

Solution Structures of *Mycobacterium tuberculosis* Thioredoxin C and Models of the Intact Thioredoxin System Suggest New Approaches to Inhibitor and Drug Design

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* Running title: *Thioredoxin system models for inhibitor design*

Supplementary Material

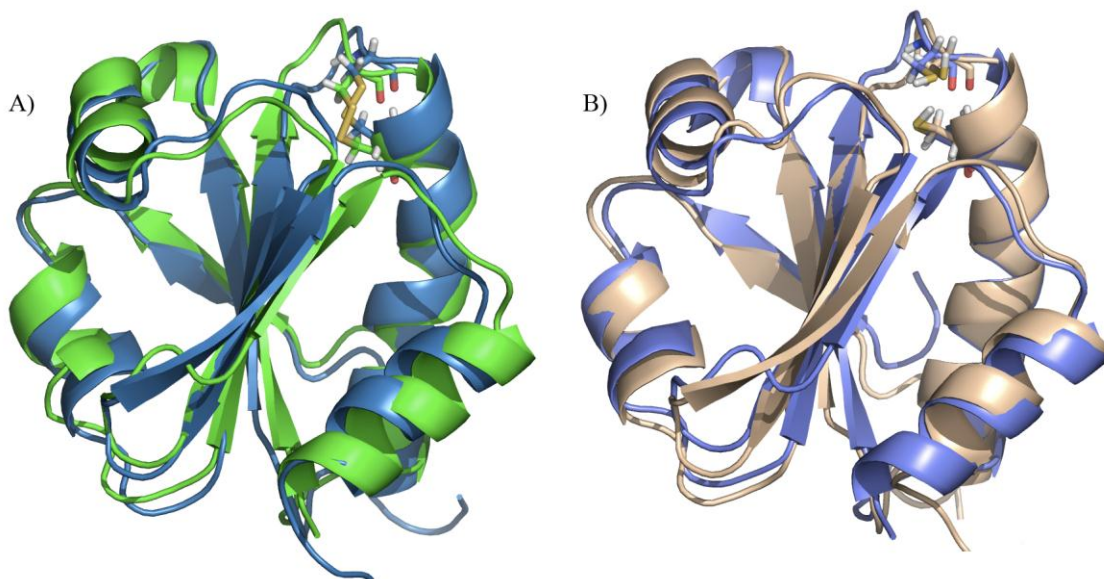


Figure S1. Overlays of both redox states of solution structures from *E. coli* Trx and *M. tuberculosis* TrxC, where A) is the overlay for the oxidized states, with blue being from *M. tuberculosis* and green from *E. coli* and B) is the overlay for the reduced states, with wheat being from *M. tb* and purple from *E. coli*.

