SUPPLEMENTAL DATA

ESCHERICHIA COLI THIOREDOXIN-LIKE PROTEIN YBBN CONTAINS AN ATYPICAL TETRATRICOPEPTIDE REPEAT MOTIF AND IS A NEGATIVE REGULATOR OF GROEL

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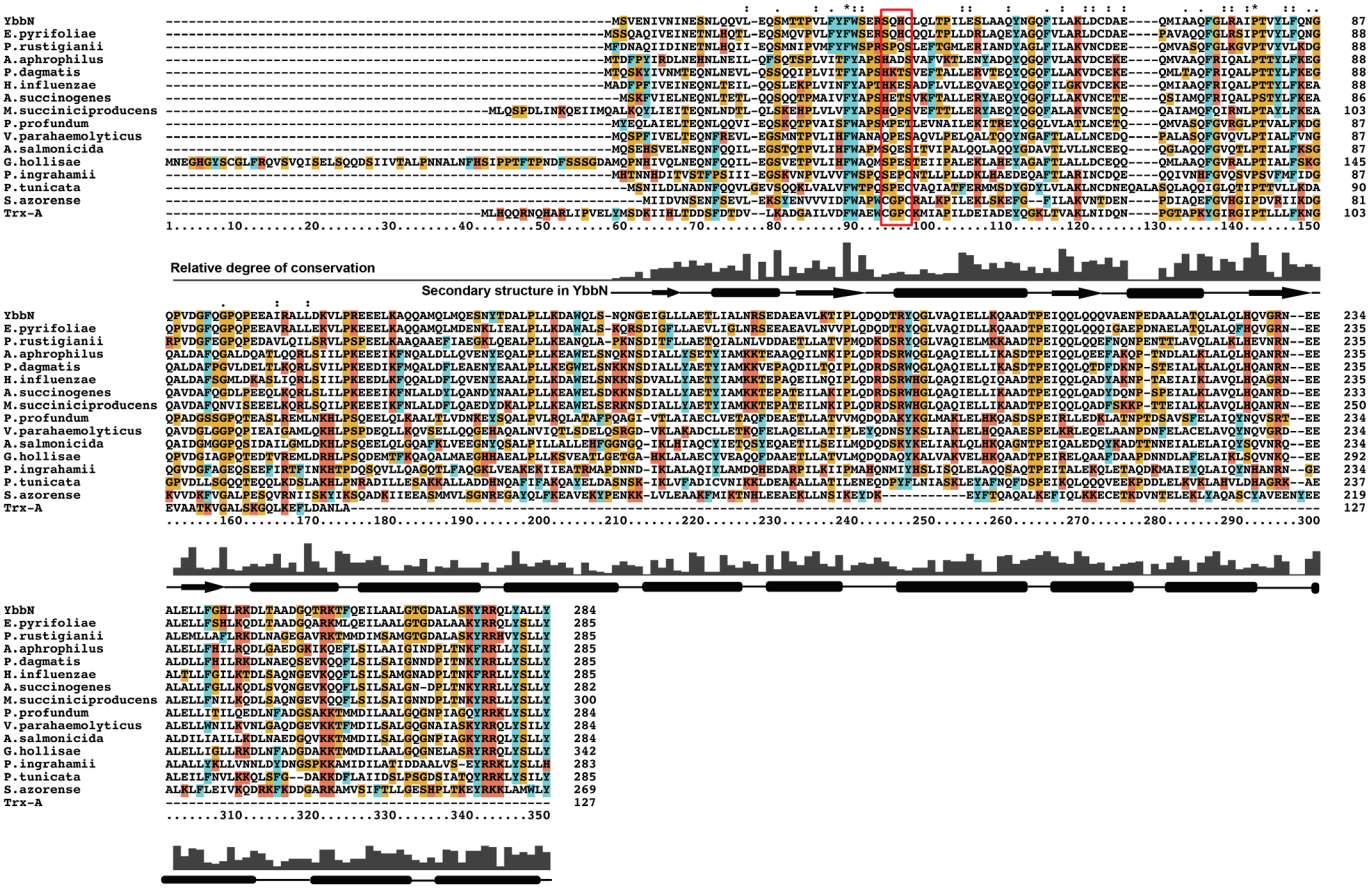
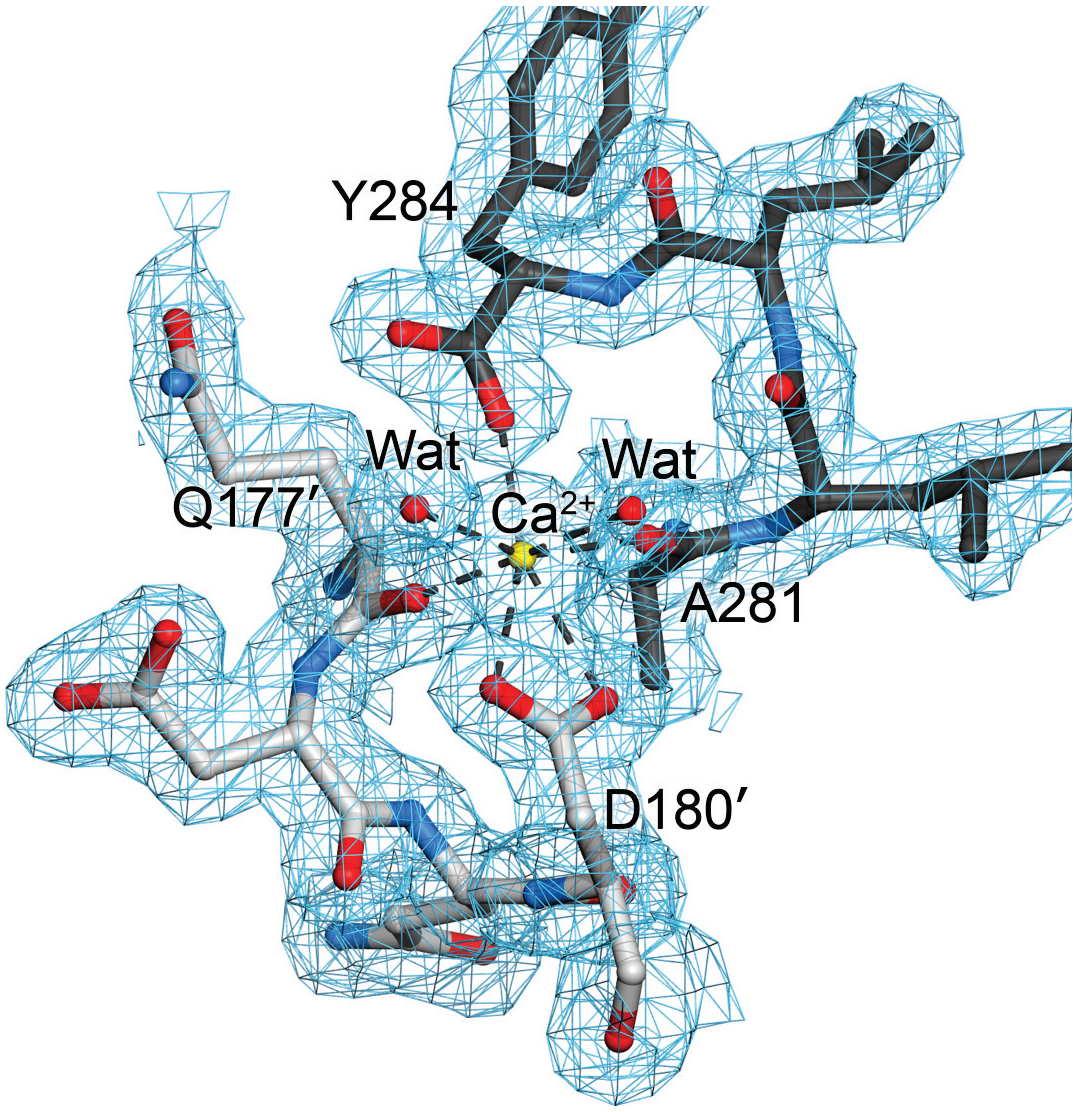
Supplemental Figure 1: Sequence alignment of YbbN homologues



