Self-assembled monolayers probed by electrochemistry: From layer properties to sensors

Tom Steentjes

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SELF-ASSEMBLED MONOLAYERS PROBED BY ELECTROCHEMISTRY: FROM LAYER PROPERTIES TO SENSORS

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Chapter 1

General introduction

Electron transfer processes are of importance in a broad range of fields, such as biosensing¹ and molecular electronics.² Gold electrodes can be readily functionalized using thiol chemistry³ for the formation of self-assembled monolayers (SAMs). SAMs constitute an excellent platform for sensing because of the possibilities to precisely tune the surface composition and the distance from the electrode surface. As such the influence of the electron transfer properties of a redox probe that is present in solution or attached at the SAM can be tuned.

Electrochemistry utilizes redox couples in solution, where the electron transfer is mostly dictated by diffusion, and attached to a surface where diffusion either plays no role or is mediated via a flexible linker. When the redox couple is in solution it can be used to probe the electron transfer through a layer, and the layer can be designed to block the electron transfer as an insulating layer, or to optimize electron transfer for the use in molecular electronics.

The work described in this thesis aims to further our understanding of the design and study of the electron transfer properties of two different types of monolayer surfaces. First, the design and characterization of several novel β -cyclodextrin (β -CD) adsorbates is presented (Chapters 3 and 4) with the aim to study the influence of the distance of the β -CD core to the electrode surface on the electron transfer properties using different redox probes. Secondly, layers of linear flexible poly(ethylene glycol) (PEG) polymers end-tagged with an electrochemically active redox group are described in Chapters 5 and 6 to study the effect of polymer length and conformation on the electron transfer characteristics, both on macroscopic electrodes as well as in nanofluidic devices.

Chapter 2 provides an overview of several common electrochemical methods and looks into their uses for the determination of the electron transfer rate constants. A further focus is on flexible probes such as DNA and their use in electrochemical biosensing devices that provide a signal upon changing the linker conformation.

Chapter 3 describes the design and characterization of six multivalent β -CD adsorbates that provide close contact of the β -CD cavity to the gold surface. Monolayers of these β -CD adsorbates were characterized and the adsorption kinetics, thickness, layer stability and number of anchoring groups bound to the surface were assessed for the different anchoring groups.

In the work described in Chapter 4, monolayers of four of the β -CD adsorbates introduced in Chapter 3 were further characterized with regard to their packing densities using electrochemical methods. The electron transfer kinetics was studied using different redox probes, both in solution and reversibly immobilized on the host monolayers. Electron transfer rates between the novel adsorbates in close proximity to the surface were compared to that of a β -CD adsorbate further away from the surface by long thioether chains.

Chapter 5 describes the electron transfer for linear PEG polymers attached to the Au electrode surface on one end and tagged with a ferrocene moiety on the other end. The effects of bounded diffusion on the electron transfer was studied and compared for different polymer lengths, together with the effect of conformational changes as a function of the surface density.

In Chapter 6, the molecules described in Chapter 5 were introduced into nanofluidic devices with nanospaced electrodes. The effect of surface-bound diffusion of the ferrocene probe on the repeated shuttling of electrons between the two electrodes was studied. Subsequently, the effect of linker length and surface density was assessed in order to validate the potential for using such a device architecture for biosensor applications.

1.1 References

- (1) Labib, M.; Sargent, E. H.; Kelley, S. O., Electrochemical Methods for the Analysis of Clinically Relevant Biomolecules. *Chem. Rev.* **2016**, *116*, 9001-9090.
- (2) Adams, D. M.; Brus, L.; Chidsey, C. E. D.; Creager, S.; Creutz, C.; Kagan, C. R.; Kamat, P. V.; Lieberman, M.; Lindsay, S.; Marcus, R. A.; Metzger, R. M.; Michel-Beyerle, M. E.; Miller, J. R.; Newton, M. D.; Rolison, D. R.; Sankey, O.; Schanze, K. S.; Yardley, J.; Zhu, X., Charge Transfer on the Nanoscale: Current Status. *J. Phys. Chem. B* **2003**, *107*, 6668-6697.
- (3) Yildiz, I.; Raymo, F. M.; Lamberto, M., Self-Assembled Monolayers and Multilayers of Electroactive Thiols. In *Electrochemistry of Functional Supramolecular Systems*, John Wiley & Sons, Inc.: **2010**; pp 185-200.

Chapter 2

Electron transfer on electrode surfaces and applications in biosensing

The increasing demand for diagnostic tools in personalized medicine calls for an increase in sensitivity and specificity for the detection devices that are developed to this purpose. Electrochemistry provides a range of tools which can meet these requirements. Metallic electrodes can be readily functionalized using thiol chemistry and provide an excellent basis for tuning the electrode surface for specific goals. This chapter reviews several common electrochemical techniques such as chronoamperometry, cyclic voltammetry and electrochemical impedance spectroscopy, and their uses for the determination of electron transfer rate constants. Redox-active moieties attached to long flexible linkers form a special group as their electron transfer partly relies on diffusion. The conformation of these linkers can be modified by changing the surface density, and in the case of DNA the stiffness can be affected upon binding with the complementary DNA chain. These strategies have been utilized for the development of biosensing devices such as E-DNA and aptamer-based detection schemes.

2.1 Introduction

Electron transfer processes play an important role in a wide range of fields, from molecular electronics¹, to the development of biosensors.²⁻⁴ The growing interest in personalized medicine and point-of-care testing demands an increase in sensitivity, specificity, miniaturization, affordability and ease of signal interpretation.⁵ Several of these requirements are met by electrochemical devices.⁶ As devices become smaller, chemical modification of electrodes provides an excellent way for control and tunability of the chemistry of the detection probe.⁷

Electrochemistry is suited for sensitive and accurate measuring of molecular redox processes both in solution and at the surface. This chapter will focus on electrochemical processes on electrode surfaces, specifically with the redox probe covalently attached to the surface via the formation of self-assembled monolayers (SAMs) to eliminate the use of additional probes to the analyte solution. The affinity of thiols to noble metals is well known as a way to attach (electroactive) molecules to surfaces.⁸

In the first part of this chapter, several commonly used electrochemical techniques will be discussed together with their uses for the determination of electron transfer kinetics, with a focus on ferrocene alkylthiols as a key example. The electron transfer plays a central part in the detection mechanism of biosensors as they rely on conformational changes upon detection. In the second part, E-DNA^{2,9} and aptamer-based sensors^{2,10-11} rely on this principle and will be discussed in further depth.

2.2 Electrochemical techniques

A redox-active SAM in its most basic form is a redox couple immobilized at, but separated from, the electrode by a short linker. The affinity of thiols for noble metals such as gold can be exploited for functionalization of the electrode. Electrochemical methods provide powerful tools for the characterization of these types of layers. A plethora of electrochemical techniques is available, a few of the more commonly used techniques will be discussed here.

2.2.1 Chronoamperometry

In chronoamperometry the potential is changed step-wise and the recorded current follows an exponential decay according to equation 2.1:¹²

$$i(t) = kQe^{-kt} \quad (\text{Equation 2.1})$$

When the natural logarithm of the current i is plotted versus the time, the slope can be used to determine the rate constant k . In addition, this analysis can be performed at varying overpotentials giving a more complete picture of the influence of the potential on the kinetics as predicted by the Butler-Volmer behavior. The Butler-Volmer formulation states that the electrode kinetics at an electrode depends on the overpotential, $E - E^{0'}$, as shown in equation 2.2, giving rise to the characteristic Tafel plot.¹²

$$k_f + k_b = k^0 \exp(-\alpha R(E - E^{0'})/T) + k^0 \exp((1 - \alpha)R(E - E^{0'})/T)$$

(Equation 2.2)

Where k_f and k_b are the forward and backward rate constants, k^0 the standard heterogeneous rate constant, R the gas constant, T the absolute temperature, E the electrode potential, $E^{0'}$ the formal potential of the electroactive group and α the transfer coefficient.

Chidsey was the first to use this method on SAMs of alkanethiols modified with ferrocene.¹³ These SAMs were made by mixing the ferrocene alkylthiols with electrochemically inactive alkylthiols to sufficiently space the ferrocene moieties and prevent cross-interactions. It was shown that for low overpotentials the Butler-Volmer formulation was followed, but curvature far from $E^{0'}$ could not be accounted for with these formulations. This was overcome by fitting the data with a prefactor for electron transfer and the metallic states, and λ , the reorganization energy, i.e., the energy needed to distort the atomic positions of the reactant and its solvation shell to the atomic positions of the product and its solvation shell. More importantly, it showed the possibility of these layers to be probed systematically on the dependence of distance, medium and spacer structure.¹³ A wide variety of studies for different alkane chain lengths have been performed to determine the electron transfer kinetics using different techniques, and these have been summarized by Eckermann *et al.*⁷ These layers will be discussed in more detail below.

2.2.2 Cyclic voltammetry

In cyclic voltammetry, the potential at an electrode is varied linearly with time while simultaneously the current is recorded. Typically, the responses of electrochemically active layers that are surface confined are different than those of diffusion-controlled processes,¹² and the former show a linear

dependence of the peak current (I_p) on the scan rate (v) (Equation 2.3). The charge (Q) under the recorded anodic and cathodic peaks is proportional to the number of redox centers contributing to the electron transfer, and consequently the surface density (Γ) can be determined (Equation 2.4).

$$i_p = \frac{n^2 F^2}{4RT} v A \Gamma \quad (\text{Equation 2.3})$$

$$Q = n F A \Gamma \quad (\text{Equation 2.4})$$

Here, n is the number of electrons involved in the electron transfer process, F the Faraday constant, R the gas constant, T the temperature and A the surface area of the electrode. The electron transfer of surface-confined electrochemical species has been described by Laviron¹⁴ and the rate constant for electron transfer (k_{ET}) can be determined from the change in peak potential (E_p) with increasing scan rate. When the difference between the anodic and cathodic peak potentials exceeds 200 mV, the electron transfer is said to be electrochemically irreversible and the peak potentials change linearly with the logarithm of the scan rate. At the intercept of the x-axis of this linear change, the scan rates v_a and v_c can be determined and the k_{ET} can be determined from Equation 2.5:

$$k_{ET} = \frac{\alpha n F v_c}{RT} = \frac{(1-\alpha) n F v_a}{RT} \quad (\text{Equation 2.5})$$

The electron transfer in ferrocene alkylthiols has been studied using a variety of methods, as mentioned earlier.⁷ Cyclic voltammetric measurements on different lengths of alkylthiols at low temperatures have shown that the electron transfer in well-packed SAMs drops exponentially according to equation 2.6.¹⁵ This effect had been previously shown for pentaaminecobalt(III) anchored to gold and indirectly reduced via $\text{Ru}(\text{NH}_3)_6^{2+}$.¹⁶

$$k_{ET}(x) = k_{ET}(x = 0) e^{-\beta x} \quad (\text{Equation 2.6})$$

Where x is the distance to the surface and β expresses the overall reaction rate sensitivity to distance. Cyclic voltammetry proved to be more suitable than potential step methods as it is not as susceptible to the effects of kinetic dispersion caused by structural disorder in the monolayer.¹⁷

Since Chidsey's first work on ferrocene alkylthiols on electrodes¹⁸ these layers have been studied widely. Ideal electrochemical behavior is found when these SAMs are diluted with electrochemically inactive alkylthiols. At high ferrocene

densities significant peak broadening and peak splitting has been observed.¹⁸⁻¹⁹ It was found that the peak splitting observed in cyclic voltammetry was consistent with local effects expected from phase separation, and the two peaks could be attributed to ferrocene moieties surrounded by neutral alkylthiols (peak I in Figure 2.1b), or by other ferrocene species (peak II in Figure 2.1b).²⁰ This property has been utilized in order to determine the homogeneity of alkylthiol layers by exposing formed SAMs to a ferrocene alkylthiol solution providing access to different types and populations of defects. Single site defects would only be filled with one ferrocene alkylthiol molecule (Figure 2.1a), i.e. surrounded by neutral alkylthiols (corresponding to peak I), whereas collapsed site and pinhole defects provided room for multiple molecules, giving rise to the faradaic signal corresponding to ferrocene moieties surrounded by other ferrocene species (corresponding to peak II).²¹

Figure 2.1: a) Three different types of defects probed by backfilling with a ferrocene alkylthiol, from left to right a single site defect, collapsed defect and a pinhole. Reproduced from Ref. 21 with permission from the PCCP Owner Societies. b) Cyclic voltammetric response of ferrocene alkanethiols on a gold electrode for different solution ratios of electrochemically inactive (C_{10}SH) and ferrocene tethered alkylthiols (FcC_{12}SH). Reprinted with permission from Ref. 20. Copyright 2006 American Chemical Society.

When the kinetics of the electroactive group is controlled by diffusion, the Laviron method is not suitable for the determination of the kinetics. Typically, diffusion-controlled electron transfer applies to species in solution, but diffusion control has also been observed when the redox moiety is attached to long flexible linkers such as poly(ethylene glycol) (PEG)²²⁻²⁴ and DNA.²⁵⁻²⁶ In these cases, at sufficiently low scan rates the current was proportional to the scan

rate, whereas cycling at increased scan rates showed that the current became proportional to the square root of the scan rate, following the Randles-Sevcik equation for diffusing species (equation 2.7):¹²

$$i_p = 0.4463nFAC \left(\frac{nFvD}{RT} \right)^{\frac{1}{2}} \quad (\text{Equation 2.7})$$

Here, D is the diffusion coefficient and C the concentration. For surface-attached species, the concentration can be transformed into surface density (Γ) using $\Gamma = LAC$, with L being the layer thickness. From this, information could be gathered about the dynamics of the flexible linker. For immobilized PEG linkers it was found that the time response of PEG (M_w of 3400 Da) linkers in a loose brush conformation, in which the surface-attached polymers are packed in a sufficiently high density to promote chain stretching, gave similar time responses as a shorter PEG (M_w of 600 Da) in a mushroom conformation, in which the individual polymer molecules are isolated from each other. This indicated that the diffusion coefficients of the longer PEGs were necessarily higher, which has been attributed to an increased contribution of the spring constant.²³

Similarly, ferrocene moieties that have been linked to single-stranded DNA show electron transfer that is controlled by diffusion at high scan rates, as could be determined both experimentally²⁵ and by modelling.²⁷ Upon hybridization of the single-stranded DNA with its complementary chain, the double-stranded DNA becomes more rigid which has been shown to affect the diffusional properties markedly. In this case the regime in which diffusion governs the electron transfer shifts to lower scan rates, while at higher scan rates the current decreases drastically, indicating that the rigidity hampers the movement of the ferrocene to such an extent that it does not reach the surface in the measured time frame.²⁵ It has been shown that the electron transfer of the double-stranded DNA in this case occurs via elastic bending of the entire double-stranded linker (Figure 2.2b).²⁶ It should be noted that this observation applies only when the DNA is bound to the surface via a short alkane linker (C_2 in this instance), while in contrast, when the linker length is increased to C_6 the electron transfer occurs via the rotational movement around the linker (Figure 2.2c), direct electron transfer through the DNA backbone (Figure 2.2a) could be excluded.²⁸

Figure 2.2: Possible types of electron transport mechanisms in Fc-labeled double-stranded DNA: (a) Direct electron transfer through the DNA backbone (can be excluded due to the presence of a diffusion regime). (b) Elastic bending of the double-stranded DNA backbone. (c) Rotational motion of the DNA rod. Reprinted with permission from Ref. 28. Copyright 2008 American Chemical Society.

The conformation of molecules on the surface has a marked influence on the electron transport: studies with ferrocene-tagged PNA on electrodes have shown that increasing the surface density causes a decrease of the diffusion constant of two orders of magnitude with an analogous decrease of k_{ET} .²⁹

If diffusion plays a role in the electron transfer process, the Nicholson method can be used for the determination of electron transfer rate constants.³⁰ Using this method, the peak separation (ΔE_p) can be transformed into a dimensionless parameter ψ , from which the rate constant can be calculated using Equation 2.8.

$$\psi = \frac{k_{\text{ET}} \left(\frac{D_{\text{O}}}{D_{\text{R}}} \right)^{\frac{\alpha}{2}}}{\sqrt{\pi \frac{nFv}{RT} D_{\text{O}}}} \quad (\text{Equation 2.8})$$

Where D_{O} and D_{R} are the diffusion coefficients of the oxidized and reduced species, respectively.

If the layer thickness is known, this method can be used for the determination of rate constants of diffusing species attached to an electrode via a flexible linker. Rate constants have in this way been determined for electrochemically active PNA surfaces to study the effect of surface density, chain length and hybridization. It was shown that with an increase in surface density of a PNA 12-mer, as shown in Figure 2.3a, the slope of the peak current vs the square root of the scan rate decreases, indicating a decrease in the diffusion coefficient. Analogously, the increase in surface density showed a decrease in the electron

transfer rate constants (Figure 2.3b). This was attributed to an increased distance of the electrochemically active ferrocene to the surface as the layer becomes more crowded. Similarly, this increase in distance from the surface resulted in a decrease in rate constants and diffusion coefficients as the linker length was increased (Figure 2.3c). An increase in kinetics and diffusion was found upon hybridization with a fully complementary DNA strand due to a decrease in elasticity of the linker and increased interactions between the positively biased surface and negatively charged PNA-DNA duplex.²⁹

Figure 2.3: a) Randles Sevcik plot of the immobilization process, a decreased slope of i_p vs $v^{1/2}$ was found for increased reaction times (or surface densities, see b) for 12-mer PNA. b) Increase in surface density (circles) and the decrease in electron transfer kinetics (squares) with increased reaction time. c) Decrease of the rate constants vs linker length for 3-, 6-, 9-, 12- and 16-mer PNA. Reprinted with permission from Ref. 29. Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.2.3 Electrochemical impedance spectroscopy

In electrochemical impedance spectroscopy (EIS), the impedance is measured by applying a small AC signal over a range of frequencies at a specified potential.⁷ The obtained data can be fitted to an equivalent circuit, the easiest being the Randles circuit, consisting of an element for solution resistance in series with a parallel combination of the double-layer capacitance (C_{DL}) and an

element for the impedance of a faradaic reaction, which for an electrode coated with an redox-active layer consists of a charge transfer resistance (R_{CT}) and an element for the adsorption pseudocapacitance (C_{AD}) (Figure 2.4).³¹⁻³²

The k_{ET} can subsequently be calculated using equation 2.9.

$$k_{ET} = \frac{1}{2R_{CT}C_{AD}} \quad (\text{Equation 2.9})$$

Impedimetric techniques are often quite time consuming and the system needs to be in equilibrium, if this is not the case the equivalent circuit needs to be expanded.⁷ As a characterization tool it is often used with a redox probe in solution, for example to characterize the quality of monolayers by way of assessing the ability to block the diffusing redox probe, which increases the R_{CT} .³³

Figure 2.4: Randles equivalent circuit for the impedance of an electrochemically active layer.³¹⁻³²

Impedimetric sensing techniques based on the change in R_{CT} , or faradaic impedance, similarly rely on the presence of an electrochemically active probe.³⁴⁻³⁵ For example, Barton immobilized antibodies for prostate specific antigen (PSA, a prostate cancer marker) on an electrode surface. Upon binding of PSA, the increase in R_{CT} was used as a detection signal.³⁶ In non-faradaic impedance, no redox probe is necessary as changes in the dielectric properties on the electrode surface are detected via a change in the capacitance.^{34, 37} If upon detection the charge distribution is changed, this will result in a change in the capacitance. For example, a zwitterionic polymer has been used to immobilize an anti-insulin antibody on electrode surfaces, and the perturbation of the double layer caused an associated change in the capacitance characteristics that provided detection in the fM range in undiluted blood serum.³⁸

In order to study the kinetics of redox active surfaces, the R_{CT} and C_{AD} need to be determined (equation 2.8), and the data needs to be fitted to an equivalent circuit. To overcome this, Bueno has shown that for any redox active surface,

directly plotting the imaginary capacitance vs the frequency (resolved for the parasitic capacitance, measured at potentials outside of the redox window) shows a peak at the characteristic frequency which is equal to the rate constant. The electron transfer rates were determined for azurin films immobilized on SAMs with increasing thiol layer thicknesses and 11-ferrocene-undecanethiol on gold electrodes (Figure 2.5a and b, respectively). The electron transfer rates of azurin films showed a decrease with increased thiol layer thickness. All determined values were compared with rate constants determined from cyclic voltammetric measurements and were shown to be similar.³⁹

Figure 2.5: a) Imaginary capacitance of azurin films on three different supporting thiol layer thicknesses (hexanethiol, decanethiol and dodecanethiol) after correction for the parasitic capacitance. The electron transfer rate can be determined directly from the characteristic frequency. b) Imaginary capacitance of 11-ferrocene-undecanethiol before (red) and after (green) subtraction of the parasitic capacitance. Reprinted with permission from Ref. 39. Copyright 2012 American Chemical Society.

2.3 Electrochemical DNA sensing

The effect of conformational changes on the electron transfer process has been exploited to develop E-DNA sensors. The principle of an E-DNA sensor is based on that of optical molecular beacons⁴⁰ in which single-stranded DNA backfolds upon itself at its extremities and forms a stem-loop bringing a fluorophore and a quencher into close proximity. This loop dissociates upon binding with a complementary strand. In an E-DNA sensor device the ss-DNA is bound with one end onto an electrode and via a similar stem-loop brings an electrochemically

active ferrocene label into close proximity of the electrode (Figure 2.6). In the presence of a complementary target, the ferrocene moiety is moved away from the surface, causing a strongly reduced electron transfer rate. For this system, target DNA concentrations of 10 pmol were detectable.⁴¹

Figure 2.6: A DNA stem-loop bound onto itself at its extremities and attached to an electrode at one end, bearing a ferrocene tag on the other end. Upon hybridization with its complementary strand, the ferrocene tag is moved away from the surface resulting in a large change in the redox current. Reprinted with permission from Ref. 41. Copyright 2003 National Academy of Sciences.

Similar to the previously mentioned ferrocene-modified PNA, which showed a decrease in k_{ET} for increased surface densities, crowding of the surface leads to an increased signal suppression if the stem-loops are hybridized, because the densely packed layer prevents collisions of the redox moiety with the electrode surface.⁴² The use of a stem-loop was shown not to be necessary for the detection of complementary DNA strands on a 27-base linear DNA probe. The binding of a complementary chain changed the rigidity, and with that, the dynamics of the probe DNA changed sufficiently to give an increased signal suppression over its stem-loop counterpart. The signal suppression could be increased even more by increasing the probe density, thereby making the movement (and subsequent electron transfer) of the hybridized chains more difficult.⁴³

These and similar E-DNA sensors⁴⁴⁻⁴⁷ based on the stem-loop are 'signal off' architectures and provide a decrease in signal upon detection which can give rise to false positives.⁴¹ Even though this can be avoided by the use of 'multicolor' redox labels (electrochemically active moieties with a different

E_0),⁴⁸⁻⁴⁹ these systems are still limited by a maximum of 100% signal suppression.⁴³ ‘Signal on’ detection schemes (Figure 2.7) have been developed based on a variety of schemes, like the pseudoknot,⁵⁰ triplex DNA interactions,⁵¹ inverted stem-loop,⁵² DNA-PEG-DNA triblock,⁵³ and electrode bound duplex,⁵⁴ in order to increase the sensitivity, resulting in decreased detection limits reported as low as 400 fM for the bound duplex.

Figure 2.7: Several schemes for ‘signal on’ detection: (a) the pseudoknot. Reprinted with permission from Ref. 50. Copyright 2009 American Chemical Society. (b) Triplex DNA interactions. Reprinted with permission from Ref. 51. Copyright 2014 American Chemical society. (c) inverted stem-loop. Reprinted with permission from Ref. 52. Copyright 2011 American Chemical Society. (d) DNA-PEG-DNA triblock. Reprinted with permission from Ref. 53. Copyright 2004 American Chemical Society. (e) electrode bound duplex. Reprinted with permission from Ref. 54. Copyright 2006 National Academy of Sciences.

Aptamer-based electronic sensors employ aptamers, which are short oligonucleotides selected for their high binding affinities with a wide variety of biomolecular targets ranging from cells⁵⁵ to ATP.⁵⁶ Aptamer-based sensors are similarly to E-DNA sensors based on the molecular beacon principle, and detection of an analyte can be based on binding of single-stranded DNA,⁵⁷ a displacement of a complementary strand upon binding,⁵⁸ or interestingly, a change in the conductivity of the DNA backbone upon binding with an analyte.⁵⁹ A universal aptamer-based detector has been developed, based on a neutralizer displacement strategy. The neutralizer is composed of PNA and cationic amino acids and neutralizes the negative charge of the DNA probe. Due to the presence of mismatches between the DNA and the neutralizer, the analyte displaces the neutralizer, causing a significant decrease in the current.⁶⁰ Figure 2.8 shows ATP as the analyte, but the method has been shown to work for ATP, cocaine, DNA, RNA and thrombin, resulting in femto- and attomolar detection limits.

Figure 2.8: Concept of the universal aptamer based detector, binding of the aptamer releases the neutralizer, thus switching on the electrochemical signal. Reprinted with permission from Ref. 60. Copyright 2012 Nature Publishing Group.

2.4 Conclusions

A wide array of electrochemical techniques is available and can be used to study the characteristics of electrochemically active surfaces. A short overview of several common techniques has been discussed here. Electron transfer rates can be determined using different techniques like cyclic voltammetry, chronoamperometry and electrochemical impedance spectroscopy. Next to the determination of specific electrochemical parameters, the quality of the layers present on the electrode can be probed. It has been shown that for flexible linkers like PEG and DNA, the electron transfer is dictated by diffusion of the

probe, which is directly influenced by the flexibility of the linker. As such, conformational changes of the linker, by changing the probe surface density or the rigidity upon binding with the complementary DNA strand, can have a significant effect on the kinetics and diffusion of the probe.

The change in conformation has been exploited for a variety of electrochemical biosensing methods, like the detection of complementary DNA strands, or other analytes via binding with DNA aptamers. The resulting change in conformation affects the signal output in the timeframe of the measurement, resulting in detection limits sometimes as low as femto-molar. The systems described here exhibit a relatively easy way of detection and thus provide an attractive alternative to laborious laboratory tests. Especially in combination with the increased interest in personalized medicine and point-of-care testing, electrochemical sensing devices are an attractive candidate for diagnostics.

2.5 References

- (1) Adams, D. M.; Brus, L.; Chidsey, C. E. D.; Creager, S.; Creutz, C.; Kagan, C. R.; Kamat, P. V.; Lieberman, M.; Lindsay, S.; Marcus, R. A.; Metzger, R. M.; Michel-Beyerle, M. E.; Miller, J. R.; Newton, M. D.; Rolison, D. R.; Sankey, O.; Schanze, K. S.; Yardley, J.; Zhu, X., Charge Transfer on the Nanoscale: Current Status. *J. Phys. Chem. B* **2003**, *107*, 6668-6697.
- (2) Labib, M.; Sargent, E. H.; Kelley, S. O., Electrochemical Methods for the Analysis of Clinically Relevant Biomolecules. *Chem. Rev.* **2016**, *116*, 9001-9090.
- (3) Grieshaber, D.; MacKenzie, R.; Vörös, J.; Reimhult, E., Electrochemical Biosensors - Sensor Principles and Architectures. *Sensors* **2008**, *8*, 1400-1458.
- (4) Plaxco, K. W.; Soh, H. T., Switch-Based Biosensors: A New Approach Towards Real-Time, in Vivo Molecular Detection. *Trends Biotechnol.* **2011**, *29*, 1-5.
- (5) Urdea, M.; Penny, L. A.; Olmsted, S. S.; Giovanni, M. Y.; Kaspar, P.; Shepherd, A.; Wilson, P.; Dahl, C. A.; Buchsbaum, S.; Moeller, G.; Hay Burgess, D. C., Requirements for High Impact Diagnostics in the Developing World. *Nature* **2006**.
- (6) Wang, J., Electrochemical Biosensors: Towards Point-of-Care Cancer Diagnostics. *Biosens. Bioelectron.* **2006**, *21*, 1887-1892.
- (7) Eckermann, A. L.; Feld, D. J.; Shaw, J. A.; Meade, T. J., Electrochemistry of Redox-Active Self-Assembled Monolayers. *Coord. Chem. Rev.* **2010**, *254*, 1769-1802.
- (8) Yildiz, I.; Raymo, F. M.; Lamberto, M., Self-Assembled Monolayers and Multilayers of Electroactive Thiols. In *Electrochemistry of Functional Supramolecular Systems*, John Wiley & Sons, Inc.: **2010**; pp 185-200.
- (9) Ricci, F.; Plaxco, K. W., E-DNA Sensors for Convenient, Label-Free Electrochemical Detection of Hybridization. *Microchim. Acta* **2008**, *163*, 149-155.

- (10) Deng, B.; Lin, Y.; Wang, C.; Li, F.; Wang, Z.; Zhang, H.; Li, X.-F.; Le, X. C., Aptamer Binding Assays for Proteins: The Thrombin Example—a Review. *Anal. Chim. Acta* **2014**, *837*, 1-15.
- (11) Jarczewska, M.; Gorski, L.; Malinowska, E., Electrochemical Aptamer-Based Biosensors as Potential Tools for Clinical Diagnostics. *Anal. Methods* **2016**, *8*, 3861-3877.
- (12) Bard, A. J.; Faulkner, L. R., *Electrochemical Methods: Fundamentals and Applications*. 2 ed.; John Wiley & Sons: New York, **2001**.
- (13) Chidsey, C. E. D., Free Energy and Temperature Dependence of Electron Transfer at the Metal-Electrolyte Interface. *Science* **1991**, *251*, 919-922.
- (14) Laviron, E. E. E., General Expression of the Linear Potential Sweep Voltammogram in the Case of Diffusionless Electrochemical Systems. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, *101*, 19-28.
- (15) Carter, M. T.; Rowe, G. K.; Richardson, J. N.; Tender, L. M.; Terrill, R. H.; Murray, R. W., Distance Dependence of the Low-Temperature Electron Transfer Kinetics of (Ferrocenylcarboxy)-Terminated Alkanethiol Monolayers. *J. Am. Chem. Soc.* **1995**, *117*, 2896-2899.
- (16) Li, T. T. T.; Weaver, M. J., Intramolecular Electron Transfer at Metal Surfaces. 4. Dependence of Tunneling Probability Upon Donor-Acceptor Separation Distance. *J. Am. Chem. Soc.* **1984**, *106*, 6107-6108.
- (17) Ravenscroft, M. S.; Finklea, H. O., Kinetics of Electron Transfer to Attached Redox Centers on Gold Electrodes in Nonaqueous Electrolytes. *J. Phys. Chem.* **1994**, *98*, 3843-3850.
- (18) Chidsey, C. E. D.; Bertozzi, C. R.; Putvinski, T. M.; Muijsce, A. M., Coadsorption of Ferrocene-Terminated and Unsubstituted Alkanethiols on Gold: Electroactive Self-Assembled Monolayers. *J. Am. Chem. Soc.* **1990**, *112*, 4301-4306.
- (19) Auletta, T.; van Veggel, F. C. J. M.; Reinhoudt, D. N., Self-Assembled Monolayers on Gold of Ferrocene-Terminated Thiols and Hydroxyalkanethiols. *Langmuir* **2002**, *18*, 1288-1293.
- (20) Lee, L. Y. S.; Sutherland, T. C.; Rucareanu, S.; Lennox, R. B., Ferrocenylalkylthiolates as a Probe of Heterogeneity in Binary Self-Assembled Monolayers on Gold. *Langmuir* **2006**, *22*, 4438-4444.
- (21) Yoon Suk Lee, L.; Bruce Lennox, R., Ferrocenylalkylthiolate Labeling of Defects in Alkylthiol Self-Assembled Monolayers on Gold. *Phys. Chem. Chem. Phys.* **2007**, *9*, 1013-1020.
- (22) Anne, A.; Demaille, C.; Moiroux, J., Elastic Bounded Diffusion. Dynamics of Ferrocene-Labeled Poly(Ethylene Glycol) Chains Terminally Attached to the Outermost Monolayer of Successively Self-Assembled Monolayers of Immunoglobulins. *J. Am. Chem. Soc.* **1999**, *121*, 10379-10388.
- (23) Anne, A.; Moiroux, J., Quantitative Characterization of the Flexibility of Poly(Ethylene Glycol) Chains Attached to a Glassy Carbon Electrode. *Macromolecules* **1999**, *32*, 5829-5835.
- (24) Anne, A.; Demaille, C.; Moiroux, J., Terminal Attachment of Polyethylene Glycol (Peg) Chains to a Gold Electrode Surface. Cyclic Voltammetry Applied to the Quantitative Characterization of the Flexibility of the Attached Peg Chains and of Their Penetration by Mobile Peg Chains. *Macromolecules* **2002**, *35*, 5578-5586.

