

A Localized Surface Plasmon Resonance Aptasensor for Rapid and Selective Detection of the SARS-CoV-2 Antigens

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Conventional Testing

Conventional methods for viral detection, such as polymerase chain reaction (PCR), can be **costly, time-consuming**, and accompanied by **high false -negative and -positive rates**.¹

Viral Target

Approaches that target viral proteins, such as the **Spike (S) protein** can lead to **higher sensitivity** and offers the potential for **early diagnostic testing** of viral infection.²

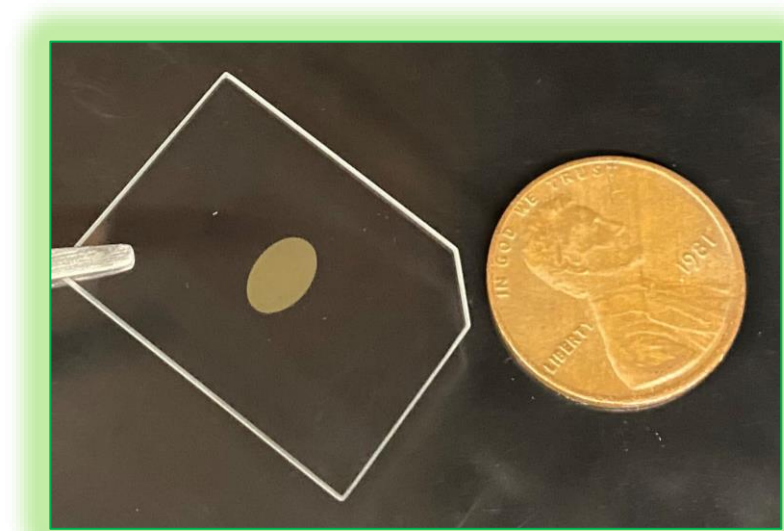
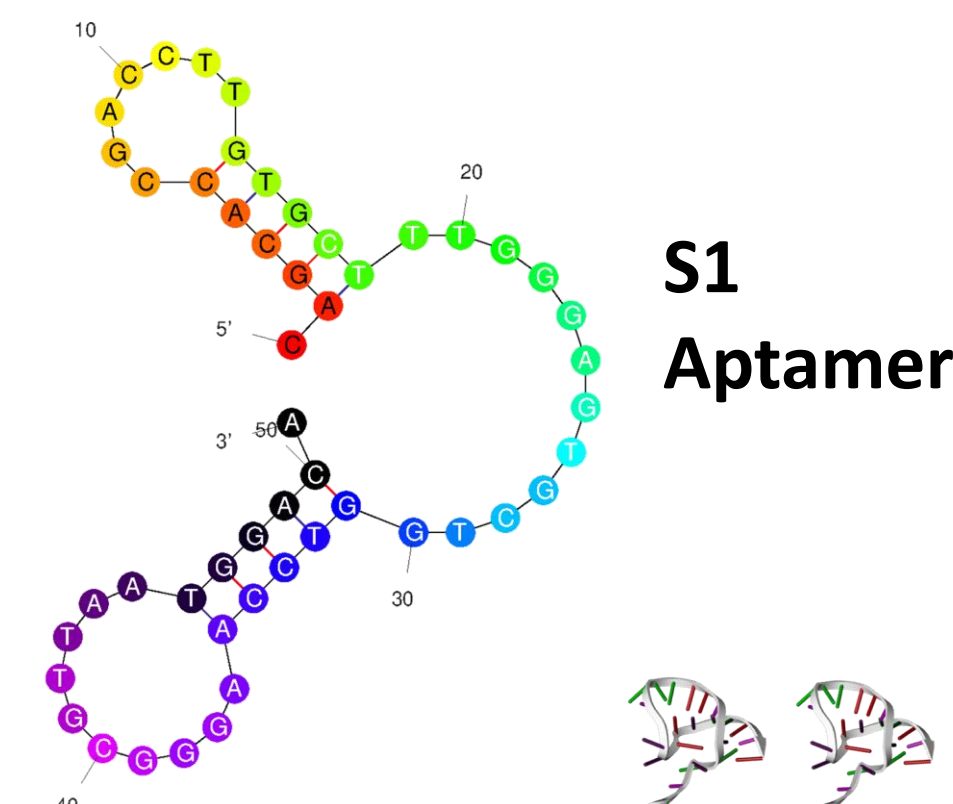
- The high demand on testing of suspected COVID-19 cases has demonstrated the need for **efficient, accurate, rapid** and **reliable solutions** for viral detection.
- The **SARS-CoV-2 spike antigen** plays a vital role in the function of the coronavirus and can be used as a diagnostic biomarker for viral infections.
- For sensing applications, **aptamers** are suitable, **high-affinity** and **cost-effective** binding partners to their targets, including viral antigens.^{1,2}

