Molecular and Nano-engineering with iron, ruthenium and carbon: Hybrid structures for sensing

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Summary, Conclusions & Outlook

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8.1 Summary

8.1.1 General Introduction (CH 1)

Monitoring and measuring is at the basis of modern healthcare, and sensitive and selective sensors are in high demand. Graphene is an all-carbon 2D semiconductor material that is very suitable for such sensing technologies due to its electronic properties. Yet for graphene-based sensors, the graphene sheet has to be functionalized with molecules that probe the environment. These functional molecules undergo changes when they react with a binding partner, and these changes can be detected by graphene, as graphene is sensitive to molecular changes near its surface. As a result of these changes, the electronic properties of the graphene sheet are altered, which can be read out electronically through standard electronic measurement equipment.

Metal complexes can fulfill the role of functional molecule on a sensor. Due to the nearly unlimited tunability of metal complexes through changing the ligand environment surrounding a metal center and the accessibility of multiple charged states of the metal center itself, metal complexes can show a wide range of chemical behaviors that can be useful for sensing technologies. For example, iron(II)-based complexes can do spin crossover (SCO), which is a rearrangement of electrons in the d^6 configuration from a high spin state to a low spin state and vice versa, changing the chemical and physical properties of the molecule and the bulk material. This molecular switching effect is technologically interesting, but technological implementation of these typically dielectric materials is difficult. Through nanotechnology and device engineering approaches, ways have been found to read-out the switching behavior in the nanoscale. Yet, scaling down SCO materials strongly influences the switching properties.

Triggered by light, ruthenium polypyridyl complexes can switch properties as well, for example by photodriven linkage isomerization or ligand exchange. Moreover, these complexes can interact with biomolecules like DNA. These interactions can be used for bio-imaging and sensing, as the complexes change their photophysical properties upon binding; they can for example become light-emissive upon binding. Furthermore, binding of ruthenium complexes to nucleotides can be used to improve sensing, for instance by labeling specific nucleotides. Ruthenium complexes are thus appealing as molecular probes and labels for sensing.

8.1.2 Contactless spin switch sensing by chemo-electric gating of graphene (CH 2)

In chapter 2, it is described how graphene senses spin switches in a dielectric molecular material that has spin crossover (SCO) behavior. Graphene field effect transistors were fabricated over single crystals of the SCO complex [Fe(bapbpy)(NCS)$_2$]. The graphene sheet and the SCO crystal were separated by a polymer spacer, so that they were electrically disconnected. Abrupt resistance variations occurred as the devices were cooled down or heated up to trigger the phase transitions, accompanied by a strong color change of the SCO crystal. The phase transitions in the SCO crystal could thus be sensed electrically by the graphene transistor.

We found that the thickness of the polymer spacer played a crucial role in the sensing mechanism for these devices, and that the mechanism of sensing involved two effects. First, mechanical effects of the phase transition (expansion and contraction of the SCO crystal) contributed to a minor amount of the resistance variations upon spin crossover. The mechanical effects, studied by a careful analysis of the 2D peak of graphene in Raman spectroscopy, were especially strong in the very first phase transition in spin phase cycling experiments. This was in agreement with the much stronger resistance variation at the first phase transition than for following phase transitions. Importantly, with the thickest spacer (0.5 μm) these mechanical effects were largely buffered by the polymer spacer.

Second, the resistance variations were mainly due to changes in the electrostatic potential of the spin crossover crystal As the molecules in the crystal lattice of the single crystal are highly ordered, the dipoles of all the molecules in the bulk crystal are directed similarly, giving the crystal an electrostatic potential. Upon SCO, the dipole of the molecules in the single crystals changes all at once, as the phase transition of the crystal occurs within seconds, and thus the electrostatic potential changes as well. In effect, the graphene that is nearby the crystal is feeling that the potential changed; the doping of the graphene sheet changes. This is a new mechanism of sensing with graphene, we called chemo-electric gating, which opens opportunities for the integration of molecular materials in electronic devices.