- (25) Anne, A.; Bouchardon, A.; Moiroux, J., 3'-Ferrocene-Labeled Oligonucleotide Chains End-Tethered to Gold Electrode Surfaces: Novel Model Systems for Exploring Flexibility of Short DNA Using Cyclic Voltammetry. *J. Am. Chem. Soc.* **2003**, *125*, 1112-1113.
- (26) Anne, A.; Demaille, C., Dynamics of Electron Transport by Elastic Bending of Short DNA Duplexes. Experimental Study and Quantitative Modeling of the Cyclic Voltammetric Behavior of 3'-Ferrocenyl DNA End-Grafted on Gold. *J. Am. Chem. Soc.* **2006**, *128*, 542-557.
- (27) Huang, K. C.; White, R. J., Random Walk on a Leash: A Simple Single-Molecule Diffusion Model for Surface-Tethered Redox Molecules with Flexible Linkers. *J. Am. Chem. Soc.* **2013**, *135*, 12808-12817.
- (28) Anne, A.; Demaille, C., Electron Transport by Molecular Motion of Redox-DNA Strands: Unexpectedly Slow Rotational Dynamics of 20-Mer Ds-DNA Chains End-Grafted onto Surfaces Via C6 Linkers. *J. Am. Chem. Soc.* **2008**, *130*, 9812-9823.
- (29) Hüsken, N.; Gëbala, M.; La Mantia, F.; Schuhmann, W.; Metzler-Nolte, N., Mechanistic Studies of Fc-Pna(-DNA) Surface Dynamics Based on the Kinetics of Electron-Transfer Processes. *Chem. - Eur. J.* **2011**, *17*, 9678-9690.
- (30) Nicholson, R. S., Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics. *Anal. Chem.* **1965**, *37*, 1351-1355.
- (31) Randles, J. E. B., Kinetics of Rapid Electrode Reactions. *Faraday Discuss.* **1947**, *1*, 11-19.
- (32) Creager, S. E.; Wooster, T. T., A New Way of Using Ac Voltammetry to Study Redox Kinetics in Electroactive Monolayers. *Anal. Chem.* **1998**, *70*, 4257-4263.
- (33) Beulen, M. W. J.; Bugler, J.; De Jong, M. R.; Lammerink, B.; Huskens, J.; Schönherr, H.; Vancso, G. J.; Boukamp, B. A.; Wieder, H.; Offenhäuser, A.; Knoll, W.; Van Veggel, F. C. J. M.; Reinhoudt, D. N., Host-Guest Interactions at Self-Assembled Monolayers of Cyclodextrins on Gold. *Chem. - Eur. J.* **2000**, *6*, 1176-1183.
- (34) Santos, A.; Davis, J. J.; Bueno, P. R., Fundamentals and Applications of Impedimetric and Redox Capacitive Biosensors. *J. Anal. Bioanal. Tech.* **2014**, *S7*, 15.
- (35) Guan, J. G.; Miao, Y. Q.; Zhang, Q. J., Impedimetric Biosensors. *J. Biosci. Bioeng.* **2004**, *97*, 219-226.
- (36) Barton, A. C.; Davis, F.; Higson, S. P. J., Labelless Immunosensor Assay for Prostate Specific Antigen with Picogram Per Milliliter Limits of Detection Based Upon an Ac Impedance Protocol. *Anal. Chem.* **2008**, *80*, 6198-6205.
- (37) Berggren, C.; Bjarnason, B.; Johansson, G., Capacitive Biosensors. *Electroanalysis* **2001**, *13*, 173-180.
- (38) Luo, X.; Xu, M.; Freeman, C.; James, T.; Davis, J. J., Ultrasensitive Label Free Electrical Detection of Insulin in Neat Blood Serum. *Anal. Chem.* **2013**, *85*, 4129-4134.
- (39) Bueno, P. R.; Mizzon, G.; Davis, J. J., Capacitance Spectroscopy: A Versatile Approach to Resolving the Redox Density of States and Kinetics in Redox-Active Self-Assembled Monolayers. *J. Phys. Chem. B* **2012**, *116*, 8822-8829.

- (40) Bonnet, G.; Tyagi, S.; Libchaber, A.; Kramer, F. R., Thermodynamic Basis of the Enhanced Specificity of Structured DNA Probes. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 6171-6176.
- (41) Fan, C.; Plaxco, K. W.; Heeger, A. J., Electrochemical Interrogation of Conformational Changes as a Reagentless Method for the Sequence-Specific Detection of DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 9134-9137.
- (42) Ricci, F.; Lai, R. Y.; Heeger, A. J.; Plaxco, K. W.; Sumner, J. J., Effect of Molecular Crowding on the Response of an Electrochemical DNA Sensor. *Langmuir* **2007**, *23*, 6827-6834.
- (43) Ricci, F.; Lai, R. Y.; Plaxco, K. W., Linear, Redox Modified DNA Probes as Electrochemical DNA Sensors. *Chem. Commun.* **2007**, 3768-3770.
- (44) Lubin, A. A.; Lai, R. Y.; Baker, B. R.; Heeger, A. J.; Plaxco, K. W., Sequence-Specific, Electronic Detection of Oligonucleotides in Blood, Soil, and Foodstuffs with the Reagentless, Reusable E-DNA Sensor. *Anal. Chem.* **2006**, *78*, 5671-5677.
- (45) Lai, R. Y.; Lagally, E. T.; Lee, S.-H.; Soh, H. T.; Plaxco, K. W.; Heeger, A. J., Rapid, Sequence-Specific Detection of Unpurified Pcr Amplicons Via a Reusable, Electrochemical Sensor. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 4017-4021.
- (46) Immoos, C. E.; Lee, S. J.; Grinstaff, M. W., Conformationally Gated Electrochemical Gene Detection. *ChemBioChem* **2004**, *5*, 1100-1103.
- (47) Mao, Y.; Luo, C.; Ouyang, Q., Studies of Temperature-Dependent Electronic Transduction on DNA Hairpin Loop Sensor. *Nucleic Acids Res.* **2003**, *31*.
- (48) Brazill, S. A.; Kim, P. H.; Kuhr, W. G., Capillary Gel Electrophoresis with Sinusoidal Voltammetric Detection: A Strategy to Allow Four-“Color” DNA Sequencing. *Anal. Chem.* **2001**, *73*, 4882-4890.
- (49) Hüsken, N.; Gębala, M.; Schuhmann, W.; Metzler-Nolte, N., A Single-Electrode, Dual-Potential Ferrocene-Pna Biosensor for the Detection of DNA. *ChemBioChem* **2010**, *11*, 1754-1761.
- (50) Cash, K. J.; Heeger, A. J.; Plaxco, K. W.; Xiao, Y., Optimization of a Reusable, DNA Pseudoknot-Based Electrochemical Sensor for Sequence-Specific DNA Detection in Blood Serum. *Anal. Chem.* **2009**, *81*, 656-661.
- (51) Idili, A.; Amodio, A.; Vidonis, M.; Feinberg-Somerson, J.; Castronovo, M.; Ricci, F., Folding-Upon-Binding and Signal-on Electrochemical DNA Sensor with High Affinity and Specificity. *Anal. Chem.* **2014**, *86*, 9013-9019.
- (52) Rowe, A. A.; Chuh, K. N.; Lubin, A. A.; Miller, E. A.; Cook, B.; Hollis, D.; Plaxco, K. W., Electrochemical Biosensors Employing an Internal Electrode Attachment Site and Achieving Reversible, High Gain Detection of Specific Nucleic Acid Sequences. *Anal. Chem.* **2011**, *83*, 9462-9466.
- (53) Immoos, C. E.; Lee, S. J.; Grinstaff, M. W., DNA-Peg-DNA Triblock Macromolecules for Reagentless DNA Detection. *J. Am. Chem. Soc.* **2004**, *126*, 10814-10815.

- (54) Xiao, Y.; Lubin, A. A.; Baker, B. R.; Plaxco, K. W.; Heeger, A. J., Single-Step Electronic Detection of Femtomolar DNA by Target-Induced Strand Displacement in an Electrode-Bound Duplex. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 16677-16680.
- (55) Hermann, T.; Patel, D. J., Adaptive Recognition by Nucleic Acid Aptamers. *Science* **2000**, *287*, 820-825.
- (56) Zuo, X.; Song, S.; Zhang, J.; Pan, D.; Wang, L.; Fan, C., A Target-Responsive Electrochemical Aptamer Switch (Treas) for Reagentless Detection of Nanomolar Atp. *J. Am. Chem. Soc.* **2007**, *129*, 1042-1043.
- (57) Radi, A.-E.; Acero Sánchez, J. L.; Baldrich, E.; O'Sullivan, C. K., Reagentless, Reusable, Ultrasensitive Electrochemical Molecular Beacon Aptasensor. *J. Am. Chem. Soc.* **2006**, *128*, 117-124.
- (58) Xiao, Y.; Piorek, B. D.; Plaxco, K. W.; Heeger, A. J., A Reagentless Signal-on Architecture for Electronic, Aptamer-Based Sensors Via Target-Induced Strand Displacement. *J. Am. Chem. Soc.* **2005**, *127*, 17990-17991.
- (59) Huang, Y. C.; Ge, B.; Sen, D.; Yu, H.-Z., Immobilized DNA Switches as Electronic Sensors for Picomolar Detection of Plasma Proteins. *J. Am. Chem. Soc.* **2008**, *130*, 8023-8029.
- (60) Das, J.; Cederquist, K. B.; Zaragoza, A. A.; Lee, P. E.; Sargent, E. H.; Kelley, S. O., An Ultrasensitive Universal Detector Based on Neutralizer Displacement. *Nat. Chem.* **2012**, *4*, 642-648.

Chapter 3

Self-assembled monolayers on gold of β -cyclodextrin adsorbates with different anchoring groups

In this chapter, the design of multivalent β -cyclodextrin-based adsorbates bearing different anchoring groups is described, with the aim to yield stable monolayers with improved packing and close contact of the cavity to the gold surface. Towards this end the primary rim of the β -cyclodextrin was decorated with several functional groups, namely iodide, nitrile, amine, isothiocyanate, methyl sulfide and isocyanide. Monolayers formed by these adsorbates were characterized by contact angle measurements, surface plasmon resonance spectroscopy, polarization-modulation infrared reflection-absorption spectroscopy, X-ray photoelectron spectroscopy, and electrochemistry. The nature of the anchoring group influenced the adsorption kinetics, thickness, layer stability, number of anchoring groups bound to the surface and packing of the resulting monolayers. Therefore, chemical manipulation of multivalent adsorbates can be used to modify the properties of their monolayers.

3.1 Introduction

Cyclodextrins (CDs)¹ are cyclic oligosaccharides constituted of 6, 7 or 8 (α , β and γ -CDs respectively) D-glucopyranose units linked by α -(1 $\rightarrow$ 4) bonds featuring a basket-like structure. These have been used extensively as a platform for the design of adsorbates that assemble to form monolayers with recognition capabilities. In this sense, they have found a wide range of versatile applications including oriented immobilization of proteins,² supramolecular thin film deposition,³ or as a valuable model for studying multivalent host-guest interactions.⁴

Nonetheless the immobilization of the CDs, mostly based on the incorporation of a varying number of thiol or thioether moieties, it is far from straightforward and the nature of the anchoring groups and linker have a great impact on the coverage, orientation and packing and by extrapolation on the host-guest chemistry due to heterogeneous orientation of the hosts.⁵⁻¹³ While inclusion of multiple, preferably symmetrically displayed head groups increases the possibility of achieving orientation of the cyclodextrin core, the strong multivalent interaction with the substrate hampers lateral mobility and self-healing.¹⁴ The use of multivalently exposed strong gold-binding groups such as thiols result in a reduced lateral mobility and thus poor packing of the resulting monolayers.¹³ Substitution of thiols with long alkyl thioethers with a lower affinity to gold improved the lateral mobility of the molecules.¹⁰ The increased mobility of the molecules and the interacting additional alkyl chains led to an increase of surface coverage and an improved order. However the incorporation of the alkyl chains creates an insulating layer that hampers their use in applications where electron transfer from or to the electrode is required.

Improving and modulating the adsorption capabilities of multivalent molecules without hampering the electrical properties remains a challenge. In this sense, tuning of the affinity of the head group for the substrate might be used for improving the lateral mobility, although comparative information about the effect of their chemical nature in the self-assembly properties is in general scarce.¹⁴⁻¹⁷ Furthermore, this concept may take advantage from the fact that variations of the nature of the head group can change the electron transport characteristics of the metal-organic interface.¹⁸⁻¹⁹

The synthesis of a series of β -CD adsorbates bearing different anchoring groups and their self-assembly on gold is described here. The anchoring groups were

selected based on their different affinities for gold and their facile implementation onto the CD scaffold to ensure the homogeneity of the final derivatives and eventually the electrode-head group contact. Concretely, β -CD adsorbates fully functionalized at their primary rim with methyl sulfide,²⁰ isocyanide,²¹ isothiocyanate,^{15, 22} nitrile,²³ amine²⁴ and iodide²⁵⁻²⁶ functionalities were chosen. These adsorbates show lower affinities for gold in comparison to thiols and provide a convenient set of derivatives well suited for comparative studies. The kinetics of the self-assembly process and the structure and electrochemical properties of the monolayer were studied for the presented set of molecules.

3.2 Results

Figure 3.1 shows the molecular structures of the set of β -CD adsorbates bearing different anchoring groups on the primary rim of the CD core. Hepta-iodo β -CD adsorbate (**1**)²⁷ provides the synthetic intermediate for the other derivatives. Adsorbates **2**²⁸, **3**²⁷ and **4**²⁹ were prepared according to published routes with some minor modifications.

Figure 3.1: Structure of the β -CD-based adsorbates used in this study.

The synthesis of the per-methylsulfide β -CD **5** was reported previously.⁹ However the final desired product was obtained only in an inseparable mixture with incompletely functionalized derivatives. In order to circumvent the separation problem, an alternative strategy based on the temporary protection of the secondary rim with acetyl groups was developed (Scheme 3.1a).

Scheme 3.1: Synthesis of adsorbates **5** and **6**: i) methyl imidothiocarbamate hydroiodide, CS_2CO_3 , DMF, overnight, 44%; ii) $MeONa/MeOH$, 2.5 h, 82%; iii) formic acid, DCC, Et_3N , DCM, 3 h, 88%; iv) PCl_3 , DIPA, DCM, 1 h, 63%.

Treatment of compound **7** with methyl imidothiocarbamate hydroiodide in the presence of CS_2CO_3 in *N,N*-dimethylformamide (DMF) afforded the fully functionalized derivative **8** after chromatographic purification in 44% yield. Deacetylation using conventional conditions gave adsorbate **5** in 88% yield after filtering off the insoluble product. The complete substitution of all seven iodines was confirmed by proton and carbon NMR showing a single fully symmetric compound due to its C_7 symmetry (see Experimental section, Figure 3.8). Electrospray ionization mass spectrometry (ESI-MS) and elemental analysis further supported this conclusion.

The preparation of the per-isocyanide- β -CD **6** was carried out according to the strategy shown in Scheme 3.1b, consisting of *N*-formylation followed by dehydration. The secondary rim was permanently protected as methyl ethers in order to avoid dehydration of the hydroxyl groups and to facilitate the purification procedure. Derivative **9** was treated with a preformed mixture of formic acid and *N,N'*-dicyclohexylcarbodiimide (DCC) in dichloromethane (DCM) to give a mixture of rotamers **10**. After dehydration with PCl_3 in the presence of

an excess of diisopropylamine (DIPA) under careful control of the pH, adsorbate **6** was isolated in a 63% yield. The presence of the isocyano groups was confirmed by a peak at 159 ppm in the ^{13}C NMR spectrum, as well as an absorption band at 2148 cm^{-1} in the infrared attenuated total reflectance (IR-ATR) spectrum corresponding to stretching of the isocyanide moiety (see Experimental section, Figures 3.10 and 3.11).

Self-assembled monolayers of adsorbates **2**, **4** and **5** were prepared by immersion of freshly cleaned gold substrates in 0.1 mM adsorbate solutions in THF- H_2O 4:1 for 12-24 h at room temperature, except for adsorbates **1**, **3**, and **6**, which were dissolved in THF-MeOH 4:1, water (pH = 7), and dry degassed DCM, respectively, for solubility reasons. The resulting monolayers were characterized by contact angle goniometry, surface plasmon resonance (SPR) spectroscopy, polarization-modulation infrared reflection-absorption spectroscopy (PM-IRRAS), X-ray photoelectron spectroscopy (XPS), and electrochemistry. Table 3.1 summarizes the most relevant SAM properties, all values are an average of measurements on at least three samples.

Table 3.1: Characterization of the self-assembled monolayers of the β -CD adsorbates on gold.

Adsorbate	θ_a/θ_r (H_2O , deg) ^a	C_{ml} (F/m^2) ^b	R_{CT} (Ω) ^c	Thickness (nm) ^d
1 (I)	45/<20	0.18	82	0.6
2 (CN)	46/<20	0.14	170	1.0
3 (NH_2)	38/<20	0.16	63	1.0
4 (NCS)	49/<20	0.14	242	1.1
5 (SMe)	36/<20	0.20	159	0.4
6 (NC)	61/<20	0.12	18	0.5

^a Advancing (θ_a) and receding (θ_r) contact angles for the monolayer with water. ^b Capacitance of the monolayer determined by cyclic voltammetry at a scan rate of 0.1 V s^{-1} at 0.15 V . ^c Charge-transfer resistance determined by electrochemical impedance spectroscopy. ^d Thickness determined by SPR.

Advancing contact angles ranged from 36 – 49° for **1**–**5**, which suggested a significant hydrophilicity of the substrates. In contrast, the methylated derivative **6** showed a contact angle of 61° reflecting the more hydrophobic

nature of the methylated secondary rim. Low values for the receding contact angle further confirm the hydrophilic character of the surfaces. As a control, bare gold immersed in the same solvent as the β -CD derivatives produced an advancing contact angle of 66° .

The chemical composition of the monolayers and the presence of the anchoring groups was confirmed by observing the corresponding vibrational bands in PM-IRRAS spectra. Figure 3.2a shows the IR-ATR spectrum of neat compound **1** as a representative example for the whole set of derivatives. The main features of the spectrum are the strong vibrational bands related to the antisymmetric glycosidic C-O-C stretching and the coupled C-C/C-O stretching found at $1180\text{--}960\text{ cm}^{-1}$. Two intense bands are observed in this range. The first band, located at lower wavenumbers is quasi-degenerate with two components essentially located at the x, y plane. The other one has an important contribution along the z axis.³⁰ C-H and O-H bending vibrations are also found in the IR-ATR spectra between $1412\text{--}1160\text{ cm}^{-1}$, as well as C-H stretching at $\text{ca } 2900\text{ cm}^{-1}$ and –OH stretching (See Experimental section, Figure 3.11). Upon adsorption of the derivatives, most of the characteristic vibrational bands observed for the neat compounds are also visible in the PM-IRRAS spectra (Figure 3.2b). Because of IRRAS selection rules,³¹ the relative peak intensities between $1190\text{--}1010\text{ cm}^{-1}$ that have different spatial contributions experience changes due to the different orientation of the CD core. Peaks corresponding to asymmetric and symmetric C-H stretching are found between 3000 and 2800 cm^{-1} respectively for **3** and **6**. The presence of a broad band at $\text{ca } 2079\text{ cm}^{-1}$ in the monolayer of compound **4** confirms the presence of the isothiocyanate moiety. Although the shape of the band is changed in the monolayer, no shift was observed in comparison to the neat compound, which suggests that the vibrational energy does not change significantly upon adsorption. Monolayers of **6** showed an adsorption band at 2217 cm^{-1} assignable to the isocyano group, shifted 69 cm^{-1} to higher wavenumbers in comparison to the neat compound.

Figure 3.2: a) IR-ATR spectra for neat **1**. b) PM-IRRAS spectra of monolayers of **1-6**.

SPR gave insight into the adsorption kinetics of the molecules and the monolayer thickness. To a first approximation, at relatively high adsorbate concentrations to avoid mass transport limitation, the adsorption kinetics depends on the anchoring group-surface interactions, and thus it can be regarded as a measure of the binding affinity. Solutions of the adsorbates in MeOH were employed, except for **3** which was dissolved in water (pH = 7). A typical process of the formation and the stability of the monolayer can be found in Figure 3.3a for the adsorption of **6**. After recording the baseline with the pure solvent, the solution of **6** was flowed until the signal leveled off (about 12 min), indicating that the adsorbate was immobilized on the surface. The sample was subsequently washed with the pure solvent to investigate the inherent stability

of the monolayer and to remove nonspecifically adsorbed molecules. The final angle shift indicates the presence of a stable monolayer with an angle shift that depends on the film thickness and dielectric constant of the adsorbate. The reflectivity curves of the stabilized monolayers in water were used to obtain an estimation of the film thickness. The experimental data were fitted to the theoretical curve generated for a five-layer model (prism/Ti/gold/CD/water) assuming an $\epsilon_{real} = 2.3$ ³² for the CD derivatives (Figure 3.3b).

The comparative adsorption profiles and subsequent rinsing steps are shown in Figure 3.3c. Adsorption of **3** was carried out in water due to solubility issues and therefore the angle shifts were higher (Figure 3.3d). Due to the slower kinetics of the adsorption of **1**, **2** and **3** to the surface, more concentrated solutions (0.3 mM) were used to achieve maximum adsorption within the same timescale as when using the 0.1 mM solutions of **4**, **5** and **6**. The adsorption of **1** did not reach clear saturation, even after an extended flowing time. The rinsing step for this compound did not provide reliable information about the removal of the physisorbed material and therefore is not shown. Instead, the sample was washed with solvent until a stable signal SPR was observed. The higher shifts observed for **3**, as well as longer washing times needed to achieve stabilization, suggests that multilayered structures are formed in the process, probably due to electrostatic interactions with the gold substrate.²⁴ Adsorption curves of **5** saturated quickly in about 40 min with low angle shifts while **4** showed a fast adsorption with high angle shifts indicating thicker monolayers. We found thickness values between 0.4 and 1.1 nm assuming that all molecular monolayers have the same refractive index (Table 3.1). Previous SPR measurements on densely packed monolayers of thiolated CDs bearing similar and shorter tethers yielded thicknesses between 0.7 and 1 nm.^{6,33} These values correspond well with the upper value 1.1 found for compound **4**, that has the longest anchoring group. The lower thickness values measured for the other compounds are attributed to the formation of an incomplete monolayer although variations of the dielectric constant cannot be excluded entirely. We ruled out multilayer formation as a contribution to the determination of the thicknesses. As a negative control, the injection of native β -CD (0.3 mM) did not produce a signal change in the SPR adsorption curve.

The adsorption kinetics of alkylthiols occurs in a two-step process. The first step is rapid, while the second is slower and is regarded as the rearrangement of thiols on the surface and the slow adsorption of molecules into newly exposed

surface in order to achieve the highest density of packing. Taking both processes as independent, the film growth can be described by the following expression:

$$T = T_1[1 - e^{-k_1 \cdot t}] + T_2[1 - e^{-k_2 \cdot t}] \quad (\text{Equation 3.1})$$

Where T is the relative thickness, T_1 and T_2 are limiting thickness of the first and second step respectively, and k_1 and k_2 are the corresponding rate constants.³³ The adsorption curves were fitted with Equation 3.1, a representative example is shown in Figure 3.3e. The kinetic data obtained are presented in Table 3.2.

Table 3.2: Rate constants and limiting SPR shift for the adsorption of adsorbates **1-6**.

Adsorbate	Conc. (mM)	T_1 (nm)	T_2 (nm)	$k_1 \times 10^{-5} \text{ (s}^{-1}\text{)}$	$k_2 \times 10^{-5} \text{ (s}^{-1}\text{)}$	χ^2
1 (I)	0.3	0.2	0.6	203 ± 3	14 ± 0.4	1.94
2 (CN)	0.3	0.2	1.1	140 ± 6	14 ± 0.5	0.68
3 (NH ₂)	0.3	1.6	2.3	383 ± 6	89 ± 1	15.1
4 (NCS)	0.1	0.8	0.4	179 ± 2	17 ± 2	1.43
5 (SMe)	0.1	0.1	0.2	1642 ± 142	91 ± 3	0.26
6 (NC)	0.1	0.4	0.1	3710 ± 130	74 ± 6	0.55

A good fitting is observed except for adsorbate **3**, probably due to the multilayering process, and thus the model might not be well suited to describe the experimental data. As expected, the second kinetic constant k_2 is slower than k_1 by about one order of magnitude which is in agreement with the fast adsorption, slow reorganization model. In comparison to alkylthiol adsorbates, the first adsorption kinetics is much slower at comparable concentrations, while k_2 is in the same order of magnitude. The former fact could be interpreted in basis of the lower affinity of the binding groups used here, as well as the slower diffusion from the solution to the surface because of the higher molecular weight of the CD-based adsorbates. On the other hand, with the exception of adsorbates **4** and **6**, the major contribution to the final coverage is given by the second step as evidenced by the higher T_2 values, in contrast to the self-assembly of thiols. This underlines the importance of the lateral diffusion in the assembly of these monolayers to allow further binding.

When comparing adsorbates **1-6**, while k_2 is on the same order of magnitude in the whole set, k_1 is about one order of magnitude higher in **5** and **6**.

Figure 3.3: a) SPR adsorption curve corresponding to the adsorption of **6**: ■ injection of 0.1 mM solution in MeOH and ▼ washing with MeOH. b) Experimental reflectivity curve (markers) and fit (solid line) after adsorption of **6** in water. c) Comparative adsorption curves and rinsing steps for adsorbates **1-5**. d) Adsorption curve and rinsing of adsorbate **3** in water. e) Experimental (dashed lines) and fitted (solid line) adsorption curve of **6**.

Electrochemical investigation of the samples provided information about the relationship between thickness and capacitance of the monolayers and their permeability towards an external ferro/ferri cyanide redox couple. The capacitance of SAM-modified electrodes is proportional to the dielectric

constant of the separation medium and inversely proportional to the film thickness, as predicted by the Helmholtz theory.³⁴ Cyclic voltammetry of the modified gold electrodes in 0.1 M K_2SO_4 showed no redox reaction in the measured range 0–0.3 V. Consequently, only non-Faradaic processes contribute to the current flow at the electrode-solution interface. The values of the capacitance of the monolayers formed by **1–5** correlate inversely with the thickness values obtained by SPR (Figure 3.4). The monolayer of **6** produced the lowest C_{ml} , probably due to a lower dielectric constant of the molecule. The order of magnitude found for our monolayers is in agreement with the values found in monolayers of CD adsorbates with short-tethers or cucurbiturils.^{6, 13, 35}

Figure 3.4: Thickness obtained by SPR experiments versus monolayer capacitance obtained by cyclic voltammetry at 0.15 V at a scan rate of 0.1 V s⁻¹ in 0.1 M K_2SO_4 in water, the error bars are the standard deviation determined over measurements on three samples.

Electrochemical impedance spectroscopy (EIS) measurements were carried out to further study the packing of these monolayers. Nyquist plots (Figure 3.5) show a semicircle at high frequencies and a straight line at lower frequencies, indicating diffusion controlled charge transport. The charge transfer resistance values ranged from 18 to 240 Ω , suggesting excellent electron conduction in comparison to long thioether β -CD monolayers, for which the resistances values are in k Ω range.¹⁰ This might be related to the packing properties but also to the inherent presence of unshielded cavities at the surface where the ionic species can permeate.

The elemental composition of the monolayers and the binding of the adsorbates was revealed by X-ray photoelectron spectroscopy (XPS) measurements (Table 3.3). For all compounds, a broad band was found for C(1s) at around 286 eV.

Although some carbon contamination was present in some cases, deconvolution of the carbon signal into three peaks (O-C*-O, C-C*-O and C-C*-C) allowed the calculation of the elemental ratios with respect to the other elements, as the majority of the signal of the CD molecules comes from the C-C*-O and O-C*-O carbons. Signals of the elements constituting the anchoring groups were observed for all SAMs. The XPS spectrum of a monolayer of per-iodide- β -CD **1** shows the characteristic I(3d_{5/2}) and I(3d_{3/2}) signals. Deconvolution of the I(3d_{5/2}) signal gave rise to two bands at 619.3 and 621.0 eV with areas of 87% and 13%, respectively (Figure 3.6a). The lower energy band is assigned to gold-bound iodide. The higher energy band showed a similar binding energy as unbound iodide. The nitrogen based adsorbates show the N(1s) nitrogen signal around 399.4 eV (Figure 3.6b). The N(1s) signal of the monolayer based on adsorbate **2** can be fitted with a single band with no apparent shift in comparison to the neat compound, suggesting that the CN-Au interaction is rather weak.

Table 3.3: XPS binding energies, elemental ratios and fractions of headgroup moieties bound to gold for SAMs prepared with **1-6**

Adsorbate (X)	Binding energy (eV)				C:X ratio ^a Exp. (Theor.)	% Head group bound
	C(1s)	N(1s)	S(2p _{3/2})	I(3d _{5/2})		
1 (I)	286.6			619.3	6:1.4 (6:1)	87
2 (CN)	286.3	399.5			7:1.1 (7:1)	n. d.
3 (NH₂)	286.5	399.4			6:0.9 (6:1)	88
4 (NCS)	286.4	399.7	161.7		7:1.1:0.8 (7:1:1)	61
5 (SMe)	286.5		163.0		7:0.6 (7:1)	100
6 (NC)	286.5	399.7			9:0.8 (9:1)	87

Figure 3.5: Representative Nyquist plots for the monolayers prepared by adsorption of **1-6** determined by electrochemical impedance spectroscopy in 1 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ at its formal potential (0.24 V)

The monolayers of **3** were deconvoluted into two peaks at 399.4 and 401.9 eV in a ratio 88:12 with the latter assignable to protonated amines.³⁶ This indicates that the adsorbed molecules interact mostly by strong coordinative interactions with the gold substrate. The nitrogen signal in the monolayer of isocyanide **6** is deconvoluted into two bands, the first at 399.7 and the second at 401.5 eV, in a

ratio of 87:13. Since PM-IRRAS did not show unbound isocyanide (Figure 3.2), we expect that the major signal at 399.7 eV corresponds to bound isocyanide. The monolayers of **4** and **5** show S(2p) signals centered at 161.7 (broad) and 163.0 eV, respectively (Figure 3.6c). Fitting the S(2p) signal for adsorbate **4** with two double peaks (for bound and unbound S(2p_{3/2}) and S(2p_{1/2})) shows that 61% of the sulfur atoms are bound to gold. The optimal fit of the presence of sulfur for adsorbate **5** was achieved with two peaks (Figure 3.6c), but due to the low intensity it can only be considered an estimate. This, together with the energy shift of 0.2 eV with respect to the neat compound, suggests that all sulfur atoms of **5** are bound to gold. In all monolayers, a reasonable agreement between the theoretical and experimental elemental ratios was found for C and N, S, or I, suggesting that the structural integrity of the molecules is preserved in the self-assembled monolayers.

