Ionic Circuits for Transduction of Electronic Signals into Biological Stimuli

Klas Tybrandt

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

Cover by Klas Tybrandt

In memory of Ann Lindén

Abstract

Modern electronics has revolutionized the way information is processed and stored in our society. In health care and in biology it is of great interest to utilize technology to regulate physiology and to control the signaling pathways. Therefore, the coupling of electronic signals to biological functions is of great importance to many fields within the life sciences. In addition to the conventional inorganic electronics, a new branch of electronics based on organic materials has emerged during the last three decades. Some of these organic materials are very attractive for interacting with living systems since they are soft, flexible and have benevolent chemical properties.

This thesis is focused on the development of ionic circuits for transduction of electronic signals into biological stimuli. By developing such an intermediate system technology between traditional electronics and biology, signals with chemical specificity may be controlled and addressed electronically. First, a technology is described that enables direct transformation of electronic signals into ionic ones by the use biocompatible conductive polymer electrodes. The ionic bio-signals are transported in lateral channel configurations on plastic chips and precise spatiotemporal delivery of neurotransmitter, to regulate signaling in cultured neuronal cells, is demonstrated. Then, in order to achieve more advanced ionic circuit functionality, ion bipolar junction transistors were developed. These ion transistors comprise three terminals, in which a small ion current through one terminal modulates a larger ion current between the other two terminals. The devices are functional at physiological salt concentrations and are utilized to modulate neurotransmitter delivery to control Ca^{2+} signaling in neuronal cells. Finally, by integrating two types of transistors into the same chip, complementary NOT and NAND ion logic gates were realized for the first time. Together, the findings presented in this thesis lay the groundwork for more complex ionic circuits, such as matrix addressable delivery circuits, in which dispensing of chemical and biological signals can be directed at high spatiotemporal resolution.

Populärvetenskaplig Sammanfattning

Den moderna elektroniken har revolutionerat vårt sätt att hantera information och banat väg för dagens informationssamhälle. Elektronik används även i stor utsträckning till att styra och reglera olika processer genom att inhämta och behandla data, för att sedan sätta lämpliga styrparametrar. Biologiska system bearbetar information genom att sammanväva en mängd komplexa signalvägar. Inom sjukvården och livsvetenskaperna finns ett stort intresse av att utnyttja modern teknologi till att styra fysiologiska förlopp och biologiska signalvägar. Därför är det av stor vikt att utveckla teknik för att koppla ihop elektriska signaler med biologiska funktioner. Parallellt med utvecklingen av den konventionella elektroniken har en ny typ av elektronik tagits fram som är helt baserad på organiska material. Denna organiska elektronik är mycket attraktiva för tillämpningar inom biologin, då materialens kemiska beståndsdelar, mjukhet och flexibilitet är mera lika biologiska material än vanlig hård elektronik. I dagsläget har organisk elektronik med framgång används till att utläsa och skapa elektriska signaler i vävnader. Elektrisk interaktion har dock sina begränsningar, både med avseende på vävnadstyper och specificitet.

Denna avhandling beskriver utvecklingen av jonkretsar och hur dessa kan användas för att översätta elektroniska signaler till biologiska stimuli. Tanken är att använda jonkretsar, vars laddningsbärare är kemiskt specifika joner och laddade biomolekyler, som en brygga mellan vanlig elektronik och biologiska system. På så sätt kan elektroniska signaler översättas till biologins egna signaler. I första delen av avhandlingen utvecklas passiva jonkretsar, med elektriskt ledande polymerelektroder, för kontrollerad leverans av stimuli. Jonkretsarna används för att styra cellsignalering i nervceller genom att leverera neurotransmittorn acetylkolin till cellerna. Vidare används jonkretsarna till att skapa amyloida proteinaggregat, som är av intresse inom Alzheimerforskning. För att kunna konstruera mer avancerade jonkretsar krävs aktiva komponenter, så som transistorer. I avhandlingens andra del utvecklades därför bipolära jontransistorer, en ny typ av transistor som kan modulera transporten av joner och laddade biomolekyler. Två typer av jontransistorer utvecklades, en sort som transporterar positiva joner och en

annan som transporterar negativa joner. Jontransistorerna fungerar i de höga saltkoncentrationer som förekommer i kroppen och det är första gången som detta rapporterats. Som en demonstration på att tekniken funderar ihop med biologiska system så används transistorena till att modulera kalciumkoncentrationen i odlade nervceller. I nästa steg byggdes logiska jongrindar, som är grundstenarna för mer komplexa jonkretsar. Genom att utveckla dessa grindar öppnas en rad nya möjligheter, t.ex. bör det gå att utveckla adresserbara leveranskretsar för laddade biomolekyler baserat på resultaten som presenterats i denna avhandling.

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List of Included Papers

Paper I

Translating Electronic Currents to Precise Acetylcholine-Induced Neuronal Signaling Using an Organic Electrophoretic Delivery Device

Klas Tybrandt, Karin C Larsson, Sindhulakshmi Kurup, Daniel Simon, Peter Kjäll, Joakim Isaksson, Mats Sandberg, Edwin Jager, Agneta Richter-Dahlfors and Magnus Berggren

Contributions: Design and fabrication of devices, large part of device characterization and analysis of data, performed simulations. Wrote large part of the first draft and contributed to the final editing of the manuscript.

Paper II

Spatially Controlled Amyloid Reactions Using Organic Electronics

Erik O Gabrielsson, Klas Tybrandt, Per Hammarström, Magnus Berggren and Peter Nilsson

Contributions: Design of devices, part in analysis of data, performed simulations. Contributed to the first draft and to the final editing of the manuscript.

Paper III

Ion bipolar junction transistors

Klas Tybrandt, Karin C Larsson, Agneta Richter-Dahlfors and Magnus Berggren

Contributions: All experimental work except cell experiments. Wrote large part of the first draft and contributed to the final editing of the manuscript.

Paper IV

Toward Complementary Ionic Circuits: The npn Ion Bipolar Junction Transistor

Klas Tybrandt, Erik O Gabrielsson and Magnus Berggren

Journal of the American Chemical Society, **2011**, 133(26), 10141-10145.

Contributions: All experimental work. Wrote the first draft and contributed to the final editing of the manuscript.

Paper V

Logic gates based on ion transistors

Klas Tybrandt, Robert Forschheimer and Magnus Berggren

Nature Communications, **2012**, 3(871).

Contributions: All experimental work. Wrote the first draft and contributed to the final editing of the manuscript.

Related Work Not Included in the Thesis

Papers

Organic electronics for precise delivery of neurotransmitters to modulate mammalian sensory function.

Daniel T Simon, Sindhulakshmi Kurup, Karin C Larsson, Ryusuke Hori, Klas Tybrandt, Michel Goiny, Edwin W H Jager, Magnus Berggren, Barbara Canlon and Agneta Richter-Dahlfors

Nature Materials, **2009**, 8(9), 742-746.

Ion diode logics for pH control

Erik O Gabrielsson, Klas Tybrandt and Magnus Berggren

Lab on a Chip, **2012**, 12, 2507-2513.

Patent Applications

Electrically controlled ion transport device 1

EP 1862799 (A1)

Electrically controlled ion transport device 2

EP 2232260 (A1)

Electrically controlled ion transport device 3

EP 2265325 (A1)

Selective ion transport device

WO 2010119069 (A1)

Table of Contents

1. Introduction

1.1. From Electronics to Organic Bioelectronics

In 1771 Luigi Galvani discovered that an electric spark could induce movement in the detached legs of a dead frog. Since then our understanding of electricity and biological systems has increased dramatically. The modern era of electronics was born in December 1947, when John Bardeen and Walter Brattain constructed the first transistor, a germanium-based point contact bipolar junction transistor.[1] Their research in inorganic semiconductor devices was considered of such importance that they together with William Shockley were awarded the Nobel Prize in Physics 1956. The progress in developing devices and circuits based on their achievements was rapid and within only a few years milestones such as the first integrated circuit (Kilby and Noyce, 1958-59) and the MOSFET (Atalla and Kahng, 1959) were reached. Together, these discoveries laid the groundwork for today's information society, as transistor circuits are the foundation of all modern electronics. During the same period of time the understanding of living systems at the molecular level started to develop, represented by the double-helix model of DNA proposed by James D. Watson and Francis Crick in 1953.[2] As the fields of electronics and biotechnology progressed there was interest in transducing signals between the electronic and biological domains. To date, a vast number of devices have been constructed to accomplish this, including pacemakers and neuronal electrodes.

Along with the development of modern electronics, polymers - commonly referred to as plastics - became an integrated part of our technology and are nowadays found in almost every product. Polymers consist of small building blocks (monomers) and by modifying the chemical composition of these monomers the polymers chemical, mechanical and thermal properties can be tailored to fit different needs. Traditionally, all polymers were regarded as electrically insulating materials. However, in 1977 Alan J. Heeger, Alan G. MacDiarmid and Hideki Shirakawa reported that conjugated polymers could be made electrically conductive via chemical doping (which rewarded them the Nobel Prize in Chemistry year 2000).[3,4]

This was the starting point for the field of organic electronics which has flourished in recent decades, much due to the possibility to achieve desired optical and electrical properties by tailor-making conjugated polymers.[5] Also, conjugated polymers allow for inexpensive processing from solution, which enables low-cost and high volume manufacturing using common printing techniques. To date most efforts have been devoted to the development of light-emitting diodes[6], organic solar cells[7] and thin-film transistors[8]. However, electrochemical devices base on conjugated polymers such as transistors[9], light-emitting electrochemical cells[10] and biosensors[11] have also received significant attention. The electrochemical properties of conjugated polymers make them attractive for conversion between electronic and ionic signals in bioelectronic applications, *e.g.* in neuronal electrodes[12]. Additionally, conjugated polymers can be soft, flexible, electronically and ionically conductive and biocompatible[13], which makes them suitable for interaction with biological systems.

1.2. Aim and Outline of the Thesis

The interaction with biological systems can be divided into two main categories; sensing and actuation of various physiological and neuronal processes. In order to regulate biological systems, both functions have to be available so that the proper actuation can be applied based on the sensor input. Such feedback-controlled systems are expected to generate many new applications and therapies in the future. Conjugated polymers have been identified as an attractive group of materials for interfacing electronics with biological systems. Sensors based on conjugated polymers are well explored, including both field effect and electrochemical sensors.[11,14] Actuation can be achieved by either neuronal electrodes[12,15], which induce local electric fields, or by the delivery of specific biologically active substances. The latter is a relatively more challenging problem, since an adequate amount of a substance has to be stored in a close proximity of the target and then be released upon an electric stimulus. The aim of this thesis is to develop electronically controlled delivery devices based on the attractive properties of electrically conducting polymers. In previous studies conjugated polymer drug delivery electrodes[16-19] have been explored to modulate the release of trapped molecules from within the electrodes. These devices operate in the time interval of minutes to several hours and are typically better suited for therapeutic use than for fast electronic signal transduction. In this thesis an alternative delivery route is presented, in which chemical messengers are transported laterally through ion exchange layers. First passive ionic circuits are reported. Then, the thesis covers the

development of active ionic circuit elements (ion transistors) and finally the construction of ion logic circuits is reported.

The first part of this thesis gives the background information necessary to understand the achieved scientific results and to put them into context. The introduction describes conjugated polymers, ion transport processes, ion exchange membranes, bioelectronic devices and the devices developed in this thesis along with the employed fabrication methods. Finally, an outlook towards future work is provided where my findings presented in this thesis are discussed with respect to possible further developments.

In the second part of the thesis, the main results are presented in five separate papers:

In papers I and II, delivery circuits based on conjugated polymers and cation selective layers are described. These circuits provide precise delivery of ions and neurotransmitters, which was utilized to regulate neuronal cell signaling and to control amyloid reactions. The devices were characterized and the spatiotemporal delivery was evaluated.

In papers III and IV, the development of ion transistors is reported. These devices provide active modulation of ionic currents. Nonlinear circuit elements, such as transistors, are necessary in order to achieve more advanced circuit features, like addressability of substance delivery. The reported ion bipolar junction transistors (IBJTs) are constructed according to the electronic bipolar junction transistor principle. Here, cation- and anion-selective layers are used instead of *p*- and *n*-doped semiconductors, respectively. Both the fabrication protocols and the device characteristics of the *pnp*- and *npn*-IBJTs are reported.

