# Supplemental Data for

**Human protein disulfide isomerase is a redox-regulated chaperone activated by oxidation of domain a'**

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**Supplemental Figure S1**

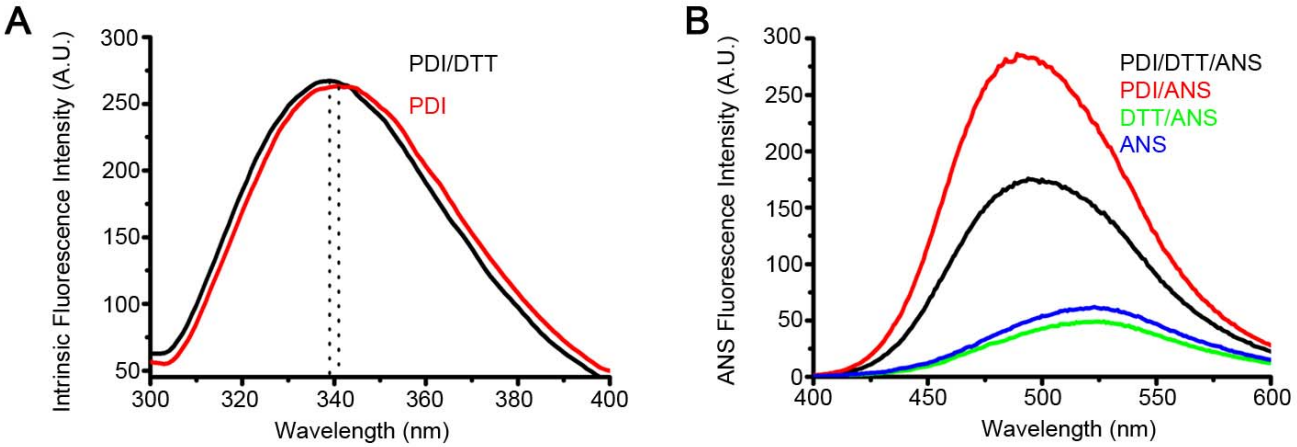


**Figure S1**: **The intrinsic and ANS fluorescence spectra of hPDI in the absence or presence of DTT.** hPDI at 5 μM was incubated without or with 1 mM DTT at 25°C for 30 min. Intrinsic (A) and ANS (B) fluorescence spectra were recorded.

**Supplemental Figure S2**

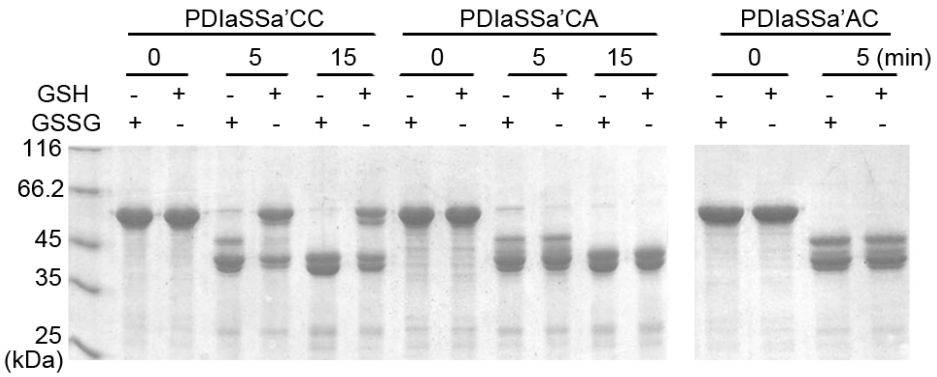


**Figure S2: The integrity of the a' active site is essential for the compact conformation of the reduced hPDI.** hPDI mutants at 1 mg/ml were incubated with 1 mM GSH or GSSG at 25$^{o}$C for 30 min and then digested by 2 μg/ml proteinase K for different time as indicated. The reactions were terminated by adding PMSF to a final concentration of 0.5 mM, and analyzed by reducing SDS-PAGE.

