniline have been demonstrated to give measurable conductivity [148]. Keren et al. have shown that a more advanced structure, such as a field effect transistor, can be fabricated by direct assembling of carbon nanotubes to DNA in combination with a metallization technique [168]. The same research group has also demonstrated how to use molecular lithography, where a part of the stretched DNA is protected by sequence specific hybridization of a short DNA probe complexed with recombinant protein A, prior to the metallization step.

4.3.1 CPE Functionalization of DNA Wires

We have chosen to functionalize the DNA with conjugated polyelectrolytes. The complexation of CPE with biological polyelectrolytes offers an attractive approach for building materials and devices where the assembly of electronic polymers is controlled by the interactions with macromolecular assemblers, in this case DNA. The CPEs used are POMT, tPOMT and POWT (Fig. 2.4). Unfortunately, these CPEs show very low conductivity and are therefore hard to use in functional devices. However, due to their luminescent properties they function as a good model system with many evaluation possibilities, and the obtained knowledge can be transferred when more appropriate CPEs has been synthesized. Synthesis of PEDOT based CPEs that might have better semiconducting properties are ongoing.

A complex of the conjugated polyelectrolyte and the λ DNA is formed in solution and then stretched into aligned photoluminescent arrays by molecular combing techniques on PDMS energy patterned surfaces. Compared to DNA stretching with bare DNA, the CPEs introduce some new phenomena to take into account (Fig. 4.8). The first is that during the mixing of CPE and DNA, cross linking can occur. In solution, DNA has a random coil structure and the polycationic CPEs can therefore cross link within the same DNA, but also between different DNA molecules. Furthermore, the CPEs can induce polymer aggregation. The result is therefore often stretched bundles of DNA. The continuous coverage along the DNA can also be an issue. You want enough CPE for continuous decoration, but not any excess that creates background and induces unnecessary aggregation. Decoration of the DNA after stretching is an interesting possibility to avoid DNA bundles, but so far the liquid environment and the CPE interaction tend to make the DNA detach from the surface [160].

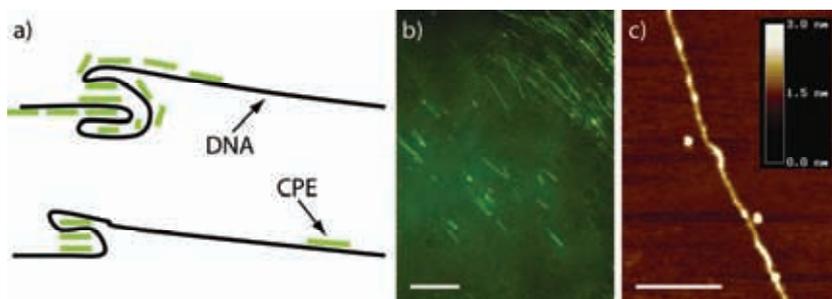


Figure 4.8: a) cartoon showing possible organization of the DNA/CPE complexes; a middle size cluster (top) and a small cluster or an individual chain that can show fluorescence blinking (bottom). Fluorescence image (b) and AFM image of the result (c). Scale bars: 10 μ m (b) and 500 nm (c).

4.3.2 Single Molecular Spectroscopy (SMS) Evaluation

Interaction studies of conjugated polyelectrolyte layers/solutions and DNA has been done using SPR, QCM-D and optical methods and is described in chapter 3. To study the fluorescence of single spots of stretched CPE/DNA complexes on surfaces, we have used a home built system based on a commercial inverted wide-field fluorescence microscope.

The fluorescence is excited with a laser at 458 nm and the fluorescence light is collected by an objective lens and projected onto a CCD camera. The sample can be kept in a home-built vacuum chamber (10^{-2} torr) to increase photostability during the measurements. Imaging of the complexes and recording of their fluorescence spectra are carried out simultaneously by using a holographic grating placed in front of the CCD camera. The distance between the CCD chip and the grating is such that zero-order and first-order of the diffraction appears on the CCD chip at the same time. The zero-order gives an image and the first order gives a spectrum. Selection of a particular place on the sample is done by a vertical slit with adjustable width, placed in an intermediate image plane. An example of the result can be seen in figure 4.9.

