

# Surface Plasmon Resonance Sensor Surfaces for Environmental Online Monitoring



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## Declaration of Collaborations

Thomas Hirsch and Antje Bäumner supervised the research. Thomas Hirsch and the author conceived and designed this work. The author prepared all samples and performed all measurements, except those mentioned in detail. Data evaluation was carried out by the author. Some of the results were obtained together with other researchers. In accordance with § 8 Abs. 1 Satz 2 Punkt 7 of the “Ordnung zum Erwerb des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.) an der Universität Regensburg vom 18. Juni 2009“, this section gives information about these collaborations.

Chapter 1: The manuscript was authored by the author and Thomas Hirsch. Thomas Hirsch and the author conceived this review. The literature survey was performed by the author as well as writing the manuscript. Thomas Hirsch revised the manuscript and is the corresponding author.

Chapter 3: This study was conceived and designed by Thomas Hirsch, Peter Hausler, Rudolf Bierl and the author and supervised by Thomas Hirsch and Rudolf Bierl. The experimental work was carried out by the author (point based and imaging SPR) and Peter Hausler (imaging SPR). The imaging setup was developed and operated by Peter Hausler. Nanoparticle synthesis and characterization was done by Susanne Märkl. Data curation was performed by Peter Hausler and the author. The original draft was created by Peter Hausler and the author, edited by Peter Hausler, Thomas Hirsch and the author. Thomas Hirsch is the corresponding author.

Chapter 4: The concluding remarks were conceived and written by the author. Christoph Bühler performed part of the SPR measurements in the course of an internship. Data evaluation was solely done by the author.

# 1. Current Challenges in Nanomaterial-based Sensors for Online Monitoring of Drinking-Water by Surface Plasmon Resonance

## 1.1. Abstract

This opinion focuses on basic considerations of environmental monitoring with surface plasmon resonance imaging (SPRi) and nanomaterials. Theoretical aspects of non-covalent interactions of nanomaterials as receptor surfaces are discussed to develop cross-reactivity sensors. It is pointed out that continuous, online, and long-term water monitoring will be best suited by a combination of different nanomaterial functionalized receptor spots that are capable of interacting with non-covalent interactions such as electrostatic interactions,  $\pi$ -stacking, or hydrophobic effects. Only a smart combination of those nanomaterials will be able to reach the desired selectivity for a comprehensive overview of water quality. Additionally, improvements in the sensor's sensitivity by nanomaterials are discussed, which can be achieved by using a nanostructured gold surface or plasmonic materials.

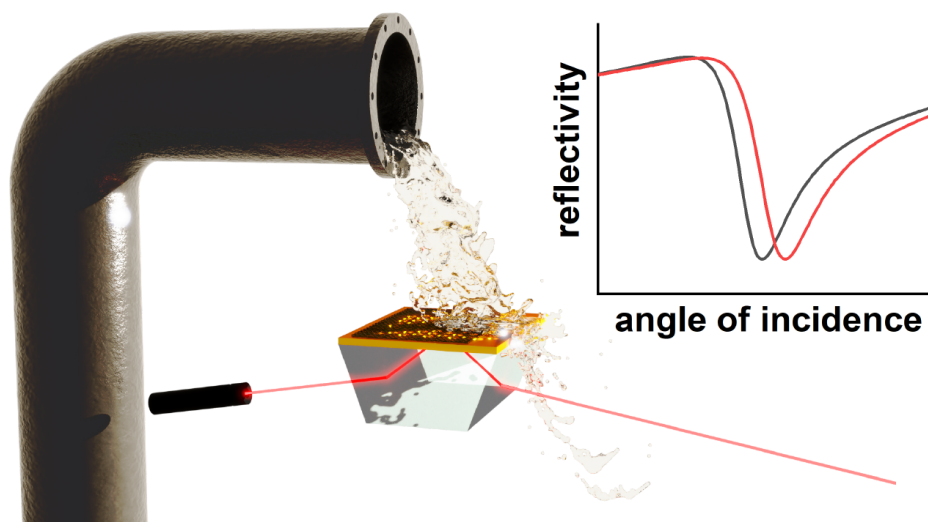


Figure 1.1: Graphical abstract of the publication "Current Challenges in Nanomaterial-based Sensors for Online Monitoring of Drinking-Water by Surface Plasmon Resonance"



Invited manuscript, currently under investigation by Current Opinion in Environmental Science & Health (Elsevier, Amsterdam). This chapter was submitted on June 14<sup>th</sup>, 2021.

**Author contributions:** This manuscript is authored by Lukas Wunderlich (LW) and Thomas Hirsch (TH). TH and LW conceived this review. The literature survey was performed by LW as well as writing the manuscript. TH revised the manuscript and is the corresponding author.

## 1.2. Introduction

The drastic consequences of environmental pollution by anthropogenic chemical substances deserve more and more attention as it is also reflected by increasing coverage of such topics in media. Besides contamination by heavy metals, those are becoming if not already are a huge problem to human health all around the world [1], widespread examples, which have come to the common public interest, are plasticizers like bisphenol A (BPA) used in consumer products [2-4], herbicides like glyphosate used in agriculture [1,5-7], or very recently, concerns from microplastics of various sources [1,8-11]. In science, there are tremendous efforts needed in developing sustainable and eco-friendly alternatives for harmful or long-persistent chemicals and find new ways to turn the translational chemistry into a chemistry of reuse as it is also addressed in the SIDE vision roadmap for 2030 with the four pillars of **Sustainable, Innovation, Diversity, and Education** [12,13]. Besides future action, the past of industrialization with all the impacts on nature needs to be taken into account by providing tools for fast and reliable detection of polluted environment and especially for monitoring the quality of indispensable resources such as water and air. The overwhelming increase in citations of articles discussing water pollution over the last ten years demonstrates the urge for taking care of these topics (Figure 1.2).

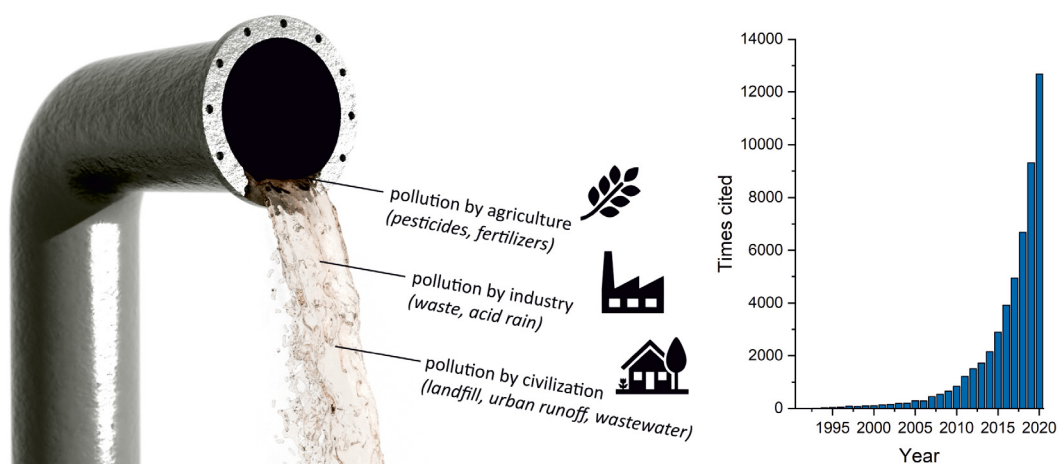


Figure 1.2: Main sources of pollution of drinking water are agriculture, industry and civilization. The increasing awareness of this issue is shown by citations regarding scientific articles concerning the topics “water” and “pollution” based on the database of Web of Science. The results were refined by the category “Chemistry Multidisciplinary”.

Regarding pollution of nature, the acute contact and intake of possible toxic compounds is only one danger. An even greater threat bears the accumulation in the environment and

living species [14]. With the extensive use of chemicals in industry, agriculture and human consumption, more and more chemicals have been brought into nature. Since not all of those substances are biodegradable, they will sooner or later accumulate in soil or air and then enter the water cycle by rain or other types of surface water [14]. Consuming water or food with low pollution which isn't harmful to the organism but can't be metabolized at all or not fast enough will accumulate these pollutants during the food chain and might reach toxic levels [1,14]. The accumulation of individual materials in water or food results in additional danger, since if a variety of compounds is present there, the people consuming them are not only exposed to a certain toxin, but to mixtures of these, which might yield in an even more toxic effect on the human health [15].

The human body can adapt to changes in the environment by evolution, but this mechanism takes too long, compared to the ever-increasing speed of human life and ingenuity. In numbers, every year a high amount of new chemicals is invented, approved and used. In the U.S. the responsible authority TSCA (Toxic Substances Control Act) lists 86,000 chemicals [16], REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) in the E.U. lists 23,316 substances [17]. The health risks to humans and the biological activity of these often are still not known nor understood completely [14]. One such example is exposure to polybrominated diphenyl ethers (PBDE) flame retardants. Their extensive use started in the 1970s right after finding those excellent properties of these chemicals in fire protection minimizing the daily risks for mankind. Later, health problems by the slow uptake of these chemicals became clear. Following that many regulations were passed, to restrict the use of these toxic compounds, which limited acute intoxication. Even if banned now, these extremely persistent materials are still present in the environment, which more and more shifts the contamination to water and yields an intoxication of animals like fish [18,19].

The acute intake of toxic compounds is of danger, but since these issues are usually identified fast and overcome by prohibition, the even greater threat is the long-term, unknown intake of small concentrations of pollutants. Thereby the monitoring of drinking water is especially important since it is involved in many aspects of human life, like food production, cleaning or various production processes and, as in the case of PBDE, performs as a kind of sewage for environmental contaminations. Quoted from the WHO Guidelines

for drinking-water quality: “Significant problems, even crises, can occur, however, when chemicals posing high health risk are widespread, but their presence is unknown because their long-term health effect is caused by chronic exposure as opposed to acute exposure” [20].

### *1.3. Surface Plasmon Resonance Sensors*

At present, the most common way to detect environmental pollutants deals with spectrometric methods, like mass spectrometry, or with sensors using complex amplification elements, like biomolecules by quantifying known individual toxic compounds [20,21]. Such sophisticated methods are needed since many anthropogenic pollutants are molecules of small size (< 1,000 Da) and hence are challenging to be detected in trace amounts [22]. On the one hand, this comes with the benefit that the contaminations can be individually identified, their concentrations are known, and hence conclusions can be drawn upon the potential origin. On the other hand, these methods usually require expensive equipment which cannot be implemented widely in the water system for automated inline monitoring [23]. The use of biomolecules, like enzymes, is limited since they lack stability over a long period and often demand special measurement conditions which are not easy to provide constantly over time [24].

To overcome these issues, label-free techniques relying on intrinsic parameters such as charge, changes in mass, or refractive index might be a solution. They do not necessarily rely on biomolecules as recognition element and additionally, they provide online information [25,26]. During the last 30 years, label-free sensors have also been extensively studied for the determination of individual analytes. Many examples of such sensors have been reviewed recently [22,27-32]. Up to now, most label-free sensors are applied in clinical diagnostics and food safety monitoring.

Not all intrinsic parameters are equally suited for the design of label-free sensors. Charge for example is heavily amenable to changes in its surroundings, *e.g.* when pH can change or the ionic strength of a media cannot be fixed. Optical parameters, like absorbance or fluorescence, come to their limits if small molecules are analytes of interest. Those often do not exhibit strong effects in the visible spectrum of light which makes it challenging to

detect them at very low concentrations. Mass sensitive transducers, such as quartz crystal microbalance (QCM) comprise an excellent accuracy and high reliability but so far this technology was not able to demonstrate its full potential mainly due to limited sensitivity as well as complex operation when it comes to online monitoring of more than one analyte in parallel. In contrast to that, the optical readout of changes in the refractive index enables the simultaneous integration of different receptors in one arrangement, when surface plasmon resonance imaging (SPRi) is used for signal readout. This can enable a cheap and miniaturized setup [33].

SPR was also only sparsely considered for environmental monitoring, due to instrumental limitations in providing the accuracy and sensitivity which is needed. Nevertheless, in recent times some approaches have been reported which enable the detection of small molecules by nanoengineering of the metal surface [34-36] as well as by instrumental improvements by the realization of better cameras for reasonable costs [37]. According to the WHO, a maximal tolerable guideline value for an insecticide in drinking water is  $1 \mu\text{g L}^{-1}$  (e.g. for dichlorodiphenyltrichloroethane (DDT)) [20]. When taking into account that a typical SPR setup can resolve changes in refractive index in the order of  $10^{-5}$  it is obvious that this is about 30 times too high to be used in online monitoring of DDT in drinking water [25]. The consequence is that the analyte needs to be concentrated at the sensor surface. Such enrichment layers commonly are used for specific analyte recognition, but in general, the combination of highly selective individual receptors for each analyte wouldn't be straightforward for online monitoring of drinking water, due to the manifold analytes which can be present [1,20]. If individual sensors were used, each new analyte demands the development of an individual receptor surface or a hardware extension which all comes for additional costs. A more elegant strategy would be the use of an array technology making use of a combination of several receptors on a single sensor, generating a signal pattern. Ideally, a single receptor within the array exhibits only moderate selectivity, as this enables to use of only a few receptors to discriminate lots of different analytes, by the so-called cross-reactivity sensor concept [38].

The combination of two hydrophilic disaccharides, lactose (neutral charge) and sulfated lactose (negatively charged), shows the potential of cross-reactivity sensors very well [38].

These two compounds deal as building blocks and were deposited on the SPR surface with different ratios, varying from 0 to 100% of each molecule, via self-assembly (Figure 1.3).