In paper V, the IBJTs are integrated into ion logic gates of both *npn*- and complementary type. The NOT and NAND gates were realized and characterized. It was found that the NOT gates exhibit proper gain characteristics. This critical feature is a requirement for further construction of more complex circuits.

2. Conjugated Polymers

Polymers consist of a large number of sub-units, monomers, which are linked together by covalent bonds. The properties of a polymer depend on the chemical nature of the monomer and also on how the monomers are bound together. In conjugated polymers, the polymer backbone comprises alternating double and single bonds, which enables electrical conduction and absorption/emission at visible wavelengths. Therefore, conjugated polymers are often referred to as conductive or electronic polymers.

2.1. Molecular and Electronic Structure

2.1.1. Orbitals

Atoms consist of a dense positively charged nucleus surrounded by a cloud of negatively charged electrons. The laws of quantum mechanics restrict the electrons to only occupy certain allowed states, which are described by a combination of quantum numbers. These states, referred to as atomic orbitals, define the probability of finding an electron at a certain location in space. Only two electrons of different spin (up or down) can occupy each orbital and in the electronic ground state of an atom the electrons occupy the orbitals with the lowest energy. The orbitals are grouped into shells based on their energy levels. The two most important atomic orbitals for organic materials are the *s* and *p* orbitals (Figure 2.1a).
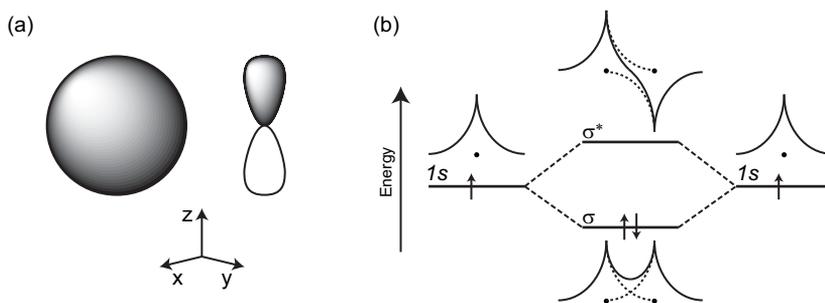
Figure 2.1 Atomic and molecular orbitals. (**a**) The *2s* (left) and *2p^z* (right) atomic orbitals. (**b**) The overlap of two *1s* atomic orbitals creates two molecular orbitals, one bonding orbital (σ) and one anti-bonding orbital (σ*).

The electrons of the outermost shell, the valence electrons, start to interact when two atoms are brought close together. This interaction can be described by molecular orbitals, which can be approximated by the linear combinations of the atomic orbitals. The interaction can be either constructive, *i.e.* a bonding molecular orbital with lower energy is formed, or destructive when an anti-bonding orbital with higher energy is generated (Figure 2.1b). A stable bond between the two atoms is formed if the total energy of the occupied molecular orbitals is lower than the energy of the two separate atoms. When electrons are shared between atoms it is referred to as covalent bonds, with the notation σ for coaxially symmetric bonds and π for non-coaxially symmetric bonds.

2.1.2. Bonds in Conjugated Polymers

The key building block of organic polymers is the carbon atom because of its ability to form four covalent bonds to neighboring atoms. In the electronic ground state of the carbon atom the *$1s^2 2s^2 2p^2$* orbitals are occupied, but this configuration is not present once covalent bonds are formed. When binding to other atoms the electrons of the carbon atom occupies a set of hybrid orbitals, *i.e.* a set of linear combinations of the atomic orbitals. In the *sp^3* hybridization the hybrid orbitals form a tetrahedral-shaped structure where each orbital forms a single σ-bond to a neighboring atom. The *$sp^2 p_z$* hybridization is composed of three hybrid orbitals oriented in the same plane, which form σ-bonds, and one *p*-orbital orthogonal to the plane which forms a π-bond. When both a σ-bond and a π-bond are formed between two atoms it is referred to as a double bond. Finally, in the linear *sp* hybridization two σ-bonds and two π-bonds are formed.

The backbone of a conjugated polymer consists of $sp^2 p_z$ hybridized carbon atoms, which form alternating single and double bonds. All σ-bonding molecular orbitals are occupied while all the σ* (anti-bonding) molecular orbitals are empty. The electrical and optical properties of these materials originate to a large extent from the arrangement and occupation of the π orbitals. The p_z atomic orbitals of all carbon atoms are oriented perpendicular to the chain and overlap, therefore the electrons in these orbitals are delocalized along the chain. The number of molecular π and π* orbitals is equal to the number of carbon atoms, thus energy levels split up with increasing chain length. (Figure 2.2). For long chains the difference between energy levels becomes so small that the levels may be treated as continuous bands. In polyacetylene the highest occupied molecular orbital (HOMO) is the top of the π band while the lowest unoccupied molecular orbital (LUMO) is the bottom of the π* band. If all bond lengths were equal in polyacetylene, the π and π* bands would have form a continuous band and the material would appear as a quasi-metal. However, this is not the case sine Peierl's theorem state that this configuration is not energetically stable. Instead, the bond length alternates between double and single bonds, which creates a band gap E_g between the HOMO and LUMO levels. A material with a relatively small band gap is referred to as a semiconductor, thus conjugated polymers are organic semiconductors with typically $E_g \in [1,4]$ eV.[20]
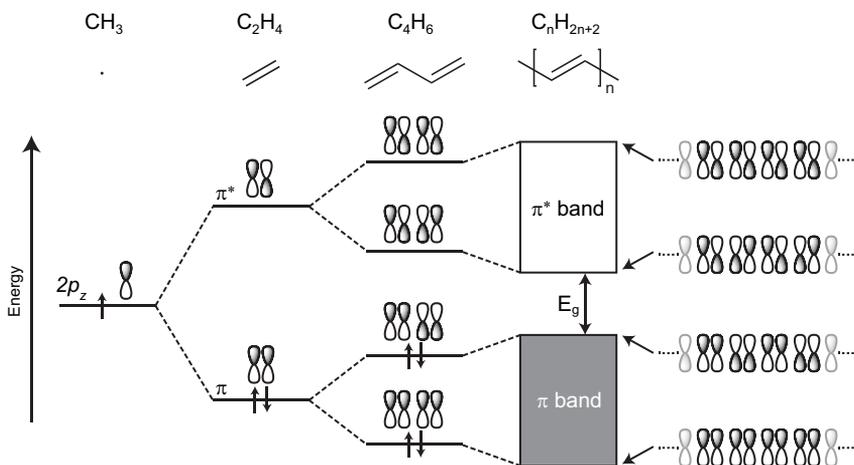
Figure 2.2 Energy level splitting and band formation in polyacetylene. With increasing chain length the number of π orbitals increase and the energy difference between neighboring orbitals decrease. For long chains π and π* energy bands form, separated by the bandgap energy E_g. The bandgap is a consequence of the alternation in bond length along the chain.

2.2. Charge Carriers and Doping

Charge carriers in conjugated polymers are introduced by removal/addition of electrons from/to the polymer chain. The introduced charge locally distorts the electronic and geometric configuration of the polymer chain and thereby creates a charged quasiparticle called polaron (soliton in the special case of a degenerate ground state). The distorted phase, the so-called quinoid form, of the polymer has a relatively higher energy per monomer as compared to the non-distorted aromatic form. Therefore, polarons are confined to a few monomer units (Figure 2.3). Two polarons may form a bipolaron, a configuration that sometimes is energetically more favorable. In an electric field, polarons can move easily along the conjugated chain, however in most devices the length of charge transport is typically on the order of micrometers. This implies that charges normally have to travel far beyond the length of a single polymer chain. Charge transport between chains is mediated by charge hopping and this is often the limiting factor for charge mobility in conjugated polymer thin films. Activation energy for hopping can be suppressed by organizing the polymer chains in a structured phase. Well-ordered materials with a high degree of crystallinity typically have a higher mobility than amorphous polymers.[21]
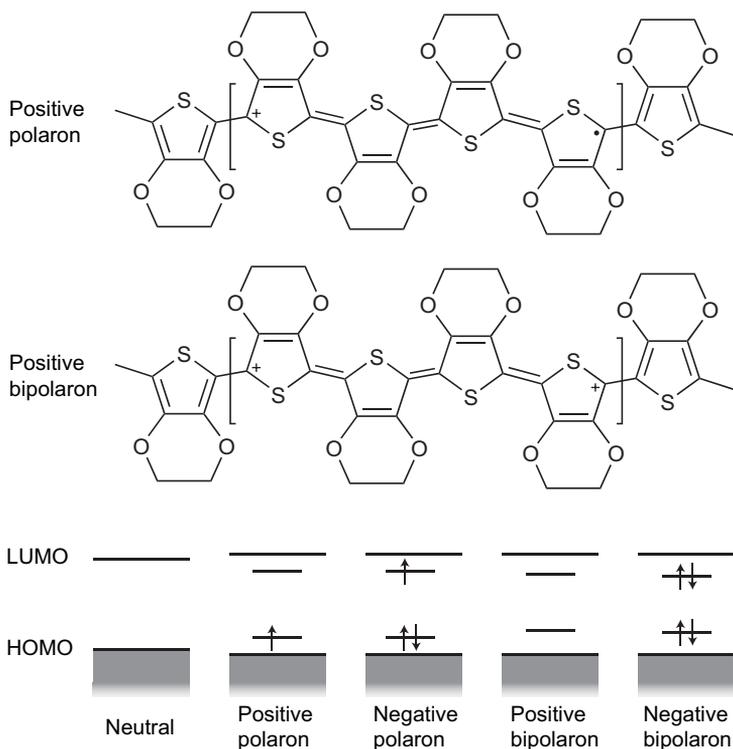
Figure 2.3 Polarons in conjugated polymers. In poly(3,4-ethylenedioxythiophene) (PEDOT) positive polarons and bipolarons are formed. Generally, both positive and negative polarons can form in conjugated materials, however the stability of these polarons varies greatly.

Since the band gap of most conjugated polymers is rather large, typically 2-3 eV,[22] there are few thermally excited charge carriers, which then results in a low conductivity. The conductivity can be improved by several orders of magnitude by introducing charge carriers by the process of doping, in which polarons are compensated by ions of the opposite charge. In chemical doping charge carriers are created in the conjugated polymer as the dopant molecules are ionized.[23] For positive polarons this is called oxidation or *p*-doping of the polymer while it is called reduction or *n*-doping for negative polarons. In electrochemical doping the conjugated polymer is in contact with a solution containing ions (electrolyte). As a voltage is applied between the polymer and a counter electrode immersed into the solution, electric charge carriers are injected into the polymer and ions will go into the film to compensate for the electric charge.[23,24] When synthesized, conjugated polymers can be prepared into a doped or undoped form, depending on the polymerization process and the properties of the specific polymer.

2.3. Conductive Polymer Electrodes

2.3.1. Doping Ions

The ability of changing the doping level of conjugated polymer electrodes is utilized in many applications, *e.g.* in supercapacitors[25,26], electrochromic displays[27,28] and in neuronal electrodes[12,15]. As the redox state of the polymer is changed, *i.e.* the doping level is controlled, ions move in or out of the conjugated polymer material. In a pure conjugated polymer film the mobile ionic species are predominantly of the opposite charge with respect to the electronic charge residing on the chains, however salt may also be present within the film (Figure 2.4).[23] When a conjugated polymer is blended with an excess of a large immobile ion or a polyelectrolyte, the mobile ionic species in the film are predominantly of the same charge as the electronic charge (Figure 2.4).[29] These different situations can be utilized to achieve the desired functionality of the electrode.
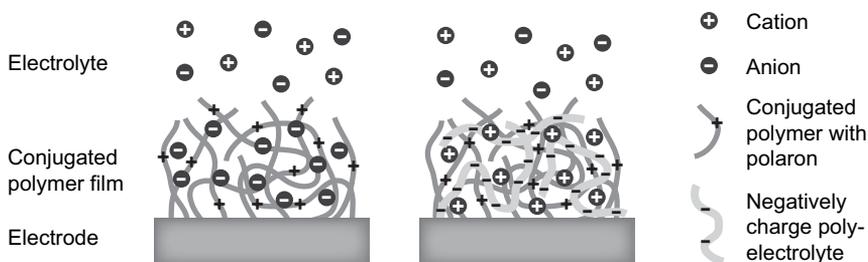
Figure 2.4 Partially doped conjugated polymer electrodes. In a pure conjugated polymer film (left) the polarons are compensated by ions of the opposite charge. In a conjugated polymer film which comprises an excess of a polyelectrolyte (right), the polarons are compensated by the fixed charges on the polyelectrolyte chains.

