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An ultrasensitive aptasensor of SARS-CoV-2 N protein based on ion current rectification with nanopipettes

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ABSTRACT
Since the outbreak of COVID-19 in the world, it has spread rapidly all over the world. Rapid and effective detection methods have been a focus of research. The SARS-CoV-2 N protein (NP) detection methods currently in use focus on specific recognition of antibodies, but the reagents are expensive and difficult to be produced. Here, aptamer-functionalized nanopipettes utilize the unique ion current rectification (ICR) of nanopipette to achieve rapid and highly sensitive detection of trace NP, and can significantly reduce the cost of NP detection. In the presence of NP, the surface charge at the tip of the nanopipette changes, which affects ion transport and changes the degree of rectification. Quantitative detection of NP is achieved through quantitative analysis. Relying on the high sensitivity of nanopipettes to charge fluctuations, this sensor platform achieves excellent sensing performance. The sensor platform exhibited a dynamic working range from 10^{-5}-10^{6} pg/mL with a detection limit of 73.204 pg/mL, which showed great potential as a tool for rapidly detecting SARS-CoV-2. As parallel and serial testing are widely used in the clinic to avoid missed diagnosis or misdiagnosis, we hope this platform can play a role in controlling the spread and prevention of COVID-19.

1. Introduction

At the end of 2019, COVID-19 spread to the whole world and caused a large number of casualties [1]. SARS-CoV-2, which causes severe acute respiratory syndrome, is the infectious agent responsible for this pandemic [2]. SARS-CoV-2 spreads from person to person through virus-containing droplets expelled when an infected person coughs, sneezes, talks, and breaths [3]. Symptoms include fever, dry cough, headache, and so on [4].

The rapid diagnosis of COVID-19 is vital for halting the virus’s spread, and it plays a key role in resuming flights, promoting the development of tourism, and normalizing economic activities. Nowadays, RT-PCR is primarily used for the clinical detection of SARS-CoV-2, while antibody-targeted ELISA is used for serological detection. Detection of SARS-CoV-2 specific antibodies is an alternative diagnostic technique. After 5–7 days of infection, the IgM antibody was discovered, and then IgG was detected [5]. It has a clear latency when compared to PCR detection and is typically employed as a backup method.

SARS-CoV-2 has a single-stranded RNA genome that is 30,000 nucleotides in length [6]. SARS-CoV-2 encodes four structural proteins in its RNA genome: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) [7]. The majority of currently used PCR-based techniques have a detection limit of 100 copies/mL or less, which corresponds to viral detection 2–3 days before symptoms appear [8]. Although very effective as the “gold standard” for SARS-CoV-2 identification, RT-PCR requires trained personnel, pricey equipment, and

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time-consuming sample preparation and analysis [9]. At present, some RNA detection methods have also been proposed, including RT-LAMP [10,11], CRISPR [12,13], and so on. These methods have their advantages over PCR, but all require sophisticated instruments, expensive reagents, and skilled instrument operators.

Meanwhile, RNA is less stable during transport and storage than protein, improper storage of RNA and improper sample handling is easy to cause false-negative results in final detection [14]. As the most expressed virus-related protein during infection [7], the detection of N protein (NP) is significant for the identification of SARS-CoV-2. Different from RNA, the detection of protein doesn’t involve the amplification process, which avoids false negatives caused by the false replication generated during the nucleic acid amplification process. Since no amplification process is involved, direct detection of proteins poses a huge challenge. Researchers have proposed electrochemical sensors [15, 16], fluorescent sensors [17], and chemiluminescence methods for the detection of NP [18].

Electrochemical detection is frequently used and benefits from quick analysis, low detection limits, and high sensitivity [19]. In 1997, ion current rectification (ICR) was found in nanopipette experiments [20]. The effect of ion current rectification was observed as asymmetric current-voltage (I–V) curves, with the current recorded for one voltage polarity higher than that for the same absolute value of the voltage of opposite polarity. Siwy referred that the ICR has an intimate relation to the charge of the inner wall of a nanopipette [21]. Changes in steric hindrance and charge density on the nanopipettes’ inner surface will have an impact on ion transit, as well as a quick electrochemical reaction. Based on this, the researchers constructed a nanopipette sensor based on ICR. According to the electrochemical characterization changes caused by the change of the surface charge of the nanopipettes before and after the capture probe captures the target are observed, the qualitative and quantitative analysis of the target is realized. This type of sensor has been used to detect nucleic acids [22–24], proteins [25–27], enzymatic activities [28], metal ions [29–31], etc.

