Supplemental Data

Isomer activation controls stereospecificity of class I fructose-1,6-bisphosphate aldolases

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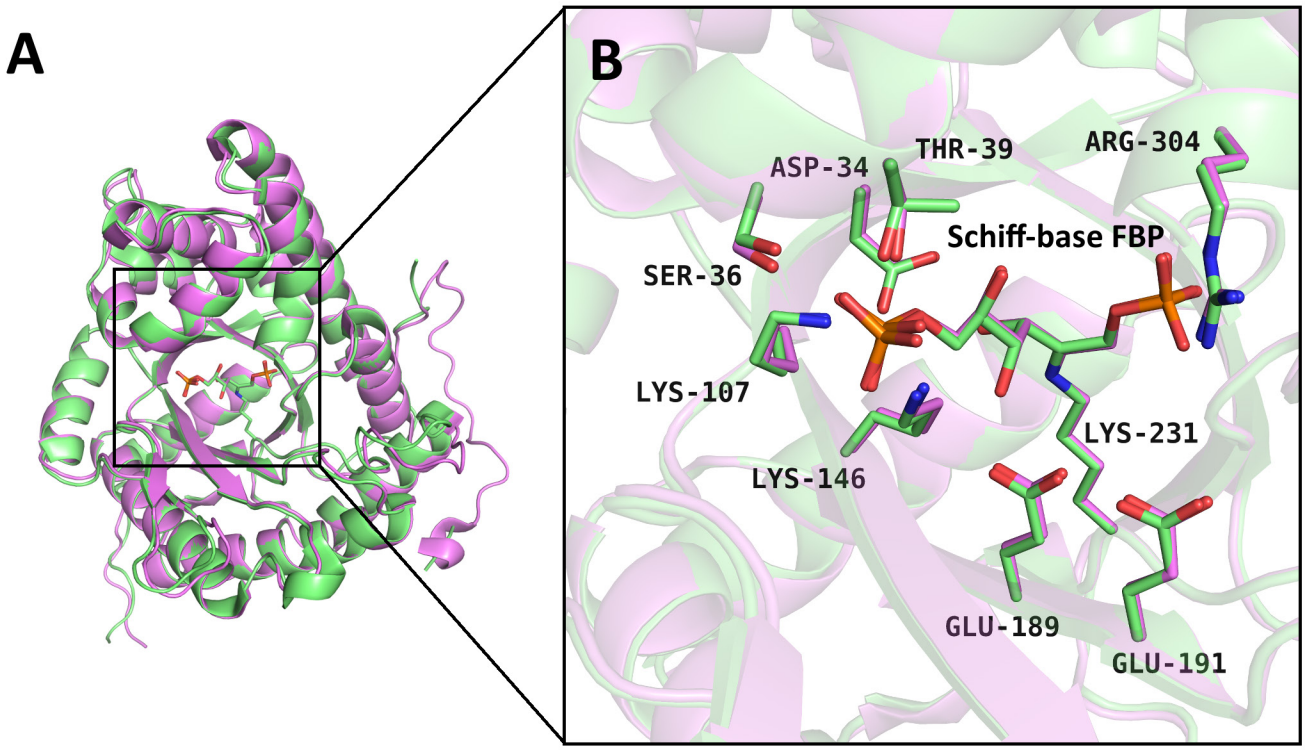

FIGURE S1. Structural comparison of the Schiff-base FBP complexes in TgALD and rabbit muscle aldolase

(*A*) Structural superposition of Schiff-base FBP TgALD (*green*) corresponding to the *30min* structure with Schiff-base FBP from rabbit muscle aldolase (*pink*) (PDB ID: 1ZAI) based on PyMol alignment of equivalent Cα atoms (r.m.s.d. = 0.40 for 273 atoms). (*B*) Active site view of key residues implicated in the reaction mechanism. Lys-231 is responsible for Schiff-base formation with ketone substrates (FBP or DHAP). TgALD Thr-39 corresponds to Ser-38 in rabbit muscle aldolase; all other key active residues are identical. Alignment of active site residues shown (9 residues) based on equivalent Cα atoms yields an r.m.s.d. = 0.14. Numbering shown is based on TgALD sequence. Equivalent residues in rabbit muscle aldolase correspond to: Asp-33, Ser-35, Ser-38, Lys-107, Lys-146, Glu-187, Glu-189, Lys-229, and Arg-303.

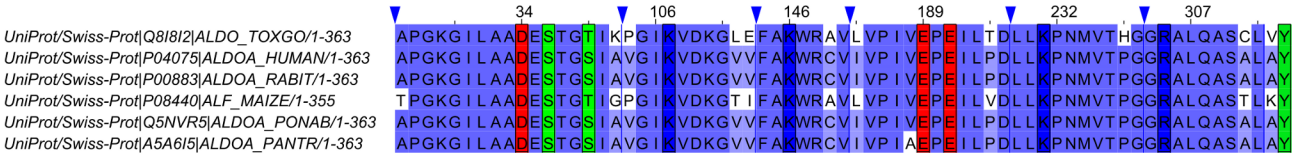

FIGURE S2. Multiple sequence alignment showing active site conservation.

