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Abstract

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A Label-Free Potentiometric Sensor Principle for the Detection of Antibody–Antigen Interactions

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Supporting Information

ABSTRACT: We report here on a new potentiometric biosensing principle for the detection of antibody–antigen interactions at the sensing membrane surface without the need to add a label or a reporter ion to the sample solution. This is accomplished by establishing a steady-state outward flux of a marker ion from the membrane into the contacting solution. The immunobinding event at the sensing surface retards the marker ion, which results in its accumulation at the membrane surface and hence in a potential response. The ion-selective membranes were surface-modified with an antibody against respiratory syncytial virus using click chemistry between biotin molecules functionalized with a triple bond and an azide group on the modified poly (vinyl chloride) group of the membrane. The bioassay sensor was then built up with streptavidin and subsequent biotinylated antibody. A quaternary ammonium ion served as the marker ion. The observed potential was found to be modulated by the presence of respiratory syncytial virus bound on the membrane surface. The sensing architecture was confirmed with quartz crystal microbalance studies, and stir effects confirmed the kinetic nature of the marker release from the membrane. The sensitivity of the model sensor was compared to that of a commercially available point-of-care test, with promising results.

Over the past few decades, important advancements in the field of diagnostics have been achieved as a result of the introduction of the enzyme-linked immune absorbent assay (ELISA) and polymerase chain reaction (PCR) based tests, which allowed large scale automation of serological testing and the amplification of nucleic acids, respectively.

Seventy percent of the in vitro diagnostic (IVD) market is currently shaped by tests based on these technologies for infectious pathogens.1 Despite their proven reliability, these tests are time-consuming and normally only performed in a laboratory environment. In case of acute infections, immediate availability of the results is often not possible, potentially resulting in a loss of precious time before a suitable therapy can be started.

It has been shown that respiratory syncytial virus (RSV) can cause lower respiratory tract disease (LRD) in infants and patients, for example, after hematopoietic cell transplantation (HCT) and result in substantial early mortality.5–6 Early disease detection and intervention, even initiated at a time when the viral load is at its highest, can improve disease outcome in previously healthy, naturally infected children.7 Indeed, early information about the infecting agent obtained from rapid diagnostic tests has been shown to significantly alter the management of the patient’s illness, resulting in a reduction in diagnostic tests performed, reduced antibiotic use, more accurate use of antivirals and better patient management in general.8

Unfortunately, current point-of-care (POC) diagnostic tests do not always meet the needs of patients and doctors since only qualitative results are obtained and the limit of detection is often insufficient.

Currently, no commercial POC tests are based on potentiometric readout. Modern potentiometric sensors are established in the determination of inorganic ions in environmental (e.g., F− and NH4+) and clinical applications (e.g., pH, Na+, K+, Ca2+ and Cl−).9 On the other hand, electrochemical biosensors for the label-free detection of antibody–antigen interaction have been researched extensively.10 The detection of bacteria by potentiometry has been reported11 and early work by Rechnitz demonstrated potentiometry to be a promising tool to probe antibody–antigen interactions.12 Still, the use of ion-selective electrodes (ISEs) as the transduction method of a label-free determination of immunobinding is in its infancy.

In this study, a potentiometric polymeric membrane electrode was investigated as a possible POC diagnostic tool for the direct detection of antibody–antigen interactions. Zero
current ion fluxes across ion-selective membranes are today well-understood and are known to often be responsible for the lower detection limit of ISEs. Such fluxes have been exploited for the design of stepstrokes and switchstrokes, where large potential changes around a critical concentration can be observed. In the approach presented here, such an ion potential changes around a critical concentration can be for the design of steptrodes and switchtrodes, where large potentiometry and QCM measurements were performed in Consort D130 using a home-built data acquisition software. All Measurements were performed at ambient temperature with a lower detection limit of ISEs. Such

Two-electrode con

at Janssen Pharmaceutica NV. (MH-SVM25, ATCC) (mAb-gp41) were all cultured in house the appropriate salts in Milli-Q-purified DPBS). All aqueous solutions were prepared by dissolving glycol (PEG)-modified PVC membrane as a working electrode. A disk of 6 mm was cut from a microporous polypropylene (Dulbecco’s Phosphate Buffered Saline, DPBS). The electrical connection was provided by means of a silver wire. The electrodes were conditioned in DPBS for 48 h prior to measurement under constant stirring (300 rpm) to hydrate the membrane.