The selection of the capture probe is important for the construction of the sensor. Some specific recognition targets based on NP detection use molecular imprinting [32,33], antigen-antibody reactions [34–38], and aptamers [15]. Aptamers display more affinity and more binding sites with lower molecular weight and are easy to synthesize/modify, and cost-effective. Benefit from the strong negative charge of the aptamer, the signal is pronounced when the immobilized aptamer captures the target allowing one to identify the captured single molecule [39]. Therefore, aptamers are prefer chosen for modifying nanopipettes. Aptamers have so far been demonstrated to be capable of binding to a variety of targets, such as metal ions [40,41], proteins [42–44], and even cells [45]. Researchers have screened aptamer by SELEX technique and constructed aptasensor for the detection of SARS-CoV-2 S1 protein [25].

In this research, we proposed a sensor based on ICR to detect NP. Aptamer-functionalized nanopipette is used for specific capture and detecting NP. The aptamer was introduced to the nanopipette via a covalent modification process. At the pH used for detecting NP, the aptamer was negatively charged while the NP (pI=10.07) was positively charged. The biospecific interaction of aptamers with proteins causes a decrease in charge density and an increase in steric hindrance. Coupled with an electrochemical technique, the protein can be sensitively identified. The sensor shows good resistance against interference thanks to its high affinity, low limit of detection (LOD), and good stability. As expected, the sensor can play a role in assisting other COVID-19 detection methods. (Fig. 1).

2. Experimental section

2.1. Nanopipette fabrication and characterization

Before pulling the nanopipette with capillaries (OD 1.0 mm, ID 0.58 mm), they should be cleaned with piranha solution (98% H_2SO_4/30% H_2O_2, V/V=3:1) in 80 °C for 2 h and rinsed by DI water. The capillaries were then dried in a stream of nitrogen. After being dried, capillaries were pulled by P-2000 (Sutter Instruments, Novato, CA) through a program as follows: Heat 325, Filament 1, Velocity 10, Delay 145, and Pull 180. The puller should be preheated for at least 15 min.

After nanopipettes were prepared, they were characterized by a JSM-7800 F scanning electron microscope (JEOL) at 5 kV accelerating voltage (Fig. S1A). The inner diameter was estimated using the I-V curves observed in a solution of 1 M KCl.
solution in the dark for 1 h. Afterward, the nanopipettes were washed in ethanol and the tips of the nanopipettes were immersed in the same filled with 5% (V/V) 3-aminopropyl-triethoxysilane (APTES) solution silicon hydroxyl terminal modifications. Firstly, the nanopipettes were then treated with PB (10 mM K\textsubscript{2}HPO\textsubscript{4}, 10 mM KH\textsubscript{2}PO\textsubscript{4}, and 1 mM KCl, pH 7.4) containing 2.5% (V/V) glutaraldehyde overnight, followed by rinsing with PB thoroughly. The aldehyde-functionalized nanopipettes were connected with 0.6 μM aptamer for 120 min at room temperature. Finally, the nanopipettes were rinsed with PB and ready for use.

Before aptamer-functionalization, the aptamer should be heated at 95 °C for 5 min and cooled down to uncoil the DNA.

2.2. Modification of nanopipette

After treatment with piranha solution, the nanopipettes finished silicon hydroxyl terminal modifications. Firstly, the nanopipettes were filled with 5% (V/V) 3-aminopropyl-triethoxysilane (APTES) solution in ethanol and the tips of the nanopipettes were immersed in the same solution in the dark for 1 h. Afterward, the nanopipettes were washed with ethanol and baked at 120 °C for 1 h subsequently. The nanopipette was then treated with PB (10 mM K\textsubscript{2}HPO\textsubscript{4}, 10 mM KH\textsubscript{2}PO\textsubscript{4} and 1 mM KCl, pH 7.4) containing 2.5% (V/V) glutaraldehyde overnight, followed by rinsing with PB thoroughly. The aldehyde-functionalized nanopipettes were connected with 0.6 μM aptamer for 120 min at room temperature. Finally, the nanopipettes were rinsed with PB and ready for use.