Highlights the extensive conservation of active site residues in TgALD with respect to other class I aldolases (including rabbit muscle aldolase). The blue gradient illustrates the level of similarity, and the different colours correspond to key active site residues (shown in Fig. 1A), colored according to their biochemical properties: *red* - acidic; *green* - polar; *blue* - basic. The numbering above the alignment corresponds to residue positions for TgALD. Alignment was performed in Jalview v.2.10.1 using the Clustal algorithm. The blue arrows are to denote the stretches of contiguous sequences lining the active site that contribute residues implicated in catalysis.

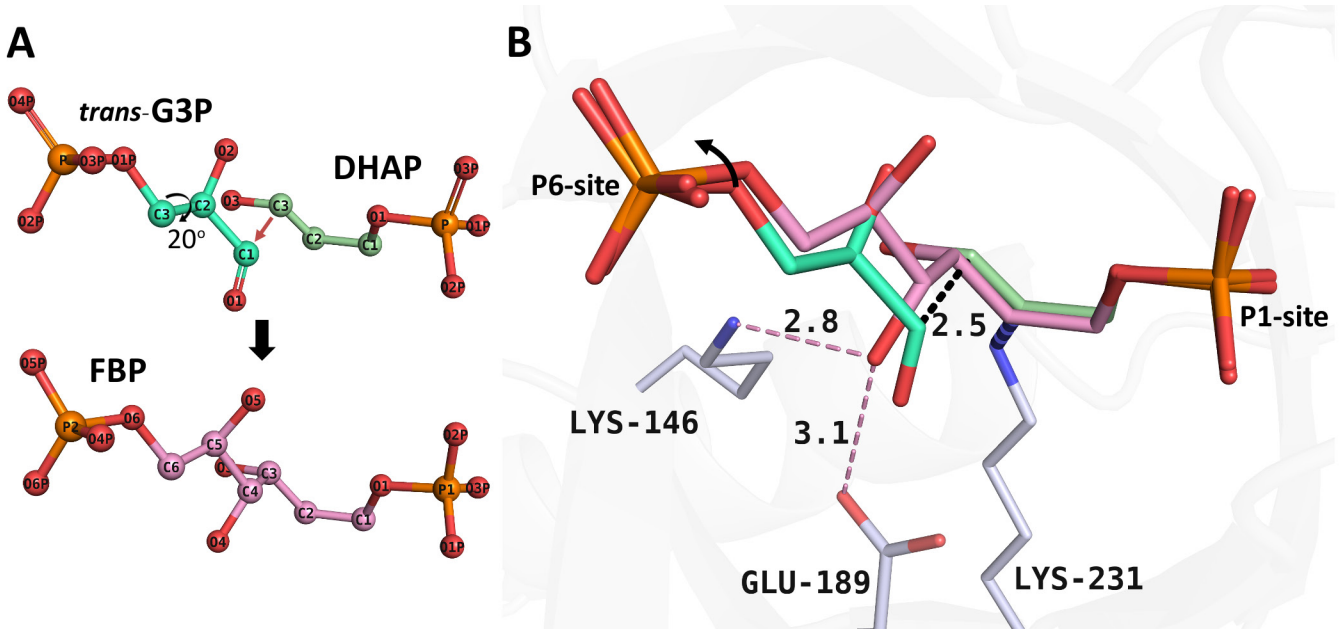

FIGURE S3. Configurational rearrangements during aldol addition.

(*A*) Panel showing nucleophilic attack on the electrophilic C1 carbonyl atom by the DHAP C3 carbanion. The modeled trans-configuration (*cyan*) of D-G3P is shown with the appropriate torsion angle at the C2-C3 bond enabling aldol addition with the DHAP-enamine to form FBP (*pink*). (*B*) The *1min* and *30min* structures were superposed (alignment of Cα atoms) to illustrate the conformational change that occurs during C3-C4 bond formation, and is consistent with least atomic motion. Binding by the phosphate groups of D-G3P and FBP at the P6-site is isostructural and the trajectory requires a slight hinge rotation perpendicular to the viewing plane (~ 10°) about the D-G3P phosphate to superpose D-G3P atoms C1, C2, and C3 with FBP atoms C4, C5, and C6. Atoms in the DHAP enamine are essentially identically positioned as equivalent FBP atoms (r.m.s.d. = 0.20 Å). The distance between DHAP C3 and D-G3P C1 prior to addition is 2.5 Å (*black dashes*). The nascent C4-hydroxyl group is stabilized by a short hydrogen bend with Glu-189 and by charged hydrogen-bonding with Lys-146 (*pink dashes*).

