Comparative Assessment of the Binding Affinities of SARS-CoV-2 Proteins to Ni-NTA Surfaces

Erin Giroux, Tyra Lewis, Anasofia Vargas, Dr. Sanela Martic
Trent University, Peterborough, Ontario, Canada, K9L 0G2
sanelamartic@trentu.ca *@MarticSanela*

Commonwealth Chemistry
Federation of Chemical Sciences Societies

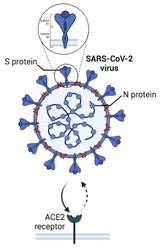
TRENT UNIVERSITY

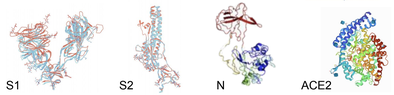
INTRODUCTION

The COVID-19 pandemic generated the urgent need worldwide for a simple, rapid, and accurate detection method for the SARS-CoV-2 virus.

Proteins of Interest

The SARS-CoV-2 spike (S) protein, consisting of the S1 and S2 subunits, is located on the envelope of the coronavirus and is responsible for binding to host cell receptors. In human cells, this receptor is the human angiotensin-converting enzyme 2 (ACE2).[1] The SARS-CoV-2 nucleocapsid (N) protein is the site of abundant RNA-binding which is crucial in packaging viral genome.[2] As such, the interactions of these proteins can be employed in developing biomarker based viral detection methods.





METHODOLOGY

Analyte Proteins

- SARS-CoV-2 S1 protein, His-tag (SinoBiological- 40591-V08B1)
- SARS-CoV-2 S2 protein, His-tag (Sino Biological- 40590-V08B)
- SARS-CoV-2 N protein, His-tag (Sino Biological- 40588-V08B)
- Human ACE2 protein, His-tag (Sino Biological- 10108-H08H)

Experimental Measurements

- A portable, two-channel, Localized Surface Plasmon Resonance (LSPR) instrument (OpenSPR, Nicoya Lifesciences) with a nickel-nitrilotriacetic acid (Ni-NTA) sensor surface was used for all measurements.

Experimental Procedure

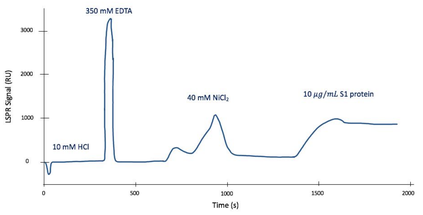
1. **Surface Cleaning:** 10 mM HCl then 350 mM EDTA

2. **Surface Activation:** 40 mM $NiCl_2$

3. **Protein Immobilization:** 10 µg/mL protein

4. **Protein Removal:** 2 regeneration methods were compared for optimizing S1 protein removal

   (a) imidazole, HCl;
   or
   (b) EDTA, HCl;



LSPR sensorgram demonstrating the procedural steps of surface cleaning, surface activation, and protein immobilization.

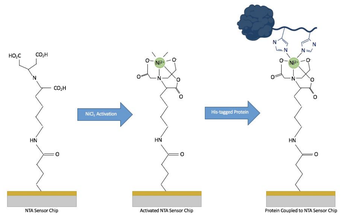
Ni-NTA SURFACE CHEMISTRY

Surface Cleaning and Activation

- The NTA sensor chip is cleaned with injections of HCl and EDTA prior to surface activation with $NiCl_2$.
- When the $NiCl_2$ interacts with the NTA on the sensor surface, the Ni^{2+} covalently bonds and acts as a chelating agent for histidine tagged analytes.

Protein Binding

- NTA sensors are generally used for binding poly-histidine (His) tagged proteins due to their binding affinity to the Ni-NTA complex.
- LSPR allows for the real time monitoring of the protein binding to the Ni-NTA complex. An increased signal is an indication of binding interactions on the sensor.
- The signal increase is representative of an analyte's binding affinity to the Ni-NTA complex.



Surface chemistry of NTA sensor chip through the $NiCl_2$ activation step and His-tagged protein immobilization.

IMMOBILIZATION OF SARS-CoV-2 PROTEINS

Comparing Binding Affinities of Proteins

- 10 µg/mL SARS-CoV-2 S1, S2, N, and human ACE2 protein were introduced to an activated Ni-NTA sensor, and replicates of each protein injections were performed to obtain an average and standard deviation of the LSPR signal obtained from each protein for comparison.

SARS-CoV-2 S1 Protein

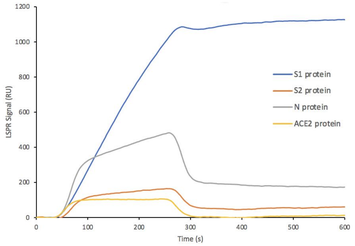
- The SARS-CoV-2 S1 protein was found to have the highest binding affinity to the Ni-NTA complex surface, with an average LSPR signal obtained of 800.7 +/- 288.2 RU.

SARS-CoV-2 N Protein

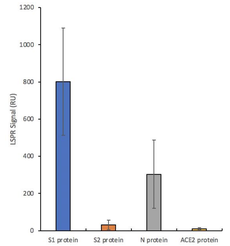
- The SARS-CoV-2 N protein was found to have a moderate binding affinity, but showed a significant degree of protein dissociation following the injection, as seen in the grey binding curve on the sensorgram below. The average LSPR signal obtained was 303.1 +/- 183.4 RU.

SARS-CoV-2 S2 and Human ACE2 Proteins

- The SARS-CoV-2 S2 protein and human ACE2 protein exhibited a low binding affinity towards the Ni-NTA surface, with average LSPR signals of 31.0 +/- 25.7 RU and 10.0 +/- 5.1 RU, respectively.



