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The role of SARS-CoV-2 ORF8 protein ARKS motif on novobiocin binding

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The role of SARS-CoV-2 ORF8 protein ARKS motif on novobiocin binding

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Abstract:

The discovery of the SARS-CoV-2 virus during the COVID-19 pandemic required scientists to develop medical solutions to reduce viral spread and symptoms, prompting novel therapeutic drug methods to be developed. This experimental project focuses on targeting the unique accessory protein, Open Reading Frame 8 (ORF8) in SARS-CoV-2 through studying its interactions with novobiocin. ORF8 specializes in helping evade immune system checks, is involved in inflammatory responses from the cytokine storm, and most importantly, was proposed to act as a histone mimic at the histone-H3 ARKS motif that causes post-translational changes in chromatin, further worsening these problems. Previous experimental work from our lab has shown that novobiocin [K_d = 54.5 ± 3.14 μM] and three other computationally verified ligands bind to ORF8. To probe the role of Arg in the histone-H3 ARKS motif, specific mutation was done in position 52 from Arg to Met, Glu and Leu respectively, resulting in drastic intermolecular force changes that affect novobiocin's ability to bind to the ORF8 pocket . *In silico* analyses for the mutagenic ORF8 found the variants still docked successfully to ORF8 according to Swissdock. Primers for the ORF8 R52 mutants were then designed, and mutagenic plasmids were sequence verified. The mutant ORF8 proteins were overexpressed, purified, and K_d values for binding to novobiocin were determined via intrinsic fluorescence spectroscopy. This data will help further understand the role of SARS-CoV-2 ORF8 protein ARKS motif and how its interactions affects novobiocin binding, potentially benefitting future studies attempting to repurpose novobiocin for treatment of the virus.

Introduction:

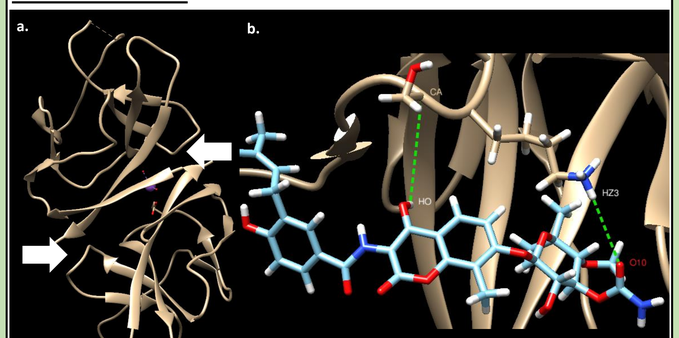

Figure 1. Previous understandings of SARS-CoV-2 ORF8 and novobiocin interaction. **Figure 1a.** Protein structure of SARS-CoV-2 ORF8. White arrows indicate location of ARKS motif and novobiocin binding pocket; **Figure 1b.** ORF8 interaction with novobiocin.

- Novobiocin was found to selectively bind to SARS-CoV ORF7a, and in SARS-CoV-2 ORF8, targets the ARKS motif engaging in histone mimicry.
- Favorable intermolecular interaction with Arg signifies key target for binding , encouraging mutagenic studies to understand the importance of the amino acid in potential ORF8 inhibition.

Mutagenesis of ORF8:

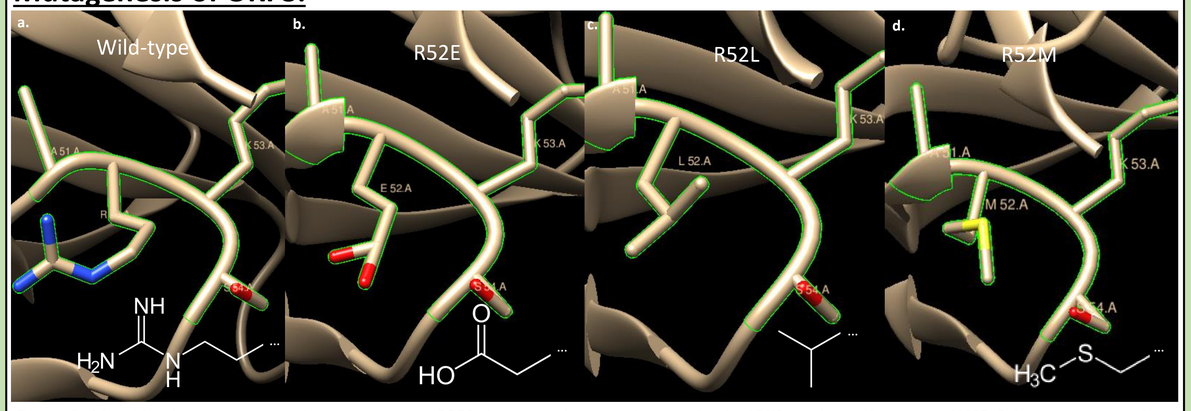

Figure 3. Magnified images of crystalline structures of ORF8 (in gold) with mutations of Arg in ARKS motif obtained from UCSF Chimera, and chemical structures of side chains of amino acid on position 52 (in white). The diagrams depict the most probable structure of the ARKS motif (highlighted in green) after mutagenesis to its respective amino acid, as well as relative change in binding pocket size. (indicated by percentage vacancy of the curve outside of green highlighted region). Mutants ORF8 R52E & R52L were further selected for *in vitro* novobiocin testing.

