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Potential regulation of deadly water-borne *Shigella* bacteria pathogenesis through the *Shigella* infection protein Spa47

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I. Introduction

- *Shigella* (Figure 1) is a gram-negative, bacterial pathogen typically found in contaminated water sources.
- Each year, *Shigella* is responsible for over 90 million infections and 100,000 deaths stemming from symptoms of severe dysentery, fever, nausea and vomiting.
- A needle-like apparatus (Figure 2) found on the surface of *Shigella* allows the bacterium to infect host cells.
- Each needle-apparatus has an associated ATPase, a protein that can hydrolyze ATP into ADP and P_i.
- The *Shigella* needle-apparatus ATPase Spa47 (Figure 2, circled red) is predicted to provide the energy for infection.
- Spa47 has been shown to be essential for infection – without Spa47, no infection will occur.
- We were able to recombinantly express, purify, and characterize Spa47 for the first time.

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II. Methods

- *Shigella* protein Spa47 was recombinantly expressed in *E. coli* expression strains with a chitin binding domain tag.
- Spa47 was purified based on protein affinity and size.
- Activity of Spa47 was measured using a radioactive ATP hydrolysis assay protocol to determine the rate of ATP hydrolysis.
- Several mutations of Spa47 were made at key amino acid residues and ATP hydrolysis activity is measured.

III. Results

- Spa47 purifies are two separate species, a monomer and a trimer (three associated Spa47 proteins) species.
- The ATP hydrolysis activity of the Spa47 trimer species is 10-fold higher than the Spa47 monomeric species (Figure 3).
- There is no ATP hydrolysis activity in Spa47 with a single amino acid mutation at the predicted active site of the protein.
- Activity of Spa47 appears to be oligomer dependent.

