Performance analysis of SARS-CoV-2 selective Optimer reagents to evolving viral variants

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Introduction

- Optimer reagents were developed to the SARS-CoV-2 virus to support research and rapid diagnostics to address the COVID-19 pandemic.
- As the pandemic has evolved mutations in SARS-CoV-2 have resulted in the emergence of variant strains with prevalent and virulent strains showing mutations in the S protein.
- To ensure accurate diagnostics all tests should recognise both the WT and subsequent strains of SARS-CoV-2.
- Binding and affinity of the SARS-CoV-2 S1 and S2 Optimer reagents was characterized for the S protein and irradiated virus of the new SARS-CoV-2 variant strains to assess the accuracy of any research or tests using these reagents.

Methods

- Previously developed Optimer reagents to the S1 and S2 subunits of the WT SARS-CoV-2 protein were used for analysis.
- A panel of SARS-CoV-2 variant S proteins and irradiated virus particles (Table 1) were assessed for Optimer binding via biolayer interferometry.

Mutation	Variant	Sample Type Analyzed	
		SARS-CoV-2 S1 Protein	Irradiated Virus Particles
S1-WT	WT	✓	✓
S1-D614G	Denmark	✓	Not tested
S1-B.1.1.7	UK 'Kent'	✓	✓
S1-B.1.351	South African	✓	✓

Table 1: The SARS-CoV-2 S protein variant forms analyzed for Optimer binding performance. Four different variants of the SARS-CoV-2 S protein were investigated representing examples of the most prevalent and virulent mutations internationally.

Results

Optimers bind the SARS-CoV-2 S protein variants

- Optimer reagents developed to the WT SARS-CoV-2 S1 protein subunit are able to bind each of the mutant SARS-CoV-2 strain S proteins by BLI (Fig. 1).
- In contrast to the WT and D14G (Fig. 1a, b) which fit a 1:1 interaction model, two phases of binding are evident for B.1.17 and B.1.351 (Fig. 1c, d), thus K_{D2} values were calculated for these interactions.