Figure 3.6: XPS spectra for (a) I(3d_{5/2}) in monolayers of **1**; (b) N(1s) signals for monolayers resulting from the assembly of **2**, **3**, **4** and **6**; (c) S(2p) signals found for the adsorption of **4** and **5**.

3.3 Discussion

There is a lack of data of self-assembled monolayers with different head groups other than thiols due to the low stability of these monolayers. In our case,

multivalent, spatially oriented exposure of the anchoring functionality can be exploited to enhance affinities^{10, 35} and increase the adsorption capabilities. Most of the head groups employed in this study yield rather poorly ordered and/or incomplete monolayers when presented in monovalent adsorbates, or even do not exhibit significant adsorption. For instance, amines can be adsorbed on gold substrates in a vapor-phase environment to give stable monolayers; however polar solvents such as ethanol disrupt the Au/N interaction because of its weak nature.³⁷ This was overcome through the multivalent exposure of amino moieties to achieve stable adsorption, as reported in polyamidoamine (PAMAM) dendrimers.^{24, 38-39} Here the β -CD core offers the additional advantage of directional exposure of the head groups and in that way the maximization of the interactions does not impose an entropic penalty.

In our comparative studies, we found different kinetics of formation and structures of monolayers depending on the anchoring group installed. Although the chemical affinity between the substrate and the head group is probably the most important contribution to the adsorption kinetics, there might be additional parameters that influence the overall phenomena, such as the gold-head group binding geometry. In this sense, the β -CD core can also restrict the possible reorientation of the head groups and to limit the probability of a binding event.

A remarkable effect was observed on the adsorption kinetics. In this study, reorganization provides the more significant contribution for the completion of the monolayer; for example adsorbate **2** shows slow adsorption kinetics that could be related to a low head group-substrate affinity. XPS seems to confirm such observations with no important shift of the N(1s) signal. Therefore, changing the nature of the head group critically determines the possibility or reorganization of the adsorbate. As an opposite example, adsorption of **5** is fast in the first stage, but the reorganization kinetics is not high enough to allow further adsorption, thus limiting the packing. Longer immersion times do not promote further packing in this case since the capacitance measurements for adsorbate **5** suggest lower film thickness, probably meaning sub-monolayer coverage.

Hydrophilicity of the surface increased upon adsorption of CDs as shown by water contact angle goniometry. This is expected due to the exposure of the hydrophilic secondary rim pointing upward the surface and eventually supports

oriented adsorption of the derivatives. PM-IRRAS and XPS further supported the presence and structural integrity of the adsorbates at the surfaces, since the most characteristic vibrational bands were observed for the whole set and the elemental composition was in good agreement with the expected values, meaning that the constitutive elements are retained at the surface after monolayer formation. Therefore, cleavage and adsorption of only the anchoring groups and concomitant release of the β -CD core was excluded. Monolayer thicknesses from 0.5 to 1.1 nm were comparable to the values reported before by SPR or AFM for cyclodextrin-based adsorbates.^{6, 32} Furthermore, SPR and capacitance measurements were in reasonable agreement. Taking into account the successful use of multivalent thioethers to yield good quality monolayers when attached to long alkyl chains,^{10, 40} adsorbate **5** gave surprisingly the lowest thickness values. This might indicate the need of interadsorbate interactions to make this anchoring group effective. Unreliable performance of methyl sulfides is not unprecedented,¹⁵ but the problem seems not to arise from the cleavage of the thioether on the surface, since the carbon contents found by XPS was still in good agreement with the theoretical values. EIS provided some information about packing; the higher packing densities were found for adsorbates **2** and **4**. Interestingly, nitrile-based adsorbates usually gave rise to better quality monolayers even though a low affinity was observed by SPR. The low values found for adsorbate **6** might be attributed to a dipole effect rather than poor packing, since the $\nu(\text{NC})$ values obtained are in agreement with >70% saturation coverages as found in bis and tridentate isocyanides.⁴¹

In general, the majority of the anchoring groups of all compounds are used in the adsorption process, as revealed by XPS and PM-IRRAS. Alkanenitriles may undergo adsorption through two basic coordination types: η^1 -type that implies σ -coordination via the nitrogen atom, or η^2 -type that involves coordination of both atoms of the functional group. Clear information about the coordination in our system is not available since the absence of evident nitrile adsorption bands on the PM-IRRAS spectra. However, η^2 -coordination to a variety of metallic substrates usually produces high shifts on the $\text{N}(1s)$ band in XPS spectra (~ 2.9 eV).⁴² Fitting of the $\text{S}(2p)$ signal for isothiocyanate-based adsorbate **4** evidenced that 4.3 of 7 isothiocyanato headgroups are involved in the interaction with gold, and thus isothiocyanates employ the sulfur with the same efficiency as in long thioether- β CD derivatives and in a more efficient way in comparison to the per-thio- β -CD.^{10, 43} The presence of $\nu(\text{NCS})$ without evident shift seems to

corroborate the presence of unbound head groups. The other sulfur-based adsorbate **5** indicated 100% of bound sulfur. Finally, PM-IRRAS spectra of monolayers prepared with isocyanide-based adsorbate **6** exhibited the characteristic isocyanide stretching band with a shift compatible with a η^1 coordination where the isocyanide group is coordinated with on-top gold atoms through a single lineal carbon-gold bond.⁴¹ Here the presence of a single vibrational band for the isocyanide stretching seems to corroborate the binding estimation obtained by XPS, with 6 out of 7 anchoring groups bound to the substrate.

3.4 Conclusions

Modulation of the self-assembly properties of β -CD-based adsorbates was achieved *via* homogeneous decoration of the primary rim with an assortment of functional groups. Methyl sulfide and isocyanide head groups can be conveniently inserted without compromising the homogeneity of the final compound through temporal or permanent protection of the secondary rim. XPS and PM-IRRAS strongly supported the non-destructive attachment to the surfaces due to the good agreement between the elemental composition found at the surface and the theoretical value, as well as the presence of the most characteristic vibrational bands of the β -CD core. The effect of the head group was evidenced in the adsorption kinetics, amount of material adsorbed and order of the resulting monolayers. Interestingly, the multivalent exposure of weakly gold binding anchoring groups such as amines can promote the assembly in a significant manner and give rise to rather stable monolayers. Good adsorption capabilities are also achieved with nitriles and isothiocyanates. Adsorbates **2** and **4** provided monolayers of better quality with higher charge transfer resistivities and thickness in the monolayer range and constitute the most promising candidates for further studies in current development in our laboratories (see also Chapter 4).

3.5 Experimental section

3.5.1 Materials

Reagents and solvents were purchased from commercial sources and used without further purification unless stated otherwise. ^1H and ^{13}C NMR were recorded at 400 and 100.6 MHz, respectively. 2D COSY and HMQC experiments were used to assist on NMR peak assignments. Thin-layer chromatography (TLC)

was carried out on aluminum sheets, with visualization by UV light and by charring with 10% H₂SO₄. Column chromatography was carried out on silica gel (230-400 mesh) or basic alumina. ESI-MS spectra were obtained for samples dissolved in DCM-MeOH, CH₃CN-H₂O-TFA or THF-MeOH mixtures at low μ M concentrations. DCM and DMF were dried with molecular sieves. Methyl imidothiocarbamate hydroiodide,⁴⁴ heptakis(6-deoxy-6-iodo)cyclomaltoheptaose,²⁷ heptakis(2,3-di-O-acetyl-6-deoxy-6-iodo)cyclomaltoheptaose,⁴⁵ heptakis(6-azido-6-deoxy)cyclomaltoheptaose,²⁷ and heptakis(6-deoxy-6-isothiocyanato)cyclomaltoheptaose²⁹ were prepared according to the known procedures. The analytical data was in a good agreement with the described data. Heptakis(6-amino-6-deoxy)cyclomaltoheptaose was prepared following the procedure of Ashton et al²⁷ and further purified by repeated precipitation of the aqueous solution of hydrochloride by adding aqueous solution of NH₄OH (30%) until reaching the pH 9-10. The precipitate was then filtered off, washed with water, MeOH and diethyl ether and dried at vacuum.

3.5.2 Synthesis of new adsorbates

Heptakis(6-cyano-6-deoxy)cyclomaltoheptaose (2):

This compound was prepared following the adapted procedure of Baer *et al*²⁸. To a solution of heptakis(6-deoxy-6-iodo)cyclomaltoheptaose (1 g, 0.52 mmol) in dry DMF (17 mL), KCN (310 mg, 4.8 mmol, 1.3 eq) was added and the reaction mixture was vigorously stirred under Ar atmosphere at 45 °C for 1 day and subsequently at rt for 1 day. The solution was concentrated to 1/3 of the initial volume and the residue was poured into 60 mL of cold water and stirred for 30 min. The solid was filtered off and washed with water (3 x 10 mL). The collected solid was suspended in EtOH (10 mL) and centrifugated (10 min, 7000 rpm) 3 times, then dried at high vacuum to give a white solid. Yield: 354 mg (57%). The characterization data was according the published data, plus: ¹H NMR (400 MHz, DMSO-d₆): δ = 6.05 (bs, 7 H, OH), 5.84 (bs, 7 H, OH), 4.98 (d, 7 H, $J_{1,2}$ = 3.4 Hz, H-1), 3.91 (dt, 7 H, H-5), 3.60 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 3.40 (m, 14 H, H-2, H-4), 3.01 (dd, 7 H, $J_{6a,6b}$ = 14.5 Hz, $J_{5,6a}$ = 2.7 Hz, H-6a), 2.90 (dd, 7 H, $J_{5,6b}$ = 8.9 Hz, H-6b); ESI-MS: m/z = 1199.4 [M + H]⁺, 1237.8 [M + K]⁺.

Heptakis(2,3-di-O-acetyl-6-methylthio)cyclomaltoheptaose (8):

To a solution of heptakis (2,3-di-*O*-acetyl-6-deoxy-6-iodo)cyclomaltoheptaose (1.1 g, 0.44 mmol) and Cs_2CO_3 (3.6 g, 11 mmol, 3.6 eq.) in dry DMF (39 mL), methyl imidothiocarbamate hydroiodide (1.4 g, 6.4 mmol, 2.1 eq.) was added and the reaction mixture was vigorously stirred overnight under Ar atmosphere. The solution was poured on ice and HCl 0.5 M (200 mL) was added. The aqueous layer was extracted with DCM (4 x 50 mL). The combined organic layers were washed with 0.5 M solution of HCl (100 mL) and brine (100 mL), dried (NaSO_4), filtered and concentrated. Traces of DMF were removed under reduced pressure and the residue was purified by column chromatography using DCM-MeOH 50:1 $\rightarrow$ 40:1 as an eluent to give **8** as a white solid. Yield: 370 mg (44%); R_f = 0.40 (DCM-MeOH 9:1); IR-ATR: 2917, 1741, 1368, 1215, 1025, 728, 601, 466 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ = 5.22 (t, 7 H, H-3), 5.06 (d, 7 H, $J_{1,2}$ = 3.9 Hz, H-1), 4.74 (dd, 7 H, $J_{2,3}$ = 9.8 Hz, H-2), 4.11 (m, 7 H, H-5), 3.73 (t, 7 H, $J_{3,4}$ = $J_{4,5}$ = 8.4 Hz, H-4), 2.97 (m, 14 H, H-6a, H-6b), 2.13 (s, 21 H, SCH_3), 2.02, 1.99 (2 s, 42 H, 2 COCH_3); ^{13}C NMR (100 MHz): δ = 170.9, 169.5 (CO), 96.9 (C-1), 79.1 (C-4), 71.6 (C-5), 71.1 (C-3), 70.8 (C-2), 36.2 (C-6), 20.9 (COCH_3), 17.6 (SCH_3); ESI-MS: m/z = 1932.9 $[\text{M}]^+$, 1954.9 $[\text{M} + \text{Na}]^+$; Anal. Calc. for $\text{C}_{77}\text{H}_{112}\text{O}_{42}\text{S}_7 \cdot 2 \text{H}_2\text{O}$: C, 46.94; H, 5.93; Found C, 46.97; H, 5.83. NMR spectra are shown in Figure 3.7.

Figure 3.7: ^1H (top) and ^{13}C NMR (bottom) spectra (400 and 100.3 MHz, CDCl_3) of compound **8**.

Heptakis(6-methylthio)cyclomaltoheptaose (**5**):

To a solution of **8** (370 mg, 0.19 mmol) in MeOH (32 mL), NaOMe 1 M (0.3 mL) was added and the reaction was stirred at rt. After 30 min a white solid precipitate appeared and the solution was further vigorously stirred for 2 h. The solvent was evaporated under reduced pressure and the residue suspended in water (10 mL), filtrated and further washed with water (3 x 5 mL) and MeOH (5

mL), then dried at high vacuum with P_2O_5 to give a white solid. Yield: 211 mg (82%); IR-ATR: 3322, 2913, 1038, 585 cm^{-1} ; ^1H NMR (400 MHz, DMSO-d_6): δ = 5.96 (d, 7 H, $J_{\text{H,H}}$ = 6.6 Hz, OH), 5.85 (d, 7 H, $J_{\text{H,H}}$ = 1.8 Hz, OH) 4.90 (d, 7 H, $J_{1,2}$ = 3.4 Hz, H-1), 3.78 (m, 7 H, H-5), 3.60 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 3.44-3.35 (m, 14 H, H-2, H-4), 3.14 (d, 7 H, $J_{6a,6b}$ = 12.6 Hz, H-6a), 3.60 (dd, 7 H, $J_{5,6b}$ = 8.6 Hz, H-6b), 2.09 (s, 21 H, SCH_3); ^{13}C NMR (100 MHz): δ = 102.1 (C-1), 85.3 (C-4), 72.6 (C-3), 72.3 (C-2), 71.0 (C-5), 35.1 (C-6), 15.9 (SCH_3); ESI-MS: m/z = 1366.9 $[\text{M} + \text{Na}]^+$; Anal. Calc. for $\text{C}_{49}\text{H}_{84}\text{O}_{28}\text{S}_7$: C, 43.71; H, 6.29. Found C, 43.69; H, 6.41. NMR spectra are shown in Figure 3.8.

Figure 3.8: ^1H (top) and ^{13}C NMR (bottom) spectra (400 and 100.3 MHz, CDCl_3) of compound 5.

Heptakis(6-deoxy-6-formamido-2,3-di-*O*-methyl)cyclomaltoheptaose (10):

A solution of DCC (1.16 g, 5.6 mmol, 2.5 eq.) in dry DCM (15 mL) under Ar atmosphere was cooled down in an ice-bath. 2 M solution of formic acid in dry DCM (6.7 mL, 13.4 mmol, 6 eq.) was added dropwise to give a white suspension, and the mixture was further stirred for 15 min. A suspension of heptakis(6-amino-6-deoxy-2,3-di-*O*-methyl)cyclomaltoheptaose⁴⁶⁻⁴⁷ (500 mg, 0.32 mmol) and Et₃N (0.31 mL, 2.2 mmol, 1 eq.) in dry DCM (10 mL) was sonicated for 15 min and then added dropwise to the previous solution, and the mixture was vigorously stirred at 0 °C → rt for 3 h. The suspension was filtered off and the solid washed with portions of DCM (4 x 5 mL). The organic solvent was washed with brine (3 x 10 mL) and the organic layer was dried, filtered and concentrated. The residue was purified by chromatography in DCM-MeOH 2:1 → MeOH to give a white powder. **10** was isolated as a mixture of rotamers. Yield: 430 mg (88%); *R*_f = 0.45 (MeOH); IR-ATR: 3295, 2929, 2832, 1657, 1530, 1382, 1014 cm⁻¹; ¹H NMR (400 MHz, CD₃OD): δ = 8.12, 8.09, 8.07, 8.05, 7.97 (5 s, 7 H, CHO), 5.20 (m, 7 H, *J*_{1,2} = 3.6 Hz, H-1), 4.20-3.95 (m, 7 H, *J*_{6a,6b} = 13.8 Hz, *J*_{6a,6b} = 2.3 Hz, H-6a), 3.88 (m, 7 H, H-5), 3.67 (m, 21 H, OCH₃), 3.51 (m, 28 H, H-3, OCH₃), 3.45-3.35 (m, 14 H, H-4, H-6b), 3.18 (dd, 7 H, *J*_{2,3} = 9.8 Hz, H-2); ¹³C NMR (100 MHz): δ = 164.2 (CO), 99.9 (C-1), 83.4, 83.2, 82.4 (C-2, C-3, C-4), 71.8 (C-5), 62.0, (OCH₃), 54.2 (OCH₃), 40.4 (C-6); ESI-MS: *m/z* = 1542.3 [M + Na]⁺. NMR spectra are shown in Figure 3.9.

Figure 3.9: ^1H (top) and ^{13}C NMR (bottom) spectra (400 and 100.3 MHz, CDCl_3) of compound **10**.

Heptakis(6-deoxy-6-isocyano-2,3-di-O-methyl)cyclomaltoheptaose (**6**):

An oven dried three-neck flask was cooled down under a stream of dry N_2 , then filled with a solution of **10** (150 mg, 0.1 mmol) in dry DCM (5 mL) and DIPA (0.26 mL, 2.6 mmol, 3.8 eq.) under Ar atmosphere. The flask was cooled down in an ice-bath and PCl_3 (0.075 mL, 0.26 mmol, 1.1 eq.) was added dropwise. The reaction mixture was stirred for 1 h. 0.2 mL of DIPA and 0.025 mL of PCl_3 were added and stirred for 30 min. Then 5% aqueous solution of NaHCO_3 was slowly added (5 mL) and the mixture was stirred for 10 min. DCM was added (30 mL) and the organic layer was washed with 5% aqueous solution of NaHCO_3 (3 x 10 mL) and the organic layer was dried, filtered and concentrated. The residue was

purified by flash column chromatography on dry basic alumina in EtOAc to give **6** as a white solid. The compound was stored at $-30\text{ }^{\circ}\text{C}$ under N_2 . Yield: 86 mg (63%); $R_f = 0.59$ (EtOAc); IR-ATR: 2928, 2830, 2148, 1108, 1040, 966, 934, 840 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): $\delta = 5.14$ (d, 7 H, $J_{1,2} = 3.8$ Hz, H-1), 3.87 (m, 7 H, $J_{6a,5} = 5.3$ Hz, H-6a), 3.87 (m, 7 H, H-5), 3.72 (d, 7 H, $J_{6a,6b} = 13.8$ Hz, H-6b), 3.63 (s, 21 H, OCH_3), 3.52 (s, 21 H, OCH_3), 3.52-3.47 (m, 14 H, H-3, H-4), 3.21 (dd, 7 H, $J_{2,3} = 9.2$ Hz, H-2); ^{13}C NMR (100 MHz): $\delta = 159.0$ (NC), 98.5 (C-1), 81.5, 81.1, 80.5 (C-2, C-3, C-4), 69.3 (C-5), 61.6 (OCH_3), 59.1 (OCH_3), 44.1 (C-6); ESI-MS: $m/z = 1394.8$ $[\text{M} + \text{H}]^+$, 1416.8 $[\text{M} + \text{Na}]^+$; Anal. Calc. for $\text{C}_{63}\text{H}_{91}\text{O}_{28}\text{N}_7 \cdot 2\text{H}_2\text{O}$: C, 52.90; H, 6.69; N, 6.85. Found C, 52.73; H, 6.38; N, 6.68. NMR spectra are shown in Figure 3.10.

Figure 3.10: ^1H (top) and ^{13}C NMR (bottom) spectra (400 and 100.3 MHz, CDCl_3) of compound **6**.

Figure 3.11: IR-ATR spectra of compounds 2-6.

3.5.3 Methods

Monolayer preparation:

All glassware used to prepare monolayers was immersed in piranha solution (conc. $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ 3:1, **warning:** *piranha should be handled with caution; it can detonate unexpectedly*), washed with copious amount of water and dried in an oven. Gold substrates were cleaned by an oxygen plasma for 10 min and the resulting oxide layer was removed by leaving the substrates immersed in EtOH for 20 min and finally transferred rapidly to 0.1 mM solutions of β -CD adsorbates in THF- H_2O 4:1 (**2**, **4** and **5**), THF-MeOH 4:1 (**1**), H_2O (pH = 7, **3**) or DCM (**6**) for 12-24 h at room temperature. Isocyanide monolayers were prepared using degassed DCM under Ar atmosphere. Subsequently, the substrates were

removed from the solution and rinsed with the solvent used in the monolayer formation, EtOH and dried with a stream of N₂.

Monolayer characterization:

Contact angles were measured on a Krüss G10 contact angle setup equipped with a CCD camera.

Surface Plasmon Resonance Spectroscopy was carried out on glass-supported gold substrates for SPR (50 nm gold, 2 nm Ti adhesion layer) obtained from SSENS (Hengelo, The Netherlands). SPR measurements were performed on a two-channel vibrating mirror angle scan set-up based on the Kretschmann configuration.⁴⁸ The instrument consist of a HeNe laser (JDS Uniphase, 10 mW, $\lambda = 632.8$ nm) whose light passes through a chopper that is connected to a lock-in amplifier. The light was coupled via a high index prism (LaSFN 9) to an optically matched glass-supported gold substrate with an index matching oil (Cargille; series B; $n_D^{25} = 1.700 \pm 0.002$). A Teflon cell was placed on the monolayer via an O-ring to avoid leakage. SPR experiments were performed in a flow cell system with Teflon tubing at a continuous flow of 0.15 mL min⁻¹). Solutions of adsorbates were dissolved in water, MeOH or by adding the minimal amount of DMSO (2% except in the per-iodide β -CD **1** solution, 10%) and diluted with MeOH. If DMSO was used, baseline and washing steps were carried out with MeOH containing the same amount of DMSO. Prior to and after the formation of the monolayer, reflectivity curves were recorded in MilliQ water after stabilization of the signal and used to obtain the film thickness. Winspall Version 3.02⁴⁹ developed by Prof. Dr. W. Knoll's group was used to simulate and fit reflectivity curves by generating a 5-layer model system (Prism/Ti/Au/ β -CD film/water), taking the film thickness as fitting parameter and assuming a dielectric constant of 2.30 for cyclodextrin derivatives.³² The following dielectric constants were used to simulate the reflectivity curves: LaSFN 9 prism, $\epsilon_{\text{real}} = 3.42$; Ti: $\epsilon_{\text{real}} = -3.44$, $\epsilon_{\text{im}} = 10.25$ ⁵⁰ and water, $\epsilon_{\text{real}} = 1.78$ The dielectric constants of gold were obtained by fitting the SPR curve before monolayer formation and were on average: $\epsilon_{\text{real}} = -12.80 \pm 0.11$, $\epsilon_{\text{img}} = 1.94 \pm 0.13$.

PM-IRRAS measurements were conducted on a Nicolet FT-IR 6700 and a TOM optical module (Thermo Scientific) equipped with a Photo Elastic Modulator (PEM, Hinds Instruments). Spectra were recorded with the p-polarized light incidence at 82° relative to the surface normal, with the PEM wavenumber set

to 1500, 2100 or 2900 cm^{-1} . 200 scans with a resolution of 4 cm^{-1} at room temperature were collected in each experiment.

XPS measurements were performed on a Quantera Scanning X-ray Multiprobe instrument from Physical Electronics, equipped with a monochromatic Al K α X-ray source producing approximately 25 W of X-ray power. Spectra were referenced to the main C 1s peak set at 284.0 eV. A surface area of 1000 μm x 300 μm was scanned with an X-ray beam about 10 μm wide.

All electrochemical measurements were performed on a CH instruments bipotentiostat 760D. All solutions used during electrochemical measurements were deaerated with N_2 for at least 30 minutes. Cyclic voltammetry measurements were performed in a three-electrode setup using the SAM-covered gold plate as the working electrode (area = 0.44 cm^2), a platinum disc as the counter electrode and a Ag/AgCl reference electrode in aqueous 0.1 M K_2SO_4 . Electrochemical impedance measurements were performed using 2 mm diameter (CH Instruments) and 1.6 mm diameter (BASi) gold disk electrodes. Before modification the electrodes were polished using 50 nm alumina particles (CH Instruments), followed by extensive rinsing with ethanol and 5 minutes of ultrasonic treatment in ethanol and 5 minutes in MilliQ water. Subsequently the electrodes were cleaned electrochemically in 0.5 M H_2SO_4 by applying an oxidizing potential of 2 V for 5 seconds followed by a reducing potential of -0.35 V for 10 seconds. Then the electrode potential was scanned from -0.25 V to 1.55 V and back for 40 cycles at a scan rate of 100 mV/s. After cleaning the electrodes were rinsed with MilliQ water and ethanol and dried under a flow of N_2 and placed immediately in the appropriate solution. The measurements were performed in an aqueous solution of 1 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in 0.1 M K_2SO_4 as supporting electrolyte and measured at the formal potential of the $\text{Fe}(\text{CN})_6^{3-/4-}$ couple (0.24 V) and an amplitude of 10 mV.

3.6 References

- (1) Szejtli, J., Introduction and General Overview of Cyclodextrin Chemistry. *Chem. Rev.* **1998**, *98*, 1743-1753.
- (2) Yang, L.; Gomez-Casado, A.; Young, J. F.; Nguyen, H. D.; Cabanas-Danés, J.; Huskens, J.; Brunsveld, L.; Jonkheijm, P., Reversible and Oriented Immobilization of Ferrocene-Modified Proteins. *J. Am. Chem. Soc.* **2012**, *134*, 19199-19206.

- (3) Crespo-Biel, O.; Dordi, B.; Reinhoudt, D. N.; Huskens, J., Supramolecular Layer-by-Layer Assembly: Alternating Adsorptions of Guest- and Host-Functionalized Molecules and Particles Using Multivalent Supramolecular Interactions. *J. Am. Chem. Soc.* **2005**, *127*, 7594-7600.
- (4) Gomez-Casado, A.; Dam, H. D.; Yilmaz, D.; Florea, D.; Jonkheijm, P.; Huskens, J., Probing Multivalent Interactions in a Synthetic Host-Guest Complex by Dynamic Force Spectroscopy. *J. Am. Chem. Soc.* **2011**, *133*, 10849-10857.
- (5) Diget, J. S.; Petersen, H. N.; Schaarup-Jensen, H.; Sørensen, A. U.; Nielsen, T. T.; Pedersen, S. U.; Daasbjerg, K.; Larsen, K. L., Synthesis of β -Cyclodextrin Diazonium Salts and Electrochemical Immobilization onto Glassy Carbon and Gold Surfaces. *Langmuir* **2012**, *28*, 16828-16833.
- (6) Perl, A.; Kumprecht, K.; Kraus, T.; Armspach, D.; Matt, D.; Reinhoudt, D. N.; Huskens, J., Self-Assembled Monolayers of α -Cyclodextrin Derivatives on Gold and Their Host-Guest Behavior. *Langmuir* **2009**, *25*, 1534-1539.
- (7) McNally, A.; Forster, R. J.; Keyes, T. E., Interfacial Supramolecular Cyclodextrin-Fullerene Assemblies: Host Reorientation and Guest Stabilization. *Phys. Chem. Chem. Phys.* **2009**, *11*, 848-856.
- (8) Kitano, H.; Taira, Y., Inclusion of Bisphenols by a Self-Assembled Monolayer of Thiolated Cyclodextrin on a Gold Electrode. *Langmuir* **2002**, *18*, 5835-5840.
- (9) Stine, K. J.; Andraukas, D. M.; Khan, A. R.; Forgo, P.; D'Souza, V. T., Electrochemical Study of Self-Assembled Monolayers of a β -Cyclodextrin Methyl Sulfide Covalently Linked to Anthraquinone. *J. Electroanal. Chem.* **1999**, *465*, 209-218.
- (10) Beulen, M. W. J.; Bügler, J.; Lammerink, B.; Geurts, F. A. J.; Biemond, E. M. E. F.; van Leerdam, K. G. C.; van Veggel, F. C. J. M.; Engbersen, J. F. J.; Reinhoudt, D. N., Self-Assembled Monolayers of Heptapodant β -Cyclodextrins on Gold. *Langmuir* **1998**, *14*, 6424-6429.
- (11) Godínez, L. A.; Lin, J.; Muñoz, M.; Coleman, A. W.; Rubin, S.; Parikh, A.; Zawodzinski, T. A. J.; Loveday, D.; Ferraris, J. P.; Kaifer, A. E., Multilayer Self-Assembly of Amphiphilic Cyclodextrin Hosts on Bare and Modified Gold Substrates: Controlling Aggregation Via Surface Modification. *Langmuir* **1998**, *14*, 137-144.
- (12) Nelles, G.; Weisser, M.; Back, R.; Wohlfart, P.; Wenz, G.; Mittler-Neher, S., Controlled Orientation of the Cyclodextrin Derivatives Immobilized on Gold Surfaces. *J. Am. Chem. Soc.* **1996**, *118*, 5039-5046.
- (13) Rojas, M. T.; Königer, R.; Stoddart, J. F.; Kaifer, A. E., Supported Monolayers Containing Preformed Binding Sites. Synthesis and Interfacial Binding Properties of a Thiolated β -Cyclodextrin Derivative. *J. Am. Chem. Soc.* **1995**, *117*, 336-343.
- (14) Chinwangso, P.; Jamison, A. C.; Lee, T. R., Multidentate Adsorbates for Self-Assembled Monolayer Films. *Accounts Chem. Res.* **2011**, *44*, 511-519.
- (15) Weidner, T.; Ballav, N.; Zharnikov, M.; Priebe, A.; Long, N. J.; Maurer, J.; Winter, R.; Rothenberger, A.; Fenske, D.; Rother, D.; Bruh, C.; Fink, H.; Siemeling, U., Dipodal Ferrocene-Based Adsorbate Molecules for Self-Assembled Monolayers on Gold. *Chem. Eur. J.* **2008**, *14*, 4346-4360.