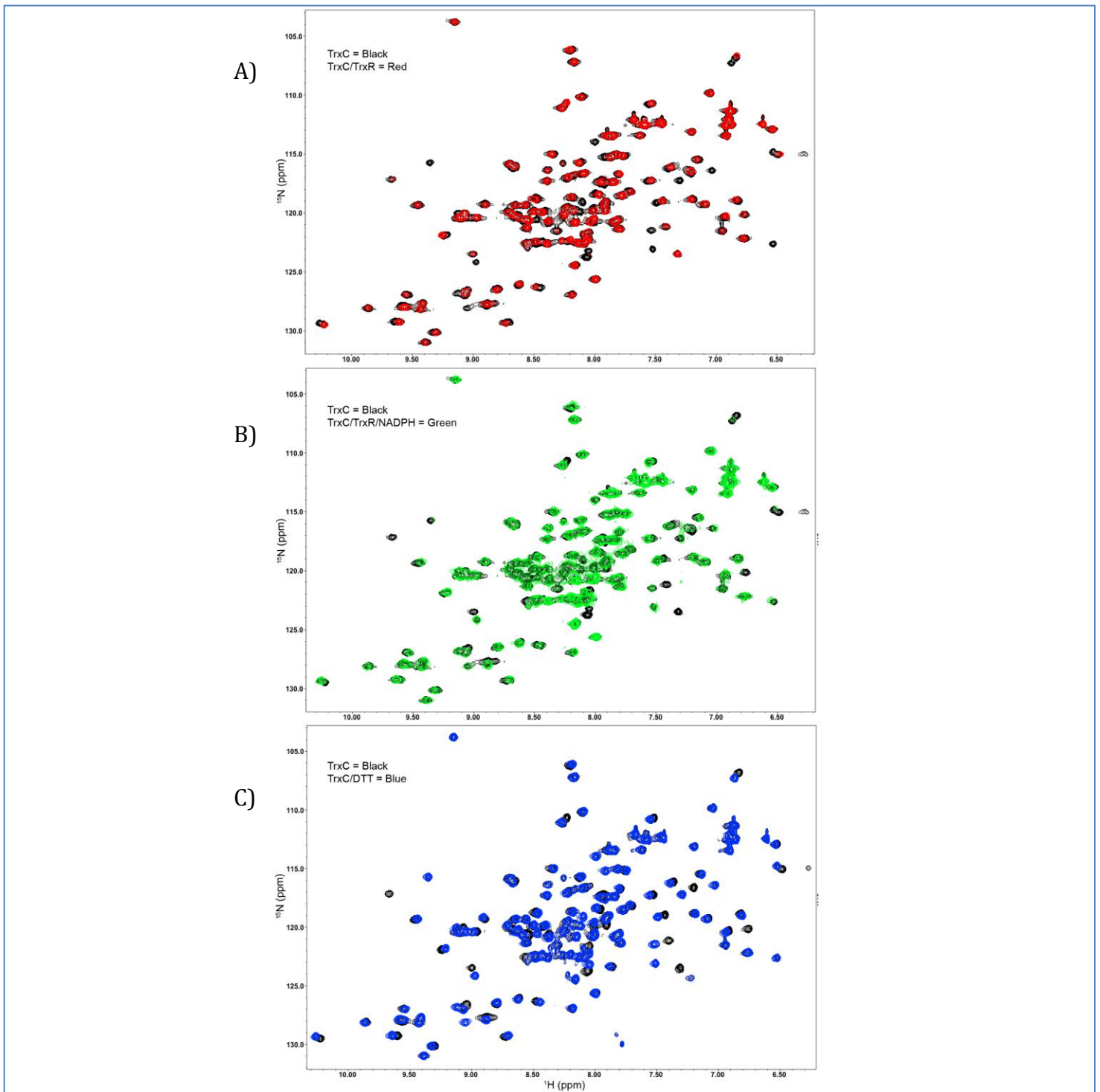


Figure S2. 2D ^1H - ^{15}N HSQC spectra where the black cross peaks in all panels are for oxidized/reduced TrxC and A) TrxR has been added to the sample (red), B) TrxR has been added to the sample and the redox activity initiated by addition of with NADPH to reduce TrxC (green), and C) DTT is added to the sample to fully reduce TrxC as a control experiment (blue). Protein concentration is 250 μM for TrxC and TrxR is slightly less than 250 μM .

