BioTechniques Rapid Dispatches

Supplementary Material For:

Tools to discriminate between targets of CK2 vs PLK2/PLK3 acidophilic kinases

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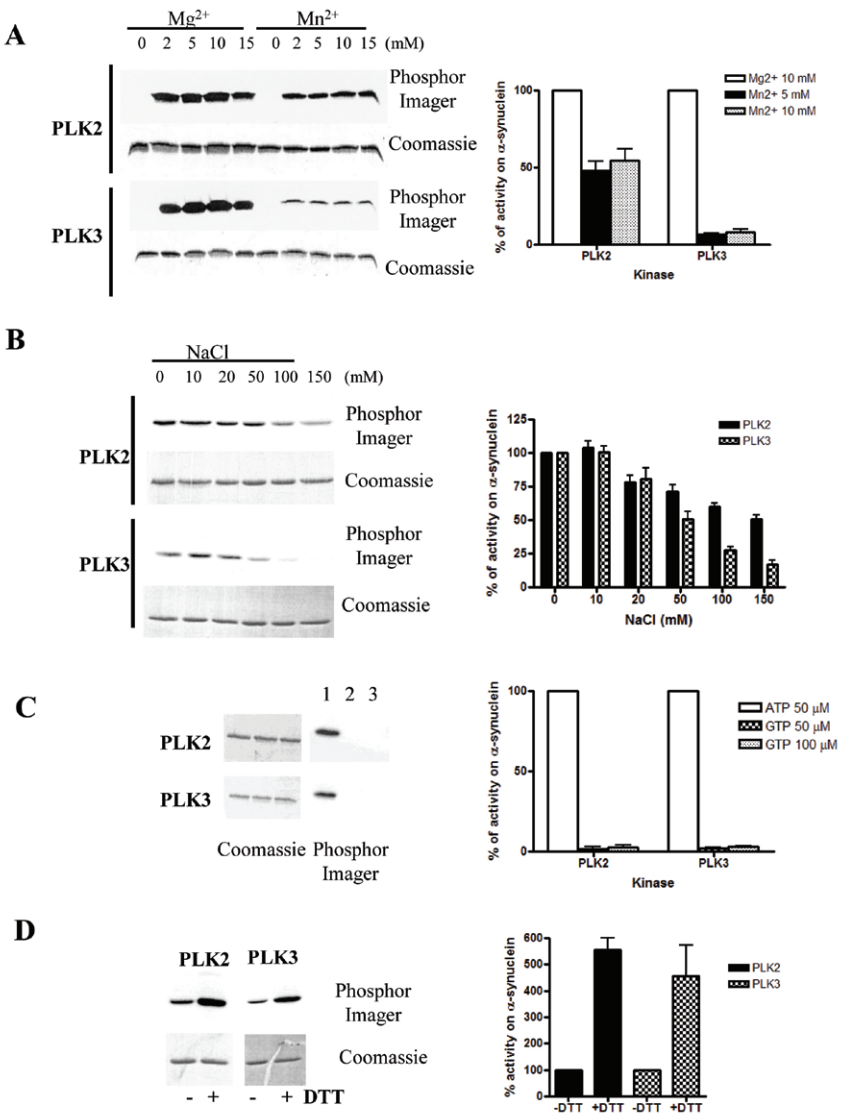

Figure S1. Characterization of PLK2 and PLK3 kinase activity on a-synuclein. (A-B) 0.3 mg of a-synuclein (~K_M for PLK, not shown) was phosphorylated by PLK2 or PLK3 in a radioactive mixture (50 mM Tris, pH 7.5, 50 mM (γ-^{33}P) ATP, specific radioactivity ~3000 cpm/pmol), in the presence or absence of increasing concentrations of Mg^{2+}, Mn^{2+} (A) and NaCl (B) as indicated. Proteins were resolved by SDS/PAGE, Coomassie stained, and analyzed by PhosphorImager. A representative picture of three independent experiments is shown. On the right is reported the effect on α-synuclein activity with 5 and 10 mM Mn^{2+} as a percentage with respect to the activity measured with 10 mM Mg^{2+} (A) and NaCl effect as a percentage with respect to the activity measured in its absence (B) (mean values ±SD of three determinations are shown). (C) Phosphorylation of α-synuclein by PLK2, PLK3, and CK2 in the presence of ATP or GTP. 0.3 mg of α-synuclein was phosphorylated by PLK2, PLK3, or CK2 in 50 mM Tris (pH 7.5), 10 mM Mg^{2+}, 50 mM (γ-^{33}P)ATP (specific radioactivity ~3000 cpm/pmol) (lane 1), or 50 (lane 2) or 100 mM (lane 3) (γ-^{33}P)GTP (with the same specific radioactivity) (mean values ±SD of three determinations are shown). (D) 0.3 mg of α-synuclein was phosphorylated by PLK2 or PLK3 in a radioactive mixture (50 mM Tris, pH 7.5, 10 mM Mg^{2+}, 50 mM (γ-33P)ATP, specific radioactivity ~3000 cpm/pmol) in the presence or absence of 1 mM DTT. Proteins were resolved by SDS/PAGE, Coomassie stained, and analyzed by PhosphorImager. A representative picture of three independent experiments is shown. On the right, the effect of DTT is quantified (mean values ±SD of three determinations are shown). Recombinant α-synuclein production was described in Negro et al. (1)