**Supplemental Figure S3**

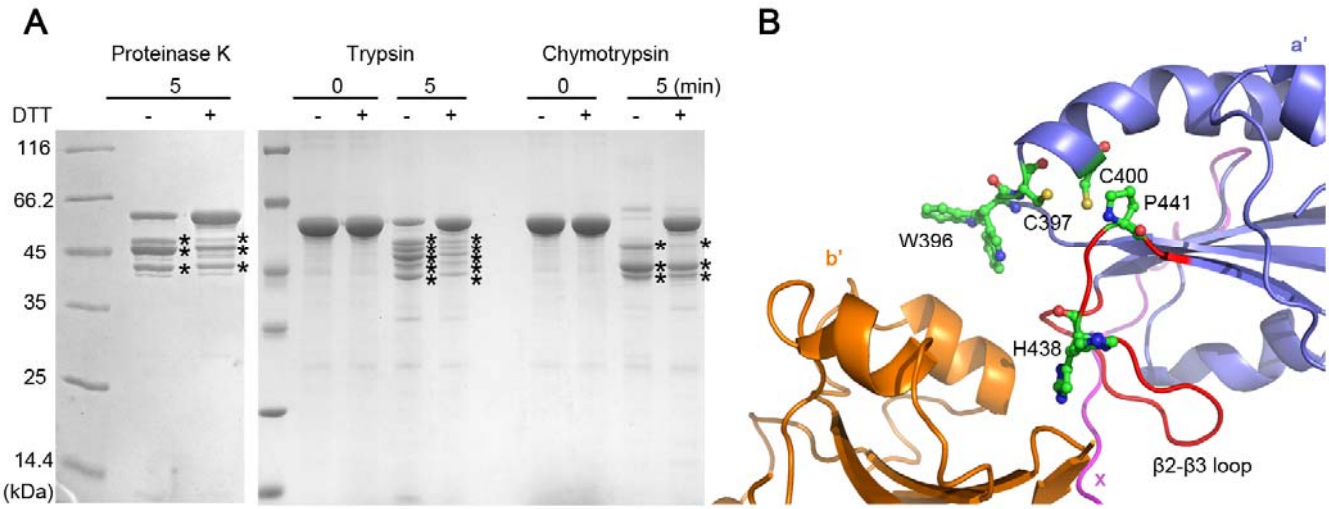


**Figure S3: Mapping the redox sensitive residues in hPDI.** (A) Protease digestion profiles of hPDI at different redox states. hPDI at 1 mg/ml was incubated with or without 1 mM DTT at 25$^{o}$C for 30 min and then digested by 0.3 μg/ml proteinase K, 2μg/ml trypsin or chymotrypsin for 5 min. The reactions were terminated by adding PMSF to a final concentration of 0.5 mM, and analyzed by reducing SDS-PAGE. The major products are indicated by asterisks. (B) Hotspots sensing the redox switch of the **a'** domain active site. The potential critical residues triggering the redox-regulated conformational changes of hPDI are shown in sticks. The **b'**, **x** and **a'** domains are colored in orange, magenta and blue, respectively, and the β2-β3 loop is colored in red.

**Supplemental Table S1**: **Mapping of the digestion sites for oxidized and reduced PDI.**

| Products[a] | Actual molecular mass | Observed mass | | | | | |
|---|---|---|---|---|---|---|---|
| | | Proteinase K | DTT/ Proteinase K | Trypsin | DTT/ Trypsin | Chymotrypsin | DTT/ Chymotrypsin |
| Full-length PDI | 56692.59 | 56691.52 | 56692.39 | 56694.55 | 56694.23 | 56694.94 | 56694.29 |
| $Met^{-12}$-$His^{438}$ | 48892.34 | 48890.82 | 488893.69 | / | / | 48891.45 | / |
| $Met^{-12}$-$Thr^{428}$ | 47815.13 | 47811.15 | / | / | / | / | / |
| $Met^{-12}$-$Asp^{426}$ | 47626.95 | 47622.91 | / | / | / | / | / |
| $Met^{-12}$-$Met^{425}$ | 47511.86 | 47509.48 | 47512.48 | / | / | / | / |
| $Met^{-12}$-$Lys^{424}$ | 47380.66 | 47378.02 | 47380.73 | 47380.97 | 47381.84 | 47381.13 | / |
| $Met^{-12}$-$Ala^{423}$ | 47252.49 | 47250.54 | 47253.40 | / | / | / | / |
| $Met^{-12}$-$Lys^{401}$ | 44716.60 | / | / | 44716.51 | 44717.25 | / | / |
| $Met^{-12}$-$Trp^{396}$ | 44187.95 | / | / | / | / | 44188.36 | 44189.36 |
| $Met^{-12}$-$Ala^{394}$ | 43904.62 | / | / | / | / | 43901.03 | / |
| $Met^{-12}$-$Lys^{375}$ | 41611.10 | / | / | 41611.06 | 41612.65 | / | / |
| $Met^{-12}$-$Leu^{372}$ | 41326.74 | 41326.18 | 41327.04 | / | / | 41326.99 | 41328.33 |
| $Met^{-12}$-$Lys^{370}$ | 41114.45 | / | / | 41115.33 | / | / | / |

[a] All the products contain a N-terminal ($MRGSH_6GS$) tag.