LSPR sensorgram of 10 µg/mL S1, S2, N, and ACE2 protein binding interaction to Ni-NTA complex surface.



LSPR signal averages and standard deviations of repeated injections of analyte proteins.

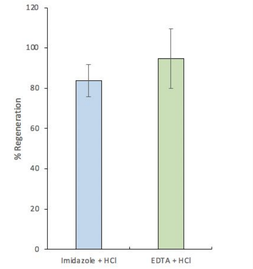
REGENERATION FOR REUSABILITY

Principles of Regeneration

- To allow for repeated use of the sensor, various regeneration methods were investigated to remove protein bound to the surface.
- The regeneration efficiency was monitored in real time as a decrease in the LSPR signal.
- In a successful regeneration, the LSPR signal returns to the baseline signal that was seen prior to the protein injection. If the signal remains above baseline, <100% regeneration, protein remains on the surface.

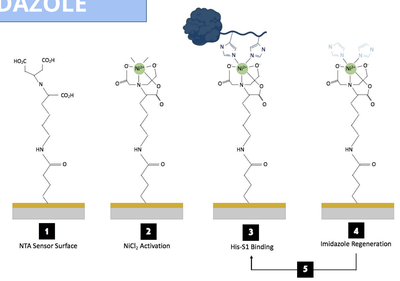
Regeneration Methods

- Imidazole breaks the His-Ni bond between the Ni-NTA complex and the His-tagged protein by replacing the histidine. Two injections of 500 mM imidazole followed by one injection of HCl to clean the surface had a regeneration efficiency of 83.9 +/- 7.9%.
- EDTA is a chelating agent, which removes the nickel from the surface. Following this method, the nickel needs to be replaced prior to another protein injection. Two injections of 350 mM EDTA followed by one injection of HCl for surface cleaning was found to have a regeneration efficiency of 94.7 +/- 14.7%.

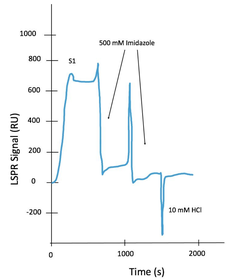


Regeneration efficiency of 500 mM imidazole and 350 mM EDTA methods compared.

IMIDAZOLE

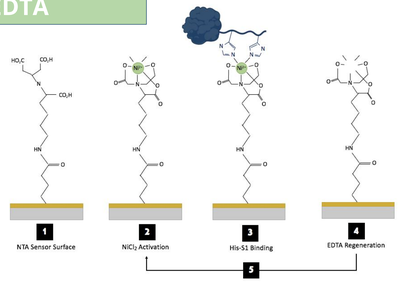


Surface chemistry of NTA sensor chip through activation (2), protein binding (3), and imidazole regeneration (4). To reuse sensor with this regeneration method, steps 3 and 4 can be repeated multiple times.

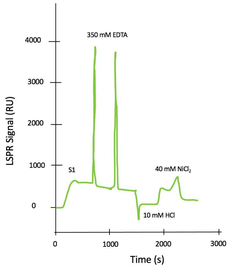


LSPR sensorgram of imidazole regeneration method following S1 protein injection.

EDTA



Surface chemistry of NTA sensor chip through activation (2), protein binding (3), and imidazole regeneration (4). To reuse sensor with this regeneration method, steps 2, 3 and 4 can be repeated multiple times.



LSPR sensorgram of EDTA regeneration method following S1 protein injection.

CONCLUSIONS

- The OpenSPR instrument provided real-time monitoring of the binding interactions between protein and the Ni-NTA surface.
- Significant binding of the SARS-CoV-2 S1 protein to the Ni-NTA surface was achieved.
- Regenerating the sensor surface with EDTA was found to be an effective method for removing bound protein, allowing for the sensor to be reused.

FUTURE WORK

- Build on developed method to investigate the binding interactions between ACE2 and the spike protein.
- Compare the binding affinities of SARS-CoV-2 proteins on Ni-NTA to sensors of other surface chemistries.
- Improve procedure and regeneration methods to obtain better consistency in LSPR signal obtained by consecutive injections of protein on the same sensor

UN SDG CONTRIBUTION

- The COVID-19 pandemic is more than a global health crisis. It has caused disruptions and setbacks in plans to improve global equality, education, and the well-being of everyone worldwide.
- Rapid testing and treatment has been identified as a key to ending the pandemic. The development of this sensor for protein-protein interactions could allow for the detection of SARS-CoV-2.
- Beyond the current pandemic, these methods may be applied to other viral targets, providing a rapid and reliable detection method for other viruses.

REFERENCES

1. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., Wang, X. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, **2020,** 581(7807), 215-220.
2. Zeng, W., Liu, G., Ma, H., Zhao, D., Yang, Y., Liu, M., Mohammed, A., Zhao, C., Yang, Y., Xie, J., Ding, C. Biochemical characterization of SARS-CoV-2 nucleocapsid protein. *Biochem Biophys Res Commun*, **2020,** 527(3), 618-623.
3. Song, Y., Song, J., Wei, X., Huang, M., Sun, M., Zhu, L., Lin, B., Shen, H., Zhu, Z., Yang, C. Discovery of Aptamers Targeting Receptor-Binding Domain of the SARS-CoV-2 Spike Glycoprotein, *Anal. Chem.* **2020**, *92*, 9895-9900.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support of Trent University and the members of the Martic Lab group, as well as the contributions from Nicoya LifeSciences and NSERC Alliance COVID-19.

NSERC CRSNG nicoya *sanelamartic@trentu.ca*