2.3.2. Energy Levels

When the counter electrode potential lies within the band gap of an undoped conjugated polymer, the applied potential, between the polymer electrode and the counter electrode, has to be above a certain threshold for *p*- or *n*-doping to occur.[22] In a partially doped system, however, the doping level changes continuously with the applied potential. Therefore, redox processes can occur for much smaller applied potentials, although the reaction rate typically increases when potentials close to the band gap edges are applied.[22] Highly doped systems often behave in a capacitive-like manner. The charging and discharging of a polymer can be studied in a cyclic voltammetry measurement set-up, in which the current through the polymer is monitored as the applied potential is cycled between two boundary potentials (Figure 2.5). This

measurement gives information about the band gap of the polymer as well as the kinetics involved in the redox processes. Conjugated polymers are attractive coatings on metal electrodes for various sensing, battery and capacitor applications. The materials dramatically increase the charge capacity per unit area of the electrode, since the entire bulk of the film is involved in electrochemical doping.[30] In many biological applications, it is desirable to only polarize the electrode to avoid any potentially toxic electrochemical reaction products.[31] With a high charge capacity electrode this can be achieved at the same time as sufficient current is passed through the electrode.
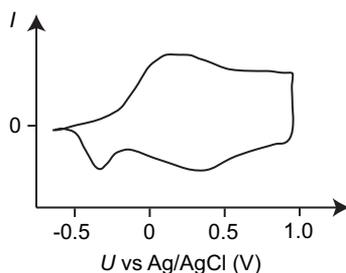
Figure 2.5 A typical cyclic voltammogram of the partially oxidized conjugated polymer PEDOT:PSS. At -0.5 V vs Ag/AgCl the doping level is low. Above 0 V the current magnitude is rather constant during the sweep, thus the electrode is capacitive in this regime.

3. Electrolytes and Ion Exchange Membranes

An electrolyte is a substance that contains free mobile ions which renders it electrically conductive. The ions usually originate from a salt which dissociates in a solvent, resulting in the ionic concentrations c_i where i denotes the different ionic species. Electrolytes are often liquids, but may also come in the form of gels or solids. The conductivity depends on the concentration of ions and how easily the ions move in the system (the mobility, μ_i).

3.1. Transport Processes

The most common theoretical description of transport phenomena is knows as the thermodynamics of irreversible processes, in which phenomenological equations are derived from the entropy production within the system.[32] By various approximations one arrive at the set of equations typically used in practice. The process is separated into movement of fluid (convection), transport due to concentration differences (diffusion) and transport in electric fields (migration). The equations of each kind of process are then coupled together to yield the final description.

3.1.1. Convection

In electrochemistry and membrane science the transport that occurs by the movement of fluid is usually labeled convection. In other fields of science the term advection is used instead. Convection can be a desired or an undesired thing, depending on the application. Controlled convection can help to establish stable concentration gradients at electrodes[33] or serve as the mechanism that provide substance delivery, *e.g.* in microfluidic systems[34]. However, in systems where fluid motion is undesired, convection arising from temperature differences, concentration differences or ionic currents may disturb the concentration gradients within the system.[35] A common approach to prevent convection is to use a highly viscous polymer gel to prevent the motion of fluid.

3.1.2. Diffusion and Migration

The flow of molecules (or particles) from high concentration to lower concentration is called diffusion. For electrically neutral molecules in ideal mixtures the diffusion flux density j (mol m^{-2}s^{-1}) is described by Fick's law:

$$j = -D\nabla c$$

where D is the diffusion coefficient (m^{2}s^{-1}) and c is the concentration. Ions and charged molecules can in addition to diffusion also be transported under the influence of an electric field, *i.e.* by migration. The flux density j_i of the ionic species i is given by the Nernst-Planck equation:

$$j_i = -D_i\nabla c_i + z_i D_i c_i f\nabla\phi \qquad (1)$$

where D_i is the ionic diffusion coefficient, z_i is the electric valence, $f = F/RT$ and ϕ is the electric potential. The first part of the equation describes the diffusion contribution and the second part the migration contribution to the transport. Even without an external electric field the difference in diffusion of the ionic species gives rise to an internal electric field, which couples the transport of the different species. Eq. 1 assumes negligible deviation from the Nernst-Einstein relation $D_i = \mu_i RT/F$ and neglects the concentration dependence of the activity coefficient. The time dependence of the system is described by $\dfrac{\partial c_i}{\partial t} = -\nabla j_i$.

3.1.3. Poisson's Equation and the Electroneutrality Assumption

The Poisson's equation of electrostatics relates the variation in electric field to the charge concentration of the media:

$$\nabla^2\phi = \frac{F}{\varepsilon}\sum_i z_i c_i$$

A common assumption when solving ion transport equations is the electroneutrality assumption, which is the special case of a negligible variation in the electric field:

$$\sum_i z_i c_i = 0$$

The electroneutrality assumption is usually a good approximation in the bulk of electrolytes, however, the situation at electrolyte interfaces is typically completely different.

3.1.4. Electrode Interfaces

A difference in electric potential between an electrode and an electrolyte causes the formation of a charged interface region; the electric double layer (EDL). Throughout the EDL electric charges and ions are polarized along the surface of the electrode.[33] According to the Goüy-Chapman-Stern model the EDL is divided into two layers; the Helmholtz layer next to the electrode and the diffuse double layer further out from the electrode (Figure 3.1). The Helmholtz layer consists of solvent molecules adsorbed onto the electrode surface and partially solvated ions of the opposite charge of the electrode. The diffuse double layer consists of both cations and anions, but with an excess of the ionic species of the opposite charge of the electrode.
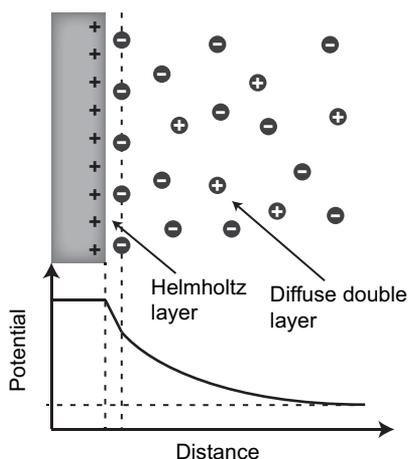
Figure 3.1 Electric double layer at the electrode-electrolyte interface. The thin Helmholtz layer comprises partially solvated ions and the potential decreases linearly in this region. In the diffuse double layer there are more anions than cations and the potential decreases exponentially.

When an electric potential is applied to a metal electrode two different situations can occur. If the electrolyte contains no electrochemically active species at the specific potential, an EDL is formed. The EDL is then capacitive in nature and once it is formed no further current flows through the system. The electrode is said to be polarizable. In the presence of electrochemically active ions or molecules the EDL is once again formed but the current remains finite due to electrochemical reactions at the electrode interface. Often a low concentration of an electrochemically active species is dissolved in an inert supporting electrolyte of higher concentration. In this situation the EDL is formed and the electric field is screened by the

supporting electrolyte.[32] This causes the current to be limited by diffusion of the reactive species; thus the frequent discussions about diffusion and its limitations in electrochemical reactions.

3.2. Electrolytes
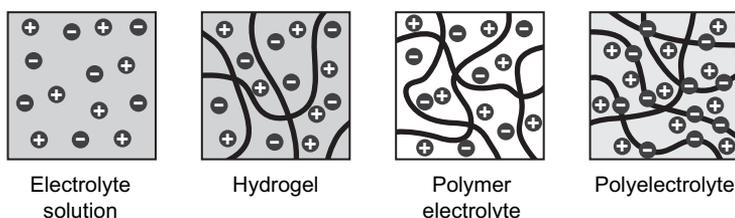
Figure 3.2 Different kinds of electrolytes. Electrolytes come in many forms, as salt can be dissolved in a liquid solvent (grey), a gelled liquid or in a polymer host. The salt may also be a polymer in itself, which is the case for polyelectrolytes.

3.2.1. Electrolyte Solutions

Electrolyte solutions are the most common type of electrolyte in which salt is dissolved in a liquid. Electrolytes, which are completely dissociated independently of concentration, are termed strong electrolytes, while partially dissociated electrolytes are called weak electrolytes. Organic solvents are sometimes preferred due to their electrochemical stability, however in biological applications water is typically used because of its natural presence in all biological systems. All dissociated ionic species are mobile in an electrolyte solution and thus contribute to the conductivity. For strong electrolytes, the conductivity typically increases linearly with the salt concentration until inter-ion interactions become so strong that the mobility of the ionic species decreases.[33] Water is a weak electrolyte in itself as it dissociates into hydronium and hydroxide ions to a concentration of approximately 0.1 µM under neutral conditions.

3.2.2. Hydrogels

In many applications, it is not convenient or possible to work with liquid electrolytes. An alternative is then to blend an aqueous electrolyte with a small fraction of a polymer which crosslinks into a hydrogel. The mechanical properties of a hydrogel can be tuned to fit certain requirements while retaining high ionic conductivity. There are numerous bio-applications based on hydrogels, *e.g.* soft cell culture scaffolds[36] or drug delivery carriers[37].

3.2.3. Polymer Electrolytes

Polymer electrolytes are solvent free systems in which salt can be dissolved. One of the most commonly used polymer electrolytes is poly(ethylene glycol) (PEG). The ion transport is coupled to the chain movement of the polymer

and therefore often require higher temperatures to be efficient. However, with the addition of water PEG acts as a viscous polymer solution.[38,39]

3.2.4. Polyelectrolytes

A polymer with fixed salt groups along the backbone, which can dissociate in a polar solvent, is called a polyelectrolyte (Figure 3.2). Polyelectrolytes are usually soluble in water and produce viscous solutions at high concentrations. A positively charged polyelectrolyte is called a polycation while the negatively charged equivalent is called polyanion. The vast difference in size between the polyelectrolyte chains and their counter ions give rise to unique charge transport properties. In the semi dry state the polymer chains are effectively immobile while some of the smaller counterions still can move. This feature can be exploited in electrolyte gated organic field effect transistors to suppress electrochemical doping of the semiconductor.[40,41]

3.3. Ion Exchange Membranes

3.3.1. Composition

Ion exchange membranes (IEMs) are polyelectrolytes, which form a membrane phase in solvents that dissociate their ionic groups. The membrane phase is kept intact by hydrophobic forces and/or crosslinking between the polyelectrolyte chains.[42] When placed in aqueous solution the IEMs water uptake is typically 10-40 % and the concentration of fixed charges inside the membrane is usually 1-3 M.[42] Mobile ions of opposite charge to the polyelectrolyte chains are called counterions while ions of the same charge as the chains are called coions. In most cases, anion exchange membranes are amines while cation exchange membranes are sulfonates.

