Exoenzyme U and Ubiquitin:
A Fatal Attraction

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Abstract
Exoenzyme U (ExoU) is a destructive "effector" encoded by the pathogen *Pseudomonas aeruginosa*, an omnipresent bacterial pathogen found in soil and water. ExoU is a potent phospholipase, a catalytic enzyme that targets membrane phospholipids. The enzyme is activated inside mammalian cells by ubiquitin. ExoU is inactive in *P. aeruginosa* as prokaryotic cells do not have ubiquitin, a regulatory protein used in post-translational modifications vital for processes in mammalian cells. During the initial stages of infection with *P. aeriginosa,* Exo U expression is postulated to destroy immune cells, which allows the bacterium to replicate *in vivo* without being attacked by the immune system. Understanding the mechanism of activation inside mammalian cells may lead to the development of inhibitors of ExoU's toxic activity. Research has highlighted the C-terminal four-helix bundle of ExoU, principally located between residues 587 and 687, because it is a probable binding site between ExoU and ubiquitin. It is also known that Tyr-619 and Arg-661 (located near the surface exposed tip of the bundle) play a role in the binding and activation of ExoU. Arginine 661 may also be important for membrane binding. ExoU is problematic in people who use artificial breathing machines and have weak immune systems. The Saint Joan Antida SMART (Students Modeling a Research Topic) Team modeled the ubiquitin binding domain of ExoU using 3D printing technology.

I. ExoU – a Cytotoxin from *Pseudomonas aeruginosa*

- *P. aeruginosa* is a soil organism.
- *P. aeruginosa* targets people with compromised immune systems.
- ExoU injection into target cells by the bacteria causes membrane destruction resulting in cell death.
- The bacterium is associated with patients who have:
  - -Cystic fibrosis
  - -Damaged tissue
  - -To be artificially ventilated

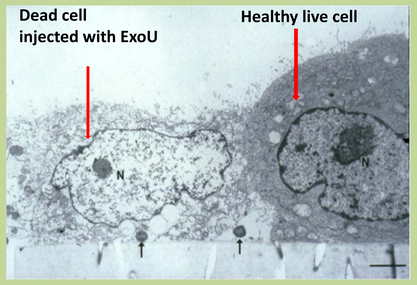

Figure 1: Comparison of Healthy and ExoU Injected Cells

II: ExoU and Ubiquitin Interactions

- Ubiquitin is a protein that is used in post-translational modification, which is vital for many cellular processes.
- ExoU is the first enzyme that requires an interaction with ubiquitin for enzymatic activation.
- ExoU activation is specific to eukaryotic target cells because bacteria do not possess the activator ubiquitin.

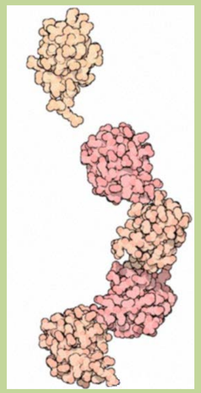
Figure 2: Ubiquitous Ubiquitin (Ubiquitin is found in all Eukaryotic Cells)

IV: Phospholipase Activity Makes ExoU a Toxin

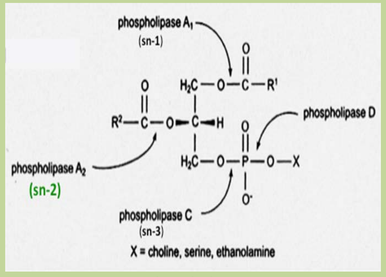

Figure 3: ExoU is a phospholipase

Properties of ExoU:
- Cleaves at sn-2 position – A_2 specificity
- Requires a eukaryotic activator, ubiquitin
- Held in the inactive state in prokaryotic cells

V: DEER Data

- DEER is an acronym that signifies double electron electron resonance.
- There are two pairs of labeled sites (see diagram B).
- The distance between these sites was measured by DEER (see diagram C).
- Ubiquitin is not the factor that changes ExoU conformation between these two particular pairs of spin labeled sites (Figure 3C).
- Membrane substrates (liposomes) appear to drastically separate the labeled sites.
- Ubiquitin may play are role in stabilizing this new, open conformation.

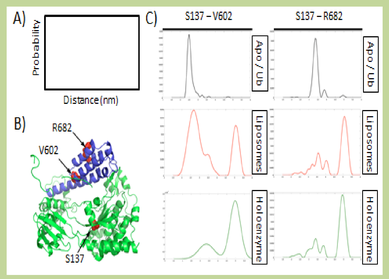

Figure 4: Labeled sites from DEER measurements

<u>Apo/Ub (ExoU only or ExoU + ubiquitin)</u>
- There is only a single peak, which indicates that there was no significant changes between the labeled sites.

<u>Liposomes (ExoU + liposomes)</u>
- There are two large peaks, which indicates that there are now at least two populations of molecules, each having its own distance distribution.

<u>Holoenzyme (ExoU + ubiquitin + liposomes)</u>
- There is a single predominant peak at the longer distance measurement.

V: Dynamic Model of ExoU

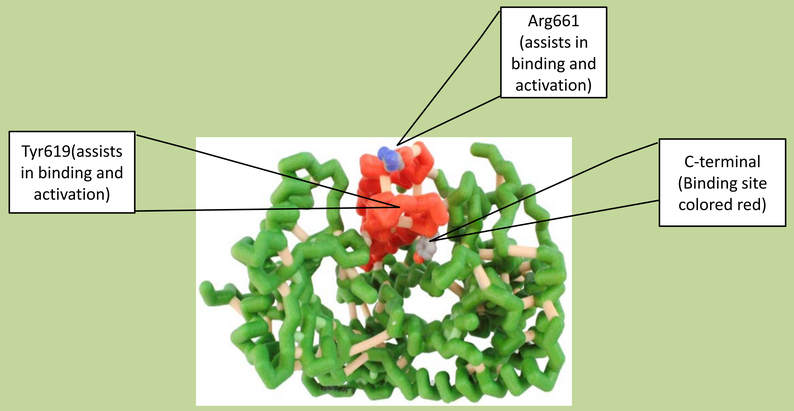

Figure 5: ExoU model based on pdb file 4akx

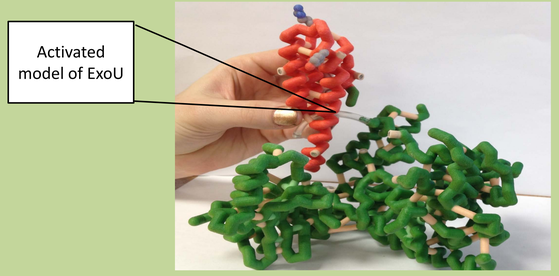

Figure 6: Activated ExoU model based on pdb file 4akx

VI: Conclusions

- ExoU changes conformation when liposome substrates or liposomes and ubiquitin are added.
  - •Ubiquitin alone does not significantly affect the structure of the C-terminal bundle in relation to the rest of the molecule.
- The presence of both liposomes causes ExoU to open up more compared to the apoenzyme state.
- The C-terminus of ExoU is important because it both with membrane substrates and the ubiquitin cofactor.
- It is likely that ubiquitin binding stabilizes the activate conformation.

References
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2. D.M. Anderson, K.M. Schmalzer, H. Sato, M. Casey, S.S. Terhune, A.L. Haas, J.B. Feix, D.W. Frank (2011). Ubiquitin and ubiquitin- modified proteins activate the *Pseudomonas aeruginosa* T3SS cytotoxin, ExoU. *Molecular Microbiology* 1454:1467.
2. Anderson, D. (2014). unpublished data.

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