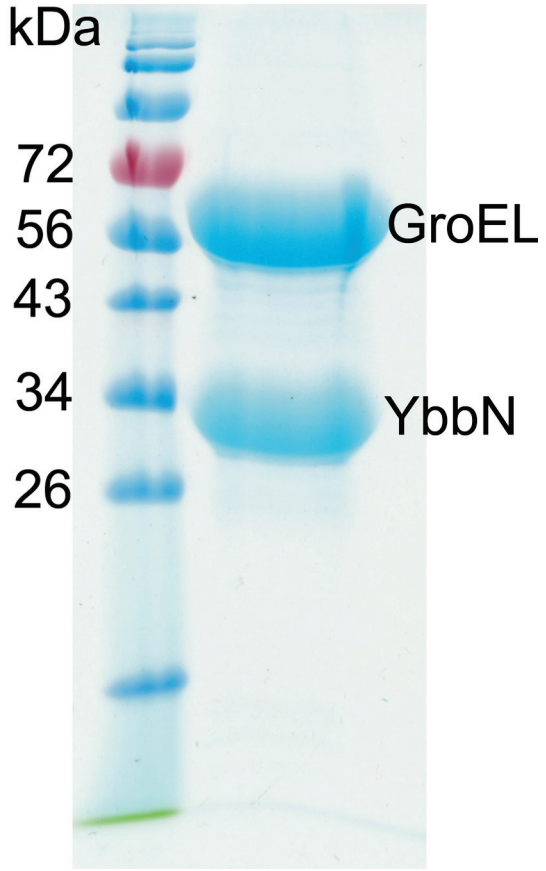
Sequence alignments performed in ClustalW for a selection of YbbN homologues, showing that the CxxC motif in the Trx domain (red box) is poorly conserved. The degree of conservation at each position is shown below the alignment in gray bars, with increased height indicating higher conservation. Below that, the secondary structure for *E. coli* YbbN determined from the crystal structure is represented with cylinders indicating helices and arrows indicating strands. A number of proteins lack both active site cysteine residues, implying either functional distinctions exist between them or that YbbN homologues are not oxidoreductases.

