# Supplement A2

## Grk2-specific variations in the CLT-inter-lobe interface and the role of CLT in PKB and PDK1 functions

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### Modulation of the αC-β4 loop-CLT interface in GRK2 by the RH domain

The AGC kinase GRK2 lacks some of the canonical interactions in the CLT-inter-lobe interface and thus appears to utilize an alternative mechanism that could involve a regulatory RGS homology (RH) domain, within which the GRK2 kinase domain and C-tail are inserted (1). In a crystal structure of GRK2 containing all of these regions, the N- and C-terminal ends of the split RH domain interact with the CLT and αC-β4 loop regions, respectively, and were proposed to modulate inter-lobe and C-helix movement (1).

*N-terminal end of the split RH domain*. In the GRK2 crystal structure the inter-lobe region assumes an unusual conformational state: the relative orientation of the N- and C-lobes is intermediate between a fully open inactive state and a closed active state (1). Notably, the relative orientation of the N- and C-lobes of Src kinase regulates catalytic activity and is controlled by the Src SH2 domain, which is structurally analogous to the N-terminal end of the RH domain (1). This end of the RH domain interacts with residues flanking the CLT basic residue position, which in GRK2 corresponds to a glutamine ($Q464^{GRK2}$ in Fig A2-E). This glutamine, unlike the CLT basic residue, lacks a canonical CH-π interaction with the histidine in the β8 strand ($H517^{PKC}$ in Fig A2-B; $H330^{GRK2}$ in Fig A2-E). Perhaps GRK2's unusual conformational state is due to these variant RH domain-associated interactions.

*C-terminal end of the split RH domain*. The C-terminal end of the split RH domain is structurally analogous to the SH2-kinase linker in Src kinases that regulate activity by controlling C-helix movement (2, 3). Thus this region of the GRK2 RH domain might regulate kinase activity in a similar manner and, indeed, it harbors an arginine ($R516^{GRK2}$ in Fig A2-E) that hydrogen bonds to the backbone of a serine conserved within the αC-β4 loop of GRK2 but not other kinases (not shown) and that was proposed to play a role in positioning the regulatory C-helix (1). This arginine also forms a hydrogen bond to an aspartate ($D272^{GRK2}$ in Fig A2-E) that is conserved within the GRK2 kinase domain. Other AGC kinases and, for that matter, other EPKs in general lack this aspartate and instead conserve a glutamate ($E459^{PKC}$ in Fig A2-B; $E121^{PKA}$ in Fig A2-C) that forms a salt bridge interaction with a conserved lysine within the β8 strand ($K519^{PKC}$ in Fig A2-B; $Q181^{PKA}$ in Fig A2-C). This lysine is absent from GRK2, which instead conserves at this position an arginine ($R332^{GRK2}$ in Fig A2-E) that, nevertheless, is still capable of forming a salt bridge with the GRK2 aspartate ($D272^{GRK2}$). Within this structural conformational state, however, GRK2 also lacks the canonical salt bridge interaction between these two residue positions. Taken together, this suggests that the C-terminal end of the RH domain regulates GRK2 kinase activity by forming these interactions and thereby disrupting both the EPK salt bridge interaction and critical interactions involving the αC-β4 loop, which is associated with C-helix movements. The atypical GRK2 salt bridge residues may have evolved to accommodate this alternative mode of regulation by the RH domain.

## Potential phosphorylation site in the CLT region of PDK1

The relative orientation of the two lobes is in an intermediate state in PDK1, which also contains a glutamine at the CLT basic residue position ($Q353^{PDK1}$ in Fig A2-F). This glutamine in the PDK1 crystal structure is pointing away from the inter-lobe region (Fig A2-F) and might be positioned by binding of regulatory proteins to the CLT. Notably, the CLT region in PDK1 contains a PDK1 specific "TP" motif ($T354^{PDK1}$ and $P355^{PDK1}$ in Fig A2-F), which is a potential phosphorylation site for a proline-directed kinase. Notably, in patients with colorectal cancer, the TP motif threonine ($T354^{PDK1}$) is mutated to a methionine, suggesting a regulatory role for the CLT region in PDK1 functions (4).

## The CLT region is an interaction site for Smad3 in PKB

A tryptophan at the beginning of the CLT (W639$^{PKC}$ in Fig A2-B and W302$^{PKA}$ in Fig A2-C), which in PKB (W414$^{PKB}$ in Fig A2-C) is important for interaction with Smad3 (5). This tryptophan, like the PxxP motif, is also not solvent exposed for interaction with Smad3 (or similar regulatory factors in other AGC kinases), but instead is buried in the hydrophobic interface formed between the CLT and inter-lobe regions (Fig A2). Thus for Smad3 to interact with the CLT of PKB, one would expect conformational changes in the CLT region.

