# Poster Presentations

**[MS7-P06] Probing Determinants of Cyclopiazonic Acid Sensitivity of Bacterial $Ca^{2+}$-ATPases** Manuela Gorgel[1,2], Aljona Kotsubei[1,2,3], Jens P. Morth[1], Poul Nissen[1,2] and Jacob L. Andersen[1,2].

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$Ca^{2+}$-ATPases are integral membrane proteins that actively extrude $Ca^{2+}$ out of the cytoplasm and thus play a crucial role in $Ca^{2+}$ homeostasis [1]. The $Ca^{2+}$-ATPase of the Sarco-Endoplasmic Reticulum (SERCA1a) is potently and specifically inhibited by the mycotoxin Cyclopiazonic acid [2, 3]. To test the sensitivity of prokaryotic $Ca^{2+}$-ATPases towards CPA and to describe their binding pocket, we have investigated different bacterial $Ca^{2+}$-ATPases [4]. Based on structural [5] and bioinformatics studies, we have identified four key residues in the CPA binding site. Our prediction was experimentally confirmed by characterizing $Ca^{2+}$-ATPases from *Bacillus cereus* (BACCA1), *Listeria monocytogenes* (LMCA1) and *Lactococcus lactis* (LLCA1); BACCA1 contains the same residues as SERCA1a in the proposed positions and is CPA sensitive, whereas LMCA1 and LLCA1 possess different amino acids and are not inhibited by CPA. When these four residues were mutated into the homologous ones of SERCA1a, LMCA1 and LLCA1 could be rendered CPA sensitive. Hence, these CPA-sensitive gain-of-function mutations further underscore the importance of the identified determinants. The differences between bacterial and mammalian binding pockets encompassing the CPA site suggest that CPA derivatives that are specific for bacteria or other pathogens can be developed. In order to describe the structural arrangement of the CPA binding pocket in bacterial $Ca^{2+}$-ATPases, we are currently aiming at co-crystallizing BACCA1 in complex with CPA in a mixture of C12E8 detergent and DOPC lipid applying the HiLiDe method [6]. Initial crystallization hits have been identified in an in-house PEG screen and crystal optimization by secondary detergents and additives are currently under way.

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