



**SUPPLEMENTARY FIG. S3. ITC experiments following  $Mn^{2+}$  binding to reduced FurA mutants.** *Top* sections show raw data obtained at 25°C in 50 mM Tris/HCl pH 9, 50 mM arginine, 1 mM DTT. Aliquots of 300  $\mu\text{M}$   $MnCl_2$  were added over 20  $\mu\text{M}$  FurA mutants: C101S (A), C104S (B), and C133S (C). *Lower* sections show the binding isotherms from the data integrated and fitted to a single-site model (A) or two independent-sites model (B, C). DTT, dithiothreitol.