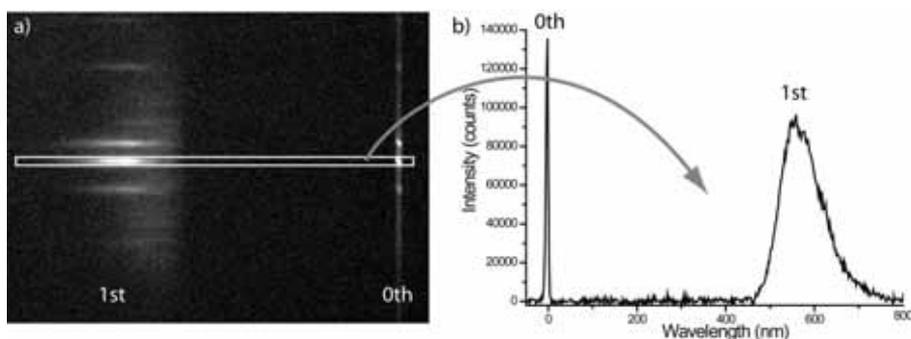


Figure 4.9: a) A SMS image recorded on a CCD. A grating splits the light and the zero-order gives an image and the first-order gives the intensity for different wavelengths. b) The CCD-image translated to a wavelength spectrum of the emission.

The setup is very sensitive and has potential for measuring on single or at least very few emitting molecules. When studying such emitting and spatially diluted polymer species, blinking can occur (Fig. 4.10). The fluorescence blinking or/and stepwise photobleaching [169] shows that those polymer clusters/molecules should be at most 10 nm in size, where 10 nm is the upper limit of Förster energy transfer distance toward a fluorescence quencher in conjugated polymers [170-172]. In the case of figure 4.10, the length of the single CPE chain (POMT) is already about 10 nm. Therefore those blinking spots are most likely individual chains or small aggregates stacking to a size less than 10 nm.

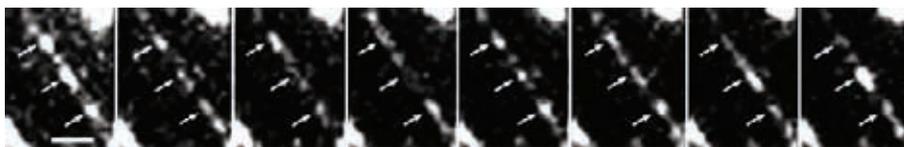


Figure 4.10: Time series of fluorescence images taken with an interval of around 1.5 s and 0.3 s exposure time for diluted concentration of POMT relative to that of DNA. Blinking spots are observed and marked with arrows. Scale bar: 2 μm .

Taking spectra of different parts on a stretched DNA decorated with POMT shows that the spectrum varies along the DNA chain. Low intensity spots tend to have a more blue shifted spectra compared to stronger emitted spots. The brighter spots probably consists of more CPE chains packed closer together, making energy transfer possible to lower energy sites. In the medium and less bright parts, the CPEs are probably more separated and can adopt more of a solution like conformation, consistent with the CPE theories described in chapter 2. The structures in the emission spectra can be enhanced by cooling down the samples to 77K. AFM (atomic force microscopy) images also confirm the variation in size distribution of CPE molecules/clusters along the DNA chain (Fig. 4.9c) [160].

The anisotropy of the CPE binding to DNA can be studied by adding rotating polarizers to the SMS-setup (Fig. 4.11). Introducing polarizers both in the excitation light path and the emission light path gives the possibility to simultaneously record the polarization dependence of the CPE in absorption and emission when attached to stretched DNA. From the measurements, it can be seen that tPOMT has some alignment to the DNA axis. However the modulation depths, $M=(I_{\text{max}}-I_{\text{min}})/(I_{\text{max}}+I_{\text{min}})$, is usually only about 0.2 in both emission and excitation. This can be compared to PTAA decorated on insulin fibrils that can have values up to over 0.9 [112]. We can not see any relation between spectral shape for the emission and size of the polarization, and there seems to be very little effect from cooling down the samples to 77K.

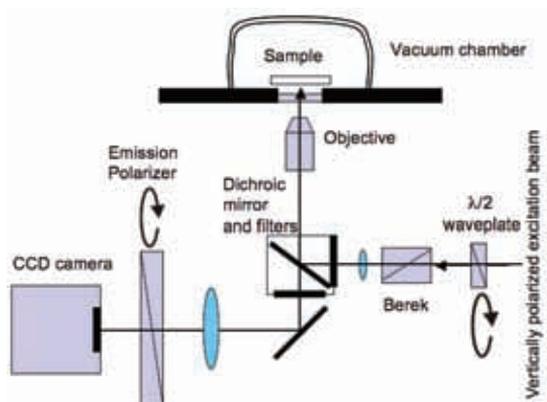


Figure 4.11: SMS-setup with rotating polarizers. The polarizers rotate with different speed to enable simultaneous recording and evaluation of the polarization dependence in emission and absorption.

