

The Na⁺/H⁺ antiporter NhaA from *Salmonella* Typhimurium (ST-NhaA) is a membrane protein that was expressed and purified in *E. coli*. For crystallization, an antibody fragment mediated approach is used. Crystallization conditions in which crystals are formed that diffract to high resolution are already elaborated. In this shift 2 different questions were addressed. (1) To determine the Na⁺ pathway in ST-NhaA, crystals were soaked with TI⁺, an alkali ion mimic. (2) In addition, different variants of ST-NhaA and of the antibody fragment were tested for their impact on the resolution of the crystals.

A total of 13 crystals obtained from the ST-NhaA in complex with one of the two antibody fragment variants were taken from an initial screen (MemGold) but did not diffract better than 10 Å. In contrast, from 57 crystals tested using the wild type antibody fragment, 12 diffracted better than 5 Å and a total of 28 datasets were collected. To determine the Na⁺-pathway with the substrate mimic TI⁺, different soaking conditions in terms of concentration (up to 50 mM) and incubation time (1 min – 4 h) were tested. Thereby it was challenging to not destroy the crystal and to wash it well, since for the elaborated crystallization condition Cl⁻ is used in the presence of which TI⁺ easily precipitates. Co-crystallization with a replacement of Cl⁻ was also tried in advance without success. The crystal with the shortest incubation time of 1 min was diffracting to the highest resolution of 2.6 Å with anomalous signal to 11 Å resolution. The following unit cell parameters were determined: space group: P2₁, cell dimensions: 112 Å, 92 Å, 139 Å, 90°, 109°, 90°. For this crystal, highly redundant datasets were collected and also helical data collection was used. One of the two tested ST-NhaA variants diffracted to 3.5 Å resolution and as for the wildtype protein, space group P2₁ and similar unit cell constants were determined.

During the beamtime, we could also collect data from YGT, a bacterial glycosyltransferase. These were the first YGT crystals tested at synchrotron sources and the best diffracted x-rays to 1.9 Å-resolution on ID30A-3. During the same shift, we had also the chance to have access to tunable beamline ID29. There, similar crystals were collected at 6keV. The structure could be determined using the Native-SAD data collected on ID29. These data had a resolution of 2.2 Å-resolution. The model is currently being built with the higher resolution data collected on ID30A-3 and a manuscript is in preparation.