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Electrostatic Read Out for Label-Free Assays Based on Kelvin Force Principle

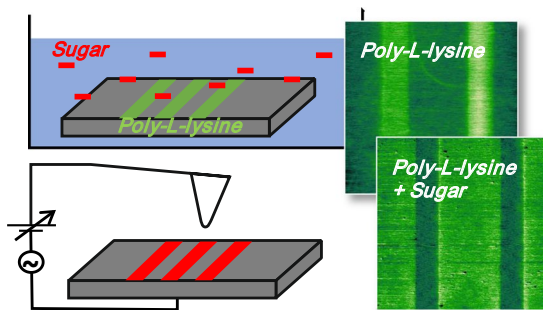
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Abstract

Detection methods and analytical devices have drawn increasing attention in recent years due to their direct impact on early detection, monitoring and diagnosis of disease in medical research. In this work, we describe a simple but so far unrecognized label-free method for surface-bound analyte detection, which could be applicable to a wide range of substances. In this respect, the feasibility and practical aspects of a micrometer-scale, poly-L-lysine-based, solid-phase assay for label-free analyte detection with electrostatic read-out are investigated. Micropatterned poly-L-lysine layers were produced using soft-lithography on mica and their electrostatic surface potential was determined using Kelvin-probe Force Microscopy. Ribose, a natural sugar, was used as analyte. Upon exposure to ribose, the surface potential changed from positive to negative in a reversible manner. We report for the first time the use of an electrostatic principle for assay read-out. This purely physical effect could be used to develop label- and marker-free assays for sugars, various other substances or, possibly, biosensors.

Graphical Abstract



Keywords Kelvin-probe · Soft-lithography · Sugar · Atomic force microscopy · Ribose

Extended author information available on the last page of the article

1 Introduction

High-throughput and parallel detection of minimal amounts of biochemical substances can be achieved using the microarray format as in DNA chips or protein microarrays [1]. These are technically a form of microscopic solid-phase assay, that is, an analyte binding-partner is attached on a solid support and then binds to the desired analyte(s) [2]. Analyte-binding is then read out using a physical quantity such as an optical (e.g. fluorescence, colourimetry, transmittance, plasmon resonance, etc.) or a radiative signal (radio-immunoassay).

A drawback of these read-out methods is that they often require some form of labelling (fluorescence, radio-labelling, etc.) or the introduction of additional materials (e.g. marker particles), which could interfere with the actual analyte binding mechanism.

A label-free read-out alternative is the use of an electrostatic signal, that is, the detection of changes of the electrostatic surface potential of the solid support upon analyte-binding. This was demonstrated using the Kelvin-probe principle on the example of the well-established avidin–biotin reaction, complementary DNA-binding [3], aptamer-binding [4], or ligand-receptor binding [5]. In these assays, the analyte-binding mechanism is highly specific, as in the example of complementary DNA-binding [3]. However, situations are conceivable where a more generic mechanism could be useful. One such mechanism could be an ionic interaction of the analyte with its binding-partner.

In the present paper, we explore the combination of highly charged poly-L-lysine (PLL) as a generic analyte binding-partner with the Kelvin-probe method as the read-out (Fig. 1). The motivation and the advantage of PLL is that it readily adsorbs on typical, solid supports such as glass or mica in a defined and fairly homogenous layer, its properties are well-known, it is biocompatible (it is a component of many foods and is routinely used for enhancing cell adhesion), and it has a high charge due to the many lysines exposing the ionised ϵ -amine group. Especially the latter aspect is important for any electrostatic read-out as the Kelvin-probe principle, which essentially detects alterations of surface charge. An additional advantage of PLL is that its amine groups could serve as attack points for a variety of more specific, chemical reactions such as glycation, which typically starts with a Schiff-base formation between amine-groups of peptides and carbonyl groups of carbohydrates.

