



# Investigating an unknown protein (3UN6) in *Staphylococcus aureus*

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## Abstract

Pathogenic bacteria wreak havoc on life all over the world and use virulent proteins which give them these destructive capabilities. Research groups all over the world investigate these proteins to understand how they operate, and what mechanisms they are incorporated in. This research project investigated an unidentified protein in *Staphylococcus aureus* (PDB: 3UN6). This bacterium is known to cause Staph infections and has the capability to become drug resistant. We hypothesize that 3UN6 is the substrate binding unit of an ATP-binding cassette (ABC) transporter that attaches to the cell within the periplasmic space and is helping transport nitrate into the cell.

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# Investigating an unknown protein (3UN6) in *Staphylococcus aureus*



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## ABSTRACT

Pathogenic bacteria wreak havoc on life all over the world and use virulent proteins which give them these destructive capabilities. Research groups all over the world investigate these proteins to understand how they operate, and what mechanisms they are incorporated in. This research project investigated an unidentified protein in *Staphylococcus aureus* (PDB: 3UN6). This bacterium is known to cause Staph infections and has the capability to become drug resistant (Taylor and Unakal). We hypothesize that 3UN6 is the substrate binding unit of an ATP-binding cassette (ABC) transporter that attaches to the cell within the periplasmic space and is helping transport nitrate into the cell.

## BACKGROUND

This project is based on a larger project being conducted by the CSGID. CSGID works to identify proteins in bacteria, capture their 3D structure, then upload them to their database. Our project builds off their work and we chose to research a protein entry present in the bacteria *Staphylococcus aureus*. We chose 3UN6 because of the type of complications the bacteria is known to cause and 3UN6 had preexisting annotations that are useful in building a stronger prediction.

## METHODS

To identify a possible protein function 3 assessments were implemented which requiring online prediction programs (BLASTp, InterPro, UniProt, PredictProtein, etc).

### 1) Sequence Analysis

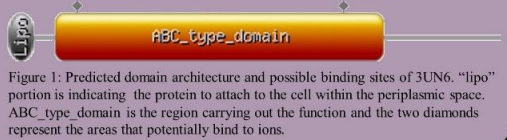


Figure 1: Predicted domain architecture and possible binding sites of 3UN6. "lipo" portion is indicating the protein to attach to the cell within the periplasmic space. ABC\_type\_domain is the region carrying out the function and the two diamonds represent the areas that potentially bind to ions.

### 2) Sequence Alignments

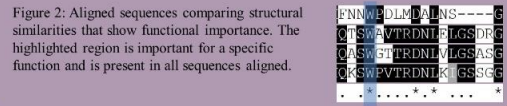


Figure 2: Aligned sequences comparing structural similarities that show functional importance. The highlighted region is important for a specific function and is present in all sequences aligned.

### 3) Structural Features

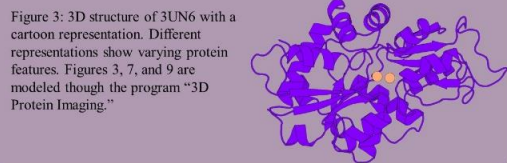


Figure 3: 3D structure of 3UN6 with a cartoon representation. Different representations show varying protein features. Figures 3, 7, and 9 are modeled through the program "3D Protein Imaging."

## RESULTS

1) For our first step, we got consensus findings for protein localization and functional regions. These analyses predicted the protein attaching to the cell within the periplasmic space and being a unit of an ABC transporter. ABC transporters have 3 proteins/ units working together to transport molecules within and out of the cell. These units are ATP Hydrolyzing (ATPase), Transmembrane (TM) Transporter, and Substrate Binding Protein (SBP). 3UN6 is predicted to be the substrate binding protein in an ABC transporter that transports nitrate into the cell.

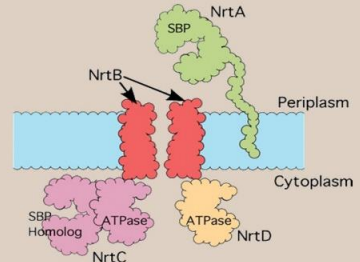


Figure 4: Depiction of the three units of a nitrate ABC transporter. The NrtA unit is the SBP, the NrtB unit transports the nitrate through the membrane, and NrtC/ NrtD units hydrolyze ATP to facilitate the reaction. (Koropatkin et al.)