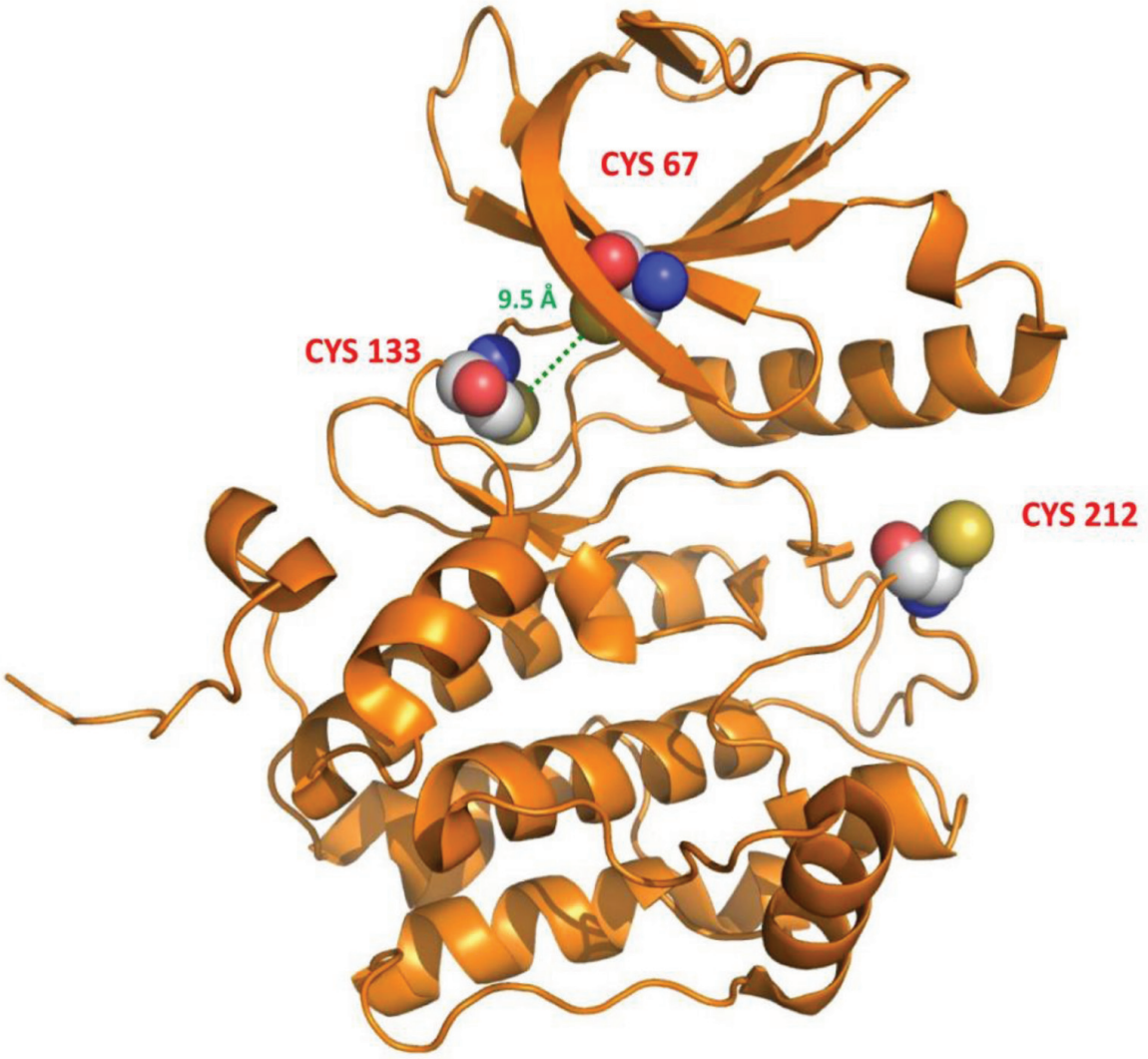

Figure S2. Crystal structure of PLK1 protein kinase showing the conserved cysteines. From the crystal structure of PLK1 (PDB ID: 2OU7), the distance between Cys 67 and Cys 133 is 9.5 Å. However, the high mobility of the ATP binding cleft could push the two residues close each other to form an internal disulfide bond. On the other hand, the cysteine located in the activation loop is water exposed and could promote the formation of dimers under oxidation conditions.

References

1. **Negro A., A.M. Brunati, A. Donella-Deana, M.L. Massimino, and L.A. Pinna.** 2002. Multiple phosphorylation of alpha-synuclein by protein tyrosine kinase Syk prevents eosin-induced aggregation FASEB J. *16*:210-2.