Before aptamer-functionalization, the aptamer should be heated at 95 °C for 5 min and cooled down to uncoil the DNA.

2.3. Electrochemical measurements

The \textit{I-V} curves were measured by CHI760E (Shanghai CHI Instrument Co. Ltd., China). Linear sweep voltammetry (LSV) was tested in PB. The scan voltage was adjusted to range from −1.0 to +1.0 V with a 0.05 V/s scan rate, and the pulse amplitude was set to 0.001 V. All measurements were performed at room temperature. The bulk solution and the infused solution were kept the same throughout the experiments. The rectification ratio \( r = \log_{2} [I^−/I^+] \), \( I^− \) is the current in −1 V and \( I^+ \) is the current in +1 V) can illustrate the charge status of the nanopipettes. When \( r < 0 \), the inner surface charge of nanopipettes is positive while \( r > 0 \) the charge in the inner wall is negative. A normalized current change (\( \Delta I/I_o \), \( \Delta I=I-I_o \), \( I_o \) and \( I \) represent the current before and after the incubation at −1 V) was used to reduce the current variation between different nanopipettes.

Different concentrations of NP are prepared in PB and used for the measurement of the respective \textit{I-V} curves.

3. Results and discussion

3.1. Nanopipettes characterization

The orifice diameter was approximately 125 nm according to the SEM picture (Fig. S1A), which was similar to the calculated value from the \textit{I-V} curves measured in 1 M KCl (Fig. S1B) using the equation below [46,47].

![Fig. 2. Nanopipette modification processes. (A) Monitoring the process of chemical alteration on the inner surface of the nanopipettes by measuring the \textit{I-V} curves in PB. (B) ICR ratio (\( r \)-value) of the modification stages (silica hydroxyl groups, amino groups, aldehyde groups, and immobilized aptamer strands (0.6 μM), respectively).](image-url)

\[ R = \frac{\gamma \cot \theta}{2a} \]

where \( R \) is the resistance of the nanopipettes, \( \gamma \) is the electrolyte’s resistivity (S/m), \( a \) is the radius of the nanopipettes, and \( \theta \) is the cone angle (° in this example).

3.2. Modification of nanopipettes

It is well understood that nanopipettes’ inner surface modification has a significant impact on the mass and charge transport parameters [21]. The surface charge changes can be characterized using the \textit{I-V} curves (Fig. 2A) and rectification ratios (Fig. 2B). Compared to the commonly used electrochemical impedance spectroscopy and cyclic voltammetry, LSV can directly obtain the current characterization related to rectification effects by virtue of simpler parameter settings.

At physiological pH, the dissociation of silanol groups makes silica surfaces acquire a charge in contact with the solution[48].

\[ \text{SiOH} = \text{OH}^- + \text{H}^+ \]

Silicate glass surfaces immersed in water are known to have a negative surface charge density in this scenario. According to the current of an undecorated nanopipette, the measured current for negative voltage is higher than that of positive voltage (Fig. 2A) and an \( r \)-value of 3.043 ± 0.190 (Fig. 2B) which suggested the nanopipettes’ inner surface was negatively charged. After APTES modification, the \( r \)-value was converted to −3.043 ± 0.190 (Fig. 2B) and the nanopipettes’ inner surface was positively changed which can be characterized by \textit{I-V} curves (Fig. 2A). On one end of APTES are three ethoxy silanes that can covalently bond to silica surfaces after undergoing a condensation reaction. A primary amine group on the other end is protonated in aqueous solutions (p\textsubscript{I} = 9.6). Therefore, an APTES-covered surface will be positively charged at pH 7.4 [49].

Aldehyde groups were introduced to the nanopipettes by cross-linking glutaraldehyde with the fixed amino groups. Because the aldehyde group is neutral, the current of positive voltage is close to the current of negative voltage (Fig. 2A) and the charged nanopipette had an \( r \)-value of −0.188 ± 0.047 (Fig. 2B), indicating a significantly lower positive charge than the APTES modified nanopipettes.