- (16) Pranger, L.; Goldstein, A.; Tannenbaum, R., Competitive Self-Assembly of Symmetrical, Difunctional Molecules on Ambient Copper Surfaces. *Langmuir* **2005**, *21*, 5396-5404.
- (17) Lee, Y.; Morales, G. M.; Yu, L., Self-Assembled Monolayers of Isocyanides on Nickel Electrodes. *Angew. Chem. Int. Ed.* **2005**, *44*, 4228-4231.
- (18) Ko, C.-H.; Huang, M.-J.; Fu, M.-D.; Chen, C.-h., Superior Contact for Single-Molecule Conductance: Electronic Coupling of Thiolate and Isothiocyanate on Pt, Pd, and Au. *J. Am. Chem. Soc.* **2010**, *132*, 756-764.
- (19) Heimel, G.; Romaner, L.; Zojer, E.; Brédas, J.-L., Toward Control of the Metal-Organic Interfacial Electronic Structure in Molecular Electronics: A First-Principles Study on Self-Assembled Monolayers of π -Conjugated Molecules on Noble Metals. *Nano Lett.* **2007**, *7*, 932-940.
- (20) Troughton, E. B.; Bain, C. D.; Whitesides, G. M., Monolayer Films Prepared by Spontaneous Self-Assembly of Symmetrical and Unsymmetrical Dialkyl Sulfides from Solution onto Gold Substrates: Structure, Properties, and Reactivity of Constituent Functional Groups. *Langmuir* **1988**, *4*, 365-385.
- (21) Angelici, R. J.; Lazar, M., Isocyanide Ligands Adsorbed on Metal Surfaces: Applications in Catalysis, Nanochemistry, and Molecular Electronics. *Inorg. Chem.* **2008**, *47*, 9155-9165.
- (22) Kang, H.; Noh, J.; Ganbold, E.-O.; Uuriintuya, D.; Gong, M.-S.; Oh, J. J.; Joo, S.-W., Adsorption Changes of Cyclohexyl Isothiocyanate on Gold Surfaces. *J. Colloid. Interf. Sci.* **2009**, *336*, 648-653.
- (23) Steiner, U. B.; Caseri, W. R.; Suter, U. W., Adsorption of Alkanenitriles and Alkanedinitriles on Gold and Copper. *Langmuir* **1992**, *8*, 2771-2777.
- (24) Rahman, K. M. A.; Durning, C. J.; Turro, N. J.; Tomalia, D. A., Adsorption of Poly(Amidoamine) Dendrimers on Gold. *Langmuir* **2000**, *16*, 10154-10160.
- (25) Yu, Y.; Dubey, M.; Bernasek, S. L.; Dismukes, G. C., Self-Assembled Monolayer of Organic Iodine on a Au Surface for Attachment of Redox-Active Metal Clusters. *Langmuir* **2007**, *23*, 8257-8263.
- (26) Hirayama, M.; Caseri, W. R.; Suter, U. W., Reaction of Long-Chain Iodoalkanes with Gold Surfaces. *J. Colloid Interface Sci.* **1998**, *202*, 167-172.
- (27) Ashton, P. R.; Königer, R.; Stoddart, J. F., Amino Acid Derivatives of β -Cyclodextrin. *J. Org. Chem.* **1996**, *61*, 903-908.
- (28) Baer, H. H.; Shen, Y.; Santoyo González, F.; Vargas Berenguel, A.; Isac García, J., Synthesis of a Cycloheptaose Consisting of (1-4)-Linked 7-Amino-6,7-Dideoxy- α -D-Gluco-Heptopyranosyl Units: A New Analog of Cyclomaltoheptaose. *Carbohydr. Res.* **1992**, *235*, 129-139.
- (29) García Fernández, J. M.; Ortiz Mellet, C.; Jiménez Blanco, J. M.; Fuentes Mota, J.; Gabelle, A.; Coste-Sarguet, A.; Defaye, J., Isothiocyanates and Cyclic Thiocarbamates of α, α' -Trehalose, Sucrose, and Cyclomaltooligosaccharides. *Carbohydr. Res.* **1995**, *268*, 57-71.
- (30) Mascetti, J.; Castano, S.; Cavagnat, D.; Desbat, B., Organization of β -Cyclodextrin under Pure Cholesterol, Dmpc, or Dmpg and Mixed Cholesterol/Phospholipid Monolayers. *Langmuir* **2008**, *24*, 9616-9622.

- (31) Frey, B. L.; Corn, R. M.; Weibel, S. C., *Polarization-Modulation Approaches to Reflection-Absorption Spectroscopy in Handbook of Vibrational Spectroscopy*. John Wiley and Sons, Ltd.: Chichester, UK, **2001**; Vol. 2.
- (32) Frasconi, M.; Mazzei, F., Electrochemical and Surface Plasmon Resonance Characterization of β -Cyclodextrin-Based Self-Assembled Monolayers and Evaluation of Their Inclusion Complexes with Glucocorticoids. *Nanotechnology* **2009**, *20*, 285502.
- (33) DeBono, R. F.; Loucks, G. D.; Manna, D. D.; Krull, U. J., Self-Assembly of Short and Long-Chain N-Alkyl Thiols onto Gold Surfaces: A Real-Time Study Using Surface Plasmon Resonance Techniques. *Can. J. Chem.* **1996**, *74*, 677-688.
- (34) Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. E. D., Spontaneously Organized Molecular Assemblies. 4. Structural Characterization of N-Alkyl Thiol Monolayers on Gold by Optical Ellipsometry, Infrared Spectroscopy, and Electrochemistry. *J. Am. Chem. Soc.* **1987**, *109*, 3559-3568.
- (35) An, Q.; Li, G.; Tao, C.; Li, Y.; Wu, Y.; Zhang, W., A General and Efficient Method to Form Self-Assembled Cucurbit[N]uril Monolayers on Gold Surfaces. *Chem. Commun.* **2008**, 1989-1991.
- (36) Graf, N.; Yegen, E.; Gross, T.; Lippitz, A.; Weigel, W.; Krakert, S.; Terfort, A.; Unger, W. E. S., Xps and Nexafs Studies of Aliphatic and Aromatic Amine Species on Functionalized Surfaces. *Surf. Sci.* **2009**, *603*, 2849-2860.
- (37) Xu, C.; Sun, L.; Kepley, L. J.; Crooks, R. M., Molecular Interactions between Organized, Surface-Confined Monolayers and Vapor-Phase Probe Molecules. 6. In-Situ Ftir External Reflectance Spectroscopy of Monolayer Adsorption and Reaction Chemistry. *Anal. Chem.* **1993**, *65*, 2102-2107.
- (38) Ujihara, M.; Imae, T., Adsorption Behaviors of Poly(Amido Amine) Dendrimers with an Azacrown Core and Long Alkyl Chain Spacers on Solid Substrates. *J. Colloid. Interf. Sci.* **2006**, *293*, 333-341.
- (39) Ito, M.; Toyoko, I.; Aoi, K.; Tsutsumiuchi, K.; Noda, H.; Okada, M., In Situ Investigation of Adlayer Formation and Adsorption Kinetics of Amphiphilic Surface-Block Dendrimers on Solid Substrates. *Langmuir* **2002**, *18*, 9757-9764.
- (40) Schierbaum, K. D.; Weiss, T.; Thoden van Velzen, E. U.; Engbersen, J. F. J.; Reinhoudt, D. N.; Göpel, W., Molecular Recognition by Self-Assembled Monolayers of Cavitand Receptors. *Science* **1994**, *265*, 1413-1415.
- (41) Ontko, A. C.; Angelici, R. J., Studies of the Adsorption of Bi- and Tridentate Isocyanides on Gold Powder. *Langmuir* **1998**, *14*, 3071-3078.
- (42) Sexton, B. A.; Avery, N. R., Coordination of Acetonitrile (CH_3CN) to Platinum(111): Evidence for an $\text{H}^2(\text{C},\text{N})$ Species. *Surf. Sci.* **1983**, *129*, 21-36.
- (43) Liu, W.; Zhang, Y.; Gao, X., Interfacial Supramolecular Self-Assembled Monolayers of C_{60} by Thiolated β -Cyclodextrin on Gold Surfaces Via Monoanionic C_{60} . *J. Am. Chem. Soc.* **2007**, *129*, 4973-4980.

- (44) Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J. A., Novel Potent Organoselenium Compounds as Cytotoxic Agents in Prostate Cancer Cells. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6853-6859.
- (45) Karginov, V. A.; Yohannes, A.; Robinson, T. M.; Fahmi, N. E.; Alibek, K.; Hecht, S. M., β -Cyclodextrin Derivatives That Inhibit Anthrax Lethal Toxin. *Bioorg. Med. Chem.* **2006**, *14*, 33-40.
- (46) Takeo, K.; Uemura, K.; Mitoh, H., Derivatives of α -Cyclodextrin and the Synthesis of 6-*O*- α -D-Glucopyranosyl- α -Cyclodextrin. *J. Carbohydr. Chem.* **1988**, *7*, 293-304.
- (47) Boger, J.; Corcoran, R. J.; Lehn, J.-M., Cyclodextrin Chemistry. Selective Modification of All Primary Hydroxyl Groups of α - and β -Cyclodextrins. *Helv. Chim. Acta* **1978**, *61*, 2190-2218.
- (48) Lenferink, A. T. M.; Kooyman, R. P. H.; Greve, J., An Improved Optical Method for Surface Plasmon Resonance Experiments. *Sens. Actuators B* **1991**, *3*, 261-265.
- (49) Knoll, W. Winspall Software V 3.02. <http://www.mpip-mainz.mpg.de/groups/knoll/software>.
- (50) Handbook of Chemistry and Physics. 93rd ed.; Haynes, W. M., Ed. CRC Press: **2013**.

Chapter 4

Electron transfer rates in host-guest assemblies at β -cyclodextrin monolayers

The effect of the distance between a β -cyclodextrin (β -CD) host core and a conductive substrate on the electron-transfer rate of complexed guests as well as of free-diffusing electrochemically active probes has been studied. First we have evaluated a set of short-tethered β -CD adsorbates bearing different anchoring groups in order to get a reliable platform for the study of short-distance electron transfer. An electrochemically active trivalent guest was immobilized on these host monolayers in a selective and reversible manner, providing information about the packing density. Iodine- and nitrile-functionalized β -CD monolayers gave coverages close to the maximum packing density. Electron transfer in the presence of $\text{Fe}(\text{CN})_6^{3-/4-}$ studied by impedance spectroscopy revealed that the electron transfer of the diffusing probe was 3 orders of magnitude faster than when the β -CD cores were separated from the surface by undecyl chains. When an electrochemically active guest was immobilized on the surface, electron-transfer rate measurements by cyclic voltammetry and capacitance spectroscopy showed differences of up to a factor of 8 for different β -CD monolayers. These results suggest that increasing the distance between the β -CD core and the underlying conductive substrate leads to a diminishing of the electron-transfer rate.

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4.1 Introduction

Electron transfer processes¹ are of great importance in a broad range of fields encompassing biochemistry²⁻³ and nanoelectronics.⁴ The understanding of mechanisms involved in such processes can unveil fundamental information which is of relevance in, among others, the performance of sensors,⁵ information storage devices⁶ or artificial photosynthesis.⁷⁻⁸ Monolayers on solid substrates offer a versatile platform for the study of such processes because most of the critical parameters can be tuned by the design of the interfacial architecture, i.e. length and chemical nature of the bridge.⁹ Typically, covalent anchoring of electroactive groups has been employed because homogeneous monolayers and a high degree of spatial control can be achieved.¹⁰ Ferrocene bound to the surface, normally through alkyl chains, has been broadly used as an electroactive self-assembled monolayer in order to study and to control electron transfer processes in a variety of surfaces.¹¹⁻¹⁶ However, covalent assemblies can only partially model the complex dynamics observed in systems of high interest, such as biological machinery, where supramolecular interactions are found ubiquitously. For instance, supramolecular organization of photosynthetic reaction centers enables electron transfer processes with a quantum yield close to unity.¹⁷

The design of building blocks that can be assembled in a supramolecular fashion to build up electroactive architectures is a way to mimic biological systems. Supramolecular complexes have attracted attention as potential constituents of molecular electronics.¹⁸⁻²⁰ A key point in this sense is the modularity, which allows structural modifications through non-covalent interactions in a selective, but reversible manner. This can be exquisitely tuned by the use of multivalency,²¹ which has been used for the development of molecular printboards onto which electroactive dendrimers can be reversibly anchored.²²⁻²⁵ However, while the distance-dependent electron transfer rate in surface-confined probes has been broadly addressed in covalently built-up monolayers, similar effects in host-guest structures have been generally disregarded. This is especially important if we take into account that when an electrochemically active guest is recognized, an additional timescale, involving host-guest complexation, is coupled with the electron transfer process.²⁶⁻²⁷ Such host-guest complexation rates have been studied even at the single molecule level.²⁸⁻³⁰ The surface architecture could thus become the rate limiting step for a variety of

processes, i.e. by limiting electron transfer rates.³¹ Therefore, the design of platforms, which require a reliable multivalent supramolecular host-guest assembly and efficient electron transfer, needs a careful evaluation of the boundaries in which such a limitation may occur.

The aim of the study in this chapter is to evaluate the effect of the distance of the β -cyclodextrin (β -CD) cavity on the electron transfer rate when the host core is positioned in close proximity to or distanced from the metallic substrate. For the latter, we used long alkyl thioether tethers³² as a reference because of their known good packing capabilities. On the other hand, assembling well-packed homogeneous β -CD monolayers close to the surface is not trivial because classical thiol-functionalized β -CDs in general yield limited coverages and order.³³⁻³⁴ First, we evaluate the packing of several of the β -CD adsorbates described in Chapter 3. This is critical because the presence of defects on the structure will influence the electron transfer capabilities. Second, electron transfer processes are investigated by using diffusing and supramolecularly anchored electrochemical probes.

4.2 Results and discussion

We have selected an assortment of β -CD-based adsorbates described in Chapter 3. Adsorbates **1-4** feature short tethers on the cyclosaccharidic core and contain iodine (**1**, I), isothiocyanate (**2**, NCS), nitrile (**3**, CN) and methyl sulfide (**4**, SMe) anchoring groups. Adsorbate **5** ($C_{11}SC_{12}$) is constituted by thioether anchoring groups embedded in a long alkylic chain (Chart 4.1).

Depending on the anchoring group employed, different layer architectures such as depicted in Figure 4.1a-c are considered. For short tethers, the varying affinities to the gold surface will translate into different host coverages on the surfaces, where low (Figure 4.1a) or high (Figure 4.1b) densities of hosts could be possible. On the other hand, a high density of adsorbates has been shown for compound **5** ($C_{11}SC_{12}$),³² where the β -CD core is separated from the electrode through alkyl chains (Figure 4.1c). It should be noted that lattice order of the CD cavities is difficult to prove and that the cartoons depicted in Figure 4.1 are meant to indicate only differences in density (and concomitantly in the fraction of exposed bare gold area) and in the distance of the cavity from the surface. For derivatives **1-4**, a detailed analysis described in Chapter 3 has confirmed multipodal binding, and therefore oriented immobilization, of the host cavities, albeit with different packing densities and layer quality.³⁵ The packing order of

the monolayer has not been observed for these monolayers. In contrast, monolayers of **5** have been shown to be well-packed, and a very similar methylated derivative has been shown by AFM to pack in an ordered fashion.³⁶

Chart 4.1: Chemical structures of β -CD-based adsorbates (**1-5**) and electrochemical guest probe **6** employed in this study.

Anchoring a ferrocene (Fc) derivative in a supramolecular fashion has a limited applicability because of a low binding constant to the β -CD cavity (e.g. ferrocenecarboxylate is complexed by β -CD with a binding constant of $K_a = 2.1 \times 10^3 \text{ M}^{-1}$),³⁷ which in combination with the low solubility of ferrocene derivatives, allows only partial coverage. To enhance the stability of the supramolecular complexes on the surface, we prepared trivalent guest **6** (Chart 4.1), which consists of two strong-binding adamantane moieties linked through tetra(ethylene glycol) spacers to a benzene ring functionalized with a ferrocene moiety. It is known that the bis-adamantane motif possesses sufficient length to bind to two different β -CD hosts (Figure 4.1d),³⁸ therefore allowing divalent binding on β -CD monolayers at micromolar concentrations, even in the presence of β -CD in solution. Our evaluation of space-filling models show that the distance between the adamantane moieties can range from 1.7 nm up to 3.3 nm, while the ferrocene moiety is less flexible, with the distance to the adamantane moieties increasing up to 2 nm. Since the diameter of β -CD is ca 1.53 nm, guest **6** could in principle bind trivalently (Figure 4.1e) when the host on the surface is

organized in a close-packed structure. Yet monolayers of **5** have been shown to pack with a periodicity of 2.1 nm,³⁶ which could be sterically problematic for binding the Fc moiety together with the adamantane moieties.

Figure 4.1: Different monolayers employed in this chapter: a) short tethered, low densely assembled β -CDs; b) short tethered, high densely assembled β -CDs; c) long tethered, high densely assembled β -CDs; d) monolayers with divalent binding or (e) monolayers with trivalent binding of guest **6**.

SPR titrations with increasing concentrations of guest **6** in the presence of 1 mM β -CD were carried out. SPR sensograms showed guest binding that was reversible after rinsing with aqueous 1 mM β -CD in the absence of the guest (a titration example is shown in Figure 4.2a), thus regenerating the surface. Monolayers made from **1** (I), **2** (NCS) and **5** ($C_{11}SC_{12}$) were studied in this way (Figure 4.2b). As is evident from the graphs, nonspecific adsorption was observed for **1** (I) and **2** (NCS) at high guest concentrations, which was also evidenced by the incomplete removal of the guest by competition with 1 mM native β -CD. In contrast, guest binding remained fully reversible for adsorbate **5**.

Figure 4.2: a) Titration of monolayers of **5** (C₁₁SC₁₂) with increasing concentrations of guest **6** in the presence of 1 mM β-CD (▲). In between guest concentration changes, the surface was rinsed with 1 mM β-CD (Δ). b) SPR titrations with guest **6** in the presence of 1 mM β-CD for monolayers prepared with adsorbates **1** (I, ▲), **2** (NCS, ■) and **5** (C₁₁SC₁₂, □). Dotted lines represent fittings to a thermodynamic multivalent model which considers the surface binding of ferrocene and adamantane moieties in competition with free host on solution.

A thermodynamic model for the binding of the trivalent guest to the surface in the presence of a competing guest in solution was elaborated on.³⁹ Briefly, this model contains the affinity constants of β-CD-ferrocene and β-CD-adamantane guests both in solution ($K_{i,FC,l}$, $K_{i,Ad,l}$) and on the surface ($K_{i,FC,s}$, $K_{i,Ad,s}$), as well as two effective concentration terms, accounting for the binding of ferrocene ($C_{eff,FC}$) and adamantane ($C_{eff,Ad}$) on the surface (for more detail, see Experimental section). The curves in Figure 4.2b very strongly resemble the binding of a divalent bisadamantyl calixarene to a β-CD monolayer of **5** described before.³⁸ Simulations of SPR sensograms for monolayers constructed with adsorbates **1** (I), **2** (NCS) and **5** (C₁₁SC₁₂) using equal affinities for binding of a guest (Ad or Fc) moiety in solution or to a surface β-CD site and using effective concentrations of 0.1 M showed predicted adsorbed masses that were too high when assuming trivalent binding to the surface. Proper fits were obtained only when assuming an order of magnitude lower affinity for Ad at the surface or an order of magnitude lower C_{eff} for binding of both the Ad and Fc units or when effective divalent binding was assumed ($K_{i,FC,s} < 10 \text{ M}^{-1}$ or $C_{eff,FC} < 1 \text{ mM}$). Of these options, the first is unlikely because of the way that the Ad group has been attached to the guest scaffold in comparison to earlier guest molecules used. The second could indicate a lower packing density, but earlier data on monolayers of **5** always gave C_{eff} values in the range of 0.1-0.4 M. Therefore, we conclude that

the Fc group is not contributing to the overall affinity of the complex with the β -CD monolayer, making the binding primarily divalent in nature, via both Ad groups. This is in line with the steric problems of the Fc group noted above. It does, however, not rule out that part of the Fc groups can show some interaction with neighboring empty β -CD sites, but only as a minor fraction (<30%).

Qualitatively, the binding of trivalent **6** to monolayers of adsorbates **1** (I), **2** (NCS) and **5** ($C_{11}SC_{12}$) as shown in Figure 4.2 indicates highly comparable binding, both in affinities and in amounts of adsorbed guests. The different apparent limiting SPR values could be attributed to differences in packing density, which would correspond to an order of **2** (NCS) > **5** ($C_{11}SC_{12}$) > **1** (I), but the higher value for **2** could also come from the higher apparent nonspecific adsorption. Overall, we conclude that the binding of **6** to the here studied β -CD monolayers is very similar and essentially divalent. This makes this guest an ideal candidate for probing the distance variation between the β -CD core and the substrate imposed by the different β -CD adsorbates and at the same time probing the effect of a guest, which can only transiently interact with a β -CD cavity, on its electron-transfer rate to the substrate.

Subsequently, self-assembly of guest **6** was probed electrochemically. Guest **6** was immobilized onto monolayers of **1-5**, after which cyclic voltammograms were measured in the absence of the guest in solution. As a representative example for short-tethered monolayers, Figure 4.3a shows a cyclic voltammogram of guest **6** bound to monolayers of **1** (I) which clearly reveals the presence of both oxidation and reduction peaks of the ferrocene/ferrocenium couple. The area of both peaks is comparable in a wide range of scan rates (up to 500 V/s), which indicates that the divalent adamantane anchor prevents the dissociation of the electroactive moiety from the surface, thus keeping the electroactive moiety in close proximity. This is in contrast with the observation of irreversible ferrocene oxidation on β -CD-modified electrodes after the dissociation from the host cavity.^{24, 26} After immersion of the guest-functionalized monolayer in an aqueous solution of 10 mM β -CD, the measured faradaic signal decreased greatly, suggesting that the binding is reversible upon competition with a host in solution, which agrees with the SPR experiments described above. Figure 4.3b shows the data for monolayers of the long-tethered **5** ($C_{11}SC_{12}$), which data resemble the observations for **1**. The current increases linearly with the scan rate, as shown for monolayers of **1** in Figure 4.3c,

which confirms that the redox process takes place at the surface of the electrode and no diffusion of the guest in solution is involved. This was observed for all monolayers described here. Table 4.1 summarizes the formal apparent potential, peak separation and full width at half maximum (FWHM) for the monolayers prepared at room temperature (**1**, I and **4**, SMe), 50 °C (**2**, NCS and **3**, CN) or 60 °C (**5**, C₁₁SC₁₂), which are the optimized conditions (*vide infra*).

Figure 4.3. Cyclic voltammograms at 0.1 V/s in 1 M NaClO₄, after functionalization with guest **6**; a) monolayers of **1** (I) before (solid line) and after (dashed line) incubating with 10 mM β-CD, and b) monolayers of adsorbate **5** (C₁₁SC₁₂) before (solid line) and after (dashed line) incubation with 10 mM β-CD. c) Current versus scan rate for monolayers of **1** (I) functionalized with guest **6**.

As a general trend, the apparent formal potential $E^{0'}$ measured in short tethered hosts is smaller value in comparison with the long tethered adsorbate **5** (C₁₁SC₁₂), and the peak separation at 0.1 V/s is below 59 mV, as expected for surface-confined species. Full width at half maximum (FWHM) values varied from 90 to 126 mV. Remarkably, the voltammogram for **5** (C₁₁SC₁₂) shows the characteristics for an ideal nernstian reaction,⁴⁰ with a small peak separation (5 mV) and a narrow FWHM (98 mV). The higher values of FWHM found in the case of guests adsorbed on monolayers of **2** (NCS) might indicate structural disorder of the host assembly, which results in a broader distribution of the formal potential.⁴¹ Indeed, XPS measurements of monolayers of **2** (NCS) revealed an

important fraction of unbound sulfur, and therefore higher levels of disorder in this monolayer (see Chapter 3).³⁵

Table 4.1: Apparent formal potential, peak separation, FWHM for anodic peak at 0.1 V/s in NaClO₄ for monolayers of guest **6** in monolayers prepared with compounds **1-5**. Potentials are referenced versus Ag/AgCl reference electrode.

Adsorbate	$E^{0'}$ (mV)	E_a-E_c (mV)	FWHM (mV)
1 (I)	496 $\pm$ 6	18 $\pm$ 2	107 $\pm$ 9
2 (NCS)	506 $\pm$ 4	55 $\pm$ 1	126 $\pm$ 12
3 (CN)	525 $\pm$ 6	21 $\pm$ 6	90 $\pm$ 8*
4 (SMe)	482 $\pm$ 8	18 $\pm$ 5	103 $\pm$ 16
5 (C ₁₁ SC ₁₂)	516 $\pm$ 6	5 $\pm$ 2	98 $\pm$ 2

*At room temperature.

We employed compound **6** as an electrochemical probe to quantify the coverage achieved by the host adsorbate series **1-5**. It has been reported that monolayer formation of macrocyclic, multidentate structures at elevated temperatures can improve the packing and increase the coverage.⁴² By increasing the desorption rate, only adsorbates with higher binding affinities remain on the surface, while structures showing poor binding affinities tend to experience desorption, leaving space for new binding events. This has resulted in an optimal adsorption temperature of 60 °C for adsorbate **5** (C₁₁SC₁₂).³⁶ After host monolayer formation, the adsorption of guest **6** was carried out at a concentration of 10 μ M guest in the absence of native β -CD by solubilization of **6** in MeOH and dilution in water (the fraction of MeOH v/v is less than 1%), in order to maximize the fraction of bound hosts. As rationalized above, we assume the binding to be divalent. We compared coverages for monolayers prepared at room temperature and at 50 °C by measuring (at room temperature) the peak areas of ferrocene in cyclic voltammograms at 0.1 V s⁻¹. The results are shown in Figure 4.4, where we include adsorbate **5** (C₁₁SC₁₂), adsorbed at 60 °C, as a reference.

Figure 4.4: Comparative coverage of adsorbates **1-5** by using the electrochemical probe **6**, assuming divalent binding of the guest. Different conditions of preparation were employed: gray bars denote preparation at room temperature, and white bars denote heated samples (50 °C for **1-4**, 60 °C for **5** ($C_{11}SC_{12}$)). The thick gray line at 45 pmol cm⁻² denotes maximum coverage determined for adsorbate **5** ($C_{11}SC_{12}$) based on the experimental lattice constant (2.06 nm).³⁶ Symbol* denotes statistical difference determined by Student's t-test ($p < 0.05$). Error margins indicate standard deviations based measurements of at least 3 independent samples

We observed that the achieved coverage depended both on the nature of the anchoring group as well as on the preparation procedure. The maximum coverage expected for adsorbate **5** ($C_{11}SC_{12}$), based on the experimental lattice length determined by AFM, is about 45 pmol cm⁻².³⁶ Reference compound **5** ($C_{11}SC_{12}$), assembled at 60 °C, showed high packing densities, even higher (about 30%) than the maximum expected. However, this calculation does not take into account the surface roughness, which might partially explain this over-estimation. Coverages of **1-4** assembled at room temperature varied from 23 to 53 pmol cm⁻² in comparison to the maximum packing of **5** ($C_{11}SC_{12}$); these values range from quasi-highly packed to half-packing coverages. An increase in the guest coverage on the surface was measured for **2** (NCS) and **3** (CN) when using an elevated assembly temperature. No significant change was found for **1** (I), and a decrease in the coverage was observed for **4** (SMe). This confirms that the good binding properties of **5** ($C_{11}SC_{12}$) are evidently imposed by the alkyl chains, since compound **4** (SMe), based also on the thioether motif, desorbs upon heating, suggesting a rather low binding affinity. Addition structural parameters, such as heterogeneous orientation of the saccharidic core, could induce a source

of error in our quantification of coverage. In Chapter 3, it was described that adsorbate **2** (NCS) shows about 60% of bound sulfur to the surface,³⁵ which indicates a disordered structure, combined with a higher film thickness than for the rest of the adsorbates. This might also suggest that tilted adsorption is involved; therefore, the binding ability of guest **5** can be strongly influenced by an irregular host distribution. We therefore conclude that adsorbates **1** (I) and **3** (CN) gave the more reliable short-tethered monolayers.

Electron transfer rates for diffusing and anchored probes. Electrochemical experiments were carried out at the optimized monolayer preparation conditions: at increased temperatures for **2** (NCS), **3** (CN) and **5** (C₁₁SC₁₂) and room temperature for **1** (I) and **4** (SMe). We set out to examine how the passivation of the surface by the assembly of a host monolayer influences the electron transfer rate of a freely diffusing electrochemical probe, such as ferrocene or the [Fe(CN)₆]^{3-/4-} redox couple. For ferrocene, the heterogeneous rate of electron transfer is determined using the Nicholson method,⁴³ for quasi-irreversible electron transfer dynamics. The peak separation in cyclic voltammograms can be related to the kinetic parameter Ψ from which k_0 can be calculated using Equation 4.1:

$$\Psi = \left(\frac{D_O}{D_R}\right)^{\frac{\alpha}{2}} \bigg/ \sqrt{\frac{\pi \nu F D_O}{RT}} \quad (\text{Equation 4.1})$$

Where D_O and D_R are the diffusion coefficients for the oxidized and reduced species, respectively, α is the electron-transfer coefficient, F is the Faraday constant, ν is the scan rate, R is the gas constant and T is the temperature.

When using 126 μM 1,1'-ferrocene-dimethanol (FDM) in aqueous 1 M NaClO₄ as the electrolyte, the peak separations were recorded for scan rates up to 200 V/s at the various β -CD adsorbate layers from which the kinetic data were extracted (Table 4.2). The results show that the heterogeneous electron transfer kinetics for compounds **1-4** are somewhat higher than for compound **5** (C₁₁SC₁₂). This low difference is attributed to FDM accessing the cavity. On the other hand, the [Fe(CN)₆]^{3-/4-} redox couple cannot diffuse to the electrode through densely packed monolayers since it is larger than the β -CD core. Cyclic voltammograms were recorded between 0.1 and 5 V/s and showed significant peak separation in this region for compounds **1-4**, indicative of quasi-irreversible electron transfer. Adsorbate **1** showed lower values than the others, which is in line with the better observed packing whereas **2** showed the highest values and a high

deviation indicative of poor (and uncontrolled) packing. The peak separations recorded for **5** (C₁₁SC₁₂) for these scan rates were too large ($\Delta E_p > 212$ mV) meaning that the electron transfer is irreversible and no kinetic data could be extracted using this method.

Table 4.2: Comparison of electron transfer rate on monolayers prepared with 1-5.

Adsorbate	k_0 (cm/s) ^a	k_0 (10 ⁻³ cm/s) ^b	$k_{ET,s}$ (10 ⁻³ cm/s) ^c
1 (I)	0.088 ± 0.013	2.42 ± 1.09	25.4 ± 29.3
2 (NCS)	0.11 ± 0.02	12.4 ± 11.9	19.6 ± 11.7
3 (CN)	0.15 ± 0.02	10.2 ± 6.5	20.8 ± 18.6
4 (SMe)	0.14 ± 0.04	11.9 ± 3.8	100 ± 59.3
5 (C ₁₁ SC ₁₂)	0.062 ± 0.011	-	0.0012 ^d

^a In the presence of FDM, using the Nicholson method. ^b For the [Fe(CN)₆]^{3-/4-} redox couple at 1 mM concentration using the Nicholson method. ^c For the [Fe(CN)₆]^{3-/4-} redox couple at 1 mM concentration using our previous electrochemical impedance spectroscopy measurements analyzed as for the determination of $k_{ET,s}$.³⁵ ^d Calculated from the literature.³⁶

In order to make a better comparison with compound **5** (C₁₁SC₁₂), $k_{ET,s}$ values were calculated from EIS measurements. EIS data was fitted a Randles equivalent circuit and the $k_{ET,s}$ values were obtained from Equation 4.2:⁴⁴

$$k_{ET,s} = \frac{RT}{n^2 F^2 A R_{ct} C} \quad (\text{Equation 4.2})$$

where R and T are the ideal gas constant and the temperature, n is the number of electrons transferred, F is the Faraday constant, A the surface area of the electrode, R_{ct} the electron transfer resistance obtained from the fit, and C the concentration of [Fe(CN)₆]^{3-/4-}. Values for the electron transfer rate obtained in this way are shown in Table 4.2, the value obtained for **5** (C₁₁SC₁₂) was calculated from the $R_{ct} = 49.2$ kΩ.³⁶

These measurements clearly confirmed that the electron transfer rate is decreased by three orders of magnitude, when the β-CD core is distanced from the surface through long alkyl chains. Complexing the cavities with an anionic guest (1-anilinonaphthalene-8-sulfonic acid, 1,8-ANS) gave rise to an increase of the resistance of the monolayer determined by impedance spectroscopy,⁴⁵ as

shown in Figure 4.5. The magnitude of the increase depended on the coverage of the host, where clear differences are observed when comparing good (**1**, I) or poorly (**4**, SMe) packed monolayers, which is in agreement with the $k_{ET,S}$ trend described above. Adsorbates **1** (I), **2** (NCS) and **3** (CN) achieved comparable coverages, which translates in similar electron transfer rates, while adsorbate **4** (SMe), which gives poorly packed monolayers and therefore a higher fraction of pinholes, showed a higher electron transfer rate.

