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# Crystal Structure Determination of Gramicidin by Microcrystal Electron Diffraction

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Microcrystal electron diffraction (micro-ED) is a cryo-TEM technique that can be used to determine the atomic structure of molecules. Micro-ED data is obtained by continuously tilting a small crystal in the cryo-TEM while recording diffraction information. This technique has been successfully used for the structure determination of proteins, peptides, metal organic frameworks, and small molecules [1-3]. X-ray diffraction is the most commonly used method for crystal structure determination but requires crystals that are at least 1 µm in each direction. Growing diffraction quality crystals of macromolecular structures such as proteins and peptides can be challenging. In contrast, micro-ED can be carried out on crystals in the nanometer size range.

In this contribution, we report the results of our study of the crystal structure of gramicidin D (gD), 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. gD itself is a highly apolar pentadecapeptide consisting of alternating D- and L-amino acids that forms dimers. It naturally occurs as a mixture of isoforms: gA (80%), gB (6%), and gC (14%). The amino acid sequence of gA is [4]:

Formyl–NH–L–Val–Gly–L–Ala–D–Leu–L–Ala–D–Val–L–Val–D–Val–L–Trp–D–Leu–L–Trp11–D–Leu–L–Trp–D–Leu–L–Trp–CO–NH–CH2–CH2–OH.

The sample for microED analysis was prepared by placing a glow-discharged quantifoil R2/2 TEM grid into manufacturer provided gramicidin powder. Excess powder was removed by shaking the grid and the TEM grid was flash frozen in liquid nitrogen. Diffraction data were collected using a Thermo Scientific Glacios cryo-TEM equipped with Ceta-D camera. Continuous rotation micro-ED datasets were collected from numerous small crystals with average rotation range from 90-120°, a rotation speed of 1°/s, and an exposure time of 1 s/frame. The data were processed using the XDS suite and dataset quality was improved through isocluster and CC1/2 analysis [5-7]. Some processing statistics can be seen in Table 1 and a diffraction pattern in Figure 1. The structure was solved through molecular replacement using Phaser [8] using the gramicidin model (PDBID: 1ALX [9]). The structure was refined using Phenix.Refine [10].

Gramicidin crystallized in space group $P2_1$ with 2 dimers per asymmetric unit (Figure 2B,C). The peptide crystallized as a novel polymorph not previously described in the literature. Gramicidin is known to adopt multiple conformations ranging from a head-to-head, single stranded helical dimer to a left- or right-handed intertwined, parallel or antiparallel, double stranded double helix [4]. The conformation that is observed in this structure is the antiparallel double helix (Figure 2A). The peptide is

packed tightly with a solvent content of approximately 13%. All side chains are observed in the Coulomb density and some amino acid side chains are shown in Figure 2D, E, F [11].

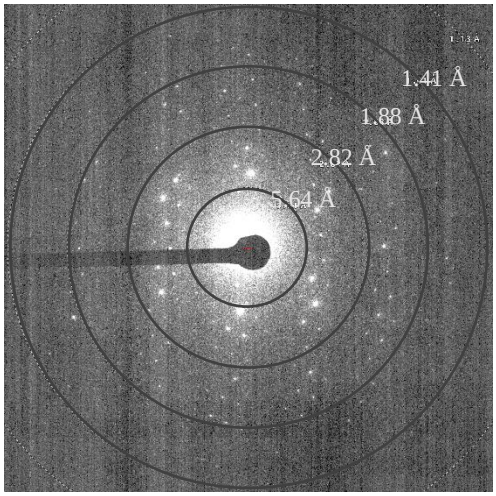


**Figure 1.** Diffraction pattern of gramicidin, resolution is indicated by rings

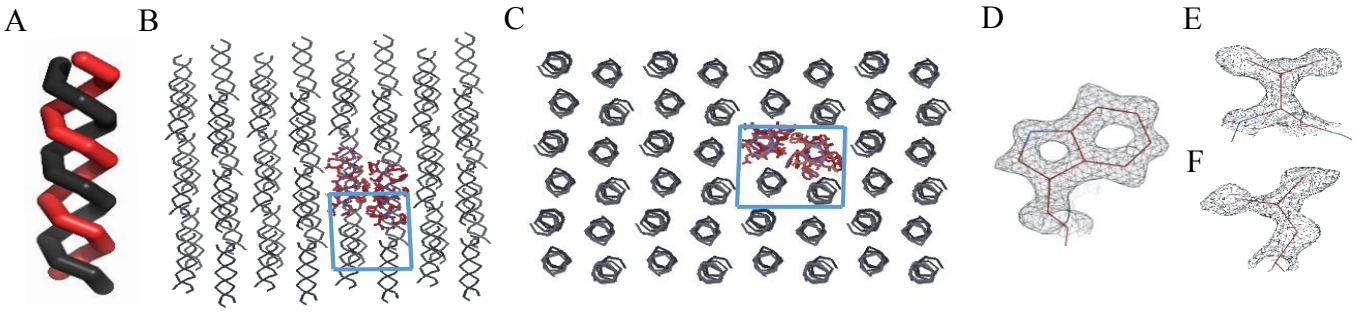


**Figure 2.** A. gD dimer in ion-free antiparallel double helix conformation; B,C. Crystal packing shown along c axis (B) and b axis (C), gD dimers in asymmetric unit are shown with sidechains in red and blue, gD dimers showing crystals packing are in grey, blue shape shows unit cell; D,E,F. Amino acid residues shown in Coulomb density; D) Trp-13 chain C, E) D-Val 8 chain A, F) D-Leu 4 chain B

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**Table 1**. Processing Statistics

| **Processing statistics** | **Value** |
|---|---|
| Wavelength (Å) | 0.0251 |
| Number of merged data sets | 14 |
| Space group | P $2_1$ |
| Unit cell (a, b, c) (Å) | 25.85 x 31.62 x 25.85 |
| (α, β, γ) (°) | 90.0 x 91.9 x 90.0 |
| Resolution range (Å) | 50.0 - 1.08 |
| No. of total reflections | 258193 (14066) |
| No. of unique reflections | 15683 (1099) |
| Multiplicity | 16.5 (12.8) |
| Completeness (%) | 97.9 (96.9) |
| Mean I/σ(I) | 4.6 (0.72) |
| CC1/2 | 0.99 (0.20) |

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