8.1.3 Large-area thin films of the spin crossover complex [Fe(bapbpy)(NCS)$_2$] grown selectively on graphene (CH 3)

Continuing on chapter 2, in chapter 3 it is described how the spin-crossover material based on [Fe(bapbpy)(NCS)$_2$] can be scaled down to thin films. We found that a film based on this complex deposited over time when methanol was evaporated slowly into a solution of the metal complex. Interestingly, the films were found to be growing with a strong preference on graphene-coated areas over clean SiO$_2$ silicon wafer surfaces, on the centimeter scale as well as on micrometer-sized disks of graphene on SiO$_2$ surfaces. A study of the film with AFM revealed that the film grew homogenously, and the thickness increased slowly in the range of nanometers per day. Raman spectroscopy showed particularly convincingly for thicker films (>20 nm) good similarity between the spectra for the films as compared to the bulk materials. Additionally, Raman and XRD showed that the film was amorphous. Lastly, XPS showed that the iron centers in the films were at least partially oxidized to Fe(III); the level of oxidation of iron depended on the means of oxygen protection during film growth.

Measuring the spin crossover properties of these films was challenging. Samples were analyzed by SQUID magnetometry; the low amount of material in the film and strong diamagnetic background signal of the wafer itself made interpretation of the SQUID results difficult. For only one out of four samples, a reversible transition was found, which could mean that this film was spin crossover active; however, no hard conclusions can be drawn from the SQUID analysis. Whether oxidation of the films (during growth and/or storage) played a role remains to be an important research question.

The films could be grown on graphene field effect transistors, with a preference again for growing on the graphene sheet. Only very thin films could be obtained however, as the electrodes of the devices were etched in the thin film solution and devices could thus be immersed for a limited time only. Functional devices were produced, yet no sign of spin crossover could be found though examining the resistance variations of the devices upon cooling and heating from room temperature down to 150 K to induce spin crossover. Altogether, no strong evidence was found that the films were spin-crossover active.

8.1.4 Polymer coated graphene-based gas sensors: chemical fingerprinting by simultaneous sensing (CH 4)

In chapter 4, a graphene-based sensor is described that is not functionalized with a metal complex or material that has a specific interaction for a binding partner. Instead, the sensors described here were functionalized with the transfer polymer layer. Graphene-based sensors (fabricated on standard silicon wafers) that were coated with a 300 nm-thick layer of the transfer polymer poly (methyl methacrylate) (PMMA) were subjected to chemical vapors of 15 compounds (single injections were carried by a nitrogen flow to the measurement chamber). Resistance variations, or responses, were observed for most compounds, and were highly reproducible. The peak intensity was different for different species, showing the sensors to discriminate between compounds.

We used this discrimination between compounds in our advantage to evaluate the response to mixtures of compounds. By lowering the concentration of methanol in diethyl ether (methanol gave strong responses, while diethyl ether gave hardly any response), we estimated that the lower detection limit of the PMMA-coated sensors for methanol was 6 ppm. Moreover, we could also use the PMMA-coated sensor for quantification of complex binary mixtures; good linear relations were found between peak intensity and the percentage of methanol in ethanol, and between the full-width half maximum of the response and the percentage of water in ethanol.

These results showed the potential of the PMMA-coated sensor, but the sensors were poorly selective and could thus not be used for compound identification. To use polymer coated sensors for identification, we produced arrays of graphene sensors, each with a different transfer polymer coating (PMMA, Nafion® 117, and cellulose acetate butyrate (CAB)). The sensors were packed in a small tube configuration that could be integrated in a gas chromatography (GC) setup, enabling auto-injection for continuous sampling to produce chemical fingerprints of the vapors: the combined information of the responses of the sensors in the array, which all responded simultaneously to the vapor in the chamber. The chemical fingerprinting (CF) array data could be used for classification and identification by supervised machined learning, reaching an accuracy of 92%. Using these chemical fingerprints, the selectivity was thus strongly increased so that the acquired data could be used for accurate compound identification.

8.1.5 Monitoring a ruthenium-based photoreaction with graphene on paper (CH 5)

In chapter 5, we introduced a new substrate for graphene sensors: ordinary paper. Using paper as a substrate has the advantage that solutions of interest can be soaked in the paper, and analytes can easily reach the graphene-paper interface, where sensing happens. We soaked these devices with solutions containing either the photosubstitutionally active ruthenium complex $[Ru(tpy)(biq)(OH_2)]^{2+}$, the ruthenium complex $[Ru(bpy)_3]Cl_2$ which is a good electron transfer agent but cannot do photosubstitution, or with KNO_3. Remarkably, when we monitored the resistance of the graphene sheet, all devices responded to light, independent of the chemical nature of the solution.