Supplemental Figure 2: A calcium binding site at the C-terminus of YbbN



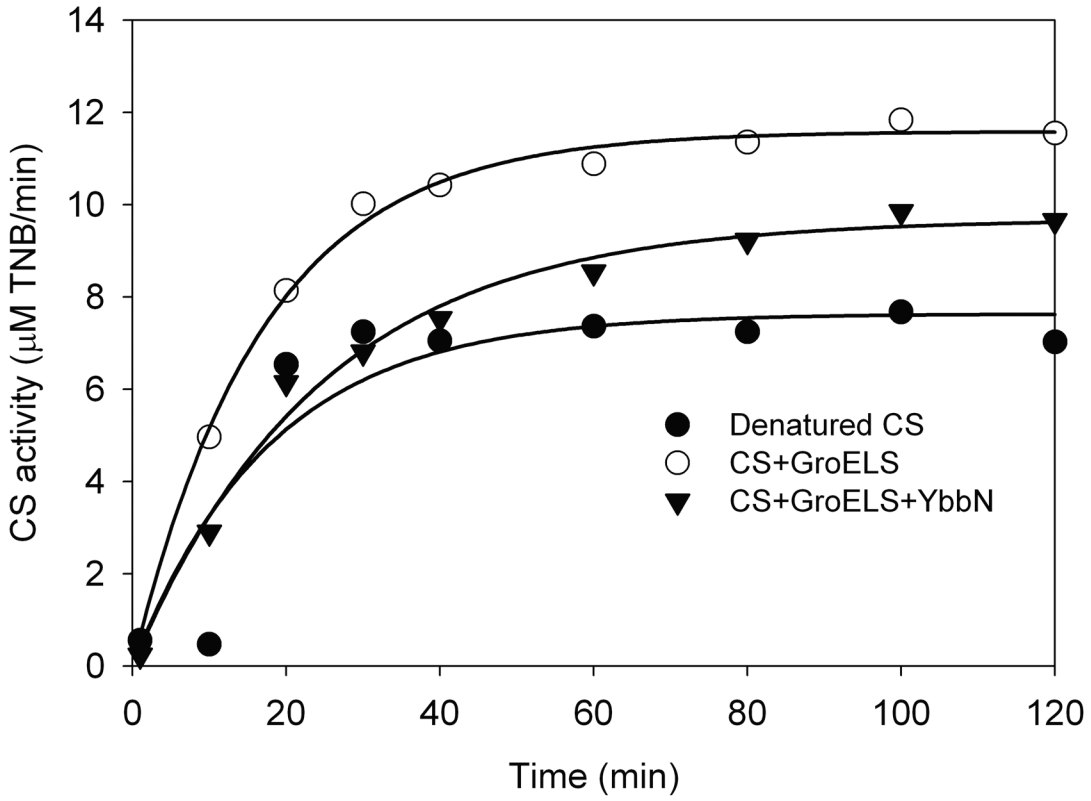
The C-terminus of one YbbN molecule (black carbons) creates a Ca^{2+} biding site with a neighboring molecule in the lattice (grey carbons). $2mF_o$-DF_c electron density contoured at 1.0 σ is shown in blue and residues that contribute a coordinating atom to Ca^{2+} (yellow) are labeled. Primes indicate a residue contributed from the neighboring molecule in the crystal. The Ca^{2+} is bound in a heptacoordinate, distorted octahedral fashion. Although this bound calcium is likely an artifact of crystallization, the corresponding region of related protein structures 3QDN and 2R5S also bind to buffer components.

Supplemental Figure 3: Resin-immobilized YbbN binds GroEL tightly



A Coomassie blue-stained SDS-PAGE gel of proteins eluted from a YbbN affinity resin by denaturation with SDS. YbbN was immobilized on cyanogen bromide-activated resin and used to reverse purify interacting proteins from clarified *E. coli* lysate as described in Experimental Procedures. After the final 1.0 M NaCl elution, a portion of the resin was heated to 95°C in SDS-containing loading buffer and the supernatant analyzed by SDS-PAGE. Two strong bands appear: one matching the molecular weight of GroEL and the other matching the migration of YbbN. Although YbbN is initially covalently attached to cyanogen bromide-activated resin *via* moderately stable isourea linkages, heating in SDS loading buffer disrupts some of these bonds, releasing a portion of the bound YbbN into the soluble fraction.

Supplemental Figure 4: YbbN suppresses refolding of citrate synthase by the GroESL chaperonin system.



Kinetics of citrate synthase (CS) refolding. The recovery of CS activity was measured as a function of time after dilution of denatured CS into refolding buffer alone (filled circles) or containing the protein(s) indicated in the inset, as described in the Experimental Procedures. GroELS significantly improves the recovery of CS activity (open circles), consistent with its role as a chaperonin. The presence of YbbN at a 2:1 molar ratio with GroEL partially inhibits CS renaturation (filled triangles) over the entire 120 minute duration of the experiment. Notably, YbbN almost completely inhibits GroESL chaperone activity at early timepoints in this experiment (up to about 40 minutes). The data are fit with exponential models (solid lines).