Potential-Dependent Stochastic Amperometry of Multiferrocyenylthiophenes in an Electrochemical Nanogap Transducer

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Supporting Information

ABSTRACT: In nanofluidic electrochemical sensors based on redox cycling, zeptomole quantities of analyte molecules can be detected as redox-active molecules travel diffusively between two electrodes separated by a nanoscale gap. These sensors are employed to study the properties of multiferrocyenyl compounds in nonpolar media, 2,3,4-triferoce

1. INTRODUCTION

There is great interest in miniaturized electrochemical sensors for their ease of integration in lab-chip applications, highly sensitive detection, the requirement of only the smallest sample volumes, direct electrical signal transduction, and cost-effectiveness when standard microfabrication is employed.1−8 We previously reported on electrochemical nanogap sensors which allow sensitive analysis in femtoliter detection volumes.9 In these systems, electrochemically active analyte molecules shuttle by diffusion between two electrodes embedded in the roof and ceiling of a nanochannel (Figure 1). Thereby, the molecules undergo redox cycling, i.e., they are repeatedly oxidized and reduced at the opposing electrodes at kilohertz frequencies. In this way, each analyte contributes a current of several thousand electrons per second, resulting in a high intrinsic signal amplification (as compared to a single-electrode configuration in which a molecule reacts only once).

Nanogap sensors are currently rare in their ability to perform stochastic amperometric sensing.10 Only a small number of molecules are present in a very limited detection volume, even at high analyte concentrations. Therefore, the system can be described as a mesoscopic regime instead of a continuum: All molecules undergo a random Brownian walk and diffuse in and out of the nanofluidic channel and a coupled reservoir. This results in pronounced fluctuations of the molecular number density in the detection volume.11 These fluctuations are reflected in the recorded electrical current; they can directly be probed amperometrically. Molecular-level information is then gleaned from fluctuations in current—time traces. In particular, diffusion coefficients as well as specific dynamic adsorptivities were determined by analysis of the amplitude12 and frequency spectrum13,14 of current fluctuations.

To date, nanogap sensors have exclusively been employed for the detection of archetypical organometallic or metal—organic compounds such as ferrocenes or catechol derivatives; experiments were limited to conditions of a polar solvent (water or acetonitrile), a high concentration of fully dissociated background electrolyte, and analytes with typically only one possible electron-transfer process.

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Electrochemical nanogap devices were fabricated by clean-room microfabrication on an oxidized silicon wafer substrate as described previously.\(^4\) They consist of a 5 μm wide, 10–20 μm long, and 70 nm high nanochannel in silicon oxide/silicon nitride; the roof and ceiling of the channel are Pt electrodes. The length and width of the nanochannel are defined by photolithography; the nanoscale height is defined by evaporation of a sacrificial Cr layer sandwiched between two Pt layers, which were also defined and fabricated by photolithography and electron-beam deposition, respectively. After these three metal deposition steps, the structure was buried in a Si\(_3\)N\(_4\)/SiO\(_2\) passivation layer by plasma-enhanced chemical vapor deposition. Access holes were subsequently plasma-etched into this layer to connect the nanodevice to a fluidic reservoir. Just before an experiment, the nanochannel was released by a selective wet-chemical etch of the sacrificial Cr layer (Cr etchant Selectipur, BASF). A micrograph and schematic view of a nanofluidic electrochemical device are shown in Figure 1.

### 2. EXPERIMENTAL SECTION

#### 2.1. Chemical Reagents and Nanofluidic Device Fabrication

2,5-diferrocenylthiophene and 2,3,4-triferrocenylthiophene (2,5-Fc\(_2\)C\(_4\)HS and 2,3,4-Fc\(_3\)C\(_4\)HS) were synthesized in a Pd-promoted Negishi C\(_2\) cross-coupling reaction of 2,5-dibromothiophene or 2,3,4-tribromo-thiophene with ferrocenyl zinc chloride, as reported previously.\(^21\) As electrolyte, an 0.1 M solution of tetrabutylammonium tetrakis-(pentfluorophenyl)borate was used, whereby \([\text{N}^+\text{Bu}_4][\text{B}(\text{C}_6\text{F}_5)_4]\) was prepared by metathesis of lithium tetrakis(pentafluorophenyl)borate etherate (Boulder Scientific) with tetrabutylammonium bromide according to a published procedure and enhanced by a filtration step of the crude product through a pad of silica using dichloromethane as solvent.\(^40\)

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#### 2.2. Electrochemical Instrumentation and Experimentation

Using a home-built potentiostat setup, two sensitive operational amplifiers (Femto DDCPA-300) were separately connected to the top and bottom electrodes of a nanofluidic device. The amplifiers were LabView-controlled and used as current meters and voltage sources (Figure 1b). A micro-manipulator was used to position a reservoir in polydimethylsiloxane (PDMS) on top of the access holes. After the wet-etch of the chromium layer, the etchant was replaced by sulfuric acid (Sigma-Aldrich), prepared as a 0.5 M solution in Milli-Q water, and both Pt electrode surfaces were cleaned by repeatedly sweeping their potential between −0.15 and 1.2 V versus a Ag/AgCl reference electrode (BASI inc.) placed in the reservoir.

