

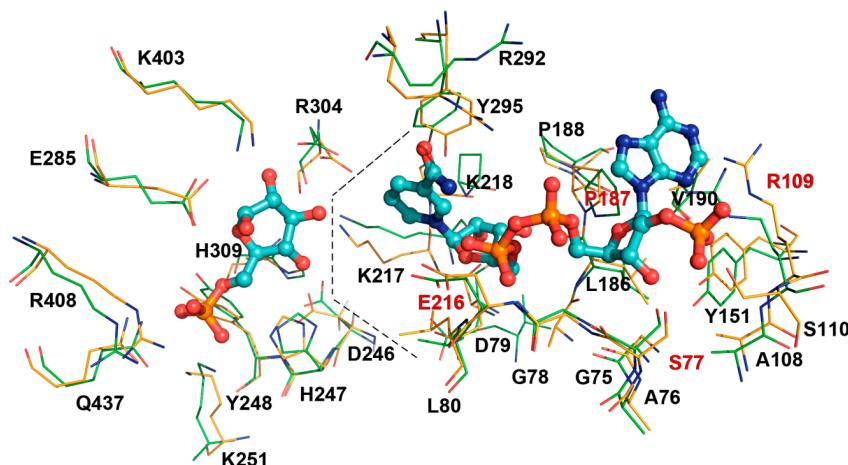
# Supplementary Materials: Binding Mode and Selectivity of Steroids towards Glucose-6-Phosphate Dehydrogenase from the Pathogen *Trypanosoma cruzi*

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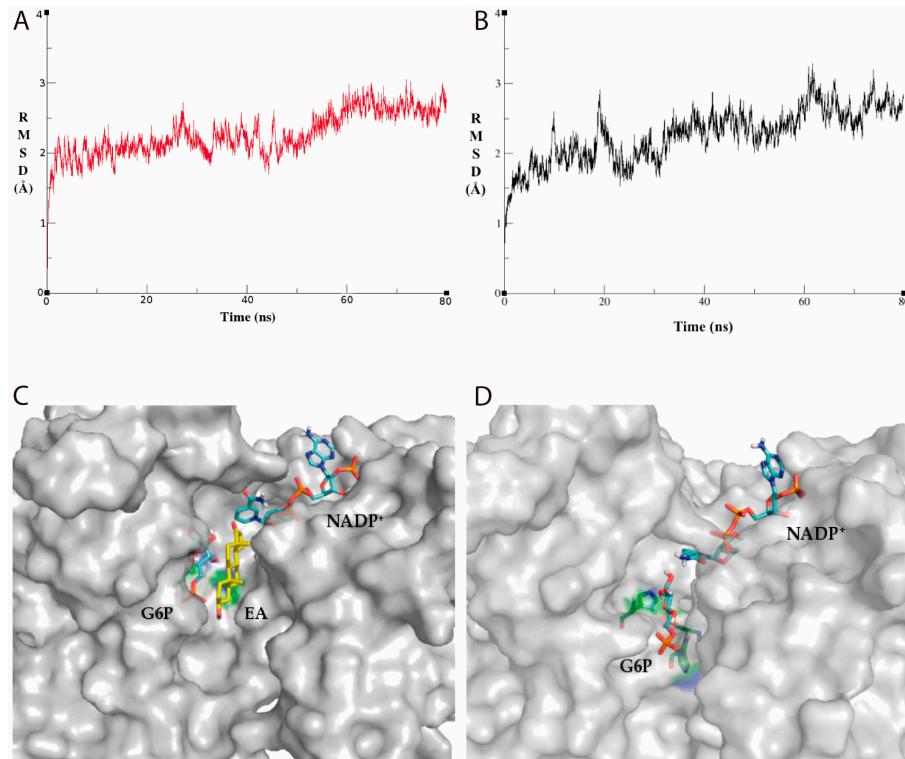
**Table S1.** Binding  $\Delta G$  values (kcal/mol) for different binding poses obtained for the respective inhibitors on the energy minimized ternary complex of TcG6PDH. In bold are highlighted the poses for the corresponding compounds shown in Figures 2, 4 and 5.