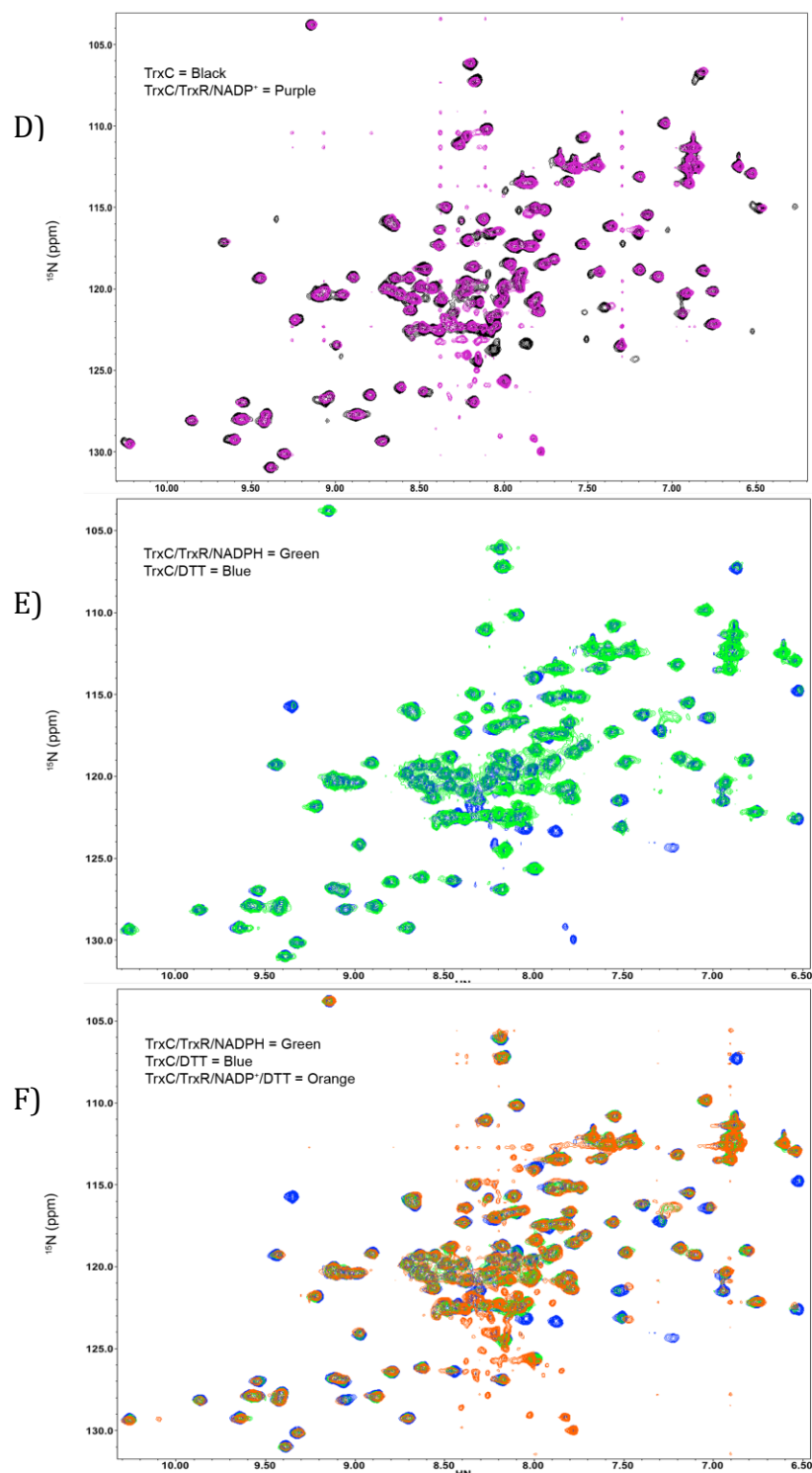


Figure S2 continued. 2D ^1H - ^{15}N HSQC spectra where D) black cross peaks are for oxidized/reduced TrxC and TrxR is added to the sample along with the oxidized cofactor NADP⁺. E) The overlay of the DTT reduced sample (blue) and the NADPH reduced sample (green), and F) is the same as panel E with the oxidized sample from panel D and DTT added to it (orange).