In vitro Novobiocin Testing:

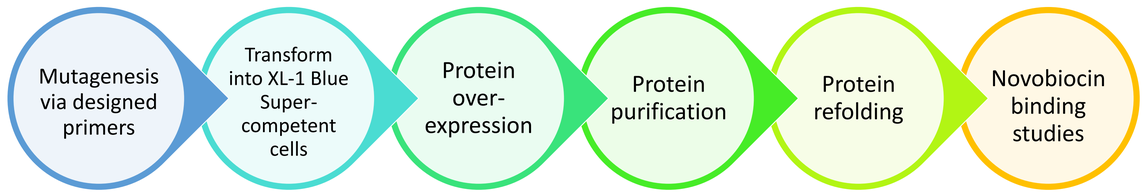

Figure 4. Experimental process of over-expression and purification of SARS-CoV-2 ORF8 (R52E and R52L mutations) for ligand binding studies.

Determination of Ligand Binding to ORF8:

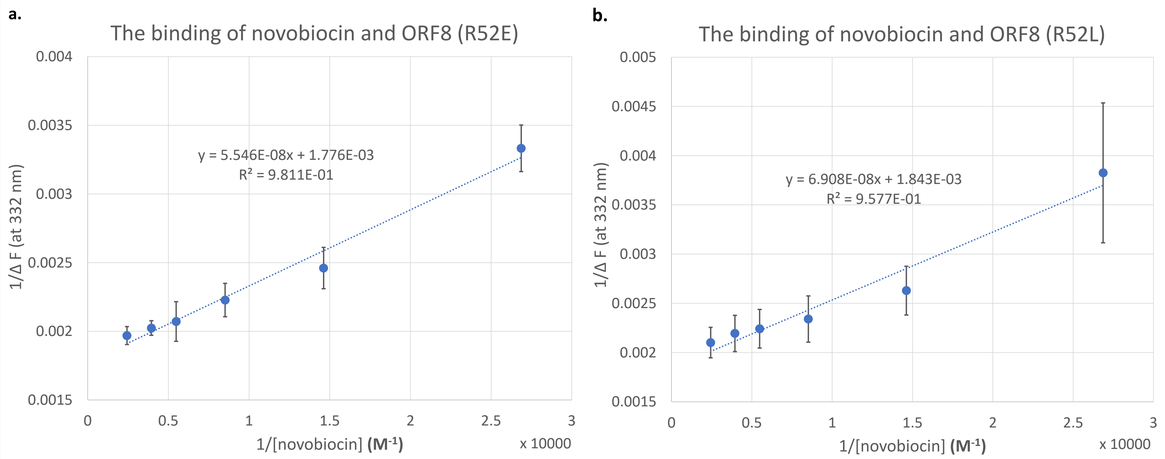

Figure 5. Fluorescence graphs of 1/Δ F (at 332 nm) versus the reciprocal of drug concentration describing the binding of novobiocin to mutant SARS-CoV-2 ORF8. K_d is determined via the Benesi-Hildebrand relationship using the equation $1/\Delta F = 1/(F\infty - Fo)*Kd*[L] + 1/ F\infty - Fo$, where the value ΔF is (F – *Fo*) and *Fo* is the fluorescence intensity in the absence of ligand, and the x-intercept of each graph is equal to $-1/K_d$. **Figure 5a** corresponds to novobiocin binding to R52E ORF8, while **Figure 5b** corresponds to novobiocin binding to R52L ORF8.

Previous Lab Work:

ORF8 Variant	R^2
WT	0.9992
K_d (μM)	**K_d Error (μM)**
54.5	3.14

Table 1. Calculated K_d of novobiocin ligand binding to SARS-CoV-2 ORF8. See **Figure 5**.

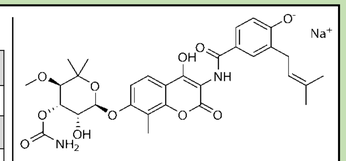

Figure 2. Structure of novobiocin. The ligand was computationally docked and verified to bind to SARS-CoV-2 ORF8 ARKS.

Results:

ORF8 Variant	K_d (μM)	K_d Error (μM)
WT	54.5	3.14
R52E	31.2	2.35
R52L	39.4	5.01

Table 2. Calculated K_d of novobiocin binding to SARS-CoV-2 ORF8 categorized by mutant variant. K_d values are calculated as described in **Figure 5**.

- K_d experienced decreases in overall values, indicating a slightly stronger binding interaction of novobiocin within the ORF8 binding pocket.
- However, accuracy of K_d decreased due to smaller R^2 values, showing a weaker regression relation to the Benesi-Hildebrand relationship compared to wild-type binding.

Conclusion:

- *In silico* analyses show that R52E, R52L and R52M mutants require less energy (FullFitness, ΔG) to dock novobiocin than wild-type ORF8.
- Arg has an overall slight negative impact on the binding of novobiocin at the ORF8 ARKS motif compared to mutants R52E and R52L. (evidenced by higher K_d, or weaker binding affinity)
- More mutagenesis data is needed to better understand how novobiocin binding at the ORF8 ARKS motif is affected compared to Arg.

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