

Abstract No. Mari514

## **MAD Data Collection on a Terbium Derivative of InIB, a Surface-attached Protein of the Bacterial Pathogen *Listeria Monocytogenes***

M. Marino and P. Ghosh (University of California, San Diego)

Beamline(s): X25

**Introduction:** InIB is a 67 kD surface-attached protein of the bacterial pathogen *Listeria monocytogenes* that promotes bacterial invasion of diverse mammalian cell types. The protein triggers an intracellular signaling cascade involving activation of phosphoinositide 3-kinase. The structure of the leucine-rich repeat domain of InIB demonstrated the presence of two unsuspected calcium binding sites (Marino et al., 1999). Based on the presence of these sites, we designed a multiwavelength anomalous dispersion (MAD) experiment utilizing terbium, a lanthanide that has been shown to substitute for calcium, to determine the X-ray crystal structure of intact InIB.

**Methods and Materials:** Crystals of intact InIB were derivatized with  $TbCl_3$ , soaked in an erythritol-containing cryobuffer, and flash-cooled at  $\sim 100$  K. Several MAD data sets utilizing three wavelengths (peak, inflection, and remote) corresponding to the terbium  $L_{III}$  edge were collected at X25A. Dataset collection times were optimized to minimize radiation damage to crystals while still getting high resolution data. Higher quality single wavelength datasets were also collected at 1.1 Å wavelength.

**Results:** Analysis of the MAD experiment using SOLVE indicated that both anomalous and dispersive difference signals yielded useful phasing information. MAD phases were calculated with Sharp and the previously solved structure of the LRR domain of InIB was placed into the resulting map. Phases from this partial solution were used to calculate anomalous and dispersive maps that yielded additional terbium sites and an improved heavy atom model. Phasing information extends to  $\sim 3.0$  Å, and solvent flattening (77 percent solvent) significantly improves these phases. Resulting electron density maps have been used in conjunction with others from previous MAD experiments at NSLS and other facilities in order to build a partial model for InIB. The model is currently 80% complete.

Single wavelength data collected at X25 extended to 2.7 Å and will be used in model refinement. This represents a significant improvement over the  $\sim 3.2$  Å data collected at the home source.

**Acknowledgments:** We thank Michael Becker for advice and help during data collection.

**References:** M. Marino, L. Braun, P. Cossart, P. Ghosh "Structure of the InIB leucine-rich repeats, a domain that triggers host cell invasion by the bacterial pathogen *L. monocytogenes*," Molecular Cell 4, 1063-1072, 1999.