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## Structural insights into the hYAP-hTEAD4 protein-protein interaction: an emerging target in cancer treatment

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The Hippo pathway is a signaling network, regulating cell growth, proliferation, and apoptosis, involved in tissue homeostasis and organ size control. A central role in this pathway is played by the Yes-Associated Protein (YAP), a DNA transcription co-activator without an intrinsic DNA binding domain. Upon activation of the Hippo pathway, a core kinase-cascade mediates the intracellular signaling, leading to phosphorylation and subsequent degradation of YAP. In its hypo-phosphorylated status, YAP moves inside the nucleus where it interacts with various DNA-binding partners. In mammalian cells, YAP primarily binds all four Transcriptional Enhancer Associate Domains (TEAD1-4) [1]. TEAD transcription factors can induce gene transcription only upon interaction with YAP [2]. This protein-protein interaction (PPI) is essential for expressing Hippo pathway-downstream genes, modulating cell proliferation and apoptosis [2]. All human TEADs (hTEADs) have an acylation binding site, physiologically occupied by palmitic/myristic acid; even so, the functional role of acylation is yet not fully understood [3]. Dysregulation of the Hippo pathway is associated with tumorigenesis, thus targeting the YAP:TEAD interaction is an emerging, attractive therapeutic strategy in the oncology field [4]. The development of new modulators of this PPI is challenging, indeed very few YAP:TEAD4 inhibitors have been reported so far [5]. A relevant problem is the poor structural information available on this complex, limited to the characterization of the C-terminal YAP-Binding Domain of hTEAD4 (hTEAD4-YBD) in complex with a 40mer peptide, a fragment of the TEAD-Binding Domain of human YAP (hYAP-TBD). Aiming to expand the current structural understanding on this PPI, we developed reliable protocols for co-expression and co-purification of hTEAD4-YBD in complex with hYAP-TBD (L-complex) and with two shorter fragments, including 70 and 90 residues (S and M complex, respectively). All three complexes were crystallized but, despite optimization, crystals showed only poor diffraction in preliminary X-ray crystallographic analyses. Meaningful improvements were achieved by the microseeding technique, allowing to obtain diffraction quality crystals for both the S and L complexes. The structure of the S complex was determined to 2.5 Å resolution, in the trigonal space group P3121 with unit cell parameters  $a, b = 164.58 \text{ \AA}$  and  $c = 258.61 \text{ \AA}$ , whereas the L complex was obtained to 3.1 Å resolution. The crystal ASU consists of 12 heterodimers, showing new structural insights on hYAP:hTEAD4 PPIs. Recently, we further expanded the study of this complex to the characterization of hTEAD4-YBD in complex with full length hYAP (XL complex). We set up a production protocol and preliminary structural characterization through bioSAXS, cryo-EM and X-ray crystallography are currently ongoing. Our results expand the current knowledge on the downstream effectors of the Hippo pathway, providing novel information to design hYAP:hTEAD4 PPI modulators.

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