Structural snapshots of *Mycobacterium tuberculosis* enolase during the reverse reaction reveal dual mode of 2PG binding and its implication in enzyme catalysis

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Enolase is a ubiquitous enzyme found in the glycolytic pathway of organisms of all three domains of life and is involved in the catalysis of the reversible conversion of 2-Phosphoglycerate (2-PG) to Phosphoenolpyruvate (PEP). It plays an important role in *Mycobacterium tuberculosis* (*Mtb*) virulence by acting as cell surface receptor of human plasminogen. Enolase in *Mtb* stands out from most of its homologs by being catalytically active only in an octameric state. While the forward reaction is well understood, not much is known about how the catalytic conversion of PEP to 2PG takes place. Here we present structural snapshots of *Mtb* enolase (MtENO) at various stages as it progresses through the reverse reaction. We found a plausible catalytic pathway involving a novel transient product bound state called ‘alternate conformation’, in addition to the canonical one. We observed two major deviations from the forward reaction: presence of Mg⁸⁶ is non-obligatory for the reaction and flipping of 2PG to an alternate conformation makes it energetically feasible to exit the site. Molecular dynamics and free energy calculation further indicate that alternate conformation may act as exit conformation and facilitate the opening of active site loops due to distortion in metal ion coordination and H-bond interactions. Additionally, P-P docking and simulation study of enolase and plasminogen helps us to understand the molecular interaction of the complex.

![Figure 1](image-url)

**Figure. 1.** (A) CryoEM structure of apo enolase (B) FoFc map at 3σ of novel alternate conformation of 2PG.

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