Investigating an uncharacterized protein (3UN6) in *Staphylococcus aureus NCTC 8325*

By: **Charles Wise** & Dr. Amanda Storm

Abstract

Pathogenic bacteria wreak havoc on life across the world and use virulent proteins which give them these destructive capabilities. Research groups investigate these proteins and try to understand how they operate. This research project investigated an uncharacterized protein in *Staphylococcus aureus NCTC 8325* (PDB: 3UN6). *Staphylococcus aureus* is known to cause staph infections and has the capability to become drug resistant. Researching how *Staphylococcus aureus* survives and infects lets researchers work towards building treatments that circumvent current drug resistance. To identify a possible protein function three assessments were implemented and required bioinformatic programs and databases (BLASTp, InterPro, UniProt, PredictProtein, etc). First, 3UN6's amino acid sequence was sent to programs similar to InterPro to compile possible domain, motif, and residue features. Second, 3UN6's sequence was aligned with similar predicted proteins to determine important features shared between them. Lastly, the tertiary structure of 3UN6 was used in programs such as ConSurf to look at structural features, and how similar 3UN6's structure is to other proteins to identify conserved function. We hypothesize that 3UN6 is the solute binding protein of an ATP-binding cassette (ABC) transporter, it attaches to the cell within the periplasmic space as a lipoprotein, and helps transport a trigonal planar molecule like nitrate, bicarbonate, and sulfonate into the cell. These molecules are important at many levels of survival within the cell. Nitrate for example is important for denitrification which can occur in *Staphylococcus aureus* when cellular respiration continues without oxygen and instead is replaced by nitrate as an electron acceptor. If an inhibitory target could be made for 3UN6 then *Staphylococcus aureus's* tolerance might decline which would make it difficult for it to survive inside a host organism. We are continuing our investigation and exploring new avenues to build upon our hypothesis, narrow down the transported molecule, and publish our findings.

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Investigating an Uncharacterized Protein (3UN6) in *Staphylococcus aureus* NCTC 8325

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ABSTRACT

Pathogenic bacteria wreak havoc on life across the world and use virulent proteins which give them these destructive capabilities. Research groups investigate these proteins and try to understand how they operate. This research project investigated an uncharacterized protein in *Staphylococcus aureus NCTC 8325* (PDB: 3UN6). *Staphylococcus aureus* is known to cause staph infections and has the capability to become drug resistant.[1] Researching how *Staphylococcus aureus* survives and infects lets researchers work towards building treatments that circumvent current drug resistance. To identify a possible protein function different assessments were implemented and required bioinformatic programs and databases (BLASTp, InterPro, UniProt, PredictProtein, etc.). We hypothesize that 3UN6 is the solute binding protein of an ATP-binding cassette (ABC) transporter, it attaches to the cell within the periplasmic space as a lipoprotein, and helps transport a trigonal planar molecule like nitrate, bicarbonate, and sulfonate into the cell with the use of a cation. These molecules are important at many levels of survival within the cell. Nitrate for example is important for denitrification which can occur in *Staphylococcus aureus* when cellular respiration continues without oxygen and instead is replaced by nitrate as an electron acceptor.[2] If an inhibitory target could be made for 3UN6 then *Staphylococcus aureus's* tolerance might decline which would make it difficult for it to survive inside a host organism. We are continuing our investigation and exploring new avenues to build upon our hypothesis, narrow down the transported molecule, and publish our findings.

BACKGROUND

This project is based on a larger project being conducted by the Center for Structural Genomics of Infectious Diseases (CSGID). CSGID works to identify peculiar proteins in infectious disease-causing agents, image their structure, then upload them to their database. Our project builds off their work and we chose to research a protein (3UN6) entry present in the bacteria *Staphylococcus aureus*. We chose 3UN6 because of the type of complications *S. aureus* is known to cause, and 3UN6 had preexisting annotations that are useful in building a stronger hypothesis.

METHODS

To identify a possible protein function three assessments were implemented which required online prediction programs (BLASTp, InterPro, UniProt, PredictProtein, etc.). First, 3UN6's amino acid sequence was sent to programs such as InterPro to compile possible domain, motif, and residue features (Figures 1,3). Second, 3UN6's sequence was aligned with similar predicted proteins to determine important features shared between them (Figure 4). Lastly, the tertiary structure of 3UN6 was used in programs such as ConSurf to look at structural features, and how similar 3UN6's structure is to other proteins to identify conserved function (Figure 5, 6, 7, 8).

RESULTS

1) For our first step we used 3UN6's sequence and ran it through different programs which predicted similar features. These analyses predicted 3UN6 attaching to the cell within the periplasmic space and being a unit of an ABC transporter F cluster, 1 subcluster.[3, 4, 5, 6] This class of ABC transporters have 4 proteins or units working together to transport molecules in and out of the cell.[6, 7] These units are ATP Hydrolyzing (ATPase), Transmembrane (TM) Transporter, and Substrate Binding Protein (SBP). 3UN6 is predicted to be the substrate binding protein that helps transport a trigonal planar molecule into the cell such as nitrate, bicarbonate, or sulfonate.

