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ORGANIC BIOELECTRONIC DEVICES TO CONTROL CELL SIGNALLING

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ABSTRACT

The nervous system consists of a network of specialized cells that coordinate the actions of the body by transmitting information to and from the brain. The communication between the nerve cells is dependent on the interplay of both electrical and chemical signals. As our understanding of nerve cell signalling increases there is a growing need to develop techniques capable of interfacing with the nervous system. One of the major challenges is to translate between the signal carriers of the nervous system (ions and neurotransmitters) and those of conventional electronics (electrons). Organic conjugated polymers represent a unique class of materials that can utilize both electrons and ions as charge carriers. Taking advantage of this combined feature, we have established a novel communication interface between electronic components and biological systems. The organic bioelectronic devices presented in this thesis are based on the organic electronic ion pump (OEIP) made of the conducting organic polymer poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS). When electronically addressed, electrochemical redox reactions in the polymer translate electronic signals into electrophoretic migration of ions. We show that the device can transport a range of substances involved in nerve cell signaling. These include positively charged ions, neurotransmitters and cholinergic substances. Since the devices are designed to be easily incorporated in conventional microscopy set-ups, we use Ca^{2+} imaging as readout to monitor cell responses. We demonstrate how electrophoretic delivery of ions and neurotransmitters with precise, spatiotemporal control can be used to modulate intracellular Ca^{2+} signaling in neuronal cells in the absence of convective disturbances. The electronic control of delivery enables strict control of dynamic parameters, such as amplitude and frequency of Ca^{2+} responses, and can be used to generate temporal patterns mimicking naturally occurring Ca^{2+} oscillations. To enable further control and fine-tuning of the ionic signals we developed the electrophoretic chemical transistor, an analogue of the traditional transistor used to amplify and/or switch electronic signals. We thereby take the first step towards integrated chemical circuits. Finally, we demonstrate the use of the OEIP in a new “machine-to-brain” interface. By encapsulating the OEIP we were able to use it *in vivo* to modulate brainstem responses in guinea pigs. This was the first successful realization of an organic bioelectronic device capable of modulating mammalian sensory function by precise delivery of neurotransmitters. Our findings highlight the potential of communication interfaces based on conjugated polymers in generating complex, high-resolution, signal patterns to control cell physiology. Such devices will have widespread applications across basic research as well as future applicability in medical devices in multiple therapeutic areas.

LIST OF PUBLICATIONS

This thesis is based on the following papers:

I. Translating electronic currents to precise acetylcholine–induced neuronal signaling using an organic electrophoretic delivery device
K. Tybrandt*, **K.C. Larsson***, S. Kurup, D.T. Simon, P. Kjäll, J. Isaksson, M. Sandberg, E.W.H. Jager, A. Richter- Dahlfors, M. Berggren.
Advanced Materials, 2009, 21,1–5 *equal contribution

II. Activation of defined cholinergic signal transduction pathways using multiplexing conductive polymer devices
K.C. Larsson, K. Tybrandt, M. Berggren, A. Richter- Dahlfors.
Manuscript.

III. Ion bipolar junction transistors
K.T. Tybrandt, **K.C. Larsson**, A. Richter-Dahlfors, M. Berggren.
Proceedings of the National Academy of Science of the United States of America, 2010, 1:107(22):9929-32

IV. Organic electronics for precise delivery of neurotransmitters to modulate mammalian sensory function
D. Simon, S. Kurup, **K.C. Larsson**, R. Hori, K.T. Tybrandt, M. Goiny, E.W. Jager, M. Berggren, B. Canlon, A. Richter-Dahlfors.
Nature Materials, 2009, Sep8(9):742-6

Additional publications produced during my PhD:

Precise neurotransmitter-mediated communication with neurons *in vitro* and *in vivo* using organic electronics
D. Simon, **K.C. Larsson**, M Berggren M, A Richter-Dahlfors A.
Journal of Biomechanical Science and Engineering, 2010, 5:208-217. Review.

Organic bioelectronics in nanomedicine
K. Svennersten, **K.C. Larsson**, M. Berggren M, A. Richter-Dahlfors.
Biochimica et Biophysica Acta, 2011,1810(3):276-85. Review.

Slice preparation, organic tissue culturing and luciferase recording of clock gene activity in the suprachiasmatic nucleus
S.A. Savelyev*, **K.C. Larsson***, A.S Johansson, G.B. Lundkvist.
Journal of visual experiments, 2011, Feb15(48) *equal contribution

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LIST OF ABBREVIATIONS

ABR	auditory brainstem response
ACh	acetylcholine
AM	amplitude modulation
ATP	adenosine-5´triphosphate
$[Ca^{2+}]_i$	intracellular calcium concentration
CaM	calmodulin
CICR	Ca^{2+} induced Ca^{2+} release
CNS	central nervous system
DAG	diacylglycerol
ER	endoplasmic reticulum
FM	frequency modulation
GABA	γ-aminobutyric acid
Glu	glutamate
HPLC	high-performance liquid chromatography
IBJT	ion bipolar junction transistor
IHC	inner hair cell
IP_3	inositol 1,4,5-trisphosphate
IP_3R	inositol 1,4,5-trisphosphate receptor
mAChR	muscarinic acetylcholine receptor
mGluR	metabotropic glutamate receptor
nAChR	nicotinic acetylcholine receptor
NCX	Na^+/Ca^{2+} ion exchanger
RWM	round window membrane
OEIP	organic electronic ion pump
OHC	outer hair cell
oxo-M	oxotremorine methiodide
PDMS	poly(dimethylsiloxane)
PEDOT	poly(3,4-ethylenedioxythiophene)
PET	polyethylene terephthalate
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKC	protein kinase C
PLC	phospholipase C
PMCA	plasma-membrane Ca^{2+}-ATPase
PNS	peripheral nervous system
PSS	poly(styrenesulfonate)
PPy	poly(pyrrole)
ROCC	receptor-operated Ca^{2+} channel
RyR	ryanodine receptor
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase
SOCC	store-operated Ca^{2+} channel
SOCE	store-operated Ca^{2+} entry
SR	sarcoplasmic reticulum
STIM	stromal interaction molecule
TRP	transient receptor potential
VOCC	voltage-operated Ca^{2+} channel

1 INTRODUCTION

Sound is a mechanical wave that oscillates through a medium. But more than a subject of physics curriculum, sound is a channel through which we perceive and communicate with the world around us. Everyone enjoys music, but we all have different musical preferences. While some people consider opera being music, others regard it as noise for acquired taste. But even though the interpersonal appreciations for the same sounds are different, the way we detect it is the same.

As sound waves hit the eardrum, the vibrations induced propagate through the fluid filled channels of the cochlea where they activate auditory sensory cells. The activated cells release chemical messengers, which relay the information to adjacent cells where electrical potentials are evoked. The electrical signals are transferred from the ear, through a bundle of nerve fibres, to the brain where the signal is interpreted. In essence, perception and interpretation of sound involves a biological signal transmission system, represented here by the nerve cell to which communication is dependent on the interplay of both electrical and chemical signals. Irrespectively of the nerve cell location, its function or morphology, the mechanism of signal transmission is the same, allowing for a constant flow of information between the brain and the body.

The brain is the central cognitive unit by which sensory input as well as all other internal and external information is processed. Illustrating with a rough analogy, the brain is like a computer, as both are information processors identical in their use of mobile charge carriers and electric signals to convey information. Neurons, the basic information processing units of the brain, can send electrical signals (action potentials) and utilize ions and ion fluxes as major charge carriers. Computers on the other hand, transfer information by moving charge in the form of electrons. Even though the mobility of electrons in silicon is 10^6 times faster in comparison to ions in a water solution, the transport of charge represents a crucial link between biological and electronic systems. In this way, the concept of electronic and ionic signal transport will be central in establishing new communication interfaces bridging the gap between hard electronic components and soft biological systems.

2 NERVE CELL SIGNAL TRANSMISSION

Cell signalling is part of a complex communication system that governs basic cellular activities. From the moment of fertilization throughout life to death, networks of intra- and extracellular signalling pathways convey information delivered by signal carriers such as biomolecules and ions. Cellular signal transduction consists of a number of steps, beginning with the arrival of a signal that activates the cell. The activation may occur by a molecule binding to a receptor on the cell surface, ions changing the membrane potential of the cell or a shear flow mechanically activating the cell. The signal is conveyed to trigger intracellular pathways of ions and molecules, propagating the signal inside the cell to its intended destination. The signal finally arrives at its downstream target and changes the state of a cell, for example by regulation of transcription factors. The following sections describe the general principles of nerve cell signalling, cholinergic signalling and the important role of calcium (Ca^{2+}) as a second messenger.

2.1 THE NERVE CELL

The nervous system is anatomically divided into central and peripheral components. The central nervous system (CNS) is comprised by the brain and spinal cord, and the peripheral nervous system (PNS) is constituted of sensory cells. The nervous system consists of billions of nerve cells, neurons, and an equally great number of supporting cells. Neurons are cells highly specialized in processing and transmitting cellular signals and they are organized in neuronal circuits controlling and coordinating functions of sensation, perception and behaviour. In other words, a vast number of events in the human body rely on neurons communicating with one another.

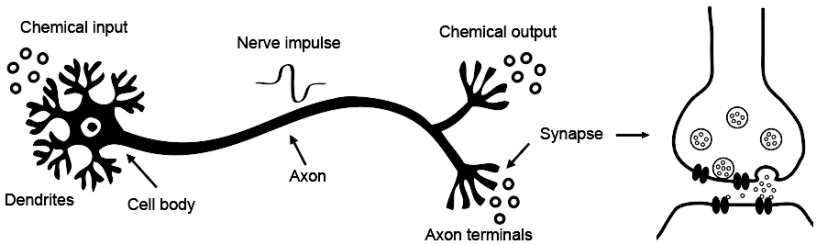


Figure 1. Nerve cell signal transmission. **A)** Information from the chemical input is translated into an electrical impulse, relaying the message along the nerve cell, and is converted back to chemical output at the axon terminals. **B)** The signal transmission in the synapse.

The neuron consists of i) a soma, the cell body ii) dendrites, processes from the cell body receiving synaptic input and iii) an axon, the process that transmits the signal from the cell body to the target cell (Figure 1). Neurons are excitable cells and their communication is dependent on both chemical and electrical signal transmission. The translation from chemical and electrical signal, and vice versa, is dependent on a number of different ions and chemical messengers, neurotransmitters (Table 1). In the resting state, ion selective pumps in the neuron's cell membrane build up an ionic concentration difference, making the inside of the cell more negatively charged compared to the outside. This results in an electrochemical potential difference over the membrane, which is known as the resting potential. When a neuron is stimulated, opening of ion channels result in ion fluxes across the cell membrane

changing the membrane potential. If the unbalance of charge causes the membrane potential to exceed a certain threshold, the neuron is activated and fires an action potential. In the rising phase of the action potential, Na^+ channels open and Na^+ flow into the cell. The depolarization results in opening of K^+ channels and an outward flux of K^+. The cell returns to its resting membrane potential level and will again become able to fire a new action potential.

The action potential is an electrical signal conducted along the axon, conveying information from one place to another in the nervous system. When the action potential reaches the axon terminals at the end of the axon, Ca^{2+} enter the cell and trigger exocytosis. Synaptic vesicles on the transmitting presynaptic neuron fuse with the cell membrane and release neurotransmitters into the extracellular space of the synaptic cleft. The neurotransmitters diffuse across the 20 nm wide gap of the synapse and activate receptors on the receiving postsynaptic cell. If the target cell is another neuron the process can start all over again.

Table 1. Examples of ions and neurotransmitters involved in cell signalling.