The binding interaction between single stranded DNA (ssDNA) aptamers and the SARS-CoV-2 S1 protein was evaluated using **localized surface plasmon resonance (LSPR)**.³

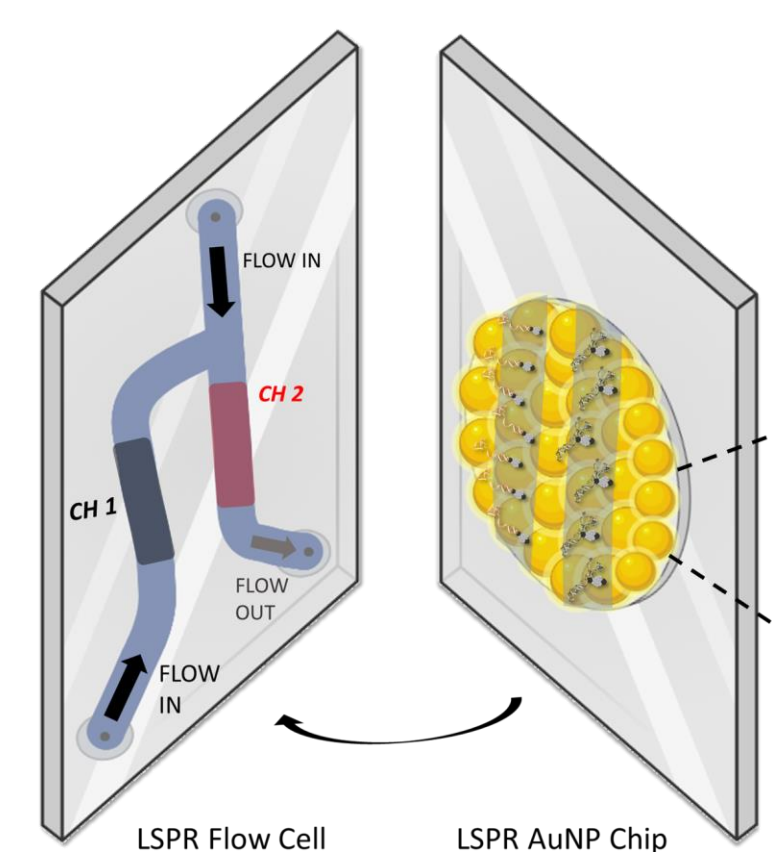
DEVELOPMENT OF THE LSPR APTASENSOR

Localized Surface Plasmon Resonance (LSPR) Measurement:

All LSPR measurements were performed using a 2-channel OpenSPR (NicoyaLifeSciences) and data analyzed using TraceDrawer Software to determine the association rate (k_a), dissociation rate (k_d), and dissociation constant (K_D).³