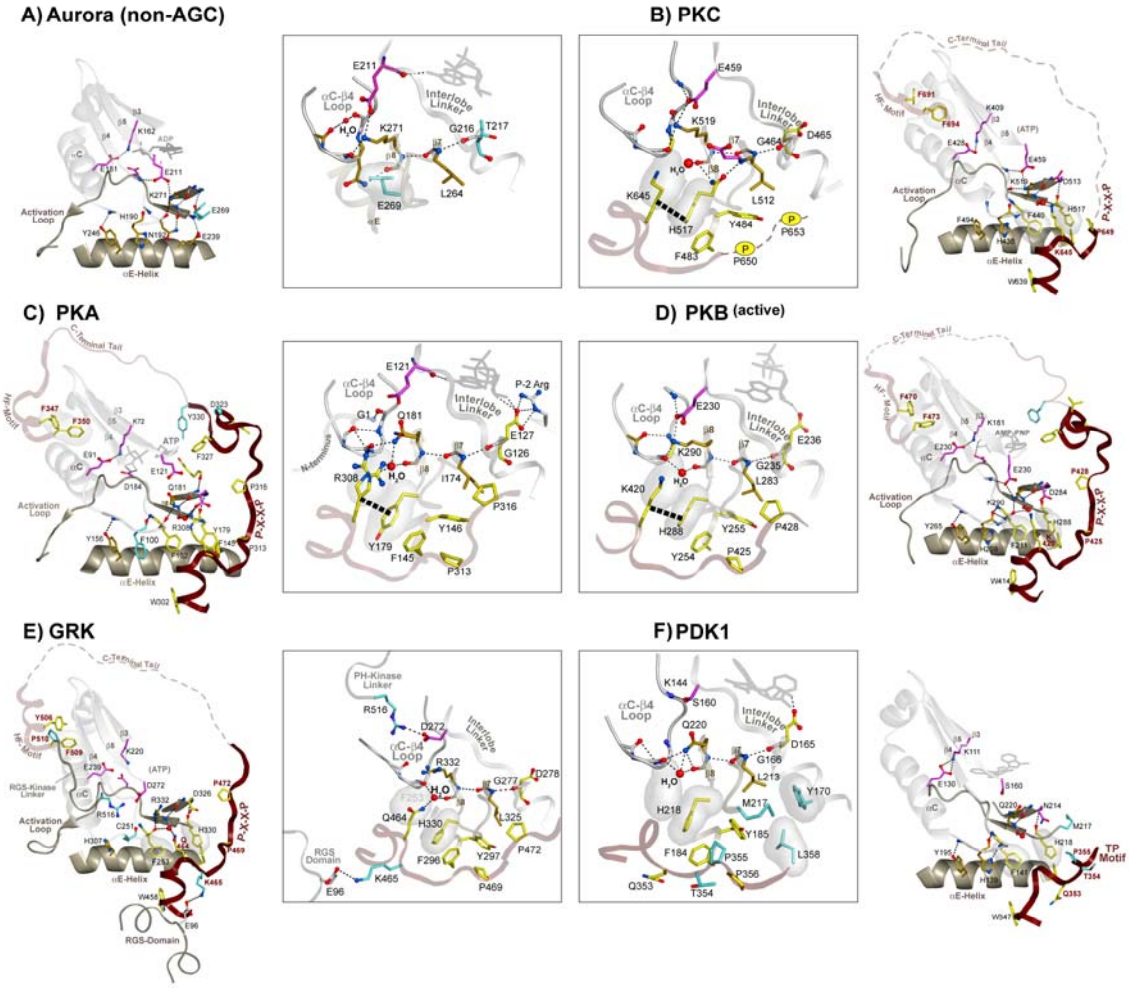

**Figure A2.** Conserved interactions between the CLT and the inter-lobe region. A) Aurora kinase (non-AGC) B-F) AGC kinases. The disordered regions in the C-terminal tail are shown by dotted lines. E) In GRK, the RGS domain and its interaction with the catalytic domain and C-terminal tail are shown. In PDK2 (Fig A2-F), the residue corresponding to the GRK-lysine ($K465^{GRK}$) in the Ct-tail is a threonine ($T354^{PDK1}$), which gets mutated to a metheonine in colorectal cancer (4). The PxxP proline in PKC (Fig A2-B) are disordered and are shown by yellow circles. The main chain is shown by dotted lines. The water molecule is labeled as $H_2O$.

## References


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