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A non-linear harmonic analysis of potential induced fluorescence modulation of a DNA self assembled monolayer

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ABSTRACT

The characterization of a mixed mercaptohexanol - fluorophore labeled DNA self-assembled monolayer (SAM) on a single crystal gold bead electrode is demonstrated using an electrochemical perturbation and optical harmonic analysis scheme. This approach relies on the non-linear characteristics of the fluorescence intensity response towards a single frequency sinusoidal potential perturbation (163 Hz) which causes the DNA SAM to reorient, changing the efficiency of fluorescence quenching by the gold electrode. This results in a modulated fluorescence intensity that is composed of first, second and third harmonic signals which were found to strongly depend on the hybrization state of the DNA SAM, the DNA packing density on the surface, and the surface crystallography. Comparing the results between the different surface crystallographies on the same electrode and between different electrodes was simplified with a self-consistent normalization method reducing the influence of the raw fluorescence intensity. A significant difference in the second harmonic response was observed for single and double stranded DNA SAMs especially on the lower coverage (100) region and surfaces with related crystallographies. The third harmonic signal was weaker, but also showed a distinction between the hybridization states. Higher coverage (311) surface regions showed no significant fluorescence modulation nor any difference based on state of hybridization. Within a limited potential range, the electrochemical response (current) from the modified electrode did not show significant non-linearity revealing that the source of the harmonic signals was due to the non-linear relationship between the fluorescence quenching and fluorophore separation from the electrode surface. Increasing the amplitude of the potential perturbation increased the harmonic signal magnitudes further differentiating the DNA hybridization state.

1. Introduction

The use of AC modulation techniques has proven to be an effective method for characterizing and analyzing electrochemical interfaces as detailed in many excellent textbooks and monographs.[1–4] A number of electrochemical processes can result in a non-linear relationship between potential and current [5–9] and therefore a small amplitude perturbation is used when analyzing the interface using linear circuit elements (e.g., resistors, capacitors) as done in electrochemical impedance spectroscopy (EIS).[3,4] Many approaches are available that test for non-linear character in the EIS measurement [10,11] one of which is measuring the magnitude of second and higher harmonic responses.[12,13] Moreover, these higher frequency harmonic responses can be purposely used for electrochemical analysis (e.g., finding kinetic parameters as well as the uncompensated resistance and double layer capacitance), [14,15] in addition to determining the electrical characteristics of a diode.[16] The magnitude of the non-linear response can be enhanced through the use of large amplitude potential perturbations [17–19] and when coupled with harmonic analysis was used in the characterization of adsorbed redox species.[20–22] Also known as non-linear EIS, it has been used to study the fractal electrode surface [7], redox kinetics [23,24,6,25] electrocatalysis relevant to fuel cells [26,27]. The accurate measurement of these signals is facilitated by the computerization of modern impedance methods so that Fourier Transform instruments, or frequency response analysis (FRA) can

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measure the impedance over a wide range of frequencies, with high fidelity measurements at the frequency of the perturbation in addition to the harmonic signals. This is well described by Lasia [3] and Macdonald [4]. In addition digital lock-in-amplifiers, in contrast to their analog counterparts, are able to measure the higher harmonic signals without contamination from other harmonics.[3]

Most impedance analyses characterize purely electrochemical systems by measuring potentials or currents, or through the variation of potential and measurement of the resulting optical signal modulation as in electroreflectance methods.[28–31] These impedance methods can also be used with an optical perturbation (modulated light intensity) generating an oscillating current (photocurrent) or potential (photovoltage) and were used to study illuminated semi-conductor or dye sensitized solar cells.[32–36] A fluorophore containing DNA self-assembled monolayer(SAM) adsorbed onto a gold surface was also characterized using an optical based electrochemical impedance method.[37,38,30] Under constant intensity illumination, modulation of the fluorescence emission from the modified DNA was accomplished through a modulated charging / discharging of the electrode surface. Electrostatic repulsion or attraction of the negative charged DNA backbone changed the distance the of fluorophore from the gold surface. This effectively changed the efficiency of the fluorescence quenching by the metal surface, resulting in a potential dependent fluorescence intensity. The nature of the DNA SAM (single or double strand) was found to have an impact on the fluorescence response changing the fluorescence - potential relationship presumably due to the stiffness of the dsDNA SAM as compared to the ssDNA SAM given the large changes in persistence length with hybridization.[39,40] The motion of the DNA SAM was shown to be significantly slowed when protein is bound to the DNA by using capture molecules on the end furthest from the surface, enabling the measurement of the hydrodynamic radius of the protein using potential steps and time resolved fluorescence measurements.[41] Using linear impedance methods, we demonstrated correction of the fluorescence response by deconvoluting the effects of the electrochemical time constant which results in an accurate measurement of the movement of the DNA in response to the electrode charge. [42,43] This study also showed that the electrochemical characteristics of the DNA SAM (within a restricted potential range) was modeled very well with a RC circuit even at the rather high voltage amplitudes used (200 mV p-p).

