**Protein-Protein Interactions Involved in the Assembly of Bacterial Nanoinjectors Defined by EPR Spectroscopy**

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**Introduction**

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| fig1-for-report.jpg  **Fig.2** Models of **(A)** ‘open’ and **(B)** ‘closed’ conformations of doubly spin-labeled LcrG. EPR data **(C)** suggest a population of LcrG in ‘closed’ conformation. |

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| fig1-for-report-2.jpg  **Fig.1** LcrG is expected to bind to LcrV in a closed conformation (image from ref. 1). |

The goal of this project is to use EPR to determine the protein-protein interactions of two proteins from Yersinia pestis: LcrV and LcrG. These proteins are essential in the pathogenesis of *Yersinia pestis*, the causative agent of bubonic plague. Contrary to what was expected for LcrG – that it forms a coiled coil, our NMR results indicated that LcrG lacks a tertiary structure and consists only of secondary alpha helical structures [1]. However, the current hypothesis in the literature is that LcrG forms a coiled coil upon binding to LcrV (**Fig.1**) [2]. Our NMR analysis could not identify if the two helices are in close contact with each other [1], hence, we are using EPR.

**Experimental**

Site-directed cysteine mutants of LcrG recombinant proteins were expressed and purified following published methods [1]. MTSL spin labels were attached to the LcrG proteins following published protocols [3]. EPR experiments have been carried out at the NHMFL using a Bruker E680 spectrometer and the HiPER spectrometer. In 2016, we extended our studies from the *Yersinia* LcrG to the *Pseudomonas* PcrG protein. In 2017, we extended our EPR studies in identifying how LcrG interacts with LcrV.

**Results and Discussion**

Our preliminary EPR data of two spin labeled sites at C34 and D65C suggest that LcrG samples a 'closed' conformation (**Fig.2**). The EPR results show that spin labels at C34 and D65C are in close proximity to each other (**Fig.2**). In 2017, we were able to use EPR to determine how LcrG interacts with LcrV. Our preliminary EPR results suggest a model where LcrG adopts an 'open' conformation – where the two helices are not in close proximity with each other – when bound to LcrV (**Fig.2**).

**Conclusions**

The EPR model of LcrG-LcrV interaction will upend the current hypothesis in the literature (**Fig.1**) that assumes a 'closed' conformation for LcrG when bound to LcrV.

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**References**

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