Supplementary Information

Dissection of the water cavity of yeast Thioredoxin 1: the effect of a hydrophobic residue in the cavity.

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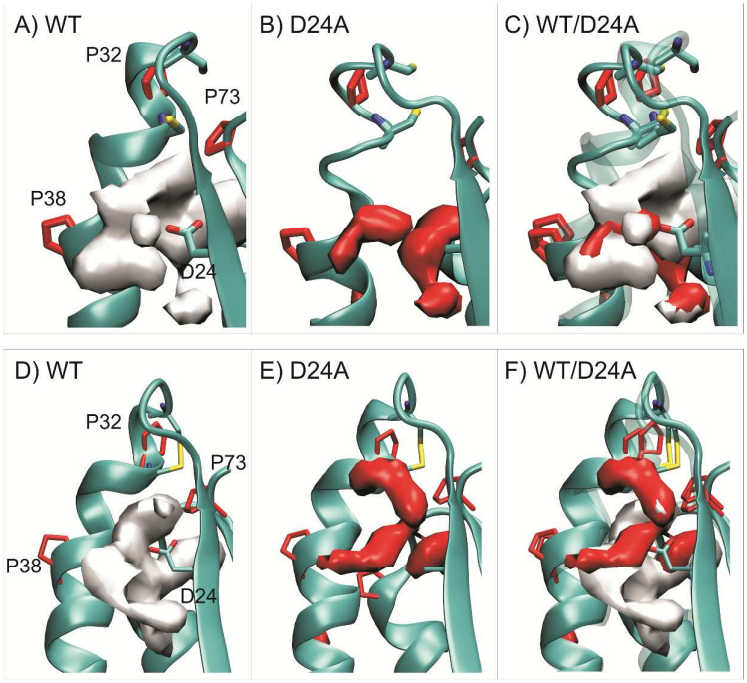

Figure S1: Water cavity of wt yTrx1 and mutant D24A. Panel A and D are the cavity of the wild type yTrx1[1] of the reduced and oxidized forms, respectively (cavity surface is in grey). Panel B and E are the cavity of the mutant D24A of the reduced and oxidized forms, respectively (cavity surface is in red). Panel C and F are the superposition of the cavities of wt yTrx1 and D24A mutant of the reduced and oxidized forms, respectively. The figure also shows the prolines (red sticks) and the amino acid residue Asp/Ala24.

We used Fpocket[2] to compute the cavities over the 200 ns MD simulation. To compute the isosurface , we used the same isovalue of 2.2 for the four simulations.