In order to investigate the basic properties and aspects of this concept, we employed Kelvin-probe Force Microscopy (KFM or KPFM) to detect alterations of the surface potential of the micropatterned PLL-structures (Fig. 1b, d). In KFM, a conductive tip is held at very close distance (a few nm) of the surface, an AC+DC voltage is applied between tip and surface (Fig. 1b), and the resulting tip oscillation is recorded [6]. There are a number of variants, such as amplitude modulation vs frequency modulation, or open-loop vs closed-loop methods [7]. However, all have in common that the electrostatic properties of the surface (surface potential or surface charge) ultimately influence the tip oscillation and, hence, the recorded signal. As our objective was to produce test structures on the

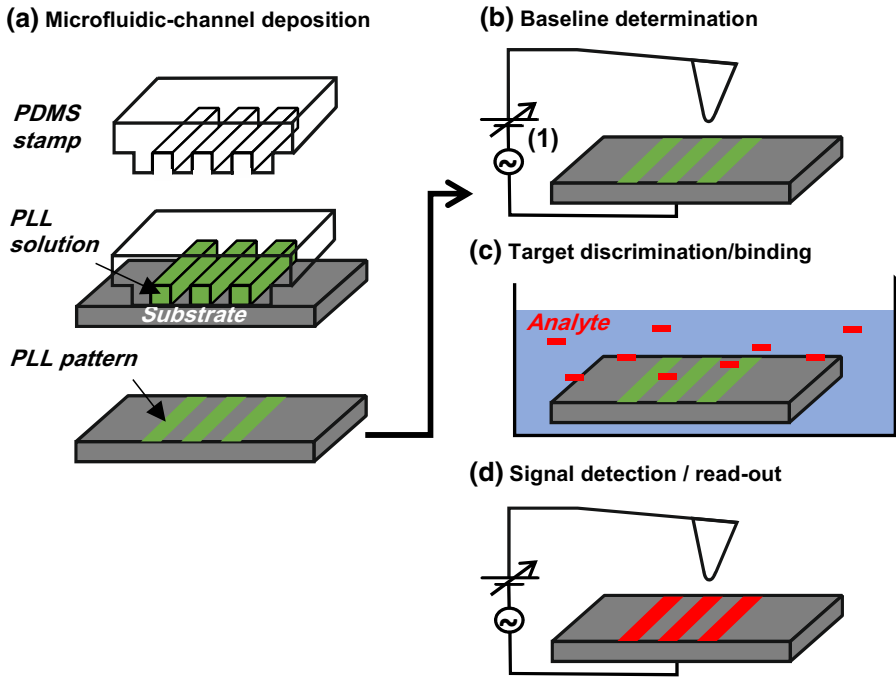


Fig. 1 General concept. **a** Pattern creation by deposition from solution in microfluidic channels, **b** determination of baseline, (1) = Kelvin-probe circuitry, **c** analyte binding, **d** electrostatic read-out of assay

micrometer scale, we used well-established soft-lithography techniques based on siloxane rubber stamps for micropatterning [8].

2 Experimental Methods

2.1 Patterning Methods

Elastomeric polydimethylsiloxane (PDMS) stamps were used to create PLL patterns. The stamps were produced using the procedure described in the literature [9] and the curing was performed by heating the polymer/curing agent mixture at 50 °C for 2 hours in a plastic petri dish on a hot plate. To deposit patterns of PLL the microfluidic-channel deposition method was used [10].

Microfluidic-channel deposition was performed by placing a fresh PDMS stamp (10 μm wide channels, 30 μm wide ridges) on the substrate (Fig. 1a). The channels were filled up by pipetting around 40 μL of poly-L-lysine solution (Sigma-Aldrich, CAS-#: 25988-63-0, 0.01% w/v in water) at the open ends of the channels. After 5 min, when the channels were visually filled up with PLL solution, deionised (DI) water was flushed into the channels and the stamp was removed. Finally, the sample was rinsed with DI-water and dried either with a gentle stream of nitrogen or a hand-held air bellows.

2.2 AFM/KFM

AFM/KFM was performed using a Dimension Icon PT AFM or a Multimode 8 AFM both with Nanoscope V controller (Bruker Corporation, Billerica MA, USA) in ambient air (relative humidity = 20–30%, temperature = 21–25 °C) (Fig. 1b, d). Conductive silicon tips (Veeco antimony (n) doped Si, NTESPA) were used with nominal spring constants of 40 N/m. The *surface potential* mode was used for KFM imaging. All images of a given data set were taken using the same imaging-parameters (lift height, controller gains, scan speeds, lines/image, etc.).

