MIP Discovery

Technical Brief

SARS-CoV-2 nanoMIP

- fg/ml spike protein analytical sensitivity in sensor applications
- No cross reactivity to other coronaviruses
- Outperforms a leading commercial antibody in a sensor device
- **PLUS** thermal stability and consistency of supply only achievable with nanoMIPs

Enabling next generation rapid sensors for COVID-19 mass-screening and diagnosis

The SARS-CoV-2 nanoMIP from MIP Discovery is a synthetic alternative to antibodies for next generation COVID-19 rapid sensors. It offers a high affinity to the virus, a low limit of detection and no cross reactivity to other coronavirus strains. It also introduces a host of unique benefits including thermal stability tested to 121°C, batch to batch consistency and security of supply.

Introduction

Since being recognized as a pandemic in March 2020, the COVID-19 outbreak has led to an unprecedented demand for rapid and accurate diagnostic solutions. In the early stages, real-time reverse transcription-polymerase chain reaction testing (RT-PCR) was the diagnostic method of choice, driven by the need for highly accurate results. However, as the virus has spread exponentially, the requirement for mass testing has shifted that driver towards volume, ease of use and speed of results. Antigen-detecting rapid diagnostic tests are now beginning to provide a solution; they can be mass produced, do not require highly skilled professionals to operate, and can give results in approximately 10 – 15 minutes.

The solution to COVID-19 mass testing

Antigen-detecting rapid diagnostic tests have introduced point-of-care testing to the fight against COVID-19. They can support the need for increased testing capacity at a reduced cost, and do not require skilled professionals to operate. This type of test is ideally placed in settings such as care homes, schools and local clinics.

However, the first wave of antigen detecting rapid diagnostic tests have come under scrutiny, with some suggesting they are too inaccurate for screening programs. Viral loads below the limit of detection have led to false negatives, and it is therefore essential that the next generation of COVD-19 rapid tests offer a low limit of detection.

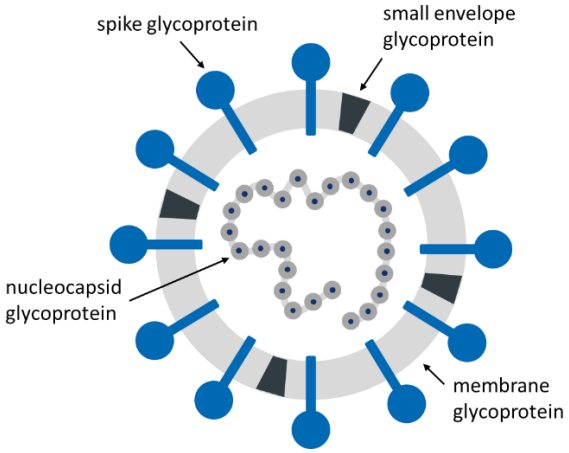

Figure 1. SARS-CoV-2 structure

The SARS-CoV-2 virus is made up of four structural proteins: spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) glycoprotein[1] (Figure 1). The spike glycoprotein is a 150 kDa transmembrane protein found on the surface of the virus. Its size and location make it an ideal target for virus detection in antigen-detecting rapid diagnostic tests.

SARS-CoV-2 nanoMIP

MIP Discovery has developed a SARS-CoV-2 nanoMIP, designed for antigen-detecting rapid diagnostic sensors. The SARS-CoV-2 nanoMIP has been imprinted against a portion of the receptor binding domain (RBD) region of the spike glycoprotein using a proprietary development process (Figure 2). The SARS-CoV-2 nanoMIP was developed in just 8 weeks, demonstrating the rapid response times achievable with MIPs. This will be of high importance should challenging new variants arise that cannot be detected using current commercial assays.