Signalling species	Selected functions	In this thesis
Ions		
Na^+	Involved in the initiation of action potentials.	Paper I and II
K^+	Maintain the resting potential in excitable cells.	
Ca^{2+}	Important second messenger in cell signalling, dictates release of neurotransmitters.	Paper I-IV
Neurotransmitters		
Acetylcholine	Neurotransmitter in the CNS and PNS.	Paper I and III
Aspartate	Excitatory neurotransmitter in the CNS.	Paper IV
Dopamine	Excitatory or inhibitory depending on type of receptor.	-
GABA	Major inhibitory neurotransmitter in CNS.	Paper IV
Glutamate	Major excitatory neurotransmitter in the CNS.	Paper IV
Glycine	Inhibitory neurotransmitter in the CNS	-

2.2 CHOLINERGIC SIGNALLING

In the first half of the 20th century, scientists knew that individual neurons carry information in the form of small electrical currents which can be passed on to the neighbouring cell. The question remained: by which mechanism does the signal cross the small gap between the two adjacent neurons? The pharmacologist Otto Loewi performed the now-famous experiment using two isolated frog hearts placed in separate chambers filled with saline. Electric stimulation of the vagus nerve of the first heart decreased its heart rate. Transferring the perfusion fluid from this heart to the second chamber, the rate of heartbeat of the second heart would also slow down. Loewi's *vagusstoff* was later shown by Henry Dale to be acetylcholine (ACh), the first neurotransmitter to be discovered, and in 1936 the two scientists were awarded the Nobel Prize in Physiology or Medicine for this discovery [1]. ACh is one of the major neurotransmitters in the nervous systems and neurons containing ACh, cholinergic cells, are involved in signalling in both the CNS and PNS. In the CNS, cholinergic neurons are located in the basal fore brain from where they project to the hippocampus, amygdala and cerebral cortex [2]. In the PNS, ACh plays an important role in the synaptic transmission at the skeletal neuromuscular junctions [3].

The cholinergic receptors are divided into nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs). The nAChR is named for its affinity to the natural occurring CNS stimulant nicotine obtained from the tobacco plant. This ionotropic nAChR is directly linked to a nonselective transmembrane cation channel that generates excitatory postsynaptic responses. The nAChRs can be divided in subgroups of neuronal and muscular types and are built up from a combination of five protein subunits (α, β, γ, δ, ε) clustering around the receptor channel. The metabotropic mAChR G-protein linked receptor

is named for its activation by muscarine, a poinsonous chemical naturally found in mushrooms. The mAChRs exist in five isoforms (M1-M5), which activate two different signalling pathways. M1, M3 and M5 activate of phospholipase C (PLC) and M2 and M4 inhibit adenylyl cyclase activity [4].

Changes in cholinergic signalling or even loss of cholinergic neurons are observed in a number of progressive neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [5, 6]. Development of therapies has therefore been targeted towards the molecular players involved, especially inhibitors of ACh esterase as well as agonists to nAChRs and mAChRs [7, 8]. Understanding of the mechanisms and functions of cholinergic signalling has been greatly benefitted from the use of endogenous ACh but also from cholinergic agonists such as natural chemical compounds, e.g. nicotine and muscarine, or synthetically produced substances, e.g. carbachol, methacholine and oxotremorine methiodide (oxo-M) [9-11].

2.3 CALCIUM SIGNALLING

Ion fluxes are important in all living systems. In particular, the Ca^{2+} ion plays a vital role as second messenger. The Ca^{2+} ion is a highly versatile second messenger that regulates many different cellular responses and functions. Ca^{2+} signalling plays an important role in signal transduction pathways, for example in muscle contractions, cell migration and fertilization [12]. Ca^{2+} signals are often organised in complex spatial and temporal patterns creating different duration and concentration ranges. The propagation of Ca^{2+} signals can be described as oscillations, waves, spikes, or puffs, and are essential for cell survival since sustained high Ca^{2+} concentrations in the cytoplasm are toxic [13]. In the synaptic junction Ca^{2+} triggers exocytosis in the range of microseconds while Ca^{2+} signalling may operate in the minutes to hours range when driving events such as gene transcription and cell proliferation [12].

Calcium regulation

The Ca^{2+} signalling systems of mammalian cells utilize an extensive signalling toolkit to both receive and present highly complex signalling patterns. This advanced machinery consists of sensory mechanisms, channels and transporters that together regulate the gradients across the plasma membrane and intracellular stores (Figure 2). In the eukaryotic cell the resting intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is maintained at approximately 100 nM while the extracellular $[Ca^{2+}]$ is over 1 mM. Cells also have access to intracellular stores located primarily in the sarcoplasmic reticulum (SR) or endoplasmic reticulum (ER), which may contain free Ca^{2+} in the range of mM. The dynamic changes in the $[Ca^{2+}]_i$ from the activities of ion channels and regulators can be visualized by fluorescent-based Ca^{2+} imaging. Today, there is a wide range of synthetic Ca^{2+} sensitive fluorophores as well as genetically encoded Ca^{2+} indicators.

Ca^{2+} channels located on the plasma membrane introduce Ca^{2+} into the cytoplasm. Channels regulating Ca^{2+} entry are either voltage operated Ca^{2+} channels (VOCCs) or receptor operated channels (ROCCs). While the VOCC opens upon depolarization of the plasma membrane, binding of a ligand activates the ROCC. The expression of ROCCs varies depending on cell type, two examples being the nAChR and the metabotropic glutamate receptors (mGluR). When the $[Ca^{2+}]_i$ increases pumps, exchangers and buffers will remove Ca^{2+} from the cytoplasm in order to avoid toxicity. Plasma-membrane Ca^{2+}-ATPases (PMCAs) use adenosine-5´triphosphate (ATP) to pump Ca^{2+} against the electrochemical gradient and in Na^+/Ca^{2+} ion exchangers (NCXs) one cytoplasmic Ca^{2+} ion is exchanged for three extracellular Na^+ ions.

The ER membranes also contain Ca^{2+} channels and Ca^{2+} pumps to regulate the cytoplasmic Ca^{2+} level. Release of Ca^{2+} from intracellular stores occurs from channels that are

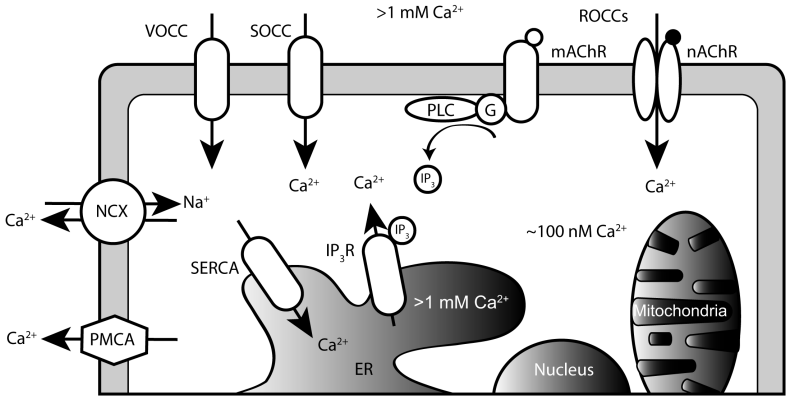


Figure 2. Cellular regulators in Ca^{2+} signalling.

classified in two families, the inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs). The IP_3R is gated by the ligand IP_3 while the RyR is a Ca^{2+}-gated channel activated by several different modulators, for example caffeine. Upon activation of PLC, the enzyme cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into two intracellular messengers, diacylglycerol (DAG) and IP_3. DAG is hydrophobic and remains in the plasma membrane where it is an activator of the Ca^{2+} sensitive enzyme protein kinase C (PKC) that is important in many signal transduction pathways. IP_3 diffuses into the cytosol where binding to its receptor IP_3R result in release of Ca^{2+} into the cytosol. IP_3R also contain a Ca^{2+} binding domain modulating the release from the intracellular stores. Moderate $[Ca^{2+}]_i$ activate while high $[Ca^{2+}]_i$ inhibit release. This Ca^{2+} dependent Ca^{2+} release from IP_3R is also known as Ca^{2+} induced Ca^{2+} release (CICR) [12, 14]. Re-filling intracellular stores with Ca^{2+} is performed by channels located on the SR/ER known as SR Ca^{2+}-ATPases (SERCAs). The $[Ca^{2+}]_i$ level is also regulated by the Ca^{2+}-binding protein calmodulin (CaM). Upon binding of Ca^{2+}, CaM changes conformation and binds to VOCCs and IP_3Rs [15, 16]. Re-filling of the stores is also accomplished by an intracellular event, triggering opening of channels in the plasma membrane, which is described in the next paragraph.

Store-operated calcium entry

Store-operated Ca^{2+}entry (SOCE), also know as capacitative Ca^{2+} entry, is a process in which emptying of intracellular Ca^{2+} stores initiates entry of Ca^{2+} over the plasma membrane. SOCE was first described in 1986 by Putney et al. from observations that emptying Ca^{2+} stores activated channels in the plasma membrane to help refill the stores [17]. The molecular basis for SOCE remained a mystery for a long time and over the years researchers have struggled to find the molecular players involved in this process. Transient receptor potential (TRP) channels were suggested to contribute to the Ca^{2+} influx upon store depletion. Among the seven TRP channels, the canonical channels TRPC1, 3, 4, 5 and 7 have been reported to be involved in SOCE [18]. In 2005 two independent research groups identified a protein liking the mechanisms of SOCE to activation of Ca^{2+} stores [19, 20]. Stromal interaction molecule 1 (STIM1) contains an EF-motif functioning as a Ca^{2+} sensor in the ER. As the intracellular Ca^{2+} stores in the ER are depleted, STIM1 is transported to the plasma membrane where it interacts with Orai1, the pore-forming subunit of the highly Ca^{2+} selective SOCCs [21]. Opening of SOCCs allow Ca^{2+} to flow into the cell and being a reversible process SOCE terminates in response to refilling of the intracellular Ca^{2+} stores [22].

Temporal dynamics of calcium fluxes

When information has to be retained over longer periods of time, the Ca^{2+} signalling system use repetitive transients, i.e. $[Ca^{2+}]_i$ elevations followed by rapid decays [12]. These types of discharges, also known as Ca^{2+} oscillations, allow the cell to avoid toxic effects of sustained high Ca^{2+} levels. Ca^{2+} oscillations have been described in analogy with the amplitude modulation (AM) and frequency modulation (FM) in electronic communication [23]. AM refers to differences in $[Ca^{2+}]_i$ signal strength whereas FM refers to the interval of $[Ca^{2+}]_i$ elevations. In 1997 Dolmetsch et al. demonstrated how downstream effectors could decode the information contained in the amplitude and frequency of Ca^{2+} signals [24]. Ca^{2+} oscillations have also been demonstrated to create specificity for diverse cellular mechanisms and activation of a range of biological processes including cytokine release in renal proximal tubule cells, oocyte activation and determination of cell neurotransmitter phenotype [25-27]. The mechanism of generating Ca^{2+}oscillations may vary with cell type and examples of agonists shown to trigger oscillations include, α-hemolysin, glutamate acting on mGluR and ATP [12, 25, 28].

Calcium signalling in SH-SY5Y cells

The human neuroblastoma cell line SH-SY5Y is a subclone originating from the neuronal cell line SK-N-SH, derived from a biopsy of metastatic neuroblastoma site in a young girl [29]. Despite being an already defined cell type, SH-SY5Y cells exhibit some of the biochemical and functional properties of neurons making this nerve like cell type a useful *in vitro* model. These electrically excitable cells possess both types of cholinergic receptors (nAChR and mAChR) and have been found to be responsive to treatments with ACh, nicotine, muscarine, carbachol, methacholine and oxo-M [9-11, 30-34]. The SH-SY5Y plasma membrane contain both L- and N-type VOCCs and express three of the major Ca^{2+} regulating ER proteins: SERCA, IP_3R and RyR [35, 36]. Consequently, the cell line is a well-established experimental model for Ca^{2+} signalling studies of e.g. voltage dependent Ca^{2+} entry, receptor-mediated Ca^{2+} homeostasis and SOCE [11, 36, 37]. Presence of SOCCs in the SH-SY5Y cell has been indicated by stimulation with carbachol, thapsigargin and oxo-M [9, 11, 37]. In 2005 SH-SY5Y cells were used by one of the two independent research groups that identified the protein STIM1, linking the mechanisms of SOCE to activation of Ca^{2+} stores [19]. The three subtypes TRPC1, 3 and 5, know to be involved in SOCE, have also been shown to be expressed in SH-SY5Y cells [38].