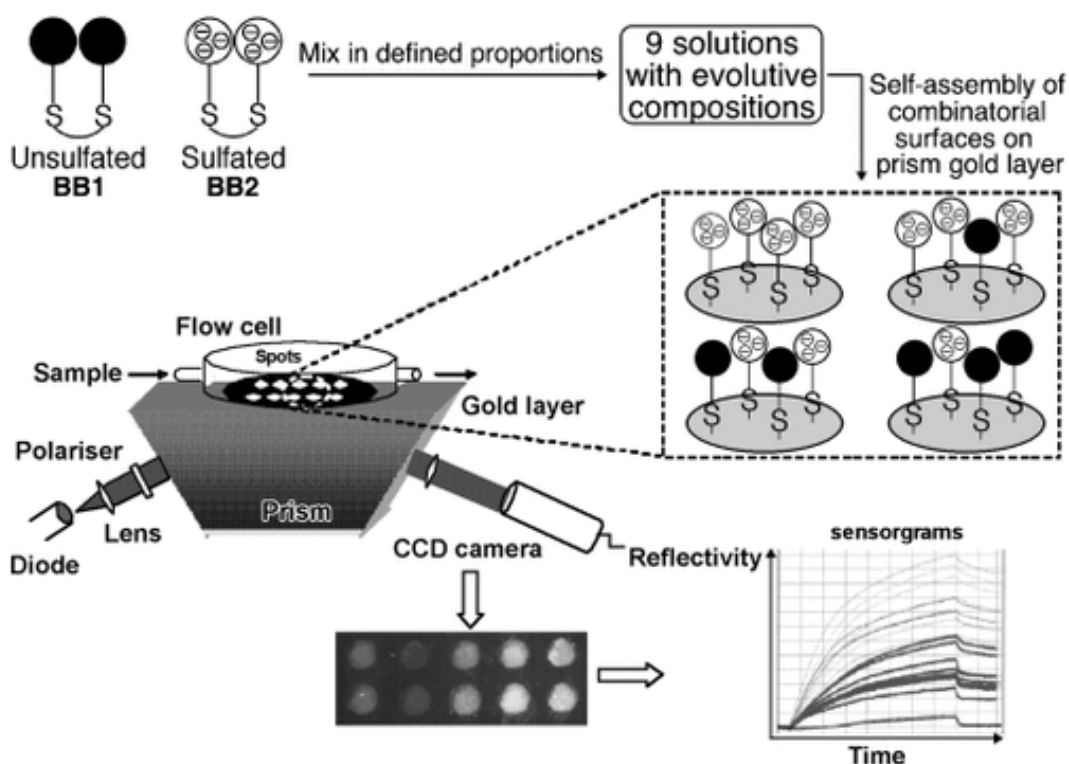


Figure 1.3: Different ratios of lactose and sulfated lactose serve as sensor spots in an SPR imaging setup. The simultaneous evaluation of all combined sensor responses enables a high selectivity using these individually low selective receptor molecules. This concept is called cross-reactivity sensing. Used with permission from [39]. Copyright © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

A single building block wouldn't yield meaningful information. In combination, different signal patterns give access to the composition of a protein mixture. These studies were followed by the investigation of the discrimination ability of what was called complex mixtures, which were drinks, such as wine, beer, and milk. The different signal patterns were able to distinguish these different types of drinks and additionally different types of milk. During the milk studies, it was noticed that the sensor array is also able to monitor the aging of the milk samples. Depending on the analyte, different solutions for regeneration were used from 0.02 M NaOH, over 1 M NaCl to 1% SDS [39] or 2% SDS [40]. This is a major drawback. Environmental, long-term, online monitoring prohibits such a regeneration protocol. In addition to that, the small molecular weights of environmental pollutants tend to decrease the overall signal change and hence sensitivity. It can be seen that these major challenges need a lot of further investigation, which receptor molecules

are suitable for environmental monitoring. A careful choice is needed to find the right combination of sensitivity and reversibility.

#### *1.4. Affinity-based Interactions*

The most established and straightforward recognition elements are based on biomolecules, like antibodies, aptamers, or enzymes which are relying on non-covalent interactions to interact with their respective counterpart [41,42]. By this, high selectivity towards single analytes is generated for large molecules, when several interactions are exploited at once, but at the same time, this concept won't be useful for a low selective cross-reactivity sensor array. A major drawback for environmental sensing is that the outstanding binding affinity generated by those receptors prevents the easy desorption of bound analyte and hence hinders recovery. It is, therefore, necessary to separate the individual interactions, following by a suitable combination and tuning of these.

Typical non-covalent interactions are ion-ion, ion-dipole, dipole-dipole, H-bonding, van-der-Waals (including dispersion) forces and  $\pi$ -stacking [41-45]. Figure 1.4 classifies these interactions regarding their energy and interaction range. One has to remember that usually these interaction energies are determined in gas phase and not in aqueous solution where the non-covalent interactions between two analytes or the analyte and the receptor have to compete with surrounding molecules from the solvent or present in the solution [45]. This has an especially large impact on the forces with the lowest energy which are van-der-Waals interactions (about  $4 \text{ kJ mol}^{-1}$ ) [41], including London dispersion forces. These appear during the very close interaction of two non-polar molecules, one with a dipole and one without (dipole-induced dipole interaction) or by two molecules without a permanent dipole (London dispersion force) [41,44]. That is why they only play a minor role in the direct detection of analytes in aqueous solution, since there the analyte interaction with water molecules is stronger than the possible recognition procedure. The same is true for dipole-dipole interactions [45]. This can be seen in literature where, to our best knowledge, these interactions are used only in gas sensing individually [46,47].

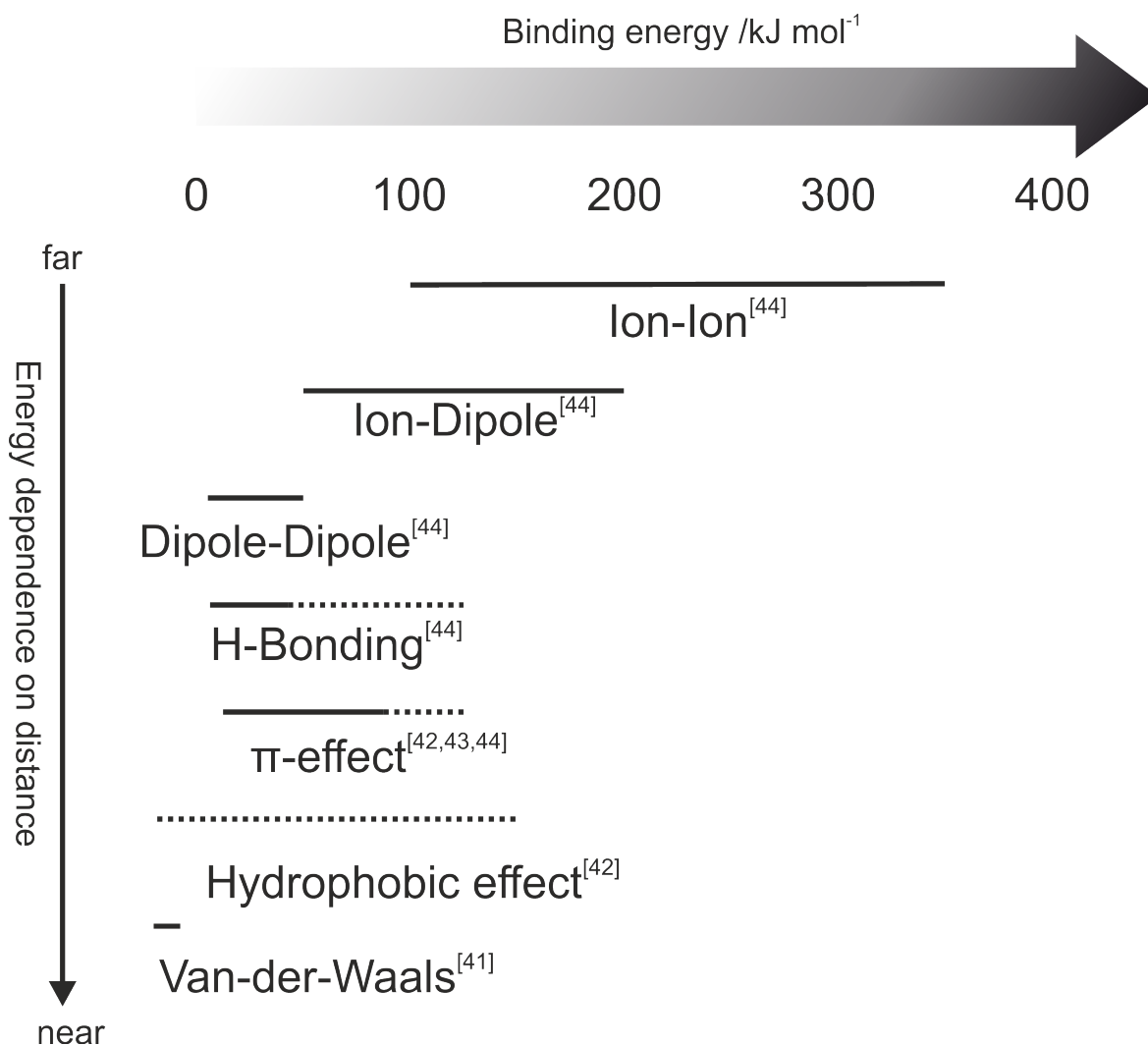


Figure 1.4: Binding energies and distance dependence of non-covalent binding energies.  $\pi$ - and hydrophobic effects are highly dependent on the area of interaction, therefore an exact range is hard to narrow.

H-bonding in principle is a dipole-dipole interaction where a hydrogen atom is attached to an electronegative atom, which interacts with a neighboring dipole [41]. Usually, the attraction occurs between the hydrogen atom and electronegative atoms such as O or N. This bonding energy usually ranges between 4 – 60 kJ mol<sup>-1</sup> [44]. Fan *et al.* exploited this behavior by using 11-mercaptoundecanoic acid as receptor layer for detection of the amino acid arginine [48]. The thiol layer was deposited via self-assembly on an SPR optical fiber probe. The detection limit of 0.005  $\mu$ M is better than competing fluorescent methods but the selectivity wasn't tested. One can assume since no particularly specific recognition element was used that a variety of other molecules will interact with this surface. The receptor surface was regenerated by using 0.12 M NaOH solution, which caused the guanidino group to be negatively charged and hence be repulsed from the surface, which



won't be applicable in an online measurement setup for drinking water. This circumstance also shows that the non-covalent interactions are highly dependent on the measurement conditions, like pH in this case.

The binding energy of stacking effects caused by  $\pi$ - $\pi$  interactions of molecules with conjugated  $sp^2$ -electron-systems depends on the size of the conjugated  $\pi$ -system and the mutual orientation of the compounds. The highest energy can be obtained if the  $\pi$ -systems are large, parallel, and, in contrast to the name, slightly displaced to each other. The energy ranges from  $8.4 \text{ kJ mol}^{-1}$  for two benzene molecules in gas phase [42] but can easily get larger if graphene-type nanomaterials interact over an extended area. Genslein *et al.* developed a sensor based on  $\pi$ -stacking to detect the plasticizer diethyl phthalate in environmental water using surface plasmon resonance with reduced graphene oxide as a nanomaterial to form a receptor layer [49]. Despite reduced graphene oxide has no perfect continuous conjugated  $\pi$ -system, it still has enough areas to provide  $\pi$ -stacking binding sites. A detection limit of 20 nM was obtained, which can cover the threshold of 3 – 40 nM for bis(2-ethylhexyl)phthalate, the most common phthalate, in fresh and drinking water. Since no other selective element was applied, it is expected that the rGO layer will interact with any molecules containing a conjugated  $\pi$ -system.

Even though in aqueous solution the use of some low energy interactions for detection purposes is limited, it facilitates a new type of interaction, the hydrophobic effect [41,44]. Due to entropic favorability, hydrophobic molecules tend to complex together in aqueous solution. This behavior can be used to enrich hydrophobic analytes on a surface or to exclude hydrophilic compounds from specific interactions. It is difficult to tell a distinct binding energy, as a result of their entropic nature, but it is suggested that the interaction is in a typical range of  $120 - 210 \text{ J mol}^{-1} \text{ \AA}^{-1}$ , for comparison a  $\text{CH}_2$  in an alkane can be assumed a surface area of  $29 \text{ \AA}^2$ , which will equal an energy of about  $50 \text{ kJ mol}^{-1}$  [42].

Yan *et al.* took benefit of this behavior by fabricating silica mesochannels perpendicular on top of an indium tin oxide electrode [50]. These mesochannels, providing a diameter of 2 – 3 nm, can be coated using a cationic surfactant, formed by self-assembly, which resulted in the formation of a hydrophobic micelle inside the channel (Figure 1.5).

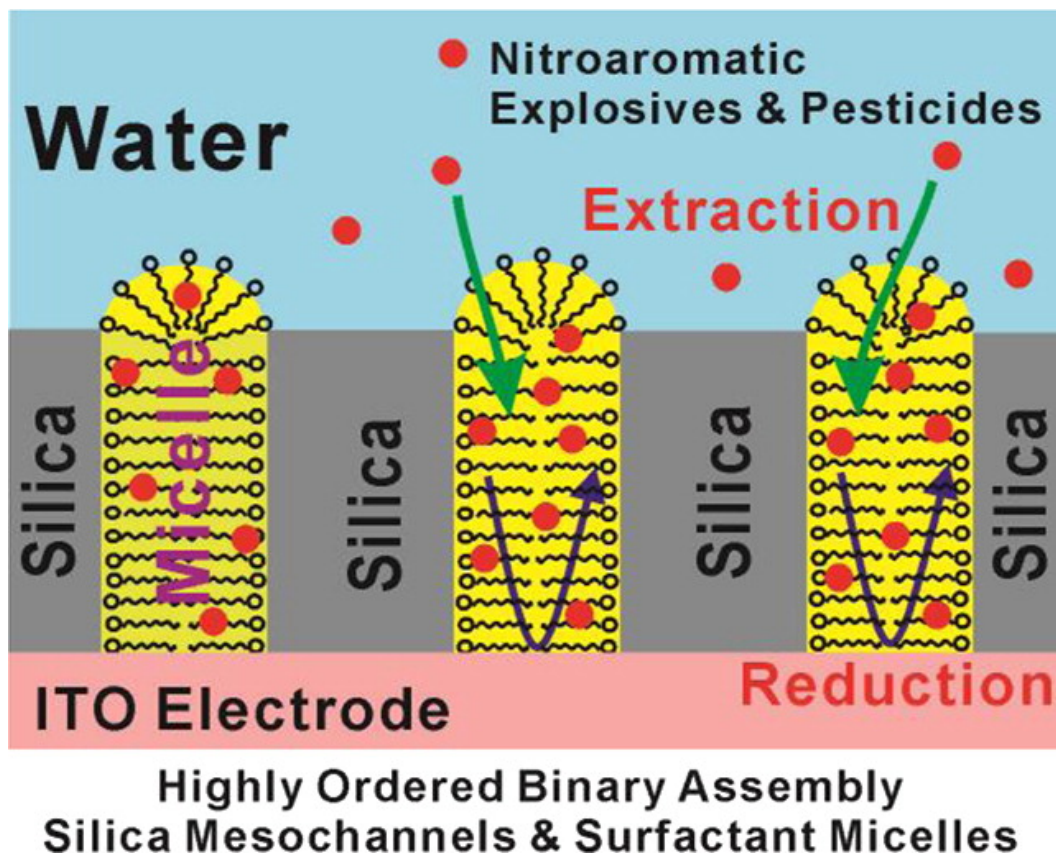


Figure 1.5: By coating silica nanopores with a cationic surfactant a hydrophobic environment is formed inside the mesochannels. Hydrophile molecules are prevented to enter the channels and reaching the sensor surface. Reprinted with permission from [50]. Copyright 2015 American Chemical Society.

