s1.m8.p2 **Structural studies on metalloregulatory protein MerR.** Anna-Maria Brandt[a], Heidi Kidron[a], Matti Karp[b] and Tiina Salminen[a]. [a]*Department of Biochemistry and Pharmacy, Åbo Akademi University, Po. Box 66, FIN-20521 Finland.* [b]*Department of Biochemistry and Food Chemistry, University of Turku, Finland. E-mail: anna.brandt@abo.fi*

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Mercury resistance regulator (MerR) is a 32 kD metalloregulatory protein that controls mercury resistance in Gram-negative bacteria by regulating transcription of the *mer*-operon. It is a member in the diverse MerR-family of transcriptional activators. In the presence of Hg (II) the regulator functions as an activator of the mer-genes while in the absence of Hg(II) it is a weak repressor.

The monomer folds into three functional domains: a N-terminal DNA-binding domain, which is homologous among the MerR family proteins, a C-terminal Hg-binding domain, and an intervening region, which plays a role in active repression and in transcriptional activation. The DNA-binding domain contains a helix-turn-helix motif that binds to DNA between positions -35 and -10 on the operon. The Hg-binding domain has three conserved cysteine residues at positions C82, C117, and C126, which form the trigonal high-affinity Hg-binding site. Every dimer binds one Hg atom, but the mechanism of binding is still unknown. High affinity and selectivity of MerR for Hg has been utilized in biosensors and has been engineered for purification of mercury-contaminated drinking water [1,2].

In the absence of Hg the inactive form of MerR causes a distortion of DNA in the middle of the *mer*-operator. In this inactive form the RNA polymerase binds adjacent to MerR in a closed complex, which has access only to the -35 site of the promoter, while the -10 site remains inaccessible. The distortion is caused by a 19 bp spacing between the -35 and -10 elements, which is unusually long compared to normal 17 bp in bacteria. Incoming Hg binds to MerR and causes unwinding and unkinking of DNA. The helix backbone is straightened out and unwound which brings the -35 and -10 promoter elements onto the same face of the DNA double helix. The reorientation of the -35 and -10 sequences allows them to interact with the RNA polymerase to form an open transcriptional complex and transcription is initiated [3].

Our aims are to solve the crystal structure of MerR in active and inactive form and study the specific protein-DNA interactions, binding mechanisms and structural changes occurring upon ligand binding.

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s1.m8.p3 **The Role of Glutamate 90 in Drug Binding by QacR, a Multidrug-Resistance Regulator in Staphylococcus aureus.** Benjamin Brooks, Kate M. Hardie, Ronald A. Skurray, Richard G. Brennan, *OHSU, Biochemistry and Molecular Biology, 3181 SW Sam Jackson Pk Rd., MS L224, Portland, USA. E-mail: brooksbe@ohsu.edu*

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The QacR binding pocket, as revealed by x-ray crystallography, is lined primarily with aromatic residues and several glutamates. The glutamates found in the binding pocket were not a suprize,surprising, as the inducing ligands for QacR are all positively charged. These glutamates, including glutamate 90 (E90), appear to make electrostatic contacts with the drug analogs, all of which are positively charged. These glutamates may function to increase the affinity of the protein for positive ligands or to decrease the affinity for negative ligands; mutation of the anionic residue, E90, to an electrostatically neutral residue would lead to a marked decrease in affinity for the drug analogs that interact with E90. Focusing on the first hypothesis, weTo study the effect of alanine and glutamine substitutions of residue E90 on drug analog binding, we measured changes in drug-binding affinity with fluorescence polarization and isothermal titration calorimetry. X-ray crystallographic structural information was used to reveal any unexpected changes in the binding pocket due to the mutation. Drug analogs that interact with E90 (R6G and malachite green (MG)) and one that does not (dequalinium (Dq)) were used in the crystallization and affinity measurement studies. By fluorescence polarization, the wild-type QacR and the mutants, E90A and E90Q, have similar affinities for the drug analog rhodamine-6-G (R6G), which makes a complementary charge interaction with E90 in the wild-type QacR. However, isothermal titration calorimetry, which measures stoichiometry as well as affinity, indicated that E90A has a drug:QacR-monomer stoichiometry of 1:1 rather than the 1:2 value that was measured for both wild-type and QacR E90Q. The QacR-E90Q-MG complex crystals diffracted to 2.6 Å resolution and crystallized in a different space group than any previous crystals of ligand complexes with either wild-type or mutant QacR proteins ($P6_2$ vs. $P4_22_12$). Though the DNA-binding domains of the QacR-E90Q-MG structure are almost identical to those of the wild-type-MG structure, they have swung closer together by 6 Å. Unexpectedly, the glutamine substitution does not affect the QacR-E90Q-MG complex. The drug-binding pocket in the QacR-E90Q-MG structure superimposes almost perfectly with the drug-binding pocket of the wild-type-QacR-MG structure. By contrast, the 3.3 Å resolution structure of the E90Q-QacR-Dq complex reveals that the side chain of Q90 makes a contact with the dequalinium where as in the wild-type-QacR-Dq structure E90 does not. This new contact may increase the affinity of the QacR-E90Q protein for dequalinium. In conclusion, the affinity measurements suggests that E90 does not contribute to drug binding affinity, but structural confirmation is required to interpret the affinity measurements. Further X-ray crystallographic structures are also necessary to determine the structural significance of the new stoichiometry of the QacR-E90A-drug complexes. Additionally, the hexagonal crystal form for QacR has captured a different conformation of the protein, thus illustrating the flexibility of the DNA-binding domains of QacR.