2.4 AUDITORY SIGNALLING

The specificity of Ca^{2+} oscillations is determined by the amplitude and frequency of the signal. Comparably, sound can be described as the propagation of an oscillation, that is, a mechanical wave, defined by its amplitude and frequency. Auditory transduction is the process where a sequence of events transforms sound waves in the air into electrical impulses interpreted by the brain. Sound waves enter the ear and are transported along the hearing duct where they set the cone shaped eardrum in vibration. Low frequency sound produces a slow rate of vibration while higher frequency sound produces faster vibrations. Similarly, low amplitude sound produces less dramatic vibrations compared to high amplitude sound. The eardrum passes on the information of amplitude and frequency over to the auditory ossicles (Figure 3a). Here, vibrations travel to the footplate of the stapes, on to the oval window membrane and into the fluid filled bony labyrinth. The piston like movement of the stapes displaces the perilymph fluid in the bony labyrinth, which is possible due to the flexibility of another membrane, the round window membrane (RWM). Consequently, vibrations are transduced into the snail shell shaped cochlea. In the cochlea, the basal membrane and the Reissner's membrane divide the space into three fluid filled compartments: scala vestibuli, scala media (also known as the cochlear duct)

and scala tympani. Vibrations from the oval window membrane travel, through the ascending passage of scala vestibuli, over the cochlear duct, and return through the descending passage of scala tympani. The basal membrane is one of the components responsible for frequency tuning. Low frequency sounds vibrate the more flexible basilar membrane towards the apex of the cochlea, while high frequency sounds induce vibrations closer to the base where the membrane is stiffer (Figure 3b). The human cochlea typically detects frequencies in the range of 20 – 20,000 Hz and this topographical mapping of frequencies is known as tonotopic organization. Within the basal membrane lays the hearing organ, the organ of Corti, containing auditory sensory cells responsible for generation of the nerve impulses sent to the brain.

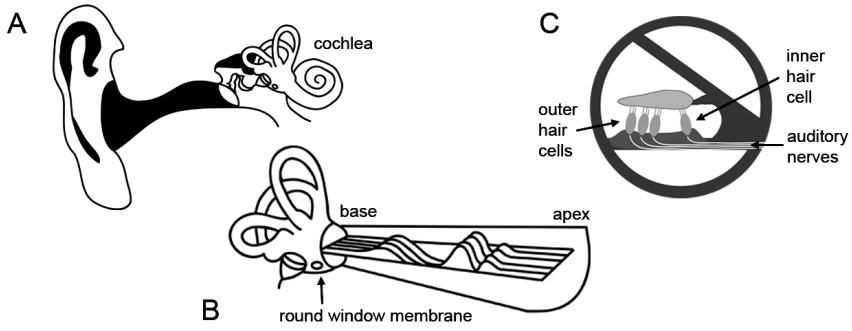


Figure 3. The ear converts sound waves into neural signals. **A)** The outer, middle and inner ear. **B)** Unrolled cochlea with a stretched basilar membrane. The vibrations generate a wave traveling on the basilar membrane in the cochlea. **C)** Cross section of the organ of Corti in the cochlea.

Inner hair cells

There are two types of auditory sensory cells, the inner and outer hair cells (Figure 3c). The outer hair cells (OHCs) amplify and tune the signal while inner hair cells (IHCs) are responsible for transferring the electric impulses to the primary auditory neurons, the spiral ganglion neurons (SGNs). As the basal membrane vibrates, stereocilia "hairs" on the apical pole of the hair cell are deflected against the tectorial membrane closely covering the hair cells. The deflection causes opening of mechanosensitive ion channels and an influx of K^+ depolarizes the cell. The altered membrane potential results in opening of VOCCs and subsequent Ca^{2+} influx causes transmitter release. Glutamate is the primary neurotransmitter for the IHC and when released it stimulates dendrites on the postsynaptic cell, the SGN [39]. The afferent SGN receives the signal and transfers it via the axon, in a bundle forming the auditory nerve, to the cochlear nuclei in the brain stem. Exposure to traumatic noise will cause overstimulation of IHCs and an excessive release of glutamate at the IHCs' afferent synapses. Glutamate overstimulation at the dendrites of postsynaptic cells will in turn cause massive entry of cations and water, subsequent dendrite swelling and loss of contact between IHCs and SGNs dendrites [40]. The excitotoxic effect of glutamate disturbs the Ca^{2+} homeostasis in the SGN and can lead to cell damage and death.

The auditory sensory system represents a good *in vivo* model for a "machine-to-brain" interface, as the cochlea provides relatively easy access to the sensory organ and allows a direct communication pathway between the device and the brain. Hence, the auditory system has been used extensively in validation of local delivery systems as the effect on the auditory nerve can be monitored [41, 42].

3 TECHNIQUES TO REGULATE CELL SIGNALLING

Cell biologists study cell communication in cell cultures, tissues and animal models and to elucidate the mechanisms behind cell signalling the cells must be exposed to stimuli. Neuronal cells can be stimulated by a number of chemical substances, e.g. neurotransmitters and drugs, and physical factors, such as pressure, temperature, light and electric fields. By inducing signalling pathways with appropriate stimuli it is possible to characterize the players participating in the dynamic cell signalling processes. These players provide clues that aid in understanding of cellular physiology and possibly in the development of treatment for neurophysiological disorders. The following section describes different methods used for stimulating cells *in vitro* and *in vivo*.

3.1 STIMULATING CELLS *IN VITRO* AND *IN VIVO*

Two of the most commonly used techniques to investigate signal transduction pathways in cells *in vitro* are live cell imaging and electrophysiology. The different types of techniques used to stimulate single or multiple cells in these set-ups can be divided into flow based and non-flow based (Table 2). Some of these methods are also valuable research tools for *in vivo* experiments, however, only a few techniques currently have the potential to be developed into new medical devices.

Table 2. Examples of delivery techniques used for stimulation of cells[1].

Technique	Possibility to calculate delivered concentration	Spatial resolution	Temporal resolution	Clinical applicability	References
Flow based					
Bath application	++[2]	n/a	+	n/a	[11, 43]
Puffer pipette	++	++	++	n/a	[44-46]
Microfluidics	++	+	+	+	[47-49]
Non-flow based					
Electric stimulation	-	n/a	++	+++	[50-52]
Uncaging	+	+++	+++	n/a	[53-55]
Optogenetics	-	+++	+++	n/a	[56, 57]
Iontophoresis	+	++	+	++	[58-60]

[1]Table compiled partly based on information from references.

[2] n/a = not applicable; - = low; + = good; ++ = very good; +++ = excellent

Flow-based delivery of stimuli

Bath application: In the majority of contemporary delivery techniques, the stimuli are dissolved in a liquid, which is introduced to the target system by liquid flow. Most commonly the liquid is manually added by a pipette. Cells can also be superfused using flow chambers and perfusion systems controlled by motor- or gravity-driven pumps. As a solution flows over surface-attached cells they are exposed to shear stress from the fluid flow. Mechanical stress from the flow may stimulate cells expressing mechanosensitive ion channels. This can be used as a tool to induce cell signalling in studies of the subsequent modulation of physiological processes at the molecular as well as cellular level [61]. On the other hand, high flow rates are likely to change cell morphology or cause cells to detach, but even at low flow rates exerted forces may induce other signalling pathways than those intended to be studied. Liquid flow will also wash away cell-secreted factors used in cell-to-cell communication, altering the cellular microenvironment and potentially changing the cell state [47]. In addition to the physiological

considerations, practical limitations such as trapped air bubbles interfering with the imaging acquisition or introduction of excess fluid in the target system can also be cumbersome in flow based delivery methods. In bath applications, the entire cell population is stimulated. To achieve higher spatial resolution the delivery has to be refined.

Puffer pipette: Local delivery of stimuli can be achieved by pressure-ejection from a fluid filled glass pipette, also known as a puffer pipette [44]. The glass pipette is filled with a solution containing the stimulus, which is released in the vicinity of the cell upon application of a brief pressure-pulse. As the delivered solution mix with the bath solution it is difficult to determine the exact concentration arriving at the cell. Leakage from the glass micropipette tip can result in problems when recording the baseline and sometimes a "push-pull" mode can be applied to avoid this. The puffer pipette technique is especially valuable when stimulating only one or a few cells in an explant, e.g. brain slice, as the local delivery allow for several experiments in the same preparation and low consumption of potentially expensive stimuli.

Microfluidics: The development of small, portable microfluidic systems has enabled further reduction of volumes and consumption of delivered stimuli in various cell signalling studies. Microfluidic systems were originally made of hard glass or silicon, but today the softer silicone-based organic polymer poly(dimethylsiloxane) (PDMS) is used for most biological applications. Examples of PDMS microfluidics are generation of chemical gradients for probing cellular Ca^{2+} dynamics and solution switching for rapidly applying brief (400 μs) neurotransmitter pulses [48, 49]. To operate microfluidic devices, they need to be connected to tubes, valves and external pumps. Such equipment makes them altogether bulky and in some perspectives unfitting for development towards implantation. Microfabricated microfluidic devices for local drug delivery have, however, been tested and evaluated for *in vivo* applications [62]. The guinea pig is an ideal animal model for the auditory system as the structure of the cochlea and the range of hearing frequencies are similar to that of the humans. But it is also a challenging target as the auditory cells are mechanosensitive and the volume of the scala tympani is only 8 μl in guinea pigs as compared to 30 μl in humans [63]. Utilizing ultra low flow rates and infuse/withdraw methods for a zero net volume transfer have been demonstrated in local delivery of stimuli in the cochlea [41].

Non-flow based delivery of stimuli

Electric stimulation: Excitable cells respond to depolarizing electric fields. As described earlier, the action potential is an important component in neural communication, and stimulation and recording of action potentials forms the basis for the functionality of modern neural probes. Some of today's most well-established neural prostheses are based on electrical stimulation of cells and tissues. The cochlear implant bypass the damaged part of the ear and provides direct electronic stimulation of the auditory nerves. In deep brain stimulation, surgically implanted probes send electrical impulses to suppress symptoms in patients with for example Parkinson's disease [51]. It is believed that electrical stimulation might be based on depolarization of the semi-permeable cell membranes at the axon of neuronal cells and subsequent generation of action potentials [52]. As electric stimulation bypass the chemical input it does not discriminate different types of cells in the vicinity of the electrode.

Photostimulation: To obtain a more specific physical activation, photostimulation techniques based on light-activation of chemically or genetically modified receptors or ion channels can be used (optogenetics). This type of optical stimulation can be used to induce hyperpolarizing or depolarizing cells responses with high spatiotemporal resolution. The channelrhodopsins are naturally occurring light-gated ion channels, which can be genetically targeted and thereby used for cell-type-specific stimulation [56]. Receptors and ion channels can also be chemically or genetically modified to become light-activated, for example by introducing a genetically

encoded synthetic photoswitch to a receptor [57]. A second type of photostimulation technique result in chemical stimulation by light-mediated release, or uncaging, of biologically inactivated compounds. In the chemical stimulation method, biologically active molecules made inert by binding of a light sensitive group are flooded over the preparation [53]. When illuminated with light of an appropriate wavelength the caged groups absorb photons, breaking the bond between the caged group and the stimuli. Caged compounds can be ions, neurotransmitters, fluorescent probes or other molecules, which will be released in the intra- or extracellular environment. Combining cages with different compounds that are activated at individual wavelengths, allow generation of highly complex multi-site activation patterns [54]. Although the photoactivation methods will remain valuable research tools both *in vitro* and *in vivo*, their inherent limitations will prevent clinical staging.

Iontophoresis: A non-flow based delivery technique that is already in clinical use is iontophoresis, a non-invasive transdermal delivery of charged molecules for both localized treatments and systemic targets [59, 60]. Iontophoresis is best described as a type of electrophoresis, where a charged field is created between two electrodes in a solution and small charged ions or molecules are delivered by electromigration and electroosmosis (current-induced convective flow of water) [64]. The same principle is applied when the technique is used for fine-tuned delivery of chemical substances *in vitro*. These types of micro-iontophoresis systems can be a glass micropipette or a container on a carbon fiber microelectrode [46, 58]. Even though the set-up is similar to the previously described puffer pipette, iontophoresis does not require a flow for delivery. Instead, applying a potential over the ion solution, a constant electric field will cause ions to move out from the tip of the micropipette. Iontophoresis requires high a concentration of the stimulus in the micropipette in order to carry the iontophoretic current [46]. Micropipettes with multiple barrels allow delivery of several compounds on the same cell and screening of a variety of substances [58].