Docking Pose	EA	16Br-EA <sup>a</sup>	1	2	3	4
1	<b>-11.458</b>	-6.509	-11.717	<b>-11.096</b>	<b>-11.325</b>	-10.595
2	-9.824	-10.750	-10.236	-7.661	-9.467	-12.191
3	-6.471	0.480	<b>-13.307</b>	-9.205	-6.108	-11.690
4	-7.420	<b>-12.069</b>	<b>-10.058</b>	<b>-14.368</b>	-11.700	<b>-11.344</b>
5	-7.670	-9.013	-9.641	-9.727	-8.224	-5.725
6	-7.767	-8.954	-3.826	-10.398	-9.268	<b>-14.421</b>
7	-8.476	-10.423	-8.357	-7.805	-9.980	-11.010
8	-11.323	-0.652	-2.281	-8.161	<b>-13.918</b>	-4.379
9	-10.838	-3.979	-6.088	-4.704	-9.925	-5.400
10	-9.768	-6.391	-9.500	-4.586	-11.675	-10.757

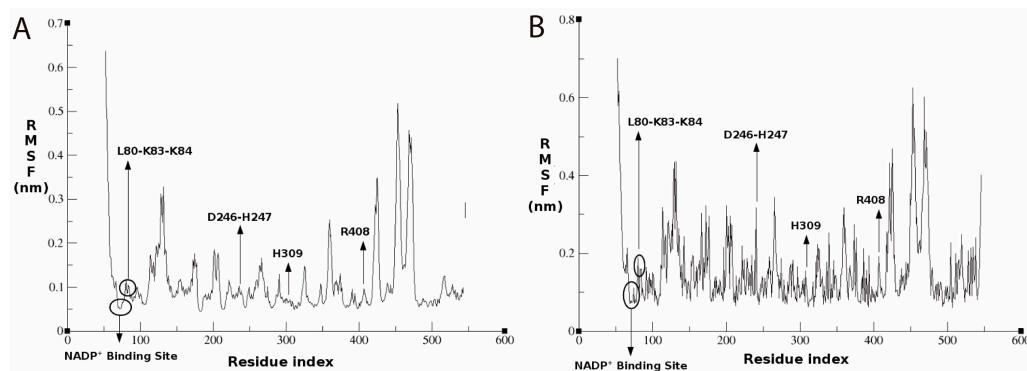
<sup>a</sup> For 16Br-EA was investigated the R-enantiomer.