When we electrically gated these devices by varying the potential underneath the graphene sheet between 0 and 1.5 V, we could see the typical Dirac peak for all devices, showing that these devices are very suitable for such gating experiments. The Dirac peak did not move for the devices wetted with the previously mentioned solutions. To initiate a chemical reaction in solution, we added a ligand, 2-deoxyguanonine monophosphate (dGMP) to allow for a coordination reaction between the ligand and $[Ru(tpy)(biq)(OH_2)]^{2+}$ in the dark and the phototriggered release of the ligand with visible light. When we irradiated devices with this solution, we could observe a small peak shift, which seemed to indicate that the graphene sheet indeed senses the chemical reaction taking place.

At the same time however, we noticed that a current was flowing from the gate electrode to the graphene sheet as we varied the gate potential. Peaks appeared in the plots of this current versus the gate potential, which indicated electrochemistry was taking place. The exact nature of the electrochemical reaction, possibly the oxidation and reduction of the ruthenium complexes, could not be determined, however. The question whether the graphene sheet has sensed the (photo)chemical conversions in the paper substrate is thus not definitively answered, as we could not exclude these electrochemical processes from a possible sensing mechanism. Further research is required to fully understand the processes taking place in the graphene on paper devices, and to develop graphene on paper as a sensing platform.

8.1.6 Reducing the translocation speed of DNA in solid-state nanopores by photo-labile ruthenium complex decoration (CH 6)

Chapter 6 takes us to a different kind of sensing with nanodevices. In a nanopore device, an ionic current flows between two chambers, separated by a membrane with a single pore. If DNA is located in one of the chambers, it is driven by an electrostatic potential to the other chamber. While the DNA strand travels through the pore, the ionic current is blocked, as the bulky DNA effectively narrows the pore. Careful analysis of the ionic current behavior in theory could be used to tell which bases are located in the pore. For solid-state nanopores, the challenge lies in slowing down the translocation of DNA through the pore, as the DNA travels too fast to determine its sequence.

To slow down DNA translocation, we proposed to attach ruthenium complexes to the DNA strand, which can be released again with visible light. When the ruthenium complex is attached, the DNA experiences more friction by the added bulk, which should decrease its motion. Upon irradiation, the DNA is released and is free to travel. In this way, the motion of DNA in a nanopore can be controlled with light irradiation.

With UV-vis, we found that the ruthenium complex [Ru(tpy)(biq)(Cl)]Cl, hydrolysed to $[Ru(tpy)(biq)(OH_2)]^{2+}$ in aqueous solutions, coordinated to ctDNA and specifically to the nucleotide dGMP. No binding was found with dAMP, dTMP, and dCMP. DNA was incubated with the ruthenium complex and translocated through a nanopore in a silicon nitride membrane of 30 nm thickness. The translocation speed of DNA was not lowered, but the ionic current drop was significantly larger with the complexes attached. This indicated that the ruthenium complex co-translocated with the DNA strand: with the ruthenium complexes attached, the DNA was more bulky, resulting in the larger current drop, but its speed was not decreased as the ruthenium complexes could not provide sufficient drag.

Further research should be done to see if the ruthenium complexes can be attached to the surface of the membrane and inhibit DNA movement, and what will be the effect of visible light irradiation on the DNA translocation speed through a nanopore in a ruthenium-modified membrane.

8.1.7 [Ru(3)(biq)(STF-31)]$^{2+}$: A lock-and-kill anticancer PACT agent (CH 7)

Moving away from sensing using electronic devices, in the final experimental chapter we describe how molecules themselves can be used for sensing. A ruthenium complex was designed and synthesized that is thought to play a dual role in anticancer therapy with light, known as photoactivated chemotherapy (PACT). We hypothesized that the complex [Ru(**3**)(biq)(STF-31)](PF$_6$)$_2$ (**3** = 3-([2,2′:6′,2″-terpyridin]-4′-yloxy)propyl-4-(pyren-1-yl)butanoate), biq = 2,2′-biquinoline and STF-31 = 4-((4-*t*-butyl)phenylsulfonamido)methyl)-N-(pyridin-3-yl)benzamide) can show that it was taken up by a cell through a fluorescence signal. This information could be very helpful for a surgeon to pinpoint where the prodrug is located in the body and thus to know where irradiation needs to be done; upon irradiation with visible light the complex releases an inhibitor for the NAMPT protein that is vital to cell survival, which eventually kills the irradiated cells.

