Microcrystal electron diffraction (micro-ED) is a cryo-TEM technique that is used to determine the atomic structure of proteins, peptides, and small molecules. Micro-ED data is obtained by continuously tilting the crystal in the cryo-TEM while recording diffraction information. This method has been successfully used for the structure determination of proteins such as proteinase K, lysozyme, adenosine A2A receptor (G-protein coupled receptor) and peptides such as an Alzheimer associated amyloid-β (20-34), SVQIVY (tau protein fragment) and others.

Growing diffraction quality crystals of proteins and peptides can be challenging. The more commonly used method of X-ray diffraction requires crystals that are at least 1 µm in each direction. Crystals smaller than that could not be utilized until the advent of micro-ED. In this technique the ideal thickness of the crystal perpendicular to the incident beam should be in the nanometer range.

We have chosen to determine the structure of gramicidin D, a peptide antibiotic produced non-ribosomally by Bacillus brevis. It acts, in part, by creating pores in membranes, rendering them incapable of supporting life-sustaining transmembranal gradients. Gramicidin is a highly apolar pentadecapeptide consisting of alternating D- and L-amino acids. Naturally occurring gramicidin is a mixture of isoforms: gA (80%), gB (6%), and gC (14%). The amino acid sequence of gA is:


In gB and gC, Trp at position 11 is replaced by L-Phe and L-Tyr, respectively. The ion conducting form of gD is generally considered to be a dimer. Gramicidin exists in two major conformations; a head-to-head, single stranded helical dimer and a left- or right-handed intertwined, parallel or antiparallel, double stranded double helix.

The peptide was dissolved in a mixture of ethanol / PEG 4000 and crystallized in batch-mode at 4 °C. Small plate-like crystals formed. The crystals in the presence of ethanol / PEG 4000 mixture were transferred onto quantifoil R 2/2 grids and access solvent was removed by vacuum suction. The grids were flash frozen in liquid nitrogen and transferred into the Glacios-TEM equipped with a Ceta-D camera. Datasets from several crystals were successfully collected. In this contribution we will discuss the data processing in XDS and our attempts to solve and refine the structure. Diffraction datasets were also collected from manufacturer-prepared powder.

The authors acknowledge funding from the Foundation For A Better World.