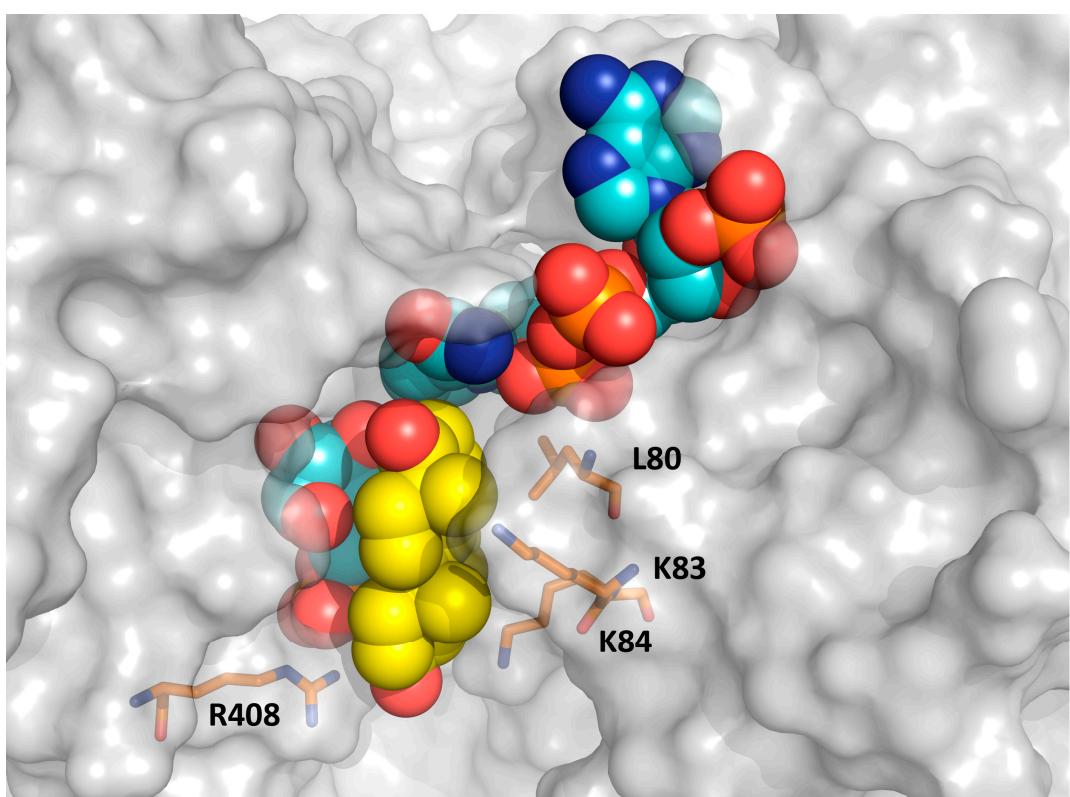
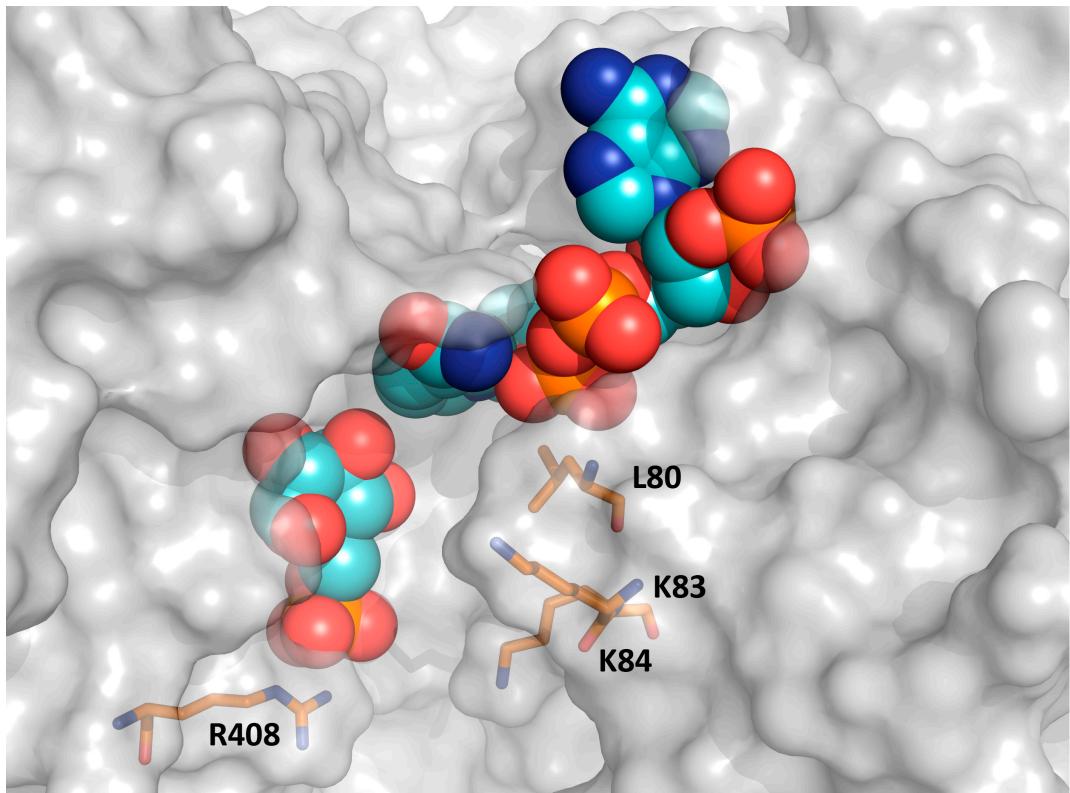
**Figure S1.** Comparison of the substrate binding sites for the crystal structure of *T. cruzi* G6PDH with bound G6P and the corresponding molecular model of the catalytic complex. The residues involved in substrate binding are shown for TcG6PDH with bound G6P (PDB 4EM5, green lines) and for the model of the ternary complex enzyme-G6P-NADP<sup>+</sup> (bright orange lines). The dashed line denotes the separation between the N- (right side) and C-terminal (left side) domain of G6PDH. Residues from PDB 4EM5 interfering with NADP<sup>+</sup> binding are labeled with red fonts (S77, R109, P187 and E216): the OH group from S77 ( $\beta$ 1-loop- $\alpha$ 1) clashes with an oxygen from the phosphate bound at C2' in the nucleotide-adenine moiety, the side chain of R109 ( $\beta$ 2-loop- $\alpha$ 3) clashes with the adenine ring from NADP<sup>+</sup>, P187 ( $\beta$ 4-loop- $\alpha$ 6) clashes with the adenine ring, and the CO from E216 ( $\beta$ 5-loop- $\alpha$ 7) clashes with the OH group from the C2' of NADP<sup>+</sup>. During MD studies, these residues underwent conformational changes that allowed binding of NADP<sup>+</sup>.