Figure 4.5: Resistance of the monolayers determined by impedance spectroscopy at increasing concentrations of 1,8-ANS for monolayers of **1** (I) (black dots) and **4** (SMe) (white boxes). Experiments were carried out in the presence of 1 mM $\text{Fe}(\text{CN})_6^{3/4-}$ in 0.1 M K_2SO_4 .

We took advantage of the high binding affinity of guest **6** to carry out electron-transfer measurements of a probe strongly anchored to the surface. In this case, diffusion from the solution to the surface is not involved. We used cyclic voltammetry at different scan rates to obtain electron-transfer rates of the ferrocene moiety, as described by Laviron.⁴⁶ To minimize the possibility of having physisorbed material, we immersed monolayers of adsorbates **1-5** in 10 μM solutions of guest **6** in the presence 1 mM of β -CD, and the functionalized substrates were rinsed and measured in 1 M aqueous NaClO_4 . Under these conditions we expect about half the coverage as under the conditions used above in the absence of β -CD in solution, so approximately half of the β -CD sites remain unbound. Figure 4.6a shows the peak separation at different scan rates of guest **6** immobilized on monolayers of **1** (I) and **5** ($\text{C}_{11}\text{SC}_{12}$). While increasing the scan rate, we found higher peak separations for monolayers of **2** (NCS) and **5** ($\text{C}_{11}\text{SC}_{12}$), which indicates slower electron-transfer rates. The increase of anodic and cathodic peak potentials with scan rate is only slightly asymmetric in

most of the range (<200 V/s), which suggest that the kinetics for ferrocene oxidation and reduction are comparable. For long-tethered adsorbate **5** ($C_{11}SC_{12}$), at sufficiently high scan rates (> 200 V s^{-1}), the increase of cathodic peak potential with scan rate was greater than that of the anodic peak potential, and in addition, the peak corresponding to the reduction of ferrocene broadens.

Figure 4.6: a) Cathodic and anodic currents of guest **6** in monolayers of **1** (I, red circles) and **5** ($C_{11}SC_{12}$, blue squares) at different scan rates (in V/s) in 1 M $NaClO_4$ (absence of guest in solution). b) Electron transfer rate of guest **6** obtained by the Laviron method for monolayers prepared with short tethers: **1** (I), **2** (NCS), **3** (CN), **4** (SMe) and long tethers **5** ($C_{11}SC_{12}$). Different points for each adsorbate corresponds to different substrates.

By application of the Laviron formalism, we obtained the electron transfer rates (k_{ET}) and the transfer coefficients. An average transfer coefficient of 0.41 ± 0.07 (short tethered, **1-4**) and 0.33 ± 0.11 (**5**, $C_{11}SC_{12}$) confirmed a little asymmetry in the activation energy for the redox process which is more pronounced in long tethered adsorbates. Electron transfer rates were compared to the coverage of guest **6**, since it is known that differences in coverage might induce differences in the electron transfer rate due to the different orientation of ferrocene (Figure 4.6b).⁴⁷ We observed that in general the electron transfer rate for the long-tethered adsorbate **5** ($C_{11}SC_{12}$) is in average of 870 s^{-1} while short-tethered monolayers **1-4** gave average values of 1124 , 715 and 984 s^{-1} for **1** (I), **2** (NCS) and **3** (CN) respectively), which is in the range of the values found for ferrocene anchored to a distance of about 12 methylene units to the surface.⁴⁸ Adsorbate **4** (SMe) gave higher k_{ET} values (1500 s^{-1}), although this measurement was not highly reliable because of the weakness of the ferrocene signal in this case. In addition the presence of uncovered host areas might facilitate the electron transfer. When disregarding adsorbate **2** (NCS), the trend in electron kinetics is

similar to the one found for the ferrocyanide couple described above. It is worth to notice in this respect: i) there is a trend with guest coverage in the k_{ET} values if we compare short-tethered adsorbates **1-4** (since all of the monolayers were immersed in the same solution, differences in surface coverage of **6** could be attributed to differences in the packing), ii) this difference is lost in some substrates for long-tethered **5** ($C_{11}SC_{12}$), and iii) the magnitude of the difference is relatively low, **2** (NCS) being the adsorbate showing the lowest electron transfer rates. However, data obtained by the Laviron method suffer from some drawbacks, such as nonfaradaic noise or an iR drop. Additionally, poor definition of the peaks at higher scan rates makes a reliable analysis difficult.

Table 4.3: Fitted equivalent circuits of impedance data shown in Figure 4.9a-c. Percentages in parentheses represent the error of the fitting.

	At formal potential		Outside redox window	
Adsorbate	R_{SAM} (k Ω)	C_{SAM} (μ F)	R_{SAM} (k Ω)	C_{SAM} (μ F)
1 (I)	5.9 (9%)	6.8 (0.8%)	14 (28%)	2 (2.5%)
3 (CN)	0.092 (80%)	6 (38%)	5.6 (12%)	3.7 (0.4%)
5 ($C_{11}SC_{12}$)	0.89 (9.2%)	2.3 (1.6%)	2.3 (232%)	0.9 (22%)

Figure 4.7: Equivalent circuits used in the fitting of impedance spectra at the formal potentials; a) used for short tethered adsorbates **1** and **3**; b) used for long tethered adsorbate **5**.

We employed capacitive spectroscopy⁴⁹ in order to solve the limitations of the Laviron method. For molecules directly adsorbed to the surface, the imaginary component C'' of the complex capacitance $C^*(\omega)$ measured within the redox potential window contains contributions from the parasitic nonfaradaic and faradaic charging of the monolayer. The nonfaradaic contribution can be identified by measuring outside of the redox window and later subtracted to C'' .

This corrected C'' function is free from effects from SAM polarization and iR drops and therefore contains solely information from characteristic frequencies of the electron transfer of the redox charging⁴⁹ by assuming that the resistance of the monolayer does not change importantly with the applied potential. Fittings of the impedance data taken in and out of the redox window to an equivalent circuit revealed small changes of the capacitance of the monolayer (Table 4.3 and Figure 4.7) and might include a source of error. However, these values as well as the changes in resistance are smaller than observed for the adsorption of dendrimers.²⁴

As an example, Figure 4.8 shows the transition from non-faradaic (300 mV vs Ag/AgCl) to mixed faradaic and non-faradaic charging (508 mV vs Ag/AgCl) of guest **6** complexed on monolayers of **5** ($C_{11}SC_{12}$) when the potential window is moved. Figure 4.9a-c shows plots for raw, parasitic and parasitic-corrected C'' for host-guest complexes of **6** in monolayers of **1** (I), **3** (CN) and **5** ($C_{11}SC_{12}$).

Figure 4.8: Capacitance spectroscopy measurements of guest **6** adsorbed on monolayers of **5** ($C_{11}SC_{12}$) at different potentials, ranging from outside de redox potential window (300 mV vs Ag/AgCl) to the redox formal potential (508 mV vs Ag/AgCl).

The observed electron-transfer rates decrease in the order k_{ET} in **1** (I) > **3** (CN) > **5** ($C_{11}SC_{12}$), the latter being up to 6-8 times slower. We notice that these lower values are comparable to those obtained for monolayers of ferroceneundecanethiol by the same technique.⁵⁰ This is an indication of a well-packed monolayer for **5**. In addition, the magnitude of C'' was higher than in short-tethered adsorbates, probably due to a higher guest coverage. In general, the ratio between the faradaic and non-faradaic contributions was less than one

order of magnitude, which might suggest poor electronic coupling, as observed for metalloproteins adsorbed on monolayers.⁵⁰ The differences observed between the values obtained by the Laviron formalism and the capacitance method have been attributed to polarization effects that cannot be corrected in the former.⁴⁹

Figure 4.9: Electron transfer rates determined by capacitance spectroscopy for the electron transfer of guest **6** in monolayers of a) **1** (I); b) **3** (CN); c) **5** ($\text{C}_{11}\text{SC}_{12}$). Data was collected at the redox potential (blue lines), outside the redox potential (red lines) and the subtraction gives the parasitic corrected C'' (black line). The electron transfer rate is indicated within the graph. d) k_{ET} measurements for monolayers of **1** (I, red triangles), **3** (CN, white circles) and **5** ($\text{C}_{11}\text{SC}_{12}$, blue squares).

Several reports have shown a decrease of the electron transfer rates of ferrocene alkyl chains upon increase of the surface coverage. At higher alkyl ferrocene surface densities, the chains tend to stand up, increasing the distance between the surface and the ferrocene moieties.⁵¹ We varied the surface coverage in order to compare electron-transfer rates at different surface densities. Immersion in solutions were performed for guest **6** at 1, 3, 7 and 10

μM for **1** (I) monolayers and 1, 5 and 10 μM for **3** (CN) and **5** ($\text{C}_{11}\text{SC}_{12}$). Figure 4.9d shows a set of measurements plotted versus the corresponding ferrocene coverage.

We observed a trend of increased electron transfer rates at lower guest coverages, and when coverages are comparable in different adsorbates, the trend is similar to the mentioned above (k_{ET} in **1** (I) > **3** (CN) > **5** ($\text{C}_{11}\text{SC}_{12}$)), which is roughly coherent with our observations using the Laviron method. A possible explanation is that higher host coverages (reflected in higher guest coverage) decrease the number of surface defects, therefore reducing the fast electron transfer through pinholes. This might explain that host samples showing similar guest coverages can present different electron-transfer rates. Additionally, the increase in guest packing might require that the Fc moieties orient in a vertical fashion rather than horizontally, therefore increasing the distance between the surface and the probe, as reported.⁵¹

The small differences observed in the electron transfer rates of adsorbed guest **6** when comparing short and long-tethered adsorbates with both methods employed is in principle surprising. When a redox moiety is anchored to a surface separated at a distance d , which is sufficiently short to allow tunneling, the dependence of the electron-transfer rate k_{ET} on d is given by:

$$k_{\text{ET}} = k_{\text{ET},0} \exp(-\beta d) \quad (\text{Equation 4.3})$$

where $k_{\text{ET},0}$ includes the effect of the covalent connection on the electronic coupling and β is the exponential decay constant, typically about 10 nm^{-1} for saturated alkyl chains.⁵² Therefore the ratio between short-tethered and long-tethered electron transfer rates ($k_{\text{ET,long}}/k_{\text{ET,short}}$) will be proportional to $\exp(\beta \cdot (d_{\text{short}} - d_{\text{long}}))$, which predicts orders of magnitude of difference if d_{short} is importantly different from d_{long} . Therefore, although distances imposed by the host adsorbate have an influence on the electron-transfer rate, they do not provide a complete picture of the charge transport across these host-guest systems. In this sense, both host and guest coverage as well as other structural or chemical features of the interfacial layer, for example, the disorder observed for **2** (NCS) host layers, might play an important role as well. Moreover, as described above, guest **6** is primarily bound in a divalent fashion, whereby the Fc moiety is, for most of the time, not close to the host layer. Thus, we can expect that the divalent binding of **6**, with the concomitantly larger distances of the Fc moiety to the surface possibly mitigated by transient moments of

interaction between the Fc moiety and the host cavities, diminishes the relative contribution of the host layer thickness to the observed differences in electron-transfer rates.

4.3 Conclusions

We have measured electron transfer rates of probes both in solution and immobilized on monolayers of β -CD hosts bound directly to the gold surface or spaced through an alkyl chain layer. For freely diffusing probes, we observed a decrease in the electron-transfer rate for long-tethered adsorbates of up to 3 orders of magnitude. We have successfully immobilized a trivalent, bis-Ad/mono-Fc guest on short- and long-tethered β -CD adsorbates by host-guest interactions. Thermodynamic modeling suggests that the binding of this guest is predominantly divalent via the stronger-binding Ad moieties. Guest binding is reversible upon competition with free host in solution, which suggests specific binding of the multivalent probe to the surface hosts. Electron-transfer rate measurements revealed faster kinetics in comparison to freely diffusing probes and a less pronounced difference between short- and long-tethered structures. While the first could be attributed to a better electronic coupling between the probe and substrate, the second might be due to the variation in the packing density (providing the presence of pinholes with facilitated electron-transfer rates) and the dynamic nature of the assembly, which influences the average position of the electrochemical probe relative to the surface and thus affects the transfer rates. This ensemble of results shows that various structural features of surface host-guest assemblies determine the temporal frame where the electron transfer takes place. These findings are of importance in the fields of electronic sensing and molecular electronics because device optimization relies on strategies to improve the kinetics of the electron transfer, which, to the best of our knowledge, has not been addressed before in multivalent host-guest systems. The dynamic nature of host-guest interactions might be an adequate tool for the future development of architectures with regulated (i.e., externally switched or induced by conditions) electron-transfer rates by modulating the affinity of the host-guest binding.

4.4 Experimental section

Materials. Reagents and solvents were purchased from commercial sources and used without further purification unless stated otherwise. ^1H and ^{13}C NMR were recorded at 400 and 100.6 MHz, respectively. 2D COSY and HMQC experiments

were used to assist on NMR peak assignments. Thin-layer chromatography (TLC) was carried out on aluminum sheets, with visualization by UV light. Column chromatography was carried out on silica gel (230-400 mesh). ESI-MS spectra were obtained for samples dissolved in dichloromethane (DCM)-MeOH, at low μM concentrations. Adsorbates **1-4**³⁵ and **5**³⁶ were prepared by reported procedures.

We additionally developed an alternative procedure for the preparation of **5** based on click thio-ene chemistry: Heptakis[6-deoxy-6-[undec-10-enamido]]cyclomaltoheptaose⁵³ was prepared as described in the literature and further purified by column chromatography in DCM-MeOH 9:1 $\rightarrow$ 5:1 to give the heptasubstituted β -CD alkene in 42 % yield. A solution of this derivative (185 mg, 0.08 mmol), AIBN (45 mg, 0.28 mmol, 0.5 eq.) and dodecanethiol (392 mg, 1.9 mmol, 3.5 eq.) in dry, degassed THF (5 mL) was refluxed overnight under argon. The solvent was evaporated and the residue was dissolved in a little portion of DCM, then slowly dropped over acetone (50 mL) to give a white precipitate. The precipitate was filtered off and washed thoroughly with acetone, then dried to give a white solid (220 mg, 74%). ¹H NMR spectra analysis is in agreement with one reported in the literature (Figure 4.10). Before monolayer preparation, the product was further purified by column chromatography in DCM-MeOH 20:1 $\rightarrow$ 9:1. Monolayers showed similar contact angle as the ones prepared following the reported synthetic procedure.

Figure 4.10: ^1H NMR spectra (400 MHz, CDCl_3) of adsorbate **5**.

Synthesis of guest **6**: To a solution of the amine precursor⁵⁴ (194 mg, 0.26 mmol) in dry DCM (2.5 mL) under Ar, ferrocenoyl chloride (82 mg, 0.33 mmol, 1.3 eq.) in dry DCM (2.5 mL) and Et_3N (45 μL , 0.33 mmol, 1.3 eq.) was added and the solution was stirred for 2 h. under inert atmosphere. The solvent was evaporated and the crude was purified twice by chromatography by elution with EtOAc-petroleum ether 3:1 $\rightarrow$ 2:1 $\rightarrow$ EtOAc to give compound **5** as brown syrup. Yield: 145 mg (57%). $R_f = 0.57$ (DCM-MeOH 9:1); IR-ATR: 2904, 2851, 1633.5 (CO amide), 1595, 1534, 1450, 1353, 1293, 1106, 1089, 731 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): $\delta = 6.51$ (d, 2 H, $^4J_{\text{H,H}} = 2.2$ Hz, H_d), 6.40 (t, 1 H, H_f), 6.06 (t, 1 H, $^3J_{\text{H,H}} = 5.3$ Hz, NH), 4.69 (t, 2 H, $J_{\text{H,H}} = 1.8$ Hz, Fc), 4.47 (d, 2 H, CH_2NH), 4.33 (t, 2 H, Fc), 4.18 (s, 5 H, Fc), 4.08 (t, 4 H, $^3J_{\text{H,H}} = 5.0$ Hz, $\text{CH}_{2,\text{g}}$), 3.81 (t, 4 H, $\text{CH}_{2,\text{h}}$), 3.66-3.53 (m, 24 H, CH_2O), 2.12 (bs, 6 H, CH_q), 1.73 (d, 12 H, $J_{\text{H,H}} = 2.7$ Hz, $\text{CH}_{2,\text{p}}$), 1.59 (m, 12 H,

CH_{2,r}); ¹³C NMR (100 MHz): δ = 171.2 (C=O), 160.3 (C_e), 107.0 (C_d), 100.6 (C_f), 72.4, 71.4, 71.2, 71.0, 70.7, 69.8, 67.8, 59.3, 43.5 (C_b), 41.6 (C_p), 36.5 (C_r), 30.6 (C_q); ESI-MS: m/z = 972.0 [M]⁺, 994.0 [M + Na]⁺; Anal. Calc. for C₅₄H₇₇FeNO₁₁: C, 66.72; H, 7.98; N, 1.44; Found C, 66.52; H, 7.76; N, 1.21. NMR spectra are shown in Figure 4.11.

Figure 4.11: ¹H and ¹³C NMR spectra (400 and 100.7 MHz, CDCl₃) of guest **6**.

Monolayers were prepared as described elsewhere.^{35–36} Gold substrates were cleaned with piranha, (conc. H₂SO₄-H₂O₂ 3:1, **warning: piranha should be handled with caution; it can detonate in contact with organic compounds**), then washed with copious amount of water, immersed in EtOH for 20 min and finally transferred rapidly to 0.1 mM solutions of β-CD adsorbates in THF-H₂O 4:1 (**2**, **3** and **4**), THF-MeOH 4:1 (**1**) or chloroform-EtOH 2:1 (**5**) for 12 h at room temperature or 50 °C (**1–4**) and at 60 °C (**5**). Subsequently, the substrates were

removed from the solution and rinsed with the solvent used in the monolayer formation, EtOH and dried with a stream of N_2 . Monolayers of **5** were rinsed with DCM, EtOH and water at least two times.

Functionalization with guest was carried out in two ways: For host coverage determination, the guest was dissolved in MeOH and diluted with H_2O to give 10 μM solutions (MeOH <1% (v/v)). Substrates were incubated for 10 min with the guest, then rinsed with H_2O , 2 mL β -CD 1 mM, H_2O and dried with a stream of nitrogen. For the rest of the experiments the guest was dissolved in aqueous β -CD 1 mM solutions with the help of sonication. Substrates were incubated for 10 min with the guest, then rinsed with H_2O and dried with a stream of nitrogen. Competition experiments were carried out by immersion of the substrates in β -CD 10 mM (twice 15 min).

Surface Plasmon Resonance Spectroscopy was carried out on glass-supported gold substrates for SPR (50 nm gold, 2 nm Ti adhesion layer) obtained from SSENS (Hengelo, The Netherlands). SPR measurements were performed on a two-channel vibrating mirror angle scan set-up based on the Kretschmann configuration. The instrument consists of a HeNe laser (JDS Uniphase, 10 mW, $\lambda = 632.8$ nm) whose light passes through a chopper that is connected to a lock-in amplifier. The light was coupled via a high index prism (LaSFN 9) to an optically matched glass-supported gold substrate with an index matching oil (Cargille; series B; $n_D^{25} = 1.700 \pm 0.002$). A Teflon cell was placed on the monolayer via an O-ring to avoid leakage. SPR experiments were performed in a flow cell system at a continuous flow of 0.15 mL min⁻¹. All the solutions were prepared in the presence of β -CD 1 mM. Baseline and rinsing steps were carried out with aqueous β -CD 1 mM.

All electrochemical measurements were performed on a CH instruments bipotentiostat 760D. All solutions used during electrochemical measurements were deaerated with N_2 for at least 30 minutes. Unless otherwise indicated, all the measurements were performed in a three-electrode setup using the SAM-covered gold plate as the working electrode (area = 0.44 cm²), a platinum disc as the counter electrode and an Ag/AgCl reference electrode in aqueous 1 M NaClO₄. Scan rates used in cyclic voltammetry experiments were 0.1 V s⁻¹ for coverage determinations and 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 300, 400 and 500 V s⁻¹ for determination of electron transfer rates. All the measurements were carried out after a first complete scan of preconditioning.

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To simplify the computational cost, first the equilibrium in solution was considered (Figure 4.13):

Figure 4.13: Thermodynamic host-guest equilibria of guest **6** in a solution of native β -CD.

The total concentration of guest, $[\text{Ad-Ad-Fc}]_{\text{tot}}$ and total native β -CD on solution $[\text{CD}]_{\text{I,tot}}$ are related with the individual species by following mass balance:

$$[\text{Ad-Ad-Fc}]_{\text{tot}} = [\text{Ad-Ad-Fc}] + [\text{Ad-Ad-CDI-Fc}] + [\text{Ad-Ad-Fc-CDI}] + [\text{Ad-CDI-Ad-CDI-Fc}] + [\text{Ad-Ad-CDI-Fc-CDI}] + [\text{Ad-CDI-Ad-CDI-Fc-CDI}] \quad (1)$$

$$[\text{CD}]_{\text{I,tot}} = [\text{Ad-Ad-CDI-Fc}] + [\text{Ad-Ad-Fc-CDI}] + 2 \cdot [\text{Ad-CDI-Ad-CDI-Fc}] + 2 [\text{Ad-Ad-CDI-Fc-CDI}] + 3 [\text{Ad-CDI-Ad-CDI-Fc-CDI}] \quad (2)$$

Where

$$[\text{Ad-Ad-CDI-Fc}] = 2 \cdot K_{i,\text{Ad,I}} [\text{Ad-Ad-Fc}][\text{CD}]_{\text{I}} \quad (3)$$

$$[\text{Ad-Ad-Fc-CDI}] = K_{i,\text{Fc,I}} [\text{Ad-Ad-Fc}][\text{CD}]_{\text{I}} \quad (4)$$

$$[\text{Ad-CDI-Ad-CDI-Fc}] = K_{i,\text{Ad,I}}^2 [\text{Ad-Ad-Fc}][\text{CD}]_{\text{I}}^2 \quad (5)$$

$$[\text{Ad-Ad-CDI-Fc-CDI}] = 2 \cdot K_{i,\text{Ad,I}} K_{i,\text{Fc,I}} [\text{Ad-Ad-Fc}][\text{CD}]_{\text{I}}^2 \quad (6)$$

$$[\text{Ad-CDI-Ad-CDI-Fc-CDI}] = K_{i,\text{Ad,I}}^2 K_{i,\text{Fc,I}} [\text{Ad-Ad-Fc}][\text{CD}]_{\text{I}}^3 \quad (7)$$

Where $K_{i,\text{Ad,I}}$ and $K_{i,\text{Fc,I}}$ denote the thermodynamic host-guest binding constants for adamantane- native β CD and ferrocene-native β CD in solution for guest **6**.

Inserting equations (3-7) into equations (1) and (2) gives:

$$[\text{Ad-Ad-Fc}]_{\text{tot}} = [\text{Ad-Ad-Fc}] (1 + [\text{CD}]_{\text{I}} (2K_{i,\text{Ad,I}} + K_{i,\text{Fc,I}} + K_{i,\text{Ad,I}} [\text{CD}]_{\text{I}} (K_{i,\text{Ad,I}} + 2K_{i,\text{Fc,I}} (1 + \frac{1}{2} K_{i,\text{Ad,I}} [\text{CD}]_{\text{I}})))) \quad (8)$$

$$[\text{CD}]_{\text{I,tot}} = [\text{CD}]_{\text{I}} (1 + [\text{Ad-Ad-Fc}] (2K_{i,\text{Ad,I}} + K_{i,\text{Fc,I}} + 2K_{i,\text{Ad,I}} [\text{CD}]_{\text{I}} (K_{i,\text{Ad,I}} + 2K_{i,\text{Fc,I}} (1 + \frac{3}{4} K_{i,\text{Ad,I}} [\text{CD}]_{\text{I}})))) \quad (9)$$

We implemented in a spreadsheet equations (3-9) to retrieve the fraction of bound species (f). Figure 4.14 shows the results of the solution speciation

assuming $K_{i,Ad,I}$ and $K_{i,Fc,I}$ equal to $3 \times 10^5 \text{ M}^{-1}$ and $1.2 \times 10^3 \text{ M}^{-1}$, respectively, at $[\text{CD}]_{I,\text{tot}} = 1 \text{ mM}$.

Figure 4.14. Thermodynamic host-guest equilibrium of guest **6** in a solution of native β -CD assuming $K_{i,Ad,I}$ and $K_{i,Fc,I}$ equal to $3 \times 10^5 \text{ M}^{-1}$ and $1.2 \times 10^3 \text{ M}^{-1}$, respectively.

The total concentration of guest is given by the following expression:

$$\begin{aligned}
 [\text{Ad-Ad-Fc}]_{\text{tot}} = & [\text{Ad-Ad-Fc}] + [\text{Ad-Ad-Fc-CD}_s] + [\text{Ad-Ad-CD}_I\text{-Fc}] + [\text{Ad-Ad-Fc-CD}_I] + \\
 & [\text{Ad-Ad-CD}_s\text{-Fc}] + [\text{Ad-Ad-CD}_I\text{-Fc-CD}_s] + [\text{Ad-Ad-CD}_s\text{-Fc-CD}_s] + [\text{Ad-CD}_I\text{-Ad-CD}_I\text{-Fc}] + \\
 & [\text{Ad-CD}_s\text{-Ad-CD}_s\text{-Fc}] + [\text{Ad-Ad-CD}_I\text{-Fc-CD}_I] + [\text{Ad-Ad-CD}_s\text{-Fc-CD}_I] + [\text{Ad-CD}_I\text{-Ad-CD}_s\text{-Fc}] + \\
 & [\text{Ad-CD}_I\text{-Ad-CD}_s\text{-Fc-CD}_s] + [\text{Ad-CD}_I\text{-Ad-CD}_I\text{-Fc-CD}_s] + [\text{Ad-CD}_s\text{-Ad-CD}_s\text{-Fc-CD}_s] + \\
 & [\text{Ad-CD}_I\text{-Ad-CD}_I\text{-Fc-CD}_I] + [\text{Ad-CD}_s\text{-Ad-CD}_s\text{-Fc-CD}_I] + [\text{Ad-CD}_I\text{-Ad-CD}_s\text{-Fc-CD}_I] \quad (10)
 \end{aligned}$$

Where the concentration of the individual species can be expressed as a function of $[\text{Ad-Ad-Fc}]$, $[\text{CD}]_s$ and $[\text{CD}]_I$:

$$[\text{Ad-Ad-Fc-CD}_s] = K_{i:Fc,S} [\text{Ad-Ad-Fc}][\text{CD}]_s \quad (11)$$

$$[\text{Ad-Ad-CD}_I\text{-Fc}] = 2 \cdot K_{i:Ad,I} [\text{Ad-Ad-Fc}][\text{CD}]_I \quad (12)$$

$$[\text{Ad-Ad-Fc-CD}_I] = K_{i:Fc,I} [\text{Ad-Ad-Fc}][\text{CD}]_I \quad (13)$$

$$[\text{Ad-Ad-CD}_s\text{-Fc}] = 2 \cdot K_{i:Ad,S} [\text{Ad-Ad-Fc}][\text{CD}]_s \quad (14)$$

$$[\text{Ad-Ad-CD}_I\text{-Fc-CD}_s] = 2 \cdot K_{i:Ad,I} K_{i:Fc,S} [\text{Ad-Ad-Fc}][\text{CD}]_s [\text{CD}]_I \quad (15)$$

$$[\text{Ad-Ad-CD}_s\text{-Fc-CD}_s] = 2 \cdot C_{\text{eff},Fc} K_{i:Ad,S} K_{i:Fc,S} [\text{Ad-Ad-Fc}][\text{CD}]_s^2 / [\text{CD}]_{s,\text{tot}} \quad (16)$$

$$[\text{Ad-CD}_I\text{-Ad-CD}_I\text{-Fc}] = K_{i:Ad,I}^2 [\text{Ad-Ad-Fc}][\text{CD}]_I^2 \quad (17)$$

$$[\text{Ad-Ad}\cdot\text{CD}_\text{I}\text{-Fc}\cdot\text{CD}_\text{I}] = 2 \cdot K_{\text{i:Fc,I}} K_{\text{i:Ad,I}} [\text{Ad-Ad-Fc}][\text{CD}]^2 \quad (18)$$

$$[\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}] = K_{\text{i:Ad,S}} K_{\text{i:Ad,I}} [\text{Ad-Ad-Fc}][\text{CD}]_1 [\text{CD}]_S \quad (19)$$

$$[\text{Ad-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{I}] = 2 \cdot K_{\text{i:Ad,S}} K_{\text{i:Fc,I}} [\text{Ad-Ad-Fc}][\text{CD}][\text{CD}]_S \quad (20)$$

$$[\text{Ad}\cdot\text{CD}_\text{S}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}] = C_{\text{eff,Ad}} K_{\text{i:Ad,S}}^2 [\text{Ad-Ad-Fc}][\text{CD}]_S^2 / [\text{CD}]_{\text{S,tot}} \quad (21)$$

$$[\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{S}] = K_{\text{i:Ad,I}} K_{\text{i:Ad,S}} K_{\text{i:Fc,S}} C_{\text{eff,Fc}} [\text{Ad-Ad-Fc}][\text{CD}]_S^2 [\text{CD}]_1 / [\text{CD}]_{\text{S,tot}} \quad (22)$$

$$[\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{I}\text{-Fc}\cdot\text{CD}_\text{S}] = K_{\text{i:Ad,I}}^2 K_{\text{i:Fc,S}} [\text{Ad-Ad-Fc}][\text{CD}]_S [\text{CD}]_1^2 \quad (23)$$

$$[\text{Ad}\cdot\text{CD}_\text{S}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{S}] = C_{\text{eff,Ad}} C_{\text{eff,Fc}} K_{\text{i:Ad,S}}^2 K_{\text{i:Fc,S}} [\text{Ad-Ad-Fc}][\text{CD}]_S^3 / [\text{CD}]_{\text{S,tot}}^2 \quad (24)$$

$$[\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{I}\text{-Fc}\cdot\text{CD}_\text{I}] = K_{\text{i:Fc,I}} K_{\text{i:Ad,I}}^2 [\text{Ad-Ad-Fc}][\text{CD}]_1^3 \quad (25)$$

$$[\text{Ad}\cdot\text{CD}_\text{S}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{I}] = K_{\text{i:Fc,I}} K_{\text{i:Ad,S}}^2 C_{\text{eff,Ad}} [\text{Ad-Ad-Fc}][\text{CD}]_S^2 [\text{CD}]_1 / [\text{CD}]_{\text{S,tot}} \quad (26)$$

$$[\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{I}] = K_{\text{i:Ad,S}} K_{\text{i:Fc,I}} K_{\text{i:Ad,I}} [\text{Ad-Ad-Fc}][\text{CD}]_1^2 [\text{CD}]_S \quad (27)$$

Mass balance of the total β CD on surface $[\text{CD}]_S$ is given by:

$$\begin{aligned} [\text{CD}]_{\text{S,tot}} = & [\text{Ad-Ad-Fc}\cdot\text{CD}_\text{S}] + [\text{Ad-Ad}\cdot\text{CD}_\text{S}\text{-Fc}] + [\text{Ad-Ad}\cdot\text{CD}_\text{I}\text{-Fc}\cdot\text{CD}_\text{S}] + 2 [\text{Ad-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{S}] \\ & + 2 [\text{Ad}\cdot\text{CD}_\text{S}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}] + [\text{Ad-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{I}] + [\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}] + 2 \\ & [\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{S}] + [\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{I}\text{-Fc}\cdot\text{CD}_\text{S}] + 3 \cdot [\text{Ad}\cdot\text{CD}_\text{S}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{S}] + 2 \\ & \cdot [\text{Ad}\cdot\text{CD}_\text{S}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{I}] + [\text{Ad}\cdot\text{CD}_\text{I}\text{-Ad}\cdot\text{CD}_\text{S}\text{-Fc}\cdot\text{CD}_\text{I}] \end{aligned} \quad (28)$$

By combining equations (28) and (11-27)

$$\begin{aligned} [\text{CD}]_S = & [\text{CD}]_{\text{S,tot}} / (1 + [\text{Ad-Ad-Fc}](K_{\text{i:Fc,S}} + 2 \cdot K_{\text{i:Ad,S}} + [\text{CD}]_1 (2 \cdot K_{\text{i:Ad,S}} K_{\text{i:Fc,I}} + K_{\text{i:Ad,S}} K_{\text{i:Ad,I}} + \\ & 2 \cdot K_{\text{i:Ad,I}} K_{\text{i:Fc,S}} + K_{\text{i:Ad,I}} [\text{CD}]_1 (K_{\text{i:Fc,S}} + K_{\text{i:Ad,S}} K_{\text{i:Fc,I}}) + 2 \cdot K_{\text{i:Ad,S}} [\text{CD}]_S) / ([\text{CD}]_{\text{S,tot}} (K_{\text{i:Ad,I}} K_{\text{i:Fc,S}} C_{\text{eff,Fc}} \\ & + K_{\text{i:Fc,I}} K_{\text{i:Ad,S}} C_{\text{eff,Ad}})) + 2 \cdot K_{\text{i:Ad,S}} [\text{CD}]_S) / ([\text{CD}]_{\text{S,tot}} (2 \cdot C_{\text{eff,Fc}} K_{\text{i:Fc,S}} + C_{\text{eff,Ad}} K_{\text{i:Ad,S}} + \frac{3}{2} C_{\text{eff,Fc}} K_{\text{i:Fc,S}} \\ & C_{\text{eff,Ad}} K_{\text{i:Ad,S}} [\text{CD}]_S) / ([\text{CD}]_{\text{S,tot}}))) \end{aligned} \quad (29)$$

Fittings of the model to the experimental data are presented in the main text. Speciation in the experimental conditions is shown in Figure 4.14.