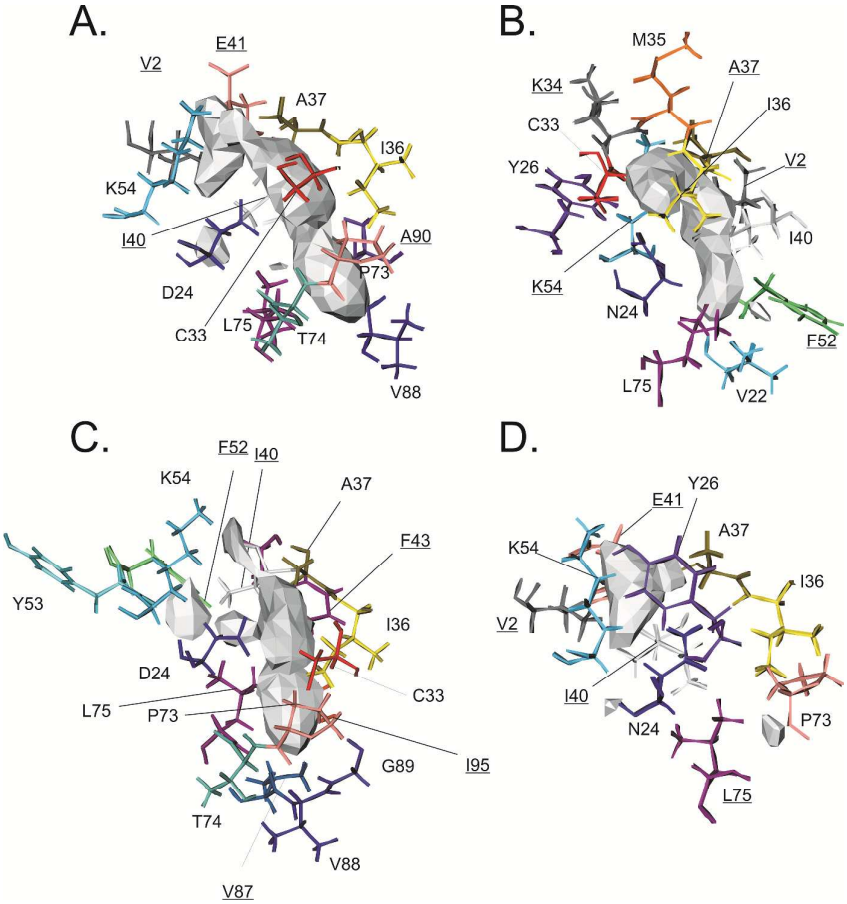

Figure S2: Water cavities of wt yTrx1 and mutant D24N. Panel A and C shows the cavity of the wild type yTrx1[1] of the reduced and oxidized forms, respectively (cavity surface is in grey). Panel B and D are the cavity of the mutant D24N of the reduced and oxidized forms, respectively. To facilitate the analysis of the figure, the residues are color coded according the residues position. The orientation of all cavities is similar, with the top view of the protein, with the interacting loops (not being shown) at the top. Note that for the wild type yTrx1, Cys33 and Pro73 are at the top of the cavity. Residues that are in the back of the cavity are underlined. All residues participating of the cavities are shown. We used Fpocket[2] (mdpocket) to compute the cavities over the

200 ns MD simulation. To compute the isosurface, we used the same isovalue of 3 for the four simulations.

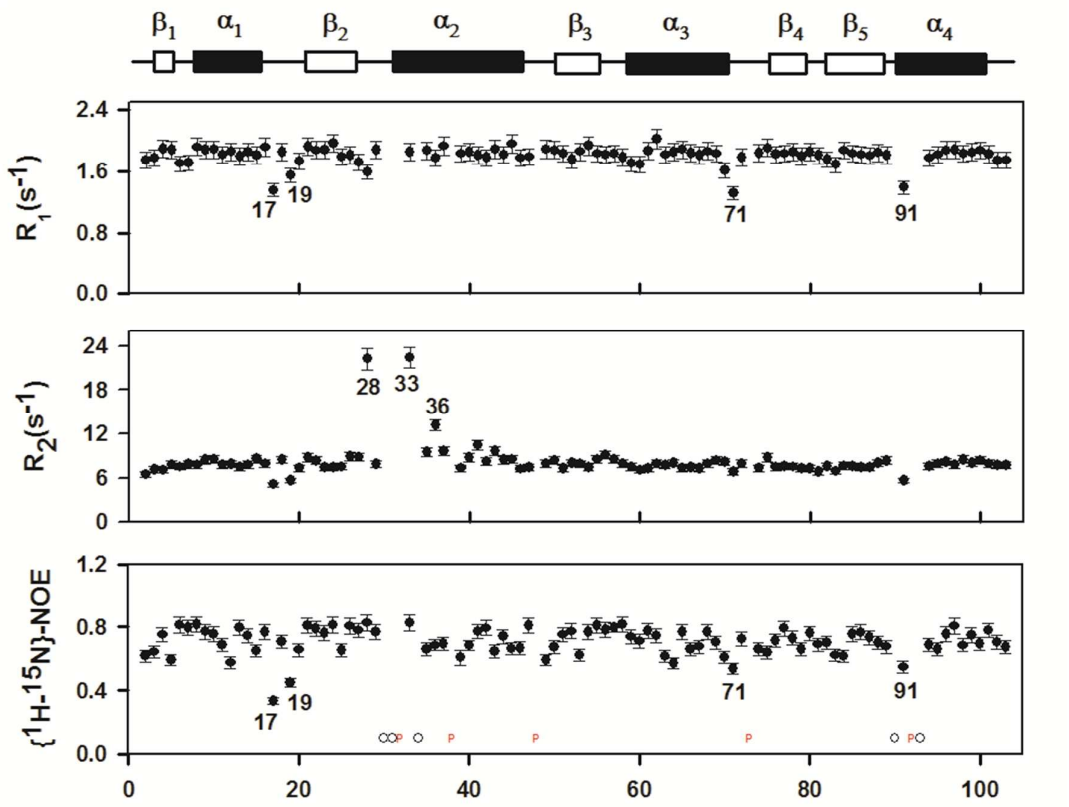

Figure S3: R1, R2 and ^{1}H-^{15}N heteronuclear NOE relaxation parameters of reduced yTrx1D24A. Open red circles represent resonances absent in the HSQC due to line broadening, and "P" represents proline. Both yTrx1 and D24A were at 200 μM, 298 K, 20 mM phosphate buffer, pH 7.0, with 30 mM of perdeuterated DTT.