First of all, the ruthenium ion – the photocage – was photoreactive and expelled the STF-31 ligand – the inhibitor – upon irradiation with green light. Kinetic studies showed that the pyrene group attached to the tridentate ligand did not affect the quantum yield of photoexpulsion, but that the quantum yield was lower because of the ether connection to the tpy ligand. The pyrene moiety did thus not affect the photochemistry of the ruthenium complex. Second, ligand **3** attaches the pyrene group – the fluorescent tag – to the ruthenium complex via an ester bond. Fluorescence studies showed that when pyrene was attached to the ruthenium complex its fluorescence was quenched. When the ester bond, which connected the ruthenium complex and the pyrene group, was broken by esterase activity the liberation of the pyrene group unlocked its fluorescence. This ester bond is expected to be cleaved quickly after entering the cell by esterase activity, yet will remain intact in the blood stream, where no esterases are present. Ester bond cleavage leads to fluorescence, and cells which have taken up the complex are thus expected to light up over cells that did not internalize the prodrug.

In the near future, confocal microscopy should point out whether cells that have taken up the ruthenium complex indeed become fluorescent. Furthermore, the potential of these complexes to kill malignant cells should be studied through in vitro tests and the effect of irradiation on cell death after treatment should be evaluated to see if the complex is indeed more toxic upon irradiation.

8.2 Conclusions and Outlook

8.2.1 General conclusions

Graphene and metal complexes have shown to be valuable tools in the design of sensing devices and molecular sensors. The various properties of these materials could be exploited in a number of sensing platforms. The options to design these platforms are numerous. We constructed graphene devices on common substrates, *i.e.* silicon wafers, but also on extraordinary substrates, including micrometer-sized single crystals grown from an iron complex with spin crossover properties, and ordinary paper. We could fabricate devices that exploited the properties of these substrates: phase transitions in the spin crossover crystal could be detected due to the electrostatic potential of this crystal, which changes as spin crossover occurred. Also, using paper as a substrate for graphene sensors allowed us to bring analytes in aqueous ionic solutions close to the graphene surface where they could be sensed, while at the same time we could efficiently gate these devices to carefully inspect the electronic properties of graphene. Thus, the ability of graphene to be transferred to various substrates by the aid of a polymer makes this material suitable for construction of graphene devices on functional substrates.

Moreover, we found that coating the surface of the graphene sheet was a fruitful approach to fabricate devices. Thin films of the spin crossover iron complex were found to grow specifically on graphene, even in a field effect transistor configuration; however we could not yet detect phase transitions in the thin films with these devices. The transfer polymer, on the other hand, turned out to be an ideal component for the sensors, crucially providing protection to the graphene sheet when it was placed on the single crystal and on paper, thus safeguarding the integrity of the devices. The transfer polymer could even be the sensitizing component of graphene-based vapor sensors: by using an array of multiple sensors with different polymer coatings, high selectivity could be obtained for a wide range of compounds. In fact, leaving the transfer polymer on graphene intact was perhaps the design principle that was the most beneficial for the devices reported in this thesis.

We also explored the possibility of using metal complexes, and in particular ruthenium complexes that are light-active, to enhance sensing and as molecular sensors. Ruthenium polypyridyl complexes which bound to DNA were found to improve DNA detection, by increasing the signal strength when a DNA passed through a nanopore by making the DNA strand more bulky, and thus better observable. Moreover, a ruthenium complex was found to be suitable to quench a fluorescent tag, pyrene, when this tag was covalently attached to the complex via an enzymatically degradable linker. Degradation of this linker unlocked the fluorescence of this tag: hence this complex, a light-activatable anticancer prodrug, may help to show where the compound is taken up as the linker is degraded and cells become fluorescent, which can help to pinpoint where irradiation should occur *in vivo*. These cases showed that the photophysical properties of ruthenium are very helpful to improve sensing techniques and to design molecular sensors.

In the end, the strategy of using metal complexes with graphene devices turned out to be an efficient one. Yet, we only explored the use of a very limited number of metal complexes, with functions that were focused on sensing. The versatility of metal complexes and the flexibility in graphene-based device architectures means that we have only revealed the tip of the iceberg. It is our belief that, through molecular and nano-engineering with graphene and metal complexes, hybrid structures can be designed, not only for sensing devices, but for virtually any type of device.