4.5 References

- (1) Launay, J.; Verdaguer, M., *Electrons in Molecules: From Basic Principles to Molecular Electronics*. Oxford University Press: **2014**.
- (2) Moser, C. C.; Keske, J. M.; Warncke, K.; Farid, R. S.; Dutton, P. L., Nature of Biological Electron Transfer. *Nature* **1992**, 355, 796-802.
- (3) Williamson, H. R.; Dow, B. A.; Davidson, V. L., Mechanisms for Control of Biological Electron Transfer Reactions. *Bioorg. Chem.* **2014**, 57, 213-221.

- (4) Adams, D. M.; Brus, L.; Chidsey, C. E. D.; Creager, S.; Creutz, C.; Kagan, C. R.; Kamat, P. V.; Lieberman, M.; Lindsay, S.; Marcus, R. A.; Metzger, R. M.; Michel-Beyerle, M. E.; Miller, J. R.; Newton, M. D.; Rolison, D. R.; Sankey, O.; Schanze, K. S.; Yardley, J.; Zhu, X., Charge Transfer on the Nanoscale: Current Status. *J. Phys. Chem. B* **2003**, *107*, 6668-6697.
- (5) Waleed Shinwari, M.; Jamal Deen, M.; Starikov, E. B.; Cuniberti, G., Electrical Conductance in Biological Molecules. *Adv. Funct. Mater.* **2010**, *20*, 1865-1883.
- (6) Lindsey, J. S.; Bocian, D. F., Molecules for Charge-Based Information Storage. *Acc. Chem. Res.* **2011**, *44*, 638-650.
- (7) Uosaki, K.; Kondo, T.; Zhang, X.-Q.; Yanagida, M., Very Efficient Visible-Light-Induced Uphill Electron Transfer at a Self-Assembled Monolayer with a Porphyrin-Ferrocene-Thiol Linked Molecule. *J. Am. Chem. Soc.* **1997**, *119*, 8367-8368.
- (8) Yamada, H.; Imahori, H.; Nishimura, Y.; Yamazaki, I.; Ahn, T. K.; Kim, S. K.; Kim, D.; Fukuzumi, S., Photovoltaic Properties of Self-Assembled Monolayers of Porphyrins and Porphyrin-Fullerene Dyads on Ito and Gold Surfaces. *J. Am. Chem. Soc.* **2003**, *125*, 9129-9139.
- (9) Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M., Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology. *Chem. Rev.* **2005**, *105*, 1103-1170.
- (10) Eckermann, A. L.; Feld, D. J.; Shaw, J. A.; Meade, T. J., Electrochemistry of Redox-Active Self-Assembled Monolayers. *Coord. Chem. Rev.* **2010**, *254*, 1769-1802.
- (11) Ruther, R. E.; Cui, Q.; Hamers, R. J., Conformational Disorder Enhances Electron Transfer through Alkyl Monolayers: Ferrocene on Conductive Diamond. *J. Am. Chem. Soc.* **2013**, *135*, 5751-5761.
- (12) Liu, B.; Bard, A. J.; Mirkin, M. V.; Creager, S. E., Electron Transfer at Self-Assembled Monolayers Measured by Scanning Electrochemical Microscopy. *J. Am. Chem. Soc.* **2004**, *126*, 1485-1492.
- (13) Smalley, J. F.; Finklea, H. O.; Chidsey, C. E. D.; Linford, M. R.; Creager, S. E.; Ferraris, J. P.; Chalfant, K.; Zawodzinski, T.; Feldberg, S. W.; Newton, M. D., Heterogeneous Electron-Transfer Kinetics for Ruthenium and Ferrocene Redox Moieties through Alkanethiol Monolayers on Gold. *J. Am. Chem. Soc.* **2003**, *125*, 2004-2013.
- (14) Napper, A. M.; Liu, H.; Waldeck, D. H., The Nature of Electronic Coupling between Ferrocene and Gold through Alkanethiolate Monolayers on Electrodes: The Importance of Chain Composition, Interchain Coupling, and Quantum Interference. *J. Phys. Chem. B* **2001**, *105*, 7699-7707.
- (15) Chidsey, C. E. D., Free Energy and Temperature Dependence of Electron Transfer at the Metal-Electrolyte Interface. *Science* **1991**, *251*, 919-922.
- (16) Chidsey, C. E. D.; Bertozzi, C. R.; Putvinski, T. M.; Majsce, A. M., Coadsorption of Ferrocene-Terminated and Unsubstituted Alkanethiols on Gold: Electroactive Self-Assembled Monolayers. *J. Am. Chem. Soc.* **1990**, *112*, 4301-4306.

- (17) Allen, J. P.; Williams, J. C., Photosynthetic Reaction Centers. *FEBS Lett.* **1998**, *438*, 5-9.
- (18) Fujii, S.; Tada, T.; Komoto, Y.; Osuga, T.; Murase, T.; Fujita, M.; Kiguchi, M., Rectifying Electron-Transport Properties through Stacks of Aromatic Molecules Inserted into a Self-Assembled Cage. *J. Am. Chem. Soc.* **2015**, *137*, 5939-5947.
- (19) Li, F.; Ito, T., Complexation-Induced Control of Electron Propagation Based on Bounded Diffusion through Nanopore-Tethered Ferrocenes. *J. Am. Chem. Soc.* **2013**, *135*, 16260-16263.
- (20) Wimbush, K. S.; Reus, W. F.; van der Wiel, W. G.; Reinhoudt, D. N.; Whitesides, G. M.; Nijhuis, C. A.; Velders, A. H., Control over Rectification in Supramolecular Tunneling Junctions. *Angew. Chem. Int. Ed.* **2010**, *49*, 10176-10180.
- (21) Huskens, J., Multivalent Interactions at Interfaces. *Curr. Opin. Chem. Biol.* **2006**, *10*, 537-543.
- (22) Nijhuis, C. A.; Huskens, J.; Reinhoudt, D. N., Binding Control and Stoichiometry of Ferrocenyl Dendrimers at a Molecular Printboard. *J. Am. Chem. Soc.* **2004**, *126*, 12266-12267.
- (23) Nijhuis, C. A.; Yu, F.; Knoll, W.; Huskens, J.; Reinhoudt, D. N., Multivalent Dendrimers at Molecular Printboards: Influence of Dendrimer Structure on Binding Strength and Stoichiometry and Their Electrochemically Induced Desorption. *Langmuir* **2005**, *21*, 7866-7876.
- (24) Nijhuis, C. A.; Boukamp, B. A.; Ravoo, B. J.; Huskens, J.; Reinhoudt, D. N., Electrochemistry of Ferrocenyl Dendrimer- β -Cyclodextrin Assemblies at the Interface of an Aqueous Solution and a Molecular Printboard. *J. Phys. Chem. C* **2007**, *111*, 9799-9810.
- (25) Yang, L.; Gomez-Casado, A.; Young, J. F.; Nguyen, H. D.; Cabanas-Danés, J.; Huskens, J.; Brunsveld, L.; Jonkheijm, P., Reversible and Oriented Immobilization of Ferrocene-Modified Proteins. *J. Am. Chem. Soc.* **2012**, *134*, 19199-19206.
- (26) Mallon, C. T.; Forster, R. J.; Keyes, T. E., Mechanism and Release Rates of Surface Confined Cyclodextrin Guests. *Analyst* **2011**, *136*, 5051-5057.
- (27) Mallon, C. T.; McNally, A.; Keyes, T. E.; Forster, R. J., Mercury-Platinum Tunneling Junctions Incorporating Supramolecular Host-Guest Assemblies. *J. Am. Chem. Soc.* **2008**, *130*, 10002-10007.
- (28) Schönherr, H.; Beulen, M. W. J.; Bügler, J.; Huskens, J.; van Veggel, F. C. J. M.; Reinhoudt, D. N.; Vancso, G. J., Individual Supramolecular Host-Guest Interactions Studied by Dynamic Single Molecule Force Spectroscopy. *J. Am. Chem. Soc.* **2000**, *122*, 4963-4967.
- (29) Zapotoczny, S.; Auletta, T.; de Jong, M. R.; Schönherr, H.; Huskens, J.; van Veggel, F. C. J. M.; Reinhoudt, D. N.; Vancso, G. J., Chain Length and Concentration Dependence of β -Cyclodextrin-Ferrocene Host-Guest Complex Rupture Forces Probed by Dynamic Force Spectroscopy. *Langmuir* **2002**, *18*, 6988-6994.
- (30) Auletta, T.; de Jong, M. R.; Mulder, A.; van Veggel, F. C. J. M.; Huskens, J.; Reinhoudt, D. N.; Zou, S.; Zapotoczny, S.; Schönherr, H.; Vancso, G. J.; Kuipers, L., β -Cyclodextrin Host-Guest Complexes Probed under Thermodynamic Equilibrium: Thermodynamics and Afm Force Spectroscopy. *J. Am. Chem. Soc.* **2004**, *126*, 1577-1584.
- (31) Slinker, J. D.; Muren, N. B.; Renfrew, S. E.; Barton, J. K., DNA Charge Transport over 34 nm. *Nat Chem* **2011**, *3*, 228-233.

- (32) Beulen, M. W. J.; Bügler, J.; Lammerink, B.; Geurts, F. A. J.; Biemond, E. M. E. F.; van Leerdam, K. G. C.; van Veggel, F. C. J. M.; Engbersen, J. F. J.; Reinhoudt, D. N., Self-Assembled Monolayers of Heptapodant β -Cyclodextrins on Gold. *Langmuir* **1998**, *14*, 6424-6429.
- (33) Liu, W.; Zhang, Y.; Gao, X., Interfacial Supramolecular Self-Assembled Monolayers of C60 by Thiolated β -Cyclodextrin on Gold Surfaces Via Monoanionic C60. *J. Am. Chem. Soc.* **2007**, *129*, 4973-4980.
- (34) Rojas, M. T.; Koeniger, R.; Stoddart, J. F.; Kaifer, A. E., Supported Monolayers Containing Preformed Binding Sites. Synthesis and Interfacial Binding Properties of a Thiolated β -Cyclodextrin Derivative. *J. Am. Chem. Soc.* **1995**, *117*, 336-343.
- (35) Méndez-Ardoy, A.; Steentjes, T.; Kudernac, T.; Huskens, J., Self-Assembled Monolayers on Gold of β -Cyclodextrin Adsorbates with Different Anchoring Groups. *Langmuir* **2014**, *30*, 3467-3476.
- (36) Beulen, M. W. J.; Bugler, J.; De Jong, M. R.; Lammerink, B.; Huskens, J.; Schönherr, H.; Vancso, G. J.; Boukamp, B. A.; Wieder, H.; Offenhäuser, A.; Knoll, W.; Van Veggel, F. C. J. M.; Reinhoudt, D. N., Host-Guest Interactions at Self-Assembled Monolayers of Cyclodextrins on Gold. *Chem. - Eur. J.* **2000**, *6*, 1176-1183.
- (37) Godínez, L. A.; Schwartz, L.; Criss, C. M.; Kaifer, A. E., Thermodynamic Studies on the Cyclodextrin Complexation of Aromatic and Aliphatic Guests in Water and Water-Urea Mixtures. Experimental Evidence for the Interaction of Urea with Arene Surfaces. *J. Phys. Chem. B* **1997**, *101*, 3376-3380.
- (38) Mulder, A.; Auletta, T.; Sartori, A.; Del Ciotto, S.; Casnati, A.; Ungaro, R.; Huskens, J.; Reinhoudt, D. N., Divalent Binding of a Bis(Adamantyl)-Functionalized Calix[4]Arene to B-Cyclodextrin-Based Hosts: An Experimental and Theoretical Study on Multivalent Binding in Solution and at Self-Assembled Monolayers. *J. Am. Chem. Soc.* **2004**, *126*, 6627-6636.
- (39) Huskens, J.; Mulder, A.; Auletta, T.; Nijhuis, C. A.; Ludden, M. J. W.; Reinhoudt, D. N., A Model for Describing the Thermodynamics of Multivalent Host-Guest Interactions at Interfaces. *J. Am. Chem. Soc.* **2004**, *126*, 6784-6797.
- (40) Bard, A. J.; Faulkner, L. R., *Electrochemical Methods: Fundamentals and Applications*. 2 ed.; John Wiley & Sons: New York, 2001.
- (41) Clark, R. A.; Bowden, E. F., Voltammetric Peak Broadening for Cytochrome C/Alkanethiolate Monolayer Structures: Dispersion of Formal Potentials. *Langmuir* **1997**, *13*, 559-565.
- (42) Thoden van Velzen, E. U.; Engbersen, J. F. J.; Reinhoudt, D. N., Self-Assembled Monolayers of Receptor Adsorbates on Gold: Preparation and Characterization. *J. Am. Chem. Soc.* **1994**, *116*, 3597-3598.
- (43) Nicholson, R. S., Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics. *Anal. Chem.* **1965**, *37*, 1351-1355.
- (44) Nkosi, D.; Pillay, J.; Ozoemena, K. I.; Nouneh, K.; Oyama, M., Heterogeneous Electron Transfer Kinetics and Electrocatalytic Behaviour of Mixed Self-Assembled Ferrocenes and Swcnt Layers. *Phys. Chem. Chem. Phys.* **2010**, *12*, 604-613.

- (45) Henke, C.; Steinem, C.; Janshoff, A.; Steffan, G.; Luftmann, H.; Sieber, M.; Galla, H.-J., Self-Assembled Monolayers of Monofunctionalized Cyclodextrins onto Gold: A Mass Spectrometric Characterization and Impedance Analysis of Host-Guest Interaction. *Anal. Chem.* **1996**, *68*, 3158-3165.
- (46) Laviron, E., General Expression of the Linear Potential Sweep Voltammogram in the Case of Diffusionless Electrochemical Systems. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, *101*, 19-28.
- (47) Roth, K. M.; Yasseri, A. A.; Liu, Z.; Dabke, R. B.; Malinovskii, V.; Schweikart, K.-H.; Yu, L.; Tiznado, H.; Zaera, F.; Lindsey, J. S.; Kuhr, W. G.; Bocian, D. F., Measurements of Electron-Transfer Rates of Charge-Storage Molecular Monolayers on Si(100). Toward Hybrid Molecular/Semiconductor Information Storage Devices. *J. Am. Chem. Soc.* **2003**, *125*, 505-517.
- (48) Weber, K.; Hockett, L.; Creager, S., Long-Range Electronic Coupling between Ferrocene and Gold in Alkanethiolate-Based Monolayers on Electrodes. *J. Phys. Chem. B* **1997**, *101*, 8286-8291.
- (49) Bueno, P. R.; Mizzon, G.; Davis, J. J., Capacitance Spectroscopy: A Versatile Approach to Resolving the Redox Density of States and Kinetics in Redox-Active Self-Assembled Monolayers. *J. Phys. Chem. B* **2012**, *116*, 8822-8829.
- (50) Góes, M. S.; Rahman, H.; Ryall, J.; Davis, J. J.; Bueno, P. R., A Dielectric Model of Self-Assembled Monolayer Interfaces by Capacitive Spectroscopy. *Langmuir* **2012**, *28*, 9689-9699.
- (51) Fabre, B.; Ababou-Girard, S.; Singh, P.; Kumar, J.; Verma, S.; Bianco, A., Noncovalent Assembly of Ferrocene on Modified Gold Surfaces Mediated by Uracil-Adenine Base Pairs. *Electrochem. Commun.* **2010**, *12*, 831-834.
- (52) Smalley, J. F.; Newton, M. D.; Feldberg, S. W., A Simple Comparison of Interfacial Electron-Transfer Rates for Surface-Attached and Bulk Solution-Dissolved Redox Moieties. *J. Electroanal. Chem.* **2006**, *589*, 1-6.
- (53) Lagrost, C.; Alcaraz, G.; Bergamini, J.-F.; Fabre, B.; Serbanescu, I., Functionalization of Silicon Surfaces with Si-C Linked β -Cyclodextrin Monolayers. *Chem. Commun.* **2007**, 1050-1052.
- (54) Mulder, A.; Onclin, S.; Péter, M.; Hoogenboom, J. P.; Beijleveld, H.; ter Maat, J.; García-Parajó, M. F.; Ravoo, B. J.; Huskens, J.; van Hulst, N. F.; Reinhoudt, D. N., Molecular Printboards on Silicon Oxide: Lithographic Patterning of Cyclodextrin Monolayers with Multivalent, Fluorescent Guest Molecules. *Small* **2005**, *1*, 242-253.

Chapter 5

Electron transfer processes in ferrocene-modified poly(ethylene glycol) monolayers on electrodes

Electrochemistry is a powerful tool to study self-assembled monolayers. Here, we modified electrodes with different lengths of linear poly(ethylene glycol) (PEG) polymers end-functionalized with a redox-active ferrocene (Fc) group. The electron transport properties of the Fc probes were studied using cyclic voltammetry. The Fc moiety attached to the shortest PEG ($M_n=250$ Da) behaved as a surface-confined species, and the homogeneous electron transfer rate constants were determined. The electron transfer of the ferrocene group on the longer PEGs ($M_n=3.4, 5$ and 10 kDa) was shown to be driven by diffusion. For low surface densities, where the polymer exists in the mushroom conformation, the diffusion coefficients (D) and rate constants were increasing with polymer length. In the loose brush conformation, where the polymers are close enough to interact with each other, the thickness of the layers (e) was unknown and a parameter $D^{1/2}/e$ was determined. This parameter showed no dependence on surface density and an increase with polymer length.

5.1 Introduction

Understanding conformations of (bio)polymers at surfaces is important since such layers are widely used for electrochemical detection methods, for example for DNA sensing.¹⁻⁷ The mechanism of so-called E-DNA sensors relies on changes in conformation upon binding with the analyte, and this conformational change results in a change in signal.³ As such, the flexibility of the linker plays an important role in the signal output.

The configuration of end-tethered polymers on surfaces is dictated by the surface density and the length of the polymers.⁸⁻⁹ The transition between the mushroom and brush conformation, where the polymers begin to overlap has been modelled¹⁰⁻¹¹ and observed experimentally¹²⁻¹³ with different results for the onset of the brush formation. If the molecules are tethered with an electrochemical probe, the behavior of the end-group can be studied using electrochemical methods. Several studies have been performed with poly(ethylene glycol) (PEG) layers end-tethered with ferrocene, both with an atomic force/scanning electrochemical microscope setup¹⁴ and on single electrodes.¹⁵⁻¹⁶ The polymer behavior in the mushroom configuration, where the polymer chains exist as isolated entities, has been modelled.¹⁷ These studies have shown that the electrochemical response of the end-group involves diffusion to the surface. As the surface density is increased, which brings about conformational changes, changes in bounded diffusion and kinetics can be expected, as have indeed been observed for DNA and PNA layers.¹⁸ A detailed experimental study on a well-defined model system in which the length and density of surface-attached PEG chains are varied is however currently lacking.

Herein we study the effect of polymer length and surface density of end-tethered PEG layers immobilized on electrodes, in order to study the electron transfer behavior of the end-group. The surface densities are varied to achieve the mushroom and loose brush conformations, and the influence of the varying surface density on the electrochemical properties of the ferrocene group is established using cyclic voltammetry.

5.2 Results and discussion

PEG chains bearing on one end a ferrocene (Fc) moiety, used for electrochemical detection, and on the other end a reactive succinimide (NHS) group, used for surface attachment, were synthesized according to known procedures.^{15, 19} Fc-

PEG-NHS derivatives of four molecular weights ($M_n = 0.25, 3.4, 5$ and 10 kDa, i.e. Fc-PEG₂₅₀-NHS, Fc-PEG_{3k}-NHS, Fc-PEG_{5k}-NHS, and Fc-PEG_{10k}-NHS, respectively) were separately grafted onto gold electrodes that were pretreated with cystamine. The PEGs (with the exception of PEG₂₅₀) used here were reported to have a polydispersity index of 1.08, and their average molecular weights correspond to fully extended lengths of $L = 2.1$ nm, 27 nm, 40 nm and 79 nm (Table 5.1) for PEG₂₅₀, PEG_{3k}, PEG_{5k} and PEG_{10k}, respectively. The conformation of the polymers is dictated by the surface density (Figure 5.1). If the density is sufficiently low (Table 5.1), the polymers exhibit a mushroom conformation. As the surface density increases and the chains start to interact, the polymers extend outwards into the solution and form a loose brush.

Figure 5.1: Surface density effect on polymer conformation. If the surface density is sufficiently low, i.e. when the Flory radius (R_f) is smaller than the distance between the grafting points (s), the polymers are in the mushroom conformation (left). When the surface density is increased, the polymers start to overlap, and a loose brush is formed (right).

Table 5.1: Overview of the used polymers, their molecular weights and standard deviations calculated from the reported polydispersity (1.08), calculated Flory radius, R_f , fully extended chain length (L), and the calculated surface density at which the chains start to interact and move into a loose brush regime (for the calculations of these densities, see the Experimental).

M_n (kDa)	R_f (nm)	L (nm)	Mushroom to brush transition density (mol/cm ²)
0.25*	1.0	2.1	1.6×10^{-10}
3.4 ± 1.0	4.7	27	7.4×10^{-12}
5.0 ± 1.4	6.0	40	4.6×10^{-12}
10 ± 2.8	9.1	79	2.0×10^{-12}

*Polydispersity not reported.

Cleaned gold electrodes were modified with cystamine, resulting in a monolayer with free amine groups onto which the Fc-PEG-NHS polymers were grafted via their succinimide groups. Cyclic voltammetry measurements of the modified electrodes were performed in a 1 M NaClO₄ solution. The results, see Figure 5.2a for a cyclic voltammogram of a the Fc-PEG₂₅₀ layer, were typical for surface-attached species, with a small peak separation (<59 mV) and a peak current that was linearly dependent on the scan rate, indicating that all the redox couples have sufficient time to contribute to the electron transfer. In this regime, the charge determined from the peak area remains constant independent of scan rate, and the surface density (Γ) of the PEG molecules can be determined according to Equation 5.1.

$$\Gamma = Q/nFA \quad (\text{Equation 5.1})$$

In Equation 5.1, Q is the charge of the integrated peak area, A the microscopic surface area as determined from sulfuric acid cleaning scans, F the Faraday constant, and n the number of electrons involved ($n=1$ for ferrocene). At scan rates above 200 V/s a significant peak separation became apparent for Fc-PEG₂₅₀, resulting in a characteristic trumpet plot (Figure 5.2b) when plotting the peak separation, η , versus the logarithm of the scan rate, ν .

Figure 5.2: a) Cyclic voltammogram of a Fc-PEG₂₅₀ monolayer, obtained upon a reaction time of 5 min, recorded in 1 M NaClO₄ at 2 V/s vs a Hg/Hg₂SO₄ reference electrode. b) Trumpet plot of the peak separation, η , versus the logarithm of the scan rate, ν .

When the peak separation increased past 200 mV and the electron transfer became electrochemically irreversible, the peak potentials changed linearly with the logarithm of the scan rate. In that case, a standard rate constant, k , can be determined using Laviron's formulation, Equation 5.2.²⁰

$$k = \frac{\alpha n F v_c}{RT} = \frac{(1-\alpha) n F v_a}{RT} \quad (\text{Equation 5.2})$$

In Equation 5.2, α is the transfer coefficient, taken as 0.5, and R and T are the ideal gas constant and the temperature, respectively, while v_c and v_a are the cathodic and anodic scan rates, respectively. From this treatment, rate constants k of $2.4 \times 10^3 \text{ s}^{-1}$ and $1.7 \times 10^3 \text{ s}^{-1}$ were found for the anodic and cathodic processes, respectively. These values are significantly larger than the standard rate constants determined for ferrocene moieties on well packed alkane monolayers of similar length,²¹ thus indicating a loosely packed layer in the case of PEG layers. This is confirmed by the measured surface densities, for which values between 2 and $3 \times 10^{-10} \text{ mol/cm}^2$ were obtained when using a surface functionalization time of 5 min.

The peak separation for the longer PEGs was less pronounced and was mostly caused by a shift of the cathodic peak and a smaller shift of the anodic peak combined with significant peak broadening (Figure 5.3a and d). At increased scan rates, the current became linear with the square root of the scan rate (Figure 5.3c) following the Randles-Sevcik equation,²² indicating an additional dependence of the electron transfer rate on the diffusion of the ferrocene head groups to and from the surface. Since the effect of diffusion on the electron transfer excludes the use of the Laviron method for the determination of rate constants, these were determined using the Nicholson method for diffusing species adjusted for surface-confined species.^{18, 23-24} In order to do this, the diffusion constants of ferrocene and oxidized ferrocenium species (D_{Fc} and D_{Fc^+} , respectively) were determined from the anodic and cathodic peak currents using the Randles-Sevcik equation (Equation 5.3).

$$i_p = 0.4463 n F A C \left(\frac{n F v D}{RT} \right)^{\frac{1}{2}} \quad (\text{Equation 5.3})$$

In Equation 5.3, the concentration, C , can be replaced by Γ/e , with e being the layer thickness. Normalizing the anodic peak current densities ($j_a = i_a/A$) to $v^{1/2}$ showed that at higher scan rates $j_a/v^{1/2}$ became independent of the scan rate, thus reaching a plateau (Figure 5.3c). From the height of this plateau the diffusion coefficients D_{Fc} and D_{Fc^+} were determined using the Randles-Sevcik equation for the polymers in the mushroom conformation. When the surface density is sufficiently low so that the polymers on the surface can be assumed to exist in the mushroom conformation (see Table 5.1) and do not interact with

each other, the average thickness of the polymer layer, e , can be taken as the Flory radius.

Figure 5.3: a) Cyclic voltammogram of a 1.5×10^{-11} mol/cm² monolayer of Fc-PEG_{10k} (obtained from a reaction time of 90 min) in 1 M NaClO₄ at 10 V/s. b) Anodic peak current, i_a , plotted versus the square root of the scan rate v ; the line is a linear fit to the data. c) Anodic peak current density ($j_a = i_a/A$), normalized to the square root of the scan rate, vs the logarithm of the scan rate. d) Peak separation vs the logarithm of the scan rate. e) The obtained surface densities as a function of the reaction time, t .

As can be seen in Table 5.2, the determined diffusion coefficients show that the bound ferrocenium cation diffuses faster (2.5 - 4 times) than the reduced ferrocene, which is also visible from the peak asymmetry (Figure 5.3a), the cathodic peak being sharper than the anodic peak.¹⁵ This effect is not present for ferrocene species in solution²⁵⁻²⁶ but has been noted before for surface-bound Fc-PEG, and has been attributed to the positively charged Fc⁺ being

‘propelled away’ from the surface, caused by electrostatic repulsion between the surface and the Fc^+ moiety directly after the oxidation step.¹⁵

The diffusion coefficients increased with increasing polymer length, which is counterintuitive. For the mushroom configuration, the concentration of ferrocene can be represented by a Gaussian distribution with the highest concentration close to the surface. The longer molecules have a higher local density due to the amount of polymer surrounding the ferrocene head, which is expected to slow down diffusion. Indeed, the opposite trend has been observed for different lengths of Fc-modified PNA strands tethered on electrodes, with the diffusion coefficients decreasing and the system becoming more surface confined as the strand length decreased.¹⁸ On the other hand, it has been shown that the diffusion coefficient for tethered PEG_{3k} is higher than for PEG_{600} in dichloromethane, even with the PEG_{3k} being in a loose brush conformation and the PEG_{6k} in a mushroom conformation, which has been attributed to an increased influence of the spring constant on the diffusion coefficient.¹⁵

Table 5.2: Diffusion coefficients and homogeneous electron transfer rate constants determined for Fc- PEG_{3k} , Fc- PEG_{5k} and Fc- PEG_{10k} in the mushroom regime.

polymer	$D_{\text{Fc}} (\times 10^{-12} \text{ cm}^2/\text{s})$	$D_{\text{Fc}^+} (\times 10^{-12} \text{ cm}^2/\text{s})$	$k_0 (\text{s}^{-1})$
Fc- PEG_{3k}	1.4 ± 1.0	6.0 ± 1.3	88 ± 23
Fc- PEG_{5k}	1.9 ± 0.4	6.2 ± 0.4	114 ± 104
Fc- PEG_{10k}	9.1 ± 6.3	29.8 ± 9.7	228 ± 54

As mentioned above, using the diffusion coefficients, the homogeneous electron transfer rate constants could be determined using the Nicholson method for diffusing species when the peak separation is between 61 and 212 mV. In short, the peak separation is related by a kinetic parameter ψ , from which the rate constant can be calculated.²³ As can be seen in Table 5.2, the determined rate constants are lower than the value (approx. $2 \times 10^3 \text{ s}^{-1}$) determined for PEG_{250} , in which case the electron transfer is not affected by diffusion. Furthermore, the rate constants increased with increasing polymer length, which could indicate that for the longer molecules the ferrocene end groups are on average closer to the surface.¹⁸

As the surface density was increased by employing longer reaction times of Fc-PEG-NHS on the cystamine surfaces (Figure 5.4a), the average distance between the anchoring points became smaller than the Flory radius, and consequently the polymer conformation moved from a mushroom into a loose brush regime. In the brush regime, the polymer chains stretch outward into the solution, therefore, the layer thickness (e) is no longer related to the Flory radius, but instead becomes proportional to the average number, N , of monomers per chain, by $e \sim pNa\sigma^{1/3}$,⁸ where a is the monomer size, σ the graft density and p a proportionality coefficient. Since the actual layer thickness is unknown in this case, the diffusion and rate constants cannot be directly determined.