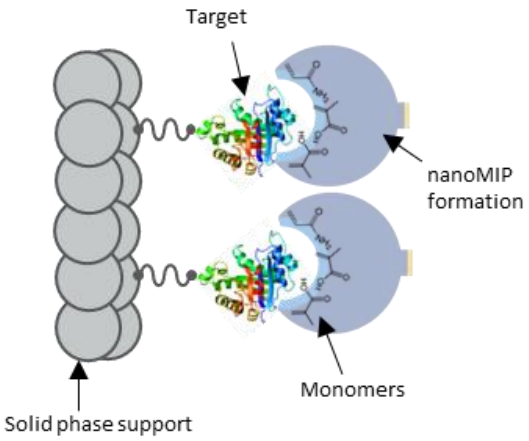

Figure 2. nanoMIP imprinting demonstrating the polymerisation step of the production process.

The first generation of antigen detecting COVID-19 tests used antibodies targeting the nucleocapsid glycoprotein, which not only means that the sample requires timely pre-treatment but would also give a positive result for all types of human coronaviruses. The RBD region of the spike glycoprotein is unique to SARS-CoV-2, and therefore by design, the nanoMIP will not detect other human coronaviruses, and could eliminate the need for any sample pre-treatment steps. The development method utilized also means the nanoMIP should detect all current variants of concern.

Could eliminate the need for sample pre-treatment

MIP Discovery has also partnered with Stream Bio to offer a ready-conjugated SARS-CoV-2 nanoMIP variant for fluorescence-based assays. This CPN-MIP (Conjugated Polymer Nanoparticle - Molecularly Imprinted Polymer) offers a significantly brighter signal than other dyes, proven to be 1000x brighter than quantum dots.[2]

High affinity and an analytical sensitivity of <5 fg/ml

SARS-CoV-2 viral loads have been shown to decline approximately 2 - 4 days after the onset of symptoms[3]. This means a low limit of detection is key to ensuring accurate testing results during the full lifecycle of the infection. SARS-CoV-2 nanoMIPs have been immobilized on an electrode surface and integrated into a thermal resistance sensor platform. Sensor sensitivity has been demonstrated to <5 fg/ml for the RBD from spike protein (Figure 3).

To demonstrate the affinity of the SARS-CoV-2 nanoMIPs to the spike glycoprotein, SPR analysis has been carried out across multiple batches with a demonstrated affinity constant (KD) ranging from 2 – 18 nM (Figure 4).

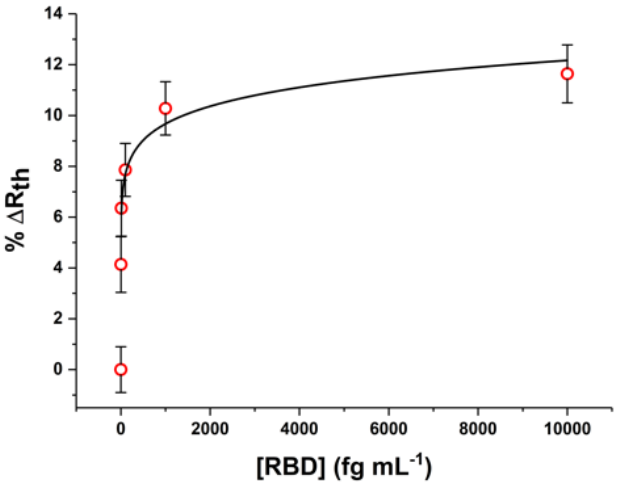

Figure 3. Dose response curve demonstrating an analytical sensitivity of <5 fg/ml. (Marloes Peeters' Group, Newcastle University) The nanoMIPs were immobilized on an electrode surface and integrated into a sensor platform that relies on monitoring the thermal resistance (R_{th}) at the solid-liquid interface. Sensor sensitivity was tested in PBS buffer spiked with SARS-CoV-2 receptor binding domain protein.

