

The Na⁺/H⁺ antiporter NhaA from *Salmonella* Typhimurium is a membrane protein that was expressed and purified from *E. coli*. The transporter is active above pH 7 and reaches highest activity around pH 9.25. The transporter was crystallized at pH 8.2 in the presence of its substrate Na⁺. For determination of the Na⁺-pathway, the crystals were soaked with the substrate mimic Tl⁺. The crystals were washed, soaked and half the crystals were also backsoaked in buffer solutions without Tl⁺. A Tl⁺ concentration of 25 mM was used but different soaking conditions were applied in terms of time. Beamline MASSIF3 allows anomalous data collection from Tl⁺ (L-III Edge at 0.9795 Å, f' and f'' of about respectively -10.9 and 9.9 at 12.8 keV). In total, 10 crystals were measured and, among them, 8 crystals diffracted X-rays to a resolution better than 4.3 Å. Crystals were belonging to space group P21 and their cell dimensions were 110 Å, 92 Å, 137 Å, 90°, 110°, 90°. To obtain anomalous data, 49 highly redundant datasets were collected taking advantage of the small beam size on MASSIF3. Resolutions of the measured crystals were ranging from 2.65 – 4.3 Å. Anomalous signals were detected only in datasets of the crystals for which no backsoaking was applied. The crystal with the longest soaking time of 3 min had the highest resolution of 2.65 Å resolution with anomalous signal extending to 5.3 Å resolution.