All the biotemplated electronics experiments shows that a complex of conjugated polyelectrolytes and DNA, formed in solution prior to stretching or after stretching, can be aligned into photoluminescent nanowire arrays as models for conducting nanowires by molecular combing techniques. The positioning of the stretched wires can also be controlled to confined areas by micro contactprinting and energy surface modification methods. Conjugated polyelectrolytes have the advantage that they can form nanowires in the true nano regime, making this approach promising for nanofabrication. Still, there are improvements needed such as more homogeneous polymer distribution and fully individual stretching of CPE/DNA complexes. Binding evaluations also shows that the anisotropy is very low for the DNA/CPE interaction, thus they probably attach as multimolecular ensembles consisting of maybe up to 10 polymer chains.

5 Future Outlook

To continue the research in the interface of biology and conjugated polymers, further understanding of the binding of CPEs to biomolecules is vital. There are still a lot of question marks of how the CPEs function and interact with the surroundings. Methods such as dynamic light scattering (DLS), fluorescence correlation spectroscopy (FCS), single molecular spectroscopy (SMS) in combination with polarization setup and ultracentrifuge can give a deeper understanding both of the photophysical properties and the binding interaction with biomolecules. Comparing different variants of similar and well defined CPEs can also give new knowledge of the interaction mechanism.

5.1 Detecting Biological Targets

The biological detection using CPEs is an enormous area and it is the human mind that sets the limits when it comes to possible applications. Everything from ion detection to in vivo has been done or is ongoing. Superlighting as detection principle is an extra interesting area because it allows very low concentration detection of biological targets. We know that our CPEs forms aggregates in most environments which is a key property for super quenching. DNA hybridization detection is the most straight forward system to test this principle. If it works and can be combined with a chip fabrication method we have the basis for sensitive high throughput screening.

Finding out what the CPE binds to in cells is an interesting and difficult task. Experiments to extract the CPE/cell complex from cell suspension via CPE functionalized magnetic particles are ongoing. After separation of the functionalized particles with the CPE/biomolecular complex from the cell suspension, the complexes are planned to be run in a gel extraction followed by identification by mass spectroscopy.

The staining of live organisms with designed CPEs for in vivo detection of amyloid has also started.

5.2 Biotemplated Electronics

The long term goal is to achieve self-assembly of functional devices where the biomolecules coordinates the electronic material, in our case conjugated polymers. However, this is a very challenging task and so far very few real functional devices been shown in the literature. This area still has many stages to pass before the goal is within reach. Probably, one of the first practical steps will be to use the biomolecule to organize the electronic material in a bulk function for i.e. photovoltaic and transistor setups.

Synthesis of new CPEs is the next step to develop DNA templated electronics. Especially crucial is to have CPEs with the possibility for conductivity measure, and for DNA as template they should preferably have a cationic charge for best interaction. CPEs built up from EDOT based monomers have been done with anionic side chain. A CPE called PEDOT-S with a sulfate group in its side chains is an example of a CPE with very promising properties for amyloid fiber decoration. Of vital importance is also to control polymer-polymer aggregation when complexing the CPE with the biotemplate in solution, without losing the ability to transport electrons between neighboring polymer chains on the final biotemplated material.

One further step for advanced assembly of biotemplates is to combine the DNA addressability with the structural rigidity of amyloid. Functionalization of amyloid fibrils opens the way for making crossings and stacked piles with defined distance between the fibers. The fibers, and possibly also the DNA, should of course be decorated with a CPEs to allow functionality to be incorporated.

We have also started to look into assembly of synthetic peptides as biotemplates [173]. The peptides are designed as a two helix loop and when mixing a negatively charged with a positively charged peptide they form a four helix bundle. By making the peptides to dimerize before mixing, a fiber can be built up by controlled folding (Fig. 5.1). To add crossing functionalities trimers or more complex mer-formations can also be constructed

as well as the introduction of peptide functionalized gold nanoparticles. These peptides are known to interact well with our CPEs and may therefore be an interesting variant for templated assembly [34, 174].



Figure 5.1: Schematic illustration of peptide fiber built up by two dimer peptides that form helix bundles with each other, and a gold nanoparticle functionalized with a peptide as a possible route for making crossings.

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