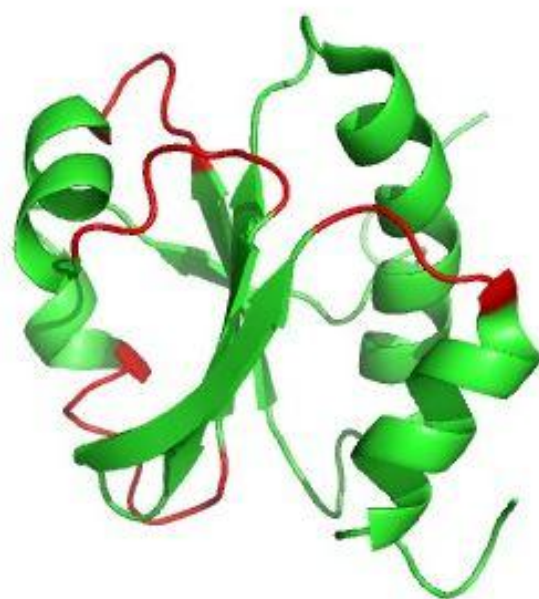
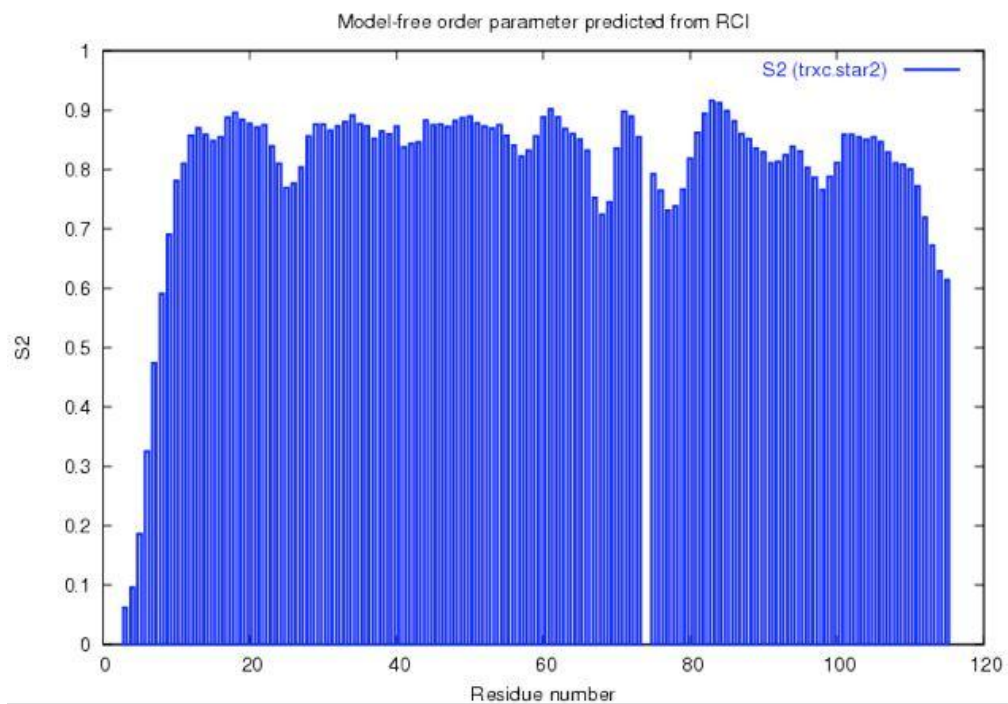


Figure S3. Top panel: Calculated order parameters, based on the Random Coil Index (RCI), from the Wishart group. Bottom panel: structure of TrxC (reduced) showing those segments (in red) with the greatest disorder, predicted to be random coil. The $\alpha 1$ and $\alpha 4$ helices are shown on the left and right sides, respectively, in the above representation.



Figure S4. Structure of TrxC showing location of last contiguously assigned residue, Asn116. The C-terminal histidine tag was present after Asn116 (some residues in the histidine tag were assigned as well).

