

Summary

Fluorescent self-assembled monolayers (SAMs) on glass surfaces have been studied as a new material for chemical sensing. The new sensing system presented in this thesis is a label-free sensing approach suitable for metal ion and inorganic anions sensing in both organic solvents and aqueous solution. The sensing SAMs are created by sequential deposition of two building blocks, a fluorophore and a ligand molecule onto an amino terminated SAM on glass slides. This produces a flat glass surface provided with a large number of binding pockets. Perturbation of the fluorophore upon binding of the analyte to the surface creates a measurable signal making the sensing of analytes possible. In this way a large number of different systems are fabricated by combinatorial techniques and parallel synthesis. The sensing SAMs displayed good sensitivity for a number of ions with detection limits of 10^{-6} M. Some of fluorescent SAMs have been found to respond specifically to the presence of Cu^{2+} and CH_3COO^- in the presence of other analytes. These sensing SAMs are also used as cross-reactive sensor arrays in which the analyte is identified by differential sensing using the collective response of a series of different SAMs to the analyte instead of the individual response of a single SAM. Arrays of fluorescent SAMs have been produced both in microtiterplate and in multichannel microfluidic chip formats.

In addition, these glass substrates coated with fluorescent SAMs have been used as substrates for chemical patterning. Different metal ion patterns have been created onto fluorescent SAMs coated glass slides. Due to the sensing properties of the substrates, modulation of fluorescence occurs in the localized areas where the metal ions are deposited. Therefore the chemical pattern is easily revealed by a luminescent pattern which is visualized by simple fluorescent microscopy.

In Chapter 2 an overview of the literature on fluorescent materials for chemical sensing is given. The last trends in the development of chemical sensors such as the use of combinatorial chemistry for discovery and development of new sensing probes, sensor

miniaturization, the use of microfluidics, and the fabrication of sensor arrays are also reviewed.

Chapter 3 describes the synthesis, characterization and sensing properties of two libraries of fluorescent SAMs on quartz for metal ions and inorganic anions. Twenty different fluorescent SAMs were fabricated by parallel synthesis. Monolayer formation was evaluated by ellipsometry, contact angle measurements, X-ray photoelectron spectroscopy (XPS) and fluorescence spectroscopy. The fluorescent SAMs showed sensitivity for metal ions (Ca^{2+} , Co^{2+} , Pb^{2+} , Cu^{2+}) and inorganic anions (HSO_4^- , NO_3^- , H_2PO_4^- , CH_3COO^-) in acetonitrile. The detection limit of the system is 10^{-6} M. Selectivity studies showed SAMs that are highly selective for Cu^{2+} in presence of other metal ions such as Pb^{2+} and Ca^{2+} . Systems highly selective for CH_3COO^- in presence of HSO_4^- were also found.

In Chapter 4, new amino-terminated SAMs stable in water were fabricated and subsequently modified with fluorophores and binding groups to create a library of fluorescent SAMs stable in aqueous environments. By using these fluorescent SAMs, the approach described in Chapter 3 was expanded to the sensing of metal ions (Ca^{2+} , Co^{2+} , Hg^{2+} , Cu^{2+}) and inorganic anions (HSO_4^- , NO_3^- , H_2PO_4^- , CH_3COO^-) in water. These sensing studies showed that the library displays a unique fluorescent “fingerprint” for the different analytes in aqueous solvents which is used for identification of the analyte.

To apply high-throughput screening techniques, the fabrication of a fluorescent SAM cross-reactive sensor array in a microtiterplate was carried out in Chapter 5. Custom designed 140 well glass microtiterplates were fabricated. A library of fluorescent SAMs was made by parallel synthesis on the microtiter plates by coating the bottom glass of each well with a different monolayer. Inspection of the properties of the array for the sensing of Pb^{2+} , Zn^{2+} , Co^{2+} , Cu^{2+} and Ca^{2+} was made by laser scanning confocal microscopy (LSCM) and by fluorescence scanning. Different fluorescent patterns were obtained for the microtiterplate after exposure to different metal ions demonstrating the good performance of the sensor array for analyte identification.

In Chapter 6 the use of microchannels for the integration of chemical sensing systems in microfluidics devices was demonstrated. Coating of the walls of glass microchannels with fluorescent sensing SAMs have been used to generate sensing channels that respond to the presence of an analyte in the fluid passing through the channel. Monolayer formation was evaluated by contact angle measurements inside the channel and fluorescence microscopy. Additionally, a sensor array has been fabricated in a custom designed multichannel chip for differential sensing of Ca^{2+} and Cu^{2+} .

In Chapter 7 the complexing properties of the fluorescent SAM-coated substrates are exploited to create and easily visualize metal ion patterns on glass substrates. Patterning of fully covered fluorescent surfaces with metal ions (Ca^{2+} , Co^{2+} , Pb^{2+} , Cu^{2+}) has been carried out by soft and probe lithography techniques. Microcontact printing (μCP) and dip pen nanolithography (DPN) of metal ion salts onto the fluorescent SAMs on glass resulted on the successful transfer of the metal ions to the surface. Additionally, the generation of fluorescent patterns on amino-terminated SAM coated glass slides by covalent attachment of fluorophores moieties to the surface by μCP has been proven. These patterns also function as sensing molds for metal ions.

In conclusion, new fluorescent materials have been developed for chemical sensing. Their performance has been demonstrated for the sensing of metal cations and inorganic anions in organic and aqueous solvents. The simple fabrication scheme allows the generation of sensor arrays on glass surfaces, allowing mass screening of a number of different systems. The possibility of using the array format and combinatorial methods for fabrication of this sensing scheme provides the new approach with a high-throughput character. In addition, these sensing SAMs are generated in any glass substrate simplifying its integration in glass microfluidic devices. The new material constitutes a label-free approach in which binding of analytes can be directly monitored by fluorescence without the need of tagging steps.

The developed materials appear as a powerful tool with affinity for metal ions in which chemical patterns can be easily created and visualized. The possibility of

patterning with an almost unlimited number of analytes and substrates expands the tool box for pattern formation towards the discovery of new strategies for nanofabrication protocols. Catalysis and electroless deposition of metals are some of the envisioned applications of these patterns.