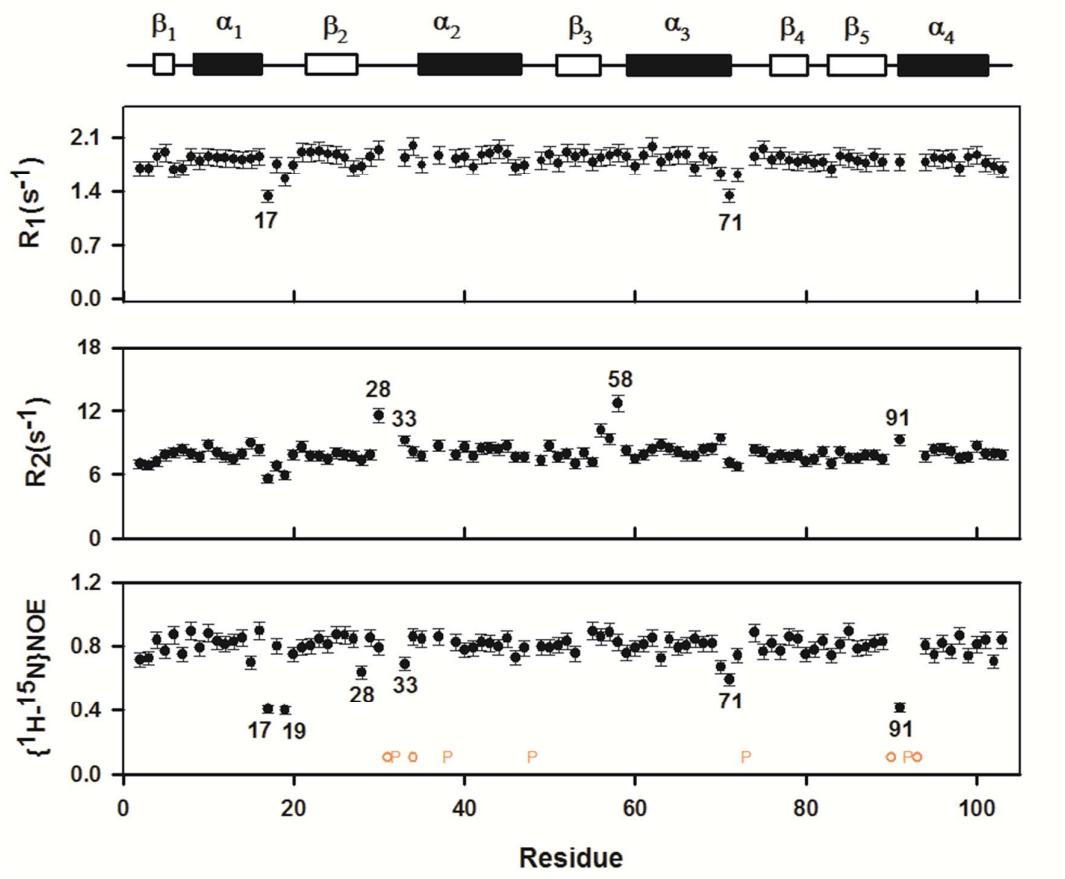

Figure S4: The R1, R2 and heteronuclear NOE relaxation parameters of reduced yTrx1D24A. Open red circles represent resonances absent in the HSQC due to line broadening, and "P" represents proline. Both yTrx1 and D24A were at 200 μM, 298 K, 20 mM phosphate buffer, pH 7.0, with 30 mM of perdeuterated DTT.

