Structural Characterization of Hypoxia Inducible Factor α - Prolyl Hydroxylase Domain 2 interaction through MD Simulations

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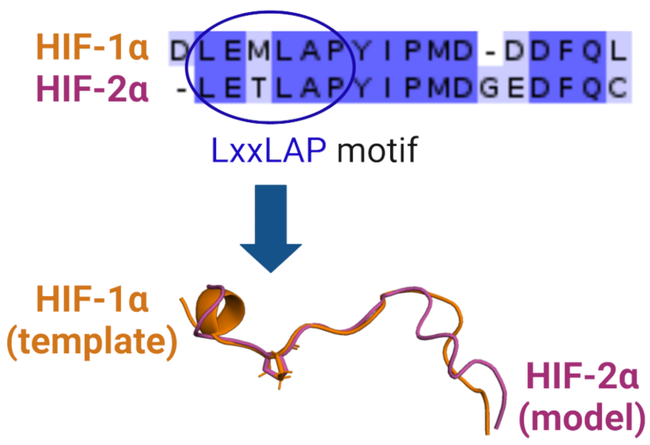
INTRODUCTION

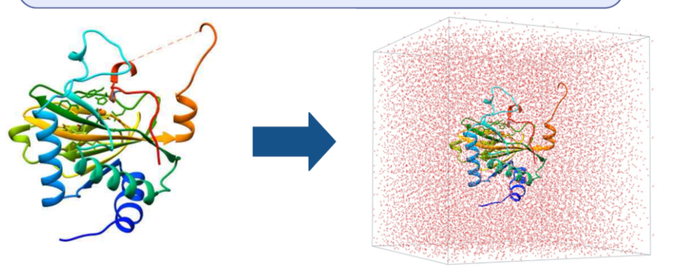
The Prolyl Hydroxylases (PHDs) are an enzymatic family, regulating the cell oxygen-sensing. Three PHDs enzymes isoforms exist in human, i.e. PHD1-3, coded by three different genes. All isoforms hydroxylate HIF-α (HIF-1,2,3α), however, have different affinities and they are thought to have a variable and cell-dependent impact on tumor progression. **What determines these differences and how they pair with tumor growth is poorly understood**. Here, molecular dynamics simulations were used to identify and characterize the PHD2 binding property in complexes with HIF-1α and HIF-2α.
We also characterized the role of the phospho-Thr405 (TPO) localizing on the PHD2 C-terminus in acting as a molecular switch. In parallel, conservation analysis and binding free energy calculations were performed for all complexes to better understand PHD2 substrate affinity.

METHODS

Homology Modeling



Molecular Dynamics Simulations



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CHARMM36 ff

EM: steepest descent algorithm

NVT: 2000ps (T=300K)
NPT: 2000ps
MD: 1μs

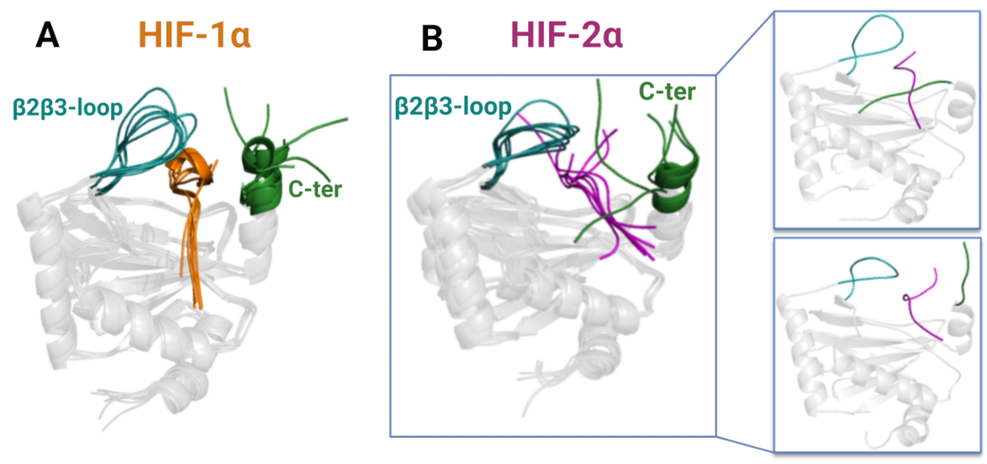
CONCLUSION

Our findings suggest that the PHD2 C-terminus may potentially act as a molecular regulator of PHDs activity. Further, the presence of a phororylation site in this region is suggestive of a fine regulative switch. Our data show that specific conserved residues allow the enzimatic discrimination between different substrates, suggesting that mutations in these sites emerging in cancer may interfer with the binding of a single substrate, ideally conferring an adaptative advantage rather than compromising the entire PHD2 enzymatic activity.