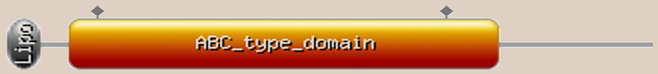

Figure 1: Predicted domain architecture and possible binding sites of 3UN6. "lipo" portion is indicating a lipidation site that lets 3UN6 attach to the cell within the periplasmic space. ABC_type_domain is the region that likely carries out the main function of the protein and the two diamonds represent the areas that potentially bind to a substrate.

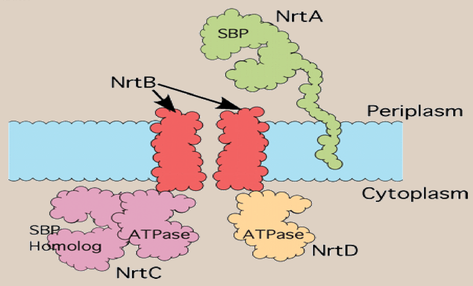

Figure 2: Depiction of the units of a nitrate ABC transporter. The NrtA unit is the SBP, the NrtB unit transports the nitrate through the membrane, and NrtC/ NrtD units hydrolyze ATP to facilitate the reaction.[7]

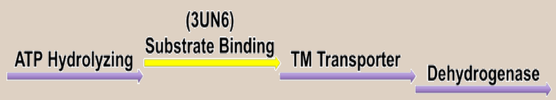

Figure 3: Predicted operon that 3UN6 is within. Operons are sections of genes within bacteria that are expressed at the same time. For this operon, 3UN6's gene neighbors are predicted to be units in an ABC transporter. This operon builds confidence in 3UN6 being a part of an ABC transporter. ATP Hydrolyzing (GI: 87201507), TM Transporter (GI: 87201509), Dehydrogenase (GI: 87201510).

2) The second step involved aligning multiple sequences (MSA) along with 3UN6 to identify functional similarities shared between SBP sequences. The SBP that are in the same predicated class of ABC transporter are the NrtA proteins which transport nitrate, and the CmpA proteins which transport bicarbonate. Certain specific positions seem to be conserved and are important for binding a substrate in these types of SBP. CmpA proteins use Ca^{2+} to bind bicarbonate while NrtA proteins do not use a cation to bind nitrate.[7, 8] 3UN6 is binding a $Zinc^{2+}$ but is still not overly similar to one or the other.

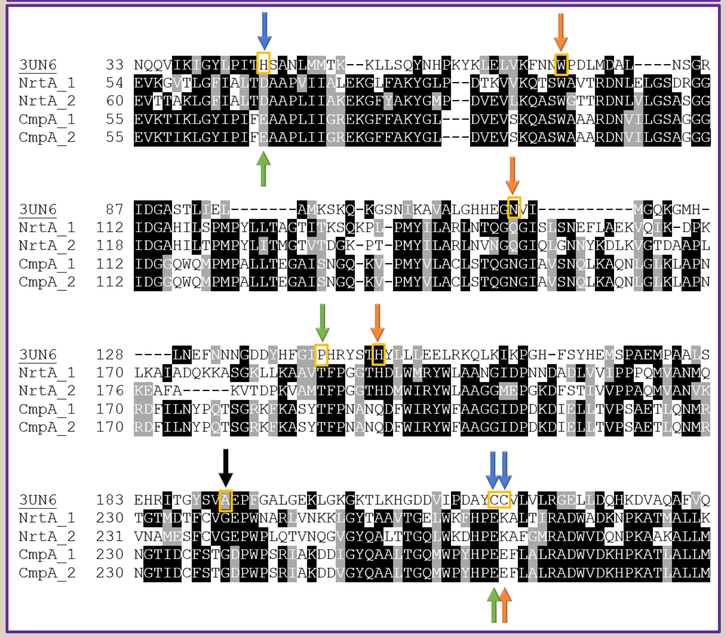

Figure 4: MSA of 3UN6 along with other annotated NrtA and CmpA SBP. Blue arrows point to the amino acid positions that are significant for binding zinc in 3UN6. Orange arrows point to significant binding amino acid positions shared by both NrtA and CmpA proteins. Green arrows point to significant binding amino acids in CmpA proteins. Black arrows point to significant binding amino acids in NrtA proteins. Yellow boxes represent highly conserved amino acids in 3UN6 based on ConSurf.[9] This figure shows how many significant binding amino acids in both NrtA and CmpA are shared in 3UN6. (3UN6 UniProt: Q2G1I5, NrtA_1 UniProt: P38043, NrtA_2 UniProt: P73452, CmpA_1 UniProt: P39660, CmpA_2 UniProt: Q5MZ56)

3) The third step was determining what information could be predicted using the structure of 3UN6. Structures that are often highly conserved usually convey a common broad function.