Figure 5.4: a) The obtained surface densities as a function of the reaction time, t . b) and c) The anodic (j_a) and cathodic (j_c) peak current densities normalized to the square root of the scan rate, vs surface density, for Fc-PEG_{3k}, Fc-PEG_{5k} and Fc-PEG_{10k}. d) The Gaussian fully extended chain length distribution for PEG_{3k}, PEG_{5k} and PEG_{10k} (represented in all graphs by the colors red, blue and black, respectively).

The anodic and cathodic peak current densities normalized to the square root of v , $j_c/v^{1/2}$, showed a linear increase upon increasing surface density (Figure 5.4b

and c). Since the slope of this line is now solely dependent on the parameter $D_{\text{Fc}}^{+1/2}/e$ (see Equation 5.3), this parameter was determined for all three PEG lengths in the brush conformation. The values for $D_{\text{Fc}}^{+1/2}/e$ were found to be 10.6 ± 1.7 , 9.2 ± 0.6 and $9.1 \pm 0.8 \text{ s}^{-1/2}$ for Fc-PEG_{3k}, Fc-PEG_{5k}, and Fc-PEG_{10k}, respectively, when using the cathodic peak current densities. The $D_{\text{Fc}}^{1/2}/e$ values, determined from the anodic peak current densities, were again lower, 4.5 ± 1.4 , 6.6 ± 0.7 , and $6.1 \pm 1.5 \text{ s}^{-1/2}$. The similarity between the PEGs can partly be attributed to the broad size distributions, which show that a large proportion of the size distributions overlap (Figure 5.4d), despite the difference in M_n and a relatively small polydispersity of 1.08. The fact that the $D^{1/2}/e$ values remain constant implies that, as the layer thickness increases, the diffusion constants must increase as well. An increase of the diffusion constant with increased surface density can be explained with an increased proximity of the redox centers to the surface.²⁷ For a surface density of $1.5 \times 10^{-12} \text{ mol/cm}^2$, and a layer thickness of 40 nm (half of the fully extended length of PEG_{10k}), the ferrocene concentration inside that layer can be calculated to be 3.8 mM, which is of the same order of magnitude as electrochemical mediators in solution.²⁸ This cannot be the sole contribution to the diffusion process, since the diffusion constant is greater for PEG_{10k}, which must mean that the local ferrocene concentration is lower than for shorter PEGs, as the layer thickness is larger.

5.3 Conclusions

Surfaces were modified with different lengths of poly(ethylene glycol) (PEG) end-tethered with a ferrocene moiety and studied using cyclic voltammetry. The shortest Fc-PEG, Fc-PEG₂₅₀, behaved as a surface-confined layer for which homogeneous rate constants could be determined using the Laviron method. These results suggest that a loosely packed layer was formed. For longer Fc-PEGs, diffusion coefficients and homogeneous electron transfer rate constants could be determined when the layers were present in the mushroom conformation, both of which increased with the polymer length. As the surface density was increased, and the polymer layers entered the loose brush regime, the determination of these parameters was not possible due to the unknown layer thickness. Instead, $D^{1/2}/e$ values were determined for both ferrocene and ferrocenium, and were shown to be constant for increasing surface densities, implying an increase in diffusion coefficient with surface density. This increase is attributed to an increased chance of electron hopping as the local ferrocene

concentration increases. However, the diffusion coefficient also appears to become larger for longer PEGs, while these form a thicker layer and thus a lower Fc concentration. More information is needed to fully explain this increase. Overall, the data presented here highlight that redox-modified polymers attached to an electrode surface show a rich and complex electrochemical behavior, that leads to currents that depend on a multitude of parameters. Unraveling these dependencies will assist in the development of electrochemical sensors that rely on redox behavior of surface-tethered probe molecules.

5.4 Experimental section

5.4.1 Materials

Reagents and solvents were purchased from Sigma-Aldrich, high-purity water (MilliQ) was used (Millipore, $R = 18.2 \text{ } \Omega$). All bis-NHS-functionalized PEGs were purchased from Nanocs and have a reported dispersity of 1.08. The Fc-PEG-NHS molecules were synthesized according to known procedures.¹⁵ Fc-PEG₂₅₀-NHS was further purified by column chromatography with dichloromethane as eluent. After the first fraction was removed, the eluent was changed to DCM/EtOH (95:5). Fc-PEG_{3k}-NHS, Fc-PEG_{5k}-NHS, and Fc-PEG_{10k}-NHS was further purified by size exclusion chromatography (Biobeads SX-1) with DCM as eluent.

2-Ferrocene-ethylamine

2-Ferrocene-ethylamine was synthesized by the reduction of ferrocene acetonitrile by LiAlH_4 as described in the literature,¹⁹ with some modifications. An amount of 0.25 g (6.5 mmol) LiAlH_4 and 0.6 g (4.5 mmol) AlCl_3 were carefully added to 10 mL dry THF while stirring in an ice bath. Ferrocene acetonitrile (0.5 g, 2.25 mmol) was dissolved in 5 mL dry THF and subsequently added to the cooled mixture and refluxed overnight under an argon atmosphere. After cooling, water was added drop wise to decompose the excess LiAlH_4 . A volume of 0.25 mL of concentrated NaOH was added to destroy the formed $\text{AlCl}_3/2$ -ferrocene-ethylamine complex. The aqueous phase was extracted three times with diethylether. The combined organic phases were dried with MgSO_4 and filtered, and the solvent was removed by rotary evaporation. The product was purified by column chromatography with dichloromethane as the eluent. After drying in vacuo a brown solid was obtained (0.13 g; 26%). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 4.2 (m, 9H, Fc), 2.82 (t, 2H, CH_2 -Fc), 2.48 (t, 2H, CH_2 -N).

PEG₂₅₀-(NHS)₂

The bis-NHS ester of the PEG₂₅₀ diacid was prepared according to a procedure described earlier.¹⁵ An amount of 0.44 g (3.8 mmol) of *N*-hydroxysuccinimide (NHS) and 0.78 g (3.8 mmol) dicyclohexylcarbodiimide (DCC) were added to a stirred solution of 0.4 g (1.6 mmol) poly(ethylene glycol) bis(carboxymethyl) ether in 30 mL 1,4-dioxane. After stirring over night at room temperature under an argon atmosphere, the mixture was filtered to remove the 1,3-dicyclohexylurea precipitate. The solvent was removed by rotary evaporation and the residue was further dried under vacuum. ¹H NMR (300 MHz, CDCl₃): δ (ppm) = 4.56 (s, 4H, CH₂C=O), 3.66 (s, 12H, C₂H₄-O), 2.81 (s, 8H, NHS).

5.4.2 Surface functionalization and electrochemistry

All electrochemical measurements were performed on a CH Instruments bipotentiostat 760D. Cyclic voltammetry measurements were performed in a three-electrode setup using 2 mm diameter (CH Instruments) or 1.6 mm diameter (BASi) gold disk electrodes, a platinum wire as the counter electrode and a Ag/AgCl reference electrode in aqueous 1 M NaClO₄.

Before modification the electrodes were polished using 50 nm alumina particles (CH Instruments), followed by extensive rinsing with ethanol and 5 min of ultrasonic treatment in ethanol and 5 min in MilliQ water. Subsequently, the electrodes were cleaned electrochemically in 0.5 M H₂SO₄ by applying an oxidizing potential of 2 V for 5 seconds followed by a reducing potential of -0.35 V for 10 s. Then the electrode potential was scanned from -0.25 V to 1.55 V and back for 40 cycles at a scan rate of 100 mV/s, the surface area of the electrodes was determined from the Au-O reduction peak, using a value of 386 $\mu\text{C}/\text{cm}^2$ to convert the charge into surface area.²⁹⁻³⁰

After cleaning, the electrodes were rinsed with MilliQ water and ethanol and dried under a flow of N₂ and placed immediately in a 2 mM cystamine solution and left over night. Subsequently, the electrodes were rinsed with MilliQ water and dried under a flow of N₂ and placed in a 0.2 mM Fc-PEG-NHS solution. The surface densities were varied by changing the reaction time between 5-120 min. The prepared electrodes were copiously rinsed with MilliQ water and subsequently cycled electrochemically at 100 mV/s in 1 M NaClO₄ until a stable signal was obtained.

5.4.3 Calculations

The Flory radii (R_f) in Table 5.1 were calculated with $R_f = aN^{3/5}$ where a is the size of the individual ethylene glycol monomer ($a = 0.35 \text{ nm}^{31}$) and N the degree of polymerization ($N = 6, 77, 114$ and 227 for PEG₂₅₀, PEG_{3k}, PEG_{5k} and PEG_{10k}, respectively). When the distance between the grafting points (s) of the PEG molecules approaches the R_f , the chains start to overlap and form a polymer brush at the critical dimensionless coverage $\sigma_{\text{critical}} = (a/R_f)^2 = (N_a \Gamma) a^2$, where N_a is Avogadro's constant. From this the critical surface densities as shown in Table 5.1 can be determined.¹⁴

5.5 References

- (1) Fan, C.; Plaxco, K. W.; Heeger, A. J., Electrochemical Interrogation of Conformational Changes as a Reagentless Method for the Sequence-Specific Detection of DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 9134-9137.
- (2) Prieto-Simón, B.; Campàs, M.; Marty, J. L., Electrochemical Aptamer-Based Sensors. *Bioanalytical Reviews* **2010**, *1*, 141-157.
- (3) Ricci, F.; Plaxco, K. W., E-DNA Sensors for Convenient, Label-Free Electrochemical Detection of Hybridization. *Microchim. Acta* **2008**, *163*, 149-155.
- (4) Xiang, Y.; Qian, X.; Jiang, B.; Chai, Y.; Yuan, R., An Aptamer-Based Signal-on and Multiplexed Sensing Platform for One-Spot Simultaneous Electronic Detection of Proteins and Small Molecules. *Chem. Commun.* **2011**, *47*, 4733-4735.
- (5) Xiao, Y.; Qu, X.; Plaxco, K. W.; Heeger, A. J., Label-Free Electrochemical Detection of DNA in Blood Serum Via Target-Induced Resolution of an Electrode-Bound DNA Pseudoknot. *J. Am. Chem. Soc.* **2007**, *129*, 11896-11897.
- (6) Xu, X.; Li, B.; Xie, X.; Li, X.; Shen, L.; Shao, Y., An I-DNA Based Electrochemical Sensor for Proton Detection. *Talanta* **2010**, *82*, 1122-1125.
- (7) Zhang, K.; Zhu, X.; Wang, J.; Xu, L.; Li, G., Strategy to Fabricate an Electrochemical Aptasensor: Application to the Assay of Adenosine Deaminase Activity. *Anal. Chem.* **2010**, *82*, 3207-3211.
- (8) De Gennes, P. G., Conformations of Polymers Attached to an Interface. *Macromolecules* **1980**, *13*, 1069-1075.
- (9) Alexander, S., Adsorption of Chain Molecules with a Polar Head. A Scaling Description. *J. Phys. France* **1977**, *38*, 983-987.
- (10) Pépin, M. P.; Whitmore, M. D., Monte Carlo and Numerical Self-Consistent Field Study of End-Tethered Polymers in Good Solvent. *J. Chem. Phys.* **1999**, *111*, 10381-10388.
- (11) Karaikos, E.; Bitsanis, I. A.; Anastasiadis, S. H., Monte Carlo Studies of Tethered Chains. *J. Polym. Sci., Part B: Polym. Phys.* **2009**, *47*, 2449-2461.

- (12) Wu, T.; Efimenko, K.; Genzer, J., Combinatorial Study of the Mushroom-to-Brush Crossover in Surface Anchored Polyacrylamide. *J. Am. Chem. Soc.* **2002**, *124*, 9394-9395.
- (13) Dumont, E. L. P.; Belmas, H.; Hess, H., Observing the Mushroom-to-Brush Transition for Kinesin Proteins. *Langmuir* **2013**, *29*, 15142-15145.
- (14) Abbou, J.; Anne, A.; Demaille, C., Probing the Structure and Dynamics of End-Grafted Flexible Polymer Chain Layers by Combined Atomic Force-Electrochemical Microscopy. Cyclic Voltammetry within Nanometer-Thick Macromolecular Poly(Ethylene Glycol) Layers. *J. Am. Chem. Soc.* **2004**, *126*, 10095-10108.
- (15) Anne, A.; Moiroux, J., Quantitative Characterization of the Flexibility of Poly(Ethylene Glycol) Chains Attached to a Glassy Carbon Electrode. *Macromolecules* **1999**, *32*, 5829-5835.
- (16) Anne, A.; Demaille, C.; Moiroux, J., Terminal Attachment of Polyethylene Glycol (Peg) Chains to a Gold Electrode Surface. Cyclic Voltammetry Applied to the Quantitative Characterization of the Flexibility of the Attached Peg Chains and of Their Penetration by Mobile Peg Chains. *Macromolecules* **2002**, *35*, 5578-5586.
- (17) Abbou, J.; Anne, A.; Demaille, C., Accessing the Dynamics of End-Grafted Flexible Polymer Chains by Atomic Force-Electrochemical Microscopy. Theoretical Modeling of the Approach Curves by the Elastic Bounded Diffusion Model and Monte Carlo Simulations. Evidence for Compression-Induced Lateral Chain Escape. *J. Phys. Chem. B* **2006**, *110*, 22664-22675.
- (18) Hüsken, N.; Gębala, M.; La Mantia, F.; Schuhmann, W.; Metzler-Nolte, N., Mechanistic Studies of Fc-Pna(-DNA) Surface Dynamics Based on the Kinetics of Electron-Transfer Processes. *Chem. - Eur. J.* **2011**, *17*, 9678-9690.
- (19) Seiwert, B.; Karst, U., Analysis of Cysteine-Containing Proteins Using Precolumn Derivatization with N-(2-Ferroceneethyl)Maleimide and Liquid Chromatography/Electrochemistry/ Mass Spectrometry. *Anal. Bioanal. Chem.* **2007**, *388*, 1633-1642.
- (20) Laviron, E. E. E., General Expression of the Linear Potential Sweep Voltammogram in the Case of Diffusionless Electrochemical Systems. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, *101*, 19-28.
- (21) Chidsey, C. E. D., Free Energy and Temperature Dependence of Electron Transfer at the Metal-Electrolyte Interface. *Science* **1991**, *251*, 919-922.
- (22) Bard, A. J.; Faulkner, L. R., *Electrochemical Methods: Fundamentals and Applications*. 2 ed.; John Wiley & Sons: New York, 2001.
- (23) Nicholson, R. S., Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics. *Anal. Chem.* **1965**, *37*, 1351-1355.
- (24) Anne, A.; Demaille, C., Dynamics of Electron Transport by Elastic Bending of Short DNA Duplexes. Experimental Study and Quantitative Modeling of the Cyclic Voltammetric Behavior of 3'-Ferrocenyl DNA End-Grafted on Gold. *J. Am. Chem. Soc.* **2006**, *128*, 542-557.

- (25) Martin, R. D.; Unwin, P. R., Theory and Experiment for the Substrate Generation/Tip Collection Mode of the Scanning Electrochemical Microscope: Application as an Approach for Measuring the Diffusion Coefficient Ratio of a Redox Couple. *Anal. Chem.* **1998**, *70*, 276-284.
- (26) Mampallil, D.; Mathwig, K.; Kang, S.; Lemay, S. G., Redox Couples with Unequal Diffusion Coefficients: Effect on Redox Cycling. *Anal. Chem.* **2013**, *85*, 6053-6058.
- (27) Akhoury, A.; Bromberg, L.; Hatton, T. A., Interplay of Electron Hopping and Bounded Diffusion During Charge Transport in Redox Polymer Electrodes. *J. Phys. Chem. B* **2013**, *117*, 333-342.
- (28) Liu, B.; Bard, A. J.; Mirkin, M. V.; Creager, S. E., Electron Transfer at Self-Assembled Monolayers Measured by Scanning Electrochemical Microscopy. *J. Am. Chem. Soc.* **2004**, *126*, 1485-1492.
- (29) Trasatti, S.; Petrii, O. A., Real Surface Area Measurements in Electrochemistry. *J. Electroanal. Chem.* **1992**, *327*, 353-376.
- (30) Tremiliosi-Filho, G.; Dall'Antonia, L. H.; Jerkiewicz, G., Limit to Extent of Formation of the Quasi-Two-Dimensional Oxide State on Au Electrodes. *J. Electroanal. Chem.* **1997**, *422*, 149-159.
- (31) Allen, C.; Dos Santos, N.; Gallagher, R.; Chiu, G. N. C.; Shu, Y.; Li, W. M.; Johnstone, S. A.; Janoff, A. S.; Mayer, L. D.; Webb, M. S.; Bally, M. B., Controlling the Physical Behavior and Biological Performance of Liposome Formulations through Use of Surface Grafted Poly(Ethylene Glycol). *Biosci. Rep.* **2002**, *22*, 225-250.

Chapter 6

Electron transfer mediated by surface-tethered redox groups in nanofluidic devices

Electrochemistry provides a powerful sensor transduction and amplification mechanism that is highly suited for use in integrated, massively parallelized assays. Here we demonstrate the cyclic voltammetric detection of flexible, linear poly(ethylene glycol) polymers that have been functionalized with redox-active ferrocene (Fc) moieties and surface-tethered inside a nanofluidic device consisting of two microscale electrodes separated by a gap of <100 nm. Diffusion of the surface-bound polymer chains in the aqueous electrolyte allows the redox groups to repeatedly shuttle electrons from one electrode to the other, resulting in a greatly amplified steady-state electrical current. Variation of the polymer length provides control over the current, as the activity per Fc moiety appears to depend on the extent to which the polymer layers of the opposing electrodes can interpenetrate each other and thus exchange electrons. These results outline the design rules for sensing devices that are based on changing the polymer length, flexibility and/or diffusivity by binding an analyte to the polymer chain. Such a nanofluidic enabled configuration provides an amplified and highly sensitive alternative to other electrochemical detection mechanisms.

6.1 Introduction

Biosensing plays an increasingly important role, in particular in the development of personalized medicine.¹ This growing importance also puts new demands on the performance of such devices, which ranges from higher sensitivities and selectivities, to the use of smaller sample volumes, the use of body fluids, an easy readout and signal processing, and the option to use disposable devices. Electrochemical devices have particular advantages that meet several of these requirements.² The combination with electrochemistry opens pathways for a multitude of benefits for biosensing strategies that allow their use in personalized medicine.³⁻⁵ A key opportunity offered by electrochemistry is an enhanced sensitivity based on signal amplification, which can be achieved when multiple electrons, *e.g.* in cyclic redox processes, amplify a single molecular recognition event.

Prime examples of sensitive electrochemical detection are redox-activated DNA (E-DNA) biosensors, which rely on conformational changes that modulate the motion and the distance of the redox moiety to an electrode, resulting in a change in signal.⁶⁻⁸ By default, the nature of the linker and the surface density of the redox-labeled polymer chains play a pivotal role in the optimization of the sensitivity of such a sensor. Long linear molecular chains such as synthetic polymers⁹⁻¹¹ and DNA strands end-capped with an electrochemically active label¹²⁻¹⁴ have been shown to give faradaic currents dictated by the diffusional motion of the redox moiety. This is in contrast with surface-immobilized systems in which the redox center is located at a fixed distance from the surface and the electron transfer rate depends exponentially on the distance to the surface.¹⁵⁻¹⁷

A further increase in sensitivity can be achieved by the use of electrochemical mediators which replenish the oxidized surface layer.¹⁸ Re-activation of the diffusing species in the presence of a second electrode providing an opposing reducing or oxidizing potential, as is performed in scanning electrochemical microscopy (SECM) and in nanofluidic devices,¹⁹ provides a significant amplification of the faradaic current due to redox cycling. This concept has been exploited for biodetection with increased sensitivity.²⁰⁻²¹ Alternatively, if the second electrode is sufficiently close to the surface to get in contact with the electrochemically active layer, the electrode can take the role of the mediator by replenishing the oxidized/reduced species directly. Such a strategy has been employed using a conductive atomic force microscopy (AFM) tip positioned on

top of poly(ethylene glycol) (PEG)²² and DNA²³ layers, which allows the direct measurement of the redox cycling current provided by the bounded diffusion of the electrode-attached redox labels.

Nanodevices with nanometric electrode spacings, comparable to the molecular lengths of the surface-attached polymer chains, provide an excellent basis for electrochemical devices in which electron shuttling can occur in the absence of a mediator. This approach further promises the well-controlled and reproducible architecture of a closed solid-state device as well as integration with microfluidics, sample handling, and signal processing.²⁴ Several device architectures have been demonstrated that lend themselves to miniaturization down to the nanometer scale, including interdigitated electrode arrays,²⁵⁻²⁷ recessed ring electrodes²⁸ and thin-layer cells.^{19, 29-30} As of yet, however, no such system has been reported in a device configuration where surface-attached redox couples are directly replenished due to the effect of the second electrode without the need of a mediator.

In this chapter we report nanofluidic devices with two opposing microscale electrodes spaced by a sub-100 nm gap and their functionalization with electrochemically active end-capped polymers in order to study their ability to transfer electrons between the electrodes across the nanogap in the absence of a mediator. We compare the effects of different polymer length and length distribution, as well as the effect of changes in surface density, on the measured current.

6.2 Results and discussion

The nanogap devices used in this chapter (Figure 6.1a) consist of two planar rectangular platinum electrodes that are separated by a distance of 65 nm. To fabricate the nanofluidic channel, a sacrificial chromium layer was sandwiched between two platinum electrodes followed by wet etching of the chromium to open up the nanochannel. Two access holes acted as the inlets to the nanochannel. The active region for redox cycling is defined by the overlapping area of the two electrodes, as indicated in Figure 6.1b. Two different types of nanofluidic devices were used (Type I and Type II), the devices differing solely in the area of the active region (30 μm^2 for Type I and 300 μm^2 for Type II). A larger active region is expected to trap a likewise larger number of redox molecules between the electrodes, leading to a linear scaling of the current with the area of the active region. This was confirmed in practice, as described further below.

The platinum electrodes were modified with cystamine, resulting in a monolayer with exposed amine groups. Onto this monolayer, bifunctional PEG chains were grafted *via* a reactive succinimide group present on one end of the chain. The other end bore a redox-active ferrocene label which was used for electrochemical detection. Three lengths of PEG, “long” (PEG_{10k}; $M_n = 10 \pm 2.8$ kDa; single standard deviation, polydispersity index PDI = 1.08; average, fully extended chain length L of 79 nm), “intermediate” (PEG_{5k}; $M_n = 5.0 \pm 1.4$ kDa, equal PDI; $L = 40$ nm), and “short” (PEG_{3k}; $M_n = 3.4 \pm 0.96$ kDa, equal PDI; $L = 27$ nm), were used in this study to probe the possible effects of chain length and surface density on the current. The PDI causes the polymers to have a rather broad length distribution (see Chapter 5, Figure 5.4d). Electron transfer is expected to occur from the reducing electrode onto an oxidized Fc (ferrocenium) group that, upon diffusion, can transfer the electron to another Fc group of another PEG chain. Because the polymer lengths used here allow interpenetration of the chains immobilized at the opposing electrodes, the Fc-Fc electron transfer may occur from a moiety attached to the reducing electrode onto one that is immobilized at the oxidizing electrode. Finally, diffusion of a reduced Fc to the oxidizing electrode allows the transfer of the electron between them. These electron transfer steps are schematically shown in Figure 6.1c. Differences in polymer length and their coverage on the electrodes are expected to influence the surface density of polymer and Fc moieties, the degree of interpenetration of the opposing immobilized layers in the nanogap device, and possibly the diffusion rate of the Fc moieties within the polymer layer. These aspects all contribute to the frequencies of electrode-Fc and Fc-Fc electron transfer events that together define the current.

The nanogap electrodes were initially functionalized with PEG for at least 3 h in order to obtain maximum surface densities. To make sure that all physisorbed PEG molecules were removed from the electrodes, the device was held in a beaker with MilliQ water and vigorously stirred for at least 10 min. Without this procedure, the redox signal decreased during flow, while the rinsing procedure resulted in signals that were stable for the duration of the experiments. Additionally, control experiments, using mercaptoethanol instead of cystamine thus prohibiting the attachment of the Fc-modified PEG, showed complete removal of redox activity upon applying the same rinsing method (see Experimental section, Figure 6.6), and thus confirmed the suitability of this method.

Figure 6.1: a) Schematic diagram of the cross-section of the nanodevice prior to sacrificial layer etching (not drawn to scale), where $z = 65$ nm represents the height of the nanogap. b) Optical microscopy image of the top view of a Type II device before the sacrificial layer was etched. The dotted white rectangle represents the active area. c) The nanogap electrodes were functionalized with ferrocene end-capped PEG chains. The PEG layers at opposing electrodes are expected to interpenetrate to allow a current to flow from the reducing to the oxidizing electrode by a sequence of electrode-Fc, Fc-Fc, and Fc-electrode electron transfer steps. Reduced ferrocene groups are depicted in purple, oxidized ferrocenium cation moieties in red, and electron transfer steps in green.

In order to measure the surface densities at the electrodes, the top and bottom electrodes were connected and cycled as one electrode as shown in Figure 6.2a.³¹ The results are illustrated in Figure 6.2b, which shows a cyclic voltammogram measured on a Type I device grafted with PEG_{10k}. The recorded voltammogram is characteristic for surface-attached species, with a peak separation of less than 59 mV for all scan rates employed, a formal potential (E_0') of 0.3 V vs. Ag/AgCl, and a peak current (I_p) that increases linearly with the scan rate (Figure 6.2c).³² For surface-attached species, the charge under the peak is proportional to the total number of electrons transferred and can be used to calculate the surface density Γ using $\Gamma = Q/nF(A_{\text{top}} + A_{\text{bottom}})$. Here Q is the measured charge (as shown in Figure 6.2b), n the number of electrons transferred per ferrocene moiety ($n = 1$), F the Faraday constant, and A_{top} and A_{bottom} are the geometric surface areas of the two electrodes exposed to the

solution. A surface density of 19 pmol cm^{-2} was calculated in this case, corresponding to an average spacing of 3 nm between the polymer chains. The latter value is smaller than the calculated Flory radius ($R_f = 9.1 \text{ nm}$) of the polymer coils; the value of R_f for good solvents can be calculated from $R_f = aN^{3/5}$, where $a = 0.35 \text{ nm}$ is the monomer length³³ and $N = 227$ is the degree of polymerization. Therefore it can be concluded that the polymer was attached to the surface in a loose brush conformation, extending upwards from the electrode surface.^{22, 34}

Figure 6.2: a) Schematic diagram of the measurement configuration when both electrodes are cycled together as one electrode. b) Cyclic voltammogram of ferrocene end-capped PEG_{10k} functionalized electrodes measured in a Type I device at both electrodes simultaneously in a 1 M NaClO₄ solution (scan rate 20 mV s^{-1}). The inset shows the baseline-corrected anodic peak, the area of which was integrated to extract the surface charge Q and the corresponding surface density ($\Gamma = 19 \text{ pmol cm}^{-2}$ in this case). c) Plot of the peak current vs. scan rate, indicating a linear increase of the peak current with the scan rate.

Subsequently, the two electrodes were addressed separately: a reducing potential (0 V) was applied to the bottom electrode, while the potential of the top electrode was cycled between 0 and 0.6 V (Figure 6.3a). When the potential of the sweeping electrode passed E_0' , oxidized ferrocenium species attached to the PEG molecules could regain an electron, either by diffusion to the reducing electrode or by electron transfer from a Fc group attached to this electrode; the opposite being true for reduced species contacting the oxidizing electrode. Combined with Fc-Fc electron transfer between moieties attached to opposite electrodes caused by layer interpenetration (Figure 6.1c), these electron transfer events gave rise to a redox cycling current. The redox cycling currents measured at the two electrodes are expected to have the same magnitude but opposite signs. The resulting cyclic voltammograms for PEG_{10k}, measured on a

Type II device with a surface density of 8.8 pmol cm^{-2} , are shown in Figure 6.3b, where the simultaneously measured currents through each of the two working electrodes are plotted. The current at the electrode being cycled includes a large capacitive contribution which is marked by a pronounced hysteresis (Figure 6.3b, red curve). This capacitance results in part from the electrical double layer in regions where the electrode is exposed to the solution as well as from the capacitance of the connecting wires, which have a relatively large area and are only protected from the solution by a thin passivating layer of $\text{SiN}/\text{SiO}_2/\text{SiN}$. For this reason we concentrate our analysis below on the current measured at the electrode held at a fixed potential, which nicely isolates the redox cycling component.

Figure 6.3: a) Schematic diagram of the measurement configuration showing that the top electrode is cycled (red) while the bottom electrode is fixed at 0 V (black). b) Simultaneously recorded voltammograms for $\text{PEG}_{10\text{k}}$ (Type II device; $\Gamma = 8.8 \text{ pmol cm}^{-2}$). c) Comparison of the redox cycling current obtained from $\text{PEG}_{10\text{k}}$ (data from panel b), $\text{PEG}_{5\text{k}}$ (Type I device; $\Gamma = 25 \text{ pmol cm}^{-2}$) and $\text{PEG}_{3\text{k}}$ (Type II device; $\Gamma = 70 \text{ pmol cm}^{-2}$). All measurements were performed at a scan rate of 50 mV s^{-1} and the currents were normalized by the area A_{active} to facilitate comparison.

Additionally, in order to probe the effect of PEG length, a Type I device was functionalized with $\text{PEG}_{5\text{k}}$ and a Type II device with a tenfold larger active area was used for $\text{PEG}_{3\text{k}}$. Figure 6.3c shows the obtained redox cycling currents normalized to the area of the active region, A_{active} , for the three different devices. The steady state redox cycling current obtained for $\text{PEG}_{10\text{k}}$ is nearly an order of magnitude higher than that obtained for $\text{PEG}_{5\text{k}}$. This is attributed to the $\text{PEG}_{5\text{k}}$ layers at the opposing electrodes having less layer interpenetration and thus a less frequent Fc-Fc electron transfer between Fc-PEGs immobilized at opposite electrodes. Only the ferrocene on the sweeping electrode is expected to oxidize, but since the ferrocene moieties on both electrodes can extend half-way

through the nanogap, the ferrocenes attached to the opposing reducing electrode can donate electrons and act as a mediator. Since the amount of ferrocene moieties attached to the electrodes is known ($\Gamma = 25 \text{ pmol cm}^{-2}$ in this case), a concentration of 7.4 mM ferrocene can be calculated to be present inside the active region. This is a concentration comparable to mediators used in SECM for measurements on monolayers.³⁵ PEG_{3k} is even shorter and was not expected to be able to exchange electrons as the average, fully extended length, $L = 27 \text{ nm}$, does not allow the ferrocene labels attached to the electrode to reach halfway across the channel. However, at the present PDI of 1.08, corresponding to a standard deviation of 0.96 kDa (or a total of 4.4 kDa) the length of a significant fraction of the polymer chains can extend beyond 34 nm, which exceeds half the gap separation. This makes it plausible that also in this case a fraction of polymer chains is capable of participating in electron transfer. Combined with the higher coverage for PEG_{3k} (70 pmol cm^{-2}), this explains the observed similar current values obtained for PEG_{5k} and PEG_{3k}.