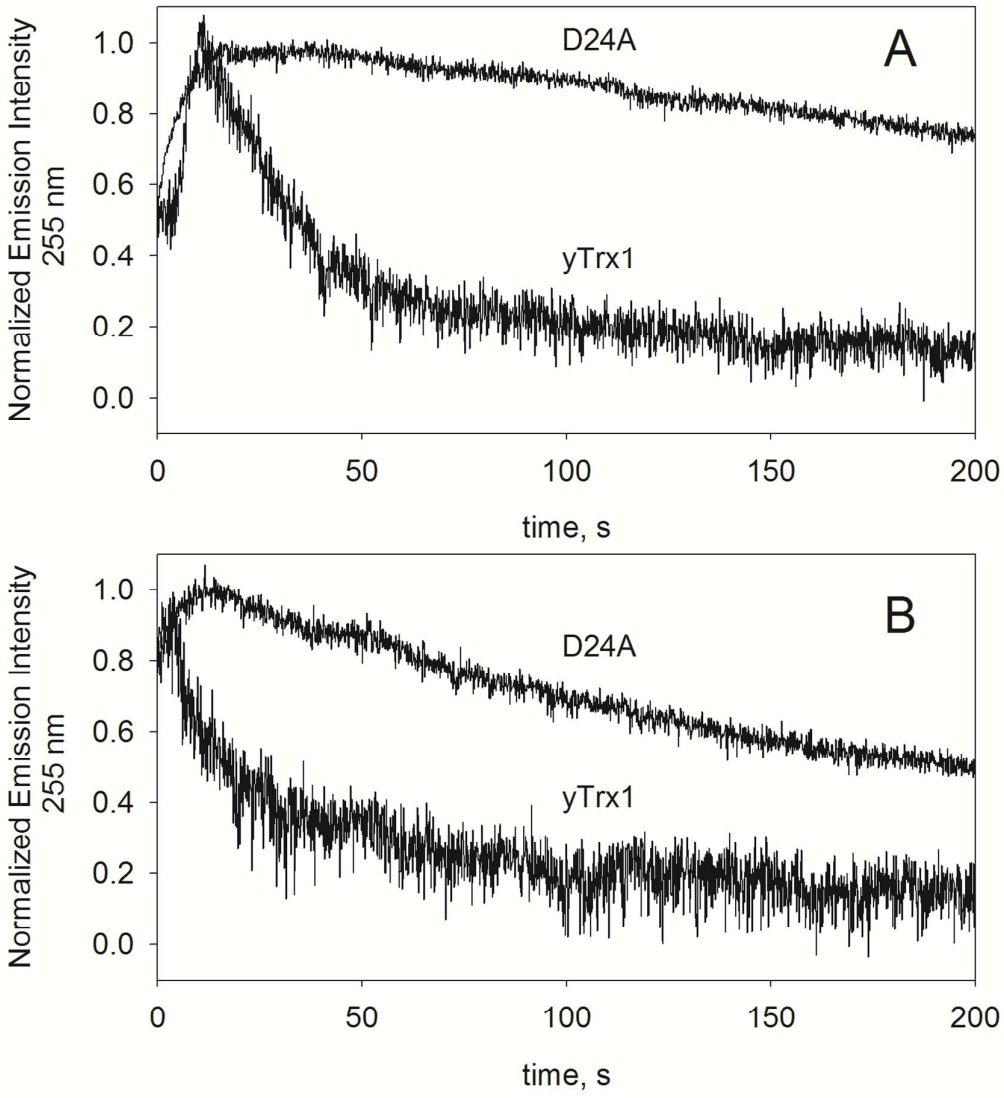

Figure S5: Fast kinetic experiment for measuring the reduction activity of yTrx1 and the mutant D24A at 295 K. It was measured the fluorescence emission of the fully reduced yTrx1 and D24A (immediately desalted to get rid of the dithiothreitol) at 20 mM exposed to 30 mM of two substrates: Insulin (A) and L-cystine (B). The reaction of yTrx1 and D24A with the substrates was measured by the reduction of fluorescence emission upon oxidation. We have used excitation at 280 nm and emission at 355 nm, with an excitation slit of 10 nm and emission slit of 10 nm. The fluorescence experiments were carried out in a Carry Eclipse (Agilent) equipped with the rapid mix

accessory SFA-20 in a manual mode. The dead time of the reaction mixer varied between 2 to 10 seconds. The reported kinetics is an average of three experiments. The cystine activity was measured by measuring of the slope of the graph ln[$Trx1^{red}$] versus time, not shown), which is the apparent rate constant k_{app}. All the experiments were done starting with 20 mM of reduced thioredoxin and 2:1 molar excess (40 mM) of the oxidized substrate. The observed k_{app} and k_1 are the following: $k_{app} = 0.0308\ s^{-1}$ (yTrx1 reducing insulin), $k_{app} = 0.00203\ s^{-1}$ (D24A reducing insulin), $k_{app} = 0.0182\ s^{-1}$ (yTrx1 reducing cystine) and $k_{app} = 0.00360\ s^{-1}$ (D24A reducing L-cystine).

