Understanding differential ribosomal interactions of Shiga Toxins via analysis of surface charge differences

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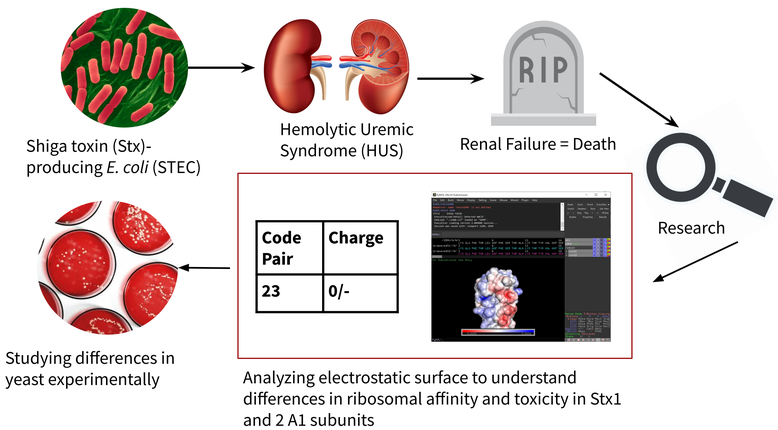
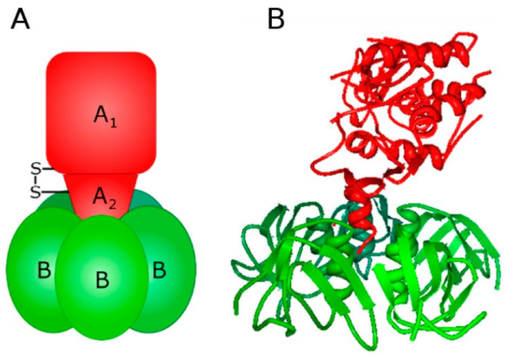
Graphical Abstract:



Introduction:

What are Shiga Toxins and why do we care?

- Shiga Toxin (Stx)-producing *Escherichia coli* produce virulence factors Stx1 and Stx 2, which are foodborne and waterborne pathogens that can cause hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS), the most common cause of acute renal failure in children worldwide[1]
- AB5 structure with A subunits (A1 and A2), and 5 B subunits as seen in image below[2]
- Stx2 is more toxic than Stx1[1]



Why study ribosomal interactions?

-The A1 subunit of Stx 1 and 2 bind to ribosomes to inhibit translation[3]

-Stx2A1 has higher ribosomal affinity compared to Stx1A1[3]

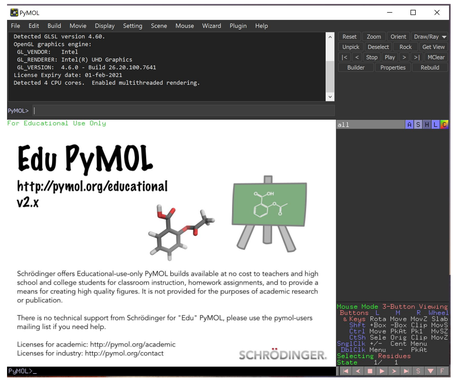
Why analyze surface charge differences?

- Interactions with the ribosome are via electrostatic interactions[4]
- Could explain the difference in levels of toxicity between Stx 1 and 2, and will give insight on how to design inhibitors

Aim: Identify surface charge differences in Stx1 and 2 A1 subunits through the use of the protein imaging software, Pymol by Schrodinger

Methodology:

- Fetch PDB code 1DM0 for Shiga toxin 1 (Stx1) onto Pymol as seen on the right
- Remove A2 subunit and B receptors, along with any extra water molecules
  - Coding sequence 1 – 251
- Create electrostatic surface
- Display coding sequence
- Analyze corresponding charge for each code
- Repeat all steps for Shiga toxin 2 (Stx2) with code 1R4P
  - Coding sequence 1 – 250
- Compare to find charge differences
  - Stx1A1 has an extra amino acid at 188
- Remove sites that were previously identified (Figure 1)[4]
- Confirm surface charge differences by comparing electrostatic surfaces



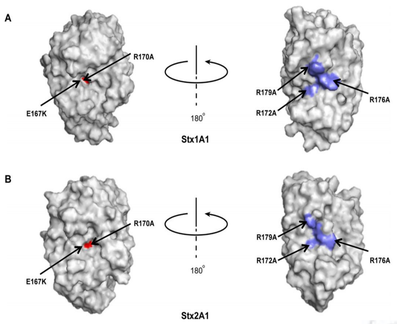


Figure 1. Crystallographic structures of Stx1A1 and Stx2A1 showing the active site and the distal face of the active site. The active site of Stx1A1 (A) and Stx2A1 (B) are labeled red, while rotation about the y axis by approximately 180° reveals the conserved arginines in blue.

Results:

Table 1. List of 18 identified surface charge differences between Stx1A1 and Stx2A1. Gray text corresponds to a neutral charge, red corresponds to negative, and blue corresponds to positive.

Amino Acid Code Pair (Stx1/Stx2)	Amino Acid Code Pair (Stx1/Stx2)
23 Ala/Glu	124 Ser/Glu
30 Thr/His	141 Asp/Ala
42 Asp/Asn	144 Ser/Glu
43 Ser/His	145 His/Phe
47 Asp/Gly	152 Gln/Arg
65 Asn/Asp	153 Ser/Asp
66 Asn/His	183 Asp/Ser
73 Arg/Gln	196/195 Glu/Gly
84 Arg/Thr	245/244 His/Gln

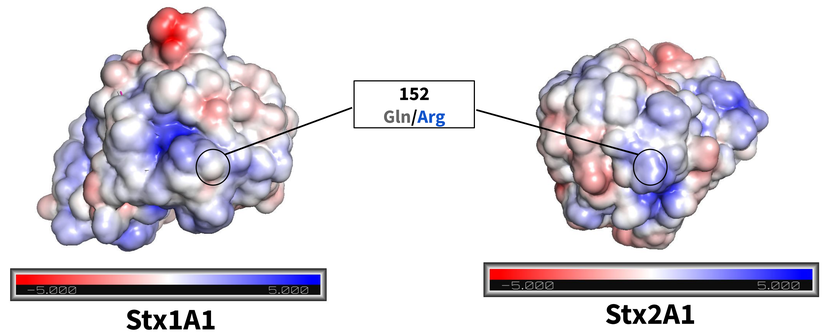


Figure 2. Generated electrostatic surface from Pymol software of Shiga Toxin 1 (Left) and Shiga Toxin 2 (Right) A1 subunits. The encircled areas illustrate the surface charge difference at the sequence code 145, where it is neutral in Stx1A1, and positive in Stx2A1.

Discussion:

Through the use of Pymol, I was able to identify 18 other pairs besides the ones that were found in previous studies (Table 1 and Figure 2). Looking at these residues computationally would provide us avenues to research experimentally to explain the difference in affinity of Stx2A1 for the ribosome compared to Stx1A1. This work lays the groundwork for truly analyzing these residues experimentally in the lab to verify their importance, such as studying these differences in yeast.

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References:

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