LSPR Sensing Chip



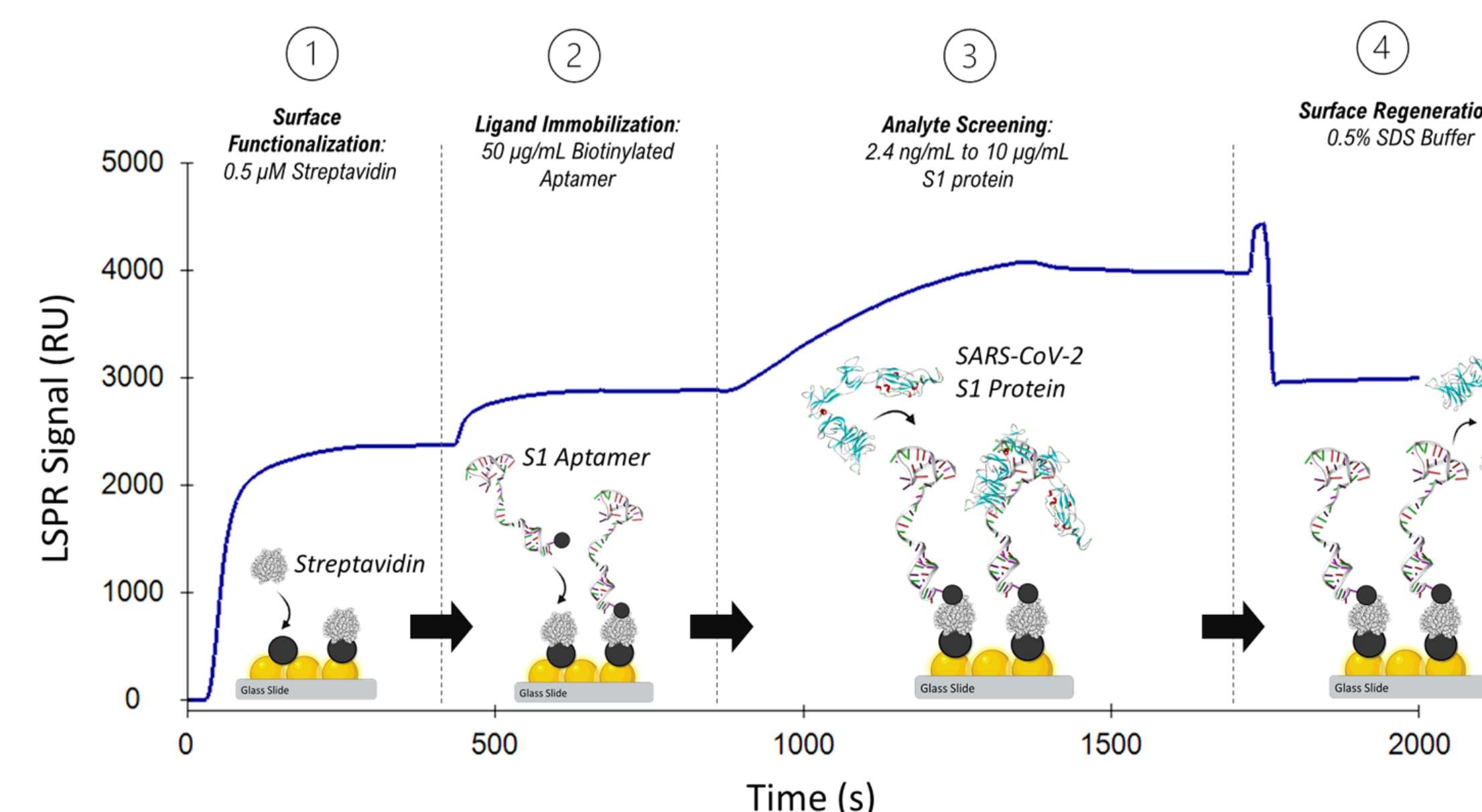
Created using biorendor.com

Biotin coated AuNP Surface

Surface Activation: 0.5 μ M Streptavidin

Ligand Immobilization: 50 μ g/mL Biotinylated Aptamer

EVALUATION OF ANTIGEN-APTAMER INTERACTIONS

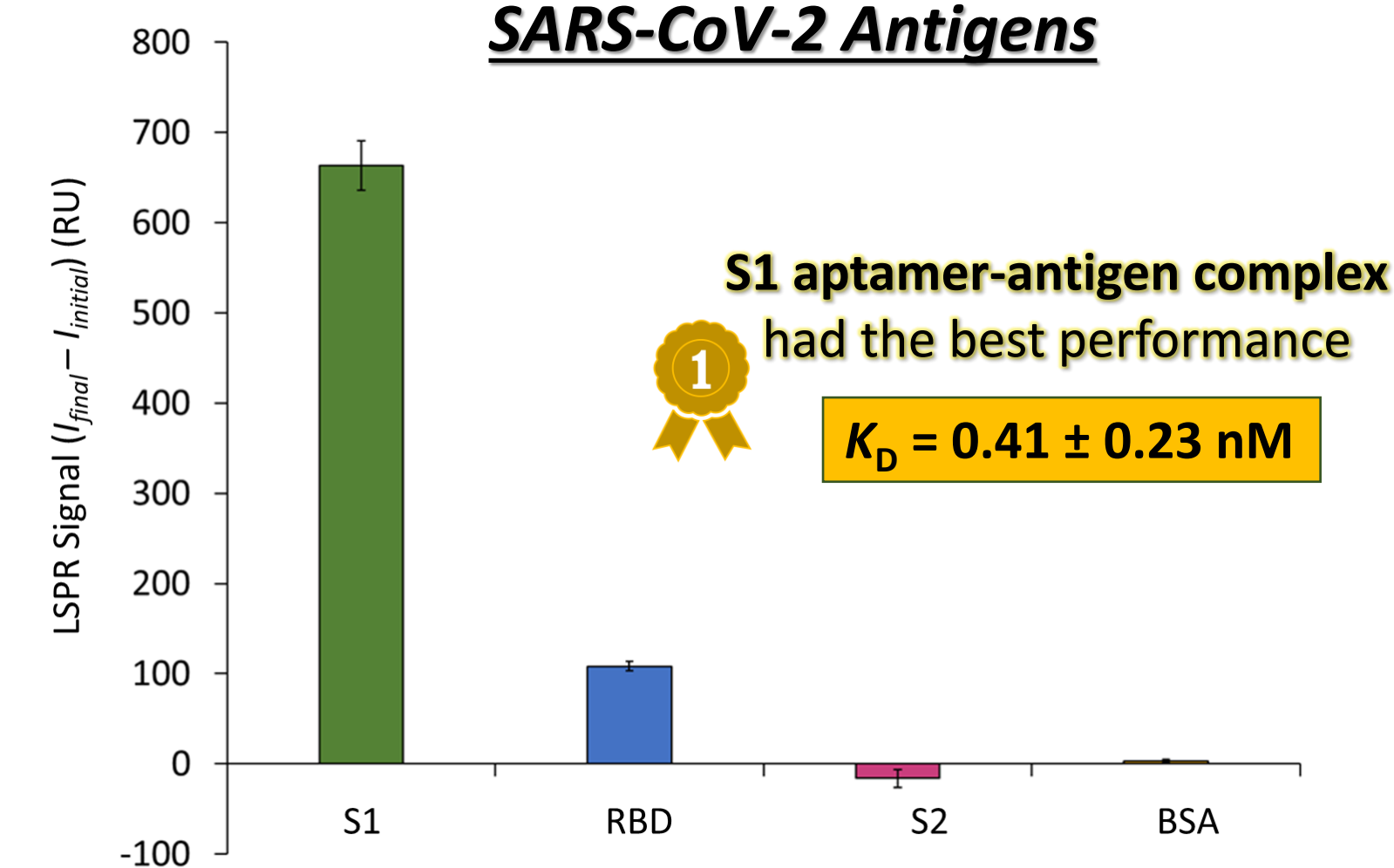


Aptasensor Design

LSPR sensorgram representing the fabrication of the S1 aptasensor including: (1) attachment of streptavidin to the biotin-AuNP surface, (2) immobilization of the biotinylated S1 aptamer, (3) 10-min S1 antigen association, and (4) surface regeneration allowing for subsequent measurement of another sample.

