Application Note 30

Reichert's Surface Plasmon Resonance (SPR) systems and XanTec's Sensor Chips in SARS-CoV-2 Research

SARS-CoV-2 is a member of a large family of viruses called coronaviruses that causes a highly contagious respiratory disease called COVID-19. This has created a world-wide public-health emergency, affecting billions of lives. The development of therapeutics and vaccines to prevent the spread of SARS-CoV-2 is an area of enormous focus for scientists worldwide. Toward this end, researchers at Sichuan University and the XtalPi AI Research Center implemented an in-silico method called Rosetta Flex ddG to predict the binding affinity changes caused by point mutations on the receptor binding domain (RBD) of the SARS-CoV- 2 virus S (spike) protein. Their aim was to determine higher affinity mutants which could become more infectious and more resistant to antibodies. Mutants with large negative predicted ddG score were taken into the lab for experimental testing using surface plasmon resonance (SPR). Researchers utilized a Reichert4SPR to determine the binding of these mutants. Specifically, equilibrium binding constants for the homo-trimeric spike glycoprotein (S protein), and various predicted mutants, binding to hACE2 (human angiotensin-converting enzyme 2) were determined and compared to the predicted values.[1]

The first step in the virus cell infection process is cell entry, so blocking the binding of S protein to hACE2 could be key to virus transmission prevention. One way to accomplish that is to use monoclonal antibodies from convalescent plasma, which provides competition to the S protein binding and thus prevents it from binding. Another way is to develop a recombinant vaccine. A third possibility is to use a protein inhibitor.[1]

Mutations on the S binding protein of the RBD can change affinity to the cell receptor thus affecting potency of potential therapeutics. Researchers in this study note that 196 mutations in the RBD domain have been found. Also noted was the fact that while some mutants are less infectious, others are more infectious and tend to be resistant to neutralizing antibodies. These potentially more infectious mutants with higher affinity were the focus of this research with the aim of looking to future treatments.[1]

Researchers initially predicted protein-protein interaction strength for each mutant using the Rosetta Flex method, then determined the actual affinity with SPR to see how accurate their predictions were. A ddG value was calculated which indicates what the difference in affinity is once a protein mutates compared to the wild type.[1]

Experimental

Background

Researchers were interested in comparing SPR results with those predicted using an in-silico affinity maturation method. Their ultimate aim was to use in-silico methods to predict which mutants should be used for vaccine and other treatment development.[1]

Conditions

- Instrument: Reichert4SPR
- Sensor Chip: planar carboxyl (also available: ready-to-use SARS-CoV-2 RBD precoated sensor chips)
- Temperature: 25°C
- Buffer: PBST (8mM Na_2HPO_4, 136 mM NaCl, 2 mM KH_2PO_4, 2.6 mM KCl, and 0.05 % (v/v) Tween 20, pH 7.4)
- Flow Rate: 25 µL/min
- Target: SARS-CoV-2 Spike Protein (RBD, His tag) and its mutants
- Analyte: hACE2
- Association: 3 minutes
- Dissociation: 5 minutes
- Regeneration: none

Results

In total, the Rosetta Flex method was applied to 475 possible single point mutants. Of those, 114 had a negative ddG score which indicated they might have higher affinity than the wild type. Mutants with a large negative ddG value were looked at manually and ultimately, 9 mutants were selected for further study using SPR. Researchers ran their SPR experiments as a kinetic titration, so no regeneration conditions needed to be determined. Of the 9 chosen, 6 targets showed improved binding to hACE2 (were higher affinity) compared to the wild type. The remaining 3 mutants showed either similar affinity as the wild type or lower. The authors reviewed the results and decided that hydrogen binding, which was not fully factored into the model for the three lower affinity binders, was the cause of the discrepancy for them.[1]

As complement, **XanTec** has developed a cross-platform, ready-to-use COVID-19 SPR sensor chip to meet the increasing demand for COVID-19-related research tools. The sensor chip consists of the SARS-CoV-2 receptor-binding domain (RBD) protein (wild-type) homogenously preimmobilized on **XanTec's** proprietary ultra-low-background polycarboxylate HC surface. This new surface is a versatile tool for various applications in clinical and pharmaceutical COVID-19 R&D and available for various SPR instruments.

The C19RBDHC30M sensor chip was primarily developed for fast and label-free detection of anti-SARS-CoV-2 antibodies from patient serum. As the sample matrix contains serum and is thus prone to nonspecific interactions, a highly bioinert chip coating, optimized reagents, and an adapted protocol are essential for highly specific and sensitive detection of anti-SARS-CoV-2 antibodies[2].

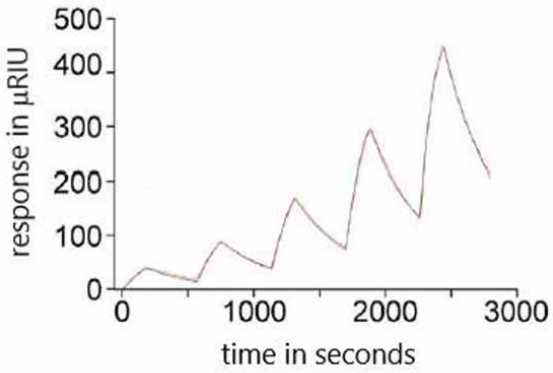

Figure 1. An example of a binding series for a higher affinity mutant called Q498W is shown here. Concentrations of hACE2 ranging from 6.25 up to 100 nM were injected, without regeneration in between, over immobilized Q498W RBD mutant. Data was fit to a 1:1 binding model in Clamp. Kinetics values obtained were as follows: association constant, k_a = 6.2e^4 M^{-1}s^{-1}, dissociation constant, k_d = 4.4e^{-4} s^{-1} and the equilibrium dissociation constant, K_D = 7.1 nM. This affinity value is about 3 times higher than that obtained for the wild type RBD which has an affinity of about 21 nM.[1]

Summary

- Predicted affinity interaction results for RBD and its mutants binding to hACE2 using the Rosetta Flex method, in general, compared favorably to those obtained from experimental testing.
- Comparison of calculated or predicted results to those obtained experimentally using SPR yielded 6 mutants that showed higher affinity than the wild type with the best (highest affinity) ones being about 3 times higher.
- Rosetta Flex ddG-based in silico affinity maturation methods could be combined with machine learning to become faster and more accurate in future applications.
- Since computational methods are relatively low cost and fast, methods such as that outlined here could play a role in helping identify virus pathogenicity at an early stage for quickly mutating viruses in the future.
- By using **XanTec's** COVID-19 sensor chips, SPR can be used as rapid high-content method, not only to quantify antibody titer in patient serum, but also to assess antibody quality. The latter correlates directly with the disease progression[3] and would therefore be an extremely valuable parameter for hospitalization decisions (triage).

References

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