2.3 Image Analysis

All image data was analysed using the free, third-party data analysis software Gwyddion (gwyddion.net). AFM topography and KFM surface potential images were 1st-order or 2nd-order line-levelled excluding the features of interest (e.g. the PLL stripes in Fig. 2b) using the *mask* function. The average potential and RMS value of a designated area were determined using the *statistical quantities* function. All potential data represents the potential value of the area of interest minus the potential value of the immediately surrounding substrate.

3 Results and Discussion

3.1 Microfluidic-Channel Deposition

Figure 2 shows the results of patterning PLL layers on mica. The channels mechanically restricted the adsorption to stripes of 10 μm width with a 30 μm gap in-between. The topography images (Fig. 2a as an example) show barely any features, which indicates that the PLL layers are very thin, whereas the potential images (Fig. 2b) show a clear, positive signal of approximately +80 mV (Fig. 2d) with respect to the surrounding mica, with the geometry expected from the patterning procedure.

In order to be useable for subsequent target-binding and read-out by KFM, the PLL patterns need to be sufficiently stable, that is, their surface potential should not change significantly upon scanning or immersion in pure water, that is, without exposure to the actual analyte. It is conceivable that repeatedly scanning PLL patterns could alter their surface potential, for example through mechanical removal of PLL from the substrate or dissipation of surface charge through the conductive AFM tip during the scanning. Furthermore, although mica is a well-established and very suitable substrate for AFM-based experiments, the fact that highly mobile cations desorb from the mica surface when exposed to water and, possibly, migrate in the thin water layer present in ambient air, could lead to an unwanted alteration of the surface charge of mica, which would then introduce an unwanted artifact.

