Appendix

Supplemental Methods

RNA extraction

Cultures were centrifuged and cell pellets were immediately stored in at 4°C RNAlater (Sigma, UK). To extract RNA, cell pellets were heated to 65°C for 10 min in 500μl sodium acetate buffer (0.1% SDS, 30mM Na-Acetate pH5.5) and 500μl hot acidic phenol (QBiogene). The aqueous phase was then extracted with phenol:chloroform:isoayml alcohol (25:24:1) (Sigma) and the RNA precipitated by addition of 500μl isopropanol and 500μl 3M Na-Acetate pH7. RNA pellets were washed with ice-cold 80% ethanol and resuspended in 50μl RNAse free water. Integrity of the RNA was assessed by spectrophotometry and agarose gel electrophoresis.

Recombinant CovR expression

Recombinant His-tagged CovR protein was expressed using pET100 TOPO cloning kit (Invitrogen). BL21 *E.coli* (Invitrogen) were ITPG induced and after 3 hours growth cells were lyzed by agitation in Bugbuster solution (Novagen, UK) and centrifuged. Pelleted cells were then resuspended in 10mls of Binding buffer (Histag purification kit, Novagen), votexed, briefly sonicated and centrifuged at 5000x*g* for 15 min. This was repeated twice. Inclusion bodies were solubilized in 6M urea-Binding buffer on ice for 1 hour and collected as supernatant from centrifugation and passage through a 0.45 micron filter. Recombinant CovR was purified using a HisTag nickel resin column (Novagen). After elution, recombinant CovR was refolded into storage buffer (50mM Tris pH8, 100mM NaCl, 1mM EDTA, 20%

glycerol). For phosphorylation, CovR protein was diluted in 20mM NaH_2PO_4, 10mM $MgCl_2$, 1mM DTT and 32mM of acetyl phosphate, and incubated at 37°C for 90 min.