This environment inside the channels enables the preconcentration of hydrophobic analytes which then were detected by voltammetry on the electrode at the bottom. Nitroaromatic compounds, including explosives, such as 2,4,6-trinitrotoluene, 2,4,6-trinitrophenol, 2,6-dinitrotoluene, 3-nitrophenol, and nitrobenzene and organophosphate pesticides, such as paraoxon, methyl parathion, and fenitrothion were analyzed. A fast response, wide linear range, high sensitivity, and low detection limit at the ppb level could be achieved. Besides the hydrophobic inside no additional selectivity element was implemented. The obtained discrimination of the different analytes was provided by their different electrochemical responses. The analytes could diffuse freely inside the micelles, which makes this setup also suitable for a measurement in a continuous fluidic system that uses surface plasmon resonance.

In summary, there are a variety of interactions to choose from, when thinking on the development of a specific sensor. For sensors suitable for online monitoring of the quality of drinking water, this is a challenging task, as ideally changes in the matrix caused by

unknown species need to be recognized quickly. Therefore, sensor arrays based on many of those kinds of interactions with only weak binding affinities come into play. Those are ideally generated by nanomaterials which can consist of several moieties combined in one element as demonstrated perfectly for graphene derivatives [51-53].

### *1.5. Nanomaterial Design and Functionalization*

Pollutants found in water are often molecules of small size. Their accumulation on the sensor surface by an enrichment layer still lacks signal changes to be high enough to detect them in a label-free manner by changes of intrinsic parameters such as mass, charge, or refractive index. To gain better sensitivity signal enhancement is necessary and nanomaterials have been demonstrated to be best suited to fulfill this task [54].

For sensing of refractive index changes by SPR the sensitivity decreases exponentially with the distance to the metallic film where the surface plasmons are generated. Therefore, it is obvious that direct modification by self-assembly of only nanometer-thin receptor monolayers was massively studied over the past decades. One, if not the, most commonly known coating of a gold surface is a self-assembled monolayer of thiols [55-57], preferable used as a surface activation layer for further functionalization. Those allow by fabrication techniques such as micro contact printing to structure a continuous gold film by stamping long-chain alkanethiols with different terminal functionalities to a sensor array [58]. The combination of thiols with different functional groups can be used to optimize analyte interaction. This was shown for streptavidin interaction, where the addition of a spacer thiol limits the influence of steric hindrance [57]. The large number of different thiols provides access to almost any kind of interaction type to analytes, but due to limited selectivity, a combination of different interaction types to an array generating signal patterns needs to be envisioned.

Similar to self-assembled monolayers, 2D nanomaterials provide a surface functionalization with the advantage of extremely low thickness for SPR sensing. Depending on the material (*e.g.* carbon materials like graphene, BN, MoS<sub>2</sub>) different interaction types can be addressed. Graphene, mainly described as a hexagonal orientation of sp<sup>2</sup>-hybridized carbons with  $\pi$ -stacking features, can also consist of defects, which are introduced during

synthesis. Depending on the harshness of fabrication the type as well as the number of such lattice defects can be tuned and materials such as graphene oxide or reduced graphene oxide are obtained (Figure 1.6) [51,59].

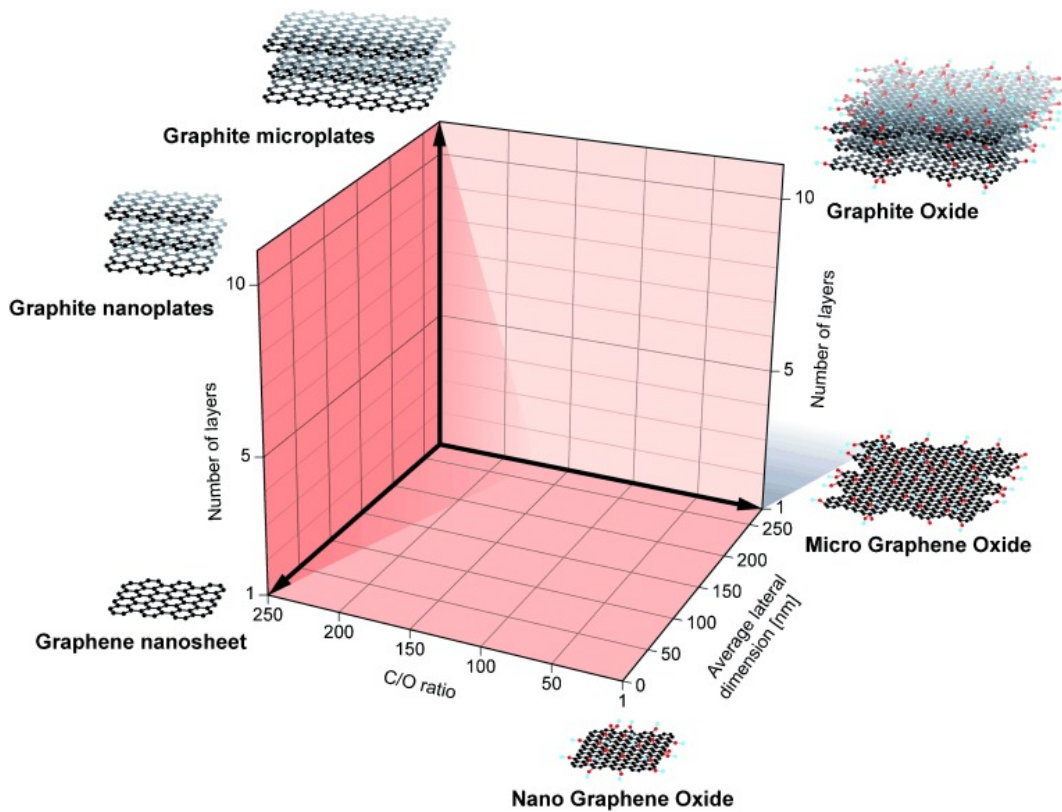


Figure 1.6: Graphene is able to provide different interactions depending on its oxidation degree. Used with permission from [60]. © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

For many applications, such defects need to be avoided, in sensing it turned out that they can bring benefits. Those defects can function as active areas for hydrogen bonding since they typically consist of oxygen atoms. Besides these features, graphene has another major benefit of providing sensitivity enhancement [61]. This happens due to the charge transfer from the nanomaterial surface to the metallic sensing film surface, which introduces a larger evanescent field enhancement [61].

For sensing of small molecules, host-guest interactions can be used by receptors forming nano- or even sub-nanodimensional cages. The most prominent representative of such receptors is cyclodextrin, made out of glucose monomers [44], but there are some more such as cucurbituril [44], made out of glycoluril monomers or calixarene [42,44], which can be based on different components. Depending on the monomer the molecules cavity

provides different interaction properties, where the bare number of monomers influences the cavity size. Additionally, the molecules can be further modified to tune the cavity interaction. An example is the functionalization of  $\beta$ -cyclodextrin with  $\text{NH}_2$ -groups to enhance its differentiation ability for six different psychotropic drugs [62].

In the regard of host-guest interactions, metal organic frameworks (MOFs) are expected to become popular sieves that can selectively enrich the sensing surface by analyte molecules [63]. This novel type of nanomaterial has already been coupled to SPR transduction for demonstrating to enhance the sensitivity towards the detection of volatile organic compounds (VOCs) in biomedical application [64]. So far there are still stability issues within this class of materials, and only a few examples of MOFs capable of sensing in water have been reported [65,66]. Nevertheless, the huge potential is demonstrated, as in the approach when MOFs are combined with SPR selectivity towards different VOCs was achieved by combining two types of MOFs, ZIF-8 a hydrophobic MOF together with ZIF-83 which is more hydrophilic [64]. As the number of available MOFs is constantly growing these materials may be promising in designing receptor patterns which can reversibly discriminate analytes by different interaction towards low-affinity interactions.

A different analyte interaction to the latter elements can be provided by the implementation of membranes or polymers on the surface. Those will require the analyte to diffuse into the fabricated material and don't just use bare surface (or cavity) interaction. Similar to self-assembled monolayer, nanomaterials, and cage-molecules, membranes or polymers can be used to accumulate analytes near the surface, or rather inside the membrane pores. In addition, membranes or polymers can be used to exclude materials with repulsive interactions and can be placed on top of other receptor elements. Thereby, the analyte recognition can be further separated. Membrane or polymer selectivity can range from using a basic material like plain methoxypolyethylene glycol (MPEG) or chitosan [67,68]. This can be further enhanced by the addition of further molecules inside the membrane to tune the provided interactions, like the implementation of zinc oxide into a chitosan membrane [68]. The highest selectivity can be obtained using molecular imprinting [69,70]. The introduction of gold nanoparticles into a polymer is reported to enhance sensitivity, due to the indirect signal response induced by the polymer swelling upon analyte binding (Figure 1.7) [71].

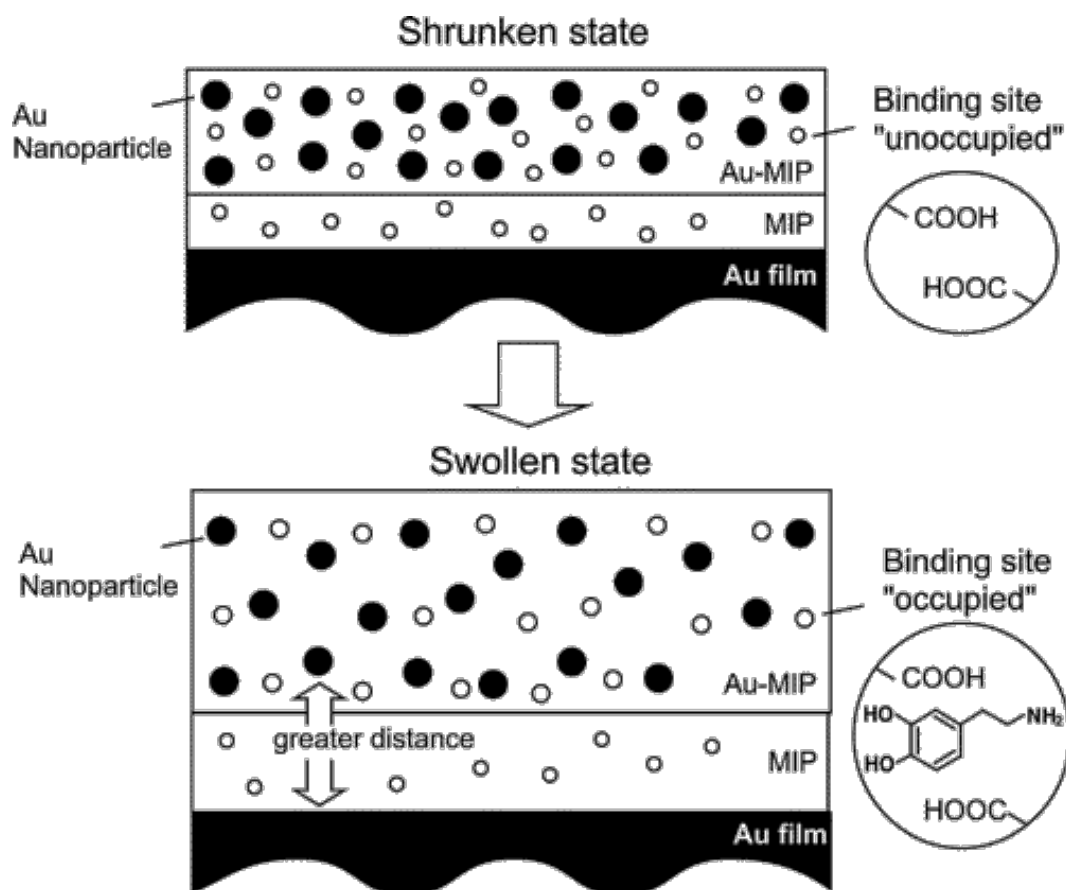


Figure 1.7: The implementation of Au nanoparticles increases the signal intensity upon polymer swelling due to analyte binding. An indirect measurement signal will be obtained. A molecular imprinted polymer (MIP) is used which shows high selectivity to the analyte. Reprinted with permission from [71]. Copyright 2005 American Chemical Society.

In principle, the enhancement by implementing heavy molecules into a membrane or polymer should be possible in different polymers, too, as long as the particles are immobilized permanently and don't leach out. Molecular imprinting polymers suffer from the drawback that in SPR sensing the receptor should be at a close distance to the sensor surface. As the sensing layer is only in the range of a few hundred nanometers in thickness it is challenging to fabricate such thin imprints. Another drawback is the slow regeneration of the sensor surface due to diffusion limitations which limits applications that demand fast online detection.

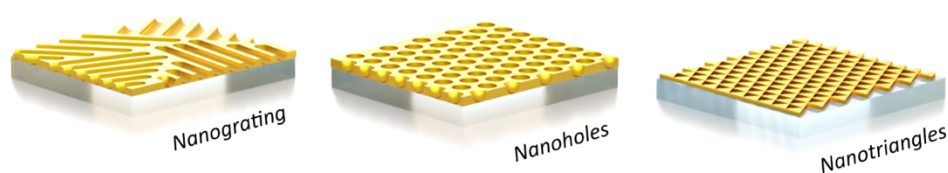
A cross-reactivity sensor will require a smart combination of receptor components to yield the most versatile and suitable sensor array and hence significance. The variety of differently functionalized self-assembled monolayers is a good base for different interaction principles and selectivity, expanded by nanomaterials and cage molecules,

whereby materials like graphene can provide sensitivity enhancement. In addition to those well-known materials, the concept of cross-reactivity further expands the possible sensor elements. Materials like nanofibers [32,72] or mxenes [73], which receive more and more attention from researchers at the moment, are promising candidates to be used for further optimization of the SPR array.

More sophisticated receptor materials with increased sensitivity can yield decreased desorption of the analytes. This makes regeneration of the sensor challenging. Most sensor setups typically require a regeneration step in their sensing protocol. The manifold regeneration steps range from using bases (*e.g.* NaOH) [39,48], salts [39,74], organic solvents [62,75] or surfactants (*e.g.* SDS) [39,40] to remove the analyte from the sensor surface. An online measurement, as desired in environmental monitoring, prohibits the use of additional chemicals, besides that the mere cost of permanently required solutions would stop its applicability. It can be assumed that some regeneration protocols aren't compulsory for the desorption of the analyte but are used to reduce the measurement time. Those wouldn't be necessary for a long-term environmental monitoring device. If this isn't the case energy has to be brought in to disrupt the interaction between analyte and receptor surface. This could be done by improving flow rate and adding kinetic energy, by heating the setup, or at least the surface and adding thermal energy, or by adding electric energy, *e.g.* by exploiting the Starck effect [76] and adding electrostatic repulsion to the surface. Besides overcoming the binding energy, photocatalytic conversion of the analyte to a form that is less attracted to the receptor material, exploiting the photocatalytic properties some (functionalized) nanomaterials provide, could be another possible regeneration step [77,78]. In all cases, the receptor itself needs to stay intact, which demands a lot of research in the future.