3.3.2. Selectivity

The high density of fixed ionic groups in the membrane electrostatically repels coins from entering into the membrane. This Donnan exclusion is effective as long as the concentration of the electrolyte surrounding the membrane is well below the concentration of fixed charges. Thus, for low to moderate electrolyte concentrations predominately counterions can move inside the membrane phase and therefore IEMs are also called cation/anion selective membranes. As the electroneutrality condition holds within the membrane phase, the concentration of counterions approximately equals that of the fixed charges inside the IEM, independently of the surrounding electrolyte concentration. Though, this is valid only up to a certain maximum electrolyte concentration. This is an attractive feature for some applications as

the transport and conductivity of the membrane is well defined. The fraction of counterions (c_1) inside a membrane immersed in a binary electrolyte is approximated by[32]:

$$\frac{c_1}{c_1 + c_2} = \frac{1}{2} + \frac{1}{4\sqrt{\frac{1}{4} + \left(\frac{c_w}{X}\right)^2}} \quad (2)$$

where c_2 is the coion concentration, c_w is the electrolyte concentration and X is the fixed charge concentration. For $X/c_w = 10$ the counterion fraction is predicted to be 99% (eqn. 2). In an ideal membrane the fraction is 100 %, however in commercial applications the fraction rarely exceeds 97%.[42]

3.3.3. Electric Potentials and Concentration Polarization

The fixed charges in an IEM give rise to a potential difference between the membrane phase and the surrounding electrolyte. The shift in potential creates a double layer (DL) at the interface, which compensates for the diffusion flux of counterions out from the membrane. The extension of the DL into the electrolyte spans from less than a nanometer at high electrolyte concentrations to tens of nanometers at low concentrations.[32] When an ionic current is driven through an IEM a phenomenon known as concentration polarization occurs, which originates from the selectivity of the membrane. At the side where counterions enter, the electrolyte concentration decreases next to the membrane while the concentration increases on the opposite side (Figure 3.3). For an ideal membrane, the concentration gradient is linear and the transport is diffusion-limited. The extension of the gradient is usually assumed to have a certain length δ due to convective disturbances. As the current through the membrane increases the concentration at the electrolyte-membrane interface decreases until a point when it reaches virtually zero. As the concentration cannot decrease any further the limiting current (I_{lim}) is reached. However, if the applied potential is further increased the current increases relatively slower into the overlimiting current regime ($I > I_{lim}$).[43] This current-voltage behavior is possible due to the formation of an extended space charge layer with low concentration but high electric field.[44,45] It should be noted that most theoretically predicted values of overlimiting currents, which neglect electro-osmotic effects, deviates somewhat from experimentally measured values.[46,47]
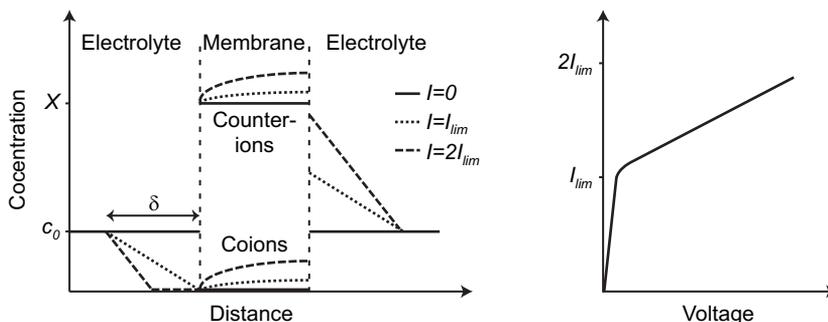
Figure 3.3 Concentration polarization around ion exchange membranes. The electrolyte concentration is polarized in the vicinity of the membrane when a current is driven through the membrane (left). The limiting current is reached when the concentration reaches zero at the feeding side of the membrane. This results in an increase in resistance for currents higher than this limiting value (right).

3.4. Bipolar Membranes

A bipolar membrane (BM) is a sandwich structure of a cation exchange membrane (CEM) and an anion exchange membrane (AEM). BMs exhibit ion current rectification similarly to that of semiconductor *pn*-junctions, with CEMs and AEMs corresponding to *p*- and *n*-doped semiconductors, respectively.[48] The junction between the two membranes is depleted of mobile ions due to the interaction of the fixed charges (Figure 3.4). The current-voltage (IV) characteristics of BMs have three distinct regions.[49] In the forward bias a positive voltage is applied to the CEM side. In this bias, counterions move into the junction, which results in high conductivity and current (Figure 3.4). Eventually, the junction becomes soaked with mobile ions which cause coions to move into the opposing membranes.[50] When a negative voltage is applied to the CEM side, the BM is in reverse bias. For voltages > -1 V the current is low as the junction is depleted of mobile ions and thus nonconductive. For elevated negative voltages the high electric field confined at the junction increases the rate of water dissociation, which creates protons and hydroxide ions. This field-enhanced water dissociation allows a high current to pass through the BM as new ions are continuously created inside the BM. The BM configuration can also be constructed with a neutral spacer in between the CEM and AEM. This layer has little effect on the forward bias characteristics, however it may suppress the field-enhanced water dissociation as the electric field is distributed in between the two interfaces across the entire neutral layer.[51,52]
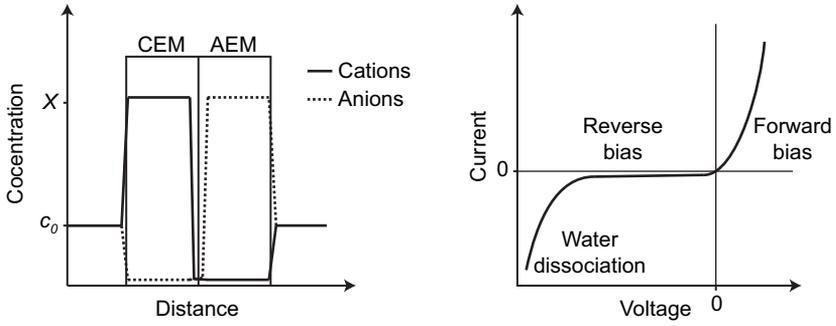
Figure 3.4 Ion distributions and electrical characteristics of bipolar membranes. In bipolar membranes, the region in between the two membrane phases is depleted of mobile ions due to the interaction of the fixed charges (left). The current rectification observed for these membranes is caused by this depletion region (right).

4. Bioelectronics

The field of bioelectronics seeks to exploit the combination of biological elements and electronics. Two of the main focuses are sensing of biological signals and actuation of biological systems. Successful implementation of these functionalities would enable communication over the technology-biology barrier, which could lead to many new applications and medical therapies. Materials applied to biological systems are subject to special requirements, as they cannot be toxic or harmful to the system, *i.e.* they should be biocompatible and bio-stable. The ambition of this chapter is to highlight some key characteristics and concepts to put the work in this thesis into context.

4.1. Electronics

4.1.1. Passive and Active Components

A component that is incapable of power gain is referred to as (incrementally) passive. Resistors, capacitors, inductors and diodes are typically included in this category. In contrast, an active component can amplify signals and thereby inject power into the circuit. This prevents the signal amplitude from degrading and allows for functionality not possible to realize with only passive components.

4.1.2. Transistors

Transistors are active components that can amplify and switch signals. Typically, a transistor has three terminals where the current between two of the terminals can be modulated by the third. Transistors are based on semiconductor materials, in which the density of charge carriers can be modified by electric fields or incorporation of dopants. There are two major types of transistors; field effect transistors (FETs) and bipolar junction transistors (BJTs).[53] In most FETs the control terminal is isolated from the semiconductor by a thin dielectric layer. The control terminal voltage modulates, via field-effect, the charge carrier concentration in the adjacent semiconductor channel. This then controls the current between the other two terminals. Today, FETs are the most commonly used transistor and they can

be found in nearly every single piece of electronic equipment. However, the first transistors made were the BJTs, but nowadays these transistors are mostly used in special applications where their specific characteristics are better suited than those of FETs. BJTs have also three terminals, labeled emitter, collector and base. In an *npn*-BJT the emitter and collector are *n*-doped while the base is *p*-doped (Figure 4.1). The operation involves both electron and holes, thereby the term bipolar. Electrons are injected from the emitter into the *p*-doped base, where they diffuse over to the collector. This process is fundamentally different from the migration of charge carriers in FETs, as the current in BJTs is the result of injection and diffusion of charge carriers in the base.
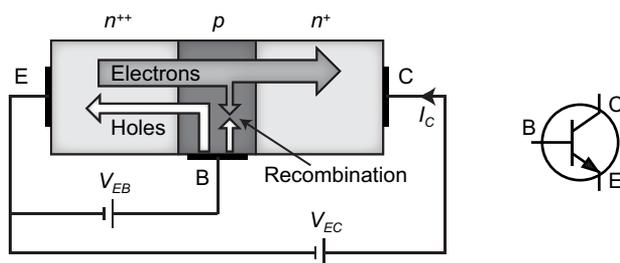
Figure 4.1 Bipolar Junction Transistors. In the *npn*-IBJT electrons are injected from the emitter into the base, where they diffuse over to the collector. Some electrons recombine with holes in the base and are therefore lost.

4.1.3. Analog and Digital Electronics

In analog electronic systems the signals are continuously variable. The input and output information of those circuits can for example be represented by the voltage, current, frequency or phase shift of the signals. Most physical signals are analog by nature and thus require at least one step of analog processing before becoming available to digital processing. The accuracy of an analog system is sensitive to non-idealities and noise of the components, which degrades the signal quality as the signal is processed.

In digital electronics the signals are represented by discrete input and output values, which are defined by analog voltage ranges. In most cases digital signals have two values, 0 and 1, which are separated by a forbidden voltage range. The functionality of digital circuits can be described by Boolean algebra, which provides a framework for designing digital systems. Another important aspect of digital signals is that they do not degrade with noise as long as the magnitude of the noise is within a certain range. Together these properties make digital systems easy to design and also robust to

operate, which explains their tremendous success during the last 50 years. The fundamental building blocks of digital circuits are logic gates. These gates represents Boolean functions such as NOT, AND, OR, NAND and NOR. Logic gates can be implemented with a single type of transistor or in a complementary fashion with two types of transistors (Figure 4.2).[54] The complementary metal–oxide–semiconductor (CMOS) technology based on metal–oxide–semiconductor-FETs (MOSFETs) is today the dominating technology. Advanced microfabrication processes that include hundreds of processing steps are used to fabricate CMOS circuits. As most physical signals are analog, digital circuits require a conversion interface to communicate with the physical world. These interfaces are analog-to-digital (A/D) and digital-to-analog (D/A) converters.
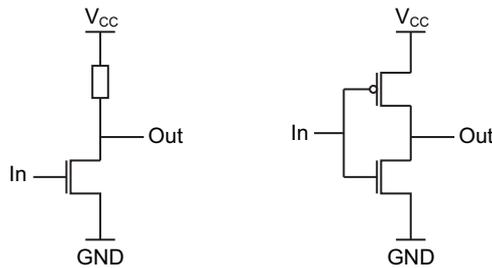
Figure 4.2 Two different implementations of inverters. The NOT gate realized with a single transistor in series with a resistor (left) and in a complementary fashion using two transistors (right).

4.2. Biological Systems

4.2.1. Composition

The cell, which is the basic building block of all life, is built up of organic materials such as carbohydrates, proteins, lipids, nucleic acids and fats. Some organisms like bacteria consist of only one cell while humans comprise trillions of cells. All cells have a membrane, composed of a phospholipid bilayer, which encloses it from the surrounding environment. This enables the cell to control its internal composition by selectively transporting ions and molecules across its membrane. Eukaryotic cells, *e.g.* animal cells, have several internal membrane enclosed compartments, each with their specific function, which together allows the cell to regulate its metabolism. The human body consists of around 210 distinct cell types. To perform a certain function, one or several cell types are organized together in what is called a

tissue. An organ carries out a specific function and comprises several types of tissues.

4.2.2. Signals in Biological Systems

Signaling in biological systems is very different from the signal processing that is performed in modern electronics. In biology information processes do not rely on large numbers of identical subunit circuits, which only utilize one type of signal carrier. Rather, biology makes use of numerous chemically different signals connected in feedback networks. Examples of such signals are ions, neurotransmitters, peptides, hormones and proteins. The specificity and selectivity of biological signaling arises from the ability to release and detect specific chemical substances. In cells signals are triggered by external or internal stimuli. The response can be fast, like in the synapse of a neuron, or slow, like the production and release of hormones into the circulatory system. On the other end of the system the signals are selectively detected, *e.g.* by specific receptors in the cell membrane.

