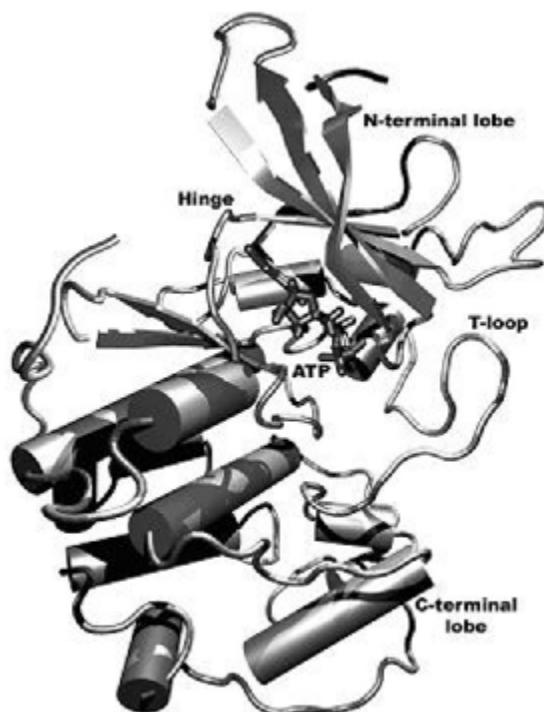


CYCLIN-DEPENDENT PROTEIN KINASE-2 REGULATION BY PHOSPHORYLATION, A MOLECULAR DYNAMICS STUDY

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Cyclin-dependent kinases (CDKs) are enzymes controlling the eukaryotic cell cycle. CDKs activity is regulated by complex mechanism that includes binding to positive regulatory subunit and phosphorylation at positive and/or negative regulatory sites [1]. CDK2 requires for activation binding to cyclinA or cyclinE. CDK2 obtains full activity by phosphorylation of the Thr160 residue in the activation segment (T-loop) [2]. CDKs activity is natively inhibited in several ways, for example, by (de)phosphorylation, interactions with various natural protein inhibitors [3], *etc.* CDK2 can be negatively regulated by phosphorylation



at Tyr15 and (theoretically) at Thr14 [4]. Human CDK2 contains the classical bi-lobal kinase fold [1]. The N-terminal domain is composed mainly of β -sheet, containing five anti-parallel β -strands, and one helix (the C-helix). The larger C-terminal domain is predominantly α -helical, and is linked to the N-terminal domain by a flexible hinge (see picture). The adenosine triphosphate is natural substrate of the CDK2. The ATP-binding site is located in the deep cleft between two lobes. The primary function of CDK is to catalyze the phosphoryl transfer of the γ -phosphate of ATP to the hydroxyl of a serine or threonine residues in the protein target substrate [1]. This study compares behavior of monomeric CDK2, CDK2/cyclinA complex, and pT160-CDK2/cyclinA complex (CDK2/cyclinA complex phosphorylated at Thr160) using the molecular dynamics simulations with the Cornell *et al.* force field as implemented in the AMBER software package [5]. The inhibited forms of CDK2 were also studied. The complexes pT14,pT160-CDK2/cyclinA and pY15,pT160-CDK2/cyclinA were prepared by phosphorylation of the pT160-CDK2/cyclinA complex at Thr14 or Tyr15 residues in the Gly-rich loop (residues 11–18). Activation and inhibition of CDK2 by phosphorylation involves various conformational changes, including the reorientation of the phosphate part of the ATP and key residues involved in the ATP binding site. Conformational change of the ATP phosphate in the pT160-CDK2/cyclinA complex is important to form substrate binding site, and is thought to be critical for catalysis. This study is aimed at a detailed description of the molecular mechanism of CDK2 activation and deactivation by phosphorylation on the activatory and inhibitory sites. Results of conformational behavior of ATP, T-loop, Gly-rich loop and key residues will be presented.

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