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Enhanced Sampling Simulations Shed Light on the Regulatory Role of the C-terminal Tail of Phosphofructokinase-1

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The key regulatory enzyme in glycolysis, phosphofructokinase-1 (PFK1), catalyzes the phosphorylation of fructose 6-phosphate (F6P) to fructose 1,6-bisphosphate (FBP). Recent studies have provided structural details of the human enzyme in its active (R-state) and inactive (T-state) forms, suggesting that the C-terminal tail (CTT) is a critical modulator of its conformational equilibrium. Using enhanced-sampling molecular dynamics simulations, we investigated the stability of the CTT binding and showed that its interaction within the binding groove on the enzyme surface is significantly weaker in the active state. Key stabilizing interactions were identified, supporting the role of the CTT as a bridge between the catalytic and regulatory domains in the inactive state. Our analysis revealed that residues 765-767 form an essential region that maintains stable CTT binding in the T-state, facilitated by hydrogen bonds with the catalytic domain and salt bridges with the regulatory domain, ensuring domain connectivity. Our findings shed new light on the regulatory mechanisms and functional dynamics of PFK1.