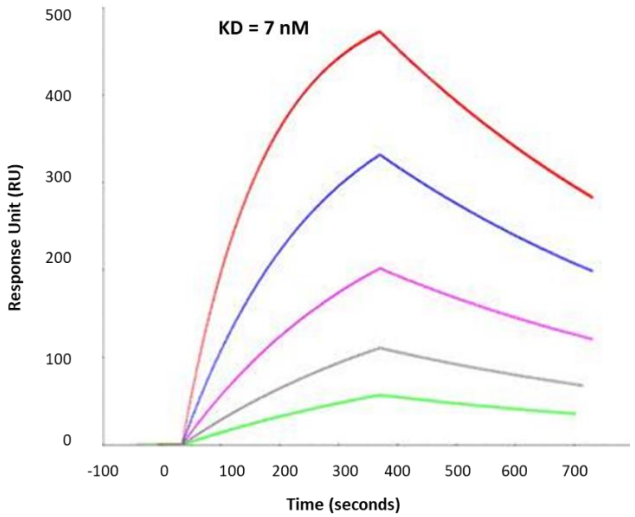

Figure 4. SPR analysis demonstrating an affinity constant (KD) of 7nM. The spike protein S1 subunit of SARS-CoV-2 was immobilised on the sensor chip and SARS-CoV-2 nanoMIPs injected at the relevant concentrations (0.15-40 nM) in PBS.

Analytical sensitivity of <5 fg/ml RBD spike protein demonstrated in a sensor

Proven to detect live virus

To simulate a finished assay, the nanoMIP was conjugated to fluorescent conjugated polymer nanoparticles (CPN™) to demonstrate real virus detection in a dot blot format (Figure 5).

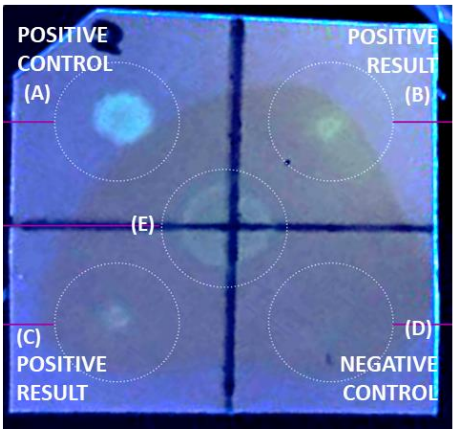

Figure 5. Dot blot demonstrating live virus detection (in collaboration with Stream Bio). Viral particles of SARS-CoV-2 ($2x10^4$ PFU) (B/C), a negative control of viral culture media only (D), positive control of SARS-CoV-2 full length spike protein trimer (A) and reference control of CPN (E) were spotted down and exposed to the MIP+CPN

Selective to SARS-CoV-2 and does not detect other coronavirus variants 299E, HKU1 or OC43

When developing any diagnostic test, it is important to ensure no cross-reactivity with similar targets to avoid false positives. This is especially true for COVID-19, where presenting symptoms are similar to those caused by other human coronaviruses [4]. Not only has the SARS-CoV-2 nanoMIP been designed to avoid this, by imprinting to a portion of the spike glycoprotein unique to SARS-CoV-2, but it has also been demonstrated experimentally. The SARS-CoV-2 nanoMIP has been shown to only detect SARS-CoV-2 spike Glycoprotein and not detect Human Coronavirus 299E Spike Glycoprotein, Human Coronavirus HKU1 Spike Glycoprotein and Human Coronavirus OC43 Spike Glycoprotein in Dot Blot (Figure 6).

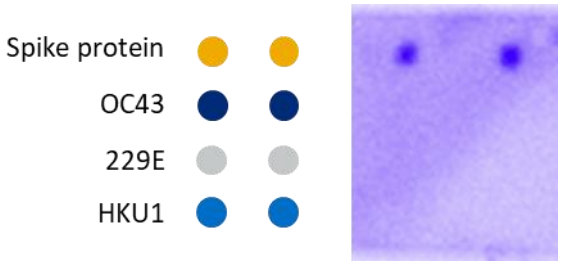

Figure 6. Dot blot demonstrating selectivity to SARS-Cov-2 Spike protein and no cross reactivity to other coronavirus variants. Spike protein, OC43 protein, 229E protein and HKU1 protein were spotted down and exposed to fluorescent tagged nanoMIP in 2 parallel runs.

No cross reactivity with coronavirus variants 29EE, HKU1 or OC43

Outperforms a leading commercial antibody in a sensor device

Antibodies have a long history of use within the IVD sector, and the industry has quickly become accustomed to working around their limitations with regards to stability and lot to lot consistency. The ability of nanoMIPs to overcome these challenges is well documented, and we can now demonstrate that nanoMIPs can also deliver superior sensitivity. Data generated by an independent group has shown that alongside their stability and repeatability characteristics, nanoMIPs also deliver superior sensitivity when tested side by side with flagship antibodies from a leading commercial supplier.