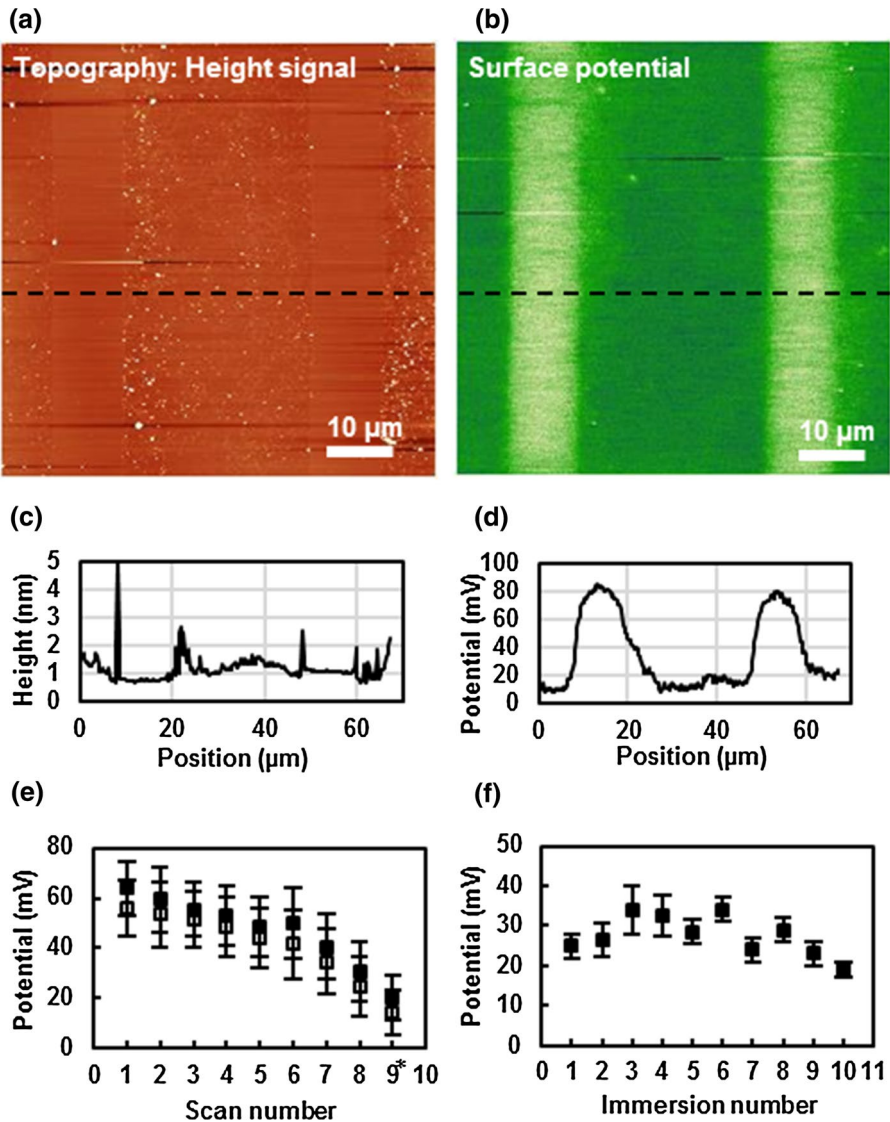


Fig. 2 PLL patterns by microfluidic-channel deposition. Nominal lift height=25 nm. Recorded with MultiMode 8 AFM. Topography (a), z-colour scale=20 nm; surface potential (b), potential colour scale=200 mV. Topography cross-section c at dashed line in a; surface potential cross-section d at dashed line in b. Diagram e shows PLL potential of subsequent scans in air; * = scan number 9 taken 48 h after scan number 8. Diagram f shows scans in air after immersion in DI-water for 20 min at each data point

Figure 2e shows the evolution of the PLL potential signal with repeated scans under the same conditions. It can be seen that, although the first 2–3 scans do not yet alter the signal significantly, it slowly decays to smaller values within 8 scans. This could be due to the aforementioned influences. Furthermore, scan number 9 was

taken 48 h after scan number 8 and the drop in signal observed indicates that there is a substantial amount of charge decay on the sample surface even when it does not come into contact with the tip. This charge decay could be due to surface migration of charge carriers, possibly caused by a thin layer of water on the sample.

The latter effect would be particularly prominent whenever immersing the substrate in the assay solution. We, therefore, investigated if repeatedly immersing the patterned substrate in DI-water caused signal decay. Figure 2f shows that the signal varies by about $\pm 20\%$ upon immersion (and subsequent drying) but no obvious trend could be discerned and, after the 10th immersion, the signal still has approximately the same value as after the 1st immersion of slightly more than +20 mV.

3.2 Application to Sugar Detection

As an assay-type application, we assessed the possibility of using PLL patterns for sugar detection. PLL patterns were produced as in Fig. 2 and were alternately immersed in DI-water for 1 min. and in 6-mM D-ribose solution for 1 min. After each immersion, the sample was dried by a gentle stream of air and then imaged by KFM.

Figure 3 shows the results of the experiment. The potential images show a clear and well-defined PLL pattern, which exhibits a positive potential of +50 mV to +100 mV with respect to mica after immersion in DI-water (Fig. 3b) and a negative potential of about -20 mV with respect to mica after immersion in ribose solution (Fig. 3d). Thus, a clear polarity reversal is observed upon exposure of the pattern to ribose.

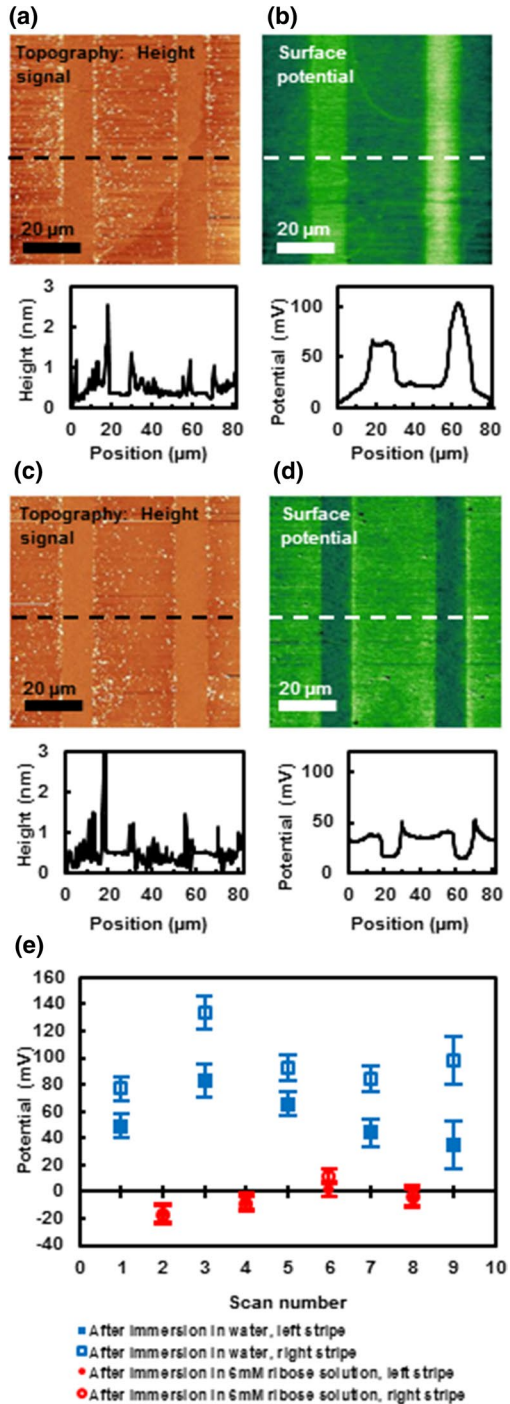
Figure 3e shows that the effect itself is reversible. Although there is a significant variation of the signal upon repeated immersions in water (blue data points in Fig. 3e), the signal drop upon immersion in the sugar solution is considerable greater and allows clear discrimination between pure water and sugar solution in a repeated manner.