Upon immobilizing aptamer, the nanopipettes exhibited an \( r \)-value of 3.039 ± 0.201 (Fig. 2B), which showed that the inner wall of the nanopipettes had accumulated more negative charges and resulted in a higher current at a negative voltage (Fig. 2A) that the phosphate
backbones of DNA were deprotonated in a neutral state [26].

All of the I-V curves (Fig. 2A) and r-values (Fig. 2B) can demonstrate the success of nanopipette modification.

3.3. The influence of experimentation conditions on sensing performance

The biosensor’s detection performance is significantly influenced by the experimental settings. As the probe is used to capture the targets, the concentration of the aptamer could have a substantial impact on how well the detection works. Different concentrations (0.1, 0.2, 0.4, 0.6, 0.8, and 1 μM) were chosen to explore the optimal concentration and the incubation time was 120 min (Fig. 3A, B). When the concentration of aptamer was higher than 0.6 μM, the I-V curves changed slightly, indicating that the binding of aptamer onto the nanopipettes had reached saturation, so we concluded that 0.6 μM aptamer was optimal for modification nanopipettes. At too high concentrations, the aptamer might cause steric hindrance. After that, the incubation time (30, 60, 90, and 120 min) of probes and NP (100 ng/mL) was studied to shorten the detection time and increase the accuracy of the results. It was observed that the I-V curves were stable after 60 min of incubation and the current change was enough to be found, so 60 min was determined as the incubation time (Fig. 3C, D).

3.4. Detection of the NP

The working principle of the sensor platform can be explained by the accumulation and dissipation of electric charge. Under neutral conditions, the surface of aptamer-functionalized nanopipettes is negatively charged, with cation selectivity, and it generates an electric current driven by an electric field. When the NP (pI=10.07) is captured by the aptamer, the positively charged NP will neutralize the negative charges, which will reduce the density of the surrounding cations and the current generated. We use standardized current change (Fig. 4B) to judge the relationship between NP concentration and current response.

To determine the analytical range, different concentrations (10^2, 10^3, 10^4, 10^5, 10^6, 10^7 pg/mL) of NP were incubated with the aptamer-functionalized nanopipettes at room temperature for 60 min, followed by the I-V measurement (Fig. 4A). As depicted in Fig. 4B, the normalized current change enhanced with the increase of NP concentration and showed a good linear relationship range from 10^2 to 10^6 pg/mL (Fig. 4D). The linear relationship for NP is \( \Delta I/I_0 = 0.255 - 0.187 \log C \) with \( R^2 = 0.992 \) and the LOD was down to 73.204 pg/mL, which exhibits better sensing performance than other reported NP detection methods as shown in Table S1.

3.5. Specificity of the nanopipette sensor

Target-free sensors using aptamer capture analysis are susceptible to
electrostatic and other non-specific bindings. The aptamer-functionalized nanopipettes were used to detect IgM (1 μg/mL), BSA (1 μg/mL), Thrombin (1 μg/mL), and a mixture of the same concentration at room temperature for 1 h to evaluate the specificity. As shown in Fig. 5, compared with 1 ng/mL of NP, the effect of a high concentration of protein on rectifying degree is almost negligible, which is due to the high specificity of aptamer and NP recognition. The experimental results show that the nanopipette sensor platform has good specificity, and the
The rectification performance of the nanopipette was tested by LSV, in which on aptamer functionalization was stored at 4℃. nonspecific interference can be ignored.

### 3.6. Stability of the nanopipette sensor

To further explore the possibility of developing and manufacturing sensors in clinical diagnosis and biological detection, it is of great significance to study the stability of sensors. The nanopipette sensor based on aptamer functionalization was stored at 4℃ for 7 days, and the rectification performance of the nanopipette was tested by LSV, in which the concentration of NP was 1μg/mL. As shown in Fig. 6, the response of the nanopipettes to the rectification change of 1μg/mL NP did not change obviously after 7 days.