Collectively, the presented techniques are examples of methods used in cell signalling studies *in vitro* and *in vivo*. Flow-based techniques provide good control over applied concentrations but induce convection in the target system and generally have less spatiotemporal resolution compared to the non-flow based methods. On the other hand, stimulating cells with highly spatiotemporal non-flow methods is often complicated and it is difficult to quantify amount of applied stimuli. Hence, there is a need for a new technology that enables electronic control of non-flow based delivery, with high spatiotemporal resolution and the capability to calculate delivered amount of stimuli. Technology, such as that presented here, will allow for development towards new medical devices, which can restore malfunctioning biological systems.

4 ORGANIC ELECTRONICS

Polymers are macromolecules composed of repeating structural subunits called monomers. Nature has created a vast number of polymers essential for life, for example nucleic acids (DNA and RNA), proteins and carbohydrates. Polymers can also be synthetically manufactured and are then commonly known as plastics. From the first commercially produced plastic (Bakelit) in the beginning of the 20th century, plastics have contributed to our quality of life in an endless number of ways. Polymers have traditionally been regarded as poor electronic conductors and consequently been frequently used as insulators in electronic equipment. In 1977 A. Heeger, A. MacDiarmid and H. Shirakawa made the revolutionary discovery that polymers can, after some modifications, be made electrically conductive [65]. In 2000 they were awarded with the Nobel Prize in chemistry *"for the discovery and development of conductive polymers"*.

4.1 CONDUCTING POLYMERS

Organic polymers are carbon based macromolecules of several repeated molecular units building up long chains. The repeating units are coupled to each other by covalent bonds and form the backbone of the polymer. Carbon backbones decorated with hydrogen form the simplest of all organic compounds, hydrocarbons. The simplest organic polymer is poly(ethylene) (Figure 4a), which can be obtained by polymerization of ethylene. In polyethylene each of the carbons' four valence electrons participates in bonds to carbons or hydrogens. With the electrons strongly localized in the bonds, poly(ethylene) makes a good insulator. To achieve electronic conductivity in a polymer all the electrons cannot be localized in bonds but some have to be delocalized (mobile) along the polymer backbone. The simplest conducting polymer is poly(acetylene), in which each monomer consists of two carbons joined by a double bond and two hydrogens (Figure 4b). In conjugated double bonds, each carbon has three of the four valence electrons occupied in the sp^2 orbitals, forming strong bonds (σ-bonds) with neighbouring atoms. The remaining electron is in the p_z orbital and forms a π-bond by overlapping p_z orbitals of neighbouring electrons. These π-bonds can then hybridize with each other, forming molecular orbitals within which electrons become relatively mobile, and this mobility of electrons is the key to the conductivity of the material.

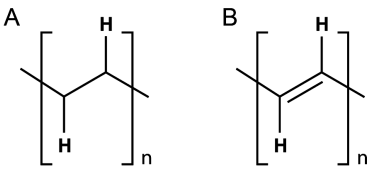


Figure 4. Chemical structures of **A)** poly(ethylene) and **B)** poly(acetylene).

Doping

In their neutral form, conjugated polymers are actually poor conductors due to a low concentration of free charge carriers. By introducing mobile charge carriers in the system, the conductivity of a polymer can be increased several orders of magnitude. This method, often referred to as doping, can be achieved by chemical and electrochemical methods. In chemical doping, a reactive doping agent is introduced which oxidizes/reduces the polymer. After the

reaction the dopant stays in the polymer system to balance the charge of the oxidized/reduced polymer. Electrochemical doping is achieved by applying a potential to the polymer while in contact with an electrolyte. When the conjugated polymer backbone is oxidized, referred to as positive doping (p-doping), electrons are removed from the valence bands creating positively charged "holes". Negative doping (n-doping) is equivalent to reducing the polymer by introducing electrons and increasing the number of negative charges. Conjugated polymer devices are mainly p-doped, since most n-doped conjugated polymers easily react with oxygen.

PEDOT:PSS

Poly(3,4-ethylenedioxythiophene) (PEDOT) is an example of a conjugated polymer which is partially oxidized in its pristine state and thus highly conductive (Figure 5). Oxidized PEDOT carry positive charges, which have to be compensated by negative ions to maintain charge neutrality and stability within the polymer system. To achieve this, poly(styrenesulfonate) (PSS) can be used as a doping agent, as the negatively charged sulfonyl group in PSS balances the positive charges of PEDOT [66]. Doping PEDOT with PSS creates the chemically stable polymer-polyelectrolyte system PEDOT:PSS (Figure 5).

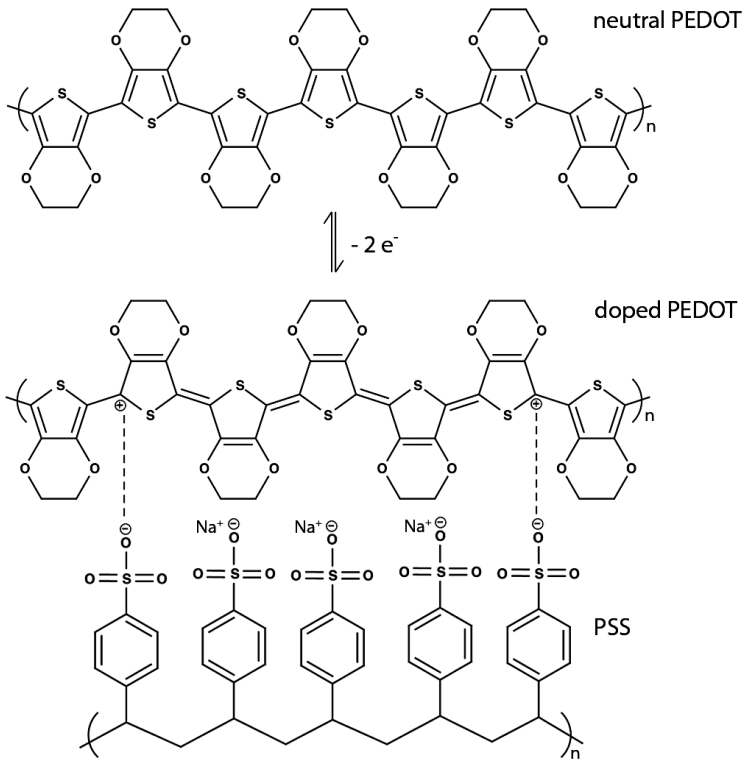


Figure 5. Chemical structures of **A)** neutral poly(3,4-ethylenedioxythiophene) (PEDOT) and **B)** PEDOT doped with poly(styrenesulfonate) (PSS) making up the polymer-polyelectrolyte blend PEDOT:PSS.

While the conductive properties of metals and inorganic electronics depend solely on electrons, conjugated polymers such as PEDOT:PSS can conduct both electrons and ions. PEDOT provides electronic conductivity, while PSS provides both enhanced electronic conductivity and cationic conductivity. When a potential is applied to PEDOT:PSS in an electrochemical cell, redox reactions dependent on transport of both mobile cations (M^+) and electrons (e^-) will take place (Reaction 1).

$$PEDOT^+PSS^- + M^+ + e^- \rightleftarrows PEDOT^0 + M^+PSS^- \qquad \text{Reaction 1.}$$

The reaction to the right represents reduction of PEDOT and the reaction to the left oxidation of PEDOT. PEDOT can be reversible switched between its conducting ($PEDOT^+$) and low-conducting ($PEDOT^0$) state.

In addition to the chemical and electrical characteristics, PEDOT:PSS also exhibit optical properties. A PEDOT:PSS film in its oxidized state is almost transparent while reduction of the material results in a dark blue (reversible) colour. All together, the chemical, electrical and optical properties of PEDOT:PSS has made it useful in numerous applications such as light emitting diodes and solar cells [67, 68]. Over the past years, the integration of the soft, flexible and organic structure of conjugated polymers with biological systems has opened for a new branch of organic electronics to emerge.

5 ORGANIC BIOELECTRONICS

Bioelectronics is an interdisciplinary research field where elements of physics, electronics, materials science and biology merge. The focus of this emerging field is on the development of interfaces between biological materials and micro- and nano-electronics. The discovery of conductive polymers was the beginning of a great advance, as scientists started to explore how the ionic and electronic properties and mechanisms of organic materials could provide a unique transition from the "hard" electronics to the "soft" biology. This was the beginning of a new field of science, *Organic Bioelectronics* [69], where organic electronic materials create novel communication interfaces for monitoring and regulating cellular functions.

5.1 CONJUGATED POLYMERS IN BIOMEDICAL APPLICATIONS

The concept of biocompatibility is central in biomaterial science. Although its definition will vary with the application, the simplest description may be found in the origin of the words. As *bio* means "life" and *compatible* means "fit right", biocompatible would mean "fit right with life". Today, there is a wide range of tests for determining biological responses to materials. Two of the most commonly studied conjugated polymers, PEDOT and polypyrrole (PPy), are considered to have good compatibility with a range of biological systems [70-72]. These properties and the fact that they are both highly conductive and straightforward to produce have promoted the use of PEDOT and PPy in various biomedical applications.

Neural applications

Electrical stimulation techniques are predominant among the treatments of neurological disorders. Stimulation of neuronal tissue is achieved by surgically implanting electrodes into a specific target tissue. The introduction of these probes often results in immunological responses and scar formations, which reduce the sensitivity of signal transmission over the electrode-neuron interface [73]. By increasing the biocompatibility of the electrode, the immunological response and subsequent scar formation can be reduced [74]. This can result in an improvement in the long-time performance of the implant and an increased sensitivity in applications for both stimulating and/or recording cell activities.

Conjugated polymer coatings have been shown to enhance both stimulation and recording at neural microelectrode interfaces. Microelectrodes of indium tin oxide have been shown to evoke a greater response when coated with PEDOT:PSS, compared to non-coated, when stimulating cellular networks *in vitro* [75]. Both PEDOT and PPy have been shown to promote neural attachment and neurite outgrowth both with and without electrical stimulation [76, 77]. The enhanced nerve generation on electrically stimulated PPy has partly been ascribed to the antioxidant properties of PPy, as scavenging of free radicals at the site of injury minimized scare formation, allowing more efficient neural regeneration [78].

To further promote neural growth and minimize immune responses the polymers can be functionalized. Decorating polymers with biomolecules can increase cell viability, as exemplified by *in vivo* biocompatibility assays of PEDOT:heparin coated platinum probes [70]. Increased cell viability and functionality is also demonstrated by the incorporation of neurotrophins in PPy-films coated on the surfaces of cochlear implant electrodes. When implanted in guinea pigs the implant showed maintained electrical stimulation together with active neurotrophin release over time [79].

Bioactuators

Bioactuators made of conjugated polymers belong to the niche of devices taking advantage of the volume change these materials undergo during oxidation and reduction. The redox reactions result in ions entering or leaving the polymer together with water that take up space and result in structural changes of the polymer. The mechanical force generated in this transformation has been used in the development of polymeric microactuators, which can be used as pumps, artificial muscles or tools for mechanical stimulation and manipulation [80, 81]. Biomedical actuators have also been demonstrated as electrically controlled drug delivery systems, often exemplified by release of the synthetic anti-inflammatory drug dexamethasone. Electrically addressing drug-loaded nanotubes of PEDOT coated on microelectrodes result in a reduction of the polymer volume and thereby drug release [82]. The same principle of release has been described for dexamethasone incorporated in the backbone as well as in nanostructures made of PPy [83, 84].

Biosensors

Conjugated polymers are commonly used as sensing elements in organic transistors, a field that has been rapidly expanded over the last decade. This section provides a brief overview of contemporary applications for organic transistors for biosensing, but first the basic function of the traditional silicon transistor will be described.

The solid-state transistor is a three terminal semiconductor device in which the current between the first and second terminal can be controlled by applying an electrical signal to the third. This way, the transistor can be used to amplify and/or switch electronic signals. There are many different types of transistors, for example the field effect transistor (FET), which is used as a building block in integrated circuits. Another example is the bipolar junction transistor, in which the main current flows from the emitter to collector and is controlled by applying a voltage to the base (Figure 6). Bipolar junction transistors come in two types, the pnp and the npn, constructed by combinations of differently doped semiconductors.