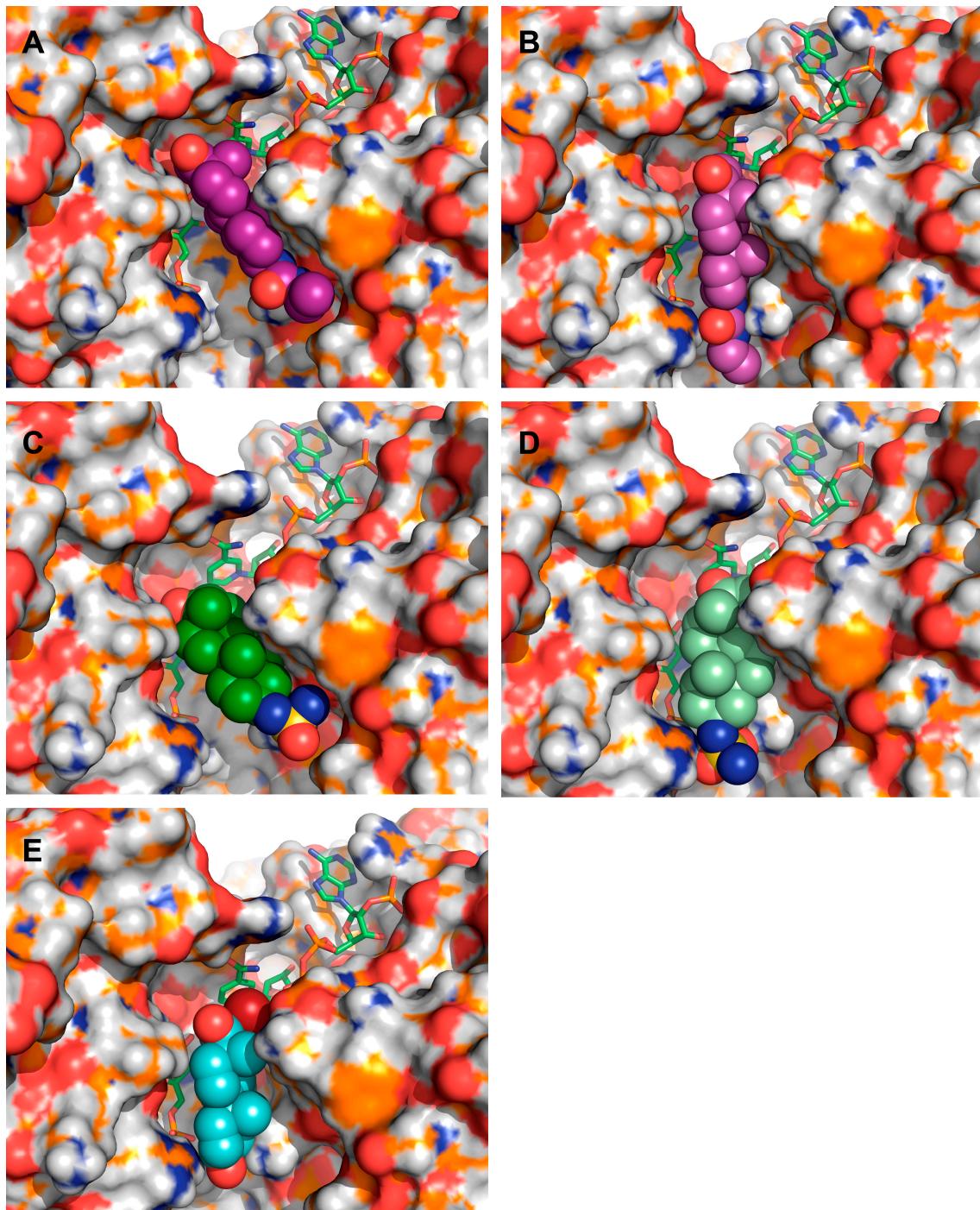
**Figure S2.** Molecular dynamics analysis of *TcG6PDH* in complex with ligands. Root mean square deviation plot over 80 ns MD simulation for (A) the protein-G6P complex and (B) the protein-G6P-NADP<sup>+</sup>-EA complex, where the deviation from the equilibrium observed after 50 ns correspond to the exit of EA from its binding site; (C) EA docking binding pose (yellow stick) in the ternary complex *TcG6PDH*/G6P/NADP<sup>+</sup> (protein surface is depicted in gray and substrates with cyan sticks); (D) Conformational change of the docking complex after 80 ns MD simulation, where EA exits the complex and G6P adopts an orientation not favorable for electron exchange with NADP<sup>+</sup>.