### 3.7. Real sample analysis

To verify the applicability of the sensor platform, the potential of the developed aptamer functionalized nanopipettes in actual samples was studied by detecting the NP added in human serum with different concentrations. Different concentrations of NP (0.01 μg/mL, 0.1 μg/mL) were added to the serum diluted by PB for 10 times, and the presence of NP was detected by the nanopipette sensor. According to the results obtained in Table S2, the sensor platform obtained recovery rates of 104% and 106% in two NP samples (0.01 μg/mL, 0.1 μg/mL) which indicated that the constructed nanopipette sensor showed high reproducibility, accuracy, and feasibility in detecting NP in human serum.

### 4. Conclusions

In this research, we have developed the NP sensor platform for the rapid and sensitive detection of NP in serum based on the unique ion rectification effect of nanopipettes. By functionalizing the inner wall of the nanopipette with aptamers, the platform takes full advantage of the high sensitivity of the nanopipette to surface charge fluctuations and the high specificity of aptamers for target recognition which can achieve highly specific capture of NP, exhibiting an extremely low detection limit of 73.204 pg/mL within a broad linear NP concentration range from 100 pg/mL to 1 μg/mL and strong specificity for NP in complex samples with good stability. Moreover, the method is short in time, low in cost and simple in operation. The developed sensor is based on a completely different approach to NP detection compared to the currently constructed approach to NP detection which is expected to play a role in COVID-19 detection. By altering the modified capture probe we anticipate that the sensitive detection capability, stability, simplicity and inexpensive fabrication will make nanopipettes a universally useful platform for epidemic prevention and control.

### CRediT authorship contribution statement

Conceptualization: Wenhao Ma, Wanyi Xie, Changjun Hou, Danqun Huo, Deqiang Wang. Methodology: Wenhao Ma, Wanyi Xie, Rong Tian, Xiaqing Zeng, Liyuan Liang. Writing – original draft: Wenhao Ma, Wanyi Xie. Funding acquisition: Changjun Hou, Danqun Huo, Deqiang Wang. Supervision: Changjun Hou, Danqun Huo, Deqiang Wang.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2022.133075.

### References

[1] I. Chakraborty, P. Maity, COVID-19 outbreak: migration, effects on society, global environment and prevention, Sci. Total Environ. 728 (2020), 138882.

[2] P. Wu, S. Zhao, B. Yu, Y. Chen, W. Wang, Z. Song, Y. Hu, Z. Tao, J. Tian, Y. Pei, M. Yuan, Y. Zhang, F. Dai, Y. Liu, Q. Wang, J. Zheng, L. Xu, E. Holmes, Y. Zhang, A new coronavirus associated with human respiratory disease in China, Nature 579 (2020) 265–268.

[3] R. Pranesh, C. Wang, R. Schoudey, Reducing transmission of SARS-CoV-2, Science 368 (2020) 1422–1424.

[4] L. Fu, B. Wang, T. Yuan, X. Chen, Y. Ao, T. Fitzpatrick, P. Li, Y. Zhou, Y. Lin, Q. Duan, G. Liu, S. Fan, Y. Lu, A. Feng, Y. Zhan, B. Liang, W. Cai, L. Zhang, X. Du, L. Li, Y. Shu, H. Zou, Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: a systematic review and meta-analysis, J. Infect. 80 (2020) 656–665.

[5] J. Zhang, X. Zhang, J. Liu, Y. Ban, N. Li, Y. Wu, Y. Liu, R. Ye, J. Liu, X. Li, L. Li, X. Qin, R. Zheng, Serological detection of 2019-nCoV respond to the epidemic: A useful complement to nucleic acid testing, Int. Immunopharmacol. 88 (2020), 106861.

[6] A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, J. Sheng, L. Quan, Z. Xia, W. Tan, G. Cheng, T. Jiang, Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China, Cell Host Microbe 27 (2020) 325–328.

[7] D. Kim, J. Lee, J. Yang, J. Kim, H. Chang, The architecture of SARS-CoV-2 transcriptome, Cell 181 (2020) 914–921.

[8] K. Ejima, K. Kim, C. Ludena, A. Bento, S. Iwamani, Y. Fujita, H. Ohashi, Y. Koizumi, K. Watashi, K. Aihara, H. Nishiura, S. Iwami, Estimation of the incubation period of COVID-19 using viral load data, Epidemiology 35 (2021), 106861.

