

Guide to study the Anthrax toxin paper

**1) Sequence variations in PA homologues suggest a functional interaction.**

Figures 1a, 1b, 1c.

- What is the %identity of the aligned sequences?
- Locate the loops in the pre-pore complex.

**2) Functional complementation of mutations at position 397 and 426**

Figures 1d and 1e

- How many nucleotides need to be mutated to change a:  
K for Q  
D for Q  
P for L

- What is a compensatory mutation?
- Define correlated mutation.

**3) K397 Interacts with D426 in a neighboring subunit**

Figure 2 Table 1

- Define  $EC_{50}$
- Discuss how they grouped the mutants based on the four phenotypes (Supporting text)

Figure 3

- Show in an heptamer diagram how the monomers interact via K/D (How do they arrange in the closed circle?)
- Discuss possibility of heterogeneous mixtures of monomers during the assays.
- How is it possible that by mixing two inactive monomers they obtained activity?

**4) The 397-426 interaction positions F427 within the pore**

Figure 4

- Describe the MTS-ET experiments.
- How does the charge on the protein complex (positive or negative) may affects the reaction of MTS (charged positively)? How does the rate of MTS-ET-mediated conductance block relate to the accessibility or the reactivity of the reagent?

Describe the potential salt bridges and hydrogen bonds formed between subunits.

How electrostatic interactions affect translocation of LF? Fig 5.