**Figure S3.** RMSF plots for backbone and side chains of the *TcG6PDH*/G6P/NADP<sup>+</sup>/EA complex. The computed root mean square fluctuation plots were obtained for the protein backbone (A) and side chains (B) of the holo-*TcG6PDH* with bound EA. The high peaks (RMSF >0.1 nm for plot A or >0.17 nm for plot B) between residues 110 to 140 and residues 420 to 470 correspond to (loop rich) protein regions not engaged in ligand-binding but exposed to the solvent and participating in subunit interactions, respectively. The fluctuations encompassing the last region will be smoothed if running a simulation of the protein tetramer rather than of a single subunit (in our case chain C), but they are not expected to affect the final results since it is located far enough from the substrates- and inhibitor-binding sites. In the plots are highlighted the positions of the main residues responsible for EA binding and/or involved in catalysis (for details see text).



**Figure S4.** Binding pocket for EA in *TcG6PDH*. Molecular model of the corresponding energy minimized quaternary complex of the enzyme (grey surface) with residues shown as bright orange sticks and substrates depicted as cyan spheres. Model without (A) and with docked EA (B), which is shown with yellow spheres.



**Figure S5.** Binding of  $3\beta$ -,  $16\beta$ - and  $17\beta$ -substituted androstanes to the catalytic complex of *T. cruzi* G6PDH. This figure is a replica of Figure 4 with *TcG6PDH* shown as surface, the substrates as light green sticks and the compounds (lacking hydrogen atoms) as colored spheres. The most stable and alternative poses are shown for (A) **1** (dark purple) and (D) **2** (pale green) and alternative binding conformations for (B) **1** (pale purple) and (C) **2** (dark green); (E) Best docking pose for 16Br-EA, shown as light blue sphere.