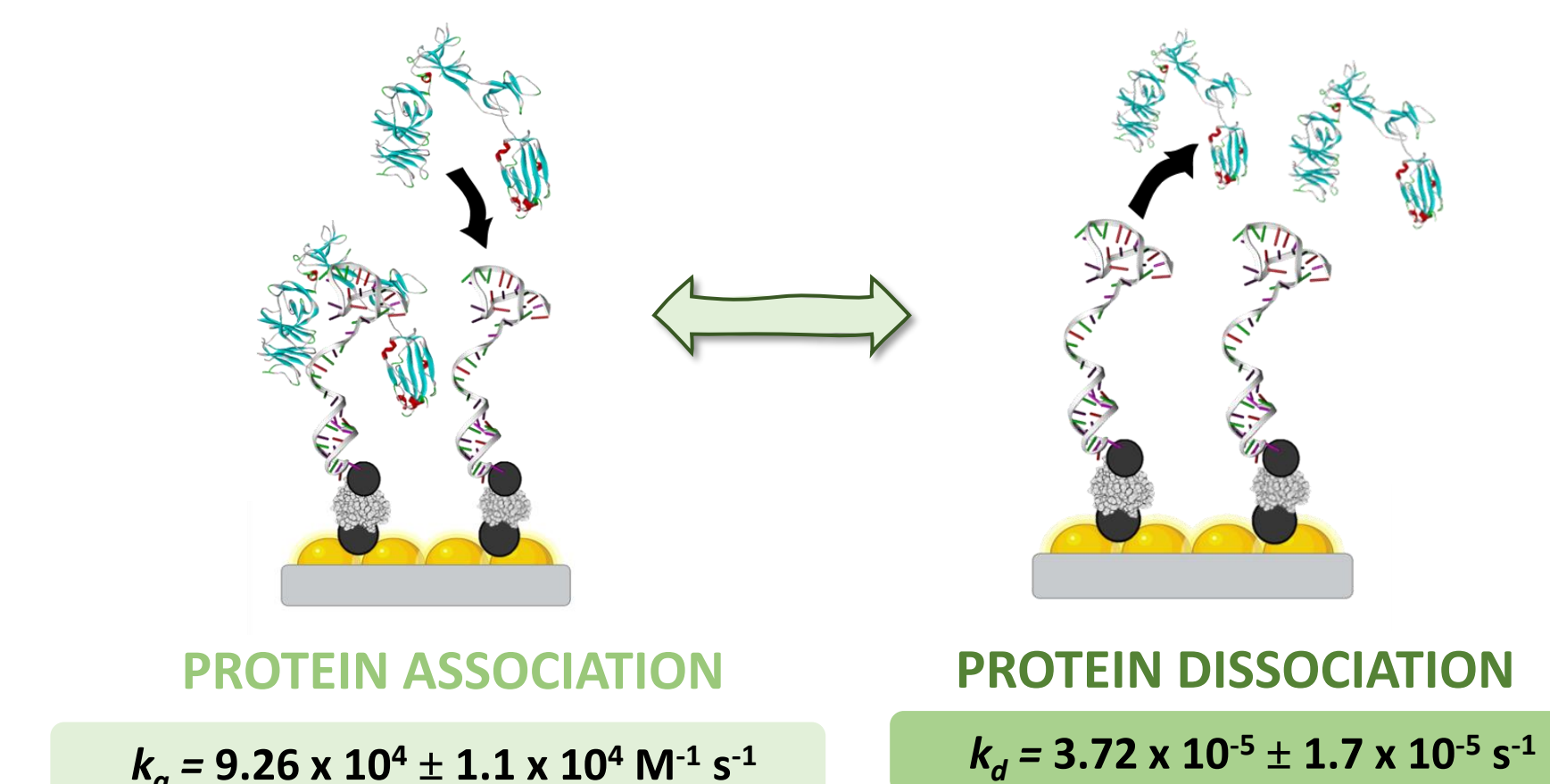
EXCELLENT S1 APTASENSOR PERFORMANCE

S1 Aptamer Selectivity Amongst SARS-CoV-2 Antigens



LSPR signal recognition of SARS-CoV-2 S1, RBD and S2 antigens or BSA to the S1- aptasensor. The S1 aptasensor showed selective to the SARS-CoV-2 S1 antigen. [Antigens] = 2.5 μ g/mL.

Kinetic Evaluation of the Antigen-Aptamer Interaction



Kinetic evaluation suggests a strong binding interaction between the SARS-CoV-2 S1 protein and the S1 aptamer and high affinity toward the SARS-CoV-2 S1 protein ($K_D = 0.41 \pm 0.23$ nM).

>90%
Signal Recovery
in artificial saliva or
serum albumin

9x
Repeats
without diminishing
sensor performance

24
Days shelf stability
with pre-fabricated
aptasensor LSPR chips

10
minutes
of the user's time for
sample testing

CONCLUSIONS

- ✓ Direct and selective detection of the SARS-CoV-2 S1 protein was achieved using the S1 aptamer with LSPR.
- ✓ Using LSPR, the **binding of the S1 protein to the immobilized aptamer** was monitored in **real-time**.
- ✓ **Surface regeneration** allows for **reuse of the aptasensor** for multiple measurements.
- ✓ OpenSPR can be used to **monitor aptamer-protein interactions** for application to coronavirus related measurements.
- ✓ Using the streptavidin-biotin chemistry, a **limit of detection** of 0.26 nM and **limit of quantification** of 1.05 nM was achieved.
- ✓ The LSPR aptasensor is a promising platform for **assessing viral protein interactions in complex solutions**, such as those that mimic biological fluids.

FUTURE WORK

- ➔ LSPR evaluation of sensor performance using clinical samples to further investigate efficiency of the aptasensor in real-life applications
- ➔ Comparison of SARS-CoV-2 sensors using other sensor chemistries such as carboxyl-amine and gold-thiol.
- ➔ Utilize LSPR to determine the ability of DNA aptamers to serve as interferents of the ACE2-RBD binding interactions; toward the development of anti-viral therapies

REFERENCES

- 1)Chen, Z., et al., *Virol. Sin.* **2020**, 35 (3), 351–354. <https://doi.org/10.1007/s12250-020-00236-z>.
- 2)Song, Y., et al., *Anal. Chem.* **2020**, 92 (14), 9895–9900. <https://doi.org/10.1021/acs.analchem.0c01394>.
- 3)Lewis T., Giroux E., Jovic M., Martic S. *Analyst*, **2021**, 146:7207-7217. doi: 10.1039/d1an01458g.

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