If the sensitivity of the composed receptor elements isn't suitable for required threshold values, which are usually very low concerning human sustenance like drinking water, the plasmonic features of the sensor itself can be adapted to improve sensitivity. The vast majority of published SPR sensor concepts or commercially available sensors use a continuous metal film as sensor surface. The surface plasmon resonance then is based on the excitation of propagating surface plasmons, which depends on the metal used [61]. Some materials, *e.g.* silver, theoretically would show a higher sensitivity than the typically

used gold, but are limited in terms of chemical inertness and long-term stability [25,61]. Fortunately, the sensitivity can be enhanced by structuring the metal surface as well. Thereby localized surface plasmons are enhanced, which occur if the dimensions of the nanostructures are lower than the wavelength of the incident light [79]. Those can be coupled with the propagating surface plasmons and hence improve the sensor performance. The excitation of localized surface plasmons is highly dependent on the size and shape of the fabricated particle or structure. That's why different possible metal structures can be used for this purpose. Commonly known is the attachment of spherical gold nanoparticles to the sensor surface [61]. Their widespread use can be explained due to their easy fabrication and easy availability respectively. A further enhancement is possible by the fabrication of gold particle nanocomposites. An Au-Ag nanoalloy benefits from the increased enhancement of silver and simultaneously remains the inertness of gold. ZnO-, or Fe<sub>3</sub>O<sub>4</sub>-Au nanocomposites were able to improve the sensor performance [61]. More complex structures like nanorod structures, nanocubes, nanocages, nanoprisms, nanoplates, and dendrimer-like shapes can even further enhance the sensitivity and become more and more available due to advancements in colloidal synthesis [61]. Besides nanoparticles, a structured metal surface can enhance localized plasmons as well. Examples are nanotriangle, nanocrescents, or nanohole arrays, to name a few (Figure 1.8) [35].



*Figure 1.8: Nanostructured metallic films can be used to tune the plasmonic field of SPR sensors which can improve sensitivity in close distance to the surface by a better signal-to-noise ratio. This is especially of interest when small molecules need to be detected.*

In addition to coupling both types of surface plasmons, those structured surfaces have the benefits of providing reproducible and scale-able manufacturing methods and easier handling if applied to a chip-based setup [34,35]. All mentioned modifications should still be able to be coated with the range of surface coatings described in this work.



### 1.6. Conclusion

Drinking water observation has high requirements on a potential monitoring device. Long-term, online, label-free measurements should detect small molecules regarding their low threshold concentrations. SPRi as sensor concept can provide these features since it is well known for its long-term, online, and label-free measurements. But the fulfillment of all requirements is only possible if a combination of sensor setup and receptor layer is found which enables optimal sensitivity and selectivity. The typically used receptor element biomolecules (*e.g.* antibodies or enzymes) are usually chosen for their high affinity to their counterparts which enables a high selectivity. This advantage will be an issue for continuous water monitoring since it prevents easy desorption of analytes. In this case, the sensor would perform as a dosimeter and would need care after detecting pollutions every time. The remote places where a water sensor would be implemented prevents this type of action. Therefore, receptor elements using interactions with lower affinity, more precisely non-covalent interactions have to be implemented in such a setup. The interactions most prominent in aqueous media are suggested to be hydrogen bonding,  $\pi$ -effects, van-der-Waals, and hydrophobic interactions.

Materials or coatings providing those can be implemented on a sensor surface and additionally be combined or tuned appropriately to the desired use case or expected analytes. There are easy to perform, yet versatile coating methods, like the self-assembly of functionalized alkanethiols on the gold surface, the addition of two-dimensional nanomaterials on the surface, or the use of cage-molecules, like cyclodextrin, providing lower selectivity. The very adaptable fabrication of membrane coatings can be fitted to the desired demands which needs a higher amount of development, but in return, its selectivity can be highly tunable. The concept of a cross-reactivity sensor additionally expands the possible receptor elements since it doesn't require high selectivity or affinity respectively. That makes nanomaterials interesting. Introduced into a cross-reactivity sensor and thereby in combination with other receptors nanomaterials can contribute to a highly selective sensor response.

In conclusion, there is a huge variety of different receptor elements which can be adapted to the desired sensing purpose. Besides the developed sensor hardware, the evaluation

software will contribute to a high sensor performance as well. The evaluation of cross-reactivity sensors demands computer-aided data processing on a complex level. Statistical analysis like principal component analysis with an automated evaluation is the simplest way for data evaluation at the moment. Artificial intelligence is currently a hot topic that will certainly improve the pattern analysis methods in array-based imaging processing. In combination with machine learning, it is envisioned to be able to generate highly meaningful statements about water quality.

### 1.7. References

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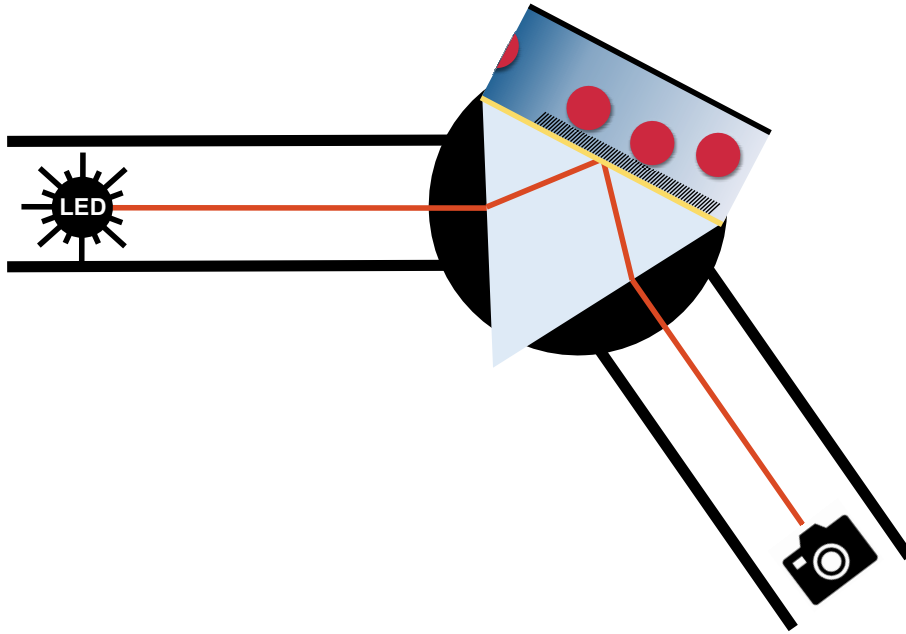
## 2. Aim of the Work

This work investigates the possibilities of surface plasmon resonance imaging (SPRi) for environmental monitoring. The increasing amount of pollution in the environment becomes a more and more severe problem. Unfortunately, the comprehensive monitoring of contaminations in environmental samples appears to be difficult. This work tackles the question if a label-free, online and long-term sensor based on SPRi is suitable for this purpose. Due to the low concentrations of pollutants, high selectivity and sensitivity are desired. A cross-reactivity sensor design for SPRi should be developed for this purpose. In combination with materials that bind analytes by non-covalent interactions, a label-free and long-term measurement is expected to be possible. To complement the sensor design suggestions are made on how to reach the required sensitivity.

## **3. Nanoparticle Determination in Water by LED-Excited Surface Plasmon Resonance Imaging**

### *3.1. Abstract*

The increasing popularity of nanoparticles in many applications has led to the fact that these persistent materials pollute our environment and threaten our health. An online sensor system for monitoring the presence of nanoparticles in fresh water would be highly desired. We propose a label-free sensor based on SPR imaging. The sensitivity was enhanced by a factor of about 100 by improving the detector by using a high-resolution camera. This revealed that the light source also needed to be improved by using LED excitation instead of a laser light source. As a receptor, different self-assembled monolayers have been screened. It can be seen that the nanoparticle receptor interaction is of a complex nature. The best system when taking sensitivity as well as reversibility into account is given by a dodecanethiol monolayer on the gold sensor surface. Lanthanide-doped nanoparticles, 29 nm in diameter and with a similar refractive index to the most common silica nanoparticles were detected in water down to  $1.5 \mu\text{g mL}^{-1}$ . The sensor can be fully regenerated within one hour without the need for any washing buffer. This sensing concept is expected to be easily adapted for the detection of nanoparticles of different size, shape, and composition, and upon miniaturization, suitable for long-term applications to monitor the quality of water.



*Figure 3.1: Graphical abstract of the publication “Nanoparticle Determination in Water by LED Excited Surface Plasmon Resonance Imaging”*

**This chapter has been published.**

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**Author Contributions:** This study was conceptualized by Lukas Wunderlich (LW), Thomas Hirsch (TH), Peter Hausler (PH) and Rudolf Bierl (RB). The authors have been invited to make this contribution. Methodology was suggested by PH, LW and Susanne Märkl (SM). Validation and investigation was done by LW, PH and SM. Data curation was performed by LW and PH. The original draft was created by LW and PH, which was reviewed and edited by LW, PH and TH. Supervision by RB and TH. All authors have read and agreed to the published version of the manuscript.

### *3.2. Introduction*

Nanoparticles are becoming more and more of an everyday material, as they find applications as catalysts in petroleum refining, as surface coatings in displays and optical glasses, in paints, as smart fabrics with antimicrobial properties in textiles, as drug delivery systems in biomedicine, or in cosmetics. The most prominent nanoparticles are made from gold, silver, iron, lanthanides, TiO<sub>2</sub>, and silica. The European Community reports a production of about 1.5 million tons per year of silica particles [1], and a mean concentration of 5.34 µg L<sup>-1</sup> is predicted to be found in fresh surface water [2]. The potential risk of nanoparticle pollution has been known for more than a decade. The sheer number of different nanoparticles [3], in addition to their prevalent use, led to the assumption of possible health-related problems [4, 5]. In addition to that, no or little information is available about the health risks of nanoparticles after their release to nature and potential subsequent degradation [3, 6]. A major problem, besides their complete removal, is the challenging detection of these small particles over a widespread area in complex media to identify the nanoparticle paths over their lifespan [5, 6]. As a result of the increasing concentration and subsequent health risks of nanoparticle pollution, different techniques have been investigated to enable the widespread monitoring of them and provide insights into their distribution and concentration. Detection methods such as inductively coupled plasma combined with mass spectrometry (ICP-MS) [7], surface enhanced Raman spectroscopy [7,8] or electrochemical techniques [7,9] have been tailored to be suitable for nanoparticle detection, all with different advantages and disadvantages. ICP-MS shows a high sensitivity but needs elaborate equipment and a high amount of labor. Measurements based on surface enhanced Raman spectroscopy and electrochemistry can enable an online and miniaturized setup, but certainly still require sample pretreatment and are confined to a selection of particles. This clearly demonstrates the need to develop sensors suitable for the online monitoring of nanoparticles in the environment.

Surface plasmon resonance (SPR) might be a sufficient tool for this purpose. The excitation of surface plasmons on a gold surface deposited on a prism by a monochromatic light source is highly sensitive to changes in its surrounding environment [10 - 13]. Nanoparticles, which are large sized analytes, compared to other pollutants such as

chemicals or pharmaceuticals, generate a larger signal change in SPR, which allows us to detect them at relative low levels [14 - 17] or use them as labels for signal enhancement [18, 19]. The advantage of using an SPR imaging (SPRi) setup compared to a single channel SPR is the ability to easily functionalize the sensor surface by a variation of receptors, enabling the determination of multiple analytes at once [20, 21]. Reflectivity-based SPRi setups have been reported to resolve about  $10^{-5}$  refractive index units (RIU) [22]. There were many tries to overcome this limit by developing advanced SPRi technologies such as spectral SPRi or phase contrast SPRi [23]. However, these technologies all had disadvantages, such as a more complex setup, which impedes a widespread sensor implementation. The sensitivity of reflectivity-based SPRi can be enhanced by the implementation of a more sensitive and temperature-stabilized camera of a high-resolution (16-bit and better) [24]. While having a more sensitive detector, the properties of the light source became more important. Point-based SPR predominantly uses lasers as a light source. This is because most semiconductor lasers are polarized and emit at a wavelength with a very narrow full width half maximum (FWHM). In addition to such an advantage, laser light sources in SPR suffer from a spatial coherence that generates diffraction patterns from every object within the light beam. Hence, every dust particle or every structure at the sensing surface will generate diffraction patterns. Moreover, the quality of the retrieved SPR image in prism-coupled sensors is affected by the creation of speckles, which arise from the roughness at the interface of the glass to the metal layer, when it is in the range of the excitation wavelength or higher. Furthermore, semiconductor lasers lack a temperature-dependent wavelength shift and they can even demonstrate mode hopping. To overcome these problems and enable the full potential of a high-resolution camera, the laser was exchanged with a high-power LED, which enables a homogenized intensity over the whole sensor surface and low noise [25]. To enable the required selectivity as well, the SPR prism was functionalized with differently capped alkanethiols, providing different surface charges, as receptor elements. Their low selectivity is ideal for an application in a cross-reactivity sensor system in the future, capable of detecting the huge variety of different nanoparticles. Lanthanide-doped yttrium fluoride particles have been chosen as model particles in this work. First, they belong to non-metallic particles, and therefore cannot be easily detected by electrochemical stripping methods [5], which makes them ideal candidates to demonstrate the power of

SPR-based sensing. Second, those particles can be tailored very reproducibly regarding their size, with extremely narrow size distribution. Their refractive index of  $\sim 1.475$  [26] is nearly identical to the widely applied silica nanoparticle ( $n_D = 1.475$ , [27]), which, in contrast to the yttrium fluoride particles, tend to form aggregates, and therefore are not as ideal as model analyte particles. With this study, we demonstrate the feasibility of the reversible detection of the presence of nanoparticles in water by using an improved SPRi setup.

### 3.3. Materials and Methods

6-Amino-1-hexanethiol hydrochloride, 16-mercaptohexadecanoic acid (90%, w/w), 1-dodecanthiol (98%, w/w) and ethanol (absolute, >99.8%, w/w) were purchased from Sigma-Aldrich (Taufkirchen, Germany). NaCl salt (>99.5%, w/w), glucose (p.a.) and ethanol (p.a.,  $\geq 99.8\%$ , w/w) were obtained from Carl Roth (Karlsruhe, Germany). The poly(dimethylsiloxane) (PDMS) stamp was fabricated using Sylgard 184 silicone elastomer base and curing agent by Dow Corning (Wiesbaden, Germany) in a ratio of 10:1 (v/v). All reagents were used without further purification. Double distilled water was used in all experiments.