The reversibility points to a physical adsorption process rather than to a (irreversible) chemical reaction between ribose and PLL. However, it is conceivable that, under different conditions, e.g. higher ribose concentration, higher temperature, longer incubation time, etc., a Maillard- or glycation-type reaction could occur as PLL offers many reactive amine groups at its surface.

4 Conclusions

In summary, we investigated practical aspects of using the Kelvin-probe principle as an electrostatic method to read out microstructured PLL assays and demonstrated the principle on the example of sugar detection. Regarding sensitivity, the serum fasting-level of ribose in human is 100 μM [11] Hence, the concentration of 6 mM we used here is still much higher. In contrast, reported values for experimental biosensors for glucose detection can range from 0.013 to 5.85 M [12] while others can reach as low as 300 nM as a detection limit [13]. Despite our system has not yet

Fig. 3 Detection of sugar by KFM. Nominal lift height = 50 nm. Recorded with Dimension Icon PT AFM. PLL pattern fabricated by microfluidic-channel deposition on mica, after immersion in DI-water: Topography (a), z-colour scale = 5 nm; surface potential (b), potential colour scale = 200 mV. The same pattern after immersion in 6-mM ribose solution: Topography (c), z-colour scale = 10 nm; surface potential (d), potential colour scale = 100 mV. Diagram e shows pattern potential with respect to mica after immersion in DI-water (blue) and after immersion in 6-mM ribose solution (red). Data points represent scans after repeated immersions in the solutions indicated (Color figure online)



been tested under low concentrations of sugar, it is well known that KFM is capable of detecting changes in surface potential in the order of a few mV [14], which is a remarkable feature to be exploited in label-free detection technology. The detection limit, linearity and sensitivity would need to be further investigated.

However, our results show that it is possible to read out a basic sugar assay and they highlight that the baselines determined in Fig. 2e, f need to form an inherent part of any assay protocol. In the present paper, we did not investigate the effect of interferents on the read-out signal, which should be performed in future works. Interference is usually minimized by ensuring high specificity between the substrate and the analyte of interest. Here, we used PLL for practical reasons. However, the baseline could easily be replaced by using different molecules in the patterning-step, favouring specificity with the analyte. Furthermore, selective membranes to minimize interference could be used prior to performing the read out.

Obviously, KFM as implemented in commercial AFMs would not be suitable for routine read-out of microarrays in a typical, analytical laboratory setting. This is trivial insofar AFM/KFM is a technique developed for high, spatial resolution microscopy and not for signal detection. How could the Kelvin-probe principle and its high sensitivity be implemented efficiently with the purpose of assay read-out? Even in a microarray design, where the spot size is typically in the 10 to 100 μm range [15], it is not necessary to image every spot with nm-resolution. In fact, for assay read-out, it is not even necessary to record any image at all. The only information that is needed is the surface potential signal before and after the analyte binding-event. Hence, AFM scanner and tip are not needed. It is conceivable that Kelvin-probe-based read-out could be achieved, for example, with a multiplexed array of tipless cantilevers hovering in close proximity to the microarray spots on the substrate. Similar set-ups were developed in the early days of AFM [16] and could be adapted to perform electrostatic detection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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