**Electrode Membrane Surface Modification.** The current setup allows working in 4 independent cells. In each measurement cell, one can place up to six electrodes and a reference electrode in 5 mL DPBS under constant stirring at 150 rpm. After reaching a stable potential (i.e., potential drift less than 1 mV/10 min), the electrodes were exposed to streptavidin for 1 h under constant stirring at a final concentration of 2.5 μg/mL.

After each incubation step, DPBS was refreshed. Once a stable baseline potential was established, which usually took up to 30 min, electrodes were incubated for 1 h with biotinylated specific or nonspecific antibodies with a final concentration 2.5 μg/mL. Before exposing the electrodes to the antigen, the buffer was refreshed.

**Sensor Working Mechanism.** The mechanism of action is based on the disturbance of the internal marker ion (TBACl) flux as a result of target molecules binding on the sensor surface. As the membrane is very sensitive to the marker ion that leaches out from the internal solution to the sample side where the virus is present, the binding of the virus to the antibody disturbs the flux of the marker ion, changing its local concentration in the vicinity of the sensor, which then results in a measurable potential change at the sensor. Schematic representation of the sensing principle is depicted in Figure 1.

**Quartz Crystal Microbalance Measurements.** QCM experiments were carried out with a Q-Sense E4 (Gothenburg, Sweden) instrument in a flow through cell to investigate whether the click chemistry modification, antibody interaction, and virus adherence were executed in accordance with the expected electrode architecture. In all QCM experiments, gold chips uniformly spin-coated with the same PVC membrane cocktail used for the potentiometric sensor. Resulting membrane layer thickness was monitored with ellipsometry and only those with desired thickness (around 100 nm) were used for the QCM measurements.
The identical virus batches were used. Potentiometric and parallel tests performed using Binax addition of the virus sample to the test strip, and a 15 min nitrocellulose membrane to form a sample line. Upon the conjugated to visualizing particles and adsorbed onto a swab specimens. The test is based on anti-RSV antibodies RSV fusion protein antigen in nasal washes and nasopharyngeal based immunochromatographic technique designed to detect POC test, Binax NOW RSV,17 was used to assess the sensitivity units)/mL each. The time between expositions was 10 min. A

Western Blot Analysis and ELISA. The affinities of the mAb-RSV and mAb-gp41 (control) antibodies against the target antigen of interest (RSV) were tested with Western Blot analysis. The binding between the anti-RSV F protein antibody and RSV was tested by means of ELISA. In these tests, the signal readout was monitored by varying the antibody concentration while keeping the viral concentration constant and vice versa.

Potentiometric Measurements. Two sets of potentiometric measurements were performed, measurements to support the working mechanism and the actual determination of antibody–antigen interactions.

Measurements Supporting the Mechanism of Action. The functionality of the electrodes, based on the outflux of the marker ion, was first tested based on a potentiometric stir effect. As the elevation of the local concentration of TBACl in the sample side is precluded as a result of constant stirring, when the stirring is absent, the concentration is expected to increase. Additionally, the mechanism of action (MOA) was tested experimentally during electrode build-up since the addition of streptavidin and antibody binding on the sensor surface are also expected to yield a signal.

Experiments were conducted in which electrodes without surface modification were exposed to BSA to ascertain the MOA. In these experiments, ion strength of the marker ion was kept constant at the same concentration on both sides of the ISE membrane. BSA solutions were tested in the presence of interfering ions.

Measurement of Virus. Electrodes were exposed to four consecutive virus spikes containing 10^4 PFU (plaque forming units)/mL each. The time between exposures was 10 min. A POC test, Binax NOW RSV,17 was used to assess the sensitivity of the proposed sensor. The Binax NOW RSV is a membrane-based immunochromatographic technique designed to detect RSV fusion protein antigen in nasal washes and nasopharyngeal swab specimens. The test is based on anti-RSV antibodies conjugated to visualizing particles and adsorbed onto a nitrocellulose membrane to form a sample line. Upon the addition of the virus sample to the test strip, and a 15 min incubation period, the signal readout was done. In all potentiometric and parallel tests performed using Binax NOW, the identical virus batches were used.