The PDMS reservoir was then replaced by a reservoir machined in polytetrafluoro-ethylene and connected to the nanochannel via an O-ring (fluorinated propylene monomer, 0.5 mm inner diameter). The reservoir and nanochannel were filled with the analyte-containing dichloromethane solution, and a double-junction Ag/Ag’ reference electrode (BASI inc.) was immersed into the reservoir. This reference electrode was constructed from a silver wire inserted into a Luggin capillary with a Vycor tip containing a solution of 0.01 M [AgNO\(_3\)] and 0.1 M of the supporting electrolyte in acetonitrile. This Luggin capillary was inserted into a second Luggin capillary with Vycor tip filled with a 0.1 M supporting electrolyte solution in dichloromethane. The whole setup was shielded from interference in a Faraday cage.

### 3. RESULTS AND DISCUSSION

#### 3.1. Cyclic Voltammetry

Cyclic voltammograms (CVs) of 2,5-diferrocenylthiophene and 2,3,4-triferrocenylthiophene (1 mM in 0.1 M \([\text{N}^+\text{Bu}_4][\text{B}(\text{C}_6\text{F}_5)_4]\) in dichloromethane) recorded at a Pt ultramicrodisk electrode (UME; BASI inc.) with a diameter of 10 μm are shown in Figure 2. In a stepwise oxidation, the analytes exhibit two (or three, respectively) well-resolved electrochemically reversible one-electron transfer processes with equal heights of the current steps. This is in good agreement with previous results.\(^37\)

By determining the magnitude of the steady-state currents, we estimate the diffusion coefficients, \(D\), of the fully reduced species to be 1.08 × 10^{-9} m^2/s for 2,5-Fc\(_2\)C\(_4\)HS and 0.96 × 10^{-9} m^2/s for 2,3,4-Fc\(_3\)C\(_4\)HS. Diffusion coefficients (\(D\)) were estimated by \(i = 4nFCDr\), where \(i\) is the height of a current step,
n the number of exchanged electrons, F Faraday constant, c the analyte concentration, and r the electrode radius. 

3.2. Cyclic Voltammetry in a Nanogap Device. Figure 3 shows voltammograms of both thiophene compounds recorded in a nanochannel device. The steps are not entirely as pronounced as for the measurements using the UME, which is most likely caused by the small nanochannel height, and f = F/RT (F, Faraday constant; R, gas constant; T, temperature). $D_{eff}$ is the effective diffusion coefficient; diffusion is effectively slowed by dynamic analyte adsorption due to the high surface-to-volume ratio in the nanochannel of 3 × 10⁷ m⁻³, typically to $D_{eff} = 0.2$–0.5 D in previous experiments. The fitting parameters were $i_{lim}$ and the dimensionless rate constant $D_{eff}/z_k$. The transfer coefficient was approximated by $\alpha = 0.5$.

Figure 2. Cyclic voltammograms of 2,5-diferrocenylthiophene (left) and 2,3,4-triferrocenylthiophene (right) detected in dichloromethane solutions (1 mM) at a Pt ultramicroelectrode with a diameter of 10 μm (supporting electrolyte 0.1 M [NBF₄][B(C₆F₅)₄]).

Figure 3. Cyclic voltammograms of both thiophene compounds recorded in a nanochannel device. The steps are not entirely as pronounced as for the measurements using the UME, which is most likely caused by the small nanochannel height, and f = F/RT (F, Faraday constant; R, gas constant; T, temperature). $D_{eff}$ is the effective diffusion coefficient; diffusion is effectively slowed by dynamic analyte adsorption due to the high surface-to-volume ratio in the nanochannel of 3 × 10⁷ m⁻³, typically to $D_{eff} = 0.2$–0.5 D in previous experiments. The fitting parameters were $i_{lim}$ and the dimensionless rate constant $D_{eff}/z_k$. The transfer coefficient was approximated by $\alpha = 0.5$.

Figure 4. (a) Effective diffusion coefficient of 2,3,4-Fc₂-C₄H₂S determined by the magnitude of fluctuations $i_{diff}$ in current–time traces. The “mean charge in $e$” corresponds to the applied potentials 0.25 V; 1 e = 0.5 V; 1.25 (“0.3”) = 0.75 V; 1.5 e (“1.2”) = 0.25–0.5 V; 2 e = 1 V. (b) Corresponding currents per molecule for 2,3,4-Fc₂-C₄H₂S. The gray lines indicate one-electron (lower line) and two-electron (upper line) transfer processes. (c) Effective diffusivities and currents per molecule for 2,5-Fc₂-C₄H₂S. Here the mean charge in $e$ corresponds to the potentials 0.25 V; 1 e = 0.5 V; 1.5 e = 0.25–0.5 V.