The experiments of Figures 6.2 and 6.3 were repeated in several additional devices for all three chain lengths, and the results are summarized in Figure 6.4. The measured surface densities decreased with increasing polymer length (Figure 6.4a), as expected since the increased Flory radius leads to steric hindrance. Some scatter is observed in the data, which we attribute primarily to surface roughness: the area values are based on the geometric surface area and averaged over both electrodes, whereas the surface roughness is not the same for both electrodes (see Experimental section) and can vary between devices. The corresponding redox cycling currents were recorded simultaneously and were similar to the examples shown in Figure 6.3. To get a clear picture of the effect of the linker length, the redox cycling currents were normalized to the surface density according to $\nu = I_{\text{RC}}/(eN_{\text{active}})$ (with e the charge of an electron and N_{active} the amount of PEG-Fc molecules in the active area) – yielding the rate of electron transfer per available ferrocene group – as presented in Figure 6.4b (real currents normalized for the active area are shown in Figure 6.4c). This analysis shows that a single PEG_{10k} chain functionalized with a ferrocene end-group is able to transfer on average 6 electrons to the opposing electrode per second, whereas the electron transfer rate drops to 1 s^{-1} per ferrocene for PEG_{5k}, reflecting the decrease in polymer length and the lower frequency of Fc-Fc electron transfer events between oppositely immobilized chains. It also becomes clear that, although the measured redox cycling currents for PEG_{3k} are

not significantly lower than for PEG_{5k}, the rate of electrons transferred per ferrocene available is only 0.3 s⁻¹ for PEG_{3k}, and thus the measured redox cycling currents for PEG_{3k} compared to PEG_{5k} are the result of a higher surface density while a smaller fraction of the polymer chains is long enough to contribute to electron transfer.

Figure 6.4: a) The effect of linker length on the obtained maximum surface density after 3 h functionalization, and b) the corresponding redox cycling currents, presented as the rate of electrons transferred per PEG chain. The measurements were performed on both types of devices, Type I = ■, Type II = ○.

When considering the mechanism by which the electrons are transferred, it was anticipated that the polymer length of PEG_{10k} is long enough to enable the attached ferrocene moiety to travel to the opposing electrode, whereas the chain length for PEG_{5k} can only provide for electron transfer between the ferrocene moieties itself inside the channel in the zone where layer interpenetration and Fc-Fc electron transfer events between oppositely immobilized chains occur. Not taking into account the dispersity of the polymers, the length of 40 nm of PEG_{5k} gives rise to a 15 nm exchange zone

inside the 65 nm channel where the polymer chains from both electrodes can overlap and the ferrocene moieties can exchange electrons. For the PEG_{10k} chains to reach the opposing electrodes they have to stretch outwards and penetrate through the polymer layer resulting in an exchange zone of 65 nm (the entire channel). Next to larger exchange zone by a factor 4, the concentration of ferrocene inside the channel is on average a factor 2 lower for PEG_{10k} than for PEG_{5k} due to the lower surface densities, which corresponds to an overall expected higher activity of a factor 2 for PEG_{10k}. Additionally, for PEG_{10k} there is no need to stretch fully to reach an opposing PEG chain. Taking all these factors into account, we attribute the observed 5-fold higher activity of PEG_{10k} compared to PEG_{5k} to electron transfer dominated by exchange between the diffusing and interpenetrating ferrocene moieties in both cases, rather than direct transfer to the opposing electrode. We expect the same mechanism to hold for PEG_{3k} as well, when taking the dispersity into account. In this case, only a fraction of the molecules is sufficiently long to allow some interpenetration of the chain ends from opposing electrodes which, combined with the higher surface density, leads to the observed 3 times lower activity compared to PEG_{5k}. When taking the polymer chain length dispersities into account for all polymers, the interpenetration and exchange zones do not have sharply defined boundaries, but the exchange extends outside of the given borders with decreasing probabilities. Since proper electron transfer rate calculations would have to take into account the polymer length distributions, probability distributions of the distance between the Fc end group and the electrode, collision frequencies, polymer surface densities, diffusion rates, and Fc-Fc and Fc-electrode electron transfer rates, we did not attempt this here. From the almost linear behavior observed for the electron transfer per Fc moiety for the polymers studied here (Figure 6.4b) and the arguments given above, we conclude that the interpenetration and Fc-Fc exchange model (as depicted in Figure 6.1c) holds for all polymer lengths studied here.

The conformation of polymers grafted to the surface depends on the surface density, with the polymers existing in a mushroom conformation at low surface densities, where the polymers are isolated from their neighboring chains, and becoming more brush-like with increasing surface density as the surface concentration passes a critical surface density.³⁴ In order to study a possible effect of the PEG density and conformation regime on the redox cycling currents, Type I devices were functionalized with different surface densities of

PEG_{10k}, by changing the time the molecules were allowed to react with the cystamine monolayer. The resulting redox cycling currents were plotted vs. the incubation time, as shown in Figure 6.5. The surface densities, especially for short reaction times, were too low to give clearly distinguishable peaks in the cyclic voltammograms, and no reliable surface densities could be recorded. Therefore the redox cycling currents were compared with surface densities determined from experiments on macro electrodes. As can be seen in Figure 6.5a, the obtained surface densities on the macro electrodes reach a plateau around 40 pmol cm⁻², which is more than a factor 2 higher than the values obtained inside the nanogap devices (Figure 6.4a). Therefore it is expected that the surface densities inside the nanogap devices are still in the linear regime (left side of Figure 6.5a) for all incubation times used, probably due to slower mass transport compared to the macro electrodes. Indeed, it can be seen (Figure 6.5b) that the recorded redox cycling currents increase linearly with the incubation time. It can thus be concluded that the increase in current with increasing reaction times is mostly the result of the increase of ferrocene density and not of a change in polymer conformation. Due to the bulky nature of the PEG_{10k} molecule, all used reaction times result in polymers present in a loose brush conformation (the maximum surface density for the mushroom configuration for PEG_{10k} is calculated as 2 pmol cm⁻²),³⁴ and no dramatic change of behavior could be observed.

Figure 6.5: a) Surface densities on macro electrodes, and b) redox cycling currents in nanogap devices for PEG_{10k} as a function of the incubation time.

6.3 Conclusions

We have shown that surface-tethered PEG polymer chains with redox-active end groups allow the transfer of electrons between the electrodes in nanogap

devices in the absence of an electrochemical mediator. The primary condition that needs to be met is that the polymer chains of the opposing electrodes can interpenetrate so that oxidized and reduced ferrocene moieties can exchange electrons. The activity per Fc unit, as defined by the average number of electrons transferred per Fc moiety, is strongly dependent on the chain length, but not on the chain density in the loose brush density range studied here. The chain length effect is interpreted primarily by the increasing thickness of the channel zone in which exchange can occur and the increasing fraction of the polymers that can reach this exchange zone. The overall measured cycling currents are additionally dependent on the surface density, which is lower for longer polymers and thus counteracts to some extent the enhanced activities observed for the longer PEGs. All polymer lengths studied here were found to obey this same interpenetration and electron exchange model.

The findings of this study can contribute to an enhanced understanding and improved performance of biosensing devices that use redox cycling in surface-tethered receptor devices as the main amplification mechanism, such as in DNA hairpin-like detection schemes. For example, if a probe DNA molecule that allows hairpin formation is inserted in an immobilized polymer chain, binding of a complementary target DNA can break up the hairpin leading to an apparent lengthening of the polymer chain and thus to an increased layer penetration and enhanced current. A single binding event is thus affecting the electron transfer of many electrons, hence causing amplification. An increase in current, and with that an increase in sensitivity, can be expected if the height of the nanochannel is more finely tuned towards the length of the linker. At the same time, the selectivity between bound and unbound receptors can be improved when the polymer length distribution is more narrow and when the extended chain length is close to half of the channel width, so that small changes in polymer stiffness and length have large effects on the probabilities of chain interpenetration and electron exchange.

6.4 Experimental section

Materials

Reagents and solvents were purchased from Sigma-Aldrich, high-purity water (MilliQ) was used (Millipore, $R = 18.2 \text{ M}\Omega$). All bis-NHS-functionalized PEGs were purchased from Nanocs and have a reported dispersity of 1.08. The Fc-PEG-NHS molecules were synthesized according to known procedures.¹⁰ The obtained Fc-

PEG_{3k}-NHS, Fc-PEG_{5k}-NHS, and Fc-PEG_{10k}-NHS was further purified by size exclusion chromatography (Biobeads SX-1) with DCM as eluent. The synthesis of the Fc-tethered adsorbates with different lengths of PEG is described in Chapter 5.

Device fabrication

A 4-inch Si wafer was isolated with 500 nm thick thermally grown SiO₂. A Pt bottom electrode, a Cr sacrificial layer and a Pt top electrode were then consecutively deposited by electron-beam evaporation of 20 nm, 60 nm and 100 nm of metal, respectively, and patterned using a lift-off process based on a positive photoresist (OIR 907-17, Arch Chemicals). Thereafter, a passivation layer consisting of 90 nm / 325 nm / 90 nm thick PECVD SiO₂/Si₃N₄/SiO₂ was deposited. Access holes were then etched through the passivation layer in by reactive ion etching, reaching the Cr sacrificial layer. Lastly, the sacrificial layer was etched by placing a drop of chromium etchant (BASF, Chromium Etch Selectipur) at the inlets of the nanochannel. Additional details of the fabrication process have been described elsewhere.³⁶⁻³⁷

Electrochemical experiments in the nanodevice

The electrodes were cleaned prior to the measurements by cycling their potential in 0.5 M H₂SO₄ between -0.15 and 1.2 V at 50 mV/s until a stable voltammogram was obtained. The devices were rinsed with MilliQ water and a polydimethylsiloxane (PDMS) reservoir was positioned above the device which and was filled with a 2 mM solution of cystamine and left overnight. Subsequently, the devices were rinsed with a copious amount of water and filled with a 0.2 mM Fc-PEG-NHS solution in water for 3 h or the incubation times indicated in Figure 6.5, after which the device was immersed in a beaker with MilliQ water which was vigorously stirred for at least 5 min (control measurements have shown that this is sufficient time to remove all physisorbed PEG chains from the channel, see Figure 6.6). Before measurements, the devices were filled with a 1M NaClO₄ supporting electrolyte solution, and a standard Ag/AgCl electrode (BASi, MF 2079, RE-5B) inserted in the PDMS reservoir was used as the reference electrode. No counter electrode was used as the current through the reference is minimal in this configuration. Two transimpedance amplifiers (Femto, DDP-300) were used to apply potentials to the two

working electrodes with respect to the reference electrode and monitor the currents through these electrodes.

Figure 6.6: Redox cycling currents of a nanodevice before (black) and after (red) rinsing in water, after functionalization with PEG10k and 2-mercaptoethanol instead of cystamine. Measurements were performed at a scan rate of 10 mV/s.

Assessment of electrode surface roughness

Devices were functionalized overnight with 2 mM 11-(ferrocenyl)undecanethiol in ethanol. Due to the high surface densities and suppression of the capacity by the long alkyl chains, surface densities could be determined for the top and bottom electrodes separately. The surface densities of the top and bottom electrodes were calculated as $\Gamma = 1.9$ and 0.59 nmol/cm^2 , respectively. Given that the surface density of a full ferrocene layer is 0.45 nmol/cm^2 ,¹ and assuming that both the electrodes are similarly functionalized, the determined surface densities imply a higher surface roughness for the top electrode than the bottom (4.2 and 1.3, respectively, for this particular device).

Macro electrode functionalization

The Fc-PEG surface densities at different incubation times were measured on macro electrodes (2 mm diameter gold disc electrodes; CH Instruments). Before modification the electrodes were polished using 50 nm alumina particles (CH Instruments), followed by extensive rinsing with ethanol and 5 min of ultrasonic treatment in ethanol and 5 min in MilliQ water. Subsequently the electrodes were cleaned electrochemically in 0.5 M H_2SO_4 by applying an oxidizing potential

of 2 V for 5 s followed by a reducing potential of -0.35 V for 10 s. Then the electrode potential was scanned from -0.25 V to 1.55 V and back for 40 cycles at a scan rate of 100 mV/s. After cleaning the electrodes were rinsed with MilliQ water and ethanol and dried under a flow of N₂ and placed immediately in a 2 mM cystamine solution and left overnight. The surface densities of PEG were varied by changing the incubation time with a 0.2 mM PEG solution and determined from cyclic voltammograms measured at scan rates between 50 and 100 mV/s, using a CH instruments bipotentiostat 760D, with 1 M NaClO₄ as electrolyte vs. a Ag/AgCl reference electrode and a platinum counter electrode.

6.5 References

- (1) Hamburg, M. A.; Collins, F. S., The Path to Personalized Medicine. *N. Engl. J. Med.* **2010**, *363*, 301-304.
- (2) Wang, J., Electrochemical Biosensors: Towards Point-of-Care Cancer Diagnostics. *Biosens. Bioelectron.* **2006**, *21*, 1887-1892.
- (3) Kirsch, J.; Siltanen, C.; Zhou, Q.; Revzin, A.; Simonian, A., Biosensor Technology: Recent Advances in Threat Agent Detection and Medicine. *Chem. Soc. Rev.* **2013**, *42*, 8733-8768.
- (4) Perumal, V.; Hashim, U., Advances in Biosensors: Principle, Architecture and Applications. *J. Appl. Biomed.* **2014**, *12*, 1-15.
- (5) Rackus, D. G.; Shamsi, M. H.; Wheeler, A. R., Electrochemistry, Biosensors and Microfluidics: A Convergence of Fields. *Chem. Soc. Rev.* **2015**, *44*, 5320-5340.
- (6) Fan, C.; Plaxco, K. W.; Heeger, A. J., Electrochemical Interrogation of Conformational Changes as a Reagentless Method for the Sequence-Specific Detection of DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 9134-9137.
- (7) Xiao, Y.; Qu, X.; Plaxco, K. W.; Heeger, A. J., Label-Free Electrochemical Detection of DNA in Blood Serum Via Target-Induced Resolution of an Electrode-Bound DNA Pseudoknot. *J. Am. Chem. Soc.* **2007**, *129*, 11896-11897.
- (8) Xiao, Y.; Lou, X.; Uzawa, T.; Plakos, K. J. I.; Plaxco, K. W.; Soh, H. T., An Electrochemical Sensor for Single Nucleotide Polymorphism Detection in Serum Based on a Triple-Stem DNA Probe. *J. Am. Chem. Soc.* **2009**, *131*, 15311-15316.
- (9) Anne, A.; Demaille, C.; Moiroux, J., Terminal Attachment of Polyethylene Glycol (Peg) Chains to a Gold Electrode Surface. Cyclic Voltammetry Applied to the Quantitative Characterization of the Flexibility of the Attached Peg Chains and of Their Penetration by Mobile Peg Chains. *Macromolecules* **2002**, *35*, 5578-5586.
- (10) Anne, A.; Moiroux, J., Quantitative Characterization of the Flexibility of Poly(Ethylene Glycol) Chains Attached to a Glassy Carbon Electrode. *Macromolecules* **1999**, *32*, 5829-5835.

- (11) Blauch, D. N.; Saveant, J. M., Dynamics of Electron Hopping in Assemblies of Redox Centers. Percolation and Diffusion. *J. Am. Chem. Soc.* **1992**, *114*, 3323-3332.
- (12) Anne, A.; Bouchardon, A.; Moiroux, J., 3'-Ferrocene-Labeled Oligonucleotide Chains End-Tethered to Gold Electrode Surfaces: Novel Model Systems for Exploring Flexibility of Short DNA Using Cyclic Voltammetry. *J. Am. Chem. Soc.* **2003**, *125*, 1112-1113.
- (13) Huang, K. C.; White, R. J., Random Walk on a Leash: A Simple Single-Molecule Diffusion Model for Surface-Tethered Redox Molecules with Flexible Linkers. *J. Am. Chem. Soc.* **2013**, *135*, 12808-12817.
- (14) Hüsken, N.; Gëbala, M.; La Mantia, F.; Schuhmann, W.; Metzler-Nolte, N., Mechanistic Studies of Fc-Pna-(DNA) Surface Dynamics Based on the Kinetics of Electron-Transfer Processes. *Chem. - Eur. J.* **2011**, *17*, 9678-9690.
- (15) Smalley, J. F.; Feldberg, S. W.; Chidsey, C. E. D.; Linford, M. R.; Newton, M. D.; Liu, Y.-P., The Kinetics of Electron Transfer through Ferrocene-Terminated Alkanethiol Monolayers on Gold. *J. Phys. Chem.* **1995**, *99*, 13141-13149.
- (16) Weber, K.; Hockett, L.; Creager, S., Long-Range Electronic Coupling between Ferrocene and Gold in Alkanethiolate-Based Monolayers on Electrodes. *J. Phys. Chem. B* **1997**, *101*, 8286-8291.
- (17) Finklea, H. O.; Hanshew, D. D., Electron-Transfer Kinetics in Organized Thiol Monolayers with Attached Pentaammine(Pyridine)Ruthenium Redox Centers. *J. Am. Chem. Soc.* **1992**, *114*, 3173-3181.
- (18) Merchant, S. A.; Meredith, M. T.; Tran, T. O.; Brunski, D. B.; Johnson, M. B.; Glatzhofer, D. T.; Schmidtke, D. W., Effect of Mediator Spacing on Electrochemical and Enzymatic Response of Ferrocene Redox Polymers. *J. Phys. Chem. C* **2010**, *114*, 11627-11634.
- (19) Zevenbergen, M. A. G.; Krapf, D.; Zuiddam, M. R.; Lemay, S. G., Mesoscopic Concentration Fluctuations in a Fluidic Nanocavity Detected by Redox Cycling. *Nano Lett.* **2007**, *7*, 384-388.
- (20) Zhao, C.; Wittstock, G., An Scsm Detection Scheme with Improved Sensitivity and Lateral Resolution: Detection of Galactosidase Activity with Signal Amplification by Glucose Dehydrogenase. *Angew. Chem. Int. Ed.* **2004**, *43*, 4170-4172.
- (21) Rassaei, L.; Mathwig, K.; Kang, S.; Heering, H. A.; Lemay, S. G., Integrated Biodetection in a Nanofluidic Device. *ACS Nano* **2014**, *8*, 8278-8284.
- (22) Abbou, J.; Anne, A.; Demaille, C., Probing the Structure and Dynamics of End-Grafted Flexible Polymer Chain Layers by Combined Atomic Force-Electrochemical Microscopy. Cyclic Voltammetry within Nanometer-Thick Macromolecular Poly(Ethylene Glycol) Layers. *J. Am. Chem. Soc.* **2004**, *126*, 10095-10108.
- (23) Wang, K.; Goyer, C.; Anne, A.; Demaille, C., Exploring the Motional Dynamics of End-Grafted DNA Oligonucleotides by in Situ Electrochemical Atomic Force Microscopy. *J. Phys. Chem. B* **2007**, *111*, 6051-6058.
- (24) Rassaei, L.; Singh, P. S.; Lemay, S. G., Lithography-Based Nanoelectrochemistry. *Anal. Chem.* **2011**, *83*, 3974-3980.

- (25) Dam, V. A. T.; Olthuis, W.; van den Berg, A., Redox Cycling with Facing Interdigitated Array Electrodes as a Method for Selective Detection of Redox Species. *Analyst* **2007**, *132*, 365-370.
- (26) Goluch, E. D.; Wolfrum, B.; Singh, P. S.; Zevenbergen, M. A. G.; Lemay, S. G., Redox Cycling in Nanofluidic Channels Using Interdigitated Electrodes. *Anal. Bioanal. Chem.* **2009**, *394*, 447-456.
- (27) Samarao, A. K.; Rust, M. J.; Ahn, C. H. In *Rapid Fabrication of a Nano Interdigitated Array Electrode and Its Amperometric Characterization as an Electrochemical Sensor*, Proc. IEEE Sens., 2007; pp 644-647.
- (28) Ma, C.; Contento, N. M.; Gibson, L. R.; Bohn, P. W., Redox Cycling in Nanoscale-Recessed Ring-Disk Electrode Arrays for Enhanced Electrochemical Sensitivity. *ACS Nano* **2013**, *7*, 5483-5490.
- (29) Kätelhön, E.; Hofmann, B.; Lemay, S. G.; Zevenbergen, M. A. G.; Offenhäusser, A.; Wolfrum, B., Nanocavity Redox Cycling Sensors for the Detection of Dopamine Fluctuations in Microfluidic Gradients. *Anal. Chem.* **2010**, *82*, 8502-8509.
- (30) Xiong, J.; Chen, Q.; Edwards, M. A.; White, H. S., Ion Transport within High Electric Fields in Nanogap Electrochemical Cells. *ACS Nano* **2015**, *9*, 8520-8529.
- (31) For the determination of the surface densities, the electrodes could not be cycled individually as the two electrodes are capacitively coupled, giving rise to a situation analogous to the positive-feedback mode of SECM with an unbiased electrode. See: Oleinick, A. I.; Battistel, D.; Daniele, S.; Svir, I.; Amatore, C., Simple and Clear Evidence for Positive Feedback Limitation by Bipolar Behavior During Scanning Electrochemical Microscopy of Unbiased Conductors. *Anal. Chem.* **2011**, *83*, 4887-4893.
- (32) Bard, A. J.; Faulkner, L. R., *Electrochemical Methods: Fundamentals and Applications*. 2 ed.; John Wiley & Sons: New York, 2001.
- (33) Allen, C.; Dos Santos, N.; Gallagher, R.; Chiu, G. N. C.; Shu, Y.; Li, W. M.; Johnstone, S. A.; Janoff, A. S.; Mayer, L. D.; Webb, M. S.; Bally, M. B., Controlling the Physical Behavior and Biological Performance of Liposome Formulations through Use of Surface Grafted Poly(Ethylene Glycol). *Biosci. Rep.* **2002**, *22*, 225-250.
- (34) De Gennes, P. G., Conformations of Polymers Attached to an Interface. *Macromolecules* **1980**, *13*, 1069-1075.
- (35) Liu, B.; Bard, A. J.; Mirkin, M. V.; Creager, S. E., Electron Transfer at Self-Assembled Monolayers Measured by Scanning Electrochemical Microscopy. *J. Am. Chem. Soc.* **2004**, *126*, 1485-1492.
- (36) Zevenbergen, M. A. G.; Wolfrum, B. L.; Goluch, E. D.; Singh, P. S.; Lemay, S. G., Fast Electron-Transfer Kinetics Probed in Nanofluidic Channels. *J. Am. Chem. Soc.* **2009**, *131*, 11471-11477.
- (37) Kang, S.; Mathwig, K.; Lemay, S. G., Response Time of Nanofluidic Electrochemical Sensors. *Lab Chip* **2012**, *12*, 1262-1267.

Summary

Electron transfer processes play an important role in the development of nanoelectronics and biosensors. Therefore it is important to understand and to optimize these processes. Self-assembled monolayers (SAMs) provide a highly tunable platform which can be implemented on metallic electrodes for the use of electrochemical techniques. The research described in this thesis considers two different types of monolayer surfaces with the aim to tune and study their electron transfer properties.

Chapter 2 provides a general overview of several electrochemical techniques and how they can be used in the determination of electron transfer rate constants. A further focus is on flexible linear probes such as DNA for the use in sensors, in which their flexibility is altered upon binding of a complementary sequence and the resulting conformational changes affect the signal output.

In the first part of this thesis (Chapters 3 and 4) six multivalent β -cyclodextrin (β -CD) adsorbates have been described. The effect of the different functional groups on the adsorption kinetics, thickness, layer stability has been evaluated (Chapter 3). Even weakly binding anchoring groups gave rise to rather stable monolayers and several of these compounds were selected for further study. These novel β -CD adsorbates were designed with their cavity in close contact to the gold electrode. The effect of this small distance on the electron transfer rates, when compared to placing the β -CD core at a distance from the surface by long alkyl tethers, was studied using different electrochemical probes (Chapter 4). Decreasing the distance between the β -CD core and the underlying substrate led to an increase in the electron transfer rates.

In the second part of this thesis (Chapters 5 and 6), the electron transfer to/from ferrocene (Fc) moieties tethered to long linear poly(ethylene glycol) (PEG) molecules attached to a gold electrode was studied. In Chapter 5, the Fc-PEGs were attached to macro electrodes. The electron transfer to/from the Fc moieties was shown to be governed by bounded diffusion, and the effects of conformational changes by means of tether length and surface density were probed. The introduction of the Fc-PEG molecules between two nanospaced electrodes (Chapter 6) showed that the bounded diffusion made it possible to

shuttle electrons between two electrodes. This provides a powerful mechanism for signal-enhanced biosensor devices.

The results described in this thesis show that the versatility of SAMs makes it possible to tune the properties of these layers to enhance or modify the electron transfer properties. Electrochemistry provides a powerful tool for the characterization of these layers. The results presented here provide a contribution to the development of electrochemical biosensing devices.

Samenvatting

Elektronenoverdrachtsprocessen spelen een belangrijke rol in de ontwikkeling van nanoelektronica en biosensoren. Daarom is het belangrijk om deze processen te begrijpen en te optimaliseren. Zelf-assemblerende monolagen (SAMs) vormen een veelzijdig platform dat toegepast kan worden op metallische elektroden voor het gebruik van elektrochemische technieken. Het in dit proefschrift beschreven onderzoek beschouwt twee verschillende typen monolaagoppervlakken met als doel de elektronoverdrachtseigenschappen te kunnen controleren en te bestuderen.

Hoofdstuk 2 geeft een algemeen overzicht van verschillende elektrochemische technieken en hoe deze gebruikt kunnen in de bepaling van elektronenoverdrachts-constanten. Verder ligt er een focus op flexibele lineaire moleculen zoals DNA voor het gebruik in sensoren, waarin de flexibiliteit verandert na binding met een complementaire sequentie. De resulterende conformationele veranderingen beïnvloeden daardoor het uitgangssignaal.

In het eerste deel van dit proefschrift (hoofdstukken 3 en 4) worden zes multivalente β -cyclodextrine- (β -CD-)adsorbaten beschreven. Het effect van de verschillende functionele groepen op de adsorptiekinetiek, laagdikte en stabiliteit is geëvalueerd (hoofdstuk 3). Zelfs zwak bindende functionele groepen bleken een redelijk stabiele monolaag te geven en een aantal van de ontwikkelde β -CD-adsorbaten werden geselecteerd voor verdere studie. Deze nieuwe β -CD-adsorbaten zijn ontworpen zodat hun holte vlakbij de goud-elektrode gepositioneerd kon worden. Het effect van deze kleine afstand op de elektronoverdrachtssnelheid, vergeleken met een β -CD-holte op afstand van het oppervlak gefaciliteerd door lange alkylketens, werd bestudeerd door middel van verschillende elektrochemische sondes (hoofdstuk 4). Het verminderen van de afstand tussen de β -CD-kern en het onderliggende substraat veroorzaakte een stijging in de elektronenoverdrachtssnelheid.

In het tweede deel van dit proefschrift (hoofdstukken 5 en 6) werd de elektronenoverdracht van en naar ferroceen- (Fc-)groepen, vastgezet op goud-elektroden via lange lineaire poly(ethyleenglycol)- (PEG-)moleculen, bestudeerd. In hoofdstuk 5 werden de Fc-PEGs vastgezet aan macro-elektroden. De elektronenoverdracht van en naar de Fc-groepen werd gestuurd door

gebonden diffusie, en de effecten van conformationele veranderingen aan de hand van het aanpassen van de PEG-lengte en de oppervlaktedichtheid werden bestudeerd. Het aanbrengen van de Fc-PEG-moleculen tussen twee elektroden met een onderlinge afstand van 65 nm (hoofdstuk 6) liet zien dat de gebonden diffusie het mogelijk maakte om elektronen tussen de twee elektroden te laten bewegen. Dit biedt een krachtig mechanisme voor signaalversterkende biosensor-apparaten.

De in dit proefschrift beschreven resultaten laten zien dat de veelzijdigheid van SAMs het mogelijk maakt de eigenschappen van deze lagen aan te passen om de elektronenoverdrachtseigenschappen te versterken of aan te passen. Elektrochemie biedt krachtige methodes voor de karakterisering van deze lagen. De hier beschreven resultaten leveren een bijdrage aan de ontwikkeling van elektrochemische biosensor-apparaten.

Acknowledgements

And so we find ourselves at the end of this thesis, the result of several years of work. Now that the end of my PhD is near and my defense is only a few weeks away it is time to reflect a bit on the past years and give credit where credit is due. Because, without a doubt none of this would have been possible without the help and support of a large number of people.

To start at the top, special thanks go out to my promotors Jurriaan en Pascal who gave me the chance to work in MnF. Thanks to you both for being inspiring and for putting me back on track when I felt a bit stuck and helping me get my first grasp on electrochemistry. You both played a big part in me pursuing this path, Pascal having been my supervisor since my master project and Jurriaan going all the way back to my bachelor project.

A lot of our early experiments were performed in the BIOS labs, thanks to Albert van den Berg for the opportunity to be part of the ELab4Life project. Maarten, you were my first collaborator, it's a shame that our devices never turned out to be useful for the purposes we had in mind, but I'm glad you got some good results out of them for a different project. Whether you liked it or not, you also became my resident expert on all things related to electrochemistry. Thanks for the many, many fruitful discussions and sharing my frustrations when we started figuring out how to measure on electrodes.

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Bernard Boukamp were also of great value and of course thanks for being part of my committee.

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Again, many thanks to everyone who was part of this journey and for the fond memories and experiences!

Tom

Curriculum Vitae

Tom Steentjes was born on the 18th of October 1982 in Doetinchem, the Netherlands. He studied Chemical Engineering at the University of Twente in Enschede, the Netherlands, where he received his Bachelor degree in 2007. His Bachelor thesis was entitled “Surface diffusion of two divalent molecular inks on a molecular printboard” and was performed in the Supramolecular Chemistry and Technology group under the supervision of prof. dr. ir. Jurriaan Huskens.

In December 2010 he received his MSc degree in Chemical Engineering. His internship, entitled “Immobilization of quantum dots on a functionalized surface”, was carried out at the Institute of Materials Research and Engineering in Singapore, under the supervision of prof. dr. G.J. Vancso and dr. N. Tomczak. His master thesis, entitled “Light-induced immobilization of a novel perylene bisimide by the thiol-ene click reaction”, was performed in the Molecular NanoFabrication group under the supervision of prof. dr. ir. Jurriaan Huskens and prof. dr. ir. Pascal Jonkheijm.

Since January 2011 he has been working as a PhD candidate in the Molecular NanoFabrication group under the supervision of prof. dr. ir. Jurriaan Huskens and prof. dr. ir. Pascal Jonkheijm. The results of this research are described in this thesis.