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Selective allostery perturbation reveals the structural mechanism of the negative regulation for hypoxia inducible factor-1α

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The unique abilities of intrinsically disordered proteins make them precise regulators of cellular processes. The interaction between the transcription factor HIF-1α and the coactivator proteins p300/CBP plays an essential role in the fast response to low oxygenation through the transcription of numerous adaptive genes associated with increased angiogenesis and glycolysis among others. Previous histological studies linked the elevated levels of HIF-1α in various types of malignancies to poor prognosis and high tumor resistance which makes HIF-1α a desirable target in cancer therapy [1]. The negative feedback regulator, CITED2, switches off the hypoxic response through a very efficient irreversible mechanism. When CITED2 binds to the HIF-1α–p300/CBP complex forming a ternary system its binding motifs induce allosteric structural changes in p300/CBP which locks it in the CITED2 bound conformation displacing HIF-1α and rendering the competition unidirectional [2]. Assessing the role of these binding motifs is invaluable to understanding the competition mechanism in molecular detail. Our strategy is to apply modifications to CITED2 that do not significantly affect the thermodynamics of p300 binding. This way, the contribution of a sequence motif to maintain high binding affinity can be decoupled from its ability to induce allosteric changes in p300/CBP, thus, the structural change is directly linked to the competition efficiency. We introduce α → β^3 amino acid replacements motif-by-motif with the goal to maintain native-like properties. Several CITED2 derivatives are synthetized, and their binding properties and competition efficiency are characterized using biophysical assays (eg. ITC, FA) and NMR measurements. Our data shows how the different binding motifs contribute to the allosteric changes necessary for unidirectional displacement of HIF-1α. Based on these results we propose a model which explains the unidirectional nature of the competition, in which the N-terminal tail of CITED2 plays the most important role. Without this sequence motif HIF-1α cannot be displaced in an irreversible manner. The motif-by-motif backbone modification approach can be advantageous for evaluating the roles of different binding motifs in other intrinsically disordered proteins in the future.

References:

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