We believe that the shape of the cyclic voltammograms recorded in the nanochannel is dictated by the finite rate for heterogeneous electron transfer, as has been reported earlier for other couples. We estimated the electrochemical rate constants $k$. As shown in the dashed lines in Figure 3, the forward sweeps of the CVs were fitted to the Butler–Volmer expression for thin layer cells, using a superposition of $j$ one-electron reactions:

$$i(E) = \sum_{j=1}^{3} \left[ 1 + \exp\left( \frac{E - E_{j}^{0}}{F \alpha} \right) \right] \exp\left[ -\left( 1 - \alpha \right)f(E - E_{j}^{0}) \right]$$

Here $i_{lim,j}$ are limiting currents for the individual waves, $E_{j}^{0}$ formal potentials, $\alpha$ the transfer coefficient, $z = 70$ nm the nanochannel height, and $f = F/RT$ (F, Faraday constant; R, gas constant; T, temperature). $D_{eff}$ is the effective diffusion coefficient; diffusion is effectively slowed by dynamic analyte adsorption due to the high surface-to-volume ratio in the nanochannel of 3 × 10⁷ m⁻³, typically to $D_{eff} = 0.2$–0.5 D in previous experiments. The fitting parameters were $i_{lim}$ and the dimensionless rate constant $D_{eff}/z_k$. The transfer coefficient was approximated by $\alpha = 0.5$. Formal potentials...
The current per molecule, $i_p$, depends only on the nanochannel height, $z$; the number of electrons transferred, $n$; and the diffusivity, $D_{eff}$ (and on the elementary charge, $e$):

$$i_p = neD_{eff}/z^2 \quad (n = 1, 2, 3) \quad (3)$$

By using eqs 2 and 3 as well as the limiting current ($i_{lim}$) and $i_p$ can directly be determined, i.e., $i_p = i_{lim}/n$. The results of this fluctuation analysis are shown in Figure 4a. Here, the effective diffusion coefficient, $D_{eff}$ is plotted as a function of applied potential represented by the nominal “mean charge in e”.

The slightly faster diffusion of 2,5-Fc$_2$·C$_4$H$_2$S compared to 2,3,4-Fc$_3$·C$_4$H$_2$S is expected because of its smaller size. The observed reduced diffusivities at higher oxidation states are also not unexpected. For example, the diffusion coefficient of ferrocenemethanethiol in aqueous solution is reduced by 20% in its oxidized state because of a changed solvation shell. In the case of the ferrocenylthiophenes, a similarly strong effect is not expected because of the very large size of the electrolyte ions in a nonpolar solvent. Several origins or a combination of effects can lead to the observed change in $D_{eff}$, namely, a change in bulk diffusivity, a change in dynamic adsorptivity, a slightly reduced limiting current due to slow kinetics, as well as a contribution by electrical migration. The increased $D_{eff}$ for the spectroscopic determination compared to the current magnitude analysis (Figure S2 in the Supporting Information) hints at an anisotropic contribution by migration which is captured in $i_{ad}$ but not in $i_p$ because only longitudinal diffusion contributes to the crossover frequency (shuttling in and out of the nanochannel), while fluctuations on all time scales (including diffusion across the channel) are captured in $i_{ad}$.

A change in electrostatic adsorption can be ruled out as a cause for the decreased effective diffusivity because more positively charged analytes would be repelled from more positively charged electrode surfaces, but a decrease in $D_{eff}$ is observed for these conditions.

Ultimately, while clear trends are observed for $D_{eff}$ and $i_p$, so far it is not possible to distinguish the contribution of adsorption and changed diffusivities to these observations. While transport is hindered in the nanochannel, very large currents per molecule of up to 15 fA at room temperature were measured for high overpotentials (Figure 4b). This shows that compounds exhibiting multielectron transfer processes can have a potential application in single-molecule electrochemistry.

4. CONCLUSIONS

We introduced a new electrochemical method to determine the diffusive properties as a function of the oxidation state of complex electrochemically active molecules in a stochastic amperometric measurement. The experiments show that electrochemical nanogap sensors are suitable to work under conditions of a very volatile solvent, enabling a more complex analysis of novel compounds. For the investigated oligoferrocenyl thiophenes, our measurements show a decrease in effective diffusivity for an increase in applied potentials and oxidation state of the compounds. Moreover, highest currents per molecule of 15 fA per molecule were determined, which compares well to previous single-molecule experiments. For future experiments, we plan to investigate the origins of changes in effective diffusivity by determining the influence of electrical migration as well as to extend the method from identifying the properties of a redox couple to determining the diffusion of a specific oxidation state.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.6b07320.

Power spectral density analysis of current–time traces (PDF)
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Notes

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