4.2.3. Neuronal Cells

The nervous system is a network of specialized cells, which receives, processes and transmits signals within the body. The central nervous system (CNS) comprises the brain and the spinal cord, while the peripheral nervous system (PNS) consists of sensory neurons and their connections. The nervous system consists of billions of neurons and supporting cells. Neurons are built up of a cell body, dendrites for receiving signals and an axon for transmitting signals (Figure 4.3). In the cell membrane there are ion selective pumps, composed of proteins, which pump K^+ into the cell and Na^+ out of the cell. This creates an electrochemical gradient across the membrane known as the resting potential. The neuron receives signals from other neurons at its dendrites through structures called synapses. At the synapse the signaling cell releases neurotransmitters that diffuse over the 20 nm wide synaptic cleft to the receiving cell. The neurotransmitters open ion channels in the cell membrane, which then creates an ion flux over the membrane. If this depolarization effect is strong enough, *i.e.* above a specific threshold, the neuronal cell is activated and fires an action potential. Initially, the potential rises as Na^+ channels are opened causing Na^+ to flow into the cell. This is then followed by opening of channels which allow K^+ to flow out and thereby restoring the resting potential of the cell. The action potential travels along the axon until it reaches the axon terminals. Here, it causes influx of Ca^{2+} into the neuron. The Ca^{2+} concentration spike causes synaptic vesicles that contain neurotransmitters to merge with the cell membrane (exocytosis) and thereby

releasing the neurotransmitters into the synaptic cleft, where they actuate signaling in the receiving cell. Thus, the signal is transferred along a neuron by changes in the potential induced by ion flows, while the signal transduction between cells is chemical in nature.
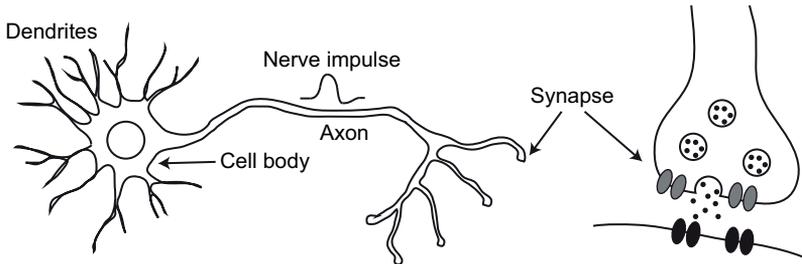
Figure 4.3 The neuronal cell. A neuron receives chemical input (neurotransmitters) at its dendrites from other neurons. If the input is strong enough the cell is depolarized and transmits a nerve impulse along its axon. The axon ends in multiple synapses where the signal is transferred chemically to receiving cells.

4.2.4. Calcium Signaling

The Ca^{2+} ion plays an important role in a wide range of cellular processes, from neurotransmitter exocytosis (microsecond concentration transients) to cell proliferation (hours).[55] The versatility of the Ca^{2+} ion is a result of the highly spatiotemporal nature of Ca^{2+} signaling. For eukaryotic cells, the resting intracellular free Ca^{2+} concentration is approximately 100 nM, while the extracellular Ca^{2+} concentration is more than 10^4 times higher. Also, the cell has access to intracellular Ca^{2+} stores, which are used to regulate the free Ca^{2+} concentration inside the cell. The temporal component in Ca^{2+} signaling is very important and signaling may occur as Ca^{2+} oscillations, spikes or waves.[56] Cells have many pathways for regulating intracellular Ca^{2+} levels and some of the most important ones are shown in Figure 4.4.

Cell membrane

VOCC

>1 mM Ca^{2+}

mAChR

Pumps

nAChR

Ca^{2+}

Cytosol

Ca^{2+}

IP$_3$

Ca^{2+}

Ca^{2+}

IP$_3$R

Ca^{2+}

~100 nM Ca^{2+}

ER
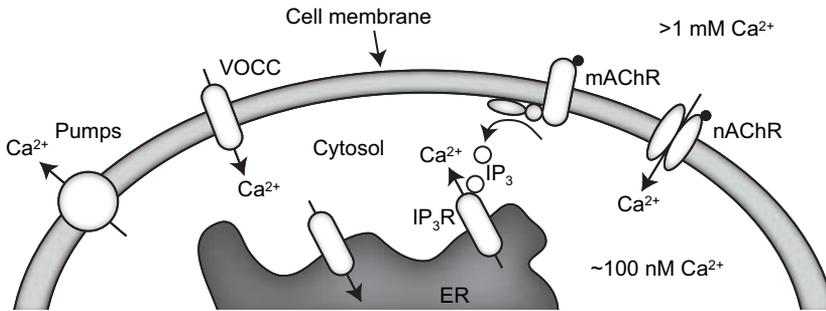
Figure 4.4 Cellular Ca^{2+} regulators. Ca^{2+} can enter into the cytosol in a number of different ways. If the cell is depolarized, the voltage-operated Ca^{2+} channels (VOCCs) are opened. Ca^{2+} can also enter if a ligand binds to the nicotinic acetylcholine receptor (nAChR), which opens a Ca^{2+} channel. Ligand binding to the muscarinic acetylcholine receptor (mAChR) starts a signaling cascade, in which the ligand 1,4,5-trisphosphate (IP$_3$) is released and diffuses to the receptor 1,4,5-trisphosphate receptor (IP$_3$R), which causes Ca^{2+} to escape the stores in the endoplasmic reticulum (ER). Ca^{2+} can also be pumped out from the cytosol, either into the ER or out through the cell membrane.

4.2.5. SH-SY5Y Cells

A cell line is a population of cells, which due to mutation maintain the ability to divide. The SH-SY5Y is a neuroblastoma cell line derived from a biopsy of a metastatic neuroblastoma site in a four-year old girl.[57] SH-SY5Y cells exhibit many of the biochemical and functional properties of neurons, which makes this cell line an attractive *in vitro* model. Specifically, SH-SY5Y cells express nicotinic acetylcholine receptors (nAChRs), muscarinic acetylcholine receptors (mAChRs), voltage-operated Ca^{2+} channels (VOCCs) and 1,4,5-trisphosphate receptors (IP$_3$Rs). Therefore, the SH-SY5Y cell line is a well-established model system for investigating calcium signaling. Acetylcholine (ACh), which is one of the major neurotransmitters in both the CNS and PNS, binds to both nAChR and mAChR.

4.3. Sensing

4.3.1. Electrodes

The simplest way of recording a local electric signal from a biological system is to place an electrode in contact with, or in close proximity to, the specific system of interest. The local shift in potential within the system couples to the electrode and a change in potential or displacement current can be read out and referenced to a second electrode also in contact with the system. Common electrode materials include platinum, gold, iridium oxide and carbon. The key

parameter of the electrode is its impedance, as this property determines how well the signal is recorded.[15] Low impedance is typically desirable as this allows for recording of high frequency signals and provides low noise levels. To achieve spatial resolution a large number of electrodes can be organized in arrays or matrixes.[58,59] The Utah and Michigan arrays are well-known examples of implantable brain micro electrode arrays.

4.3.2. Transistors

Transistors are attractive in sensing applications because of their ability to amplify signals. By coupling the signal of interest to the control terminal of the transistor the signal can be amplified, thereby increasing the sensitivity of the measurement. Moreover, transistors can be connected to form various dedicated sensor circuits, such as the differential amplifier circuits, that even further increases the overall sensitivity. Silicon FETs have been developed for neural activity recording and later integrated into CMOS sensor arrays.[60,61] Further, carbon nanotube FETs have been reported for detection of proteins and DNA.[62,63]

4.3.3. Specificity

Without modifications, most electrodes lack the specificity to differentiate between different chemical signals. In some fortunate circumstances the species of interest dominates the oxidation reaction at an electrode and can therefore be detected, *e.g.* the detection of dopamine in the brain.[64] Under normal circumstances, the specificity is usually achieved by incorporation of biological recognition elements into the devices.[65] A common approach to detect metabolites is to utilize enzymes that creates electrochemically active species like hydrogen peroxide, which can then be detected at electrodes or by transistors. The most well known and commercially successful biosensor is the glucose sensor, based on the enzyme glucose oxidase, which is used by millions of diabetes patients.[66] Another approach is to immobilize molecules with high affinity for the biomolecule of interest on the surface of a device. The binding of the biomolecule can then be detected as a change in surface charge/potential.[67] A third way is to incorporate a lipid membrane with ion channels and measure the impedance over the membrane.[68]

4.4. Actuation

4.4.1. Electrical Stimulation

Some cell types, *e.g.* neurons and muscle cells, may be stimulated by electric fields induced by electrodes. The field depolarizes the cell membrane, which

triggers ion flows across the membrane and causes firing of neurons or contraction of muscle tissue. Electrical stimulation affects all susceptible cells within the vicinity of the electrode. The higher potential that is applied, the larger is the affected volume within the tissue.[15] However, to avoid potentially toxic electrochemical side reactions, electrodes are typically operated in the polarization regime, which limits the magnitude of the applied voltage. Cochlea implants, pacemakers and deep brain stimulation systems are examples of commercial electrodes utilized in medicine today.

4.4.2. Chemical Stimulation

Stimulation with chemical compounds has a huge potential due to the specificity of the stimuli. The simplest and most common form is the administration of drugs through pills or injections; however, these are rather rough approaches, which lack both temporal and spatial control. Both forms affect the whole or a large part of the body and the concentration of the administered drugs deviates from the optimal therapeutic level over time. An additional problem with the drugs taken as pills is that they are prone to be removed by the kidneys and liver. Electronically controlled release of biologically active substances with miniaturized devices may circumvent some of the mentioned problems above. The electronic control gives temporal resolution while the miniaturization allows for spatial control. Further, by incorporating biosensors, feedback-regulated release may be achieved. Therefore such bioelectronics systems have received much attention lately and are expected to improve therapy and treatment in health care, as described in chapter 4.6 below.

4.5. Organic Bioelectronics

4.5.1. Properties

The use of conjugated organic materials in bioelectronic applications is a fairly novel field of research, which currently is expanding rapidly. The chemical composition and mechanical properties of organic materials make them promising for integration with soft biological systems.[69] Suitable device geometries may be obtained as conjugated polymers can be cast and printed from solution, which enables fabrication of devices onto nonplanar and/or flexible substrates.[70] Also, the chemical structure of these materials can be optimized for various needs and many conjugated polymers are also found to be biocompatible and biostable. Additionally, biomolecules may be incorporated into/onto the films to promote adhesion or biocompatibility.[71]

4.5.2. Sensing

Conductive polymer coatings for neuronal electrodes is one of the main application areas of organic bioelectronics and has been extensively explored for more than a decade.[12,72] The coatings improved the impedance of the electrode and allowed for prolonged times of *in vivo* recording. Polymer electrodes may also be used as electrochemical sensors of dopamine release from single cells.[73] Recently, conductive polymer electrodes have even been polymerized in living neural tissue.[74,75]

There are two major types of organic transistors that are used for sensing applications; organic field effect transistors (OFETs) and organic electrochemical transistors (OECTs, Figure 4.5).[14] By coupling recognition elements to the gate of the OFETs small variations in binding can be detected.[14] OECTs have the advantage of being more robust and can operate in direct contact with aqueous media at low voltages, which makes them suitable as enzymatic sensors.[76,77]
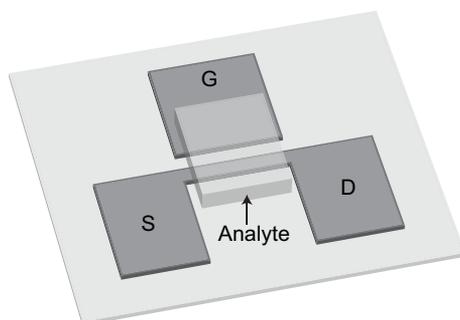
Figure 4.5 Organic electrochemical transistors for sensing applications. OECTs can be fabricated in one layer and used for enzymatic sensing. The current between the source (S) and drain (D) is modulated by the gate (G), which can be made sensitive to the analyte.