The side-by-side analysis has shown the SARS-CoV-2 nanoMIPs outperform antibodies with regards to sensitivity, demonstrating a limit of detection of <5 fg/ml compared to 105 fg/ml respectively (Figure 7). This evidences that nanoMIPs have the ability to generate a high-performance antigen-detecting rapid test.

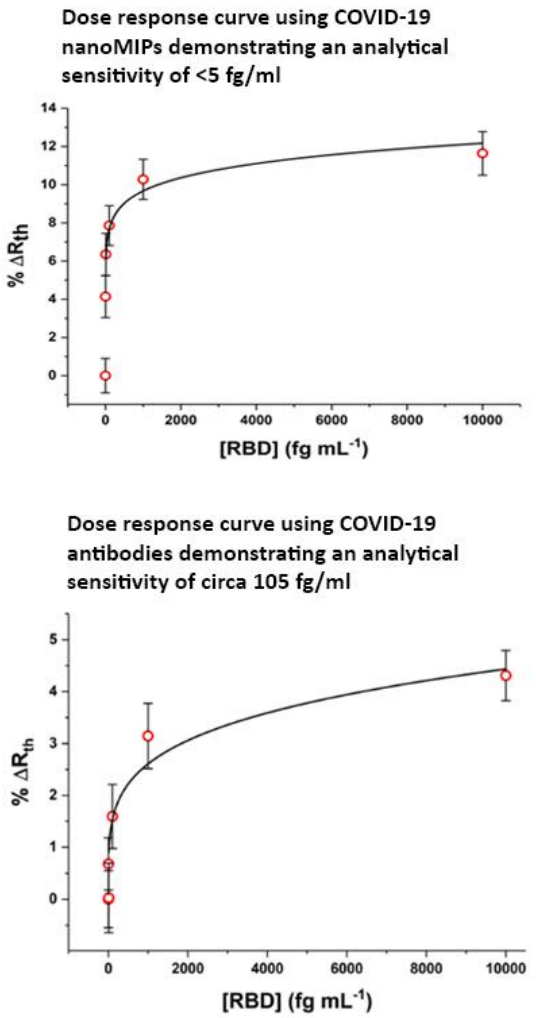

Figure 7. **Dose response curve of COVID nanoMIPs vs leading commercially available COVID antibody** (Marloes Peeters' Group, Newcastle University) The nanoMIPs and antibodies were immobilized on separate electrode surfaces and integrated into a sensor platform that relies on monitoring the thermal resistance (R_{th}) at the solid-liquid interface. Sensor sensitivity was tested in PBS buffer spiked with SARS-CoV-2 receptor binding domain protein.

NanoMIPs outperform a leading commercial antibody in a sensor device

Highly robust at extreme temperatures

One of the unique benefits of nanoMIPs when compared to antibodies is their ability to withstand harsh conditions. Their resistance to temperature, pH and organic solvents eliminates any restrictions around buffer selection and storage conditions, making them ideal for cost effective mass production of point-of-care assays.

The SARS-CoV-2 nanoMIP has been analyzed via SPR pre- and post-autoclaving to 121°C and has demonstrated consistent affinity within experimental error with an affinity constant of 7nM pre-autoclaving and 3nM post-autoclaving. This level of stability will allow for extended assay shelf life and can also reduce the need for complex stabilizers in the finished sensor device.

Thermal stability demonstrated to 121°C.

Batch to batch consistency for supply assurance

The chemical manufacturing process employed ensures batch to batch consistency and reproducibility of the SARS-CoV-2 nanoMIP. This allows for a repeatable production methodology and controlled process designed to meet size and affinity specifications; particles are within the 40 – 80 nm range and affinity constants (KD) are ≤18 nM. Table 1 demonstrates the consistency of particle size and affinities for three separate batches of SARS-CoV-2 nanoMIPs. Figure 7 shows an example SEM image of nanoMIPs produced using MIP Discovery's proprietary solid-phase imprinting process.