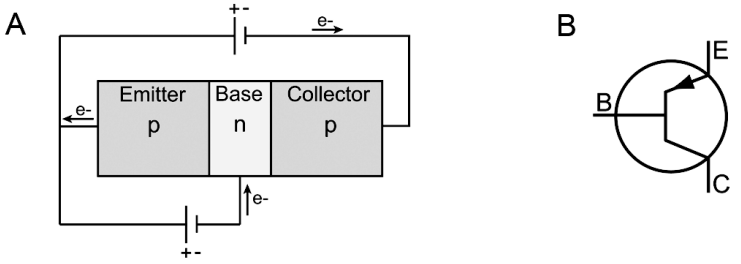


Figure 6. The pnp bipolar junction transistor. **A)** The materials in the emitter and collector are p-doped whereas the base is n-doped. In operating mode, the major current carriers between the emitter and collector are holes while electrons flow in the external circuit. A small emitter-base bias controls a large emitter-to-collector current. **B)** Schematic symbol for the pnp-type bipolar junction transistor.

Transistors can also be produced with organic semiconductors as the active component of the channel. The two major groups of organic transistors are organic field effect transistors (OFETs) and the organic electrochemical transistors (OECTs). OECTs have been applied extensively for biological and chemical sensing applications of redox active compounds [85]. Applying PEDOT:PSS as the active material has been demonstrated for detection of ions, glucose and DNA [86-88] but also for monitoring and regulating cell growth [89-91]. The OFET and OECT have different working principles in gating of the channel. In OFETs an

electric field modulates the conductivity of the semiconducting material whereas OECTs apply electrochemical redox reactions of the semiconducting material. Both methods result in modulation of the electric current and are based on the transport of electrons as the main charge carriers. Today, there are only a few examples of transistors utilizing ions as charge carriers to control ion flows. The majority of these devices are based on inorganic materials and the transistors produced by organic materials do not operate well under biological conditions [92-94].

5.2 THE ORGANIC ELECTRONIC ION PUMP (OEIP)

Some conjugated polymers are soft and flexible as well as function well in wet environments, which all are properties that make them suitable for integration with biological systems. Taking advantage of the combined electronic and ionic conductivity of PEDOT:PSS a new type of bioelectronic device was developed in 2007 - the organic electronic ion pump (OEIP) [71]. The OEIP is based on a PEDOT:PSS film patterned onto a polyethylene terephthalate (PET) substrate (Figure 7).

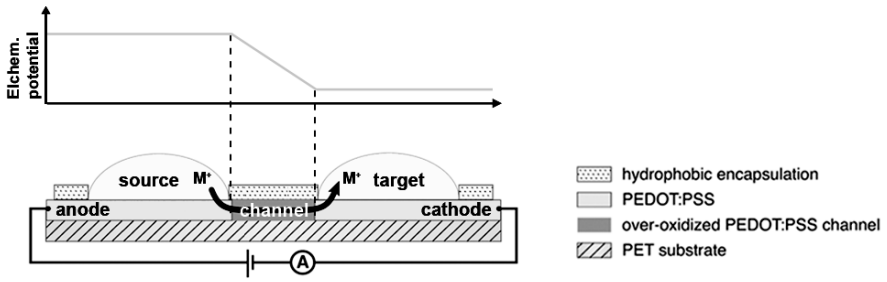


Figure 7. Schematic side view of the OEIP. The black arrow represents the transport of charged ions M^+ upon electrical addressing of the device. M^+ migrates from the source electrolyte, into the anode, through the over-oxidized channel, and out into the target electrolyte on top of the cathode. The electrochemical potential fall is confined to the over-oxidized channel.

The pattern consists of two electrodes, a source and a target, connected by a polymer channel. Over-oxidation of the channel with sodium hypochlorite disrupts the conjugation pathway of the PEDOT backbone, rendering the channel electronically insulating. As the over-oxidation process leaves the PSS phase intact, this creates a channel that conducts ions but not electrons. This is an important feature as it circumvents stimulating the cells with applied voltage/current. The channel and electrodes are covered by a hydrophobic photoresist, which provides openings for application of electrolytes on the two electrodes as well as for contact points for control electronics. The source electrolyte contains the positively charged ion to be delivered into the target electrolyte. When addressing the OEIP, the source electrode is oxidized and the target electrode is reduced according to the electrochemical half-reactions:

$$PEDOT^0 + M^+PSS^- \longrightarrow PEDOT^+PSS^- + M^+ + e^- \qquad \text{(Reaction 2a)}$$

$$PEDOT^+PSS^- + M^+ + e^- \longrightarrow PEDOT^0 + M^+PSS^- \qquad \text{(Reaction 2b)}$$

Oxidation of the source electrode (Reaction 2a) will result in M^+ from the source electrolyte to enter the source electrode (anode). The fall of potential over the ionically (but not electronically) conductive channel will result in migration of M^+ towards the reduced target

electrode (cathode, Reaction 2b). As the M^+ reaches the rim of the hydrophobic resist by the channel outlet, M^+ will diffuse up into the target electrolyte. Since the delivery of M^+ does not rely on aqueous flow, no convective disturbances are induced in the target electrolyte. Given by the electrochemical relationships of the equations, each transported ionic charge M^+ is compensated by an equal amount of transported electronic charge transferred between the electrodes. Hence, the current measured in the electronic branch of the circuit is directly proportional to the delivery rate of cations in the target electrolyte. This novel technique enables a unique way to electronically control the lateral transport and delivery of positively charged ions to an electrolyte.

The OEIP signal translation, converting an electronic input signal to chemical output, resembles the signal transmission in excitable cells (Figure 1). As nerve cells utilize ions in fluids as their major charge carriers the OEIP could be integrated with a biological system to induce signalling in excitable cells. The first ion demonstrated to be transported through the OEIP was the monovalent metal ion K^+ [71]. A solution containing K^+ was placed on top of the source electrode whereas neuronal cells were cultured on the target electrode in cell media. Applying a potential between the source and target electrodes initiated delivery of K^+ through the 4 mm wide polymer-channel. K^+ diffuse out from the channel in a high concentration that depolarizes the cell membrane and activate VOCCs. The resulting influx of Ca^{2+} was monitored using the Ca^{2+}-sensitive probe Fura-2 AM in microscopy-based real-time imaging. Turning the OEIP off stops K^+ transport and as delivered K^+ diffuses out in the bulk of the target electrolyte the cell re-establishes its Ca^{2+} homeostasis. As the OEIP delivery is spatially confined to the point of the channel outlet, the diffusion of delivered ions creates a concentration gradient in the target electrolyte. This was shown by transporting H^+ to the target electrolyte where a standard pH indicator paper was placed on top of the target electrode [95]. Within 2 min after application of the voltage a H^+ gradient was established from the outlet where a decrease of 2 pH units was observed. In addition to stable gradients, dynamic H^+ oscillations were achieved by utilizing the strict electronic on/off control of delivery. Cycling the voltage on/off resulted in similar behavior in H^+ transport [95].

The OEIP is produced from a transparent, flexible PEDOT:PSS film, commercially available from AGFA (Orgacon^{TM}) which is patterned using standard photolithography and printing techniques. Production of devices using microfabrication techniques enables integration with other organic or inorganic solid-state systems. Such combinations will allow for further development of an interface where complex, high-resolution, signal patterns are generated to control cell signalling. The precise electronic control of the OEIP makes it useful as a modulated – and potentially automated – chemical delivery system. In addition to its ease of fabrication, and thus its potential for easy incorporation into therapeutic devices, the OEIP technique provides several sought after features for cell signalling studies, listed in Table 3.

Table 3. Capabilities of the OEIP.

OEIP capabilities:	References:
1. Non flow-based delivery system with diffusive release.	[71]
2. Electronic addressing with on/off control.	[71]
3. Easy integration of the soft and flexible organic material in biological systems.	[71]
4. Delivery of the cations H^+, K^+ and Ca^{2+}.	[71] [95]
5. Delivery of single stimuli to cells *in vitro*.	[71]
6. Quantification of total amount of delivered ions and calculations of transport efficiencies.	[71]
7. Generation of temporal concentration gradients and oscillations.	[95]

6 AIM OF THE THESIS

The overall aim of this thesis is to design, develop and integrate organic bioelectronic delivery devices with biological systems.

Specific aims are to:

1. Characterize which compounds, such as ions, neurotransmitters and other biosubstances, that can be transported using the OEIP technology (Paper I, II and IV).

2. To develop a device that enables electronically controlled delivery of single or multiple stimuli with high spatiotemporal precision to study the dynamics of cholinergic signaling in nerve cells *in vitro* (Paper I, II and III).

3. To develop an organic bioelectronic communication interface, according to the "machine-to-brain" concept, to chemically modulate the nervous system *in vivo* (Paper IV).

7 RESULTS AND DISCUSSION

7.1 OEIP TRANSPORT OF IONS AND BIOMOLECULES (PAPER I, II, IV)

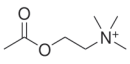
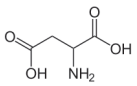
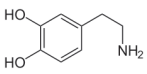
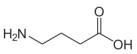
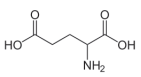
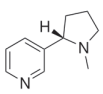
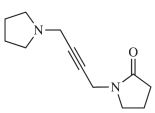
The first OEIP was published in 2007 and demonstrated electronically controlled transport and delivery of the smallest positive ion, the proton H^+, and the metal ions K^+ and Ca^{2+} [71] [95]. The successful integration with nerve cells inspired us to investigate if other biomolecules relevant for nerve cell signalling could be transported through the OEIP. The papers presented in this thesis describe the expansion of the OEIP transport repertoire with positively charged ions, neurotransmitters and other signalling molecules (Table 4). In paper I, we show successful transport of the neurotransmitter ACh. Paper II describes transport of Na^+, Ca^{2+} and Mn^{2+} as well as cholinergic substances carbachol, methacholine, muscarine, oxo-M and nicotine. In paper IV, we demonstrate transport of the neurotransmitters glutamate, GABA and aspartate.

Transport efficiency

Planar OEIP devices were used to investigate which ions/biomolecules could be transported (Figure 7). When PEDOT:PSS undergo a redox reaction (Reaction 1), the electronic charge transferred in the reduction of the polymer is balanced by an equal amount of ionic charge transported through the over-oxidized polymer-channel. The mobility of each transported ion in the channel is reflected by its net-charge, physical size and chemical structure. Together these parameters determine the transport efficiency of the ion and accordingly how effectively it will be delivered. The transport efficiency can be defined as the molecule-to-electron ratio, in which the total number of ions transported through the channel is compared to the total number of electrons transferred through the electronic branch of the circuit. The total number of electrons is calculated from the total charge transported in the driving circuit, which is obtained from recorded currents. To determine the total number of molecules delivered through the channel we applied different methods. These methods were high-performance liquid chromatography (HPLC), a fluorometric enzyme assay and liquid scintillation counting for measuring ^{3}H-labelled substances. Transport efficiencies for the different signaling species are presented in Table 4.

If every electron measured in the electronic part of the circuit corresponds to the delivery of one of the ions intended to be transported, this would represent a molecule-to-electron ratio of 1 and a transport efficiency of 100 %. Our data, however, show that calculated transport efficiencies vary widely depending on the substance. The low transport efficiencies of the neurotransmitters glutamate and aspartate can be explained by the fact that these two amino acids are transported in an excess of H^+ in the source solution. Dissolving glutamic/aspartic acid in water will result in an acidic solution and Glu/Asp molecules will exist in both protonated and de-protonated form. The Glu/Asp molecules with a net positive charge will have to compete with the small H^+ ions, which have higher mobility in the channel and therefore will be more readily transported [95]. Also, the electrochemical reactions in the polymer have shown to affect transport of certain substances, for example dopamine (unpublished data). Dopamine easily oxidizes to dopamine quinone, a reaction that takes place even at low potentials [96]. When the device, containing dopamine as source electrolyte, is addressed the recorded current is close to zero and the source electrolyte turns into a brown colour, a reaction indicating that dopamine is oxidized.

Table 4. Delivery repertoire and efficiencies of the OEIP.

