Self-assembly is a very powerful tool for surface modification, providing solutions for a large number of applications from water repellent car windshields to the inhibition of corrosion in industry. This thesis presents the development of a new strategy to achieve controlled self-assembly of charged and uncharged thiolated molecules on gold surfaces in a very fast manner. The main envisioned application is the development of a low-cost, yet powerful electrochemical technique for the production of DNA chips as point-of-care devices. The explored strategy consists of the potential-pulse assisted acceleration of the surface modification process by applying a pulse-type potential modulation, using carefully selected potential pulse intensities and an optimized pulse duration. Once optimized, this strategy increases significantly the immobilization kinetics in a reproducible way as compared to passive self-assembly or immobilization supported by the application of constant potentials.

The quality and cleanliness of the material used for surface modification is highly important for the reproducibility and sensitivity of the envisaged sensing devices. Therefore, special attention was given to the preparation of the surface used for modification. This resulted in a surface preparation protocol defining certain criteria as a prerequisite to achieve a reproducible surface architecture, reflected by the reproducible roughness factor and signals obtained from the characterization via electrochemical impedance spectroscopy and cyclic voltammetry.

In order to tailor optimal DNA sensing surfaces, it is important to understand the processes occurring at the electrode surface during the DNA assay build-up, taking into consideration the physico-chemical properties of the investigated molecules, electrode polarization and the surrounding solution. EIS was used to sequentially follow each step of the build-up of DNA assays and understand how the variation of different parameters alters the quality of the surface modification. The influence of surface modification by DNA on the value of the potential of zero charge of polycrystalline gold was investigated by determining the pzc of bare gold before and upon its modification with DNA. It was observed that the pzc shifts towards more negative values due to the surface modification with DNA. The importance of this shift was evident in the study for the selection of appropriate potential-pulse profiles to achieve high immobilization rates of both DNA and alkylthiol derivatives as representatives of intrinsically charged and uncharged molecules, respectively.

Based on previous findings showing that in solutions of high ionic strength charge screening of DNA is significant and the potential profile in front of an electrode surface upon electrode polarization is fairly steep, it is evident that the widely accepted model explaining the influence
of the polarized electrode on the behavior of DNA in its proximity is far too simple. Attraction/repulsion of the DNA by the electric field in front of the polarized electrode can very unlikely be responsible for an improved immobilization rate due to the shortness of the Debye length. Thus, a new model is proposed, suggesting that the polarized electrode rather affects the ions in the vicinity of the electrified interface. When the electrode is polarized to negative values with respect to the pzc, cations move towards the electrified interface while anions move towards the bulk of the solution. In contrast, the opposite behavior is obtained when the electrode is polarized to more positive potentials with respect to the pzc. Switching fast enough between these two situations creates a “stirring effect” that effectively exceeds the Debye length in front of the electrified interface and pulls along DNA strands present in close proximity to the electrode surface including their condensed ion cloud. Hence, immobilization is not diffusion controlled but driven by the migration of ions in front of the electrode. Parameters that are playing a crucial role in achieving a significant improvement in the immobilization kinetics are the applied potential intensities and the duration of an individual pulse. The applied potential intensities need to be on the one hand within the stable potential window of the Au-S bond and on the other hand high enough to evoke an efficient stirring to bring the DNA towards the surface. The potential-pulse duration needs to be long enough to allow for an appropriate concentration gradient to form and for a whole molecule to be pulled down to the electrode surface, hence allowing for the formation of the Au-S bond regardless of the orientation of the molecule. On the other hand, it needs to be simultaneously short enough to allow a high number of potential pulse cycles.

Using the developed strategy, alkythiol SAM formation can also be significantly accelerated, leading to the formation of compact SAMs within minutes. It was shown that, besides the potential-pulse intensities and the pulse duration, the length of a molecule influences the efficiency of the immobilization. Optimization of the potential difference does not depend on the molecule length, as long as the appropriate pulse duration is chosen, since longer potential pulses are needed to bring down longer molecules to the electrode. The fact that this strategy tremendously accelerates the immobilization of uncharged molecules as well supports the conclusion that only DNA attraction/repulsion by the polarized electrode is a far too simple model to explain the mechanism of DNA immobilization supported by applied potentials.

The developed strategy was subsequently implemented into the development of a DNA sensor, using the developed potential-pulse assisted method for both DNA immobilization and thiol
passivation steps to create a DNA sensing platform. The optimization of the protocol was performed by investigating the influence of subsequent passivation step and the potential pulsing itself on the stability of DNA-modified surfaces and by choosing the right thiol for passivation.

Furthermore, the applicability of the developed method for the production of DNA chips was investigated using a 32-electrode array chip. Using this approach, the whole chip is exposed to each DNA solution used for the modification of a certain number of electrodes, avoiding the need for a localized positioning of DNA solutions on individual electrodes as in light-directed synthesis or spotting procedures that require expensive and sophisticated equipment. Due to the goal of making multiple probe DNA chips, it is clear that electrodes need to be cleaned prior to the modification with a selected DNA sequence. Thus, a method for potential-pulse assisted cleaning of Au-modified surfaces was developed, which allows a very fast and efficient regeneration of individual electrodes without causing any damage to the surface. The fact that by using our approach the Au surface can be easily regenerated without jeopardizing the quality of the following surface modification, points also to chip recycling as another advantage of the proposed approach. Until now research in this field did not address this topic, but relatively soon, in the world of private medicine, where everyone will own point-of-care devices, the question of reusability will become an important issue. Furthermore, construction of DNA chips in this manner opens the door towards the use of even smaller electrodes, since their size does not need to be limited by the size of the droplet used for modification or its evaporation rate.

Finally, the developed technique was implemented into the development of a new DNA sensing platform based on signal amplification via an enzyme-conjugated intercalating compound (glucose oxidase-acridine orange) as hybridization indicator. Using the potential-pulse assisted surface modification strategy, DNA/thiol surfaces were fabricated to obtain lower probe DNA coverage and very efficient blocking of unspecific adsorption, which resulted in a significant contrast between ss- and dsDNA. The developed DNA sensing strategy finds its application in multiple probe DNA chips, since the synthesized intercalating compound can universally interact with all DNA sequences present on the chip and, by this, simultaneously increase the sensitivity on all electrodes.

Even though this work is primarily orientated towards DNA chip development, the developed potential-pulse assisted surface modification method is an equally promising concept for applications in many other research fields, such as protein binding or investigation of cells.