Electronic Supplementary information (ESI) for:

The catalytic mechanism of glyceraldehyde 3-phosphate dehydrogenase from *Trypanosoma cruzi* elucidated via the QM/MM approach

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1 - Details of the Potential of mean force (PMF).

The procedure for the PMF calculation is straightforward and requires a series of molecular dynamics simulations in which the distinguished reaction coordinate variable, ξ, is constrained around particular values [62]. The values of the variables sampled during the simulations are then pieced together to construct a distribution function from which the PMF is obtained as a function of the distinguished reaction coordinate ($W(\xi)$). The PMF is related to the normalised probability of finding the system at a particular value of the chosen coordinate by equation (1) as follows:

$$W(\xi) = C - kTln\int \rho(r^N)\delta(\xi(r^N) - \xi)dr^N \quad (1)$$

The activation free energy can be then expressed as proposed by Shenter, Garret, and Truhlar (2003) [64] as follows:

$$\Delta G^{\ddagger}(\xi) = W(\xi^{\ddagger}) - \left[W(\xi^R) + G_{\xi}(\xi^R)\right] \qquad (2)$$

where the superscripts indicate the value of the reaction coordinate at the reactants (R) and TS, and $G_{\xi}(\xi^R)$ is the free energy associated with setting the reaction coordinate to a specific value at the reactant state. Normally this last term makes a small contribution [65], and the activation free energy is directly estimated from the PMF change between the maximum of the profile and the reactant's minimum as follows:

$$\Delta G^{\ddagger}(\xi) \approx W(\xi^{\ddagger}) - W(\xi^R) = \Delta W^{\ddagger}(\xi) \qquad (3)$$

The selection of the reaction coordinate is usually trivial when the mechanism can be driven by a single internal coordinate or a simple combination (as the anti-symmetric combination of two interatomic distances). However, this is not the case for the reaction subject of study in this paper where several coordinates are participating. Instead, we were compelled to obtain a much more computationally demanding 2D-PMF using two coordinates: $\xi_1$and ξ_2. The 2D-PMF is related to the probability of finding the system at particular values of these two coordinates as follows:

$$W(\xi_1,\xi_2) = C' - kTln\int \rho(r^N)\delta(\,\xi_1(r^N) - \xi_1)\delta(\xi_2(r^N) - \xi_2)dr^N$$

Figure S1 – AM1/MM Potential energy surface (PES). R1 corresponds to the nucleophilic attack of S atom of the Cys166 on the C1 atom of the aldehydic group of G3P (*trans* conformation) and R2-R3 corresponds to coordinates proton transfer to His194.