Signalling species	Chemical structure	Transport efficiency	Reference
Ions			
Na^+		80 %	[97] Paper I
K^+		100 %	[71]
Ca^{2+}		100 %	[71] Paper II
Mn^{2+}		n.m.[2]	Paper II
Neurotransmitters			
Acetylcholine		100 %	Paper I
Aspartate		16 %	Paper IV
Dopamine		n.d.[1]	Unpublished
GABA		77 %	Paper IV
Glutamate		37 %	Paper IV
Glycine		ca 10 %	Unpublished
Cholinergic substances			
Nicotine		90 %	Paper II
Oxotremorine-M		99 %	Paper II

[1]not detected
[2]not measured

OEIP operation

OEIP delivery can be performed by operating the device in two different modes. In constant voltage mode a DC voltage is applied between the two electrodes and in a constant current mode a DC current is applied to the device. When the OEIP is turned on recordings of potentials/currents during operation of devices demonstrate that the OEIP technique provides a stable delivery over time. The results also show that the electronic addressing does not result in burst release, a phenomenon otherwise commonly encountered in different types of nanostructured delivery systems. In for example drug-loaded conjugated polymer actuator systems, which contract when electrically addressed, an initial rapid release is often followed

by a sustained slower delivery profile [82, 84]. One important factor to consider when deciding on delivery systems for a specific application is the amount of stimuli leakage from the system in the off-mode. When the OEIP is turned off, i.e. no potential or current is applied, the diffusion-mediated leakage from the source electrolyte to the target electrolyte is very small. The leakage of ACh was found to be below the detection level of used measuring method (0.3 μM). In previously reported paper, K^+ delivery showed an on/off ratio in delivery rate higher than 300 [71]. Hence, turning the OEIP to the off-mode the leakage is negligible, and compared to other delivery techniques the OEIP can easily be switched back on for another round of delivery. The straightforward on/off control through the OEIP software can be compared to measures taken to prevent interference of stimuli leakage in other delivery systems. Different push-and-pull methods are often used in several types of techniques. In iontophoresis, the leakage of stimuli from the micropipette is usually circumvented by applying a retaining current, i.e. reversing the current [46]. In puffer pipette application, the pipette can be moved from a stand-by position, some 100 μm away from the cell, towards the cell where the stimuli is delivered where after it is pulled back to the standby position [44]. In microfluidics systems, leakage problems are often solved by reciprocal pumping systems. By reversing the pressure pushing out the stimuli, leakage, as well as an increased target volume, is circumvented [98].

7.2 USING THE OEIP IN ACH-INDUCED CA^{2+} SIGNALLING (PAPER I)

One of the major neurotransmitters of the nervous system is ACh, a quaternary ammonium compound that maintains a positive charge across the full pH range. The combined structural and functional properties made ACh an interesting candidate for OEIP transport. Successful ACh transport was first confirmed using the original OEIP (Figure 7). This discovery inspired us to utilize conjugated polymer devices as a communication interface between electronic components and the nervous system.

Design of the 10 μm-OEIP

The original OEIP was designed with a 4 mm wide channel outlet to enable delivery of K^+ in the concentration range of 20-50 mM [71]. High K^+ concentrations are required to change the neurons' membrane potential resulting in a depolarization. Compared to K^+, ACh binds to specific receptors on the cell membrane and trigger cells at about 1000 times lower concentrations. Transport of ACh in a 4 mm wide channel resulted in extremely high ACh concentration. To reduce the number of transported ACh molecules and direct them to a single point of delivery, we miniaturized the polymer channel and created a 10 μm narrow outlet point (Figure 8).

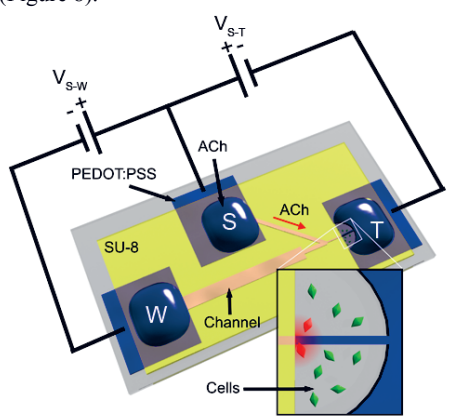


Figure 8. Design of the 10 μm-OEIP. The source (S), waste (W) and target (T) electrodes of electronically conducting PEDOT:PSS are connected by the cation selective channel (pink). ACh (red) is delivered at the 10 μm channel outlet where it spreads by diffusion to the SH-SY5Y cells (green) cultured on top of the target electrode.

The 10 μm outlet point was equal to or smaller than the dimensions of a single cell and resulted in spatial control of ACh delivery. The original OEIP design had a lag time of K^+ delivery of 2-4 minutes after the device was turned on [71]. To improve the delivery delay time through the channel, we incorporated a third electrode, the waste electrode. The waste electrode was used for pre-filling the channel system with ACh before the delivery to the target system.

Delivery of ACh to SH-SY5Y cells

To analyze whether the biological activity of the ACh was retained after transport, human SH-SY5Y neuroblastoma cell expressing AChRs were cultured on the target electrode and used as biosensors (Figure 8). The transport channel was pre-filled with ACh by applying $V_{S\text{-}W} = 20$ V for 10 min. The pre-filling systems resulted in a reduction of the initial delivery time, from minutes down to seconds, and thereby greatly improved the temporal resolution. In addition, the pre-filling system decreased delivery of undesired residual ions from the channel. ACh-delivery to the cells was then initiated by applying a potential between the source and target electrodes ($V_{S\text{-}T} = 5$ V). Delivery of biological active ACh was confirmed by monitoring the intracellular Ca^{2+} responses in cells located by the 10 μm outlet.

Turning delivery off stops ACh-transport and the local ACh concentration rapidly decreases as delivered ACh diffuse out in the bulk of the target electrolyte. As ACh spreads by diffusion it is possible to establish gradients in the target electrolyte. We used computer analyses to predict the evolution of the local [ACh] from the 10 μm outlet of the OEIP at application of a constant voltage ($V_{S\text{-}T} = 5$ V). Calculations were performed for concentration profiles ranging from a distance of 0 to 200 μm from the outlet over times of 5 to 100 seconds (Paper I, Fig 2c). To test the predicted [ACh] profiles, we again observed Ca^{2+} responses in cells (Figure 9a). Applying 5 V to the device initiated delivery of ACh at the outlet. Ca^{2+} responses were first observed in cells located closest to the outlet (50 μm), followed by responses in cells further away (150 μm) a few seconds later (Paper I, Fig 3a). These results were all in agreement with theoretically calculated concentration profiles. Control experiments delivering Na^+ do not result in Ca^{2+} responses and this implies that neither the electric field nor the potential affect the cells on the target electrode (Paper I, Fig 3B). This can be explained by the device design, as the electrochemical potential fall is confined to the over-oxidized channel (Figure 7).

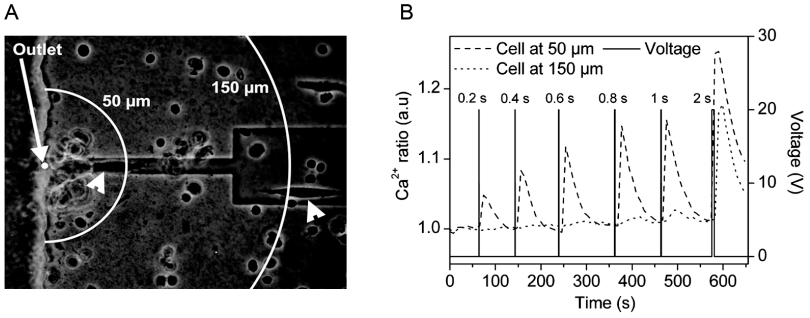


Figure 9. Temporal dynamics of Ca^{2+} signalling. **A)** Light microscopy image of SH-SY5Y cells located at 50 μm and 150 μm (arrow heads) from the 10-μm channel outlet. **B)** Ca^{2+} imaging of in cells in A) on delivery of ACh. Voltage pulses of 20 V for indicated times generated Ca^{2+} oscillations.

Controlling the amplitude of Ca^{2+} signals

The electronic nature of the OEIP delivery allows control of both the magnitude of applied voltage/current as well as the time of applied pulses. For a given pulse length, an increase in applied voltage results in an increase in delivery rate of ACh. As long as the cell is not desensitized, this will lead to an increase in the amplitude of the Ca^{2+} response (Paper I, Fig 3c). Correspondingly, prolonging the pulse length at a constant voltage can increase the amplitude of the Ca^{2+} response. The latter was demonstrated by modulating the amplitudes of Ca^{2+} response of cells located 50 μm and 150 μm from the outlet (Figur 9b). Whereas a cell located closest to the channel outlet was triggered by a 20 V pulse of 0.2 s, the cell located 150 μm from the outlet showed a prompt response first when the 20 V pulse was 2 s long. These results also demonstrate how pulsatile ACh-delivery enabled single cell stimulation with a spatial resolution of 100 μm (Figur 9b).

Controlling Ca^{2+} oscillations

Exposing cells to sustained high concentrations of ions or stimuli can be toxic. To avoid the toxic effects of high Ca^{2+} levels over a longer period of time cells utilize temporal patterns of $[Ca^{2+}]_i$. The 10 μm-OEIP functionality was tested in generation of temporal patterns mimicking the naturally occurring Ca^{2+} oscillations. The electronic on/off control of the 10 μm-OEIP makes it possible to adjust the release rate over time and entirely shut off the release of ACh, without any leakage. When the OEIP is turned off, ACh transport stops and cells are able to recover their intracellular Ca^{2+} homeostasis. By matching the length of the applied pulse of ACh-delivery with the Ca^{2+} response we were able to create Ca^{2+} oscillations. Brief pulses (0.2, 0.4 and 0.6 s) resulted in Ca^{2+} response in cells closest to the outlet (<50 μm), while extending the time of the applied pulses (0.8, 1.0 and 2.0 s) stimulated cells further away (>150 μm) (Figure 9b). Our data shows that the generated Ca^{2+} responses oscillated with a periodicity of about 100 s. This suggests that the OEIP technique will be useful in applications where modulation of amplitude and frequency components of Ca^{2+} responses in the second to minute range is required. Examples of Ca^{2+} fluctuations determining biological processes in this time range include oocyte activation at fertilization, determination of cell neurotransmitter phenotype and neuronal cell migration [26, 27, 99, 100].

Taken together, the results from the 10 μm-OEIP show tremendous improvements of both spatial and temporal resolution in the delivery of ACh as compared to delivery by the first design with a 4 mm channel. Comparing the performance of 10 μm-OEIP technique to other delivery methods, for example uncaging, there are differences and similarities. One feature in common for both techniques is tuning of delivered stimuli. Varying the strength of applied voltage/current pulse in the OEIP is comparable to varying the intensity of the activating light in uncaging, as more photolysis result in a higher concentration of stimuli [101]. The uncaging technique is still very beneficial for obtaining high spatiotemporal resolution of delivery with activation areas of a few μm^2 and speeds down to ms [55]. The OEIP technique is, however, not accompanied by the drawbacks of photolysis, such as possible effects of precursor, by-products and direct light.

Iontophoresis is a delivery technique that shares many similarities with the electrophoretic function of the OEIP as it utilizes an electric field for transport of charged ions. However, as iontophoresis generally involves transport of charged species through a liquid both electromigration and electroosmosis are observed [64]. Furthermore, the delivery depends on the molecular conductivity of the stimuli, so it can be difficult to quantify delivered stimuli [45, 64]. One of the important findings in paper I is that the electronic control of electromigration through the cation selective OEIP channel provides control over the number of delivered

ACh molecules. This enables predictions of concentrations at the outlet and allows for calculations of the ion bulk concentration of the target electrolyte. Hypothetically, the electronic control of OEIP transport could be used to fine-tune the delivery down to release of single molecules. In conclusion, the 10 µm-OEIP set the stage for delivery of neurotransmitter regulated Ca^{2+} signalling and development towards more complex addressing schemes.

7.3 MULTIPLEX DELIVERY ACTIVATE DEFINED CHOLINERGIC SIGNAL TRANSDUCTION PATHWAYS (PAPER II)

Paper II followed up on the findings from ACh-delivery in paper I by increasing the complexity of the OEIP delivery technique. Today, most cell signaling studies are performed by introduction of a single type of stimuli that initiates a cascade of signalling events. Cell signalling processes, however, often depends on multiple inputs with signalling pathways that act in synergy [102]. Detailed studies of such pathways are hampered due to the limited number of stimuli that can be applied with existing delivery techniques. To address this limitation, we developed a device for delivery of multiple stimuli, the OEIP multiplexer, enabling simultaneous and/or sequential delivery of appropriate combinations of ions and/or biomolecules (Paper II).