Figure 5: Predicted operon that 3UN6 is within. Operons are sections of genes within bacteria that are created into proteins at the same time. For this operon, 3UN6's gene neighbors both 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 sequences.

| Sequence  | 33        | 54        | 60        | 57        | 61        | 96     | 111   | 117   | 115   | 121   | 129       | 171     | 176    | 169     | 181       | 184     | 231      | 232      | 229      | 241      |
|-----------|-----------|-----------|-----------|-----------|-----------|--------|-------|-------|-------|-------|-----------|---------|--------|---------|-----------|---------|----------|----------|----------|----------|
| 3UN6      | -NQQVIR   | EVKGVH    | EVKAKLGE  | VEIKAKLGE | VEIKAKLGE | RFDSDH | FDSDH | FDSDH | FDSDH | FDSDH | NE--FNNDG | KALDQNR | KEAFAR | KALDQNR | NE--FNNDG | HRFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH |
| NrtA_3    | EVKGVH    | EVKGVH    | EVKGVH    | EVKGVH    | EVKGVH    | FDSDH  | FDSDH | FDSDH | FDSDH | FDSDH | FNNDG     | KALDQNR | KEAFAR | KALDQNR | FNNDG     | HRFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH |
| NrtA_1    | EVKAKLGE  | EVKAKLGE  | EVKAKLGE  | EVKAKLGE  | EVKAKLGE  | FDSDH  | FDSDH | FDSDH | FDSDH | FDSDH | FNNDG     | KALDQNR | KEAFAR | KALDQNR | FNNDG     | HRFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH |
| NrtA_2    | VEIKAKLGE | VEIKAKLGE | VEIKAKLGE | VEIKAKLGE | VEIKAKLGE | FDSDH  | FDSDH | FDSDH | FDSDH | FDSDH | FNNDG     | KALDQNR | KEAFAR | KALDQNR | FNNDG     | HRFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH | GVNFDSDH |
| consensus | 61        |           |           |           |           |        |       |       |       |       |           |         |        |         |           |         |          |          |          |          |

Figure 6: MSA of functional region in 3UN6 along with other annotated NrtA proteins. The highlighted regions are what publications (Maeda and Omata) have identified to be important binding regions for NrtA proteins. Only 2 regions in 3UN6 are not the same with the other 3 being similar or the same. These NrtA proteins are in cyanobacteria which is evolutionarily distinct from *S. aureus*, so the conservation of these regions are extremely significant for function. NrtA\_1 (UniProtKB: P73452), NrtA\_2 (UniProtKB: Q44292), NrtA\_3 (UniProtKB: P38043)

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.

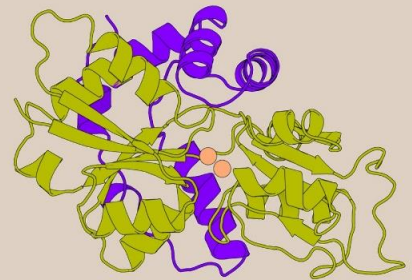


Figure 7: 3D structure of 3UN6 with the functional region highlighted in gold. The two spheres represent bound zinc ions and could indicate the binding site. This shows that most of the protein is being taken up by the functional region and is also the region that potentially binds ions.

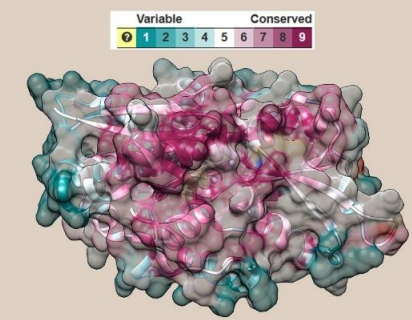


Figure 8: 3D structure of the protein with ConSurf annotation of conserved regions along with a guide. Higher conservation means it is crucial for function and less likely to change due to its importance. The highest conservation is predicted in the functional region/ domain.

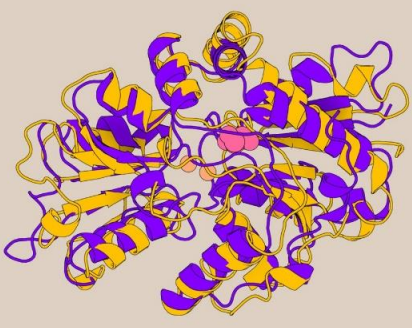


Figure 9: 3D structure of 3UN6 in purple with a superimposition structure of a SsuA protein from *E. coli* (PDB: 2X26) in yellow. SsuA proteins are annotated to be similar in broad function to NrtA proteins since it is also a SBP for an ABC transporter. A msd of 3.3 for this superimposition indicates a strong structural similarity between 3UN6 and a known SBP that belongs to an ABC transporter.

## CONCLUSION

Based on sequence analyses, alignments, and structural features we hypothesize 3UN6 to be a SBP of an ABC transporter. 3UN6 attaches to the cell within the periplasmic space and has a nitrate binding region present within the functional region of the protein. 3UN6 would be helping in transporting nitrate into the cell.

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.

A growing health concern that is caused by antibiotic overuse is drug resistant *S. aureus* known as MRSA. 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