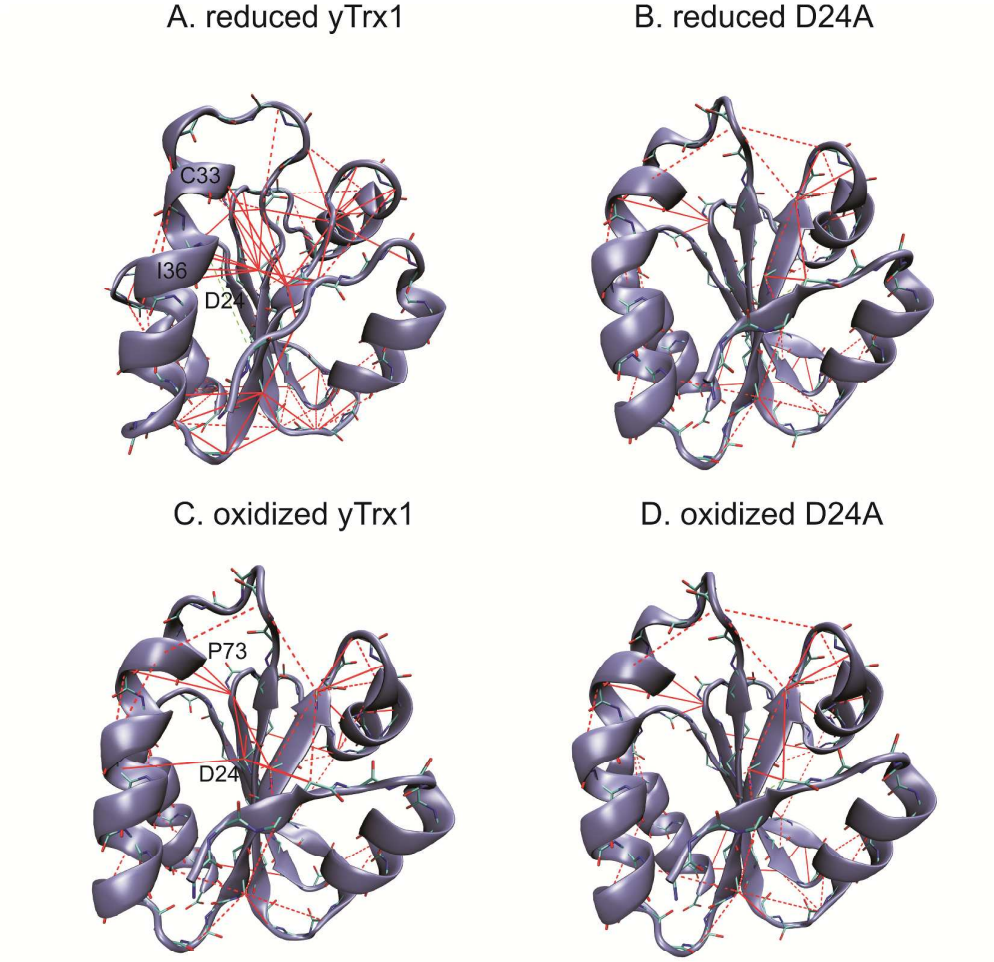

Figure S6: Degree of local degree of mutational frustration manifested for reduced (A) and oxidized yTrx1 (C) and the reduced (B) and oxidized mutant (D).The lines connects the highly frustrated contacts, as measured by the Frustratrometer (http:// http://www.frustratometer.tk/[3]). The mutational frustration index measures how favorable are the contacts of the native residue relative to other residues in the same location. The high frustration contacts within the water cavity shows that for these residues there are evolutionary pressure other than folding for these residues. It shows that the water cavity is highly frustrated because of the presence of a set o unfavorable

contacts responsible for the formation of the cavity. The mutation D24A reduces significantly the frustration level.

Table S1: FoldX stabilization free energy ($\Delta\Delta G_u = \Delta G_u^{mutant} - \Delta G_u^{yTrx1}$) calculated using FoldX, v3.0[4]. For FoldX calculations of the oxidized protein and mutants D24A and D24N, we have used the structure 3FQ3[5] stripped of the crystallographic water and the N-terminal Histidine tag. For FoldX calculations of the reduced protein and mutants D24A and D24N, we have used the structure 2I9H[6]. In these manner, the sequences of both calculations were identical. Before running the calculation of the unfolded free energy of the wild type yTrx1 (ΔG_u^{yTrx1}) and mutants (ΔG_u^{mutant}) we run the command RepairPDB, which prevent incorrect rotamer assignments of Asn, Gln and His residues. This command also optimizes side chains to eliminate small Van der Waal´s clashes. We then used the command Mutate to perform the mutations D24A and D24N.

	$\Delta\Delta G_u = \Delta G_u^{mutant} - \Delta G_u^{yTrx1}$ **Kcal/mol**	
	D24A	**D24N**
yTrx1 oxidized	-0.623	-0.610
yTrx1 reduced	-2.65	-0.800

References

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