Figure 1 – A depiction of *Shigella*, a deadly water-borne pathogen



<http://www.cdc.gov/shigella/>

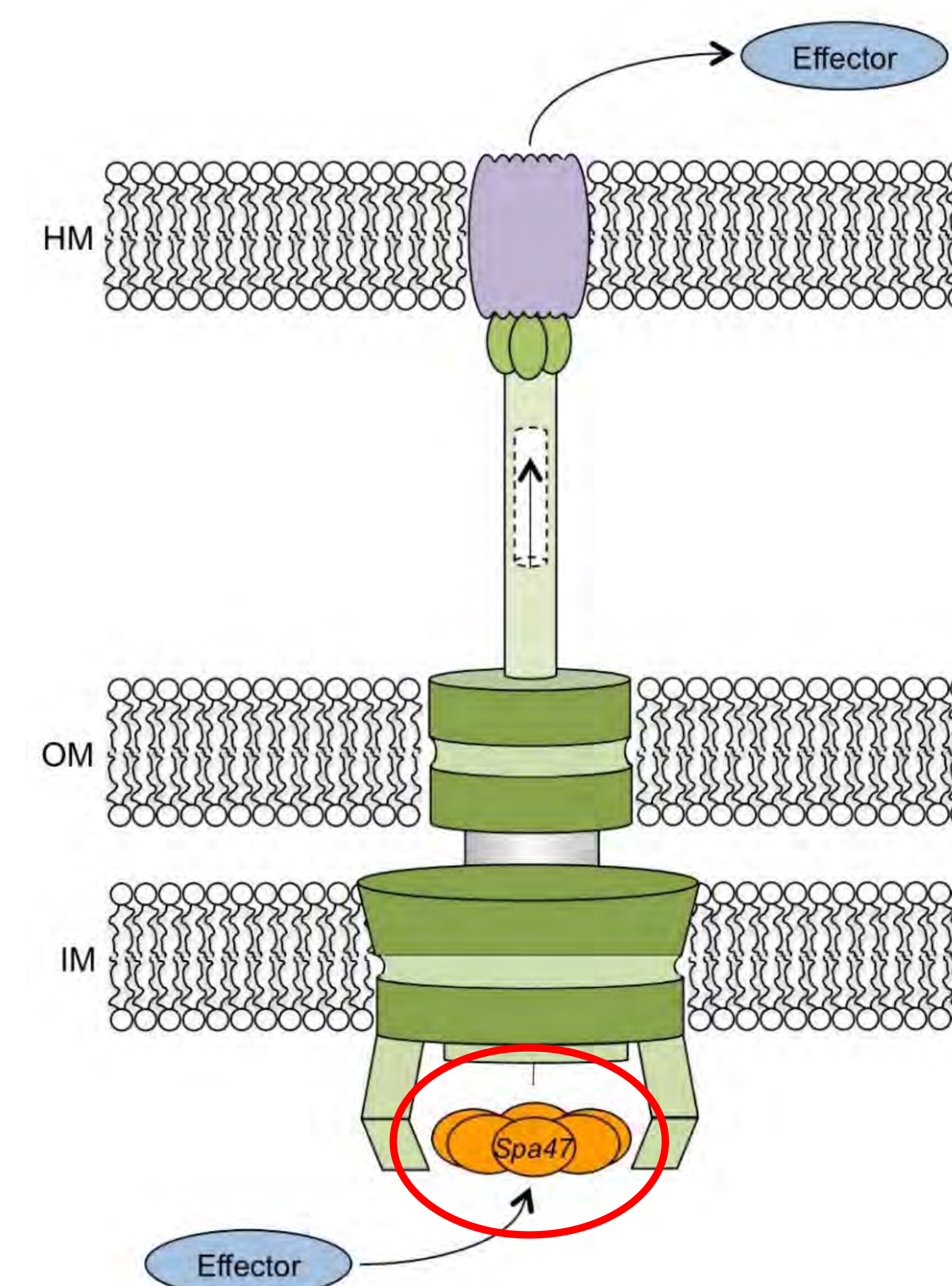
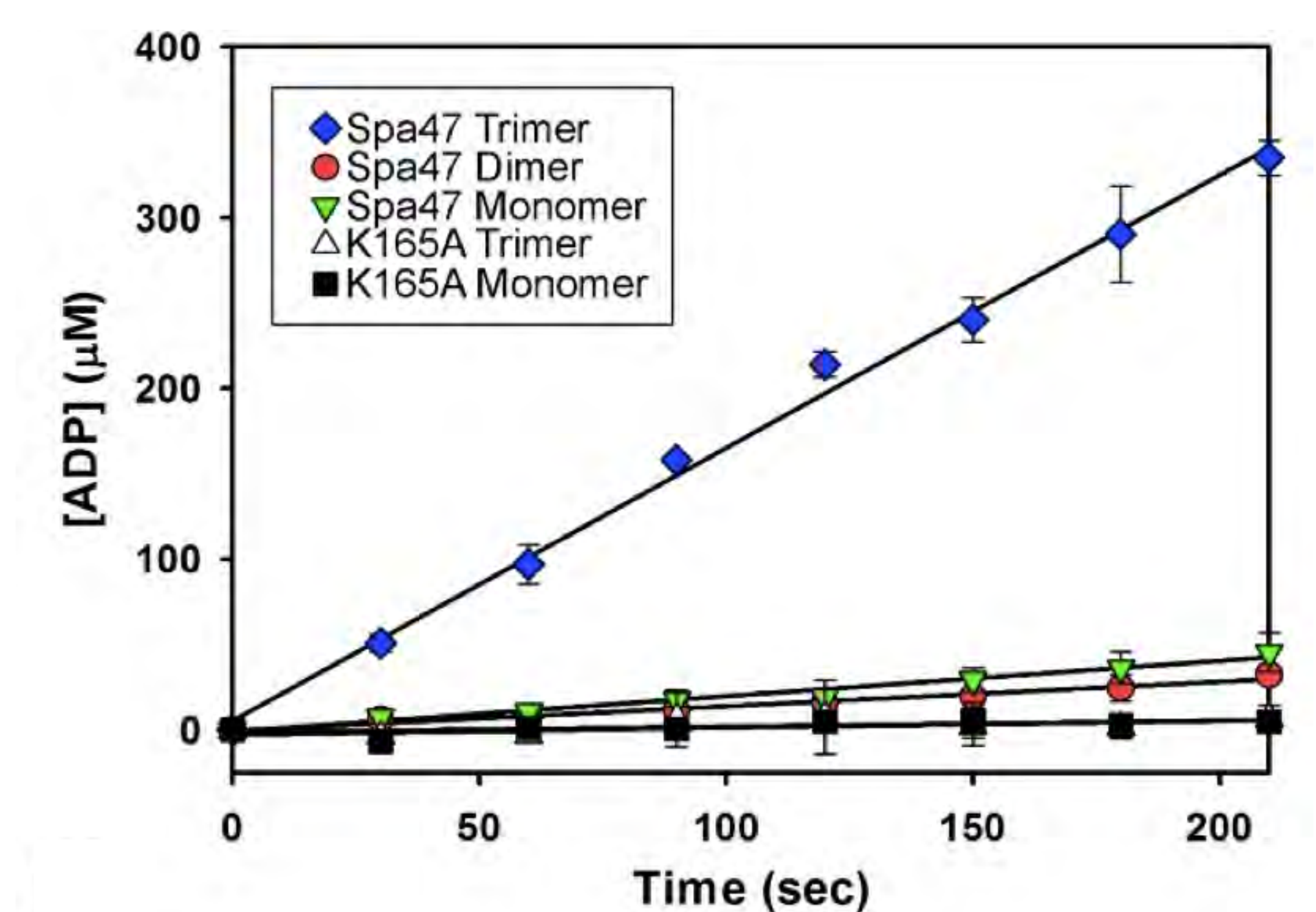


Figure 2 – A cartoon rendition of the *Shigella* needle-like infection system

Figure 3 – The Spa47 oligomer species is 10-fold more active than monomeric or mutant Spa47



IV. Conclusions

- Spa47 has been successfully purified using recombinant protein expression.
- Single point mutations at key residues in Spa47 eliminate ATP hydrolysis activity.
- The trimeric Spa47 oligomer species was greater than 10 times that of the monomer, suggesting oligomerization may play a role in infection regulation.

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