Proposal code MX-1566

Proposal title

Structural basis of substrate recognition by the E. coli Rhomboid intramembrane protease GlpG

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Experiment report

During our beamtime at ID29 (December 3rd-December 4th) we could collect various complete datasets of the *E. coli* intramembrane protease GlpG in complex with a substrate-derived inhibitor. Complexes of both the wildtype enzyme and a mutant carrying a mutation in the binding pocket were used. The substrate derived inhibitors bind covalently to the enzyme and the complexes therefore crystallize in conditions similar to the unliganded enzyme. Knowing that the wt crystals suffer from problems with twinning we assumed that this might also be true for the complexes. Therefore we measured ~40 crystals from many slightly different crystallization conditions. Indeed many of our crystals showed a high twinning factor, which we determined by analyzing each dataset with phenix Xtriage. We were finally able to identify 6 datasets (wt and mutant) with no or low twinning. From those we selected a 2.5 Å dataset for the wt complex and a 2.9 Å for the mutant. Both datasets show good overall statistics and we were able to solve and refine the structures. In both structures the density for the inhibitor substrate is excellent, which enabled us to characterize the interactions in the S1-S4 position. These results will soon be part of a high impact publication.