RESULTS

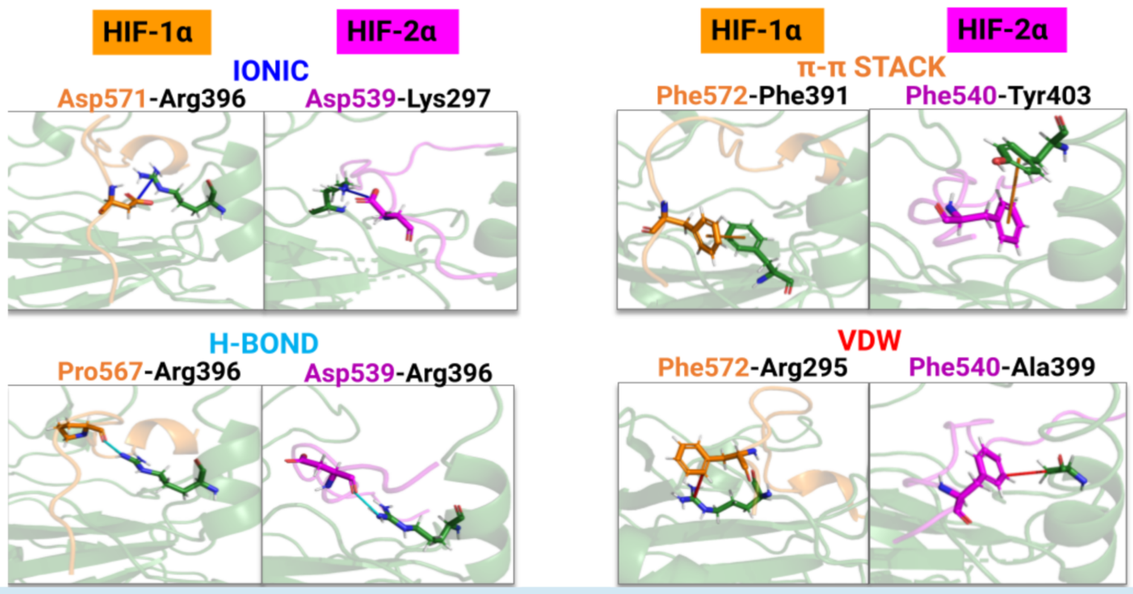
PHD2/HIF-1α v.s. PHD2/HIF-2α

Cluster Analysis



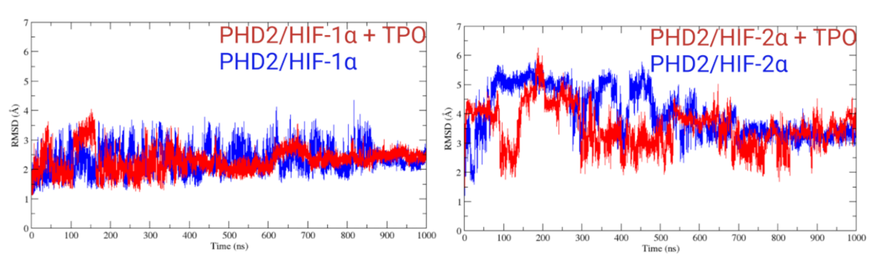
The cluster's main conformations that the protein assumes during the simulations were identified.
(A) PHD2/HIF-1α cluster.
As expected, the regions with the most significant movement are the disordered regions β2β3-loop and C-terminus. The complex remains stable throughout the entire simulation.
(B) PHD2/HIF-2α cluster.
The protein mainly assumes two conformations: one in which the C-terminus interacts with the substrate and the other in which it does not, as a PHD2/HIF-1α complex suggesting a role of the C-terminus in the binding process.

Interaction Analysis

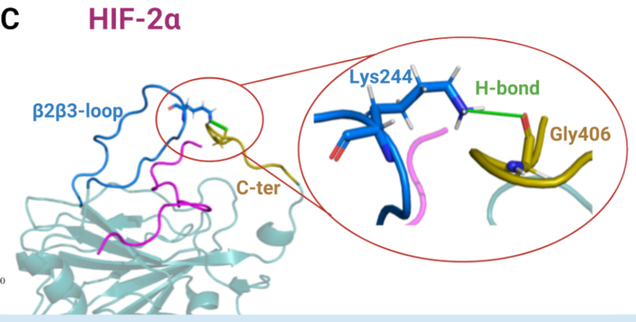


PHD2/HIF-1α v.s. PHD2/HIF-2α *Thr405-P (TPO)*

RMSD Analysis



Interaction Analysis



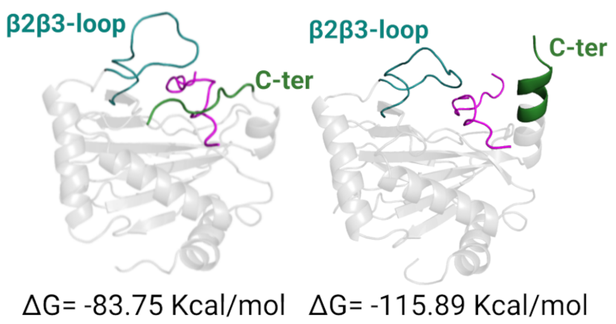
From the RMSD analysis (A),(B), it is observed that the phosphorylation on threonine residue (red) has a modest effect on the stability of both complexes compared to when it is not present (blue).
(C) However, in PHD2/HIF-2a complex, the C-terminus interacts with the β2β3-loop, closing the binding pocket but not interacting with the substrate.
This data is not observed in the PHD2/HIF-1α complex.

BINDING ENERGY (ΔG)

A

HIF-1α
$\Delta G_{average}$= -115.13 Kcal/mol
HIF-1α + TPO
$\Delta G_{average}$= -117.73 Kcal/mol
HIF-2α
$\Delta G_{average}$= -90.63 Kcal/mol
HIF-2α + TPO
$\Delta G_{average}$= -82.24 Kcal/mol

B HIF-2α



ΔG= -83.75 Kcal/mol ΔG= -115.89 Kcal/mol

All complexes' binding free energy was calculated to better understand the PHD2 substrate affinity.
(A) PHD2 has a higher affinity for HIF-1α, as indicated in the literature. TPO is causative of a variation in binding energy, albeit this PTM has only a limited structural impact on PHD2/HIFs-α complexes.
(B) it was observed that the ΔG value is more positive when the C-terminus interacts with HIF-2α and β2β3-loop indicating that the closure of the binding pocket destabilizes the complex.