Isolating Proteins that Neutralize the Adaptive Immune System in Bacteria
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Hypothesis
Using affinity chromatography, a purified Strep-tagged Csy complex bound to a Strep-Tactin column can be used as a “hook” to “fish out” novel, virally encoded anti-CRISPR proteins that bind the Csy complex.

Abstract
Bacteria have an adaptive immune system which evades invasive bacteriophage DNA by utilizing clustered regularly interspaced short palindromic repeats (CRISPR) separated by copies of small segments of viral DNA, which are transcribed and combine with proteins to form a surveillance complex (Figure 1). This ribonucleoprotein surveys the cell to identify viral DNA with which a base-pair interaction can be formed, subsequently degrading the viral genome. Bacteriophage have evolved a defense mechanism against the CRISPR-Cas immune system through proteins that bind to, and thereby inactivate the CRISPR-Cas system. Here we demonstrate that anti-CRISPR (ACR) proteins produced by bacteriophages isolated from environmental samples can be eluted from a Step-Tactin column in tandem with a Strep-tagged Csy complex due to binding of the ACR proteins to the complex. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the eluted protein fractions shows all four subunits of the Csy complex, along with a band of approximately 11kDa, consistent with ACR F1 protein.

Methods
Isolate Phages from Environmental Samples
Individual Phage Enrichment in PA01
Anti-CRISPR protein production in PA01
Affinity Column Chromatography with pre-bound Csy complex
SDS-PAGE

Phage isolates from plaque assays conducted with environmental samples from the Bozeman Water Treatment Plant were enriched overnight in P. aeruginosa strain PA14, then 5 samples were pooled and inoculated into P. aeruginosa strain PA01 for one hour to produce free anti-CRISPR proteins. Samples were chemically lysed and sonicated, then the lysate was applied to a Strep-Tactin column pre-bound with a Strep-tagged Csy complex, and eluted over eight fractions. SDS-PAGE was used to visualize the proteins.

Results
The binding affinity between ACR F1 and ACR F2 to the type I-F Csy surveillance complex in P. aeruginosa (Figure 2) was utilized in an attempt to isolate novel ACR proteins. Through binding a strep-tagged Csy complex to a Strep-Tactin Column, ACR proteins were eluted together with the Csy complex and were visualized using SDS-PAGE.

Figure 3: Coomassie stained 15% SDS polyacrylamide gel from positive control of experiment using phage DMS1m:35 which produces the ACR F1 protein with molecular weight of 11kDa. Lane 1 is BioRad Kaledioscope Ladder. Lane 2 and 3 are concentrated elution fractions 1-3; lane 4 is from the pellet post incubation in PA01, lane 5 is from the supernatant.

This data confirms the hypothesis that the technique described can be used to isolate novel ACR proteins.

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