8.2.2 Outlook

Of course, the principles that are described in this work are not final. The lines that we have presented could be continued in various directions, and thereby offer a plethora of possible roadmaps for further research.

Using switchable substrates like the spin crossover single crystals for graphene field effect transistors could go two ways. First of all, other types of crystals may be used instead of the spin crossover crystal. For example, crystals of ruthenium DMSO complexes could be used that can undergo linkage isomerization,[1] so switching can be achieved specifically with light instead of with temperature. Moreover, single crystals of gas-sensitive metal-organic frameworks (MOFs) could be used as well, to provide devices that detect a gas by responding to the phase transitions in the MOF crystal that are triggered by the gas.[2] Second, heading towards device engineering, one could think of constructing nano-sized devices on the single crystals, using for example (e-beam) lithographic methods.[3] The crystals are micrometers in size, giving enough space to design complex architectures for nanodevices. By intentionally damaging the crystal with laser ablation,[4] (nano)sized domains may be produced in the SCO crystal itself as well, creating SCO domains that can be switched independently. These small domains, together with scaled-down GFETs, could possibly leads to a large array of switching devices produced on one crystal. This could be useful for data storage.

In fact, further study of the thin films based on the $[Fe(bapbpy)(NCS)_2]$ may go into the same direction. Because the films grow selectively on graphene, 'traditional' techniques may be used to design complex architectures for graphene-based electronic devices, which may then be coated with the thin film grown selectively on the graphene areas. Possible applications of devices that are coated with spin crossover active thin films would be again data storage, but other types of thin films are envisioned for sensing, as the molecules of the thin film are expected to be sensitive to their environment.[5]

In line with this sensing application, of course, are the chemical fingerprint arrays. The CF arrays presented in this work were 1st generation prototypes; further research should be focused on producing high-quality data that can be used to reliably detect and identify chemical species using machine learning techniques.[6] The results we found for the 1st generation were encouraging; yet, a challenge is to overcome device-to-device variations. Producing devices that always respond in the same way gives the advantage of using a machine-generated learning model from one CF array onto the data produced by other CF arrays. In that way, one optimized algorithm can be used for all CF arrays, thus maximizing the reliability of the CF array. Eventually, CF arrays may be used as a breath analysis device for patient screening and diagnosis in the clinics.

Similarly, graphene on paper has been explored as a sensing platform. Seemingly due to interference by electrochemical processes, the platform has not reached its full potential in our opinion. Studying these electrochemical processes should help understand which processes occur during the GFET measurements; a deeper understanding of these processes could help to further develop the GFETs on paper. On the other hand, efforts to sense a reaction involving strictly non-redox active species (within the gate potential range, *i.e.* 0 – 1.5 V) could be very successful, and may provide a simplified and more conclusive view on the sensing capabilities of the GFETs on paper.

We have shown that ruthenium complexes can be useful to improve DNA detection using solid-state nanopores, but we have yet only made a start to show how useful they can be. To continue on this line, crucially, the effect of light on ruthenium-decorated DNA translocation should be examined. The photoreactivity of ruthenium may be exploited to control the binding and unbinding of the complex to DNA: using an anchored complex, *i.e.* a complex that is fixed to the surface of the nanopore, the complex may provide the friction that is required to slow down DNA when it is bound. Irradiation may break the Ru-DNA bond, thus releasing the DNA and allowing the DNA to travel. With light, control can be gained over the translocation speed of DNA in this way. A complex that can do this is presented in fact in chapter 7; the pyrene group on this complex can be used as an anchor on graphene-based nanopore devices.

The complex described in chapter 7 may of course also be studied further as a PACT agent and uptake indicator at the same time. We could see the fluorescence of the pyrene tag upon degradation of the ester connection, but not yet *in vitro*, which is required to see if indeed cells light up when they internalize the complex. Moreover, the cytotoxicity in the dark and under irradiation should be evaluated. To circumvent the poor solubility of the complex, liposomes may be used as a drug carrier, as the complex is amphiphilic and should easily be incorporated in a lipid membrane.[7] Eventually this complex may be an effective PACT agent while showing where irradiation should take place to kill malicious tissue.

8.2.3 References and notes

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