This fluorophore labeled DNA SAM electrochemical interface is an ideal system for investigating the non-linear response of the optical signal that results from perturbing a linear electrochemical system because of the nature of the quenching behavior of a fluorophore near a metal surface.[44] The fluorescence intensity - potential characteristics are non-linear and we show that the optical response is composed of multiple harmonics which can be used to characterize the electrochemical interface. In essence, this system has linear electrochemical response characteristics but responds non-linearly with regards to the optical response. So unlike the typical electrochemical systems that are studied by non-linear EIS, the potential that drops across the interface is well defined as is the current response, which significantly simplifies the interpretation of the optical-electrochemical harmonic response. In this work, a sinusoidal voltage perturbation is used to generate a modulated fluorescence emission intensity response from a fluorophore labelled DNA self assembled monolayer (SAM). The response is non-linear and harmonic analysis can be performed to characterize the biosensor surface. We compare the response from ssDNA, dsDNA and hybridized ssDNA SAMs prepared on a Au electrode at a low surface density using the ligand-exchange method.[45,46] In addition, the use of a single crystal Au bead electrode enables a study of the influence of surface crystallography on the environment of the assembled DNA SAM. A static picture of the DNA SAM surface previously showed that the coverage was quite dependent on the nature of the surface in a systematic manner, corresponding to systematic changes in the surface crystallography.[46] The harmonic analysis of the dynamics of the potential induced DNA SAM movement is measured and is shown to depend on the DNA surface density, the structure of the adsorbed layer and underlying surface atomic arrangement, and on the extent of hybridization. A self-consistent approach to comparing these different surfaces on the same electrode and on different electrodes is outlined. Surface hybridization of the ssDNA SAMs and their denaturation are then compared revealing a possible sensing methodology based on harmonic analysis.

2. Experimental

2.1. Preparation of MCH-DNA SAMs

The MCH-DNA SAM surfaces were prepared by the thiol exchange method which results in a two-component SAM modified surface with a low density of DNA in a mercaptohexanol (MCH, Aldrich, ≥99%) modified gold surface [45,47,42,43]. These modified surfaces are prepared on a flame annealed single crystal Au bead electrode (prepared as detailed in [46]). The doubly modified DNA HS-C6-DNA-AlexaFluor647 was initially synthesized as a disulfide and purified with HPLC (by Integrated DNA Technologies). The DNA sequence is 5'-CTG-TAT-TGA-GTT-GTA-TCG-TGT-GGT-GTA-TTT-3', the same sequence used in [46]. Before use, the disulfide DNA was dissolved in a buffer solution containing 10 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol (Tris, Sigma, ≥99.9%) and 100 mM NaCl (Sigma-Aldrich, BioXtra, ≥99.5%) and incubated with an excess (>100 fold) of tris(2-carboxyethyl) phosphine (TCEP, Aldrich, ≥98%) to reduce the disulfide DNA to a thiol. This solution of thiol modified DNA was purified by passing through a MicroSpin^{TM} G-50 column (GE Healthcare) following the manufacturer's protocol and further diluted with an immobilization buffer (IB) containing 10 mM Tris, 100 mM NaCl and 50 mM $MgCl_2$ (Sigma-Aldrich, BioXtra, ≥99.0%) (pH = 7.5 (± 0.05) adjusted with HCl) to a designated concentration. To prepare a low DNA coverage MCH-DNA SAM, a clean substrate Au bead electrode was first modified by immersion in 1 mM MCH (in MeOH (Fisher, HPLC grade, ≥99.9%)) for 120 min. After being rinsed with MeOH and water, the electrode was immediately immersed in a 0.5 μM HS-C6-DNA-AlexaFluor647 solution for only 120 min. to ensure a low DNA coverage SAM. The resultant MCH-DNA SAM-modified electrode was stored in IB for at least 48 h prior to further characterizations. This was sufficient to remove all nonspecifically adsorbed DNA present on these SAMs and no potential stepping pretreatments were required.[48] All water used was ultrapure MilliQ water (18.3 MΩ cm, <3 ppb TOC).

This preparation method was used for both MCH-ssDNA and MCH-dsDNA SAMs except that for the MCH-dsDNA SAMs the deposition solution containing dsDNA was prepared ahead of time. This was achieved by heating a 1:2 mixture of HS-C6-DNA-AlexaFluor647 and its corresponding complementary strand in IB to about 90 °C and then slowly cooling it down to room temperature over 1 h. Hybridization and dehybridiation of the SAM surfaces were also performed so as to make MCH-dsDNA and MCH-ssDNA SAMs, respectively. Hybridization of the MCH-ssDNA SAM surface was performed by immersing the modified electrode into either a 10 or 100 nM complementary strand in IB for 24 h. Dehybridization of the MCH-dsDNA SAM surface was performed by immersing the modified electrode into 8 M urea in water for 2 min.