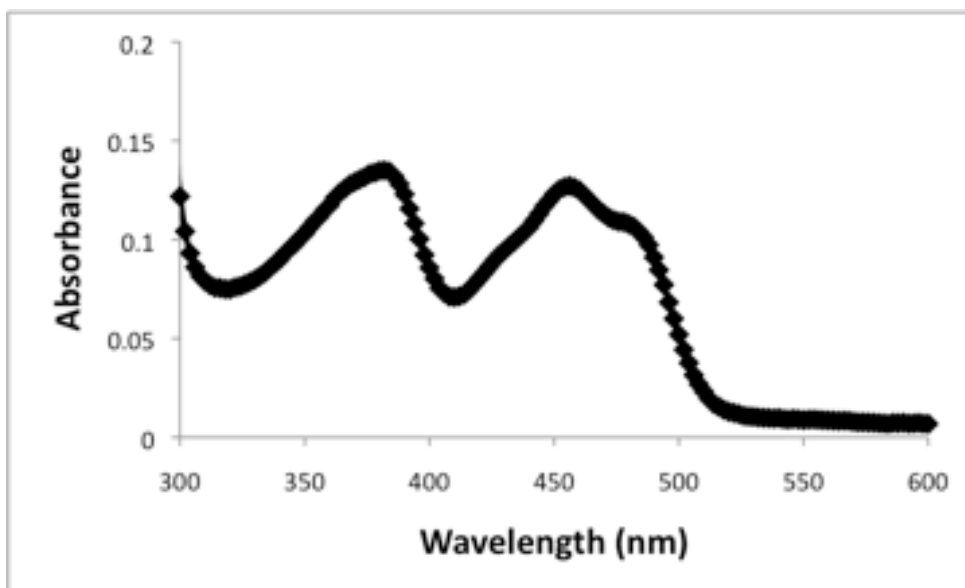


Figure S5. Absorbance spectrum of a TrxR sample (28 μ M) at 25 degrees in a 50 mM phosphate buffer at pH 7.6, obtained using an HP 8452 diode array UV-Vis spectrophotometer. Relative band intensity and shape matches that which was previously reported for the *E. coli* thioredoxins (Prongay, A.J., Engelke, D.R., and Williams, C.H., Jr. (1989) *J. Biol. Chem.* 264, 2656-2664).

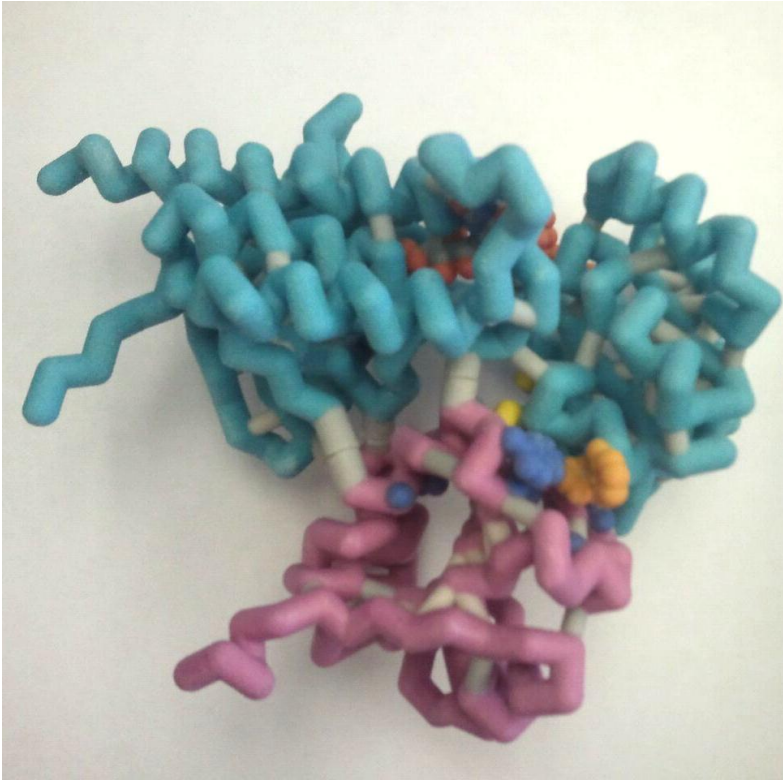


Figure S6. Physical model of the TrxR/TrxC structural that was built with students from Messmer High School (Giovanni Rodriguez, Kevonna Nathaniel, Anwuri Osademe and David Gonzalez), and their teacher Carol Johnson, to introduce students to structural biology research. This then led to production of a molecular animation of the TrxR/TrxC catalytic cycle in partnership with Milwaukee School of Engineering, as a teaching tool (under the NSF-funded CReST program).