### THEORY

The sensing principle, put forward here for the first time, involves the continuous release of a label ion from an ion-selective membrane into the aqueous solution. The system is here understood by a steady-state concentration profile across the membrane that is driven by the extraction of marker ion salt at the backside of the membrane. A surface confined immunoreaction is here understood to result in an intermediate diffusion layer between the membrane and the aqueous diffusion layer. The concentration profile across the three diffusion layers is schematically shown in Figure 2.

The flux of marker ion j across the membrane is described by Fick’s first law as

\[
J_j^m = -D_j^m \frac{c_j^m(\delta^m) - c_j^m(0)}{\delta^m}
\]

where the phase labels are shown as m superscripts, \(D_j^m\) is the diffusion coefficient, \(c_j^m(\delta^m)\) the molar concentration at the backside of the membrane (position \(\delta^m\), see Figure 2), \(c_j^m(0)\) its concentration at the sample side of the membrane, and \(\delta^m\) is the membrane thickness.

The flux across the diffusion layer where immunoreaction occurs is written in analogy as

\[
J_j^d = -D_j^d \frac{c_j^d(0) - c_j^d(\delta^d)}{\delta^d}
\]

where position 0 refers to the membrane surface and \(\delta^d\) to the end of this intermediate layer in contact with the sample solution. Finally, the flux across the aqueous diffusion layer is written similarly as

\[
J_j^a = -D_j^a \frac{c_j^a(\delta^a) - c_j^a(\text{bulk})}{\delta^a}
\]

where \(\delta^a\) is the aqueous diffusion layer thickness at steady state. It can be altered by the stirring rate of the solution. At position...
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\[ E_{PB} = \Delta_{m}^{m} + \frac{RT}{zF} \ln \left( \frac{c_{m}^{m}(0)}{c_{j}^{m}(0)} \right) \]

where \( \Delta_{m}^{m} \) is the standard potential of ion transfer across this interface (a constant) and \( R, T, \) and \( F \) have their established meanings. The charge of the marker ion, \( z \), is taken as 1.

At steady-state, all three fluxes in eqs 1, 2, and 3 are equal. Eliminating \( c_{i}^{m}(d) \) and \( f_{i}^{m} \), solving the result for \( c_{j}^{m}(0) \), and inserting it into eq 4 gives the potential response as

\[ E_{PB} = \Delta_{m}^{m} + \frac{RT}{zF} \ln \left( \frac{c_{m}^{m}(\text{bulk})}{c_{j}^{m}(0)} \right) \]

\[ \left\{ c_{m}^{m}(d) - c_{m}^{m}(0) \right\} \]

In a label-free sensing approach, one aims to avoid addition of the label to the sample solution. Consequently, we use \( c_{m}^{m}(\text{bulk}) = 0 \) and keep the diffusion coefficients and the membrane thickness and compositions constant to obtain from eq 5:

\[ E_{PB} = A + \frac{RT}{zF} \ln \left( \frac{D_{aq}^{m}}{D_{f}^{m}} \right) \]

where \( A \) is a constant. A reduction of the apparent diffusion coefficient in the surface-confined layer, for example by a surface blocking event, results in a potential increase. This change is a direct function of the diffusion coefficient and the additional diffusion layer thickness. Figure 3 illustrates expected potential changes on the basis of eq 6 as a function of binding layer thickness and reduction in diffusion coefficient in that layer.

Convective stirring of the solution is expected to give smaller signals (reduced value of \( \Delta_{aq}^{m} \)). In accordance with Figure 3, surface binding events on the scale of a few hundred nanometers are detectable with this approach if it induces an important retardation of the marker ion.