4.5.3. Actuation

Conductive polymer electrodes with large capacitance and low impedance have successfully been explored as stimulating electrodes in neuronal applications.[15] Further, various peptides and growth factors have been incorporated into these coatings.[71,78] For electrically controlled release, polymer drug delivery electrodes have been developed.[16,17,19,79,80] In those, the drug is typically incorporated during the electropolymerization process as the polymer is manufactured. When a voltage is applied to the electrode, a controlled release of the drug is achieved. A limitation of this technique is that only charged drugs may be delivered in this way. Conjugated polymers can

also bee used to switch the properties of a surface. By electrically changing the redox state of the polymer, protein conformation, cell adhesion and surface energy may be controlled.[81-86] Also, the volume of conjugated polymers change with the redox state (Figure 4.6). Such effect has been exploited to construct microactuators for biological and medical applications.[87-90]
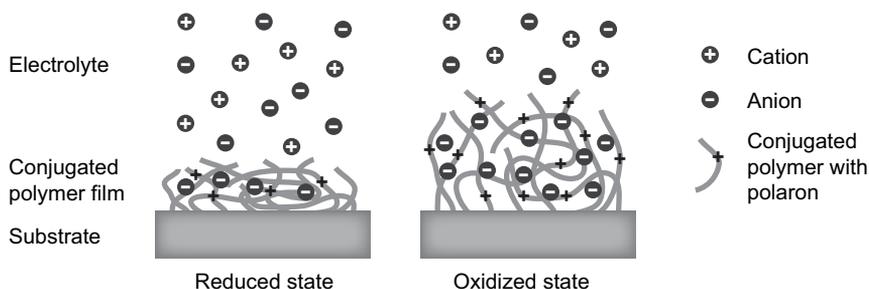
Figure 4.6 Volume change of conjugated polymers. The volume of conjugated polymers varies with the redox state. As the polymer is further oxidized (right), anions and water enter the film and thereby increase its volume.

4.6. Electronically Controlled Delivery Devices

Electronic signals are often the preferred and most versatile way of mediating control of a function. Therefore the use of electronic signals in drug delivery devices has been investigated by several groups. Different ways of delivering the desired substance may be electronically controlled; each with its own advantages and drawbacks. Generally, stimulation with chemical substances is more complicated than stimulation with electric fields or potential, as the substance has to be stored, prevented from leaking out and then physically transported to the site of delivery. Also, a large issue with implantable drug delivery units is the limited storage capacity, meaning you have to either reoperate the patient to refill or have an external container, meaning a tube thought the skin, with infection risk.

4.6.1. Convective Delivery

The delivery of stimuli by the movement of fluid is a versatile method with respect to the substances to deliver, since any compound that is soluble may be delivered in this way. There are many ways to create micro-pumps, including mechanical pumps based on piezoelectric, electrostatic or pneumatic effects and non-mechanical pumps based on electro-osmotic, electro-hydrodynamic or electrochemical effects.[91] Manipulation of the flow

of fluid requires that there is a large enough receiving reservoir volume to deliver to. This is not always the case for various *in vivo* applications.

4.6.2. Delivery by Diffusion

Delivery by diffusion is versatile with respect to the substances and where to deliver it. Here instead the challenge lies in modulating the rate of diffusion. A closed membrane can be disintegrated electrochemically, although only one (or a limited series of) burst of substance is then possible.[92] Electromechanical valves may be used to regulate the delivery in microfluidic or micro-container systems.[93,94] Another approach would be to modulate the diffusion rate within the material, *e.g.* by changing the pH electrochemically that then controls the swelling of the material. Electrochemically actuated layer-by-layer assemblies have also been used to release biomolecules,[95] recently in combination with vesicles.[96] It is also possible to deliver substances through membranes while regulating the feeding solution by convective means.[97]

4.6.3. Electrophoretic Delivery

Charged substances can be delivered by migration in an electric field. The advantage of this technique is the direct electronic control over the delivery rate, however large biomolecules like proteins are less suitable for this kind of delivery because of their low mobility. In iontophoresis charged drugs are administrated through the skin by an ionic current established between two electrodes.[98] In microiontophoresis a miniaturized device, typically a thin glass needle with multiple compartments, can be used to deliver substances to neuronal cells in the brain.[99] Both migration and electro-osmosis contribute to the delivery in these kinds of devices, which sometimes makes it difficult to dictate the exact doses of delivery.

4.7. Ion Diodes and Ion Transistors

Transistor and diode functionality can be achieved based on ions as the charge carriers and lately such ionic devices have received significant attention. There is a great challenge and interest to establish ionic (chemical) circuits that mimic some of the non-linear signal processing features of ion channels and pumps of cells. Further, development of ionic circuits could have a significant impact on biotechnology and chemical engineering as a tool to address and deliver chemicals in a highly complex manner. A parameter of great importance in ionic components is the electrolyte concentration, as high concentrations effectively screen charges, which might affect device performance. This may be an issue, since many suggested applications within the life sciences require physiological salt concentrations.

4.7.1. Ion Diodes

To date three major classes of ion diodes have been reported. In all those cases the ion current rectification is achieved by depletion or enrichment of mobile ions within a defined region. The mechanism to achieve concentration modulation, however, varies between the three different types of diodes. In electrolytic diodes based on hydrogels, the suppression of reverse current is a consequence of the recombination of protons and hydroxide ions under reverse voltage bias.[100,101] These diodes require acidic and alkali electrolytes, which thus limits their applicability. Nanofluidic diodes are the most studied system, in which surface charges in narrow channels (often cone shaped structures) are utilized to achieve ion current rectification (Figure. 4.7).[102,103] Unfortunately, high electrolyte concentrations tend to deteriorate the characteristics of these devices. The third class is bipolar membrane diodes, which essentially consist of a BM. BM diodes have been fabricated into microfluidic systems and ionic AND gates have been realized.[104] However, simple BM diodes suffer from large hysteresis effects and also water splitting at elevated voltages in reverse bias.[105] Recently, an improved version of BM diodes was reported which suppresses the previously mentioned issues.[106]
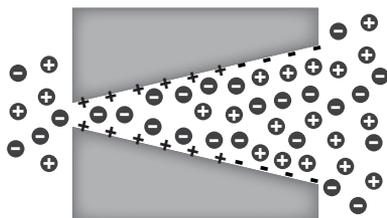
Figure. 4.7 Nanofluidic diode. The fixed positive charges close to the pore opening renders the diode anion selective. When a current passes through the opening of the pore, the electrolyte is polarized at both sides. Because of the difference in geometry of the two sides of the pore opening, rectification is achieved.

4.7.2. Ion Transistors

In similarity to their electronic counterpart, ion transistors can be divided into field effect transistors and bipolar junction transistors. Nanofluidic transistors are currently the dominating type of field effect ion transistors and they comprise narrow channels with at least one dimension in the nanometer range (Figure 4.8).[107,108] The ion current in these devices is modulated by changing the surface charge of the channels, but like nanofluidic diodes, nanofluidic transistors do not operate well at physiological salt concentrations.[109] Recently, polysaccharide[110] and polyelectrolyte junction[111] field effect ion

transistors have also been reported, however, it is still unclear if these devices work at elevated salt concentrations, such as at physiological conditions. The other type of ion transistors, ion bipolar junction transistors (IBJTs), is the class of transistors explored in this thesis (papers III-V). The fundamental difference between IBJTs and field effect ion transistors is that the ion conduction occurs within the bulk of the materials instead of at the interfaces. This allows for higher densities of fixed charges and thus operation at relatively much higher salt concentrations.
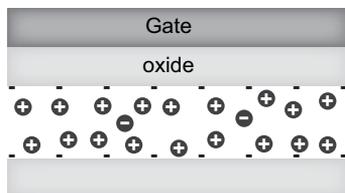
Figure 4.8 Nanofluidic transistor. The gate of the nanofluidic transistor can modulate the surface charge of the channel and thereby the ion concentration within the channel.

5. Experimental Methods

5.1. Fabrication Methods

The devices developed within this thesis are manufactured in multiple sequential microfabrication steps. This chapter describes the techniques employed to deposit and pattern the functional layers of the devices; spin coating, photolithography, reactive ion etching and inkjet printing. Also, a summary of the processing flow is given.

5.1.1. Spin Coating

Spin coating is a well-established method to produce thin uniform films onto flat surfaces and it is widely used in microfabrication of electronic systems. The material to deposit is typically dissolved in a volatile organic solvent and the solution is spread out onto the substrate by rotating the substrate at a high speed (Figure 5.1). The centrifugal force creates a thin layer of solution, the solvent starts to evaporate away and the spinning can be stopped when a solid thin film has been formed. Film thicknesses between 10 nm and 100 µm can readily be achieved depending on which materials, solvents, concentrations and spinning speeds that are used.
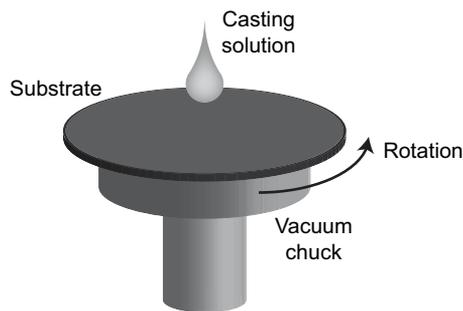
Figure 5.1 Spin coating. The substrate is fixed on a vacuum chuck and rotated in order to spread the solution. The solution is typically applied before the rotation is started.

5.1.2. Photolithography

Photolithography is a patterning method widely used in microfabrication of electronics and micromechanical systems. A light-sensitive chemical (photoresist) is typically spin coated onto a substrate and then exposed with UV-light in a mask-aligner system through a photomask (Figure 5.2). For positive photoresists, the exposed parts become soluble in a developer. Negative photoresists on the other hand becomes insoluble when they are exposed to UV light. The generated photoresist pattern can be used as an integral part of the device or just utilized in a subsequent patterning step. When the patterning step has been performed, the photoresist is then removed, either chemically or by etching. Often a fabrication process includes several photolithographic patterning steps before the final device is achieved. The patterns of the different layers usually have to be aligned, which may induce alignment errors, especially if flexible substrates are used. The resolution of the pattern is limited by the wavelength of the UV-light among other things. With the Suss MA/BA 6 mask aligner system used in this work resolutions slightly below 1 µm are possible to achieve.
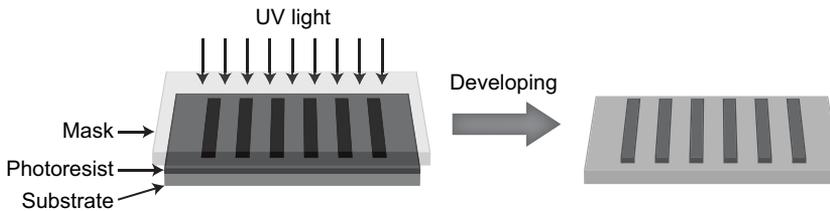
Figure 5.2 Photolithography. The photoresist is exposed by UV light through a mask in a mask aligner. For a positive resist, the exposed material is removed during a developing step.

5.1.3. Reactive Ion Etching

In reactive ion etching (RIE) a chemically reactive plasma is used to etch the substrate. The plasma is generated by a strong RF electromagnetic field between two plates at low gas pressure. The gas molecules are ionized and hit the substrate vertically which makes it possible to obtain highly anisotropic etching. Material can be etched by two processes, either sputtered away by the kinetic energy of the ions or chemically reacted by the reactive ionic species of the plasma. The latter effect can be controlled by the supply of different gases to the plasma. Polymers are typically etched in an oxidizing O_2 plasma, sometimes comprising a fraction of CF_4 to increase the etch rate or modify the etch chemistry.

5.1.4. Inkjet Printing

An inkjet printer is an electronically controlled device that propels droplets of ink onto a substrate. There exists a vast array of different inkjet printers for various purposes ranging from conventional document printing to circuit fabrication. The Dimatix inkjet printer (DMP-2800) used in this work differs from standard desktop printers in a number of ways which gives the user much greater flexibility with respect to choice of substrate and ink formulation. The DMP-2800 uses piezoelectrics to eject droplets, through nozzles, from an ink-filled chambers. Each replaceable cartridge comprises 16 nozzles, which can be individually controlled. The ink container of the cartridge can be loaded with 1-2 ml of ink and it is possible to set the temperature of the nozzles. The viscosity and wetting properties of the ink formulation have to be within a certain range, however the ability to change the waveform and temperature of the nozzles relaxes the requirements of the ink formulation. The substrates are fixated on a flat surface, which enables printing on various kinds of substrates, *e.g.* plastic foils and silicon wafers. The drop volume is 1-10 pl and the typical resulting dot size on the substrate measure a diameter of around 50 μm for 10 pl drops.

