ESRF	**Experiment title:** **The Colicin E9 Receptor-Binding Domain**	**Experiment number**: LS-1607
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Shifts: 3	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

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Report:

Background.

Colicins are protein antibiotics produced by strains of *E. coli* and closely related bacteria. They are classified into groups corresponding to the cell surface receptor to which they bind in *E.coli* cells; for example the E colicins bind to the BtuB receptor, which is an essential component of the high affinity vitamin B_{12} transport system. Killing of *E.coli* cells by E colicins requires three stages, receptor-binding, translocation and cytotoxicity. Commensurate with this E colicin proteins have three functional domains, each of which is implicated in one of the stages. The central (R) domain is responsible for receptor-binding activity, whilst the N-terminal (T) domain mediates translocation, the process by which the C-terminal cytotoxic domain is transported from the receptor to the site of its cytotoxicity. We have recently solved the crystal structure of the DNase domain of colicin E9 with immunity protein bound (1). This represents the first structure of a member of the HNH family of homing endonucleases that are found in both procaryotes and eucaryotes. We now

wish to determine the crystal structure of the BtuB receptor-binding domain of the colicin E9 by Se-MAD at BM14 of the ESRF.

Results.

We have overexpressed in *E.coli* a fragment corresponding to the minimal BtuB receptor-binding domain of the DNase colicin E9. This 6xHis-tagged protein has been crystallized and the wild type crystals shown previously to diffract to around 2.8Å resolution. The space group is I23 and cell parameters a = b = c = 233.8Å with an estimated 12-16 molecules (monomer molecular weight is 9.5 kDa) in the asymmetric unit. We subsequently prepared a selenomethionyl derivative of the protein which crystallized under similar conditions to the wild-type protein. There are 4 non-N-terminal methionine residues in the R-domain sequence. An X-ray fluorescence scan was taken around the selenium K-edge using a single crystal of the R-domain. Calculated f' (inflexion point) and f" (peak) values were -11.9 and 8.5 electrons, respectively. A 3-wavelength MAD experiment was then performed using the same crystal and data collected and processed to 3.2Å resolution. The program SOLVE was then used to locate selenium sites. This process is not complete but the results so far are as follows for a 28-site model calculated using all data to 3.5Å resolution.

	Peak-remote	**Inflexion point-remote**	**Peak-remote**
Dispersive differences	4.8%	5.9%	2.7%
	Remote	**Peak**	**Inflexion Point**
Anomalous differences	5.9%	10.1%	4.9%

The current mean FOM from SOLVE is 0.83 and a map calculated from this data shows clear solvent boundaries. Map improvement by non-crystallographic symmetry averaging and solvent flattening is in progress.

(1) ”Structural and mechanistic basis of immunity towards endonuclease colicins” Kleanthous .C., Kühlmann,U.C., James,R. , Moore,G.R. and **Hemmings,A.M.** (1999) *Nature Str.Biol.*, 6, 243-252.