**RESULTS AND DISCUSSION**

Figure 4 shows the QCM data acquired from different sensors. The signal at the top panel was acquired from a gold crystal coated with an antibody recognizing RSV F protein, whereas the middle was from a gold crystal modified with an antibody recognizing HIV-1 gp41 protein (as a negative control). The bottom signal was obtained from a crystal coated only with PEG, serving as another control in the experiments. This experiment confirms the build-up of the immunoreagents at the ion-selective membrane surface. The exposure of the biotinylated membranes to streptavidin and the subsequent step of binding the biotinylated antibodies were clearly visualized by QCM. PEG-modified membranes (bottom) show no response to streptavidin, as expected. As can be seen from Figure 4, the QCM data showing the change in resonance frequency occurring only for the (A) specific electrode (top) upon RSV exposure, whereas the (B) middle (modified with an antibody recognizing HIV-1 gp41 protein as a negative control) and the (C) bottom signal (coated only with PEG) show no response to RSV spikes. (A and B) respond to streptavidin and antibody injections as a result of streptavidin binding to the biotinylated surfaces (PEG-B) and subsequent biotinylated antibody binding to streptavidin while (C) PEG electrode shows no response, as expected.
seen from Figure 4, a decrease in the resonance frequency upon exposure to $10^3$ PFU RSV was observed only for the specific electrode (top). This indicates that the sensor surface was modified as desired.

The specificity of the anti-RSV F antibody was tested with Western Blot analysis. The data confirmed a specific interaction of this antibody with the denatured F1 part of the RSV F protein (Figure 5, left). The binding between the anti-RSV F antibody and the F protein in its denatured and native pretriggered conformation. Dark-blue and light blue represent the responses with and without mAb-RSV primary antibody, respectively.

The proposed response mechanism of the potentiometric biosensor principle was initially confirmed based on a stir-effect. As seen in Figure 6A, when the solution was stirred, the signal read-out was low. This is a consequence of the fact that constant stirring prevented the ion flux building up the local concentration, which was increased when the stirring was off (Figure 6B). Clear signal responses, following stir-effect, recorded potentiometrically from a set of two electrodes in the same measurement cell as a result of BSA binding to the sensor surface. Potential of each electrode, indicated by red and blue, respectively, increases after BSA injection.

The mechanism laid out in the theoretical part was independently supported with BSA as a model analyte. In this experiment two different electrodes, placed in the same measurement cell, were exposed to BSA to test their responses to any possible interaction with this molecule (Figure 6B). In this case the membrane consisted of a PVC, which was not modified with any antibody. The electrodes were first exposed to a BSA filtrate (i.e., not containing any BSA) to confirm the lack of potential increase in the absence of BSA and to rule out any impact of sample impurities. Indeed, no potential increase was observed in response to the BSA filtrate (data not shown). This was followed by a stir effect, as shown in Figure 6B, to test electrode response according to eq 5. Signals increased when the stirring was off, whereas they all decreased as the stirring was on. A BSA spike of (50 mg/mL) caused electrode signals to rise. The fact that BSA filtrate injections did not give rise to a measurable potential change indicates that the observed signal changes were brought about by the BSA binding to the membrane surface. It is important to emphasize that no response was observed when the marker ion was already present in the sample solution. This in accordance with the principle set forth above and indicates that a potential response is indeed induced by retarding the marker ion and increasing its accumulation at the membrane surface. Figure 7 (top) presents
the experimental data on RSV detection, using a set of (A) specific electrodes and three sets of control electrodes were used [i.e., total number of 39 electrodes, 16 of which were specific (A)]. As seen in this graph, while (B, C, D) control groups show no response to the lowest virus concentration of 10 PFU/mL, the (A) specific electrodes respond to the virus. A schematic of different sensor architectures can be seen in Figure 7 (bottom).

Among three control groups (B, C, and D), two control groups (C and D) showed no response to 10 and 100 PFU/mL RSV injections. It is also observed in Figure 7 that the difference in signal response between the specific (A) and control electrodes (B, C, D) becomes larger as the virus concentration increases (e.g., 1000 PFU/mL). It should be noted that control group B was identical to the specific set A except for the monoclonal antibody used (i.e., mAb-gp41 instead of mAb-RSV), so as to ascertain that different signal responses correspond to the electrode specificity. Additionally, control groups C and D were employed to monitor any interaction which might be due to mAbs binding on the membrane surface despite the PEG layers used.

A comparison of the potentiometric signal readouts of a specific and nonspecific electrode pair recorded within the same measurement cell is given in Figure 8. As shown in this figure, the signal amplitude of the specific electrode increases as the amount of viral particles in the cell is increased. The potential recorded from the nonspecific electrode follows a steady baseline except for the highest viral load.