Oleate-coated core-shell nanoparticles  $\text{NaYF}_4(\text{Yb,Er})@\text{NaYF}_4$  were prepared by means of a protocol published recently [28] in a two-step approach. Firstly, core particles were synthesized from the rare earth trichlorides in high boiling solvents with 78%  $\text{YCl}_3$ , 20%  $\text{YbCl}_3$  and 2%  $\text{ErCl}_3$ . Cubic  $\text{NaYF}_4$  particles were used to grow shells around the core particles through a stepwise injection of the cubic particles to a boiling dispersion of the hexagonal particles. The oleate coating of the core-shell nanoparticles  $\text{NaYF}_4(\text{Yb,Er})@\text{NaYF}_4$  was exchanged for  $\text{BF}_4^-$  [29], and the  $\text{BF}_4^-$ -coated nanoparticles were dispersed in  $\text{H}_2\text{O}$ . The concentration was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) with a Spectro Flame-EOP (Kleve, Germany).

A commercially available BioSuplar SPR 521i instrument (Mivitec GmbH, Sinzing, Germany) was used, equipped with an F1-65 glass prism installed on a swivel carriage. F1 gold-coated glass slides ( $20 \times 20$  mm) were obtained from Mivitec GmbH (Sinzing, Germany).

The homemade SPRi setup was built with a V-shaped light path with fixed arms and a movable middle edge on a linear stage, to adjust the measurement angle (step size  $0.001^\circ$ ). An OSOLON SQUARE 660 nm LED was purchased from Osram GmbH (Berlin, Germany). The

LED was driven by a 2636B SourceMeter by Keithley (Cologne, Germany) at a current of 10 mA. Further implemented optical components were an f40 aspheric plastic lens and a 1:9000 ratio polarizer, from Edmund Optics (York, UK), and a 2 nm bandwidth filter. The laser reference measurements were performed using a 660 nm, 75 mW miniLas from RGB Lasersystems (Kelheim, Germany), which was run at a power of 10 mW. The image is acquired by a 16-bit sCMOS Camera Edge 4.2 from PCO AG (Kelheim, Germany).

Schott F2 glass prisms were installed in this setup. Before usage, the prism surface was polished to grade P4, and an approximately 1 nm chromium adhesive layer and an approximately 50 nm gold layer were deposited on top by electron beam deposition.

The microfluidics consisted of a micropump, purchased from Bartels Mikrotechnik GmbH (Dortmund, Germany) and a custom-made microfluidic chip, made of stainless steel.

The refractive index of the solutions was determined with a DR6300-T refractometer from Krüss (Hamburg, Germany). Dynamic light scattering and zeta potential measurements have been performed with a Nano ZS from Malvern (Worcestershire, UK) at 20 °C. Transmission electron microscopy (TEM) was used to estimate the nanoparticle size. Samples on carbon-coated copper grids (400 mesh) were investigated by a 120 kV Philips CM12 microscope (FEI, Munich, Germany).

The gold-coated glass slide or prism was functionalized with the respective alkanethiol monolayer by self-assembly, using 200  $\mu\text{M}$  alkanethiol solution in ethanol. A structured self-assembled monolayer was obtained by microcontact printing with a PDMS-stamp of a squared structure.

Before measurement, the SPR prism or slide was cleaned with ethanol and dried under nitrogen flow. A stable SPR signal was obtained after flowing degassed water for about one hour. Each nanoparticle solution was cycled over the surface for six minutes. Next, degassed water was applied to the surface to remove the excess of nanoparticles from the slide. All steps were performed at a constant flow rate of 0.2 mL min<sup>-1</sup>. After the highest nanoparticle concentration was applied, the slide was extensively washed, at a flow rate of 0.4 mL min<sup>-1</sup>, until the signal intensity was back to its original level. At last, the SPR signal was calibrated to RIU with NaCl solutions of a known refractive index.

### 3.4. Results

In SPR imaging, the sensitivity is mostly influenced by the choice of a proper light source and by a camera system with a high resolution. The wavelength sensitivity of the SPR signal demands a nearly monochromatic, wavelength-stabilized light source. Typical monochromatic, polarized light sources are lasers. However, lasers do have spatial coherence, which causes speckles on rough surfaces and interference patterns from every dust particle and the edges of optical components in its light path [25]. Hence, an LED-based light source was implemented to overcome these limitations. The optimized setup uses a 16-bit camera which yields a relative signal change of 1545 counts by measuring two aqueous reference solutions with a refractive index change of  $1.2 \times 10^{-4}$  RIU, which equals a resolution of  $7.8 \times 10^{-8}$  RIU per count. When taking into account the signal's standard deviation ( $3\sigma$ ) of 2.36 during the 3 min measurement time, a sensitivity of  $5.5 \times 10^{-7}$  RIU can be achieved.

Such a low limit of detection enables SPRi as a sensing tool for the label-free detection of nanoparticles in water samples. To prove this, lanthanide-doped yttrium fluoride particles were studied in this work. The reason for choosing these particles is due to the possibility to synthesize them in a controlled size with high uniformity, as demonstrated by the low polydispersity, which allows one to calculate concentrations in a more comfortable. These particles have a surface that is known to be highly attractive to negatively charged ligands, which saturate vacancies at the nanoparticle interface. As the surface, ligand  $\text{BF}_4^-$  was chosen, which increases their dispersibility in water. The nanoparticles have a size of  $29 \pm 2$  nm, estimated by averaging the diameter of 3603 particles from transmission electron micrographs. This size was chosen as a perfect average size, as most nanoparticles in practical applications currently are in a range from 10 to 50 nm in diameter. The zeta-potential of these particles, measured at a concentration of  $12.1 \pm 0.2$  mg mL<sup>-1</sup> in water, is  $-3.63 \pm 0.09$  mV, which is close to neutral, expecting that electrostatic interaction will not be the main force for binding to any surface.

To enable controllable interaction of the nanoparticles with the sensor surface, a suitable surface modification has to be found. Besides the surface coating, but according to the zeta-potential, their affinity was highest for the bare gold surface and a dodecanethiol-modified gold surface, with a neutral surface charge (Figure 3.2).



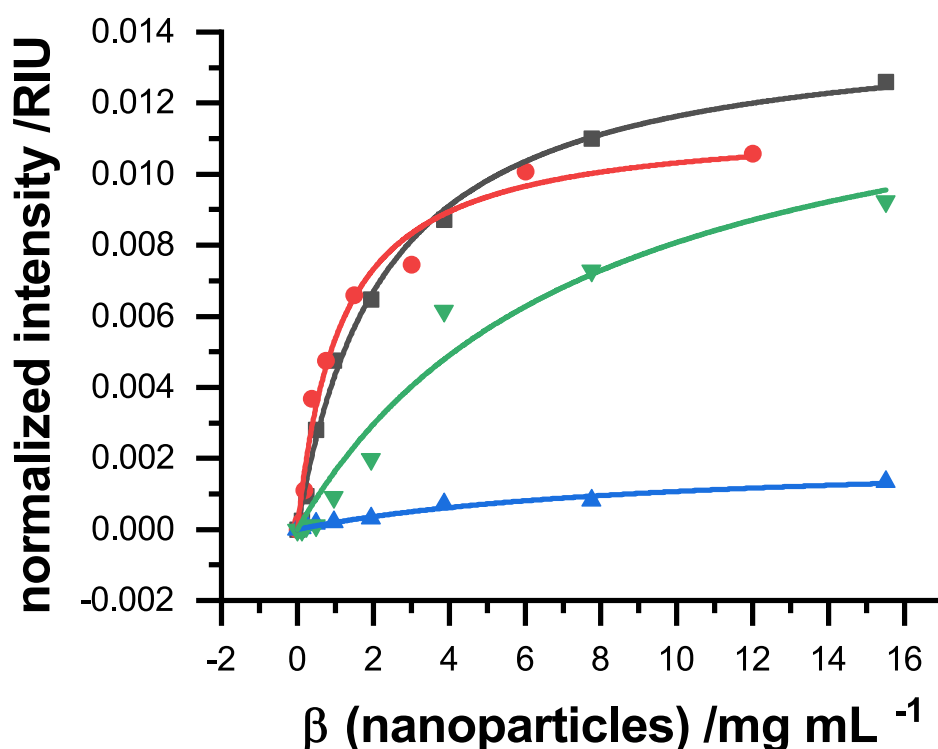


Figure 3.2: Dose-response curves of lanthanide-doped nanoparticles of  $\text{NaYF}_4$  type measured by a point-based SPR setup ( $N = 1$ ). The gold slides were functionalized by a self-assembled monolayer of dodecanethiol (red), 16-mercaptohexadecanoic acid (green) or aminohexanethiol (blue). The signal response to a non-modified, blank gold slide (black) is shown as a reference. The data have been fit by a Langmuir isotherm.

Thereby, the overall binding capacity was higher for blank gold, but the affinity was superior towards the self-assembled monolayer. The binding constants for all surface modifications are summarized in Table 3.1. In comparison, if surface charges are applied, negative *via* a coating of 16-mercaptohexadecanoic acid and positive *via* a coating of 6-aminohexanethiol, the binding performance was severely decreased. A negative surface charge decreases binding affinity but still enables a relatively high binding capacity, whereas a positive surface charge decreases both to a minimum.

Table 3.1 Binding constants of the different self-assembled monolayer-modified SPR surfaces and a blank gold surface to lanthanide-doped nanoparticles of  $\text{NaYF}_4$  type. A Langmuir isotherm was applied to the respective dose-response curves for fitting.

Surface	$K_D$ [mg mL <sup>-1</sup> ]	$K_a$ [mg <sup>-1</sup> mL]	$R_{eq}$ [ $10^{-3}$ RIU]
blank	$2.2 \pm 0.2$	$0.44 \pm 0.04$	$14.3 \pm 0.4$
dodecanethiol	$1.1 \pm 0.2$	$0.9 \pm 0.2$	$11.5 \pm 0.7$
16-mercaptohexadecanoic acid	$7.7 \pm 3$	$0.13 \pm 0.05$	$14 \pm 3$
aminohexanethiol	$9.6 \pm 3$	$0.10 \pm 0.03$	$2.1 \pm 0.3$

The order of the different binding affinities cannot be explained by electrostatic interactions between the sensor surface and the nanoparticle only. Here, one would expect that amino-functionalized surface would lead to good binding. Indeed, binding can be found, but the other surfaces are superior. For surfaces with carboxy functionality at the surface, the particles show a better adsorption behavior as their surface consists of lanthanide ions which do form a stable coordinative binding to carboxy groups, which has been reviewed by Wolfbeis *et al.* [30]. Surprisingly, dodecanethiol was also able to bind the nanoparticles. This finding is interesting as it suggests that the surface modification of those particles performed by ligand exchange, where oleate molecules at the particle surface get stripped off, does result in a complete loss of the original surface capping. The reason for the binding might be that some oleate is still present at the particle surface which interacts by the intercalation of the long hydrophobic tail of the oleate with the long hydrophobic chain of the dodecanethiol. Better insights into nanoparticle surface interaction are currently under investigation and are out of scope of this work. It seems reasonable to continue with the dodecanethiol coating in further measurements. Figure 3.3 shows a typical measurement sequence. After each binding step, a short washing step was applied to exclude the unbound particles from the SPR signal. The particles show a high overall signal change, which is due to their high mass and size.

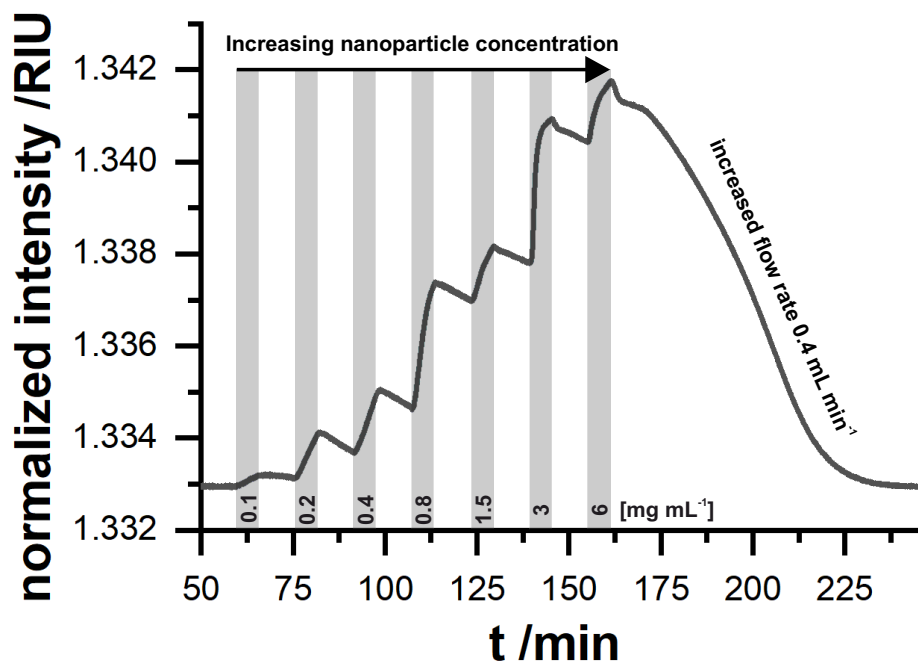


Figure 3.3: Time course of a SPR measurement sequence. The grey areas indicate the presence of lanthanide-doped nanoparticles of  $\text{NaYF}_4$  type in the measurement solution, whereas in the white areas, the system was rinsed with water. In the first part, until  $t = 160$  min, a flow rate of  $0.2 \text{ mL min}^{-1}$  was applied, which was then increased to  $0.4 \text{ mL min}^{-1}$ . The gold surface was modified by a dodecanethiol monolayer.

For an online sensing system of environmental samples such as freshwater, reversibility is one of the key requirements. Gold slides modified with a dodecanethiol monolayer can achieve this. SPR measurements at a flow rate of  $0.2 \text{ mL min}^{-1}$  allow us to easily discriminate the presence of  $0.1 \text{ mg mL}^{-1}$  nanoparticles in water. In contrast to changes in the bulk refractive index, the slope in the change in the signal is concentration-dependent, which also indicates that the particles adsorb to the surface by means of a weak interaction. The desorption at the same flow rate is somehow slower compared to the absorption of the particles to the surface. When doubling the flow rate, the sensor surface can be regenerated within about one hour. This would be attractive for stop flow sensing systems, which would be able to get one data point in less than 1.5 hours. Such a behavior is beneficial for a long-term continuous sensor, since a high signal response can be obtained and the adsorption is faster than the desorption which makes a regeneration step expendable. An optimization of the microfluidic flow cell together with the flow rate is expected to improve the sample rate, but this is out of scope of this study. The discrimination of the signal change caused by fluctuations in the refractive index of the media itself and the presence of nanoparticles can be achieved by exploiting the binding

behavior of different surface modifications together with pattern analysis. This requires the use of sensor arrays and SPR imaging.