5.1.5. Fabrication of IBJTs

The IBJTs are fabricated in several sequential microfabrication steps. The processing scheme for the devices reported in papers IV and V are shown in Figure 5.3. For details, see the papers.
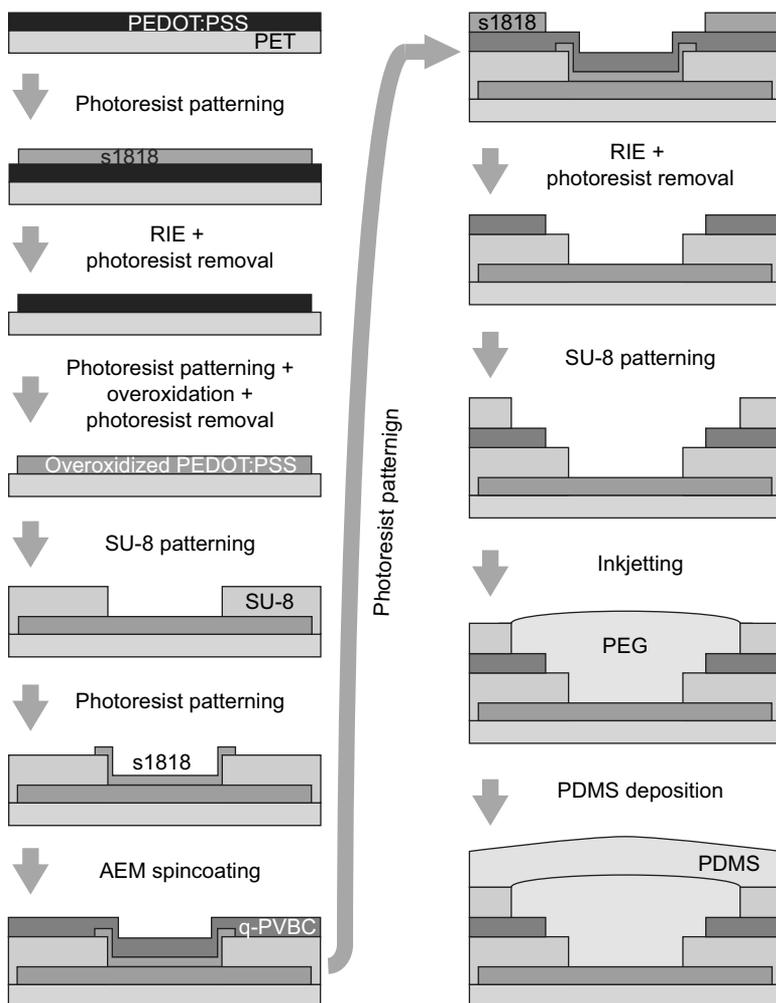
Figure 5.3 The fabrication steps of IBJTs. The starting substrate is a circular 4 inch PET foil coated with PEDOT:PSS. The plastic substrate is attached to a silicon wafer in many of the processing steps in order to facilitate the handling.

5.2. Electric Characterization

Applying a voltage and measuring the resulting current is one of the most fundamental ways of electrically characterizing a device. For electrochemical devices the electric current equals the ionic current, thus electric measurements are also a convenient way of characterizing the ion transport in materials and devices. From the electrical measurements it is typically not possible to distinguish between anion transport occurring in one direction and cation transport occurring in the opposite direction. In the special case of

selective membranes, however, counter ions account for the large majority of the transported ions. Therefore, electric characterization of devices based on these materials is very useful. A three-electrode configuration is often utilized in electrochemical measurements, in which the current passes between the studied electrode (working electrode) and a counter electrode. The potential of the working electrode is measured with respect to a reference electrode, through which ideally no current passes through. The advantage of this approach is that there is no current induced voltage drop over the reference electrode, thus it is possible to exclusively measure the shift in potential drop over the working electrode with respect to the electrolyte.

In this work electric characterization was carried out with Keithley sourcemeters (K2400 and K2602(A)). These sourcemeters can source a specified voltage and measure the current or source a specified current and then measure the applied voltage. Currents in the nA range are readably measurable with these instruments. The sourcemeters were controlled via LabView.

5.3. Transport Characterization

By correlating the measured transport rate of a substance with an electrically measureable quantity, *e.g.* the voltage or current, it is possible to control the transport from electric parameters. Feasible measurement techniques have been developed to identify and quantify a vast number of different chemical substances. Enzymatic reactions can be utilized to specifically detect a wide range of biomolecules. In paper I the Amplex® Red Acetylcholine/-Acetylcholinesterase Assay Kit was used to measure the amount of transported acetylcholine in our devices. The enzyme acetylcholinesterase converts acetylcholine into choline, which in turn is oxidized by choline oxidase. This process creates hydrogen peroxide, which reacts with the Amplex Red reagent. The final product is then detected in a fluorescence microplate reader. In paper II the transported amount of radioactive $^{22}Na^+$ was measured. A NaCl solution that partially contained $^{22}Na^+$ was used as source electrolyte and the target solution was collected for different transporting times. The samples were analyzed with a Beckman LS 6500 instrument.

5.4. *in vitro* Cell Experiments

The Latin term *in vitro* means "in glass" and refers to experiments on biological elements which have been isolated. Common examples of *in vitro* studies are cell experiments carried out in a Petri dish. In this work, several

different *in vitro* cell experiments were carried out using IPs and IBJTs to regulate Ca^{2+} cell signaling in neuroblastoma SH-SY5Y cells. SH-SY5Y cells have previously been used for Ca^{2+} signaling studies and express both nAChR and mAChR. The cells were typically cultured on the devices in an incubator for 1-2 days before use. Before the experiments were carried out, the cells were loaded with the membrane-permeable Ca^{2+} sensitive dye Fura-2 AM. As the cells were stimulated with the neurotransmitter ACh, the receptor operated calcium channels were opened and the influx of Ca^{2+} was recorded in a fluorescence microscope setup.

6. Devices in Papers I-V

6.1. Organic Electronic Ion Pumps (Papers I,II)

The organic electronic ion pump (OEIP) was first reported by Isaksson *et al* in 2007.[112] The OEIP was utilized to control the transport of various cations to trigger voltage operated calcium channels in neuronal cells. Importantly, it was found that the PEDOT:PSS material was biocompatible with the cultured neuronal cells. Based on these results the development of the OEIP continued in two directions; one in which the OEIP was redesigned for *in vivo* experiments and another direction aimed at achieving high spatiotemporal precision of the delivery for *in vitro* experiments. The latter direction is covered in this thesis and is reported in papers I and II.

6.1.1. Device Architecture

The basic structure of the OEIP includes two conductive polymer PEDOT:PSS electrodes connected by a thin cation selective channel (Figure 6.1). An insulating layer covers the structure, with openings for electrolytes and electrical connectors at the electrodes. The cationic substance to be delivered is placed on the source electrode while the system to be stimulated, *e.g.* cells, is placed in the close vicinity of the channel outlet at the target electrode. The devices presented in both paper I and II also include one additional electrode that is connected to each channel system. This additional electrode enables preloading before the start of delivery.
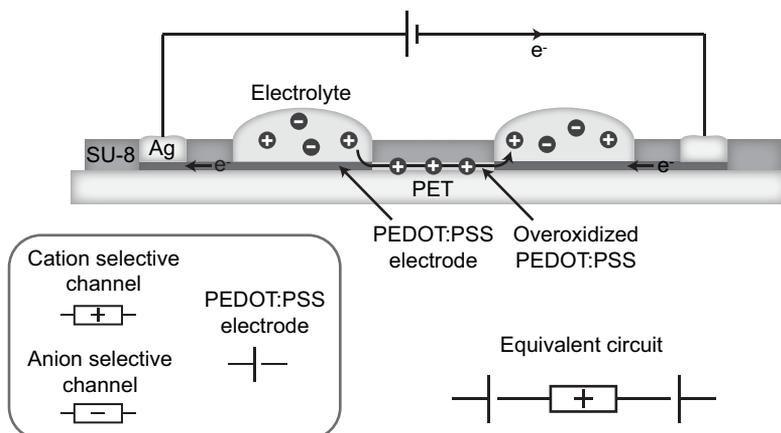
Figure 6.1 The organic electronic ion pump. The reduction/oxidation of the PEDOT:PSS electrodes drives a current of cations through the channel (top). The equivalent circuit of the OEIP is two electrodes connected by a ionic resistor (bottom right).

6.1.2. Operation

Before operation, the OEIP must be soaked in water, typically for 24 h, in order to hydrate the channel system. When a voltage is applied between two electrodes the following electrochemical reaction occurs in the PEDOT:PSS system:

$$PEDOT^+:PSS^- + e^- + M^+ \leftrightarrow PEDOT^0 + PSS^-:M^+$$

This reaction requires that the transported electric charge is compensated by an equal amount of ionic charge of the opposite polarity. Since the channel is cation selective, predominately cations will migrate through the channel, from the positively addressed source electrolyte into the target electrolyte. This is confirmed by measurements of the neurotransmitter acetylcholine (ACh) transport efficiency that reaches close to 100% (paper I). At the outlet, diffusion takes over the transport of the cationic substance as the electric field is screened by the electrolyte. One issue with a two-electrode delivery system is that the substance must travel the entire distance from the source to the target, which induces a significant delay between addressing and actual turn on of the delivery (~100 s). By incorporating a waste electrode, the delivery channel can be pre-loaded up to a point close to the outlet before the actual delivery is turned on, thereby reducing the delay in delivery to ~1 s.

6.1.3. Applications

OEIPs have been utilized to stimulate cultured neuronal cells with various neurotransmitters and metal cations.[112,113] Further, controlled delivery of

protons can induce pH oscillations and also catalyze the formation of amyloid fibrils.[114,115] Possible future applications include formation of functionalized amyloid fibril nanomaterials, *in vitro* experiments with multiple stimuli and gradients, as well as incorporation of biosensors with feedback regulation of the OEIP.

6.2. Ion Bipolar Junction Transistors (Papers III,IV)

OEIPs are passive linear components and the channel basically perform as a resistive load. To obtain multiple individually controlled outlets, one therefore has to assign a separate electrode and reservoir to each outlet. There are several disadvantages associated with such an approach. First, each electrode must have sufficient capacity, *i.e.* volume, which puts a limit on the minimum spacing between outlets. Secondly, the large number of separate reservoirs probably have to be prefilled, as it is hard to fill the reservoirs once the device has been assembled. It is known that nonlinear components such as diodes and transistors are necessary to achieve more advanced circuit functionality, like matrix addressing. Transistors are especially attractive since a small control signal can modulate a relatively much larger output signal, *i.e.* they exhibit gain. In the ion bipolar junction transistor (IBJT) all currents are ionic in nature; compare with conventional transistors that modulate electronic currents. This allows for delivery of charged biomolecules, like in the OEIP, but with the additional possibility of constructing more complex and addressable delivery circuits.

6.2.1. Device Architecture

The IBJT can be designed in two complementary forms, the *npn*-IBJT and the *pnp*-IBJT (Figure 6.2). The *npn* version comprises two AEM channels (emitter and collector) meeting one CEM channel (base) in a neutral polymer junction, which is sealed by a drop of PDMS (Figure 6.2). The *pnp*-IBJT instead comprises two CEM channels and one AEM.
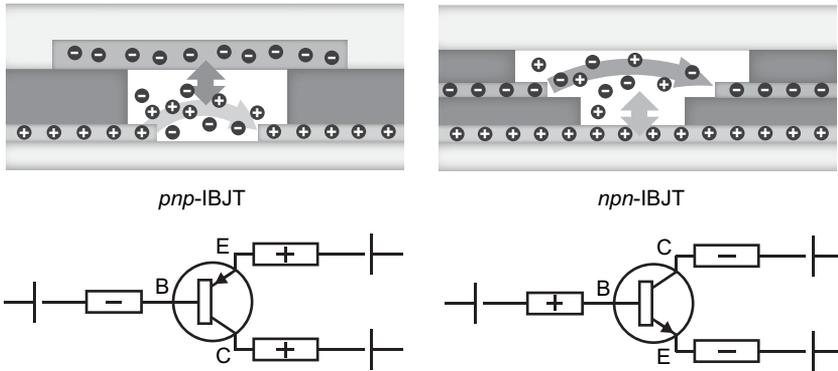
Figure 6.2 Ion bipolar junction transistors. The *pnp*-IBJT conducts cations beween the emitter and collector (left) while the *npn*-IBJT conducts anions (right). The equivalent circuits are shown below respective device.

