# Hormonal Steroids Bind MtrR to Induce Multidrug Resistance and Stress Response Genes in *Neisseria gonorrhoeae*

Grace M. Hooks[1], Julio C. Ayala[2*], Concerta L. Holley[2], Vijaya Dhulipala[2], Grace A. Beggs[3], John R. Perfect[4], Maria A. Schumacher[1], William M. Shafer[2,5,6], and Richard G. Brennan[1]

[1.] Department of Biochemistry, Duke University School of Medicine, Durham, North Carolina, USA [2.] Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia, USA [3.] Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA [4.] Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, North Carolina, [5.] Laboratories of Microbial Pathogenesis, VA Medical Research Service, Veterans Affairs Medical Center, Decatur, Georgia, USA [6.] Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta, Georgia, USA.
* Present address: STD Laboratory Reference and Research Branch, Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

## Abstract

Overexpression of the multidrug efflux pump operon *mtrCDE,* encoding a critical factor of multidrug-resistance in *Neisseria gonorrhoeae*, the causative agent of gonorrhea, is repressed by the transcriptional regulator, MtrR (Multiple transferable resistance Repressor). Here, we report the results from a series of *in vitro* and cellular experiments to identify innate, human inducers of MtrR and to understand the biochemical and structural mechanisms of the gene regulatory function of MtrR. Isothermal titration calorimetry experiments reveal that MtrR binds the hormonal steroids progesterone, β-estradiol, and testosterone, all of which are present at significant concentrations at female and male urogenital infection sites as well as ethinyl estrogen, a component of some birth control pills. Binding of these steroids results in decreased affinity of MtrR for cognate DNA, as demonstrated by fluorescence polarization-based assays, and an *in vivo* increased antimicrobial resistance and expression of the MtrR-repressed *mtrCDE* and *rpoH* genes. The crystal structures of MtrR bound to each steroid provided insight into the flexibility of the binding pocket, elucidated residue-ligand interactions, and revealed the conformational consequences of the induction mechanism of MtrR. Three residues, D171, W136 and R176 are key to the specific binding of these gonadal steroids. These studies provide a molecular understanding of the transcriptional regulation by MtrR that promotes *N. gonorrhoeae* survival in its human host when challenged by innate steroidal antimicrobials.