Table S1. Table of Circular Dichroism Spectra Deconvolution Data.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Enzyme | HsTPI-WT | HsTPI-C217K | HsTPI-N16D | HsTPI-E104D |
|  | Control | +G3P | Control | +G3P | Control | +G3P | Control | +G3P |
| Wavelength | 200-250 nm | 200-250 nm | 200-250 nm | 200-250 nm |
| (Scala-factor) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Helix 1 (regular) | 55.4 | 58.4 | 71.4 | 18.4 | 59.1 | 51.7 | 54.3 | 10 |
| Helix 1 (distorted) | 22.8 | 12.6 | 11 | 14.4 | 21.5 | 22.1 | 19.3 | 6.8 |
| Anti 1 (left-twisted) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anti 2 (left-twisted) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anti 3 (right-twisted) | 14.7 | 15.6 | 12.1 | 0 | 16.2 | 15.9 | 15.2 | 3.6 |
| Parallels | 6.7 | 13.3 | 5.5 | 17.2 | 0 | 1.4 | 9.2 | 18.8 |
| Turns | 0.4 | 0 | 0 | 7 | 3.1 | 3.7 | 2.1 | 8.9 |
| Others | 0 | 0 | 0 | 42.9 | 0 | 6.2 | 0 | 51.9 |
| Helix | 78.2 | 71.1 | 82.4 | 32.8 | 80.6 | 72.8 | 73.5 | 16.8 |
| Antiparallels | 14.7 | 15.6 | 12.1 | 0 | 16.2 | 15.9 | 15.2 | 3.6 |
| Parallels | 6.7 | 13.3 | 5.5 | 17.2 | 0 | 1.4 | 9.2 | 8.9 |
| Turns | 0.4 | 0 | 0 | 7 | 3.1 | 3.7 | 2.1 | 8.9 |
| Others | 0 | 0 | 0 | 42.9 | 0 | 6.2 | 0 | 51.9 |

The percentage changes of the secondary structure components of HsTPI-WT, C217K, N16D and E104D are shown. The spectra were processed with BeStSel Protein Circular Dichroism Spectra Analysis Online Software [59]. The table shows the percentage of the secondary structure obtained for the controls of WT, C217K, and N16D enzymes and plus-G3P 1 mM for 24 h at 37 °C.

Table S2. Table of intrinsic fluorescence parameters of HsTPI-WT, C217K, N16D, and E104D mutants.

|  |  |
| --- | --- |
| *Intrinsic fluorescence* Wavelength (exc.) | HsTPI |
|  WT |  C217K |  N16D |  E104D |
| 280 nm |  | *IFmax* (a.u.) | 225.6 | 215.3 | 207.3 | 155.9 |
|  | max (nm) | 332.5 | 332.5 | 332 | 333 |
|  | SCM (nm) | 346.9 | 347.1 | 350 | 347.45 |
| 295 nm |  | *IFmax* (a.u.) | 64.7 | 63.8 | 52.9 | 47.14 |
|  | max (nm) | 336 | 335 | 335.5 | 336.5 |
|  | SCM (nm) | 350.5 | 350.7 | 356 | 350.5 |



**Figure S1.** Standard curve of fluorescentARGpadduct formation.(**A**)The 20 mM Arg-MGO equimolar reaction was included in the TE buffer. Samples at 1:20 dilution were excited at exc. 325 nm, the signal of *IFmax* was plotted to be obtained from a scanning of em. 340 - 600 nm from 0 to 421 h at 37 °C.(**B**)Michaelis-Menten equation fit of the ARGp formation,plotting the *IFmax* at each time at 395 nm. Curve fitting showed a mean saturation time of 87 h in forming the ARGp adduct. Experimental blanks were subtracted; each spectrum was the mean of three replicate scans.



**Figure S2.** Kinetics of ARGp adduct formation in HsTPI-C217K**.** IFmax changes in HsTPI-C217K induced by G3P or MGO were recorded over a 0-500 h incubation in TE buffer at pH 7.4 at 37 °C. Both samples containing 1 mg/mL of HsTPI-C217K plus-G3P 20 mM or plus-MGO 20 mM were incubated. For each time, 1:20 dilutions were made in the same buffer, and spectrofluorometric readings were performed in 600 L at 25 °C. FI at each time was obtained with 340-600 nm scans after exc. at 325 nm. **(A)** C217K plus-G3P. **(B)** C217K plus-MGO. The black arrows show the wavelength signal at 495 nm at which data were collected to construct the graphs (Figures 6A, 7A).

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**Figure S3.** Analysis of structural effects exerted by G3P on TPIs. **(1A-1B)** Fluorescence emission spectra were obtained for the enzyme controls and plus-G3P 2 mM with  exc at 280 and 295 nm, with readings from 2, 28, 48, 48, 72, and 96 h at 37 °C. The maximum FI was substantially modified in the mutants at each time, with strongly decreased plus-G3P relative to the -WT enzyme. **(2A-2B)** the max was plotted, red-shift was observed at 280 and 295 nm, and the same behaviour was observed in both  exc. This inversion of max differed among C217K, N16D and E104D, being markedly higher than the WT signal **(3A-3B)**. Spectral centres of mass (SCM) showed the far red shift of three mutants against WT; however, it was higher at 295 nm. Enzymes: (WT, black squares); mutants, (C217K, red circles) (N16D, blue triangles) and (E104D, green diamonds); empty squares show incubation controls; filled squares represent enzymes exposed to G3P 2 mM.



**Figure S4.** Analysis of structural effect exerted by MGO on TPIs. **(1A-1B)** Fluorescence of enzymes exposed to MGO 1 mM readings from 2, 28, 48, 72, and 96 h at 37 °C at exc at 280 and 295 nm. The IFmax at exc 280-295 nm was modified in the mutants and decreased to the WT enzyme. **(2A)** The  max shows a red shift at  exc—280 nm. The same behaviour was observed at 295 nm. **(2B)** This inversion of the  max was different in C217K and N16D, being markedly higher than the signal of the WT. **(3A-3B)** SCM showed the far red-shift of both mutants against WT. However, it was higher with excitation at 295 nm. (WT, black squares); mutants, (C217K, red circles) (N16D, blue triangles) and (E104D, green diamonds).



**Figure S5.** Refractory proteolysis in C217K glycated by MGO.In HsTPI-C217K; **A),** the control lanes were loaded with 5 g and protein plus MGO 20 mM and glycated by 500 h at 37°C with 10 g/lane. 16% von Jagow electrophoresis SDS-PAGE was performed to prove the change in the proteolysis pattern. Under the same condition (MGO-free), the digestion pattern is similar in both enzymes WT and C217K. At 500 h, it did not decrease its residual activity against the controls; the enzymes retained their activity (Supplementary Figure S5A and S5C, respectively). However, proteolysis limited of C217K plus MGO showed no residual activity and many resistant aggregates to proteolysis with a sweep on SDS-PAGE observed originating from MGO adducts formation (Supplementary Figure S5C)—a slight disappearance of oligomeric species. Decreasing C217K monomer (27 kDa) increased Mr <monomer fragments. The residual activity was determined after incubation times (0, 30, 65, 130, 180, 210, 240, 270, and 300 min.) at 30 °C. This assay shows that under the same condition, at 5 h of proteolysis, their residual activity did not decrease vs controls. However, control C217K proved to be catalytically competent and showed a residual activity of 4260.4 mol∙min-1∙mg-1 that was similar to WT, but C217K incubated plus-MGO did not show residual activity (Supplementary Figure S5D). C217K (red open circles) and C217K plus-MGO (blue open triangles), WT Ctrol (black open squares).