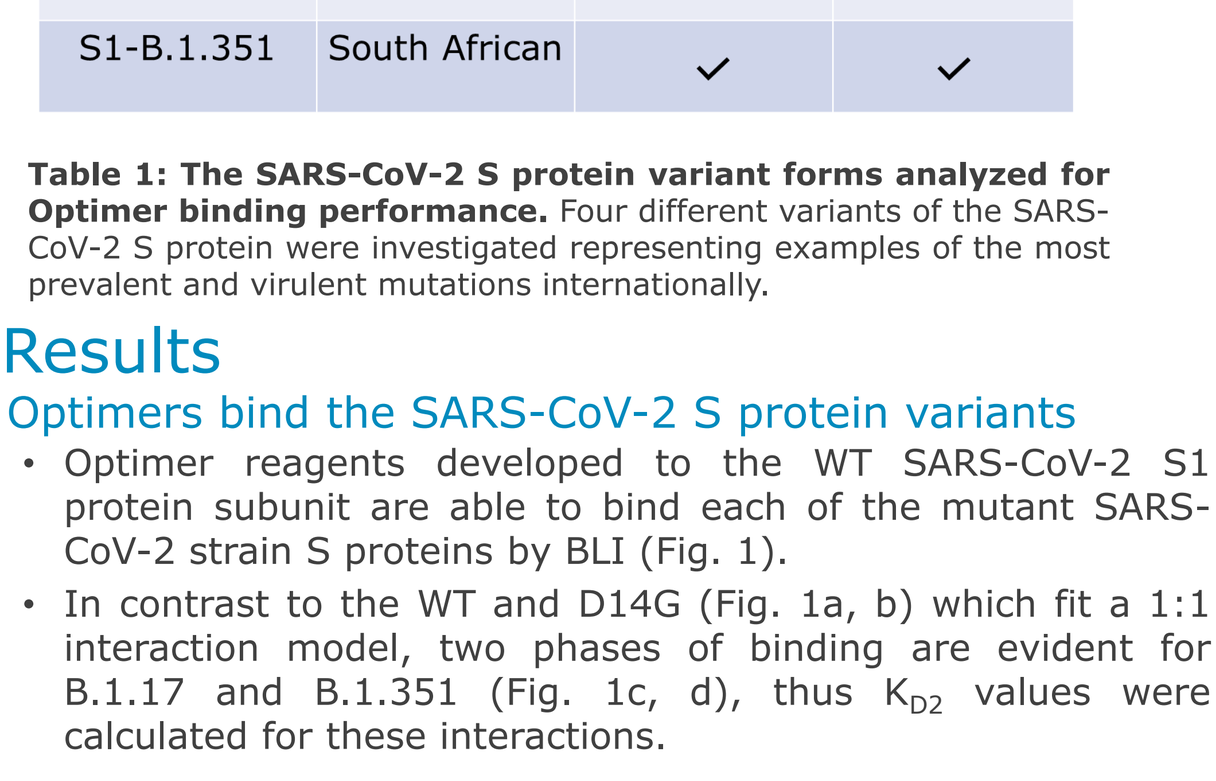

Figure 1: SARS-CoV-2 S1 Optimers bind S protein variants by BLI. BLI strepatavidin probes were coated with 20 nM biotinylated Optimer, washed and interaction measured in 80 µL of (a) WT (b) D614G (c) B.1.17 (d) B.1.351 protein in buffer over the S protein concentration range shown.

Equivalent binding to SARS-CoV-2 variants

- The S1 Optimer showed approximately equivalent binding affinity by BLI for each of the SARS-CoV-2 variants (Table 2).

Protein	K_D (nM)
S1-WT	10.7 ± 0.057
S1-D614G	19.8 ± 0.35
S1-B.1.1.7	62.6 ± 1.22
S1-B.1.351	43.7.6 ± 1.24

Table 2: Affinity of the S1 Optimer to the SARS-CoV-2 variants show similar binding kinetics to the WT and all three variant S proteins. Values determined by BLI analysis of Optimer-coated probes monitored across concentration gradients of each of the SARS-CoV-2 variant S proteins. K_D values were determined for the WT and D614G mutants. K_{D2} values were determined for the B.1.17 and B.1.351 mutants (shown in blue).

Optimers bind SARS-CoV-2 irradiated virus particles

- Both the S1 and S2 Optimer reagents bind to irradiated virus particles of SARS-CoV-2.
- Optimer reagents show similar binding performance to the B.1.1.7 and B.1.351 SARS-CoV-2 variants as to the WT SARS-CoV-2 virus.