[9] R. Wang, C. Qian, Y. Pang, M. Li, Y. Yang, H. Ma, M. Zhao, F. Qian, H. Yu, Y. Liu, T. Ni, Y. Zheng, Y. Wang, opvCRISPR: One-pot visual RT-LAMP-CRISPR platform for SARS-cov-2 detection, Biosens. Bioelectron. 172 (2021), 113276.

[10] Y. He, L. Wang, X. An, Y. Tong, All-in-one in situ colorimetric RT-LAMP assay for point-of-care testing of SARS-CoV-2, Analyst 146 (2021) 6026–6034.

[11] Y. He, T. Xie, Y. Tong, Rapid and highly sensitive one-tube colorimetric RT-LAMP assay for visual detection of SARS-CoV-2 RNA, Biosens. Bioelectron. 187 (2021), 113330.
W. Ma et al.

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[12] B. Yang, X. Zeng, J. Zhang, J. Kong, X. Fang, Accurate identification of SARS-CoV-2 variant delta using graphene/Cr2S3-pADC electrochemical biosensor, Talanta 2103 (2021) 121629.

[13] X. Ding, K. Yin, Z. Li, M. Sfer, C. Liu, Sensitive quantitative detection of SARS-CoV-2 in clinical samples using digital warm-start CRISPR assay, Biosens. Bioelectron. 184 (2021), 112518.

[14] J. Lee, M. Chey, Y. Jung, S. Lee, C. Lee, J. Kim, J. Kim, N. Kim, B. Kim, H. Kim, A novel rapid detection for SARS-CoV-2 spike 1 antigens using human angiotensin converting enzyme 2 (ACE2), Biosens. Bioelectron. 171 (2021), 112715.

[15] S. Ramanathan, S. Gopinath, Z. Ismail, M. Md Arshad, P. Poopalan, Aptasensing nucleic acidaptasensing nucleic acid aptasensor in a single glass conical nanopore, Biosens. Bioelectron. 71 (2015), 21620.

[16] X. Hou, Tannic acid modified single nanopore with multivalent metal ions phosphatase activity with a functionalized nanopipette, Electrochem. Commun. 99 (2008) 746.

[17] S. Zhang, G. Liu, H. Chai, H. Chen, Y. Zhao, B. Hu, M. Wang, L. He, D. Peng, Z. Zhang, Aptamer-templated silver nanoclusters embedded in zirconium metal-organic framework for bifunctional electrochemical and SPR aptasensors toward caricoembrionic antigens, Biosens. Bioelectron. Interfaces 9 (2017) 4188–4199.

[18] Z. Zhang, F. Duan, J. Tian, J. He, X. Yang, H. Zhao, S. Zhang, C. Liu, L. He, M. Chen, D. Chen, M. Du, Aptamer-embedded zirconium-based metal-organic-framework composites prepared by de novo bio-inspired approach with enhanced biosensing for detecting trace analytes, ACS Sens. 2 (2017) 982–989.

[19] Y. Li, M. Hu, X. Huang, M. Wang, L. He, Y. Song, Q. Jia, N. Zhou, Z. Zhang, M. Du, Multicomponent zirconium-based metal-organic frameworks for impedimetric aptasensing of living cancer cells, Sens. Actuators B. Chem. 306 (2020), 127608.

[20] H. Tang, H. Wang, C. Yang, D. Zhao, Y. Qian, Y. Li, Nanopore-based strategy for selective detection of single caricoembrionic antigen (CEA) molecules, Anal. Chem. 92 (2020) 3043–3049.

[21] M. Cao, H. Wang, H. Tang, D. Zhao, Y. Li, Enzyme-encapsulated zeolitic imidazolate frameworks formed inside the single glass nanopore: catalytic performance and sensing application, Anal. Chem. 93 (2021) 12257–12264.

[22] S. Behrens, D. Greiner, The charge of glass and silica surfaces, J. Chem. Phys. 115 (2001) 6716–6721.

[23] C. Pick, C. Argento, G. Drazer, J. Frechet, Microsized charge heterogeneities via vapor deposition of aminalosides, Langmuir 31 (2015) 10725–10733.

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