SPR imaging relying on an LED and a camera shows the characteristic of an angle shift over the image in x-direction, which was observable during this measurement (Figure 3.4). This behavior would exclude certain regions to be used for signal generation if the whole imaging surface area should be used for only one receptor. However, this would contravene the potential of SPRi. If multiple receptors are used in SPRi, this circumstance can be even useful. Each recognition element has a different SPR angle, as seen for dodecanethiol and aminohexanethiol in Figure 3.4c. The angle shift can be exploited to place both receptors in a region where the complete linear intensity range from the minimum to total internal reflection can be used.

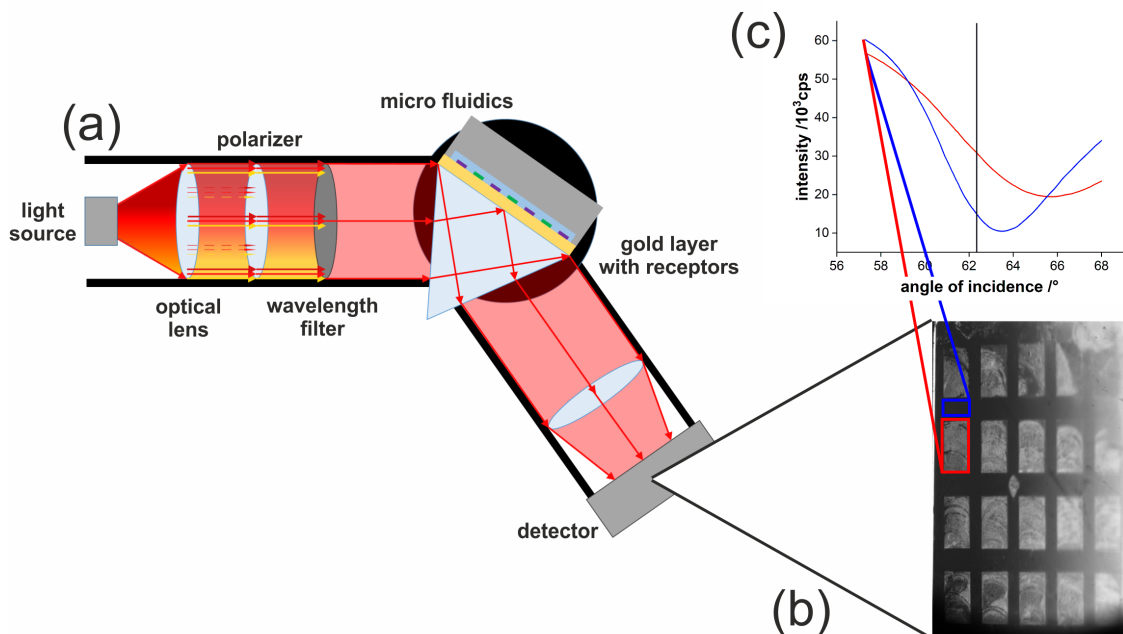


Figure 3.4: a) Scheme of the SPRi setup. b) SPR image obtained when coated with dodecanethiol squares (blue) and surrounded by aminohexanethiol (red). The irregular coating is caused by the stamping procedure to form a patterned surface. c) SPR curves from the two respective thiols. Both were obtained within the region of interest with the same dimensions at the same x-position. The marked positions only show the principal division for illustration purposes and do not equal the actual positions.

SPR imaging is needed to further minimize the detection limit as well. The association curves measured by a commercially available SPR device, shown in Figure 3.5, feature a limit of detection of  $29 \pm 9 \mu\text{g mL}^{-1}$ , which still is too high for a practical application. In

contrast to this, the optimized home-built SPRi system with an LED light source was able to improve the limit of detection under identical measurement conditions by a factor of 20 (Figure 3.5). It should be said that, due to measurement, there was a reduction in the number of nanoparticles of around 50% after multiple measurement cycles, due to experimental limitations, which were both identical for both measurements systems. For a fair comparison, the limit of detection was calculated based on the ICP-OES-estimated concentration of the nanoparticle applied to each system without prior application.

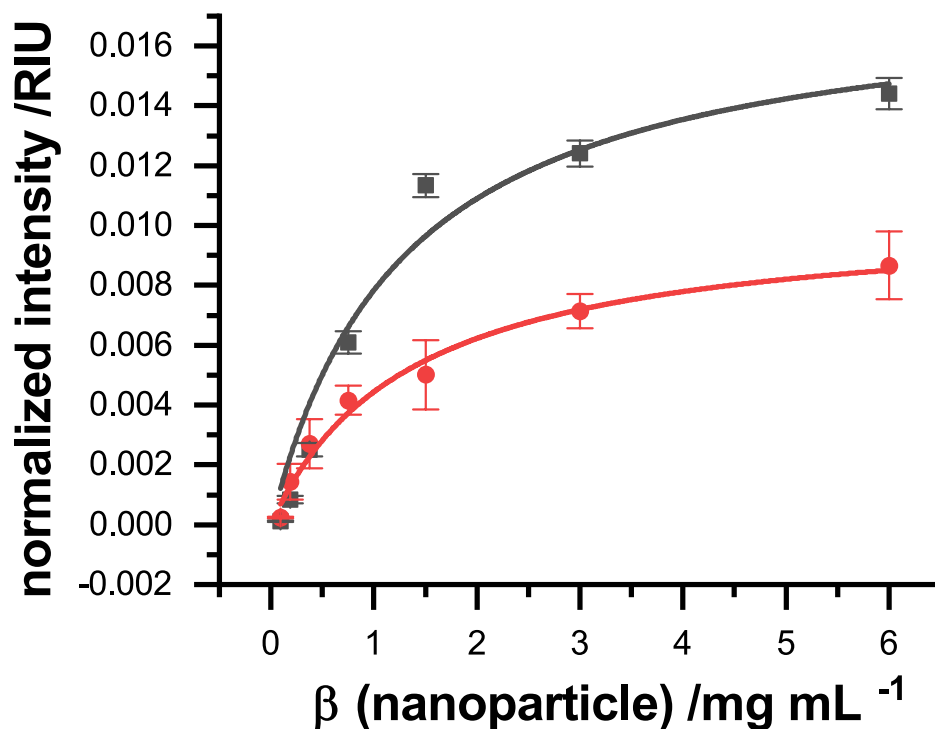


Figure 3.5: Dose-response curve of NaYF<sub>4</sub>-based nanoparticles measured by a point-based SPR setup (red) and an SPR imaging setup with LED illumination (black) (N = 4).

Detailed analysis found that the equilibrium dissociation constant stayed the same, but aside from that, the equilibrium binding capacity was increased (Table 3.2), leading to a limit of detection (LOD) of 1.5  $\mu\text{g mL}^{-1}$ . In contrast to a point-based SPR, where the signal is averaged from the whole illuminated area on the gold, the imaging setup allows us to select only those pixels on the camera image which are best suited in terms of sensitivity and noise due to irregularities and artifacts such as small air bubbles, which get stuck over time when analyzing real samples.

Table 3.2: LODs and binding constants of the point-based SPR and the optimized SPRi setup of NaYF<sub>4</sub> nanoparticle on a dodecanethiol-coated surface. A Langmuir isotherm was applied to the respective dose-response curves for fitting.

Setup	LOD [ $\mu\text{g mL}^{-1}$ ]	K <sub>D</sub> [ $\text{mg mL}^{-1}$ ]	R <sub>eq</sub> [ $10^{-3}$ RIU]
Point-based SPR	29 ± 9	1.3 ± 0.2 *	10.4 ± 0.6 *
SPR imaging	1.5 ± 0.7	1.3 ± 0.4	17.9 ± 0.6

\* Value differs from that in Table 1, due to another batch of similar produced nanoparticles was used.

### 3.5. Discussion

In this work, we demonstrated that the major drawback of reflectivity-based SPRi - its insufficient sensitivity - can be overcome by improving the camera system for detection, as well as the light source. For further development, such a system is capable for miniaturization, and therefore for a wide range of sensing applications. The spreading of the angle of incidence in one direction of the image collected by LED-based SPRi is not necessarily a drawback, as it enables the simultaneous use of different angles of incidence (in a range of up to 3° in our setup) within one sensor chip, without the utilization of any moving component. This allows us to place every receptor with its individual refractive index to be placed at its most sensitive angle position. Temperature drift and fluctuations in the light source can be overcome by using internal reference spots placed on the gold slide. This work is currently under investigation.

By the modification of the sensor surface with different self-assembled monolayers, it became apparent that the interaction of nanoparticles with a sensor surface is of a complex nature, as nanoparticles will be coated by additional surface ligands. Therefore, sensor arrays with cross-reactivity receptors are suggested to tackle this challenge by means of pattern recognition. Surface plasmon resonance imaging might be capable of handling a mix of different particles in composition and size, since it can measure multiple analytes simultaneously. This is made possible by the combination of different receptors and by confining the plasmonic field in its penetration depth by applying nanostructured gold surfaces [31,32]. Thereby, the key will not be to find the most selective receptor for each particle, but to find a smart combination of low selective receptors, which can detect particles by their specific signal pattern. Using LED-based SPRi for this approach, each receptor can be positioned at the angle of highest sensitivity, due to the tunable angle shift.

The limit of detection still needs to be improved when applications in the quality control of environmental samples are envisioned.

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## 4. Conclusion and Further Perspectives for Cross-Sensitivity based SPR Sensing

In the future humanity faces a lot of problems, which need to be overcome. One of them is the ever-increasing worldwide pollution. There are the unsettling pictures everyone has seen already showing plastics in the ocean, uncontrolled landfills, or beaches covered in oil. Besides those highly visible pollutions, a more hidden threat is the contamination of the environment by anthropogenic compounds, like medicine, fertilizers, plasticizers, and all the chemicals broadly used by humanity [1]. Classical chemical sensors focus on the highly selective detection of individual, known analytes. They are unbeatable in their narrow use cases, as they provide high selectivity and sensitivity. This is usually achieved by using (bio)recognition elements that are highly selective and highly tunable to specific analytes or by the addition of labels providing signal amplification [2]. This approach will come to its limits if essential environmental resources such as water or air should be monitored, investigated, and understood over long-time or large areas. The number of sensors that would be required to have a comprehensive view over all contaminated areas in the environment would be innumerable not to mention the effort needed to develop all these sensors. Besides that, many sensors wouldn't be suitable for implementation in remote areas as they require constant maintenance or aren't long-term stable. That is why solutions need to be found how to solve this issue and be able to provide a widespread sensor network with low-maintenance devices measuring in an online manner. A concept used by nature comes into one's mind: the human nose. It is using only a limited number of receptors yet can distinguish a huge number of different odoriferous substances. There are already sensor concepts using such an approach called electric nose but they never achieved a wide breakthrough [3]. A combination with modern techniques like miniaturization, powerful new (nano)materials, increasing computer power, and concepts like big data and artificial intelligence, paves the road, to unleash their full potential and provide the necessary requirements.

Electronic noses, or more scientifically cross-reactivity sensors, don't necessarily rely on the mentioned highly selective biorecognition elements. Instead, a smart combination of semi-selective receptors based on non-covalent interactions can be used to detect a variety of different analytes based on their response pattern [3]. These receptors should be based on non-covalent interactions (hydrogen bonding,  $\pi$ -stacking, van-der-Waals, and hydrophobic interactions [4,5]), which should be addressed carefully, to enable easy desorption and hence regeneration. This behavior will be crucial for long-term sensing.

The application of labels can be overcome by using a label-free sensor technique. Intrinsic parameters of every molecule are for example size, mass, charge, and refractive index. Among those, refractive index, which can be measured by surface plasmon resonance, is of high interest. Surface plasmon resonance is obtained if a monochromatic light source illuminates a gold surface attached to a prism. Thereby surface plasmons are excited which are highly sensitive to nearby changes of refractive index [6]. A selective response can be obtained if a receptor layer is attached. Preferably very thin, since the surface plasmon resonance is limited to an evanescent field of around 300 nm, depending on the excitation wavelength.

SPR is a perfect platform for a cross-reactivity sensor. If a camera is used as detector an image can be obtained which can monitor multiple receptor areas at once [7]. Such a setup is called SPR imaging (SPRi). Until recently reflectivity based SPRi was limited in sensitivity [8]. A reason therefore has been the low bit resolution of the cameras. As cameras were getting better and the bit resolution increased the influence of the illumination by the light source gained a higher amount of influence in sensor sensitivity [9]. Typically used lasers have an issue with spatial coherence which adds speckles and noise to the SPR image and hence decreases sensitivity. That's why the implementation of an LED light source was done to reduce noise and lower sensitivity [9]. It was seen, that a very low theoretical LOD of  $5.5 \cdot 10^{-7}$  RIU can be obtained with this setup, which means that the sensitivity is about 2 to 3 orders better compared to systems excited by laser light.

To get even better - for being able to detect the low threshold value required - these optimized technical components can subsequently be combined with SPR specific sensitivity enhancements, like graphene. Besides this nanostructured gold surfaces can be used. In contrast to the propagating surface plasmons, excited at a continuous gold surface,

plasmonic nanoparticles or nanostructured surfaces can excite localized surface plasmons [10]. Those two can be coupled together and a more sensitive response of the plasmons to changes in their surrounding is obtained.

But all in all, the instrumental optimizations will come to a limit, if not already are close to this limit, where no further sensitivity improvement can be achieved by further optimization. The reason for this border is the circumstance that even if a lower analyte concentration can be resolved the analyte diffusion will then become the limiting factor. This can be seen in the following equation, which describes the dependence of time  $t_s$  needed by an analyte to approach the sensor surface and limit of detection for planar sensors [11]:

$$\rho_0 \sqrt{t_s} \sim N_s \sqrt{\frac{2}{D}}$$

Thereby  $\rho_0$  is the analyte concentration,  $N_s$  the minimum number of analytes to be captured for the generation of a measurable signal, hence the limit of detection and  $D$  the diffusion coefficient of the analyte. Sensor optimization only has an influence on  $N_s$ , whereas all other parameters are tuned either by the analyte itself (concentration  $\rho_0$  and its diffusion coefficient  $D$ ) or the remaining settling time  $t_s$ . Using an exemplary setup, it could be calculated that the switch from nanomolar to picomolar will increase the required settling time from seconds to days or weeks, in the case of a planar setup.