COVID-19 nanoMIPs	Diameter (nm)	Affinity KD (nM)
Batch 1	56.9	5
Batch 2	53.9	9
Batch 3	59.3	3

Table 1. Batch to batch consistency across three SARS-CoV-2 nanoMIP batches. Particle size was measured on a NanoSight NS300 and affinity was measured on a Biacore 3000.

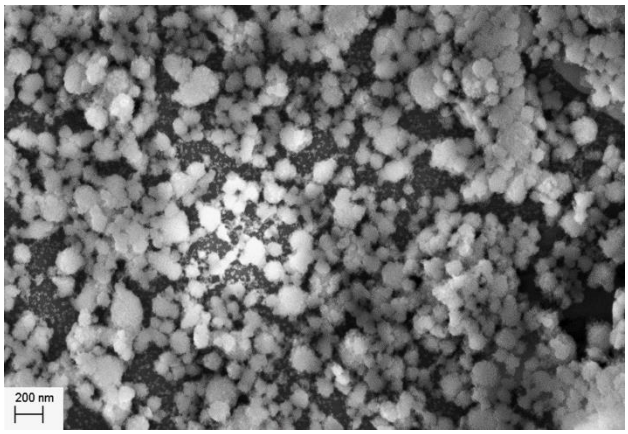

Figure 7. Representative SEM image of nanoMIPs produced using MIP Discovery's proprietary solid-phase imprinting process. (Dr. E. Mazzotta Group, University of Salento)

Ideal for sensor integration

Alongside their exceptional sensitivity performance, SARS-CoV-2 nanoMIPs also enable simple and successful integration into sensor platforms when compared with natural receptors such as antibodies or nucleic acids. nanoMIPs easily couple to the solid surface of an electrode, whereas natural receptors will deform or denature during the coupling process.

The SARS-CoV-2 nanoMIPs from MIP Discovery come ready-functionalized with amine groups to enable attachment to a label of choice or a sensor electrode surface.

Highly stable nanoMIPs do not denature during coupling to the electrode surface

Summary

The SARS-CoV-2 nanoMIP from MIP Discovery can offer superior sensitivity to antibodies, plus the added benefits of thermal stability and continuity of supply. Proven to have a high affinity, selectivity to SARS-CoV-2 and a low limit of detection, nanoMIPs are a clear choice for innovative scientists developing the next generation of COVID-19 diagnostic assays.

References

(1) Astuti, I. et al. 2020, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response, Diabetes Metab Syndr. 14, 4, 407–412.
(2) https://www.streambio.co.uk/
(3) Muge, C. et al. 2020, Virology, transmission, and pathogenesis of SARS-CoV-2, BJM, 371, m3862
(4) Gaunt, E. R. et al. 2010, Epidemiology and Clinical Presentations of the Four Human Coronaviruses 229E, HKU1, NL63, and OC43 Detected over 3 Years Using a Novel Multiplex Real-Time PCR Method, J Clin Microbiol, 48, 8, 2940–294

Samples available now for evaluation

Product Name	Product Code	Specification
SARS-CoV-2 nanoMIP	N108-SC2S-F6AU	Affinity KD ≤18nM | Particle size 40 - 80nm | concentration ≥100µg/ml
SARS-CoV-2 nanoMIP-CPN	N108-SC2S-F6AU-CPN	Product available on request

Why choose nanoMIPs for your next sensor?

- Exceptional sensitivity in sensor platforms, superior to antibodies as demonstrated in a comparison study
- Unlike natural receptors, nanoMIPs do not denature on coupling to an electrode surface
- Proven to perform at extreme temperatures, pressures and pH
- nanoMIPs can easily be used to functionalise screen-printed electrodes
- Rapid development in as little as 8 – 12 weeks
- Custom development service tailors nanoMIP to your target marker and application needs

To request our SARS-CoV-2 nanoMIP product specification and evaluation sample, please contact:
enquiries@mipdiscovery.com or telephone +44 1234 589 725

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