![Figure 8](image_url)

**Figure 8.** A comparison of the potentiometric signal readouts of an electrode treated with mAb-RSV (solid line) and a control electrode with mAb-gp41 (dashed line), recorded within the same measurement cell. The signal amplitude of the specific electrode increases as the virus concentration in the cell is increased, whereas that from the nonspecific electrode follows a steady baseline except for the highest viral load.

The detection limit of the proposed sensor system was found to be approximately 3 logarithmic units higher than that of the qRT-PCR (data not shown). We used identical RSV batches in order to compare the response and sensitivity of the proposed sensor with the commercial RSV test. Table 1 shows the responses recorded from a pair of specific (i.e., PEG-B membranes modified with mAb-RSV) as well as nonspecific electrodes (i.e., PEG-B membranes modified with mAb-gp41) to a set of RSV spikes of varying concentrations. Plus and minus signs indicate the presence and absence of a signal, respectively. Only the electrode treated with mAb-RSV responded to virus concentrations of 10⁴ and 10⁵ PFU.

| RSV (PFU) | PEG-B Ab-RSV | PEG-B Ab-gp41 | Binax NOW
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*A Plus and minus signs indicate the presence and absence of a signal, respectively. The majority of the electrodes were found to be clearly more responsive to Influenza A virus than to RSV, although nonspecific interactions were also observed, especially at high virus concentrations.*

## CONCLUSIONS

A new potentiometric biosensor principle has been evaluated to explore the concept of modulating the mass transport of a marker ion to detect antigen–antibody interactions at the ion-selective membrane surface. This was demonstrated by an infectious disease model for RSV. The proposed mechanism of action was evidenced by potentiometric experiments. The electrode architecture was verified with QCM measurements.

Most electrochemical measurement systems require the addition of an indicator ion to the sample solution (e.g., Ca²⁺) or a redox couple (e.g., amperometry) to recognize the antibody–antigen interaction. In this sensor principle, the marker ion is delivered by the membrane in direction of the analyte solution by diffusion through the membrane. Consequently, the evaluated sensing system renders label-free detection of antibody–antigen interactions possible. This can in turn make the sample preparation process less cumbersome for point-of-care applications.

The sensor principle requires effective coverage of the sensing surface upon immunoreaction. The area of the current sensor is very large (a few millimeters in diameter) compared to the 100 nm virus one wishes to detect. Potentiometric microelectrodes with size ranges in the submicrometer range have been known for many years and should provide a much more favorable membrane to virus area than the systems studied here. Miniaturization of the sensing system is expected to increase the sensitivity of the sensor and hence lower the limit of detection (LOD).
The delivery of the target molecules to the sensor surface achieved by stirring in the current study can be facilitated by using a microfluidic channel which can transport the target molecule to the sensor surface more effectively.

Despite the promising results presented in this work, nonspecific interactions on the polymer surface remain a potential limitation of the current design. Signals recorded from PEG-only membranes treated with mAb-RSV (Figure 7) indicate a suboptimum shielding of the membrane surface which needs to be overcome before the technique can be applied in real application with more complex biological samples.

Although not investigated in our current study, the Ab isotype (e.g., use of IgG versus IgM) may have an impact on the sensitivity of the sensor as a result of different avidity of the Abs. Using multimeric antibodies as compared to their monomeric counterparts could improve sensitivity as more antigen binding possibly results in more pronounced changes of the ion flux at the surface of the sensor. Additionally, the size of the antigen of interest can affect signal transfer as larger molecules can be expected to cause larger flux changes of the marker ion the membrane surface and hence yield larger signals.

This early study aimed at exploring the concept of an incorporated marker ion flux in potentiometric ion-selective sensors as a new biosensor approach. The data confirm the feasibility of detecting antibody–antigen interactions by potentiometry. To our knowledge, this is the first reported use of potentiometry on the basis of passive ion fluxes in probing antibody–antigen interactions. Such an apparently label-free approach may become an attractive platform for future progress in bioaffinity sensor research.

**ASSOCIATED CONTENT**

Supporting Information

Synthetic details and additional experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Kalorama Information Worldwide POC Diagnostic Test Markets.