Transport of cholinergic substances in the OEIP multiplexer

The OEIP multiplexer is designed with five electrodes and two separate channel systems (Paper II, Figure 1b). Each channel system connects a source and a waste electrode, and the two channel outlets are confined to the common target electrode. The two 30 µm wide channel outlets are designed to enable local exposure of cells to both ions and cholinergic substances in relevant concentration ranges. Cholinergic substances are agonists to the AChRs and trigger elevations in the cytosolic free $[Ca^{2+}]$ from two primary sources: the extracellular medium and/or intracellular stores, primarily in the endoplasmic reticulum. To investigate the effect of multiple stimuli on Ca^{2+} signalling events, we used the SH-SY5Y cell line as model system, as it express both nAChRs and mAChRs. First, we examined the transport of a number of cholinergic substances targeting both or one type of the AChRs. Nicotine and oxo-M were both transported with high efficiencies and could be used to specifically target the nAChRs respective the mAChRs. Operating the OEIP with two separate source electrolytes, nicotine in source 1 and oxo-M in source 2, we were able to perform sequential delivery to cells cultured on the target electrode (Paper II, Fig 4b). Our results show that it is possible to repeatedly switch between two types of stimuli and how Ca^{2+} signalling pathways induced by the activation of either the ionotropic nAChR or the metabotropic mAChR can be differentiated.

Agonist-induced store depletion and store operated Ca^{2+} entry

To further dissect the metabotropic Ca^{2+} signalling pathway, we examined the different contributions of Ca^{2+} to the elevated $[Ca^{2+}]_i$ by applying combinations of small cations (Na^+, Ca^{2+} and Mn^{2+}) and oxo-M. First, cells cultured on the target electrode were placed in a Ca^{2+} free solution. Monitoring the cells in this Ca^{2+} free environment while delivering oxo-M from the source 1 result in a signalling event, which only can be a result from Ca^{2+} release from intracellular stores. Keeping oxo-M transport constant and turning on Ca^{2+} delivery from source 2 induced a massive influx of Ca^{2+} over the plasma membrane. The entry of divalent cations over the plasma membrane can be studied using the Mn^{2+} quenching technique. Mn^{2+} has higher affinity for the Ca^{2+} dye Fura-2, compared to Ca^{2+}, and quenches Fura-2 fluorescence irreversibly at the excitation wavelength 360 nm. Using this technique we were able to perform the same experiment with the cells maintained in physiologic concentrations of extracellular

Ca^{2+}. Transporting Mn^{2+} from source 1 to cells result in a slow entry of Mn^{2+} into the cell due to the cell membrane's basal permeability. Keeping Mn^{2+} transport constant and turning on oxo-M transport from source 2 resulted a more rapid Fura-2 quench rate (Paper II, Fig 4g). This increased quenching-rate is a result of an enhanced Mn^{2+} influx over the plasma membrane. This entry takes place primarily over SOCCs and demonstrates the extracellular contribution to the mAChR activation. In summary, these results demonstrate how dynamic OEIP multiplexer delivery can be utilized in agonist-induced Ca^{2+} depletion of intracellular stores and the studies of opening SOCCs. We have also shown how the OEIP multiplexer enables simultaneous or sequential delivery of two stimuli with independently modulated delivery rates.

Delivery of multiple stimuli is often necessary to fully understand the mechanisms behind cross-talk between signalling pathways. Here, presented as a delivery system for two stimuli, the OEIP multiplexer can easily be expanded for delivery of a number of stimuli. Patterning of additional sources and channel outlets is straightforward with the microfabrication techniques used to produce the OEIPs. The majority of devices developed for multiplexing are based on microfluidic systems, some of which are able to generate complex combinations of stimuli [103]. As previously stated, the main drawback of such microfluidic delivery is the fluid flow and its potential for physiological side effects. Although microfluidic devices are getting smaller and smaller they necessitate a lot of peripheral equipment to operate, e.g. tubes, pumps and power supplies, and these parts remain bulky. In contrast, the OEIP multiplexer is easily addressed using small contact probes directly connected to a single power supply, which can be located at a convenient, arbitrary distance away from the set-up. For cell signaling studies of physiological functions at a subcellular level, two-photon, two-color uncaging is a powerful tool. However, for multiplexing with two stimuli these have to be attached to two different inactivating caging groups and uncaging must be performed at different wavelengths [54]. In summary, paper II outlined how the OEIP multiplexer delivering two stimuli can be used to target specific cell signaling pathways. To further improve the control of the ions migrating through the OEIP channel we need to develop a control component in the channel that allows on/off switching of the ion flows.

7.4 SWITCHING ACH-DELIVERY ON/OFF USING AN ION BIPOLAR JUNCTION TRANSISTOR (PAPER III)

The performance of the OEIP multiplexer in cell signalling experiments inspired us to develop a component that may allow for fine-tuning of delivery and construction of even more advanced delivery schemes. To achieve this, new methods of controlling ion flows are necessary. In conventional electronics, transistors are active circuit elements used to amplify and/or switch electronic signals. In analogy to the electronic transistor, paper III describes the development of an electrophoretic chemical transistor, the ion bipolar junction transistor (IBJT).

Design and operation of the IBJT

We have developed the planar IBJT consisting of three channels, the emitter, collector and base, which are connected to terminals of PEDOT:PSS (Figure 10a). The cation selective emitter and collector channels consist of over-oxidized PEDOT:PSS, while the base channel is made of an anion selective membrane. The three channels meet in a junction where they all interface a neutral cross-linked gel layer. Transport of ACh from the emitter to the collector requires that voltage is applied across the emitter-collector ($V_{EC} > 0$ V) and that the junction is conductive. The conductivity of the junction is modulated by varying the salt concentration within it. In the active mode ($V_{EB} > 0$ V), chloride ions (Cl^-) migrate through the base into the junction where they get compensated by cations (ACh) from the emitter. This increases the

ionic conductivity between the emitter and collector, allowing positively charged ACh molecules to be transported from the emitter, through the gel and to the collector. Hence, the ionic current between the emitter and collector, i.e. the amount of delivered ACh, becomes a function of the potential applied to the base.

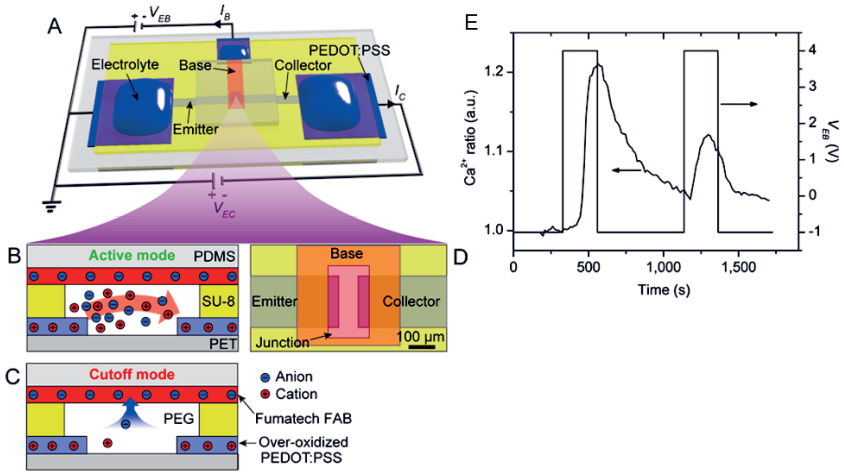


Figure 10. The pnp-IBJT. **A)** The emitter, collector and base channels meet in a junction consisting of a neutral polymer gel electrolyte. The conductive PEODT:PSS electrodes are covered by electrolytes and inject and/or extract ions from the terminals. **B)** In the active mode, the base supplies the junction with Cl^- Increased conductivity results in ACh transport from emitter to collector. **C)** In the off-mode, the base depletes the junction of Cl^- and ACh delivery stops due to decreased conductivity. **D)** Top view of the IBJT junction. The emitter and collector channels are separated by 100 µm and the base channel covers the entire junction. **E)** Intracellular Ca^{2+} recording of ACh stimulated SH-SY5Y cells cultured on the collector terminal. Turning the base on/off regulates ACh-delivery.

The IBJT in regulation of ACh-induced Ca^{2+} signalling

The functionality of the IBJT was demonstrated using real-time Ca^{2+} imaging measurements in SH-SY5Y cells cultured by the outlet on the collector electrode. Baselines of $[Ca^{2+}]_i$ were recorded in the IBJT off state (V_{EC} = 10 V and V_{EB} = -1 V). Reversing the biased voltage of the base switch the device to its active mode (V_{EC} = 10 and V_{EB} = 4 V). Cl^- migrate through the base channel to the junction and enables ACh migration from emitter to collector. Delivery of ACh at the outlet on the collector electrode results in an increase of $[Ca^{2+}]_i$ (Figure 10e). Turning the IBJT off, Cl^- migrate from the junction and back to the base. This terminates ACh-delivery and fluorescence from the Ca^{2+}-indicator decrease to a lower level. This experiment demonstrates how an anionic (Cl^-) base-current can be used to turn ACh-delivery on/off in the purpose of controlling ACh-delivery to modulate Ca^{2+} signalling. The delay in the Ca^{2+} response after turning the base on can be explained by the time required for building up the concentration of Cl^- in the junction. Correspondingly, the slow decay in fluorescence is a result of the time required to stop ACh-transport by depleting the junction from Cl^-. This experiment demonstrates how the inherent biological specificity of the ionic charge carrier (ACh) enables targeted on/off cell stimulation based on elementary transistor principles.

The pnp-IBJT represents an interesting development towards all-organic addressable X-Y matrixes with controlled release of chemical compounds. Such a matrix system would be beneficial in cell signalling studies of neuronal networks, circuits of connected neurons where information is exchanged via electrical and chemical signals. Recordings of network activities

has been demonstrated by multichannel electrodes integrated in perfusion chambers [104]. Efforts to create a matrix systems for delivery of stimuli have been made primarily with microfluidics, for example in an "artificial synaps chip" with multiple delivery points [105]. The pnp-IBJT was recently followed up by the development of the anionic equivalent, the npn-IBJT, controlling delivery of negatively charged ions [106]. The two types of IBJTs promise for the development of chemical circuits with both positively and negatively charged ions and biomolecules. By connecting several IBJTs in a circuit, the chemical equivalent to a light emitting display could also be possible to achieve. Instead of each pixel emitting light in one colour, an IBJT-circuit would allow "pixelated" delivery of specific signalling substances. Taken together, the OEIPs and the IBJTs create a toolbox of bioelectronic devices which can be connected in an immense number of combinations. This sets the stage for developments towards addressable matrixes and integrated chemical circuits, technologies we believe will have immense potential in the future of medical devices.

7.5 MODULATION OF NERVE FUNCTION *IN VIVO* (PAPER IV)

The OEIPs' electronic on/off control of non-flow based delivery of biomolecules, without leakage or burst release, together with the biocompatible properties of PEDOT:PSS makes this technique attractive for *in vivo* applications. In Paper IV, we demonstrate the use of the OEIP in a new "machine-to-brain" interface. Using the auditory system of the guinea pig as a model for biological signal transmission we show how this technique can be used for chemical modulation of the nervous system. Local delivery to the cochlea is challenging as the delicate mechanosensitive hair cells in the small fluid-filled structure are easily damaged. However, the structure provides easy access for modulating one of the brain's senses.

Design of the *in vivo* OEIP

To achieve this, the planar OEIP is re-designed to a more flexible, tube-like shape with a potential to be surgically implanted. The planar device was separated into two encapsulated electrode-electrolyte systems, the source and target compartments (anode respective cathode) (Paper IV, Fig 1). This allowed filling of the source compartment (anode) with the substance to be delivered and the cathode system with a NaCl electrolyte. By cutting the over-oxidized and electronically insulated ion channel in half, the tip from the source and target electrodes (outlet/inlet) comes in direct contact with the target system. When used *in vitro*, the target system can be cell media or a buffer, while *in vivo* the bio-fluid can be of any type. From the bio-fluid, cations are extracted into the target compartment (cathode) to complete the electrochemical circuit.