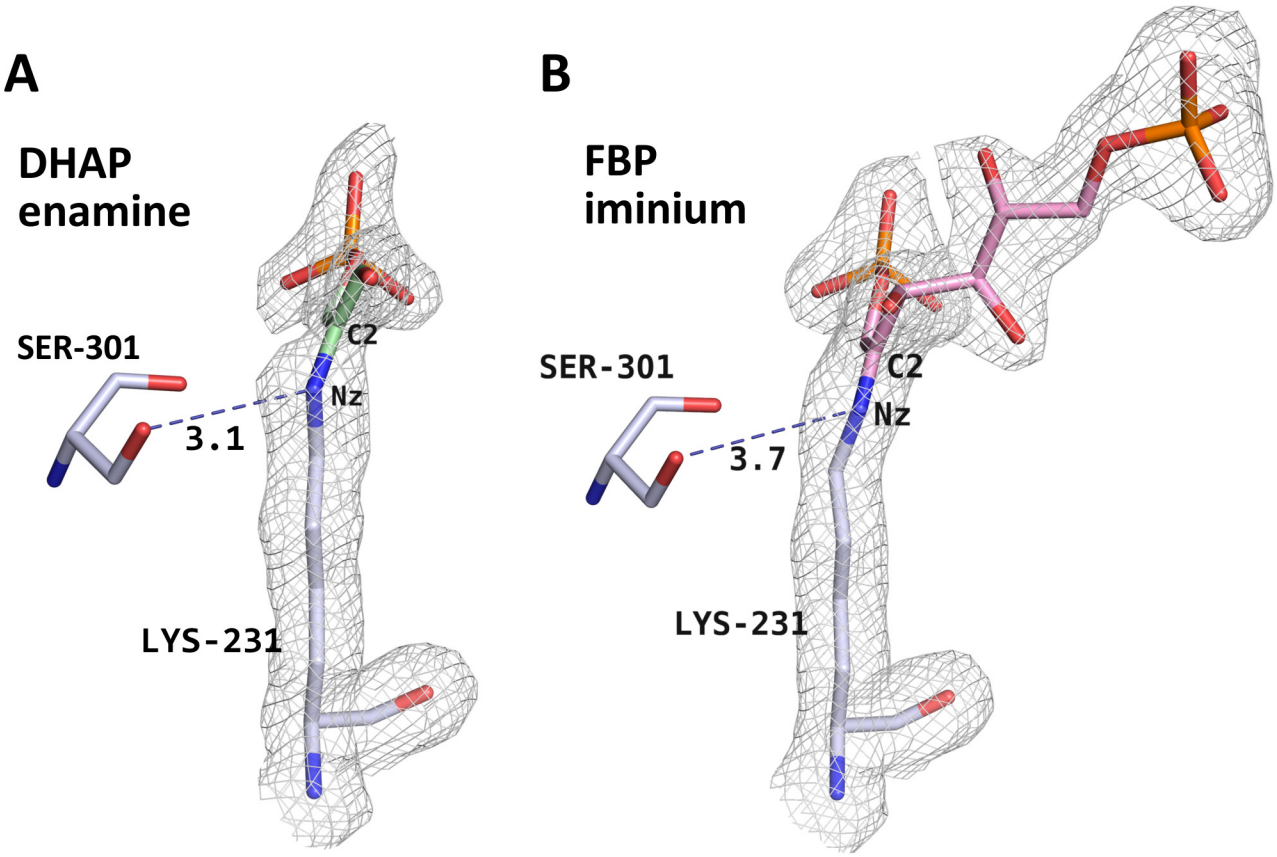

FIGURE S4. Discrimination of enzymatic intermediates formed with FBP in *T. gondii* aldolase active site.

Geometry of enzymatic intermediates was used to identify the chemical identity of the trapped intermediate-aldolase complex. (*A*) DHAP-enamine intermediate (green) was identified in the *0.5min*, *1min* and *2min* structures of native TgALD soaked with substrate. The observed hydrogen bond formation (*blue dash* – 3.1 Å) between the Ser-301 hydroxyl and Lys-231 Nζ is consistent with *sp3* hybridization of Lys-231 Nζ in the enamine intermediate. Planar stereochemistry is observed about the DHAP C2 atom, consistent with enamine formation. Structure shown illustrates the *1min* structure. (*B*) The iminium FBP intermediate was identified in the *10min* and *30min* structures corresponding to the Schiff base complex with FBP (pink). *sp2* hybridization of Lys-231 Nζ would preclude hydrogen bond formation with Ser-301 (*blue dash* – 3.7 Å) and is indicated by the planarity of the electron density about the Nζ atom. Structure shown illustrates the *10min* structure. Both maps depict difference electron density maps calculated from simulated annealing $F_o - F_c$ omit maps encompassing Lys-231 and bound substrate and contoured at 3σ.