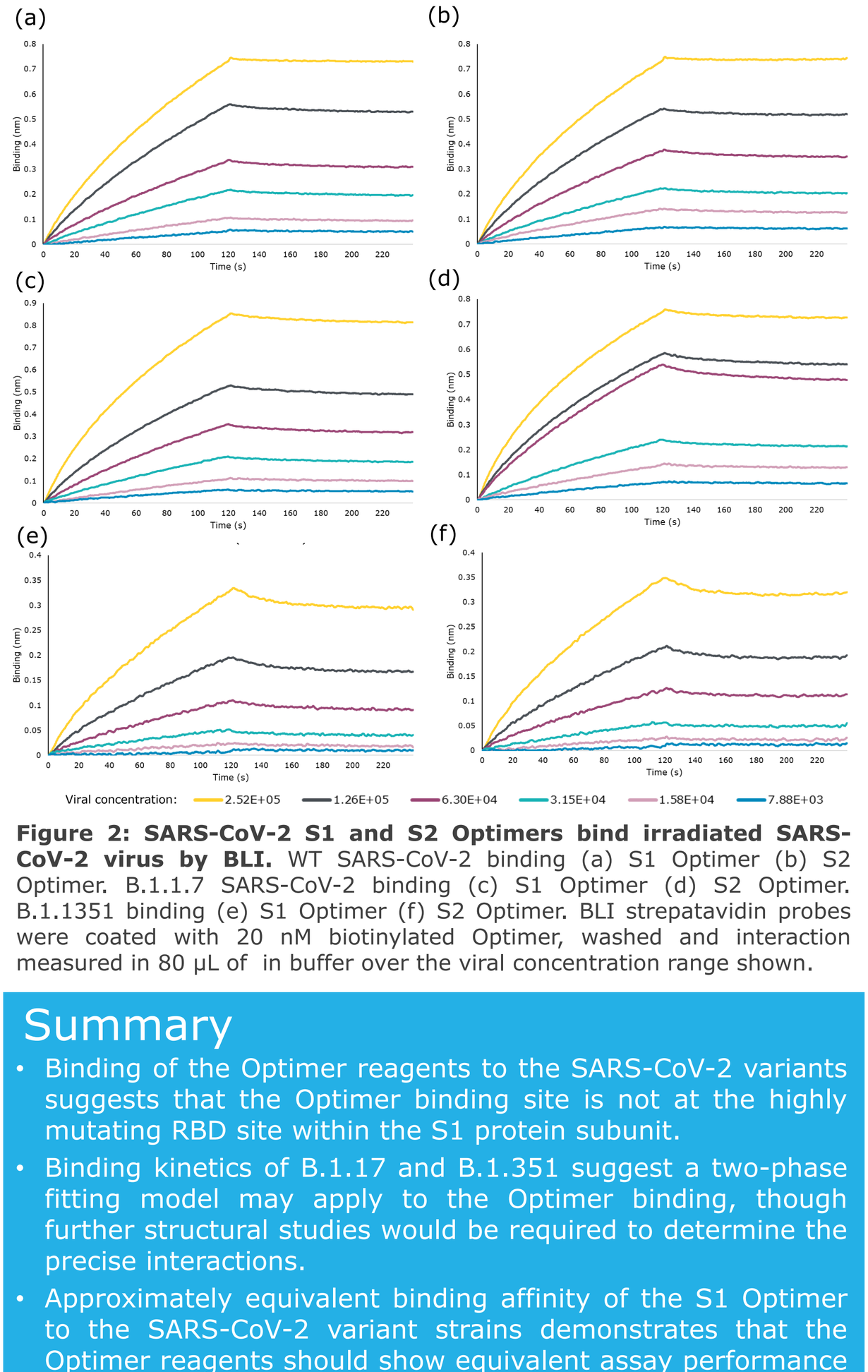

Figure 2: SARS-CoV-2 S1 and S2 Optimers bind irradiated SARS-CoV-2 virus by BLI. WT SARS-CoV-2 binding (a) S1 Optimer (b) S2 Optimer. B.1.1.7 SARS-CoV-2 binding (c) S1 Optimer (d) S2 Optimer. B.1.1351 binding (e) S1 Optimer (f) S2 Optimer. BLI strepatavidin probes were coated with 20 nM biotinylated Optimer, washed and interaction measured in 80 µL of in buffer over the viral concentration range shown.

Summary

- Binding of the Optimer reagents to the SARS-CoV-2 variants suggests that the Optimer binding site is not at the highly mutating RBD site within the S1 protein subunit.
- Binding kinetics of B.1.17 and B.1.351 suggest a two-phase fitting model may apply to the Optimer binding, though further structural studies would be required to determine the precise interactions.
- Approximately equivalent binding affinity of the S1 Optimer to the SARS-CoV-2 variant strains demonstrates that the Optimer reagents should show equivalent assay performance to detect each of the SARS-CoV-2 variants.
- Optimer performance is maintained with regards to the SARS-CoV-2 variants for reliable research and diagnostic performance.