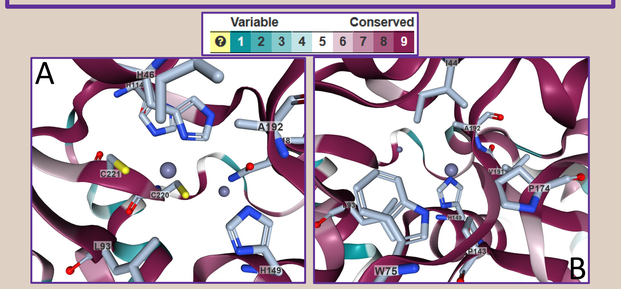

Figure 5AB: Tertiary structure of 3UN6's binding site with ConSurf's annotation of conservation along with a guide.[9] Higher conservation means it is crucial for function and less likely to change due to its importance. The highest conservation is annotated in the domain and possible binding site. Figure 5A shows the inner binding site while figure 5B shows the outer binding site.

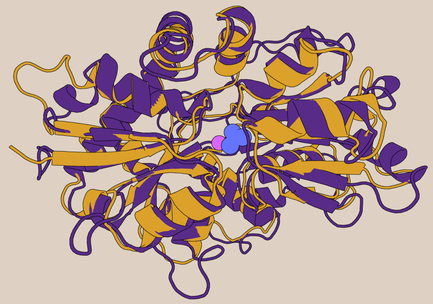

Figure 6: Tertiary superimposition of 3UN6 and a NrtA protein, gold and purple respectively. Both 3UN6's and NrtA's structure are similar with a calculated RMSD 5.071. This is showing how 3UN6 is likely a SBP along with being highly similar to an NrtA protein. Calculated in Chimera MatchMaker and visualized in 3D Protein Imager.[10, 11] (NrtA PDB: 2G29)

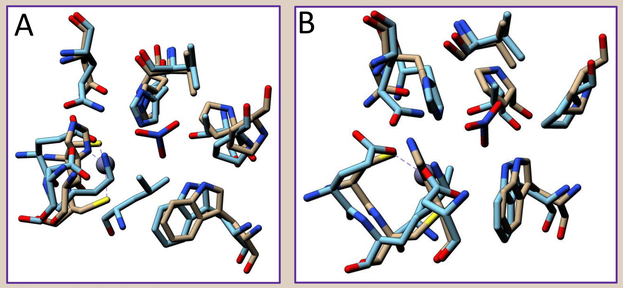

Figure 7AB: Binding site superimposition of 3UN6 and a NrtA protein, tan and blue respectively. **A** and **B** are different angles. Both binding sites are similar with many conserved amino acids present in both. This also shows how 3UN6 is binding zinc while NrtA is not using a cation to bind nitrate. Likely 3UN6 is using these similar or same amino acids seen in NrtA to bind a small trigonal planar molecule such as nitrate. Calculated and visualized in Chimera MatchMaker.[10] (NrtA PDB: 2G29)

A

Compound	Binding Affinity (kcal/mol)
Nitrate	-2.8
Bicarbonate ion	-2.6
Choline Ion	-2.6
Trimethyl Glycine	No Binding
Glutamic Acid	No Binding
Nitric Acid	No Binding
Phosphate	No Binding
Sulphate	No Binding
Glycerol	No Binding
Molybdate ion	Failed

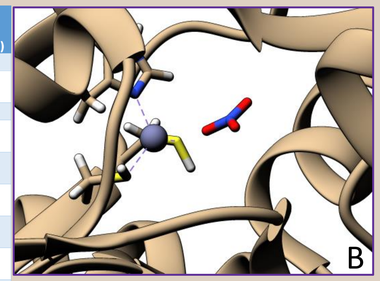

Figure 8AB: Figure 8A is a table of ligand scoring results from Chimera Autodock Vina.[10, 12] Figure 8B is showing 3UN6 with the nitrate prediction modeled in a similar fashion to what is seen in Figure 7AB. Even though the scores are not high the nitrate it is still being placed in a similar conformation as nitrate is in a NrtA protein as seen in figure 7AB.

CONCLUSION

Based on sequence analyses, alignments, and structural features we hypothesize 3UN6 to be a SBP of an ABC transporter that is helping transport nitrate or a similar trigonal planar molecule into the cell with the use of a cation. 3UN6 would be attaching to the cell within the periplasmic space where it would use a functional binding region to bind a trigonal planar molecule and align it into the TM transporter.

ABC transporters are commonly used to intake essential nutrients and expel toxins from the bacteria. Bacteria need to intake essential nutrients or precursor molecules from the environment to synthesize vitamins and other important molecules.[13]

A growing heath concern that is caused by antibiotic overuse is drug resistant *S. aureus* strains, the most renown is Methicillin-resistant *Staphylococcus aureus* (MRSA).[1] Finding a target molecule to inhibit this protein could potentially limit the survivability of the bacterium and help fight this drug resistant bacteria.

Moving forward, experimental analyses testing nitrate binding in 3UN6 would be required to build confidence in these results.

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Affiliate logos

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