6.2.2. Operation

When a voltage is applied between the emitter and collector of a *npn*-IBJT, the potential drop is divided over the emitter, junction and collector. By changing the base voltage, the salt concentration within the junction, *i.e.* the ion conductivity, can be modulated, which in turn affects the ionic current through the collector. It appears that the current within the junction is diffusion limited, which also is the case for conventional bipolar junction transistors. The IBJTs show good transistor characteristics and well behaving devices have collector current on-off ratios up to 100.

6.3. Ion Transistor Logics (Paper V)

In Boolean algebra the only allowed values are 0 and 1. Logic circuits can be described by Boolean functions and the physical implementations of the basic boolean functions are called logic gates. In devices logic 0 is represented by voltages below a certain threshold level and logic 1 by voltages above another threshold level. A first step towards more complex ionic circuits based on IBJTs is to implement some of these logic gates. This could open up for the construction of delivery circuits based on well-known concepts from conventional electronics, *e.g.* demultiplexers.
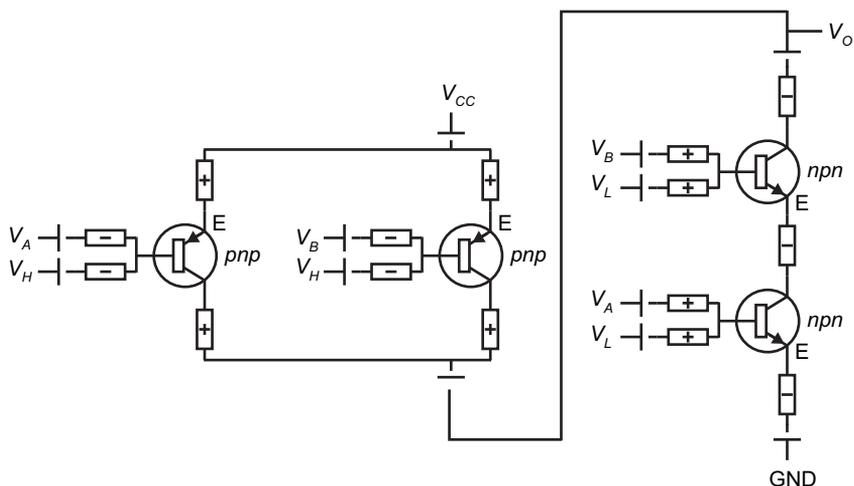
Figure 6.3 A complementary NAND gate based on IBJTs. By combining four IBJTs a complementary NAND gate can be achieved. Pull up (V_H) and pull down (V_L) voltages are required for the IBJTs to operate in the appropriate regime.

6.3.1.   Circuit Implementations

The simplest logic gate is the NOT gate (inverter), a component in which the output signal is the inversion of the input signal. The NOT gate can be implemented with a single *npn*-IBJT in series with a resistor or by replacing the resistor with a *pnp*-IBJT in a complementary version. The complementary version is expected to have higher gain and lower currents, which is also found for our IBJT NOT gates. Another fundamental gate is the NAND gate, which includes two input signals. The output signal is logic 0 only when both input signals are logic 1. The NAND gate is attractive since it is sufficient to realize any other logic function. As with the NOT gate, the NAND gate can be realized with only *npn*-IBJTs or in a complementary fashion.

7. Concluding Remarks

7.1. Collaborations

This thesis is the result of the multidisciplinary work carried out within the Strategic Research Center for Organic Bioelectronics (OBOE). Projects have been run together with neuroscientist, protein chemists and electrical engineers. The primary collaboration in this thesis has been with Prof. Agneta Richter-Dahlfors group at the Department of Neuroscience, Karolinska Institute. Within the OBOE center most projects have been organized in a twinning fashion with at least one person each from the physical and biological sciences, respectively. My twinning partner was recently appointed Dr Karin C. Larsson and we have worked together within projects during the full extent of this thesis work. Other collaborators were Prof. Per Hammarström and Ass. Prof. Peter Nilsson, Department of Physics, Biology and Chemistry at Linköping University, with whom we explored ion pumps to controlled amyloid formation. For the development of ion logics we collaborated with Prof. Robert Forshheimer, Department of Electrical Engineering at Linköping University. Finally, our lab facilities are shared with Acreo AB, and their expertise aided greatly in the development of fabrication processes .

7.2. The Development from Ion Pumps to Ion Transistors

When this work started the first reports on the ion pump had just been published. They generated a lot of excitement as this device combined several attractive features to create a chip technology for conversion from electronic to chemical signals. Initially the technology was immature and the device physics was not fully understood. My first contributions came in the understanding of the ion transport processes involved in the ion pump. This resulted in the elimination of the two redundant electrodes in the original design and also explained why some early attempts to achieve addressability failed. These insights eventually lead to the development of the ion pumps reported in paper I and II. The outlet dimensions were shrunk, a loading

channel configuration was added to decrease the delay time of delivery and an additional source electrode was added to enable delivery of multiple substances. During this time the repertoire of transported substances was extended from metal cations and protons to neurotransmitters and amino acids.

Quite soon it became clear that it was not possible to build fully matrix-addressable delivery systems based on the ion pump alone. The reason is that the channel in the ion pump is the equivalent of a resistor. Even before my work began my supervisor Prof. Magnus Berggren and Prof. Robert Forshheimer had discussed the advantages of building ion transistors based on conjugated polymers and polyelectrolytes, however no successful implementations had been made. The first progress came after the Material Research Society spring conference in 2008 when I started to think about the ion transport in cationic and anionic polyelectrolytes. I realized that the stack of the two materials should behave similarly to a diode and immediately tried it out in the lab and it worked! The disappointment was great, however, when I soon after found out that the so-called bipolar membrane had been discovered some 30 years earlier. The disappointment did not stop me from continue thinking about similar ionic structures and I got interested in bipolar junction transistors. The first implemented ion bipolar junction transistor was rather different from the published one. It was an *pnp*-IBJT structure with photolithography patterned PSS emitter and collector and an encapsulating SU-8 layer on top. The neutral material was cellulose triacetate (CTA), dissolved in chloroform and painted on the opening in the SU-8. The base was another painted layer of CTA mixed with a bulky quaternary amine (Aliquat 336). Luckily, the first device showed significant promise, however the following devices did not. This lead to a series of changes until a final device configuration was achieved, reported in paper III. The neutral material was a crosslinked PEG gel and the base comprised a piece of cut membrane laminated on top. The fabrication of these devices included several manual steps and the yield and reproducibility was modest at best. Nevertheless, the transistor characteristics of these devices together with the successful cell experiments represented a big leap forward compared to the ion pump technology.

The next step was to develop the *npn* version of the IBJT. This activity was inspired mainly by two reasons; the possibility to construct complementary circuits and the need to remove manual fabrication steps. In the *npn* structure the base is in the bottom and comprises overoxidized PSS. A first progress was made by starting to inkjet print the PEG electrolytes instead

of applying them manually onto the substrate. As the anion selective emitter and collector must be patterned, the previous approach with cut membrane pieces was not a viable option. Erik Gabrielsson and I started to work on a patternable anion selective membrane which could be deposited from solution. This turned out to be quite hard and many failed attempts with photopolymerization followed. We finally abandoned that approach and instead quartanized a polymer, which could be crosslinked once a film was formed. Unfortunately it was very hard to pattern this layer on top of the PEG gel. Some functional devices were fabricated but their characteristics were poor. After many attempts this approach was abandoned and instead the anion selective layer was patterned on top of the first SU-8 layer and then sealed with a second SU-8 layer. Finally the gel was inkjet printed and sealed with PDMS. This is the device reported in paper IV and the fabrication only includes one manual step; the sealing with PDMS.

With both *pnp*-and *npn*-IBJTs at hand the aim was to realize some of the fundamental logic gates. To enable fabrication of both types of IBJTs on the same substrate, the *pnp*-IBJT structure had to be modified. Apart from that the major challenges were to match the high channel resistances to obtain good characteristics. Eventually, complementary inverters and NAND gates were successfully demonstrated.

7.3. Scientific Contributions

I believe my main scientific contribution lies in the development and demonstration of novel ionic transistors and circuits for transduction of electric currents into chemical signals. In the first two papers we showed that passive components can be used for precise spatiotemporal control of neurotransmitter release to regulate functions in neuronal cells. The use of cation selective material enables direct electronic control over the delivery of substance and also prevents any undesired major liquid flow. The following move from passive to active ionic components is what I personally regard as my major scientific contribution, as to my knowledge this is the first time an ion transistor has been successfully operated in physiological salt concentrations. In order to formulate a model of the ionic transport processes occurring within the IBJTs, several advanced concepts from membrane science has been invoked and combined. The subsequent development of the *npn* version of the ion bipolar junction transistor and the implementation of complementary ionic circuits was more straight forward on an intellectual level, however nevertheless presented plenty of technical challenges. On the technical side the contributions comprise development of the processing

schemes which eventually enabled fabrication of integrated chemical circuits. The developed ionic transistor and circuit technology is a semi-solid-state technology, which in many cases is attractive compared to various microfluidic approaches where materials are formed and stored in the liquid phase.

7.4. Future Outlook

In the work leading up to this thesis, our ion circuit technology has evolved from passive components to ion logic gates based on active components. The longstanding goal of addressable delivery circuits, however, has yet to be realized. Hopefully, these circuits will materialize in the near future as the components and knowledge necessary to construct such circuits have been developed within this thesis. Preferably, the delivery should occur in the vertical direction with a horizontal addressing backplane. Such addressable membrane design would allow for swift and accurate delivery as the substance only has to be transported a short distance vertically.

Focusing on individual ion transistors, the obvious area for improvement is the switching speed. Based on our current understanding of the device physics, the RC constant for transistors in circuits should scale with $\sim L^{-2}$ where L is the dimension. Thus, by scaling down the dimensions it should be possible to achieve ion circuits that operate at a frequency above 1 Hz. For many delivery circuits this may be sufficient performance as other factors are limiting the time form addressing to actual delivery.

It is always hard to predict the impact of emerging technologies but a discussion about strong points and weaknesses may put some light into the matter. For the ion circuit technology developed in this thesis the strength and limitations originates from the same property; the selectivity of the materials. This selectivity gives control over the ion flows, thereby enabling the construction of active elements and circuits. This may lead to features not possible to realize with other types of technologies. The fact that the transport is electronically controlled is very attractive as it eliminates the need for fail prone mechanical components. The drawback of relaying on ion selectivity is that it limits the number of substances possible to transport within the circuits. Only transport of charged substances may be controlled with this technology. Also, the required concentration of fixed ionic charge within selective materials put limitations on the porosity of these materials, which results in sieving effects on larger molecules. This is a sever limitation which might impact the range of possible applications for this technology drastically. To

date neurotransmitters, amino acids and small peptides have successfully been delivered with these devices, however no effort has been directed into optimizing this material aspect within this thesis.

Finally, one should keep in mind that this is a young technology and many expected and unexpected improvements may be provided in the future. From an application perspective, a single ion transistor is not an improvement over the simpler ion pump. However, many ion transistors put together may provide functionalities not possible to achieve with only the passive ion pumps. Therefore, I believe it is important to push this technology to a certain point where chemical circuits readily can be realized. Once at this point it will be possible to reach out to the life science community and investigate if chemical circuits can play a major role in the future. Hopefully, new applications will emerge based on the concept presented in this thesis.

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