This model shows that the approach of online sensors, providing non-stop information every second, which requires a continuous flow isn't suitable for the low concentrations present and required threshold values in the environmental samples, like water [12]. At a specific ratio, no analyte will be bound on the surface anymore. The changes in refractive index in the bulk solution will still be visible, but for the predicted small concentrations of analytes by far not suitable. In addition to that, a bare bulk detection would cancel any selectivity provided by receptor elements. No meaningful information can then be obtained. But, in the case of environmental monitoring it won't be required to obtain a signal every second. Therefore, one can think of measuring in semi-continuous flow, i.e. an online setup is chosen, but instead of measuring the analyte in continuous flow, a defined measurement volume is enclosed for an specific amount of time. With this settling time

can be increased, where the analyte(s) has time to diffuse to the surface and interact with the receptors. Afterward a SPR image is taken, compared to the state prior to the period and the measurement cell is flushed again to remove the analyte. The increased time will yield in an increased adsorption on the surface, an increased signal and subsequently an increased sensitivity. The sensor still will be online and can deliver a continuous signal, with a lower resolution but a tremendously increased sensitivity. Thereby, the best combination of settling time and signal density must be found.

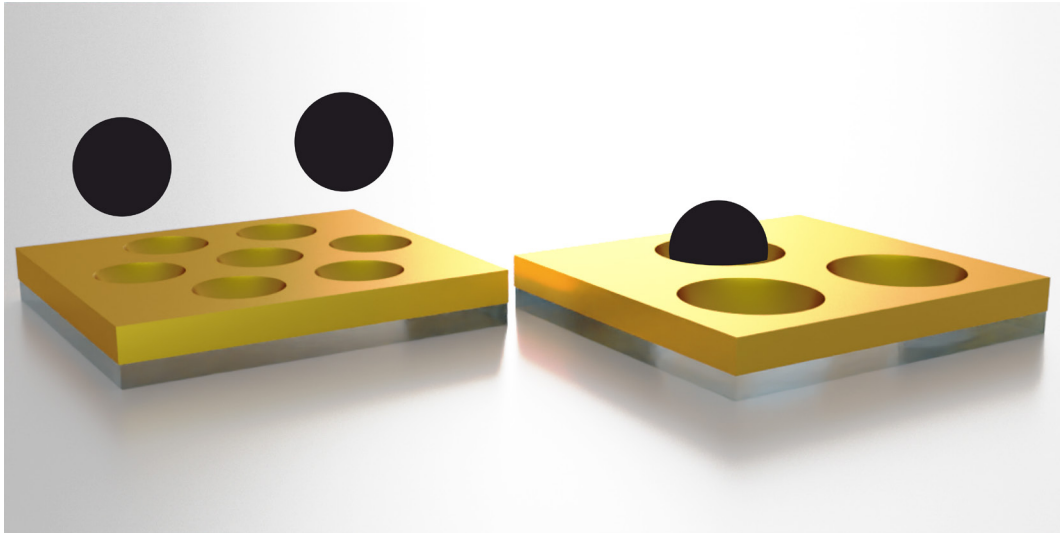
In such a sensor the time delay between two data points is the sum of measurement interval and the necessary time needed for sensor regeneration. The second should be as short as possible to yield a preferably high time resolution. This makes considerations inevitable about a suitable regeneration protocol. As already mentioned, the addition of chemicals to the system isn't applicable. Everything that would need maintenance isn't suitable for the remote areas where environmental sensor should be installed. Therefore, bonding need to be counteracted with addition energy brought into the system. This can happen by introducing thermal energy (rising the temperature), kinetic energy (increasing the flow rate) or electric energy (charging the surface by a current to repulse oppositely charge molecules [13]).

Many different materials can come into one's mind for the implementation in cross-reactivity sensors, providing a broad range of affinities to form a meaningful signal pattern. A coating by self-assembled monolayers, with the ability to vary the head group by moieties changing the interaction forces with its surroundings, is one of the most known methods to functionalize a surface [14]. Usually, they are used to activate the surface for subsequent functionalization. This is again due to the fact, that traditionally high selective coatings were desired. Besides their wide range of different materials and a decisive advantage using these, is their high stability if attached on the surface.

To prove the capability of such rather basic coating by self-assembled monolayers, it was tried to detect nanoparticles using an alkanethiol modified SPR surface. Nanoparticles are becoming more and more of a threat to human health, as their usage is increasing, their removal is limited and hence their concentration in the environment is increasing as well [15]. The high variety of different nanoparticles, providing different surface functionalities, the, now, low concentration and the complex sample matrixes limit a widespread

investigation of occurrence and concentration. A cross-reactivity sensor would be an ideal platform for this purpose. The adsorption of lanthanide-based particles on a 1-dodecanethiol coated surface showed perfect properties for a non-selective interaction needed. The nanoparticles adsorbed on the surface and an increased signal, compared to the bulk phase, could be obtained. At the same time the particles weren't bound permanently and desorbed from the surface over time. This makes the sensor usable over a long period of time without further maintenance. In addition to that, the coating with differently end capped alkanethiols, providing different surface charges yielded in varying affinities of the nanoparticles to the surface. In principle this already is a kind of signal pattern generation. It can be suggested that particles with a different surface charge would interact differently, with the, in this case very basic, surface coatings and hence can be discriminated by their interaction pattern. A low LOD of  $1.5 \text{ mg mL}^{-1}$  could be obtained. This is still too high compared to the predicted concentration of  $\text{SiO}_2$  nanoparticles, which are one of the most common used particles, in surface fresh water which is  $5.34 \text{ ng mL}^{-1}$  [16]. But this initial study used a continuous gold layer and measured in flow, which still leaves high potential in further optimization of the setup, by the already mentioned sensitivity enhancement techniques.

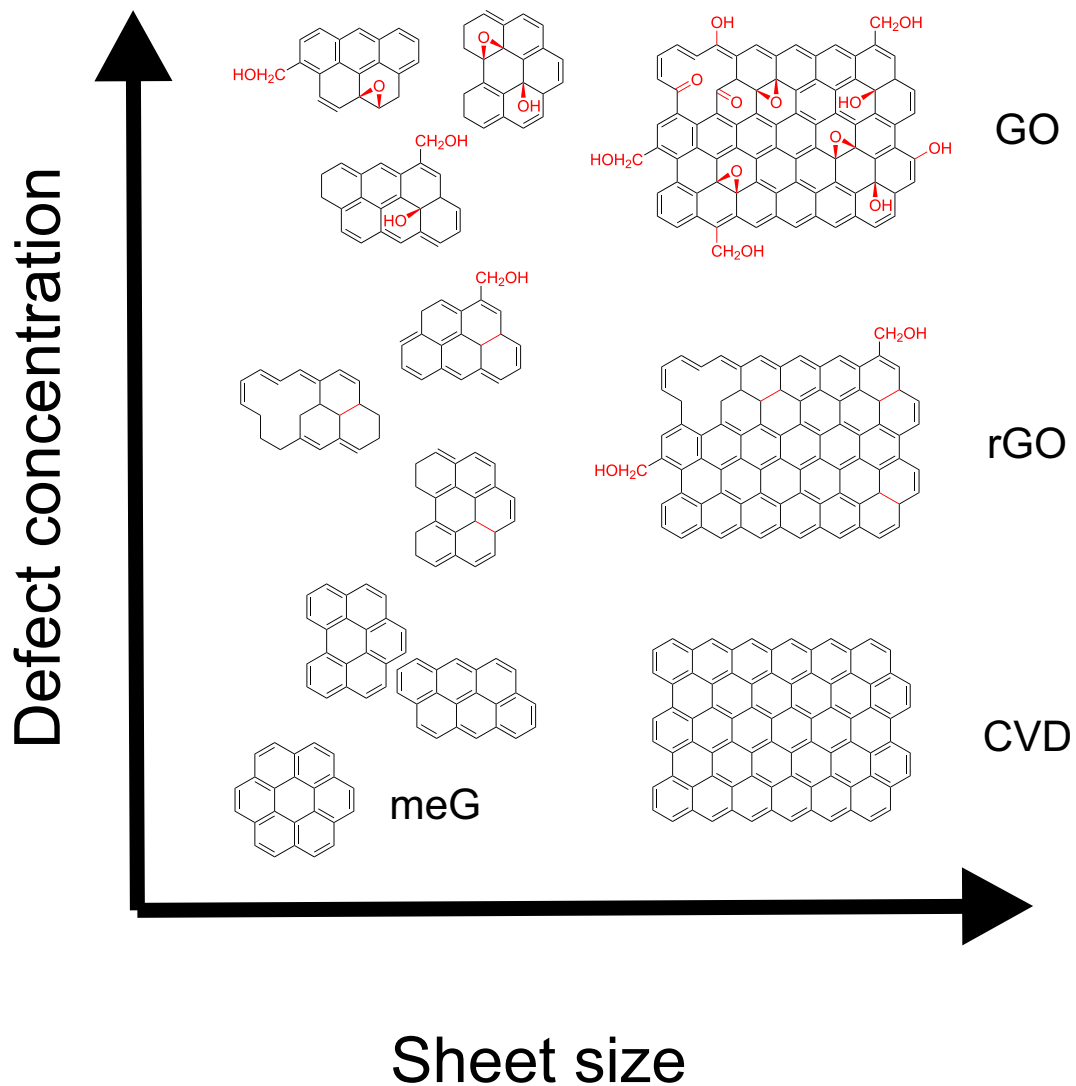
Besides that, the question remains how differently sized nanoparticles can be distinguished since the nanoparticle size plays a role in their access into the human body and hence their toxicity. By finetuning the plasmonic field, using nanostructured gold surfaces, a differently intense signal response could be obtained depending on the particle size to evanescent field expansion ratio. Another possible approach would be to implement differently sized cavities on the gold surface, by lithographic techniques (Figure 4.1). Like host-guest-interaction provided by cage molecules, cavities in the gold surface can be fabricated with a spectrum of different sizes which can then be equipped with functionality by adding receptor materials. Thereby, the analyte interaction has to be tuned exactly to this amount, that only inside the cavity, the interactions add up to increase the residence time of the particle near the sensor surface. Mixtures of different self-assembled monolayer are a straightforward way to do so. To best knowledge such a technique hasn't been investigated yet, hence it needs further development.



*Figure 4.1:* Cavities in the gold surface can be used to distinguish different nanoparticle sizes. Only particles smaller than the cavity can enter. There a finetuned functionalization enables a selective interaction and yields an increased residence time of those.

In general, this technique would be useful for any type of analyte, providing the capability of fine-tuning affinities by cavity formation.

A more sophisticated surface coating than self-assembles monolayers can be provided by two-dimensional nanomaterials or nanomaterials in general. They tend to be low in size as well and can provide various surface interactions. Graphene, first time fabricated by Geim and Novoselov in 2004 [17], is the most prominent representative of those materials. It has been suggested for a huge variety of different applications, which includes SPR where it has been implemented as receptor layer [18]. It is gladly used due to its thin layers, which nevertheless can provide interaction areas for  $\pi$ -stacking due to its conjugated  $\pi$ -electron system. Besides that, it is a welcome surface coating, due to its additional sensitivity enhancement, by charge transfer from graphene to the metallic sensing film, which introduces a larger evanescent field [10]. The  $\pi$ -stacking capability is widely known. What hasn't gained much interest up to now is the influence of defects to its binding capabilities. Depending on its synthesis protocol different number of lateral defects in the carbon layer and, more important, oxygen defects are present in this material (Figure 4.2).



*Figure 4.2:* Different graphene materials arranged regarding their defect concentration and sheet size. Chemically vapor deposited graphene (CVD) and mechanically exfoliated graphene (meG) can be fabricated without oxygen defects. In contrast to that the fabrication of graphene oxide (GO) introduces lateral and oxygen defects, with the number depending on the fabrication harshness. After subsequent reduction to reduced graphene oxide (rGO) a number of those remains in the material.

Both limit  $\pi$ -stacking capability, but an increasing oxygen content provides new interaction areas for hydrogen bonding. To verify this hypothesis, it was tried to detect four purine bases with graphene oxide (GO), presumably providing hydrogen bonding and to a minor amount  $\pi$ -stacking, and a reduced graphene oxide (rGO), preferably providing  $\pi$ -stacking.



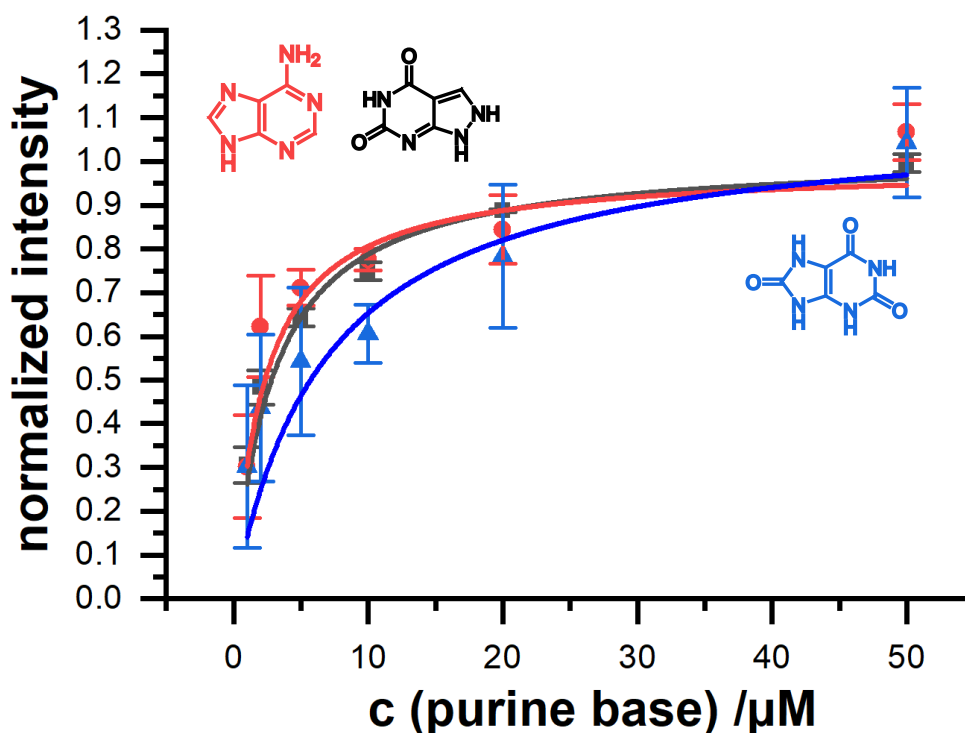


Figure 4.3: Dose-response curves of purine bases measured on a surface coated with reduced graphene oxide ( $N = 3$ ). Oxipurinol (black) and adenine (red) show a similar binding affinity, whereas uric acid (black) affinity is decreased. The intensity was normalized to the fitted value for  $S_{\text{max}}$  to highlight the different dissociation constants.