Confirming biological activity of delivered glutamate *in vitro*

The functionality of the new design was first tested *in vitro*. The biological activity of glutamate (Glu) delivered through the OEIP was evaluated on primary cultures of astrocytes expressing receptors for glutamate. First, the source compartment of the syringe-like device was loaded with Glu solution and mounted next to astrocytes cultured in a dish placed in an upright microscope. Turning the device on by applying 20 V between the two electrodes initiated Glu-transport. Glu migrates through the source electrode and the over-oxidized channel to the tip. The working principle is the same as in the planar devices where the voltage drop occurs primarily across the channel resulting in negligible electric fields in the target system (Figure 7). Hence, the cells will not be triggered by the applied potential or electric field. When Glu reach the tip of the device, it spreads by diffusion to the astrocytes in cell culture medium. Glu-responsive receptors are activated and this results in opening of Ca^{2+} channels, which promote a robust Ca^{2+} influx into the cells. By transporting Glu to the cells while at the same time

monitoring the real-time Ca^{2+} responses we demonstrated how the device could be operated *in vitro* (Paper IV, Fig 3). Turning Glu-delivery on, the lag time of delivery to actual Ca^{2+} responses is in the range of one minute. This is due to the fact that the syringe-like device does not contain the type of pre-filling system described in paper I and II. Turning the device off, Glu-transport stops and the cellular Ca^{2+} homeostasis is re-established.

Modulation of nerve function *in vivo*

The new design was explored in non-invasive delivery of Glu to the small volume of perilymph in the cochlea. The tip of the syringe-like OEIP was mounted at the bone beside the round window membrane (RWM) of the cochlea. Using the RWM as a port of diffusive entry to the cochlea, continuous Glu-delivery was carried out at a constant voltage for one hour. Glu is the primary neurotransmitter of the inner hair cells, transmitting the signal to the spiral ganglion neurons [39]. Overstimulation with Glu result in swollen dendrites of the spiral ganglion neurons and loss of contact with the inner hair cells [40]. By monitoring the brain's ability to perceive sound of specific frequencies, auditory brainstem responses (ABR), the hearing sensitivity was assessed before and after Glu-delivery. ABR measurements showed a significant loss of higher frequencies at the base of the cochlea where Glu enters (Paper IV, Fig 4c). While this finding confirm the excitotoxic effect of Glu, the control group exposed to H^+ showed no change in hearing sensitivity. This data show that electronically controlled delivery of Glu can be used for targeted activation of the nervous system, without introducing additional liquid into an already limited volume. After completion of the experiment, histological analysis of the cochlea confirmed the cellular and molecular details of the mechanism for auditory impact (Paper IV, Fig 4d). Thus, we used Glu-delivery as a proof-of-principle for the modulatory effect of local neurotransmitter delivery to the cochlea. In accordance with our results, Glu overstimulation and delivery of ototoxic medications damaging the auditory nerve have previously been used in validation of local delivery systems as their effect can be monitored by reduced hearing sensitivity [41, 42].

The next step for the OEIP technique is to enable delivery to restore or replace missing biological functions. Researchers are making efforts to incorporate delivery techniques into cochlear implants to further prevent and restore hearing functions. Local delivery to the cochlea has been demonstrated by several microsystem technologies. Examples of flow-based techniques are reciprocating microfluidic systems, drug-filled cannulas in cochlear implants and osmotic pumps [41, 98, 107, 108]. Moving liquids in these devices requires tubes, pumps and valves, which have to be controlled by fragile mechanical parts. The OEIP technique is a soft and flexible delivery system in which small cables and electrical contact points are used. Hence, the OEIP technique could be an alternative to todays' flow-based methods and, in combination with electric stimulation, potentially contribute to functional recovery of hearing loss. Other non-flow techniques based on polymers that have been evaluated for local delivery *in vivo* are biodegradable polymers and functionalized polymers coated on electrodes [42, 79]. While such passive techniques often are accompanied by burst release and/or leakage, the OEIP provides a strict on/off control of the delivered stimuli. The other possible non-flow delivery technique, ionotophoresis, has already been established in clinical settings. It is used for drug delivery over the skin barrier, preliminarily for treatment of inflammations in skin, muscles and joints [59]. As previously stated are the high drug concentrations required and the difficulty in calculating delivered concentrations considerable drawbacks. In conclusion, this is the first successful report of an organic bioelectronic implant used to chemically modulate the nervous system *in vivo*. This could open up multiple possibilities for prevention and treatment of hearing loss and other neurological conditions.

7.6 SUMMARY OF THE RESULTS

Collectively, data presented in paper I-IV demonstrate a communication interface that can be used in cell signalling research *in vivo* and *in vitro*. Through this thesis work I have contributed to the development of the OEIP. This development is summarized as additional capabilities of the OEIP listed in Table 4 (7-13).

Table 4. Capabilities of the OEIP.

OEIP capabilities:	References:
1. Non flow-based delivery system with diffusive release.	[71]
2. Electronic addressing with on/off control.	[71]
3. Easy integration of the soft and flexible organic material in biological systems.	[71]
4. Delivery of the cations H^+, K^+ and Ca^{2+}.	[71] [95]
5. Delivery of single stimuli to cells *in vitro*.	[71]
6. Quantification of total amount of delivered ions and calculations of transport efficiencies.	[71]
7.* Generation of temporal concentration gradients and oscillations.	[95] Paper I, II
8.* Transport of ions, neurotransmitters and cholinergic substances.	Paper I, II, IV
9.* Delivery of multiple stimuli with individually controlled delivery rates *in vitro*.	Paper II
10.* Modulation of the amplitude of Ca^{2+} responses by regulating applied voltage or time.	Paper I
11.* Generation of Ca^{2+} oscillations by regulating applied time.	Paper I
12.* Regulation of ion flows in electrophoretic transistors.	Paper III
13.* Delivery of single stimuli to cells *in vivo*.	Paper IV

* My contribution

8 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This thesis describes the results from an interdisciplinary project, merging organic electronics with biology/medicine with the aim to establish a novel communication interface between electronic components and biological systems. By taking advantage of the electronic and ionic properties of conjugated polymers, we have developed a diverse toolbox of organic bioelectronic devices that can be used to control cell signalling *in vitro* and *in vivo*. This novel technique enables a unique way to electronically control the lateral transport and delivery of signalling substances of the nervous system without any convective disturbances. I have described the development of the organic electronic ion pumps and the ion bipolar junction transistor, which together make up a toolbox of organic bioelectronics devices. The results presented in this thesis include the successfully transport of a number of ions, neurotransmitters and cholinergic substances. The continuous expansion of the OEIP transport repertoire may make this technique useful in a number of therapeutic areas. With the fast advances in the field of organic bioelectronics, this technique is likely to succeed as a candidate for the next generation of implantable biomedical delivery devices. It can also be envisaged that the incorporation of a bio-sensing component with the OEIP would allow chemical input signals to regulate the on/off control of the delivery system. This would enable OEIP delivery to restore the balance of ions/biomolecules or activate specific cell types. Such a device, combining the OEIP function with a sensor, could represent an “artificial neuron” with signal transmission resembling that of excitable cells: from chemical input, through electrical signals, to chemical output.

I feel truly privileged to have been part of this exciting time in mapping out the next stages of the field of organic bioelectronics and I am hopeful that this work fulfils a small part of the big effort in developing organic bioelectronic devices that can be used in biological systems.

9 MY SCIENTIFIC CONTRIBUTION

This thesis represents a multidisciplinary work with the aim to develop a new communication interface between electronic components and biological systems. The project has been a part of the strategic research center for Organic Bioelectronics (OBOE) and has been performed in close collaborations with specialists from different disciplines, collaborations that have been essential for the findings presented in this thesis.

My most prominent role in the projects has been in the integration the organic bioelectronic devices with existing techniques used for studying and analyzing biological responses. By taking conjugated polymer devices, produced by microfabrication technologies, into conventional microscopy set-ups we have been able to demonstrate translation of electronic signals into chemical messengers regulating intracellular Ca^{2+} signaling in nerve cells *in vitro*. Re-designing the planar device into a syringe-like device, utilizing the same principle of transport, we were able to demonstrate modulation of nerve cell signaling and auditory responses *in vivo*. I would like to think that this thesis contributes to broadening the perspectives of how conjugated polymers can be used to control biological systems, not only in cell signaling research but also as a future potential resource for new therapeutics.

This thesis highlights how to develop and implement a new technology for addressing biological questions. To achieve this, it is important that scientist within differential disciplines communicate their results and discoveries. Hence, our intention has been to make the papers presented in this thesis understandable to scientists of different fields. When scientists from various backgrounds understand and recognize each other's strengths and limitations interdisciplinary research will result in new scientific and technological breakthroughs.

10 POPULÄRVETENSKAPLIG SAMMANFATTNING

Hjärnan fungerar som en stor informationscentral som tar emot, sorterar och skickar iväg meddelanden ut i kroppen. Hjärnan kan liknas vid en dator då båda använder sig av laddningsbärare för att överföra information. Till skillnad från datorn, som fungerar elektriskt, är hjärnan mer komplex då dess nervceller, vilka kan liknas vid små processorer, kan utnyttja både elektriska och kemiska signaler. Nervcellerna använder främst joner och jonflöden för att överföra information då istället datorn utnyttjar förflyttningar av elektroner. Trots att elektronerna i datorn rör sig ca en miljon gånger snabbare än jonerna i nervcellen representerar dessa båda laddningsbärare en viktig länk mellan biologiska och elektroniska system.

Den här avhandlingen beskriver hur ledande polymerer (plaster), vilka utnyttjar både joner och elektroner som laddningsbärare, kan användas för att kontrollera cellsignalering i nervsystemet *in vitro* och *in vivo*. Vi har använt dessa ledande polymerer för att utveckla organiska elektroniska jonpumpar genom vilka det är möjligt att elektroniskt styra frisättningen av kemiska substanser som stimulerar celler. De första tre artiklarna som ingår i avhandlingen påvisar transport av signalsubstansen acetylkolin (ACh), samt en rad substanser som binder till acetylkolinreceptorerna, med olika utvecklingsgrader av jonpumpen i *in vitro* experiment. Genom att odla nervceller på den elektriskt ledande plasten kan signaler levereras direkt till cellerna med hög rums- och tidsupplösning. När signalsubstanser aktiverar nervceller ökar koncentrationen av kalcium (Ca^{2+}) inne i cellerna. Om cellerna först laddats med en Ca^{2+}-känslig färg kan denna koncentrationsökning studeras i ett mikroskop. Ca^{2+} har många viktiga funktioner i kroppen och reglerar bland annat de nervimpulser som styr signaleringen mellan hjärnan och olika kroppsfunktioner.

I den första artikeln visas hur jonpumpens elektroniska på/av funktion kan användas för att stimulera nervceller med ACh och därigenom inducera olika typer av Ca^{2+} svar. I artikel II utnyttjas möjligheten att med en mer komplicerad jonpump leverera två substanser, parallellt eller i sekvens, för att påvisa AChs olika signaleringsvägar via Ca^{2+}. I artikel III har jonpumpen modifierats ytterligare för att uppnå ännu en nivå av kontroll på leveransen av signalsubstanser. Genom att förvandla jonpumpen till en jontransistor skapade vi den kemiska analogen till den elektroniska transistorn som används för att styra och förstärka strömmar i elektriska kretsar. Jontransistorn kan användas för att sätta på och stänga av transporten av acetylkolin till celler och är därmed ett första steg i utvecklingen mot att använda ledande plaster i kemiska kretsar.

För att ta steget att fortsättningsvis möjliggöra leverans av signalsubstanser *in vivo* förändrades designen på jonpumpen i artikel IV till en smal sprutliknande form som användes i experiment med marsvinsöron. Glutamat, som är en av de främsta aktiverande signalsubstanserna i hjärnan och örat, levererades med den modifierade jonpumpen i marsvinets hörselsnäcka. Den nya designen av jonpumpen möjliggjorde leverans av glutamat till hörselceller utan att tillföra någon extra vätska till den redan begränsade volymen i hörselsnäckan. Genom att leverera glutamat kontinuerligt, samtidigt som hörselnervens funktion undersöktes, kunde vi demonstrera att tekniken fungerade. Detta är första gången ett organisk bioelektroniskt implantat använts för att kemiskt modulera nervsystemet *in vivo*. Sammanfattningsvis visar den här avhandlingen hur ledande plaster kan användas som ett kommunikationsgränssnitt för celler *in vitro* och *in vivo*. Förhoppningen är att dessa tekniker även ska kunna användas i medicinska implantat.

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