

# A Molecular View Of Stress Relief In Bartonella Quintana: Crystal Structures PhyR, PhyR Complexed With Nepr, And RpoE Complexed With Nepr

David Dranow<sup>1</sup>

<sup>1</sup>UCB/SSGCID

David.Dranow@ucb.com

*Bartonella quintana* is a species of pathogenic  $\alpha$ -proteobacteria that causes fatal disease in humans. *B. quintana* was originally identified as the causative agent of trench fever, which infected over 1 million soldiers in the European theater of World War I. This infectious organism, which is carried by the human body louse, *Pediculus humanus humanus*, has since made a major resurgence in the last two decades. Infection can present as relapsing fever, endocarditis, and vascular proliferative lesions, the latter two of which can be fatal if not treated with antibiotics. *B. quintana* alternates between two very different environments, the hemin-restricted human bloodstream at higher temperature (37°C), and the hemin-rich body louse gut at lower temperature (28°C).

Bacteria use varied sets of regulatory proteins to respond to stress caused by changing environments. Two major classes of transcriptional regulators are two-component signaling (TCS) systems and alternative sigma factors ( $\sigma$ ). Recently in  $\alpha$ -proteobacteria, both of these mechanisms have been found in a single protein, PhyR, which contains an N-terminal  $\sigma$ -like (SL) domain and a C-terminal TCS receiver domain. The SL domain in *B. quintana* is not a true  $\sigma$  domain that binds DNA, but instead functions in a partner-switching mechanism with the true  $\sigma$  factor RpoE, exchanging the anti-sigma factor NepR. In non-stress conditions, the SL domain of PhyR is not phosphorylated and bound by the TCS receiver domain, while NepR is bound to RpoE, preventing RpoE from binding to the RNA polymerase core enzyme and to DNA. Upon sensing stress, PhyR is phosphorylated, allowing NepR to outcompete the TCS receiver domain and bind the SL domain. In this way, PhyR functions as an anti-anti-sigma factor. This frees RpoE to then associate with RNA polymerase core enzyme and bind DNA to regulate transcription of genes in response to changes in temperature and hemin concentration.

To better understand the interaction between the anti-anti-sigma factor PhyR, the anti-sigma factor NepR, and the sigma factor RpoE in *Bartonella quintana*, we have determined the x-ray crystal structures of apo PhyR, PhyR complexed with NepR, and RpoE complexed with NepR. In apo PhyR, we observe the TCS receiver domain binding the SL domain, which is consistent with a recently determined PhyR structure from *Brucella abortus* (4G97). Upon binding NepR, the PhyR undergoes a conformational change that moves the receiver domain away from the SL domain, where NepR binds. Most interestingly, the PhyR SL domain/ NepR binding mode is nearly identical to the binding mode between RpoE and NepR. These structures together provide a clear molecular view of this critical stress response mechanism in *Bartonella quintana*.