In the case of reduced graphene oxide adenine and oxipurinol both show a rather similar binding behavior (Figure 4.3). Their undisrupted molecular structures provide the best qualification for  $\pi$ -stacking. The carbonyl groups of oxipurinol stay in the same plane and hence don't hinder  $\pi$ -stacking, which is very dependent on distance (suggested to be similar to London Dispersion,  $r^{-6}$  [5]). That's the reason why no significant binding could be observed in the case of caffeine since its methyl groups sterically hinder the interaction. The decreased interaction of uric acid is justified by the reduced conjugated  $\pi$ -system. This circumstance shows, how susceptible those interactions are. If the surface is exchanged to graphene oxide, the observed binding behaviors change completely.

Table 4.1: Dissociation constants and maximal signal change obtained from oxipurinol, adenine, uric acid and caffeine interacting with reduced graphene oxide (rGO) and graphene oxide (GO). Caffeine didn't show significant binding on rGO, which is why no meaningful values could be obtained.

Analyte	rGO		GO	
	$K_d$ [ $\mu\text{M}$ ]	$S_{\text{max}}$ [RIU]	$K_d$ [ $\mu\text{M}$ ]	$S_{\text{max}}$ [RIU]
Oxipurinol	$2.5 \pm 0.1$	$3.6 \cdot 10^{-4} \pm 0.7 \cdot 10^{-4}$	$1.1 \pm 0.3$	$1.6 \cdot 10^{-4} \pm 0.6 \cdot 10^{-4}$
Adenine	$1.9 \pm 0.1$	$4 \cdot 10^{-4} \pm 3 \cdot 10^{-4}$	$1.0 \pm 0.6$	$1.9 \cdot 10^{-4} \pm 0.3 \cdot 10^{-4}$
Uric Acid	$4 \pm 4$	$1.6 \cdot 10^{-4} \pm 0.5 \cdot 10^{-4}$	$1.0 \pm 0.3$	$2.6 \cdot 10^{-4} \pm 0.5 \cdot 10^{-4}$
Caffeine	-	-	$1.0 \pm 0.7$	$2 \cdot 10^{-4} \pm 2 \cdot 10^{-4}$

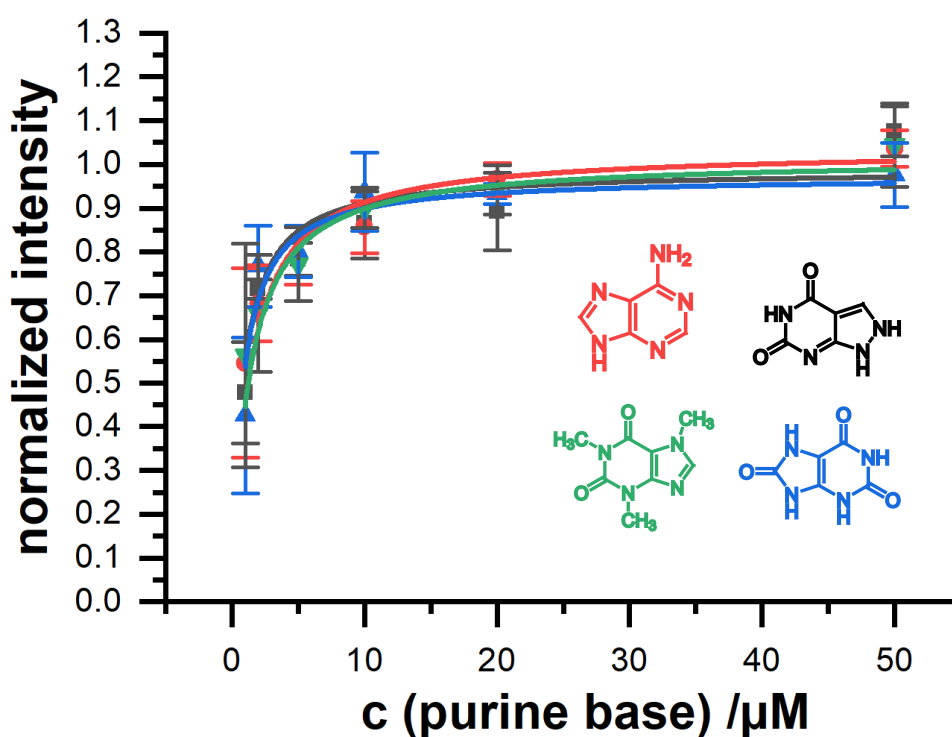


Figure 4.4: Dose-response curves of purine bases measured on a surface coated with graphene oxide ( $N = 3$ ). Oxipurinol (black), adenine (red), uric acid (black) and caffeine (green) all show a similar binding affinity. The intensity was normalized to the fitted value for  $S_{\text{max}}$  to highlight the different dissociation constants.

With GO as receptor, it is suggested that  $\pi$ -stacking only is present to a minor part, due to its oxygen moieties. As clearly visible in Figure 4.4 the dissociation constants all are in a range of about  $1 \mu\text{M}$  (Table 4.1). It seems that hydrogen bonding is less selective, in the case of these four purine bases, than  $\pi$ -stacking provided by rGO. As suggested GO's internal oxygen functional groups limit  $\pi$ -stacking. At the same time hydrogen bonding is available

at higher distances (around  $r^{-2}$  [19]) which is why the methyl groups in caffeine don't show the same impact as in the case of rGO.

The introduction of functional groups highly influences the interaction properties of graphene. This can be further expanded if CVD graphene is used, which is even more likely to provide  $\pi$ -stacking interaction areas [20] or on the other end a higher oxidized graphene oxide, using a harsher oxidation technique [21]. Other functional groups can be introduced as well, which will enable further tuning capability [21]. But one can as well think of differently ratiometric mixtures of two or more materials. These mixtures might provide fine nuances of binding affinities.

Besides graphene, in theory other two-dimensional materials, such as BN [22] or MoS<sub>2</sub> and other transition metal dichalcogenides [23], qualify as receptor layer as well. A major drawback of graphene and two-dimensional nanomaterials in general, compared to a self-assembled monolayer, is their more complicated handling. From synthesis and purification over characterization to deposition, all steps show a lower reproducibility and a higher complexity compared to self-assembly. This can be seen in the measurements shown here as well, where a relatively high standard deviation is present throughout the measurements.

Similar to self-assembled monolayers, the absent widespread use of nanomaterials in sensing is suggested to be due to their limited selectivity which has been essential for traditional sensor design. Cross-reactivity sensors requires precisely this behavior. With this premise the range of coatings can be further expanded to materials like mxenes, nanofibers or metal organic frameworks. In addition to that already established coatings as cage molecules, like cyclodextrins, or a membrane or polymer coatings still are suitable for a cross-reactivity design. With this library a combination can be found to enable a pattern generation to provide high selectivity.

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## Summary

This thesis focuses on the challenges which are faced developing cross-reactivity for surface plasmon resonance imaging (SPRi).

In Chapter one basic requirements for environmental monitoring with surface plasmon resonance imaging (SPRi) and (nano)materials are considered. Theoretical aspects of non-covalent interactions of materials as receptor surfaces are discussed to develop cross-reactivity sensors. The concept of cross-reactivity sensing is explained, which is based on the detection of analytes by a signal pattern provided by a combination of different sensor spots that can interact with varying binding affinities to generate a signal pattern. It is pointed out that label-free, continuous, online, and long-term water monitoring will be best fulfilled by a combination of different material functionalized receptor spots providing non-covalent interactions such as electrostatic interactions,  $\pi$  stacking, or hydrophobic effects. Different materials providing these features were suggested, e.g. self-assembled monolayers or two-dimensional nanomaterials. Only a smart combination of those materials will be able to reach the desired selectivity for a comprehensive overview of water quality. Additionally, improvements in the sensor's sensitivity by nanomaterials are discussed, which can be achieved by using a nanostructured gold surface or plasmonic materials (e.g. gold nanoparticles or graphene).

Chapter two proposes a label-free sensor based on SPR imaging. A high sensitivity can be obtained by using a high-resolution camera. Its application revealed that the light source needed to be improved as well, by using LED excitation instead of a laser light source. As a receptor different self-assembled monolayers have been screened. It could be shown that the nanoparticle receptor interaction is of a complex nature. The best system when taking sensitivity as well as reversibility into account is given by a 1-dodecanethiol monolayer on the gold sensor surface. Nanoparticles of 29 nm in diameter and with a similar refractive index to the most common silica nanoparticles have been detected in water down to  $1.5 \mu\text{g mL}^{-1}$ . This sensing concept is expected to be easily adapted for the detection of

nanoparticles of different size, shape, and composition, and upon miniaturization, suitable for long-term applications to monitor the quality of water.

Future perspectives of cross-reactivity sensing based on SPRi were proposed and discussed in Chapter three. The potential of SPR imaging to form a cross-reactivity sensor for environmental monitoring was further consolidated. It can detect online, continuous, and label-free and can display multiple receptors at once. A lot of different surface coatings qualify for the sensor design, but further research is inevitable. It was pointed out, that many materials haven't been considered yet and even established ones have been underestimated in respect to providing semi-selective interactions. Graphene was shown as an example where minor changes in oxygen concentration provided different binding affinities to four purine bases (adenine, oxipurinol, uric acid, and caffeine). The necessity of overall further and material research in particular, regarding semi-selectivity, is pointed out but it is suggested that in combination with new technologies, like artificial intelligence, a high selectivity could be obtained. In contrast to that sensitivity enhancement of SPRi by instrumental optimization (like optical components or highly sensitive sensor surfaces) is promised to reach a plateau sooner or later. Therefore, new sensing protocols need to be established, e.g. a stop-flow system, with trades time resolution for a higher sensitivity.

## Zusammenfassung

Diese Arbeit beschäftigt sich mit den Herausforderungen bei der Entwicklung eines Kreuzreaktionssensors, mit dem Fokus auf bildgebender Oberflächenplasmonenresonanzspektroskopie (eng. surface plasmon resonance imaging – SPRi).

Das erste Kapitel erklärt die zugrundeliegenden Überlegungen welche bei Umweltüberwachung mit SPRi und (Nano-)Materialien in Betracht gezogen werden müssen. Es wird kurz das generelle Konzept eines Kreuzreaktionssensors (welches auch als elektronische Nase bzw. Zunge bekannt ist) erklärt. Dabei wird versucht Analyten mit einem Signalmuster zu erkennen, welches von verschiedenen Rezeptoren mit verschiedenen Affinitäten erzeugt wird. Um einen solchen Sensor zu entwerfen, der Wasser Label frei, kontinuierlich, online und für lange Zeit überwachen kann, wird eine Kombination aus verschiedenen Materialien vorgeschlagen, die auf nicht-kovalenten Wechselwirkungen basieren. Dafür in Frage kommen elektrostatische Wechselwirkungen,  $\pi$ - und hydrophobe Effekte, welche theoretisch erklärt wurden. Eine Auswahl an möglichen Rezeptormaterialien wurde näher beschrieben, darunter unter anderem selbst organisierende Monolagen und zweidimensionale Nanomaterialien. Nur eine sinnvolle Kombination solcher Materialien ist in der Lage, die nötige Selektivität zu erreichen um ein umfassendes Bild über z.B. die Wasserqualität zu erhalten. Des weiteren wurden Techniken vorgestellt um auch die Sensitivität weiter zu optimieren. Dabei wurde die Verwendung von nanostrukturierten Goldoberflächen und weiteren plasmonischen Materialien angesprochen.

In Kapitel zwei wird ein Label freier, auf SPRi basierender Sensor vorgestellt. Durch die Verwendung einer hochauflösenden Kamera konnte erhöhte Sensitivität erreicht werden. Dabei wurde festgestellt, dass sich der üblicherweise verwendete Laser, wegen verschiedener Störeffekte, dann nicht mehr als Lichtquelle eignet. Die Verwendung einer LED stellte eine gute Alternative dar, da dadurch eine homogene Belichtung erzielt werden konnte. Als Rezeptor wurden verschiedene selbst organisierende Monoschichten verwendet. Die besten Ergebnisse konnten mit einer Schicht aus 1-Dodecanthiol erzielt



werden. Die verwendeten Nanopartikel, mit einer Größe von 29 nm, konnten dabei sensitiv, mit einer Nachweisgrenze von  $1.5 \mu\text{g mL}^{-1}$ , und noch dazu reversibel gemessen werden. Dieses Sensorkonzept kann in Zukunft für weitere Nanopartikel, unterschiedlicher Größe, Form oder Zusammensetzung angepasst werden und eignet sich, in Kombination mit Miniaturisierung, für die Langzeit Überwachung von Wasserqualität.

Im dritten und letzten Kapitel wurden die bisherigen Ergebnisse zusammengefasst, eingeordnet und weiter zukünftige Möglichkeiten von kreuzreaktionsbasierten SPRi Sensoren aufgezeigt. Das bisher schon erkennbare Potential von SPRi für diesen Anwendungszweck wurde weiter vertieft. Zusammenfassend ist SPRi in der Lage online, kontinuierlich und Label frei, viele Rezeptoren gleichzeitig abbilden. Für die hierfür in Frage kommenden Rezeptoren, ist allerdings weitere Forschung unumgänglich, da bisher der Fokus zu sehr auf spezifischer Selektivität gelegen hatte und nicht auf der Verwendung als semi-selektive Rezeptoroberfläche. Dies wurde beispielhaft an einer Graphenrezeptoroberfläche aufgezeigt. Dieses Nanomaterial konnte schon durch minimale Änderung an dessen Sauerstoffanteil so verändert werden, dass veränderte Affinitäten zu vier Purin Basen (Adenin, Oxipurinol, Harnsäure und Koffein) erzielt werden konnten. Nach weiterer Forschung und in Kombination mit neuen Technologien, wie künstlicher Intelligenz, sollten dieses und weitere (Nano)Materialien in einem Kreuzselektivitätssensor ein hohes Maß an Selektivität erzeugen können. Ergänzend dazu wurde festgestellt, dass die Verbesserung der Sensitivität, durch apparative Optimierungen langsam zu einem Ende zu kommen scheint. Es müssen deshalb neue Messkonzepte entwickelt werden, wie z.B. Intervallflusssysteme, welche niedrigere zeitliche Auflösung zeigen würden dafür eine weitaus höhere Sensitivität. Ein solches kann dann bei den technisch optimierten Geräten verwendet werden.

# Curriculum Vitae

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2018 KAUST Sensor Initiative Meeting, KAUST, Saudi Arabien  
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## Publikationen

**Wunderlich L**, Hausler P, Märkl S, Bierl R, Hirsch T: Nanoparticle Determination in Water by LED-Excited Surface Plasmon Resonance Imaging. *Chemosensors* 2021, 9:175. <https://doi.org/10.3390/chemosensors9070175>.

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## Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Weitere Personen waren an der inhaltlich-materiellen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe eines Promotionsberaters oder anderer Personen in Anspruch genommen. Niemand hat von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

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Ort, Datum

Unterschrift